Loss of β-cell identity in diabetes: Significance of miR-7-mediated chromatin remodelling

by

Yorrick von Ohlen

Cellular Identity and Metabolism Group

Institute of Clinical Sciences

Faculty of Medicine

MRC – London Institute of Medical Sciences

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Declaration of Originality

I, Yorrick von Ohlen, declare that the work presented in this thesis is my own and that any work carried out by others has been acknowledged and appropriately referenced in the text or the figure legends. The following people contributed to work presented in this thesis.

Briefly:

Tracy Mak: Lineage tracing experiments for the identification of β- to δ-cell transdifferentiation in Tg7a2 tdTomato$^{RIP\text{-}Cre}$ mice

Yi-Fang Wang: Bioinformatical analysis of the ATAC- and RNA-Seq raw data

Mathieu Latreille: Isolated mouse islet preparations, and performed and analysed the RNA-Seq and the ATAC-Seq

Copyright Declaration

Abstract

Type 2 diabetes (T2D) affects 10% of the adult global population and is a multifactorial disease characterised by insulin resistance of peripheral organs. Insulin resistance results eventually in failure of pancreatic β-cells and subsequent apoptosis. Recent research revealed that β-cell dedifferentiation is contributing to decreasing their function in T2D. Dedifferentiated β-cells express atypical non-β-cell hormones, suggesting transdifferentiation into other cells. Our group previously showed that miR-7 levels are increased in T2D. Upregulation of miR-7a2 in β-cells of mice resulted in a diabetic phenotype and loss of key β-cell identity markers.

The aims of this study were to 1) investigate if miR-7a2 triggers the dedifferentiation of β-cells, 2) identify messenger RNA (mRNA) targets of miR-7 which contribute to induce β-cell dedifferentiation in T2D, and 3) assess how miR-7 targets impact on the functional identity of mature β-cells.

Our results showed that elevated miR-7a2 levels impair β-cell identity in mouse and human, accompanied by the occurrence of polyhormonal β-cells. Furthermore, we demonstrated that miR-7a2 is a regulator of the chromatin remodelling mSWI/SNF complexes, through repression of the catalytic ATPase subunit Brahma-related gene 1 (Brg1) both *in vitro* and *in vivo*. Additionally, we revealed that miR-7a2 represses the expression of BAF60A, a regulatory subunit of mSWI/SNF complexes.

β-cell-specific miR-7a2 overexpression in mice resulted in the closure of chromatin around genes involved in maintaining β-cell identity. Those regions overlapped with binding sites of β-cell specific transcription factors (TF). Knockdown of Brg1 in β-cell lines and β-cell specific Brg1 deletion in mice resulted in the loss of β-cell identity as revealed by the downregulation of β-cell markers, reduction of pancreatic islet insulin protein content and an impairment in glucose stimulated insulin secretion (GSIS).

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Abbreviations

2w	2 weeks
12w	12 weeks
RPLP0/36B4	Ribosomal phosphoprotein P0
37°C incubator	37°C, 95% O_2/5% CO_2 incubator
αKG	α-ketoglutarate
Ad-7a2	MiR-7a2 overexpressing adenovirus
Ad-Ctrl	Control adenovirus
ADP	Adenosine diphosphate
AG	Aminoguanidine
Ago	Argonaute
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
APS	Ammonium persulfate
ATP	Adenosine-5'-triphosphate
ATAC-Seq	Assay for Transposase-Accessible Chromatin with high-throughput sequencing
BAF	Brm/Brg1-associated factor
BG	Blood glucose
BMI	body mass index
Brg1	Brahma-related gene 1
$Brg1^{fl/fl}$	Floxed Brg1
Brm	Brahma
BSA	Bovine serum albumin
BW	Body weight
cAMP	Cyclic AMP
cDNA	Complementary DNA
CHD	Chromodomain-helicase-DNA-binding protein
ChREBP	Carbohydrate response element binding protein
CPE	Carboxypeptidase E
Cre	Cre recombinase
CREB	cAMP-responsive element-binding protein
CTCF	CCCTC-binding factor
db/+	heterozygous control for *db/db* diabetic mice
DAG	Diacylglycerol
Dapi	4′,6-diamidino-2-phenylindole
DAR(s)	Differential accessible region(s)
DMEM	Dulbecco's Modified Eagle Medium with 4.5 g/L glucose and phenol red
DMSO	Dimethyl sulfoxide
Dnmt	DNA methyltransferases
E. coli	*Escherichia coli*
Epac	Exchange protein directly activated by cAMP

ER	Endoplasmic reticulum
ES	embryonic stem (cells)
Exoc7	Exocyst complex component 7
Exp-5	Exportin-5
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum
Fbxo32	F-Box Protein 32
FFA	Free fatty acids
FGF	Fibroblast growth factor
Foxa2	Forkhead box A2
Foxo1	forkhead box O1
G6PC2	Glucose-6-phosphatase 2
GATA	GATA binding protein
Gcg	Glucagon
Gck	Glucokinase
GDH	Glutamate dehydrogenase
GDP	Guanosine diphosphate
GFP	Green fluorescent protein
GLP-1	glucagon-like peptide-1
GLP-1R	GLP-1 receptor
Glut	Glucose transporter
GNAS	Guanine Nucleotide Binding Protein (G Protein), Alpha Stimulating Activity Polypeptide 1
Grhl2	Grainyhead like 2
GSIS	Glucose stimulated insulin secretion
GTP	Guanosine-5'-triphosphate
H3K9ac	Histone 3 Lysine 9 acetylation
H3K14ac	Histone 3 Lysine 14 acetylation
H3K27me3	Histone 3 Lysine 27 trimethylation
HDAC	Histone deacetylase
HEK293T	Human embryonic kidney cells expressing the large T antigen
Hhex	Hematopoietically expressed homeobox
Hnf1β	Hepatocyte nuclear factor 1 homeobox β
Hnf6	Hepatocyte nuclear factor 6
IAPP	Islet amyloid polypeptide
IC_{50}	Half maximal inhibitory concentration
Il-1R1	IL-1 receptor type 1
Inpp5k	Inositol Polyphosphate-5-Phosphatase K
Ins+	Ins positive
Ins1E	Immortalised rat pancreatic β-cell line
Ins1-Cre	Cre recombinase behind the mouse Ins1 promoter
Ins	Insulin
IP	Intra-peritoneal
IPGTT	Intraperitoneal glucose tolerance test

IPITT	Intraperitoneal insulin tolerance test
IR	Insulin receptor
IRS	Insulin receptor substrate
IRTK	Insulin receptor tyrosine kinase
K^+	Potassium ion
K-ATP	ATP-sensitive potassium (channel)
KLF4	Krüppel-like factor 4
LDB1	LIM domain-binding protein 1
Lipofectamine	Lipofectamine 2000
LMS	MRC London Institute of Medical Sciences
LNA	Locked Nucleic Acid
LNA-ctrl	Control for LNA-7a, non-targeting LNA
LNA-7a	LNA-7 miRCURY LNA Inhibitor has-miR-7-5p
L-VDCC	Dihydropyridine-sensitive L-type Ca^{2+} channels
MafA/MafB	MAF BZIP Transcription Factor A/B
MCT2	Monocarboxylate Transporter 2
Mg	Magnesium
Min6	Immortalised mouse pancreatic β-cell line
miRNA(s)	microRNA(s)
miR-x	miRNA-x (e.g. miR-7)
miR-NT	Si-non-targeting
MODY	Maturity onset diabetes of the young
MPC(s)	Multipotent pancreatic progenitor cell(s)
mRNA	Messenger RNA
C-Myc	V-Myc Avian Myelocytomatosis Viral Oncogene Homolog
NAC	N-acetyl-L-cysteine
NBDs	Nucleotide binding domains
Neurod1	Neuronal Differentiation 1
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
Ngn3	Neurogenin 3
Nkx2.2	Homeobox Protein NK-2 Homolog B
Nkx6.1	Homeobox protein NK-6 homolog 1
ON	Overnight
Optimem	Optimem-media, reduced serum, no phenol red
P/S	Penicillin-Streptomycin
padj	adjusted p value
PAHSA	Palmitic-acid-9-hydroxy-stearic-acid
Pax4	Paired box 4
Pax6	Paired Box 6
PBS	Phosphate buffered saline
PC2 and PC1/3	Prohormones convertases 2 and 1/3
PCR	Polymerase chain reaction
PDK-1	3-phosphoinositide-dependent protein kinase 1
Pdx-1	Pancreatic and duodenal homeobox 1

PFA	Paraformaldehyde
PI3K	Phosphatidylinositol 3-kinase
PIP_2	Phosphorylates phosphatidylinositol 4,5-bisphosphate
PIP_3	Phosphatidylinositol (3,4,5)-triphosphate
PKA and PKC	Protein kinase A and C
Ppy	Pancreatic polypeptide
PRC1 and PRC2	Polycomb Repressive Complex 1 and 2
pre-miRNAs	precursor miRNAs
pri-miRNAs	primary miRNAs
Ptf1a	Pancreas Associated Transcription Factor 1a
qPCR	Quantitative polymerase chain reaction
TdTomato+ or -	TdTomato positive or negative
Rae1	Retinoic acid early transcript 1
Rim2	Rab3 interacting molecule 2
RISC	RNA-induced silencing
RIP	Rat insulin II promoter
RIP-Cre miR-7a2$^{fl/fl}$	Knockout of miR-7a2 in β-cells by RIP-Cre
RNA-Seq	RNA-Sequencing
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute medium 1640
RTK(s)	Receptor tyrosine kinase(s)
SDS	Sodium dodecyl sulfate
shRNA	Small hairpin RNA
siRNA	Small interfering RNA
Slc30a8	Solute carrier family 30
SLS	Scientific laboratory supplies
SNARE	Soluble NSF attachment protein receptor
Sno202	Small nucleolar RNA MBII-202
Sst	Somatostatin
SUR-1	Sulfonylurea receptor-1
SWI/SNF	switch/sucrose non-fermentable
T2D	Type 2 diabetes
TBS	TRIS-buffered saline
TBS-T	TBS with 0.1% Tween20
TCA	Tricarboxylic acid cycle
tdTomato$^{RIP\text{-}Cre}$	Rosa26-floxed-Stop-tdTomato transgene + RIP
TF(s)	Transcription factor(s)
Tg7a2	Transgenic-miR-7a2
Tris-HCl	Trizma ®-base – hydrogen chloride
Tsc1	Tuberous sclerosis complex 1
TXNIP	Thioredoxin-interacting protein
Ucn3	Urocortin 3
UTR	Untranslated region
VDCC	Voltage-dependent calcium channel

VGLUT	Vesicular glutamate transporter
t-SNARE	Target-SNARE
v-SNARE	Vesicles-SNARE
Wt	Wildtype
Zn^{2+}	Zinc

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Chapter 1: Introduction

In 2011, the estimated number of people suffering from diabetes world-wide was 366 million, which is approximately 1 out of every 11 people in the adult population which is expected to rise to 550 million by 2030 (DiabetesUK 2011). Diabetes comes in two forms, named type 1 (T1D) and type 2 diabetes (T2D), with T2D accounting for over 90% of diabetes cases (Tripathi and Srivastava 2006). While T1D is mainly described as an autoimmune disease, leading to the destruction of insulin (Ins) producing pancreatic β-cells (Tripathi and Srivastava 2006), T2D is characterised by insulin resistance in peripheral organs, like the muscle, which results in hyperglycaemia and eventually impaired insulin secretion by β-cells (Kahn 1998). T2D is characterised as a complex and multifactorial disease, which includes not only genetic, but also environmental and behavioural risk factors (Chen, Magliano and Zimmet 2011). The hyperglycaemic phenotype observed in individuals suffering from T2D leads to a heightened risk in developing retinopathy (Fong 2003), vasculopathy (Cooper 2001), neuropathy (Bansal, Kalita and Misra 2006), renal (Iseki et al. 2003) and cardiac disease (Sowers, Epstein and Frohlich 2001), as well as cancer (Satija et al. 2015). Together these morbidities affect the quality of life and lead to premature death.

1.1.1 The pancreas

The pancreas is a composite organ, with exocrine, performed by acinar cells, and endocrine function (Chandra and Liddle 2013). The majority of endocrine cells are organised within islets of Langerhans, first described by Paul Langerhans in 1969 (Ceranowicz et al. 2015). In human, it is estimated that, on average, 3.2 million islets are found within the pancreas (Ionescu-Tirgoviste et al. 2015). The majority of the pancreatic islet is comprised of insulin producing β-cells (~60% in human), together with glucagon (Gcg) producing α-cells (~30% in human), somatostatin (Sst) producing δ-cells (<10%), pancreatic polypeptide (Ppy) producing γ-cells (<5%) and ghrelin producing ε-cells (Fig. 1.1A) (Orci et al. 1976, Brissova et al. 2005, Cabrera 2006). Contrary to human islets, where no specific arrangement of endocrine cells is seen, mouse islets, not only consist of an increased number of pancreatic β-cells (70%) and a decrease in the number of pancreatic α-cells, but are also arranged in a particular manner, with β-cells forming the core of the islets and the remaining endocrine cells a surrounding mantle (Fig. 1.1B) (Cabrera 2006, Steiner 2010). Interestingly, islet size and cell numbers are highly conserved, ranging from 50-400 μm in diameter and 2000-4000 cells per islet (Jo, Choi and

Koh 2007, Steiner 2010). In mammals, β-cells are the only known cells to produce and release insulin into circulation (Kulkarni 2004), opposite to invertebrates like *Drosophila melanogaster*, where a cluster of 14 insulin producing cells, located in the pars intercerebralis of the brain, produce three different insulin-like peptides (Brogiolo et al. 2001, Cao and Brown 2001, Rulifson, Kim and Nusse 2002, Kapan et al. 2012). In response to a rise in glycaemia, β-cells secrete insulin into the circulation which binds to its receptor in peripheral tissues, mainly the liver, skeletal muscle and adipose tissue, and promotes cellular glucose uptake, regulating lipid, protein and carbohydrate metabolism (Prentki, Matschinsky and Madiraju 2013). In carbohydrate metabolism, insulin increases the rate of glucose transport across the cell membrane, induces hexokinase and 6-phosphofructokinase to increase glycolysis and promotes the rate of glycogen synthesis while inhibiting the breakdown of glycogen (Dimitriadis et al. 2011). Insulin increases the rate of transport of certain amino acids across the cell membrane and promotes protein synthesis while inhibiting protein breakdown (Dimitriadis et al. 2011). Insulin effects lipid metabolism by decreasing the rate of lipolysis in adipose tissue, stimulates the uptake of triglycerides into adipose tissue and muscle and increases triacylglycerol and fatty acid generation while inhibiting breakdown of fatty acids (Dimitriadis et al. 2011). In addition, insulin is able to induce the proliferation of cells, for example by activation of farnesyltransferase. Through this, insulin potentiates the mitogenic response of cells to growth factors (Tennagels and Werner 2013, Goalstone et al. 1997).

In contrast, Gcg secretion is directly stimulated by hypoglycaemia (Quesada, Todorova and Soria 2006), and counteracts the function of insulin by binding directly to the Gcg receptor, found highly expressed in the liver, thus promoting hepatic glucose output by stimulating glycogenolysis and gluconeogenesis but inhibiting glycogenesis and glycolysis (Dunphy, Taylor and Fuller 1998, Jiang and Zhang 2003). In the islet Sst regulates the other endocrine hormones through binding to the Sst receptor, found in multiple pancreatic endocrine cells, with subtype 1, 3 and 4 found in all endocrine cells, subtype 2 found in α- and β-cells and some in δ-cells and subtype 5 in most β- and δ-cells (Portela-Gomes et al. 2000). Sst inhibits the release of insulin and Gcg from β- and α-cells respectively (Mortimer et al. 1974, Alberti et al. 1973, Gerich et al. 1974) and is regulated by Ppy in a negative manner (Kim et al. 2014).

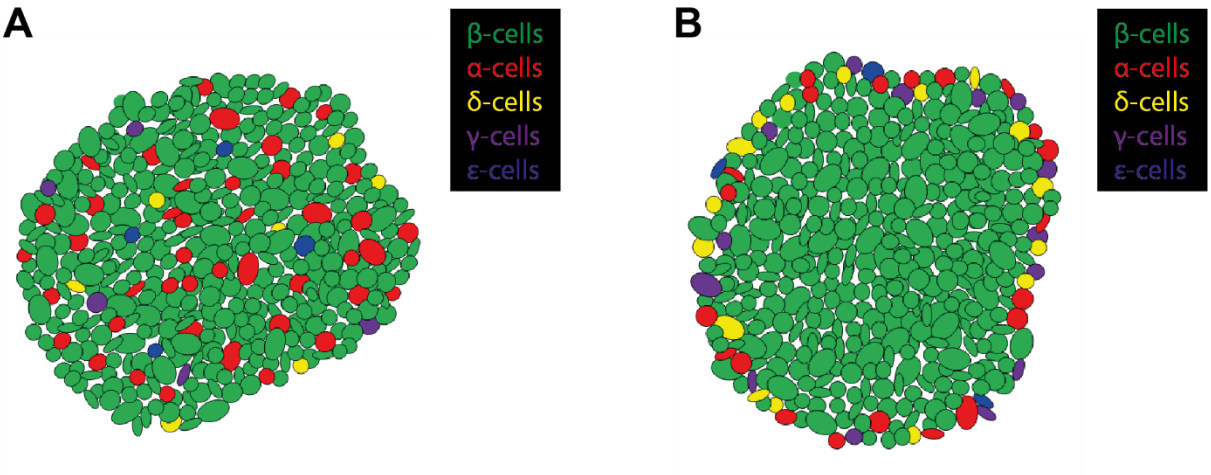


Figure 1.1: Schematic illustration of pancreatic islets of Langerhans. (A) Human islets. (B) pancreatic islets. While mouse islets show a strict organisation of β-cells forming the core of the islets surrounded by a mantle of other pancreatic endocrine cells, human islets miss this organisation.

1.1.2 Pancreatic β-cell differentiation during development

β-cells play a key role in the regulation of glucose homeostasis due to their ability to synthesise and secrete insulin. Therefore, many studies investigated the differentiation of pancreatic β-cells during development, which led to the identification of specific sets of TFs directing the specification of pancreatic endocrine progenitor cells (Kaneto and Matsuoka 2015).

Human pancreas development starts at around 4 weeks of gestational age with the formation of the pancreatic bud, followed by the ventral bud (Conrad, Stein and Hunter 2014). At this stage, specific TFs are activated (Fig. 1.2). High mobility group box TF *SOX17* is an example of a TF that is expressed in a specific time window of formation of the pancreatic bud in pancreatobiliary progenitor cells from immediately before 4 weeks and is excluded from pancreatic cells at around the start of week 5 (Jennings et al. 2013, Spence et al. 2009). Similar to *SOX17*, *SOX9* is expressed in the pancreatic bud in pancreatic and duodenal homeobox 1 (Pdx-1) positive cells from about 4 weeks onwards, but is excluded from mature pancreatic endocrine cells (Piper et al. 2002, Piper et al. 2004, McDonald et al. 2012, Jennings et al. 2013). On the other hand, TFs like forkhead box A2 (*FOXA2*), or *PDX-1* start being expressed in pancreatic budding from 4 weeks onwards (Lyttle et al. 2008, Jeon et al. 2009, Jennings et al. 2013) and are maintained in the mature pancreatic endocrine cells (Pan and Wright 2011). During early development in rodents *PDX-1* is expressed in the entire pancreatic epithelium and an increase of Pdx-1 is essential for commitment to β-cells in endocrine formation (Jonsson

et al. 1994, Holland et al. 2002, Pan and Wright 2011, Bernardo, Hay and Docherty 2008, Gao et al. 2008). In the islet in adulthood Pdx-1 is found in β-cells and δ-cells (Gao et al. 2014, Ahlgren et al. 1998). *FOXA2* is detectable in the entirety of pancreatic progenitor cells in early development, becomes progressively restricted to endocrine progenitor cells and is essential for β-cell maintenance (Willmann et al. 2016, Lantz et al. 2004, Gao et al. 2010). The TFs involved in the development of the pancreas and β-cells are involved in a crosstalk regulating the expression of each other. For example, studies in mice, reported the regulation of *PDX-1* by *SOX9*, Hepatocyte nuclear factor 1 homeobox β (*HNF1β*) and *FOXA2* in the early stages of pancreas development (Haumaitre et al. 2005, Lynn et al. 2007b, Gao et al. 2008). Additionally, *SOX9* regulates the expression of *FOXA2* and *HNF1β* and, in turn, both TFs regulate *SOX9* expression (Lynn et al. 2007b). The expression of homeobox protein NK-6 homolog 1 (*NKX6.1*) starts in early multipotent pancreatic progenitors cells (MPCs) after 4 weeks, when Sox17 levels retract, and is maintained in β-cells (Sander et al. 2000, Jennings et al. 2013).

Continued expression of GATA binding protein (*GATA*) 4, which starts to be expressed in early pancreatic budding between weeks 4 and 5 and remains only in mature acinar cells (Jennings et al. 2013), *FOXA2*, *PDX-1*, *SOX9* and *NKX6.1* leads to MPCs formation. MPCs will then be further specified into pancreatic ductal, exocrine and endocrine cell lineages (Cano et al. 2014). With the formation of MPCs, the expression of the TF *GATA6* begins (Conrad et al. 2014). *GATA6*, together with *GATA4*, plays an important role in differentiating MPCs into acinar and endocrine cell lineages and is involved in β-cell differentiation, as shown in rodents (Watt et al. 2007, Carrasco et al. 2012). In the analysis of human pancreas at weeks 7-21, Hepatocyte nuclear factor 6 (*HNF6*) was shown to be constantly expressed in the developing pancreas, supporting MPCs formation (Lyttle et al. 2008, Jeon et al. 2009). Similar results are seen in mice where *HNF6* expression eventually becomes restricted to pancreatic α- and acinar cells right before birth (Zhang et al. 2009). Additionally, *HNF1β* is expressed at high levels in the pre-pancreatic foregut endoderm and in MPCs, where it is essential for proliferation and survival of MPCs (Poll et al. 2006). Hnf1β is essential for early and late duct morphogenesis and required for endocrine progenitor specification (De Vas et al. 2015). In experimental setups, Hnf1β was measured at high levels in human from 7 weeks onwards and throughout pancreas development (Haumaitre et al. 2005, Jeon et al. 2009). Pancreas-specific transcription factor 1A (*PTF1A*) expression starts in MPCs in the early budding pancreas slightly after PDX-1 expression is detected and becomes progressively restricted to acinar cells (Cras-Méneur et al. 2009). *PTF1A* regulates *PDX-1* expression in a positive manner in pancreatic precursors

(Miyatsuka et al. 2007). Additionally, *PTF1A* plays a role in the endocrine versus exocrine cell fate decision, with low levels of *PTF1A* promoting the endocrine fate, whereas high levels promote the exocrine fate (Dong et al. 2008).

For the commitment of MPCs into endocrine cells, another set of specific TFs is required. Coincidental with the loss of *SOX9*, Neurogenin 3 (*NGN3*) expression is induced in pancreatic epithelial MPCs committing to the endocrine cell fate (Lyttle et al. 2008, Jennings et al. 2013, Memon et al. 2018). In mice, a first wave of *NGN3* expression takes place in early pancreas budding, recedes, and reoccurs in a second wave during endocrine cell lineage differentiation (Rukstalis and Habener 2009). In human, *NGN3* expression is shown to start at around 8 weeks, is highly expressed around 11 weeks and declines to low levels at around 19 weeks (Jeon et al. 2009, Capito et al. 2013, Jennings et al. 2013, Memon et al. 2018). *NGN3* expression precedes the induction of the TFs involved in late endocrine cell differentiation starting around 15 weeks (*ISL1*, *NEUROD1*, *MAFB*, *NKX2.2* and *PAX6*, discussed later) (Jeon et al. 2009). Other TFs involved in the endocrine cell lineage commitment are Regulatory factor X 6 (*RFX6*) (Smith et al. 2010, Taleb and Polychronakos 2011), Paired box gene 4 (*PAX4*) (Jeon et al. 2009), GLIS family zinc finger 3 (*GLIS3*) (Senée et al. 2006) and MAF BZIP TF B (*MAFB*) (Jeon et al. 2009, Hang and Stein 2011, Dai et al. 2012). *PAX4* is experimentally shown to be expressed from 9 weeks onwards (Jeon et al. 2009) and, while to be confirmed in human, is found in mouse endocrine progenitors and later in pancreatic β-cell precursors (Sosa-Pineda et al. 1997). In mice, the expression of *MAFB* diminishes in mature β-cells, opposite to in human where the expression of *MAFB* starts in MPCs at 7 weeks, increases during pancreatic endocrine cell differentiation to 21 weeks and then remains in pancreatic α- and β-cells (Jeon et al. 2009, Hang and Stein 2011, Dai et al. 2012).

While the correct development of the pancreas relies on a crosstalk between TF, various signalling pathways contribute as well. For example, pancreas formation prior pancreatic budding is induced by morphogenic signals, including activin-βB and fibroblast growth factor (FGF) 2, released by the notochord (Kim et al. 2000, Kumar and Melton 2003, Martín et al. 2005). Those signals suppress sonic hedgehog signalling which activates pancreatic gene expression (Hebrok, Kim and Melton 1998, Xuan and Sussel 2016). Sonic hedgehog signalling has also been reported to be inhibited by the TFs *GATA4* and *GATA6* in pancreatic development (Xuan and Sussel 2016). The pancreatic epithelium and the surrounding mesenchyme are also involved in signalling processes involved in pancreas development (Golosow and Grobstein 1962). Factors like FGF10, produced by the mesenchyme, has been shown to act through its

receptor FGFR2 to enhance and maintain *PDX-1* expression by activation of Notch signalling (Bhushan et al. 2001, Norgaard, Jensen and Jensen 2003). FGF10, FGF2R and Sox9 have been shown to regulated pancreatic cell differentiation during pancreatic bud formation, and disruption results in a change to the liver cell fate (Seymour et al. 2012). FGF10 maintains the expression of *SOX9*, and Sox9 induces *FGF2R* expression, which enables FGF10 signalling in a feed-forward loop (Seymour et al. 2012).

The first insulin positive cells occur in human at a gestation age of 8 weeks (Polak et al. 2000, Jeon et al. 2009). In contrast to humans, mice show two waves of endocrine cell development with insulin and Gcg co-expressing cells in a first wave from about embryonic day (E) 9.5 to E12.5 and, in a second wave from E12.5 to birth, the generation of endocrine cells that form the mature islet (Herrera 2000, Cano et al. 2014, Jennings et al. 2013). In the differentiation of pancreatic progenitor cells into β-cells, additional TFs are involved Homeobox Protein NK-2 Homolog B (*Nkx2.2*) expression in human starts at around 8 weeks with an increased expression by 14-16 weeks (Jennings et al. 2013, Lyttle et al. 2008). In mice, *Nkx2.2* is expressed from in MPCs at E9.5 and restricted to β-cells and a subset of α- and γ-cells in the mature islet (Sussel et al. 1998). A nonsense mutation of Insulin gene enhancer protein ISL-1 (Isl1) in human T2D patients showed its involvement in the development of the pancreas and its function in the maturation of β-cells (Shimomura et al. 2000). Neuronal Differentiation 1 (*NEUROD1*) expression starts at endocrine lineage commitment at around week 15 and is found in all endocrine cell types of pancreatic islets (Jennings et al. 2013, Lyttle et al. 2008, Jeon et al. 2009). Mutations of *NEUROD1* in pancreatic β-cells show its importance for the maturation of β-cells, as mutants remained in an immature state (Gu et al. 2010). Paired Box 6 (*PAX6*) is required for the spatial organisation and correct development of islet endocrine cell precursors and is induced after endocrine progenitor cell commitment triggered by NGN3 expression (St-Onge et al. 1997, Sander et al. 1997, Gradwohl et al. 2000). In experiments, *PAX6* expression starts around week 14-16 in the human pancreas and is continued to be expressed in all pancreatic endocrine cells (Lyttle et al. 2008). Knockout of *PAX6* in mice shows a reduction in islet cell mass, hormone synthesis and an impaired islet morphogenesis in the embryo (Sander et al. 1997). The expression of MAF BZIP Transcription Factor A (*MAFA*) arises relatively late in the pancreatic development, with MafA only detectable in the second wave of insulin positive cells in mice and after 21 weeks in human in β-cells (Jeon et al. 2009, Hang and Stein 2011, Guo et al. 2013). *MAFA* is specifically expressed in mature β-

cells and controls the glucose responsiveness of β-cells through the regulation of *INS* and *SLC2A2* genes (Dai et al. 2012, Hang and Stein 2011).

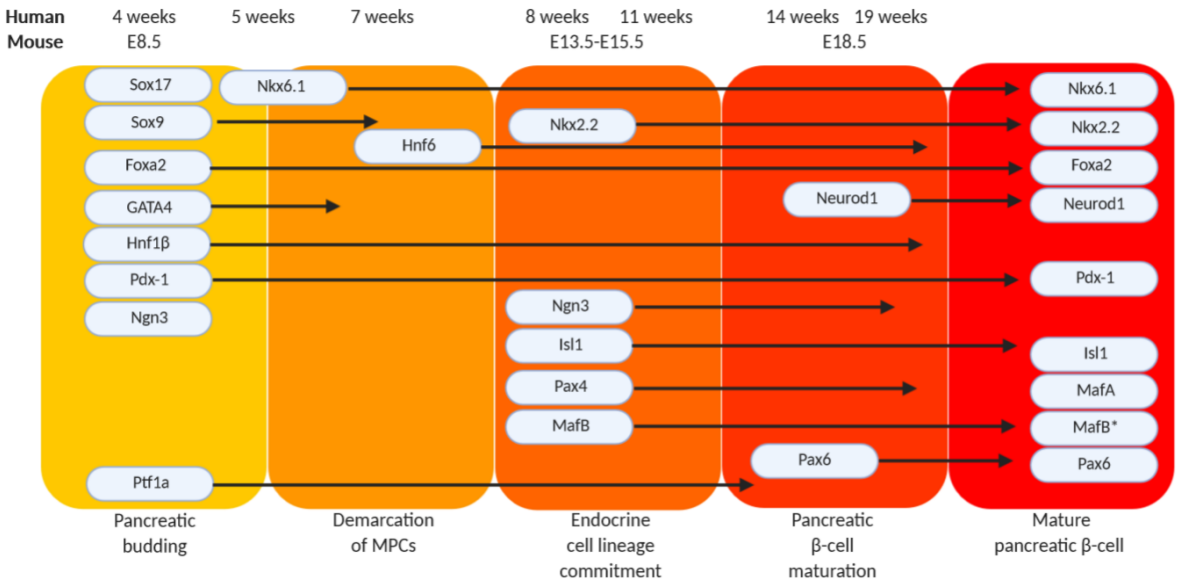


Figure 1.2: Schematic illustration of the expression profile of TFs involved in pancreatic β-cell differentiation. Specific sets of TFs regulate the differentiation and maintain the identity of β-cells. Arrows indicate ongoing TF expression. MafB is not found in mouse mature β-cells. Adapted from Conrad et al. 2014.

1.1.3 Insulin biosynthesis

Insulin is synthesised from a 110 amino acid long preproinsulin polypeptide with a weight of 5.8 kDa, which, upon processing, leads to the production of mature insulin composed of 51 amino acids (Fu 2013) (Figure 1.3). Preproinsulin contains a hydrophobic N-terminal signal peptide which interacts with ribonucleoprotein signal recognition particles, found on the cytoplasmic side of the rough endoplasmic reticulum (ER), thus allowing preproinsulin to cross the membrane of the rough ER through the peptide conducting channel (Fu 2013, Egea, Stroud and Walter 2005, Chan, Keim and Steiner 1976, Lomedico et al. 1977). Upon translocation in the ER, signal peptidases cleave off the signal peptide, producing proinsulin from preproinsulin (Patzelt et al. 1978). The tertiary structure matures, as found for the final insulin peptide, by folding of the proinsulin, as well as forming its three disulphide bonds through chaperone proteins, such as protein-thiol reductase (Munro and Pelham 1987, Huang and Arvan 1995). The immature form of insulin, proinsulin, has only a low affinity to the insulin receptor and requires additional proteolytic processing to become the bioactive hormone (Steiner 1969,

Galloway et al. 1992). Proinsulin enters the Golgi apparatus where packaging of proinsulin into immature secretory vesicles occurs (Huang and Arvan 1994). Cleavage of the connecting domain (C-domain) from the A- and B-domain of proinsulin (Fig. 1.3) does not take place within the trans Golgi network, but inside the immature secretory vesicles by prohormones

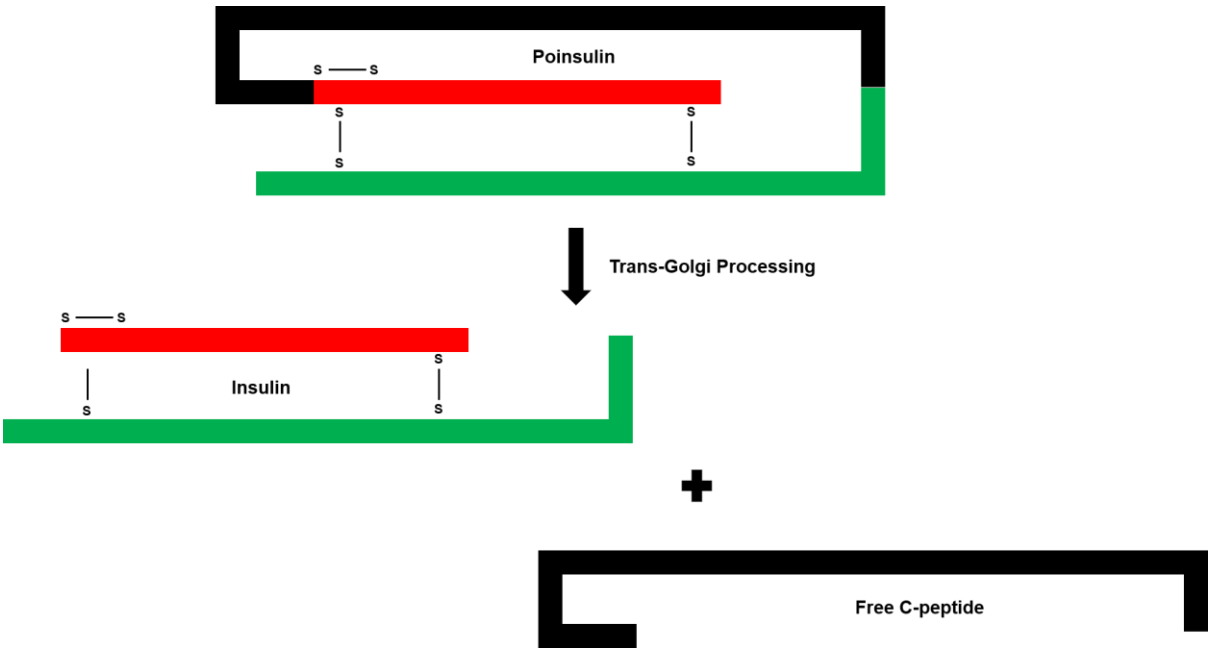


Figure 1.3: Schematic illustration of processing of proinsulin into mature insulin. Cleavage by prohormone convertases and carboxypeptidase E between the A-domain (red), C-domain (black) and B-domain (green) forms the mature insulin and free C-peptide. Adapted from Weiss 2009.

convertases 2 and 1/3 (PC2, PC1/3) and carboxypeptidase E (CPE) (Huang and Arvan 1994, Steiner 1998). PC1/3 cleaves between the B- and C-domain at the C-terminal side of the basic amino acid pair Arg^{31}-Arg^{32} and PC2 cleaves the bond between the A- and C-domain at the C-terminal side of the amino acid pair Lys^{64}-Arg^{65} (Davidson, Rhodes and Hutton 1988). CPE then removes the leftover C-terminal basic residues generated by the cleavage of proinsulin, forming the mature insulin hormone and free C-peptide (Davidson and Hutton 1987). Insulin is then stored in specialised secretory vesicles in the form of hexamers stabilised by zinc (Zn^{2+}), together with the free C-peptide, islet amyloid polypeptide (IAPP) and other pancreatic β-cell secretary products like glutamate (Nishi et al. 1990, Huang and Arvan 1995, Gheni et al. 2014). The Zn^{2+}-insulin-hexamers are thought to be a prevention against toxic protein misfolding of insulin inside β-cells since the insulin monomer is susceptible to fibrillation (Brange et al. 1997, Dodson and Steiner 1998). Upon vesicle exocytosis, the hexamers dissociate freeing insulin

monomers which can then mediate its biological effects on glucose homeostasis (Gold and Grodsky 1984).

1.1.4 Glucose-stimulated insulin secretion

GSIS (Fig. 1.4) is initiated by the uptake of glucose into β-cells by the constitutively expressed glucose transporter (Glut) 2 (Newgard and McGarry 1995, Thorens et al. 2000). Glut2 displays a high capacity, but low affinity (K_M ~17 mM), for glucose (Thorens et al. 2000), which ensures the controlled release of insulin in response to rising blood glucose levels and prevents inappropriate insulin release that could result in life threatening hypoglycaemia (Güemes and Hussain 2015). Upon its transport in the β-cell, glucose is phosphorylated by glucokinase (Gck, K_M 6 mM), a pancreas and liver specific hexokinase (Dipietro, Sharma and Weinhouse 1962, Matschinsky and Ellerman 1968, Ashcroft and Randle 1970), which initiates metabolization through glycolysis and acts as the rate-limiting enzyme in glucose metabolism and insulin secretion (Iynedjian 1993, De Vos et al. 1995, Newgard and McGarry 1995, Matschinsky 1996). In glycolysis, glucose gets converted to the metabolic substrate pyruvate, which was discovered among others by G. Embden, O. Meyerhof and J.K. Parnas (Kresge, Simoni and Hill 2005). Pyruvate is then transported into the mitochondria where it gets oxidised through the tricarboxylic acid (TCA) cycle to produce adenosine-5'-triphosphate (ATP) from adenosine diphosphate (ADP) (Berg, Tymoczko and Stryer 2002). The rise in cytosolic ATP concentration inside the β cells, and, subsequently, a decrease in magnesium (Mg) ADP that shifts the ATP:ADP ratio in favour of ATP, leads to the closure of plasma membrane localised ATP-sensitive potassium (K-ATP) channels (Tarasov, Dusonchet and Ashcroft 2004, Henquin 2009). The K-ATP channel is composed of two tetramer forming subunits (Tarasov et al. 2004). Four inward-rectifier potassium channel subunits (Kir6.2) form the centric pore of the K-ATP channel (Inagaki et al. 1995, Sakura et al. 1995). Each Kir6.2 subunit has an integrated ATP-binding site, enabling four possible ATP binding events to occur simultaneously (Tucker et al. 1997, Craig, Ashcroft and Proks 2008). The Kir6.2 subunits bind one regulatory sulfonylurea receptor (SUR)-1 subunit each, which are arranged in a tetramer and form the outer part of the K-ATP channel (Aguilar-Bryan et al. 1995). The binding sites for ATP on the Kir6.2 subunits have a high selectivity for the adenine base and, although possible, is guanosine-5'-triphosphate (GTP) binding less potent than ATP binding events as the half maximal inhibitory concentration (IC_{50}) for GTP is 6 mM compared to the IC_{50} of ATP being

~100 µM (Trapp, Tucker and Ashcroft 1997, Tucker et al. 1998). A minimum of two phosphate groups are required for channel inhibition, as binding of ATP and ADP lead to similar K-ATP channel closures while adenosine monophosphate (AMP) is less effective (Tucker et al. 1998). In contrast to the nucleotide binding sites of SUR-1, Kir6.2's binding sites do not require the presence of Mg (e.g. MgADP or MgATP) for inhibition of the K-ATP channel (Ashcroft and Rorsman 1989, Gribble, Tucker and Ashcroft 1997, Gribble et al. 1998). In the absence of ATP the K-ATP channel is spontaneously and ligand independently active in form of short bursts of opening and closing, with long closed states in between, allowing potassium (K^+) to flow out of β-cells (Craig et al. 2008). ATP can bind to Kir6.2 in the open and closed state of the K-ATP channel reducing the duration of the opening bursts and prolonging the overall closed state preventing K^+ to exit the cell (Craig et al. 2008).

Opposite to the inhibition of K-ATP channels through binding of ATP to Kir6.2, the regulatory subunit SUR-1, member of the ATP-binding cassette transporter family (Tarasov et al. 2004), activates K-ATP channels through binding of MgATP/MgADP via two cytosolic nucleotide binding domains (NBDs) (Nichols et al. 1996, Gribble et al. 1997, Shyng, Ferrigni and Nichols 1997, Tucker et al. 1997). In contrast to MgADP, MgATP does not directly activate the K-ATP channel, but hydrolysis of MgATP at the NBD2 results in the production of MgADP which then binds NBDs and activates the K-ATP channel (Zingman et al. 2001). In the presence of Mg^{2+} the half maximal inhibitory concentration (IC_{50}) for ATP induced channel closure increases from 6-10 µmol/l to 10-30 µmol/l (Gribble et al. 1998). Contrary to the Kir6.2 subunits, MgGTP/MgGDP are able to activate SUR-1 to a similar extent as MgATP/MgADP and guanosine diphosphate (GDP). Opposite to ATP, GTP is able to activate K-ATP channels to an even higher extent than MgGDP (Trapp et al. 1997). Although, according to literature, mainly involved in the activation of K-ATP channels, mutations in the SUR-1 encoding gene *ABCC8* resulted in a loss of ATP sensitivity of the K-ATP channel and an impairment of glucose sensing, showing that SUR-1 is also involved in the ATP induced closure of K-ATP channels (Tarasov et al. 2008). In summary, the activity of the K-ATP channel does not only depend on the ATP:ADP ratio, but rather on the ratio of inhibitory binding events of ATP and ADP to the Kir6.2 subunits and activating events of MgADP and MgATP binding at the NBDs of the SUR-1 subunits (Tarasov et al. 2004).

Glucose triggers closure of K-ATP channels resulting in a cytoplasmic increase of K^+ levels. Such increase in intracellular K^+ concentration leads to the depolarisation of the β-cell plasma membrane leading to an opening of voltage-dependent calcium channels (VDCC) including

dihydropyridine-sensitive L-type Ca^{2+} channels (L-VDCC) (Tarasov et al. 2004, Henquin 2009, Tsien et al. 1988, Schulla et al. 2003). These are activated by strong membrane depolarisation events and are inhibited by low concentrations of Ca^{2+} antagonist drugs like dihydropyridines (Catterall and Striessnig 1992, Striessnig, Berger and Glossmann 1993). L-VDCC consist of four subunits (α1, α2δ, β and γ) (Curtis and Catterall 1984, Curtis and Catterall 1986, Flockerzi et al. 1986, Hosey et al. 1987, Leung, Imagawa and Campbell 1987, Striessnig et al. 1987, Takahashi et al. 1987). The α1 subunits of L-VDCC are part of the Ca_V1 type family (Snutch and Reiner 1992, Ertel et al. 2000, Catterall 2011) and consist of four homologous domains forming the pore of the channel (Takahashi et al. 1987, Tanabe et al. 1987). Each domain contains six transmembrane segments (S1-6). Between S5 and S6 is a membrane associated loop (Takahashi et al. 1987). Depolarisation creates an electric field in which the S4 segments move outward and rotate, inducing the conformational changes required for L-VDCC opening (Catterall 2011). In contrast to the K^+ concentration, intracellular levels of Ca^{2+} are lower than the extracellular environment of the β-cell. Opening of L-VDCC results in Ca^{2+} influx into the cell and a subsequent rise in the cytosolic Ca^{2+} concentration (Rorsman, Braun and Zhang 2012). Cytosolic Ca^{2+} concentrations can also be influenced by the release of Ca^{2+} from intracellular stores like the ER controlled by ryanodine and inositol 1,4,5-triphosphate receptors (Gilon et al. 2014, Yang et al. 2014). Increases in Ca^{2+} levels result in exocytosis of secretory vesicles transporting insulin to the plasma membrane (Hou, Min and Pessin 2009).

Cytosolic Ca^{2+} is sensed by synaptotagmin III and VII found on the membrane of insulin vesicles (Gao et al. 2000, Gut et al. 2001, Fukuda et al. 2004, Wang et al. 2005). The family of synaptotagmins is made of two C2 regions which form the majority of its cytoplasmic region and are homologues of the regulatory C2 domain of protein kinase C (Perin et al. 1990, Fernandez et al. 2001). Both C2 subunits of synaptotagmins are involved in binding Ca^{2+} leading to exocytosis of insulin vesicles through the formation of the large soluble NSF attachment protein receptor (SNARE) complexes between the secretory vesicles and the plasma membrane (Chapman 2002). The SNARE complex is arranged as four SNARE motifs in a twisted parallel bundle of four helices by the interaction of vesicles-SNARE (v-SNARE) and target-SNARE (t-SNARE) proteins found on the vesicles and the plasma membrane, respectively (Hong 2005, Hou et al. 2009). In the β-cell VAMP2 (Regazzi et al. 1995) is identified as a v-SNARE protein and is shown to interact with the t-SNARE proteins syntaxin

1 (Martín et al. 1995) and SNAP25 (Gonelle-Gispert et al. 1999) to trigger fusion of the plasma membrane with the insulin vesicle, thus leading to insulin release in the circulation.

Insulin secretion can be amplified by different signals, like glutamate (Maechler and Wollheim 1999, Gheni et al. 2014) or the generation of cyclic AMP (cAMP) after activation of the Gcg-like peptide-1 (GLP-1) receptor (GLP-1R) (Drucker 2006, Holst 2007). GLP-1, an incretin secreted by L-cells of the gut, promotes insulin gene transcription, the secretion of insulin and the loading of glutamate into insulin-containing secretory vesicles (Renström, Eliasson and Rorsman 1997, Mortensen et al. 2003, Gheni et al. 2014, Fu, Gilbert and Liu 2013). Studies have shown that oral glucose administration triggers a stronger insulin response than intravenous administration of glucose through the secretion of GLP-1, known as the incretin effect (Nauck et al. 1986, Nauck et al. 1993). Activation of the GLP-1R leads to an increase of cytosolic cAMP, generated by the α-subunit of the G-protein of the receptor (Drucker et al. 1987, Yang, Lee Sr and Wand 1997), which can augment insulin secretion in protein kinase A (PKA) dependent and independent manners (Taylor, Buechler and Yonemoto 1990, Renström et al. 1997).

PKA is made of a catalytic subunit and a regulatory subunit containing the cAMP binding site and inhibiting PKA function in the absence of cAMP (Taylor et al. 1990, Cheng et al. 2008). cAMP binding to the regulatory subunit of PKA induces intra-kinase conformational changes leading to its activation and phosphorylation of specific protein substrates (Taylor et al. 1990). Among these are the SUR-1 subunits of the K-ATP channel which promotes inhibition of the channel by ADP, thus facilitating insulin secretion (Light et al. 2002). Additionally, L-VDCC are phosphorylated by PKA, leading to an increase in Ca^{2+} influxes into the β-cell (Ämmälä, Ashcroft and Rorsman 1993, Rorsman et al. 2012).

On the other hand, cAMP can regulate insulin secretion in a PKA independent manner through the activation of exchange protein directly activated by cAMP (Epac), also known as cAMP-regulated guanine exchange factor (cAMP-GEF) (Kawasaki et al. 1998, de Rooij et al. 1998). Two isoforms of the Epac protein exist, classified by the number of cAMP binding sites, with Epac1 having one and Epac2 having two cAMP binding domains (de Rooij et al. 1998, Cheng et al. 2008). Both isoforms are expressed in pancreatic β-cells, but only Epac2 regulates glucose-stimulated insulin vesicle exocytosis (Leech et al. 2000, Tengholm 2012). The GEF-function of Epac2 is induced by cAMP binding to the cAMP binding domain of Epac2, which activates Rap1 (de Rooij et al. 1998). Rap1 is a member of the Ras superfamily of small

GTPases (de Rooij et al. 1998) and, while not directly inducing exocytosis of insulin vesicles, is involved in promoting the trafficking of insulin containing secretory vesicles to the plasma membrane and increases the number of insulin vesicles recruited and immediately docked to the plasma membrane (Shibasaki et al. 2007). Alongside this, Epac2 is able to inhibit the function of the K-ATP channels by interacting with SUR-1 subunits, thereby increasing cytosolic Ca^{2+} concentration (Kang et al. 2006). Epac2 is also involved in Ca^{2+} sensing in β-cells through the formation of a complex with Rab3 interacting molecule 2 (Rim2) (Ozaki et al. 2000) and the cytoskeletal matrix associated with the active zone protein Piccolo (Fenster et al. 2000). In the Epac2-Rim2-Piccolo complex, the subunit Piccolo plays the role of the Ca^{2+} sensor, facilitating GLP-1R activated insulin secretion (Fujimoto et al. 2002), while the Epac2-Rim2 subunits promotes the SNARE complex formation at the plasma membrane leading to fusion and exocytosis of insulin vesicles (Ozaki et al. 2000, Kwan et al. 2007). Furthermore, cAMP activates the TF cAMP-responsive element-binding protein (CREB), which is involved in promoting of β-cell survival by Akt activation, through generation of insulin receptor substrate 2, an intermediate of the insulin secretion pathway (Withers et al. 1998, Tuttle et al. 2001, Jhala et al. 2003).

Glutamate is described as a major excitatory neurotransmitter with modulatory function within pancreatic islets, where it acts as an intracellular messenger linking multiple stimuli for insulin secretion together, as well as participating in the exchange between β-cells and other endocrine cells of the pancreas (Fahien and Macdonald 2011, Maechler 2013, Prentki et al. 2013, Otter and Lammert 2016). Glutamate is produced from either deamination of glutamine by glutaminase 2 (Jenstad and Chaudhry 2013, Zhou et al. 2014), or from α-ketoglutarate (αKG) within the Malate-aspartate shuttle (Feldmann et al. 2011, Gheni et al. 2014). Alternatively, glutamate dehydrogenase (GDH) generates glutamate within the mitochondria from αKG by addition of an ammonium ion (Maechler and Wollheim 1999). The transport of glutamate into secretory vesicles requires the vesicular glutamate transporters VGLUT1 and VGLUT3 (Gammelsaeter et al. 2011, Gheni et al. 2014).

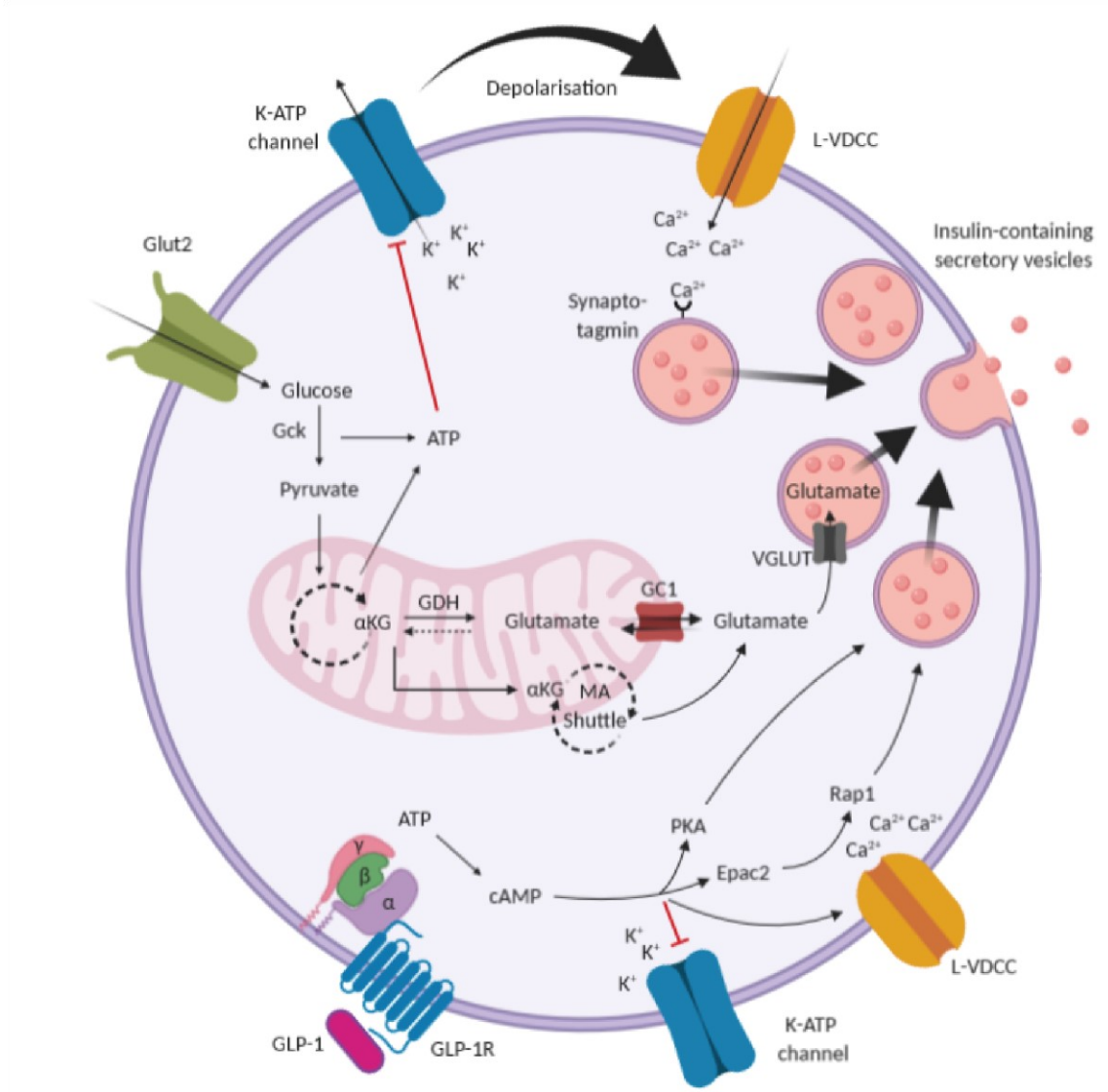


Figure 1.4: Schematic illustration of insulin secretion from pancreatic β-cells. Glucose metabolism increases intracellular ATP levels leading to the closure of K-ATP channels, depolarisation of the plasma membrane and opening of voltage-gated L-VDCC . The resulting Ca^{2+} influx leads to insulin-containing secretory vesicle exocytosis. Glutamate, generated from the TCA cycle, gets incorporated into insulin-containing secretory vesicles, promoting insulin maturation and secretion. Activation of GLP-1R generates cAMP from ATP, which facilitates insulin release by β-cells via PKA and Epac2 activation in a glucose dependent manner. Adapted from Otter and Lammert 2016.

Inside the secretory vesicles, glutamate is thought to contribute to the acidification of those vesicles, essential for the exocytosis through vesicles fusion with the plasma membrane, as well as the maturation of insulin from proinsulin (Maechler and Wollheim 1999, Gammelsaeter et al. 2011, Jenstad and Chaudhry 2013). In summary, while glucose metabolism remains the major contributor of insulin secretion, many factors, like incretin signalling and glutamate processing, contribute to regulate the release of insulin from β-cells.

1.1.5 Peripheral effects of insulin signalling

The insulin receptor (IR) belongs to the superfamily of receptor tyrosine kinases (RTKs) (Ebina et al. 1985, Ullrich et al. 1985) and is part of a subfamily which also comprises the insulin-like growth factors I and II receptors and the orphan insulin receptor-related receptor (Jui, Accili and Taylor 1996, Pandini et al. 2002, Massoner et al. 2010). The subfamily of insulin receptors is an exception to the RTKs, as they exist as a dimer linked by covalent disulphide bonds in the absence of the ligand (i.e. insulin) (Berg, J.L. and Stryer 2002). The IR is organised as a tetrameric transmembrane protein consisting of two extracellular α-subunits, with ligand binding domains (like insulin), and two transmembrane β-subunits (Boucher, Kleinridders and Kahn 2014). Insulin binding to the IR induces a conformational change of the receptor, resulting in the activation of the kinase activity associated to β-subunits and subsequent transphosphorylation of the two β-subunits (Boucher et al. 2014). Phosphorylation allows the recruitment of receptor substrates, including the insulin receptor substrates (IRSs) (Sun et al. 1991, Sun et al. 1995, Lavan et al. 1997, Cai et al. 2003, White 2006, Shaw 2011). IRS proteins are tyrosine phosphorylated and induce a cascade of phosphorylation events including the activation of the phosphatidylinositol 3-kinase (PI3K) / Akt pathway (Sun et al. 1993). IRS recruits and activates PI3K through the binding of the two Src-homology 2 (SH2) domains found in the regulatory domain of PI3K (Sun et al. 1993). Binding of IRS to the SC2 domains activates the catalytic subunit of PI3K, which phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP_2) to phosphatidylinositol (3,4,5)-triphosphate (PIP_3) (Myers et al. 1992, Alessi et al. 1997, Shaw 2011). PIP_3 recruits 3-phosphoinositide-dependent protein kinase 1 (PDK-1) to the plasma membrane. PDK-1 activates Akt through phosphorylation (Alessi et al. 1997). Active Akt mediates the majority of metabolic effects of insulin, including glucose transport, lipid synthesis, gluconeogenesis and glycogen synthesis (Boucher et al. 2014). For instance, Akt induces phosphorylation of glycogen synthase 3, which results in glycogen synthesis in skeletal muscle and liver (Friedrichsen et al. 2013), or the translocation of glucose transporter type 4 (Glut4) from intracellular vesicles to the plasma membrane to facilitate glucose uptake in fat and muscle cells (Sano et al. 2003)

Glucose homeostasis is controlled by two different, but interconnected mechanisms: 1) uptake of glucose by the liver, muscle and gastrointestinal, and 2) suppression of glucose release by the liver (Aronoff et al. 2004). The muscle is the major contributor of insulin stimulated glucose uptake in the postprandial state (Yang 2014).

In fasting condition, Gcg signals to the liver to promote the release of glucose to meet the demands of other tissues (Hancock et al. 2010). Gcg binds to its receptor, a member of the G protein-coupled receptor family. Activation of the Gcg receptor activates the Gα-cAMP-PKA pathway and increases cellular cAMP levels, leading to PKA activation (Jiang and Zhang 2003). PKA then phosphorylates and activates p44/p42 mitogen-activated protein kinase (ERK1/2) which in turn phosphorylates and activates CREB (Dalle et al. 2004). Activation of the TF CREB promotes hepatic gluconeogenesis by inducing the expression of peroxisome proliferator-activated receptor-γ coactivator 1α (*PGC1α*) (Herzig et al. 2001). In response to feeding, increased circulating insulin and decreased circulating Gcg leads to the inhibition of hepatic glucose production (HPG) to prevent hyperglycaemia (Girard 2006). Glucose enters hepatocytes mostly through Glut2 and is subsequently phosphorylated by glucokinase to produce glycogen (Gould and Holman 1993, Polonsky and Burant 2016).

The muscle, the major contributor of glucose disposal, is able to take up to 11 ± 0.1 mg of glucose per kg leg weight per min, as measured in the leg as the product of arterial-femoral venous blood glucose concentration difference and the leg blood flow (DeFronzo et al. 1985). Glucose is taken up by Glut4, found on the sarcolemma and transverse tubules (t-tubules) of myofibril bundles making the muscle cells (Marette et al. 1992, Wang et al. 1996a). Insulin, sensed by insulin receptors, increases the density of Glut4 in sarcolemma and t-tubules. In an insulin stimulated state 90% of the Glut4 occurrence is being accounted to the t-tubules (Wang et al. 1996a). Glucose is stored as glycogen in skeletal muscles (DeFronzo et al. 1981, Shulman et al. 1990).

While the Adipose tissue only plays a minor role in the direct uptake of glucose, the release of free fatty acids (FFA) and adipocytokines by adipocytes play an essential role in preserving the insulin sensitivity of the muscle and liver (Bays, Mandarino and DeFronzo 2004, Groop et al. 1989, Bergman 2000, Boden 1997). Increases in circulating insulin decrease lipolysis in adipocytes, resulting in lowered FFA levels in the plasma (Groop et al. 1989). Lower FFA plasma concentrations stimulate the uptake of glucose by the muscle and suppresses HPG (Randle et al. 1963, Wititsuwannakul and Kim 1977, Cherrington 1999).

1.2 The pathogenesis of T2D

T2D is characterised by insulin resistance of peripheral tissues, relative insulin deficiency due to failing β-cells and individuals therefore develop chronic hyperglycaemia as a result of a combination and interaction of genetic, environmental and behavioural risk factors (Maitra and Abbas 2005, Chen, Magliano and Zimmet 2012). In T2D the ability of the body to maintain normal glucose levels is impaired, resulting in the development of hyperglycaemia the characteristic of T2D patients (Kahn 1998). Maintaining normoglycemia is not only dependent on insulin secretion by pancreatic β-cells, but also relies on peripheral tissues being responsive to a rise in circulating insulin levels which promotes uptake of glucose and suppression of glucose production (Kahn et al. 1993, Kahn, Cooper and Del Prato 2014). Indeed, in the progression of T2D, it is generally believed that T2D is initiated by an insulin resistance of peripheral tissues associated with obesity which results in the need of the β-cells to compensate through increases in β-cell mass and elevated insulin secretion (Prentki and Nolan 2006, Weir 2004).

1.2.1 The aetiology of T2D

T2D results from insulin resistance and impaired insulin secretion. This malfunction may be acquired, hereditary or a mixed form of both (Freeman, Soman-Faulkner and Pennings 2019). Acquired factors include aging (Rowe et al. 1983), physical inactivity and nutritional imbalance leading to obesity (Aronne and Segal 2002), air pollution (Rao et al. 2015) and excessive consumption of alcohol (Lindtner et al. 2013).

Obesity is the leading risk factor of T2D in humans (Tsigos et al. 2011, Frühbeck et al. 2013). The increase in adipose tissue, and in particular visceral adipose tissue, in obese individuals leads to an increase in circulating FFA (Giorgino, Laviola and Eriksson 2005). In contrast to subcutaneous fat, the visceral adipose tissue has an increased rate of lipolysis, the source of FFA (Giorgino et al. 2005). FFA uptake by muscle and liver promotes the accumulation of triglycerides (Perseghin, Petersen and Shulman 2003). The metabolites of triglycerides (i.e. fatty acyl-CoAs) have been shown to inhibit insulin function in muscle and liver, leading to insulin resistance and preventing the uptake of glucose (DeFronzo 1988, DeFronzo 1997, Prentki and Corkey 1996). While obesity and the other acquired risk factors apply to a large

portion of individuals practicing similar, unhealthy, lifestyles, T2D develops only in a proportion of those individuals (Franks 2011).

It is believed that a concept of "gene x environment interaction" (GEI) plays a crucial role in the development of T2D (Franks 2011) (Fig. 1.5). The concept of GEI refers to differentiating susceptibilities of individuals to develop T2D coupled to genetic variations (The InterAct 2011).

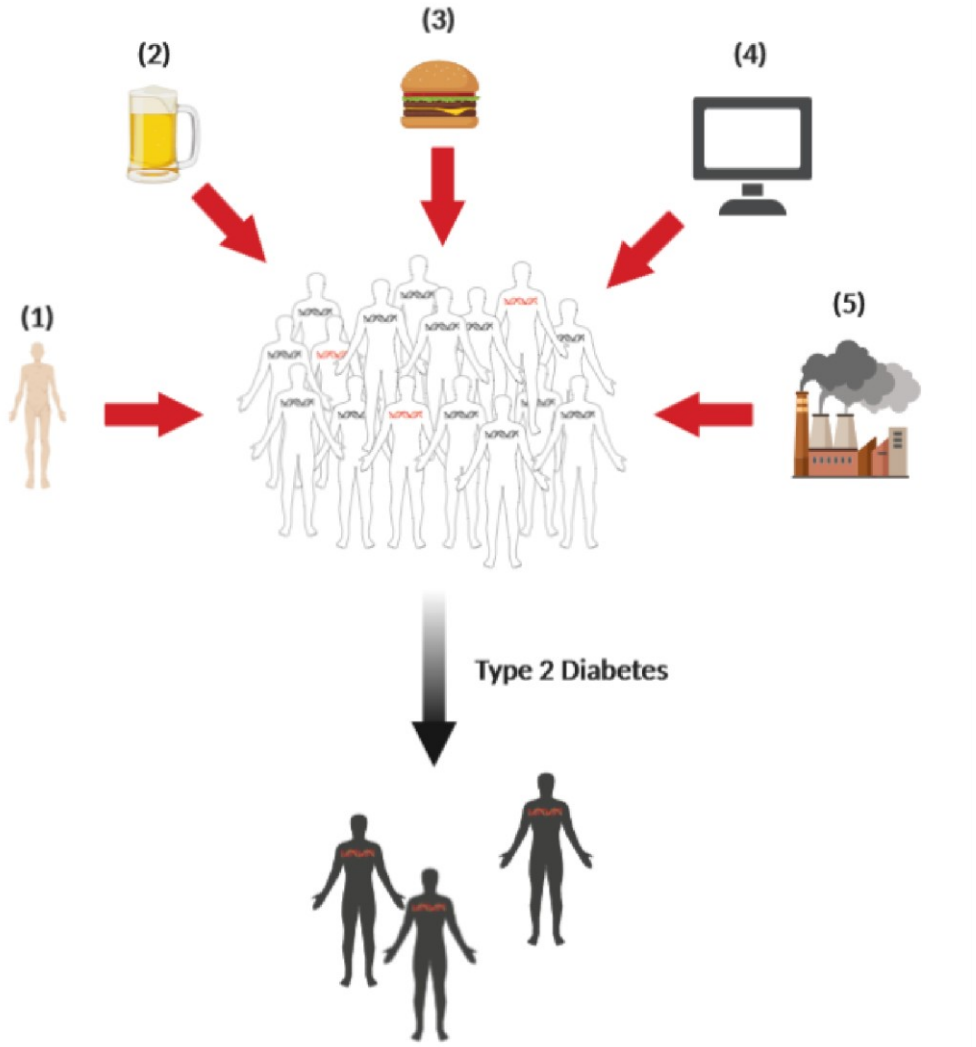


Figure 1.5: Schematic illustration of GEI in the progression of T2D. Genetic variations in certain individuals make them more susceptible to T2D. This leads to the development of T2D in a subset of the population even though the environmental risk factors are similar. Environmental risk factors include (1) age, (2) excessive alcohol consumption, (3) fatty and high sugar diets, (4) sedentary lifestyle and (5) pollution.

1.2.2 Linkage, Candidate gene and GWAS studies on T2D

The estimates for the heritability of T2D vary between 20-80% (Poulsen et al. 1999, Meigs, Cupples and Wilson 2000), and the risk to develop T2D in a lifetime is increased in individuals with one (40% risk to develop T2D) or two parents (70%) with T2D (Tillil and Köbberling 1987).

Linkage studies look at the relation between the inheritance of a locus and the disease (Dawn Teare and Barrett 2005). Linkage analyses identified multiple T2D risk genes, including Calpain 10 (*CAPN10*) (Hanis et al. 1996) and TF 7-like 2 (*TCF7L2*) (Duggirala et al. 1999, Reynisdottir et al. 2003, Grant et al. 2006). The function of *CAPN10* in glucose metabolism in β-cells is still unknown (Song et al. 2004b). Mouse studies revealed many roles of *TCF7L2* in β-cells including its role as a TF and regulator of the Wnt signalling pathway, that is associated with maintaining β-cell mass and the expression of β-cell identity genes such as insulin receptor substrate 1 and 2 (*IRS-1/IRS-2*), the insulin genes *INS1* and *INS2* and TFMAF BZIP TF A (*MAFA*) (Takamoto et al. 2014). Further evidence showed the involvement of *TCF7L2* in regulating insulin exocytosis and through regulating the VDCC plasma membrane distribution (da Silva Xavier et al. 2009).

Candidate gene studies investigate mutations and polymorphisms of pre specified genes of interest that are already suspected to be involved in the development of a disease. Candidate gene studies identified a range of genes associated with T2D, although with a minor role in the global burden. These include Peroxisome Proliferator Activated Receptor Gamma (*PPARG*) (Ringel et al. 1999, Clément et al. 2000, Ruchat et al. 2009), *IRS-1* and *IRS-2* (Clausen et al. 1995, Le Fur, Le Stunff and Bougnères 2002), Wolframin (*WFS-1*) (Sandhu et al. 2007, Franks et al. 2008) and the Hepatic Nuclear Factors 1A, 1B and 4F (*HNF1A, HNF1B* and *HNF4A)* (Furuta et al. 2002, Zhu et al. 2003, Muller et al. 2005). Additionally, *KCNJ11*, the gene encoding for the Kir6.2 subunit of the K-ATP channel, was identified to be associated with T2D (Hani et al. 1998, Gloyn et al. 2003, Florez et al. 2004, Nielsen et al. 2003).

T2D genome wide association studies (GWAS), linking commonly occurring single nucleotide polymorphisms with a common trait and are able to scan entire genomes for common genetic variants, enabled high throughput identification of genes associated to T2D (Ali 2013). GWAS studies confirmed the association genes like *TCF7L2* to T2D and identified a range of new genes including haematopoietically expressed homeobox (*HHEX*) or the solute carrier family 30 (*SLC30A8*) (Strawbridge et al. 2011, Manning et al. 2012, Xu, Wang and Chen 2012).

SLC30A8 encodes for the zinc transporter 8, responsible for the transport of Zn^{2+} into insulin-containing secretory vesicles (Davidson, Wenzlau and O'Brien 2014).

GWAS studies identified many gene variants associated with T2D. However, most of these were found in non-coding regions of the genome (Voight et al. 2010, Morris et al. 2012, Consortium et al. 2014). The findings imply a regulatory function for these regions. Indeed, single nucleotide polymorphisms associated with T2D were mapped closely to long non-coding RNAs (lncRNAs) that could affect the function of surrounding genes (Morán et al. 2012).

In contrast to T2D which involves multiple dysfunctional genes and environmental factors, a small percentage (≤ 5%) of non-autoimmune diabetic cases are caused by a mutation within a single gene (Ledermann 1995, Shih and Stoffel 2002) also known as monogenic diabetes or maturity onset diabetes of the young (MODY) (Shih and Stoffel 2002, Ali 2013). MODY is characterised by an early onset of diabetes (< 25 years of age), an inheritance that is autosomal-dominant and impaired insulin secretion (Shih and Stoffel 2002). The most common forms of MODY are mutations in the genes *HNF1A* (MODY1)(Stride et al. 2005) and *GCK (MODY2)* (Froguel et al. 1993, Pearson et al. 2001). Other genes include human insulin (*INS*) (MODY10) (Edghill et al. 2008, Meur et al. 2010), *PDX-1 (MODY4)* (Wright et al. 1993, Staffers et al. 1997, Fajans et al. 2010) and neuronal differentiation 1 (*NEUROD1,* MODY6) (Malecki et al. 1999, Kristinsson et al. 2001), a TF essential for β-cell maturation and maintenance of mature β-cell functionality (Gu et al. 2010). Although MODY only makes up for a small percentage of overall non-autoimmune diabetic cases, polymorphisms in or near those MODY genes contribute to the progression of T2D (Vaxillaire, Bonnefond and Froguel 2012).

1.2.3 Alteration of glucose metabolism by insulin resistance in obesity and T2D

Insulin resistance is defined as the reduced biological response, like glucose disposal, by peripheral tissues like the skeletal muscle, liver and adipose tissue, to normal or elevated insulin levels (Cefalu 2001, Reaven 2004). The link between obesity, insulin resistance and T2D is well established (Choi and Kim 2010).

Although intramyocellular lipid (IMCL) content is a predictor of muscle insulin resistance, triglycerides are causative for insulin resistance in the muscle (Goodpaster et al. 2001, Russell

et al. 2003). Muscle insulin resistance is associated with an increase in cytosolic diacylglycerol (DAG) (Szendroedi et al. 2014). DAGs bind to and activate protein kinase C (PKC)-θ (Hayashi and Altman 2007). PKCθ phosphorylates IRS-1 at serine 1101, therefore blocking tyrosine phosphorylation by the insulin receptor tyrosine kinase (IRTK). This inhibits the function of IRS-1 and prevents downstream Akt activation (Li et al. 2004). Similar findings were reported for PKCε in skeletal muscles of obese rats after feeding (Qu, Seale and Donnelly 1999). In T2D patients the uptake of insulin stimulated glucose into the muscle is delayed by up to 40 min and decreased up to 50% in comparison to healthy controls (Butterfield and Whichelow 1965, Jackson et al. 1973, DeFronzo et al. 1985, Campbell, Mandarino and Gerich 1988, Mitrakou et al. 1990, Utriainen et al. 1998).

In hepatic insulin resistance similar observations were made. In obese rats and patient samples of non-insulin-dependent diabetes mellitus PKCε, the primary liver PKC, was induced by DAGs (Considine et al. 1995, Samuel et al. 2004). Increases in PKCε were associated with a decrease in the activation of IRTK (Samuel et al. 2007). Deletion of the PRKCE gene, coding for PKCε, protected mice from diet induced insulin resistance in the liver (Raddatz et al. 2011). In T2D patients, HPG is upregulated compared to healthy controls, despite hyperglycaemia and elevated insulin levels are present, showing insulin resistance and glucose resistance of the liver as part of the progression of T2D (Sharabi et al. 2015). Additionally, hepatic glucose uptake after oral glucose administration is decreased in T2D (DeFronzo and Ferrannini 1987, Firth, Bell and Rizza 1987, Ferrannini et al. 1988).

In adipose tissues, the deletion of PKCλ or PKCζ have been shown to inhibit insulin stimulated glucose transport and the translocation of Glut4 (Kotani et al. 1998, Bandyopadhyay et al. 1997). The entry of glucose into adipocytes activates the carbohydrate response element binding protein (ChREBP). Adipose-ChREBP expression is directly associated with insulin stimulated glucose uptake, therefore improving systemic insulin sensitivity (Herman et al. 2012). Those findings were confirmed by the implication of adipose-ChREBP to the synthesis of branched fatty acid esters of hydroxy fatty acids (FAHFAs) (Yore et al. 2014). The FAHFA palmitic-acid-9-hydroxy-stearic-acid (PAHSA) is reduced in adipose tissue and serum of insulin resistant humans (Yore et al. 2014). Administration of PAHSA in mice improved glycaemia, glucose tolerance and stimulated GLP-1 and insulin secretion (Yore et al. 2014). In T2D patients, circulating levels of FFA and lipolysis are increased to failure of insulin to mediate its action (Golay et al. 1984, Golay et al. 1988, Felber et al. 1981).

1.2.4 The three stages of pancreatic β-cell failure

Although initially β-cells can compensate for insulin resistance by secreting higher amounts of insulin, eventually hyperglycaemia and hyperlipidaemia associated with insulin resistance induces β-cell failure through glucose desensitisation, β-cell exhaustion and gluco- and lipotoxicity (Fig. 1.6) (Leahy 2005).

Glucose desensitisation describes a rapid process in which β-cells lose their ability to sense changes in glycaemia after prolonged hyperglycaemia (Rustenbeck 2002, Björklund, Lansner and Grill 2000). Desensitised β-cells have a diminished response to additional glucose exposures, or other stimuli, like tolbutamide, as seen in human and rat islets (Bedoya and Jeanrenaud 1991a, Bedoya and Jeanrenaud 1991b, Davalli et al. 1992). In contrast, desensitised β-cells maintain their ability to respond to L-arginine (Bedoya and Jeanrenaud 1991a, Bedoya and Jeanrenaud 1991b, Davalli et al. 1992). These findings show, the difference between exhausted and desensitised β-cells, as the desensitised β-cells maintain insulin content, but lose their ability to respond to stimuli like glucose. The process of glucose desensitisation in β-cells is reversible *in vivo* and *in vitro* through normalisation of glucose concentrations (Grill, Westberg and Ostenson 1987, Anello et al. 1996).

Exhaustion of β-cells relates to the depletion of intracellular insulin stores induced by exposure to prolonged hyperglycaemia (Sako and Grill 1990, Leahy, Bumbalo and Chen 1994). Exhaustion of β-cells is a reversible process, as shown on pancreatectomised rats treated with diazoxide, an activator of the K-ATP channel (Leahy et al. 1994). Pancreatectomy requires the remaining β-cells to compensate through an increase in insulin secretion, eventually exhausting the β-cell. Diazoxide was administrated 8-15 days post operation and prevents the secretion of insulin in the remaining β-cells. Diazoxide was removed 48h prior *in vitro* insulin secretion assays, allowing the degradation of diazoxide with an half-life of 28h. Islets treated with diazoxide maintained their ability to secrete insulin in the *in vitro* insulin secretion assay, opposite to the exhausted control islets (Leahy et al. 1994). The exhausted islet has no defect in insulin synthesis, in contrast to the stages of gluco- and lipotoxicity (Robertson et al. 2003).

Prolonged exposure of β-cells to hyperglycaemia drives them through glucose desensitisation, β-cell exhaustion and eventually loss of β-cell function through glucotoxicity (Moran et al. 1997, Gleason et al. 2000, Poitout and Robertson 2002). Chronic hyperglycaemia is sufficient to induce apoptosis of β-cells (Pick et al. 1998, Donath et al. 1999). Moreover, the major functional change of β-cells exposed to prolonged hyperglycaemia is the loss of insulin gene

expression. In the setting of glucotoxicity, loss of insulin gene expression is associated with a decline in the activity and expression of *PDX-1* (Olson et al. 1993, Olson et al. 1995) and *MAFA* (Sharma et al. 1995, Poitout, Olson and Robertson 1996). Additionally, reports show the activation of the transcriptional repressor of insulin CCAAT/enhancer-binding protein β (Lu, Seufert and Habener 1997, Seufert, Weir and Habener 1998) and the proto-oncogene *C-MYC* (Jonas et al. 1999) in response to prolonged hyperglycaemia. Biochemically, glucotoxicity is thought to induce β-cell failure through reactive oxygen species (ROS) generated in the glycation reaction step of glycolysis (Kaneto et al. 1996, Matsuoka et al. 1997, Tajiri, Möller and Grill 1997, Ihara et al. 1999, Tanaka et al. 1999). *In vitro*, treatment of insulin secreting HIT-T15 cells with the antioxidant N-acetyl-L-cysteine (NAC), or aminoguanidine (AG), an inhibitor of the glycation reaction, rescues insulin gene expression induced by hyperglycaemia. In isolated rat islets, treatment with NAC and AG after exposure to increased glucose levels *in vitro* prevented the loss of function and apoptosis of β-cells (Kaneto et al. 1996, Tajiri et al. 1997). *In vivo*, NAC- or AG-treated Zucker diabetic fatty rats show a recovery of glycemia and insulin gene expression, insulin content and insulin secretion in and from β-cells (Tanaka et al. 1999).

The effects of prolonged exposure to hyperlipidaemia result in irreversible deterioration of the β-cells, similar to the effects of prolonged hyperglycaemia. While FFA are an essential energy source for β-cells in the healthy individual, ongoing exposure increases basal insulin secretion by β-cells and inhibits GSIS (McGarry and Dobbins 1999). In the presence of elevated glucose levels, FFA suppress insulin gene expression (Ritz-Laser et al. 1999, Gremlich et al. 1997, Jacqueminet et al. 2000). This is partially due to the downregulation of *PDX-1* by increased FFA levels (Martinez et al. 2008). Loss of *PDX-1* expression in response to FFA resulted in the decrease of Glut2 and Gck levels additionally to insulin levels, showing that FFA also induce glucose desensitisation of β-cells (Gremlich et al. 1997). Hyperlipidaemia promotes β-cell apoptosis both *in vitro* in isolated rat islets (Maedler et al. 2001a, Cnop et al. 2001), as well as *in vivo* in rats (Pick et al. 1998, Shimabukuro et al. 1998). Biochemically, it shown that elevated FFA and glucose levels increase the generation of cytosolic citrate, a precursor of malonyl-CoA (Prentki and Corkey 1996, Prentki et al. 2000). Malonyl-CoA inhibits the carnitine-palmitoyl-transferase-1 which is responsible for the transport of FFA into the mitochondrion (Cook, Otto and Cornell 1983). Without the transport of FFA through the carnitine-palmitoyl-transferase-1 long chain fatty acyl-CoAs accumulate in the cytosol, thus impairing β-cell function and survival (Prentki and Corkey 1996).

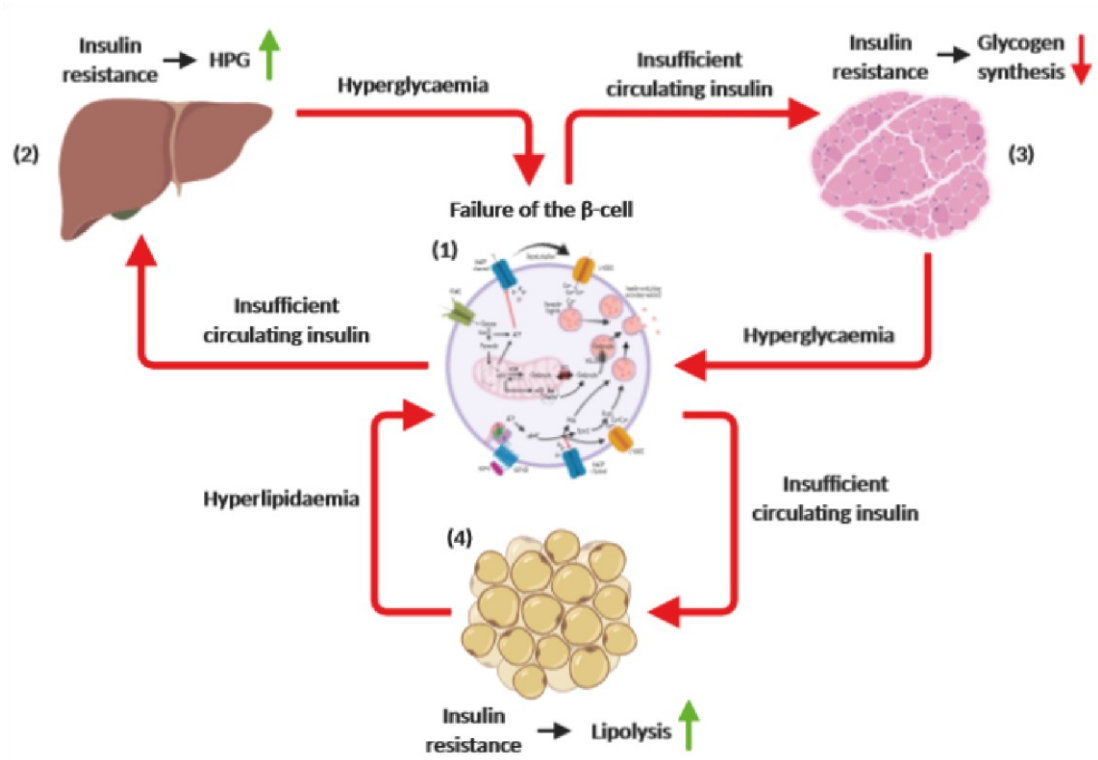


Figure 1.6: Schematic illustration of the vicious cycle of insulin resistance and pancreatic β-cell failure. Insulin resistance in the liver (2) and peripheral tissues such as the muscle (3) leads to hyperglycaemia. Hyperglycaemia induces compensating insulin secretion by the β-cells (1). Insulin resistance in the adipose tissue (4) increases lipolysis and circulating FFA. Hyperglycaemia and hyperlipidaemia tip the β-cell over the edge. The failing β-cell is not able to secrete sufficient amounts of insulin to maintain euglycemia and normal lipid levels.

Studies showed that malonyl-CoA/acyl-CoA accumulation has a lipotoxic effect in the presence of elevated glucose levels. This lead to the idea of glucolipotoxicity being the driving force of β-cell failure instead of glucotoxicity or lipotoxicity (Prentki and Corkey 1996, Poitout and Robertson 2002, Poitout et al. 2010). Indeed it has been shown that β-cells are able to compensate for elevated glucose levels (Roche et al. 1997, Roche et al. 1998) or increases in FFA concentrations (Prentki et al. 2002), explaining why hyperlipidaemia or hyperglycaemia by themselves do not cause clinical relevant β-cell failure according to the glucolipotoxicity hypothesis (Prentki and Nolan 2006).

The decline of β-cell function induced by glucolipotoxicity, combined with hyperglycaemia-induced apoptosis creates an imbalance in the available insulin to required insulin. The increased demand on the remaining β-cells drives them into failure. T2D is classified by this

loss of functional β-cell mass and if T2D progression is not treated, insulin replacement therapy eventually becomes unavoidable (Leahy 2005, Sakuraba et al. 2002).

1.2.5 Apoptosis of β-cells in T2D

In T2D, apoptosis has been reported in the pancreas of lean and obese patients (Butler et al. 2003). Additionally, apoptosis is the main form of β-cell death in T2D as detected by TUNEL staining in human donor pancreatic section (Butler et al. 2003).

Elevated glucose concentrations can cause the apoptosis of β-cells as seen in multiple animal models (Donath et al. 1999, Pick et al. 1998, Zini et al. 2008). In human islets, prolonged exposure to hyperglycaemia induced the expression of the pro apoptotic BH3-only genes *BID* and *BAD* and the repression of the anti-apoptotic gene *BCL-XL* (Federici et al. 2001). Interestingly, *BAD* has a dual role in apoptosis and insulin secretion, as deletion of *BAD* in mice resulted in impaired insulin secretion in response to glucose (Danial et al. 2008). Other studies showed the ability of high glucose concentrations to induce the generation of intracellular ROS, also through the NOX family of NADPH oxidases, that lead to apoptosis (Tanaka et al. 2002, Nakayama et al. 2005, Guichard et al. 2008). Glucose has also been reported to induce the gene expression thioredoxin-interacting protein (*TXNIP*), that induces β-cell apoptosis through activating caspase 3 (Chen et al. 2008). Studies in human islets showed a release of interleukin (IL)-1β induced by high glucose from intra-islet macrophages and β-cells. IL-1β induces Fas-triggered apoptosis through activation of the TF Nuclear Factor kappa-light-chain-enhancer of activated B cells (*NF-κB*) and increases in Fas receptor levels (Maedler et al. 2001b, Maedler et al. 2002).

Fatty acids also have been shown to induce apoptosis of β-cells. Elevated levels of saturated fatty acids, like palmitate, activate ER stress pathways and induce the apoptosis of β-cells (Laybutt et al. 2007, Eizirik, Cardozo and Cnop 2008). Additionally, FFA in Zucker diabetic fatty rats have been shown to induce apoptosis through de novo ceramide formation and nitric oxide generation (Shimabukuro et al. 1998). ER stress can also be induced by overexpression of IAPP (Huang et al. 2007). Additionally, elevated IAPP levels have been associated to increase in ROS (Zraika et al. 2009).

β-cells have a highly developed ER due to their role in synthesis and secretion of insulin and are therefore susceptible to ER stress (Eizirik et al. 2008). ER stress induces apoptosis through

activation of the pro-apoptotic BH3-only protein Bim (Puthalakath et al. 2007), as well as induction of the TF CCAAT-enhancer-binding protein homologous protein (*CHOP*) (Zinszner et al. 1998) and homo-oligomerisation of Bak and Bax, resulting in the release of cytochrome c from the mitochondria (Wei et al. 2001).

1.2.6 Loss of β-cell identity in T2D

In T2D, hyperglycaemia occurs as a result of a reduction in the functional β-cell mass (Chen et al. 2017). Reduced functional β-cell mass can be due to a reduction in the number or impaired identity of β-cells (Pipeleers et al. 2008, Guo et al. 2013). For a long time, β-cell death has been proposed to be the cause for the reduction in β-cell mass (Butler et al. 2003). More recent studies indicated that β-cell dedifferentiation represents a novel pathological mechanism in T2D (Talchai et al. 2012, Spijker et al. 2013). The first indication for loss of β-cell identity induced by chronic hyperglycaemia was found in Sprague-Dawley rats. These animals displayed a reduction in the expression of key β-cell marker genes including insulin, *GLUT2* and *Kir6.2* and the downregulation of a range of β-cell specific TFs including *PDX-1* and *NKX6.1* (Jonas et al. 1999). Years later, the concept of β-cell dedifferentiation as a cause of T2D was brought back to the scene and shown to be an intrinsic pathological mechanism in several diabetic mouse models as well as in biopsies form human T2D patients (Talchai et al. 2012, Spijker et al. 2015, Cinti et al. 2016a). Controversially, other reports, while not rejecting the existence of endocrine cells with altered identity, conclude that this dedifferentiated β-cells can't account for the deficit in functional β-cell mass in T2D (Butler et al. 2016).

Dedifferentiation of β-cells is characterised by a downregulation of insulin gene expression induced by prolonged exposure to hyperglycaemia (Olson, Qian and Poitout 1998, Briaud et al. 1999, Marshak et al. 1999). Loss of insulin gene expression has been attributed to reduced expression of *PDX-1* and *MAFA* and binding of those TFs to the insulin gene promoter (Olson et al. 1998, Poitout et al. 1996, Pino et al. 2005, Matsuoka et al. 2010).

In other studies, loss of insulin expression was prevented by treatment with antioxidants, prompting the idea of oxidative stress being causative for β-cell dedifferentiation (Tanaka et al. 1999, Robertson and Harmon 2006). Those findings are supported by reports showing the loss of PDX-1 and MAFA expression and DNA-binding activity by hydrogen peroxide (Kaneto et al. 2002b, Harmon, Stein and Robertson 2005, Guo et al. 2013). Further evidence was

presented in studies with transgenic expression of the antioxidant glutathione peroxidase showing rescue of reduced *MAFA* expression levels in *db/db* mice (Harmon et al. 2009, Guo et al. 2013) and prevention of loss of PDX-1 and MAFA expression in diabetic rat islets by administration of the glutathione peroxidase mimetic ebselen (Mahadevan et al. 2013). Additionally, it suspected that ER-stress affects the specificity of inositol-requiring enzyme 1 (IRE1), resulting in the cleavage of RNAs of the ER, including insulin (Hollien and Weissman 2006, Pirot et al. 2007, Lipson, Ghosh and Urano 2008, Han et al. 2009, Hollien et al. 2009).

Recent studies showed the involvement of microRNAs (miRNAs) in the dedifferentiation of β-cells. The upregulation of miRNA (miR)-204 by high glucose induced TXNIP resulted in reduced *MAFA* expression (Shalev et al. 2002, Saxena, Chen and Shalev 2010, Xu et al. 2013). Other examples are the downregulation of *NEUROD1* expression by upregulation of miR-30a-5p (Kim et al. 2013b) and the reduction of insulin biosynthesis by miR-133 (Fred et al. 2010). Both, miR-30a-5p and miR-133, were induced by elevated glucose levels in cultured cells and human islets, respectively (Kim et al. 2013b, Fred et al. 2010). In islets of T2D patients miR-124a was upregulated and shown to target *FOXA2* and *NEUROD1* in Min6 cells (Sebastiani et al. 2015).

The expression of insulin gene expression has also been shown to be affected through activity of transcriptional repressor proteins. Elevated glucose levels have been demonstrated to induce the expression of V-Myc Avian Myelocytomatosis Viral Oncogene Homolog (C-*MYC*), which inhibited Neurod1 mediated activation of the transcription of insulin (Kaneto et al. 2002a). C-*MYC* is a response gene of oxidative stress (Elouil et al. 2005, Jonas et al. 2009) that is associate with β-cell dedifferentiation and has been proposed to be negatively regulated by Pdx-1 (Jonas et al. 2001, Laybutt et al. 2002, Chen et al. 2007, Pascal et al. 2008, Robson et al. 2011). In recent studies, *PDX-1* repression by V-ets avian erythroblastosis virus E26 oncogene homolog 1 (*ETS1*) in a setting of glucotoxicity was observed (Chen et al. 2016). ETS1 was suggested to interact with transcription factor forkhead box O1 (Foxo1) to increase *PDX-1* promoter binding (Chen et al. 2016). Previously, Foxo1 has been shown to repress Foxa2-dependent *PDX-1* expression and Pdx-1 translocation in the setting of oxidative stress (Kitamura et al. 2002, Kawamori et al. 2006). Interestingly, the role of FOXO1 is multifunctional. Contrary to the previous discussed role of ETS1/Foxo1, evidence shows a protective role for Foxo1 under oxidative stress through promoting *NEUROD1* and *MAFA* expression (Kitamura et al. 2005). Those findings are supported by studies of FOXO1 deletion in β-cells of mice resulting in the dedifferentiation of β-cells (Talchai et al. 2012).

Dedifferentiation is not solely induced by TF action upstream of insulin gene expression. Indeed, studies have shown the involvement of various β-cell key TFs in inter-regulation and in maintaining β-cell enriched genes, like *GLUT2* and *GCK*. In addition to *PDX-1*, *MAFA* and *NEUROD1*, TFs including *HNF1α*, *HNF4α*, *FOXA2, NKX6.1* and *PAX6* were demonstrated to play an important role in maintaining β-cell identity (Cha et al. 2000, Ben-Shushan et al. 2001, Shih et al. 2001, Sund et al. 2001, Samaras et al. 2002, Shih et al. 2002, Samaras et al. 2003, Pedersen et al. 2005).

Due to the close relation between TFs expression patterns and the maintenance of β-cell identity, the effects of TF deletion, additionally to Foxo1 knockout (Talchai et al. 2012), were subsequently thoroughly investigated. Deletion of *PDX-1* in mouse β-cells resulted in the loss of β-cell identity, severe hyperglycaemia within days and β-cells acquired α-cell features, including the expression of mouse α-cell TF *MAFB* (Gao et al. 2014). Similar to *PDX-1* deletion, genetic knockout of *NKX2.2* in β-cells of mice resulted in the loss of β-cell enriched genes, like *GLUT2* and *NKX6.1*, and occurrence of atypical hormones (Gcg, Sst and Ppy) in β-cells (Gutiérrez et al. 2017). The selective deletion of *PAX6* in β-cells of mice resulted in lethal hyperglycaemia and ketosis and were attributed to a loss of β-cell function, a switch from insulin to ghrelin synthesis and expansion of α-cells (Swisa et al. 2017)

The correct activation of β-cell enriched gene expression is not only dependent on the expression of upstream TFs, but also in the correct assembly of transcriptional complexes. LIM domain-binding protein 1 (LDB1), regulator of the transcriptional activity of TFs with a LIM-domain, was demonstrated to be enriched at DNA regions bound by Pdx-1, Nkx2.2, Nkx6.1 and Foxa2 (Ediger et al. 2017). LDB1 enrichment in an active enhancer near *GLUT2* was reduced in islets from T2D patients and deletion of LDB1 in mouse β-cells has been reported to decrease the expression of β-cell enriched genes including *GLUT2*, *MAFA* and the Kir6.2 encoding gene *KCNJ11* (Ediger et al. 2017). Additionally, increases in the repressive DNA methylation mark in T2D were reported at promoter and enhancer regions of *PDX-1*, as well as in the *GLP-1R* promoter (Yang et al. 2012, Hall et al. 2013). Together, the research shows the importance of correct TF expression and their regulation in maintaining the functional identity of β-cells.

Additionally, inflammation is a potential factor in β-cell dedifferentiation. Proinflammatory cytokines, released in chronic hyperglycaemia by immune cells, endothelial cells and adipose tissue, have been proposed to be involved in the dedifferentiation of β-cells (Donath and

Shoelson 2011, Nordmann et al. 2017). In islets of T2D patients, immune cell infiltration is increased (Nordmann et al. 2017). Mouse and human islets treated with pro-inflammatory cytokines, like IL-1β, IL-6 and TNFα, showed reduced mRNA levels of β-cell genes like insulin or Foxo1 (Nordmann et al. 2017). *Db/db* mice treated with anti-inflammatories (e.g. anti-IL-1β antibody) improved islet insulin content and glucose clearance of those animals compared to controls (Nordmann et al. 2017).

Studies in mice showed that viral infections, like Coxsackievirus B4, induces a diabetic phenotype (Gerling, Nejman and Chatterjee 1988). Later, transfection of the human β-cell line (EndoC-βH1) with double strand RNA (PolyI:C) that mimics a by-product of viral replication, revealed the downregulation of β-cell gene expression in transfected cells (Oshima et al. 2018). This dedifferentiation of β-cells was accompanied with de-repression of progenitor cell markers, such as *SOX9* (Oshima et al. 2018). Together, these studies show the involvement of viral infections in the dedifferentiation of β-cells.

1.2.7 Current treatments for T2D

While insulin treatment is only necessary for patients suffering from late stage T2D, the majority of T2D patients can be treated with oral drugs and changes in lifestyle (e.g. improved diet and increase in exercise)(Nathan 2002). Four common, of all currently available drug classes used for the treatment of T2D, are: 1) biguanides, 2) sulfonylureas, 3) incretin mimetics and 4) DPP-4 inhibitors (Tahrani et al. 2011). The most commonly prescribed drug, due to its cost effectiveness and safety, is the biguanide metformin (Rena, Hardie and Pearson 2017). Metformin improves insulin sensitivity in peripheral tissues including the muscle by increasing activity of the insulin receptor tyrosine kinase, elevated glycogen synthesis and an upregulation of glucose transporter 4 (Glut4) recruitment and activity (Giannarelli et al. 2003). Additionally, metformin activates AMP-activated protein kinase (AMPK) (Zhou et al. 2001). In hepatocytes, metformin-induced AMPK activation inhibits acetyl-CoA Carboxylase activity, which promotes the oxidation of fatty acids, thus decreasing HGP (Zhou et al. 2001). In skeletal muscles, activation of AMPK coincides with the increase of glucose uptake (Zhou et al. 2001). However, biguanides like metformin and phenformin have been shown to cause lactic acidosis (Blackshear 1977, Misbin 1977, DeFronzo et al. 2016). Therefore phenformin has been withdrawn in many countries, whereas metformin, only reported to affect a subset of susceptible individuals, is still available (Misbin 2004, Olokoba, Obateru and Olokoba 2012).

Sulfonylureas, like tolbutamide, inhibit the SUR-1 regulatory subunit of the K-ATP channel to induce a closure of K-ATP channels and resulting in the depolarisation of the plasma membrane, thereby promoting insulin secretion by β-cells (Proks et al. 2002). The risks associated with sulfonylurea usage include hypoglycaemia due sulfonylurea's ability to effectively stimulation insulin secretion (Chiniwala and Jabbour 2011) as well as undesirable weight gain (Nathan et al. 2008).

Similar to the incretin GLP-1, incretin mimetics potentiate glucose-stimulated insulin secretion, induce weight loss through appetite regulation and improve β-cell viability through their anti-apoptotic effects (Campbell and Drucker 2013). In contrast to tolbutamide, incretin mimetics have lower risks to induce hypoglycaemia due to their dependency on glucose (Jackson et al. 2010). However, incretin mimetics have adverse effects, including disturbance of gastrointestinal tract, nausea and an increase risk to develop pancreatitis (Buse et al. 2009, Meier 2012, Meier and Nauck 2014). DPP-4 inhibitors, like sitagliptin, block the function of the enzyme dipeptidyl peptidase-4 (DPP-4) and increase the half-life of GLP-1 in the circulation (Deacon 2011). An increase in active circulating GLP-1 enhances glucose-dependent insulin secretion and therefore improves glycaemic control in T2D patients (Ahrén et al. 2002). Adverse side effects reported for sitagliptin include upper respiratory tract infections, nasopharyngitis and headache (Pathak and Bridgeman 2010).

1.3.1 Epigenetics and the development of pancreatic β-cells

The differentiation of a cell requires a precise order of gene expression patterns to ensure the correct lineage commitment and cell fate determination required for organ function (Boland, Nazor and Loring 2014). Epigenetics, defined as "a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence" (Berger et al. 2009), enable this kind of temporal control over the expression of specific genes. Epigenetic regulation of cell differentiation is mediated by DNA methylation of deoxycytidine residues, the posttranslational modification of histone tails, nucleosome remodelling and regulation of chromatin structure and chromosome organisation through long non-coding RNAs (Boland et al. 2014).

DNA methylation is an epigenetic mark associated with transcriptional repression and was mainly studied at methylation of cytosine at the C5 position by DNA methyltransferases

(Bestor et al. 1988, Okano, Xie and Li 1998, Yoder and Bestor 1998, Bestor 2000, Cheng and Blumenthal 2008, Moore, Le and Fan 2013). Although methylation of the C5 position of cytosine is the most studied, methylation of other nucleobases like adenine at the N6 and guanine at the N7 position to be involved in gene transcription regulation has been shown (Herron and Shank 1981, Thomas et al. 2013, Iyer, Zhang and Aravind 2016). Additionally, while transcriptional repression is to date the more common function of DNA methylation, activation of gene transcription has been reported, as for example for *FoxA2* in β-cells (Bahar Halpern, Vana and Walker 2014).

C5 cytosine DNA methylation occurs at cytosines preceding a guanine nucleotide, so called CpG sites (Jaenisch and Bird 2003, Antequera 2007, Portela and Esteller 2010). DNA methylation in intergenic regions, like transposable and viral elements, results in their inactivation, preventing gene disruption and DNA mutation (Michaud et al. 1994, Kuster et al. 1997, Wu et al. 1997, Gwynn et al. 1998, Ukai et al. 2003, Schulz, Steinhoff and Florl 2006). CpG islands are likely to enhance binding of TFs to transcriptional start sites, and approximately 70% of gene promoters are within CpG islands (Saxonov, Berg and Brutlag 2006, Moore et al. 2013). Methylation of CpG islands is suggested to impair TF binding and recruit transcription repressing methyl binding proteins, like MeCP2 (Wade 2001, Moore et al. 2013). DNA methylation in the gene body is, as in promoter regions, associated with transcriptional repression, particularly in differentiated cells (Aran et al. 2010, Brenet et al. 2011, Guo et al. 2011, Xie et al. 2012). Studies propose that gene body DNA methylation is associated with increased gene expression in highly dividing cells (Hellman and Chess 2007, Ball et al. 2009, Aran et al. 2010).

DNA methylation interacts with various other epigenetic modulators, including histone modifications (Suzuki and Bird 2008). Histone modifications are associated with either repressive or activating functions on transcriptional expression. For instance Histone 3 Lysine 27 trimethylation (H3K27me3), catalysed by the SET domain of the Ezh2 component of the Polycomb Repressive Complex 2 (PRC2), catalyses the trimethylation of H3K27 to the repressive H3K27me3 mark (Wiles and Selker 2017). H3K27me3 can be recognised by the Polycomb Repressive Complex 1 (PRC1), which is proposed to be involved in transcriptional repression through monoubiquitinylation of H2AK119 and chromatin compaction (Cao et al. 2002, Fischle et al. 2003, Min, Zhang and Xu 2003, de Napoles et al. 2004, Wang et al. 2004, Eskeland et al. 2010).

Acetylation of histones is controlled by histone acetyl transferases (HATs) and histone deacetylases (HDACs). H3K9 and H3K14 are present at active promoters and enhancers, as well as bivalent promoters and often occur in the same regions of the genome (Wang et al. 2009, Anamika et al. 2010, Karmodiya et al. 2012)

H3K4 is trimethylated by family members of the COMPASS methylases (Shilatifard 2012). H3K4me3 is associated with transcriptional activation through recruitment of the chromatin remodelling factors Chromodomain-helicase-DNA-binding protein (CHD) 1 (Flanagan et al. 2005) and BPTF (Li et al. 2006) and prevents the binding of the repressive INHAT (Schneider et al. 2004) and nucleosome remodelling and deacetylase activities (NuRD) (Nishioka et al. 2002a) complexes.

Indeed, previous studies showed that epigenetic regulation of gene expression is crucial in early development of the pancreas and enables commitment of cells into either pancreatic or hepatic cell fates (Fig. 1.7) (Xie, Carrano and Sander 2015). Both the pancreas and the liver are formed from a subpopulation of cells in the ventral foregut during development (Xie et al. 2015). Specific chromatin modifications near pancreas specific regulatory sequences control the lineage commitment of the ventral foregut cells, including deposition of the repressive H3K27me3 and the active H3K9K14Ac (Xu et al. 2011). The combination of both marks results in a poised chromatin state for potential future activation (Xu et al. 2011). However, those marks are poorly represented in regulatory sequences driving hepatocyte differentiation. Inactivation of the histone methyltransferase Ezh2 favours the pancreatic fate (Xu et al. 2011). Additionally, inactivation of acetyltransferase p300 activity prevents the required *de novo* acetylation at liver specific elements and results in an increase in pancreatic lineage commitment (Xu et al. 2011). These findings demonstrate how epigenetics regulate cell lineage commitment regulation to ensure correct organ formation.

In pluripotent embryonic stem (ES) cells, a large number of developmental genes are found in a so-called bivalent state marked by repressive H3K27me3 and the active H3K4me3 (Bernstein et al. 2006). Recent studies showed that during the differentiation of ES cells into β-cells, the bivalent sate is resolved in favour of H3K4me3 at core β-cell genes leading to the activation of β-cell gene programs (Xie et al. 2013a, Bramswig et al. 2013, van Arensbergen et al. 2010). This epigenetic plasticity enables the reprogramming of pancreatic α-cells, which exhibit an increase in bivalent genes compared to the monovalent state of H3K4me3 modification at β-cell genes in β-cells (Bramswig et al. 2013).

Other groups revealed the importance of HDAC in the differentiation of the different endocrine cell types. Indeed, different classes of HDAC were found to be essential for the development of pancreatic endocrine cells. HDAC class I (HDAC-I) members favour the development of pancreatic β- and δ-cells, while inhibiting the differentiation to α- and γ-cells (Haumaitre, Lenoir and Scharfmann 2008). In contrast, genetic deletion of HDAC class IIa (HDAC-IIa) members prompts the formation of β- and δ-cells (Lenoir et al. 2011).

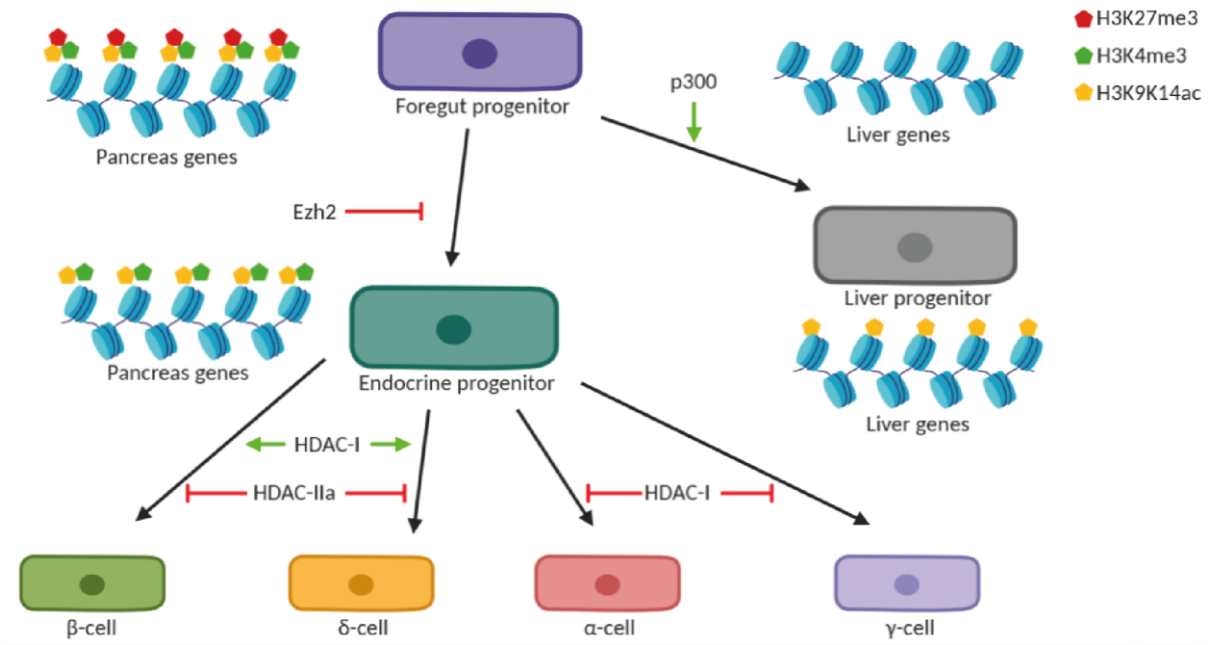


Figure 1.7: Schematic illustration of epigenetic factors involved in pancreas development. Loss of the repressive H3K27me^3 marks drives foregut progenitor cells into the pancreatic and eventually endocrine progenitor cell fate. *De* novo acetylation in foregut progenitors favours the liver lineage fate. Histone acetylation/deacetylation dynamics determine the specification of the endocrine progenitors. Adapted from Xie et al. 2015.

1.3.2 Epigenetics, pancreatic β-cell identity and T2D

As discussed earlier, epigenetic regulation plays a crucial role in the differentiation of the β-cell during development. Therefore, it is not surprising that dysregulation of key epigenetic modifiers is associated with the loss of mature β-cell identity genes and the development of T2D. For example, β-cell-specific Dnmt3a knockout mice show the de-repression of the developmental genes hexokinase 1 (HK1) and lactate dehydrogenase A (LDHA), which results in the loss of GSIS (Dhawan et al. 2015). Aging is a risk factor of T2D (Yakaryılmaz and Öztürk 2017) and DNA demethylation plays an essential role in the β-cell of aging mice (Unnikrishnan et al. 2019). When comparing young to old mice, reports showed a

demethylation of enhancers of key β-cell genes, such as insulin, and target genes of β-cell TFs like Pdx-1, Neurod1 and Foxa2 (Avrahami et al. 2015).

Epigenetic regulation plays an important role in maintaining functional identity of mature β-cells. The β-cell specific inactivation of Dnmt1 triggers the transdifferentiation of β- to α-cells and therefore the loss of functional β-cell mass (Dhawan et al. 2011). The α-cell marker *Arx* is repressed in β-cells through methylation of the promoter by the DNA methyltransferases Dnmt3a and Dnmt1 (Dhawan et al. 2011, Papizan et al. 2011). Dnmt3a also promotes silencing of *Arx* through the recruitment of HDAC-1 to the promoter, as it removes the activating histone acetylation mark (Papizan et al. 2011). In addition, methylation of the DNA enables methyl-DNA binding protein MeCP2 to be recruited to the promoter of *Arx*. MeCP2 mediates the recruitment of a complex of proteins including the methyltransferase Prmt6, which mediates repression through histone arginine methylation (Dhawan et al. 2011).

In previous studies, it has been demonstrated that the histone methyltransferase Set7/9 is required to maintain gene expression of genes involved in GSIS like *INS1*, *INS2*, *GLUT2* and *MAFA* (Deering et al. 2009). Set7/9 is thought to interact with factors like Pdx-1 and its depletion results in the loss of the gene transcription activating H3K4me2 mark at GSIS genes like *INS1*, *INS2*, *GLUT2* and *MAFA* (Deering et al. 2009). Studies have also shown an age-dependent decrease in the Polycomb group of proteins Bmi1 and Ezh2 (Chen et al. 2009, Dhawan, Tschen and Bhushan 2009). Loss of Bmi1 and Ezh2 is associated with the de-repression of *Cdkn2a* (Chen et al. 2009, Dhawan et al. 2009). *Cdkn2a* encodes for the cyclin-dependent kinase inhibitor $p16^{INK4a}$ which has been shown to inhibit β-cell proliferation (Krishnamurthy et al. 2004).

Recent studies investigating the DNA methylation profile in T2D patients compared to healthy controls identified a list of genes with alternated methylation patterns in the promoter of key β-cell genes (Volkmar et al. 2012, Dayeh et al. 2014). These results were confirmed by Lu *et al*. which they also extended into diabetic mouse models (Lu et al. 2018). In these studies, the authors show that the loss of β-cell markers, such as *PDX-1*, *MAFA* and *NKX6.1* is associated with reduced levels of H3K27me3 due to the dysfunction of PRC2 (Lu et al. 2018). β-cell specific knockout of embryonic ectoderm development (Eed) PRC2 subunit in mice resulted in the loss of several β-cell markers, de-repression of pancreatic progenitor markers, such as *GATA6* and *GATA4*, and the expression of the dedifferentiation marker GLI Family Zinc Finger 2 (Lu et al. 2018).

1.4 Chromatin remodelling complexes

Epigenetic control of gene expression is further achieved through nucleosomal repositioning by ATP-dependent chromatin remodelling complexes (Wu 1997). ATP- dependent chromatin remodelling complexes have an ATPase subunit that belongs to the family of SNF2 DNA helicases (Eisen, Sweder and Hanawalt 1995). These can be divided into four subfamilies based on sequence similarities of the ATPase subunit: 1) mammalian switch/sucrose non-fermentable (mSWI/SNF), 2) imitation SWI (ISWI), 3) chromodomain helicase DNA-binding (CHD, e.g. in the NuRD complexes) and 4) INO80 complexes (Hota and Bruneau 2016, Hoffmeister et al. 2017). In addition to the catalytic ATPase subunit, several regulatory subunits are found to interact together and form large molecular complexes. (Hota and Bruneau 2016). The regulatory subunits modulate the ATPase activity and are involved in their recruitment to specific genomic regulatory sequences (Cairns et al. 1996, Clapier and Cairns 2012, Chen, Conaway and Conaway 2013, Hota et al. 2013, Sen et al. 2013).

1.4.1 The mSWI/SNF complexes

The mSWI/SNF complexes family was first discovered in yeast in genetic screens aimed at unravelling mutations in genes involved in mating-type switching (SWI) and sucrose fermentation (SNF) (Workman and Kingston 1998, Sudarsanam and Winston 2000). mSWI/SNF complexes are around 1.5-2 MDa large complexes (Workman and Kingston 1998, Vignali et al. 2000, Hargreaves and Crabtree 2011), and are in general thought to stimulate transcriptional gene activity (Ryan, Jones and Morse 1998), although few reports have also shown a role in transcriptional repression (Wang et al. 1996b, Ho and Crabtree 2010). mSWI/SNF complexes are composed of evolutionary conserved subunits (Tang, Nogales and Ciferri 2010). In higher eukaryotes, the increasing complexity of the chromatin through an increase in genome size, DNA methylation and a more complex organisation of the genome required for the mSWI/SNF complexes to adapt through substitution or the addition of regulatory subunits (Tang et al. 2010). In mammals, the mutual exclusive Brahma (Brm) and Brg1 proteins form the ATPase core subunit of the mSWI/SNF complexes (Fig. 1.8) (Khavari et al. 1993, Muchardt et al. 1996, Tang et al. 2010).

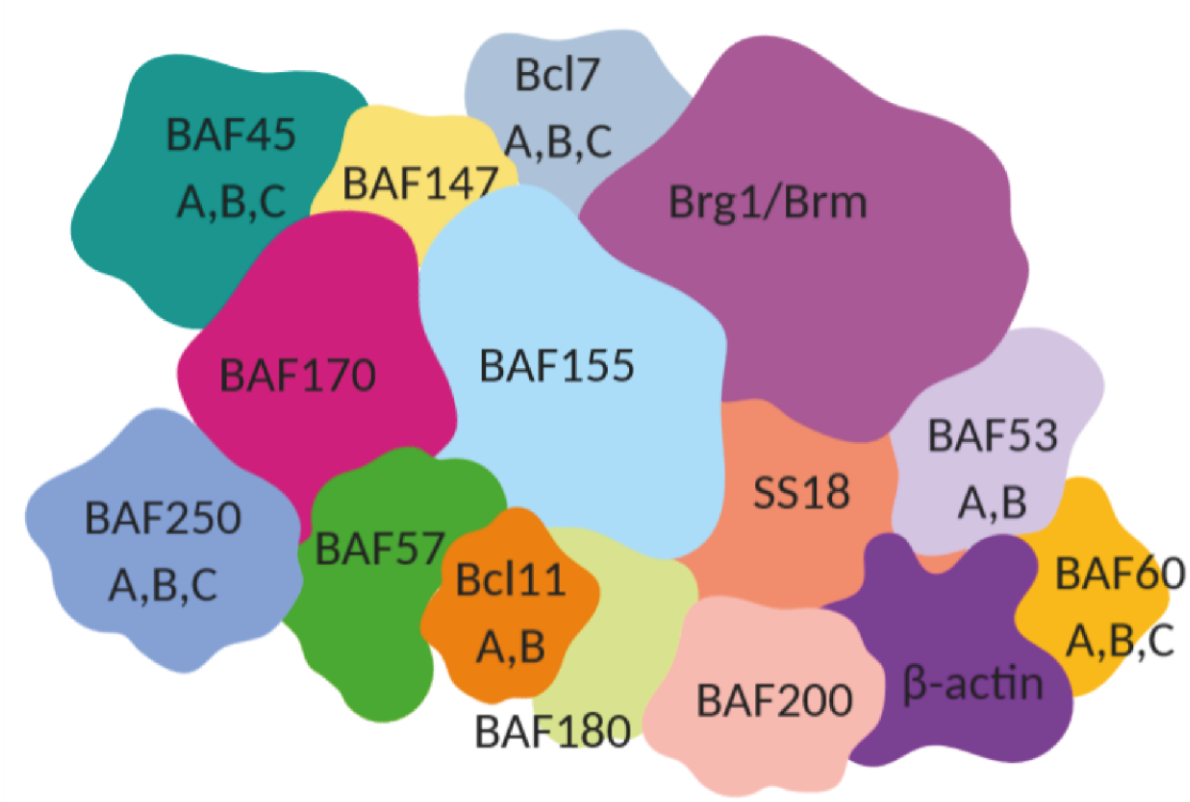


Figure 1.8: Schematic illustration of the arrangement of the mSWI/SNF complex subunits in mammals. The potential different exclusive isoforms of the regulatory subunits are indicated (e.g. BAF60 A or B or C). The catalytic ATPase core subunit is either Brg1 or Brm. Adapted from Spaeth et al. 2019.

Around the Brg1 or Brm core subunit are at least 14 different regulatory subunits arranged to form the mSWI/SNF complexes, also known as Brm/Brg1-associated factor (BAF) complexes in mammals (Fig. 1.8) (Hota and Bruneau 2016). A range of those regulatory subunits (BAF60, BAF45, BAF53, BAF250, Bcl7 and Bcl11) exist in different exclusive isoforms (Kaeser et al. 2008, Tang et al. 2010, Middeljans et al. 2012, Kadoch et al. 2013, Wischhof et al. 2017). mSWI/SNF complex function depends on the incorporation of specific isoforms of regulatory subunits (Goljanek-Whysall et al. 2014, Saccone et al. 2014).

mSWI/SNF complexes have essential roles in regulating cell differentiation during development in many different tissues, including hair follicles and skin (Xiong et al. 2013, Indra et al. 2005), heart (Takeuchi et al. 2011, Li et al. 2013), skeletal muscle (Albini et al. 2015, Mallappa et al. 2010), immune cells (Choi et al. 2012, Chi et al. 2003) and brain (Kim et al. 2001, Marathe et al. 2013). Interestingly, most of the mentioned studies, investigating the role of mSWI/SNF complexes in the development of different tissues revealed a dominating

role for Brg1 versus Brm to be the driver of differentiation. In those studies, deletion of Brg1 affected the correct formation of the specific tissues

1.4.2 Role of mSWI/SNF in cancer

The role of the mSWI/SNF complexes in tumorigenesis is well confirmed, with recent studies showing a mutation in genes encoding for mSWI/SNF complex members in approximately 20% of all human tumours (Kadoch et al. 2013). Extended research established the mSWI/SNF complexes as critical epigenetic regulators of metabolism, cell cycle and oncogenic pathways in cancer development (Wiegand et al. 2010, Li et al. 2011, Varela et al. 2011, Wilson and Roberts 2011, Shain et al. 2012, Shain and Pollack 2013, Masliah-Planchon et al. 2015).

Pancreatic ductal adenocarcinoma (PDAC) accounts for 90% of pancreatic cancer cases (He et al. 2018). Activating mutations of KRAS are present in 95% of PDAC cases and often accompanied with loss of *CDKN2A*, *TP53*, *SMAD4*, and *BRCA2* expression (Rozenblum et al. 1997, Gharibi, Adamian and Kelber 2016). *KRAS* expression together with loss of *GATA6* has been associated with dedifferentiation and stem cell-like properties in PDAC and acinar cells (Hermann et al. 2014). Additionally, dedifferentiation of pancreatic cancer cells has been associated through Dnmt1 induced silencing of Krüppel-like factor 4 (*KLF4*) (Xie et al. 2017b). Induction of miR-152 expression inhibited DNMT1 expression and subsequently induced *KLF4* expression. Re-expression of *KLF4* resulted in reduced cell growth in vitro and tumorigenesis mouse models of pancreatic cancer (Xie et al. 2017b).

The regulatory subunit isoform BAF250A has been reported to be mutated in pancreatic cancer cells (Biankin et al. 2012). In a study assessing the clinical relevance of the mSWI/SNF in pancreatic cancer, high expression levels of *BRG1* and *BRM* correlated with a worsened phenotype, including larger tumour size and increased invasiveness (Numata et al. 2013). Those findings suggest a similar role of Brg1 and Brm in pancreatic cancer. On the other hand, high levels of *BAF180* were associated with a decrease in tumour size and *BAF47* was related with negative lymphatic invasiveness. Additionally, high Brm and low BAF180 levels were independent indicators of poor survival (Numata et al. 2013). Those findings were supported by studies presenting intraepithelial neoplasia–derived pancreatic ductal adenocarcinoma (PDAC) that originated from acinar cells required positive regulation of *SOX9* expression by Brg1 (Tsuda et al. 2018). Interestingly, other studies showed a tumour suppressive role for the

mSWI/SNF complexes, where re-expression of *BRG1* and *BRM* in pancreatic cancer cell lines deficient for both genes show reduced cell growth and induction of senescence (Shain et al. 2012).

1.4.3 Role of mSWI/SNF in metabolic and insulin responsive tissues

Surprisingly, not much evidence is found for the involvement of the mSWI/SNF complexes in the regulation of β-cell function in adulthood. McKenna et al. first showed the involvement of the mSWI/SNF complexes in glucose homeostasis, postulating an opposing role of Brm and Brg1 mSWI/SNF complexes in the regulation of genes involved in GSIS (McKenna et al. 2015a). According to their studies, elevation of glucose levels induces a switch in the recruitment from the Brm to the Brg1 containing mSWI/SNF complexes. Following its interaction with the Brg1 subunit, mSWI/SNF induce transcription of insulin and other genes involved in insulin biosynthesis and secretion, such as *GLUT2* and *MAFA* (McKenna et al. 2015a). They identified Pdx-1 as a major TF recruited with the mSWI/SNF complexes. While *BRG1* and *BRM* expression were not altered in T2D patient samples, interaction of Pdx-1 with Brg1 was perturbed in accordance with loss of *PDX-1* expression. Interestingly, Pdx-1:Brm interaction was not altered in T2D patients, suggesting that the switch from Pdx-1:Brm to Pdx-1:Brg1 is dysfunctional due to decreased Pdx-1 levels (McKenna et al. 2015a).

Very recent studies revealed that embryonic deletion of Brg1 reduced the proliferation capability of pancreatic progenitor cells and resulted in pancreas hypoplasia (Spaeth et al. 2019). In adult mice, the combination of Brg1 and Brm β-cell specific deletion was necessary to perturb β-cell function, suggesting a redundant role of Brg1 and Brm in β-cells (Spaeth et al. 2019). Deletion of Brg1 and Brm resulted in reduced expression of insulin and other key metabolic genes (Spaeth et al. 2019).

In the liver, Brg1 is involved in proliferation processes, as seen by upregulation of BRG1 expression in mice subjected to partial hepatectomy. Hepatocyte specific deletion of BRG1 resulted loss of liver to body weight ratio and decreases in hepatocellular proliferation 48 hours after partial hepatectomy compared to Wt mice (Wang et al. 2019a). Other studies show the involvement of BAF60A in fatty acid β-oxidation in the liver (Li et al. 2008) and determine it as a diet-sensitive subunit of the mSWI/SNF complexes in the liver controlling whole body cholesterol homeostasis (Meng et al. 2015a).

In brown adipose tissue, inhibition of the histone reader regulatory subunit BAF45C resulted in the decrease of brown adipocyte specific gene expression, including *UCP2* and *DIO2* (Shapira et al. 2017). BAF45C has been shown to interact with transcription factor early B-cell factor 2 (EBF2) in brown adipocytes and supports Brg1 binding to brown adipose genes *UCP1* and *PPARA* (Shapira et al. 2017).

1.4.4 The mechanism of mSWI/SNF chromatin remodelling

mSWI/SNF complexes utilise the energy liberated from hydrolysis of ATP into ADP to move the position of nucleosomes (Fig. 1.9) (Wu 1997). In the first step, the mSWI/SNF complex binds to the nucleosome. This process of binding occurs with a nanomolar dissociation constant (Lorch et al. 1998) in a way that prevents nucleosomal DNA to be digested by nucleases (Saha, Wittmeyer and Cairns 2005). The catalytic ATPase subunit, also named translocase domain, of the mSWI/SNF complexes is proposed to consist of a torsion and a tracking subdomain and binds approximately two turns from the dyad axis, the central axis of the nucleosome core particle (Schwanbeck, Xiao and Wu 2004, Zhang et al. 2006, Zofall et al. 2006). Hydrolysis of ATP induces loss of DNA – histone contacts and creates a transient DNA loop that transmits through the first DNA gyre onto the second DNA gyre and the loop resolves upon reaching the exit site on the other side of the nucleosome (Havas et al. 2000). This process shifts the nucleosome unidirectional, ensured by the tracking domain, along the DNA (Havas et al. 2000). Afterwards the mSWI/SNF complex resets its original position and is ready for a new cycle (Havas et al. 2000).

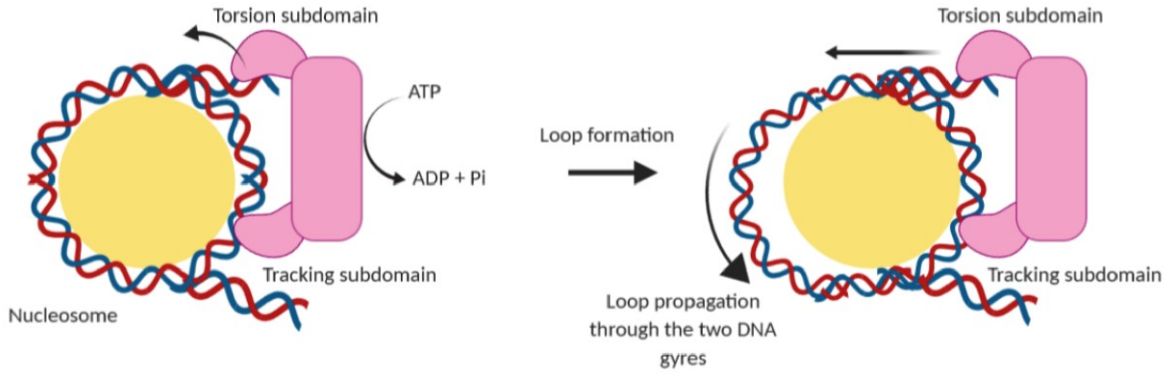


Figure 1.9: Schematic illustration of the mechanism of chromatin remodelling by mSWI/SNF complexes. Hydrolysis of ATP induces DNA loop formation by the torsion subdomain of the translocase domain. The DNA loop

propagates through the two DNA gyres. The tracking subdomain ensures unidirectional movement of the DNA loop. Adapted from Tang et al. 2010.

1.5 MicroRNAs (miRNA)

1.5.1 miRNA identification and biogenesis

MiRNAs are 18-25 nucleotide long single stranded RNA molecules (Lujambio and Lowe 2012). The first discovered miRNA, *lin-4*, was found in *Caenorhabditis elegans* and shown to inhibit translation of the heterochronic protein lin-14, encoded by the gene *lin-14*, by interacting with a partial complementary sequence found in the 3' untranslated region (UTR) of the *lin-14* transcript (Lee, Feinbaum and Ambros 1993, Wightman, Ha and Ruvkun 1993, Ambros 2001).

MiRNA biogenesis is initiated in the nucleus by the transcription of the miRNA gene into a long, capped and polyadenylated primary RNA transcript (pri-miRNAs) by RNA polymerase II (Fig. 1.10) (Lujambio and Lowe 2012). These RNA molecules are processed in the nucleus by the ribonuclease Drosha (Denli et al. 2004). In complex with the RNA binding protein DGCR8, Drosha cleaves the pri-miRNAs into hairpin RNAs of 60-100 nucleotides (Denli et al. 2004), also known as precursor miRNAs (pre-miRNAs), which are then transported by the Ras-GTP-dependent Exportin-5 transporter into the cytoplasm (Bohnsack, Czaplinski and Gorlich 2004, Yi et al. 2003). In the cytoplasm, the pre-miRNAs are further processed by the RNAse III endonuclease Dicer into 18-25 nucleotide long double stranded miRNAs (Bernstein et al. 2001, Ketting et al. 2001, Knight and Bass 2001). Double stranded miRNA is loaded onto an argonaute (Ago) protein to form the RNA-induced silencing (RISC) complex, where a single miRNA strand is selected, while the other one is degraded (Martinez et al. 2002). Loading of the miRNA duplex onto the Ago protein is supported by ATP-hydrolysis by heat shock 70 kDa protein 8 (Hsc70) and heat shock protein 90 (Hsp90) (Iwasaki et al. 2010, Miyoshi et al. 2010). The Ago protein consists of the N, PAZ, MID and PIWI domains and two linker regions (L1 and L2) (Wang et al. 2008, Elkayam et al. 2012). The loading of the strand is asymmetric, as the strand with the less stable 5' end binding to its complementary strand, also known as guide strand, is kept (Khvorova, Reynolds and Jayasena 2003, Schwarz et al. 2003). The 5' end of the guide strand is hold between the MID and PIWI domain, whereas the 3' end is anchored at the PAZ domain (Elkayam et al. 2012, Nakanishi et al. 2012, Schirle and MacRae 2012). The N domain and the L1 linker are the main binding sites for Hsc70/Hsp90 (Tahbaz, Carmichael

and Hobman 2001, Nakanishi 2016). The family of Ago proteins consists of 8 members and is divided into two subfamilies in human. One is the AGO subfamily, consisting of *AGO1*, *AGO2*, *AGO3* and *AGO4* with similarities to *Arabidopsis thaliana AGO1* and the other is the PIWI subfamily, consisting of *HIWI1*, *HIWI2*, *HIWI3* and *HIWI4* with similarities to *Drosophila PIWI* (Schirle and MacRae 2012, Meister 2013). While the PIWI subfamily is only found to be expressed in germ-line cells, the AGO subfamily is broadly expressed in the majority of tissues (Schirle and MacRae 2012, Meister 2013). In human, AGO2 has been shown to be the only member with catalytic activity and an essential role in the RISC complex (Liu et al. 2004, Meister et al. 2004, Song et al. 2004a, Rivas et al. 2005). The miRNA incorporated into the RISC complex guides Ago2 to the mRNA target by complementary binding of the 6-8 nucleotide seed sequence found at the 5' end of the miRNA to its target site found in the 3'UTR of the mRNA (Lewis et al. 2003). The hybridisation of the RISC complex with the target mRNA results in degradation or translational repression (Lujambio and Lowe 2012). Glycine-tryptophan protein of 182 kDa (GW182), is recruited to the RISC complex and facilitates translational repression and recruitment of the mRNA degrading CCR4-NOT deadenylase complex (Huntzinger and Izaurralde 2011, Iwakawa and Tomari 2015, Jonas and Izaurralde 2015).

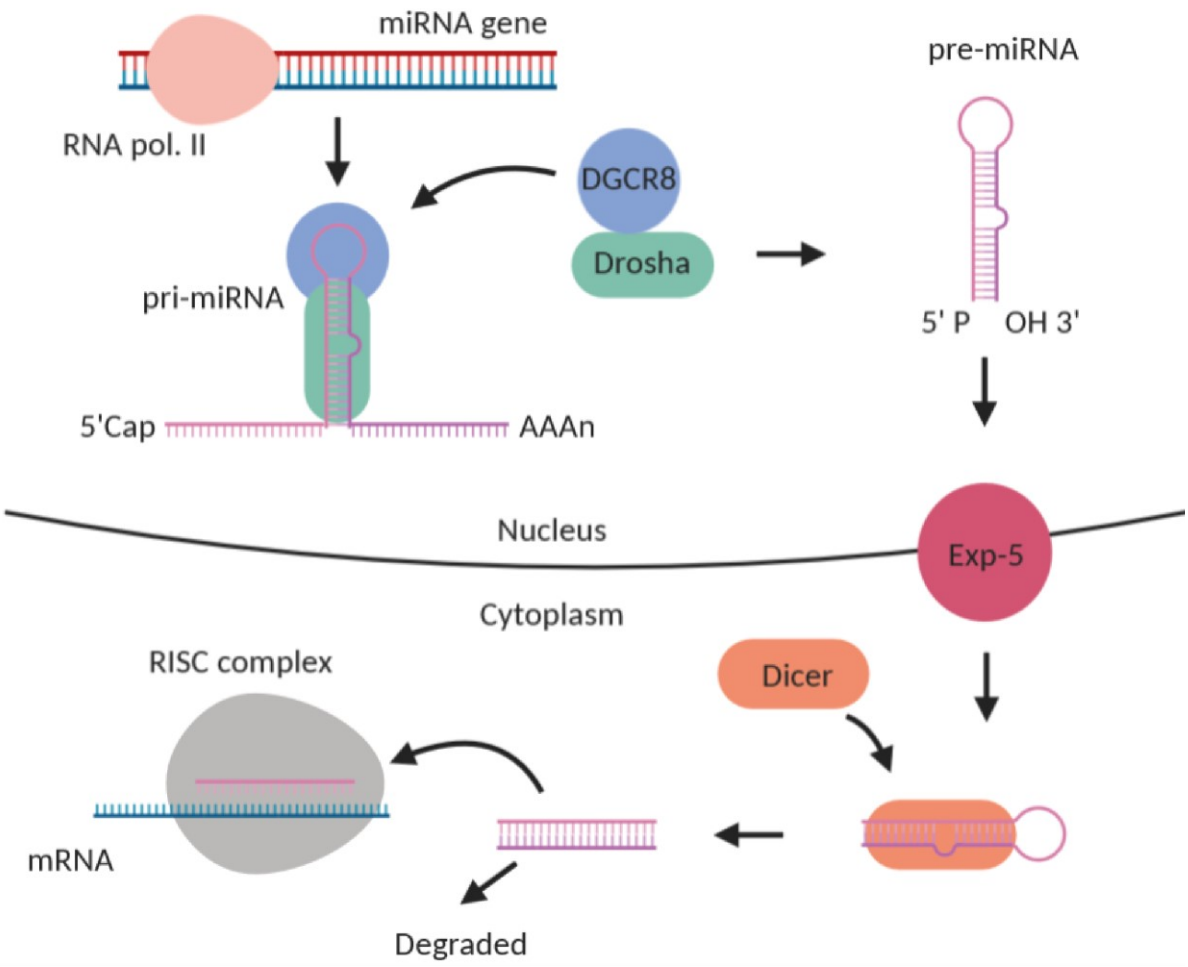


Figure 1.10: Schematic illustration of miRNA biogenesis. Pri-miRNAs are transcribed by RNA polymerase II and processed by the Drosha enzyme into pre-miRNAs. Pre-miRNAs are then transported out of the nucleus by Exportin-5 (Exp-5). Processing of the pre-miRNA by Dicer results in mature miRNAs. The guiding strand is incorporated into the RISC complex to initiate mRNA degradation or translational repression through complementary binding of the seed sequence on the miRNA with the target sequence found in the 3'UTR of the mRNA target. Adapted from Yao, Chen and Zhou 2019.

1.5.2 Biological function of miRNAs

The role of miRNAs in cellular differentiation and tissue development is well understood (Song and Tuan 2006). Similar to the expression profiles of TFs, miRNA expression is spatially and/or temporally controlled at specific stages of cell and tissue development (Lagos-Quintana et al. 2002, Aravin et al. 2003). For instance, while the miRNAs of the miR-290-295 cluster are expressed in undifferentiated ES cells, their expression is repressed in embryoid bodies of 14 day cultured ES cells (Houbaviy, Murray and Sharp 2003).

Global knockout of the pre-miRNA processing Dicer enzyme in mice, which processes pre-miRNAs, results in early lethality of the embryos and the lack of multipotent stem cells (Murchison et al. 2005). In accordance with that, deletion of Dicer enzyme in early pancreas development through a Cre recombinase under control of the *PDX-1* promoter resulted in gross defect of all pancreatic lineages with more severe effects in the endocrine cell lineage and β-cells in particular (Lynn et al. 2007a). Dicer deletion resulted in an increase in *HES1* and a subsequent downregulation of *NGN3* (Lynn et al. 2007a).

Dicer deletion in insulin expressing cells through a Cre recombinase under control of the rat insulin II promoter (*RIP*) resulted in normal β-cells and insulin secretion at two weeks of age, suggesting no changes in β-cell development (Kalis et al. 2011). Contrary, in the adult animal, the RIP-Cre Dicer knockout resulted in hyperglycaemia and impaired insulin secretion, including reduced insulin gene expression, decreased β-cell mass and reduced insulin granules and granule docking events (Kalis et al. 2011).

Martinez-Sanchez *et al.* deleted Dicer in β-cells of adult mice utilising a tamoxifen inducible Pdx1-Cre (Martinez-Sanchez, Nguyen-Tu and Rutter 2015). 2 weeks post recombination, β-cell dysfunction was demonstrated by the de-repression of β-cell disallowed genes, including *OAT*, *PDGFRA*, and *SLC16A1*, preceding a decrease in islet insulin content and loss of β-cell mass (Martinez-Sanchez et al. 2015).

MiR-375 is highly expressed in pancreatic islets and its global knockout in mice resulted in a 30-40% reduction in β-cell mass as a result of impaired proliferation, as seen in the upregulation of genes of negative regulators of proliferation like *RASD1* and *CADM1* (Poy et al. 2009). Mir-375 knockout mice show an increase in α-cell mass and increases in circulating Gcg levels resulted in chronic hyperglycaemia and diabetes (Poy et al. 2009). Interestingly, follow up studies revealed, that the rescue of miR-375 expression in β-cells was sufficient to normalise β- and α-cell mass and restored the glucose clearance ability in comparison to miR-375 global knockout mice (Latreille et al. 2015).

1.5.3 Regulation of epigenetic modulators by miRNAs

The research into miRNA and their epigenetic regulation of pancreatic β-cell differentiation processes is mostly unknown. The first evidence has been provided by Mutskov and Felsenfeld, that showed a correlation between the abundance of non-coding RNAs and the activating $H3K4me^2$ on the insulin gene in human donors (Mutskov and Felsenfeld 2009).

MiRNAs have been reported to repress the expression of enzymes associated to the epigenetic regulation in cells. In recent studies, Zhang et al. showed that miR-29b inhibition in porcine early embryo development upregulated the expression of *Dnmt3a/b* and the ten eleven translocation (TET) enzyme *TET1*, while downregulating *TET2/3*, which increased the levels of global DNA methylation (Zhang et al. 2018). Similar results were seen in mouse models of bladder cancer, where suppression of miR-29 and subsequent upregulation of *DNMT3A* expression, resulted in gene silencing through DNA methylation at the promoter of the tumour suppressor *PTEN* (Palmbos et al. 2015). In lung cancer cells, miR-101 has been shown to suppress the proliferation of those cells by targeting the mRNA of *Dnmt3a* (Wang et al. 2017). The mRNA of *Dnmt1* is targeted by miR-377 in human skin fibroblasts, where the upregulation of miR-377 promotes senescence (Xie et al. 2017a).

Genes encoding subunits of histone modifying complexes are also subjected to miRNA regulation. For instance, Zhao *et al*. showed that miR-34a directly targets *HDAC-I* mRNA levels in foam cells. Loss of HDAC-1 increased global H3K9Ac levels, which was associated with increased lipid accumulation (Zhao et al. 2017). Additionally, bioinformatical analysis of the gene expression datasets obtained from hepatitis B virus infected hepatocellular carcinomas tissues, compared to hepatitis B virus tissue samples with no fibrosis revealed the altered expression of multiple miRNAs in the regulation of H3K9 methylation. Out of those, miRNAs 14 were overexpressed and 16 downregulated compared with the control samples (Ding et al. 2017).

Furthermore, miRNAs have been reported to be involved in the regulation of chromatin remodelling processes involving the mSWI/SNF complexes by regulating the incorporation of specific isoforms of regulatory subunits. Studies demonstrated the switch of BAF60A and BAF60B, through the repression by microRNA (miR) – 133 (BAF60A) and miR-1/206 (BAF60B), to BAF60C (Goljanek-Whysall et al. 2014, Saccone et al. 2014). The switch from BAF60A/B to BAF60C induces muscle specific gene expression (Goljanek-Whysall et al. 2014, Saccone et al. 2014). Similar to the BAF60 switch in myogenesis, occurs a switch from

BAF53A, found in neural progenitor cells, to BAF53B, expressed in mature neuronal cells (Yoo et al. 2009). This switch is, again, mediated by miRNAs. MiR-9* and miR-124 repress the expression of BAF53A and therefore facilitate BAF53B expression (Yoo et al. 2009).

1.5.4 The miR-7 gene family

MiR-7 was first discovered in *Drosophila melanogaster*, where it was shown to be present only during embryogenesis and absent in later developmental stages (Lagos-Quintana et al. 2001). Later studies showed that miR-7 is highly conserved throughout the whole animal kingdom (Prochnik, Rokhsar and Aboobaker 2007). In mice, miR-7 is encoded and processed from three different genes named miR-7a1, miR-7a2 and miR-7b. The seed sequence in all three miR-7 family members is conserved and miR-7a1 and miR-7a2 share the exact same sequence. MiR-7b only differs in the 10th nucleotide compared to miR-7a1 and miR-7a2 (Fig. 1.11) (Lee, Palkovits and Young 2006, Pollock et al. 2014). MiR-7a1, 7a2 and 7b are transcribed from unlinked genomic loci on chromosomes 13, 7 and 17, respectively. Similar to the mouse, the human miR-7 is generated from three different precursors (pri-miR-7-1, pri-miR-7-2, pri-miR-7-3) located on chromosomes 9, 15 and 19 (Kalinowski et al. 2014). Pri-miR-7-1, pri-miR-7-2, pri-miR-7-3 are processed into pre-miR-7-1, pre-miR-7-2, pre-miR-7-3 and further into one mature, 23 nucleotide, miR-7, that is incorporated into the RISC complex (Fig. 1.11) (Kalinowski et al. 2014, Ahmed et al. 2017).

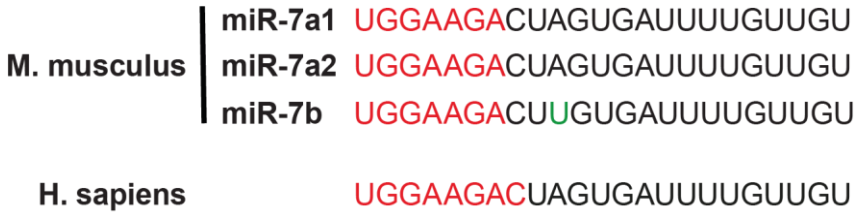


Figure 1.11: Schematic illustration of the mature miR-7 sequence in mouse and human. The miR-7 seed sequence is highlighted in red. Mouse miR-7a1 and miR-7a2 and human miR-7 share the same sequence. The sequence for mouse miR-7b differs at the 10th nucleotide (highlighted in green) compared to mouse miR-7a1/7a2 and human miR-7.

1.5.5 Physiological functions of miR-7

Although miR-7 is mainly expressed in neuroendocrine cells of the pituitary gland, adrenal gland, hypothalamus and islet of Langerhans (Landgraf et al. 2007, Joglekar, Joglekar and Hardikar 2009, Bravo-Egana et al. 2008, Kredo-Russo et al. 2012b) others reported expression in other tissues including brain cortex, muscle and liver (Yan et al. 2014). MiR-7 is involved in the correct development of the cerebral cortex. Blocking of miR-7 function, utilising miR-7 sponge transgenic mice, resulted in brain defects similar to those observed in microcephaly, which was associated with increased of *AK1* and *CDKN1A* expression of the p53 pathway (Pollock et al. 2014). Knockdown of miR-7, utilising the same mouse miR-7 sponge, in proopiomelanocortin (POMC)-expressing neurons in the arcuate nucleus of the hypothalamus resulted in sex specific aggravation of high fat diet induced obesity in female mice (Gao et al. 2019). Interestingly, miR-7 blockage temporally improved glucose clearance in female mice at 18 weeks. In females of 35 weeks and male mice (both timepoints) no differences were observed (Gao et al. 2019).

Global miR-7a2 knockout in mice disturbs sexual maturation and infertility in both male and females. Loss of miR-7a2 results in reduced gonadotropic and sex steroid hormones, small testes and ovaries and impaired spermatogenesis and ovulation in males and females, respectively. MiR-7a2 affects gonadotropin synthesis by suppressing golgi glycoprotein 1 *GLG1*) and prostaglandin F2a receptor negative regulator (*PTGFRN)* in the pituitary gland, resulting in dysregulation of prostaglandin signalling as well as that of follicle-stimulating hormone (*FSH*) and luteinizing hormone (*LH*) (Ahmed et al. 2017).

MiR-7 is one of the major miRNAs found in islets (Bravo-Egana et al. 2008) and has also been shown to be involved in the development of the pancreas and differentiation of β-cells. Stimulation of miR-7 expression in early pancreatic development in mice was addressed in studies utilising Pdx-1-Cre induced miR overexpression, resulting in miR-7 overexpression from E13.5 onwards (Kredo-Russo et al. 2012a). Pdx-1-Cre overexpressing miR-7 mice showed a decrease in mRNA and protein levels of the endocrine markers Ins and Gcg at E15.5, as well as the decreases in the expression of endocrine TFs *ARX*, *PAX4* and the direct target of miR-7 *PAX6*. Of note, markers of exocrine cells, like *CPA1* and *PTF1A* were not affected (Kredo-Russo et al. 2012a).

In human embryonic stem (hES) cells miR-7 overexpression resulted in upregulation of key β-cell genes including *INS*, *FOXA2* and *PDX-1* (López-Beas et al. 2018). miR-7 levels were

observed to be highly expressed in early differentiation stages of hES cells (5 days) and decreased progressively with maturation (11 days and 22 days), showing the importance of miR-7 in development, whereas the role of miR-7 in differentiated β-cell remained to be determined (López-Beas et al. 2018).

In β-cells, miR-7 expression was reported to be under the control of NeuroD/Beta2 and Ngn3 binding to conserved genomic DNA blocks, that exhibit promoter activity, upstream of pre-miR-7a2 and pre-miR-7b (Kredo-Russo et al. 2012b). Additionally, recent studies showed a significant enrichment of miR-7 inside human T2D patients serum when compared to control samples (Wan et al. 2017)

In previous work, our group discovered the involvement of miR-7 in regulating β-cell function. Conditional knockout mice with deletion of miR-7a2 in β-cells using the RIP-Cre (RIP-Cre miR-7a2$^{fl/fl}$) show improved glucose clearance compared to control animals due to increased insulin secretion (Latreille et al. 2014). Overexpression of miR-7a2 in dissociated mouse β-cells resulted in no changes in plasma membrane depolarisation but demonstrated reduced insulin granule exocytosis. Gene ontology analysis in miR-7a2 overexpressing immortalised mouse β-cells (Min6) showed clusters of downregulated genes in “Membrane”, “Cell-to-Cell signalling” and “Secretion” pathways. Among the genes repressed by miR-7 were several regulators of vesicle fusion and SNARE activity (*SNCA*, *CSPA* and *CPLX1*), calcium-regulated vesicular trafficking (*PKCB*), cytoskeleton rearrangement (*PFN2*, *WIPF2*, *BASP1* and *PHACTR1*) and membrane targeting (*ZDHHC9*), supporting a role for mIR-7 in regulating insulin vesicle trafficking and fusion with the membrane. MiR-7a expression levels were initially decreased in normoglycemic and compensating *db/db* animals at 5 weeks but progressively increased in *db/db* mice compared to Wt mice reaching a 4-fold increase at 12 weeks (Latreille et al. 2014). Moreover, human islets, grafted under the kidney capsule of mice, showed a decrease in miR-7 expression at 6 weeks and an increase at 10 weeks in mice on a high fat diet compared to chow diet controls. In accordance with that, gene expression of SNCA, SOX9, MAFA, PDX-1, NKX6.1, PAX6 and INS was initially increased (6 weeks) but decreased with time (10 weeks) (Latreille et al. 2014). Those results indicate that initially miR-7 is downregulated in the compensating islets, facilitating the upregulation of genes involved in insulin synthesis and secretion. In the progression of the disease, miR-7 expression is upregulated, resulting in the loss of key β-cell genes.

Tg7a2 mice constitutively overexpressing miR-7a2 under the control of the rat Ins2 promoter (RIP) showed a 3-fold increase in miR-7a expression levels. Tg7a2 animals show a severe hyperglycaemic phenotype starting at 3 weeks of age. Hyperglycaemia was accompanied with reduced circulating insulin levels and a reduction in GSIS in 12w Tg7a2 mice. The expression of genes involved in vesicular trafficking and exocytosis, including *CSPA*, *CPLX1* and *PKCB*, was decreased in Tg7a2 mice. Mutant animals also showed reduced expression of *INS1* and *INS2*, as well as *PDX-1*, *NKX6.1*, *MAFA*, *PAX6*, *GATA6* and *NEUROD1*. Those results were confirmed via immunostaining at 15 weeks, showing a reduction in detected Nkx6.1, MafA, Glut2 and insulin protein levels in Tg7a2, which mirrored the expression profile of islets from *db/db* animals. *PAX6* and *GATA6* were validated as targets of miR-7a. Interestingly, Tg7a2, and *db/db*, mice show a de-repression of the progenitor marker *SOX9* at 15 weeks of age in immunostainings. These results strongly indicate a dedifferentiation of β-cells following overexpression of miR-7a2 in diabetes (Latreille et al. 2014).

Recent gene set enrichment analysis (GSEA) (Subramanian et al. 2005) on RNA-Sequencing (RNA-Seq) performed by our group shows that downregulated genes in islets from Tg7a2 animals compared to Wt mice are enriched in classes of core β-cell components, including "unfolded protein response" (like *DNAJC3*, *ERO1B* and *ATF6*), "pancreatic β-cell identity" (like *UCN3*, *INS2* and *NKX6.1*) and "protein secretion" (like *RABA2A*, *SNAP23* and *VAMP3*) (Fig. 1.12A, Mak et al. under review, data from M. Latreille). On the other hand, we found that upregulated genes in Tg7a2 islets enriched in classes like "epithelial to mesenchymal transition" (EMT), "inflammation" and "Kras activation" (Fig. 1.12B, Mak et al. under review). The loss of β-cell core components and the upregulation of EMT (luminal stem cells, breast cancer), inflammation (T2D, β-cells) and Kras (PDAC, acinar cells) have all been associated to dedifferentiation processes (Bai et al. 2014, Hermann et al. 2014, Nordmann et al. 2017)

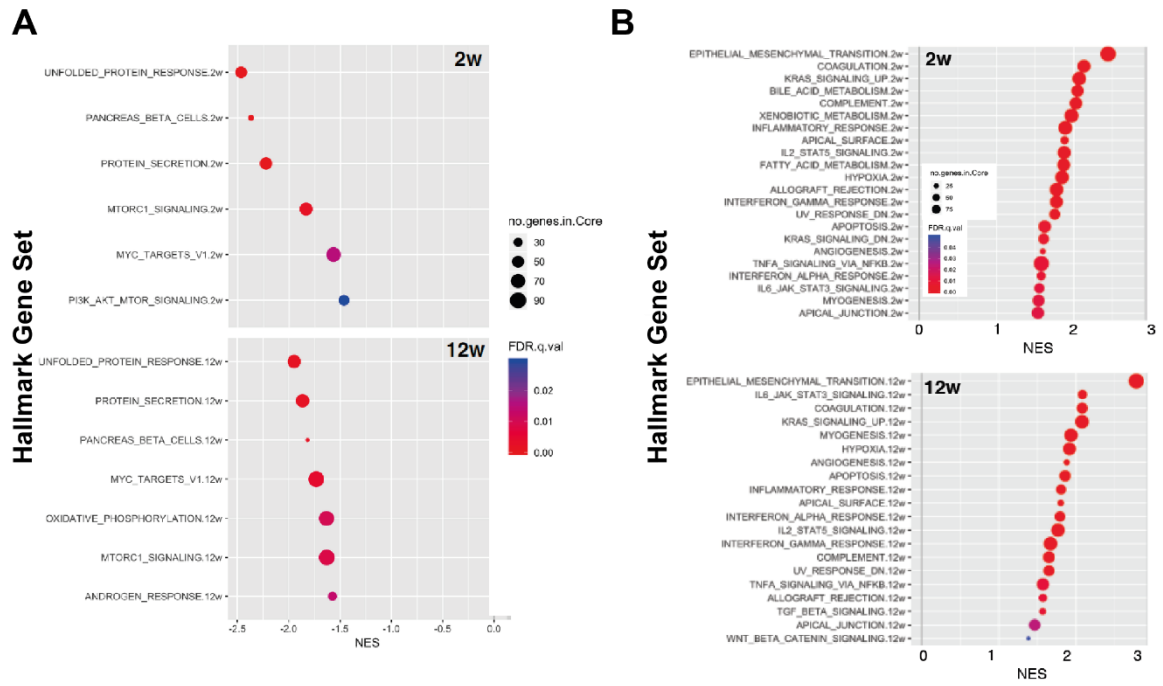


Figure 1.12: GSEA in RNA-Seq of islets from Tg7a2 mice compared to Wt controls. (A) GSEA of MSigDB Hallmark genes in downregulated pre-ranked gene ratios (Tg7a2:Wt) from 2w (top) and 12w (bottom) Tg7a2 islets. (B) GSEA) of MSigDB Hallmark genes in upregulated pre-ranked gene ratios (Tg7a2:Wt) from 2w (top) and 12w (bottom) islets. Gene sets with indicated FDR are plotted relative to normalized enrichment scores (NES). Circle size denotes the number of genes in each category and circle colour indicates FDR q values. Adapted from Mak *et al.* under review.

The process of dedifferentiation to a progenitor-like cell state can proceed the transdifferentiation into other cell types, as seen in *FOXO1* knockout mice, showing a subpopulation of dedifferentiated β-cells to acquire an α-cell fate (Talchai et al. 2012).

We found further evidence of β-cell transdifferentiation in Tg7a2 animals crossed with tdTomato$^{RIP\text{-}Cre}$ animals. tdTomato$^{RIP\text{-}Cre}$ mice carry a Rosa26-floxed-Stop-tdTomato transgene (Luche et al. 2007), as well as the Cre recombinase behind the RIP (Herrera 2000). Consequently, Tg7a2 tdTomato$^{RIP\text{-}Cre}$ express miR-7a2 and tdTomato in a β-cell specific manner, allowing lineage tracing of β-cells. Quantification showed an increase in the Sst / tdTomato co-stained area in islets of Tg7a2 animals compared tdTomato$^{RIP\text{-}Cre}$ controls, indicative for transdifferentiation of β-cells into δ-like cells (Fig. 1.13, Mak et al. under review). In fact, increased expression of δ-cell markers is also found in islet of Tg7a2 mice further supporting the transdifferentiation of β-cells into δ-cells in diabetes.

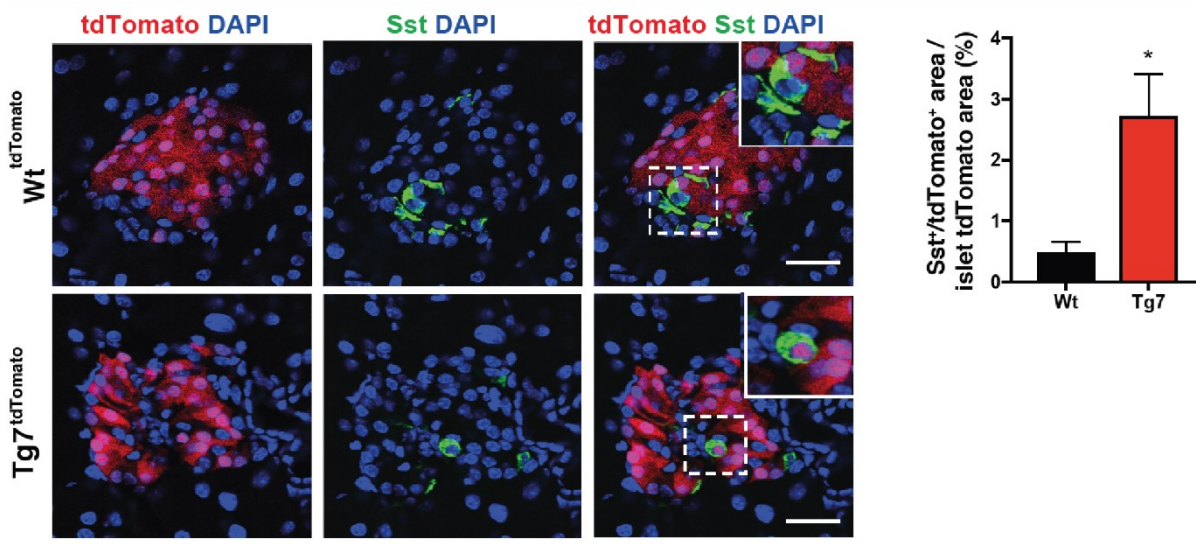


Figure 1.13: Transdifferentiation of β-cell into δ-cells in islet of Tg7a2 mice. animals show an increase in Sst / tdTomato expressing cells, indicating a transdifferentiation β-cells and to δ-cells. Adapted from Mak *et al*. under review.

1.6 Aims of the project

Our previous findings indicated a dedifferentiation and transdifferentiation of β-cells in δ-cells following induction of miR-7a in mice. In human, insulin, glucagon and somatostatin expressing cells in the developing, as well as adult pancreas, show similar levels of miR-7 (Correa-Medina et al. 2009). We now aimed to address if elevated miR-7 levels trigger the dedifferentiation, as well as potential transdifferentiation, of mouse and human β-cells *in vitro* and *ex vivo* independently of circulating factors associated with hyperglycaemia and diabetes. Therefore, our first goal was to:

Investigate if miR-7a2 triggers the dedifferentiation, and acquisition of an atypical cell fate, of β-cells in an β-cell only, as well as an islet, environment in both mouse and humans.

Dedifferentiation of β-cells by miR-7a must involve one or multiple targets regulated by miR-7a. In previous work our group identified *PAX6,* a β-cell TF and target of miR-7a2 in *in vitro* reporter assays (Latreille et al. 2014). However, the repression of expression of *PAX6* is relatively modest in islets form Tg7a2 mice, indicating that the repression of other mRNA targets mediates the effect of miR-7 in β-cells. Therefore, our second goal was to:

Identify mRNA targets of miR-7 which contribute in inducing β-cell dedifferentiation in T2D.

Following up on the identification of novel miR-7 targets, we wanted to investigate whether those targets effect the identity of β-cells. Therefore, our last goal was to:

Assess how miR-7 targets impact on the functional identity of mature β-cell.

The majority of the presented results in this thesis are under revision at the Journal of Clinical Investigation in which T. Mak and I have co-first authorship (Mak et al. under review) and also uploaded to bioRvix: https://doi.org/10.1101/789461

Chapter 2: Material and Methods

2.1 Cell culture

In the studies the following cell lines were used: Immortalised mouse (Min6) and rat (Ins1E) pancreatic β-cell lines and human embryonic kidney cells expressing the large T antigen (HEK293T), which improves the viral replication of plasmids containing a SV40 origin of replication. Cells were cultured in a 37°C, 95% O_2/5% CO_2 incubator (37°C incubator, Triple red, Nuare ISO13485) and their specific media. Min6 cells were kept in Dulbecco's Modified Eagle Medium with 4.5 g/l glucose and phenol red (DMEM, Thermo Fisher, UK) supplemented with 15% fetal bovine serum (FBS, Euro-Bio, UK), 1% Penicillin-Streptomycin (P/S, Thermo Fisher), 1% GlutamaX (Gx, Thermo Fisher), 1% sodium pyruvate 100mM (Thermo Fisher) and 0.0005% β-Mercaptoethanol (Sigma Aldrich, UK). Ins1E cells were kept in Roswell Park Memorial Institute medium 1640 (RPMI, Thermo Fisher) supplemented with 10% FBS, 1% P/S, 1% Gx, 1% sodium pyruvate, 0.0005% β-Mercaptoethanol and 0.2% 1M Hepes pH 7.3 (Thermo Fisher). HEK293T cells were kept in DMEM with 5% FBS (Sigma Aldrich), 1% P/S and 1% Gx.

2.2 Adenoviral transduction of microRNA-7a2 (miR-7a2)

For overexpression of miR-7a2, transduction of Min6/Ins1E/pancreatic islets with adenovirus carrying mouse miR-7a2 expressing vectors as described in (Latreille et al. 2014) was performed. MiR-7a2 (Ad-7a2) (Viraquest, VQAd-mir-7a2 250) and control adenoviruses (Ad-Ctrl) (Viraquest, VQAd EMPTY-eGFP) also carried the green fluorescent protein (GFP) under an independent promoter. Min6/Ins1E cells were plated in a 6-well plate at $1*10^6$ cells/well and incubated overnight (ON) in a 37°C incubator. For whole and dissociated islets, we calculated for an average of 1000 cells per islet. The media was changed to one with 2% FBS and 1% P/S, the adenovirus added at a multiplicity of infection (MOI) of 200 and left in a 37°C incubator ON. Afterwards the media was changed to the full one used for the specific cell type and the cells were left in a 37°C incubator for an additional 36 h (48 h total). Successful transduction was verified by fluorescence microscopy and miR-7a2 mRNA expression in quantitative polymerase chain reaction (qPCR).

2.3 Transfection of Locked Nucleic Acid (LNA)

For downregulation of miR-7 in cells, the miRCURY LNA Inhibitor has-miR-7-5p (LNA-7a, Exiqon, USA) was used. Transfection was done via lipofection. Cells were seeded into 6-well plates at a density of $1*10^6$ cells/well (6-well plate) the day before the transfection in their respective media. For the transfection, the media was aspirated and replaced by 1.5 ml media without P/S. 244 µl of Optimem-media, reduced serum, no phenol red (Optimem, Thermo Fisher) buffer were combined with 6 µl Lipofectamine 2000 (Lipofectamine, Thermo Fisher) per well and incubated at room temperature (RT) for 5 min. The LNA-7a and control LNA (LNA-ctrl, non-targeting LNA) were pipetted into Optimem buffer at the concentration of 20 nM and to a total volume of 250 µl. LNA and Lipofectamine were then combined and incubated at RT for 20 min. Afterwards 500 µl of the LNA/Lipofectamine mix were pipetted into each well for a total volume of 2 ml. After 48 h the cells were lysed/Trizol RNA extraction for further analysis.

2.4 Transfection of small interfering RNA (siRNA)

siRNAs were transfected into $8*10^5$ cells/well (6-well plate) following the protocol of Lipofectamine transfection described in Chapter 2.3 at a concentration of 50 nM for 72h total. A list of the utilised siRNA can be found in Table 2.1.

Table 2.1: List of used siRNAs in this thesis.

Name	Company	Identifier
si-Non targeting (siCtrl)	Dharmacon	D-001810-10-05
si-Brg1	Dharmacon	L-100544-02-0005
si-Brm	Dharmacon	L-085701-02-0005

2.5 Lentiviral small hairpin RNA (shRNA) production and applications

The different shRNAs used in the experiment are listed below (Table 2.2) and were provided by the shRNA service unit of the MRC London Institute of Medical Sciences (LMS). shRNAs were transduced via the replication incompetent pGIPZ lentiviral vector (Shimada 1995) into Ins1E cells. PGIPZ lentiviral vector information can be found in the GIPZ Lentiviral shRNA

Technical Manual provided by the shRNA service unit of the LMS. In short: Ampicillin and Zeocin™ resistance allowed selection of bacterial colonies expressing the vector (Ampicillin 100 µg/ml and Zeocin™ 25 µg/ml (Scientific Laboratory Supplies Ltd (SLS), UK). Successful introduction into the cells was verified by fluorescence microscopy (expression of GFP through the vector) and Puromycin selection (Puromycin resistance carried by the vector). DNA was extracted from *Escherichia coli* (E. coli) bacteria via Miniprep and Maxiprep. 10 µl DNA were digested with 2 µl KpnI or double digested with 1 µl XhoI / 1 µl NotI 2 µl 10x Buffer and 6 µl H_2O (New England Biolabs, UK) for 1-3 h to verify a positive product. For the lentiviral production HEK293T cells were used. HEK293T cells were plated into 6-well plates at $1*10^6$ cells/well the night before. For the transfection the media on the HEK293T cells was replaced with 1.8 ml Optimem buffer. Lipofectamine was prepared at 4 µl Lipofectamine in 96 µl Optimem per well and incubated for 5 min. A volume of 100 µl/well, topped up with Optimem, with 1 µg lentiviral vector DNA, 100 ng pCMV-VSV-G envelopment plasmid and 900 ng psPAX2 packaging plasmid were combined with the 100 µl Lipofectamine/Optimem, inverted 5 times and incubated for 20min at RT. The 200 µl of DNA/Lipofectamine mix were then added to the HEK293T cells and incubated at 37°C for 3.5 h. Afterwards the solution was replaced by Ins1E media and the cells left ON in a 37°C incubator. The following day the media, containing the lentivirus, was taken from the HEK293T cells, replaced with new Ins1E media, and filtered through a 22mm, 45µm Corning acetate filters (Sigma Aldrich, CLS431220). Polybrene (Sigma Aldrich) was added to the filtered media at 8 µg/ml and added to Ins1E cells plated out the previous day at $8*10^5$ cells/well in a 6-well plate. HEK293T and Ins1E cells were left ON in a 37°C incubator. The following day the lentivirus containing media of the HEK293T cells was removed, treated as previously described and used to replace the media on the Ins1E cells. The Ins1E cells were then incubated at 37°C for 6 h before the media was replaced by Ins1E media and left in a 37°C incubator ON. The next day Puromycin was added at 100 µg/ml and the cells incubated at 37°C ON. The media was replaced by Ins1E-media with Puromycin and the cells were left in a 37°C incubator for 48h before lysis/RNA extraction.

Table 2.2: List of used shRNAs in this thesis. Packaged into the replication incompetent pGIPZ lentiviral vector, from the shRNA service unit of the LMS.

Name	OBS Clone ID	Vendor ID	Library name	Sense Sequence
shnon-silencing (shNS)	x	x	x	ATCTCGCTTGGGCGAGAGTAAG
shBAF60A	200392635	V3LMM_422699	CSHL Mm shRNAmir 7.18 (Lenti)	GCAAGAGACCTGCACCTCA
shBrg1	200392891	V3LMM_427686	CSHL Mm shRNAmir 7.18 (Lenti)	AGCGACTCACTGACAGAGA

2.6 Miniprep

E. coli were grown in 3ml Lysogeny broth (LB, LMS) culture with specific antibiotics with one colony ON at 37°C. The next day 1.5 ml of the culture were transferred to a microtube and spun down at 13000 rounds per minute (rpm) at RT for 1min. The supernatant was discarded. The pellet was resuspended via vortexing in 250 µl resuspension buffer containing RNase. 250 µl of lysis buffer were added and the tube 10x inverted before another 350 µl of neutralisation buffer were added. All buffers were taken from the PureLink™ HiPure Plasmid Maxiprep Kit (Thermo Fisher). Afterwards the solution was spun down at 13000 rpm, RT for 5min and the supernatant transferred into a new microtube. The precipitate was discarded. The tubes were topped up to 1.5 ml with 100% Ethanol (Fisher, UK), 10x inverted and spun down at 13000 rpm, RT for 5 min. The supernatant was discarded, 70% Ethanol were added and the microtube vortexed. The solution was then spun down at 13000 rpm, RT for 5min, the supernatant discarded, and the pellet dried on a dry bath at 50-60°C. The DNA was resuspended in 35 µl H_2O. DNA concentration was determined via nanodrop (Thermo Scientific, NanoDrop One).

2.7 Maxiprep

Maxipreps were carried out following the protocol and with the materials provided in the PureLink™ HiPure Plasmid Maxiprep Kit. DNA concentration was determined via nanodrop.

2.8 Cell-lysis, Protein quantification and Western blot

Cells lysis was done with RIPA Lysis buffer containing in 1 ml: 50 µl TRIS-HCl pH 8.0, 30 µl NaCl 5M, 4 µl Ethylenediaminetetraacetic acid (EDTA) 0.5 M pH 8.0, 100 µl NP-40 10%, 100 µl Na-deoxycholate 10%, 20 µl protease inhibitor (PI, Roche) and 716 µl H_2O (Sigma Aldrich). The media was aspirated from the cells and the cells were washed with 2 ml of 1x phosphate buffered saline (PBS, LMS) before 200 µl of ice cold RIPA Lysis buffer were added. The cells were scraped (immortalised cell lines, for islets not necessary) and transferred inside the RIPA Lysis buffer into chilled microtubes and left on ice for 10 min. The lysed cells were spun down at 15000 rpm, 4°C for 10 min and the supernatant was transferred into a new microtube. For protein quantification to 2 µl of samples or standard (Bovine serum albumin (BSA, Sigma)), pipetted into a 96 well plate, flat bottom, 200 µl of BCA working solution were added, containing Bicinchoninic Acid solution and Copper(II) sulfate solution (1:49, Sigma Aldrich). The plate was closed, incubated at 37°C for 30 min and read at a set wavelength of 562 nm. SDS-PAGE gels percentage used in Western blot analysis depended on the size of the target protein. Acrylamide / Bis solution (30%/0.8%, Bio-Rad Laboratories, UK) determined the percentage of the gel. For example, an 8% gel contained 2.7 ml of Acryl. / Bis solution in a total volume of 10 ml. To this 2.5 ml 1.5M TRIS pH 8.8, 100 µl 10% sodium dodecyl sulfate (SDS, AppliChem Panreac), 50 µl 10% Ammonium Persulfate (APS, Sigma Aldrich) and 5 µl TEMED (Bio-Rad Laboratories) and filled up to 10 ml with H_2O. A 4% stacking gel contained 3 ml H_2O, 0.7 ml Acryl. / Bis, 1.3 ml 0.5M TRIS pH 6.8, 50 µl 10% SDS, 50 µl 10% APS and 5 µl TEMED. The Running Buffer consisted of 3g TRIS-Base, 14.4 g Glycine and 1 g SDS dissolved in 1 l H_2O (Sigma Aldrich). Lysates were supplemented with Laemmli and the gels were run at 25 mAmp per gel. For the transfer on the membrane a transfer buffer was used containing 3 g TRIS-Base, 14.4 g Glycine, 200 ml Methanol (Fisher) and the transfer was run at 100 V for 2 h in an ice bucket. Membranes were blocked with 10% milk (Sigma Aldrich) in TRIS-buffered saline (TBS, LMS) with 0.1% Tween20 (TBS-T) (National diagnostics, USA), washed 3x for 10 min each with TBS-T and incubated with the primary antibody in 5% milk ON at 4°C. The following day the membranes were washed 3x with TBS-T for 10 min each and incubated with secondary antibody (Goat-anti-mouse) in 5% milk for 1 h at 37°C. Membranes were treated for 5 min with Clarity™ Western ECL Substrate (Bio-Rad Laboratories) and subsequently images were taken with an Image Quant LAS 4000 mini (GElifesciences, USA). The antibodies used in Western blot assays are listed in Table 2.3.

Table 2.3: List of used antibodies in Western blot.

Name	Dilution	Company	Identifier
Mouse anti-BAF60A	1/1000	BD Bioscience	611728
Mouse anti-Brg1	1/200	SantaCruz	Sc-17796
Mouse anti-γ-Tubulin	1/6000	Sigma	T6557
Goat-anti-Mouse HRP	1/3000	Calbiochem	401253

2.9 Luciferase Assay

Luciferase assays were performed on HEK293T at $5*10^4$ cells / well (24-well plate) in 500 µl 10% FBS DMEM without P/S. 1 µl of 100 ng/µl commercially available mouse Brg1 3'UTR reporter vector (GeneCopoeia™, MmiT075618-MT06) and 6 µl of 20 µM siRNA mimics (Table 2.4) were added into Optimem buffer to a total volume of 50 µl (per well). This results in a siRNA concentration of 50 nM in the final incubation volume in the wells. 0.75 µl Lipofectamine were added to 49.25 µl Optimem per well. Lipofectamine mix and Brg1 3'UTR / siRNA mix were combined and incubated at RT for 15 min. 100 µl of the mix were added to each well and incubated for 48 h at 37°C. Transfected HEK293T cells were processed with and following the instructions of the Dual-Glo® Luciferase Assay System (Promega) and analysed in a FLUOstar Omega filter-based multi-mode microplate reader (BMG Labtech, FLUOstar OMEGA 415-1641).

Table 2.4: List of siRNA miRNA mimics used for Luciferase Assay in this thesis. Forward (F) and Reverse (R) primer pairs are shown with according sequences.

Name	Company	Identifier
si-non-targeting (miR-NT)	Dharmacon	D-001810-10-05
si-223 (miR-223)	Sigma	HA03868326
si-7a (miR-7a)	Sigma	HA03868320
si-7b (miR-7b)	Sigma	HA03868323

2.10 Animals

All mice were derived from the C57BL/6 background. Transgenic-miR-7a2 (Tg7a2) animals express consistently miR-7a2 under the control of the rat Ins2 promoter (RIP), resulting in pancreatic β-cell specific overexpression of miR-7a2, and have been previously described by M. Latreille (Latreille et al. 2014). $Brg1^{fl/fl;Ins1\text{-}Cre}$ mice were generated by crossing floxed Brg1($Brg1^{fl/fl}$) animals, kindly provided by Dr. P. Chambon via M. Goetz (Helmholtz Institute Munich) (Sumi-Ichinose et al. 1997) with mice expressing the Cre recombinase behind the mouse Ins1 promoter (Ins1-Cre) (Thorens et al. 2015) for pancreatic β-cell specific Brg1 knockout. $tdTomato^{RIP\text{-}Cre}$ mice carry a Rosa26-floxed-Stop-tdTomato transgene (Luche et al. 2007), as well as the Cre recombinase behind the RIP (Herrera 2000), resulting in the pancreatic β-cell specific expression of red fluorescent protein (RFP). *db/db* mice (Hummel, Dickie and Coleman 1966) were purchased from Jackson Laboratory.

2.11 Housing conditions

All animals were kept and bred in a pathogen-free animal facility according to the Home Office regulations as defined by the Animal (Scientific Procedures) Act, 1986. Mice were maintained at a maximum density of 7 animals per cage, or at a maximum of 4 animals per cage with a maximum bodyweight of over 30g in Allentown XJ individually ventilated cages. The animals were housed on a 12-hour light/dark cycle with constant environmental conditions (temperature: 21°C ± 2°C, humidity: 55% ± 10%) and had free access to standard rodent chow if not otherwise indicated. Dox was administered through the drinking water at a concentration of 2 g/l.

2.12 Genotyping

Ear clipping samples were used for identification of individual mice as well as the source of DNA for genetic identification. For post-mortem genotyping tail tips were used. DNA was obtained by heating the samples at 96°C for 10 minutes (min) in 300 µl of 0.06 M sodium hydroxide (NaOH, Sigma Aldrich). The samples were then neutralised with 50 µl of 1 M Trizma ®-base – hydrogen chloride (Tris-HCl), pH 8 (Sigma Aldrich). Samples were then used in polymerase chain reaction (PCR) experiments with Kapa Taq polymerase with dye (Kapa Biosciences/Roche, UK) and 0.2 mM dNTPS (Promega, UK) for Tg7a2 genotyping. GoTaq

Hot Start Green Master mix (Promega) with 5% dimethyl sulfoxide (DMSO) (Sigma Aldrich) was used for the remaining genotyping PCR. Primers were used at 0.5 mM and are listed below together with the used TProfessional TRIO Thermocycler (Thermocycler, Biometra, UK) programs. Example genotyping gels of 1.5% agarose (Appleton) TBE buffer (Fig. 2.1). A list of genotyping primers is provided in Table 2.5 (Appendices).

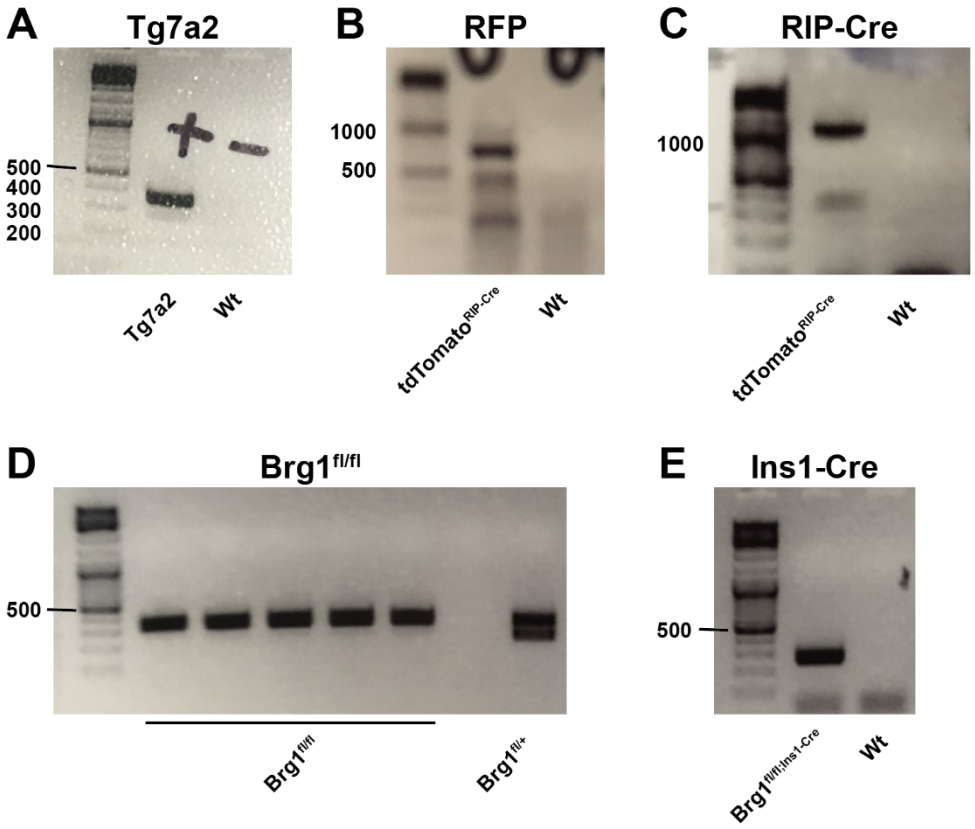


Figure 2.1: Genotyping of mouse lines used in this thesis. (A-E) On top of the gel is the gene analysed and below the gel are the animals. Genotyping of mice with (A) Tg7a2, (B) RFP, (C) RIP-Cre, (D) $Brg1^{fl/fl}$ and (E) Ins1-Cre primer pairs, shown in Table 2.5.

2.13 Blood glucose measurements, intra-peritoneal (IP) glucose/insulin tolerance test (IPGTT/IPITT)

For IPGTTs, prior to the experiment, mice were fasted ON. 2g/kg of body weight of D-(+)-glucose (Sigma Aldrich) were administered by IP injection. Blood samples were taken from the tail at 0, 15, 30, 60, 90 and 120 min after glucose administration. For IPITT, prior to the experiment, mice were fasted for 6h. 1U/kg of body weight of Insulin (Sigma, I9278-5ML) were administered by IP injection. Blood samples were taken from the tail every 15min starting at 0 min to 120 min. Blood glucose levels in random fed animals as well as IPGTT /IPITT were

measured using a Contour glucometer (Bayer, UK). Body weights were taken after blood glucose levels were measured, for IPGTT/IPITT at 0 min.

2.14 Insulin serum assay

For the measurements of Insulin serum content, experiments were carried out following the protocol and with the materials provided in the Insulin Mouse Serum Assay kit (Cisbio, 62IN3PEF). Blood samples were taken from mice after a 6 h fast. Blood volumes were taken for approximately 10-15 µl of serum, taken off as supernatant after blood samples were spun down at 8000 rpm, RT for 8 min.

2.15 Islet isolation

Mice were culled by cervical dislocation. Islets were isolated by perfusion of the pancreas via the bile duct with ice cold Liberase (Roche, 5401127001), administered with a 30G needle on a 2 ml syringe. The recovered pancreas was digested at 37°C for 17 min in 1 ml Liberase in a 50 ml falcon tube. The tube was filled to 40 ml with RPMI with 10% FBS and homogenised by shaking. The samples were then spun down quickly at 2700 rpm and the supernatant was discarded. The pellet was resuspended in 20 ml RPMI (10% FBS) via vortexing. The suspension was filtered and again quick spun at 2700 rpm, supernatant was trashed. The pellet was resuspended in 5 ml Histopack 1077 (Sigma Aldrich) via vortexing and another 5 ml Histopack 1077 were added. The suspension was overlaid with 10 ml RPMI and spun for 20 mins at 2200 rpm without break up/down. The islets were collected into 40 ml RPMI (10% FBS) and quick spun at 2700 rpm, the supernatant was discarded. The islets were then resuspended in 5 ml RPMI (10% FBS) and filtered through a 70 µM strainer (Falcon, 352350). Islets were cultured in 6-well plates in 3ml RPMI (10% FBS, 1% P/S) in a 37°C incubator (Thermo Scientific, Midi40).

2.16 Fluorescence-activated cell sorting (FACS)

The isolated islets were transferred into 1.5 ml microtubes with 1 ml 1x PBS (no Ca^{2+}/Mg^{2+}) (Sigma Aldrich), for dissociation, and incubated at 37°C for 9 min. The islets were pipetted 10 times up and down with a P1000 pipette for better dissociation. FACS islet dissociation was non-enzymatic. The whole solution was then transferred into 15 ml falcon tubes and 9 ml RPMI were added. The falcon tubes were spun down for 5 min at 200 g, the supernatant discarded, and the cells resuspended in 500 µl 1x PBS (1% BSA). The samples were transferred through a strainer into FACS tubes (pluriSelect, 352235) and sorted by the LMS FACS facility in an FACS ARIA III machine. Sorted cells were stained for viability utilising 1 µM Calcein-AM (Santa Cruz, sc-203865).

2.17 Dissociation of pancreatic islets (excluding dissociation for FACS, see 2.16)

For the dissociation of islets, islets were transferred to a 1.5 ml Eppendorf tube up to a maximum of 600 islets per tube. Pancreatic islets were washed three times with 1x PBS by spinning them down at 3000 rpm, RT for 2 min and removing the supernatant. 1 ml of Accutase (Thermo Fisher) were added per tube and incubated in a water bath at 37°C for 10 min. Islets were pipetted up/down 5 times with a P1000 pipette to support dissociation. 1 ml of 100% FBS was added to stop the reaction and everything was transferred in a 15 ml falcon tube. The dissociated islets were spun down at 1200 rpm, RT for 5 min. The pellet was resuspended in RPMI (10% FBS, 1% P/S), cells were counted in a Neubauer chamber (Hawkslex, BS748).

2.18 Immunofluorescence staining on dissociated pancreatic islets and cultured cells

Dissociated islets and cultured cells were plated out in their respective media at a maximum density of $2*10^5$ cells / well (24-well plate) on 804G extra cellular matrix covered coverslips (VWR, 631-0149) the day before immunofluorescence staining. 804G cell conditioned media (Riddelle, Hopkinson and Jones 1992) was provided by M. Latreille and was added to coverslips the night before plating out cells. Before cells were plated out the 804G cell conditioned media was taken off and the coverslips were washed three times with sterile H_2O.

On the day, the media was removed, and the cells washed three times with 1x PBS. The cells were fixed in 4% paraformaldehyde (PFA, Sigma) in 1x PBS at RT for 15 min. The PFA was removed and the cells were washed twice with 1x PBS. Cells can be stored in 1x PBS at 4°C until used. The cells were permeabilised with 0.1% Triton X-100 in 1x PBS solution at RT for 15 min, followed by three washes with 1x PBS. Blocking buffer (1% BSA, 5% donkey or goat serum (SLS) in 1x PBS, serum depends on species of secondary antibody) was added and incubated at RT for 1 h. Primary antibodies were added in blocking buffer and incubated ON at 4°C. Cells were washed four times with 1x PBS and the secondary antibodies in blocking buffer were added and incubated at RT for 1 h, followed by four 1x PBS washing steps. 4′,6-diamidino-2-phenylindole (Dapi, Sigma, D9543) diluted 1:10000 in 1x PBS was added for 5 min and coverslips were mounted on slides with VECTASHIELD® Antifade Mounting Media (Vector Laboratories). A list of the utilised antibodies, primary and secondary, can be found in Table 2.6.

2.19 Agar embedding of whole pancreatic islets

For embedding of whole isolated islets, the islets were fixed for 15 min in 4% PFA. Islets were then spun down at 3000 rpm, RT for 3 min and the supernatant removed. Islets were resuspended in 10 µl of 3% UltraPure™ Low Melting Point Agarose (Thermo Fisher), kept at 55-60°C, in PCR strip tubes. The agarose containing the fixed whole islets was left set at RT for 10 min before the PCR strip tubes were cut away. The islet containing agarose pellet was embedded in OCT compound (VWR) and left to set in dry ice. 10 µm sections were cut on a Leica cryostat (LEICA CM1950, Leica). Sections were stored at -20°C.

2.20 Immunofluorescence staining on cryostat slides

Slides were thawed at RT for 5 min and incubated at RT for 15 min in permeabilization buffer (0.1% Triton X-100 in 1x PBS, Perm buffer). Hydrophobic rings were drawn around the sections on the slide with a Pap pen (Invitrogen, 00-8899). Slides were blocked in blocking buffer (no BSA) at RT for 1 h. Primary antibody diluted in blocking buffer (no BSA) was put on sections and incubated ON at 4°C. The following day slides were washed three times in 1x PBS for 15 min each before hydrophobic rings were redrawn and secondary antibody diluted in blocking buffer (no BSA) was added and incubated at RT for 1 h. Secondary antibody was

removed and Dapi, diluted 1/10000 in 1x PBS, was added at RT for 5 min. Slides were washed three times at RT for 15 min each in 1x PBS and sections were mounted with VECTASHIELD® Antifade Mounting Media. A list of the utilised antibodies, primary and secondary, can be found in Table 2.6.

2.21 Paraffin embedding of mouse pancreata and immunofluorescence staining

Pancreata were removed from sacrificed mice and fixed ON at 4°C in 4% PFA. The following day the pancreata were moved into 70% ethanol. Pancreata were paraffinized in a Tissue-Tek VIP® tissue embedder (Sakura) and 4 µm sections were cut on a microtome (Microm HM355S, Thermo Scientific). Slides were incubated at RT two times in xylene (Honeywell, 534056-4L) for 10 min each, twice in 100% ethanol for 3 min each, in 95% ethanol for 3 min, 85% ethanol 3 min, 70% ethanol 3 min, 50% ethanol 3 min and in H_2O for 5 min. Antigen retrieval was performed in the LMS Decloaking Chamber NxGen (BioCare Medical) at 95°C for 5 min in 0.01 M NaCitrate (Sigma), pH 6.0. Slides were left to cool at 4°C for 15 min before being washed three times in H_2O for 5 min each. Pancreas sections were permeabilised at RT for 30 min with Perm buffer. Hydrophobic rings were drawn around the sections on the slide. Sections were blocked in blocking buffer at RT for 1 h. Primary antibody was diluted in blocking buffer, applied to sections and incubated ON at 4°C. Slides were washed three times at RT for 15 min each in Perm buffer, the hydrophobic rings redrawn and sections incubated in secondary buffer diluted in blocking buffer at RT for 1 h in the dark. Secondary antibody was removed and Dapi, diluted 1/10000 in 1x PBS, was added for 5 min. Slides were washed three times for 7 min each in Perm buffer followed by five brief washes (approximately 10 sec) in H_2O. Sections were mounted with VECTASHIELD® Antifade Mounting Media. A list of the utilised antibodies, primary and secondary, can be found in Table 2.6.

Table 2.6: List of antibodies used for immunofluorescence staining in this thesis. Primary and secondary antibodies with used dilution factors and source.

Name	Dilution	Source	Identifier
Mouse anti-BAF60A	1/50 (sections) 1/200 (cells)	BD Bioscience	611728
Mouse anti-Brg1	1/200 (both)	Santa Cruz	Sc-17796
Rabbit anti-Glucagon	1/1000 (cells)	Millipore	AB932
Mouse anti-IAPP	1/100 (sections)	Abcam	Ab115766
Guinea-pig anti Insulin	1/500 (both)	Millipore	AB3440
Rabbit anti-Ppy	1/150 (cells)	Abcam	ab77192
Rabbit anti-RFP	1/200 (sections)	Abcam	ab62341
Rabbit anti-Somatostatin	1/1000 (cells) 1/200 (sections)	Dako	a0566
Donkey Anti-Guinea-pig Alexa Fluor 488	1/500	Jackson	706-545-148
Donkey anti-Guinea-pig 647	1/200	Millipore	AP193SA6
Donkey Anti-Mouse IgG Cy3	1/200	Jackson	715-166-150
Donkey Anti-Rabbit Alexa Fluor 567	1/500	Life Tech	A21207
Donkey Anti-Rabbit Alexa Fluor 647	1/500	Jackson	711-605-152

2.22 Trizol RNA extraction

Islets or cells were suspended in 1 ml Trizol (Lifetechnology; ambion, 15596018). The samples were vortexed for 20 seconds (sec) at RT and incubated at RT for 5 min. At this point samples can be stored in Trizol at -80°C. 200 µl Chloroform (Sigma Aldrich) were added and the solution vortexed for 15 sec and incubated for 2-3 min at RT. The samples were spun down for 15 min at 15000 rpm, 4°C. The aqueous phase, containing the RNA, was transferred into a fresh 1.5 ml microtube. For islets 5-10 µg Glycogen blue (Thermo Fisher) were added. 1 ml

Isopropanol (VWR, UK) were added and the microtubes inverted 10x. Samples were precipitated at -20 °C for a minimum of 15 min and up to ON for a better RNA yield. Samples were then spun down for 15 min at 15000 rpm, 4°C, the supernatant was discarded. The pellet was washed once for islets and twice for cells. Washing was done with 75% Ethanol, vortexing, followed by spinning at 15000 rpm, 4 °C. The supernatant was discarded and the pellet air dried. The RNA was resuspended in H_2O (10 µl for islets, 25 µl for cells) and left at RT for 5 min. RNA concentration was determined via nanodrop.

2.23 DNAse I treatment (for mRNA)

DNAse I treatment was done using the RNAse-free DNAse set (Thermo Fisher). 10 µg of RNA were incubated for 20 min at 37°C with 5 µl 10x Buffer, 1 µl DNAse I and filled up to 50 µl with H_2O. After incubation 5 µl of inactivation reagent were added and incubated at RT with occasional flicking for 2 min. The samples were spun down at 10000 g for 2 min and 48 µl of the supernatant were recovered into fresh tubes.

2.24 Reverse Transcription (RT) of mRNA

RT of mRNA was done using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher). To 10 µl of the DNAse I treated RNA-solution were 10 µl of the RT mix (following the protocol) added and incubated for 10 min at 25 °C, 120 min at 37 °C, 5 min 85 °C and hold at 4 °C in a Thermocycler.

2.25 Reverse Transcription of micro RNA (miRNA)

RT of miRNA was done using the Taqman™ MicroRNA Reverse Transcription Kit (Thermo Fisher). To 5 µl of the RT mix (following the protocol) 2.5 µl of RNA (4 ng/µl) were added and incubated in a Thermocycler for 30 min at 16°C, 30 min at 42°C, 5min at 85 °C and hold at 4 °C. Taqman primers for SnoRNA202 (RT: 001232, PN4440887, lot: P170515-005 A06), dme-miR-7 (RT: 000268, PN4440887, lot: P170515-005 A06) and mmu-miR-7b (RT: 002555, PN4427975, lot: P170301-000 E11) were used.

2.26 Quantitative PCR (qPCR) of RT of mRNA (RT-qPCR)

For qPCR, 4 µl of RT complementary DNA (cDNA) at differing dilutions (1:10 – 1:100, depending on gene abundance in cell type) were used. 6 µl of Kapa Sybr fast 2x mix (Kapa Biosystems, KK4611), 1 µl of a 6 µM primer mix and 1 µl of H_2O were added. qPCR was performed in a LightCycler® 480 Instrument (Roche) using the following program: 95°C 5 min, 45x (95°C 10 sec, 60°C 10 sec, 72°C 10sec), 95°C 5 sec, 65°C 1 min followed by a 97°C melt curve. Table 2.7, 2.8 and 2.9 (Appendices) show the cDNA qPCR primers pairs for mouse, rat and human, respectively, used in this thesis.

2.27 qPCR of miRNA

For qPCR of miRNAs, 4 µl of 1:4 diluted miRNA RT were used. 0.5 µl of 20x Taqman probes, 5 µl 2x Master mix and 0.5 µl H_2O were added and qPCR was performed in a LightCycler® 480 Instrument using the following program: 95°C 10 min, 40x (95°C 15 sec, 60°C 1min), 40°C 30 sec. Taqman probes for snoRNA202 (TM: 001232, PN4440887, lot: P170515-005 A06), dme-miR-7 (TM: 000268, PN4440887, lot: P170515-005 A06) and mmu-miR-7b (TM: 002555, PN4427975, lot: P170301-000 E11) were used.

2.28 Statistical Analysis

Data are represented as mean ± SEM and tested with Student t-test or ANOVA if not otherwise indicated. All figures and analyses were generated using GraphPad Prism 7. $p<0.05$ was used to indicate statistical significance.

2.29 Human donor information

Table 2.10: List of human donors of pancreatic islets used in this thesis. Shown are the figures the in which the islets were used in, where they come from (source), age, sex, body mass index (BMI) and cause of death of the donor.

Figure	Source	Age	Sex	BMI (kg/m^2)	Cause of death
3.5	Pisa	76	Male	26.6	Stroke
3.8	Milan	60	Male	26.3	Cerebral bleeding
4.7	Pisa	66	Female	26.4	Trauma
4.12	Edmonton (McDonald)	66	Female	18.5	Unknown
4.17	Oxford	44	Male	36	Unknown
5.9	Edmonton (Shapiro)	48	Female	38.5	Unknown

2.30 Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-Seq) library preparation from mouse pancreatic islets

ATAC-Seq library preparation was performed based on published instructions (Buenrostro et al. 2015b). For the preparation of ATAC-Seq libraries, 50 islets per sample were used. Pancreatic islets were picked into low binding 1.5 ml Eppendorf tubes (Eppendorf, 022431021) and placed on ice. The samples were spun down at 200 g, 4°C for 1 min and the supernatant was removed. The samples were washed twice in 500 µl of ice-cold 1x PBS by resuspending them through five up and down pipetting steps with a P200 pipette and low binding tips (Starlab, S1183 - 1810) and spinning them down at 200 g, 4°C for 1 min. The samples were resuspended in in 300 µl of ice cold ATAC lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$ (Sigma), 0.1% NP-40 in H_2O) and incubated on ice for 20 min with gentle shaking at 25 rpm (Stuart^{TM} Gyratory rocker, SSL3, Sigma). The lysed islets were resuspended with a 1 ml syringe and 30G needle to free the nuclei (approximately 15 times up and down). The nuclei were spun down at 500 g, 4°C for 10 min and the supernatant carefully removed. The nuclei pellet was resuspended in 100 µl of ice-cold ATAC lysis buffer and passed through a FACS cell strainer. The nuclei were spun down at 500 g, 4°C for 10 min and the supernatant carefully removed. The nuclei pellet was resuspended in transposase reaction mix (per sample: 25 µl 2x TD buffer, 2.5 µl Transposase, 22.5 µl nuclease-free H_2O) (Illumina Nextera Library

Prep kit #15028212) and incubated at 300 rpm, 37°C for 30 min. After the incubation the samples were put on ice and proceeded with DNA purification using the Qiagen MinElute Reaction Cleanup kit (Qiagen, 28204), following the kit's instruction, but eluting in 11 µl. At this point samples can be stored at -20°C. For amplification of the transposed DNA fragments to 10 µl of transposed DNA, 10 µl nuclease-free H_2O, 2.5 µl 25 µM Primer-Adapter 1, 2.5 µl 25 µM Primer-Adapter 2.x (Table 2.11) and 25 µl NEB Next High-Fidelity 2x PCR Master Mix (New England Biolabs, M0541S) were added. Samples were put in a thermocycler at 72°C for 5 min, 98°C for 30 sec, 5 cycles of 98°C for 10 sec, 63°C for 30 sec and 72°C for 1min and 4°C on hold. After the 5 cycles samples were put on ice and prepared for qPCR that allows calculation how many additional PCR runs per sample are required to even out sample concentrations and to stop amplification before the saturation is reached. To calculate the number of cycles needed the relative fluorescence is plotted against the number of cycles in qPCR. The optimal number of additional cycles corresponds to the cycle number at a third of when the maximum relative fluorescence is reached, subtracted by the 5 cycles already run previously. To 5 µl of previously PCR-amplified DNA 4.41 µl nuclease-free H_2O, 0.25 µl 25 µM Primer-Adapter 1, 0.25 µl 25 µM Primer-Adapter 2.x, 0.09 µl 100x SYBR Green I (Thermo Fisher, #S7563) and 5 µl NEB Next High-Fidelity 2x PCR Master Mix. qPCR was performed in a LightCycler® 480 Instrument at 98°C for 30 sec, 20 cycles of 98°C for 10 sec, 63°C for 30 sec and 72°C for 1 min. The amplified library was purified with the Qiagen MinElute Reaction Cleanup kit and eluted twice with 10 µl elute buffer provided in the kit. 15 µl per sample were used for sequencing, 1 µl for determining the concentration and the rest for analysing fragment size distribution in a Bioanalyser (Genomic Facility of the LMS, Machine 1 and 2, Type: G2938B and G2939A, High Sensitivity DNA Assay). Concentration was determined via qPCR and an 80 µl 10 nM pool of all samples was submitted.

Table 2.11: List of adapters used in ATAC-Seq.

Sample	Identifier	Sequence 5'-'3	Source
All samples	Ad1_noMX	AATGATACGGCGACCACCGAGATCTACACTCG TCGGCAGCGTCAGATGTG	Sigma
WT-12A	Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTA GTCTCGTGGGCTCGGAGATGT	Sigma
WT-12B	Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACG GTCTCGTGGGCTCGGAGATGT	Sigma
WT-12C	Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCT GTCTCGTGGGCTCGGAGATGT	Sigma
WT-12E	Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTC CGTCTCGTGGGCTCGGAGATGT	Sigma
Tg7a2-12G	Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATACGAGATGTAGAGA GGTCTCGTGGGCTCGGAGATGT	Sigma
Tg7a2-12I	Ad2.9_GCTACGCT	CAAGCAGAAGACGGCATACGAGATAGCGTAG CGTCTCGTGGGCTCGGAGATGT	Sigma
Tg7a2-12J	Ad2.10_CGAGGCTG	CAAGCAGAAGACGGCATACGAGATCAGCCTC GGTCTCGTGGGCTCGGAGATGT	Sigma
Tg7a2-12K	Ad2.11_AAGAGGCA	CAAGCAGAAGACGGCATACGAGATTGCCTCTT GTCTCGTGGGCTCGGAGATGT	Sigma
WT-2A	Ad2.13_GTCGTGAT	CAAGCAGAAGACGGCATACGAGATATCACGAC GTCTCGTGGGCTCGGAGATGT	Sigma
WT-2B	Ad2.14_ACCACTGT	CAAGCAGAAGACGGCATACGAGATACAGTGGT GTCTCGTGGGCTCGGAGATGT	Sigma
WT-2E	Ad2.17_TGCTGGGT	CAAGCAGAAGACGGCATACGAGATACCCAGC AGTCTCGTGGGCTCGGAGATGT	Sigma
WT-2F	Ad2.18_GAGGGGTT	CAAGCAGAAGACGGCATACGAGATAACCCCTC GTCTCGTGGGCTCGGAGATGT	Sigma
Tg7a2-2G	Ad2.19_AGGTTGGG	CAAGCAGAAGACGGCATACGAGATCCCAACCT GTCTCGTGGGCTCGGAGATGT	Sigma
Tg7a2-2I	Ad2.21_TGGGTTTC	CAAGCAGAAGACGGCATACGAGATGAAACCCA GTCTCGTGGGCTCGGAGATGT	Sigma
Tg7a2-2J	Ad2.22_TGGTCACA	CAAGCAGAAGACGGCATACGAGATTGTGACCA GTCTCGTGGGCTCGGAGATGT	Sigma
Tg7a2-2K	Ad2.23_TTGACCCT	CAAGCAGAAGACGGCATACGAGATAGGGTCA AGTCTCGTGGGCTCGGAGATGT	Sigma

2.31 ATAC-Seq analysis (performed by Y. Wang, Genomic Facility MRC LMS and part of a manuscript under review, Mak *et al.* under review)

The ATAC-Seq libraries were submitted to the LMS Genomics facility and sequencing was done with Hiseq2500, paired end, 100 bp reads. Basecalling and demultiplexing was one with Illumina CASAVA 1.8.4 software and raw reads were trimmed with Trim Galore! (trim_galore_v0.4.4; https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)

afterwards. With bowtie2 the trimmed reads were aligned to Ensembl mus musculus genome reference sequence assembly (mm9) (bowtie2/2.1.0, with –very-sensitive -X 2000; (Langmead and Salzberg 2012)) and R Bioconductor package ARACseqQC (Ou et al. 2018) was used to remove duplicated reads, as well as reads mapped to mitochondrial chromosomes. The reads were further split into nucleosome-free (fragment length ≤ 100 bp), mononucleosome, dinucleosome and trinucleosome bins and the nucleosome-free bin was used for downstream analysis. Peak calling was done with the MACS2 peak-calling function (with -f BAMPE; (Zhang et al. 2008) and peaks overlapping with the ENCODE Project Consortium blacklist (Dunham et al. 2012) were removed. Only peaks appearing in all biological replicates were used. The featureCounts function (Liao, Smyth and Shi 2013a) from the Rsubread Bioconductor package (Liao, Smyth and Shi 2013b) was used to obtain peak-based read counts. The DESeq2 Bioconductor package (Love, Huber and Anders 2014a) was used to find differentially accessible chromatin regions. The ChIPseeker Bioconductor package ((Yu, Wang and He 2015) was used for peak annotation and HOMER (Hypergeometric Optimization of Motif EnRichment) findMotifsGenome.pl (with -size 200 -mask) was performed to find enriched TF binding motifs.

2.32 RNA-Sequencing analysis (RNA-Seq, performed by M. Latreille, analysed by Y. Wang, Mak *et al.* under review)

RNA-Seq was performed on 2 and 12-week-old Tg7a2 animals and aged matched Wt controls. Pancreatic islets were isolated, and 10 ng RNA total were used to prepare RNA-Seq libraries with a NEB Ultra II RNA library kit (Illumina). As with the ATAC-Seq, RNA-Seq was done with Hiseq2500, paired end, 100 bp reads by the LMS Genomics facility. For base calling and demultiplexing, the Illumina CASAVA 1.8.4 software was utilised and Trimmomatic was used on raw RNA-Seq reads to trim, removing adaptor and low-quality reads. (v.0.33) (Bolger, Lohse and Usadel 2014). The RNA-Seq data was aligned against Ensembl mus musculus genome reference sequence assembly (mm9) and transcripts were annotated with Tophat2 (2.0.11) (Kim et al. 2013a), followed by performing the featureCounts function (Liao et al. 2013a) from the Rsubread Bioconductor package (Liao et al. 2013b) to obtain gene-based read counts. Differential gene expression of Tg7a2 vs Wt mice was analysed with the DESeq2 Bioconductor package (Love et al. 2014a) and a list of ranged genes, based on Wald statistics, of those results was set up. GSEA was performed on this list with MSigDB gene sets from the

'H' collections (Mootha et al. 2003, Subramanian et al. 2005). A list of stemness genes was put together from the Cancer stem cell database (CSCdb) at http://bioinformatics.ustc.edu.cn/cscdb/. Literature review and web research done to generate the epithelial gene list.

2.33 Software

Cellprofiler 2.2.0 (Carpenter et al. 2006) was used to analyse relative intensities in immunofluorescence stainings, pipelines were mainly written by C. Whilding of the LMS Microscopy facility. ImageJ (Schneider, Rasband and Eliceiri 2012) was used to prepare images of immunofluorescence stainings. TargetScan (Agarwal et al. 2015) was utilised for predicting miR-7a2 targets. Integrative Genomics Viewer (IGV) (Robinson et al. 2011) was used to view ATAC-Seq traces of chromatin regions. CLC Main Workbench 7 (https://www.qiagenbioinformatics.com/) was used to investigate gene sequences, e.g. for primer design. Adobe Illustrator CC 2018 (Adobe) was used to make the figures. Primers were designed with the ncbi primer blast tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Schematic illustrations in the introduction were generated utilising the biorender app (https://app.biorender.com/)

2.34 Microscopes

Images of Figure 3.1, 3.3, 5.1, 5.2 and 5.5 were taken with LEICA DMIRE2 (Leica). All other images were taken with a Leica TCS SP5 confocal microscope (Leica, DM6000 CS).

Chapter 3-5: Results

3. Regulation of pancreatic β-cell identity by miR-7a

3.1 Introduction

Previous studies investigating the pathophysiological mechanism underlying type 2 diabetes showed that not only apoptosis, but also dedifferentiation of pancreatic β-cells results in insufficient amounts of Ins to maintain euglycemia (Talchai et al. 2012, Dor and Glaser 2013). β-cell dedifferentiation is accompanied with the reduction in the expression of β-cell enriched genes as well as biosynthesis of atypical hormones, resulting in transdifferentiation (Talchai et al. 2012, Dor and Glaser 2013). This dedifferentiation of β-cells was confirmed in mouse models (Blum et al. 2014, Brereton et al. 2014, Wang et al. 2014), and more recently in human T2D (Cinti et al. 2016a, Md Moin et al. 2016). Our group previously showed increased miR-7 levels in islets of several mouse models of diabetes as well as in human islet transplanted in mice exposed to an obesogenic diet (Latreille et al. 2014). Mice with β-cell-specific overexpression of miR-7a2 display elevated blood glucose levels associated with the loss of key β-cell identity markers (e.g. Ins and Glut2), thus revealing induction of miR-7a expression as a novel mechanism triggering the dedifferentiation of β-cells (Latreille et al. 2014). Furthermore, our laboratory recently showed that β-cells from Tg7a2 mice started expressing the non-β-cell hormone Sst, thus indicative of β- to δ-cell transdifferentiation. Although Tg7a2 indicate a dedifferentiation, it is still unclear if this process is induced by the hyperglycaemic phenotype or an effect of miR-7a2 overexpression. Additionally, further research is required to clarify if miR-7a2 overexpression induces dedifferentiation in human islets. In the presented thesis, we investigated how miR-7a expression affects β-cell identity. Moreover, we have profiled the genetic signature and epigenetic landscape of islets isolated from Tg7a2 mice and identified new miR-7 targets causing β-cell dedifferentiation in diabetes. To accomplish this, we first manipulated the expression of miR-7a in different experimental models (cell lines, mouse islets, human islets and mice) to model miR-7a-mediated β-cell dedifferentiation. We utilised our different models of β-cell dedifferentiation to confirm those findings of potential transdifferentiation induced by miR-7a.

3.2.1 Alteration of miR-7a levels in immortalised pancreatic β-cell lines

To identify the miR-7a targets responsible for the dedifferentiation of β-cells, we first sought to establish an *in vitro* model of miR-7a-mediated β-cell dedifferentiation. To accomplish this, we overexpressed miR-7a in the Min6 mouse immortalized β-cell line using miR-7a2 overexpressing (Ad-7a2) or control adenoviruses (Ad-ctrl). Both adenoviral vectors carried an EGFP reporter gene under an independent promoter, which enabled us to control for infection efficiency. As an alternative approach, we inhibited miR-7a function using locked nucleic acid inhibitor (LNA-7a). qPCR was then utilized to measure mRNA levels of genes maintaining β-cell identity in cells in which we manipulated miR-7a levels. We also used immunofluorescence microscopy to determine the expression of Ins and β-cell markers at a subcellular resolution.

Infection of Min6 cells with Ad-7a2 resulted in a significant increase in *MIR-7a* ($p=0.0001$) expression in Ad-7a2 infected Min6 cells compared to Ad-Ctrl measured in qPCR (Fig. 3.1A). Min6 cells infected with Ad-7a2 show significant downregulation of the β-cell identity markers *INS1* ($p=0.0064$), *INS2* ($p=0.0004$), *GLUT2* ($p\leq0.0001$), *NKX6.1* ($p=0.0456$) and *PDX-1* ($p\leq0.0001$) in comparison to Ad-Ctrl (Fig. 3.1B). Immunofluorescence microscopy was then used to determine if changes in mRNA levels correlate with alteration of protein levels. Quantification of images via CellProfiler showed a significant decrease in Ins intensity ($p=0.0500$) in Min6 cells infected with Ad-7a2 in comparison to Ad-Ctrl (Fig. 3.1E,F). This indicates, that miR-7a2 induces the dedifferentiation of β-cells through suppressing the expression of key β-cell enriched genes.

In contrast, loss-of-function of studies revealed a significant decrease of miR-7a mRNA levels ($p=0.0014$) in Min6 cells in transfected with miR-7a LNA compared to LNA-Ctrl transfected cells. (Fig. 3.1C). LNA-7a Min6 cells displayed a significant increase in Ins2 ($p=0.0482$) and Glut2 ($p=0.0024$) mRNA levels, whereas a tendency for increased expression of *NKX6.1* ($p=0.2260$) and *PDX-1* ($p=0.0630$) could be observed, although the effect did not reach statistical significance (Fig. 3.1D).

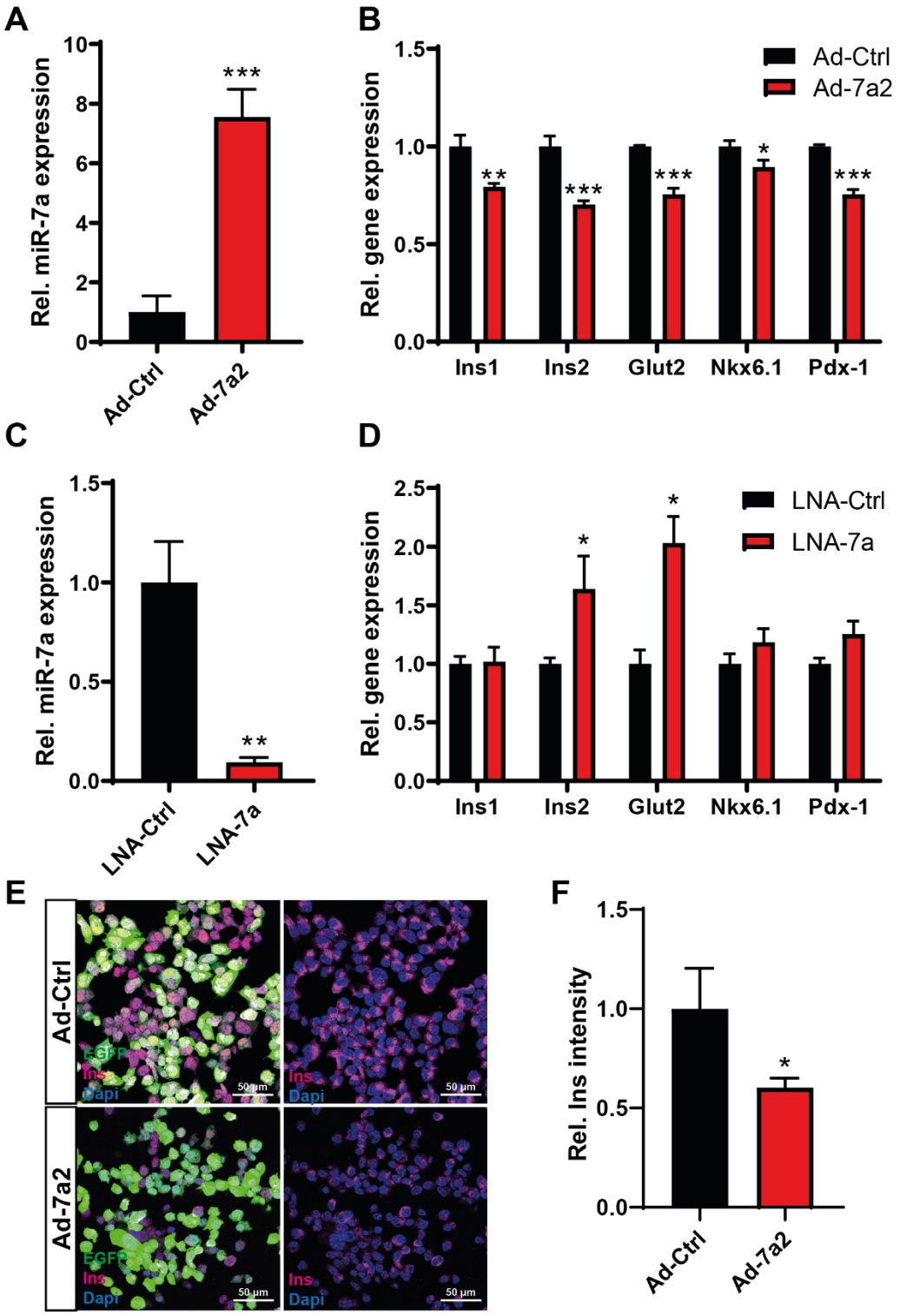


Figure 3.1: Alterations of miR-7a lead to changes in β-cell markers in Min6 cells. (A) Relative miR-7a expression in Min6 cells infected with Ad-7a2 compared to Ad-Ctrl for 48h. Data was normalised to Small nucleolar RNA MBII-202 (*Sno202*) *expression*. (B) Relative expression of β-cell identity genes in Min6 cells infected with Ad-7a2 compared to Ad-Ctrl. Data was normalised to Ribosomal phosphoprotein P0 (*RPLP0/36B4*). (C) Relative *miR-7a* expression in Min6 cells transfected with LNA-7a compared to LNA-Ctrl. Data was normalised to *Sno202*. (D) Relative expression of β-cell identity genes in Min6 cells transfected with LNA-7a compared to LNA-Ctrl. Data was normalised to *RPLP0/36B4*. (E) Representative immunofluorescence

microscopy images of Min6 cells infected with Ad-7a2/Ad-Ctrl and subjected to immunofluorescence for Ins expression. EGFP shows successful infections. (F) Quantification of Ins intensity in EGFP positive cells obtained in E (A-D) n = 6; (F) n = 3 (Ad-Ctrl), n = 5 (Ad-7a2) images of 3 independent biological replicates. Data are means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, unpaired Student's t-test.

To confirm these results, we infected rat Ins1E cells with Ad-7a2, resulting in a significant increase in miR-7a mRNA levels (p=0.0101) in comparison to Ad-Ctrl (Fig. 3.2A). Ins1E cells infected with Ad-7a2 show a significant decrease in the β-cell identity markers *GLUT2* (p=0.0008), *NKX6.1* (p=0.0020) and *PDX-1* (p=0.0350), whereas the expression of *INS1* (p=0.1172) and *INS2* (p=0.4158) tend to be decreased but did not reach statistical significance (Fig. 3.2B). Together these results show that overexpression of miR-7a2 is sufficient to induce loss of β-cell identity in the course of 48h. Conversely, inhibition of miR-7a upregulates gene involved in maintaining β-cell identity. Both gain- and loss-of function *in vitro* models were used to investigate mRNA targeted by miR-7 and triggering β-cell dedifferentiation.

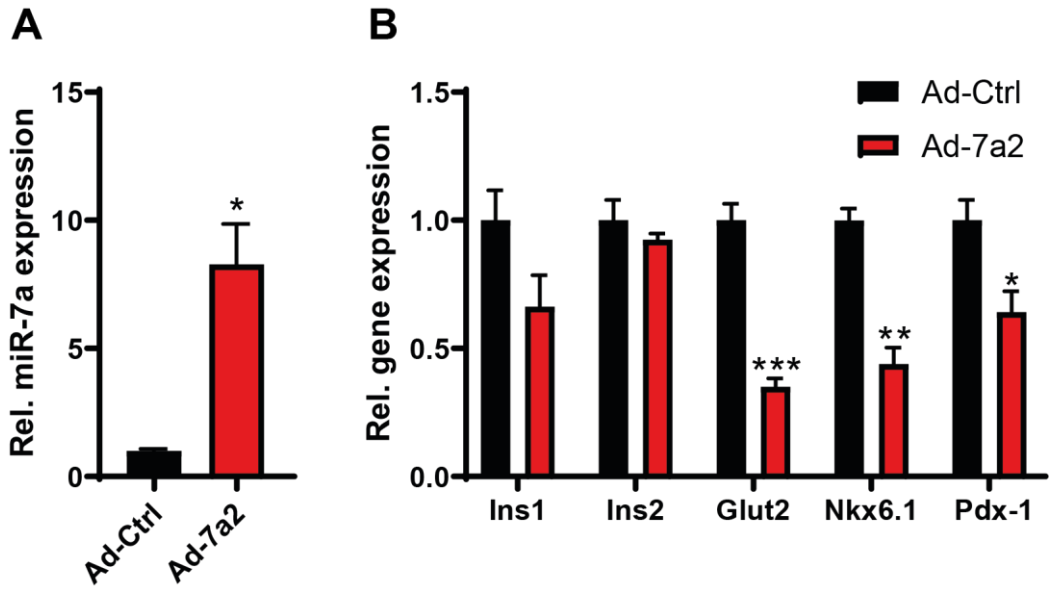
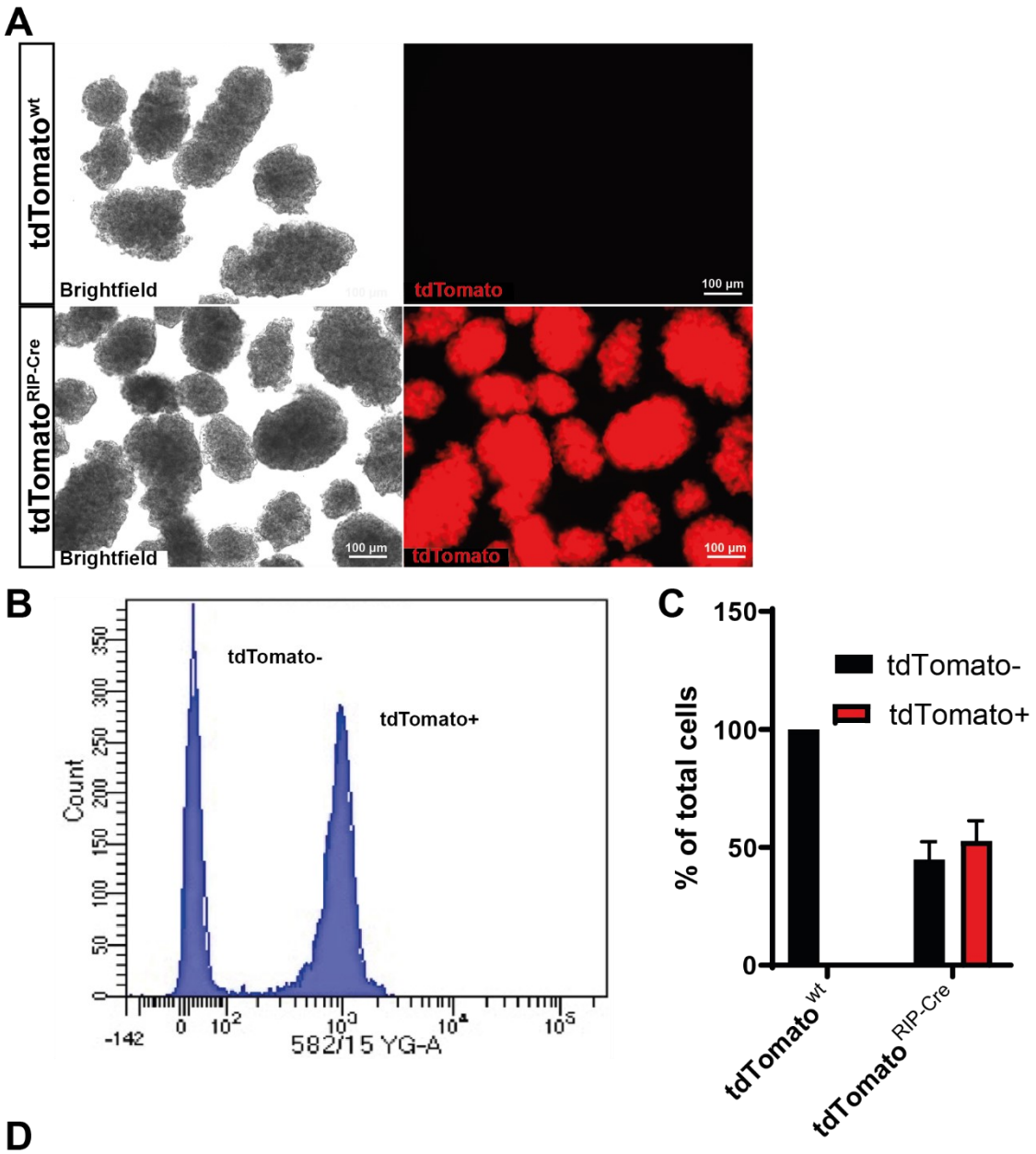


Figure 3.2: Overexpression of miR-7a2 leads to loss of β-cell identity in Ins1E cells. (A) Relative miR-7a expression in Min6 cells infected with Ad-7a2 in comparison to Ad-Ctrl, normalised to *Sno202*. (B) Relative expression of β-cell identity genes in Min6 cells infected with Ad-7a2 in comparison to Ad-Ctrl, normalised to *RPLP0/36B4*. n = 3. Data are means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, unpaired Student's t-test.

3.2.2 Overexpression of miR-7a2 in isolated mouse islets

Most models of diabetes are affected by secondary effects of metabolic stress, resulting in dysregulation of β-cell enriched genes (Zhao et al. 2016, Ottosson-Laakso et al. 2017). Additionally, *in vivo* work can be influenced by secondary effects of other organs. We therefore isolated islets from non-diabetic mice tdTomato$^{RIP\text{-}Cre}$ and infected them with Ad-7a2 / Ad-Ctrl to trigger the dedifferentiation. tdTomato$^{RIP\text{-}Cre}$ label β-cells specifically through the expression of tdTomato under control of the rat Ins II promoter (Fig. 3.3A). Isolated islets from tdTomato$^{RIP\text{-}Cre}$ show red fluorescence under the microscope in comparison to tdTomato^{wt} control mice (Fig. 3.3A), indicating the successful expression of tdTomato in β-cells. We then confirmed those results by sorting islet endocrine cells from tdTomato$^{RIP\text{-}Cre}$/ tdTomato^{wt} islets using FACS. For FACS a yellow green (561nm) laser was used with a 582/15nm bandpass. The representative histogram of the FACS experiment, sorted by tdTomato, shows a clear differentiation between tdTomato positive (tdTomato+) and tdTomato negative (tdTomato-) cells (Fig. 3.3B). When sorted, around 50% of the islet endocrine cells were classified as tdTomato+ cells in tdTomatoRIP^{-Cre} mice, compared to 0% in the tdTomato^{wt} (Fig. 3.3C). Importantly, tdTomato+ cells from tdTomato$^{RIP\text{-}Cre}$ show a significant enrichment in gene expression levels for β-cell markers like *INS2* ($p \leq 0.0001$), *PDX-1* ($p = 0.0010$) and *UCN3* ($p \leq 0.0001$), while the α-cell markers *GCG* ($p \leq 0.0001$), *MAFB* ($p = 0.0013$) and *ARX* ($p = 0.0009$) are significantly less abundant (Fig. 3.3D), indicating that sorting successfully separated β-cells from other endocrine cells, as indicated by α-cell markers.


A
tdTomato^wt
Brightfield
tdTomato
100 µm
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Brightfield
100 µm
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100 µm
B
tdTomato-
tdTomato+
Count
582/15 YG-A
C
% of total cells
tdTomato-
tdTomato+
tdTomato wt
tdTomato RIP-Cre
D
Rel. gene expression
tdTomato-
tdTomato+
Ins2
Pdx-1
Ucn3
Gcg
MafB
Arx
β-cell marker
α-cell marker

Figure 3.3: tdTomato allows tracing of β-cells in tdTomato$^{RIP\text{-}Cre}$ mice. (A) Representative microscopy images (left: Brightfield, right: Red fluorescence) of tdTomato$^{RIP\text{-}Cre}$ mice in comparison to tdTomato^{wt} mice. (B) Representative histogram of FACS sorted dissociated islets of tdTomato^{wt} / tdTomato$^{RIP\text{-}Cre}$ mice showing the cell count on the y-axis and red fluorescence intensity on the x-axis. (C) Percentage of tdTomato- / tdTomato + cells of the total of FACS sorted cells from dissociated islets isolated from tdTomato^{wt} / tdTomato$^{RIP\text{-}Cre}$ mice. (D) Relative gene expression of β-cell and α-cell identity markers in tdTomato+ cells compared to tdTomato- cells from FACS sorted tdTomato$^{RIP\text{-}Cre}$ dissociated islets, normalised to *RPLP0/36B4*. (C) n = 5 (tdTomato^{wt}), n = 6 (tdTomato$^{RIP\text{-}Cre}$); (D) n = 7 (tdTomato-), n = 6 (tdTomato+); n = animal number. Data are means ± SEM, **p < 0.01, ***p < 0.001, unpaired Student's t-test.

Successful labelling of β-cells with tdTomato allowed us to trace these cells in studies of miR-7a2 overexpression, where typical β-cell markers, like Ins, are lost. To investigate the effect of miR-7a2 overexpression on β-cells in islets, we infected whole islets from tdTomato$^{RIP\text{-}Cre}$ mice (Fig. 3.4A). We analysed the intensity of Ins in the tdTomato-labelled β-cells that were successfully infected with Ad-7a2/Ad-Ctrl (EGFP+). Quantitative analyses revealed a significant decrease in Ins intensity (p=0.0002) in Ad-7a2 infected islets compared to Ad-Ctrl (Fig. 3.4B). Together with the results found in immortalised β-cell lines, our results show that we can dedifferentiate β-cells *ex vivo* and that miR-7a2 induced dedifferentiation is independent of metabolic stress and/or of intrinsic signals from other organs and tissues.

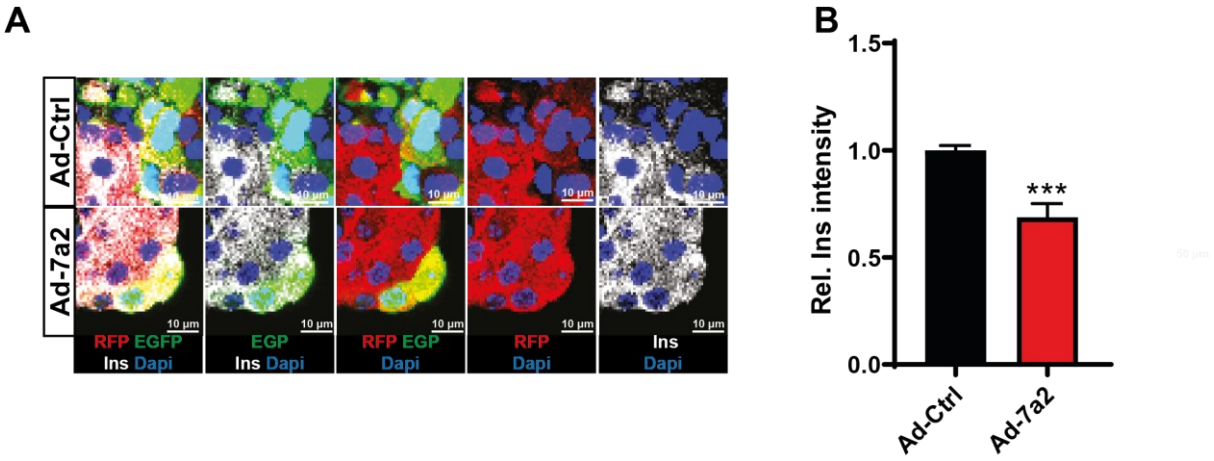

Figure 3.4: miR-7a2 overexpression decreases Ins content in β-cells of tdTomato$^{RIP\text{-}Cre}$ mice. (A) Representative microscopy images of whole isolated islets from tdTomato$^{RIP\text{-}Cre}$ mice infected with Ad-Ctrl/Ad-7a2 and stained for Ins. (B) Quantification of the measured intensity of Ins in the images from (A). (B) n = 9 (Ad-Ctrl), n = 7 (Ad-7a2); n = number of islets. ***p < 0.001, unpaired Student's t-test.

3.2.3 Overexpression of miR-7a2 in human islets

While cell lines come with the advantage of quantity, allowing to perform wide screens in the identification of targets, factors or pathways related to the investigated question and mouse work gives a valuable insight into the effects *in vivo* and in the complexity of a whole organism, the overall goal of every medical related study is to expand its research into man. Until now, the knowledge of miR-7 inside the human islets in relation to T2D is uncharted. Recent studies show a significant enrichment of miR-7 inside human T2D patients serum when compared to control samples (Wan et al. 2017). Together with the findings relating miR-7a2 elevation inside islets to diabetes in mice (Latreille et al. 2014), it encouraged us to investigate if miR-7 is actively involved in the progression of T2D in human. We therefore sought to continue our experiments in islets of human donors, kindly provided by Prof. G. A. Rutter. We utilised IAPP as a marker for β-cells, since we observed no significant regulation of *IAPP* in Tg7a2 mice at 2 and 12 weeks of age in RNA-Seq performed by M. Latreille and analysed by Y. Wang.

We continued with infecting human donor islets with Ad-7a2/Ad-Ctrl and investigated Ins expression using immunofluorescence microscopy (Fig.3.5A). Ad-7a2 infected β-cells (EGFP+, IAPP+) show a significant downregulation of measured Ins intensity (p=0.0366) when compared to Ad-Ctrl samples (Fig. 3.5B). Our findings indicate elevated miR-7a2 levels can trigger the dedifferentiation of both mouse and human primary β-cells.

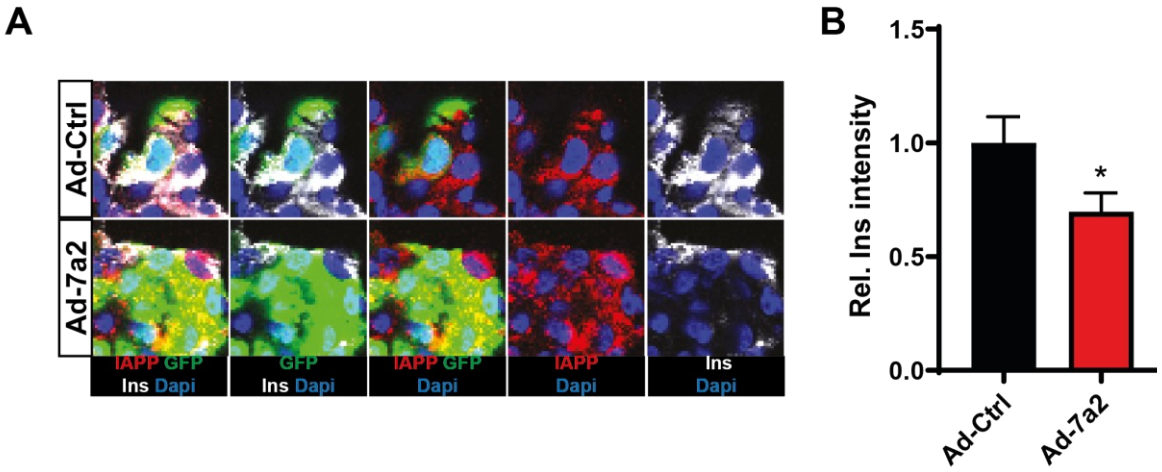

Figure 3.5: miR-7a2 overexpression decreases Ins content in human β-cells (A) Representative microscopy images of whole isolated islets from human donors infected with Ad-Ctrl/Ad-7a2 and stained for Ins and IAPP. (B) Quantification of the measured intensity of Ins in IAPP+/EGFP+ cells in the images from (A). (B) n = 14 (Ad-Ctrl), n = 17 (Ad-7a2); n = number of islets. *p < 0.05, unpaired Student's t-test.

3.2.4 Insulin islet content in mouse models of diabetes

Our group previously showed that Ins expression, as well as circulating Ins, is reduced in Tg7a2 mice at 12 weeks of age (Latreille et al. 2014). However, Ins content in islets of Tg7a2 animals at 2 weeks of age is still unexplored. Onset of hyperglycaemia in Tg7a2 animals is from 3 weeks onwards (Latreille et al. 2014). Therefore 2-week-old (2w) animals allow the investigation of miR-7a2 effects on β-cells without secondary effects by metabolic stress. Tg7a2 animals of 2 and 12 weeks of age were used for ATAC-Seq studies presented in Chapter 4.4.

We investigated Ins content in islets of Tg7a2 animals at 2 and 12 weeks of age using immunofluorescence microscopy. We hypothesised that miR-7a2 overexpression reduces Ins content in Tg7a2 animals from 2 weeks onwards. Immunostaining in 2 weeks of age did not shows any significant difference in Ins content in Tg7a2 and Wt littermate controls. (Fig. 3.6A,B). Interestingly, we were able to observe that 12-week-old (12w) Wt animals contain significantly higher amounts of Ins when compared to 2w Wt mice ($p=0.0090$), while there is no significant difference between Tg7a2 mice of 2 and 12 weeks of age. At 12 weeks of age, Tg7a2 animals show a significant reduction in Ins content compared to Wt animals ($p=0.0157$) (Fig. 3.6A,B). Our results show, that miR-7a2 overexpression induces loss of Ins content after 2 weeks of age. While Ad-7a2 and Tg7a2 induced overexpression of miR-7a2 provide interesting models for the investigation of T2D, we sought to confirm our results in *db/db* mice, a well-established mouse model of diabetes with increased miR-7 levels (Hummel et al. 1966, Latreille et al. 2014, Burke et al. 2017). Interestingly, we found that 12w diabetic *db/db* animals displayed decreased islet Ins content in comparison to age matched *db/+* control animals (Fig. 3.6C).

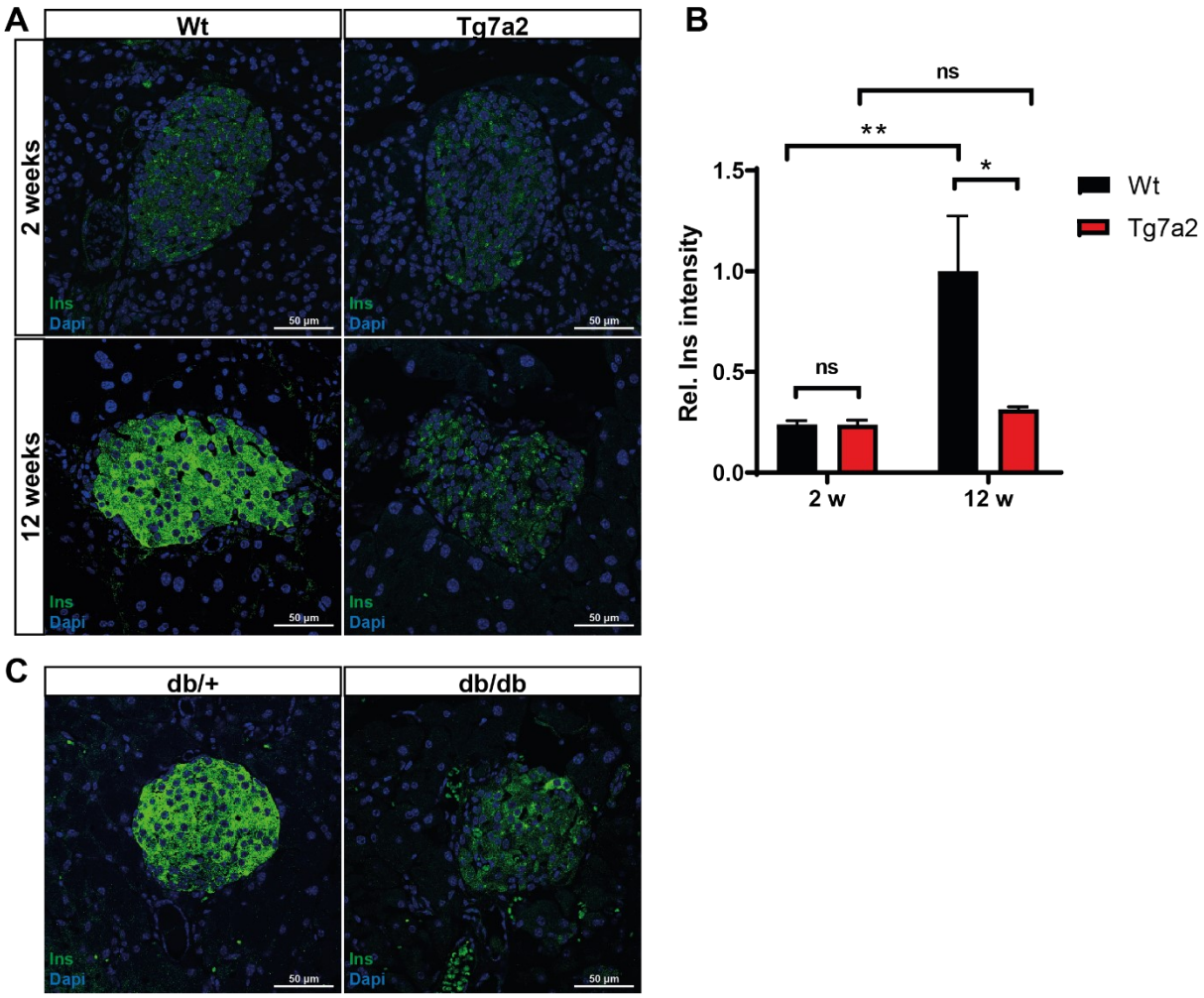

Figure 3.6: Decreased Ins content in Tg7a2 and *db/db* islets. (A) Representative microscopy images of islets from Wt control and Tg7a2 animals at 2 and 12 weeks of age stained for Ins. (B) Quantification of Ins staining from mice represented in (A). (C) Representative microscopy images of islets from *db/+* control and db/db animals at 12 weeks of age stained for Ins. (B) n = 3. Data are means ± SEM, *p<0.05, **p < 0.01, Two-way ANOVA.

3.2.5 Analysis of polyhormonal cells in miR-7a overexpressing islets

Following up on the findings by T. Mak, showing that elevated miR-7 levels cause a transdifferentiation of β-cell into δ-cells (Mak et al. under review), we sought to investigate if miR-7a2 overexpression *ex vivo* is sufficient to induce transdifferentiation, therefore excluding secondary effects induced by metabolic stress and/or other organs and tissues. Our first approach was to dissociate the islets of tdTomato$^{RIP\text{-}Cre}$ mice, infect the cells with Ad-7a2/Ad-Ctrl and stain them with a cocktail of antibodies against non-β-cell hormones (Sst/Gcg/Ppy) (Fig. 3.7A). Although not significant, our data raised our interest whether miR-7a2 increases Sst/Gcg/Ppy positive cells in Ad-7a2 infected β-cells (tdTomato+, EGFP+) compared to Ad-Ctrl infected samples of tdTomato$^{RIP\text{-}Cre}$ mice (Fig. 3.7A,B), leading us to continue investigating the imminent effect of miR-7a2 to induce a polyhormonal β-cell state in human islets (Fig. 3.8) We utilised the *db/db* mouse model as an independent model of diabetes, without artificially increasing miR-7a2 expression, to further investigate the occurrence of polyhormonal cells in T2D. We stained 12w *db/db* and *db/+* control animals for Ins and Sst (Fig. 3.7C). When comparing *db/db* mice to *db/+* control animals our data shows a significant increase in the number of Sst positive cells ($p=0.0229$) in comparison to age matched *db/+* controls (Fig. 3.7D), confirming our results, as well as previous studies (Talchai et al. 2012), stating an occurrence of polyhormonal cells in T2D mice.

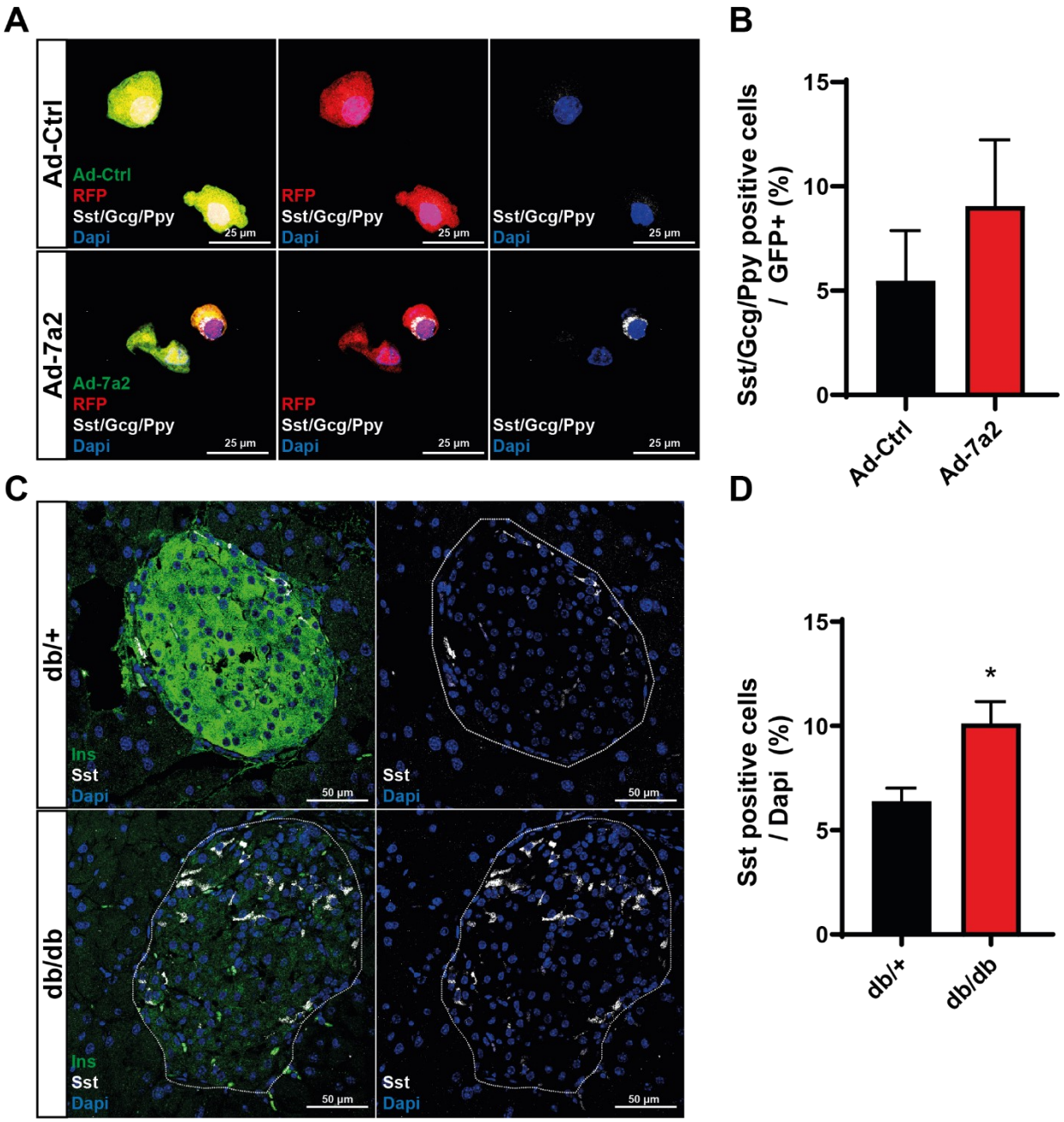

Figure 3.7: Occurrence of polyhormonal cells and increases in δ-cell mass in mice. (A) Representative microscopy images of dissociated islets from tdTomato$^{RIP\text{-}Cre}$ infected with Ad-Ctrl/Ad-7a2 and stained for an antibody cocktail containing anti- Sst/Gcg/Ppy. (B) Quantification of Sst/Gcg/Ppy positive cells in the EGFP positive cell population in the images from (A). (C) Representative microscopy images of islets from ~12w *db/+* and *db/db* mice stained for Ins and Sst. (D) Quantification of the proportion of Sst positive cells inside the islets of ~12w *db/db* animals in comparison to *db/+* shown in (C). (B) n = 14 (Ad-Ctrl, 81 EGFP+ cells), n = 13 (Ad-7a2, 78 EGFP+ cells); n = number of images. (D) n = 4 (*db/+*), n = 3 (*db/db*); n = number of animals. *p < 0.05, unpaired Student's t-test.

To recapitulate our findings in man we dissociated human donor islets and infected them with Ad-7a2/Ad-Ctrl (Fig. 3.8A). Dissociated islets were used to increase the effectiveness of infection. Infected islets were stained for Ins and a Sst / Gcg antibody cocktail to mark

polyhormonal cells (Fig 3.8A). Confirming our previously results in whole human islets infected with Ad-7a2/Ad-Ctrl (Fig. 3.5), quantification revealed a significant reduction in the percentage of Ins positive cells ($p \leq 0.0001$) in Ad-7a2 infected cells (EGFP+) in comparison to Ad-Ctrl samples (Fig. 3.8B). In contrast to that the number of Sst/Gcg positive cells in the infected cell population (EGFP+) is significantly increased ($p=0.0008$) when comparing Ad-7a2 to Ad-Ctrl samples (Fig. 3.8C). Next we wanted to address, if the increase in Gcg/Sst positive cells we observe (Fig. 3.8C) is due to an increase in Sst/Gcg cell proliferation or β-cells losing their identity. We therefore quantified the number of Ins/Sst/Gcg positive cells in the total number of Ins/EGFP positive cells, resulting in a significant increase in polyhormonal Ins/Sst/Gcg positive cells ($p \leq 0.0001$) in Ad-7a2 samples in comparison to Ad-Ctrl samples (Fig. 3.8D), showing that the increase in non-β-cell specific hormones observed is due to a loss of mature β-cells, rather than an increase of α and δ cells.

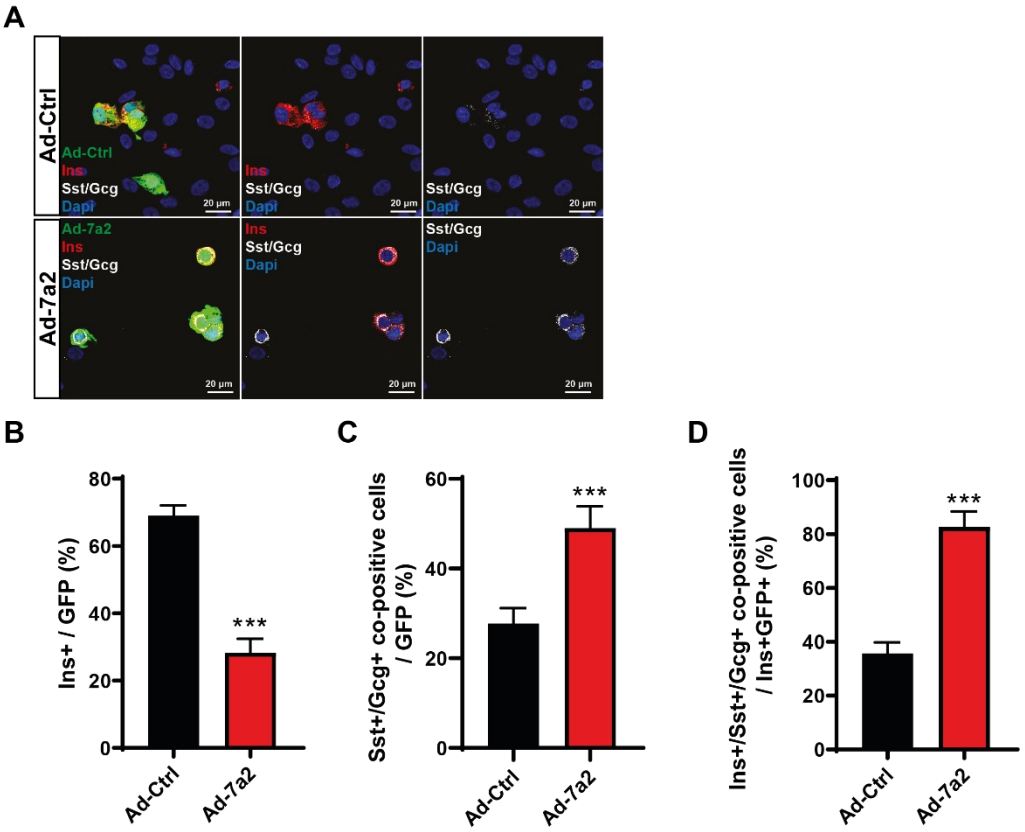

Figure 3.8: miR-7a2 overexpression increases polyhormonal cells in human islets *in vitro* (A) Representative microscopy images of dissociated human islets infected with Ad-Ctrl/Ad-7a2 and stained for Ins and an antibody cocktail containing anti- Sst/Gcg. (B) Quantification of Ins positive cells in the EGFP positive cell population in the images from (A). (C) Quantification of Sst/Gcg positive cells in the EGFP positive cell population in the images from (A). (D) Quantification of Ins/Sst/Gcg positive cells in the Ins/EGFP positive cell population in the images from (A). (B-D) n = 97 (Ad-Ctrl, 456 EGFP+ cells), n = 41 (Ad-7a2, 554 EGFP+ cells); n = number of images. ***p < 0.001, unpaired Student's t-test.

3.3 Discussion

Throughout this thesis an adenovirus based miR-7a2 overexpression system was utilised which allows the effective manipulation of miR-7a2 levels, but also comes with potential drawbacks that need to be kept in mind. In general, the overexpression of the miRNA in those systems can be potentially unnatural and therefore needs to be carefully optimised to avoid unwanted effects. In previous studies it has been shown that oversaturation of miRNA pathways by consistent overexpression can lead to fatality in mice (Grimm et al. 2006). Therefore, it is to assume that wrong dosage, while not necessarily fatal, leads to side effects. In previous studies we showed that *db/db* mice have 4fold increased miR-7 levels compared to wt control mice at 12 weeks of age and human islets grafted under the kidney capsule of high fat diet fed mice express approximately 1.7fold higher miR-7 compared to chow fed mice after 10 weeks of diet. Obese mice and human showed no significant changes (Latreille et al. 2014). In the adenovirus-based system, inducing a 7fold increase in miR-7a2 levels, those differences could affect the results and need to be considered when comparing Ad-7a2 overexpression and other forms of T2D models. Of note, Tg7a2 animals have an approximately 3fold increase in miR-7a2 levels. Additionally, while we assume that miR-7a1/miR-7a2/miR-7b function is redundant, due to their similarity in their sequence, we previously showed that the expression of the different miR-7 family members is different inside the pancreatic islets, showing an enrichment for miR-7a2, and differs between different organs, with a 2fold higher expression in the pituitary compared to the hypothalamus or islet (Latreille et al. 2014). Due to the enrichment of miR-7a2 in the islet we utilised a miR-7a2 overexpressing adenovirus, but while we focused on miR-7a and miR-7a2 in particular, it would be interesting to investigate how miR-7b is affected in T2D and if overexpression of miR-7a2 alternates the balance between the miR-7 family members. Another drawback of adenovirus based delivery systems is, that they are not able to integrate into the host genome and induce an immune response, which not only can cause additional side effects, but also limits their usage for long term miRNA effect studies (Bessis, GarciaCozar and Boissier 2004). An alternative would be the adeno-associated virus, which can integrate into the host genome and induces a milder immune response (Rutledge and Russell 1997, Grimm and Kay 2003).

Historically the loss of functional β-cells was thought to be caused by apoptosis, therefore decreasing the overall β-cell mass of the pancreas (Butler et al. 2003). While apoptotic β-cells are irreversibly lost in diabetes, dedifferentiated β-cells, although non-functional, may be targeted by agents improving their functional identity through re-differentiation into functional

β-cells (Talchai et al. 2012, Wang et al. 2014, Cinti et al. 2016a). Interestingly, while there are studies showing the loss of β-cell identity through selected knockout of key genes in mice, for example *FOXO1* (Talchai et al. 2012), little evidence has been provided on the mutation triggering β-cell dedifferentiation in humans. In previous work we showed, that miR-7a levels are increased in *db/db* mice, as well as human islets grafted under the kidney capsule of mice fed a high fat diet and provided first evidence that miR-7a2 is able to trigger β-cell dedifferentiation (Latreille et al. 2014). MiR-7a2 could therefore be one of the first reported, in human T2D naturally elevated occurring, factors causing β-cell dedifferentiation (Wan et al. 2017).

The initial goal of my study was to establish experimental models to study miR-7a mediated β-cell dedifferentiation which could be used to identify mRNA targets underlying the effect of miR-7a. In our first models, we used immortalised mouse and rat β-cell lines, Min6 and Ins1E, respectively. On top of the obvious advantages like cost efficiency, their ease to culture and unlimited supply, these cell lines also present us the opportunity to research the effects of miR-7a2 on a pure population of β-cells, eliminating potential effects of other cells. When we overexpressed miR-7a2, in both β-cell lines (Fig. 3.1A and 3.2A) we observed a loss of key β-cell identity genes via mRNA levels (Min6 + Ins1E) and on a protein level (Min6) (Fig. 3.1B,E,F and 3.2B). The loss of these key β-cell identity genes: *GLUT2* (Thorens 2015), *NKX6.1*(Taylor, Liu and Sander 2013), *PDX-1* (Ahlgren et al. 1998, Brissova et al. 2002) and the Ins genes (Duvillié et al. 1997) have all been associated with the progression of diabetes. Additionally, dedifferentiation of human β-cells through fibroblast growth factor 2, showed reduced expression of key master β-cell markers, including *INS*, *MAFA*, *PAX6* and *GLUT2* (Diedisheim et al. 2018). While miR-7 is known to be present in the development of the pancreas and β-cells and is upregulated in diabetic models (Wienholds et al. 2005, Nieto et al. 2012, Liao et al. 2013, Wei et al. 2013, Nesca et al. 2013, Latreille et al. 2014), it would be interesting to investigate in future experiments, whether an induced dedifferentiation of β-cells, by e.g. fibroblast growth factor 2, results in an upregulation of miR-7.

Decrease in mRNA levels of those genes provided us with the necessary evidence for the occurrence of β-cell dedifferentiation following miR-7a2 overexpression. On the other hand, we were interested, if downregulation of miR-7a2 in Min6 cells, could improve the function of the β-cell. In previous work, we demonstrated that β-cell specific knockout of miR-7a2 improved glucose tolerance in mice (Latreille et al. 2014). We aimed to exclude any secondary effects by other organs and tissues. We therefore utilised the miR-7a2 inhibitor LNA-7a to

reduce miR-7a2 levels in Min6 cells (Fig. 3.1C). The observed changes in mRNA levels of β-cell identity genes were not as strong as for the miR-7a2 overexpression, which could be explained by the fact, that the levels of miR-7a2 in normal-state Min6 cells are too low to affect regulators of β-cell key genes in the first place. Therefore, a downregulation of miR-7a2 has no significant effect, whereas upregulation of miR-7a2 shows severe effects on the expression of these genes.

For our next step, we sought to investigate miR-7a2 overexpression on the whole islets, while excluding secondary effects induced by metabolic stress or other organs and tissues. To do so, we tdTomato labelled β-cells using the tdTomato$^{RIP\text{-}Cre}$ mouse model (Fig. 3.3). Although the accuracy of the RIP-Cre recombinase has been reported before (Kulkarni et al. 1999), evidence shows that leakage of the labelling efficiency can occur (Estall and Screaton 2015). To ensure the faithfulness of the system we FACS sorted the cells via tdTomato intensity. Our results confirm, due to an enrichment of β-cell identity markers in the tdTomato+ population, and lower levels of α-cell markers, that tdTomato expression is in indeed specifically inside the β-cell. Interestingly, while it has been reported, that miR-7 is selectively expressed in neuroendocrine tissues (pituitary glands, pancreatic islets, hypothalamus and adrenal glands) (Landgraf et al. 2007, Bravo-Egana et al. 2008, Joglekar et al. 2009, Kredo-Russo et al. 2012b, Latreille et al. 2014), in situ hybridisation demonstrated that miR-7 is expressed preferentially in endocrine cells and the embryonic neural tube (Nieto et al. 2012). Infection of whole tdTomato$^{RIP\text{-}Cre}$ mouse islets with Ad-Ctrl/Ad-7a2, shows that the dedifferentiation of β-cells *ex vivo* is reproducible in whole islets (Fig. 3.4). Differentiating results between single cells (Min6) and whole islets is necessary, as function of the endocrine cells relies on the structural integrity of the islets (Cirulli et al. 1994, Esni et al. 1999, Olofsson et al. 2009, Aamodt and Powers 2017). These results exclude that the dissociation plays aa role in miR-7 mediated β-cell dedifferentiation. We furthermore confirmed that our results from Ad-7a2 infected tdTomato+ β-cells are indeed an effect of the miR-7a2 overexpression and not an artefact of the infection with viral particles itself, by comparing the effect of Ad-7a2 to Ad-Ctrl (Fig. 3.4A,B).

In human islets we utilised the pancreatic β-cell marker IAPP (Blodgett et al. 2015) to identify β-cells after Ad-7a2 infection since our RNA-Seq analysis performed on isolated islets from Tg7a2 animals did not show any changes in IAPP expression in comparison to Wt controls. This also shows that the diabetic phenotype induced by miR-7a2 is not a developmental defect of the whole islet, but rather a quite specific process leading to decreased expression of only a

subset of β-cell markers. Our results in human islets confirm our findings in cells and mice. Those findings are important for the progression of the research project, due to the fact that it has been reported that, while there are many mouse models available, none model the pathophysiological characteristics of human T2D in its entirety (Cefalu 2006), showing the need of human samples in diabetic research.

While *in vitro* work provides insights into cellular aspects of the β-cell dysfunction in diabetes, we also wanted to explore how miR-7a impacts on the identity of β-cells *in vivo*. For this, we used Tg7a2 and *db/db* which both overexpress miR-7a2 in β-cells (Fig. 3.6) (Latreille et al. 2014). While Tg7a2 animals express a hyperglycaemic phenotype from 3 weeks onwards, and the islets in 15-week-old Tg7a2 animals show a decrease in Ins content and other β-cell markers (MafA, Nkx6.1, Glut2), 2w normoglycaemic mice allowed us to investigate how miR-7a2 induces β-cell dedifferentiation, while excluding potential effects induced by the diabetic phenotype itself (e.g. hyperglycaemia). In future experiments, the glucose response of Tg7a2 animals should be investigated at 2 weeks of age to determine if miR-7a2 overexpression has a pathological effect on pre-weaned mice.

Our results suggest that, as Ins content is reduced in 2w Wt mice compared to 12w WT mice, but not between 2w Tg7a2 and Wt mice, overexpression of miR-7a2 does not affect the remaining Ins synthesis. It has been previously reported, that maturation of the β-cells and establishment of β-cell mass is triggered by the change in diet composition at the weaning period (Tarussio et al. 2014, Stolovich-Rain et al. 2015). Those changes are most likely due to the switch from a high fat milk diet to the carbohydrate rich chow diet, coming with the need for functional islets to compensate changing blood glycaemia levels. This enables β-cells to respond to changes in circulating glucose levels and secrete appropriate amounts of Ins in the circulation. Additionally, single cell transcriptomic analysis of β-cells shows significant differences between β-cells at different stages of development in mice, including genes crucial for insulin synthesis and secretion (e.g. Ins2 and Ucn3) (Qiu et al. 2017). As miR-7a2 induces dedifferentiation to a more progenitor-like cell type (e.g. *SOX9* expression, Latreille et al. 2014), the effects on the "premature" β-cell pre-weaning are not affecting Ins content.

Insulin content analysis was performed utilising immunofluorescence approaches only. Other, more conventional, methods can be used in combination with immunofluorescence to assess insulin content and provide further confirmation, including insulin secretion assays, western blots or ELISA.

The *db/db* mouse is a well-established model of diabetes (Hummel et al. 1966, Burke et al. 2017). The reduced Ins content compared in diabetic *db/db* animals (Fig. 3.6C), was not unexpected, as *db/db* animals have previously been reported to have reduced insulin content in islets. Nevertheless, *db/db* mice confirm our findings so far and represents another valuable diabetic mouse model with miR-7a overexpression.

While we established the loss of key β-cell identity markers through the overexpression of miR-7a2, we also sought to address the occurrence of polyhormonal cells, indicating transdifferentiation of β-cells, in a diabetic mouse models as previously reported (Talchai et al. 2012, Blum et al. 2014, Brereton et al. 2014, Wang et al. 2014, Cinti et al. 2016a, Md Moin et al. 2016). Contrary to expectations, we could not detect an increase in the number of polyhormonal cells in tdTomato$^{RIP\text{-}Cre}$ mice (Fig. 3.7.A,B). These experiments will need to be repeated with larger number of islet samples to uncover its significance.

The increase in polyhormonal cells in human endocrine cells, induced by upregulation of miR-7a2, confirms our expectations that miR-7a2 is able to not only dedifferentiate endocrine and in particular β-cells, but also induces the expression of atypical hormones and potential transdifferentiation. Our results prove that β-cell dedifferentiation is not exclusively found in mouse models of diabetes but expands into human and may underlie an important pathological mechanism in T2D. These observation, and previous data obtained in mice indicating the β-cell that have lost their identity can redifferentiate into functional β-cells (Blum et al. 2014, Wang et al. 2014), may open up novel therapeutic possibilities in humans. Surprisingly, the number of polyhormonal cells in our controls is higher than expected (Fig. 3.7/3.8). This could be an artefact of the isolation (and transport) of the mouse and human islets, resulting in increased cell stress. Additionally, the infection of the islet cells with adenovirus itself could result in an increase in the overall number of polyhormonal cells. Indeed it has been reported that stress is able to induce the dedifferentiation and therefore potential transdifferentiation of cells (Shoshani and Zipori 2011).

We utilised *db/db* mice to confirm T. Mak's finding in Tg7a2 animals describing a transdifferentiation of β- to δ-cells, induced by miR-7a2 overexpression (Mak et al. under review). The increase in the number of Sst+ δ-cells in islets of *db/db* mice could be due to transdifferentiation or increases in δ-cell proliferation. Previous work suggests an increase in α- but not δ-cell mass in T2D (Saito, Yaginuma and Takahashi 1979, Rahier, Goebbels and Henquin 1983, Kilimnik et al. 2011). Therefore, our results in *db/db* indicate to confirm our

findings in Tg7a2 showing that miR-7a2 overexpression triggers the transdifferentiation of β-cell into δ-cells. Analysis of the co-expression of Ins and Sst, or lineage tracing experiments in *db/db* animals, will be required. The experiments in *db/db* animals used antibodies against Sst and Ins, in opposite to the experiment performed on isolated islets (mouse and human) infected with Ad-7a2/Ad-GFP, where antibodies against Ins, Sst, Gcg and Ppy were used. Further experiments with Ins, Sst, Gcg and Ppy antibodies in *db/db* animals or a separate quantification of Ins+, Sst+ positive cells in isolated islets would be needed to allow comparison between those experiments. Initial studies were performed with a cocktail of Ins, Sst, Gcg and Ppy antibodies. Later findings of our group suggested that Sst+ cell counts increase in Tg7a2 animals, resulting in our following experiments on *db/db* mice to use the combination of Ins and Sst antibodies.

In summary, the data presented in this chapter shows that miR-7a2 overexpression is able to induce the dedifferentiation of β-cells and additionally trigger a polyhormonal state in those cells opening up potential new avenues for the treatment of T2D by targeting the dedifferentiation or inducing re-differentiation processes through miR-7a2 inhibitors. Further research in the function of miR-7a2 and the mRNA target it represses in β-cells is required to fully understand the pathways inducing β-cell dedifferentiation and ensure a risk-free admission of miR-7a2 inhibitors as a potential treatment option.

4. Epigenetic modulation of β-cell identity by miR-7a2

4.1 Introduction

The findings presented in Chapter 3 provide evidence for the involvement of miR-7a2 in the dedifferentiation of mouse and human β-cells which is associated with cellular polyhormonality. However, the molecular mechanisms through which miR-7a induces these changes have yet to be completely elucidated. Given that miRNAs repress genetic expression on a post-transcriptional level through binding to its target 3'UTR (Cannell, Kong and Bushell 2008) we sought to identify mRNA targets repressed by miR-7a2. Interestingly, miR-7a2 induces a loss of β-cell identity and a reduction of insulin biosynthesis, without directly targeting the majority of β-cell identity marker genes. For example, *INS1* and *INS2* show no potential miR-7 binding sites in their respective 3'UTRs (Fig. 4.1).

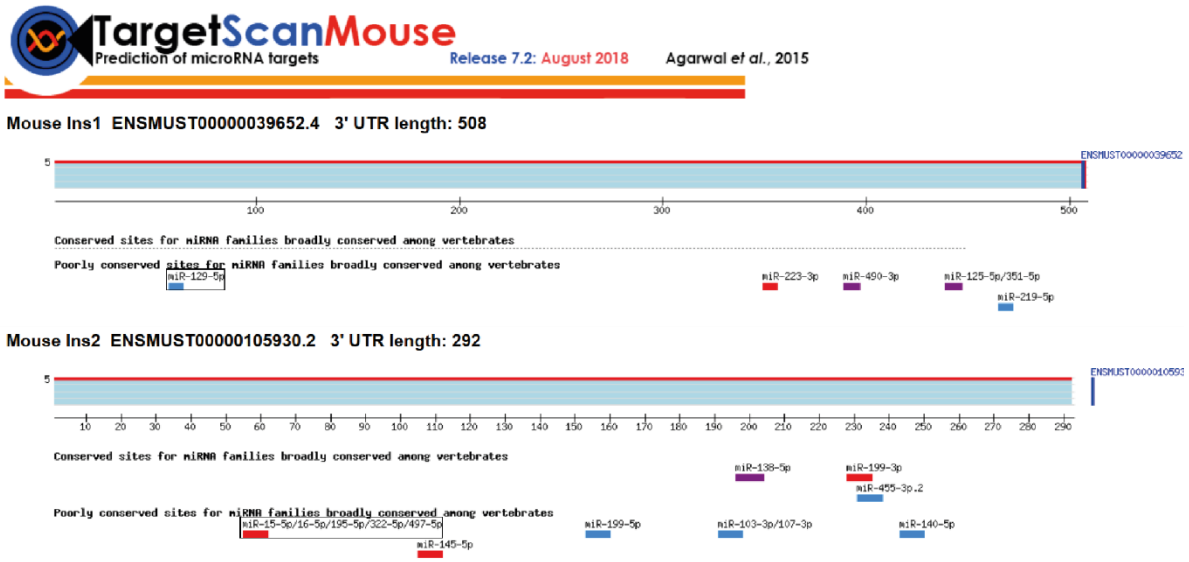

Figure 4.1: Predicted miRNA targets in the 3'UTR of *INS1* and *INS2* in mice. Screening of the 3'UTR of mouse *INS1* and *INS2* via TargetScan shows no miR-7a binding sites.

Therefore, we hypothesised that miR-7a2 must regulate the expression of an intermediary mRNA target responsible for β-cell dedifferentiation. During development, it is well established that cell differentiation is accompanied by epigenetic changes (Atlasi and Stunnenberg 2017).

Furthermore, active remodelling of epigenetic marks has been reported in the development of β-cells (Xie et al. 2013b) and the specific distribution of epigenetic marks exert a pivotal function in maintaining the identity of islet endocrine cells (Bramswig et al. 2013). Recent,

findings have demonstrated the involvement of a loss-of-function of the Polycomb complex leading to the dedifferentiation of mouse and human β-cells (Lu et al. 2018).

As a result, we hypothesize that miR-7 alters β-cell identity through regulation of epigenetic modulators. Another focus of the present investigation was to further explore changes in the epigenetic landscape induced by miR-7a2 in islets from Tg7a2 mice, in order to gain an improved understanding of the epigenetic processes involved in the dedifferentiation of β-cells.

4.2 Identification of potential epigenetic regulators targeted by miR-7a

To identify epigenetic regulators as miR-7a2 targets, we used the publicly available TargetScan database predicting epigenetic targets of miR-7 (Table 4.1). The different epigenetic modulators were grouped into different categories related to their function which included:

1) "Histone Acetylation": Brd3 (LeRoy, Rickards and Flint 2008), Phf21a (Iwase et al. 2004), Phf17 (Panchenko, Zhou and Cohen 2004), Tada2b (Gamper, Kim and Roeder 2009) and Rest (Huang, Myers and Dingledine 1999),

2) "Histone Methylation": CHD5 (Paul et al. 2013), Setd8 (Nishioka et al. 2002b) and JHDM1D (Klose, Kallin and Zhang 2006),

3) "Histone Ubiquitination": Rnf20 (Hwang et al. 2003),

4) "DNA Methylation": Dnmt3a (Masaki, Shaoping and En 1998), MeCP2 (Lewis et al. 1992), Zbtb4 and Zbtb38 (Filion et al. 2006) and

5) members of the mSWI/SNF complexes "Chromatin remodelling": Arid2 (Xu, Flowers and Moran 2012), Brm, Brg1 and BAF60A (Tang et al. 2010) (Fig. 4.2A,B).

Table 4.1: Identification of potential miR-7a targets. Predicted bindings sites of miR-7a in the 3'UTR of epigenetic regulators and log_2 fold-changes of predicted genes in RNA-Seq of Tg7a2 animals in comparison to Wt controls at 2w and 12w of age. Conserved sites are conserved broadly among vertebrates and poorly conserved sites are found in m = mouse, r = rat, h = human as predicted by TargetScan. RNA-Seq: n = 3, Wald test with adjusted p values. Initial TargetScan search by M. Latreille, reanalysed by me; RNA-Seq performed by M. Latreille (Mak et al. under review).

Gene	Predicted sites		Tg7a2 2w log_2 fold	Adjusted p-value	Tg7a2 12w log_2 fold	Adjusted p-value
	Conserved	Poorly conserved				
Brd3	1	1 (m)	0.079949518	0.65517198	0.126588	0.385948
Phf21a/ BHC80	1	0	0.109169643	0.693687247	0.352727	0.06058
Phf17/ Jade1	1	0	-0.826688118	0.00038255	-0.66326	0.003734
Tada2b	1	0	-0.578181404	0.04238607	-0.8013	0.001231
Rest	1	1 (m/r)	0.709632324	0.032710444	0.625771	0.071548
CHD5	0	1 (m/r)	1.610789658	1.98e-08	1.282118	7.34e-06
Setd8	1	1 (m/r/h)	0.079932557	0.690289891	-0.34444	0.007591
JHDM1D/ KDM7a	1	1 (m/r/h)	-0.185645667	0.583196723	-0.46783	0.052807
Rnf20	1	2 (m/r)	-0.287681182	0.147093254	-0.27398	0.137286
Dnmt3a	0	1 (m)	-0.403246738	0.057197346	0.0357	0.910328
MeCP2	0	1 (m/r/h)	-0.186273371	0.495317945	-0.65171	0.00048
Zbtb4	0	1 (m/r/h)	-0.321833147	0.078532806	-0.45489	0.00387
Zbtb38	1	0	-0.066774439	0.828722078	-0.50838	0.004288
Arid2	1	0	0.060010874	0.851718706	-0.08466	0.757444
Brm/ Smarca2	0	0	0.121062632	0.608978592	0.213593	0.248624
Brg1/ Smarca4	1 (not r)	2 (2m/1r)	-0.371894451	0.015731945	-0.4703	0.000666
BAF60A/ Smarcd1	1	2 (m/r/h)	-0.048240116	0.860381703	-0.22357	0.214249

We then screened the epigenetic targets in Min6 cells infected with Ad-7a2/Ad-Ctrl followed by RT-qPCR. Overexpression of miR-7a2 by Ad-7a2 revealed a significant decreased mRNA levels of Tada2b ($p=0.0132$), Setd8 ($p=0.0003$), MeCP2 ($p=0.0034$), Zbtb4 ($p\leq0.0001$) in

addition to members of the chromatin remodelling mSWI/SNF complexes: Brm ($p \leq 0.0001$), Brg1 ($p=0.0338$) and BAF60A ($p=0.0002$) (Fig. 4.2A,B) when compared to Ad-Ctrl samples. Additionally, a significant upregulation of *CHD5* ($p=0.0008$) and *DNMT3A* ($p=0.0145$) expression levels was observed in Ad-7a2 samples compared with Ad-Ctrl (Fig. 4.2A,B).

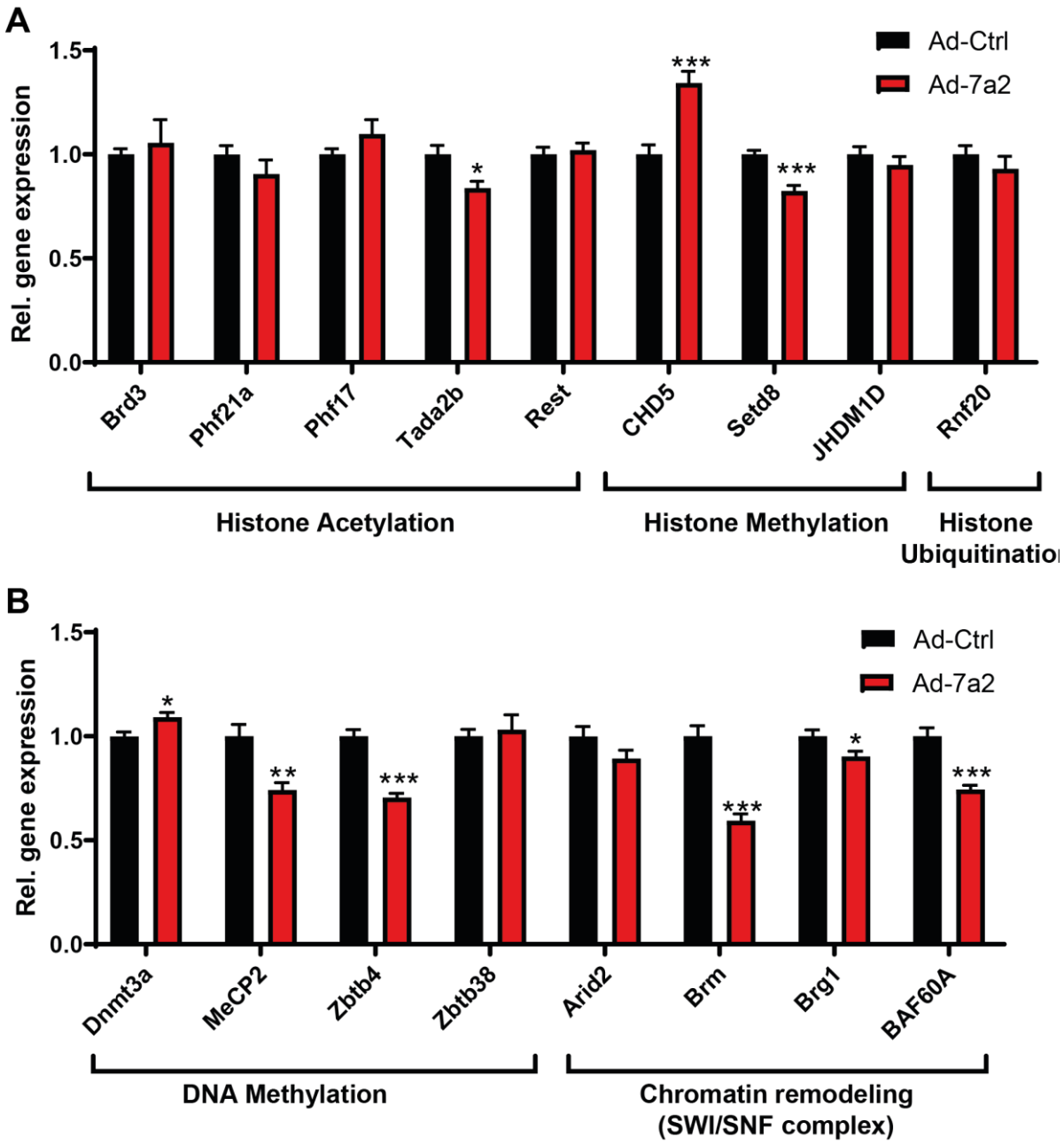

Figure 4.2: Identification of epigenetic regulators in Min6 cells overexpressing miR-7a2. (A) qPCR results of epigenetic regulators involved in Histone Acetylation, Methylation and Ubiquitination processes in Min6 cells infected with Ad-7a2 compared to Ad-Ctrl, normalised to *RPLP0/36B4*. (B) qPCR results of epigenetic regulators involved in DNA Methylation and Chromatin remodelling processes in Min6 cells infected with Ad-7a2 compared

to Ad-Ctrl. normalised to *RPLP0/36B4*. n = 6. Data are means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, unpaired Student's t-test.

To complement these studies, we determined whether loss-of-function experiments would upregulate miR-7a targets. Therefore, miR-7a inhibitors were utilized to block miR-7a function levels in Min6, and its effect on the mRNA levels of epigenetic modulators described in Table 4.1 was measured by qPCR (Fig. 4.3A,B)

Inhibition of miR-7a by LNA-7a led to a significant upregulation of the mRNA levels of Brd3 (p=0.0093), Tada2b (p=0.0248), Setd8 (p=0.0405), Brm (p=0.00998) and BAF60A (p=0.0241) in comparison to LNA-Ctrl samples (Fig. 4.3A,B). This confirmed the previous findings of Tada2b, Setd8, Brm and BAF60A mRNA levels in Ad-7a2 overexpression samples (Fig. 4.2A,B). The results also revealed that there was a significant downregulation of Phf21a (p=0.0133) and Rest (p=0.0145) mRNA levels in LNA-7a samples compared to LNA-Ctrl (Fig 4.3A).Surprisingly no significant differences were observed in the mRNA levels of CHD5, Dnmt3a, MeCP2, Zbtb4, or Brg1 in LNA-7a, when compared with LNA-Ctrl (Fig. 4.3A,B), which was the opposite of the findings in Ad-7a2 infected Min6 cells (Fig. 4.2A,B). Of note, a significant regulation of *BRM* expression was observed in the Ad-7a2 (Fig. 4.2B) and LNA-7a (Fig. 4.3B) experiments, despite Brm not carrying any predicted miR-7a target sites in its 3'UTR (Table 4.1).

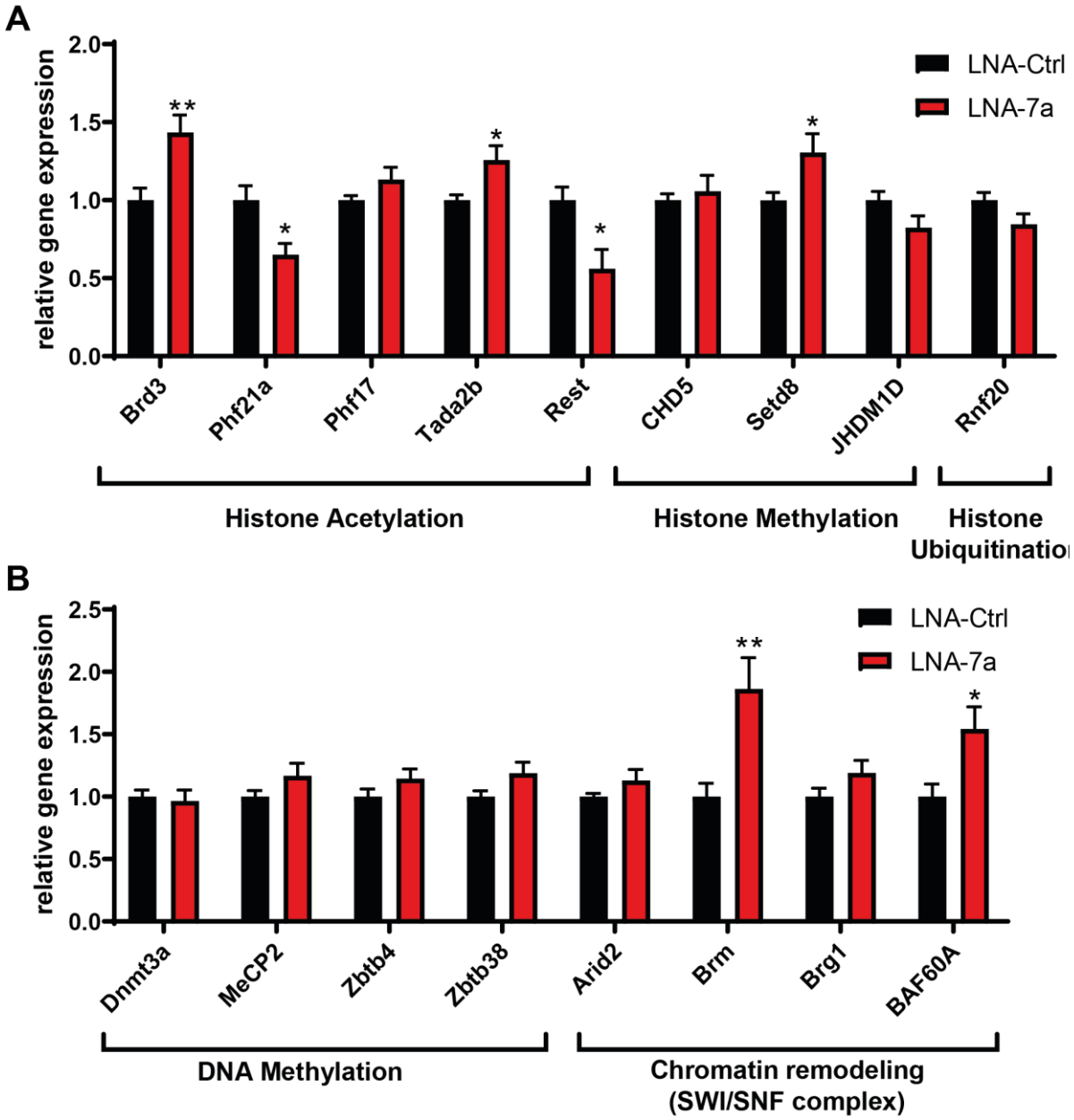

Figure 4.3: Identification of epigenetic regulators in Min6 with inhibited miR-7a function. (A) qPCR results of epigenetic regulators involved in Histone Acetylation, Methylation and Ubiquitination processes in Min6 cells transfected with LNA-7a compared to LNA-Ctrl, normalised to *RPLP0/36B4*. (B) qPCR results of epigenetic regulators involved in DNA Methylation and Chromatin remodelling processes in Min6 cells infected with LNA-7a compared to LNA-Ctrl, normalised to *RPLP0/36B4*. n = 6. Data are means ± SEM, *p < 0.05, **p < 0.01, unpaired Student's t-test.

We compared our findings of Ad-7a2 and LNA-7a treated Min6 cells with RNA-Seq in 2 and 12w old Tg7a2 animals (Mak et al. under revision). This allowed to investigate the effects of miR-7a2 on potential target expression levels without secondary effects induced through metabolic stress or other organs and tissues (Min6 experiments) and to address if the repressive

effect of miR-7a2 on potential targets translates to *in vivo*. Our findings obtained from the RNA-Seq in islets of Tg7a2 animals at 2 and 12 weeks of age compared to age matched Wt controls are listed in Table 4.1. The entirety of the RNA-Seq results will be published in Mak *et* al. under revision. Significant changes were observed for the expression of *PHF17* (2w: padj=0.0004; 12w: padj=0.0037), *TADA2B* (2w: padj=0.0424; 12w: padj=0.0012), *ZBTB4* (2w: padj=0.0785; 12w: padj=0.0039) and *BRG1* (2w: padj=0.0157; 12w: padj=0.0007) being downregulated at both timepoints and *CHD5* (2w: padj=$1.98e^{-08}$; 12w: padj=$7.34e^{-06}$) and *REST* (2w: padj=0.0327; 12w: padj=0.0715), which were upregulated at both time points in Tg7a2 mice compared age matched Wt controls. With the exception of Phf17 and Rest, the results in Min6 cells infected with Ad-7a2 (Fig. 4.2A,B) were in accordance with the data in Tg7a2 animals.

Taken together, the results show that miR-7a regulates the expression of several epigenetic regulators *in vitro* and *in vivo*.

4.3 Identification of the mSWI/SNF complex subunits BAF60A and Brg1 as targets of miR-7a

We decided to follow up on the members of the mSWI/SNF complexes carrying miR-7 target sites: BAF60A and Brg1. The mSWI/SNF complexes are one of the four major protein families responsible for chromatin remodelling and therefore exert pivotal functions in epigenetic regulation (Längst and Manelyte 2015). They are hypothesised to be responsible for the dedifferentiation of β-cells, as mSWI/SNF has been reported to be involved in the differentiation processes in muscle (Puri and Mercola 2012). Furthermore, mSWI/SNF complexes are thought to be involved mainly in promoting transcriptional activation (Ryan et al. 1998), whereas dysfunction of the mSWI/SNF complex may explain the loss of the expression of key β-cell markers, as observed in the progression of T2D. BAF60A has also been demonstrated to act in a diet sensitive manner, resulting in elevated *BAF60A* expression and protein levels in the liver of mice fed a western diet, compared to chow fed animals (Meng et al. 2015b) and its regulation by miRNAs has previously been reported (Goljanek-Whysall et al. 2014).

However, there is little data regarding the function of mSWI/SNF complexes within β-cells. Recent findings have reported a switch between Brg1 and Brm in β-cells, which is dependent

on glucose concentration and the perturbation of this switch is observed in T2D (McKenna et al. 2015b). Although BAF60A mRNA levels were not differentially altered in the RNA-Seq obtained in islets from Tg7a2 mice (Table 4.1), we pursued this target due to the clear regulation of BAF60A mRNA levels in Min6 cells infected with Ad-7a2 or treated with LNA-7a (Fig. 4.2 and 4.3).

4.3.1 Effects of miR-7a alterations on BAF60A and Brg1 protein levels in Min6

Since mRNA and protein levels are not automatically correlated, we next sought to test if alteration of miR-7a levels trigger changes in BAF60A protein levels using western blotting, with γ-tubulin serving as a loading control (Fig. 4.4). Min6 cells infected with Ad-7a2 presented with a decrease in detected BAF60A protein levels when compared to Ad-Ctrl infected cells, while γ-Tubulin was not affected (Fig. 4.4). With our research focussing on Brg1 rather than BAF60A, while BAF60A protein levels were investigated in Ad-7a2 treated Min6 cells, we decided to not analyse BAF60A protein levels in LNA-7 treated Min6 cells. Future work should investigate whether BAF60A protein levels are changed also in miR-7a downregulating experiments.

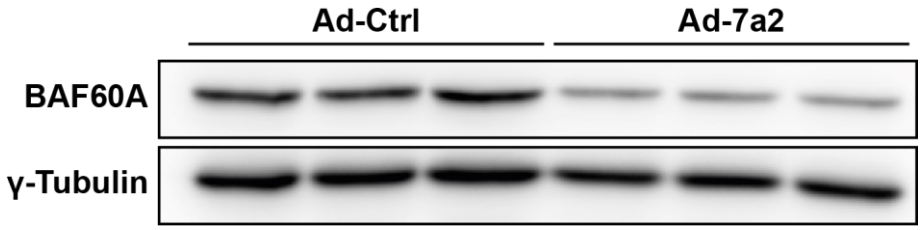

Figure 4.4: BAF60A protein levels in Min6 cells overexpressing miR-7a2. Min6 cells infected with Ad-7a2/Ad-Ctrl and levels of BAF60A and γ-Tubulin determined by western blotting.

In contrast, we did not find any difference in Brg1 protein levels (Fig. 4.5A). Conversely, miR-7a inhibition through the transfection of LNA-7a increased Brg1 protein levels in comparison to LNA-Ctrl (Fig. 4.5B). While the infection with Ad-7a2 had no effect on Brg1 levels in Min6 cells, the downregulation of miR-7a clearly affects the protein level of Brg1. This could be due to the half-life of Brg1 exceeding the length of the experiment in Min6 cells. Indeed, recent findings demonstrated that the half-life of Brg1 in HEK293T cells exceeded 32h (Tatarskiy et al. 2017). In comparison, while only data was available in *Arabidopsis thaliana*, the half-life

of BAF60A in a light dark cycle was less than 9h (Jégu et al. 2017). Of note, the switch between light and dark could induce BAF60A degrading proteins in *Arabidopsis thaliana*.

Taken together, those results show that BAF60A and Brg1 protein levels are regulated by alterations in miR-7a expression Min6 cells.

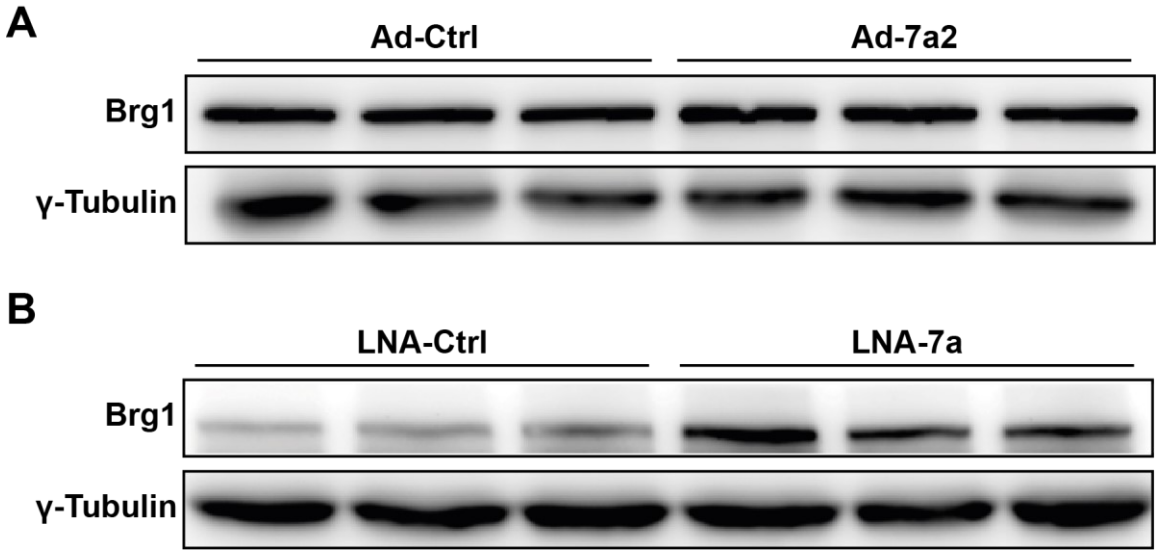

Figure 4.5: Brg1 protein levels in Min6 cells with alternated miR-7a expression. (A) Western Blot of Min6 cells infected with Ad-7a2/Ad-Ctrl and stained for Brg1 and γ-Tubulin. (B) Western Blot of Min6 cells transfected with LNA-7a/LNA-Ctrl and stained for Brg1 and γ-Tubulin.

4.3.2 Effects of miR-7a2 overexpression on BAF60A and Brg1 protein levels in dissociated pancreatic islets

After establishing the regulation of BAF60A and Brg1 by miR-7a at the mRNA and protein levels in immortalised β-cell lines, we next aimed to confirm our findings in the islets of mice. Therefore, islets were isolated from Wt animals at 12 weeks of age, dissociated and plated before being infected with Ad-7a2 or Ad-Ctrl. When infected with Ad-7a2, a downregulation of protein levels for BAF60A (Fig. 4.6A) and Brg1 (Fig. 4.6B) were observed when compared to Ad-Ctrl samples. γ-tubulin was used as a loading control (Fig. 4.6). This shows, that miR-7a2 overexpression is sufficient to inhibit BAF60A and Brg1 protein translation in isolated islets of mice.

While islets of mice provide a promising insight into the effects of miR-7a2 on the expression levels of BAF60A and Brg1, we next sought to establish the relevance of BAF60A/Brg1 regulation by miR-7a2 in human islets (Fig. 4.7). For this, we dissociated and infected human

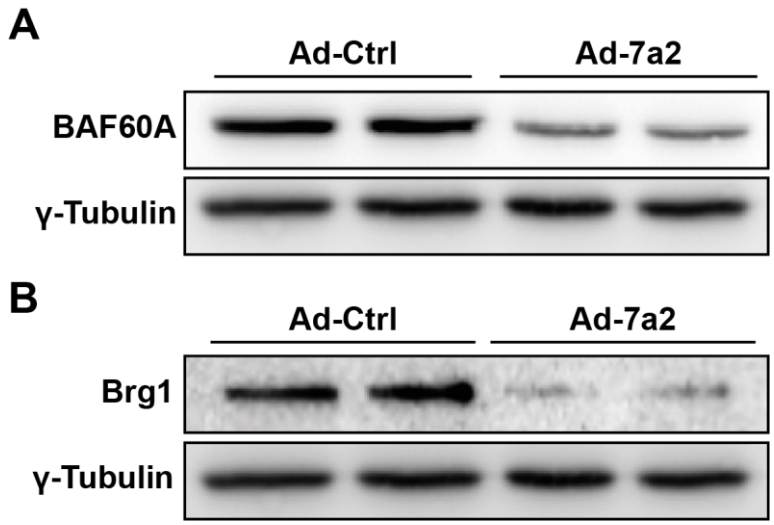

Figure 4.6: BAF60A/Brg1 protein levels in 12w Wt mouse pancreatic islets overexpressing miR-7a2. (A) Western Blot of 12w Wt mouse islets infected with Ad-7a2/Ad-Ctrl and stained for BAF60A and γ-Tubulin. (B) Western Blot of 12w Wt mouse islets infected with Ad-7a2/Ad-Ctrl and stained for Brg1 and γ-Tubulin. n = 2 animals.

islets kindly provided by Prof. G. A. Rutter (Fig. 4.7). Dissociated human islets infected with Ad-7a2 presented a downregulation of BAF60A protein levels in comparison to Ad-Ctrl infected samples (Fig. 4.7A).

Similar to the findings for BAF60A, our results showed a decrease of Brg1 protein levels in Ad-7a2 infected human islets in comparison to Ad-Ctrl samples (Fig. 4.7B). The cytoarchitecture of islets, which consist of multiple types of endocrines cells, prevents cellular resolution of protein levels in β-cells in western blotting. We therefore used immunofluorescence microscopy to assess how overexpression of miR-7a2 impacts on Brg1 protein levels in dissociated human islets (Fig. 4.7C). The quantification of GFP+ (infected with Ad-7a2/Ac-Ctrl) / insulin positive (Ins+) cells (β-cells) revealed a notable downregulation of insulin intensity ($p=0.055$) (Fig. 4.7D) and a significant downregulation of Brg1 intensity ($p\leq0.0001$) (Fig. 4.7E) in Ad-7a2 infected samples in comparison to Ad-Ctrl samples. Together, these results show, that the regulation of BAF60A and Brg1 protein levels by miR-7a2 is conserved in humans.

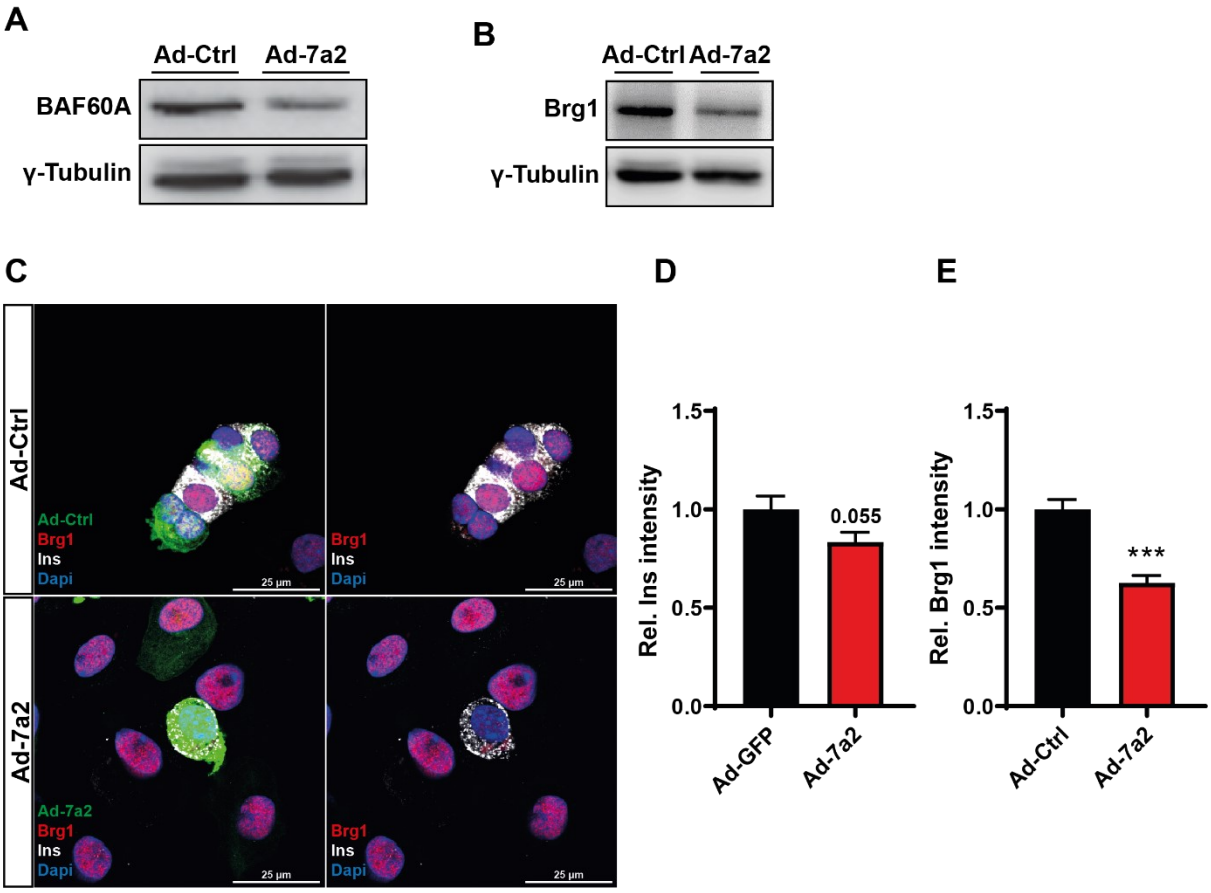

Figure 4.7: BAF60A/Brg1 protein levels in human pancreatic islets overexpressing miR-7a2. (A) Western Blot of dissociated human islets infected with Ad-7a2/Ad-Ctrl and stained for BAF60A and γ-Tubulin. (B) Western Blot of dissociated human islets infected with Ad-7a2/Ad-Ctrl and stained for Brg1 and γ-Tubulin. (C) Representative microscopy images of dissociated human islets infected with Ad-7a2/Ad-Ctrl and stained for Brg1 and Ins. (D) Quantification of the relative Ins intensity in GFP+/Ins+ cells of Ad-7a2 infected samples compared to Ad-Ctrl samples represented in (C). (E) Quantification of the relative Brg1 intensity in GFP+/Ins+ cells of Ad-7a2 infected samples compared to Ad-Ctrl samples represented in (C). (D) n = 129 (Ad-Ctrl), n = 268 (Ad-7a2); (E) n = 56 (Ad-Ctrl), n = 84 (Ad-7a2); n = number of GFP+/Ins+ cells. Data are means ± SEM, ***p < 0.001, unpaired Student's t-test.

4.3.3 mRNA and protein levels of BAF60A and Brg1 in mouse models of diabetes

Following up on the findings of miR-7a2 overexpression *in vitro* in immortalised β-cells as well as in dissociated mouse and human islets, our interest was subsequently focussed on the regulation of BAF60A and Brg1 *in vivo* in the diabetic mouse models Tg7a2 and *db/db* (Hummel et al. 1966). Based on our RNA-Seq data, we predicted that islets of Tg7a2 would show reduced levels of mRNA and protein of BAF60A and Brg1. We previously showed that miR-7a is also induced in islets from diabetic *db/db* mice (Latreille et al. 2014).We then used

this diabetic model to measure the expression of BAF60A and Brg1. We performed RT-qPCR to address BAF60A and Brg1 mRNA levels and utilised immunofluorescence microscopy to investigate BAF60A and Brg1 protein levels inside islets.

Based on our RNA-Seq data, 12-well-old Tg7a2 animals exhibited no significant changes in BAF60A mRNA levels in comparison to age matched Wt controls. We confirmed those findings in RT-qPCR, showing no significant difference in BAF60A mRNA levels (Fig. 4.8A). For Brg1 we detected a notable decrease in its mRNA levels ($p=0.1052$) in Tg7a2 mice compared to age matched Wt controls (Fig. 4.8A). In order to investigate BAF60A and Brg1 protein levels in Tg7a2 animals and age matched Wt controls at 2 weeks and 12 weeks of age, the animals were stained for Ins and BAF60A (Fig. 4.8B) or Brg1 (Fig. 4.8D). Quantification of the relative BAF60A intensity in β-cells revealed no significant differences between Tg7a2 and Wt controls at 2 weeks and 12 weeks of age (Fig. 4.8C). Of note, we observed a significant increase in BAF60A intensity ($padj=0.0215$) in 12w Wt animals compared to 2w Wt animals (Fig. 4.8C), indicating that upregulation of BAF60A is involved in the postnatal maturation of β-cells. Finally, Brg1 intensity was notably reduced ($padj=0.0599$) in 2w Tg7a2 animals compared to age matched Wt controls (Fig. 4.8E). However, we measured a significant reduction in Brg1 intensity ($padj=0.0035$) in 12w Tg7a2 mice compared to age matched Wt controls (Fig. 4.8E). No significant change in Brg1 intensity was observed between 2 weeks and 12 weeks in Tg7a2 or Wt animals (Fig. 4.8E). Together these results indicate that Brg1, but not BAF60A protein levels are regulated by miR-7a2 *in vivo*. Additionally, our results suggest that BAF60A, not Brg1, upregulation is induced in postnatal β-cell maturation.

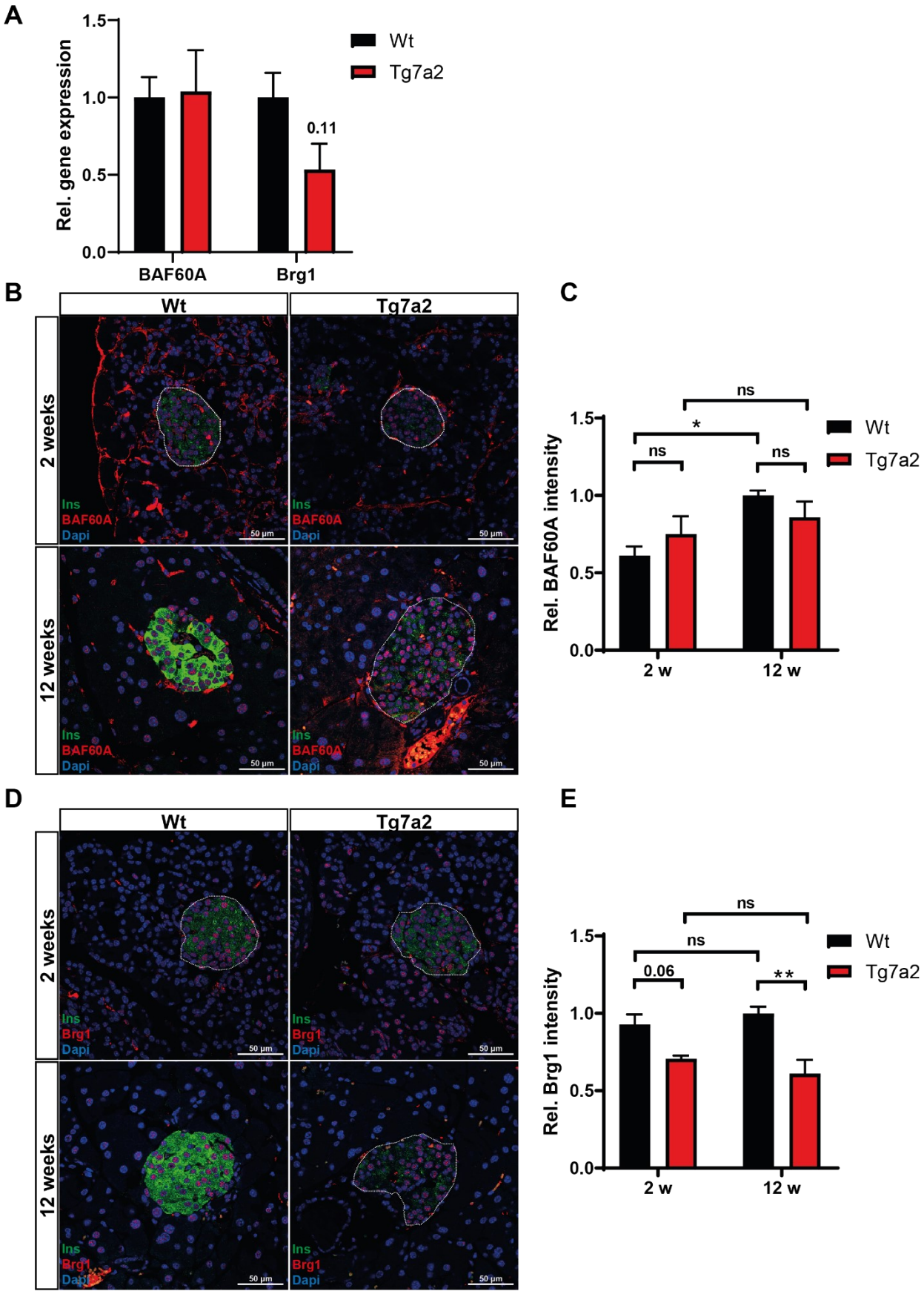

Figure 4.8: BAF60A/Brg1 levels in the diabetic Tg7a2 mouse model. (A) qPCR results of *BAF60A* and *Brg1* mRNA expression levels in 12w Tg7a2 mice compared to age matched Wt controls, normalised to *RPLP0/36B4*. (B)

Representative microscopy images of 2w and 12w Wt and Tg7a2 mice stained for BAF60A and Ins. (C) Quantification of the relative BAF60A intensity in 2w and 12w Tg7a2 and their age matched Wt controls represented in (B). (D) Representative microscopy images of 2w and 12w Wt and Tg7a2 mice stained for Brg1 and Ins. (E) Quantification of the relative Brg1 intensity in 2w and 12w Tg7a2 and their age matched Wt controls represented in (D). (A) n = 5 (Wt), n = 3 (Tg7a2), unpaired Student's t-test; (C,E) n = 3, Two-way ANOVA. (A,C,E) n = number of animals. Data are means ± SEM, *p < 0.05, **p < 0.01.

We subsequently quantified the expression of *BAF60A* and *BRG1* in *db/db* mice using RT-qPCR (Fig. 4.9). Our RT-qPCR data showed a significant downregulation of Brg1 (p=0.0374) and a notable reduction of BAF60A (p=0.0627) mRNA levels in *db/db* animals compared to *db/+* controls (Fig. 4.9). Taken together, our results show that unlike BAF60a, Brg1 expression is regulated on a mRNA level in both Tg7a2 and *db/db* mice.

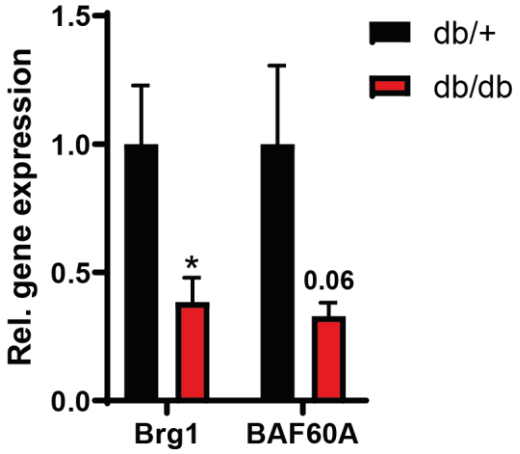

Figure 4.9: BAF60A/Brg1 mRNA levels in the diabetic mouse model *db/db*. qPCR results of BAF60A and Brg1 mRNA levels in ~12w *db/db* mice compared to age matched *db/+* controls, normalised to *RPLP0/36B4*. (A) n = 5; n = number of animals. Data are means ± SEM, *p < 0.05, unpaired Student's t-test.

4.3.4 Luciferase Assay of Brg1 3'UTR

We next sought to investigate if the regulation of Brg1 by miR-7a is mediated through its 3'UTR. To demonstrate this, we transfected HEK293T cells with pmirGLO plasmid containing the 3'UTR of Brg1 cloned downstream of the Firefly luciferase gene together with miR-7a, miR-7b, miR-223 and a non-targeting scramble control miRNA (miR-NT) (Fig. 4.10). In accordance with our data in Min6 and islets we expected miR-7 to reduce luciferase activity in comparison to miR-223 and miR-NT controls.

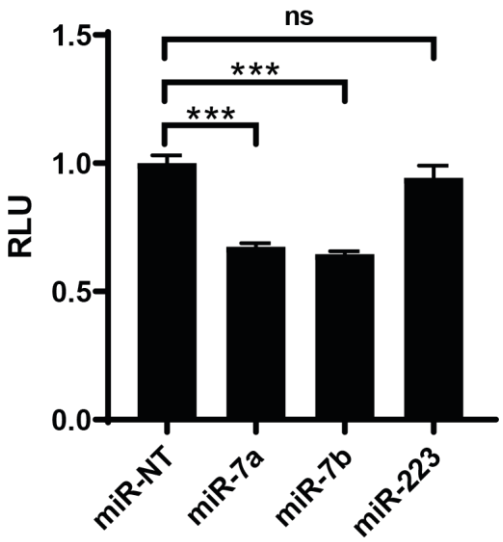

Figure 4.10: Luciferase assay of the 3'UTR of Brg1 with miR-7. Relative Luciferase activity, as measured by relative light units (RLU) of a pmirGLO plasmid containing with the 3'UTR of Brg1 cloned downstream of the Firefly gene, co-transfected with miR-NT control, miR-7a, miR-7b and miR-223 mimics inside HEK293T cells. n = 3. Data are means ± SEM, ***p < 0.001, One-way ANOVA.

Analysis of the luciferase assay revealed that there was a significant downregulation of Luciferase activity in samples transfected with miR-7a (padj=0.0002) and miR-7b (padj≤0.0001) compared to miR-NT controls (Fig. 4.10). However, transfection with miR-223 had no significant differences in Luciferase activity when compared to miR-NT (padj=0.4335) (Fig. 4.10). In summary, the Luciferase assay proved that miR-7a regulates Brg1 mRNA levels through its 3'UTR. Additionally, it confirms the functional redundancy between miR-7a and miR-7b, as both mimics regulated the activity of the luciferase reporter similarly. To show, that the 3'UTR of Brg1 is a direct target of miR-7a, additional methods should be done. One way is the mutation of the putative miR-7a binding site in the 3'UTR of Brg1, followed by a luciferase assay. Another method would be the crosslinking of RNA to RNA associated proteins by ultraviolet irradiation followed by immunoprecipitation of miRNA and deep sequencing to identify bound mRNAs, known as high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP), or crosslinking immunoprecipitation (CLIP)-seq (Chi et al. 2009, Zisoulis et al. 2010, Thomson, Bracken and Goodall 2011).

4.3.5 BAF60A/Brg1 distribution in the pancreas

In addition to the regulation of BAF60A and Brg1 mRNA and protein levels by miR-7a, we were also interested in determining the distribution of the mSWI/SNF complex subunits in the

exocrine pancreas, consisting of acinar cells surrounding the islets, and endocrine islets. Therefore, we investigated BAF60A and Brg1 using immunofluorescence on 12w mouse pancreatic sections (Fig. 4.11A,C). Quantification of the relative BAF60A intensity via CellProfiler showed a significant increase in the measured BAF60A intensity within the nuclei of islet endocrine cells in comparison to the surrounding exocrine cells (p=0.0007; Fig. 4.11B). As with the findings for BAF60A, quantification of the relative Brg1 intensity demonstrated a significant enrichment in the endocrine cells in comparison to the exocrine portion of the pancreas (p=0.0004; Fig. 4.11D).

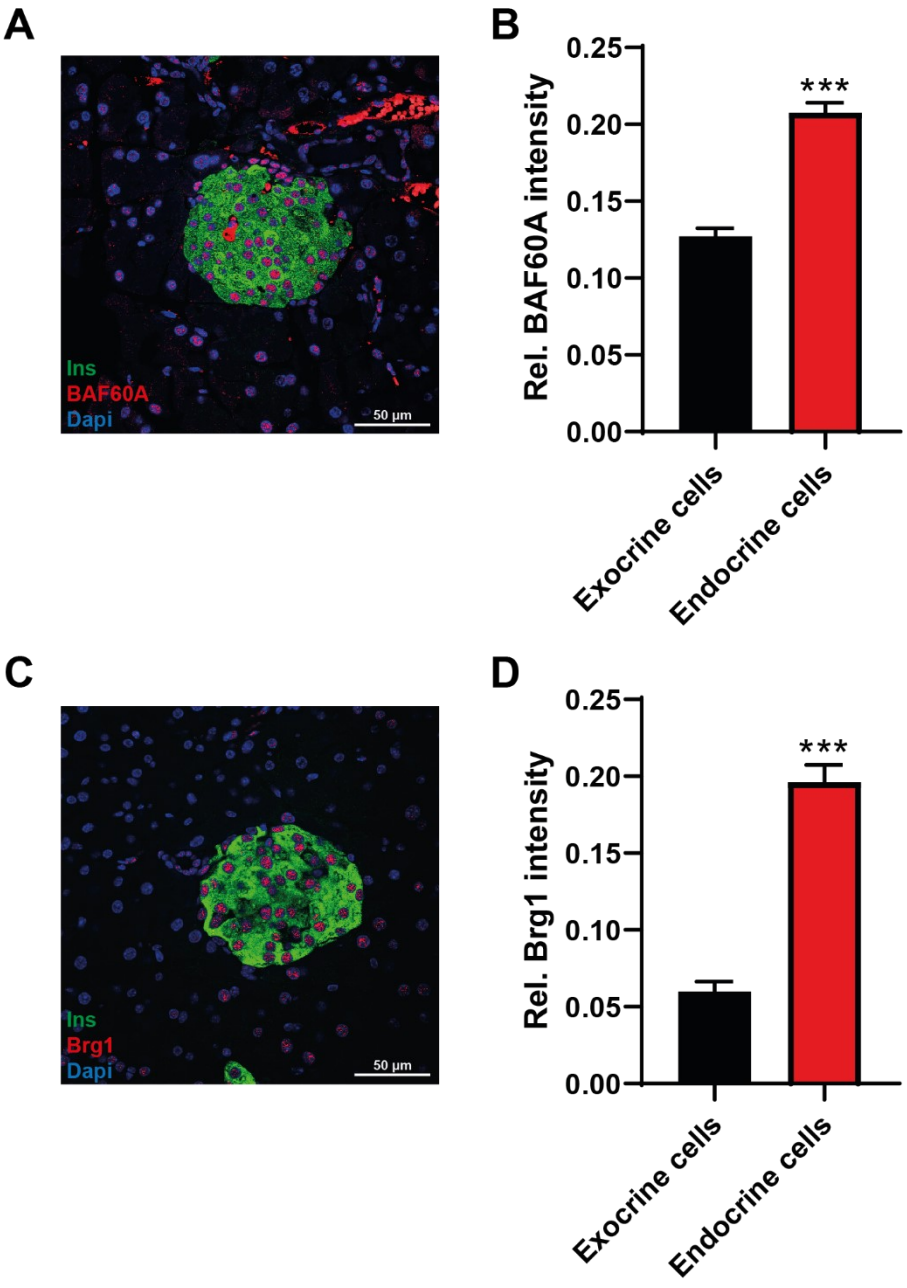

Figure 4.11: Distribution of BAF60A/Brg1 inside the pancreas. (A) Representative microscopy images of Wt mouse islets stained for BAF60A and Ins. (B) Quantification of the relative BAF60A intensity in exocrine and endocrine cells of the pancreas represented in (A). (C) Representative microscopy images of Wt mouse islets stained for Brg1 and Ins. (D) Quantification of the relative Brg1 intensity in exocrine and endocrine cells of the pancreas represented in (C). n = 3; n = number of animals. Data are means ± SEM, ***p < 0.001, unpaired Student's t-test.

Additionally, whole mouse islets were dissociated and stained for BAF60A, as well as the markers of β-, δ-, α- and γ-cells, namely Ins, Sst, Gcg and Ppy, respectively (Fig. 4.12A). Quantification of the microscopy images revealed that BAF60A is expressed in β-, δ-, α- and γ-cells (Fig. 4.12B). We confirmed our results in dissociated human islets (Fig. 4.12C). Quantification of the stained human pancreatic endocrine cells revealed a similar proportion of BAF60A positive cells in the different endocrine cells in human (Fig. 4.12D) when compared with mouse cells (Fig. 4.12B). Furthermore, RT-qPCR was performed on sorted islet cells from tdTomato$^{RIP\text{-}Cre}$ mice. The results indicated that the mRNA levels of Brm, Brg1 and BAF60A was similar in non-β-cells versus tdTomato-labelled β-cells, indicating that *BRM*, *BRG1* and *BAF60A* are evenly expressed in β- and non-β-cells (Fig. 4.12E). Of note, those results allow no comparison of *BRM*, *BRG1* and *BAF60A* expression levels in between the non-β-cells.

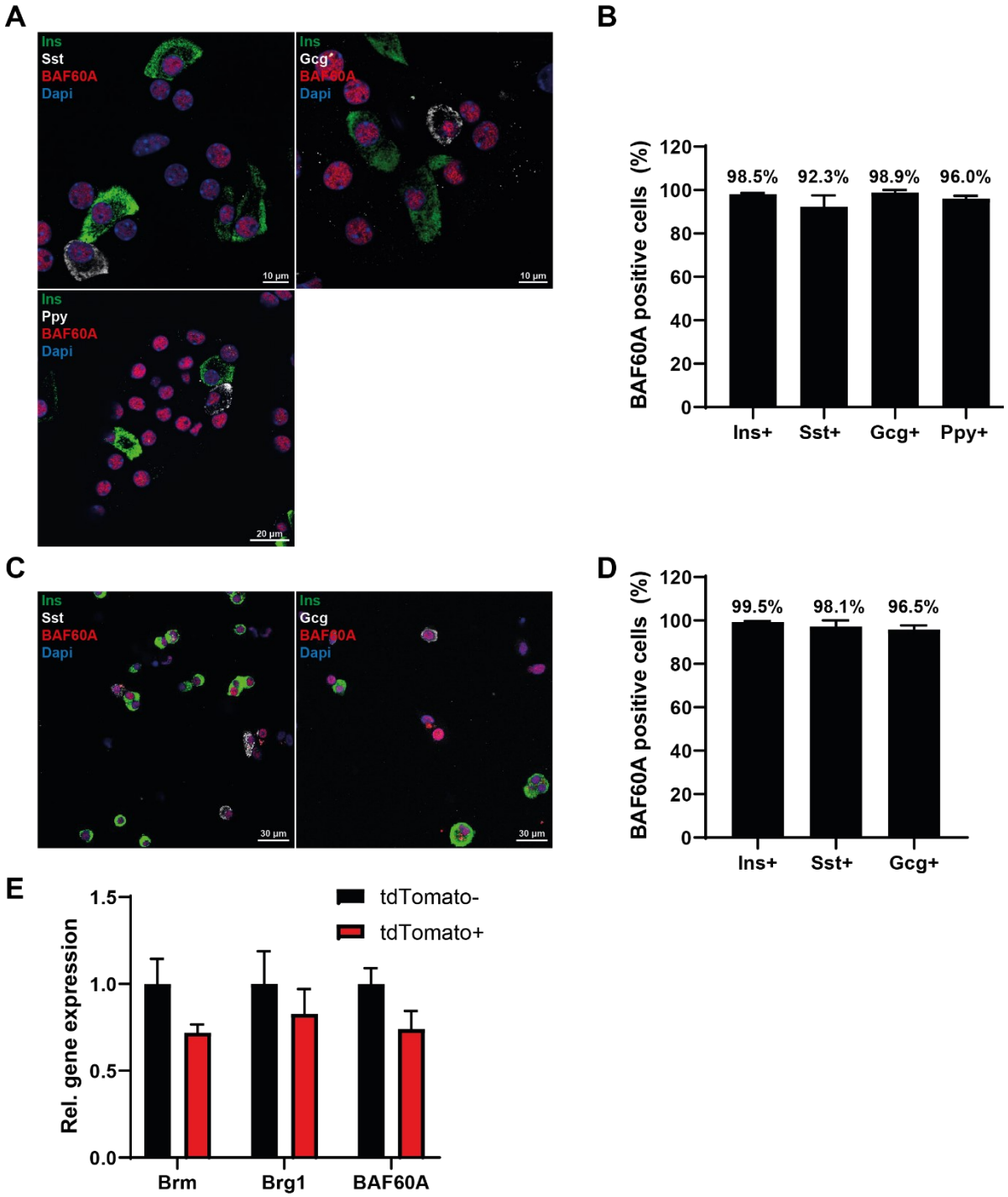

Figure 4.12: Distribution of mSWI/SNF subunits inside the pancreatic islets. (A) Representative microscopy images of dissociated mouse islets stained for BAF60A and Ins, Sst (top left), Gcg (top right) and Ppy (bottom left). (B) Quantification of the number of BAF60A positive cells in the Ins+, Sst+, Gcg+ and Ppy+ cell populations represented in (A). (C) Representative microscopy images of dissociated human islets stained for BAF60A and Ins, SST and Gcg. (D) Quantification of the number of BAF60A positive cells in the Ins+,Sst+ and Gcg+ cell populations represented in (C). (E) qPCR results of *Brm*, *Brg1* and *BAF60A* mRNA expression levels in the RFP+ and RFP- cell populations of FACS sorted islets from tdTomato$^{RIP\text{-}Cre}$ mice. (B,D) n = 1 ((B) Ins = 975 cells, SST = 18 cells, Gcg = 61 cells, Ppy = 171 cells; (D) Ins = 349 cells, SST = 51 cells, Gcg = 142 cells); (E) n = 7 (RFP-), n = 5 (RFP+), n = number of animals / human donors. Data are means ± SEM, unpaired Student's t-test.

4.4 ATAC-Seq in islets of Tg7a2 mice

After identifying Brg1 and BAF60A subunits as targets of miR-7a in β-cells, we next sought to investigate whether this triggers changes in the chromatin state in islets of Tg7a2 animals. ATAC-Seq was performed in islet isolated from 2w non diabetic and 12w diabetic Tg7a2 and Wt controls (Latreille et al. 2014). ATAC-Seq measures the accessibility of chromatin regions genome-wide, through a hyperactive Tn5 transposase which integrates sequencing adapters into accessible regions in the chromatin. DNA sequencing then allows to infer for regions that are more accessible, as well as map regions of nucleosome position and transcription factor binding (Buenrostro et al. 2015a). Chromatin regions with increased accessibility are here referred to as "open", whereas chromatin regions with lowered accessibility are referred to as "closed" throughout this thesis.

Principal Component Analysis (PCA) of our ATAC-Seq data showed that Tg7a2 and Wt islets demonstrate a higher variance from each other at 12 weeks compared to 2 weeks of age (Fig. 4.13). Furthermore, the PCA plot illustrates, that Tg7a2 animals at 2 weeks are less variant to Tg7a2 at 12 weeks, than 2w Wt animals to 12w Wt animals. Overall, the clustering of the four sample groups into four different clusters inside the PCA plot supported the continued analysis of the ATAC-Seq of Tg7a2 compared to Wt animals at 2 and 12 weeks of age.

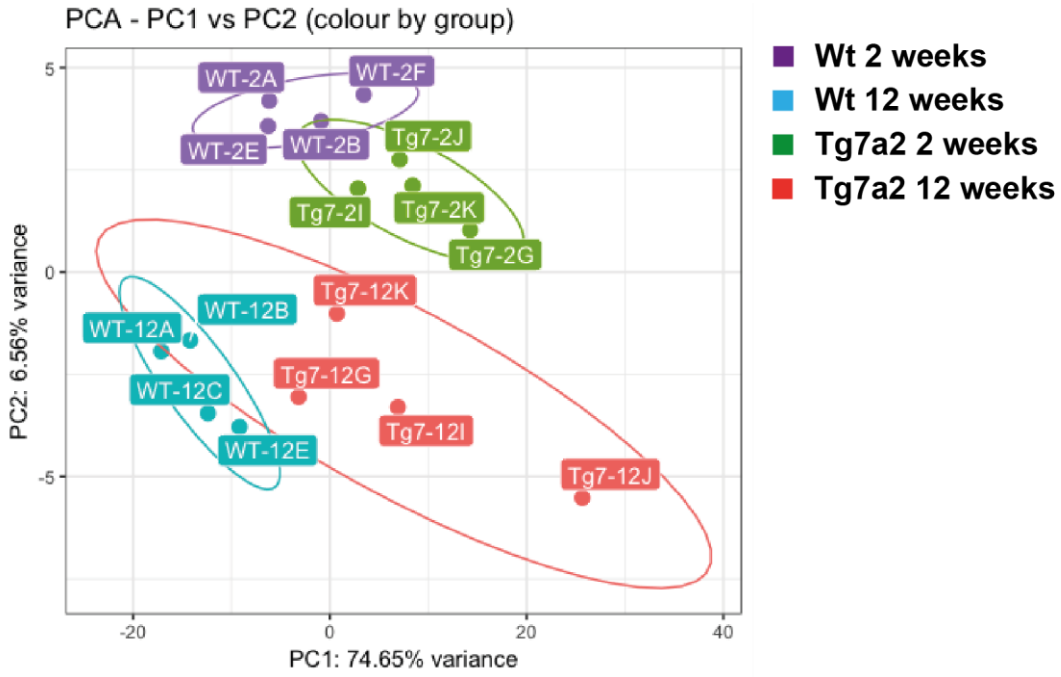

Figure 4.13: Principal Component Analysis (PCA) plot of the ATAC-Seq in Tg7a2. Relatedness among the Tg7a2 and Wt mouse samples at 2 and 12 weeks of age. n = 4.

4.4.1 Chromatin state inside pancreatic islets of Tg7a2 animals

In order to continue, the genome-wide differences in accessible chromatin regions detected by ATAC-Seq in Tg7a2 animals compared to Wt animals required further investigation. Dr. Yi-Fang Wang from the LMS bioinformatic facility contributed at providing us with a list of differentially open chromatin segments in Tg7a2 islets. Analysis of the chromatin regions amplified was done by read alignment to the reference genome through a so-called seed-and-vote method. Read alignment is the alignment of read sequences, obtained from the sequencing experiment to a common reference genome, in our case the mm9 mouse genome. In seed-and-vote, small regions of the reads, called seeds or subreads, are used to map their respective read to chromatin regions and alignment algorithms are then used to fill detailed mismatches or indel information (Liao et al. 2013b). The featureCounts program then allowed us to summarise those reads aligning to the same regions on the reference genome to generate peaks of accessible chromatin regions (Liao et al. 2013a). When the number of reads summarised to peaks is higher in Tg7a2 animals compared to Wt controls the chromatin is in a more accessible or open state in Tg7a2 vs Wt controls. On the other hand, when the number of reads summarised to peaks is higher in Wt controls compared to Tg7a2 animals the chromatin is in a less accessible or close state in Tg7a2 vs Wt controls. The DeSeq2 was then used to find statistically significant differences in the number of reads between Tg7a2 and Wt controls. DeSeq2 tests whether the null hypothesis, meaning whether the logarithmic fold change between two groups is zero, is true or not. DeSeq2 is a generalised linear model with three parameters: 1) normalisation parameter, 2) variance parameter and 3) group differences parameter that need to be fitted and is based on a count matrix with a row for each gene (as summarised reads by the featureCounts program are allocated to certain gene regions) and a column for each sample. The 1) normalisation parameter accounts for differences in library size which can be a result of sequencing of the samples to different depths. In the 2) variance parameter, which describes the dispersion of a gene in a group of samples, a likelihood estimation is performed which finds the value that makes the likelihood the largest and is the middle value of all the values of a certain genes. Likelihood describes how well a set of observations fits a statistical model. Through Bayes theorem the values are then moved to that middle value depending on the amount of information available for that gene. Low information for the gene means that the value is moved more towards the middle and high information means little movement towards the middle. Bayes theorem describes the probability of an event. The fit of the 3) group differences parameter is the same as for the 2) variance parameter

but between the different groups rather than within a group (Anders and Huber 2010, Love, Huber and Anders 2014b).

The cut-off point for a significant change in the accessibility of chromatin regions was at an adjusted p-value of 0.05. Interestingly, the total number of differential accessible regions (DARs) increased from 539 to 9840 in 2w and 12w animals in Tg7a2 compared to Wt islets (Fig. 4.14A). Those results show that with progressing age of the Tg7a2 animals the number of DARs in comparison to Wt controls increases. Assessment of the data revealed that DARs in Tg7a2 islets are to a higher proportion in a closed state than an opened state compared to Wt islets at 2 and 12 weeks of age. At 2w, 450 of the total 539 DARs and at 12w 6432 out of 9840 DARs are in a closed state in Tg7a2 compared to Wt islets (Fig. 4.14A). We next looked into β-cell enriched genes involved in maintaining the mature β-cell. We investigated the chromatin state of markers of pancreatic β-cell identity utilising the Integrative Genomics Viewer (IGV), significant regions are marked with a green border (Fig. 4.14B-F). Our results showed a significant difference in chromatin accessibility for regions mapping for Syt4 at 2 and 12 weeks of age in favour of a more closed state in Tg7a2 animals compared to Wt animals (2w padj=0.0069; 12w padj=0.0209 (Fig. 4.14B and Table 4.2). For Glut2 and urocortin 3 (Ucn3) chromatin regions similar observations were made, where a significant difference in favour of a closed chromatin state in Tg7a2 animals at 12 weeks of age was observed (Glut2: padj=0.0050; Ucn3: padj=0.0053) (Fig. 4.14C,D and Table 4.2). At 2 weeks, no significant DARs for Glut2 and Ucn3 were observed (Fig. 4.14C,D and Table 4.2). While the statistics do not show any significant differences for Glut2 and Ucn3 in 2 weeks old mice, the visualisation of the chromatin accessibility by IGV indicated a similar regulation as at 12 weeks of age (Fig. 4.14C,D). Interestingly, our results showed no significant differences for the chromatin regions of the two mouse insulin genes Ins1 and Ins2 (Fig. 4.14E,F). However, similar to the findings for Glut2 and Ucn3, the differences in chromatin accessibility visualised by IGV, indicated that the chromatin structure for the Ins1 and Ins2 chromatin regions is in a more closed state in Tg7a2 animals compared to Wt animals at 2 and 12 weeks of age.

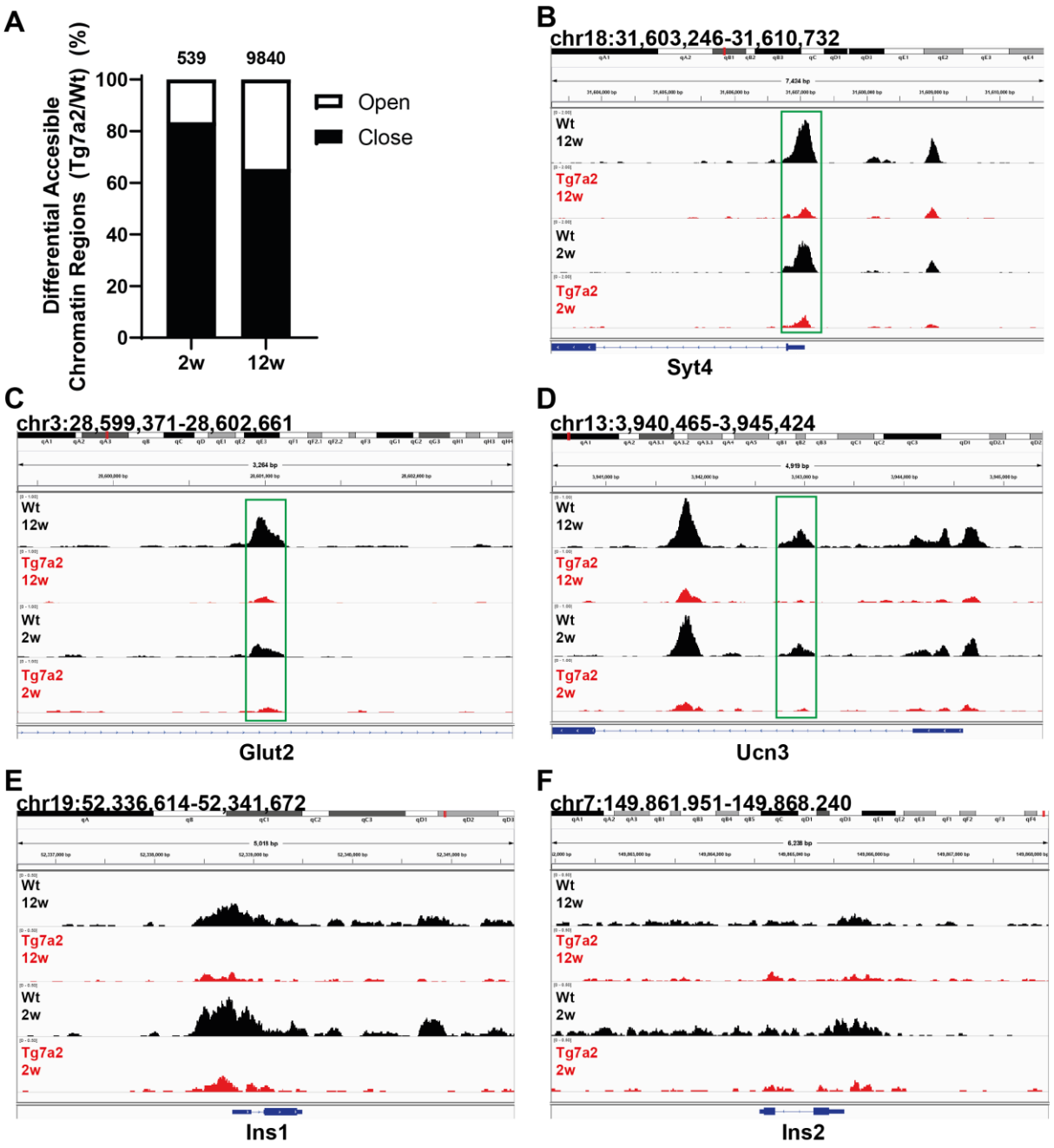

Figure 4.14: Differential accessible chromatin regions (DARs) in Tg7a2 animals. (A) Percentage of open and closed and total number of DARs in Tg7a2 islets compared to Wt controls at 2 and 12 weeks of age. (B-F) Visualisation of Syt4 (B), Glut2 (C), Ucn3 (D), Ins1 (E) and Ins2 (F) chromatin accessibility in IGV of Tg7a2 islets compared to Wt controls at 2 and 12 weeks of age. Significant regions are marked with a green border. n = 4, Data processed in IGV.

Table 4.2: Differential Accessible Region data of genes presented in Figure 4.14. Focus on genes involved in β-cell identity, n = 4. Aligned to mm9 via bowtie 2.

Gene	id4IGV	log_2 fold	Adjusted p-value	Age	Region
Syt4	chr18:31606762-31607217	-1.30447753	0.006921184	2w	Promoter (<=1kb)
Syt4	chr18:31636274-31636394	-2.22911852	0.020944873	12w	Distal Intergenic
Glut2	chr3:28600912-28601120	-1.815063936	0.005035982	12w	Intron
Ucn3	chr13:3942877-3943012	-2.552950183	0.005309078	12w	Promoter (1-2kb)

Our bioinformatician then utilised the Genomic Regions Enrichment of Annotations Tool (GREAT) binomial approach (McLean et al. 2010) to identify potential pathways affected in islets ATAC-Seq from 12w Tg7a2 compared to Wt islets. GREAT associates genomic regions, in our case the reads from our ATAC-Seq, with specific genes through the definition of a regulatory domain for each gene in the genome. The regulatory domain for each gene consists of a basal domain that reaches 5 kb up- and 1 kb downstream from its respective transcription start site and extends to the basal regulatory regions of the next gene within 1 Mb up- and downstream. With an ontology of gene annotations, GREAT then calculates ontology term enrichments using a binomial test. The binomial test measures the fraction of the genome that falls under any given ontology term and counts how many of the input reads align to these fractions, while taking the total number of genomic regions into account (McLean et al. 2010).

Those results allow the identification of novel, yet unravelled genes potentially contributing to T2D and being regulated by miR-7a2. Analysed pathways included: “GO Biological Processes”, “GO Cellular Components”, “Disease Ontology” and “MSigDB Pathway” (Table 4.3).

Closed DARs were located in genes associated with "*diabetes mellitus type 1*" (padj=0.0033), in the "**Disease Ontology**" pathway, including Glucose-6-phosphatase 2 (*G6PC2*). While G6PC2 has been reported to be a negative regulator of glucose stimulated insulin secretion, other studies show, that genetic variances of *G6PC2* resulted in decreased first phase insulin secretion and are associated with risk for T2D (Pound et al. 2013, Heni et al. 2010, Hu et al. 2009). Other genes involved were the IL-1 receptor type 1 (*Il-1R1*) and *NEUROD1*. *Il-1* signalling has been shown to be lost in the islets of obese T2D patients and *Il-1R1* knockout mice become glucose intolerant (Hajmrle et al. 2016). *NEUROD1* is a known MODY gene (Horikawa et al. 2018).

Another hit was "*Genes involved in Peptide hormone biosynthesis*" (padj=0.0010) in the "**MSigDB Pathway**", including the gene *PCSK1*, which encodes for the prohormone convertase 1/3. Prohormone convertase 1/3 which cleaves proinsulin. Downregulation of *PCSK1* in mouse models and patients resulted in increased circulating proinsulin, a reduction in their glucose clearing ability and early onset of obesity (O'Rahilly et al. 1995, Zhu et al. 2002, Farooqi et al. 2007, Liu et al. 2007). Other genes involved in this pathway were *INHBA*, that has been reported to be overexpressed in pancreatic cancer (Kleeff et al. 1998) and *INHBB*, which promotes β-cell proliferation and is induced by Pdx-1 (Hayes et al. 2016).

The hit for "*transport vesicle membrane"* (padj=0.0002) and "*exocytic vesicle"* (padj=0.0095) in "**GO Cellular Components**" included genes involved in the insulin containing secretory vesicles, like *SNCA* and *VAMP2*. VAMP2 and SNCA are known to be part of the SNARE complex involved in fusion of insulin containing secretory vesicles with the plasma membrane of β-cells (Latreille et al. 2014, Jahn and Scheller 2006). In previous studies we reported the downregulation of SNCA by miR-7a through SNCA's 3'UTR (Latreille et al. 2014). Our results suggest, that SNCA is also regulated on a chromatin level.

In open DARs we found hits for "*DNA methylation*" (padj=0.049) in "**GO Biological Processes**", including the gene CCCTC-binding factor (*CTCF*), which has been reported to regulate the expression of Pax6 in a negative manner. *CTCF* overexpression affects β-cell survival and proliferation (Tsui, Dai and Lu 2014). Other genes included *DNMT3A*, Guanine Nucleotide Binding Protein (G Protein), Alpha Stimulating Activity Polypeptide 1 (*GNAS*) and grainyhead like 2 (*GRHL2*). While increases in *DNMT3A* expression correlate with adipose insulin resistance (You et al. 2017) and *GNAS* expression is upregulated in diabetic cells in T2D (Segerstolpe et al. 2016, Wang et al. 2016, Xin et al. 2016), *GRHL2*, a transcription factor

involved in hearing loss, has yet no relation to diabetes and could present a novel factor in T2D (Van Laer et al. 2007).

Other hits of open DARs in the "**MSigDB Pathway**" was the "*Insulin signalling pathway*" (padj=0.0024), that included genes like Tuberous sclerosis complex 1 (*TSC1*), a tumour suppressor, shown to inhibit β-cell proliferation in Pdx-1 deficient diabetes models (Sun et al. 2018), whose knockout results in improved glycaemic control as well as insulin production (Mori and Guan 2012). Other genes included exocyst complex component 7 (*EXOC7*), which is reported to be involved in the insulin response, but not documented in relation to β-cells in T2D (Wang et al. 2019b, Hansen et al. 2015) and Inositol Polyphosphate-5-Phosphatase K (*INPP5K*). While there is no direct relation of *INPP5K* and T2D, *INPP5K* has been reported to be an glycogen binding protein in β-cells and it is hypothesised that increases in glycogen content improves β-cell function (Nagy et al. 2018).

We further found a hit for "*FoxO family signalling*" (padj=0.0201) in open DARs. This pathway included genes like F-Box Protein 32 (*FBXO32*). *FBXO32* is regulated by FoxO and, while mainly associated to muscle atrophy in diabetes, has been reported to show increased expression in islets of Islet-1 knockout diabetic mice (O'Neill et al. 2019, Ediger et al. 2014). Another gene was *EP300*. Knockout of *EP300* in mice resulted in impaired β-cell proliferation and glucose tolerance (Qiu et al. 2004, Wong et al. 2018). The increased accessibility of chromatin regions associated to *EP300* in Tg7a2 mice could be a potential compensatory mechanism.

Also, in open DARs was a significant hit for "*Genes involved in Regulation of Glucokinase by Glucokinase Regulatory Protein*" (padj=0.0415)". One of the genes was retinoic acid early transcript 1 (*RAE1*), a ligand of NKG2D on cytotoxic T lymphocytes. Overexpression of *RAE1* has been shown to be sufficient for the recruitment of cytotoxic T lymphocytes into the islets, as seen in the pathogenesis of T1D (Ogasawara et al. 2004, Markiewicz et al. 2012). Other genes with increased accessibility were nuclear pore complex proteins like NUP50, NUP153 or NUP210, although no relation diabetes is currently known.

Together our results show that the function of the β-cell is impaired on a chromatin level at genes involved in glucose sensing, β-cell maturity, insulin expression and processing and secretion of insulin, but also unravel potential new genes involved in T2D and miR-7a2 regulation.

Table 4.3: Genomic Regions Enrichment of Annotations Tool (GREAT) analysis of ATAC-Seq differential accessible regions. Analysis of the pathways: "GO Biological Processes", "Disease Ontology", "MSigDB Pathway" and "GO Cellular Component", in the Close and Open differential accessible regions ATAC-Seq on 12w Tg7a2 compared to age matched Wt controls. Open refers to the genes of those pathways to map to chromatin regions with significantly increased accessibility, whereas Close refers to the genes of those pathways to map to chromatin regions with significantly decreased accessibility, analysed by Y. Wang, n = 4, GREAT binomial test.

GO Biological Processes: Open		
Pathway	**Fraction of Genome**	**Adjusted p value**
DNA methylation	0.001498732	0.04904896
Disease Ontology: Close		
Diabetes mellitus type 1	0.001053735	0.003303884
MSigDB Pathway: Close		
Genes involved in Peptide hormone biosynthesis	0.001553598	0.000953895
MSigDB Pathway: Open		
Insulin signalling pathway	0.01116009	0.002444824
FoxO family signalling	0.004775695	0.020105205
Genes involved in Regulation of Glucokinase by Glucokinase Regulatory Protein	0.001611978	0.041540491
GO Cellular Component: Close		
Transport vesicle membrane	0.004261	0.000192
Exocytic vesicle	0.00016466	0.00948494

4.4.2 Identification of TF motifs at closed and open DARs

Transcription factors serve an important role in the regulation of gene expression essential for determining cell identity in development (Lee and Young 2013). The mSWI/SNF complexes interact with various TFs, including Pdx-1, to translocate nucleosomes (Neely et al. 2002, McKenna et al. 2015b). Therefore, we determined the TF motifs located in the closed and open DARs in the ATAC-Seq dataset. Motif identification was performed by Yi-Fang Wang from the bioinformatic facility utilising the Hypergeometric Optimization of Motif EnRichment (HOMER) software. Due to the nature of the software, motifs were considered significant from a p-value of $p=1e^{-10}$. Identified motifs at 2 weeks of age in DAR are displayed (Fig. 4.15). At 2w, Tg7a2 animals overexpress miR-7a2 but do not show a hyperglycaemic phenotype. Therefore, TF binding is only affected by the overexpression of miR-7a2 and not impaired by glucotoxicity. Our results showed significance for the β-cell TFs Nkx6.1 and Pdx-1 ($p=1e^{-62}$) in 60.92% of the target sequences (Fig. 4.15A). Motifs matching the β-cell TFs Neurod1 ($p=1e^{-20}$, 25.00%) and Foxa1 / Foxa2 ($p=1e^{-15}$, 8.98%) were also identified (Fig. 4.15A). Interestingly, a match for MafB was observed ($p=1e^{-18}$, 22.82%) (Fig. 4.15A), a TF that is known to be involved in the development of β-cells, but which is exclusively found in glucagon producing α-cells in adult organisms. (Nishimura et al. 2006, Artner et al. 2006).

While the TF motifs found in closed DARs revealed multiple hits with high significance, our results analysing the open DARs revealed fewer binding motifs. At 2 weeks of age two different motifs were detected, a motif matching Zic3/Zic4 ($p=1e^{-10}$, 7.69%) and one matching Znf354C/Foxh1 ($p=1e^{-9}$, 12.82%) (Fig. 4.15B).

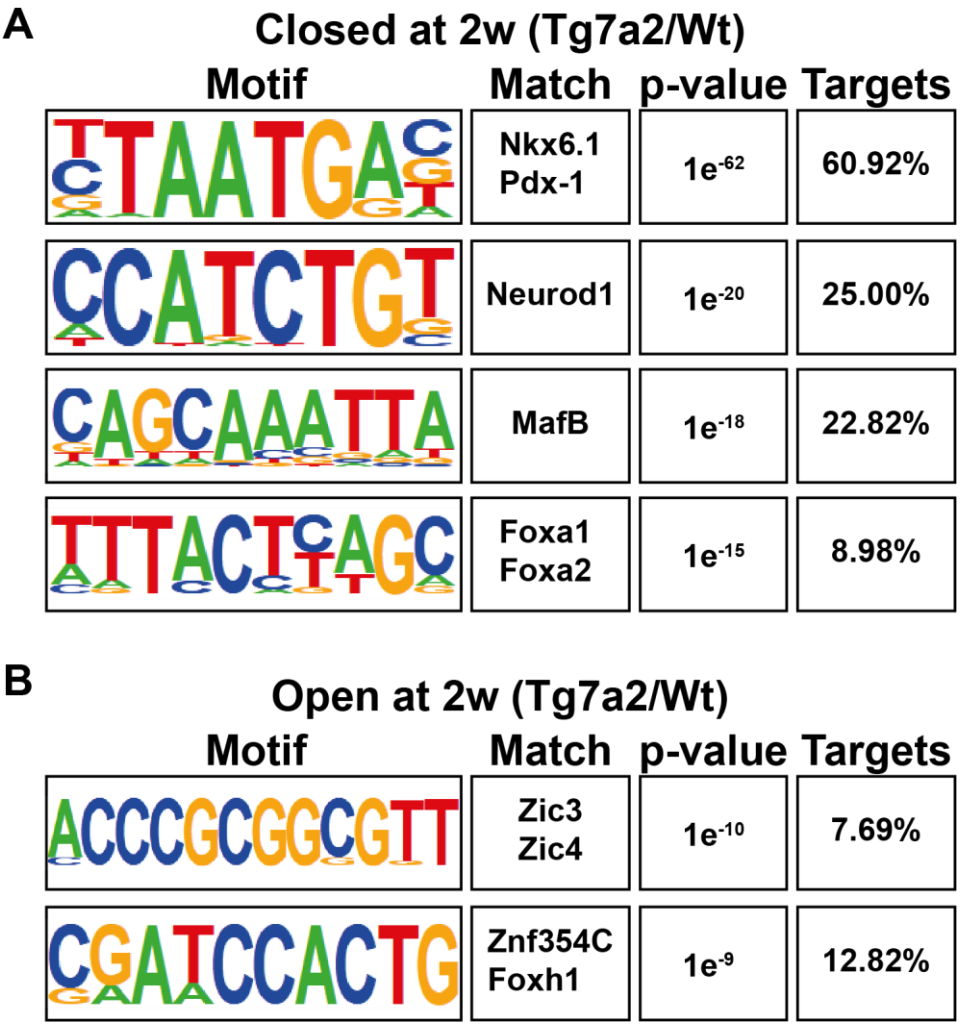

Figure 4.15: Transcription factor (TF) motif identification via HOMER in ATAC-Seq of Tg7a2. (A) Identified TF motifs in closed DARs of 2w Tg7a2 compared to age matched Wt controls selected for their involvement in β-cell identity. (B) Identified TF motifs in open DARs of 2w Tg7a2 compared to age matched Wt controls selected for their involvement in EMT. n = 4, HOMER, analysed by Y. Wang.

Overall, our data shows a bias towards the closed state in DARs and identified TF motifs, indicating that miR-7a2 overexpression, through the downregulation of Brg1 induces transcriptional repression through decreasing the accessibility of chromatin regions associated to genes involved in β-cell identity or transcription factors regulating those genes.

4.5 Discussion

Several studies indicated that the identity of cells is modulated by epigenetic factors impacting on the expression of lineage defining genes (Atlasi and Stunnenberg 2017, Bramswig et al. 2013, Xie et al. 2013b, Lu et al. 2018). In light of this, we investigated whether potential epigenetic regulators targeted and repressed by miR-7a2 may be responsible for β-cell dedifferentiation in T2D. A list of predicted miR-7 targets was generated following inspection of the TargetScan database and were compared with the RNA-Seq dataset obtained from the miR-7a2 overexpressing mice. At the beginning of my project, the majority of the candidate targets of miR-7a2 showed no links to diabetes according to the literature review. Since then, McKenna et al. demonstrated that Brg1 and Brm play important roles in controlling the expression of β-cell specific genes. Brg1 and Brm were shown to have opposing functions in β-cells (McKenna et al. 2015b). However, how the activity of both epigenetic regulators is regulated in β-cells remains to be determined. Here, we found that the expression of *BRG1*, *BAF60A* and *BRM* is regulated in Min6 cells where miR-7a2 expression was modulated. (Fig. 4.2 and 4.3), providing three regulated subunits in a single complex. While Brg1 (Fig. 4.16) and BAF60A mRNAs carry predicted miR-7a2 sites in their 3'UTR, none is found in that of Brm (Table 4.1). As TargetScan only predicts potential targets of selected miRNA those findings need to be assessed carefully. Indeed, our results suggest that Brm, while not carrying predicted miR-7a2 sites, is regulated by miR-7a2 in Min6 cells. Interestingly, similar to BAF60A, although it has a predicted miR-7a2 site, those results do not translate into islets of Tg7a2, as seen in our RNA-Seq. On the other hand, while Brg1 shows an upregulation on the protein level by LNA-7, no effect is seen on the gene expression level. Whether this is an artifact of the experiment, or other factors contribute to the downregulation of Brg1 protein synthesis that are affected in the LNA-7 experiment, opposite to the gene expression of Brg1, remains to be determined.

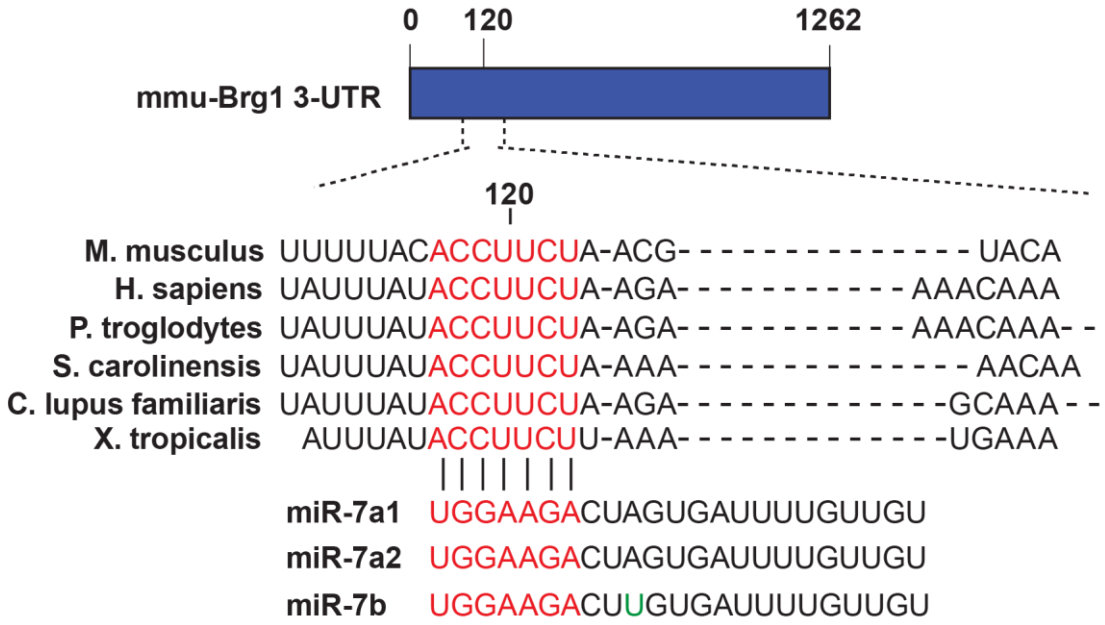

Figure 4.16: Schematic illustration the 3'UTR of mouse Brg1 including miR-7 binding sites. The binding sites for miR-7 in the 3'UTR is preserved throughout the animal kingdom. Red marks the miR-7 seed sequence. MiR-7a1 and miR-7a2 share the same sequence. The sequence for miR-7b differs at the 10th nucleotide (marked in green) compared to miR-7a1/7a2. Adapted from Mak *et al.* under review

Given the functional compensation between Brg1 and Brm (McKenna et al. 2015b), the compensatory downregulation of Brm in response to miR-7a2-mediated repression of Brg1 expression could be a possibility (Fig. 4.2 and 4.3). Other possibilities are either additional miR-7a2 targets that are involved in the regulation of *BRM* expression or additional factors, such as the loss of β-cell TFs, trigger the compensatory response. Conversely, our results showing downregulation of Brm mRNA in Min6 overexpressing miR-7a2 were not supported by the RNA-Seq results in Tg7a2 animals, suggesting that Brm downregulation in Min6 cells is an artefact of the experiment (Table 4.1). While *BAF60A* expression also showed no change in the RNA-Seq of Tg7a2 islets, it was our continued focus of investigation due to the presence of three miR-7 sites (Table 4.1). We confirmed our findings regarding the mRNA expression levels of *BAF60A* in β-cells with altered miR-7a2 expression levels, by showing that protein levels of BAF60A were reduced in Min6 cells infected with Ad-7a2 in comparison to Ad-Ctrl, therefore demonstrating that repression of BAF60A mRNA levels by miR-7a2 leads to decreased BAF60A protein (Fig. 4.4). We confirmed the regulation of BAF60A *in vitro* in mouse (Fig. 4.6A) and human (Fig. 4.7A) islets. Surprisingly, our results in Tg7a2 animals again showed no regulation of BAF60A by miR-7a2 on a mRNA (Fig. 4.8A), as well as protein

levels (Fig. 4.8B,C). *db/db* mice showed a notable difference in BAF60A mRNA levels (Fig. 4.9A).

Taken together, these results lead to the conclusion that miR-7a2 can regulate BAF60A levels *in vitro* but not *in vivo*. This could be due to compensatory mechanisms present in islets that maintain BAF60A levels. As changes in BAF60A mRNA and protein in islets were observed only *in vitro*, the question remains as to whether time plays an important factor in the compensatory effect, since comparisons were made for a 2-day infection with Ad-7a2 compared to the experiment to islets from 2 or 12 weeks Tg7a2 mice. Alternatively, the environment within islets of Tg7a2 mice may provide signals positively influencing the maintenance of BAF60A levels.

Interactions between the brain and pancreas are crucial for the secretion of insulin (Osundiji et al. 2012) and factors released by the islet have been reported to be involved in controlling the secretion by acinar cells (Petrella et al. 2012). These factors may serve a role in maintaining a functional islet under stressful conditions as observed in response to induction of miR-7a2 expression in diabetes.

For Brg1, our initial findings looking at protein levels in Min6 cells with increased miR-7a2 expression (Fig. 4.5A) were surprising, as we did not detect any changes, whereas inhibition of miR-7a increased Brg1 protein levels in Min6 cells (Fig. 4.5B). This could be due to higher endogenous miR-7 levels in Min6 cells compared to mouse and human islets which masks the overexpression of miR-7a2 by Ad-7a2. Studies have shown that target competition inside a miRNA regulated network is influenced by the miRNA target ratios, in addition to the affinity of the miRNA to the specific target (Bosson, Zamudio and Sharp 2014). Given that decreased Brg1 protein levels were observed in mouse and human dissociated islets infected with Ad-7a2 (Fig. 4.6B and 4.7B-D), we verified miR-7a and miR-7b levels in Min6, Isn1E cells and mouse islets and human islets (Fig. 4.17). miR-7a levels are actually significantly higher in mouse islets ($padj \leq 0.0001$) and human islets ($padj = 0.0341$) compared to Min6 cells, rejecting the hypothesis that increased basal miR-7 levels inside Min6 cells nullify the effects of miR-7a2 induction by Ad-7a2 on Brg1 protein levels.

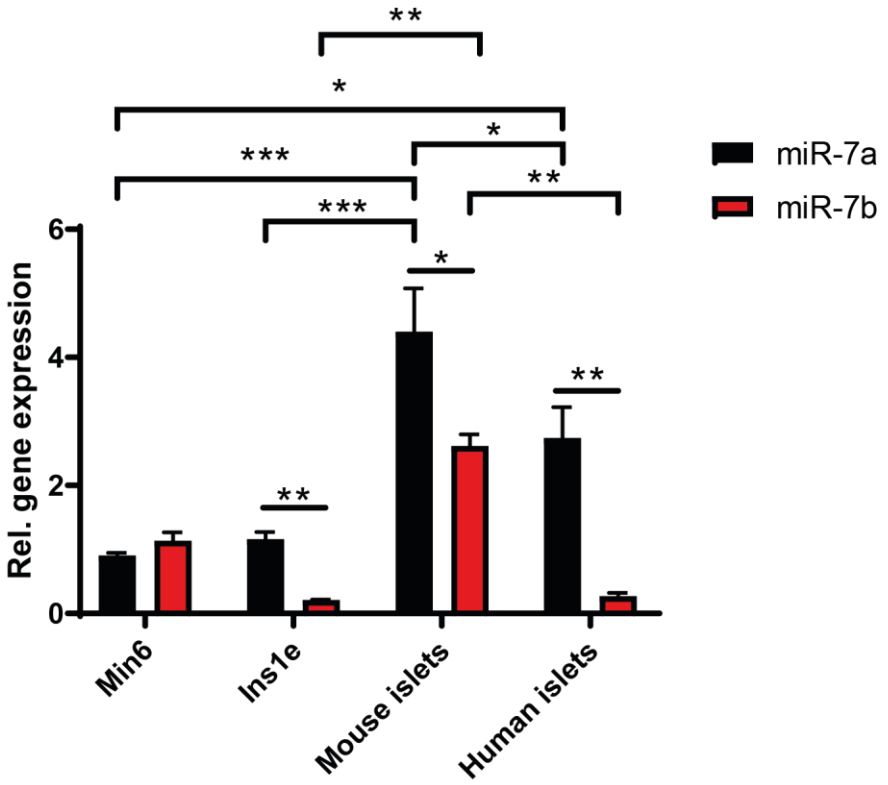

Figure 4.17: miR-7a/miR-7b expression in Min6, Isn1E, mouse islets and human islets. (A) n = 3 (Min6, Isn1E, human islets), n = 5 (mouse islets). Data was normalised to Small nucleolar RNA MBII-202 (*Sno202*) expression. Data are means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, Two-way ANOVA (stats between models), unpaired Student's t-test (stats intra model).

Another explanation may be a decreased number of miR-7a2 targets in Min6 cells compared to mouse/human islets. The heterogeneity of the islet, including β-cell hubs, are involved in the function of β-cells to respond to glucose (Johnston et al. 2016, Avrahami et al. 2017, Rutter and Hodson 2013). Therefore, Min6 cells present a different cellular context to β-cells from islets.

While we were unable to explain the differences in Brg1 protein regulation by miR-7a2 overexpression in Min6 vs mouse and human islets, our results clearly demonstrate the differences between those models and the importance to expand studies beyond immortalised cell lines and toward primary dissociated islet cells and whole animals.

Our findings showing a downregulation of Brg1 mRNA and protein levels *in vivo* in Tg7a2 and Brg1 mRNA in *db/db* animals confirmed our pervious results and therefore encouraged us to believe that Brg1 is a true target of miR-7a2, which was proven by Luciferase assay (Fig. 4.10). While our results showed no difference in the distribution of mSWI/SNF complexes in the different islet endocrine cells, as seen on BAF60A occurrence in multiple endocrine

pancreatic cell types (Fig. 4.12), we observed an increase in the abundance of BAF60A and Brg1 protein levels in the endocrine cells of the islets compared to the surrounding pancreatic exocrine cells (Fig. 4.11). This additionally demonstrated the relevance of the role of the mSWI/SNF complex in islets.

Our ATAC-Seq samples (2w and 12w, Tg7a2 and Wt) clustered into four distinct groups (Fig. 4.13). The larger variance between Tg7a2 and Wt animals with age (2w vs 12w) can be explained by the fact that hyperglycaemia is observed at 3 weeks of age, after the 2-week time point of our ATAC-Seq cohort and worsens with age (e.g. 12w). Changes in chromatin accessibility have been reported in islets of T2D patients compared to non-diabetic donors (Bysani et al. 2019). Furthermore, Tg7a2 islets show less variance at 2 and 12 weeks of age to each other than Wt animals (Fig. 4.13). This may be due to the regulation of genes by miR-7a2 that are found at both time points, since miR-7a2 is upregulated from early embryonic development in Tg7a2 animals. The overexpression of miR-7a2 is independent of the diabetic hyperglycaemic phenotype, therefore reinforcing the importance of the 2-week animals as a crucial time point to decipher miR-7a2 action from secondary changes in chromatin conformation arising in response to hyperglycaemia.

Contrary to our findings herein, ATAC-Seq on T2D donors revealed only 1078 DARs compared to non-diabetic donors, with the majority of those to be open in T2D patients (Bysani et al. 2019). Those findings contradict our results in Tg7a2 animals compared to Wt animals, were the majority of DARs are in a closed state at 2 and 12 weeks of age (Fig. 4.14A). Interestingly, judging from the data available, typical β-cell markers, like *GLUT2*, appear to not be severely affected (Bysani et al. 2019), a surprising finding given that a major hallmark of T2D is decrease in expression of those genes. While we observed closure of chromatin in regulatory regions of β-cell-specific genes (Fig. 4.14B-F), it must be taken into consideration that diabetic patients are usually receiving anti-diabetic treatment, potentially impacting on the chromatin conformation. Bysani et al. identified a that a number of open DAR were located in GWAS risk genes for T2D, including *MTNR1B*, *HHEX*, *HMGA2*, *GLIS3* and *WFS1* (Tuomi et al. 2016, Hall et al. 2014, Dayeh et al. 2013, Rönn et al. 2009, Bysani et al. 2019). Our findings showed that, besides *MTNR1B*, which was in a closed state (2w: padj=0.0155; 12w: padj=$7.42e^{-8}$), there were no significant differences in DARs of those genes. Furthermore, Bysani et al. found *SLC16A7*, encoding the Monocarboxylate Transporter 2 (MCT2), chromatin regions in an open state. A previous study reported that MCT2 was expressed in any type of pancreatic cell (Zhao et al. 2001) and *SLC16A7* silencing increased insulin content and

secretion in β-cells (Bysani et al. 2019). Interestingly, our results, while not showing any significant DARs at 2 weeks of age, revealed a closed DAR (padj=0.0238) and an open DAR (padj=0.0078) for Slc16a7 at 12 weeks of age in Tg7a2 animals compared to Wt animals.

Similar to our results, only a limited number of disease pathways associated with diabetes were identified in ATAC-Seq of islets of T2D individuals (Bysani et al. 2019), indicating that pathway analysis, while well established for the analysis of other Sequencing Assays (e.g. GSEA in RNA-Seq) is still a novel application in ATAC-Seq and that potential novel pathways are involved in the development of T2D. The pathways involving vesicles exocytosis were expected to be found in closed DARs, as Tg7a2 animals show an impairment of insulin exocytosis, including the gene *SNCA* (Latreille et al. 2014). The closure of genes involved in peptide biosynthesis, including insulin, was not unexpected either. Interestingly, *INHBA* is part of this pathway. *INHBA* overexpression is involved in pancreatic cancer formation. That we observed it in a transcriptional inactive state indicates that the EMT processes we showed in Tg7a2 animals (Fig. 1.12) are related to β-cell dedifferentiation as seen in diabetes and not the induction of a cancer phenotype. For the open DARs we found genes involved in DNA methylation to be identified. As DNA methylation induces transcriptional repression, those finding fit without hypothesis of β-cell gene repression in the development of diabetes in Tg7a2. Interestingly, we found pathways relating to insulin signalling, FOXO, glucose transport and glucokinase regulation to be in an open state in Tg7a2 animals. This could be explained by potential compensatory mechanisms of the β-cell to meet the hyperglycaemia induced increasing demand in insulin.

TF motif analysis by Bysani *et al.* determined the islet specific TFs Nkx6.1, Pdx-1, Foxa2 and MafB, as well as Fra1 as the most significantly one, enriched in ATAC-Seq peaks (Bysani et al. 2019). Interestingly, in the closed DARs of our ATAC-Seq results in Tg7a2 animals, Nkx6.1, Pdx-1, Foxa2 and MafB (Fig. 4.15A). as well as Fra1 were the most enriched TF motif ($p=1e^{-91}$). Fra1 is involved in MAPK signalling (Vial and Marshall 2003), however, its role in the pancreas is mostly unknown. Reports have shown the involvement of Fra1 in pancreatic cancer (Hanson et al. 2016). This makes it an interesting target for future studies. In contrast, the involvement of Nkx6.1 (Taylor et al. 2013), Pdx-1 (Ahlgren et al. 1998, Brissova et al. 2002) and Foxa1 and Foxa2 (Gao et al. 2008, Gao et al. 2010) in mature β-cell function is well described.

MafB, a TF involved in β-cells differentiation during development has been reported to be absent in mature β-cells (Nishimura et al. 2006, Artner et al. 2006) In contrast to MafA, MafB does not induce *INS* or *PDX-1* expression when artificially introduced in β-cells (Artner et al. 2006) (Samaras et al. 2003). The Maf family consist of large (MafA and MafB) and small (MafK, MafF) protein family members (Katsuoka and Yamamoto 2016) that recognise e Maf-recognition element (MARE) (Hang and Stein 2011, Eychène, Rocques and Pouponnot 2008, Kataoka, Noda and Nishizawa 1994). Small Maf family members lack a transactivation domain and compete with the large Maf proteins for MARE sites (Motohashi et al. 2002). Studies overexpressing MafK in β-cells of mice inhibited the function of large Maf members by suppressing known β-cell genes leading to developmental defect in islet and bringing hyperglycaemia (Shimohata et al. 2006, Abdellatif et al. 2016). If MafK functions as a negative counterpart to MafA function in β-cells, one can assume that the other small Maf family members may exhibit similar repressive functions for other large Maf proteins. Interestingly, MafF has been reported to show "extensive homology" to MafB in the DNA-binding domain (Kataoka et al. 1994) and our results show that, while MafB being the principal identified motif, MafF also appears in the list of motifs (Fig. 4.18). This suggests, that MafF acts as a counterpart to MafB that may potentially inhibit the expression of genes regulated by MafB in the development of the pancreas in mature β-cells. Reactivation of the repressed genes, through loss of MafF binding, may be involved in the dedifferentiation processes of β-cells into a more progenitor-like, poly-hormonal cell state observed in Tg7a2 animals.

Matches to Known Motifs

PB0041.1_Mafb_1/Jaspar

Match Rank: 1
Score: 0.74
Offset: -7
Orientation: reverse strand
Alignment: -------CAGCAAATTA
NCTANGTCAGCAAATTT

MafF(bZIP)/HepG2-MafF-ChIP-Seq(GSE31477)/Homer

Match Rank: 2
Score: 0.72
Offset: -5
Orientation: forward strand
Alignment: -----CAGCAAATTA
HWWGTCAGCAWWTTT

Figure 4.18: MafB and MafF TF identification in closed DARs of 2w Tg7a2. (A) Identified TF motif of MafB and MafF in closed DARs of Tg7a2 animals of 2 weeks of age compared to age matched Wt controls in Motif 5. n = 4, HOMER, analysed by Y. Wang.

In addition to its crucial function in the secretion in β-cells, the loss of Foxa1 and Foxa2 has been reported to trigger an EMT in pancreatic cancer (Song, Washington and Crawford 2010). Furthermore, Foxa1 has been reported to be enriched in regions that are bound by Brg1 and not by Brm (Raab et al. 2017), which is in accordance with our findings showing the closure of Foxa1 motif regions in Tg7a2 islets displaying decreased expression of Brg1. This would provide an explanation for the upregulation of EMT genes in 2w and 12w old Tg7 islet (Fig. 1.12).

Interestingly, *ZIC3* and *ZIC4* and *ZNF354C* and *FOXH1* motifs were found in open DARs of 2w Tg7a2 mice (Fig. 4.15B). Zic3 has been proven to be upregulated in cells expressing an EMT phenotype (Kong et al. 2010) and Zic4 is associated with an involvement in cancer (Kandimalla et al. 2012). In DNA binding motif analysis of Zeb1 ChIP, a regulator of EMT in cancer (Oh et al. 2019), Znf354C was identified as being centrally enriched, suggesting a co-function of those TFs (Maturi et al. 2018), while Foxh1 plays a central role in early mouse development (Yamamoto et al. 2001) and is enriched in human embryonic stem cells (Brandenberger et al. 2004).

With the identification of the miR-7a2-Brg1-axis we shed light on the regulation of a major chromatin remodeller in a diabetic setting. The results this far, only allow for a correlation between the loss of β-cell function and the downregulation of Brg1 by miR-7a2. Therefore, chapter 5 will focus on addressing how inactivation of Brg1 in immortalised β-cell lines as well as in β-cells of mice impacts on β-cell identity and glucose metabolism.

5. Modulation of BAF60A and Brg1 expression in pancreatic β-cells

5.1 Introduction

In the previous chapters, we established that miR-7a2 triggers the dedifferentiation of mouse and human β-cells (Chapter 3). We further identified the chromatin remodelling complex subunits BAF60A and Brg1 as targets of miR-7a2 and reported changes in chromatin accessibility taking place in miR-7a2-overexpressing Tg7a2 mice (Chapter 4). In this chapter we sought to link the loss of β-cell function with the regulation of BAF60A/Brg1. We therefore investigated the effects of BAF60A/Brg1 expression changes/functional inhibition on islets and β-cells function. The role of the mSWI/SNF complex in islets is mostly unknown. Studies have shown the recruitment of Brg1 and Brm in a glucose dependent manner (McKenna et al. 2015a). Exposure of Ins1E cells to elevated levels of glucose appears to cause a switch from the Brm complex to the Brg1 complex at genes essential for insulin biosynthesis and secretion (McKenna et al. 2015a). Those findings suggest an antagonistic role of the mutually exclusive subunits Brg1 and Brm in β-cells, with Brg1 inducing, while Brm is repressing gene transcription. The involvement of the mSWI/SNF complexes in islet development and mature function has been recently demonstrated. However, in contrast to previous observation made by McKenna et al., loss of both Brg1 and Brm is required to impair the function of mature β-cells (Spaeth et al. 2019). Previous studies on BAF60A focussed on its dysregulation in non-pancreatic tissues like liver, adipose tissue or skeletal muscle in diabetes mouse models (Li et al. 2008, Li et al. 2018, Meng et al. 2017). In contrast, the function of BAF60A in mature β-cell has not yet been described. In this chapter, we investigated if depletion of Brg1, Brm and BAF60A impacts on β-cell identity.

5.2 Alteration of BAF60A and Brg1 expression *in vitro*

First, we investigated how knockdown of BAF60A or Brg1 effects the identity of immortalised β-cells. We hypothesised that loss of BAF60A or Brg1 decreases the expression of β-cell identity genes.

5.2.1 Lentiviral shRNA inhibition of BAF60A in Ins1E cells

For the investigation of selective BAF60A regulation and its effect on β-cell identity, we utilised the replication incompetent pGIPZ lentiviral vector carrying small hairpin RNA (shRNA) targeting BAF60A (shBAF60A) or a non-silencing control (shNS). Ins1E cells were transfected ON, followed by another 6h transfection with fresh lentiviral particles and a 48h Puromycin selection the following day.

Successful transduction of Ins1E cells with lentiviral particles was confirmed by fluorescence microscopy of GFP independently encoded by the pGIPZ vector (Fig. 5.1A). RT-qPCR analysis of Ins1E cells transduced with shBAF6A shows a significant downregulation of *BAF60A* gene expression levels (p=0.0009) compared to shNS transduced samples (Fig. 5.1B). We confirmed the regulation of BAF60A by shRNAs by Western Blot analysis, showing a loss in BAF60A protein content in cells transduced with shBAF60A compared to shNS control lentiviruses (Fig. 5.1C).

After establishing the successful downregulation of BAF60A, we measured the expression of β-cell markers (Fig. 5.1D). Our results show no significant regulation of insulin mRNA levels in shBAF60A cells compared to shNS controls (Fig. 5.1D). mRNA levels of the β-cell marker Ucn3 were significantly downregulated (p=0.0423), whereas we observed a notable decrease in Glut2 (p=0.0845) and Pax6 (p=0.0861) mRNA levels. In contrast, the expression of *PDX-1*, *NKX6.1* and *MAFA* was not affected (Fig. 5.1D). Overall, those results led us to the conclusion that, while effects on certain β-cell genes were detected, knockdown of BAF60A is not sufficient to drive loss of β-cell identity.

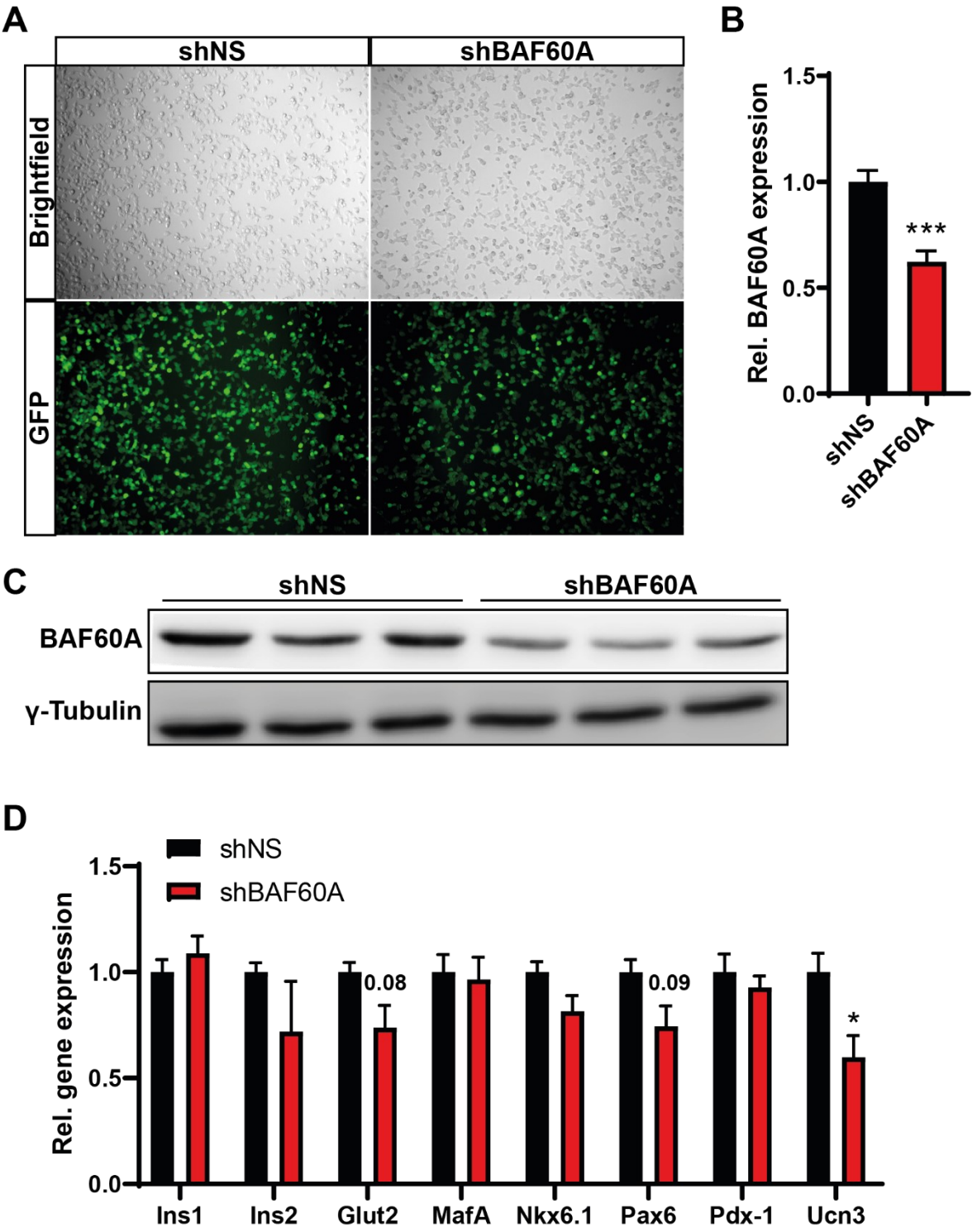

Figure 5.1: Gene silencing of BAF60A via shRNA in Ins1E cells. (A) Microscopy images of Ins1E transduced with shBAF60A/shNS. (B,C) qPCR results, normalised to *HNRNPK*, (B) and Western blot (C) of BAF60A levels in Ins1E cells transduced with shBAF60A/shNS. (D) qPCR results of β-cell identity markers in Ins1E cells transduced with shBAF60A/shNS, normalised to *HNRNPK*. n = 3. Data are means ± SEM, *p < 0.05, ***p < 0.001, unpaired Student's t-test.

5.2.2 Lentiviral shRNA inhibition of Brg1 in Ins1E cells

Next we tested how shRNA against Brg1 affect levels of β-cell markers in Ins1E cells. Successful transduction of cells was confirmed following GFP fluorescence microscopy (Fig. 5.2A). qPCR results show a significant downregulation (p=0.0023) of Brg1 mRNA levels in shBrg1 transduced Ins1E cells compared to shNS controls (Fig. 5.2B). We observed a significant decrease in Ins2 (p=0.0183) mRNA levels in shBrg1 cells compared to shNS controls, while Ins1 was not affected (Fig. 5.2C). Furthermore, we observed a significant decreased in the expression of *GLUT2* (p=0.0044), *NKX6.1* (p=0.0040), *UCN3* (p=0.0060) and *PAX6* (p=0.0222) and a notable decrease in *MAFA* expression (p=0.0712) (Fig. 5.2C). Interestingly, Brg1 inhibition seems to not affect Pdx-1 gene expression (Fig. 5.2C).

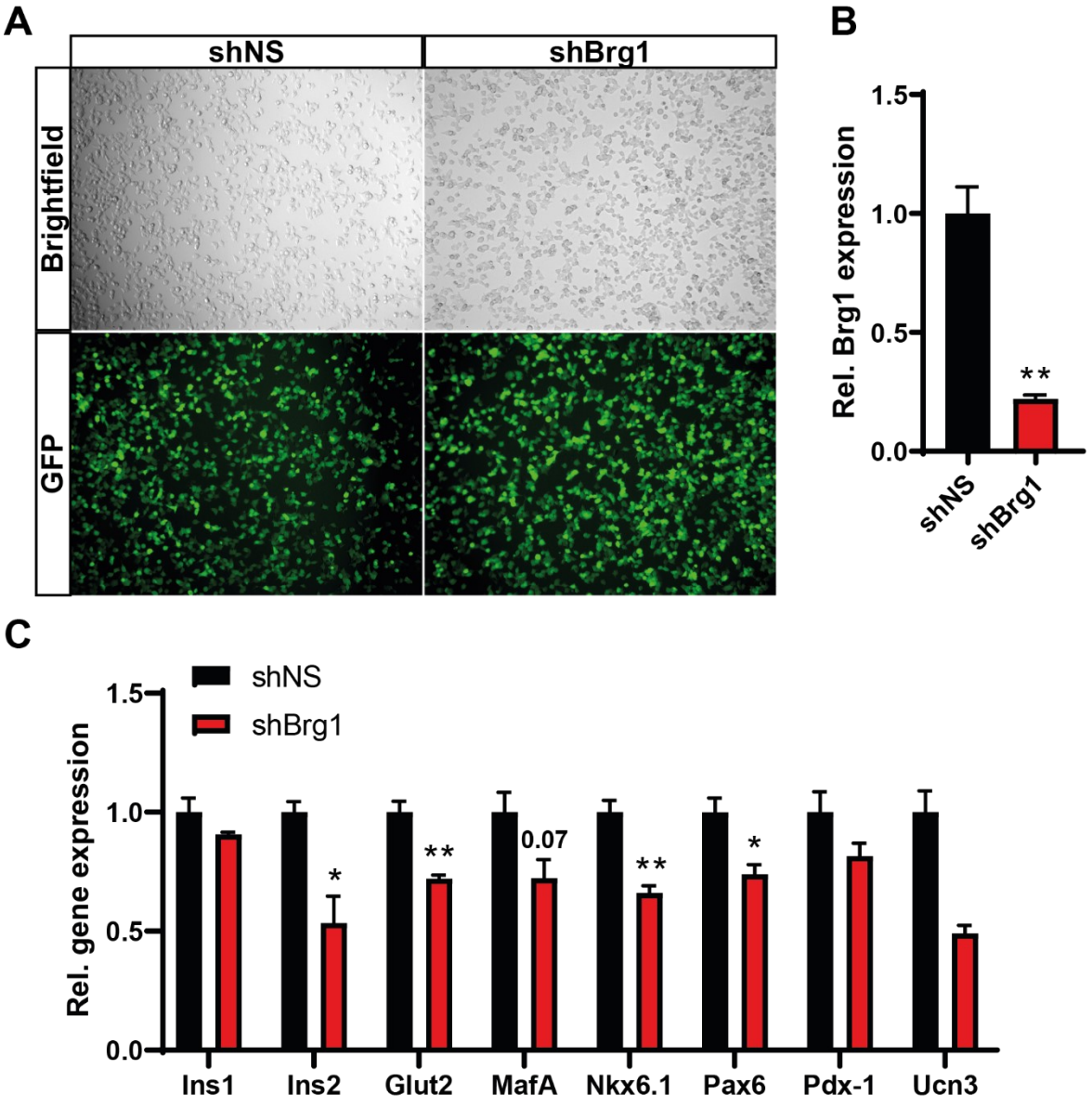

Figure 5.2: Gene silencing of Brg1 via shRNA in Ins1E cells. (A) Microscopy images of Ins1E transduced with sBrg1/shNS. (B) qPCR results of *Brg1* levels in Ins1E cells transduced with shBrg1/shNS, normalised to *HNRNPK*. (C) qPCR results of β-cell identity markers in Ins1E cells transduced with shBrg1/shNS, normalised to *HNRNPK*. n = 3. Data are means ± SEM, *p < 0.05, **p < 0.01, unpaired Student's t-test.

5.2.3 Inhibition of Brg1 and Brm by siRNA in Min6 cells

We next sought to confirm our results utilising siRNA targeting Brg1 (siBrg1) or a scrambled control (siCtrl). We decided to continue our work in Min6 cells, given that Brg1 repression by miR-7a2 was more pronounced in Min6 compared to Ins1E cells (Chapter 4.2). Additionally, Min6 cells, as an immortalised mouse β-cell line, are a better model preceding our *in vivo* work in mice.

Our results show a significant decrease in Brg1 mRNA levels (p=0.0004) in Min6 cells transfected with siBrg1 compared to siCtrl (Fig. 5.3A). Investigation of the expression levels of insulin shows a significant downregulation of *INS1* (p=0.0318) and *INS2* (p=0.0372) (Fig. 5.3B). Moreover, we found decreased expression of *GLUT2* (p=0.0332) and *NKX6.1* (p≤0.0001) in siBrg1 treated samples compared to siCtrl (Fig. 5.3B). Opposite to our previous findings, showing a downregulation of Ucn3 and Pax6 mRNA levels (Fig. 5.2C), those two genes were not affected (Fig. 5.3B). We were able to confirm that Pdx-1 mRNA levels are not regulated *in vitro* by silencing of *BRG1* expression (Fig. 5.3B).

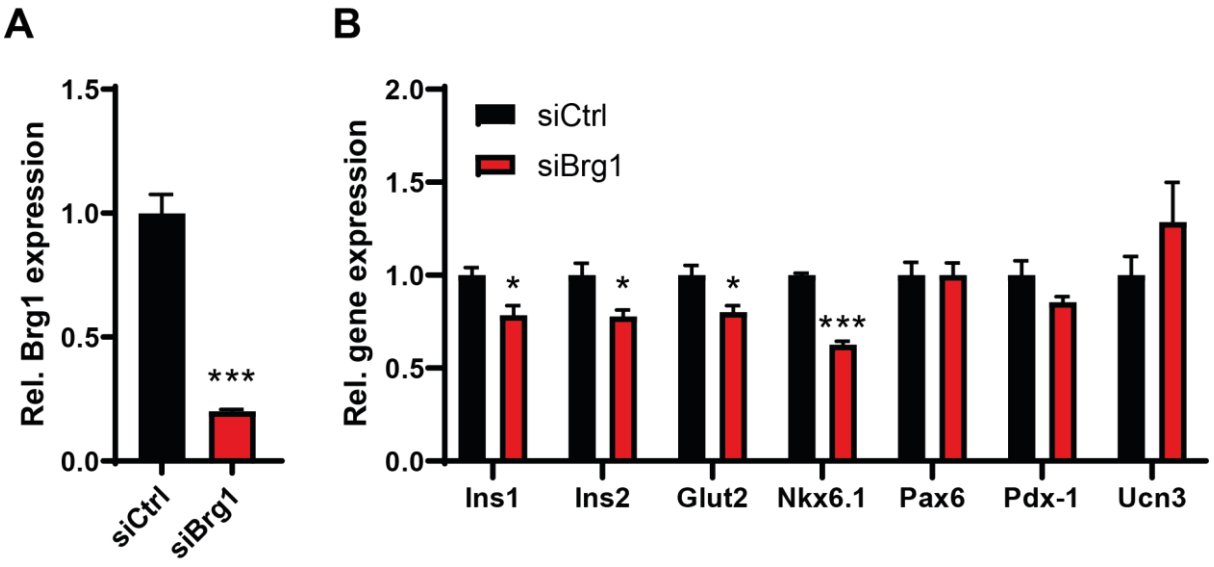

Figure 5.3: Gene silencing of Brg1 via siRNA in Min6 cells. (A,B) qPCR results of Min6 cells transfected with siBrg1/siCtrl, normalised to *RPLP0/36B4*: (A) *Brg1* and (B) β-cell identity marker gene expression levels. n = 3. Data are means ± SEM, *p < 0.05, ***p < 0.001, unpaired Student's t-test.

If Brg1 and Brm display antagonistic functions in β-cells (McKenna et al. 2015a), it would be expected that depletion of Brm with siRNA increases the expression of β-cell-specific genes. When siRNA against Brm were transfected in Min6 cells, we observed that Brm mRNA levels were significantly decreased (p=0.0060) compared to siCtrl (Fig. 5.4A). In contrast to Brg1 knockdown, depletion of Brm did not result in changes in the expression of insulin genes (Fig. 5.4B). In fact, we observed a significant downregulation of Nkx6.1 (p=0.0025), a notable decrease in Pdx-1 (p=0.0538) and a significant upregulation of Ucn3 (p=0.0480) mRNA levels in siBrm treated Min6 cells compared to siCtrl (Fig. 5.4B).

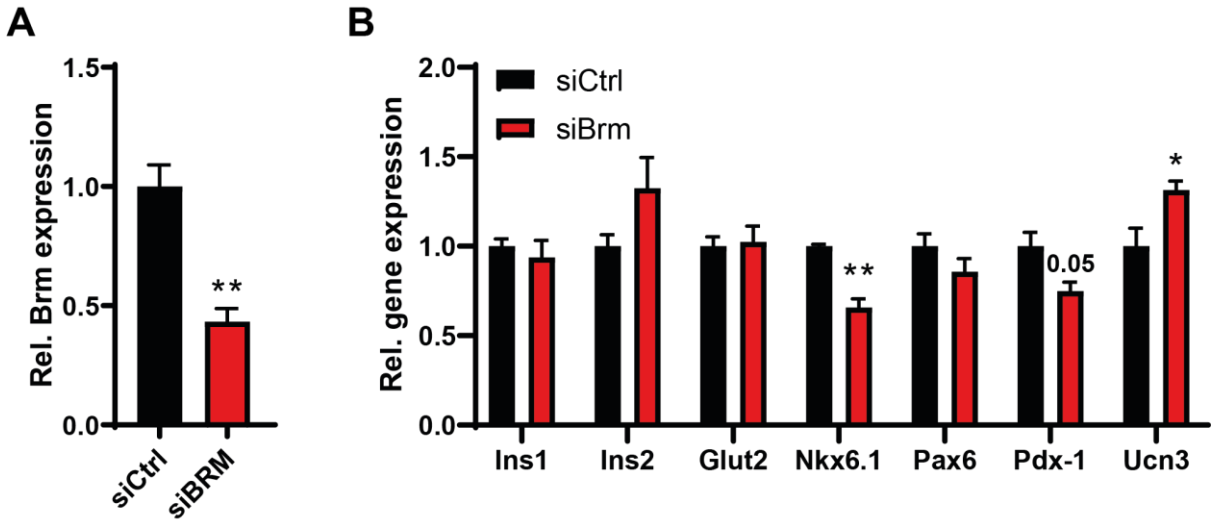

Figure 5.4: Gene silencing of Brm via siRNA in Min6 cells. (A,B) qPCR results of Min6 cells transfected with siBrm/siCtrl, normalised to *RPLP0/36B4*: (A) *Brg1* and (B) β-cell identity marker gene expression levels. n = 3. Data are means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, unpaired Student's t-test.

Taken together, our results were not able to replicate the improvement of β-cell identity by Brm knockdown that has been shown in previous studies (McKenna et al. 2015a), but rather observed a decrease of β-cell identity. This is in accordance with studies by Spaeth et al. suggesting a synergistic effect of Brg1 and Brm, where double knockout in mature β-cells is required to induce a diabetic phenotype (Spaeth et al. 2019). In contrast, our results show that Brg1 knockdown in Min6 cells is sufficient to induce a decrease in the expression of β-cell genes, including *INS1* and *INS2*. This indicates, that Brg1 may have a more important role in maintaining β-cell genes than Brm.

5.3 Characterisation of the pancreatic β-cell specific Brg1 knockout mice: Brg1$^{fl/fl;Ins1\text{-}Cre}$

While we were able to show that Brg1 knockdown leads to loss of β-cell identity *in vitro*, we next sought to demonstrate the significance of our findings *in vivo*. We hypothesised that the specific deletion of Brg1 in β-cells of mice will impair β-cell identity and reproduce the diabetic phenotype of Tg7a2 mice. We therefore generated a β-cell specific Brg1 knockout mouse line where Brg1$^{fl/fl}$ were crossed with Ins1-Cre (Thorens et al. 2015) mice, thus regenerating Brg1$^{fl/fl;Ins1\text{-}Cre}$. In these experiments, Brg1$^{fl/fl}$ animals were used as controls.

5.3.1 Validation of Brg1 knockout in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice

The first step in analysing Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice was to validate the successful knockout of the Brg1 gene in β-cells. We therefore isolated islets from Brg1$^{fl/fl;Ins1\text{-}Cre}$ and Brg1$^{fl/fl}$ mice and performed RT-qPCR. qPCR results of the Brg1 gene expression shows a significant downregulation ($p \leq 0.0001$) in 24-week-old Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice compared to age matched Brg1$^{fl/fl}$ controls, while Brm levels are not affected (Fig. 5.5A).

We then sacrificed Brg1$^{fl/fl;Ins1\text{-}Cre}$ and Brg1$^{fl/fl}$ mice at 16 weeks of age to address protein levels of Brg1 via immunofluorescence with Brg1 antibodies (Fig. 5.5B). Images of Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice show a clear decrease in nuclear Brg1 within the core of islets, while Brg1 is retained in the exocrine pancreas (Fig. 5.5B). Counting of the Brg1 positive cells with a signal for insulin shows a significant decrease ($p = \leq 0.0001$) in Brg1$^{fl/fl;Ins1\text{-}Cre}$ pancreatic sections compared to controls (Fig. 5.5C). Additionally, our results suggest a decrease in insulin content in islets of Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to controls (Fig. 5.5B). Together those results made us confident, that Brg1 was successfully knocked out in β-cells.

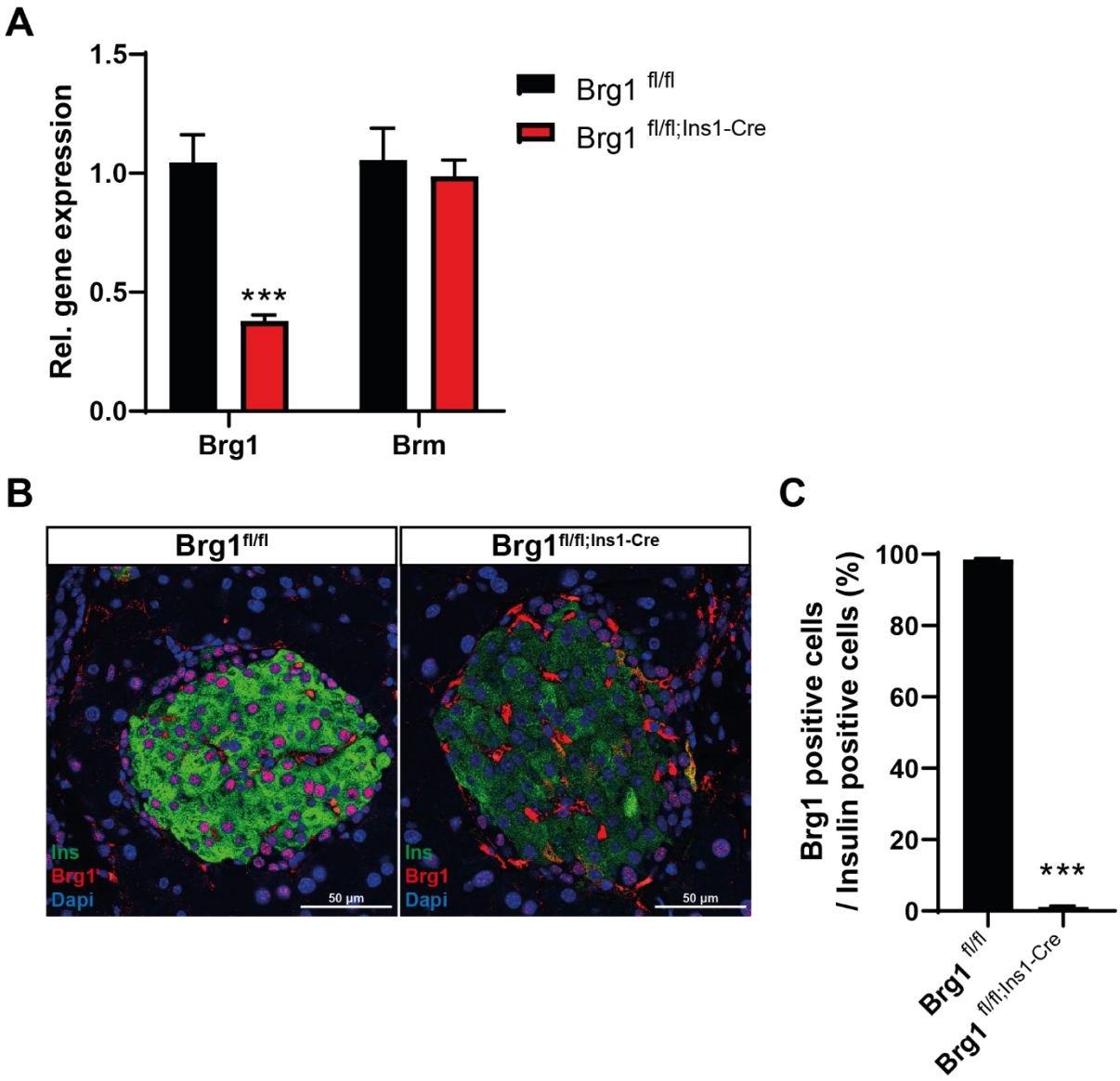

Figure 5.5: Validation of Brg1 knockout in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. (A) qPCR results of *Brg1* and *Brm* gene expression levels in Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice at 24 weeks of age, normalised to *RPLP0/36B4*. (B) Representative microscopy images of in Brg1$^{fl/fl;Ins1\text{-}Cre}$ and Brg1fl/fl mice of 16 weeks of age stained for Brg1 and Ins. (C) Count of Brg1 positive cells in insulin positive cells of Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice of 16 weeks of age represented in (B). (A) n = 9 (Brg1$^{fl/fl}$), n = 11 (Brg1$^{fl/fl;Ins1\text{-}Cre}$); (C) n = 3. Data are means ± SEM, ***p < 0.001, unpaired Student's t-test.

5.3.2 The metabolic phenotype of Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice

Next, we addressed the metabolic phenotype, including random fed, fasted, glucose and insulin tolerance test blood glucose levels and weight measurements of Brg1$^{fl/fl;Ins1\text{-}Cre}$ before investigating potential changes in insulin secretion and content in islets. Blood glucose (BG) levels and body weight (BW) were measured weekly in *ad libitum* fed Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. This revealed no significant differences in BG and BW from 2 to 24 weeks of age in Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice (Fig. 5.6).

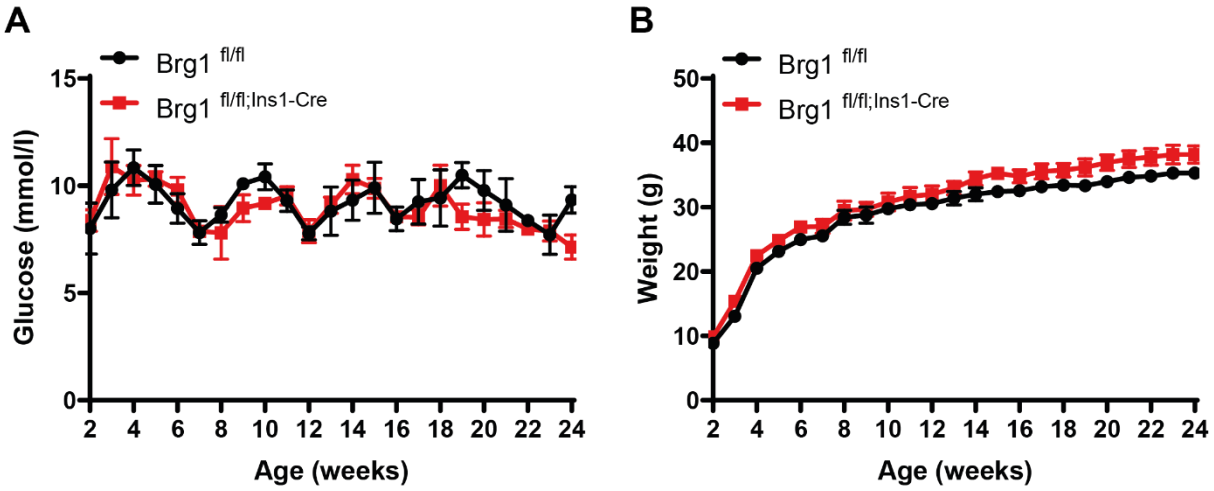

Figure 5.6: The metabolic phenotype of *ad libitum* fed Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. (A) Weekly sampling of *ad libitum* fed BG levels in Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice. (B) Weekly sampling of *ad libitum* fed BW levels in Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice. n = 4. Data are means ± SEM, Two-way ANOVA.

Although BG levels were unchanged in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice, we investigated the glucose tolerance of mutant mice in an intraperitoneal glucose tolerance test (IPGTT)(Fig. 5.7). Statistical analysis of the IPGTT curve showing measured BG values (Fig. 5.7A) and the area under the curve of the measured BG levels (Fig. 5.7B), performed at 16 weeks of age, showed overall no significant differences between Brg1$^{fl/fl;Ins1\text{-}Cre}$ and Brg1$^{fl/fl}$ mice. Statistical analysis at the single time points showed significantly reduced BG levels at 0 min (padj=0.0386), prior the glucose injection, in Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice (Fig. 5.7A). Fasted BG levels at multiple timepoints showed significant reduced BG levels after an ON fast in Brg1$^{fl/fl;Ins1\text{-}Cre}$ at 16 (p=0.0073, Fig. 5.7C), 16.5 (p=0.0012, Fig. 5.7D) and 23 (p=0.0261, Fig. 5.7E) weeks of age compared to Brg1$^{fl/fl}$ mice. Those results prompted us to calculate the percentile changes in BG compared to timepoint (t) 0 min, as is commonly done in the analysis of intraperitoneal insulin tolerance test (IPITT) experiments, to present the actual changes in BG levels rather

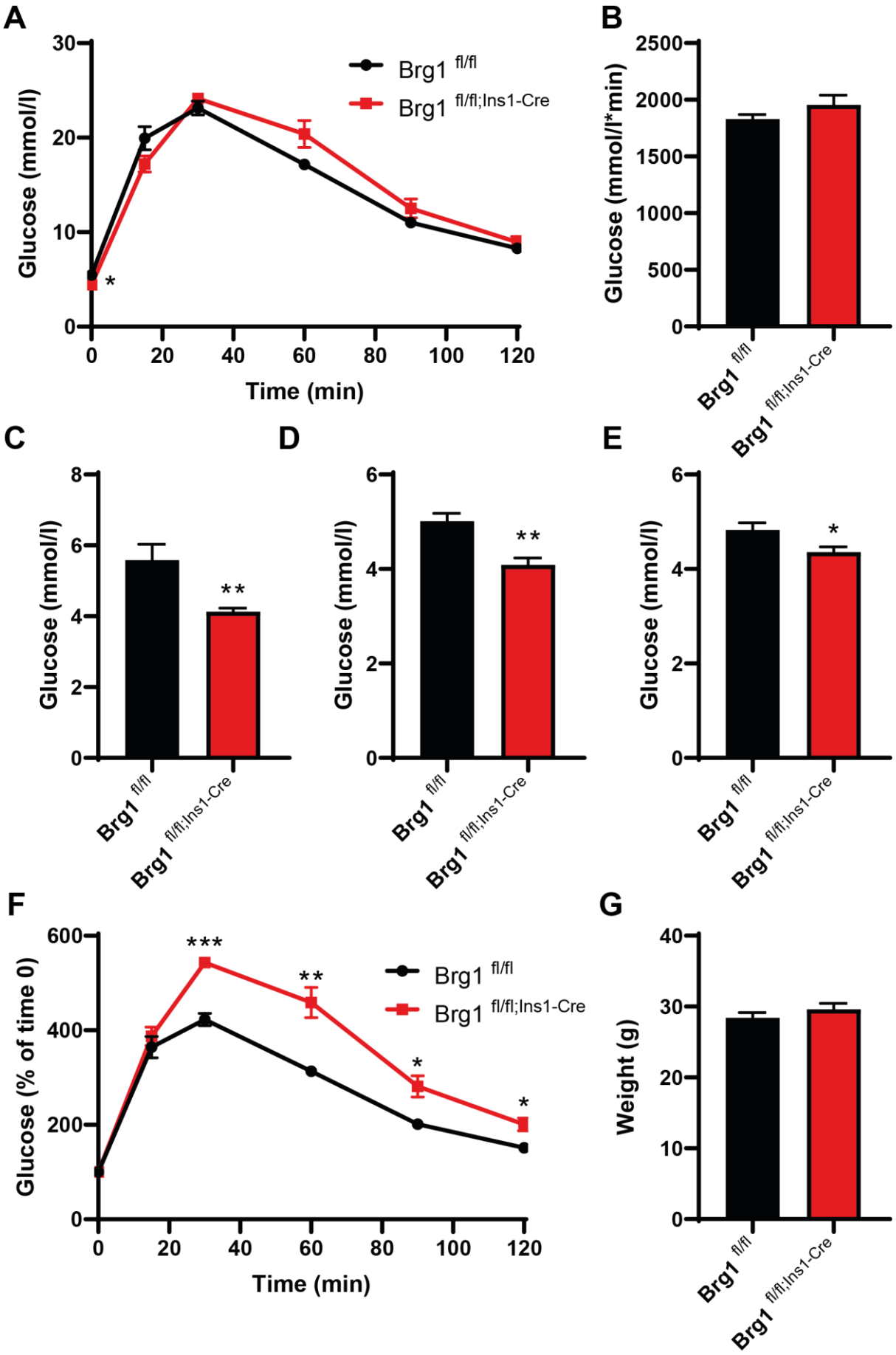
A
Glucose (mmol/l)
Time (min)
Brg1 fl/fl
Brg1 fl/fl;Ins1-Cre
B
Glucose (mmol/l*min)
C
Glucose (mmol/l)
D
Glucose (mmol/l)
E
Glucose (mmol/l)
F
Glucose (% of time 0)
Time (min)
G
Weight (g)

Figure 5.7: Glucose tolerance test on overnight fasted Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. (A) Raw BG values measured in IPGTT performed on Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice. (B) Area under the curve of raw BG values measured in IPGTT performed on Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice shown in (A). (C-E) BG values after an ON fast in Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice at (C) 16, (D) 16.5 and (E) 23 weeks of age. (F) Percental changes compared to t = 0 min of BG values measured in IPGTT (A) performed on Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice. (G) BW values measured in IPGTT (A) performed on Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice. (A,B,F,G) n = 11, (C-E) n = 7. Data are means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, (A,F) Two-way ANOVA; (B-E,G) unpaired Student's t-test.

than the absolute value, giving a better representation of the ability of the mice to compensate for changes in BG levels (Fig. 5.7F).

Statistical analysis of the percentile changes of Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice shows an overall significant increase ($p \leq 0.0001$) in measured BG levels, as well as in multiple comparisons of the single timepoints at 30 (padj$\leq$0.0001), 60 (padj=0.0060), 90 (padj=0.0341) and 120 min (padj=0.0333) (Fig. 5.7F). No significant differences were observed for the BW of Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice at the time of the IPGTT (Fig. 5.7G).

The results observed in the IPGTT gave us the first indication that inactivation of the Brg1 gene in β-cells of mice induces a diabetic phenotype by promoting an impairment of the animal's ability to compensate for sudden elevation in BG levels, and encouraged us to continue investigating the undergoing changes in gene and protein expression that the diabetic phenotype can be accounted for.

Failure to compensate for hyperglycaemia can also be caused by insulin resistance of peripheral organs. We therefore performed IPITT in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice at 17 weeks of age (Fig. 5.8). Injection of insulin did not result in a significant change in BG levels in Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice (Fig. 5.8A). No significant changes in BW were observed in the Brg1$^{fl/fl;Ins1\text{-}Cre}$ and Brg1$^{fl/fl}$ mice used for the IPITT (Fig. 5.8B). Those results show that the peripheral tissues of Brg1$^{fl/fl;Ins1\text{-}Cre}$ respond similar to those of Brg1$^{fl/fl}$ to insulin.

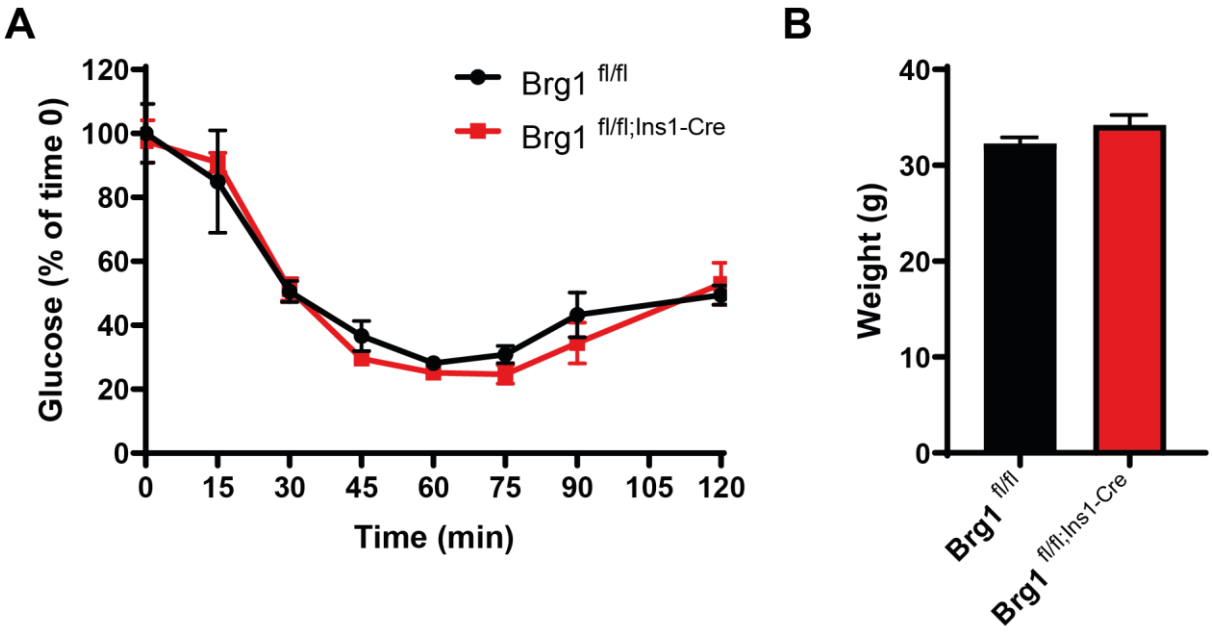

Figure 5.8: Insulin tolerance test on 6h fasted Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. (A) BG values measured in IPITT as percental of t = 0 min performed on Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice. (B) BW of Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice used for IPITT in (A). n = 3 (Brg1$^{fl/fl}$), n = 4 (Brg1$^{fl/fl;Ins1\text{-}Cre}$). Data are means ± SEM, (A) Two-way ANOVA; (B) unpaired Student's t-test.

5.3.3 Circulating and pancreatic islet insulin content of Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice

Glucose stimulated insulin secretion is the primary mechanism underlying compensation for hyperglycaemia and is impaired in diabetic subjects (Perley and Kipnis 1967). We next sought to investigate if the inability of Brg1$^{fl/fl;Ins1\text{-}Cre}$ to metabolise glucose compared to Brg1$^{fl/fl}$ mice can be attributed to reduced insulin secretion and/or synthesis. Blood samples were taken from Brg1$^{fl/fl;Ins1\text{-}Cre}$ and Brg1$^{fl/fl}$ mice at 16 weeks of age after a 6h fast. Plasma insulin of Brg1$^{fl/fl;Ins1\text{-}Cre}$ indicated a reduction in circulating insulin levels (p=0.0558)(Fig. 5.9A). Next, we examined β-cell insulin content in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. We therefore stained sections of Brg1$^{fl/fl;Ins1\text{-}Cre}$ and Brg1$^{fl/fl}$ mice at 16 weeks of age for insulin and Brg1, to confirm the successful deletion of Brg1 (Fig. 5.9B). Quantification of the relative insulin intensity in microscopic images (Fig. 5.9B), showed a significant decrease (p=0.0097) in insulin intensity in Brg1$^{fl/fl;Ins1\text{-}Cre}$ animals compared to Brg1$^{fl/fl}$ controls (Fig. 5.9C).

Together those results reveal that the failure of Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice to compensate hyperglycaemia, can be attributed to a reduced insulin content in the islets of those animals resulting in decreases in circulating insulin levels.

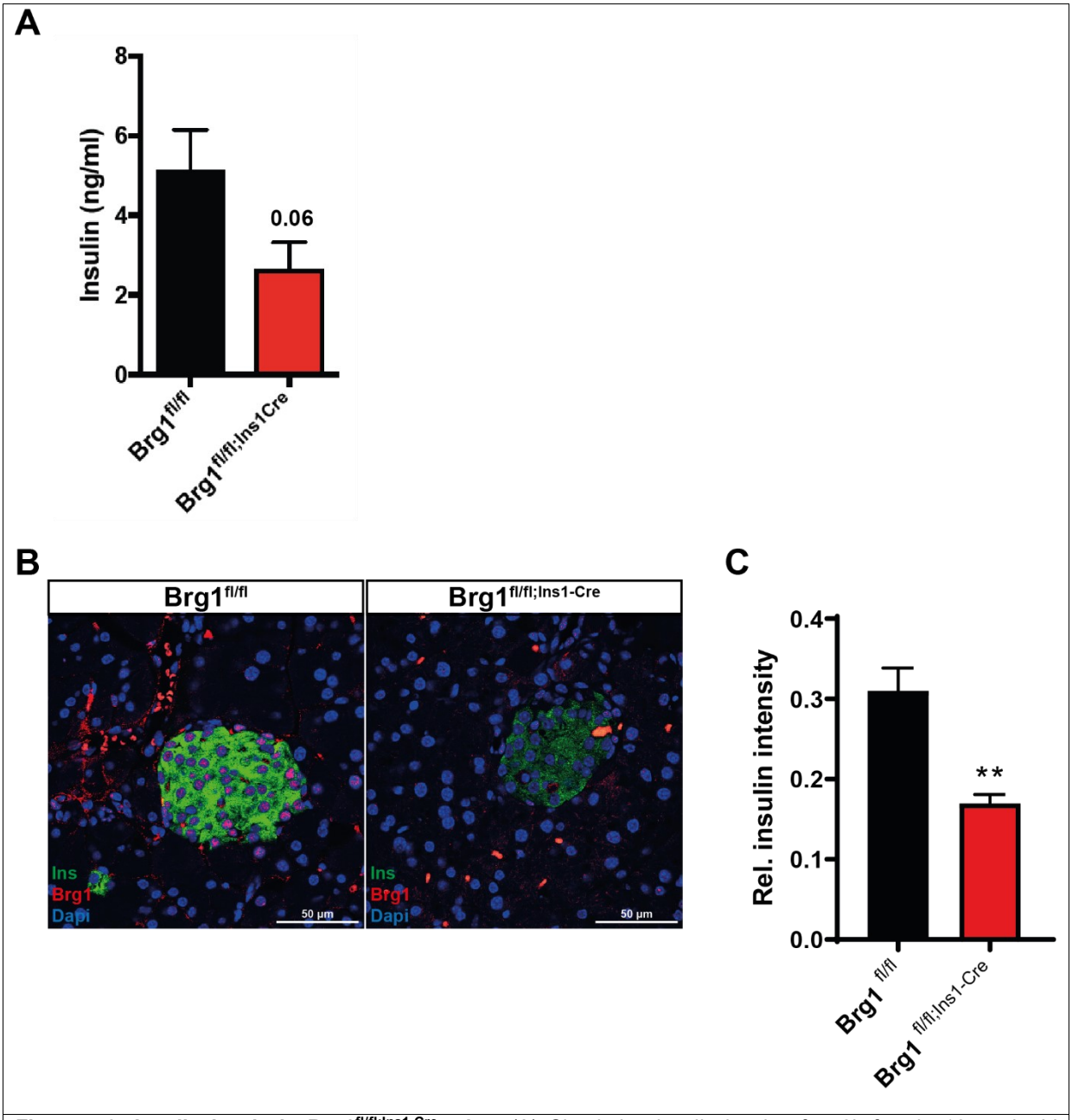

Figure 5.9: Insulin levels in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. (A) Circulating insulin levels after 6h fast in 16-week-old Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice. (B) Representative microscopy images of in Brg1$^{fl/fl;Ins1\text{-}Cre}$ and Brg1$^{fl/fl}$ mice of 16 weeks of age stained for Brg1 and Ins. (D) Quantification of the relative Ins intensity of Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice of 16 weeks of age represented in (B). (A) n = 11 (Brg1$^{fl/fl}$), n = 9 (Brg1$^{fl/fl;Ins1\text{-}Cre}$); (C) n = 3. Data are means ± SEM, **p < 0.01, (A-C,E) unpaired Student's t-test.

5.3.4 Gene expression changes in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice

After showing that a decrease in the available insulin synthesised by the islets of Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice is causative for the failure of those animals to compensate for elevated BG levels (Fig. 5.7 and 5.9), our next goal was to deepen our understanding of what triggers the loss of insulin in those animals. According to our ATAC-Seq data, miR-7a2 overexpression in Tg7a2 animals induces epigenetic changes favouring a closed chromatin state, including genes involved in insulin synthesis (Fig. 4.14). A closed chromatin state results in the inability of β-cells to transcribe those genes. We hypothesised that the decrease in insulin content can be accounted to the loss of key β-cell identity genes. We performed RT-qPCR to address β-cell gene expression in islets isolated from Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice via RT-qPCR. Our results showed a loss in β-cell identity, with significant downregulation in mRNA levels of Ins1 ($p \leq 0.0001$), Ins2 ($p=0.0004$), Nkx6.1 ($p=0.0156$) and Syt4 ($p=0.0015$) and a notable decrease in MafA ($p=0.0737$) (Fig. 5.10).

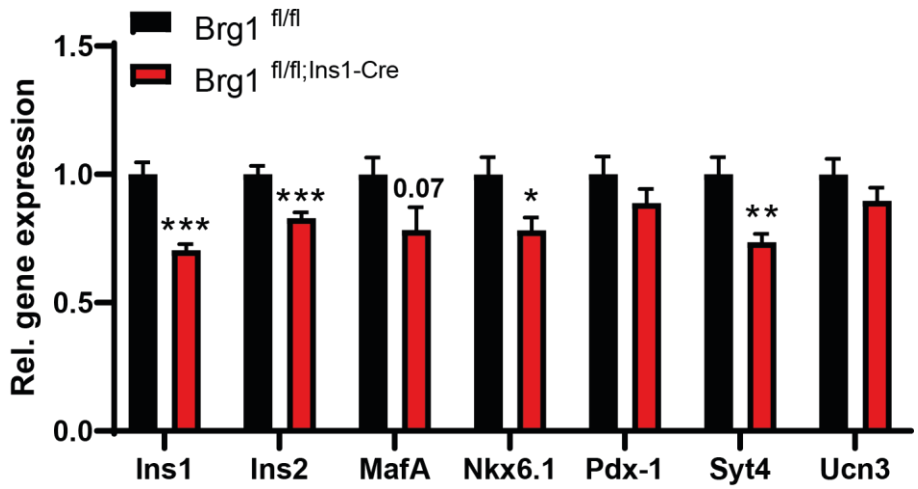

Figure 5.10: Gene expression of pancreatic β-cell markers in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. qPCR results for β-cell identity genes in in Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to in Brg1$^{fl/fl}$ mice at 24 weeks of age, normalised to *RPLP0/36B4*. n = 9 (Brg1$^{fl/fl}$), n = 11 (Brg1$^{fl/fl;Ins1\text{-}Cre}$). Data are means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, unpaired Student's t-test.

In work performed by T. Mak (Fig. 1.13) (Mak et al. under review), we observed an increase in Ins and Sst polyhormonal expressing cells that originated from β-cells in Tg7a2 animals compared to Wt controls, indicating that those cells undergo a process where they not only lose the β-cell identity but also acquire characteristics of other cells types possibly through transdifferentiation. To test if repression of Brg1 by miR-7 leads to the induction of δ-cell markers, we measured the expression of δ-cell specific markers in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice (Fig. 5.11). We observed a significant increase in Lepr ($p=0.0270$) mRNA levels, while other genes,

including Sst, were not affected (Fig. 5.11). We conclude that Brg1 knockout alone is not sufficient to induce the transdifferentiation of pancreatic β-cells into δ-cells. Other mechanisms than loss of Brg1 function must underlie the acquisition of a δ-cell identity in β-cells.

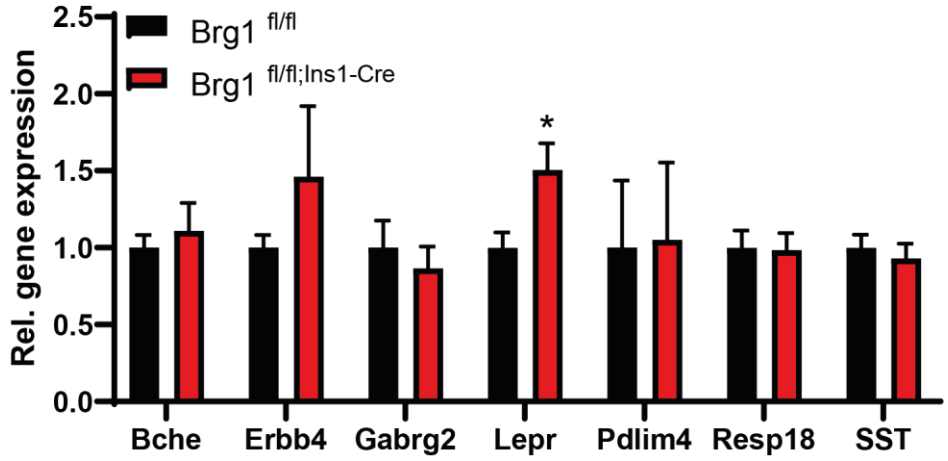

Figure 5.11: Gene expression of pancreatic δ-cell markers in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. qPCR results for δ-cell genes in Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to in Brg1$^{fl/fl}$ mice at 24 weeks of age, normalised to *36B4*. n = 9 (Brg1$^{fl/fl}$), n = 11 (Brg1$^{fl/fl;Ins1\text{-}Cre}$). Data are means ± SEM, *p < 0.05, unpaired Student's t-test.

5.4 Pharmacological inhibition of mSWI/SNF function *in vitro*

We next tested whether treatment of human islets with a mSWI/SNF complex inhibitor affects the expression of β-cell identity genes. For these studies we used PFI-3, identified by the Structural Genomic Consortium and in collaboration with the biopharmaceutical company Pfizer. PFI-3 has been shown to inhibit Brg1/Brm/Pb1 with high specificity as tested in a selective screening against 48 family members with a C-terminal bromodomain (BRD), as well as in a commercial screening of 102 cellular receptors and 30 enzyme assays showing no significant interactions outside the BRD family (Fedorov et al. 2015). We treated human islets, with PFI-3 at a concentration of 100 µM and looked at gene expression levels via RT-qPCR. Treatment with PFI-3 did not alter the expression of *BAF60A*, *BRG1* and *BRM*, showing that PFI-3 blocks their function without affecting their gene expression (Fig. 5.12A). In contrast, we measured downregulation of MafA (p=0.000989), Nkx2.2 (p=0.0041), Nkx6.1 (p=0.0081), Pdx-1 (p=0.0047) and Ucn3 (p=0.0275) mRNA levels (Fig. 5.12B).

Together those results demonstrate that inhibition of Brg1 triggers β-cell dedifferentiation in humans.

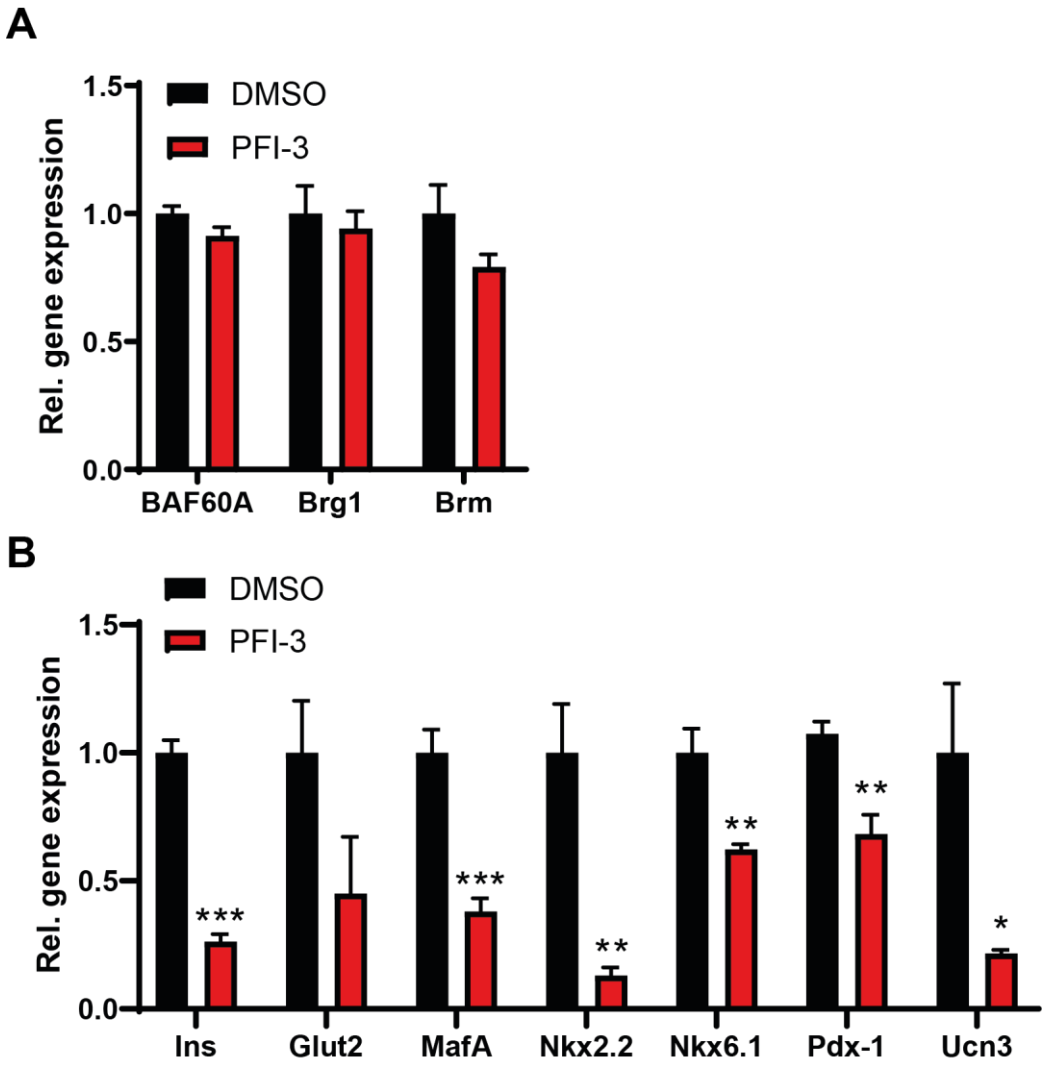

Figure 5.12: PFI-3 treatment of human pancreatic islets. (A,) qPCR results of human islets treated with 100 μM PFI-3 or an equal volume of DMSO, normalised to *36B4:* (A) mSWI/SNF member and (B) β-cell identity marker expression levels. n = 4. Data are means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, unpaired Student's t-test.

5.5 Discussion

With the data accumulated in the chapters 3 and 4, we were able to show a correlation between miR-7a2 induced loss of β-cell identity, epigenetic remodelling and the downregulation of the chromatin remodelling mSWI/SNF complexes subunit Brg1 (and BAF60A). However, our study was missing the link between those events, connecting the loss of Brg1 (and BAF60A) expression with the changes in dedifferentiating β-cells. Therefore, we next focused on the selective knockdown of Brg1 and BAF60A expression inside β-cells. In accordance with our previous findings showing that downregulation of BAF60A by miR-7a2, while found in *in vitro* experiments, does not expand into our studies *in vivo* (Chapter 4.2 and 4.3), selective knockdown of BAF60A only shows minor changes in the expression patterns of β-cell markers (Fig. 5.1). The selective knockdown of Brg1 (Fig. 5.2 and 5.3) showing loss of β-cell identity prompted us to further investigate the selective regulation of Brg1. At this point we mostly stopped the investigation of BAF60A as a target of miR-7a2 causative for a diabetic phenotype.

We tried to generate a BAF60A knockout mice utilising the CRISPR-Cas9 approach. None of the offspring showed deletion of BAF60A. This means that either knockout of BAF60A is lethal or the deletion was not successful. Additionally, we tried to establish a collaboration with Z. Meng from the Zhejiang University (ZJU) who had BAF60A floxed mice. Sadly, this collaboration was not established. With that being said, while our results show no major effects on the selective knockdown of BAF60A in relation to β-cell identity, its role in T2D should not be discarded as its co-dysregulation together with other genes, like Brg1, could have amplifying effects on the progression of the disease. Indeed, with the current research finding multiple genes regulated in diseases like T2D, it is very unlikely that a single gene is the sole trigger of the diabetic phenotype. Rather a combination of the dysregulation of multiple genes seems plausible. This is in some ways similar to the debunked hypothesis that obesity alone triggers T2D. This idea has been largely abandoned in favour of the assumption that individuals who are genetically predisposed can develop T2D when exposed to diabetes promoting environmental factors, like obesity or glucolipotoxicity (Kahn 2003, Cnop 2008). It can be generally assumed that single genetic predispositions can be compensated by the individual, and multiple dysregulations are more likely to promote the progression of severe T2D. Exceptions are obviously the MODY-genes, able to trigger diabetes based on single gene failure (Naylor , Johnson and del Gaudio 2018). Indeed, we detected a major difference in the loss of β-cell identity not only *in vitro* between miR-7a2 overexpression and selective Brg1/BAF60A knockdown, but also *in vivo* between Tg7a2 and $Brg1^{fl/fl;Ins1\text{-}Cre}$ mice. While

To ensure that Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice have a selective knockout of Brg1 without affecting the rest of the mSWI/SNF complexes, additional stainings for e.g. Brm and BAF60A should be performed in future experiments, to ensure that other subunits are not affected. Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice show no changes in *ad libitum* fed glycaemia levels (Fig. 5.6), previous results from our group described a significant hyperglycaemic phenotype in Tg7a2 animals (Latreille et al. 2014). Lastly, while Tg7a2 and other models of miR-7a2 overexpression show the expression of δ-cell markers in β-cells, no observations of this kind were made in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice (Fig. 5.11).

Together those results show that overexpression of miR-7a2 has a more severe effect on the loss of β-cell identity compared to the selective knockout of Brg1. As previously discussed, this is most likely due to the fact of multiple genes being affected in miR-7a2 overexpression models, as miRNAs have multiple targets (Hashimoto, Akiyama and Yuasa 2013). When looking back at the miR-7a2 target identification chapters of this thesis, especially the screen in immortalised β-cells (Chapter 4.2), we see that indeed miR-7a2 seems to have multiple potential targets (e.g. Tada2b, Setd8, etc.). While this study focussed on the effects of the mSWI/SNF complex subunits Brg1 and BAF60A, those other targets can have potential in deciphering the genetic changes undergoing in T2D.

Indeed, in previous studies, Tada2b has been reported to interact with Brg1 in HEK293T cells to activate Pax5 (Barlev et al. 2003), a TF associated with B cell differentiation (Kaneko et al. 1998, Pridans et al. 2008). Furthermore, we demonstrated a regulation of Brm in miR-7a2 overexpressing models, which we did not observe in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice, where knockout of Brg1 does not affect Brm mRNA levels (Fig. 5.5A), suggesting that other miR-7a2 targets than Brg1 affect the expression of Brm. Those phenomena would result in a double knockdown of Brg1 and Brm in miR-7a2 overexpressing models compared to the selective knockdown of Brg1 in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. Although previous studies recounted an antagonistic role for Brg1 and Brm (McKenna et al. 2015a), recent results report that a dual knockout of Brg1 and Brm in β-cells is needed to induce fasted hyperglycaemia, glucose intolerance and reduced serum insulin content (Spaeth et al. 2019). This confirms the hypothesis that multiple targeting of the miR-7a2 overexpression is more potent than single knockout of Brg1. While the effects are more severe in Tg7a2 animals, our results still show a diabetic phenotype in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. It may be prudent to mention that the studies in Spaeth et al. investigated an adult induced knockout of Brg1 on β-cell function, whereas our Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice lose Brg1 expression in early development. Indeed Spaet et al. show that E15.5 Brg1 knockout results in the reduction

of all pancreatic cell lineages, confirming that loss of Brg1 function affects β-cells. Therefore, miR-7a2 induced loss of Brg1 could represent a form of genetic susceptibility in individuals, making them prone to develop T2D in the future. Although not quantified, our results don't suggest a difference in pancreatic islet numbers or size. Opposite to our findings, Spaet et al. reported a reduction in pancreas size in Brg1 knockout mice in comparison to control animals. Additionally, they report a hypoplasia in mice with an embryonic specific removal of Brg1, and while we see an impairment of the islets to produce insulin, we detect no other signs of hypoplasia, but further studies are required to fully confirm/oppose those findings. It is to mention, that those findings were achieved utilising a Ptf1a-Cre to knockout Brg1 in early development, including MPCs. MPCs in Brg1 knockout animals also show reduced proliferation. We did not investigate the proliferation of β-cells in our mouse model. Knockout of Brg1 in adult animals resulted in no changes in fasting glycaemia, glucose tolerance, serum insulin levels or GSIS (Spaeth et al. 2019). Although their adult knockout is as well under control of the MIP-Cre, opposite to our studies they utilised a tamoxifen inducible system. Opposing findings between our groups can potentially be explained by the timepoint of deletion. A inducible Brg1 knockout mouse model would be required to investigate if different timepoints of Brg1 deletion result in different outcomes and if there is an age which protects the animals from developing a diabetic phenotype following Brg1 deletion.

Spaet et al. showed that many typical β-cell TFs are not affected by the loss of Brg1 and Brm. While this is true for Pdx-1 in our results (indicating that Brg1 knockout does not trigger a feedback on the expression of Pdx-1) we found other TFs important for β-cell function to be negatively affected by loss of Brg1, like Nkx6.1, MafA or Syt4 (Fig. 5.10). The closest we got to a selective double knockout of Brg1 and Brm in this study was the application of PFI-3. Indeed, our results utilising PFI-3 on human islets showed a significant decrease in multiple β-cell factors (Fig. 5.12). Those results confirm the severe effect of Brg1 and Brm double knockdown in β-cells reported by Spaeth et al.

With insulin content inside the islets and of 6h fast serum from $Brg1^{fl/fl;Ins1\text{-}Cre}$ mice being decreased (Fig. 5.9), we were quite surprised to observe a decrease in BG in $Brg1^{fl/fl;Ins1\text{-}Cre}$ compared to $Brg1^{fl/fl}$ mice after an ON fast (Fig. 5.7), as our expectations were for a hyperglycaemic phenotype. Although not further investigated, the hypoglycaemic phenotype in ON fasted $Brg1^{fl/fl;Ins1\text{-}Cre}$ compared to $Brg1^{fl/fl}$ mice could be explained by an inability of the β-cells to react to changes in glycaemia in either way, being neither able to compensate for hyper- nor hypoglycaemia. In healthy animals an ON fast triggers the release of glucose

storages into the bloodstream. This appears to be impaired in Brg1$^{fl/fl;Ins1\text{-}Cre}$. In future experiments, insulin levels should be measured after an ON fast to investigate if insulin levels are increased, as in this study only insulin levels after an 6h fast were measured. Additionally, insulin levels after an IPGTT would be required to confirm the results of an impaired glucose response in our IPGTT in Brg1$^{fl/fl;Ins1\text{-}Cre}$. Indeed other studies have shown an increase in basal insulin levels in immature β-cells, as well as their inability to react to glycaemic changes (Huang et al. 2018). They explain those changes by the switch of a repression to activation of Syt4 in maturation of β-cells, compensating the insulin secretion promoting effect of Syt7 expressed in immature and mature β-cells (Huang et al. 2018). Syt4 and Syt7 are both found on insulin vesicles inside β-cells with opposite effects based on their Ca^{2+} sensitivity. Syt7, being a high affinity Ca^{2+} sensor, promotes the fusion of insulin vesicles with the cell membrane promoting exocytosis, while the low Ca^{2+} affinity of Syt4 represses vesicles membrane fusion (Huang et al. 2018). Since one of our hypotheses in the lab is the loss of β-cell identity into a more immature type by miR-7a2, we investigated the expression profile of Syt4 in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. Interestingly, Brg1$^{fl/fl;Ins1\text{-}Cre}$ compared to Brg1$^{fl/fl}$ mice show a decrease in the gene expression of Syt4 (Fig. 5.10). This would confirm our hypothesis that miR-7a2 triggers immaturity in β-cells in accordance with the findings of Huang et al., showing that Syt4 is a marker of the mature β-cell.

This immaturity could explain the inability of Brg1$^{fl/fl;Ins1\text{-}Cre}$ β-cells to compensate for hypoglycaemia in an ON fasted state (Fig. 5.7) and the observed lack of insulin content stored in β-cells (Fig. 5.9) prevents the compensation of the induced hyperglycaemia in glucose tolerance tests (Fig. 5.7). As Brg1 is selectively knocked out in β-cells of Brg1$^{flfl;Ins1\text{-}Cre}$ mice, our insulin tolerance tests results, showing no significant difference (Fig. 5.8), were expected. This shows that other tissues (like the liver or muscles) are not affected by an unspecific knockout of Brg1, and that the knockout in β-cells does not affects those peripheral tissues.

In summary the Brg1$^{fl/fl;Ins1\text{-}Cre}$ shows the importance of Brg1 in maintaining β-cell identity through reducing available insulin content in the islet and therefore loss in circulating insulin, an impairment in glucose tolerance and the loss of expression of key β-cell identity markers, as similarly seen in Tg7a2 animals. To consolidate our findings, rescue experiments that re-establish Brg1 expression in cells or islets exposed to elevated miR-7a2 levels would be required to fully elucidate the role Brg1 plays in the miR-7a2 induced diabetic phenotype. Additionally, ATAC-Seq should be performed on Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. Although Tg7a2 animals show a closure of DARs in comparison to Wt controls, and Brg1 is involved in

chromatin remodelling and associated to transcriptional activation, this experiment would allow us to compare the data with our ATAC-Seq findings in Tg7a2 animals.

Our results utilising the Brg1/Brm/Pb1 inhibitor PFI-3, showing a downregulation of β-cell genes in human islets, confirmed the translation of our findings into human (Fig. 5.12). Interestingly there is no evidence yet in the literature showing that miR-7 is directly involved in the progression of T2D. The only relation between miR-7 and T2D was presented by Wan et al. suggesting serum miR-7 levels as a promising biomarker for T2D (Wan et al. 2017). Opposite to that, dedifferentiation of β-cells in human T2D is well reported (Cinti et al. 2016b, Efrat 2019), confirming the possibility of our results showing a loss of β-cell identity in β-cells from human donor islets through miR-7a2 and impairment of Brg1/Brm/Pb1.

In summary, our results in this chapter help us further understand the biology of miR-7a2 induced T2D by deciphering the mechanisms of how overexpression of miR-7a2 induces a diabetic phenotype. We were able to show that compromised hyperglycaemia compensation and the loss of β-cell identity we observe in miR-7a2 overexpressing models can be attributed to the miR-7a2 regulated knockdown of Brg1. Breaking down pathways of T2D progression will improve our understanding of this disease and support future drug development.

Chapter 6: Summary and future directions

The data presented in this study provides new information about the involvement of miR-7 in the progression of T2D. With the rising incidence of T2D in the global population, new insights are necessary to better understand the characteristics of the disease and therefore treat patients.

In chapter 3, we showed that miR-7a2 upregulation induces a loss in β-cell identity in different models, both *in vitro* and *in vivo*. This loss of β-cell identity is characterised by the downregulation of β-cell specific markers like insulin, Nkx6.1 or MafA. This loss of β-cell identity is seen in immortalised pancreatic β-cell lines, isolated islets of mice and human donors, as well as in different mouse models of diabetes. Loss of β-cell identity is accompanied with the de-repression of atypical hormones, including somatostatin or glucagon. Those results suggest that miR-7a2 overexpression triggers the dedifferentiation and transdifferentiation of β-cells into other cell types. Therefore miR-7a2 may represent a novel target in the treatment of T2D. Modulating miR-7a2 levels in patients comes nonetheless with complications, especially in the specific application to β-cells. Gene therapy utilising viral vectors with a high specificity for β-cells could provide an approach to introduce miR-7a2 inhibitors, like the circular RNA miR-7 sponge Cdr1as. Photoactivatable drugs could provide an approach to apply miR-7 inhibitors (e.g. LNAs) with a selective activity in the pancreas. In previous work on Liraglutide, we confirmed the control over the activity of the drug through UV light (Broichhagen et al. 2015). Small UV light emitting probes could be put into the pancreas and therefore similar approaches for miR-7a2 inhibitors could be applied.

In future work it would be interesting to investigate the effects of miR-7 inhibitors on the progression of T2D. Additionally, investigating if inhibition of miR-7 could redifferentiate β-cells that have lost their identity could provide a new avenue to increase the functional β-cell mass in individuals with T2D. Overall the research in the dedifferentiation of β-cells, to which this study contributes, has made much progress. Therefore, future studies should focus on progressing the research towards the actual goal to utilise the dedifferentiated β-cells that are still alive and use them to replenish the diminished functional β-cell mass.

Dedifferentiated β-cells, including those in Tg7a2 mice, display endocrine progenitor-like characteristics (Wang et al. 2014, Oshima et al. 2018, Lu et al. 2018, Talchai et al. 2012, Latreille et al. 2014). Therefore, it would be interesting to investigate if it is possible to induce proliferation of those progenitor-like cells and induce their differentiation into mature β-cells. This therapy would provide an approach to counteract the loss of functional β-cell mass

induced by dedifferentiation and restore normal islet composition. With miR-7 being involved in the development of many different tissues (Horsham et al. 2015), alteration of miR-7 levels in those endocrine progenitor-like cells could provide the required tool to induce proliferation and/or differentiation.

Chapter 4 focussed on the identification of epigenetic regulators targeted by miR-7. We showed that the chromatin remodelling complex mSWI/SNF complex is regulated by miR-7 through the repression of BAF60A *in vitro* and Brg1 *in vitro* and *in vivo*. We investigated the chromatin landscape in isolated islets of β-cell specific miR-7a2 overexpressing mice (Tg7a2) by ATAC-Seq. Our results show a closed chromatin state in Tg7a2 animals compared to Wt animals at key β-cell markers, as well as at binding sites for β-cell specific TFs. These results indicate that the diabetic phenotype induced by miR-7a2 overexpression, is caused by changes in the chromatin state preventing transcription activation of genes maintaining β-cell identity and function.

In future work it would be interesting to investigate the chromatin landscape by ATAC-Seq in Brg1 knockout mice and compare it to the ATAC-Seq obtained in islets from Tg7a2 animals. The comparison would show if the chromatin remodelling seen in Tg7a2 can be fully accounted to miR-7a2's repression of the mSWI/SNF complexes or if other targets genes of miR-7 also impact on chromatin structure. Additionally, future work should address if rescue of Brg1 expression in β-cells overexpressing miR-7a2 can reverse the chromatin state and/or diabetic phenotype. Those results would give insight into whether Brg1 is the major chromatin remodeller affected by miR-7a2 and if the loss of β-cell identity can be solely accounted to epigenetic changes. The identification of Brg1 and BAF60A as targets of miR-7a2 sheds new light on the mechanism by which miR-7a2 induces T2D.

Since miR-7a2 is present in many tissues, and has been shown to prevent cancer formation (Zhang et al. 2014), its inhibition could have severe side effects. Therefore, understanding the function of miR-7a2 inside β-cells helps developing potential future treatments. The further research deciphers the pathways involved in T2D progression, the more specific can potential drugs target the causative factors in T2D while avoiding side effects inside the same or different organs.

The results presented in chapter 5 showed that deletion of Brg1 in β-cells of mice ($Brg1^{fl/fl;Ins1\text{-}Cre)}$ results in loss of β-cell markers. Deletion of Brg1 in β-cells reduces the islet insulin content,

secretion of insulin into circulation and impairs GSIS. Those results show the involvement of the mSWI/SNF complexes to maintain functional β-cell mass *in vivo*.

Interestingly, the diabetic phenotype in Tg7a2 animals is stronger than in Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice. This suggests that more factors are targeted by miR-7a2 that promote β-cell dedifferentiation in T2D. It would be interesting to investigate other miR-7 targets and determine if they interact with the mSWI/SNF complexes. For example, Tada2b, a transcriptional adapter protein that potentiates transcription through coordination of HAT activity, has been shown to interact with Brg1 (Barlev et al. 2003). According to our qPCR results in Min6 cells infected with Ad-7a2/Ad-Ctrl, miR-7a2 repressed Tada2b. The loss of multiple epigenetic factors could have a more severe effect on the identity of the β-cells. Our results show the involvement of Brg1 in maintaining β-cell identity, as knockout of Brg1 impairs β-cell identity genes and the glucose response of Brg1$^{fl/fl;Ins1\text{-}Cre}$ mice, but not as severe as in Tg7a2 animals overexpressing miR-7a2. Therefore, the research of additionally miR-7a2 targets interacting with Brg1 could present valuable insight in how miR-7a2 triggers β-cell dedifferentiation.

Since epigenetic regulation is essential for the correct development of cells and organs, it would be interesting to investigate the effects of the miR-7-Brg1 axis in the development of the pancreas and β-cells. The TF Pdx-1 is involved in the development of β-cells from approximately E8.5 (Conrad et al. 2014). Since Brg1 and Pdx-1 have been shown to interact (McKenna et al. 2015a), it is to assume that the differentiation of β-cells would be disturbed. Pdx-1 is only one of many TFs in the development of β-cells. Future work could also focus to identify additional TFs interacting with Brg1 in the pancreas in development and the adult animal.

Overall, our findings reveal a new regulatory pathway in the progression of T2D. This opens new avenues in researching the biology of the diseases and will hopefully contribute in the development of new treatments for T2D focussing on re-establishing β-cell identity.

Bibliography

Aamodt, K. I. & A. C. Powers (2017) Signals in the pancreatic islet microenvironment influence β-cell proliferation. *Diabetes, Obesity and Metabolism,* 19**,** 124-136.

Abdellatif, A. M., H. Oishi, T. Itagaki, Y. Jung, H. H. Shawki, Y. Okita, Y. Hasegawa, H. Suzuki, S. E. El-Morsy, M. A. El-Sayed, M. B. Shoaib, F. Sugiyama & S. Takahashi (2016) β-Cell-Specific Mafk Overexpression Impairs Pancreatic Endocrine Cell Development. *PLOS ONE,* 11**,** e0150010.

Agarwal, V., G. W. Bell, J. Nam & D. P. Bartel (2015) Predicting effective microRNA target sites in mammalian mRNAs. *eLife,* 4:e05005.

Aguilar-Bryan, L., C. G. Nichols, S. W. Wechsler, J. P. Clement, A. E. Boyd, G. Gonzalez, H. Herrera-Sosa, K. Nguy, J. Bryan & D. A. Nelson (1995) Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science,* 268**,** 423.

Ahlgren, U., J. Jonsson, L. Jonsson, K. Simu & H. Edlund (1998) β-Cell-specific inactivation of the mouseIpf1/Pdx1 gene results in loss of the β-cell phenotype and maturity onset diabetes. *Genes & Development,* 12**,** 1763-1768.

Ahmed, K., M. P. LaPierre, E. Gasser, R. Denzler, Y. Yang, T. Rülicke, J. Kero, M. Latreille & M. Stoffel (2017) Loss of microRNA-7a2 induces hypogonadotropic hypogonadism and infertility. *The Journal of clinical investigation,* 127**,** 1061-1074.

Ahrén, B., E. Simonsson, H. Larsson, M. Landin-Olsson, H. Torgeirsson, P.-A. Jansson, M. Sandqvist, P. Båvenholm, S. Efendic, J. W. Eriksson, S. Dickinson & D. Holmes (2002) Inhibition of Dipeptidyl Peptidase IV Improves Metabolic Control Over a 4-Week Study Period in Type 2 Diabetes. *Diabetes Care,* 25**,** 869.

Alberti, K. G. M. M., N. Juel Christensen, S. Engkjær Christensen, A. A. Prange Hansen, J. Iversen, K. Lundbæk, K. Seyer-Hansen & H. O/rskov (1973) INHIBITION OF INSULIN SECRETION BY SOMATOSTATIN. *The Lancet,* 302**,** 1299-1301.

Albini, S., P. Coutinho Toto, A. Dall'Agnese, B. Malecova, C. Cenciarelli, A. Felsani, M. Caruso, S. J. Bultman & P. L. Puri (2015) Brahma is required for cell cycle arrest and late muscle gene expression during skeletal myogenesis. *EMBO reports,* 16**,** 1037-1050.

Alessi, D. R., S. R. James, C. P. Downes, A. B. Holmes, P. R. J. Gaffney, C. B. Reese & P. Cohen (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bα. *Current Biology,* 7**,** 261-269.

Ali, O. (2013) Genetics of type 2 diabetes. *World journal of diabetes,* 4**,** 114-123.

Ambros, V. (2001) microRNAs: Tiny Regulators with Great Potential. *Cell,* 107**,** 823-826.

Anamika, K., A. R. Krebs, J. Thompson, O. Poch, D. Devys & L. Tora (2010) Lessons from genome-wide studies: an integrated definition of the coactivator function of histone acetyl transferases. *Epigenetics & chromatin,* 3**,** 18-18.

Anders, S. & W. Huber (2010) Differential expression analysis for sequence count data. *Genome Biology,* 11**,** R106.

Anello, M., A. M. Rabuazzo, C. Degano, V. Caltabiano, G. Patanè, R. Vigneri & F. Purrello (1996) Fast Reversibility of Glucose-Induced Desensitization in Rat Pancreatic Islets: Evidence for an Involvement of Ionic Fluxes. *Diabetes,* 45**,** 502.

Antequera, F. (2007) CpG Islands and DNA Methylation. *eLS*.

Aran, D., G. Toperoff, M. Rosenberg & A. Hellman (2010) Replication timing-related and gene body-specific methylation of active human genes. *Human Molecular Genetics,* 20**,** 670-680.

Aravin, A. A., M. Lagos-Quintana, A. Yalcin, M. Zavolan, D. Marks, B. Snyder, T. Gaasterland, J. Meyer & T. Tuschl (2003) The Small RNA Profile during Drosophila melanogaster Development. *Developmental Cell,* 5**,** 337-350.

Aronne, L. J. & K. R. Segal (2002) Adiposity and Fat Distribution Outcome Measures: Assessment and Clinical Implications. *Obesity Research,* 10**,** 14S-21S.

Aronoff, S. L., K. Berkowitz, B. Shreiner & L. Want (2004) Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. *Diabetes Spectrum,* 17**,** 183.

Artner, I., J. Le Lay, Y. Hang, L. Elghazi, J. C. Schisler, E. Henderson, B. Sosa-Pineda & R. Stein (2006) MafB: an activator of the glucagon gene expressed in developing islet alpha- and beta-cells. *Diabetes,* 55**,** 297.

Ashcroft, F. M. & P. Rorsman (1989) Electrophysiology of the pancreatic β-cell. *Progress in Biophysics and Molecular Biology,* 54**,** 87-143.

Ashcroft, S. J. H. & P. J. Randle (1970) Enzymes of glucose metabolism in normal mouse pancreatic islets. *Biochemical Journal,* 119**,** 5.

Atlasi, Y. & H. G. Stunnenberg (2017) The interplay of epigenetic marks during stem cell differentiation and development. *Nature Reviews Genetics,* 18**,** 643.

Avrahami, D., A. Klochendler, Y. Dor & B. Glaser (2017) Beta cell heterogeneity: an evolving concept. *Diabetologia,* 60**,** 1363-1369.

Avrahami, D., C. Li, J. Zhang, J. Schug, R. Avrahami, S. Rao, Michael B. Stadler, L. Burger, D. Schübeler, B. Glaser & Klaus H. Kaestner (2015) Aging-Dependent Demethylation of Regulatory Elements Correlates with Chromatin State and Improved β Cell Function. *Cell Metabolism,* 22**,** 619-632.

Bahar Halpern, K., T. Vana & M. D. Walker (2014) Paradoxical role of DNA methylation in activation of FoxA2 gene expression during endoderm development. *Journal of Biological Chemistry*.

Bai, F., H. L. Chan, A. Scott, M. D. Smith, C. Fan, J. I. Herschkowitz, C. M. Perou, A. S. Livingstone, D. J. Robbins, A. J. Capobianco & X.-H. Pei (2014) BRCA1 Suppresses Epithelial-to-Mesenchymal Transition and Stem Cell Dedifferentiation during Mammary and Tumor Development. *Cancer Research,* 74**,** 6161.

Ball, M. P., J. B. Li, Y. Gao, J.-H. Lee, E. M. LeProust, I.-H. Park, B. Xie, G. Q. Daley & G. M. Church (2009) Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. *Nature biotechnology,* 27**,** 361-368.

Bandyopadhyay, D., A. Kusari, K. A. Kenner, F. Liu, J. Chernoff, T. A. Gustafson & J. Kusari (1997) Protein-Tyrosine Phosphatase 1B Complexes with the Insulin Receptor in Vivo and Is Tyrosine-phosphorylated in the Presence of Insulin. *Journal of Biological Chemistry,* 272**,** 1639-1645.

Bansal, V., J. Kalita & U. K. Misra (2006) Diabetic neuropathy. *Postgrad Med J,* 82**,** 95-100.

Barlev, N. A., A. V. Emelyanov, P. Castagnino, P. Zegerman, A. J. Bannister, M. A. Sepulveda, F. Robert, L. Tora, T. Kouzarides, B. K. Birshtein & S. L. Berger (2003) A novel human Ada2 homologue functions with Gcn5 or Brg1 to coactivate transcription. *Molecular and cellular biology,* 23**,** 6944-6957.

Bays, H., L. Mandarino & R. A. DeFronzo (2004) Role of the Adipocyte, Free Fatty Acids, and Ectopic Fat in Pathogenesis of Type 2 Diabetes Mellitus: Peroxisomal Proliferator-Activated Receptor Agonists Provide a Rational Therapeutic Approach. *The Journal of Clinical Endocrinology & Metabolism,* 89**,** 463-478.

Bedoya, F. J. & B. Jeanrenaud (1991a) Evolution of Insulin Secretory Response to Glucose by Perifused Islets From Lean (FA/FA) Rats Chronically Infused With Glucose. *Diabetes,* 40**,** 7.

--- (1991b) Insulin Secretory Response to Secretagogues by Perifused Islets From Chronically Glucose-Infused Rats. *Diabetes,* 40**,** 15.

Ben-Shushan, E., S. Marshak, M. Shoshkes, E. Cerasi & D. Melloul (2001) A Pancreatic β-Cell-specific Enhancer in the HumanPDX-1 Gene Is Regulated by Hepatocyte Nuclear Factor 3β (HNF-3β), HNF-1α, and SPs Transcription Factors. *Journal of Biological Chemistry,* 276**,** 17533-17540.

Berg, J. M., T. J.L. & L. Stryer. 2002. *Some Receptors Dimerize in Response to Ligand Binding and Signal by Cross-phosphorylation*. New York: W H Freeman.

Berg, J. M., J. L. Tymoczko & L. Stryer. 2002. *Biochemistry*. New York: W. H. Freeman and Company.

Berger, S. L., T. Kouzarides, R. Shiekhattar & A. Shilatifard (2009) An operational definition of epigenetics. *Genes & development,* 23**,** 781-783.

Bergman, R. N. (2000) Non-esterified fatty acids and the liver: why is insulin secreted into the portal vein? *Diabetologia,* 43**,** 946-952.

Bernardo, A. S., C. W. Hay & K. Docherty (2008) Pancreatic transcription factors and their role in the birth, life and survival of the pancreatic β cell. *Molecular and Cellular Endocrinology,* 294**,** 1-9.

Bernstein, B. E., T. S. Mikkelsen, X. Xie, M. Kamal, D. J. Huebert, J. Cuff, B. Fry, A. Meissner, M. Wernig, K. Plath, R. Jaenisch, A. Wagschal, R. Feil, S. L. Schreiber & E. S. Lander (2006) A Bivalent Chromatin Structure Marks Key Developmental Genes in Embryonic Stem Cells. *Cell,* 125**,** 315-326.

Bernstein, E., A. A. Caudy, S. M. Hammond & G. J. Hannon (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature,* 409**,** 363-366.

Bessis, N., F. J. GarciaCozar & M. C. Boissier (2004) Immune responses to gene therapy vectors: influence on vector function and effector mechanisms. *Gene Therapy,* 11**,** S10-S17.

Bestor, T., A. Laudano, R. Mattaliano & V. Ingram (1988) Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells: The carboxyl-terminal domain of the mammalian enzymes is related to bacterial restriction methyltransferases. *Journal of Molecular Biology,* 203**,** 971-983.

Bestor, T. H. (2000) The DNA methyltransferases of mammals. *Human Molecular Genetics,* 9**,** 2395-2402.

Bhushan, A., N. Itoh, S. Kato, J. P. Thiery, P. Czernichow, S. Bellusci & R. Scharfmann (2001) Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis. *Development,* 128**,** 5109.

Biankin, A. V., N. Waddell, K. S. Kassahn, M.-C. Gingras, L. B. Muthuswamy, A. L. Johns, D. K. Miller, P. J. Wilson, A.-M. Patch, J. Wu, D. K. Chang, M. J. Cowley, B. B. Gardiner, S. Song, I. Harliwong, S. Idrisoglu, C. Nourse, E. Nourbakhsh, S. Manning, S. Wani, M. Gongora, M. Pajic, C. J. Scarlett, A. J. Gill, A. V. Pinho, I. Rooman, M. Anderson, O. Holmes, C. Leonard, D. Taylor, S. Wood, Q. Xu, K. Nones, J. Lynn Fink, A. Christ, T. Bruxner, N. Cloonan, G. Kolle, F. Newell, M. Pinese, R. Scott Mead, J. L. Humphris, W. Kaplan, M. D. Jones, E. K. Colvin, A. M. Nagrial, E. S. Humphrey, A. Chou, V. T. Chin, L. A. Chantrill, A. Mawson, J. S. Samra, J. G. Kench, J. A. Lovell, R. J. Daly, N. D. Merrett, C. Toon, K. Epari, N. Q. Nguyen, A. Barbour, N. Zeps, I. Australian Pancreatic Cancer Genome, M.-A. L. Brancato, S. J. Rowe, S. L. Simpson, M. Martyn-Smith, M. T. Thomas, J. Pettit, J. Tao, R. DiPietro, C. Watson, R. Wong, M. Giry-Laterriere, E. A. Musgrove, R. L. Sutherland, S. M. Grimmond, J. V. Pearson, N. Pavlakis, A. Guminski, R. Asghari, D. A. Pavey, A. Das, P. H. Cosman, K. Ismail, C. O'Connor, V. W. Lam, D. McLeod, H. C. Pleass, A. Richardson, V. James, C. L. Cooper, D. Joseph, C. Sandroussi, M. Crawford, J. Gallagher, M. Texler, C. Forrest, A. Laycock, K. P. Epari, M. Ballal, et al. (2012) Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature,* 491**,** 399.

Björklund, A., A. Lansner & V. E. Grill (2000) Glucose-induced [Ca2+]i abnormalities in human pancreatic islets: important role of overstimulation. *Diabetes,* 49**,** 1840.

Blackshear, P. J. (1977) Biguanides and lactic acidosis in diabetics. *British medical journal,* 2**,** 1436-1436.

Blodgett, D. M., A. Nowosielska, S. Afik, S. Pechhold, A. J. Cura, N. J. Kennedy, S. Kim, A. Kucukural, R. J. Davis, S. C. Kent, D. L. Greiner, M. G. Garber, D. M. Harlan & P. diIorio (2015) Novel Observations From Next-Generation RNA Sequencing of Highly Purified Human Adult and Fetal Islet Cell Subsets. *Diabetes,* 64**,** 3172-3181.

Blum, B., A. N. Roose, O. Barrandon, R. Maehr, A. C. Arvanites, L. S. Davidow, J. C. Davis, Q. P. Peterson, L. L. Rubin & D. A. Melton (2014) Reversal of β cell de-differentiation by a small molecule inhibitor of the TGFβ pathway. *eLife,* 3**,** e02809-e02809.

Boden, G. (1997) Role of Fatty Acids in the Pathogenesis of Insulin Resistance and NIDDM. *Diabetes,* 46**,** 3.

Bohnsack, M. T., K. Czaplinski & D. Gorlich (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA (New York, N.Y.),* 10**,** 185-191.
Boland, M. J., K. L. Nazor & J. F. Loring (2014) Epigenetic regulation of pluripotency and differentiation. *Circulation research,* 115**,** 311-324.
Bolger, A. M., M. Lohse & B. Usadel (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics (Oxford, England),* 30**,** 2114-2120.
Bosson, A. D., J. R. Zamudio & P. A. Sharp (2014) Endogenous miRNA and target concentrations determine susceptibility to potential ceRNA competition. *Molecular cell,* 56**,** 347-359.
Boucher, J., A. Kleinridders & C. R. Kahn (2014) Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harbor perspectives in biology,* 6**,** a009191.
Bramswig, N. C., L. J. Everett, J. Schug, C. Dorrell, C. Liu, Y. Luo, P. R. Streeter, A. Naji, M. Grompe & K. H. Kaestner (2013) Epigenomic plasticity enables human pancreatic alpha to beta cell reprogramming. *J Clin Invest,* 123**,** 1275-84.
Brandenberger, R., H. Wei, S. Zhang, S. Lei, J. Murage, G. J. Fisk, Y. Li, C. Xu, R. Fang, K. Guegler, M. S. Rao, R. Mandalam, J. Lebkowski & L. W. Stanton (2004) Transcriptome characterization elucidates signaling networks that control human ES cell growth and differentiation. *Nature Biotechnology,* 22**,** 707-716.
Brange, J., L. Andersen, E. D. Laursen, G. Meyn & E. Rasmussen (1997) Toward Understanding Insulin Fibrillation. *Journal of Pharmaceutical Sciences,* 86**,** 517-525.
Bravo-Egana, V., S. Rosero, R. D. Molano, A. Pileggi, C. Ricordi, J. Dominguez-Bendala & R. L. Pastori (2008) Quantitative differential expression analysis reveals miR-7 as major islet microRNA. *Biochem Biophys Res Commun,* 366**,** 922-6.
Brenet, F., M. Moh, P. Funk, E. Feierstein, A. J. Viale, N. D. Socci & J. M. Scandura (2011) DNA methylation of the first exon is tightly linked to transcriptional silencing. *PloS one,* 6**,** e14524-e14524.
Brereton, M. F., M. Iberl, K. Shimomura, Q. Zhang, A. E. Adriaenssens, P. Proks, I. I. Spiliotis, W. Dace, K. K. Mattis, R. Ramracheya, F. M. Gribble, F. Reimann, A. Clark, P. Rorsman & F. M. Ashcroft (2014) Reversible changes in pancreatic islet structure and function produced by elevated blood glucose. *Nature communications,* 5**,** 4639-4639.
Briaud, I., C. Rouault, G. Reach & V. Poitout (1999) Long-term exposure of isolated rat islets of langerhans to supraphysiologic glucose concentrations decreases insulin mRNA levels. *Metabolism - Clinical and Experimental,* 48**,** 319-323.
Brissova, M., M. J. Fowler, W. E. Nicholson, A. Chu, B. Hirshberg, D. M. Harlan & A. C. Powers (2005) Assessment of Human Pancreatic Islet Architecture and Composition by Laser Scanning Confocal Microscopy. *Journal of Histochemistry & Cytochemistry,* 53**,** 1087-1097.
Brissova, M., M. Shiota, W. E. Nicholson, M. Gannon, S. M. Knobel, D. W. Piston, C. V. E. Wright & A. C. Powers (2002) Reduction in Pancreatic Transcription Factor PDX-1 Impairs Glucose-stimulated Insulin Secretion. *Journal of Biological Chemistry,* 277**,** 11225-11232.
Brogiolo, W., H. Stocker, T. Ikeya, F. Rintelen, R. Fernandez & E. Hafen (2001) An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. *Current Biology,* 11**,** 213-221.
Broichhagen, J., T. Podewin, H. Meyer-Berg, Y. von Ohlen, N. R. Johnston, B. J. Jones, S. R. Bloom, G. A. Rutter, A. Hoffmann-Röder, D. J. Hodson & D. Trauner (2015) Optical Control of Insulin Secretion Using an Incretin Switch. *Angewandte Chemie (International ed. in English),* 54**,** 15565-15569.
Buenrostro, J. D., B. Wu, H. Y. Chang & W. J. Greenleaf (2015a) ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Current protocols in molecular biology,* 109**,** 21.29.1-21.29.9.
--- (2015b) ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Curr Protoc Mol Biol,* 109**,** 21 29 1-9.

Burke, S. J., H. M. Batdorf, D. H. Burk, R. C. Noland, A. E. Eder, M. S. Boulos, M. D. Karlstad & J. J. Collier (2017) db/db Mice Exhibit Features of Human Type 2 Diabetes That Are Not Present in Weight-Matched C57BL/6J Mice Fed a Western Diet. *Journal of diabetes research,* 2017**,** 8503754-8503754.

Buse, J. B., J. Rosenstock, G. Sesti, W. E. Schmidt, E. Montanya, J. H. Brett, M. Zychma & L. Blonde (2009) Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). *The Lancet,* 374**,** 39-47.

Butler, A. E., S. Dhawan, J. Hoang, M. Cory, K. Zeng, H. Fritsch, J. J. Meier, R. A. Rizza & P. C. Butler (2016) β-Cell Deficit in Obese Type 2 Diabetes, a Minor Role of β-Cell Dedifferentiation and Degranulation. *The Journal of Clinical Endocrinology & Metabolism,* 101**,** 523-532.

Butler, A. E., J. Janson, S. Bonner-Weir, R. Ritzel, R. A. Rizza & P. C. Butler (2003) β-Cell Deficit and Increased β-Cell Apoptosis in Humans With Type 2 Diabetes. *Diabetes,* 52**,** 102.

Butterfield, W. J. H. & M. J. Whichelow (1965) Peripheral glucose metabolism in control subjects and diabetic patients during glucose, glucose-insulin and insulin sensitivity tests. *Diabetologia,* 1**,** 43-53.

Bysani, M., R. Agren, C. Davegårdh, P. Volkov, T. Rönn, P. Unneberg, K. Bacos & C. Ling (2019) ATAC-seq reveals alterations in open chromatin in pancreatic islets from subjects with type 2 diabetes. *Scientific Reports,* 9**,** 7785.

Cabrera, O., Berman, D. M., Kenyon, N. S., Ricordi, C., Berggren, P.-O. and Caicedo, A. (2006) The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proceedings of the National Academy of Sciences of the United States of America,* 103**,** 2334-2339.

Cai, D., S. Dhe-Paganon, P. A. Melendez, J. Lee & S. E. Shoelson (2003) Two New Substrates in Insulin Signaling, IRS5/DOK4 and IRS6/DOK5. *Journal of Biological Chemistry,* 278**,** 25323-25330.

Cairns, B. R., R. S. Levinson, K. R. Yamamoto & R. D. Kornberg (1996) Essential role of Swp73p in the function of yeast Swi/Snf complex. *Genes & Development,* 10**,** 2131-2144.

Campbell, Jonathan E. & Daniel J. Drucker (2013) Pharmacology, Physiology, and Mechanisms of Incretin Hormone Action. *Cell Metabolism,* 17**,** 819-837.

Campbell, P. J., L. J. Mandarino & J. E. Gerich (1988) Quantification of the relative impairment in actions of insulin on hepatic glucose production and peripheral glucose uptake in non-insulin-dependent diabetes mellitus. *Metabolism - Clinical and Experimental,* 37**,** 15-21.

Cannell, Ian G., Yi W. Kong & M. Bushell (2008) How do microRNAs regulate gene expression? *Biochemical Society Transactions,* 36**,** 1224.

Cano, D. A., B. Soria, F. Martín & A. Rojas (2014) Transcriptional control of mammalian pancreas organogenesis. *Cellular and Molecular Life Sciences,* 71**,** 2383-2402.

Cao, C. & M. R. Brown (2001) Localization of an insulin-like peptide in brains of two flies. *Cell and Tissue Research,* 304**,** 317-321.

Cao, R., L. Wang, H. Wang, L. Xia, H. Erdjument-Bromage, P. Tempst, R. S. Jones & Y. Zhang (2002) Role of Histone H3 Lysine 27 Methylation in Polycomb-Group Silencing. *Science,* 298**,** 1039.

Capito, C., M.-T. Simon, V. Aiello, A. Clark, Y. Aigrain, P. Ravassard & R. Scharfmann (2013) Mouse muscle as an ectopic permissive site for human pancreatic development. *Diabetes,* 62**,** 3479-3487.

Carpenter, A. E., T. R. Jones, M. R. Lamprecht, C. Clarke, I. H. Kang, O. Friman, D. A. Guertin, J. H. Chang, R. A. Lindquist, J. Moffat, P. Golland & D. M. Sabatini (2006) CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biology,* 7**,** R100.

Carrasco, M., I. Delgado, B. Soria, F. Martín & A. Rojas (2012) GATA4 and GATA6 control mouse pancreas organogenesis. *The Journal of clinical investigation,* 122**,** 3504-3515.

Catterall, W. A. (2011) Voltage-gated calcium channels. *Cold Spring Harbor perspectives in biology,* 3**,** a003947-a003947.

Catterall, W. A. & J. Striessnig (1992) Receptor sites for Ca²⁺ channel antagonists. *Trends in Pharmacological Sciences,* 13**,** 256-262.

Cefalu, W. T. (2001) Insulin Resistance: Cellular and Clinical Concepts. *Experimental Biology and Medicine,* 226**,** 13-26.
--- (2006) Animal Models of Type 2 Diabetes: Clinical Presentation and Pathophysiological Relevance to the Human Condition. *ILAR Journal,* 47**,** 186-198.
Ceranowicz, P., J. Cieszkowski, Z. Warzecha, B. Kuśnierz-Cabala & A. Dembiński (2015) The Beginnings of Pancreatology as a Field of Experimental and Clinical Medicine. *BioMed research international,* 2015**,** 128095-128095.
Cha, J.-Y., H.-i. Kim, K.-S. Kim, M.-W. Hur & Y.-h. Ahn (2000) Identification of Transacting Factors Responsible for the Tissue-specific Expression of Human Glucose Transporter Type 2 Isoform Gene: COOPERATIVE ROLE OF HEPATOCYTE NUCLEAR FACTORS 1α AND 3β. *Journal of Biological Chemistry,* 275**,** 18358-18365.
Chan, S. J., P. Keim & D. F. Steiner (1976) Cell-free synthesis of rat preproinsulins: characterization and partial amino acid sequence determination. *Proceedings of the National Academy of Sciences of the United States of America,* 73**,** 1964-1968.
Chandra, R. & R. A. Liddle (2013) Modulation of pancreatic exocrine and endocrine secretion. *Current opinion in gastroenterology,* 29**,** 517-522.
Chapman, E. R. (2002) Synaptotagmin: A Ca2+ sensor that triggers exocytosis? *Nature Reviews Molecular Cell Biology,* 3**,** 498-508.
Chen, C., C. M. Cohrs, J. Stertmann, R. Bozsak & S. Speier (2017) Human beta cell mass and function in diabetes: Recent advances in knowledge and technologies to understand disease pathogenesis. *Molecular metabolism,* 6**,** 943-957.
Chen, F., M. Sha, Y. Wang, T. Wu, W. Shan, J. Liu, W. Zhou, Y. Zhu, Y. Sun, Y. Shi, D. Bleich & X. Han (2016) Transcription factor Ets-1 links glucotoxicity to pancreatic beta cell dysfunction through inhibiting PDX-1 expression in rodent models. *Diabetologia,* 59**,** 316-324.
Chen, H., X. Gu, I. h. Su, R. Bottino, J. L. Contreras, A. Tarakhovsky & S. K. Kim (2009) Polycomb protein Ezh2 regulates pancreatic beta-cell Ink4a/Arf expression and regeneration in diabetes mellitus. *Genes & development,* 23**,** 975-985.
Chen, J., G. Saxena, I. N. Mungrue, A. J. Lusis & A. Shalev (2008) Thioredoxin-Interacting Protein. *Diabetes,* 57**,** 938.
Chen, L., R. C. Conaway & J. W. Conaway (2013) Multiple modes of regulation of the human Ino80 SNF2 ATPase by subunits of the INO80 chromatin-remodeling complex. *Proceedings of the National Academy of Sciences,* 110**,** 20497.
Chen, L., D. J. Magliano & P. Z. Zimmet (2011) The worldwide epidemiology of type 2 diabetes mellitus--present and future perspectives. *Nat Rev Endocrinol,* 8**,** 228-36.
--- (2012) The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. *Nature Reviews Endocrinology,* 8**,** 228-236.
Chen, L., H.-X. Yan, J. Chen, W. Yang, Q. Liu, B. Zhai, H.-F. Cao, S.-Q. Liu, M.-C. Wu & H.-Y. Wang (2007) Negative Regulation of c-Myc Transcription by Pancreas Duodenum Homeobox-1. *Endocrinology,* 148**,** 2168-2180.
Cheng, X. & R. M. Blumenthal (2008) Mammalian DNA methyltransferases: a structural perspective. *Structure (London, England : 1993),* 16**,** 341-350.
Cheng, X., Z. Ji, T. Tsalkova & F. Mei (2008) Epac and PKA: a tale of two intracellular cAMP receptors. *Acta biochimica et biophysica Sinica,* 40**,** 651-662.
Cherrington, A. D. (1999) Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. *Diabetes,* 48**,** 1198.
Chi, S. W., J. B. Zang, A. Mele & R. B. Darnell (2009) Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. *Nature,* 460**,** 479-486.
Chi, T. H., M. Wan, P. P. Lee, K. Akashi, D. Metzger, P. Chambon, C. B. Wilson & G. R. Crabtree (2003) Sequential Roles of Brg, the ATPase Subunit of BAF Chromatin Remodeling Complexes, in Thymocyte Development. *Immunity,* 19**,** 169-182.

Chiniwala, N. & S. Jabbour (2011) Management of diabetes mellitus in the elderly. *Current Opinion in Endocrinology, Diabetes and Obesity,* 18.
Choi, J., M. Ko, S. Jeon, Y. Jeon, K. Park, C. Lee, H. Lee & R. H. Seong (2012) The SWI/SNF-like BAF Complex Is Essential for Early B Cell Development. *The Journal of Immunology,* 188**,** 3791.
Choi, K. & Y.-B. Kim (2010) Molecular mechanism of insulin resistance in obesity and type 2 diabetes. *The Korean journal of internal medicine,* 25**,** 119-129.
Cinti, F., R. Bouchi, J. Y. Kim-Muller, Y. Ohmura, P. R. Sandoval, M. Masini, L. Marselli, M. Suleiman, L. E. Ratner, P. Marchetti & D. Accili (2016a) Evidence of beta-Cell Dedifferentiation in Human Type 2 Diabetes. *J Clin Endocrinol Metab,* 101**,** 1044-54.
--- (2016b) Evidence of β-Cell Dedifferentiation in Human Type 2 Diabetes. *The Journal of Clinical Endocrinology & Metabolism,* 101**,** 1044-1054.
Cirulli, V., D. Baetens, U. Rutishauser, P. A. Halban, L. Orci & D. G. Rouiller (1994) Expression of neural cell adhesion molecule (N-CAM) in rat islets and its role in islet cell type segregation. *Journal of Cell Science,* 107**,** 1429.
Clapier, C. R. & B. R. Cairns (2012) Regulation of ISWI involves inhibitory modules antagonized by nucleosomal epitopes. *Nature,* 492**,** 280.
Clausen, J. O., T. Hansen, C. Bjørbaek, S. M. Echwald, S. A. Urhammer, S. Rasmussen, C. B. Andersen, L. Hansen, K. Almind, O. Pedersen, K. Borch-Johnsen, K. Winther & J. Haraldsdòttir (1995) Insulin resistance: interactions between obesity and a common variant of insulin receptor substrate-1. *The Lancet,* 346**,** 397-402.
Clément, K., S. Hercberg, B. Passinge, P. Galan, M. Varroud-Vial, A. Shuldiner, B. Beamer, G. Charpentier, B. Guy-Grand, P. Froguel & C. Vaisse (2000) The Pro115Gln and Pro12Ala PPAR gamma gene mutations in obesity and type 2 diabetes. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity,* 24**,** 391-3.
Cnop, M. (2008) Fatty acids and glucolipotoxicity in the pathogenesis of Type 2 diabetes. *Biochemical Society Transactions,* 36**,** 348.
Cnop, M., J. C. Hannaert, A. Hoorens, D. L. Eizirik & D. G. Pipeleers (2001) Inverse Relationship Between Cytotoxicity of Free Fatty Acids in Pancreatic Islet Cells and Cellular Triglyceride Accumulation. *Diabetes,* 50**,** 1771.
Conrad, E., R. Stein & C. S. Hunter (2014) Revealing transcription factors during human pancreatic β cell development. *Trends in endocrinology and metabolism: TEM,* 25**,** 407-414.
Considine, R. V., M. R. Nyce, L. E. Allen, L. M. Morales, S. Triester, J. Serrano, J. Colberg, S. Lanza-Jacoby & J. F. Caro (1995) Protein kinase C is increased in the liver of humans and rats with non-insulin-dependent diabetes mellitus: an alteration not due to hyperglycemia. *The Journal of clinical investigation,* 95**,** 2938-2944.
Consortium, D. G. R. A. M.-a., C. Asian Genetic Epidemiology Network Type 2 Diabetes, C. South Asian Type 2 Diabetes, C. Mexican American Type 2 Diabetes, C. Type 2 Diabetes Genetic Exploration by Nex-generation sequencing in muylti-Ethnic Samples, A. Mahajan, M. J. Go, W. Zhang, J. E. Below, K. J. Gaulton, T. Ferreira, M. Horikoshi, A. D. Johnson, M. C. Y. Ng, I. Prokopenko, D. Saleheen, X. Wang, E. Zeggini, G. R. Abecasis, L. S. Adair, P. Almgren, M. Atalay, T. Aung, D. Baldassarre, B. Balkau, Y. Bao, A. H. Barnett, I. Barroso, A. Basit, L. F. Been, J. Beilby, G. I. Bell, R. Benediktsson, R. N. Bergman, B. O. Boehm, E. Boerwinkle, L. L. Bonnycastle, N. Burtt, Q. Cai, H. Campbell, J. Carey, S. Cauchi, M. Caulfield, J. C. N. Chan, L.-C. Chang, T.-J. Chang, Y.-C. Chang, G. Charpentier, C.-H. Chen, H. Chen, Y.-T. Chen, K.-S. Chia, M. Chidambaram, P. S. Chines, N. H. Cho, Y. M. Cho, L.-M. Chuang, F. S. Collins, M. C. Cornelis, D. J. Couper, A. T. Crenshaw, R. M. van Dam, J. Danesh, D. Das, U. de Faire, G. Dedoussis, P. Deloukas, A. S. Dimas, C. Dina, A. S. Doney, P. J. Donnelly, M. Dorkhan, C. van Duijn, J. Dupuis, S. Edkins, P. Elliott, V. Emilsson, R. Erbel, J. G. Eriksson, J. Escobedo, T. Esko, E. Eury, J. C. Florez, P. Fontanillas, N. G. Forouhi, T. Forsen, C. Fox, R. M. Fraser, T. M. Frayling, P. Froguel, P. Frossard, Y. Gao, K. Gertow, C. Gieger, B. Gigante, H. Grallert, G. B. Grant, L. C.

Grrop, C. J. Groves, E. Grundberg, et al. (2014) Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. *Nature genetics,* 46**,** 234-244.
Cook, G. A., D. A. Otto & N. W. Cornell (1983) Malonyl-CoA inhibition of carnitine palmitoyltransferase: interpretation of I50 and K1 values. *The Biochemical journal,* 212**,** 525-527.
Cooper, M. E., Bonnet, F., Oldfield, M. and Jandeleit-Dahm, K. (2001) Mechanisms of diabetic vasculopathy: an overview. *American Journal of Hypertension,* 14**,** 475-486.
Correa-Medina, M., V. Bravo-Egana, S. Rosero, C. Ricordi, H. Edlund, J. Diez & R. L. Pastori (2009) MicroRNA miR-7 is preferentially expressed in endocrine cells of the developing and adult human pancreas. *Gene Expression Patterns,* 9**,** 193-199.
Craig, T. J., F. M. Ashcroft & P. Proks (2008) How ATP inhibits the open K(ATP) channel. *The Journal of general physiology,* 132**,** 131-144.
Cras-Méneur, C., L. Li, R. Kopan & M. A. Permutt (2009) Presenilins, Notch dose control the fate of pancreatic endocrine progenitors during a narrow developmental window. *Genes & development,* 23**,** 2088-2101.
Curtis, B. M. & W. A. Catterall (1984) Purification of the calcium antagonist receptor of the voltage-sensitive calcium channel from skeletal muscle transverse tubules. *Biochemistry,* 23**,** 2113-2118.
--- (1986) Reconstitution of the voltage-sensitive calcium channel purified from skeletal muscle transverse tubules. *Biochemistry,* 25**,** 3077-3083.
da Silva Xavier, G., M. K. Loder, A. McDonald, A. I. Tarasov, R. Carzaniga, K. Kronenberger, S. Barg & G. A. Rutter (2009) TCF7L2 regulates late events in insulin secretion from pancreatic islet beta-cells. *Diabetes,* 58**,** 894-905.
Dai, C., M. Brissova, Y. Hang, C. Thompson, G. Poffenberger, A. Shostak, Z. Chen, R. Stein & A. C. Powers (2012) Islet-enriched gene expression and glucose-induced insulin secretion in human and mouse islets. *Diabetologia,* 55**,** 707-718.
Dalle, S., C. Longuet, S. Costes, C. Broca, O. Faruque, G. Fontés, E. H. Hani & D. Bataille (2004) Glucagon Promotes cAMP-response Element-binding Protein Phosphorylation via Activation of ERK1/2 in MIN6 Cell Line and Isolated Islets of Langerhans. *Journal of Biological Chemistry,* 279**,** 20345-20355.
Danial, N. N., L. D. Walensky, C.-Y. Zhang, C. S. Choi, J. K. Fisher, A. J. A. Molina, S. R. Datta, K. L. Pitter, G. H. Bird, J. D. Wikstrom, J. T. Deeney, K. Robertson, J. Morash, A. Kulkarni, S. Neschen, S. Kim, M. E. Greenberg, B. E. Corkey, O. S. Shirihai, G. I. Shulman, B. B. Lowell & S. J. Korsmeyer (2008) Dual role of proapoptotic BAD in insulin secretion and beta cell survival. *Nature Medicine,* 14**,** 144-153.
Davalli, A. M., A. E. Pontiroli, C. Socci, F. Bertuzzi, B. Fattor, S. Braghi, V. Di Carlo & G. Pozza (1992) Human islets chronically exposed in vitro to different stimuli become unresponsive to the same stimuli given acutely: evidence supporting specific desensitization rather than beta-cell exhaustion. *The Journal of Clinical Endocrinology & Metabolism,* 74**,** 790-794.
Davidson, H. W. & J. C. Hutton (1987) The insulin-secretory-granule carboxypeptidase H. Purification and demonstration of involvement in proinsulin processing. *The Biochemical journal,* 245**,** 575-582.
Davidson, H. W., C. J. Rhodes & J. C. Hutton (1988) Intraorganellar calcium and pH control proinsulin cleavage in the pancreatic β cell via two distinct site-specific endopeptidases. *Nature,* 333**,** 93-96.
Davidson, H. W., J. M. Wenzlau & R. M. O'Brien (2014) Zinc transporter 8 (ZnT8) and β cell function. *Trends in endocrinology and metabolism: TEM,* 25**,** 415-424.
Dawn Teare, M. & J. H. Barrett (2005) Genetic linkage studies. *The Lancet,* 366**,** 1036-1044.
Dayeh, T., P. Volkov, S. Salö, E. Hall, E. Nilsson, A. H. Olsson, C. L. Kirkpatrick, C. B. Wollheim, L. Eliasson, T. Rönn, K. Bacos & C. Ling (2014) Genome-wide DNA methylation analysis of

human pancreatic islets from type 2 diabetic and non-diabetic donors identifies candidate genes that influence insulin secretion. *PLoS genetics,* 10**,** e1004160-e1004160.
Dayeh, T. A., A. H. Olsson, P. Volkov, P. Almgren, T. Rönn & C. Ling (2013) Identification of CpG-SNPs associated with type 2 diabetes and differential DNA methylation in human pancreatic islets. *Diabetologia,* 56**,** 1036-1046.
de Napoles, M., J. E. Mermoud, R. Wakao, Y. A. Tang, M. Endoh, R. Appanah, T. B. Nesterova, J. Silva, A. P. Otte, M. Vidal, H. Koseki & N. Brockdorff (2004) Polycomb Group Proteins Ring1A/B Link Ubiquitylation of Histone H2A to Heritable Gene Silencing and X Inactivation. *Developmental Cell,* 7**,** 663-676.
de Rooij, J., F. J. T. Zwartkruis, M. H. G. Verheijen, R. H. Cool, S. M. B. Nijman, A. Wittinghofer & J. L. Bos (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature,* 396**,** 474-477.
De Vas, M. G., J. L. Kopp, C. Heliot, M. Sander, S. Cereghini & C. Haumaitre (2015) Hnf1b controls pancreas morphogenesis and the generation of Ngn3⁺ endocrine progenitors. *Development,* 142**,** 871.
De Vos, A., H. Heimberg, E. Quartier, P. Huypens, L. Bouwens, D. Pipeleers & F. Schuit (1995) Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. *The Journal of clinical investigation,* 96**,** 2489-2495.
Deacon, C. F. (2011) Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. *Diabetes, Obesity and Metabolism,* 13**,** 7-18.
Deering, T. G., T. Ogihara, A. P. Trace, B. Maier & R. G. Mirmira (2009) Methyltransferase Set7/9 maintains transcription and euchromatin structure at islet-enriched genes. *Diabetes,* 58**,** 185-193.
DeFronzo, R., G. A. Fleming, K. Chen & T. A. Bicsak (2016) Metformin-associated lactic acidosis: Current perspectives on causes and risk. *Metabolism,* 65**,** 20-29.
DeFronzo, R. A. (1988) The Triumvirate: β-Cell, Muscle, Liver: A Collusion Responsible for NIDDM. *Diabetes,* 37**,** 667.
--- (1997) **Pathogenesis of type 2 diabetes mellitus: metabolic and molecular implications for identifying diabetes genes**. *Diabetes,* 5**,** 177-269.
DeFronzo, R. A. & E. Ferrannini (1987) Regulation of hepatic glucose metabolism in humans. *Diabetes/Metabolism Reviews,* 3**,** 415-459.
DeFronzo, R. A., R. Gunnarsson, O. Björkman, M. Olsson & J. Wahren (1985) Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *The Journal of clinical investigation,* 76**,** 149-155.
DeFronzo, R. A., E. Jacot, E. Jequier, E. Maeder, J. Wahren & J. P. Felber (1981) The Effect of Insulin on the Disposal of Intravenous Glucose: Results from Indirect Calorimetry and Hepatic and Femoral Venous Catheterization. *Diabetes,* 30**,** 1000.
Denli, A. M., B. B. J. Tops, R. H. A. Plasterk, R. F. Ketting & G. J. Hannon (2004) Processing of primary microRNAs by the Microprocessor complex. *Nature,* 432**,** 231-235.
Dhawan, S., S. Georgia, S.-I. Tschen, G. Fan & A. Bhushan (2011) Pancreatic β cell identity is maintained by DNA methylation-mediated repression of Arx. *Developmental cell,* 20**,** 419-429.
Dhawan, S., S.-I. Tschen & A. Bhushan (2009) Bmi-1 regulates the Ink4a/Arf locus to control pancreatic beta-cell proliferation. *Genes & development,* 23**,** 906-911.
Dhawan, S., S.-I. Tschen, C. Zeng, T. Guo, M. Hebrok, A. Matveyenko & A. Bhushan (2015) DNA methylation directs functional maturation of pancreatic β cells. *The Journal of Clinical Investigation,* 125**,** 2851-2860.
DiabetesUK. 2011. Diabetes in the UK 2011/2012: Key statistics on diabetes.
Diedisheim, M., M. Oshima, O. Albagli, C. W. Huldt, I. Ahlstedt, M. Clausen, S. Menon, A. Aivazidis, A.-C. Andreasson, W. G. Haynes, P. Marchetti, L. Marselli, M. Armanet, F. Chimienti & R.

Scharfmann (2018) Modeling human pancreatic beta cell dedifferentiation. *Molecular metabolism,* 10**,** 74-86.
Dimitriadis, G., P. Mitrou, V. Lambadiari, E. Maratou & S. A. Raptis (2011) Insulin effects in muscle and adipose tissue. *Diabetes Research and Clinical Practice,* 93**,** S52-S59.
Ding, W., H. Yang, S. Gong, W. Shi, J. Xiao, J. Gu, Y. Wang & B. He (2017) Candidate miRNAs and pathogenesis investigation for hepatocellular carcinoma based on bioinformatics analysis. *Oncology letters,* 13**,** 3409-3414.
Dipietro, D. L., C. Sharma & S. Weinhouse (1962) Studies on Glucose Phosphorylation in Rat Liver*. *Biochemistry,* 1**,** 455-462.
Dodson, G. & D. Steiner (1998) The role of assembly in insulin's biosynthesis. *Current Opinion in Structural Biology,* 8**,** 189-194.
Donath, M. Y., D. J. Gross, E. Cerasi & N. Kaiser (1999) Hyperglycemia-induced beta-cell apoptosis in pancreatic islets of Psammomys obesus during development of diabetes. *Diabetes,* 48**,** 738.
Donath, M. Y. & S. E. Shoelson (2011) Type 2 diabetes as an inflammatory disease. *Nature Reviews Immunology,* 11**,** 98-107.
Dong, P. D. S., E. Provost, S. D. Leach & D. Y. R. Stainier (2008) Graded levels of Ptf1a differentially regulate endocrine and exocrine fates in the developing pancreas. *Genes & development,* 22**,** 1445-1450.
Dor, Y. & B. Glaser (2013) Beta-Cell Dedifferentiation and Type 2 Diabetes. *New England Journal of Medicine,* 368**,** 572-573.
Drucker, D. J. (2006) The biology of incretin hormones. *Cell Metabolism,* 3**,** 153-165.
Drucker, D. J., J. Philippe, S. Mojsov, W. L. Chick & J. F. Habener (1987) Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proceedings of the National Academy of Sciences of the United States of America,* 84**,** 3434-3438.
Duggirala, R., J. Blangero, L. Almasy, T. D. Dyer, K. L. Williams, R. J. Leach, P. O'Connell & M. P. Stern (1999) Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. *American journal of human genetics,* 64**,** 1127-1140.
Dunham, I., A. Kundaje, S. F. Aldred, P. J. Collins, C. Davis, F. Doyle, C. Epstein, S. Frietze, J. Harrow, R. Kaul, J. Khatun, B. Lajoie, S. Landt, b.-k. Lee, F. Pauli Behn, K. Rosenbloom, P. Sabo, A. Safi, A. Sanyal & E. Birney (2012) The ENCODE Project Consortium: An integrated encyclopedia of DNA elements in the human genome. 2012. Nature 489: 57–74. *Nature,* 489**,** 57-74.
Dunphy, J. L., R. G. Taylor & P. J. Fuller (1998) Tissue distribution of rat glucagon receptor and GLP-1 receptor gene expression1This work was supported by a grant from the Crohn's and Colitis Foundation of America.1. *Molecular and Cellular Endocrinology,* 141**,** 179-186.
Duvillié, B., N. Cordonnier, L. Deltour, F. Dandoy-Dron, J.-M. Itier, E. Monthioux, J. Jami, R. L. Joshi & D. Bucchini (1997) Phenotypic alterations in insulin-deficient mutant mice. *Proceedings of the National Academy of Sciences,* 94**,** 5137.
Ebina, Y., L. Ellis, K. Jarnagin, M. Edery, L. Graf, E. Clauser, J.-h. Ou, F. Masiarz, Y. W. Kan, I. D. Goldfine, R. A. Roth & W. J. Rutter (1985) The human insulin receptor cDNA: The structural basis for hormone-activated transmembrane signalling. *Cell,* 40**,** 747-758.
Edghill, E. L., S. E. Flanagan, A.-M. Patch, C. Boustred, A. Parrish, B. Shields, M. H. Shepherd, K. Hussain, R. R. Kapoor, M. Malecki, M. J. MacDonald, J. Støy, D. F. Steiner, L. H. Philipson, G. I. Bell, A. T. Hattersley & S. Ellard (2008) Insulin Mutation Screening in 1,044 Patients With Diabetes. *Diabetes,* 57**,** 1034.
Ediger, B. N., A. Du, J. Liu, C. S. Hunter, E. R. Walp, J. Schug, K. H. Kaestner, R. Stein, D. A. Stoffers & C. L. May (2014) Islet-1 Is essential for pancreatic β-cell function. *Diabetes,* 63**,** 4206-4217.
Ediger, B. N., H.-W. Lim, C. Juliana, D. N. Groff, L. T. Williams, G. Dominguez, J.-H. Liu, B. L. Taylor, E. R. Walp, V. Kameswaran, J. Yang, C. Liu, C. S. Hunter, K. H. Kaestner, A. Naji, C. Li, M. Sander, R. Stein, L. Sussel, K.-J. Won, C. L. May & D. A. Stoffers (2017) LIM domain–binding 1

maintains the terminally differentiated state of pancreatic β cells. *The Journal of Clinical Investigation,* 127**,** 215-229.
Efrat, S. (2019) Beta-Cell Dedifferentiation in Type 2 Diabetes: Concise Review. *STEM CELLS,* 37**,** 1267-1272.
Egea, P. F., R. M. Stroud & P. Walter (2005) Targeting proteins to membranes: structure of the signal recognition particle. *Current Opinion in Structural Biology,* 15**,** 213-220.
Eisen, J. A., K. S. Sweder & P. C. Hanawalt (1995) Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Research,* 23**,** 2715-2723.
Eizirik, D. c. L., A. K. Cardozo & M. Cnop (2008) The Role for Endoplasmic Reticulum Stress in Diabetes Mellitus. *Endocrine Reviews,* 29**,** 42-61.
Elkayam, E., C.-D. Kuhn, A. Tocilj, A. D. Haase, E. M. Greene, G. J. Hannon & L. Joshua-Tor (2012) The structure of human argonaute-2 in complex with miR-20a. *Cell,* 150**,** 100-110.
Elouil, H., A. K. Cardozo, D. L. Eizirik, J. C. Henquin & J. C. Jonas (2005) High glucose and hydrogen peroxide increase c-Myc and haeme-oxygenase 1 mRNA levels in rat pancreatic islets without activating NFκB. *Diabetologia,* 48**,** 496-505.
Ertel, E. A., K. P. Campbell, M. M. Harpold, F. Hofmann, Y. Mori, E. Perez-Reyes, A. Schwartz, T. P. Snutch, T. Tanabe, L. Birnbaumer, R. W. Tsien & W. A. Catterall (2000) Nomenclature of Voltage-Gated Calcium Channels. *Neuron,* 25**,** 533-535.
Eskeland, R., M. Leeb, G. R. Grimes, C. Kress, S. Boyle, D. Sproul, N. Gilbert, Y. Fan, A. I. Skoultchi, A. Wutz & W. A. Bickmore (2010) Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. *Molecular cell,* 38**,** 452-464.
Esni, F., I. B. Täljedal, A. K. Perl, H. Cremer, G. Christofori & H. Semb (1999) Neural cell adhesion molecule (N-CAM) is required for cell type segregation and normal ultrastructure in pancreatic islets. *The Journal of cell biology,* 144**,** 325-337.
Estall, J. L. & R. A. Screaton (2015) To Be(ta Cell) or Not to Be(ta cell): New Mouse Models for Studying Gene Function in the Pancreatic β-Cell. *Endocrinology,* 156**,** 2365-2367.
Eychène, A., N. Rocques & C. Pouponnot (2008) A new MAFia in cancer. *Nature Reviews Cancer,* 8**,** 683.
Fahien, L. A. & M. J. Macdonald (2011) The complex mechanism of glutamate dehydrogenase in insulin secretion. *Diabetes,* 60**,** 2450-2454.
Fajans, S. S., G. I. Bell, V. P. Paz, J. E. Below, N. J. Cox, C. Martin, I. H. Thomas & M. Chen (2010) Obesity and hyperinsulinemia in a family with pancreatic agenesis and MODY caused by the IPF1 mutation Pro63fsX60. *Translational research : the journal of laboratory and clinical medicine,* 156**,** 7-14.
Farooqi, I. S., K. Volders, R. Stanhope, R. Heuschkel, A. White, E. Lank, J. Keogh, S. O'Rahilly & J. W. M. Creemers (2007) Hyperphagia and Early-Onset Obesity due to a Novel Homozygous Missense Mutation in Prohormone Convertase 1/3. *The Journal of Clinical Endocrinology & Metabolism,* 92**,** 3369-3373.
Federici, M., M. Hribal, L. Perego, M. Ranalli, Z. Caradonna, C. Perego, L. Usellini, R. Nano, P. Bonini, F. Bertuzzi, L. N. J. L. Marlier, A. M. Davalli, O. Carandente, A. E. Pontiroli, G. Melino, P. Marchetti, R. Lauro, G. Sesti & F. Folli (2001) High Glucose Causes Apoptosis in Cultured Human Pancreatic Islets of Langerhans. *Diabetes,* 50**,** 1290.
Fedorov, O., J. Castex, C. Tallant, D. R. Owen, S. Martin, M. Aldeghi, O. Monteiro, P. Filippakopoulos, S. Picaud, J. D. Trzupek, B. S. Gerstenberger, C. Bountra, D. Willmann, C. Wells, M. Philpott, C. Rogers, P. C. Biggin, P. E. Brennan, M. E. Bunnage, R. Schüle, T. Günther, S. Knapp & S. Müller (2015) Selective targeting of the BRG/PB1 bromodomains impairs embryonic and trophoblast stem cell maintenance. *Science advances,* 1**,** e1500723.
Felber, J. P., H. U. Meyer, B. Curchod, H. U. Iselin, J. Rousselle, E. Maeder, P. Pahud & E. Jéquier (1981) Glucose storage and oxidation in different degrees of human obesity measured by continuous indirect calorimetry. *Diabetologia,* 20**,** 39-44.

Feldmann, N., R. M. del Rio, A. Gjinovci, J. Tamarit-Rodriguez, C. B. Wollheim & A. Wiederkehr (2011) Reduction of plasma membrane glutamate transport potentiates insulin but not glucagon secretion in pancreatic islet cells. *Molecular and Cellular Endocrinology,* 338**,** 46-57.

Fenster, S. D., W. J. Chung, R. Zhai, C. Cases-Langhoff, B. Voss, A. M. Garner, U. Kaempf, S. Kindler, E. D. Gundelfinger & C. C. Garner (2000) Piccolo, a Presynaptic Zinc Finger Protein Structurally Related to Bassoon. *Neuron,* 25**,** 203-214.

Fernandez, I., D. Araç, J. Ubach, S. H. Gerber, O.-h. Shin, Y. Gao, R. G. W. Anderson, T. C. Südhof & J. Rizo (2001) Three-Dimensional Structure of the Synaptotagmin 1 C2B-Domain: Synaptotagmin 1 as a Phospholipid Binding Machine. *Neuron,* 32**,** 1057-1069.

Ferrannini, E., D. C. Simonson, L. D. Katz, G. J. Reichard, S. Bevilacqua, E. J. Barrett, M. Olsson & R. A. DeFronzo (1988) The disposal of an oral glucose load in patients with non-insulin-dependent diabetes. 37**,** 75-85.

Filion, G. J. P., S. Zhenilo, S. Salozhin, D. Yamada, E. Prokhortchouk & P.-A. Defossez (2006) A family of human zinc finger proteins that bind methylated DNA and repress transcription. *Molecular and cellular biology,* 26**,** 169-181.

Firth, R., P. Bell & R. Rizza (1987) Insulin action in non-insulin-dependent diabetes mellitus: The relationship between hepatic and extrahepatic insulin resistance and obesity. *Metabolism - Clinical and Experimental,* 36**,** 1091-1095.

Fischle, W., Y. Wang, S. A. Jacobs, Y. Kim, C. D. Allis & S. Khorasanizadeh (2003) Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes & development,* 17**,** 1870-1881.

Flanagan, J. F., L.-Z. Mi, M. Chruszcz, M. Cymborowski, K. L. Clines, Y. Kim, W. Minor, F. Rastinejad & S. Khorasanizadeh (2005) Double chromodomains cooperate to recognize the methylated histone H3 tail. *Nature,* 438**,** 1181-1185.

Flockerzi, V., H.-J. Oeken, F. Hofmann, D. Pelzer, A. Cavalié & W. Trautwein (1986) Purified dihydropyridine-binding site from skeletal muscle t-tubules is a functional calcium channel. *Nature,* 323**,** 66-68.

Florez, J. C., N. Burtt, P. I. W. de Bakker, P. Almgren, T. Tuomi, J. Holmkvist, D. Gaudet, T. J. Hudson, S. F. Schaffner, M. J. Daly, J. N. Hirschhorn, L. Groop & D. Altshuler (2004) Haplotype Structure and Genotype-Phenotype Correlations of the Sulfonylurea Receptor and the Islet ATP-Sensitive Potassium Channel Gene Region. *Diabetes,* 53**,** 1360.

Fong, D. S., Aiello, L., Gardner, T. W., King, G. L., Blankenship, G., Cavallerano, J. D., Ferris, F. L. and Klein, R. (2003) Retinopathy in Diabetes. *Diabetes Care,* 27**,** s84-s87.

Franks, P. W. (2011) Gene × Environment Interactions in Type 2 Diabetes. *Current Diabetes Reports,* 11**,** 552.

Franks, P. W., O. Rolandsson, S. L. Debenham, K. A. Fawcett, F. Payne, C. Dina, P. Froguel, K. L. Mohlke, C. Willer, T. Olsson, N. J. Wareham, G. Hallmans, I. Barroso & M. S. Sandhu (2008) Replication of the association between variants in WFS1 and risk of type 2 diabetes in European populations. *Diabetologia,* 51**,** 458-463.

Fred, R. G., C. H. Bang-Berthelsen, T. Mandrup-Poulsen, L. G. Grunnet & N. Welsh (2010) High Glucose Suppresses Human Islet Insulin Biosynthesis by Inducing miR-133a Leading to Decreased Polypyrimidine Tract Binding Protein-Expression. *PLOS ONE,* 5**,** e10843.

Freeman, A. M., K. Soman-Faulkner & N. Pennings. 2019. Insulin Resistance. StatPearls Publishing.

Friedrichsen, M., J. B. Birk, E. A. Richter, R. Ribel-Madsen, C. Pehmøller, B. F. Hansen, H. Beck-Nielsen, M. F. Hirshman, L. J. Goodyear, A. Vaag, P. Poulsen & J. F. P. Wojtaszewski (2013) Akt2 influences glycogen synthase activity in human skeletal muscle through regulation of NH_2-terminal (sites 2 + 2a) phosphorylation. *American journal of physiology. Endocrinology and metabolism,* 304**,** E631-E639.

Froguel, P., H. Zouali, N. Vionnet, G. Velho, M. Vaxillaire, F. Sun, S. Lesage, M. Stoffel, J. Takeda, P. Passa, M. A. Permutt, J. S. Beckmann, G. I. Bell & D. Cohen (1993) Familial Hyperglycemia

Due to Mutations in Glucokinase -- Definition of a Subtype of Diabetes Mellitus. *New England Journal of Medicine,* 328**,** 697-702.

Frühbeck, G., H. Toplak, E. Woodward, V. Yumuk, M. Maislos, J.-M. Oppert & O. Executive Committee of the European Association for the Study of (2013) Obesity: the gateway to ill health - an EASO position statement on a rising public health, clinical and scientific challenge in Europe. *Obesity facts,* 6**,** 117-120.

Fu, Z., E. R. Gilbert & D. Liu (2013) Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. *Current diabetes reviews,* 9**,** 25-53.

Fu, Z., Gilbert, E. R. and Liu, D. (2013) Regulation of Insulin Synthesis and Secretion and Pancreatic Beta-Cell Dysfunction in Diabetes. *Current diabetes reviews,* 9**,** 25-53.

Fujimoto, K., T. Shibasaki, N. Yokoi, Y. Kashima, M. Matsumoto, T. Sasaki, N. Tajima, T. Iwanaga & S. Seino (2002) Piccolo, a Ca2+ Sensor in Pancreatic β-Cells: INVOLVEMENT OF cAMP-GEFII·Rim2·PICCOLO COMPLEX IN cAMP-DEPENDENT EXOCYTOSIS. *Journal of Biological Chemistry,* 277**,** 50497-50502.

Fukuda, M., E. Kanno, M. Satoh, C. Saegusa & A. Yamamoto (2004) Synaptotagmin VII Is Targeted to Dense-core Vesicles and Regulates Their Ca2+-dependent Exocytosis in PC12 Cells. *Journal of Biological Chemistry,* 279**,** 52677-52684.

Furuta, H., M. Furuta, T. Sanke, K. Ekawa, T. Hanabusa, M. Nishi, H. Sasaki & K. Nanjo (2002) Nonsense and Missense Mutations in the Human Hepatocyte Nuclear Factor-1β Gene (TCF2) and Their Relation to Type 2 Diabetes in Japanese. *The Journal of Clinical Endocrinology & Metabolism,* 87**,** 3859-3863.

Galloway, J. A., S. A. Hooper, C. T. Spradlin, D. C. Howey, B. Frank, R. R. Bowsher & J. Anderson (1992) Biosynthetic Human Proinsulin: Review of Chemistry, in Vitro and in Vivo Receptor Binding, Animal and Human Pharmacology Studies, and Clinical Trial Experience. *Diabetes care,* 15**,** 666-92.

Gammelsaeter, R., T. Coppola, P. Marcaggi, J. Storm-Mathisen, F. A. Chaudhry, D. Attwell, R. Regazzi & V. Gundersen (2011) A Role for Glutamate Transporters in the Regulation of Insulin Secretion. *PLOS ONE,* 6**,** e22960.

Gamper, A. M., J. Kim & R. G. Roeder (2009) The STAGA Subunit ADA2b Is an Important Regulator of Human GCN5 Catalysis. *Molecular and Cellular Biology,* 29**,** 266.

Gao, N., J. Le Lay, W. Qin, N. Doliba, J. Schug, A. J. Fox, O. Smirnova, F. M. Matschinsky & K. H. Kaestner (2010) Foxa1 and Foxa2 maintain the metabolic and secretory features of the mature beta-cell. *Molecular endocrinology (Baltimore, Md.),* 24**,** 1594-1604.

Gao, N., J. LeLay, M. Z. Vatamaniuk, S. Rieck, J. R. Friedman & K. H. Kaestner (2008) Dynamic regulation of Pdx1 enhancers by Foxa1 and Foxa2 is essential for pancreas development. *Genes & development,* 22**,** 3435-3448.

Gao, T., B. McKenna, C. Li, M. Reichert, J. Nguyen, T. Singh, C. Yang, A. Pannikar, N. Doliba, T. Zhang, D. A. Stoffers, H. Edlund, F. Matschinsky, R. Stein & B. Z. Stanger (2014) Pdx1 maintains β cell identity and function by repressing an α cell program. *Cell metabolism,* 19**,** 259-271.

Gao, Y., J. Li, Z. Zhang, R. Zhang, A. Pollock & T. Sun (2019) MicroRNA miR-7 and miR-17-92 in the Arcuate Nucleus of Mouse Hypothalamus Regulate Sex-Specific Diet-Induced Obesity. *Molecular Neurobiology*.

Gao, Z., J. Reavey-Cantwell, R. A. Young, P. Jegier & B. A. Wolf (2000) Synaptotagmin III/VII Isoforms Mediate Ca2+-induced Insulin Secretion in Pancreatic Islet β-Cells. *Journal of Biological Chemistry,* 275**,** 36079-36085.

Gerich, J. E., M. Lorenzi, V. Schneider, C. W. Kwan, J. H. Karam, R. Guillemin & P. H. Forsham (1974) Inhibition of Pancreatic Glucagon Responses to Arginine by Somatostatin in Normal Man and in Insulin-Dependent Diabetics. *Diabetes,* 23**,** 876.

Gerling, I., C. Nejman & N. K. Chatterjee (1988) Effect of Coxsackievirus B4 Infection in Mice on Expression of 64,000-M_r Autoantigen and Glucose Sensitivity of Islets Before Development of Hyperglycemia. *Diabetes,* 37**,** 1419.

Gharibi, A., Y. Adamian & J. A. Kelber (2016) Cellular and molecular aspects of pancreatic cancer. *Acta histochemica,* 118**,** 305-316.
Gheni, G., M. Ogura, M. Iwasaki, N. Yokoi, K. Minami, Y. Nakayama, K. Harada, B. Hastoy, X. Wu, H. Takahashi, K. Kimura, T. Matsubara, R. Hoshikawa, N. Hatano, K. Sugawara, T. Shibasaki, N. Inagaki, T. Bamba, A. Mizoguchi, E. Fukusaki, P. Rorsman & S. Seino (2014) Glutamate acts as a key signal linking glucose metabolism to incretin/cAMP action to amplify insulin secretion. *Cell reports,* 9**,** 661-673.
Giannarelli, R., M. Aragona, A. Coppelli & S. Del Prato (2003) Reducing insulin resistance with metformin: the evidence today. *Diabetes & Metabolism,* 29**,** 6S28-6S35.
Gilon, P., H.-Y. Chae, G. A. Rutter & M. A. Ravier (2014) Calcium signaling in pancreatic β-cells in health and in Type 2 diabetes. *Cell Calcium,* 56**,** 340-361.
Giorgino, F., L. Laviola & J. W. Eriksson (2005) Regional differences of insulin action in adipose tissue: insights from in vivo and in vitro studies. *Acta Physiologica Scandinavica,* 183**,** 13-30.
Girard, J. (2006) The Inhibitory Effects of Insulin on Hepatic Glucose Production Are Both Direct and Indirect. *Diabetes,* 55**,** S65.
Gleason, C. E., M. Gonzalez, J. S. Harmon & R. P. Robertson (2000) Determinants of glucose toxicity and its reversibility in the pancreatic islet β-cell line, HIT-T15. *American Journal of Physiology-Endocrinology and Metabolism,* 279**,** E997-E1002.
Gloyn, A. L., M. N. Weedon, K. R. Owen, M. J. Turner, B. A. Knight, G. Hitman, M. Walker, J. C. Levy, M. Sampson, S. Halford, M. I. McCarthy, A. T. Hattersley & T. M. Frayling (2003) Large-Scale Association Studies of Variants in Genes Encoding the Pancreatic β-Cell K_{ATP} Channel Subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) Confirm That the KCNJ11 E23K Variant Is Associated With Type 2 Diabetes. *Diabetes,* 52**,** 568.
Goalstone, M., K. Carel, J. W. Leitner & B. Draznin (1997) Insulin Stimulates the Phosphorylation and Activity of Farnesyltransferase via the Ras-Mitogen-Activated Protein Kinase Pathway*. *Endocrinology,* 138**,** 5119-5124.
Golay, A., J. P. Felber, E. Jequier, R. A. DeFronzo & E. Ferrannini. 1988. Metabolic basis of obesity and noninsulin-dependent diabetes mellitus., 727–747. Diabetes Metab Rev.
Golay, A., J. P. Felber, H. U. Meyer, B. Curchod, E. Maeder & E. Jéquier (1984) Study on lipid metabolism in obesity diabetes. *Metabolism,* 33**,** 111-116.
Gold, G. & G. M. Grodsky (1984) Kinetic aspects of compartmental storage and secretion of insulin and zinc. *Experientia,* 40**,** 1105-1114.
Goljanek-Whysall, K., G. F. Mok, A. Fahad Alrefaei, N. Kennerley, G. N. Wheeler & A. Munsterberg (2014) myomiR-dependent switching of BAF60 variant incorporation into Brg1 chromatin remodeling complexes during embryo myogenesis. *Development,* 141**,** 3378-87.
Golosow, N. & C. Grobstein (1962) Epitheliomesenchymal interaction in pancreatic morphogenesis. *Developmental Biology,* 4**,** 242-255.
Gonelle-Gispert, C., P. A. Halban, H. Niemann, M. Palmer, S. Catsicas & K. Sadoul (1999) SNAP-25a and -25b isoforms are both expressed in insulin-secreting cells and can function in insulin secretion. *The Biochemical journal,* 339 (Pt 1)**,** 159-165.
Goodpaster, B. H., J. He, S. Watkins & D. E. Kelley (2001) Skeletal Muscle Lipid Content and Insulin Resistance: Evidence for a Paradox in Endurance-Trained Athletes. *The Journal of Clinical Endocrinology & Metabolism,* 86**,** 5755-5761.
Gould, G. W. & G. D. Holman (1993) The glucose transporter family: structure, function and tissue-specific expression. *The Biochemical journal,* 295 (Pt 2)**,** 329-341.
Gradwohl, G., A. Dierich, M. LeMeur & F. Guillemot (2000) neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proceedings of the National Academy of Sciences of the United States of America,* 97**,** 1607-1611.
Grant, S. F. A., G. Thorleifsson, I. Reynisdottir, R. Benediktsson, A. Manolescu, J. Sainz, A. Helgason, H. Stefansson, V. Emilsson, A. Helgadottir, U. Styrkarsdottir, K. P. Magnusson, G. B. Walters, E. Palsdottir, T. Jonsdottir, T. Gudmundsdottir, A. Gylfason, J. Saemundsdottir, R. L. Wilensky,

M. P. Reilly, D. J. Rader, Y. Bagger, C. Christiansen, V. Gudnason, G. Sigurdsson, U. Thorsteinsdottir, J. R. Gulcher, A. Kong & K. Stefansson (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nature Genetics,* 38**,** 320-323.

Gremlich, S., C. Bonny, G. Waeber & B. Thorens (1997) Fatty Acids Decrease IDX-1 Expression in Rat Pancreatic Islets and Reduce GLUT2, Glucokinase, Insulin, and Somatostatin Levels. *Journal of Biological Chemistry,* 272**,** 30261-30269.

Gribble, F. M., S. J. Tucker & F. M. Ashcroft (1997) The essential role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide. *The EMBO journal,* 16**,** 1145-1152.

Gribble, F. M., S. J. Tucker, T. Haug & F. M. Ashcroft (1998) MgATP activates the beta cell KATP channel by interaction with its SUR1 subunit. *Proceedings of the National Academy of Sciences of the United States of America,* 95**,** 7185-7190.

Grill, V., M. Westberg & C. G. Ostenson (1987) B cell insensitivity in a rat model of non-insulin-dependent diabetes. Evidence for a rapidly reversible effect of previous hyperglycemia. *The Journal of Clinical Investigation,* 80**,** 664-669.

Grimm, D. & M. A. Kay (2003) From Virus Evolution to Vector Revolution: Use of Naturally Occurring Serotypes of Adeno-associated Virus (AAV) as Novel Vectors for Human Gene Therapy. *Current Gene Therapy,* 3**,** 281-304.

Grimm, D., K. L. Streetz, C. L. Jopling, T. A. Storm, K. Pandey, C. R. Davis, P. Marion, F. Salazar & M. A. Kay (2006) Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. *Nature,* 441**,** 537-541.

Groop, L. C., R. C. Bonadonna, S. DelPrato, K. Ratheiser, K. Zyck, E. Ferrannini & R. A. DeFronzo (1989) Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. *The Journal of clinical investigation,* 84**,** 205-213.

Gu, C., G. H. Stein, N. Pan, S. Goebbels, H. Hörnberg, K.-A. Nave, P. Herrera, P. White, K. H. Kaestner, L. Sussel & J. E. Lee (2010) Pancreatic beta cells require NeuroD to achieve and maintain functional maturity. *Cell metabolism,* 11**,** 298-310.

Guichard, C., R. Moreau, D. Pessayre, Terry K. Epperson & K.-H. Krause (2008) NOX family NADPH oxidases in liver and in pancreatic islets: a role in the metabolic syndrome and diabetes? *Biochemical Society Transactions,* 36**,** 920.

Guo, J. U., D. K. Ma, H. Mo, M. P. Ball, M.-H. Jang, M. A. Bonaguidi, J. A. Balazer, H. L. Eaves, B. Xie, E. Ford, K. Zhang, G.-l. Ming, Y. Gao & H. Song (2011) Neuronal activity modifies the DNA methylation landscape in the adult brain. *Nature neuroscience,* 14**,** 1345-1351.

Guo, S., C. Dai, M. Guo, B. Taylor, J. S. Harmon, M. Sander, R. P. Robertson, A. C. Powers & R. Stein (2013) Inactivation of specific beta cell transcription factors in type 2 diabetes. *J Clin Invest,* 123**,** 3305-16.

Gut, A., C. E. Kiraly, M. Fukuda, K. Mikoshiba, C. B. Wollheim & J. Lang (2001) Expression and localisation of synaptotagmin isoforms in endocrine (β)-cells: their function in insulin exocytosis. *Journal of Cell Science,* 114**,** 1709.

Gutiérrez, G. D., A. S. Bender, V. Cirulli, T. L. Mastracci, S. M. Kelly, A. Tsirigos, K. H. Kaestner & L. Sussel (2017) Pancreatic β cell identity requires continual repression of non–β cell programs. *The Journal of Clinical Investigation,* 127**,** 244-259.

Gwynn, B., K. Lueders, M. S. Sands & E. H. Birkenmeier (1998) Intracisternal A-particle element transposition into the murine beta-glucuronidase gene correlates with loss of enzyme activity: a new model for beta-glucuronidase deficiency in the C3H mouse. *Molecular and cellular biology,* 18**,** 6474-6481.

Güemes, M. & K. Hussain (2015) Hyperinsulinemic Hypoglycemia. *Pediatric Clinics of North America,* 62**,** 1017-1036.

Hajmrle, C., N. Smith, A. F. Spigelman, X. Dai, L. Senior, A. Bautista, M. Ferdaoussi & P. E. MacDonald (2016) Interleukin-1 signaling contributes to acute islet compensation. *JCI insight,* 1**,** e86055-e86055.

Hall, E., T. Dayeh, C. L. Kirkpatrick, C. B. Wollheim, M. Dekker Nitert & C. Ling (2013) DNA methylation of the glucagon-like peptide 1 receptor (GLP1R) in human pancreatic islets. *BMC Medical Genetics,* 14**,** 76.

Hall, E., P. Volkov, T. Dayeh, K. Bacos, T. Rönn, M. D. Nitert & C. Ling (2014) Effects of palmitate on genome-wide mRNA expression and DNA methylation patterns in human pancreatic islets. *BMC medicine,* 12**,** 103-103.

Han, D., A. G. Lerner, L. Vande Walle, J.-P. Upton, W. Xu, A. Hagen, B. J. Backes, S. A. Oakes & F. R. Papa (2009) IRE1α Kinase Activation Modes Control Alternate Endoribonuclease Outputs to Determine Divergent Cell Fates. *Cell,* 138**,** 562-575.

Hancock, A. S., A. Du, J. Liu, M. Miller & C. L. May (2010) Glucagon deficiency reduces hepatic glucose production and improves glucose tolerance in adult mice. *Molecular endocrinology (Baltimore, Md.),* 24**,** 1605-1614.

Hang, Y. & R. Stein (2011) MafA and MafB activity in pancreatic β cells. *Trends in endocrinology and metabolism: TEM,* 22**,** 364-373.

Hani, E. H., P. Boutin, E. Durand, H. Inoue, M. A. Permutt, G. Velho & P. Froguel (1998) Missense mutations in the pancreatic islet beta cell inwardly rectifying K+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of Type II diabetes mellitus in Caucasians. *Diabetologia,* 41**,** 1511-1515.

Hanis, C. L., E. Boerwinkle, R. Chakraborty, D. L. Ellsworth, P. Concannon, B. Stirling, V. A. Morrison, B. Wapelhorst, R. S. Spielman, K. J. Gogolin-Ewens, J. M. Shephard, S. R. Williams, N. Risch, D. Hinds, N. Iwasaki, M. Ogata, Y. Omori, C. Petzold, H. Rietzsch, H. E. Schröder, J. Schulze, N. J. Cox, S. Menzel, V. V. Boriraj, X. Chen, L. R. Lim, T. Lindner, L. E. Mereu, Y. Q. Wang, K. Xiang, K. Yamagata, Y. Yang & G. I. Bell (1996) A genome–wide search for human non–insulin–dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. *Nature Genetics,* 13**,** 161-166.

Hansen, J. S., X. Zhao, M. Irmler, X. Liu, M. Hoene, M. Scheler, Y. Li, J. Beckers, M. Hrabě de Angelis, H.-U. Häring, B. K. Pedersen, R. Lehmann, G. Xu, P. Plomgaard & C. Weigert (2015) Type 2 diabetes alters metabolic and transcriptional signatures of glucose and amino acid metabolism during exercise and recovery. *Diabetologia,* 58**,** 1845-1854.

Hanson, R. L., R. B. Brown, M. M. Steele, P. M. Grandgenett, J. A. Grunkemeyer & M. A. Hollingsworth (2016) Identification of FRA-1 as a novel player in pancreatic cancer in cooperation with a MUC1: ERK signaling axis. *Oncotarget,* 7**,** 39996-40011.

Hargreaves, D. C. & G. R. Crabtree (2011) ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. *Cell Research,* 21**,** 396-420.

Harmon, J. S., M. Bogdani, S. D. Parazzoli, S. S. Mak, E. A. Oseid, M. Berghmans, R. C. Leboeuf & R. P. Robertson (2009) beta-Cell-specific overexpression of glutathione peroxidase preserves intranuclear MafA and reverses diabetes in db/db mice. *Endocrinology,* 150**,** 4855-4862.

Harmon, J. S., R. Stein & R. P. Robertson (2005) Oxidative Stress-mediated, Post-translational Loss of MafA Protein as a Contributing Mechanism to Loss of Insulin Gene Expression in Glucotoxic Beta Cells. *Journal of Biological Chemistry,* 280**,** 11107-11113.

Hashimoto, Y., Y. Akiyama & Y. Yuasa (2013) Multiple-to-multiple relationships between microRNAs and target genes in gastric cancer. *PloS one,* 8**,** e62589-e62589.

Haumaitre, C., E. Barbacci, M. Jenny, M. O. Ott, G. Gradwohl & S. Cereghini (2005) Lack of TCF2/vHNF1 in mice leads to pancreas agenesis. *Proceedings of the National Academy of Sciences of the United States of America,* 102**,** 1490-1495.

Haumaitre, C., O. Lenoir & R. Scharfmann (2008) Histone deacetylase inhibitors modify pancreatic cell fate determination and amplify endocrine progenitors. *Molecular and cellular biology,* 28**,** 6373-6383.

Havas, K., A. Flaus, M. Phelan, R. Kingston, P. A. Wade, D. M. J. Lilley & T. Owen-Hughes (2000) Generation of Superhelical Torsion by ATP-Dependent Chromatin Remodeling Activities. *Cell,* 103**,** 1133-1142.

Hayashi, K. & A. Altman (2007) Protein kinase C theta (PKCtheta): a key player in T cell life and death. *Pharmacological research,* 55**,** 537-544.
Hayes, H. L., L. Zhang, T. C. Becker, J. M. Haldeman, S. B. Stephens, M. Arlotto, L. G. Moss, C. B. Newgard & H. E. Hohmeier (2016) A Pdx-1-Regulated Soluble Factor Activates Rat and Human Islet Cell Proliferation. *Molecular and cellular biology,* 36**,** 2918-2930.
He, X., Y. Li, T. Su, S. Lai, W. Wu, L. Chen, J. Si & L. Sun (2018) The impact of a history of cancer on pancreatic ductal adenocarcinoma survival. *United European gastroenterology journal,* 6**,** 888-894.
Hebrok, M., S. K. Kim & D. A. Melton (1998) Notochord repression of endodermal Sonic hedgehog permits pancreas development. *Genes & Development,* 12**,** 1705-1713.
Hellman, A. & A. Chess (2007) Gene Body-Specific Methylation on the Active X Chromosome. *Science,* 315**,** 1141.
Heni, M., C. Ketterer, L. M. t. Hart, F. Ranta, T. W. van Haeften, E. M. Eekhoff, J. M. Dekker, D. I. Boomsma, G. Nijpels, M. H. Kramer, M. Diamant, A. M. Simonis-Bik, R. J. Heine, E. J. de Geus, S. A. Schäfer, F. Machicao, S. Ullrich, C. Thamer, N. Stefan, H. Staiger, H.-U. Häring & A. Fritsche (2010) The Impact of Genetic Variation in the G6PC2 Gene on Insulin Secretion Depends on Glycemia. *The Journal of Clinical Endocrinology & Metabolism,* 95**,** E479-E484.
Henquin, J. C. (2009) Regulation of insulin secretion: a matter of phase control and amplitude modulation. *Diabetologia,* 52**,** 739.
Herman, M. A., O. D. Peroni, J. Villoria, M. R. Schön, N. A. Abumrad, M. Blüher, S. Klein & B. B. Kahn (2012) A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. *Nature,* 484**,** 333-338.
Hermann, P. C., P. Sancho, M. Cañamero, P. Martinelli, F. Madriles, P. Michl, T. Gress, R. de Pascual, L. Gandia, C. Guerra, M. Barbacid, M. Wagner, C. R. Vieira, A. Aicher, F. X. Real, B. Sainz, Jr. & C. Heeschen (2014) Nicotine Promotes Initiation and Progression of KRAS-Induced Pancreatic Cancer via Gata6-Dependent Dedifferentiation of Acinar Cells in Mice. *Gastroenterology,* 147**,** 1119-1133.e4.
Herrera, P. L. (2000) Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development,* 127**,** 2317.
Herron, D. C. & R. C. Shank (1981) In Vivo Kinetics of O⁶-Methylguanine and 7-Methylguanine Formation and Persistence in DNA of Rats Treated with Symmetrical Dimethylhydrazine. *Cancer Research,* 41**,** 3967.
Herzig, S., F. Long, U. S. Jhala, S. Hedrick, R. Quinn, A. Bauer, D. Rudolph, G. Schutz, C. Yoon, P. Puigserver, B. Spiegelman & M. Montminy (2001) CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature,* 413**,** 179-183.
Ho, L. & G. R. Crabtree (2010) Chromatin remodelling during development. *Nature,* 463**,** 474-484.
Hoffmeister, H., A. Fuchs, F. Erdel, S. Pinz, R. Gröbner-Ferreira, A. Bruckmann, R. Deutzmann, U. Schwartz, R. Maldonado, C. Huber, A.-S. Dendorfer, K. Rippe & G. Längst (2017) CHD3 and CHD4 form distinct NuRD complexes with different yet overlapping functionality. *Nucleic acids research,* 45**,** 10534-10554.
Holland, A. M., M. A. Hale, H. Kagami, R. E. Hammer & R. J. MacDonald (2002) Experimental control of pancreatic development and maintenance. *Proceedings of the National Academy of Sciences of the United States of America,* 99**,** 12236-12241.
Hollien, J., J. H. Lin, H. Li, N. Stevens, P. Walter & J. S. Weissman (2009) Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *The Journal of Cell Biology,* 186**,** 323.
Hollien, J. & J. S. Weissman (2006) Decay of Endoplasmic Reticulum-Localized mRNAs During the Unfolded Protein Response. *Science,* 313**,** 104.
Holst, J. J. (2007) The Physiology of Glucagon-like Peptide 1. *Physiological Reviews,* 87**,** 1409-1439.
Hong, W. (2005) SNAREs and traffic. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research,* 1744**,** 120-144.

Horikawa, Y., M. Enya, H. Mabe, K. Fukushima, N. Takubo, M. Ohashi, F. Ikeda, K.-i. Hashimoto, H. Watada & J. Takeda (2018) NEUROD1-deficient diabetes (MODY6): Identification of the first cases in Japanese and the clinical features. *Pediatric Diabetes,* 19**,** 236-242.

Horsham, J. L., C. Ganda, F. C. Kalinowski, R. A. M. Brown, M. R. Epis & P. J. Leedman (2015) MicroRNA-7: A miRNA with expanding roles in development and disease. *The International Journal of Biochemistry & Cell Biology,* 69**,** 215-224.

Hosey, M. M., J. Barhanin, A. Schmid, S. Vandaele, J. Ptasienski, C. O'Callahan, C. Cooper & M. Lazdunski (1987) Photoaffinity labelling and phosphorylation of a 165 kilodalton peptide associated with dihydropyridine and phenylalkylamine-sensitive calcium channels. *Biochemical and Biophysical Research Communications,* 147**,** 1137-1145.

Hota, S. K., S. K. Bhardwaj, S. Deindl, Y.-c. Lin, X. Zhuang & B. Bartholomew (2013) Nucleosome mobilization by ISW2 requires the concerted action of the ATPase and SLIDE domains. *Nature Structural &Amp; Molecular Biology,* 20**,** 222.

Hota, S. K. & B. G. Bruneau (2016) ATP-dependent chromatin remodeling during mammalian development. *Development,* 143**,** 2882.

Hou, J. C., L. Min & J. E. Pessin (2009) Insulin granule biogenesis, trafficking and exocytosis. *Vitamins and hormones,* 80**,** 473-506.

Houbaviy, H. B., M. F. Murray & P. A. Sharp (2003) Embryonic Stem Cell-Specific MicroRNAs. *Developmental Cell,* 5**,** 351-358.

Hu, C., R. Zhang, C. Wang, X. Ma, Q. Fang, Y. Bao, K. Xiang & W. Jia (2009) A genetic variant of G6PC2 is associated with type 2 diabetes and fasting plasma glucose level in the Chinese population. *Diabetologia,* 52**,** 451-456.

Huang, C., E. M. Walker, P. K. Dadi, R. Hu, Y. Xu, W. Zhang, T. Sanavia, J. Mun, J. Liu, G. G. Nair, H. Y. A. Tan, S. Wang, M. A. Magnuson, C. J. Stoeckert, Jr., M. Hebrok, M. Gannon, W. Han, R. Stein, D. A. Jacobson & G. Gu (2018) Synaptotagmin 4 Regulates Pancreatic β Cell Maturation by Modulating the Ca(2+) Sensitivity of Insulin Secretion Vesicles. *Developmental cell,* 45**,** 347-361.e5.

Huang, C.-j., C.-y. Lin, L. Haataja, T. Gurlo, A. E. Butler, R. A. Rizza & P. C. Butler (2007) High Expression Rates of Human Islet Amyloid Polypeptide Induce Endoplasmic Reticulum Stress–Mediated β-Cell Apoptosis, a Characteristic of Humans With Type 2 but Not Type 1 Diabetes. *Diabetes,* 56**,** 2016.

Huang, X. F. & P. Arvan (1994) Formation of the insulin-containing secretory granule core occurs within immature beta-granules. *Journal of Biological Chemistry,* 269**,** 20838-20844.

--- (1995) Intracellular Transport of Proinsulin in Pancreatic β-Cells: STRUCTURAL MATURATION PROBED BY DISULFIDE ACCESSIBILITY. *Journal of Biological Chemistry,* 270**,** 20417-20423.

Huang, Y., S. J. Myers & R. Dingledine (1999) Transcriptional repression by REST: recruitment of Sin3A and histone deacetylase to neuronal genes. *Nature Neuroscience,* 2**,** 867-872.

Hummel, K. P., M. M. Dickie & D. L. Coleman (1966) Diabetes, a New Mutafton in the Mouse. *Science,* 153**,** 1127.

Huntzinger, E. & E. Izaurralde (2011) Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nature Reviews Genetics,* 12**,** 99-110.

Hwang, W. W., S. Venkatasubrahmanyam, A. G. Ianculescu, A. Tong, C. Boone & H. D. Madhani (2003) A Conserved RING Finger Protein Required for Histone H2B Monoubiquitination and Cell Size Control. *Molecular Cell,* 11**,** 261-266.

Ihara, Y., S. Toyokuni, K. Uchida, H. Odaka, T. Tanaka, H. Ikeda, H. Hiai, Y. Seino & Y. Yamada (1999) Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. *Diabetes,* 48**,** 927.

Inagaki, N., T. Gonoi, J. P. Clement, N. Namba, J. Inazawa, G. Gonzalez, L. Aguilar-Bryan, S. Seino & J. Bryan (1995) Reconstitution of I_{KATP}: An Inward Rectifier Subunit Plus the Sulfonylurea Receptor. *Science,* 270**,** 1166.

Indra, A. K., V. Dupé, J.-M. Bornert, N. Messaddeq, M. Yaniv, M. Mark, P. Chambon & D. Metzger (2005) Temporally controlled targeted somatic mutagenesis in embryonic surface ectoderm and fetal epidermal keratinocytes unveils two distinct developmental functions of BRG1 in limb morphogenesis and skin barrier formation. *Development,* 132**,** 4533.

Ionescu-Tirgoviste, C., P. A. Gagniuc, E. Gubceac, L. Mardare, I. Popescu, S. Dima & M. Militaru (2015) A 3D map of the islet routes throughout the healthy human pancreas. *Scientific reports,* 5**,** 14634-14634.

Iseki, K., Y. Ikemiya, C. Iseki & S. Takishita (2003) Proteinuria and the risk of developing end-stage renal disease. *Kidney Int,* 63**,** 1468-74.

Iwakawa, H.-o. & Y. Tomari (2015) The Functions of MicroRNAs: mRNA Decay and Translational Repression. *Trends in Cell Biology,* 25**,** 651-665.

Iwasaki, S., M. Kobayashi, M. Yoda, Y. Sakaguchi, S. Katsuma, T. Suzuki & Y. Tomari (2010) Hsc70/Hsp90 Chaperone Machinery Mediates ATP-Dependent RISC Loading of Small RNA Duplexes. *Molecular Cell,* 39**,** 292-299.

Iwase, S., A. Januma, K. Miyamoto, N. Shono, A. Honda, J. Yanagisawa & T. Baba (2004) Characterization of BHC80 in BRAF–HDAC complex, involved in neuron-specific gene repression. *Biochemical and Biophysical Research Communications,* 322**,** 601-608.

Iyer, L. M., D. Zhang & L. Aravind (2016) Adenine methylation in eukaryotes: Apprehending the complex evolutionary history and functional potential of an epigenetic modification. *BioEssays : news and reviews in molecular, cellular and developmental biology,* 38**,** 27-40.

Iynedjian, P. B. (1993) Mammalian glucokinase and its gene. *The Biochemical journal,* 293 (Pt 1)**,** 1-13.

Jackson, R. A., G. Perry, J. Rogers, U. Advani & T. R. E. Pilkington (1973) Relationship Between the Basal Glucose Concentration Glucose Tolerance and Forearm Glucose Uptake in Maturity-onset Diabetes. *Diabetes,* 22**,** 751.

Jackson, S. H., T. S. Martin, J. D. Jones, D. Seal & F. Emanuel (2010) Liraglutide (victoza): the first once-daily incretin mimetic injection for type-2 diabetes. *P & T : a peer-reviewed journal for formulary management,* 35**,** 498-529.

Jacqueminet, S., I. Briaud, C. Rouault, G. Reach & V. Poitout (2000) Inhibition of insulin gene expression by long-term exposure of pancreatic β cells to palmitate is dependent on the presence of a stimulatory glucose concentration. *Metabolism - Clinical and Experimental,* 49**,** 532-536.

Jaenisch, R. & A. Bird (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature Genetics,* 33**,** 245-254.

Jahn, R. & R. H. Scheller (2006) SNAREs — engines for membrane fusion. *Nature Reviews Molecular Cell Biology,* 7**,** 631-643.

Jennings, R. E., A. A. Berry, R. Kirkwood-Wilson, N. A. Roberts, T. Hearn, R. J. Salisbury, J. Blaylock, K. Piper Hanley & N. A. Hanley (2013) Development of the human pancreas from foregut to endocrine commitment. *Diabetes,* 62**,** 3514-3522.

Jenstad, M. & F. A. Chaudhry (2013) The Amino Acid Transporters of the Glutamate/GABA-Glutamine Cycle and Their Impact on Insulin and Glucagon Secretion. *Frontiers in endocrinology,* 4**,** 199-199.

Jeon, J., M. Correa-Medina, C. Ricordi, H. Edlund & J. A. Diez (2009) Endocrine cell clustering during human pancreas development. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society,* 57**,** 811-824.

Jhala, U. S., G. Canettieri, R. A. Screaton, R. N. Kulkarni, S. Krajewski, J. Reed, J. Walker, X. Lin, M. White & M. Montminy (2003) cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2. *Genes & development,* 17**,** 1575-1580.

Jiang, G. & B. B. Zhang (2003) Glucagon and regulation of glucose metabolism. *American Journal of Physiology-Endocrinology and Metabolism,* 284**,** E671-E678.

Jo, J., M. Y. Choi & D.-S. Koh (2007) Size distribution of mouse Langerhans islets. *Biophysical journal,* 93**,** 2655-2666.
Joglekar, M. V., V. M. Joglekar & A. A. Hardikar (2009) Expression of islet-specific microRNAs during human pancreatic development. *Gene Expr Patterns,* 9**,** 109-13.
Johnston, N. R., R. K. Mitchell, E. Haythorne, M. P. Pessoa, F. Semplici, J. Ferrer, L. Piemonti, P. Marchetti, M. Bugliani, D. Bosco, E. Berishvili, P. Duncanson, M. Watkinson, J. Broichhagen, D. Trauner, G. A. Rutter & D. J. Hodson (2016) Beta Cell Hubs Dictate Pancreatic Islet Responses to Glucose. *Cell metabolism,* 24**,** 389-401.
Jonas, J.-C., D. R. Laybutt, G. M. Steil, N. Trivedi, J. G. Pertusa, M. Van de Casteele, G. C. Weir & J.-C. Henquin (2001) High Glucose Stimulates Early Response Gene c-Myc Expression in Rat Pancreatic β Cells. *Journal of Biological Chemistry,* 276**,** 35375-35381.
Jonas, J.-C., A. Sharma, W. Hasenkamp, H. Ilkova, G. Patanè, R. Laybutt, S. Bonner-Weir & G. C. Weir (1999) Chronic Hyperglycemia Triggers Loss of Pancreatic β Cell Differentiation in an Animal Model of Diabetes. *Journal of Biological Chemistry,* 274**,** 14112-14121.
Jonas, J. C., M. Bensellam, J. Duprez, H. Elouil, Y. Guiot & S. M. A. Pascal (2009) Glucose regulation of islet stress responses and β-cell failure in type 2 diabetes. *Diabetes, Obesity and Metabolism,* 11**,** 65-81.
Jonas, S. & E. Izaurralde (2015) Towards a molecular understanding of microRNA-mediated gene silencing. *Nature Reviews Genetics,* 16**,** 421.
Jonsson, J., L. Carlsson, T. Edlund & H. Edlund (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature,* 371**,** 606-609.
Jui, H.-Y., D. Accili & S. I. Taylor (1996) Characterization of a Hybrid Receptor Formed by Dimerization of the Insulin Receptor-Related Receptor (IRR) with the Insulin Receptor (IR): Coexpression of cDNAs Encoding Human IRR and Human IR in NIH-3T3 Cells. *Biochemistry,* 35**,** 14326-14330.
Jégu, T., A. Veluchamy, J. S. Ramirez-Prado, C. Rizzi-Paillet, M. Perez, A. Lhomme, D. Latrasse, E. Coleno, S. Vicaire, S. Legras, B. Jost, M. Rougée, F. Barneche, C. Bergounioux, M. Crespi, M. M. Mahfouz, H. Hirt, C. Raynaud & M. Benhamed (2017) The Arabidopsis SWI/SNF protein BAF60 mediates seedling growth control by modulating DNA accessibility. *Genome biology,* 18**,** 114-114.
Kadoch, C., D. C. Hargreaves, C. Hodges, L. Elias, L. Ho, J. Ranish & G. R. Crabtree (2013) Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nature genetics,* 45**,** 592-601.
Kaeser, M. D., A. Aslanian, M.-Q. Dong, J. R. Yates & B. M. Emerson (2008) BRD7, a Novel PBAF-specific SWI/SNF Subunit, Is Required for Target Gene Activation and Repression in Embryonic Stem Cells. *Journal of Biological Chemistry,* 283**,** 32254-32263.
Kahn, B. B. (1998) Type 2 Diabetes: When Insulin Secretion Fails to Compensate for Insulin Resistance. *Cell,* 92**,** 593-596.
Kahn, S. E. (2003) The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia,* 46**,** 3-19.
Kahn, S. E., M. E. Cooper & S. Del Prato (2014) Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet (London, England),* 383**,** 1068-1083.
Kahn, S. E., R. L. Prigeon, D. K. McCulloch, E. J. Boyko, R. N. Bergman, M. W. Schwartz, J. L. Neifing, W. K. Ward, J. C. Beard & J. P. Palmer (1993) Quantification of the Relationship Between Insulin Sensitivity and β-Cell Function in Human Subjects: Evidence for a Hyperbolic Function. *Diabetes,* 42**,** 1663.
Kalinowski, F. C., R. A. M. Brown, C. Ganda, K. M. Giles, M. R. Epis, J. Horsham & P. J. Leedman (2014) microRNA-7: A tumor suppressor miRNA with therapeutic potential. *The International Journal of Biochemistry & Cell Biology,* 54**,** 312-317.
Kalis, M., C. Bolmeson, J. L. S. Esguerra, S. Gupta, A. Edlund, N. Tormo-Badia, D. Speidel, D. Holmberg, S. Mayans, N. K. S. Khoo, A. Wendt, L. Eliasson & C. M. Cilio (2011) Beta-cell

specific deletion of Dicer1 leads to defective insulin secretion and diabetes mellitus. *PloS one,* 6**,** e29166-e29166.

Kandimalla, R., A. A. G. van Tilborg, L. C. Kompier, D. J. P. M. Stumpel, R. W. Stam, C. H. Bangma & E. C. Zwarthoff (2012) Genome-wide Analysis of CpG Island Methylation in Bladder Cancer Identified TBX2, TBX3, GATA2, and ZIC4 as pTa-Specific Prognostic Markers. *European Urology,* 61**,** 1245-1256.

Kaneko, H., T. Ariyasu, R. Inoue, T. Fukao, K. Kasahara, T. Teramoto, E. Matsui, S. Hayakawa & N. Kondo (1998) Expression of Pax5 gene in human haematopoietic cells and tissues: comparison with immunodeficient donors. *Clinical and experimental immunology,* 111**,** 339-344.

Kaneto, H., J. Fujii, T. Myint, N. Miyazawa, K. N. Islam, Y. Kawasaki, K. Suzuki, M. Nakamura, H. Tatsumi, Y. Yamasaki & N. Taniguchi (1996) Reducing sugars trigger oxidative modification and apoptosis in pancreatic beta-cells by provoking oxidative stress through the glycation reaction. *The Biochemical journal,* 320 (Pt 3)**,** 855-863.

Kaneto, H. & T.-a. Matsuoka (2015) Role of pancreatic transcription factors in maintenance of mature β-cell function. *International journal of molecular sciences,* 16**,** 6281-6297.

Kaneto, H., A. Sharma, K. Suzuma, D. R. Laybutt, G. Xu, S. Bonner-Weir & G. C. Weir (2002a) Induction of c-Myc Expression Suppresses Insulin Gene Transcription by Inhibiting NeuroD/BETA2-mediated Transcriptional Activation. *Journal of Biological Chemistry,* 277**,** 12998-13006.

Kaneto, H., G. Xu, N. Fujii, S. Kim, S. Bonner-Weir & G. C. Weir (2002b) Involvement of c-Jun N-terminal Kinase in Oxidative Stress-mediated Suppression of Insulin Gene Expression. *Journal of Biological Chemistry,* 277**,** 30010-30018.

Kang, G., O. G. Chepurny, B. Malester, M. J. Rindler, H. Rehmann, J. L. Bos, F. Schwede, W. A. Coetzee & G. G. Holz (2006) cAMP sensor Epac as a determinant of ATP-sensitive potassium channel activity in human pancreatic beta cells and rat INS-1 cells. *The Journal of physiology,* 573**,** 595-609.

Kapan, N., O. V. Lushchak, J. Luo & D. R. Nässel (2012) Identified peptidergic neurons in the Drosophila brain regulate insulin-producing cells, stress responses and metabolism by coexpressed short neuropeptide F and corazonin. *Cellular and Molecular Life Sciences,* 69**,** 4051-4066.

Karmodiya, K., A. R. Krebs, M. Oulad-Abdelghani, H. Kimura & L. Tora (2012) H3K9 and H3K14 acetylation co-occur at many gene regulatory elements, while H3K14ac marks a subset of inactive inducible promoters in mouse embryonic stem cells. *BMC Genomics,* 13**,** 424.

Kataoka, K., M. Noda & M. Nishizawa (1994) Maf nuclear oncoprotein recognizes sequences related to an AP-1 site and forms heterodimers with both Fos and Jun. *Molecular and cellular biology,* 14**,** 700-712.

Katsuoka, F. & M. Yamamoto (2016) Small Maf proteins (MafF, MafG, MafK): History, structure and function. *Gene,* 586**,** 197-205.

Kawamori, D., H. Kaneto, Y. Nakatani, T.-a. Matsuoka, M. Matsuhisa, M. Hori & Y. Yamasaki (2006) The Forkhead Transcription Factor Foxo1 Bridges the JNK Pathway and the Transcription Factor PDX-1 through Its Intracellular Translocation. *Journal of Biological Chemistry,* 281**,** 1091-1098.

Kawasaki, H., G. M. Springett, N. Mochizuki, S. Toki, M. Nakaya, M. Matsuda, D. E. Housman & A. M. Graybiel (1998) A Family of cAMP-Binding Proteins That Directly Activate Rap1. *Science,* 282**,** 2275.

Ketting, R. F., S. E. J. Fischer, E. Bernstein, T. Sijen, G. J. Hannon & R. H. A. Plasterk (2001) Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. *Genes & Development,* 15**,** 2654-2659.

Khavari, P. A., C. L. Peterson, J. W. Tamkun, D. B. Mendel & G. R. Crabtree (1993) BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. *Nature,* 366**,** 170-174.

Khvorova, A., A. Reynolds & S. D. Jayasena (2003) Functional siRNAs and miRNAs Exhibit Strand Bias. *Cell,* 115**,** 209-216.

Kilimnik, G., B. Zhao, J. Jo, V. Periwal, P. Witkowski, R. Misawa & M. Hara (2011) Altered Islet Composition and Disproportionate Loss of Large Islets in Patients with Type 2 Diabetes. *PLOS ONE,* 6**,** e27445.

Kim, D., G. Pertea, C. Trapnell, H. Pimentel, R. Kelley & S. L. Salzberg (2013a) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biology,* 14**,** R36.

Kim, J. K., S.-O. Huh, H. Choi, K.-S. Lee, D. Shin, C. Lee, J.-S. Nam, H. Kim, H. Chung, H. W. Lee, S. D. Park & R. H. Seong (2001) Srg3, a Mouse Homolog of Yeast SWI3, Is Essential for Early Embryogenesis and Involved in Brain Development. *Molecular and Cellular Biology,* 21**,** 7787.

Kim, J. W., Y. H. You, S. Jung, H. Suh-Kim, I. K. Lee, J. H. Cho & K. H. Yoon (2013b) miRNA-30a-5p-mediated silencing of Beta2/NeuroD expression is an important initial event of glucotoxicity-induced beta cell dysfunction in rodent models. *Diabetologia,* 56**,** 847-855.

Kim, S. K., M. Hebrok, E. Li, S. P. Oh, H. Schrewe, E. B. Harmon, J. S. Lee & D. A. Melton (2000) Activin receptor patterning of foregut organogenesis. *Genes & Development,* 14**,** 1866-1871.

Kim, W., J. L. Fiori, Y.-K. Shin, E. Okun, J. S. Kim, P. R. Rapp & J. M. Egan (2014) Pancreatic polypeptide inhibits somatostatin secretion. *FEBS letters,* 588**,** 3233-3239.

Kitamura, T., J. Nakae, Y. Kitamura, Y. Kido, W. H. Biggs, III, C. V. E. Wright, M. F. White, K. C. Arden & D. Accili (2002) The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic β cell growth. *The Journal of Clinical Investigation,* 110**,** 1839-1847.

Kitamura, Y. I., T. Kitamura, J.-P. Kruse, J. C. Raum, R. Stein, W. Gu & D. Accili (2005) FoxO1 protects against pancreatic β cell failure through NeuroD and MafA induction. *Cell Metabolism,* 2**,** 153-163.

Kleeff, J., T. Ishiwata, H. Friess, M. W. Büchler & M. Korc (1998) Concomitant over-expression of activin/inhibin β subunits and their receptors in human pancreatic cancer. *International Journal of Cancer,* 77**,** 860-868.

Klose, R. J., E. M. Kallin & Y. Zhang (2006) JmjC-domain-containing proteins and histone demethylation. *Nature Reviews Genetics,* 7**,** 715-727.

Knight, S. W. & B. L. Bass (2001) A Role for the RNase III Enzyme DCR-1 in RNA Interference and Germ Line Development in Caenorhabditis elegans. *Science,* 293**,** 2269.

Kong, D., S. Banerjee, A. Ahmad, Y. Li, Z. Wang, S. Sethi & F. H. Sarkar (2010) Epithelial to Mesenchymal Transition Is Mechanistically Linked with Stem Cell Signatures in Prostate Cancer Cells. *PLOS ONE,* 5**,** e12445.

Kotani, K., W. Ogawa, M. Matsumoto, T. Kitamura, H. Sakaue, Y. Hino, K. Miyake, W. Sano, K. Akimoto, S. Ohno & M. Kasuga (1998) Requirement of atypical protein kinase clambda for insulin stimulation of glucose uptake but not for Akt activation in 3T3-L1 adipocytes. *Molecular and cellular biology,* 18**,** 6971-6982.

Kredo-Russo, S., A. D. Mandelbaum, A. Ness, I. Alon, K. A. Lennox, M. A. Behlke & E. Hornstein (2012a) Pancreas-enriched miRNA refines endocrine cell differentiation. *Development,* 139**,** 3021-31.

Kredo-Russo, S., A. Ness, A. D. Mandelbaum, M. D. Walker & E. Hornstein (2012b) Regulation of pancreatic microRNA-7 expression. *Exp Diabetes Res,* 2012**,** 695214.

Kresge, N., R. D. Simoni & R. L. Hill (2005) Otto Fritz Meyerhof and the Elucidation of the Glycolytic Pathway. *Journal of Biological Chemistry,* 280**,** e3-e3.

Krishnamurthy, J., C. Torrice, M. R. Ramsey, G. I. Kovalev, K. Al-Regaiey, L. Su & N. E. Sharpless (2004) Ink4a/Arf expression is a biomarker of aging. *The Journal of clinical investigation,* 114**,** 1299-1307.

Kristinsson, S. Y., E. T. Thorolfsdottir, B. Talseth, E. Steingrimsson, A. V. Thorsson, T. Helgason, A. B. Hreidarsson & R. Arngrimsson (2001) MODY in Iceland is associated with mutations in HNF-1α and a novel mutation in NeuroD1. *Diabetologia,* 44**,** 2098-2103.

Kulkarni, R. N. (2004) The islet β-cell. *The International Journal of Biochemistry & Cell Biology,* 36**,** 365-371.

Kulkarni, R. N., J. C. Brüning, J. N. Winnay, C. Postic, M. A. Magnuson & C. R. Kahn (1999) Tissue-Specific Knockout of the Insulin Receptor in Pancreatic β Cells Creates an Insulin Secretory Defect Similar to that in Type 2 Diabetes. *Cell,* 96**,** 329-339.

Kumar, M. & D. Melton (2003) Pancreas specification: a budding question. *Current Opinion in Genetics & Development,* 13**,** 401-407.

Kuster, J. E., M. H. Guarnieri, J. G. Ault, L. Flaherty & P. J. Swiatek (1997) IAP insertion in the murine LamB3 gene results in junctional epidermolysis bullosa. *Mammalian Genome,* 8**,** 673-681.

Kwan, E. P., L. Xie, L. Sheu, T. Ohtsuka & H. Y. Gaisano (2007) Interaction Between Munc13-1 and RIM Is Critical for Glucagon-Like Peptide-1–Mediated Rescue of Exocytotic Defects in Munc13-1–Deficient Pancreatic β-Cells. *Diabetes,* 56**,** 2579.

Lagos-Quintana, M., R. Rauhut, W. Lendeckel & T. Tuschl (2001) Identification of Novel Genes Coding for Small Expressed RNAs. *Science,* 294**,** 853-858.

Lagos-Quintana, M., R. Rauhut, A. Yalcin, J. Meyer, W. Lendeckel & T. Tuschl (2002) Identification of Tissue-Specific MicroRNAs from Mouse. *Current Biology,* 12**,** 735-739.

Landgraf, P., M. Rusu, R. Sheridan, A. Sewer, N. Iovino, A. Aravin, S. Pfeffer, A. Rice, A. O. Kamphorst, M. Landthaler, C. Lin, N. D. Socci, L. Hermida, V. Fulci, S. Chiaretti, R. Foa, J. Schliwka, U. Fuchs, A. Novosel, R. U. Muller, B. Schermer, U. Bissels, J. Inman, Q. Phan, M. Chien, D. B. Weir, R. Choksi, G. De Vita, D. Frezzetti, H. I. Trompeter, V. Hornung, G. Teng, G. Hartmann, M. Palkovits, R. Di Lauro, P. Wernet, G. Macino, C. E. Rogler, J. W. Nagle, J. Ju, F. N. Papavasiliou, T. Benzing, P. Lichter, W. Tam, M. J. Brownstein, A. Bosio, A. Borkhardt, J. J. Russo, C. Sander, M. Zavolan & T. Tuschl (2007) A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell,* 129**,** 1401-14.

Langmead, B. & S. L. Salzberg (2012) Fast gapped-read alignment with Bowtie 2. *Nature methods,* 9**,** 357-359.

Lantz, K. A., M. Z. Vatamaniuk, J. E. Brestelli, J. R. Friedman, F. M. Matschinsky & K. H. Kaestner (2004) Foxa2 regulates multiple pathways of insulin secretion. *The Journal of clinical investigation,* 114**,** 512-520.

Latreille, M., J. Hausser, I. Stutzer, Q. Zhang, B. Hastoy, S. Gargani, J. Kerr-Conte, F. Pattou, M. Zavolan, J. L. Esguerra, L. Eliasson, T. Rulicke, P. Rorsman & M. Stoffel (2014) MicroRNA-7a regulates pancreatic beta cell function. *J Clin Invest,* 124**,** 2722-35.

Latreille, M., K. Herrmanns, N. Renwick, T. Tuschl, M. T. Malecki, M. I. McCarthy, K. R. Owen, T. Rülicke & M. Stoffel (2015) miR-375 gene dosage in pancreatic β-cells: implications for regulation of β-cell mass and biomarker development. *Journal of molecular medicine (Berlin, Germany),* 93**,** 1159-1169.

Lavan, B. E., V. R. Fantin, E. T. Chang, W. S. Lane, S. R. Keller & G. E. Lienhard (1997) A Novel 160-kDa Phosphotyrosine Protein in Insulin-treated Embryonic Kidney Cells Is a New Member of the Insulin Receptor Substrate Family. *Journal of Biological Chemistry,* 272**,** 21403-21407.

Laybutt, D. R., A. M. Preston, M. C. Åkerfeldt, J. G. Kench, A. K. Busch, A. V. Biankin & T. J. Biden (2007) Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. *Diabetologia,* 50**,** 752-763.

Laybutt, D. R., G. C. Weir, H. Kaneto, J. Lebet, R. D. Palmiter, A. Sharma & S. Bonner-Weir (2002) Overexpression of c-Myc in β-Cells of Transgenic Mice Causes Proliferation and Apoptosis, Downregulation of Insulin Gene Expression, and Diabetes. *Diabetes,* 51**,** 1793.

Le Fur, S., C. Le Stunff & P. Bougnères (2002) Increased Insulin Resistance in Obese Children Who Have Both 972 IRS-1 and 1057 IRS-2 Polymorphisms. *Diabetes,* 51**,** S304.

Leahy, J. L. (2005) Pathogenesis of type 2 diabetes mellitus. *Arch Med Res,* 36**,** 197-209.
Leahy, J. L., L. M. Bumbalo & C. Chen (1994) Diazoxide Causes Recovery of β-Cell Glucose Responsiveness in 90% Pancreatectomized Diabetic Rats. *Diabetes,* 43**,** 173.
Ledermann, H. M. (1995) Maturity-onset diabetes of the young (MODY) at least ten times more common in Europe than previously assumed? *Diabetologia,* 38**,** 1482-1482.
Lee, H.-J., M. Palkovits & W. S. Young, 3rd (2006) miR-7b, a microRNA up-regulated in the hypothalamus after chronic hyperosmolar stimulation, inhibits Fos translation. *Proceedings of the National Academy of Sciences of the United States of America,* 103**,** 15669-15674.
Lee, R. C., R. L. Feinbaum & V. Ambros (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell,* 75**,** 843-854.
Lee, T. I. & R. A. Young (2013) Transcriptional regulation and its misregulation in disease. *Cell,* 152**,** 1237-1251.
Leech, C. A., G. G. Holz, O. Chepurny & J. F. Habener (2000) Expression of cAMP-regulated guanine nucleotide exchange factors in pancreatic beta-cells. *Biochemical and biophysical research communications,* 278**,** 44-47.
Lenoir, O., K. Flosseau, F. X. Ma, B. Blondeau, A. Mai, R. Bassel-Duby, P. Ravassard, E. N. Olson, C. Haumaitre & R. Scharfmann (2011) Specific control of pancreatic endocrine β- and δ-cell mass by class IIa histone deacetylases HDAC4, HDAC5, and HDAC9. *Diabetes,* 60**,** 2861-2871.
LeRoy, G., B. Rickards & S. J. Flint (2008) The double bromodomain proteins Brd2 and Brd3 couple histone acetylation to transcription. *Molecular cell,* 30**,** 51-60.
Leung, A. T., T. Imagawa & K. P. Campbell (1987) Structural characterization of the 1,4-dihydropyridine receptor of the voltage-dependent Ca2+ channel from rabbit skeletal muscle. Evidence for two distinct high molecular weight subunits. *Journal of Biological Chemistry,* 262**,** 7943-7946.
Lewis, B. P., I. h. Shih, M. W. Jones-Rhoades, D. P. Bartel & C. B. Burge (2003) Prediction of Mammalian MicroRNA Targets. *Cell,* 115**,** 787-798.
Lewis, J. D., R. R. Meehan, W. J. Henzel, I. Maurer-Fogy, P. Jeppesen, F. Klein & A. Bird (1992) Purification, sequence, and cellular localization of a novel chromosomal protein that binds to Methylated DNA. *Cell,* 69**,** 905-914.
Li, H., S. Ilin, W. Wang, E. M. Duncan, J. Wysocka, C. D. Allis & D. J. Patel (2006) Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. *Nature,* 442**,** 91-95.
Li, M., H. Zhao, X. Zhang, L. D. Wood, R. A. Anders, M. A. Choti, T. M. Pawlik, H. D. Daniel, R. Kannangai, G. J. A. Offerhaus, V. E. Velculescu, L. Wang, S. Zhou, B. Vogelstein, R. H. Hruban, N. Papadopoulos, J. Cai, M. S. Torbenson & K. W. Kinzler (2011) Inactivating mutations of the chromatin remodeling gene ARID2 in hepatocellular carcinoma. *Nature Genetics,* 43**,** 828-829.
Li, S., C. Liu, N. Li, T. Hao, T. Han, D. E. Hill, M. Vidal & J. D. Lin (2008) Genome-wide coactivation analysis of PGC-1alpha identifies BAF60a as a regulator of hepatic lipid metabolism. *Cell metabolism,* 8**,** 105-117.
Li, S., T. Liu, L. I. N. Mi, Z. Meng & J. Lin (2018) BAF60a Orchestrates Thermogenic Chromatin Accessibility in Brown Fat. *Diabetes,* 67**,** 275-OR.
Li, W., Y. Xiong, C. Shang, K. Y. Twu, C. T. Hang, J. Yang, P. Han, C.-Y. Lin, C.-J. Lin, F.-C. Tsai, K. Stankunas, T. Meyer, D. Bernstein, M. Pan & C.-P. Chang (2013) Brg1 governs distinct pathways to direct multiple aspects of mammalian neural crest cell development. *Proceedings of the National Academy of Sciences,* 110**,** 1738.
Li, Y., T. J. Soos, X. Li, J. Wu, M. DeGennaro, X. Sun, D. R. Littman, M. J. Birnbaum & R. D. Polakiewicz (2004) Protein Kinase C θ Inhibits Insulin Signaling by Phosphorylating IRS1 at Ser1101. *Journal of Biological Chemistry,* 279**,** 45304-45307.
Liao, X., H. Xue, Y.-C. Wang, K. L. Nazor, S. Guo, N. Trivedi, S. E. Peterson, Y. Liu, J. F. Loring & L. C. Laurent (2013) Matched miRNA and mRNA signatures from an hESC-based in vitro model of

pancreatic differentiation reveal novel regulatory interactions. *Journal of cell science,* 126**,** 3848-3861.
Liao, Y., G. K. Smyth & W. Shi (2013a) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics,* 30**,** 923-930.
--- (2013b) The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. *Nucleic acids research,* 41**,** e108-e108.
Light, P. E., J. E. Manning Fox, M. J. Riedel & M. B. Wheeler (2002) Glucagon-Like Peptide-1 Inhibits Pancreatic ATP-Sensitive Potassium Channels via a Protein Kinase A- and ADP-Dependent Mechanism. *Molecular Endocrinology,* 16**,** 2135-2144.
Lindtner, C., T. Scherer, E. Zielinski, N. Filatova, M. Fasshauer, N. K. Tonks, M. Puchowicz & C. Buettner (2013) Binge drinking induces whole-body insulin resistance by impairing hypothalamic insulin action. *Science translational medicine,* 5**,** 170ra14-170ra14.
Lipson, K. L., R. Ghosh & F. Urano (2008) The Role of IRE1α in the Degradation of Insulin mRNA in Pancreatic β-Cells. *PLOS ONE,* 3**,** e1648.
Liu, J., M. A. Carmell, F. V. Rivas, C. G. Marsden, J. M. Thomson, J.-J. Song, S. M. Hammond, L. Joshua-Tor & G. J. Hannon (2004) Argonaute2 Is the Catalytic Engine of Mammalian RNAi. *Science,* 305**,** 1437.
Liu, M., I. Hodish, C. J. Rhodes & P. Arvan (2007) Proinsulin maturation, misfolding, and proteotoxicity. *Proceedings of the National Academy of Sciences,* 104**,** 15841.
Lomedico, P. T., S. J. Chan, D. F. Steiner & G. F. Saunders (1977) Immunological and chemical characterization of bovine preproinsulin. *Journal of Biological Chemistry,* 252**,** 7971-7978.
Lorch, Y., B. R. Cairns, M. Zhang & R. D. Kornberg (1998) Activated RSC–Nucleosome Complex and Persistently Altered Form of the Nucleosome. *Cell,* 94**,** 29-34.
Love, M. I., W. Huber & S. Anders (2014a) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol,* 15**,** 550.
--- (2014b) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology,* 15**,** 550.
Lu, M., J. Seufert & J. F. Habener (1997) Pancreatic β-Cell-specific Repression of Insulin Gene Transcription by CCAAT/Enhancer-binding Protein β: INHIBITORY INTERACTIONS WITH BASIC HELIX-LOOP-HELIX TRANSCRIPTION FACTOR E47. *Journal of Biological Chemistry,* 272**,** 28349-28359.
Lu, T. T.-H., S. Heyne, E. Dror, E. Casas, L. Leonhardt, T. Boenke, C.-H. Yang, Sagar, L. Arrigoni, K. Dalgaard, R. Teperino, L. Enders, M. Selvaraj, M. Ruf, S. J. Raja, H. Xie, U. Boenisch, S. H. Orkin, F. C. Lynn, B. G. Hoffman, D. Grün, T. Vavouri, A. M. Lempradl & J. A. Pospisilik (2018) The Polycomb-Dependent Epigenome Controls β Cell Dysfunction, Dedifferentiation, and Diabetes. *Cell metabolism,* 27**,** 1294-1308.e7.
Luche, H., O. Weber, T. Nageswara Rao, C. Blum & H. J. Fehling (2007) Faithful activation of an extra-bright red fluorescent protein in "knock-in" Cre-reporter mice ideally suited for lineage tracing studies. *Eur J Immunol,* 37**,** 43-53.
Lujambio, A. & S. W. Lowe (2012) The microcosmos of cancer. *Nature,* 482**,** 347-355.
Lynn, F. C., P. Skewes-Cox, Y. Kosaka, M. T. McManus, B. D. Harfe & M. S. German (2007a) MicroRNA Expression Is Required for Pancreatic Islet Cell Genesis in the Mouse. *Diabetes,* 56**,** 2938.
Lynn, F. C., S. B. Smith, M. E. Wilson, K. Y. Yang, N. Nekrep & M. S. German (2007b) Sox9 coordinates a transcriptional network in pancreatic progenitor cells. *Proceedings of the National Academy of Sciences,* 104**,** 10500.
Lyttle, B. M., J. Li, M. Krishnamurthy, F. Fellows, M. B. Wheeler, C. G. Goodyer & R. Wang (2008) Transcription factor expression in the developing human fetal endocrine pancreas. *Diabetologia,* 51**,** 1169-1180.
Längst, G. & L. Manelyte (2015) Chromatin Remodelers: From Function to Dysfunction. *Genes,* 6**,** 299-324.

López-Beas, J., V. Capilla-González, Y. Aguilera, N. Mellado, C. C. Lachaud, F. Martín, T. Smani, B. Soria & A. Hmadcha (2018) miR-7 Modulates hESC Differentiation into Insulin-Producing Beta-like Cells and Contributes to Cell Maturation. *Molecular therapy. Nucleic acids,* 12**,** 463-477.

Maechler, P. (2013) Mitochondrial function and insulin secretion. *Molecular and Cellular Endocrinology,* 379**,** 12-18.

Maechler, P. & C. B. Wollheim (1999) Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. *Nature,* 402**,** 685-689.

Maedler, K., P. Sergeev, F. Ris, J. Oberholzer, H. I. Joller-Jemelka, G. A. Spinas, N. Kaiser, P. A. Halban & M. Y. Donath (2002) Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *The Journal of clinical investigation,* 110**,** 851-860.

Maedler, K., G. A. Spinas, D. Dyntar, W. Moritz, N. Kaiser & M. Y. Donath (2001a) Distinct Effects of Saturated and Monounsaturated Fatty Acids on β-Cell Turnover and Function. *Diabetes,* 50**,** 69.

Maedler, K., G. A. Spinas, R. Lehmann, P. Sergeev, M. Weber, A. Fontana, N. Kaiser & M. Y. Donath (2001b) Glucose Induces β-Cell Apoptosis Via Upregulation of the Fas Receptor in Human Islets. *Diabetes,* 50**,** 1683.

Mahadevan, J., S. Parazzoli, E. Oseid, A. V. Hertzel, D. A. Bernlohr, S. N. Vallerie, C.-q. Liu, M. Lopez, J. S. Harmon & R. P. Robertson (2013) Ebselen treatment prevents islet apoptosis, maintains intranuclear Pdx-1 and MafA levels, and preserves β-cell mass and function in ZDF rats. *Diabetes,* 62**,** 3582-3588.

Maitra, A. & A. Abbas. 2005. Endocrine system. In *Robbins and Cotran Pathologic basis of disease,* eds. V. Kumar, N. Fausto & A. Abbas, 1156-1226. Philadelphia: Saunders.

Mak, T. C. S., Y. von Ohlen, Y. F. Wang, E. Kane, K. Jurgaityte, P. Ervilha, P. Chabosseau, W. Distaso, V. Salem, A. Tomas, M. Stoffel, P. Marchetti, A. M. J. Shapiro, G. A. Rutter & M. Latreille (under review) b-cell dedifferentiation is associated with epithelial-mesenchymal transition triggered by miR-7-mediated repression of mSwi/Snf complex. *J Clin Invest*.

--- (under revision) b-cell dedifferentiation is associated with epithelial-mesenchymal transition triggered by miR-7-mediated repression of mSwi/Snf complex. *J Clin Invest*.

Malecki, M. T., U. S. Jhala, A. Antonellis, L. Fields, A. Doria, T. Orban, M. Saad, J. H. Warram, M. Montminy & A. S. Krolewski (1999) Mutations in NEUROD1 are associated with the development of type 2 diabetes mellitus. *Nature Genetics,* 23**,** 323-328.

Mallappa, C., B. T. Nasipak, L. Etheridge, E. J. Androphy, S. N. Jones, C. G. Sagerström, Y. Ohkawa & A. N. Imbalzano (2010) Myogenic MicroRNA Expression Requires ATP-Dependent Chromatin Remodeling Enzyme Function. *Molecular and Cellular Biology,* 30**,** 3176.

Manning, A. K., M.-F. Hivert, R. A. Scott, J. L. Grimsby, N. Bouatia-Naji, H. Chen, D. Rybin, C.-T. Liu, L. F. Bielak, I. Prokopenko, N. Amin, D. Barnes, G. Cadby, J.-J. Hottenga, E. Ingelsson, A. U. Jackson, T. Johnson, S. Kanoni, C. Ladenvall, V. Lagou, J. Lahti, C. Lecoeur, Y. Liu, M. T. Martinez-Larrad, M. E. Montasser, P. Navarro, J. R. B. Perry, L. J. Rasmussen-Torvik, P. Salo, N. Sattar, D. Shungin, R. J. Strawbridge, T. Tanaka, C. M. van Duijn, P. An, M. de Andrade, J. S. Andrews, T. Aspelund, M. Atalay, Y. Aulchenko, B. Balkau, S. Bandinelli, J. S. Beckmann, J. P. Beilby, C. Bellis, R. N. Bergman, J. Blangero, M. Boban, M. Boehnke, E. Boerwinkle, L. L. Bonnycastle, D. I. Boomsma, I. B. Borecki, Y. Böttcher, C. Bouchard, E. Brunner, D. Budimir, H. Campbell, O. Carlson, P. S. Chines, R. Clarke, F. S. Collins, A. Corbatón-Anchuelo, D. Couper, U. de Faire, G. V. Dedoussis, P. Deloukas, M. Dimitriou, J. M. Egan, G. Eiriksdottir, M. R. Erdos, J. G. Eriksson, E. Eury, L. Ferrucci, I. Ford, N. G. Forouhi, C. S. Fox, M. G. Franzosi, P. W. Franks, T. M. Frayling, P. Froguel, P. Galan, E. de Geus, B. Gigante, N. L. Glazer, A. Goel, L. Groop, V. Gudnason, G. Hallmans, A. Hamsten, O. Hansson, T. B. Harris, C. Hayward, S. Heath, S. Hercberg, A. A. Hicks, A. Hingorani, A. Hofman, J. Hui, J. Hung, et al. (2012) A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. *Nature genetics,* 44**,** 659-669.

Marathe, H. G., G. Mehta, X. Zhang, I. Datar, A. Mehrotra, K. C. Yeung & I. L. de la Serna (2013) SWI/SNF enzymes promote SOX10- mediated activation of myelin gene expression. *PloS one,* 8**,** e69037-e69037.

Marette, A., J. M. Richardson, T. Ramlal, T. W. Balon, M. Vranic, J. E. Pessin & A. Klip (1992) Abundance, localization, and insulin-induced translocation of glucose transporters in red and white muscle. *American Journal of Physiology-Cell Physiology,* 263**,** C443-C452.

Markiewicz, M. A., E. L. Wise, Z. S. Buchwald, A. K. Pinto, B. Zafirova, B. Polic & A. S. Shaw (2012) RAE1ε ligand expressed on pancreatic islets recruits NKG2D receptor-expressing cytotoxic T cells independent of T cell receptor recognition. *Immunity,* 36**,** 132-141.

Marshak, S., G. Leibowitz, F. Bertuzzi, C. Socci, N. Kaiser, D. J. Gross, E. Cerasi & D. Melloul (1999) Impaired beta-cell functions induced by chronic exposure of cultured human pancreatic islets to high glucose. *Diabetes,* 48**,** 1230.

Martinez, J., A. Patkaniowska, H. Urlaub, R. Lührmann & T. Tuschl (2002) Single-Stranded Antisense siRNAs Guide Target RNA Cleavage in RNAi. *Cell,* 110**,** 563-574.

Martinez, S. C., K. Tanabe, C. Cras-Méneur, N. A. Abumrad, E. Bernal-Mizrachi & M. A. Permutt (2008) Inhibition of Foxo1 Protects Pancreatic Islet β-Cells Against Fatty Acid and Endoplasmic Reticulum Stress–Induced Apoptosis. *Diabetes,* 57**,** 846.

Martinez-Sanchez, A., M.-S. Nguyen-Tu & G. A. Rutter (2015) DICER Inactivation Identifies Pancreatic β-Cell "Disallowed" Genes Targeted by MicroRNAs. *Molecular endocrinology (Baltimore, Md.),* 29**,** 1067-1079.

Martín, F., F. Moya, L. M. Gutierrez, J. A. Reig & B. Soria (1995) Role of syntaxin in mouse pancreatic beta cells. *Diabetologia,* 38**,** 860-863.

Martín, M., J. Gallego-Llamas, V. Ribes, M. Kedinger, K. Niederreither, P. Chambon, P. Dollé & G. Gradwohl (2005) Dorsal pancreas agenesis in retinoic acid-deficient Raldh2 mutant mice. *Developmental Biology,* 284**,** 399-411.

Masaki, O., X. Shaoping & L. En (1998) Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nature Genetics,* 19**,** 219.

Masliah-Planchon, J., I. Bièche, J.-M. Guinebretière, F. Bourdeaut & O. Delattre (2015) SWI/SNF Chromatin Remodeling and Human Malignancies. *Annual Review of Pathology: Mechanisms of Disease,* 10**,** 145-171.

Massoner, P., M. Ladurner-Rennau, I. E. Eder & H. Klocker (2010) Insulin-like growth factors and insulin control a multifunctional signalling network of significant importance in cancer. *British journal of cancer,* 103**,** 1479-1484.

Matschinsky, F. M. (1996) A Lesson in Metabolic Regulation Inspired by the Glucokinase Glucose Sensor Paradigm. *Diabetes,* 45**,** 223.

Matschinsky, F. M. & J. E. Ellerman (1968) Metabolism of Glucose in the Islets of Langerhans. *Journal of Biological Chemistry,* 243**,** 2730-2736.

Matsuoka, T., Y. Kajimoto, H. Watada, H. Kaneto, M. Kishimoto, Y. Umayahara, Y. Fujitani, T. Kamada, R. Kawamori & Y. Yamasaki (1997) Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. *The Journal of Clinical Investigation,* 99**,** 144-150.

Matsuoka, T.-a., H. Kaneto, T. Miyatsuka, T. Yamamoto, K. Yamamoto, K. Kato, I. Shimomura, R. Stein & M. Matsuhisa (2010) Regulation of MafA Expression in Pancreatic β-Cells in db/db Mice With Diabetes. *Diabetes,* 59**,** 1709.

Maturi, V., S. Enroth, C.-H. Heldin & A. Moustakas (2018) Genome-wide binding of transcription factor ZEB1 in triple-negative breast cancer cells. *Journal of cellular physiology,* 233**,** 7113-7127.

McDonald, E., J. Li, M. Krishnamurthy, G. F. Fellows, C. G. Goodyer & R. Wang (2012) SOX9 regulates endocrine cell differentiation during human fetal pancreas development. *The International Journal of Biochemistry & Cell Biology,* 44**,** 72-83.

McGarry, J. D. & R. L. Dobbins (1999) Fatty acids, lipotoxicity and insulin secretion. *Diabetologia,* 42**,** 128-138.
McKenna, B., M. Guo, A. Reynolds, M. Hara & R. Stein (2015a) Dynamic recruitment of functionally distinct Swi/Snf chromatin remodeling complexes modulates Pdx1 activity in islet beta cells. *Cell Rep,* 10**,** 2032-42.
--- (2015b) Dynamic recruitment of functionally distinct Swi/Snf chromatin remodeling complexes modulates Pdx1 activity in islet β cells. *Cell reports,* 10**,** 2032-2042.
McLean, C. Y., D. Bristor, M. Hiller, S. L. Clarke, B. T. Schaar, C. B. Lowe, A. M. Wenger & G. Bejerano (2010) GREAT improves functional interpretation of cis-regulatory regions. *Nature biotechnology,* 28**,** 495-501.
Md Moin, A. S., S. Dhawan, M. Cory, P. C. Butler, R. A. Rizza & A. E. Butler (2016) Increased Frequency of Hormone Negative and Polyhormonal Endocrine Cells in Lean Individuals With Type 2 Diabetes. *The Journal of clinical endocrinology and metabolism,* 101**,** 3628-3636.
Meier, J. J. (2012) GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nature Reviews Endocrinology,* 8**,** 728.
Meier, J. J. & M. A. Nauck (2014) Risk of pancreatitis in patients treated with incretin-based therapies. *Diabetologia,* 57**,** 1320-1324.
Meigs, J. B., L. A. Cupples & P. W. Wilson (2000) Parental transmission of type 2 diabetes: the Framingham Offspring Study. *Diabetes,* 49**,** 2201.
Meister, G. (2013) Argonaute proteins: functional insights and emerging roles. *Nature Reviews Genetics,* 14**,** 447.
Meister, G., M. Landthaler, A. Patkaniowska, Y. Dorsett, G. Teng & T. Tuschl (2004) Human Argonaute2 Mediates RNA Cleavage Targeted by miRNAs and siRNAs. *Molecular Cell,* 15**,** 185-197.
Memon, B., M. Karam, S. Al-Khawaga & E. M. Abdelalim (2018) Enhanced differentiation of human pluripotent stem cells into pancreatic progenitors co-expressing PDX1 and NKX6.1. *Stem cell research & therapy,* 9**,** 15-15.
Meng, Z.-X., W. Tao, J. Sun, Q. Wang, L. Mi & J. D. Lin (2017) Uncoupling Exercise Bioenergetics from Systemic Metabolic Homeostasis by Conditional Inactivation of Baf60 in Skeletal Muscle. *Diabetes***,** db170367.
Meng, Z.-X., L. Wang, L. Chang, J. Sun, J. Bao, Y. Li, Y. E. Chen & J. D. Lin (2015a) A Diet-Sensitive BAF60a-Mediated Pathway Links Hepatic Bile Acid Metabolism to Cholesterol Absorption and Atherosclerosis. *Cell reports,* 13**,** 1658-1669.
Meng, Z. X., L. Wang, L. Chang, J. Sun, J. Bao, Y. Li, Y. E. Chen & J. D. Lin (2015b) A Diet-Sensitive BAF60a-Mediated Pathway Links Hepatic Bile Acid Metabolism to Cholesterol Absorption and Atherosclerosis. *Cell Rep,* 13**,** 1658-69.
Meur, G., A. Simon, N. Harun, M. Virally, A. Dechaume, A. Bonnefond, S. Fetita, A. I. Tarasov, P.-J. Guillausseau, T. W. Boesgaard, O. Pedersen, T. Hansen, M. Polak, J.-F. Gautier, P. Froguel, G. A. Rutter & M. Vaxillaire (2010) Insulin gene mutations resulting in early-onset diabetes: marked differences in clinical presentation, metabolic status, and pathogenic effect through endoplasmic reticulum retention. *Diabetes,* 59**,** 653-661.
Michaud, E. J., M. J. van Vugt, S. J. Bultman, H. O. Sweet, M. T. Davisson & R. P. Woychik (1994) Differential expression of a new dominant agouti allele (Aiapy) is correlated with methylation state and is influenced by parental lineage. *Genes & Development,* 8**,** 1463-1472.
Middeljans, E., X. Wan, P. W. Jansen, V. Sharma, H. G. Stunnenberg & C. Logie (2012) SS18 Together with Animal-Specific Factors Defines Human BAF-Type SWI/SNF Complexes. *PLOS ONE,* 7**,** e33834.
Min, J., Y. Zhang & R.-M. Xu (2003) Structural basis for specific binding of Polycomb chromodomain to histone H3 methylated at Lys 27. *Genes & development,* 17**,** 1823-1828.

Misbin, R. I. (1977) Phenformin-Associated Lactic Acidosis: Pathogenesis and Treatment. *Annals of Internal Medicine,* 87**,** 591-595.
--- (2004) The Phantom of Lactic Acidosis due to Metformin in Patients With Diabetes. *Diabetes Care,* 27**,** 1791.
Mitrakou, A., D. Kelley, T. Veneman, T. Jenssen, T. Pangburn, J. Reilly & J. Gerich (1990) Contribution of Abnormal Muscle and Liver Glucose Metabolism to Postprandial Hyperglycemia in NIDDM. *Diabetes,* 39**,** 1381.
Miyatsuka, T., T.-a. Matsuoka, T. Shiraiwa, T. Yamamoto, I. Kojima & H. Kaneto (2007) Ptf1a and RBP-J cooperate in activating Pdx1 gene expression through binding to Area III. *Biochemical and Biophysical Research Communications,* 362**,** 905-909.
Miyoshi, T., A. Takeuchi, H. Siomi & M. C. Siomi (2010) A direct role for Hsp90 in pre-RISC formation in Drosophila. *Nature Structural & Molecular Biology,* 17**,** 1024-1026.
Moore, L. D., T. Le & G. Fan (2013) DNA methylation and its basic function. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology,* 38**,** 23-38.
Mootha, V. K., C. M. Lindgren, K. F. Eriksson, A. Subramanian, S. Sihag, J. Lehar, P. Puigserver, E. Carlsson, M. Ridderstråle, E. Laurila, N. Houstis, M. J. Daly, N. Patterson, J. P. Mesirov, T. R. Golub, P. Tamayo, B. Spiegelman, E. S. Lander, J. N. Hirschhorn, D. Altshuler & L. C. Groop (2003) PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet,* 34**,** 267-73.
Moran, A., H. J. Zhang, L. K. Olson, J. S. Harmon, V. Poitout & R. P. Robertson (1997) Differentiation of glucose toxicity from beta cell exhaustion during the evolution of defective insulin gene expression in the pancreatic islet cell line, HIT-T15. *The Journal of Clinical Investigation,* 99**,** 534-539.
Mori, H. & K.-L. Guan. 2012. Tissue-Specific Ablation of Tsc1 in Pancreatic Beta-Cells. In *mTOR: Methods and Protocols,* ed. T. Weichhart, 407-419. Totowa, NJ: Humana Press.
Morris, A. P., B. F. Voight, T. M. Teslovich, T. Ferreira, A. V. Segrè, V. Steinthorsdottir, R. J. Strawbridge, H. Khan, H. Grallert, A. Mahajan, I. Prokopenko, H. M. Kang, C. Dina, T. Esko, R. M. Fraser, S. Kanoni, A. Kumar, V. Lagou, C. Langenberg, J. a. Luan, C. M. Lindgren, M. Müller-Nurasyid, S. Pechlivanis, N. W. Rayner, L. J. Scott, S. Wiltshire, L. Yengo, L. Kinnunen, E. J. Rossin, S. Raychaudhuri, A. D. Johnson, A. S. Dimas, R. J. F. Loos, S. Vedantam, H. Chen, J. C. Florez, C. Fox, C.-T. Liu, D. Rybin, D. J. Couper, W. H. L. Kao, M. Li, M. C. Cornelis, P. Kraft, Q. Sun, R. M. van Dam, H. M. Stringham, P. S. Chines, K. Fischer, P. Fontanillas, O. L. Holmen, S. E. Hunt, A. U. Jackson, A. Kong, R. Lawrence, J. Meyer, J. R. B. Perry, C. G. P. Platou, S. Potter, E. Rehnberg, N. Robertson, S. Sivapalaratnam, A. Stančáková, K. Stirrups, G. Thorleifsson, E. Tikkanen, A. R. Wood, P. Almgren, M. Atalay, R. Benediktsson, L. L. Bonnycastle, N. Burtt, J. Carey, G. Charpentier, A. T. Crenshaw, A. S. F. Doney, M. Dorkhan, S. Edkins, V. Emilsson, E. Eury, T. Forsen, K. Gertow, B. Gigante, G. B. Grant, C. J. Groves, C. Guiducci, C. Herder, A. B. Hreidarsson, J. Hui, A. James, A. Jonsson, W. Rathmann, N. Klopp, J. Kravic, K. Krjutškov, C. Langford, K. Leander, E. Lindholm, S. Lobbens, S. Männistö, et al. (2012) Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nature genetics,* 44**,** 981-990.
Mortensen, K., L. L. Christensen, J. J. Holst & C. Orskov (2003) GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. *Regulatory Peptides,* 114**,** 189-196.
Mortimer, C. H., D. Carr, T. Lind, S. R. Bloom, C. N. Mallinson, A. V. Schally, W. M. G. Tunbridge, L. Yeomans, D. H. Coy, A. Kastin, G. M. Besser & R. Hall (1974) EFFECTS OF GROWTH-HORMONE RELEASE-INHIBITING HORMONE ON CIRCULATING GLUCAGON, INSULIN, AND GROWTH HORMONE IN NORMAL, DIABETIC, ACROMEGALIC, AND HYPOPITUITARY PATIENTS. *The Lancet,* 303**,** 697-701.
Morán, I., I. Akerman, M. van de Bunt, R. Xie, M. Benazra, T. Nammo, L. Arnes, N. Nakić, J. García-Hurtado, S. Rodríguez-Seguí, L. Pasquali, C. Sauty-Colace, A. Beucher, R. Scharfmann, J. van

Arensbergen, P. R. Johnson, A. Berry, C. Lee, T. Harkins, V. Gmyr, F. Pattou, J. Kerr-Conte, L. Piemonti, T. Berney, N. Hanley, A. L. Gloyn, L. Sussel, L. Langman, K. L. Brayman, M. Sander, M. I. McCarthy, P. Ravassard & J. Ferrer (2012) Human β cell transcriptome analysis uncovers lncRNAs that are tissue-specific, dynamically regulated, and abnormally expressed in type 2 diabetes. *Cell metabolism,* 16**,** 435-448.

Motohashi, H., T. O'Connor, F. Katsuoka, J. D. Engel & M. Yamamoto (2002) Integration and diversity of the regulatory network composed of Maf and CNC families of transcription factors. *Gene,* 294**,** 1-12.

Muchardt, C., J. C. Reyes, B. Bourachot, E. Leguoy & M. Yaniv (1996) The hbrm and BRG-1 proteins, components of the human SNF/SWI complex, are phosphorylated and excluded from the condensed chromosomes during mitosis. *The EMBO journal,* 15**,** 3394-3402.

Muller, Y. L., A. M. Infante, R. L. Hanson, L. Love-Gregory, W. Knowler, C. Bogardus & L. J. Baier (2005) Variants in hepatocyte nuclear factor 4alpha are modestly associated with type 2 diabetes in Pima Indians. *Diabetes,* 54**,** 3035-3039.

Munro, S. & H. R. B. Pelham (1987) A C-terminal signal prevents secretion of luminal ER proteins. *Cell,* 48**,** 899-907.

Murchison, E. P., J. F. Partridge, O. H. Tam, S. Cheloufi & G. J. Hannon (2005) Characterization of Dicer-deficient murine embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America,* 102**,** 12135-12140.

Mutskov, V. & G. Felsenfeld (2009) The human insulin gene is part of a large open chromatin domain specific for human islets. *Proceedings of the National Academy of Sciences,* 106**,** 17419.

Myers, M. G., Jr., J. M. Backer, X. J. Sun, S. Shoelson, P. Hu, J. Schlessinger, M. Yoakim, B. Schaffhausen & M. F. White (1992) IRS-1 activates phosphatidylinositol 3'-kinase by associating with src homology 2 domains of p85. *Proceedings of the National Academy of Sciences of the United States of America,* 89**,** 10350-10354.

Nagy, L., J. Márton, A. Vida, G. Kis, É. Bokor, S. Kun, M. Gönczi, T. Docsa, A. Tóth, M. Antal, P. Gergely, B. Csóka, P. Pacher, L. Somsák & P. Bai (2018) Glycogen phosphorylase inhibition improves beta cell function. *British journal of pharmacology,* 175**,** 301-319.

Nakanishi, K. (2016) Anatomy of RISC: how do small RNAs and chaperones activate Argonaute proteins? *Wiley interdisciplinary reviews. RNA,* 7**,** 637-660.

Nakanishi, K., D. E. Weinberg, D. P. Bartel & D. J. Patel (2012) Structure of yeast Argonaute with guide RNA. *Nature,* 486**,** 368-374.

Nakayama, M., T. Inoguchi, T. Sonta, Y. Maeda, S. Sasaki, F. Sawada, H. Tsubouchi, N. Sonoda, K. Kobayashi, H. Sumimoto & H. Nawata (2005) Increased expression of NAD(P)H oxidase in islets of animal models of Type 2 diabetes and its improvement by an AT1 receptor antagonist. *Biochemical and Biophysical Research Communications,* 332**,** 927-933.

Nathan, D. M. (2002) Initial Management of Glycemia in Type 2 Diabetes Mellitus. *New England Journal of Medicine,* 347**,** 1342-1349.

Nathan, D. M., J. B. Buse, M. B. Davidson, E. Ferrannini, R. R. Holman, R. Sherwin & B. Zinman (2008) Medical management of hyperglycaemia in type 2 diabetes mellitus: a consensus algorithm for the initiation and adjustment of therapy. *Diabetologia,* 52**,** 17.

Nauck, M. A., M. M. Heimesaat, C. Orskov, J. J. Holst, R. Ebert & W. Creutzfeldt (1993) Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *The Journal of clinical investigation,* 91**,** 301-307.

Nauck, M. A., E. Homberger, E. G. Siegel, R. C. Allen, R. P. Eaton, R. Ebert & W. Creutzfeldt (1986) Incretin Effects of Increasing Glucose Loads in Man Calculated from Venous Insulin and C-Peptide Responses*. *The Journal of Clinical Endocrinology & Metabolism,* 63**,** 492-498.

Naylor , R., A. K. Johnson & D. del Gaudio (2018) Maturity-Onset Diabetes of the Young Overview. https://www.ncbi.nlm.nih.gov/books/NBK500456/ (last accessed.

Neely, K. E., A. H. Hassan, C. E. Brown, L. Howe & J. L. Workman (2002) Transcription activator interactions with multiple SWI/SNF subunits. *Molecular and cellular biology,* 22**,** 1615-1625.
Nesca, V., C. Guay, C. Jacovetti, V. Menoud, M.-L. Peyot, D. R. Laybutt, M. Prentki & R. Regazzi (2013) Identification of particular groups of microRNAs that positively or negatively impact on beta cell function in obese models of type 2 diabetes. *Diabetologia,* 56**,** 2203-2212.
Newgard, C. B. & J. D. McGarry (1995) Metabolic Coupling Factors in Pancreatic β-Cell Signal Transduction. *Annual Review of Biochemistry,* 64**,** 689-719.
Nichols, C. G., S. L. Shyng, A. Nestorowicz, B. Glaser, J. P. Clement, G. Gonzalez, L. Aguilar-Bryan, M. A. Permutt & J. Bryan (1996) Adenosine Diphosphate as an Intracellular Regulator of Insulin Secretion. *Science,* 272**,** 1785.
Nielsen, E.-M. D., L. Hansen, B. Carstensen, S. M. Echwald, T. Drivsholm, C. Glümer, B. Thorsteinsson, K. Borch-Johnsen, T. Hansen & O. Pedersen (2003) The E23K Variant of Kir6.2 Associates With Impaired Post-OGTT Serum Insulin Response and Increased Risk of Type 2 Diabetes. *Diabetes,* 52**,** 573.
Nieto, M., P. Hevia, E. Garcia, D. Klein, S. Alvarez-Cubela, V. Bravo-Egana, S. Rosero, R. D. Molano, N. Vargas, C. Ricordi, A. Pileggi, J. Diez, J. Domínguez-Bendala & R. L. Pastori (2012) Antisense miR-7 Impairs Insulin Expression in Developing Pancreas and in Cultured Pancreatic Buds. *Cell Transplantation,* 21**,** 1761-1774.
Nishi, M., T. Sanke, S. Nagamatsu, G. I. Bell & D. F. Steiner (1990) Islet amyloid polypeptide. A new beta cell secretory product related to islet amyloid deposits. *Journal of Biological Chemistry,* 265**,** 4173-4176.
Nishimura, W., T. Kondo, T. Salameh, I. El Khattabi, R. Dodge, S. Bonner-Weir & A. Sharma (2006) A switch from MafB to MafA expression accompanies differentiation to pancreatic beta-cells. *Developmental biology,* 293**,** 526-539.
Nishioka, K., S. Chuikov, K. Sarma, H. Erdjument-Bromage, C. D. Allis, P. Tempst & D. Reinberg (2002a) Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. *Genes & development,* 16**,** 479-489.
Nishioka, K., J. C. Rice, K. Sarma, H. Erdjument-Bromage, J. Werner, Y. Wang, S. Chuikov, P. Valenzuela, P. Tempst, R. Steward, J. T. Lis, C. D. Allis & D. Reinberg (2002b) PR-Set7 Is a Nucleosome-Specific Methyltransferase that Modifies Lysine 20 of Histone H4 and Is Associated with Silent Chromatin. *Molecular Cell,* 9**,** 1201-1213.
Nordmann, T. M., E. Dror, F. Schulze, S. Traub, E. Berishvili, C. Barbieux, M. Böni-Schnetzler & M. Y. Donath (2017) The Role of Inflammation in β-cell Dedifferentiation. *Scientific Reports,* 7**,** 6285.
Norgaard, G. A., J. N. Jensen & J. Jensen (2003) FGF10 signaling maintains the pancreatic progenitor cell state revealing a novel role of Notch in organ development. *Developmental Biology,* 264**,** 323-338.
Numata, M., S. Morinaga, T. Watanabe, H. Tamagawa, N. Yamamoto, M. Shiozawa, Y. Nakamura, Y. Kameda, S. Okawa, Y. Rino, M. Akaike, M. Masuda & Y. Miyagi (2013) The clinical significance of SWI/SNF complex in pancreatic cancer. *International journal of oncology,* 42**,** 403-410.
O'Rahilly, S., H. Gray, P. J. Humphreys, A. Krook, K. S. Polonsky, A. White, S. Gibson, K. Taylor & C. Carr (1995) Impaired Processing of Prohormones Associated with Abnormalities of Glucose Homeostasis and Adrenal Function. *New England Journal of Medicine,* 333**,** 1386-1391.
Ogasawara, K., J. A. Hamerman, L. R. Ehrlich, H. Bour-Jordan, P. Santamaria, J. A. Bluestone & L. L. Lanier (2004) NKG2D Blockade Prevents Autoimmune Diabetes in NOD Mice. *Immunity,* 20**,** 757-767.
Okano, M., S. Xie & E. Li (1998) Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nature Genetics,* 19**,** 219-220.
Olofsson, C. S., J. Håkansson, A. Salehi, M. Bengtsson, J. Galvanovskis, C. Partridge, M. SörhedeWinzell, X. Xian, L. Eliasson, I. Lundquist, H. Semb & P. Rorsman (2009) Impaired

insulin exocytosis in neural cell adhesion molecule-/- mice due to defective reorganization of the submembrane F-actin network. *Endocrinology,* 150**,** 3067-3075.
Olokoba, A. B., O. A. Obateru & L. B. Olokoba (2012) Type 2 diabetes mellitus: a review of current trends. *Oman medical journal,* 27**,** 269-273.
Olson, L. K., J. Qian & V. Poitout (1998) Glucose Rapidly and Reversibly Decreases INS-1 Cell Insulin Gene Transcription via Decrements in STF-1 and C1 Activator Transcription Factor Activity. *Molecular Endocrinology,* 12**,** 207-219.
Olson, L. K., J. B. Redmon, H. C. Towle & R. P. Robertson (1993) Chronic exposure of HIT cells to high glucose concentrations paradoxically decreases insulin gene transcription and alters binding of insulin gene regulatory protein. *The Journal of Clinical Investigation,* 92**,** 514-519.
Olson, L. K., A. Sharma, M. Peshavaria, C. V. Wright, H. C. Towle, R. P. Rodertson & R. Stein (1995) Reduction of insulin gene transcription in HIT-T15 beta cells chronically exposed to a supraphysiologic glucose concentration is associated with loss of STF-1 transcription factor expression. *Proceedings of the National Academy of Sciences,* 92**,** 9127.
Orci, L., D. Baetens, M. Ravazzola, Y. Stefan & F. Malaisse-Lagae (1976) Pancreatic polypeptide and glucagon: Non-random distribution in pancreatic islets. *Life Sciences,* 19**,** 1811-1816.
Oshima, M., K.-P. Knoch, M. Diedisheim, A. Petzold, P. Cattan, M. Bugliani, P. Marchetti, P. Choudhary, G.-C. Huang, S. R. Bornstein, M. Solimena, O. Albagli-Curiel & R. Scharfmann (2018) Virus-like infection induces human β cell dedifferentiation. *JCI insight,* 3**,** e97732.
Osundiji, M. A., D. D. Lam, J. Shaw, C.-Y. Yueh, S. P. Markkula, P. Hurst, C. Colliva, A. Roda, L. K. Heisler & M. L. Evans (2012) Brain glucose sensors play a significant role in the regulation of pancreatic glucose-stimulated insulin secretion. *Diabetes,* 61**,** 321-328.
Otter, S. & E. Lammert (2016) Exciting Times for Pancreatic Islets: Glutamate Signaling in Endocrine Cells. *Trends in Endocrinology & Metabolism,* 27**,** 177-188.
Ottosson-Laakso, E., U. Krus, P. Storm, R. B. Prasad, N. Oskolkov, E. Ahlqvist, J. Fadista, O. Hansson, L. Groop & P. Vikman (2017) Glucose-Induced Changes in Gene Expression in Human Pancreatic Islets: Causes or Consequences of Chronic Hyperglycemia. *Diabetes,* 66**,** 3013.
Ou, J., H. Liu, J. Yu, M. A. Kelliher, L. H. Castilla, N. D. Lawson & L. J. Zhu (2018) ATACseqQC: a Bioconductor package for post-alignment quality assessment of ATAC-seq data. *BMC genomics,* 19**,** 169-169.
Ozaki, N., T. Shibasaki, Y. Kashima, T. Miki, K. Takahashi, H. Ueno, Y. Sunaga, H. Yano, Y. Matsuura, T. Iwanaga, Y. Takai & S. Seino (2000) cAMP-GEFII is a direct target of cAMP in regulated exocytosis. *Nature Cell Biology,* 2**,** 805-811.
O'Neill, B. T., G. Bhardwaj, C. M. Penniman, M. T. Krumpoch, P. A. Suarez Beltran, K. Klaus, K. Poro, M. Li, H. Pan, J. M. Dreyfuss, K. S. Nair & C. R. Kahn (2019) FoxO Transcription Factors Are Critical Regulators of Diabetes-Related Muscle Atrophy. *Diabetes,* 68**,** 556.
Palmbos, P. L., L. Wang, H. Yang, Y. Wang, J. Leflein, M. L. Ahmet, J. E. Wilkinson, C. Kumar-Sinha, G. M. Ney, S. A. Tomlins, S. Daignault, L. P. Kunju, X.-R. Wu, Y. Lotan, M. Liebert, M. E. Ljungman & D. M. Simeone (2015) ATDC/TRIM29 Drives Invasive Bladder Cancer Formation through miRNA-Mediated and Epigenetic Mechanisms. *Cancer Research,* 75**,** 5155.
Pan, F. C. & C. Wright (2011) Pancreas organogenesis: From bud to plexus to gland. *Developmental Dynamics,* 240**,** 530-565.
Panchenko, M. V., M. I. Zhou & H. T. Cohen (2004) von Hippel-Lindau Partner Jade-1 Is a Transcriptional Co-activator Associated with Histone Acetyltransferase Activity. *Journal of Biological Chemistry,* 279**,** 56032-56041.
Pandini, G., F. Frasca, R. Mineo, L. Sciacca, R. Vigneri & A. Belfiore (2002) Insulin/Insulin-like Growth Factor I Hybrid Receptors Have Different Biological Characteristics Depending on the Insulin Receptor Isoform Involved. *Journal of Biological Chemistry,* 277**,** 39684-39695.
Papizan, J. B., R. A. Singer, S.-I. Tschen, S. Dhawan, J. M. Friel, S. B. Hipkens, M. A. Magnuson, A. Bhushan & L. Sussel (2011) Nkx2.2 repressor complex regulates islet β-cell specification and prevents β-to-α-cell reprogramming. *Genes & development,* 25**,** 2291-2305.

Pascal, S. M., Y. Guiot, S. Pelengaris, M. Khan & J. C. Jonas (2008) Effects of c-MYC activation on glucose stimulus-secretion coupling events in mouse pancreatic islets. *American Journal of Physiology: Endocrinology and Metabolism,* 295**,** E92-E102.

Pathak, R. & M. B. Bridgeman (2010) Dipeptidyl Peptidase-4 (DPP-4) Inhibitors In the Management of Diabetes. *P & T : a peer-reviewed journal for formulary management,* 35**,** 509-513.

Patzelt, C., A. D. Labrecque, J. R. Duguid, R. J. Carroll, P. S. Keim, R. L. Heinrikson & D. F. Steiner (1978) Detection and kinetic behavior of preproinsulin in pancreatic islets. *Proceedings of the National Academy of Sciences of the United States of America,* 75**,** 1260-1264.

Paul, S., A. Kuo, T. Schalch, H. Vogel, L. Joshua-Tor, W. R. McCombie, O. Gozani, M. Hammell & A. A. Mills (2013) Chd5 requires PHD-mediated histone 3 binding for tumor suppression. *Cell reports,* 3**,** 92-102.

Pearson, E. R., G. Velho, P. Clark, A. Stride, M. Shepherd, T. M. Frayling, M. P. Bulman, S. Ellard, P. Froguel & A. T. Hattersley (2001) beta-cell genes and diabetes: quantitative and qualitative differences in the pathophysiology of hepatic nuclear factor-1alpha and glucokinase mutations. *Diabetes,* 50**,** S101.

Pedersen, J. K., S. B. Nelson, M. C. Jorgensen, K. D. Henseleit, Y. Fujitani, C. V. E. Wright, M. Sander & P. Serup (2005) Endodermal expression of Nkx6 genes depends differentially on Pdx1. *Developmental Biology,* 288**,** 487-501.

Perin, M. S., V. A. Fried, G. A. Mignery, R. Jahn & T. C. Südhof (1990) Phospholipid binding by a synaptic vesicle protein homologous to the regulatory region of protein kinase C. *Nature,* 345**,** 260-263.

Perley, M. J. & D. M. Kipnis (1967) Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic sujbjects. *The Journal of clinical investigation,* 46**,** 1954-1962.

Perseghin, G., K. Petersen & G. I. Shulman (2003) Cellular mechanism of insulin resistance: potential links with inflammation. *Int J Obes Relat Metab Disord,* 27**,** Suppl 3:S6-11.

Petrella, C., M. Broccardo, R. Possenti, C. Severini & G. Improta (2012) TLQP-21, a VGF-derived peptide, stimulates exocrine pancreatic secretion in the rat. *Peptides,* 36**,** 133-136.

Pick, A., J. Clark, C. Kubstrup, M. Levisetti, W. Pugh, S. Bonner-Weir & K. S. Polonsky (1998) Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. *Diabetes,* 47**,** 358.

Pino, M. F., D. Z. Ye, K. D. Linning, C. D. Green, B. Wicksteed, V. Poitout & L. K. Olson (2005) Elevated Glucose Attenuates Human Insulin Gene Promoter Activity in INS-1 Pancreatic β-Cells via Reduced Nuclear Factor Binding to the A5/Core and Z Element. *Molecular Endocrinology,* 19**,** 1343-1360.

Pipeleers, D., M. Chintinne, B. Denys, G. Martens, B. Keymeulen & F. Gorus (2008) Restoring a functional β-cell mass in diabetes. *Diabetes, Obesity and Metabolism,* 10**,** 54-62.

Piper, K., S. G. Ball*, J. W. Keeling, S. Mansoor, D. I. Wilson & N. A. Hanley (2002) Novel SOX9 expression during human pancreas development correlates to abnormalities in Campomelic dysplasia. *Mechanisms of Development,* 116**,** 223-226.

Piper, K., S. Brickwood, L. W. Turnpenny, I. T. Cameron, S. G. Ball, D. I. Wilson & N. A. Hanley (2004) Beta cell differentiation during early human pancreas development. *Journal of Endocrinology,* 181**,** 11-23.

Pirot, P., N. Naamane, F. Libert, N. E. Magnusson, T. F. Ørntoft, A. K. Cardozo & D. L. Eizirik (2007) Global profiling of genes modified by endoplasmic reticulum stress in pancreatic beta cells reveals the early degradation of insulin mRNAs. *Diabetologia,* 50**,** 1006-1014.

Poitout, V., J. Amyot, M. Semache, B. Zarrouki, D. Hagman & G. Fontés (2010) Glucolipotoxicity of the pancreatic beta cell. *Biochimica et biophysica acta,* 1801**,** 289-298.

Poitout, V., L. K. Olson & R. P. Robertson (1996) Chronic exposure of betaTC-6 cells to supraphysiologic concentrations of glucose decreases binding of the RIPE3b1 insulin gene transcription activator. *The Journal of Clinical Investigation,* 97**,** 1041-1046.

Poitout, V. & R. P. Robertson (2002) Minireview: Secondary β-Cell Failure in Type 2 Diabetes—A Convergence of Glucotoxicity and Lipotoxicity. *Endocrinology,* 143**,** 339-342.
Polak, M., L. Bouchareb-Banaei, R. Scharfmann & P. Czernichow (2000) Early pattern of differentiation in the human pancreas. *Diabetes,* 49**,** 225.
Poll, A. V., C. E. Pierreux, L. Lokmane, C. Haumaitre, Y. Achouri, P. Jacquemin, G. G. Rousseau, S. Cereghini & F. P. Lemaigre (2006) A vHNF1/TCF2-HNF6 Cascade Regulates the Transcription Factor Network That Controls Generation of Pancreatic Precursor Cells. *Diabetes,* 55**,** 61.
Pollock, A., S. Bian, C. Zhang, Z. Chen & T. Sun (2014) Growth of the Developing Cerebral Cortex Is Controlled by MicroRNA-7 through the p53 Pathway. *Cell Reports,* 7**,** 1184-1196.
Polonsky, K. S. & C. F. Burant. 2016. Chapter 31 - Type 2 Diabetes Mellitus. In *Williams Textbook of Endocrinology (Thirteenth Edition),* eds. S. Melmed, K. S. Polonsky, P. R. Larsen & H. M. Kronenberg, 1385-1450. Philadelphia: Content Repository Only!
Portela, A. & M. Esteller (2010) Epigenetic modifications and human disease. *Nature Biotechnology,* 28**,** 1057.
Portela-Gomes, G. M., M. Stridsberg, L. Grimelius, K. Öberg & E. T. Janson (2000) Expression of the Five Different Somatostatin Receptor Subtypes in Endocrine Cells of the Pancreas. *Applied Immunohistochemistry & Molecular Morphology,* 8.
Poulsen, P., K. Ohm Kyvik, A. Vaag & H. Beck-Nielsen (1999) Heritability of Type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance – a population-based twin study. *Diabetologia,* 42**,** 139-145.
Pound, L. D., J. K. Oeser, T. P. O'Brien, Y. Wang, C. J. Faulman, P. K. Dadi, D. A. Jacobson, J. C. Hutton, O. P. McGuinness, M. Shiota & R. M. O'Brien (2013) G6PC2: A Negative Regulator of Basal Glucose-Stimulated Insulin Secretion. *Diabetes,* 62**,** 1547.
Poy, M. N., J. Hausser, M. Trajkovski, M. Braun, S. Collins, P. Rorsman, M. Zavolan & M. Stoffel (2009) miR-375 maintains normal pancreatic α- and β-cell mass. *Proceedings of the National Academy of Sciences,* 106**,** 5813.
Prentki, M. & B. E. Corkey (1996) Are the β-Cell Signaling Molecules Malonyl-CoA and Cystolic Long-Chain Acyl-CoA Implicated in Multiple Tissue Defects of Obesity and NIDDM? *Diabetes,* 45**,** 273.
Prentki, M., E. Joly, W. El-Assaad & R. Roduit (2002) Malonyl-CoA Signaling, Lipid Partitioning, and Glucolipotoxicity. *Diabetes,* 51**,** S405.
Prentki, M., Franz M. Matschinsky & S. R. M. Madiraju (2013) Metabolic Signaling in Fuel-Induced Insulin Secretion. *Cell Metabolism,* 18**,** 162-185.
Prentki, M. & C. J. Nolan (2006) Islet beta cell failure in type 2 diabetes. *The Journal of clinical investigation,* 116**,** 1802-1812.
Prentki, M., R. Roduit, N. Davis-Lameloise, B. Corkey & F. Assimacopoulos-Jeannet (2000) Glucotoxicity, lipotoxicity and pancreatic β-cell failure: A role for malonyl-CoA, PPARα and altered lipid partitioning? *Can J Diabetes Care,* 25**,** 36-46.
Pridans, C., M. L. Holmes, M. Polli, J. M. Wettenhall, A. Dakic, L. M. Corcoran, G. K. Smyth & S. L. Nutt (2008) Identification of Pax5 Target Genes in Early B Cell Differentiation. *The Journal of Immunology,* 180**,** 1719.
Prochnik, S. E., D. S. Rokhsar & A. A. Aboobaker (2007) Evidence for a microRNA expansion in the bilaterian ancestor. *Development Genes and Evolution,* 217**,** 73-77.
Proks, P., F. Reimann, N. Green, F. Gribble & F. Ashcroft (2002) Sulfonylurea Stimulation of Insulin Secretion. *Diabetes,* 51**,** S368.
Puri, P. L. & M. Mercola (2012) BAF60 A, B, and Cs of muscle determination and renewal. *Genes & development,* 26**,** 2673-2683.
Puthalakath, H., L. A. O'Reilly, P. Gunn, L. Lee, P. N. Kelly, N. D. Huntington, P. D. Hughes, E. M. Michalak, J. McKimm-Breschkin, N. Motoyama, T. Gotoh, S. Akira, P. Bouillet & A. Strasser (2007) ER Stress Triggers Apoptosis by Activating BH3-Only Protein Bim. *Cell,* 129**,** 1337-1349.

Qiu, W.-L., Y.-W. Zhang, Y. Feng, L.-C. Li, L. Yang & C.-R. Xu (2017) Deciphering Pancreatic Islet β Cell and α Cell Maturation Pathways and Characteristic Features at the Single-Cell Level. *Cell Metabolism,* 25**,** 1194-1205.e4.

Qiu, Y., M. Guo, S. Huang & R. Stein (2004) Acetylation of the BETA2 Transcription Factor by p300-associated Factor Is Important in Insulin Gene Expression. *Journal of Biological Chemistry,* 279**,** 9796-9802.

Qu, X., J. P. Seale & R. Donnelly (1999) Tissue and isoform-selective activation of protein kinase C in insulin-resistant obese Zucker rats - effects of feeding. *Journal of Endocrinology,* 162**,** 207-214.

Quesada, I., M. G. Todorova & B. Soria (2006) Different metabolic responses in alpha-, beta-, and delta-cells of the islet of Langerhans monitored by redox confocal microscopy. *Biophysical journal,* 90**,** 2641-2650.

Raab, J. R., J. S. Runge, C. C. Spear & T. Magnuson (2017) Co-regulation of transcription by BRG1 and BRM, two mutually exclusive SWI/SNF ATPase subunits. *Epigenetics & Chromatin,* 10**,** 62.

Raddatz, K., N. Turner, G. Frangioudakis, B. M. Liao, D. J. Pedersen, J. Cantley, D. Wilks, E. Preston, B. D. Hegarty, M. Leitges, M. J. Raftery, T. J. Biden & C. Schmitz-Peiffer (2011) Time-dependent effects of Prkce deletion on glucose homeostasis and hepatic lipid metabolism on dietary lipid oversupply in mice. *Diabetologia,* 54**,** 1447-1456.

Rahier, J., R. M. Goebbels & J. C. Henquin (1983) Cellular composition of the human diabetic pancreas. *Diabetologia,* 24**,** 366-71.

Randle, P. J., P. B. Garland, C. N. Hales & E. A. Newsholme (1963) THE GLUCOSE FATTY-ACID CYCLE ITS ROLE IN INSULIN SENSITIVITY AND THE METABOLIC DISTURBANCES OF DIABETES MELLITUS. *The Lancet,* 281**,** 785-789.

Rao, X., P. Patel, R. Puett & S. Rajagopalan (2015) Air pollution as a risk factor for type 2 diabetes. *Toxicological sciences : an official journal of the Society of Toxicology,* 143**,** 231-241.

Reaven, G. (2004) The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. *Endocrinology and Metabolism Clinics of North America,* 33**,** 283-303.

Regazzi, R., C. B. Wollheim, J. Lang, J. M. Theler, O. Rossetto, C. Montecucco, K. Sadoul, U. Weller, M. Palmer & B. Thorens (1995) VAMP-2 and cellubrevin are expressed in pancreatic beta-cells and are essential for Ca(2+)-but not for GTP gamma S-induced insulin secretion. *The EMBO journal,* 14**,** 2723-2730.

Rena, G., D. G. Hardie & E. R. Pearson (2017) The mechanisms of action of metformin. *Diabetologia,* 60**,** 1577-1585.

Renström, E., L. Eliasson & P. Rorsman (1997) Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells. *The Journal of physiology,* 502 (Pt 1)**,** 105-118.

Reynisdottir, I., G. Thorleifsson, R. Benediktsson, G. Sigurdsson, V. Emilsson, A. S. Einarsdottir, E. E. Hjorleifsdottir, G. T. Orlygsdottir, G. T. Bjornsdottir, J. Saemundsdottir, S. Halldorsson, S. Hrafnkelsdottir, S. B. Sigurjonsdottir, S. Steinsdottir, M. Martin, J. P. Kochan, B. K. Rhees, S. F. A. Grant, M. L. Frigge, A. Kong, V. Gudnason, K. Stefansson & J. R. Gulcher (2003) Localization of a Susceptibility Gene for Type 2 Diabetes to Chromosome 5q34–q35.2. *The American Journal of Human Genetics,* 73**,** 323-335.

Riddelle, K. S., S. B. Hopkinson & J. C. Jones (1992) Hemidesmosomes in the epithelial cell line 804G: their fate during wound closure, mitosis and drug induced reorganization of the cytoskeleton. *Journal of Cell Science,* 103**,** 475.

Ringel, J., S. Engeli, A. Distler & A. M. Sharma (1999) Pro12Ala Missense Mutation of the Peroxisome Proliferator Activated Receptor γ and Diabetes Mellitus. *Biochemical and Biophysical Research Communications,* 254**,** 450-453.

Ritz-Laser, B., P. Meda, I. Constant, N. Klages, A. Charollais, A. Morales, C. Magnan, A. Ktorza & J. Philippe (1999) Glucose-Induced Preproinsulin Gene Expression Is Inhibited by the Free Fatty Acid Palmitate1. *Endocrinology,* 140**,** 4005-4014.

Rivas, F. V., N. H. Tolia, J.-J. Song, J. P. Aragon, J. Liu, G. J. Hannon & L. Joshua-Tor (2005) Purified Argonaute2 and an siRNA form recombinant human RISC. *Nature Structural & Molecular Biology,* 12**,** 340-349.

Robertson, R. P., J. Harmon, P. O. Tran, Y. Tanaka & H. Takahashi (2003) Glucose Toxicity in β-Cells: Type 2 Diabetes, Good Radicals Gone Bad, and the Glutathione Connection. *Diabetes,* 52**,** 581.

Robertson, R. P. & J. S. Harmon (2006) Diabetes, glucose toxicity, and oxidative stress: A case of double jeopardy for the pancreatic islet β cell. *Free Radical Biology and Medicine,* 41**,** 177-184.

Robinson, J. T., H. Thorvaldsdóttir, W. Winckler, M. Guttman, E. S. Lander, G. Getz & J. P. Mesirov (2011) Integrative genomics viewer. *Nature biotechnology,* 29**,** 24-26.

Robson, S. C., L. Ward, H. Brown, H. Turner, E. Hunter, S. Pelengaris & M. Khan (2011) Deciphering c-MYC-regulated genes in two distinct tissues. *BMC Genomics,* 12**,** 476.

Roche, E., F. Assimacopoulos-Jeannet, L. A. Witters, B. Perruchoud, G. Yaney, B. Corkey, M. Asfari & M. Prentki (1997) Induction by Glucose of Genes Coding for Glycolytic Enzymes in a Pancreatic β-Cell Line (INS-1). *Journal of Biological Chemistry,* 272**,** 3091-3098.

Roche, E., S. Farfari, L. A. Witters, F. Assimacopoulos-Jeannet, S. Thumelin, T. Brun, B. E. Corkey, A. K. Saha & M. Prentki (1998) Long-term exposure of beta-INS cells to high glucose concentrations increases anaplerosis, lipogenesis, and lipogenic gene expression. *Diabetes,* 47**,** 1086.

Rorsman, P., M. Braun & Q. Zhang (2012) Regulation of calcium in pancreatic α- and β-cells in health and disease. *Cell calcium,* 51**,** 300-308.

Rowe, J. W., K. L. Minaker, J. A. Pallotta & J. S. Flier (1983) Characterization of the insulin resistance of aging. *The Journal of clinical investigation,* 71**,** 1581-1587.

Rozenblum, E., M. Schutte, M. Goggins, S. A. Hahn, S. Panzer, M. Zahurak, S. N. Goodman, T. A. Sohn, R. H. Hruban, C. J. Yeo & S. E. Kern (1997) Tumor-suppressive Pathways in Pancreatic Carcinoma. *Cancer Research,* 57**,** 1731.

Ruchat, S. M., S. J. Weisnagel, M. C. Vohl, T. Rankinen, C. Bouchard & L. Pérusse (2009) Evidence for Interaction between PPARG Pro12Ala and PPARGC1A Gly482Ser Polymorphisms in Determining Type 2 Diabetes Intermediate Phenotypes in Overweight Subjects. *Exp Clin Endocrinol Diabetes,* 117**,** 455-459.

Rukstalis, M. & J. Habener (2009) Neurogenin3 A master regulator of pancreatic islet differentiation and regeneration. *Islets,* 1**,** 177-84.

Rulifson, E. J., S. K. Kim & R. Nusse (2002) Ablation of Insulin-Producing Neurons in Flies: Growth and Diabetic Phenotypes. *Science,* 296**,** 1118.

Russell, A. P., G. Gastaldi, E. Bobbioni-Harsch, P. Arboit, C. Gobelet, O. Dériaz, A. Golay, J. L. Witztum & J.-P. Giacobino (2003) Lipid peroxidation in skeletal muscle of obese as compared to endurance-trained humans: a case of good vs. bad lipids? *FEBS Letters,* 551**,** 104-106.

Rustenbeck, I. (2002) Desensitization of insulin secretion. *Biochemical Pharmacology,* 63**,** 1921-1935.

Rutledge, E. A. & D. W. Russell (1997) Adeno-associated virus vector integration junctions. *Journal of Virology,* 71**,** 8429.

Rutter, G. A. & D. J. Hodson (2013) Minireview: intraislet regulation of insulin secretion in humans. *Molecular endocrinology (Baltimore, Md.),* 27**,** 1984-1995.

Ryan, M. P., R. Jones & R. H. Morse (1998) SWI-SNF complex participation in transcriptional activation at a step subsequent to activator binding. *Molecular and cellular biology,* 18**,** 1774-1782.

Rönn, T., J. Wen, Z. Yang, B. Lu, Y. Du, L. Groop, R. Hu & C. Ling (2009) A common variant in MTNR1B, encoding melatonin receptor 1B, is associated with type 2 diabetes and fasting plasma glucose in Han Chinese individuals. *Diabetologia,* 52**,** 830-833.

Saccone, V., S. Consalvi, L. Giordani, C. Mozzetta, I. Barozzi, M. Sandoná, T. Ryan, A. Rojas-Muñoz, L. Madaro, P. Fasanaro, G. Borsellino, M. De Bardi, G. Frigè, A. Termanini, X. Sun, J. Rossant, B. G. Bruneau, M. Mercola, S. Minucci & P. L. Puri (2014) HDAC-regulated myomiRs control BAF60 variant exchange and direct the functional phenotype of fibro-adipogenic progenitors in dystrophic muscles. *Genes & Development,* 28**,** 841-857.

Saha, A., J. Wittmeyer & B. R. Cairns (2005) Chromatin remodeling through directional DNA translocation from an internal nucleosomal site. *Nature Structural & Molecular Biology,* 12**,** 747-755.

Saito, K. E. N., N. Yaginuma & T. Takahashi (1979) Differential Volumetry of A, B and D Cells in the Pancreatic Islets of Diabetic and Nondiabetic Subjects. *The Tohoku Journal of Experimental Medicine,* 129**,** 273-283.

Sako, Y. & V. E. Grill (1990) Coupling of β-Cell Desensitization by Hyperglycemia to Excessive Stimulation and Circulating Insulin in Glucose-Infused Rats. *Diabetes,* 39**,** 1580.

Sakura, H., C. Ammälä, P. A. Smith, F. M. Gribble & F. M. Ashcroft (1995) Cloning and functional expression of the cDNA encoding a novel ATP-sensitive potassium channel subunit expressed in pancreatic β-cells, brain, heart and skeletal muscle. *FEBS Letters,* 377**,** 338-344.

Sakuraba, H., H. Mizukami, N. Yagihashi, R. Wada, C. Hanyu & S. Yagihashi (2002) Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese Type II diabetic patients. *Diabetologia,* 45**,** 85-96.

Samaras, S. E., M. A. Cissell, K. Gerrish, C. V. E. Wright, M. Gannon & R. Stein (2002) Conserved Sequences in a Tissue-Specific Regulatory Region of the pdx-1 Gene Mediate Transcription in Pancreatic β Cells: Role for Hepatocyte Nuclear Factor 3β and Pax6. *Molecular and Cellular Biology,* 22**,** 4702.

Samaras, S. E., L. Zhao, A. Means, E. Henderson, T.-a. Matsuoka & R. Stein (2003) The Islet β Cell-enriched RIPE3b1/Maf Transcription Factor Regulates pdx-1 Expression. *Journal of Biological Chemistry,* 278**,** 12263-12270.

Samuel, V. T., Z.-X. Liu, X. Qu, B. D. Elder, S. Bilz, D. Befroy, A. J. Romanelli & G. I. Shulman (2004) Mechanism of Hepatic Insulin Resistance in Non-alcoholic Fatty Liver Disease. *Journal of Biological Chemistry,* 279**,** 32345-32353.

Samuel, V. T., Z.-X. Liu, A. Wang, S. A. Beddow, J. G. Geisler, M. Kahn, X.-m. Zhang, B. P. Monia, S. Bhanot & G. I. Shulman (2007) Inhibition of protein kinase Cepsilon prevents hepatic insulin resistance in nonalcoholic fatty liver disease. *The Journal of clinical investigation,* 117**,** 739-745.

Sander, M., A. Neubüser, J. Kalamaras, H. C. Ee, G. R. Martin & M. S. German (1997) Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. *Genes & Development,* 11**,** 1662-1673.

Sander, M., L. Sussel, J. Conners, D. Scheel, J. Kalamaras, F. Dela Cruz, V. Schwitzgebel, A. Hayes-Jordan & M. German (2000) Homeobox gene Nkx6.1 lies downstream of Nkx2.2 in the major pathway of beta-cell formation in the pancreas. *Development,* 127**,** 5533.

Sandhu, M. S., M. N. Weedon, K. A. Fawcett, J. Wasson, S. L. Debenham, A. Daly, H. Lango, T. M. Frayling, R. J. Neumann, R. Sherva, I. Blech, P. D. Pharoah, C. N. A. Palmer, C. Kimber, R. Tavendale, A. D. Morris, M. I. McCarthy, M. Walker, G. Hitman, B. Glaser, M. A. Permutt, A. T. Hattersley, N. J. Wareham & I. Barroso (2007) Common variants in WFS1 confer risk of type 2 diabetes. *Nature genetics,* 39**,** 951-953.

Sano, H., S. Kane, E. Sano, C. P. Mîinea, J. M. Asara, W. S. Lane, C. W. Garner & G. E. Lienhard (2003) Insulin-stimulated Phosphorylation of a Rab GTPase-activating Protein Regulates GLUT4 Translocation. *Journal of Biological Chemistry,* 278**,** 14599-14602.

Satija, A., D. Spiegelman, E. Giovannucci & F. B. Hu (2015) Type 2 diabetes and risk of cancer. *BMJ,* 350**,** g7707.
Saxena, G., J. Chen & A. Shalev (2010) Intracellular shuttling and mitochondrial function of thioredoxin-interacting protein. *Journal of Biological Chemistry,* 285**,** 3997-4005.
Saxonov, S., P. Berg & D. L. Brutlag (2006) A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of promoters. *Proceedings of the National Academy of Sciences of the United States of America,* 103**,** 1412-1417.
Schirle, N. T. & I. J. MacRae (2012) The crystal structure of human Argonaute2. *Science (New York, N.Y.),* 336**,** 1037-1040.
Schneider, C. A., W. S. Rasband & K. W. Eliceiri (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature methods,* 9**,** 671-675.
Schneider, R., A. J. Bannister, C. Weise & T. Kouzarides (2004) Direct Binding of INHAT to H3 Tails Disrupted by Modifications. *Journal of Biological Chemistry,* 279**,** 23859-23862.
Schulla, V., E. Renström, R. Feil, S. Feil, I. Franklin, A. Gjinovci, X.-J. Jing, D. Laux, I. Lundquist, M. A. Magnuson, S. Obermüller, C. S. Olofsson, A. Salehi, A. Wendt, N. Klugbauer, C. B. Wollheim, P. Rorsman & F. Hofmann (2003) Impaired insulin secretion and glucose tolerance in beta cell-selective Ca(v)1.2 Ca2+ channel null mice. *The EMBO journal,* 22**,** 3844-3854.
Schulz, W. A., C. Steinhoff & A. R. Florl. 2006. Methylation of Endogenous Human Retroelements in Health and Disease. In *DNA Methylation: Development, Genetic Disease and Cancer,* eds. W. Doerfler & P. Böhm, 211-250. Berlin, Heidelberg: Springer Berlin Heidelberg.
Schwanbeck, R., H. Xiao & C. Wu (2004) Spatial Contacts and Nucleosome Step Movements Induced by the NURF Chromatin Remodeling Complex. *Journal of Biological Chemistry,* 279**,** 39933-39941.
Schwarz, D. S., G. Hutvágner, T. Du, Z. Xu, N. Aronin & P. D. Zamore (2003) Asymmetry in the Assembly of the RNAi Enzyme Complex. *Cell,* 115**,** 199-208.
Sebastiani, G., A. Po, E. Miele, G. Ventriglia, E. Ceccarelli, M. Bugliani, L. Marselli, P. Marchetti, A. Gulino & E. Ferretti (2015) MicroRNA-124a is hyperexpressed in type 2 diabetic human pancreatic islets and negatively regulates insulin secretion. *Acta Diabetologica,* 52**,** 523-530.
Segerstolpe, Å., A. Palasantza, P. Eliasson, E.-M. Andersson, A.-C. Andréasson, X. Sun, S. Picelli, A. Sabirsh, M. Clausen, M. K. Bjursell, D. M. Smith, M. Kasper, C. Ämmälä & R. Sandberg (2016) Single-Cell Transcriptome Profiling of Human Pancreatic Islets in Health and Type 2 Diabetes. *Cell metabolism,* 24**,** 593-607.
Sen, P., P. Vivas, M. L. Dechassa, A. M. Mooney, M. G. Poirier & B. Bartholomew (2013) The SnAC Domain of SWI/SNF Is a Histone Anchor Required for Remodeling. *Molecular and Cellular Biology,* 33**,** 360.
Senée, V., C. Chelala, S. Duchatelet, D. Feng, H. Blanc, J.-C. Cossec, C. Charon, M. Nicolino, P. Boileau, D. R. Cavener, P. Bougnères, D. Taha & C. Julier (2006) Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism. *Nature Genetics,* 38**,** 682-687.
Seufert, J., G. C. Weir & J. F. Habener (1998) Differential expression of the insulin gene transcriptional repressor CCAAT/enhancer-binding protein beta and transactivator islet duodenum homeobox-1 in rat pancreatic beta cells during the development of diabetes mellitus. *The Journal of Clinical Investigation,* 101**,** 2528-2539.
Seymour, P. A., H. P. Shih, N. A. Patel, K. K. Freude, R. Xie, C. J. Lim & M. Sander (2012) A Sox9/Fgf feed-forward loop maintains pancreatic organ identity. *Development,* 139**,** 3363.
Shain, A. H., C. P. Giacomini, K. Matsukuma, C. A. Karikari, M. D. Bashyam, M. Hidalgo, A. Maitra & J. R. Pollack (2012) Convergent structural alterations define SWItch/Sucrose NonFermentable (SWI/SNF) chromatin remodeler as a central tumor suppressive complex in pancreatic cancer. *Proceedings of the National Academy of Sciences,* 109**,** E252.
Shain, A. H. & J. R. Pollack (2013) The Spectrum of SWI/SNF Mutations, Ubiquitous in Human Cancers. *PLOS ONE,* 8**,** e55119.

Shalev, A., C. A. Pise-Masison, M. Radonovich, S. C. Hoffmann, B. Hirshberg, J. N. Brady & D. M. Harlan (2002) Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGFbeta signaling pathway. *Endocrinology,* 143**,** 3695-3698.

Shapira, S. N., H.-W. Lim, S. Rajakumari, A. P. Sakers, J. Ishibashi, M. J. Harms, K.-J. Won & P. Seale (2017) EBF2 transcriptionally regulates brown adipogenesis via the histone reader DPF3 and the BAF chromatin remodeling complex. *Genes & development,* 31**,** 660-673.

Sharabi, K., C. D. J. Tavares, A. K. Rines & P. Puigserver (2015) Molecular pathophysiology of hepatic glucose production. *Molecular aspects of medicine,* 46**,** 21-33.

Sharma, A., L. K. Olson, R. P. Robertson & R. Stein (1995) The reduction of insulin gene transcription in HIT-T15 beta cells chronically exposed to high glucose concentration is associated with the loss of RIPE3b1 and STF-1 transcription factor expression. *Molecular Endocrinology,* 9**,** 1127-1134.

Shaw, L. M. (2011) The insulin receptor substrate (IRS) proteins: at the intersection of metabolism and cancer. *Cell cycle (Georgetown, Tex.),* 10**,** 1750-1756.

Shibasaki, T., H. Takahashi, T. Miki, Y. Sunaga, K. Matsumura, M. Yamanaka, C. Zhang, A. Tamamoto, T. Satoh, J.-I. Miyazaki & S. Seino (2007) Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. *Proceedings of the National Academy of Sciences of the United States of America,* 104**,** 19333-19338.

Shih, D. Q., M. Heimesaat, S. Kuwajima, R. Stein, C. V. E. Wright & M. Stoffel (2002) Profound defects in pancreatic β-cell function in mice with combined heterozygous mutations in Pdx-1, Hnf-1α, and Hnf-3β. *Proceedings of the National Academy of Sciences,* 99**,** 3818.

Shih, D. Q., S. Screenan, K. N. Munoz, L. Philipson, M. Pontoglio, M. Yaniv, K. S. Polonsky & M. Stoffel (2001) Loss of HNF-1α Function in Mice Leads to Abnormal Expression of Genes Involved in Pancreatic Islet Development and Metabolism. *Diabetes,* 50**,** 2472.

Shih, D. Q. & M. Stoffel (2002) Molecular etiologies of mody and other early-onset forms of diabetes. *Current Diabetes Reports,* 2**,** 125-134.

Shilatifard, A. (2012) The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. *Annual review of biochemistry,* 81**,** 65-95.

Shimabukuro, M., M. Higa, Y.-T. Zhou, M.-Y. Wang, C. B. Newgard & R. H. Unger (1998) Lipoapoptosis in Beta-cells of Obese Prediabeticfa/fa Rats: ROLE OF SERINE PALMITOYLTRANSFERASE OVEREXPRESSION. *Journal of Biological Chemistry,* 273**,** 32487-32490.

Shimada, T., et al. (1995) Development of vectors utilized for gene therapy for AIDS. *AIDS 4*.

Shimohata, H., K. Yoh, N. Morito, H. Shimano, T. Kudo & S. Takahashi (2006) MafK overexpression in pancreatic β-cells caused impairment of glucose-stimulated insulin secretion. *Biochemical and Biophysical Research Communications,* 346**,** 671-680.

Shimomura, H., T. Sanke, T. Hanabusa, K. Tsunoda, H. Furuta & K. Nanjo (2000) Nonsense mutation of islet-1 gene (Q310X) found in a type 2 diabetic patient with a strong family history. *Diabetes,* 49**,** 1597.

Shoshani, O. & D. Zipori (2011) Mammalian Cell Dedifferentiation as a Possible Outcome of Stress. *Stem Cell Reviews and Reports,* 7**,** 488-493.

Shulman, G. I., D. L. Rothman, T. Jue, P. Stein, R. A. DeFronzo & R. G. Shulman (1990) Quantitation of Muscle Glycogen Synthesis in Normal Subjects and Subjects with Non-Insulin-Dependent Diabetes by 13C Nuclear Magnetic Resonance Spectroscopy. *New England Journal of Medicine,* 322**,** 223-228.

Shyng, S., T. Ferrigni & C. G. Nichols (1997) Regulation of KATP channel activity by diazoxide and MgADP. Distinct functions of the two nucleotide binding folds of the sulfonylurea receptor. *The Journal of general physiology,* 110**,** 643-654.

Smith, S. B., H.-Q. Qu, N. Taleb, N. Y. Kishimoto, D. W. Scheel, Y. Lu, A.-M. Patch, R. Grabs, J. Wang, F. C. Lynn, T. Miyatsuka, J. Mitchell, R. Seerke, J. Désir, S. Vanden Eijnden, M. Abramowicz, N.

Kacet, J. Weill, M.-E. Renard, M. Gentile, I. Hansen, K. Dewar, A. T. Hattersley, R. Wang, M. E. Wilson, J. D. Johnson, C. Polychronakos & M. S. German (2010) Rfx6 directs islet formation and insulin production in mice and humans. *Nature,* 463**,** 775-780.

Snutch, T. P. & P. B. Reiner (1992) Ca2+ channels: diversity of form and function. *Current Opinion in Neurobiology,* 2**,** 247-253.

Song, J.-J., S. K. Smith, G. J. Hannon & L. Joshua-Tor (2004a) Crystal Structure of Argonaute and Its Implications for RISC Slicer Activity. *Science,* 305**,** 1434.

Song, L. & R. S. Tuan (2006) MicroRNAs and cell differentiation in mammalian development. *Birth Defects Research Part C: Embryo Today: Reviews,* 78**,** 140-149.

Song, Y., T. Niu, J. E. Manson, D. J. Kwiatkowski & S. Liu (2004b) Are variants in the CAPN10 gene related to risk of type 2 diabetes? A quantitative assessment of population and family-based association studies. *American journal of human genetics,* 74**,** 208-222.

Song, Y., M. K. Washington & H. C. Crawford (2010) Loss of FOXA1/2 is essential for the epithelial-to-mesenchymal transition in pancreatic cancer. *Cancer research,* 70**,** 2115-2125.

Sosa-Pineda, B., K. Chowdhury, M. Torres, G. Oliver & P. Gruss (1997) The Pax4 gene is essential for differentiation of insulin-producing β cells in the mammalian pancreas. *Nature,* 386**,** 399-402.

Sowers, J. R., M. Epstein & E. D. Frohlich (2001) Diabetes, Hypertension, and Cardiovascular Disease: An Update. *Hypertension,* 37**,** 1053-1059.

Spaeth, J. M., J.-H. Liu, D. Peters, M. Guo, A. B. Osipovich, F. Mohammadi, N. Roy, A. Bhushan, M. A. Magnuson, M. Hebrok, C. V. E. Wright & R. Stein (2019) The Pdx1 Bound Swi/Snf Chromatin Remodeling Complex Regulates Pancreatic Progenitor Cell Proliferation and Mature Islet β Cell Function. *Diabetes***,** db190349.

Spence, J. R., A. W. Lange, S.-C. J. Lin, K. H. Kaestner, A. M. Lowy, I. Kim, J. A. Whitsett & J. M. Wells (2009) Sox17 regulates organ lineage segregation of ventral foregut progenitor cells. *Developmental cell,* 17**,** 62-74.

Spijker, H. S., R. B. G. Ravelli, A. M. Mommaas-Kienhuis, A. A. van Apeldoorn, M. A. Engelse, A. Zaldumbide, S. Bonner-Weir, T. J. Rabelink, R. C. Hoeben, H. Clevers, C. L. Mummery, F. Carlotti & E. J. P. de Koning (2013) Conversion of Mature Human β-Cells Into Glucagon-Producing α-Cells. *Diabetes,* 62**,** 2471.

Spijker, H. S., H. Song, J. H. Ellenbroek, M. M. Roefs, M. A. Engelse, E. Bos, A. J. Koster, T. J. Rabelink, B. C. Hansen, A. Clark, F. Carlotti & E. J. P. de Koning (2015) Loss of β-Cell Identity Occurs in Type 2 Diabetes and Is Associated With Islet Amyloid Deposits. *Diabetes,* 64**,** 2928.

St-Onge, L., B. Sosa-Pineda, K. Chowdhury, A. Mansouri & P. Gruss (1997) Pax6 is required for differentiation of glucagon-producing α-cells in mouse pancreas. *Nature,* 387**,** 406-409.

Staffers, D. A., J. Ferrer, W. L. Clarke & J. F. Habener (1997) Early-onset type-ll diabetes mellitus (MODY4) linked to IPF1. *Nature Genetics,* 17**,** 138-139.

Steiner, D. F. (1969) Proinsulin and the Biosynthesis of Insulin. *New England Journal of Medicine,* 280**,** 1106-1113.

--- (1998) The proprotein convertases. *Current Opinion in Chemical Biology,* 2**,** 31-39.

Steiner, D. J., Kim, A., Miller, K. and Hara, M. (2010) Pancreatic islet plasticity: Interspecies comparison of islet architecture and composition. *Islets,* 2**,** 135-145.

Stolovich-Rain, M., J. Enk, J. Vikesa, Finn c. Nielsen, A. Saada, B. Glaser & Y. Dor (2015) Weaning Triggers a Maturation Step of Pancreatic β Cells. *Developmental Cell,* 32**,** 535-545.

Strawbridge, R. J., J. Dupuis, I. Prokopenko, A. Barker, E. Ahlqvist, D. Rybin, J. R. Petrie, M. E. Travers, N. Bouatia-Naji, A. S. Dimas, A. Nica, E. Wheeler, H. Chen, B. F. Voight, J. Taneera, S. Kanoni, J. F. Peden, F. Turrini, S. Gustafsson, C. Zabena, P. Almgren, D. J. P. Barker, D. Barnes, E. M. Dennison, J. G. Eriksson, P. Eriksson, E. Eury, L. Folkersen, C. S. Fox, T. M. Frayling, A. Goel, H. F. Gu, M. Horikoshi, B. Isomaa, A. U. Jackson, K. A. Jameson, E. Kajantie, J. Kerr-Conte, T. Kuulasmaa, J. Kuusisto, R. J. F. Loos, J. a. Luan, K. Makrilakis, A. K. Manning, M. T. Martínez-Larrad, N. Narisu, M. Nastase Mannila, J. Ohrvik, C. Osmond, L. Pascoe, F. Payne, A. A. Sayer,

B. Sennblad, A. Silveira, A. Stancáková, K. Stirrups, A. J. Swift, A.-C. Syvänen, T. Tuomi, F. M. van 't Hooft, M. Walker, M. N. Weedon, W. Xie, B. Zethelius, D. Consortium, G. Consortium, T. C. Mu, C. A. Consortium, C. D. Consortium, H. Ongen, A. Mälarstig, J. C. Hopewell, D. Saleheen, J. Chambers, S. Parish, J. Danesh, J. Kooner, C.-G. Ostenson, L. Lind, C. C. Cooper, M. Serrano-Ríos, E. Ferrannini, T. J. Forsen, R. Clarke, M. G. Franzosi, U. Seedorf, H. Watkins, P. Froguel, P. Johnson, P. Deloukas, F. S. Collins, M. Laakso, E. T. Dermitzakis, M. Boehnke, M. I. McCarthy, N. J. Wareham, L. Groop, F. Pattou, A. L. Gloyn, G. V. Dedoussis, et al. (2011) Genome-wide association identifies nine common variants associated with fasting proinsulin levels and provides new insights into the pathophysiology of type 2 diabetes. *Diabetes,* 60**,** 2624-2634.

Stride, A., S. Ellard, P. Clark, L. Shakespeare, M. Salzmann, M. Shepherd & A. T. Hattersley (2005) β-Cell Dysfunction, Insulin Sensitivity, and Glycosuria Precede Diabetes in Hepatocyte Nuclear Factor-1α Mutation Carriers. *Diabetes Care,* 28**,** 1751.

Striessnig, J., W. Berger & H. Glossmann (1993) Molecular Properties of Voltage-Dependent Ca²⁺ Channels in Excitable Tissues. *Cellular Physiology and Biochemistry,* 3**,** 295-317.

Striessnig, J., H.-G. Knaus, M. Grabner, K. Moosburger, W. Seitz, H. Lietz & H. Glossmann (1987) Photoaffinity labelling of the phenylalkylamine receptor of the skeletal muscle transverse-tubule calcium channel. *FEBS Letters,* 212**,** 247-253.

Subramanian, A., P. Tamayo, V. K. Mootha, S. Mukherjee, B. L. Ebert, M. A. Gillette, A. Paulovich, S. L. Pomeroy, T. R. Golub, E. S. Lander & J. P. Mesirov (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences,* 102**,** 15545.

Sudarsanam, P. & F. Winston (2000) The Swi/Snf family: nucleosome-remodeling complexes and transcriptional control. *Trends in Genetics,* 16**,** 345-351.

Sumi-Ichinose, C., H. Ichinose, D. Metzger & P. Chambon (1997) SNF2beta-BRG1 is essential for the viability of F9 murine embryonal carcinoma cells. *Molecular and cellular biology,* 17**,** 5976-5986.

Sun, J., L. Mao, H. Yang & D. Ren (2018) Critical role for the Tsc1-mTORC1 pathway in β-cell mass in Pdx1-deficient mice. *The Journal of endocrinology,* 238**,** 151-163.

Sun, X. J., D. L. Crimmins, M. G. Myers, Jr., M. Miralpeix & M. F. White (1993) Pleiotropic insulin signals are engaged by multisite phosphorylation of IRS-1. *Molecular and cellular biology,* 13**,** 7418-7428.

Sun, X. J., P. Rothenberg, C. R. Kahn, J. M. Backer, E. Araki, P. A. Wilden, D. A. Cahill, B. J. Goldstein & M. F. White (1991) Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature,* 352**,** 73-77.

Sun, X. J., L.-M. Wang, Y. Zhang, L. Yenush, M. G. Myers Jr, E. Glasheen, W. S. Lane, J. H. Pierce & M. F. White (1995) Role of IRS-2 in insulin and cytokine signalling. *Nature,* 377**,** 173-177.

Sund, N. J., M. Z. Vatamaniuk, M. Casey, S.-L. Ang, M. A. Magnuson, D. A. Stoffers, F. M. Matschinsky & K. H. Kaestner (2001) Tissue-specific deletion of Foxa2 in pancreatic β cells results in hyperinsulinemic hypoglycemia. *Genes & Development,* 15**,** 1706-1715.

Sussel, L., J. Kalamaras, O. Hartigan, D. J. Connor, J. J. Meneses, R. A. Pedersen, J. L. Rubenstein & M. S. German (1998) Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic beta cells. *Development,* 125**,** 2213.

Suzuki, M. M. & A. Bird (2008) DNA methylation landscapes: provocative insights from epigenomics. *Nature Reviews Genetics,* 9**,** 465-476.

Swisa, A., D. Avrahami, N. Eden, J. Zhang, E. Feleke, T. Dahan, Y. Cohen-Tayar, M. Stolovich-Rain, K. H. Kaestner, B. Glaser, R. Ashery-Padan & Y. Dor (2017) PAX6 maintains β cell identity by repressing genes of alternative islet cell types. *The Journal of clinical investigation,* 127**,** 230-243.

Szendroedi, J., T. Yoshimura, E. Phielix, C. Koliaki, M. Marcucci, D. Zhang, T. Jelenik, J. Müller, C. Herder, P. Nowotny, G. I. Shulman & M. Roden (2014) Role of diacylglycerol activation of PKCθ in lipid-induced muscle insulin resistance in humans. *Proceedings of the National Academy of Sciences of the United States of America,* 111**,** 9597-9602.

Tahbaz, N., J. B. Carmichael & T. C. Hobman (2001) GERp95 Belongs to a Family of Signal-transducing Proteins and Requires Hsp90 Activity for Stability and Golgi Localization. *Journal of Biological Chemistry,* 276**,** 43294-43299.

Tahrani, A. A., C. J. Bailey, S. Del Prato & A. H. Barnett (2011) Management of type 2 diabetes: new and future developments in treatment. *The Lancet,* 378**,** 182-197.

Tajiri, Y., C. Möller & V. Grill (1997) Long Term Effects of Aminoguanidine on Insulin Release and Biosynthesis: Evidence That the Formation of Advanced Glycosylation End Products Inhibits B Cell Function1. *Endocrinology,* 138**,** 273-280.

Takahashi, M., M. J. Seagar, J. F. Jones, B. F. Reber & W. A. Catterall (1987) Subunit structure of dihydropyridine-sensitive calcium channels from skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America,* 84**,** 5478-5482.

Takamoto, I., N. Kubota, K. Nakaya, K. Kumagai, S. Hashimoto, T. Kubota, M. Inoue, E. Kajiwara, H. Katsuyama, A. Obata, Y. Sakurai, M. Iwamoto, T. Kitamura, K. Ueki & T. Kadowaki (2014) TCF7L2 in mouse pancreatic beta cells plays a crucial role in glucose homeostasis by regulating beta cell mass. *Diabetologia,* 57**,** 542-553.

Takeuchi, J. K., X. Lou, J. M. Alexander, H. Sugizaki, P. Delgado-Olguín, A. K. Holloway, A. D. Mori, J. N. Wylie, C. Munson, Y. Zhu, Y.-Q. Zhou, R.-F. Yeh, R. M. Henkelman, R. P. Harvey, D. Metzger, P. Chambon, D. Y. R. Stainier, K. S. Pollard, I. C. Scott & B. G. Bruneau (2011) Chromatin remodelling complex dosage modulates transcription factor function in heart development. *Nature Communications,* 2**,** 187.

Talchai, C., S. Xuan, H. V. Lin, L. Sussel & D. Accili (2012) Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. *Cell,* 150**,** 1223-34.

Taleb, N. & C. Polychronakos (2011) RFX6 is needed for the development and maintenance of the β-cell phenotype. *Islets,* 3**,** 291-293.

Tanabe, T., H. Takeshima, A. Mikami, V. Flockerzi, H. Takahashi, K. Kangawa, M. Kojima, H. Matsuo, T. Hirose & S. Numa (1987) Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature,* 328**,** 313-318.

Tanaka, Y., C. E. Gleason, P. O. T. Tran, J. S. Harmon & R. P. Robertson (1999) Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proceedings of the National Academy of Sciences,* 96**,** 10857.

Tanaka, Y., P. O. T. Tran, J. Harmon & R. P. Robertson (2002) A role for glutathione peroxidase in protecting pancreatic β cells against oxidative stress in a model of glucose toxicity. *Proceedings of the National Academy of Sciences,* 99**,** 12363.

Tang, L., E. Nogales & C. Ciferri (2010) Structure and function of SWI/SNF chromatin remodeling complexes and mechanistic implications for transcription. *Progress in biophysics and molecular biology,* 102**,** 122-128.

Tarasov, A., J. Dusonchet & F. Ashcroft (2004) Metabolic Regulation of the Pancreatic Beta-Cell ATP-Sensitive K⁺ Channel. *Diabetes,* 53**,** S113.

Tarasov, A. I., T. J. Nicolson, J.-P. Riveline, T. K. Taneja, S. A. Baldwin, J. M. Baldwin, G. Charpentier, J.-F. Gautier, P. Froguel, M. Vaxillaire & G. A. Rutter (2008) A Rare Mutation in ABCC8/SUR1 Leading to Altered ATP-Sensitive K⁺ Channel Activity and β-Cell Glucose Sensing Is Associated With Type 2 Diabetes in Adults. *Diabetes,* 57**,** 1595.

Tarussio, D., S. Metref, P. Seyer, L. Mounien, D. Vallois, C. Magnan, M. Foretz & B. Thorens (2014) Nervous glucose sensing regulates postnatal β cell proliferation and glucose homeostasis. *The Journal of clinical investigation,* 124**,** 413-424.

Tatarskiy, V. V., Y. P. Simonov, D. S. Shcherbinin, A. V. Brechalov, S. G. Georgieva & N. V. Soshnikova (2017) Stability of the PHF10 subunit of PBAF signature module is regulated by phosphorylation: role of β-TrCP. *Scientific Reports,* 7**,** 5645.

Taylor, B. L., F.-F. Liu & M. Sander (2013) Nkx6.1 is essential for maintaining the functional state of pancreatic beta cells. *Cell reports,* 4**,** 1262-1275.

Taylor, S. S., J. A. Buechler & W. Yonemoto (1990) cAMP-DEPENDENT PROTEIN KINASE: FRAMEWORK FOR A DIVERSE FAMILY OF REGULATORY ENZYMES. *Annual Review of Biochemistry,* 59**,** 971-1005.

Tengholm, A. (2012) Cyclic AMP dynamics in the pancreatic β-cell. *Upsala journal of medical sciences,* 117**,** 355-369.

Tennagels, N. & U. Werner (2013) The metabolic and mitogenic properties of basal insulin analogues. *Archives of physiology and biochemistry,* 119**,** 1-14.

The InterAct, C. (2011) Design and cohort description of the InterAct Project: an examination of the interaction of genetic and lifestyle factors on the incidence of type 2 diabetes in the EPIC Study. *Diabetologia,* 54**,** 2272.

Thomas, B., S. Matson, V. Chopra, L. Sun, S. Sharma, S. Hersch, H. D. Rosas, C. Scherzer, R. Ferrante & W. Matson (2013) A novel method for detecting 7-methyl guanine reveals aberrant methylation levels in Huntington disease. *Analytical biochemistry,* 436**,** 112-120.

Thomson, D. W., C. P. Bracken & G. J. Goodall (2011) Experimental strategies for microRNA target identification. *Nucleic acids research,* 39**,** 6845-6853.

Thorens, B. (2015) GLUT2, glucose sensing and glucose homeostasis. *Diabetologia,* 58**,** 221-232.

Thorens, B., M. T. Guillam, F. Beermann, R. Burcelin & M. Jaquet (2000) Transgenic reexpression of GLUT1 or GLUT2 in pancreatic beta cells rescues GLUT2-null mice from early death and restores normal glucose-stimulated insulin secretion. *J Biol Chem,* 275**,** 23751-8.

Thorens, B., D. Tarussio, M. A. Maestro, M. Rovira, E. Heikkilä & J. Ferrer (2015) Ins1(Cre) knock-in mice for beta cell-specific gene recombination. *Diabetologia,* 58**,** 558-565.

Tillil, H. & J. Köbberling (1987) Age-Corrected Empirical Genetic Risk Estimates for First-Degree Relatives of IDDM Patients. *Diabetes,* 36**,** 93.

Trapp, S., S. J. Tucker & F. M. Ashcroft (1997) Activation and inhibition of K-ATP currents by guanine nucleotides is mediated by different channel subunits. *Proceedings of the National Academy of Sciences of the United States of America,* 94**,** 8872-8877.

Tripathi, B. K. & A. K. Srivastava (2006) Diabetes mellitus: complications and therapeutics. *Med Sci Monit.,* 12**,** RA130-47.

Tsien, R. W., D. Lipscombe, D. V. Madison, K. R. Bley & A. P. Fox (1988) Multiple types of neuronal calcium channels and their selective modulation. *Trends in Neurosciences,* 11**,** 431-438.

Tsigos, C., V. Hainer, A. Basdevant, N. Finer, E. Mathus-Vliegen, D. Micic, M. Maislos, G. Roman, Y. Schutz, H. Toplak, V. Yumuk, B. Zahorska-Markiewicz & O. Obesity Management Task Force of the European Association for the Study of (2011) Criteria for EASO-collaborating centres for obesity management. *Obesity facts,* 4**,** 329-333.

Tsuda, M., A. Fukuda, N. Roy, Y. Hiramatsu, L. Leonhardt, N. Kakiuchi, K. Hoyer, S. Ogawa, N. Goto, K. Ikuta, Y. Kimura, Y. Matsumoto, Y. Takada, T. Yoshioka, T. Maruno, Y. Yamaga, G. E. Kim, H. Akiyama, S. Ogawa, C. V. Wright, D. Saur, K. Takaori, S. Uemoto, M. Hebrok, T. Chiba & H. Seno (2018) The BRG1/SOX9 axis is critical for acinar cell–derived pancreatic tumorigenesis. *The Journal of Clinical Investigation,* 128**,** 3475-3489.

Tsui, S., W. Dai & L. Lu (2014) CCCTC-binding factor mediates effects of glucose on beta cell survival. *Cell proliferation,* 47**,** 28-37.

Tucker, S. J., F. M. Gribble, P. Proks, S. Trapp, T. J. Ryder, T. Haug, F. Reimann & F. M. Ashcroft (1998) Molecular determinants of KATP channel inhibition by ATP. *The EMBO Journal,* 17**,** 3290-3296.

Tucker, S. J., F. M. Gribble, C. Zhao, S. Trapp & F. M. Ashcroft (1997) Truncation of Kir6.2 produces ATP-sensitive K+ channels in the absence of the sulphonylurea receptor. *Nature,* 387**,** 179-183.

Tuomi, T., C. L. F. Nagorny, P. Singh, H. Bennet, Q. Yu, I. Alenkvist, B. Isomaa, B. Östman, J. Söderström, A.-K. Pesonen, S. Martikainen, K. Räikkönen, T. Forsén, L. Hakaste, P. Almgren, P. Storm, O. Asplund, L. Shcherbina, M. Fex, J. Fadista, A. Tengholm, N. Wierup, L. Groop & H. Mulder (2016) Increased Melatonin Signaling Is a Risk Factor for Type 2 Diabetes. *Cell Metabolism,* 23**,** 1067-1077.

Tuttle, R. L., N. S. Gill, W. Pugh, J.-P. Lee, B. Koeberlein, E. E. Furth, K. S. Polonsky, A. Naji & M. J. Birnbaum (2001) Regulation of pancreatic β-cell growth and survival by the serine/threonine protein kinase Akt1/PKBα. *Nature Medicine,* 7**,** 1133-1137.

Ukai, H., H. Ishii-Oba, M. Ukai-Tadenuma, T. Ogiu & H. Tsuji (2003) Formation of an active form of the interleukin-2/15 receptor β-chain by insertion of the intracisternal A particle in a radiation-induced mouse thymic lymphoma and its role in tumorigenesis. *Molecular Carcinogenesis,* 37**,** 110-119.

Ullrich, A., J. R. Bell, E. Y. Chen, R. Herrera, L. M. Petruzzelli, T. J. Dull, A. Gray, L. Coussens, Y. C. Liao, M. Tsubokawa, A. Mason, P. H. Seeburg, C. Grunfeld, O. M. Rosen & J. Ramachandran (1985) Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature,* 313**,** 756-761.

Unnikrishnan, A., W. M. Freeman, J. Jackson, J. D. Wren, H. Porter & A. Richardson (2019) The role of DNA methylation in epigenetics of aging. *Pharmacology & Therapeutics,* 195**,** 172-185.

Utriainen, T., T. Takala, M. Luotolahti, T. Rönnemaa, H. Laine, U. Ruotsalainen, M. Haaparanta, P. Nuutila & H. Yki-Järvinen (1998) Insulin resistance characterizes glucose uptake in skeletal muscle but not in the heart in NIDDM. *Diabetologia,* 41**,** 555-559.

van Arensbergen, J., J. García-Hurtado, I. Moran, M. A. Maestro, X. Xu, M. Van de Casteele, A. L. Skoudy, M. Palassini, H. Heimberg & J. Ferrer (2010) Derepression of Polycomb targets during pancreatic organogenesis allows insulin-producing beta-cells to adopt a neural gene activity program. *Genome research,* 20**,** 722-732.

Van Laer, L., E. Van Eyken, E. Fransen, J. R. Huyghe, V. Topsakal, J.-J. Hendrickx, S. Hannula, E. Mäki-Torkko, M. Jensen, K. Demeester, M. Baur, A. Bonaconsa, M. Mazzoli, A. Espeso, K. Verbruggen, J. Huyghe, P. Huygen, S. Kunst, M. Manninen, A. Konings, A. N. Diaz-Lacava, M. Steffens, T. F. Wienker, I. Pyykkö, C. W. R. J. Cremers, H. Kremer, I. Dhooge, D. Stephens, E. Orzan, M. Pfister, M. Bille, A. Parving, M. Sorri, P. H. Van de Heyning & G. Van Camp (2007) The grainyhead like 2 gene (GRHL2), alias TFCP2L3, is associated with age-related hearing impairment. *Human Molecular Genetics,* 17**,** 159-169.

Varela, I., P. Tarpey, K. Raine, D. Huang, C. K. Ong, P. Stephens, H. Davies, D. Jones, M.-L. Lin, J. Teague, G. Bignell, A. Butler, J. Cho, G. L. Dalgliesh, D. Galappaththige, C. Greenman, C. Hardy, M. Jia, C. Latimer, K. W. Lau, J. Marshall, S. McLaren, A. Menzies, L. Mudie, L. Stebbings, D. A. Largaespada, L. F. A. Wessels, S. Richard, R. J. Kahnoski, J. Anema, D. A.Tuveson, P. A. Perez-Mancera, V. Mustonen, A. Fischer, D. J. Adams, A. Rust, W. Chan-on, C. Subimerb, K. Dykema, K. Furge, P. J. Campbell, B. T. Teh, M. R. Stratton & P. A. Futreal (2011) Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. *Nature,* 469**,** 539-542.

Vaxillaire, M., A. Bonnefond & P. Froguel (2012) The lessons of early-onset monogenic diabetes for the understanding of diabetes pathogenesis. *Best Practice & Research Clinical Endocrinology & Metabolism,* 26**,** 171-187.

Vial, E. & C. J. Marshall (2003) Elevated ERK-MAP kinase activity protects the FOS family member FRA-1 against proteasomal degradation in colon carcinoma cells. *Journal of Cell Science,* 116**,** 4957.

Vignali, M., A. H. Hassan, K. E. Neely & J. L. Workman (2000) ATP-dependent chromatin-remodeling complexes. *Molecular and cellular biology,* 20**,** 1899-1910.

Voight, B. F., L. J. Scott, V. Steinthorsdottir, A. P. Morris, C. Dina, R. P. Welch, E. Zeggini, C. Huth, Y. S. Aulchenko, G. Thorleifsson, L. J. McCulloch, T. Ferreira, H. Grallert, N. Amin, G. Wu, C. J. Willer, S. Raychaudhuri, S. A. McCarroll, C. Langenberg, O. M. Hofmann, J. Dupuis, L. Qi, A. V. Segrè, M. van Hoek, P. Navarro, K. Ardlie, B. Balkau, R. Benediktsson, A. J. Bennett, R. Blagieva, E. Boerwinkle, L. L. Bonnycastle, K. Bengtsson Boström, B. Bravenboer, S. Bumpstead, N. P. Burtt, G. Charpentier, P. S. Chines, M. Cornelis, D. J. Couper, G. Crawford, A. S. F. Doney, K. S. Elliott, A. L. Elliott, M. R. Erdos, C. S. Fox, C. S. Franklin, M. Ganser, C. Gieger, N. Grarup, T. Green, S. Griffin, C. J. Groves, C. Guiducci, S. Hadjadj, N. Hassanali, C. Herder, B. Isomaa, A. U. Jackson, P. R. V. Johnson, T. Jørgensen, W. H. L. Kao, N. Klopp, A. Kong, P. Kraft, J. Kuusisto, T. Lauritzen, M. Li, A. Lieverse, C. M. Lindgren, V. Lyssenko, M. Marre, T. Meitinger, K. Midthjell, M. A. Morken, N. Narisu, P. Nilsson, K. R. Owen, F. Payne, J. R. B. Perry, A.-K. Petersen, C. Platou, C. Proença, I. Prokopenko, W. Rathmann, N. W. Rayner, N. R. Robertson, G. Rocheleau, M. Roden, M. J. Sampson, R. Saxena, B. M. Shields, P. Shrader, G. Sigurdsson, T. Sparsø, K. Strassburger, H. M. Stringham, Q. Sun, A. J. Swift, B. Thorand, et al. (2010) Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nature genetics,* 42**,** 579-589.

Volkmar, M., S. Dedeurwaerder, D. A. Cunha, M. N. Ndlovu, M. Defrance, R. Deplus, E. Calonne, U. Volkmar, M. Igoillo-Esteve, N. Naamane, S. Del Guerra, M. Masini, M. Bugliani, P. Marchetti, M. Cnop, D. L. Eizirik & F. Fuks (2012) DNA methylation profiling identifies epigenetic dysregulation in pancreatic islets from type 2 diabetic patients. *The EMBO journal,* 31**,** 1405-1426.

Wade, P. A. (2001) Methyl CpG-binding proteins and transcriptional repression*. *BioEssays,* 23**,** 1131-1137.

Wan, S., J. Wang, J. Wang, J. Wu, J. Song, C.-Y. Zhang, C. Zhang, C. Wang & J.-J. Wang (2017) Increased serum miR-7 is a promising biomarker for type 2 diabetes mellitus and its microvascular complications. *Diabetes Research and Clinical Practice,* 130**,** 171-179.

Wang, B., B. Kaufmann, T. Engleitner, M. Lu, C. Mogler, V. Olsavszky, R. Öllinger, S. Zhong, C. Geraud, Z. Cheng, R. R. Rad, R. M. Schmid, H. Friess, N. Hüser, D. Hartmann & G. von Figura (2019a) Brg1 promotes liver regeneration after partial hepatectomy via regulation of cell cycle. *Scientific reports,* 9**,** 2320-2320.

Wang, H., L. Wang, H. Erdjument-Bromage, M. Vidal, P. Tempst, R. S. Jones & Y. Zhang (2004) Role of histone H2A ubiquitination in Polycomb silencing. *Nature,* 431**,** 873-878.

Wang, L., J. Yao, H. Sun, K. He, D. Tong, T. Song & C. Huang (2017) MicroRNA-101 suppresses progression of lung cancer through the PTEN/AKT signaling pathway by targeting DNA methyltransferase 3A. *Oncology letters,* 13**,** 329-338.

Wang, P., M. C. Chicka, A. Bhalla, D. A. Richards & E. R. Chapman (2005) Synaptotagmin VII is targeted to secretory organelles in PC12 cells, where it functions as a high-affinity calcium sensor. *Molecular and cellular biology,* 25**,** 8693-8702.

Wang, S., L. Crisman, J. Miller, I. Datta, D. R. Gulbranson, Y. Tian, Q. Yin, H. Yu & J. Shen (2019b) Inducible Exoc7/Exo70 knockout reveals a critical role of the exocyst in insulin-regulated GLUT4 exocytosis. *Journal of Biological Chemistry,* 294**,** 19988-19996.

Wang, W., P. A. Hansen, B. A. Marshall, J. O. Holloszy & M. Mueckler (1996a) Insulin unmasks a COOH-terminal Glut4 epitope and increases glucose transport across T-tubules in skeletal muscle. *The Journal of cell biology,* 135**,** 415-430.

Wang, W., Y. Xue, S. Zhou, A. Kuo, B. R. Cairns & G. R. Crabtree (1996b) Diversity and specialization of mammalian SWI/SNF complexes. *Genes & Development,* 10**,** 2117-2130.

Wang, Y., G. Sheng, S. Juranek, T. Tuschl & D. J. Patel (2008) Structure of the guide-strand-containing argonaute silencing complex. *Nature,* 456**,** 209-213.

Wang, Y. J., J. Schug, K.-J. Won, C. Liu, A. Naji, D. Avrahami, M. L. Golson & K. H. Kaestner (2016) Single-Cell Transcriptomics of the Human Endocrine Pancreas. *Diabetes,* 65**,** 3028-3038.

Wang, Z., N. W. York, C. G. Nichols & M. S. Remedi (2014) Pancreatic β cell dedifferentiation in diabetes and redifferentiation following insulin therapy. *Cell metabolism,* 19**,** 872-882.
Wang, Z., C. Zang, K. Cui, D. E. Schones, A. Barski, W. Peng & K. Zhao (2009) Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell,* 138**,** 1019-1031.
Watt, A. J., R. Zhao, J. Li & S. A. Duncan (2007) Development of the mammalian liver and ventral pancreas is dependent on GATA4. *BMC developmental biology,* 7**,** 37-37.
Wei, M. C., W.-X. Zong, E. H. Y. Cheng, T. Lindsten, V. Panoutsakopoulou, A. J. Ross, K. A. Roth, G. R. MacGregor, C. B. Thompson & S. J. Korsmeyer (2001) Proapoptotic BAX and BAK: A Requisite Gateway to Mitochondrial Dysfunction and Death. *Science,* 292**,** 727.
Wei, R., J. Yang, G.-q. Liu, M.-j. Gao, W.-f. Hou, L. Zhang, H.-w. Gao, Y. Liu, G.-a. Chen & T.-p. Hong (2013) Dynamic expression of microRNAs during the differentiation of human embryonic stem cells into insulin-producing cells. *Gene,* 518**,** 246-255.
Weir, G. C. a. B.-W., S. (2004) Five Stages of Evolving Beta-Cell Dysfunction During Progression to Diabetes. *Diabetes,* 53**,** S16-S21.
Weiss, M. A. (2009) Proinsulin and the genetics of diabetes mellitus. *The Journal of biological chemistry,* 284**,** 19159-19163.
White, M. F. (2006) Regulating insulin signaling and β-cell function through IRS proteinsThis paper is one of a selection of papers published in this Special Issue, entitled Second Messengers and Phosphoproteins—12th International Conference. *Canadian Journal of Physiology and Pharmacology,* 84**,** 725-737.
Wiegand, K. C., S. P. Shah, O. M. Al-Agha, Y. Zhao, K. Tse, T. Zeng, J. Senz, M. K. McConechy, M. S. Anglesio, S. E. Kalloger, W. Yang, A. Heravi-Moussavi, R. Giuliany, C. Chow, J. Fee, A. Zayed, L. Prentice, N. Melnyk, G. Turashvili, A. D. Delaney, J. Madore, S. Yip, A. W. McPherson, G. Ha, L. Bell, S. Fereday, A. Tam, L. Galletta, P. N. Tonin, D. Provencher, D. Miller, S. J. M. Jones, R. A. Moore, G. B. Morin, A. Oloumi, N. Boyd, S. A. Aparicio, I.-M. Shih, A.-M. Mes-Masson, D. D. Bowtell, M. Hirst, B. Gilks, M. A. Marra & D. G. Huntsman (2010) ARID1A Mutations in Endometriosis-Associated Ovarian Carcinomas. *New England Journal of Medicine,* 363**,** 1532-1543.
Wienholds, E., W. P. Kloosterman, E. Miska, E. Alvarez-Saavedra, E. Berezikov, E. de Bruijn, H. R. Horvitz, S. Kauppinen & R. H. A. Plasterk (2005) MicroRNA Expression in Zebrafish Embryonic Development. *Science,* 309**,** 310.
Wightman, B., I. Ha & G. Ruvkun (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell,* 75**,** 855-862.
Wiles, E. T. & E. U. Selker (2017) H3K27 methylation: a promiscuous repressive chromatin mark. *Current opinion in genetics & development,* 43**,** 31-37.
Willmann, S. J., N. S. Mueller, S. Engert, M. Sterr, I. Burtscher, A. Raducanu, M. Irmler, J. Beckers, S. Sass, F. J. Theis & H. Lickert (2016) The global gene expression profile of the secondary transition during pancreatic development. *Mechanisms of Development,* 139**,** 51-64.
Wilson, B. G. & C. W. M. Roberts (2011) SWI/SNF nucleosome remodellers and cancer. *Nature Reviews Cancer,* 11**,** 481-492.
Wischhof, L., S. Maida, A. Piazzesi, A. Gioran, K. Barragan Sanz, S. Irsen, M. Beyer, J. L. Schultze, M. J. Dyer, P. Salomoni, D. Ehninger, P. Nicotera & D. Bano (2017) The SWI/SNF subunit Bcl7a contributes to motor coordination and Purkinje cell function. *Scientific Reports,* 7**,** 17055.
Withers, D. J., J. S. Gutierrez, H. Towery, D. J. Burks, J.-M. Ren, S. Previs, Y. Zhang, D. Bernal, S. Pons, G. I. Shulman, S. Bonner-Weir & M. F. White (1998) Disruption of IRS-2 causes type 2 diabetes in mice. *Nature,* 391**,** 900-904.
Wititsuwannakul, D. & K. H. Kim (1977) Mechanism of palmityl coenzyme A inhibition of liver glycogen synthase. *Journal of Biological Chemistry,* 252**,** 7812-7817.

Wong, C. K., A. K. Wade-Vallance, D. S. Luciani, P. K. Brindle, F. C. Lynn & W. T. Gibson (2018) The p300 and CBP Transcriptional Coactivators Are Required for β-Cell and α-Cell Proliferation. *Diabetes,* 67**,** 412.

Workman, J. L. & R. E. Kingston (1998) ALTERATION OF NUCLEOSOME STRUCTURE AS A MECHANISM OF TRANSCRIPTIONAL REGULATION. *Annual Review of Biochemistry,* 67**,** 545-579.

Wright, N. M., D. L. Metzger, S. M. Borowitz & W. L. Clarke (1993) Permanent Neonatal Diabetes Mellitus and Pancreatic Exocrine Insufficiency Resulting From Congenital Pancreatic Agenesis. *JAMA Pediatrics,* 147**,** 607-609.

Wu, C. (1997) Chromatin Remodeling and the Control of Gene Expression. *Journal of Biological Chemistry,* 272**,** 28171-28174.

Wu, M., E. M. Rinchik, E. Wilkinson & D. K. Johnson (1997) Inherited somatic mosaicism caused by an intracisternal A particle insertion in the mouse tyrosinase gene. *Proceedings of the National Academy of Sciences of the United States of America,* 94**,** 890-894.

Xie, H.-f., Y.-z. Liu, R. Du, B. Wang, M.-t. Chen, Y.-y. Zhang, Z.-l. Deng & J. Li (2017a) miR-377 induces senescence in human skin fibroblasts by targeting DNA methyltransferase 1. *Cell Death & Disease,* 8**,** e2663-e2663.

Xie, R., A. C. Carrano & M. Sander (2015) A systems view of epigenetic networks regulating pancreas development and β-cell function. *Wiley interdisciplinary reviews. Systems biology and medicine,* 7**,** 1-11.

Xie, R., L. J. Everett, H.-W. Lim, N. A. Patel, J. Schug, E. Kroon, O. G. Kelly, A. Wang, K. A. D'Amour, A. J. Robins, K.-J. Won, K. H. Kaestner & M. Sander (2013a) Dynamic chromatin remodeling mediated by polycomb proteins orchestrates pancreatic differentiation of human embryonic stem cells. *Cell stem cell,* 12**,** 224-237.

Xie, R., L. J. Everett, H. W. Lim, N. A. Patel, J. Schug, E. Kroon, O. G. Kelly, A. Wang, K. A. D'Amour, A. J. Robins, K. J. Won, K. H. Kaestner & M. Sander (2013b) Dynamic chromatin remodeling mediated by polycomb proteins orchestrates pancreatic differentiation of human embryonic stem cells. *Cell Stem Cell,* 12**,** 224-37.

Xie, V. K., Z. Li, Y. Yan, Z. Jia, X. Zuo, Z. Ju, J. Wang, J. Du, D. Xie, K. Xie & D. Wei (2017b) DNA-Methyltransferase 1 Induces Dedifferentiation of Pancreatic Cancer Cells through Silencing of Krüppel-Like Factor 4 Expression. *Clinical Cancer Research,* 23**,** 5585.

Xie, W., C. L. Barr, A. Kim, F. Yue, A. Y. Lee, J. Eubanks, E. L. Dempster & B. Ren (2012) Base-resolution analyses of sequence and parent-of-origin dependent DNA methylation in the mouse genome. *Cell,* 148**,** 816-831.

Xin, Y., J. Kim, H. Okamoto, M. Ni, Y. Wei, C. Adler, Andrew J. Murphy, George D. Yancopoulos, C. Lin & J. Gromada (2016) RNA Sequencing of Single Human Islet Cells Reveals Type 2 Diabetes Genes. *Cell Metabolism,* 24**,** 608-615.

Xiong, Y., W. Li, C. Shang, Richard M. Chen, P. Han, J. Yang, K. Stankunas, B. Wu, M. Pan, B. Zhou, Michael T. Longaker & C.-P. Chang (2013) Brg1 Governs a Positive Feedback Circuit in the Hair Follicle for Tissue Regeneration and Repair. *Developmental Cell,* 25**,** 169-181.

Xu, C.-R., P. A. Cole, D. J. Meyers, J. Kormish, S. Dent & K. S. Zaret (2011) Chromatin "prepattern" and histone modifiers in a fate choice for liver and pancreas. *Science (New York, N.Y.),* 332**,** 963-966.

Xu, F., S. Flowers & E. Moran (2012) Essential Role of ARID2 Protein-containing SWI/SNF Complex in Tissue-specific Gene Expression. *Journal of Biological Chemistry,* 287**,** 5033-5041.

Xu, G., J. Chen, G. Jing & A. Shalev (2013) Thioredoxin-interacting protein regulates insulin transcription through microRNA-204. *Nature Medicine,* 19**,** 1141-1146.

Xu, J., J. Wang & B. Chen (2012) SLC30A8 (ZnT8) variations and type 2 diabetes in the Chinese Han population. *Genet Mol Res.,* 11.

Xuan, S. & L. Sussel (2016) GATA4 and GATA6 regulate pancreatic endoderm identity through inhibition of hedgehog signaling. *Development,* 143**,** 780.

Yakaryılmaz, F. D. & Z. A. Öztürk (2017) Treatment of type 2 diabetes mellitus in the elderly. *World journal of diabetes,* 8**,** 278-285.
Yamamoto, M., C. Meno, Y. Sakai, H. Shiratori, K. Mochida, Y. Ikawa, Y. Saijoh & H. Hamada (2001) The transcription factor FoxH1 (FAST) mediates Nodal signaling during anterior-posterior patterning and node formation in the mouse. *Genes & Development,* 15**,** 1242-1256.
Yan, B., Z.-H. Wang, C.-D. Zhu, J.-T. Guo & J.-L. Zhao (2014) MicroRNA repertoire for functional genome research in tilapia identified by deep sequencing. *Molecular Biology Reports,* 41**,** 4953-4963.
Yang, B. T., T. A. Dayeh, P. A. Volkov, C. L. Kirkpatrick, S. Malmgren, X. Jing, E. Renström, C. B. Wollheim, M. D. Nitert & C. Ling (2012) Increased DNA Methylation and Decreased Expression of PDX-1 in Pancreatic Islets from Patients with Type 2 Diabetes. *Molecular Endocrinology,* 26**,** 1203-1212.
Yang, J. 2014. Chapter Five - Enhanced Skeletal Muscle for Effective Glucose Homeostasis. In *Progress in Molecular Biology and Translational Science,* ed. Y.-X. Tao, 133-163. Academic Press.
Yang, S.-N., Y. Shi, G. Yang, Y. Li, J. Yu & P.-O. Berggren (2014) Ionic mechanisms in pancreatic β cell signaling. *Cellular and Molecular Life Sciences,* 71**,** 4149-4177.
Yang, X., F. Y. G. Lee Sr & G. S. Wand (1997) Increased Expression Of Gsα Enhances Activation Of The Adenylyl Cyclase Signal Transduction Cascade. *Molecular Endocrinology,* 11**,** 1053-1061.
Yao, Q., Y. Chen & X. Zhou (2019) The roles of microRNAs in epigenetic regulation. *Current Opinion in Chemical Biology,* 51**,** 11-17.
Yi, R., Y. Qin, I. G. Macara & B. R. Cullen (2003) Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes & Development,* 17**,** 3011-3016.
Yoder, J. A. & T. H. Bestor (1998) A Candidate Mammalian DNA Methyltransferase Related to pmt1p of Fission Yeast. *Human Molecular Genetics,* 7**,** 279-284.
Yoo, A. S., B. T. Staahl, L. Chen & G. R. Crabtree (2009) MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. *Nature,* 460**,** 642-646.
Yore, M. M., I. Syed, P. M. Moraes-Vieira, T. Zhang, M. A. Herman, E. A. Homan, R. T. Patel, J. Lee, S. Chen, O. D. Peroni, A. S. Dhaneshwar, A. Hammarstedt, U. Smith, T. E. McGraw, A. Saghatelian & B. B. Kahn (2014) Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. *Cell,* 159**,** 318-332.
You, D., E. Nilsson, D. E. Tenen, A. Lyubetskaya, J. C. Lo, R. Jiang, J. Deng, B. A. Dawes, A. Vaag, C. Ling, E. D. Rosen & S. Kang (2017) Dnmt3a is an epigenetic mediator of adipose insulin resistance. *eLife,* 6**,** e30766.
Yu, G., L.-G. Wang & Q.-Y. He (2015) ChIPseeker: an R/Bioconductor package for ChIP peak annotation, comparison and visualization. *Bioinformatics,* 31**,** 2382-2383.
Zhang, H., E. T. Ables, C. F. Pope, M. K. Washington, S. Hipkens, A. L. Means, G. Path, J. Seufert, R. H. Costa, A. B. Leiter, M. A. Magnuson & M. Gannon (2009) Multiple, temporal-specific roles for HNF6 in pancreatic endocrine and ductal differentiation. *Mechanisms of development,* 126**,** 958-973.
Zhang, H., K. Cai, J. Wang, X. Wang, K. Cheng, F. Shi, L. Jiang, Y. Zhang & J. Dou (2014) MiR-7, Inhibited Indirectly by LincRNA HOTAIR, Directly Inhibits SETDB1 and Reverses the EMT of Breast Cancer Stem Cells by Downregulating the STAT3 Pathway. *STEM CELLS,* 32**,** 2858-2868.
Zhang, Y., T. Liu, C. A. Meyer, J. Eeckhoute, D. S. Johnson, B. E. Bernstein, C. Nusbaum, R. M. Myers, M. Brown, W. Li & X. S. Liu (2008) Model-based analysis of ChIP-Seq (MACS). *Genome biology,* 9**,** R137-R137.
Zhang, Y., C. L. Smith, A. Saha, S. W. Grill, S. Mihardja, S. B. Smith, B. R. Cairns, C. L. Peterson & C. Bustamante (2006) DNA Translocation and Loop Formation Mechanism of Chromatin Remodeling by SWI/SNF and RSC. *Molecular Cell,* 24**,** 559-568.

Zhang, Z., Y. Cao, Y. Zhai, X. Ma, X. An, S. Zhang & Z. Li (2018) MicroRNA-29b regulates DNA methylation by targeting Dnmt3a/3b and Tet1/2/3 in porcine early embryo development. *Development, Growth & Differentiation,* 60**,** 197-204.

Zhao, C., M. C. Wilson, F. Schuit, A. P. Halestrap & G. A. Rutter (2001) Expression and Distribution of Lactate/Monocarboxylate Transporter Isoforms in Pancreatic Islets and the Exocrine Pancreas. *Diabetes,* 50**,** 361.

Zhao, J., T. B. M. Hakvoort, A. M. Willemsen, A. Jongejan, M. Sokolovic, E. J. Bradley, V. C. J. de Boer, F. Baas, A. H. C. van Kampen & W. H. Lamers (2016) Effect of Hyperglycemia on Gene Expression during Early Organogenesis in Mice. *PloS one,* 11**,** e0158035-e0158035.

Zhao, Q., S. Li, N. Li, X. Yang, S. Ma, A. Yang, H. Zhang, S. Yang, C. Mao, L. Xu, T. Gao, X. Yang, H. Zhang & Y. Jiang (2017) miR-34a Targets HDAC1-Regulated H3K9 Acetylation on Lipid Accumulation Induced by Homocysteine in Foam Cells. *Journal of Cellular Biochemistry,* 118**,** 4617-4627.

Zhou, G., R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M. F. Hirshman, L. J. Goodyear & D. E. Moller (2001) Role of AMP-activated protein kinase in mechanism of metformin action. *The Journal of clinical investigation,* 108**,** 1167-1174.

Zhou, Y., L. F. Waanders, S. Holmseth, C. Guo, U. V. Berger, Y. Li, A.-C. Lehre, K. P. Lehre & N. C. Danbolt (2014) Proteome analysis and conditional deletion of the EAAT2 glutamate transporter provide evidence against a role of EAAT2 in pancreatic insulin secretion in mice. *The Journal of biological chemistry,* 289**,** 1329-1344.

Zhu, Q., K. Yamagata, A. Miura, N. Shihara, Y. Horikawa, J. Takeda, J. Miyagawa & Y. Matsuzawa (2003) T130I mutation in HNF-4α gene is a loss-of-function mutation in hepatocytes and is associated with late-onset Type 2 diabetes mellitus in Japanese subjects. *Diabetologia,* 46**,** 567-573.

Zhu, X., L. Orci, R. Carroll, C. Norrbom, M. Ravazzola & D. F. Steiner (2002) Severe block in processing of proinsulin to insulin accompanied by elevation of des-64,65 proinsulin intermediates in islets of mice lacking prohormone convertase 1/3. *Proceedings of the National Academy of Sciences,* 99**,** 10299.

Zingman, L. V., A. E. Alekseev, M. Bienengraeber, D. Hodgson, A. B. Karger, P. P. Dzeja & A. Terzic (2001) Signaling in channel/enzyme multimers: ATPase transitions in SUR module gate ATP-sensitive K+ conductance. *Neuron,* 31**,** 233-245.

Zini, E., M. Osto, M. Franchini, F. Guscetti, M. Y. Donath, A. Perren, R. S. Heller, P. Linscheid, M. Bouwman, M. Ackermann, T. A. Lutz & C. E. Reusch (2008) Hyperglycaemia but not hyperlipidaemia causes beta cell dysfunction and beta cell loss in the domestic cat. *Diabetologia,* 52**,** 336.

Zinszner, H., M. Kuroda, X. Wang, N. Batchvarova, R. T. Lightfoot, H. Remotti, J. L. Stevens & D. Ron (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes & Development,* 12**,** 982-995.

Zisoulis, D. G., M. T. Lovci, M. L. Wilbert, K. R. Hutt, T. Y. Liang, A. E. Pasquinelli & G. W. Yeo (2010) Comprehensive discovery of endogenous Argonaute binding sites in Caenorhabditis elegans. *Nature structural & molecular biology,* 17**,** 173-179.

Zofall, M., J. Persinger, S. R. Kassabov & B. Bartholomew (2006) Chromatin remodeling by ISW2 and SWI/SNF requires DNA translocation inside the nucleosome. *Nature Structural & Molecular Biology,* 13**,** 339-346.

Zraika, S., R. L. Hull, J. Udayasankar, K. Aston-Mourney, S. L. Subramanian, R. Kisilevsky, W. A. Szarek & S. E. Kahn (2009) Oxidative stress is induced by islet amyloid formation and time-dependently mediates amyloid-induced beta cell apoptosis. *Diabetologia,* 52**,** 626-635.

Ämmälä, C., F. M. Ashcroft & P. Rorsman (1993) Calcium-independent potentiation of insulin release by cyclic AMP in single β-cells. *Nature,* 363**,** 356-358.

Appendices

Table 2.5: List of genotyping primers used in this thesis. Forward (F) and Reverse (R) primers pairs are shown with according sequences and the used PCR programs.

Name	F Sequence 5'-3'	R Sequence 5'-'3	Source	Program
Tg7a2	CTATAAAGCTAGTGG GGATTCAGTAAC	CATGTGGCAGACTG GGACTTGTTGTTG	Sigma	94°C 5min, 35x (94°C 30sec, 65°C 30sec, 72°C 45sec), 72°C 7min
RIP-Cre	CTATAAAGCTAGTGG GGATTCAGTAAC	CCTGTTTTGCACGTT CACCG	Sigma	94°C 5min, 35x (94°C 30sec, 60°C 30sec, 72°C 45sec), 72°C 7min
tdTomato	GTACAGGAACAGGTG GTGG	CTGTTCCTGGGGCA TGGC	Sigma	94°C 5min, 40x (94°C 60sec, 57°C 60sec, 72°C 60sec), 72°C 7min
Brg1$^{fl/fl}$	GCCTTGTCTCAAACTG ATAAG	GTCATACTTATGTCA TAGCC	Sigma	94°C 5min, 35x (94°C 30sec, 55°C 30sec, 72°C 30sec), 72°C 7min
Ins1-Cre Cre internal	ATGCTTCTGTCCGTTT GCCGT	CCTGTTTTGCACGTT CACCG	Sigma	94°C 5min, 35x (94°C 30sec, 58°C 30sec, 72°C 60sec), 72°C 7min

Table 2.7: List of mouse qPCR primers used for cDNA qPCR in this thesis. Forward (F) and Reverse (R) primer pairs are shown with according sequences.

Name	F Sequence 5'-3'	R Sequence 5'-3'	Source
m36B4	GCCGTGATGCCCAGGGAAGACA	CATCTGCTTGGAGCCCACGTTG	Sigma
mActn4	TTTCAGGAGCGCAGAAGG	CCATCAGGTGCTCATTTTCC	Sigma
mAdam12	GAGCTGGTTATTGTGGCAGAC	GCGATCTCTATTAATCGCTGCT	Sigma
mArid2	AAGTGCTGCTCAGCAAAAGC	GGTGAAGGTGGCATAGGAGT	Sigma
mArx	TTTTCTAGGAGCAGCGGTGT	AGTGGAAAAGAGCCTGCCAA	Sigma
mBAF60A	GGGAGCTCCGGGTAGAAG	TGGCGTCATATTTGGACAAG	Sigma
mBche	TGCTCCCCAATGTCAGTTCT	ATTGCTCCAGCGATGAAATC	Sigma
mBcl2	AGTACCTGAACCGGCATCTG	GGGGCCATATAGTTCCACAAA	Sigma
mBdnf	GCATCTGTTGGGGAGACAAG	TCACCTGGTGGAACATTGTG	Sigma
mBrd3	TTTCCTTTCCCCTTTTCCTC	TTGGCATCCCATTTCTGG	Sigma
mBrg1	CGTGGCCTGCAGTCTTACTA	CATGAGGGCGGACTGCTTAT	Sigma
mBrm	TCAGCCAGCACACACTGCTC	TGTGGGTGTGGACATCTAGG	Sigma
mC-kit	GATCTGCTCTGCGTCCTGTT	CTTGCAGATGGCTGAGACG	Sigma
mCD44	CTCCTTCTTTATCCGGAGCAC	TGGCTTTTTGAGTGCACAGT	Sigma
mCdh8	CTGGACAGAGAACTAAGTGTTTGG	CGAGATATCTGACTGTGGTTCCT	Sigma
mCelsr1	GGCAGTCATGACCTTGGACTA	AGCTGATTCCCAATCTGCAC	Sigma
mCHD5	GCAAGAGGAAGAAGGGTTCC	TGGCATTGTCCAAGTCTGAG	Sigma
mCldn11	TGGAGTGGCCAAGTACAGG	GACAATGGCGCAGAGAGC	Sigma
mCldn3	GTGGCCACTGCAGCTACTT	GTTTCATGGTTTGCCTGTCTC	Sigma
mCldn4	TTTTGTGGTCACCGACTTTG	TGTAGTCCCATAGACGCCATC	Sigma
mClock	CAGCTTCCTTCAGTTCAGCA	CCGTGGAGCAACCTAGATGT	Sigma
mCol1a2	GCAGGTTCACCTACTCTGTCCT	CTTGCCCCATTCATTTGTCT	Sigma
mCol4a1	GGCCCTTCATTAGCAGGTG	TCTGAATGGTCTGACTGTGTACC	Sigma
mCol5a2	GAAAGGCTGGTGATCAAGGT	TTTCTCCCCGAGGTCCTAAT	Sigma
mCyfip2	GTGGGTACAGCGCAGAGC	GGCGCCTGTCTTTCACAC	Sigma
mCyr61	GGATCTGTGAAGTGCGTCCT	CTGCATTTCTTGCCCTTTTT	Sigma
mDapk2	GACGTGGTGCTCATCCTTG	GGCTTCCTCCTCACTTAACGA	Sigma
mDnmt3a	AAACGGAAACGGGATGAGT	ACTGCAATTACCTTGGCTTTCT	Sigma
mE-cad	ATCCTCGCCCTGCTGATT	ACCACCGTTCTCCTCCGTA	Sigma
mEhf	TGTGATAGCTTCCTGCCTTCT	CACCACTTCCTTCCAGAATCA	Sigma
mErbb4	AATGCTGATGGTGGCAAGA	CATCACTTTGATGTGTGAATTTCC	Sigma
mFas	AAACCAGACTTCTACTGCGATTCT	GGGTTCCATGTTCACACGA	Sigma
mFlnb	CACCGGCCTGCATAAAGT	CATTCACTTCAAATGGGCTCT	Sigma
mFoxa1	GAACAGCTACTACGCGGACA	CGGAGTTCATGTTGCTGACA	Sigma
mFoxo1	GAGAAGAGGCTCACCCTGTC	ACAGATTGTGGCGAATTGAA	Sigma
mFn1	CGGAGAGAGTGCCCCTACTA	CGATATTGGTGAATCGCAGA	Sigma

mGabrg2	ACAGAAAATGACGCTGTGGA	CATCTGACTTTTGGCTTGTGAA	Sigma
mGcg	ACCTGGACTCCCGCCGTGC	TCGCCTTCCTCGGCCTTTCACCAGCC	Sigma
mGck	CTGGATGACAGAGCCAGGAT	GCTGGAACTCTGCCAGGAT	Sigma
mGjd2	GGGGGAATGGACCATCTT	TCACCACCACAGTCAACAGG	Sigma
mGlut2	TCTTCACGGCTGTCTCTGTG	AATCATCCCGGTTAGGAACA	Sigma
mHadh	TTCTTAAAGACACCACAGTGACG	CTTCTTCACTTTGTCGTTCAGC	Sigma
mHnf1a	TTACACCAAGTATCCCCCACA	CAAGCTGTGCAGTGCTGTC	Sigma
mIL6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA	Sigma
mIL6R	ATCCTCTGGAACCCCACAC	GAACTTTCGTACTGATCCTCGTG	Sigma
mIns1	CAGAGAGGAGGTACTTTGGACTATAAA	GCCATGTTGAAACAATGACCT	Sigma
mIns2	GCTGGTGCAGCACTGATCTA	GCTGGTGCAGCACTGATCTA	Sigma
mJHDM1D	GCAGCTCTACACGGCTCTTC	TCTGTGTAGTCATGCCTGTGC	Sigma
mlepr	GTTCCAAACCCCAAGAATTG	GACTTCAAAGAGTGTCCGTTCTC	Sigma
mlif	AAACGGCCTGCATCTAAGG	AGCAGCAGTAAGGGCACAAT	Sigma
mMafA	AGGAGGAGGTCATCCGACTG	CTTCTCGCTCTCCAGAATGTG	Sigma
mMafB	AGGACCTGTACTGGATGGC	CACTACGGAAGCCGTCGAAG	Sigma
mMapk14	GACCTTCTCATAGATGAGTGGAAGA	CAGGACTCCATTTCTTCTTGGT	Sigma
mMarveld3	GGATTGCCACAAATGCAGAT	AGGCCAAGATCAGCAAGTTC	Sigma
mMeCP2	CAGCTCCAACAGGATTCCAT	TCTTCTGACTTTTCCTCCCTGA	Sigma
mMef2C	ATGAAGTGAAGCGTGGAAGG	CAAATCCCTGCATTCGTTC	Sigma
mMmp14	GAGAACTTCGTGTTGCCTGA	CTTTGTGGGTGACCCTGACT	Sigma
mMpzl1	TCTAGTAAAGAGTCCGCCTTCC	TCCGCCAGAGTGGTCTAACT	Sigma
mMycn	CCTCCGGAGAGGATACCTTG	TCTCTACGGTGACCACATCG	Sigma
mN-cad	GCCATCATCGCTATCCTTCT	CCGTTTCATCCATACCACAAA	Sigma
mNestin	TCCCTTAGTCTGGAAGTGGCTA	GGTGTCTGCAAGCGAGAGTT	Sigma
mNeurod1	GACCCAGAAACTGTCTAAAATAGAGACA	AAGGAGACCAGATCAGGGCTTT	Sigma
mNf2	AGCATAATACCATTAAAAAGCCTCA	TCTTCAAAGAAGGCCACTCG	Sigma
mNgn3	TCTCAAGCATCTCGCCTCTTC	ACAGCAAGGGTACCGATGAGA	Sigma
mNkx2.2	TGTCCCAGAGTCACGGAGT	AGGGCTTAAGATGCCTGGAT	Sigma
mNkx6.1	TCAGTCAAGGTCTGGTTCC	CGATTTGTGCTTTTTCAGCA	Sigma
mOat	CAATTACCATCCTTTGCCTGTA	GTACTGCCTGCCTTCCACAT	Sigma
mOcln	GTCCGTGAGGCCTTTTGA	GGTGCATAATGATTGGGTTTG	Sigma
mOvol2	CGATTTAAGGCATAGGTGTCG	GGTACTCTCCCCCTCACTCC	Sigma
mPard6G	TCGGCTATGCTGATGTGC	GCTGTAATGGTCTGCTTCTTCTC	Sigma
mPax6	ACTTCAGTACCAGGGCAACC	GAACTGATGGAGTTGGTGTTCTC	Sigma
mPdgfra	CGGAGCCTGAGCTTTGAG	GCCCTGTGAGGAGACAGC	Sigma
mPdgfrb	TCAAGCTGCAGGTCAATGTC	CCATTGGCAGGGTGACTC	Sigma
mPdlim4	TCCACATTGACCCTGAGTCC	CCTCCAGACTAATCCCAGAGAC	Sigma
mPdx-1	TTCCCGAATGGAACCGAGC	GTAGGCAGTACGGGTCCTCT	Sigma

mPhf17	CAGCTGTTCACGCACCTG	GGGTCACCATGTAAGTGAGGTT	Sigma
mPhf21a	GCCTAGTCTAACTGCATCACAGG	TTTGGGCGTGGAGTGAGT	Sigma
mResp18	TGCTTCAGTCTTCCAGTACTTACAA	GCTATGTCATCTGCCCAGAAC	Sigma
mRest	AGCAACAAAGAAAAGGAGTTGG	ACCTGGGTGGCCATAACTG	Sigma
mRGS4	TCCCTCAGTTAAACAAGATGTGC	GTTTCATGTCCTTTGCACTCC	Sigma
mRnf20	AGCTGATCAGGTTTTGACACTG	TGCTCCTTCTCTTCCAGTTTTCT	Sigma
mRobo1	AGGGAAGCCTACGCAGATG	TGGACAGTGGGCGATTTTAT	Sigma
mSerpine1	CGTGGCAGCAGGACTGATA	AGGCCTCTGGGTCATCTACA	Sigma
mSerpine2	TTGGGTCAAAAATGAGACCAG	CCTTGAAATACACTGCATTAACGA	Sigma
mSetd8	CGGAGATGGTGGAGCAGA	GCAGAGCACTTGTTCGGACT	Sigma
mSipa1|3	TGCTCCCTTACACTCCCAAC	ACGATGTCATTCCCTATGTGC	Sigma
mSlit2	CGGGGACAGCTGTGATAGAG	CCAAGCGAGATACTTTCTTAGTTGTT	Sigma
mSnail1	CTTGTGTCTGCACGACCTGT	CAGGAGAATGGCTTCTCACC	Sigma
mSnail2	CATTGCCTTGTGTCTGCAAG	AGAAAGGCTTTTCCCCAGTG	Sigma
mSox9	GAGCCGGATCTGAAGAGGGA	GCTTGACGTGTGGCTTGTTC	Sigma
mSyt4	GACAGAGCACGCAGAAAACA	AGTGAAGACGAGGCCAAAAG	Sigma
mSyt7	GAGGTGTCCATCCCTCTGAA	GCAGAGGGGTTGTAGCAGAG	Sigma
mSST	CCCAGACTCCGTCAGTTTCT	GGGCATCATTCTCTGTCTGG	Sigma
mTada2b	GACGCCATCGAGCAGTTC	GTCCGTGAAGCACCAACAT	Sigma
mTagln	GCCCAGACACCGAAGCTA	GTAGGATGGACCCTTGTTGG	Sigma
mTGFBR3	TGGCTGTGGTACTAGACATAGGAG	GGAGCCTGCACCACAATAG	Sigma
mThbs1	CACCTCTCCGGGTTACTGAG	GCAACAGGAACAGGACACCTA	Sigma
mThy1	GAAAACTGCGGGCTTCAG	CCAAGAGTTCCGACTTGGAT	Sigma
mTjp1	TGCAGACCCAGCAAAGGT	GGTTTTGTCTCATCATTTCTTCAG	Sigma
mTjp2	CCGTTCAGCAGCTTAGGAAA	GAACCTTCCGGGGTCTCTT	Sigma
mTmod1			Sigma
mUcn3	CCAGAGCAAAGTCCACTTACAG	GCTTGTCCTTGGACCTCCT	Sigma
mVcam	TCTTACCTGTGCGCTGTGAC	ACTGGATCTTCAGGGAATGAGT	Sigma
mVim	TGCGCCAGCAGTATGAAA	GCCTCAGAGAGGTCAGCAAA	Sigma
mZbtb4	CACCAAGCACGAGGTGTG	TGGGTCTTCAGGTTATAATAGGTGA	Sigma
mZbtb38	AGGCTGGCGTGTTCTGAG	CACTGTGAAAGTCGTCCTTGAG	Sigma
mZeb2	CCAGAGGAAACAAGGATTTCAG	AGGCCTGACATGTAGTCTTGTG	Sigma

Table 2.8: List of rat qPCR primers used for cDNA qPCR in this thesis. Forward (F) and Reverse (R) primer pairs are shown with according sequences.

Name	F Sequence 5'-3'	R Sequence 5'-'3	Source
r36B4	AGCTTTGGGCATCACCACTA	CTCCCACCTTGTCTCCAGTC	Sigma
rArid2	CAGGCTATAGAGCAGGTCCAA	CGTGCTGTGCTACAACTGCT	Sigma

rBAF60A	CCAGTCAGGGATGGACCAAT	TTTCGATTTTGGACCGCCTG	Sigma
rBrg1	AAGATGTTCGGCCGTGGTT	CTCGATAGCCTTGAGCCACT	Sigma
rBrm	AGGAGCAGGATGAGAACGAA	GAAGAAAGCATTCGGTTTGG	Sigma
rBrd3	CAGACCACGAAGTGGTAGCC	TTGGCAAACCTCATCTCAAA	Sigma
rCHD5	CTGTTCGCGGAGGAGATG	TCAAGGCCACCGTCTTCTT	Sigma
rDnmt3a	AGAAGTGCCGAAACATCGAG	CATTCCACCAATGAAGAGTGG	Sigma
rGcg	TGAGATGAACACGATTCTCGAT	AAGATGGTTGTGAATGGTGAAA	Sigma
rGlut2	AAAGCCCCAGATACCTTTACCT	TGCCCCTTAGTCTTTTCAAGC	Sigma
rHNRNPK	TGAAGAAAATCATCCCTACTTTGG	TCCACAGCATCAGATTCGAG	Sigma
rIns1	CTCCCACCTTGTCTCCAGTC	CTTGGGCTCCCAGAGGAC	Sigma
rIns2	CGAAGTGGAGGACCCACA	TGCTGGTGCAGCACTGAT	Sigma
rJHDM1D	CATGGAGAATGAACAAAGTTCG	TTGAGGCTGGAAACCATCTT	Sigma
rMafA	GACTTGCACAAGGGTCAAAGA	CCGGGTTCAAAGGTGAGTTA	Sigma
rMeCP2	TGGTAGCTGGGATGTTAGGG	AACTTCAGGGGTTTCTCTTTGAG	Sigma
rNkx6.1	GAGAAGACTTTCGAACAAACGAA	CGATTCTGGAACCAGACCTT	Sigma
rPax6	GCCCTCACCAACACGTACA	AGGTCTGACTGGGGACTGG	Sigma
rPdx-1	CTCTCGTGCCATGTGAACC	TTCTCTAAATTGGTCCCAGGAA	Sigma
rPhf17	ATTTGGGGCCTCCATACAG	ACGGTCAAAAGCACAAGTCA	Sigma
rPhf21a	GGAGCCCACTACCTCATAAGC	TCTGCTAATTCTACAATGTCTTCAGC	Sigma
rRest	TGTTCAACACGTTCGAACTCA	AGAACTTGAGTAAGGACACAGTTCAC	Sigma
rRnf20	AAAGAGAACAGCGGCTCAAC	CACTTTATAGCCTTTTGAGTTCACC	Sigma
rSetd8	TTTCTACCCCGTGCGAAG	CATCTATCTTCTTCCTTTCTTCAGACT	Sigma
rSST	AGCCCAACCAGACAGAGAAC	CCTCATCTCGTCCTGCTCA	Sigma
rTada2b	GACGCCATCGAGCAGTTC	AGTCCGAGAAGCACCAACAT	Sigma
rUcn3	TACAGGGAGCGATGCTGAT	GAACTTGTGGGAGAGGCTTG	Sigma
rZbtb38	AAAAGAGGGAGAAAACCCAAGT	GTCACTCTCCCGAGGCAAT	Sigma

Table 2.9: List of human qPCR primers used for cDNA qPCR in this thesis. Forward (F) and Reverse (R) primer pairs are shown with according sequences.

Name	F Sequence 5'-3'	R Sequence 5'-'3	Source
h36B4	TCTACAACCCTGAAGTGCTTGAT	CAATCTGCAGACAGACACTGG	Sigma
hArx	GCACCACGTTCACCAGCTA	CAGCCTCATGGCCAGTTC	Sigma
hBAF60A	TGGGAGCTTCGGGTAGAAG	TGGCATCATATTTGGACAAGG	Sigma
hBrg1	TGGACCAGCACTCCCAAG	CTGGCTGGAACTGGACTAGAG	Sigma
hBrm	GATTCAGCCAGCACACTCCT	GGCGTGGACATCTACCTCTC	Sigma
hGcg	GTACAAGGCAGCTGGCAAC	TGGGAAGCTGAGAATGATCTG	Sigma
hGlut2	GATGGGGTTTTCAAAATTGG	AACCAGGCCTGAAATTAGCC	Sigma

hIns	AGGCTTCTTCTACACACCCAAG	CACAATGCCACGCTTCTG	Sigma
hMafA	AGAGCGAGAAGTGCCAACTC	TTGTACAGGTCCCGCTCTTT	Sigma
hNkx2.2	CGAGGGCCTTCAGTACTCC	GGGGACTTGGAGCTTGAGT	Sigma
hNkx6.1	CGTTGGGGATGACAGAGAGT	CGAGTCCTGCTTCTTCTTGG	Sigma
hPdx-1	AAGCTCACGCGTGGAAAG	GCCGTGAGATGTACTTGTTGAA	Sigma
hPPY	TGGCTGTGAACTGGAATGTG	CCCAGCAGTGGCTGTAGTAAC	Sigma
hSST	ACCCCAGACTCCGTCAGTTT	ACAGCAGCTCTGCCAAGAAG	Sigma
hUcn3	ACAAGTTCATGGGGACGTG	CGGCATCAGCATCTCTCC	Sigma