EXAMINING THE ROLES OF MAFB IN THE PANCREATIC ISLET

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To my mother and father, for their bold spirits and enduring love.

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Chapter I

INTRODUCTION

My thesis enhances our understanding of transcriptional control of pancreatic islet β- and α-cell identity and function. I focus on two transcription factors, v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog A, (MafA) and MafB. The Introduction will begin with a broad overview of the pancreas, briefly presenting the exocrine pancreas in relation to the endocrine pancreas, and then will hone in on the composition and function of the endocrine pancreas. The pathologies of different diabetic classifications and therapeutic options will be described. Then, pancreas development will be reviewed with a focus on key transcription factors that are crucial to islet cell formation and β-cell establishment and utilized in this thesis work. Finally, the Maf transcription factors will be introduced. Furthermore, I will address the necessity of identifying differences between humans and model systems used for pancreatic islet studies, including cell lines, rodents, nonhuman primates, and *ex vivo* human samples.

Pancreas Overview

Composition and Physiology

The pancreas is situated behind the stomach and is connected to the duodenum via the Ampulla of Vater, where the pancreatic duct also joins the common bile duct (Slack, 1995) (Figure 1). The pancreas is comprised of two major types of glandular tissue and fulfills two major functions. The endocrine tissue regulates glucose homeostasis and the exocrine tissue aids in food digestion. The exocrine component, including acinar and ductal cells, comprises 95% of the pancreatic mass. By contrast, the endocrine component only makes up about 1-2% of the pancreas, forming clusters

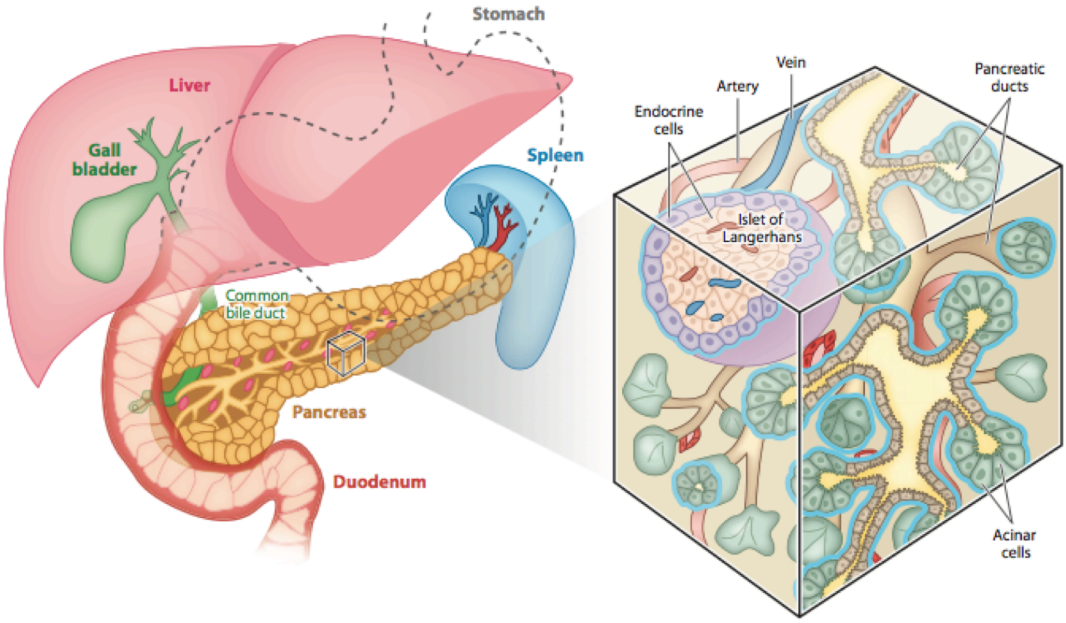

Figure 1. Adult pancreas anatomy and composition. The pancreas sits within the duodenum beneath the liver. It is composed of ductal, acinar, and endocrine cells. From Shi, Wang and Sander 2013.

of cells or islets of Langerhans throughout the pancreas (Murtaugh and Melton, 2003). Notably, the exocrine and endocrine pancreas are interconnected, with blood flow from the islets entering the capillaries of the surrounding exocrine tissue before it enters the general circulation (Ballian and Brunicardi, 2007). Although the impact of this high concentration of endocrine hormones on the exocrine tissue is not entirely understood, the acinar cells express insulin receptors that are involved in exocrine digestive enzyme synthesis (Korc et al., 1981; 1978; Sankaran et al., 1981). More detail regarding exocrine structure and function will be provided before I focus on the endocrine tissue.

Exocrine Cells

The exocrine cells are grouped into acini at the end of ducts. The secretory cells of the acini are pyramidal in shape and consist of basal nuclei, rough endoplasmic reticulum in regular arrays for protein synthesis, a prominent Golgi complex, and secretory (zymogen) granules that store digestive enzymes (Pandol, 2010; Slack, 1995) (Figure 2). Small exocrine ducts are distributed throughout the lobules of the pancreas and unite to form the large pancreatic duct. The smaller exocrine ducts are lined with columnar epithelial cells with the large duct containing some goblet and brush cells, much like those found in the intestine (Slack, 1995). The duct cell epithelium in general is comprised of cuboidal and pyramidal cells that are rich in mitochondria that provide energy needed for ion transport (Pandol, 2010). At the junction of acini and ducts are the centroacinar cells. These cells exhibit ductal characteristics, but they are also potentially progenitors for different mature pancreatic cell types (Pandol, 2010). The structure of the exocrine pancreas facilitates its secretory function.

Exocrine cells secrete a “pancreatic juice” that is comprised of a variety of digestive enzymes. The pancreatic juice is first secreted into the acinar lumen where it

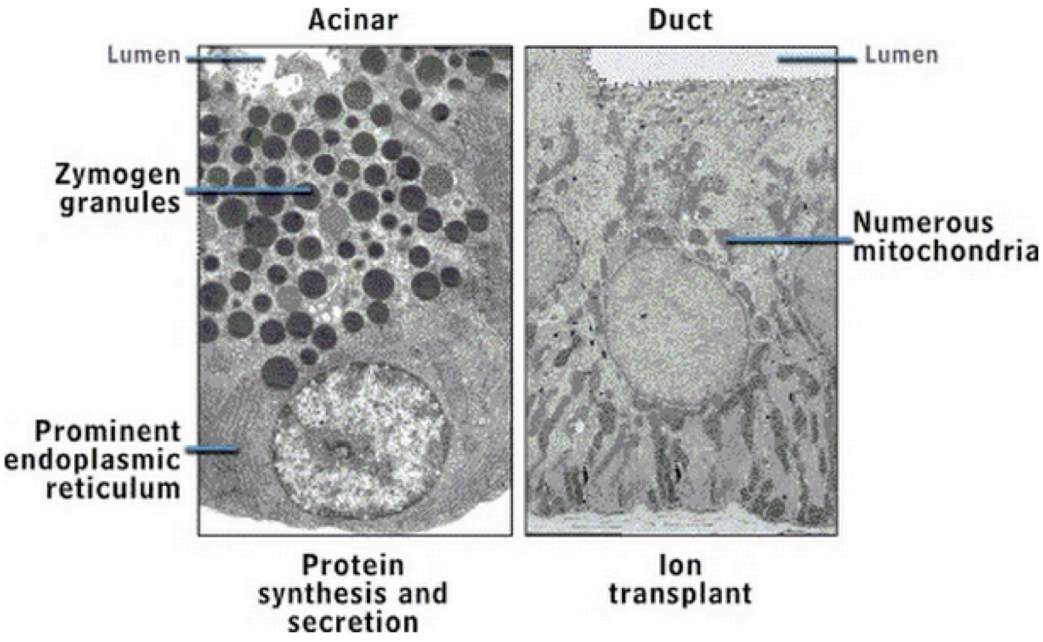

Figure 2. Acinar and ductal cell ultrastructure of the exocrine pancreas. From Pandol et al., 2010.

drains into the ductal network that eventually connects to the common bile duct (Murtaugh and Melton, 2003). These enzymes are then shunted into the gut to assist in digestion and nutrient absorption (Murtaugh and Melton, 2003; Slack, 1995). Secretion of pancreatic juice from acinar cells is regulated by hormones (e.g. by secretin, cholecystokinin, and gastrin), including some regulation by endocrine hormones and neural stimuli (Pandol, 2010; Slack, 1995). Receptors for hormones and neurotransmitters that stimulate enzyme secretion are located on the basolateral membrane of the acinar cells (Williams, 2001). Although the pancreatic exocrine cells are not directly studied in this thesis work, this brief overview facilitates the understanding of their interrelationship and proximity to the cells of interest, the endocrine pancreatic cells.

Endocrine Islet Cell Identity and Function

The 5 cell types of the endocrine pancreas are clustered into islets of Langerhans throughout the pancreatic tissue (Figure 3). This includes insulin-secreting β-cells, glucagon-secreting α-cells, somatostatin-secreting δ-cells, pancreatic polypeptide-secreting cells, and ghrelin-secreting ε-cells. Pancreatic islet β- and α-cells secrete the two main hormones that maintain glucose homeostasis, insulin and glucagon, respectively.

Endocrine hormone secretion is highly regulated. Insulin is secreted from β-cells in response to an increase in circulating glucose levels. Insulin promotes glucose uptake in peripheral tissue (e.g. liver, adipose, and skeletal muscle). Additionally, insulin acts on the liver to inhibit glucose production, known as gluconeogenesis, and to promote glucose storage in the form of glycogen (Figure 4) (Berne et al., 2008). Insulin also inhibits lipolysis, or the breakdown of fatty acids. Along with glucose, elevated free fatty acid levels, amino acids, potassium, and neurotransmitters act as secretagogues

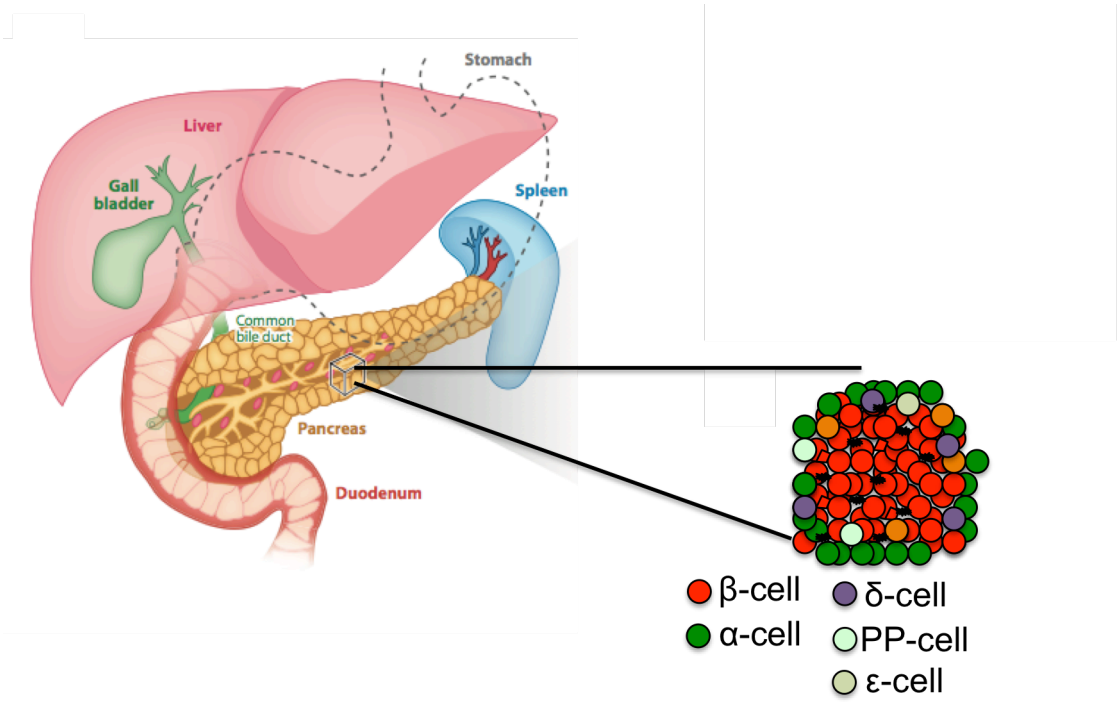

Figure 3. Pancreatic islet of Langerhans. The islets make up 1-2% of the entire pancreas, forming small cell clusters throughout the pancreas. These cell clusters are comprised of insulin-secreting β-cells, glucagon-secreting α-cells, somatostatin-secreting δ-cells, pancreatic polypeptide-secreting cells, and the few ghrelin-secreting ε-cells. Modified from Shi, Wang and Sander 2013

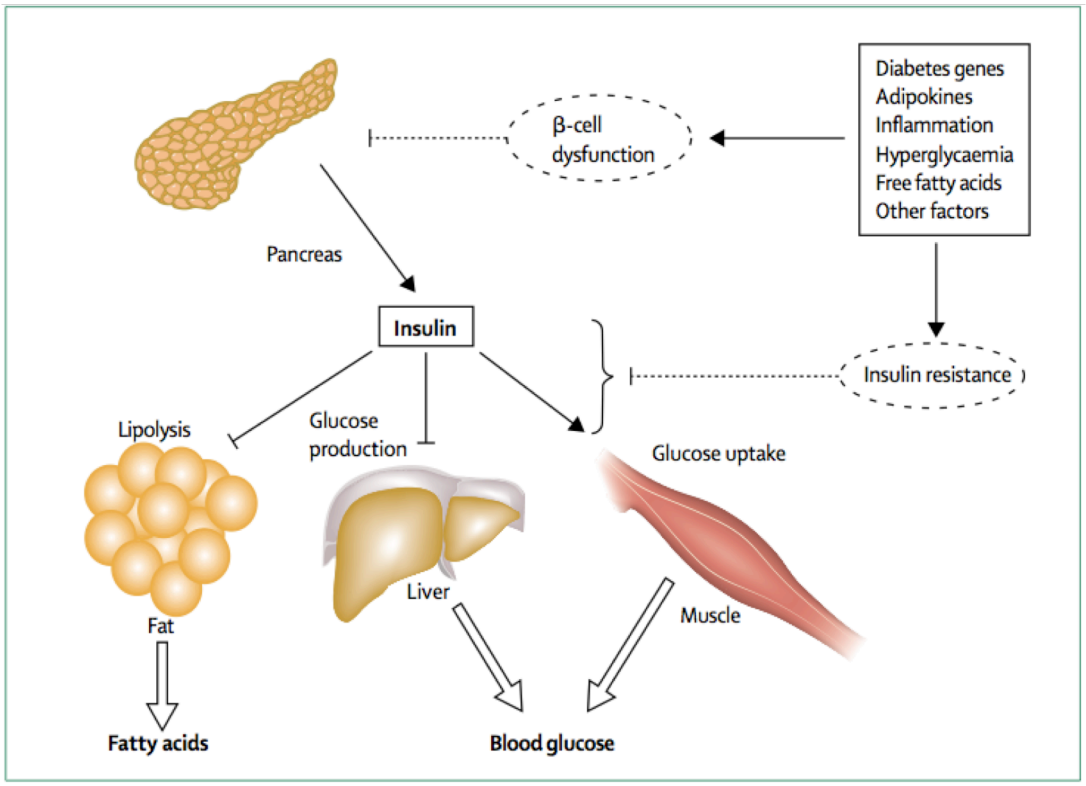

Figure 4. Schematic of insulin action and factors contributing to dysfunction. Insulin secreted from the pancreas promotes glucose uptake in peripheral tissue such as muscle or adipose tissue, inhibits glucose production by the liver and inhibits lipolysis to lower circulating blood glucose levels and free fatty acids. β-cell dysfunction leads to insufficient insulin secreted, whereas insulin resistance results in the inability of insulin to effectively act on peripheral tissue. From Stumvoll et al., 2005.

that promote insulin release (Berne et al., 2008). The counterregulatory hormone, glucagon, is secreted from α-cells in response to low blood glucose concentrations that can result from fasting or exercise. Accordingly, glucagon promotes glycogenolysis (breakdown of glycogen into glucose) and gluconeogenesis to release glucose into the bloodstream. Both glycogenolysis and gluconeogenesis primarily take place in the liver. Notably, insulin inhibits islet α-cell glucagon secretion (Figure 5) (Samols et al., 1966). In the absence of insulin, amino acids, such as arginine and alanine, can act as glucagon secretagogues. Although less studied, other islet hormones also play a role in glucose homeostasis. Somatostatin, secreted by δ-cells, inhibits insulin and glucagon secretion and also impedes nutrient absorption throughout the gastrointestinal tract (Corleto, 2010; Lins et al., 1980; Patel, 1999; Strowski et al., 2000). Similarly, ghrelin from ε-cells can inhibit insulin secretion, demonstrating the complicated regulation of these hormones (Broglio et al., 2001; Sun et al., 2007; Tong et al., 2010).

In particular, insulin secretion from the β-cell has been thoroughly examined. Different proteins with key functional roles in the β-cell insulin secretion mechanism will be examined in this thesis work (Figure 6). Specifically, glucose enters through the β-cell glucose transporter (high K_m GLUT-2 in rodents, low K_m GLUT-1 in humans) and phosphorylated by glucokinase, the rate-limiting enzyme of glucose metabolism (De Vos et al., 1995; Heimberg et al., 1995; Sweet and Matschinsky, 1995). Glucose metabolism in the β-cell mitochondria results in a rise in adenosine triphosphate (ATP) levels and a fall in adenosine diphosphate (ADP) levels (Schuit et al., 1997). This shift in adenosine nucleotide concentrations causes ATP-dependent potassium (K_{ATP}) channel closure. This channel is made up of 2 proteins encoded by the *SUR1* and *Kir6.2* genes. K_{ATP} channel closure leads to β-cell depolarization and activation of voltage-gated L-type Ca^{2+} channels. The Ca^{2+} influx then stimulates insulin granule exocytosis (Rorsman and Braun, 2013). Although glucose and ATP generation are the main stimulators of insulin

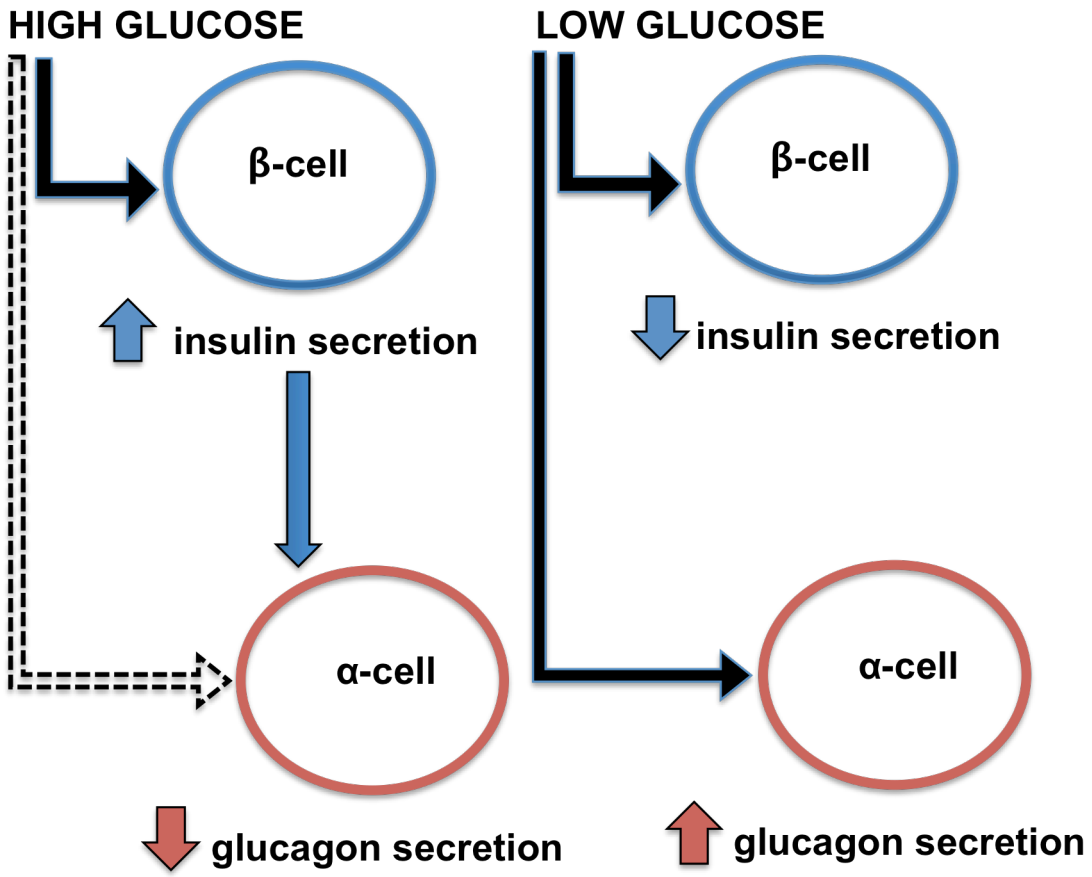

Figure 5. β-cell and α-cell hormone secretion in response to high and low glucose. Overview of β-cell insulin secretion in response to high glucose β-cell insulin secretion is stimulated. Both high glucose and insulin are reported to impact and repress glucagon secretion from α-cells. Low glucose results in inhibited insulin secretion from β-cells allows for α-cell glucagon secretion to promote liver glucose output.

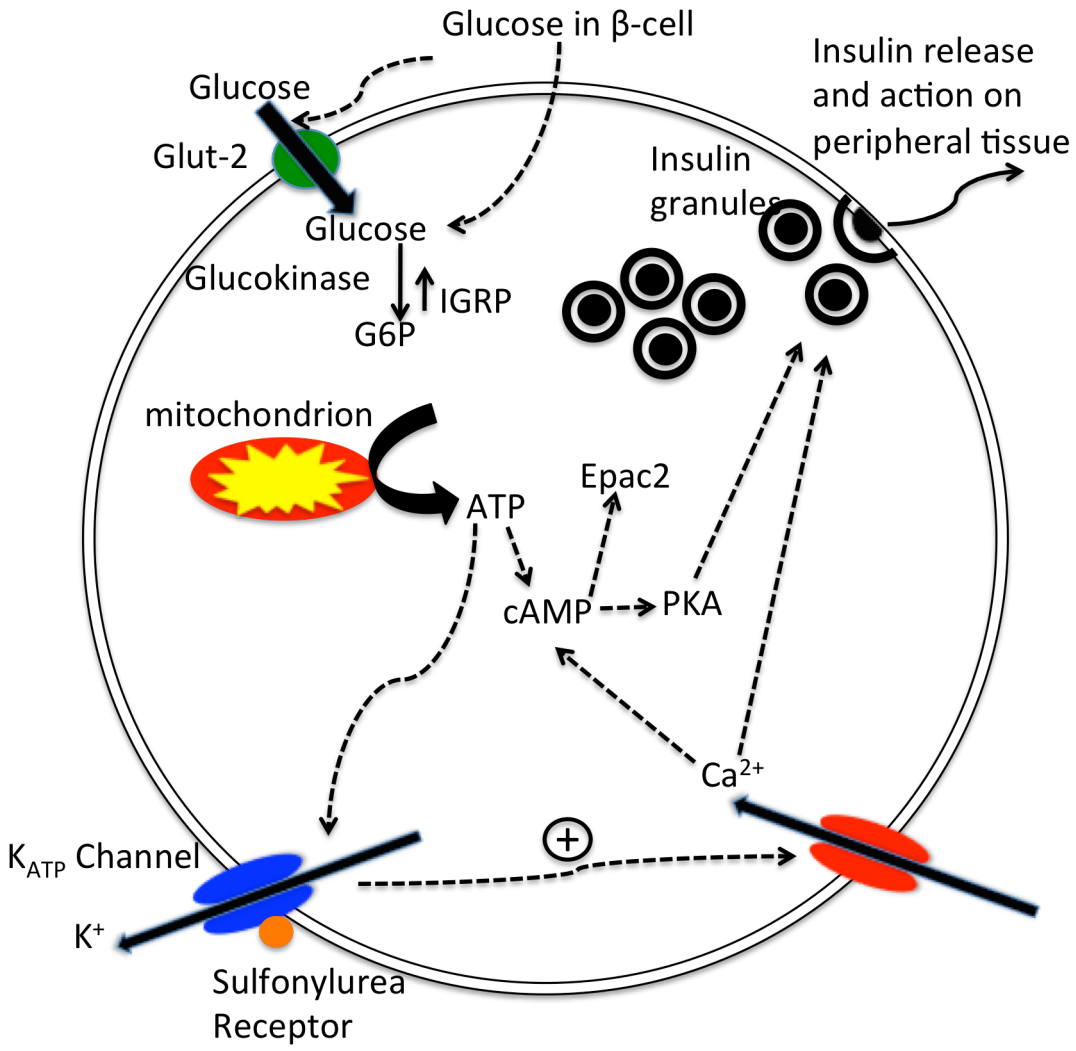

Figure 6. β-cell physiology. Mechanism of glucose-stimulated insulin secretion in the β-cell from glucose uptake from the bloodstream, glucose metabolism in the mitochondria, K_{ATP} channel closure, cell depolarization, Ca^{2+} influx into the intercellular space to promote insulin vesicle mobilization and release.

secretion, it is further amplified by a rise in cyclic adenosine monophosphate (cAMP), due to both an increase in adenylyl cyclase-mediated cAMP production and a decrease in phosphodiesterase (PDE)-mediated cAMP degradation. This regulation of cAMP is mediated by Ca^{2+} and other processes involved in cell metabolism. cAMP then mediates its impact on insulin secretion through Protein kinase A (PKA) and an exchange protein directly activated by cAMP (Epac2) (Tengholm, 2012). Taken together, these different proteins have been studied for their integral role in the β-cell insulin secretion mechanism.

Regulation of α-cell glucagon secretion is also important, since normal glucose homeostasis is largely dependent on the balanced secretion of both insulin and glucagon. Many of the same proteins that are expressed in the β-cell are also produced in α-cells and mediate glucagon secretion. For example, α-cells take up glucose through a GLUT transporter to metabolize it and the K_{ATP} channel closes in response to the shift in the ATP to ADP ratio. In contrast to β-cells, α-cells mostly express the lower K_m glucose transporter, GLUT-1, and have similar glucokinase activity to the β-cell but additionally express a lower K_m hexokinase isoform that becomes saturated at a lower glucose concentration (Gylfe and Gilon, 2014; Heimberg et al., 1996). This more sensitive isoform is likely involved in sensing the low glucose concentrations that trigger glucagon secretion. Much like in β-cells, a rise in $[Ca^{2+}]$ is believed to trigger α-cell glucagon exocytosis; but unlike β-cells, α-cells are electrically active in the absence of glucose. The presence of T-type Ca^{2+} channels in α-cells, which activate at low threshold potentials, also allow action potentials to start at more negative potentials than in β-cells (Barg et al., 2000).

The mechanism by which α-cell glucagon secretion is inhibited in high glucose has been a longstanding question in pancreatic islet studies (Gromada et al., 2007). Notably, glucagon secretion at high glucose concentrations is not inhibited in isolated α-

cells. This suggests that paracrine effects, like insulin and somatostatin secreted from nearby β- and δ-cells, primarily mediate islet glucagon inhibition. Furthermore, α-cell Ca^{2+} activity was unaffected in experiments with candidate paracrine inhibitors that led to decreased glucagon secretion, indicating that paracrine inhibition in intact islets decouples glucagon secretion from Ca^{2+} influx (Le Marchand and Piston, 2010). A recent study by Elliot et al. indicates glucagon secretion in high glucose is primarily inhibited by insulin and somatostatin, which mediate this inhibition by promoting degradation or inhibiting production of cyclic adenosine monophosphate (cAMP), respectively (Elliott et al., 2015).

Diabetes Mellitus

Glucose intolerance is defined as the inability to sufficiently clear glucose from the bloodstream. A lack of islet β-cells or dysfunctional β- or α-cells lead to glucose intolerance, elevated blood glucose (hyperglycemia), and diabetes mellitus. Maintaining normal glucose homeostasis is also dependent on the ability of peripheral tissue to sense and take up glucose in response to insulin; this measure is known as peripheral insulin sensitivity (Figure 4). When insulin is secreted normally but fails to promote normal glucose uptake, this is called insulin resistance. Defects that result in insulin deficiency, including loss of β-cells or dysfunctional β-cells, can also lead to diabetes mellitus.

Although the β-cell has historically been the focus of diabetes research, this disease is actually deemed a bihormonal defect with α-cell dysfunction also believed to play a prominent role in the diabetic state (Unger and Orci, 1977; Unger and Cherrington, 2012). Insulin inhibits α-cell glucagon secretion such that lack of insulin is then accompanied by glucagon excess. This hyperglucagonemia results in glucose release

from the liver that contributes to the hyperglycemia that characterizes diabetes (Unger and Cherrington, 2012).

Diabetes affects over 382 million people worldwide, thus much research is dedicated to understanding more about this epidemic and the cells that are impacted (International Diabetes Federation website, http://www.idf.org/worlddiabetesday/toolkit/gp/facts-figures). Transcription factors are central to many of these studies as these factors serve as critical regulators of islet cell development and function (Oliver-Krasinski and Stoffers, 2008). My thesis work contributes to diabetes research endeavors with new information on two transcription factors, MafA and MafB, which impact the identity and function of the pancreatic α- and β-cells. These are the cells that are affected in all forms of diabetes.

Diabetes mellitus is most generally defined as a metabolic disorder characterized by insufficient insulin action and hyperglucagonemia that results in chronic hyperglycemia. This occurs with the inability of β-cells to produce sufficient amounts of insulin, frequently coupled with insulin resistance. This defect is often accompanied by an increase in α-cells and/or dysregulated glucagon secretion. Diabetes is further classified by etiology, as type 1, type 2, gestational, and other specific types of diabetes (IDF website, http://www.idf.org/about-diabetes).

T1DM

Type 1 Diabetes (T1DM, insulin-dependent, childhood-onset, or juvenile diabetes) results from near complete autoimmune destruction of an individual's β-cell population and accounts for 5% of all diabetes cases. Also playing into the resulting hyperglycemia, plasma glucagon levels are elevated substantially in cases of uncontrolled T1DM (Müller et al., 1973). This form of diabetes occurs due to abnormal activation of the individual's T-cell-mediated immune system. This causes an

inflammatory response in islets and a humoral response that produces antibodies against β-cell antigens launching an attack on the β-cells (Daneman, 2006).

The β-cell destruction in T1DM is set off by environmental triggers that alter immune function. These triggers include viruses (e.g. enteroviruses, coxsackie, congenital rubella), bacteria, environmental toxins, or even foods early in life (e.g. cow's milk proteins, wheat proteins, vitamin D) (Daneman, 2006; van Belle et al., 2011). T1DM susceptibility is largely inherited, with the *DA* and *DQ* variants of the human leukocyte antigen (*HLA*) locus conferring about 50% of the genetic susceptibility (Daneman, 2006). In addition to insulin (*INS*), other important genes are associated with T1DM susceptibility, including cytotoxic T lymphocyte antigen 4 (*CTLA-4*) (Anjos et al., 2004), interleukin (IL)-2 receptor-α (*IL2Rα*) (Lowe et al., 2007), and lymphoid tyrosine phosphatase (*PtPN22*) (Zheng and She, 2005).

Transcription factors have also been linked to the genetic susceptibility of T1DM. For example, single nucleotide polymorphisms (SNPs) in the noncoding region of the *Insulin gene enhancer protein 1* (*ISL1)* transcription factor have been associated with T1DM in Swedish families (Holm et al., 2004). Notably, MAFB expression appears to be selectively lost in T1DM human islets, indicating a likely crucial role in human islet cells (Marcela Brissova, personal communication). Oxidative stress also appears to contribute to the β-cell dysfunction observed in T1DM with antioxidant treatment able to prevent T1DM onset in animal models of T1DM (Bottino et al., 2002; Gurgul et al., 2004; Tanaka et al., 1999). Posttranslational loss of MafA and Pancreatic and duodenal box 1 (Pdx1) by oxidative stress was also attributed to loss of *Insulin* gene expression in β-cells, whereas antioxidant treatment preserved MafA in the nucleus (Harmon et al., 2005; Robertson, 2004). These studies provide evidence for the integral role of transcription factors in loss of β-cell identity in T1DM.

T2DM and Gestational Diabetes

Type 2 diabetes (T2DM, non-insulin dependent, or adult-onset diabetes) results from β-cell dysfunction and a more modest decrease in β-cell mass than T1DM. This form of diabetes accounts for about 95% of all diabetes cases. Moreover, T2DM is most commonly associated with insulin resistance. Clinically, T2DM presents with an initial increase in β-cell mass and insulin secretion before the individual's β-cells can no longer meet the increasing demands of the insulin resistance. In T2DM, glucose intolerance is caused by β-cell dysfunction and β-cell death, and in most cases includes α-cell hyperplasia and abnormal glucagon secretion (Butler et al., 2003a; Dunning and Gerich, 2007; Unger and Cherrington, 2012; Weir et al., 2001). Obesity, aging, and genetics are known to predispose an individual to T2DM (Kahn et al., 2014). Similar to T1DM, oxidative stress also appears to be associated with the β-cell dysfunction and death in this form of diabetes, with elevated levels of oxidative stress markers found in human T2DM islets (Del Guerra et al., 2005; Lupi et al., 2007). Loss of transcription factor expression is similarly associated with the pathophysiology of T2DM (Harmon et al., 2009; Shirakawa and Terauchi, 2014). Moreover, PDX1, NK6 HOMEOBOX 1 (NKX6.1), MAFA, and MAFB were selectively lost in human T2DM islets (Guo et al., 2013b).

Gestational diabetes (GDM) is a special case of T2DM that occurs in 3-7% of all pregnancies (Perkins et al., 2007). In GDM, mothers develop glucose intolerance due to inadequate β-cell mass expansion and/or insufficient levels of insulin secretion during pregnancy, often leading to birth complications and postpartum issues for both the mother and offspring (Buchanan and Xiang, 2005). β-cell mass expansion and increased insulin secretion are necessary adaptations to pregnancy for mothers to respond to the modest insulin resistance that arises at mid gestation, likely due to increased maternal adiposity and placental hormones (Buchanan and Xiang, 2005; Sorenson and Brelje, 1997). Although GDM usually resolves itself after birth, it is

associated with an increased risk of T2DM later in life for both the mother and the child (Buchanan and Xiang, 2005). Notably, transcription factors have been linked to this form of diabetes. For example, a common variant of the hepatocyte nuclear factor-4-α (HNF4α) is associated with a higher risk of developing GDM (Robitaille and Grant, 2008). The forkhead transcription factor, FoxM1, has a crucial role in the adaptive maternal β-cell proliferation as revealed by studies with pregnant *FoxM1* mouse mutants that develop GDM (Zhang et al., 2009b).

Monogenic Diabetes

Monogenic diabetes accounts for 2-5% of all diabetes cases. This class represents a very heterogeneous group of disorders that results from either β-cell dysfunction or a reduction in the number of β-cells (Schwitzgebel, 2014). Monogenic diabetes is further broken into neonatal diabetes and maturity onset diabetes of the young (MODY) (Schwitzgebel, 2014). Neonatal diabetes manifests before 6 months and can be transient or permanent, whereas MODY appears before 25 years of age. Because monogenic diabetes is due to an autosomal dominant inheritance of a gene, we have learned much about specific gene roles in human islet function from this particular form of diabetes (Schwitzgebel, 2014). Some monogenic forms are actually missed or wrongly diagnosed as T1DM or T2DM. Heterozygous carriers of MODY mutations present with late-onset diabetes, and so these patients are often misdiagnosed with T2DM (Schwitzgebel, 2014). However, the generally early presentation of monogenic diabetes provides clear evidence for the functional significance of key factors.

Of the eleven genes identified as MODY genes, seven are transcription factors that regulate gene targets crucial to pancreatic islet cell identity and function. For example, homozygous and compound heterozygous mutations of *PDX1* (MODY4) can

result in pancreatic agenesis (failure of the pancreas to develop at birth). At the least, *PDX1* mutations produce a drastic phenotype with neonatal diabetes and exocrine pancreatic insufficiency (Schwitzgebel, 2014; Stoffers et al., 1997a; 1997b). Other MODY gene transcription factors include *Hepatic nuclear factor 4-α* (*HNF4α*) (MODY1), *HNF1α* (MODY3), *HNF1β* (MODY5), *Neurogenic differentiation factor 1* (*NEUROD1*) (MODY6), and *Paired box gene 4* (*PAX4*) (MODY9) (Horikawa et al., 1997; Malecki et al., 1999; Plengvidhya et al., 2007; Yamagata et al., 1996a; 1996b). Many monogenic diabetes cases have shed light on the critical roles of different transcription factors, although they are not all classified as MODY genes. For example, homozygous mutations in the *pancreas-specific transcription factor 1A gene* (*PTF1A*) and heterozygous mutations in *GATA-binding protein 6* (*GATA6*) can also lead to pancreatic agenesis (Allen et al., 2011; Sellick et al., 2004). Transcription factor expression and function in human islet cell function and pathophysiology will be discussed in more detail in the next chapter.

Therapeutic Measures and Limitations

Over the past century, the prognosis for diabetes has improved immensely with the discovery and refinement of insulin therapy. Before Banting's discovery of insulin in 1921, diabetic patients were starved to avoid hyperglycemia and to prolong their lives. The discovery of insulin allowed T1DM individuals to live with exogenous sources of insulin, which are now widely used to help diabetic individuals maintain relatively normal lives. Another more recent therapeutic strategy has been that of glucagon suppression, as glucagon hypersecretion is a major cause of the metabolic abnormalities in diabetes. Notably, the concentration of insulin from exogenous treatments that reaches the α-cells in diabetic patients is much lower than insulin secreted from neighboring islet β-cells in non-diabetic patients. This lower insulin concentration fails to suppress α-cell glucagon

secretion and hyperglucagonemia still contributes to the elevated blood glucose levels in these treated diabetic individuals (Unger and Cherrington, 2012). For this reason, along with risks associated with insulin dosage errors, exogenous insulin treatment remains an incomplete treatment for diabetes.

T2DM, at least initially, is not treated with insulin. Lifestyle changes, including increased physical activity and healthy eating, can have a great impact on T2DM patients, even reversing this disease in some cases. When drugs are necessary, Metformin is usually the first line of action for glycemic control in T2DM (Nathan et al., 2008). This drug works primarily by reducing liver glucose output, but complete understanding of Metformin function remains elusive (Gelling et al., 2003; Pernicova and Korbonits, 2014). The unknown effects of such a widely prescribed drug clearly demonstrate why a more thorough understanding of the endocrine cells that secrete the hormones antagonized or mimicked by such drugs is necessary. Glucagon-like peptide 1 (GLP-1) receptor agonists are also used for diabetic treatment, enhancing insulin secretion and reducing glucagon secretion (Cho and Kieffer, 2011; D'Alessio, 2011). Although the exact mechanism by which GLP-1 reduces plasma glucagon is not known, indirect mediation by neural regulation or via pancreatic somatostatin acting to reduce glucagon has been proposed (D'Alessio, 2011). Inhibitors of DPP-4, a ubiquitous protease that quickly deactivates GLP-1, are also used to treat diabetes with the ability of these inhibitors to lower blood glucose being attributed to GLP-1 (D'Alessio, 2011). Additionally, sulfonylureas and meglitinides are prescribed to enhance insulin secretion in T2DM individuals (Blicklé, 2006). These drugs bind the SUR1 component of the β-cell K_{ATP} channels and block K^+ efflux, causing depolarization and downstream insulin release (Figure 6). Notably, these medications are not ideal in that they work in a glucose-independent manner and do not function without side effects, including potential ketosis and coma (Juhl et al., 2001; Nathan et al., 2008). Furthermore, as T2DM

progresses, β-cell exhaustion, apoptosis, and a more extreme β-cell deficiency like that observed in T1DM manifests and insulin treatment is prescribed. Evidently, existing therapeutic options for diabetes fail to recapitulate the highly regulated work of a healthy pancreas.

Future efforts to understand better how endocrine cells produce and secrete hormones could help with the development of more complete and effective diabetes therapies. The integral role of transcription factors in islet cell formation and function makes these proteins a valid focus for research endeavors that could enhance future treatment of diabetes. Moreover, the ability to make β-cells that could replace cells that are lacking or dysfunctional in diabetes may be possible with the guided expression of transcription factors (Pagliuca and Melton, 2013).

Alternative β-cell Sources

The inconvenience, complications, and limitations of insulin therapy make cell-based replacement therapy of particular interest to clinicians and patients alike. The work presented in my thesis enhances our knowledge of these cells that are lacking or dysfunctional in diabetes, particularly with information on how the Maf factors contribute to islet cell maturation and adult islet cell function. Understanding transcriptional control of islet cell formation and function could improve existing and future therapies, including generation of new β-cells via proliferation of existing β-cells, differentiation from human embryonic stem cells (hESCs) or induced pluripotent stem (iPS) cells, or transdifferentiation from another related pancreatic cell type (Ackermann and Gannon, 2007) (Figure 7). The next sections will provide more detail and examples of these alternative β-cell sources.

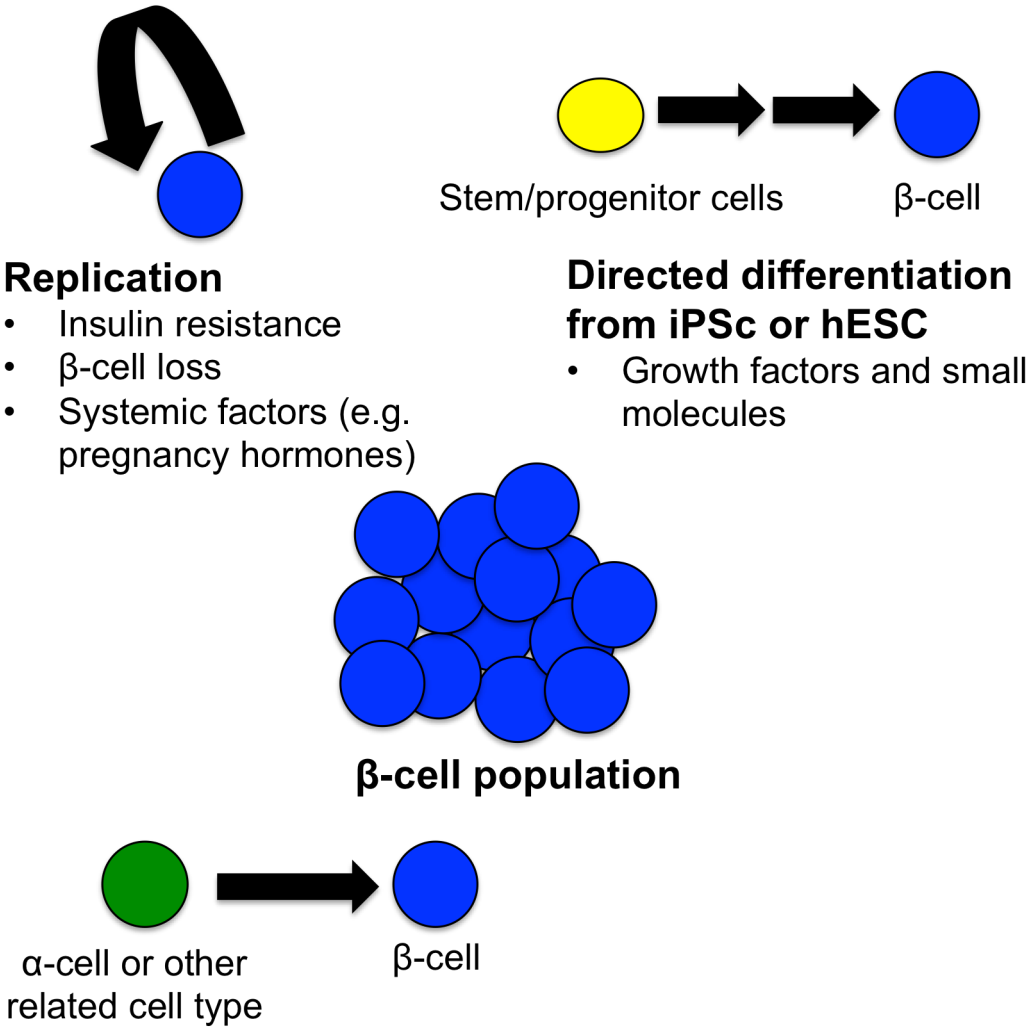

Figure 7. Adult β-cell sources. Different mechanisms by which β-cells could be generated and triggers known to induce each process.

Alternative β-cell Sources: Replication

Replication of pre-existing β-cells has been considered as a potential for β-cell replacement therapy. For this reason, a more thorough grasp of how these cells form and proliferate is needed. Evidence supports replication as the primary source of new β-cells in adult rodents and humans, although β-cell proliferation markedly decreases with aging (Dor et al., 2004; Georgia and Bhushan, 2004; Meier et al., 2008; Reers et al., 2008). Though generally not a very proliferative population in adults, β-cells exhibit potential for expansion, especially during times of physiological challenge, such as with the increased metabolic demands of pregnancy, obesity, and in response to pancreatic injury. Interestingly, factors and pathways that drive embryonic β-cell expansion appear distinct from those that direct β-cell replication in the adult context (Gunasekaran et al., 2012). Specifically, roles for cell cycle genes and transcription factors like *FoxM1*, *Pdx1, Foxo1*, and *Ngn3* have been implicated in the adaptive adult β-cell expansion (Ackermann and Gannon, 2007; Fiaschi-Taesch et al., 2010; Golson et al., 2010; Kitamura et al., 2005; Kulkarni et al., 2004; Kushner et al., 2005; Lee and Nielsen, 2009; Rane et al., 1999; Van De Casteele et al., 2013). Knowledge of how these and other transcription factors function in β-cell proliferation could improve therapeutic measures to replace this cell population by replicative expansion in the future. Whereas many of these studies were conducted in mice, the adaptive proliferation in humans is not nearly as extensive. For example, a 10-fold increase in β-cell proliferation is reported in obese mouse models versus a mere 0.5-fold increase observed in obese human samples (Butler et al., 2003a; 2003b; Meier et al., 2008). Still, the possibility of inducing regulated β-cell proliferation has driven a research focus on identifying factors that could replenish β-cells in T1DM and T2DM via replication (Yi et al., 2013). A recent study identified a novel mitogen in human islets that is mediated by the NFAT transcription factor family, indicating a crucial role for transcription factors in β-cell proliferation. Furthermore,

MAFA, PDX1, and NKX6.1 mRNA and protein levels were increased in human islets treated with this mitogen (Wang et al., 2015). Such studies indicate that the possibility of expanding the β-cell population by replication could become a therapeutic reality.

Alternative β-cell Sources: hESC- or iPS-cell-Derived

Much research has also been dedicated to directing hESC or IPS-cells to a β-cell fate. Recent protocols for differentiation of these cells into β- or β-like cells have been promising (D'Amour et al., 2006; Pagliuca et al., 2014; Rezania et al., 2014; 2012). Recently, Pagliuca et al. and Rezania et al. were able to generate insulin^{+} cells that respond to high glucose in a manner similar to primary human β-cells (Pagliuca et al., 2014; Rezania et al., 2014). In addition, insulin secretion in these newly generated β-like cells is inhibited as quickly as in endogenous β-cells in low glucose conditions. Notably, one of these studies identified MAFA expression in the glucose-responsive β-like cells, noting that MAFA could be driving maturation into glucose-responsive cells (Aguayo-Mazzucato et al., 2011; Rezania et al., 2014). Pagliuca et al. highlights that PDX1 and NKX6.1 transcription factor expression is also likely contributing to the success of these differentiation protocols into β-like cells (Pagliuca et al., 2014). Although very promising, the β-like cells produced in these protocols still fail to secrete comparable insulin levels to endogenous β-cells in response to glucose, but a more thorough analysis of β-cell identity and function could facilitate endeavors to make fully functioning β-cells by directed differentiation protocols.

Immunologic rejection of β-like cells derived from hESC or IPS-cells still remains a challenge. Even with the production of functional β-cells to replace the lost population in T1DM, cells need to be protected from the same autoimmune destruction that occurred initially (Eizirik and Darville, 2001). Recent work has been directed towards encapsulation of stem cell-derived β-cells, in addition to modifications to the cells

themselves. This work could then allow stem cell-derived β-cells to evade immune detection. These remarkable advancements in hESC- or iPS-cell-derived β-cell protocols could make β-cell replacement therapy a feasible option in the near future.

Alternative β-cell Sources: Transdifferentiation from a Related Cell Type

The redirection of a non-β-cell to a β-cell, or transdifferentiation, remains another possible source for new β-cells. Transdifferentiation into β- or β-like cells has been studied in multiple cell types, including endocrine cells, acinar cells, ductal cells, and hepatocytes. Notably, transcription factors have been identified as pivotal drivers of many of these protocols.

The closely related α-cell, which develops from an endocrine islet progenitor origin shared with β-cells, is a very compelling source for transdifferentiated β-cells. Pdx1 may be an integral component to this process. For example, forced Pdx1 expression in endocrine-committed cells during development results in α- to β-like reprogramming, although these cells maintain some key α-cell transcription factor expression (Yang et al., 2011). Remarkably, Dr. Pedro Herrera's group discovered that δ-cells reprogram to β-cells with early and extreme β-cell destruction by diphtheria toxin, whereas adult β-cell destruction results in α-cell conversion into β-cells (Chera et al., 2014; Thorel et al., 2010).

The exocrine cells also have a common developmental origin to the endocrine β-cells within the pancreas and have been another focus for β-cell transdifferentiation. Researchers have examined scenarios under which acinar or ductal cells can produce insulin (Baeyens et al., 2005; Bonner-Weir et al., 2000; Minami et al., 2005; Wang et al., 1995). Zhou and colleagues were able to direct acinar cells to β-like cells that secrete insulin *in vivo* by driving expression of islet transcription factors Pdx1, Neurogenin 3 (Ngn3), and MafA in these exocrine cells (Zhou et al., 2008). This study provides

compelling evidence for the plasticity of pancreatic cells and for the central role of transcription factors in guiding islet cell fate.

Whereas transdifferentiation of other cell types within the pancreas to endocrine cells is an obvious potential and has indeed been attempted, there are also shared developmental origins between the liver and the pancreas. This suggests that hepatocytes might also be a reasonable cell source for transdifferentiation into β-cells (Grompe, 2003; Pandol, 2010; Shen et al., 2000). Liver cells have been shown to transform into insulin-producing cells with the addition of key pancreas transcription factors, like Pdx1 and NeuroD1, even having the capacity to rescue diabetic mice (Ber, 2003; Ferber et al., 2000; Kojima et al., 2003; Yang et al., 2002). Other promising examples of transcriptional control driving a change to β-like cells in mice and humans are provided in the next chapter. Such observations indicate that transdifferentiation protocols could be an attainable source of new β-cells.

Development

The ultimate goal of potential β-cell replacement strategies is to treat human patients. Before such therapies can be successfully and safely applied to humans, a more thorough understanding of islet cell formation and function is essential. In particular, transcriptional regulation of development can shed light on how these cells become specified and form mature β-cells.

Pancreatic Morphogenesis Overview

Pancreatic organogenesis is a coordinated process that involves specific timing of signaling molecule and transcription factor expression. For the purpose of the work presented in this thesis, I will provide an overview of pancreas and endocrine cell formation. Transcriptional regulation of pancreas development will be highlighted with

brief descriptions of transcription factors that are involved in islet cell development and function.

The onset of pancreatogenesis is referred to as the "primary transition," initiating the changes that distinguish the pancreatic region from the developing gut (Jensen, 2004). The appearance of pancreatic precursor cells is first evident around embryonic day (e) 9.5 in mice and gestational or embryonic day (e) 25 in humans (Pan and Wright, 2011; Pictet et al., 1972). The pancreas forms from two buds from the endoderm that then fuse to form one organ, with the dorsal bud arising first and the ventral bud appearing about 12 hours later in mice and 6 days later in humans (Gittes, 2009; Kim et al., 2002; Slack, 1995). Early signals directing the pancreatic fate are highly regulated. For example, specification of the pancreatic domain from the rest of the gut endoderm requires repression of sonic hedgehog (Shh) signaling (Hebrok et al., 1998). Additionally, retinoic acid signaling is required for induction of dorsal pancreatic development (Molotkov et al., 2005; Stafford and Prince, 2002). Notch signaling within the epithelium is also crucial for early pancreatic growth (Shih et al., 2013). After specification, pancreatic mesodermal cells tightly associate with the pancreatic epithelium to guide development, primarily with proliferative signaling (Jensen, 2004). During this early epithelial expansion, the pancreas undergoes a branching process to form the early ductal network (Jensen, 2004).

The "secondary transition" of pancreatic development, from e13.5 to e15.5 in mice, is characterized by the appearance of most of the endocrine hormone$^+$ cells that populate the mature islet (Jensen, 2004). These different endocrine cells form from a common progenitor within the pancreatic progenitor cell population that express the transcription factor, Ngn3 (Gu et al., 2002). Notably, the insulin$^+$ cells that arise during the secondary transition still must undergo many gene expression changes postnatally before they are mature β-cells (Aguayo-Mazzucato et al., 2011; Jermendy et al., 2011).

Much of what we know of the molecular mechanisms leading to pancreas development and endocrine cell formation has been revealed with genetic manipulation in model systems, particularly in transgenic and knockout mice. The next section will highlight what is known from model systems of key transcription factors. These transcription factors direct islet cell formation and are used in my thesis studies.

Key Transcription Factors in the Pancreas

Transcription factors have a vital role in regulating lineage determination, development, and function. They do this by binding to enhancers, recruiting enzymes that modify and remodel chromatin, and ultimately regulating expression of target genes. Using different genetic mouse models, investigators have identified roles for many of these transcription factors. Studies have further examined the stage-specific transcription factor expression in the highly coordinated process of pancreas development (Cano et al., 2013; Shih et al., 2013) (Figure 8). Moreover, specific transcription factors are responsible for particular developmental stages, including directing the pancreatic cell fate, the pancreatic endocrine fate, and the even specific endocrine islet cell types. I will focus on the role of various transcription factors in endocrine cell development during the secondary transition, the stage at which my factors of interest, MafA and MafB, are known to be important.

Many of the islet-enriched factors presented in this section are necessary for both islet cell development and mature β-cell function, as first identified by functional and biochemical analyses in rodent studies. Though our knowledge of human β-cell development is more limited, mouse development generally recapitulates the process of human β-cell development (Dai et al., 2011). This will be further discussed in the next chapter. Most notably, many of these transcription factors are either not expressed or dysregulated in the context of diabetes. While some have been identified for their role in

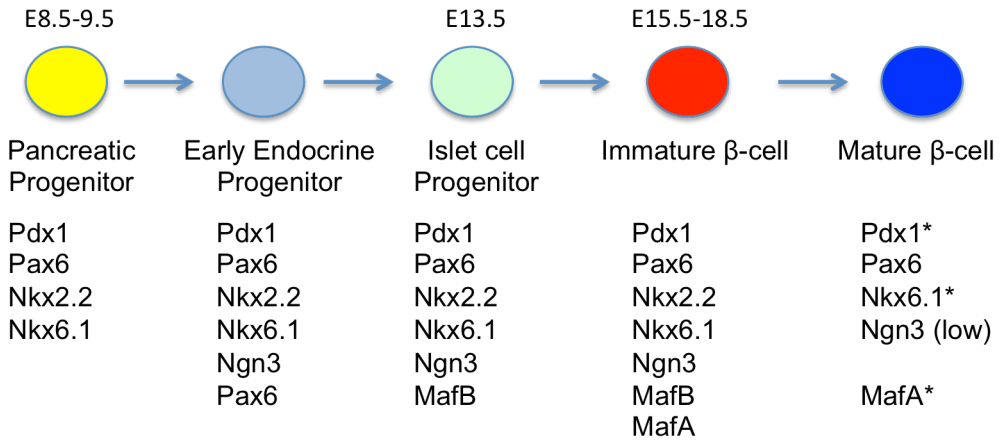

Figure 8. Transcription Factors in rodent islet β-cell development. Stage-specific expression of islet-enriched transcription factors in rodent β-cell development. * demarcates β-cell-specific in the adult population.

human β-cell function from MODY cases, transcription factor loss of expression has recently been observed in T1DM and T2DM human islet samples (Marcela Brissova, personal communication) (Guo et al., 2013b).

Pdx1

The homeodomain transcription factor, Pdx1, is referred to as a pancreatic "master regulator," with all pancreatic cell types derived from $Pdx1^+$ progenitors (Gu et al., 2002; Ohlsson et al., 1993). Pdx1 is detected as early as e8.5 in rodent development and, although it is detected slightly later in human development, it is similarly produced in the pre-pancreatic endoderm (Ahlgren et al., 1996; Jennings et al., 2013; Lyttle et al., 2008). This factor is produced in all cells that eventually comprise the pancreas, as well as some cells in the stomach and duodenum (Ohneda et al., 2000). Indeed, mice and humans lacking *PDX1* exhibit pancreatic agenesis; however the mouse mutants do form an early dorsal bud with some insulin- and glucagon-producing cells (Ahlgren et al., 1996; Jonsson et al., 1994; Offield et al., 1996). Thus, Pdx1 is not essential for early endocrine hormone expression, but it is crucial for later phases in pancreas formation.

As the pancreas develops, Pdx1 high expression is restricted to β-cells and, thus, this factor is used as a marker of β-cell identity (Pan and Wright, 2011). The β-cell-specific *Pdx1* knockout mice demonstrate the crucial role of Pdx1 in β-cells. These mutants exhibit reduced $insulin^+$ cell numbers, decreased expression of key β-cell factors, and derepressed expression of α-cell factors (Ahlgren et al., 1998; Gannon et al., 2008; Gao et al., 2014). Pdx1 also regulates distinct stages of pancreas development in a dose-dependent manner, as demonstrated by rodent studies with hypomorphic alleles created by conserved enhancer region deletions (Fujitani et al., 2006). Duct-cell-specific removal of Pdx1 after development indicated that Pdx1 is not essential for postnatal β-

cell formation, but it is required for the maturation of these newly-formed adult β-cells (Guo et al., 2013a) Furthermore, when Pdx1 production is delayed until after pancreatic budding, β- and acinar cells fail to form and only small ductal structures develop (Holland et al., 2002). These studies indicate that Pdx1 is important in early pancreatic development, endocrine and exocrine commitment, endocrine lineage differentiation, and even postnatal β-cell function. As aforementioned, forced Pdx1 expression in endocrine progenitors reprograms α-cells to β-like cells (Yang et al., 2011). Pdx1 expression has also been implicated in iPS-cell pancreatic stem cell line differentiation protocols (Kuise et al., 2014). Indeed, Pdx1, along with transcription factors Ngn3 and MafA, can reprogram adult exocrine cells to β-cells (Zhou et al., 2008). These studies demonstrate the pivotal role of transcription factors in islet cell development and β-cell identity.

Ngn3

Ngn3 is a basic helix-loop-helix (bHLH) transcription factor that is first detected at e9.5 and peaks around e15.5 in all endocrine progenitors. It is then repressed and produced at nearly undetectable levels after e17.5 in mice (Gu et al., 2002). Similarly, *NGN3* is first detected at 8 weeks in humans and transiently expressed at high levels from 11 to 19 weeks (Jeon et al., 2009). Ngn3 is expressed in all endocrine progenitor cells (Gradwohl et al., 2000; Gu et al., 2002; Magenheim et al., 2011). Mice lacking *Ngn3* fail to develop endocrine hormone$^+$ cells aside from a few glucagon$^+$ cells, just as human *NGN3* null mutations report no detectable islets (Gradwohl et al., 2000; Rubio-Cabezas et al., 2011).

Early ectopic expression of Ngn3 within the developing pancreas domain (Pdx1$^+$) drives the endocrine differentiation program in cells that would have otherwise been exocrine cells, specifically directing these to be glucagon$^+$ cells (Schwitzgebel et al.,

2000). As aforementioned, Ngn3 is one of the key factors, along with Pdx1 and MafA, necessary to direct exocrine cells to a β-like cell fate (Zhou et al., 2008). Ngn3 directs cells to the endocrine lineage, whereas Pdx1 initially directs the pancreatic cell fate, and MafA is integrally involved in β-cell identity, to be discussed in a later section.

Pax6

Appearing early in pancreas development, Pax6 is a paired box transcription factor that is produced by e9.0 in cells committed to the endocrine lineage. Pax6 is necessary for endocrine population expansion during the secondary transition (Sander et al., 1997; St-Onge et al., 1997). *Pax6* knockout mice exhibit a significant reduction in α- and β-cells (Sander et al., 1997; St-Onge et al., 1997). Recent efforts to convert human pancreatic ductal cells to insulin$^+$ cells utilized Pax6 expression along with the key β-cell transcription factors Pdx1, Ngn3, and MafA (Lee et al., 2013). Pax6 is used to mark the endocrine cell population in the studies presented in this thesis, as it is expressed early and maintained in developing and mature endocrine cells.

Nkx6.1

Nkx6.1, a member of the homeodomain NK subfamily, is also used in the ensuing studies as a marker of β-cell identity. Although it is broadly expressed prior to Ngn3 production in the pancreatic epithelium, Nkx6.1 eventually becomes restricted to mature β-cells. *Nkx6.1* mutant mice reveal a loss of only β-cells after the secondary transition (Sander et al., 2000). β-cell-specific removal of *Nkx6.1* in adult mice demonstrates the role of Nkx6.1 in maintaining the functionally mature β-cell phenotype (Taylor et al., 2013). Additional conditional inactivation and misexpression studies indicate that Nkx6.1 is both necessary and sufficient to direct the β-cell lineage (Schaffer et al., 2013). Indeed, NKX6.1 levels are reduced in human T2DM islets (Guo et al.,

2013b). Taken together, these studies indicate that Nkx6.1 expression is a specific marker for mature β-cell identity and function.

Maf factors

My thesis focuses on the large Maf factors expressed in the pancreas, MafA and MafB. Although our lab and others have learned much about how these crucial factors are involved in islet cell maturation and function, there is still much we do not yet understand. This section will outline what is known about these Maf factors.

MafA and MafB are the only Maf factors identified in the pancreas, but the first Maf family member discovered was viral Maf (v-Maf). V-Maf was isolated from a spontaneous musculoaponeurotic fibrosarcoma in a chicken and identified as the avian retrovirus AS42 transforming gene (Blank and Andrews, 1997). This factor, like all Mafs, possesses a basic leucine zipper (bZIP) domain that is crucial for its transforming activity (Blank and Andrews, 1997). The leucine zipper component is specifically responsible for the dimerization property of these bZIP proteins. Other bZIP domain-containing proteins include the cAMP response element-binding protein (CREB) transcription factor, cyclic AMP-dependent transcription factor (ATF-1), and the AP-1 fos/jun heterodimer that forms a transcription factor (Ellenberger, 1994). In addition to the C-terminal leucine-zipper dimerization motif possessed by all Maf family members, these factors also have a conserved basic domain, sharing up to 40% homology among all the Mafs, and an extended homology region (EHR). These components allow Maf factors to homo- or heterodimerize with other bZIP-containing proteins and bind to specific cis-control element sequences to regulate transcription (Blank and Andrews, 1997) (Figure 9).

Maf factor binding to cis-regulatory sequences is specified by Maf-type consensus sequences that are 11-14 base pairs and palindromic, containing either an AP-1-like (TRE) or an ATF/CREB-like (CRE) motif (Blank and Andrews, 1997). These

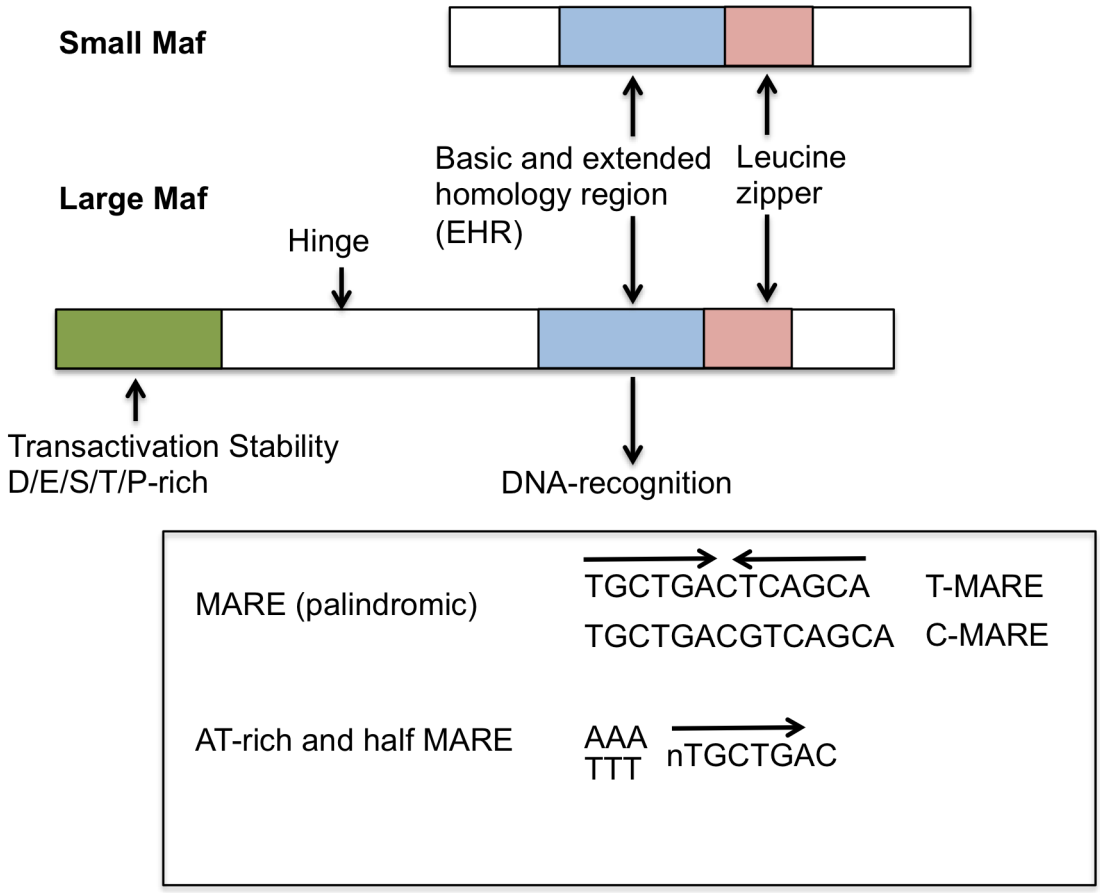

Figure 9. Maf Structure. Structural layout of small and large Mafs and the Maf recognition element (MARE). Adapted from Kataoka et al., 2007.

binding motifs (TGCTGACTCAGCA and TGCTGACGTCAGCA) are referred to as Maf recognition elements (MARES) (Kataoka et al., 1993). Each member of the dimer pair binds and interacts with adjacent half-sites, thus the symmetry of these full binding sites (Yoshida, 2005). Yoshida and colleagues found that Mafs can bind the palindromic MARE, as well as the 5'-AT-rich MARE half-site (Yoshida, 2005) (Figure 9). The unique binding specificity conferred by Maf dimers can result in similar and distinct regulation by different Maf dimer pairs (Blank and Andrews, 1997). This concept of different Maf dimers producing unique regulation will be further explored later with a review of MafA and MafB studies in the pancreas.

Maf factors are classified into two subgroups, large and small Maf factors (Kataoka et al., 2002). Small Mafs (MafF, MafG, MafK, MafT and Maf-S) range in size from 149-162 amino acids and lack the N-terminal activation domain of the large Mafs that is responsible for transcriptional activation (Blank and Andrews, 1997; Kataoka, 2007) (Figure 9). Small Mafs can heterodimerize with many other bZIP proteins, but they do not dimerize with large Mafs (Blank and Andrews, 1997). Although the small Mafs homodimerize to repress target genes, small Mafs have also been demonstrated to heterodimerize with a subunit of the NF-E2 transcription factor known to regulate erythroid gene expression (Igarashi et al., 1994). Furthermore, small Mafs can heterodimerize with Cap'n'collar and Bach family bZip proteins to repress or activate gene transcription (Kataoka, 2007). Recent studies have implicated a role for small Mafs in the hypoxic response and diabetes, among other cellular processes and perturbations (Kannan et al., 2012; Martínez-Hernández et al., 2014; Tanigawa et al., 2013). Although small Mafs lack an N-terminal activation domain, biochemical analyses with these proteins have revealed more about how Mafs dimerize.

MafA and MafB, the particular proteins of interest in my thesis work, are members of the large Maf transcription factor subfamily (MafA, MafB, c-Maf, and NRL)

that is characterized by a conserved N-terminal activation domain, rich in Asp, Glu, Ser, Thr, and Pro residues (Blank and Andrews, 1997; Kataoka, 2007) (Figure 9). Phosphorylation within this region regulates Maf activity (Benkhelifa et al., 2001; Guo et al., 2009; 2010; Ochi, 2002). Large Mafs can homo- and heterodimerize with each other to potentially regulate different target DNA (Kataoka et al., 1994; Kataoka, 2007). The next sections will introduce each of the large Mafs with thorough backgrounds provided for MafA and MafB, particularly outlining what is known in the pancreas.

NRL and c-Maf

Unlike MafA and MafB, the other large Mafs, neural retina-specific leucine zipper protein (NRL) and cellular Maf (c-Maf), are either not produced in the pancreas or not important to pancreatic function. NRL, unlike the other large Mafs, lacks the glycine and histidine repeats between the bZIP motif and the acidic N-terminal activation domain; although the function of these repeats is unknown (Blank and Andrews, 1997). NRL is only present in neurons and the retina, acting as a regulator of *rhodopsin* in photoreceptor cells of the retina (Blank and Andrews, 1997; Kumar et al., 1996; Rehemtulla et al., 1996). Interestingly though, insulin receptor signaling, known to provide neuroprotection to cone cells in the eye, is altered within cones lacking Nrl (Rajala et al., 2013).

c-Maf, the cellular counterpart of v-Maf, has been implicated in immune function and was independently identified as a T helper 2 (Th2) lineage-specific gene product. c-Maf was also shown to regulate Th2-specific interleukin-4 expression (Ho et al., 1996). *In vitro*, the binding capacity of MafA, MafB, and c-Maf is identical (Matsuoka et al., 2003). Although c-Maf expression has been reported in the pancreas by immunofluorescence, antibody cross-reaction with MafB in α-cells probably led to this conclusion as c-Maf is not detected by mRNA analysis and the *c-Maf* null mice exhibit no

pancreatic phenotype (Abdellatif et al., 2015; Artner et al., 2007; Kataoka et al., 2004). By contrast, both MafA and MafB have been examined for their crucial roles as pancreatic β-cell maturation factors.

MafA

Lens-specific Maf (L-Maf) is the chicken ortholog to the mammalian MafA and was first identified in a chicken lens development study (Ogino and Yasuda, 1998). Mammalian MafA, on the other hand, appears to have very little impact on lens development (Olbrot et al., 2002). Instead, MafA has been well studied for its role in pancreatic β-cell function as a crucial insulin regulator (Kataoka et al., 2002; Matsuoka et al., 2004; Olbrot et al., 2002). Mammalian MafA was identified and cloned as the transcription factor that binds to one of the three critical insulin enhancer elements. This element is known as the rat insulin promoter element 3b1 (RIPE3b1), or the C1 element, that regulates β-cell-specific and glucose-responsive insulin expression (Kataoka et al., 2002; Matsuoka et al., 2003; 2004; Olbrot et al., 2002; Sharma and Stein, 1994). Purification and isolation by high performance liquid chromatography and mass spectrometry were conducted from hamster insulinoma cell line (HIT T-15) and mouse insulinoma cell line (βTC-3) extracts, respectively (Matsuoka et al., 2003; Olbrot et al., 2002). This RIPE3b1 binding factor was identified as mammalian MafA (Matsuoka et al., 2003; Olbrot et al., 2002). Pax6, Pdx1, and NeuroD1 were found to bind other insulin gene enhancer elements with roles in pancreas development and β-cell formation and function (Matsuoka et al., 2004; Naya et al., 1995; Offield et al., 1996; Ohlsson et al., 1993; Sander et al., 1997; Serup et al., 1996).

Mouse and cell line studies have demonstrated that MafA is crucial for β-cell maturation and adult β-cell GSIS function. MafA is first expressed in insulin$^+$ cells around e13.5, at the onset of the secondary transition in mouse pancreas development

(Matsuoka et al., 2004). *MafA* mutant mice (*MafA*$^{-/-}$) are glucose intolerant and develop diabetes postnatally with reductions in *Insulin 1, Insulin 2, Pdx1, Neurod1,* and *solute carrier family 2 facilitated glucose transporter member* (*Slc2a2*, also known as GLUT-2, the glucose transporter of the β-cell*)*. These mice exhibit severely impaired insulin secretion, although the neonates appear healthy (Zhang et al., 2005). Experiments with an inducible rat insulinoma INS-1 β-cell line to drive *MafA* overexpression and a dominant negative *MafA (DN-MafA)* construct reveal that MafA controls *Slc2a2, Pdx1, Nkx6.1, Glucagon-Like Peptide 1 Receptor (Glp1r), Prohormone Convertase 1/3 (Pcsk1),* and *Pyruvate Carboxylase* (*Pc)* (Wang et al., 2007). Dysregulation of these β-cell genes in the *DN-MafA* line likely contribute to the insulin secretory defect observed in these cells (Wang et al., 2007). Pancreas-specific *MafA* mutant (*MafA*$^{\Delta panc}$) mice studies refined earlier *in vivo* studies and demonstrate that MafA becomes important to rodent β-cells at 3 weeks of age (Artner et al., 2010; Hang et al., 2014). Moreover, MafA is regulated by many other transcription factors that are studied for their role in pancreas development and β-cell identity, including FoxA2, Pdx1, NeoroD1, Isl1, Nkx6.1, Pax6, and Nkx2.2 (Du et al., 2009; Raum et al., 2006; 2010). Supporting this evidence, the insulin^{+} cells of the *Pdx1*, *Nkx2.2,* and *Nkx6.1* mutants lack MafA expression (Matsuoka et al., 2004; Raum et al., 2006; 2010). MafA likely mediates many of the β-cell functions attributed to these factors. Taken together, these analyses indicate the vital role of MafA in β-cell maturation and adult function.

The regulation of MafA has been examined with six highly conserved 5' *cis* regions of the *MafA* promoter identified (Raum et al., 2006; 2010). Region 3 (R3: base pairs -8118 to -7750 relative to transcriptional start site) not only shares 94% conservation between the mouse and human sequence, but also is essential for the β-cell-specific *MafA* activity (Raum et al., 2006). Moreover, transgenic *MafA* promoter reporter mice experiments confirmed R3 is necessary but not sufficient for β-cell-specific

MafA expression (Raum et al., 2010). The other five regions were also found to be necessary for β-cell-specific *MafA* activity, as transgenic reporter mice of R3 alone or of R1-6 but lacking R3 cannot drive β-cell-specific *MafA* activity. Furthermore, Pdx1 and Pax6 bind to R1 and R6, potentially regulating *MafA* activity through these regions (Raum et al., 2010). Conversely, follow up studies demonstrated that Hnf6 negatively regulates *MafA* through its FoxA2 binding cis-element, demonstrating the regulated control of this key transcription factor (Raum et al., 2006; Yamamoto et al., 2013). The transgenic *MafA* promoter R1-6 reporter mice also reveal that *MafA* activity is stimulated by glucose.

In fact, MafA mRNA and protein are impacted by glucose (Kataoka et al., 2002; Poitout et al., 2006; Raum et al., 2006; 2010; Vanderford et al., 2007; Wang et al., 2007; Zhang et al., 2005; Zimmet, 1999). Conversely, prolonged glucose exposure is deleterious to MafA, with reductions observed in models of hyperglycemia (Harmon et al., 2005; Kitamura et al., 2005; Poitout et al., 1996). Moreover MAFA is one of the few transcription factors selectively reduced in T2DM human islet samples (Guo et al., 2013b). Other metabolic effectors of β-cell activity also impact MafA levels, including fatty acids and insulin itself (Hagman et al., 2005; Poitout et al., 2006; Ueki et al., 2006; Vanderford et al., 2007). This sensitivity suggests that MAFA loss has a pivotal role in β-cell dysfunction.

MafB

MafB is the other large Maf expressed in the pancreas, as detected by mRNA and immunofluorescent analysis. In the rodent pancreas, MafB is detected as early as e10.5 in developing insulin$^+$ and glucagon$^+$ cells and precedes MafA expression in developing rodent β-cells (Figure 10) (Artner et al., 2006). MafB was initially studied as the gene mutated in an X-ray-induced recessive mutation known as the *kreisler* (*kr*)

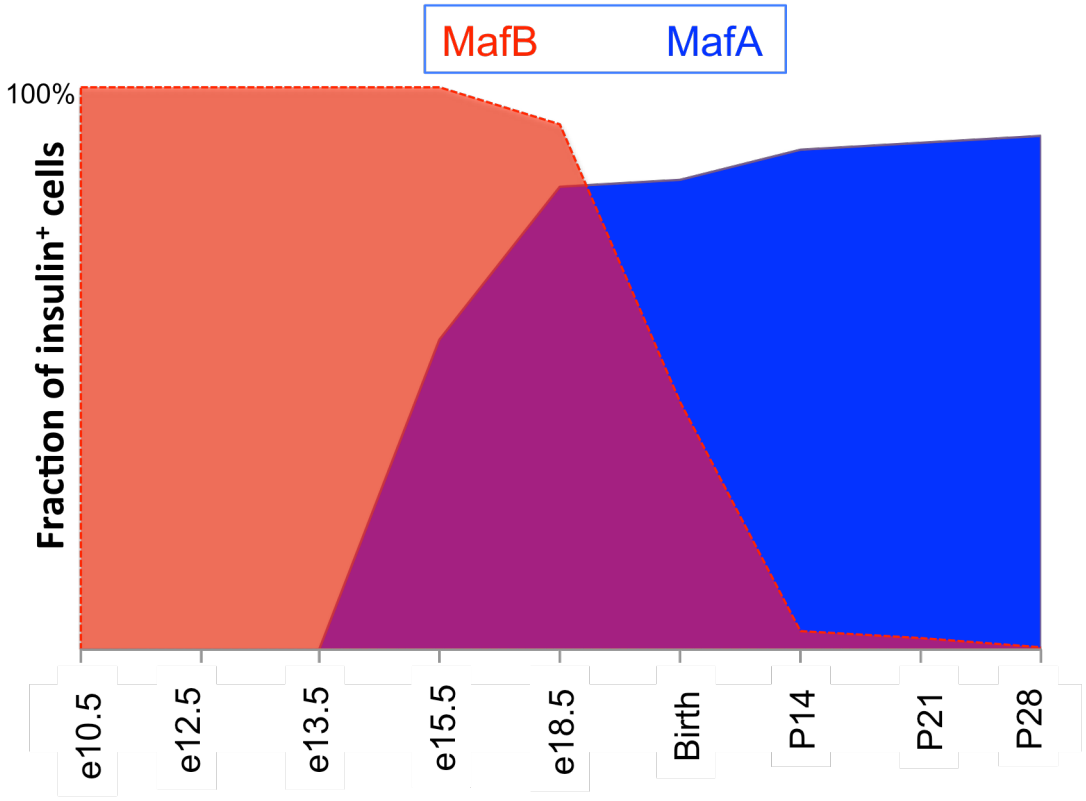

Figure 10. MafA and MafB are dynamically expressed in developing rodent β-cells. Proportion of insulin$^+$ cells producing either MafA or MafB throughout rodent pancreas development. Adapted from Artner and Hang et al., *Diabetes,* 2010.

mutation (Artner et al., 2006; Deol, 1964). This mutation leads to segmentation alterations in the caudal hindbrain and inner ear developmental defects that produce altered behavior in mice, including running in circles (Cordes and Barsh, 1994; Deol, 1964). MafB is also expressed and functions in developmental programs in other tissues, including the kidney podocytes (Morito et al., 2014; Sadl et al., 2002), macrophages (Aziz et al., 2009; 2006; Moriguchi et al., 2006; Sarrazin et al., 2009), neurons (Blanchi et al., 2003), osteoclasts (Zankl et al., 2012), chondrocytes (Sakai et al., 1997; Zhang and Ross, 2013), the inner root sheath of hair follicles (Miyai et al., 2010), foot process formation (Moriguchi et al., 2006; Suda et al., 2014), and the pancreas (Artner et al., 2006). Although MafB has been studied in the pancreas, the early death of the *MafB* null mice has limited the analysis to developmental MafB roles *in vivo*.

MafB is expressed in developing α- and β-cells before being restricted to the α-cells of adult rodent islets (Artner et al., 2006). MafB binds the *Glucagon* promoter and regulates transcription of this hormone (Artner et al., 2006). MafB likely plays an important role in the adult rodent α-cell, but no postnatal analysis of MafB loss in α-cells has been possible yet. Although the *MafB* null mutant mice die at birth due to central apnea or renal failure (Blanchi et al., 2003; Sadl et al., 2002), there is a 50% overall decrease in the number of insulin$^+$ and glucagon$^+$ cell numbers by e15.5 and a delay in the appearance of these hormone$^+$ cells (Artner et al., 2007; Nishimura et al., 2006). The *MafB* null embryonic pancreata also exhibit reductions in *Pdx1, MafA, Nkx6.1*, and *Slc2a2* by e18.5 (Artner et al., 2007). Furthermore, with no overall change in endocrine cell number but a loss of α- and β-cell maturity markers, the *MafB* global knockout suggests that MafB is essential for islet α- and β-cell maturation in mice (Artner et al., 2007).

A crucial developmental switch, from MafB to MafA expression, has been characterized in mouse β-cell differentiation that is believed to be necessary for β-cell maturation (Figure 10) (Artner et al., 2006; 2010; Nishimura et al., 2006). Beyond the temporal observation that MafB is expressed before MafA and then turned off postnatatlly in β-cells, MafB shares many gene targets with MafA (Artner et al., 2010). MafB regulates many β-cell factors in embryonic pancreata that are later regulated by MafA in adult β-cells (Artner et al., 2010). Interestingly, MafB is retained in about 30% of the remaining adult *MafA*$^{\Delta panc}$ β-cells, and lack of one *MafB* allele in *MafA* mutants (*MafA*$^{\Delta panc}$*; MafB*$^{+/-}$ versus *MafA*$^{\Delta panc}$) results in earlier onset of defects. Additionally, MafA production correlates with insulin^{+} cell formation in the *MafB*$^{-/-}$ animals (Artner et al., 2007). These observations indicate that MafB has some compensatory role for MafA (Artner et al., 2010; Hang et al., 2014).

It is important to note, that although similar, MafA and MafB are not biochemically equivalent. MafA is dependent on phosphorylation to dimerize and effectively bind DNA, whereas MafB is not (Guo et al., 2010). Additionally, whereas both MafA and MafB can bind the *Insulin* promoter, only MafA promotes insulin production in chick *in ovo* electroporation experiments (Table 1) (Artner et al., 2008). Interestingly, most of the regulation analysis has been conducted to compare MafB properties to the better-studied MafA that is produced in the mature rodent β-cell. Like MafA, MafB can bind and regulate *Pdx1* through its Area II control region (Vanhoose et al., 2008). A recent study, in which our lab collaborated, found that Pdx1 binds the *MafB* promoter and represses *MafB,* with deletion of Pdx1 in adult rodent β-cells contributing to the de-repression of MafB as part of the rodent α-cell phenotype (Gao et al., 2014). The role of MafB in α-cell identity and the developmental switch from MafB to MafA expression in rodent β-cells warrants more attention. Moreover, how the developmental loss of MafB impacts mature β-cells remains unknown.

	MafA	MafB
Role in rodent β-cell development	No	Yes (and islet α-cell)
Role in mature rodent β-cell function	Yes	?
Role in mature rodent α-cell function	Not produced	?
Phosphorylation-dependent dimerization and DNA binding (βTC3 cells)	Yes	No
Can bind promoter region of *Insulin* and *Glucagon*	Yes	Yes
Produced in adult rodent β-cell during pregnancy	Yes	A subset (20%)
Role in maternal rodent β-cells	?	?
Produced in adult rodent and human β-cell	Yes	No; rodent, Yes; human
Important to human β-cell function	Yes	?

Table 1. Previously known roles of MAFA and MAFB in pancreatic islet cell development and function. Zhang et al., 2005. Artner et al., 2010. Hang et al., 2014. Artner et al., 2007. Guo et al., 2010. Artner et al., 2008. Dai et al., 2011. David Scoville and Dr. Holly Cyphert, personal communication.

Unlike MafA alone in rodents, recent studies have found that MAFA and MAFB are coexpressed in the mature human β-cell (Dai et al., 2011; Wang et al., 2007). Notably, other differences in islet structure and function have been identified between rodents and humans. Some of which could be attributed to this unique MAF expression. With the limited access to human samples, model systems have remained our best means for MAF islet studies. Especially, in light of the differences between rodent and human MAF expression, there is still much to left to understand about MAFA and MAFB and their contribution to human islet cell identity and function.

Interpreting Observations from Mouse Models and Human Islet Samples

Recent studies have shown that human islets exhibit some unique functional characteristics from the more widely studied rodent model, such as higher insulin secretion levels at lower glucose concentrations (Cabrera et al., 2006; Dai et al., 2011; Henquin et al., 2006). Murine islets also exhibit a distinct architecture, with a dense core of β-cells and the other endocrine cells around the periphery of the islet in contrast to the more interspersed endocrine cell architecture of the human islet (Bosco et al., 2010; Brissova, 2005). Furthermore, β-cells make up most of the murine islet mass (60-80%), with α-cells (15-20%), PP cells, and δ-cells comprising a smaller proportion of the islets (Murtaugh and Melton, 2003), whereas human islet composition is more variable with β-cells comprising 30-75% and α-cells comprising 10-65% of the islet (Brissova, 2005). In addition to this different islet cell composition, the unique GSIS profiles of rodents and humans could be impacted by different factor expression, for example T- and P/Q-type Ca^{2+} channels appear less important to mice than in human islets, whereas R-type Ca^{2+} channels appear less important in human β-cells than suggested by previous work in mice (Braun et al., 2008). Another different example is that Glut-2 is the primary transporter for glucose uptake into rodent β-cells, versus GLUT-1 in human β-cells (De

Vos et al., 1995). Moreover, islet-enriched transcription factors (*PDX1, MAFA, MAFB*) were differentially impacted by glucose in human and mouse islet experiments, with human PDX1 and MAFA unaffected by high glucose versus the MafA and Pdx1 induction observed in mouse islets culture in high glucose concentrations. These differences may reflect unique mechanisms by which MAFA or MAFB is linked with glucose uptake and metabolism in human versus rodent islets (Dai et al., 2011).

Although the human MAFB expression pattern is different from that of rodents, the mouse model remains a useful experimental tool for MAF factor islet studies. Human islets and human β-cell lines, like the EndoC-βH1 line used in Chapter IV, are informative; but they fail to recapitulate the development and function of a whole organism. Limited availability and heterogeneity of human islets could preclude elaborate biochemical experimentation in these human samples. Additionally, specific developmental timepoints of human islet samples are even more difficult to obtain and *ex vivo* manipulation of human islets could confound experimental results. For example, islet cells need to be dissociated to efficiently knock down a gene of interest. Islet dissociation disrupts normal islet structure and, thus, islet function. Moreover, the ethical limitations of *in vivo* human islet cell experiments preclude these analyses, making genetically manipulatable rodent models most valuable for whole body assessments in future studies.

Intricate genetic cell line studies and *in vivo* rodent studies provide novel mechanistic information, but these discoveries are only useful for clinical applications if we can translate these findings to human patients. Among the different translational tools, examination of more closely related species to humans, like other primates, could facilitate interpretations from rodent studies. Ultimately, identifying key differences in human and rodent islet function and what drives these differences is a necessary step

towards developing better tools to study human β-cells and applying what we know from our rodent model system to humans.

Dissertation Overview

The studies presented in this body of work are directed towards further understanding how the large MAF factors impact human islet cell identity, function, and pathophysiology. Chapter II provides a comparative overview of what is known of transcription factors in developing human and rodent β-cells. In Chapter IV, I present my work with mouse models that reveal not only the impact of MafB alone on postnatal β- and α-cells, but also the overall Maf requirement with a model lacking both MafA and MafB in the pancreas. Then I examine different MAF expression observed in nonhuman primates (NHP) with further comparative analyses of NHP to rodent and human islets. I then use a human β-cell line to provide evidence for how this distinct MAFB expression, observed in NHP and humans alike, affects human β-cell function. Chapter V introduces a different mouse model used to examine how MafB could be contributing to the β-cell adaptations of pregnancy. In Chapter VI, I present ideas and experimental continuation for this work that could advance our understanding of MAFA and MAFB function. This thesis elucidates the islet cell Maf requirement, further examining the interrelationship of MafA and MafB, and the specific impact of MAFB on human β-cell function.

Chapter II

REVEALING TRANSCRIPTION FACTORS DURING HUMAN PANCREATIC β-CELL DEVELOPMENT

This section contains the review published in *Trends in Endocrinology & Metabolism* by Conrad, Stein, and Hunter.

Conrad, E., Stein, R., Hunter C.S. 2014. Revealing Transcription Factors During Human Pancreatic β-cell Development. *Trends Endocrinol Metab* 25(8):407-14.

Introduction

To reverse diabetes, transplantation of β-cells is a promising replacement therapy, but limitations that include tissue rejection and low donor availability pose a challenge to widespread application (Muoio and Newgard, 2008). With the need for innovative therapies to better treat the growing numbers of patients with diabetes, research has focused on understanding the molecular mechanisms promoting β-cell formation and identity. Transcription factors are gene regulatory proteins that play an integral role in islet cell development, directing cell fates by regulating transcription of genes involved in specification and ultimately mature function. Much of what is known of transcription factors in β-cell development has been revealed in rodent model systems, such as genetically manipulated mouse models and cell lines (reviewed in (Cano et al., 2013)). Although these models remain our best tools for study, there are notable distinctions between mouse and human pancreas with implications in development and function.

We focus the discussion on human β-cell transcription factors, and provide an overview of what is known about their expression and function during development and how this parallels the expression profile in rodents (Figure 11). Human loss-of-function mutations that have revealed similar roles for many transcription factors in β-cells will be discussed. We will also address how knowledge of human β-cell transcriptional regulation is being applied toward generating therapeutic β-cells.

Transcriptional Regulation of Human β-Cell Development

Human and rodent pancreatic islets are comprised of five hormone-secreting endocrine cell types. During human and rodent development, early endodermal tissue becomes specified toward a pancreatic fate prior to evagination of dorsal and ventral pancreas buds (Pan and Wright, 2011). These buds are populated with multipotent pancreatic progenitor cells (MPCs). Signaling and transcriptional events then promote the MPCs towards acinar, endocrine, or ductal fates. Once specified, endocrine progenitors undergo further differentiation with lineage-specific transcription factors promoting final maturation steps.

Importantly, several key differences have been observed between species. Human islets comprise a lower proportion of β-cells, with more α- and δ-cells than mouse islets (Brissova, 2005). Islet architecture differs, with human β-cells being dispersed among α- and δ- cells, whereas mouse islets maintain a β-cell core surrounded by the four other endocrine cell types (Cabrera et al., 2006). Moreover, two genes transcribe *Insulin* in rodents (*Ins1, Ins2*), whereas only one is present in humans (Melloul et al., 2002). Human islets were found to secrete more insulin at baseline glucose levels, but less in response to a stimulatory glucose challenge, compared to mouse islets (Dai et al., 2011). As we will discuss further, human and rodent β-cells share many transcription

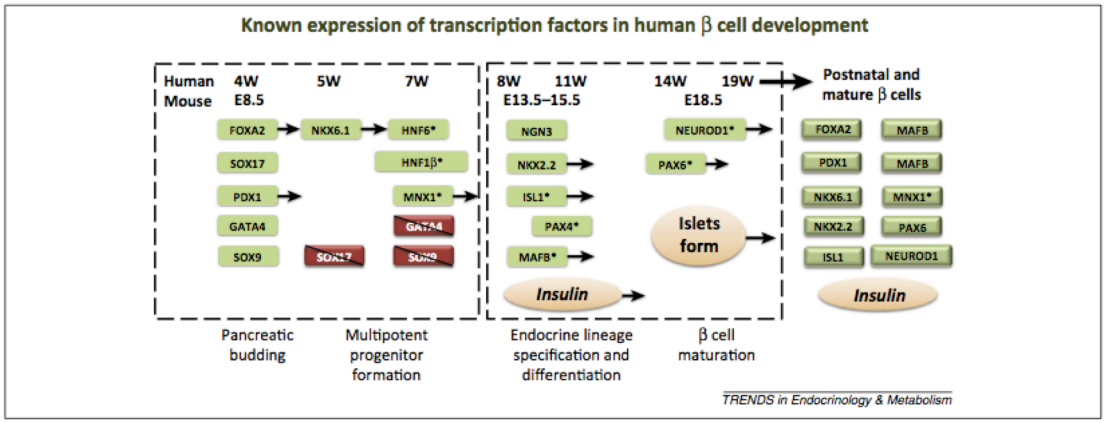

Figure 11. Known expression of transcription factors in human β-cell development. Approximate timeline of transcription factor expression during human β-cell development. A relative course of human and mouse β-cell development is depicted. Transcription factors (in shaded boxes) are listed under each time-point where their expression is first observed, or lost, according to the literature. For comparison, approximate mouse developmental points are included under the human timeline. Early [i.e. pancreatic budding and multipotent pancreatic progenitor cell (MPC) formation] and late (i.e. lineage specification and β-cell maturation) stages of pancreas formation are approximated by labels and dashed boxes. Some transcription factors are marked with an *, denoting that whole pancreas mRNA was used to characterize expression, thus cell type-specificity was not determined. Transcription factors with arrows extending denote expression that persists into postnatal and/or mature β-cells. Abbreviations: E, embryonic day; W, weeks gestation.

factors during development and in adult function, but their expression pattern, timing, and overall activities may differ (Figure 11).

In addition to fetal studies describing human transcription factor expression, functional roles for many transcription factors have been revealed in genome-wide analyses of various forms of diabetes. For example, Maturity Onset Diabetes of the Young (MODY) genes are responsible for rare forms of diabetes that are caused by single gene mutations (Ashcroft and Rorsman, 2012). Although MODY accounts for only 1-2% of diagnosed cases, their monogenic nature links these factors to roles in β-cell identity, having implications for T2DM and T1DM. Neonatal diabetes mellitus is a more rare monogenic form of non-autoimmune diabetes that can be permanent or transient after presenting in the first six months of life. Several recent reviews have focused on genes studied in these contexts (Ashcroft and Rorsman, 2012), therefore here we will briefly note transcription factors that are most commonly involved and which appear to act by affecting human β-cell development and/or function.

Foregut Development and Budding

About 4 weeks into human gestation, the dorsal pancreatic bud emerges, followed by the ventral bud. This early phase of pancreas specification is marked by several transcription factors that are also linked to early mouse development (Piper et al., 2004). Many of these factors are expressed throughout organogenesis, and have unique roles in distinct temporal windows and populations. Early human pancreas studies are particularly limited, thus many additional factors are likely involved in this early process.

Transcription Factors Involved in Early Stages of Development

Forkhead box A2 (FOXA2): During early pancreas development, the *FOXA2* transcription factor is consistently expressed from week four forward, as examined in recent fetal pancreatic human studies (Jennings et al., 2013; Jeon et al., 2009; Lyttle et al., 2008). This expression profile is similar to broad mouse FoxA2 expression throughout pancreas development, acting as a pioneer factor to regulate *Pdx1*, a relationship that has not been determined in humans (Gao et al., 2008; Pan and Wright, 2011). FOXA2 persists in all mature pancreatic cell types of both mice and humans (Cano et al., 2013; Pan and Wright, 2011).

SOX17: In contrast to FOXA2, expression of the HMG box transcription factor SOX17 is observed just before four weeks in humans and is then excluded from pancreatic cells about a week later, similar to the down-regulation of *Sox17* during mouse pancreas development (Jennings et al., 2013; Piper et al., 2004). Studies in mice have indicated that while early Sox17 expression is necessary for endoderm formation, it later represses the pancreatic fate (Spence et al., 2009).

Hepatocyte nuclear factor (HNF6): Also known as *Onecut1* or *Oc1,* mRNA analysis of human pancreas aged 7-21 weeks demonstrated that *HNF6* is consistently expressed (Jeon et al., 2009; Lyttle et al., 2008). This parallels mouse *Hnf6* expression at e8.5 with broad expression throughout development, directing endocrine allocation until just before birth when it becomes restricted to duct and acinar cells (Zhang et al., 2009a).

Hepatocyte nuclear factor (HNF1β): A high level of *HNF1β* expression begins as early as seven weeks in humans, and persists throughout pancreatic development (Jeon et al., 2009). Interestingly, heterozygous loss-of-function *HNF1β* mutations (termed MODY5)

result in diabetes in humans but only homozygous mutations produced diabetes in mice (Horikawa et al., 1997). This could be due to a potentiated single wave of human endocrine differentiation versus the two phases observed in rodents, rendering these human cells more sensitive to HNF1β dosage (Cano et al., 2013). *Hnf1β*-deficient mice, exhibit pancreatic agenesis by e13.5, suggesting the role for Hnf1β in pancreas development is evolutionarily conserved (Haumaitre et al., 2005).

Pancreatic and duodenal homeobox 1 (PDX1): Also known as insulin promoter factor 1 (IPF1), PDX1 has been studied for its role throughout all phases of pancreatic development. PDX1 is broadly expressed at around four weeks with a high level of expression restricted later to adult human β-cells (Jennings et al., 2013; Lyttle et al., 2008). Pdx1 high expression is specific to rodent β-cells by e15.5, where it regulates *Insulin 1* (*Ins1*), *MafA,* and *Pdx1,* whereas only low-level expression is observed in the exocrine pancreas (reviewed in (Pan and Wright, 2011)). Based on the staging of the surrounding tissue morphology, PDX1 appears slightly later in human development than in mice. Expression is only evident after the notochord and aorta are separate from the dorsal foregut in humans (Jennings et al., 2013; Jeon et al., 2009). PDX1 expression is also observed in human adult duct cells, thus it remains unclear if human *PDX1* is regulated in a similar manner (Castaing et al., 2005; Heimberg et al., 2000). Otherwise, PDX1 spatial expression compares with mouse Pdx1 first seen in the pre-pancreatic endoderm around e8.5 (Ahlgren et al., 1996). Mouse lineage-tracing studies demonstrated that $Pdx1^+$ cells mark progenitors of all the mature pancreatic cell types including endocrine, acinar, and ductal cells (Gu et al., 2002). Similar to mice with targeted disruption of *Pdx1,* homozygous inactivating mutations of *PDX1/IPF1* result in pancreatic agenesis (termed MODY4) (Stoffers et al., 1997a; 1997b). Autosomal

recessive mutations in the *PDX1* locus have also been reported to cause permanent neonatal diabetes, comparable to the *Pdx1*$^{+/-}$ mice that likewise exhibit a diabetic phenotype (Brissova, 2004). PDX1 levels were compromised in rodent models of T2DM and human T2DM islets, suggesting conservation in adult islet β-cells (Guo et al., 2013b).

Pancreas transcription factor 1A (PTF1A): *PTF1A* is barely detectable by quantitative RT-PCR until midgestation in whole human fetal pancreas, presumably due to its enriched expression at that timepoint in acinar cells. It is better characterized in mice, with broad expression at e9 in dorsal and ventral pancreatic buds that is later restricted to acinar cells (Jeon et al., 2009; Obata et al., 2001). Mutations in the *PTF1A* locus result in autosomal recessive cases of permanent neonatal diabetes that require insulin for survival (Sellick et al., 2004). This is similar to *Ptf1a*$^{-/-}$ mice, which die postnatally with impaired pancreatic development (Krapp et al., 1998). A recent study identified mutations in the human *PTF1A* enhancer resulting in pancreatic agenesis (Weedon et al., 2013). These human and mouse mutant phenotypes support an evolutionarily conserved role during early pancreatic development.

GATA binding protein 4 (GATA4): This transcription factor is expressed during early human pancreas budding between 4 and 5 weeks of age, but then becomes drastically reduced in pancreatic progenitors, remaining only in mature acinar cells (Jennings et al., 2013). This pattern is comparable to mice (Xuan et al., 2012). Although human and mouse *GATA4/Gata4* mutations have been associated with congenital heart defects and a pancreatic phenotype in mice, it was not until recently that a human pancreatic phenotype was observed (Cano et al., 2013; Garg et al., 2003; Shaw-Smith et al., 2014).

SOX9: SOX9 is found in PDX1^{+} cells in early human and mouse pancreas by about 4 weeks and e9, respectively, and is then excluded from mature endocrine cells (Cano et al., 2013; Jennings et al., 2013; McDonald et al., 2012; Pan and Wright, 2011). Sox9 is necessary for the maintenance of multipotent progenitor populations in mice (Jennings et al., 2013; McDonald et al., 2012; Pan and Wright, 2011; Piper et al., 2002). The mouse *Sox9*$^{+/-}$ model phenocopies *SOX9* haploinsufficiency in humans, with islet hypoplasia from failed maintenance of endocrine progenitors (Piper et al., 2002; Seymour et al., 2008; Sosa-Pineda et al., 1997).

Homeobox Protein NK-6 Homolog (NKX6.1): Human NKX6.1 is expressed in early multipotent pancreatic progenitors after 4 weeks, once SOX17 is excluded from the pancreatic buds(Jennings et al., 2013). Then its expression becomes restricted to β-cells by 14-16 weeks (Brissova, 2004; Jennings et al., 2013). Similarly, early rodent Nkx6.1 expression is broad, then gradually becomes β-cell-specific (Jennings et al., 2013; Sander et al., 2000; Sosa-Pineda et al., 1997). *Nkx6.1* null mice exhibit a severe reduction in β-cells, and conditional *Nkx6.1* mutants reveal its requirement for specifying endocrine precursors toward a β-cell lineage (Hang and Stein, 2011; Sander et al., 2000; Schaffer et al., 2013). In adult pancreas, NKX6.1 is a key β-cell identity factor with severely reduced expression in diabetic and obese *Db/Db* mice and human T2DM islets (Dai et al., 2011; Guo et al., 2013b).

Motor neuron and pancreas homeobox 1 (MNX1): *MNX1*, also known as Homeobox HB9 (HLXB9), is expressed as early as 7 weeks in the developing pancreas, then its expression is reduced to lower levels by 14 to 16 weeks into gestation (Jeon et al., 2009; Lyttle et al., 2008). *MNX1* transcripts have been identified in the adult human pancreas, although the cell-type distribution has yet to be characterized (Jeon et al., 2009; Pan and

Wright, 2011). It is unknown whether MNX1 becomes progressively restricted to β-cells, as found in mice. Detailed expression analysis found mouse Mnx1 in the e9.5 endoderm, which is then gradually restricted to the $Pax6^+$ endocrine population by e15.5, and finally only in adult β-cells (Cano et al., 2013; Li et al., 1999). Recently, a patient presenting with permanent neonatal diabetes was found to harbor a homozygous mutation within the DNA-binding homeodomain of MNX1 (Bonnefond et al., 2013; Flanagan et al., 2014). Similar to the null mouse model, these patients had no obvious exocrine deficits but exhibited reduced β-cell numbers and likely dorsal pancreatic lobe agenesis (Cano et al., 2013; Flanagan et al., 2014; Li et al., 1999).

Multipotent Pancreatic Progenitor Cells

Continued expression of the transcription factors FOXA2, PDX1, SOX9, NKX6.1 and GATA4 in developing human pancreatic cells likely demarcates the MPCs that will be further restricted to ductal, endocrine, and exocrine compartments (Cano et al., 2013). The MPC population also controls ultimate pancreas size (Stanger et al., 2007). Most of the factors described above also have distinct functional roles in later differentiated acinar, islet, and ductal populations.

GATA6: Another MPC-expressed transcription factor, GATA6, appears more important during human pancreas development than in mice, with $Gata6^{+/-}$ mice exhibiting no obvious phenotype (De Franco et al., 2013). However, *de novo* heterozygous human *GATA6* mutations, often in the DNA-binding domain, cause pancreatic agenesis (Allen et al., 2011; Bonnefond et al., 2012). Also important for rodent β-cell generation, Gata6 is expressed in multipotent pancreatic progenitors and $Gata6^{-/-}$ embryos have fewer $Pdx1^+$ cells compared to heterozygous controls (Watt et al., 2007). Strikingly, *Gata6/Gata4* compound mouse mutants present with similar pancreatic agenesis as seen in *GATA6*

patients (Xuan et al., 2012). The human fetal temporal GATA6 expression pattern has yet to be determined (Cano et al., 2013; Jennings et al., 2013).

Endocrine Cell Specification

After MPCs commit to pancreatic acinar, ductal, or endocrine fate, a host of transcription factors are required in mice for production of islet endocrine cell lineages, discussed below. There is evidence that these factors are also important in human endocrine commitment.

Transcription Factors Involved in the Production of Islet Endocrine Cell Lineages

Neurogenin 3 (NGN3): Coincident with SOX9 loss**,** endocrine commitment in pancreatic epithelial cells initiates with NGN3 expression, a factor also required for endocrine cell specification in mouse (Gradwohl et al., 2000; Jennings et al., 2013; Lyttle et al., 2008). NGN3 is seen as early 8 weeks and becomes more highly expressed around 11 weeks, then expression subsequently declines to only low levels at 19 weeks (Capito et al., 2013; Gradwohl et al., 2000; Jennings et al., 2013; Jeon et al., 2009). Later induction of human transcription factors including *ISL1*, *NEUROD1, MAFB, NKX2.2,* and *PAX6* near week 15 indicates that *NGN3* expression precedes the expression of these factors that are implicated in late endocrine cell differentiation (Jeon et al., 2009). Similarly, these islet-enriched factors act downstream of Ngn3 in mice (Cano et al., 2013; Gradwohl et al., 2000). A rare *NGN3* null mutation resulted in permanent neonatal diabetes with no histologically detectable islets, although the patient maintained low C-peptide levels (Rubio-Cabezas et al., 2011). Similarly, *Ngn3*$^{-/-}$ mice develop diabetes and die a few days after birth with a complete lack of endocrine cells (Gradwohl et al., 2000).

Regulatory factor X 6 (RFX6): By quantitative real-time PCR, pancreatic *RFX6* expression is limited to adult human islet cells, and autosomal recessive mutations at this locus result in neonatal diabetes, with absence of insulin^{+}, glucagon^{+}, and somastostatin^{+} cells (Smith et al., 2010). Similarly, *Rfx6*-deficient mice exhibit impaired formation of all endocrine cell types except for pancreatic polypeptide (Smith et al., 2010). Although Rfx6 is expressed more broadly and earlier than Ngn3 during mouse development, Rfx6 expression is not detected in Ngn3$^{-/-}$ mice. Interestingly, human NGN3 mutations result in milder diabetes cases than *RFX6* mutations (Taleb and Polychronakos, 2011). Thus, RFX6 could act either upstream or downstream of NGN3 in coordinating production of a subset of islet cell types (Smith et al., 2010; Taleb and Polychronakos, 2011).

Paired box gene 4 (PAX4): Human *PAX4* expression is evident by 9 weeks in whole fetal pancreatic mRNA analysis (Jeon et al., 2009). Although its spatial pattern has yet to be reported in humans, Pax4 is found in mouse endocrine progenitors and later in β-cell precursors, as a regulator of β-cell commitment (Sosa-Pineda et al., 1997). Further, *PAX4* (termed MODY9) mutations result in diabetic symptoms resembling those of *Pax4*$^{+/-}$ mice (Plengvidhya et al., 2007). Mutant mice also exhibit a severe reduction in β-cells and abnormal α-cell clustering, similar to *Pdx1*$^{+/-}$ mice (Brissova, 2004; Sosa-Pineda et al., 1997).

GLIS family zinc finger 3 (GLIS3): Patients with *GLIS3* mutations present with autosomal recessive diabetes and impaired islet cell development (Senée et al., 2006). The *Glis3*-deficient mouse pancreatic phenotype is similar (Watanabe et al., 2009). This is further supported by a study indicating that Glis3 may interact with Hnf6 to regulate *Ngn3*

expression (Kim et al., 2012). The expression pattern has yet to be determined in humans.

MAFB: Unlike in mice where β-cell *MafB* expression diminishes postnatally, human *MAFB* increases from 7 to 21 weeks then remains in mature α- and β-cells (Dai et al., 2011; Hang and Stein, 2011; Jeon et al., 2009). Sustained MAFB expression may have functional implications in β-cell development and identity. Indeed, severe reductions in MAFB were found in human T2DM islet α- and β-cells, suggesting a role in their functional maintenance (Guo et al., 2013b).

Endocrine Cell Differentiation and Maturation

Pancreatic hormone expression first occurs about 8 weeks into human gestation, with the appearance of insulin$^+$ cells, which become more abundant by week 9 when glucagon$^+$ cells also appear (Jeon et al., 2009; Polak et al., 2000). In rodents, two waves of endocrine development have been observed. A first wave from about e9.5-12.5 is characterized by insulin and glucagon coexpressing cells, whereas the second wave, from about e12.5 to birth, produces endocrine cells that will populate mature islets (Herrera, 2000). By contrast, human development lacks two waves of endocrine cell formation (Cano et al., 2013; Jennings et al., 2013). Several islet-enriched transcription factors have been implicated in mouse and human β-cells differentiation. Here we will discuss these factors in relative order of expression during human development.

Transcription Factors Involved in β-Cell Differentiation

NKX2.2: Another key difference between mice and humans is seen with NKX2.2 expression (Jennings et al., 2013). Although its expression is only observed later in human α- and β-cells by 14-16 weeks, Nkx2.2 expression is observed earlier in rodent

development around e9.5 (Jennings et al., 2013; Lyttle et al., 2008; Sussel et al., 1998). Only later in development does NKX2.2/Nkx2.2 expression overlap, with rodent Nkx2.2 restricted to β-cells and a subset of α- and PP cells (Jennings et al., 2013; Lyttle et al., 2008; Sussel et al., 1998). This implies a more limited role for NKX2.2 in human β-cell differentiation.

Insulin gene enhancer protein ISL-1 (ISL1): ISL1, also called ISLET1, appears to be required for pancreatic development in humans and mice (Shimomura et al., 2000). Isl1 is a pan-endocrine cell marker, with endocrine *Isl1* mouse mutants becoming diabetic, exhibiting impaired islet cell maturation and reduced postnatal islet mass expansion (Ahlgren et al., 1997; Du et al., 2009). A nonsense mutation in a Japanese T2DM patient implicated a role for ISL1 in the maturation of functional human β-cells (Shimomura et al., 2000). In humans, its expression has been observed in the fetal pancreas at age 8-10 weeks, and expression then gradually increases from midgestation (Jeon et al., 2009; Lyttle et al., 2008). This is similar to the situation in mice, where Isl1 is first expressed broadly in the pancreatic mesenchyme at e9 and is then maintained in the mature hormone$^+$ endocrine cells (Ahlgren et al., 1997).

Neurogenic differentiation 1 (NEUROD1): NEUROD1 is expressed at week 15 and is then found in all endocrine cell types of adult islets (Jennings et al., 2013; Jeon et al., 2009; Lyttle et al., 2008). However, *NeuroD1* expression occurs relatively earlier mouse in development-by e10.5-but is similarly restricted to the endocrine compartment (Gu et al., 2010; Naya et al., 1995). Although rare cases of heterozygous *NEUROD1* (termed MODY6) mutations have been reported, the phenotype of homozygous *NEUROD1* mutations appear similar to the mutant mouse phenotype, causing autosomal recessive neonatal diabetes (Malecki et al., 1999; Rubio-Cabezas et al., 2010). β-cell-specific

mouse mutants revealed that *NeuroD1* is required for β-cell maturation because the β-cells formed are immature, with increased glycolytic gene expression, Neuropeptide Y overexpression (a hormone whose expression in islets normally decreases after birth), and elevated basal insulin secretion (Gu et al., 2010).

PAX6: PAX6 is induced by 14-16 weeks in the human pancreas and is then maintained in all adult islet cells (Lyttle et al., 2008). This is similar to the known Pax6 expression pattern in mice (Sander et al., 1997). *Pax6* null mice die at birth from brain abnormalities, but embryos have reduced islet cell numbers, impaired hormone synthesis, and defective islet morphogenesis, indicating a role in endocrine cell allocation and differentiation (Sander et al., 1997). The only study linking human *PAX6* to β-cell function identified a common single-nucleotide polymorphism that resulted in reduced PAX6 mRNA associated with reduced insulin response and sensitivity (Ahlqvist et al., 2011).

MAFA: Mouse MafA is expressed relatively late in development and is found only in second wave insulin$^+$ cells that will become mature β-cells (Hang and Stein, 2011). In adults, MafA is known as a maturation marker, crucial for glucose-responsive β-cells through regulation of *Insulin* and *Glut2* (also known as Slc2a2, solute carrier family 2 facilitated glucose transporter, member 2) (Hang and Stein, 2011). Similar to the late onset of expression in developing rodent β-cells, MAFA is nearly undetectable in embryonic human samples from 7-21 weeks (Guo et al., 2013b; Hang and Stein, 2011; Jeon et al., 2009). Later, in mice and humans, MAFA is specifically expressed in mature adult β-cells (Dai et al., 2011). Recently, reductions in MAFA levels were found in *db/db* mice and in human T2DM islets, potentially a signature of dysfunctional β-cells (Guo et al., 2013b).

Applying Knowledge of Transcriptional Control to Promote Human β-cell Fates

A collective goal of islet biologists is to apply what has been learned of transcription factor expression and function toward creating renewable and transplantable therapeutic β-cells. Several strategies have been developed, including *in vitro* human ES cell directed differentiation (D'Amour et al., 2006; Kroon et al., 2008) and modulation of existing β-cell proliferation (reviewed in (Ackermann and Gannon, 2007)). Transcription factors important during β-cell development and function can be used in approaches to convert non β-cells into β- (or β-like) cells. Several examples of mouse cell conversion have been described, which can guide studies in human cells. Zhou et al. converted mouse acinar cells to β-like cells using an adenovirally-delivered combination of MafA, Pdx1 and Ngn3 (Zhou et al., 2008), whereas α-to- β-cell conversion was observed after forced Pdx1 (Yang et al., 2011) and Pax4 (Collombat et al., 2009) overexpression, or *Arx* (aristaless related homeobox) inactivation (Courtney et al., 2013) in the developing pancreas. Further, conversion of non-pancreatic cells has been promising, including converting liver cells to insulin$^+$ cells (Banga et al., 2012; Ber, 2003; Kojima et al., 2003). However, it is possible that the cells produced still retain non β-cell phenotypes, an obvious impediment for use in human studies.

Recent studies have demonstrated that the epigenetic landscapes of β- and non β-cells are unique, suggesting that transcription factor-mediated cell type conversion is impacted by chromatin modifications. Dhawan and colleagues reported that mouse β-cell loss of the Dnmt1 DNA methyltransferase induces de-repression of the *Arx* transcription factor gene and subsequent conversion into α-cells (Dhawan et al., 2011). These findings lend evidence the plasticity of these cells and the potential for interconversion of endocrine cells for the generation of β-cells. Histone acetylation/deacetylation also influences cell type specification. Haumaitre and colleagues treated cultured rat embryonic pancreas rudiments with histone deacetylase

inhibitors, finding an increase in the Ngn3$^+$ endocrine pool and enhanced β- and δ-cell lineage allocation (Haumaitre et al., 2008). The above studies suggest plasticity exists between the various foregut or posterior foregut endoderm-derived cell populations. Mouse studies appear to serve as proof-of-principal that cells can undergo functional β-cell conversion. However, given differences between mouse and human islet development, architecture, and glucose-sensing properties, a next step is to employ available human samples for conversion experiments.

Several recent reports utilized human tissue and cell lines to demonstrate similar cellular plasticity as observed in mice. Reprogramming non-pancreatic cell types into insulin$^+$ cells was achieved using ectopic PDX1 expression in human liver cells (Zalzman et al., 2005; 2003) and keratinocytes (Mauda-Havakuk et al., 2011). Pennarossa et al. utilized a DNA methyltransferase inhibitor to convert skin fibroblasts into cells that expressed pancreatic transcription factors and insulin C-peptide, and could reverse streptozotocin (STZ)-induced diabetes in SCID mice (Pennarossa et al., 2013). These studies indicate that altering the transcription factor expression profile and chromatin modification state of a human cell may enhance conversion to insulin$^+$ cells. However, much like the mouse studies described above, it remains unclear how closely the trans-differentiated cells resemble true β-cells.

To this end, many are focusing on examining the level of plasticity between closely related human pancreatic cell types. Recently, human exocrine tissue was directly converted into a ductal phenotype, with lineage-traced exocrine cells expressing ductal markers CK-19, HNF1β, and SOX9 (Houbracken et al., 2011). Similarly, human ductal cells are also able to be converted to β-like because Ngn3 alone or added with MafA, Pdx1, and Pax6 was able to activate an endocrine program and produce insulin$^+$ cells, although these cells are only β-like and are likely to lack the glucose-sensing and insulin secretion properties of endogenous β-cells (Heremans, 2002; Lee et al., 2013;

Swales et al., 2012). Perhaps more tantalizing is the direct conversion between human α- and β-cell phenotypes without addition of exogenous transcription factors. As proof-of-principle for human cell conversion, Spijker et al. used dispersed human donor islets and lineage-tracing methods to illustrate conversion of β-cells to α-like cells (Spijker et al., 2013). This was shown to be mediated by ARX, an essential regulator of α-cell development (Spijker et al., 2013). Recently, Bramswig et al. established that human α-cells possess an epigenetic profile more amenable to conversion than other pancreatic cell types, including β-cells (Bramswig et al., 2013). α-cells retain bivalently marked histones (i.e. activating H3K4me3 and repressing H3K27me3), particularly at β-cell signature genes such as *MAFA* and *PDX1*, suggesting the human α-cell epigenetic landscape is primed for cellular conversion (Bramswig et al., 2013). Treatment with a histone methyltransferase inhibitor impacted H3K27me3 levels at β-cell genes within α-cells, allowing for partial α-to-β conversion (Bramswig et al., 2013). Consideration of epigenetics during reprogramming or in hES cell differentiation was suggested in another recent study. The authors utilized the Novocell protocol to direct hES cells while monitoring epigenetic and gene expression profiles (D'Amour et al., 2006; Kroon et al., 2008; Xie et al., 2013). The key findings included the dysfunctional *in vitro*-produced polyhormonal cells having inappropriately remodeled chromatin, as compared to primary human islets. This dysfunction was linked to a failure to eliminate polycomb group-mediated repression of endocrine-specific genes. These studies collectively suggest careful modification of transcription factors and epigenetic profiles allows for conversion of pancreatic and non-pancreatic cells into *bona fide* β-cells.

Concluding Remarks and Future Perspectives

Future studies of human β-cell transcription factors will not only yield insight into β-cell conversion protocols, but will also provide a foundation for development of novel *in*

vitro tools for studying β-cell development (Cano et al., 2013). Although transcription factor profile manipulation will certainly facilitate efforts toward protocols that convert non β-cells to β-cells, it is important to note that insulin expression alone does not yield a functional β-cell with the appropriate secretory machinery to maintain glucose homeostasis. Regardless, there is a need for innovative genetic systems that enable deeper analysis of human β-cells, as compared to mouse. For example, iPS-cells derived from MODY patients have the potential to elucidate protein interactions and expression cascades dysregulated in patients (Teo et al., 2013). Functionally immortalized human β-cell lines have recently become available, and these will allow for numerous *in vitro* studies of human transcription factor function (McCluskey et al., 2011; Ravassard et al., 2011). Although rodent models have been essential to understanding transcription factor roles in pancreatic organogenesis, key differences in human islet expression and function place limits on what can be learned without consideration of the human context (Ashcroft and Rorsman, 2012). Greater comprehension of the transcriptional regulation that defines a human β-cell will benefit from more sensitive genetic tools, better markers for purifying human islet cells, and increased availability of human samples and cell lines (Cano et al., 2013; Pagliuca and Melton, 2013).

Chapter III

MATERIALS AND METHODS

Generation of the Pancreas-Specific, Endocrine-Specific, and β-Cell-Specific Knockout Mice

Pancreas-wide deletion mutants of *MafA* and *MafB* were generated by crossing $MafA^{fl/fl}$ (Artner et al., 2010) and $MafB^{fl/fl}$ (Yu et al., 2013) mice with $Pdx1^{5.5}$-*Cre* mice (Gu et al., 2002), which produces Cre in pancreatic progenitor cells prior to MafA and MafB expression. $MafA^{fl/fl}$; $MafB^{fl/fl}$; $Pdx1^{5.5}$-*Cre* and $MafB^{fl/fl}$; $Pdx1^{5.5}$-*Cre* were referred to as $MafAB^{\Delta panc}$ and $MafB^{\Delta panc}$ mice, respectively. $MafAB^{fl/fl}$ and $MafB^{fl/fl}$ mice were used as controls. Pancreatic endocrine-cell-specific *MafA* and *MafB* deletion mutant mice were generated with *Neurogenin 3 (Ngn3)-Cre* mice (Schonhoff et al., 2004); referred to as $MafAB^{\Delta endo}$. Chi^2 analysis of expected versus actual genotypes at time of genotyping was performed on $MafAB^{\Delta panc}$ and $MafAB^{\Delta endo}$ litters. β-cell-specific *MafB* deletion mice were generated by crossing and $MafB^{fl/fl}$ (Yu et al., 2013) mice with *Rat Insulin* $Promoter^{Mgn}$*(RIP)-Cre* mice (Postic et al., 1999). $MafB^{fl/fl}$; *RIP-Cre* mice were referred to as $MafB^{\Delta\beta}$ mice. For embryonic samples, day 0.5 was counted as the day the vaginal plug was observed. All studies with mice were in compliance with protocols approved by the Vanderbilt Institutional Animal Care and Use Committee.

Islet Isolation Conditions

Two-week-old mouse pancreata were partially digested with 1mg/mL collagenase followed by handpicking of islets. 10- to 12-week-old male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) and 8-week-old $MafB^{\Delta panc}$ islets were collected from

mice that were anesthetized and killed via cervical dislocation. Collagenase P (Roche) (1mg/mL) in PBS was injected into the common bile duct with the end connecting to the duodenum clamped. The pancreas was then dissected and further digested in collagenase P in a shaking 37°C water bath before the reaction was quenched with 0.5% Bovine Serum Albumin (Sigma) in PBS. Islets were handpicked under 10x magnification. NHP (rhesus macaque) pancreata were obtained from 5 females and 5 males (average age 8.8±1.3 years; range 0.32-13 years) as excess material under unrelated protocols approved by the Oregon National Primate Research Center Institutional Animal Care and Use Committee. Islet isolation was initiated within 10-15 minutes of exsanguination by cannulation and perfusion through the pancreatic duct with collagenase/neutral protease solution in a Ricordi apparatus. When islet release was evident by dithizone staining, islets in digestion solution were concentrated and washed by centrifugation and purified using a COBE 2991 cell processor. Human islets were provided by the Integrated Islet Distribution Network (http://iidp.coh.org/; 30 total preparations, 11 female and 19 male donors, age 38.4±2.4 years [range, 17-60], BMI 25.99±0.55 kg/m^2 [range 18.8-29.7]). Cause of death was head trauma (n=11), neurological events (stroke, subarachnoid hemorrhage etc., n=10), anoxia (n=2), or unknown (n=7). The cold ischemia time before pancreas isolation was 9.9±1.1 hours (range, 1.5-24.9 hours). Because the purity of these preparations varied greatly (30–90% as designated by the isolation center), human and NHP islets were handpicked on the day of arrival prior to further analysis. Some islet preparations were incubated in dithizone for 30 min and then viewed by light microscopy for purity and islet count. All studies with human and NHP islets were in compliance with the Vanderbilt Institutional Animal Care and Review Board Committee.

RNA Analysis

For Quantitative real time-PCR (qPCR), *MafB*$^{\Delta panc}$ and *MafB*$^{fl/fl}$ control islet RNA was isolated using Trizol (Invitrogen) and then the DNA-Free RNA Kit (Zymo Research). Samples found to have RINs greater than 7 by the FSGR were used. The RNA was transcribed using iScript reverse transcriptase and iScript reaction mix (Bio-Rad) and qPCR was conducted on a Light Cycler 480 II using SYBR Green (with dissociation curve program). Primer sequences are provided (Table 2A). Isolated islets from NHP, human, and mouse (e.g. C57BL/6J) (100-200 islets) were added to 350-700 uL Lysis/Binding Solution in the RNAqueous Small Scale Phenol-Free Total RNA isolation kit (Ambion), with trace DNA contamination removed with TURBO DNA-free (Ambion). RNA bioanalysis, quality control, and quantity assessment (QC/QA) was conducted in the Vanderbilt Functional Genomics Shared Resource (FGSR) core lab with only samples having a 28S/18S ratio from 1.4 to 2.0 and an RNA integrity number (RIN) greater than 7 used in experiments. Preloaded arrays (Applied Biosystems) of 16 genes were used to determine their expression levels in NHP, human, and C57BL/6J mouse islets, with 6 of these being stably expressed in islets from all species. Four of these genes (*18S/18s, ACTB/ActB, TFRC/Tfrc, TBP/Tbp*) were used in the study for normalization of genes of interest to enable comparison of the mRNA levels between NHP, human, and mouse islets. Primer sets are provided (Table 2B). All reactions were performed in duplicate with reference gene normalization and median cycling threshold values were used for analysis. The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) was followed for assessing all gene expression data (Bustin et al., 2009).

A.

Gene	Forward	Reverse
MafA	CCT GTA GAG GAA GCC GAG GAA	CCT CCC CCA GTC GAG TAT AGC
MafB	ACC TCG CCG GCT TCT CTA C	GAG CTG CCC CGG AGC TT
Ins2	GGC TTC TTC TAC ACA CCC AT	CCA AGG TCT GAA GGT CAC CT
PreIns2	GGG GAG CGT GGC TTC TTC TA	GGG GAC AGA ATT CAG TGG CA
Pax6	AAA CAA ACG CCC TAG CTC TCC	CCG CCC TTG GTT AAA GTC TTC
CyclinD2	GAG TGG GAA CTG GTA GTG TTG	CGC ACA GAG CGA TGA AGG T
Pdx1	CGG CTG AGC AAG CTA AGG TT	TGG AAG AAG CGC TCT CTT TGA
Nkx6.1	CCT CTG GAC CCG AAC TCT C	GCT GCC ACC GCT CGA TT
Slc30a8	AGC TTC CTG TGT TTC CTA GGC CAT	AAT CTA TTC CGA CGG CTG CCT CAT
G6PC2	ACC TGG TCC TTT CTG TGG AGT GTT	TTCAAAGGCCTCGGCTACTAGCAT
Atp2a2	AGC CCA GAG AGA TGC CTG CTT AAA	AGA GCA GGA GCA TCA TTC ACA CCA
Glucagon	CAT TCA CCA GCG ACT ACA GCA A	TCA TCA ACC ACT GCA CAA AAT CT
Nnat	TGA TCT GGA CCA AGT CGG AAC A	ATT CTG GTT GAT GGG CTG T
Glut2	GTT AAT GGC AGC TTT CCG GTC	CAG TTC GGC TAT GAC ATC GGT
Arx	TCC GGA TAC CCC ACT TAG CTT	GAC GCC CCT TTC CTT TAA GTG
cMaf	AGC AGT TGG TGA CCA TGT CG	TGG AGA TCT CCT GCT TGA GG
Pdk4	AGGGAGGTCGAGCTGTTCTC	GGAGTGTTCACTAAGCGGTCA
Ndst4	ACTTTTTGCTTGGTGAGCATCC	CCGATAAGGGAGGTCTTTGATGT
Higd1a	TAAGGAGACACCGTTTGTCCC	TGATACATGGAGTAGCCCATACC
Ptprd	TGCCAGGTGGAAGTGTAAATATC	CGGCATATCATCTTCTGGTGTC
Brn4	CTGCCTCGAATCCCTACAGC	CTGCAAGTAGTCACTTTGGAGAA
Pcsk2	AGAGAGACCCCAGGATAAAGATG	CTTGCCCAGTGTTGAACAGGT
Somatostatin	AACGCAAAGCTGGCTGCAAGAA	TCAGCGGTCTGGCTAGGACAACAA
Ghrelin	AGCCCAGCAGAGAAAGGAATC	GGGAGCATTGAACCTGATCTC
PP	TTGCAGCCTCTCTTGTCTTCA	TAGTTTGCAAGGGAGCAGGTT
Kir6.2	TGTGCAGAATATCGTCGGGCTGAT	GCATGCTTGCTGAAGATGAGGGTT

B.

Gene	Catalog #	Gene	Catalog #
MAFB	Hs00534343_s1	*MafB*	Mm00627481_s1
MAFA	Hs01651425_s1	*MafA*	Mm00845206_s1
ARX	Hs00292465_m1	*Arx*	Mm00545903_m1
PDX1	Hs00426216_m1	*Pdx1*	Mm00435565_m1
NEUROD1	Hs00159598_m1	*Neurod1*	Mm01280117_m1
INS	Hs02741908_m1	*Ins2*	Mm00731595_gh
GCG	Hs01031536_m1	*Gcg*	Mm01269055_m1
SST	Hs00356144_m1	*Sst*	Mm00436671_m1
18S	Hs99999901_s1	*18s*	Hs99999901_s1
TBP	Hs99999910_m1	*Tbp*	*Mm00446973_m1*

Table 2. qPCR primer information.
(A) Primer sequences used for *MafB*$^{\Delta panc}$ gene expression studies of Figure 15.
(B) Source information for NHP, human, and mouse primers used in Figure 20.

Tissue Collection and Immunohistochemistry

NHP, human, and C57BL/6J mouse islets were embedded in collagen I (Bectin-Dickinson) and then fixed in 4% (wt/volume) paraformaldehyde in 1X PBS for 20 minutes at 4° C. After three 20-minute 1x PBS washes and a 3-hour equilibration in 30% sucrose/1X PBS, islets were cryopreserved in Optimum Cutting Temperature Compound (VWR Scientific Products). Mouse pancreata (*MafAB*$^{\Delta panc}$, *MafB*$^{\Delta panc}$, *MafB*$^{\Delta \beta}$, and controls) were fixed in 4% paraformaldehyde for 4 hours at 4° C followed by three 10-minute 1X PBS washes at 4° C, serial dehydration using the organic solvent, Citrasolv, and embedded in paraffin. Double immunofluorescence was performed with all samples. Primary antibodies and dilutions are provided (Table 3). Briefly, the primary antibody–antigen complex was visualized on 12μm- (cryopreserved) or 6μm-sections (paraffin) by immunofluorescence using secondary antibodies conjugated with Cy2, Cy3, or Cy5 fluorophores (Jackson ImmunoResearch, West Grove, PA, USA; 1:500). Nuclear costaining was conducted with DAPI Fluoromount G (Southern Biotech). Immunofluorescent images were acquired with a Zeiss LSM510 confocal microscope or a Zeiss Axioimager M2 microscope.

Islet Cell Population Analysis

Mouse pancreatic sections at 133 μm, 288 μm, and 354 μm spacing were prepared from e15.5, postnatal day (P) 1, and 2-week-old samples, respectively. Insulin$^+$, glucagon$^+$, Pax6$^+$, Pdx1$^+$, and Nkx6.1$^+$ cell images were counted manually and divided by the total number of pancreatic DAPI$^+$ nuclei. At least 10,000, 20,000, and 100,000 pancreatic nuclei were counted for e15.5, P1, and 2-week-old samples, respectively.

Mouse samples			NHP and human samples		
Insulin, guinea pig	Dako	1:1000	Insulin, guinea pig	Dako	1:1000
Glucagon, mouse	Sigma	1:4000	Glucagon, mouse	Sigma	1:4000
Glucagon, rabbit	Linco	1:1000	MafB, rabbit	Novus	1:1000
MafB, rabbit	Bethyl	1:1000	Somatostatin, goat	Santa Cruz	1:500
MafA, rabbit	Novus	1:1000	MAFB, rabbit (EndoC-βH1 cells)	Bethyl	1:1000
Pax6, rabbit	Covance	1:300	Beta actin, rabbit (EndoC-βH1 cells)	Cell Signaling	1:1000
Pdx1, goat	Provided by Dr. Chris Wright	1:20,000			
Nkx6.1, rabbit	Beta Cell Biology Consortium	1:1000			
Slc30a8, rabbit	Pierce	1:1000			
Glut2, goat	Santa Cruz	1:100			
Ghrelin, goat	Santa Cruz	1:200			
Ki67, mouse		1:200			

Table 3. Source information for antibodies used in analyses.

Intraperitoneal Glucose Tolerance Tests

Three-, six-, eight-week-old mice, and GD15.5 female mice (n ≥ 7) were fasted for 6 hours, and blood glucose level from tail blood was determined using a FreeStyle glucometer (Abbott Diabetes Care). The mice were then weighed, and 2 mg dextrose (0.75 mg for submaximal dose for GD15.5 females)/g body weight (FisherBiotech) in sterile PBS was injected intraperitoneally. Blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 minutes post injection. Fed blood glucose levels were determined prior to fasting.

Stimulated Hormone Secretion

Handpicked islets from 2- and 8-week-old *MafB*$^{\Delta panc}$ and control *MafB*$^{fl/fl}$ mice were cultured overnight in RPMI1640, 10% FBS, Pen/Strep, supplemented with 5.6 mM (for insulin secretion) or 11 mM D-glucose (for glucagon secretion). The next day, islets were subjected to hormone secretion conditions in KRBH buffer (1.25 mM $CaCl_2$, 0.6 mM $MgS0_4$, 0.6 mM KH_2PO_4, 2.4 mM KCl, 64.0 mM NaCl, 20 mM HEPES pH7.9, 5 mM $NaHCO_3$) with either 2.8 mM or 16.7 mM glucose for insulin secretion and 1 mM glucose or 1 mM glucose and 10 mM arginine for glucagon secretion. Following 45 minutes of stimulation at 37º C, secretion media was collected and islets were lysed in 1.5% HCl, 70% ethanol. Secreted (media) and cell content hormone levels were determined by ELISA (Insulin-ultra sensitive EIA ALPCO; Glucagon-RayBiotech). Hormone secretion was expressed as the percentage of total hormone content. The NHP and human perifusion studies were conducted in the Vanderbilt Islet Isolation and Analysis Core under standard conditions. Following isolation, human islets were usually cultured overnight at the islet isolation center before overnight shipment to Vanderbilt. Human and mouse islets were cultured in RPMI-1640 with 10% FBS and 5 mmol/l glucose at 37° C. Then mRNA or protein was collected. The time for human islet shipment

(approximately 24 hours) was included in the culture time. Human islets were perifused on the day of arrival or after 24 hours of culture at Vanderbilt. Mouse islets were perifused after 48 or 72 hours of culture; GSIS at both time points was similar. Perifusion responses were measured using size-matched mouse and human islets. Human islet preparations were designated as having intact insulin secretion based on the following: stable baseline response at 5.6 mmol/l glucose, at least a threefold response to 16.7 mmol/l glucose, and at least a 5-fold response to 16.7 mmol/l glucose + 100 μmol/l3-isobutyl-1-methyl-xanthine (IBMX) for stimulating insulin secretion. To assess the impact of shipping on function, C57BL/6J mouse islets were isolated at Vanderbilt then shipped by overnight courier to the University of Massachusetts and then back to Vanderbilt.

MAFB Knockdown and GSIS Analysis in Human EndoC-βH1 Cells

The Dharmafect #1 transfection reagent was used to introduce 50 picomoles of either *MAFB* (#L-009018-00; GE Dharmacon) or scrambled control siRNAs (#D001810; GE Dharmacon) into EndoC-βH1 cells (Ravassard et al., 2011). Knockdown efficiency was assessed by immunoblot analysis of whole cell extracts, using RIPA buffer (100 mM NaCl, 1% Nonidet P40, 0.5% deoxycholic acid, 0.1% SDS, 50 mM Tris/HCl [pH 8.0], and 1 mM EDTA) and quantitated using NIH ImageJ software. Transfected cells (~2 million cells per well) were then incubated for an additional 36 hours in normal media, which was then replaced with low glucose media the night prior to GSIS studies (i.e. 1.1 mM Glucose, 2% bovine serum albumin, 50 μM 2-mercaptoethanol, 10 mM nicotinamide, 5.5μ g/mL transferrin, 6.7 ng/ml selenite and penicillin-streptomycin at 100 units/mL). After a 30 minute preincubation in 5.5 mM glucose, GSIS was performed with medium containing 5.5 mM glucose, 15.5 mM glucose, or 15.5 mM glucose and 500 uM IBMX, which was supplied in a time-sensitive manner. Secreted insulin was normalized to

insulin content following cell lysis (cell lysis buffer: 1 M Tris HCl, Triton X-100, 10% glycerol, 5 M NaCl, 0.2 M EGTA, protease inhibitor tablet).

Statistics

Statistical significance was determined by one-way ANOVA between NHP, human, and mouse samples. The data with *MafAB*$^{\Delta panc}$, *MafB*$^{\Delta panc}$, and *MafB*$^{\Delta \beta}$ mice were presented as mean ± SEM and statistical significance assessed by the Student's two-tailed unpaired t-test.

Chapter IV

THE MAFB TRANSCRIPTION FACTOR HAS A DISTINCT ROLE IN PRIMATE ISLET β-CELLS

This chapter contains data submitted for publication.

Introduction

Here we produced pancreas-wide deletion mutants of *MafB* (*MafB*$^{\Delta panc}$) and *MafA/B* (*MafAB*$^{\Delta panc}$) to compare the postnatal contributions of these transcription factors to islet cell formation and function. Elevated blood glucose levels were observed just after birth in *MafB*$^{\Delta panc}$ mice, which soon resolved upon normal induction of MafA production within the insulin^{+} cell population. In addition, there was a profound reduction in glucagon secretion levels from adult islet □-cells, and decreased *Ghrelin* mRNA levels in □-cells. In contrast, *MafAB*$^{\Delta panc}$ mice died from hyperglycemia shortly after birth due to loss of insulin^{+} cells. These results demonstrated that MafB individually had a transient effect on developing mouse islet □-cells. Notably, MAFB was coexpressed with MAFA in NHP islet β-cells, suggesting that this factor imparts distinct control properties in primates. Experiments conducted in the EndoC-βH1 human β-cell line reveal a novel function of MAFB in cAMP-mediated GSIS.

Results

Loss of MafB Alone Results in Delayed β-Cell Maturation

The role of MafB in postnatal α- and β-cells remains unclear, since *MafB*$^{-/-}$ mice die at birth (Sadl et al., 2002). As a result, floxed *MafB* mice were crossed with early

pancreas-driven *Pdx1-Cre* (*MafB*$^{\Delta panc}$) transgenic mice to determine the postnatal impact of MafB loss in developing islet cells. MafB was effectively removed from *MafB*$^{\Delta panc}$ α- and β-cells by e15.5 (Figure 12, 3.65 ± 2.15% remain MafB^{+}insulin^{+} and 6.62 ± 1.63% MafB^{+}glucagon^{+}), and there was no effect on mortality. However, *MafB*$^{\Delta panc}$ mice were mildly hyperglycemic at P1, a situation that resolved by 2 weeks (Figure 13A and 13C). Hormone^{+} and pan-endocrine Pax6^{+} cell numbers were quantified at e15.5 and 2 weeks to determine if insulin^{+} and/or glucagon^{+} cell production was impacted in *MafB*$^{\Delta panc}$ mice. There was a significant decrease in the insulin^{+} (~75%) and glucagon^{+} (~50%) cell numbers at e15.5, while Pax6^{+} cell numbers were unchanged (Figure 13B). These results were consistent with the analysis of the *MafB*$^{-/-}$ mutant (Artner et al., 2007).

Notably, *MafB*$^{\Delta panc}$ insulin^{+} and glucagon^{+} cell numbers were indistinguishable from wild type littermate controls by 2 weeks (Figure 13B). However, the islet β-cell-enriched Pdx1 and Nkx6.1 transcription factors were aberrantly detected in hormone-negative cells in P1 and P4 *MafB*$^{\Delta panc}$ pancreata, and insulin staining intensity was reduced appreciably (Figure 14A). In addition to insulin (Olbrot et al., 2002) and glucagon (Artner et al., 2006), the levels of two other known MafA- and MafB-activated gene targets were examined, Slc30a8 and Glut2 (Artner et al., 2010; Hang et al., 2014). The expression of both was transiently compromised in neonatal *MafB*$^{\Delta panc}$ islets (Figure 14B).

Our results suggested that embryonic loss of MafB delays islet β-cell maturation. Consequently, we analyzed whether GSIS was compromised in 2-week compared to 8-week-old *MafB*$^{\Delta panc}$ islets (Figure 15A), when essentially no MafB^{+} β- or α-cells were present (Figure 12, data not shown). Insulin secretion was slightly elevated under non-stimulating 2.8 mM glucose conditions in 2-week-old *MafB*$^{\Delta panc}$ islets, a basal secretion pattern of immature β-cells (Boschero et al., 1990; Jermendy et al., 2011; Rozzo et al., 2009). Conversely, the insulin secretion profile was normal in 8-week-old *MafB*$^{\Delta panc}$

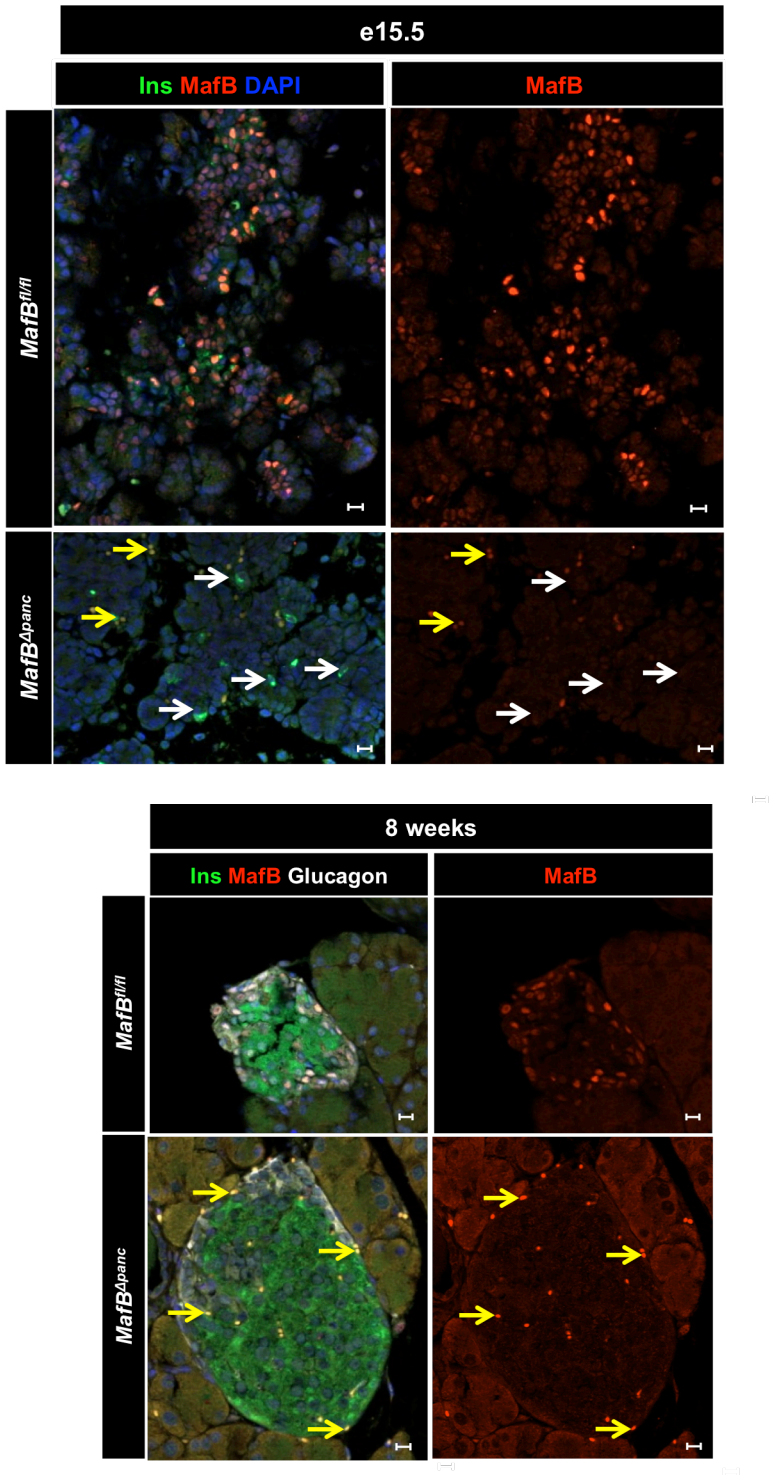

Figure 12. MafB is efficiently removed from *MafB*$^{\Delta panc}$ α- and β-cells. Representative image of *MafB*$^{\Delta panc}$ and *MafB*$^{fl/fl}$ pancreata at e15.5 and 8 weeks; Ins = Insulin (green), Glucagon (white), MafB (red), DAPI (blue). White arrows denote insulin^{+}MafB^{-} cells. Yellow arrows mark blood cell autofluorescence. Scale bars = 10 μm.

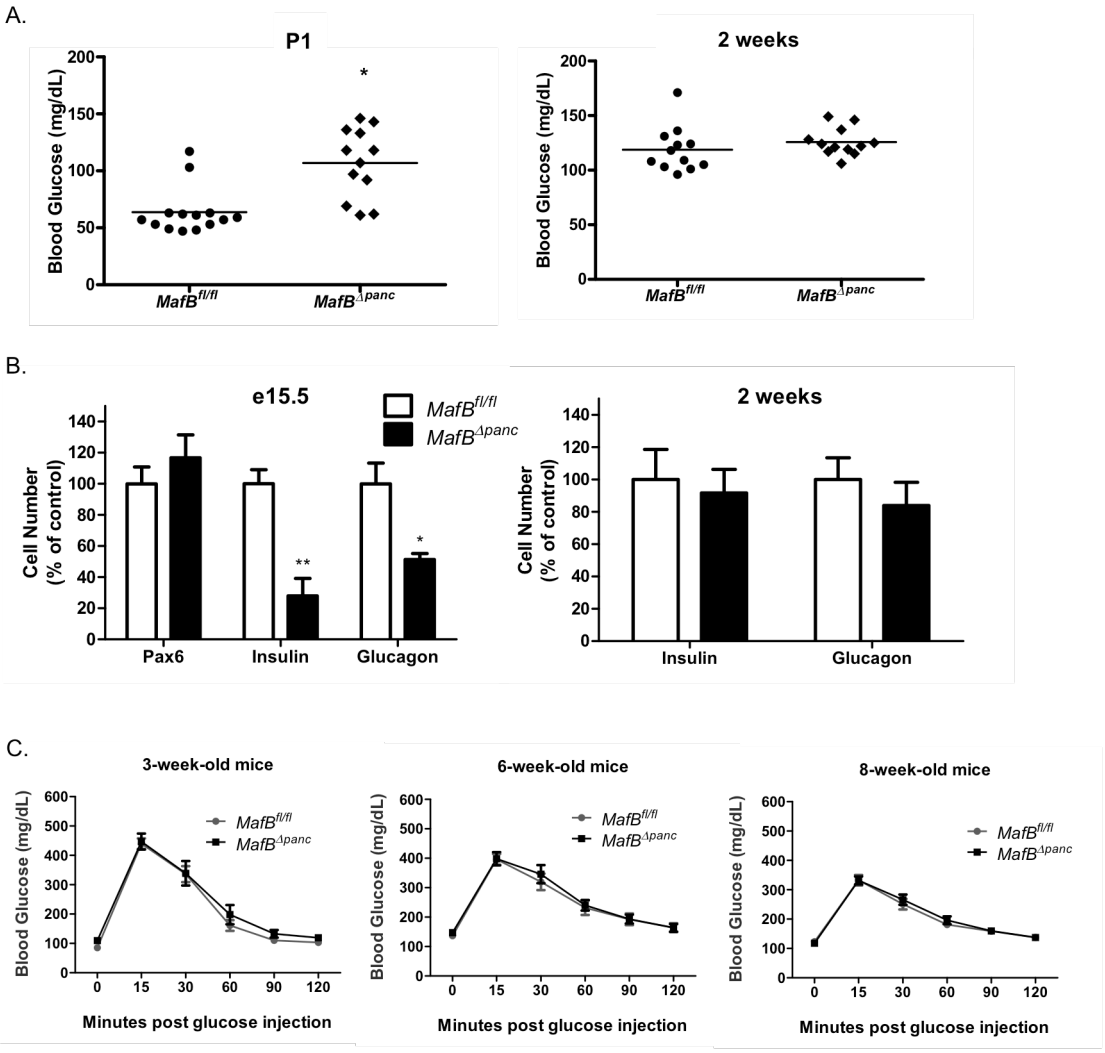

Figure 13. Postnatal β-cell maturation is delayed in *MafB*$^{\Delta panc}$ mice. (A) P1 and 2-week-old random blood glucose measurements in *MafB*$^{\Delta panc}$ and control *MafB*$^{fl/fl}$ littermates. * $p < 0.05$ compared with *MafB*$^{fl/fl}$ (n=12-14). (B) The reduction in e15.5 *MafB*$^{\Delta panc}$ insulin^{+} and glucagon^{+} cell numbers is ameliorated within 2 weeks after birth. Approximately 10,000 and 100,000 pancreatic nuclei were counted at e15.5 and 2 weeks, respectively. * $p < 0.05$; ** $p < 0.01$ compared with *MafB*$^{fl/fl}$ (n=3). (C) *MafB*$^{\Delta panc}$ animals exhibit normal glucose tolerance by 3 weeks. Glucose tolerance tests were performed at 3, 6, and 8 weeks (n=11-21).

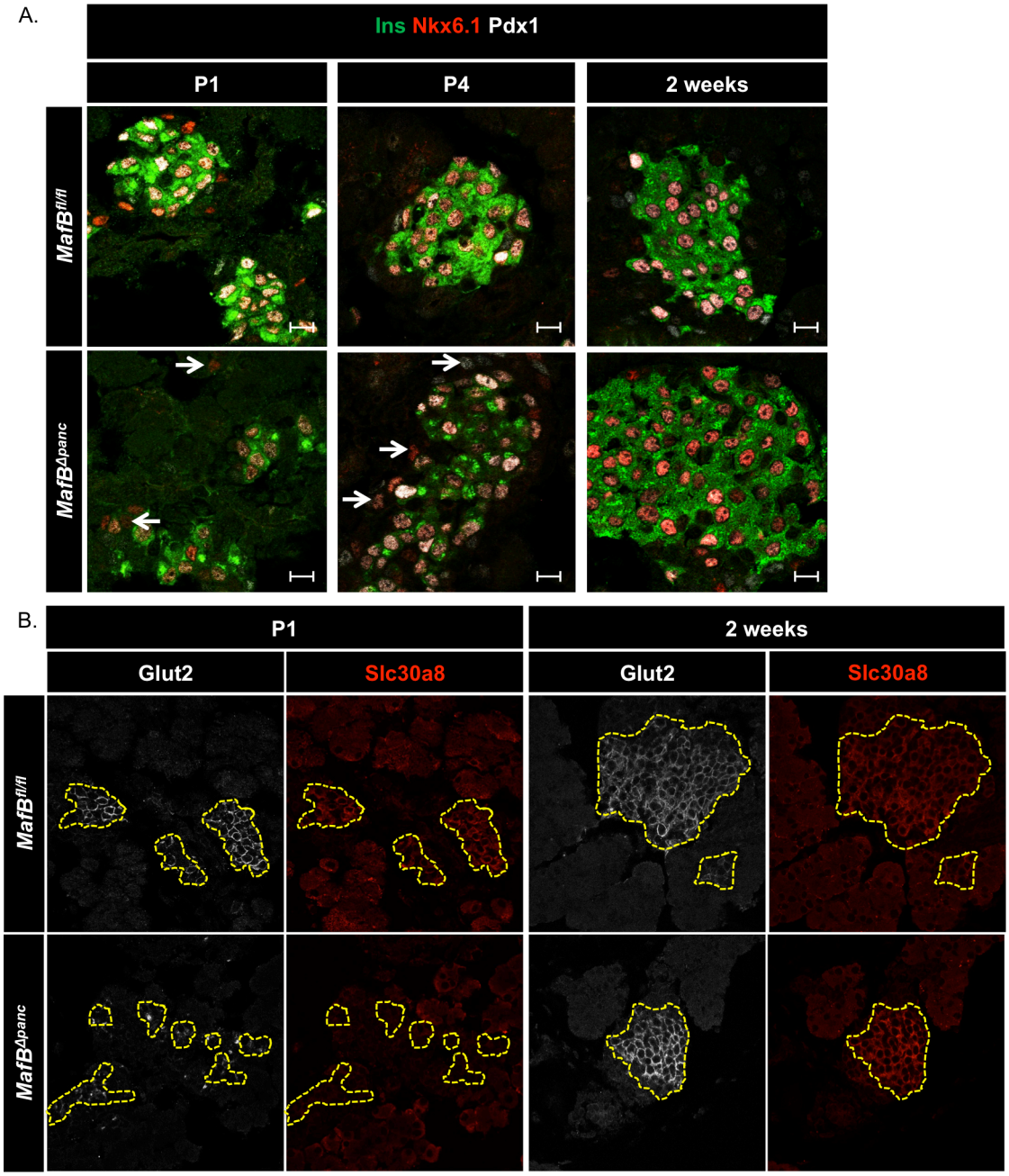

Figure 14. Islet-enriched transcription factor distribution and Glut2 and Slc30a8 levels are transiently altered in neonatal *MafB*$^{\Delta panc}$ islet β-cells. (A) The white arrows denote the abnormal presence of Pdx1^{+} Nkx6.1^{+} nuclei in non-hormone expressing *MafB*$^{\Delta panc}$ islet cells at P1 and P4. Ins = Insulin (green), Nkx6.1 (red), and Pdx1 (white). (B) Islet-enriched Slc30a8 and Glut2 levels are reduced at P1 and restored by 2 weeks in *MafB*$^{\Delta panc}$ islets; Glut2 (white) and Slc30a8 (red). Yellow-hashed lines mark the endocrine^{+} cells. Scale bars = 10 μm. The same confocal settings were used to produce these images.

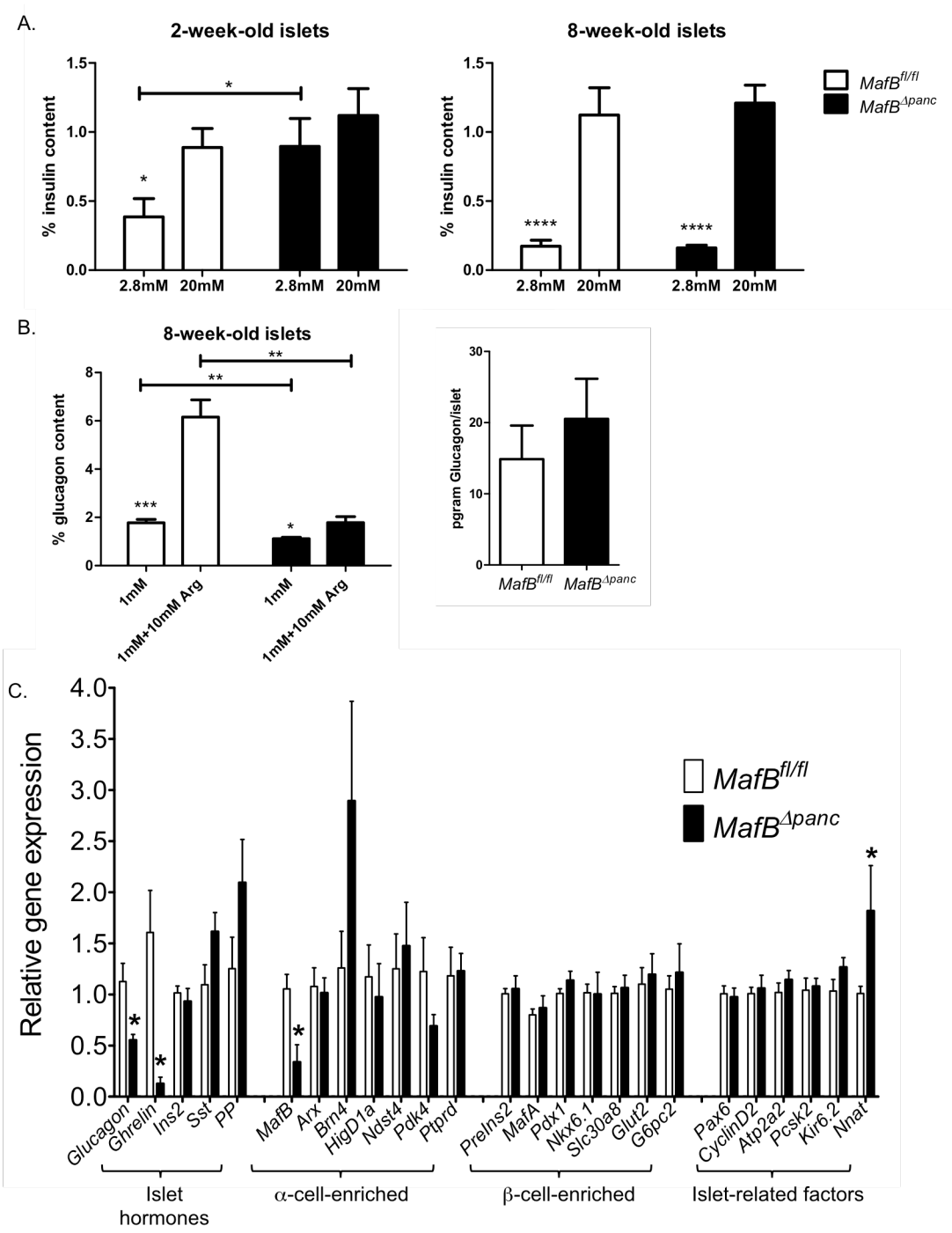

Figure 15. Glucagon secretion is compromised in adult *MafB*$^{\Delta panc}$ islets, but not insulin secretion. Static culture analysis of insulin (A) and glucagon (B) secretion in *MafB*$^{\Delta panc}$ (black bars) and *MafB*$^{fl/fl}$ (white bars) islets. In (B), the ratio of 1 mM glucose to 1 mM glucose + 10 mM Arg is 3.5 in *MafB*$^{fl/fl}$ and 1.6 in *MafB*$^{\Delta panc}$. * $p < 0.05$, ***$p < 0.001$, **** $p< 0.0001$, basal versus stimulating conditions and * $p < 0.05$, **$p < 0.01$, *MafB*$^{\Delta panc}$ versus *MafB*$^{fl/fl}$ controls (n=4-6). (C) *Glucagon* and *Ghrelin* mRNA levels are reduced in 8-week-old *MafB*$^{\Delta panc}$ islet cells ($n \geq 4$). In contrast, many other α -, β - and islet-related factors are unchanged. * $p < 0.05$ compared with *MafB*$^{fl/fl}$ controls.

islets. Furthermore, glucose clearance rates *in vivo* were similar between *MafB*$^{\Delta panc}$ and controls at 3, 6, and 8 weeks (Figure 13C). The expression levels of a variety of MafA- and MafB-regulated genes were also indistinguishable from controls in 8-week-old *MafB*$^{\Delta panc}$ islets, including *Insulin, Nkx6.1, Glut2, Pdx1,* and *Slc30a8* (Figure 15C).

Since MafB is normally produced in embryonic insulin^{+} cells prior to MafA (Artner et al., 2006; Matsuoka et al., 2004), we examined whether MafA or another related large Maf transcription factor, c-Maf (Matsuoka et al., 2003; Nishimura et al., 2006), could be acting in a compensatory manner in *MafB*$^{\Delta panc}$ mice. MafA was only detected in *MafB*$^{\Delta panc}$ insulin^{+} cells (Figure 16), whereas c-Maf was undetectable in these samples (data not shown). Considering the importance of MafA to mouse β-cell function after birth (Hang et al., 2014; Zhang et al., 2005), we conclude that MafA compensates for MafB in *MafB*$^{\Delta panc}$ mice, with the later expression of MafA influencing the temporal production of insulin^{+} cell numbers and β-cell function.

MafB Is Important to Islet α-Cell Function and ε-Cell Ghrelin Expression

Interestingly, *Glucagon* and *Ghrelin* mRNA levels were significantly decreased in 8-week-old *MafB*$^{\Delta panc}$ islets, whereas *Somatostatin, Pancreatic Polypeptide,* and *Insulin* levels were similar to controls (Figure 15C). The impact on *Glucagon* expression was not surprising, since MafB binds to and activates at the G1 element found between -71 to -55 base pairs relative to the transcription start site (Artner et al., 2006). In contrast, it is unclear how MafB regulates *Ghrelin* expression. Unfortunately, we could not determine if there was a change in ghrelin staining levels due to the very few □-cells in adult *MafB*$^{fl/fl}$ or *MafB*$^{\Delta panc}$ islets. Immunostaining confirmed that MafB is present in wild type ghrelin^{+} cells at P1 (Figure 17A), when □-cells make up a larger proportion, albeit still small, of the islet cell population (Prado et al., 2004). There was no obvious change

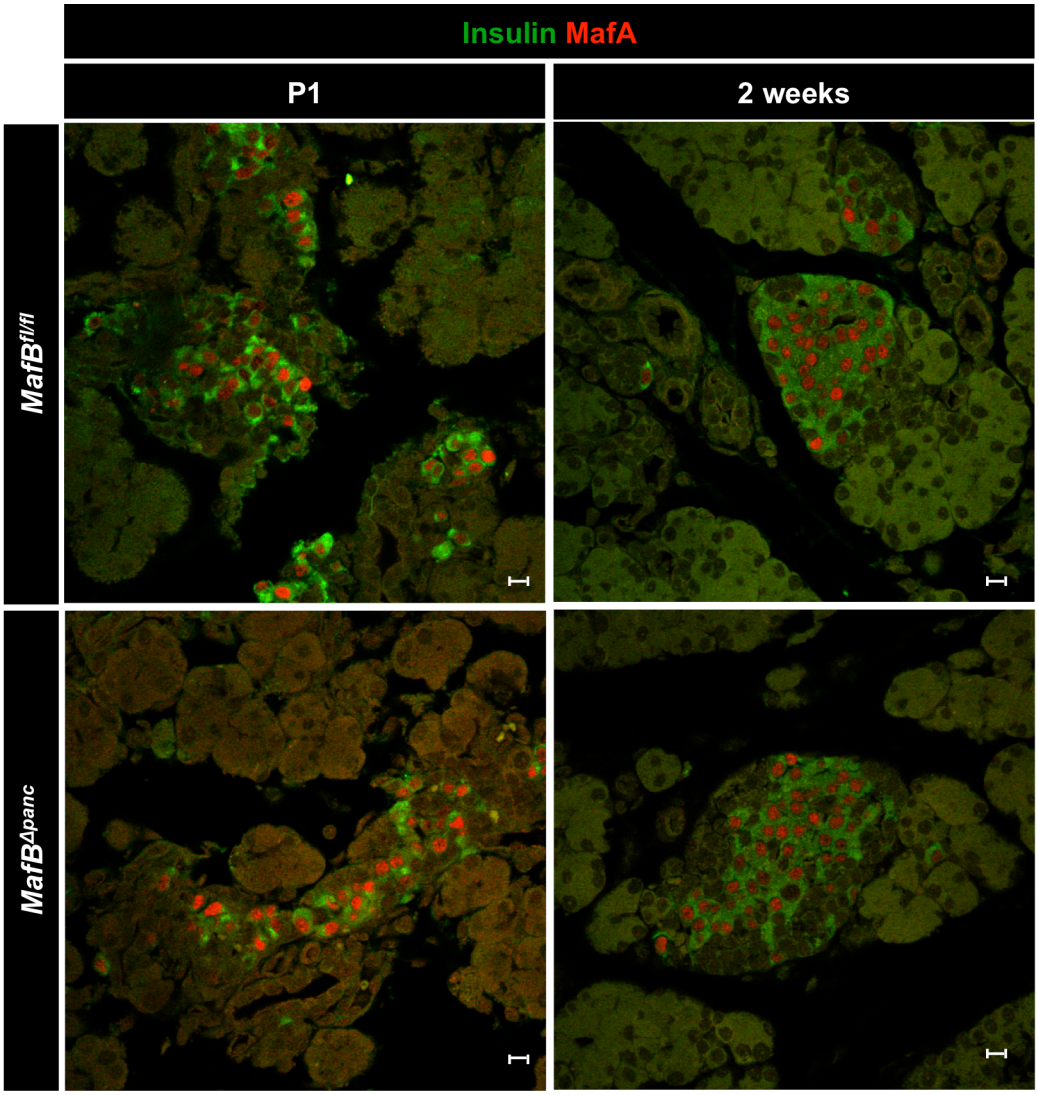

Figure 16. MafA expression is restricted to insulin$^+$ cells in *MafB$^{\Delta panc}$* animals. Representative images of P1 and 2-week-old *MafB$^{\Delta panc}$* and *MafB$^{fl/fl}$* islets; Insulin (green) and MafA (red). Scale bars = 10 μ m.

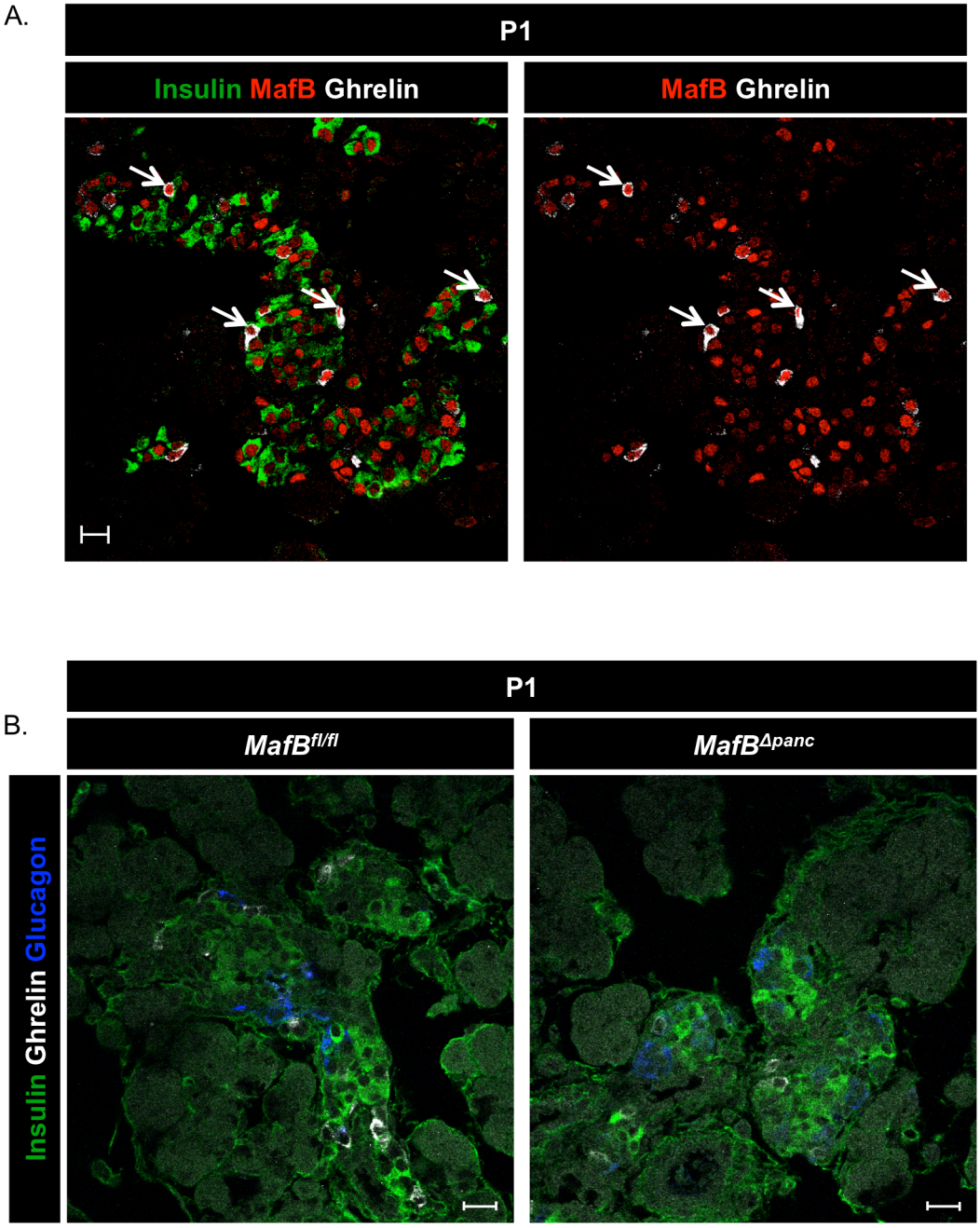

Figure 17. MafB is made in mouse P1 islet ghrelin^{+} ε-cells, although there is no apparent change in the P1 *MafB$^{\Delta panc}$* ghrelin^{+} cell population. Ghrelin (white), MafB (red), Insulin (green), and Glucagon (blue). White arrows denote MafB^{+}Ghrelin^{+} cells. Scale bars = 20 μm.

in the P1 islet ghrelin cell population (Figure 17B). Although, MafB expression was observed in the gut, it was not present in ghrelin^{+} cells of the gut (Figure 18), the principal location of this hormone-expressing population.

Glucose-stimulated glucagon secretion was measured in 8-week-old *MafB*$^{\Delta panc}$ islets to determine if secretion capacity was altered. Both glucagon content (Figure 15B) and □-cell numbers (Figure 13B) were unchanged from controls, although glucagon secretion was reduced in *MafB*$^{\Delta panc}$ islets upon exposure to a stimulating low glucose concentration and the potent secretagogue, arginine (Figure 15B). There were no changes in islet □-cell-enriched transcription factors (i.e. *Arx* (Collombat, 2003) and *Brn4* (Scott Heller et al., 2004) in Figure 15C) or several other α-cell-enriched gene products in *MafB*$^{\Delta panc}$ islets (i.e. *HigD1a, Ndst4, Pdk4, Ptprd* of (Gao et al., 2014) in Figure 15C). These analyses demonstrate that MafB controls postnatal islet α-cell function, which is quite interesting considering that MAFB levels are reduced in T2DM islet □-cells and glucagon secretion is dysregulated in this state (Guo et al., 2013b; Unger et al., 1970).

Loss of MafA and MafB in β-Cells Results in Death Soon after Birth Due to Hyperglycemia

Floxed *MafA* and *MafB* mice were crossed with early pancreas-driven *Pdx1-Cre* (*MafAB*$^{\Delta panc}$) transgenic mice to compare their phenotype to the individual mutants. In contrast to *MafA*$^{\Delta panc}$ (Artner et al., 2010) or *MafB*$^{\Delta panc}$ mutants, no viable *MafAB*$^{\Delta panc}$ mice were found within 2.5 weeks of birth (Table 4), although these mice were born at the predicted frequencies (data not shown). This same property was found in *MafAB*$^{\Delta endo}$ animals, where expression of *Ngn3*-driven *Cre* removes each transcription factor gene prior to expression in embryonic islet cell progenitors (Table 5). The blood glucose levels in *MafAB*$^{\Delta panc}$ P1 mice were extremely high (Figure 19A), strongly indicating that deficiencies in β-cell mass and/or function led to their early death. In

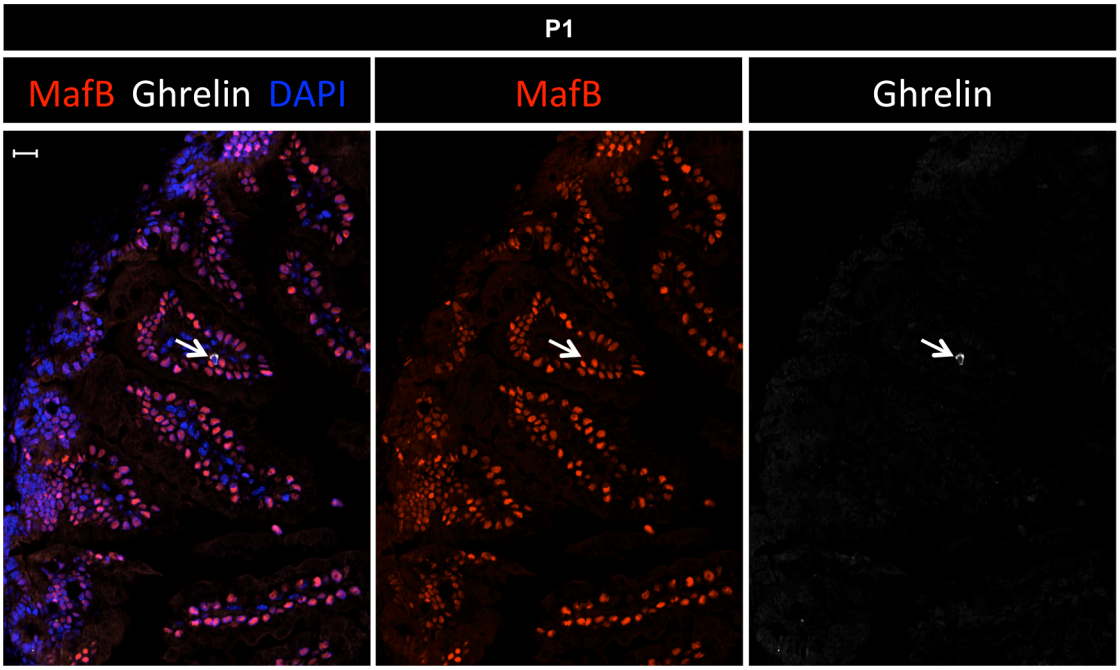

Figure 18. MafB is produced in the developing gut, although not in gut ghrelin^{+} cells. Representative image of P1 wild type mouse duodenum; MafB (red), Ghrelin (white) and DAPI (blue). White arrow denotes ghrelin^{+}MafB^{-} cell. Scale bar = 20 μm.

$MafA^{fl/fl}MafB^{fl/fl}$ x $MafA^{\Delta panc/+}MafB^{\Delta panc/+}$				
Potential Genotypes	Mendelian Ratio	# pups expected out of 54	Actual # pups	Actual ratio
$MafAB^{\Delta panc}$	0.125	6.75	0*	0
$MafA^{\Delta panc/+}B^{\Delta panc}$ or $MafA^{\Delta panc}B^{\Delta panc/+}$	0.25	13.50	16	0.30
$MafA^{\Delta panc/+}MafB^{\Delta panc/+}$	0.1	6.75	12	0.22
$Pdx1\text{-}Cre^{-}$	0.50	27.00	26	0.48
Total			54	

Table 4. $MafAB^{\Delta panc}$ mice die soon after birth. The expected and actual number of animals obtained from the $MafA^{fl/fl}MafB^{fl/fl}$ x $MafA^{\Delta panc/+}MafB^{\Delta panc/+}$ mating at 2.5 weeks. * p = 0.0438.

$MafA^{fl/fl}MafB^{fl/fl}$ x $MafA^{\Delta endo/+}MafB^{\Delta endo}$				
Potential Genotypes	Mendelian Ratio	# pups expected out of 59	Actual # pups	Actual ratio
$MafAB^{\Delta endo}$	0.25	14.75	0*	0
$MafA^{\Delta endo/+}MafB^{\Delta endo}$	0.25	14.75	21	0.36
$MafA^{fl/+}B^{fl/fl}$	0.25	14.75	18	0.31
$MafA^{fl/fl}B^{fl/fl}$	0.25	14.75	20	0.34
Total			59	

Table 5. *MafAB*$^{\Delta endo}$ animals die within 3 weeks of birth. Expected and actual genotypes obtained from the $MafA^{fl/fl}MafB^{fl/fl}$ x $MafA^{\Delta endo/+}MafB^{\Delta endo}$ mating. * p = 0.000171.

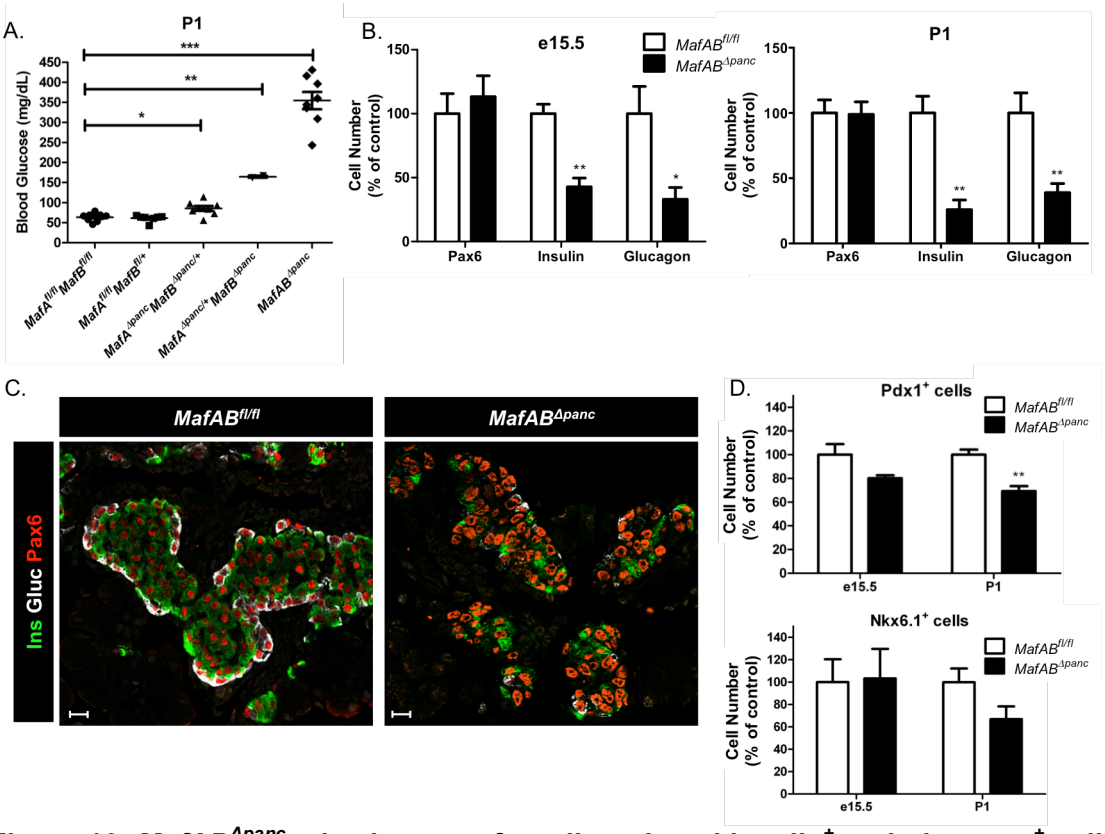

Figure 19. *MafAB*$^{\Delta panc}$ mice have profoundly reduced insulin$^+$ and glucagon$^+$ cell numbers. (A) Neonatal *MafAB*$^{\Delta panc}$ animals are severely hyperglycemic at P1. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0001$ compared with *MafAB*$^{fl/fl}$ controls. (B) Insulin$^+$ and glucagon$^+$ cell numbers are decreased in *MafAB*$^{\Delta panc}$ animals (n=3). * $p < 0.05$; ** $p < 0.01$ compared with *MafAB*$^{fl/fl}$ controls. (C) Most *MafAB*$^{\Delta panc}$ Pax6$^+$ cells do not produce insulin at P1; Ins = Insulin (green), Gluc = Glucagon (white), and Pax6 (red). Scale bars = 10 μm. (D) *MafAB*$^{\Delta panc}$ animals have fewer β-cell-enriched Pdx1$^+$ and Nkx6.1$^+$ cells (n=3). Approximately 10,000 and 20,000 pancreatic nuclei were counted at e15.5 and P1, respectively. ** $p < 0.01$ compared with *MafAB*$^{fl/fl}$ control.

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addition, blood glucose levels were elevated in *MafA*$^{\Delta panc}$*MafB*$^{\Delta panc/+}$ and *MafA*$^{\Delta panc/+}$*MafB*$^{\Delta panc}$ mice.

To investigate how insulin$^+$ and glucagon$^+$ cell formation was affected in *MafAB*$^{\Delta panc}$ mice, the number of hormone$^+$ and Pax6$^+$ cells was quantified at e15.5 and P1. The *MafAB*$^{\Delta panc}$ insulin$^+$ and glucagon$^+$ cell population was significantly reduced (e.g. ~75% change of insulin$^+$ cells in Figure 19B), while total Pax6$^+$ endocrine cell numbers were unchanged. Note that very few Pax6$^+$ cells produce insulin in *MafAB*$^{\Delta panc}$ islets (Figure 19C). However, β-cell-enriched Nkx6.1$^+$ and Pdx1$^+$ cell numbers were only decreased by ~25% (Figure 19D). These results suggest that MafA and MafB are the principal drivers of *Insulin* gene expression, and not Nkx6.1 (Sander et al., 2000) and Pdx1 (Ohlsson et al., 1993). It is important to note that the overall impact on hormone and transcription factor levels in *MafAB*$^{\Delta panc}$ was analogous to *MafB*$^{-/-}$ (Artner et al., 2007) islets, even though the *MafAB*$^{\Delta panc}$ is more severe. Collectively, these data not only demonstrate that the combined actions of MafA and MafB are required for postnatal β-cell formation, but that MafB alone (unlike MafA) only has an early and transient impact on mouse β-cells.

MAFB Is Expressed in NHP Islet β-Cells, with Islet Architecture and Function Analogous to Human

Human islets are dissimilar to mouse in several ways, including the presence of MAFB in human β-cells, GSIS properties, and islet cell composition and distribution (Brissova, 2005; Cabrera et al., 2006; Dai et al., 2011). We next determined if these islet properties were shared with NHPs. Like humans, MAFB was present in β-cells as well as α-cells (Figure 20A, B). *MAFB* mRNA expression was relatively high in NHP and human islets compared to mouse, whereas NHP *MAFA* was present at a level similar to mouse (Figure 20A). *PDX1* and pan-endocrine *NEUROD1* transcription factor levels

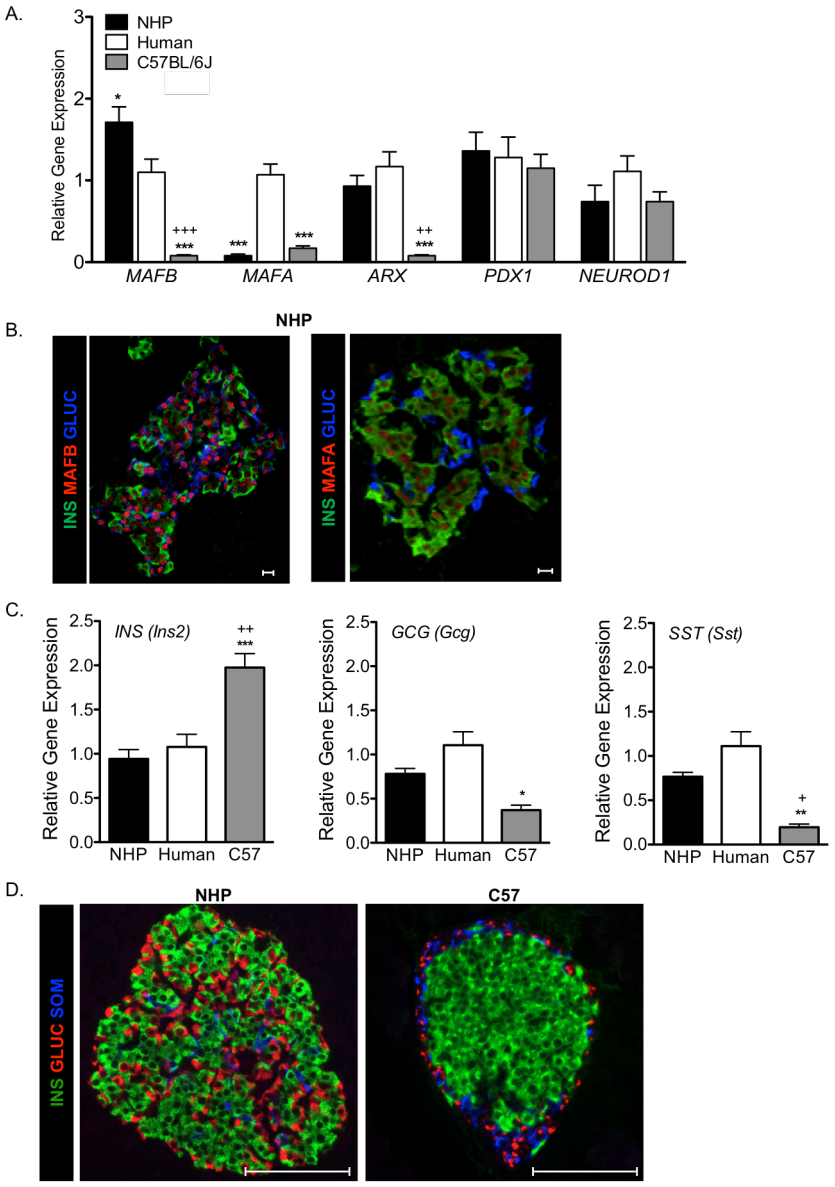


Figure 20. MAFB is expressed with MAFA in adult NHP islet β-cells. (A) Relative gene expression of various transcription factors in NHP (n=5), human (n=10), and mouse (C57BL/6J) (n=6) islets. * $p < 0.05$, *** $p < 0.001$, NHP and mouse versus human; ++ $p < 0.01$, +++ $p < 0.001$, mouse versus NHP. (B) Representative images of a NHP islets; MAFB (red), MAFA (red), and INS = INSULIN (green), and GLUC = GLUCAGON (blue). Scale bars = 10 μm (C) The endocrine hormone mRNA expression pattern is analogous between human and NHP islets, whereas mouse is different. NHP (n=5), human (n=10), and mouse (C57BL/6J) (n=6) islets. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, mouse versus human; + $p < 0.05$, ++ $p < 0.01$, mouse versus NHP. No significant difference seen between NHP and human ($p > 0.05$). (D) Representative image of a NHP and mouse (C57BL/6J) islet; INS = INSULIN (green), GLUC = GLUCAGON (red), and SOM = SOMATOSTATIN (blue). Scale bars = 100 μm.

were comparable across species, although *ARX* levels closely paralleled the increased proportion of α-cells observed in human and NHP islets.

The relative mRNA levels of NHP *INSULIN*, *GLUCAGON,* and *SOMATOSTATIN* were similar to human islet levels (Figure 20C). The distribution of human (Brissova, 2005) and NHP α-, β-, and δ-cells were distributed within the islet core, different from mouse islets where the α- and δ-cells surround the β-cell-rich core (Figure 20D). Insulin secretion from NHP and human islets was indistinguishable at 5.6 mmol/l glucose, 16.7 mmol/l glucose, and 16.7 mmol/l glucose plus IBMX, and significantly different from mouse islets (Figure 21). The higher basal and lower insulin secretion response to high glucose is a distinguishing feature of human and NHP islets. These results suggest that MAFB β-cell expression represents a primate islet feature, unique from the rodent model.

MAFB Regulates GSIS in Human β-Cells

The glucose-responsive human EndoC-βH1 cell line was used to investigate the contribution of MAFB to primate β-cell function (Ravassard et al., 2011). Insulin secretion was monitored from scrambled control and MAFB siRNA knockdown cells treated with 5.5 mM glucose, 15.5 mM glucose, or 15.5 mM glucose plus IBMX (Figure 22). The former is a phosphodiesterase inhibitor that simulates high glucose-induced secretion by stabilizing intracellular cAMP levels. Insulin secretion was compromised in MAFB knockdown cells treated with IBMX (Figure 22C), with no difference found in the presence of only 15.5 mM glucose. These results illustrate the impact of MAFB on cAMP-mediated glucose-induced insulin release, and support a significant role for the MAFB homodimer and/or the MAFA-MAFB heterodimer in human and NHP islet β-cell function.

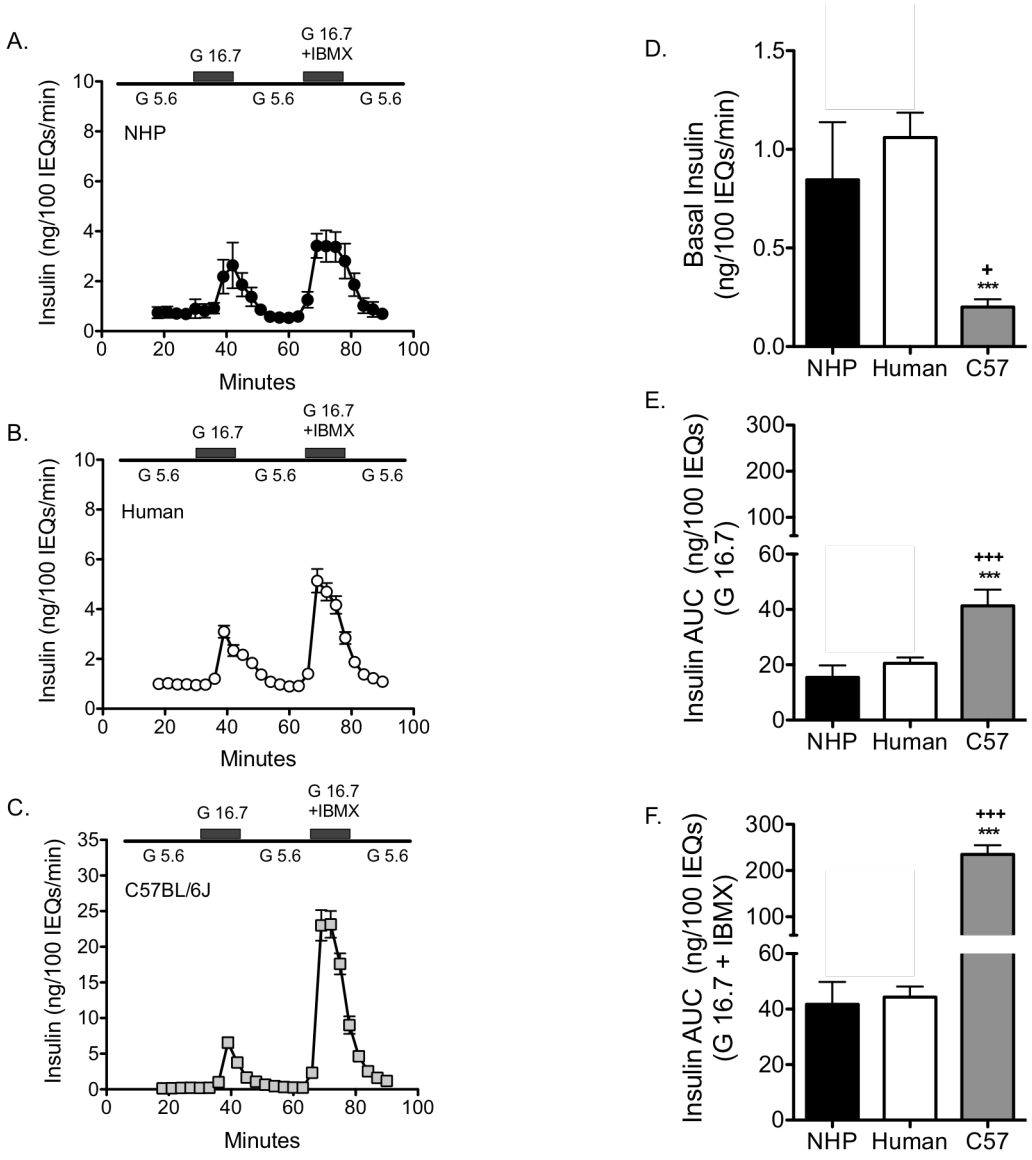


Figure 21. Insulin secretion properties of NHP and human islets are indistinguishable. GSIS characteristics of (A) NHP (n=9), (B) Human (n=30), and (C) mouse (C57BL/6J) (n=12) islets in perifusion assays. (D) Basal insulin levels. (E-F) Area under the curve analyses of (A) - (C). *** $p < 0.001$, mouse versus human; + $p < 0.05$, +++ $p < 0.001$, mouse versus NHP. No significant differences were found between NHP and human.

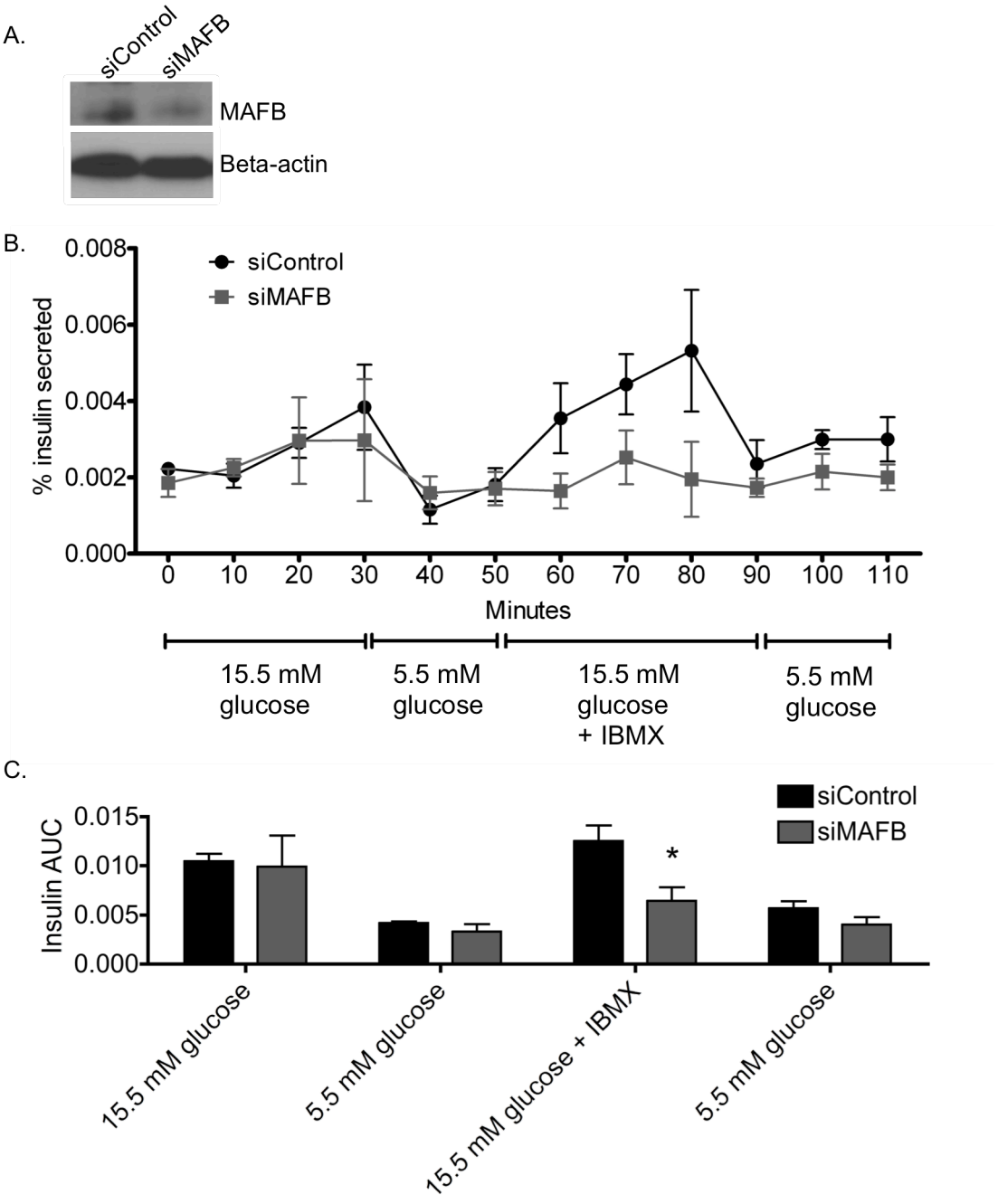


Figure 22. MAFB mediates cAMP-induced glucose-stimulated insulin secretion in human EndoC-βH1 cells. (A) Immunoblot analysis of whole cell extracts from scrambled control and MAFB siRNA treated EndoC-bH1 cells (46.7% knockdown) (n=3). (B) Following MAFB knockdown, secreted insulin was measured every 10 minutes in EndoC-β H1 cells cultured in 15.5 mM glucose, 5.5 mM glucose, and 15.5 mM glucose + 500 μ M IBMX (n=3). (C) AUC analysis of (B). * p = 0.0438 for high glucose + IBMX, siMAFB vs. siControl.

Discussion

Mouse MafA and/or MafB were removed specifically in early pancreatic progenitor cells to obtain insight into the contribution of MafB individually and MafA and MafB in combination to postnatal islet □- and □-cells. Only transient deficiencies in *MafB*$^{\Delta panc}$ □-cell activity were found, although adult islet α-cell glucagon secretion, *Glucagon* mRNA levels as well as *Ghrelin* were compromised. Compensation by MafA likely explains the limited effect on *MafB*$^{\Delta panc}$ □-cells, as supported by the tight expression linkage with mutant insulin^{+} cells (Figure 16). This proposal is also consistent with the substantially reduced islet insulin^{+} cell mass in *MafAB*$^{\Delta panc}$ islets, which caused overt hyperglycemia and death. We conclude that MafB is only essential to postnatal mouse □-cell activity. This seems paradoxical since MAFB in human islet □-cells represents a unique transcription factor signature. Here we show that NHP islet □-cells also produce MAFB, and islet cell composition and □-cell function is analogous to human and distinct from mice. Moreover, and in contrast to regulation in mice, we provide evidence using the human EndoC-βH1 cell line that MAFB is essential to human islet □-cell activity.

MafB was broadly expressed in the P1 duodenum (Figure 18), wherein a variety of metabolically interesting hormones are produced (e.g. Cholecystokinin (CCK), Gastric Inhibitory Peptide (GIP), Glucagon-Like Peptide (GLP-1, mainly produced in the jejunum and ileum, where MafB was not detected), and Ghrelin (Gunawardene et al., 2011). Notably, MafB was not expressed in ghrelin^{+} cells in the duodenum, presumably because these cells are not derived from Ngn3^{+} progenitors (Jenny et al., 2002), as in the pancreas. If so, it will be of interest to determine if MafB is necessary for regulating Ngn3-derived CCK, GIP, and/or GLP-1 expression, with positive results supporting a wider role for MafB in defining cell identity decisions.

MafA and MafB primarily activate as homodimers in rodents, with analyses *in vivo* demonstrating that MafB-MafB is essential to postnatal α-cell function (*MafB*$^{\Delta panc}$ (Figure 15)) and MafA-MafA in β-cells (*MafA*$^{-/-}$ (Zhang et al., 2005), *MafA*$^{\Delta panc}$ mice (Artner et al., 2010)) (Figure 23). Here we found that MAFB, potentially acting as MAFA-MAFB or MAFB-MAFB, is significant to GSIS in human EndoC-βH1 cells (Figure 22). Based upon the relative abundance of *MAFB* to *MAFA* in NHP islets and similar functional properties to human (Figures 20, 21), much of this regulation could be mediated by $MAFB_2$ in primate β-cells (Figure 23). Notably, while MafA-MafA and MafB-MafB influence transcription of genes associated with glucose sensing and hormone secretion in mouse islet α- and β-cells (Artner et al., 2010; Hang et al., 2014), both have distinct effects on cell activity (Aguayo-Mazzucato et al., 2011; Artner et al., 2010) and hormone gene expression (Artner et al., 2008). For example, induction of glucose-responsive insulin secretion is obtained upon mis-expression of MafA in neonatal rat islets, with high MafB expression associated with immature rodent GSIS properties (Aguayo-Mazzucato et al., 2011). Consequently, there are presumably regulatory differences between MAFB-MAFB and MAFA-MAFB, as (for example) in controlling the relatively high basal insulin secretion levels associated with the primate islet (Figure 21) (Figure 23) (Dai et al., 2011).

Human T2DM islets have reduced levels of MAFA and MAFB in □-cells, as well as decreased MAFB in □-cells. These proteins are more sensitive to stress conditions associated with T2DM islet dysfunction than other key islet-enriched transcriptional regulators (Guo et al., 2013b). Reduced MAFA and/or MAFB activity/levels is proposed to cause the compromised GSIS in pre-diabetic patients and found in *MafA*$^{\Delta panc}$ mice (Artner et al., 2010; Guo et al., 2013b). Our studies in the human □-cell line imply that MAFB influences cAMP-mediated GSIS (i.e. 15.5 mM glucose plus IBMX in Figure 22), which is noteworthy, since the IBMX response is unaffected in *MafA*$^{\Delta panc}$ islets (Hang et

al., 2014). Further experimentation is necessary to determine how this occurs, although this will probably involve cellular effectors like the cAMP response element (CRE) binding protein (i.e. CREB) that promotes □-cell function in response to increasing cytoplasmic cAMP levels (e.g. induced by GLP-1 (Shin et al., 2014)).

Our data further suggests that reduced MAFB levels in islet □-cells would contribute to dysregulated glucagon secretion associated with T2DM patients (Unger and Cherrington, 2012). However, we predict that T2DM α-cell dysfunction does not solely derive from MAFB loss, rather from a compilation of changes that occur in the islet populations, including counter-regulatory insulin signaling to the α-cell. It will be of value to explore if MAFB controls cAMP-induced glucagon secretion in □-cells (Elliott et al., 2015) using similar effector mechanisms to those found in □-cells.

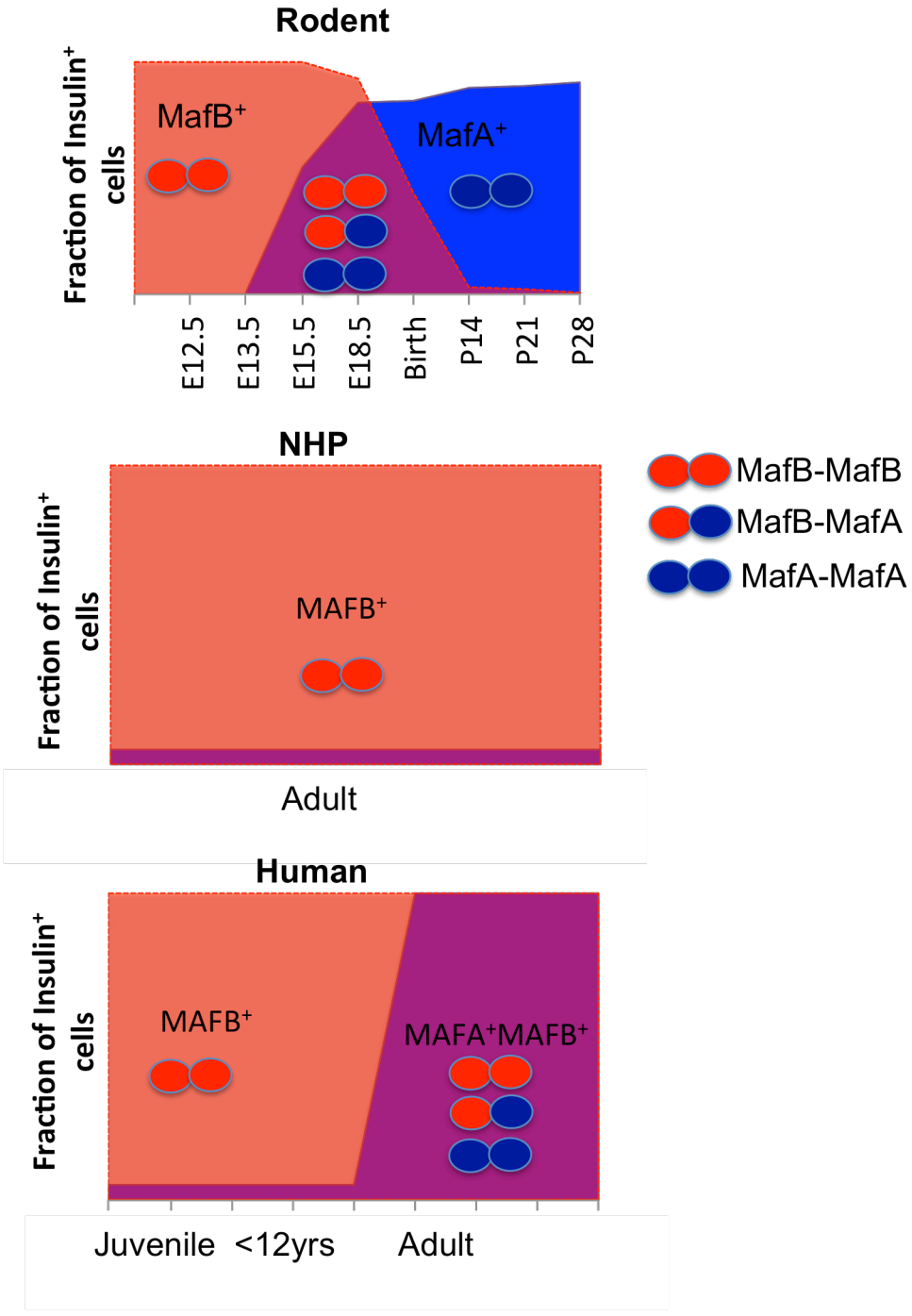


Figure 23. MAF expression pattern in rodent versus NHP and human β-cells. MAF dimer presence in respective species and temporal windows.

Chapter V

EXAMINING THE ROLE OF MAFB IN REGULATING MATERNAL β-CELL MASS AND/OR ACTIVITY

This chapter contains some data that has been submitted for publication by Dr. Sueng Kim's laboratory at Stanford University in the manuscript entitled, "Gestational diabetes from inactivation of the prolactin receptor and MafB in islet β-cells."

Introduction

Pregnancy is a unique period during which pancreatic β-cells must respond to the increased metabolic demands of carrying a fetus, regulating blood glucose levels of both self and the baby. GDM results from the inability of the maternal islet β-cells to adapt and secrete sufficient insulin. These mothers also exhibit compromised ability to adapt to the normal insulin resistance that arises during the third trimester. During this time, increased hepatic gluconeogenesis and lipolysis are stimulated by placental lactogen and growth hormones (Alsat et al., 1997; Buchanan and Xiang, 2005; Sorenson and Brelje, 1997). The mechanisms by which β-cells adapt to this increased insulin demand during pregnancy, including β-cell mass expansion and increased insulin secretion, are still only partially understood (Buchanan and Xiang, 2005; Rieck and Kaestner, 2010; Sorenson and Brelje, 1997). The majority of GDM cases are believed to result from a combination of β-cell dysfunction and preexisting chronic insulin resistance in the mother. This preexisting insulin resistance does not produce glucose intolerance until the woman becomes pregnant and the insulin resistance inherent to pregnancy is then additive (Buchanan and Xiang, 2005).

GDM is defined as glucose intolerance arising specifically during pregnancy (Perkins et al., 2007). Risk factors for GDM include T2DM family history, certain ethnic backgrounds (Hispanic, Native American, African American, and Asian), and obesity (Cypryk et al., 2008; Savitz et al., 2008). GDM is usually resolved after birth, but risks associated with this disorder include preeclampsia, higher chance of a Cesarean section, and an increased risk of developing T2DM later in life for both mother and child (Cheung and Byth, 2003; Perkins et al., 2007). Offspring of mothers with GDM also face higher risks of respiratory distress syndrome, macrosomia, jaundice, polycythemia, hypocalcemia, hypoglycemia, and obesity (Frías et al., 2007; Perkins et al., 2007).

During a healthy pregnancy, the increased need for insulin is met with an increase in β-cell mass and enhanced β-cell insulin secretion. The β-cell mass expansion occurs by an increase in β-cell size and β-cell number. Notably, this increase in β-cell mass then returns to pre-pregnancy size with parturition (Scaglia et al., 1995). This adaptation is observed in rodents and humans, with a 2-fold β-cell mass increase in rodents and a more modest increase of about 1.4-fold in humans (Butler et al., 2010; Sorenson and Brelje, 1997). In nonpregnant adult rodents, about 0.5% of β-cells proliferate, but during pregnancy β-cell replication increases to about 2-3% (Karnik et al., 2007).

Although the mechanisms by which these highly regulated adaptations occur are not completely known, studies have implicated particular signaling pathways. For example, lactogens are implicated in enhanced maternal β-cell replication, with the height of enhanced β-cell proliferation coinciding with the highest placental lactogen levels that arise during pregnancy (Karnik et al., 2007; Parsons et al., 1992; Rieck and Kaestner, 2010). Placental lactogen also contributes to the functional β-cell adaptations of pregnancy by enhancing glucose sensitivity. With this adaptation, a lower glucose stimulation threshold then allows β-cells to secrete more insulin at a lower glucose

concentration (Parsons et al., 1992). Additionally, prolactin is integral to the β-cell adaptations of pregnancy. Prolactin and placental lactogen signal through the prolactin receptor (PRLR) and heterozygous null *Prolactin receptor* mice (*Prlr*$^{+/-}$) develop glucose intolerance during pregnancy (Huang et al., 2009; Sorenson and Brelje, 1997). Although this defect is at least partially mediated by failure of maternal β-cell mass expansion, whether GSIS is impacted during pregnancy in these mutants was not determined (Huang et al., 2009). Serotonin also appears to function in the pregnancy-induced β-cell adaptations. Two of the most upregulated genes identified in pregnant islets code for two isoforms of the rate-limiting enzyme of serotonin synthesis, *Tph1* and *Tph2* (Kim et al., 2010). Another study demonstrates that FoxM1 is specifically induced by placental lactogen in adult β-cells during pregnancy and is involved in the adaptive β-cell proliferation during this time (Zhang et al., 2009b). Similarly, MafB is induced in rodent β-cells during pregnancy, albeit in a subset of the β-cell population (Pechhold et al., 2009). Previously found to possess oncogenic activity, MafB could have a role in the enhanced maternal β-cell proliferation (Pouponnot et al., 2006). Moreover, a recent study examined the role of MafB in adult β-cell proliferation. This study identified MafB β-cell induction under different conditions and in different models known to result in increased β-cell proliferation, including pregnancy (Lu et al., 2011).

Interestingly, we find that the height of MafB induction that we observed is coincident with the height of enhanced maternal β-cell proliferation (Karnik et al., 2007; Pechhold et al., 2009), although we do not see MafB in proliferating maternal β-cells. Additionally, MafB is still induced in maternal insulin^{+} cells in the pancreas-specific *FoxM1* mutants. Without ruling out that MafB has some impact on maternal β-cell proliferation, we generated a β-cell-specific *MafB* mutant mouse (*MafB*$^{\Delta\beta}$) with a *Rat Insulin promoter* driven Cre recombinase (*RIP-Cre*) to examine if MafB is functionally important in maternal β-cells. We hypothesized that blocking MafB in maternal insulin^{+}

cells would lead to gestational diabetes in *MafB*$^{\Delta\beta}$ mothers. *MafB*$^{\Delta\beta}$ adult mice exhibit normal glucose tolerance until the onset of pregnancy, but glucose intolerance is observed in these mutant mothers in comparison to *MafB*$^{fl/fl}$ and *RIP-Cre* alone mothers. In collaboration with Drs. Ron Banerjee and Sueng Kim, Dr. Holly Cyphert has found that MafB induction in maternal β-cells is blocked in β-cell-specific *Prlr* knockout mothers, and so Prlr may mediate this induction. Upon further investigation, pregnant *MafB*$^{\Delta\beta}$ females exhibit reduced β-cell proliferation. My studies indicate that MafB may be uniquely affecting maternal β-cells by contributing to the increase in β-cell mass and perhaps the enhanced β-cell insulin secretion function (Figure 24).

Results

MafB Induction in Maternal β-cells May Contribute to Adaptive Proliferation

Although our lab was unable to observe MafB induction in the adult β-cells of obese mouse models as seen in Lu et al., we have pursued the role of MafB induction in the β-cell during pregnancy with possible proliferative implications (Pechhold et al., 2009). The MafB^{+}insulin^{+} cells that arise throughout wildtype rodent pregnancy were quantified to find that the highest level of MafB induction observed in the pregnant samples examined coincided with height of enhanced β-cell proliferation (Figure 25). Interestingly, MafB was still induced in maternal insulin^{+} cells in the pancreas-specific *FoxM1* mutants that develop GDM due to insufficient β-cell expansion (Zhang et al., 2009b) (Figure 26, samples provided by Dr. Maureen Gannon). Despite recent reports of MafB production in proliferating maternal β-cells, we did not observe colocalization of MafB and the global cell cycle proliferative marker, Ki67, in islets of wild type pregnant pancreatic samples (Lu et al., 2011) (Figure 27). This discrepancy between our findings and Lu et al. could be attributed to a different genetic background, as different mouse

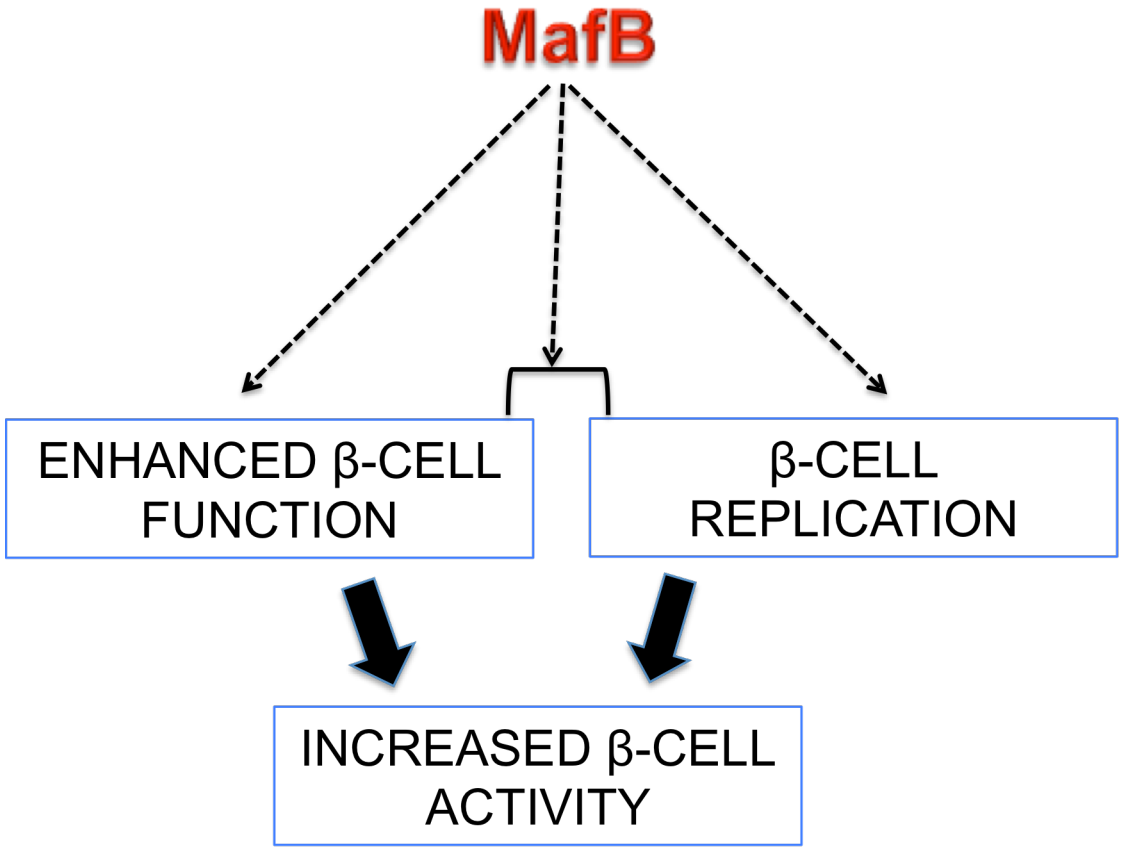


Figure 24. Model of MafB function in maternal rodent β-cells. Proposed mechanism by which MafB impacts rodent β-cells during pregnancy.

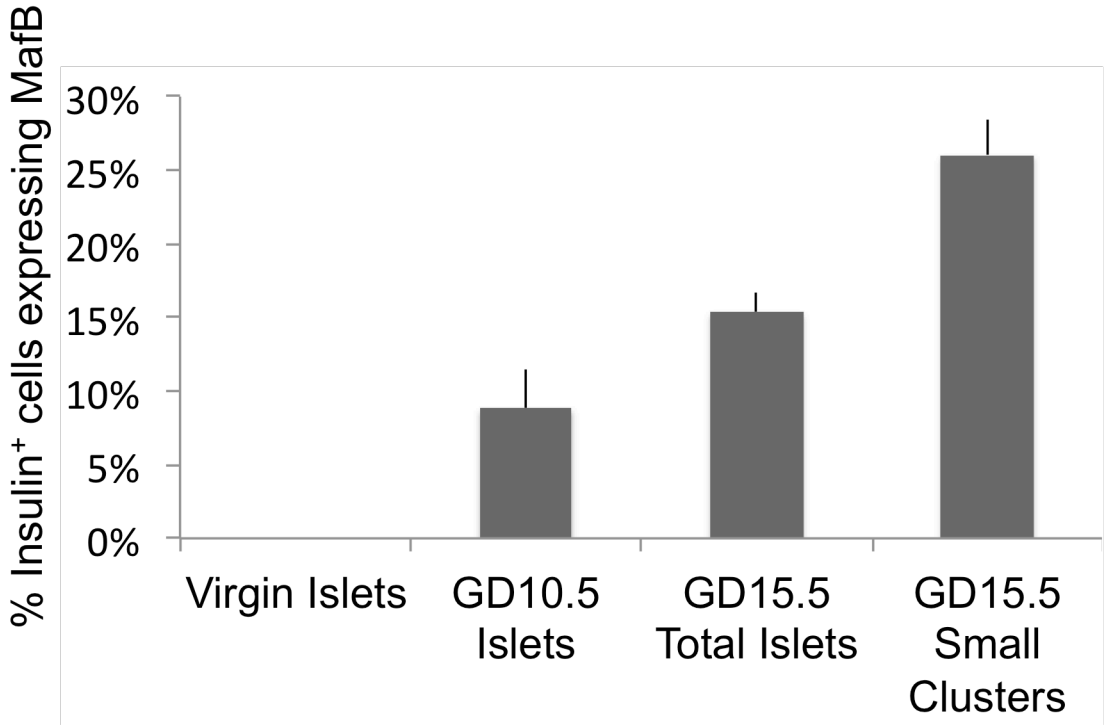


Figure 25. Of the samples examined, the height of MafB induction observed occurs at GD15.5. Quantitation of $MafB^{+}insulin^{+}$ cells detected in immunohistochemical islet samples from nonpregnant and pregnant female mice.

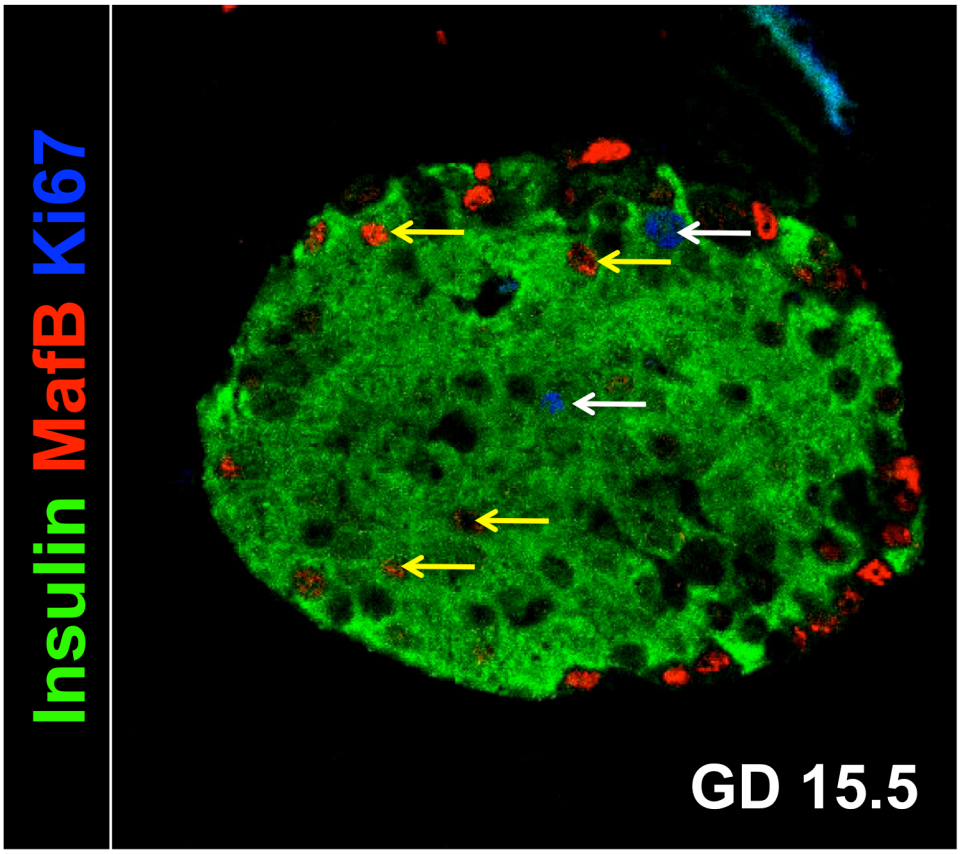


Figure 26. MafB is not induced in proliferating wild type β-cells during rodent pregnancy. Representative GD15.5 islet. Insulin (green), MafB (red), Ki67 (blue). White arrows denote Ki-67^{+}, thus proliferating cells; yellow arrows denote MafB^{+}insulin^{+} cells. Also confirmed with BrdU.

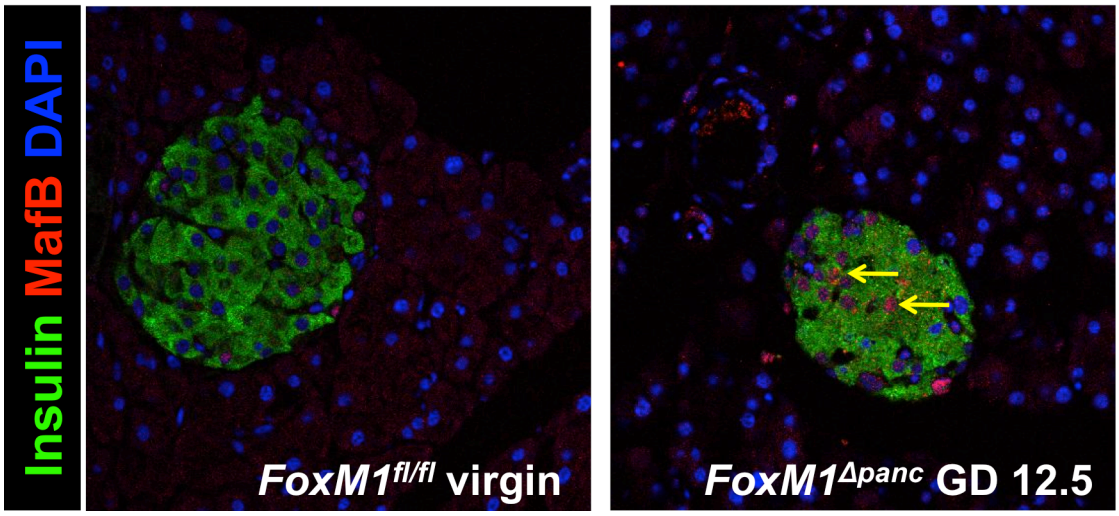


Figure 27. MafB is still produced in insulin$^+$ cells of *FoxM1*$^{\Delta panc}$ mothers. Representative GD12.5 *FoxM1*$^{\Delta panc}$ islet. Insulin (green), MafB (red), DAPI (blue). Yellow arrows denote MafB$^+$ insulin$^+$ cells.

strains were examined in these studies. Regardless, lack of MafB in proliferating maternal β-cells does not preclude its involvement in this adaptation. MafB could still impact adaptive maternal β-cell proliferation in the case that these MafB^{+}insulin^{+} cells are pre- or post-proliferative β-cells or that these cells are acting in a non-autonomous manner to promote proliferation in nearby β-cells.

MafB$^{\Delta\beta}$ Adult Mice Exhibit Normal Glucose Tolerance

MafB is no longer expressed in the rodent β-cell after 2 weeks postnatally (Artner et al., 2006). The *MafB$^{\Delta\beta}$* animals, similar to the *MafB$^{\Delta panc}$* animals (Figure 13B), are only glucose intolerant until 3 weeks of age and are able to clear glucose as effectively as *MafB$^{fl/fl}$* controls by 4 weeks (Figure 28). Additionally, these mice have no significant reduction in β-cell numbers at 4 weeks of age (Figure 29).

Notably, the *RIP-Cre* transgene alone reportedly produces a GDM-like phenotype, so we verified that *RIP-Cre* alone did not produce glucose intolerance in our colony (Brouwers et al., 2014; Lee, 2005) (Figure 30). In addition to neural *RIP-Cre* transgene expression, recent findings also report that a *human growth hormone* (*hGH*) minigene, inserted downstream of the *Cre recombinase* to facilitate efficient *RIP-Cre* transgene expression, produces a metabolic phenotype (Brouwers et al., 2014; Lee, 2005). This *hGH* minigene activates PRLR and downstream effects that simulate similar transcriptional activation and responses seen in pregnancy, like serotonin production, even without pregnancy (Brouwers et al., 2014; Schraenen et al., 2010). Glucose tolerance tests on animals with *RIP-Cre* alone, in nonpregnant and pregnant mice (Dr. Holly Cyphert) alike, indicate that the *MafB$^{\Delta\beta}$* model could be an effective model to determine how MafB impacts rodent β-cells during pregnancy.

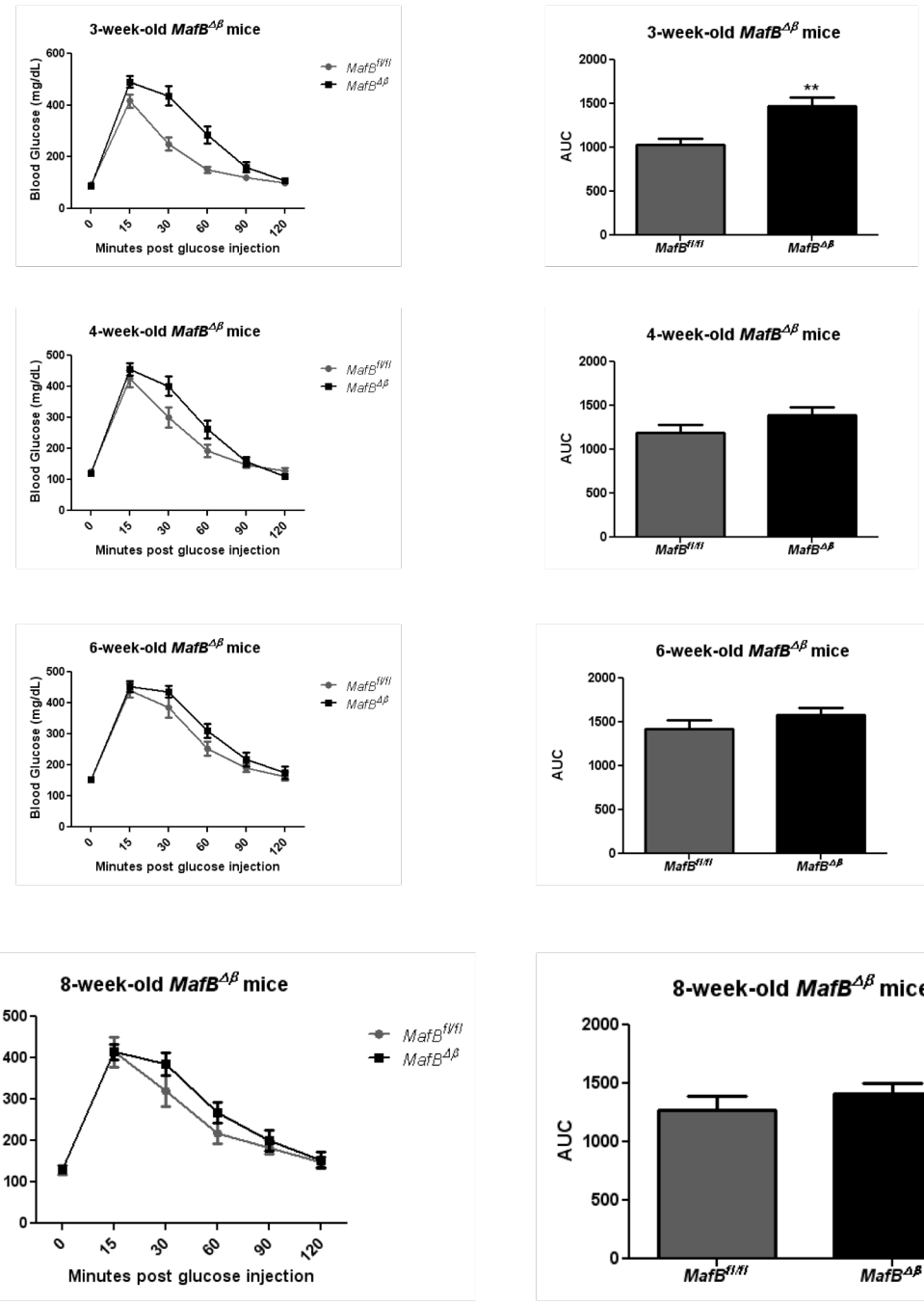


Figure 28. *MafB*$^{\Delta\beta}$ adults display normal glucose tolerance. Glucose tolerance tests performed at 3, 4, 6, and 8 weeks on *MafB*$^{\Delta\beta}$ and *MafB*$^{fl/fl}$ control littermates. (n ≥ 9).

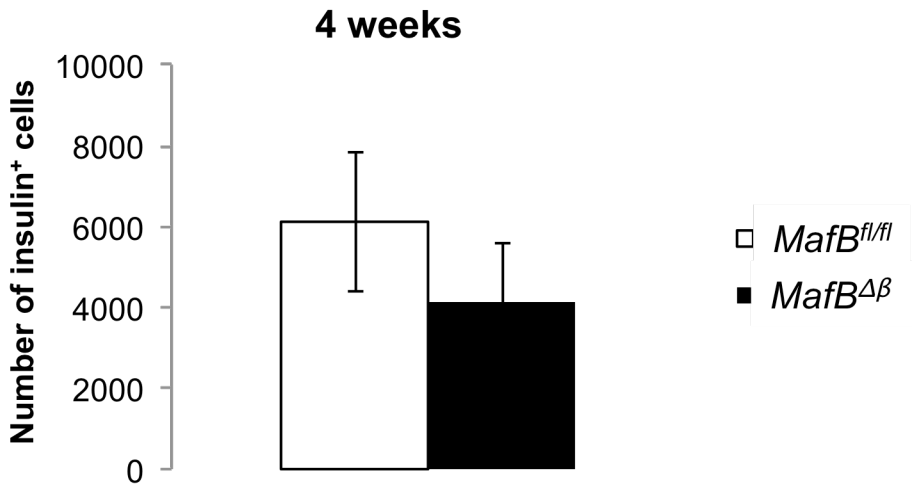
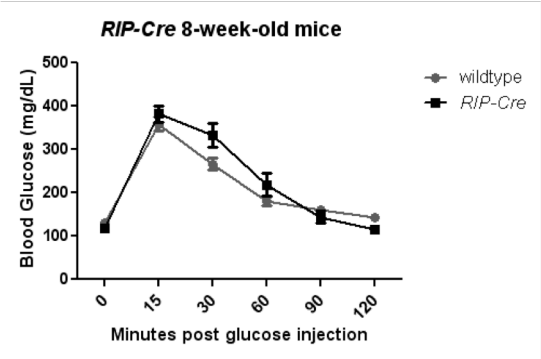


Figure 29. *MafB*$^{\Delta\beta}$ animals have no significant change in β-cell numbers. 4-week-old *MafB*$^{\Delta\beta}$ and *MafB*$^{fl/fl}$ islets quantified. (n=3).



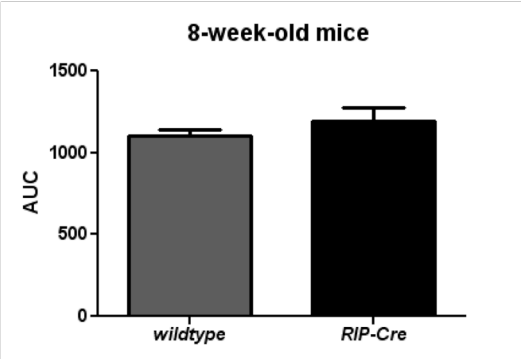


Figure 30. *RIP-Cre* adults display normal glucose tolerance. Glucose tolerance tests performed at 8 weeks on *RIP-Cre* and wildtype littermates. (n = 6).

Impaired GSIS Is Observed in MafB$^{\Delta\beta}$ Islets

Although the *MafB*$^{\Delta\beta}$ animals exhibit normal glucose tolerance, GSIS is impaired in *MafB*$^{\Delta\beta}$ islets (Figure 31). Specifically at 3, 6, and 9 weeks, *MafB*$^{\Delta\beta}$ islets are less responsive to glucose in comparison to control *MafB*$^{fl/fl}$ islets. With previous studies reporting glucose intolerance in C57BL/6J mice with the *RIP-Cre* transgene alone, this GSIS defect could be attributed to the *hGH* minigene in the *RIP-Cre* transgene (Brouwers et al., 2014; Schraenen et al., 2010). These experiments need to be repeated with *RIP-Cre* control islets to confirm the impact, if any, of the *RIP-Cre* transgene on islet insulin secretion function.

Pregnant MafB$^{\Delta\beta}$ Animals Are Glucose Intolerant

The pregnant *MafB*$^{\Delta\beta}$ mothers display a delayed glucose clearance in comparison to their pregnant *MafB*$^{fl/fl}$ littermates. This is observed with a maximal glucose dose (2 mg/kg body weight) in glucose tolerance tests (Figure 32A). Glucose intolerance in *MafB*$^{\Delta\beta}$ mothers was also confirmed with a submaximal dose (0.75 mg/kg), which was used to account for gestational weight gain and potential masking of leftward shift in the dose response for GSIS in *MafB*$^{\Delta\beta}$ mothers with a maximal glucose (Pound et al., 2013) (Figure 32B).

MafB in Maternal β-cells Affects β-cell Proliferation and Requires PRLR

Glucose intolerance in *MafB*$^{\Delta\beta}$ mothers could be due to either an insufficient number of functional β-cells or impaired function of β-cells. To examine the impact of blocking MafB induction in maternal β-cells on the adaptive β-cell mass expansion, proliferation was assessed to determine that pregnant *MafB*$^{\Delta\beta}$ animals have reduced β-cell proliferation in contrast to pregnant *MafB*$^{fl/fl}$ controls (Figure 33, Dr. Holly Cyphert).

Interestingly, β-cell-specific *Prlr* knockout mice (βPRLKO) develop GDM with

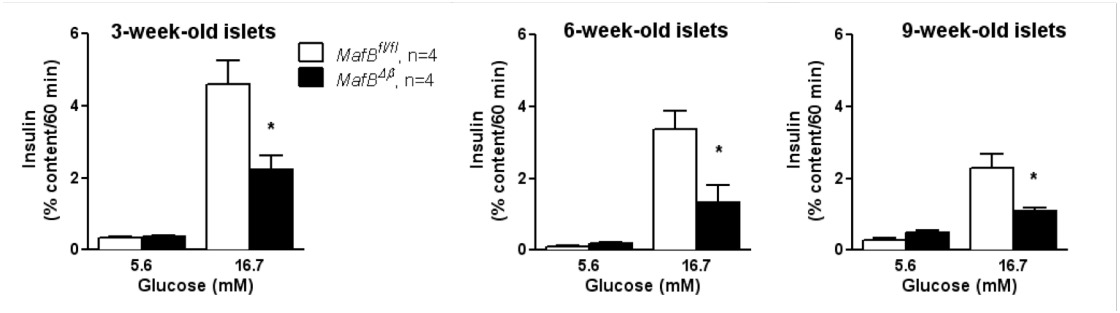


Figure 31. *MafB*$^{\Delta\beta}$ islets display slightly impaired GSIS compared to *MafB*$^{fl/fl}$ islets. Static islet culture performed on at 3-, 6-, and 9-week-old islets at low (5.6 mM) and high (16.7 mM) glucose. (n=4).

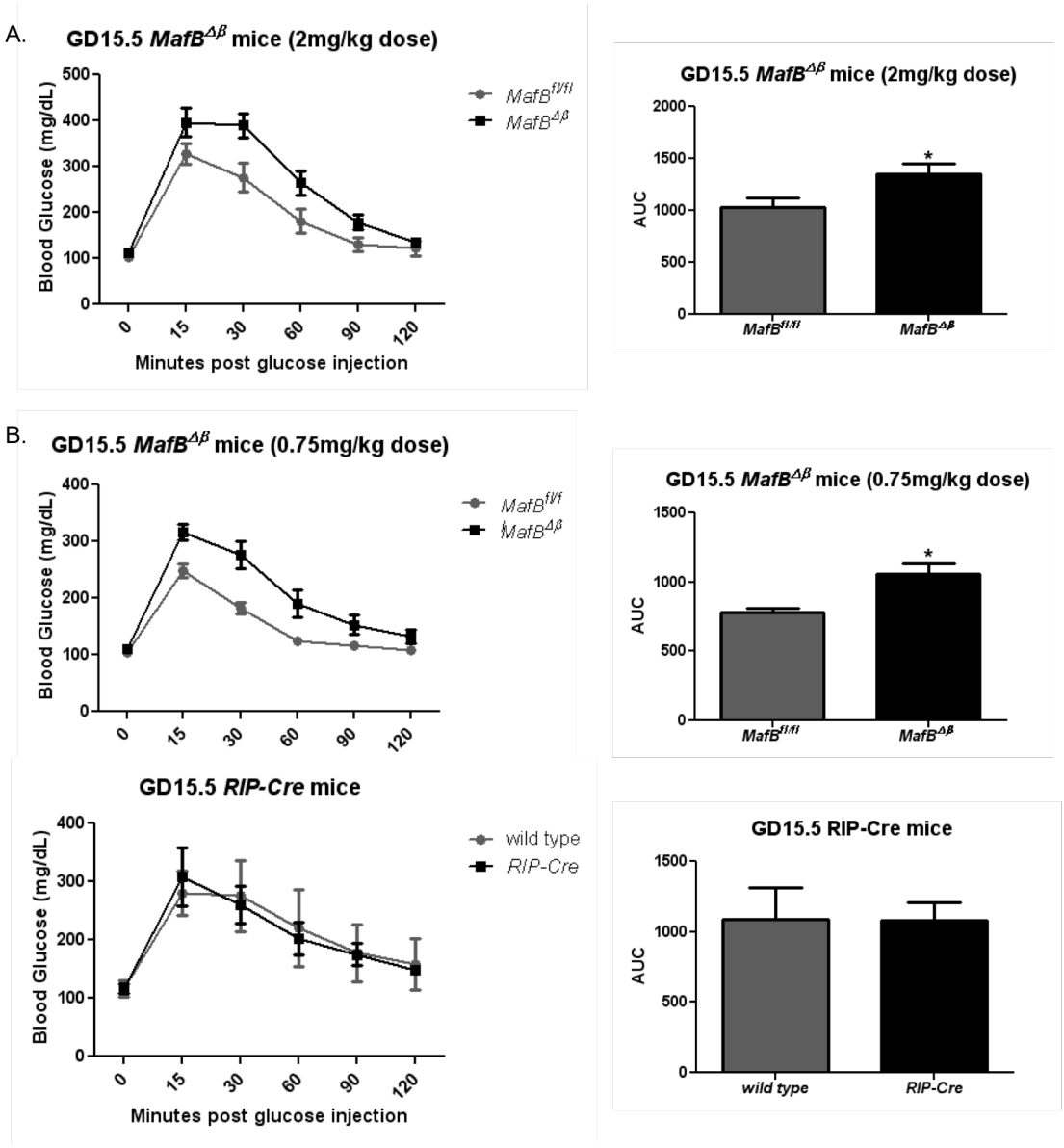


Figure 32. Pregnant *MafB*$^{\Delta\beta}$ animals are glucose intolerant. Glucose tolerance tests performed on GD15.5 *MafB*$^{\Delta\beta}$ and *MafB*$^{fl/fl}$ littermates and area under the curve (AUC) analyses with (A) 2mg/kg glucose dosage (n ≥ 11) and (B) submaximal 0.75mg/kg dosage (n ≥7). * $p < 0.05$ compared with *MafB*$^{fl/fl}$ controls. (C) Glucose tolerance tests performed on GD15.5 *RIP-Cre* and wild type mothers (Dr. Holly Cyphert) (n=4).

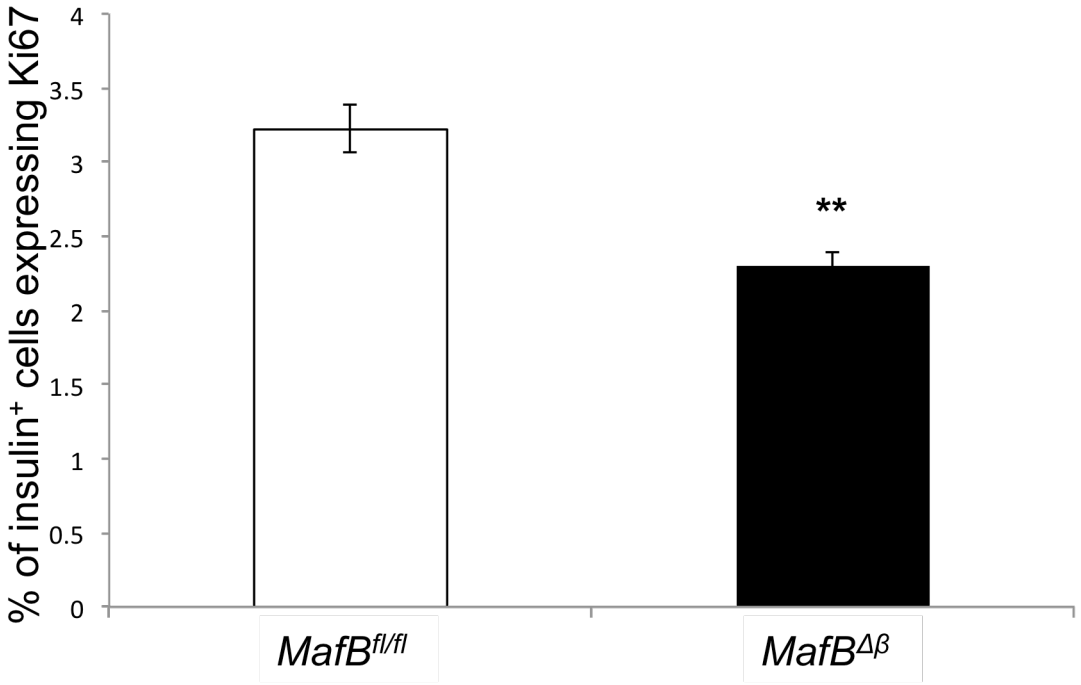


Figure 33. *MafB*$^{\Delta\beta}$ mothers exhibit impaired proliferation during pregnancy. (Holly Cyphert). Quantitation of GD15.5 *MafB*$^{\Delta\beta}$ and *MafB*$^{fl/fl}$ islets. * $p < 0.05$ compared with GD15.5 *MafB*$^{fl/fl}$ controls.

impaired β-cell proliferation and mass expansion (Drs. Ron Banerjee and Sueng Kim, personal communication). In collaboration with Drs. Ron Banerjee and Sueng Kim, it was found that MafB induction is blocked in maternal βPRLKO β-cells. Additionally, similar gene expression changes were observed in the *MafB*$^{\Delta\beta}$ and βPRLKO maternal islets, including failure to induce genes that promote β-cell cycle progression like *FoxM1, Ccna2*, *Ccnb1*, and *Ccnb2* (Dr. Holly Cyphert, personal communication). These observations indicate that MafB lies downstream of PRLR in a pathway that mediates the adaptive maternal β-cell mass expansion and perhaps enhanced maternal β-cell insulin secretion. Further investigation of β-cell function is warranted to determine whether MafB contributes to enhanced maternal GSIS.

Discussion

This work establishes a role for MafB in the maternal β-cell adaptations. Although MafB induction in β-cells during pregnancy coincides with the height of adaptive proliferation, we fail to detect MafB in proliferating maternal β-cells. With the *MafB*$^{\Delta\beta}$ mouse model, we find that lack of MafB induction results in glucose intolerance that is, at least in part, due to impaired maternal β-cell proliferation. Moreover, blockage of MafB induction in the βPRLKO maternal β-cells indicates that PRLR lies upstream of MafB in a pathway that mediates some of the proliferative pregnancy adaptations that are impaired in βPRLKO mothers.

We propose that MafB induction, downstream of prolactin receptor activation, contributes to the enhanced maternal β-cell proliferation and potentially the enhanced GSIS as well (Figure 34). This work establishes that MafB is important to the adaptive β-cell proliferation of pregnancy, likely impacting β-cell mass during this time. β-cell mass analysis of pancreata from pregnant *MafB*$^{\Delta\beta}$ animals would need to be conducted to

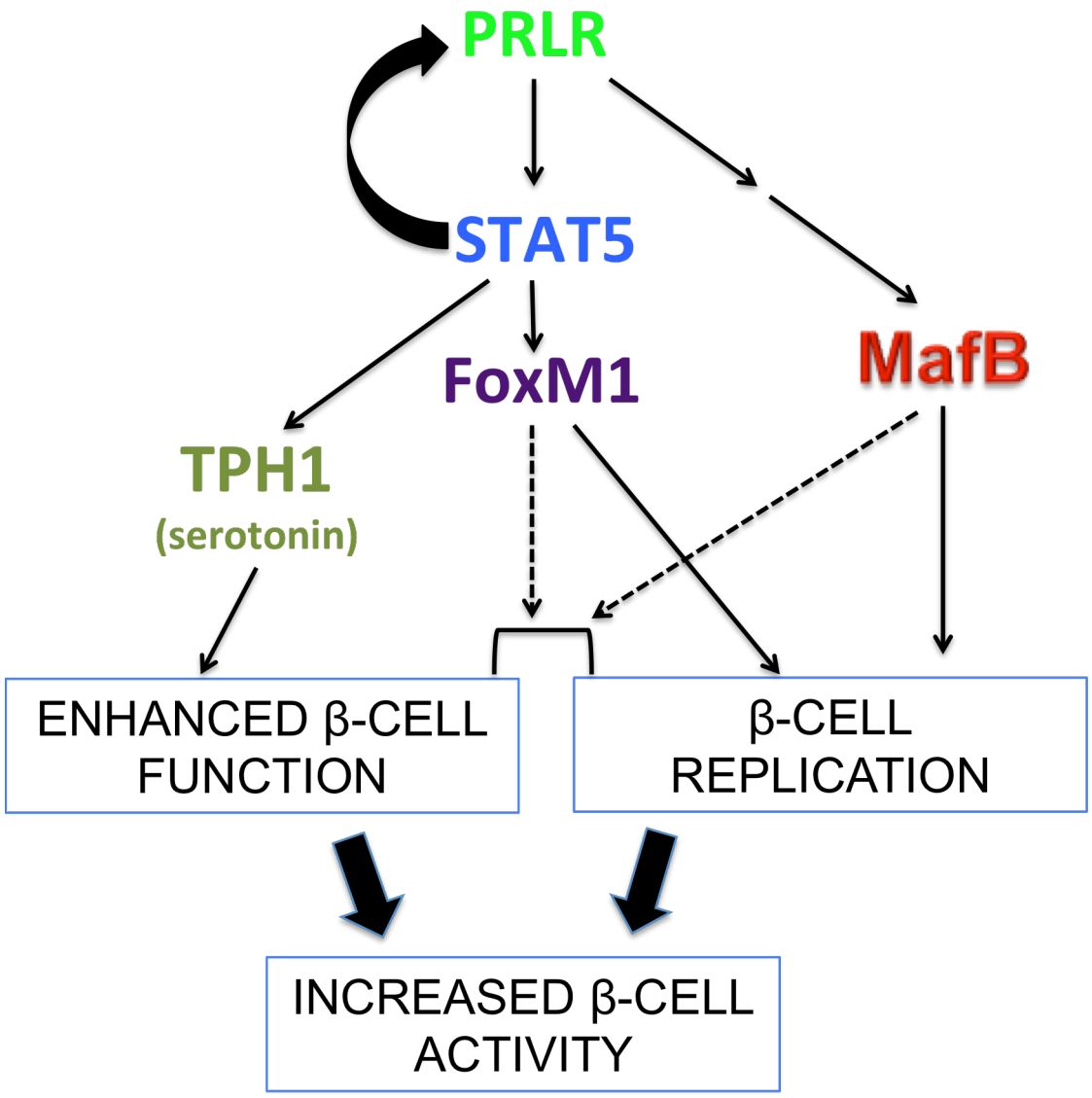


Figure 34. Model of MafB function in maternal rodent β-cells. Proposed mechanism by which MafB impacts rodent β-cells during pregnancy. Solid arrows = established, dotted = not yet determined.

confirm that the observed GD15.5 *MafB*$^{\Delta\beta}$ proliferation defect results in reduced β-cell mass. Additionally, the effect of MafB on maternal β-cell function is still unclear. GSIS experiments (static culture or islet perifusion) on islets from *MafB*$^{\Delta\beta}$, *MafB*$^{fl/fl}$, and *RIP-Cre* mothers could clarify whether MafB has a role in maternal β-cell insulin secretion or just in the proliferative β-cell adaptation. Although our collaborative study with the Kim lab focused on the maternal β-cell population expansion, PRLR signaling is also thought to be required for other β-cell adaptations to pregnancy, including enhanced GSIS (Sorenson and Brelje, 1997). If GSIS function is impaired in *MafB*$^{\Delta\beta}$ maternal islets, a similar defect would be anticipated in maternal βPRLRKO islets. If this were confirmed, then the adaptive increase in insulin secretion and in β-cell mass would be mediated by PRLR induction of MafB, at least partially.

Unfortunately, *MafB*$^{\Delta\beta}$ islet GSIS may be slightly impaired even before pregnancy making this a less valid model of GDM. *RIP-Cre* control experiments still need to be conducted for this GSIS analysis. Furthermore, previous reports indicate that the *Cre* transgene alone could be producing the impaired *MafB*$^{\Delta\beta}$ islet GSIS (Brouwers et al., 2014; Lee, 2005). Notably, the *MafB*$^{\Delta panc}$ islets from the previous chapter lack MafB in the α- and β-cell population, yet exhibit normal glucose tolerance, β-cell GSIS, and β-cell-enriched gene expression at 8 weeks of age. This supports the possibility that the *MafB*$^{\Delta\beta}$ GSIS phenotype is driven by the *RIP-Cre* transgene *hGH* minigene. Under the scenario that the *RIP-Cre* transgene alone produces transcriptional changes that impact the metabolic phenotype, a better model for these studies could be *RIP-Cre*^{Herr} (Herrera, 2000); *MafB*$^{fl/fl}$ mice. This transgene has less neural Cre expression and lacks the *hGH* minigene in the 3' region (Brouwers et al., 2014; Herrera, 2000; Wicksteed et al., 2010). Follow-up functional islet experiments with the appropriate *RIP-Cre* controls could be informative for MafB impact on maternal β-cell function.

The β-cell adaptations to pregnancy are notable and important to future endeavors to generate or modify β-cells therapeutically. Maternal β-cells behave much like neonatal β-cells in that they are proliferative and exhibit higher basal insulin secretion (Lain and Catalano, 2007). Moreover, cell cycle regulators and factors like Foxm1 and MafB are more highly expressed in the β-cells of neonates and pregnant mothers alike (Artner et al., 2006; Goodyer et al., 2012; Zhang et al., 2009b). If the mechanisms by which β-cells proliferate and secrete more insulin were more completely understood, they could be driven in preexisting or hESC protocols for clinical applications in diabetics. Future studies to determine MafB function in maternal β-cells, with this model and in human cells, could determine how MafB could impart these β-cell adaptions.

Chapter VI

SUMMARY AND FUTURE DIRECTIONS

Thesis Summary

Prior to this work, the only *in vivo* rodent studies of MafB impact on islet cells resulted in early death due to global *MafB* removal (Artner et al., 2007). With the generation of a viable *MafB* mutant mouse in Chapter IV, we determined that MafB is crucial to adult α-cell function and also regulates islet *Ghrelin* expression; although, it not as significant to the postnatal rodent β-cell as previously anticipated (Artner et al., 2007). We also demonstrate that both Mafs are collectively required for β-cell formation, as *MafAB*$^{\Delta panc}$ animals die a few days after birth. However, NHP comparative analyses and EndoC-βH1 GSIS analysis demonstrate that, contrary to the rodent, MAFB is important to the adult primate β-cell. This difference in MAFB β-cell impact between rodents and humans indicates that there are essential considerations moving forward with rodent models for human islet studies. In Chapter V, the *MafB*$^{\Delta\beta}$ model was utilized to examine the impact of MafB induction on maternal rodent β-cells. These results demonstrate that MafB is induced by PRLR in the maternal β-cell and is involved in adaptive proliferation during pregnancy. This thesis work establishes the significance of MAFB in human β-cell function and provides new information on the role of MAFB in postnatal islet cells (Table 6).

	MafA	MafB
Role in rodent β-cell development	No	Yes (and islet α-cell)
Role in mature rodent β-cell function	Yes	No, but crucial to α-cells
Role in mature rodent α-cell function	Not produced	Yes
Phosphorylation-dependent dimerization and DNA binding (βTC3 cells)	Yes	No
Binds promoter region of *Insulin* and *Glucagon*	Yes	Yes
Produced in adult rodent β-cell during pregnancy	Yes	A subset (20%)
Role in maternal rodent β-cell adaptations	?	Yes, at least involved in proliferation
Produced in adult rodent and human β-cell	Yes	No; rodent, Yes; human
Important to human β-cell function	Yes	Yes, in cAMP-mediated GSIS

Table 6. Known roles of MAFA and MAFB in pancreatic islet cell development and function. Zhang et al., 2005. Artner et al., 2010. Hang et al., 2014. Artner et al., 2007. Guo et al., 2010. Artner et al., 2008. Dai et al., 2011. David Scoville and Dr. Holly Cyphert, Personal communication. Findings from this thesis work are in red text.

Future Directions

Now that we have established that MAFB is important for human β-cell GSIS, when it is most important and what MAFB targets are most impactful are not known. Additionally, we demonstrate that MafB is necessary for α-cell function, but more mechanistic investigations could indicate what α-cell factors are mediated by MafB and if this regulation is recapitulated in human α-cells. Earlier chapters have briefly proposed future experiments for the respective studies. Here, I propose future studies with mouse models, human β-cells, and human islets that could advance our understanding of MAF function and human islet cell function and pathophysiology.

Examining Spatial and Temporal MAF expression in Human Islet Development

MAFB expression is maintained in the adult human β-cell, unlike in rodents where it is only produced in the developing β-cell before being restricted to adult rodent α-cells (Artner et al., 2010; Dai et al., 2011; Nishimura et al., 2006). Notably, MAFB appears to be produced at much higher levels than MAFA in developmental and early postnatal human islets (Marcela Brissova, personal communication). This finding is supported by the high NHP *MAFB* levels reported in Chapter IV and changes in *MAF* expression in the human β-cell EndoC-βH2 line after immortalization gene excision (Scharfmann et al., 2014). This cell line utilizes the Cre/lox system to excise the immortalization gene products, SV40 large Tag and hTERT. Strikingly, this leads to cessation of cell proliferation, massively enhanced β-cell differentiation, and physiological features that normally occur *in vivo* after birth. Such changes include adult-like levels of *INSULIN* mRNA, INSULIN protein, and glucose-induced insulin secretion. Significantly, profiling of islet-enriched transcription factor expression suggests that these effects are mediated by a profound and selective increase in *MAFA* over other regulators (Scharfmann et al., 2014). These results indicate that

immortalization gene excision in the EndC-βH2 cells generally mimics the human β-cell MAF expression.

To supplement previous observations that MAFB precedes MAFA in developing human islet cells, gene expression and IHC analyses could determine when MAFB and MAFA are each first detected. Ghrelin^{+} cells of the human islet and gut could be assessed to determine whether MAFB expression colocalizes with this hormone in the islet, like in our rodent model. This islet expression information could determine how MAF expression, spatially and temporally, changes as human development progresses. Knowledge of when and in which islet cell populations MAFB and MAFA are expressed in human islet cell development will help define the interrelationship of these factors and how they function in human islet cell maturation.

Investigating How and When MafB Impacts the Adult α- and β-Cells *In Vivo*

To identify unique MafB targets in α-cells versus β-cells and at different timepoints in these islet populations, our *MafB*$^{\Delta panc}$ model could be crossed with *Mouse Insulin Promoter-GFP* (*MIP-GFP*) transgenic or *Glucagon*^{GFP} knock-in mice (Hara et al., 2003; Hayashi et al., 2009) to sort the mutant α- and β-cell populations by fluorescence-activated cell sorting (FACS). RNA-seq assays could be conducted with early postnatal (prior to 2 weeks) and adult (8 week) *MafB*$^{\Delta panc}$ α- and β-cells to determine which genes are impacted by MafB loss in each population. Notably, this data would include genes that are primarily and secondarily impacted by lack of MafB. Follow up chromatin immunoprecipitation (ChIP) experiments could then validate direct MafB targets from these candidate genes from the RNA-Seq. This data would not only supplement the earlier *MafB*$^{-/-}$ E15.5 pancreatic microarray, but also refine our knowledge of MafB gene targets in these specific islet populations.

To determine exactly when MafB is no longer important for the regulation of *Insulin* expression in rodents, altered gene expression (including *Insulin* and genes identified in the early postnatal *MafB*$^{\Delta panc}$ β-cell RNA-seq) could be assessed in 1-, 2-, and 3-week-old *MafB*$^{\Delta panc}$ islets. This information could shed light on the shift from MafB expression alone to MafA expression in β-cell maturation. The shift from MafB alone in early rodent development could be reflective of human β-cell maturation, as the general pattern of MAFB preceding MAFA in developing and early postnatal human β-cells is preserved.

The adult α-cell *MafB*$^{\Delta panc}$ RNA-seq analysis could provide novel mechanistic gene targets involved in α-cell identity and function. Not only would *Glucagon* expression likely be reduced, but there might also be a reduction in factors implicated in hormone secretion that are shared with β-cells, such as *Atp2a2, Pcsk2, Kir6.2*. Because the β-cells comprise most of the islet, alterations in the α-cell population may have been masked in the earlier whole islet *MafB*$^{\Delta panc}$ analysis in Chapter IV.

A potential issue with sorting islet cells for gene expression analysis that should be noted is that both MafA and MafB are sensitive to oxidative stress (Guo et al., 2013b). The process of dissociating islets could impact gene expression and confound expression results. Under this scenario, whole islet analysis would still be valuable to identify novel MafB targets.

Targets identified from these different *MafB*$^{\Delta panc}$ RNA-seq analyses could then be examined in the more precious human islet samples to verify that similar MAFB regulation occurs in human islet cells. Moreover, identifying novel MAFB gene targets could explain how the different GSIS function between humans and rodents could be mediated by sustained MAFB expression in adult human β-cells. Taken together, this information would enhance our understanding of Maf hormone regulation and the

MafA/MafB interrelationship that could shed light on MAFA and MAFB function in human islet cells by identifying new MAFB β- and α-cell targets.

Investigating a Rodent Model to Determine the Role of MafB in α-Cell Development and Adult Function *In Vivo*

The α-cell population is known to be dysfunctional in T1DM and T2DM, yet is less widely studied than the islet β-cell population. Moreover, many T2DM therapeutics, like Metformin, at least in part, function by antagonizing the actions of glucagon. Still, how α-cell glucagon secretion is regulated remains a topic of debate (Gylfe and Gilon, 2014). Previous work has indicated that MafB is likely important to the α-cell population as a potent *Glucagon* activator (Artner et al., 2006; 2007). In addition, $MafB^{\Delta panc}$ analysis presented in Chapter IV supports the significance of MafB for *Glucagon* expression and α-cell function (Figure 15). Generating an α-cell-specific *MafB* mutant mouse with the *MafB floxed allele* and a *Glucagon-Cre* transgene (Herrera, 2000), or even an inducible *Glucagon-rtA; TetO-Cre* (Perl et al., 2002; Thorel et al., 2010) line would specifically examine the role of MafB in this population and the impact on the whole organism and islet function when MafB is absent from α-cells. A collaborator, Dr. Sueng Kim, is presently pursuing this MafB α-cell investigation.

Investigating a Rodent Model to Determine How MafA/MafB Coexpression Impacts the Adult β-Cell *In Vivo*

MAFA and MAFB are coexpressed in the adult human β-cell and evidence from this thesis and the work of others indicates that expression of MAFB in adult β-cells drives some of the observed islet differences between rodents and humans (Dai et al., 2011). Our lab has generated a transgenic mouse model that maintains MafB (*MafB-Tg*) in the adult rodent β-cell to assess *in vivo* functional analysis of MafA/B coexpression

(Drs. Yan Hang and Holly Cyphert, personal communication). These animals have normal rodent islet architecture and normal glucose tolerance. Gene expression analysis indicates that sustained MafB expression in rodent β-cells drives higher levels of *Insulin, G6PC2*, and *CyclinD2*. From these results, rodent islets with $MafA^{+}MafB^{+}$ β-cells could have some advantage over MafA alone; but in young, lean, and nonpregnant mice, the impact is not obvious. Interestingly, islet perifusion experiments with the *MafB-Tg* animals crossed with the β-cell-specific *MafA* mutant mice indicate that MafB expression in the rodent β-cell can partially rescue the defects of the *MafA* mutant model and that MafB impacts first phase insulin secretion (David Scoville and Dr. Holly Cyphert, personal communication).

In vivo MafA/MafB coexpression in adult β-cells could be further investigated to determine if *MafB-Tg* animals are more or less susceptible to hyperglycemia. Under physiologically stressed conditions, β-cells adapt, at least initially, to increased metabolic demands. *MafB-Tg* aging and high fat diet (HFD) studies could be conducted with glucose tolerance tests and the following analyses: α- to β-cell ratio, islet architecture, islet gene expression, β-cell mass, and β-cell proliferation rates. Based on increased *Insulin* expression in *MafB-Tg* animals and the impact of MafB on maternal rodent β-cells, *MafB-Tg* animals are anticipated to be more resilient than wildtype rodents. $MafA^{+}MafB^{+}$ cells are predicted to have more proliferative potential such that *MafB-Tg* mice on HFD would have improved glucose tolerance and an increased β-cell mass versus wild type mice on HFD. These experiments could reveal the impact of sustained MAFB β-cell expression and if MAFB expression in human β-cells could confer some resilience under stress. Moreover, these analyses would examine whether MafA/MafB coexpression alone confers the human GSIS profile or if there are other variables, such as different cofactors for the MAFs that would drive the distinct human and mouse GSIS profiles.

Biochemical Analysis of MAFA and MAFB Coexpression in Human β-Cells

We can learn a lot about how MafA and MafB function from our rodent models. However, observations from our mouse models need to be confirmed in human cells because ultimately knowing how MAFA and MAFB function in humans is necessary for therapeutic advancements. Our studies identify MAFB significance to adult β-cell function as a key distinction between human and mouse islets, but this work only begins to explore how sustained MAFB expression impacts human islet β-cell function. Unfortunately, human *in vivo* studies cannot be conducted. "Humanized" models are only beginning to be utilized. These models are generated by transplantation of human islet cells or β-like cells from hESC or IPS-cell differentiation protocols into mice (Brissova et al., 2014; Kroon et al., 2008; Rezania et al., 2012).

To circumnavigate some of the human islet caveats, glucose-responsive human β-cell lines, like the EndoC-βH1 human β-cell line used in Chapter IV, have been used to determine how MAFA and MAFB affect gene expression and β-cell function in experiments that utilize short hairpin interfering RNA (shRNA) or siRNA to knockdown MAFs (Pasquali et al., 2014)(David Scoville, personal communication). Static culture experiments demonstrate that both MAFA and MAFB impact GSIS in this human β-cell line (David Scoville, personal communication). Additionally, gene expression analyses after each MAF knockdown reveals that MAFA and MAFB impact many, but not all, of the same targets previously reported in mice. The effect on only a subset of genes identified as Maf targets with MAFA or MAFB knockdown in EndoC-βH1 cells may be due to compensation by the other MAF, uniquely both present in these human cells. Notably, siRNA knockdown of both MAFA and MAFB in these cells results in very low knockdown efficiency, so whether more of these targets are impacted with reduction in both MAFs is unknown.

Even more compelling than the Endo-βH1 cells, the human EndoC-βH2 exhibits an expression profile more reflective of mature β-cells. The EndoC-βH1 cells are unlike primary β-cells in that they are continuously proliferating and are less glucose-responsive, and so behave more like immature β-cells. With Cre-mediated excision of the immortalization genes, the newly developed $MAFA^{+}$ $MAFB^{+}$, glucose-responsive, human Endo-βH2 β-cell line can be used to substantially extend our knowledge of these transcription factors in the mature human β-cell. Experiments could be performed on EndoC-βH2 cells after transduction with a Cre-expressing lentiviral vector to excise the immortalization genes and lentiviral delivery of shRNAs that target MAFA and MAFB. A recent analysis in EndoC-βH1 cells achieved about a 60% knockdown efficiency with lentivirally delivered shRNAs that target MAFB (Pasquali et al., 2014). Moving forward, the EndoC-βH2 cells could even be transplanted under the kidney capsule in mice for a novel "humanized" model.

Identifying Unique and Shared Targets of MAFA and MAFB

Studies have revealed that MafA and MafB are not biochemically equivalent (Artner et al., 2010; Guo et al., 2010). However, they have some shared targets and there is evidence of compensatory expression or function in mutant mouse models (i.e. $MafA^{\Delta panc}$, $MafB^{\Delta panc}$, and $MafAB^{\Delta panc}$) (Artner et al., 2010; Hang et al., 2014). RNA-seq analysis after Cre-mediated immortalization gene excision and MAF knockdown in the EndoC-βH2 cells could provide novel information and be compared to the mouse islet cell RNA-seq analyses proposed earlier in this chapter (Wang et al., 2009). MAFA and MAFB knockdown, in combination and separately, could identify shared and independent targets. Different experimental knockdown would have to be explored to overcome reduced efficiency with knockdown of both MAFs.

I would anticipate more shared targets of MAFA and MAFB in the human β-cells versus adult rodent Maf regulation. This would result in not all of the Maf targets identified in the rodent studies being impacted with knockdown of just one of the MAFs in human β-cells. These results would indicate that these target genes could be regulated by the other MAF in humans. This analysis could then be compared to MAFA and MAFB knockdown in sorted human islet cells to determine how closely the cell line mimics human MAF regulation. Identifying human MAF targets from these experiments could indicate how MAFA and MAFB impact adult human β-cell identity and function and how MAFA/MAFB coexpression could drive some of these key species islet distinctions.

Chromatin immunoprecipitation-sequencing (ChIP-seq) analysis of MAFA and MAFB in the EndoC-βH2 cells after Cre-mediated immortalization gene excision could indicate which cis-regulatory elements are occupied by these two factors in human β-cells. These results could be compared to existing ChIP-seq data sets, including MAFB in human islets and MafA in mouse islets (Hoffman et al., 2008; Johnson et al., 2007; Pasquali et al., 2014). MAFA ChIP-seq analysis in human islets has yet to be conducted. MAFB ChIP-seq in sorted human α-cells and β-cells could reveal distinct MAFB occupancy in β-cells and α-cells. Furthermore, direct targets could be identified if MAFA or MAFB are found to bind cis-regulatory elements of targets identified from the RNA-seq analyses proposed in an earlier section. Based on a recent study, enhancer sites associated with MAF target gene expression are anticipated to have the most impact on islet cell type-specific transcriptional activity. This is in contrast to binding of islet-specific transcription factors at promoters (Pasquali et al., 2014).

Cis-regulatory elements of targets bound by MAFA and MAFB from each respective ChIP-seq analysis would likely include different MAF dimer partners. These dimers would include the MAFA-MAFB heterodimer, as well as the $MAFA_2$ and $MAFB_2$ homodimers. Re-chip of the promoters identified from the ChIP-seq analyses could

confirm which elements are occupied by the MAFA-MAFB heterodimer or the homodimers (Buck and Lieb, 2004) (Figure 35). These experiments could elucidate how these different MAF dimers impact changes in gene expression, islet function, and islet pathophysiology.

Examining the Mechanism of MAFB in GSIS with the EndoC-βH2 Cells

The GSIS EndoC-βH1 cell culture experiment in Chapter IV demonstrates that loss of MAFB impacts the cAMP-mediated amplification of GSIS in human β-cells. This experiment should be repeated in the EndoC-βH2 cell line after Cre-mediated immortalization gene excision to confirm that these cells, with more of the mature β-cell attributes, are similarly impacted by MAFB loss. Moreover, these results would suggest that MAFB impacts GSIS downstream of cAMP. Investigation of different proximal cAMP targets, such as PKA and Epac, could provide more detail to the mechanism of MAFB-mediated GSIS.

Investigating the Role of MafB in Islet Cell Adaptations to Pregnancy

The functional impact of MafB induction in maternal rodent β-cells has been established by work included in this thesis, but the mechanism by which MafB functions in the maternal β-cell adaptations is not yet understood. Moreover, animal models, like the *MafB*$^{\Delta\beta}$ model in Chapter V, are particularly valuable for pregnancy studies because pregnant human islet samples are extremely rare and the whole body changes inherent to pregnancy are impossible to mimic in a cell line.

Interestingly, the *MafB-Tg* mothers, presented in a previous section, exhibit improved glucose tolerance from wild type pregnant mice (Dr. Holly Cyphert, personal communication). This suggests that having many more MafA^{+}MafB^{+}insulin^{+} cells has a physiological advantage over wild type pregnant mice with fewer MafA^{+}MafB^{+}insulin^{+}

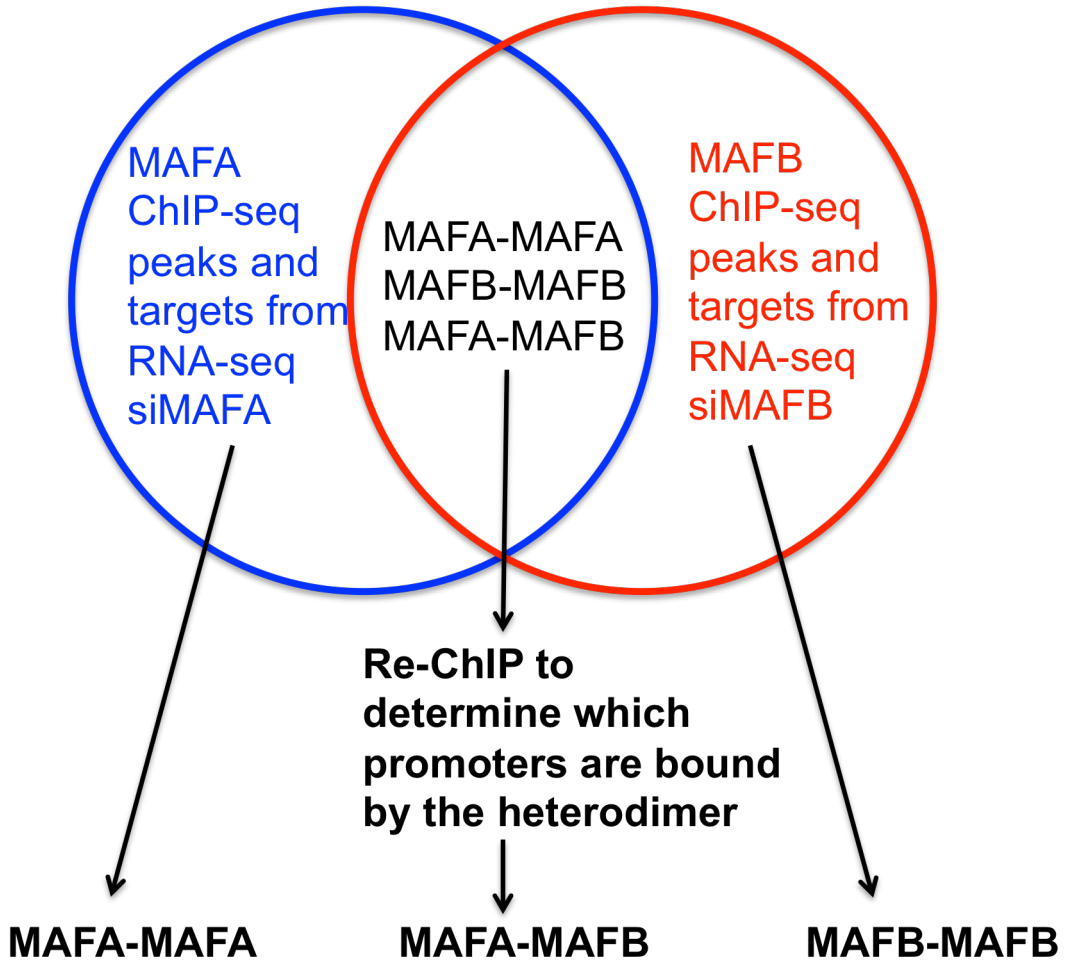


Figure 35. Model of experimental analysis to determine MAFA and MAFB dimer targets identified from ChIP-seq, RNA-seq, and Re-Chip analysis in EndoC-βH2 cells after Cre excision.

cells. Based on the *MafB*$^{\Delta\beta}$ pregnancy studies, β-cell proliferation and mass increases would be anticipated in *MafB-Tg* mothers. Changes in islet gene expression of *MafB-Tg* pregnant mice and wild type controls would confer any unique impact of the larger MafB^{+} β-cell population in these mutant mothers. GSIS functional assays, including static islet culture and islet perifusion, could also establish if MafB improves insulin secretion function during pregnancy to contribute to β-cell pregnancy adaptations.

Although very little is known about islet α-cells in the context of pregnancy, α-cell dysregulation could somehow also impact maternal islet function. Dysregulated glucagon secretion could occur by paracrine disinhibition of glucagon signaling or cell-autonomous α-cell dysfunction that could contribute to the hyperglycemia of GDM. MafB in α-cells could have a role in maternal α-cell function. This possibility could be investigated with the inducible pregnant α-cell-specific *MafB* mutant mothers.

These findings could be extended to humans to determine if MAFB similarly plays an integral role in human maternal β-cells and perhaps α-cells. Islets from pregnant humans could be assayed to determine if MAFB is produced at even higher levels in maternal human islet cells. Furthermore, females with lower levels of MAFB could be more susceptible to GDM. These studies could link MAFB function to human islet pregnancy adaptations.

Concluding Remarks

This thesis work utilizes novel mouse models of Maf ablation, comparative NHP islet analyses, and a human β-cell line functional assay to interpret how different MAF expression impacts human islets. These findings demonstrate that MAFB is a key factor in human β-cell function that could be driving differences between rodent and human islet function. Although this work demonstrates that MafB only impacts the developing and early postnatal rodent β-cell, it remains important to adult α-cell function and it may become important to rodent β-cells during pregnancy. This work establishes that MAFB expression in human β-cells is distinguishing and impactful to human β-cells, in contrast to the commonly used rodent model.

Moving forward, a more thorough understanding of how MAFB impacts cAMP levels, and thus insulin secretion, in the human β-cell is warranted. Furthermore, investigations into MAFB function in the α-cell will be crucial for a more complete understanding into the role of MAFB in healthy and diabetic islets, where its levels are reduced in both α- and β-cells (Guo et al., 2013b). Moreover, interpretations from mouse models remain valuable to islet studies as long as researchers identify and acknowledge key differences found between these experimental models and humans.

REFERENCES

Abdellatif, A.M., Ogata, K., Kudo, T., Xiafukaiti, G., Chang, Y.-H., Katoh, M.C., El-Morsy, S.E., Oishi, H., and Takahashi, S. (2015). Role of large MAF transcription factors in the mouse endocrine pancreas. Exp. Anim.

Ackermann, A.M., and Gannon, M. (2007). Molecular regulation of pancreatic -cell mass development, maintenance, and expansion. Journal of Molecular Endocrinology *38*, 193–206.

Aguayo-Mazzucato, C., Koh, A., Khattabi, El, I., Li, W.-C., Toschi, E., Jermendy, A., Juhl, K., Mao, K., Weir, G.C., Sharma, A., et al. (2011). Mafa expression enhances glucose-responsive insulin secretion in neonatal rat beta cells. Diabetologia *54*, 583–593.

Ahlgren, U., Jonsson, J., and Edlund, H. (1996). The morphogenesis of the pancreatic mesenchyme is uncoupled from that of the pancreatic epithelium in IPF1/PDX1-deficient mice. Development *122*, 1409–1416.

Ahlgren, U., Jonsson, J., Jonsson, L., Simu, K., and Edlund, H. (1998). beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. Genes & Development *12*, 1763–1768.

Ahlgren, U., Pfaff, S.L., Jessell, T.M., Edlund, T., and Edlund, H. (1997). Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. Nature *385*, 257–260.

Ahlqvist, E., Turrini, F., Lang, S.T., Taneera, J., Zhou, Y., Almgren, P., Hansson, O., Isomaa, B., Tuomi, T., Eriksson, K., et al. (2011). A common variant upstream of the PAX6 gene influences islet function in man. Diabetologia *55*, 94–104.

Allen, H.L., Flanagan, S.E., Shaw-Smith, C., De Franco, E., Akerman, I., Caswell, R., Ferrer, J., Hattersley, A.T., and Ellard, S. (2011). Allen, 2012 GATA6. Nature Publishing Group *44*, 20–22.

Alsat, E., Guibourdenche, J., Luton, D., Frankenne, F., and Evain-Brion, D. (1997). Human placental growth hormone. Am. J. Obstet. Gynecol. *177*, 1526–1534.

Anjos, S.M., Tessier, M.-C., and Polychronakos, C. (2004). Association of the cytotoxic T lymphocyte-associated antigen 4 gene with type 1 diabetes: evidence for independent effects of two polymorphisms on the same haplotype block. Journal of Clinical Endocrinology &Amp; Metabolism *89*, 6257–6265.

Artner, I., Blanchi, B., Raum, J.C., Guo, M., Kaneko, T., Cordes, S., Sieweke, M., and Stein, R. (2007). MafB is required for islet beta cell maturation. Proc. Natl. Acad. Sci. U.S.a. *104*, 3853–3858.

Artner, I., Hang, Y., Guo, M., Gu, G., and Stein, R. (2008). MafA is a dedicated activator of the insulin gene in vivo. J. Endocrinol. *198*, 271–279.

Artner, I., Hang, Y., Mazur, M., Yamamoto, T., Guo, M., Lindner, J., Magnuson, M.A., and Stein, R. (2010). MafA and MafB regulate genes critical to beta-cells in a unique temporal manner. Diabetes *59*, 2530–2539.

Artner, I., Le Lay, J., Hang, Y., Elghazi, L., Schisler, J.C., Henderson, E., Sosa-Pineda, B., and Stein, R. (2006). MafB: an activator of the glucagon gene expressed in developing islet alpha- and beta-cells. Diabetes *55*, 297–304.

Ashcroft, F.M., and Rorsman, P. (2012). Diabetes Mellitus and the β Cell: The Last Ten Years. Cell *148*, 1160–1171.

Aziz, A., Soucie, E., Sarrazin, S., and Sieweke, M.H. (2009). MafB/c-Maf deficiency enables self-renewal of differentiated functional macrophages. Science *326*, 867–871.

Aziz, A., Vanhille, L., Mohideen, P., Kelly, L.M., Otto, C., Bakri, Y., Mossadegh, N., Sarrazin, S., and Sieweke, M.H. (2006). Development of macrophages with altered actin organization in the absence of MafB. Molecular and Cellular Biology *26*, 6808–6818.

Baeyens, L., De Breuck, S., Lardon, J., Mfopou, J.K., Rooman, I., and Bouwens, L. (2005). In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. Diabetologia *48*, 49–57.

Ballian, N., and Brunicardi, F.C. (2007). Islet vasculature as a regulator of endocrine pancreas function. World J Surg *31*, 705–714.

Banga, A., Akinci, E., Greder, L.V., Dutton, J.R., and Slack, J.M.W. (2012). In vivo reprogramming of Sox9+ cells in the liver to insulin-secreting ducts. Proc. Natl. Acad. Sci. U.S.a. *109*, 15336–15341.

Barg, S., Galvanovskis, J., Göpel, S.O., Rorsman, P., and Eliasson, L. (2000). Tight coupling between electrical activity and exocytosis in mouse glucagon-secreting alpha-cells. Diabetes *49*, 1500–1510.

Benkhelifa, S., Provot, S., Nabais, E., Eychene, A., Calothy, G., and Felder-Schmittbuhl, M.P. (2001). Phosphorylation of MafA Is Essential for Its Transcriptional and Biological Properties. Molecular and Cellular Biology *21*, 4441–4452.

Ber, I. (2003). Functional, Persistent, and Extended Liver to Pancreas Transdifferentiation. Journal of Biological Chemistry *278*, 31950–31957.

Berne, R.M., Koeppen, B.M., and Stanton, B.A. (2008). Berne and Levy Physiology, 6th ed, Philidelphia Mosby Elsevier (Philadelphia: Mosby/Elsevier).

Blanchi, B., Kelly, L.M., Viemari, J.-C., Lafon, I., Burnet, H., Bévengut, M., Tillmanns, S., Daniel, L., Graf, T., Hilaire, G., et al. (2003). MafB deficiency causes defective respiratory rhythmogenesis and fatal central apnea at birth. Nat. Neurosci. *6*, 1091–1100.

Blank, V., and Andrews, N.C. (1997). The Maf transcription factors: regulators of differentiation. Trends Biochem. Sci. *22*, 437–441.

Blicklé, J.F. (2006). Meglitinide analogues: a review of clinical data focused on recent

trials. Diabetes Metab. *32*, 113–120.

Bonnefond, A., Sand, O., Guerin, B., Durand, E., Graeve, F., Huyvaert, M., Rachdi, L., Kerr-Conte, J., Pattou, F., Vaxillaire, M., et al. (2012). GATA6 inactivating mutations are associated with heart defects and, inconsistently, with pancreatic agenesis and diabetes. Diabetologia *55*, 2845–2847.

Bonnefond, A., Vaillant, E., Philippe, J., Skrobek, B., Lobbens, S., Yengo, L., Huyvaert, M., Cavé, H., Busiah, K., Scharfmann, R., et al. (2013). Transcription factor gene MNX1 is a novel cause of permanent neonatal diabetes in a consanguineous family. Diabetes Metab. *39*, 276–280.

Bonner-Weir, S., Taneja, M., Weir, G.C., Tatarkiewicz, K., Song, K.H., Sharma, A., and O'Neil, J.J. (2000). In vitro cultivation of human islets from expanded ductal tissue. Proc. Natl. Acad. Sci. U.S.a. *97*, 7999–8004.

Boschero, A.C., Bordin, S., Sener, A., and Malaisse, W.J. (1990). D-glucose and L-leucine metabolism in neonatal and adult cultured rat pancreatic islets. Mol. Cell. Endocrinol. *73*, 63–71.

Bosco, D., Armanet, M., Morel, P., Niclauss, N., Sgroi, A., Muller, Y.D., Giovannoni, L., Parnaud, G., and Berney, T. (2010). Unique arrangement of alpha- and beta-cells in human islets of Langerhans. Diabetes *59*, 1202–1210.

Bottino, R., Balamurugan, A.N., Bertera, S., Pietropaolo, M., Trucco, M., and Piganelli, J.D. (2002). Preservation of human islet cell functional mass by anti-oxidative action of a novel SOD mimic compound. Diabetes *51*, 2561–2567.

Bramswig, N.C., Everett, L.J., Schug, J., Dorrell, C., Liu, C., Luo, Y., Streeter, P.R., Naji, A., Grompe, M., and Kaestner, K.H. (2013). Epigenomic plasticity enables human pancreatic α to β cell reprogramming. J. Clin. Invest. *123*, 1275–1284.

Braun, M., Ramracheya, R., Bengtsson, M., Zhang, Q., Karanauskaite, J., Partridge, C., Johnson, P.R., and Rorsman, P. (2008). Voltage-gated ion channels in human pancreatic beta-cells: electrophysiological characterization and role in insulin secretion. Diabetes *57*, 1618–1628.

Brissova, M. (2004). Reduced PDX-1 expression impairs islet response to insulin resistance and worsens glucose homeostasis. AJP: Endocrinology and Metabolism *288*, E707–E714.

Brissova, M. (2005). Assessment of Human Pancreatic Islet Architecture and Composition by Laser Scanning Confocal Microscopy. Journal of Histochemistry and Cytochemistry *53*, 1087–1097.

Brissova, M., Aamodt, K., Brahmachary, P., Prasad, N., Hong, J.-Y., Dai, C., Mellati, M., Shostak, A., Poffenberger, G., Aramandla, R., et al. (2014). Islet microenvironment, modulated by vascular endothelial growth factor-A signaling, promotes β cell regeneration. Cell Metabolism *19*, 498–511.

Broglio, F., Arvat, E., Benso, A., Gottero, C., Muccioli, G., Papotti, M., van der Lely, A.J.,

Deghenghi, R., and Ghigo, E. (2001). Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. Journal of Clinical Endocrinology &Amp; Metabolism *86*, 5083–5086.

Brouwers, B., de Faudeur, G., Osipovich, A.B., Goyvaerts, L., Lemaire, K., Boesmans, L., Cauwelier, E.J.G., Granvik, M., Pruniau, V.P.E.G., Van Lommel, L., et al. (2014). Impaired Islet Function in Commonly Used Transgenic Mouse Lines due to Human Growth Hormone Minigene Expression. Cell Metabolism *20*, 979–990.

Buchanan, T.A., and Xiang, A.H. (2005). Gestational diabetes mellitus. J. Clin. Invest. *115*, 485–491.

Buck, M.J., and Lieb, J.D. (2004). ChIP-chip: considerations for the design, analysis, and application of genome-wide chromatin immunoprecipitation experiments. Genomics *83*, 349–360.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., et al. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. *55*, 611–622.

Butler, A.E., Cao-Minh, L., Galasso, R., Rizza, R.A., Corradin, A., Cobelli, C., and Butler, P.C. (2010). Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. Diabetologia *53*, 2167–2176.

Butler, A.E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R.A., and Butler, P.C. (2003a). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes *52*, 102–110.

Butler, A.E., Janson, J., Soeller, W.C., and Butler, P.C. (2003b). Increased beta-cell apoptosis prevents adaptive increase in beta-cell mass in mouse model of type 2 diabetes: evidence for role of islet amyloid formation rather than direct action of amyloid. Diabetes *52*, 2304–2314.

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. Proc. Natl. Acad. Sci. U.S.a. *103*, 2334–2339.

Cano, D.A., Soria, B., Martín, F., and Rojas, A. (2013). Transcriptional control of mammalian pancreas organogenesis. Cell. Mol. Life Sci.

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

Castaing, M., Duvillié, B., Quemeneur, E., Basmaciogullari, A., and Scharfmann, R. (2005). Ex vivo analysis of acinar and endocrine cell development in the human embryonic pancreas. Dev. Dyn. *234*, 339–345.

Chera, S., Baronnier, D., Ghila, L., Cigliola, V., Jensen, J.N., Gu, G., Furuyama, K., Thorel, F., Gribble, F.M., Reimann, F., et al. (2014). Chera 2013. Nature *514*, 503–507.

Cheung, N.W., and Byth, K. (2003). Population health significance of gestational diabetes. Diabetes Care *26*, 2005–2009.

Cho, Y.M., and Kieffer, T.J. (2011). New aspects of an old drug: metformin as a glucagon-like peptide 1 (GLP-1) enhancer and sensitiser. Diabetologia *54*, 219–222.

Collombat, P. (2003). Opposing actions of Arx and Pax4 in endocrine pancreas development. Genes & Development *17*, 2591–2603.

Collombat, P., Xu, X., Ravassard, P., Sosa-Pineda, B., Dussaud, S., Billestrup, N., Madsen, O.D., Serup, P., Heimberg, H., and Mansouri, A. (2009). The Ectopic Expression of Pax4 in the Mouse Pancreas Converts Progenitor Cells into α and Subsequently β Cells. Cell *138*, 449–462.

Cordes, S.P., and Barsh, G.S. (1994). The mouse segmentation gene kr encodes a novel basic domain-leucine zipper transcription factor. Cell *79*, 1025–1034.

Corleto, V.D. (2010). Somatostatin and the gastrointestinal tract. Curr Opin Endocrinol Diabetes Obes *17*, 63–68.

Courtney, M., Gjernes, E., Druelle, N.M., Ravaud, C., Vieira, A., Ben-Othman, N., Pfeifer, A., Avolio, F., Leuckx, G., Lacas-Gervais, S., et al. (2013). The Inactivation of Arx in Pancreatic Î±-Cells Triggers Their Neogenesis and Conversion into Functional Î²-Like Cells. PLoS Genet *9*, e1003934.

Cypryk, K., Szymczak, W., Czupryniak, L., Sobczak, M., and Lewiński, A. (2008). Gestational diabetes mellitus - an analysis of risk factors. Endokrynol Pol *59*, 393–397.

D'Alessio, D. (2011). The role of dysregulated glucagon secretion in type 2 diabetes. Diabetes Obes Metab *13 Suppl 1*, 126–132.

D'Amour, K.A., Bang, A.G., Eliazer, S., Kelly, O.G., Agulnick, A.D., Smart, N.G., Moorman, M.A., Kroon, E., Carpenter, M.K., and Baetge, E.E. (2006). Production of pancreatic hormone–expressing endocrine cells from human embryonic stem cells. Nature Biotechnology *24*, 1392–1401.

Dai, C., Brissova, M., Hang, Y., Thompson, C., Poffenberger, G., Shostak, A., Chen, Z., Stein, R., and Powers, A.C. (2011). Islet-enriched gene expression and glucose-induced insulin secretion in human and mouse islets. Diabetologia *55*, 707–718.

Daneman, D. (2006). Type 1 diabetes. Lancet *367*, 847–858.

De Franco, E., Shaw-Smith, C., Flanagan, S.E., Shepherd, M.H., International NDM Consortium, Hattersley, A.T., and Ellard, S. (2013). GATA6 mutations cause a broad phenotypic spectrum of diabetes from pancreatic agenesis to adult-onset diabetes without exocrine insufficiency. Diabetes *62*, 993–997.

De Vos, A., Heimberg, H., Quartier, E., Huypens, P., Bouwens, L., Pipeleers, D., and Schuit, F. (1995). Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. J. Clin. Invest. *96*, 2489–2495.

Del Guerra, S., Lupi, R., Marselli, L., Masini, M., Bugliani, M., Sbrana, S., Torri, S., Pollera, M., Boggi, U., Mosca, F., et al. (2005). Functional and molecular defects of pancreatic islets in human type 2 diabetes. Diabetes *54*, 727–735.

Deol, M.S. (1964). THE ABNORMALITIES OF THE INNER EAR IN KREISLER MICE. J Embryol Exp Morphol *12*, 475–490.

Dhawan, S., Georgia, S., Tschen, S.-I., Fan, G., and Bhushan, A. (2011). Pancreatic β Cell Identity Is Maintained by DNA Methylation-Mediated Repression of Arx. Developmental Cell *20*, 419–429.

Dor, Y., Brown, J., Martinez, O.I., and Melton, D.A. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature *429*, 41–46.

Du, A., Hunter, C.S., Murray, J., Noble, D., Cai, C.-L., Evans, S.M., Stein, R., and May, C.L. (2009). Islet-1 is Required for the Maturation, Proliferation, and Survival of the Endocrine Pancreas. Diabetes *58*, 2059–2069.

Dunning, B.E., and Gerich, J.E. (2007). The role of alpha-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. Endocr. Rev. *28*, 253–283.

Eizirik, D.L., and Darville, M.I. (2001). beta-cell apoptosis and defense mechanisms: lessons from type 1 diabetes. Diabetes *50 Suppl 1*, S64–S69.

Ellenberger, T. (1994) Getting a grip on DNA recognition: structures of the basic region leucine zipper, and the basic region helix-loop-helix DNA-binding domains. Current Opinion in Structural Biology *4*, 12–21.

Elliott, A.D., Ustione, A., and Piston, D.W. (2015). Somatostatin and insulin mediate glucose-inhibited glucagon secretion in the pancreatic alpha-cell by lowering cAMP. Am. J. Physiol. Endocrinol. Metab. *308*, E130–E143.

Ferber, S., Halkin, A., Cohen, H., Ber, I., Einav, Y., Goldberg, I., Barshack, I., Seijffers, R., Kopolovic, J., Kaiser, N., et al. (2000). Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat. Med. *6*, 568–572.

Fiaschi-Taesch, N.M., Salim, F., Kleinberger, J., Troxell, R., Cozar-Castellano, I., Selk, K., Cherok, E., Takane, K.K., Scott, D.K., and Stewart, A.F. (2010). Induction of Human -Cell Proliferation and Engraftment Using a Single G1/S Regulatory Molecule, cdk6. Diabetes *59*, 1926–1936.

Flanagan, S.E., De Franco, E., Allen, H.L., Zerah, M., Abdul-Rasoul, M.M., Edge, J.A., Stewart, H., Alamiri, E., Hussain, K., Wallis, S., et al. (2014). Analysis of Transcription Factors Key for Mouse Pancreatic Development Establishes NKX2-2 and MNX1 Mutations as Causes of Neonatal Diabetes in Man. Cell Metabolism *19*, 146–154.

Frías, J.L., Frías, J.P., Frías, P.A., and Martínez-Frías, M.L. (2007). Infrequently studied congenital anomalies as clues to the diagnosis of maternal diabetes mellitus. Am. J. Med. Genet. A *143A*, 2904–2909.

Fujitani, Y., Fujitani, S., Boyer, D.F., Gannon, M., Kawaguchi, Y., Ray, M., Shiota, M., Stein, R.W., Magnuson, M.A., and Wright, C.V.E. (2006). Targeted deletion of a cis-regulatory region reveals differential gene dosage requirements for Pdx1 in foregut organ differentiation and pancreas formation. Genes & Development *20*, 253–266.

Gannon, M., Tweedie Ables, E., Crawford, L., Lowe, D., Offield, M.F., Magnuson, M.A., and Wright, C.V.E. (2008). pdx-1 function is specifically required in embryonic β cells to generate appropriate numbers of endocrine cell types and maintain glucose homeostasis. Developmental Biology *314*, 406–417.

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

Gao, T., McKenna, B., Li, C., Reichert, M., Nguyen, J., Singh, T., Yang, C., Pannikar, A., Doliba, N., Zhang, T., et al. (2014). Pdx1 maintains β cell identity and function by repressing an α cell program. Cell Metabolism *19*, 259–271.

Garg, V., Kathiriya, I.S., Barnes, R., Schluterman, M.K., King, I.N., Butler, C.A., Rothrock, C.A., Eapen, R.S., Hirayama-Yamada, K., Joo, K., et al. (2003). GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. Nature *424*, 438–443.

Gelling, R.W., Du, X.Q., Dichmann, D.S., Rømer, J., Huang, H., Cui, L., Obici, S., Tang, B., Holst, J.J., and Fledelius, C. (2003). Lower blood glucose, hyperglucagonemia, and pancreatic α cell hyperplasia in glucagon receptor knockout mice. Proc. Natl. Acad. Sci. U.S.a. *100*, 1438–1443.

Georgia, S., and Bhushan, A. (2004). Beta cell replication is the primary mechanism for maintaining postnatal beta cell mass. J. Clin. Invest. *114*, 963–968.

Gittes, G.K. (2009). Developmental biology of the pancreas: a comprehensive review. Developmental Biology *326*, 4–35.

Golson, M.L., Misfeldt, A.A., Kopsombut, U.G., Petersen, C.P., and Gannon, M. (2010). High Fat Diet Regulation of β-Cell Proliferation and β-Cell Mass. Open Endocrinol J *4*.

Goodyer, W.R., Gu, X., Liu, Y., Bottino, R., Crabtree, G.R., and Kim, S.K. (2012). Neonatal β Cell Development in Mice and Humans Is Regulated by Calcineurin/NFAT. Developmental Cell *23*, 21–34.

Gradwohl, G., Dierich, A., LeMeur, M., and Guillemot, F. (2000). neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc. Natl. Acad. Sci. U.S.a. *97*, 1607–1611.

Gromada, J., Franklin, I., and Wollheim, C.B. (2007). Alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. Endocr. Rev. *28*, 84–116.

Grompe, M. (2003). Pancreatic-hepatic switches in vivo. Mechanisms of Development *120*, 99–106.

Gu, C., Stein, G.H., Pan, N., Goebbels, S., HOrnberg, H., Nave, K.-A., Herrera, P., White, P., Kaestner, K.H., Sussel, L., et al. (2010). Pancreatic β Cells Require NeuroD to Achieve and Maintain Functional Maturity. Cell Metabolism *11*, 298–310.

Gu, G., Dubauskaite, J., and Melton, D.A. (2002). Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. Development *129*, 2447–2457.

Gunasekaran, U., Hudgens, C.W., Wright, B.T., Maulis, M.F., and Gannon, M. (2012). Differential regulation of embryonic and adult β cell replication. Cell Cycle *11*, 2431–2442.

Gunawardene, A.R., Corfe, B.M., and Staton, C.A. (2011). Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. Int J Exp Pathol *92*, 219–231.

Guo, L., Inada, A., Aguayo-Mazzucato, C., Hollister-Lock, J., Fujitani, Y., Weir, G.C., Wright, C.V.E., Sharma, A., and Bonner-Weir, S. (2013a). PDX1 in ducts is not required for postnatal formation of β-cells but is necessary for their subsequent maturation. Diabetes *62*, 3459–3468.

Guo, S., Burnette, R., Zhao, L., Vanderford, N.L., Poitout, V., Hagman, D.K., Henderson, E., Ozcan, S., Wadzinski, B.E., and Stein, R. (2009). The stability and transactivation potential of the mammalian MafA transcription factor are regulated by serine 65 phosphorylation. J. Biol. Chem. *284*, 759–765.

Guo, S., Dai, C., Guo, M., Taylor, B., Harmon, J.S., Sander, M., Robertson, R.P., Powers, A.C., and Stein, R. (2013b). Inactivation of specific β cell transcription factors in type 2 diabetes. J. Clin. Invest. *123*, 3305–3316.

Guo, S., Vanderford, N.L., and Stein, R. (2010). Phosphorylation within the MafA N terminus regulates C-terminal dimerization and DNA binding. Journal of Biological Chemistry *285*, 12655–12661.

Gurgul, E., Lortz, S., Tiedge, M., Jörns, A., and Lenzen, S. (2004). Mitochondrial catalase overexpression protects insulin-producing cells against toxicity of reactive oxygen species and proinflammatory cytokines. Diabetes *53*, 2271–2280.

Gylfe, E., and Gilon, P. (2014). Glucose regulation of glucagon secretion. Diabetes Research and Clinical Practice *103*, 1–10.

Hagman, D.K., Hays, L.B., Parazzoli, S.D., and Poitout, V. (2005). Palmitate Inhibits Insulin Gene Expression by Altering PDX-1 Nuclear Localization and Reducing MafA Expression in Isolated Rat Islets of Langerhans. Journal of Biological Chemistry *280*, 32413–32418.

Hang, Y., and Stein, R. (2011). MafA and MafB activity in pancreatic β cells. Trends Endocrinol. Metab. *22*, 364–373.

Hang, Y., Yamamoto, T., Benninger, R.K.P., Brissova, M., Guo, M., Bush, W., Piston, D.W., Powers, A.C., Magnuson, M., Thurmond, D.C., et al. (2014). The MafA transcription factor becomes essential to islet β-cells soon after birth. Diabetes *63*, 1994–2005.

Hara, M., Wang, X., Kawamura, T., Bindokas, V.P., Dizon, R.F., Alcoser, S.Y., Magnuson, M.A., and Bell, G.I. (2003). Transgenic mice with green fluorescent protein-labeled pancreatic beta -cells. Am. J. Physiol. Endocrinol. Metab. *284*, E177–E183.

Harmon, J.S., Stein, R., and Robertson, R.P. (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.

Harmon, J.S., Bogdani, M., Parazzoli, S.D., Mak, S.S.M., Oseid, E.A., Berghmans, M., LeBoeuf, R.C., and Robertson, R.P. (2009). β-Cell-Specific Overexpression of Glutathione Peroxidase Preserves Intranuclear MafA and Reverses Diabetes in db/dbMice. Endocrinology *150*, 4855–4862.

Haumaitre, C., Barbacci, E., Jenny, M., Ott, M.O., Gradwohl, G., and Cereghini, S. (2005). Lack of TCF2/vHNF1 in mice leads to pancreas agenesis. Proc. Natl. Acad. Sci. U.S.a. *102*, 1490–1495.

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

Hayashi, Y., Yamamoto, M., Mizoguchi, H., Watanabe, C., Ito, R., Yamamoto, S., Sun, X.-Y., and Murata, Y. (2009). Mice deficient for glucagon gene-derived peptides display normoglycemia and hyperplasia of islet {alpha}-cells but not of intestinal L-cells. Mol. Endocrinol. *23*, 1990–1999.

Hebrok, M., Kim, S.K., and Melton, D.A. (1998). Notochord repression of endodermal Sonic hedgehog permits pancreas development. Genes & Development *12*, 1705–1713.

Heimberg, H., Bouwens, L., Heremans, Y., Van De Casteele, M., Lefebvre, V., and Pipeleers, D. (2000). Adult human pancreatic duct and islet cells exhibit similarities in expression and differences in phosphorylation and complex formation of the homeodomain protein Ipf-1. Diabetes *49*, 571–579.

Heimberg, H., De Vos, A., Moens, K., Quartier, E., Bouwens, L., Pipeleers, D., Van Schaftingen, E., Madsen, O., and Schuit, F. (1996). The glucose sensor protein glucokinase is expressed in glucagon-producing alpha-cells. Proc. Natl. Acad. Sci. U.S.a. *93*, 7036–7041.

Heimberg, H., De Vos, A., Pipeleers, D., Thorens, B., and Schuit, F. (1995). Differences in glucose transporter gene expression between rat pancreatic alpha- and beta-cells are correlated to differences in glucose transport but not in glucose utilization. J. Biol. Chem. *270*, 8971–8975.

Henquin, J.-C., Dufrane, D., and Nenquin, M. (2006). Nutrient control of insulin secretion in isolated normal human islets. Diabetes *55*, 3470–3477.

Heremans, Y. (2002). Recapitulation of embryonic neuroendocrine differentiation in adult human pancreatic duct cells expressing neurogenin 3. The Journal of Cell Biology *159*, 303–312.

Herrera, P.L. (2000). Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. Development *127*, 2317–2322.

Ho, I.C., Hodge, M.R., Rooney, J.W., and Glimcher, L.H. (1996). The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. Cell *85*, 973–983.

Hoffman, B.G., Zavaglia, B., Witzsche, J., Ruiz de Algara, T., Beach, M., Hoodless, P.A., Jones, S.J.M., Marra, M.A., and Helgason, C.D. (2008). Identification of transcripts with enriched expression in the developing and adult pancreas. Genome Biol. *9*, R99.

Holland, A.M., Hale, M.A., Kagami, H., Hammer, R.E., and Macdonald, R.J. (2002). Experimental control of pancreatic development and maintenance. Proc. Natl. Acad. Sci. U.S.a. *99*, 12236–12241.

Holm, P., Rydlander, B., Luthman, H., Kockum, I., European Consortium for IDDM Genome Studies (2004). Interaction and association analysis of a type 1 diabetes susceptibility locus on chromosome 5q11-q13 and the 7q32 chromosomal region in Scandinavian families. Diabetes *53*, 1584–1591.

Horikawa, Y., Iwasaki, N., Hara, M., Furuta, H., Hinokio, Y., Cockburn, B.N., Lindner, T., Yamagata, K., Ogata, M., Tomonaga, O., et al. (1997). Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. Nat Genet *17*, 384–385.

Houbracken, I., de Waele, E., Lardon, J., Ling, Z., Heimberg, H., Rooman, I., and Bouwens, L. (2011). Houbracken 2011. Ygast *141*, 731–741.e734.

Huang, C., Snider, F., and Cross, J.C. (2009). Prolactin receptor is required for normal glucose homeostasis and modulation of beta-cell mass during pregnancy. Endocrinology *150*, 1618–1626.

Igarashi, K., Kataoka, K., Itoh, K., Hayashi, N., Nishizawa, M., and Yamamoto, M. (1994). Regulation of transcription by dimerization of erythroid factor NF-E2 p45 with small Maf proteins. Nature *367*, 568–572.

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

Jenny, M., Uhl, C., Roche, C., Duluc, I., Guillermin, V., Guillemot, F., Jensen, J., Kedinger, M., and Gradwohl, G. (2002). Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. Embo J. *21*, 6338–6347.

Jensen, J. (2004). Gene regulatory factors in pancreatic development. Dev. Dyn. *229*, 176–200.

Jeon, J., Correa-Medina, M., Ricordi, C., Edlund, H., and Diez, J.A. (2009). Endocrine Cell Clustering During Human Pancreas Development. Journal of Histochemistry and Cytochemistry *57*, 811–824.

Jermendy, A., Toschi, E., Aye, T., Koh, A., Aguayo-Mazzucato, C., Sharma, A., Weir,

G.C., Sgroi, D., and Bonner-Weir, S. (2011). Rat neonatal beta cells lack the specialised metabolic phenotype of mature beta cells. Diabetologia *54*, 594–604.

Johnson, D.S., Mortazavi, A., Myers, R.M., and Wold, B. (2007). Genome-wide mapping of in vivo protein-DNA interactions. Science *316*, 1497–1502.

Jonsson, J., Carlsson, L., Edlund, T., and Edlund, H. (1994). Insulin-promoter-factor 1 is required for pancreas development in mice. Nature *371*, 606–609.

Juhl, C.B., Pørksen, N., Pincus, S.M., Hansen, A.P., Veldhuis, J.D., and Schmitz, O. (2001). Acute and short-term administration of a sulfonylurea (gliclazide) increases pulsatile insulin secretion in type 2 diabetes. Diabetes *50*, 1778–1784.

Kahn, S.E., Cooper, M.E., and Del Prato, S. (2014). Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet *383*, 1068–1083.

Kannan, M.B., Solovieva, V., and Blank, V. (2012). The small MAF transcription factors MAFF, MAFG and MAFK: current knowledge and perspectives. Biochim. Biophys. Acta *1823*, 1841–1846.

Karnik, S.K., Chen, H., McLean, G.W., Heit, J.J., Gu, X., Zhang, A.Y., Fontaine, M., Yen, M.H., and Kim, S.K. (2007). Menin controls growth of pancreatic beta-cells in pregnant mice and promotes gestational diabetes mellitus. Science *318*, 806–809.

Kataoka, K., Nishizawa, M., and Kawai, S. (1993). Structure-function analysis of the maf oncogene product, a member of the b-Zip protein family. J. Virol. *67*, 2133–2141.

Kataoka, K., Noda, M., and Nishizawa, M. (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.

Kataoka, K., Shioda, S., Ando, K., Sakagami, K., Handa, H., and Yasuda, K. (2004). Differentially expressed Maf family transcription factors, c-Maf and MafA, activate glucagon and insulin gene expression in pancreatic islet alpha- and beta-cells. Journal of Molecular Endocrinology *32*, 9–20.

Kataoka, K. (2007). Multiple mechanisms and functions of maf transcription factors in the regulation of tissue-specific genes. J. Biochem. *141*, 775–781.

Kataoka, K., Han, S.-I., Shioda, S., Hirai, M., Nishizawa, M., and Handa, H. (2002). MafA is a glucose-regulated and pancreatic beta-cell-specific transcriptional activator for the insulin gene. J. Biol. Chem. *277*, 49903–49910.

Kim, C., Newton, K.M., and Knopp, R.H. (2002). Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care *25*, 1862–1868.

Kim, H., Toyofuku, Y., Lynn, F.C., Chak, E., Uchida, T., Mizukami, H., Fujitani, Y., Kawamori, R., Miyatsuka, T., Kosaka, Y., et al. (2010). Serotonin regulates pancreatic beta cell mass during pregnancy. Nat. Med. *16*, 804–808.

Kim, Y.-S., Kang, H., Tahkeda, Y., Hom, L., Song, H.-Y., Jensen, J., and Jetton, A.

(2012). Glis3 regulates neurogenin 3 expression in pancreatic beta-cells and interacts with its activator, Hnf6. Molecules and Cells *34*, 193–200.

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

Kojima, H., Fujimiya, M., Matsumura, K., Younan, P., Imaeda, H., Maeda, M., and Chan, L. (2003). NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. Nat. Med. *9*, 596–603.

Korc, M., Iwamoto, Y., Sankaran, H., Williams, J.A., and Goldfine, I.D. (1981). Insulin action in pancreatic acini from streptozotocin-treated rats. I. Stimulation of protein synthesis. Am. J. Physiol. *240*, G56–G62.

Korc, M., Sankaran, H., Wong, K.Y., Williams, J.A., and Goldfine, I.D. (1978). Insulin receptors in isolated mouse pancreatic acini. Biochemical and Biophysical Research Communications *84*, 293–299.

Krapp, A., Knofler, M., Ledermann, B., Burki, K., Berney, C., Zoerkler, N., Hagenbuchle, O., and Wellauer, P.K. (1998). The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. Genes & Development *12*, 3752–3763.

Kroon, E., Martinson, L.A., Kadoya, K., Bang, A.G., Kelly, O.G., Eliazer, S., Young, H., Richardson, M., Smart, N.G., Cunningham, J., et al. (2008). Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nature Biotechnology *26*, 443–452.

Kuise, T., Noguchi, H., Tazawa, H., Kawai, T., Iwamuro, M., Saitoh, I., Kataoka, H.U., Watanabe, M., Noguchi, Y., and Fujiwara, T. (2014). Establishment of a pancreatic stem cell line from fibroblast-derived induced pluripotent stem cells. Biomed Eng Online *13*, 64.

Kulkarni, R.N., Jhala, U.S., Winnay, J.N., Krajewski, S., Montminy, M., and Kahn, C.R. (2004). PDX-1 haploinsufficiency limits the compensatory islet hyperplasia that occurs in response to insulin resistance. J. Clin. Invest. *114*, 828–836.

Kumar, R., Chen, S., Scheurer, D., Wang, Q.L., Duh, E., Sung, C.H., Rehemtulla, A., Swaroop, A., Adler, R., and Zack, D.J. (1996). The bZIP transcription factor Nrl stimulates rhodopsin promoter activity in primary retinal cell cultures. J. Biol. Chem. *271*, 29612–29618.

Kushner, J.A., Ciemerych, M.A., Sicinska, E., Wartschow, L.M., Teta, M., Long, S.Y., Sicinski, P., and White, M.F. (2005). Cyclins D2 and D1 are essential for postnatal pancreatic beta-cell growth. Molecular and Cellular Biology *25*, 3752–3762.

Lain, K.Y., and Catalano, P.M. (2007). Metabolic Changes in Pregnancy. Clinical Obstetrics and Gynecology *50*, 938–948.

Le Marchand, S.J., and Piston, D.W. (2010). Glucose suppression of glucagon secretion: metabolic and calcium responses from alpha-cells in intact mouse pancreatic

islets. Journal of Biological Chemistry *285*, 14389–14398.

Lee, J.Y. (2005). RIP-Cre Revisited, Evidence for Impairments of Pancreatic beta-Cell Function. Journal of Biological Chemistry *281*, 2649–2653.

Lee, J., Sugiyama, T., Liu, Y., Wang, J., Gu, X., Lei, J., Markmann, J.F., Miyazaki, S., Miyazaki, J.-I., Szot, G.L., et al. (2013). Expansion and conversion of human pancreatic ductal cells into insulin-secreting endocrine cells. eLife *2*, e00940–e00940.

Lee, Y.C., and Nielsen, J.H. (2009). Regulation of beta cell replication. Mol. Cell. Endocrinol. *297*, 18–27.

Li, H., Arber, S., Jessell, T.M., and Edlund, H. (1999). Selective agenesis of the dorsal pancreas in mice lacking homeobox gene Hlxb9. Nat Genet *23*, 67–70.

Lins, P.E., Efendić, S., Meyers, C.A., Coy, D.H., Schally, A., and Luft, R. (1980). Selective effect of some somatostatin analogs on glucagon as opposed to insulin release in rats in vivo. Metab. Clin. Exp. *29*, 728–731.

Lowe, C.E., Cooper, J.D., Brusko, T., Walker, N.M., Smyth, D.J., Bailey, R., Bourget, K., Plagnol, V., Field, S., Atkinson, M., et al. (2007). Large-scale genetic fine mapping and genotype-phenotype associations implicate polymorphism in the IL2RA region in type 1 diabetes. Nat Genet *39*, 1074–1082.

Lu, J., Hamze, Z., Bonnavion, R., Herath, N., Pouponnot, C., Assade, F., re, S.F.E., Bertolino, P., Cordier-Bussat, M., and Zhang, C.X. (2011). Reexpression of oncoprotein MafB in proliferative β-cells and Men1 insulinomas in mouse. 1–8.

Lupi, R., Del Guerra, S., Mancarella, R., Novelli, M., Valgimigli, L., Pedulli, G.F., Paolini, M., Soleti, A., Filipponi, F., Mosca, F., et al. (2007). Insulin secretion defects of human type 2 diabetic islets are corrected in vitro by a new reactive oxygen species scavenger. Diabetes Metab. *33*, 340–345.

Lyttle, B.M., Li, J., Krishnamurthy, M., Fellows, F., Wheeler, M.B., Goodyer, C.G., and Wang, R. (2008). Transcription factor expression in the developing human fetal endocrine pancreas. Diabetologia *51*, 1169–1180.

Magenheim, J., Klein, A.M., Stanger, B.Z., Ashery-Padan, R., Sosa-Pineda, B., Gu, G., and Dor, Y. (2011). Ngn3(+) endocrine progenitor cells control the fate and morphogenesis of pancreatic ductal epithelium. Developmental Biology *359*, 26–36.

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

Martínez-Hernández, A., Gutierrez-Malacatt, H., Carrillo-Sánchez, K., Saldaña-Alvarez, Y., Rojas-Ochoa, A., Crespo-Solis, E., Aguayo-González, A., Rosas-López, A., Ayala-Sanchez, J.M., Aquino-Ortega, X., et al. (2014). Small MAF genes variants and chronic myeloid leukemia. Eur. J. Haematol. *92*, 35–41.

Matsuoka, T.-A., Artner, I., Henderson, E., Means, A., Sander, M., and Stein, R. (2004).

The MafA transcription factor appears to be responsible for tissue-specific expression of insulin. Proc. Natl. Acad. Sci. U.S.a. *101*, 2930–2933.

Matsuoka, T.-A., Zhao, L., Artner, I., Jarrett, H.W., Friedman, D., Means, A., and Stein, R. (2003). Members of the large Maf transcription family regulate insulin gene transcription in islet beta cells. Molecular and Cellular Biology *23*, 6049–6062.

Mauda-Havakuk, M., Litichever, N., Chernichovski, E., Nakar, O., Winkler, E., Mazkereth, R., Orenstein, A., Bar-Meir, E., Ravassard, P., Meivar-Levy, I., et al. (2011). Ectopic PDX-1 Expression Directly Reprograms Human Keratinocytes along Pancreatic Insulin-Producing Cells Fate. PLoS ONE *6*, e26298.

McCluskey, J.T., Hamid, M., Guo-Parke, H., McClenaghan, N.H., Gomis, R., and Flatt, P.R. (2011). Development and Functional Characterization of Insulin-releasing Human Pancreatic Beta Cell Lines Produced by Electrofusion. Journal of Biological Chemistry *286*, 21982–21992.

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

Meier, J.J., Butler, A.E., Saisho, Y., Monchamp, T., Galasso, R., Bhushan, A., Rizza, R.A., and Butler, P.C. (2008). Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. Diabetes *57*, 1584–1594.

Melloul, D., Marshak, S., and Cerasi, E. (2002). Regulation of insulin gene transcription. Diabetologia *45*, 309–326.

Minami, K., Okuno, M., Miyawaki, K., Okumachi, A., Ishizaki, K., Oyama, K., Kawaguchi, M., Ishizuka, N., Iwanaga, T., and Seino, S. (2005). Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. Proceedings of the ….

Miyai, M., Tanaka, Y.G., Kamitani, A., Hamada, M., Takahashi, S., and Kataoka, K. (2010). c-Maf and MafB transcription factors are differentially expressed in Huxley“s and Henle”s layers of the inner root sheath of the hair follicle and regulate cuticle formation. J. Dermatol. Sci. *57*, 178–182.

Molotkov, A., Molotkova, N., and Duester, G. (2005). Retinoic acid generated by Raldh2 in mesoderm is required for mouse dorsal endodermal pancreas development. Dev. Dyn. *232*, 950–957.

Moriguchi, T., Hamada, M., Morito, N., Terunuma, T., Hasegawa, K., Zhang, C., Yokomizo, T., Esaki, R., Kuroda, E., Yoh, K., et al. (2006). MafB is essential for renal development and F4/80 expression in macrophages. Molecular and Cellular Biology *26*, 5715–5727.

Morito, N., Yoh, K., Ojima, M., Okamura, M., Nakamura, M., Hamada, M., Shimohata, H., Moriguchi, T., Yamagata, K., and Takahashi, S. (2014). Overexpression of Mafb in Podocytes Protects against Diabetic Nephropathy. Journal of the American Society of Nephrology.

Muoio, D.M., and Newgard, C.B. (2008). Mechanisms of disease: molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. Nat. Rev. Mol. Cell Biol. *9*, 193–205.

Murtaugh, L.C., and Melton, D.A. (2003). Genes, signals, and lineages in pancreas development. Annu. Rev. Cell Dev. Biol. *19*, 71–89.

Müller, W.A., Faloona, G.R., and Unger, R.H. (1973). Hyperglucagonemia in diabetic ketoacidosis. Its prevalence and significance. Am. J. Med. *54*, 52–57.

Nathan, D.M., Buse, J.B., Davidson, M.B., Ferrannini, E., Holman, R.R., Sherwin, R., and Zinman, B. (2008). Medical Management of Hyperglycemia in Type 2 Diabetes: A Consensus Algorithm for the Initiation and Adjustment of Therapy: A consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care *32*, 193–203.

Naya, F.J., Stellrecht, C.M., and Tsai, M.J. (1995). Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. Genes & Development *9*, 1009–1019.

Nishimura, W., Kondo, T., Salameh, T., Khattabi, El, I., Dodge, R., Bonner-Weir, S., and Sharma, A. (2006). A switch from MafB to MafA expression accompanies differentiation to pancreatic beta-cells. Developmental Biology *293*, 526–539.

Obata, J., Yano, M., Mimura, H., Goto, T., Nakayama, R., Mibu, Y., Oka, C., and Kawaichi, M. (2001). p48 subunit of mouse PTF1 binds to RBP-Jkappa/CBF-1, the intracellular mediator of Notch signalling, and is expressed in the neural tube of early stage embryos. Genes Cells *6*, 345–360.

Ochi, H. (2002). The Stability of the Lens-specific Maf Protein is Regulated by Fibroblast Growth Factor (FGF)/ERK Signaling in Lens Fiber Differentiation. Journal of Biological Chemistry *278*, 537–544.

Offield, M.F., Jetton, T.L., Labosky, P.A., Ray, M., Stein, R.W., Magnuson, M.A., Hogan, B.L., and Wright, C.V. (1996). PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development *122*, 983–995.

Ogino, H., and Yasuda, K. (1998). Induction of lens differentiation by activation of a bZIP transcription factor, L-Maf. Science *280*, 115–118.

Ohlsson, H., Karlsson, K., and Edlund, T. (1993). IPF1, a homeodomain-containing transactivator of the insulin gene. Embo J. *12*, 4251–4259.

Ohneda, K., Mirmira, R.G., Wang, J., Johnson, J.D., and German, M.S. (2000). The homeodomain of PDX-1 mediates multiple protein-protein interactions in the formation of a transcriptional activation complex on the insulin promoter. Molecular and Cellular Biology *20*, 900–911.

Olbrot, M., Rud, J., Moss, L.G., and Sharma, A. (2002). Identification of beta-cell-specific insulin gene transcription factor RIPE3b1 as mammalian MafA. Proc. Natl. Acad. Sci. U.S.a. *99*, 6737–6742.

Oliver-Krasinski, J.M., and Stoffers, D.A. (2008). On the origin of the beta cell. Genes & Development *22*, 1998–2021.

Pagliuca, F.W., and Melton, D.A. (2013). How to make a functional β-cell. Development *140*, 2472–2483.

Pagliuca, F.W., Millman, J.R., Gürtler, M., Segel, M., Van Dervort, A., Ryu, J.H., Peterson, Q.P., Greiner, D., and Melton, D.A. (2014). Generation of Functional Human Pancreatic β Cells In Vitro. Cell *159*, 428–439.

Pan, F.C., and Wright, C. (2011). Pancreas organogenesis: From bud to plexus to gland. Dev. Dyn. *240*, 530–565.

Pandol, S.J. (2010). The Exocrine Pancreas (San Rafael (CA): Morgan & Claypool Life Sciences).

Parsons, J.A., Brelje, T.C., and Sorenson, R.L. (1992). Adaptation of islets of Langerhans to pregnancy: increased islet cell proliferation and insulin secretion correlates with the onset of placental lactogen secretion. Endocrinology *130*, 1459–1466.

Pasquali, L., Gaulton, K.J., Rodríguez-Seguí, S.A., Mularoni, L., Miguel-Escalada, I., Akerman, I., Tena, J.J., Morán, I., Gómez-Marín, C., van de Bunt, M., et al. (2014). Pancreatic islet enhancer clusters enriched in type 2 diabetes risk-associated variants. Nature Publishing Group.

Patel, Y.C. (1999). Somatostatin and its receptor family. Front Neuroendocrinol *20*, 157–198.

Pechhold, S., Stouffer, M., Walker, G., Martel, R., Seligmann, B., Hang, Y., Stein, R., Harlan, D.M., and Pechhold, K. (2009). Transcriptional analysis of intracytoplasmically stained, FACS-purified cells by high-throughput, quantitative nuclease protection. Nature Biotechnology *27*, 1038–1042.

Pennarossa, G., Maffei, S., Campagnol, M., Tarantini, L., Gandolfi, F., and Brevini, T.A.L. (2013). Brief demethylation step allows the conversion of adult human skin fibroblasts into insulin-secreting cells. Proc. Natl. Acad. Sci. U.S.a. *110*, 8948–8953.

Perkins, J.M., Dunn, J.P., and Jagasia, S.M. (2007). Perspectives in gestational diabetes mellitus: a review of screening, diagnosis, and treatment. Clinical Diabetes *25*, 57–62.

Perl, A.-K.T., Wert, S.E., Nagy, A., Lobe, C.G., and Whitsett, J.A. (2002). Early restriction of peripheral and proximal cell lineages during formation of the lung. Proc. Natl. Acad. Sci. U.S.a. *99*, 10482–10487.

Pernicova, I., and Korbonits, M. (2014). Metformin--mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol *10*, 143–156.

Pictet, R.L., Clark, W.R., Williams, R.H., and Rutter, W.J. (1972). An ultrastructural analysis of the developing embryonic pancreas. Developmental Biology *29*, 436–467.

Piper, K., Ball, S.G., Keeling, J.W., Mansoor, S., Wilson, D.I., and Hanley, N.A. (2002).

Novel SOX9 expression during human pancreas development correlates to abnormalities in Campomelic dysplasia. Mechanisms of Development *116*, 223–226.

Piper, K., Brickwood, S., Turnpenny, L.W., Cameron, I.T., Ball, S.G., Wilson, D.I., and Hanley, N.A. (2004). Beta cell differentiation during early human pancreas development. J. Endocrinol. *181*, 11–23.

Plengvidhya, N., Kooptiwut, S., Songtawee, N., Doi, A., Furuta, H., Nishi, M., Nanjo, K., Tantibhedhyangkul, W., Boonyasrisawat, W., Yenchitsomanus, P.T., et al. (2007). PAX4 Mutations in Thais with Maturity Onset Diabetes of the Young. Journal of Clinical Endocrinology &Amp; Metabolism *92*, 2821–2826.

Poitout, V., Olson, L.K., and Robertson, R.P. (1996). Chronic exposure of betaTC-6 cells to supraphysiologic concentrations of glucose decreases binding of the RIPE3b1 insulin gene transcription activator. J. Clin. Invest. *97*, 1041–1046.

Poitout, V., Hagman, D., Stein, R., Artner, I., Robertson, R.P., and Harmon, J.S. (2006). Regulation of the insulin gene by glucose and fatty acids. J. Nutr. *136*, 873–876.

Polak, M., Bouchareb-Banaei, L., Scharfmann, R., and Czernichow, P. (2000). Early pattern of differentiation in the human pancreas. Diabetes *49*, 225–232.

Postic, C., Shiota, M., Niswender, K.D., Jetton, T.L., Chen, Y., Moates, J.M., Shelton, K.D., Lindner, J., Cherrington, A.D., and Magnuson, M.A. (1999). Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. J. Biol. Chem. *274*, 305–315.

Pound, L.D., Oeser, J.K., O'Brien, T.P., Wang, Y., Faulman, C.J., Dadi, P.K., Jacobson, D.A., Hutton, J.C., McGuinness, O.P., Shiota, M., et al. (2013). G6PC2: a negative regulator of basal glucose-stimulated insulin secretion. Diabetes *62*, 1547–1556.

Pouponnot, C., Sii-Felice, K., Hmitou, I., Rocques, N., Lecoin, L., Druillennec, S., Felder-Schmittbuhl, M.P., and Eychene, A. (2006). Cell context reveals a dual role for Maf in oncogenesis. Oncogene *25*, 1299–1310.

Prado, C.L., Pugh-Bernard, A.E., Elghazi, L., Sosa-Pineda, B., and Sussel, L. (2004). Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. Proc. Natl. Acad. Sci. U.S.a. *101*, 2924–2929.

Rajala, A., Dighe, R., Agbaga, M.P., Anderson, R.E., and Rajala, R.V.S. (2013). Insulin Receptor Signaling in Cones. Journal of Biological Chemistry *288*, 19503–19515.

Rane, S.G., Dubus, P., Mettus, R.V., Galbreath, E.J., Boden, G., Reddy, E.P., and Barbacid, M. (1999). Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in beta-islet cell hyperplasia. Nat Genet *22*, 44–52.

Raum, J.C., Gerrish, K., Artner, I., Henderson, E., Guo, M., Sussel, L., Schisler, J.C., Newgard, C.B., and Stein, R. (2006). FoxA2, Nkx2.2, and PDX-1 Regulate Islet -Cell-Specific mafA Expression through Conserved Sequences Located between Base Pairs -8118 and -7750 Upstream from the Transcription Start Site. Molecular and Cellular Biology *26*, 5735–5743.

Raum, J.C., Hunter, C.S., Artner, I., Henderson, E., Guo, M., Elghazi, L., Sosa-Pineda, B., Ogihara, T., Mirmira, R.G., Sussel, L., et al. (2010). Islet -Cell-Specific MafA Transcription Requires the 5'-Flanking Conserved Region 3 Control Domain. Molecular and Cellular Biology *30*, 4234–4244.

Ravassard, P., Hazhouz, Y., Pechberty, S., Bricout-Neveu, E., Armanet, M., Czernichow, P., and Scharfmann, R. (2011). A genetically engineered human pancreatic β cell line exhibiting glucose-inducible insulin secretion. J. Clin. Invest. *121*, 3589–3597.

Reers, C., Erbel, S., Esposito, I., Schmied, B., Büchler, M.W., Nawroth, P.P., and Ritzel, R.A. (2008). Impaired islet turnover in human donor pancreata with aging. Eur. J. Endocrinol. *160*, 185–191.

Rehemtulla, A., Warwar, R., Kumar, R., Ji, X., Zack, D.J., and Swaroop, A. (1996). The basic motif-leucine zipper transcription factor Nrl can positively regulate rhodopsin gene expression. Proc. Natl. Acad. Sci. U.S.a. *93*, 191–195.

Rezania, A., Bruin, J.E., Arora, P., Rubin, A., Batushansky, I., Asadi, A., O'Dwyer, S., Quiskamp, N., Mojibian, M., Albrecht, T., et al. (2014). Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nature Biotechnology.

Rezania, A., Bruin, J.E., Riedel, M.J., Mojibian, M., Asadi, A., Xu, J., Gauvin, R., Narayan, K., Karanu, F., O'Neil, J.J., et al. (2012). Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. Diabetes *61*, 2016–2029.

Rieck, S., and Kaestner, K.H. (2010). Expansion of beta-cell mass in response to pregnancy. Trends Endocrinol. Metab. *21*, 151–158.

Robertson, R.P. (2004). Chronic Oxidative Stress as a Central Mechanism for Glucose Toxicity in Pancreatic Islet Beta Cells in Diabetes. Journal of Biological Chemistry *279*, 42351–42354.

Robitaille, J., and Grant, A.M. (2008). The genetics of gestational diabetes mellitus: evidence for relationship with type 2 diabetes mellitus. Genet. Med. *10*, 240–250.

Rorsman, P., and Braun, M. (2013). Regulation of Insulin Secretion in Human Pancreatic Islets. Annu. Rev. Physiol. *75*, 155–179.

Rozzo, A., Meneghel-Rozzo, T., Delakorda, S.L., Yang, S.-B., and Rupnik, M. (2009). Exocytosis of insulin: in vivo maturation of mouse endocrine pancreas. Ann. N. Y. Acad. Sci. *1152*, 53–62.

Rubio-Cabezas, O., Jensen, J.N., Hodgson, M.I., Codner, E., Ellard, S., Serup, P., and Hattersley, A.T. (2011). Permanent Neonatal Diabetes and Enteric Anendocrinosis Associated With Biallelic Mutations in NEUROG3. Diabetes *60*, 1349–1353.

Rubio-Cabezas, O., Minton, J.A.L., Kantor, I., Williams, D., Ellard, S., and Hattersley, A.T. (2010). Homozygous Mutations in NEUROD1 Are Responsible for a Novel Syndrome of Permanent Neonatal Diabetes and Neurological Abnormalities. Diabetes *59*, 2326–2331.

Sadl, V., Jin, F., Yu, J., Cui, S., Holmyard, D., Quaggin, S., Barsh, G., and Cordes, S. (2002). The mouse Kreisler (Krml1/MafB) segmentation gene is required for differentiation of glomerular visceral epithelial cells. Developmental Biology *249*, 16–29.

Sakai, M., Imaki, J., Yoshida, K., Ogata, A., Matsushima-Hibaya, Y., Kuboki, Y., Nishizawa, M., and Nishi, S. (1997). Rat maf related genes: specific expression in chondrocytes, lens and spinal cord. Oncogene *14*, 745–750.

Samols, E., Marri, G., and Marks, V. (1966). Interrelationship of glucagon, insulin and glucose. The insulinogenic effect of glucagon. Diabetes *15*, 855–866.

Sander, M., Neubuser, A., Kalamaras, J., Ee, H.C., Martin, G.R., and German, M.S. (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., Sussel, L., Conners, J., Scheel, D., Kalamaras, J., Cruz, Dela, F., Schwitzgebel, V., Hayes-Jordan, A., and German, M. (2000). Homeobox gene Nkx6.1 lies downstream of Nkx2.2 in the major pathway of beta-cell formation in the pancreas. Development *127*, 5533–5540.

Sankaran, H., Iwamoto, Y., Korc, M., Williams, J.A., and Goldfine, I.D. (1981). Insulin action in pancreatic acini from streptozotocin-treated rats. II. Binding of 125I-insulin to receptors. Am. J. Physiol. *240*, G63–G68.

Sarrazin, S., Mossadegh-Keller, N., Fukao, T., Aziz, A., Mourcin, F., Vanhille, L., Kelly Modis, L., Kastner, P., Chan, S., Duprez, E., et al. (2009). MafB restricts M-CSF-dependent myeloid commitment divisions of hematopoietic stem cells. Cell *138*, 300–313.

Savitz, D.A., Janevic, T.M., Engel, S.M., Kaufman, J.S., and Herring, A.H. (2008). Ethnicity and gestational diabetes in New York City, 1995-2003. Bjog *115*, 969–978.

Scaglia, L., Smith, F.E., and Bonner-Weir, S. (1995). Apoptosis contributes to the involution of beta cell mass in the post partum rat pancreas. Endocrinology *136*, 5461–5468.

Schaffer, A.E., Taylor, B.L., Benthuysen, J.R., Liu, J., Thorel, F., Yuan, W., Jiao, Y., Kaestner, K.H., Herrera, P.L., Magnuson, M.A., et al. (2013). Nkx6.1 Controls a Gene Regulatory Network Required for Establishing and Maintaining Pancreatic Beta Cell Identity. PLoS Genet *9*, e1003274.

Scharfmann, R., Pechberty, S., Hazhouz, Y., Bülow, von, M., Bricout-Neveu, E., Grenier-Godard, M., Guez, F., Rachdi, L., Lohmann, M., Czernichow, P., et al. (2014). Development of a conditionally immortalized human pancreatic β cell line. J. Clin. Invest.

Schonhoff, S.E., Giel-Moloney, M., and Leiter, A.B. (2004). Neurogenin 3-expressing progenitor cells in the gastrointestinal tract differentiate into both endocrine and non-endocrine cell types. Developmental Biology *270*, 443–454.

Schraenen, A., Lemaire, K., de Faudeur, G., Hendrickx, N., Granvik, M., Van Lommel, L.,

Mallet, J., Vodjdani, G., Gilon, P., Binart, N., et al. (2010). Placental lactogens induce serotonin biosynthesis in a subset of mouse beta cells during pregnancy. Diabetologia *53*, 2589–2599.

Schuit, F., De Vos, A., Farfari, S., Moens, K., Pipeleers, D., Brun, T., and Prentki, M. (1997). Metabolic fate of glucose in purified islet cells. Glucose-regulated anaplerosis in beta cells. J. Biol. Chem. *272*, 18572–18579.

Schwitzgebel, V.M., Scheel, D.W., Conners, J.R., Kalamaras, J., Lee, J.E., Anderson, D.J., Sussel, L., Johnson, J.D., and German, M.S. (2000). Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. Development *127*, 3533–3542.

Schwitzgebel, V.M. (2014). Many faces of monogenic diabetes. J Diabetes Investig *5*, 121–133.

Scott Heller, R., Stoffers, D.A., Liu, A., Schedl, A., Crenshaw, E.B., III, Madsen, O.D., and Serup, P. (2004). The role of Brn4/Pou3f4 and Pax6 in forming the pancreatic glucagon cell identity. Developmental Biology *268*, 123–134.

Sellick, G.S., Barker, K.T., Stolte-Dijkstra, I., Fleischmann, C., J Coleman, R., Garrett, C., Gloyn, A.L., Edghill, E.L., Hattersley, A.T., Wellauer, P.K., et al. (2004). Mutations in PTF1A cause pancreatic and cerebellar agenesis. Nat Genet *36*, 1301–1305.

Senée, V., Chelala, C., Duchatelet, S., Feng, D., Blanc, H., Cossec, J.-C., Charon, C., Nicolino, M., Boileau, P., Cavener, D.R., et al. (2006). Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism. Nat Genet *38*, 682–687.

Serup, P., Jensen, J., Andersen, F.G., Jørgensen, M.C., Blume, N., Holst, J.J., and Madsen, O.D. (1996). Induction of insulin and islet amyloid polypeptide production in pancreatic islet glucagonoma cells by insulin promoter factor 1. Proc. Natl. Acad. Sci. U.S.a. *93*, 9015–9020.

Seymour, P.A., Freude, K.K., Dubois, C.L., Shih, H.-P., Patel, N.A., and Sander, M. (2008). A dosage-dependent requirement for Sox9 in pancreatic endocrine cell formation. Developmental Biology *323*, 19–30.

Sharma, A., and Stein, R. (1994). Glucose-induced transcription of the insulin gene is mediated by factors required for beta-cell-type-specific expression. Molecular and Cellular Biology *14*, 871–879.

Shaw-Smith, C., De Franco, E., Allen, H.L., Batlle, M., Flanagan, S.E., Borowiec, M., Taplin, C.E., van Alfen-van der Velden, J., Cruz-Rojo, J., Perez de Nanclares, G., et al. (2014). GATA4 mutations are a cause of neonatal and childhood-onset diabetes. Diabetes.

Shen, C.N., Slack, J.M., and Tosh, D. (2000). Molecular basis of transdifferentiation of pancreas to liver. Nat. Cell Biol. *2*, 879–887.

Shih, H.-P., Wang, A., and Sander, M. (2013). Pancreas organogenesis: from lineage determination to morphogenesis. Annu. Rev. Cell Dev. Biol. *29*, 81–105.

Shimomura, H., Sanke, T., Hanabusa, T., Tsunoda, K., Furuta, H., and Nanjo, K. (2000). Nonsense mutation of islet-1 gene (Q310X) found in a type 2 diabetic patient with a strong family history. Diabetes *49*, 1597–1600.

Shin, S., Le Lay, J., Everett, L.J., Gupta, R., Rafiq, K., and Kaestner, K.H. (2014). CREB mediates the insulinotropic and anti-apoptotic effects of GLP-1 signaling in adult mouse β-cells. Mol Metab *3*, 803–812.

Shirakawa, J., and Terauchi, Y. (2014). Selective and sequential loss of transcriptional factors: A hallmark of β-cell failure in type 2 diabetes? J Diabetes Investig *5*, 359–361.

Slack, J.M. (1995). Developmental biology of the pancreas. Development *121*, 1569–1580.

Smith, S.B., Qu, H.-Q., Taleb, N., Kishimoto, N.Y., Scheel, D.W., Lu, Y., Patch, A.-M., Grabs, R., Wang, J., Lynn, F.C., et al. (2010). Smith 2010. Nature *463*, 775–780.

Sorenson, R.L., and Brelje, T.C. (1997). Adaptation of islets of Langerhans to pregnancy: beta-cell growth, enhanced insulin secretion and the role of lactogenic hormones. Horm. Metab. Res. *29*, 301–307.

Sosa-Pineda, B., Chowdhury, K., Torres, M., Oliver, G., and Gruss, P. (1997). The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. Nature *386*, 399–402.

Spence, J.R., Lange, A.W., Lin, S.-C.J., Kaestner, K.H., Lowy, A.M., Kim, I., Whitsett, J.A., and Wells, J.M. (2009). Sox17 Regulates Organ Lineage Segregation of Ventral Foregut Progenitor Cells. Developmental Cell *17*, 62–74.

Spijker, H.S., Ravelli, R.B.G., Mommaas-Kienhuis, A.M., van Apeldoorn, A.A., Engelse, M.A., Zaldumbide, A., Bonner-Weir, S., Rabelink, T.J., Hoeben, R.C., Clevers, H., et al. (2013). Conversion of mature human β-cells into glucagon-producing α-cells. Diabetes *62*, 2471–2480.

St-Onge, L., Sosa-Pineda, B., Chowdhury, K., Mansouri, A., and Gruss, P. (1997). Pax6 is required for differentiation of glucagon-producing alpha-cells in mouse pancreas. Nature *387*, 406–409.

Stafford, D., and Prince, V.E. (2002). Retinoic acid signaling is required for a critical early step in zebrafish pancreatic development. Curr. Biol. *12*, 1215–1220.

Stanger, B.Z., Tanaka, A.J., and Melton, D.A. (2007). Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. Nature *445*, 886–891.

Stoffers, D.A., Ferrer, J., Clarke, W.L., and Habener, J.F. (1997a). Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. Nat Genet *17*, 138–139.

Stoffers, D.A., Zinkin, N.T., Stanojevic, V., Clarke, W.L., and Habener, J.F. (1997b). Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet *15*, 106–110.

Strowski, M.Z., Parmar, R.M., Blake, A.D., and Schaeffer, J.M. (2000). Somatostatin inhibits insulin and glucagon secretion via two receptors subtypes: an in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice. Endocrinology *141*, 111–117.

Suda, N., Itoh, T., Nakato, R., Shirakawa, D., Bando, M., Katou, Y., Kataoka, K., Shirahige, K., Tickle, C., and Tanaka, M. (2014). Dimeric combinations of MafB, cFos and cJun control the apoptosis-survival balance in limb morphogenesis. Development *141*, 2885–2894.

Sun, Y., Asnicar, M., and Smith, R.G. (2007). Central and peripheral roles of ghrelin on glucose homeostasis. Neuroendocrinology *86*, 215–228.

Sussel, L., Kalamaras, J., Hartigan-O'Connor, D.J., Meneses, J.J., Pedersen, R.A., Rubenstein, J.L., and German, M.S. (1998). Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic beta cells. Development *125*, 2213–2221.

Swales, N., Martens, G.A., Bonné, S., Heremans, Y., Borup, R., Van de Casteele, M., Ling, Z., Pipeleers, D., Ravassard, P., Nielsen, F., et al. (2012). Plasticity of Adult Human Pancreatic Duct Cells by Neurogenin3-Mediated Reprogramming. PLoS ONE *7*, e37055.

Sweet, I.R., and Matschinsky, F.M. (1995). Mathematical model of beta-cell glucose metabolism and insulin release. I. Glucokinase as glucosensor hypothesis. Am. J. Physiol. *268*, E775–E788.

Taleb, N., and Polychronakos, C. (2011). RFX6 is needed for the development and maintenance of the β-cell phenotype. Islets *3*, 291–293.

Tanaka, Y., Gleason, C.E., Tran, P.O., Harmon, J.S., and Robertson, R.P. (1999). Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. Proc. Natl. Acad. Sci. U.S.a. *96*, 10857–10862.

Tanigawa, S., Lee, C.H., Lin, C.S., Ku, C.C., Hasegawa, H., Qin, S., Kawahara, A., Korenori, Y., Miyamori, K., Noguchi, M., et al. (2013). Jun dimerization protein 2 is a critical component of the Nrf2/MafK complex regulating the response to ROS homeostasis. Cell Death Dis *4*, e921.

Taylor, B.L., Liu, F.-F., and Sander, M. (2013). Nkx6.1 Is Essential for Maintaining the Functional State of Pancreatic Beta Cells. Cell Reports.

Tengholm, A. (2012). Cyclic AMP dynamics in the pancreatic β-cell. Ups J Med Sci *117*, 355–369.

Teo, A.K.K., Windmueller, R., Johansson, B.B., Dirice, E., Njolstad, P.R., Tjora, E., Raeder, H., and Kulkarni, R.N. (2013). Derivation of Human Induced Pluripotent Stem Cells from Patients with Maturity Onset Diabetes of the Young. Journal of Biological Chemistry *288*, 5353–5356.

Thorel, F., Népote, V., Avril, I., Kohno, K., Desgraz, R., Chera, S., and Herrera, P.L.

(2010). thorel 2010. Nature *464*, 1149–1154.

Tong, J., Prigeon, R.L., Davis, H.W., Bidlingmaier, M., Kahn, S.E., Cummings, D.E., Tschöp, M.H., and D'Alessio, D. (2010). Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humans. Diabetes *59*, 2145–2151.

Ueki, K., Okada, T., Hu, J., Liew, C.W., Assmann, A., Dahlgren, G.M., Peters, J.L., Shackman, J.G., Zhang, M., Artner, I., et al. (2006). Total insulin and IGF-I resistance in pancreatic β cells causes overt diabetes. Nat Genet *38*, 583–588.

Unger, R.H., and Orci, L. (1977). The role of glucagon in the endogenous hyperglycemia of diabetes mellitus. Annu. Rev. Med. *28*, 119–130.

Unger, R.H., Aguilar-Parada, E., Müller, W.A., and Eisentraut, A.M. (1970). Studies of pancreatic alpha cell function in normal and diabetic subjects. J. Clin. Invest. *49*, 837–848.

Unger, R.H., and Cherrington, A.D. (2012). Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. J. Clin. Invest. *122*, 4–12.

van Belle, T.L., Coppieters, K.T., and Herrath, von, M.G. (2011). Type 1 diabetes: etiology, immunology, and therapeutic strategies. Physiol. Rev. *91*, 79–118.

Van De Casteele, M., Leuckx, G., Baeyens, L., Cai, Y., Yuchi, Y., Coppens, V., De Groef, S., Eriksson, M., Svensson, C., Ahlgren, U., et al. (2013). Neurogenin 3+ cells contribute to β-cell neogenesis and proliferation in injured adult mouse pancreas. Cell Death Dis *4*, e523.

Vanderford, N.L., Andrali, S.S., and Ozcan, S. (2007). Glucose Induces MafA Expression in Pancreatic Beta Cell Lines via the Hexosamine Biosynthetic Pathway. Journal of Biological Chemistry *282*, 1577–1584.

Vanhoose, A.M., Samaras, S., Artner, I., Henderson, E., Hang, Y., and Stein, R. (2008). MafA and MafB Regulate Pdx1 Transcription through the Area II Control Region in Pancreatic Cells. Journal of Biological Chemistry *283*, 22612–22619.

Wang, H., Brun, T., Kataoka, K., Sharma, A.J., and Wollheim, C.B. (2007). MAFA controls genes implicated in insulin biosynthesis and secretion. Diabetologia *50*, 348–358.

Wang, P., Alvarez-Perez, J.-C., Felsenfeld, D.P., Liu, H., Sivendran, S., Bender, A., Kumar, A., Sanchez, R., Scott, D.K., Garcia-Ocaña, A., et al. (2015). A high-throughput chemical screen reveals that harmine-mediated inhibition of DYRK1A increases human pancreatic beta cell replication. Nat. Med. *21*, 383–388.

Wang, R.N., Klöppel, G., and Bouwens, L. (1995). Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. Diabetologia *38*, 1405–1411.

Wang, W., Walker, J.R., Wang, X., Tremblay, M.S., Lee, J.W., Wu, X., and Schultz, P.G. (2009). Identification of small-molecule inducers of pancreatic beta-cell expansion. Proc.

Natl. Acad. Sci. U.S.a. *106*, 1427–1432.

Watanabe, N., Hiramatsu, K., Miyamoto, R., Yasuda, K., Suzuki, N., Oshima, N., Kiyonari, H., Shiba, D., Nishio, S., Mochizuki, T., et al. (2009). A murine model of neonatal diabetes mellitus in Glis3-deficient mice. FEBS Letters *583*, 2108–2113.

Watt, A.J., Zhao, R., Li, J., and Duncan, S.A. (2007). Development of the mammalian liver and ventral pancreas is dependent on GATA4. BMC Developmental Biology *7*, 37.

Weedon, M.N., Cebola, I., Patch, A.-M., Flanagan, S.E., De Franco, E., Caswell, R., Rodríguez-Seguí, S.A., Shaw-Smith, C., Cho, C.H.-H., Allen, H.L., et al. (2013). Recessive mutations in a distal *PTF1A* enhancer cause isolated pancreatic agenesis. Nature Publishing Group *46*, 61–64.

Weir, G.C., Laybutt, D.R., Kaneto, H., Bonner-Weir, S., and Sharma, A. (2001). Beta-cell adaptation and decompensation during the progression of diabetes. Diabetes *50 Suppl 1*, S154–S159.

Wicksteed, B., Brissova, M., Yan, W., Opland, D.M., Plank, J.L., Reinert, R.B., Dickson, L.M., Tamarina, N.A., Philipson, L.H., Shostak, A., et al. (2010). Conditional gene targeting in mouse pancreatic ß-Cells: analysis of ectopic Cre transgene expression in the brain. Diabetes *59*, 3090–3098.

Williams, J.A. (2001). Intracellular signaling mechanisms activated by cholecystokinin-regulating synthesis and secretion of digestive enzymes in pancreatic acinar cells. Annu. Rev. Physiol. *63*, 77–97.

Xie, R., Everett, L.J., Lim, H.-W., Patel, N.A., Schug, J., Kroon, E., Kelly, O.G., Wang, A., D'Amour, K.A., Robins, A.J., et al. (2013). Dynamic Chromatin Remodeling Mediated by Polycomb Proteins Orchestrates Pancreatic Differentiation of Human Embryonic Stem Cells. Stem Cell *12*, 224–237.

Xuan, S., Borok, M.J., Decker, K.J., Battle, M.A., Duncan, S.A., Hale, M.A., Macdonald, R.J., and Sussel, L. (2012). Pancreas-specific deletion of mouse Gata4 and Gata6 causes pancreatic agenesis. J. Clin. Invest. *122*, 3516–3528.

Yamagata, K., Furuta, H., Oda, N., Kaisaki, P.J., Menzel, S., Cox, N.J., Fajans, S.S., Signorini, S., Stoffel, M., and Bell, G.I. (1996a). Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). Nature *384*, 458–460.

Yamagata, K., Oda, N., Kaisaki, P.J., Menzel, S., Furuta, H., Vaxillaire, M., Southam, L., Cox, R.D., Lathrop, G.M., Boriraj, V.V., et al. (1996b). Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). Nature *384*, 455–458.

Yamamoto, K., Matsuoka, T.-A., Kawashima, S., Takebe, S., Kubo, F., Kubo, N., Miyatsuka, T., Kaneto, H., and Shimomura, I. (2013). A novel function of Onecut1 protein as a negative regulator of MafA gene expression. Journal of Biological Chemistry *288*, 21648–21658.

Yang, L., Li, S., Hatch, H., Ahrens, K., Cornelius, J.G., Petersen, B.E., and Peck, A.B. (2002). In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. Proc. Natl. Acad. Sci. U.S.a. *99*, 8078–8083.

Yang, Y.P., Thorel, F., Boyer, D.F., Herrera, P.L., and Wright, C.V.E. (2011). Context-specific -to- -cell reprogramming by forced Pdx1 expression. Genes & Development *25*, 1680–1685.

Yi, P., Park, J.-S., and Melton, D.A. (2013). Betatrophin: A Hormone that Controls Pancreatic β Cell Proliferation. Cell 1–12.

Yoshida, T. (2005). The 5'-AT-rich half-site of Maf recognition element: a functional target for bZIP transcription factor Maf. Nucleic Acids Res. *33*, 3465–3478.

Yu, W.-M., Appler, J.M., Kim, Y.-H., Nishitani, A.M., Holt, J.R., and Goodrich, L.V. (2013). A Gata3-Mafb transcriptional network directs post-synaptic differentiation in synapses specialized for hearing. eLife *2*, e01341.

Zalzman, M., Anker-Kitai, L., and Efrat, S. (2005). Differentiation of human liver-derived, insulin-producing cells toward the beta-cell phenotype. Diabetes *54*, 2568–2575.

Zalzman, M., Gupta, S., Giri, R.K., Berkovich, I., Sappal, B.S., Karnieli, O., Zern, M.A., Fleischer, N., and Efrat, S. (2003). Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells. Proc. Natl. Acad. Sci. U.S.a. *100*, 7253–7258.

Zankl, A., Duncan, E.L., Leo, P.J., Clark, G.R., Glazov, E.A., Addor, M.-C., Herlin, T., Kim, C.A., Leheup, B.P., McGill, J., et al. (2012). Multicentric Carpotarsal Osteolysis Is Caused by Mutations Clustering in the Amino-Terminal Transcriptional Activation Domain of MAFB. The American Journal of Human Genetics *90*, 494–501.

Zhang, C., Moriguchi, T., Kajihara, M., Esaki, R., Harada, A., Shimohata, H., Oishi, H., Hamada, M., Morito, N., Hasegawa, K., et al. (2005). MafA Is a Key Regulator of Glucose-Stimulated Insulin Secretion. Molecular and Cellular Biology *25*, 4969–4976.

Zhang, H., Ables, E.T., Pope, C.F., Washington, M.K., Hipkens, S., Means, A.L., Path, G., Seufert, J., Costa, R.H., Leiter, A.B., et al. (2009a). Multiple, temporal-specific roles for HNF6 in pancreatic endocrine and ductal differentiation. Mechanisms of Development *126*, 958–973.

Zhang, H., Zhang, J., Pope, C.F., Crawford, L.A., Vasavada, R.C., Jagasia, S.M., and Gannon, M. (2009b). Gestational diabetes mellitus resulting from impaired beta-cell compensation in the absence of FoxM1, a novel downstream effector of placental lactogen. Diabetes *59*, 143–152.

Zhang, Y., and Ross, A.C. (2013). Retinoic acid and the transcription factor MafB act together and differentially to regulate aggrecan and matrix metalloproteinase gene expression in neonatal chondrocytes. J. Cell. Biochem. *114*, 471–479.

Zheng, W., and She, J.-X. (2005). Genetic association between a lymphoid tyrosine phosphatase (PTPN22) and type 1 diabetes. Diabetes *54*, 906–908.

Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J., and Melton, D.A. (2008). In vivo reprogramming of adult pancreatic exocrine cells to β-cells. Nature *455*, 627–632.

Zimmet, P.Z. (1999). Diabetes epidemiology as a tool to trigger diabetes research and care. Diabetologia *42*, 499–518.