THE ROLES OF TRANSCRIPTIONAL COREGULATOR SIN3 IN PANCREATIC β-CELL DIFFERENTIATION, FUNCTION, AND SURVIVAL

By

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Arx	Aristaless-related homeobox
ATAC-seq	Assay for Transposase-Accessible Chromatin using sequencing
bp	base pairs
Brn4/Pou3f4	Brain-specific Homeobox/POU Domain Protein 4
CBP	CREB-binding protein
cCasp3	cleaved Caspase-3
ChIP	Chromatin Immunoprecipitation
DB	dorsal pancreatic bud
Dnmt1	DNA methyltransferase 1
dpc	post-conception
E8.5	embryonic day 8.5
Ecad	E-cadherin
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
ERRγ	estrogen-related receptor γ
ES cells	embryonic stem cells
FBS	fetal bovine serum
FDR	false discovery rate
Foxa2	Forkhead box protein a2
GABA	gamma aminobutyric acid

Gcgr	glucagon receptor
GLP-1	glucagon-like peptide 1
GLPR	GLP-1 receptor
GLUT4	glucose transporter 4
GO	Gene Ontology
Grg3	Groucho-related protein 3
GSEA	Gene-set enrichment assays
GSIS	Glucose-stimulated insulin secretion
H&E staining	haemotoxylin and eosin staining
HAT	histone acetyltransferase
HDAC	histone deacetylase
Hhex	Hematopoietically expressed homeobox
HID	histone deacetylase interaction domain
IF	immunofluorescence
Insm1	Insulinoma-associated 1
IPGTT	Intraperitoneal glucose tolerance tests
IR	insulin receptor
IRS	insulin receptor substrate
Isl1	Isl LIM Homeobox 1
ITT	Insulin tolerance test
KRB	Krebs-Ringer Bicarbonate

Lbd1	LIM domain-binding protein 1
logFC	log-fold change
Mafa	Maf BZIP Transcription Factor a
miR	microRNA
Mll3	mixed-lineage leukemia 3
Mnx1	Motor Neuron And Pancreas Homeobox 1
MODY	maturity onset diabetes of the young
MPC	multipotent pancreatic progenitor cell
MS	mass spectrometry
Myt1	Myelin Transcription Factor 1
NDM	neonatal diabetes mellitus
NeuroD1	Neuronal Differentiation 1
Neurog3	Neurogenin3
Nkx6.1	NK6 Homeobox 1
P1	postnatal day 1
PAH1	paired amphipathic helix 1
Pax4	Paired box gene 4
PCIF1	Pdx-1 C terminus-interacting factor 1
PCR	Polymerase chain reaction
Pdx1	pancreatic and duodenal homeobox 1
PI3K	phosphoinositide 3-kinase

PP	pancreatic polypeptide
Ptf1a	pancreas-specific transcription factor 1a
qRT-PCR	Quantitative reverse-transcription PCR
Ras-MAPK	rat sarcoma-mitogen-activated protein kinase
Re-CLIP	reversible cross-link immunoprecipitation
REST	RE1-Silencing Transcription factor
Rfx6	Regulatory Factor x6
ROI	Region-of-Interest
RR	Regulatory region
Sap30	Sin3a Associated Protein 30
scRNA-seq	Single-cell RNA sequencing
SHC	SH2 domain-containing adaptor
Sin3a	Swi-independent 3a
Sox9	SRY-Box Transcription Factor 9
T1D	type 1 diabetes
T2D	type 2 diabetes
T3	tri-iodothyronine
TCA	tricarboxylic acid
tdT	tdTomato
TEM	Transmission electron microscopy
TET1	Ten-eleven translocation methylcytosine dioxygenase 1

TF	transcription factor
Ucn3	urocortin 3
UMI	unique molecular identifier
VB	ventral pancreatic bud

CHAPTER 1

INTRODUCTION

Abstract

This dissertation focuses on the roles of the transcriptional coregulator Swi-independent 3a (Sin3a) in pancreatic β-cell differentiation, function, and survival. In the introduction, I will first present the information about the pancreas structure, the functions of the endocrine pancreas, and the main disorder of the endocrine pancreas, diabetes. Then I will discuss the transcriptional regulation of the pancreas development and β-cell maturation and function. After that, I will discuss the roles of transcriptional coregulators in pancreas development and function and then focus on the roles of the coregulator Sin3a in gene expression.

Pancreas structure

The pancreas contains both exocrine and endocrine tissues that regulate food digestion and nutrient homeostasis, respectively (**Figure 1**) (Bastidas-Ponce et al., 2017; Longnecker, 2014). The exocrine pancreas makes up the majority of the pancreas (more than 95%) and contains acinar cells and ductal cells (Longnecker, 2014). The acinar cells secrete digestive enzymes via their apical surface. The digestive enzymes include proteases (peptidase), lipase, and amylase that digest the proteins, lipids, and carbohydrates from the food (Motta et al., 1997). They are secreted as inactive zymogens into the ductiles and activated in the duodenum (Motta et al., 1997).

The polarized acinar cells form rosette-like acinar structures that open into small ductiles (Motta et al., 1997). The duct is a closed tree-like structure, with multiple ductiles joining each other to form intermediate ducts, which eventually fuse to form the large ducts that connect with the common bile duct and empty into the duodenum (Longnecker, 2014). The duct cells serve two functions. They form the closed ductal tree that serves as a route for acinar secretes transportation. They also secrete sodium bicarbonate-rich electrolytes, which facilitate the flow of acinar secretions while also neutralizing stomach acids (Grapin-Botton, 2005). The diseases associated with the exocrine pancreas include pancreatitis (inflammation of the pancreas) and pancreatic cancer (especially pancreatic ductal adenocarcinoma) (Saluja and Maitra, 2019). Understanding pancreatic exocrine cell differentiation and growth is essential to combat these diseases and have attracted much attention (Perbtani and Forsmark, 2019; Saluja and Maitra, 2019). Yet these are not the focuses of this dissertation research and will not be discussed in more detail.

The endocrine pancreas makes up only 1-2% of the whole pancreas (Longnecker, 2014). There are five endocrine cell types in the endocrine pancreas, including insulin-secreting β-cells, glucagon-secreting α-cells, somatostatin-secreting δ-cells, pancreatic polypeptide-secreting PP cells, and ghrelin-secreting ε-cells (Bastidas-Ponce et al., 2017). The endocrine cells form cell clusters called the islets of Langerhans. The islets of Langerhans are scattered throughout the exocrine pancreas. The organization of the endocrine cells in the islets varies in different species (**Figure 2**) (Cabrera et al., 2006). Monkey islets and human islets have similar architecture (**Figure 2A, B**), which is different from the mouse islets. In mouse islets, β-cells are in the core,

and other non-β-cells in the periphery (**Figure 2C**) (Brissova et al., 2005; Cabrera et al., 2006). Pig islets have smaller subunits (as shown in **Figure 2D**) and the architecture of each unit resembles that of mouse islets. Previous studies found that β-cells, α-cells, and δ-cells are scattered throughout the human islets, without obvious segregation among different cell types (Brissova et al., 2005; Cabrera et al., 2006). Another study found that human islets form trilaminar epithelial plates, with the middle layer β cells sandwiched by two α-cell layers (Bosco et al., 2010). However, the trilaminar epithelial plate structure was not observed in three-dimensional imaging (Dybala and Hara, 2019). Nevertheless, the above studies all supported that there are more β cell-α cell contacts in human islets than in mouse islets (Bosco et al., 2010; Brissova et al., 2005; Cabrera et al., 2006; Dybala and Hara, 2019).

Besides the islet architecture, the cell type composition also varies according to species. In the adult mouse endocrine pancreas, there are 60-80% β-cells, 15-20% α-cells, 5-10% δ-cells, and the rest PP-cells and ε-cells (Edlund, 2002; Steiner et al., 2010). The adult human endocrine pancreas contains different proportions of the endocrine cells, with ~50% β-cells, ~40% α-cells, and ~10% δ-cells and the rest other endocrine cells (Brissova et al., 2005; Cabrera et al., 2006; Steiner et al., 2010). The composition of the endocrine cells in the pancreas changes over development and under different physiological and pathophysiological conditions, such as obesity and diabetes. In the non-diabetic obese mice, β-cells can account for ~90% of pancreatic endocrine cells (Kim et al., 2009), while in the diabetic mice, β cells may only account for ~50% of pancreatic endocrine cells (Kim et al., 2009). Islet architecture and islet-cell proportions may affect intra-islet communications, which will be discussed later.

Functions of the endocrine pancreas

The endocrine cells in the pancreas secrete hormones, including insulin, glucagon, somatostatin, pancreatic polypeptide (PP), and ghrelin, to maintain the nutrient homeostasis (Brass et al., 2010).

Insulin is the key regulator of glucose homeostasis and energy storage (Wilcox, 2005). It stimulates peripheral tissues to update glucose from the bloodstream and promotes glucose metabolism and energy storage (Molina, 2013). Its main targets are liver, skeletal muscle, and fat tissues (Molina, 2013). By binding to the insulin receptors (IRs), belonging to tyrosine kinase receptor superfamily, on the cell membrane, insulin activates the intracellular signaling networks through insulin receptor substrates (IRS) and the phosphoinositide 3-kinase (PI3K)/AKT and extracellular signal-regulated kinase (ERK) pathways (Beale, 2013; Poloz and Stambolic, 2015). Insulin also recruits the SH2 domain-containing adaptor (SHC) to IRs and activates of the rat sarcoma-mitogen-activated protein kinase/ERK (Ras-MAPK/ERK) pathway (Poloz and Stambolic, 2015). Activation of insulin signaling pathways promotes glucose transport through translocation of glucose transporter 4 (GLUT4) in the skeletal muscle and fat tissues, facilitates glucose storage into glycogen, reduces glucose output from the liver, and facilitates fatty acid uptake and triglyceride formation in the fat tissues (Brass et al., 2010; De Meyts, 2016). Besides the short-term effects on glucose homeostasis, insulin also has long-term effects on regulating cell proliferation, differentiation, and apoptosis (Haeusler et al., 2018). Insulin secretion is regulated by several factors, including plasma nutrients (glucose and amino acids), hormones

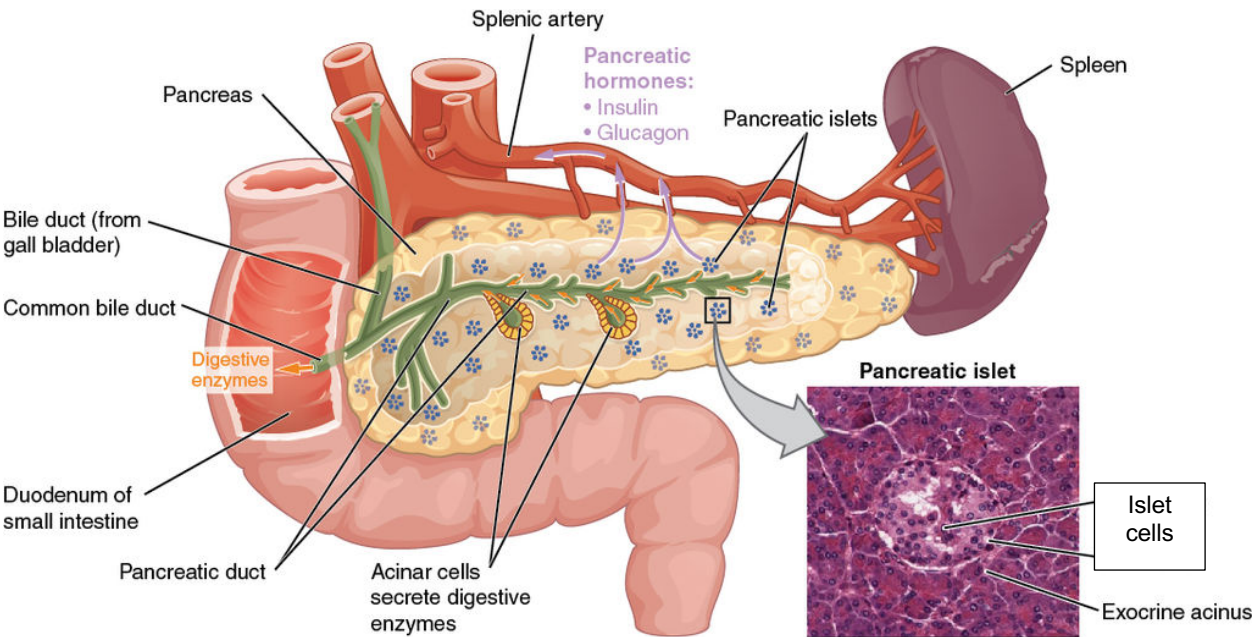


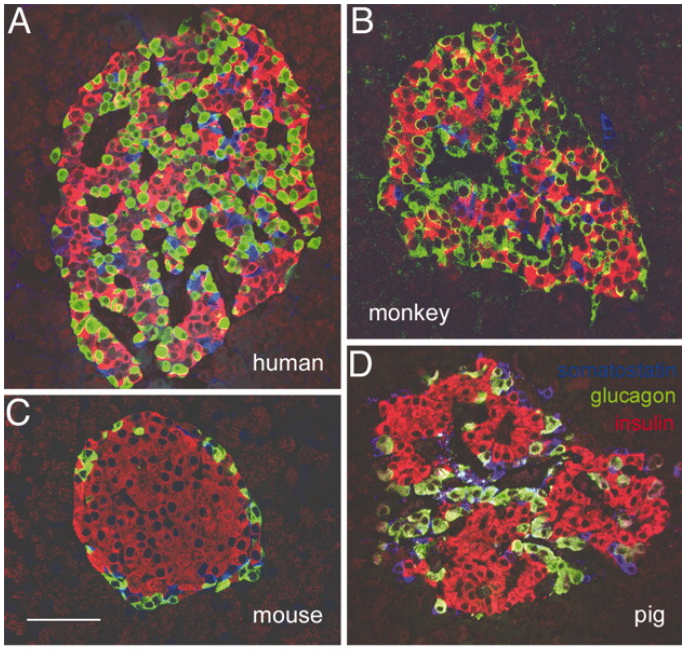
Figure 1: Human pancreas localization and structure.

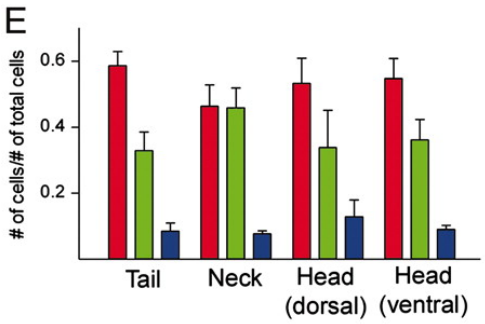
The pancreas is behind the stomach, its head and body near the duodenum, and its tail stretching close to the spleen. The pancreas contains both exocrine and endocrine tissues. The exocrine pancreas makes up the majority of the pancreas (more than 95%) and contains acinar cells and ductal cells. The acinar cells form rosette-like acinar structures that open into small ductiles. The ductiles join each other to form intermediate ducts, which eventually fuse to form the large ducts that connect with the common bile duct and eventually empty into the duodenum. The endocrine pancreas makes up only 1-2% of the whole pancreas. The endocrine cells form the islets of Langerhans. The image in the right lower corner shows haemotoxylin and eosin (H&E) staining of the islet cells in light pink surrounded by exocrine cells in dark pink. Figure is adapted from OpenStax College, Anatomy & Physiology, Connexions Web site (http://cnx.org/content/col11496/1.6/).

(insulin, somatostatin, glucagon-like peptide 1), and neurotransmitters, with glucose as the principal stimulus and a permissive effector (Molina, 2013). The process of glucose-stimulated insulin secretion will be discussed later.

Glucagon has opposite roles to insulin in nutrient homeostasis. At low blood glucose levels, glucagon facilitates the release of stored glycogen in the liver, which is its principal target organ (Brass et al., 2010). It promotes hepatic glucose production through the breakdown of glycogen and gluconeogenesis using amino acids, glycerol, or lactate to increase plasma blood glucose level (Molina, 2013).

Somatostatin secreted from pancreatic δ-cells inhibits both insulin and glucagon secretion (Strowski et al., 2000). Pancreatic polypeptide secreted from pancreatic PP-cells inhibits pancreatic exocrine secretion and gastrointestinal motility and may regulate feeding behavior (Molina, 2013; Śliwińska-Mossoń et al., 2017). Ghrelin secreted from pancreatic ε-cells may affect food intake and increase fat mass (Molina, 2013). The endocrine hormone(s) secreted by one islet cell type affect the functions of other islet cell types. Intra-islet communications are mainly mediated by paracrine actions (Kelly et al., 2011). In islets, insulin inhibits glucagon secretion by inducing intra-islet somatostatin release (Vergari et al., 2019). Besides insulin, β-cells secrete urocortin 3 (Ucn3, a small peptide hormone) and gamma aminobutyric acid (GABA) to promote somatostatin secretion from δ-cells to mediate the timely reduction of insulin secretion (Hartig and Cox, 2020; van der Meulen et al., 2015). The GABA secreted from β-cells inhibits glucagon secretion from α-cells (Bailey et al., 2007). Moreover, insulin also affects β-cell function and proliferation through autocrine actions (Beith et al., 2008; Jiang et al., 2018).


A
human
B
monkey
C
mouse
D
somatostatin
glucagon
insulin
pig


E
# of cells/# of total cells
0.6
0.4
0.2
Tail
Neck
Head (dorsal)
Head (ventral)

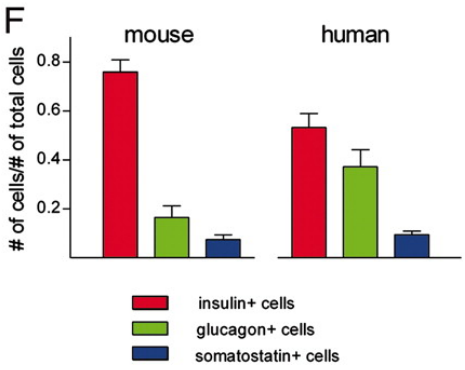

F
mouse
human
# of cells/# of total cells
0.8
0.6
0.4
0.2
insulin+ cells
glucagon+ cells
somatostatin+ cells

Figure 2: Islets of different species have different architectures and cell-type compositions.

(A-D) The islet architecture of human (A), monkey (B), mouse (C), and pig (D). Glucagon is in green labeling α-cells and insulin in red labeling β-cells. Human islets (A) and monkey islets (B) have similar architecture, with β-cells, α-cells, and δ-cells scattered throughout the islets and without obvious segregation among different cell types. In mouse islets (C), β-cells are in the core and other non-β-cells in the periphery. Pig islets (D) have smaller subunits and the architecture of each unit resembles that of mouse islets. (E) The composition of islet cell types in different regions of the human pancreas. (F) The different compositions of islet cell types between mouse and human. Figure is reprinted from (Cabrera et al., 2006) (Copyright (2006) National Academy of Sciences, U.S.A.).

The endocrine hormone(s) secreted by one islet cell type affect the functions of other islet cell types. Intra-islet communications are mainly mediated by paracrine actions (**Figure 3**) (Kelly et al., 2011). The GABA secreted from β-cells inhibits glucagon secretion from α-cells (Bailey et al., 2007). Glucagon and glucagon-like peptide 1 (GLP-1) secreted from α-cells have been shown to stimulate β-cell insulin secretion through a paracrine mechanism both *in vitro* and *in vivo* (Ahrén, 2015; Svendsen et al., 2018; Zhu et al., 2019), which is mediated by β-cell GLP-1 receptor (GLPR) and glucagon receptor (GCGR) (Svendsen et al., 2018; Zhu et al., 2019). More β-cell-α-cell contacts and a larger proportion of α-cells in human islets indicate that glucagon may have more paracrine effects on β-cells in human islets than in mouse islets (Bosco et al., 2010; Brissova et al., 2005; Cabrera et al., 2006). Somatostatin secreted from pancreatic δ-cells inhibits insulin and glucagon secretion by binding to somatostatin receptors expressed on β-cells and α-cells, respectively, and activating an inhibitory Gi protein pathway, which inhibits the cAMP dependent pathway (Cejvan et al., 2003; Hartig and Cox, 2020; Hauge-Evans et al., 2015; Strowski et al., 2000). The intra-islet communications ensure that proper levels of insulin and glucagon are secreted to maintain glucose homeostasis (Kelly et al., 2011).

Diabetes

Diabetes is the most common disorder of the endocrine pancreas, which afflicts approximately 463 million adults (20-79 years) worldwide in 2019 (IDF, 2019). This number will likely grow to 700 million by 2045 (IDF, 2019). Diabetes occurs when the blood glucose level is higher than normal (Molina, 2013). There are two main types of

diabetes, type 1 diabetes (T1D) and type 2 diabetes (T2D). T1D is caused by the immune system-mediated destruction of insulin-secreting β-cells (Warshauer et al., 2020), which may occur at any age (Thomas et al., 2018). People with T1D produce low or no insulin (i.e. absolute insufficiency). The risk factors of T1D are still not fully understood. The known risk factors include family history, genetic susceptibilities in immune function and regulation, and virus infections (Warshauer et al., 2020). T2D is caused by the failure of β-cells to produce enough insulin to regulate blood glucose because of insulin resistance (i.e. impaired insulin action) (Kasuga, 2006; Prentki and Nolan, 2006). It is the most common type of diabetes, accounting for ~90% of all diabetes around the world (IDF, 2019). Obesity is one of the most important risk factors for T2D, which causes insulin resistance (Kasuga, 2006). Besides obesity, age is another important risk factor for T2D (Selvin and Parrinello, 2013). As a result, the prevalence of T2D is higher in older adults (Selvin and Parrinello, 2013). Other types of diabetes include 1) gestational diabetes, which is diabetes first diagnosed during pregnancy due to the failure of β-cells to meet the increased insulin demand (Beale, 2013), 2) monogenic diabetes, caused by mutations in a single gene essential for β-cell production and/or function (Misra and Owen, 2018), including maturity onset diabetes of the young (MODY) and neonatal diabetes mellitus (NDM), and 3) diabetes caused by drugs or chemicals that impair β-cell function and/or survival (IDF, 2019).

In diabetic patients, chronic hyperglycemia leads to vascular complications and damages of the microvasculature of the heart, retina, neurons, and kidney (Forbes and Cooper, 2013), which cause severe complications, including cardiovascular diseases,

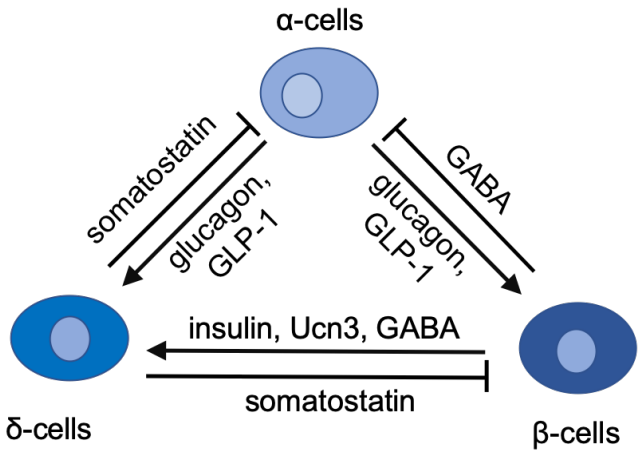


Figure 3: Intra-islet communications.

Insulin, Ucn3, and GABA secreted from β-cells promote somatostatin secretion from δ-cells and GABA secreted from β-cells promotes glucagon secretion from α-cells. Glucagon and GLP-1 secreted from α-cells stimulate β-cell insulin secretion and δ-cell somatostatin secretion. Somatostatin inhibits both insulin and glucagon secretion. (Hartig and Cox, 2020; Vergari et al., 2019)

eye disease, and nerve and kidney damage (IDF, 2019). As a result, diabetic patients need to manage their blood glucose and control the complications.

For T1D, the current treatments include insulin replacement (the main practice), immunotherapy (Warshauer et al., 2020), and transplantation of islets and potential stem cell-derived insulin-producing β-cells (Gamble et al., 2018). For T2D, exercise, diet, and behavior modification are effective management strategies (Kirwan et al., 2017; Nelson et al., 2002). Many T2D patients need diabetes medications due to β-cell failure. Several factors control glucose homeostasis, including β-cell insulin secretion, glucose release from the liver, and glucose uptake by skeletal muscles and adipose tissues (Molina, 2013). All the factors have been drug targets to treat T2D (Molina, 2013). For example, sulfonylureas close K+-ATP channels to increase β-cell insulin release (Sola et al., 2015). GLP-1 receptor agonists amplify glucose-stimulated insulin release and inhibit glucagon release (Garber, 2011). Biguanides, such as metformin, reduce glucose output from the liver and increase glucose uptake in skeletal muscles and adipose tissues (Luna and Feinglos, 2001; Molina, 2013). Thiazolidinediones increase insulin sensitivity in adipose and skeletal muscle (Luna and Feinglos, 2001; Molina, 2013). However, the current treatments for T2D do not prevent β-cell loss and eventually, the T2D patients will need insulin treatment to maintain normal blood glucose (DeFronzo et al., 2015; Kahn et al., 2014). The progressive loss of β-cells in T2D may be caused by glucolipotoxicity (Poitout and Robertson, 2008) and amyloid deposition (Jurgens et al., 2011), which promote β-cell apoptosis through oxidative stress and endoplasmic reticulum stress (Hasnain et al., 2016).

More studies are needed to better understand the mechanisms of β-cell loss in T2D patients and how to maintain functional β-cell mass to treat T2D.

Pancreas development

The pancreas is derived from the endoderm and its development is tightly regulated by different transcriptional programs and signaling pathways (Bastidas-Ponce et al., 2017). Pancreas development has been very well studied in mouse, generating a wealth of data to help understand the roles of genes in human pancreatic disorders (Larsen and Grapin-Botton, 2017; Pan and Wright, 2011) and generate pancreatic cells, especially β-like-cells, using human pluripotent stem cells *in vitro* because the transcriptional programs directing pancreas development are mostly conserved between mouse and human (Gaertner et al., 2019; Nair and Hebrok, 2015). However, there are important differences in the pancreas development between mouse and human (Gaertner et al., 2019; Jennings et al., 2015; Nair and Hebrok, 2015). I will mainly discuss the studies on mouse pancreas development and then briefly discuss the findings of human pancreas development.

The mouse pancreas organogenesis starts at embryonic day 8.5 (E8.5) upon the induction of the key transcription factor (TF) pancreatic and duodenal homeobox 1 (Pdx1) in the dorsal and ventral regions of the posterior foregut endoderm, forming dorsal and ventral pancreatic buds, respectively (Bastidas-Ponce et al., 2017; Larsen and Grapin-Botton, 2017). The two pancreatic buds later form the adult pancreas through fusion (Pan and Wright, 2011). The induction signals of early pancreas development are mainly derived from the notochord, aortic endothelium and the

surrounding mesenchyme (Bastidas-Ponce et al., 2017; Larsen and Grapin-Botton, 2017). At E9.5, the activation of pancreas-specific transcription factor 1a (Ptf1a) in a portion of the Pdx1+ cells promotes these cells to acquire a pancreatic fate (Fujitani, 2017). The Pdx1+Ptf1a+ cells are multipotent pancreatic progenitor cells (MPCs) from E9.5 to E12.5 (Burlison et al., 2008), which generate all endocrine and exocrine cells in the pancreas (Bastidas-Ponce et al., 2017).

Mouse pancreas development can be divided into two transitions. The primary transition is from E9.5 to E12.5 and the secondary transition from E13.5 to E17.5 (**Figure 4**) (Larsen and Grapin-Botton, 2017; Pan and Wright, 2011). In the primary transition, pancreatic progenitors actively replicate to evaginate the pancreatic epithelium to the surrounding mesenchyme (Larsen and Grapin-Botton, 2017). A small portion of pancreatic progenitors transiently express Neurogenin3 (Neurog3), a key TF to drive the endocrine cell formation (Gradwohl et al., 2000; Gu et al., 2002), and differentiate into mainly α-cells. In the secondary transition, the pancreatic epithelium undergoes plexus remodeling and is gradually segregated into ‘‘tip’’ and ‘‘trunk’’ domains (Bankaitis et al., 2015). Cells in the tip domains have sustained high expression of *Ptf1a* and differentiate to acinar cells (Schaffer et al., 2010). Cells in the trunk domains with activation of *NK6 Homeobox 1* (*Nkx6.1*) and downregulation of *Ptf1a* generate bipotent progenitors to give rise to SRY-Box Transcription Factor 9+ (Sox9+) duct progenitors and Neurog3+ endocrine progenitors (Schaffer et al., 2010), differentiating to ductal cells and endocrine cells, respectively. The Neurog3+ progenitors mainly give rise to β-cells in the secondary transition but also give rise to the other four endocrine cell types (Larsen and Grapin-Botton, 2017).

The specification of each endocrine cell type from Neurog3+ endocrine progenitors is driven by different TF networks (Murtaugh, 2007). The endocrine progenitors with low levels of Neurog3 maintain the progenitor pool or generate Neurog3 high progenitors, while high levels of Neurog3 promotes the endocrine progenitors to exit the cell cycle and differentiate into endocrine cells (Bechard et al., 2016; Miyatsuka et al., 2009). As a key TF, Neurog3 activates a combination of pan-endocrine TFs, including *Insulinoma-associated 1* (*Insm1*) (Mellitzer et al., 2006), *Neuronal Differentiation 1* (*NeuroD1*) (Huang et al., 2000; Naya et al., 1997), *Regulatory Factor x6* (*Rfx6*) (Smith et al., 2010; Soyer et al., 2010), and *Myelin Transcription Factor 1* (*Myt1*) (Wang et al., 2007), which are required for establishing the endocrine lineage and proper differentiation of all endocrine cell types in the pancreas (Larsen and Grapin-Botton, 2017). The differentiation of each endocrine cell type is tightly regulated by activating high expression of lineage-specific TFs while decreasing the expression of other TFs (Bastidas-Ponce et al., 2017). β-cell differentiation relies on the activation and/or maintenance of *Pdx1*, *Nkx6.1*, *Paired box gene 4* (*Pax4*), *Nkx2.2*, *Motor Neuron And Pancreas Homeobox 1* (*Mnx1*), and *Maf BZIP Transcription Factor a* (*Mafa*) (Murtaugh, 2007). During pancreas development, *Pdx1* expression gradually restricts to the β-cell lineage and part of the δ-cell lineage. *Nkx6.1* expression gradually restricts to the β-cell lineage (Bastidas-Ponce et al., 2017). α-cell differentiation relies on the activation of *Aristaless-related homeobox* (*Arx*), *Pax6*, *Brain-specific Homeobox/POU Domain Protein 4* (*Brn4*/*Pou3f4*), *Forkhead box protein a2* (*Foxa2*) and *Mafb* (Larsen and Grapin-Botton, 2017). *Pax4* and *Arx* mutually repress each other to establish β-cell and α-cell lineages, respectively (Collombat et al., 2005; Collombat et al., 2003). TFs

essential for proper δ-cell differentiation include Pdx1, Pax4, and Hematopoietically expressed homeobox (Hhex) (Bastidas-Ponce et al., 2017; Zhang et al., 2014). TFs essential for proper PP-cell differentiation include Arx, Rfx3, and Isl LIM Homeobox 1 (Isl1) (Bastidas-Ponce et al., 2017).

The process of human pancreas development is not well understood because access to human samples is limited. The data of human pancreas development are generated from human embryonic and fetal tissues (Jennings et al., 2015) and lately modeling from human pluripotent stem cells (Gaertner et al., 2019). Key TFs have been identified in the human fetal pancreas, including PDX1 and NEUROG3, which show a similar spatiotemporal pattern to mouse pancreas (Pan and Wright, 2011). The multipotent progenitors in the human pancreas are also marked by PDX1 in the foregut endoderm (Jennings et al., 2013). PDX1 is first detected in the presumptive pancreatic endoderm on 29-31 days post-conception (dpc) (Jennings et al., 2015), which is required for the human pancreas organogenesis (Stoffers et al., 1997). The transient expression of *NEUROG3* is first detected on 49-52 dpc (Jennings et al., 2015). Impairments of the endocrine pancreas development in patients with NEUROG3 mutations vary because different NEUROG3 variant has distinct molecular defect(s) (Zhang et al., 2019). Endocrine cell progenitors also express *PAX6*, *NKX2.2*, *NKX6.1*, *ISLET1*, *NEUROD*, and *PAX4*, indicating part of the regulatory networks of pancreas development may be conserved between mouse and human (Jennings et al., 2015). However, there are important differences in human pancreas development from that of mouse pancreas, including only one phase of endocrine generation and β-cells as the first endocrine cell type to appear in the human pancreas (Nair and Hebrok, 2015). As a

result, it is important to better understand the signaling pathways regulating human pancreas development, especially by using modeling from human pluripotent stem cells (Gaertner et al., 2019).

The maturation and function of β-cells

Pancreatic β-cells increase insulin secretion at high glucose to facilitate glucose uptake by peripheral tissues while suppressing insulin secretion at low glucose to maintain basal functions of the body (Liu and Hebrok, 2017). At embryonic stages, pancreatic endocrine progenitors differentiate into immature β-cells, which are lineage-specified and express the canonical β-cell TFs Pdx1, Nkx6.1, Isl1, Pax6 and Nkx2.2 (Liu and Hebrok, 2017). The process is termed β-cell differentiation (Salinno et al., 2019). The immature β-cells gain glucose-responsive insulin secretion during the first postnatal weeks in mice (Liu and Hebrok, 2017; Salinno et al., 2019), termed β-cell matuation. Immature β-cells secrete insulin at low concentrations of glucose, while mature β-cells have a higher glucose threshold for insulin secretion (Blum et al., 2012).

The process of glucose-stimulated insulin secretion (GSIS) starts with glucose transportation into the β-cells (**Figure 5**) (De León and Stanley, 2007). After glucose metabolism, ATP is generated to close ATP-sensitive potassium channels, which depolarizes the cell membrane and opens voltage-dependent Ca^{2+} channels. The influx of Ca^{2+} triggers insulin secretion (De León and Stanley, 2007). Several gene expression

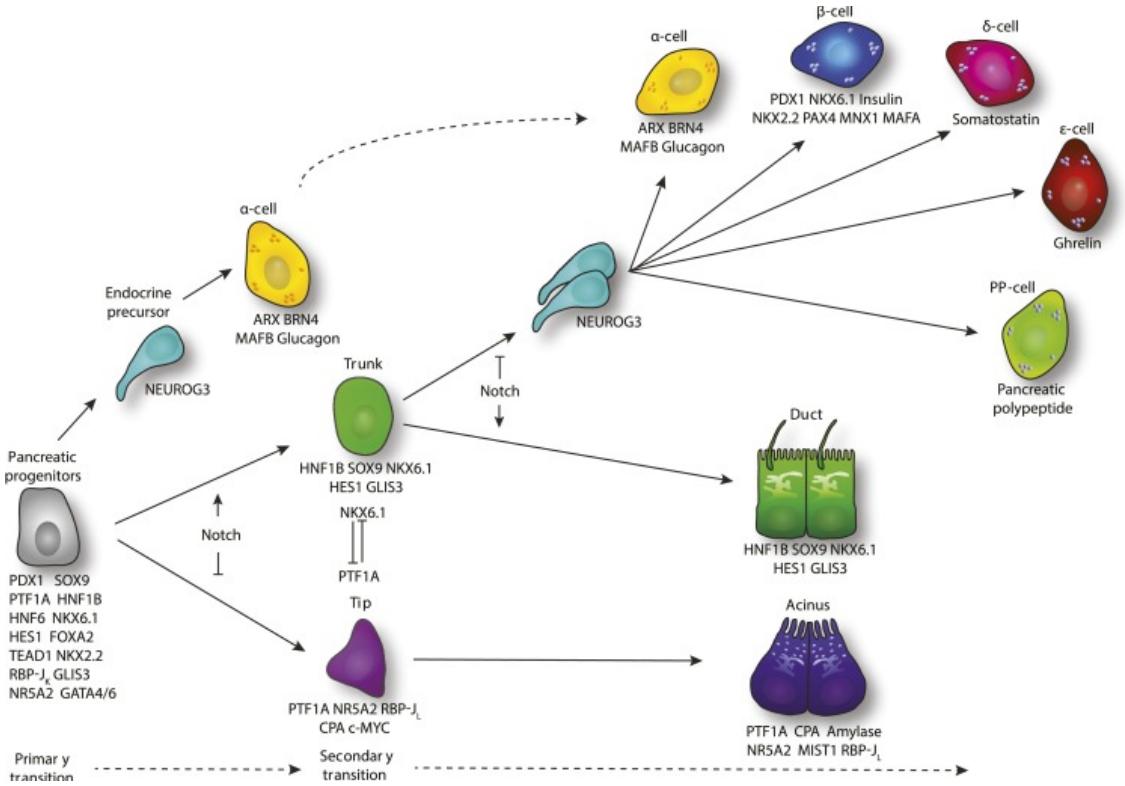


Figure 4: Mouse pancreas development.

Mouse pancreas development can be divided into two transitions, the primary transition from E9.5 to E12.5 and the secondary transition from E13.5 to E17.5. In the primary transition, pancreatic progenitors actively replicate. A small portion of pancreatic progenitors transiently express Neurog3 and differentiate into mainly α-cells. In the secondary transition, the pancreatic epithelium is gradually segregated into ‘‘tip’’ and ‘‘trunk’’ domains. Cells in the tip domains have sustained high expression of *Ptf1a* and differentiate to acinar cells. Cells in the trunk domains with activation of *Nkx6.1* and downregulation of *Ptf1a* generate bipotent progenitors to give rise to Sox9+ duct progenitors and Neurog3+ endocrine progenitors, differentiating to ductal cells and endocrine cells, respectively. Figure is reprinted from (Larsen and Grapin-Botton, 2017).

changes in the GSIS process during the maturation process promote immature β-cells to gain glucose-responsive insulin secretion, including downregulation of hexokinase, microRNAs (miRs) 130b, 203, 17/92, and Mafb, and upregulation of glucokinase, miRs 204, 29, 129, Mafa, and genes involved in β-cell metabolism (**Figure 6**) (Hang and Stein, 2011; Liu and Hebrok, 2017).

Several factors have been identified as maturation drivers, including Mafa, tri-iodothyronine (T3), the nuclear receptor estrogen-related receptor γ (ERRγ), and NeuroD, which are sufficient to promote β-cell maturation by inducing mature GSIS (Liu and Hebrok, 2017). Ucn3 is identified as a maturation marker, which is correlated with β-cell maturation but not sufficient to promote β-cell maturation (Blum et al., 2012; Liu and Hebrok, 2017).

Both immature and mature β-cells express key β-cell TFs (Conrad et al., 2014), including Pdx1 (Gao et al., 2014; Pan and Wright, 2011), Nkx2.2 (Doyle and Sussel, 2007; Sussel et al., 1998), Nkx6.1 (Nelson et al., 2007; Schaffer et al., 2013; Taylor et al., 2013), Isl1 (Ediger et al., 2014; Hunter et al., 2013), and Pax6 (Sander et al., 1997; Swisa et al., 2017), establishing β-cell transcriptional profiles and maintaining β-cell identity. For example, Pdx1 is a key TF to maintain β-cell identity and function by activating β-cell key functional genes, such as *Insulin 1* and *Mafa*, and repressing α-cell related genes, such as *glucagon* and *Mafb* (Gao et al., 2014). The deletion of *Pdx1* in mature β-cells caused severe hyperglycemia and β-cell to α-cell conversion (Gao et al., 2014).

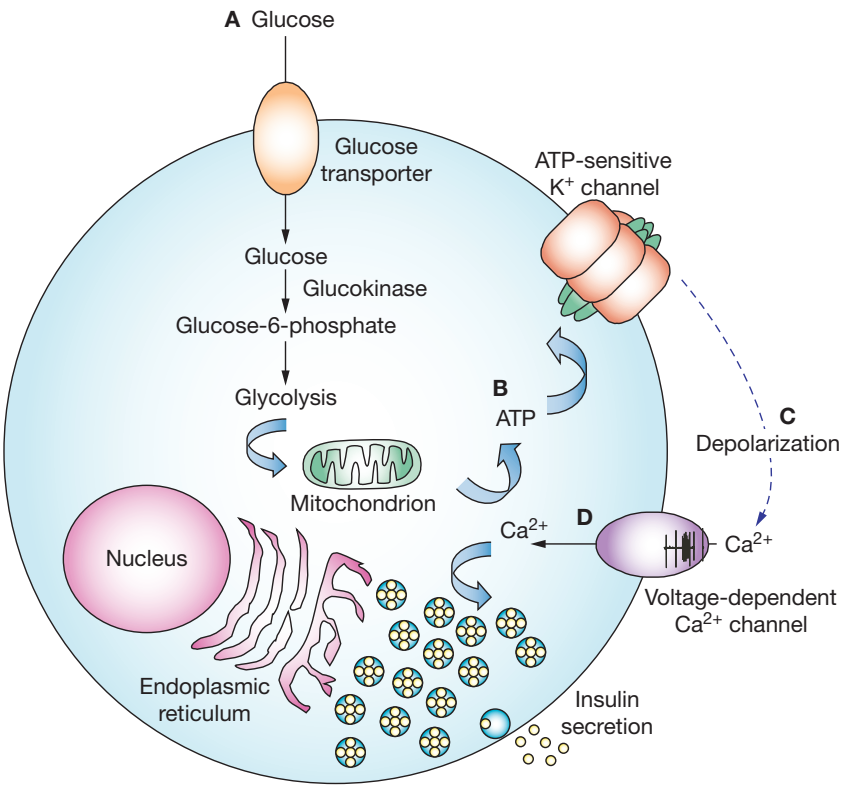


Figure 5: Pancreatic β-cell glucose-stimulated insulin secretion.

In the mature β-cells, glucose transported into the cells and metabolized by glucokinase (A). Glucose metabolism generates more ATP to close ATP-sensitive potassium channels (B), which depolarizes the cell membrane and opens voltage-dependent Ca2+ channels (C). The influx of Ca2+ triggers insulin secretion (D). Figure is reprinted from (De León and Stanley, 2007).

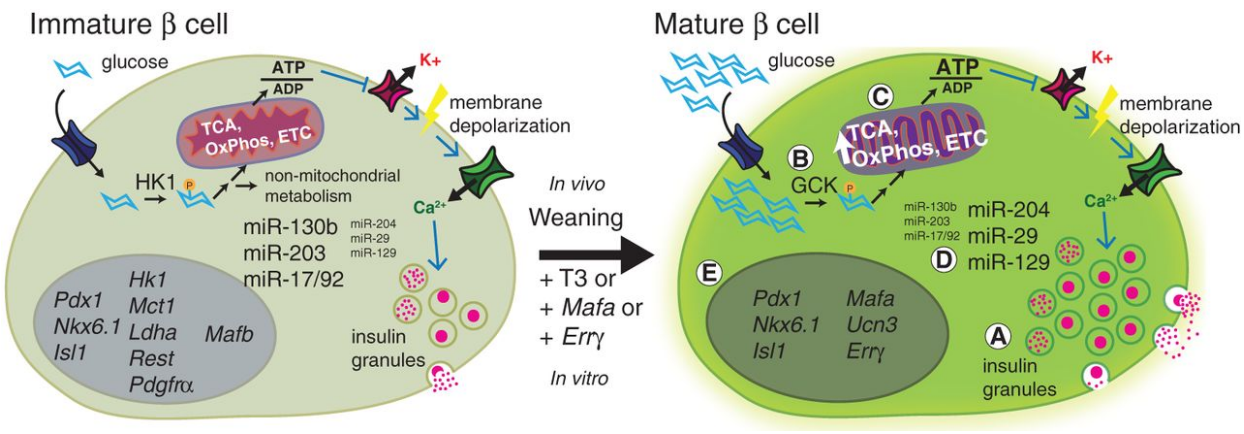


Figure 6: Pancreatic β-cell maturation.

Both immature and mature β-cells express key β-cell TFs, including Pdx1, Nkx6.1, and Isl1. In mature β-cells, the expression of hexokinase, miRs 130b, 203, 17/92, and *Mafb* is downregulated, while the expression of glucokinase, miRs 204, 29, 129, *Mafa*, and genes involved in β-cell metabolism are upregulated. The gene expression changes during the maturation process promote immature β-cells to gain glucose-responsive insulin secretion. Figure is reprinted from (Liu and Hebrok, 2017).

The roles of coregulators in pancreas development and function

During the differentiation and in the differentiated pancreatic endocrine cells, TFs recruit coregulators to control gene expression. The coregulators act as functional effectors to add ("writers") or remove ("erasers") the epigenetic marks to modify DNA, histones, and chromatin structures to regulate gene expression (**Figure 7**) (Falkenberg and Johnstone, 2014; Spaeth et al., 2016). There are around 320 identified coregulators so far (www.nursa.org), but the functions of many of the coregulators are not known in the pancreas.

The function of some coregulators have been examined in the pancreas. p300 and CREB-binding protein (CBP) contain histone acetyltransferase (HAT) domains and act as transcriptional coactivators. They interact with β-cell TFs, such as Pdx1 and NeuroD, and are required for postnatal β-cell proliferation (Wong et al., 2018) and function by acetylating histone tails and activating the expression of target genes (Qiu et al., 1998; Stanojevic et al., 2004). Methyltransferase Set7/9 interacts with Pdx1 to enhance the ability of Pdx1 to regulate β-cell functional genes, such as *Insulin 1/Insulin 2* and *Mafa*, by methylating the N-terminus of Pdx1 (Maganti et al., 2015). Pdx1 recruits a different set of coregulators, including Brm:Swi/Snf complex and histone deacetylases 1 and 2 (HDACs 1 and 2) to reduce the expression of *Ins1/Ins2* under low glucose conditions (**Figure 8**) (Spaeth et al., 2016). Pdx-1 C terminus-interacting factor 1 (PCIF1, also known as SPOP) increases Pdx1 degradation and inhibits Pdx1 transactivation activities (Claiborn et al., 2010; Liu et al., 2004).

The mixed-lineage leukemia 3 (Mll3) and Mll4 methyltransferases are recruited by Mafa in β-cells to activate target gene expression and maintain β-cell function (Scoville et al., 2015).

Other coregulators important for pancreas development and/or function include DNA methyltransferase 1 (Dnmt1) (Georgia et al., 2013), Dnmt3a (Dhawan et al., 2015; Papizan et al., 2011), HDACs 1 and 2 (Mosley and Ozcan, 2004), regulating histone acetylation, Bridge-1 (Stanojevic et al., 2005), Swi/Snf complex (McKenna et al., 2015; Spaeth et al., 2019), Groucho-related protein 3 (Grg3) (Metzger et al., 2012), Grg1 (Metzger et al., 2014), LIM domain-binding protein 1 (Lbd1) (Ediger et al., 2017; Hunter et al., 2013). The coregulators regulate DNA methylation, histone acetylation, or the formation of chromatin-modifying protein complex to regulate gene expression (Spaeth et al., 2016). The coregulators usually have stage- and cell-type-specific roles (Spaeth et al., 2016) and they may serve as potential drug targets (Bishop et al., 2019). As a result, it is important to study the roles of other coregulators in pancreatic endocrine cells, especially in β-cells, to better understand the transcriptional regulatory networks in pancreatic endocrine cell development and function.

The roles of coregulator Sin3a in gene expression

Sin3 was first identified in budding yeast *Saccharomyces cerevisiae* (Nasmyth et al., 1987; Sternberg et al., 1987) when the two groups examined mating-type switching using genetic screens. Since then, the homologs of Sin3 have been identified in *Caenorhabditis elegans*, drosophila, zebrafish, and mammals (Chaubal and Pile, 2018; Kadamb et al., 2013).

The Sin3 protein structure is conserved across different species (Kadamb et al., 2013). Sin3 has no DNA binding domain or enzymatic activity. It acts as a scaffold protein and contains five protein-protein interacting domains (**Figure 9**), including paired amphipathic helix 1 (PAH1), PAH2, PAH3, histone deacetylase interaction domain (HID), and Sin3_C domain (Chaubal and Pile, 2018; Kadamb et al., 2013). The three PAH domains are important protein-protein interacting sites and mediate the interactions with other proteins, especially TFs (Adams et al., 2018). The Sin3-interacting TFs recruit Sin3 protein complexes to specific loci to regulate target gene expression. Sin3 interacts with HDAC1 and HDAC2 through the HID domain, which provide enzymatic activities for the Sin3 protein complexes (Hassig et al., 1997; Laherty et al., 1997; Zhang et al., 1997). Other associated proteins, such as Sin3a Associated Protein 30 (Sap30), Sap130 and Inhibitor Of Growth Family Member 1/2 (Ing1/2), interact with Sin3 through the PAH3 and HID domains and help to stabilize and support the Sin3-HDAC1/2 protein complexes (Adams et al., 2018; Chaubal and Pile, 2018; Fleischer et al., 2003). The protein-protein interactions mediated by Sin3_C domain are not clear (Chaubal and Pile, 2018).

Sin3 was first identified as a corepressor (Nasmyth et al., 1987; Sternberg et al., 1987). HDAC1 and HDAC2 attribute to the corepressor activities of Sin3 by removing acetyl groups from histone tails and leading to chromatin condensation (Silverstein and Ekwall, 2005). Other enzymes contributing to the corepressor activities of Sin3 include histone and DNA methyltransferases (Silverstein and Ekwall, 2005). Sin3 can also act as a coactivator but the mechanisms are not very clear. One possible mechanism is that Sin3 has been found to interact with the DNA demethylase Ten-eleven translocation

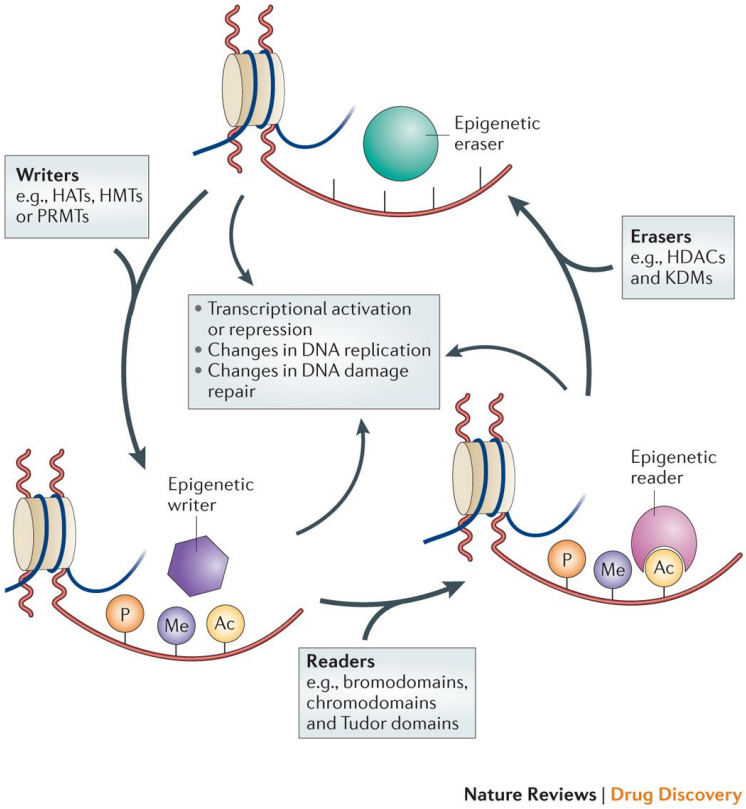


Figure 7: Epigenetic writer, reader, and eraser.

The coregulators act as functional effectors to add ("writers") or remove ("erasers") the epigenetic marks to modify DNA, histones, and chromatin structures to regulate gene expression. For example, HATs act as epigenetic writers to add acetyl groups to histone tails, HDACs as epigenetic erasers to remove acetyl groups from histone tails, and Sin3a as scaffold proteins binding epigenetic writers and erasers. DNA binding TFs act as epigenetic readers to recruit the coregulators to regulate the expression of target genes. Figure is reprinted from (Falkenberg and Johnstone, 2014).

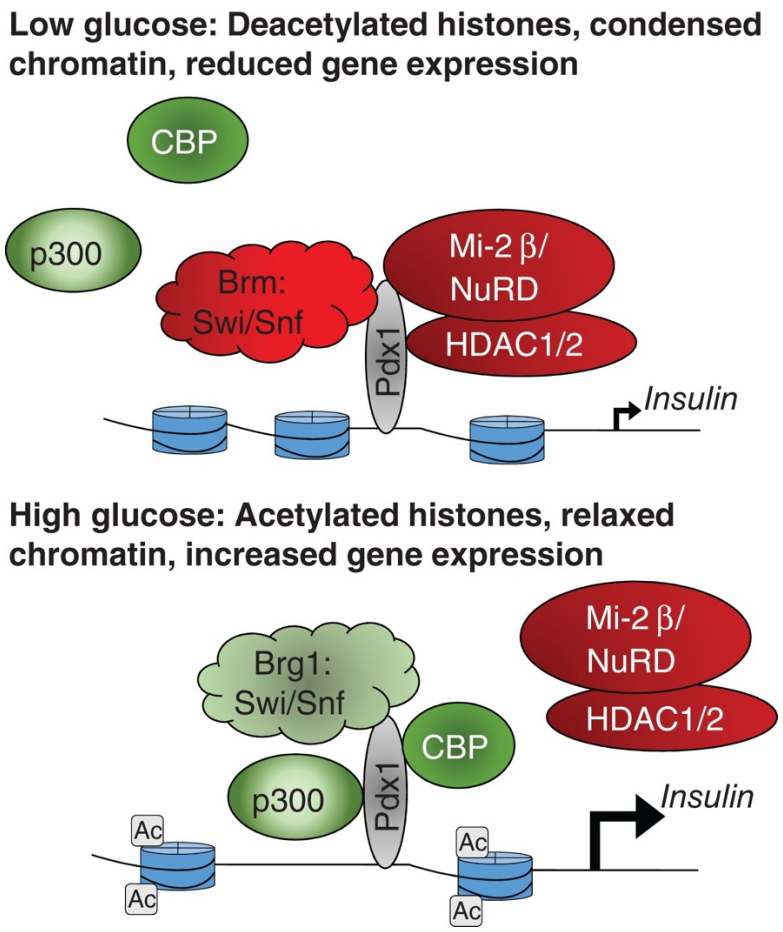


Figure 8: Pdx1 recruits different coregulators under low and high glucose conditions to regulate insulin gene expression.

Under low glucose conditions, Pdx1-recruited coregulators Brm:Swi/Snf complex and HDAC1/2 deacetylate histones and condense the chromatin landscape, which reduces *insulin* gene expression. Under high glucose conditions, Pdx1-recruited coregulators Brg1:Swi/Snf complex, p300, and CBP, acetylate histones, and relax chromatin, which enhances *insulin* gene expression. Figure is reprinted from (Spaeth et al., 2016).

methylcytosine dioxygenase 1 (TET1) (Williams et al., 2011), which can reduce the levels of DNA methylation and activate gene expression.

In mammals, there are two paralogs of Sin3, Sin3a and Sin3b. They share 57% similarity in their protein sequences and have overlapped and also different protein interaction partners and functions (Kadamb et al., 2013). The functions of Sin3a and Sin3b have been investigated in embryonic stem cells (Cowley et al., 2005; Dannenberg et al., 2005; David et al., 2008) and muscle cells (van Oevelen et al., 2010) using loss-of-function studies. In mouse embryonic development, the *Sin3a* null embryos cannot survive beyond E6.5 (Cowley et al., 2005; Dannenberg et al., 2005), while the *Sin3b* null embryos can survive to late gestation but not beyond postnatal day 1 (P1) (David et al., 2008). The data indicate that Sin3a is required for early embryonic development, while Sin3b is required for late organ function. In mouse skeletal muscles, loss of *Sin3a* causes severe defects in sarcomere structure, which are enhanced upon loss of both *Sin3a* and *Sin3b* (van Oevelen et al., 2010). Moreover, Sin3a is also required for the survival of male germ cells (Pellegrino et al., 2012), lung progenitor cells (Yao et al., 2017), and the maintenance of skin tissue homeostasis (Nascimento et al., 2011).

The transcriptional target-selection of Sin3 is mainly mediated by its association with TFs. The interactions between mammalian Sin3a/Sin3b with a large number of TFs have been identified, including RE1-Silencing Transcription factor (REST), Mad1, p53, Myc, ESET, Mafa, Foxo1, Myt1, and Mafa (Barnes et al., 2018; Kadamb et al., 2013; Langlet et al., 2017; Romm et al., 2005; Scoville et al., 2015). REST represses the transcription of neuronal genes in nonneuronal cells by recruiting Sin3a (Huang et al., 1999). Mad1 recruits Sin3a to repress gene expression (Brubaker et al., 2000; Laherty

et al., 1997). p53 recruits both Sin3a and Sin3b to repress target gene expression (Kadamb et al., 2013). Myc interacts with Sin3b in human and rat cells and the interaction causes Myc deacetylation and degradation to attenuate cell proliferation (Garcia-Sanz et al., 2014). Sin3a is recruited by Foxo1 in liver cells to repress glucokinase gene expression (Langlet et al., 2017). Sin3a is found in Mafa-containing protein complex in mouse β cells (Scoville et al., 2015). Sin3b directly interacts with Myt1 to repress gene expression in neurons (Romm et al., 2005).

The close interactions between Sin3a/Sin3b and widely expressed TFs and islet-enriched TFs indicate the potential roles of Sin3a/Sin3b in β-cell production and function. It is thought that specific combinations of coregulators Sin3a/Sin3b and DNA-binding TFs, widely expressed TFs and islet-enriched TFs, organize the chromatin landscape to shape the transcriptomic profiles to regulate β-cell development and function.

Dissertation overview

TFs recruit transcriptional coregulators to regulate gene expression. Some coregulators have been found to be required for pancreas development and/or function, but the roles of many important coregulators have not been studied in the pancreas. Sin3a and Sin3b are paralogous transcriptional coregulators that direct cellular differentiation, survival, and function. I found that Sin3a and Sin3b are co-produced in most pancreatic cells during embryogenesis but become much enriched in endocrine cells in adults, implying the potential roles in pancreas development and in mature-cell endocrine function.

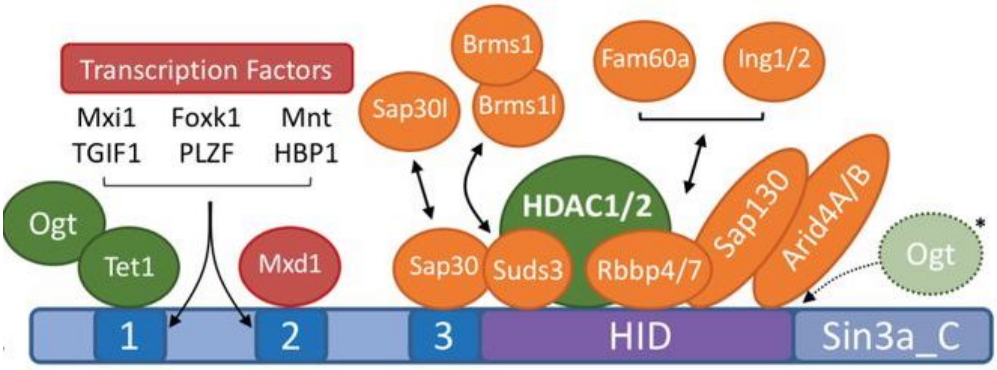


Figure 9: Sin3 protein structure and its interacting proteins.

Sin3 acts as a scaffold protein and contains five protein-protein interacting domains, including PAH1, PAH2, PAH3, HID, and Sin3_C domains. The three PAH domains are important protein-protein interacting sites and mediate the interactions with other proteins, especially TFs. Sin3 interacts with HDAC1 and HDAC2 through the HID domain, which provide enzymatic activities for the Sin3 protein complexes. Some accessory factors, such as Sap30, Sap130 and Ing1/2, interact with Sin3 through the PAH3 and HID domains and stabilize the Sin3-HDAC1/2 interaction. Sin3_C domain lacks protein-protein interaction structures. Figure is adapted and reprinted from (Adams et al., 2018) (https://portlandpress.com/biochemj/article-lookup/doi/10.1042/BCJ20170314).

Moreover, Sin3a and/or Sin3b have been found to interact with TFs, including REST, Mad1, p53, Myc, ESET, Mafa, Foxo1, and Myt1, that are either ubiquitously expressed in all cells or highly expressed in pancreatic islet cells. The evidence further indicates the potential roles of Sin3a/Sin3b in pancreas development and/or function.

I first examined the role of Sin3a in β-cell differentiation, function, and survival. I found that mice with loss of *Sin3a* in endocrine progenitors were normal at early postnatal stages but gradually developed diabetes before weaning, preceded by defective survival, insulin-vesicle packaging, nutrient-induced Ca2+ influx, and insulin secretion from *Sin3a*-deficient β cells. RNA-seq coupled with candidate chromatin-immunoprecipitation assays revealed several genes that could be directly repressed or activated by Sin3a in β cells, regulating cell survival, glucose metabolism, stress responses, vesicle/membrane trafficking, and Ca2+/ion transport. Next, I examined the potential redundant functions of Sin3a and Sin3b in mouse pancreas development. Loss of both *Sin3a* and *Sin3b* in endocrine progenitors further reduced the generation of endocrine cells at early postnatal stages, which caused severe postnatal diabetes. Moreover, mice with loss of both *Sin3a* and *Sin3b* in Pdx1-expressing multipotent pancreatic progenitors had significantly reduced islet-cell mass at birth, preceded by decreased endocrine-progenitor production and postnatal β-cell death.

In conclusion, the findings of the dissertation highlight the specific temporal requirements for the presumed “general” coregulators Sin3a and Sin3b in islet β-cells, being dispensable for differentiation but required for postnatal function and survival.

CHAPTER 2

THE ROLES OF SIN3 IN PANCREATIC β-CELL DIFFERENTIATION, FUNCTION, AND SURVIVAL

(Adapted from (Yang et al., 2020))

Abstract

Sin3a and Sin3b are paralogous transcriptional coregulators that direct cellular differentiation, survival, and function. Mouse Sin3a and Sin3b are co-produced in most pancreatic cells during embryogenesis but become much more enriched in endocrine cells in adults, implying continued essential roles in mature endocrine-cell function. Mice with loss of *Sin3a* in endocrine progenitors were normal during early postnatal stages but gradually developed diabetes before weaning. These physiological defects were preceded by the compromised survival, insulin-vesicle packaging, insulin secretion, and nutrient-induced Ca^{2+} influx of *Sin3a*-deficient β-cells. RNA-seq coupled with candidate chromatin-immunoprecipitation assays revealed several genes that could be directly regulated by Sin3a in β-cells, which modulate Ca^{2+}/ion transport, cell survival, vesicle/membrane trafficking, glucose metabolism, and stress responses. Lastly, mice with loss of both *Sin3a* and *Sin3b* in multipotent embryonic pancreatic progenitors had significantly reduced islet-cell mass at birth, caused by decreased endocrine-progenitor production and increased β-cell death. These findings highlight the stage-specific requirements for the presumed “general” coregulators Sin3a and Sin3b in islet β-cells,

with Sin3a being dispensable for differentiation but required for postnatal function and survival.

Introduction

Islet β-cell differentiation starts from early embryogenesis when a subset of pancreatic progenitor cells transiently activate the expression of a key TF *Neurog3*. Depending on the DNA-methylation landscape (Liu et al., 2019a), Neurog3 then activates a combination of islet-enriched TFs including *Isl1*, *Insm1*, *Mnx1*, *NeuroD1*, *Nkx2.2*, *Nkx6.1*, *Pax4*, *Pdx1*, and *Rfx6* to initiate β-cell differentiation (Bastidas-Ponce et al., 2017; Pan and Wright, 2011). Inactivating any of these genes compromises β-cell production, underscoring their importance for β-cell differentiation (Larsen and Grapin-Botton, 2017). At postnatal stages, the progenitor-driven β-cell production ceases while the differentiated β cells proliferate to increase the bulk β-cell mass, a process that depends on several pathways, including PI3K-AKT, Ca2+-Calcineurin, Ras-ERK/MAPK, Wnt, Jak-stat, G-proteins, and growth factor-mediated processes (Bernal-Mizrachi et al., 2014; Jiang et al., 2018; Kulkarni et al., 2012; Stewart et al., 2015; Wang et al., 2015). Meanwhile, the postnatal β cells fine-tune their gene-expression networks including TFs (e.g., MafA), paracrine hormones (e.g., Ucn3), metabolic genes (e.g., Ldha) (Liu and Hebrok, 2017), and Ca2+ sensors (e.g., Syt4) (Huang et al., 2018) to become mature, functional β cells.

Besides TFs, several transcriptional coregulators have also been shown to regulate the production and function of β cells (Spaeth et al., 2016). These factors do not bind DNA directly but, by being recruited to specific DNA regulatory elements via

TFs, they play essential roles in transcriptional regulation. For example, Grg3 (Metzger et al., 2012; Metzger et al., 2014), Lbd1 (Ediger et al., 2017), Set7/9 (Deering et al., 2009), Bridge 1 (Thomas et al., 2009), Mll3 and Mll4 methyltransferases (Scoville et al., 2015), HDAC 1 and 2 (Mosley and Ozcan, 2004), Swi/Snf complexes (McKenna et al., 2015; Spaeth et al., 2019), and p300/CBP (Wong et al., 2018) have been shown essential for the production and/or function of β cells. The standard model (Spaeth et al., 2016) is that the number and specific combinations of coregulators act as functional effectors of location-dependent TF binding.

Sin3 is a well-established coregulator found in yeast to human cells (Kadamb et al., 2013). It does not bind DNA, but uses several highly conserved amphipathic α-helices to interact with a variety of TFs or other coregulators including P53, Mad1, Myc, REST, and ESET (Barnes et al., 2018; Kadamb et al., 2013). In addition, Sin3 contains binding sites for HDACs and histone lysine methylases or demethylases (Liu et al., 2019b; Yang et al., 2018; Zhu et al., 2018a). Thus, Sin3 primarily acts as a scaffold protein to assemble chromatin-modifying complexes to regulate gene transcription, with target-gene selectivity determined by the DNA-binding TFs that recruit Sin3 (Liu et al., 2019a; Yang et al., 2018; Zhu et al., 2018a). Notably, although Sin3 was commonly known as a "corepressor", several studies suggest that it can coactivate genes in some cellular contexts (Chaubal and Pile, 2018).

Sin3 has two paralogs in mammalian cells, Sin3a and Sin3b, with evidence for overlapping yet distinct functions (Chaubal and Pile, 2018; Kadamb et al., 2013). In mice, Sin3a, but not Sin3b, is essential in cell survival and/or differentiation during embryogenesis. Nullizygous *Sin3a*$^{-/-}$ mouse embryos died shortly after implantation,

whereas *Sin3b*-null mice were born with roughly normal-looking organs but they die immediately after birth (Kadamb et al., 2013). Consequently, Sin3a was reported to be required for the development and/or survival of embryonic stem cells (Saunders et al., 2017; Streubel et al., 2017), muscle cells (van Oevelen et al., 2010), male germ cells (Pellegrino et al., 2012), lung progenitors (Yao et al., 2017), and some skin cells (Nascimento et al., 2011). In addition, gene expression and protein-DNA interaction studies showed that Sin3a could directly regulate molecules involved in cell proliferation, survival, metabolism, and stress responses (Dannenberg et al., 2005; Tiana et al., 2018; Yang et al., 2018). Yet how Sin3a functions in postnatal organs has not been examined.

Sin3a was detected in transcriptional complexes containing Myt, Mafa, and/or Foxo TFs, all required for β-cell production and/or function (Langlet et al., 2017; Romm et al., 2005; Scoville et al., 2015). In this dissertation, I assessed the roles and working mechanism of Sin3, focusing on Sin3a, in embryonic development and postnatal function of mouse islet β-cells. I found that Sin3a is dispensable for islet-cell differentiation but required for β-cell function and survival – that is, their postnatal "fitness". In addition, although Sin3 activity, sufficiently provided by either Sin3a or Sin3b, is essential for endocrine specification from MPCs, it is dispensable for the differentiation of endocrine progenitors into islet hormone-positive cells. Thus, the data reveal stage- and cell-type specific roles of the Sin3 complex, with Sin3a being particularly important for postnatal β-cell fitness.

Materials and Methods

Mice

Mouse usage was supervised by the Vanderbilt University IACUC in compliance with AAALAC regulations. *Sin3a*^{F}, *Sin3a*$^{-}$, *Sin3b*^{F}, and *Sin3b*$^{-}$ mice were described in (Dannenberg et al., 2005; David et al., 2008), *Pdx1*^{Cre} [Tg(Pdx1Cre)89.1Dam], *Ai9* [Cg-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J], and *Neurog3-Cre* [Tg(Neurog3-Cre)C1Able/J] mice were from the Jackson Laboratory. *Sin3a*$^{F/-}$*; Neurog3-Cre was termed Sin3a*$^{\Delta endo}$, *Sin3a*$^{F/-}$*; Sin3b*$^{F/-}$*; Neurog3-Cre termed Sin3a/3b*$^{\Delta endo}$, *Sin3a*$^{F/F}$*; Sin3b*$^{F/F}$*; Pdx1*^{Cre} *termed Sin3a/3b*$^{\Delta Panc}$. ICR (CD1) mice were from Charles River Laboratories. All analyzed mice have a mixed genetic background (estimated ~25 % CD1, ~37.5 % C57BL/6, and ~37.5 % 129, estimated from crossing history). Polymerase chain reaction (PCR)-based genotyping used oligos listed in **Table 1**. The diagnostic PCR fragments are: *Sin3a*$^{+}$, 267 base pairs (bp). *Sin3a*^{F}, 350 bp. *Sin3a*$^{-}$, 625 bp. *Sin3b*$^{+}$, 250 bp. *Sin3b*^{F}, 330 bp. *Sin3b*$^{-}$, 175 bp. *Pdx1*^{Cre}: 470 bp. *Ai9*, 400 bp. *Neurog3-Cre*, ~200 bp. The day of vaginal plug appearance was counted as E0.5, the day of birth as P1.

Physiological phenotyping

Ad lib-fed blood glucose levels were measured via tail snipping. 16-hour fasting and 1-hour re- fed plasma insulin levels were measured with plasma collection via retro-orbital blood samples using a mouse insulin ELISA kit (Alpco, Salem, NH). Plasma

glucagon measurement was performed by VUMC Hormone Assay and Analytical Services Core using a glucagon ELISA kit (Mercodia, Uppsala, Sweden).

Tissue preparation and immunofluorescence (IF) detection

Tissue preparation and IF analysis followed established protocols (Pan et al., 2015; Wang et al., 2007). Pancreata were fixed in 4% (w/v) paraformaldehyde at 4°C for 4 hours, washed in cold phosphate-buffered saline (PBS, pH = ~7.2) twice, equilibrated in 30% sucrose in PBS at 4°C overnight, frozen in OCT (Sakura Finetek USA, Inc., Torrance, CA), and sectioned at 10 μm thickness. Slides were washed 3 x 5 minutes in PBS, permeabilized in PBS with 0.2% (v/v) Triton X-100 for 30 minutes, blocked with blocking buffer [(PBS + 0.1% Tween-20, PBST) + 0.1% BSA + 5% donkey serum; all v/v] for 30 minutes at room temperature, and incubated with primary antibodies diluted in blocking buffer at 4°C overnight. After washing in PBST for 3 x 5 minutes, sections were incubated with secondary antibodies in blocking buffer, washed in PBST, and co-stained with DAPI. Primary antibodies (1:500 to 1:2000 dilution) and secondary antibodies (1:500 dilution) are listed in **Table 2**.

Confocal microscopy and image analysis

Images were taken using Nikon spinning-disk confocal microscope (Nikon Instruments Inc., Melville, NY) and quantified with ImageJ 1.51j14 (NIH) under double-blind settings using 16-bit images. The presented results are the average intensity per pixel within the Region-of-Interest (ROI). The ROI was manually selected to encircle the plasma membrane (for Glut2), cytoplasm (Ucn3), or nuclei (Mafa) of β-cells. The relative

levels of control cells were then normalized to 1.0 for comparison. For each sample, 12-15 islet sections were examined.

Islet-cell mass measurement

One of every twelve sections (~120 μm apart) of entire P1 and P7 pancreata or one of every eighteen sections (~180 μm apart) of P14 pancreata were scored. The sections were co-stained with DAPI and hormone antibodies to identify all cells and islet cells, respectively. Tissue sections were scanned/analyzed using Aperio ImageScope (Leica Biosystems Inc.). The islet-cell mass was calculated as: islet-cell mass (mg) = (hormone+ area / DAPI-positive area) X pancreas weight (mg).

Transmission electron microscopy and image analysis

Transmission electron microscopy (TEM) followed published protocols (Zhao et al., 2010). Isolated P7 islets were fixed overnight in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at room temperature, washed for one hour in 1% osmium tetroxide in 0.1 M cacodylate buffer, and then washed, dehydrated, embedded, and thin-sectioned for TEM imaging. The TEM images were analyzed manually using the double-blind setting in ImageJ 1.51j14 (NIH).

Glucose/KCl-stimulated insulin secretion assay

Islet isolation and secretion assays were as described in (Huang et al., 2018). Pancreata were digested using 0.5 mg/ml Type IV collagenase (MilliporeSigma) in Hanks' balanced salt solution (Thermo Fisher Scientific) and islets were handpicked.

Table 1: DNA oligos used in genotyping

Gene	Primer Sequences (from 5' to 3')	Diagnostic size
Sin3a	GTGTCCTCAGGGAAGACGTTGA	Wildtype allele: 267 bp Floxed allele: 350 bp Null allele: 625 bp
	ACGCCCTGTCCTATCTTGACCAG	
	CAGGACCACCAAAGTTCACCAG	
Sin3b	TACAACGGCTTCCTGGAGATCA	Wildtype allele: 250 bp Floxed allele: 330 bp Null allele: 175 bp
	ACACCCAACACTCCCTGTTCAG	
	CCCTCGAGGTCGACCCCGGGAAGC	
	CCAACACTCCCTGTTCAGG CTC	
Pdx1-Cre	TTGAAACAAGTGCAGGTGTTCG	450 bp
	TTCCGGTTATTCAACTTGCACC	
Ai9	GCGGCCACTACCTGGTGGAGT	400 bp
	CCACGCCACGTTGCCTGACAA	
Neurog3-Cre	CGAACCTCATCACTCGTTGCATC	~200 bp
	TGCAGTGACCTCTAAGTCAGAGGCT	

Table 2: Primary and secondary antibodies used in the study

Antibody	Source	Identifier
Rabbit anti-Sin3a	LifeSpan BioSciences	LS-C331555
Rabbit anti-Sin3b	Abcam	ab101841
Rabbit anti-Mafa	Novus Biologicals	NBP1-00121
Rabbit anti-Mafb	Bethyl Laboratories	IHC-00351
Rabbit anti-Nkx6.1	Gift from Dr. Palle Serup, University of Copenhagen, Denmark	N/A
Rabbit anti-Myt1	This lab	N/A
Goat anti-Pdx1	Gift from Dr. Chris Wright, Vanderbilt University	N/A
Goat anti-Neurog3	This lab	N/A
Rabbit anti-Glut2	Alpha Diagnostic	GT21-A
Rabbit anti-Ucn3	Abcam	ab79121
Guinea pig anti-insulin	Dako	A0564
Rabbit anti-glucagon	Abcam	ab92517
Mouse anti-glucagon	MilliporeSigma	MABN238
Goat anti-somatostatin	Santa Cruz Biotechnology	sc-7819
Rabbit anti-cleaved Caspase-3	Cell Signaling Technology	9661
Rabbit anti-Ki67	Abcam	ab15580
Alexa Fluor® 488 AffiniPure Donkey Anti-Guinea Pig IgG (H+L)	Jackson Immunoresearch	706-545-148
Alexa Fluor® 488 AffiniPure Donkey Anti-Mouse IgG (H+L)	Jackson Immunoresearch	715-545-150
Cy™3 AffiniPure Donkey Anti-Rabbit IgG (H+L)	Jackson Immunoresearch	711-165-152
Cy™3 AffiniPure Donkey Anti-Goat IgG (H+L)	Jackson Immunoresearch	705-165-147
Alexa Fluor® 647 AffiniPure Donkey Anti-Guinea Pig IgG (H+L)	Jackson Immunoresearch	706-605-148
Alexa Fluor® 647 AffiniPure Donkey Anti-Rabbit IgG (H+L)	Jackson Immunoresearch	711-605-152
Alexa Fluor® 647 AffiniPure Donkey Anti-Goat IgG (H+L)	Jackson Immunoresearch	705-605-147

Islets were recovered overnight in RPMI 1640 medium (10% v/v fetal bovine serum (FBS) and 11.0 mM glucose). Then the islets were incubated in Krebs-Ringer Bicarbonate (KRB) (111 mM NaCl, 4.8 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 2.3 mM CaCl2, 25 mM NaHCO3, 10 mM HEPES, 0.2% BSA; pH=7.2) containing 2.8 mM glucose for 1 hour. For secretion assay, islets were incubated in KRB Buffer with 2.8 mM glucose for 45 minutes, then in KRB with 20 mM high glucose for 45 minutes, then in KRB with 25 mM KCl for 30 minutes. Insulin was measured using a mouse insulin ELISA kit (Alpco).

Insulin tolerance test (ITT)

Mice were fasted for four hours starting in the morning. Then insulin was intraperitoneally injected at 1.0 unit/kg body weight. Blood glucose levels were measured from tail vein blood collection.

Intraperitoneal glucose tolerance tests (IPGTT)

Mice were fasted for six hours starting in the morning. Then glucose was intraperitoneally injected at 2.0 g/kg body weight. Blood glucose levels were measured from tail vein blood collection at 0, 30, 60, 120 min after injection.

Ca^{2+} imaging

Ca^{2+} imaging and analysis followed published protocols (Dickerson et al., 2018; Jacobson et al., 2010). In brief, handpicked P5 islets were attached to poly-lysine coated dishes in RPMI 1640 medium (supplemented with 10% FBS and 11.0 mM

glucose) by overnight incubation. Islets were loaded with 2.0 mM Fura-2 AM (Invitrogen, Carlsbad, CA) for 20 min in RPMI 1640/10% FBS/2.8 mM glucose) followed by 20 min washing in RPMI 1640/10% FBS/2.8 mM glucose). Ca^{2+} imaging was performed in REC solution (119 mM NaCl, 2.5 mM $CaCl_2$, 4.7 mM KCl, 10 mM HEPES, 1.2 mM $MgSO_4$ and 1.2 mM KH_2PO_4). The islets were perfused at 2.0 mL/min at 37 °C with 2.8 mM glucose (0-195 second (s), 20 mM glucose (196-915 s), 2.8 mM glucose (916-1800 s), and 25 mM KCl with 2.8 mM glucose (1801-2090 s). Images were taken every 5 seconds with a Nikon Eclipse Ti2 microscope and Photometrics Prime 95B 25mm sCMOS Camera. The ratios of Fura-2 AM fluorescence excited at 340 and 380 nm (F340/F380) were measured.

Quantitative reverse-transcription PCR (qRT-PCR)

Total RNA was extracted from isolated P4 and P7 islets using TRIzol (Life Technologies, Carlsbad, CA) and purified using RNA Clean & Concentrator (Zymo Research). Total RNA was used for cDNA preparation using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). qPCR was performed using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA) on a Bio-Rad CFX96 Thermocycler. Transcript abundance was normalized to *Gapdh*. Primers used are listed in **Table 3**.

Single-cell RNA sequencing (scRNA-seq)

Two batches of P4 *Sin3a*$^{F/+}$ and *Sin3a*$^{\Delta endo}$ islets (two to four mice used for each batch and each genotype) were isolated and dissociated into single cells using Trypsin-

EDTA. After live-cell selection, the inDrop platform (1CellBio) was used to encapsulate and barcode single cells, used for CEL-Seq-based library preparation and sequencing (Nextseq 500, Illumina). Reads were assigned to individual cells. After adaptor sequences were trimmed and cell-doublet correction, batch alignment assigned the reads to specific gene loci, or unique molecular identifier (UMI). We next identified β-cells based on *Insulin 1* and *Insulin 2* expression, which were then compared with Mouse Cell Atlas to verify their cell identity (Consortium et al., 2018). Afterwards, the number of reads of each UMI within each sample were combined and analyzed as one bulk sample using Deseq2 (Love et al., 2014). Log(FC) was calculated as Log(2) [(level in mutant) - (level in control)]/(level in control). Statistical analyses used Wilcoxon Rank Sum test and the adjusted *p*-values were derived using Bonferroni post-test correction based on the total number of genes in the data set. Gene-set enrichment assays (GSEA) followed that in (Subramanian et al., 2005). Gene Ontology (GO) based DAVID analysis of potential Sin3a-binding genes followed that in (Huang et al., 2009).

Chromatin Immunoprecipitation (ChIP) assays

A Magna ChIPTM-HiSens Chromatin Immunoprecipitation kit (Millipore Sigma) was used. MIN6 cells (passage # 38-43) were fixed for 20 minutes using 2.0 mM disuccinimidyl glutarate followed by an additional 12 minutes together with 1% formaldehyde (Nowak et al., 2005; Zeng et al., 2006). Sheared chromatin (200-500 bp) was prepared using a Pico Bioruptor® (Diagenode) [~30 cycles of (30s sonication + 30s rest)]. For each immunoprecipitation, 2.0 µg of chromatin and 2.0 µg antibodies were used. Normal rabbit IgG was from Cell Signaling Technology (Cat# 2729). Rabbit anti-

Table 3: DNA oligos used in qRT-PCR

	Gene	Primer Sequences (from 5' to 3')
qRT-PCR	*Pdx1*	CCAGGTTGTCTAAATTGG
		GTTGGGTATAGCCGGAGAGA
	Glut2	AAGAACACGTAAGGCCCAAG
		AGCAACTGGGTCTGCAATTT
	Mafa	ACCACGTGCGCTTGGAGGAG
		ACCTCCTCCTTGCTGAA
	Ucn3	AAGCCTCTCCCACAAGTTCTA
		GAGGTG CGTTTGGTTGTCATC
	Casp3	GAGTCTGTAGCATGCTGACTTT
		CCCTTTCGTTTCTAGCACAGTAT
	Rab11a	AAGCACTTACCCTGACGTTT
		TTTGCAGGTATCCCACCATC
	Cltb	GAGCAGCAAGCAGTGTAAAG
		GACAGTGGTGTTTGCTTCAG
	Calr	TCCCTCCCTTTCTCCATCTT
		CCTAGGGCTTCTCCTCTACAC
	Aldoa	GCAGCCAGTGAATCTCTCTT
		GTTGATGGAGCAGCCTTAGT
	Esyt1	GCCTGTTACCTCTTCCTTCTTC
		AGCGAATGCCACTGCTAAT
	Kcnh2	AGCATCTCCTCCTGCAATAC
		GGGCAGTTAGACCAGCTAAT
	Bnip3	CACTGTCCCACACTTAACTCA
		CTTGCTCCAAATGAAAGGGATATAG
	Ing1	TGCCAGCAAATACATGTCAAAG
		CTTAGCTCACAGGCCATACAA
	Gapdh	AACTTTGGCATTGTGGAAGG
		GGATGCAGGGATGATGTTCT
	Sin3a	CTCCAGGAGTGATTAGCCGAG
		GGCAAGAAGGTGTTAAAGCCC

Sin3a (LS-C331555) was from LifeSpan-Biosciences, Inc. The primers used for ChIP-PCR are listed in **Table 4**.

Statistics

BM SPSS Statistics 25 (IBM, Armonk, NY) was used for the statistical analysis. Data are shown as mean ± standard deviation. Student's *t*-test was used to compare the means. One-way repeated measurement ANOVA was used to compare IPGTT and ITT data. $p < 0.05$ was considered significant.

Data availability

The datasets generated and/or analyzed during the current study were deposited in GEO under GSE146474, freely available for download.

Results

Pan-pancreatic Sin3a/Sin3b expression during embryogenesis becomes enriched in adult endocrine cells

I first used IF to examine the normal expression pattern of Sin3a and Sin3b at both embryonic and postnatal stages in mouse pancreas. At E10.5, E15.5, and P1, both Sin3a and Sin3b proteins were detected in most pancreatic cells (**Figure 10A-C, 11A-C**), including Pdx1+ pancreatic progenitors (**Figure 10A, 11A**), Neurog3+ endocrine

Table 4: DNA oligos used in ChIP-PCR

	Gene	Regulatory region (RR) #:	Sequence (5' to 3')	Location	Near-by sites (within 100 bp)
ChIP-PCR	*Insulin*	RR1	GGCCATCTGGTCCCTTATTA	-113	3 P53 (sites)
			ACACTTGCCTGGTGCTAGGT		
	Glucagon	RR1	AAGGCTAAACAGCCTGGAGA	-47	3 P53 sites
			CCCTTGGGAACTTTGAGTGT		
	Slc2a2	RR1	CAGCTGTCACTCCTGATCG	-1856	2 Myc, 5 P53
			AGATGCCCTGACAACTGTTT		
		RR2	CTCACACAGATAACCGATGCT	-340	3 P53
			GAGGGCACAGAGCAGTTT		
	Mafa	RR1	CAATCTGCTTGCCACTTGTC	-812	2 Myc, 5 P53
			CACAAGGAATCTCAGACCCA		
		RR2	GACATGTCACTGGCTGCTAC	-4010	4 P53
			GTGGGTGCACACTCCATAAA		
	Ucn3	RR1	GTATGAGTAGCTGGCTAACACTG	-139	
			GGTGGTTTGGAGTTCATCTGT		
		RR2	AGCACAGCAGGAAACCTT	-2856	
			TGAGCAACAAGAGGATGATTAGT		
	Kcnh2	RR1	CCAGGACTGTAAGTTGTGAAGAA	-5404	
			ACTTCACTTTCCTTACCTGCAA		
		RR2	TTCCACTTGGAACAAGGATACA	-4551	2 Myc, 4 P53
			AACAGCTTCCTAAAGCCAGAT		
	Esyt1	RR1	CAGGGTTTCTTTGGAACTTTGG	-4770	
			GGCAGTTGGATCTGTGAATTTG		
		RR2	GCGGTGCTGACTTCCTT	162	2 Myc, 13 P53
			GCACGAAACCTACGCTAAGA		
	Bnip3	RR1	CTCTTCCCTACCCGCCTA	-245	5 P53
			TTAAGGTCTAATGCAGAAGTGGA		
		RR2	GCCTTTCACATCATGCTTCAAT	-3834	
			AATGTAGATGGCTTGGCTCTC		
	Casp3	RR1	TCCCTAGACGCCCATCTT	-222	2 Myc, 2 P53
			TGCGCCACTTGAGGTAAAT		

		RR2	GTGCATGAGTGTGCATGTG	-2730	
			AAACAGAGGGATTGAGGAAGATAC		
	Ing1	RR1	CAGAGCGCCATCACCAG	-1271	3 P53
			TGAGTCCCAGGTCCTCAAT		
		RR2	ACTAGGGCTGAAAGCAGTTG	-1693	
			TTTCTGCAGGAGGAAGTTCG		
	Cltb	RR1	GCAGGTGACCCTGATTCAA	-3474	3 Myc
			GTTTGGGTGTGTGTGTGTTC		
		RR2	CCTCCTACGCAGGAAGAGTA	-210	3 P53
			AAGTTTCTCCCAGAGCTGATTT		
	Rab11a	RR1	CCGGAAGTGACGCATACA	-146	6 P53
			CGAGCGAGGAGCTTCAG		
		RR2	TGGCCTTTCTTGTGCTATGT	-1911	3 Myc
			CTACTTGGGAGGAAGAGGTAAATG		
	Aldoa	RR1	CCCACCTCTCTCTTCTCCTTTA	-1839	5 P53
			CACAGGCACAGTCACACA		
		RR2	GGAGGAGGGATCGTGTTCTA	-186	4 P53
			AATTGAAGGTGGGCACTGT		
	Hsp90b1	RR1	GTTACATCCTGAACTTGCACTTAT	-3383	3 P53
			GGTGCTCCTGAGTGTTAGAA		
		RR2	CCCATGCGTCTCCATTG	-137	9 P53
			GGGACGGGAAACACGAA		
	Hspe1	RR1	ACCCTCCCACAGAGTCC	-643	5 Myc, 7 P53
			GCTAGGGTGGAAGCAACTT		
		RR2	CACCAACAAGTATTGGCAACTT	-2829	1 Myc
			ACCTGCATCTTTAAGAACTGTTTAC		
	Albumin-2	RR1	GAACCAATGAAATGCGAGGTAAG	-59	
			GTGCAGAAAGACTCGCTCTAATA		
	Gapdh	RR1	CTTGGTGCGTGCACATTTCAA	-50	
			GCTACGTGCACCCGTAAAGC		
	Kcnh2	RR3	CTTGAGCATCCTCAGACCTCTTT	-988	8 P53
			AACCCTGGCCTTGCCTT		
		RR4	TGAGGCCTCTCAGGCTAA	-1673	2 P53
			CAGACCCTTTCCACCAACA		
	Ucn3	RR3	TCTTCAGAAGTTCTCCTCCCT	-1433	

			GAGAGTAGGTGGTTAGAAACTTGA		
	Calr	RR1	CCCATAGTGCGACCAATAGAAA	-143	8 P53
			CCAGTTGGCTCAGCAAGTTA		
		RR2	TGACCAGAGAGGATGGGAAAG	-379	
			GGCTCGCTAGGAGTCGTTTA		
		RR3	CTTACCAGCCTTGTCCTTAGTT	-5889	
			TACTGAGCATTGTGTGGAGTG		
		RR4	ATGCATGTGTGCATGTGTTAAT	-6345	5 Myc
			TCGTTAGCCAGGGAAGAGATA		
	Esyt1	RR3	GGACATGTTAGCCAAGTGATGA	-5700	3 Myc
			CATGTGGCCACTGACTCTTT		
		RR4	GGATTAAAGGCATGTGCTCTATTG	-4872	
			CTTCCCTCCCAACTATCAAGAC		
	Bnip3	RR3	TAACTCAGCTGGCCTGGAA	-2215	2 Myc 3 P53
			GAGGCAGGTGGATCTCTGT		
		RR4	GGTTTCATGTAGTCCAGGCT	-2621	
			GATGTAGAGCCAGAAGGATCAG		
	Casp3	RR3	TCGCCAAATGGAAGAGAGTG	-1410	7 P53
			TTCCAGGAGCTGGAGTAAGA		
		RR4	TTTATGGTTGCCCAGGCT	-3018	2 Myc 1 P53
			TGTAGTGGCACATGCCTAAA		
		RR5	GTCTATTAAGGGCTGTGGATGG	-3701	1 Myc, 1 P53
			TGAGTGACTGGTGACTACAGA		
	Ing1	RR3	GCCTAGGCAGGAAGAGG	-1086	8 P53
			GCCGGATTTAGGGCTTTCT		
	Arl6	RR1	TAATCGGCCAGCTTCCTTAAC	-857	
			CTTGTTAGCATAAATGTGGGATGTT		
		RR2	TCAAGTATTGCTCACTGAATAATGG	-4969	
			TCAAAGGGAACACTCTAGACAAA		
	Cltb	RR3	CAACCCTGCTGTGAATATTTGTATAG	-5412	
			AGATGACCCAGCAACTTGAAT		
		RR4	GGGCTCATCACACAGATAGG	-316	2 P53
			ACAGCAGAGCCTTGTTCTC		
	Ergic3	RR1	CGGAGCACTGCCCAATC	-89	8 P53
			CTCACCTGCCTCCTCAAAG		

		RR2	ATGCCTTTAATTACAGCACTTGG	-3063	2 Myc
			CTGGTTGTCCTGGAACTTGT		
		RR3	GAAGCAGAACCAGTTACATTGC	-4224	2 Myc, 2 P53
			GCCTGTACCTGACACATGAA		
		RR4	GCAAAGTTAACACCCACTCTCT	-717	
			GCGTGCCCTTGACTACAC		
	Rab11a	RR3	TTGAACTGCTGGTCCTCTTG	-2899	3 Myc
			CAGGACAAAGGGATCGAGTG		
		RR4	CACCACATAGAACAGGGTACAG	-4730	3 Myc, 2 P53
			CTGCACTTGGGATGTAGTCAA		
	AldoA	RR3	TGTGTGCATCTGATCCTGTC	-5052	1 P53
			ACCCTTGGTCACTCTACATTTC		
		RR4	GCTACTGCACTGGAGCTATG	-1619	2 P53
			GAACTCAAGGTTCCCAGGTAAT		
		RR5	CCTTAGTCCTTTCGCCTACC	75	2 Myc, 2 P53
			CCGGCCCAAGATCCTAAC		
	Idh3a	RR1	TGGGTTTGATGCCCAGAAT	-5014	3 Myc
			CAGATCACACAGGTAGCACAG		
		RR2	CAAGCTCTCCAAAGAGAGATAGAG	-3873	
			TGTCATAGCAACTTCTTTCAAATCC		
		RR3	ATGTAAGAATCAAGTTAGGCGAGGTG	-566	
			TTTCTTGCCTCTATCTCCCAGGTA		
		RR4	GATCGAGAGACCCGAGCTA	-126	7 P53
			CATGCGCATAGCCCTCTT		
	Hsp90b1	RR3	CACAGATGACAGGTGGACAT	-6098	2 Myc
			AGAACTCACATGATGGAAGGAG		
		RR4	TCACCACATTATGTAAACACCAAACC	-1544	
			CCCTTTGACAACTTCAAGAATTTAGACT		
	Hspe1	RR3	CCGCAATGAGCCCGAGT	-292	5 P53
			GCGCCGCATGCAGATTG		
		RR4	CATGGTGGTAGAATGACCAATAGA	-1584	1 Myc
			CAACTTAGTGGTCTAGAGGTGTTATT		

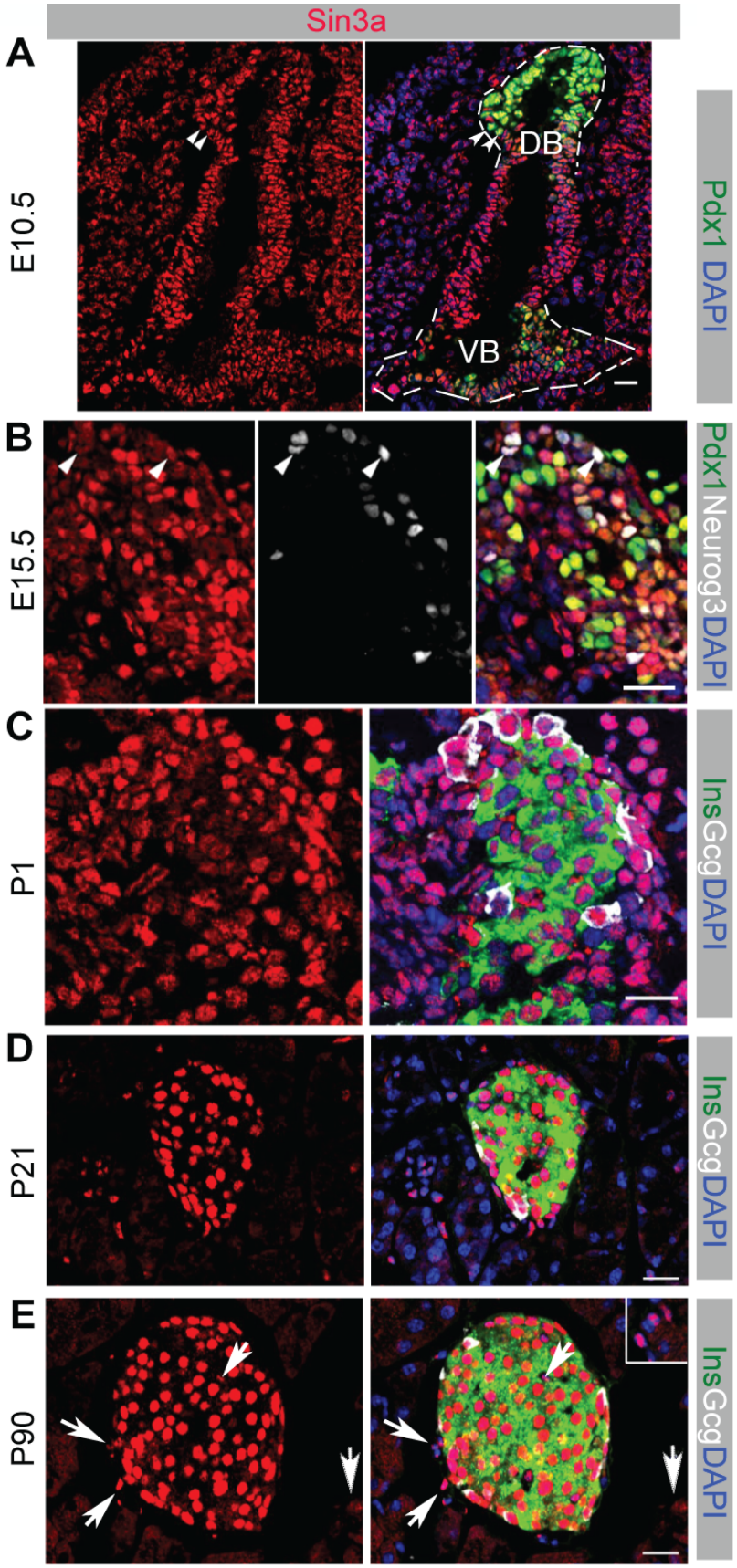

Sin3a
A
E10.5
DB
VB
Pdx1 DAPI
B
E15.5
Pdx1Neurog3DAPI
C
P1
InsGcgDAPI
D
P21
InsGcgDAPI
E
P90
InsGcgDAPI

Figure 10: Sin3a is produced in pancreatic progenitors and islet cells.
DAPI was used to mark nuclei in some panels. (A) IF of Sin3a, co-stained with Pdx1 at E10.5. Both dorsal pancreatic bud (DB) and ventral pancreatic bud (VB) were shown. Arrowheads, Pdx1+ cells that also co-express Sin3a. (B) Co-staining of Sin3a, Neurog3, and Pdx1 at E15.5. Arrowheads, Neurog3+ cells that also express Sin3a. (C-E) IF of Sin3a, insulin, glucagon in P1 (C), P21 (D), and P90 (E) pancreata. Arrows in E point to several non-islet cells that express Sin3a. Scale bars = 20 μm.

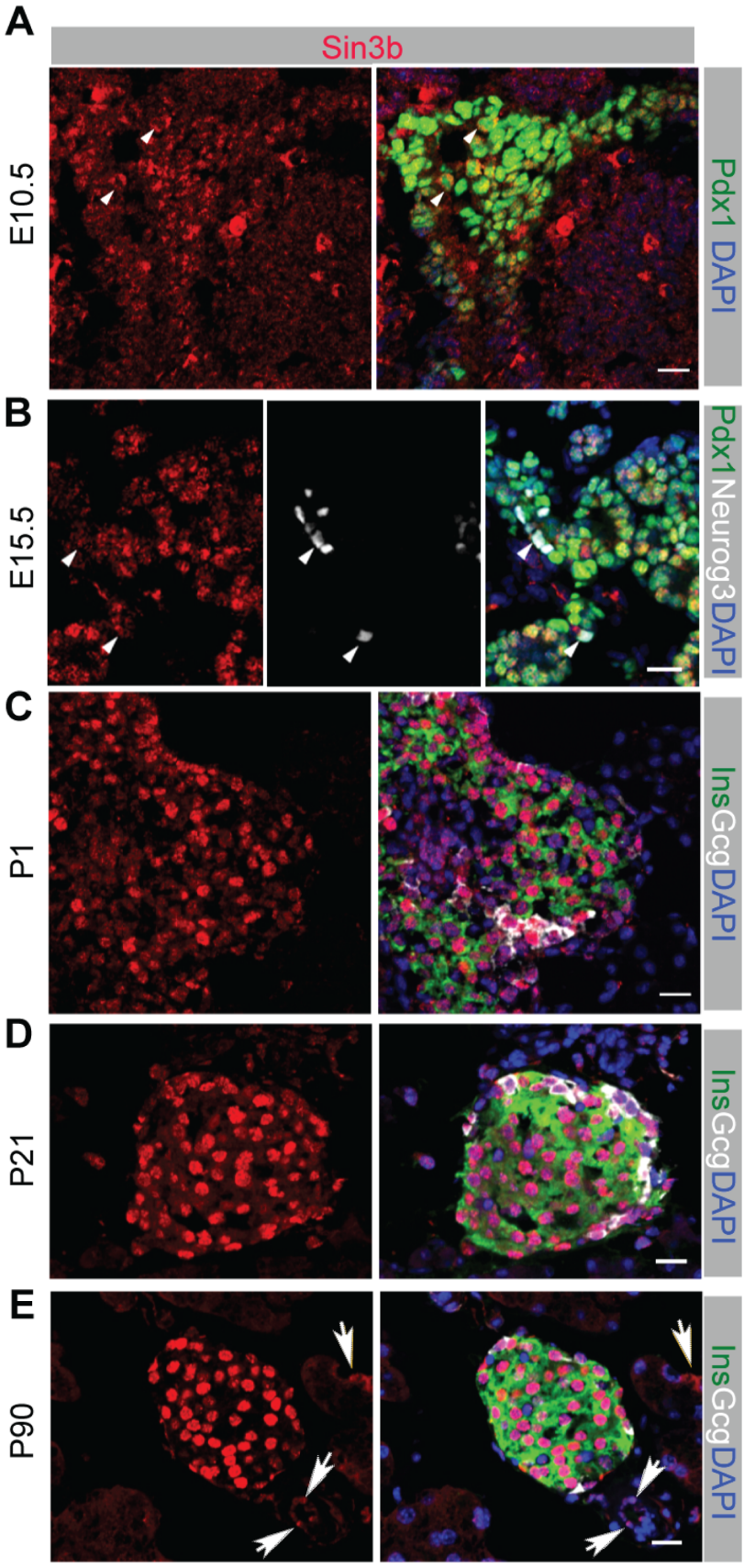

A
Sin3b
E10.5
Pdx1 DAPI
B
E15.5
Pdx1Neurog3DAPI
C
P1
InsGcgDAPI
D
P21
InsGcgDAPI
E
P90
InsGcgDAPI

Figure 11: Sin3b is produced in pancreatic progenitors and islet cells.
DAPI was used to mark nuclei in some panels. (A) IF of Sin3b, co-stained with Pdx1 at E10.5. Only DB was shown. Arrowheads, Pdx1+ cells that also co-express Sin3b. (B) Co-staining of Sin3b, Neurog3, and Pdx1 at E15.5. Arrowheads, Neurog3+ cells that also express Sin3b. (C-E) IF of Sin3b, insulin, glucagon in P1 (C), P21 (D), and P90 (E) pancreata. Arrows in E point to several non-islet cells that express Sin3b. Scale bars = 20 μm.

progenitors (**Figure 10B, 11B**), and hormone+ islet cells (**Figure 10C, 11C**). After P1, their expression increased further and became highly enriched in islet cells (**Figure 10D-E, 11D-E**). Yet both Sin3a and Sin3b remained detectable in some non-islet cells in 3-month-old pancreas (**Figure 10E, 11E**). These data suggest that Sin3a and Sin3b may be required for pancreas development and the function of postnatal endocrine pancreas.

Neurog3-Cre has no detectable toxicity

Sin3a$^{F/-}$*; Neurog3-Cre* mice (termed *Sin3a*$^{\Delta endo}$) were derived to inactivate *Sin3a* in the endocrine progenitors. However, because *Neurog3* is also expressed in gut endocrine cells and neuronal cells, we expect that *Sin3a* will also be inactivated in small intestinal enteroendocrine cells and some neuronal cells (Magnuson and Osipovich, 2013; Schonhoff et al., 2004; Song et al., 2010). We therefore used different approaches to examine the islet specific effects. First, to examine the deletion efficiency of *Sin3a* in the islet cells, IF and qRT-PCR assays were used. The results supported the efficient deletion of *Sin3a* in *Sin3a*$^{\Delta endo}$ islets (**Figure 12**). Second, to examine whether there are detrimental effects of *Neurog-Cre* or haploinsufficiency of *Sin3a*, I examined the several physiological phenotypes of both male and female *Sin3a*$^{F/-}$, *Sin3a*$^{F/+}$, and *Sin3a*$^{F/+}$*; Neurog3-Cre* mice. No significant differences were observed in body weights (**Figure 13A, C**), ad lib-fed blood glucose (**Figure 13B, D**), islet structure (**Figure 13E**), or expression of several diagnostic endocrine markers at postnatal stages (e.g., Mafa, Pdx1, Nkx6.1, and hormones, **Figure 13F**), except that *Sin3a*$^{F/-}$ female mice had slightly

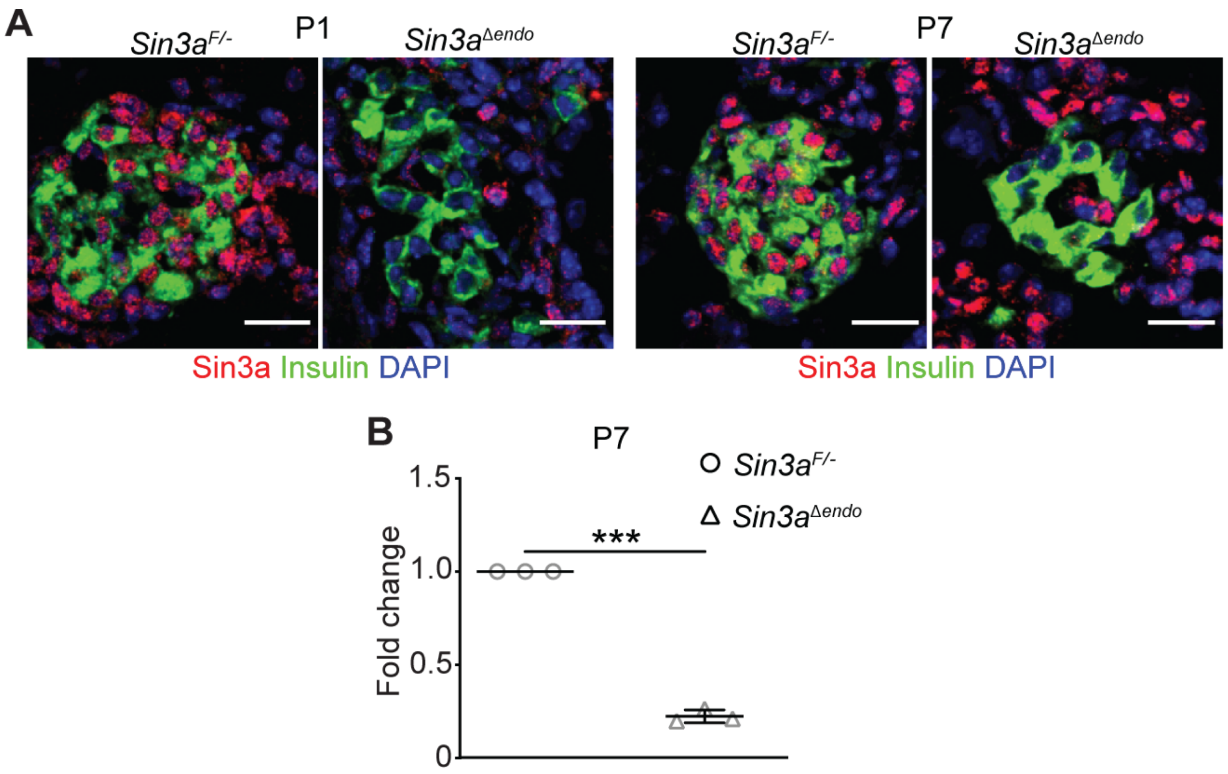


Figure 12: High *Sin3a* deletion efficiency in islet cells mediated by *Ngn3-Cre*. (A) IF detection of Sin3a in P1 and P7 control and *Sin3a*$^{\Delta endo}$ pancreata, highlighting insulin+ β-cells. DAPI counterstaining marked nuclei. Scale bars = 20 μm. (B) qRT-PCR detection of *Sin3a* transcripts in P7 control and *Sin3a*$^{\Delta endo}$ islets, amplifying the floxed exon that should be deleted. Note that the islet samples include mesenchymal and endothelial cells as well, which also express *Sin3a*. ***p<0.001.

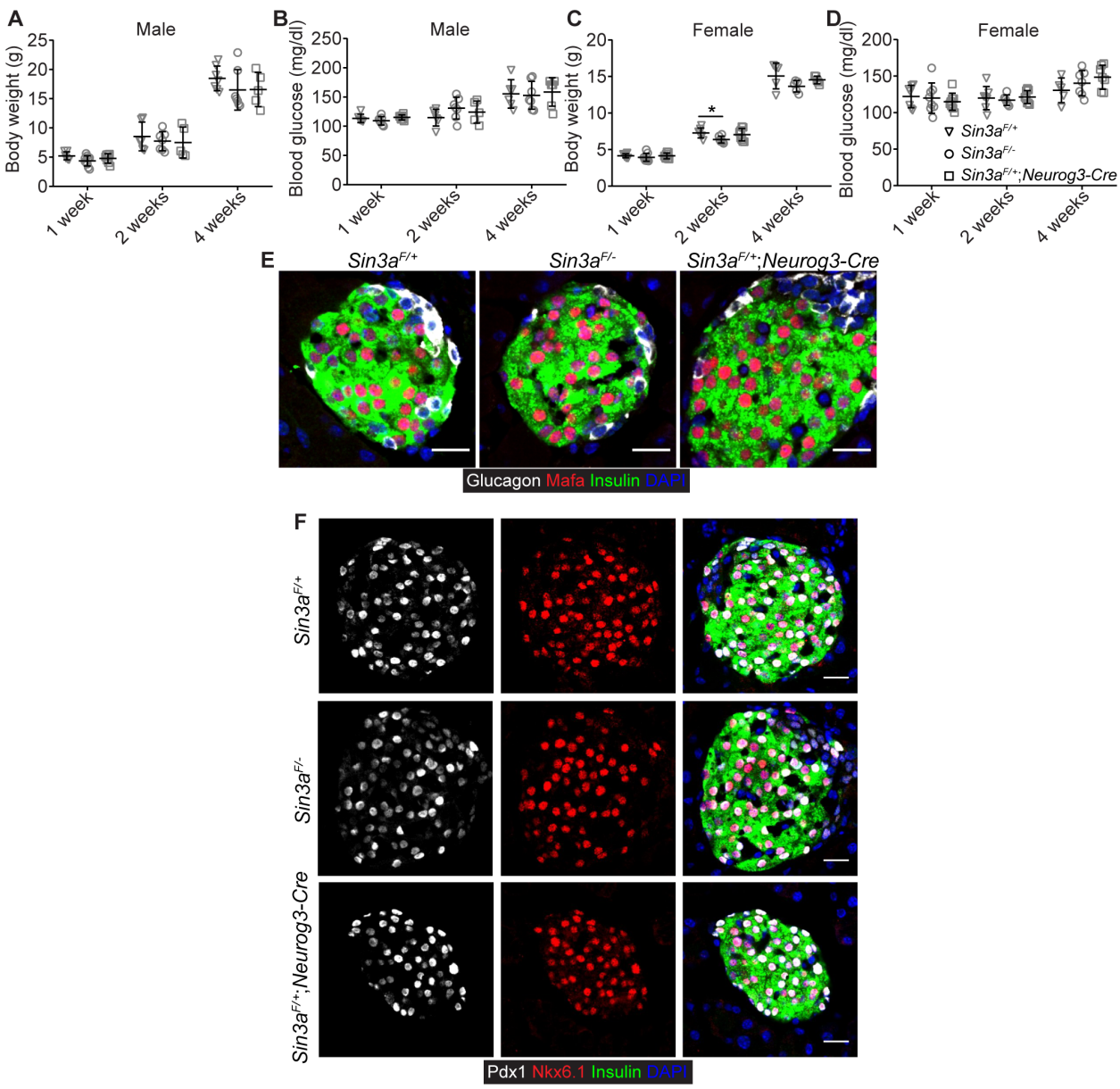


Figure 13: *Neurog3-Cre* did not have detectable toxicity.

(A-D) The body weights and *ad lib*-fed blood glucose levels of male and female mice as indicated (n = 5-10 mice). The annotation of genotypes is the same as described in panel D. (E) IF staining of insulin, glucagon, Mafa, and DAPI in 5-week pancreata. (F) IF staining of insulin, Pdx1, Nkx6.1, and DAPI using 5-week pancreata. Scale bars = 20 μm. *p<0.05.

lower body weights compared to *Sin3a*$^{F/+}$ females at two weeks of age (**Figure 13C**). The data indicate that there is no detrimental effect of *Neurog-Cre* or negative effects of *Sin3a* haploinsufficiency on the function of the endocrine pancreas. Thus, *Sin3a*$^{F/-}$ mice were used as controls for most studies unless noted.

Loss of *Sin3a* causes postnatal diabetes

Up to 2 weeks of age, both male and female *Sin3a*$^{\Delta endo}$ mice had indistinguishable body weights from control littermates but showed significant growth retardation afterward (**Figure 14A**). There was normal glycemia at 1 week of age but significantly higher blood glucose levels afterward in *Sin3a*$^{\Delta endo}$ mice (**Figure 14B**). The temporal phenotypic development is similar in both male and female mice (**Figure 14A, B**). Therefore, both sexes were used interchangeably and the findings were presented together for the rest of the studies unless noted. There were significantly higher fasting glucose levels in *Sin3a*$^{\Delta endo}$ mice on P28 but not on P14 (**Figure 15A**). There was a trend of compromised glucose tolerance in P14 *Sin3a*$^{\Delta endo}$ mice (**Figure 15B**), which had normal insulin sensitivity on P14 (**Figure 15C, E**), but increased sensitivity on P21 (**Figure 15D, F**). There was reduced plasma insulin in P28 (**Figure 16A**) and P7 *Sin3a*$^{\Delta endo}$ mice (**Figure 16B**). The plasma glucagon levels in P7 *Sin3a*$^{\Delta endo}$ mice were reduced (**Figure 16C**). These combined findings suggest that reduced circulating insulin but not increased glucagon or compromised insulin response causes the hyperglycemic phenotype of *Sin3a*$^{\Delta endo}$ mice. Thus, our following studies focused on β-cells.

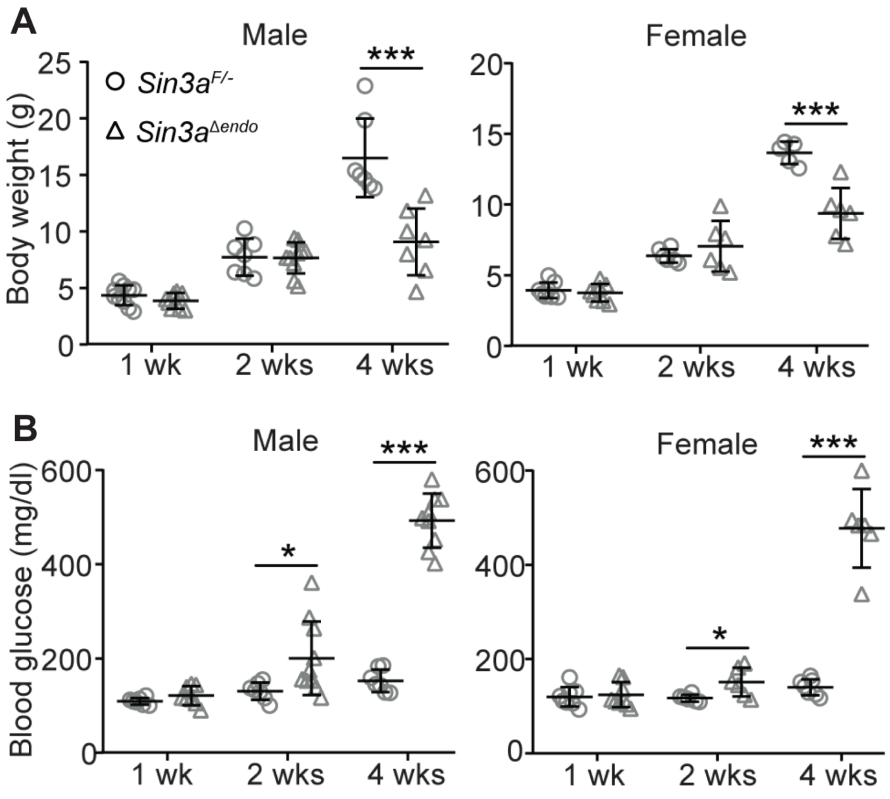


Figure 14: Loss of *Sin3a* causes late-onset diabetes.

(A) Up to 2 weeks of age, both male and female *Sin3a*$^{\Delta endo}$ mice had indistinguishable body weights from control littermates but showed significant growth retardation afterward. (B) There was normal glycemia at 1 week of age but significantly higher blood glucose levels afterward in *Sin3a*$^{\Delta endo}$ mice. The temporal phenotypic development is similar in both male and female mice (A, B)**.** A, n = 7-10. B, n = 7-10. **p*<0.05, ****p*<0.001.

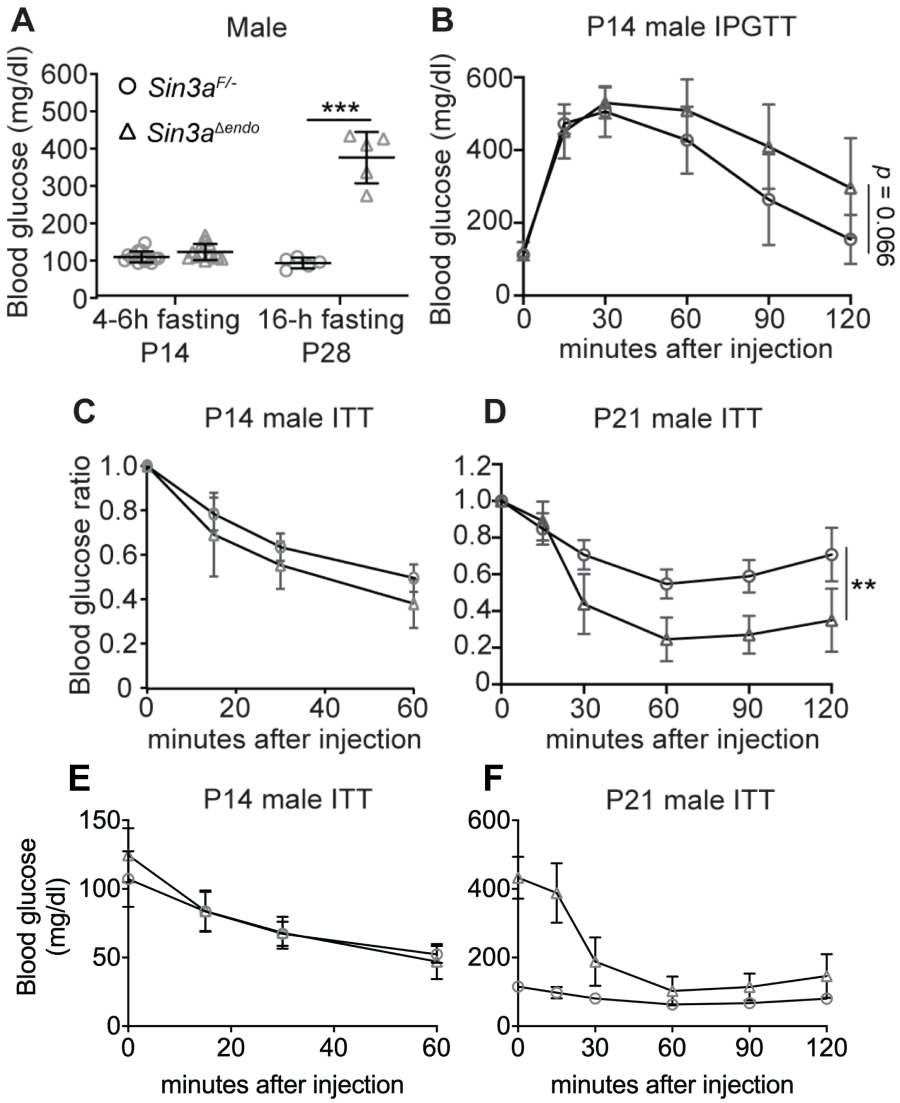


Figure 15: Phenotyping of control and *Sin3a*$^{\Delta endo}$ mice, including fasting blood glucose, glucose tolerance, and insulin tolerance.

(A) There were significantly higher fasting glucose levels in *Sin3a*$^{\Delta endo}$ mice on P28 but not on P14. (B-F) Compromised glucose tolerance was obvious in P14 *Sin3a*$^{\Delta endo}$ mice (B), which had normal insulin sensitivity on P14 (C, E), but increased sensitivity on P21 (D, F). A, n = 5-14. B, n = 7-8. C, D, n = 5-6. **p<0.01, ***p<0.001.

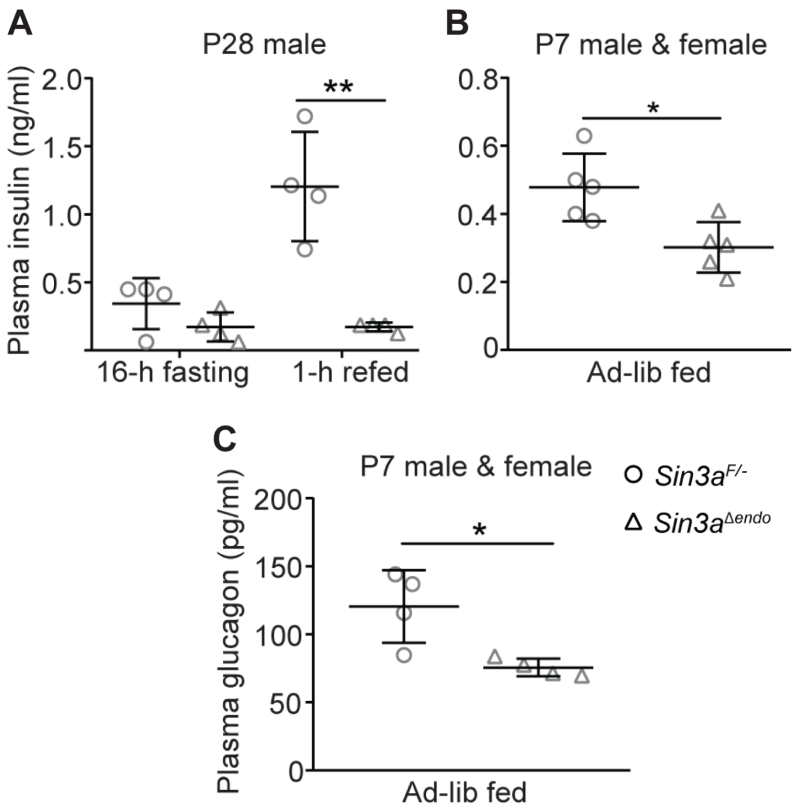


Figure 16: Phenotyping of control and *Sin3a*$^{\Delta endo}$ mice, including plasma insulin or glucagon.

(A, B) There was reduced plasma insulin in P28 (A) and P7 *Sin3a*$^{\Delta endo}$ mice (B). (C) The plasma glucagon levels in P7 *Sin3a*$^{\Delta endo}$ mice were reduced (C). A, B, n = 4-5. C, n = 4. **p*<0.05, ***p*<0.01.

Sin3a is required for postnatal β-cell function

I assayed insulin secretion of P7 *Sin3a*$^{\Delta endo}$ islets, the oldest stage when intact mutant islets could be readily isolated. These islets secreted more insulin under 2.8 mM glucose (**Figure 17A**), but less under 20 mM glucose (**Figure 17B**) or 25 mM KCl (**Figure 17C**). There was a compromised Ca^{2+} influx induced by glucose or KCl, with higher levels of basal but lower stimulated Ca^{2+} influx (**Figure 18**). In addition, *Sin3a*$^{\Delta endo}$ β-cells had more vesicles per unit cytoplasmic area, decreased insulin dense-core size within each vesicle, and more vesicle-like membrane structures that lack detectable insulin crystals (**Figure 19**). The data indicate that Sin3a may regulate genes regulating β-cell insulin secretion and insulin vesicle packaging.

Sin3a is required for postnatal β-cell and α-cell survival

P7 and P14, but not P1 *Sin3a*$^{\Delta endo}$ mice had significantly reduced β-cell mass (**Figure 20**), accompanied by increased apoptosis starting from P1 (**Figure 21A, B**) but no change of proliferation (**Figure 21C, D**). In addition, *Sin3a*$^{\Delta endo}$ mice had reduced α-cell mass but no change in δ-cell mass on P14 (**Figure 22**). These combined findings suggest that Sin3a is not required for β-cell differentiation (i.e., producing insulin+ cells) during embryogenesis, but required for their fitness after birth. This conclusion led us to examine the detailed molecular mechanism(s) on how Sin3a regulates this fitness.

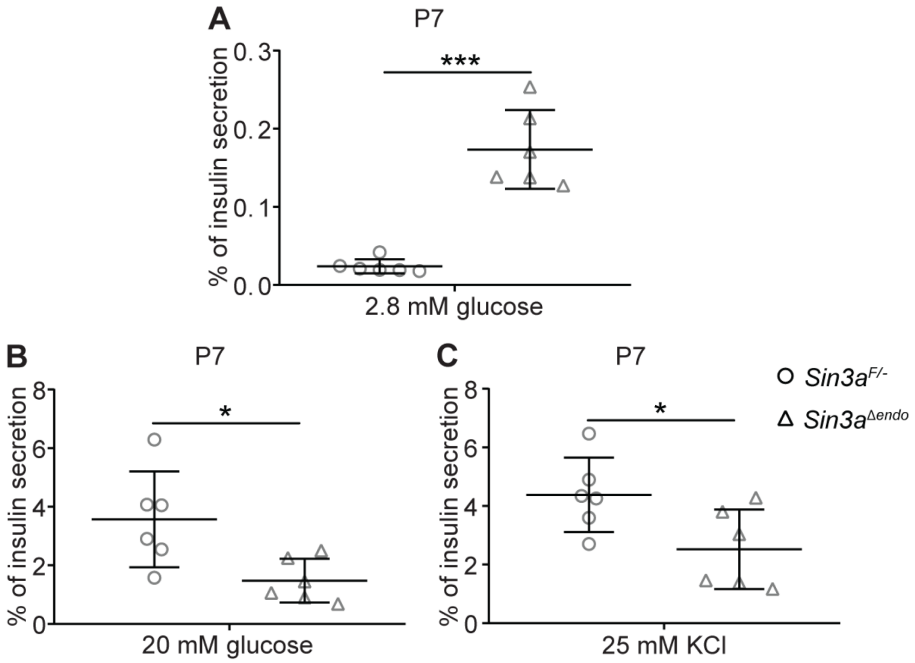


Figure 17: Sin3a promotes insulin secretion.

(A-C) Insulin secretion in P7 *Sin3a*$^{F/-}$ control and *Sin3a*$^{\Delta endo}$ islets (n = 6), presented as % insulin secretion within a 45-minutes window. *Sin3a*$^{\Delta endo}$ islets secreted more insulin under 2.8 mM glucose (A), but less under 20 mM glucose (B) or 25 mM KCl (C). **p*<0.05, ****p*<0.001.

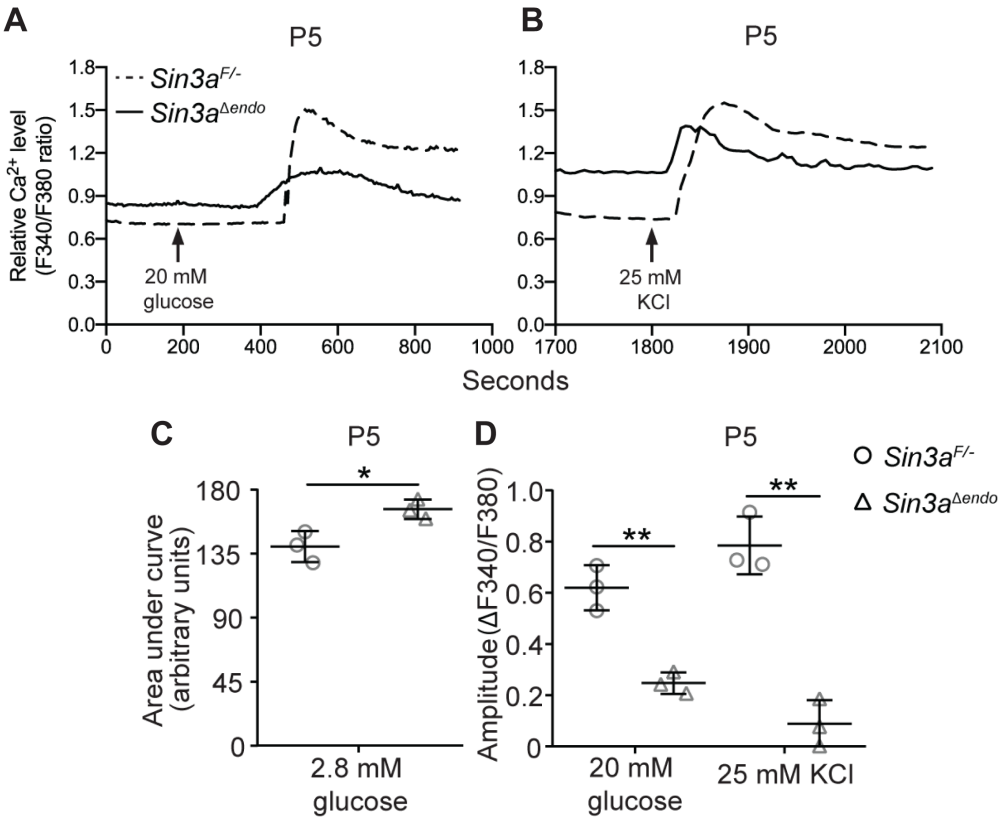


Figure 18: Sin3a is required for β-cell Ca^{2+} homeostasis.

(A-C) Quantification of Ca^{2+} influx from P5 control and *Sin3a*$^{\Delta endo}$ islets, with representative Ca^{2+} recording (A, B), the overall Ca^{2+} influx at 2.8 mM glucose (C, area under the curve from 0-195 seconds) and the highest Ca^{2+} influx amplitude at 20 mM glucose and 25 mM KCl stimulation (D) (n = 3, the number of mice). *p<0.05, **p<0.01.

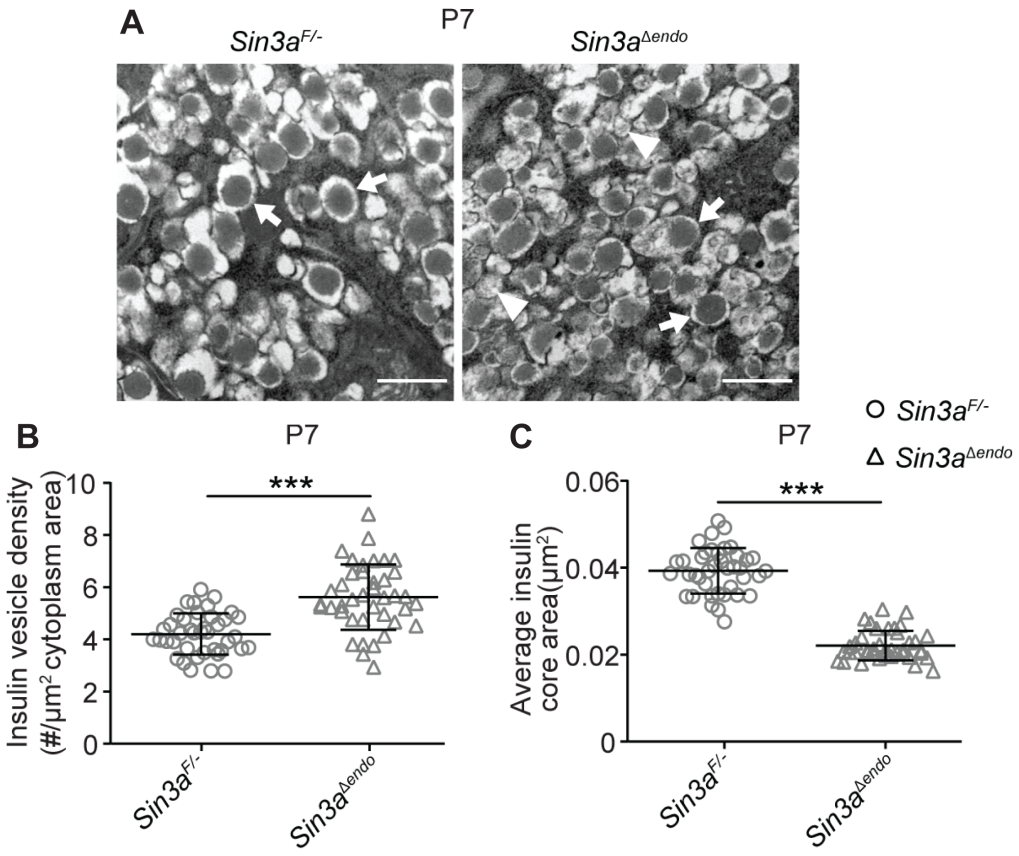


Figure 19: Sin3a promotes insulin granule biosynthesis.

(A) TEM images of P7 β-cells. White arrows, normal insulin vesicles. White arrowheads, empty vesicles. (B, C) Vesicular quantification of P7 control and mutant β-cells (n = 40 images from 2 batches of mice), including vesicle density (B) and size (C). Scale bars = 500 nm. ***p<0.001.

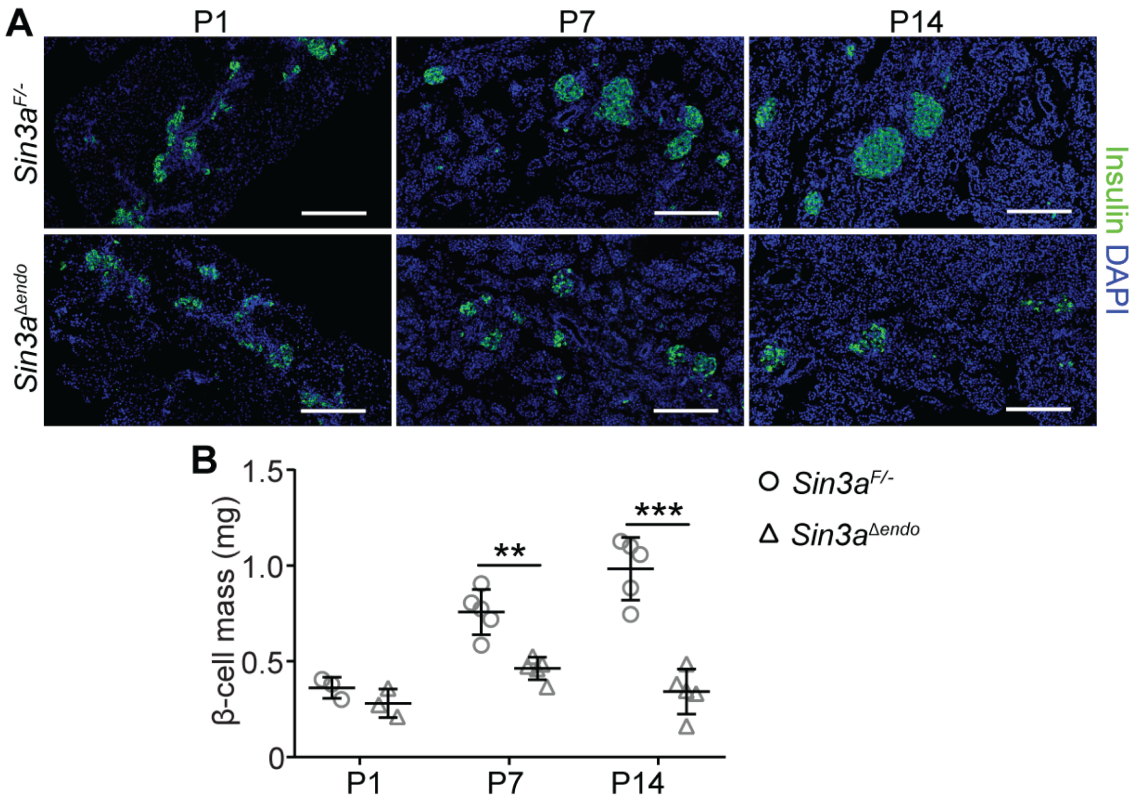


Figure 20: Loss of *Sin3a* reduced postnatal β-cell mass.

(A, B) Quantification of β-cell mass using IF assays. P7 and P14, but not P1 *Sin3a*$^{\Delta endo}$ mice had significantly reduced β-cell mass (n = 3-5). Scale bars = 200 μm. $^{**}p<0.01$, $^{***}p<0.001$.

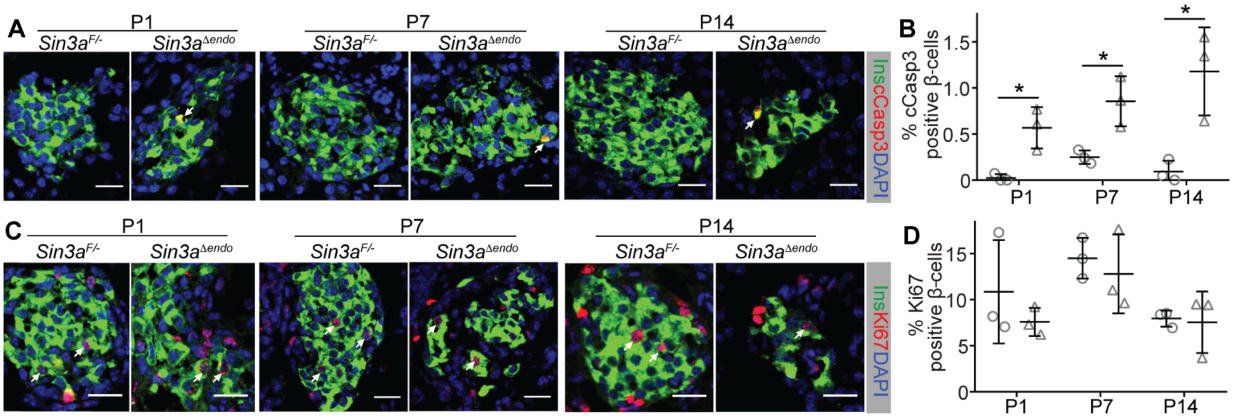


Figure 21: Sin3a is required for postnatal β-cell survival.

(A, B) Co-IF staining of insulin with cleaved Caspase-3 (cCasp3) (A) and the quantification (B) (n = 3). The white arrows indicate cCasp3+ (A). (C, D) Co-IF staining of insulin with Ki67 (C) and the quantification (D) (n = 3). The white arrows indicate Ki67^{+} β cells (C). Scale bars = 20 µm. *p<0.05.

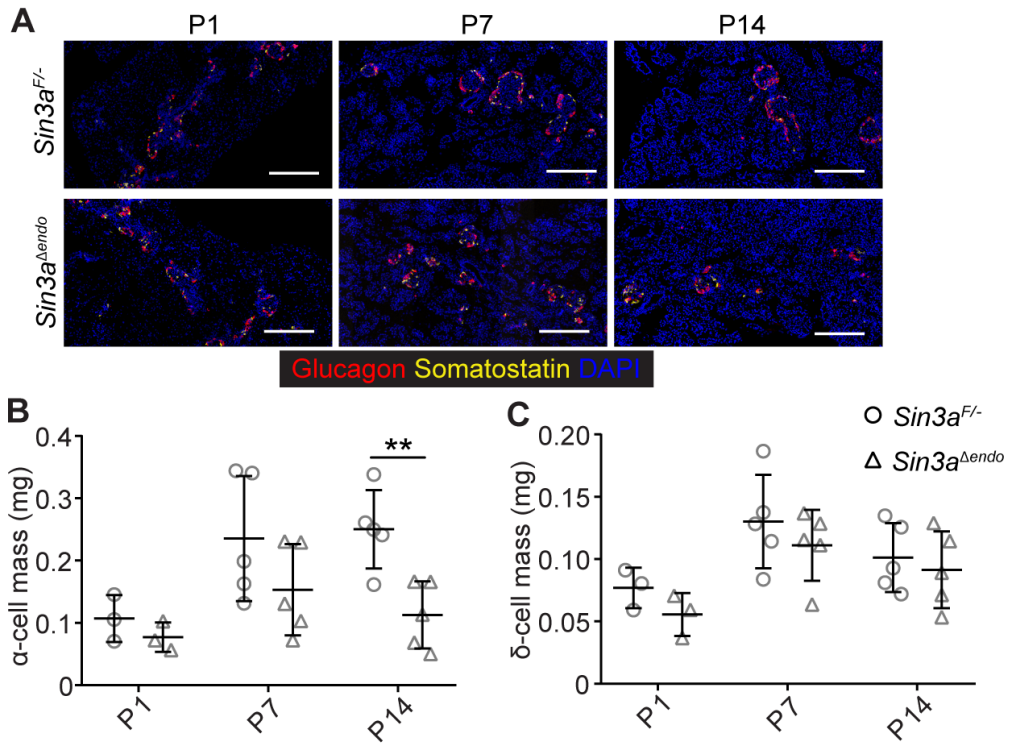


Figure 22: Loss of *Sin3a* in endocrine progenitors reduces α-cell mass, but not δ-cell mass, on P14.

(A) IF staining of glucagon and somatostatin to measure α-cell and δ-cell mass, respectively, on P1, P7, and P14. DAPI was used for counterstaining. Scale bars = 200 μm. (B, C) Quantification of α-cell mass (B) and δ-cell mass (C) (n = 3-5, the number of mice.). ***p*<0.01.

Sin3a is required for the expression of function and maturation genes in postnatal β-cells

I started with a candidate approach by examining the expression of several TFs Mafa, Mafb, Pdx1, and Nkx6.1 that are important for β-cell differentiation and Glut2 and Ucn3 for function. There was no difference in Pdx1, Nkx6.1, or Mafb levels (**Figure 23**), but progressive reductions of Glut2, Ucn3, and Mafa expression in *Sin3a*$^{\Delta endo}$ β-cells compared with controls. Specifically, Ucn3 expression was significantly down-regulated by P4 while that of Glut2 and Mafa by P7 at both protein and mRNA levels (**Figure 24, 25**). However, because the glycemic defects of *Ucn3* or *Mafa* mutant mice developed much later than the *Sin3a*$^{\Delta endo}$ animals (Hang and Stein, 2011; Li et al., 2007) and the increased β-cell death was observed before the down-regulation of *Ucn3*, *Mafa*, and *Slc2a2*, these three genes are unlikely the major mediators of Sin3a function. Thus, we comprehensively defined the Sin3a-regulated genes in β-cells using RNA-seq approaches.

Sin3a regulates genes involved in ion transport, cell death, vesicular production/secretion, glucose metabolism, and stress response

P4 *Sin3a*$^{F/+}$ and *Sin3a*$^{\Delta endo}$ islets were used for scRNA-seq, allowing us to distinguish β-cells from other islet-cell types. P4 *Sin3a*$^{\Delta endo}$ mice had no recognizable physiological defects, avoiding complications imposed by hyperglycemia. Yet they had

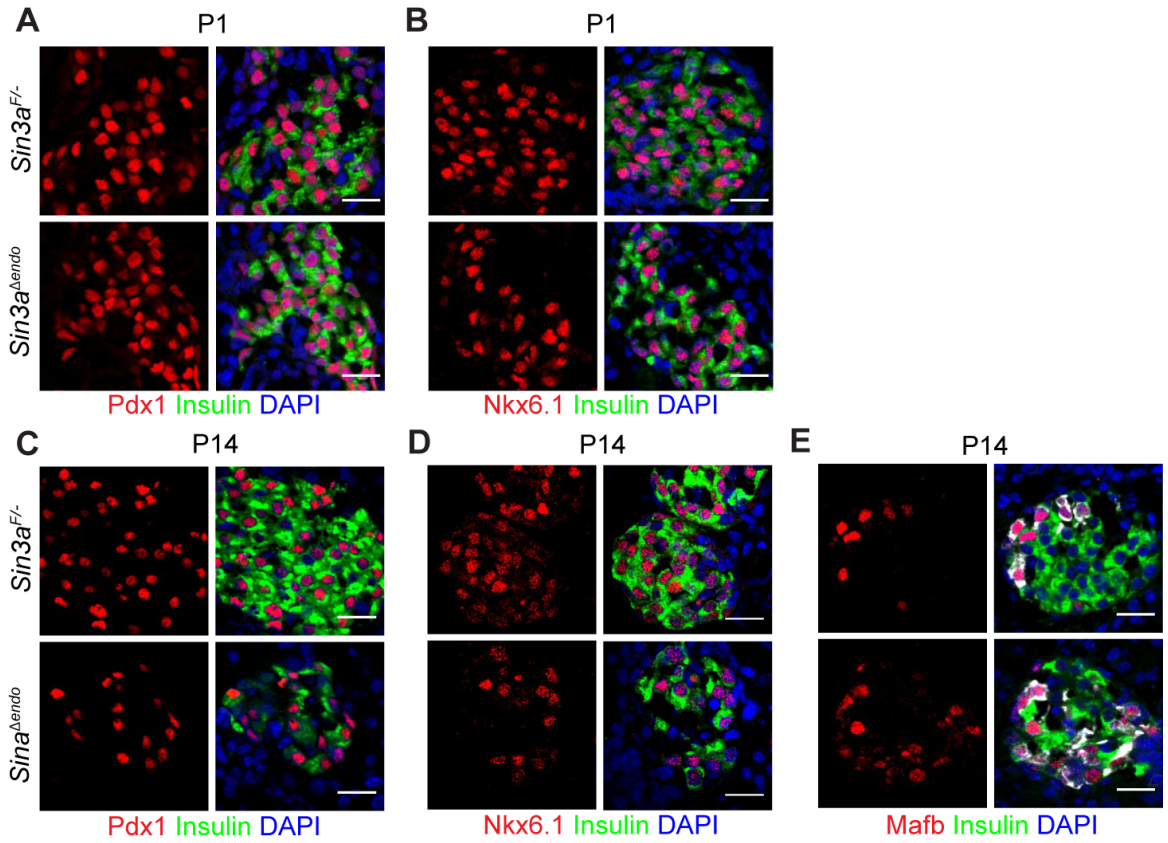


Figure 23: Loss of Sin3a does not affect the expression of Pdx1, Nkx6.1, or Mafb in *Sin3a$^{\Delta endo}$* β cells.

(A, B) IF staining of insulin with Pdx1 (A), Nkx6.1 (B) using P1 pancreata. (C, D) IF staining of insulin, Pdx1 (C), Nkx6.1 (D) using P14 pancreatic sections. (E) IF staining of insulin, glucagon and Mafb using P14 pancreatic sections. Scale bars = 20 μm.

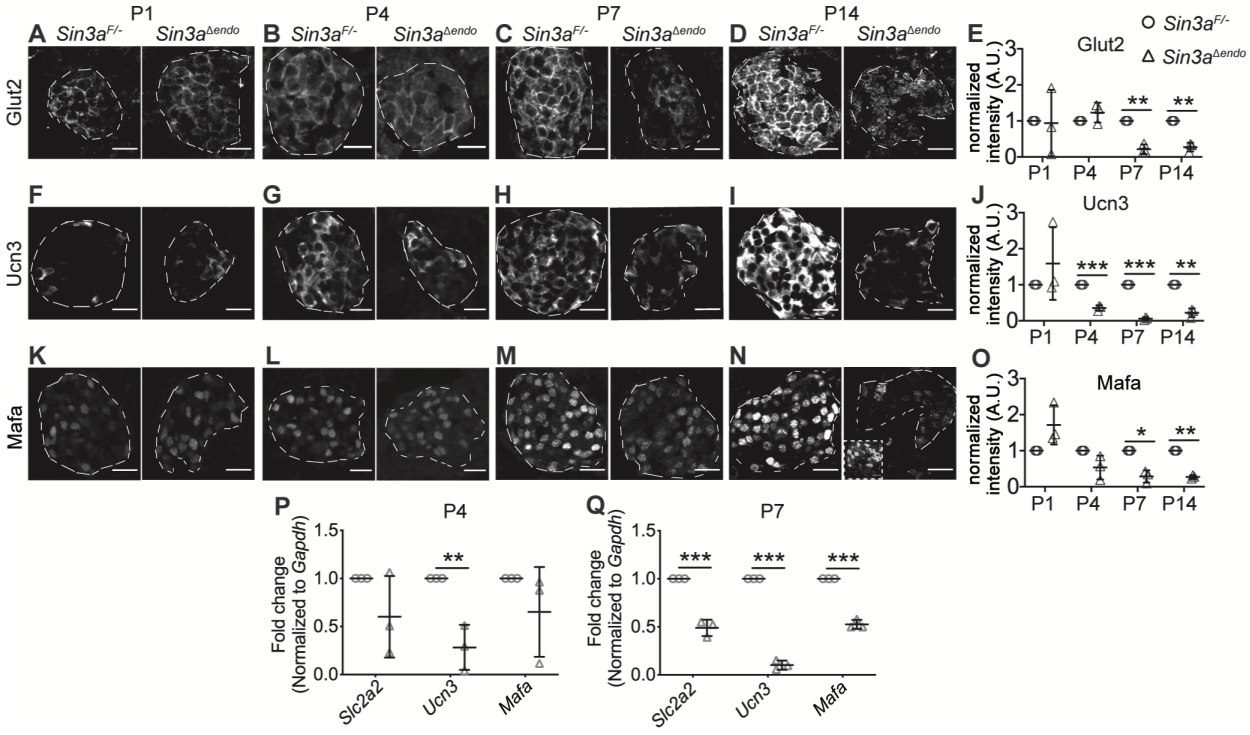


Figure 24: Sin3a regulates the expression of several functional genes in postnatal β-cells.

(A-E) Glut2 staining and quantification in islets of different ages with or without *Sin3a* inactivation. (F-J) and (K-O), the same as above expect Ucn3 and MafA protein levels were measured, respectively. n = 3 mice for all assays. Scale bars = 20 μm. Broken lines encircle the islet areas, highlighting the signal intensity. Refer to **Figure 25** to see the corresponding insulin staining to locate β cells. Inset in N-mutant panel also showed an example of correcponding insulin signals. (P, Q) Relative expression of *Slc2a2* (encoding Glut2), *Ucn3*, and *MafA* in P7 islets, assayed using qRT-PCR. The gene expression levels were normalized to *Gapdh* (n = 3 mice). **p*<0.05, ***p*<0.01, ****p*<0.001.

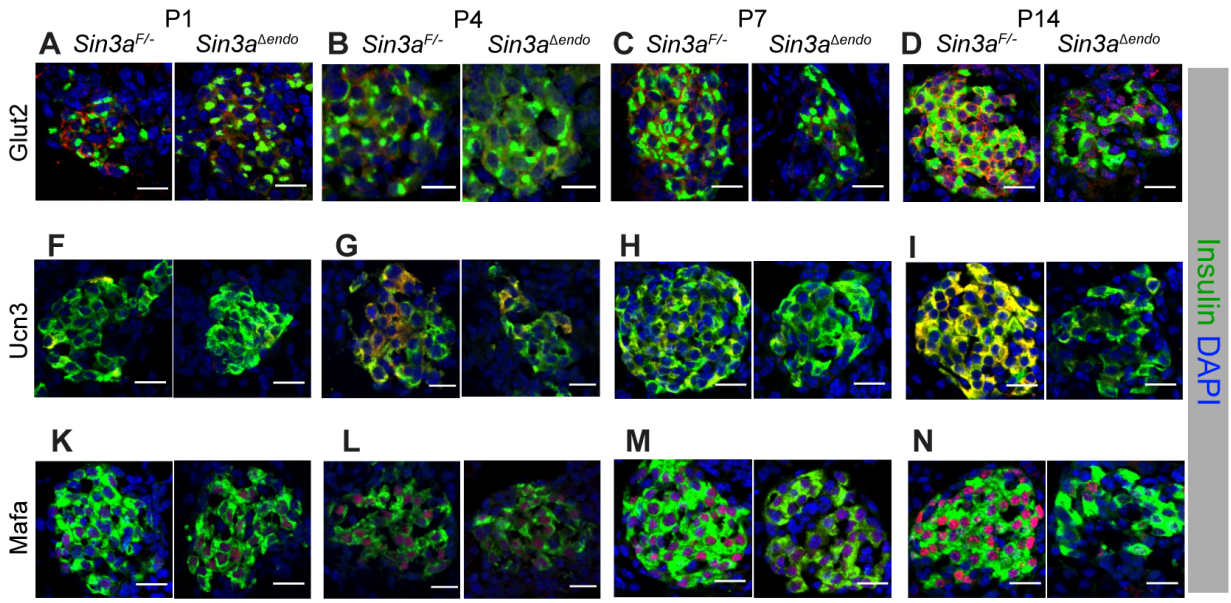


Figure 25: IF showing changed Glut2, Mafa, and Ucn3 expression in late-postnatal *Sin3a$^{\Delta endo}$* β-cells.

Shown are co-staining results of insulin with Glut2 (A-D), Ucn3 (F-I), or Mafa (K-N) in control and *Sin3a$^{\Delta endo}$* pancreas of different stages. The insulin signals mark β-cell areas that were quantified in **Figure 24**. Scale bars = 20 μm.

increased β-cell death, underscoring the existing molecular defects in the P4 *Sin3a*$^{\Delta endo}$ β-cells.

Two highly reiterative scRNA-seq datasets (**Figure 26A, 27A**) were obtained, which showed clear islet-cell type separations (**Figure 26B-G, 27B**). Expression differences were seen between control and *Sin3a*$^{\Delta endo}$ β-cells, with both downregulated and upregulated genes (**Figure 27B**). For example, *Ucn3* transcripts were downregulated, a pattern that recapitulates qRT-PCR results. *Hspe1* was upregulated that represents a newly identified Sin3a-regulated gene.

In order to minimize the saturation and scarcity issues inherent to scRNA-seq (Mawla and Huising, 2019), we analyzed the scRNA-seq results by combining identical cell types into bulk expression data within each sample (Svensson et al., 2017). This revealed 772 down-regulated and 3,668 up-regulated genes in *Sin3a*$^{\Delta endo}$ β-cells, with adjusted $p < 0.05$ and at least two-fold expression differences. **Table 5** shows the top 100 upregulated and downregulated genes in *Sin3a*$^{\Delta endo}$ β-cells and table “Sin3 candidate gene list.xlsm” in GEO (GSE146474) shows all the differentially expressed genes. The data indicate that Sin3a may act as both a coactivator and a corepressor in β-cells. We observed changed transcription of *Ucn3* but not *Mafa*, *Nkx6.1*, or *Pdx1*, consistent with IF (**Figure 23, 24**) and qRT-PCR results (**Figure 24**). GSEA of the scRNA-seq data set revealed several altered molecular pathways (**Figure 28**). The terms include protein/membrane transport/ endoplasmic reticulum (ER) function, tricarboxylic acid (TCA) cycle/mitochondrial activities, and oxidative stress responses. Defects in the altered molecular pathways may contribute to the impairments in insulin-

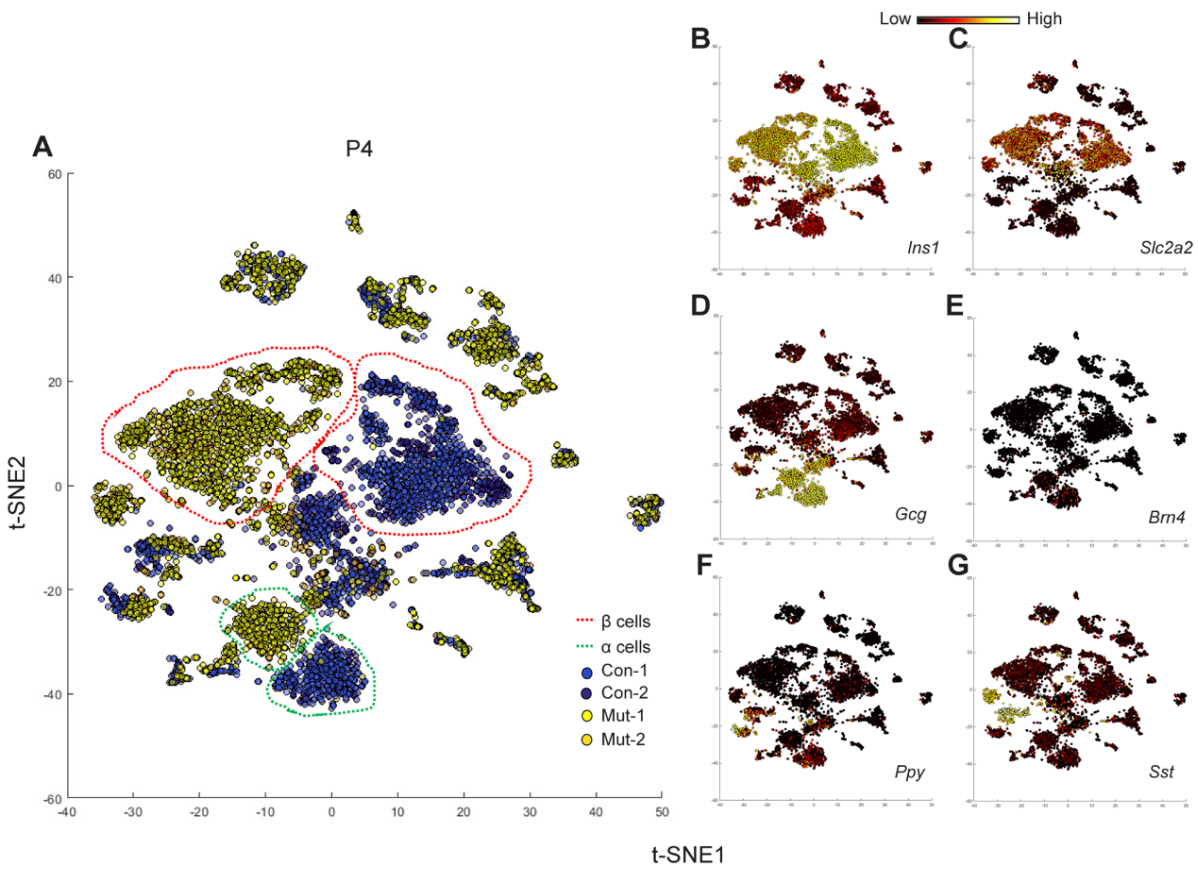


Figure 26: scRNA-seq data of β-cells, α-cells, and other cells.

(A) t-SNE visualization of pancreatic scRNA-seq data of P4 islets. (B-G) Marker gene expression in different cell clusters on the t-SNE map (black to white, gene expression levels low to high), including β-cell marker genes, Ins1 (B) and Slc2a2 (C), α-cell marker genes, Gcg (D) and Brn4 (E), PP-cell marker gene, Ppy (F), and δ-cell marker gene, Sst (G). Con, control cells from *Sin3A*$^{F/+}$ islets. Mut, mutant islets from *Sin3a*$^{\Delta endo}$ islets.

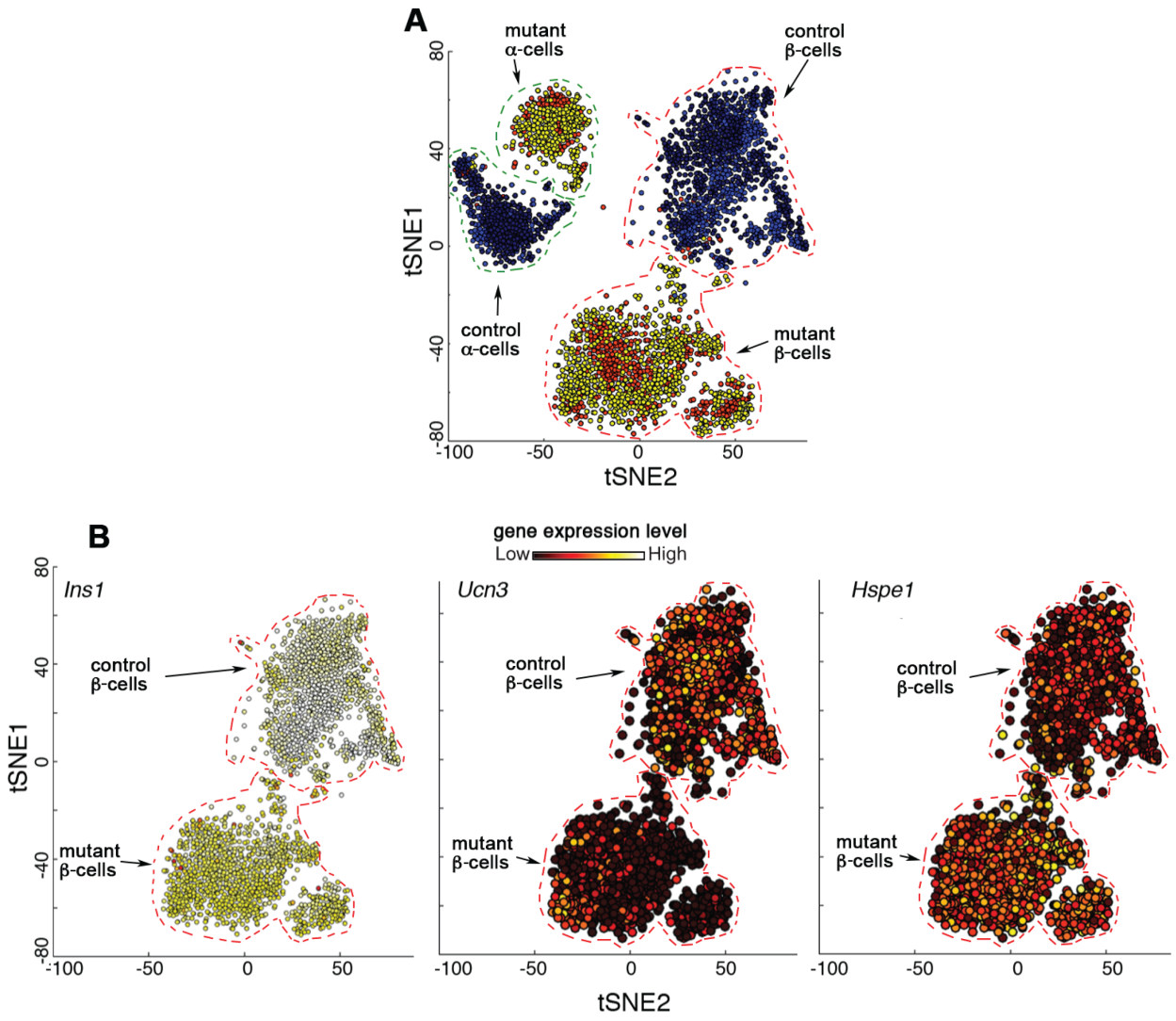


Figure 27: ScRNA-seq revealed Sin3a-regualted genes.

(A) t-SNE-aided visualization of α and β cell clusters in two duplicate experiments, with both control and *Sin3a*$^{\Delta endo}$ cells. Note that the same cell types from two duplicate expression assays were not separable but can be recognized by the color of dots. (B) The expression of Ins1, Ucn3, and Hspe1 in each cell cluster on the t-SNE map in panel A. Note that in this panel, the color of dots indicates the relative gene expression level.

Table 5: Top 100 upregulated and downregulated genes in *Sin3a*-deficient β-cells assayed by scRNA-seq

Upregulated genes	average_logFC	pct.1	pct.2	p_val	p_val_adj
Pyy	1.325	0.952	0.758	9.00E-161	3.46E-156
Cldn11	1.193	0.274	0.067	1.87E-54	7.21E-50
Spp1	0.931	0.353	0.077	9.97E-80	3.83E-75
Aass	0.929	0.397	0.026	2.21E-137	8.48E-133
Slc38a5	0.901	0.593	0.341	1.27E-68	4.89E-64
Nt5dc2	0.785	0.919	0.602	5.22E-192	2.01E-187
Cox7c	0.766	0.957	0.707	1.80E-144	6.91E-140
mt-Nd2	0.761	0.994	0.963	2.99E-224	1.15E-219
mt-Nd1	0.756	0.998	0.962	7.88E-173	3.03E-168
Serf1	0.755	0.715	0.234	2.47E-162	9.51E-158
Tspan7	0.746	0.974	0.834	2.32E-190	8.92E-186
Marcksl1	0.733	0.524	0.113	8.98E-132	3.45E-127
Nnat	0.730	0.971	0.886	9.50E-76	3.65E-71
Hspe1	0.709	0.885	0.552	9.06E-156	3.48E-151
mt-Nd3	0.708	0.991	0.933	6.35E-222	2.44E-217
Tmeff1	0.702	0.577	0.117	4.82E-151	1.85E-146
Ybx3	0.698	0.835	0.487	8.47E-124	3.26E-119
Mtfr1l	0.697	0.755	0.303	8.70E-149	3.34E-144
Rpl23	0.689	0.987	0.913	6.82E-136	2.62E-131
Ing1	0.683	0.77	0.332	9.50E-145	3.65E-140
Prdx3	0.678	0.802	0.39	3.42E-152	1.32E-147
Aplp1	0.674	0.975	0.826	5.50E-196	2.12E-191
Fhl1	0.650	0.475	0.067	4.02E-140	1.55E-135
Hsd17b12	0.650	0.836	0.419	3.74E-160	1.44E-155
Dcx	0.648	0.626	0.252	2.50E-108	9.62E-104
Synpr	0.645	0.502	0.134	4.01E-107	1.54E-102
Spc25	0.624	0.914	0.585	4.09E-104	1.57E-99
Casp3	0.617	0.723	0.277	4.91E-136	1.89E-131
Aldoa	0.609	0.757	0.368	1.54E-127	5.93E-123
Hsp90b1	0.596	0.982	0.883	2.51E-175	9.66E-171

Snrpf	0.595	0.815	0.458	1.28E-115	4.93E-111
Gsto1	0.594	0.552	0.122	1.69E-137	6.50E-133
Map2k1	0.593	0.694	0.285	1.09E-112	4.19E-108
Esyt1	0.588	0.764	0.355	1.07E-137	4.10E-133
Fuca1	0.586	0.649	0.232	1.90E-131	7.31E-127
Tph1	0.583	0.282	0.028	1.27E-84	4.90E-80
Dlk1	0.574	0.789	0.532	6.00E-85	2.31E-80
Gas5	0.574	0.848	0.509	4.83E-109	1.86E-104
Ndrg4	0.574	0.547	0.245	7.82E-77	3.01E-72
Rps23	0.572	0.99	0.931	1.13E-104	4.36E-100
Bnip3	0.572	0.777	0.363	3.44E-118	1.32E-113
Gng5	0.572	0.893	0.616	3.91E-118	1.51E-113
Sod2	0.570	0.827	0.454	9.96E-125	3.83E-120
mt-Nd4	0.569	0.998	0.974	4.33E-152	1.67E-147
Dynll1	0.561	0.93	0.668	1.16E-114	4.47E-110
Cct2	0.561	0.882	0.559	5.80E-128	2.23E-123
Tbca	0.558	0.911	0.599	2.73E-105	1.05E-100
Pkib	0.554	0.578	0.234	1.04E-90	3.99E-86
Sgpl1	0.551	0.788	0.398	4.13E-105	1.59E-100
Mycbp	0.551	0.588	0.178	7.84E-120	3.02E-115
Sod1	0.550	0.902	0.669	6.86E-126	2.64E-121
Tmod2	0.550	0.796	0.542	7.28E-83	2.80E-78
Myc	0.546	0.369	0.076	5.37E-82	2.06E-77
Sesn3	0.543	0.788	0.449	4.96E-100	1.91E-95
Bri3bp	0.542	0.604	0.174	1.70E-127	6.55E-123
Idh3a	0.542	0.704	0.318	4.12E-110	1.59E-105
Rack1	0.539	0.978	0.875	4.05E-160	1.56E-155
Hsph1	0.538	0.719	0.343	1.24E-91	4.76E-87
Scarb1	0.529	0.627	0.229	7.69E-111	2.96E-106
Eef1b2	0.528	0.964	0.793	8.32E-142	3.20E-137
Grb10	0.520	0.745	0.393	2.49E-97	9.58E-93
Nrep	0.514	0.878	0.663	6.59E-72	2.53E-67
Pfn2	0.511	0.587	0.178	7.61E-115	2.93E-110
Tubb5	0.503	0.904	0.6	3.19E-97	1.23E-92
Igf2r	0.503	0.725	0.358	4.35E-102	1.67E-97

mt-Cytb	0.501	0.999	0.982	2.50E-81	9.63E-77
Ctnnbip1	0.498	0.86	0.537	3.71E-103	1.43E-98
Tsen34	0.495	0.834	0.503	2.31E-105	8.87E-101
Glb1l2	0.495	0.834	0.517	3.53E-91	1.36E-86
Ucp2	0.494	0.786	0.539	3.18E-77	1.22E-72
Wipi1	0.493	0.941	0.691	1.74E-91	6.69E-87
Pdia6	0.492	0.98	0.857	1.71E-123	6.59E-119
Pabpc1	0.492	0.939	0.762	2.18E-119	8.38E-115
Paics	0.491	0.754	0.395	1.45E-92	5.58E-88
Glud1	0.489	0.877	0.589	2.78E-100	1.07E-95
Lbh	0.484	0.718	0.431	2.51E-70	9.64E-66
Psma4	0.480	0.801	0.46	1.22E-94	4.68E-90
Cyb5a	0.479	0.767	0.417	4.43E-93	1.70E-88
Rps11	0.478	0.958	0.793	6.53E-116	2.51E-111
Atp5g3	0.476	0.845	0.633	2.95E-58	1.13E-53
Prr13	0.475	0.68	0.351	5.18E-78	1.99E-73
Reep6	0.475	0.588	0.252	4.20E-81	1.62E-76
Ap3s1	0.473	0.845	0.522	4.09E-96	1.57E-91
Tmod1	0.473	0.615	0.256	1.19E-95	4.56E-91
Rbm15b	0.471	0.624	0.287	2.85E-81	1.10E-76
mt-Co2	0.466	1	0.977	7.82E-92	3.01E-87
Chp1	0.465	0.782	0.409	5.21E-100	2.00E-95
Txndc17	0.463	0.751	0.4	3.32E-97	1.28E-92
Nefm	0.463	0.362	0.046	3.28E-102	1.26E-97
Tes	0.463	0.789	0.445	2.06E-90	7.93E-86
Cpd	0.462	0.768	0.417	2.31E-90	8.88E-86
Sec11c	0.461	0.789	0.492	1.64E-73	6.31E-69
Pcyox1	0.461	0.638	0.271	5.49E-93	2.11E-88
Pno1	0.460	0.647	0.287	2.11E-80	8.13E-76
Cox8a	0.459	0.97	0.866	5.27E-134	2.03E-129
Cpne3	0.459	0.644	0.298	2.52E-77	9.69E-73
Gm10053	0.458	0.817	0.543	1.54E-75	5.91E-71
Man2a1	0.455	0.423	0.184	9.45E-48	3.63E-43
Hcfc1r1	0.455	0.7	0.379	2.68E-79	1.03E-74
Bdh1	0.455	0.548	0.16	1.19E-107	4.56E-103

Downregulated genes	average_logFC	pct.1	pct.2	p_val	p_val_adj
Klk1	-1.294	0.68	0.816	4.26E-65	1.64E-60
Ucn3	-1.041	0.373	0.715	4.28E-111	1.65E-106
Prss53	-1.027	0.297	0.702	1.77E-159	6.79E-155
Hspa1b	-1.025	0.189	0.371	1.86E-34	7.17E-30
Plag1	-1.013	0.417	0.549	1.12E-37	4.32E-33
Rnase4	-1.013	0.233	0.672	6.11E-163	2.35E-158
Gm42418	-0.966	0.543	0.592	1.36E-13	5.23E-09
Gm15927	-0.961	0.383	0.55	2.62E-50	1.01E-45
4930523C07Rik	-0.961	0.478	0.604	1.15E-44	4.43E-40
Gcg	-0.935	0.934	0.902	5.00E-80	1.92E-75
Trp73os	-0.871	0.334	0.486	5.71E-40	2.20E-35
Defb9	-0.866	0.543	0.715	1.20E-93	4.61E-89
Pcdhb22	-0.853	0.349	0.509	5.15E-46	1.98E-41
Ttc28	-0.843	0.261	0.592	2.38E-108	9.15E-104
Hist1h1c	-0.839	0.579	0.74	6.51E-66	2.50E-61
Ins1	-0.838	0.998	1	1.09E-124	4.20E-120
Ctrb1	-0.838	0.157	0.083	2.36E-09	9.09E-05
Abi3	-0.798	0.322	0.443	4.46E-30	1.72E-25
Hspa1a	-0.782	0.11	0.285	2.22E-37	8.52E-33
Tfcp2l1	-0.750	0.42	0.559	5.21E-46	2.00E-41
Tmsb4x	-0.742	0.394	0.69	4.93E-82	1.90E-77
Ier2	-0.732	0.494	0.697	2.59E-77	9.98E-73
Kcnh2	-0.728	0.394	0.541	2.31E-44	8.89E-40
4930570G19Rik	-0.722	0.293	0.448	5.24E-39	2.02E-34
Fos	-0.687	0.932	0.923	1.41E-121	5.43E-117
Insrr	-0.632	0.094	0.412	4.10E-92	1.58E-87
Cntfr	-0.627	0.129	0.386	1.77E-64	6.80E-60
Hbb-bs	-0.618	0.107	0.17	1.24E-07	0.004767212
Ubc	-0.595	0.825	0.839	8.75E-75	3.36E-70
Palm3	-0.590	0.221	0.441	9.44E-54	3.63E-49
Gm42556	-0.586	0.248	0.365	2.15E-25	8.25E-21
Ins2	-0.571	0.997	1	1.47E-101	5.67E-97
Acss1	-0.569	0.228	0.398	4.78E-41	1.84E-36

Malat1	-0.559	0.841	0.834	4.77E-52	1.84E-47
Zbtb48	-0.555	0.43	0.471	1.18E-12	4.54E-08
Serpinh1	-0.551	0.159	0.409	1.94E-59	7.47E-55
Prlr	-0.551	0.548	0.698	3.53E-47	1.36E-42
Msln	-0.528	0.037	0.234	5.56E-56	2.14E-51
Ppy	-0.516	0.159	0.339	7.08E-36	2.72E-31
Coro2b	-0.514	0.308	0.495	4.32E-40	1.66E-35
Zbtb44	-0.501	0.389	0.507	1.56E-30	6.01E-26
Pex26	-0.488	0.278	0.403	7.19E-27	2.77E-22
Atf5	-0.485	0.261	0.445	3.26E-37	1.26E-32
Gm16835	-0.480	0.191	0.332	3.61E-29	1.39E-24
Tceal6	-0.479	0.416	0.466	3.00E-13	1.15E-08
Fosb	-0.476	0.768	0.797	6.46E-22	2.49E-17
Tmem67	-0.475	0.231	0.379	2.13E-29	8.18E-25
Vstm2l	-0.474	0.105	0.382	2.29E-76	8.82E-72
Arc	-0.472	0.136	0.339	2.19E-45	8.43E-41
Ffar2	-0.471	0.204	0.404	1.47E-39	5.65E-35
Nfkbia	-0.466	0.419	0.487	9.79E-14	3.77E-09
Isg20	-0.456	0.261	0.441	5.37E-35	2.07E-30
Hexim1	-0.454	0.198	0.421	5.17E-46	1.99E-41
Aatk	-0.449	0.016	0.239	4.06E-73	1.56E-68
Cebpb	-0.446	0.238	0.417	2.28E-32	8.77E-28
Psap	-0.440	0.872	0.843	1.63E-47	6.29E-43
Wnt4	-0.438	0.064	0.306	1.53E-66	5.90E-62
Nr4a1	-0.438	0.533	0.618	1.34E-21	5.14E-17
Dpp4	-0.435	0.074	0.302	3.25E-60	1.25E-55
Cpq	-0.432	0.221	0.401	7.53E-35	2.90E-30
Nfic	-0.431	0.308	0.454	1.64E-26	6.31E-22
Ak1	-0.430	0.084	0.311	1.98E-57	7.61E-53
E330034L11Rik	-0.424	0.137	0.324	4.45E-42	1.71E-37
Hist1h2bc	-0.418	0.425	0.518	3.48E-13	1.34E-08
Atf4	-0.418	0.727	0.744	5.79E-29	2.23E-24
Emilin1	-0.416	0.046	0.267	4.24E-63	1.63E-58
Dusp1	-0.416	0.505	0.599	6.59E-22	2.53E-17
H2-Q4	-0.414	0.839	0.822	2.97E-42	1.14E-37

5330417C22Rik	-0.413	0.058	0.304	2.63E-69	1.01E-64
Dusp5	-0.413	0.322	0.443	9.38E-18	3.61E-13
Pold3	-0.411	0.288	0.38	4.27E-17	1.64E-12
Zfp36	-0.410	0.686	0.69	6.34E-10	2.44E-05
Fam167a	-0.408	0.073	0.232	1.55E-34	5.96E-30
Mrps31	-0.406	0.188	0.339	5.95E-30	2.29E-25
Ufc1	-0.401	0.35	0.529	1.51E-38	5.81E-34
Ms4a6c	-0.399	0.429	0.54	6.69E-25	2.57E-20
Igfbp7	-0.395	0.449	0.556	5.45E-20	2.10E-15
Yipf2	-0.389	0.261	0.395	3.74E-21	1.44E-16
RP23-235J18.1	-0.388	0.17	0.328	5.67E-32	2.18E-27
Gm45159	-0.386	0.218	0.36	4.95E-27	1.90E-22
Comt	-0.385	0.175	0.381	2.49E-42	9.56E-38
Papss2	-0.384	0.808	0.805	6.64E-32	2.55E-27
Irak1	-0.383	0.611	0.653	3.61E-17	1.39E-12
Rpl41	-0.383	0.782	0.741	4.82E-09	0.000185194
Dhrs4	-0.381	0.24	0.407	1.01E-30	3.89E-26
Srxn1	-0.366	0.107	0.303	7.64E-42	2.94E-37
H3f3b	-0.365	0.94	0.9	1.02E-35	3.94E-31
Sv2a	-0.364	0.342	0.441	1.55E-13	5.96E-09
Rnf150	-0.363	0.166	0.32	4.23E-30	1.63E-25
Celf4	-0.363	0.286	0.393	1.09E-15	4.18E-11
Gm20379	-0.362	0.468	0.539	3.83E-11	1.47E-06
Gm42428	-0.360	0.175	0.277	2.51E-14	9.65E-10
Ccnd1	-0.360	0.065	0.199	5.48E-28	2.11E-23
Auts2	-0.359	0.178	0.347	1.08E-29	4.15E-25
Ift122	-0.359	0.272	0.445	1.25E-34	4.80E-30
Lpp	-0.357	0.643	0.657	7.43E-13	2.86E-08
Slc50a1	-0.355	0.139	0.306	3.07E-31	1.18E-26
Npy	-0.354	0.297	0.467	5.17E-24	1.99E-19
Trp53inp2	-0.352	0.624	0.633	1.80E-11	6.93E-07
1500011B03Rik	-0.352	0.393	0.512	5.32E-21	2.05E-16

The results were from two controls and two mutant β-cell preparations. The positive level changes indicate increased expression in *Sin3a*-deficient β cells. The negative

level changes indicate reduced expression in *Sin3a*-deficient β cells. Log(FC) was calculated as Log(2) [(level in mutant) - (level in control)]/(level in control). Differentially expressed genes are those with adjusted $p < 0.05$ and at least two-fold expression differences. Note that Pct. 1 and Pct.2 indicate the % of control or mutant β cells that express the indicated gene, respectively.

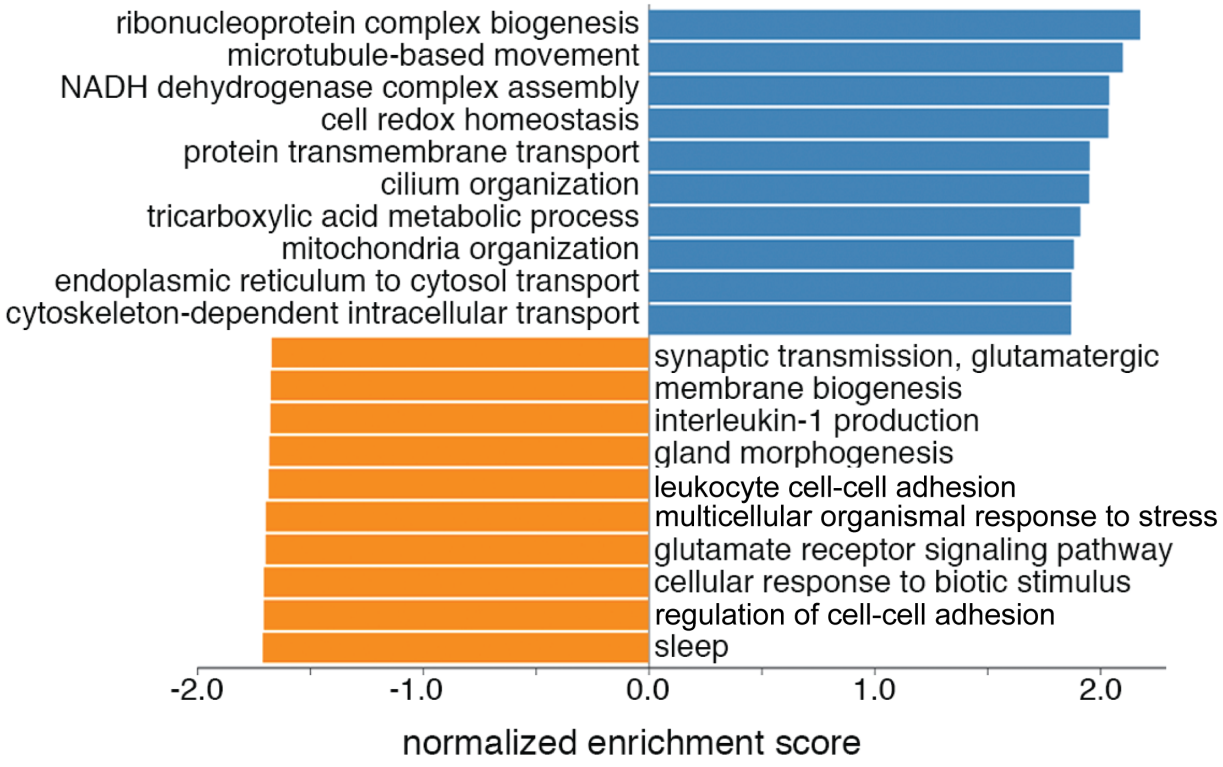


Figure 28: GSEA results of all differentially expressed genes in control and *Sin3a*$^{\Delta endo}$ β cells.

The terms include protein/membrane transport/ER function, TCA cycle/mitochondrial activities, and oxidative stress responses. Only pathways with false discovery rate (FDR) < 0.05 were shown.

vesicle packaging, nutrient-induced Ca2+ influx, insulin secretion, and the survival of *Sin3a*-deficient β cells.

Sin3a is enriched in 5' regulatory regions of several β-cell function/survival genes

Sin3a was reported to associate with several widely-expressed DNA binding proteins, including P53, Myc, Mad1, and Foxo1 (Bansal et al., 2016; Langlet et al., 2017). It was also detected in a Mafa-containing transcriptional complex in islet β-cells (Scoville et al., 2015). We postulated that Sin3a shares common target genes among different cell types. Bioinformatic analyses were used to identify putative Sin3-target genes in β-cells. This was achieved by comparing our list of Sin3a-dependent genes with published Sin3a-bound genes, identified based on ChIP-seq results in mouse ES cells (Williams et al., 2011), epiblast stem cells (Seki et al., 2014), and muscle cells (van Oevelen et al., 2010). We also included the Mafa-bound enhancers in mouse islets, postulating the Sin3a associates with these sites via Mafa (Tennant et al., 2013). This analysis revealed 2,847 gene loci having at least one reported Sin3a/Mafa-binding site, with 335 down-regulated and 2,512 up-regulated in *Sin3a*$^{\Delta endo}$ β-cells (see table "Sin3 candidate gene list.xlsm" in GSE146474). GO-based analyses [the Database for Annotation, Visualization and Integrated Discovery (DAVID)] revealed that these Sin3a-associated genes likely regulate processes in mitochondrion, endoplasmic reticulum/protein transport, ribosome, cell redox homeostasis, and synapse (**Figure 29**), similar to those by all differentially expressed genes between control and *Sin3a*$^{\Delta endo}$ β-cells.

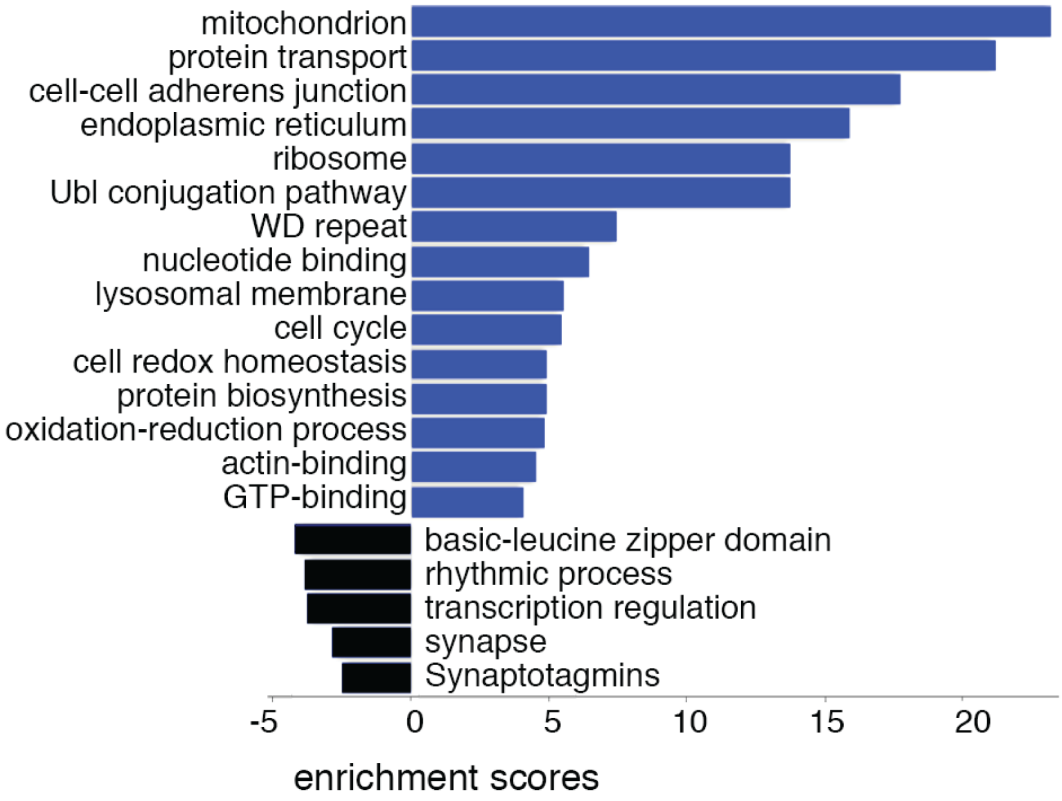


Figure 29: GO clustering of differentially expressed genes that were also reported to be direct Sin3a targets.

Only the top 15 up-regulated terms (enrichment score > 4.8) and top 5 down regulated terms (enrichment score > 2.4) were listed.

ChIP-PCR was used to verify if some of these predicted genes are direct Sin3a targets in MIN6 β-cells. Primary β-cells were not used because it is difficult to collect enough cells for ChIP. We prioritized several genes that have established molecular functions that correlate with the cellular defects of *Sin3a*$^{\Delta endo}$ β-cells (**Table 6**). These include down-regulated *Ins1* and *Kcnh2*, required for insulin production and cell membrane polarization (Hardy et al., 2009). They also include up-regulated genes *Esyt1* and *Calr* [related to defective Ca^{2+} homeostasis (Wang et al., 2017; Yu et al., 2016)]; *Bnip3*, *Casp3*, and *Ing1* [cell death (Bose et al., 2013; Ma et al., 2017)]; *Arl6*, *Cltb*, *Ergic3*, and *Rab11a* [lipid transport and vesicular biosynthesis (Pearse, 1976; Price et al., 2012; Sato and Nakano, 2007; Zhu et al., 2018b)]; *Aldoa* and *Idh3a* [glucose metabolism]; and *Hsp90b1* and *Hspe1* [stress responses (Kim et al., 2018)]. *Mafa* was also included, because Sin3a enrichment was detected on its putative regulatory elements (**Table 6**). *Ucn3*, *Slc2a2, Gcg*, *Gapdh*, and *Albumin* were included as controls because Sin3a was not reported to directly regulate these genes.

For ChIP-PCR, we selected several putative Regulatory-Regions (noted as RR) in the 5' region of each gene with putative Myc/P53 binding sites (Messeguer et al., 2002) (**Table 4**). We found significantly enriched Sin3a occupancy in the 5' regulatory regions of *Kcnh2*, *Esyt1*, *Bnip3*, *Casp3*, and *Ing1*, *Cltb*, *Rab11a*, *Aldoa*, *Hsp90b1*, and *Hspe1* (**Figure 30**). Corresponding to these data, qRT-PCR assays detected significantly decreased transcription of *Kcnh2* but increased expression of *Bnip3*, *Casp3*, *Ing1*, *Rab11a*, *Aldoa*, and *Hspe1* in P4 *Sin3a*$^{\Delta endo}$ islets, matching the scRNA-seq results (**Figure 32 and Table 6**). We did not detect Sin3a enrichment in the tested regions of *Ins1*, *Gcg*, *Calr*, *Arl6*, *Ergic3*, and *Ucn3* genes (**Figure 31**). Intriguingly, we

also found enriched Sin3a binding to the 5' regions of *Slc2a2* and *Mafa*, although their expression levels remain unchanged in P4 *Sin3a*$^{\Delta endo}$ β-cells (**Table 6**). It is not clear if Sin3a directly regulates these two genes in older β-cells.

Loss of both *Sin3a* and *Sin3b* in the endocrine lineage causes diabetes and reduces β-cell mass by P5

Sin3a and Sin3b have been found to have some redundant functions in different cell types (Chaubal and Pile, 2018; Kadamb et al., 2013). Sin3a and Sin3b are co-produced in the pancreas and *Sin3b* was upregulated upon loss of *Sin3a* in pancreatic β-cells, indicating there may be redundant functions of Sin3a and Sin3b in the pancreas. To examine the roles of Sin3a and Sin3b in the pancreatic endocrine progenitors, I generated *Sin3a*$^{F/-}$*; Sin3b*$^{F/-}$*; Neurog3-Cre* (termed *Sin3a/3b*$^{\Delta endo}$) mice. I found that *Sin3a/3b*$^{\Delta endo}$ mice became diabetic before P5 (**Figure 33A**), at least a week earlier than *Sin3a*$^{\Delta endo}$ mice (**Figure 14B**), accompanied by largely reduced β-cell and α-cell production (**Figure 33B**). These findings support the redundant function of *Sin3a* and *Sin3b* for the production/survival of islet β cells.

Table 6: Several candidate genes studied by ChIP-PCR and qRT-PCR assays

Function category	Genes	LogFC	Pct. mut	Pct. con	P_value_adj	Sin3a binding				Mafa sites
						Epiblast (Seki et al., 2014)	**ES Ab1*** (Williams et al., 2011)	**ES Ab2*** (Williams et al., 2011)	**Muscle** (van Oevelen et al., 2010)	**Islets** (Tennant et al., 2013)
hormone	*Ins1*	-0.838	0.998	1.00	4.20E-120					yes
Function/ maturation	*Slc2a2*	-0.002	0.911	0.843	1.00					
	Mafa	-0.007	0.002	0.005	1.00	yes	yes		yes	
	Ucn3	-1.041	0.373	0.715	1.65E-106					
	Pdx1	-0.143	0.531	0.544	1.00					
channel	*Kcnh2*	-0.728	0.394	0.541	8.89E-40	yes	yes	yes		
Ca^{2+} homeostasis	*Esyt1*	0.588	0.764	0.355	4.10E-133		yes	yes		
	Calr	0.344	0.985	0.923	3.09E-70		yes	yes		
cell death	*Bnip3*	0.572	0.777	0.363	1.32E-113		yes			
	Casp3	0.617	0.723	0.277	1.89E-131		yes			
	Ing1	0.683	0.770	0.332	3.65E-140		yes		yes	
lipid transport	*Arl1*	0.324	0.397	0.125	2.32E-57	yes	yes	yes		
	Cltb	0.346	0.854	0.608	3.02E-55		yes	yes		
	Ergic3	0.335	0.760	0.491	8.56E-48		yes	yes	yes	
	Rab11a	0.324	0.790	0.522	7.11E-49		yes	yes		
metabolism	*Aldoa*	0.609	0.757	0.368	5.93E-123	yes	yes	yes		
	Idh3a	0.542	0.794	0.318	1.59E-105		yes	yes	yes	
Stress response	*Hsp90b1*	0.596	0.982	0.883	9.66E-171			yes	yes	
	Hspe1	0.709	0.885	0.552	3.48E-151		yes	yes		
Paralog	*Sin3b*	0.198	0.437	0.225	2.85E-33					

Shown are the reported function, log-fold change (logFC) of mutant over control β-cells, percentage of mutant (Pct. Mut) or control (Pct. Con) β-cells that expressed each gene, and adjusted p-values (P_value_adj). Also indicated are if Sin3a or Mafa was enriched in putative cis-regulatory regions of each gene, from published data, with references number provided in Row 1. (*: the data were from a single publication, with two antibodies used for ChIP-seq).

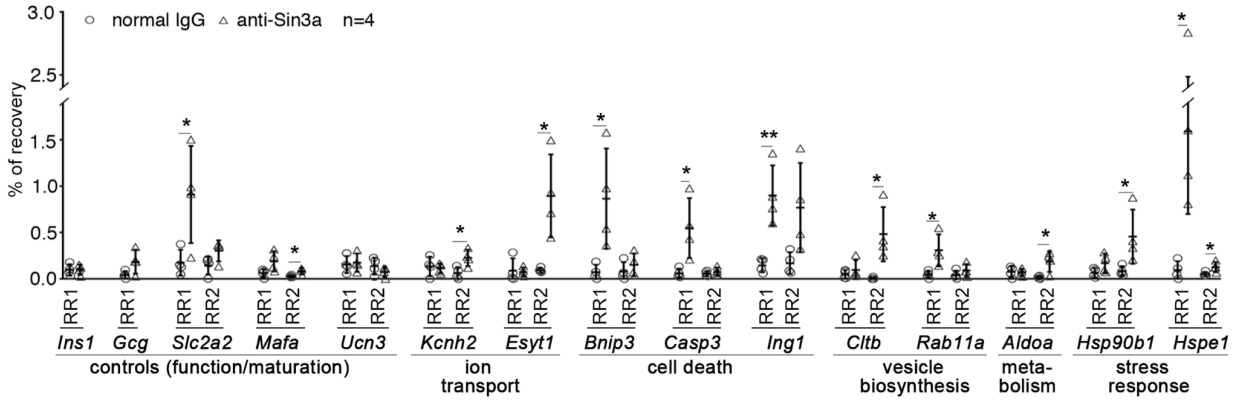


Figure 30: ChIP-PCR reveals several potential Sin3a target genes in MIN6 β-cells. ChIP-PCR assays of Sin3a-associated DNA RR of several genes. % of chromatin recovery was shown (n = 4, batches of chromatin preparations). Results of two RRs for each gene were presented. Normal IgG was used as control. The selected genes were grouped according to their reported functions (see text). *p<0.05, **p<0.01.

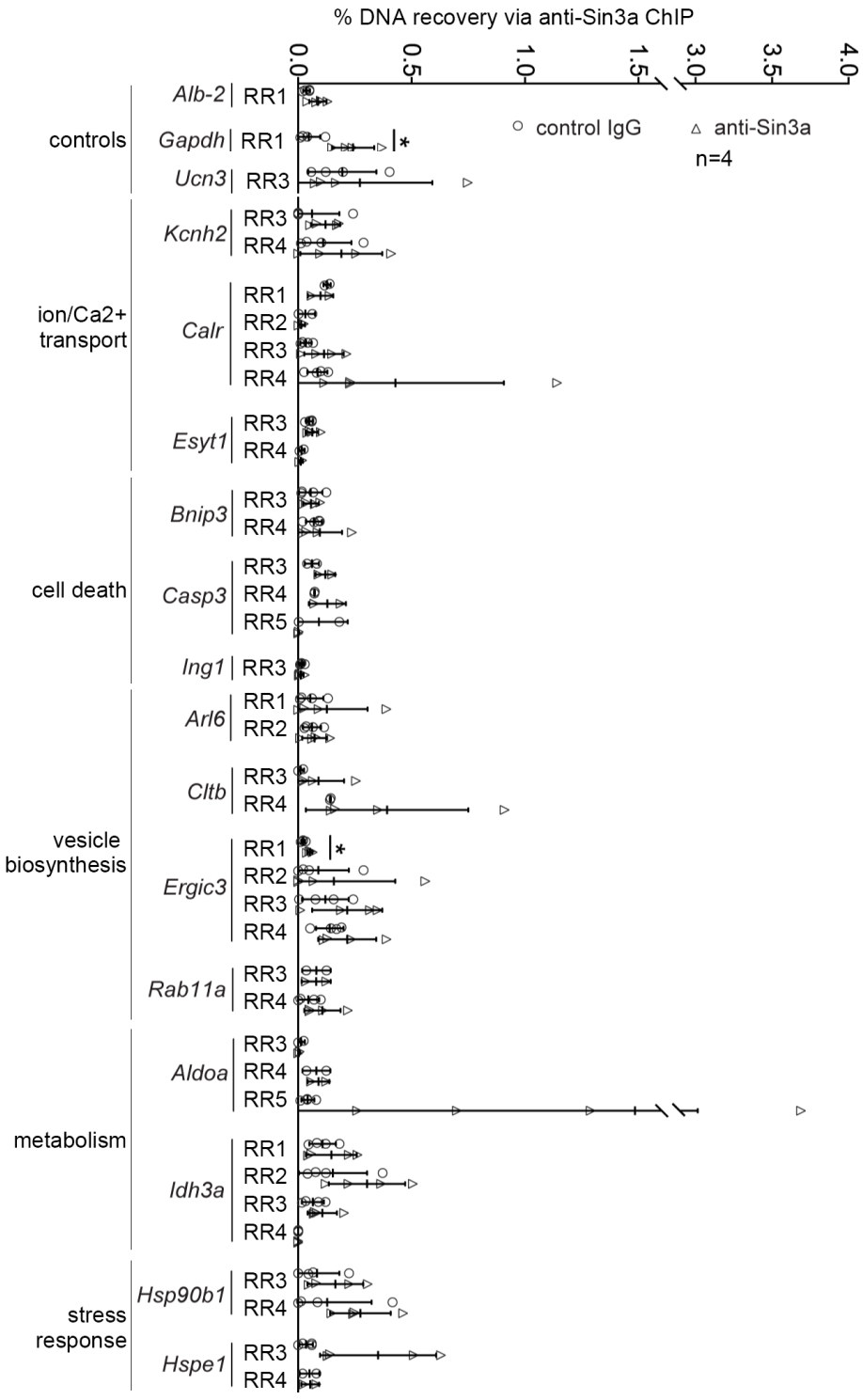


Figure 31: ChIP-PCR assays reveal several Sin3a target genes.

ChIP-PCR experiments were performed to identify Sin3a targets in Min6 cells. n = 4, four batches of chromatin preparations. *p<0.05.

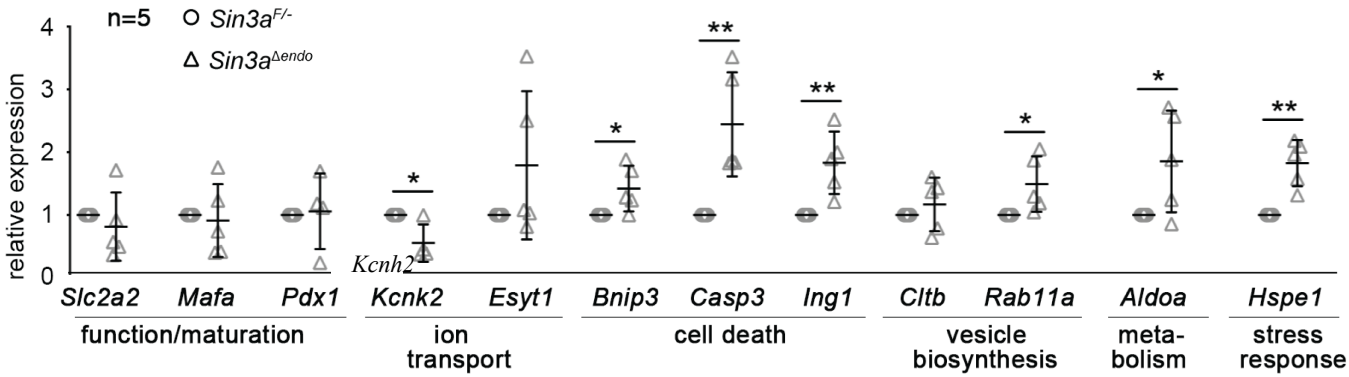


Figure 32: QRT-PCR assays of gene transcription in P4 islets.

The results were normalized against that of *Gapdh*. We then artificially set the relative expression level in control islets at 1.0 for comparison. n=5, batches of islets, including 1-3 individual mice for each batch. **p*<0.05, ***p*<0.01.

Loss of both *Sin3a* and *Sin3b* in the pancreatic progenitors reduces the generation of endocrine progenitors and endocrine cells

I then examined the roles of Sin3a and Sin3b in the pancreatic cells. *Sin3a*$^{F/F}$; *Sin3b*$^{F/F}$; *Pdx1*^{Cre} (termed *Sin3a/3b*$^{\Delta Panc}$ mice) and *Sin3a*$^{F/F}$; *Sin3b*$^{F/F}$; *Pdx1*^{Cre}; *Ai9* mice were derived. In the former, *Sin3a* and *Sin3b* were inactivated in the majority of pancreatic β-cells on P1 (**Figure 34A**). In the latter, the *Ai9* Cre-reporter allele in the latter marked *Sin3a/Sin3b*-deficient pancreatic cells with tdTomato (tdT) (**Figure 34E**) (Liu et al., 2019a). At E15.5 and P1, there was substantially reduced production of β- and α-cells in *Sin3a/3b*$^{\Delta Panc}$ pancreata (**Figure 34B-C**), accompanied by reduced Neurog3+-cell production (E15.5, **Figure 34D**) and increased β-cell death (P1, **Figure 34E**). Notably, the majority of pancreatic cells in *Sin3a*$^{F/F}$; *Sin3b*$^{F/F}$; *Pdx1*^{Cre}; *Ai9* mice expressed tdT, suggesting that *Sin3a/3b*-deficient pancreatic cells do not die immediately after *Sin3a/3b* inactivation (**Figure 34F**).

Discussion

The mammalian Sin3a and Sin3b paralogs are scaffold proteins for the overall Sin3 coregulator complex that can associate with common TFs, such as Foxo, Mad1, Myc, and P53 (Kadamb et al., 2013; Langlet et al., 2017), and β-cell factors Mafa and Myt TFs (Scoville et al., 2015). These associations recruit HDACs and histone lysine methylases or demethylase to regulate gene transcription in a highly cell-context-dependent manner (Kadamb et al., 2013). Inactivating *Sin3a* singly in the early mouse

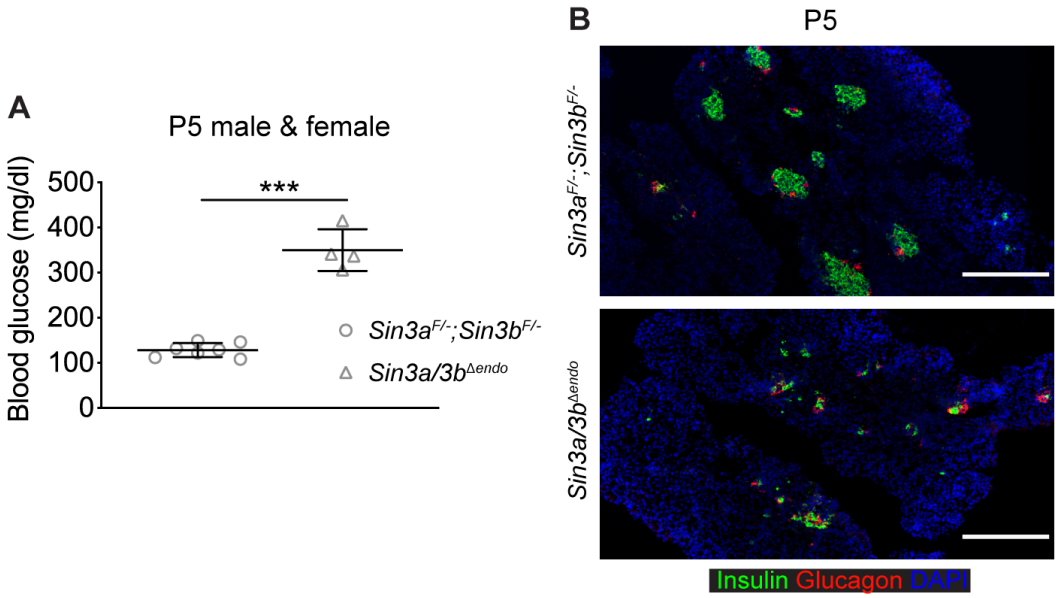


Figure 33: Loss of both *Sin3a* and *Sin3b* in the endocrine lineage causes diabetes and reduced β-cell mass by P5.

(A) Random-fed blood glucose levels of *Sin3a*$^{F/-}$*; Sin3b*$^{F/-}$ control and *Sin3a/3b*$^{\Delta endo}$ mice (n = 4-7). (B) IF staining of insulin and glucagon in control and double mutant pancreata on P5. DAPI was used for counterstaining. Scale bar = 200 μm. ***p<0.001.

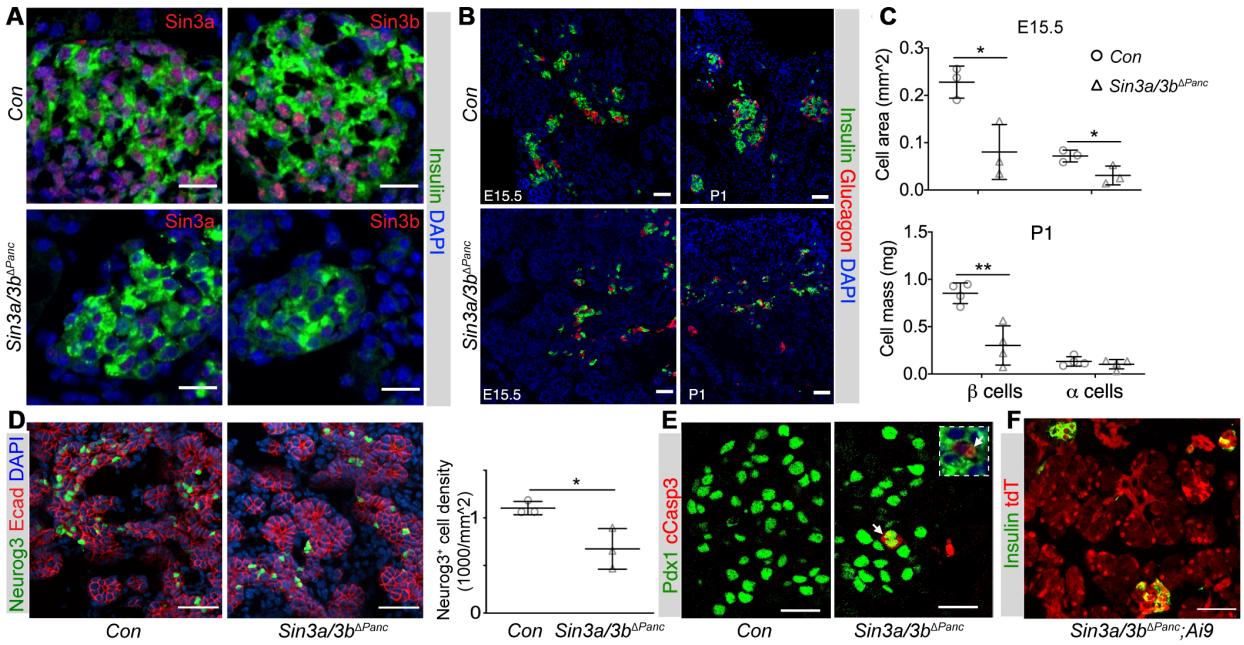


Figure 34: *Sin3a* and *Sin3b* have redundant function in protecting pancreatic cell survival.

DAPI was used to label nuclei in all panels except E and F. Controls (Con) used were *Sin3a*$^{F/F}$*; Sin3b*$^{F/F}$ mice/embryos. (A) IF assays showing effective *Sin3a* and *Sin3b* inactivation in P1 *Sin3a/3b*$^{\Delta Panc}$ pancreata. B) Insulin/glucagon expression in E15.5 and P1 control or *Sin3a/3b*$^{\Delta Panc}$ pancreata. (C) Quantification of islet cells in E15.5 and P1 pancreata. (D) *Neurog3* expression and quantification in E15.5 control and *Sin3a/3b*$^{\Delta Panc}$ pancreata. E-cadherin (Ecad) was used to label all pancreatic cells. (E) The presence of dying β cells in *Sin3a/3b*$^{\Delta Panc}$ pancreas, stained for Pdx1. The inset at the top-right corner was stained for insulin (green). (I) The presence of tdT^{+} pancreatic cells in *Sin3a*$^{F/F}$*; Sin3b*$^{F/F}$*; Pdx1*^{Cre}*; Ai9* (*Sin3a/3b*$^{\Delta Panc}$*; Ai9*) pancreas. A, E, scale bars = 20 μm. B, D, F scale bars = 50 μm. *p<0.05, **p<0.01, ***p<0.001.

pancreatic endocrine lineage has little effect on islet-cell differentiation, but substantially reduces postnatal β-cell function and survival without affecting the survival of δ-cells. The *Sin3a*-deficient β-cells showed defective insulin secretion, cell survival, Ca^{2+} influx, and insulin-vesicle biogenesis. Moreover, Sin3a associates with putative enhancers of several genes involved in ion transport/Ca^{2+} homeostasis, cell death, membrane trafficking, glucose metabolism, and stress response in β-cells. Intriguingly, inactivating both *Sin3a* and *Sin3b* in the same endocrine lineage resulted in further reduction of endocrine-cell numbers by birth, while co-inactivating both *Sin3a* and *Sin3b* in the early pancreatic MPCs substantially reduced production of endocrine progenitors without preventing their differentiation into hormone^{+} islet cells.

Sin3a is not required for β-cell differentiation

Sin3a inactivation compromises early steps in the differentiation and/or survival of several cell types including ES cells (Cowley et al., 2005; Dannenberg et al., 2005), muscle cells (van Oevelen et al., 2010), male germ cells (Pellegrino et al., 2012), lung progenitors (Yao et al., 2017), but, as shown here, does not appear to affect most of the process of islet β-cell differentiation. Pancreatic β-cells could use the Sin3a-TF complexes in a different manner from those other progenitor-cell types, such that they are only essential after most of the differentiation pathway has been completed. The known Sin3a-interacting TFs such as Mafa (Scoville et al., 2015), Myt TFs (Romm et al., 2005; Scoville et al., 2015), and Foxo1 (Langlet et al., 2017) fit into this latter profile. Alternatively, it is possible that Sin3a is also required for islet-cell differentiation from endocrine progenitors. In this case, *Neurog3-Cre*-mediated *Sin3a* gene inactivation

might have still allowed relatively long-lived Sin3a protein to function in driving differentiation. Sin3a protein levels could be measured in Pax6+ endocrine precursors using immunofluorescence staining to examine Sin3a protein perdurance after gene inactivation. However, we consider the latter possibility unlikely because inactivating *Sin3a* and *Sin3b* in all pancreatic progenitors using an earlier-acting *Pdx1-Cre* transgene also did not prevent the differentiation of Neurog3+ cells into hormone+ islet cells.

Sin3a is required for postnatal β-cell survival

Loss of *Sin3a* did not affect postnatal β-cell proliferation but increased their apoptosis. β-cell actual proliferation rate (%/hour) can be calculated as Ki67+ frequency (%) X 0.025 (the conversion factor), and β-cell actual apoptotic rate (%/hour) can be calculated as cCasp3+ frequency (%) X 0.41 (the conversion factor) (Saisho et al., 2009; Yang and Johnson, 2013). If only taking proliferation and apoptosis into account, net β-cell generation rate (%/hour) can be calculated as [Ki67+ frequency (%) X 0.025] – [cCasp3+ frequency (%) X 0.41], which was around 0.26, 0.26, 0.16 in control mice on P1, P7, and P14, respectively, and around -0.04, -0.03, -0.29 in $Sin3a^{\Delta endo}$ mice on P1, P7, and P14, respectively. As a result, the reduced β-cell mass in $Sin3a^{\Delta endo}$ mice was mainly caused by the negative postnatal β-cell generation rate. Moreover, the reduced β-cell mass might also be caused by β-cell dedifferentiation, which can be examined by immunostaining aldehyde dehydrogenase 1a3 (ALDH1A3) and gastrin, the markers of failing or dedifferentiated β-cells (Gómez-Banoy et al., 2019).

Sin3a regulates β-cell function and survival by interacting with different TFs

The absence of *Sin3a* resulted in β-cell dysfunction and late-onset diabetes. These phenotypes are consistent with Sin3a being the coregulator of Mafa (Scoville et al., 2015), Myt TFs (Romm et al., 2005; Scoville et al., 2015), and Foxo1 (Langlet et al., 2017), factors that are mainly essential for maintaining the mature β-cell state and function but not for most of the β-cell differentiation program (Buteau and Accili, 2007; Hang and Stein, 2011; Huang et al., 2018; Liu et al., 2019a; Mall et al., 2017; Nishimura et al., 2015; Wang et al., 2007).

However, it is important to point out that the *Sin3a*-deficient mice developed overt diabetes before weaning, while the individual Mafa-, Myt TF-, or Foxo1-deficient mice all do so a significant period afterward. An implication is that *Sin3a* inactivation may be considered equivalent to inducing the concurrent inactivation of all these identified Sin3A-interacting TFs. Alternatively, Sin3a may also mediate the function of additional TFs required for β-cell function and/or survival, e.g., Myc and P53, which are ubiquitous in most cell types. Our findings are consistent with the latter possibility. ChIP-PCR assays have shown that Sin3a is enriched in putative regulatory regions of several genes in β cells that contain recognizable Myc/P53 binding sites. Moreover, products of these target genes regulate cell death, ion transport, lipid trafficking/vesicular biosynthesis, metabolism, and stress responses (**Table 6**). Thus, our collective findings are consistent with the idea that Myc/P53 could recruit Sin3a in β-cells to promote β-cell function and survival by ensuring overall survival as well as insulin-vesicle biosynthesis, and efficient stimulus-secretion coupling.

Sin3a has been functionally diagnosed as both a coactivator and corepressor. Consistent with this notion, we found both downregulated and upregulated genes in *Sin3a*-deficient β-cells, with many being reported as directly regulated by Sin3a in other cell types. Our candidate ChIP-PCR assays corroborated enrichment of Sin3a in putative regulatory regions of Sin3-repressed (e.g., *Hspe1*, *Casp3* etc.) and -activated genes (*Kcnh2*) in β-cells, underscoring the bi-directional regulatory roles of Sin3a. The changed expression of non-Sin3a targets in *Sin3a*-deficient β-cells, such as *Ucn3*, is probably caused by secondary effects.

Sin3a plays stage and/or cell type-specific roles in the pancreas

Sin3a is required for postnatal α- and β-cell, but not δ-cell, survival. It is possible that Sin3a regulates similar gene sets in all islet-cell types but that the cellular context dictates resistance to cell-death pathways. Alternatively, different islet-cell types may use different Sin3a-TF complexes to regulate transcription. Follow-up identification of these complexes using proteomic studies could address this question as discussed in Chpater 3.

Although Sin3 (either Sin3a or Sin3b) is required in MPCs to promote endocrine specification, their overall activities are dispensable for a large portion of the differentiation of committed endocrine progenitors, at least into the stage when they have progressed forward into hormone-expressing islet cells. Therefore, our collective findings, combined with the reported roles of Sin3a in the differentiation of several non-pancreatic cells (Cowley et al., 2005; Dannenberg et al., 2005; Pellegrino et al., 2012; van Oevelen et al., 2010; Yao et al., 2017), highlight the idea that pancreatic cells use the Sin3-TF

complexes differentially in a stage and/or cell type-specific manner for differentiation, function, and survival.

Sin3a and Sin3b may share redundant functions in the pancreas

Although Sin3a but not Sin3b is required for maintaining postnatal β-cells function, both paralogs are required for β-cell survival. Thus, inactivating *Sin3b* expedited islet-cell loss in *Sin3a-null* islets. It is possible that Sin3a and Sin3b regulate a similar set of molecular targets, yet Sin3a contributes a higher proportion of such activity because of a higher affinity for transcriptional effectors. Alternatively, Sin3a and Sin3b may regulate different sets of target genes that have similar function(s). In either case, it appears that the total Sin3 activity has to achieve a certain threshold to maintain normal β-cell fitness. Note that the complete list of key Sin3a/Sin3b targets in β-cells remains unknown. Future genome-wide ChIP-seq studies in primary β-cells using specific Sin3a and Sin3b antibodies, combined with examining the transcriptomes of the few *Sin3a/Sin3b*$^{\Delta endo}$ and/or *Sin3a/Sin3b*$^{\Delta panc}$ β-cells that are formed, may shed more light on how Sin3a/Sin3b regulate β-cell genes in preventing diabetes.

In summary, our findings show that the Sin3 coregulator plays essential roles in islet-cell production and postnatal β-cell function and survival, with Sin3a being the major contributor. Thus, modulating Sin3a levels or activities could be explored to protect β-cell fitness and to control diabetes initiation or progression.

CHAPTER 3

CONCLUSIONS AND FUTURE DIRECTIONS

Overall conclusions of my dissertation

TFs in pancreas development and function have been very well studied. To better understand the intricate and probably highly cross-interacting gene regulatory networks that drive pancreas development and maintaining pancreas function, the roles of the coregulators that mediate the intricate combined functions of TFs in the pancreas need to be better examined. The mammalian Sin3a is an important transcriptional coregulator that directs cellular differentiation, survival, and function, but its roles in the pancreas were not clear before my studies. The main questions that I addressed in my dissertation were: 1) is Sin3a required for the development and function of the pancreas, especially β-cells? 2) If so, how does Sin3a regulate the processes? I found that Sin3a and its close paralog Sin3b are produced in most cells of the embryonic pancreas, and gradually become enriched in the postnatal endocrine pancreas in mice, indicating their potential roles in the pancreas development and function. Inactivating *Sin3a* singly in the early mouse pancreatic endocrine lineage has little effect on islet-cell differentiation, but substantially reduces postnatal β-cell function and survival. The *Sin3a*-deficient β-cells showed defective insulin secretion, cell survival, Ca^{2+} influx, and insulin-vesicle biogenesis. Sin3a associates with putative enhancers of several genes involved in ion transport/Ca^{2+} homeostasis, cell death, membrane trafficking, glucose metabolism, and stress response in β-cells. Inactivating both Sin3a and Sin3b in the same endocrine lineage resulted in further reduction of endocrine-cell numbers by birth,

while co-inactivating both Sin3a and Sin3b in the early pancreatic MPCs substantially reduced production of endocrine progenitors without preventing their differentiation into hormone+ islet cells. The data support that Sin3a and Sin3b may have redundant functions in the production/survival of islet β-cells. They may regulate a similar set of molecular targets or different sets of targets that have similar function(s) that are required for β-cell production/survival. The findings highlight the specific temporal requirements for the presumed "general" coregulators Sin3a and Sin3b in the production and/or survival of islet β-cells, with Sin3a being dispensable for differentiation but required for postnatal function and survival.

Future directions

Are there additional unknown TFs interacting with Sin3a in mouse β-cells and other islet cell types?

Knocking out *Sin3a* in the endocrine progenitors caused more severe defects on β-cell maturation and function than caused by the individual inactivation of *Mafa* or *Foxo1*. One implication is that *Sin3a* inactivation may be equivalent to the co-inactivation of *Mafa* and *Foxo1*. Another possibility, which we consider more likely, is that Sin3a is a broadly used coregulator, also interacting with additional TFs required for β-cell function and/or survival, such as Myc and P53. Indeed, ChIP-PCR assays detected Sin3a enrichment in putative Myc/P53-binding enhancers of several genes in β-cells. Identifying Sin3a-interacting TFs in mouse β-cells may reveal novel TFs that are required for β-cell maturation, function, and survival, and I discuss approaches towards

this goal below. Moreover, I discovered that different islet cell types have different levels of Sin3a dependency, with α- and β-cells, but not δ-cells, requiring Sin3a for postnatal survival. Sin3a may interact with different sets of TFs that vary or overlap between different islet cell types and thus control the initiation, stabilization, and maintenance of gene regulatory networks. The stage-specific effects of Sin3a (the requirement of Sin3a to maintain gene regulatory networks of β-cells at postnatal stages) may be due to postnatal metabolic, exocytotic, and other physiological changes of β-cells (Liu and Hebrok, 2017).

The reversible cross-link immunoprecipitation (Re-CLIP) and mass spectrometry (MS) is one method that could be re-applied towards identifying Sin3a-interacting TFs in mouse β-cells and other islet cell types (Scoville et al., 2015; Smith et al., 2011). It may be difficult to collect enough materials for RE-CLIP experiments using primary cells. As a result, cell lines could be used. For β-cells, MIN6 cells are good cell line models, which are derived from a mouse insulinoma and have relatively high glucose-responsive insulin secretion and high expression of key β-cell TFs (Ishihara et al., 1993). The results may reveal different sets of TFs interacting with Sin3a in different islet cell types. But even then, within the same cell types, the Sin3a interactions with TFs could also vary by developmental and maturation stages, or under different physiological and pathophysiological conditions. Moreover, heterogeneity of endocrine cell types also arises as an important consideration, and so understanding the exact Sin3a-TF couplings that operate dynamically might be a difficult challenge. Nonetheless, “bulk” clues as to function and complex sub-types might be gained, including novel Sin3a-interacting TFs that may be identified, which may shed light on the gene regulatory

networks of postnatal β-cell maturation processes. Modulating the interactions between Sin3a and TFs in β-cells could be explored to promote the maturation of β-cells generated *in vitro* and maintain β-cell function and survival to treat diabetes.

Direct and indirect Sin3a and Sin3b targets in mouse β-cells

ChIP-PCR assays detected Sin3a enrichment in putative Myc/P53-binding enhancers of several genes in β-cells, including genes regulating cell membrane polarization, Ca^{2+} homeostasis, cell death, lipid transport and vesicular biosynthesis, glucose metabolism, and stress responses. The identified Sin3a targets indicate that Sin3a directly genes that are involved in key β-cell functions. Our findings and previous studies suggested that Sin3a and Sin3b may have redundant functions and have shared targets. However, the complete list of Sin3a/Sin3b targets in β-cells remains unknown. To better understand the functions of Sin3a and Sin3b in β-cells, future studies could use genome-wide ChIP-seq studies to examine the Sin3a and Sin3b targets. As transcriptional coregulators, Sin3a and Sin3b do not have DNA binding activity (Silverstein and Ekwall, 2005). They are recruited by TFs to regulate the expression of target genes (Silverstein and Ekwall, 2005). Because the interactions between coregulators and TFs are hyper-dynamic and labile, a two-step crosslinking method using disuccinimidyl glutarate and formaldehyde (Tian et al., 2012) can be used for Sin3a/Sin3b ChIP-seq to protect both Sin3a/Sin3b-TF and TF-chromatin interactions. Compared to conventional formaldehyde-mediated protein-chromatin fixation, the two-step crosslinking method first uses disuccinimidyl glutarate to crosslink proteins and then uses formaldehyde to crosslink protein-chromatin (Tian et al., 2012),

which can conserve Sin3a/Sin3b-TF-chromatin interactions and achieve better signal-to-noise ratio.

As Sin3a and Sin3b can act as both coactivators and corepressors, it will provide more information on how Sin3a and Sin3b regulate the expression of their targets in β-cells by combining the ChIP-seq data with the transcriptomic data of β-cells deficient in *Sin3a* and/or *Sin3b*. *De novo* motif analysis of the ChIP-seq data will indicate the TFs interacting with Sin3a/Sin3b in β-cells, which, together with the Re-CLIP method, will provide more information about Sin3a/Sin3b-TF interactions. In addition, to better understand how Sin3a/Sin3b affect the expression of their targets, auxin-inducible degron system (Li et al., 2019) can be used to induce the degradation of Sin3a/Sin3b. An auxin receptor and Sin3a/Sin3b tagged with an auxin-inducible destabilizing domain will be expressed in β-cells. The controlled and fast deletion of Sin3a/Sin3b proteins can be achieved by adding auxin class small molecules (Li et al., 2019). Then the expression of their targets at different time points of the treatment can be examined using RNA-seq.

How do Sin3a and Sin3b regulate the chromatin landscapes, histone markers, and target gene availability in β-cells?

Sin3 binds chromatin-modifying enzymes, including HDACs, demethylases, and methyltransferases, which would modify histone and DNA structures to change chromatin structure and gene expression. Future studies should examine how loss of *Sin3a/Sin3b* affects histone markers, chromatin accessibility, and DNA methylation using histone marker ChIP-seq, Assay for Transposase-Accessible Chromatin using

sequencing (ATAC-seq), and bisulfite sequencing, respectively. The experiments may reveal the important roles of Sin3a and Sin3b in regulating histone markers and chromatin accessibility due to the interactions between Sin3 and HDACs. Identifying the epigenetic mechanisms maintaining β-cell function and survival would provide information for designing better drugs to treat diabetes.

Sin3a and Sin3b function in mature mouse β-cells

Both Sin3a and Sin3b are highly enriched in the endocrine pancreas at adult stages, indicating a role not just in guiding differentiation and proper proliferative properties of cells over the postnatal-to-weaning transition period, but also likely potential ongoing functions for Sin3a and Sin3b in mature islet cells, especially β-cells. Sin3a and Sin3b may regulate different sets of genes in mature β-cells (in this context, I mean post-weaning and adult) from those in immature β cells because the Sin3-interacting TFs, or additional coregulators, may vary between mature and immature β-cells. As a result, it would be interesting to examine how Sin3a and Sin3b regulate the functions of mature β-cells by inactivating *Sin3a/Sin3b* only in β-cells at adult stages using the temporally controlled inducible conditional inactivation, for example using the Lox/CreER system. Based on the functions of Sin3a-interacting TFs in β-cells, such as Mafa, Foxo1, Myc, loss of *Sin3a/Sin3b* in mature β-cells may impair their maturity, insulin secretion, stress responses, and survival. The defects may be caused by changed β-cell chromatin landscapes and the expression of target genes. The data may reveal how Sin3a and Sin3b regulate genes maintaining mature β-cell function, identity, and survival.

Sin3a and Sin3b regulation of gene regulatory networks in human β-cells

There are both similarities and differences in the expression patterns of key TFs between mouse and human β-cells. For example, Mafb is expressed in adult β-cells in human but not in the adult mouse, and Slc2a2 is highly expressed in mouse β cells but not in human β cells (Benner et al., 2014). The differences indicate that there are some limitations of using mice to model human β-cells. As a result, it would be important to examine the expression pattern of Sin3a and Sin3b in the human pancreas under physiological and pathophysiological conditions, such as obesity and diabetes. Short interfering RNAs could be used to knock down *Sin3a/Sin3b* in human β-cell lines and human islets to examine whether and how Sin3a and Sin3b regulate the function and survival of human β cells. The data may improve our understanding of how Sin3a/Sin3b mediate the gene regulatory networks of human β-cells. If Sin3a/Sin3b is required for human β-cell function and/or survival, modulating Sin3a/Sin3b levels or their interactions with TFs could be explored to protect β-cell function and/or survival to control diabetes initiation and progression.

How is the spatiotemporal pattern of expression of Sin3a and Sin3b in the pancreas achieved?

Sin3a and Sin3b are ubiquitously expressed in the embryonic pancreas but their expression is gradually enriched in the postnatal endocrine pancreas, but it is not clear how the spatiotemporal pattern of Sin3a and Sin3b production, which might be critical in allowing context-specific development of functions, is controlled at the embryonic and

postnatal stages. Future studies could identify the *cis*-regulatory regions of Sin3a and Sin3b, the transcriptional machinery operating through these regions and sequence motifs, and the changes of the chromatin landscapes of Sin3a and Sin3b during pancreas development. The knowledge of the regulation of Sin3a and Sin3b may identify upstream factors that may be required for pancreas development and function. Gain-of-function and loss-of-function studies could be used to examine the roles of the identified upstream factors in β-cell production, function, and survival. Moreover, the upstream factors could be used to modulate Sin3a/Sin3b levels to maintain β-cell function and survival.

Concluding Remarks

The goal of my dissertation is to examine whether and how Sin3a regulates mouse pancreas, especially β-cell, development, and functions. I found that Sin3a is required for postnatal β-cell function and survival by directly regulating key β-cell functional pathways. Moreover, there are redundant functions of Sin3a and its close paralog Sin3b in the production/survival of islet β-cells. The findings show that coregulators Sin3a and Sin3b play essential roles in islet-cell production and postnatal β-cell fitness, with Sin3a being the major contributor. Modulating Sin3a levels or activities could be explored to protect β-cell fitness and to control diabetes initiation or progression.

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