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MAFB in Pancreatic β-Cell Biology:

From Developmental Regulation to Diabetes Risk

SARA BSHARAT
DEPARTMENT OF LABORATORY SCIENCE | FACULTY OF MEDICINE | LUND UNIVERSITY

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About the Author

SARA BSHARAT is originally from Palestine and holds a master's degree in Molecular Biology from Lund University, where she continued her PhD studies in pancreatic biology and diabetes research. The overall aim of this thesis is to define the role of the transcription factor MAFB in pancreatic development and endocrine cell specification and function, with a particular focus on its contribution to β-cell maturation and pathways relevant to diabetes risk.

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Sara Bsharat

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University. To be publicly defended on 10th of February 2026 at 10.00 am in Agardhsalen at Clinical Research Centre, Department of Laboratory Medicine, Jan Waldenströms gata 35, 214 28 Malmö, Sweden.

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Abstract: Diabetes mellitus is the most common chronic metabolic disease, and loss or dysfunction of pancreatic β-cells is a central cause of impaired glucose homeostasis. However, the transcriptional mechanisms that control endocrine cell development and function remain incompletely understood. The MAF family of transcription factors, including MAFA and MAFB, plays critical roles in β-cell maturation and hormone secretion, and their expression is reduced in islets from individuals with Type 2 diabetes(T2D). During pancreatic development, MAFB is expressed not only in α- and β-cell progenitors but also in NEUROG3^{+} endocrine progenitors, suggesting an important role in lineage specification and islet formation. This thesis primarily investigates the regulatory functions of MAFB in three key contexts: regulation of insulin exocytosis, control of β-cell clustering and islet morphogenesis, and gene networks in endocrine progenitors, while MAFA is examined alongside MAFB only in the regulation of insulin exocytosis.

Results: In paper I the regulatory roles of MAFA and MAFB in controlling exocytosis-related genes were investigated. Analysis of MafA-deficient mouse islets, human islet transcriptomes, and β-cell knockdown models identified a conserved network of exocytotic genes, including STX1A and STXBP1, that are dependent on MAFA/MAFB. Loss of these transcription factors resulted in impaired insulin secretion, linking reduced MAFA/MAFB expression in T2D to defects in vesicle docking and fusion. In paper II, we demonstrated that loss of MafB in developing α- and β-cells disrupted islet and duct formation and reduced expression of neurotransmitter and axon guidance receptor genes. Single-cell sequencing confirmed that these receptors are co-expressed with MAFB in developing human β-cells and downregulated in both mouse and human MafB deficient progenitor cells. Functional inhibition of nicotinic acetylcholine receptors further impaired β-cell migration and clustering, demonstrating a novel role for neuronal signaling in islet morphogenesis. In paper III, single-cell transcriptomic analysis of MafB-deficient endocrine progenitors identified novel MAFB target genes, including AUTS2 and ETV1, which harbor T2D risk alleles in enhancer regions that are activated by MAF transcription factors. Functional validation of these targets will be important to define how MAFB influences lineage allocation and early endocrine cell differentiation.

Conclusion: This thesis establishes MAFB as a central regulator of endocrine progenitor cell specification, islet morphogenesis, and β-cell function, with MAFA contributing specifically to insulin exocytosis. These findings provide new insight into diabetes pathogenesis and inform strategies to generate functional β-cells for therapeutic purposes.

Key words: T2D, MAFA, MAFB, Pancreatic β-Cell, Endocrine progenitors, islet morphogenesis, transcriptional regulation, insulin exocytosis, neuronal signaling, single-cell transcriptomics.

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To my family

وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا

"And the bounty of Allah upon you is great"

Holy Quran. Surah An-Nisa (4:113)

Table of Contents

Popular Science Summary

Diabetes is one of the most rapidly increasing health challenges worldwide, affecting millions of people each year. At the centre of this disease are the insulin-producing β-cells in the pancreas, which are responsible for keeping blood sugar levels within a healthy range. When these cells fail to release enough insulin, reflecting an early loss of function, blood glucose rises, and diabetes develops. Although much progress has been made in understanding β-cell biology, we still do not fully understand how these cells are formed during development, how they maintain their function throughout life, and why they are particularly vulnerable in T2D.

This thesis focuses on a gene called MAFB, a transcription factor that acts as a molecular switch to turn other genes on or off. MAFB, together with a related gene called MAFA, plays key roles in building and maintaining healthy β-cells. Reduced levels of these factors have been observed in people with T2D, suggesting that problems in these regulatory networks could contribute to β-cell dysfunction and disease.

The first paper of this thesis investigates how MAFA and MAFB help β-cells release insulin. Insulin release depends on a finely tuned process called exocytosis, in which insulin-containing granules dock to the cell membrane and fuse to release their contents into the bloodstream. We showed that both transcription factors regulate many of the genes needed for this process, including *STX1A, STXBP1,* and *SYT7*. When MAFA or MAFB levels were experimentally reduced, β-cells secreted less insulin and key exocytosis genes were downregulated, resembling defects seen in T2D. We also identified a genetic variant in MAFA that lowers its expression in human islets and reduces the expression of several exocytosis genes, suggesting that some individuals may carry a genetic predisposition to weaker insulin secretion.

The second paper of the thesis focuses on the development pancreas. Before birth, endocrine cells (the hormone-producing cells of the pancreas) must migrate from the pancreatic ducts and cluster into small groups called islets of Langerhans. This is a highly coordinated process guided by signals from the surrounding tissue, including autonomic nerves. We found that embryos lacking MafB show major defects in islet formation: β-cells fail to migrate properly, remain attached to ducts, and do not form normal islet structures. These cells also lose many of the receptors needed to sense and respond to nerve-derived signals, such as nicotinic

acetylcholine receptors. Inhibiting these receptors in experimental systems impaired β-cell migration and clustering, demonstrating that nerve communication is essential for organizing the developing pancreas.

The final part of the thesis examines the earliest cells that will eventually become hormone-producing cells in the pancreas. Using single-cell analysis, we studied thousands of developing cells and found that without MafB, many of them become stuck and cannot decide whether to become β-cells or α-cells. Instead of maturing normally, they remain in an in-between state. We also identified several important genes, such as *AUTS2, ETV1, MEIS2*, and others, that MafB normally switches on to guide these cells toward their final identity. Some of which contain genetic variants linked to T2D. More studies on these genes are needed to understand exactly how MAFB helps early pancreatic cells choose whether to become β-cells or α-cells. These findings suggest that small changes in how MAFB works during early development could influence a person's risk of developing diabetes later in life.

Taken together, the findings in this thesis reveal that MAFB is a central regulator of β-cell development, communication, and function. It shapes the earliest endocrine progenitor cells, coordinates nerve-mediated signals that organize the developing pancreas and maintains insulin secretion in adult β-cells. MAFA and MAFB act together to support the genetic programs required for healthy β-cells, and disruptions in these pathways may contribute to the onset and progression of T2D. By clarifying the roles of these transcription factors, this work provides deeper insight into diabetes biology and supports the development of new strategies to generate or protect β-cells for therapeutic purposes.

Populärvetenskaplig sammanfattning

Diabetes är en av de snabbast växande globala hälsoutmaningarna och drabbar miljontals människor varje år. I centrum för denna sjukdom finns de insulinproducerande β-cellerna i bukspottkörteln, som har till uppgift att hålla blodsockernivåerna på en hälsosam nivå. När dessa celler inte längre kan frisätta tillräckligt med insulin, eller gradvis förlorar sin funktion, stiger blodsockret och diabetes utvecklas. Trots stora framsteg inom diabetesforskningen förstår vi fortfarande inte fullt ut hur β-celler bildas under utvecklingen, hur de behåller sin funktion genom livet och varför de är särskilt sårbara vid typ 2-diabetes (T2D).

Denna avhandling fokuserar på en gen som heter MAFB, en transkriptionsfaktor som fungerar som en molekylär strömbrytare som kan slå på eller av andra gener. MAFB, tillsammans med den närbesläktade genen MAFA, spelar viktiga roller i att bygga upp och upprätthålla friska β-celler. Minskade nivåer av dessa faktorer har observerats hos personer med T2D, vilket antyder att störningar i dessa regleringsnätverk kan bidra till β-cellsdysfunktion och sjukdom.

Den första delen av avhandlingen undersöker hur MAFA och MAFB hjälper β-celler att frisätta insulin. Insulinfrisättning beror på en finreglerad process som kallas exocytos**,** där insulinfyllda vesikler dockar mot cellmembranet och smälter samman med det för att tömma sitt innehåll i blodet. Vi visade att båda transkriptionsfaktorerna reglerar många av de gener som behövs för denna process, bland annat *STX1A***,** *STXBP1* och *SYT7*. När nivåerna av MAFA eller MAFB minskades experimentellt, frisatte β-cellerna mindre insulin och flera viktiga exocytosgener nedreglerades – ett mönster som liknar det som ses vid T2D. Vi identifierade också en genetisk variant i MAFA som minskar genens uttryck i mänskliga Langerhanska öar och samtidigt sänker uttrycket av flera exocytosgener. Detta tyder på att vissa individer kan ha en genetisk benägenhet för svagare insulinfrisättning.

Den andra delen av avhandlingen handlar om bukspottkörtelns utveckling. Innan födseln måste endokrina celler (de hormonproducerande cellerna i bukspottkörteln) migrera från gångarna och samlas i små grupper som kallas Langerhanska öar**.** Denna process styrs av signaler från omgivande vävnader, bland annat från det autonoma nervsystemet. Vi fann att embryon som saknar MafB uppvisar allvarliga defekter i ö-bildningen: β-cellerna migrerar inte som de ska, fastnar vid gångarna och bildar inte normala ö-strukturer. Dessa celler förlorar också många av de

receptorer som krävs för att uppfatta och svara på nervsignaler, exempelvis nikotinacetylkolinreceptorer. När dessa receptorer hämmades i experimentella modeller försämrades β-cellernas migration och klustring, vilket visar att nervsignalering är avgörande för hur bukspottkörteln organiseras under utvecklingen.

Den sista delen av avhandlingen undersöker de tidigaste cellerna som så småningom kommer att bli bukspottkörtelns hormonproducerande celler. Med hjälp av cellsanalys studerade vi tusentals utvecklande celler och fann att i frånvaro av MafB fastnar många av dem i ett mellanläge och kan inte bestämma sig för om de ska bli β-celler eller α-celler. Vi identifierade också flera viktiga gener – bland annat *AUTS2*, *ETV1* och *MEIS2* – som MafB normalt aktiverar för att styra cellerna mot deras slutliga identitet. Vissa av dessa gener innehåller dessutom genetiska varianter som är kopplade till T2D. Fler studier av dessa gener behövs för att förstå exakt hur MAFB hjälper tidiga pankreasceller att välja om de ska utvecklas till β-celler eller α-celler. Dessa fynd tyder på att små förändringar i hur MAFB fungerar under utvecklingen kan påverka risken att drabbas av diabetes senare i livet.

Sammanfattningsvis visar resultaten i denna avhandling att MAFB är en central regulator av β-cellsutveckling, kommunikation och funktion. MAFB formar de tidigaste endokrina progenitor cellerna, samordnar nervmedierade signaler som organiserar den utvecklande bukspottkörteln och upprätthåller insulinfrisättning i vuxna β-celler. MAFA och MAFB arbetar tillsammans för att stödja de genetiska program som krävs för friska β-celler, och störningar i dessa nätverk kan bidra till uppkomsten och utvecklingen av T2D. Genom att klargöra hur dessa transkriptionsfaktorer fungerar bidrar denna avhandling till en djupare förståelse av diabetes och kan stödja utvecklingen av nya strategier för att skapa eller bevara funktionella β-celler i framtida behandlingar.

Papers included in the thesis

I. Cataldo, L. R., Singh, T., Achanta, K., **Bsharat, S**., Prasad, R. B., Luan, C., Renström, E., Eliasson, L., & Artner, I. (2022). MAFA and MAFB regulate exocytosis-related genes in human β-cells. Acta physiologica (Oxford, England), 234(2).

II. **Bsharat, S**., Monni, E., Singh, T., Johansson, J. K., Achanta, K., Bertonnier-Brouty, L., Schmidt-Christensen, A., Holmberg, D., Kokaia, Z., Prasad, R. B., & Artner, I. (2023). MafB-dependent neurotransmitter signaling promotes β cell migration in the developing pancreas. Development (Cambridge, England), 150(6), dev201009.

III. **Bsharat, S.,** Achanta, K., Bertonnier-Brouty, L., Prasad, R., & Artner, I. Novel MAFB-regulated genes contribute to the genetic architecture of type 2 diabetes (Unpublished manuscript).

Author contribution to the papers

Paper I

For this paper, I was responsible for performing several of the experimental procedures, including immunohistochemistry and Western blot analyses, which contributed to the characterization and validation of key findings. In addition, I assisted in interpreting the experimental results and contributed to the manuscript, particularly by helping to prepare and refine the methodology section.

Paper II

In this study, I contributed by performing immunohistochemistry and by scanning and imaging the slides using confocal microscopy and the slide scanner. I also carried out the quantitative analysis of the imaging data. In addition, I participated in the interpretation of the results and contributed to discussions regarding the

scientific conclusions. I further supported the manuscript preparation by helping to write and revise selected sections, and I performed most of the additional experiments required during the revision of the manuscript.

Paper III

For this study, I contributed to the experimental design and performed several of the key laboratory procedures, including embryo dissections, cell culture, and qPCR analyses. I was responsible for preparing the samples for fluorescence-activated cell sorting (FACS) and for the subsequent single-cell RNA sequencing workflow. I also carried out immunohistochemistry to support the characterization of developmental phenotypes. In addition to these experimental contributions, I was actively involved in interpreting the results and leading scientific discussions throughout the project. Based on this work, I also wrote the manuscript.

Papers not included in the thesis

I. Huang, P., Venskutonytė, R., Wilson, C. J., **Bsharat, S**., Prasad, R. B., Gourdon, P., Artner, I., de Groot, B. L., & Lindkvist-Petersson, K. (2025). Structural insights into AQP3 channel closure upon pH and redox changes reveal an autoregulatory molecular mechanism. Nature Communications, 16, Article 10997. https://doi.org/10.1038/s41467-025-67144-2.

II. Bertonnier-Brouty, L., **Bsharat, S**., Achanta, K., Andersson, J., Pranomphon, T., Singh, T., Kaprio, T., Hagström, J., Haglund, C., Seppänen, H., Prasad, R. B., & Artner, I. (2025). Homeobox protein B6 and homeobox protein B8 control immune-cancer cell interactions in pancreatic cancer. Molecular biomedicine, 6(1), 48.

III. Bertonnier-Brouty, L., Andersson, J., Kaprio, T., Hagström, J., **Bsharat, S.**, Asplund, O., Hatem, G., Haglund, C., Seppänen, H., Prasad, R. B., & Artner, I. (2024). E2F transcription factors promote tumorigenicity in pancreatic ductal adenocarcinoma. *Cancer medicine*, *13*(9), e7187

Abbreviations

A	Alpha cell
ADA	American Diabetes Association
ADRA2A	Alpha-2A adrenergic receptor
ANS	Autonomic nervous system
Ach	Acetylcholine
B	Beta cell
ChIP	Chromatin immunoprecipitation
δ	Delta cell
E	Embryonic day
EP	Endocrine progenitor
FACS	Fluorescence-activated cell sorting
GLP-1	Glucagon-like peptide-1
GLUT	Glucose transporter
GSIS	Glucose-stimulated insulin secretion
GWAS	Genome-wide association studies
HbA1c	Haemoglobin A1c
MAREs	Maf Recognition Elements
MP	Multipotent progenitor
MafA	v-maf musculoaponeurotic fibrosarcoma oncogene homolog A
MafB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B
NGN3	Neurogenin 3
OGTT	Oral Glucose Tolerance Test
PSCs	Pluripotent stem cells
PCW	post-conception week

RNA-seq	Ribonucleic acid sequencing
RT-qPCR	Real-time quantitative polymerase chain reaction
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SNP	Single nucleotide polymorphism
SiRNA	Small interfering RNA
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TH	Tyrosine hydroxylase
VAChT	Vesicular acetylcholine transporter
VGCC	Voltage-gated calcium (Ca^{2+}) channel
WT	Wild type
eQTL	expression quantitative trait locus
nAChR	nicotinic acetylcholine receptor

Introduction

Diabetes mellitus

Diabetes mellitus is the most prevalent chronic metabolic disorder worldwide, characterized by impaired glucose homeostasis and often causing long-term complications. According to the World Health Organization, the global prevalence of diabetes has increased dramatically, rising from approximately 200 million cases in 1990 to an estimated 830 million in 2022, significantly contributing to the global disease burden (1). People diagnosed with diabetes have a two- to threefold increase in overall mortality risk compared with non-diabetic individuals (2). Beyond its impact on health, diabetes also imposes a substantial economic burden. In 2019, global health spending on diabetes reached approximately USD 760 billion, a figure projected to increase to USD 825 billion by 2030 and USD 845 billion by 2045 (3). This escalating economic pressure, together with the long-term complications of the disease, poses a major challenge to healthcare systems worldwide.

The etiology of diabetes mellitus remains incompletely understood. However, current evidence suggests that it is a multifactorial disease, arising from interactions between genetic susceptibility and environmental factors. A significant genetic component has been well established in the predisposition to disease. Genome-wide association studies (GWAS) have uncovered many genetic loci associated with T2D risk, revealing the polygenic nature of the disease (4). In parallel, environmental factors such as diet, physical activity, sleep patterns, psychological stress, and socioeconomic status have been recognized as major contributors that modulate individual susceptibility to the disease (5).

Developing diabetes mellitus significantly increases the risk of various complications that impact both health and life expectancy. Chronic hyperglycemia contributes to damage in small and large blood vessels, leading to microvascular complications such as retinopathy, nephropathy, and neuropathy, as well as macrovascular issues like cardiovascular disease and stroke (6). Clinically, diabetes is diagnosed when fasting plasma glucose is ≥7.0 mmol/L on two separate occasions, when the two-hour plasma glucose concentration during an oral glucose tolerance test (OGTT) is ≥11.1 mmol/L, or glycated hemoglobin A1c (HbA1c) is ≥6.5%. These diagnostic criteria provide essential insights into an individual's glycaemic status and guide appropriate clinical management (7). Typical manifestations of diabetes include fatigue, excessive thirst, frequent urination,

weight loss, blurry vision, recurring infections, and delayed wound healing. These signs result from the body's inability to regulate glucose levels effectively, causing dehydration and impairing immune responses (8).

Diabetes is commonly categorized into several types, with the two main ones being Type 1 diabetes(T1D) and Type 2 diabetes (T2D). T2D accounts for approximately 90-95% of all diabetes cases globally, while T1D comprises about 5-10% of cases. The American Diabetes Association (ADA) categorizes diabetes into four primary types: T1D, T2D, gestational diabetes mellitus (GDM), and specific forms of diabetes resulting from other causes. Table 1 provides an overview of prevalence, etiology, and key diagnostic criteria for various types of diabetes mellitus as classified by the ADA (9).

Table 1: Diabetes mellitus classification according to the ADA

Diabetes Type	Prevalence	Etiology	Key Diagnostic Criteria
Type 1 Diabetes	~5-10% of diabetes cases.	Autoimmune destruction of pancreatic β-cells leads to insulin deficiency.	High fasting glucose levels and presence of autoantibodies against pancreatic islet antigens.
Type 2 Diabetes	~90-95% of diabetes cases.	Insulin resistance combined with progressive β-cell dysfunction. Risk factors include obesity, physical inactivity, and genetic predisposition.	Elevated fasting glucose, increased HbA1c percentages, and abnormal results from OGTT.
Gestational Diabetes Mellitus (GDM)	Varies; approximately 8.3% of U.S. pregnancies.	Glucose intolerance was first detected during pregnancy.	OGTT, typically performed during the third trimester of pregnancy.
Other specific types	Less common.	Includes: Monogenic diabetes syndromes; diseases of the exocrine pancreas (e.g., cystic fibrosis); drug- or chemical-induced diabetes	Diagnostic approaches vary based on the specific subtype; genetic testing is frequently required.

Type 1 Diabetes

T1D is an autoimmune disease characterized by immune driven destruction of insulin-producing beta cells (β-cells) in the pancreas, leading to insulin deficiency (10). Insulin deficiency in T1D leads to elevated blood glucose levels, typically confirmed by the presence of diabetes-related autoantibodies and low C-peptide levels. T1D is recognized as a polygenic autoimmune disease, wherein multiple genetic variants contribute to an individual's susceptibility. Among of these, the human leukocyte antigen (HLA) region stands out as the most significant genetic factor, with certain HLA haplotypes markedly increasing the risk (11). T1D usually manifests during childhood or early adulthood and is managed with lifelong exogenous insulin treatment (12). In recent years, islet transplantation has emerged as an effective treatment for T1D (13). In this procedure, functional islets are isolated from the pancreas of a deceased donor and transplanted into the recipient's liver, where they can re-establish endogenous insulin production (14). While islet transplantation has shown clinical success in reducing or eliminating insulin dependence, the limited availability of donor pancreata significantly restricts its widespread application. This limitation underscores the urgent need for alternative cell-based therapies, such as the generation of functional islet-like cells from *in vitro* differentiation protocols.

Type 2 Diabetes

T2D is primarily characterized by a combination of insulin resistance and progressive β-cell dysfunction (15). Insulin resistance refers to the diminished responsiveness of peripheral metabolic tissues, specifically skeletal muscle, adipose tissue, and hepatocytes to insulin, resulting in impaired glucose uptake and utilization (16). Although insulin resistance is highly prevalent in the general population, affecting approximately 25% of adults, normoglycemia is often maintained because pancreatic β-cells compensate by increasing insulin secretion (17). However, insulin resistance alone does not cause T2D (18). The disease develops when this compensatory capacity fails, driven by a progressive decline in β-cell function and mass, thus β-cells can no longer secrete enough insulin to overcome the prevailing insulin resistance (19). Consequently, the interplay between insulin resistance and β-cell dysfunction forms the core pathophysiological basis of T2D, with β-cell failure being the critical determinant of disease onset and progression (20).

As previously discussed, hyperglycemia is associated with various environmental and lifestyle factors. Obesity significantly influences the development of T2D by impairing insulin sensitivity in peripheral tissues. Excess adipose tissue, particularly visceral fat, secretes pro-inflammatory cytokines and free fatty acids, which disrupt

insulin signalling pathways and reduce glucose uptake in muscle and adipose cells. This dysfunction contributes to the pathogenesis of insulin resistance and T2D (21).

Aging also is a significant risk factor for T2D. It adversely affects β-cell mass, proliferation, and regenerative capacity, resulting in impaired insulin secretion. These age-related changes hinder the pancreas's ability to meet metabolic demands, thereby increasing the risk of developing T2D in older adults (22).

Emerging evidence indicates that epigenetic modifications- heritable changes in gene expression without alterations in the DNA sequence, play a pivotal role in the pathogenesis of T2D. Environmental factors such as diet, physical activity, and aging can influence epigenetic mechanisms, including DNA methylation and histone modifications, thereby affecting genes involved in glucose metabolism and insulin signalling (23).

In addition to environmental influences, genetic predisposition plays a significant role in the development of T2D, particularly through its impact on pancreatic β-cell function. GWAS have identified numerous genetic variants associated with T2D susceptibility, with the *TCF7L2* gene being the most statistically significant. Each copy of the risk allele in *TCF7L2* increases the risk of developing T2D by approximately 41%, highlighting its substantial role in disease pathogenesis (24). T2D is recognized as a polygenic disorder, where variants across multiple genetic loci collectively determine an individual's overall genetic risk for developing the disease (4).

Recognizing T2D as a heterogeneous condition comprising various subtypes with distinct characteristics, underlying mechanisms, and complications is increasingly important. This heterogeneity significantly affects both disease progression and therapeutic outcomes. To better address this complexity, researchers have developed refined classification systems that go beyond the traditional binary classification of diabetes. In a key study, Ahlqvist and colleagues used data driven cluster analysis to identify five subgroups of adult-onset diabetes: severe autoimmune diabetes (SAID), severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD), and mild age-related diabetes (MARD) (25). Importantly, this classification was later shown in a large prospective study of the ANDIS cohort to be associated with distinct risks of microvascular and macrovascular complications, as well as mortality, independent of traditional risk factors, underscoring its clinical relevance (26). Implementing such classifications could help improve personalized treatment strategies by allowing healthcare providers to tailor interventions more effectively and identify patients at higher risk of complications early in the disease course.

Pancreatic islet

The pancreas is an abdominal organ with both exocrine and endocrine functions, classifying it as a heterocrine gland. Anatomically, it is situated in the upper abdomen, posterior to the stomach, and extends from the duodenum to the spleen (27).

The exocrine component constitutes approximately 85% of the pancreatic tissue and comprises acinar cells organized into clusters. These cells synthesize and secrete digestive enzymes, including trypsin, lipase, and amylase, which are essential for the breakdown of proteins, fats, and carbohydrates. These enzymes are delivered into the duodenum through a branched ductal system, enabling efficient nutrient digestion (28).

Scattered among the exocrine acinar cells are clusters of endocrine tissue known as the islets of Langerhans, which comprise approximately 1–2% of the total pancreatic mass. The estimated number of islets in the human pancreas ranges from 1 to 1.5 million (29, 30), whereas the mouse pancreas contains approximately 1,000–5,000 islets, a difference that reflects the proportional ratio between the pancreatic masses of the two species (31). Within these islets, endocrine cells are organized into distinct populations responsible for the secretion of different hormones. Approximately 60% insulin-producing β cells, 30% glucagon-secreting alpha (α) cells, and about 10% somatostatin-producing delta (δ) cells, along with smaller populations of pancreatic polypeptide (PP)-producing cells and ghrelin-secreting epsilon (ε) cells (32). While human and mouse pancreatic islets share a similar cellular composition, they differ in architecture and cellular distribution (31). In mouse islets, β-cells are predominantly located at the core, surrounded by a mantle of α-, δ-, and other endocrine cells. In contrast, human islets exhibit a more heterogeneous and intermingled structure, with α- and β-cells more evenly distributed throughout the islet (Figure1) (33). Additionally, mouse islets contain a higher proportion of β-cells, while human islets have a relatively higher abundance of α-cells. These structural differences are accompanied by species-specific variations in gene expression profiles and glucose responsiveness (34).

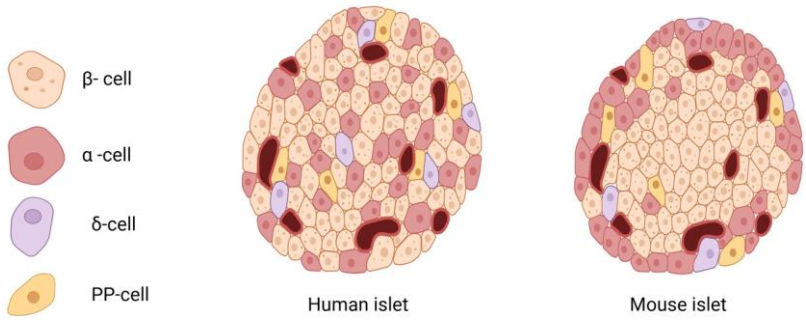

Figure1: Schematic illustration of human and mouse islet. Created with BioRender.com.

Pancreatic β-cells secrete insulin in response to elevated blood glucose levels, a process regulated by both intrinsic cellular mechanisms and extrinsic signals from other physiological systems. One key regulatory factor is the incretin hormone glucagon-like peptide-1 (GLP-1), which is secreted by intestinal L-cells upon nutrient ingestion. GLP-1 enhances glucose-stimulated insulin secretion (GSIS) from β-cells in a glucose-dependent manner, thereby reducing the risk of hypoglycaemia (35). Additionally, GLP-1 suppresses glucagon secretion from α-cells, contributing to the maintenance of glucose homeostasis (36).

In addition to hormonal and metabolic cues, the autonomic nervous system (ANS) comprising sympathetic and parasympathetic branches play a critical role in regulating islet function. The parasympathetic nervous system, primarily through the vagus nerve, promotes insulin secretion by releasing acetylcholine, which acts on muscarinic receptors on β-cells (37). Conversely, the sympathetic nervous system releases catecholamines like norepinephrine, which can inhibit insulin secretion via α2-adrenergic receptors and stimulate glucagon release from α-cells, particularly during stress or hypoglycaemia (38). The ANS can also participate in the control of insulin secretion indirectly via the regulation of pancreatic and islet blood flow (39). Moreover, pancreatic islet function is influenced by circulating signals from peripheral metabolic organs, including the liver, adipose tissue, bone, and skeletal muscle. These inter-organ communications play a crucial role in maintaining metabolic homeostasis and can influence islet hormone secretion and β-cell survival (38).

The β-cell and Insulin granules

Pancreatic β-cells are the most abundant endocrine cell type in the islets across species. They are responsible for the production, storage, and regulated release of insulin which is the only hormone capable of lowering blood glucose levels. Human insulin is encoded by the *INS* gene, located on chromosome 11. With glucose acting as the primary regulator of INS mRNA expression.

Insulin biosynthesis begins with the translation of INS mRNA into preproinsulin, a 110-amino-acid precursor that includes a signal peptide, a B-chain, a connecting peptide (C-peptide), and an A-chain (40). The signal peptide directs the nascent polypeptide into the endoplasmic reticulum, where it is cleaved to produce proinsulin. Within the endoplasmic reticulum, proinsulin undergoes folding into its native conformation, facilitated by the formation of three disulfide bonds between specific cysteine residues. Proinsulin is then transported to the Golgi apparatus and sorted into immature secretory granules within the trans-Golgi network. In these granules, proinsulin undergoes enzymatic cleavage, resulting in the production of biologically active insulin and free C-peptide (41).

The mature insulin, composed of 51 amino acids, is stored in dense-core granules until β-cells are stimulated primarily by elevated glucose levels to secrete insulin through regulated exocytosis (42). A typical β-cell contains approximately 8,000 to 13,000 insulin-containing secretory granules and, upon nutrient stimulation, can secrete around 5–10% of its insulin content per hour (43). Interestingly, around 60% of the insulin released over a 24-hour period is newly synthesized, despite individual insulin granules having a lifespan of approximately 2.7 days (44). The molecular basis for the selective secretion of newly synthesized insulin is still not fully clarified.

Each insulin granule measures approximately 200–300 nanometers in diameter and contains a dense crystalline core primarily composed of insulin complexed with zinc ions. This core is surrounded by a less dense halo and enclosed within a phospholipid bilayer membrane (45). In addition to insulin, granules contain over 150 distinct protein species, including prohormone processing enzymes, granins, membrane trafficking regulators, and ion transporters, all of which contribute to granule maturation, stability, and regulated secretion (46).

Insulin Secretion

Insulin secretion by pancreatic β-cells is a highly regulated process initiated by elevated blood glucose levels. In humans, glucose enters β-cells primarily via the GLUT1 transporter, whereas in mice, GLUT2 serves as the principal glucose

transporter (46). Notably, GLUT2 is also expressed in human β-cells, and its knockdown has been shown to impair insulin secretion (47). After entering the β-cell, glucose is phosphorylated by glucokinase and converted to pyruvate through glycolysis. Subsequent mitochondrial oxidation of pyruvate in the tricarboxylic acid cycle leads to an accumulation of intracellular ATP. This increase in ATP triggers the closure of ATP-dependent potassium (K^+) channels, resulting in plasma membrane depolarization. Consequently, voltage-gated calcium (Ca^{2+}) channel (VGCC) open, leading to an influx of Ca^{2+} ions. The resulting elevation in cytoplasmic Ca^{2+} concentration triggers the exocytosis of insulin-containing secretory granules, ultimately leading to insulin release into the bloodstream (48). These steps in insulin secretion are summarized in Figure 2.

To sustain insulin release, β-cells must continuously replenish and prepare insulin granules through several coordinated steps, including transporting granules to the plasma membrane, assembling the exocytotic machinery, docking, priming, and eventual fusion for release (49). Insulin exocytosis is triggered within milliseconds by a glucose-induced rise in intracellular Ca^{2+} and initially involves granules from the readily releasable pool, which consists of granules docked at the plasma membrane and primed for immediate release upon stimulation. Although the readily releasable pool represents a small fraction of the total insulin granule population, it is essential for the swift β-cell response to acute glucose elevation (50). This first phase is often markedly reduced or absent in people with impaired glucose tolerance or early T2D (18).

Following this, the second phase is characterized by a sustained and gradual release of insulin and depends on the recruitment of granules from a deeper cytoplasmic reserve pool. To become competent for secretion, these granules are progressively mobilized from internal stores and prepared at the plasma membrane before fusion can occur. The priming step prepares these granules for Ca^{2+}-dependent fusion through the assembly of the SNARE complex: plasma membrane-associated syntaxin 1A (STX1A) and SNAP25 bind to the vesicle-associated VAMP2, forming a tight complex that positions the vesicle close to the membrane (51) . This process is further regulated by Ca^{2+}-sensing synaptotagmins (e.g., SYT 1–3, 5–7, 9–10) and accessory proteins including RIM1/2, STXBP1 (MUNC-18), and UNC-13 (MUNC-13) (52, 53). In T2D, several studies have reported reduced expression levels of key SNARE proteins, which may contribute to defective insulin granule exocytosis (54-56).

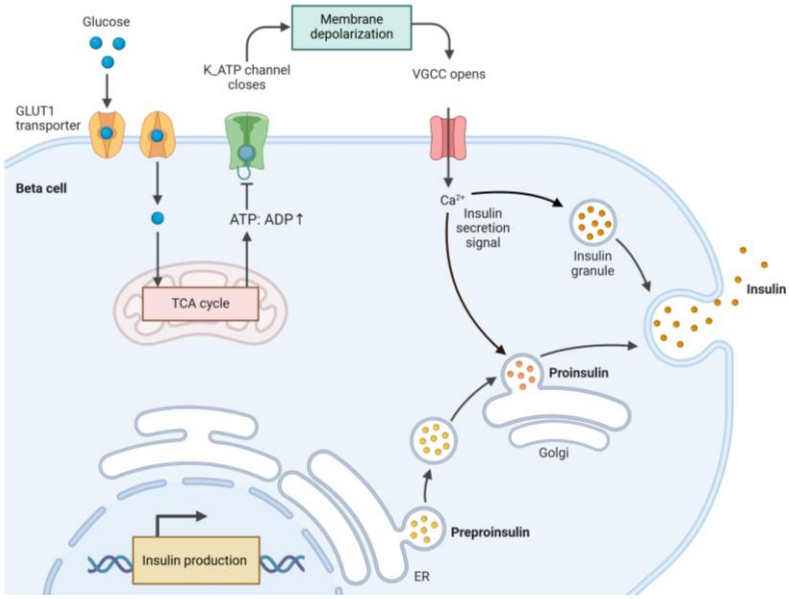

Figure 2: Schematic illustration of GSIS in pancreatic β-cells**.** Glucose uptake via GLUT1 increases ATP, closing ATP-dependent potassium (K^+) channels and depolarizing the membrane. This opens voltage-gated Ca^{2+} channels, causing Ca^{2+} influx and triggering insulin granule exocytosis. Created with BioRender.com.

Pancreatic development

Pancreatic development has been extensively studied to guide the development of regenerative strategies for pancreatic disorders, particularly diabetes. Insights into the molecular mechanisms governing *in vivo* pancreatic cell fate decisions have significantly improved protocols for *in vitro* differentiation of pluripotent stem cells (PSCs) into pancreatic lineages. The pancreas originates from both dorsal and ventral domains of the foregut endoderm**,** with pancreatic organogenesis initiating at approximately post-conception week (PCW) 4 in humans (day 27–29), corresponding to embryonic day (E) 8.5 in mice (57). At this first stage, the emerging pancreatic domain is marked by the onset of PDX1 expression, which defines the pancreatic anlage. The indispensable requirement of PDX1 for pancreas formation is demonstrated by global Pdx1 deletion in mice, resulting in complete pancreatic agenesis at birth. Moreover, hypomorphic Pdx1 alleles generated by deletion of regulatory regions required for optimal PDX1 expression produce only a small remnant pancreas containing ductal and acinar cells but severely reduced endocrine development, with α-cells being the least affected (58, 59). In humans, rare homozygous or compound-heterozygous mutations in PDX1 have similarly

been associated with pancreatic dysgenesis, ranging from partial to complete absence of the pancreas, typically presenting with endocrine and exocrine insufficiency and often causing permanent neonatal diabetes (60).

As development progresses, rotation of the primitive gut tube facilitates the convergence and fusion of the dorsal and ventral pancreatic buds, resulting in the formation of a single, unified organ. Both pancreatic buds harbour multipotent progenitor (MP) cells that differentiate to all epithelial lineages of the pancreas.

During branching morphogenesis, MP cells diverge into two major progenitor cell populations: tip cells, which are committed to the acinar (exocrine) lineage, and trunk cells, which retain the capacity to generate both ductal and endocrine cells. This cell fate divergence is governed by key transcription factors: PTF1A**,** promoting tip cell identity, and NKX6-1**,** driving trunk specification (61). In addition, Notch signalling exerts a critical influence on trunk progenitor cells through lateral inhibition, maintaining the balance between ductal and endocrine lineages. High Notch activity preserves progenitors in a ductal state by inducing Hes1, whereas reduced Notch activity relieves this repression and permits endocrine cell commitment (62, 63). Recent studies further indicate that Notch acts in a level-dependent manner rather than an on/off switch, fine-tuning progenitor self-renewal and lineage allocation (64, 65).

Subsequently, trunk progenitors progressively give rise to endocrine progenitor cells (EPs) marked by the expression of NEUROG3 (also known as NGN3), a master regulator of endocrine cell differentiation (66). Following NEUROG3 expression, endocrine progenitor cells delaminate from the ductal epithelium and migrate into the surrounding mesenchyme, where they cluster and form the islets of Langerhans (67). Once specified, EPs undergo further differentiation driven by a set of lineage-defining transcription factors that establish the major endocrine cell types.

Among these lineage-specifying factors, the mutually antagonistic transcription regulators Pax4 and Arx play a central role in directing endocrine subtype allocation (68). The essential role of Pax4 is evident from *Pax4*-null mice, which die shortly after birth due to a profound loss of insulin-producing β-cells and resulting hyperglycaemia. These animals also lack somatostatin-secreting δ-cells, while α-cells are disproportionately increased and exhibit abnormal clustering (69). In humans, several *PAX4* polymorphisms and mutations have been associated with increased susceptibility to T2D, including variants identified in Japanese and African American populations (70). Conversely, *Arx* expression restricted to developing α- and PP-cells downstream of *Ngn3*. Loss of *Arx* results the reciprocal phenotype, with a complete absence of α-cells and a compensatory expansion of β- and δ-cells, leading to severe hypoglycaemia, weakness, and dehydration (71).

In mice, committed EPs undergo spatial reorganization to form a structure known as the “peninsula,” where newly formed precursor cells displace older cells outward.

In this process, α-cells appear first at the tip of the peninsula, followed by β-cells, which localize to an intermediate layer, and δ-cells, forming the innermost region near the epithelial cord. Around birth, as NEUROG3^{+} cell production declines, differentiated endocrine cells re-enter the cell cycle, and the islets begin to adopt a more spherical morphology. By late gestation and early postnatal life, endocrine cells migrate inward and cluster near pancreatic ducts, ultimately forming the mature islets of Langerhans that regulate hormone secretion in the adult pancreas (72).

Importantly, there are significant species-specific differences between mouse and human pancreatic development that influence both developmental biology and endocrine maturation. In humans, pancreatic hormone expression begins around 8 PCW with the appearance of insulin-producing β-cells, which increase in number by week 9. Glucagon-expressing α-cells appear shortly thereafter (73). In contrast, in mice, the α-cells emerge first, with glucagon^{+} cells detectable as early as E9.5, followed by insulin accumulation begins slightly later, around E11, and then rapidly increases during the "second wave" of insulin differentiation, around E15.5 (74). Unlike the biphasic pattern observed in mice, human pancreatic development appears to follow a single, continuous wave of β-cell formation. Although β-cells in both species utilize many of the same transcriptional regulators throughout development and into maturity, the timing, expression dynamics, and functional roles of these factors can differ significantly.

Neurogenin-3

Neurogenin-3 (Neurog3) is a proendocrine transcription factor essential for establishing the endocrine lineage during pancreatic development. It is transiently expressed in endocrine progenitor cells and is critical for initiating the differentiation of all major pancreatic endocrine cell types. In mice, *Neurog3* expression begins around E8.5 and peaks between E15.5 and E16.5, marking the critical window for endocrine lineage specification (75). In humans, *NEUROG3* expression is detectable in the developing pancreas by 7–8 PCW, with peak expression observed around week 11 (76).

Importantly, Neurog3-positive cells do not co-express mature endocrine hormones, highlighting their identity as early endocrine progenitors (66). Mice deficient in *Neurog3* fail to develop pancreatic endocrine cells and die postnatally due to diabetes (77). Conversely, overexpression of *Neurog3* has been shown to accelerate endocrine differentiation, driving premature commitment of progenitor cells to the endocrine fate (78). In humans, mutations in *NEUROG3*, whether homozygous or compound heterozygous, have been linked to early-onset diabetes(79, 80). The timing of disease manifestation varies, from the neonatal period to childhood, depending on the severity of *NEUROG3* dysfunction. Patients often also develop congenital malabsorptive diarrhoea, caused by a failure of intestinal endocrine cells

to form in the absence of functional *NEUROG3*. Studies using human pluripotent stem cells have further demonstrated that NEUROG3 is essential for the differentiation of pancreatic endocrine cells, including insulin-producing β-cells, confirming its critical role in human endocrine development (81).

Innervation of the islets

The adult pancreas is densely innervated from the autonomic nervous system (ANS), including sympathetic, parasympathetic, sensory fibers, and contains intrapancreatic ganglia (82). Islet innervation is essential for proper islet function and plays a critical role in islet development and the maintenance of β-cell mass (83, 84). Upon activation, nerve terminals release neurotransmitters and neuropeptides near islet cells, enabling rapid neural regulation of hormone secretion and glucose homeostasis. In addition to this direct neural input, the ANS indirectly modulates hormone release by regulating islet blood flow, reflecting the close integration of vascularization and innervation (85). Importantly, islet innervation is altered in animal models of insulin resistance and T2D. In addition to the extrinsic neural input, mature β-cells themselves secrete neurotransmitters such as γ-aminobutyric acid (GABA), serotonin, and dopamine, which contribute to local autocrine and paracrine regulation within the islet (86, 87).

Vascularization and innervation of islets begin in the embryo and continue postnatally (88). Pancreatic innervation begins with the development of intrinsic neurons that form intrapancreatic ganglia. These ganglia are believed to originate from neural crest (NC) cells that migrate into the pancreas during development. In the adult pancreas, these ganglia are diffusely distributed throughout the pancreas, located near nerve trunks and adjacent to the islets (89). Their axons extend to the islets of the endocrine pancreas, ducts, and acinar tissue, establishing complex neural networks that regulate pancreatic function. These intrapancreatic ganglia are parasympathetic postganglionic ganglia that receive parasympathetic preganglionic input from the brain (via the vagus nerve), as well as modulatory sympathetic postganglionic input (90). Although the precise function of these ganglia remains unclear, evidence suggests that they stimulate insulin secretion (91).

Mouse and human islets exhibit distinct but related patterns of innervation. Earlier studies suggested that pancreatic islets in mice are densely innervated, with nerve fibres in close contact with endocrine cells, whereas human islets contain relatively few large nerve fibres, mainly located around intra-islet blood vessels. This led to the conclusion that neural regulation of hormone secretion in humans is largely indirect and mediated through changes in blood flow rather than direct neurotransmission (92, 93). However, recent three-dimensional (3D) imaging studies have challenged this view, demonstrating that both human and mouse islets

receive extensive sympathetic and parasympathetic innervation, although the overall density of innervation is lower in human islets (90). Comparative studies further indicate that, in adult mice, innervation is enriched within the endocrine compartment relative to the exocrine pancreas, whereas in humans the endocrine and exocrine compartments display a more comparable degree of innervation(90). Despite these quantitative differences, the overall organization and functional role of pancreatic innervation are more similar between human and mouse than previously assumed. Interestingly, islet nerve density has been shown to increase in non-obese diabetic (NOD) mice, and in pancreatic tissue from human donors with T2D (90).

Sympathetic innervation

The sympathetic nerves of the pancreas arise from the dorsal root ganglia located in the thoracolumbar region of the spinal cord (94). Sympathetic preganglionic neurons project their axons via the splanchnic nerves to the celiac and superior mesenteric ganglia. From there, sympathetic postganglionic fibres extend to the pancreas, where they innervate the intrapancreatic ganglia, islets, acinar tissue, and ducts (82). These postganglionic fibres express tyrosine hydroxylase (TH), an enzyme required for noradrenaline synthesis, and release noradrenaline as their primary neurotransmitter. In addition to noradrenaline, sympathetic nerve terminals may co-release neuropeptides such as neuropeptide Y and galanin, which can modulate sympathetic signalling. In the adult pancreas, sympathetic neural activity plays a key role in maintaining blood glucose homeostasis by inhibiting insulin secretion from β cells (95)and stimulating glucagon release from α cells, thereby increasing circulating glucose levels during stress and exercise (96). Sympathetic signalling also causes vasoconstriction of pancreatic blood vessels, reducing islet perfusion and contributing indirectly to decreased insulin secretion and hormone release into the circulation.

During development, sympathetic fibres can be detected in the mouse pancreas as early as E12.5 and at 8 PCW in humans (94) (97). Disruption of sympathetic nerves during development, whether through genetic manipulation or pharmacological methods, leads to changes in islet structure, decreased insulin secretion, and impaired glucose tolerance in mice, despite no observed defects in β-cell differentiation (98). Importantly, a study in a global tyrosine hydroxylase (TH) knockout mouse demonstrated a marked decrease in β-cell numbers and lower expression of the pro-endocrine transcription factors Neurog3 and Ins1 at E13.5 (99).

In mice, TH-positive sympathetic axons are densely distributed around the periphery of the islets and make preferential contacts with α-cells, facilitating glucagon release. In contrast, in humans, sympathetic axons are less abundant and exhibit a different distribution pattern. Most TH-labelled fibres are located around

intra-islet blood vessels, where they likely regulate hormone secretion indirectly by modulating islet blood flow rather than through direct synaptic signalling (92, 100). However, advanced three-dimensional imaging analyses have identified a minor population of sympathetic axons near α- and β-cells, suggesting that direct innervation of endocrine cells may complement the predominant indirect vascular regulation (97, 101). Alterations in sympathetic islet innervation have been linked to diabetes in a disease-specific manner. Loss of local sympathetic innervation has been consistently observed in animal models of T1D and in individuals with T1D (102, 103). In contrast, sympathetic neuronal loss is not observed in people with T2D, suggesting distinct autonomic alterations between the two conditions.

Parasympathetic innervation

Parasympathetic innervation emerges predominantly from the dorsal motor nucleus of the vagus (DMV) and to a lesser extent from the nucleus ambiguus (NA), both situated within the brain stem. Preganglionic vagal fibers project to the intrapancreatic ganglia, where they synapse with postganglionic neurons that innervate acinar tissue and the islets of Langerhans (104). The primary neurotransmitter released is acetylcholine (ACh**);** however, nitric oxide (NO)**,** vasoactive intestinal polypeptide (VIP)**,** and gastrin are also co-released (105). Parasympathetic nerves, like their sympathetic counterparts, play an important role in blood glucose regulation. Following food intake, activation of parasympathetic neurons stimulates the secretion of digestive enzymes from acinar cells (106) and promotes insulin release from pancreatic β-cells (107). In addition, during hypoglycaemic conditions, parasympathetic signalling enhances glucagon secretion from α-cells, which helps increase blood glucose levels when necessary (108).

On embryonic day 12.5 (E12.5), only a small population of parasympathetic fibres can be observed in the developing mouse pancreas. and by E18.5, these axons appear to establish synaptic contacts with endocrine cells (109). The specific role of parasympathetic innervation in the embryonic mouse pancreas remains incompletely understood. However, some studies have demonstrated that ablation of parasympathetic nerves leads to a selective reduction in pancreatic δ cell populations (110).

In both humans and mice, parasympathetic fibres run alongside blood vessels, forming neurovascular networks that extend toward the acinar–islet interface and link the exocrine and endocrine compartments. In human islets, scattered $VAChT^{+}$ (vesicular acetylcholine transporter) cells are also observed, although their identity remains uncertain since both neurons and endocrine cells can take up and decarboxylate amine precursors. This highlights the close anatomical integration of vascular and neural pathways within the pancreas and suggests potential species-specific features of cholinergic signalling (111).

Maf Transcription Factors

MAF transcription factors belong to the basic leucine zipper (bZIP) superfamily and are defined by a conserved DNA-binding domain, which enables sequence-specific interaction with target genes, and a leucine zipper motif that mediates protein dimerization required for functional activity. Members of the MAF family play essential roles in the development, differentiation, and functional specification of various organs and tissues, including the pancreas, lens, myeloma cells, and cartilage (112).

Based on their molecular size and domain structure, MAF transcription factors are classified into two subgroups: small MAFs (MAFF, MAFG, and MAFK), consisting of approximately 150–160 amino acids, and large MAFs (MAFA, MAFB, c-MAF, and NRL), which range from 240 to 340 amino acids in length. Small MAFs lack intrinsic transcriptional activation domains, leading their homodimers to function primarily as transcriptional repressors. However, they can form heterodimers with other bZIP proteins to regulate gene expression in various biological contexts (113).

In contrast, Large MAF transcription factors possess a transcriptional activation domain that enables them to act as transcriptional activators. They typically form homodimers and bind to specific DNA motifs known as Maf Recognition Elements (MAREs) which are conserved sequences located within the promoter or enhancer regions of target genes. This interaction allows large MAFs to regulate gene expression programs essential for cell differentiation and tissue-specific function (114).

MafA

MafA (v-maf musculoaponeurotic fibrosarcoma oncogene homolog A) is a key marker of β-cell identity and a critical transcription factor in the regulation of β cell function and maturation (115). In mice, MafA expression is first detected at E13.5 in insulin producing cells and remains exclusively restricted to adult β-cells(116). MafA binds to a conserved insulin enhancer element, RIPE3, where it acts as a transcriptional activator of the insulin gene in β-cells (117, 118). While MafA can activate the insulin promoter independently, its co-expression and synergistic interaction with Pdx1 and Beta2 (NeuroD1) further enhance insulin gene transcription (119). In mice, maximal GSIS is typically not achieved until approximately three months after birth, coinciding with the upregulation of *MafA* and insulin expression (115, 120). Interestingly, ectopic MafA expression in pancreatic endocrine progenitor cells disrupts their differentiation into hormone producing cells, underscoring the need for tightly regulated MafA levels during β-cell maturation (121).

In humans, MAfA expression begins around gestational week 21 and increases postnatally (122). During fetal and early postnatal stages, MAFA expression in human β-cells remains low, but it increases markedly with maturation, reaching levels approximately six times higher in adult compared to fetal purified β-cells (123). This postnatal, age-dependent increase closely mirrors the MAFA expression pattern observed in rodents, where MAFA upregulation is similarly linked to β-cell maturation.

Beyond its developmental functions, MafA plays a crucial role in maintaining glucose homeostasis and β-cell identity in adult animals. MafA-deficient mice develop glucose intolerance after weaning as a result of impaired GSIS and display progressive, age-dependent onset of diabetes, along with reduced expression of key β-cell genes such as *Ins1, Ins2, Pdx1, Neurod1,* and *Slc2a2* (124, 125), underscoring its critical role in sustaining the mature β-cell transcriptional program. More recent evidence demonstrates that MafA deficiency in adult β-cells also triggers aberrant hormone gene expression, including ectopic activation of *Gast* (gastrin), a hormone normally absents from β-cells**.** These MafA-deficient β-cells acquire a transcriptional profile resembling *Gast*$^{+}$ cells that emerge under conditions of chronic hyperglycemia and obesity, highlighting MafA's central role in preserving β-cell identity and functional stability (126).

Reduced expression of MafA has been strongly associated with the progression of diabetes in both murine models and human patients. In db/db diabetic mice, MafA levels are significantly downregulated, largely due to oxidative stress caused by chronic hyperglycaemia (127). Likewise, pancreatic islets from individuals with T2D show a substantial reduction in both *MAFA* mRNA and protein expression when compared to non-diabetic controls (128).

Given its critical role in mature β-cell function, MAFA has emerged as a key target in regenerative strategies aimed at producing fully functional β-cells from pluripotent stem cells. Timely upregulation of MAFA expression is essential for promoting β-cell maturation *in vitro*. In human embryonic stem cells (hESCs), inducing MAFA at appropriate stages of differentiation has been shown to enhance GSIS, highlighting its importance in diabetes cell therapy strategies (129).

MafB

Although MafA and MafB both belong to the large Maf transcription factor family, their expression patterns and functional roles are distinct. In mice, MafB is expressed in mature α-cells and is commonly used as α-cell marker (130)Its expression begins earlier than that of MafA, first appearing around E10.5 in glucagon-positive cells and subsequently at E12.5 in insulin-positive cells. MafB is also detected in a small subset of Neurog3^{+} endocrine progenitors, indicating a role in early endocrine cell differentiation and islet morphogenesis (131). During

embryogenesis, MafB is expressed in both α- and β-cell progenitors; however, its expression in β-cells progressively declines after E18.5 and is completely lost by approximately three weeks postnatally. This downregulation coincides with the postnatal upregulation of MafA in insulin-producing β-cells (116).

Mice with a global deletion of MafB exhibit severe physiological defects, including respiratory and kidney abnormalities, and typically die at birth because of respiratory failure (132). In these knockout models, insulin- and glucagon-producing cells are reduced during embryogenesis, while the total number of endocrine cells remains unchanged. This suggests that MafB is specifically required for α- and β-cell differentiation. Mechanistically, MafB binds directly to the regulatory regions of the insulin and glucagon genes, playing a critical role in their transcription during fetal development. Notably, insulin-expressing cells appear with a delay in MafB-deficient mice, emerging around E13.5, in parallel with the initiation of MafA expression (133). MafB also promotes the transcription of MafA in developing β-cells, indicating a sequential and cooperative relationship between these two factors. Moreover, MafB is required to maintain expression of essential β-cell genes such as *Pdx1* and *Slc2a2* (encoding the glucose transporter GLUT2). In the absence of MafB, the expression of these gene products remains at normal levels until approximately E15.5 but is markedly reduced by E18.5 (133, 134).

In contrast to mice, the expression pattern of MAFB in humans is different. MAFB expression begins around gestational week 7 and continues to increase through week 21, where it is detected in both α- and β-cells. Unlike in mice, MAFB remains expressed in mature human β-cells postnatally, suggesting that human β-cells co-express both MAFA and MAFB.

Functionally, MafB plays a crucial role in regulating glucagon gene expression in pancreatic α-cells by binding to the G1 regulatory element within the promoter region (131, 135). This regulation is essential for proper glucagon secretion during hypoglycaemia, thereby contributing to glucose homeostasis. Importantly, MAFB expression is downregulated in both α- and β-cells of individuals withT2D, suggesting that impaired MAFB function may contribute to dysregulated insulin and glucagon secretion in the diabetic state (136).

Developmental Regulators

As described earlier in the preceding sections, endocrine lineage specification relies on a core set of regulators, including NEUROG3, PDX1, NKX6-1, PAX4, and ARX. In addition to these canonical regulators, several transcription factors that have been primarily studied in other biological systems have been proposed as candidates with potential relevance for early pancreatic development. These include

AUTS2, ETV1, RUNX1T1, MEIS2, and MLXIPL, each of which carries regulatory functions that could influence how endocrine progenitor cells acquire their fate.

AUTS2 is known for its role as a transcriptional activator acting through its interactions with chromatin-modifying complexes and its enrichment in regions of open, transcriptionally active chromatin (137). AUTS2 has also emerged as an important regulator of lineage specification. Studies using cerebral organoids show that loss of AUTS2 disrupts normal neuronal differentiation, reducing the number of neuron-committed cells while increasing cells that adopt a choroid plexus–like identity (138). These findings suggest that AUTS2 safeguards proper differentiation by preventing progenitor cells from diverting toward inappropriate cell fates, highlighting its broader developmental importance.

ETV1 is a transcription factor with important roles in early development and tissue morphogenesis (139, 140). It is required for proper heart formation and for the maturation of both murine and human stem-cell–derived cardiomyocytes (141, 142). Recent work has shown that ETV1 also influences pancreatic differentiation: loss of ETV1 impairs cell–cell and cell–matrix adhesion during *in vitro* pancreatic development, leading to reduced formation of endocrine progenitor cells (143). These observations position ETV1 as a regulator of mechanosignaling and cellular organization, supporting proper endocrine lineage allocation and 3D tissue architecture.

RUNX1T1 has been implicated in midgut development and in several epithelial and solid-organ neoplasms, including those of the lung and breast (144, 145). Importantly, expression analyses reveal that *Runx1t1* mRNA is markedly reduced in *Neurog3*-deficient fetal pancreas (146), strongly suggesting that RUNX1T1 functions downstream of this master endocrine regulator and may play a previously underappreciated role in endocrine lineage specification.

MEIS2 is a TALE-homeodomain transcription factor with diverse developmental functions. It is a direct regulator of PAX6 during lens development and cooperates with PDX1 to enhance transcriptional activation of target genes (147, 148). MEIS2 expression has been reported in adult human islets, primarily in β-cells and ε-cells (149). Single-cell transcriptomic studies indicate that it is also present in endocrine progenitor cells and, to a lesser extent, in ductal populations (150). Despite this expression pattern, the specific functions of this gene in human pancreatic development remain largely unknown, suggesting a potential role in endocrine progenitor cell maturation that warrants further investigation.

MLXIPL (ChREBP) is expressed in pancreatic β-cells, where its α and β isoforms are required for glucose-stimulated β-cell proliferation (151). MLXIPL expression is elevated in diabetic mouse models, linking its activity to nutrient-driven β-cell stress (152). Under conditions of chronic caloric excess, MLXIPL promotes lipid accumulation and reduces mitochondrial fatty acid β-oxidation in β-cells, contributing to metabolic dysfunction (153).

Aims

The MAF family of transcription factors, including MAFA and MAFB, are critical regulators of pancreatic islet development and function, and their expression is reduced in β cells from individuals with T2D. However, the MAF-regulated genes required for insulin granule exocytosis in human β cells remain poorly characterized. During pancreas development, MAFB is present in Neurog3^{+} endocrine progenitors as well as in insulin- and glucagon-producing cells, suggesting broader functions in endocrine differentiation and islet formation. The overall aim of this thesis is to define the role of MAFB during pancreatic development and endocrine cell specification and function.

Specific aims of the thesis

1. To investigate how MAFA and MAFB regulate genes required for insulin exocytosis in human β cells, and how their loss may contribute to impaired insulin secretion in T2D.
2. To investigate the role of MAFB in regulating neural signaling pathways during pancreatic development and to determine how its loss affects β-cell clustering and islet formation.
3. To identify direct target genes of MAFB in endocrine progenitor cells, in order to define the gene networks that MAFB controls during early endocrine lineage specification.

Ethical Consideration

All research presented in this thesis was conducted in accordance with relevant ethical guidelines, legislation, and approved permits. Experiments involving mouse embryos and genetically modified mouse lines, including the MafA used to study β-cell function and the MafB mutant models used to investigate endocrine cell development, were carried out at accredited animal facilities at Lund University and approved by the regional animal ethics committee, with all procedures designed to minimize suffering and performed in accordance with the 3R principles of Replace, Reduce, and Refine.

Studies involving human pancreatic islets were conducted using tissue obtained from ethically approved biobanks, where donors had provided informed consent in accordance with national regulations; all samples were fully anonymized before being received by the laboratory, ensuring that no identifiable information was accessible to researchers.

The EndoC-βH1 cell line used in this thesis was originally derived from human fetal pancreatic tissue obtained after elective termination of pregnancy, for which written informed consent was provided. The generation of this cell line was approved by the French Biomedical Agency and conducted in accordance with national bioethics legislation. The purpose of creating EndoC-βH1 was to establish a physiologically relevant human β-cell model for diabetes research, and subsequent studies have shown that it closely mirrors many features of adult human β-cells. This makes it a valuable and ethically well-regulated model system for studying β-cell function and diabetes pathophysiology. Genomic analyses, including single-cell RNA sequencing, were performed on anonymized samples and stored on secure servers in compliance with Lund University's data protection policies. Analyses involving human fetal pancreatic tissue were likewise conducted using samples obtained through approved ethical procedures, with informed consent from donors and complete anonymization before use. Throughout the project, scientific integrity was maintained through rigorous documentation, appropriate experimental controls, and transparent reporting of methods and results. Overall, the ethical considerations of this work focus on the responsible use of animals, human islets, human-derived cell lines, and genomic data, with the overarching aim of advancing scientific understanding of pancreatic development and diabetes pathophysiology while upholding ethical and societal standards.

Material and Methods

This thesis combines several experimental approaches. In this chapter, the primary models and methods are presented and discussed, with particular attention to their respective strengths and limitations. Complete and detailed descriptions of each method are provided in the appended papers.

Animal models

In this thesis, various transgenic mouse models were employed. All animal procedures were pre-approved and performed in accordance with Swedish national guidelines.

MafB-GFP knock-in (*MafB*$^{-/-}$) mice

Generation of *MafB*$^{-/-}$ mice was previously described (132). In this model, the coding region of the *MafB* gene was replaced by a GFP reporter cassette through homologous recombination in embryonic stem cells, thereby disrupting *MafB* expression while allowing GFP to serve as a reporter for *MafB* promoter activity. Embryonic pancreata were collected at stages E14.5, E15.5, and E18.5 (paper II), or at E13.5, E14.5, and E15.5 (Paper III), and processed for cDNA microarray, single-cell RNA-seq, and immunohistochemistry. These stages represent critical windows of endocrine progenitor specification and β-cell differentiation.

MIP-GFP transgenic mice

The MIP-GFP mouse line, originally developed by Hara et al (154), expresses GFP under the control of the mouse insulin promoter, restricting expression to insulin-producing β-cells. No alterations in islet formation were observed, allowing its use as a reliable model for β-cell identification. In Paper II, embryonic β-cells from MIP-GFP mice were isolated and co-cultured with superior cervical ganglia, which provide a source of acetylcholine, to assess whether inhibition of nAChR activity affected β-cell migration toward autonomic nerves.

MafA-deficient (*MafA−/−*) mice

MafA$^{-/-}$ mice were used in Paper I to investigate whether loss of MafA in β-cells contributes to T2D progression by regulating genes essential for insulin exocytosis. Global deletion of *MafA* was achieved by crossing *MafA*$^{fl/fl}$ mice (155) with *Sox2*-Cre transgenic animals (156), resulting in complete knockout of *MafA* in all tissues.

Cell lines

EndoC-βH1 cells

EndoC-βH1 cells were maintained as previously described (157) on flasks coated with Matrigel (100 µg mL^{-1}) and fibronectin (2 µg mL^{-1}). Cells were cultured in DMEM containing 5.6 mmol L^{-1} glucose, 2% BSA, 10 mmol L^{-1} nicotinamide, 50 µmol L^{-1} β-mercaptoethanol, 5.5 mg mL^{-1} transferrin, 6.7 ng mL^{-1} sodium selenite, and antibiotics (100 IU mL^{-1} penicillin, 100 µg mL^{-1} streptomycin) at 37 °C in a humidified atmosphere with 5% CO_2.

Human and Mouse Islets

Human pancreatic islets from 191 donors of European ancestry were obtained through the Nordic Islet Transplantation Program, Uppsala, coordinated by Prof. Olle Korsgren. Collection and use of islets were conducted under full ethical clearance (Uppsala Regional Ethics Board, Pro00001754) with written informed consent from donor families. The islets were provided in collaboration with the Excellence of Diabetes Research in Sweden and the Lund University Diabetes Centre and distributed by the Human Tissue Lab at the Clinical Research Centre, Malmö, Sweden.

To complement the human data, mouse pancreatic islets from 6-month-old wild-type and *MafA*$^{-/-}$ mice were isolated using standard collagenase digestion and hand-picking procedures. Both human and mouse islets were processed for RNA extraction and subsequent RNA sequencing (RNA-seq), with RNA extraction, quality control, and sequencing.

Molecular and Genomic Techniques

Real-time quantitative polymerase chain reaction (RT-qPCR)

RT-qPCR was used in all the papers included in this thesis. In this method, total RNA is extracted, reverse-transcribed into cDNA, which then served as a template for amplification. During amplification, fluorescence dyes enabled real-time detection of the accumulating product. The fluorescence signal increased proportionally with the amount of amplified DNA, and the cycle threshold (Ct) was defined as the number of cycles required for the fluorescence to exceed a set threshold, reflecting the starting quantity of the target transcript.

To assess relative expression, the ΔΔCt method was applied. First, Ct values of target genes were normalized to those of endogenous reference genes (ΔCt). Then, differences between experimental and control samples were calculated (ΔΔCt), and relative fold change in gene expression was determined using the 2^(-ΔΔCt) formula. The choice of stable endogenous controls is essential for accurate normalization and reliable interpretation of RT-qPCR results (158).

cDNA Microarray Analysis

For global gene expression profiling in MafB-deficient endocrine progenitors, cDNA microarray analysis was performed in paper II. Total RNA was extracted from fluorescence-activated cell-sorted (FACS) GFP^+ endocrine cells, reverse-transcribed into fluorescently labelled cDNA, and hybridized to DNA microarrays containing probes for thousands of known mouse genes. Fluorescence intensity at each probe site was quantified to determine relative transcript abundance between MafB-deficient and control samples. While this technique provides robust detection of gene expression changes, it is limited to predefined probe sets and does not capture novel transcripts or isoforms, in contrast to bulk RNA sequencing used in later studies.

Bulk RNA-sequencing

Bulk RNA sequencing was used to profile gene expression in whole pancreatic islet preparations. Total RNA was extracted from a heterogeneous cell population, converted into cDNA, and sequenced. In Paper I, bulk RNA-seq was used to compare transcriptomes of wild-type and *MafA−/−* mouse islets, determining how long-term loss of MafA affects β-cell gene expression. In Paper II, it was used to assess whether neurotransmitter and axon guidance signaling genes are expressed in the developing human pancreas. While bulk RNA-seq provides an averaged expression profile across all cells, it does not resolve cell-to-cell heterogeneity.

Single-cell qPCR analysis (Fluidigm panel)

To assess cell-to-cell variation in gene expression, single GFP^+ pancreatic cells from $MafB^{+/-}$ and $MafB^{-/-}$ embryos were isolated by FACS and subjected to targeted single-cell gene expression profiling using the Fluidigm BioMark HD system. After reverse transcription and pre-amplification, a selected panel of genes related to α- and β-cell differentiation and neurotransmitter signalling was quantified by microfluidic qPCR. This approach enabled us to determine the distribution of specific endocrine progenitor subtypes in heterozygous versus knockout sample.

Single-cell RNA-seq

In contrast to targeted qPCR panels, scRNA-seq provides genome-wide transcriptional profiles of individual cells, enabling the identification of distinct cell populations and transcriptional heterogeneity. In Paper II and Paper III, scRNA-seq was performed on human embryonic pancreas and MafB-deficient mouse pancreata, respectively, to characterize endocrine progenitor cells, β-cell precursors, and other cell types during development. Cells were processed using a droplet-based microfluidic system (10x Genomics Chromium), sequenced, and analyzed using standard pipelines for dimensionality reduction, clustering, and differential gene expression. This approach provided high-resolution insights into cell-specific transcriptional programs that are not detectable by bulk RNA-seq.

Dual Luciferase Reporter Assay

In principle, a luciferase assay involves cloning a regulatory element, such as a promoter or enhancer, upstream of a luciferase reporter gene in an expression plasmid. The construct is then transfected into cells, where reporter activity provides a readout of the regulatory element's activity. In paper III, this approach was used to directly assess the functional impact of genetic variation at the *AUTS2* and *ETV1* loci. Genomic fragments carrying either reference or risk alleles of T2D-associated SNPs were subcloned into the pFOXLucPRL.GB firefly luciferase vector. HEK293 cells were transfected with these constructs together with MAFA-WT/pcDNA3.1 or MAFB-WT/pcDNA3.1 expression plasmids, along with a Renilla luciferase plasmid for normalization. An illustration of the luciferase assay design used in Paper III is shown in Figure 3.

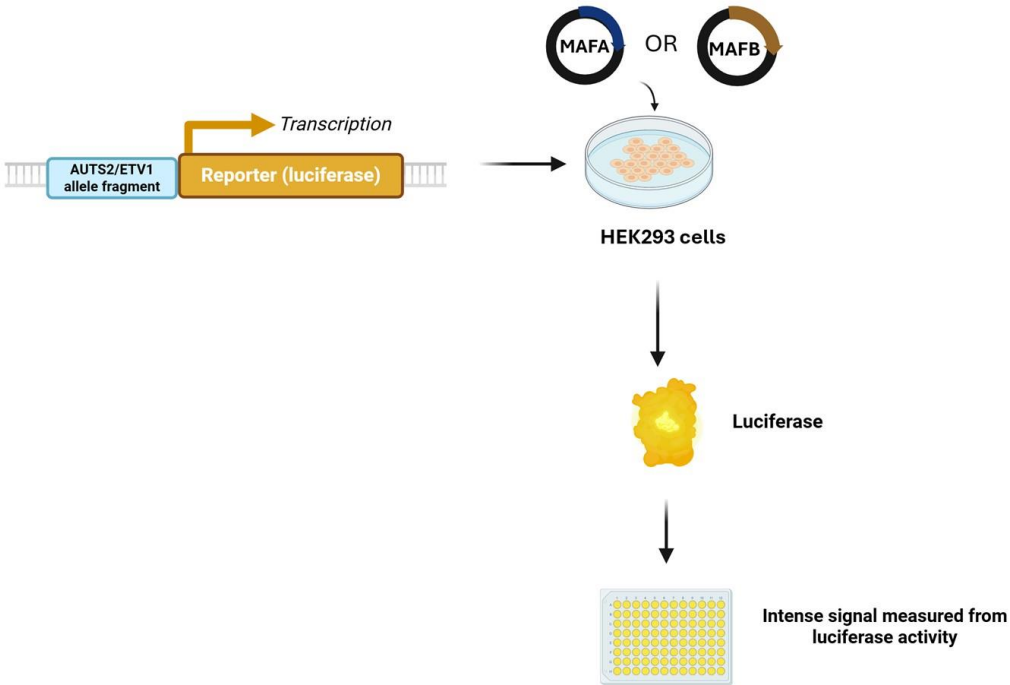

Figure 3: Luciferase reporter assay design for AUTS2 and ETV1 regulatory variants. Genomic fragments from the *AUTS2* and *ETV1* loci containing either reference or risk alleles of T2D-associated SNPs were cloned upstream of a Firefly luciferase reporter in the pFOXLucPRL.GB vector. HEK293 cells were co-transfected with these constructs together with MAFA-WT/pcDNA3.1 or MAFB-WT/pcDNA3. Created with BioRender.com.

Papers In Summary

Paper I

MAFA and MAFB regulate exocytosis-related genes in human β-cells

T2D develops when pancreatic β-cells can no longer secrete sufficient insulin to meet metabolic demand, reflecting a progressive decline in β-cell function. One of the earliest defects is the loss of first-phase insulin secretion, a process that depends on efficient granule docking, priming, and exocytosis at the plasma membrane (159). Reduced expression of exocytosis-related genes and impaired granule docking have been consistently observed in human T2D islets, implicating defective exocytosis as a key contributor to β-cell dysfunction (160). MAFA expression is markedly reduced in β-cells from individuals with T2D, and both MAFA and MAFB regulate genes required for glucose-stimulated insulin secretion (136, 161, 162). However, the specific MAF target genes that control insulin granule exocytosis in human β-cells remain poorly defined. This study aims to identify MAFA- and MAFB-regulated exocytosis genes in mouse and human β-cells and to determine how their dysregulation may contribute to β-cell failure in T2D.

Results and discussion:

Our first objective was to identify novel target genes of MafA involved in insulin granule exocytosis. RNA sequencing of wild-type and *MafA*$^{-/-}$ mouse islets revealed 62 exocytosis-related genes that were significantly altered upon MafA loss, with 30 being downregulated, further supporting MafA as a major activator of the β-cell exocytotic program. Because adult human β-cells co-express both MAFA and MAFB, we next examined whether these factors regulate similar pathways in humans. Correlation analyses in human islet RNA-seq datasets showed that 29 exocytosis genes were positively associated with both MAFA and MAFB, whereas additional genes displayed MAFA-specific or MAFB-specific correlations, indicating both shared and divergent regulatory roles for the two transcription factors. Cross-species comparison identified eight conserved MAF-regulated exocytosis genes: *STXBP1, UNC13A, SYT5, VAMP2, STX16, RAB3A, CAMK2N2,* and *ADRA2A* with *NAPA* showing MAFA-specific and *NSF* showing MAFB-specific regulation.

To define the functional contribution of MAFA and MAFB to β-cell exocytosis, we performed siRNA-mediated knockdown of each factor in EndoC-βH1 cells. *MAFA*

silencing significantly impaired both GSIS and KCl-induced insulin secretion (K-SIS), whereas *MAFB* knockdown produced only mild, non-significant effects, indicating a predominant role for MAFA in maintaining the exocytotic machinery. Because the K-SIS assay bypasses glucose metabolism and directly triggers insulin release via depolarization and Ca^{2+} influx, these data indicate that MAFA directly supports the Ca^{2+}-dependent exocytosis. This observation is further supported by independent studies combining single-cell RNA sequencing and patch-clamp electrophysiology, which demonstrated a strong positive correlation between *MAFA* transcript levels and insulin exocytosis in human β-cells (163).

To explore whether MAFA and MAFB regulate specific components of the exocytosis process, we quantified transcripts of candidate exocytosis genes identified in our earlier analyses. *MAFA* knockdown markedly reduced the expression of *STX1A, STXBP1,* and *SYT7*, whereas *MAFB* knockdown caused a more subtile decrease in *STX1A* and *SYT7*. Reduced STXBP1 protein levels after MAFA knockdown confirmed these transcriptional effects. In primary human islets, partial knockdown of *MAFA* or *MAFB* similarly lowered *STX1A* expression and showed trends toward impaired insulin secretion, although variability among donors likely masked statistical significance.

We next assessed whether these pathways are altered in diabetes. In pancreatic sections from donors with T2D, β-cells exhibited reduced MAFA, STXBP1, and STX1A protein levels, whereas SYT7 was unchanged. MafA-deficient mouse islets also showed reduced STXBP1 protein expression, supporting MAF-dependent regulation. ChIP-seq data further demonstrated MAFB binding to regulatory regions upstream of *STX1A* and *STXBP1* (164), and previous human studies have linked reduced levels of these proteins to impaired GSIS and granule docking (55). Together, these findings highlight STX1A and STXBP1 as critical downstream mediators of MAF-dependent exocytotic function.

To extend these findings, we examined whether naturally occurring genetic variants in *MAFA* influence its own expression and that of downstream exocytosis genes. eQTL analysis in 188 human islet donors identified a *MAFA* SNP (rs61731375) where carriers of the alternate allele showed reduced *MAFA* transcript levels and a suggestive association with T2D risk (165). Importantly, this variant was also associated with lower expression of the exocytosis genes *STXBP1, STX1A,* and *SYT7*, mirroring the effects observed after *MAFA* knockdown. Given that STX1A and STXBP1 are essential for insulin granule docking, these data further support that individuals with genetically reduced MAFA expression may possess a predisposed impairment in exocytosis. A summary of Paper I findings is illustrated in Figure 4.

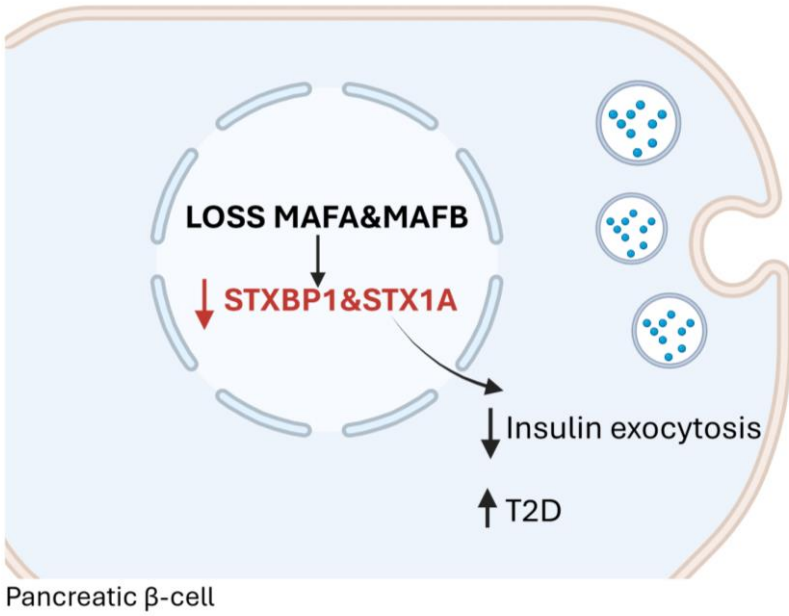

Figure 4: Summary of the main findings from Paper I. Loss of MAFA and MAFB in pancreatic β-cells leads to reduced expression of the exocytosis genes STXBP1 and STX1A, two core components of the insulin-granule docking and fusion machinery. Their downregulation impairs insulin exocytosis, contributing to defective insulin secretion and increased susceptibility to T2D. Created with BioRender.com.

Key findings:

- MAFA and MAFB regulate many exocytosis-related genes in mouse and human β-cells.
- *MAFA* knockdown reduces insulin secretion (GSIS and K-SIS) and lowers *STX1A, STXBP1*, and *SYT7* expression.
- The *MAFA* SNP rs61731375 reduces *MAFA* and key exocytosis gene expression, potentially impairing β-cell function and increasing T2D risk.

Paper II

MafB-dependent neurotransmitter signalling promotes β cell migration in the developing pancreas

During pancreatic development, mouse islets arise through the budding of endocrine progenitor cells from the pancreatic epithelium and the subsequent aggregation of migrating endocrine cells. This process is orchestrated by a complex interplay of signalling cues from the pancreatic mesenchyme, vasculature, and sympathetic neurons, all of which promote endocrine differentiation and islet assembly (98, 166, 167). Adrenergic signals from sympathetic nerves have been shown to stimulate β-cell expansion and contribute to islet formation. Understanding how MafB regulates pancreatic development provides key insight into the transcriptional and structural mechanisms governing islet morphogenesis. MafB is expressed in α- and β-cell progenitors and is required for the terminal differentiation of both cell types. Its loss leads to an approximately 50% reduction in α- and β-cell mass at E18.5, despite the presence of endocrine progenitors (133). MafB is also expressed in a subset of Neurog3^{+} endocrine progenitor cells, suggesting that MafB regulates early processes of endocrine cell specification and differentiation prior to their terminal maturation.

Results and discussion:

Loss of MafB in developing endocrine cells disrupts islet and ductal morphogenesis

We examined whether the loss of MafB affects pancreatic and islet morphology at E18.5, a stage when islet structures are well established. In *MafB*$^{-/-}$ pancreata, islet cells failed to form the typical clusters observed in wild-type embryos and instead remained closely associated with the ductal epithelium. In addition, ductal morphology was altered, with fewer large ducts and an increased number of smaller luminal structures, and these lumina contained fewer branching points (Figure 5). Importantly, the number of branching points within the exocrine compartment did not differ, suggesting that the observed defects are specific to endocrine-associated ducts.

To determine whether these abnormalities occur earlier in development, we next analysed pancreatic morphology at E12.5. At this stage, *MafB*$^{-/-}$ pancreatic anlagen appeared indistinguishable from wild-type littermates, indicating that MafB is not required for early pancreatic bud formation and that the observed defects arise later in development.

These results were somewhat unexpected, as *MafB* expression is initially detected in the endocrine lineage from E10.5 onward, with no reported expression in ductal cells (131, 168). During pancreatic development, endocrine progenitors delaminate from the epithelium to form the pancreatic islets, a process regulated by several transcription factors (169). Notably, epithelial and ductal abnormalities similar to

those observed here have been reported in *Hnf1b*, *Neurog3*, and *Prox1* knockout models (170-172). Since ductal branching defects were only found in islet and ductal regions near mutant islets, it is likely that the altered ductal organization in *MafB*$^{-/-}$ pancreata arises indirectly from impaired α- and β-cell migration or epithelial exit, rather than from a direct effect on ductal cells. Thus, MafB appears to coordinate endocrine differentiation and migration with proper ductal morphogenesis, ensuring the structural integrity of the developing pancreatic epithelium.

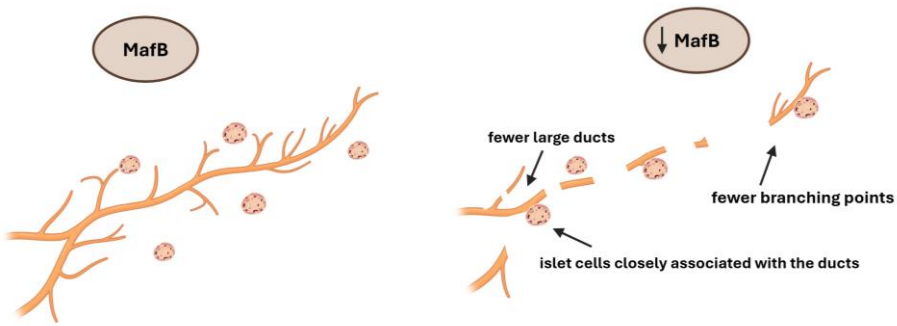

Figure 5: Schematic illustration showing that loss of *MafB* disrupts islet organization and pancreatic ductal morphology, resulting in fewer large ducts, reduced branching, and islet cells remaining closely associated with the ducts. Created with BioRender.com.

MafB regulates neurotransmitter and axon guidance receptor genes required for β-cell–nerve interactions

Next, we investigated how loss of *MafB* affects the transcriptional programs governing β-cell differentiation, focusing particularly on neurotransmitter and axon guidance signalling pathways. We showed that MafB promotes the maturation of α- and β-progenitor cells into functional endocrine cells and supports proper islet formation. In *MafB*$^{-/-}$ pancreata, expression of β-cell maturation genes including *Ins1*, *Ins2*, *Slc30a8*, *Slc2a2*, *Pcsk2*, and *G6pc2* were markedly downregulated, while many cells retained an immature transcriptional profile and co-expressed α- and β-cell marker genes such as *Pdx1* and *Arx*. These findings demonstrate that loss of *MafB* hinders the timely differentiation and maturation of endocrine cells, thereby disrupting normal islet development. A comparable accumulation of immature endocrine progenitor cells was also reported in β-like cells derived from *MAFB*-

deficient human embryonic stem cells (173). However, in this in *vitro* model, *MAFB* loss additionally led to an increased proportion of somatostatin-producing δ-cells, a phenotype not observed in the developing mouse pancreas. This difference may reflect species-specific roles of *MafB* or differences between in *vitro* differentiation systems and in *vivo* pancreatic development.

Beyond its role in endocrine differentiation, MafB also regulates the expression of genes involved in neurotransmitter and axon guidance signalling, which are essential for communication between developing β-cells and their microenvironment. Loss of *MafB* resulted in a marked downregulation of several neurotransmitter receptor genes (*ChrnA3*, *ChrnA4*, *ChrnB4*, *P2ry1*, *Adra2a*, *MaoB*) as well as axon guidance receptors (*Robo1*, *Robo2*, *Nrp1*, *Nrp2*, *PlxnA3*, *PlxnA4*). These pathways are known to influence β-cell migration and islet morphogenesis, suggesting that MafB not only promotes β-cell maturation but also coordinates neuronal signalling mechanisms that guide islet formation.

Analysis of human fetal pancreas (7–14 PCW) revealed that $MAFB^{+}/PDX1^{+}$ endocrine progenitor cells co-express neurotransmitter and axon guidance genes such as *ADRA2A*, *CHRNB1*, and *ROBO1*, with autonomic nerves positioned nearby, indicating early nerve–islet interactions. These findings challenge earlier reports suggesting that parasympathetic acetylcholine signalling contributes to β-cell function only in rodents (92) and are supported by observations of nerve–islet contacts in human fetal pancreas (174). Consistently, loss of *MAFB* in human β-like cells reduced the expression of these genes, while *MAFB* levels in adult islets correlated with neurotransmitter and axon guidance pathways. Moreover, eQTL analyses linked variants in *ADRA2A (175)* and *CHRNB4 (176)* to altered gene expression and T2D risk, suggesting that *MAFB*-dependent neuronal signalling contributes to both islet development and β-cell function.

Finally, cell culture experiments demonstrated that inhibition of nicotinic receptor activity in co-cultures of embryonic β-cells and autonomic ganglia impaired β-cell migration and clustering, demonstrating that acetylcholine signalling contributes to islet innervation and organization (Figure 6). A similar effect was observed following nicotine treatment, indicating that finely tuned, localized receptor activation is required to provide directional cues for β-cell migration, whereas uniform activation abolishes this guidance. These observations, together with previous findings on β-adrenergic signalling (98), suggest that both adrenergic and nicotinic pathways play complementary roles in regulating β-cell positioning and islet morphogenesis. Such mechanisms likely involve intracellular calcium signalling, as seen in other epithelial systems (177), and underscore the importance of MafB-dependent regulation of neurotransmitter receptor expression in coordinating neuronal cues during islet development.

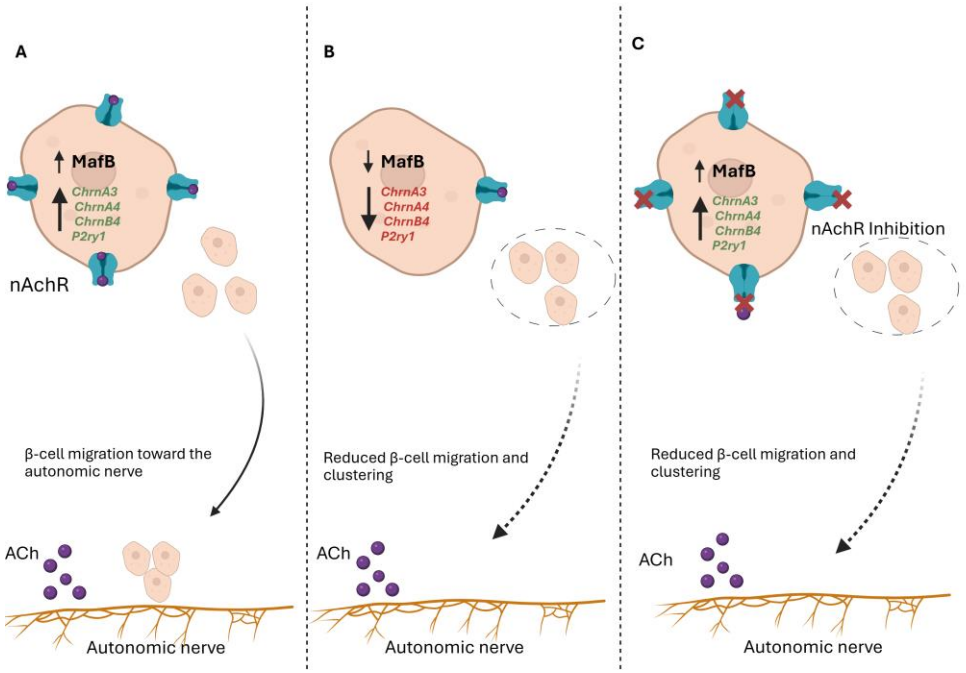

Figure 6: Schematic summary of the main findings from Paper II. (A) In wild-type β-cells, MafB enhances the expression of neurotransmitter and axon-guidance receptor genes (e.g., *ChrnA3, ChrnA4, ChrnB4, P2ry1*), enabling nAchR-mediated signaling and promoting directed β-cell migration toward autonomic nerves. (B) In MafB-deficient β-cells, these receptor genes are markedly downregulated, resulting in impaired nAchR signaling and reduced β-cell migration and clustering near autonomic nerves. (C) Pharmacological inhibition of nAChR similarly disrupts acetylcholine-mediated signaling, leading to reduced β-cell migration and impaired cluster formation, demonstrating that nAchR activity is required for β-cell–nerve interactions during islet formation. Created with BioRender.com.

Key findings:

- MafB is essential for islet and ductal morphogenesis during pancreatic development.
- MafB is critical for proper lineage specification, promoting timely commitment of progenitors to the α- and β-cell fates.
- MafB is required for the expression of multiple neurotransmitter and axon guidance receptor genes in developing β cells in both mouse and human.
- Inhibition of nicotinic receptor activity reduces β-cell migration toward autonomic nerves and impairs β-cell clustering.

Paper III

Novel MAFB-Regulated Genes Contribute to the Genetic Architecture of T2D

Single-cell transcriptomic studies have mapped pancreatic development at high resolution, defining major cell types and uncovering significant heterogeneity within endocrine progenitor populations (149, 178, 179). These analyses have shown that transcription factors can exert subtype-specific functions even among closely related islet cells, highlighting the complexity of endocrine lineage regulation. However, they don't resolve the mechanistic basis by which individual transcription factors coordinate progenitor cell progression and endocrine cell fate decisions. MafB, expressed in both α- and β-cell precursors during early pancreatic development, is a strong candidate regulator of these processes, yet its transcriptional targets in endocrine progenitor cells remain incompletely defined.

Result and discussion:

Our first aim was to define the transcriptional dynamics underlying endocrine lineage transitions. To address this, we performed single-cell RNA sequencing on GFP^+ cells isolated from MafB-GFP knock-in embryos at embryonic days E13.5, E14.5, and E15.5, while E15.5 wild-type embryos were included as controls. Comparative analysis of wild-type, heterozygous, and MafB-deficient pancreata revealed that $MafB^+$ cells were enriched within endocrine progenitor, α-, and β-cell clusters. In contrast, MafB-mutant cells accumulated in a transitional 'pro-α/β' progenitor cell population, a state rarely observed in wild-type E15.5 samples and previously noted in MafB-deficient tissue using single-cell qPCR (180). Transcriptomic profiling revealed extensive gene expression changes during the transition from endocrine progenitors to pro-α/β progenitors, whereas relatively few changes occurred during subsequent maturation into α- or β-cells. These findings suggests that the pro-α/β cell state represents a late differentiation stage immediately

before lineage commitment, consistent with earlier reports showing that early α- and β-cells share a common transcriptional program (181).

To examine how MafB loss affects differentiation, we compared $MafB^+$ and $MafB^-$ cells within each cluster. In both α- and β-cell populations, $MafB^-$ cells showed reduced expression of key identity genes (e.g., Slc30a8, Pcsk2, Gcg, Ins1, Syt4), indicating that MafB reinforces transcriptional programs required for terminal endocrine cell differentiation. Only minor differences were detected in late endocrine progenitor cells.

The most pronounced transcriptional effects were observed in the $Neurog3^+$ early endocrine progenitor cell cluster, where 143 genes (many of these genes encoded transcription factors) were differentially expressed between $MafB^+$ and $MafB^-$ cells. Cross-species comparison using published scRNA-seq data from MAFB-deficient human ES-derived β-like cells (173) showed that most of these transcription factors were similarly dysregulated in human cells, suggesting a conserved role for MafB. Furthermore, comparison with published gene regulatory networks revealed that approximately 75% of these genes are likely direct MafB targets (181). Integration with fetal human pancreas datasets further showed that key MafB-dependent transcription factors including: AUTS2, ETV1, MEIS2, RUNX1T1, MLXIPL, and TOX3 are expressed in human endocrine progenitor cells. While some factors such as TOX3 and RUNX1T1 were enriched in early progenitor cells in both species, ETV1 consistently marked pro-α lineage cells. In contrast, AUTS2 and MEIS2, which align with α-cell differentiation in mice, were predominantly expressed in β-cell progenitor cells in humans, indicating conserved regulatory network with species-specific deployment of individual factors.

Several MafB-regulated transcription factors have established roles in chromatin remodeling, neuronal differentiation, and cell fate control, indicating that MafB engages conserved regulatory mechanisms across tissues. For example, MEIS2 cooperates with PDX1 to enhance pancreatic gene expression and regulates PAX6 in other developmental contexts (147, 148). Our data further show that its expression in $Neurog3^+$ progenitor cells depends on MafB and is reduced in T2D islets (182), suggesting a role in endocrine cell maturation and adult β-cell function. Other factors appear to link MafB to calcium-responsive transcriptional pathways.TOX3, a Ca^{2+}-regulated transcription factor that interacts with the CREB–CBP complex (183) may couple MafB activity to Ca^{2+}-dependent maturation signals. Consistent with this model, AUTS2, a regulator of neuronal differentiation and PRC1-mediated chromatin activation (137, 184), may act downstream of MAFB to initiate lineage-specific transcriptional programs. ETV1 which contributes to cytoskeletal organization and tissue morphogenesis (143), may similarly function at the interface of transcriptional and structural aspects of endocrine cell differentiation. Together, these findings suggest that MafB controls a network of transcription factors that integrate chromatin regulation, Ca^{2+}-dependent signaling, and cell organization, linking developmental programs to pathways relevant for T2D susceptibility. An

overview of the MafB-dependent transcriptional network regulating endocrine progenitor progression and α/β-cell differentiation is shown in Figure 7.

Analysis of adult human islets further supports these connections. ETV1 and MEIS2 are expressed in mature endocrine cells, whereas AUTS2 shows transient expression that is elevated in T2D islets. Genetic and islet eQTL analyses identified T2D-associated variants in putative enhancer regions of ETV1 (downstream) and AUTS2 (intronic). The AUTS2 intronic regions containing T2D risk alleles, islet eQTLs, and MAFB-binding sites were highly conserved among donors; sequencing an 800 bp fragment showed that the only difference in the sequence was the T2D-associated SNP, implying functional importance. Reporter assays confirmed this, showing that MAFA and MAFB differentially regulate enhancer activity between risk and reference alleles in a pattern consistent with islet eQTL effects. These findings demonstrate that AUTS2 and ETV1 regulatory regions are directly responsive to islet transcription factors and that these noncoding variants disrupt transcriptional regulation.

In conclusion, our data identify MafB as a key regulator of endocrine progenitor cell specification and α/β-cell lineage commitment. Beyond its developmental role, MafB coordinates a broader transcriptional network that links endocrine differentiation to pathways implicated in T2D genetics. MafB-dependent factors such as AUTS2, ETV1, and MEIS2 emerge as critical nodes connecting developmental transcriptional programs with adult β-cell function and disease vulnerability. Future work using human ES-cell–derived models will be important to delineate how disruption of this MAFB-centred regulatory network contributes to defective islet formation and β-cell dysfunction in diabetes.

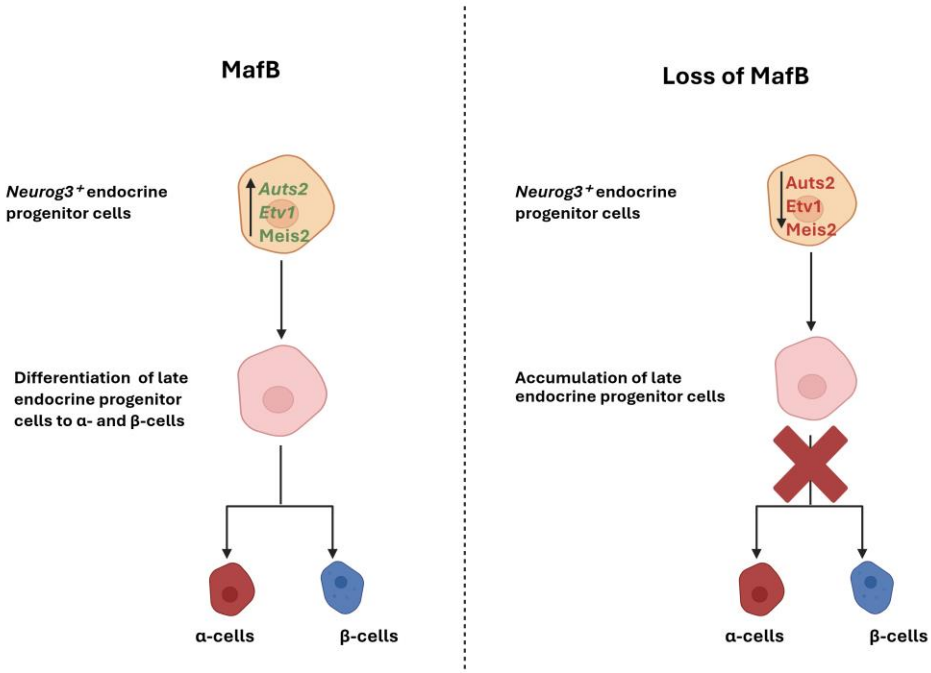

Figure 7: Overview of the main findings from Paper III. Left: With *MafB*, early *Neurog3*$^{+}$ progenitors upregulate *Auts2, Etv1, and Meis2* progress to late endocrine progenitor cells, and differentiate into α- and β-cells. Right: Loss of *MafB* reduces expression of these transcription factors, causing accumulation of late endocrine progenitor cells and impaired α- and β-cell differentiation. Created with BioRender.com.

Key findings:

- Loss of MafB results in the accumulation of late endocrine progenitor cells
- MafB expression is required in early endocrine progenitor cells
- MAFB participates in a transcriptional network regulating human endocrine cell development
- AUTS2 and ETV1 harbour T2D risk alleles and regulate enhancer activity in a MAF-dependent manner.

Concluding Remarks

This thesis provides new insights into the role of the MAFB transcription factor in pancreatic islet development and function. By combining studies on insulin exocytosis, neural signaling, and transcriptional regulation of endocrine progenitor cell differentiation the work highlights the diverse and stage-specific contributions of MAFB to endocrine biology.

The first study demonstrates that MAFA and MAFB jointly regulate genes essential for insulin granule exocytosis, linking their dysfunction to impaired β-cell secretion and T2D. The second study reveals that MafB deficiency impairs β-cell clustering and islet formation, coinciding with the loss of neurotransmitter and axon guidance receptor gene expression, thereby uncovering an unexpected role for MAFB in coordinating neural signaling events required for islet morphogenesis. The third study identifies MAFB target genes in endocrine progenitors, advancing our understanding of how this factor shapes early lineage specification and gene regulatory networks.

Together, these findings establish MAFB as a key regulator across multiple stages of islet biology from progenitor cell specification to islet morphogenesis and β-cell functional maturation. They also suggest that MAFB dysfunction may contribute to diabetes through both developmental and functional mechanisms. Importantly, a thorough understanding of the molecular mechanisms that regulate islet development will be essential to recreate islet architecture *in vitro* and to generate fully functional islets for transplantation, and the knowledge gained from this thesis contributes toward that long-term goal.

General Discussion and Future Perspectives

The findings presented in this thesis provide new insight into how MAFB transcription factors regulate β-cell function, islet morphogenesis, and endocrine cell lineage specification. Although MAF transcription factors have long been associated with β-cell maturation, the work in this thesis reveals that their roles extend across multiple developmental stages and biological processes. Together, the three papers demonstrate that MAFB is not only essential for insulin secretion but also plays key roles during pancreatic development, including β-cell migration and the transcriptional control of endocrine progenitor cells. By integrating mouse models, human islet datasets, and comparative datasets from stem-cell–derived β-like cells, this work connects developmental biology with genetic mechanisms relevant to T2D.

Paper I introduce an important conceptual advance by identifying a conserved set of MAFA and MAFB regulated exocytosis genes in human and mouse β-cells. Prior studies linked MAFA to mature β-cell identity, but its direct downstream targets involved in insulin granule docking and fusion had not been systematically characterized. The findings show that MAFA and MAFB regulate core components of the exocytotic machinery, linking reduced MAF expression which was observed in β-cells from individuals with T2D to impaired insulin secretion. The discovery of a MAFA genetic variant associated with lower expression of exocytosis genes suggests a mechanism by which genetic variation may predispose individuals to defective insulin secretion and increased diabetes risk.

Paper II uncovers a previously unrecognized developmental function of MafB by demonstrating that β-cell migration and islet formation rely on neurotransmitter and axon guidance receptor pathways that are MafB-dependent. Although MafB was known to influence α- and β-cell differentiation, its role in coordinating neural-derived signals that guide islet morphogenesis had not been explored. This study shows that MafB deficiency disrupts nerve-dependent signaling pathways and impairs β-cell clustering, revealing that endocrine progenitor cells rely on autonomic cues to assemble into structured islets. In addition, the findings from Paper II indicate that MafB also affects β-cell–associated ductal morphogenesis, as loss of MafB results in fewer large ducts and reduced branching specifically in

regions where endocrine cells fail to delaminate from the epithelium. This suggests that proper β-cell migration is necessary to maintain normal ductal structure.

A key novel aspect of this thesis emerges from Paper III, where we uncover a set of previously uncharacterized MAFB-dependent genes in endocrine progenitor cells, with *AUTS2* and *ETV1* representing the most prominent examples. The integration of transcriptional profiling, cross-species comparison, and genetic association analyses demonstrates that these MAFB-regulated factors coincide with T2D-associated risk loci**,** thereby establishing a mechanistic link between early developmental gene networks and later-life diabetes susceptibility. This finding significantly extends the current understanding of endocrine lineage commitment and identifies new molecular pathways for future investigation.

The results presented here collectively deepen our understanding of how transcription factors integrate developmental programs with functional outcomes in β-cells, but they also raise important questions for future research. An important next step will be to delineate the complete MAFA- and MAFB-dependent gene regulatory networks in human β-cells. Genome-wide binding assays such as CUT&RUN or ChIP-seq, integrated with single-cell transcriptomic would help establish whether STX1A and STXBP1 are part of broader MAF-dependent networks essential for vesicle docking and fusion. To establish causality, MAFA and/or MAFB could be repressed in human β-cell models (EndoC-βH1 and primary islets) followed by re-expression of STX1A and/or STXBP1. If restoring these effectors normalizes glucose- and KCl-stimulated insulin secretion, this would demonstrate that STX1A and STXBP1 are critical downstream effectors linking MAFA/MAFB activity to insulin exocytosis. Complementary loss of function experiments targeting STX1A and STXBP1, combined with rescue using siRNA-resistant constructs or enCRISPRa, would further confirm pathway specificity and clarify the relative contributions of each gene.

The findings in Paper II also open new directions regarding how neuronal cues regulate islet morphogenesis. Identifying which receptor subunits are most critical, and imaging how β-cells respond to acetylcholine in real time, could provide important mechanistic insight. Moving these studies into human stem cell–derived islets combined with neuronal co-culture systems will be particularly valuable, as it will establish whether these pathways are conserved in humans. Such approaches could ultimately be applied to improve stem cell differentiation protocols so that *in vitro*–derived islets develop an architecture that more closely resembles their *in vivo* counterparts.

Paper III focuses on the early stages of endocrine lineage specification. This work validates MAFB target genes in endocrine progenitor cells, particularly those overlapping with T2D risk loci. CRISPR-based editing of these genomic regions in stem cell lines, followed by differentiation into α- and β-cells, will provide a direct way to test whether genetic variation influences endocrine lineage allocation

through MAFB. Complementary studies in human pancreatic tissue from risk allele carriers, combined with analysis of large donor transcriptomic datasets, can connect these mechanistic insights to human physiology and disease.

Beyond the direct extensions of this work, broader questions remain. Are the neural signalling roles of MAFB specific to the pancreas, or do they reflect a more general developmental mechanism? Comparative studies across species, alongside advanced human stem cell–derived models, will be important to address these questions.

Finally, this work carries important translational implications. A thorough understanding of the molecular mechanisms that regulate islet development and maturation will be crucial to optimize the generation of functional β-cells from stem cells. By integrating MAFB-dependent transcriptional networks and neuronal signalling pathways into differentiation protocols, it may become possible to engineer islets that replicate native architecture and function. In the long term, such advances could help bring cell replacement therapies for diabetes closer to reality.

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