UNIVERSITY OF CALIFORNIA SAN DIEGO

Myt1l Contributes to Reprogramming of Mature Hepatocytes to a Beta-cell Like State

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Melissa Tran

Committee in charge:

Marc Montminy, Chair
Gen-Sheng Feng, Co-Chair
Stephanie Mel

2020

The Thesis of Melissa Tran is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Co- Chair

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Chair

University of California San Diego

2020

DEDICATION

I dedicate this thesis to my family and friends. I am so grateful for all of the love and support that I have received during this process. Your ongoing encouragement has motivated me to push through every difficult moment.

To my parents, I wouldn't be where I am and who I am without you both. You have taught me so much and you have molded me into a person who is hardworking, thoughtful, and smart. Your sacrifices have given me, Emily and Bryan a better life and there are not enough words to thank you. I love you both so much and I hope that my accomplishments make you proud.

To Peter, thank you for everything that you do for me. Your support and love has pushed me to accomplish so much. I can always count on you. You kept me focused and you always made sure that I saw the finish line. I couldn't have done it without you.

To Joanne, my best friend, you have been an amazing friend to me and I can never repay you for all of the support that you have given me. Thank you for everything.

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LIST OF ABBREVIATIONS

NeuroD1: Neurogenic Differentiation 1

Pdx1: Pancreatic and duodenal homeobox 1

MafA: V-maf musculoaponeurotic fibrosarcoma oncogene homolog A

Pax6: Paired Box 6

Isl1: ISL LIM Homeobox 1

Insm1: Insulinoma-associated 1

Myt1l: Myelin Transcription Factor 1 Like

Ngn3: Neurogenin 3

IAPP: Islet Amyloid Polypeptide

Ins1: Insulin 1

Ins2: Insulin II

qPCR: Quantitative Polymerase Chain Reaction

LDTF: Lineage Determining Transcription Factor

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ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge Dr. Marc Montminy for allowing me to be a part of his lab. For the past 5 years I have been lucky enough to find a home in Dr. Montminy's lab. I have not only grown as a scientist but also as an individual, where I have learned that diligence and preparedness are essential to not only research, but life itself. I cannot express enough gratitude towards Dr. Montminy and the rest of the members of the lab.

I would also like to acknowledge Gen-Sheng Feng and Stephanie Mel for taking the time and effort to be a part of my committee. I am very appreciative of all of the encouragement and support that I have received.

My sincere thanks also goes to Dr. Sam Van de Velde for allowing me to work alongside him on this project. I am extremely grateful for his patience, encouragement and mentorship. He has provided me with invaluable guidance. It was a privilege to work under and with Dr. Van de Velde.

ABSTRACT OF THE THESIS

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Master of Science in Biology

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Professor Marc Montminy, Chair

Professor Gen-Sheng Feng, Co-Chair

The goal of regenerative medicine is to replenish damaged and diseased cells in the body. In order to do so, mature cells are encouraged to convert towards another cell type. One way of doing this is by overexpressing specific genes using viral vector delivery. Previous research has shown that the beta cell specific transcription factors *Pdx-1, NeuroD1, MafA, Insm1, Isl1,* and *Pax6* are able to shift liver cells and exocrine pancreas cells towards a beta cell-like state. In this experiment, we analyze different combinations of these lineage determining transcription factors

and their reprogramming effects on mature hepatocytes. We were also prompted to investigate *Myt1l*, a gene that has previously only been found in neuronal tissue. Previous work done in the lab, however, has shown it to be highly enriched in islets, which may suggest that the gene plays a critical role in beta cell function. Here we show that combining the transcription factors *Pdx-1*, *NeuroD1*, *MafA*, *Insm1*, *Isl1*, *Pax6* and *Myt1l* leads to a substantial shift towards a beta cell-like state based on the increased expression of IAPP, a beta cell marker that is co-expressed with insulin to regulate blood glucose levels with satiety signals. Furthermore, our results suggest that *Myt1l* is an important factor converting mature hepatocytes towards a more beta cell-like state. Based on transcriptomic characterization, experimental conditions in the presence of *Myt11* when compared to the experimental conditions without M*yt1l* display an upregulation of beta cell markers that are closely related to insulin secretion. These observations provide evidence that *Myt1l* is a key beta cell reprogramming factor.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease that has become the seventh leading cause of death in the United States. Long-term complications of the disease can lead to increased health difficulties, leading to a decreased quality of life. Diabetes is characterized by hyperglycemia, which is caused by auto-immune destruction of pancreatic beta cells (Type 1) or resistance in insulin action followed by decreased beta cell function (Type 2). Healthy beta cells are critical for maintaining blood glucose levels and metabolism. Without beta cells, sugar accumulates in the blood and causes damage to various organs, particularly in the heart, kidneys, eyes, nerves, and blood vessels (American Diabetes Association, 2013).

Several strategies for diabetes therapy have been developed and proven to help increase the quality of life for diabetes patients. Insulin injections, administered daily, and insulin pumps, that are surgically implanted, are the most prominent techniques to replenish insulin levels into the body (Wang et al., 2007). Beta cell transplantation is another option to replenish healthy insulin secreting beta cells from cadaveric pancreases. Alternative options for those with diabetes include replenishing beta cells and generating beta-like cells. There are several methods to encourage the expansion of beta cells. Islet neogenesis and beta cell replication can both increase existing beta cell numbers with the help of different stimuli (Bonner-Weir et al., 2012)). Another strategy to increase beta cell numbers is to differentiate human embryonic stem (ES) cells and induced pluripotent stem cells (iPS). With specific culture conditions and sequential addition of growth and differentiation factors, new beta-like cells are able to secrete insulin in the presence of glucose and correct hyperglycemia (Zhang et al., 2009). All of these techniques look like promising treatments for diabetes, however they all have different limitations which prevent them from being long term solutions.

Transdifferentiation transforms mature, specialized cell types into another while bypassing a pluripotent stage. This technique requires an ectopic expression of transcription factors and it works without the risk of teratoma genesis or an immune rejection response. This technique changes the chromatin accessibility and activates endogenous genes that are important for islet development and function. Previous studies have investigated the reprogramming abilities of a number of different lineage determining transcription factors that are critical for beta cell maturation and function. Research on *NeuroD1, Pdx1, and MafA* (NPM) have confirmed that these three transcription factors bind to the insulin promoter and promote transcription of insulin mRNA. Their individual functions are stated in Table 1. A study done by Guo et al., investigated how the combined transfection of NPM causes bone marrow mesenchymal stem cells to transdifferentiate into insulin secreting cells. They found that the triple infection had a stronger effect in converting stem cells than a single or double infection. Combining these three genes leads to an upregulation of the insulin gene and induces biosynthesis and secretion of insulin in the converted stem cells. However, the study also indicates that the triple infection is not enough to make the change permanent (Guo et al. 2012). In order to successfully convert mature liver cells into beta cells, additional genes may be necessary.

Studies on *Pax6, Isl1* and *Insm1* have demonstrated that these individual genes play an important role in directing various cell lines towards a beta cell lineage (Lee et al., 2013). Research on *Pax6* indicates that it is an important lineage determining transcription factor. Additional details on the function of *Pax6* can be found in Table 1. A previous study conducted by Lee et al., shows that they were able to convert exocrine ductal cells into beta-like cells after infected with *Pax6, MafA, Neurog3*, and *Pdx1*. Expression of these four transcription

factors gave the cells the ability to synthesize, process and store insulin, as well as secrete it in response to glucose. However, without the presence of *Pax6*, insulin expression was significantly downregulated (Lee et al., 2013).

Similar to *Pax6*, *Isl1* plays a significant role in beta cell development. *Isl1* binds to the enhancer region of the insulin gene, where it activates insulin expression with the help of *NeuroD1*, and regulates transcription of *Pdx1* and *MafA* (Jung et al., 2018). There is a strong interaction between *Isl1* and NPM. Jung et al. investigated the ectopic expression of *Isl1* in conjunction with NPM, and why it leads to a stronger and longer transdifferentiation of hepatic cells. The data reveals that the hepatocytes infected with NPM and *Isl1* could produce and secrete insulin. This virus combination improved glucose tolerance and responsiveness, and reverses hyperglycemia in diabetic mice for 28 days. When compared to NPM alone, the addition of *Isl1* made a significant shift towards more of a beta cell-like state. This experiment indicates the importance of *Isl1* as a key factor in generating glucose-responsive insulin-producing cells (Jung et al., 2018).

Along with the other lineage determining factor that we researched, we found *Insm1* to be a promising addition to the project. The transcription factor regulates the downstream target *NeuroD1* and insulin during beta cell maturation (Zhang et al., 2010). Zhang et al. was able to increase insulin expression with the overexpression *of Insm1*, *Pdx1*, and *NeuroD1* in a pancreatic duct cell. They also discovered that *Insm1* alone can begin beta cell reprogramming by modifying the expression of key islet transcription factors. In another study done by Jia et. al, *Insm1* is conditionally removed from beta cells. They found that these cells do not secrete insulin in the presence of glucose, indicating its important role in mature beta cell function (Jia et al., 2015). Alone, *Insm1* has proven to be capable of reprogramming cells towards a beta cell fate

(Zhang et al., 2010). Adding this factor, along with other key transcription factors that are previously mentioned, we hypothesize that there will be a stronger shift towards beta cell differentiation.

Along with known beta cell-specific transcription factors, we are interested in looking at *Myt1l*, a gene whose function is unknown in beta cell development. A genome-wide comparison of primary islet and hepatocyte data previously conducted in the lab has revealed that *Myt1l* is highly enriched in islet tissue. *Myt1l* was recently reported as a master repressor, in which its main purpose is to establish and maintain the identity of neurons. The gene represses expression of non-neuronal genes during neuronal differentiation in order to establish neuron development. Some genes that are suppressed are ones that that are important for the development of the lung, heart, liver, cartilage (Mall et al., 2017).

In order to further this research, we were looking to combine the known lineage determining transcription factors with *Myt1l* to see if there is a bigger, synergistic effect on hepatocyte to beta cell transdifferentiation. Recent work suggests that adenoviral infection with multiple lineage determining factors will produce a stronger effect than the expression of one factor alone. Liver and pancreas are derived from a common endodermal lineage (Wang et al., 2007). Thus we chose to use liver cells to test our hypothesis, and attempted to differentiate liver cells towards a beta cell state. By utilizing mature hepatocytes as a source for transdifferentiation, we work with an abundant amount of cells. The goal of this experiment is to test different combinations of known lineage determining factors, as well as *Myt1l*, to see if these different combinations can induce the shift of mature hepatocytes to more of a beta cell like state. The data from this experiment reveals that *Myt1l* is in fact a key lineage determining factor, and plays a strong role in reprogramming mature hepatocytes into a beta-like cell. In its

presence, there is a significant conversion of mature hepatocytes into beta like cells, and genes that are related to islet function are significantly upregulated. Through our experiments, we also found that there is a stronger shift when all of the transcription factors in the study were co-expressed.

MATERIALS & METHODS

Adenovirus Production

In order to induce the expression of GFP, *NeuroD1, Pdx1, MafA, Pax6, Myt1l, Insm1*, and *Isl1*, individual adenoviral vectors were generated for each transcription factor. The gene of interest was amplified in Ins-1 cells and then cloned into the ADTrack(+CMV) vector. The vector was linearized with PmeI and then recombined with the AdEASY vector in BJ5183 cells by electroporation. The recombinants are transformed to a RecA strain and then transfected into MGH-293 cells. Purification by ultracentrifugation was done over CsCl gradients.

Preparation of Mouse Hepatocyte Cells

Collagenase perfusion of wild-type C57BL/6J mice (The Jackson Laboratory 000664) liver was executed to isolate primary hepatocytes. The mice were anesthetized with 7% PBS chloral hydrate. The liver was washed with HBSS with 0.25mM of EGTA and then perfused with a fresh, sterile mixture of HBSS, collagenase and 5mM of CaC2l. After the liver was removed it was shredded and filtered with a 250nm Nylon mesh. The cells are spun and washed twice with DBF media (M199 Media mixed with 1% Penicillin streptomycin, 0.2% BSA, and 2% Fetal Bovine Serum). The cells are plated in DBF and after 4 to 5 hours, the media is replaced with M199 media with 1% Penicillin Streptomycin.

RNA isolation, quantitative polymerase chain reaction (qPCR)

RNA was extracted from tissue with Trizol (Invitrogen 15596026). Utilizing 1ug of total RNA, cDNA was synthesized with the Transcriptor First Strand cDNA Synthesis Kit (Roche 04897030001) following the manufacturer's instructions. Quantitative PCR was then performed

in a LightCycler 480 II (Roche) using LightCycler 480 SYBR Green I Master mix (Roche 04887352001). Values were normalized against L32.

RNA Sequencing

Total RNA was extracted from hepatocytes 36 hours after treatment with Trizol. Each sample was quantified using a Quibit 2.0. The RNA library was prepared following the NEBNext Ultra II Directional RNA Library Prep Kit from Illumina (NEB #E7760S/L). The mRNA is fragmented and then prepared for first strand cDNA synthesis, followed by the second strand cDNA synthesis. The double stranded cDNA is purified using NEBNext Sample Purification Beads. Adaptors are then ligated to the cDNA and the reaction is purified using the NEBNext Sample Purification Beads. The library quality is asses with a Qubit 2.0 and tapestation (Agilent Tape Station 2200) for library quantity and quality.

RNA Sequencing Analysis

FASTQ files were aligned against the mouse genome (mm10) using STAR (Dobin et al., 2013). For the differential expression analysis in Figure 2 and 3, raw read count tables of two biological replicates from hepatocytes treated with control-GFP and indicated experimental condition, were generated using Homer (Heinz et al., 2010) (analyzeRepeats.pl with option –raw). Differential expression was calculated with Homer (getDiffExpression.pl using DESeq2 (Love et al., 2014)).

RESULTS

Selection of transcription factors

Previous work in the lab has identified a number of transcription factors that are enriched in primary mouse islets, but not in hepatocytes (Table 1). A genome wide comparison of primary mouse islets and hepatocytes has revealed that there are several genes that are highly enriched in only islet cells, and not in hepatocyte cells.

The growing expression of the Islet Amyloid Polypeptide gene increases with the number of lineage determining transcription factors expressed in each experimental condition.

Primary mouse hepatocytes were infected with distinct adenovirus vector combinations in order to analyze the cells' shift towards a beta-cell state. The mRNA expressed from the hepatocyte cells of wild-type C57BL/6J mice were analyzed by qPCR. To determine whether the cells were converting to more of a beta cell-like state, we tested the expression of the gene, Islet Amyloid Polypeptide (IAPP). This gene is specifically expressed in beta cells and produces a hormone that is co-secreted with insulin after food intake. It also regulates blood glucose levels and acts as a satiation signal. We tested six conditions: (1) Control-GFP, (2) NPM, (3) NPM with *Pax6*, (4) NPM,*Pax6* and *Myt1l*, (5) NPM, *Pax6, Insm1* and *Isl1*, (6) NPM, *Pax6, Myt1l*, *Insm1*, and *Isl1 (*Figure 1). The gene expression levels of the six conditions in mature hepatocytes are shown in Fig. 1. The results demonstrate that IAPP was upregulated in conditions 2 through 6. As expected, the control condition has undetectable expression of IAPP. Triple infection with NPM, slightly increases expression of IAPP, and we found that it is further upregulated with each additional transcription factor that is added. Conditions 5 and 6 show a significantly higher expression of IAPP compared to conditions 1-4, which are expressing fewer

transcription factors. When comparing condition 2 with 5 and 6, there is a greater than seventy-four fold increase.

RNA sequencing

RNA sequencing was utilized to analyze and compare the transcriptome of the different experimental conditions against the control condition, GFP. RNA was collected from primary hepatocytes that were infected with (1) GFP, (2) NPM, (3) NPM with *Pax6*, (4) NPM,*Pax6* and *Myt1l*, (5) NPM, *Pax6*, *Insm1* and *Isl1*, (6) NPM, *Pax6*, *Myt1l*, *Insm1*, and *Isl1*. This experiment was duplicated, and the values from both experiments were averaged together and analyzed.

A surprising amount of genes are shown to be significantly up and downregulated after infection for all conditions tested (Fig. 2,3). In condition 2, which is infected with NPM alone, there are 229 genes that are upregulated and 130 genes that are downregulated (Fig. 2A). With the addition of *Pax6* (condition 3), there are 250 genes that are upregulated and 96 genes that are down regulated (Fig. 2B). In condition 4, *Myt1l* is introduced into the virus combination, which leads to a jump to 610 total genes that are upregulated, and also an increase to 249 genes that are downregulated (Fig. 3A). In condition 5, with the absence of *Myt1l* and the addition of *Insm1* and *Isl1*, the number of genes that are upregulated drops to 503 and similarly 181 genes are down regulated (Fig. 2C). In condition 6, with all seven genes expressed, there is the greatest amount of genes upregulated at 869 and 292 genes downregulated (Fig. 3B).

Extending this analysis to gene ontology, we listed the top five gene sets that are upregulated and downregulated for each condition (Fig. 2D-F; 3C-D). We found that genes that encode for cytochrome P450 and chemical carcinogenesis are consistently downregulated in all conditions. When we analyze the upregulated gene sets between the experimental conditions,

there are similar trends between the two conditions with *Myt1l* present and likewise there are similarities between the conditions without *Myt1l* present.

In condition 2 and 3, the top upregulated ontology group is Maturity and Onset of Diabetes in the Young. Three out of the four genes in that set are NPM, which was ectopically expressed, and the fourth gene in that group is *IAPP*. Further examination of condition 3 revealed that there is an upregulation of genes that encode for complement and coagulation cascades, as well as insulin secretion (Fig. 2E). In condition 4, with the addition of *Myt1l*, all five gene groups are significantly upregulated and these genes correlate with insulin secretion, cholinergic synapse, oxytocin pathway, axon guidance, and glutamatergic synapse (Fig. 3B). In condition 5, which has all the transcription factors minus *Myt1l,* there is only one gene ontology group that is significantly upregulated: complement and coagulation cascade. The other gene groups that are moderately upregulated are involved in axon guidance, focal adhesion, and the calcium signaling pathway (Fig. 2F). In the last condition, with all of the transcription factors present, insulin secretion is the top gene set that is enriched. Other significantly upregulated gene sets include those involved in the calcium signaling pathway, Rap1 signaling pathway, axon guidance and cGMP-PKG signaling pathway (Fig. 3D). In conditions 4 and 6, the only conditions to include Myt1l, the majority of the genes that are significantly upregulated relate to signaling molecules that increase intrinsic cAMP and calcium levels (ADCY2-4, 7,8, GCG, CACNA1D, ATP1B1).

DISCUSSION

Every year, the number of individuals that are diagnosed with diabetes increases. As this disease becomes more prevalent, there is a greater need in finding new strategies that will treat the root issue of faulty beta cells that cannot properly secrete insulin. A great deal of research on various lineage determining factors has established several important genes that play a key role in reprogramming mature cells into insulin secreting beta cells. Some of the factors that have been studied include *NeuroD1, Pdx1, MafA, Pax6, Insm1,* and *Isl1*. These studies demonstrate that individually, these six factors can assist with cell reprogramming at different degrees. However, in order to completely reprogram a mature cells into a beta cell, additional transcription factors or different combinations of the factors are needed.

In this study, we overexpress different combinations of *NeuroD1, Pdx1, MafA, Pax6, Insm1, Isl1*, and *Myt1l* in mature mouse hepatocytes. Our goal is to identify which combination of factors will stimulate the conversion of hepatocytes towards a beta cell-like state. Our first experimental condition contained NPM, this condition was used as a reference because these three transcription factors have been proven to initiate the reprogramming of mature cells to beta cells. The second experimental condition contained NPM with *Pax6* and the third condition, almost identical to condition 2, also expressed *Myt1l* in addition to the other four factors. Similarly, the fourth condition contained NPM, *Pax6, Insm1,* and *Isl1* while the last condition contained the same factors as the fourth experimental condition with *Myt1l* as well. Through a combination of qPCR and RNA sequencing, we identified *Myt1l* as a key transcription factor for beta cell reprogramming. *Myt1l* contributes to the conversion away from a liver cell, towards an insulin secreting beta cell. In addition to these findings, we discovered that when the seven factors are co-expressed, there is a synergistic effect. The seven transcription factors together, produce a stronger effect, whether it be the *IAPP* expression level or the number of genes up and

down regulated. The data indicates that the interaction between the seven transcription factors leads to a stronger beta cell reprogramming.

My1l has not been studied in relation to beta cell development. Several studies have identified this gene to be exclusively expressed in neurons. A study done by Mall *et al.* classified *Myt1l* as a master repressor to non-neuronal lineages, and contributes to helping establish and maintain the identity of neurons. However, based on previous work done in the lab, we found that *Myt1*l is highly enriched in primary islet cells. This probed us to investigate the gene's role in beta cell development. Our RNA sequencing experiment revealed that the experimental conditions that include *Myt1l,* are shifting away from a liver cell and towards a beta cell-like state. In these conditions, there are a significant amount of upregulated genes that encode for insulin secretion and various mechanisms that relate to beta cell development. There is also a downregulation of genes coding for cytochrome P450, an enzyme highly expressed in liver. These genes are associated with enzymes that detoxify chemical compounds (Guengerich, 2008). Similarly, all of the experimental conditions demonstrate that they are all transitioning away from a hepatocyte state. However, only in the conditions with *Myt1l* present, is there a significant shift towards a beta cell-like state. Genes that encode for Ca2+ channels (CACNA1C, CACNA1D), K+ channels (KCNN2, KCNMB4, ATP1B1), and adenylate cyclases (ADCY2-4, 7, 8), which are essential to the insulin secretion pathway in beta cells, are upregulated. These components are essential in amplifying second messenger signaling; intrinsic cAMP and Ca2+ levels are increased leading to insulin secretion by exocytosis. We also found that conditions with *Myt1l* also have the highest amount of genes up and down regulated which illustrates the importance of the gene in assisting with a stronger conversion. *Myt1l'*s presence stimulates hepatocytes to move away from a liver cell and more importantly, towards an insulin secreting

beta cell. This data indicates that *Myt1l*'s presence will advance the reprogramming effects toward a beta cell-like state.

In addition to these findings, we demonstrated that the delivery of the adenoviral combination with all seven transcription factors had the greatest reprogramming effect. The last condition had the highest expression of IAPP and the greatest number of genes that were up and down regulated. Adding Myt1l to the combination of known transcription factors enhanced the conversion and stimulated a larger reprogramming effect. Each transcription factor has a specific function and role in beta cell development, and when all expressed simultaneously, shifted the cells towards an insulin secreting beta cell-like state. These results add to the growing recognition that more transcription factors may be needed in order to fully convert mature cells into beta cells.

One key finding was that even with all seven factors, the hepatocytes were not able to fully convert to beta cells. Our qPCR data indicates that when compared to the GFP control condition, the other 5 conditions had low to no expression of *Ins1* and *Ins2*, which both encode for insulin expression in mice. Our RNA sequencing data revealed that genes related to insulin secretion are upregulated, but the insulin gene itself is not being activated. Additional transcription factors may be necessary to activate the transcription of insulin.

Further analyses investigating the mechanism of the chromatin acetylation states of known beta cell regions will be necessary. Experiments studying the chromatin accessibility in mouse hepatocytes using an Assay for Transposase-Accessible Chromatin using sequencing (ATAC sequencing), may reveal if there is a chromatin transition towards an open state across beta cell-specific genes as the cells reprogram into a beta cell-like state. Combining this technique with Chromatin Immunoprecipitation sequencing (ChIP sequencing), a correlation

between enhancer histone modifications and open chromatin could reveal specific lineage-determining transcription factors that are bound to lineage-specific open chromatin. Additional transcription factors could be uncovered that may contribute in discovering how to fully reprogramming hepatocytes into insulin secreting beta cells.

FIGURES

Table 1.
Function and relevance of beta cell-specific lineage determining transcription factors used in this study.

Gene	**Function**
NeuroD1	A member of the NeuroD family of basic helix-loop-helix (bHLH) transcription factors. In immature islets, the gene colocalizes with glucagon and insulin. Regulates expression of the insulin gene. Vital for development and maintenance of mature glucose responsive beta cell (Itkin-Ansari et al., 2005).
Pdx1	Master regulator of pancreas development. Required for early embryonic pancreatic formation, specification of different endocrine lineages, and mature beta cell function (Zhu et al., 2017).
MafA	Specifically binds to a conserved insulin enhancer element RIPE3b/C1-A2 and activates insulin gene in response to glucose. Assists with beta cell function and maturation (Zhu et al., 2017).
Isl1	Isl1 is apart of the LIM-homeodomain family, which is an important activator of the insulin gene. The transcription factor binds to the enhancer region of the insulin gene, activates expression with the help of *NeuroD1*, and regulates transcription of *Pdx1* and *MafA*. The protein is essential to the development of pancreatic cell lineages (Jung et al. 2018).
Insm1	Encodes a zinc finger factor that controls differentiation of β-cells and other endocrine cell types in the pancreas and other tissues. It is also in charge of mature β-cell function and is required for correct glucose-stimulated insulin secretion (Jia et al., 2015).
Pax6	Pax6 is involved in the maturation of beta-cells and also in the control of glucose utilization and β-cell response to incretins (Gosmain et al., 2012). This transcription factor directly activates beta cell genes but also binds to promoters and enhancers to repress alternative islet cell genes (Swisa et al., 2017).
Myt1l	Encodes a member of the zinc finger superfamily of transcription factors that are commonly found in neuronal tissue. It is involved in neuronal differentiation and function. Myt1l is described as a master repressor in which its main purpose is to establish and maintain the identity of neurons (Mall et al., 2017)

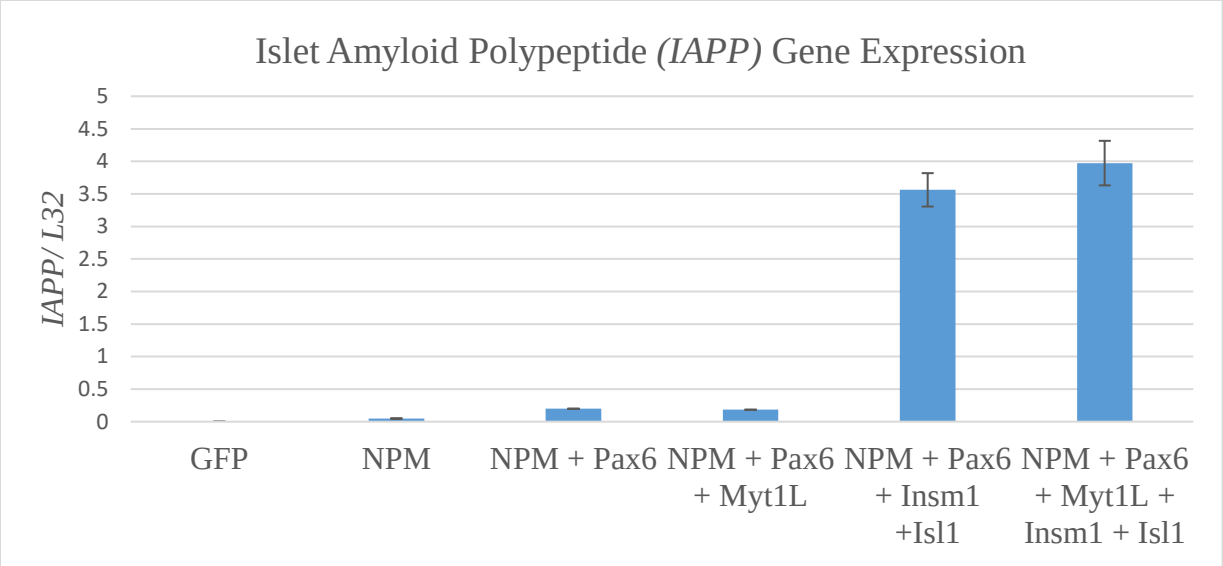

Figure 1. Mature hepatocyte reprogramming and measurement of IAPP gene expression.

qPCR analysis of mouse IAPP gene expression in mature hepatocytes. One day after harvesting, hepatocytes were infected with the indicated reprogramming factors. Virus was left on the cells for 36 hours and IAPP mRNA was measured by QPCR.

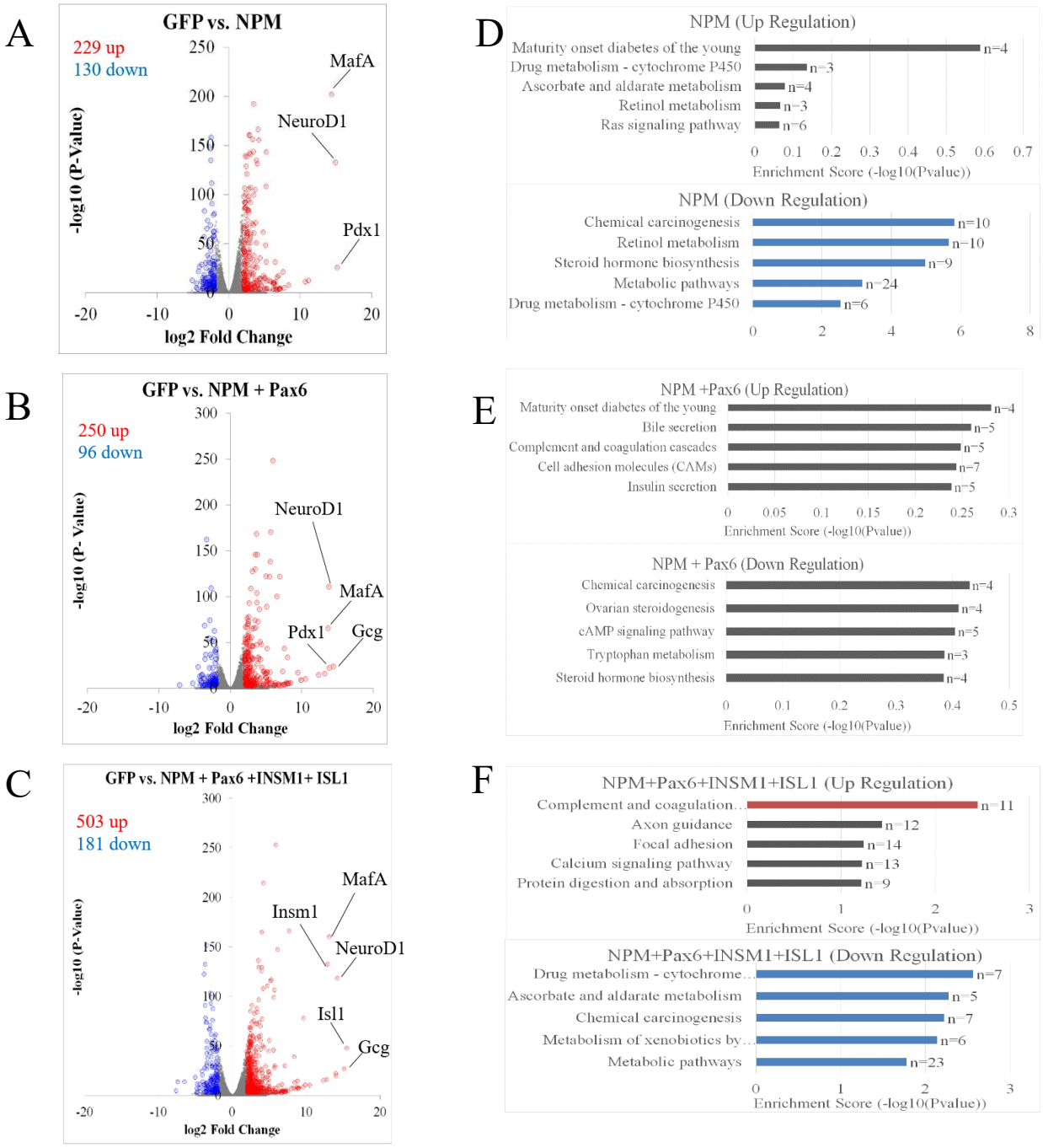

Figure 2. Transcriptomic Analysis of mature hepatocytes infected with beta cell-specific LDTFs excluding Myt1l.

(A-C) Volcano plot for RNA sequencing data between independent replicates of GFP versus indicated condition in hepatocytes, comparing -log10 (p value) versus log2 (fold change), for differential gene expression analysis. Blue data points: treated versus control log2 fold change ≤ -2, Adj. p ≤ 0.001. Red data points: treated versus control log2 fold change ≥ 2, Adj. p ≤ 0.001.

(D-F) Gene set enrichment analysis of mature hepatocytes infected with indicated transcription factors. The top five up and down regulated gene sets are indicated. Red indicates that these gene sets are significantly upregulated and blue indicates that these gene sets are significantly downregulated. Adj. p ≤ 0.01

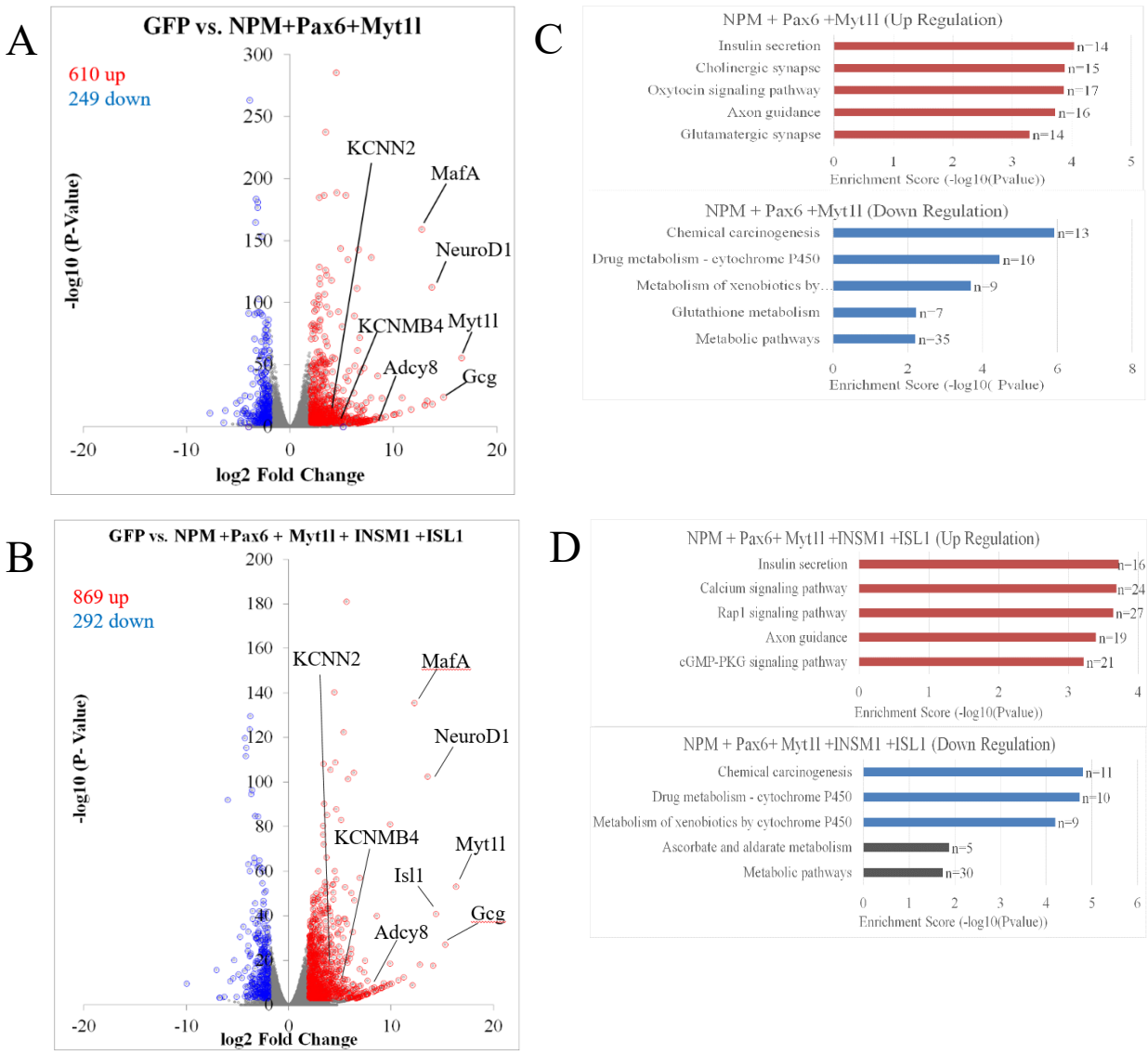

Figure 3. Transcriptomic Analysis of mature hepatocytes infected with beta cell-specific LDTFs including Myt1l.

(A-B) Volcano plot for RNA sequencing data between independent replicates of GFP versus indicated condition in hepatocytes, comparing -log10 (p value) versus log2 (fold change), for differential gene expression analysis. Blue data points: treated versus control log2 fold change ≤ -2, Adj. $p \leq 0.001$. Red data points: treated versus control log2 fold change ≥ 2, Adj. $p \leq 0.001$.

(C-D) Gene set enrichment analysis of mature hepatocytes infected with indicated transcription factors. The top five up and down regulated gene sets are indicated. Red indicates that these gene sets are significantly upregulated and blue indicates that these gene sets are significantly downregulated. Adj. $p \leq 0.01$

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