Pancreatic β-Cell Regeneration in TIF-IA Knockout Mice

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Affidavit

Here I declare that my doctoral thesis entitled **"Pancreatic β-Cell Regeneration in TIF-IA Knockout Mice"** has been written independently with no other sources and aids than quoted.

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Farnaz Shamsi, Göttingen, May 2014

To my Family,

and Mehdi

Contents

1 Acknowledgement

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2 Abbreviations and nomenclature

TIF-IA	Transcription initiation factor 1A
RIP	Rat insulin promoter
Pdx1	Pancreatic and duodenal homeobox 1
Ptf1a	Pancreas transcription factor 1 subunit alpha
Ngn3	Neurogenin 3
PDL	Partial duct ligation
MPCs	Facultative multipotent progenitors
MafA	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein A
STZ	Streptozotocin
Pax4	Paired box gene 4
Rrn3	RRN3 RNA polymerase I transcription factor homolog (S. cerevisiae)
UBF	Upstream binding factor
JNK pathway	c-Jun N-terminal kinase pathway
MDM2	Mouse double minute 2 homolog
UTR	Untranslated region
RISC	RNA-induced silencing complex
mTOR	mammalian target of rapamycin
GLP-1	Glucagon-like peptide 1
lncRNAs	long non-coding RNAs
Glut2	glucose transporter 2
Nkx6.1	NK6 homeobox 1
PPAR	Peroxisome proliferator-activated receptor
MSP-RON	Macrophage Stimulating Protein/ RON Protein Tyrosine Kinase
TREM1 signaling	Triggering receptor expressed on myeloid cells 1 signaling
IL	Interleukin
LXR/RXR	Liver X receptor/Retinoid X receptor
Chga	Chromogranin A
Acvr1c	Activin A receptor, type IC
Limk2	LIM domain kinase 2
Yars	Tyrosyl-tRNA synthetase
Trib2	Tribbles homolog 2
Arfip2	ADP-ribosylation factor interacting protein 2
H3f3b	H3 histone, family 3B

3 Summary

As the sole source of insulin production in body, pancreatic β-cells play an essential role in glucose homeostasis. Diabetes mellitus is a chronic disease characterized by loss, reduction, and/or malfunction of β-cell mass. The putative induction of adult β-cell regeneration represents a promising approach for the treatment of type 1 diabetes. Towards this ultimate goal, it is essential to develop an inducible model mimicking the long-lasting disease progression. In the current study, we have established a novel β-cell ablation mouse model, in which the β-cell mass progressively declines, as seen in type 1 diabetes. The model is based on the β-cell specific genetic ablation of the transcription initiation factor 1A, TIF-IA, essential for RNA Polymerase I activity (TIF-IA$^{\Delta/\Delta}$). Using this approach, we induced a slow apoptotic response that eventually leads to a protracted β-cell death. In this model, we observed β-cell regeneration that resulted in a complete recovery of the β-cell mass and normoglycemia. In addition, we showed that adaptive proliferation of remaining β-cells is the prominent mechanism acting to compensate for the massive β-cell loss in young but also aged mice. Interestingly, at any age, we also detected β-like cells expressing the glucagon hormone, suggesting a transition between α and β-cell identities or vice versa. Taken together, the (TIF-IA$^{\Delta/\Delta}$) mouse model can be used to investigate the potential therapeutic approaches for type 1 diabetes targeting β-cell regeneration. RNA-sequencing of (TIF-IA$^{\Delta/\Delta}$) pancreas provided the possibilty to look at β-cell regeneration at the molecular level and dissect out the cellular pathways induced in pancreas upon β-cell ablation. These signaling pathways work together to mediate β-cell stress response, metabolic adaptations, and tissue homeostasis. Using these data, we have identified some novel regulators of β-cell regeneration.

4 Introduction

4.1 Pancreas structure and function

Pancreas is a compound organ composed of exocine and endocrine compartments. The exocrine compartment consists of acinar and duct cells, which are involved in secretion of zymogens for digestive purposes (acinar cells) and their transport to the duodenum (duct cells). The pancreatic duct also secrete bicarbonate for neutralization of stomach acid in the duodenum. The adult endocrine pancreas is organized in the islets of Langerhans. Islets of Langerhans are made of five different hormone-producing cell types β-cells producing insulin, α-cells producing glucagon, δ-cells producing somatostatin, PP cells producing pancreatic polypeptide, and ε-cell producing ghrelin. The ε-cell represents about 1% of the embryonic endocrine pancreas, but disappears after birth [1, 2, 3]. Glucose homeostasis is controlled by the endocrine pancreas through the secretion of insulin and glucagon by β- and α-cells, respectively.

4.2 Pancreas development and β-cells differentiation

During mouse development, early pancreas arises as dorsal thickening in the foregut endoderm to form the dorsal pancreatic bud. The thickening is first evident around day 9 of embryonic development (E9.0-E9.5). Early expression of transcription factors Pdx1 and Ptf1a marks the developing pancreas. This is followed by emergence of ventral bud along the ventral surfaces of the posterior foregut around day 10 (E10-E10.5). Afterwards, pancreatic epithelium starts to expand, branch, and differentiate to different cell types present in the mature pancreas. Gut rotation brings the two pancreatic lobes together and they fuse to form one interconnected organ by day 12.5 (E12.5) [3, 2, 1]. Rodent pancreas development has been classified in two temporal waves of events called first and secondary transitions [4, 5]. During the first transition, occurring between E9.5–E12.5 in the mouse, early endocrine cells expressing glucagon are formed. A small number of cells co-expressing insulin and glucagon, pancreatic polypeptide or peptide YY are also observed at this time [6, 7]. The most important event in the first transition is rapid proliferation of pancreatic progenitors that serve as precursors of different pancreatic cell types. In the secondary transition (E13.5–E15.5), massive differentiation and lineage allocation of acinar, duct, and endocrine lineages occurs. As a result, single hormone-expressing cell types are formed (β-cells, α-cells, δ-cells, ε-cells and PP cells). Following the secondary transition, acinar cell proliferation continues that leads to the further expansion of pancreatic epithelium. Endocrine cells leave the epithelium and assemble into islet structures during late gestation and few weeks after birth [8, 1]. Neurogenin 3 (Ngn3) is a bHLH transcription factor that is essential for endocrine cell specification and develop-

ment of pancreatic islets [9]. Ngn3 has a biphasic expression pattern during early pancreatic development, and its expression is correlated with first and secondary waves of endocrine differentiation (Figure 1) [8].

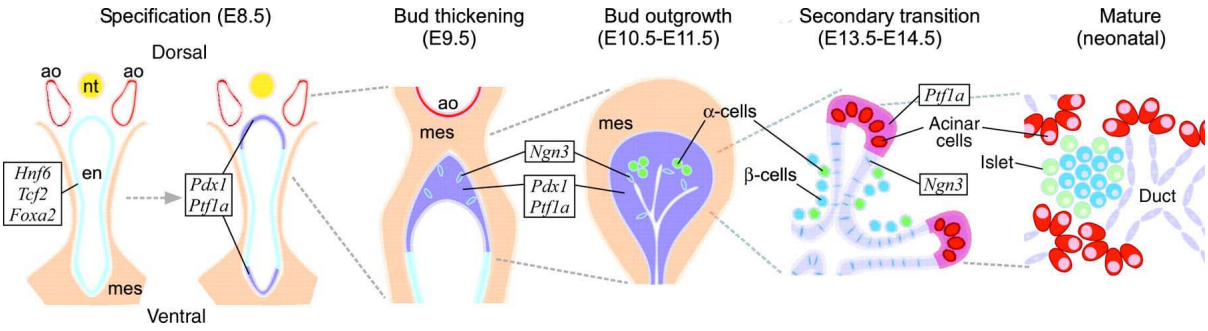

Figure 1: Pancreas development and β-cells differentiation. Induction of Pdx1 and Ptf1a transcription factors in dorsal and ventral gut endoderm (en) initiates during pancreas specification. Signaling from the nearby tissue, notochord (nt) and aorta (ao), helps the specification process. Early pancreatic buds (dorsal and ventral) evaginate from gut endoderm, and are surrounded by mesoderm (mes). Ngn3-expressing endocrine progenitor cells proliferate and differentiate to produce different cell types of endocrine lineage, which later cluster together to form islet structure (Figure from Murtaugh L., 2007).

4.3 β-cell function in glucose homeostasis

As the one and only source of insulin production in the body, pancreatic β-cells play a pivotal role in the regulation of fuel metabolism. The presence of a sufficient number of functional glucose responsive β-cells is indispensable for normal glucose homeostasis. Diabetes mellitus is a chronic disease associated with loss or reduction malfunction of β-cell mass, and it is characterized by chronic elevation of blood glucose levels (hyperglycemia) [10].

4.4 Diabetes mellitus

Diabetes mellitus is a group of metabolic disorders caused by insulin insufficiency, impaired insulin action due to the peripheral insulin resistance, or both. Different classes of diabetes include type 1 diabetes, type 2 diabetes, gestational diabetes and other types of diabetes such as monogenic diabetes. Type 1 and type 2 diabetes are the most common forms of diabetes [11]. Type 1 diabetes is considered as an autoimmune disease caused by immune-mediated destruction of the β-cells, which eventually leads to absolute insulin deficiency [12]. Type 2 diabetes is an age-related disease characterized by inadequate response of pancreatic β-cells to insulin resistance. Insulin resistance is a condition in which the insulin-responsive tissues including liver, fat, and muscle, become resistant and fail to trigger a proper biological response

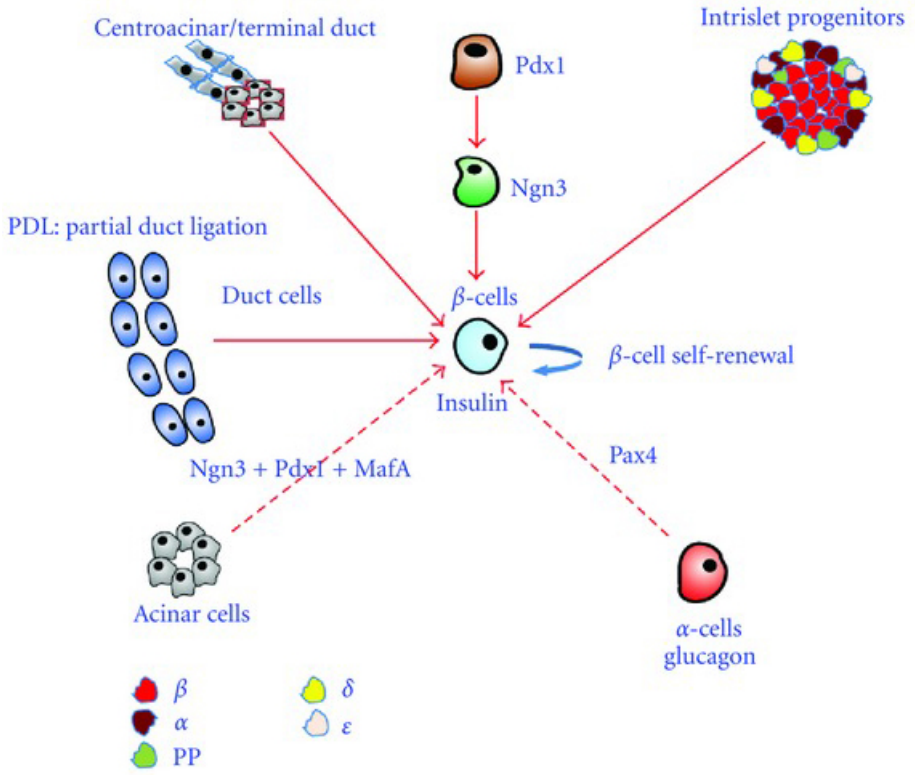

Figure 2: Different routes to β-cell regeneration. During development, β-cells are differentiated from Ngn3-expressing progenitors, which are a small proportion of Pdx1 positivie cells in embryonic pancreas. In adult rodent, β-cell mass can be regenerated by different mechanisms, depending on the type and extent of β-cell loss or injury (Figure from Mansouri A., 2012).

to normal or elevated insulin level [13]. The failure to compensate for insulin resistance rises from incapability in elevating β-cell mass, functional disruptions, or both [11].

4.5 β-cell regeneration

It has been shown that the adult pancreas can regenerate in several species of mammals following surgical insult or disease [14]. This organ has also the potential to increase its β-cell content in response to metabolic demand, as seen during pregnancy and in obesity [15]. Several animal models have been used to investigate the mechanisms underlying β-cell regeneration, and it is now believed that the regenerative capacity, and the mechanisms through which it takes place, are highly dependent on the extent of β-cell loss, and type of injury. Different cellular sources are proposed to contribute to β-cell regeneration in adult rodent pancreas (Figure 2).

4.5.1 Adaptive β-cell proliferation

β-cell self-renewal has been shown to be the major mechanism for the turnover of pancreatic β-cell under physiological condition and β-cell regeneration in response to different types of pancreatic injury [16, 17, 18, 19] as well as increased metabolic demands in pregnancy and

obesity [20, 21]. Using genetic lineage tracing, it was confirmed that following 70-80 % ablation of β-cell mass, proliferation of pre existing insulin positive cells is responsible for complete regeneration of β-cells [22].

4.5.2 Duct and centroacinar-derived progenitors

Presence of stem/progenitor cells in the duct epithelium and their contribution to endocrine lineage has been proposed by several studies in pancreas injury models. Presence of insulin and glucagon positive cells, as well as induction of proendocrine transcription factor Ngn3 in duct after PDL (partial duct ligation) induced injury suggested that regeneration may recapitulate embryonic development of β-cells and duct epithelium may comprise progenitor cells capable of differentiating to different endocrine cell types [23, 24, 25]. However, contribution of duct cells to endocrine lineage is challenged by several lineage tracing experiments using different duct/centroacinar specific CreER lines such as Hnf1B, Hes1, and Sox9 [26, 27, 28, 29]. Although PDL has been used for years as a model to study β-cells regeneration, a recent study has questioned the validity of this model for studying β-cell regenration by showing that β-cells are not generated in response to PDL in adult mice [30].

4.5.3 Facultative facultative multipotent progenitors (MPCs) in acini

Acinar cells are another cellular source that might replenish β-cell mass. It has been shown that ectopic over expression of three transcription factors Pdx1, Ngn3, and MafA can convert acinar cells to functional β-cells [31]. Furthermore, lineage tracing of Ptf1a labeled acinar cells confirmed that facultative multipotent progenitors in acini can contribute to duct and rarely to endocrine lineages following PDL. Interestingly, their contribution to endocrine cells (acinar to duct to endocrine transdifferentiation) was facilitated by combination of PDL and Streptozotocin (STZ)-induced β-cell depletion, but it was not observed in STZ only treated mice, where only β-cell mass is reduced [32].

4.5.4 α- to-β-cell transdifferentiation

α- to β-like cell conversion was shown to be the major mechanism underlying β-cell regeneration in condition of extreme β-cell loss [33] and in a PDLmodel combined with alloxan-induced β-cell ablation [34]. Moreover, the forced expression of Pax4 in α-cells, promotes their conversion into functional β-cells that counter chemically induced diabetes [35, 36]. Interestingly, the conversion of α-cells revealed their regeneration capacity, and the propensity of duct/duct lining to contribute to β-cell neogenesis by epithelial mesenchymal transition mechanism [35].

4.6 TIF-IA

Transcription initiation factor TIF-IA, the mammalian homolog of yeast Rrn3p, interacts with and activates RNA polymerase I and regulates the growth-dependent transcription of

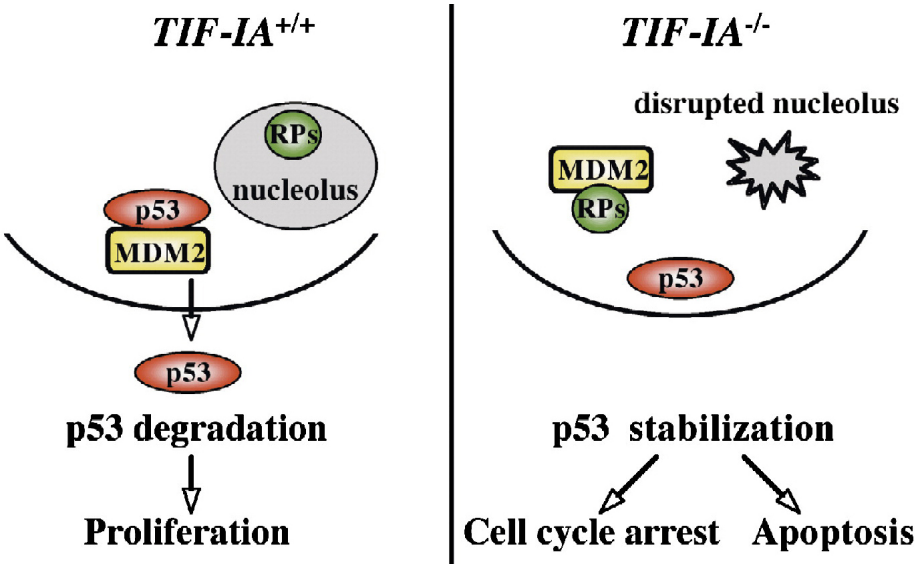

Figure 3: Depletion of TIF-IA activates apoptotic pathways. In the presence of TIF-IA, interaction of MDM2 with p53 controls its abundance in the cells. Inactivation of TIF-IA and nucleolar stress increases the interaction of MDM2 with ribosomal proteins, causing MDM2 inhibition and p53 stabilization. Elevated level of p53 induces cell cycle arrest and apoptosis in TIF-IA knockout cells (Figure from Yuan X., 2005).

ribosomal DNA (rDNA) and ribosome synthesis [37]. TIF-IA colocalizes with nucleolar transcription factor UBF (upstream binding factor) in nucleolus [38]. TIF-IA is known as a key mediator of transducing extracellular signals to RNA polymerase I machinery and thereby linking external stimuli to rRNA synthesis, ribosome biogenesis, and cellular proliferation [39]. TIF-IA is a downstream target of c-Jun N-terminal kinase (JNK) pathway. Under stress condition, JNK phosphorylates TIF-IA and impairs its binding to RNA polymerase I and formation of transcription initiation complex [39]. In the normal cells, MDM2 protein (Mouse double minute 2 homolog) bind to p53 protein and regulates the level and function of p53 by inhibiting its transcriptional activity, exporting into the cytoplasm, and/or by promoting its degradation [40, 41, 42, 43]. Loss of TIF-IA and subsequent perturbation of nucleolar structure causes disruption of the p53-MDM2 complex, elevation of p53 protein level, and activation of p53-dependent pathways. Genetic inactivation of TIF-IA eventually leads to cell cycle arrest and apoptosis mediated by a p53-dependent pathway (Figure 3). Mouse embryos lacking TIF-IA gene die at embryonic day 9.5 (E9.5) due to the massive growth retardation and developmental defects [44]. Targeted deletion of TIF-IA in adult mouse hippocampus neurons induces a protracted neuronal degeneration, whereas in embryonic neural progenitors, it triggers a rapid apoptosis [45, 46]. These observations suggested that the conditional ablation of TIF-IA could be used as a genetic tool to induce a protracted suicide response in

slowly dividing or post-mitotic cells.

4.7 Non-coding RNAs

4.7.1 microRNAs

This part is adapted from Rabe and Shamsi, 2014 [47].
microRNA are small non-coding RNAs that function in post-transcriptional regulation of gene expression. The first two miRNAs were identified in Caenorhbditis elegans, containing sequences complementary to a part of the 3'-untranslated region (3'-UTR) of lin-14 mRNA [48]. miRNAs act as negative regulators of gene expression through base pairing between miRNA seed sequence at the 5' ends of miRNAs (nucleotides 2-8) and miRNA-binding site that is mostly located in mRNA 3'-UTR. miRNAs are first transcribed as pri-miRNAs, and then cleaved by Drosha RNAse III endonuclease to generate the pre-miRNAs that are about 60-70 nucleotides long [49]. The pre-miRNAs are transported to the cytoplasm, where they are processed by Dicer to generate the miRNA/miRNA* double strand [50]. The mature miRNA will be incorporated into the miRNA-induced silencing complex (miRISC) that finally binds to the 3'-UTR of their target sequence, resulting in either translational repression and/or mRNA deadenylation or decay [51, 49, 50].
In the past few years, the significance of miRNAs function in regulation of several developmental processes and human diseases has become more and more evident. Several miRNAs have been shown to play role in pancreas development, β-cell function and regeneration, as well as both type I, and type 2 diabetes pathogenesis.

4.7.2 microRNAs in β-cell development and regeneration

Emerging evidences show that cellular stress can affect the biogenesis and function of miRNAs. Under stress conditions, miRNAs can fine-tune the expression program of cells, and consequently, regulate cellular behavior by activation and inhibition of different signaling pathways [52]. There have been few studies on the function of miRNAs in β-cell stress response and regeneration (Figure 4)
In the islet of type 2 diabetes mouse model, ob/ob mice, expression of miR-375 is upregulated. Both basal proliferation and the adaptive proliferation of β-cells induced by insulin resistance and obesity are impaired in miR-375 KO mice. In the normal conditions, minor reduction in β-cell mass did not affect a drastic effect on plasma insulin level. However, lack of compensatory proliferation in miR-375 knockout insulin resistant obese mice caused severe reduction of β-cell mass and insulin level compared to the ob/ob littermates [53].
miR-7 is another miRNA highy expressed in pancreatic islets [54, 55]. It was recently shown that mir-7 suppresses β-cell proliferation in mouse and human islets through targeting different components of the mammalian target of rapamycin (mTOR) signaling pathway. mTOR

signaling pathway plays the central role in sensing the external and internal stimuli and linking them to cell growth and proliferation, cell survival, and metabolism [56, 57]. However, a recently published study has challenged the role of miR-7 in β-cell proliferation using miR-7a2 loss and gain of function mouse models and showed that miR-7a2 is involved in functional compensation of pancreatic β-cells in obesity and type 2 diabetes [58].

4.7.2.1 miRNA-mediated adaptions in pregnancy and obesity

A recent study has identified a set of miRNAs that are differentially expressed in the islets of pregnant rats [59]. miR-218, miR-338-3p, and miR-874 are significantly downregulated at day 14 of gestation in pregnant rats compared to the age-matched non-pregnant females. In these animals, miR-144 and miR-451 levels are significantly upregulated. Inhibition of miR-338-3p promotes cellular proliferation in INS832/13 cells and dissociated rat β-cells, but not human β-cells. Additionally, reduction of miR-338-3p exerts a protective effect in rat and human islets against pro-inflammatory cytokines [59].

4.7.3 Long non-coding RNAs

Long non-coding RNAs (lncRNAs) belong to a class of transcripts that are longer than 200 nucleotides, and have poor or no protein-coding potential [60]. lncRNAs can also be classified by their functions into four category including signals, decoys, guides and scaffolds [61]. lncRNAs form a significant layer of genomic regulation, as they play critical roles in establishing the epigenetic signature of different cell types mainly through regulation of gene transcription, translation, chromatin modification, imprinting, genomic rearrangement, nuclear factor trafficking, and protein degradation [61, 62]. Misregulation of lncRNAs is associated with several complex human diseases [62].

4.7.3.1 lncRNAs expression in pancreatic islets

Interestingly, lncRNAs are extremely tissue and cell type specific compared to the protein-coding genes [63], which makes them highly valuable candidates for therapeutic targeting. Systematic identification of lncRNAs expressed in human pancreatic islets showed that more than 1100 lncRNAs are expressed in human islet, many of which are islet-specific [64]. Expression of some of these islet-specific lncRNAs is dynamically regulated during β-cell development that suggests a potential role for lncRNAs in β-cell differentiation program [64]. Genetic variations of ANRIL, a lncRNA transcribed from Cdkn2a locus, have been associated with type 2 diabetes in different ethnic populations [65, 66]. ANRIL is shown to be involved in transcriptional silencing of genes at the INK locus (p14, p15 and p16) [67, 68]. Ink4a/Arf locus, encoding cyclin-dependent kinase inhibitor p16INK4a and tumor suppressor p19Arf, plays an important role in regulation of β-cell proliferation and aging [69].

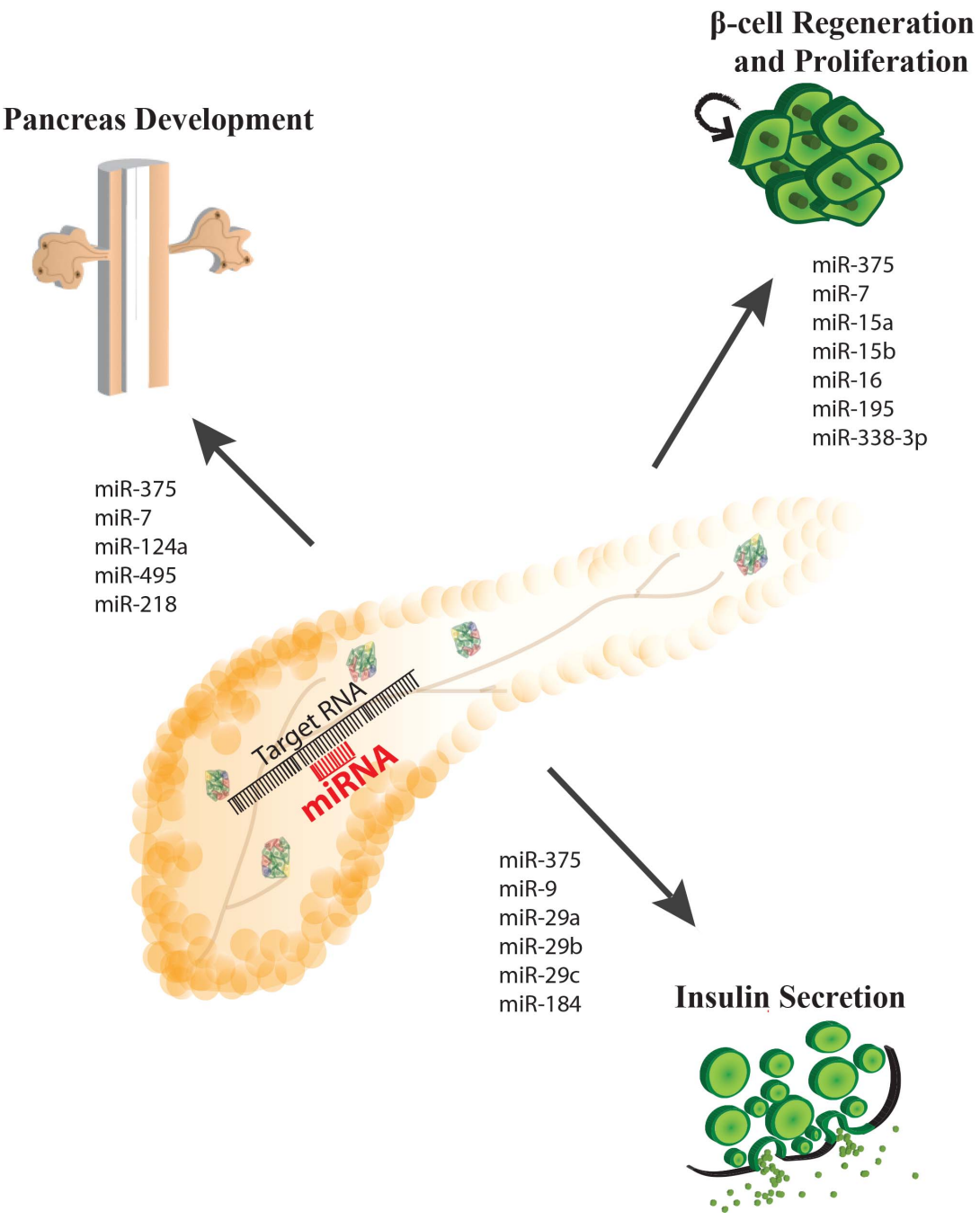

Figure 4: miRNAs involved in pancreas development, β-cell regeneration, and insulin secretion (Figure from Rabe and Shamsi, 2014).

5 Aims

The existing data for progression of type 1 diabetes describe this disease as a chronic progressive autoimmune disorder, in which the loss of the β-cell mass occurs in a slow and gradual manner [12, 10, 70]. Additionally, it is shown that the β-cell mass falls gradually over time in rodent models of type 1 diabetes. However, in all of the existing models of β-cell regeneration, β-cell ablation occurs very rapidly within days after initial induction [19, 22, 33, 34]. To better understand the potential of β-cell regeneration processes that might be induced in diabetic islets, it is important to use a model mimicking the slow progression and extent of β-cell loss seen in type 1 diabetes. This study was initiated in order to develop a novel inducible β-cell loss model, based on the conditional ablation of TIF-IA, in which β-cell death occurs gradually and over a longer period of time compared to previously used animal models. Additionally, we used this novel mouse model to address the following questions:

1. What are the mechanisms of β-cell regeneration in (TIF-IA$^{\Delta/\Delta}$) mouse model?
2. How does aging affect different mechanisms of β-cell regeneration in (TIF-IA$^{\Delta/\Delta}$) mouse model?
3. How does gene expression program of adult pancreas change in response to β-cell ablation?
4. Which genes and signaling pathways are induced in adult pancreas upon β-cell loss that are involved in stress response and tissue homeostasis processes?

6 Materials and methods

6.1 Ethics statement

All animal works have been conducted according to the German animal welfare law (LAVES Niedersachsen).

6.2 Animals

Knockout and transgenic mouse lines TIF-IA$^{fl/fl}$::RIPCreERT2::Rosa26$^{YFP/YFP}$ mice were generated by crossing TIF-IA$^{fl/fl}$ line [44] with RIPCreERT2 line [16] and Rosa26$^{YFP/YFP}$ reporter line [71]. One- to three-month old male mice were used for experiments.

TIF-IA Forward	CCGGTGGTCCTGCTTACACTAGAGATGTGG
TIF-IA Reverse	AATATAATTTGCAGCAGCCTGCCTGATGATGG
Cre Forward	ATG CTT CTG TCC GTT TGC CG
Cre Reverse	CCT GTT TTG CAC GTT CAC CG
YFP Forward	ACCCTGAAGTTCA TCTGCACCA
YFP Reverse	TGGGTGCTCAGGTAGTGGTTGT

Table 1: List of genotyping primers

6.3 Animal treatments

Tamoxifen (Sigma-Aldrich) was injected to mice for seven days (2mg/day per 20gr body weight). For BrdU labeling experiment, 5-bromo-2'-deoxyuridine (Sigma-Aldrich) was administrated in drinking water (0.8 mg/ml) for six weeks, and changed every five days. For double thymidine analogues experiments, CldU and IdU (Sigma-Aldrich) were given in drinking water each for four days (1mg/ml) with one-day washout interval.

6.4 Blood glucose and insulin measurements

Blood glucose levels were measured with AccuChek glucose monitoring system using tail tip blood. Insulin ELISA was performed using Mercodia Ultrasensitive Mouse Insulin ELISA according to according to manufacturer's instruction.

6.5 Tissue preparation and immunohistochemistry

Pancreata were isolated and immediately fixed in 4% paraformaldehyde for 2 hours at 4 °C. Fixed tissues were then washed four time, 30 minutes each, and incubated overnight in 30% (w/v) sucrose solution in PBS. Tissues were cryopreserved in Jung tissue freezing medium (Leica Microystems, Nussloch, Germany). 8 µm cryosections were air-dried, washed in PBS and then blocked in 10

For BrdU staining, sections were treated in 2N HCl for 30 minutes at 37 °C and then washed in PBS prior to blocking step.

6.6 CldU/IdU immunohistochemistry

The staining of CldU/IdU labeled tissue was done according to the established protocol [18] with minor modifications. Briefly, sections were treated with 1.5 N HCl, blocked with 10% FCS, and incubated overnight with mouse anti-BrdU (BD Biosciences, Franklin Lakes, NJ) and guinea-pig anti-insulin (Dako). The next day, sections were washed with high salt PBST (0.5M NaCl, 36mM Tris-HCl pH8.0, 0.5% Tween20) and again incubated overnight with rat anti-BrdU antisera (BU1/75; Accurate Chemical, Westbury, NY). Secondary antibodies were incubated for one hour at room temperature.

Following antibodies were used in this study:

Antibody	**Host**	**Dilution**	**Source/Provider**
Insulin	Guinea-pig	1:1000	Dako
Insulin	Mouse	1:1000	Sigma-Aldrich
Glucagon	Rabbit	1:200	Abcam
Ki67	Rat	1:100	Dako
GFP	Chicken	1:800	Abcam
Glut2	Rabbit	1:500	Abcam
MafA	Rabbit	1:500	BETHYL Laboratories
C-peptide	Rabbit	1:100	Cell signaling
BrdU	Mouse	1:100	Roche-applied-science
BrdU	Mouse	1:250	BD Biosciences
BrdU	Rat	1:250	Accurate Chemical
Arx	Rabbit	1:1000	Millipore
Pdx1	Rabbit	1:2000	kindly provided by C.Wright
Nkx6.1	Rabbit	1:100	kindly provided by C.Wright
Ngn3	Guinea-pig	1:1000	kindly provided by M.Sander
Sox9	Rabbit	1:1000	Millipore

Table 2: List of primary antibodies

Antibody	Dilution	Source/Provider
Alexa Fluor 594 anti-guinea pig	1:1000	Invitrogen
Alexa Fluor 594 anti-mouse	1:1000	Invitrogen
Alexa Fluor 594 anti-rabbit	1:1000	Invitrogen
Alexa Fluor 594 anti-rat	1:1000	Invitrogen
Alexa Fluor 488 anti-mouse	1:1000	Invitrogen
Alexa Fluor 488 anti-rabbit	1:1000	Invitrogen
Alexa Fluor 488 anti-chicken	1:1000	Invitrogen
Alexa Fluor 488 anti-rat	1:1000	Invitrogen
Alexa Fluor 488 anti-guinea pig	1:1000	Invitrogen
Cy5 anti-mouse	1:50	Jackson Immunoresearch Laboratories

Table 3: List of secondary antibodies

6.7 TUNEL assay

TUNEL assay was performed using Apoptag apoptosis detection kit (Millipore) according to manufacturer's protocol.

6.8 Quantifications and Statistics

For each experiments, at least 40 islets from four equally separated sections were counted per animal. The values are presented as mean ± SEM from at least three animals at the same experimental condition. Statistical significance between the mean values was analyzed using ANOVA, Tukey's Multiple Comparison Test, Kruskal-Wallis test, Dunn's Multiple Comparison Test, and Student t-test. P-values less than 0.05 were considered as statistically significant.

6.9 RNA isolation from murine pancreas

For isolation of high quality RNA from adult pancreas tissue, a perfusion-based method that has been developed for porcine pancreas was adapted [72]. In order to immediately stabilize RNA in the whole tissue, the RNase inhibitor reagent, RNAlater (Qiagen) was perfused in the tissue using a 1-mL syringe and a 27 gauge × 1/2 inch needle. Swollen tissue was immediately cut into small pieces, and immersed in 5 mL TRIzol reagent. Total RNA was isolated using RNeasy mini kit (Qiagen).

6.10 RNA-sequencing and data analysis

RNA-sequencing experiment was performed in collaboration with DNA microarray and deep-sequencing facility in university clinic Goettingen. Candidate genes were filtered based on a threshold of minimum 2x fold change and FDR-corrected p-value of lower than 0.05. For functional characterization of the differentially expressed transcripts, gene ontology (GO) and pathway enrichment analysis was performed using the webtool DAVID and IPA software

Gene	Forward Primer	Reverse Primer
Abcc8	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
BMP7	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
Plk2	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
Chga	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
Gatm	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
Pnpla3	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
Acvr1c	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
Syt13	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
Gck	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
Limk2	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
Thrsp	QuantiTect Primer Assay (Qiagen)	QuantiTect Primer Assay (Qiagen)
Yars	CAAGTCTGAGTTTGTGATCCT	AATTCTTTAGGTCTCCAGGGT
Cdkn1a	CCAGCCTGACAGATTTCTATCAC	ACACAGAGTGAGGGCTAAGG
TRIB2	CCGACTGTTCTACCAGATTGC	TGTAAGCGTCTTCCAAACTCTC
Arfip2	CGCAAGTATGAAAGTGTCCTG	AGCCAAATTCCTCCTGAAGC
Mthfd2	CTCCCAGAGCACATTGATGAG	TAATTATCTCCCACACGCCC
Abs6	CTCACTCTCCATTCTACCAGG	TAGTAGGTAACAGGGTCTTCG
Rapgefl1	ATCCTCAAAGACCTGACCTTCC	ATTGTCCTCACTTTCTCGGC
Vldlr	CGAGAAGAACTGTGTAAAGAAGAC	TCACACTGCCATCTGTTAGG
Tlk2	TACTTTGAGTTTGCTGGGGGAAG	TGCTGCACTGCCATCTAAAC
Wasf1	CAGCTTTTCGACCGCAAGAC	CCTTCCTTACCATCATCCCTGT
NeuroD1	CACGCAGAAGGCAAGGTGTC	GTCATGTTTCCACTTCCTGTTGTT
H3f3b	GGAAGCTGCCATTCCAGAGAT	TTCGCTAGCCTCCTGAAGGG
Map3k12	AGCTGAGTGACAAGAGCACC	CCACCCCAAAGGACCAGATG
Il13ra1	GTGGCAGTCATAATCCTCCTTT	TCTTCCAGTGCAGGGTATCA
Esr1	GATGAAAGGCGGCATACGGA	TCGGCCTTCCAAGTCATCTC
Ppargc1a	GAAGTCCCATACACAACCGC	TGGGGTCATTTGGTGACTCT
Ppara	CAGCCTCAGCCAAGTTGAAGT	CAGAGCGCTAAGCTGTGATG
Sirt1	CGGCTACCGAGGTCCATATAC	CAGCTCAGGTGGAGGAATTGT
Pten	ACAATCTATGTGCTGAGAGACATT	GCTGTGGTGGGTTATGGTCT
Sp1	ACACCCCAGGTGATCATGGA	CCTTCTCCACCTGCTGTCTC

Table 4: List of qPCR primers

(Ingenuity Systems, www.ingenuity.com).

6.11 MIN6 cell culture and transfection

Mouse Insulinoma cell line (MIN6) [73] were cultured in DMEM medium (Life technologies) supplemented with 10% Fetal Calf Serum, penicillin/streptomycin (Gibco, 100U/mL penicillin and 100 µg/L) and β-mercaptoethanol and maintained in humidified incubator at 37 °C with 5% CO2. For miRNA mimic transfection experiment, MIN6 cells were transfected with either mmu-miR-22-3p mimic or miRNA Mimic Negative Control #1 (mirVana miRNA mimic, Life technologies) using Lipofectamine 2000 (Life technologies). Cells were harvested 48 hours after transfection.

6.12 RNA isolation from MIN6 cells

RNA was isolated from the cells using TRIzol reagent (Life technologies) according to the manufacturer's instruction. Briefly, after removing growth media, cells were lyzed and homogenized by adding 1mL TRIzol per 10 cm of culture dish surface area. Homogenized samples were incubated at room temperature for 5 minutes, followed by addition of 0.2 mL chloroform per 1 mL of TRIzol Reagent used. Samples were vigorously shaken, incubated at room temperature, and centrifuged at 12,000 × g for 15 minutes at 4 °C. The upper aqueous phase was removed and RNA was precipitated by adding 0.5 mL of 100% isopropanol per 1 mL of TRIzol. Following 10 minutes incubation at room temperature, samples were centrifuged at 12,000 × g for 8 minutes at room temperature to form a gel-like RNA pellet. RNA was washed by 1 mL of 75% ethanol, air-dried, and finally dissolved in nuclease-free water.

6.13 Reverse transcription and Real-Time qRT-PCR

cDNA was synthesized from 1 µg RNA using QuantiTect Reverse Transcription kit (Qiagen) according to the manufacturer's instruction. Real-Time qRT-PCRs were performed using 25 ng cDNA per reaction and KAPA SYBR FAST qPCR Master Mix (KAPA biosystems). Eppendorf realplex2 real-time system was used for amplification and detection. The expression levels of genes in different samples were normalized to Rn18s, Gapdh, or Actb.

7 Results

7.1 Efficient and specific ablation of β-cells is induced by deletion of TIF-IA

To generate an inducible β-cell ablation model, TIF-IA$^{fl/fl}$ mouse line was crossed with

RipCreERT2 transgenic animals (Figure 5A). Upon systemic administration of tamoxifen, TIF-IA gene is specifically deleted in pancreatic β-cells in double transgenic mice (TIF-IA$^{\Delta/\Delta}$). In order to specifically label and trace β-cells upon tamoxifen injection and TIF-IA deletion, Rosa$^{YFP/YFP}$ reporter line was crossed with TIF-IA$^{fl/fl}$l;RipCreERT2 animals (YFP::TIF-IA$^{\Delta/\Delta}$ (Figure5A). TIF-IA deletion induced a protracted apoptosis in β-cells, as detected by the appearance of TUNEL-positive cells in pancreatic islets (Figure 5B) The number of TUNEL-positive cells per islet at different time points following tamoxifen treatment was significantly higher in the mutant mice in comparison to wild type littermates and it increased over time. This was in consistency with a previous study that showed the apoptotic effects of TIF-IA perturbation in postmitotic neurons is slower in comparison with fast dividing cells [46].
Furthermore, the fraction of YFP-labeled and unlabeled β-cells were quantified at different time points upon tamoxifen injection (Figure 5C and D). 95% of β-cells were found YFP$^+$ in YFP::TIF-IA$^{+/+}$ controls two weeks after injection, while the labeled fraction was slightly smaller in mutant mice. In both TIF-IA$^{+/+}$ and TIF-IA$^{\Delta/\Delta}$ mice, YFP labeling was exclusively observed in β-cells and never detected in non-β endocrine cells. This shows that the tamoxifen treatmen can induce an efficient and β-cell specific recombination in this mouse line. Over time, the YFP-labeled fraction decreased so that three months after tamoxifen injection only few YFP$^+$ β-cells could be detected in some islets (Figure 5C and D). The continuous shift from YFP$^+$ β-cells to YFP$^-$ β-cells reflects the loss of β-cells and also their progressive replacement through regeneration from unlabeled cells. It was concluded that in TIF-IA$^{\Delta/\Delta}$ model, about 95% of β-cells lost TIF-IA expression, and eventually died. Additionally, the massive and gradual loss of β-cell observed in this model resemble the slow-progression and extent of β-cell loss observed in type 1 diabetes. Therefore TIF-IA$^{\Delta/\Delta}$ model could provide a valuable tool to study β-cells regeneration in the context of type 1 diabetes.

7.2 β-cell regeneration results in complete recovery of mice upon massive protracted β-cell loss

One-month old mice were injected with tamoxifen for seven days. Ten days after the last injection, blood glucose levels started to raise in TIF-IA$^{\Delta/\Delta}$ mice and it reached to the highest level one month after injection (Figure 6A). A slow and gradual decrease of hyperglycemia was subsequently observed. Eventually, glycemic condition went back to normal levels after eight months, and remained within normal ranges for longer than one month. In the parallel experiments, the number of β-cells was quantified at different time points after tamoxifen treatment. Consistent with the changes in blood glucose level , a continuous increase in the number of insulin-expressing cells was observed (Figure 6B and C). Eight months after tamoxifen induction, the number of β-cells became similar to wild type controls and islet structure was indistinguishable from wild type islets (Figure 6C). This was further confirmed by circulating insulin measurement showing a drastic decrease in TIF-IA$^{\Delta/\Delta}$ mice one month post-injection. Eight months post-injection, insulin concentration in TIF-IA$^{\Delta/\Delta}$ mice was

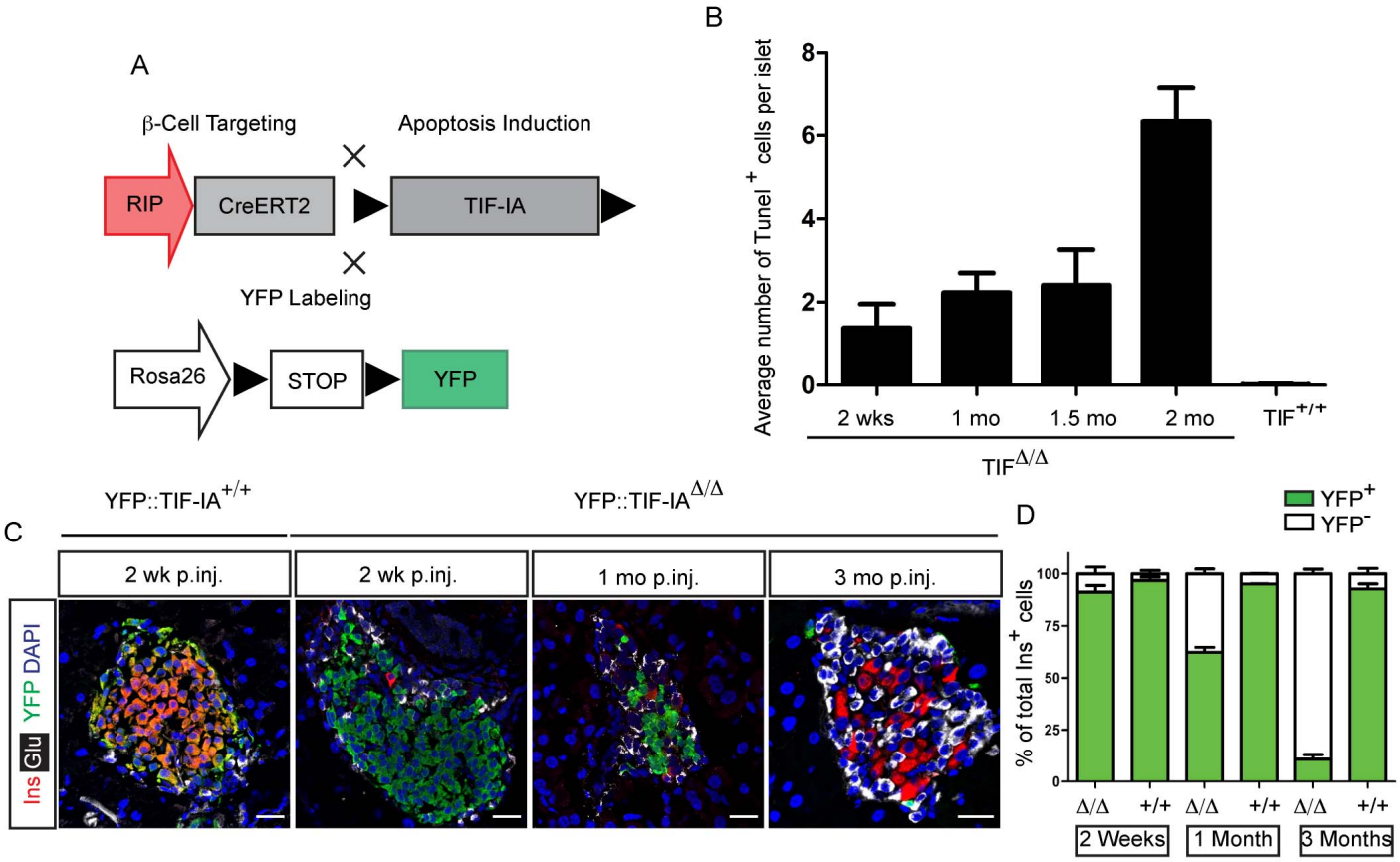

Figure 5: Generation of TIF-IA$^{\Delta/\Delta}$ mouse model to study β-cell regeneration. A: β-cell specific deletion of TIF-IA is used to induce apoptosis. YFP labeling marks the recombined cells. B: Apoptotic β-cells are detected in the islet of TIF-IA$^{\Delta/\Delta}$ mice. C: Representative immunostaining of insulin (red), glucagon (gray), and YFP (green) in the pancreata of TIF-IA$^{+/+}$ and TIF-IA$^{\Delta/\Delta}$ mice at different time points after tamoxifen injection. D: Quantification of insulin and YFP expressing cells shows 95% YFP-labeling upon Cre-mediated recombination. In TIF-IA$^{\Delta/\Delta}$ mutants, YFP-labeled cells are lost gradually, and replaced with non-labeled cells (D). mo p.inj. : months post injection. Scale bar : 25 µm. Values are presented by mean ± SEM. N >3 animals for each experimental condition.

found comparable to age-matched controls (Figure 6D). Glut2 and MafA are two genes which their expression mark functionally mature β-cells. Immunohistochemistry againts these two markers revealed that after recovery, the majority of insulin+ cells express Glut2 and MafA, and therefore they have a mature β-cell phenotype (Figure 7). These mature β-cells were able to release insulin and maintain normoglycemia (Figure 6A and D). In conclusion, these results show that the β-cell mass can be spontaneously regenerated in adult mice upon the protracted loss of 95% of β-cells. β-like cell regeneration leads to a complete recovery from long-lasting

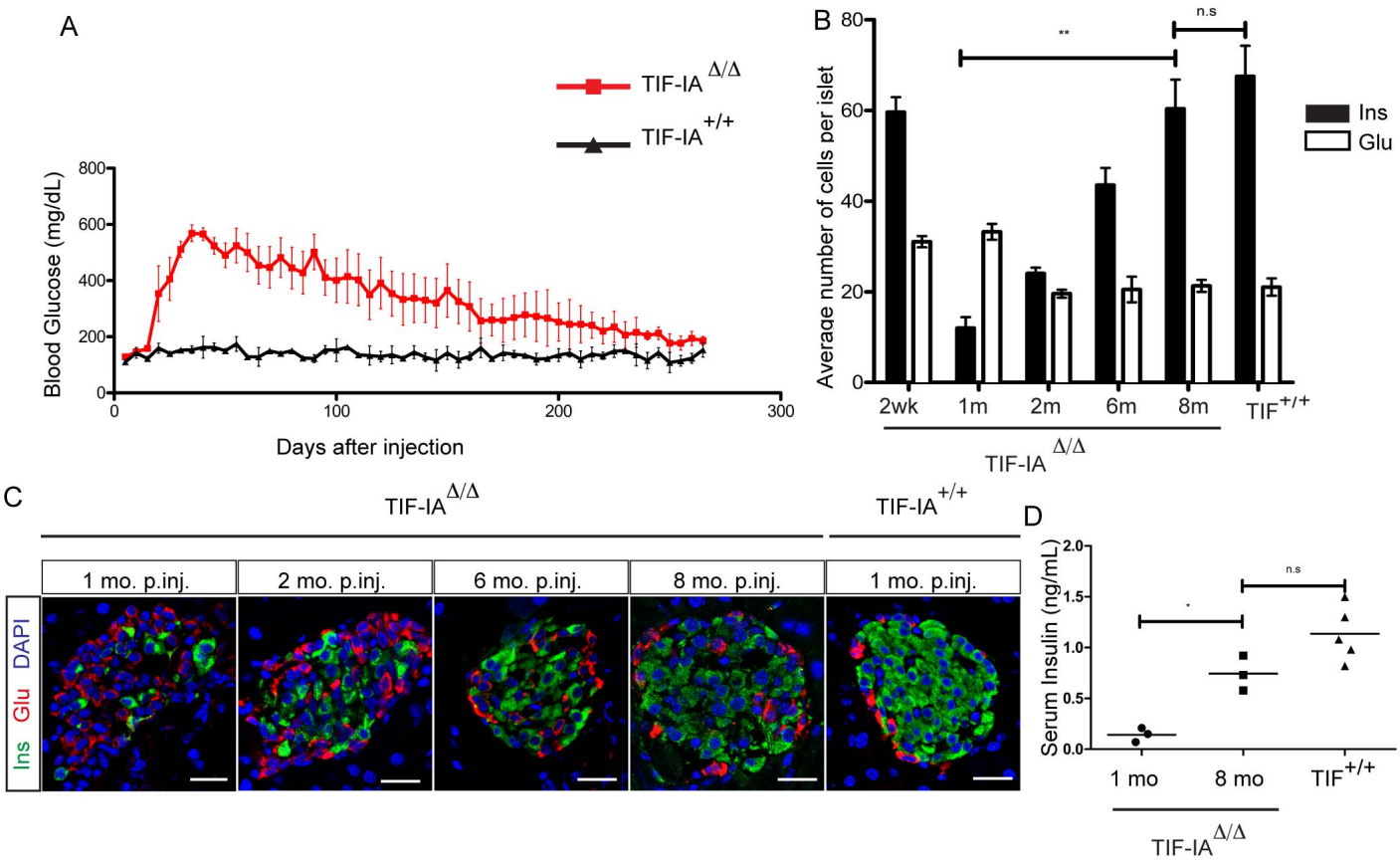

Figure 6: β-cell ablation and regeneration in TIF-IA$^{\Delta/\Delta}$ mice. A: Massive β-cell ablation leads to development of severe and long-lasting hyperglycemia in TIF-IA$^{\Delta/\Delta}$ mice. Mutant mice gradually recover from hyperglycemia. B and C: Immunostaining and quantification of insulin (green) and glucagon (red) expressing cells in the pancreata of TIF-IA$^{+/+}$ and TIF-IA$^{\Delta/\Delta}$ mice at different time points post injection (A). β-cell number is increased gradually in mice injected at one-month (B) D: Measurement of blood insulin level confirms the changes in functional β-cell number. mo p.inj. : months post injection.: months post injection. Scale bar : 25 µm. Values are presented by mean ± SEM.P-value < 0.05 : *, P-value < 0.01 : **. N>3 animals for each experimental condition.

and severe hyperglycemia in TIF-IA$^{\Delta/\Delta}$ mice.

7.3 Adaptive β-cell proliferation contributes to β-cell regeneration in TIF-IA$^{\Delta/\Delta}$ model

To investigate the cellular source of newly generated β-like cells in TIF-IA$^{\Delta/\Delta}$ mice, the contribution of different regenerative mechanisms upon tamoxifen induction was studied. First, the self-replication of β-cells was analyzed. The β-cell proliferation rate was measured as a fraction of Ki67-expressing cells in YFP^{-} β-cells in TIF-IA$^{\Delta/\Delta}$ (both Cre-escaper and newly-formed β-cells) as compared to wild type animals. The β-cells proliferation rate was significantly higher in TIF-IA$^{\Delta/\Delta}$ mice at different time points following β-cell ablation (Figure 8A). At any given age, a three-fold increase was observed in TIF-IA$^{\Delta/\Delta}$, as compared to controls. Additionally, to montior the accumulation of new β-cells, long-term BrdU labeling analysis

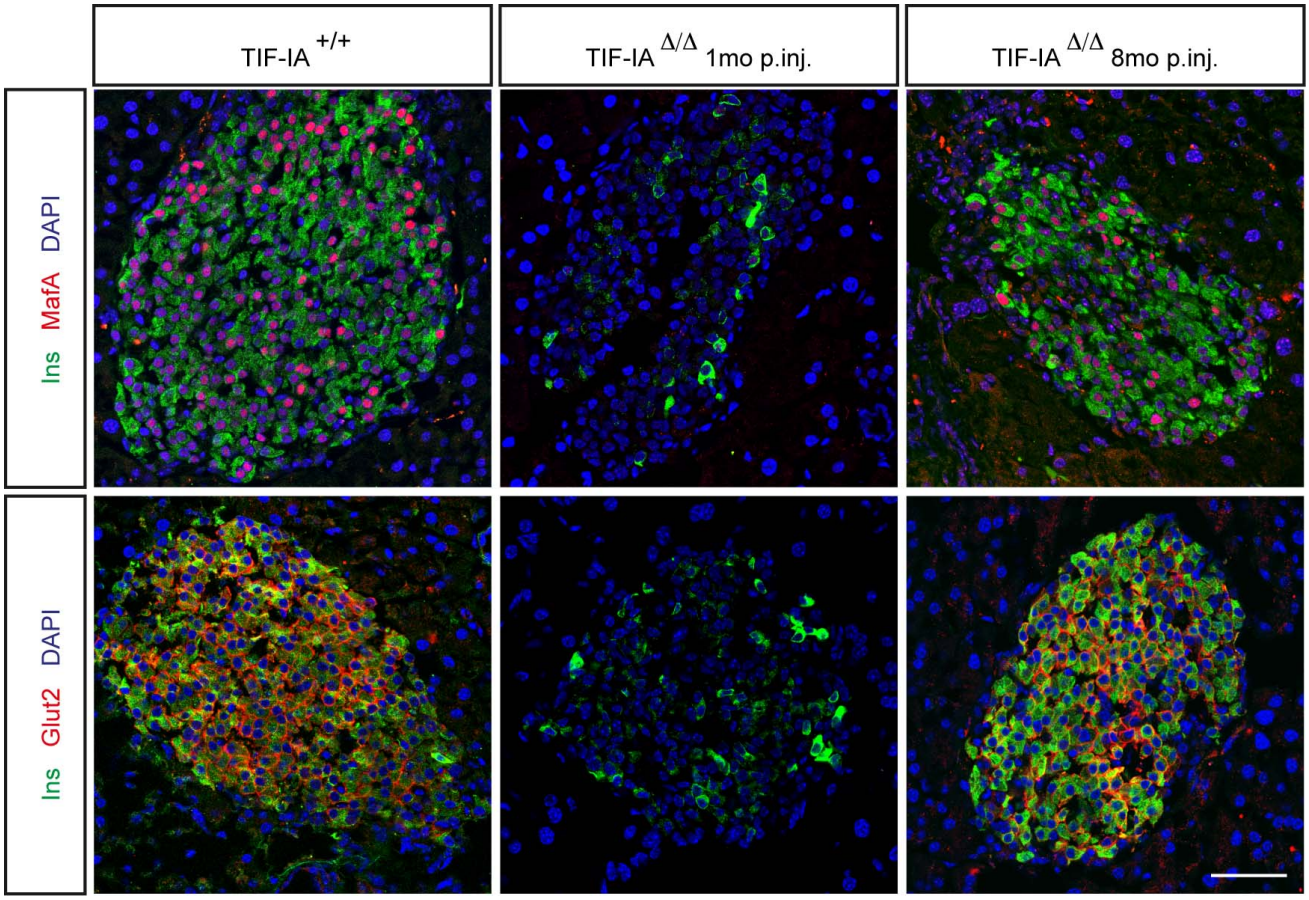

Figure 7: Newly formed β-cell display functional maturation in TIF-IA$^{\Delta/\Delta}$ mice. Immunostaining of insulin and either MafA (upper panel) or Glut2 (lower panel) as β-cell functional markers. Scale bar : 50 µm

(initiated 2 weeks after tamoxifen treatment and continued for 6 weeks) was performed. Consistent with the results of ki67 quantification, a significantly elevated BrdU incorporation was observed in TIF-IA$^{\Delta/\Delta}$ β-cells compared to their wild type counterparts (Figure 8B and C). This confirmed that neo-generated β-cells went through a proliferative phase. However, BrdU labeled β-cells could be formed either by self-replication of preexisting β-cells or they could be derived from other cells having undergone replication prior to their conversion into β-cells.

7.4 α- to β-Cell transdifferentiation is induced in response to β-cell ablation

Pancreatic α-cells are considered as another cellular source that contributes to β-cell mass increase upon extreme β-cell loss [33, 34]. Interestingly, the number of glucagon expressing α-cells increased in the islets of TIF-IA$^{\Delta/\Delta}$ mice upon TIF-IA deletion and β-cell ablation (Figure 6B and C). Shortly after tamoxifen treatment, α-cells became the major cell types in the islets. After recovery, this elevated number of α-cells appeared attenuated as the number of β-cells increased. This suggests that α-cells may play a role in β-cell mass replenishment in response to hyperglycemia, or β-cell injury. There are several evidences supporting the

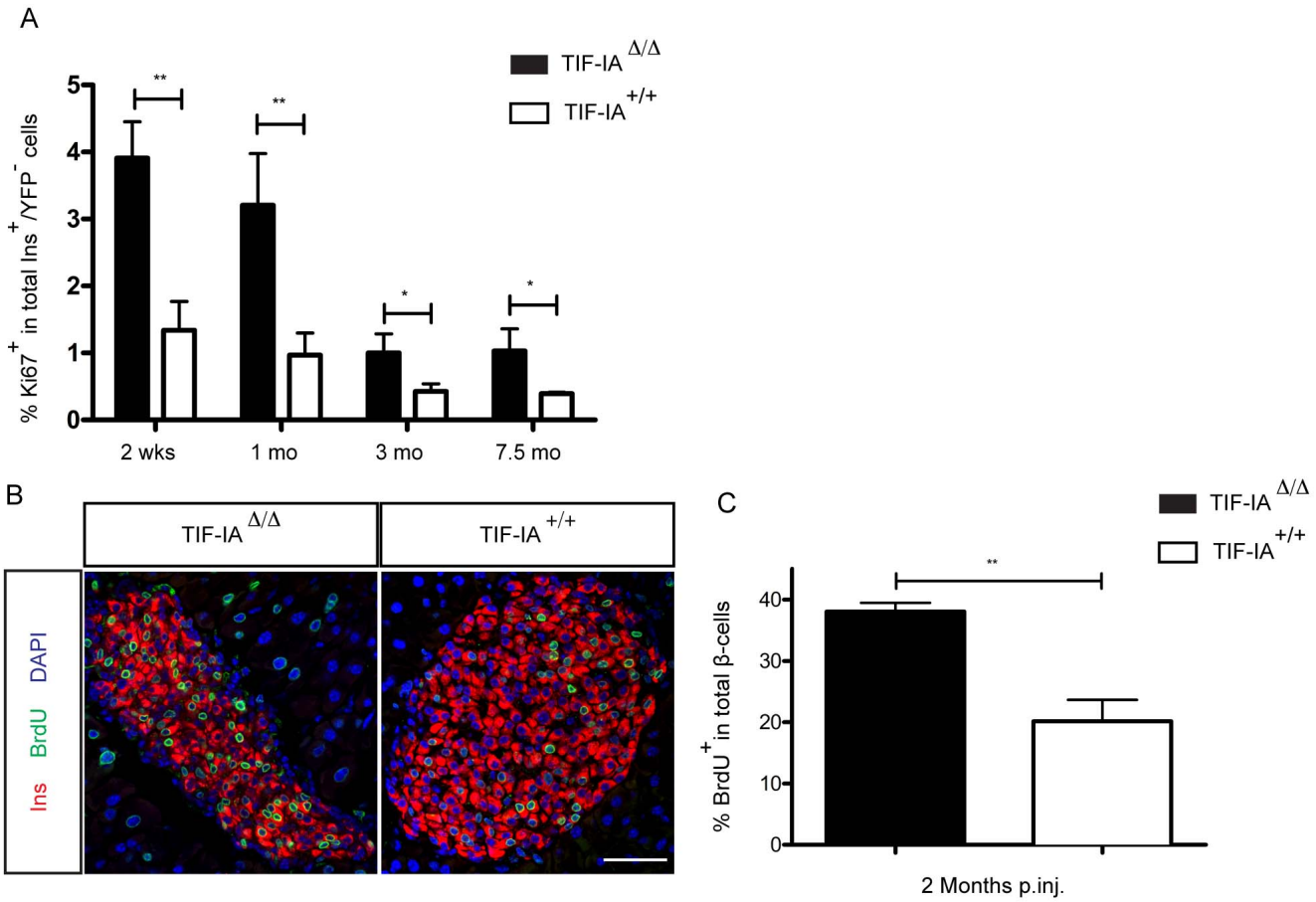

Figure 8: Increased β-cell proliferation rate upon massive β-cell loss in TIF-IA$^{\Delta/\Delta}$ mice. A: Quantification of Ki67-expressing β-cells shows higher proliferative fraction of β-cells in TIF-IA$^{\Delta/\Delta}$ mice compared to the wild types at different time points following β-cell loss. B and C: Quantification of BrdU labeled β-cells shows 2-fold increase in the BrdU labeled β-cell population in TIF-IA$^{\Delta/\Delta}$ mice compared to the wild types after two months BrdU treatment. mo.: months post injection. Scale bar : 50 µm. Values are presented by mean ± SEM. P-value < 0.05 : * , P-value < 0.01 : **. N >3 animals for each experimental condition.

contribution of α-cell to β-cell regeneration occurring in the TIF-IA$^{\Delta/\Delta}$ model.

First, at different time points following β-cell loss, some cells co-expressing insulin and glucagon (bi-hormonal cells) were found in the islets of TIF-IA$^{\Delta/\Delta}$ mice (Figure 9 A and B). These bihormonal cells could represent the transition of an α- to β-cells [33]. Further characterization of these bi-hormonal cells revealed that they have an intermediate gene expression signiture. They expressed both Arx and Pdx1, which are α and β-cell specific transcription factors, respectively (Figure 10C). Additionally, C-peptide, which is a side product of pre-insulin processing, was detected in these cells, implying that pre-insulin processing is effectively taking place in these cells (Figure 9C). But as expected, such bi-hormonal cells never expressed MafA, which is a mature β-cells transcription factor, indicating a rather immature transient nature for these cells (Figure 9C).

Second, in the islets of TIF-IA$^{\Delta/\Delta}$ mice, some α-cells were detected that express important β-

cell transcription factors, such as Pdx1 or Nkx6.1 (Figure 9D and E). These two transcription factors, along with some others, are essential regulators of β-cell development and function. The induction of these factors in α-cells upon β-cell suggests that α-cells are acquring a β-cell signiture, which mediates their fate conversion.
All together, these results indicate that the massive and protracted β-cell depletion in the TIF-IA$^{\Delta/\Delta}$ model activate, albeit at a low frequency, reprogramming of some α-cells toward β-cell fate. However, to confirm the extent of contribution of α-cells to β-cell regeneration, establishment of an advanced lineage-tracing strategy is essential.

7.5 Adult progenitors do not contribute to β-cell regeneration in TIF-IA$^{\Delta/\Delta}$ model

Having the YFP reporter system, it was possible to distinguish the pre-existing islets and newly formed ones. The protracted nature of the β-cell death induced by TIF-IA deletion, enabled the detection of some YFP^{+} cells in the islets up to 3 months after tamoxifen injection (Figure 6C). This feature of the system was used to assess the potenial contribution of precursor cells to β-cell regeneration in TIF-IA$^{\Delta/\Delta}$ model. Three months after tamoxifen injection, most islets contained few YFP labeled cells. This suggested that regeneration replenished the β-cell content of the pre-existing islets and new islets are not formed. In this case, it is possible that some intra-islet precursors proliferate and then differentiation to β-cells in response to β-cell loss. To search for this potential intra-islet progenitors, the sequential incorporation of two different thymidine analogues was used to monitor more than one round of β-cell division. They mice were first treated with CldU and then IdU in drinking water each for four days, with one-day washout interval. Pancreatic tissue sections were stained against CldU and IdU. Interstingly, β-Cells were exclusively labeled with either CldU or IdU, and no β-Cell was double-labeled with CldU and IdU in neighter TIF-IA$^{\Delta/\Delta}$ nor TIF-IA$^{+/+}$ mice (Figure 10A and B). This result clearly demonstrate that proliferation rate is evenly distributed among all the β-cells in normal tissue homeostasis, and even in regenerating β-cells following severe β-cell loss.
Furthermore, the association of insulin-positive cells with duct epithelium was analyzed using a duct specific marker, Sox9. Insulin expressing cells inside the duct structures were rare and their frequency did not change in the TIF-IA$^{\Delta/\Delta}$ compared to wild type littermates (Figure 10C and D). It was also asked whether Neurogenin3 expression is induced in TIF-IA$^{\Delta/\Delta}$ pancreata. Ngn3 positive cells were not detected in the TIF-IA$^{\Delta/\Delta}$ and TIF-IA$^{+/+}$ pancreata (Figure 10E), neither Ngn3 mRNA level was changed at any time point following β-cell loss (data not shown). Based on these results, adult precursors are less likely to be involved in β-cell regeneration in response to the protracted β-cell loss condition induced in the TIF-IA$^{\Delta/\Delta}$

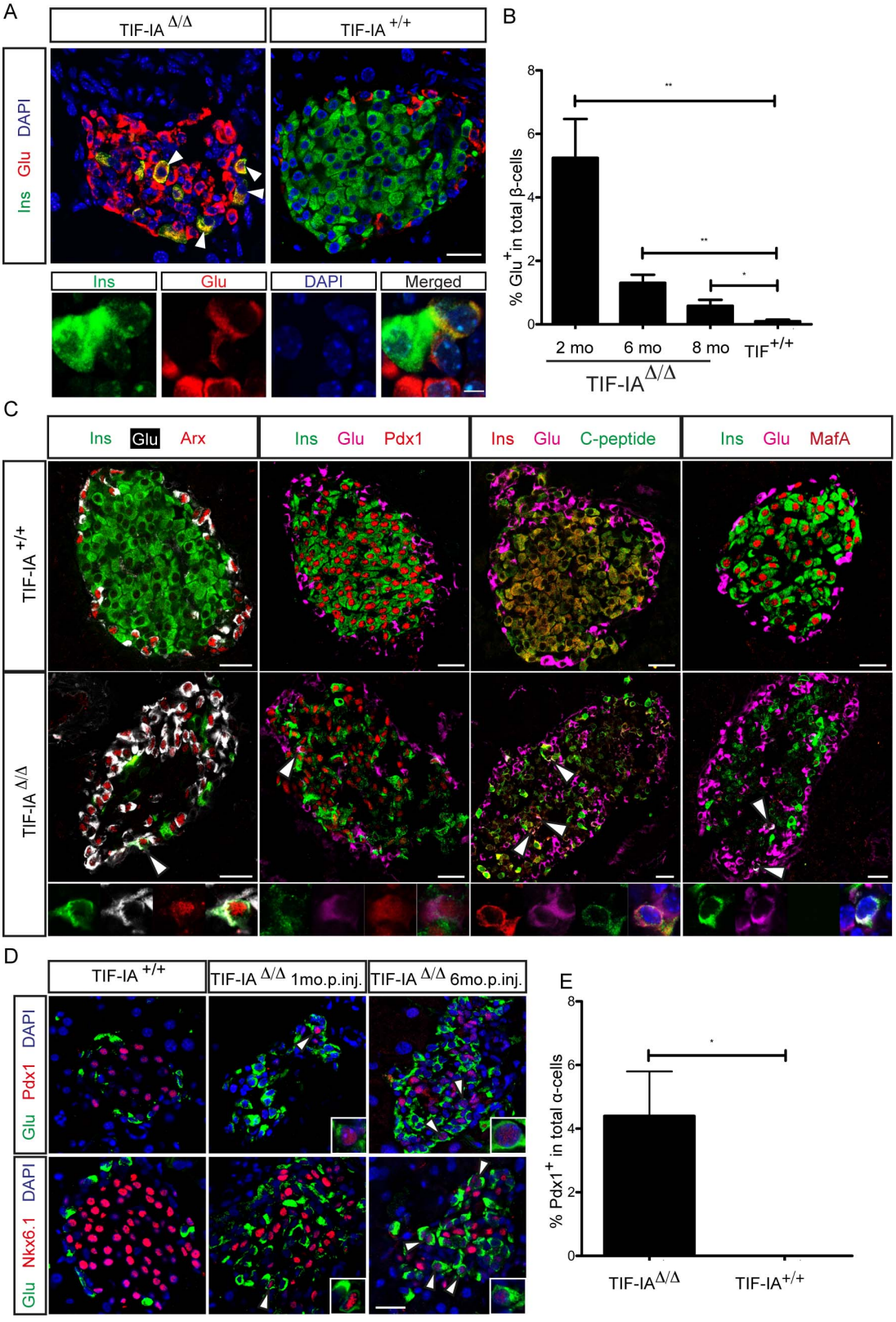

Figure 9: α-to β-cell transdifferentiation is induced in TIF-IA$^{\Delta/\Delta}$ model. A: Immunostaining of insulin (green) and glucagon (red) reveals the presence of cells co-expressing insulin and glucagon inside the islets of TIF-IA$^{\Delta/\Delta}$ mice. B: The relative abundance of these bihormonal cells is decreased over time. C: Bihormonal cells express a combination of α- and β-cell markers. Arrows point to the bihormonal cells. D and E: Expression of Pdx1 and Nkx6.1 is induced in glucagon expressing α-cells. m.p.inj. : months post injection. Scale bar : 25 µm, 25 µm for 4A lower images. Values are presented by mean ± SEM.P-value <0.05 : * , P-value <0.01 : **. N>3 animals for each experimental condition.

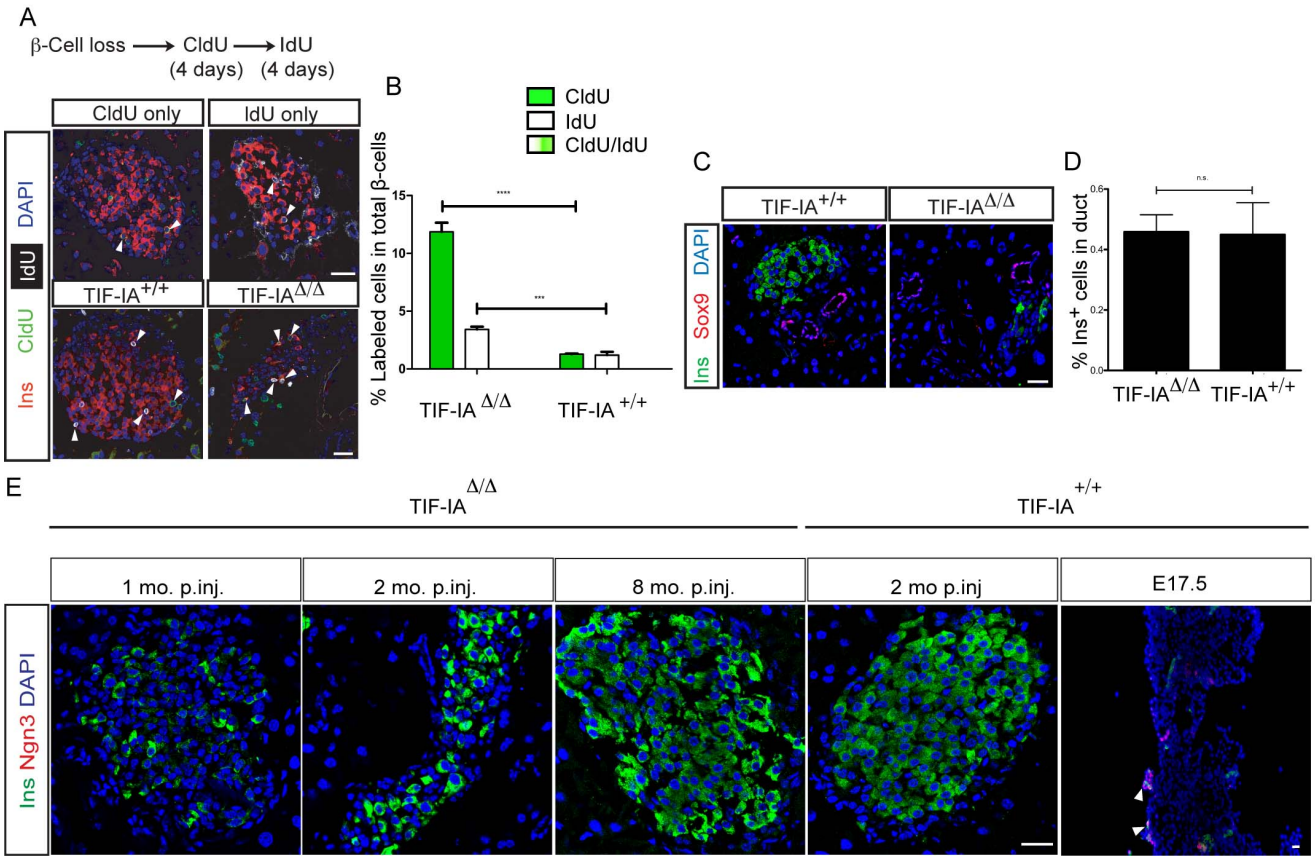

Figure 10: The new β-cells do not arise from a rapidly proliferating progenitor cell type. A and B: double thymidine analogue labeling shows even distribution of proliferation in the β-cell population. C and D: The frequency of insulin positive cells inside the duct structures, marked by Sox9 expression, does not change in response to β-cell loss in TIF-IA$^{\Delta/\Delta}$ mice. E: Ngn3 expression is not induced at several time points after tamoxifen injection. Scale bar : 25 µm. Values are presented by mean ± SEM. P-value <0.001: *** , P-value <0.0001 :****. N>3 animals for each experimental conditions.

mouse model.

7.6 β-Cell regeneration in aged mice

To understand how aging affects the induction of different regenerating pathways, β-cell ablation was induced in older mice. Three-months old mice were treated with tamoxifen to induce β-cell ablation. The pattern of β-cell loss and subsequent increase in β-cell numbers was similar to their younger animals (Figure 11A and B). The changes in blood glucose levels were also consistent with the number of insulin positive β-cells at different time points (Figure 11C). However, β-cell regeneration and, consequently, recovery rate was slower in older mice. β-cell proliferation rate was lower in three-months old injected TIF-IA$^{\Delta/\Delta}$ animals compared to one-month old injected TIF-IA$^{\Delta/\Delta}$ mice analyzed at similar time points following ablation initiation, but it was always higher than in the wild type littermates with similar fold-changes compared to the basal level at any given age (Figure 11C). These results show that although the basal β-cell proliferation rate is reduced in older mice, it will increase in response of β-cell

loss, supporting the activation of an adaptive proliferative response.
Interestingly, similar amount of bihormonal α-cells appeared in both yong and old mice. Quantification of bihormonal cells at different time points following β-cell ablation showed a similar fraction of β-cells expressing glucagon at two- and six-months post injection (Figure 11E). It was concluded that although the basal and adaptive proliferation rate of β-cells is reduced by age, massive β-cell depletion in the TIF-IA model appears to still activate some α-cells to reprogram and acquire β-cell characteristics.

7.7 Transcriptome Analysis of Regenerating Pancreas

To investigate the molecular events happening in the pancreas following β-cell ablation and regeneration, transcriptome profiles of TIF-IA$^{\Delta/\Delta}$ and TIF-IA$^{+/+}$ pancreata was compared at one and eight months upon tamoxifen injection, using RNA-sequencing (Figure 13A). One month after tamoxifen injection, the expression level of 1326 transcripts was significantly different in pancreata of TIF-IA$^{\Delta/\Delta}$ mice compared to TIF-IA$^{+/+}$ mice. Among those, 179 transcripts were downregulated and 1147 transcripts were upregulated in TIF-IA$^{\Delta/\Delta}$ pancreata. Expression of many and β-cell specific genes such as Ins1, Ins2, Glp1r, MafA, Glp1r, Iapp, Foxa2, and Nkx6.1 was significantly downregulated in TIF-IA$^{\Delta/\Delta}$ pancreata (Figure 14). This was consistent with results observed using immunohistochemistry on pancreas tissue. It provided the proofs of principle that sequencing the RNAs obtained from whole pancreas tissues reliably reflects the alteration of pancreatic islet gene expression.
Interestingly, analyses of eight-month post injection TIF-IA$^{\Delta/\Delta}$ and TIF-IA$^{+/+}$ pancreata showed that most of the differentially expressed genes at one month post injection are no longer different after complete β-cell regeneration, and in fact the tissue is effectively recovered at a genome-wide level (Figure 13B).
To understand the molecular mechanisms underlying pancreas regeneration, functional enrichment analysis of biological processes and pathways was performed, using the list of all differentially transcripts between one-month post injection TIF-IA$^{\Delta/\Delta}$ and TIF-IA$^{+/+}$ pancreata. The top enriched biological processes were related to cellular growth and proliferation, lipid metabolism, inflammatory response, cell-to cell interaction, endocrine system development and function, hepatic system diseases and development, and cellular compromise (Figure 15). The most significant enriched pathways were acute phase response signaling, complement and coagulation systems, TREM1 signaling, IL-10 and IL-6 signaling, NFkB signaling, Toll-like receptor signaling, cytokine-cytokine receptor interaction, chemokine signaling pathway, and MSP-RON (Macrophage Stimulating Protein/ RON Protein Tyrosine Kinase) signaling. Additionally, pathways related to glucose and fatty acid homeostasis including LXR/RXR (liver X receptor/Retinoid X receptor) Activation and PPAR (Peroxisome proliferator-activated re-

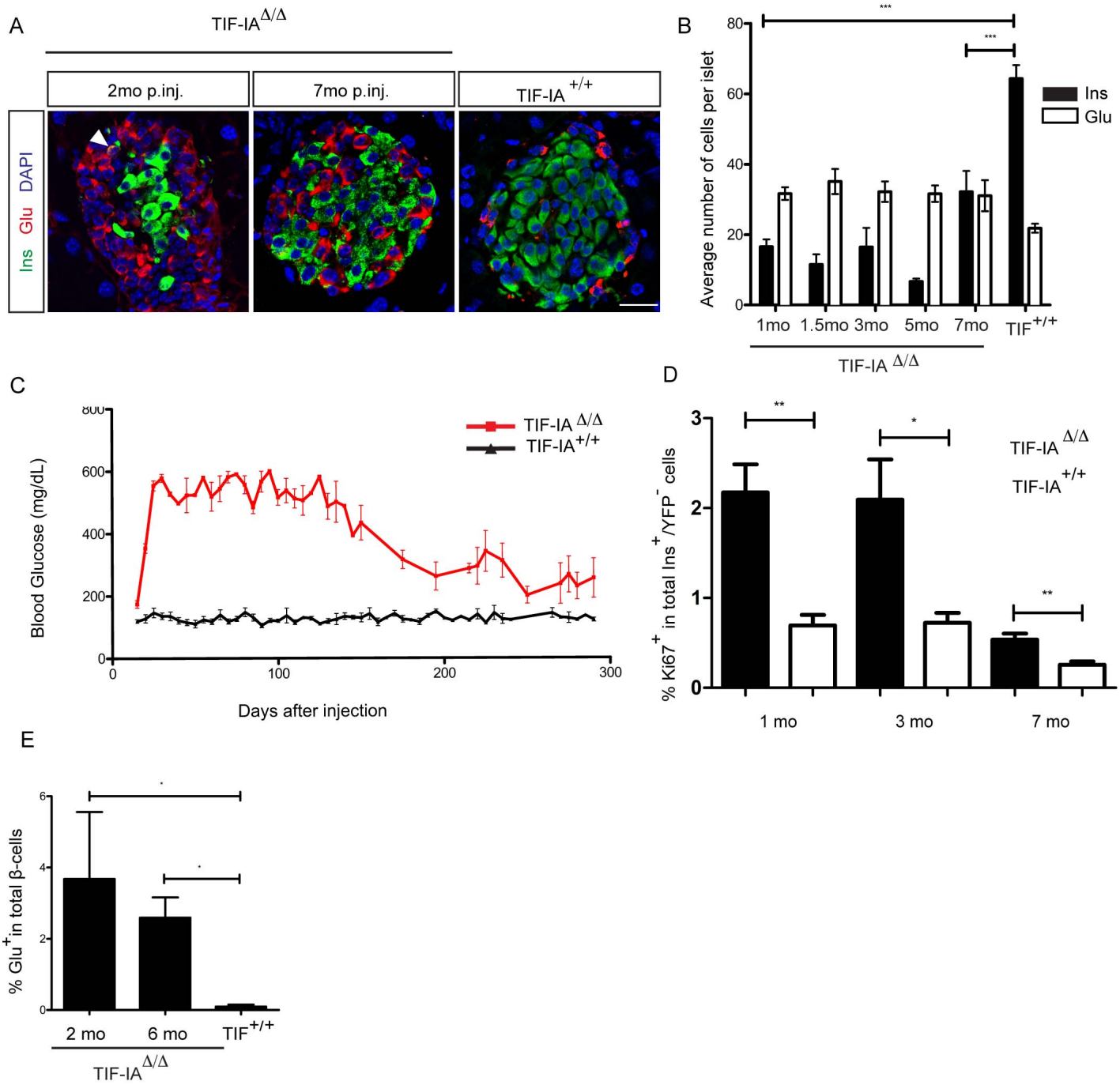

Figure 11: Declined regenerative rate in TIF-IA$^{\Delta/\Delta}$ mice with older onset of β-cell loss. A and B. Immunostaining and quantification of insulin (green) and glucagon (red) expressing cells in the pancreata of TIF-IA$^{+/+}$ and TIF-IA$^{\Delta/\Delta}$ mice at different time points post injection (A). β-cell number is increased gradually in mice injected at three-months, but at a slower rate compared tot he younger mice (B). C: Reduction of blood glucose level takes place at a slower manner in older mice. D: β-cell proliferation rate is increased in mice with the older onset ablation. E: The relative abundance of bihormonal cells is similar in one- and three-months old injected mice. In both cases, their frequencies decrease by time following initial ablation. mo.: months post injection. Scale bar : 25 µm. Values are presented by mean ± SEM. P-value <0.05 : *, P-value <0.01 : **, P-value <0.001 : ***. N >3 animals for each experimental conditions.

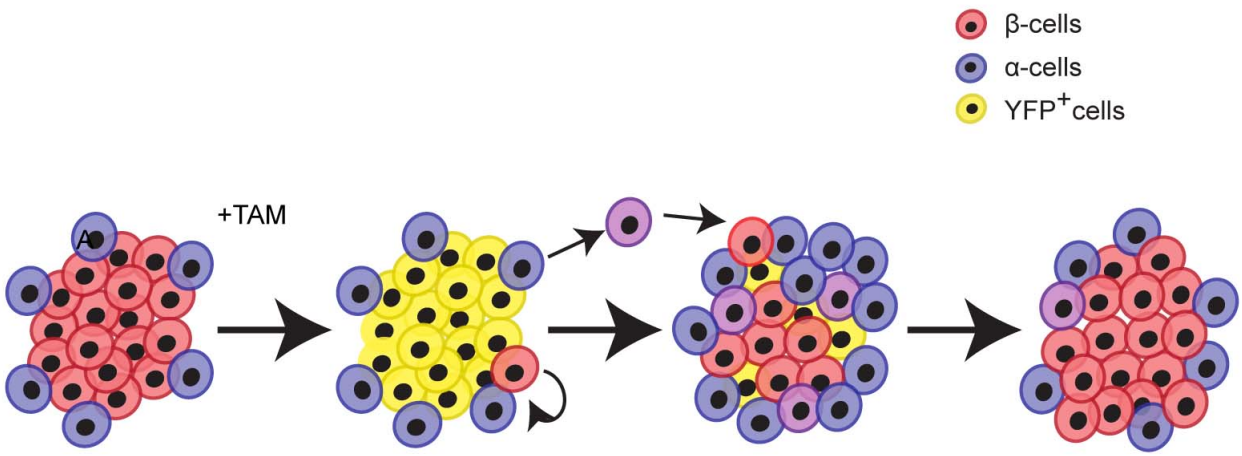

Figure 12: Proposed model for β-cell regeneration inTIF-IA$^{\Delta/\Delta}$ model. Adaptive β-cell proliferation and α- to β-cell transdifferentiation contribute to β-cell regeneration in TIF-IA$^{\Delta/\Delta}$ model. Bi-hormonal cells are shown in violet.

ceptor) signaling were also enriched in the differentially expressed genes (Figure 16).

7.8 Several non-coding RNAs are differentially expressed in TIF-IA$^{\Delta/\Delta}$ pancreas

Furthermore, the expression level of seven non-coding RNAs was significantly up or down-regulated in TIF-IA$^{\Delta/\Delta}$ pancreata compared to TIF-IA$^{+/+}$ controls one month following β-cell ablation (Figure 17). Expression of miR-22 host gene (miR-22hg) was upregulated in TIF-IA$^{\Delta/\Delta}$. miR-22hg encodes a non-coding transcript that is further processed and gives rise to mature miR-22.
Using In-situ hybridization against miR-22, the induction of mature miR-22 in pancreatic islet of TIF-IA$^{\Delta/\Delta}$ mice was confirmed (Figure 18). In the wildtype islets, miR-22 was expressed at a low level, but its expression is increased in the regenerating islets. Combining miR-22 *In-situ* hybridization with immunohistochemistry against insulin and glucagon showed that miR-22 is expressed in both α- and β-cells (Figure 19). Interestingly, miR-22 expression was normalized in TIF-IA$^{\Delta/\Delta}$ islets after eight months and the recovered islets were indistinguishable from TIF-IA$^{+/+}$ islets (Figure 18).

7.8.1 miR-22 target genes

In the light of the above results, potential miR-22 target genes that convey its functional impacts in regenerating islets were searched. The expression of target genes should be consequently decreased as a consequence of such increased expression. For selection of potential microRNA targets, several online prediction algorithms (including miRanda, miRDB, miRWalk, RNA22, and Targetscan) and the results from previous publications in different tissues and cell types was used. In the next step, the candidate gene list was compared to the list of

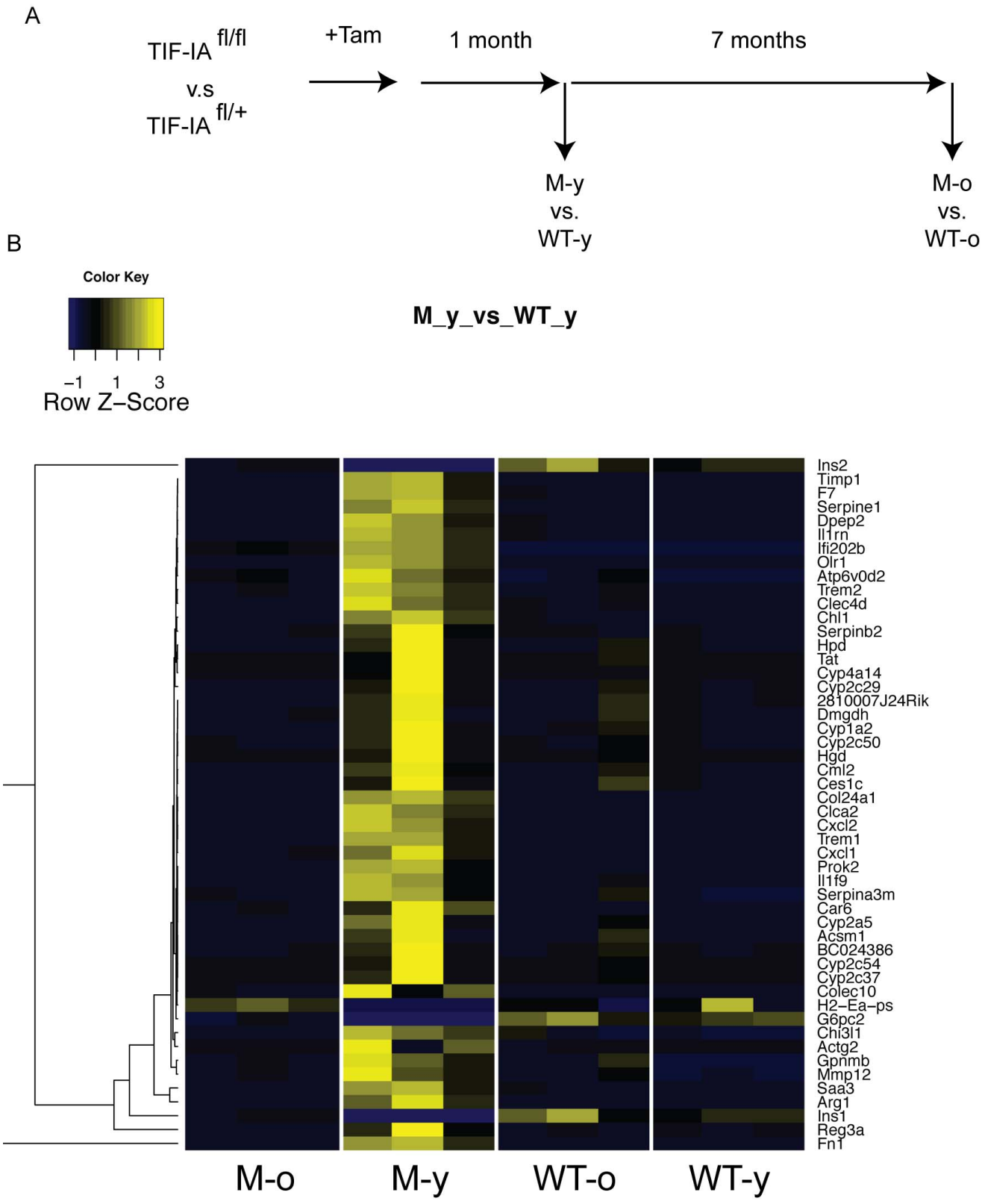

Figure 13: Transcriptome Analysis of Regenerative Pancreas. A: Pancreata were harvested from TIF-IA$^{\Delta/\Delta}$ and TIF-IA$^{+/+}$ one and eight months upon tamoxifen injection. B: Heatmap visualisation of the top 50 candidate genes with normalised values (M-y vs. WT-y). M: mutant, WT: wildtype, y: young, o: old.

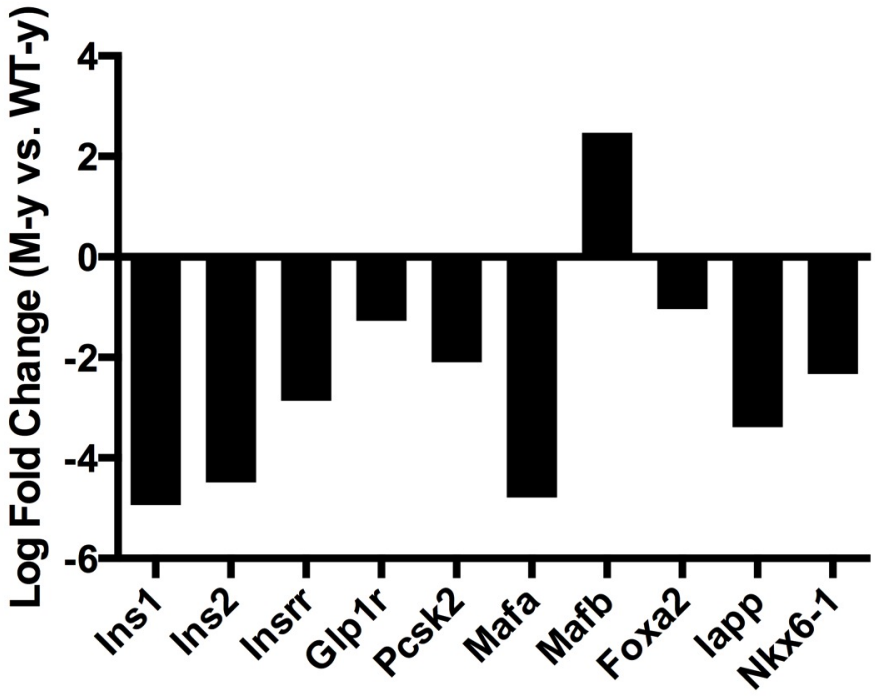

Figure 14: Expression of β-cell specific genes was significantly downregulated in TIF-IA$^{\Delta/\Delta}$ pancreata compared to TIF-IA$^{+/+}$ controls. Expression of MafB, a transcription factor expressed in α-cells and immature β-cells, was significantly increased in TIF-IA$^{\Delta/\Delta}$ pancreata.

genes significantly downregulated in TIF-IA$^{\Delta/\Delta}$ mice.

To identify the miR-22 target genes in pancreatic β-cells, Min6 cells were transfected with miR-22 mimic. The impact of miR-22 induction on the mRNA level of potential target genes was analyzed by quantitative real-time PCR (qPCR). Among 31 potential target genes analyzed by qPCR, the expression of seven genes showed significant downregulation at 48 hours after transfection (Figure 20). miR-22 target genes are involved in regulation of pancreatic islet function, cell cycle, and apotosis. Therefore, it was concluded that miR-22 is induced in pancreatic islet in response to β-cell loss and it mediate the functional adaptions associated with stress response.

8 Discussion

8.1 TIF-IA$^{\Delta/\Delta}$ model is a valuable tool to study β-cell regeneration

In this study, TIF-IA conditional knockout mice was used to establish a novel model of progressive β-cell ablation enabling the monitoring of β-cell regeneration in the endocrine pancreas. Following 95% β-cell loss, adaptive proliferation of pre-existing β-cells and α- to β-cell transdifferentiation contribute to the recovery of the β-cell mass (Figure 12). In contrast to the previously described extreme β-cell ablation model [33], the low number of non-ablated

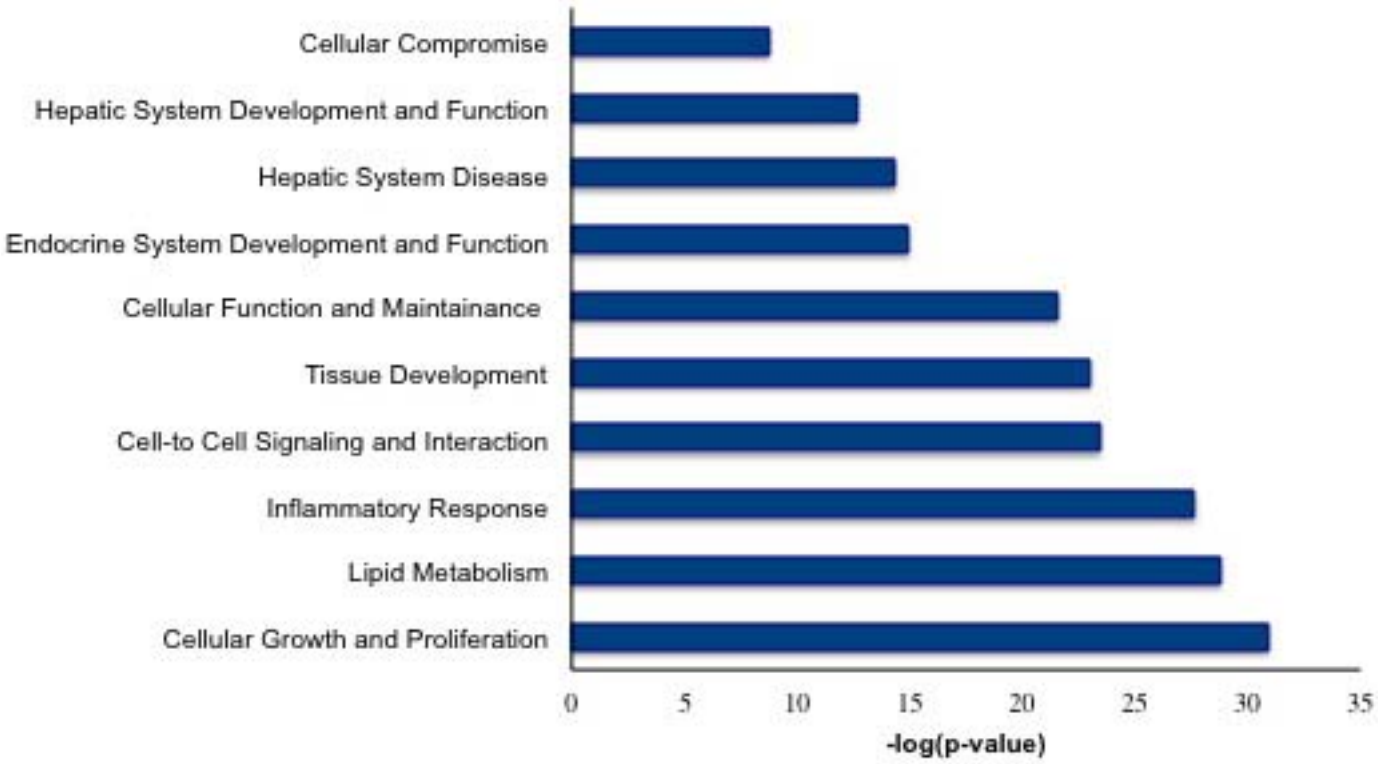

Figure 15: List of the top ten biological processes enriched in genes differentially expressed in TIF-IA$^{\Delta/\Delta}$ pancreata compared to TIF-IA$^{+/+}$ controls at one month post-injection.

β-cells in the TIF-IA$^{\Delta/\Delta}$ model is sufficient to drive the adaptive proliferation, and contribute significantly to β-cell regeneration.

Using TIF-IA$^{\Delta/\Delta}$mouse model in β-cell regeneration studies offers several advantages compared to previously studied models:

First, the deletion of TIF-IA triggers a protracted endogenous cell death in insulin producing cells that results in a gradual ablation of the β-cell mass, which mimics type 1 diabetes disease progression [12, 10, 70].

Second, the efficient recombination induced by tamoxifen treatment induces apoptosis in most of β-cells, but keeps a number of residual insulin-producing cells intact. This aspect is particularly interesting in light of the finding that such residual β-cells might have a critical function in response to therapeutic approaches [12].

Third, since blood glucose starts to elevate as early as 10 days upon tamoxifen induction, the regeneration procedure is initiated while islets are still not completely depleted of insulin producing β-cells (Figure 6D and 7A). The gradual loss and presence of the healthy residual populations of β-cells help to sustain animal survival without exogenous insulin administration, which has been used to improve animal survival rate in another reported genetic model of extreme β-cell loss [33], the effect of such treatment on regeneration remaining to be characterized. For instance, in the diphtheria toxin-inducible system [33], all β-cells are lost within 15 days upon induction. In such conditions, mice have to be treated with insulin to survive the extreme β-cells loss. This will indeed influence glucose metabolism, which plays the central

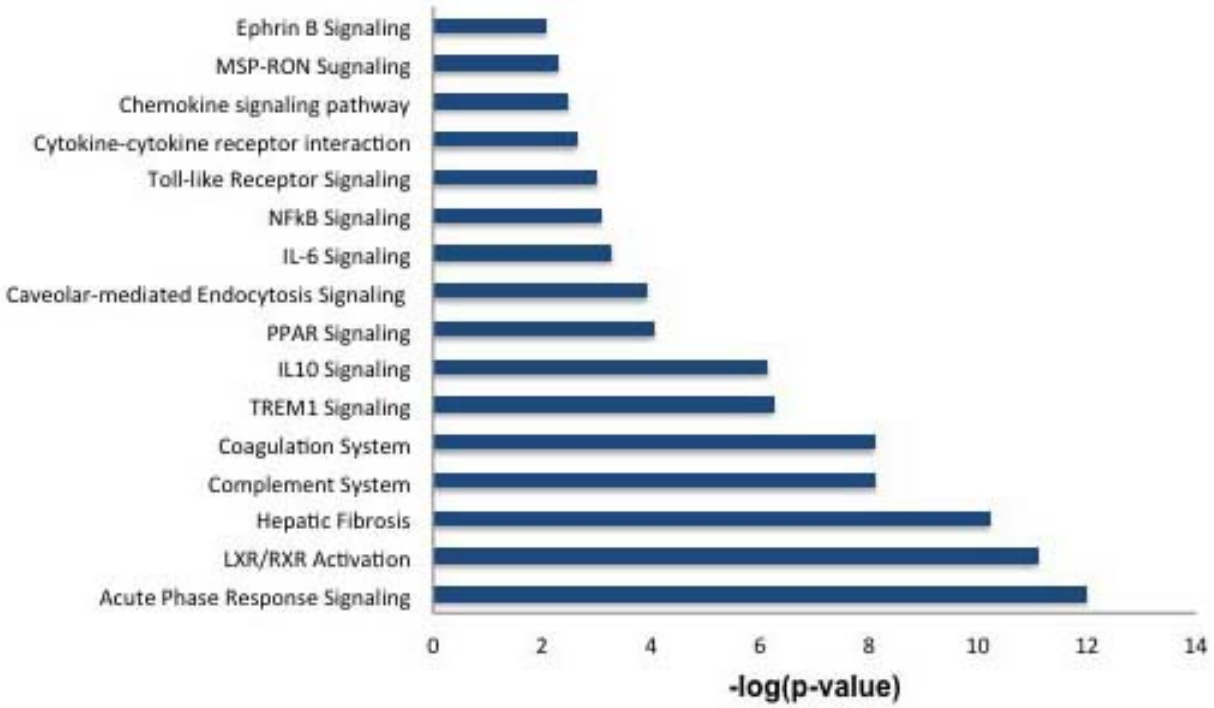

Figure 16: List of the top canonical pathways enriched in genes differentially expressed in TIF-IA$^{\Delta/\Delta}$ pancreata compared to TIF-IA$^{+/+}$ controls at one month post-injection.

role in stimulating β-cells proliferation and regeneration [74]. Exogenous insulin administration is suggested to reduce β-cell proliferation in mice lacking normal β-cell mass by lowering the glucose metabolism rate and individual β-cells workload [74]. This can explain why β-cell proliferation never increased neither in the remaining β-cells population shortly after ablation nor after partial increment of β-cell mass [33]. In can be postulated that in the absence of exogenous insulin treatment, newly formed β-cells could increase their proliferation rate and gain control of regeneration in later stages, as it was observed in the TIF-IA$^{\Delta/\Delta}$ model.
In conclusion, this study revealed that TIF-IA$^{\Delta/\Delta}$ model can be used in a great spectrum of investigations addressing different aspects of β-cell regeneration in type 1 diabetes.

8.2 Adaptive β-cell proliferation is the major regenerative mechanism in TIF-IA$^{\Delta/\Delta}$ model

In the different ages analyzed in this study, the proliferation rate of β-cell seems to be the major compensatory responses induced following β-cell loss. These findings are consistent with previous reports [16, 17, 18, 19], showing that increased proliferation of pre-existing β-cells can compensate, at least partially, for a massive β-cell loss, as found in the TIF-IA$^{\Delta/\Delta}$ model. These findings have led to the conclusion that drugs that are able to stimulate endogenous human pancreatic β-cell proliferation can offer great therapeutic potential for treatment and management of both type 1 and type 2 diabetes. In this regard, it is important to notice that adult human β-cells harbor lower proliferation capacity compared to the rodents. Therefore,

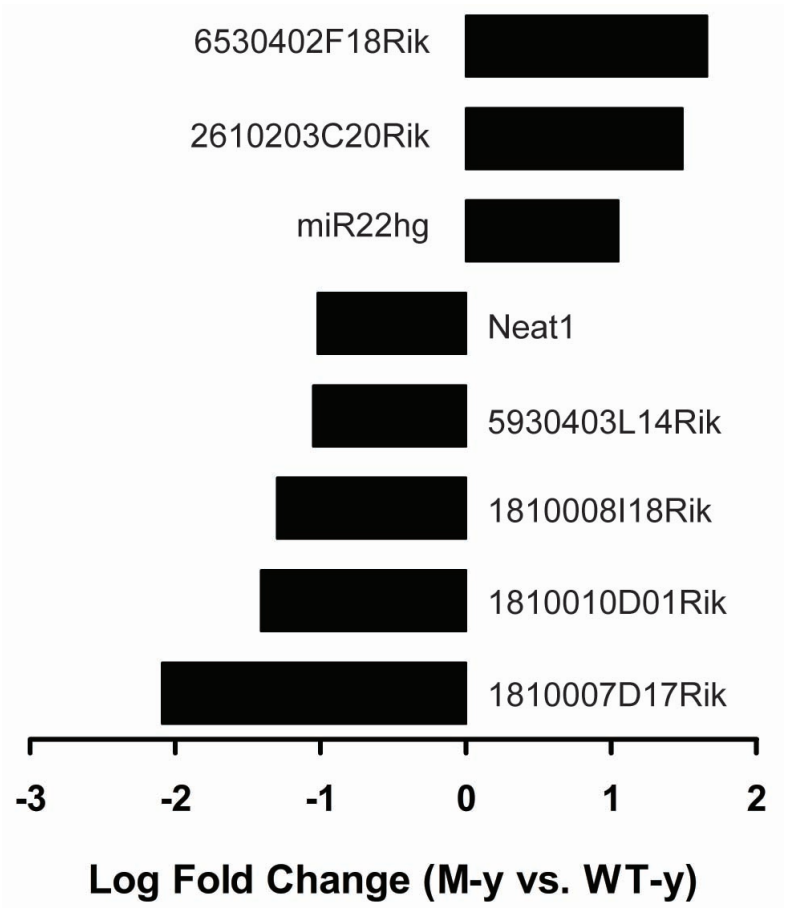

Figure 17: Differentially expressed non-coding RNAs in TIF-IA$^{\Delta/\Delta}$ pancreata compared to TIF-IA$^{+/+}$ controls at one month post-injection.

it is essential to find the regulatory mechanisms that induce human β-cell proliferation, while maintaining the differentiated status of these cells.

8.3 α-cells play an essential role in maintenance of β-cell mass

The presence of α-cells displaying some β-cell markers, such as insulin, Pdx1, and Nkx6.1, but lacking MafA, suggests that these cells may represent α-cells transitioning towards a β-cell identity. However, a definitive proof of the contribution of these cells to the regenerated β-cell mass will await lineage tracing experiments. As TIF-IA is expressed ubiquitously in different cell types, it was not possible to incorporate a Cre-LoxP based lineage tracing system into this model. In the future, other types of "hit and run" recombination systems could be used to determine the extent of the contribution from the two cellular sources, that is pre-existing β-cell and α-cells, to the regenerated β-cell mass. In addition, it is possible that due to the existing diabetic condition induced by TIF-IA knockout, the observed bihormonal cells may emanate from a dedifferentiation of β-cells into α-cells, as reported by Talchai et al., 2012 [75]. ChIP-sequencing analyses have shown that several genes involved in the regulation of the β-cell identity are bivalently marked by activating and repressing histone modifications in human α-cells [76]. These findings confer an innate epigenetic plasticity to α-cells, which allows their

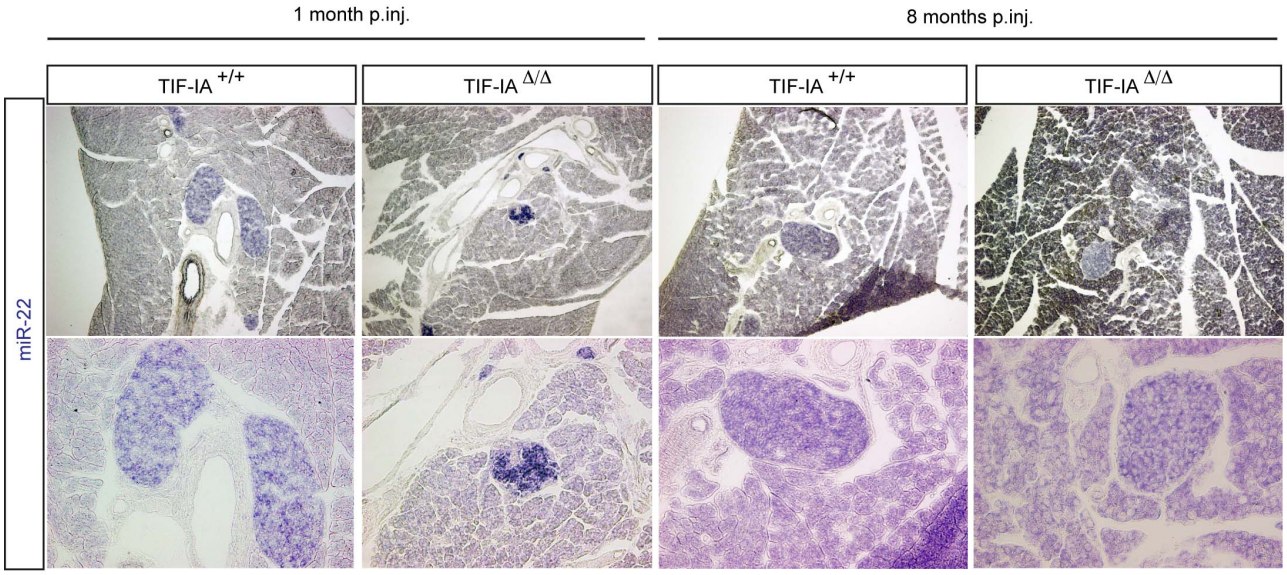

Figure 18: miR-22 expression is upregulated in TIF-IA$^{\Delta/\Delta}$ islets. *In-situ* hybridization of miR-22 in mouse pancreas tissues showed that miR-22 is exclusively expressed in islets, and it was absent in the exocrine tissue. In wildtype islet, miR-22 is expressed at a low level. The expression is increased upon β-cell loss in TIF-IA$^{\Delta/\Delta}$ islets, but it has normal expression level in the recovered islets. (*In-situ* hybridization was performed by Dr Rabe (RG Molecular Cell Differentiation, Max-Planck institute for biophysical chemistry)).

reprograming into β-like cells in extreme conditions. This point to the action of supportive mechanisms of α-cells for β-cell mass maintenance, through allowing β-cell regeneration even in extreme conditions, when β-cells are completely diminished. In this context, it is essential to understand the mechanisms underlying the epigenetic reorganization leading to identity switch in condition of massive β-cell loss. Towards this goal, it is worth mentioning the suggestion that the cytokine(s) and chemokine(s) released from injured β-cells, such as stromal cell factor-1 (SDF-1) and interleukin-6 (IL-6), could mediate β-cell hyperplasia and α to-β-cell transdifferentiation [77, 78, 79].

8.4 Pancreatic progenitor cells do not contribute to β-cell regeneration in adult mice

The contribution of adult progenitor cells to endocrine lineages is highly controversial. In TIF-IA$^{\Delta/\Delta}$ model, there was no evidence supporting their contribution to the regenerated β-cell mass. Adult progenitors have been shown to play a role in β-cell regeneration, when the pancreas tissue is surgically damaged in pancreatic duct ligation and pancreatectomy models [32], and in Pax4-misexpressing mice. Following ectopic expression of Pax4, a transcription factor essential for β-cell development, in β-cells of transgenic mice, duct-lining cells were shown to contribute to β-cell regeneration [35, 36]. The involvement of progenitor cells was not confirmed in any specific β-cell ablation model.

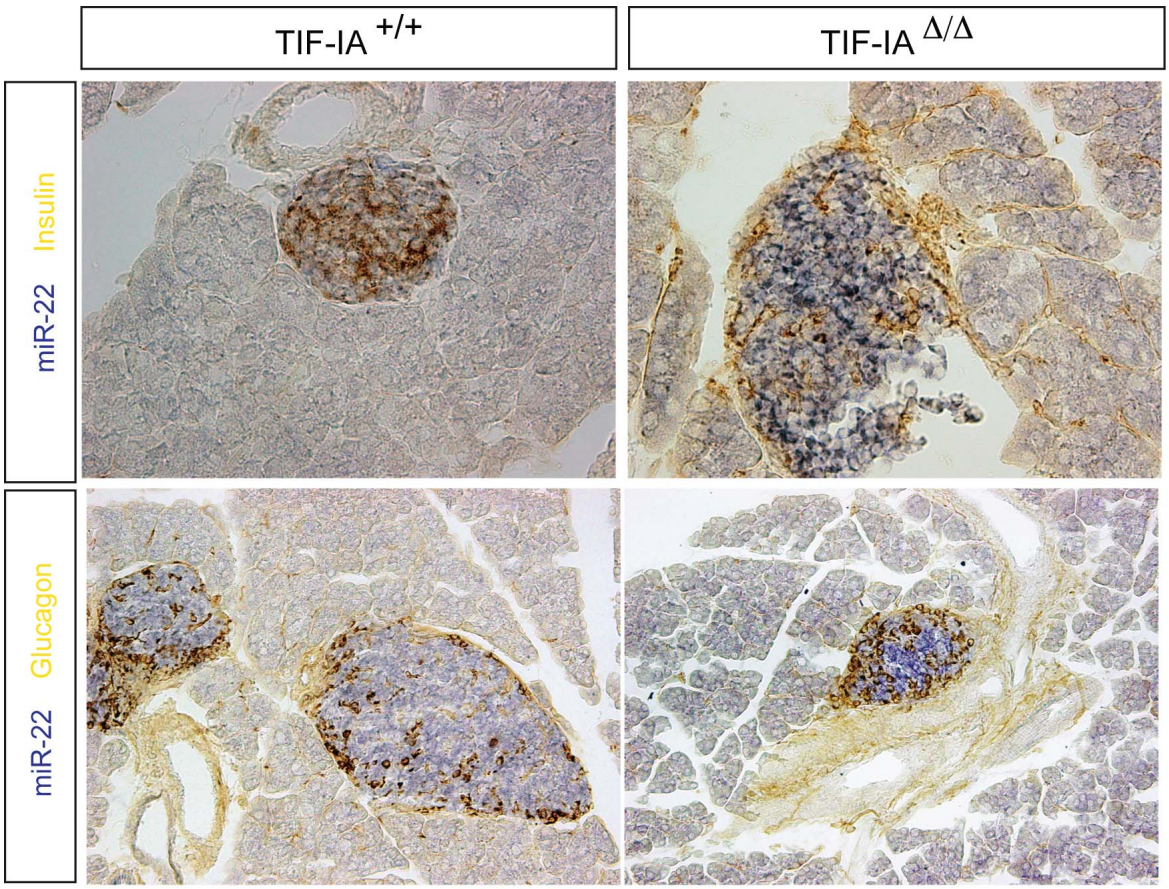

Figure 19: miR-22 is expressed in pancreatic α- and β-cells. Immunohistochemistry and in situ hybridization showed that miR-22 colocalizes with insulin and glucagon-expressing cell in islets (*In-situ* hybridization was performed by Dr Rabe (RG Molecular Cell Differentiation, Max-planck institute for biophysical chemistry)).

8.5 Aging delays β-cell regeneration

In this study, the mice with older onset of β-cell ablation showed regeneration delay; however, their regenerative capacity is not altered. Although the basal β-cell proliferation rates decline with age, their proliferation rate still enhances with a similar fold-change relative to the basal level in response to challenge, which is consistent with an earlier report [80]. These findings sustain the notion that the declined proliferation rate reduces β-cell regeneration capacity in aged mice [80, 81]. α- to β-cell conversion was demonstrated to contribute to β-cell regeneration in condition of extreme β-cell loss [33] and in a PDL (pancreatic duct ligation) model combined with alloxan-induced β-cell ablation [34]. Moreover, it was shown that adult α-cells can be reprogramed into β-cell following ectopic overexpression of Pax4 with the similar efficiency at different ages, suggesting that aging does not restrict α-cells plasticity and ability to transdifferentiate [35, 82]. Interestingly, the appearance and the low abundance of transdifferenting α-cells is age independent in TIF-IA$^{\Delta/\Delta}$ model.

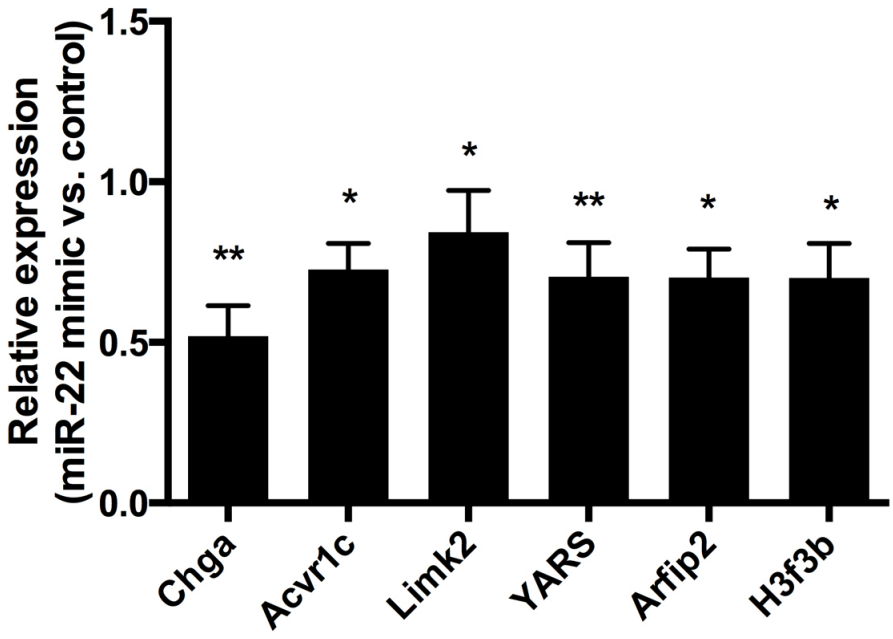

Figure 20: miR-22 target genes in MIN6 cells. miR-22 mimic transfection downregulated the expression of its target mRNAs in MIN6 cells. Relative expression level was analyzed 48 hours after transfection compared to MIN6 cells transfected with a negative control mimic. P-value <0.05 : *, P-value <0.01 : **.

8.6 Transcriptome profiling of regenerating pancreas provides a resource to identify the key regulatos of β-cell and pancreas regeneration

The change in the protein level results from coordinated regulation at different layers of gene expression, including transcription, mRNA processing and translation. Transcriptional profiling and quantification of transcripts abundance enable us to analyze the dynamic transcriptional changes underlying developmental and disease-associated transitions. In this study, next-generation sequencing (NGS) technology was used to provide a transcriptome map of regenerating mouse pancreas. Comparative RNA-sequencing of regenerating pancreas helped us to get insight into the molecular mechanisms that lead to functional responses of tissues to β-cell ablation and its consequent effects.

8.7 Several signaling pathways are involved in β-cell regeneration

These results shows that integration of several signaling pathways induced by systemic metabolic changes and Inflammation regulate pancreatic β-cell regeneration. Inflammatory responses seem to be the major pathways induced in pancreas upon β-cell ablation. Acute phase response signaling, IL-10 and IL-6 signaling, Caveolar-mediated Endocytosis Signaling, NFkB signaling, cytokine-cytokine receptor interaction, chemokine signaling pathway, MSP-RON signaling, LXR/RXR Activation, and PPAR signaling are the top pathways induced in

TIF-IA$^{\Delta/\Delta}$ pancreas, which all are related to tissue injury and inflammatory response and many of them are shown to be important mediators of stress response and are critical for normal regeneration in variety of tissues.

8.7.1 Acute phase response

Among the enriched canonical pathways, the highest p-value was observed for acute phase response signaling (Figure 17). Acute phase response is a systemic early defense mechanism induced in response to infection, stress, and inflammation. In conditions of tissue injury, acute phase response is involved in initiation of inflammatory processes, as well as tissue repair and regeneration [83].

8.7.2 IL-10 and IL-6 signaling

Member of interleukin 6 (IL-6) family of cytokines have both pro- and anti-inflammatory properties. IL-6 type cytokines are involved in variety of cellular processes including differentiation, survival, apoptosis and proliferation [84]. They are also shown to play role in haematopoiesis [85], liver [86] and neuronal regeneration [87, 88], embryonic development [89, 90], and fertility [90].
Interleukin 10 (IL-10) is a potent anti-inflammatory cytokine that represses the expression of inflammatory cytokines such as TNF-α, IL-6 and IL-1 [91]. Binding of IL-6 and IL-10 to their receptors triggers the activation of the JAK/STAT (Janus kinase/signal transducer and activator of transcription) and MAPK (mitogen-activated protein kinase) cascades [92]. Interleukin-6 and interleukin-10 both activate the same signaling mediator, STAT3 [93].
It is shown that IL-6 increases insulin secretion through enhancing the expression of GLP-1 (glucagon-like peptide-1) from intestinal L cells and pancreatic α-cells [94]. Additionally, IL-6 enhances α-cell mass expansion [77]. Therefore, it is postulated that IL-6 might be involved in α-cell-mediated β-cell regeneration [95]. The genes encoding for receptors and signaling molecules downstream of these two cytokines are mostly upregulated in regenerating pancreas. For example, expression of Socs3 (Suppressor of cytokine signaling 3) is upregulated in TIF-IA$^{\Delta/\Delta}$ pancreas. Socs3 is induced by both IL-6/STAT3 and IL-10/STAT3 pathways, and suppresses the uncontrolled expansion of inflammation [93]. Suppression of Socs3 expression in the pancreatic β-cell leads to resistance to type 1 diabetes [96]. Overexpression of Socs3 leads to reduced β-cell volume in female transgenic mice and suppress growth hormone-mediated replication of primary β-cells [97].

8.7.3 LXR/RXR Activation

Liver X receptors (LXRs) are members of a nuclear hormone receptor family of transcription factors, which play important roles in regulation of cholesterol, fatty acids, and glucose homeostasis [98, 99]. The retinoid X receptors (RXRs) are nuclear receptors that are involved

in the retinoic acid-mediated gene activation. Binding of oxysterol ligands to LXRs receptors leads to their activation and formation of a heterodimer with RXR [100, 101]. Following heterodimerization, LXRs activate transcription of target genes by binding to the genomic LXR response element [101]. LXR/RXR is involved in the regulation of lipid metabolism, inflammation, and cholesterol to bile acid catabolism. Both isoforms of LXRs (LXRα and LXRβ) are expressed in rodent and human islets. Incubation of cultured islet with T0901317, the synthetic LXR agonist, enhances insulin secretion and insulin biosynthesis via stimulation of glucose and lipid metabolism [102]. Another study also showed direct effect of LXR agonists on islet function and insulin secretion and expression through increased Pdx-1 nuclear protein levels and increased Pdx-1 binding to the insulin promoter [103]. Considering the observed function of LXR/RXR signaling pathway in different contexts, its activation in TIF-IA$^{\Delta/\Delta}$ islets may offer beneficial effects on islet function in response to elevated glucose and fatty acid levels.

8.7.4 Caveolar-mediated Endocytosis Signaling

Caveolae are a special type of lipid rafts that are enriched in cholesterol- and sphingolipid and form flask-shaped invaginations of the plasma membrane. Caveolae play role in the internalization of membrane components, extracellular ligands (insulin, albumin, EGF), viruses, and bacterial toxins. Caveolar-mediated endocytosis is involved in a broad range of cellular processes such as endocytosis, cellular signaling and lipid recycling [104].
Caveolin-1 is the main component of caveolae in non-muscle cells [105]. In Caveolin-1 knockout mice, liver regeneration is severely impaired. In response to tissue injury, Caveolin-1$^{-/-}$ hepatocytes cannot accumulate lipid droplets and fail to proceed through the cell division cycle [106]. Additionally, it is shown that Caveolin-1 can function as a Cdc42 guanine nucleotide dissociation inhibitor in islets or MIN6 β-cells, and its loss of function leads to an enhanced basal level insulin release [107]. In contrast to this, the Caveolin-1 knockout mice are reported to have normal fasting plasma insulin levels [108]. Interestingly, expression of Caveolin-1 and other important genes of Caveolar-mediated endocytosis signaling and lipid recycling pathways such as fatty acid transporter CD36/FAT and many enzymes involved in fatty acid esterification and oxidation are upregulated in regenerating pancreas. These observations point to the importance of adaptive changes of lipid metabolism pathways in β-cell regeneration. In response to systemic alteration in the fatty acid level, β-cells seems to increase their lipid internalization and processing rate to cope with the harmful effects of high free fatty acid levels and to incorporate them as cell membrane building blocks and energy source.

8.8 Expression of miR-22 is induced in pancreatic islets and mediate the stress response

It is shown that miR-22 is essential for hypertrophic cardiac growth in response to stress, and loss of miR-22 in heart impairs cardiac remodeling and hypertrophy and leads to cardiac dysfunction in the animals exposed to different types of stressors [109, 110]. miR-22 is also shown to function as a tumor suppressor by induction of cellular senescence and growth inhibition in cancer cell lines [111, 112]. The miR-22 targets identified in this study belong to diverse functional categories ranging from β-cells function and insulin secretion to cell cycle regulation and apoptosis.

8.8.1 Chromogranin A

Chromogranin A (Chga) encodes a neuroendocrine hormone that is expressed in variety of neuronal and endocrine tissues including different endocrine cell types of pancreatic islets [113]. Chga protein is localized in the secretory granules of these cells [114]. Although the biological function of ChgA is not well understood yet, but it is suggested that it act as a precursor of several biologically active peptide hormones, which regulate the neuroendocrine functions of cells in autocrine and paracrine manner [115]. In ChgA null mutants, islet size, number, and the proportion of β-cells in the islet were decreased. Additionally, ChgA loss of function resulted in decreased β-cell function and increased α-cell function in the mutants [116]. These results showed that ChgA expression is involved in development and function of endocrine pancreas cell types.

8.8.2 Activin A receptor, type IC

Activin A receptor, type IC (Acvr1c) is a member of the type I receptor serine/threonine kinases of the TGF-β superfamily that function as a receptor for Nodal and Activin AB and B [117]. Acvr1c expressed in pancreatic islets and β-cell lines, and it negatively regulates β-cell function and glucose-stimulated insulin release. While in the mutant mice lacking Acvr1c pancreas organogenesis is not affected, they developed an age-dependent syndrome involving progressive hyperinsulinemia, reduced insulin sensitivity, impaired glucose tolerance, and islet enlargement. Loss of Acvr1c resulted in enhanced insulin secretion under sustained glucose stimulation in mutant islet [118]. Overexpression of a constitutively active form of Acvr1c in β-cells led to increased apoptosis and reduced proliferation rate by inhibition of Akt signaling and activation of Smad2-dependent pathway [119].

8.8.3 LIM motif-containing protein kinase 2

LIM motif-containing protein kinase 2 (Limk2) belongs to small family of LIM kinases.

LIM domains are highly conserved cysteine-rich structure composed of two zing fingers, and mediate the protein-protein interactions. Both members of LIM kinase family, LIM kinase-1 and LIM kinase-2 are composed of a C-terminal protein kinase domain and two LIM motifs at their N-terminal [120, 121]. Both Limk1 and Limk2 act downstream of RhoA and ROCK, and are involved in actin cytoskeleton reorganization by phosphorylating and inactivating the actin depolymerizing factors ADF/cofilin [122]. The LIM kinases are overexpressed in some tumor types and have been shown to be involved in cancer metastasis and cell cycle progression [123, 124, 125].

8.8.4 Tyrosyl-tRNA synthetase

Tyrosyl-tRNA synthetase (Yars) belongs to class I aminoacyl-tRNA synthetase family that catalyze the transfer of amino acids to tRNA [126]. The enzyme can be splited into two fragments that harbor distinct cytokine activities [127, 128, 129]. It is shown that this protein is secreted under apoptotic conditions and it might be involved in apoptotic response through arresting translation and cytokine functions [129].

8.8.5 ADP-ribosylation factor interacting protein 2

ADP-ribosylation factor interacting protein 2 (Arfip2) is a Rac1-interacting protein. Studies have shown that Arfip2 directly binds to Rac1 in a GTP-dependent manner [134], and might be involved in regulation of cytoskeletal reorganization at the cell periphery [135].

8.8.6 H3 histone, family 3B

H3 histone, family 3B (H3f3b) encodes a member of the histone H3 family. H3.3 is a replacement histone subtype encoded by two genes that their expression is cell cycle independent (H3.3A and H3.3B). These two genes code the same protein sequence, but their gene organization and genomic sequence is different [136, 137]. H3.3 plays critical roles in regulation of chromatin structure and functions, and thereby controls cell proliferation, genome integrity, and development [138]. Interestingly, the expression of H3f3b was shown to be upregulated in miR-22 knockout heart [109].
Based on the results obtained in this study, miR-22 mediates the compensatory response of β-cells to pathological stress by targeting several genes regulating β-cell function, proliferation and apoptosis. This will extend the function of miR-22 as a key regulator of tissue homeostasis. Some of the miR-22 target genes seem to be different in myocytes and pancreatic β-cells, which suggests that miR-22 can mediate the essential cell-type specific adaptions depending on the physiologic function of each cell type.

9 Outlook

In conclusion, this study has established a novel genetic tool to induce progressive β-cell loss in mice, and revealed that TIF-IA$^{\Delta/\Delta}$ model can be used in a great spectrum of investigations addressing different aspects of β-cell regeneration in type I diabetes. Using this model, it was shown that several signaling pathways are induced in response to β-cell ablation and its pathophysiological effects. Integration of these pathways in β-cells and other pancreatic cell type, including α-cells, enforces the compensatory responses of these cells, such as increased proliferation rate, enhanced function and survival, and cell fate switching.

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