The Hippo pathway terminal effector YAP modulates β-cell regeneration and diabetes

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Summary

Diabetes, characterized by chronic hyperglycemia and severe metabolic disorders, is a major public health problem worldwide. In type 1 diabetes (T1D), β-cell loss is caused by immune-mediated destruction, whereas in type 2 diabetes (T2D), β-cell loss is initially caused by an increase in β-cell insulin requirements. Pancreatic β-cell apoptosis and loss of β-cell mass is a common feature of both T1D and T2D. Identifying the key regulatory mechanisms and active signaling pathways involved in β-cell failure is required for future strategies to prevent β-cell destruction and promote β-cell regeneration in diabetes. I show in this thesis that a critical downstream component of the Hippo pathway, the co-transcription factor Yes-associated protein (YAP), has a dual role in T1D. YAP potently promoted β-cell proliferation, protected from β-cell apoptosis and restored β-cell mass. Conversely, in the presence of enteroviruses, YAP was pro-apoptotic; it potentiated enterovirus induced inflammation and cell death.

In the first part of my dissertation I investigated the potential role of YAP induction in β-cells *in vivo* in mice with β-cell specific YAP overexpression. In this study, I found that YAP overexpression induced robust proliferation in both male and female mice, and subsequently increased β-cell mass. Overexpression of YAP in a model of T1D, namely through specific destruction of β-cells by streptozotocin (STZ) prevented diabetes induction. In both control and STZ-injected mice, YAP promoted β-cell mass, proliferation and protected β-cells from apoptosis. In the STZ mice, YAP's protective effect was mainly based on its negative transcriptional and translational effect on the expression of the glucose transporter GLUT2, a critical component of glucose metabolism and insulin secretion in β-cells.

YAP, the downstream transcriptional effector of the Hippo pathway, acts through its major transcription factor TEAD. In the second part of my thesis, I tested a chemical TEAD activator, for its efficacy to promote β-cell regeneration. TT-10 has been previously reported to induce cardiomyocyte regeneration during cardiac infarction. TT-10 promoted the proliferation of β-cells in both mouse and human islets, in an organ donor with T2D, as well as *in vivo* in mouse pancreatic islets, together with the restoration of functional gene expression in β-cells. This effected was mediated through TEAD.

In the last part of my studies, I overexpressed YAP in β-cells *in vivo* and investigated the effects on β-cells. The expression of YAP correlated with Coxsackieviruses B (CVB) infection, and many YAP-expressing cells show virus positivity or are localised in the immediate vicinity of virus-infected cells in the pancreas of organ donors with T1D and with T1D associated autoantibodies (AAb+). YAP was highly upregulated in both exocrine and endocrine pancreas

in T1D and AAb^+ donors. My studies focused on identifying the pathological connection between diabetes and YAP levels in β-cells in pancreas, revealed that chronic and homozygous induction of YAP expression was associated with impaired glucose tolerance, abolished insulin secretion and dedifferentiation of the β-cells in mice.

Together, my findings highlight β-cell regeneration for the therapy of diabetes and demonstrate the functional importance of YAP and its transcription factor TEAD in restoring pancreatic islet β-cell mass and proliferation, while YAP's protective effect becomes opposite in the presence of enteroviral infection.

ZUSAMMENFASSUNG

Diabetes ist durch chronische Hyperglykämie und schwere Stoffwechselstörungen gekennzeichnet und stellt weltweit ein großes Gesundheitsproblem dar.
Im Typ-1-Diabetes (T1D) wird der β-Zellverlust in den Inselzellen des Pankreas durch immunvermittelte Zerstörung verursacht, während im Typ-2-Diabetes (T2D) der β-Zellverlust durch einen erhöhten Insulinbedarf vermittelt ist. Die Apoptose der β-Zellen und der Verlust der β-Zellmasse ist ein gemeinsames Merkmal von T1D und T2D. Die Identifizierung der wichtigsten Regulationsmechanismen und aktiven Signalwege, die am β-Zellversagen beteiligt sind, ist für zukünftige Strategien zur Verhinderung der β-Zellzerstörung und zur Förderung der β-Zellregeneration bei Diabetes essentiell. In dieser Arbeit zeige ich, dass eine kritische nachgeschaltete Komponente des Hippo-Signalwegs, der Co-Transkriptionsfaktor Yes-associated protein (YAP), eine paradoxe Rolle im Diabetes hat. YAP förderte die Proliferation von β-Zellen, schützte vor der Apoptose von β-Zellen und stellte die β-Zellmasse wieder her. Doch in Gegenwart von Enteroviren war YAP pro-apoptotisch; es verstärkte die durch Enteroviren induzierte Entzündung und den Zelltod.

Im ersten Teil meiner Dissertation untersuchte ich die mögliche Rolle der YAP-Induktion in β-Zellen in vivo in Mäusen mit β-Zell-spezifischer YAP-Überexpression. In dieser Studie stellte ich fest, dass die Überexpression von YAP sowohl bei männlichen als auch bei weiblichen Mäusen eine starke Proliferation induzierte und anschließend die β-Zellmasse erhöhte. Die Überexpression von YAP in einem Modell von T1D, nämlich durch die spezifische Zerstörung von β-Zellen durch Streptozotocin (STZ), verhinderte die Induktion von Diabetes. Sowohl bei Kontrollmäusen als auch bei Mäusen, denen STZ injiziert wurde, förderte YAP die β-Zellmasse und die Proliferation und schützte die β-Zellen vor Apoptose. Bei den STZ-Mäusen beruhte die schützende Wirkung von YAP hauptsächlich auf seiner negativen Transkriptions- und Translationswirkung auf die Expression des Glukosetransporters GLUT2, einer kritischen Komponente des Glukosestoffwechsels und der Insulinsekretion in β-Zellen.

YAP, der nachgeschaltete transkriptionelle Effektor des Hippo-Wegs, wirkt über seinen Haupttranskriptionsfaktor TEAD. Im zweiten Teil meiner Arbeit habe ich einen chemischen TEAD-Aktivator auf seine Wirksamkeit zur Förderung der β-Zell-Regeneration getestet. Es wurde bereits berichtet, dass TT-10 die Regeneration von Kardiomyozyten bei Herzinfarkten fördert. TT-10 förderte die Proliferation von β-Zellen sowohl in Mäusen als auch in menschlichen Inseln, in einem Organspender mit T2D, sowie in vivo in Mäuse-Pankreasinseln, zusammen mit der Wiederherstellung der funktionellen Genexpression in β-Zellen. Dieser Effekt wurde durch TEAD vermittelt.

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Im letzten Teil meiner Studien habe ich YAP in β-Zellen in vivo überexprimiert und die Auswirkungen auf β-Zellen untersucht. Die Expression von YAP korrelierte mit der Infektion mit Coxsackieviren B (CVB), und viele YAP-exprimierende Zellen zeigten Viruspositivität oder waren in unmittelbarer Nähe von virusinfizierten Zellen im Pankreas von Organspendern mit T1D und mit T1D-assoziierten Autoantikörpern (AAb+) lokalisiert. YAP war sowohl im exokrinen als auch im endokrinen Pankreas von T1D- und AAb+-Spendern stark hochreguliert. Meine Studien konzentrierten sich auf die Identifizierung des pathologischen Zusammenhangs zwischen Diabetes und YAP-Spiegeln in β-Zellen in der Bauchspeicheldrüse und zeigten, dass eine chronische und homozygote Induktion der YAP-Expression mit einer gestörten Glukosetoleranz, einer aufgehobenen Insulinsekretion und einer Dedifferenzierung der β-Zellen in Mäusen verbunden war.

Zusammengenommen unterstreichen meine Ergebnisse die β-Zell-Regeneration für die Therapie von Diabetes und zeigen die funktionelle Bedeutung von YAP und seinem Transkriptionsfaktor TEAD bei der Wiederherstellung der β-Zellmasse und -proliferation der Pankreasinseln, während sich die schützende Wirkung von YAP in Gegenwart einer enteroviralen Infektion ins Gegenteil verkehrt.

Abbreviations

Aldh1A3	Aldehyde dehydrogenase 1 isoform A3
AMP	Adenosine monophosphate
AMPK	AMP-activated kinase
Ankard1	Ankyrin Repeat Domain 1
ATP	Adenosine triphosphate
CD4	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
CTGF	Connective Tissue Growth Factor
DPP1	Dipeptidyl peptidase 1
EGFR	Epidermal Growth Factor Receptor
FOXM1	Forkhead boxM1
GCK	Glucokinase
Glis3	GLI-similar zinc finger 3
GLP-1	Glucogen like peptide-1
GLUT2	Glucose transporter 2
GPCR	G-protein coupled receptor
GSIS	Glucose Stimulated Insulin Secretion
GSK	Glucogen synthase kinase
INS1-E	Rat-insulinoma cell line INS-1E
INS1	Insulin 1
INS2	Insulin 2
i.pGTT	i.p glucose tolerance test
i.pITT	i.p insulin tolerance test
JNK	c-Jun N-terminal kinase
LATS1/2	Large tumor suppressor kinase 1/2
MafA	MAF BZIP Transcription Factor A
MAP4K	Mitogen activated protein 4 kinase
MOB	Mps1binder related 1
MST1/2	Mammalian sterile 20 kinase 1/2
mTORC1	Mechanistic target of rapamycin complex 1
MYC	Myc proto-oncogene
NeuroD1	Neurogeneic Differentiation1
NF2	Neurofibromatosis 2
Nkx2.2	NK2 homeobox 2
Nkx6.1	NK6 homeobox 1
NGN3	Neurogenin 3
oGTT	oral glucose tolerance test
PAX4	Paired box 4
PDAC	Pancreatic ductal adenocarcinoma
PDX1	Pancreatic and Duodenal Homeobox 1
PGC1-α	PPARG coactivator 1 alpha
PPAR	Peroxisomal proliferator activated receptor
Rag	Ras related GTPase
RASSF	Ras association domain family member
Rho GTPase	Rho family of small GTP-binding proteins
ROCK	Rho associated protein kinases
ROS	Reactive oxygen species
SAV1	Salvador homologue 1
SNARE	Snap receptor
STAT	Signal transducer and activator of transcription
STK	Serine/threonine kinase
STZ	Streptozotocin
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes

TAZ	Transcriptional coactivator with PDZ binding motif
TEAD	Transcriptional enhancer factor TEF-1
TNF	Tumor necrosis factor
UPR	Unfolded protein response
UPS	Ubiquitin proteasome system
VGLL4	Vestigial Like Family Member 4
YAP	Yes Associated Protein

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1 Introduction

1.1. Diabetes Mellitus: A Health Emergency

The history of diabetes is a fascinating journey through centuries of medical observations, discovery, and innovation. Clinical features resembling diabetes mellitus were described by the ancient Egyptians approximately 3000 years ago. Aretaeus Cappadocia is credited with coining the term “diabetes,” derived from the Greek word meaning “to pass through,” likely describing the frequent urination characteristic of the condition in the 1st to 2nd century AD. In 1675, Thomas Willis added “mellitus” to the term “diabetes,” signifying the sweet taste of the urine and blood of patients. Claude Bernard, in 1875, proposed a hypothesis that diabetes results from excess glucose production. This was a significant step toward understanding the metabolic basis of the disease. Von Mering and Minkowski 1889 discovered the role of the pancreas in diabetes by observing that removal of the pancreas in dogs led to symptoms resembling diabetes. This highlighted the importance of the pancreas in regulating blood sugar levels. One of the significant milestones in the history of diabetes was isolation of insulin from the pancreas by Frederick Banting and Charles Best in 1921. This discovery revolutionized the treatment of diabetes, allowing patients to manage their condition with exogenous insulin injections [1], [2], [3].

Diabetes is a prevalent metabolic disorder that can be classified majorly into two types, mainly Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D) [4]. T2D is the most common, accounting for approximately 90% of all diabetes cases, whereas T1D accounts for 5-10% [5], [6]. As per the 10th edition of the International Diabetes Federation, around 537 million adults are diagnosed with diabetes globally. Projections indicate that this figure is predicted to escalate to 783 by 2045 [7]. T1D is primarily caused by cellular-mediated autoimmune destruction of pancreatic β-cells. In this condition, autoreactive T-cells target and destroy these cells, leading to insulin deficiency [8]. Conversely, T2D is a complicated metabolic disorder characterized by a progressive decline in insulin secretion coupled with insulin resistance [9], [10].

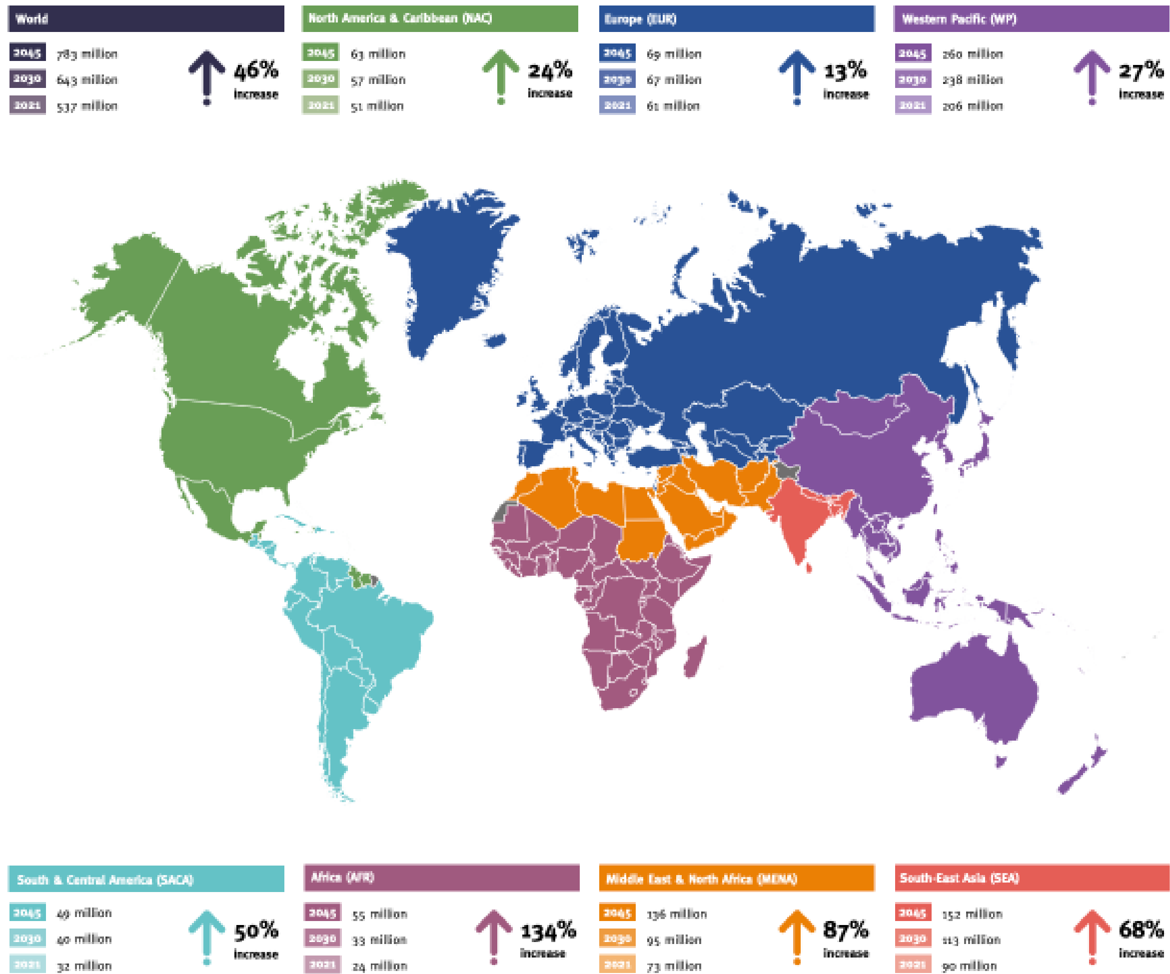

Figure 1: Diabetes statistics around the world. 10th edition of the International Diabetes Federation highlights that diabetes predominantly impacts low and middle-income countries (From [7]).

The leading cause of diabetes is the insufficient production of insulin by pancreatic β-cells, which leads to elevated blood sugar levels. Environmental and genetic factors work together to impact this development [11]. $CD4^+$ and $CD8^+$ T cells are essential for the immunological response in T1D, which destroys β-cells. $CD4^+$ T cells release immune signals, whereas $CD8^+$ T cells directly attack and kill β-cells [12], [13]. The immunological response results in a considerable reduction in β-cell mass, leading to decreased insulin production. Although genetic predisposition and environmental triggers, among other factors, contribute to the onset of T1D, the exact causes are not fully understood. [8], [14], [15], [16]. Insulin resistance is a common trigger for T2D; in response, the pancreas increases insulin production to maintain glucose homeostasis.

Eventually, β-cells become exhausted and unable to meet the increased insulin demand, leading to β-cell destruction [17]. The progression of insulin resistance and T2D involves multifactorial factors, including genetics, lifestyle choices, and environmental influences such as obesity and physical inactivity [9], [10], [11], [18].

The relationship between insulin resistance and β-cell failure is complex and needs to be fully understood. However, it appears that chronic insulin resistance and the subsequent increased demand for insulin put significant strain on β-cells, ultimately leading to their dysfunction and eventual destruction [16], [19]. Insulin therapy is indeed a crucial treatment for individuals with diabetes because it helps manage blood glucose levels and prevents acute complications. However, it is essential to note that insulin therapy is not a cure for diabetes [20], [21]. It addresses the symptoms by supplementing the body with insulin but does not address the underlying cause of the condition [22]. Moreover, insulin therapy alone may not prevent long-term complications associated with persistently elevated blood glucose levels. These can include damage to the small blood vessels, resulting in an elevated risk of macrovascular disease and stroke, as well as ailments like retinopathy, nephropathy, and neuropathy. In addition, insulin treatment can indeed be complex and demanding [23], [24], [25].

New directions for β-cell replacement treatment have been made possible by the discoveries of pluripotent embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) [25], [26], offering potential treatment options for diabetes, particularly T1D. ESCs and iPSCs can differentiate into various cell types, including insulin-producing β-cells, making them promising candidates for generating renewable sources of functional β-cells for transplantation [27], [28]. Through directed differentiation protocols, ESCs or iPSCs can be guided to develop into β-cells in large quantities, offering a potential solution to the shortage of donor β-cells. This approach holds particular promise for T1D, where β-cell destruction is a crucial feature. An advantage of using iPSCs is their ability to be derived from an individual's cells, such as skin cells. This personalized approach reduces the risk of immune rejection after transplantation because the transplanted cells are genetically matched to the recipient, potentially eliminating the need for lifelong immunosuppression [29], [30]. Despite the promise of ESCs and iPSCs for β-cell replacement therapy, several challenges must

be addressed. These include ensuring the differentiated cells' effectiveness and safety, enhancing the differentiation process's efficiency, and devising strategies to protect transplanted cells from immune attack [31], [32].

Islet transplantation therapy is a current diabetes treatment that involves the transplantation of insulin-producing islets into the liver via the hepatic portal vein [33], [34], [35]. However, although this method has shown promise, the hepatic environment may not provide an optimal diabetic milieu for the long-term function and survival of transplanted islets [36], [37]. To address this limitation, exploring the development of biomaterials and alternative transplantation sites could enhance the viability and long-term survival of transplanted insulin-producing cells. By providing a more supportive environment, these advancements aim to improve the effectiveness of islet transplantation therapies in the future [37], [38], [39]. Incretins are an essential area of research for the treatment of T2D and are under great attention and hype because they are used as anti-obesity drugs [40], [41], [42]. Drugs such as GLP-1 agonists and DPP-4 inhibitors have been used in diabetes therapy for many years. GLP-1 agonists reproduce GLP-1's effect of stimulating insulin release response to glucose consumption. DPP-4 inhibitors, on the other hand, prevent the breakdown of GLP-1, thus prolonging its activity [43], [44], [45], [46], [47].

1.2.1. Pancreas Development

The pancreas is a complex organ with two compartments: the endocrine contains the islets of Langerhans, which secrete insulin to regulate glucose homeostasis, and the exocrine, which comprises acinar and ductal cells that produce digestive enzymes [48]. The endocrine cell types are: α, β, δ, ε, and PP- (Pancreatic polypeptide) cells that secrete insulin, glucagon, somatostatin, and ghrelin, respectively [49], [50], [51].

Primary and secondary transitions are two stages of the developing pancreas in the embryo. The first transition occurs between E9.5 and E12.5 days. It is distinguished by the proliferation of multipotent pancreatic progenitor cells (MPC) and morphogenic alterations in the epithelium that result in the formation of a continuous tubular network [52], [53], [54]. Specification and maintenance of MPCs in the pancreas require several transcription factors, including Pdx1, Ptf1a, Mnx1, Sox9, and HNFβ [55], [56], [57].

MPCs produce all pancreatic cell types [58], [59], [60], [61]. It has been shown that regulating the proliferation and maintenance of MPCs in the early stages of the pancreas is crucial in determining organ size by assessing the number of mature pancreatic cells [52]. Due to these considerations, a strict framework is needed to regulate MPC differentiation and proliferation. Experimental evidence from mice models deficient in Notch components has shown that the MPC pool is diminished during early endocrine development [62], [63], [64]. Moreover, inhibiting Notch signaling in MPCs through pharmacological compounds has resulted in abnormalities in epithelial branching and a reduced pancreas size [65].

The secondary transition happens between E12.5 and E16.5 [57]. During this phase, the pancreatic epithelium branches out differentiate and designates a pancreatic cell lineage [66]. MPCs near the branching epithelium's tip are restricted to the exocrine cell fate after E13. These cells can self-renew or differentiate into acinar progenitor cells by E13.5. Between E13.5 and E15.5, the epithelial stem fully differentiated into α and β-cells [53]. Early in development, acinar and ductal cells share certain transcription factors that control their differentiation and maturation [52]. Somatostatin-expressing cells appear the following day, and endocrine cells group into tiny aggregates between E14.5 and E18.5 [53], [57]. Eventually, exocrine and endocrine gene expression increases and thus is accompanied by a marked increase in cellular mass [49], [50], [66].

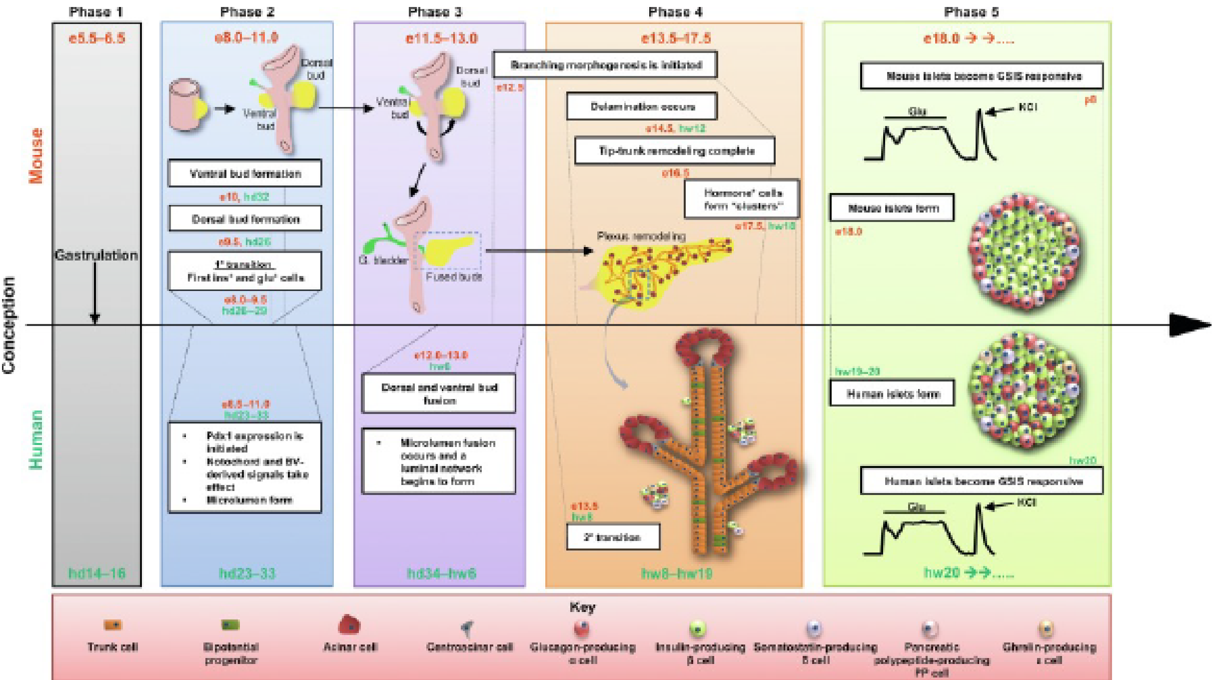

Figure 2: Timeline of mouse and human pancreatic development. During the five phases of pancreatic development in mice and humans, clusters of hormone-producing cells gradually emerge. They ultimately emerge into islets by E18 in mice and around week 20 in humans at the last phase (From [53]).

1.2.2. β-cell differentiation

As pancreatic cells differentiate from a multipotent precursor cell line to full maturity [67], [68]. By secreting hormones into the bloodstream in a balanced manner, pancreatic β-cells are intricate endocrine micro-organs vital to maintaining glucose homeostasis [69]. Pancreatic β-cells produce insulin, the primary hormone that lowers blood sugar [70]. Changes in β-cell function and insulin secretion perturb the balance of glucose, leading to diabetes if insulin secretion is insufficient or prolonged hypoglycemia when insulin is secreted in excess [71]. Neogenesis, the process of differentiation from ductal precursor cells to mature β-cells, and replication of differentiated cells are the two processes that remain intact during the embryonic development and postnatal stage [72]. During the postnatal stage, β-cell differentiation occurs by activating a distinct transcriptional network that maintains the expression of genes specific to β-cells.

The helix-loop-helix transcription factor Ngn3 originates from bipotent pancreatic progenitors at the secondary transition stage and drives the endocrine specification [60], [73]. Ngn3, a master regulator of pancreatic islet development, progressively starts differentiating endocrine cell migration and determining cell destiny. The Hippo terminal regulator YAP transcriptional activity is directly suppressed by Ngn3 [60], [74], [75], [76]. During development, the size of an organ is determined by the regulation of Hippo effectors, YAP, by all mechanical stimuli. This indicates that YAP is essential for the formation of β-cell; it is the primary focus of this thesis.

Postnatally, β-cells have lost their proliferative capacity. Because of the inverse correlation between replication and specialization [77], adult β-cells appear quiescent for most of their lives. This makes it difficult to functionally replenish lost or dysfunctional β-cells when needed [6], [10]. A proportion of adult β-cells in mice can be induced to increase because of the demands placed on them by obesity or

pregnancy. A small percentage of these cells have been observed to form by trans-differentiation of α-cells, δ-cells, and even exocrine cells to β-cells [78]. Many of these adult proliferative cells are referred to as "virgin" β-cells, accounting for ~1.5% of the total β-cell population [79]. Neogenesis is the process by which new β-cells are generated from pre-existing stem/progenitor cells. Virgin β-cells lack essential markers for the maturation of β-cells, such as Ucn3, G6pc2, and MafA [79], [80]. They are found in a neogenic niche at the periphery of the islets. However, recent studies have shown that these cells are also found throughout the islet cross-section [81], [82]. It appears that they retain a certain degree of stem cell quality.

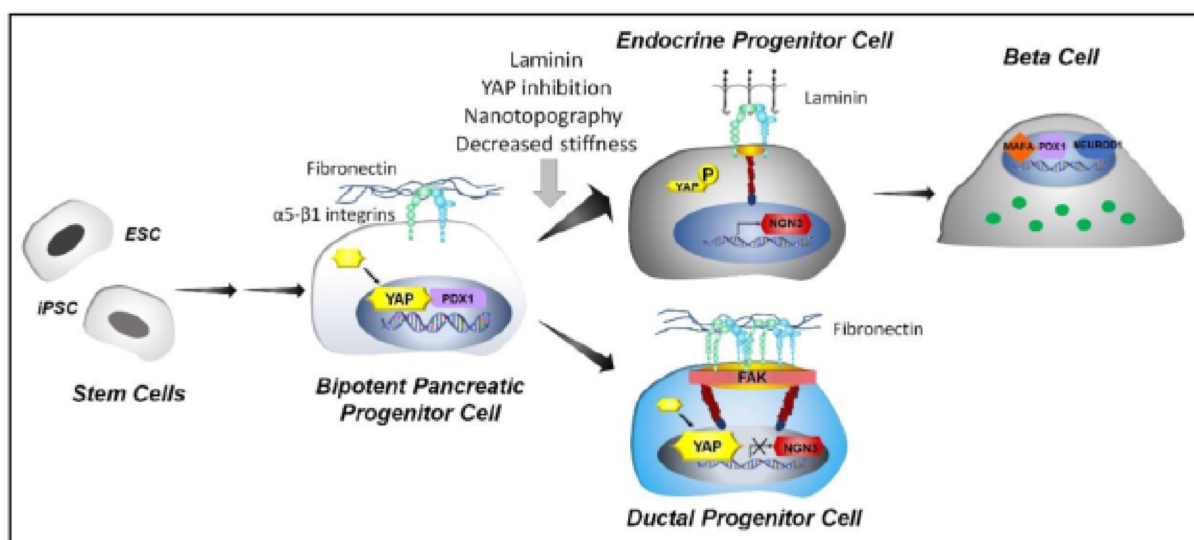

Figure 3: YAP signaling regulates β-cell differentiation. YAP greatly influences bipotent pancreatic progenitor cell fate. When YAP is activated, and Ngn3 is inhibited, it promotes progenitor cell development towards ductal cells and blunt endocrinogenesis. Conversely, Ngn3 expression promotes differentiation towards an endocrine cell fate characterized by transcription factors for insulin, Pdx1, NeuroD1, and MafA when YAP is suppressed (From [67]).

1.2.3. β-cell dedifferentiation

One of the leading causes of β-cell dedifferentiation in diabetes is the loss of functioning β-cells. Dedifferentiation entails downregulating β-cell genes, such as insulin, glucose metabolism, secretory pathway genes, and so on [83], [84]. Simultaneously, genes that are suppressed or expressed at low levels in adult β-cells

are upregulated, which are β-cell disallowed genes (YAP is one of those genes) [85], [86], and potentially progenitor cell genes [87]. These alterations finally cause an insulin secretion deficiency and phenotypic reconfiguration of the β-cells [88]. While glucotoxicity in β-cell dedifferentiation is mainly caused by oxidative stress, ER stress, inflammation, and hypoxia [89], [90] β-cell impairment may also be caused by the activation of disallowed genes. Theoretically, these differences in gene expression could result from altered transcription rates, changes in the repertoire of transcription factors, or epigenetic modifications [91], [92]. β-cells dedifferentiated and reverted to a state condition resembling that of progenitor cells in response to hyperglycemia [93], [94], [95], [96]. Moreover, β-cells can develop into various endocrine cells, including α-like cells that produce glucagon, which may account for the hyperglucagonemia linked to diabetes [94], [97], [87], [92].

In T2D, β-cells undergo dedifferentiation or trans-differentiation [87], [98], [99], [100]. Recent studies reported that β-cell dedifferentiation in T2D is relatively slight, meaning it cannot account for the β-cell deficiency on its own [101]. Only a cross-sectional characterization of human T2D has been studied [87], [102]. Consequently, it is challenging to conclude that the loss of β-cell mass associated with T2D is not due to β-cell dedifferentiation [87], [91]. β-cells revert to progenitor-like cells with L-Myc, Nanog, Pou5f1, and Ngn3 expression. Essential mature-cell genes like Nkx6.1, Pdx1, Slc2a2, and Ins2 are all correlated with decreased expression in this context [93] and upregulation of β-cell repressed genes such as Hexokinases I-III, ALDH1A3, and LDHA [103]. Foxo1 appears crucial in controlling this identity loss, ultimately leading to cells' inability to secrete insulin [83], [104]. A related investigation demonstrated that hypoxia contributes to β-cell dedifferentiation by promoting the expression of mature β--cell-restricted genes in human islets, including ALDH1A3 and LDHA [105].

ALDH1A3 is an alternate marker of progenitor cells in failing pancreatic islets and a marker for β-cell dedifferentiation in rodents and human T2D [106]. Above all, whether ALDH1A3 activity causes β-cell dysfunction is raised by the substantial negative connection between ALDH1A3 expression and β-cell function [103]. Whether ALDH1A3 activity plays a role in β-cell failure, which leads to chronic diabetes, is yet unknown [105], [107]. Moreover, viral infection and inflammation exacerbate β-cell

dedifferentiation. For example, IL-1β can decrease the expression of functional genes of β-cells in both mouse and human islets and increase the expression of progenitor cell marker genes [107], [108]. Pancreatic cancer tissues show strong immunoreactivities for ALDH1A3 [109], supporting the knowledge that cancer is associated with the proliferation of dedifferentiated cells.

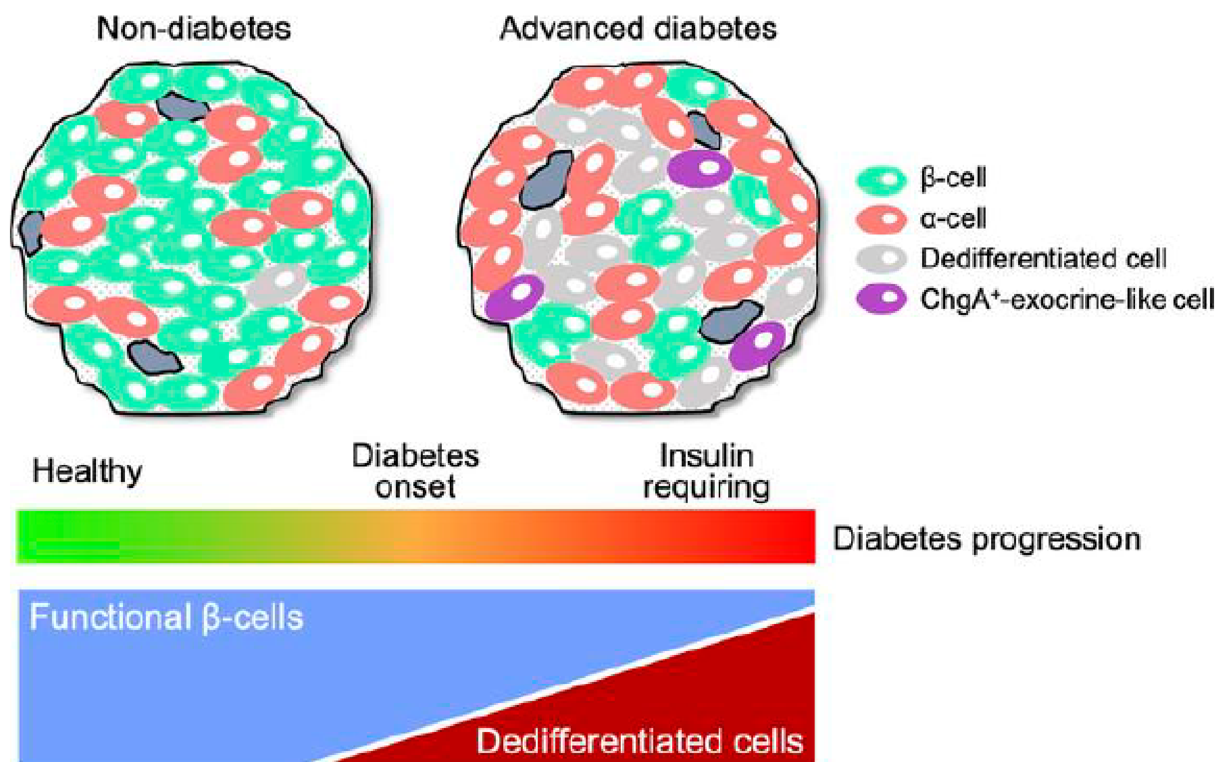

Figure 4: β-Cell dedifferentiation is a crucial feature of diabetes. Dedifferentiation is one mechanism of β-cell failure, characterized by losing cell identity and differentiated state. β-cells can undergo reprogramming into a different cell type or revert to a progenitor-like state (From [110]).

1.2.4. β-cell regeneration therapy

Regenerating or replacing the pancreatic β-cells that produce insulin is the primary goal of β-cell regeneration [111]. This is particularly important in diabetes, where the βcells malfunction or are destroyed [112]. Increasing β-cell mass through endogenous expansion is one of the main areas of diabetes research [113]. This can be achieved by promoting the replication of existing β-cells or by recruiting new β-cells through processes such as neogenesis (formation of new β-cells) or trans-differentiation

(converting of other cell types into β-cells) (Fig. 5) [113], [114]. Knowing the signaling pathways and other components that support β-cell regeneration is the primary goal of this research, as well as developing therapeutic strategies to enhance the β-cell mass in individuals with diabetes.

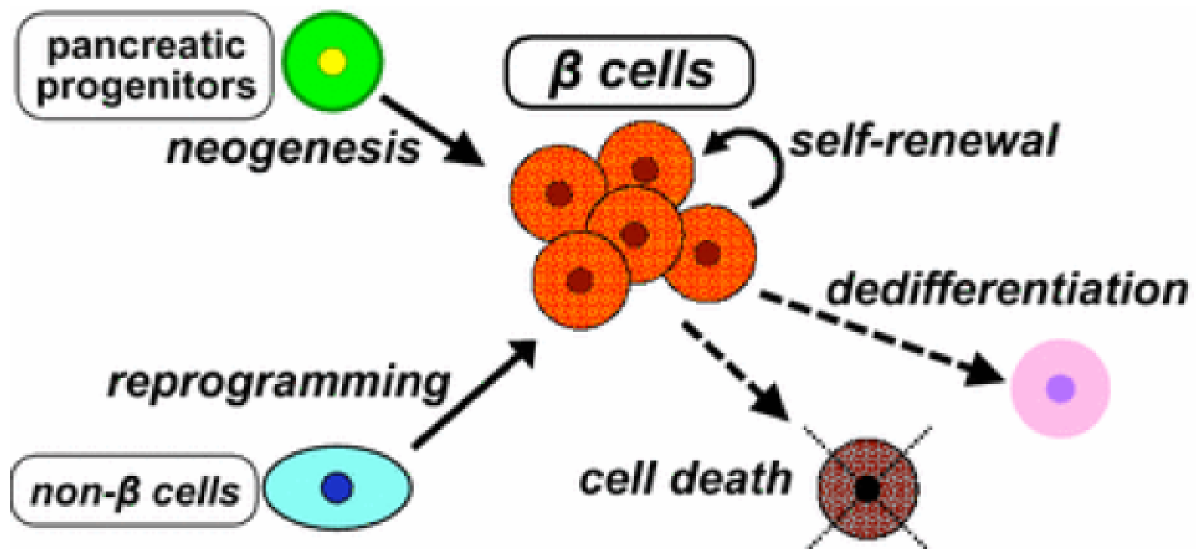

Figure 5: Strategies for β-cell regeneration: Several methods are expected to increase β-cell mass. Solid arrows signify approaches that promote β-cell mass, whereas dashed lines represent adverse effects that may result in the depletion of β-cells (From [115]).

One strategy for regeneration is the induction of β-cell neogenesis from progenitor cells. The cell of origin in neogenesis, especially in regenerating pancreatic β-cells, has been controversial [116]. Many reports suggest that the cell of origin is located within the pancreatic duct epithelium or in its vicinity [117], [118], [119]. However, the specific nature of this cell remains a subject of investigation. It could be a simple stem cell, a differentiated duct epithelial cell capable of re-differentiation into various cell types, or a different kind of cell altogether [120]. If it is a duct cell, it remains uncertain whether all duct cells can regenerate or if only a specific subpopulation possesses this ability, which remains to be investigated [121]. One study has shown that Ngn3 is activated in cells inside or next to the ducts [122]. The triggering mechanism of β-cell neogenesis is obscure and contentious, and the rate of $Ngn3^+$ cell production could be

higher. Consequently, a method for regenerating functional β-cells is represented by better comprehending this process [123], [124].

β-cell replication, the process of β-cells dividing to form new cells, occurs in humans but at a low rate of ~2% [125]. This replication activity is limited to the early years of life. As a person ages, the replication capacity of β-cells declines significantly. Despite numerous attempts, adult human β-cells to replicate through pharmacological attempts have been unsuccessful [126]. The fact that β-cells still exist in individuals with both T1D and T2D suggests the potential for β-cell mass restoration if drugs could be developed to induce replication [127], [128], [129]. To promote β-cell regeneration pharmacologically, comprehending the molecular pathways via which regular physiological inputs drive β-cell proliferation is crucial. Various growth factors have been implicated in the replication control [130].

For instance, prolactin is involved in β-cell hyperplasia during pregnancy, and gastrin has also been linked to β-cell replication since β-cell hyperplasia occurs adjacent to gastrinomas [128], [131]. The SGLT2 inhibitor dapagliflozin may have various direct protective effects on β-cells and its glucose-lowering effect [132], [133]. Treatment of diabetic mice with dapagliflozin promotes several processes that contribute to the regeneration of β-cells, promotes β-cell self-replication, induces α-cell to β-cell conversion, and activates endocrine progenitors and stimulates these cells to differentiate to β-cells [134], [135]. Moreover, dapagliflozin regulates the expression of pancreatic endocrine markers and induces α-cells to secrete GLP-1, which may be one of the mechanisms of β-cell regeneration [126], [136], [137], [138]. There may be a relationship between glucagon and glucagon-like peptide-1 and α- to β-cell trans-differentiation [139].

Acinar ductal trans-differentiation into functional β-cells is another strategy for inducing β-cell regeneration. Digestive enzymes secreting acinar cells can develop into ductal cells [140]. When acinar cells experience damage or re-express developmental transcription factors (Ngn3, Pdx1, Glis3, and MafA), they demonstrate their ability to reprogram and acquire β-cell features [141], [142], [143]. One specific example of cell trans-differentiation is the ability of glucagon-secreting α-cells to differentiate into

insulin-secreting β-cells [144], [145]. Genetic lineage tracking with a glucagon-TetO system revealed that α-cells converted into β-cells, for instance, employing diphtheria toxin-induced diabetes in mice in which the insulin promoter was conjugated with the diphtheria toxin receptor [146], [147], [148], [149]. Somatostatin-producing α-cells can differentiate into insulin-producing cells; this process occurs in the juvenile stage and is connected to the Foxo1 network [150]. On the other hand, Pdx1, Nkx2.2, and Foxo1 caused the β-cells to dedifferentiate into α-cells [148], [151].

Before regenerative approaches can be applied clinically, the regulatory mechanisms for switching on regenerative, which are also pro-oncogenic pathways, must be thoroughly investigated. Below are some examples of potent pharmacological strategies (Table 1):

Compound	Mechanism of action	Effect on β-cell Proliferation	Other effects	Specificity
Harmine	It inhibits DYRK1A, MAOs, and CLKs and reduces the abundance of SMAD protein [152], [153].	Increase β-cell proliferation rate by ~3.5% in human islets [152].	Mice transplanted with human islets showed increased β-cell differentiation markers (PDX1, NKX6.1, and MAFA) and enhanced glucose tolerance [154].	Mitogenetic effects were not specific to β-cells [153], [155], [156].
5-IT	Inhibition of DYRK1A and adenosine kinase [152].	Proliferation effect ~10 fold more potent than Harmine [152].	In human islets, elevated GSIS, raised DLC1A2 expression, and C-peptide and insulin levels in mice transplanted with human islets [155].	Mitogenetic effects were not specific to β-cells [155].
Harmine + TGFβ inhibitors	Modulation of cell cycle activators and repression of cell cycle inhibitors [157].	Increase β-cell proliferation rate by 5-8% in human islets, ~5% in T2D donors, and ~2% in mouse *in vivo* [157].	Increased PDX, NKX6.1, MAFA. MAFB, SLC2A2, and PCSK1 expression in human islets and T2D donors [157].	Mitogenetic effects were not specific to β-cells [157].
Harmine + GLP-1	DYRK1A inhibition and GLP-1 action	Increase β-cell proliferation rate ~5% in	Increased expression of PDX1, NKX6.1, MAFB, SLC2A2,	The GLP-1 family is relatively

	on cAMP (PKA and EPAC2 pathways) [138], [156].	human islets, 1.1% in mice transplanted with human islets [138].	PCSK1, and GLP1R in T2D human islets donors. Enhanced blood glucose and circulating insulin in mice transplanted with human islets [138].	specific β-cell [138].
GNF-9228	Unknown.	Increased EdU incorporation by ~10 fold in human islets [158].	Improved insulin secretion in human islets, of 75-140%. In a rat β-cell line, protected cells against cytotoxicity (ER stress-induced by of IL-1β and IFN-γ) [158].	
SKP2	Reduce p27kip1 expression, enhance cdk6 and cyclin D3, and stimulate Myc monoubiquitination [159].	In β-cell from T2D donors overexpressing SKP2, the proliferation response was 8.1 ±1.0% [159].		

Table 1: Compounds are found to promote the growth and regeneration of human islets (Adapted from [160]).

1.3.1. The Hippo signaling pathway in pancreas development

A wide range of sophisticated molecular signaling pathways are involved in a complicated process of pancreas development [62], [161]. The Hippo pathway has emerged as a critical regulator of cell proliferation, differentiation, and organ size control [162]. In recent years, research efforts have focused on unraveling the intricate role of the Hippo pathway in pancreas development, shedding light on its significance in both normal organogenesis and disease pathogenesis. The pancreas requires continuous maintenance and regeneration throughout life to ensure proper function [163], [164]. The balance between cell division, apoptosis, and proliferation in response to multiple signals and stresses is regulated by the Hippo pathway, which helps maintain tissue homeostasis [165], [166].

The Hippo signaling pathway is a highly conserved signaling cascade found in many organisms, from fruit flies to humans. Initially identified in Drosophila melanogaster, it was named after the enlarged organs observed in mutant flies, resembling a hippopotamus [167], [168]. Its roles are more intricate and context-specific in mammals, including tissue homeostasis regulation [161], [162]. This has significant implications for several developmental processes, including pancreas development, both in cell lineage differentiation and pancreatic morphogenesis [171], as well as the regeneration of the heart, liver, and intestine after damage [172], [173]. The major components of the Hippo pathway include several proteins, among which the key players are the kinases MST1/2, SAV1, LATS1/2, MOB1, and the transcriptional co-activators YAP and TAZ. These proteins function together to regulate the activity of transcription factors such as TEAD (TEA domain family members 1-4), which control the expression of genes involved in cell proliferation and survival [174], [175], [176], [177].

Cell-cell contact, mechanical cues, and various upstream signals from the cellular environment primarily regulate the Hippo signaling. For instance, cell polarity, cytoskeletal tension, and extracellular matrix stiffness can influence the activity of MST1/2 and LATS1/2 kinases, thereby modulating downstream events [178]. MST1/2 phosphorylates and activates LATS1/2, which phosphorylates and inhibits the transcriptional coactivators YAP/TAZ. Phosphorylated YAP/TAZ undergoes cytoplasmic sequestration or ubiquitin-mediated proteasomal degradation, which suppresses YAP/TAZ-mediated gene transcription [179], [180], [181]. YAP and TAZ are not phosphorylated when the Hippo pathway is inhibited, which allows them to translocate into the nucleus, interacting with TEAD to stimulate gene expression in proliferation, survival, and growth [181], [182] (Fig 6). The Hippo pathway intersects with various other signaling networks, including the Wnt, Notch, TGF-β, and Hedgehog pathways, forming a complex network that orchestrates tissue development, regeneration, and homeostasis [62], [183], [184], [185].

MST1/2 and LATS1/2 serve as the primary upstream kinases responsible for the phosphorylation, the most prevalent post-translational modification of YAP. Additionally, AMPK can directly phosphorylate specific residues on YAP, including S61 and S94 [186], [187], [188]. The Hippo target genes, including CTGF, CYR61,

ANKARD1, BIRC5, and AXL, are transcriptionally mediated by the YAP/TAZ in the nucleus [180], [189], [190]. Several studies have shown that the transcriptional activities of YAP and TAZ are significantly enhanced [191], [192], while LATS1/2 inactivation diminishes, indicating a robust promotion of their functions [193], [194]. Constitutive overexpression of the YAP results in accelerated proliferation and protection against apoptosis, and when combined with the negative regulation of MST1/2, MOB1, LATS1/2, and merlin, eventually leads to overgrowth across multiple tissues [195], [196], [197], [198]. This underscores the Hippo pathway's critical role as a regulator of organ size. Alterations in Hippo pathway components have been observed in cancer cells, where aberrant YAP/TAZ activity promotes tumor growth and metastasis. Moreover, perturbations in Hippo signaling have been linked to β-cell dysfunction and impaired regeneration in diabetes [199], [200], [201].

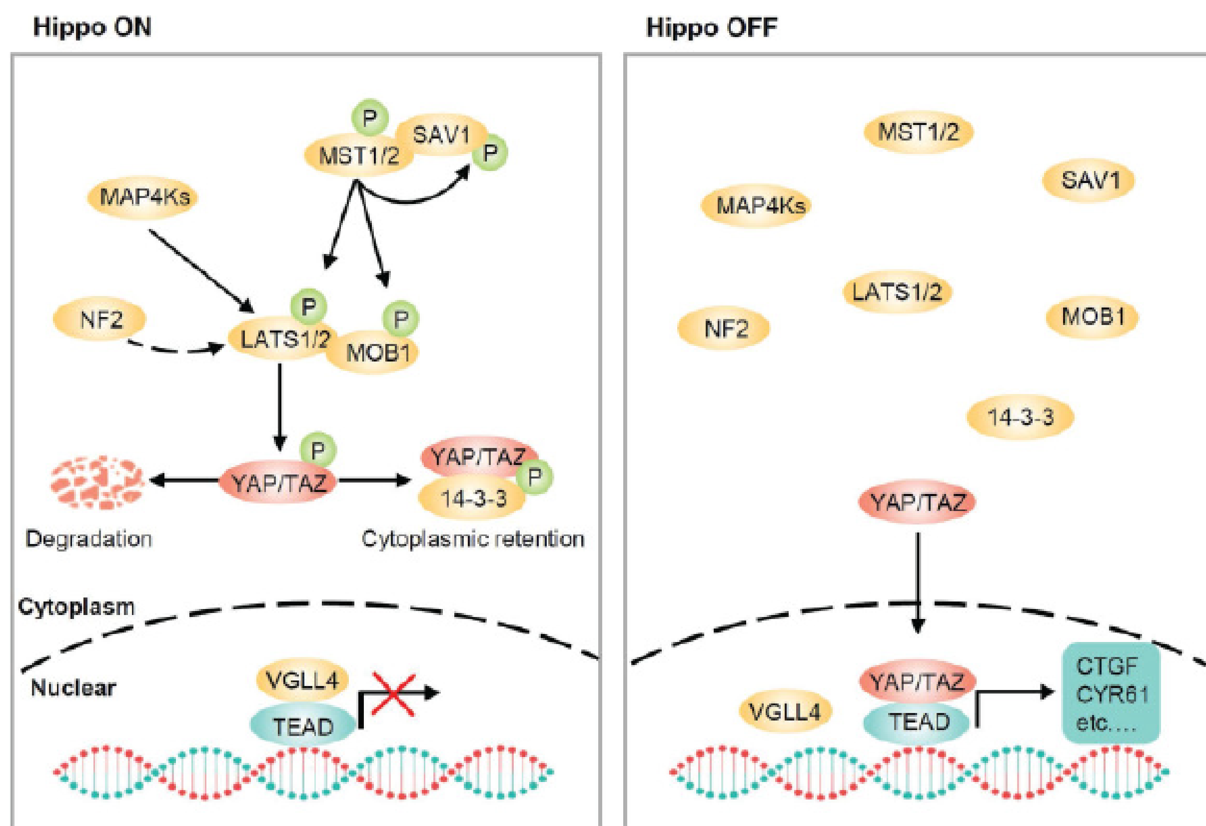

Figure 6: Hippo pathway core components. The Hippo pathway comprises MST1/2, LATS1/2, and YAP/TAZ. MST1/2 phosphorylates LATS1/2 and MOB1 in concert with SAV1 to initiate the Hippo pathway. Upon activation, LATS1/2 phosphorylates YAP and TAZ, which leads to their ubiquitin-mediated proteasomal destruction and 14-3-3-mediated sequestration in the cytoplasm. YAP/TAZ competes with VGLL4 for TEAD

binding when the Hippo pathway is deactivated, which in turn causes the transcription of transcription downstream target genes crucial for proliferation, growth, and survival (from [189], [202]).

1.3.2. β-cell proliferation and regeneration

The Hippo pathway is a significant player in pancreas development, particularly in governing stem and progenitor cells [203], [204]. It regulates pancreas development and cell fate specifications in humans and mice during early prenatal stages [205], [206]. In β-cells, the Hippo pathway exerts profound effects on proliferation dynamics. In recent years, its significance in the field of β-cell biology has garnered considerable attention. β-cells play a significant role in glucose homeostasis by secreting insulin, and understanding the mechanisms governing their proliferation and regeneration holds immense therapeutic potential for diabetes treatment. Several studies have revealed that genetic manipulation of critical components of this pathway influences β-cell mass and function. For instance, inactivation of MST1/2 or LATS1/2 promotes β-cell proliferation, highlighting the inhibitory role of the Hippo pathway in this context [129], [207]. Conversely, activation of YAP/TAZ enhances β-cell proliferation by modulating the expression of cell cycle regulators and anti-apoptotic factors [208], [209].

Interestingly, throughout pancreas development, YAP is initially expressed in pancreas progenitor cells. However, during cell type specifications, it is limited to exocrine cells and subsequently disappears in endocrine cells following differentiation [204], [210]. Constitutively active YAP (YAPS127A) has been re-expressed in human β-cells to investigate how YAP can promote proliferation. It maintained the identity of genes and insulin secretory function while significantly fostering proliferation [209], [211]. Remarkably, YAP-positive β-cells were the most replicating ones, indicating that YAP primarily acts on cells independently [209]. It is important to remember that growth factors released near islets or the proteins activated by YAP expression could potentially, via paracrine processes, aid in the growth of YAP-negative cells. In human islets reconstructed YAP, there is a significant upregulation of CTGF, a known target of the Hippo-YAP pathway [212], [213]. Furthermore, CTGF overexpression during pancreatic development enhances β-cell proliferation and mass, leading to

regeneration [214], [215]. CTGF is also necessary for proliferation during embryogenesis. [216], [217].

Hyperactivation of YAP, induced by the deletion of MST1 and MST2 proteins in the pancreas, triggers enhanced proliferation of acinar and ductal cells [218], [219]. MST1/2 loss of function mice have a disorganized, highly infiltrated pancreas with immune cells and are smaller than normal mice [197]. Research on mice has demonstrated that genetically blocking MST1 or both MST1/2 alone is not enough to stimulate the proliferation of β-cells, most likely because the Hippo effector YAP is missing [86]. Nonetheless, in mouse models of T1D and T2D, deletion of MST1 has demonstrated therapeutic potential through stimulating hyperplasia and restoring β-cell function [220]. Inactivation of LATS1/2 by various components leads to YAP-mediated transcriptional activity, which promotes β-cell proliferation and survival, ultimately leading to regeneration [221], [222].

These findings suggest that during the formation of the mouse pancreas, there is a context-dependent and cell-type-specific response [215], [216]. They also demonstrate how crucial the Hippo pathway, precisely its control over YAP, is to the pancreas's development [223]. In conclusion, these results emphasize the critical role of the Hippo pathway, particularly in regulating YAP and pancreas development. While the Hippo pathway has been extensively studied in multiple tissues, its significance on β-cell proliferation and regeneration remains unclear [224]. Pancreatic β-cells typically exhibit quiescence, with limited capacity for replication and regeneration, particularly with advancing age. A comprehensive understanding of the regulation of β-cell proliferation and regeneration is not well-studied in terms of proteins, complexes, and signaling pathways [225].

1.3.3. β-cell survival

Apoptosis of β-cells stands as a significant contributor to diabetes pathogenesis. Recent studies unveiled the Hippo pathway's profound influence on β-cell survival. Under stress conditions, such as oxidative damage or inflammatory conditions, activation of the Hippo pathway promotes β-cell apoptosis through downstream effectors like pro-apoptotic Bcl-2 family proteins and p73 [221], [226]. Conversely,

inhibition of Hippo signaling enhances β-cell survival by suppressing apoptotic pathways and promoting expression of anti-apoptotic factors. Research on the Hippo pathway components, including Merlin, MST1, LATS2, and YAP, has revealed their significance in regulating β-cell apoptosis in response to stress and metabolic demand, both *in vivo* and *in vitro* [209], [220], [227], [228], [229].

Among these components, MST1 is a key kinase, an essential apoptotic agent in response to diabetic stimuli. MST1 is highly activated in β-cells in many diabetic models, including human and mouse islets, pancreatic postmortem sections from T2D patients, β-cell lines, and diabetic mice [220], [230], [231]. Overexpression of MST1 in both human and rodent β-cells prompts apoptosis and functional impairment [232]. Deleting MST1 from β-cells *in vivo* or its deficiency in human islets and β-cell lines *in vitro* protects β-cells from apoptosis, restoring function and normoglycemia [220], [233]. Furthermore, RASSF1, a protein associated with MST1, stimulates MST1 pro-apoptotic actions and is significantly elevated in diabetic mice [234], [235], suggesting its potential to modulate MST1 mediated β-cell death [220], [228], [236]. Similarly, defects or deletion of LATS2 in mice reduce β-cell apoptosis and promote the progression of diabetes [229], [237]. Merlin, a critical Hippo component, plays a crucial role in regulating β-cell survival. In human islets and INS-1E β-cells, Merlin's loss protects against apoptosis triggered by proinflammatory cytokines and glucose and lipid toxicity [227].

Recent research has emphasized the significance of the Hippo terminal effector YAP for β-cell survival. YAP functions as a signal that protects β-cells from apoptosis by giving them an innate resistance. Active YAP protects human islets and INS-1E β-cells against apoptosis after extended exposure to cytokines IL-1β and IFN-γ, as well as high glucose and palmitate [209], [227]. Alterations in the Hippo components considerably impact β-cell survival in humans and rodents, revealing a tightly controlled survival program that maintains β-cell homeostasis. The Hippo pathway, including MST1, LATS2, Merlin, and YAP, is crucial for controlling β-cell survival and function (Fig 7) [220], [227], [228], [238].

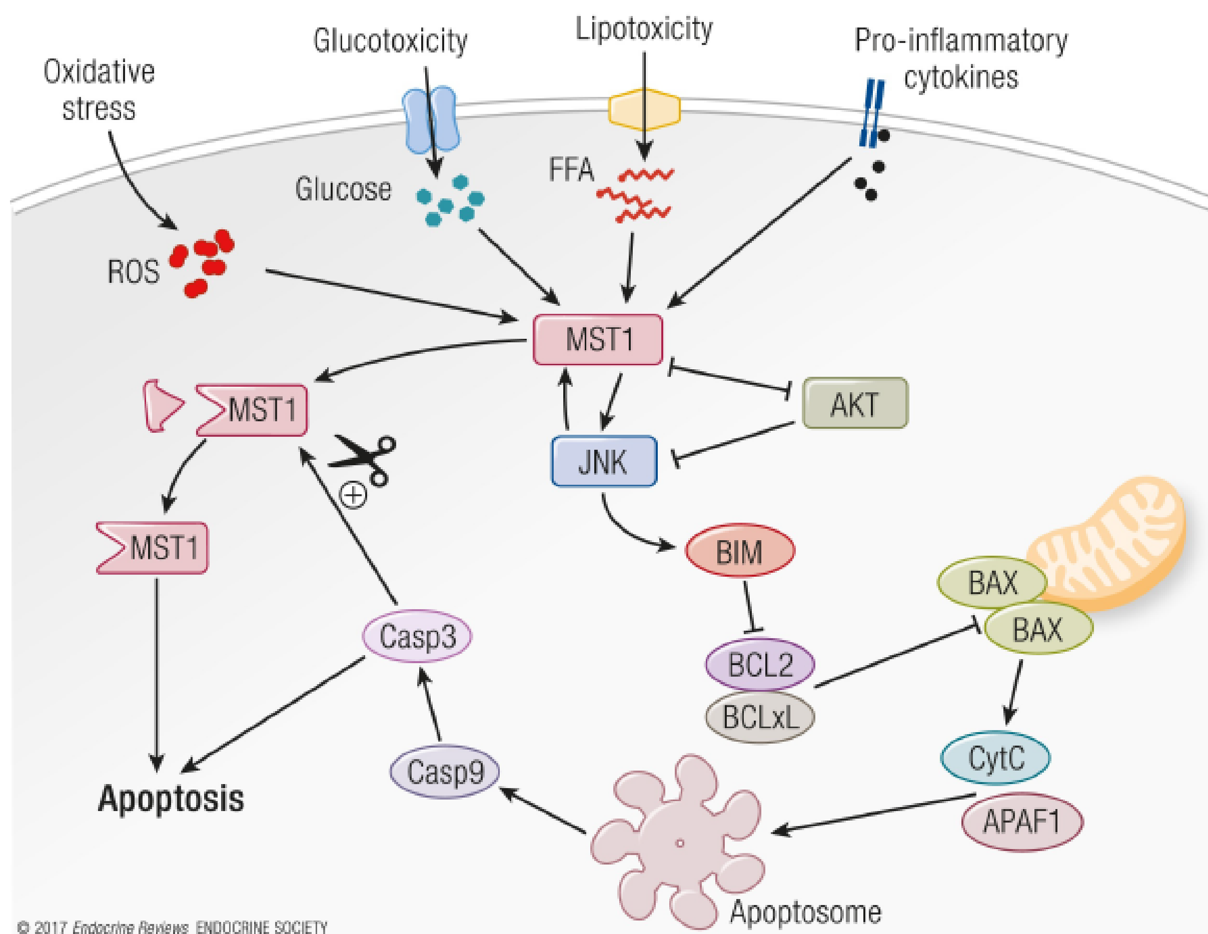

Figure 7: A mechanistic hypothesis explaining how β-cell survival is impacted by the Hippo kinase MST1. Diabetic stressors activate MST1 via unidentified upstream mechanisms by blocking the anti-apoptotic BCL-2 family of proteins BCL-2 and BCL-xL; MST1 upregulates the mitochondrial pro-apoptotic protein BIM, resulting in the BAX-dependent release of cytochrome C. Apoptosomes develop when cytochrome C binds to APAF1, cleaves the initiator caspase-9, and activates. Caspase-3 cleaves MST1 into constitutively active fragments, ultimately leading to β-cell apoptosis (from [188]).

1.3.4. YAP in β-cell development

Over the past twenty years, the Hippo pathway and its nuclear effectors YAP/TAZ have been discovered and characterized, leading to an unprecedented understanding of the links between islet size control, regeneration, and β-cell development [239]. Because YAP and TAZ lack a DNA binding domain, other transcription factors are required to control the expression of particular genes [169]. YAP and TAZ binding to the TEAD

regulate the expression of downstream genes involved in proliferation and tissue architecture [240]. Although YAP is repressed during β-cell differentiation [86], it has been demonstrated that YAP re-expression boosts replication of β-cells, supports their ability to respond to stress, and promotes anti-apoptotic response under diabetic conditions [241], [242]. The exact mechanisms YAP stimulates β-cell proliferation are still being researched. Re-expressing YAP in human islets upregulates its transcriptional partner TEAD [209], [243], [244].

In human islets, YAP overexpression strongly activates mTORC1 and promotes YAP-induced cell proliferation. The effects of this activation are somewhat offset by the mTOR inhibitor [245], [246]. In another pathway, YAP overexpression increases FOXM1 mRNA and protein levels. Inhibiting FOXM1 completely prevents YAP-induced β-cell replication [247]. FOXM1 is one of the known positive regulators of β-cell proliferation [248], and its abolition in the pancreas leads to diabetes due to a progressive decline in β-cell mass [249], [250]. The YAP/TEAD complex binds directly to FOXM1 and stimulates the expression of proliferative genes that encode positive cell cycle regulators [248].

YAP transcriptional partner TEAD1 is prominently expressed in β-cells, essential to its function. Disrupting TAED1 in β-cells leads to the progressive development of diabetes and hyperglycemia, highlighting the distinct, unique function of TEAD1 [251]. Conversely, PGC1-α, an essential regulator of gluconeogenesis, is effectively suppressed by YAP in the liver [252], [253], [254]. By inhibiting PGC1-α, YAP effectively reduces the gluconeogenesis in rodent models [255]. Because of this, enhancing YAP expression in the liver alone can suppress the activity of the hepatic gluconeogenic gene, resulting in reduced blood sugar levels and improved glucose tolerance [243], [246]. These findings highlight the critical role of YAP in modulating overall glucose metabolism.

1.3.5. Potential of YAP in the proliferation and regeneration

The absence of YAP expression in mature islets presents a barrier to targeted proliferation induction, as YAP plays a protective and promoting proliferation role in β-cell survival and proliferation [211]. MST1/2 inhibition promotes YAP re-expression,

preventing YAP degradation and supporting its nuclear localization [256]. It is essential to investigate the evolutionary processes and interacting molecules that cause YAP silencing in mature β-cells [210]. Comprehending these pathways can aid in creating complex pharmacological and molecular tactics to induce YAP expression [211] temporarily. The oncogenic potential of YAP should be noted [257]. Therefore, the design, dosing, and timing of YAP induction need to be carefully considered. While avoiding prolonged activation that could lead to the initiation of neoplastic growth, the level of YAP expression should be regarded as activating β-cell proliferation [258], [259], [260].

YAP prevents endogenous cells from undergoing apoptosis, which is essential for the survival of β-cells. Several studies have shown the constitutive expression of YAP to increase proliferation and repair in rodent models of T1D and T2D [108], [261]. Remarkably, MPCs exhibit significant expression of TEAD, a binding partner for YAP/TAZ [244], [262], [263]. It has been investigated that TEAD1, TEAD homolog, is essential to the transcription factor combinatorial enhancer model in MPCs [263], [264], [265]. TEAD1 is limited to MPCs and inactivates throughout pancreatic lineage differentiation, even though it does have YAP-dependent roles in early pancreatic development. In conclusion, YAP silencing in adult β-cell homeostasis is necessary. However, by fostering protection under various diabetic conditions, it may be able to transform quiescent cells into proliferative cells and preserve β-cell survival.

1.4.1. Rescue of the β-cell phenotype

To maintain normoglycemia, the production and secretion of insulin by pancreatic β-cells is carefully regulated. The maturity of β-cells is inversely correlated with their capacity for replication due to the separation of mature, glucose-sensing insulin production and replication potential [77], [266], with the overall function of β-cells deteriorating, leading to dysregulated insulin processing and release [267]. Therefore, extensive research is required to protect the viability of β-cells and to identify compounds that help to maintain the maturity and function of β-cells.

1.4.2. The ROCKII inhibitor H1152

Many chemical substances have been investigated for their potential to affect stem cell fates and to act at the initial phases of β-cell lineage specification to promote the differentiation of hESC into the ultimate endoderm and pancreatic progenitors [268], [269]. While numerous methods for glucose-responsive cells have been studied, the effectiveness of various hESC lines varies significantly. This is challenging, particularly given the ultimate origin of specific cells [270], [271]. H1152, a ROCKII inhibitor, increases the formation and development of β-cell-like structure during differentiation [272]. H1152 treatment promotes hESC-derived β-cell maturation and glucose homeostasis [273].

Most of the molecular processes control β-cell proliferation and maturation (Fig.8). The discovery of H1152 demonstrates that ROCK pathway regulation plays a role in some of the mechanisms. There is evidence that the loss of ROCKII but not ROCKI caused the modulation of β-cells [272], [274]. Multiple studies employing β-cell lines and mouse islets have consistently revealed that ROCK inhibitors augment GSIS. It has been suggested that the GLP-1-dependent ROCK signaling pathway is a protective mechanism against the stress caused by glucotoxicity [275], [276]. PDX1, NGN3, MAFA, PAX4, ROCK, and TGF1β inhibitors can resuscitate the human exocrine to secrete insulin [273], [277]. Furthermore, using WNT-conditioned media, ROCK inhibitors have been increased β-cell maturation [278]. However, the role of ROCK inhibition in controlling the maturation of β-cells and pancreatic development has not been studied.

Regardless of the absence of GSIS or proliferation of β-cells, ROCKII inhibition is essential for the growth and maturation of β-cells, as demonstrated by genetic and pharmacological approaches. Moreover, it unveils noteworthy molecular pathways that govern the development and maturation of β-cells [273], [278]. ROCK signaling can alter the integrity of self-renewal and survival competency by modulating YAP's dual role as proliferation and apoptosis [279], [280].

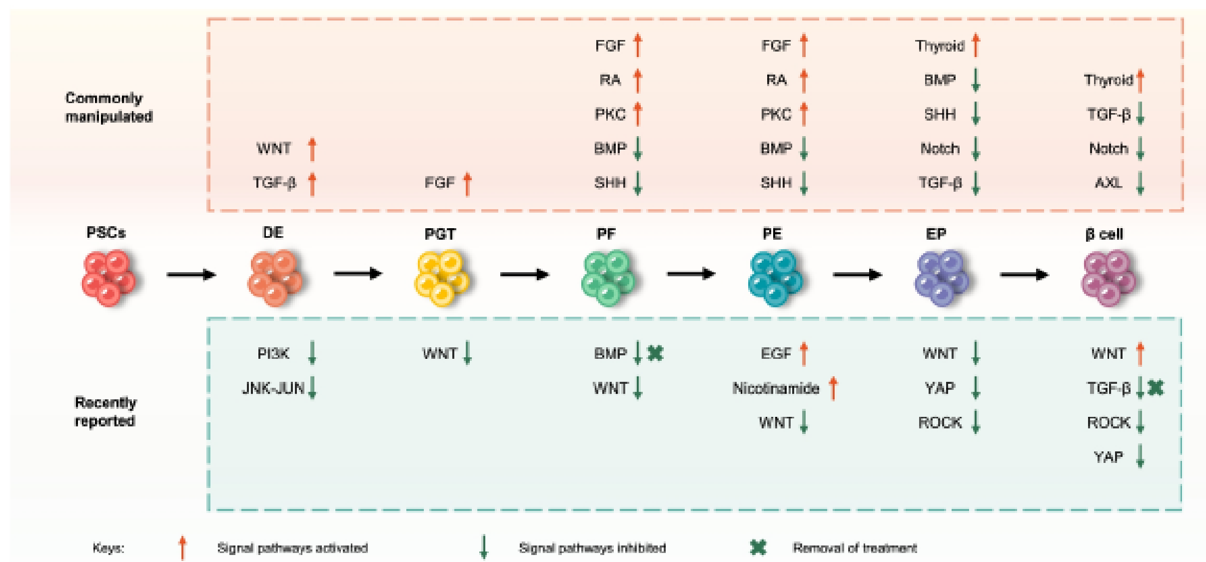

Figure 8: Human pluripotent stem cells turn into functional islets. The latest advances in effective differentiation are primarily directed toward exploring signaling pathways. The top represents frequently altered signaling pathways, whereas the bottom displays newly discovered chemical compounds or pathways to increase differentiation efficiency (From [281]).

1.5.1. Drugging the Hippo pathway as a strategy for β-cell regeneration

One possible therapeutic strategy for the treatment of diabetes is to stimulate the innate proliferation ability of the existing β-cells [262], [282]. The Hippo-YAP/TAZ-TEADs pathway has recently been shown to exert potent growth-regulatory activity in cardiomyocytes (CMs) [283], [284]. A large-scale cell-based chemical screen identified small molecule activators of TEADs. To enhance CM proliferation *in vitro* and *in vivo*, a fluoro substituent (called TT-10) derived from a bioactive compound (TAZ-12) was successfully developed [285], [286], which induced CM proliferation in both CMs after myocardial infarction and controls. In addition, TT-10 also prevented apoptosis by activating the transcription factor NRF2 in cultured CMs exposed to oxidative stress. Cardiac remodeling after myocardial infarction was improved by treatment with TT-10 [287]. This has been attributed to the proliferation of CMs and the direct antioxidant and apoptotic effects facilitated by TT-10 *in vivo* [285].

The effect of TT-10 treatment on cell cycle progression and apoptosis in cultured cardiomyocytes was shown to be mediated by the Hippo signaling pathway. The

TEAD-dependent activity was increased in cardiac myocytes without YAP or TAZ expression changes. Still, YAP nuclear levels were increased, and TAZ nuclear expression was not altered in cardiomyocytes [286]. Because the induction of proliferation by TT-10 was mainly mediated by Hippo-YAP signaling in cardiomyocytes, proliferation and anti-apoptosis were induced by YAP [209], [211]. These studies suggest TT-10 as a potential but unclear strategy to stimulate endogenous β-cell regeneration, as here, YAP is repressed.

The transcription factor TEAD promotes Hippo pathway activation, which drives the transcription of downstream genes, including cell cycle progression and cell proliferation throughout many tissues. However, its function in pancreatic β-cells is still unknown [288], [289], [290]. Previous research highlights the significance of Hippo signaling in maintaining tissue homeostasis and cell growth. It has been reported that the TEAD transcription network downstream of the Hippo pathway is down-regulated during differentiation and inhibits the proliferation of β-cells [167], [288]. TEAD plays a crucial role in maintaining glucose homeostasis in adult β-cells by coordinating proliferation, identity, and functional competence. Modulating this reciprocal regulation may help overcome the obstacles to induce β-cell proliferation without loss of function.

1.6.1. YAP's role in inflammation during diabetes pathophysiology

The Hippo pathway involves many inflammation responses, including antibacterial, antiviral, and macrophage polarization. Multiple Hippo-YAP interacting components regulate the innate immune response, whereas the activation of innate immune signaling can also modulate the Hippo-YAP pathway [291], [292]. Clinical cases have reported associations between deficiencies in Hippo signaling and immunodeficiency symptoms, such as recurrent bacterial and viral infections, indicating the critical role of this pathway in immune modulation [293], [294]. It is important to note that an overall understanding of the reciprocal regulation between the Hippo-YAP pathway and the innate immune response needs to be improved.

By regulating the macrophage response after myocardial infarction (MI) injury, YAP/TAZ controls the pro-inflammatory response to MI in the heart [295]. Overexpression of YAP in mice increases pro-inflammatory macrophage polarization.

This leads to proinflammatory cytokine secretion, collagen synthesis, cardiac dysfunction, and fibrosis [295]. Similarly, fibroblast-specific overexpression of YAP/TAZ increases pro-inflammatory cytokines and cardiac fibrosis, whereas the gain of function of YAP decreases it [295], [296]. Studies in mouse and human patients have demonstrated a pro-inflammatory role of YAP/TAZ in liver disease. Overexpression of YAP in hepatocytes promotes the infiltration of immune cells into the liver following fibrosis. Hepatocyte activation of TAZ is associated with fibrosis in non-alcoholic steatohepatitis (NASH) [297], [298]. YAP activation in resident hepatic macrophages or Kupffer cells causes inflammation, and the production of inflammatory cytokines in the liver of mice fed a high-fat diet [299]. Evidence suggests that upregulated YAP expression is involved in developing inflammatory diseases [300], [301].

The Hippo-YAP/TAZ pathway involves many metabolic processes, including glycolysis, gluconeogenesis, fatty acid accumulation, and amino acid metabolism [302]. YAP has been the subject of increasing research interest, with some studies reporting that the renal cortex of diabetic mice has increased phosphorylation and expression of the YAP [303]. Recent studies in patients with diabetic kidney disease have also shown that the expression of YAP is upregulated and that of TAZ is down-regulated [251], [304]. Based on these findings, YAP expression has been one of the most critical research targets for understanding the progression of diabetes and its treatment. In this thesis, I explored the effects of YAP overexpression on postnatal stage pancreatic β-cells *in vivo*.

Aim of the thesis

Diabetes is characterized primarily by the dysfunction of β-cells caused by increased β-cell apoptosis and dedifferentiation. YAP is strongly expressed during the early stages of pancreatic development, where it promotes the proliferation and expansion of multipotent pancreatic progenitor cells and regulates the generation of viable β-cells. Our previous studies demonstrated that reintroducing YAP into human pancreatic islet cells robustly induces β-cell proliferation and protects against apoptosis without altering functional cells' identity genes.

The main aim of this study was to characterize how the Hippo pathway can be used to stimulate β-cells for proliferation *in vivo* and to identify a strategic pharmacological β-cell regeneration therapy.

In the first part of the thesis, I investigated the effect of YAP overexpression *in vivo* under physiological conditions as well as during β-cell regeneration on STZ-induced diabetes model mice:

- Does YAP induce β-cell proliferation and function in mice?
- Can short-term re-expression of YAP in adult mice improve functional cell proliferation, regeneration, and survival under basal conditions and increased cell stress and metabolic demand *in vivo*?
- Does overexpression of YAP protect against glycemia and apoptosis in a diabetes mouse model? And does it promote β-cell proliferation to compensate for the β-cell loss?

The second part focused on TT-10, a drug that stimulates the downstream effectors of the Hippo pathway to induce β-cell proliferation for regeneration:

- Does the TEAD activator TT-10 affect islet proliferation in experimental models of diabetes?
- Does TT-10 promote β-cell regeneration, and what are the mechanisms?
- Does TT-10 phenocopy YAP function in β-cells?

The last part of my thesis focused on how homozygous YAP overexpression chronically affects mouse β-cells:

- Does overexpressing YAP alter β-cell phenotype in mice *in vivo*?
- Does re-expression of YAP promote dedifferentiation?

References:

[1] M. Karamanou, "Milestones in the history of diabetes mellitus: The main contributors," *World J Diabetes*, vol. 7, no. 1, 2016, doi: 10.4239/wjd.v7.i1.1.

[2] R. M. M. Khan, Z. J. Y. Chua, J. C. Tan, Y. Yang, Z. Liao, and Y. Zhao, "From pre-diabetes to diabetes: Diagnosis, treatments and translational research," *Medicina (Lithuania)*, vol. 55, no. 9. 2019. doi: 10.3390/medicina55090546.

[3] A. M. Egan and S. F. Dinneen, "What is diabetes?," *Medicine (United Kingdom)*, vol. 50, no. 10. 2022. doi: 10.1016/j.mpmed.2022.07.001.

[4] N. Y. Kang, A. A. P. Soetedjo, N. S. Amirruddin, Y. T. Chang, O. Eriksson, and A. K. K. Teo, "Tools for Bioimaging Pancreatic β Cells in Diabetes," *Trends in Molecular Medicine*, vol. 25, no. 8. 2019. doi: 10.1016/j.molmed.2019.05.004.

[5] N. G. Forouhi and N. J. Wareham, "Epidemiology of diabetes," *Medicine (United Kingdom)*, vol. 50, no. 10. 2022. doi: 10.1016/j.mpmed.2022.07.005.

[6] M. G. White, J. A. M. Shaw, and R. Taylor, "Type 2 diabetes: The pathologic basis of reversible β-cell dysfunction," *Diabetes Care*, vol. 39, no. 11. 2016. doi: 10.2337/dc16-0619.

[7] H. Sun *et al.*, "IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045," *Diabetes Res Clin Pract*, vol. 183, 2022, doi: 10.1016/j.diabres.2021.109119.

[8] J. A. Todd, M. Knip, and C. Mathieu, "Strategies for the prevention of autoimmune Type 1 diabetes," *Diabetic Medicine*, vol. 28, no. 10. 2011. doi: 10.1111/j.1464-5491.2011.03400.x.

[9] F. Zhong and Y. Jiang, "Endogenous pancreatic β cell regeneration: A potential strategy for the recovery of β cell deficiency in diabetes," *Front Endocrinol (Lausanne)*, vol. 10, no. FEB, 2019, doi: 10.3389/fendo.2019.00101.

[10] T. Mezza, F. Cinti, C. M. A. Cefalo, A. Pontecorvi, R. N. Kulkarni, and A. Giaccari, "Β-cell fate in human insulin resistance and type 2 diabetes: A perspective on islet plasticity," *Diabetes*, vol. 68, no. 6. 2019. doi: 10.2337/db18-0856.

[11] K. I. Rother, "Diabetes Treatment — Bridging the Divide," *New England Journal of Medicine*, vol. 356, no. 15, 2007, doi: 10.1056/nejmp078030.

[12] Y. Li *et al.*, "Revisiting the Antigen-Presenting Function of β Cells in T1D Pathogenesis," *Frontiers in Immunology*, vol. 12. 2021. doi: 10.3389/fimmu.2021.690783.

[13] T. Delong *et al.*, "Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion," *Science (1979)*, vol. 351, no. 6274, 2016, doi: 10.1126/science.aad2791.

[14] C. Kopan, T. Tucker, M. Alexander, M. R. Mohammadi, E. Pone, and J. R. T. Lakey, "Approaches in immunotherapy, regenerative medicine, and bioengineering for type 1 diabetes," *Frontiers in Immunology*, vol. 9, no. JUN. 2018. doi: 10.3389/fimmu.2018.01354.

[15] S. M. Rodrigues Oliveira, A. Rebocho, E. Ahmadpour, V. Nissapatorn, and M. de Lourdes Pereira, "Type 1 Diabetes Mellitus: A Review on Advances and Challenges in Creating Insulin Producing Devices," *Micromachines*, vol. 14, no. 1. 2023. doi: 10.3390/mi14010151.

[16] A. Helman and D. A. Melton, "A stem cell approach to cure type 1 diabetes," *Cold Spring Harb Perspect Biol*, vol. 13, no. 1, 2021, doi: 10.1101/cshperspect.a035741.

[17] A. E. Butler, J. Janson, S. Bonner-Weir, R. Ritzel, R. A. Rizza, and P. C. Butler, "β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes," *Diabetes*, vol. 52, no. 1, 2003, doi: 10.2337/diabetes.52.1.102.

[18] D. M. Nathan, "Diabetes: Advances in diagnosis and treatment," *JAMA - Journal of the American Medical Association*, vol. 314, no. 10. 2015. doi: 10.1001/jama.2015.9536.

[19] M. Tahbaz and E. Yoshihara, "Immune Protection of Stem Cell-Derived Islet Cell Therapy for Treating Diabetes," *Frontiers in Endocrinology*, vol. 12. 2021. doi: 10.3389/fendo.2021.716625.

[20] B. Silver *et al.*, "EADSG Guidelines: Insulin Therapy in Diabetes," *Diabetes Therapy*, vol. 9, no. 2, 2018, doi: 10.1007/s13300-018-0384-6.

[21] A. L. McCall, "Insulin therapy and hypoglycemia," *Endocrinology and Metabolism Clinics of North America*, vol. 41, no. 1. 2012. doi: 10.1016/j.ecl.2012.03.001.
[22] A. Peloso *et al.*, "Regenerative medicine and diabetes: Targeting the extracellular matrix beyond the stem cell approach and encapsulation technology," *Frontiers in Endocrinology*, vol. 9, no. AUG. 2018. doi: 10.3389/fendo.2018.00445.
[23] P. Chhabra and K. L. Brayman, "Stem Cell Therapy to Cure Type 1 Diabetes: From Hype to Hope," *Stem Cells Transl Med*, vol. 2, no. 5, 2013, doi: 10.5966/sctm.2012-0116.
[24] B. Okere, L. Lucaccioni, M. Dominici, and L. Iughetti, "Cell therapies for pancreatic beta-cell replenishment," *Italian Journal of Pediatrics*, vol. 42, no. 1. 2016. doi: 10.1186/s13052-016-0273-4.
[25] F. Boscari and A. Avogaro, "Current treatment options and challenges in patients with Type 1 diabetes: Pharmacological, technical advances and future perspectives," *Reviews in Endocrine and Metabolic Disorders*, vol. 22, no. 2. 2021. doi: 10.1007/s11154-021-09635-3.
[26] B. Johannesson, L. Sui, D. O. Freytes, R. J. Creusot, and D. Egli, "Toward beta cell replacement for diabetes," *EMBO J*, vol. 34, no. 7, 2015, doi: 10.15252/embj.201490685.
[27] M. Okada, M. Oka, and Y. Yoneda, "Effective culture conditions for the induction of pluripotent stem cells," *Biochim Biophys Acta Gen Subj*, vol. 1800, no. 9, 2010, doi: 10.1016/j.bbagen.2010.04.004.
[28] K. Takahashi and S. Yamanaka, "Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors," *Cell*, vol. 126, no. 4, 2006, doi: 10.1016/j.cell.2006.07.024.
[29] P. Mali and L. Cheng, "Reprogramming somatic cells without fusion or ethical confusion," *Regenerative Med*, vol. 1, no. 6, 2006, doi: 10.2217/17460751.1.6.837.
[30] K. Okita, T. Ichisaka, and S. Yamanaka, "Generation of germline-competent induced pluripotent stem cells," *Nature*, vol. 448, no. 7151, 2007, doi: 10.1038/nature05934.
[31] E. Latres, D. A. Finan, J. L. Greenstein, A. Kowalski, and T. J. Kieffer, "Navigating Two Roads to Glucose Normalization in Diabetes: Automated Insulin Delivery Devices and Cell Therapy," *Cell Metabolism*, vol. 29, no. 3. 2019. doi: 10.1016/j.cmet.2019.02.007.
[32] W. Rust, "Regenerative medicine technologies applied to beta cell replacement: The industry perspective," in *Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas: Volume 2*, 2019. doi: 10.1016/B978-0-12-814831-0.00037-3.
[33] F. T. AlRashidi and K. M. Gillespie, "Biomarkers in Islet Cell Transplantation for Type 1 Diabetes," *Current Diabetes Reports*, vol. 18, no. 10. 2018. doi: 10.1007/s11892-018-1059-4.
[34] A. Agarwal and K. L. Brayman, "Update on islet cell transplantation for type 1 diabetes," *Semin Intervent Radiol*, vol. 29, no. 2, 2012, doi: 10.1055/s-0032-1312569.
[35] I. Fujita, R. Utoh, M. Yamamoto, T. Okano, and M. Yamato, "The liver surface as a favorable site for islet cell sheet transplantation in type 1 diabetes model mice," *Regen Ther*, vol. 8, 2018, doi: 10.1016/j.reth.2018.04.002.
[36] T. Anazawa, H. Okajima, and S. Uemoto, "Pancreatic islet transplantation," *Nihon Rinsho*, vol. 73, no. 12, 2015, doi: 10.4240/wjgs.v1.i1.16.
[37] F. Cayabyab, L. R. Nih, and E. Yoshihara, "Advances in Pancreatic Islet Transplantation Sites for the Treatment of Diabetes," *Frontiers in Endocrinology*, vol. 12. 2021. doi: 10.3389/fendo.2021.732431.
[38] J. Gołębiewska *et al.*, "Pancreatic islet transplantation in a simultaneous pancreas and kidney transplant recipient — a case report," *Clinical Diabetology*, vol. 9, no. 5, 2020, doi: 10.5603/DK.2020.0034.
[39] A. M. J. Shapiro, M. Pokrywczynska, and C. Ricordi, "Clinical pancreatic islet transplantation," *Nature Reviews Endocrinology*, vol. 13, no. 5. 2017. doi: 10.1038/nrendo.2016.178.
[40] E. Lazzaroni *et al.*, "Anti-diabetic drugs and weight loss in patients with type 2 diabetes," *Pharmacological Research*, vol. 171. 2021. doi: 10.1016/j.phrs.2021.105782.

[41] T. D. Müller, M. Blüher, M. H. Tschöp, and R. D. DiMarchi, "Anti-obesity drug discovery: advances and challenges," *Nature Reviews Drug Discovery*, vol. 21, no. 3. 2022. doi: 10.1038/s41573-021-00337-8.
[42] D. M. Williams, A. Nawaz, and M. Evans, "Drug Therapy in Obesity: A Review of Current and Emerging Treatments," *Diabetes Therapy*, vol. 11, no. 6. 2020. doi: 10.1007/s13300-020-00816-y.
[43] X. Zhao *et al.*, "GLP-1 Receptor Agonists: Beyond Their Pancreatic Effects," *Frontiers in Endocrinology*, vol. 12. 2021. doi: 10.3389/fendo.2021.721135.
[44] H. Cena, L. Chiovato, and R. E. Nappi, "Obesity, Polycystic Ovary Syndrome, and Infertility: A New Avenue for GLP-1 Receptor Agonists," *Journal of Clinical Endocrinology and Metabolism*, vol. 105, no. 8. 2020. doi: 10.1210/clinem/dgaa285.
[45] B. Gallwitz, "Clinical use of DPP-4 inhibitors," *Frontiers in Endocrinology*, vol. 10, no. JUN. 2019. doi: 10.3389/fendo.2019.00389.
[46] M. Ishida *et al.*, "DPP-4 inhibitor impedes lipopolysaccharide-induced osteoclast formation and bone resorption in vivo," *Biomedicine and Pharmacotherapy*, vol. 109, 2019, doi: 10.1016/j.biopha.2018.10.052.
[47] J. M. Trujillo, W. Nuffer, and B. A. Smith, "GLP-1 receptor agonists: an updated review of head-to-head clinical studies," *Therapeutic Advances in Endocrinology and Metabolism*, vol. 12. 2021. doi: 10.1177/2042018821997320.
[48] A. Bastidas-Ponce, K. Scheibner, H. Lickert, and M. Bakhti, "Cellular and molecular mechanisms coordinating pancreas development," *Development (Cambridge)*, vol. 144, no. 16. 2017. doi: 10.1242/dev.140756.
[49] M. Bakhti, A. Bastidas-Ponce, and H. Lickert, "Sorting Out Fate Determination," *Developmental Cell*, vol. 49, no. 1. 2019. doi: 10.1016/j.devcel.2019.03.020.
[50] F. C. Pan and C. Wright, "Pancreas Organogenesis: From Bud to Plexus to Gland OVERVIEW OF PANCREAS DEVELOPMENT," *Developmental Dynamics*, vol. 240, 2011.
[51] M. Lasierra Losada *et al.*, "Pancreas morphogenesis and homeostasis depends on tightly regulated Zeb1 levels in epithelial cells," *Cell Death Discov*, vol. 7, no. 1, 2021, doi: 10.1038/s41420-021-00522-z.
[52] G. Nair and M. Hebrok, "Islet formation in mice and men: Lessons for the generation of functional insulin-producing β-cells from human pluripotent stem cells," *Current Opinion in Genetics and Development*, vol. 32. 2015. doi: 10.1016/j.gde.2015.03.004.
[53] S. R. Andrei and M. Gannon, "Embryonic development of the endocrine pancreas," in *Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas: Volume 2*, 2019. doi: 10.1016/B978-0-12-814831-0.00012-9.
[54] M. C. Jørgensen, J. Ahnfelt-Rønne, J. Hald, O. D. Madsen, P. Serup, and J. Hecksher-Sørensen, "An illustrated review of early pancreas development in the mouse," *Endocr Rev*, vol. 28, no. 6, 2007, doi: 10.1210/er.2007-0016.
[55] M. Reichert and A. K. Rustgi, "Pancreatic ductal cells in development, regeneration, and neoplasia," *Journal of Clinical Investigation*, vol. 121, no. 12. 2011. doi: 10.1172/JCI57131.
[56] A. I. Romer and L. Sussel, "Pancreatic islet cell development and regeneration," *Current Opinion in Endocrinology, Diabetes and Obesity*, vol. 22, no. 4. 2015. doi: 10.1097/MED.0000000000000174.
[57] R. Sancho, R. Gruber, G. Gu, and A. Behrens, "Loss of Fbw7 reprograms adult pancreatic ductal cells into α, δ, and β cells," *Cell Stem Cell*, vol. 15, no. 2, 2014, doi: 10.1016/j.stem.2014.06.019.
[58] B. K. Baumgartner, G. Cash, H. Hansen, S. Ostler, and L. C. Murtaugh, "Distinct requirements for beta-catenin in pancreatic epithelial growth and patterning," *Dev Biol*, vol. 391, no. 1, 2014, doi: 10.1016/j.ydbio.2014.03.019.
[59] F. C. Pan and C. Wright, "Pancreas organogenesis: From bud to plexus to gland," *Developmental Dynamics*, vol. 240, no. 3. 2011. doi: 10.1002/dvdy.22584.

[60] J. Magenheim *et al.*, "Ngn3+ endocrine progenitor cells control the fate and morphogenesis of pancreatic ductal epithelium," *Dev Biol*, vol. 359, no. 1, 2011, doi: 10.1016/j.ydbio.2011.08.006.
[61] L. Flasse, C. Schewin, and A. Grapin-Botton, "Pancreas morphogenesis: Branching in and then out," in *Current Topics in Developmental Biology*, vol. 143, 2021. doi: 10.1016/bs.ctdb.2020.10.006.
[62] X. Y. Li, W. J. Zhai, and C. B. Teng, "Notch signaling in pancreatic development," *International Journal of Molecular Sciences*, vol. 17, no. 1. 2016. doi: 10.3390/ijms17010048.
[63] M. Rubey *et al.*, "DlL1- And Dll4-mediated Notch signaling is essential for adult pancreatic islet homeostasis," *Diabetes*, vol. 69, no. 5, 2020, doi: 10.2337/db19-0795.
[64] D. S. Lorberbaum and L. Sussel, "A Notch in Time: Spatiotemporal Analysis of Notch Signaling during Pancreas Development," *Developmental Cell*, vol. 52, no. 6. 2020. doi: 10.1016/j.devcel.2020.02.018.
[65] M. L. Golson *et al.*, "Jagged1 is a competitive inhibitor of Notch signaling in the embryonic pancreas," *Mech Dev*, vol. 126, no. 8–9, 2009, doi: 10.1016/j.mod.2009.05.005.
[66] N. Ebrahim, K. Shakirova, and E. Dashinimaev, "PDX1 is the cornerstone of pancreatic β-cell functions and identity," *Frontiers in Molecular Biosciences*, vol. 9. 2022. doi: 10.3389/fmolb.2022.1091757.
[67] A. Galli *et al.*, "Shaping Pancreatic β-Cell Differentiation and Functioning: The Influence of Mechanotransduction," *Cells*, vol. 9, no. 2. 2020. doi: 10.3390/cells9020413.
[68] V. Vanhorenbeeck *et al.*, "Role of the Onecut transcription factors in pancreas morphogenesis and in pancreatic and enteric endocrine differentiation," *Dev Biol*, vol. 305, no. 2, 2007, doi: 10.1016/j.ydbio.2007.02.027.
[69] D. Al Rijjal and M. B. Wheeler, "A protocol for studying glucose homeostasis and islet function in mice," *STAR Protoc*, vol. 3, no. 1, 2022, doi: 10.1016/j.xpro.2022.101171.
[70] A. S. Bernardo, C. W. Hay, and K. Docherty, "Pancreatic transcription factors and their role in the birth, life and survival of the pancreatic β cell," *Molecular and Cellular Endocrinology*, vol. 294, no. 1–2. 2008. doi: 10.1016/j.mce.2008.07.006.
[71] J. Juan-Mateu *et al.*, "Pancreatic microexons regulate islet function and glucose homeostasis," *Nat Metab*, vol. 5, no. 2, 2023, doi: 10.1038/s42255-022-00734-2.
[72] S. Bonner-Weir, "Perspective: Postnatal pancreatic β cell growth," *Endocrinology*, vol. 141, no. 6. 2000. doi: 10.1210/endo.141.6.7567.
[73] J. Soyer *et al.*, "Rfx6 is an Ngn3-dependent winged helix transcription factor required for pancreatic islet cell development," *Development*, vol. 137, no. 2, 2010, doi: 10.1242/dev.041673.
[74] M. G. De Vas, J. L. Kopp, C. Heliot, M. Sander, S. Cereghini, and C. Haumaitre, "Hnf1b controls pancreas morphogenesis and the generation of Ngn3+ endocrine progenitors," *Development (Cambridge)*, vol. 142, no. 5, 2015, doi: 10.1242/dev.110759.
[75] A. Villasenor, D. C. Chong, and O. Cleaver, "Biphasic Ngn3 expression in the developing pancreas," *Developmental Dynamics*, vol. 237, no. 11, 2008, doi: 10.1002/dvdy.21740.
[76] C. Gribben *et al.*, "Ductal Ngn3-expressing progenitors contribute to adult β cell neogenesis in the pancreas," *Cell Stem Cell*, vol. 28, no. 11, 2021, doi: 10.1016/j.stem.2021.08.003.
[77] S. Puri *et al.*, "Replication confers β cell immaturity," *Nat Commun*, vol. 9, no. 1, 2018, doi: 10.1038/s41467-018-02939-0.
[78] H. Zhao *et al.*, "Pre-existing beta cells but not progenitors contribute to new beta cells in the adult pancreas," *Nat Metab*, vol. 3, no. 3, 2021, doi: 10.1038/s42255-021-00364-0.
[79] S. Lee *et al.*, "Virgin b-cells at the neogenic niche proliferate normally and mature slowly," *Diabetes*, vol. 70, no. 5, 2021, doi: 10.2337/db20-0679.
[80] K. Katsumoto *et al.*, "Wnt4 is heterogeneously activated in maturing β-cells to control calcium signaling, metabolism and function," *Nat Commun*, vol. 13, no. 1, 2022, doi: 10.1038/s41467-022-33841-5.

[81] T. van der Meulen *et al.*, "Virgin Beta Cells Persist throughout Life at a Neogenic Niche within Pancreatic Islets," *Cell Metab*, vol. 25, no. 4, 2017, doi: 10.1016/j.cmet.2017.03.017.
[82] M. O. Huising, S. Lee, and T. van der Meulen, "Evidence for a Neogenic Niche at the Periphery of Pancreatic Islets," *BioEssays*, vol. 40, no. 11. 2018. doi: 10.1002/bies.201800119.
[83] A. Swisa, B. Glaser, and Y. Dor, "Metabolic stress and compromised identity of pancreatic beta cells," *Frontiers in Genetics*, vol. 8, no. FEB. 2017. doi: 10.3389/fgene.2017.00021.
[84] E. Ishida, J. Y. Kim-Muller, and D. Accili, "Pair feeding, but not insulin, phloridzin, or rosiglitazone treatment, curtails markers of β-cell dedifferentiation in db/db mice," *Diabetes*, vol. 66, no. 8, 2017, doi: 10.2337/db16-1213.
[85] T. Gao *et al.*, "Hippo signaling regulates differentiation and maintenance in the exocrine pancreas," *Gastroenterology*, vol. 144, no. 7, 2013, doi: 10.1053/j.gastro.2013.02.037.
[86] N. M. George, C. E. Day, B. P. Boerner, R. L. Johnson, and N. E. Sarvetnick, "Hippo Signaling Regulates Pancreas Development through Inactivation of Yap," *Mol Cell Biol*, vol. 32, no. 24, 2012, doi: 10.1128/mcb.01034-12.
[87] P. P. Khin, J. H. Lee, and H. S. Jun, "A brief review of the mechanisms of β-cell dedifferentiation in type 2 diabetes," *Nutrients*, vol. 13, no. 5. 2021. doi: 10.3390/nu13051593.
[88] T. Sun and X. Han, "Death versus dedifferentiation: The molecular bases of beta cell mass reduction in type 2 diabetes," *Seminars in Cell and Developmental Biology*, vol. 103. 2020. doi: 10.1016/j.semcdb.2019.12.002.
[89] M. Bensellam, J. C. Jonas, and D. R. Laybutt, "Mechanisms of β;-cell dedifferentiation in diabetes: Recent findings and future research directions," *Journal of Endocrinology*, vol. 236, no. 2. 2018. doi: 10.1530/JOE-17-0516.
[90] W. Wang and C. Zhang, "Targeting β-cell dedifferentiation and transdifferentiation: Opportunities and challenges," *Endocrine Connections*, vol. 10, no. 8. 2021. doi: 10.1530/EC-21-0260.
[91] K. Lemaire, L. Thorrez, and F. Schuit, "Disallowed and Allowed Gene Expression: Two Faces of Mature Islet Beta Cells," *Annual Review of Nutrition*, vol. 36. 2016. doi: 10.1146/annurev-nutr-071715-050808.
[92] S. Efrat, "Beta-Cell Dedifferentiation in Type 2 Diabetes: Concise Review," *Stem Cells*, vol. 37, no. 10, 2019, doi: 10.1002/stem.3059.
[93] S. Guo *et al.*, "Inactivation of specific β cell transcription factors in type 2 diabetes," *Journal of Clinical Investigation*, vol. 123, no. 8, 2013, doi: 10.1172/JCI65390.
[94] C. Talchai, S. Xuan, H. V. Lin, L. Sussel, and D. Accili, "Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure," *Cell*, vol. 150, no. 6, 2012, doi: 10.1016/j.cell.2012.07.029.
[95] S. Puri, H. Akiyama, and M. Hebrok, "VHL-mediated disruption of Sox9 activity compromises β-cell identity and results in diabetes mellitus," *Genes Dev*, vol. 27, no. 23, 2013, doi: 10.1101/gad.227785.113.
[96] B. L. Taylor, F. F. Liu, and M. Sander, "Nkx6.1 Is Essential for Maintaining the Functional State of Pancreatic Beta Cells," *Cell Rep*, vol. 4, no. 6, 2013, doi: 10.1016/j.celrep.2013.08.010.
[97] M. F. Brereton *et al.*, "Reversible changes in pancreatic islet structure and function produced by elevated blood glucose," *Nat Commun*, vol. 5, 2014, doi: 10.1038/ncomms5639.
[98] P. Marchetti *et al.*, "A direct look at the dysfunction and pathology of the β cells in human type 2 diabetes," *Seminars in Cell and Developmental Biology*, vol. 103. 2020. doi: 10.1016/j.semcdb.2020.04.005.
[99] P. Marchetti, "Islet inflammation in type 2 diabetes," *Diabetologia*, vol. 59, no. 4. 2016. doi: 10.1007/s00125-016-3875-x.
[100] P. Marchetti, M. Bugliani, V. D. De Tata, M. Suleiman, and L. Marselli, "Pancreatic beta cell identity in humans and the role of type 2 diabetes," *Frontiers in Cell and Developmental Biology*, vol. 5, no. MAY. 2017. doi: 10.3389/fcell.2017.00055.

[101] R. R. Huang and Y. Ou, "Research Advances in Islet β Cells Dedifferentiation in Type 2 Diabetes," *Chinese Journal of Pharmaceutical Biotechnology*, vol. 28, no. 3. 2021. doi: 10.19526/j.cnki.1005-8915.20210316.
[102] J. Sun *et al.*, "β-Cell Dedifferentiation in Patients with T2D with Adequate Glucose Control and Nondiabetic Chronic Pancreatitis," *Journal of Clinical Endocrinology and Metabolism*, vol. 104, no. 1, 2020, doi: 10.1210/jc.2018-00968.
[103] J. Son *et al.*, "Genetic and pharmacologic inhibition of ALDH1A3 as a treatment of β-cell failure," *Nat Commun*, vol. 14, no. 1, 2023, doi: 10.1038/s41467-023-36315-4.
[104] T. Zhang *et al.*, "Foxo1 plays an important role in regulating β-cell compensation for insulin resistance in male mice," *Endocrinology*, vol. 157, no. 3, 2016, doi: 10.1210/en.2015-1852.
[105] R. Liang *et al.*, "Cytohistologic analyses of β cell dedifferentiation induced by inflammation in human islets," *Eur J Inflamm*, vol. 19, 2021, doi: 10.1177/20587392211014416.
[106] J. Y. Kim-Muller *et al.*, "Aldehyde dehydrogenase 1a3 defines a subset of failing pancreatic β cells in diabetic mice," *Nat Commun*, vol. 7, Aug. 2016, doi: 10.1038/ncomms12631.
[107] T. M. Nordmann *et al.*, "The Role of Inflammation in β-cell Dedifferentiation," *Sci Rep*, vol. 7, no. 1, 2017, doi: 10.1038/s41598-017-06731-w.
[108] A. Benito-Vicente, S. Jebari-Benslaiman, U. Galicia-Garcia, A. Larrea-Sebal, K. B. Uribe, and C. Martin, "Molecular mechanisms of lipotoxicity-induced pancreatic β-cell dysfunction," in *International Review of Cell and Molecular Biology*, vol. 359, 2021. doi: 10.1016/bs.ircmb.2021.02.013.
[109] C. Ginestier *et al.*, "ALDH1 Is a Marker of Normal and Malignant Human Mammary Stem Cells and a Predictor of Poor Clinical Outcome," *Cell Stem Cell*, vol. 1, no. 5, 2007, doi: 10.1016/j.stem.2007.08.014.
[110] K. Amo-Shiinoki *et al.*, "Islet cell dedifferentiation is a pathologic mechanism of long-standing progression of type 2 diabetes," *JCI Insight*, vol. 6, no. 1, 2021, doi: 10.1172/jci.insight.143791.
[111] G. G. Nair, E. S. Tzanakakis, and M. Hebrok, "Emerging routes to the generation of functional β-cells for diabetes mellitus cell therapy," *Nature Reviews Endocrinology*, vol. 16, no. 9. 2020. doi: 10.1038/s41574-020-0375-3.
[112] C. Liu and H. Wu, "From beta cell replacement to beta cell regeneration: Implications for antidiabetic therapy," *J Diabetes Sci Technol*, vol. 8, no. 6, 2014, doi: 10.1177/1932296814540611.
[113] G. Basile *et al.*, "Emerging diabetes therapies: Bringing back the β-cells," *Molecular Metabolism*, vol. 60. 2022. doi: 10.1016/j.molmet.2022.101477.
[114] J. R. Benthuysen, A. C. Carrano, and M. Sander, "Advances in β cell replacement and regeneration strategies for treating diabetes," *Journal of Clinical Investigation*, vol. 126, no. 10. 2016. doi: 10.1172/JCI87439.
[115] T. Miyatsuka, "Uncovering the mechanisms of beta-cell neogenesis and maturation toward development of a regenerative therapy for diabetes," *Diabetol Int*, vol. 6, no. 4, 2015, doi: 10.1007/s13340-015-0233-z.
[116] S. Bonner-Weir *et al.*, "Islet neogenesis: A possible pathway for beta-cell replenishment," *Review of Diabetic Studies*, vol. 9, no. 4. 2012. doi: 10.1900/RDS.2012.9.407.
[117] S. Bonner-Weir, W. C. Li, L. Ouziel-Yahalom, L. Guo, G. C. Weir, and A. Sharma, "β-cell growth and regeneration: Replication is only part of the story," *Diabetes*, vol. 59, no. 10. 2010. doi: 10.2337/db10-0084.
[118] N. Ben-Othman *et al.*, "From pancreatic islet formation to beta-cell regeneration," *Diabetes Res Clin Pract*, vol. 101, no. 1, pp. 1–9, Jul. 2013, doi: 10.1016/J.DIABRES.2013.01.013.
[119] N. Kerper, S. Ashe, and M. Hebrok, "Pancreatic β-Cell Development and Regeneration," *Cold Spring Harb Perspect Biol*, vol. 14, no. 5, 2022, doi: 10.1101/cshperspect.a040741.
[120] J. E. Campbell and C. B. Newgard, "Mechanisms controlling pancreatic islet cell function in insulin secretion," *Nature Reviews Molecular Cell Biology*, vol. 22, no. 2. 2021. doi: 10.1038/s41580-020-00317-7.

[121] S. H. Lee, E. Hao, and F. Levine, "β-cell replication and islet neogenesis following partial pancreatectomy," *Islets*, vol. 3, no. 4, 2011, doi: 10.4161/isl.3.4.16338.
[122] X. Xu *et al.*, "β Cells Can Be Generated from Endogenous Progenitors in Injured Adult Mouse Pancreas," *Cell*, vol. 132, no. 2, 2008, doi: 10.1016/j.cell.2007.12.015.
[123] C. W. Cheng *et al.*, "Fasting-Mimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes," *Cell*, vol. 168, no. 5, 2017, doi: 10.1016/j.cell.2017.01.040.
[124] R. Azzarelli *et al.*, "Multi-site Neurogenin3 Phosphorylation Controls Pancreatic Endocrine Differentiation," *Dev Cell*, vol. 41, no. 3, 2017, doi: 10.1016/j.devcel.2017.04.004.
[125] E. Karakose, C. Ackeifi, P. Wang, and A. F. Stewart, "Advances in drug discovery for human beta cell regeneration," *Diabetologia*, vol. 61, no. 8. 2018. doi: 10.1007/s00125-018-4639-6.
[126] H. Sasaki, Y. Saisho, J. Inaishi, and H. Itoh, "Revisiting Regulators of Human β-cell Mass to Achieve β-cell-centric Approach Toward Type 2 Diabetes," *Journal of the Endocrine Society*, vol. 5, no. 10. 2021. doi: 10.1210/jendso/bvab128.
[127] S. Tian Yang, "Advances in Human Pluripotent Stem Cells for Regenerative Medicine and Drug Discovery," *J Tissue Sci Eng*, vol. 05, no. 01, 2014, doi: 10.4172/2157-7552.1000e127.
[128] C. Aguayo-Mazzucato and S. Bonner-Weir, "Pancreatic β Cell Regeneration as a Possible Therapy for Diabetes," *Cell Metab*, vol. 27, no. 1, pp. 57–67, Jan. 2018, doi: 10.1016/J.CMET.2017.08.007.
[129] A. Ardestani *et al.*, "Neratinib protects pancreatic beta cells in diabetes," *Nat Commun*, vol. 10, no. 1, 2019, doi: 10.1038/s41467-019-12880-5.
[130] G. Basile, R. N. Kulkarni, and N. G. Morgan, "How, When, and Where Do Human β-Cells Regenerate?," *Current Diabetes Reports*, vol. 19, no. 8. 2019. doi: 10.1007/s11892-019-1176-8.
[131] S. Yamada and I. Kojima, "Regenerative medicine of the pancreatic β cells," *Journal of Hepato-Biliary-Pancreatic Surgery*, vol. 12, no. 3. 2005. doi: 10.1007/s00534-005-0983-2.
[132] R. Wei *et al.*, "Dapagliflozin promotes beta cell regeneration by inducing pancreatic endocrine cell phenotype conversion in type 2 diabetic mice," *Metabolism*, vol. 111, 2020, doi: 10.1016/j.metabol.2020.154324.
[133] D. Giugliano *et al.*, "The effect of DPP-4 inhibitors, GLP-1 receptor agonists and SGLT-2 inhibitors on cardiorenal outcomes: a network meta-analysis of 23 CVOTs," *Cardiovasc Diabetol*, vol. 21, no. 1, 2022, doi: 10.1186/s12933-022-01474-z.
[134] A. Welters and E. Lammert, "Novel Approaches to Restore Pancreatic Beta-Cell Mass and Function," in *Handbook of Experimental Pharmacology*, vol. 274, 2022. doi: 10.1007/164_2021_474.
[135] P. Collombat, X. Xu, H. Heimberg, and A. Mansouri, "Pancreatic beta-cells: From generation to regeneration," *Seminars in Cell and Developmental Biology*, vol. 21, no. 8. 2010. doi: 10.1016/j.semcdb.2010.07.007.
[136] A. Hosseini, R. Shafee-Nick, and A. Ghorbani, "Pancreatic beta cell protection/regeneration with phytotherapy," *Brazilian Journal of Pharmaceutical Sciences*, vol. 51, no. 1, 2015, doi: 10.1590/S1984-82502015000100001.
[137] H. Zhao and B. Zhou, "Pancreatic beta cells regeneration," *Yi chuan = Hereditas*, vol. 44, no. 5. 2022. doi: 10.16288/j.yczz.22-072.
[138] C. Ackeifi *et al.*, "GLP-1 receptor agonists synergize with DYRK1A inhibitors to potentiate functional human β cell regeneration," *Sci Transl Med*, vol. 12, no. 530, 2020, doi: 10.1126/scitranslmed.aaw9996.
[139] L. Ye, M. A. Robertson, D. Hesselson, D. Y. R. Stainier, and R. M. Anderson, "Glucagon is essential for alpha cell transdifferentiation and beta cell neogenesis," *Development (Cambridge)*, vol. 142, no. 8, 2015, doi: 10.1242/dev.117911.
[140] A. Criscimanna, G. M. Coudriet, G. K. Gittes, J. D. Piganelli, and F. Esni, "Activated macrophages create lineage-specific microenvironments for pancreatic acinar- and β-cell

regeneration in mice," *Gastroenterology*, vol. 147, no. 5, 2014, doi: 10.1053/j.gastro.2014.08.008.
[141] S. Miyazaki, F. Tashiro, and J. I. Miyazaki, "Transgenic expression of a single transcription factor Pdx1 induces transdifferentiation of pancreatic acinar cells to endocrine cells in adult mice," *PLoS One*, vol. 11, no. 8, 2016, doi: 10.1371/journal.pone.0161190.
[142] Q. Zhou, J. Brown, A. Kanarek, J. Rajagopal, and D. A. Melton, "In vivo reprogramming of adult pancreatic exocrine cells to β-cells," *Nature*, vol. 455, no. 7213, 2008, doi: 10.1038/nature07314.
[143] Y. Yang, B. H. J. Chang, and L. Chan, "Sustained expression of the transcription factor GLIS3 is required for normal beta cell function in adults," *EMBO Mol Med*, vol. 5, no. 1, 2013, doi: 10.1002/emmm.201201398.
[144] M. Kuljanin, R. M. Elgamal, G. I. Bell, A. Xenocostas, G. A. Lajoie, and D. A. Hess, "Human Multipotent Stromal Cell Secreted Effectors Accelerate Islet Regeneration," *Stem Cells*, vol. 37, no. 4, 2019, doi: 10.1002/stem.2976.
[145] M. G. Amer, A. S. Embaby, R. A. Karam, and M. G. Amer, "Role of adipose tissue derived stem cells differentiated into insulin producing cells in the treatment of type I diabetes mellitus," *Gene*, vol. 654, 2018, doi: 10.1016/j.gene.2018.02.008.
[146] C. Dai *et al.*, "Stress-impaired transcription factor expression and insulin secretion in transplanted human islets," *Journal of Clinical Investigation*, vol. 126, no. 5, 2016, doi: 10.1172/JCI83657.
[147] P. Bhagchandani *et al.*, "Islet cell replacement and transplantation immunology in a mouse strain with inducible diabetes," *Sci Rep*, vol. 12, no. 1, 2022, doi: 10.1038/s41598-022-13087-3.
[148] H. S. Kim and M. K. Lee, "β-Cell regeneration through the transdifferentiation of pancreatic cells: Pancreatic progenitor cells in the pancreas," *J Diabetes Investig*, vol. 7, no. 3, 2016, doi: 10.1111/jdi.12475.
[149] C. Bonal, F. Thorel, A. Ait-Lounis, W. Reith, A. Trumpp, and P. L. Herrera, "Pancreatic Inactivation of c-Myc Decreases Acinar Mass and Transdifferentiates Acinar Cells Into Adipocytes in Mice," *Gastroenterology*, vol. 136, no. 1, 2009, doi: 10.1053/j.gastro.2008.10.015.
[150] S. Chera *et al.*, "Diabetes recovery by age-dependent conversion of pancreatic δ-cells into insulin producers," *Nature*, vol. 514, no. 7253, 2014, doi: 10.1038/nature13633.
[151] K. Lee *et al.*, "XBP1 maintains beta cell identity, represses beta-to-alpha cell transdifferentiation and protects against diabetic beta cell failure during metabolic stress in mice," *Diabetologia*, vol. 65, no. 6, 2022, doi: 10.1007/s00125-022-05669-7.
[152] C. Ackeifi *et al.*, "Pharmacologic and genetic approaches define human pancreatic β cell mitogenic targets of DYRK1A inhibitors," *JCI Insight*, vol. 5, no. 1, 2020, doi: 10.1172/jci.insight.132594.
[153] J. Shirakawa and R. N. Kulkarni, "Novel factors modulating human β-cell proliferation," *Diabetes, Obesity and Metabolism*, vol. 18. 2016. doi: 10.1111/dom.12731.
[154] P. Wang *et al.*, "Induction of human pancreatic beta cell replication by inhibitors of dual specificity tyrosine regulated kinase," *Nat Med*, vol. 21, no. 4, 2015.
[155] E. Dirice *et al.*, "Inhibition of DYRK1A stimulates human β-cell proliferation," *Diabetes*, vol. 65, no. 6, 2016, doi: 10.2337/db15-1127.
[156] Y. Guo, L. Li, Y. Yao, and H. Li, "Regeneration of Pancreatic β-Cells for Diabetes Therapeutics by Natural DYRK1A Inhibitors," *Metabolites*, vol. 13, no. 1. 2023. doi: 10.3390/metabo13010051.
[157] P. Wang *et al.*, "Combined Inhibition of DYRK1A, SMAD, and Trithorax Pathways Synergizes to Induce Robust Replication in Adult Human Beta Cells," *Cell Metab*, vol. 29, no. 3, 2019, doi: 10.1016/j.cmet.2018.12.005.

[158] H. E. Hohmeier *et al.*, "Identification of a small molecule that stimulates human β-cell proliferation and insulin secretion, and protects against cytotoxic stress in rat insulinoma cells," *PLoS One*, vol. 15, no. 3, 2020, doi: 10.1371/journal.pone.0224344.

[159] S. Tiwari *et al.*, "Definition of a Skp2-c-Myc pathway to expand human beta-cells," *Sci Rep*, vol. 6, 2016, doi: 10.1038/srep28461.

[160] M. F. Desentis-Desentis, "Regenerative approaches to preserve pancreatic β-cell mass and function in diabetes pathogenesis," *Endocrine*, vol. 75, no. 2. 2022. doi: 10.1007/s12020-021-02941-5.

[161] P. Collombat *et al.*, "Opposing actions of Arx and Pax4 in endocrine pancreas development," *Genes Dev*, vol. 17, no. 20, 2003, doi: 10.1101/gad.269003.

[162] N. M. George, C. E. Day, B. P. Boerner, R. L. Johnson, and N. E. Sarvetnick, "Hippo Signaling Regulates Pancreas Development through Inactivation of Yap," *Mol Cell Biol*, vol. 32, no. 24, 2012, doi: 10.1128/mcb.01034-12.

[163] F. C. Pan and M. Brissova, "Pancreas development in humans," *Current Opinion in Endocrinology, Diabetes and Obesity*, vol. 21, no. 2. 2014. doi: 10.1097/MED.0000000000000047.

[164] Z. Ma *et al.*, "Deciphering early human pancreas development at the single-cell level," *Nat Commun*, vol. 14, no. 1, 2023, doi: 10.1038/s41467-023-40893-8.

[165] V. Fu, S. W. Plouffe, and K. L. Guan, "The Hippo pathway in organ development, homeostasis, and regeneration," *Current Opinion in Cell Biology*, vol. 49. 2017. doi: 10.1016/j.ceb.2017.12.012.

[166] I. M. Moya and G. Halder, "The Hippo pathway in cellular reprogramming and regeneration of different organs," *Current Opinion in Cell Biology*, vol. 43. 2016. doi: 10.1016/j.ceb.2016.08.004.

[167] S. Wu, Y. Liu, Y. Zheng, J. Dong, and D. Pan, "The TEAD/TEF family protein Scalloped mediates transcriptional output of the Hippo growth-regulatory pathway.," *Dev Cell*, vol. 14, no. 3, 2008, doi: 10.1016/j.devcel.2008.01.007.

[168] R. W. Justice, O. Zilian, D. F. Woods, M. Noll, and P. J. Bryant, "The Drosophila tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation," *Genes Dev*, vol. 9, no. 5, 1995, doi: 10.1101/gad.9.5.534.

[169] F. Deng, Z. Wu, F. Zou, S. Wang, and X. Wang, "The Hippo–YAP/TAZ Signaling Pathway in Intestinal Self-Renewal and Regeneration After Injury," *Frontiers in Cell and Developmental Biology*, vol. 10. 2022. doi: 10.3389/fcell.2022.894737.

[170] R. Johnson and G. Halder, "The two faces of Hippo: Targeting the Hippo pathway for regenerative medicine and cancer treatment," *Nature Reviews Drug Discovery*, vol. 13, no. 1. 2014. doi: 10.1038/nrd4161.

[171] F. X. Yu, B. Zhao, and K. L. Guan, "Hippo Pathway in Organ Size Control, Tissue Homeostasis, and Cancer," *Cell*, vol. 163, no. 4. 2015. doi: 10.1016/j.cell.2015.10.044.

[172] D. Mugahid, M. Kalocsay, X. Liu, J. S. Gruver, L. Peshkin, and M. W. Kirschner, "YAP regulates cell size and growth dynamics via non-cell autonomous mediators," *Elife*, vol. 9, Jan. 2020, doi: 10.7554/eLife.53404.

[173] G. Sorrentino *et al.*, "Metabolic control of YAP and TAZ by the mevalonate pathway," *Nat Cell Biol*, vol. 16, no. 4, pp. 357–366, 2014, doi: 10.1038/ncb2936.

[174] " Hippo Limits Cell Growth ," *Science's STKE*, vol. 2003, no. 198, 2003, doi: 10.1126/stke.2003.198.tw338.

[175] S. Wu, J. Huang, J. Dong, and D. Pan, "hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts," *Cell*, vol. 114, no. 4, 2003, doi: 10.1016/S0092-8674(03)00549-X.

[176] H. Kwon, J. Kim, and E. hoon Jho, “Role of the Hippo pathway and mechanisms for controlling cellular localization of YAP/TAZ,” *FEBS Journal*, vol. 289, no. 19. 2022. doi: 10.1111/febs.16091.
[177] V. Rausch and C. G. Hansen, “The Hippo Pathway, YAP/TAZ, and the Plasma Membrane,” *Trends in Cell Biology*, vol. 30, no. 1. 2020. doi: 10.1016/j.tcb.2019.10.005.
[178] Z. Meng, T. Moroishi, and K. L. Guan, “Mechanisms of Hippo pathway regulation,” *Genes and Development*, vol. 30, no. 1. 2016. doi: 10.1101/gad.274027.115.
[179] W. Su *et al.*, “Overexpressed WDR3 induces the activation of Hippo pathway by interacting with GATA4 in pancreatic cancer,” *Journal of Experimental and Clinical Cancer Research*, vol. 40, no. 1, 2021, doi: 10.1186/s13046-021-01879-w.
[180] D. Ansari *et al.*, “The hippo signaling pathway in pancreatic cancer,” *Anticancer Research*, vol. 39, no. 7. 2019. doi: 10.21873/anticanres.13474.
[181] S. W. Plouffe *et al.*, “Characterization of Hippo Pathway Components by Gene Inactivation,” *Mol Cell*, vol. 64, no. 5, 2016, doi: 10.1016/j.molcel.2016.10.034.
[182] P. C. Calses, J. J. Crawford, J. R. Lill, and A. Dey, “Hippo Pathway in Cancer: Aberrant Regulation and Therapeutic Opportunities,” *Trends in Cancer*, vol. 5, no. 5. 2019. doi: 10.1016/j.trecan.2019.04.001.
[183] T. Heallen *et al.*, “Hippo pathway inhibits wnt signaling to restrain cardiomyocyte proliferation and heart size,” *Science (1979)*, vol. 332, no. 6028, 2011, doi: 10.1126/science.1199010.
[184] M. Kuljanin, G. I. Bell, S. E. Sherman, G. A. Lajoie, and D. A. Hess, “Proteomic characterisation reveals active Wnt-signalling by human multipotent stromal cells as a key regulator of beta cell survival and proliferation,” *Diabetologia*, vol. 60, no. 10, 2017, doi: 10.1007/s00125-017-4355-7.
[185] F. Deng *et al.*, “YAP triggers the Wnt/β-catenin signalling pathway and promotes enterocyte self-renewal, regeneration and tumorigenesis after DSS-induced injury,” *Cell Death Dis*, vol. 9, no. 2, 2018, doi: 10.1038/s41419-017-0244-8.
[186] M. deRan *et al.*, “Energy stress regulates Hippo-YAP signaling involving AMPK-mediated regulation of angiomotin-like 1 protein,” *Cell Rep*, vol. 9, no. 2, 2014, doi: 10.1016/j.celrep.2014.09.036.
[187] W. Wang *et al.*, “AMPK modulates Hippo pathway activity to regulate energy homeostasis,” *Nat Cell Biol*, vol. 17, no. 4, 2015, doi: 10.1038/ncb3113.
[188] A. Ardestani and K. Maedler, “The Hippo signaling pathway in pancreatic β-cells: Functions and regulations,” *Endocrine Reviews*, vol. 39, no. 1. 2018. doi: 10.1210/er.2017-00167.
[189] W. Mao, J. Mai, H. Peng, J. Wan, and T. Sun, “YAP in pancreatic cancer: Oncogenic role and therapeutic strategy,” *Theranostics*, vol. 11, no. 4, 2021, doi: 10.7150/thno.53438.
[190] D. Shome, T. Von Woedtke, K. Riedel, and K. Masur, “The HIPPO Transducer YAP and Its Targets CTGF and Cyr61 Drive a Paracrine Signalling in Cold Atmospheric Plasma-Mediated Wound Healing,” *Oxid Med Cell Longev*, vol. 2020, 2020, doi: 10.1155/2020/4910280.
[191] T. Azad *et al.*, “A LATS biosensor screen identifies VEGFR as a regulator of the Hippo pathway in angiogenesis,” *Nat Commun*, vol. 9, no. 1, 2018, doi: 10.1038/s41467-018-03278-w.
[192] G. T. K. Boopathy and W. Hong, “Role of Hippo Pathway-YAP/TAZ signaling in angiogenesis,” *Frontiers in Cell and Developmental Biology*, vol. 7, no. APR. 2019. doi: 10.3389/fcell.2019.00049.
[193] Y. Wu, P. Aegerter, M. Nipper, L. Ramjit, J. Liu, and P. Wang, “Hippo Signaling Pathway in Pancreas Development,” *Frontiers in Cell and Developmental Biology*, vol. 9. 2021. doi: 10.3389/fcell.2021.663906.
[194] H. E. Arda, C. M. Benitez, and S. K. Kim, “Gene regulatory networks governing pancreas development,” *Developmental Cell*, vol. 25, no. 1. 2013. doi: 10.1016/j.devcel.2013.03.016.
[195] Q. Chen *et al.*, “Homeostatic control of Hippo signaling activity revealed by an endogenous activating mutation in YAP,” *Genes Dev*, vol. 29, no. 12, 2015, doi: 10.1101/gad.264234.115.

[196] M. Nishio *et al.*, "Cancer susceptibility and embryonic lethality in Mob1a/1b double-mutant mice," *Journal of Clinical Investigation*, vol. 122, no. 12, 2012, doi: 10.1172/JCI63735.
[197] H. Song *et al.*, "Mammalian Mst1 and Mst2 kinases play essential roles in organ size control and tumor suppression," *Proc Natl Acad Sci U S A*, vol. 107, no. 4, 2010, doi: 10.1073/pnas.0911409107.
[198] K. P. Lee *et al.*, "The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis," *Proc Natl Acad Sci U S A*, vol. 107, no. 18, 2010, doi: 10.1073/pnas.0912203107.
[199] A. Kulkarni, M. T. Chang, J. H. A. Vissers, A. Dey, and K. F. Harvey, "The Hippo Pathway as a Driver of Select Human Cancers," *Trends in Cancer*, vol. 6, no. 9. 2020. doi: 10.1016/j.trecan.2020.04.004.
[200] Z. He, R. Li, and H. Jiang, "Mutations and Copy Number Abnormalities of Hippo Pathway Components in Human Cancers," *Frontiers in Cell and Developmental Biology*, vol. 9. 2021. doi: 10.3389/fcell.2021.661718.
[201] L. Wu and X. Yang, "Targeting the hippo pathway for breast cancer therapy," *Cancers*, vol. 10, no. 11. 2018. doi: 10.3390/cancers10110422.
[202] J. Cai, K. Choi, H. Li, K. D. P. Prieto, Y. Zheng, and D. Pan, "YAP-VGLL4 antagonism defines the major physiological function of the Hippo signaling effector YAP," *Genes Dev*, vol. 36, no. 21–24, 2022, doi: 10.1101/gad.350127.122.
[203] B. Gaertner, A. C. Carrano, and M. Sander, "Human stem cell models: lessons for pancreatic development and disease," *Genes & development*, vol. 33, no. 21–22. 2019. doi: 10.1101/gad.331397.119.
[204] T. L. Mastracci and L. Sussel, "The endocrine pancreas: Insights into development, differentiation, and diabetes," *Wiley Interdisciplinary Reviews: Developmental Biology*, vol. 1, no. 5. 2012. doi: 10.1002/wdev.44.
[205] R. E. Jennings *et al.*, "Development of the human pancreas from foregut to endocrine commitment," *Diabetes*, vol. 62, no. 10, 2013, doi: 10.2337/db12-1479.
[206] E. Conrad, R. Stein, and C. S. Hunter, "Revealing transcription factors during human pancreatic β cell development," *Trends in Endocrinology and Metabolism*, vol. 25, no. 8. 2014. doi: 10.1016/j.tem.2014.03.013.
[207] N. Kastan *et al.*, "Small-molecule inhibition of Lats kinases may promote Yap-dependent proliferation in postmitotic mammalian tissues," *Nat Commun*, vol. 12, no. 1, 2021, doi: 10.1038/s41467-021-23395-3.
[208] J. H. Koo *et al.*, "Induction of AP-1 by YAP/TAZ contributes to cell proliferation and organ growth," *Genes Dev*, vol. 34, no. 1–2, 2020, doi: 10.1101/gad.331546.119.
[209] T. Yuan *et al.*, "Proproliferative and antiapoptotic action of exogenously introduced YAP in pancreatic β cells," *JCI Insight*, vol. 1, no. 18, 2016, doi: 10.1172/jci.insight.86326.
[210] A. Pocaterra, P. Romani, and S. Dupont, "YAP/TAZ functions and their regulation at a glance," *J Cell Sci*, vol. 133, no. 2, Jan. 2020, doi: 10.1242/jcs.230425.
[211] N. M. George, B. P. Boerner, S. U. R. Mir, Z. Guinn, and N. E. Sarvetnick, "Exploiting expression of hippo effector, yap, for expansion of functional islet mass," *Molecular Endocrinology*, vol. 29, no. 11, 2015, doi: 10.1210/me.2014-1375.
[212] R. C. Pasek, J. C. Dunn, J. M. Elsakr, M. Aramandla, A. R. Matta, and M. Gannon, "Vascular-derived connective tissue growth factor (Ctgf) is critical for pregnancy-induced β cell hyperplasia in adult mice," *Islets*, vol. 9, no. 6, 2017, doi: 10.1080/19382014.2017.1356963.
[213] L. A. Crawford *et al.*, "Connective tissue growth factor (CTGF) inactivation leads to defects in islet cell lineage allocation and β-cell proliferation during embryogenesis," *Molecular Endocrinology*, vol. 23, no. 3, 2009, doi: 10.1210/me.2008-0045.
[214] A. Ardestani, B. Lupse, and K. Maedler, "Hippo Signaling: Key Emerging Pathway in Cellular and Whole-Body Metabolism," *Trends in Endocrinology and Metabolism*, vol. 29, no. 7. 2018. doi: 10.1016/j.tem.2018.04.006.

[215] W. Kim and E. H. Jho, "The history and regulatory mechanism of the Hippo pathway," *BMB Reports*, vol. 51, no. 3. 2018. doi: 10.5483/BMBRep.2018.51.3.022.
[216] K. G. Riley *et al.*, "Connective tissue growth factor modulates adult β-cell maturity and proliferation to promote β-cell regeneration in mice," *Diabetes*, vol. 64, no. 4, 2015, doi: 10.2337/db14-1195.
[217] T. Tamura *et al.*, "Dysregulation of PI3K and Hippo signaling pathways synergistically induces chronic pancreatitis via CTGF upregulation," *Journal of Clinical Investigation*, vol. 131, no. 13, 2021, doi: 10.1172/JCI143414.
[218] V. S. W. Li, "Yap in regeneration and symmetry breaking," *Nat Cell Biol*, vol. 21, no. 6, pp. 665–667, Jun. 2019, doi: 10.1038/s41556-019-0334-1.
[219] L. García-Gutiérrez, E. Fallahi, N. Aboud, N. Quinn, and D. Matallanas, "Interaction of LATS1 with SMAC links the MST2/Hippo pathway with apoptosis in an IAP-dependent manner," *Cell Death Dis*, vol. 13, no. 8, 2022, doi: 10.1038/s41419-022-05147-3.
[220] A. Ardestani *et al.*, "MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes," *Nat Med*, vol. 20, no. 4, 2014, doi: 10.1038/nm.3482.
[221] Y. Deng, Y. Matsui, W. Pan, Q. Li, and Z. C. Lai, "Yap1 plays a protective role in suppressing free fatty acid-induced apoptosis and promoting beta-cell survival," *Protein Cell*, vol. 7, no. 5, 2016, doi: 10.1007/s13238-016-0258-5.
[222] A. Sato *et al.*, "Pharmacological Activation of YAP/TAZ by Targeting LATS1/2 Enhances Periodontal Tissue Regeneration in a Murine Model," *Int J Mol Sci*, vol. 24, no. 2, 2023, doi: 10.3390/ijms24020970.
[223] A. Sharma, V. G. Yerra, and A. Kumar, "Emerging role of Hippo signalling in pancreatic biology: YAP re-expression and plausible link to islet cell apoptosis and replication," *Biochimie*, vol. 133. 2017. doi: 10.1016/j.biochi.2016.12.009.
[224] D. Kong, Y. Zhao, T. Men, and C. B. Teng, "Hippo signaling pathway in liver and pancreas: The potential drug target for tumor therapy," *Journal of Drug Targeting*, vol. 23, no. 2. 2015. doi: 10.3109/1061186X.2014.983522.
[225] I. M. Moya and G. Halder, "Hippo–YAP/TAZ signalling in organ regeneration and regenerative medicine," *Nature Reviews Molecular Cell Biology*, vol. 20, no. 4. Nature Publishing Group, pp. 211–226, Apr. 01, 2019. doi: 10.1038/s41580-018-0086-y.
[226] B. Soria and B. R. Gauthier, "Dual trade of Bcl-2 and Bcl-xL in islet physiology balancing life and death with metabolism secretion coupling," *Diabetes*, vol. 62, no. 1. 2013. doi: 10.2337/db12-1023.
[227] T. Yuan, K. D. D. Gorrepati, K. Maedler, and A. Ardestani, "Loss of Merlin/NF2 protects pancreatic β-cells from apoptosis by inhibiting LATS2," *Cell Death and Disease*, vol. 7, no. 2. 2016. doi: 10.1038/cddis.2016.21.
[228] A. Ardestani and K. Maedler, "MST1: a promising therapeutic target to restore functional beta cell mass in diabetes," *Diabetologia*, vol. 59, no. 9. 2016. doi: 10.1007/s00125-016-3892-9.
[229] T. Yuan *et al.*, "The Hippo kinase LATS2 impairs pancreatic β-cell survival in diabetes through the mTORC1-autophagy axis," *Nat Commun*, vol. 12, no. 1, 2021, doi: 10.1038/s41467-021-25145-x.
[230] W. Wen *et al.*, "MST1 promotes apoptosis through phosphorylation of histone H2AX," *Journal of Biological Chemistry*, vol. 285, no. 50, 2010, doi: 10.1074/jbc.M110.151753.
[231] F. Yuan *et al.*, "MST1 promotes apoptosis through regulating Sirt1-dependent p53 deacetylation," *Journal of Biological Chemistry*, vol. 286, no. 9, 2011, doi: 10.1074/jbc.M110.182543.
[232] S. Gezginci-Oktayoglu and S. Bolkent, "Ras signaling in NGF reduction and TNF-α-related pancreatic β cell apoptosis in hyperglycemic rats," *Apoptosis*, vol. 17, no. 1, 2012, doi: 10.1007/s10495-011-0657-2.
[233] A. Ardestani and K. Maedler, "MST1 deletion protects β-cells in a mouse model of diabetes," *Nutr Diabetes*, vol. 12, no. 1, 2022, doi: 10.1038/s41387-022-00186-3.

[234] M. Praskova, A. Khoklatchev, S. Ortiz-Vega, and J. Avruch, "Regulation of the MST1 kinase by autophosphorylation, by the growth inhibitory proteins, RASSF1 and NORE1, and by Ras," *Biochemical Journal*, vol. 381, no. 2, 2004, doi: 10.1042/BJ20040025.
[235] H. Donninger *et al.*, "The RASSF1A Tumor Suppressor Regulates XPA-Mediated DNA Repair," *Mol Cell Biol*, vol. 35, no. 1, 2015, doi: 10.1128/mcb.00202-14.
[236] H. J. Oh *et al.*, "Role of the tumor suppressor RASSF1A in Mst1-mediated apoptosis," *Cancer Res*, vol. 66, no. 5, 2006, doi: 10.1158/0008-5472.CAN-05-2951.
[237] M. Gao *et al.*, "Deletion of Lats 1 and 2 in mouse pancreatic acinar cells induces pancreatic fibrosis and inflammation," *bioRxiv*, 2019.
[238] C. M. Braitsch *et al.*, "LATS1/2 suppress NFκB and aberrant EMT initiation to permit pancreatic progenitor differentiation," *PLoS Biol*, vol. 17, no. 7, 2019, doi: 10.1371/journal.pbio.3000382.
[239] L. LeBlanc, N. Ramirez, and J. Kim, "Context-dependent roles of YAP/TAZ in stem cell fates and cancer," *Cellular and Molecular Life Sciences*, vol. 78, no. 9. 2021. doi: 10.1007/s00018-021-03781-2.
[240] M. Santucci *et al.*, "The Hippo Pathway and YAP/TAZ-TEAD Protein-Protein Interaction as Targets for Regenerative Medicine and Cancer Treatment," *Journal of Medicinal Chemistry*, vol. 58, no. 12. 2015. doi: 10.1021/jm501615v.
[241] M. Al-Mathkour, A. Dwead, E. Alp, A. Boston, J. Owens, and B. Cinar, "The Hippo Pathway Effector YAP/TEAD Regulates EPHA3 Expression and Functions," *The FASEB Journal*, vol. 35, no. S1, 2021, doi: 10.1096/fasebj.2021.35.s1.05157.
[242] L. Spanehl, D. Revskij, K. Bannert, L. Ehlers, and R. Jaster, "YAP activates pancreatic stellate cells and enhances pancreatic fibrosis," *Hepatobiliary and Pancreatic Diseases International*, vol. 21, no. 6, 2022, doi: 10.1016/j.hbpd.2022.06.004.
[243] M. Fan *et al.*, "Covalent Disruptor of YAP-TEAD Association Suppresses Defective Hippo Signaling," *Elife*, vol. 11, 2022, doi: 10.7554/eLife.78810.
[244] Y. Sun *et al.*, "Pharmacological blockade of TEAD–YAP reveals its therapeutic limitation in cancer cells," *Nat Commun*, vol. 13, no. 1, 2022, doi: 10.1038/s41467-022-34559-0.
[245] F. F. Diehl *et al.*, "Nucleotide imbalance decouples cell growth from cell proliferation," *Nat Cell Biol*, vol. 24, no. 8, 2022, doi: 10.1038/s41556-022-00965-1.
[246] C. G. Hansen, Y. L. D. Ng, W. L. M. Lam, S. W. Plouffe, and K. L. Guan, "The Hippo pathway effectors YAP and TAZ promote cell growth by modulating amino acid signaling to mTORC1," *Cell Res*, vol. 25, no. 12, 2015, doi: 10.1038/cr.2015.140.
[247] J. M. M. Kwok, S. S. Myatt, C. M. Marson, R. C. Coombes, D. Constantinidou, and E. W. F. Lam, "Thiostrepton selectively targets breast cancer cells through inhibition of forkhead box M1 expression," *Mol Cancer Ther*, vol. 7, no. 7, 2008, doi: 10.1158/1535-7163.MCT-08-0188.
[248] D. B. Davis *et al.*, "FoxM1 is up-regulated by obesity and stimulates β-cell proliferation," *Molecular Endocrinology*, vol. 24, no. 9, 2010, doi: 10.1210/me.2010-0082.
[249] K. Tumaneng *et al.*, "YAP mediates crosstalk between the Hippo and PI(3)K-TOR pathways by suppressing PTEN via miR-29," *Nat Cell Biol*, vol. 14, no. 12, 2012, doi: 10.1038/ncb2615.
[250] N. Liang *et al.*, "Regulation of YAP by mTOR and autophagy reveals a therapeutic target of tuberous sclerosis complex," *Journal of Experimental Medicine*, vol. 211, no. 11, 2014, doi: 10.1084/jem.20140341.
[251] Y. Sun *et al.*, "The critical role of the Hippo signaling pathway in kidney diseases," *Frontiers in Pharmacology*, vol. 13. 2022. doi: 10.3389/fphar.2022.988175.
[252] Y. Hu *et al.*, "YAP suppresses gluconeogenic gene expression through PGC1α," *Hepatology*, vol. 66, no. 6, 2017, doi: 10.1002/hep.29373.
[253] X. Bian, H. Jiang, Y. Meng, Y. ping Li, J. Fang, and Z. Lu, "Regulation of gene expression by glycolytic and gluconeogenic enzymes," *Trends in Cell Biology*, vol. 32, no. 9. 2022. doi: 10.1016/j.tcb.2022.02.003.

[254] C. Luo *et al.*, "H3K27me3-mediated PGC1α gene silencing promotes melanoma invasion through WNT5A and YAP," *Journal of Clinical Investigation*, vol. 130, no. 2, 2020, doi: 10.1172/JCI130038.

[255] A. W. Hong, Z. Meng, S. W. Plouffe, Z. Lin, M. Zhang, and K. L. Guan, "Critical roles of phosphoinositides and NF2 in Hippo pathway regulation," *Genes Dev*, vol. 34, no. 7–8, 2020, doi: 10.1101/gad.333435.119.

[256] D. Zhou *et al.*, "Mst1 and Mst2 Maintain Hepatocyte Quiescence and Suppress Hepatocellular Carcinoma Development through Inactivation of the Yap1 Oncogene," *Cancer Cell*, vol. 16, no. 5, 2009, doi: 10.1016/j.ccr.2009.09.026.

[257] T. Panciera *et al.*, "Induction of Expandable Tissue-Specific Stem/Progenitor Cells through Transient Expression of YAP/TAZ," *Cell Stem Cell*, vol. 19, no. 6, 2016, doi: 10.1016/j.stem.2016.08.009.

[258] Y. Yuan *et al.*, "YAP1/TAZ-TEAD transcriptional networks maintain skin homeostasis by regulating cell proliferation and limiting KLF4 activity," *Nat Commun*, vol. 11, no. 1, 2020, doi: 10.1038/s41467-020-15301-0.

[259] B. J. Pepe-Mooney *et al.*, "Single-Cell Analysis of the Liver Epithelium Reveals Dynamic Heterogeneity and an Essential Role for YAP in Homeostasis and Regeneration," *Cell Stem Cell*, vol. 25, no. 1, 2019, doi: 10.1016/j.stem.2019.04.004.

[260] S. Noguchi, A. Saito, and T. Nagase, "YAP/TAZ signaling as a molecular link between fibrosis and cancer," *International Journal of Molecular Sciences*, vol. 19, no. 11. 2018. doi: 10.3390/ijms19113674.

[261] C. Jain, Ansarullah, S. Bilekova, and H. Lickert, "Targeting pancreatic β cells for diabetes treatment," *Nature Metabolism*, vol. 4, no. 9. 2022. doi: 10.1038/s42255-022-00618-5.

[262] C. Stein *et al.*, "YAP1 Exerts Its Transcriptional Control via TEAD-Mediated Activation of Enhancers," *PLoS Genet*, vol. 11, no. 8, 2015, doi: 10.1371/journal.pgen.1005465.

[263] K. C. Lin, H. W. Park, and K. L. Guan, "Regulation of the Hippo Pathway Transcription Factor TEAD," *Trends in Biochemical Sciences*, vol. 42, no. 11. 2017. doi: 10.1016/j.tibs.2017.09.003.

[264] F. Li *et al.*, "TEAD1 regulates cell proliferation through a pocket-independent transcription repression mechanism," *Nucleic Acids Res*, vol. 50, no. 22, 2022, doi: 10.1093/nar/gkac1063.

[265] J. Lee *et al.*, "Tead1 Reciprocally Regulates Adult β-Cell Proliferation and Function to Maintain Glucose Homeostasis," *SSRN Electronic Journal*, 2020, doi: 10.2139/ssrn.3704009.

[266] A. Bartolome, C. Zhu, L. Sussel, and U. B. Pajvani, "Notch signaling dynamically regulates adult β cell proliferation and maturity," *Journal of Clinical Investigation*, vol. 129, no. 1, 2019, doi: 10.1172/JCI98098.

[267] A. Khadra and S. Schnell, "Development, growth and maintenance of β-cell mass: Models are also part of the story," *Molecular Aspects of Medicine*, vol. 42. 2015. doi: 10.1016/j.mam.2015.01.005.

[268] S. Chen *et al.*, "A small molecule that directs differentiation of human ESCs into the pancreatic lineage," *Nat Chem Biol*, vol. 5, no. 4, 2009, doi: 10.1038/nchembio.154.

[269] M. Borowiak *et al.*, "Small Molecules Efficiently Direct Endodermal Differentiation of Mouse and Human Embryonic Stem Cells," *Cell Stem Cell*, vol. 4, no. 4, 2009, doi: 10.1016/j.stem.2009.01.014.

[270] T. J. Kieffer, "Closing in on Mass Production of Mature Human Beta Cells," *Cell Stem Cell*, vol. 18, no. 6. 2016. doi: 10.1016/j.stem.2016.05.014.

[271] S. Zhu *et al.*, "Human pancreatic beta-like cells converted from fibroblasts," *Nat Commun*, vol. 7, 2016, doi: 10.1038/ncomms10080.

[272] E. Hammar, A. Tomas, D. Bosco, and P. A. Halban, "Role of the Rho-ROCK (Rho-associated kinase) signaling pathway in the regulation of pancreaticβ-cell function," *Endocrinology*, vol. 150, no. 5, 2009, doi: 10.1210/en.2008-1135.

[273] Z. Ghazizadeh *et al.*, "ROCKII inhibition promotes the maturation of human pancreatic beta-like cells," *Nat Commun*, vol. 8, no. 1, 2017, doi: 10.1038/s41467-017-00129-y.

[274] X. Liu *et al.*, "Involvement of RhoA/ROCK in insulin secretion of pancreatic β-cells in 3D culture," *Cell Tissue Res*, vol. 358, no. 2, 2014, doi: 10.1007/s00441-014-1961-2.
[275] X. Kong *et al.*, "Glucagon-like peptide 1 stimulates insulin secretion via inhibiting RhoA/ROCK signaling and disassembling glucotoxicity-induced stress fibers," *Endocrinology (United States)*, vol. 155, no. 12, 2014, doi: 10.1210/en.2014-1314.
[276] L. Sakhneny *et al.*, "The postnatal pancreatic microenvironment guides β cell maturation through BMP4 production," *Dev Cell*, vol. 56, no. 19, 2021, doi: 10.1016/j.devcel.2021.08.014.
[277] M. J. Lima *et al.*, "Suppression of epithelial-to-Mesenchymal transitioning enhances ex vivo reprogramming of human exocrine pancreatic tissue toward functional insulin-Producing β-Like cells," *Diabetes*, vol. 62, no. 8, 2013, doi: 10.2337/db12-1256.
[278] H. Aly *et al.*, "A Novel Strategy to Increase the Proliferative Potential of Adult Human β-Cells While Maintaining Their Differentiated Phenotype," *PLoS One*, vol. 8, no. 6, 2013, doi: 10.1371/journal.pone.0066131.
[279] M. Ohgushi, M. Minaguchi, and Y. Sasai, "Rho-Signaling-Directed YAP/TAZ Activity Underlies the Long-Term Survival and Expansion of Human Embryonic Stem Cells," *Cell Stem Cell*, vol. 17, no. 4, 2015, doi: 10.1016/j.stem.2015.07.009.
[280] D. E. Mason *et al.*, "YAP and TAZ limit cytoskeletal and focal adhesion maturation to enable persistent cell motility," *Journal of Cell Biology*, vol. 218, no. 4, 2019, doi: 10.1083/jcb.201806065.
[281] W. Jin and W. Jiang, "Stepwise differentiation of functional pancreatic β cells from human pluripotent stem cells," *Cell Regeneration*, vol. 11, no. 1. 2022. doi: 10.1186/s13619-022-00125-8.
[282] K. I. Watt, K. F. Harvey, and P. Gregorevic, "Regulation of tissue growth by the mammalian Hippo signaling pathway," *Frontiers in Physiology*, vol. 8, no. NOV. 2017. doi: 10.3389/fphys.2017.00942.
[283] M. Xin *et al.*, "Hippo pathway effector Yap promotes cardiac regeneration," *Proc Natl Acad Sci U S A*, vol. 110, no. 34, 2013, doi: 10.1073/pnas.1313192110.
[284] A. Von Gise *et al.*, "YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy," *Proc Natl Acad Sci U S A*, vol. 109, no. 7, 2012, doi: 10.1073/pnas.1116136109.
[285] W. Chen, D. Pretorius, Y. Zhou, Y. Nakada, J. Yang, and J. Zhang, "TT-10–loaded nanoparticles promote cardiomyocyte proliferation and cardiac repair in a mouse model of myocardial infarction," *JCI Insight*, vol. 6, no. 20, 2021, doi: 10.1172/jci.insight.151987.
[286] H. Hara *et al.*, "Discovery of a Small Molecule to Increase Cardiomyocytes and Protect the Heart After Ischemic Injury," *JACC Basic Transl Sci*, vol. 3, no. 5, pp. 639–653, Oct. 2018, doi: 10.1016/j.jacbts.2018.07.005.
[287] J. Kim *et al.*, "YAP/TAZ regulates sprouting angiogenesis and vascular barrier maturation," *Journal of Clinical Investigation*, vol. 127, no. 9, 2017, doi: 10.1172/JCI93825.
[288] I. Osman *et al.*, "YAP1/TEAD1 upregulate platelet-derived growth factor receptor beta to promote vascular smooth muscle cell proliferation and neointima formation," *J Mol Cell Cardiol*, vol. 156, 2021, doi: 10.1016/j.yjmcc.2021.03.005.
[289] Z. Wang, M. Liu, H. Lei, S. Xiao, and Y. Zheng, "TEAD1 Silencing Regulates Cell Proliferation and Resistance to 5-Fluorouracil in Cutaneous Squamous Cell Carcinoma," *Clin Cosmet Investig Dermatol*, vol. 15, 2022, doi: 10.2147/CCID.S386547.
[290] F. Li *et al.*, "VGLL4 and MENIN function as TEAD1 corepressors to block pancreatic β cell proliferation," *Cell Rep*, vol. 42, no. 1, 2023, doi: 10.1016/j.celrep.2022.111904.
[291] M. M. Mia and M. K. Singh, "Emerging roles of the Hippo signaling pathway in modulating immune response and inflammation-driven tissue repair and remodeling," *FEBS Journal*, vol. 289, no. 14. 2022. doi: 10.1111/febs.16449.

[292] J. Chen *et al.*, "AYAP modRNA reduces cardiac inflammation and hypertrophy in a murine ischemia-reperfusion model," *Life Sci Alliance*, vol. 3, no. 1, 2020, doi: 10.26508/lsa.201900424.
[293] Q. Zhang *et al.*, "Hippo signalling governs cytosolic nucleic acid sensing through YAP/TAZ-mediated TBK1 blockade," *Nat Cell Biol*, vol. 19, no. 4, 2017, doi: 10.1038/ncb3496.
[294] K. Song *et al.*, "Yes-Associated Protein in Kupffer Cells Enhances the Production of Proinflammatory Cytokines and Promotes the Development of Nonalcoholic Steatohepatitis," *Hepatology*, vol. 72, no. 1, 2020, doi: 10.1002/hep.30990.
[295] M. M. Mia *et al.*, "YAP/TAZ deficiency reprograms macrophage phenotype and improves infarct healing and cardiac function after myocardial infarction," *PLoS Biol*, vol. 18, no. 12, 2020, doi: 10.1371/journal.pbio.3000941.
[296] M. M. Mia *et al.*, "Loss of Yap/Taz in cardiac fibroblasts attenuates adverse remodelling and improves cardiac function," *Cardiovasc Res*, vol. 118, no. 7, 2022, doi: 10.1093/cvr/cvab205.
[297] X. Wang *et al.*, "Hepatocyte TAZ/WWTR1 Promotes Inflammation and Fibrosis in Nonalcoholic Steatohepatitis," *Cell Metab*, vol. 24, no. 6, 2016, doi: 10.1016/j.cmet.2016.09.016.
[298] M. Mooring *et al.*, "Hepatocyte Stress Increases Expression of Yes-Associated Protein and Transcriptional Coactivator With PDZ-Binding Motif in Hepatocytes to Promote Parenchymal Inflammation and Fibrosis," *Hepatology*, vol. 71, no. 5, 2020, doi: 10.1002/hep.30928.
[299] M. V. Machado *et al.*, "Accumulation of duct cells with activated YAP parallels fibrosis progression in non-alcoholic fatty liver disease," *J Hepatol*, vol. 63, no. 4, 2015, doi: 10.1016/j.jhep.2015.05.031.
[300] Q. Zhang *et al.*, "Influenza A virus NS1 protein hijacks YAP/TAZ to suppress TLR3-mediated innate immune response," *PLoS Pathog*, vol. 18, no. 5, 2022, doi: 10.1371/journal.ppat.1010505.
[301] A. Dong, A. Gupta, R. K. Pai, M. Tun, and A. W. Lowe, "The human adenocarcinoma-associated gene, AGR2, induces expression of Amphiregulin through hippo pathway co-activator YAP1 activation," *Journal of Biological Chemistry*, vol. 286, no. 20, 2011, doi: 10.1074/jbc.M110.215707.
[302] J. H. Koo and K. L. Guan, "Interplay between YAP/TAZ and Metabolism," *Cell Metabolism*, vol. 28, no. 2. 2018. doi: 10.1016/j.cmet.2018.07.010.
[303] A. W. Stitt *et al.*, "The progress in understanding and treatment of diabetic retinopathy," *Progress in Retinal and Eye Research*, vol. 51. 2016. doi: 10.1016/j.preteyeres.2015.08.001.
[304] J. Chen and R. C. Harris, "Interaction of the EGF receptor and the hippo pathway in the diabetic kidney," *Journal of the American Society of Nephrology*, vol. 27, no. 6, 2016, doi: 10.1681/ASN.2015040415.

2 Results

Publication 1:

In vivo β-cell-specific YAP overexpression promotes β-cell regeneration in a mouse model of diabetes

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Designed, performed experiments, analysed data and assembled the figures for:

Figure 1 A, B; Figure 2 A, B, C, D, E, F, G, H, I, J, K; Figure 3 A, B, C, D, E, F, G, H, I; and Figure 4 A, B, C, D, E, F.

Wrote the paper.

***In vivo* β-cell-specific YAP overexpression promotes β-cell regeneration in a mouse model of diabetes**

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Abstract

All forms of diabetes are characterized by a significant decline in pancreatic β-cell function and mass, making the restoration and maintenance of β-cell mass a primary focus in diabetes research. Yes-Associated Protein (YAP), transcriptional co-regulator in the Hippo pathway, controls proliferation and survival but is silenced in mature adult pancreatic β-cells. Reintroducing YAP into adult human islets demonstrated a potent induction of human β-cell proliferation, preserving insulin secretory function and key β-cell identity genes. The question of whether YAP re-expression specifically in β-cells in mice could reverse diabetes *in vivo* remained unanswered. Here, we show that mice overexpressing YAP specifically in β-cells exhibit a robust increase in β-cell proliferation and an expansion of β-cell mass under physiological conditions. In a mouse model of streptozotocin (STZ)-induced β-cell destruction and diabetes, transient YAP induction resulted in reduced glucose levels and improved glucose tolerance. Moreover, these interventions led to substantial enhancements in β-cell area, mass, survival, and proliferation. Notably, YAP overexpression was associated with the suppression of glucose and STZ transporter GLUT2 mRNA and protein expression, indicating that its potential protective effect against STZ-induced diabetes was linked to reduced GLUT2 expression. In conclusion, our findings underscore the robust pro-proliferative activity of YAP in mice *in vivo*. Nevertheless, optimizing the regenerative potential of YAP warrants careful consideration of timing, dosage, and specificity, especially given its impact on functional genes, such as GLUT2 expression.

Introduction

Both type 1 diabetes (T1D) and type 2 diabetes (T2D) arise from a significant decline in pancreatic β-cell function and/or mass. Therefore, a key focus in this field is the development of therapeutic strategies aimed at restoring and sustaining β-cell mass in individuals with diabetes. β-cell apoptosis, dedifferentiation and impaired regeneration of insulin-producing β-cells are hallmarks of β-cell dysfunction and underlying causes of diabetes. Consequently, the improvement of β-cell survival and/or the enhancement of β-cell regenerative capacity emerge as promising therapeutic approaches aiming to increase functional β-cell mass, either *in vitro* (before transplantation) or *in vivo* (within the existing β-cell population), in patients with diabetes [1; 2; 3].

To maintain glucose homeostasis and prevent the onset of diabetes, the expansion of β-cells in response to insulin resistance is required. Several studies have shown that the maintenance of β-cell mass predominantly occurs through replication of existing β-cells, rather than by differentiation from pancreatic progenitors or other cell types [4; 5]. In this context, considerable efforts have been made to characterize the complex molecular pathways that regulate or trigger β-cell replication. This proliferation process is critical in preserving β-cell mass in adults, as well as in response to various proliferative stimuli. The identification of signaling pathways and regulatory networks governing β-cell proliferation and regeneration, coupled with a deeper understanding of their mechanisms of action, will play a crucial role in uncovering novel targets for β-cell regeneration in diabetes [6; 7; 8].

Several signaling cascades such as PI3K-AKT, MAPK, and Wnt/β-catenin pathways have been investigated for their roles in cell cycle control and β-cell proliferation [6; 7; 8]. The Hippo pathway has emerged as key regulator of organ size as well as tissue development, homeostasis and regeneration under physiological conditions; its dysregulation is extensively linked to cancer and metabolic disorders [9; 10]. Originally identified in fruit flies, the evolutionary conservation of this pathway in mammals has been firmly established. At the center of the mammalian Hippo pathway lies the kinase core, consisting of Mammalian Sterile 20-like kinases (MST1/2) and Large-tumor suppressors (LATS1/2). The key terminal effectors of the Hippo pathway are the transcriptional coactivator known as Yes-associated protein (YAP) and TAZ. MST1/2, in conjunction with the adaptor protein Salvador (Sav1), phosphorylates and activates LATS1/2 kinases. LATS1/2 control YAP's turnover by directly phosphorylating it at S127, among other sites. This modification results in the sequestration of YAP in the

cytoplasm (via binding to 14-3-3 proteins) and/or its degradation through the ubiquitin-proteasome pathway. These processes ultimately lead to the suppression of YAP's transcriptional activity. In the absence of this regulatory module, or in the presence of stimuli that activate YAP/TAZs independently of Hippo core kinases, YAP/TAZs translocate to the nucleus and interact with different transcription factors to mediate the expression of target genes. YAP predominantly exerts its influence through TEA domain (TEAD) family transcription factors, which in turn regulate the expression of target genes responsible for modulating various cellular functions, including proliferation and apoptosis [9]. Importantly, the direct targets of YAP are closely linked to cell cycle progression, replication licensing, and DNA synthesis [11]. Disruption of any component within the kinase core culminates in a YAP-dependent increase in proliferation and resistance to apoptosis across multiple tissues, underscoring the potent regulatory role of the Hippo pathway in controlling tissue/cell size [12; 13; 14].

In our recent investigations, we have identified various components of the Hippo signaling pathway as pivotal regulators of β-cell survival, proliferation, and insulin secretion [15; 16; 17; 18; 19; 20; 21; 22]. YAP is highly expressed in the pancreatic progenitor cells during the early stages of pancreas development, where it play a role in promoting the proliferation and expansion of multipotent pancreatic progenitors cells [23; 24]. However, YAP exhibits extremely low or no expression in terminally differentiated mature human and mouse endocrine islet cells [25; 26; 27] and is therefore one of the “disallowed” genes in β-cells [28]. Notably, YAP’s expression maintains in the exocrine compartments including ducts and acinar cells [25; 26; 27]. The loss in YAP expression correlates with the extremely low rate of β-cell proliferation after birth.

In our previous study, we demonstrated the potent pro-proliferative and anti-apoptotic effects of YAP in isolated human islets and β-cells through adenoviral-mediated YAP reconstitution which was accompanied with preservation of insulin secretory function and β-cell identity genes [29]. In this current investigation, we selectively re-expressed the active form of YAP specifically in β-cells in mice in an inducible manner. We determined whether YAP-induced responses; enhanced β-cell proliferation and resistance to apoptosis, can potentially contribute to the regeneration of endogenous β-cells and protection from hyperglycemia in the context of the streptozotocin (STZ) model of β-cell destruction and subsequent regeneration.

Results and discussion

Generation of inducible β-cell-YAP overexpressing mice

To validate our *in vitro* findings in isolated human islets [29] and assess the potential benefits of YAP induction in β-cells *in vivo*, we created doxycycline (dox)-inducible β-cell-specific Rip-Ins2-TetO-hYAP1-S127A mice, referred to as "β-YAP". This was achieved by crossbreeding mice that overexpress active YAP under doxycycline control (TetO-$YAP^{Ser127A}$) [30] with mice carrying the tetracycline transactivator (tTA) under the regulation of the insulin promoter (RIP-rtTA mice) [31] (Figure 1A). Upon the administration of dox to adult mice, we observed a substantial and selective induction of YAP in β-cells within pancreatic islets, confirmed through immunostaining (Figure 1B).

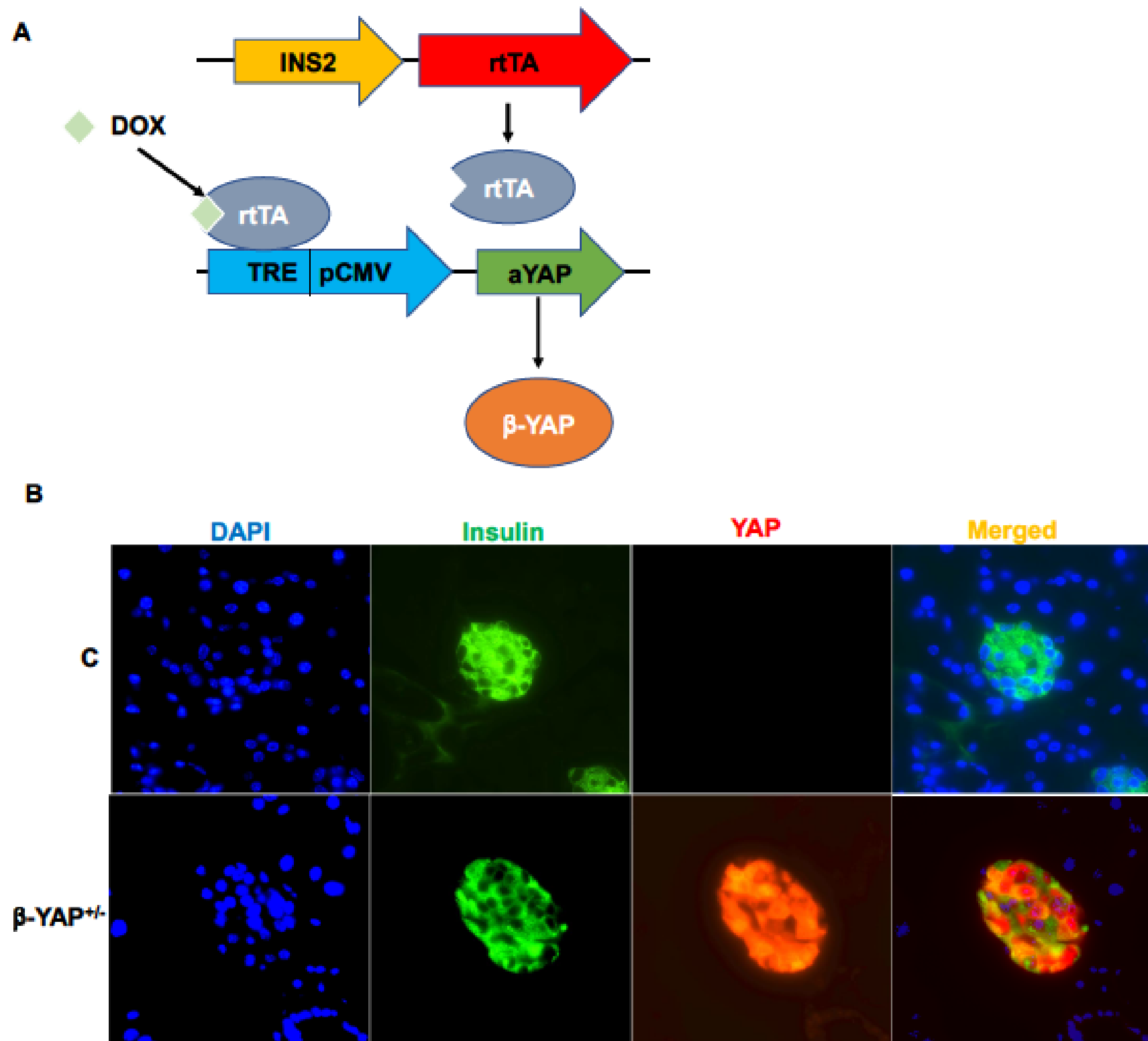

Figure 1

Figure 1. Generation of inducible β-cell YAP-overexpressing mice. (A) Scheme how β-YAP-OE mice were generated by crossing RIP-rtTA with TetO-YAP^{Ser127A} mice. **(B)** IHC confirmation of YAP induction in pancreatic β-Cells after 2 weeks treatment with dox.

YAP overexpression promotes β-cell regeneration *in vivo* and restores glycemia in streptozotocin (STZ)-induced diabetes in mice

The STZ model is a widely used experimental model to study β-cell regeneration in the context of diabetes. In this model, STZ, a chemical compound that selectively damages insulin-producing β-cells in the pancreas, is administered to induce diabetes-like conditions in rodents. After the initial destruction of β-cells, the model allows to investigate the mechanisms and interventions that promote β-cell regeneration, with the aim of restoring insulin production and understanding the regeneration capacity of β-cells [32; 33].

In a first strategy, a single high dose of STZ injection (200 mg/kg body weight) was given to the mice, and β-YAP was induced constitutively for two weeks after STZ (referred to as β-YAP; ON; Figure 2A). Following the injection of STZ, hyperglycemia became evident as it gradually increased to over 400 mg/dl within one week. This elevated glucose level remained stable for an additional 2 weeks in the control mice that received STZ ($YAP^{+/-}$ STZ Dox-) (Figure 2B). In contrast, when YAP was induced specifically in β-cells by administering dox in the drinking water, glucose levels were significantly reduced in STZ mice already after 3 days of YAP induction in β-$YAP^{+/-}$ heterozygous as well as in β-$YAP^{+/+}$ homozygous mice, an effect which remained stable over the 2 weeks of the experiment (Figure 2B). In control non-diabetic mice, $YAP^{+/-}$ heterozygous expression had no effect on glycemia (Figure 2B). At the end of the experiment, intraperitoneal glucose (GTT) and insulin tolerance tests (ITT) were carried out. The results of the GTT confirmed elevated glucose levels in the STZ control mice at all time points during the test (Figure 2C). In contrast, β-YAP-OE mice displayed a dose-dependent effect, leading to a reduction in glucose levels. Specifically, glucose levels were reduced in β-$YAP^{+/-}$, and this protective effect was further potentiated in β-$YAP^{+/+}$ mice when compared to the heterozygous STZ-injected control mice (Figure 2C). Furthermore, insulin tolerance, as measured by the ITT, remained unaffected by β-cell YAP induction (Figure 2D). However, the STZ-induced increase in glucose levels after 4 hours of fasting was confirmed (Figure 2D).

Importantly, in nondiabetic β-$YAP^{+/-}$ control mice, an augmentation in percentage of β-cell mass (Figure 2E) and β-cell area (Figure 2F) was evident indicating a cell-autonomous impact of YAP on β-cell mass induction under normal conditions. In line with the observed metabolic improvements in β-$YAP^{+/-}$ and β-$YAP^{+/+}$ mice, there was

a significant increase in both the percentage of β-cell mass (Figure 2G) and β-cell area (Figure 2H) in β-YAP$^{+/-}$ and β-YAP$^{+/+}$ mice, when compared to STZ-treated control mice. Of note, under the STZ-induced diabetic conditions, the β-cell area in β-YAP$^{+/+}$ mice nearly reached the area seen in the non-diabetic control mice. While there was a substantial increase in β-cell mass in STZ-treated β-YAP$^{+/+}$ mice, it remained notably lower than that observed in the nondiabetic mice likely due to a reduced pancreatic weight in the STZ-treated mice.

To assess the impact of β-YAP overexpression, whether the increase in β-cell mass resulted from augmented β-cell replication and/or reduced β-cell apoptosis, we performed a comprehensive examination of β-cell proliferation and survival. Quantification of double labelled-Ki67/insulin (Figure 2I) and pHH3/insulin+ co-positive cells (Figure 2J) clearly demonstrated that a substantial increase in the number of proliferating β-cells in both β-YAP$^{+/-}$ and β-YAP$^{+/+}$ mice, compared to their respective controls in diabetic as well as in nondiabetic mice. TUNEL staining for survival studies showed that the apoptotic β-cells were robustly abolished in both β-YAP$^{+/-}$ and β-YAP$^{+/+}$ mice in comparison to the STZ-treated control mice (Figure 2K). β-cell viability was also improved in non-diabetic β-YAP$^{+/-}$ mice under physiological conditions (Figure 2K).

In a second strategy, the mice were administered a single high dose of STZ injection. Subsequently, β-YAP was induced one week after STZ for a duration of one week, followed by an interim 1-week pause, and another week of YAP-induction (referred to as β-YAP; ON-OFF-ON; Figure 3A). As expected, STZ rapidly induced hyperglycemia within the first week of injection. Upon the induction of β-YAP, blood glucose levels significantly decreased, similarly in both STZ-injected β-YAP$^{+/-}$ and β-YAP$^{+/+}$ mice (Figure 3B). However, when β-YAP induction was turned OFF, glucose levels promptly rose back to diabetic levels, only to mildly normalize during last week of the experiment when β-YAP was reinduced (Figure 3B). This clearly demonstrated a YAP-dependent effect: YAP induction immediately lowered glucose levels, but a one-week intervention was not sufficient for sustained restoration. The subsequent reactivation of YAP led to the normalization of glucose levels. Random glucose levels were similarly reduced in both STZ-treated β-YAP$^{+/-}$ and β-YAP$^{+/+}$ mice compared to STZ-control mice, and the results of the GTT showed a YAP-dosage dependent glycemia effect with β-YAP$^{+/+}$ mice having greater and more significant improvement in glycemia (Figure 3C).

Similar to the restoration of β-regenerative capacity observed in the 2-week YAP-induction experiment (Figure 2), there was a significant enhancement in β-cell mass and area (Figures 3D,E) as a result of decreased apoptosis (TUNEL^{+} -β-cells) and increased proliferation (Ki67^{+} and pHH3^{+} β-cells) (Figures 3F-I), compared to STZ-control mice. The combined metabolic and morphological results suggest that overexpression of YAP specifically in pancreatic β-cells promotes the regeneration of endogenous β-cells by restoring the β-cell mass, enhancing proliferation, and reducing β-cell apoptosis in STZ-induced diabetic mice. Although there was a tendency towards a more pronounced improvement in regeneration in β-YAP$^{+/+}$ vs. β-YAP$^{+/-}$ mice, such a dose-dependent effect did not achieve statistical significant. The convergence of metabolic and morphological improvements indicates that the targeted re-expression of YAP in pancreatic β-cells promotes β-cell regeneration, ultimately leading to restoration of β-cell mass in the STZ-induced model of β-cell destruction and diabetes.

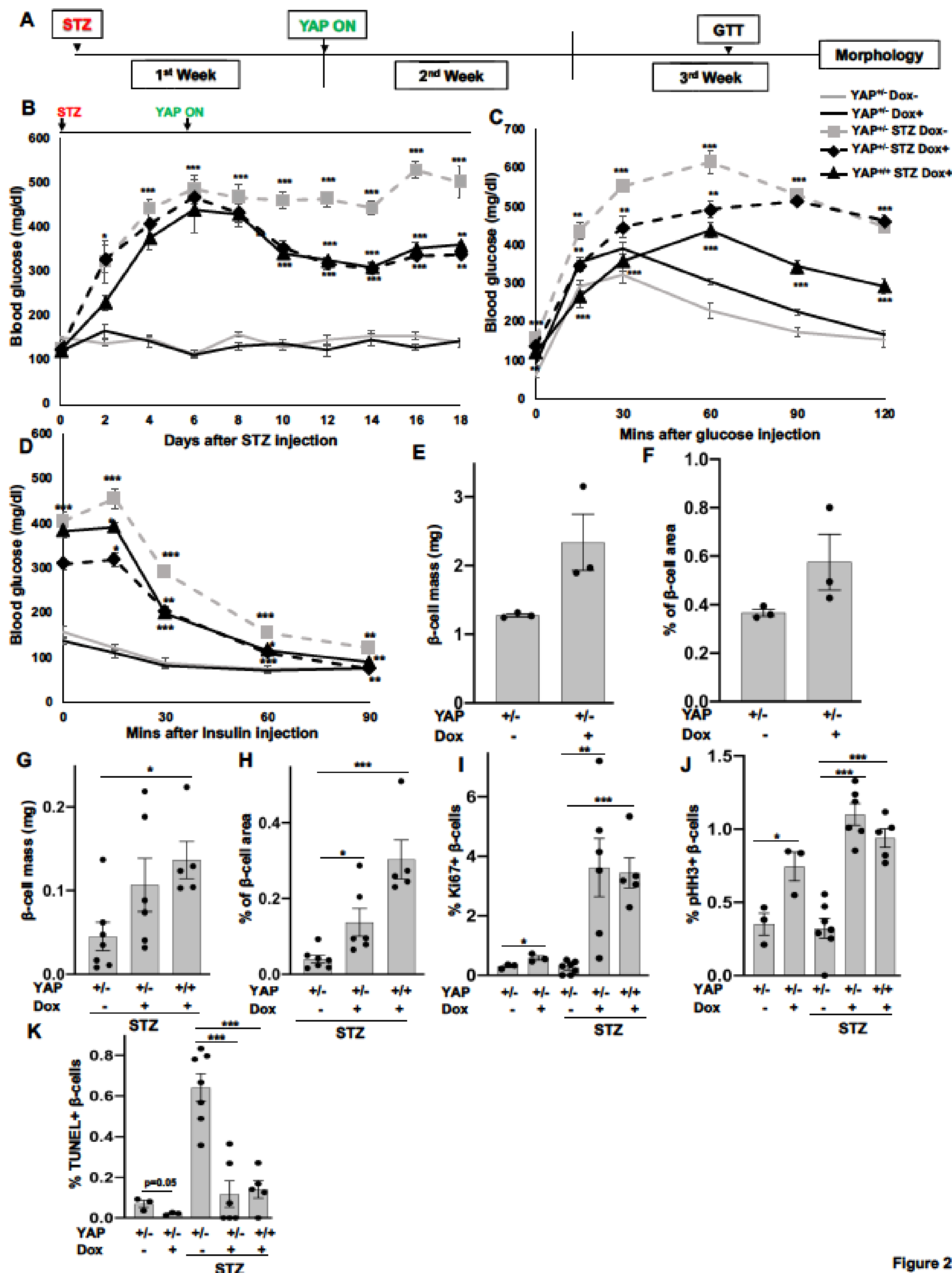

Figure 2. Constitutive YAP overexpression restores glycemia and induce β-cell mass in STZ induced diabetic mice. (A-K) Metabolic and morphological analyses of YAP overexpression in STZ-injected and control mice using the following mice: YAP$^{+/-}$ without dox (n=3), YAP$^{+/-}$ with dox (n=3), YAP$^{+/-}$ with STZ, without dox (n=7), YAP$^{+/-}$ with both STZ, dox (n=6-7) and YAP$^{+/+}$ with both STZ, dox (n=5). **(A)** Scheme of

experimental strategy. **(B)** Fed random blood glucose measurements after a single high dose of STZ (200mg/kg body weight) injection (day 0) over 18 days. **(C)** i.p. Glucose Tolerant Test (ipGTT) performed on day 19. **(D)** i.p. Insulin Tolerant Test (ipITT) performed on day 21. **(E,F)** β-cell mass and percentage (%) of β-cell area for the control mice with and without dox. **(G,H)** β-cell mass and % of β-cell area for the STZ mice with and without dox. **(I,J,K)** Quantitative analysis of triple staining for Ki67 or pHH3 or TUNEL, insulin and DAPI expressed as percentage of Ki67 or pHH3 or TUNEL positive β-cells. Data are expressed as means ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$; by two tailed unpaired t-test.

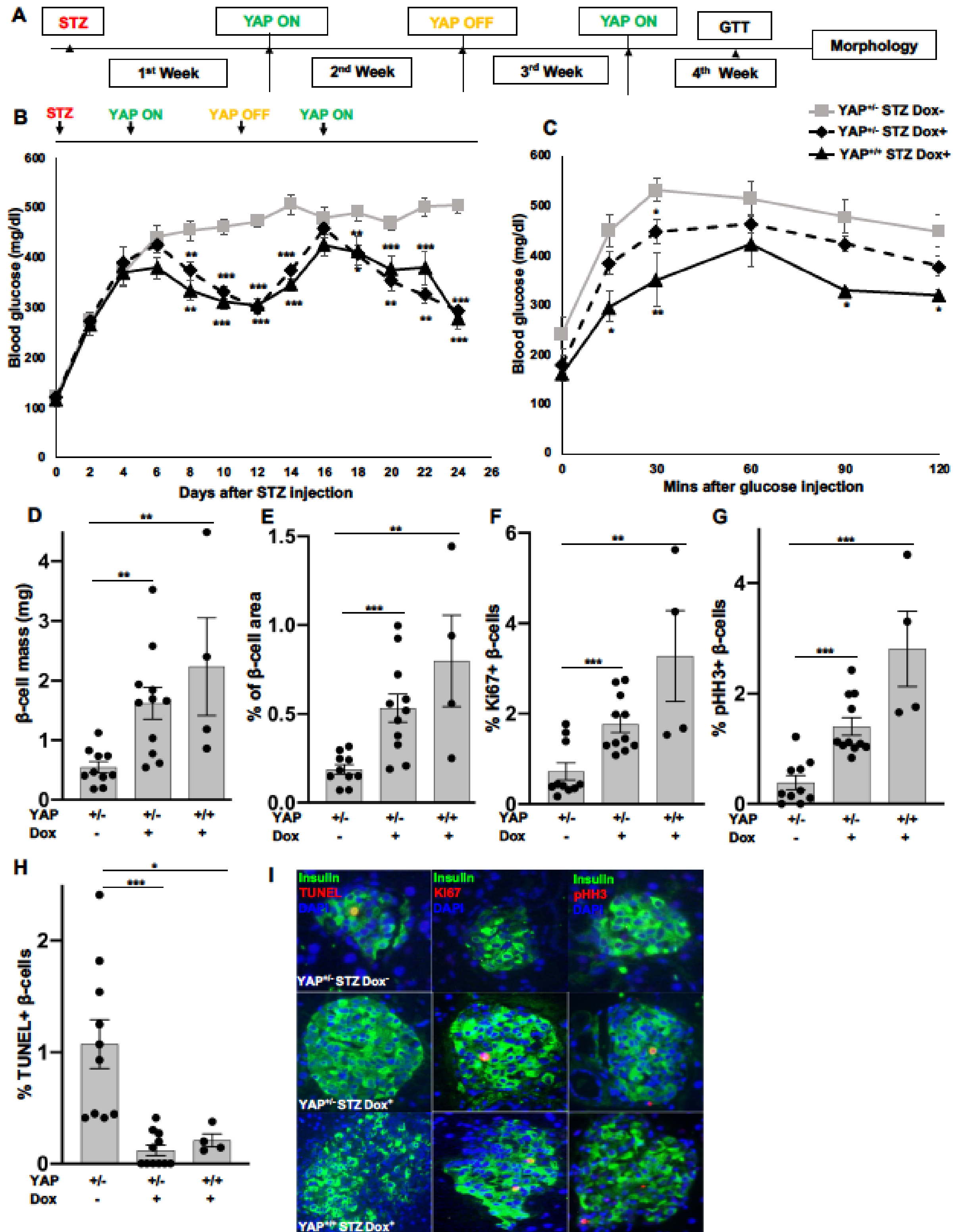

Figure 3

Figure 3. Bi-phasic YAP overexpression restores glycemia and induce β-cell mass in STZ-induced diabetic mice. (A-I) Metabolic and morphological analyses of YAP overexpression in STZ-injected and control mice using the following mice: $YAP^{+/-}$ with STZ, without dox (n=10-11), $YAP^{+/-}$ with both STZ, dox (n=11) and $YAP^{+/+}$ with both STZ, dox (n=4-5). **(A)** Scheme of experimental strategy. **(B)** Fed random blood

glucose measurements after a single high dose of STZ (200mg/kg body weight) injection (day 0) over 24 days. **(C)** i.p. GTT (ipGTT) performed on day 28. **(D,E)** β-cell mass and percentage (%) of β-cell area for the STZ mice with and without dox. **(F,G,H)** Quantitative analysis of triple staining for Ki67 or pHH3 or TUNEL, insulin and DAPI expressed as percentage of Ki67 or pHH3 or TUNEL positive β-cells. **(I)** Representative images of triple staining for (Ki67 or pHH3 or TUNEL in red, insulin in green and DAPI in blue) $YAP^{+/-}$ STZ Dox-, $YAP^{+/-}$ STZ Dox+, $YAP^{+/+}$ STZ Dox+ mice. Data are expressed as means ± SEM. *p<0.05, **p<0.01, ***p<0.001; by two tailed unpaired t-test.

YAP overexpression provides protection against STZ-induced β-cell injury by reducing GLUT2 expression

STZ is a cytotoxin that selectively targets pancreatic β-cells and is widely used to induce experimental diabetes in rodent models. As a glucose analogue, STZ is transported to β-cells via the low affinity glucose transporter 2 (GLUT2) in the plasma membrane [34; 35]. After undergoing chemical transformations, STZ triggers DNA and oxidative damage, ultimately resulting in an increase in β-cell death [36; 37]. Importantly, it has been demonstrated that both homozygous and heterozygous GLUT2-knockout (KO) mice are resistant to STZ-induced hyperglycemia. This finding underscores the vital role of GLUT2 in STZ's entry and action, as even a 50% reduction in GLUT2 gene expression confers full protection against STZ-induced diabetes [38]. A significant fraction of highly proliferating β-cells exhibits metabolic immaturity [39], as they must simultaneously downregulate numerous metabolic and functional genes, including those related to glucose metabolism as well as insulin expression and secretion, in order to allocate energy and cellular resources toward increasing their mass for replication. This process carries the risk of losing functional markers such as GLUT2 during YAP-induced robust β-cell proliferation. Given this knowledge, we embarked on an investigation to assess whether YAP re-expression in β-cells could influence the expression of GLUT2 in pancreatic islets. Notably, the transcript levels of the GLUT2 transporter (encoded by the *Slc2a2* gene) exhibited a substantial reduction of 95% in β-$YAP^{+/+}$ mice and 60% in β-$YAP^{+/-}$ animals. As GLUT2 mRNA levels declined in YAP-overexpressing islets, we further probed whether this reduction would translate into a decrease in GLUT2 protein expression on the surface of β-cells. Immunostaining of GLUT2 in combination with insulin revealed that GLUT2 protein

expression was nearly absent in pancreatic β-cells of β-YAP$^{+/+}$ mice and highly downregulated in β-YAP$^{+/-}$ mice. These findings provide insight into the mechanism by which YAP overexpression shields β-cells from STZ-induced entry and the subsequent cell death. Consistent with our findings, the conditional overexpression of PAX4 in adult β-cells protected against STZ-induced hyperglycemia by reducing the expression of GLUT2 regulator, transcription factor MafA, and subsequent decline in GLUT2 expression levels [38].

A key feature of diabetes is the insufficient functional β-cell mass. Identifying signaling molecules capable of stimulating β-cell mass regeneration within the context of diabetes is of paramount importance. It has been proposed that a successful therapy targeting β-cells for diabetes should prioritize the simultaneous prevention of β-cell apoptosis and enhancement of β-cell proliferation [40; 41]. Recent research has illuminated the significance of YAP in tissue regeneration. It has been observed that experimentally increasing YAP activity results in the entry of nondividing or poorly dividing cells into the cell cycle, a phenomenon noted in both liver [30; 42] and heart [43; 44; 45]. In this study, we show that the re-expression of the Hippo pathway terminal effector YAP has a strong capacity to promote β-cell regeneration by inducing proliferation of endogenous β-cells and protecting them from apoptosis. This is in line with our previous data in isolated human islets, where we demonstrated the YAP-overexpression could foster the replication of human β-cells and protect them from apoptosis under pro-inflammatory cytokines and glucolipotoxic conditions [29]. It is important to note that, unlike the current data on YAP overexpression in mouse β-cells, the expression of GLUT2 and other functional genes remained unaltered in isolated human islets upon YAP overexpression. This discrepancy could be attributed to species differences and may also stem from the chronic nature of YAP overexpression in mice (lasting several weeks) as compared to the relatively short duration of YAP overexpression in human islets *in vitro* (lasting several days).

In conclusion, our study highlights the potential of YAP modulation as a promising avenue for influencing both β-cell replication and survival. This holds great promise for regenerative therapies aimed at restoring β-cell mass in diabetes. However, it is crucial to acknowledge the potential negative impact of the robust proliferation induced by YAP on essential metabolic and functional genes. Our findings clearly demonstrate that YAP-induced proliferation can negatively affect GLUT2 gene expression, a critical component of glucose metabolism and insulin secretion in β-cells. Given the inverse

relationship between β-cell replication and function, any regenerative approach should be meticulously optimized in terms of timing, dosage, and specificity. It is imperative that these strategies do not compromise glucose metabolism and insulin secretion in β-cells. Achieving the optimal balance between mature functionality and the capacity for expansion is a critical factor in ensuring the overall success of regenerative therapies for diabetes.

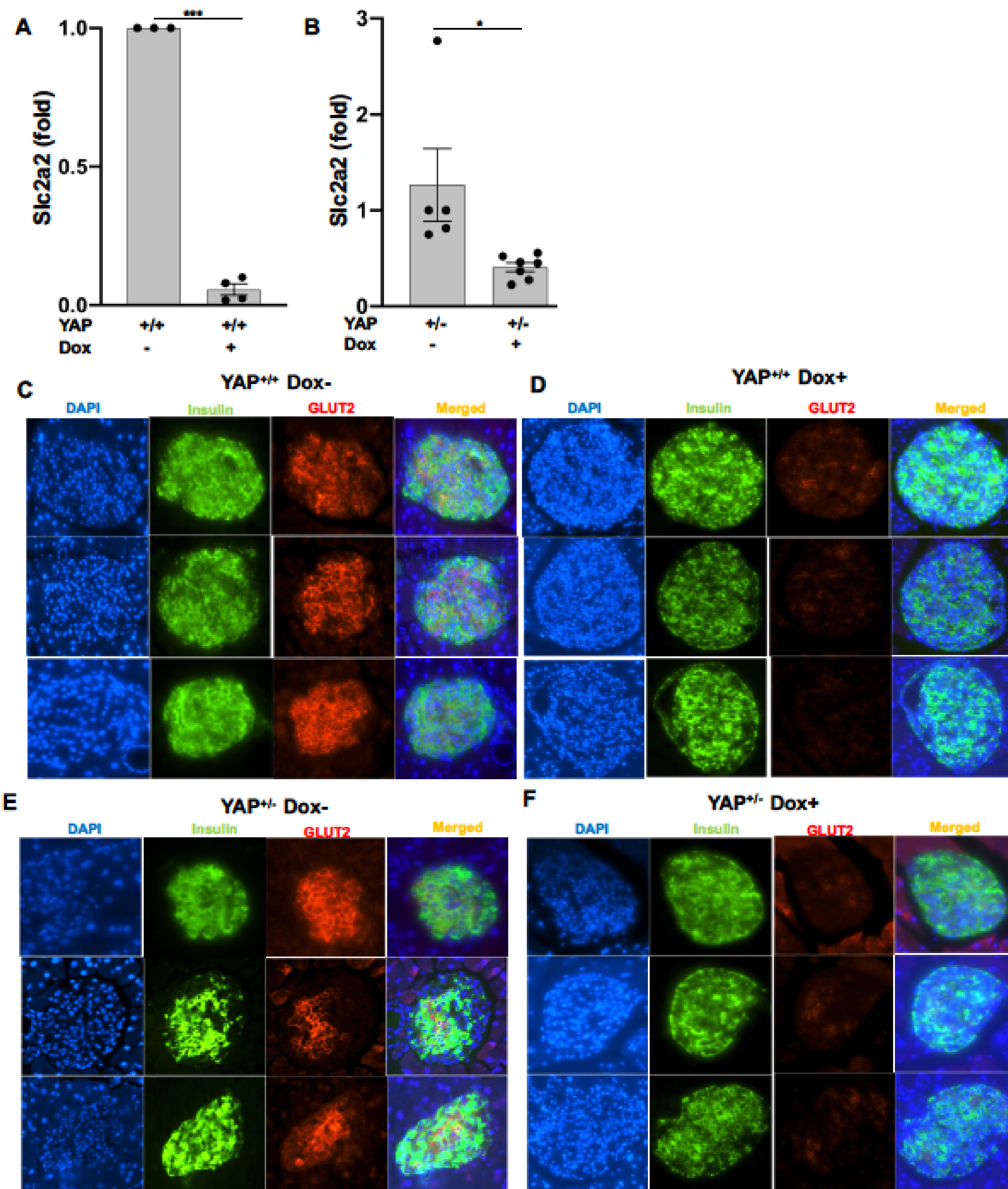

Figure 4. YAP overexpression reduces GLUT2 expression in pancreatic β-cells. (A,B) qPCR for *slc2a2* mRNA expression normalized to actin in isolated mouse islets from **(A)** β-YAP$^{+/+}$ and **(B)** β-YAP$^{+/-}$ mice (n=3-7). **(C-F)** Representative immunohistochemistry images of triple staining for GLUT2 in red, insulin in green and

DAPI in blue in **(C,D)** β-YAP$^{+/+}$ and **(E,F)** β-YAP$^{+/-}$ mice. Data are expressed as means ± SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$; by two tailed unpaired t-test.

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Author contributions

Conceptualization AA.; Experimental design MKM., KM., and AA.; Investigation MKM., LS., FL., SB., RCN., IK., MY., SR., SG., HL., HP., KM and A.A.; Formal Analysis MKM., KM., and AA.; Writing MKM., KM., and AA.; Funding Acquisition and Supervision KM and AA.

Declaration of interests

The authors declare no competing interests.

Methods

Mice

β-Cell specific YAP overexpressing mice (Rip-Ins2-TetO-hYAP1-A127A; referred to as β-YAP-OE) were obtained by crossing inducible aYAP overexpressing mice (TetO-YAPser127A, provided to our laboratory by Fernando Camargo, Boston Children Hospital, Boston, MA, USA) with mice carrying the tetracycline trans-activator (tTA) under the control of the insulin promoter (RIP-rtTA mice, provided by Al Powers, Vanderbilt University medical Center, Nashville, TN, USA). Following treatment with doxycycline (1mg/ml) through drinking water, the rtTA gene is specifically activated in β-cells by the Ins2 promoter. The rtTA protein can then bind to the Tet response element (TRE) and subsequently trigger transcription of the constitutively active form of the YAP gene, which is located under a CMV promoter element. This system allows for a precise spatiotemporal control of YAP gene expression in pancreatic β-cells. All experiments were performed using mice aged 8 to 10 weeks, with homozygous (β-

YAP$^{+/+}$) and heterozygous (β-YAP$^{+/-}$) genotypes. In single high-dose streptozotocin (STZ) experiments, 8 to 10-week-old male mice injected with a single high-dose STZ (200 mg/kg body weight) freshly dissolved in 50 mM sodium citrate buffer. One week later, doxycycline was administered. At the end of the metabolic studies, mice were sacrificed, and the pancreas was harvested and sectioned for further morphological analysis. All mice used in this experiment were housed in a temperature-controlled room with a 12h light-dark cycle and had free access to food and water in accordance with NIH animal care guidelines, §8 of the German Animal Protection Act, the German Welfare Act, and the guidelines of the Society of Laboratory Animals (GV-SOLAS) and the Federation of Laboratory Animal Science Associations (FELASA). All protocols have been approved by the Bremen Senate (Senator for Science, Health and Consumer Protection) and we have complied with all relevant ethical regulations for animal experiments and research.

Islet isolation

Mouse pancreatic islets were isolated after (or without; controls) doxycycline induction by pancreatic perfusion with Liberase™ (#05401119001, Roche, Mannheim, Germany) according to the manufacturer's instructions and digested at 37°C, followed by washing and hand picking. Mouse islets were cultured in complete RPMI-1640 medium (Sigma Aldrich, Missouri, MO, USA) medium containing 11.1 mM glucose. All media were supplemented with L-glutamate, 1% penicillin-streptomycin, and 10% fetal bovine serum (FBS).

Glucose and insulin tolerance tests

For blood glucose measurements, blood was drawn from the tail vein, and glucose was measured using a glucometer (FreeStyle; Abbott, IL, USA). Intraperitoneal glucose tolerance tests (ipGTT) were performed by fasting the mice overnight for 12 hours, followed by i.p. injection of glucose (B. Braun, Germany) at a dose of 1g/kg body weight. Blood samples were collected at 0, 15, 30, 60, 90, and 120 min. For i.p. Insulin Tolerance Testing (ipITT), mice were fasted for 4 hours and then injected with recombinant human insulin (Novo Nordisk, Denmark) at a dose of 0.75 U/kg body weight. The glucose concentration was determined using a glucometer at 0, 15, 30, 60, and 90 minutes.

qPCR analysis

Total RNA was isolated from cultured isolated mouse islets cells using TriFast (PEQLAB Biotechnologie, Germany). 500-1000ng of RNA were reverse transcribed to

cDNA (RevertAid reverse transcriptase, Thermo Fisher Scientific (TFS), MA, USA). Quantitative RT-PCR was carried out as previously described using Biosystems StepOne Real-Time PCR system (Applied Biosystems, CA, USA) with TaqMan assays. TaqMan® Gene Expression Assays were used for *Slc2A2* (#Mm00446229_m1), and *Actin* (#Mm00607939_s1).

Immunohistochemistry

Mouse pancreases were dissected, fixed in 4% formaldehyde at 4°C for 8h, and dehydrated before embedding in paraffin. Mouse pancreatic sections (4μm) were deparaffinized, rehydrated, and blocked with a blocking buffer containing 3% BSA. Paraffin-embedded pancreatic tissues were incubated overnight at 4°C with guinea pig anti-insulin (#IR002, Flex polyclonal DAKO), rabbit anti-YAP (#4912, CST), rabbit anti-Ki67 (#AB16667, Abcam), rabbit anti GLUT2 (#07-1402-I, Millipore), rabbit anti-pHH3 (#06-570, Merck-millipore), followed by fluorescein isothiocyanate (FITC; #715-096-148) - conjugated donkey anti-guinea pig or CY3 (#715-165-150) conjugated donkey anti-rabbit/mouse and Biotin-conjugated donkey anti-guinea pig secondary antibodies (Jackson Immuno Research Laboratories, PA, USA). Slides were fixed with Vectashield containing 406-diamidino-2-phenylindole (DAPI) (Vector Labs, CA, USA). Pancreatic β-cell apoptosis was analyzed using the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique according to the manufacturer's instructions (In situ Cell Death Detection Kit, TMR red; Roche, Switzerland) and double-stained with insulin. Fluorescence was analyzed using a Nikon MEA53200 microscope (Nikon GmbH, Germany), and images were captured using the Nikon's NIS -Elements software.

Morphometric analysis

Ten sections (across the width of the pancreas) per mouse were analyzed for morphometric data. The pancreatic tissue area and insulin-positive area (detected using the VECTASTAIN ABC kit; Vector Labs, USA) were determined by computer-assisted measurements using a Nikon MEA53200 microscope (Nikon GmbH, Germany), and images were acquired using Nikon's NIS -Elements software. The mean percentage of β-cell content per pancreas was calculated as the ratio of insulin-positive to the total pancreatic area. The pancreatic β-cell mass was determined by multiplying the β-cell fraction by the weight of the pancreas [18].

Statistical analyses

Statistical comparisons between groups were analyzed for significance by unpaired two-tailed Student's t-test. p value<0.05 was considered statistically significant. Data are presented as means ± SEM. The exact values of *n* (refers to number of mice, or number of independent biological experiments), and statistical significance are reported in the figure legends.

References:

[1] N.S. Wilcox, J. Rui, M. Hebrok, and K.C. Herold, Life and death of beta cells in Type 1 diabetes: A comprehensive review. J Autoimmun 71 (2016) 51-8.

[2] P.C. Butler, J.J. Meier, A.E. Butler, and A. Bhushan, The replication of beta cells in normal physiology, in disease and for therapy. Nat Clin Pract Endocrinol Metab 3 (2007) 758-68.

[3] E. Karakose, C. Ackeifi, P. Wang, and A.F. Stewart, Advances in drug discovery for human beta cell regeneration. Diabetologia 61 (2018) 1693-1699.

[4] Y. Dor, J. Brown, O.I. Martinez, and D.A. Melton, Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 429 (2004) 41-6.

[5] G. Basile, R.N. Kulkarni, and N.G. Morgan, How, When, and Where Do Human beta-Cells Regenerate? Curr Diab Rep 19 (2019) 48.

[6] R.N. Kulkarni, E.B. Mizrachi, A.G. Ocana, and A.F. Stewart, Human beta-cell proliferation and intracellular signaling: driving in the dark without a road map. Diabetes 61 (2012) 2205-13.

[7] E. Bernal-Mizrachi, R.N. Kulkarni, D.K. Scott, F. Mauvais-Jarvis, A.F. Stewart, and A. Garcia-Ocana, Human beta-cell proliferation and intracellular signaling part 2: still driving in the dark without a road map. Diabetes 63 (2014) 819-31.

[8] A.F. Stewart, M.A. Hussain, A. Garcia-Ocana, R.C. Vasavada, A. Bhushan, E. Bernal-Mizrachi, and R.N. Kulkarni, Human beta-cell proliferation and intracellular signaling: part 3. Diabetes 64 (2015) 1872-85.

[9] A. Dey, X. Varelas, and K.L. Guan, Targeting the Hippo pathway in cancer, fibrosis, wound healing and regenerative medicine. Nat Rev Drug Discov 19 (2020) 480-494.

[10] A. Ardestani, B. Lupse, and K. Maedler, Hippo Signaling: Key Emerging Pathway in Cellular and Whole-Body Metabolism. Trends Endocrinol Metab 29 (2018) 492-509.

[11] F. Zanconato, M. Forcato, G. Battilana, L. Azzolin, E. Quaranta, B. Bodega, A. Rosato, S. Bicciato, M. Cordenonsi, and S. Piccolo, Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. Nature cell biology 17 (2015) 1218-27.

[12] K.F. Harvey, X. Zhang, and D.M. Thomas, The Hippo pathway and human cancer. Nat Rev Cancer 13 (2013) 246-57.

[13] G. Halder, and R.L. Johnson, Hippo signaling: growth control and beyond. Development 138 (2011) 9-22.

[14] K. Harvey, and N. Tapon, The Salvador-Warts-Hippo pathway - an emerging tumour-suppressor network. Nature reviews. Cancer 7 (2007) 182-91.

[15] A. Ardestani, B. Lupse, and K. Maedler, Hippo Signaling: Key Emerging Pathway in Cellular and Whole-Body Metabolism. Trends Endocrinol Metab (2018).

[16] A. Ardestani, and K. Maedler, MST1: a promising therapeutic target to restore functional beta cell mass in diabetes. Diabetologia 59 (2016) 1843-9.

[17] A. Ardestani, and K. Maedler, The Hippo Signaling Pathway in Pancreatic beta-Cells: Functions and Regulations. Endocr Rev 39 (2018) 21-35.

[18] A. Ardestani, F. Paroni, Z. Azizi, S. Kaur, V. Khobragade, T. Yuan, T. Frogne, W. Tao, J. Oberholzer, F. Pattou, J.K. Conte, and K. Maedler, MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes. Nat Med 20 (2014) 385-397.
[19] T. Yuan, S. Rafizadeh, Z. Azizi, B. Lupse, K.D. Gorrepati, S. Awal, J. Oberholzer, K. Maedler, and A. Ardestani, Proproliferative and antiapoptotic action of exogenously introduced YAP in pancreatic beta cells. JCI Insight 1 (2016) e86326.
[20] T. Yuan, K.D. Gorrepati, K. Maedler, and A. Ardestani, Loss of Merlin/NF2 protects pancreatic beta-cells from apoptosis by inhibiting LATS2. Cell Death Dis 7 (2016) e2107.
[21] T. Yuan, K. Maedler, and A. Ardestani, Pancreatic β-cell rescue in diabetes by targeting Merlin. Expert Review of Endocrinology & Metabolism 12 (2017) 97-99.
[22] T. Yuan, K. Annamalai, S. Naik, B. Lupse, S. Geravandi, A. Pal, A. Dobrowolski, J. Ghawali, M. Ruhlandt, K.D.D. Gorrepati, Z. Azizi, D.S. Lim, K. Maedler, and A. Ardestani, The Hippo kinase LATS2 impairs pancreatic beta-cell survival in diabetes through the mTORC1-autophagy axis. Nat Commun 12 (2021) 4928.
[23] I. Cebola, S.A. Rodriguez-Segui, C.H. Cho, J. Bessa, M. Rovira, M. Luengo, M. Chhatriwala, A. Berry, J. Ponsa-Cobas, M.A. Maestro, R.E. Jennings, L. Pasquali, I. Moran, N. Castro, N.A. Hanley, J.L. Gomez-Skarmeta, L. Vallier, and J. Ferrer, TEAD and YAP regulate the enhancer network of human embryonic pancreatic progenitors. Nat Cell Biol 17 (2015) 615-626.
[24] A. Mamidi, C. Prawiro, P.A. Seymour, K.H. de Lichtenberg, A. Jackson, P. Serup, and H. Semb, Mechanosignalling via integrins directs fate decisions of pancreatic progenitors. Nature 564 (2018) 114-118.
[25] N.M. George, C.E. Day, B.P. Boerner, R.L. Johnson, and N.E. Sarvetnick, Hippo signaling regulates pancreas development through inactivation of Yap. Mol Cell Biol 32 (2012) 5116-28.
[26] T. Gao, D. Zhou, C. Yang, T. Singh, A. Penzo-Mendez, R. Maddipati, A. Tzatsos, N. Bardeesy, J. Avruch, and B.Z. Stanger, Hippo signaling regulates differentiation and maintenance in the exocrine pancreas. Gastroenterology 144 (2013) 1543-53, 1553 e1.
[27] N.M. George, B.P. Boerner, S.U. Mir, Z. Guinn, and N.E. Sarvetnick, Exploiting Expression of Hippo Effector, Yap, for Expansion of Functional Islet Mass. Mol Endocrinol 29 (2015) 1594-607.
[28] T.J. Pullen, M.O. Huising, and G.A. Rutter, Analysis of Purified Pancreatic Islet Beta and Alpha Cell Transcriptomes Reveals 11beta-Hydroxysteroid Dehydrogenase (Hsd11b1) as a Novel Disallowed Gene. Front Genet 8 (2017) 41.
[29] T. Yuan, S. Rafizadeh, Z. Azizi, B. Lupse, K.D.D. Gorrepati, S. Awal, J. Oberholzer, K. Maedler, and A. Ardestani, Proproliferative and antiapoptotic action of exogenously introduced YAP in pancreatic beta cells. JCI Insight 1 (2016) e86326.
[30] F.D. Camargo, S. Gokhale, J.B. Johnnidis, D. Fu, G.W. Bell, R. Jaenisch, and T.R. Brummelkamp, YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol 17 (2007) 2054-60.
[31] Q. Cai, M. Brissova, R.B. Reinert, F.C. Pan, P. Brahmachary, M. Jeansson, A. Shostak, A. Radhika, G. Poffenberger, S.E. Quaggin, W.G. Jerome, D.J. Dumont, and A.C. Powers, Enhanced expression of VEGF-A in beta cells increases endothelial cell number but impairs islet morphogenesis and beta cell proliferation. Developmental biology 367 (2012) 40-54.
[32] M. Kleinert, C. Clemmensen, S.M. Hofmann, M.C. Moore, S. Renner, S.C. Woods, P. Huypens, J. Beckers, M.H. de Angelis, A. Schurmann, M. Bakhti, M. Klingenspor, M. Heiman, A.D. Cherrington, M. Ristow, H. Lickert, E. Wolf, P.J. Havel, T.D. Muller, and M.H. Tschop, Animal models of obesity and diabetes mellitus. Nat Rev Endocrinol 14 (2018) 140-162.

[33] S. Sachs, A. Bastidas-Ponce, S. Tritschler, M. Bakhti, A. Bottcher, M.A. Sanchez-Garrido, M. Tarquis-Medina, M. Kleinert, K. Fischer, S. Jall, A. Harger, E. Bader, S. Roscioni, S. Ussar, A. Feuchtinger, B. Yesildag, A. Neelakandhan, C.B. Jensen, M. Cornu, B. Yang, B. Finan, R.D. DiMarchi, M.H. Tschop, F.J. Theis, S.M. Hofmann, T.D. Muller, and H. Lickert, Targeted pharmacological therapy restores beta-cell function for diabetes remission. Nat Metab 2 (2020) 192-209.
[34] Z. Wang, and H. Gleichmann, GLUT2 in pancreatic islets: crucial target molecule in diabetes induced with multiple low doses of streptozotocin in mice. Diabetes 47 (1998) 50-6.
[35] Z. Wang, and H. Gleichmann, Glucose transporter 2 expression: prevention of streptozotocin-induced reduction in beta-cells with 5-thio-D-glucose. Exp Clin Endocrinol Diabetes 103 Suppl 2 (1995) 83-97.
[36] E. Horwitz, L. Krogvold, S. Zhitomirsky, A. Swisa, M. Fischman, T. Lax, T. Dahan, N. Hurvitz, N. Weinberg-Corem, A. Klochendler, A.C. Powers, M. Brissova, A. Jorns, S. Lenzen, B. Glaser, K. Dahl-Jorgensen, and Y. Dor, beta-Cell DNA Damage Response Promotes Islet Inflammation in Type 1 Diabetes. Diabetes 67 (2018) 2305-2318.
[37] J.W. Cardinal, G.P. Margison, K.J. Mynett, A.P. Yates, D.P. Cameron, and R.H. Elder, Increased susceptibility to streptozotocin-induced beta-cell apoptosis and delayed autoimmune diabetes in alkylpurine-DNA-N-glycosylase-deficient mice. Mol Cell Biol 21 (2001) 5605-13.
[38] K.H. Hu He, P.I. Lorenzo, T. Brun, C.M. Jimenez Moreno, D. Aeberhard, J. Vallejo Ortega, M. Cornu, F. Thorel, A. Gjinovci, B. Thorens, P.L. Herrera, P. Meda, C.B. Wollheim, and B.R. Gauthier, In vivo conditional Pax4 overexpression in mature islet beta-cells prevents stress-induced hyperglycemia in mice. Diabetes 60 (2011) 1705-15.
[39] S. Puri, N. Roy, H.A. Russ, L. Leonhardt, E.K. French, R. Roy, H. Bengtsson, D.K. Scott, A.F. Stewart, and M. Hebrok, Replication confers beta cell immaturity. Nat Commun 9 (2018) 485.
[40] C. Jain, Ansarullah, S. Bilekova, and H. Lickert, Targeting pancreatic beta cells for diabetes treatment. Nat Metab 4 (2022) 1097-1108.
[41] C. Aguayo-Mazzucato, and S. Bonner-Weir, Pancreatic beta Cell Regeneration as a Possible Therapy for Diabetes. Cell Metab 27 (2018) 57-67.
[42] J. Dong, G. Feldmann, J. Huang, S. Wu, N. Zhang, S.A. Comerford, M.F. Gayyed, R.A. Anders, A. Maitra, and D. Pan, Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell 130 (2007) 1120-33.
[43] M. Xin, Y. Kim, L.B. Sutherland, M. Murakami, X. Qi, J. McAnally, E.R. Porrello, A.I. Mahmoud, W. Tan, J.M. Shelton, J.A. Richardson, H.A. Sadek, R. Bassel-Duby, and E.N. Olson, Hippo pathway effector Yap promotes cardiac regeneration. Proc Natl Acad Sci U S A 110 (2013) 13839-44.
[44] A. von Gise, Z. Lin, K. Schlegelmilch, L.B. Honor, G.M. Pan, J.N. Buck, Q. Ma, T. Ishiwata, B. Zhou, F.D. Camargo, and W.T. Pu, YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy. Proc Natl Acad Sci U S A 109 (2012) 2394-9.
[45] T. Heallen, M. Zhang, J. Wang, M. Bonilla-Claudio, E. Klysik, R.L. Johnson, and J.F. Martin, Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. Science 332 (2011) 458-61.

Publication 2:

The TEAD activator TT-10 promotes β-cell regeneration in human and mouse islets

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My contribution:

Designed, performed experiments, analyzed data and assembled the figures for: Figure 1 A-I, Figure 2 A-F, Figure 3 A-E, H, I-M, and Figure 4 A-G.

Wrote the paper.

The TEAD activator TT-10 promotes β-cell regeneration in human and mouse islets

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Abstract

Pancreatic β-cell regenerative therapy is crucial for addressing the loss of highly functional β-cells in both type 1 and type 2 diabetes (T1D/T2D). Despite a small subpopulation of cells within islets with a rather progenitor-like character, which are especially capable to regenerate, human β-cells have lost their proliferative capacity during maturation. One of the genes controlling β-cell proliferation is the Yes Associated Protein (YAP), a transcriptional co-activator of the Hippo pathway; it classically acts through TEAD family of transcription factors (TEAD1–4), but is lost during β-cell differentiation. In our search for a small molecule capable of activating the YAP-TEAD proliferative pathway, we find TT-10, a stimulator of TEAD transcription factors, which robustly induces β-cell proliferation. Our data demonstrate that TT-10 treatment significantly promotes β-cell proliferation in islets isolated islets from both wild-type and leptin receptor deficient db/db diabetic mice. In db/db mouse islets, TT-10 also inhibits β-cell apoptosis. Consistent with the potent pro-proliferative effect of YAP on human β-cells, *TT-10 profoundly induces* β-cell proliferation in both healthy human islets and those from donors with T2D, compared to the untreated controls. The TT-10-induced β-cell proliferation is completely abolished by both genetic and pharmacological inhibition of TEAD function suggesting the TEAD-dependent action of TT-10. Notably, TT-10-induced β-cell replication does not adversely affect insulin secretion or the expression of β-cell functional and maturation genes. Instead, it significantly upregulates β-cell identity genes such as *MAFA*, *SLC2A2, ABCC8, KCNJ11, GLIS3 and PDX1*. Importantly, TT-10 markedly elevates the expression of TEAD-related proliferative genes *FoxM1 and Myc,* which are specifically associated with β-cell proliferation in human islets. Intriguingly, a short 7-day course of TT-10 injections promotes β-cell proliferation in mice *in vivo*. Our results show that TT-10, through downstream TEAD signaling, exhibits strong pro-proliferative activity in mouse and human β-cells *in vitro* and in mice *in vivo.* These findings suggest that TT-10 could be a novel candidate for β-cell regenerative therapy to restore functional mature β-cells in diabetes.

Introduction

The loss of functional pancreatic β-cells is central to the pathology of both in type 1 diabetes (T1D) and type 2 diabetes (T2D). Generating new stable and highly functional β-cells is an essential goal for diabetes therapy. Human β-cells have a very limited capacity to proliferate after birth, and this further declines with age. The highest proliferation rates are observed in new-borns [1], where it substantially decreases to below 1% in adolescents and becomes almost undetectable by middle age [2,3]. Despite these low proliferation levels, β-cell mass expands from birth to adulthood by replication of the existing β-cells suggesting that there is a certain degree of human β-cell proliferative capacity still exists [1,4].

Understanding the cellular signaling and molecular mechanisms responsible for β-cell quiescence is critical for designing regenerative strategies. Several signaling cascades have been identified as critical regulators of β-cell proliferation [5], including PI3K-AKT [6], TGFβ, ERK/MAPK [7], mammalian rapamycin complex 1 (mTORC1), and Wnt/β-catenin [8]. Theses cascades ultimately activates ocell cycle regulators, i.e. cyclin Ds, and cyclin-dependent kinases (CDKs) [9,10]. While modulating these pathways is potent in inducing rodent β-cell replication, they present less potential in human β-cells. In the search for pharmacological activators of human β-cell proliferation, several promising targets and pathways have been identified [5,11]. For example, the activation of calcium-dependent signaling, PI3K/AKT and CREB-IRS-2 pathways through GABA has been shown to promote the proliferation of transplanted human β-cell [12]. Similarly, GSK3β inhibitors stimulate β-cell proliferation in both cultured and transplanted human islets [13]. Modulating a AKT-GSK3β axis is also the mechanism through which osteoprotegerin (OPG) and denosumab, a monoclonal antibody that mimics OPG [14], as well as Serpin B1 [15] enhance human β-cell proliferation. OPG, responsible for adaptive proliferation in rodent β-cells during pregnancy, exerts a dual role by inducing proliferation and inhibiting apoptosis through the TNFα/RANK/p38-MAPK axes, making OPG a potent drug for β-cell protective therapy [14]. SerpinB1, a liver-derived secretory protein which inhibits β-cell proteases, also functions by activating key growth/survival pathways, including the insulin/IGF-1 in the β-cell [15].

Direct repression of the cell cycle inhibitor p16INK4a promotes human β-cell proliferation through interfering with TGFβ signaling [16]. Blocking TGFβ is also able to potentiate β-cell proliferation by harmine, an inhibitor of the dual-specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A). Inhibition of DYRK1A alone restores β-cell mitogenic activity, especially in human β-cells, through fostering the nuclear translocation of nuclear factor of activated T cell (NFAT) [17]. While previous studies have demonstrated a residual inducible proliferative capacity in human β-cells, the molecular reason why specifically human β-cells lose their proliferative capacity during maturation remains an open question. Several studies have found

that a subpopulation of cells within islets, particularly capable of regeneration, do exist [18-20]. These cells, with a progenitor-like character, have been subsequently named "virgin" β-cells [18] and are differ from mature β-cells, specifically in the expression of proliferative genes. It is possible that such cells could be a source of newly formed β-cells, proliferating and subsequently reverting to mature, differentiated β-cells.

As soon as an islet progenitor cell becomes a mature β-cell, characterized by its specific expression of Neurogenin 3 (NGN3), many genes associated with proliferation are lost. This is most likely to ensure tightly regulated β-cell function and to avoid hyperinsulinemia. One such gene whose repression coincides with NGN3 expression, is the Hippo pathway transcriptional co-activator Yes associated protein (YAP). YAP and its paralog TAZ are key downstream effectors of the Hippo signaling pathway, regulated negatively by core kinases MST1/2 and LATS1/2. When activated, YAP and TAZ accumulate in the nucleus, where they physically interact with TEA domain transcription factors (TEADs). This interaction is crucial for mediating the transcription of genes essential for cell proliferation and survival, thereby playing a significant role in cellular growth and regulatory processes [21,22].

YAP is strongly expressed in the pancreatic precursor cells at early stage of development of the pancreas, where it fosters the proliferation and expansion of multipotent pancreatic progenitor cells [23-26]. Its expression, while maintained in the exocrine compartments including the ducts, is low or undetectable, and "disallowed" in mature functional β-cells [27]. However, they do functionally express TAZ, as well as the transcription factor TEAD1, which is the predominant TEAD isoform in both mouse and human islets [28-30]. YAP, in cooperation with TEAD transcription factors, is essential for cell proliferation, growth and survival [26]. We have previously demonstrated that genetic re-expression of YAP highly promotes the proliferation of functional human β-cells [31], highlighting the Hippo pathway as a promising target for stimulating β-cell.

Recently, a versatile fluorinated compound known as TT-10 demonstrated its ability to induce proliferation of cardiomyocytes and protecting them from apoptosis in a myocardial infarction model both *in vivo* and *in vitro* by directly enhancing YAP-TEAD signaling output [32,33]. In this study, we investigated whether a chemical activation of TEAD signaling by TT-10 could promote β-cell replication in both humans and mice. The potent activation of YAP/TAZ-TEAD downstream signaling by TT-10 suggests that its potential as a candidate to stimulate β-cell regeneration through the Hippo signaling pathway.

Results

TT-10 promotes β-cell proliferation in mouse and human islets

The efficacy of TT-10 to induce β-cell regeneration was comprehensively tested using mouse and human islet models. To begin with, mouse islets were cultured with two different

concentrations of TT-10, specifically 10µM and 25µM, for a duration of 72 hours. This treatment significantly elevated the number of replicating β-cells as represented by the proliferation markers Ki67 and pHH3, as well as BrdU incorporation, in comparison to the vehicle control mouse islets (Figures 1A-D). This finding underscores the potential of TT-10 in enhancing β-cell proliferation in mouse islets.

Similarly, human islets were also subjected to the same experimental conditions. In line with mouse β-cells, TT-10 treatment significantly induced human β-cell proliferation (Figures 1E-G). Furthermore, the efficacy of TT-10 was tested on islets obtained from a human T2D donor. Consistently, TT-10 treatment resulted in increased β-cell proliferation in a human T2D islet donor (Figures 1H,I)). These results collectively indicate that TT-10 is capable of inducing a high level of proliferation in both mouse and human islets, phenocopying the mitogenic effect of YAP overexpression seen previously in the human primary β-cells [31].

Extending the scope of the study to a more pathological context, TT-10's impact was tested on islets isolated from 10-week-old hyperglycemic db/db mice, a model of severe diabetes characterized by significant β-cell destruction. Notably, TT-10 not only induced β-cell proliferation (Figures 1J,K) but also achieved a substantial reduction in β-cell apoptosis (Figure 1L). This dual effect of TT-10 in both promoting β-cell growth and inhibiting cell death highlights its potential as a multifaceted therapeutic drug, particularly in conditions where β-cell mass is critically compromised.

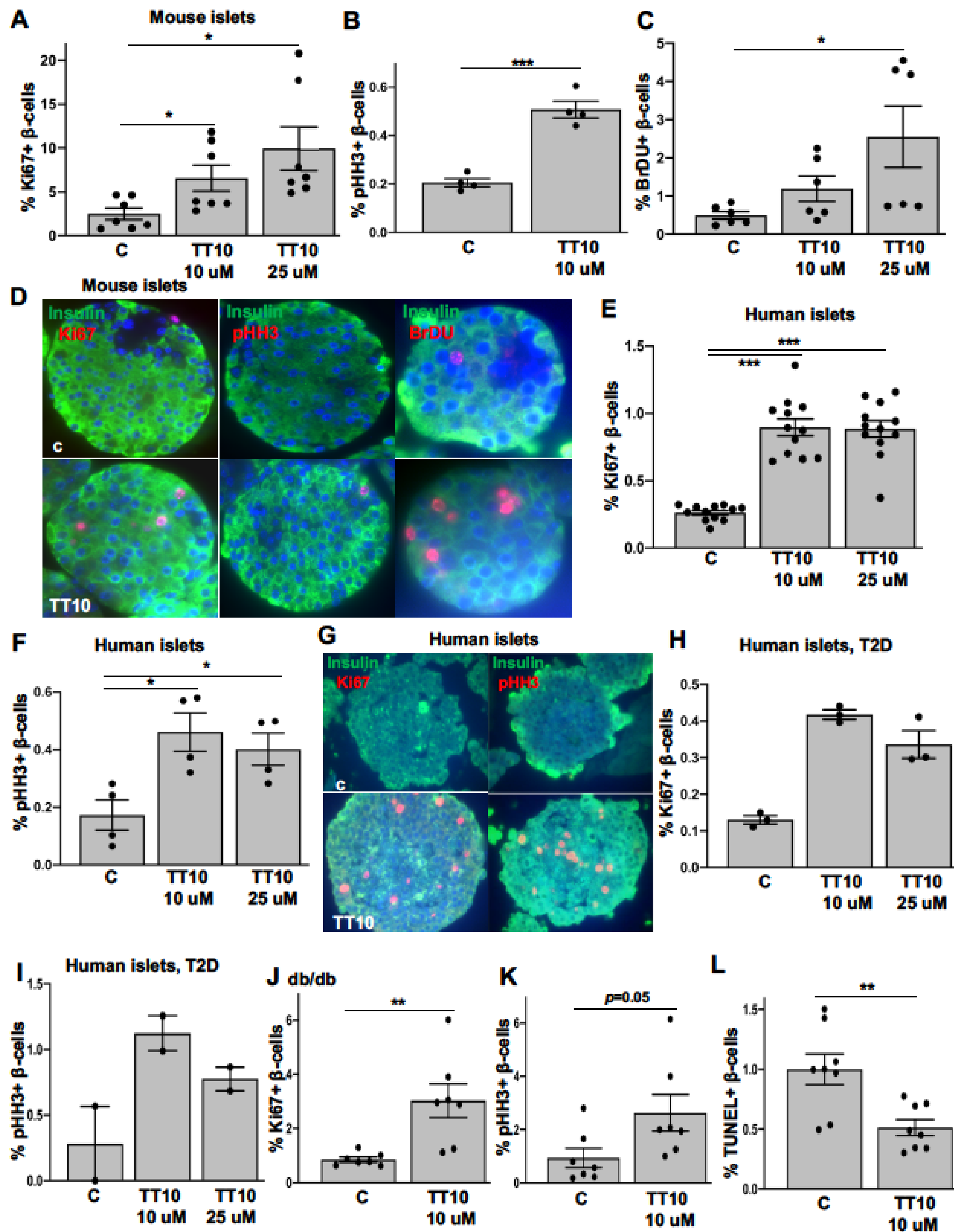

Figure 1. The TEAD activator TT-10 promotes β-cell proliferation in mouse and human islets. Isolated mouse **(A-D)** and human islets **(E-G)**, human islets from a donor with type 2 diabetes (T2D; **H,I**) and from 7-week old db/db mice **(J-L)** were cultured overnight and then treated with TT-10 (10 and 25μM) for 72h. Quantitative analysis of triple staining for Ki67 **(A,E,H,J)**, pHH3 **(B,F,I,K)** or BrDU **(C)** presented as %mean of all β-cells. n=7 for each

condition (from 20 mice; A), n=4 for each condition (from 12 mice; B), n=6 for each condition (from 18 mice; C), n=12 from four independent organ donors (E), n=4 from two independent organ donors (F), n=3 from 1 single donor with T2D (H), n=2 from 1 single donor with T2D (I), and n=7-8 for each condition (from 14 db/db mice; J-L). **(H)** Representative images were taken from triple staining of Ki67 or pHH3 in red, insulin in green and DAPI in blue from mouse (D) and human islets (G). Data are expressed as means ± SE. *p<0.05, **p<0.01, ***p<0.001; by two-tailed t-test.

TT-10 induces genes for β-cell proliferation and identity and maintains β-cell function

We then examined gene expression profiles related to β-cell proliferation, function and identity (Figures 2A,B). While expression of most of the β-cell functional and maturation genes remained stable by TT-10 treatment in human islets from both nondiabetic and T2D donors, *MafA, Slc2A2, ABCC8, KCNJ11, Glis3* and *Pdx1* were significantly upregulated, with the highest upregulation seen in *MafA* (11-fold upregulated) in nondiabetic isolated human islets (Figures 2A,B). Importantly, YAP/TEAD targets *FoxM1* [34] and *Myc* [35], previously associated with β-cell proliferation [36-39], showed significant upregulation following TT-10 treatment (Figure 2A), which was also confirmed in T2D islets (Figure 2B). Functional studies, including the evaluation of glucose stimulated insulin secretion (GSIS) *in vitro,* revealed that TT-10 treatment maintained β-cell function. This was evident in both basal and glucose stimulated insulin secretion (Figures 2C,E), as well as in the stimulatory index (Figures 2D,F), in both human islets from control donors and from a donor with T2D. These results suggest that TT-10 fosters β-cell proliferation and β-cell functional gene expression in human islets without harming β-cell identity and insulin secretion.

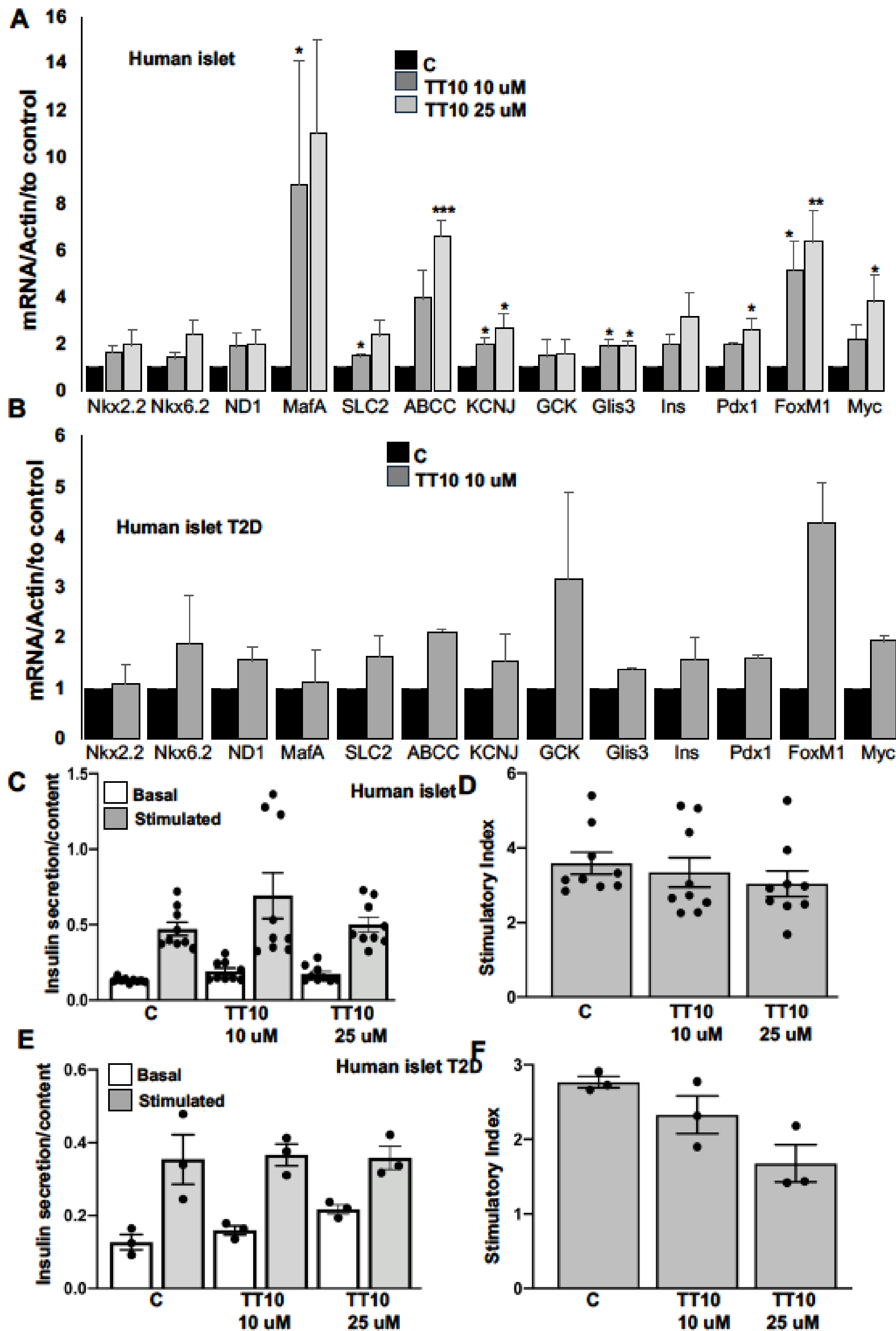

Figure 2. TT-10 induces genes for β-cell proliferation and identity and maintains β-cell function. (A,B) RT-PCR analysis of a panel of proliferative and β-cell functional/identity markers including *MafA, Nkx6.1, Slc2a2, NeuroD1, GCK, Ins, Pdx1, Nkx2.2, Glis3, Abcc8, Kcnj11, Foxm1 and Myc* in normal human islets (A) or from T2D donors (B) . mRNA expression normalized to housekeeping gene (actin) shown as change from control. n=4 from four

independent organ donors (A), and n=2 from two independent organ donors with T2D (B), each in duplicate. **(C-F)** Islets were cultured for 48h and treated with TT-10 (10μM, 25μM) for 48h; Insulin secretion during 1 h incubation with 2.8mM (basal) and 16.7mM (stimulated) glucose normalized to insulin content from isolated human islets (**C**,**E**) and the insulin stimulatory index denotes the ratio of secreted insulin during 1h incubation with 16.7mM glucose to secreted insulin at 2.8mM glucose **(D,F).** n=9 from three independent organ donors (C,D), and n=3 from a single organ donor with T2D (E,F). Data are expressed as means ± SE. *p<0.05, **p<0.01, ***p<0.001 to control; by two-tailed t-test.

TT-10 acts through TEAD to induce β-cell proliferation

TT-10 has been shown to selectively enhance the YAP/TAZ-TEAD interaction and activate TEAD target genes in cardiomyocytes [40]. To uncover TT-10's regulatory network and to investigate its TEAD-dependent effect on β-cell proliferation, we employed several strategies to block TEAD function. These included verteporfin, a pharmacological small-molecule inhibitor of the YAP-TEAD interaction [41], a TEAD1-specific siRNA pool and, a genetically encoded fluorescently tagged competitive inhibitor that blocks the binding of YAP and TEAD (hereafter referred to as TEAD inhibitor: TEADi) [42]. While TT-10 induced a substantial increase in β-cell proliferation (Ki67 & pHH3) in mouse (Figures 3A,B) as well as in both non-diabetic (Figures 3C-E) and T2D (Figures 3F-H) human islets, this effect was completely abolished by additional exposure to verteporfin in all experimental models (Figures 3A-H). In line with these data, genetic inhibition of TEAD1 by TEAD1-specific siRNA significantly abrogated the TT-10 effect of β-cell proliferation (Figures 3I,J) and reduced TEAD1 protein expression in mouse islets (Figure 3K). Similarly, transfection with TEADi prevented TT-10-induced β-cell proliferation in both mouse and human islets (Figures 3L,M). All these results collectively demonstrate that TT-10 acts through TEAD in pancreatic β-cells to induce endogenous β-cell proliferation.

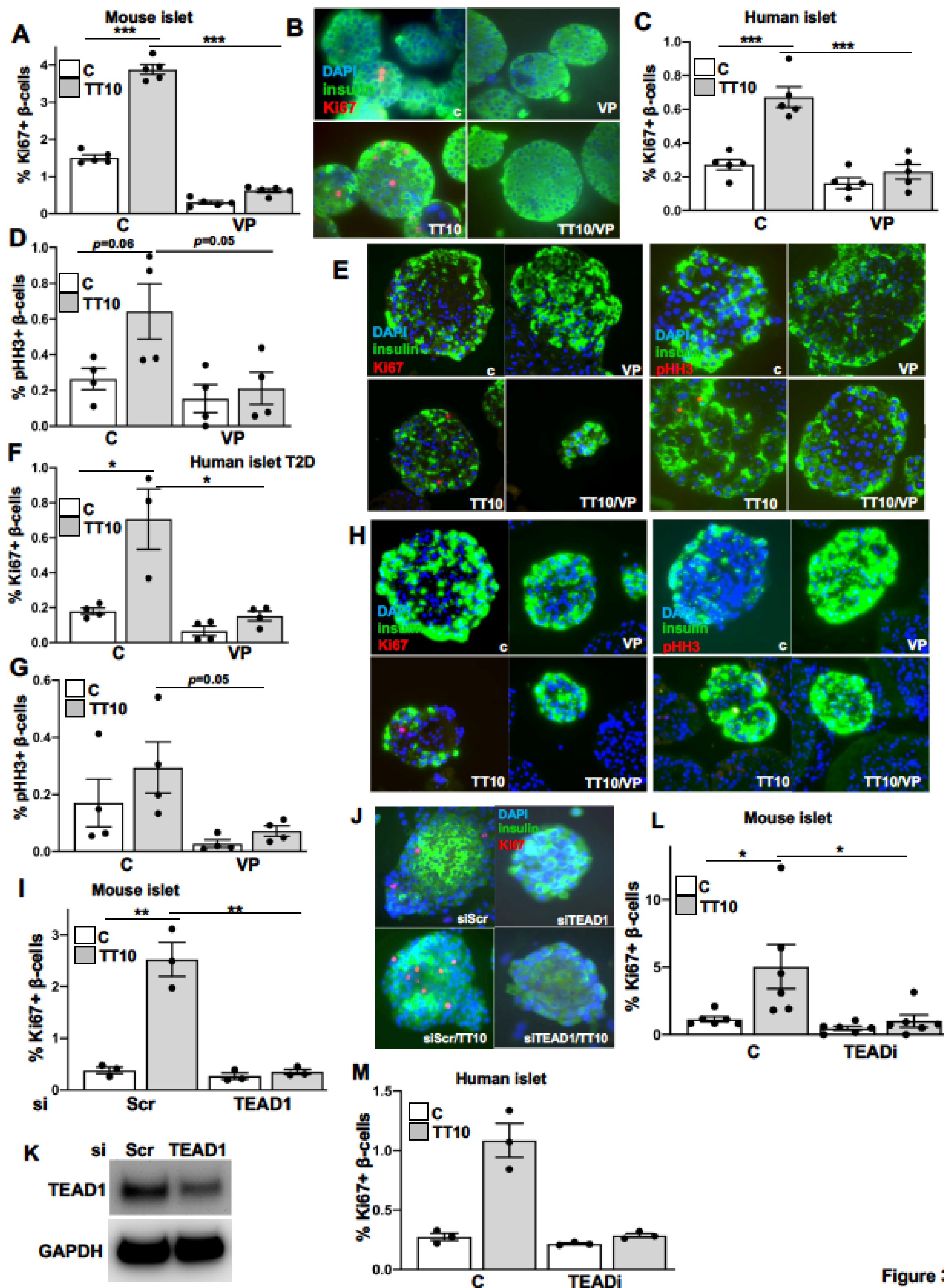

Figure 3. TT-10 acts through TEAD in pancreatic β-cells to induce proliferation. Mouse **(A,B)** and human control islets **(C-E)** and islets from donors with T2D **(F-H)** were cultured overnight and treated with or without TT-10 (10µM) for 72h; 1µM verteporfin (VP) was added to the culture as indicated during the last 24h. **(A,C,D,F,G)** Quantitative analysis of % Ki67 or pHH3-positive β-cells from triple staining for Ki67/pHH3, insulin and DAPI; representative

images were taken from mouse islets **(B),** human islets **(E)** and human T2D islets **(H).** n=5 for each condition (from 21 mice; A), n=5 from three organ donors (C), n=4 from two organ donors (D), n=3-4 from two organ donors with T2D (F,G). **(I-K)** Isolated mouse islets were transfected with TEAD1 siRNA (siTEAD1) or control siScr and treated with TT-10 for 72h. Quantitative analysis (**I**) and representative images (**J**) of triple staining for Ki67, insulin and DAPI positive β-cells and (**K**) representative Western Blot analysis for TEAD1 and GAPDH. n=3 for each condition (from 8 mice; I). Mouse (**L**) and human (**M**) islets were transfected with 3μg of eGFP-TEADi or control plasmids and treated with TT-10 for 72h, quantitative analysis of triple staining for Ki67, insulin and DAPI. N=6 for each condition (from 15 mice; L) and n=3 from 1 single organ donor (M). Data are expressed as means ± SE. *p<0.05, **p<0.01, ***p<0.001; by two-tailed t-test.

TT-10 enhances β-cell proliferation *in vivo*

As TT-10 has been shown to enhance cardiomyocyte regeneration and heart repair *in vivo* [32,33], we tested its efficacy on the pancreas in C57Bl6/J mice by daily intraperitoneal (i.p.) injection of 10 mg/kg body weight for seven consecutive days. TT-10 had no effect on body weight or metabolic parameters, including non-fasting random glycemia and glucose tolerance (Figures 4A,B). Interestingly, just after seven days, there was a marked increase in β-cell proliferation, shown by Ki67 and pHH3 immunolabeling (Figures 4C-E). Additionally, there was a tendency of increased percentage of insulin-positive area and β-cell mass (Figures 4F,G) without signs of β-cell dedifferentiation in islets, evaluated by ALDH1A3 immunostaining (Figure 4H). These results establish TT-10 as a potential drug for the regeneration of endogenous β-cells.

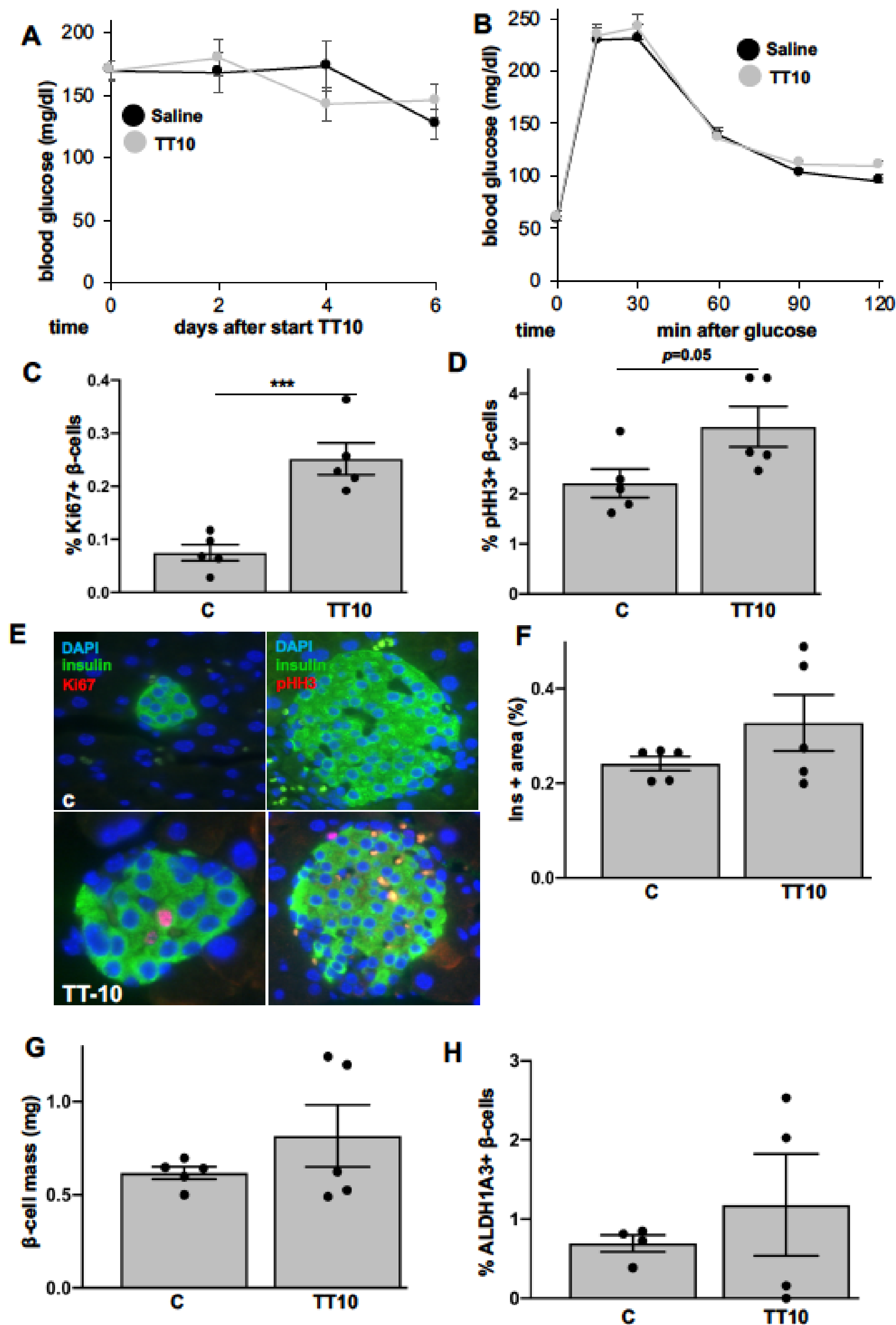

Figure 4

Figure 4. TT-10 *in vivo* injection promotes β-cell proliferation. C57Bl/6J mice were i.p. injected with saline or TT-10 (10mg/kg body weight) for 7 consecutive days. **(A)** Random fed glycemia measurements and **(B)** ipGTT was performed at day 7. Quantitative analysis of pancreases **(C,D)** and representative images **(E)** of triple staining for Ki67 or pHH3, insulin and DAPI. Insulin-positive are **(F)** and β-cell mass **(G)** (given as percentage of insulin^{+} cells of the

whole pancreatic section from 10 sections spanning the width of the pancreas multiplied by the pancreas mass. Quantitative analysis of pancreases for ALDH1A3-positive β-cells. n=5 for each condition (A-G) and n=4 for each condition (H). Data are expressed as means ± SE. *p<0.05, **p<0.01, ***p<0.001; by two-tailed t-test.

Discussion

Given the high clinical demand for inducing pancreatic islet β-cell proliferation for the treatment of diabetes, and the potential of YAP as a robust regulator of human β-cell proliferation [31,43], this study tested TT-10, a potent activator of the YAP/TAZ-TEAD interaction, which had been identified through extensive screening for its ability to promote cardiomyocyte proliferation and protect the heart after myocardial injury [32,33]. TT-10 led to robust proliferation, as evidenced by upregulation of several antigens active during cell division including Ki67, and pHH3 as well as the higher rate of BrdU incorporation in human and mouse islets, as well as in two *ex vivo* models of T2D; specifically, human isolated islets from organ donors with T2D diabetes and in islets from hyperglycemic db/db mice. Functional studies on isolated human pancreatic islets treated with TT-10 did not show any changes in insulin secretion and insulin content, nor was there a loss in β-cell identity and maturation. This was confirmed by gene expression analyses of established β-cell markers, indicating that the proliferating β-cells were both mature and functional [30,31,44].

TT-10 highly increased expression of FoxM1 and Myc in human islets, including those from an organ donor with T2D, showing that islets in a T2D environment retain the capacity to proliferate. Both FoxM1 and Myc are key regulator of β-cell proliferation [36-39]. Overexpression of FoxM1 or Myc alone is sufficient to induce β-cell proliferation in both mice and humans, underscoring the central role of these proliferative genes in β-cell proliferation [45,46]. Thus, the TT-10-dependent induction of FoxM1 and Myc could be critical signaling elements during β-cell proliferation.

Upon TT-10 treatment in human islets, genes important for β-cell function and identity, including *MAFA, SLC2A2, ABCC8*, *KCNJ11, GLIS3* and *PDX-1,* were significantly upregulated. While TT-10 induced β-cell proliferation in a TEAD-dependent manner, both TEAD1 and TEAD2, which are constitutively expressed in β-cells, as well as their direct target genes connective tissue growth factor (*CTGF)* and *ANKARD1* [21] remained unchanged (data not shown). Previous research has shown that TEAD1 alone directly controls and promotes critical β-cell transcription factors, such as *PDX1, NKX6.1* and *MAFA*. Its β-cell specific deletion in mice leads to diabetes induction with severe hyperglycemia and blunted insulin secretion [30]. Surprisingly, TEAD1 deficiency in mouse β-cells also leads to increased proliferation, contrary to the classical observations that its activation promotes proliferation. Mechanistically, TEAD1 critically regulates Cdkn2a (p16), maintaining β-cell quiescence. Thus, the absence of YAP in

adult β-cells, coupled with the simultaneous expression of other transcriptional co-regulators such as Menin [47], could redirect the TEAD-dependent transcriptional program toward specific genes, such as *Cdkn2a*. This ensures keeping proliferation in highly functional β-cells at a minimal level to prevent unnecessary proliferation and hyperinsulinemia, while simultaneously preserving β-cell identity and functionality to maintain glucose homeostasis.

Pioneering studies by Maureen Gannon's lab on the function of YAP-TEAD target gene *CTGF* in β-cells have established its role in β-cell proliferation, promoting β-cell regeneration in mice [48-50], which is in line with our current study, although the YAP target *CTGF* expression levels remained unaffected by TT-10. Additionally, the other co-transcriptional activator of TEAD, TAZ, has been shown to directly interact with PDX1, promoting insulin gene transcription and thus β-cell function [44], which indicates that TAZ can also act independently of TEAD. This might represent a secondary mechanism allowing proliferation without loss-of-function, potentially increasing PDX1 activity. Numerous studies have highlighted a tightly regulated balance between β-cell's replication and functionality [18,51]. Proliferating β-cells typically show reduced insulin expression [52]. In line with this, β-cells primed to enter cell cycle are functionally less mature [46].

Tight control over the regulation of mitogenic pathways is essential to achieve β-cell proliferation for regenerative therapy. Firstly, induction of proliferation must by controllable to avoid a shift toward oncogenic growth [11]. Secondly, proliferating cells must retain functionality, and thirdly, prevention of apoptosis in newly regenerating β-cells must be achieved. The compound TT-10 therefore emerges as a key in achieving this triple goal. It targets pathways which control growth and proliferation (TEAD), together with upregulation of functionality markers (promotion of PDX1 activation) as well as prevention of apoptosis in a model of T2D. Such simultaneous prevention of β-cell apoptosis and enhancement of β-cell proliferation should be a primary focus in successful β-cell targeted therapy for diabetes [29,53]. Under diabetogenic stressors like inflammation and gluco- and lipotoxicity, regenerating β-cells are highly susceptible to apoptosis, which exacerbates the loss of β-cell mass [54,55].

In this study, we show that TT-10, by activating the transcription factors TEADs, fosters β-cell regeneration by inducing proliferation of endogenous β-cells, protects them from apoptosis and maintains their maturity and functionality. Consequently, TT-10 represents a novel and promising therapeutic strategy for the treatment of diabetes.

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Author contributions

Conceptualization AA.; Experimental design MKM., KM., and AA.; Investigation MKM., HL., SG., HP., SB., GR., IR., DBK., SKY., KM and AA.; Formal Analysis MKM., KM., and AA.; Writing MKM., KM. and AA.; Funding Acquisition and Supervision AA and KM.

Declaration of interests

The authors declare no competing interests.

Methods

Mice

For the *in vivo* experiments involving TT-10, C57Bl/6J wild-type mice were injected daily with TT-10 at a dose of 10 mg/kg body weight or vehicle (for controls) for one week. At the end of metabolic studies, the mice were sacrificed, and their pancreas were harvested and sectioned for further morphological analysis. All mice used in this experiment were housed in a temperature-controlled room with a 12h light-dark cycle and had free access to food and water in accordance with NIH animal care guidelines, §8 of the German Animal Protection Act, the German Welfare Act, and the guidelines of the Society of Laboratory Animals (GV-SOLAS) and the Federation of Laboratory Animal Science Associations (FELASA). All protocols have been approved by the Bremen Senate (Senator for Science, Health and Consumer Protection), and we have complied with all relevant ethical regulations for animal experiments and research.

Islets isolation and treatment

Mouse pancreatic islets were isolated by pancreatic perfusion with Liberase™ (#05401119001, Roche, Mannheim, Germany) according to the manufacturer's instructions and digested at 37°C, followed by washing and hand picking. Human islets were isolated from the pancreas of non-diabetic and T2D organ donors (male and female) at the University of Lille and ProdoLabs and the islets were cultured on dishes coated with Biocoat Collagen I (#356400, Corning, ME, USA). Human islets were cultured in complete CMRL-1066 medium (Invitrogen) containing 5.5 mM glucose. Mouse islets were cultured in complete RPMI-1640 medium (Sigma Aldrich, Missouri, MO, USA) medium containing 11.1 mM glucose. All media were supplemented with L-glutamate, 1% penicillin-streptomycin, and 10% fetal bovine serum (FBS). In some experiments, human and mouse islets were additionally cultured with 10-25 μM TT-10 (#AOB17167, AOBIOUS) for 48-72h or 1μM YAP/TEAD inhibitor verteporfin (#SML0534, Sigma Aldrich, USA) for 24h or transiently transfected with 3μg TEADi plasmid for 24h or 100nM TEAD1 siRNA pool for 4h.

All islet experiments were performed at the Islet Biology Laboratory, University of Bremen. This study was in compliance with all relevant ethical regulations for work with human cells for research purposes. The organ donors are not identifiable and are anonymous; such approved experiments using human islet cells for research are covered by the NIH Exemption 4 (Regulation PHS 398). Human islets were distributed by the approved coordination program in Europe (Islet for Basic Research program; European Consortium for Islet Transplantation ECIT) and permission has been granted by the Ethical Committee at the University of Bremen.

Glucose tolerance test

For blood glucose measurements, blood was drawn from the tail vein and glucose measured using a glucometer (FreeStyle; Abbott, IL, USA). For intraperitoneal glucose tolerance tests (ipGTT), mice were fasted overnight for 12 hours and injected i.p. with glucose (B. Braun, Germany) at a dose of 1g/kg body weight. Glycemia was measured at 0, 15, 30, 60, 90, and 120 min time points.

Glucose-stimulated insulin secretion (GSIS)

Human islets were preincubated in Krebs-Ringer bicarbonate buffer (KRB) containing 2.8 mM glucose for 30 minutes, followed by 1 hour in fresh KRB containing 2.8 mM glucose (basal) and another 1 hour in KRB containing 16.7 mM glucose (stimulated). Islets were washed with PBS and lysed with RIPA buffer to measure total insulin content. Insulin was determined using an ELISA assay for human and mouse insulin (ALPCO Diagnostics, NH USA). Secreted insulin was normalized to insulin content.

Plasmids and siRNAs

The SMARTpool technology was used to knockdown TEAD1 (Dharmacon, CO, USA). A mixture of ON -TARGET plus siRNAs J-048419-09 targeted against the following sequences: AGACGGAGUAUGCGAGGUU, CCGAAUAAACCGCUCGCCA, GUCUAAGCUUACAACGUUA, GGAUGAGCGACUCUCGGCAGA. ON-TARGET plus nontargeted siRNA pool (scramble; siScr) served as a control. pCEFL EGFP-TEADi was a gift from Ramiro Iglesias-Bartolome (Addgene plasmid # 140144; http://n2t.net/addgene:140144; RRID: Addgene_140144).

Transfection

3μg EGFP-TEADi plasmid or 100 nM TEAD1-siRNA or Scr-siRNA were used for islet transfection. To achieve silencing, the jetPRIME® transfection reagent (#114-75; Polyplus transfection, France) was used to introduce the desired plasmids or siRNAs into human or mouse islets according to the manufacturer's instructions. Briefly, jetPRIME buffer was mixed with the siRNA/plasmid and vortexed for 10 seconds, and then jetPRIME® transfection reagent was added and vortexed for 1 second. The mixture was allowed to stand at room temperature (RT) for 10 minutes and then shaken rapidly. The jetPRIME-siRNA or plasmid complexes were then added to the complete culture medium and transfected into Accutase (#A6954, Sigma) -

treated dispersed mouse and human islets. Transfection efficiency was estimated using fluorescence microscopy of GFP and Western Blot.

Western Blot analysis

Mouse islets were washed three times with PBS after removal of the medium and lysed with ice-cold RIPA lysis buffer (50 mM Tris HCl pH 8, 150 mM NaCl, 1% NP -40, 0.5% sodium deoxycholate, and 0.1% SDS) supplemented with protease and phosphatase inhibitors (Thermo Fisher Scientific, MA, USA). The samples were subjected to a series of freeze-thaw cycles and finally incubated for 30 min on ice with intermittent vortexing. Cell lysates were centrifuged at 16000 x g for 20 min at 4°C, and the clear supernatant containing the extracted proteins was stored at -80°C. The protein concentrations were measured using the BCA protein assay (Thermo Fisher Scientific). Equivalent amounts of protein from each condition were loaded onto a NuPAGE 4-12% Bis-Tris gel (Invitrogen; CA, USA) and the gel was electro transferred to PVDF membranes. Membranes were blocked for one hour at RT with a mixture of 2.5% milk (Cell Signaling Technology/ CST, MA, USA) and 2.5% BSA (SERVA Electrophoresis GmbH, Heidelberg, Germany) and incubated overnight at 4°C with mouse anti-TEAD1 (#610922, BD Biomedicine), and rabbit anti-GAPDH (#2118, Cell Signaling Technology (CST)). All primary antibodies were used at 1:1,000 dilution in 1xTris-buffered saline plus Tween-20 (1xTBS-T) containing 5% BSA and 0.5% NaN3. Membranes were then incubated with horseradish peroxidase-bound rabbit or mouse secondary antibodies (Jackson ImmunoResearch, PA, USA) and developed using the Immobilon Western Chemiluminescence Assay System (Millipore, MA, USA). Immunoblot analysis was performed using the Vision Works LS Image Acquisition and Analysis Software version 6.8 (UVP BioImaging Systems, CA, USA).

qPCR analysis

Total RNA was isolated from cultured isolated human islets cells using TriFast (PEQLAB Biotechnologie, Germany). 500-1000ng of RNA were reverse transcribed to cDNA (RevertAid reverse transcriptase, Thermo Fisher Scientific (TFS), MA, USA). Quantitative RT-PCR was carried out as previously described using Biosystems StepOne Real-Time PCR system (Applied Biosystems, CA, USA) with TaqMan assays. TaqMan® Gene Expression Assays were used for *Nkx2.2* (#Hs00159616_m1), *Nkx6.1* (#Hs00232355_m1C12), *NeuroD1* (#Hs01922995_s1), *MafA* (#Hs01651425_s1C18), *Slc2A2* (#Hs01096905_m1), *Abcc8* (#Hs01093761_m1), *Glis3* (#Hs00541450_m1C33), *Gck* (#Hs01564555_m1), *KcnJ11* (#Hs00265026_s1), *Ins* (#Hs02741908_m1), *PDX1* (#Hs00236830_m1B20), *FoxM1* (#Hs00231106_m1), *Myc* (#Hs00153408_m1), and *Actin* (#Hs99999903_m1).

Immunohistochemistry

Mouse pancreases were dissected, fixed in 4% formaldehyde at 4°C for 8h, and dehydrated before embedding in paraffin. Mouse pancreatic sections (4μm) were deparaffinized,

rehydrated, and blocked with blocking buffer containing 3% BSA and paraffin-embedded, bouin-fixed islets were deparaffinized and rehydrated. Cultured mouse or human islets cells were seeded in glass bottom dishes allowing them to attach to the surface for 48h and treated with respective compounds and fixed with 4% PFA for 30 min followed by 4 min permeabilization with 0.5 % Triton-X-100, blocked with blocking buffer containing 3% BSA. Fixed pancreatic tissues or islets were then incubated overnight at 4°C with the following antibodies (single or double): guinea pig anti-insulin (#IR002, Flex polyclonal DAKO)(1:50), rabbit anti-Ki67 (#AB16667, Abcam) (1:100), rabbit anti-pHH3 (#06-570, Merck-millipore) (1:200), mouse anti-BrdU (#NA61; Calbiochem) (1:100), and rabbit anti-ALDH1A3 (#NBP2-15339) (1:100), followed by fluorescein isothiocyanate (FITC; #715-096-148) - conjugated donkey anti-guinea pig (1:100) or CY3 (#715-165-150) conjugated donkey anti-rabbit/mouse (1:100) or Biotin-conjugated donkey anti-guinea pig (1:100) secondary antibodies (Jackson Immuno Research Laboratories, PA, USA). Slides were fixed with Vectashield containing 406-diamidino-2-phenylindole (DAPI) (Vector Labs, CA, USA). β-cell apoptosis in Bouin's fixed isolated mouse islets sections was analyzed by terminal deoxynucleotidyl transferase-linked dUTP nick-end labeling (TUNEL) technique according to the manufacturer's instructions (In Situ Cell Death Detection Kit, TMR red; Roche) and dual staining for insulin. Fluorescence was analyzed using a Nikon MEA53200 microscope (Nikon GmbH, Germany), and images were captured using the Nikon's NIS -Elements software.

Morphometric analysis

Ten sections (across the width of the pancreas) per mouse were analyzed for morphometric data. The pancreatic tissue area and insulin-positive area (VECTASTAIN ABC kit; Vector Labs, USA) were determined by computer-assisted measurements using a Nikon MEA53200 microscope (Nikon GmbH, Germany), and images were acquired using Nikon's NIS -Elements software. The mean percentage β-cell content per pancreas was calculated as the ratio of insulin-positive to total pancreatic area. The pancreatic β-cell mass was determined by multiplying the β-cell fraction by the weight of the pancreas [56].

Statistical analyses

Statistical comparisons between groups were analyzed for significance by a paired or unpaired two-tailed Student's t-test. p value<0.05 was considered statistically significant. Data are presented as means ± SEM. The exact values of *n* (refers to independent replicas or mice, or number of independent biological experiments), number of organ donors and statistical significance are reported in the figure legends.

Reference:

1. Meier, J.J., *et al.* Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. *Diabetes* **57**, 1584-1594 (2008).
2. Kushner, J.A. The role of aging upon beta cell turnover. *J Clin Invest* **123**, 990-995 (2013).
3. Maedler, K., *et al.* Aging correlates with decreased beta-cell proliferative capacity and enhanced sensitivity to apoptosis: a potential role for Fas and pancreatic duodenal homeobox-1. *Diabetes* **55**, 2455-2462 (2006).
4. Butler, P.C., Meier, J.J., Butler, A.E. & Bhushan, A. The replication of beta cells in normal physiology, in disease and for therapy. *Nat Clin Pract Endocrinol Metab* **3**, 758-768 (2007).
5. Basile, G., Kulkarni, R.N. & Morgan, N.G. How, When, and Where Do Human beta-Cells Regenerate? *Curr Diab Rep* **19**, 48 (2019).
6. Tuttle, R.L., *et al.* Regulation of pancreatic beta-cell growth and survival by the serine/threonine protein kinase Akt1/PKBalpha. *Nat Med* **7**, 1133-1137 (2001).
7. Hanley, S. & Rosenberg, L. Transforming growth factor beta is a critical regulator of adult human islet plasticity. *Mol Endocrinol* **21**, 1467-1477 (2007).
8. Rulifson, I.C., *et al.* Wnt signaling regulates pancreatic beta cell proliferation. *Proc Natl Acad Sci U S A* **104**, 6247-6252 (2007).
9. Wang, P., *et al.* Diabetes mellitus--advances and challenges in human beta-cell proliferation. *Nat Rev Endocrinol* **11**, 201-212 (2015).
10. Fatrai, S., *et al.* Akt induces beta-cell proliferation by regulating cyclin D1, cyclin D2, and p21 levels and cyclin-dependent kinase-4 activity. *Diabetes* **55**, 318-325 (2006).
11. Karakose, E., Ackeifi, C., Wang, P. & Stewart, A.F. Advances in drug discovery for human beta cell regeneration. *Diabetologia* **61**, 1693-1699 (2018).
12. Purwana, I., *et al.* GABA promotes human beta-cell proliferation and modulates glucose homeostasis. *Diabetes* **63**, 4197-4205 (2014).
13. Shen, W., *et al.* Inhibition of DYRK1A and GSK3B induces human beta-cell proliferation. *Nat Commun* **6**, 8372 (2015).
14. Kondegowda, N.G., *et al.* Osteoprotegerin and Denosumab Stimulate Human Beta Cell Proliferation through Inhibition of the Receptor Activator of NF-kappaB Ligand Pathway. *Cell Metab* **22**, 77-85 (2015).
15. El Ouaamari, A., *et al.* SerpinB1 Promotes Pancreatic beta Cell Proliferation. *Cell Metab* **23**, 194-205 (2016).
16. Dhawan, S., Dirice, E., Kulkarni, R.N. & Bhushan, A. Inhibition of TGF-beta Signaling Promotes Human Pancreatic beta-Cell Replication. *Diabetes* **65**, 1208-1218 (2016).
17. Wang, P., *et al.* A high-throughput chemical screen reveals that harmine-mediated inhibition of DYRK1A increases human pancreatic beta cell replication. *Nat Med* **21**, 383-388 (2015).
18. van der Meulen, T., *et al.* Virgin Beta Cells Persist throughout Life at a Neogenic Niche within Pancreatic Islets. *Cell Metab* **25**, 911-926 e916 (2017).
19. Wang, Y.J., *et al.* Single-Cell Mass Cytometry Analysis of the Human Endocrine Pancreas. *Cell Metab* **24**, 616-626 (2016).
20. Tritschler, S., Theis, F.J., Lickert, H. & Bottcher, A. Systematic single-cell analysis provides new insights into heterogeneity and plasticity of the pancreas. *Mol Metab* **6**, 974-990 (2017).
21. Zhao, B., *et al.* TEAD mediates YAP-dependent gene induction and growth control. *Genes Dev* **22**, 1962-1971 (2008).
22. Dey, A., Varelas, X. & Guan, K.L. Targeting the Hippo pathway in cancer, fibrosis, wound healing and regenerative medicine. *Nat Rev Drug Discov* **19**, 480-494 (2020).
23. Cebola, I., *et al.* TEAD and YAP regulate the enhancer network of human embryonic pancreatic progenitors. *Nat Cell Biol* **17**, 615-626 (2015).
24. George, N.M., Boerner, B.P., Mir, S.U., Guinn, Z. & Sarvetnick, N.E. Exploiting Expression of Hippo Effector, Yap, for Expansion of Functional Islet Mass. *Mol Endocrinol* **29**, 1594-1607 (2015).
25. George, N.M., Day, C.E., Boerner, B.P., Johnson, R.L. & Sarvetnick, N.E. Hippo signaling regulates pancreas development through inactivation of Yap. *Mol Cell Biol* **32**, 5116-5128 (2012).
26. Johnson, R. & Halder, G. The two faces of Hippo: targeting the Hippo pathway for regenerative medicine and cancer treatment. *Nat Rev Drug Discov* **13**, 63-79 (2014).

27. Pullen, T.J., *et al.* Identification of genes selectively disallowed in the pancreatic islet. *Islets* **2**, 89-95 (2010).
28. Ardestani, A., Lupse, B. & Maedler, K. Hippo Signaling: Key Emerging Pathway in Cellular and Whole-Body Metabolism. *Trends Endocrinol Metab* (2018).
29. Ardestani, A. & Maedler, K. The Hippo Signaling Pathway in Pancreatic beta-Cells: Functions and Regulations. *Endocr Rev* **39**, 21-35 (2018).
30. Lee, J., *et al.* Tead1 reciprocally regulates adult β-cell proliferation and function. *bioRxiv* (2020).
31. Yuan, T., *et al.* Proproliferative and antiapoptotic action of exogenously introduced YAP in pancreatic beta cells. *JCI Insight* **1**, e86326 (2016).
32. Chen, W., *et al.* TT-10-loaded nanoparticles promote cardiomyocyte proliferation and cardiac repair in a mouse model of myocardial infarction. *JCI Insight* **6**(2021).
33. Hara, H., *et al.* Discovery of a Small Molecule to Increase Cardiomyocytes and Protect the Heart After Ischemic Injury. *JACC Basic Transl Sci* **3**, 639-653 (2018).
34. Mizuno, T., *et al.* YAP induces malignant mesothelioma cell proliferation by upregulating transcription of cell cycle-promoting genes. *Oncogene* **31**, 5117-5122 (2012).
35. Nishimoto, M., *et al.* Transformation of normal cells by aberrant activation of YAP via cMyc with TEAD. *Sci Rep* **9**, 10933 (2019).
36. Davis, D.B., *et al.* FoxM1 is up-regulated by obesity and stimulates beta-cell proliferation. *Mol Endocrinol* **24**, 1822-1834 (2010).
37. Zhang, H., *et al.* The FoxM1 transcription factor is required to maintain pancreatic beta-cell mass. *Mol Endocrinol* **20**, 1853-1866 (2006).
38. Karslioglu, E., *et al.* cMyc is a principal upstream driver of beta-cell proliferation in rat insulinoma cell lines and is an effective mediator of human beta-cell replication. *Mol Endocrinol* **25**, 1760-1772 (2011).
39. Rosselot, C., *et al.* Myc Is Required for Adaptive beta-Cell Replication in Young Mice but Is Not Sufficient in One-Year-Old Mice Fed With a High-Fat Diet. *Diabetes* **68**, 1934-1949 (2019).
40. Ito, M., *et al.* Characterization of a small molecule that promotes cell cycle activation of human induced pluripotent stem cell-derived cardiomyocytes. *J Mol Cell Cardiol* **128**, 90-95 (2019).
41. Liu-Chittenden, Y., *et al.* Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. *Genes & development* **26**, 1300-1305 (2012).
42. Yuan, Y., *et al.* YAP1/TAZ-TEAD transcriptional networks maintain skin homeostasis by regulating cell proliferation and limiting KLF4 activity. *Nat Commun* **11**, 1472 (2020).
43. Wu, Y., *et al.* Hippo Signaling Pathway in Pancreas Development. *Front Cell Dev Biol* **9**, 663906 (2021).
44. Jeong, M.G., *et al.* TAZ promotes PDX1-mediated insulinogenesis. *Cellular and molecular life sciences : CMLS* **79**, 186 (2022).
45. Shirakawa, J., *et al.* Insulin Signaling Regulates the FoxM1/PLK1/CENP-A Pathway to Promote Adaptive Pancreatic beta Cell Proliferation. *Cell Metab* **25**, 868-882 e865 (2017).
46. Puri, S., *et al.* Replication confers beta cell immaturity. *Nat Commun* **9**, 485 (2018).
47. Li, F., *et al.* VGLL4 and MENIN function as TEAD1 corepressors to block pancreatic beta cell proliferation. *Cell Rep* **42**, 111904 (2023).
48. Riley, K.G., *et al.* Connective tissue growth factor modulates adult beta-cell maturity and proliferation to promote beta-cell regeneration in mice. *Diabetes* **64**, 1284-1298 (2015).
49. Guney, M.A., *et al.* Connective tissue growth factor acts within both endothelial cells and beta cells to promote proliferation of developing beta cells. *Proc Natl Acad Sci U S A* **108**, 15242-15247 (2011).
50. Townsend, S.E., Fuhr, J.D. & Gannon, M. Context-dependent effects of CCN2 on beta-cell mass expansion and indicators of cell stress in the setting of acute and chronic stress. *American journal of physiology. Endocrinology and metabolism* **325**, E280-E290 (2023).
51. Yang, C., Galivo, F. & Dorrell, C. Is a beta cell a beta cell? *Curr Opin Endocrinol Diabetes Obes* **24**, 92-97 (2017).
52. Yuan, T., *et al.* The Hippo kinase LATS2 impairs pancreatic beta-cell survival in diabetes through the mTORC1-autophagy axis. *Nat Commun* **12**, 4928 (2021).

53. Ardestani, A. & Maedler, K. Deathly triangle for pancreatic beta-cells: Hippo pathway-MTORC1-autophagy. *Autophagy*, 1-3 (2021).
54. Meier, J.J., Ritzel, R.A., Maedler, K., Gurlo, T. & Butler, P.C. Increased vulnerability of newly forming beta cells to cytokine-induced cell death. *Diabetologia* **49**, 83-89 (2006).
55. Ardestani, A. & Maedler, K. MST1: a promising therapeutic target to restore functional beta cell mass in diabetes. *Diabetologia* **59**, 1843-1849 (2016).
56. Ardestani, A., *et al.* MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes. *Nat Med* **20**, 385-397 (2014).

Publication 3:

The Hippo terminal effector YAP boosts enterovirus replication in type 1 diabetes

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My contribution:

Designed, performed experiments, analyzed data and assembled the figures for: Figure 4 I, J, K, L, M, P, Q, T, V.

Supplementary Figures: SF 4 A, B, C, D, E, F and SF 5 A.

Partial contribution in writing the paper.

The Hippo terminal effector YAP boosts enterovirus replication in type 1 diabetes

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Key words: Type 1 diabetes; Pancreas; Exocrine; Islet; Beta cells; Enteroviruses; Hippo pathway; YAP; MST1; Inflammation; Apoptosis.

Abstract

Type 1 diabetes (T1D) risk has been associated with enteroviral infections, especially with those from coxsackieviruses B (CVBs). Cellular host factors and intrinsic signaling mechanisms may contribute to the initiation or acceleration of virus-induced islet autoimmunity and consequent β-cell destruction, but these remain unclear. Here we show that the Hippo pathway terminal effector Yes-associated Protein (YAP) is highly upregulated in both the exocrine and endocrine pancreas of T1D and at-risk autoantibody-positive (AAb^+) organ donors. YAP expression is correlated with CVB infections and many YAP-expressing cells show virus positivity or localization in close proximity to virus-infected cells in T1D and AAb^+ pancreases. YAP over-expression enhances CVB replication and fosters CVB-induced islet inflammation and β-cell apoptosis, whereas its inhibition halts viral replication in both primary and immortalized pancreatic cells. YAP re-expression in β-cells in mice is associated with impaired glucose tolerance, abolished insulin secretion, and β-cell dedifferentiation. Mechanistically, we found that YAP, in complex with its transcription factor TEAD, directly induces its own negative regulator, MST1 kinase. MST1 inhibition increases viral replication and diminishes β-cell apoptosis. This constitutes a negative feedback loop in which the reciprocal antagonism between YAP and MST1 regulates viral replication and β-cell death during CVB infections. Our work uncovers an integral role for YAP as key host factor for enteroviral amplification in the pancreas, and has an important translational impact for inhibiting viral replication, which may be beneficial in T1D.

Introduction

Type 1 diabetes (T1D) is a multi-factorial inflammatory disorder characterized by the autoimmune destruction of insulin-producing pancreatic β-cells, mediated by immune cell recruitment and infiltration (insulitis) of the pancreatic islets and the local release of pro-inflammatory cytokines and chemokines. Eventually, this process leads to β-cell apoptosis, impaired insulin secretion and development of hyperglycemia [1]. Although genetic predisposition is universally accepted as a key determinant in the development of T1D, environmental factors play their part, either as potential triggers, or accelerators. Enteroviruses, and especially Coxsackievirus B (CVB) strains, have been linked to increased T1D risk, and are suspected to play a role in the initiation and progression of islet autoimmunity [2,3]. Enteroviruses are small, non-enveloped, positive single-stranded RNA viruses of the Picornaviridae family [4]. CVBs are highly effective in infecting isolated human islet cells; their RNA and capsid protein were found in both the endocrine and exocrine pancreas of biopsies from living adults with recent-onset T1D as well as in organ donor pancreata from individuals with T1D. The presence of viral proteins and RNA is associated with MHCI-hyperexpression by islet cells, local inflammation and β-cell destruction [5-12]. Collectively, epidemiological and tissue studies suggest that persistent, low-grade enteroviral infections may contribute to T1D pathogenesis [13].

While most studies exclusively investigated enteroviral expression within islets, CVB infection has also been reported in the exocrine pancreas in donors with T1D [7,8]. By using a single molecule-based fluorescent in situ hybridization (smFISH) method, we have recently shown that enteroviral RNA is substantially increased in pancreases from organ donors with T1D and with disease-associated autoantibodies (AAb^+) with the majority of virus-positive cells scattered in the exocrine pancreas [8]. Infected regions outside of islets are wired by immune cells and may constitute a potential reservoir for the virus itself or for the ongoing inflammation to spread to islets.

Enteroviruses may contribute to the development of T1D by various mechanisms, including direct destruction of β-cells due to virus infection; viral persistence and chronic stimulation and recruitment of immune cells to the islets to promote local inflammation, β-cell injury and subsequent release of autoantigens, which then trigger autoreactive T-cell responses ultimately mediating β-cell death [2,13]. Another possible mechanism is "molecular mimicry", in which immune reactivity is driven by similarity of viral and β-cell epitopes. Similar hypotheses are applicable to autoimmune diseases in general, but presently it is unclear whether viruses directly initiate autoimmunity and target cell destruction or only accelerate this process [14].

In order to efficiently replicate, viruses hijack the cellular machinery and signaling pathways. While external and internal receptors for enterovirus entry and sensing are known [15,16], the endogenous host factor(s), their regulation in response to virus infections, and the

molecular mechanisms which lead to excessive stimulation of the immune system remain elusive. Pathways which regulate host's cellular survival and proliferation may allow a virus to attack the cell replication machinery.

Hippo signaling represents an evolutionarily conserved pathway that controls organ size, tissue homeostasis, and cellular survival; it has been linked to the pathophysiology of cancer and metabolic diseases [17,18]. Yes-associated protein (YAP) is the transcriptional co-regulator and major terminal effector of the Hippo pathway. The activity of YAP is mainly regulated through a phosphorylation-dependent inhibition mechanism by the Hippo central kinases, mammalian STE20-like protein kinase 1 and 2 (MST1/2) and large tumor suppressor 1 and 2 (LATS1/2). Upon MST1/2 activation by physiological or pathological signals, MST1/2 phosphorylate and activate the LATS1/2 kinases, which in turn directly phosphorylate YAP on multiple sites, leading to YAP inactivation through its cytoplasmic retention and/or its degradation by the proteasome machinery. In contrast, when Hippo signaling is inhibited, YAP can freely translocate into the nucleus where it interacts with several different transcription factors such as the TEA domain family members (TEAD) and stimulates the expression of genes responsible for cell turnover, differentiation and regeneration [17]. The Hippo pathway has major control over pancreas development and β-cell survival, regeneration and function [19-21]. YAP is broadly expressed in pancreatic progenitor cells in the developing pancreas and is indispensable for pancreatic cell identity through directing cell fate decisions and organ morphogenesis [22,23]. While YAP's presence maintains in the exocrine pancreas and is essential for its function and plasticity, its expression is extremely low or undetectable in terminally differentiated, adult endocrine islet cells [24-26]. Importantly, we and others have previously shown that re-expression of active YAP induces human β-cell proliferation, indicating that the absence of YAP in adult human β-cells correlates with their low-replication capacity and β-cell quiescence [26,27]. Further, previous studies have linked YAP with innate immunity to balance host antiviral immune responses [28,29]. Thus, we investigated YAP as potentially dysregulated factor and initiator of the immune disbalance in T1D, and determined the functional significance and molecular mechanisms of YAP in enteroviral replication starting in the exocrine pancreas and promoting islet inflammation and β-cell apoptosis.

Results

YAP is highly upregulated in the pancreas of T1D and AAb$^+$ organ donors

Based on the fact that YAP is expressed in the human exocrine pancreas and directly linked to innate immunity and host inflammatory responses, we first examined the endogenous expression of YAP in the exocrine pancreas. Immunohistochemistry (IHC) for YAP was performed and analyzed in paraffin-embedded pancreatic tissue from organ donors with T1D (n=15), AAb$^+$ (n=15) and age and BMI-matched non-diabetic controls (n=13) from the well-

characterized cohort of organ donors from nPOD (Network for Pancreatic Organ Donors with Diabetes; Table S1) [30]. YAP protein expression, represented as %YAP-positive area in the exocrine pancreas, was significantly higher in T1D (mean 19.95%) than in AAb^+ (mean 14.09%) and nondiabetic individuals (mean 11.97%) (Figures 1A,B). Moreover, a modest but significant increase in YAP-positive area in exocrine regions was also observed in AAb^+ donors compared to nondiabetic controls (Figures 1A,B). Consistent with previous findings [24,31], ductal and terminal-duct centro-acinar cells expressed the highest levels of YAP in the exocrine pancreas (Figure S1A). The majority of AAb^+ and T1D donors abundantly expressed YAP within centro-acinar and ductal cells, while much less ductal YAP expression was observed in non-diabetic controls (Figure S1A).

Endocrine cells, including β-cells, do not express YAP [26,27]. To investigate whether intra-islet expression of YAP in T1D is induced, we quantified the number of YAP-positive cells within the islet area. YAP-positive cells were markedly higher in islets from T1D (mean 3.05%) donors than from nondiabetic AAb^+ (mean 1.78%) and from control (mean 0.64%) donors (Figures 1C,D and S1B). To determine which cell types were YAP-positive in AAb^+ and T1D islets, tissue sections were stained for YAP and chromogranin, a late endocrine marker also expressed by islet cells which have lost hormone expression [32]. Consistent with the higher intra-islet YAP expression observed in T1D donors, also the percentage of YAP/chromogranin double-positive cells was significantly higher in islets from T1D donors (mean 0.62%) than in AAb^+ (mean 0.10%) or nondiabetic (mean 0.04%) donors (Figures 1E,F).

The higher YAP protein abundance was paralleled by elevated *Yap* mRNA expression. As determined using the highly sensitive in situ hybridization (ISH) RNAscope method, *Yap1* mRNA levels were significantly increased in donors with AAb^+ (mean 1.8 puncta per cell) and T1D (mean 3.01) compared with nondiabetic controls (mean 1.37) (Figures 1G,H). In addition, the expression of *Yap1* was higher in pancreases from T1D compared to AAb^+ donors (Figures 1G,H). Importantly, exocrine YAP levels highly correlated with endocrine YAP expression in T1D ($r=0.6964$; $p=0.005$) donors, while there was a similar trend in AAb^+ ($r=0.6242$; $p=0.060$) (Figure 1I). These data indicate an association of YAP upregulation as common modulator in both pancreas compartments with T1D; not only in islets but also in the exocrine pancreas. YAP expression and patients' clinical parameters revealed no correlation between YAP and age, BMI or Hb1AC in AAb^+ and T1D donors (Figures S1C-E).

YAP colocalizes and correlates with enteroviral RNA expression in the pancreas

Recent research has shown that YAP seems to play a highly complex and bidirectional role in the control of innate immunity. On one hand, it balances inflammation and host's antiviral immune responses and is vital for cellular survival during infection [28,29]. On the contrary, YAP can be pro-inflammatory and drive inflammation [31,33-36]. To determine YAP's complex role in

pancreatic inflammation and its association with enteroviral infection in the pancreas of AAb^+ and T1D donors, we analyzed YAP's cellular colocalization with two diabetogenic β-cell-tropic strains of CVB; CVB3 and CVB4 (CVB3/4) RNA. Double ISH-RNA analysis of *Yap* and CVB3/4 RNAs allowed us to systematically localize and quantify RNA throughout the whole pancreas sections. Due to the expected absent/very low number of virus-positive cells in the control group [8], such analysis was only possible in AAb^+ and T1D donors. Using single-cell analysis of CVB3/4 RNA and *Yap* mRNA staining, we categorized infected cells into three groups: 1) cells with both YAP and viral RNA present in the same cell ("YAP^+/CVB^+"), 2) cells with viral RNA present in cells in close proximity of neighbor YAP-positive cells ("n-YAP^+/CVB^+") and 3) cells with no YAP but positive for viral RNA ("YAP^-/CVB^+"; Figure S2). *Yap* mRNA and enteroviral RNA mainly colocalized in the same cell, or Yap-positive cells were in close proximity to infected cells (Figures 2A-C). Quantification of double-positive pancreatic cells revealed the percentage of YAP/virus-co-positive cells were significantly higher in both patients with T1D as well as AAb^+ donors compared to YAP-negative/virus-positive cells (Figure 2C). Importantly, the mean number of YAP/virus-positive cells with both single (5-10 puncta per cell) and cluster (>10 puncta per cell) infections were markedly higher in T1D than AAb^+ donors (mean, 56 and 29 in T1D versus 31 and 7 in AAb^+ for single and cluster infections, respectively; Figure 2D) similar to what has been previously reported by us using the single-molecule *in situ* hybridization (smFISH) approach [8]. In addition to their cellular co-expression, YAP expression in the exocrine pancreas showed trends of positive correlation with the number of virus-expressing cells within the same region in AAb^+ donors ($r=0.6193$; $p=0.08$; Figure 2E) and with the number of virus-expressing cells in the pancreas of T1D donors ($r=0.5149$; $p=0.06$).

To confirm YAP-virus colocalization in the pancreas of AAb^+ and T1D donors at a single cell level, we complemented classical YAP-IHC staining with enteroviral RNA smFISH, which we have previously established to identify and localize enteroviral RNA in pancreata [37]. In line with CVB3/4-YAP RNA expression, YAP-protein/viral RNA double-positive cells were detected in AAb^+ and T1D donors (representative images shown in Figure 2F); most of the infected pancreatic cells expressed YAP. This suggests a pathological association between YAP and enteroviruses and raises the question, whether the presence of YAP rather induces than balances enteroviral replication, and/or whether the infection *per se* may be a principal inducer of *Yap* transcription.

YAP enhances coxsackievirus replication and potentiates coxsackievirus- induced islet inflammation and β-cell apoptosis

To investigate a link between YAP and CVB infection and its functional significance on β-cells, they were infected with CVB3 and CVB4 (MOI of 5 and 10 for INS-1E β-cells and human islets,

respectively) [38,39], together with the adenoviral mediated transduction of a constitutively active form of YAP (YAP-S127A). YAP overexpression was sufficient to enhance viral replication seen by the substantially increased CVB3 and CVB4 genomic RNA, relative to the control LacZ transduced INS-1E cells (Figure 3A) and human islets (Figure 3B). The pro-viral effect of YAP was also confirmed by the increased level of the enterovirus-specific viral capsid protein VP1 upon YAP overexpression, compared to the control LacZ group in both INS-1E β-cells (Figures 3C,D) and human islets (Figures 3E,F). Immunofluorescence of VP1 and insulin verified the significant increase in the number of the VP1-positive β-cells by YAP overexpression in CVB-infected human islets, in comparison to control LacZ overexpression (Figures 3G,H). This supports the hypothesis that YAP hyper-activation potentiates viral replication. Further, microscopy analysis of infected cells revealed the abundant YAP/VP1/insulin triple-positive cells in primary human islets suggesting the cell-autonomous action of YAP (Figure S3A).

Besides β-cells, pancreatic exocrine cells and ductal cells in particular are highly susceptible to CVB infections [40]. As adult ductal cells naturally express YAP, we investigated whether endogenous YAP has a similar pro-viral effect. We used verteporfin (VP), a chemical inhibitor of the YAP-TEAD complex [41], which blocked downstream actions of YAP. Immunofluorescent staining for VP1 and the ductal marker CK19 showed VP1-CK19 co-positive cells in both CVB3 and CVB4 infected human ductal cells (Figure 3I). The inhibition of YAP by VP led to the reduction in CVB3 and CVB4 replication as determined by the quantification of VP1/CK19 double-positive cells (Figures 3I,J). Similarly, YAP blockade by VP significantly abolished CVB4 RNA genome replication in the infected human ductal cell line PANC1 (Figure S3B). The efficiency of VP to inhibit YAP signaling was verified by mRNA analysis of YAP's target gene connective tissue growth factor (CTGF) [42], which was reduced by VP (Figure S3C). Consistently, VP also attenuated the number of VP1-positive cells, compared to control PANC1 cells only exposed to CVB4 (Figures S3D,E). These results suggested that YAP was indispensable for CVBs replication in both primary and immortalized ductal cells.

As YAP potentiates CVBs replication in both primary and immortalized β-cells, we further investigated whether this higher virus replication also increases apoptosis. CVBs highly induce β-cell apoptosis [16]. YAP overexpression promoted a significant increase in CVB-mediated β-cell apoptosis as determined by caspase-3 cleavage, a universal marker of apoptosis, in INS-1E β-cells (Figures 3K,L) as well as in human islets (Figures 3M,N). TUNEL staining together with insulin confirmed the increased level of β-cell apoptosis in human islets upon YAP overexpression compared to LacZ-overexpressed controls (Figures 3O,P). Our data indicate an increase in viral replication together with virus-induced cell death, which would then lead to a higher rate of viral spread and a vicious cycle with viral progeny. Such hypothesis was confirmed by using polyinosinic-polycytidylic acid poly(I:C), a synthetic analog of double stranded RNA which mimics viral infection. YAP overexpression even declined poly(I:C)-

induced β-cell apoptosis compared to the LacZ-transduced control group in INS-1E cells (Figures S3F,G) suggesting that the pro-apoptotic function of YAP fully depends on CVB replication.

As inflammatory/innate immunity responses mediate the pathophysiological mechanisms from enteroviral infection to T1D [43,44] and YAP was shown to be linked to inflammatory reactions [45], we next assessed the impact of YAP on islet inflammation during CVB infections. In line with previous data [10,43,44], infection of human islets with CVB3 and CVB4 induced a strong type I interferon response represented by the mRNA upregulation of IFN-β (*IFNB1*) and the consequent production of IFN-stimulated genes (ISGs) including *CXCL10* and *OSA1* (Figures S3H-J) as well as by the Pattern recognition receptors (PRRs) and enteroviral sensors, such as RIG-I (*DDX58*), MDA-5 (*IFIH1*) and *TLR3* (Figures S3K-M). Indeed, YAP overexpression further enhanced not only *IFNB1* mRNA expression but also IFN-induced expression of *CXCL10* and *OSA1* (Figures 3Q-S) as well as of the PRRs *DDX58*, *IFIH1* and *TLR3*, compared to LacZ-transduced control cells (Figures 3T-V; the magnitude of the response varied between individual donors). In line with the gene expression data, overexpression of YAP potentiated the secretion of CXCL10 by infected human islets (Figure 3W). All these data indicate that YAP-overexpressing islets presented higher levels of antiviral response components.

Chronic YAP re-expression induces diabetes by impairing insulin secretion and inducing β-cell dedifferentiation

To establish a pathological connection between diabetes and elevated YAP levels in β-cells of organ donors with T1D, we have generated doxycycline (dox)-inducible β-cell specific homozygous ($YAP^{+/+}$) Rip-Ins2-TetO-hYAP1-S127A mice ("β-YAP";) through crossing inducible active YAP overexpressing mice ($TetO\text{-}YAP^{Ser127A}$) [46] with mice carrying the tTA tetracycline transactivator under the control of the insulin promoter [47] (Figure 4A). Dox administration in adult mice led to robust β-cell selective induction of YAP in isolated islets confirmed by IHC and Western Blot (Figures 4B,C). YAP was then transiently induced over 2 weeks (Figure 4D), which led to glucose intolerance and impaired insulin secretion during an i.p. glucose challenge in both male and female mice (Figures 4E-J). To determine whether the defect was β-cell autonomous, we isolated islets from β-YAP and control adult mice under the same conditions and performed *ex vivo* glucose stimulated insulin secretion (GSIS) studies. Clearly, the extent of insulin secretion in response to a glucose was significantly abolished in β-YAP islets compared with controls (Fig.4K,L).

We further investigated whether YAP overexpression caused a loss of β-cell identity or dedifferentiation. We analyzed the expression of functional genes, including endocrine hormone (Ins1, Ins2), key β-cell transcription factors (Pdx1, NeuroD1, MafA, Nkx2.2, Nkx6.1, and Glis3), as well as critical genes involved in glucose sensing and metabolism (GCK, Slc2a2,

ABCC8, KCNJ11) using RT-PCR in islets isolated from β-YAP and control adult mice. We found that the expression level of all genes was highly downregulated in β-YAP islets (Figure 4M), suggesting loss of β-cell identity and functionality upon YAP re-expression in islets. We further investigated β-cell dedifferentiation by labeling ALDH1A3, a universal marker of β-cell dedifferentiation [48], which showed a significant induction of ALDH1A3/insulin double-positive cells (Figures S4A,B), as well as upregulation of ALDH1A3 in isolated islets from β-YAP islets (Figure S4C), compared to controls. These findings collectively indicate β-cell dedifferentiation by homozygous YAP overexpression.

Consistent with our previous findings in human islets [27], YAP activation possessed a strong pro-proliferative capacity in both male and female mice, as determined by the quantification of double-positive insulin and Ki67 or pHH3 cells, compared to non-Dox-treated YAP-negative littermates (Figures 4N-Q). The induction in β-cell replication was accompanied by a significant increase in the insulin-positive area and β-cell mass in both male and female mice (Figures 4R-V). A significant fraction of highly proliferating β-cells exhibits metabolic immaturity [49], as they simultaneously downregulate numerous metabolic and functional genes, including those related to glucose metabolism, as well as insulin expression and secretion, to allocate energy and cellular resources toward increasing their mass for replication. Thus, YAP-induced β-cell immaturity and dedifferentiation could be a consequence of YAP-induced proliferation.

To investigate whether enhancing β-cell maturation could reverse impaired insulin secretion and the loss of β-cell identity upon YAP overexpression, we used H1152, a chemical inhibitor of ROCK, which has been shown to increase insulin secretion and β-cell maturation [50]. While YAP activation abolished glucose-induced insulin release, H1152 treatment significantly restored insulin secretion in YAP-overexpressing islets (Figures S4D,E). In line with this, H1152 exposure of YAP-overexpressing mouse islets elevated the gene expression of most β-cell identity and functionality markers, although it was significant for a subset of genes (Nkx6.1, NeuroD1, Slc2a2, ABCC8, KCNJ11, Glis3) (Figure S4F). All these findings indicate that YAP-induced impaired insulin secretion and compromised cellular identity are reversible and could be restored by enhancing β-cell maturation.

Taken together, these experiments from mice with β-cell-specific homozygous overexpression of YAP demonstrated deleterious metabolic consequences associated with long-term selective overexpression of YAP in pancreatic β-cells.

A YAP-TEAD-MST1 feedback loop controls CVB replication and cell death

Dynamic and precise control of YAP activity by the upstream Hippo components is important to ensure proper cell stress response under physiological condition or upon invasion of pathogen. In the course of analyzing the Hippo pathway, we have surprisingly noticed an increase in total MST1 protein level in the YAP-overexpressing β-cells (Figures 5A-D),

suggesting a novel Hippo feedback loop, in which YAP in its function as transcriptional co-regulator induces *STK4* (gene encoding MST1) transcription. Indeed, the amount of *STK4* mRNA was substantially increased in INS-1E cells overexpressing active YAP compared to control cells (Figure 5E). We then examined whether this feedback mechanism operates *in vivo* using β-cell specific YAP-overexpressing (β-YAP-OE) transgenic mice. In line with data in cultured cells, a significant increase of *STK4* expression was evident in islets isolated from β-YAP-OE mice (Figure S5A) further supporting a role for YAP in *STK4* transcriptional regulation. Importantly, CVB4 infection itself triggered the induction of MST1 in both INS-1E cells and human islets (Figures S5B-E) suggesting that there may be a YAP-mediated feedback mechanism that occurs during CVB infection.

As YAP mostly acts through TEAD transcription factors (TEAD1-4) to regulate gene expression, we sought to mechanistically uncover the transcriptional regulatory activity of YAP/TEAD on MST1 (Figure S5F). The YAP-TEAD inhibitor VP reduced the transcriptional upregulation of *STK4* induced by YAP, compared to untreated INS-1E cells (Figure 5F). Consistently, VP fully reversed the induction of MST1 protein expression in YAP-overexpressing cells in both INS-1E cells (Figures 5G,H) and human islets (Figures 5I,J) in a dose-dependent manner. VP also triggered degradation of exogenous YAP as mechanism to block YAP downstream signaling (Figures 5G,I). The loss-of-function form of YAP with a S94A mutation abolishes its interaction with TEADs and therefore is transcriptionally inactive [42] and helps to further dissect the molecular basis of YAP/TEAD-mediated MST1 induction. Unlike the active form of YAP, overexpression of YAP-S94A mutant failed to induce MST1 at both mRNA and protein levels compared to the GFP-transfected INS-1E cells demonstrating that YAP stimulated MST1 in a TEAD-dependent manner (Figures S5G-I). Also, a genetically encoded fluorescently-tagged competitive inhibitor that blocks binding between YAP and TEAD ("TEAD inhibitor (TEADi") [51], attenuated *STK4* mRNA and MST1 protein levels in YAP-overexpressing cells (Figures S5J-L). Altogether, we conclude that a YAP-TEAD mediated transcriptional induction of *STK4* and consequently elevated MST1 protein abundance constitute a negative feedback loop.

We then examined whether *STK4* is a direct transcriptional target of the YAP/TEAD complex. Two putative TEAD1-binding motifs were identified in the rat *STK4* promoter region by using a transcription factor-binding site prediction platform, the Eukaryotic Promoter Database (ED) [52] (Figure S5M). To experimentally confirm this, we used a luciferase reporter assay to examine whether the transcriptional rate of the *STK4* promoter could be stimulated by YAP. The *STK4* promoter region including a 1.5 kb sequence proximal to the transcription start site was cloned into an pEZX-PG04.1 reporter vector and transfected into Hela cells. We then generated a Hela cell line stably expressing conditional Gaussia Luciferase (GLuc) reporter located downstream of the *STK4* promoter and constitutively secreted Alkaline Phosphatase (SEAP)

which was used as internal control for normalization. Dual reporter analysis showed that YAP overexpression significantly increased luciferase activity- as indicated by the ratio of secreted Gluc and SEAP-, compared to LacZ control, and this response was abolished by VP (Figure 5K). Chromatin immunoprecipitation (ChIP) coupled with qPCR (using two pairs of primers to amplify *STK4* promoter region) in INS-1E cells transduced with YAP or corresponding LacZ control was conducted to check whether the YAP/TEAD transcriptional complex directly interacts with the promoter region of *STK4* gene. ChIP data using anti-YAP antibody and specific primers for the *STK4* promoter showed that YAP specifically binds to the *STK4* proximal promoter- as represented by fold enrichment in YAP occupancy- in INS-1E cells overexpressing YAP but not in the LacZ-overexpressing cells, which was again blocked by VP (Figures 5L,M). Positive control primers to amplify *ANKRD1*, a well-established direct target gene of the YAP/TEAD complex [53], and a negative control IgG verified ChIP specificity (Figures 5L-N). All these complementary methods indicate that the YAP/TEAD complex occupies the *STK4* promoter and exerts *STK4* expression induction in β-cells, confirming the postulated negative feedback loop.

To test the functional relevance of this YAP-MST1 loop during CVB infection, we performed MST1 knockdown experiments. SiRNA- mediated depletion of endogenous MST1 enhanced VP1 production, whereas at the same time attenuated apoptosis in CVB4-infected YAP-transduced cells, compared to control siScr transfected counterparts (Figures 6A,B). Consistently, immunofluorescence and qPCR analyses revealed that MST1 silencing in INS-1E cells resulted in significantly higher CVB4 replication as represented by increased VP1-positive infected cells in the siMST1-YAP-CVB4 group compared to the corresponding siScr-YAP-CVB4 control (Figures 6C,D) as well as by increased intracellular CVB4 RNA genome (Figure 6E). To further confirm the anti-viral action of MST1, we used the dominant-negative form of MST1. Amino acid substitution mutation of the critical lysine within the ATP binding site (K59 for MST1) with alanine compromises MST1 kinase activity, thus MST1 is inhibited [54]. Infection of INS-1E cells transfected with MST1-K59 led to a marked enhancement of intracellular VP1 accumulation compared to the GFP-overexpressing cells, while inhibition of MST1 markedly attenuated the level of cleaved caspase-3 in YAP-overexpressing cells upon CVB4 infection (Figures 6F,G). Also, microscopy analysis of VP1-positive cells showed that MST1-K59 introduction stimulated an increase in CVB4 replication in INS-1E cells (Figures 6H,I). Similar to the immunofluorescent staining, genetic MST1 antagonism largely induced the viral copies of CVB4 RNA compared to the GFP-transfected control group (Figure 6J) further indicating that MST1 blocks CVB4 replication. Given that MST1 is an upstream inhibitor of YAP in the classical Hippo cascade, and activated YAP induced the expression of MST1, YAP-mediated MST1 upregulation might at the end serve as a negative feedback loop to limit excessive YAP hyper-activation and subsequent CVB replication and amplification; thus, the

YAP-MST1 feedback mechanism plays an important role in regulating the viral replication machinery.

Discussion

While there is abundant support for an association of enterovirus infections with T1D, little is known about the complex enteroviral-host interactions which ultimately may determine the outcome of viral infections in the pancreas. Dysregulated interactions may trigger islet autoimmunity and T1D. In this study, we show that YAP, a principal transcriptional effector of the Hippo pathway, was highly upregulated in the exocrine pancreas of AAb^+ and T1D organ donors. This suggests, that pathological disturbance in T1D starts in the whole pancreas. Few YAP^+ cells were also observed within islets of AAb^+ and T1D donors, where they have never been seen in controls and are physiologically disallowed. Such YAP expression was associated with enteroviral infections; the majority of CVB-infected pancreatic cells were either colocalized with YAP or located in close proximity to YAP-positive cells in AAb^+ and T1D pancreases. Cell-culture models of β-cells, human islets as well as human exocrine pancreatic cells showed that YAP hyperactivation directly fostered CVB replication, potentiated β-cell apoptosis and enhanced the expression of genes involved in innate immunity and antiviral defense. Conversely, pharmacological targeting of YAP blocked CVBs replication in YAP-expressing primary and immortalized pancreatic exocrine cells. Experiments involving transgenic mice with inducible β-cell-specific overexpression of YAP clearly demonstrated the metabolic consequences associated with long-term selective overexpression of YAP in pancreatic β-cells. These data directly link the pathological YAP upregulation observed in the pancreas and islets of patients with T1D to β-cell failure and metabolic deregulation.

Our detailed mechanistic work is the first to report MST1 as a direct YAP/TEAD target forming a cell-intrinsic feedback loop. This YAP-MST1 bidirectional interaction may act as "molecular brake" to restrict excessive YAP-driven viral replication and amplification, to promote discarding of infected host cells and to finally put the viral replication machinery on hold (Figure 7). Thus, we identified YAP as a pro-viral, and MST1 as an anti-viral factor. This seems neither specific to pancreatic exocrine and endocrine cells nor to CVBs. YAP also promotes viral replication and production during SARS-CoV-2 or influenza infections [55,56], while MST1 inhibits SARS-CoV-2 replication [55]. Accordingly, MST1 genetic deficiency enhances the susceptibility to pathogen infections as well as presents autoimmune symptoms (e.g., hypergammaglobulinemia and autoantibody production) [57-60].

An imbalance between immune activation and immune protection is a key pathological element of autoimmune diseases such as T1D. Previous investigations highlighted the important regulatory function of YAP in inflammatory signaling. While highly complex and context- and cell type-dependent, its dysregulation is connected to inflammatory-related disorders such as

atherosclerosis, non-alcoholic steatohepatitis (NASH), inflammatory bowel disease, pancreatitis and pancreatic cancer [45]. For example, YAP balances inflammation and supports tissue regeneration and repair, as *Yap* mRNA therapy improves cardiac function through anti-inflammatory mechanism in ischemia-reperfusion injury [61], or it blocks antiviral signaling to balance the host response which is vital for cellular survival during infection [28,29]. On the contrary, YAP can also be pro-inflammatory i.e., YAP drives hepatic inflammation in NASH [33], and as we show, YAP was a positive regulator of islet inflammation during CVB infection with an exaggerated interferon response that could initiate autoimmunity and loss of pancreatic cells as well as β-cells in T1D. In that line, YAP genetic loss in pancreatic neoplastic epithelial cells results in a decrease in the number of $CD45^+$ immune cells in the pancreas, together with the progression of pancreatic ductal adenocarcinoma (PDAC) [31]. Likewise, YAP antagonism blocks the secretion of pro-inflammatory cytokines by neoplastic cells [31]. Whether YAP controls inflammatory cell infiltration and inflammatory cytokine activation in preclinical mouse or human T1D requires further investigations, such as using YAP-loss-of-function intervention approaches.

The innate antiviral immunity i.e., the IFN response, is a key event in the course of autoimmunity and β-cell destruction. Type I IFN in islets triggers human leukocyte antigen I (HLA-I) [62], and HLA-I hyperexpression is a hallmark of pancreas pathology in T1D [63]. The transcriptional signature of IFN responses precedes islet autoimmunity [64], and several polymorphisms within the interferon signature are genetic risk factors for T1D [65,66]. In fact, incubation of islets with type I and III IFNs or boosting IFN response limits viral replication and associated cell injury in pancreatic islets [67,68]. If the activation of the IFN response is excessively prolonged or intense, it can also trigger autoimmune reactions in the islets and cause damage to β-cells. Interestingly, YAP has been implicated in innate immunity and was previously shown to negatively regulate the type I IFN response through blockade of antiviral signaling proteins TBK1 and/or IRF3 [28,29]. Thus, our finding here, that YAP upregulated the interferon response during CVB infection in the pancreas is somewhat paradoxical, given YAP's inhibitory action on the antiviral response. One explanation for this paradox could be that the higher innate immune/antiviral response observed in YAP-overexpressing cells is primarily derived from an insufficient eradication of the virus (possibly through existing genetic polymorphisms in the interferon signature). Another possibility is that MST1, YAP's target gene identified in our study, enhances the antiviral response by (1) classical inactivation of YAP which would relieve the TBK1/IRF3 suppression, (2) direct activation of IRF3 as reported before in a different context [69], or (3) degradation of IRAK1, a negative regulator of type 1 IFN signaling [70]. In any case, such boosted antiviral response is unable to protect YAP-overexpressing cells against cell death caused by massive viral replication indicating that the classical intrinsic regulatory function of YAP/MST1 in antiviral signaling is overridden by the

YAP-driven CVB amplification. In support of this argument, unlike in actual infections, YAP did not potentiate β-cell apoptosis in poly(I:C)-treated cells which confirms that cell death and lysis during CVB infection is a consequence of high viral replication. Similarly, UV-inactivated CVB virus is not able to kill β-cells [16], and the induction of proinflammatory cytokines and chemokines depends on viral replication [43].

Aberrant upregulation of YAP- marked by robust cytoplasmic and nuclear localization of YAP in ductal and centro-acinar cells- is not limited to the pancreas in T1D; other pancreatic disorders, including PDAC and pancreatitis present elevated expression of YAP [71-73]. YAP and its well-known target gene CTGF are robustly increased in pancreatitis [25,73-75], an inflammatory disease of the exocrine pancreas manifested by extensive loss of the normal exocrine parenchyma, fibrosis and inflammation, and both exocrine and endocrine functional failure. Commonly upregulated YAP in T1D as well as in PDAC and pancreatitis suggests that the Hippo/YAP pathway may play a general and central role in the pathogenesis of pancreatic disorders. Supported by using genetically engineered mouse models, pancreas-specific deletion of MST1/2 or LATS1/2, which is functionally equivalent to YAP activation, recapitulate T1D, PDAC or pancreatitis in terms of robust immune cell infiltration, widespread inflammation, fibrosis, reduced pancreas mass, exocrine dysfunction and disrupted islet architecture [24,25,74]. Importantly, genetic loss of YAP or CTGF neutralization is sufficient to rescue the phenotype [25,74] indicating that YAP is a key driver of such pancreatic structural and functional abnormalities. Notably, various environmental and metabolic factors, e.g., viral infections, inflammation, obesity, or diabetes have the potential to induce PDAC or pancreatitis [76,77]. Also, a significant number of patients diagnosed with PDAC or pancreatitis have impaired glucose tolerance or diabetes [78,79]. Although these pancreatic disorders differ mechanistically and phenotypically in many ways, YAP may function as a major hub of transcriptional convergence in the crosstalk between pancreatic cells and immune cells in response to microenvironmental cues such as infections or cellular transformation upon injury. YAP signaling could therefore be an important therapeutic target for pancreatic comorbidity disorders.

While the classical perspective regards T1D as a β-cell specific disease, recent findings indicate that T1D is a disorder that involves the entire pancreas in which the loss of functional β-cell mass is most evident [80,81], together with the decreased pancreas mass [80-84], immune cell infiltration and inflammation of the exocrine pancreas [85,86], and exocrine dysfunction/insufficiency [87,88]. An abnormal exocrine-endocrine cell interplay has previously been linked to the development of MODY8, a monogenic form of diabetes inherited in a dominant manner, in which a mutant gene expressed selectively in acinar cells induces impaired β-cell function and loss [89]. In a recent study, we have systematically shown the predominant presence of enteroviral RNA in the exocrine pancreas in patients with T1D [8]. This suggests that enteroviruses do not primarily target islet cells but the whole pancreas providing

a pathological connection between T1D-related changes in the exocrine pancreas and the development of disease. Enteroviral infections in the exocrine pancreas can induce fulminant T1D marked by extensive inflammation with inflamed (CXCL10-positive) and/or infected (VP1-positive) ductal and acinar cells surrounded by immune cells such as T-cells indicating the existence of non-neglectable immune responses to enteroviral infection and subsequent cell injury in the exocrine pancreas [90]. In line with this, previous studies reported that, in addition to islets [91], CXCL10 expression is induced in the exocrine tissue in T1D [92] and gene expression analyses show the robust antiviral signature mainly in the exocrine pancreas in T1D [93].

A dysregulated/abnormal crosstalk between the exocrine and endocrine pancreas may have a more important role in the development of T1D than previously believed. Persistently infected exocrine cells in the pancreas, where viral replication is promoted by YAP, could be a trigger for a chronic immune cell attack and the subsequent development of T1D in two ways: firstly, the persistently infected exocrine cells may act as "cellular reservoirs" that enhance viral replication in the pancreas, leading to higher viral loads and more efficient spread of the virus to the islet cells; and secondly, local inflammation triggered by the infected exocrine cells may directly harm β-cells and attract immune cells to infiltrate the islets, ultimately leading to the destruction of β-cells. Such complex exocrine-islet interactions require further mechanistic investigations- with major emphasis on immune cell responses and paracrine factors, in analogy with other pancreatic diseases. They will be key for targeted interventions for T1D.

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Author contributions

Conceptualization, Experimental design A.A.; Methodology S.G., K.M. and A.A.; Investigation S.G., H.L., M.K.M., D.G., S.R., M.E., A.M.G., D.B., B.L. and A.A.; Formal Analysis S.G., H.L., M.K.M., K.M. and A.A.; Writing - Original Draft A.A.; Writing - Review & Editing S.G., H.L., M.K.M., Z.A., A.P. and K.M.; Resources A.P.; Funding Acquisition and Supervision K.M. and A.A.

Declaration of interests

The authors declare no competing interests.

Methods

Cell culture, treatment and islet isolation

Human islets were isolated from pancreases of nondiabetic organ donors (both male and female) at University of Lille and ProdoLabs and cultured on Biocoat Collagen I coated dishes (#356400, Corning, ME, USA). The clonal rat β-cell line INS-1E was kindly provided by Claes Wollheim (Geneva & Lund University). The immortalized cell line Hela was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The human pancreatic exocrine ductal cell line PANC-1 was generously provided by Manfred Radmacher (Institute of Biophysics, University of Bremen). PANC-1 cells were cultured in complete DMEM (Invitrogen, CA, USA) medium at 25 mM glucose. Human islets were cultured in complete CMRL-1066 (Invitrogen) medium at 5.5 mM glucose. Hela and INS-1E cells were cultured in complete RPMI-1640 (Sigma Aldrich, Missouri, MO, USA) medium at 11.1 mM glucose. All media included with L-glutamate, 1% penicillin-streptomycin and 10% fetal bovine serum (FBS). INS-1E medium was supplemented with 10 mM HEPES, 1 mM sodium pyruvate and 50-μM β-mercaptoethanol. In some experiments, human islets, INS-1E cells and PANC-1 cells were additionally cultured with 1-5 μM YAP/TEAD inhibitor verteporfin (#SML0534, Sigma Aldrich, USA) for 6h-24h. INS-1E cells were cultured with 2 μg poly(I:C) for 24h (#P9582; Sigma

Aldrich). Hela cells were cultured with 2 µg puromycin-dihydrochlorid (P9620, Sigma, USA) for positive clonal selection.

All human islet experiments were performed in the islet biology laboratory, University of Bremen. Ethical approval for the use of human islets and formalin-fixed paraffin-embedded (FFPE) pancreatic tissue sections obtained from well-characterized organ donors from the network for Pancreatic Organ Donors with diabetes (nPOD) [30] had been granted by the Ethics Committee of the University of Bremen. The study complied with all relevant ethical regulations for work with human cells for research purposes. Organ donors are not identifiable and anonymous, such approved experiments using human islet cells for research is covered by the NIH Exemption 4 (Regulation PHS 398). Human islets were distributed by the two JDRF and NIH supported approved coordination programs in Europe (Islet for Basic Research program; European Consortium for Islet Transplantation ECIT) and in the US (Integrated Islet Distribution Program IIDP).

YAP-transgenic mice and islet isolation

β-cell-specific YAP overexpressing (YAP-OE) mice were generated by crossing inducible aYAP overexpressing mice (TetO-YAPSer127A, provided to our lab in collaboration with Fernando Camargo, Boston Children's Hospital, Boston, MA) [94] with mice carrying the tetracycline transactivator (tTA) under the control of the insulin promoter (RIP-rtTA mice, kindly provided by Al Powers, Vanderbilt University Medical Center, Nashville, TN, USA) [95]. In the Rip-Ins2-TetO-hYAP1-S127A mice, rtTA gene becomes activated specifically in the islet β-cells due to the Ins2 promoter. Upon doxycycline (a tetracycline analog) treatment, the rtTA protein in these cells can bind to the tet-response element (TRE) and subsequently causing the transcription of the constitutively active form of YAP gene which is under a CMV promoter element. This system enables a fine-tuned spatio-temporal control over the expression of the aYAP gene in the pancreatic β-cells. All the experiments were done on 8-10 weeks old mice and genotype of the mice is in homozygous condition. Pancreatic islets were isolated after 2 weeks doxycycline induction through drinking water in the mice. Islets from β-cell specific YAP-OE and respective control mice were isolated by pancreas perfusion with a Liberase TM (#05401119001, Roche, Mannheim, Germany) solution [91] according to the manufacturer's instructions and digested at 37°C, followed by washing and handpicking.

All mice used in this experiment were housed in a temperature-controlled room with a 12-h light-dark cycle and were allowed free access to food and water in agreement with NIH animal care guidelines, §8 German animal protection law, German animal welfare legislation and with the guidelines of the Society of Laboratory Animals (GV-SOLAS) and the Federation of Laboratory Animal Science Associations (FELASA). All protocols were approved by the Bremen Senate (Senator for Science, Health and consumer protection) and we have complied with all relevant ethical regulations for animal testing and research.

Glucose tolerance test and insulin secretion

For intraperitoneal glucose tolerance tests (ipGTT), mice were fasted overnight for 12 hours and injected intraperitoneally with glucose (B.Braun, Germany) at a dose of 1 g/kg body weight. Blood samples were collected at time points 0, 15, 30, 60, 90, and 120 minutes for glucose measurements using a Glucometer (FreeStyle; Abbott, IL, USA). Blood samples for insulin secretion were collected before (0 minutes) and after (15 and 30 minutes) intraperitoneal injection of glucose (2 g/kg body weight) and measured using an ultrasensitive mouse ELISA kit (ALPCO Diagnostics, NH, USA).

Viruses and virus purification and titration

Enteroviruses CVB3 (Nancy) and CVB4 (JVB) were kindly provided by Andreas Dotzauer (University of Bremen, Germany). Fetal Rhesus Kidney-4 (FRhk-4) cell line was used for the preparation and isolation of virus stocks. FRhk-4 cells were infected with CVB3 or CVB4 viruses for 2h and were cultured for 2-3 days until visualization of the cytopathic effect. The supernatant from these cells was harvested after 3 rounds of freezing and thawing followed by centrifugation for 10min at 720xg to precipitate cell debris. Virus purification was carried out by the sucrose gradient method using an ultracentrifuge. First supernatant was centrifuged at 4500 x g for 10min. Further, it was centrifuged for 12h at 120000 x g in 40% sucrose gradient buffer (40% sucrose, 10 mM Tris pH 7.5 100 mM NaCl and 1 mM EDTA). The invisible pellet was resuspended in 1x PBS. Aliquoted viral stocks were stored at −80 ℃. The TCID50 (tissue culture infectious dose 50%) was determined using serial dilutions. Briefly, FRhK-4 cells were seeded in duplicates in 96-well plates. They were infected for 2h in serum-free media with serial dilutions of viral stocks. The cytopathic effect was determined under a light microscope and the TCID50 was calculated accordingly to Spearman-Kärber.

Virus infection of human islets or cell line

INS-1E or PANC-1 cells were infected with CVB4 virus at MOI (multiplicity of infection) of 5 in 35 mm dishes. Virus stocks were diluted in FCS free medium and cells were inoculated with 750 µl at 37°C and 5% CO_2. Control cells were incubated only with 750 µl of FCS-free medium. After 2h infection, cells were washed three times with 1xPBS and media was replaced by 10% FCS supplemented media for 24h. Infection of Human islets was performed with CVB3 or CVB4 viruses at MOI 10 under the same condition. For human islets 48h post-infection endpoint was chosen and then cells were harvested for staining as well as protein or RNA analysis. The culture supernatants were collected for measuring secreted CXCL10.

Adenovirus transduction

The adenoviruses control Ad-CMV-b-Gal/LacZ (#1080) and Ad-CMV-h-YAP1-S127 (custom production) were purchased from VECTOR BIOLABS, PA, USA. Isolated human islets or INS-1E cells were infected with Ad-LacZ or Ad-YAP at a multiplicity of infection (MOI) of 100 (for human islets) or 10 (for INS-1E) for 4h in CMRL-1066 or RPMI-1640 medium without FBS

respectively. After 4h incubation, adenoviruses were washed off with PBS and replaced by fresh complete medium which contains 10% FBS. Human islets or INS-1E cells were collected for staining, as well as RNA and protein isolation after 48-72h transduction.

Plasmids and siRNAs

To knock down MST1, SMARTpool technology was used (Dharmacon, CO, USA). A mix of ON-TARGETplus siRNAs directed against the following sequences: rat MST1 (#L-093629-02) sequences CUCCGAAACAAGACGUUAA; CGGCAGAAAUACCGCUCCA; CGAGAUAUCAAGGCGGGAA; GGAUGGAGACUACGAGUUU. An ON-TARGETplus nontargeting siRNA pool (Scramble; siScr) served as controls.

Following plasmids have been used: Kinase-dead (MST1-K59; dnMST1) was kindly provided by Dr. Junichi Sadoshima and Dr. Yasuhiro Maejima (UMDNJ, New Jersey Medical School). pCMV-Flag-YAP-S94A was a gift from Kunliang Guan (Addgene plasmid # 33102; http://n2t.net/addgene:33102; RRID: Addgene_33102) [42]. pCEFL EGFP-TEADi was a gift from Ramiro Iglesias-Bartolome (Addgene plasmid # 140144; http://n2t.net/addgene:140144; RRID: Addgene_140144) [51]. pCMV-flag S127A YAP was a gift from Kunliang Guan (Addgene plasmid # 27370; http://n2t.net/addgene:27370; RRID: Addgene_27370) [96]. GFP plasmid was used as a control.

Transfection

GFP, EGFP-TEADi, MST1-K59, YAP-S94A, and pCMV-flag S127A YAP plasmids were used to overexpress these proteins in INS-1E cells. 100 nM MST1 or scr siRNAs were used for the transfection in INS-1E cells. To achieve silencing and overexpression, jetPRIME® transfection reagent (#114-75; Polyplus transfection, France) was used to deliver desired siRNA or DNA into INS-1E cells according to manufacturer's instructions. In brief, jetPRIME buffer was mixed with siRNA/DNA and vortexed for 10s, then jetPRIME® transfection reagent was added and vortexed for 1s. The mixture was stand at room temperature (RT) for 10 minutes after quick spin. The jetPRIME-siRNA or DNA complexes were then added to complete RPMI-1640 to transfect INS-1E cells. Transfection efficiency was estimated by fluorescent microscopy of GFP.

Glucose-stimulated insulin secretion (GSIS)

Insulin secretion in response to glucose stimulation (GSIS) was assessed in isolated mouse islets. The islets were initially pre-incubated in Krebs-Ringer bicarbonate buffer (KRB) with 2.8 mM glucose for 30 minutes, followed by exposure to fresh KRB containing 2.8 mM glucose for 1 hour (basal) and an additional 1 hour in KRB with 16.7 mM glucose (stimulated). After washing with 1xPBS, the islets were lysed using RIPA buffer to measure total insulin content, and insulin levels were quantified using human and mouse insulin ELISA kits (ALPCO Diagnostics, NH, USA). The secreted insulin values were normalized to the insulin content.

Western Blot analysis

Human islets or INS-1E cells were washed three times with ice-cold PBS after medium removal and lysed with RIPA lysis buffer (50 mM Tris HCl pH 8, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with Protease and Phosphatase Inhibitors (Thermo Fisher Scientific (TFS), MA, USA). Samples went under multiple freeze-thaw cycles and finally incubated on ice for 30 minutes with intermittent vortexing. The cell lysates were centrifuged at 16000 x g for 20 minutes at 4°C and the clear supernatant containing the extracted proteins were kept at -80°C for storage. Protein concentrations were measured by the BCA protein assay (TFS). Equivalent amounts of protein from each condition were run on a NuPAGE 4-12% Bis-Tris gel (Invitrogen; CA, USA) and electrically transferred into PVDF membranes. Membranes were blocked at RT using mixture of 2.5% milk (Cell Signaling Technology/CST, MA, USA) and 2.5% BSA (SERVA Electrophoresis GmbH, Heidelberg, Germany) for 1h and incubated overnight at 4°C with rabbit anti-cleaved caspase-3 (#9664), rabbit anti-YAP(D8H1X; #14074), rabbit anti-MST1 (#3682), rabbit anti-ALDH1A3 (#NBP2-15339), rabbit anti-GAPDH (#2118), rabbit anti-β-actin (#4967; all CST), and mouse anti-Enterovirus/VP1 (clone 5-D8/1 #M7064, Dako). All primary antibodies were used at 1:1,000 dilution in 1xTris-buffered saline plus Tween-20 (1xTBS-T) containing 5% BSA and 0.5% NaN_3. Later, membranes were incubated with horseradish-peroxidase-linked anti-rabbit or anti-mouse secondary antibodies (Jackson ImmunoResearch, PA, USA) and developed using Immobilon Western chemiluminescence assay system (Millipore, MA, USA). Analysis of the immunoblots was performed using Vision Works LS Image Acquisition and Analysis software Version 6.8 (UVP BioImaging Systems, CA, USA).

Measurement of CXCL10 release

CXCL10 secretion into culture media from controls and virus infected isolated human islets was measured by Human CXCL10/IP-10 DuoSet ELISA kit (#DY266-05, R&D Systems, MN, USA) according to the manufacturer's instructions.

qPCR analysis

Total RNA was isolated from cultured isolated islets or INS-1E/PANC-1 cells using TriFast (PEQLAB Biotechnologie, Germany). 500-1000ng of RNA were reverse transcribed to cDNA (RevertAid reverse transcriptase, Thermo Fisher Scientific (TFS), MA, USA). Quantitative RT-PCR was carried out as previously described [20] using Biosystems StepOne Real-Time PCR system (Applied Biosystems, CA, USA) with TaqMan assays or SybrGreen (Applied Biosystems). TaqMan® Gene Expression Assays were used for Stk4 (#Hs00178979), CTGF (#Hs01026927-g1), CXCL10 (#Hs00171042), IFNB1 (#Hs02621180), OAS1 (#Hs00973637), DDX58 (#Hs01061436), TLR3 (#Hs01551078), IFIH1 (#Hs00223420), ACTB (#Hs99999903), Stk4 (#Mm00451755), Tuba1a (#Mm00846967), Stk4 (#Rn01750112), ACTB (#Rn00667869), Nkx2.2 (#Mm00839794_m1), Nkx6.1 (#Mm00454961_m1), NeuroD1 (#Mm01946604_s1),

MafA (#Mm00845206_s1), Slc2A2 (#Mm00446229_m1), Abcc8 (#Mm00803450_m1), Glis3 (#Mm00615386_m1), Gck (#Mm00439129_m1C41), KcnJ11 (#Mm00440050_s1), Ins1 (#Mm04207513_g1), Ins2 (#Mm00731595_g1), PDX1 (#Mm00435565_m1), and), and ACT (#Mm00607939_s1). EV-RNA was detected by using a SybrGreen primer pair (forward: 5′-CGGCCCCTGAATGCGGCTAA-3′; reverse: 5′-GAAACACGGACACCCAAAGTA-3′). The relative changes in gene expression were analyzed by ΔΔCT method.

Chromatin immunoprecipitation (ChIP) assay

4 × 10^6 INS-1E cells were dual-cross-linked consecutively with 2mM disuccinimidyl glutarate (DSG, #20593, TFS) for 45 min and 1% formaldehyde for 10 min. ChIP was performed according to the user's instructions for SimpleChIP Enzymatic Chromatin IP Kit (#9003, CST). In brief, chromatin DNA was digested with micrococcal nuclease (MNase). Immunoprecipitation reactions were carried out with chromatin extracts using IgG negative control or YAP antibodies (both CST) overnight at 4°C. Proteinase K was added for de-crosslinking, and samples were incubated for 4 h in a water bath at 65°C. Precipitated DNA was quantitated by real-time PCR analysis. The SybrGreen primers used in this study to amplify the promoter regions were: STK4#1 fw 5´ CCTCGACTTCCTCATGGCTG 3´, rev 5´ ACTAGGGACCCAATGAGCCT 3´; STK4#2 fw 5´ GCCAGCCTGTTTCTTCCTCT 3´, rev 5´ CTCCACGACTGGTGAGGTTT 3´; ANKRD1 fw 5´ GTGTGATGCACAATGCTTGC 3´, rev 5´CTTATCGGGAAGCCAGGGAC 3´. ANRD1, a YAP target gene, was used as a positive control. All ChIP signals were expressed as a fold enrichment (as a ratio of the YAP signal to the IgG signal for each respective condition).

Dual reporter assay

Hela cells were seeded into 6-well plates and transiently transfected with pEZX-PG04.1 reporter construct (#RPRM55953-PG04, Genecopoiea, MD, USA) using jetPRIME® transfection reagent. After 48h post-transfection, stable Hela cells expressing conditional Gaussia Luciferase (GLuc) reporter located downstream of rat *STK4* promoter and constitutive Secreted Alkaline Phosphatase (SEAP) was generated by puromycin sections. After selection, Hela cells were maintained in culture medium containing 2µg/ml puromycin. Hela stable cells were then transduced with Ad-LacZ or Ad-YAP treated with or without VP. After 48h, medium was analyzed for activities of both GLuc and SEAP using the Secrete-PairTM Dual Luminescence and Gaussia Luciferase Assay Kit (Genecopoiea) per manufacturer's instructions. The data are presented as the relative change in normalized GLuc activities to SEAP.

Immunofluorescence

Paraffin-embedded bouin-fixed human islets or human primary pancreatic cells were deparaffinized and rehydrated. INS-1E or PANC-1 cells were fixed with 4% PFA for 30 min followed by 4 min permeabilization with 0.5 % Triton-X-100. Fixed or embedded cells were

then blocked with blocking buffer containing 3% BSA and then incubated overnight at 4°C with the following antibodies (single or double): guinea pig anti-insulin (#IR002, FLEX polyclonal DAKO), mouse, mouse anti-Enterovirus/VP1 (clone 5-D8/1 #M7064, Dako), mouse anti-chromogranin (#ab715, Abcam), mouse anti-cytokeratin 19/CK-19 (#15463-1, Abcam), rabbit anti-ALDH1A3 (#NBP2-15339) and rabbit anti-YAP (#14074, CST). The next day sections were incubated with Cy3-conjugated donkey anti-mouse (#715-165-150), FITC-conjugated donkey anti-guinea pig (706-096-148) or FITC- conjugated donkey anti-mouse (#715-095-150) or anti-rabbit secondary antibodies (all from Jackson Immuno Research Laboratories, West Grove, PA; 1:100 dilution) for 1h at RT or 37°C. β-cell apoptosis in fixed human islet sections were performed by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique according to the manufacturer's instructions (In Situ Cell Death Detection Kit, TMR red; Roche) and double stained for insulin. Slides were mounted with Vectashield with 4'6-diamidino-2-phenylindole (DAPI, #H-1200-10, Vector Labs).

YAP immunohistochemistry

Detection of YAP protein in pancreatic tissue was carried out by classical immunohistochemistry (IHC) coupled with SuperBoost™ tyramide signal amplification (#B40931, Biotin XX Tyramide SuperBoost™ Kit, Streptavidin, TFS). After tissues deparaffinization and dehydration, endogenous peroxidase was quenched by 3% hydrogen peroxidase for 1h at RT. Tissues were blocked by applying the blocking buffer for 1h at RT and subsequently were incubated with rabbit anti-YAP (D8H1X, #14074, CST) antibody alone or in combination with mouse anti-chromogranin (#ab715, Abcam) antibody overnight. A day after, sections were washed with PBS and were incubated with rabbit poly-HRP-conjugated secondary antibody for 1h at RT. To amplify the signal, a Tyramide working solution was prepared according to the manufacturer's instructions by adding the Tyramide solution and hydrogen peroxide into the reaction buffer. Sections were incubated for 10min at RT followed by applying reaction stop regent for 3min. The chromogenic detection was completed by applying ABC (Avidin/Biotin) system (VECTASTAIN® ABC-HRP Kit, Peroxidase-Standard, #PK-4000) for 1h and DAB substrate (3,3'-diaminobenzidine-DAB Substrate Kit, Peroxidase-HRP, #SK-4100, all Vector Laboratories) for 5 min; both at RT. For the YAP-chromogranin double labeling, staining continued by using fluorescein isothiocyanate (FITC)-conjugated secondary donkey anti-mouse antibody (#715-095-150, Jackson Immuno Research Laboratories, West Grove, PA) for 1h at RT. Counterstaining was performed by either DAPI or Hematoxylin.

RNAscope mRNA in situ hybridization assay

YAP or YAP/CVB3-4 double staining was performed using the RNAscope 2.5 HD Detection Duplex Reagent RNAscope kit (#322430, Advanced Cell Diagnostics) according to the

manufacturer's instructions. Human Yap1 (#419131-C2; ACD), and human CVB (#409301, V-CVB4; #409291, V-CVB3) probes were used to detect Yap1 or CVB mRNAs. Briefly, tissue sections were incubated for 1 h at 60 °C, deparaffinized and rehydrated by xylene and 100% ethanol for 10 and 2min, respectively. Target retrieval was performed for 15 min at 95-97 °C, followed by protease treatment for 15 min at 40 °C. Probes were then hybridized for 2h at 40 °C followed by repeated washing with wash buffer and then kept in 5x Saline-sodium citrate (SSC) buffer overnight. RNAscope amplification was carried out using two independent signal amplification systems based on HRP and AP labeled probes and ultimately visualized by red and green chromogenic substrates. At the end, sections were counterstained with Hematoxylin.

Single molecule fluorescence in situ hybridization (smFISH)

smFISH was used to detect enterovirus mRNA in pancreatic tissue sections by using single-molecule oligonucleotide probes carried out according to the highly sensitive protocol that was previously established in our lab [97]. FISH Probes were synthesized by Stellaris® (Biosearch Technologies, Inc.; Petaluma, CA, USA), and labeled with Quasar 570 [8,37]. The three probes sets recognizes various enteroviral strains for positive strand enteroviral RNA based on sequence similarities. FFPE sections were deparaffinized with Xylene for 30 min at 70°C and 10 min at room temperature then rehydrated in 100, 95, and 70% ethanol for 20, 10, and a minimum 60 min respectively. Sections were covered with 0.2M HCL for 20 min followed by washing in prewarmed 2xSSC for 15min in a shaking water bath at 70°C. For antigen retrieval, pepsin was used for 10min in 37°C humidified chamber and washed two times with PBS. Before hybridization, samples were equilibrated 2 times with buffer made by 10% formamide and 2XSSC. Probes hybridized overnight at 37 °C. Next day slides underwent several times of washing at 37°C in a shaking water bath including 2xSSC plus 10% formamide for 40 min, 2xSSC 30 min, 1xSSC 30 min, 0.1xSSC for 20 min. Thereafter, classical immunostaining was performed for YAP and DAPI as detailed above. A 60x oil-immersion objective was used to acquire images by a Nikon Ti MEA53200 (NIKON GmbH, Düsseldorf, Germany) microscope.

Morphometric analysis

Morphometric analysis involved the examination of ten sections per mouse, spanning the pancreas width. Pancreatic tissue area and insulin-positive area were quantified through computer-assisted measurements using a Nikon MEA53200 microscope (Nikon GmbH, Germany), and images were captured with NIS-Elements software from Nikon. The average percentage of β-cell fraction per pancreas was computed as the ratio of insulin-positive area to the entire pancreatic tissue area. Pancreatic β-cell mass was obtained by multiplying the β-cell fraction by the weight of the pancreas [20].

Image analysis and quantification

Images were obtained using an inverse Nikon Ti2-A MEA54100 (NIKON GmbH, Düsseldorf, Germany) microscope with NIS-Elements Software (BR-ML). To quantify the YAP-positive area in the human exocrine pancreas, 229 different fields (in dependent positions) from 13 control donors, 223 from 15 AAb^+ donors, and 284 from 15 T1D donors were analyzed for YAP intensity by Image J.JS (v0 5.6) and data presented as % of YAP-positive area. The YAP-positive fraction in the islet was quantified manually by counting the number of YAP-positive cells in the pancreatic islet normalized to the number of all chromogranin-positive cells in the pancreas. % double YAP/chromogranin-positive cells were quantified by the number of double positive cells normalized to the number of chromogranin-positive cells. The infection rate in INS-1E cells was calculated by counting the number of VP1-positive cells divided by all cells from 40-50 randomly captured images under the 60x objective throughout the well. In PANC-1 cells, the same analysis was carried out with 12 randomly captured images under the 20x objective. Total number of cells in each image was quantified by manually counting all DAPI-stained nuclei using NIS-elements and used for normalization and to calculate the percentage of VP1-positive cells in the respective images. To quantify YAP-CVB3/4 double positive cells from RNAScope, infected cells were classified into two categories, low or single infection (5-10 puncta/cell) and full or cluster infection (>10 puncta/cell). Neighboring YAP-positive cells were the cells located exactly next to the infected cell. RNAScope YAP-mRNA was quantified by counting cells with YAP^+ puncta normalized to the number of all nuclei. Apoptosis and infection in isolated human islets were quantified by double-positive TUNEL/insulin or VP1/insulin cells normalized to all insulin-positive cells for each islet.

Statistical analyses

All statistics were performed using GraphPad Prism software (GraphPad Software Inc.). Gaussian distribution was determined by using a D'Agostino-Pearson normality test. Statistical comparisons between groups were analyzed for significance by a paired or unpaired two-tailed Student's t-test and a one-way or two-way analysis of variance (ANOVA) with Holm-Sidak multiple comparisons correction for a parametric test, or a Kruskall-Wallis test followed by Dunn post-test correction for a nonparametric test. A Spearman correlation analysis was used to assess the correlation between YAP protein expression and other markers. P value<0.05 was considered statistically significant. Data are presented as means ± SEM. The exact values of *n* (refers to number of donors or mice, or number of independent biological experiments or independent measurements/positions), and statistical significance are reported in the figure legends.

References

1. Atkinson, M.A., Eisenbarth, G.S. & Michels, A.W. Type 1 diabetes. *Lancet* **383**, 69-82 (2014).
2. Geravandi, S., Liu, H. & Maedler, K. Enteroviruses and T1D: Is It the Virus, the Genes or Both which Cause T1D. *Microorganisms* **8**(2020).
3. Rodriguez-Calvo, T. Enterovirus infection and type 1 diabetes: unraveling the crime scene. *Clin Exp Immunol* **195**, 15-24 (2019).
4. Zell, R. Picornaviridae-the ever-growing virus family. *Arch Virol* **163**, 299-317 (2018).
5. Dotta, F., *et al.* Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. *Proc Natl Acad Sci U S A* **104**, 5115-5120 (2007).
6. Richardson, S.J., Willcox, A., Bone, A.J., Foulis, A.K. & Morgan, N.G. The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. *Diabetologia* **52**, 1143-1151 (2009).
7. Ylipaasto, P., *et al.* Enterovirus infection in human pancreatic islet cells, islet tropism in vivo and receptor involvement in cultured islet beta cells. *Diabetologia* **47**, 225-239 (2004).
8. Geravandi, S., Richardson, S., Pugliese, A. & Maedler, K. Localization of enteroviral RNA within the pancreas in donors with T1D and T1D-associated autoantibodies. *Cell Rep Med* **2**, 100371 (2021).
9. Krogvold, L., *et al.* Detection of a low-grade enteroviral infection in the islets of langerhans of living patients newly diagnosed with type 1 diabetes. *Diabetes* **64**, 1682-1687 (2015).
10. Anagandula, M., *et al.* Infection of human islets of Langerhans with two strains of Coxsackie B virus serotype 1: assessment of virus replication, degree of cell death and induction of genes involved in the innate immunity pathway. *J Med Virol* **86**, 1402-1411 (2014).
11. Roivainen, M., *et al.* Mechanisms of coxsackievirus-induced damage to human pancreatic beta-cells. *J Clin Endocrinol Metab* **85**, 432-440 (2000).
12. Frisk, G. & Diderholm, H. Tissue culture of isolated human pancreatic islets infected with different strains of coxsackievirus B4: assessment of virus replication and effects on islet morphology and insulin release. *Int J Exp Diabetes Res* **1**, 165-175 (2000).
13. Nekoua, M.P., Alidjinou, E.K. & Hober, D. Persistent coxsackievirus B infection and pathogenesis of type 1 diabetes mellitus. *Nat Rev Endocrinol* **18**, 503-516 (2022).
14. Rojas, M., *et al.* Molecular mimicry and autoimmunity. *J Autoimmun* **95**, 100-123 (2018).
15. Hodik, M., *et al.* Coxsackie-adenovirus receptor expression is enhanced in pancreas from patients with type 1 diabetes. *BMJ Open Diabetes Res Care* **4**, e000219 (2016).
16. Marroqui, L., *et al.* Differential cell autonomous responses determine the outcome of coxsackievirus infections in murine pancreatic alpha and beta cells. *Elife* **4**, e06990 (2015).
17. Dey, A., Varelas, X. & Guan, K.L. Targeting the Hippo pathway in cancer, fibrosis, wound healing and regenerative medicine. *Nat Rev Drug Discov* **19**, 480-494 (2020).
18. Ardestani, A., Lupse, B. & Maedler, K. Hippo Signaling: Key Emerging Pathway in Cellular and Whole-Body Metabolism. *Trends Endocrinol Metab* **29**, 492-509 (2018).
19. Ardestani, A. & Maedler, K. The Hippo Signaling Pathway in Pancreatic beta-Cells: Functions and Regulations. *Endocr Rev* **39**, 21-35 (2018).
20. Ardestani, A., *et al.* MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes. *Nat Med* **20**, 385-397 (2014).
21. Yuan, T., *et al.* The Hippo kinase LATS2 impairs pancreatic beta-cell survival in diabetes through the mTORC1-autophagy axis. *Nat Commun* **12**, 4928 (2021).

22. Cebola, I., *et al.* TEAD and YAP regulate the enhancer network of human embryonic pancreatic progenitors. *Nat Cell Biol* **17**, 615-626 (2015).
23. Mamidi, A., *et al.* Mechanosignalling via integrins directs fate decisions of pancreatic progenitors. *Nature* **564**, 114-118 (2018).
24. George, N.M., Day, C.E., Boerner, B.P., Johnson, R.L. & Sarvetnick, N.E. Hippo signaling regulates pancreas development through inactivation of Yap. *Mol Cell Biol* **32**, 5116-5128 (2012).
25. Gao, T., *et al.* Hippo signaling regulates differentiation and maintenance in the exocrine pancreas. *Gastroenterology* **144**, 1543-1553, 1553 e1541 (2013).
26. George, N.M., Boerner, B.P., Mir, S.U., Guinn, Z. & Sarvetnick, N.E. Exploiting Expression of Hippo Effector, Yap, for Expansion of Functional Islet Mass. *Mol Endocrinol* **29**, 1594-1607 (2015).
27. Yuan, T., *et al.* Proproliferative and antiapoptotic action of exogenously introduced YAP in pancreatic beta cells. *JCI Insight* **1**, e86326 (2016).
28. Zhang, Q., *et al.* Hippo signalling governs cytosolic nucleic acid sensing through YAP/TAZ-mediated TBK1 blockade. *Nat Cell Biol* **19**, 362-374 (2017).
29. Wang, S., *et al.* YAP antagonizes innate antiviral immunity and is targeted for lysosomal degradation through IKKvarepsilon-mediated phosphorylation. *Nat Immunol* **18**, 733-743 (2017).
30. Kaddis, J.S., Pugliese, A. & Atkinson, M.A. A run on the biobank: what have we learned about type 1 diabetes from the nPOD tissue repository? *Curr Opin Endocrinol Diabetes Obes* **22**, 290-295 (2015).
31. Zhang, W., *et al.* Downstream of mutant KRAS, the transcription regulator YAP is essential for neoplastic progression to pancreatic ductal adenocarcinoma. *Sci Signal* **7**, ra42 (2014).
32. Cinti, F., *et al.* Evidence of beta-Cell Dedifferentiation in Human Type 2 Diabetes. *J Clin Endocrinol Metab* **101**, 1044-1054 (2016).
33. Song, K., *et al.* Yes-Associated Protein in Kupffer Cells Enhances the Production of Proinflammatory Cytokines and Promotes the Development of Nonalcoholic Steatohepatitis. *Hepatology* **72**, 72-87 (2020).
34. Lv, Y., *et al.* YAP Controls Endothelial Activation and Vascular Inflammation Through TRAF6. *Circ Res* **123**, 43-56 (2018).
35. Morvaridi, S., Dhall, D., Greene, M.I., Pandol, S.J. & Wang, Q. Role of YAP and TAZ in pancreatic ductal adenocarcinoma and in stellate cells associated with cancer and chronic pancreatitis. *Sci Rep* **5**, 16759 (2015).
36. Wang, D., *et al.* YAP promotes the activation of NLRP3 inflammasome via blocking K27-linked polyubiquitination of NLRP3. *Nat Commun* **12**, 2674 (2021).
37. Busse, N., *et al.* Detection and localization of viral infection in the pancreas of patients with type 1 diabetes using short fluorescently-labelled oligonucleotide probes. *Oncotarget* **8**, 12620-12636 (2017).
38. Colli, M.L., *et al.* Coxsackievirus B Tailors the Unfolded Protein Response to Favour Viral Amplification in Pancreatic beta Cells. *J Innate Immun* **11**, 375-390 (2019).
39. Bernard, H., *et al.* Coxsackievirus B Type 4 Infection in beta Cells Downregulates the Chaperone Prefoldin URI to Induce a MODY4-like Diabetes via Pdx1 Silencing. *Cell Rep Med* **1**, 100125 (2020).
40. Sane, F., *et al.* Coxsackievirus B4 can infect human pancreas ductal cells and persist in ductal-like cell cultures which results in inhibition of Pdx1 expression and disturbed formation of islet-like cell aggregates. *Cell Mol Life Sci* **70**, 4169-4180 (2013).
41. Liu-Chittenden, Y., *et al.* Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. *Genes Dev* **26**, 1300-1305 (2012).

42. Zhao, B., *et al.* TEAD mediates YAP-dependent gene induction and growth control. *Genes Dev* **22**, 1962-1971 (2008).
43. Schulte, B.M., *et al.* Cytokine and chemokine production by human pancreatic islets upon enterovirus infection. *Diabetes* **61**, 2030-2036 (2012).
44. Domsgen, E., *et al.* An IFIH1 gene polymorphism associated with risk for autoimmunity regulates canonical antiviral defence pathways in Coxsackievirus infected human pancreatic islets. *Sci Rep* **6**, 39378 (2016).
45. Mia, M.M. & Singh, M.K. Emerging roles of the Hippo signaling pathway in modulating immune response and inflammation-driven tissue repair and remodeling. *FEBS J* **289**, 4061-4081 (2022).
46. Camargo, F.D., *et al.* YAP1 increases organ size and expands undifferentiated progenitor cells. *Curr Biol* **17**, 2054-2060 (2007).
47. Cai, Q., *et al.* Enhanced expression of VEGF-A in beta cells increases endothelial cell number but impairs islet morphogenesis and beta cell proliferation. *Developmental biology* **367**, 40-54 (2012).
48. Son, J., *et al.* Genetic and pharmacologic inhibition of ALDH1A3 as a treatment of beta-cell failure. *Nat Commun* **14**, 558 (2023).
49. Puri, S., *et al.* Replication confers beta cell immaturity. *Nat Commun* **9**, 485 (2018).
50. Ghazizadeh, Z., *et al.* ROCKII inhibition promotes the maturation of human pancreatic beta-like cells. *Nat Commun* **8**, 298 (2017).
51. Yuan, Y., *et al.* YAP1/TAZ-TEAD transcriptional networks maintain skin homeostasis by regulating cell proliferation and limiting KLF4 activity. *Nat Commun* **11**, 1472 (2020).
52. Dreos, R., Ambrosini, G., Perier, R.C. & Bucher, P. The Eukaryotic Promoter Database: expansion of EPDnew and new promoter analysis tools. *Nucleic Acids Res* **43**, D92-96 (2015).
53. Stein, C., *et al.* YAP1 Exerts Its Transcriptional Control via TEAD-Mediated Activation of Enhancers. *PLoS Genet* **11**, e1005465 (2015).
54. Lupse, B., *et al.* Inhibition of PHLPP1/2 phosphatases rescues pancreatic beta-cells in diabetes. *Cell Rep* **36**, 109490 (2021).
55. Garcia, G., Jr., *et al.* Hippo signaling pathway activation during SARS-CoV-2 infection contributes to host antiviral response. *PLoS Biol* **20**, e3001851 (2022).
56. Zhang, Q., *et al.* Influenza A virus NS1 protein hijacks YAP/TAZ to suppress TLR3-mediated innate immune response. *PLoS Pathog* **18**, e1010505 (2022).
57. Crequer, A., *et al.* Inherited MST1 deficiency underlies susceptibility to EV-HPV infections. *PLoS One* **7**, e44010 (2012).
58. Nehme, N.T., *et al.* MST1 mutations in autosomal recessive primary immunodeficiency characterized by defective naive T-cell survival. *Blood* **119**, 3458-3468 (2012).
59. Halacli, S.O., *et al.* STK4 (MST1) deficiency in two siblings with autoimmune cytopenias: A novel mutation. *Clin Immunol* **161**, 316-323 (2015).
60. Dang, T.S., *et al.* Defective Leukocyte Adhesion and Chemotaxis Contributes to Combined Immunodeficiency in Humans with Autosomal Recessive MST1 Deficiency. *J Clin Immunol* **36**, 117-122 (2016).
61. Chen, J., *et al.* aYAP modRNA reduces cardiac inflammation and hypertrophy in a murine ischemia-reperfusion model. *Life Sci Alliance* **3**(2020).
62. Marroqui, L., *et al.* Interferon-alpha mediates human beta cell HLA class I overexpression, endoplasmic reticulum stress and apoptosis, three hallmarks of early human type 1 diabetes. *Diabetologia* **60**, 656-667 (2017).
63. Richardson, S.J., *et al.* Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. *Diabetologia* **59**, 2448-2458 (2016).

64. Ferreira, R.C., *et al.* A type I interferon transcriptional signature precedes autoimmunity in children genetically at risk for type 1 diabetes. *Diabetes* **63**, 2538-2550 (2014).
65. Nejentsev, S., Walker, N., Riches, D., Egholm, M. & Todd, J.A. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. *Science* **324**, 387-389 (2009).
66. Looney, B.M., Xia, C.Q., Concannon, P., Ostrov, D.A. & Clare-Salzler, M.J. Effects of type 1 diabetes-associated IFIH1 polymorphisms on MDA5 function and expression. *Curr Diab Rep* **15**, 96 (2015).
67. Flodstrom, M., *et al.* Target cell defense prevents the development of diabetes after viral infection. *Nat Immunol* **3**, 373-382 (2002).
68. Hultcrantz, M., *et al.* Interferons induce an antiviral state in human pancreatic islet cells. *Virology* **367**, 92-101 (2007).
69. Boro, M., Singh, V. & Balaji, K.N. Mycobacterium tuberculosis-triggered Hippo pathway orchestrates CXCL1/2 expression to modulate host immune responses. *Sci Rep* **6**, 37695 (2016).
70. Li, W., *et al.* STK4 regulates TLR pathways and protects against chronic inflammation-related hepatocellular carcinoma. *J Clin Invest* **125**, 4239-4254 (2015).
71. Guo, J., *et al.* Yes-associated protein (YAP65) in relation to Smad7 expression in human pancreatic ductal adenocarcinoma. *Int J Mol Med* **17**, 761-767 (2006).
72. Diep, C.H., *et al.* Down-regulation of Yes Associated Protein 1 expression reduces cell proliferation and clonogenicity of pancreatic cancer cells. *PLoS One* **7**, e32783 (2012).
73. Hu, C., *et al.* Yes-Associated Protein 1 Plays Major Roles in Pancreatic Stellate Cell Activation and Fibroinflammatory Responses. *Front Physiol* **10**, 1467 (2019).
74. Liu, J., *et al.* Activation of the intrinsic fibroinflammatory program in adult pancreatic acinar cells triggered by Hippo signaling disruption. *PLoS Biol* **17**, e3000418 (2019).
75. di Mola, F.F., *et al.* Connective tissue growth factor is involved in pancreatic repair and tissue remodeling in human and rat acute necrotizing pancreatitis. *Ann Surg* **235**, 60-67 (2002).
76. Parenti, D.M., Steinberg, W. & Kang, P. Infectious causes of acute pancreatitis. *Pancreas* **13**, 356-371 (1996).
77. Eibl, G. & Rozengurt, E. KRAS, YAP, and obesity in pancreatic cancer: A signaling network with multiple loops. *Semin Cancer Biol* **54**, 50-62 (2019).
78. Cui, Y. & Andersen, D.K. Diabetes and pancreatic cancer. *Endocr Relat Cancer* **19**, F9-F26 (2012).
79. Hart, P.A., *et al.* Diabetes following acute pancreatitis. *Lancet Gastroenterol Hepatol* **6**, 668-675 (2021).
80. Gepts, W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* **14**, 619-633 (1965).
81. Williams, A.J., *et al.* Pancreatic volume is reduced in adult patients with recently diagnosed type 1 diabetes. *J Clin Endocrinol Metab* **97**, E2109-2113 (2012).
82. Campbell-Thompson, M., Rodriguez-Calvo, T. & Battaglia, M. Abnormalities of the Exocrine Pancreas in Type 1 Diabetes. *Curr Diab Rep* **15**, 79 (2015).
83. Campbell-Thompson, M.L., *et al.* Relative Pancreas Volume Is Reduced in First-Degree Relatives of Patients With Type 1 Diabetes. *Diabetes Care* **42**, 281-287 (2019).
84. Campbell-Thompson, M.L., *et al.* The influence of type 1 diabetes on pancreatic weight. *Diabetologia* **59**, 217-221 (2016).
85. Rodriguez-Calvo, T., Ekwall, O., Amirian, N., Zapardiel-Gonzalo, J. & von Herrath, M.G. Increased immune cell infiltration of the exocrine pancreas: a possible contribution to the pathogenesis of type 1 diabetes. *Diabetes* **63**, 3880-3890 (2014).
86. Valle, A., *et al.* Reduction of circulating neutrophils precedes and accompanies type 1 diabetes. *Diabetes* **62**, 2072-2077 (2013).

87. Hardt, P.D., *et al.* High prevalence of exocrine pancreatic insufficiency in diabetes mellitus. A multicenter study screening fecal elastase 1 concentrations in 1,021 diabetic patients. *Pancreatology* **3**, 395-402 (2003).
88. Alexandre-Heymann, L., Mallone, R., Boitard, C., Scharfmann, R. & Larger, E. Structure and function of the exocrine pancreas in patients with type 1 diabetes. *Rev Endocr Metab Disord* **20**, 129-149 (2019).
89. Kahraman, S., *et al.* Abnormal exocrine-endocrine cell cross-talk promotes beta-cell dysfunction and loss in MODY8. *Nat Metab* **4**, 76-89 (2022).
90. Takita, M., *et al.* Unique Inflammatory Changes in Exocrine and Endocrine Pancreas in Enterovirus-Induced Fulminant Type 1 Diabetes. *J Clin Endocrinol Metab* **104**, 4282-4294 (2019).
91. Schulthess, F.T., *et al.* CXCL10 impairs beta cell function and viability in diabetes through TLR4 signaling. *Cell Metab* **9**, 125-139 (2009).
92. Sarkar, S.A., *et al.* Expression and regulation of chemokines in murine and human type 1 diabetes. *Diabetes* **61**, 436-446 (2012).
93. Valikangas, T., *et al.* Pancreas Whole Tissue Transcriptomics Highlights the Role of the Exocrine Pancreas in Patients With Recently Diagnosed Type 1 Diabetes. *Front Endocrinol (Lausanne)* **13**, 861985 (2022).
94. Camargo, F.D., *et al.* YAP1 increases organ size and expands undifferentiated progenitor cells. *Current biology* **17**, 2054-2060 (2007).
95. Cai, Q., *et al.* Enhanced expression of VEGF-A in β cells increases endothelial cell number but impairs islet morphogenesis and β cell proliferation. *Developmental biology* **367**, 40-54 (2012).
96. Zhao, B., *et al.* Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev* **21**, 2747-2761 (2007).
97. Busse, N., *et al.* Detection and localization of viral infection in the pancreas of patients with type 1 diabetes using short fluorescently-labelled oligonucleotide probes. *Oncotarget* **8**, 12620 (2017).

Figure legends

Figure 1. YAP is highly upregulated in pancreas of T1D and AAb$^+$ organ donors. YAP protein and *Yap1* mRNA labeling were analyzed in FFPE sections of pancreases from 13 control and 15 AAb$^+$ organ donors without diabetes and 15 donors with T1D from the nPOD pancreas collection. **(A,B)** Representative images from different donors **(A)** and quantification **(B)** of the percentage of YAP$^+$ area in the exocrine pancreas from FFPE sections of control donors without diabetes (n=229 independent positions throughout 13 pancreas slides from 13 donors), donors without diabetes but expressing T1D-associated autoantibodies (AAb$^+$) (n=223 independent positions from 15 donors), and donors with T1D (n=284 independent positions from 15 donors). **(C,D)** Representative images **(C)** and quantification **(D)** of the percentage of YAP$^+$ cells within islets of controls (n=10), AAb$^+$ (n=10), and donors with T1D (n=15) of the number of islet cells. **(E,F)** Representative images **(E)** and quantification **(F)** of YAP (brown), and late endocrine marker chromogranin (green) double-positive cells from controls (n=3; 16671 islet cells), AAb$^+$ donors (n=3; 14237 islet cells), and donors with T1D (n=6; 15116 islet cells). **(G,H)** Representative images **(G)** and quantification **(H)** of *Yap1* mRNA (pink) by RNAscope in situ hybridization of controls (n=30 independent positions from 3

donors), AAb^+ donors (n=30 independent positions from 3 donors), and donors with T1D (n=33 independent positions from 3 donors). **(I)** Association of YAP protein expression between endocrine islets and exocrine pancreas in AAb^+ (n=10; grey circles) and in donors with T1D (n=15; black circles). Box plots showing single analytes and median (min to max). **(A,C,G)** sections were counterstained with Hematoxylin. Data are expressed as means ± SEM. *p<0.05, **p<0.001, ***p<0.0001; by Kruskall-Wallis test followed by Dunn post-test correction for B, and one-way ANOVA with Holm-Sidak multiple comparisons correction for D,F and H. Scale bars depict 50 μm (A,C,G-upper panel) and 10 μm (E,G-lower panel).

Figure 2. YAP colocalizes and correlates with enteroviral RNA expression in the pancreas

(A-D) Detection and quantification of *Yap1* mRNA (pink) and viral RNA-CVB3/4 (turquois) by RNAscope in situ hybridization from FFPE nPOD pancreas sections of AAb^+ donors (n=9) and donors with T1D (n=10). **(A)** Representative images of *Yap1*/CVB-RNA double labelling from AAb^+ and T1D pancreatic sections **(B,C)** and total distribution and quantification throughout the whole pancreas section differentiated in YAP-viral RNA double positive cells (YAP^+/CVB^+; purple) CVB-positive cells in close proximity of YAP-positive neighbor cells (n-YAP^+/CVB^+; blue) or YAP-negative but CVB-RNA-positive cells (YAP^-/CVB^+; gray). **(D)** Quantification of all viral RNA-positive cells throughout the whole pancreas section in AAb^+ and T1D donors presented as the mean number of single (white; 5-10 single puncta/cell) or cluster (black; >10 single puncta/cell) infected cells. **(E;F)** Association between YAP protein expression and number of enterovirus-positive cells by smFISH for enteroviral RNA detection in AAb^+ donors (n=9) and donors with T1D (n=10). **(F)** Representative microscopical images of enteroviral RNA (red; Stellaris probes) and YAP protein (brown; IHC) expression in the pancreas showing YAP^+/$Enterovirus^+$ cells (YAP^+/V^+) and enteroviral positive cells in close proximity of YAP-positive neighbor cells (n-YAP^+/V^+). Data are expressed as means ± SEM. *p<0.05, **p<0.01, ***p<0.001; by one-way ANOVA with Holm-Sidak multiple comparisons correction for C, and two-tailed unpaired Student *t*-test for D. Scale bars depict 10 μm.

Figure 3. YAP enhances coxsackievirus replication and potentiates coxsackievirus-induced islet inflammation and β-cell apoptosis. (A,C,D,K,L) INS-1E cells and **(B,E-H,M-W)** human pancreatic islets transduced with Ad-YAP or Ad-LacZ control and then infected with CVB4 (MOI=5) for 24h (INS-1E) or CVB3 and -4 (MOI=10) for 48h (human islets). **(A,B)** Intracellular CVB3 or -4 RNA genome of **(A)** INS-1E cells (n=3 independent experiments) and **(B)** human pancreatic islets (n=4 organ donors). **(C-F)** Representative Western blots and pooled quantitative densitometry analysis of VP1 in **(C,D)** INS-1E cells (n=7 independent experiments) and **(E,F)** human islets (n=6 organ donors). **(G,H)** Representative images **(G)**

and quantitative percentage of VP1-positive β-cells **(H)** are shown (n=4 organ donors). **(I,J)** Human primary pancreatic cells transduced infected with CVB3 and -4 (MOI=10) for 48h treated with or without 2.5 uM verteporfin (VP) for the last 24h. Representative images **(I)** and quantitative percentage of CK19-positive ductal cells **(J)** are shown (n=6 independent positions from two organ donors). **(K-N)** Representative Western blots and pooled quantitative densitometry analysis of cleaved caspase 3 in **(K,L)** INS-1E cells (n=7 independent experiments) and **(M,N)** human islets (n=6 organ donors). **(O,P)** Representative images **(O)** and quantitative percentage of TUNEL-positive β-cells **(P)** are shown (n=3 organ donors). **(Q-V)** qPCR for **(Q)** IFNB1, **(R)** CXCL10, **(S)** OSA1, **(T)** IFIH1, **(U)** DDX58, and **(V)** TLR3 mRNA expression in isolated human islets normalized to actin (n=3-5 organ donors). **(W)** Secreted CXCL10 analyzed by ELISA in the culture media (n=8 independent samples from five organ donors). Data are expressed as means ± SEM. *p<0.05, **p<0.01, ***p<0.001; by two-tailed paired (A,B,D,F,H,J,L,N,P,W) or ratio paired (Q-V) Student *t*-test. Scale bars depict 50 µm (G,O) and 10 µm (I).

Figure 4: YAP re-expression induces diabetes by impairing insulin secretion and inducing β-cell dedifferentiation. **(A)** Scheme how β-YAP mice were generated by crossing RIP-rtTA with TetO-YAP^{Ser127A} mice. **(B)** IHC and **(C)** Western Blot confirmation of YAP induction in pancreatic islets after 2 days i.p injection of Dox. **(D)** YAP was transiently induced by doxycycline (DOX) administration in drinking water for 2 weeks (β-YAP) and results compared to -DOX/-YAP (control; C). **(E-H)** i.p. glucose tolerance test (GTT) and respective AUC analyses in β-YAP and control male **(E,F)** and female **(G,H)** mice (n=12-18 mice/group). **(I,J)** Insulin levels during an i.p.GTT measured before (0min) and 15/30 min after glucose injection in β-YAP and control male **(I)** and female **(J)** mice (n=9-11 mice/group). **(K,L)** Islets were isolated from β-YAP and control mice, cultured overnight and subjected to an *in vitro* GSIS. **(K)** Insulin secretion during 1h-incubation with 2.8 mM (basal) and 16.7 mM glucose (stimulated), normalized to insulin content and **(L)** stimulatory index denotes the ratio of stimulated to basal insulin secretion (n=11). **(M)** RT-PCR for *MafA, Nkx6.1, Slc2a2, NeuroD1, GCK, Ins1, Ins2, Pdx1, Nkx2.2, Glis3, Abcc8, and Kcnj11* (*n*=3-11). Microscopical analyses of β-proliferation by Ki67 **(N,O)** and pHH3 **(P,Q)** in both **(N,P)** male and **(O,Q)** female mice expressed as percentage of Ki67- or pHH3-positive β-cells (n=5-6 mice/group). Insulin-positive area **(R,T)** and β-cell mass **(S,V)** in both **(R,S)** male and **(T,V)** female mice (n=6 mice/group). Data are expressed as means ± SEM (SD for F,H). *p<0.05, **p<0.01, ***p<0.001 compared to control; by two-tailed unpaired Student *t*-test.

Figure 5. A YAP-TEAD axis regulates MST1 level. **(A,B,E)** INS-1E cells and **(C,D)** human pancreatic islets transduced with Ad-YAP or Ad-LacZ control for 48h. **(A-D)** Representative

Western blot and pooled quantitative densitometry analysis of MST1 in **(A,B)** INS-1E cells (n=6 independent experiments) and **(C,D)** human islets (n=7 organ donors). **(E)** qPCR for *STK4* mRNA expression in INS-1E cells normalized to actin (n=3 independent experiments). **(F-I)** INS-1E cells and human islets transduced with Ad-YAP or Ad-LacZ control for 48h treated with or without 1-5 uM verteporfin (VP) for last 6h (INS-1E) or 24h (human islets). Western blots and pooled quantitative densitometry analysis of MST1 in **(F,G)** INS-1E cells (n=3 independent experiments) and **(H,I)** human islets (n=3 organ donors). **(K-N)** Hela cells or INS-1E cells transduced with Ad-YAP or Ad-LacZ control for 48h treated with or without 1 uM verteporfin (VP) for the last 24h. **(K)** Hela cells culture media was analyzed for activities of both GLuc and SEAP and data presented as the relative change in normalized GLuc to SEAP. **(L-N)** ChIP from INS-1E cells was performed with control IgG, or YAP antibody as indicated. The presence of **(L,M)** *STK4* and **(N)** *ANKRD1* promoters was detected by PCR. Data presented as fold enrichment in which ChIP signals are divided by the IgG-antibody signals, representing the fold increase in signal relative to the background signal. Data are expressed as means ± SEM. *p<0.05, **p<0.01, ***p<0.001; by one-way and two-way ANOVA with Holm-Sidak multiple comparisons correction for K,L,M,N and F,H,J respectively, and two-tailed paired Student *t*-test for B,D,E. Scale bar depicts 10 μm.

Figure 6. A YAP-TEAD-MST1 feedback loop controls CVB replication and cell death. (A-E) INS-1E cells transfected with siMST1 or control siScr and then transduced with Ad-YAP or Ad-LacZ control for 48h. All cells were infected with CVB4 (MOI=5) for last 24h. **(A,B)** Representative Western blot and pooled quantitative densitometry analysis of MST1, VP1 and cleaved caspase 3 in INS-1E cells (n=3 independent experiments). **(C,D)** Representative images **(C)** and quantitative percentage of VP1-positive cells **(D)** are shown (n=28-30 independent positions). **(E)** Intracellular CVB4 RNA genome of INS-1E cells (n=4 independent experiments). **(F-J)** INS-1E cells transfected with MST1-K59 or control GFP constructs and then transduced with Ad-YAP or Ad-LacZ control for 48h. All cells were infected with CVB4 (MOI=5) for last 24h. **(F,G)** Representative Western blot **(F)** and pooled quantitative densitometry analysis **(G)** of MST1, VP1 and cleaved caspase 3 in INS-1E cells (n=3 independent experiments). **(H,I)** Representative images **(H)** and quantitative percentage of VP1-positive cells **(I)** are shown (n=40-50 independent positions). **(J)** Intracellular CVB4 RNA genome of INS-1E cells (n=3 independent experiments). Data are expressed as means ± SEM. *p<0.05, **p<0.01, ***p<0.001; by one-way with Holm-Sidak multiple comparisons correction for B,D, by Kruskall-Wallis test followed by Dunn post-test correction for I, and by two-tailed paired Student *t*-test for G,J. Scale bar depicts 10 μm.

Figure 7. Our model how a vicious cycle of YAP expression and CVB replication in the human pancreas may lead to T1D.
YAP is highly elevated in the pancreas of patients with T1D; it boosts enteroviral replication, induces a strong IFN response and islet inflammation ultimately leading to β-cell apoptosis and destruction. At the molecular level, YAP, through a feedback mechanism, induces the expression of its own negative regulator MST1 limiting YAP-driven viral replication and induces apoptosis of infected cells. Viruses and a local inflammatory milieu remain in the pancreas and T1D develops.

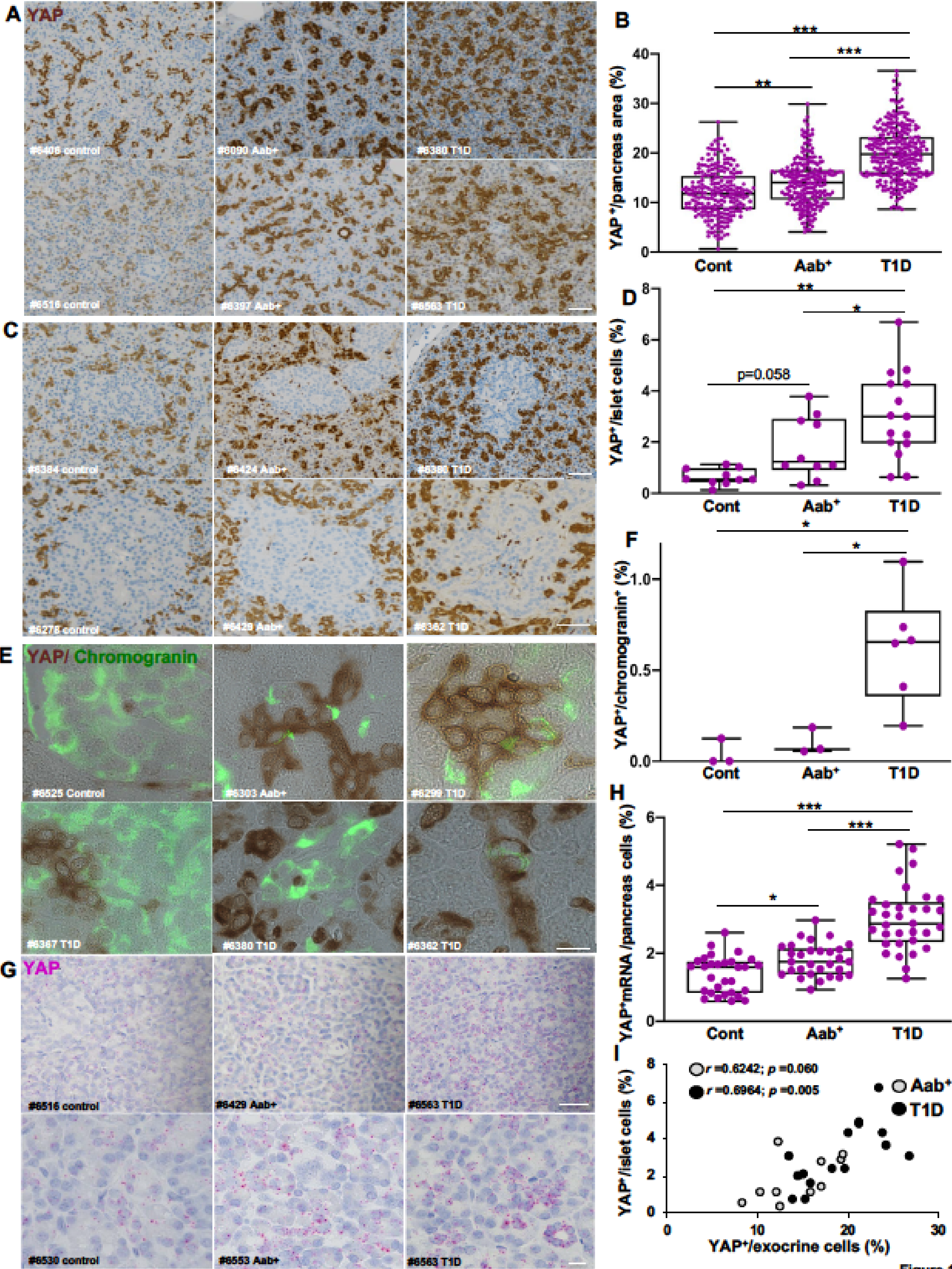
Figure S1. YAP is elevated in pancreases of T1D and AAb$^+$ organ donors. (A,B) Representative images from FFPE pancreas sections of control donors without diabetes, donors without diabetes but expressing T1D-associated autoantibodies (AAb$^+$), and donors with T1D of **(A)** YAP in exocrine pancreas and **(B)** of YAP (brown) and chromogranin (green) in the islet area. **(C-E)** Association between YAP protein expression in pancreases and **(C)** age and **(D)** BMI in AAb$^+$ donors (n=15, grey) and donors with T1D (n=15, black) and **(E)** with HbA1C in AAb$^+$ donors (n=12) and donors with T1D (n=13). Scale bars depict 50 µm (A) and 10 µm (B).
Figure S2. Various categories of infected pancreatic cells. Representative images of single (upper panel; 5-10 puncta of CVB-RNA expression/cell) and cluster (lower panel, >10 puncta of CVB-RNA expression/cell) infections of three different categories of YAP-viral RNA double positive cells (YAP$^+$/CVB$^+$), CVB-positive cells in close proximity of YAP-positive neighbor cells (n-YAP$^+$/CVB$^+$) or YAP-negative but CVB-RNA-positive cells (YAP$^-$/CVB$^+$) in human pancreases from AAb$^+$ and T1D donors. Scale bar depicts 10 µm.
Figure S3. YAP regulates coxsackieviruses replication and β-cell apoptosis. (A) Representative image of triple VP1-, YAP-, and insulin-positive β-cells is shown (n=4 organ donors). **(B-E)** PANC-1 cells infected with CVB4 for 48h treated with or without 0-5 µM verteporfin (VP) for last 24h. **(B)** Intracellular CVB4 RNA genome of PANC1 cells (n=3 independent experiments). **(C)** qPCR for CTGF mRNA expression in PANC1 cells (n=3 independent experiments). **(D,E)** Representative images **(D)** and quantitative percentage of VP1-positive cells **(E)** are shown (n=12 independent positions). **(F,G)** INS-1E cells transduced with Ad-YAP or Ad-LacZ control and then treated with Poly I:C for 24h. **(F)** Representative Western blot and **(G)** pooled quantitative densitometry analysis of cl caspase 3 in INS-1E cells (n=3 independent experiments). **(H-M)** qPCR for **(H)** *IFNB1*, **(I)** *CXCL10*, **(J)** *OSA1*, **(K)** *IFIH1*, **(L)** *DDX58*, and **(M)** *TLR3* mRNA expression in isolated human islets normalized to actin (n=3-5 organ donors). Data are expressed as means ± SEM. *p<0.05, **p<0.01, ***p<0.001; by one-way ANOVA with Holm-Sidak multiple comparisons correction for B and C, by two-tailed

unpaired Student *t*-test for E, and by two-tailed paired (G) or ratio paired (H-M) Student *t*-test. Scale bars depict 10 µm (A) and 50 µm (D).

Figure S4: YAP induces β-cell dedifferentiation. YAP was transiently induced by doxycycline administration in drinking water for 2 weeks (β-YAP) and results compared to -DOX/-YAP (control; C). **(A,B)** Quantitative analyses **(A)** and representative images **(B)** from triple stainings for ALDH1A3, insulin and DAPI expressed as percentage of ALDH1A3-positive β-cells (n=4). **(C)** Representative Western blot of ALDH1A3 in mouse islets (n=2). **(D-F)** Isolated mouse islets from β-YAP mice and respective control were left untreated or treated with 10 µM H1152 for 48 hours. **(D)** Insulin secretion during 1h-incubation with 2.8 mM (basal) and 16.7 mM glucose (stimulated), normalized to insulin content and **(E)** stimulatory index denotes the ratio of stimulated to basal insulin secretion (n=9-10). **(F)** RT-PCR for *MafA, Nkx6.1, Slc2a2, NeuroD1, GCK, Ins1, Ins2, Pdx1, Nkx2.2, Glis3*, *Abcc8, and Kcnj11* (n=5-10). Data are expressed as means ± SEM. *p<0.05, **p<0.01, ***p<0.001 by two-tailed unpaired Student *t*-test.

Figure S5. YAP-TEAD-MST1 negative feedback loop controls CVB replication and associated cell death. (A) qPCR for *STK4* mRNA expression in islets isolated from β-YAP and control mice recovered after isolation overnight (n=8-9 mice). **(B,C)** INS-1E cells and **(D,E)** human pancreatic islets infected with CVB4 (MOI=5) for 24h (INS-1E) or CVB4 (MOI=10) for 48h (human islets). **(B)** Representative Western blot and **(C)** pooled quantitative densitometry analysis of MST1 in INS-1E cells (n=5 independent experiments). **(D)** Representative Western blot and **(E)** pooled quantitative densitometry analysis of MST1 in human islets (n=5 organ donors). **(F)** A schematic which presents complementary approaches to block YAP/TEAD signaling. **(G-I)** INS-1E cells transfected with GFP, active YAP or YAP-S94A constructs for 48h. **(G)** qPCR for STK4 mRNA expression in INS-1E cells (n=3 independent experiments). **(H)** Representative Western blot and **(I)** pooled quantitative densitometry analysis of MST1 in INS-1E cells (n=4 independent experiments). **(J-L)** INS-1E cells transfected with GFP, or TEADi constructs and then transduced with Ad-YAP or Ad-LacZ control for 48h. **(J)** qPCR for STK4 mRNA expression in INS-1E cells (n=5 independent experiments). **(K)** Representative Western blot and **(L)** pooled quantitative densitometry analysis of MST1 in INS-1E cells (n=4 independent experiments). **(M)** Predicted TEAD1 binding sites in the rat *STK4* promoter identified by EPD (https://epd.epfl.ch/index.php). **(N-R)** INS-1E cells transfected with MST1-K59 or control GFP constructs and then transduced with Ad-YAP or Ad-LacZ control for 48h. All cells were infected with CVB4 (MOI=5) for last 24h. **(N,O)** Representative Western blot **(N)** and pooled quantitative densitometry analysis **(O)** of MST1, VP1 and cleaved caspase 3 in INS-1E cells (n=3 independent experiments). **(P,Q)** Representative images **(P)** and quantitative percentage of VP1-positive cells **(Q)** are shown (n=40-50 independent positions). **(R)** Intracellular CVB4 RNA genome of INS-1E cells (n=3 independent experiments). Data are

expressed as means ± SEM. *p<0.05, **p<0.01, ***p<0.001; by one-way ANOVA with Holm-Sidak multiple comparisons correction for G and I, by two-tailed unpaired Student *t*-test for A,L and by two-tailed paired Student *t*-test for C,E,J. Scale bar depicts 10 µm.



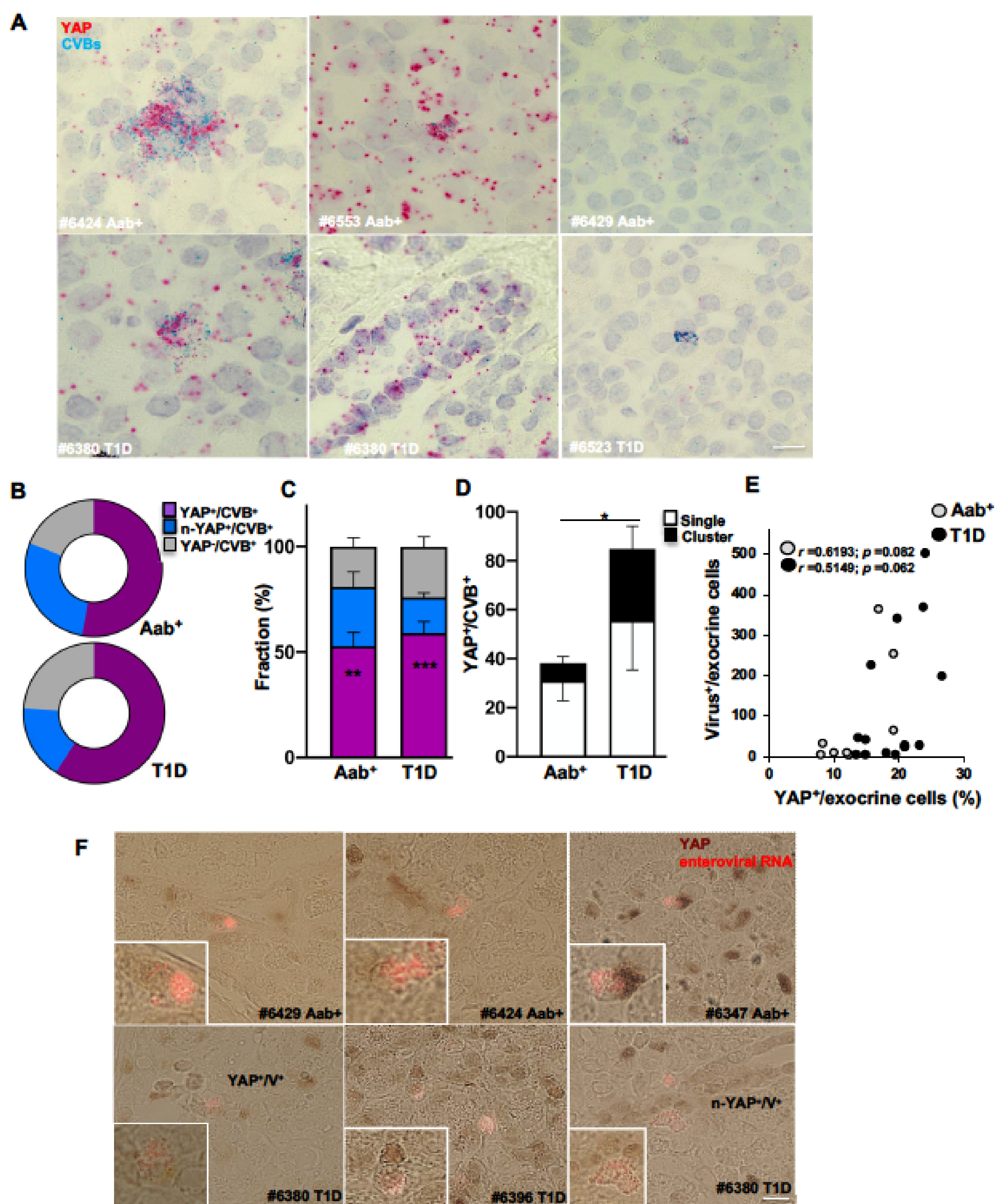
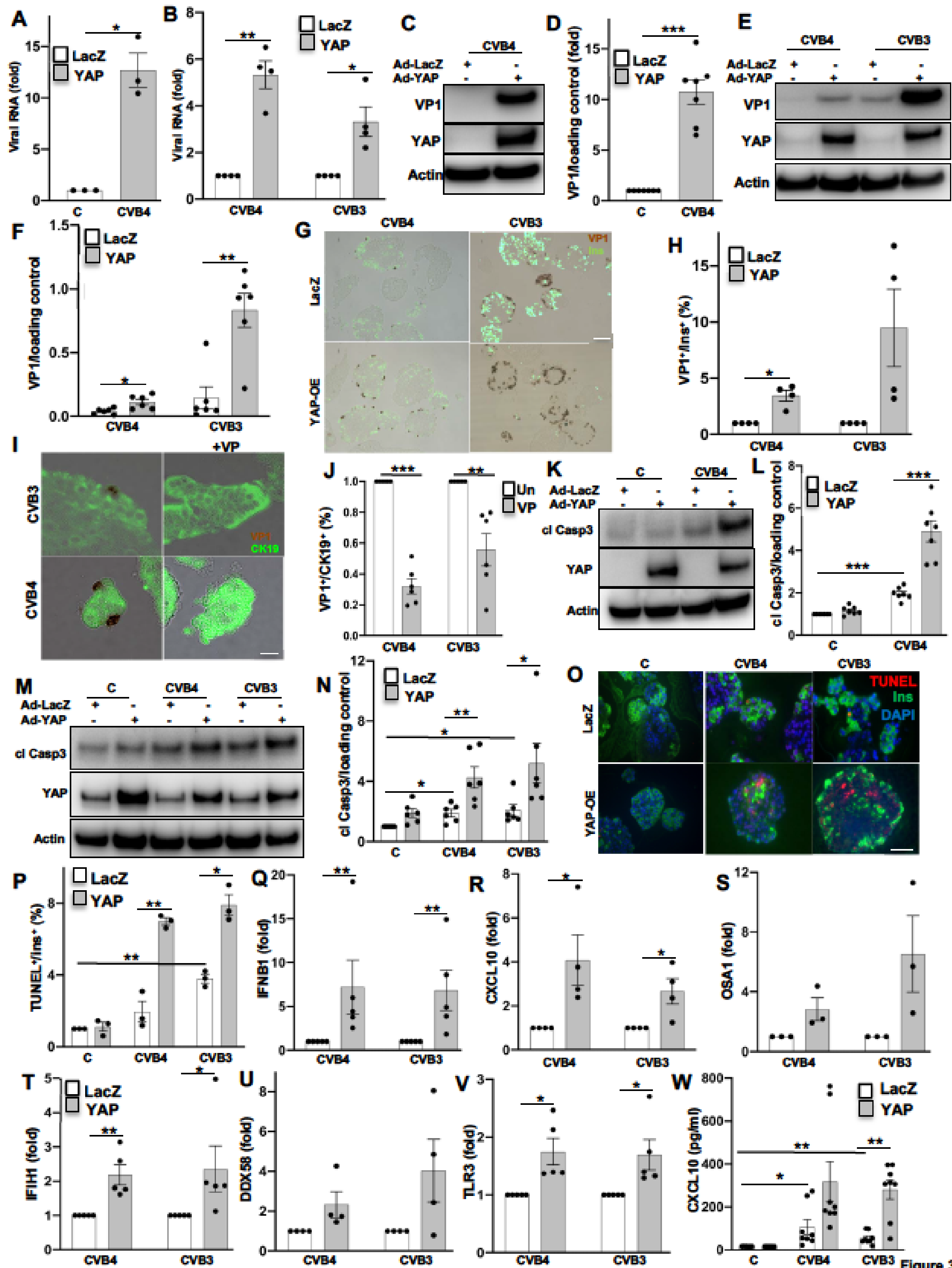


Figure 2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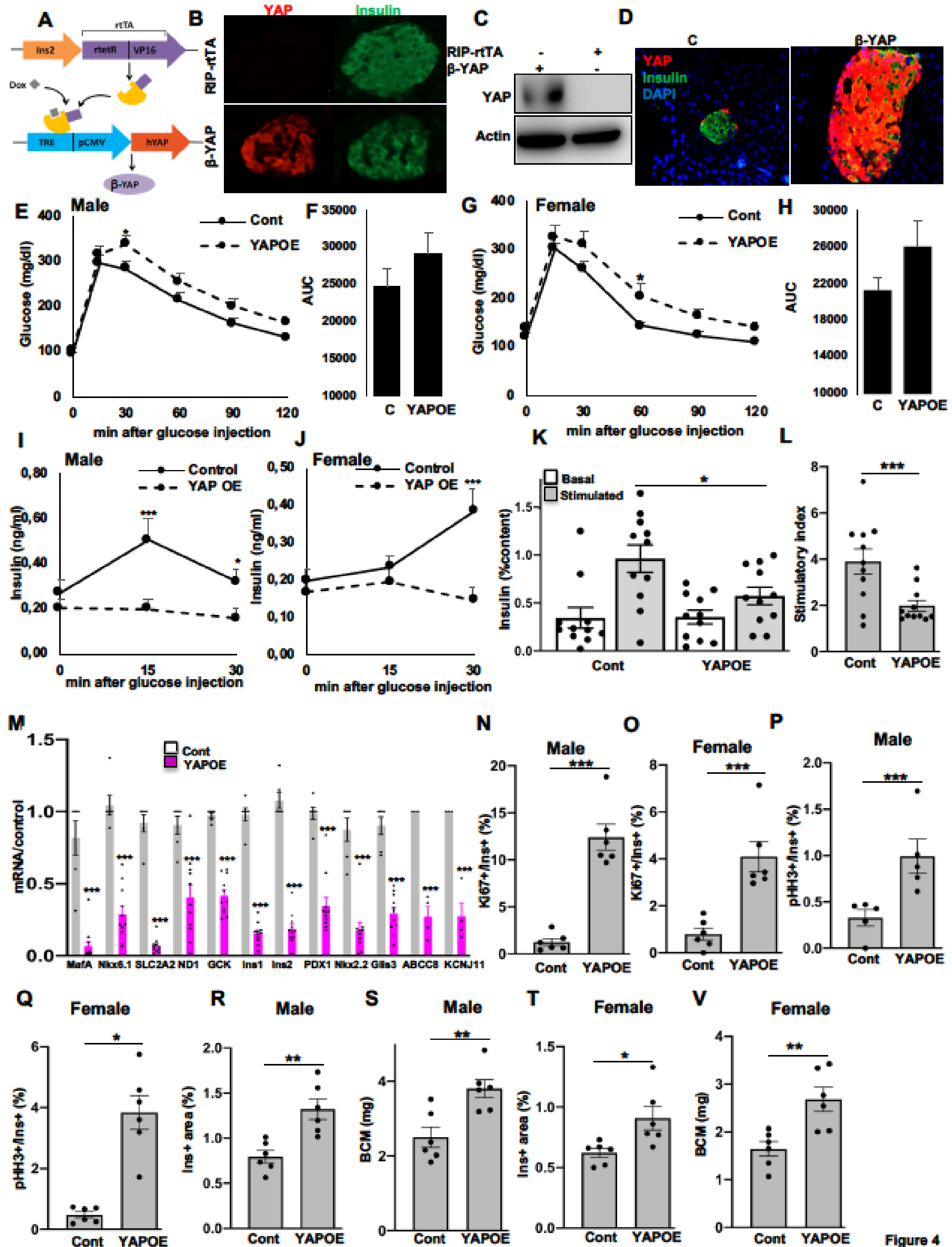

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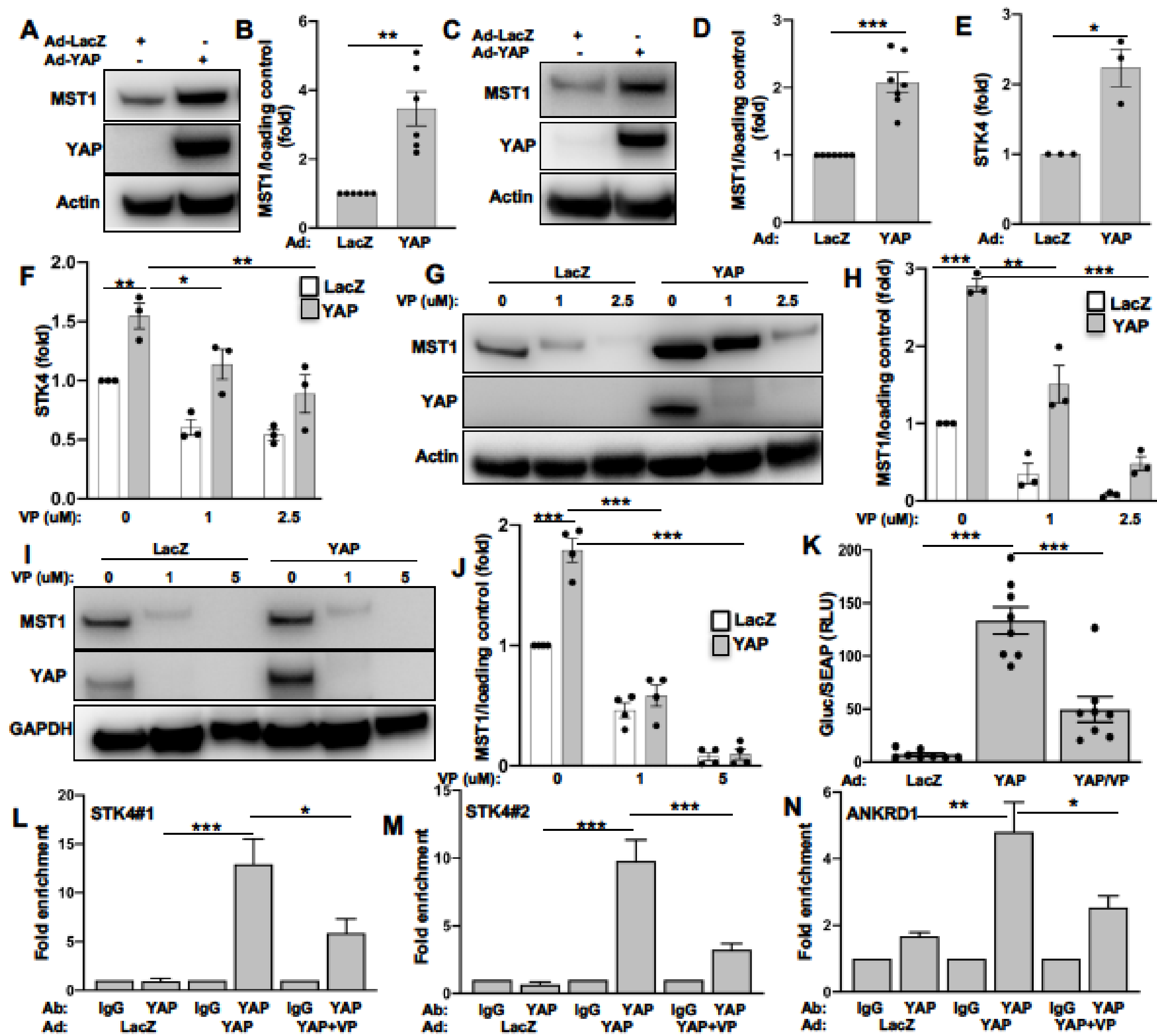

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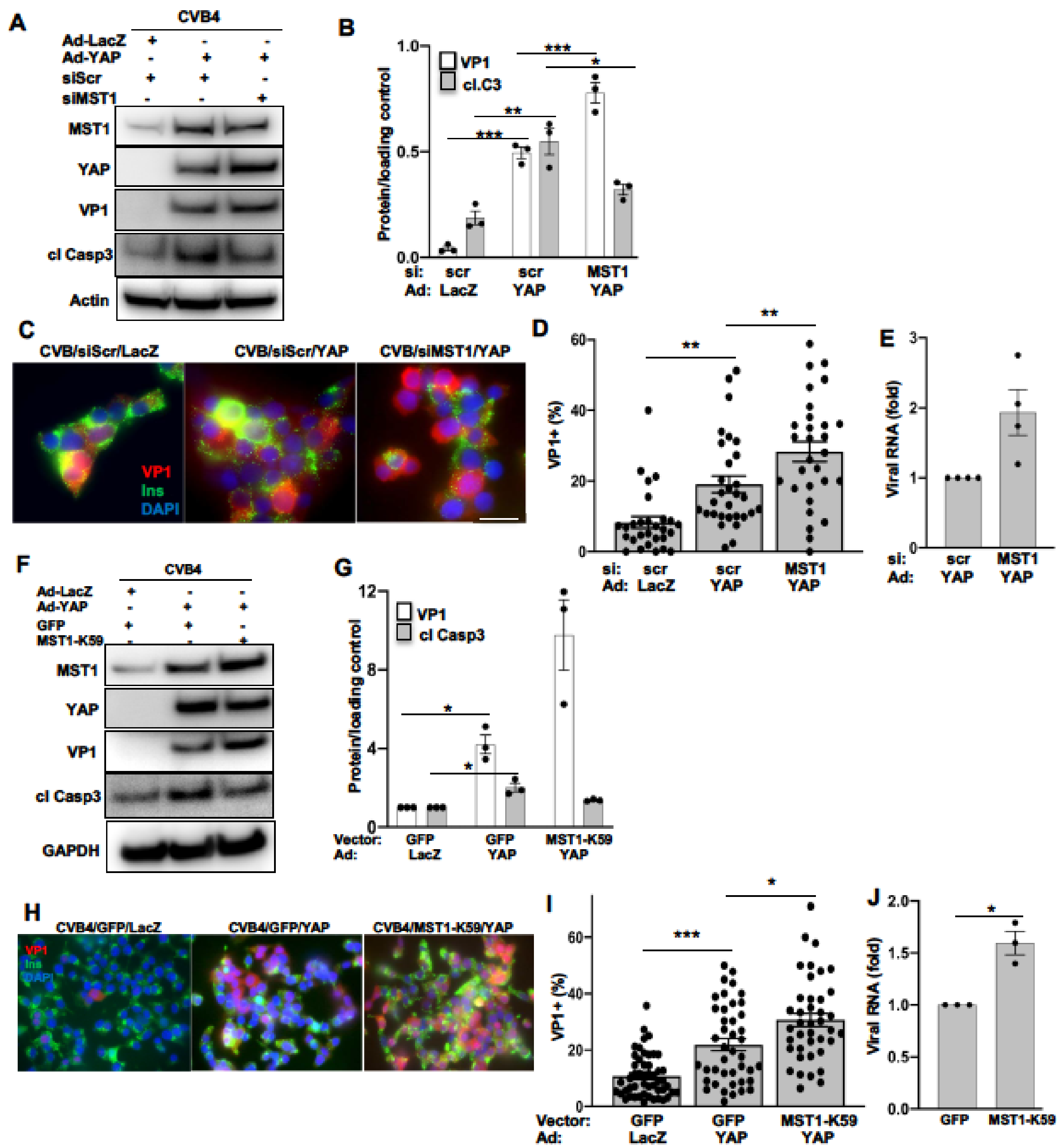

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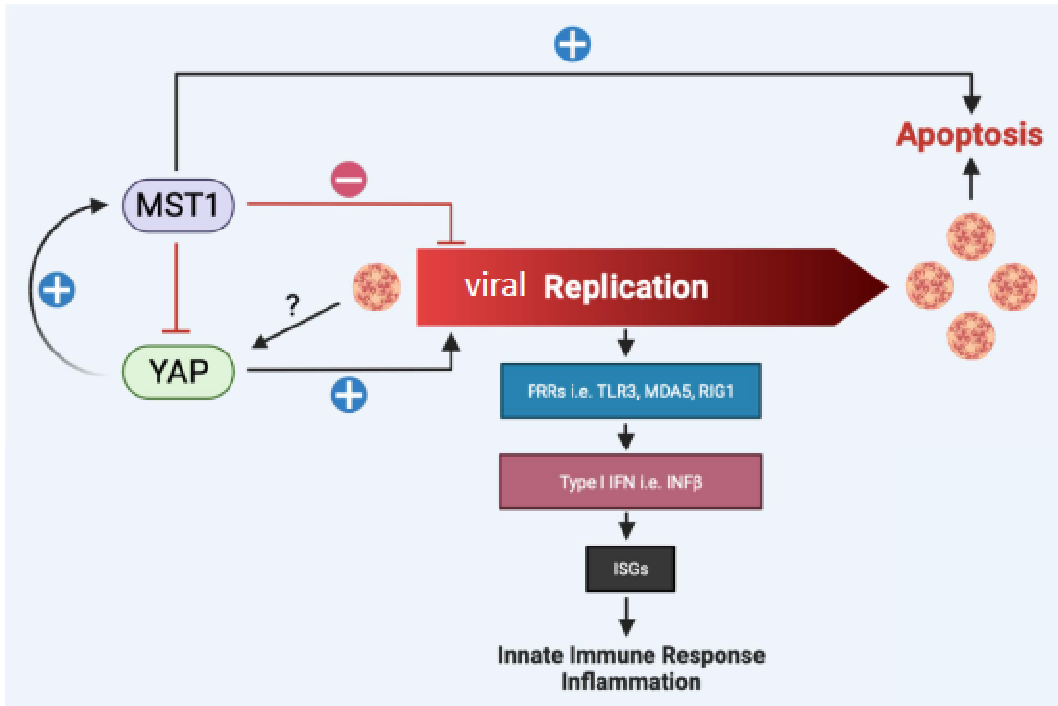

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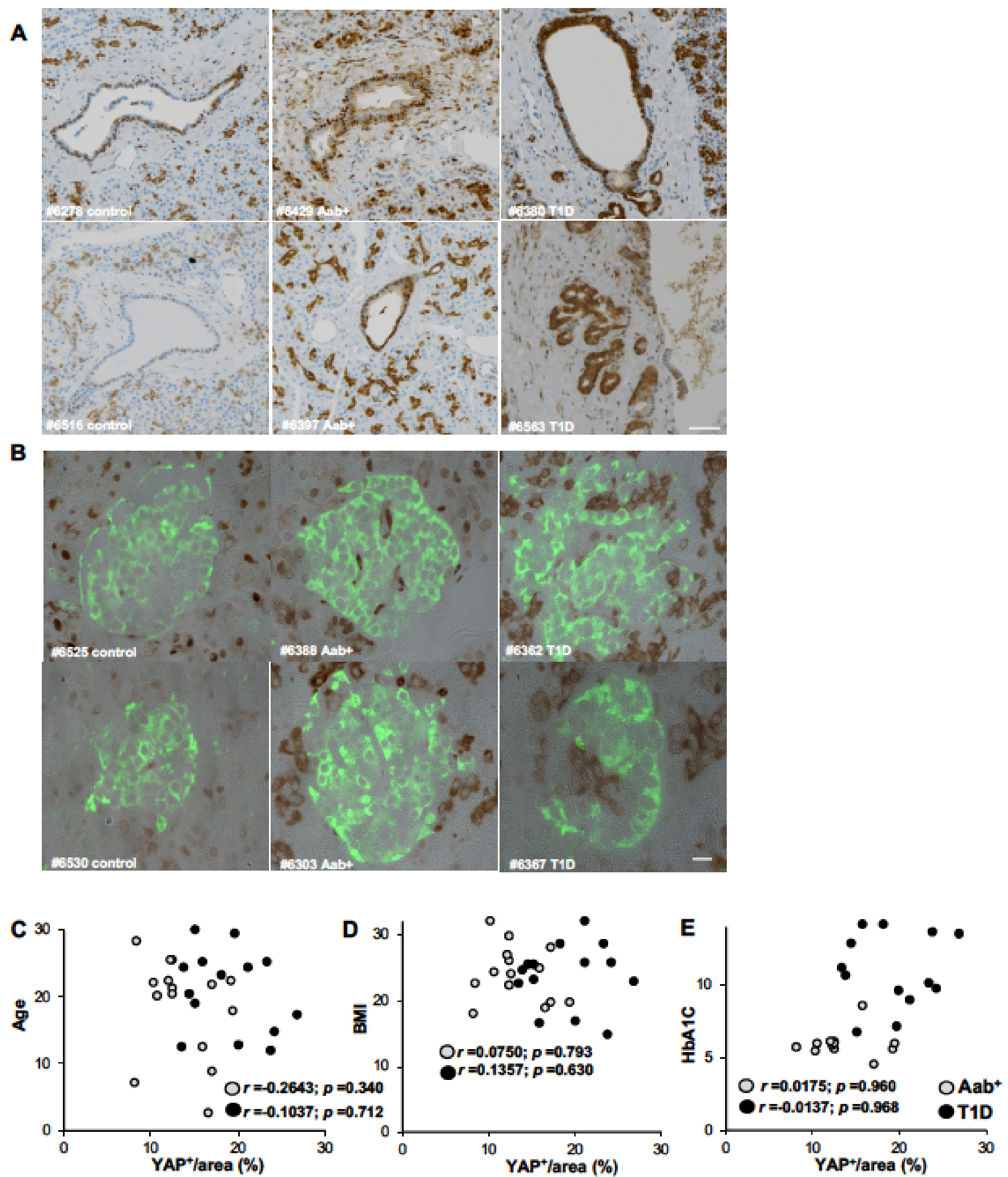

Figure S1

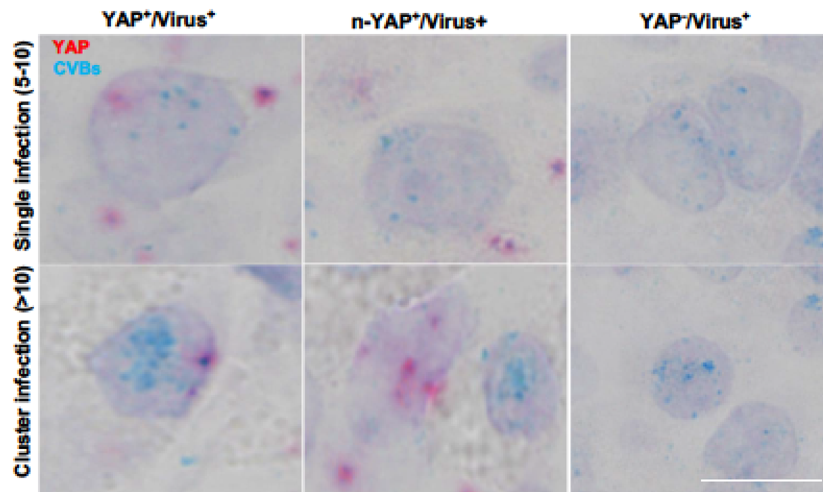

Figure S2

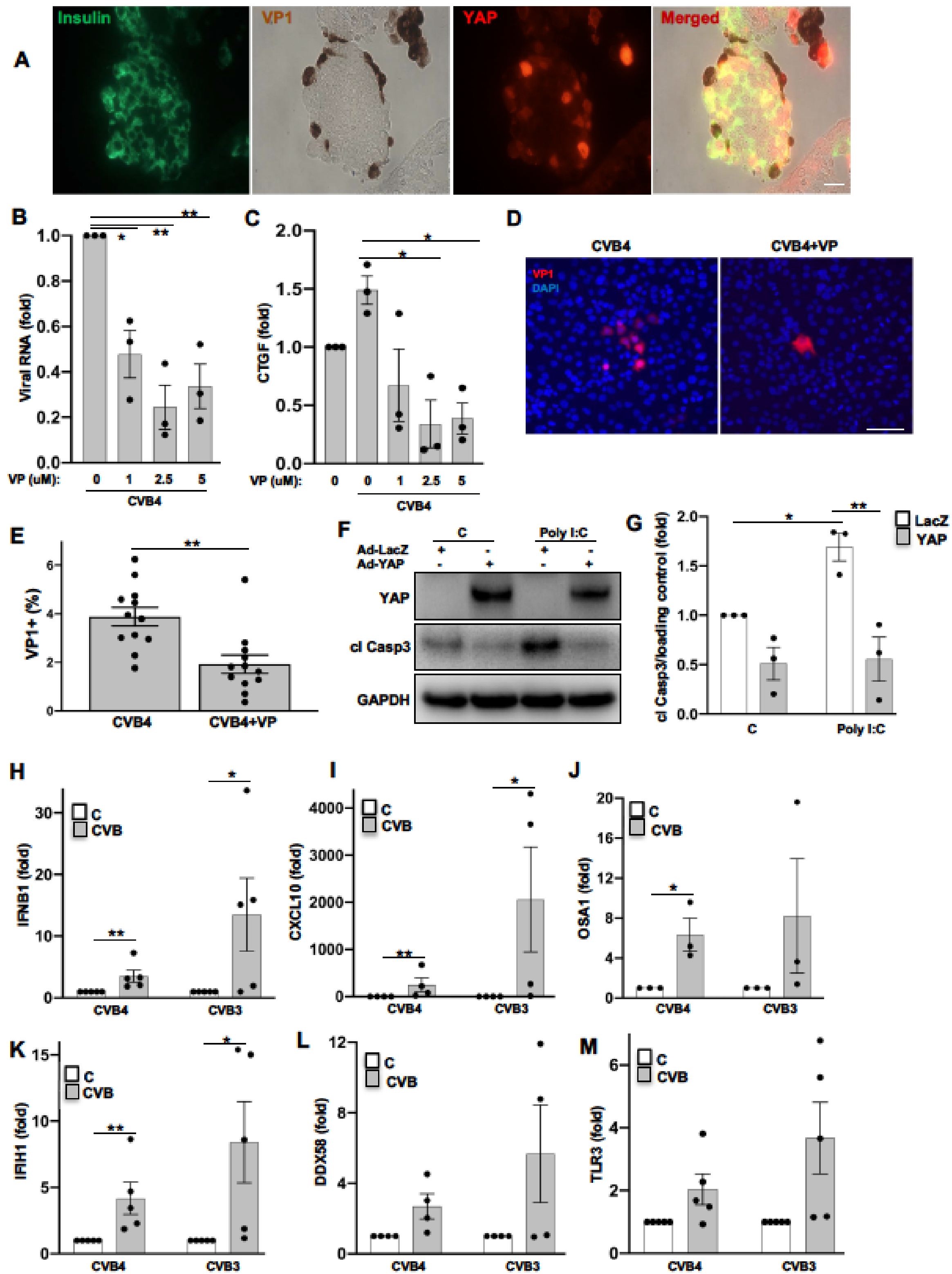

Figure S3

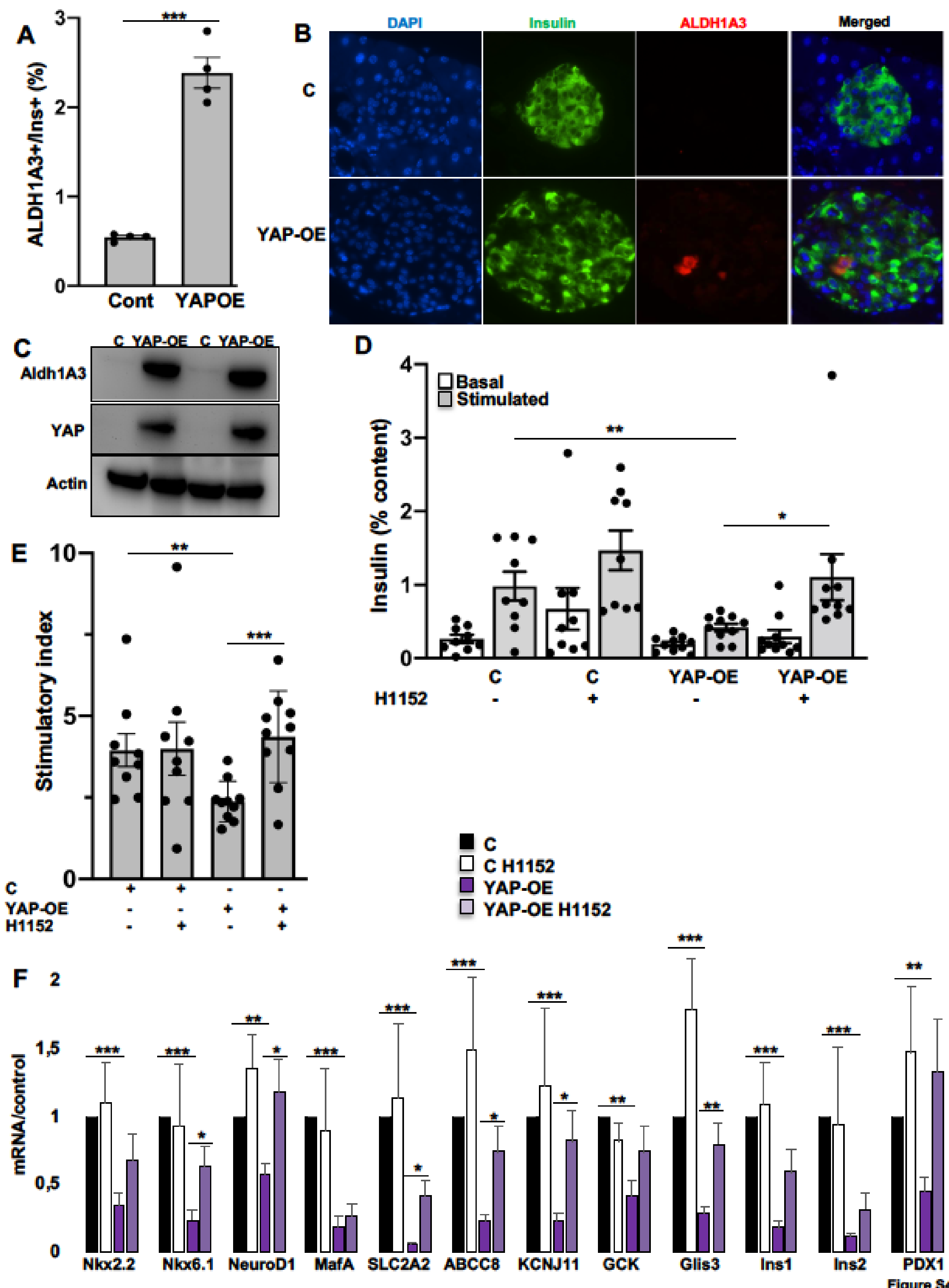

Figure S4

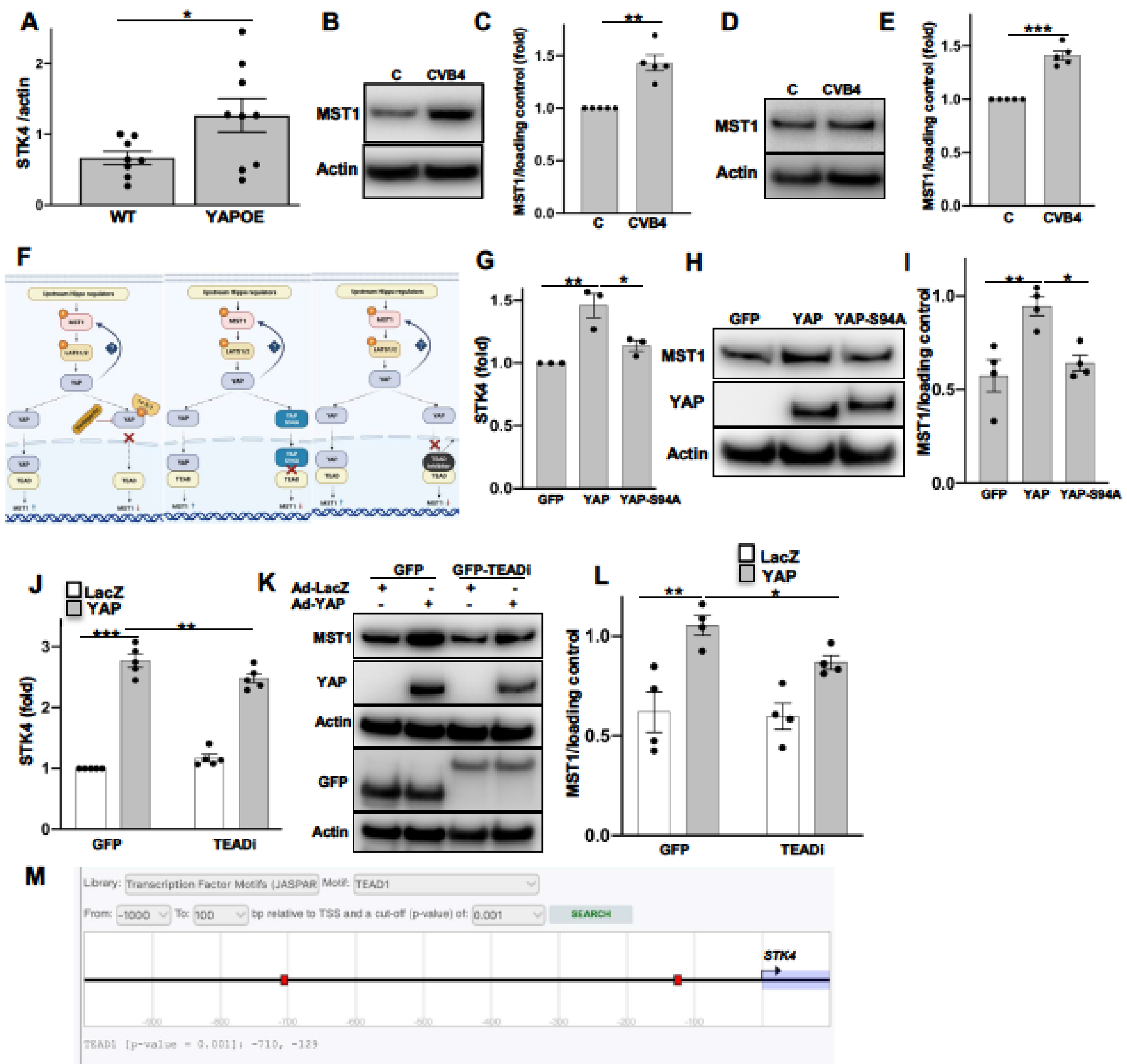

Figure S5

3 Discussion

Diabetes pathophysiology includes β-cell depletion, function, identity, proliferation alterations, and β-cell-specific transcription factor expression anomalies. Diabetes is multifactorial and can vary regardless of the type of diabetes [1], [2], [3]. Factors such as obesity, genetics, and sedentary lifestyle interact to produce the characteristic metabolic abnormalities seen in diabetes. To compensate for the loss of β-cells and to maintain adequate insulin production, the remaining β-cells must regenerate. However, β-cell proliferation is minimal and in diabetes even more impaired, leading to further β-cell loss and worsening of the disease. One of its causes is the dysregulation of the Hippo pathway. Its downstream effector YAP fosters β-cell proliferation and inhibits apoptosis. In this work, I identified YAP as a pancreatic β-cell regeneration regulator in diabetic mice. The TEAD activator TT-10 promoted β-cell proliferation and β-cell regeneration in both mouse and human islets. On the other hand, overexpressing YAP increases Coxsackieviruses B (CVB) replication and promotes CVB-induced islet inflammation and β-cell apoptosis, whereas inhibiting YAP stops virus replication in both primary and immortalized pancreatic cells. My investigation on establishing a pathological connection between diabetes and YAP expression in β-cells in vivo has shown that impaired glucose tolerance, abolished insulin secretion, and β-cell dedifferentiation in mice is associated with the induction of YAP expression in β-cells.

3.1 *In vivo* β-cell-specific YAP overexpression promotes β-cell regeneration in a mouse model of diabetes

One of the critical features of diabetes is the lack of functional β-cell mass. It is paramount to identify signaling molecules capable of stimulating the regeneration of β-cell mass in the context of diabetes [4], [5]. The simultaneous prevention of β-cell apoptosis and enhancement of β-cell proliferation should be a priority for a successful β-cell-targeted therapy for diabetes. To answer this question, our laboratory has carried out extensive research towards the treatment of diabetes by stimulating the components of the Hippo signaling pathway [6], [7], [8], [9], [10], [11], [12]. In addition, it has been shown that YAP can regulate the expression of specific genes involved in cell fate determination and tissue regeneration [13]. Experimental increases in YAP

activity were observed to induce non or badly dividing cells to enter the cell cycle, a phenomenon observed in both pancreatic human islets, liver, and heart [8], [14], [15]. The overexpression of YAP in hepatocytes promotes the infiltration of immune cells into the liver in the aftermath of fibrosis [16], [17], [18].

In this study, we show that re-expression of the Hippo pathway terminal effector YAP is highly capable of promoting regeneration of endogenous β-cells by inducing their proliferation and protecting them against apoptosis. This study aligns with our previous data in isolated human pancreatic islets, where we have shown that overexpression of YAP can promote the proliferation of human β-cells and protect them from apoptosis under inflammatory and glucolipotoxicity conditions [8], [19].

Metabolic studies of β-cell specific YAP overexpression in mice for two weeks show that it does not affect body weight and random glycemia, but shows a mild impairment against GTT in endogenous control mice. In the STZ-induced diabetic mouse model, overexpression of YAP reduced random glycaemia when YAP was 'ON' in both the heterozygous and homozygous allele conditions, indicating that the reduction in blood glucose levels was due solely to the activation of YAP and it was confirmed by two different strategies. Against GTT, YAP showed a protective effect in the heterozygous allele and an even more protective effect was observed in the homozygous condition, which means that YAP acts in a dose-dependent manner. Interestingly, there is no improvement in insulin secretion in either STZ-induced YAP overexpression or endogenous control mice. According to my findings, metabolically YAP overexpression had a significant effect on ameliorating STZ-induced hyperglycemia in mice and protecting against GTT, but did not affect ITT. In endogenous control mice, YAP overexpression had no effect on glycaemia and ITT. A certain degree of dedifferentiation may be the possible reason for the insulin secretion defect, and several investigations also suggest that insulin secretion defect is related to dedifferentiation and lack of maturity of β-cells [20], [21].

I further performed morphological analysis on pancreatic sections of both endogenous control and STZ mouse for β-cell mass, percentage of β-cell area, proliferation and apoptosis analysis. β-cell mass and β-cell area percentage was increased in control mice, this was consistent and significantly increased in STZ mice in a YAP dosage

dependent allele. For proliferation and apoptosis analysis, as I detected increase in the number of Ki67 and pHH3 co-positive β-cells and a significant decline in TUNEL positive β-cells, this was consistent and significant induction in proliferation and reduction in apoptosis was observed in STZ mice. With our data from STZ model corroborating that β-cells specific YAP overexpression is subsiding the diabetes development in STZ induced mouse model. These results confirm that overexpression of YAP promotes β-cell regeneration in diabetic mouse.

To answer the question how YAP controlled glycemia and glucose tolerance, but no improvement in insulin secretion, I further analyzed transporter gene expression, which maintains glucose homeostasis in β-cells. When YAP was overexpressed in pancreatic β-cells in mice, it inhibits the expression of glucose transporter gene Slc2A2 (gene for GLUT2), in both transcriptionally and translationally (GLUT2) and dependent on dosage (whether YAP was overexpressed in a single or both alleles). Assessment of β-cell morphology revealed that GLUT2 expression on the cell membrane was disturbed in both homozygous and heterozygous YAP overexpressing mice, which probably led to STZ unable to enter the β-cells. This also explains that insulin was not secreted during a glucose tolerance test and that β-cell mass was improved in the STZ model. Also, other studies support that GLUT2 controls β-cell morphology, insulin secretion and STZ entering in to β-cells [22], [23], [24].

The combined metabolic and morphological results suggest that overexpression of YAP specifically in pancreatic β-cells promotes the regeneration of endogenous β-cells by restoring the β-cell mass, enhancing proliferation, and reducing β-cell apoptosis in STZ-induced diabetic mice. Although there was a tendency towards a more pronounced improvement in regeneration in β-YAP homozygous vs. β-YAP heterozygous mice, such a dose dependent effect did not achieve statistical significance. The convergence of metabolic and morphological improvements indicates that the targeted re-expression of YAP in pancreatic β-cells promotes β-cell regeneration, ultimately leading to restoration of β-cell mass in the STZ-induced model of β-cell destruction and diabetes.

It is important to note that the expression of GLUT2 and other functional genes in isolated human islets remained unchanged after YAP overexpression [8], in contrast

to my data on YAP overexpression in mouse β-cells *in vivo*. This difference may reflect species differences and the chronic nature of murine YAP expression (several weeks) compared to the relatively short duration of YAP expression in human islets *in vitro* (a few days). Another study had observed GLUT2 reduction by Pax4. As it preserves the survival of the most robust cell population inside the islets, Pax4 might be considered a selfish gene [25]. Because the dormant β-cell mass would remain quiescent and functioning, the functional barrier associated with Pax-4-dependent re-entry into the cell cycle would have little short-term consequences on glucose homeostasis [23], [26].

Our study highlights the potential of controlled and short-term YAP modulation in human β-cells as a promising avenue for influencing both the replication and the survival of β-cells in diabetes. This has great potential for regenerative therapies in diabetes. β-cell proliferation is a significant contributor to the dynamic nature of adult β-cell mass. Nevertheless, the potential adverse effects of YAP on genes involved in β-cells maturation, e.g., on SLC2A2/GLUT2, a critical component of glucose metabolism and insulin secretion in β-cells. In addition, given the inverse relationship between β-cell proliferation and function, any regenerative approach should be carefully optimized regarding timing, dose, and specificity. Ensuring these strategies do not compromise β-cell glucose metabolism and insulin secretion is imperative. The achievement of an optimal balance between mature functional and expansion capacity will be critical to the overall success of regenerative therapies for diabetes.

Overexpression of YAP in human pancreatic β-cells has led to promising results, including improved function, increased proliferation and suppressed apoptosis. Therefore, ongoing research should be continued to determine the optimal therapeutic window for the enhancement of regeneration, while considering β-cell functionality and identity. Additionally, targeting YAP in combination with other regenerative approaches, such as in stem cell therapy, may further improve β-cell regeneration and function. As YAP is by far not the only pathway involved in β-cell regeneration, a better understanding of the crosstalk between other pathways, including PDX1, GLP-1, and BMP [27], [28], [29] signaling and potential interaction with YAP is necessary to develop effective therapeutic strategies.

3.2 The TEAD activator TT-10 promotes β-cell regeneration in human and mouse islets

The tightly in time and concentration-controlled activation of YAP to increase β-cell proliferation and survival is a strategy for the treatment of both T1D and T2D. Targeting the downstream YAP effector could be a potential strategy for β-cell regeneration. In search of compounds that activate TEAD, we come across a fluorine substituent (TT-10) derived from the biologically active compound TAZ (TAZ-12). YAP shares 60% amino acid sequence similarity with TAZ [30]; both are redundant in regulating cell proliferation and migration. However, TAZ increases the proliferation of cells by altering the cell cycle in a manner also independent of YAP [31], [32]. TT-10 strongly promotes proliferation of cardiomyocytes and has antioxidant and anti-apoptotic effects *in vitro*, it enhances YAP-TEAD activities and WNT/β-catenin signaling and protects cardiomyocytes from apoptosis by activating NRF2 transcription factor, which serves as TEAD activator [33], also in infarcted hearts [33], [34]. These findings suggest that TEAD plays a surprising role in controlling mature cell identity and function while maintaining proliferative quiescence by activating a network of key transcription factors and genes that confer functional competence and proliferative potential.

In my research, TT-10 demonstrated robust proliferation in mouse and human primary islets, subsequently confirmed in human T2D islets *ex vivo*. Functional studies on human pancreatic islets treated with TT-10 showed no changes in insulin secretion and content compared to untreated samples. These studies indicate that TT-10 had no effect on pancreatic islet identity and functionality, but at the same time, the proliferation increase did not negatively affect function. These results were in line with several studies that have shown that TEAD activation has no effect on β-cell identity and functionality, but it regulates the proliferation of pancreatic islets [8], [35], [36].

In addition, I detected the transcription of proliferative genes such as *FoxM1* and *Myc* in human islets, consistent with T2D islets treated with TT-10. *FoxM1* is an essential regulator of cell proliferation, controlling transitions between the G1/S, S phase, and G2/M phases [37], [38]. FoxM1 deficit in β-cells can block compensatory β-cell proliferation [39]. Pancreas-specific FoxM1 deletion shows increasing β-cell loss eventually leading to diabetes [39], [40]. On the other hand, human and mouse primary

islets can proliferate β-cells alone by overexpressing FoxM1, indicating the critical function of FoxM1 [41], [42]. In human islets, MafA, *Slc2A2, Abcc8, Kcnj11*, *Glis3, and Pdx1* were significantly up-regulated by TT-10 treatment, restoring functional gene expression. While TT-10 induced β-cell proliferation in a TEAD-dependent manner, expression of TEAD1 and TEAD2 itself, constitutively expressed in β-cells, remained unaffected. Previous research has shown that TEAD1 alone directly controls and promotes critical β-cell transcription factors, such as *Pdx1, Nkx6.1,* and *MafA*. Its β-cell specific deletion in mice leads to diabetes induction with severe hyperglycemia and blunted insulin secretion [36].

The over-expression of YAP and the activation of TEAD by TT-10 had different effects on GLUT2 (Slc2A2) expression in pancreatic islets. Re-expression of YAP reduced GLUT2 expression, so β-cell functionality was not restored. However, when I treated human donor islets with TT-10, all the transporter genes Slc2A2, Abcc8, Kcnj11, and Glis3 expression was significantly up-regulated, and β-cell maturation and functional genes MafA and Pdx1 expression were also significantly increased. These results suggest that treating pancreatic islets with TT-10 improved the functionality and identity of β-cells. Therefore, TT-10 may be a better strategy than YAP for β-cell functionality and identity. Several recent investigations have also stated that these glucose transporter genes also support the robust induction of proliferation and improve cell function [43], [44], [45]. The other co-transcription factor of TEAD, TAZ, has been shown to directly interact with Pdx1, promoting insulin gene expression and, thus, β-cell function [46], which indicates that TAZ can also act independently of TEAD. This might represent a secondary mechanism allowing proliferation without loss of function, potentially increasing Pdx1 activity.

TEAD-dependent activity is increased in TT-10 treated cardiomyocytes without altering YAP or TAZ protein expression, but nuclear YAP levels are increased, and nuclear expression of TAZ is not altered in cardiomyocytes [33], [34], [47]. YAP expression is repressed in the nuclei of mature β-cells and our experiments showed that treatment of pancreatic islets with TT-10 did not alter expression of YAP; i.e. YAP remained absent. Thus, excluding the possibility that YAP mediates the action of TT-10 in pancreatic β-cells. Here, we investigated the action of TT-10 through TEAD pharmacologically using verteporfin, which inhibits the YAP-TEAD interaction [48],

[49], and resulted a significant reduction of proliferation. Similar reductions in proliferation were also observed with genetically fluorescence tagged TEADi plasmid inhibition [50] and the pool of siRNA's mediated silencing of TEAD1 [51]. These results suggest that TT-10 acted via TEAD to enhance proliferation in pancreatic β-cells, and that the WNT pathway may be less critical. However, this has not been investigated in our study. Activating TEAD controls proliferation and improves maturation of β-cells [52], [53].

TT-10 significantly increased β-cell proliferation in vivo within 7 days in proof-of-concept investigations. After mitosis, β-cells enter a short quiescence period of ~7 days before resuming the cell cycle [54]. Therefore, by i.p injection of TT-10 for a period of 7 days, I investigated whether TT-10 could regulate the islet proliferation *in vivo*. Similarly, body weight, glycemia, and GTT were not affected by TT-10 treatment. The compound TT-10 was the key to triple achievement; it targets a pathway that controls growth and proliferation (TEAD), upregulation of functionality markers (promotion of PDX1 activation), and prevention of apoptosis in a model of T2D. Such simultaneous prevention of β-cell apoptosis and enhancement of β-cell proliferation should be a primary focus for a successful β-cell targeted therapy for diabetes.

By directly activating a critical network of genes regulating these processes of β-cell proliferation, maturity, and survival through TEAD, I identified TEAD activation as a mediator of what is currently classified as reciprocal regulation of proliferation and maintenance of mature β-cell function. These results suggest that TT-10 may be a potential compound for the regeneration of β-cells in the pancreas.

3.3 The Hippo terminal effector YAP boosts enterovirus replication in type 1 diabetes

During viral infection, YAP may be modulated by viruses to benefit cell survival and viral replication, e.g. some viruses, like hepatitis B virus (HBV) [55] and hepatitis C virus (HCV) [56], activate YAP to promote viral replication. YAP activation in HBV-infected hepatocytes enhances viral gene expression and replication [57]. Oppositely, YAP can influence the host immune response to viral infection, e.g. it can modulate the production of pro-inflammatory cytokines, such as interleukin-6 (IL6) [58], which

affects viral clearance and immune cell activation. This suggests that YAP may shape the immune response to viral infections. Similarly, other studies have shown that deletion of Lats1/2 and double knockout of MST1 result in severe inflammation in a YAP-dependent manner [59], [60]. Classically, YAP controls cell and organ development. Only in recent studies, it has been shown to be implicated in metabolism and in the modulation of the host response during viral infection [61]. YAP activation in resident hepatic macrophages or Kupffer cells causes inflammation and production of inflammatory cytokines in the liver of high-fat diet mice [62], [63], and promotes the development of non-alcoholic steatohepatitis (NASH) [63]. In a recent investigation, acinar specific Lats1/2 deletion mice developed severe pancreatic fibrosis and inflammation, this unique phenotype is mediated by the YAP1/TAZ [64].

YAP expression is repressed in islets during the postnatal development. Surprisingly, we have observed the expression of YAP in the pancreatic islets of patients with T1D, and the pro-inflammatory effect of YAP during CVB 3/-4 virus infection of human islets *in vitro.* We do not know where these YAP-expressing islets come from or how they develop. Very few YAP-expressing cells in islets stained positive for the endocrine hormone chromogranin. They could be endocrine cells that have become dedifferentiated during the course of the disease. Alternatively, they may be acinar, endothelial or immune cells that have migrated into the islets. In order to establish a pathological connection between diabetes and elevated YAP levels in the pancreas, I have used β-cell-specific doxycycline-inducible transgenic mice that overexpress YAP. I found that homozygous YAP overexpression resulted in glucose intolerance and impaired glucose stimulated insulin secretion (GSIS) *in vivo* and *ex vivo*, defined as the best functional characteristic of mature β-cells [65]. In mutant islets, the level of insulin secretion in response to glucose was significantly reduced, compared to controls.

I further investigated whether the overexpression of YAP leads to a loss of β-cell identity or to dedifferentiation. Although the *in vitro* studies of YAP overexpression showed no change in β-cell maturity or function [8], *in vivo* studies in YAP overexpressing mice revealed reduced functionality. I analyzed gene expression of β-cell functional genes, encompassing endocrine hormone (Ins1, Ins2), crucial β-cell transcription factors (Pdx1, NeuroD1, MafA, Nkx2.2, Nkx6.1, and Glis3), and critical

genes for glucose sensing and metabolism (GCK, Slc2a2, ABCC8, KCNJ11) using islets isolated from β-YAP and control adult mice. The expression level of all genes was strongly downregulated in YAP-overexpressing β-cells, suggesting a loss of β-cell identity and functionality upon YAP re-expression in pancreatic islets. Dysfunctional β-cells are characterized by the reduced expression of β-cell genes and impaired glucose-induced insulin secretion [66], [67]. These results suggest that overexpression of YAP in adult β-cells leads to dysfunction through the repression of identity genes.

YAP activation was strongly pro-proliferative in both males and females compared to control littermates, as determined by quantification of double-positive insulin and Ki67 or pHH3 cells, consistent with our previous findings in human islets [8]. Induction of β-cell replication was associated with a significant increase in insulin positivity and β-cell mass in both males and females. A significant fraction of highly proliferating β-cells shows metabolic immaturity [68], simultaneously downregulating numerous metabolic and functional genes, including those related to glucose metabolism and insulin expression and secretion, in order to allocate energy and cellular resources to increase their mass and replication. This YAP-induced β-cell immaturity could be a possibility for dedifferentiation.

By labeling ALDH1A3, a universal marker of β-cell dedifferentiation [69], I further investigated β-cell dedifferentiation. In isolated islets from β-cell specific YAP-overexpressing mice, I observed a significant induction of ALDH1A3/insulin double-positive cells by immunofluorescence and an upregulation of ALDH1A3 levels were observed in western blot analysis. Several studies indicated that Aldh1A3 is one of the key markers for β-cell dedifferentiation and its expression correlates with diabetes progression [69], [70], [71]. Among several cell biological mechanisms of cell dysfunction, β-cell dedifferentiation may explain the slowly progressive onset and partial reversibility of β-cell failure [72], [73], [74].

As replication and specialization tend to follow opposite paths during the development of the pancreatic islet, the functionality of the β-cell is equally important [75]. In order to rescue the phenotype of loss of maturity and functionality of β-cells, it would be important to understand its mechanism. Unlike apoptosis, loss of β-cell identity is a potentially reversible mechanism, meaning that re-differentiation, β-cell function and

glucose homeostasis could potentially be restored [76], [77]. In the last phase of stem-cell conversion towards β-cells, ROCK II signaling is inhibited [78], that helps cells to mature and function like β-cells [79]. H1152 is an inhibitor of the ROCK II pathway; it was previously used to develop mature and functional β-cells from human iPSCs [79], [80]. This also occurred in the presence of YAP activation, which abolished glucose-induced insulin release, while H1152 treatment significantly restored insulin secretion and expression of a subset of β-cell identity genes such as Nkx6.1, NeuroD1, Slc2a2, ABCC8, KCNJ11, and Glis3. All these results suggest that the impaired insulin secretion and the compromised cell identity induced by YAP are reversible and could be restored by enhancing the maturation of β-cells. These findings have important implications for potential therapeutic strategies targeting YAP in treating diabetes.

The metabolic consequences of long-term selective overexpression of YAP in pancreatic β-cells were demonstrated in transgenic mice with inducible β-cell specific overexpression of YAP. These data directly link the pathological upregulation of YAP in the pancreas and islets of patients with T1D and the failure of β-cells and metabolic deregulation. In conclusion, our findings suggest that YAP overexpression in pancreatic β-cells may induce their dedifferentiation, leading to impaired glucose tolerance and insulin secretion. The exact mechanisms underlying this process are poorly understood but may involve activating specific signaling pathways and transcription factors. Further studies are needed to fully unravel the molecular mechanisms involved in this process and to identify potential therapeutic targets that may prevent or reverse β-cell dedifferentiation in the context of inflammation and diabetes progression.

3.4 Two sides of the same coin – β-cell impairment and regeneration by YAP

I identified a dual role of YAP in pancreatic β-cells: on the one hand, it promotes the regeneration of β-cells in the STZ-induced diabetes mouse model through the induction of proliferation and the inhibition of apoptosis; this ultimately improved glycemia. On the other hand, YAP induces β-cell impairment, dedifferentiation, and loss of the β-cell phenotype, but these effects can be reduced. Regeneration is an important process that contributes in the restoration of tissue integrity after substantial trauma. This ability to repair organ damage and maintain homeostasis is a basic

feature of many multicellular organisms. Recent evidence suggests that YAP expression is crucial for regeneration during injury, repair, or malfunction [81], [82]. Like cardiac-specific deletion of the Hippo pathway effector, YAP impairs neonatal heart regeneration, whereas forced expression of YAP stimulates cardiac regeneration in adult heart [34], [83]. YAP is transiently activated by hepatic injury during liver regeneration [18], [84]. These studies confirm that YAP is a valuable therapeutic tool for studying regeneration.

Regenerative and oncogenic pathways have many of the same signaling components, with the primary differences being in the intensity and duration of activation and the safety mechanisms that tightly control the "ON" and "OFF" states. Since YAP is an oncogene and a pivotal effector of the Hippo pathway, the following factors must be considered: 1) the extent of YAP activation, 2) the specificity of cell targeting, and 3) effects on target cell functionality are significant to consider before it could be considered for therapy. As a result, careful consideration must be given to the dose and timing of YAP induction. YAP expression levels should promote β-cell proliferation without causing neoplastic development. Therefore, clinical research should be continued to find the best therapeutic window for enhancing regeneration while minimizing unwanted adverse effects.

Overexpression of YAP in the pancreatic β-cells of adult mice *in vivo* strongly promotes proliferation and causes β-cell impairment by disrupting the functionality and maturation of the islets through dedifferentiation into different cell types. However, recent research has shown retentiveness in gene compounds affects β-cell-like cells from induced pluripotent stem cells. The compound H1152 inhibits the ROCK signaling pathway and promotes the maturation of β-cells. Based on my findings, the effects of YAP-induced impaired insulin secretion and compromised cellular identity are reversible and could be restored by enhancing β-cell maturation through the YAP-ROCKII signaling pathway.

The development of diabetes is associated with defects in the Hippo pathway and hyperactivation of its downstream effector YAP. Conversely, YAP may also be beneficial in stimulating β-cell proliferation and regeneration after diabetes, and its activation may be therapeutically valuable in these contexts.

In conclusion, YAP has emerged as a promising target for the enhancement of β-cell regeneration and the improvement of β-cell function in diabetes. It provides a potent tool for regeneration; however, it becomes evil in the community with infectious viruses. The findings of my thesis offer new opportunities for developing new regenerative treatments for diabetes and especially a novel understanding of autoimmunity and T1D progression.

Future prospects

Proliferation of human β-cells is very limited, and this contributes to the loss of β-cells and no natural rescue of β-cell death in diabetes. Dysregulation of the Hippo pathway is one of the causes for β-cell apoptosis, and re-expression of its downstream effector YAP promotes proliferation of β-cell and inhibits apoptosis. I have used β-cell-specific inducible YAP overexpression mice to study the effect of YAP on a postnatal stage and on models of diabetes. My studies revealed that YAP has a potential role in β-cell regeneration *in vivo* by inducing proliferation and inhibiting apoptosis. I also investigated TEAD activator TT-10, which induce β-cell proliferation in both mouse and human islets. Conversely, YAP can be manipulated by viruses to facilitate their replication and influence the host immune response during viral infection and it has been shown to interact with other signaling pathways involved in immune response and inflammation, such as Hippo pathway and the T-cell receptor signaling pathway. The immune dysregulation seen in T1D may be affected by YAP activity. The results of my study suggest that overexpression of YAP in adult mice induces mild diabetes and loss of functionality and maturation by disrupting the phenotype of pancreatic islets. Furthermore, it promotes the dedifferentiation of β-cells into different cell types. Using chemical compounds that interfere in the signaling pathway, the phenotype of pancreatic β-cells was rescued.

Several aspects are open for future studies:

- In the case of the YAP-STZ model, we can study the rescue of GLUT2 expression, which will help us better understand the effects of YAP.
- TAZ and TAED tissue distribution needs to be studied to further investigate the role of TT-10 *in vivo* in pancreatic β-cell regeneration.

- Gluconeogenesis in hepatic and adipose tissues after treatment with TT-10 *in vivo* needs to be investigated to better understand TT-10's effect on glycemia, proliferation, and glucose-stimulated insulin secretion.
- The lab already has very promising results on TT-10's in vivo effect on β-cell regeneration in STZ-induced and genetically induced db/db diabetic mouse models.
- Overexpressing YAP for the long term will show whether this transforms the β-cell into fully dedifferentiated cells or whether function may be maintained.
- One of the most important studies would be to evaluate the *in vivo* effect of H1152 on YAP-overexpressing mice. Can the ROCK pathway rescue the β-cell phenotype, β-cell maturity, and function *in vivo*?

Conclusion

My primary goal was to understand the cellular and molecular mechanisms underlying the loss of β-cells in diabetes. As a result, successful methods for β-cell regeneration have been identified. My study has contributed to our understanding of the pathophysiology of pancreatic β-cells and the signaling mechanisms involved in diabetes and β-cell regeneration.

<u>YAP promotes β-cell regeneration:</u> My studies have shown that YAP is critical in regulating β-cell proliferation and apoptosis, ultimately resulting in β-cell regeneration. YAP activation increased β-cell mass and β-cell area, leading to normoglycemia in diabetic mice. Thus, targeting the Hippo pathway, specifically, YAP may be a potential therapeutic strategy for promoting β-cell regeneration in diabetes.

<u>TEAD activator TT-10 promotes β-cell regeneration:</u> TEAD is a transcriptional factor that works together with YAP to regulate proliferation and survival. TT-10 is a small molecule activator of TEAD that has been shown to increase β-cell proliferation. In my study, I investigated the effect of TT-10 on β-cell proliferation in both mouse and human islets *ex vivo*. I found that TT-10 significantly increased β-cell proliferation in both models, highlighting the potential of TEAD activation as a therapeutic approach for promoting β-cell regeneration in diabetes.

Homozygous YAP overexpression induces β-cell impairment: Overexpression of YAP in mice, specifically in β-cells, leads to robust proliferation and increase in β-cell mass, but it also causes dedifferentiation, lack of functionality and maturation, ultimately leading to loss of β-cell phenotype and impairment. These effects can be rescued. In this case, postnatal β-cells has been clever enough to inhibit the expression of YAP epigenetically or by some other pathway, so that they don't become dysfunctional, but they need to pay this with inability to proliferate. In situations when compensation is needed, β-cells and diabetes progress.

The data presented in my thesis have a strong impact on the Hippo pathway in pancreatic β-cells. YAP is an important driver of β-cell proliferation and apoptosis. My study points out that regeneration of β-cells needs the Hippo pathway's attention during pro-diabetic conditions.

References:

[1] D. M. Nathan, "Diabetes: Advances in diagnosis and treatment," *JAMA - Journal of the American Medical Association*, vol. 314, no. 10. 2015. doi: 10.1001/jama.2015.9536.

[2] B. Johannesson, L. Sui, D. O. Freytes, R. J. Creusot, and D. Egli, "Toward beta cell replacement for diabetes," *EMBO J*, vol. 34, no. 7, 2015, doi: 10.15252/embj.201490685.

[3] N. Y. Kang, A. A. P. Soetedjo, N. S. Amirruddin, Y. T. Chang, O. Eriksson, and A. K. K. Teo, "Tools for Bioimaging Pancreatic β Cells in Diabetes," *Trends in Molecular Medicine*, vol. 25, no. 8. 2019. doi: 10.1016/j.molmed.2019.05.004.

[4] A. Welters and E. Lammert, "Novel Approaches to Restore Pancreatic Beta-Cell Mass and Function," in *Handbook of Experimental Pharmacology*, vol. 274, 2022. doi: 10.1007/164_2021_474.

[5] B. Okere, L. Lucaccioni, M. Dominici, and L. Iughetti, "Cell therapies for pancreatic beta-cell replenishment," *Italian Journal of Pediatrics*, vol. 42, no. 1. 2016. doi: 10.1186/s13052-016-0273-4.

[6] A. Ardestani and K. Maedler, "MST1: a promising therapeutic target to restore functional beta cell mass in diabetes," *Diabetologia*, vol. 59, no. 9. 2016. doi: 10.1007/s00125-016-3892-9.

[7] A. Ardestani and K. Maedler, "The Hippo signaling pathway in pancreatic β-cells: Functions and regulations," *Endocrine Reviews*, vol. 39, no. 1. 2018. doi: 10.1210/er.2017-00167.

[8] T. Yuan *et al.*, "Proproliferative and antiapoptotic action of exogenously introduced YAP in pancreatic β cells," *JCI Insight*, vol. 1, no. 18, 2016, doi: 10.1172/jci.insight.86326.

[9] A. Ardestani *et al.*, "MST1 is a key regulator of beta cell apoptosis and dysfunction in diabetes," *Nat Med*, vol. 20, no. 4, 2014, doi: 10.1038/nm.3482.

[10] T. Yuan *et al.*, "The Hippo kinase LATS2 impairs pancreatic β-cell survival in diabetes through the mTORC1-autophagy axis," *Nat Commun*, vol. 12, no. 1, 2021, doi: 10.1038/s41467-021-25145-x.

[11] A. Ardestani *et al.*, "Neratinib protects pancreatic beta cells in diabetes," *Nat Commun*, vol. 10, no. 1, 2019, doi: 10.1038/s41467-019-12880-5.

[12] B. Lupse *et al.*, "Inhibition of PHLPP1/2 phosphatases rescues pancreatic β-cells in diabetes," *Cell Rep*, vol. 36, no. 5, 2021, doi: 10.1016/j.celrep.2021.109490.

[13] N. M. George, B. P. Boerner, S. U. R. Mir, Z. Guinn, and N. E. Sarvetnick, "Exploiting expression of hippo effector, yap, for expansion of functional islet mass," *Molecular Endocrinology*, vol. 29, no. 11, 2015, doi: 10.1210/me.2014-1375.

[14] K. P. Lee *et al.*, "The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis," *Proc Natl Acad Sci U S A*, vol. 107, no. 18, 2010, doi: 10.1073/pnas.0912203107.

[15] A. Von Gise *et al.*, "YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy," *Proc Natl Acad Sci U S A*, vol. 109, no. 7, 2012, doi: 10.1073/pnas.1116136109.

[16] X. Wang *et al.*, "Hepatocyte TAZ/WWTR1 Promotes Inflammation and Fibrosis in Nonalcoholic Steatohepatitis," *Cell Metab*, vol. 24, no. 6, 2016, doi: 10.1016/j.cmet.2016.09.016.

[17] M. Mooring *et al.*, "Hepatocyte Stress Increases Expression of Yes-Associated Protein and Transcriptional Coactivator With PDZ-Binding Motif in Hepatocytes to Promote Parenchymal Inflammation and Fibrosis," *Hepatology*, vol. 71, no. 5, 2020, doi: 10.1002/hep.30928.

[18] M. V. Machado *et al.*, "Accumulation of duct cells with activated YAP parallels fibrosis progression in non-alcoholic fatty liver disease," *J Hepatol*, vol. 63, no. 4, 2015, doi: 10.1016/j.jhep.2015.05.031.

[19] C. G. Hansen, T. Moroishi, and K. L. Guan, "YAP and TAZ: A nexus for Hippo signaling and beyond," *Trends in Cell Biology*, vol. 25, no. 9. 2015. doi: 10.1016/j.tcb.2015.05.002.

[20] M. Bensellam, J. C. Jonas, and D. R. Laybutt, "Mechanisms of β;-cell dedifferentiation in diabetes: Recent findings and future research directions," *Journal of Endocrinology*, vol. 236, no. 2. 2018. doi: 10.1530/JOE-17-0516.
[21] C. Dumayne *et al.*, "Klf6 protects β-cells against insulin resistance-induced dedifferentiation," *Mol Metab*, vol. 35, 2020, doi: 10.1016/j.molmet.2020.02.001.
[22] J. M. Mellado-Gil *et al.*, "PAX4 preserves endoplasmic reticulum integrity preventing beta cell degeneration in a mouse model of type 1 diabetes mellitus," *Diabetologia*, vol. 59, no. 4, 2016, doi: 10.1007/s00125-016-3864-0.
[23] P. I. Lorenzo *et al.*, "PAX4 defines an expandable β-cell subpopulation in the adult pancreatic islet," *Sci Rep*, vol. 5, 2015, doi: 10.1038/srep15672.
[24] M. T. Guillam, P. Dupraz, and B. Thorens, "Glucose uptake, utilization, and in GLUT2-null islets," *Diabetes*, vol. 49, no. 9, 2000, doi: 10.2337/diabetes.49.9.1485.
[25] T. Napolitano *et al.*, "Pax4 acts as a key player in pancreas development and plasticity," *Seminars in Cell and Developmental Biology*, vol. 44. 2015. doi: 10.1016/j.semcdb.2015.08.013.
[26] K. H. H. He *et al.*, "In vivo conditional pax4 overexpression in mature islet β-cells prevents stress-induced hyperglycemia in mice," *Diabetes*, vol. 60, no. 6, 2011, doi: 10.2337/db10-1102.
[27] J. Chmielowiec *et al.*, "Human pancreatic microenvironment promotes β-cell differentiation via non-canonical WNT5A/JNK and BMP signaling," *Nat Commun*, vol. 13, no. 1, 2022, doi: 10.1038/s41467-022-29646-1.
[28] Y. Zhu, Q. Liu, Z. Zhou, and Y. Ikeda, "PDX1, Neurogenin-3, and MAFA: Critical transcription regulators for beta cell development and regeneration," *Stem Cell Research and Therapy*, vol. 8, no. 1. 2017. doi: 10.1186/s13287-017-0694-z.
[29] Y. Xi *et al.*, "Glucagon-receptor-antagonism-mediated β-cell regeneration as an effective anti-diabetic therapy," *Cell Rep*, vol. 39, no. 9, 2022, doi: 10.1016/j.celrep.2022.110872.
[30] M. G. Jeong, H. K. Kim, and E. S. Hwang, "The essential role of TAZ in normal tissue homeostasis," *Archives of Pharmacal Research*, vol. 44, no. 3. 2021. doi: 10.1007/s12272-021-01322-w.
[31] Q.-Y. Lei *et al.*, "TAZ Promotes Cell Proliferation and Epithelial-Mesenchymal Transition and Is Inhibited by the Hippo Pathway," *Mol Cell Biol*, vol. 28, no. 7, 2008, doi: 10.1128/mcb.01874-07.
[32] N. Yang *et al.*, "TAZ induces growth factor-independent proliferation through activation of EGFR ligand amphiregulin," *Cell Cycle*, vol. 11, no. 15, 2012, doi: 10.4161/cc.21386.
[33] H. Hara *et al.*, "Discovery of a Small Molecule to Increase Cardiomyocytes and Protect the Heart After Ischemic Injury," *JACC Basic Transl Sci*, vol. 3, no. 5, pp. 639–653, Oct. 2018, doi: 10.1016/j.jacbts.2018.07.005.
[34] W. Chen, D. Pretorius, Y. Zhou, Y. Nakada, J. Yang, and J. Zhang, "TT-10–loaded nanoparticles promote cardiomyocyte proliferation and cardiac repair in a mouse model of myocardial infarction," *JCI Insight*, vol. 6, no. 20, 2021, doi: 10.1172/jci.insight.151987.
[35] F. Li *et al.*, "TEAD1 regulates cell proliferation through a pocket-independent transcription repression mechanism," *Nucleic Acids Res*, vol. 50, no. 22, 2022, doi: 10.1093/nar/gkac1063.
[36] J. Lee *et al.*, "Tead1 Reciprocally Regulates Adult β-Cell Proliferation and Function to Maintain Glucose Homeostasis," *SSRN Electronic Journal*, 2020, doi: 10.2139/ssrn.3704009.
[37] A. A. Misfeldt, R. H. Costa, and M. Gannon, "β-cell proliferation, but not neogenesis, following 60% partial pancreatectomy is impaired in the absence of FoxM1," *Diabetes*, vol. 57, no. 11, 2008, doi: 10.2337/db08-0878.
[38] D. B. Davis *et al.*, "FoxM1 is up-regulated by obesity and stimulates β-cell proliferation," *Molecular Endocrinology*, vol. 24, no. 9, 2010, doi: 10.1210/me.2010-0082.
[39] J. Yamamoto *et al.*, "Neuronal signals regulate obesity induced β-cell proliferation by FoxM1 dependent mechanism," *Nat Commun*, vol. 8, no. 1, 2017, doi: 10.1038/s41467-017-01869-7.

[40] H. Zhang *et al.*, "Gestational diabetes mellitus resulting from impaired β-cell compensation in the absence of FoxM1, a novel downstream effector of placental lactogen," *Diabetes*, vol. 59, no. 1, 2010, doi: 10.2337/db09-0050.

[41] H. Zhang *et al.*, "The FoxM1 transcription factor is required to maintain pancreatic β-cell mass," *Molecular Endocrinology*, vol. 20, no. 8, 2006, doi: 10.1210/me.2006-0056.

[42] J. Shirakawa *et al.*, "Insulin Signaling Regulates the FoxM1/PLK1/CENP-A Pathway to Promote Adaptive Pancreatic β Cell Proliferation," *Cell Metab*, vol. 25, no. 4, 2017, doi: 10.1016/j.cmet.2017.02.004.

[43] W. J. Jiang, Y. C. Peng, and K. M. Yang, "Cellular signaling pathways regulating β-cell proliferation as a promising therapeutic target in the treatment of diabetes (Review)," *Experimental and Therapeutic Medicine*, vol. 16, no. 4. 2018. doi: 10.3892/etm.2018.6603.

[44] L. J. McCulloch, M. van de Bunt, M. Braun, K. N. Frayn, A. Clark, and A. L. Gloyn, "GLUT2 (SLC2A2) is not the principal glucose transporter in human pancreatic beta cells: Implications for understanding genetic association signals at this locus," *Mol Genet Metab*, vol. 104, no. 4, 2011, doi: 10.1016/j.ymgme.2011.08.026.

[45] B. Sun, H. Chen, J. Xue, P. Li, and X. Fu, "The role of GLUT2 in glucose metabolism in multiple organs and tissues," *Molecular Biology Reports*, vol. 50, no. 8. 2023. doi: 10.1007/s11033-023-08535-w.

[46] M. G. Jeong, H. K. Kim, G. Lee, H. Y. Won, D. H. Yoon, and E. S. Hwang, "TAZ promotes PDX1-mediated insulinogenesis," *Cellular and Molecular Life Sciences*, vol. 79, no. 3, 2022, doi: 10.1007/s00018-022-04216-2.

[47] T. Heallen *et al.*, "Hippo pathway inhibits wnt signaling to restrain cardiomyocyte proliferation and heart size," *Science (1979)*, vol. 332, no. 6028, 2011, doi: 10.1126/science.1199010.

[48] Y. Liu-Chittenden *et al.*, "Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP," *Genes Dev*, vol. 26, no. 12, 2012, doi: 10.1101/gad.192856.112.

[49] Y. Sun *et al.*, "Pharmacological blockade of TEAD–YAP reveals its therapeutic limitation in cancer cells," *Nat Commun*, vol. 13, no. 1, 2022, doi: 10.1038/s41467-022-34559-0.

[50] Y. Yuan *et al.*, "YAP1/TAZ-TEAD transcriptional networks maintain skin homeostasis by regulating cell proliferation and limiting KLF4 activity," *Nat Commun*, vol. 11, no. 1, 2020, doi: 10.1038/s41467-020-15301-0.

[51] Z. Wang, M. Liu, H. Lei, S. Xiao, and Y. Zheng, "TEAD1 Silencing Regulates Cell Proliferation and Resistance to 5-Fluorouracil in Cutaneous Squamous Cell Carcinoma," *Clin Cosmet Investig Dermatol*, vol. 15, 2022, doi: 10.2147/CCID.S386547.

[52] F. Li *et al.*, "VGLL4 and MENIN function as TEAD1 corepressors to block pancreatic β cell proliferation," *Cell Rep*, vol. 42, no. 1, 2023, doi: 10.1016/j.celrep.2022.111904.

[53] S. Porat *et al.*, "Control of pancreatic β cell regeneration by glucose metabolism," *Cell Metab*, vol. 13, no. 4, 2011, doi: 10.1016/j.cmet.2011.02.012.

[54] S. J. Salpeter, A. M. Klein, D. Huangfu, J. Grimsby, and Y. Dor, "Glucose and aging control the quiescence period that follows pancreatic beta cell replication," *Development*, vol. 137, no. 19, 2010, doi: 10.1242/dev.054304.

[55] Y. Wu, J. Zhang, H. Zhang, and Y. Zhai, "Hepatitis B virus X protein mediates yes-associated protein 1 upregulation in hepatocellular carcinoma," *Oncol Lett*, vol. 12, no. 3, 2016, doi: 10.3892/ol.2016.4885.

[56] C. Zou *et al.*, "Hepatitis C virus nonstructural protein 4B induces lipogenesis via the Hippo pathway," *Arch Virol*, vol. 168, no. 4, 2023, doi: 10.1007/s00705-023-05743-4.

[57] X. Luo *et al.*, "Hippo Pathway Counter-Regulates Innate Immunity in Hepatitis B Virus Infection," *Front Immunol*, vol. 12, 2021, doi: 10.3389/fimmu.2021.684424.

[58] X. Zhou *et al.*, "YAP Aggravates Inflammatory Bowel Disease by Regulating M1/M2 Macrophage Polarization and Gut Microbial Homeostasis," *Cell Rep*, vol. 27, no. 4, 2019, doi: 10.1016/j.celrep.2019.03.028.

[59] N. M. George, C. E. Day, B. P. Boerner, R. L. Johnson, and N. E. Sarvetnick, "Hippo Signaling Regulates Pancreas Development through Inactivation of Yap," *Mol Cell Biol*, vol. 32, no. 24, 2012, doi: 10.1128/mcb.01034-12.
[60] J. Liu *et al.*, "Activation of the intrinsic fibroinflammatory program in adult pancreatic acinar cells triggered by Hippo signaling disruption," *PLoS Biol*, vol. 17, no. 9, 2019, doi: 10.1371/journal.pbio.3000418.
[61] Q. Zhang *et al.*, "Influenza A virus NS1 protein hijacks YAP/TAZ to suppress TLR3-mediated innate immune response," *PLoS Pathog*, vol. 18, no. 5, 2022, doi: 10.1371/journal.ppat.1010505.
[62] M. M. Mia *et al.*, "YAP/TAZ deficiency reprograms macrophage phenotype and improves infarct healing and cardiac function after myocardial infarction," *PLoS Biol*, vol. 18, no. 12, 2020, doi: 10.1371/journal.pbio.3000941.
[63] K. Song *et al.*, "Yes-Associated Protein in Kupffer Cells Enhances the Production of Proinflammatory Cytokines and Promotes the Development of Nonalcoholic Steatohepatitis," *Hepatology*, vol. 72, no. 1, 2020, doi: 10.1002/hep.30990.
[64] M. Gao *et al.*, "Deletion of Lats 1 and 2 in mouse pancreatic acinar cells induces pancreatic fibrosis and inflammation," *bioRxiv*, 2019.
[65] C. Salinno, P. Cota, A. Bastidas-Ponce, M. Tarquis-Medina, H. Lickert, and M. Bakhti, "β-Cell Maturation and Identity in Health and Disease," *Int J Mol Sci*, vol. 20, no. 21, 2019, doi: 10.3390/ijms20215417.
[66] A. Bartolome, C. Zhu, L. Sussel, and U. B. Pajvani, "Notch signaling dynamically regulates adult β cell proliferation and maturity," *Journal of Clinical Investigation*, vol. 129, no. 1, 2019, doi: 10.1172/JCI98098.
[67] F. Ortis *et al.*, "Cytokines interleukin-1β and tumor necrosis factor-α regulate different transcriptional and alternative splicing networks in primary β-cells," *Diabetes*, vol. 59, no. 2, 2010, doi: 10.2337/db09-1159.
[68] S. Puri *et al.*, "Replication confers β cell immaturity," *Nat Commun*, vol. 9, no. 1, 2018, doi: 10.1038/s41467-018-02939-0.
[69] J. Son *et al.*, "Genetic and pharmacologic inhibition of ALDH1A3 as a treatment of β-cell failure," *Nat Commun*, vol. 14, no. 1, 2023, doi: 10.1038/s41467-023-36315-4.
[70] R. R. Huang and Y. Ou, "Research Advances in Islet β Cells Dedifferentiation in Type 2 Diabetes," *Chinese Journal of Pharmaceutical Biotechnology*, vol. 28, no. 3. 2021. doi: 10.19526/j.cnki.1005-8915.20210316.
[71] C. Talchai, S. Xuan, H. V. Lin, L. Sussel, and D. Accili, "Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure," *Cell*, vol. 150, no. 6, 2012, doi: 10.1016/j.cell.2012.07.029.
[72] T. M. Nordmann *et al.*, "The Role of Inflammation in β-cell Dedifferentiation," *Sci Rep*, vol. 7, no. 1, 2017, doi: 10.1038/s41598-017-06731-w.
[73] M. Diedisheim *et al.*, "Modeling human pancreatic beta cell dedifferentiation," *Mol Metab*, vol. 10, 2018, doi: 10.1016/j.molmet.2018.02.002.
[74] T. Sun and X. Han, "Death versus dedifferentiation: The molecular bases of beta cell mass reduction in type 2 diabetes," *Seminars in Cell and Developmental Biology*, vol. 103. 2020. doi: 10.1016/j.semcdb.2019.12.002.
[75] K. Lemaire, L. Thorrez, and F. Schuit, "Disallowed and Allowed Gene Expression: Two Faces of Mature Islet Beta Cells," *Annual Review of Nutrition*, vol. 36. 2016. doi: 10.1146/annurev-nutr-071715-050808.
[76] N. Kerper, S. Ashe, and M. Hebrok, "Pancreatic β-Cell Development and Regeneration," *Cold Spring Harb Perspect Biol*, vol. 14, no. 5, 2022, doi: 10.1101/cshperspect.a040741.
[77] H. S. Kim and M. K. Lee, "β-Cell regeneration through the transdifferentiation of pancreatic cells: Pancreatic progenitor cells in the pancreas," *J Diabetes Investig*, vol. 7, no. 3, 2016, doi: 10.1111/jdi.12475.

[78] D. Esposito *et al.*, "ROCK1 mechano-signaling dependency of human malignancies driven by TEAD/YAP activation," *Nat Commun*, vol. 13, no. 1, 2022, doi: 10.1038/s41467-022-28319-3.
[79] Z. Ghazizadeh *et al.*, "ROCKII inhibition promotes the maturation of human pancreatic beta-like cells," *Nat Commun*, vol. 8, no. 1, 2017, doi: 10.1038/s41467-017-00129-y.
[80] E. Hammar, A. Tomas, D. Bosco, and P. A. Halban, "Role of the Rho-ROCK (Rho-associated kinase) signaling pathway in the regulation of pancreaticβ-cell function," *Endocrinology*, vol. 150, no. 5, 2009, doi: 10.1210/en.2008-1135.
[81] I. M. Moya and G. Halder, "Hippo–YAP/TAZ signalling in organ regeneration and regenerative medicine," *Nature Reviews Molecular Cell Biology*, vol. 20, no. 4. Nature Publishing Group, pp. 211–226, Apr. 01, 2019. doi: 10.1038/s41580-018-0086-y.
[82] F. Deng, Z. Wu, F. Zou, S. Wang, and X. Wang, "The Hippo–YAP/TAZ Signaling Pathway in Intestinal Self-Renewal and Regeneration After Injury," *Frontiers in Cell and Developmental Biology*, vol. 10. 2022. doi: 10.3389/fcell.2022.894737.
[83] M. Xin *et al.*, "Hippo pathway effector Yap promotes cardiac regeneration," *Proc Natl Acad Sci U S A*, vol. 110, no. 34, 2013, doi: 10.1073/pnas.1313192110.
[84] D. Kong, Y. Zhao, T. Men, and C. B. Teng, "Hippo signaling pathway in liver and pancreas: The potential drug target for tumor therapy," *Journal of Drug Targeting*, vol. 23, no. 2. 2015. doi: 10.3109/1061186X.2014.983522.

Appendix:

Inhibition of PHLPP1/2 phosphatases rescues pancreatic β-cells in diabetes

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My contribution:
Performed experiments and analysed data for Figure 3 (A,B – partially)

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Inhibition of PHLPP1/2 phosphatases rescues pancreatic β-cells in diabetes

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SUMMARY

Pancreatic β-cell failure is the key pathogenic element of the complex metabolic deterioration in type 2 diabetes (T2D); its underlying pathomechanism is still elusive. Here, we identify pleckstrin homology domain leucine-rich repeat protein phosphatases 1 and 2 (PHLPP1/2) as phosphatases whose upregulation leads to β-cell failure in diabetes. PHLPP levels are highly elevated in metabolically stressed human and rodent diabetic β-cells. Sustained hyper-activation of mechanistic target of rapamycin complex 1 (mTORC1) is the primary mechanism of the PHLPP upregulation linking chronic metabolic stress to ultimate β-cell death. PHLPPs directly dephosphorylate and regulate activities of β-cell survival-dependent kinases AKT and MST1, constituting a regulatory triangle loop to control β-cell apoptosis. Genetic inhibition of PHLPPs markedly improves β-cell survival and function in experimental models of diabetes *in vitro*, *in vivo*, and in primary human T2D islets. Our study presents PHLPPs as targets for functional regenerative therapy of pancreatic β cells in diabetes.

INTRODUCTION

Type 2 diabetes (T2D) is a heterogeneous multifactorial metabolic disease, characterized by insulin resistance and progressive loss of functional β-cell mass. Pancreatic β-cell failure finally results from decreased insulin secretory function and/or β-cell death (Alejandro et al., 2015; Ashcroft and Rorsman, 2012; Butler et al., 2003; Weir et al., 2020), hallmarks of T2D; however, underlying molecular mechanisms are still not fully characterized, and there is currently no β-cell-specific therapy for a cure (Donath et al., 2019). In addition to β-cell death and dysfunction, other mechanisms, such as β-cell dedifferentiation (Cinti et al., 2016; Jeffery and Harries, 2016; Talchai et al., 2012) and failure of adaptive expansion because of impaired regeneration (Aguayo-Mazzucato and Bonner-Weir, 2018; Tiwari et al., 2016), have been proposed as possible causes for β-cell failure in T2D.

The coordinated cellular stress response and enormous metabolic adaptation are necessary for normal β-cell insulin-secretory function, glucose homeostasis, and prevention of T2D; these are largely directed by the highly complex dynamics of signal transduction pathways. Perturbations in β-cell signaling have complex consequences leading to imbalanced and improper transcriptional and post-transcriptional alterations, metabolic deterioration, continuous decline in β-cell function and viability and the cumulative development of diabetic complications. Thus, comprehensive understanding of cell-fate decisions during stress and metabolic overload will provide new targets for the development of therapeutic approaches aiming at prevention and repair of β-cell failure in T2D.

Serine-threonine phosphatases (STPs) are important components of multiple cell signaling nodes and serve as potential targets for drug development. The pleckstrin homology (PH) domain leucine-rich repeat protein phosphatases (PHLPPs) enzymes are members of the protein phosphatase 2C (PP2C) grouped in the protein phosphatase metal-dependent (PPM) family of STP (Brognard and Newton, 2008). The PHLPP family includes two isozymes, PHLPP1 (also referred to as suprachiasmatic nucleus circadian oscillatory protein [SCOP]) and PHLPP2 (Grzechnik and Newton, 2016). PHLPP1/2 are ubiquitously expressed and involved in several cellular processes, such as proliferation, survival, stress response, inflammation, memory formation, and T cell development (Brognard and Newton, 2008; Chen et al., 2013; Cohen Katsenelson et al., 2019; Gao

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Declaration on the contribution of the candidate to a multi-author articles which are included in Chapter 2 in the submitted doctoral thesis

Publication I: *In vivo* β-cell specific YAP overexpression promotes β-cell regeneration in a mouse model of diabetes

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 60%
Experimental work and/or acquisition of (experimental) data:	ca. 90%
Data analysis and interpretation:	ca. 80%
Preparation of Figures and Tables:	ca. 80%
Drafting of the manuscript:	ca. 40%

Publication II: The TEAD activator TT-10 promotes beta cell regeneration in human and mouse islets.

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 60%
Experimental work and/or acquisition of (experimental) data:	ca. 80%
Data analysis and interpretation:	ca. 80%
Preparation of Figures and Tables:	ca. 80%
Drafting of the manuscript:	ca. 40%

Publication III: The Hippo terminal effector YAP boosts enterovirus replication in type 1 diabetes.

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 20%
Experimental work and/or acquisition of (experimental) data:	ca. 20%
Data analysis and interpretation:	ca. 20%
Preparation of Figures and Tables:	ca. 20%
Drafting of the manuscript:	ca. 5%

Appendix: Publication 1: Inhibition of PHLPP1/2 phosphatases rescues pancreatic β - cells in diabetes

Contribution of the candidate in % of the total work load (up to 100% for each of the following categories):

Experimental concept and design:	ca. 5%
Experimental work and/or acquisition of (experimental) data:	ca. 5%
Data analysis and interpretation:	ca. 5%
Preparation of Figures and Tables:	ca. 5%
Drafting of the manuscript:	ca. 0%

Date:

Signature:

Acknowledgments:

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