Tead1 reciprocally regulates adult β-cell proliferation and function

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Summary

The low proliferative rate of adult β-cells presents a challenge for diabetes therapy to increase β-cell numbers while maintaining mature function. Although proliferative quiescence in β-cells is required to maintain functional competence, exemplified by glucose stimulated insulin secretion, the molecular underpinnings of this reciprocal relationship remain enigmatic. Here, we demonstrate that Tead1, the transcription effector of the mammalian-Hippo pathway drives developmental stage-specific β-cell proliferative capacity in conjunction with its functional maturation. Tead1 promotes adult β-cell mature identity by direct transcriptional control of a network of critical β-cell transcription factors, including, *Pdx1, Nkx6.1 and MafA,* while its regulation of *Cdkn2a* maintains proliferative quiescence. Consequently, mice with either constitutive or inducible genetic deletion of Tead1 in β-cells developed overt diabetes due to a severe loss of secretory function despite induction of proliferation. Furthermore, we show that Tead1 has a similar regulatory role in human β-cells. We propose that Tead1 is an essential intrinsic molecular switch coordinating adult β-cell proliferative quiescence with mature identity and functional competence to maintain glucose homeostasis.

Diabetes has assumed epidemic proportions with over 422 million people affected by it worldwide (Roglic and World Health Organization, 2016). A reduction of functional β-cell mass underlies all forms of diabetes. In type 1 diabetes, there is an autoimmune destruction of insulin-producing β-cells, while type 2 diabetes (T2D) is characterized by significant insulin resistance with varying degrees of reduction in β-cell mass and function (Butler et al., 2003). Current strategies to promote proliferative capacity in adult β-cells are often limited by concurrent loss of mature function (Bensellam et al., 2018; Efrat, 2008; Puri et al., 2018). Hence, better understanding of the balance between proliferation and functional competence in mature β-cells is critical to enhancing functional β-cell mass to prevent and treat diabetes.

Tead family of transcription factors (Tead1-4) and their upstream modulators of the mammalian Hippo-Tead pathway are important regulators of cell proliferation to control organ size in embryonic development and tissue remodeling (Dong et al., 2007; Heallen et al., 2011; Karpowicz et al., 2010; Lee et al., 2010; Liu et al., 2019; Lu et al., 2010; Yu et al., 2015). Tead1, a canonical downstream transcriptional effector of the Hippo pathway is expressed ubiquitously, but its physiological function in mature differentiated cells is largely unknown. The Hippo signaling pathway consists of a core kinase cascade involving Mst1&2 kinases that phosphorylate Lats1&2, which in turn phosphorylate coactivators, Yap and Taz. Phosphorylated Yap/Taz are retained in the cytoplasm and subsequently targeted for degradation. In the absence of this kinase signaling Yap and Taz remain dephosphorylated, translocate to the nucleus and bind to Tead to regulate transcription (Meng et al., 2016; Yu et al., 2015). Interestingly, although various Hippo signaling components have been studied in pancreas biology, the role of Tead transcription factors in β-cells is unknown. Deletion of Mst1/2 in pancreatic progenitors resulted in Yap activation, ductal metaplasia and pancreatitis without overt β-cell defects in proliferation or function (Gao et al., 2013; George et al., 2012). During early pancreatic development Yap-Tead1 activate transcriptional events to control pancreatic progenitor (Cebola et al., 2015; Zhang et al., 2013), while Yap functions as an inhibitor of endocrine lineage specification, both in vivo (Mamidi et al., 2018) and during differentiation of iPS cells into β-cells (Rosado-Olivieri et al., 2019). In contrast, though Yap is normally absent in adult β-cells (Ardestani and Maedler, 2018; Gao et al., 2013; George et al., 2012), its overexpression promotes β-cell proliferation in human islets ex vivo (George et al., 2015; Yuan et al., 2016), while CTGF/CCN2, a transcriptional target of Hippo-Tead1 pathway, also regulates β-cell proliferation (Guney et al., 2011; Riley et al., 2015). All this strongly suggest that the Hippo-Tead1 pathway regulates β-cell proliferation. However, the role of Tead1 in adult β-cell proliferation and function has remained unexplored.

In this study, employing cell- type and developmental stage-specific genetic tools to systematically probe Tead1 function in β-cells, we uncover its surprising role in driving mature β-cell identity and glucose stimulated insulin secretion (GSIS), while maintaining proliferative

quiescence by direct transcriptional activation of a network of critical transcription factors and genes that confer functional competence and limit proliferation. Our study thus identifies Tead1, the Hippo effector, as a non-redundant reciprocal regulator of the proliferative drive and functional competence in β-cells. Differential modulation of these activities may allow an increase in functional β-cell mass without a loss of critical glucose homeostatic function as a therapeutic approach to diabetes.

Results

Tead1 is expressed in embryonic and adult beta cells

Tead family of transcription factors regulate proliferative pathways and determines organ size in many tissues during development (Yu et al., 2015; Zheng and Pan, 2019). However, their role in β-cell development and function are unknown. Examination of islets at different developmental stages revealed that Tead1 was preferentially expressed in adult mouse islets, and highly enriched as compared to other members of the Tead family that are present at 10-fold lower level (Tead2) or not detected (Tead 3 &4) (**Figure. 1A**). Of the two canonical Tead1 co-activators Yap and Taz, only Taz was robustly expressed (**Figure. 1A**), as were many upstream signaling components of Hippo pathway (Mst1&2, Lats1&2 and others), suggesting the Hippo-Tead pathway is functional in islets. Expression dynamics of Tead1 by immunostaining during islet development revealed it was most abundant at e15.5 in all pancreatic tissues, but its high expression was restricted to islets around weaning and persisted at a high level in adult β-cells (**Figure. 1B**). While Taz was robustly expressed throughout, from e15.5 into adult stage, Yap was absent in the islets at all stages examined (**Figure. 1B**), a finding that is consistent with a previous study (George et al., 2012). Human islets also expressed robust levels of TEAD1 and Taz but not YAP **(Figure 1C)**. In contrast to mouse islets, human islets express higher levels of Tead4. Based on the robust, yet restricted expression of Tead1 in mature β-cells, we selectively deleted Tead1 in a developmental stage-specific manner by crossing mice with Tead1 floxed (Tead1$^{F/F}$) alleles carrying a floxed exon 3 that encodes the DNA binding TEA domain (**Figure S1A-C**) (Liu et al., 2017) with three distinct Cre lines, namely Rip-Cre, Ins1-Cre (Thorens), and Mip-CreERT.

Tead1 deletion in β-cells leads to diabetes

Early constitutive deletion at e15.5 (Rip-β-Tead1$^{-/-}$), using Rip-Cre deletor mice, led to a loss of β-cell Tead1 expression, as indicated by >80% reduction in transcript (**Figure 1D**) and near absence Tead1 protein (**Figure 1E**) in whole islets. Loss of Tead1 immunostaining was specific to

β-cells (**Figure 1F**), but not other hormone-producing islet cells (data not shown). These Rip-β-Tead1$^{-/-}$ mice with embryonic deletion of Tead1 developed progressive hyperglycemia (fasting and fed) within 2 weeks of weaning at 5 weeks of age. At 2 months of age, they became diabetic with fed plasma glucose >500mg/dl (**Figure 1G**). Indicative of a severe defect in β-cell secretory function, the Rip-β-Tead1$^{-/-}$ mice lacked the first phase insulin secretion (**Figure 1H)**, a feature that is lost very early in type 2 diabetes (Cerasi et al., 1972; Davies et al., 1994), and displayed marked glucose intolerance during an intraperitoneal glucose tolerance testing (GTT) due to a blunted GSIS (**Figure 1I-J**). These results were not secondary to changes in body weight (**Figure S1D**) or insulin sensitivity (**Figure S1E**). Overall energy balance was similar to controls as indicated by food intake and whole body energy expenditure, VC02, V02, and RER (**Figure S1F-J**). To exclude potential confounding effect of hypothalamic expression or potential secretory dysfunction due to the Rip-Cre transgene (Lee et al., 2006; Tamarina et al., 2014), we applied another Ins1-Cre deletor line (Thorens et al., 2015) for specific genetic targeting of Tead1 in β-cells by generating the Ins1-β-Tead1$^{-/-}$mice. These Ins1-β-Tead1$^{-/-}$ phenocopied the Rip-β-Tead1$^{-/-}$ indicated by marked glucose intolerance with a significant blunting of GSIS in vivo during GTT **(Figure 1K-L).**

While the findings in Ins1-β-Tead1$^{-/-}$ and Rip-β-Tead1$^{-/-}$mice demonstrated the requirement of Tead1 function in β-cells in maintaining insulin secretion and glucose homeostasis in vivo, these models that involve embryonic deletion starting at e15.5 could also engender chronic compensatory changes. Hence, we tested whether Tead1 modulates adult β-cell function using Mip-CreERT mice (Tamarina et al., 2014) to achieve tamoxifen (TM)-inducible deletion (Mip-β-Tead1$^{-/-}$ mice). Tamoxifen administration (100mg/kg every other day for 5 doses by gavage) in 5-week-old Mip-β-Tead1$^{F/F}$ mice largely abolished-Tead1 protein expression in whole islet lysates when examined after 6 weeks, (**Figure 1M**), along with an absence of Tead1 nuclear immunostaining in ~85% of β-cells (**Figure 1N**). Strikingly, this restricted inactivation of Tead1 only in mature β-cells led to a marked glucose intolerance due to a blunting of in vivo GSIS with a loss of early phase insulin secretion (**Figure 1O-P**), demonstrating severe β-cell secretory deficits within 2 months post-TM treatment. Taken together, the three loss-of-function models of Tead1, during β-cell development (Rip-β-Tead1$^{-/-}$ and Ins1-β-Tead1$^{-/-}$) or after maturation (Mip-β-Tead1$^{-/-}$) demonstrate the essential role of Tead1 in maintaining β-cell secretory capacity and glucose homeostasis.

Tead1 transcriptional control of a regulatory network is critical for maintaining functional mature β-cell identity

Using isolated Rip-β-Tead1$^{-/-}$ islets, we next determined the specific functional deficits induced by a loss-of-Tead1 function. Despite normal basal insulin secretion these islets display significantly impaired GSIS with a markedly lower insulin secretion index (**Figure 2A**), as compared to either Floxed or Rip-Cre controls. Furthermore, in Ins-1 (832/13) cells, Tead1 knockdown yielded a similar blunting of GSIS response (**Figure S2A-B**), suggesting the cell-autonomous role of Tead1 for GSIS in β-cells. Consistent with the GSIS deficits, there were ~50-80% reductions of insulin content in Rip-β-Tead1$^{-/-}$ mice, whether measured in the whole pancreas (**Figure 2B**), normalized to islet size **(Figure 2C**) or by cell number (reflected by DNA content) as compared to controls (**Figure 2D**).

To determine the regulatory pathways mediating Tead1 function in islets, we performed global transcriptome analysis in Rip-β-Tead1$^{-/-}$ islets that revealed 259 (down) and 296 (up) differentially regulated genes as compared to age-matched floxed controls (**Figure S3C and Supplementary table 3**). In agreement with the profound defects in GSIS, among the differentially regulated genes by RNA-seq analysis distinct components of the insulin secretory pathway including the glucose transported Glut2 (Slc2a2) and glucokinase (Gck) were down-regulated in Tead1-deficient islets. In addition, key transcription factors known to specify and maintain adult β-cell function were suppressed, such as Pdx1, MafA. (**Figure 2E**). Further analysis using an unbiased Gene Set Enrichment Analysis (GSEA) revealed a global down-regulation in adult β-Tead1$^{-/-}$ islets of genes significantly enriched in mature adult as compared to immature neonatal islets (GSE47174) (Martens et al., 2014) (**Figure 2F)**. Interestingly, many disallowed genes in normal adult β-cells that comprise the neonatal islet transcriptome signature (Lemaire et al., 2016; Pullen et al., 2010; Pullen and Rutter, 2013; Thorrez et al., 2011) and are highly expressed in immature neonatal islets were not altered in Tead-1 deficient islets (**Figure S2D**). These findings suggest a loss of mature β-cell characteristics, rather than de-differentiation (Puri et al., 2018) of Tead1-null β-cells. Further support of this notion was found in the transcriptomic signatures of key processes that are critical for acquisition and maintenance of mature secretory function in β-cells, including OXPHOS, substrate metabolism, protein secretion and export, vesicular transport and UPR were consistently suppressed with loss of Tead1 (GSEA, $q<0.25$) **(Figure 2G**). We further validated that in Rip-β-Tead1$^{-/-}$ islets, transcript (**Figure 2H)** of Ins1, Glut2, Pdx1, NeuroD, MafA and Nkx6.1 were all significantly decreased along with Ucn3, a hallmark of mature β-cells (Blum et al., 2012; van der Meulen et al., 2012). The protein levels of many of these genes were also significantly reduced by immunostaining (**Figure 2I**) or western blotting **(Figure 2J & 2L)** with respective quantifications (**Figure 2K & 2M**). These data collectively demonstrated Tead1 transcriptional control of a regulatory network critical for maintaining functional mature β-cell identity.

We interrogated whether Tead1 exerts direct transcriptional regulation of this gene network driving β-cell function and identity. Analysis of Tead1 occupancy by ChIP-seq in adult mouse islets on regulatory regions of transcription factors that are critical for β-cell function, including Pdx1, MafA, Nkx6.1, revealed its enrichment on these genes that were downregulated by loss of Tead1 (**Figure 3A**). ATAC-seq further confirmed that the identified promoter regions with Tead1 occupancy in islets occurred in open chromatin regions that are transcriptionally active (**Figure 3A)**. ChIP-qPCR using either endogenous Tead1 (**Figure 3B**) or tagged Myc-Tead1 (**Figure S3A**) in mouse insulinoma cell line further validated Tead1 binding to cis-promoter elements (containing Tead consensus binding sequences of GGAATG) of Pdx1, MafA, Nkx6.1, Ucn3.

Since Pdx1 is a master regulator of mature β-cell function, we undertook a detailed analysis of Tead1 regulation of Pdx1 transcription. Interestingly, we found multiple Tead1 responsive elements in the Pdx1 promoter region as represented in **Figure 3C,** including the Tead1 consensus sequence present in Area IV region known to be critical for β-cell specific Pdx1 expression and postnatal mature β-cell function (Spaeth et al., 2017). In ChIP pull down with Myc-Tead1 in Ins2 cells, this site exhibited the highest enrichment (**Figure 3D**). In a promoter luciferase assay, expression of Tead1 together with its co-activator, Taz, activated transcription of the native Pdx1-promoter containing Tead1 response elements, demonstrating Tead1 functional regulation of Pdx1 transcription (**Figure 3E**). Furthermore, Tead1 knockdown decreased Pdx1 protein level in Ins2 cells (**Figure 3F)**; whereas its overexpression by adenoviral vector in Ins2 cells increased Pdx1 expression (**Figure 3G-H**). Overexpression of TEAD1 in additional human β-cell models including human β-cell line (EndoC-βh2) **(Figure 3I**) or human cadaveric islets (**Figure 3J**) augmented PDX1 expression. It is noteworthy that TEAD1 and PDX1 protein levels detected by western blotting in individual human islet samples exhibited significant linear correlation (**Figure 3K)**. Thus, TEAD1 transcriptional control of PDX1 is conserved in human β-cells. Taken together, these data provide direct support of Tead1 as a direct transcriptional regulator that orchestrates a transcription factor network regulatory network critical to acquire and maintain mature β-cell function.

Tead1 mediates proliferative quiescence in adult β-cells

Tead1 drives cell proliferation in various tissue development processes (Liu et al., 2019; Meng et al., 2016; Yu et al., 2015). We postulated that Tead1 deficiency during islet development may limit β-cell proliferation to reduce β-cell mass. Surprisingly, Rip-β-Tead1$^{-/-}$ islets from 12 week old mice contained increased number of β-cells (**Figure 4A**), which was also reflected by increased

DNA content in similar-sized islets (**Figure S4A**). However, these β-cells were significantly smaller (**Figure 4B-C)**, which offset the increase in numbers, so β-cell mass in Rip-β-Tead1$^{-/-}$ mice remained normal compared to that of the floxed controls (**Figure 4D**). Similar findings were observed in the inducible line of 9 month old adult Mip-β-Tead1$^{-/-}$ mice, wherein Tead1 loss-of-function in adult β-cells did not affect β-cell size (**Figure S4B**), total β-cell area (**Figure S4C**) or islet size (**Figure S4D**). Further analysis indicated that increased β-cell number in Rip-β-Tead1$^{-/-}$ islets was not due to apoptosis (**Figure S4E-F**), but rather because of higher proliferation rate, as reflected by a significantly elevated Ki67 positive β-cells (2% vs. 0.62% in 12 week old Rip-β-Tead1$^{-/-}$ and Tead1$^{F/F}$ controls, respectively; $p<0.01$) (**Figure 4E-F**).

To test if Tead1 regulates β-cell proliferation at distinct developmental stages, we analyzed in vivo BrdU incorporation into β-cells in neonatal (postnatal day 12), post-weaning (4 weeks) and adult (12 weeks) mice. Notably, while there was no significant difference in proliferation at postnatal day 12 between Rip-β-Tead1$^{-/-}$ and Tead1$^{F/F}$ littermate controls, higher rate of BrdU incorporation was observed at 4 (5.31% vs. 4.08%; $p<0.03$) and 12 weeks of age (0.5% vs. 0.2%; $p<0.05$) respectively (**Figure 4G-H)**. This data indicate that Tead1 did not contribute physiologic neonatal β-cell proliferative expansion when the proliferative drive is high, but rather was required for attaining proliferative quiescence as β-cells mature in adult mice.

β-cells have a short quiescence phase of ~7 days after mitosis before they can re-enter cell cycle (Salpeter et al., 2010). Hence, we assessed if Tead1 could regulate total pool of actively proliferating β-cells by labelling all cycling β-cells over a 6 day period with BrdU via drinking water and found that active proliferation was ~4 fold higher in Tead1-deficient β-cells than that of controls (17.1% vs. 4.46%) (**Figure 4I-J**). However, the fraction of proliferating β-cells in S-phase (BrdU-positive) to actively cycling Ki67-positive cells did not differ between the Rip-β-Tead1$^{-/-}$ and controls (**Figure 4K**) indicating that loss of Tead1 did not affect cell cycle kinetics, once the cells entered cell cycle. Together, these data supports the notion that higher proliferation in Tead1-deficient β-cells likely occurred as a result of more cells entering cell cycle (G1) from the G0 quiescent state. We then asked if Tead1 affects age-associated proliferative quiescence when most β-cells become resistant to proliferative signals (Zhou et al., 2013). Tead1 deletion in β-cells in 9 months old TM-treated Mip-β-Tead1$^{-/-}$ mice showed a 2-fold increase in proliferation (**Figure 4L)**, similar as young adult mice. Thus, Tead1 was required to maintain quiescence in adult β-cells, while its inactivation releases cells from age-associated quiescence into active cell cycle.

The augmented β-cell proliferation in 4 week old Rip-β-Tead1$^{-/-}$ mice (**Figure 4G**) preceded the onset of hyperglycemia at 5 weeks of age (**Figure 1F**), suggesting that the observed increase in proliferation is not secondary to hyperglycemia-induced compensation. However, to conclusively exclude non-cell autonomous effects, such as hyperglycemia, as a potential cause

of proliferation, we leveraged the mosaic β-cell Tead1 deletion often seen with Mip-CreERT (Tamarina et al., 2014; Wicksteed et al., 2010). Using this model, we pulse labelled cycling β-cells for 2 hours using BrdU and analyzed islets in which ~50% of β-cells had deletion of Tead1. We found that within the same pool of islets in Mip- β-Tead1$^{-/-}$ mice with Tead1-positive and Tead1-negative β-cells, the proliferative rate was ~ 5 fold higher in Tead1-negative than that of the Tead1-positive β-cells (0.58% vs. 0.11%; $p<0.05$) (**Figure 4M-N**), indicative of cell-autonomous effect of Tead1 on proliferation.

Tead1 is a direct transcriptional activator of p16 in adult β-cells

Corroborating the unexpected increased proliferation of Tead1-deficient β-cells, global transcriptome analysis of Tead1-deficient islets revealed significant upregulation of pathways involved in cell cycle control, including many G1-S checkpoint regulators (**Figure 5A**). Expression of Cyclin A2, critical for G1-S phase transition in β-cells (Song et al., 2008) was significantly elevated in Rip-β-Tead1$^{-/-}$ islets (**Figure 5B**). Though absence of Tead1 reduced Cyclin D1 transcript, its protein level was not altered (data not shown). In contrast, there were marked reductions of p16^{INK4a} and p19^{ARF}, key cell cycle inhibitors critical for limiting β-cell proliferation in adult mice (Chen et al., 2009; Dhawan et al., 2009; Krishnamurthy et al., 2006) (**Figure 5B**). p16^{INK4a} protein was nearly absent in Tead1-deficient islets (**Figure 5C-D**) as well as in Tead1-depleted Ins2 cells (**Figure 5E**), while Tead1 overexpression robustly increased p16^{INK4a} protein levels (**Figure 5F**). Examination of the *Cdkn2a* locus that encodes both p16^{INK4a} and p16^{ARF} demonstrated Tead1 occupancy in multiple Tead1 consensus motifs (**Figure 5G**). ChIP-qPCR in Ins2 cells demonstrated Tead1 binding to these motifs within the promoter of p16^{INK4a} in the *Cdkn2a* locus (**Figure 5H**). This was consistent with Chip-seq and ATAC-seq data from adult islets demonstrating Tead1 occupancy within Tead1 consensus motif containing regions of open chromatin in *Cdnk2a* locus (**Figure 5I**). Furthermore, Tead1 binding to these sites are functional as it can activate the native *Cdkn2a* (p16INK4a) promoter when co-expressed with Taz (**Figure 5J**).

p16 represents a key cell cycle entry barrier to transition from a quiescent state, and Ezh2 has an established role as its critical repressor in β-cells (Chen et al., 2009; Krishnamurthy et al., 2006; Zhou et al., 2013). To explore whether Tead1 transcriptional regulation of p16^{INK4a} determines the balance between proliferation and quiescence, and if β-cell proliferation and its mature function were inter-dependent we tested if re-expressing p16 in Tead1-null β-cells in vivo through genetic ablation of its repressor Ezh2 could restore β-cell quiescence and insulin-secretory function. We inactivated Ezh2 in β-cells of Rip-β-Tead1$^{-/-}$ mice (DKO) by crossing Ezh2 flox mice with Rip-β-Tead1$^{-/-}$ mice. As expected of Ezh2 regulation of p16, the loss of Ezh2 in Tead1-null β-cells restored the level of p16 to a comparable level in wild type controls (**Figure**

6A-B). Notably, 6 month old Rip-β-Tead1$^{-/-}$ β-cells without Ezh2 showed much lower proliferation rates than that seen in Rip-β-Tead1$^{-/-}$ mice (Figure 4G-H) and at a level comparable to the control flox mice, (**Figure 6C-D**). Therefore, restoring p16 through the modulation of Ezh2 was sufficient to achieve/rescue proliferative quiescence in Tead1-null β-cells. Interestingly, the restoration of proliferative rates to normal was not sufficient in restoring glucose tolerance in these DKO mice, as these mice exhibited persistence glucose intolerance as compared to the floxed controls though there was some improvement as compared to littermate Rip-β-Tead1$^{-/-}$ mice (**Figure 6E**). Thus, Tead1 regulation of proliferative quiescence was mediated by its direct transcriptional regulation of p16^{INK4a} expression.

Discussion

Proliferative quiescence often accompanies the acquisition of mature function in many cell types, including β-cells (Puri et al., 2018; Teta et al., 2007). The molecular mechanisms that underpin this reciprocal regulation remain largely unexplored and this knowledge gap limits therapeutic approaches in regenerative medicine. Here, we identify Tead1 as mediating this reciprocal regulation of proliferative quiescence and maintenance of mature function in β-cells by direct transcriptional activation of a critical network of genes regulating these processes (schematically shown in **Figure 7**) in both mouse and human β-cells. We demonstrate that in adult β-cells Tead1 functions to limit proliferation and maintain mature identity and functional competence and its loss-of-function leads to β-cell failure and diabetes. Recent studies have highlighted limitations of individual insulin-promoter driven Cre-lines, such as potential secretory defects and hypothalamic expression in Rip-Cre, Gh minigene effects in Mip-Cre/ERT and possibility of low efficiency in Ins1-Cre and Mip-Cre/ERT lines (Brouwers et al., 2014; Carboneau et al., 2016; Lee et al., 2006; Mosleh et al., 2020). To overcome these limitations we have used a combination of multiple β-cell Cre-line derived genetic models to decipher the critical and non-redundant function that Tead1 exerts in adult β-cells.

β-cells are highly proliferative before weaning, with proliferative rates as high as 4-6% (Stolovich-Rain et al., 2015). After weaning, they acquire GSIS with robust expression of gene networks regulating glucose sensing, transport, mitochondrial function, oxidative phosphorylation, and secretory machinery. However, as they gain functional competence, adult β-cells repress their proliferative program, lose their high basal proliferative rate having largely exited from cell cycle and self-renew at low rates that decline further with aging (Dor et al., 2004; Meier et al., 2008; Teta et al., 2005). While many pathways that regulate proliferation (Bernal-Mizrachi et al., 2014; Kulkarni et al., 2012; Stewart et al., 2015) and others that determine functional competence have been identified, mechanisms that evolved to coordinate these two programs remain poorly understood. In this study, we identify Tead1, as

the critical mediator of this coordination of proliferative quiescence and functional competence in adult β-cells.

In the mature state, p16^{INK4A} (encoded by *Cdkn2a*) and its regulators in the polycomb repressive complex (PRC 1 and 2) are one of the primary enforcers of proliferative quiescence in adult β-cells (Chen et al., 2009; Dhawan et al., 2009; Krishnamurthy et al., 2006; Krishnamurthy et al., 2004; Lu et al., 2018). These exhibit a significant age-dependent regulation of proliferation (Chen et al., 2011; Chen et al., 2009; Dhawan et al., 2009; Lu et al., 2018; Qiu et al., 2017; Tschen et al., 2009; Zhou et al., 2013). The unexpected finding that Tead1 is required for maintaining proliferative quiescence in mature β-cells led us to identify Tead1 as a novel transcriptional regulator of p16 and a loss of this regulation led to a reacquisition of a high proliferative rate. Deletion of Ezh2 to restore p16 expression in Tead1-deficient β-cells demonstrated that modulation p16 mediated the Tead1 enforced proliferative quiescence in mature β-cells. It is possible that Tead1 also interacts with other regulators of p16 including Ezh2, Bmi1 among others, especially in an age-related manner to restrict β-cell proliferation. How to modulate these Tead1 related regulatory mechanisms to enhance functional β-cell mass will be a subject for future studies.

Neonatal β-cells express many genes that are 'disallowed' in mature β-cells, including hexokinase1 (HK1), AldolaseB, Ldha, Acot7, MCT1 (Dhawan et al., 2015; Lemaire et al., 2016; Pullen et al., 2010; Thorrez et al., 2011), many of which have been associated with immature function characterized by blunted GSIS. In rodent β-cells, high expression of a network of critical β-cell transcription factors, including Pdx1, Neurod1, Nkx6.1, a switch from MafB to high MafA expression along with and their target genes including Glut2, Glucokinase, Ucn3 and others, establishes functional competence and mature identity exemplified by robust GSIS (Martens et al., 2014; Nishimura et al., 2006). Repression of these genes is associated with a loss of mature function accompanied by a fetal/immature gene expression phenotype with expression of 'disallowed 'genes, with high MafB and loss of MafA expression. Interestingly, loss of Tead1 function leads to a decrease in expression of this gene network and loss of functional competence, but is not associated with re-expression of many of the disallowed genes, such as *Ldha*, nor a re-expression of MafB, hallmarks of de-differentiation to an immature state (Nishimura et al., 2006). This is in contrast to that seen in proliferating β-cells with activation of c-Myc (Puri et al., 2018) or Notch (Bartolome et al., 2019), suggesting that the loss of mature function with Tead1 deletion may be independent of the proliferation phenotype. Interestingly, the β-cell deletion of Eed, an interactor of Ezh2 in PRC2, was reported to have a severe β-cell phenotype (Lu et al., 2018) with βEedKO mice having significant diabetes along with a significant loss of mature identity and increased expression of immature markers, although no proliferative rates were reported. While there are similarities in the loss of mature identity in these studies, the salient distinction with loss of Tead1 function is that the markers of de-

differentiation are not increased, supporting the assertion that Tead1 promotes maintenance of mature identity.

While the requirement of Tead1 for normal β-cell function is demonstrable in these studies, it would be interesting to speculate whether this is dysregulated in disease states. While factors that regulate upstream hippo kinase cascade can certainly play a role, other stimuli may also play a direct role in regulating Tead1 function, independent of the hippo kinase cascade. Recent studies have shown that Tead1 subcellular localization could be altered by p38 Map kinase activation and glucose itself (Lin et al., 2017) or its stability of co-factor binding can be regulated by palmitoylation (Chan et al., 2016; Kim and Gumbiner, 2019; Noland et al., 2016). This raises the intriguing possibility that stress pathways and glucolipotoxicity could affect β-cell function via changes in Tead1 localization and activity. Could then glucotoxicity, lipotoxicity or other stress-induced induced β-cell dysfunction be modulated by regulation of Tead1 activity and provide novel therapeutic targets? Answers to these questions in future studies could uncover new druggable targets for therapy of diabetes.

This reciprocal regulation of mature function and proliferative quiescence enforced in β-cells, by Tead1, could underpin the hurdles in inducing β-cell proliferation without loss of function. Intriguingly, recent studies in human islets demonstrate that although YAP is not normally expressed in adult β-cells, its overexpression and activation of TEAD1 pathway could lead to proliferation without loss of function, (George et al., 2015; Yuan et al., 2016). This is also consistent with Tead1 regulation of PDX1 observed in this study. These observations suggest that Tead1 activity could be determined by the specificity of upstream coactivators and open the possibility that there are as yet undetermined pathways that are regulated by Tead1. While the regulators of Tead1 action in β-cells need further exploration, identification of these would allow therapeutic modulation of Tead1 activity to enhance β-cell replacement therapy.

In summary, Tead1 is a critical β-cell transcription factor required for coordinating adult β-cell proliferative quiescence, mature identity and functional competence to maintain glucose homeostasis. Modulation of this reciprocal regulation could help overcome hurdles in inducing β-cell proliferation without loss of function.

Methods

Methods and any associated references are presented in Supplementary Information.

Expression data have been deposited in GEO: 12 weeks (GSE139228) and 1 year (GSE139152). Chip-seq and ATAC-seq data have been deposited in GEO under GEO accession GSE157513

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Author Contributions

J.L. designed and conducted the experiments, performed data analyses, interpreted data and helped write the manuscript; R.L., B.S.K., H.P. helped with specific experiments and data analysis. P.K.S., O.S., M.O.H., H.P., F.L., R.J., P.Y., V.N., E.M.P-G., R.B., Y.Z., C.C., C.J.C., K.M., and M.M. provided critical scientific input, data analysis, and logistical support. R.L., M.O.H, K.M. and M.M. provided critical input into editing the manuscript. V.K.Y supervised the project, designed experiments, interpreted data and wrote the manuscript.

Competing Financial interests

The authors declare no competing financial interests.

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Figure 1: β-cell Tead1 deletion leads to diabetes.

(A) Relative gene expression assessed by RT-qPCR of total RNA from isolated islets of 4 month old wild type mice, normalized to housekeeping gene, *Tbp*. n=4 (B) Representative immunofluorescence staining of Tead1, Taz and Yap in mouse islets at different ages. Scale bar - 50µm. (C) Relative gene expression assessed by RT-qPCR of total RNA from isolated human islets, normalized to housekeeping gene, *TBP.* n=4 (D) RT-qPCR (normalized to control Tead1$^{F/F}$) n=4-6; and (E) western blotting for Tead1 in isolated islets from Tead1$^{F/F}$ and Rip-β-Tead1$^{-/-}$mice. (F) Representative images of immunofluorescence staining of Tead1$^{F/F}$ and Rip-β-Tead1$^{-/-}$ pancreas. Scale bar - 50µm. (G) Plasma glucose level with overnight fasting and after 1 hour refeeding in Tead1$^{F/F}$ and Rip-β-Tead1$^{-/-}$mice. n=4-6. w-weeks; M-months; F-fasting; RF-refeeding. (H) Acute insulin secretion after glucose stimulation in 10-week-old mice. n=5. (I) Plasma glucose and (J) insulin during glucose tolerance testing (GTT) in overnight fasted 8 weeks old male mice. n=5-6. (K) Plasma glucose and (L) insulin during GTT in overnight fasted 9 weeks old male mice. n=3-4. (M) Western blotting of isolated islets from Tead1$^{F/F}$ and Tead1$^{F/F}$Mip-CreERT^{+} mice treated with oil or Tamoxifen (TM). (N) Nuclear Tead1 staining is absent from most insulin expressing β-cell in TM-treated Mip-β-Tead1$^{-/-}$ islets. Scale bar – 50µm. (O) Plasma glucose level and (P) insulin levels during the GTT in overnight fasted 5 month old Tead1$^{F/F}$ and Tead1$^{F/F}$Mip-CreERT^{+} (Mip-β-Tead1$^{-/-}$) mice treated with oil or TM. n=4. TM, Tamoxifen. All values are mean ± SEM, * - p≤ 0.05. For additional related information see Supplementary Figure S1.

Figure 2: Tead1 is required for mature β-cell function. (A) GSIS from isolated islets (ten islets each from 5-6 individual mice of each genotype) on exposure to increasing glucose concentrations. Insulin secretion index is expressed as a fold change over basal secretion in

2.8mM glucose. Insulin content in (B) total pancreas, n=4; in isolated islets expressed as insulin content (C) per equally sized islet or (D) per µg DNA, n=5. (E) Volcano plot from RNA-seq data from differentially expressed genes from isolated islets of 1 year old Rip-β-Tead1$^{-/-}$ compared to Tead1$^{F/F}$. Some of the genes critical in mature β-cell function are highlighted. (F) GSEA analysis of the differentially expressed downregulated genes in isolated islets of Rip-β-Tead1$^{-/-}$ compared to Tead1$^{F/F}$ as compared to the published gene set (GSE47174) that was upregulated in adult β-cells when compared to neonatal β-cells. Details in the accompanying text. NES – normalized enrichment score. FDR Q value is also shown. (G) Enriched pathways in transcriptome analysis of Rip-β- Tead1$^{-/-}$ compared to Tead1$^{F/F}$ isolated islets. x-axis shows the Normalized enrichment score. Note that in this representation the red bars indicate the enrichment of Rip- β-Tead1$^{-/-}$ over control Tead1$^{F/F}$ indicating these pathways are suppressed in islets from Rip- β-Tead1$^{-/-}$ mice, while the blue bars indicate that the same pathways are upregulated in adult over neonatal β-cells. (H) Expression, by RT-qPCR, of genes critical to mature β-cell function in Tead1$^{F/F}$ and Rip-β-Tead1$^{-/-}$ islets, normalized to housekeeping gene, *TopI.* n=4. (I) Representative immunofluorescent images of Pdx1, MafA, Ucn3 and Glut2 staining in Tead1$^{F/F}$ and Rip-β-Tead1$^{-/-}$ islets. Scale bar - 50 µm. (J-M) Western blot and quantification of (J and K) Pdx1 protein and (L and M) MafA from isolated islets from Rip-β-Tead1$^{-/-}$ and Tead1$^{F/F}$ mice. All values are mean ± SEM, * - p≤ 0.05. For additional related information see Supplementary Figure S2.

Figure 3: Tead1 is direct transcriptional regulator of genes for mature β-cell function.

(A) Tead1 occupancy in the active promoters of displayed genes that have open chromatin in mouse islets by ATAC-seq and ChIP-seq. Red boxes indicate overlapping regions of Tead1 occupancy (Tead1 Chip-seq) and open chromatin (ATAC-seq). Input control is also shown. (B)

ChIP in Ins-2 cells with Tead1 or control IgG antibody and qPCR with primers flanking putative Tead1 response elements. The y-axis represents the ratio of pulldown DNA to input DNA. n=3. (C) Tead1 response elements (Tead1 RE) in promoter region of mouse *Pdx1* is shown schematically and (D) ChIP in Myc-Tead1 over-expressed Ins-2 cells with Myc or control IgG antibody and qPCR with putative Tead1 response element on mouse Pdx1 promoter. (E) Promoter luciferase assay with native Pdx1 promoter-Tead1 response element (RE:Luc) reporter in Ins-2 cells. y-axis represents the relative luminescence units (RLU). n=3. (F) Western blotting for Pdx1 protein in Tead1 knock down Ins-1 cell line. (G and H) Western blotting and quantification for PDX1 protein in TEAD1 overexpressing (Tead1 O/E) in mouse ins2 cell line, in (I) human EndoC-β-h2 cell line and in (J) human islets. Control (Ctrl) cells are transfected with empty vector. (K) Correlation analysis of PDX1 protein and TEAD1 in human islets. Analysis of 8 samples. All values are mean ± the standard errors of the means, * - $p \leq 0.05$. For additional related information see Supplementary Figure S3.

Figure 4: Tead1 deletion enhances β-cell proliferation.

(A) β-cell number in 1000 μm^2 in Rip-β-Tead1$^{-/-}$ mice and Tead1$^{F/F}$ mouse islet. N=3. (B) Single β-cell area in Rip-β-Tead1$^{-/-}$ mice and Tead1$^{F/F}$ mouse pancreas at 3 months age. n=3. (C) β-cell size distribution in Tead1$^{F/F}$ mice (blue) and Rip-β-Tead1$^{-/-}$ mice (red) at 3 month age. All values are means ± SEM. n=3. (D) Total pancreatic β-cell area from Rip-β-Tead1$^{-/-}$ mice and Tead1$^{F/F}$ mice at 3 months age. n=3. (E-H) Quantification of β-cell proliferation in Tead1$^{F/F}$ and Rip-β-Tead1$^{-/-}$ mice at 3 months. n=3. BrdU was injected (G and H) 2 hrs prior to sacrifice, or (I and J) administered continuously by

water for 6 days prior to sacrifice. Representative microscopy images of Rip-β-Tead1$^{-/-}$ mice and Tead1$^{F/F}$ mouse pancreas at 3 months age and quantification of (E and F) Ki67 expressing and (G and I) BrdU positive β-cells. n=4. Scale bar – 50µm. (K) The fraction of BrdU-positive β-cells to Ki67-positive β-cells in the Rip-β-Tead1$^{-/-}$ and controls. n=3-4 (L) Quantification of Brdu-positive β-cells in in 11 month old TM-treated Mip-β-Tead1$^{-/-}$ mice and controls. n=4. (M) Brdu-positive cells in Tead-negative and Tead1-positive β-cell subpopulations in 11 month old TM-treated Mip-β-Tead1$^{-/-}$ mice. n=4. All values are mean ±the standard errors of the means, * - $p \leq 0.05$. N.S.-not significant. (N) Mosaic deletion of Tead1 in TM-treated Mip-β-Tead1$^{-/-}$ β-cells leads to intra-islet mosaicism of Tead1 expression in β-cells. Representative confocal microscopy images of immunofluorescent staining of pancreas sections from Mip-β-Tead1$^{-/-}$ mice indicating Tead1-neg (red arrow) and Tead1-pos (white arrow) β-cells in 3 different mice. For additional related information see Supplementary Figure S4.

Figure 5: Tead1 is direct transcriptional regulator of genes for β-cell proliferation.

(A) Transcriptome analysis of isolated Rip-β-Tead1$^{-/-}$ and control Tead1$^{F/F}$ islets from 12 weeks old male mice with significantly enriched pathways by Gene Ontology pathway analysis. All proliferation related pathways are highlighted in red. Details in the accompanying text. NES – normalized enrichment score. FDR Q value is also shown. (B) Relative expression of cell cycle related genes in Rip-β-Tead1$^{-/-}$ and Tead1$^{F/F}$ islets, normalized to housekeeping gene, *Topl*. n=4. (C) Western blot of p16^{INK4a} protein and (D) quantification in isolated islets from Rip-β-Tead1$^{-/-}$ and Tead1$^{F/F}$ mice. n=4-5. (E and F) Western blotting for Tead1 in (K) shRNA Tead1

knockdown and scrambled Ins-1 cells and (F) Tead1 overexpression and control in Ins-2 cells. (G) Tead1 response elements (Tead1 RE) in promoter region of mouse *Cdkn2a*. The *Cdkn2a* locus encoding $p16^{INK4a}$ and $p19^{ARF}$ is shown schematically. (H) ChIP of Myc-Tead1 over-expressing Ins-2 cells with Myc or control IgG antibody and qPCR with primers flanking putative Tead1 response elements. The y-axis represents the ratio of pulldown DNA to input DNA. n=3. (I) Tead1 occupancy in the active promoter/intronic region of *Cdkn2a* that have open chromatin in mouse islets by ATAC-seq and ChIP-seq. Red box indicate overlapping regions of Tead1 occupancy (Tead1 Chip-seq) and open chromatin (ATAC-seq). Input control is also shown. (J) Promoter luciferase assay with native Cdkn2a promoter-Tead1 response element (RE:Luc) reporter in Ins-2 cells. y-axis represents the relative luminescence units (RLU). n=3. All values are mean ±the standard errors of the means, * - $p \leq 0.05$.

Figure 6: Tead1 regulates proliferative quiescence by its direct transcriptional regulation of $p16^{INK4a}$ expression.

(A) western blotting for Tead1, Ezh2 and p16 and (B) quantification of p16 protein in isolated islets from $Tead1^{F/F}Ezh2^{F/F}$ and Rip-β-$Tead1^{-/-}Ezh2^{-/-}$ mice. n=4-6. (C) Representative images of immunofluorescence staining for BrdU and insulin in $Tead1^{F/F}Ezh2^{F/F}$ and Rip-β-$Tead1^{-/-}Ezh2^{-/-}$ pancreas. BrdU was injected 2 hrs prior to sacrifice. (D) Quantification of β-cell proliferation in $Tead1^{F/F}Ezh2^{F/F}$ and Rip-β-$Tead1^{-/-}Ezh2^{-/-}$ mice at 10-11 weeks. n=3. (E) Blood glucose during GTT in overnight fasted 9-10 weeks old male mice. n=4-6.

Figure 7: Schematic model of reciprocal regulation of β-cell proliferation and mature function by Tead1.

Figure S1: Generation of Tead1 floxed mouse strain and β-cell specific Tead1 knock out mice. (A) Generation of Tead1 conditional knockout allele, and β-cell specific knockout mouse models. (B) Genomic PCR showing the recombined fragment of the Tead1 allele in islet and heart from Tead1$^{F/F}$ and Rip-β-Tead1$^{-/-}$mice. (C) Tead1 flox allele and its deletion in Rip-β-Tead1$^{-/-}$, verified by long-range PCR (right) in Tead1$^{F/F}$ and Rip-β-Tead1$^{-/-}$islet. (D) Body weight of Tead1$^{F/F}$ and Rip-β-Tead1$^{-/-}$mice. n=4-6. (E) Plasma glucose represented as a percent drop from baseline during insulin tolerance test in 12 weeks old male mice. n=5. All values are mean ± SEM. (F) Food intake measured over a 3-day period. n=4. Energy expenditure and VO2 (G and H), VCO2 (I), and RER (J) were assessed as described in Methods in supplementary material. n=4. All values are mean ±SEM.

Figure S2: Cell-autonomous regulation of GSIS and Pdx1 by Tead1. (A,B) Tead1-depleted β-cells have impaired GSIS in vitro. (A) Western blotting for Tead1 in shRNA Tead1 knockdown and scrambled Ins-1 (832/13) cells. (B) GSIS in response to increasing glucose concentration in Tead1-knockdown and scrambled control 832/13 cells. n= 4. All values are mean ± SEM, * - $p \leq 0.05$. (C) Transcriptome analysis heat map showing up and downregulated genes in isolated islets from 12 week old Rip-β-Tead1$^{-/-}$ compared to Tead1$^{F/F}$ mice. (D) GSEA analysis of the differentially expressed upregulated genes in isolated islets of Rip-β-Tead1$^{-/-}$ compared to

Tead1$^{F/F}$ as compared to the published gene set (GSE47174) that was upregulated in adult β-cells when compared to neonatal β-cells. Details in the accompanying text. FDR Q value did not show any significance.

Figure S3: Tead1 regulates mature β-cell function related genes. (A) ChIP of Myc-Tead1 over-expressing Ins-2 cells with Myc or control IgG antibody and qPCR with primers flanking putative Tead1 response elements in promoters of displayed genes. The y-axis represents the ratio of pulldown DNA to input DNA. n=3.

Figure S4: Tead1 regulates β-cell proliferative quiescence. (A) Genomic DNA content in similar sized islets. n=5. (B) β- cell size, (C) β-cell area and (D) average islet size in Mip- β-Tead1$^{-/-}$ and control mice n=3-4. (E and F) Apoptosis is not altered in Tead1-deficient β-cells. Representative image of (E) TUNEL staining and (F) immunohistochemistry for Caspase-3 and cleaved Caspase-3 in Rip-β-Tead1$^{-/-}$ and control islet. DNase I treatment was used as a positive control for TUNEL staining. Scale bar – 50μm.

Figure 1

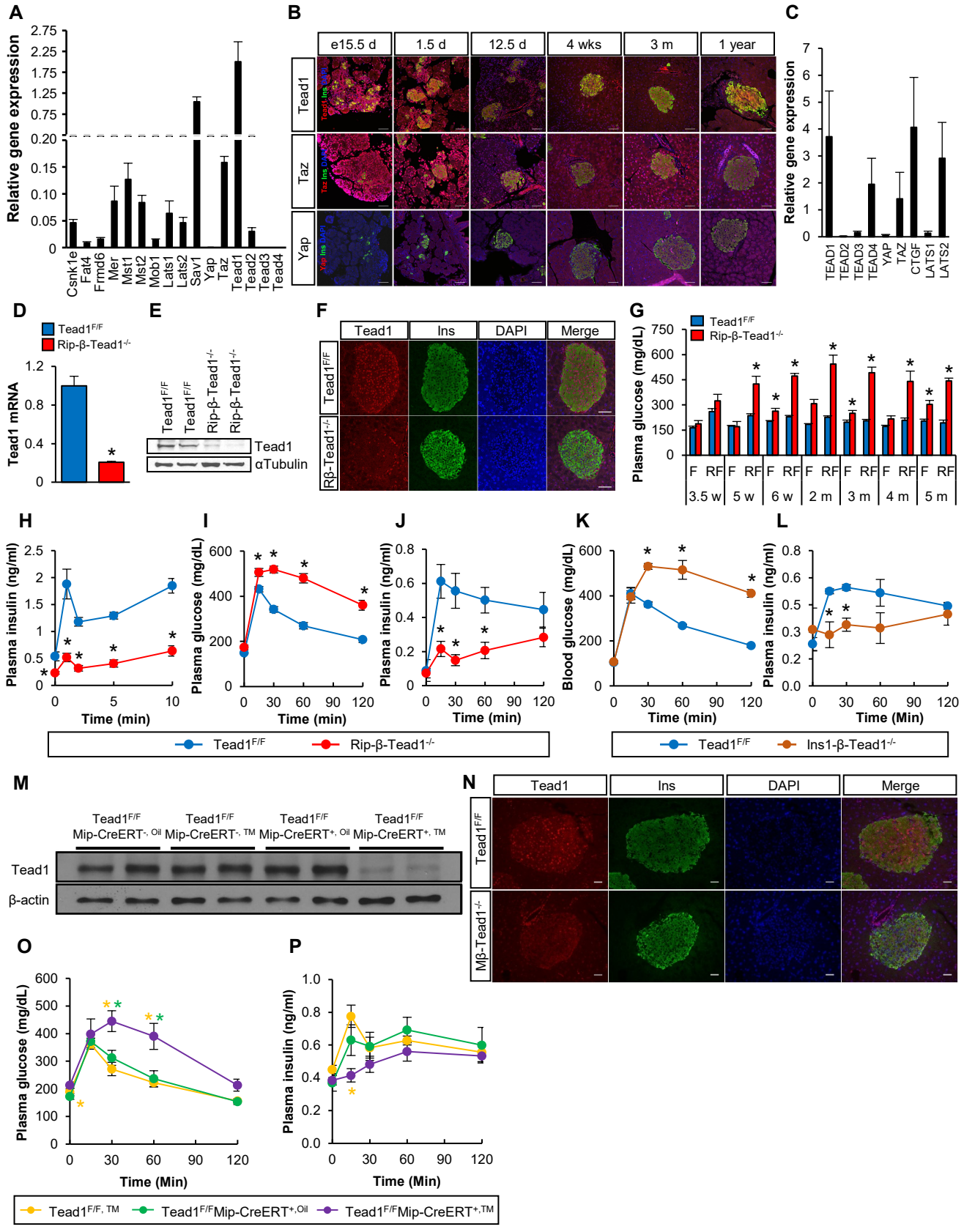

A
Relative gene expression
Csnk1e
Fat4
Frmd6
Mer
Mst1
Mst2
Mob1
Lats1
Lats2
Sav1
Yap
Taz
Tead1
Tead2
Tead3
Tead4
B
e15.5 d
1.5 d
12.5 d
4 wks
3 m
1 year
Tead1
Taz
Yap
C
Relative gene expression
TEAD1
TEAD2
TEAD3
TEAD4
YAP
TAZ
CTGF
LATS1
LATS2
D
Tead1F/F
Rip-β-Tead1-/-
Tead1 mRNA
E
Tead1F/F
Tead1F/F
Rip-β-Tead1-/-
Rip-β-Tead1-/-
Tead1
αTubulin
F
Tead1
Ins
DAPI
Merge
Tead1F/F
Rβ-Tead1-/-
G
Tead1F/F
Rip-β-Tead1-/-
Plasma glucose (mg/dL)
F
RF
3.5 w
5 w
6 w
2 m
3 m
4 m
5 m
H
Plasma insulin (ng/ml)
Time (min)
I
Plasma glucose (mg/dL)
Time (min)
J
Plasma insulin (ng/ml)
Time (min)
K
Blood glucose (mg/dL)
Time (min)
L
Plasma insulin (ng/ml)
Time (Min)
Tead1F/F
Rip-β-Tead1-/-
Tead1F/F
Ins1-β-Tead1-/-
M
Tead1F/F Mip-CreERT-, Oil
Tead1F/F Mip-CreERT-, TM
Tead1F/F Mip-CreERT+, Oil
Tead1F/F Mip-CreERT+, TM
Tead1
β-actin
N
Tead1
Ins
DAPI
Merge
Tead1F/F
Mβ-Tead1-/-
O
Plasma glucose (mg/dL)
Time (Min)
P
Plasma insulin (ng/ml)
Time (Min)
Tead1F/F, TM
Tead1F/F Mip-CreERT+,Oil
Tead1F/F Mip-CreERT+,TM

Figure 2

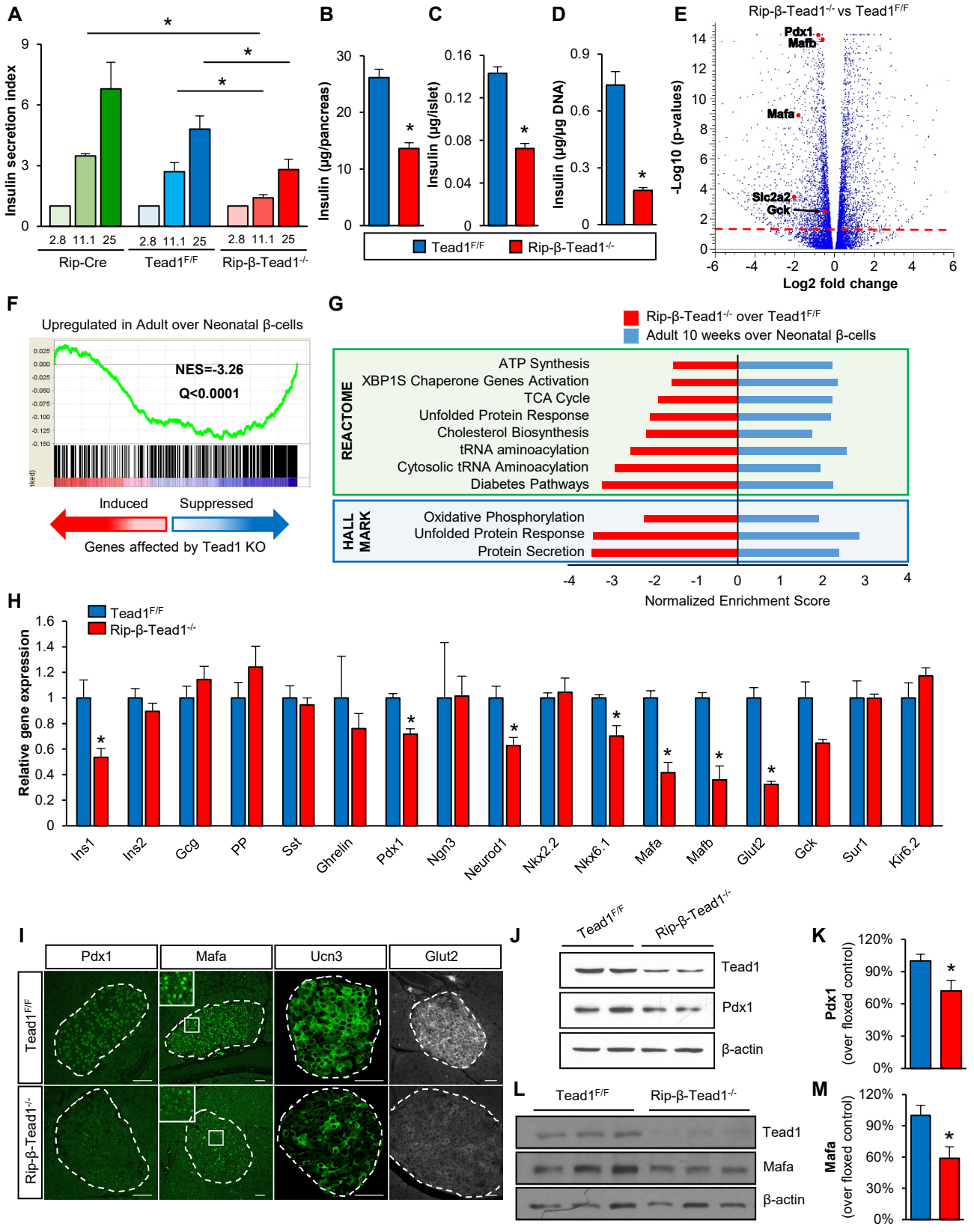
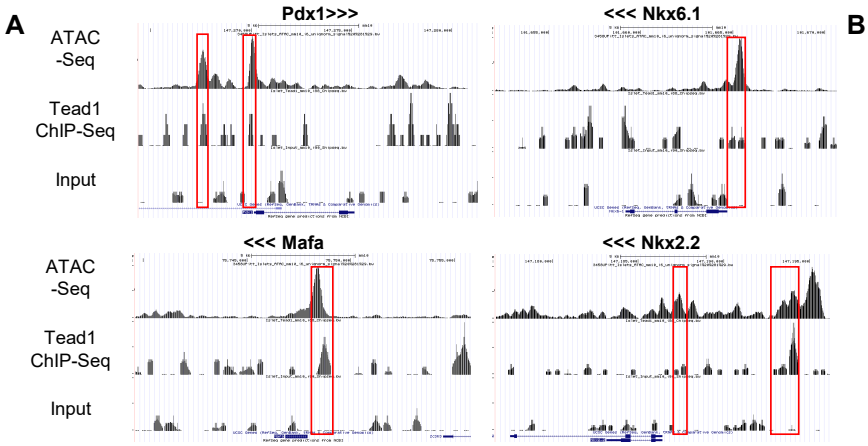
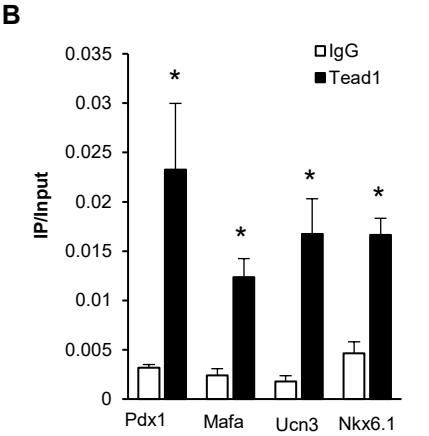
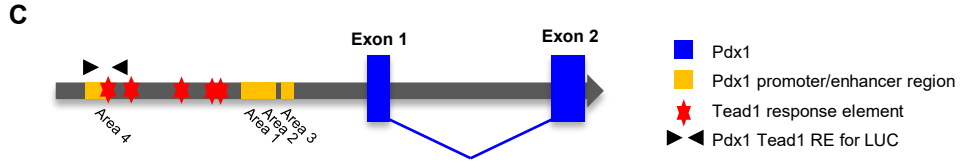
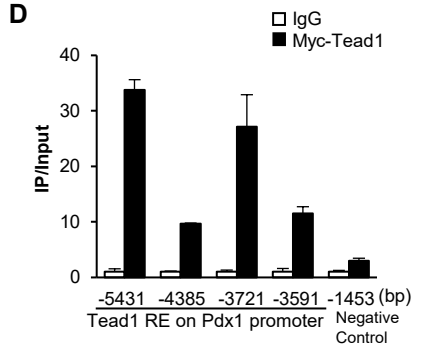

A
Insulin secretion index
2.8 11.1 25
Rip-Cre
Tead1F/F
Rip-β-Tead1-/-
B
Insulin (µg/pancreas)
C
Insulin (µg/islet)
D
Insulin (µg/µg DNA)
Tead1F/F
Rip-β-Tead1-/-
E
Rip-β-Tead1-/- vs Tead1F/F
Pdx1
Mafb
Mafa
Slc2a2
Gck
-Log10 (p-values)
Log2 fold change
F
Upregulated in Adult over Neonatal β-cells
NES=-3.26
Q<0.0001
Induced
Suppressed
Genes affected by Tead1 KO
G
Rip-β-Tead1-/- over Tead1F/F
Adult 10 weeks over Neonatal β-cells
REACTOME
ATP Synthesis
XBP1S Chaperone Genes Activation
TCA Cycle
Unfolded Protein Response
Cholesterol Biosynthesis
tRNA aminoacylation
Cytosolic tRNA Aminoacylation
Diabetes Pathways
HALL MARK
Oxidative Phosphorylation
Unfolded Protein Response
Protein Secretion
Normalized Enrichment Score
H
Tead1F/F
Rip-β-Tead1-/-
Relative gene expression
Ins1
Ins2
Gcg
PP
Sst
Ghrelin
Pdx1
Ngn3
Neurod1
Nkx2.2
Nkx6.1
Mafa
Mafb
Glut2
Gck
Sur1
Kir6.2
I
Pdx1
Mafa
Ucn3
Glut2
Tead1F/F
Rip-β-Tead1-/-
J
Tead1F/F
Rip-β-Tead1-/-
Tead1
Pdx1
β-actin
K
Pdx1
(over floxed control)
L
Tead1F/F
Rip-β-Tead1-/-
Tead1
Mafa
β-actin
M
Mafa
(over floxed control)

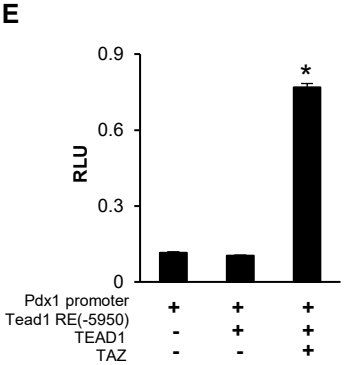
Figure 3

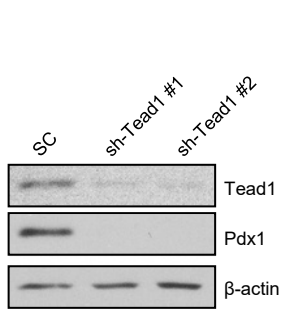

A
Pdx1>>>
<<< Nkx6.1
<<< Mafa
<<< Nkx2.2
ATAC -Seq
Tead1 ChIP-Seq
Input

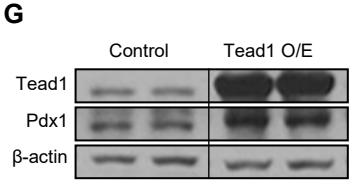

B
IgG
Tead1
IP/Input
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
Pdx1
Mafa
Ucn3
Nkx6.1

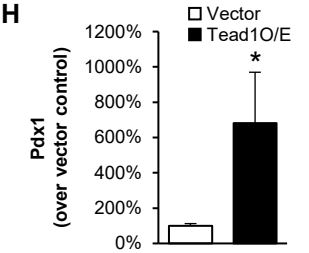

C
Exon 1
Exon 2
Area 4
Area 1
Area 2
Area 3
Pdx1
Pdx1 promoter/enhancer region
Tead1 response element
Pdx1 Tead1 RE for LUC

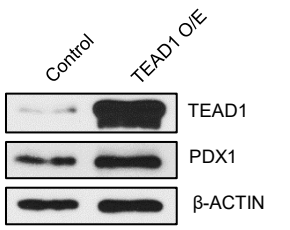

D
IgG
Myc-Tead1
IP/Input
40
30
20
10
0
-5431
-4385
-3721
-3591
-1453 (bp)
Tead1 RE on Pdx1 promoter
Negative Control

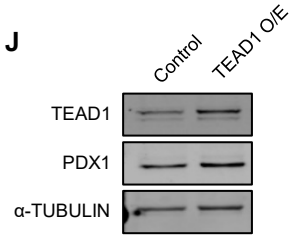

E
RLU
0.9
0.6
0.3
0
Pdx1 promoter
Tead1 RE(-5950)
TEAD1
TAZ


F
SC
sh-Tead1 #1
sh-Tead1 #2
Tead1
Pdx1
β-actin


G
Control
Tead1 O/E
Tead1
Pdx1
β-actin


H
Vector
Tead1O/E
Pdx1 (over vector control)
1200%
1000%
800%
600%
400%
200%
0%


I
Control
TEAD1 O/E
TEAD1
PDX1
β-ACTIN


J
Control
TEAD1 O/E
TEAD1
PDX1
α-TUBULIN

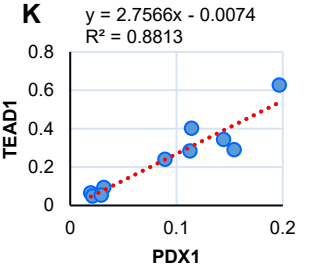

K
y = 2.7566x - 0.0074
R² = 0.8813
TEAD1
0.8
0.6
0.4
0.2
0
0
0.1
0.2
PDX1

Figure 4

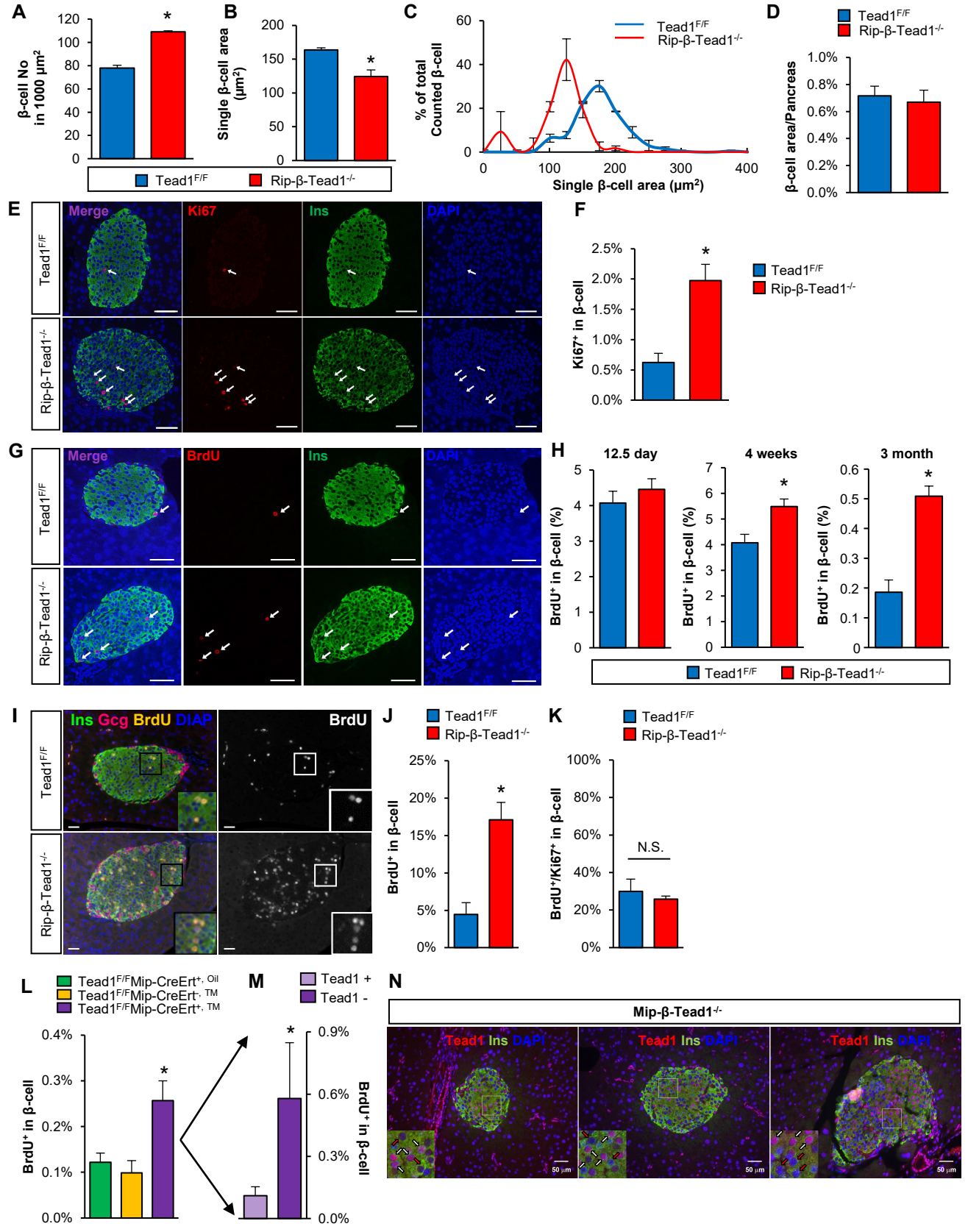
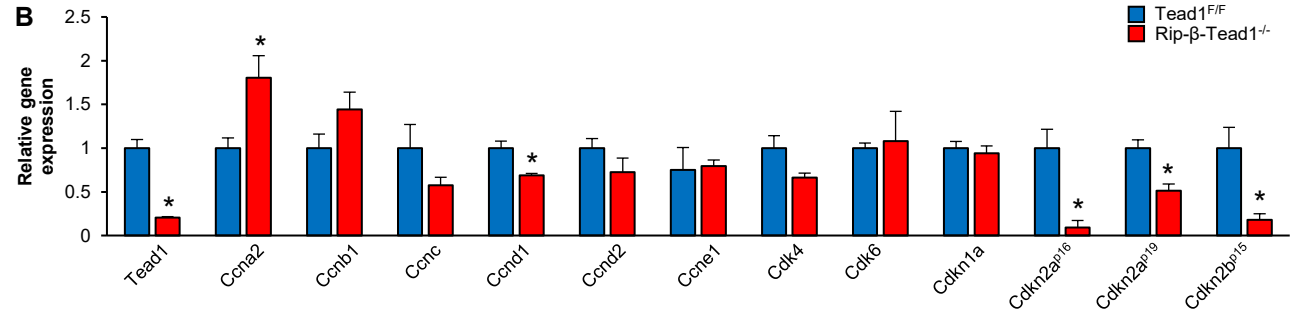
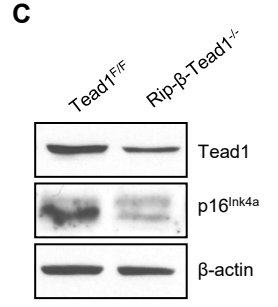

A
β-cell No in 1000 μm²
B
Single β-cell area (μm²)
C
% of total Counted β-cell
Single β-cell area (μm²)
Tead1F/F
Rip-β-Tead1-/-
D
β-cell area/Pancreas
E
Merge
Ki67
Ins
DAPI
F
Ki67+ in β-cell
G
BrdU
H
12.5 day
4 weeks
3 month
BrdU+ in β-cell (%)
I
Ins Gcg BrdU DIAP
BrdU
J
BrdU+ in β-cell
K
BrdU+/Ki67+ in β-cell
N.S.
L
Tead1F/FMip-CreErt+, Oil
Tead1F/FMip-CreErt+, TM
Tead1F/FMip-CreErt+, TM
BrdU+ in β-cell
M
Tead1 +
Tead1 -
BrdU+ in β-cell
N
Mip-β-Tead1-/-
Tead1 Ins DAPI
50 μm

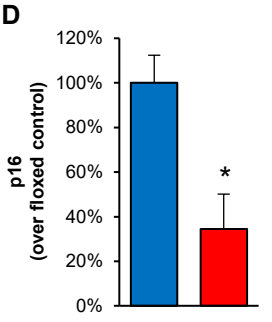
Figure 5

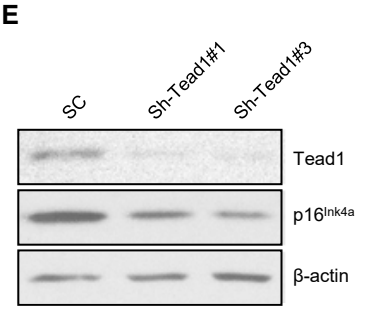
A

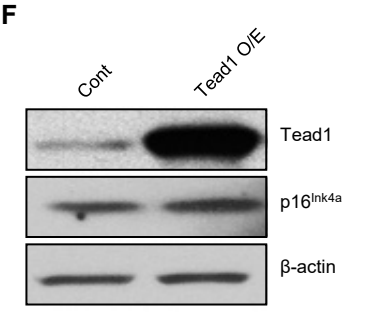
Category	Term	Count in selected genes	Count in total population	P-value
GO	regulation of DNA replication initiation	3	4	9.85E-06
GO	DNA replication checkpoint	2	2	0.000184
GO	negative regulation of DNA replication initiation	2	2	0.000184
GO	proteinaceous extracellular matrix	11	217	0.000199
GO	extracellular matrix	11	224	0.000262
GO	regulation of DNA replication	3	11	0.000379
GO	G1/S transition of mitotic cell cycle	4	27	0.000459

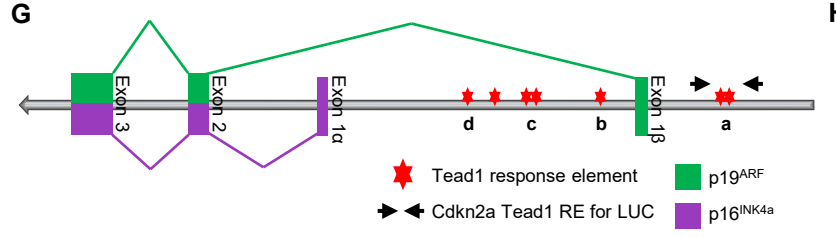


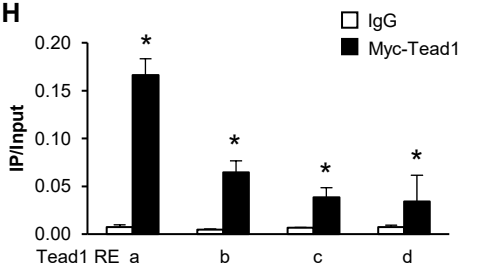


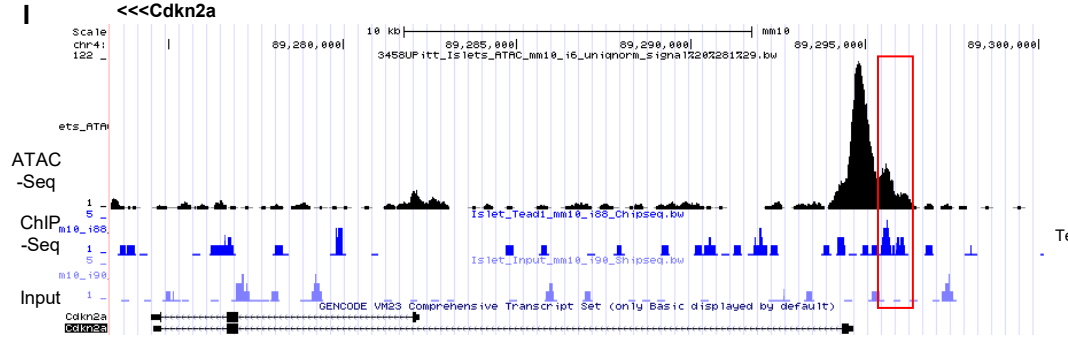












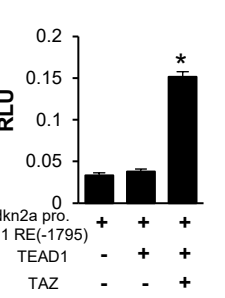


Figure 6

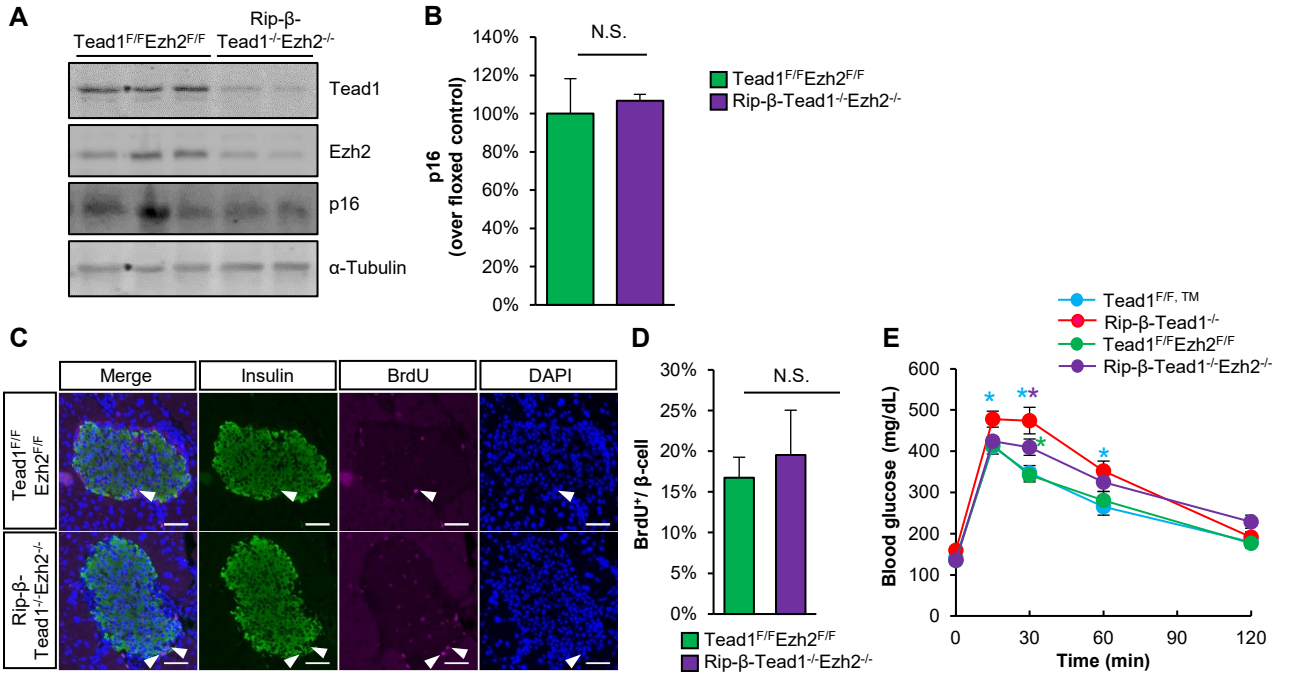
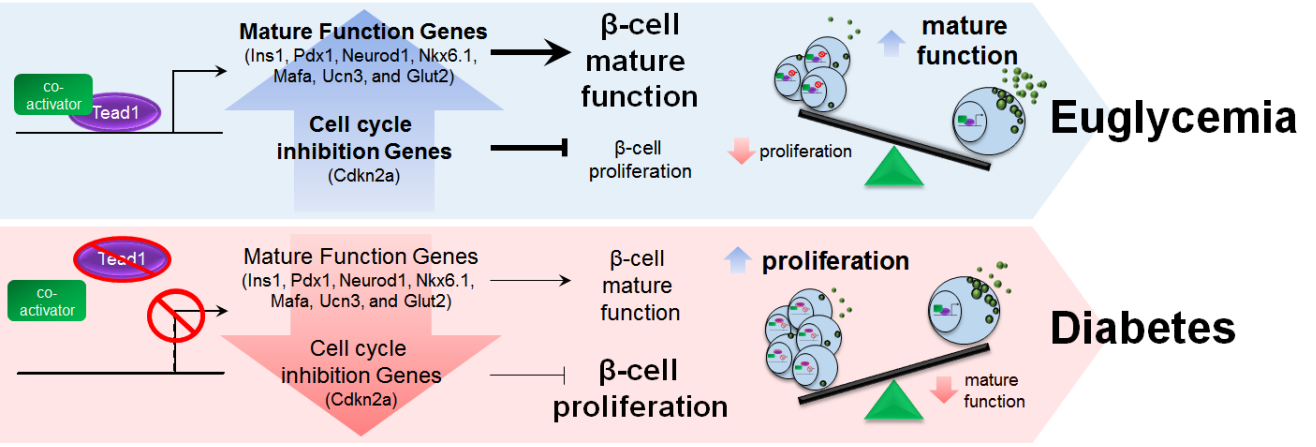

A
Tead1F/FEzh2F/F
Rip-β-Tead1-/-Ezh2-/-
Tead1
Ezh2
p16
α-Tubulin
B
N.S.
p16 (over floxed control)
140%
120%
100%
80%
60%
40%
20%
0%
Tead1F/FEzh2F/F
Rip-β-Tead1-/-Ezh2-/-
C
Merge
Insulin
BrdU
DAPI
Tead1F/F Ezh2F/F
Rip-β-Tead1-/-Ezh2-/-
D
N.S.
BrdU+/ β-cell
30%
25%
20%
15%
10%
5%
0%
Tead1F/FEzh2F/F
Rip-β-Tead1-/-Ezh2-/-
E
Tead1F/F, TM
Rip-β-Tead1-/-
Tead1F/FEzh2F/F
Rip-β-Tead1-/-Ezh2-/-
Blood glucose (mg/dL)
600
500
400
300
200
100
0
0
30
60
90
120
Time (min)

Figure 7


co-activator
Tead1
Mature Function Genes
(Ins1, Pdx1, Neurod1, Nkx6.1, Mafa, Ucn3, and Glut2)
Cell cycle inhibition Genes
(Cdkn2a)
β-cell mature function
β-cell proliferation
mature function
proliferation
Euglycemia
Tead1
co-activator
Mature Function Genes
(Ins1, Pdx1, Neurod1, Nkx6.1, Mafa, Ucn3, and Glut2)
Cell cycle inhibition Genes
(Cdkn2a)
β-cell mature function
β-cell proliferation
proliferation
mature function
Diabetes