



Beta-cell dysfunction induced by non-cytotoxic concentrations of Interleukin-1 β is associated with changes in expression of beta-cell maturity genes and associated histone modifications

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ABSTRACT

Decreased insulin secretory capacity in Type 2 diabetes mellitus is associated with beta-cell dedifferentiation and inflammation. We hypothesize that prolonged exposure of beta-cells to low concentrations of IL-1 β induce beta-cell dedifferentiation characterized by impaired glucose-stimulated insulin secretion, reduced expression of key beta-cell genes and changes in histone modifications at gene loci known to affect beta-cell function.

Ten days exposure to IL-1 β at non-cytotoxic concentrations reduced insulin secretion and beta-cell proliferation and decreased expression of key beta-cell identity genes, including *MafA* and *Ucn3* and decreased H3K27ac at the gene loci, suggesting that inflammatory cytokines directly affects the epigenome. Following removal of IL-1 β , beta-cell function was normalized and mRNA expression of beta-cell identity genes, such as insulin and *Ucn3* returned to pre-stimulation levels.

Our findings indicate that prolonged exposure to low concentrations of IL-1 β induces epigenetic changes associated with loss of beta-cell identity as observed in Type 2 diabetes.

1. Introduction

Pro-inflammatory cytokines are considered important mediators of progressive beta-cell destruction and dysfunction in both Type 1 diabetes (T1D) and Type 2 diabetes (T2D) (Donath and Shoelson, 2011; Mathis et al., 2001). However, the mechanisms involved in the development of beta-cell dysfunction differ between T1D and T2D, as reflected in the vastly distinct degrees of beta-cell failure observed in each disease. In T1D beta-cell mass and function are almost absent at diagnosis, due to the elevated concentration of inflammatory cytokines and cytotoxic T-cells (Eizirik et al., 2009; Mathis et al., 2001). In contrast, in T2D the loss of beta-cell function and mass occurs more slowly, as the concentrations of circulating and intra-islets cytokines are believed to be lower (Cnop et al., 2005). Both metabolic stress and low grade inflammation as observed in obesity, are known to have deleterious effects on beta-cell function and have been proposed as important components in the development of beta-cell dysfunction in T2D (Donath

and Shoelson, 2011; Wellen and Hotamisligil, 2005). The pro-inflammatory cytokine interleukin-1 β (IL-1 β) is a contributor of beta-cell dysfunction in T2D (Donath and Shoelson, 2011) but the importance and mechanism of action of IL-1 β in the pathogenesis is debated (Cnop et al., 2005).

Although the effects of IL-1 β on beta-cells have been studied extensively *in vitro*, most studies have used concentrations of IL-1 β that activate apoptotic pathways, resulting in beta-cell death (Mandrup-Poulsen et al., 1986; Palmer et al., 1989). However, IL-1 β effects are dependent on concentration and exposure time: high concentrations and long incubation periods cause inhibition of insulin secretion and beta-cell death (Mandrup-Poulsen et al., 1986), whereas low concentrations and short exposure periods stimulate insulin secretion and beta-cell proliferation (Dror et al., 2017; Dula et al., 2010; Palmer et al., 1989; Spinass et al., 1986). The effects of chronic exposure to low IL-1 β concentrations, which might better resemble the low-grade inflammation observed in T2D, have not been previously studied.

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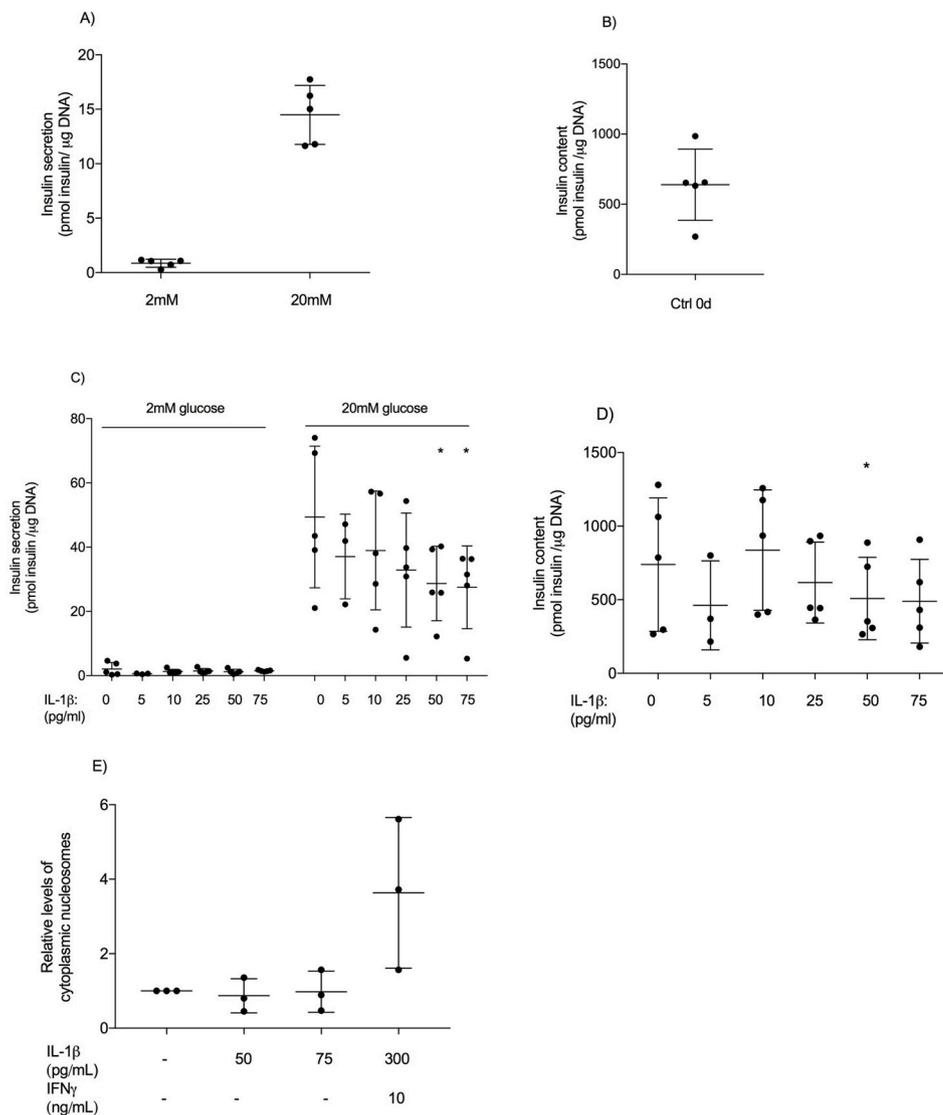


Fig. 1. Low doses of IL-1 β inhibit glucose-induced insulin secretion in mouse islets. (A) Insulin secretion of freshly isolated mouse islets was measured by static batch incubations in response to 2 mmol/l glucose or 20 mmol/l glucose and normalized to DNA content. (B) Total insulin content of freshly isolated islets was measured post-assay. (C) Mouse islets were exposed to increasing doses of IL-1 β (5–75 pg/ml) or non-exposed (Ctrl) for 10 days. Insulin secretion was measured by static batch incubations in response to 2 mmol/l glucose or 20 mmol/l glucose and normalized to DNA content. (D) Total insulin content was measured post-assay. Data are shown as means. * $p < 0.05$ 2-sided t -test vs. 20 mM glucose control (E) Apoptosis was determined in lysates from mouse islets exposed to IL-1 β (50 or 75 pg/ml) for 10 days. Two day stimulation with a mixture of 300 pg/ml IL-1 β + 10 ng/ml IFN- γ functioned as positive control for induction of apoptosis, $n = 3$.

The loss of beta-cell identity, rather than apoptosis, has been suggested to be the main contributing factor to the reduced functional beta-cell mass observed in T2D (Cinti et al., 2015; Guo et al., 2013; Ishida et al., 2017; Jonas et al., 1999; Kjørholt et al., 2005; Talchai et al., 2012; Taylor et al., 2013). A decrease in expression of key beta-cell transcription factors has been identified to be associated with the reduced glucose-stimulated insulin secretion (Cinti et al., 2015; Talchai et al., 2012). The exact mechanisms and causative stimuli behind beta-cell identity loss or dedifferentiation as it has also been termed are still not fully elucidated (Brereton et al., 2014; Guo et al., 2013; Jonas et al., 1999; Nordmann et al., 2017; Wang et al., 2014). Beta-cell dysfunction has been shown to be largely reversible upon the stress cessation both *in vivo* and *in vitro* (Blum et al., 2014; Brereton et al., 2014; Ishida et al., 2017; Kjørholt et al., 2005; Wang et al., 2014). Since the T2D beta-cells appear to remain viable, it might be possible to recover their identity and function by re-differentiating the dormant cells. Characterizing the stimuli that could induce such reversion is crucial for the development of new strategies in diabetes therapy (Bonner-Weir and Weir, 2005).

Together, these observations suggest that beta-cell dedifferentiation drives beta-cell failure during the pathogenesis of T2D (Bensellam et al., 2018; Cinti et al., 2015; Talchai et al., 2012).

The underlying molecular mechanisms of beta-cell identity loss are still not elucidated, but it might occur due to a dysregulation of several members of the transcriptional network responsible for maintaining

beta-cell identity, following inflammation and metabolic stress. Since pro-inflammatory cytokines play an important role in diabetes development, it is relevant to address the question of whether beta-cell dysfunction and identity loss are regulated by long term exposure to pro-inflammatory cytokines like IL-1 β . Here we analyze the effect of prolonged exposure of IL-1 β in the function, gene expression and epigenome *in vitro* culture of isolated islets.

2. Material and methods

2.1. Mouse islet isolation and culture

Pancreatic islets from 12 week-old male C57BL/6NRj mice (Janvier, Le Genest-Saint-Isle, France) or 12 week-old male C57BL/6NTAC mice (Taconic, Lille Skensved, Denmark) were isolated by perfusion via the bile duct of the pancreas with Liberase (Roche, Hvidovre, Denmark) as previously described (Bruun et al., 2014). Experiments involving animals were approved by the local ethics committee and animals were housed according to the Principles of Laboratory Care.

After isolation islets were cultured for 1 day in RPMI 1640 medium (Lonza) supplemented with 10% fetal bovine serum (FBS; Biosera) and 1% penicillin/streptomycin (100 U/ml penicillin, 100 μ g/ml streptomycin) (P/S; Gibco, Life Technologies) prior to treatment. For further culture RPMI 1640 medium supplemented with 2% human serum (HS;

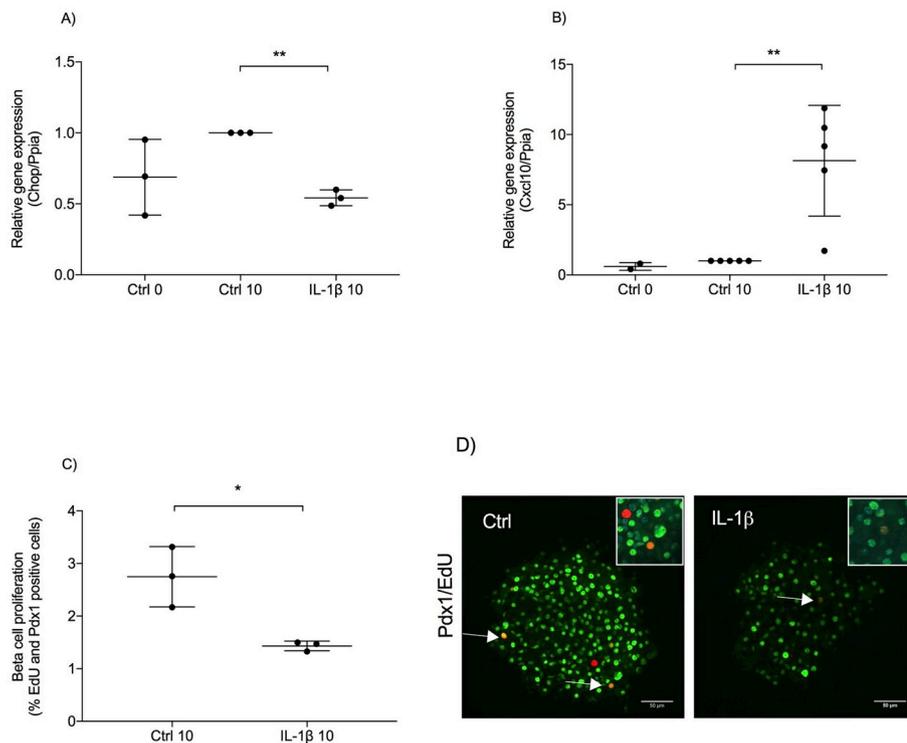


Fig. 2. Low dose of IL-1 β reduces mouse islet proliferation. Mouse islets were exposed to 50 pg/ml of IL-1 β or non-exposed (Ctrl) for 10 days. Relative *Chop* mRNA expression (A) and *Cxcl10* mRNA expression (B) were normalized to *Ppia* expression and Ctrl 10 data was set to 1. Data are mean of $n = 3-5$. * $p < 0.05$, ** $p < 0.01$ 2sided *t*-test vs. control 10 days (C) Beta-cell proliferation was examined in whole mouse islets. Proliferation was determined by immunocytochemical staining for Pdx-1 and EdU. Results are shown as percentage proliferating beta-cells. Data are shown as means for $n = 3$. * $p < 0.05$ 2sided *t*-test vs. control 10 days. (D) Immunocytochemical staining of whole mouse islets. Cells were stained for Pdx-1 (green) and EdU (red). Data shown is representative. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

BioWhittaker, Lonza) and 1% P/S was used.

For immunostaining, islets were dispersed into single cells using 0.2% trypsin (Gibco, Thermo Scientific, Waltham, MA USA) in HBSS. Dispersed islets cells (aprox. 30.000 cells per coverslip) were plated on coverslips coated with extracellular matrix (ECM; Biological Industries, Kibbutz Beit-Haemek, Israel).

2.2. Gene expression analysis

Intact mouse islets were cultured as described and exposed to 50 pg/ml IL-1 β or left untreated for 10 days. Islets were lysed in Trizol and mRNA was extracted using Direct-zol RNA-mini prep kit according to the manufacturer's protocol (Zymo Research). cDNA was synthesized using the qScript cDNA Super mix kit (Quanta Biosciences). Quantitative expression levels of genes of interest were evaluated by the use of TaqMan Gene Expression probes and performed on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). Each sample was determined in triplicate and expression was normalized to the internal control, *Ppia*.

2.3. Glucose-stimulated insulin secretion

Intact mouse islets were cultured as described and exposed to 50 pg/ml IL-1 β or left untreated for 10 or 20 days. GSIS was performed (25 islets per condition) by pre-starvation for 1.5 h in the absence of glucose followed by 30 min of incubation in the presence of 2 mM D-glucose and then 30 min of incubation in 20 mM D-glucose in Krebs-Ringer HEPES buffer. The buffer was then collected from each experimental group and insulin content was determined using an in-house insulin ELISA. Results were corrected for DNA content using Quant-IT PicoGreen dsDNA Reagent and Kit (Invitrogen).

2.4. Cell Death Detection assay

Apoptotic cell death was measured by the detection of DNA histone complexes released from the nucleus to the cytosol of cells using Cell Death Detection ELISA PLUS (Roche) as described by the manufacturer.

Intact mouse islets were cultured as described in the presence or absence of 50 pg/ml IL-1 β for 10 days. As a positive control for apoptosis we exposed intact mouse islets to 300 pg/ml IL-1 β + 10 ng/ml IFN- γ (R&D System, Minneapolis, MN, USA) for 48 h. To adjust for differences in cell number among different conditions, islet lysates were sonicated and DNA was quantified by Quant-IT PicoGreen dsDNA Reagent and Kit.

2.5. Proliferation

Beta cell proliferation was analyzed in intact mouse islets cultured on ECM in the absence or presence of 50 pg/ml IL-1 β for 10 or 20 days by the addition of 10 μ M 5-ethynyl-2'-deoxyuridine (EdU; Invitrogen, Paisley, UK) 24 h prior fixation. Proliferating beta-cells were detected by EdU incorporation with Click-iT EdU Proliferation Assay (Invitrogen, Thermo Scientific), followed by staining of pancreatic duodenal homeobox 1 (Beta Cell Biology Consortium (goat-anti Pdx1; 1:5000) and DNA (Hoescht 33342)). Islet cells positive for both Pdx1 and EdU were counted as proliferating beta-cells. Whole islets were examined by capturing z-stack images using Zeiss Axio Observer Z1 with spinning disk and counted using the ZEN software. A minimum of 700 cells were examined per experiments in each condition. For representation, a median filter with binding 2x2 pixels was applied (Image J).

2.6. Immunostaining

Dispersed islets were cultured on ECM as described and stained for insulin MafA, Ucn3, Pdx1, FoxO1 and Hoechst3342. Images were acquired using Zeiss LSM710 confocal microscope. A minimum of 20 cells were examined per experiment in each condition. Integrated Density was quantified using Image J.

2.7. ChIP seq

Mouse islets were cultured as described in the presence or absence of 50 pg/ml IL-1 β . Islets were cross-linked with formaldehyde, quenched with glycine, lysed in 1% SDS and sheared with a BioRuptor

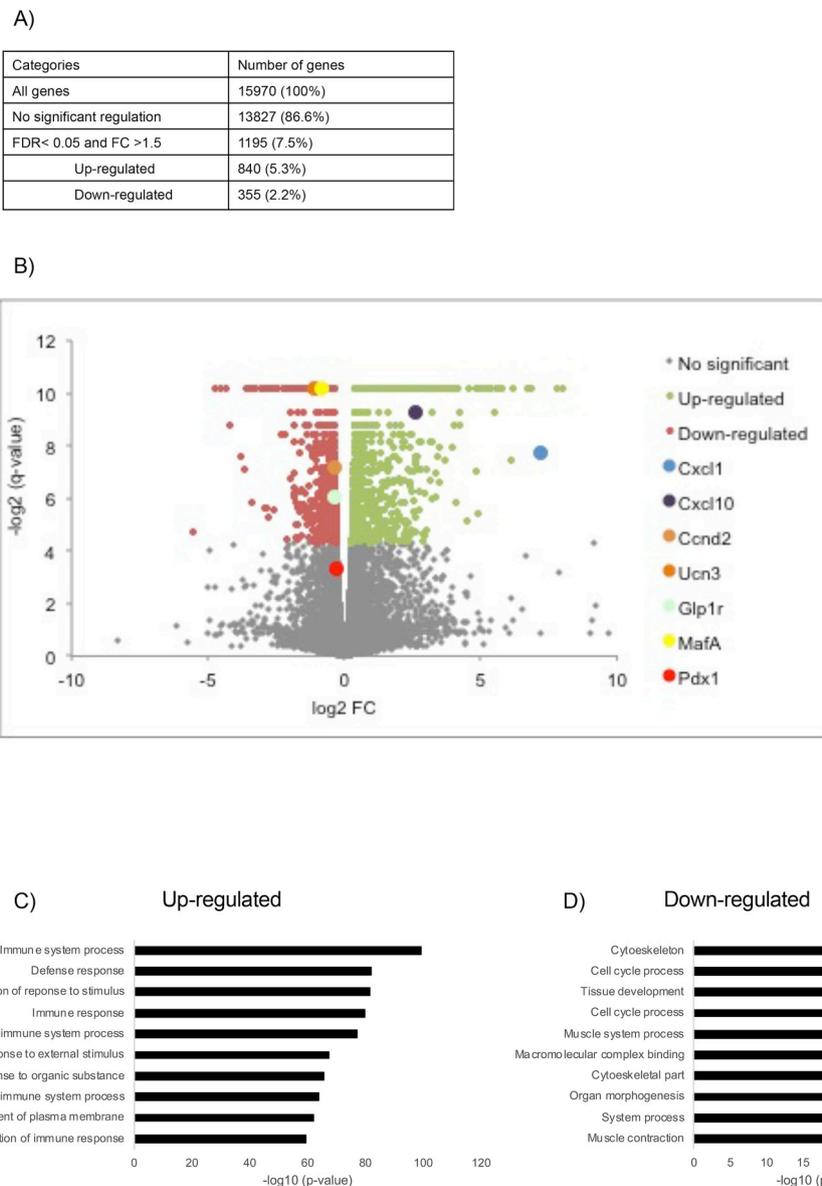


Fig. 3. IL-1 β down-regulates the expression of genes involved in the regulation of the cell cycle and beta-cell markers. Mouse islets were exposed to 50 pg/ml of IL-1 β for 10 days. (A) Table showing summary of genes regulated by IL-1 β . (B) X-axis: log₂ gene expression fold change and Y-axis: log₂ q-value. Grey not significantly regulated genes, green FDR < 0.05 up-regulated genes and red FDR < 0.05 down-regulated genes (n = 3). (C and D) Gene ontology performed by GSEA of IL-1 β up-regulated and down-regulated genes, respectively. . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Sonicator (Diagenode). Chromatin immunoprecipitation was performed according to the manufacturer's instructions (ChIP-IT High Sensitivity, cat# 53040, Active Motif) using 10–30 μ g of sonicated chromatin. ChIP-seq libraries were prepared using KAPA DNA Library Preparation Kits for Illumina® (Kapa Biosystems) and the library sequencing was performed on the HiSeq 400 System (Illumina®). All ChIP-seq data was mapped to the UCSC mouse genome NCBI37/mm9. ChIP-seq analysis was performed by HOMER.

2.8. RNA-seq

Intact mouse islets were cultured as described and exposed to 50 pg/ml IL-1 β or left untreated for 10 days. RNA was isolated from islets using the RNeasy® Micro Kit (Qiagen) according to the manufacturer instructions. For each condition 500 islets were collected for RNA extraction. For RNA sequencing (RNA-seq), stranded, single-end sequencing libraries were constructed from 35 ng of isolated RNA using the

TruSeq® Stranded mRNA Library Prep Kit (Illumina®) and library sequencing was performed on a HiSeq 4000 System (Illumina®). Sequence files were mapped to the UCSC mouse genome NCBI37/mm9.

2.9. Statistical analysis

Results are given as mean. Comparison vs. the respective control group was made by Student's paired *t*-test. P-values less than 0.05 were considered statistically significant. Statistic on RT-qPCR data was carried out using log-transformed data.

3. Results

3.1. IL-1 β effects on function and proliferation of primary mouse beta-cells

To examine the effect of long-term low dose IL-1 β on beta-cell function and proliferation we established a protocol for *in vitro* culture

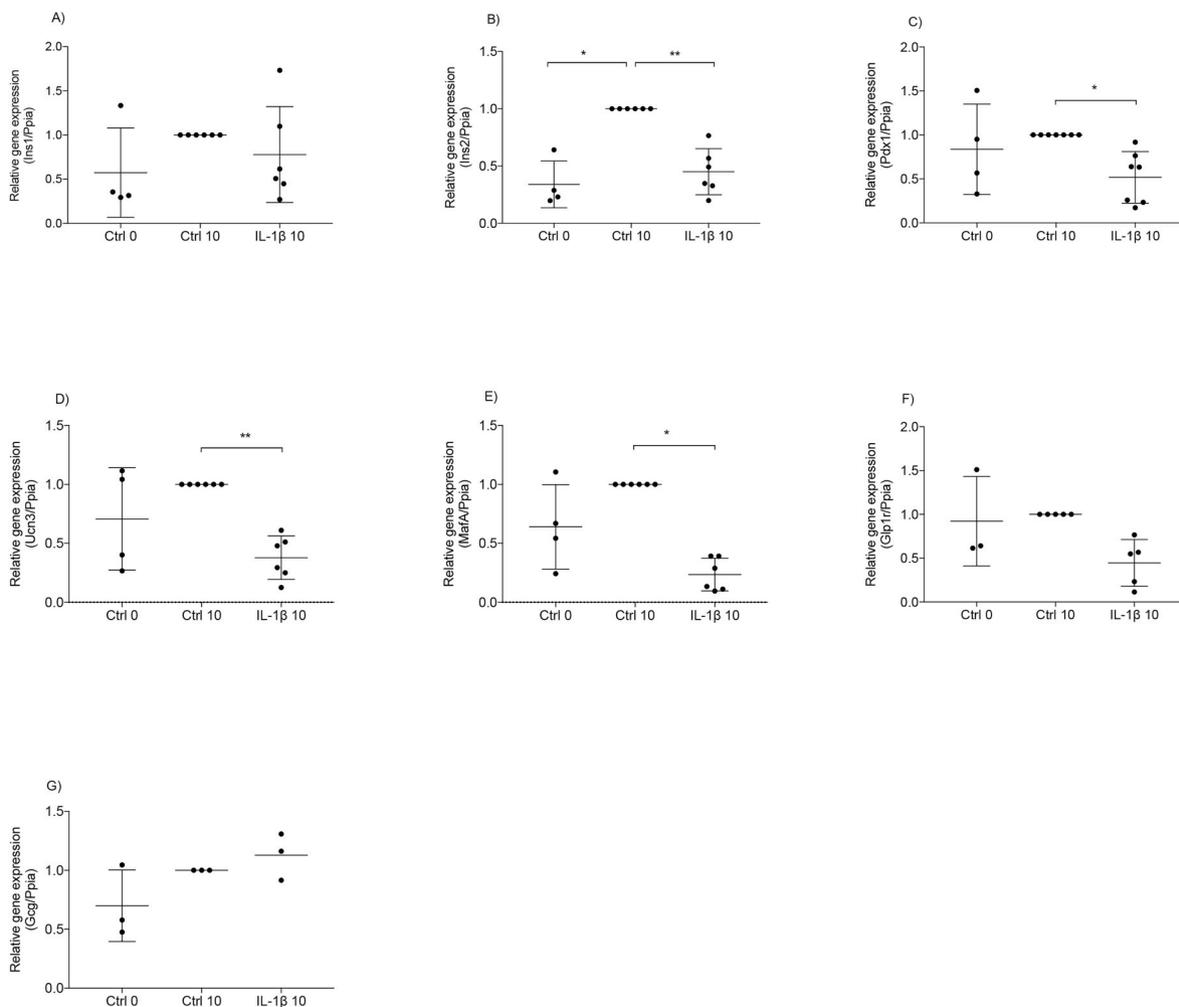


Fig. 4. Mature beta-cell gene expression is regulated by IL-1 β . Mouse islets were exposed to 50 pg/ml of IL-1 β or non-exposed for 0 days (Ctrl 0) or 10 days (Ctrl 10). Relative mRNA expression of (A) *Ins1* (B) *Ins2* (C) *Pdx1* (d) *Ucn3* (E) *MafA* (F) *Glp1r* and (G) *Gcg* were analyzed using real-time PCR. Expression levels are normalized to *Ppia* and Ctrl 10 data was set to 1. Data are shown as means (n = 3–7). *p < 0.05, **p < 0.01 2sided t-test vs. control 10 days.

of isolated mouse islets for up to 10 days. Glucose stimulated insulin secretion (GSIS) as well as insulin content was analyzed using freshly isolated islets for comparison (Fig. 1 A and B). GSIS was not affected by 10 days of culture under control conditions, while exposure of islets to 50 or 75 pg/ml IL-1 β for 10 days resulted in a significant reduction of GSIS (Fig. 1C). IL-1 β stimulation did not affect total islet insulin content (Fig. 1D).

Low dose IL-1 β did not induce apoptosis when measured by the detection of DNA histone complexes released from the nucleus. As a positive control for apoptosis islets were exposed to 300 pg/ml IL-1 β and 10 ng/ml IFN- γ (Fig. 1E). To investigate whether 50 pg/ml IL-1 β induces ER stress, we analyzed C/EBP homologous protein (*Chop*) mRNA expression following IL-1 β exposure. No increase in *Chop* mRNA expression in response to 50 pg/ml IL-1 β was observed, rather a significant reduction of *Chop* mRNA resulted from IL-1 β stimulation (Fig. 2A). IL-1 β significantly increased *Cxcl10* mRNA expression (Fig. 2B) indicating that the 50 pg/ml IL-1 β stimulates expression of IL-1 β responsive genes, without inducing ER stress or pro-apoptotic signaling in mouse islets. The 50 pg/ml IL-1 β dose was determined as the lowest effective dose and used in the subsequent experiments.

Proliferation in response to 10 days of IL-1 β stimulation was determined based on EdU incorporation in Pdx-1 expressing beta-cells. Under basal conditions, beta-cell proliferation rate was 2.7%. Exposure to IL-1 β for 10 days significantly reduced beta-cell proliferation rate to 1.4% (Fig. 2C and D). IL-1 β exposure for 10 days results in beta-cell

dysfunction and inhibition of proliferation without inducing apoptosis or ER stress marker expression.

3.2. Regulation of gene expression by IL-1 β in mouse islets

We hypothesized that beta-cell dysfunction and inhibition of proliferation is associated with changes in the global expression of genes induced by IL-1 β .

To investigate the global expression of islet mRNA we performed RNA-seq analysis on RNA isolated from control or IL-1 β treated mouse islets. Analysis of gene expression profiles revealed significant differences in the expression of 1195 genes (FDR < 0.05 and FC > 1.5) of which 840 were significantly up-regulated and 355 down-regulated by IL-1 β (Fig. 3A). The expression of *MafA* and *Ucn3* was significantly reduced, as was the expression of other genes associated with mature murine beta-cell function, like *Slc2a2* (*Glut2*) and *Slc30a8* (Zn²⁺ transporter 8) consistent with the real-time qPCR (Fig. 3B and suppl. 1). Expression of genes involved in the regulation of cell cycle such as *Mki67*, *Ccnd2* and *FoxM1* was also significantly reduced in accordance with the inhibition of beta-cell proliferation by IL-1 β (Fig. 3B and suppl. 1).

To identify biological processes affected by IL-1 β exposure, we performed a Gene Ontology analysis of the differentially expressed genes using Gene Set Enrichment Analysis (GSEA). IL-1 β up-regulated genes were enriched for pathways involved in immune responses, while

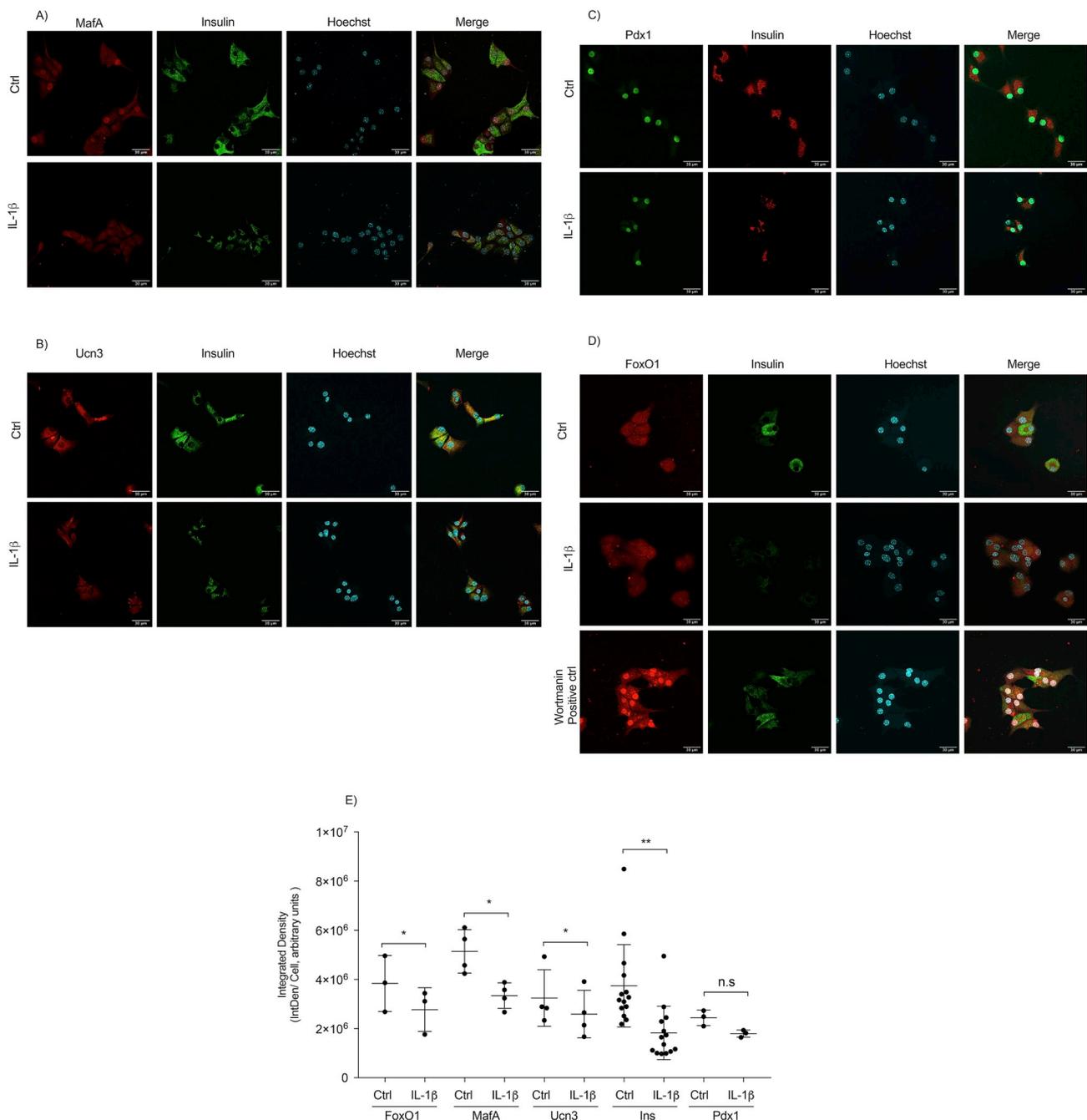


Fig. 5. Mature beta-cell protein expression is regulated by IL-1 β . Dispersed islets cultures were exposed to 50 pg/ml IL-1 β (IL-1 β) or non-exposed (Ctrl 10) for 10 days. MafA, FoxO1, Ucn-3 and Pdx1 staining were examined by immunocytochemical co-staining with insulin and Hoechst33342. (A) MafA staining. (B) Ucn3 staining. (C) Pdx1 staining. (D) FoxO1 staining. (E) Quantification of MafA, Ucn3, Pdx1 FoxO1 and insulin staining. Data are shown as means of the Integrated Density per cell, n = 3 FoxO1 and Pdx1; n = 4 MafA and Ucn3 and n = 14 Insulin. *p < 0.05, **p < 0.01 2sided t-test vs. control 10 days. Quantification was performed using Image J (Fiji).

the down-regulated genes were associated with functional categories of cell proliferation (Fig. 3C and D). (List of categories see Suppl.2 and 3).

In order to investigate and confirm changes in selected beta cell relevant genes we performed qRT-PCR analysis on independent samples (Fig. 4A–G). None of the analyzed genes were reduced by 10 days culture under control conditions, rather a significant increase in *Ins2* mRNA was observed (Fig. 4B), indicating that mouse beta-cells do not dedifferentiate spontaneously in our culture conditions. *Ins2*, *Pdx1*, *Ucn3*, *MafA* and *Glp1r* mRNA expression was significantly reduced by IL-1 β exposure (Fig. 4B–C–D–E–F). No significant differences were observed in the expression of *Ins1* or *Gcg* (Fig. 4A and G).

In agreement with the mRNA expression, MafA, Ucn3 and insulin

staining was significantly reduced (Fig. 5A–B–E). Although modestly reduced in IL-1 β exposed cells, the change in Pdx1 staining was not statistically significant (Fig. 5C and E). Although previously described in beta-cell dedifferentiation (Talchai et al., 2012), no nuclear-cytoplasmic shuttling of FoxO1 was observed in response to IL-1 β , but a significant reduction in total FoxO1 protein was observed after IL-1 β exposure (Fig. 5D and E). Cells stimulated with 500 nM wortmanin for 30 min as positive control showed FoxO1 translocation from the cytoplasm to nucleus (Fig. 5D).

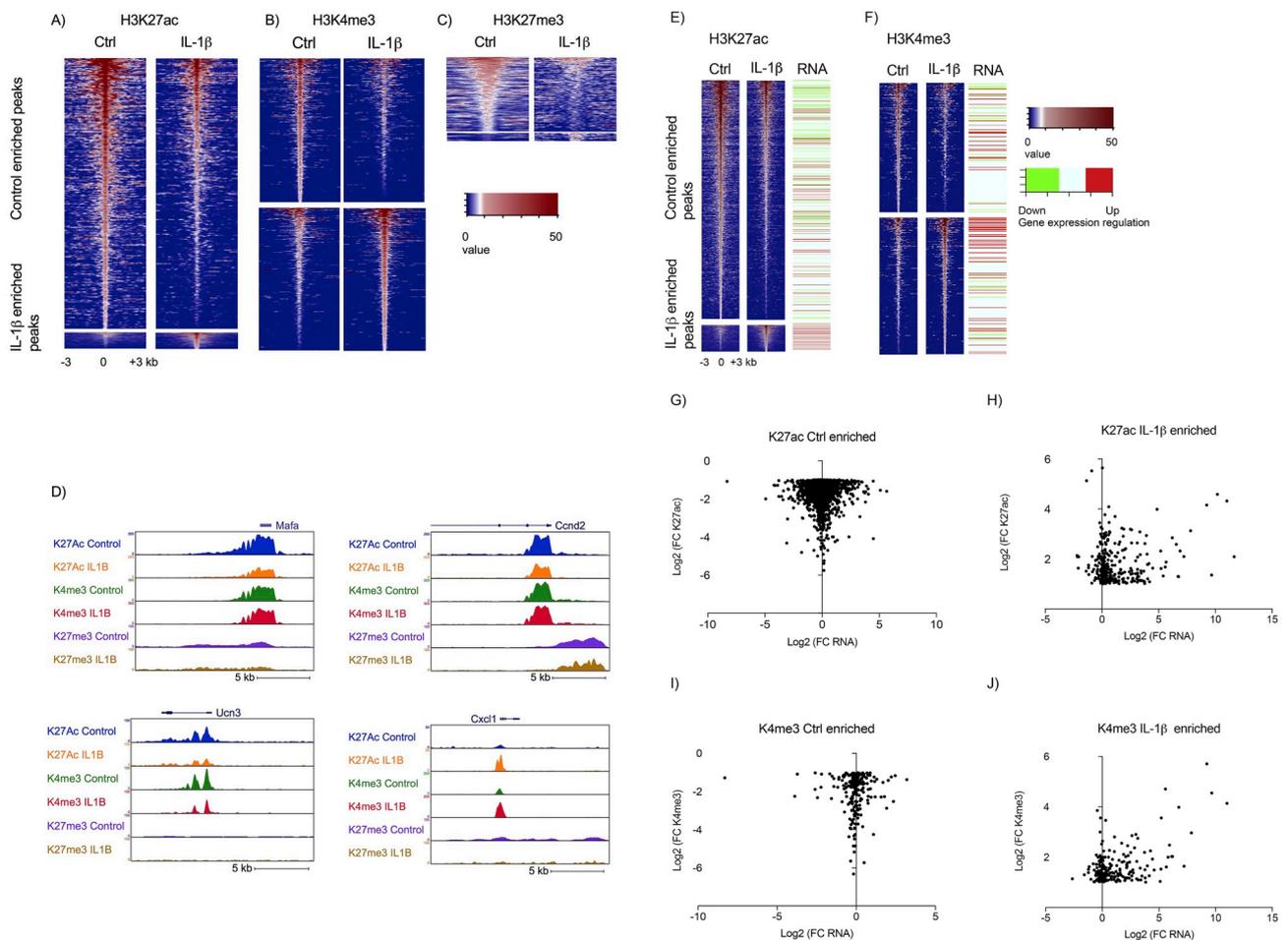


Fig. 6. Low dose of IL-1 β leads to a reduction of the active chromatin marker H3K27ac. Heat map of (A) H3K27 acetylation, (B) H3K4 tri-methylation and (C) H3K27 tri-methylation from Control (left) and IL-1 β treated (right) from -3 kb to $+3$ kb surrounding the center of the peak. Each line represents a single histone 3 binding site (peak) and the color scale indicates the H3K27ac, H3K4me3 and H3K27me3 ChIP-seq tags per bp, respectively. Upper panel: control enriched peaks (down-regulated by IL-1 β). Lower panel: IL-1 β enriched peaks (up-regulated by IL-1 β) ($n = 2$). (D) Genome browser snapshot of ChIP-seq for selected genes for the 3 histone modifications (H3K27ac, H3K4me3, and H3K27me3)

Heat map of (E) H3K27 acetylation and associated RNA-seq genes and (F) H3K4 tri-methylation and associated RNA-seq genes (FRD < 0.05). Control (left) and IL-1 β treated (middle) from -3 kb to $+3$ kb surrounding the center of the peaks. RNA expression (IL-1 β /Control) (right). Each line represents a single gene associated with the indicated histone modification peak and the color scale indicates the H3K27ac and H3K4me3 signal per million reads. RNA expression color scale indicates up-regulated (red), down-regulated (green) and not significant or not RNA association (light blue). Scale for H3K27ac ChIP seq is 0.001cm/gene and for H3K4me3 0.01cm/gene. Non-parametrical Spearman correlation between mRNA expression and histone modification fold-change: (G) H3K27ac Control enriched peaks associated with TSS (IL-1 β down-regulated) (H) H3K27ac IL-1 β enriched (IL-1 β up-regulated) (I) H3K4me3 Control enriched (IL-1 β down-regulated) and (J) H3K4me3 IL-1 β enriched (IL-1 β up-regulated). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. Regulation of histone modifications by IL-1 β in mouse islets

To investigate whether prolonged IL-1 β stimulation could also lead to changes in histone modifications and thus induce lasting changes in the beta-cell differentiation, ChIP-seq was carried out on chromatin preparations isolated from islets cultured in the absence or presence of 50 pg/ml IL-1 β for 10 days. For H3K27 acetylation, 10626 differential peaks (FC > 2) (40.17%) of shared peaks between control and IL-1 β were found to be down-regulated by IL-1 β whereas 621 (2.35% of shared peaks) were enriched in the IL-1 β treated groups, showing that IL-1 β primarily reduced H3K27ac (Fig. 6A). For H3K4 tri-methylation, comparable numbers of differential peaks (FC > 2) were found to be reduced (386, 1.77%) or increased (393, 1.81%) by IL-1 β (Fig. 6B). The effects of IL-1 β on H3K27 tri-methylation were less pronounced, as 197 (0.69%) differential peaks (FC > 2) were reduced and 24 (0.08%) increased by IL-1 β (Fig. 6C) (List of differential peaks see suppl. 4–9).

We hypothesized that IL-1 β exposure leads to a reduction of H3K27ac around the transcription start site (TSS) of mature beta-cell genes, leading to decreased transcription. We found the beta-cell

markers *Mafa* and *Ucn3* as well as the proliferation marker *Ccnd2* had reduced level of H3K27ac in the vicinity of their TSS 2.6, 2.2 and 2.9-fold respectively while the IL-1 β induced chemokine *Cxcl1* showed increased H3K27ac marks at the TSS in agreement with the increased mRNA expression. *Ucn3* also had reduced levels of H3K4me3 (approximately 2.5-fold) near its TSS whereas the levels of H3K4me3 were not altered in the proximity of the TSS of *Mafa* and *Ccnd2*. Peaks that were differentially enriched between control and IL-1 β were identified using HOMER and peaks were required to have 2-fold enrichment between experimental groups and Poisson P-value of < 0.0001 . (Fig. 6D).

The association between changes in H3K27ac associated a TSS and mRNA expression is shown in Fig. 6E. Most genes up-regulated by IL-1 β (red in RNA lane) show increased H3K27ac, while most down-regulated genes (green in RNA lane) showed decreased H3K27ac. Associations were also identified for IL-1 β regulation of H3K4me3 and expression of the corresponding mRNAs (Fig. 6F). Examples are stimulation of *Cxcl1* mRNA expression (approximately 7-fold) and increase in H3K4me3 and H3K27ac marks 3 and 4-fold respectively. Reduction in the expression of *Nkx6.1*, *Ucn3* and *Slc30a8* mRNAs was associated with a decrease in

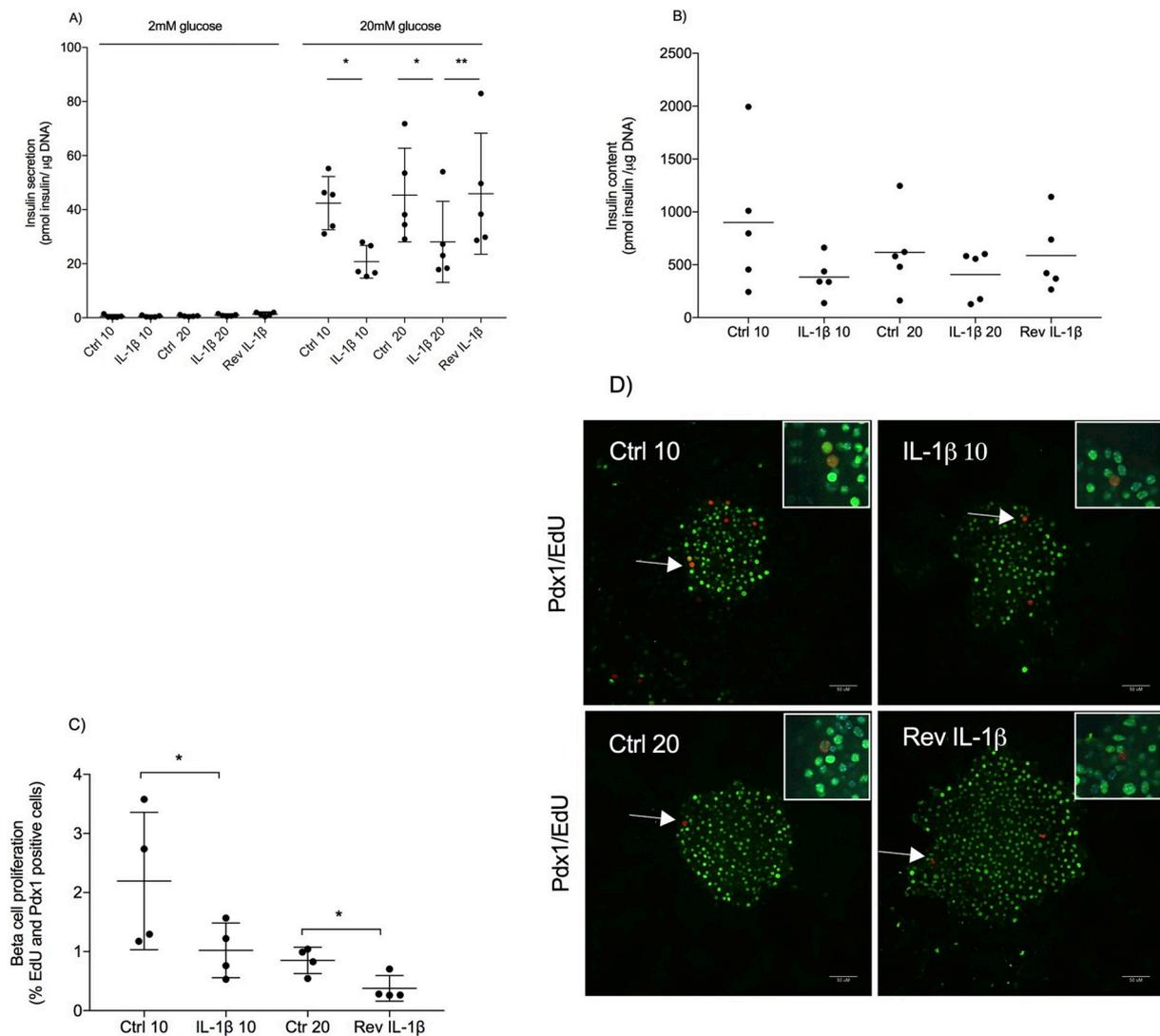


Fig. 7. Recovery of beta-cell function after IL-1 β removal. Mouse islets were exposed to 50 pg/ml IL-1 β for 10 (IL-1 β 10) or 20 (IL-1 β 20) days, non-exposed (Ctrl 10) for 10 or 20 (Ctrl 20) days or exposed to 50 pg/ml IL-1 β for 10 days and thereafter left non-exposed for another 10 days (Rev IL-1 β). (A) Accumulated insulin secretion was measured by static batch incubations in response to 2 mmol/l glucose followed by 20 mmol/l and normalized to DNA content. * $p < 0.05$, ** $p < 0.01$ 2-sided t -test (B) Total insulin content was measured post-assay and normalized to DNA content. Data are shown as means for $n = 5$. (C) Proliferation was determined by immunocytochemical staining for Pdx-1 and EdU. Results are shown as percentage proliferating beta-cells. Data are shown as means for $n = 4$. * $p < 0.05$ 2-sided t -test vs. control 10 days or control 20 days. (D) Immunocytochemical staining of whole mouse islets. Cells were stained for Pdx-1 (green) and EdU (red). Data shown is representative. Relative mRNA expression of (E) *Ins2*, (F) *Ucn3*, (G) *Pdx-1*, (H) *Glp1r*, (I) *MafA*, (J) *Ins1*, (K) *Mki67* and (L) *Cnd2* were analyzed using real-time PCR. Expression levels are normalized to control *Ppia* and Ctrl 10 data was set to 1. Data are shown as means for $n = 3-6$. * $p < 0.05$, ** $p < 0.01$ 2-sided t -test. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

H3K27ac and H3K4me3 marks. Non-parametrical Spearman correlation analysis between fold-change H3K27ac and mRNA expression (Fig. 6G and H) was not statistically significant. Correlation between fold-change H3K4me3 and mRNA expression was statistically significant in H3K4me3 IL-1 β enriched group (Fig. 6J) while Control enriched group was not correlated (Fig. 6I). Boxplot graphs showing the absolute values of mRNA expression of Control and IL-1 β samples for genes with TSS associated with H3K27ac and H3K4me3 in control and IL-1 β enriched peaks showed the same tendency (Suppl. Fig. 1A–D).

3.4. Reversibility of beta-cell function after IL-1 β removal

Exposure to IL-1 β for 10 days, as previously shown, or 20 days significantly reduced insulin secretion compared to their respective controls (Fig. 7A). When islets were cultured for 10 days in the presence of IL-1 β and left to recover for 10 days in absence of IL-1 β (Rev IL-1 β), GSIS was normalized, indicating reversibility of the IL-1 β effects on

insulin secretion. Analysis of insulin content showed no significant differences between treatments, however IL-1 β shows a trend to reduce insulin content (Fig. 7B).

To assess whether IL-1 β -induced inhibition of proliferation was also reversible, islets were cultured for 10 days in the presence of IL-1 β plus another 10 days in absence of IL-1 β (Rev IL-1 β). Under basal conditions after 10 days of culture (Ctrl 10) beta-cell proliferation rate was approximately 2%. After 20 days of culture the proliferation rate was to approximately 1%. IL-1 β exposure for 10 days reduced the proliferation to 1% of beta-cells. Rev IL-1 β showed further inhibition of beta-cell proliferation (Fig. 7C and D).

We next examined whether IL-1 β effects on expression of selected beta-cell genes were reversible. Exposure to IL-1 β for 10 or 20 days significantly reduced *Ins2* and *Ucn3* mRNA expression compared to their respective controls. Removing IL-1 β caused the return to control levels (Fig. 7E and F). *Pdx1* and *Glp1r* mRNA expression was also reduced by IL-1 β treatment for 10 and 20 days. Removal of IL-1 β did not

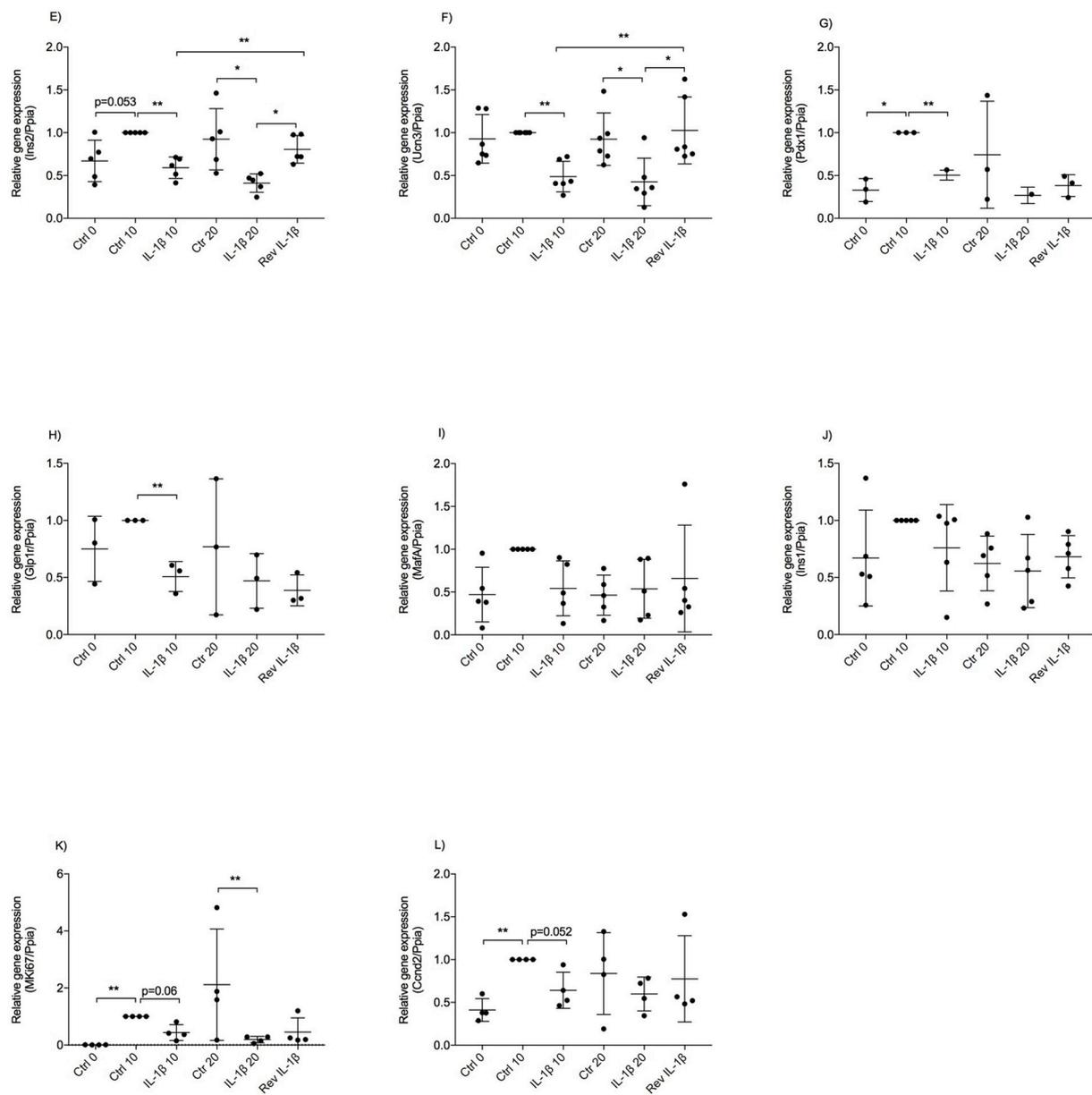


Fig. 7. (continued)

significantly increase the *Pdx1*, *Glp1r* or *MafA* mRNA expression (Fig. 7G–I). As previously observed, *Ins1* mRNA expression was not affected by any of the conditions (Fig. 7J). Expression of the cell cycles markers *Ki67* and *Ccn2* mRNA's showed a tendency to be reduced by IL-1 β , but no significant reversal after removal of IL-1 β (Fig. 7K and L). Together, these observations suggest that IL-1 β inhibitory effect on beta-cell gene expression is reversible for certain genes (*Ins2* and *Ucn3*) but not for others (*Glp1r*, *Mki67* and *Ccn2*).

4. Discussion

In this study we have characterized the function, gene expression and histone modifications in mouse islets exposed for a prolonged period to low concentrations of the pro-inflammatory cytokine IL-1 β , in order to characterize the mechanism behind beta-cell failure. IL-1 β has previously been proposed to induce beta-cell failure in both T1D and T2D. However, therapies targeting IL-1 β have shown diverse results in different clinical trials (van Asseldonk et al., 2011; Larsen et al., 2009; Ramos-Zavala et al., 2011).

During the 10 days of culture under control conditions we observed

a significant increase in *Ins2* mRNA. While we do not know the mechanism behind the up-regulation of the *Ins2* mRNA it could be speculated that culture in 11 mM glucose would increase transcription of the *Ins2* gene as glucose has been shown to enhance *Ins* gene expression (Docherty and Clark, 1994). Interestingly, the expression of the other beta cell marker mRNA's shown in Fig. 4 were also increased after 10 days of culture although this did not reach statistical significance. These observations strongly indicate that beta-cells do not undergo spontaneous dedifferentiation during the culture period. We found decreased secretion of insulin in response to glucose from islets exposed to the low dose of IL-1 β for 10 days. Moreover, beta-cell proliferation was reduced. The dysfunction and reduced proliferation was associated with significant changes in expression of genes associated with beta-cell identity such as *Ins2*, *MafA*, *Pdx1*, *Ucn3* and *Glp1r*. In agreement with our results, a recent study by Nordmann et al. (2017) have shown that exposure to pro-inflammatory cytokines at pro-apoptotic concentrations, in particular IL-1 β , compromises beta-cell identity. Our global analysis of gene expression revealed a subset of genes that were up-regulated by low dose IL-1 β exposure comprising classical immune-regulated genes. Thus, it appears that there is a form of bias in

the signaling from IL-1R, as low doses of IL-1 β affect beta-cell function and proliferation, but apoptosis is not induced. Thirty percent of regulated genes showed reduced expression in response to the low doses of IL-1 β . This group was comprised of many beta-cell identity genes and included beta-cell genes previously identified as being down-regulated in human T2D, including Slc30A8 (Zn²⁺ transporter 8), Pdk1, Arl15 and Slc2A2 (Glut2) (Fadista et al., 2014). Single cell sequencing from control and T2D donor beta-cells also revealed decreased expression of Slc2A2, Glp1r and Ins in T2D (Xin et al., 2016). Although a small number of genes were regulated similarly in human T2D and in our IL-1 β exposed mouse islets, there are also many differences. We used a single cytokine to induce beta-cell dysfunction compared with the complex exposure of islet cells to different cytokines, high glucose and free fatty acids in the T2D individuals. Despite these differences, gene ontology analysis reveal up-regulation of immune response genes in our study and in human T2D (Segerstolpe et al., 2016; Xin et al., 2016) as well as regulation of cell proliferation and mitosis-associated genes. The overlap between our study and the human studies indicate a role for IL-1 β in the pathogenesis of T2D. The significant down-regulation of several key beta-cell genes such as Mafa and Ucn3 after exposure to IL-1 β for 10 days indicates that IL-1 β can directly reduce beta-cell function and maturity status. We did not observe an increase in glucagon or *Arx* mRNA, which would have been expected if trans-differentiation into alpha-cells was occurring. In contrast, a small (1.4 fold) but significant ($p = 0.0035$) increase in *Neurog3* gene expression was observed after IL-1 β exposure, suggesting the dedifferentiation into a precursor-like state. More detailed analysis at the single cell level is required to adequately address the question of whether beta-cell dedifferentiation occurs after IL-1 β stimulation.

Beta-cell proliferation was reduced by IL-1 β exposure. In agreement with these results our RNA-seq analysis revealed that *Ccnd2*, *Mki67* and *FoxM1*, regulators in beta-cell proliferation, are down-regulated after IL-1 β exposure. It has been described that beta-cells from *Nkx6.1* knockout mice exhibit a reduced *Ccnd2* protein level as well as reduced expression of the proliferation marker *Mki67* (Taylor et al., 2013). Moreover, deletion of *FoxM1* as well as inactivation of cyclin D2 results in gradual decrease in beta-cell mass (Georgia and Bhushan, 2004; Kushner et al., 2005; Zhang et al., 2006).

Cell identity is the result of a specific pattern of gene expression obtained through cellular differentiation and adaptation. Since epigenetic regulation is essential in cell differentiation and maintenance of cell identity, we analyzed the IL-1 β -induced changes in three histone marks known to alter chromatin structure and gene transcription in islet cells. The H3K27ac was previously found to be associated with both active promoters and enhancers in human islet cells (Pasquali et al., 2014) and together with the H3K4me3 mark would allow islet-specific gene regulatory networks. The H3K4me3 mark was associated with several beta-cell-specific genes such as *SLC30A8* and *Ucn3* in human islets, but was not present in the promoter region of the *Ins* gene (Stitzel et al., 2010). In mouse islets the H3K27ac mark was found in 45% of active enhancers including the *Ins*, *Ucn3* and *Nkx6.1* indicating an important role in the regulation of transcription of these genes (Tennant et al., 2013). The observed reduction in H3K27ac marks following IL-1 β exposure and the association with beta-cell marker genes, suggests that this histone modification is significant and possibly plays a role in the induction of beta-cell dysfunction by IL-1 β . Overall an increase in H3K27 acetylation is predictive of transcriptionally active sites. Despite this trend, a substantial number of genes that have reduced H3K27ac show increased mRNA expression. This may indicate that other mechanisms (e.g. other histone markers, DNA methylation) dominate the regulation of these genes. The mechanism by which IL-1 β regulates histone modifications is not known. However, IL-1 β is known to signal through the activation of NF κ B in beta-cells and NF κ B can recruit and regulate the activity of both histone acetyl transferases (HAT's) and histone deacetylases (HDAC's) to promoters of IL-1 β regulated genes (Bhatt and Ghosh, 2014). IL-1 β -induced beta-cell

dysfunction was partially reversible once IL-1 β exposure was terminated and islets were allowed to recover. Although the IL-1 β -induced dysfunction and repression of *Ins2* and *Ucn3* was reversed, the proliferative capacity and expression of markers such as *Pdx1*, *Glp1r* and *Mafa* was not. This may be a result of the long *in vitro* culture, as the expression of these markers also decreased in the control setting after 20 days. The role of epigenetic regulation in this particular process is not known. However, since histone modifications are dynamic, it is likely that gene expression and function in IL-1 β -exposed beta-cells returns to a fully functional state due to reversal of the changes in histone modification. Furthermore, it has been described that the reversal of beta-cell function in various models of T2D is possible by achieving normoglycemia (Brereton et al., 2014; Wang et al., 2014) or by treatment with a TGF- β pathway inhibitor (Blum et al., 2014), suggesting that novel therapies for T2D could target induction of beta-cell maturity and normalization of function.

IL-1 β has been suggested to play a major role in the pathogenesis of both T1D and T2D (Cnop et al., 2005; Dinarello et al., 2010). The mechanism responsible for this has been associated with the initiation of apoptotic signaling pathways. These pathways include direct activation of apoptosis through JNK as well as transcriptional regulation of genes such as iNOS mediated by NF κ B (Cnop et al., 2005). Little is known about the potential role of low doses of IL-1 β on histone modifications. However, inhibition of HDAC3 seems to protect beta-cells from IL-1 β induced apoptosis (Remsberg et al., 2017) suggesting a possible role for histone acetylation in mediating or regulating IL-1 β induced signaling in beta-cells. In this study we have exposed islets to very low concentrations of IL-1 β compared to previous studies. Interestingly, we do observe induction of classical IL-1 β regulated genes such as *Cxcl1*, *Ccl20* and *Nos2* even after 10 days of IL-1 β stimulation, but with no evidence of cell death. Compared to low dose IL-1 β exposed beta-cells, high doses of IL-1 β -induce gene expression profiles associated with beta-cell apoptosis, thereby indicating a point of no return leading to beta-cell death.

The observation that the IL-1 β induced beta-cell dysfunction is reversible and associated with re-expression of key beta-cell genes could present novel therapeutic opportunities. Our data, together with other observations (Blum et al., 2014; Brereton et al., 2014; Wang et al., 2014) indicate that beta-cell dedifferentiation might be a reversible process. After release of beta-cell stressors, such as hyperglycemia or inflammatory cytokines, the function of beta-cells returns to pre-stress levels. It is interesting that the expression of some genes is reversible, whereas others are not. The potential role of histone modifications or other epigenetic marks in this reversal is interesting. The *in vitro* system presented here provides a simple framework to test possible therapeutic agents for their ability to revert beta-cell identity loss.

In conclusion, we have shown that prolonged exposure to very low doses of IL-1 β is associated with beta-cell dedifferentiation, as indicated by changes in the expression of several key transcription factors and other genes crucial for beta-cell function. This contrasts with the effects of short-term exposure to higher concentrations of IL-1 β that induce apoptotic pathways and beta-cell death. Our finding of changes in histone modification provides a plausible link between chronic inflammation in T2D and changes in gene transcription patterns. The observation that IL-1 β -induced beta-cell dysfunction is reversible could present novel therapeutic targets for the preservation of beta cell mass and function in T2D.

CRediT authorship contribution statement

Adriana Ibarra Urizar: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Michala Prause:** Data curation, Formal analysis, Investigation, Visualization, Writing - review & editing. **Matthew Wortham:** Data curation, Formal analysis, Investigation, Visualization, Writing - review & editing. **Yinghui Sui:** Formal analysis, Investigation,

Methodology, Visualization, Writing - review & editing. **Peter Thams:** Data curation, Formal analysis, Writing - review & editing. **Maike Sander:** Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Writing - review & editing. **Gitte Lund Christensen:** Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Nils Billestrup:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mce.2019.110524>.

Contribution statement

A.I.U., M.P. and G.L.C. planned the project, researched data, contributed to the discussion, and wrote, reviewed and edited the manuscript. M.W., P.T. and Y.S. researched data, contributed to the discussion, and reviewed and edited the manuscript. M.S. contributed to the discussion; and reviewed and edited the manuscript. N.B. conceived the study, and contributed to the discussion; and wrote, reviewed, and edited the manuscript. N.B. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

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