Figures and figure supplements

Reversal of β cell de-differentiation by a small molecule inhibitor of the TGFβ pathway

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Figure 1. Loss of Ucn3 expression is an early marker for β cell de-differentiation in diabetes. (A) Immunostaining with antibodies against insulin (red) and Ucn3 (green) in pancreata from T2D (Lepr<sup>Ob/Ob</sup> and Lepr<sup>Db/Db</sup>), insulin-dependent diabetic (Ins2<sup>Akita</sup>), and healthy control (C57BL/6) mice. Ucn3 protein but not insulin protein is downregulated in diabetic pancreata compared to the healthy control. (B) Quantitative Real-Time PCR analysis of Ins1 and Ucn3 gene expression in islets from C57BL/6 (n = 10), Lepr<sup>Ob/Ob</sup> (n = 9), Lepr<sup>Db/Db</sup> (n = 8), and Ins2<sup>Akita</sup> (n = 11) mice. Ucn3 mRNA is significantly reduced in all diabetes models, while insulin mRNA is significantly reduced only in the most diabetic model (Ins2<sup>Akita</sup>). (C) Quantitative Real-Time PCR analysis of Ins1 and Ucn3 gene expression in islets from non-diabetic control mice (n = 10; average blood glucose 167 ± 5 mg/dl), mildly diabetic (n = 16; average blood glucose 381 ± 17 mg/dl) and severely diabetic mice (n = 11; average blood glucose 588 ± 8 mg/dl). Error bars represent ±SEM. ***p < 0.001.

DOI: 10.7554/eLife.02809.003
Figure 2. Insulin resistance-induced β cell de-differentiation is reversible. (A) Immunostaining with antibodies against insulin (red) and Ucn3 (green) in pancreata from wild-type C57BL/6 mice treated with either vehicle (PBS) or S961 (insulin receptor antagonist) for 7 days (upper and middle panels) or treated with S961 for 7 days followed by a 7-day-recovery period in the absence of S961 (lower panel). Ucn3 protein expression is down regulated in β cells following 7 days S961 treatment but returns to normal expression levels upon remission to normoglycaemia (see text). Nuclei are stained with DAPI (blue). (B) Quantitative Real-Time PCR analysis of Ins1 and Ucn3 gene expression in islets from ICR lean mice taken at different time points during S961-induced de-differentiation and post S961 withdrawal recovery (n = 3 mice for each stage). S961 osmotic pumps are transplanted on day 0 and removed on day 7. Control designates mice not treated with S961. Error bars represent ±SEM. *p < 0.05; ***p < 0.005. DOI: 10.7554/eLife.02809.004
Figure 2—figure supplement 1. Expression of β cell genes during S961-induced de-differentiation and subsequent recovery. Quantitative Real-Time PCR analysis of MafA, Nkx6.1, and Pdx1 gene expression in islets from ICR lean mice taken at different time points during S961-induced de-differentiation and post S961 withdrawal recovery (n = 3 mice for each stage). S961 osmotic pumps are transplanted on day 0 and removed on day 7. Control designates mice not treated with S961. Error bars represent ±SEM. *p < 0.05; ***p < 0.005.
DOI: 10.7554/eLife.02809.005
**Figure 3.** Adherent culture-induced β cell de-differentiation is reversible.

(A) RCU reporter mice are made by crossing mice homozygous for the *Insulin2-Cre* transgene with mice doubly-homozygous for Rosa26-lox-stop-lox-H2BmCherry and *Ucn3-GFP*. *Insulin* expression in RCU progeny is permanently marked by red nuclear fluorescence, and *Ucn3* expression is marked by green cytoplasmic fluorescence. (B) Pancreas sections of PBS-treated control and S961-treated diabetic RCU mice. *Ucn3*-GFP is reduced in diabetic mice, but not in controls, and *Ucn3* expression returns after remission from diabetes. All images show live (unstained) reporter fluorescence. (C) De-differentiation and re-differentiation of RCU islets cultured in vitro. Islets from adult RCU mice were isolated and plated on 804G matrix for 1 week (left and middle panel). Note islet spreading and loss of *Ucn3*-GFP in the de-differentiated islets (middle panel). After 7 days, the de-differentiated islets were transplanted into euglycemic SCID mice for 3 weeks (right panel) after which time the transplants show the return of *Ucn3* expression in β cells.

DOI: 10.7554/eLife.02809.006
Figure 3—figure supplement 1. RCU mice show nuclear insulin expression-coupled mCherry and Ucn3-derived cytoplasmic GFP. Shown are confocal images of an islet from an adult RCU mouse. Note co-localization of nuclear H2BmCherry (red) and cytoplasmic GFP (green).
DOI: 10.7554/eLife.02809.007

Figure 3—figure supplement 2. Ucn3 and insulin expression are down regulated in islets grown in adherent culture. Shown are quantitative Real-Time PCR analyses of Ins1 and Ucn3 from islets of wild-type C57BL/6 mice grown on 804G matrix for 1 week. Each bar represents average gene expression in three independent experiments.
DOI: 10.7554/eLife.02809.008
Figure 3—figure supplement 3. β cells lose glucose-stimulated insulin secretion upon de-differentiation in culture. Shown are static GSIS analyses of adult islets de-differentiated on 804G for 1 week. Each bar represents average insulin secretion of three biological repeats.
DOI: 10.7554/eLife.02809.009
Figure 4. TGFβ pathway inhibitors and Artemin signaling reverse β cell de-differentiation. (A) Islets from adult RCU mice are isolated and plated on 804G matrix for 1 week in a 384-well plate format during which time the β cells de-differentiate. A compound library is added on day 7, and islets are cultured for an additional week in the presence of compounds. Each compound is tested in duplicates of two or three concentrations. Fresh un-manipulated RCU islets are used as a positive control, and DMSO- or untreated islets are used as negative controls. Islets are fixed on day 11 for automated imaging and subsequent analysis. Percentages of mCherry positive cells that co-express GFP are calculated for each well and used to identify conditions that significantly increase the number of GFP positive cells over negative (DMSO- or non-treated) controls. Positive hits are selected according to their statistical significance (p value) over the negative control. (B) Results of screen with 114 growth factor proteins. Factors are ordered from left to right based on the statistical p-value of their Ucn3-GFP fluorescence over the negative (non-treated) control. For convenience, values on the Y axis are presented as 1/p-value. Red bar represents the threshold for statistical significance (p < 0.01). (C) Results of screen with 19 TGFβ pathway inhibitors, 18 RET/GFRα3 inhibitors, and 42 known T2D drugs. Factors are ordered from left to right based on the statistical p-value of their Ucn3-GFP fluorescence over the negative (DMSO-treated) control as above. For convenience, values on the Y axis are presented as 1/p-value. Red bar represents the threshold for statistical significance (p < 0.01). A full list of the factors tested is presented in the Supplementary file 1.

DOI: 10.7554/eLife.02809.010
Figure 5. Alk5 inhibitor II restores β cell maturation in 804G-induced β cell de-differentiation. (A) Islets from adult RCU mice were isolated and plated on 804G matrix for 14 days with or without the addition of Alk5i at day 7 (right and middle panels, respectively). Live fluorescence images of H2BmCherry and Ucn3-GFP were taken on day 14, and compared to fresh RCU islets cultured on 804G for 24 hr. (B) H2BmCherry-positive cells from the above cultured were sorted by FACS and subjected to qRT-PCR analysis for the expression of various mature β cell genes. Statistical significance relates to the difference between Alk5i-treated and non-treated islets for each gene. Expression levels are normalized to the levels of freshly isolated islets (dashed line). Error bars represent ±SEM of three biological repeats. **p < 0.001. B.G. (C) Immunostaining with antibodies against insulin (red) and Ucn3 (green) in islets from ICR mice treated as above. DOI: 10.7554/eLife.02809.011
Figure 5—figure supplement 1. Alk5 inhibitor II induces Ucn3-GFP in RCU islets in a dose-dependent manner. Shown is a dose-curve for the induction of Ucn3-GFP in RCU islets de-differentiated on 804G matrix for 1 week, following by 1 week treatment with the indicated concentration of ALK5 inhibitor II. Bars represent ±S.D. DOI: 10.7554/eLife.02809.012

Figure 6. Alk5 inhibitor II induces expression of mature β cell transcription factors and prevents their reduction under cytokine stress. Quantitative Real-Time PCR analysis of gene expression in wild-type islets treated with cytokines as shown (A) IL-β, (B) TNFa, (C) INFγ. Each bar represents average gene expression in three independent experiments. Expression levels are normalized to the levels of control islets not treated with any cytokine (dashed line). Statistical significance relates to the difference between Alk5i-treated and DMSO-treated islets for each gene. Error bars represent ±SEM. *p < 0.05; ***p < 0.005. DOI: 10.7554/eLife.02809.013
Figure 6—figure supplement 1. β cells lose glucose-stimulated insulin secretion upon cytokine treatment. Shown are static GSIS analyses of adult islets treated with a combination of IL-1β, TNFα, and INFγ (10 ng/ml each). Each bar represents insulin secretion in three biological repeats.
DOI: 10.7554/eLife.02809.014
Figure 7. Alk5 inhibitor II induces expression of mature β cell transcription factors even in β cells that were exposed to extreme diabetic conditions for several months. (A–D) Alk5 inhibitor II (Alk5i) induces expression of specific β cell genes in islets from healthy and severely diabetic mice. Shown are quantitative Real-Time PCR analysis of gene expression in islets of healthy control (C57BL/6) and diabetic mice (Lepr<sup>Db/Db</sup>, Lep<sup>Ob/Ob</sup>, and Ins2<sup>Akita</sup>). Each bar represents average gene expression in three independent experiments for each group. Statistical significance relates to the difference between Alk5i-treated and DMSO-treated islets for each gene. Expression levels are normalized to the levels of C57BL/6 islets treated with DMSO (dashed line). Error bars represent ±SEM. *p < 0.05; ***p < 0.005. B.G. = Blood glucose level at time of sacrifice. (E) Alk5 inhibitor II (Alk5i) induces expression of specific β cell transcription factors in human islets. Shown are quantitative Real-Time PCR analyses of gene expression. Error bars represent three technical repeats on islets from a single donor. Error bars represent ±SEM. DOI: 10.7554/eLife.02809.015