ONLINE APPENDIX

Supplementary Methods

Cell isolation and culture

Rat pancreatic beta cells were isolated from 6 weeks old male Wistar rats as described before (25). Purity was always above 90%. Cells were cultured adherently in Ham's F10 medium (26). Culture wells and coverslips were coated with 10 µg/ml poly-D-lysine (Sigma-Aldrich, St. Louis, MO). After overnight culture at 37°C and 5% $C0_2$, beta cells were transduced with adenovirus and cultured until analysis. In some cultures, BrdU (100 µM) was added to the culture medium during the last 16 hours of culture.

Mouse islets were isolated from 8 week old Balb/c mice by collagenase digestion of dissected pancreases and handpicking of the islets. Using mild trypsinization the islets were partly dissociated. The pancreatic endocrine cell preparations, which consisted for more than 75% of beta cells, were cultured overnight in 24-well suspension plates (Greiner Bio-One, Kremsmuenster, Austria) and transduced with adenovirus at the multiplicity of infection (MOI) mentioned in the text. At different time points after transduction, cells were harvested for RNA or protein isolation. Islets isolated from tamoxifen-treated transgenic mice were harvested by handpicking and immediately processed for RNA or protein extraction.

Immunohistochemistry and cytochemistry

For detection of BrdU incorporation, cells (in 96-well plates or on coverslips) were fixed using 4% formalin (Sigma-Aldrich) for 30 minutes, and washed 2 times with PBS. Cells were then permeabilized using 0.2% Triton X-100 for 10 minutes and washed again 2 times with PBS. The DNA was denatured by incubating the cells with 2M HCl for 15 min at 37 °C. After washing with PBS, the acid was neutralized by 2 repeated incubations (5 min) with 0.1M borate buffer pH 8.5. Cells were blocked for 30 min with normal goat serum. Incubation with mouse anti-BrdU antibody (Progen Biotechnik GmbH, Heidelberg, Germany) was done overnight at 4 °C. After washing 3 times with PBS, cells were incubated 1 h at room temperature with fluorescently labeled goat secondary antibody (Molecular Probes, Invitrogen). After washing with PBS, nuclei were counterstained with Hoechst33342 and coverslips were mounted with mounting medium (Dako, Glostrup, Denmark).

Visualization and imaging

Cells in 96-well plates were visualized using an Axiovert 135 M inverted microscope (Carl Zeiss Jena, Jena, Germany), the images were acquired by an AxioCam digital camera and analysed using AxioVision software (Carl Zeiss). Cells on coverslips were visualized using an Axioplan 2 microscope (Carl Zeiss) and images acquired by Sensys camera (Photometrics, Tucson, AZ) using Smartcapture software (Digital Scientific UK, Cambridge UK). Confocal images were acquired with a Leica TCS SP LSM microscope (Leica, Heidelberg, Germany). Post-acquisition visualisation was performed using Volocity LE Imaging Software (Improvision, CA, USA).

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Cell cycle analysis

For study of cell cycle, cultured cells were lysed in PI-lysis buffer (0.1% Triton X-100, 0.1% Na-citrate, 50 µg/ml PI in H_2O), incubated for 2h at 4°C, and analysed by flow cytometry. Histograms for PI-fluorescence intensities were obtained using FlowJo software (Treestar, Ashland, OR). The curves were deconvoluted for separate cell cycle phases using the Watson-Pragmatic program.

Sorting of live G0/G1 and S/G2/M phase beta cells

Rat beta cells were cultured overnight in 24-well tissue culture plates at a density of 150,000 cells per well and transduced with AdE2F1 and AdAkt both at MOI 10 the next day. A minimum of 450,000 beta cells was used per sorting experiment. 24 hours after transduction, and after 1 hour labelling with BrdU (100 µM), the cells were stained 30 min with Hoechst (10 µg/ml) and detached from the plate using 0.125% trypsin + 2mM EDTA. After adding PI to exclude dead cells, G0/G1 and S/G2/M phase beta cells were sorted according to DNA-content (Hoechst-fluorescence). Sorted cells were plated on coverslips for BrdU or Ki67 immunostainings or collected for insulin content determination.

Insulin release measurement

Insulin release by AdE2F1- and AdNull-transduced rat beta cells or by islets from transgenic mice (20 freshly isolated islets per condition) was measured by static incubation. Cells or islets were first pre-incubated in Ham's F10 medium without glucose for 2 hours at 37°C and 5% CO_2. Then insulin release was tested by incubating for 2 hours with the above medium containing 2 mM Ca^{2+}, and 2 mM glucose (G2), 10 mM glucose (G10), or 20 mM glucose (G20). Insulin concentrations in the medium and total insulin levels were measured by RIA. Insulin biosynthesis was measured in adenovirus-transduced rat beta cells cultured in Ham's F10 medium containing G10. ^{3}H-labeled tyrosine was added to the medium at 20h post-infection, and 4h later, radiolabeled insulin was measured in both culture medium and beta cells, using immunoprecipitation of insulin and scintillation counting.

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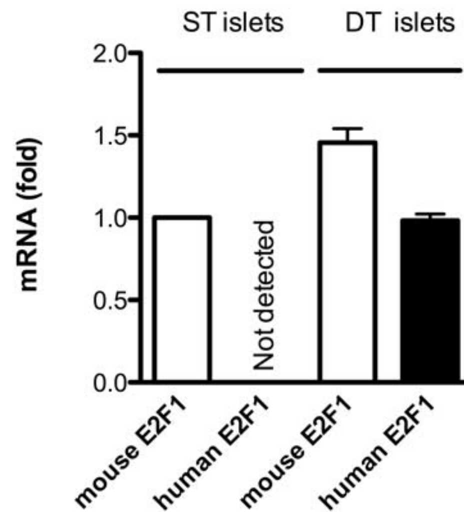
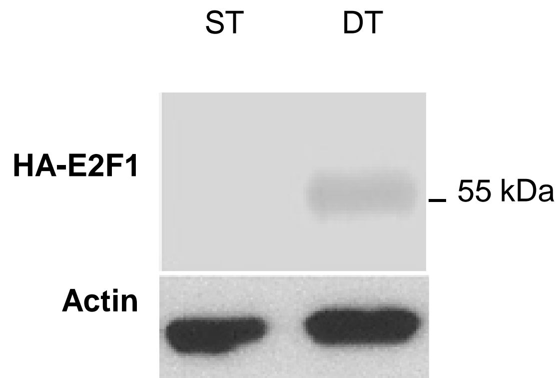
Supplementary Fig. 1. Expression level of E2F in ST and DT mouse islets. Islets were isolated from RIPCreERT-/- x R26-E2F1+/- mice (ST) and from RIPCreERT+/- x R26-E2F1+/- mice (DT) 2 weeks after tamoxifen injection. mRNA expression of recombinant human E2F1, endogenous mouse E2F1, and cyclophylin A, was determined by QRT-PCR. mRNA levels were expressed relative to cyclo A and normalized to E2F1 in ST islets. (n=3-4)



Supplementary Fig. 2. Expression of E2F protein in ST and DT mouse islets. Islets were isolated from RIPCreERT-/- x R26-E2F1+/- mice (ST) and from RIPCreERT+/- x R26-E2F1+/- mice (DT) 2 weeks after tamoxifen injection. Recombinant human HA-tagged E2F1 was detected by immunoblotting using anti-HA antibody. Lysate of 200 islets pooled from 3 mice was loaded in each lane.



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