Electronic supplementary material

Methods

Mice All mice were maintained under conditions approved by the Institutional Animal Care and Use Committee of the Joslin Diabetes Center. MIP-GFP mice were obtained from M. Hara (The University of Chicago, Chicago, IL, USA) and were backcrossed ten times with C57BL/6J mice. The B6.Cg-Tg(InsII-cre)25Mgn/J (rInsII-cre), B6;129-Gt(ROSA)26Sor^{tm2Sho}/J (ROSA-loxP-stop-loxP-GFP), C57BL/6-Tg(ACTB-EGFP)1^{Osb}/J and C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Embryos were considered to be 0.5 days of gestation (E0.5) at noon of the day plugs were detected; neonates were considered to be postnatal day 0 (P0) on the day of birth.

DNA isolation and PCR analysis for genotyping Genomic DNA of peripheral blood was extracted with a kit (Temp/Nucleospin Blood Quick Pure Kit; BD, Franklin Lakes, NJ, USA). The presence of MIP-GFP transgene was screened by PCR using primers that recognise the GFP construct as follows:
forward 5′-TGGAAACTGCAGCTTCAG-3′
reverse 5′-GTCCAGCTCGACCAGGATGG-3′ (280 bp).

Immunocytochemistry Dispersed islet cells or double-sorted cells were cultured for 8 h on gelatin-coated dishes for attachment in DMEM supplemented with 10% (wt/vol.) fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C with 5% CO_2. Then, the cells were fixed with 4% (wt/vol.) paraformaldehyde for 15 min, permeabilised for 15 min with 0.3% (wt/vol.) Triton X-100 in PBS and immunostained. After blocking with PBS containing 10% (wt/vol.) BSA for 30 min, separate dishes of cells were incubated overnight with primary antibody (guinea pig anti-insulin 1:200; guinea pig anti-glucagon, 1:200 [both Linco]; rabbit anti-somatostatin, 1:100 [Chemicon International]; or guinea pig anti-pancreatic polypeptide, 1:100 [Linco]) in PBS containing 3% (wt/vol.) BSA, washed with PBS and incubated with secondary antibody (Texas red-conjugated anti-rabbit or anti-guinea pig IgG [Jackson ImmunoResearch; Bar Harbor, ME, USA]) in PBS containing 3% (wt/vol.) BSA for 1 h. This was followed by washing with PBS and mounting. To determine the proportion of different islet cell types in unsorted single islet cells of adult male MIP-GFP mice, we counted 379 cells for insulin, 800 for glucagon, 800 for somatostatin and 700 for pancreatic polypeptide. For double-sorted GFP^+ cells we counted 300 cells immunostained separately for each islet hormone. The experiment was performed twice.

Staining of peripheral blood B lymphocytes For the negative control, peripheral blood was collected from the retro-orbital vein of adult mice. After the treatment with erythrocyte lysis buffer, cell surface molecules were stained with PE-conjugated anti-mouse CD45R/B220 (RA3-6B2) and FITC-conjugated anti-mouse IgM (AF6-78) (Pharmingen, Franklin Lakes, NJ, USA), and the B lymphocytes were FACS sorted.

Threshold for detection (copies per cell) of hormone mRNA for single-cell nested RT-PCR analysis The template cDNA fragment, which includes the entire target sequence of the external primers of the single-cell nested RT-PCR for *InsI*, *Gcg*, *Sst* and *Ppy*, was prepared by conventional PCR using: 1 × GeneAmp PCR Gold Buffer (Applied Biosystems), 1.5 mmol/l $MgCl_2$, AmpliTaq Gold (Applied Biosystems) 1.25 U/50μl and 0.2 μmol/l forward and reverse primers (ESM Table 4). We used 10 ng mouse islet cDNA as template. The PCR

reaction was performed with the following variables: 1 cycle of 10 min at 95°C, then 36 cycles of 30 s at 94°C, 90 s at 60°C and 90 s at 72°C. After measuring the weight/volume concentrations (ng/μl) of PCR product, respectively, we converted them to the copy/volume concentrations (copy/μl) with the molecular weight of each PCR product and Avogadro constant. Then we prepared a set of PCR reaction tubes, which included 1×10^6, 1×10^5, 1×10^4, 1×10^3, 1×10^2, 1×10^1, 1×10^0 and 0 copies of the template cDNA fragment of each islet hormone gene, respectively, and performed nested PCR as described above using the primers for the single-cell nested RT-PCR analysis. Aliquots of second-round PCR products were then subjected to 2% gel electrophoresis.

Quantitative RT-PCR Quantitative RT-PCR was performed using the ABI 7300 real-time PCR system (Applied Biosystems) according to the manufacturer's instructions. Total RNA was isolated from the total cultured population using a kit (RNeasy Mini; Qiagen, Valencia, MD, USA). Reverse-transcription was performed using a high-capacity cDNA kit (Applied Biosystems). Quantitative PCR reactions were performed with TaqMan Universal PCR Master Mix (Applied Biosystems) or Power SYBR Green PCR Master Mix (Applied Biosystems) with 0.2 μmol/l of forward and reverse primers. The probe and primer sets of mouse β-actin (assay ID Mm00607939_s1) were purchased from Applied Biosystems. The primers for mouse *Gcg*, *Sst* and *Ppy* are listed in ESM Table 5. Expression of each mRNA was normalised to that of the internal control β-actin. All experiments were repeated three times.