Electronic supplementary material

Results

Analysis of mRNA contamination from non-beta cells We examined the possibility that the dispersion process might lead to release of RNA that could adhere to the surface of or be endocytosed into beta cells to create an artefact. We prepared GFP^+ splenocytes from the chicken beta-actin promoter-driven enhanced GFP cDNA transgenic mice (C57BL/6-Tg[ACTB-EGFP]1^{Osb}/J), in which all of the tissues, with the exception of erythrocytes and hair, appear green under excitation. Separately, we isolated islets from wild-type C57/BL/6J mice, which were mixed with the GFP^+ splenocytes and dispersed into single cells with trypsin. Then, GFP^+ splenocytes were separated by FACS-sorting from the cell mixture and single-cell nested RT-PCR analysis was performed. In 56 sorted GFP^+ splenocytes, no PCR amplification was detected for insulin I or II, glucagon, somatostatin or pancreatic polypeptide. Therefore, even if some sorted beta cells were contaminated with mRNA released from neighbouring non-beta islet cells, their frequency must have been very rare or absent (less than 1.8% or 1/56). To further eliminate the possibility of mRNA contamination from the other cells, we checked whether mouse homologue of human trypsin1 mRNA (Accession no. NM_023333.4), which is highly expressed in acinar cells, could be detected with *InsI* mRNA in double-sorted GFP^+ cell from adult MIP-GFP islets by single-cell nested RT-PCR. In 63 single GFP^+ cells examined, all expressed *InsI* mRNA, but none co-expressed *Try1* mRNA. In this analysis of GFP^- cells sorted from non-islet fraction during islet isolation procedure, the same mice were used as positive controls, since this fraction should be enriched with non-beta islet and exocrine cells. We found that 14 out of 19 GFP^- cells (74%) were *Try1*-positive. These data confirm that the possibility of mRNA contamination from other cells was low enough to neglect.

The sensitivity of single-cell nested RT-PCR system To estimate the sensitivity of the single-cell nested RT-PCR analysis, we determined the minimum copy number of each islet hormone mRNA that could be detected. First we prepared 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* gene by conventional PCR. The primers are listed in ESM Table 4. We then prepared a set of PCR reaction tubes that 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 using the primers for the single-cell nested RT-PCR analysis. The nested PCR for *InsI* was able to detect from 1×10^6 through to 1×10^1 copies of template cDNA fragment all the others were able to detect 1×10^0 copy of template. Therefore, we estimate that the single-cell nested RT-PCR system for *InsI* used in

this study is sensitive enough to detect at least ten copies of cDNA, while those for *Gcg, Sst* and *Ppy* can detect one copy (ESM Fig. 2).

Quantitative RT-PCR analysis of other islet hormone mRNA expression in double-sorted GFP$^+$ cells from MIP-GFP mice To evaluate the expression levels of islet hormones in GFP$^+$ cells sorted from adult MIP-GFP islets, we performed quantitative RT-PCR analysis. The primers are listed in ESM Table 5. The ratios of *Gcg*, *Sst* and *Ppy* mRNA expression levels in pooled GFP$^+$ cells sorted from six adult male MIP-GFP mice to those in pooled isolated islets from three adult male MIP-GFP mice were: $3.9\times10^{-3}\pm2.1\times10^{-6}$, $2.6\times10^{-2}\pm3.7\times10^{-4}$ and $2.3\times10^{-2}\pm6.8\times10^{-4}$ (mean±SE), respectively. All quantitative RT-PCR determinations were performed in triplicate. To assess the percentage of beta, alpha, delta and pancreatic polypeptide cells in the dispersed islet cells, we stained separate aliquots after attachment for 8 h with anti-insulin, anti-glucagon, anti-somatostatin or anti-pancreatic polypeptide antibodies. As a result, the dispersed islet cells were 76% (of 379 counted cells) insulin-positive, 6.6% glucagon-positive (of 800 cells), 6.6% somatostatin-positive (of 800 cells) and 8.3% pancreatic polypeptide-positive (of 700 cells). The percentage of non-beta cells was a little lower than what is known to be present in islets in the pancreas, presumably the result of selective cell loss during the islet isolation and cell dispersion processes. We determined the relative islet hormone mRNA expression data of double-sorted GFP$^+$ cells versus whole islets cells as a function of the percentage of each islet cell type in islet. Then, we calculated that the expression levels of glucagon, somatostatin and pancreatic polypeptide in the sorted beta cells were $2.6\times10^{-4}\pm1.4\times10^{-7}$, $1.7\times10^{-3}\pm2.5\times10^{-5}$ and $1.9\times10^{-3}\pm5.7\times10^{-5}$ times lower than those in true alpha, delta and pancreatic polypeptide cells, respectively (estimated relative *Gcg*, *Sst* and *Ppy* mRNA expression level [vs alpha, delta and pancreatic polypeptide cell, respectively] = *Gcg*, *Sst* and *Ppy* mRNA expression level [vs whole isolated islet] × percentage of alpha, delta and pancreatic polypeptide cells in the dispersed islet cells/100). Thus, in beta cells the expression of islet hormone mRNA other than insulin is estimated to be extremely low and not meaningful for hormone production.