Online supplemental Methods

Identification of β-cells with DsRed

To target DsRed to β-cells, we constructed the recombinant adenovirus AdRIPBgliDsRed, using the pAdEasy system (Stratagene, La Jolla, CA). The 410 base pairs rat insulin promoter (RIP, kind gift of Dr. M. German, San Francisco, CA) was digested with *BglII* and *HindIII* and subcloned into pDsRed-Express (Clontech, Mountain View, CA) allowing generation of the pRIPDsRed plasmid. The rabbit β-globin1 intron (kind gift of Dr. J. Philippe, Geneva, Switzerland) was digested with *XhoI* and *SmaI* and subcloned into the *SalI-SmaI* digested pRIPDsRed (pRIPBgliDsRed). The RIPBgliDsRed fragment was digested with *BglII* and then partially digested with *NotI* and subcloned into pShuttle-polyA (pShuttle-RIPBgliDsRed). The adenoviral vector pAdRIPBgliDsRed was produced by recombination between pAdEasy and the *PmeI* linearized pShuttleRIPBgliDsRed in BJ5183-AD-1 cells. Ad-293 cells were then transfected with the *PacI* digested pAdRIPBgliDsRed allowing the generation of the AdRIPBgliDsRed virus. The recombinant adenovirus was then amplified in Ad-293 cells and purified on CsCl gradient. Overnight cultured islet cells were infected for 2h using this purified AdRIPBgliDsRed. Patch-clamp experiments were performed 2-3 days after infection. Control $[Ca^{2+}]_c$ measurements revealed that infected β-cells labelled with DsRed responded normally to various insulin secretagogues, including glucose.

To verify the β-cell specificity of DsRed expression in islet cells infected with AdRIPBgliDsRed, cultured cells were fixed for 3h with 4% paraformaldehyde, which preserves endogenous DsRed fluorescence. Cells were immunostained for insulin with a mouse monoclonal anti-porcine insulin antibody (Chemicon, Temecula, CA). To permit simultaneous observation of DsRed, insulin was detected by the green fluorescence of FITC-conjugated goat anti-mouse IgG (Dako, Glostrup, Denmark).

The distinct cell type specificity of EYFP and DsRed expression in GYY islets infected with AdRIPBgliDsRed was evaluated with a LSM5 Pascal laser scanning microscope (Zeiss, Jena, Germany).

Online supplemental Figure 1: DsRed is specifically expressed in β-cells infected with AdRIPBgliDsRed. ***A-C***: In islet cells from C57BL/6J mice infected with AdRIPBgliDsRed, anti-insulin immunofluorescence using fluorescein isothiocyanate (FITC, ***C***) shows that, among the three islet cells visualized in bright field (***A***), the cell expressing the DsRed protein (***B***) is labelled for insulin, but that not all β-cells are fluorescent for DsRed. ***D***. In an islet from GYY mice infected with the AdRIPBgliDsRed adenovirus, single plane projection of a Z-stack of confocal images reveals expression of EYFP (in green, α-cells) and DsRed (in red, β-cells) in distinct cell types. Scale bars: ***A-C***. 10 μm; ***D***. 20 μm.