Supplemental data

Supplemental method

Assay of intracellular cAMP content

Min6 cells were treated with various concentrations of liraglutide (0, 10, 100, and 1,000 nM) for 15 minutes. After two washes with ice-old PBS, the cells were lysed in 400 μl lysis buffer followed by 3 freeze/thaw cycles. The crude lysate of Min6 cells was pipetted into sterile tubes and centrifuged for 10 minutes at 3,000 g. The cAMP levels were measured with cAMP assessment kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.
Supplemental figure legends

Supplemental figure 1. Immunofluorescent staining of glucagon and PC1/3 in the pancreas of male diabetic Pax6<sup>−/−</sup> mice (A) and db/db mice (B) treated with PBS or liraglutide (Lira) for 2 weeks. Age-matched heterozygous db/m mice were used as a normal control of db/db mice. Scale bar = 100 μm.

Supplemental figure 2. Involvement of cAMP in the regulation of PC1/3- and PC2-dependent proinsulin processing by liraglutide (Lira). (A) Min6 cells were treated with various concentrations of Lira for 15 minutes, and intracellular cAMP content was assessed by ELISA. (B-D) Min6 cells were treated with Lira (100 nM), forskolin (Fsk, 10 μM), or 8pCPT-2′-O-Me-cAMP (8pCPT, 100 μM) for 24 hours. Cell culture supernatant was collected to measure the proinsulin/insulin ratio by ELISA (B). Cells were lysed and analyzed for PC1/3 (C) and PC2 (D) expression by western blot. The western blot data was normalized to the control protein, GAPDH. Each value represents mean ± SD of three independent experiments. * p < 0.05 (treatment vs. control).

Supplemental figure 3. Only one clear protein band was detected by the PC1/3 or PC2 antibody used in this study. The molecular weight of these protein bands was consistent with the known sites of PC1/3 (87 kDa) and PC2 (70 kDa), respectively. (A) the original gel in a representative experiment of Figure 3C; (B) the original gel in a representative experiment of Supplemental figure 2D.
A. cAMP (pM/1x10^6 cells) levels in response to Lira (nM).

B. Ratio of proinsulin : insulin across different treatments.

C. PC1/3 : GAPDH relative protein levels for each treatment.

D. PC2 : GAPDH relative protein levels for each treatment.

* indicates statistical significance.