

# Protein kinase C (Pkc)- $\delta$ mediates arginine-induced glucagon secretion in pancreatic $\alpha$ -cells

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**Supplementary Figure 1.** Glucagon staining with rat monoclonal anti-glucagon antibody in GFP-positive  $\alpha$ -cells in *Gcg<sup>gfp/+</sup>* and *Gcg<sup>gfp/gfp</sup>* mice. Pancreatic sections of *Gcg<sup>gfp/+</sup>* and *Gcg<sup>gfp/gfp</sup>* mice were stained with rat monoclonal anti-glucagon antibody. Although glucagon signals perfectly colocalized with GFP in the *Gcg<sup>gfp/+</sup>* mice, glucagon was undetected in GFP-positive  $\alpha$ -cells in *Gcg<sup>gfp/gfp</sup>* mice, indicating a high specificity of glucagon antibody used in this study. Glucagon is shown in red and DAPI in blue. Scale bar represents 50  $\mu$ m. These studies were performed under blinded analysis.

**Supplementary Figure 2.** Characterization of pancreatic  $\alpha$ -cell-specific Pkc $\delta$  knockout mice. **a–c** Body weights (a) and blood glucose levels during intraperitoneal glucose (1 g/kg) tolerance test (b) and intraperitoneal insulin (0.75 U/kg) tolerance test (c) were similar to  $\alpha$ Pkc $\delta$ KO and control mice.  $n = 10$ , each group. \*  $p < 0.05$ ; t-test. Data are expressed as a box plot.  $\alpha$ Pkc $\delta$ KO indicates *Gcg<sup>CreERT2/+</sup>; Prkcd<sup>flox/flox</sup>* mice and Cont indicates *Gcg<sup>+/+</sup>; Prkcd<sup>flox/flox</sup>* mice. **d** Pancreatic sections of  $\alpha$ Pkc $\delta$ KO and control mice were subjected to Immunohistochemistry with rat monoclonal anti-glucagon and anti-insulin antibodies. glucagon is shown in red, insulin is shown in green, and DAPI is shown in blue. Scale bars represent 30  $\mu$ m. These studies were performed under blinded analysis. **e–g** Pancreatic  $\alpha$ -cell area (e),  $\beta$ -cell area (f), and the percentage of the  $\alpha$ -cell area against the  $\beta$ -cell area (g) were similar to  $\alpha$ Pkc $\delta$ KO and control mice.  $n = 4$ , each group. \*  $p < 0.05$ ; t-test. Data are expressed as means  $\pm$  SEM.  $\alpha$ Pkc $\delta$ KO indicates *Gcg<sup>CreERT2/+</sup>; Prkcd<sup>flox/flox</sup>* mice and Cont indicates *Gcg<sup>+/+</sup>; Prkcd<sup>flox/flox</sup>* mice.

**Supplementary Figure 3.** Plasma insulin levels in pancreatic  $\alpha$ -cell-specific Pkc $\delta$  knockout mice. **a–c** No significant differences in fasting (a), random-fed (b), and arginine-induced (c, 15 min after 3 g/kg arginine intraperitoneal administration) plasma insulin levels between  $\alpha$ Pkc $\delta$ KO and control mice.  $n = 10$ , each group. \*  $p < 0.05$ ; t-test. Data are expressed as a box plot.  $\alpha$ Pkc $\delta$ KO indicates *Gcg<sup>CreERT2/+</sup>; Prkcd<sup>flox/flox</sup>* mice and Cont indicates *Gcg<sup>+/+</sup>; Prkcd<sup>flox/flox</sup>* mice.