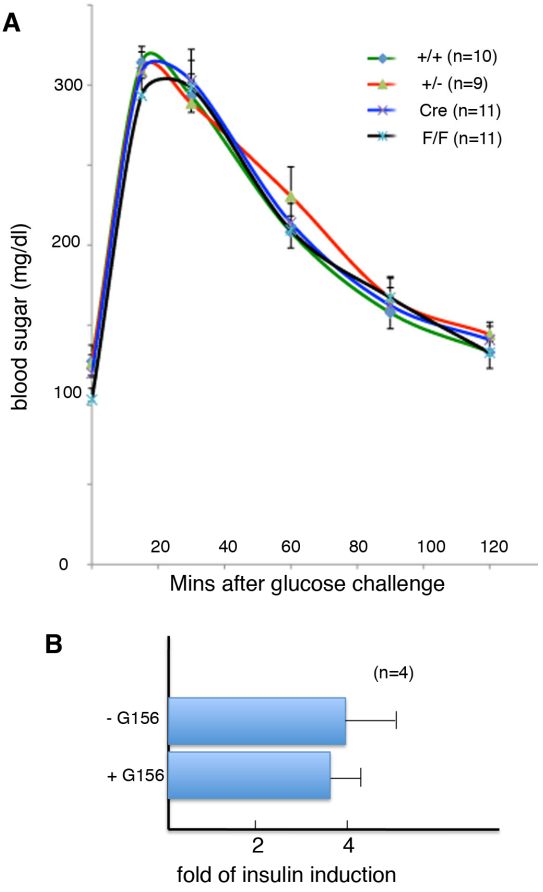
ONLINE APPENDIX—Supplementary materials:

Most materials and methods utilized are described in the manuscript. In order to test the effect of Gαo fragment to insulin secretion, an expression vector that codes for the 1-156 amino acid of the hamster Gαo was cloned into expression vector pCDNA3. The plasmid was co-transfected into Ins1 cells with pCIG vector, which expresses EGFP. Transfected cells were cultured for 3 days and were then sorted into eGFP+ (which are expected to express G1-156 as well) and eGFP- control cell populations. Then 20,000 sorted cells were cultured overnight in RMPI-1066 medium with 10% fetal bovine serum overnight. The cells were then conditioned with Ringer's solution with 2 mM glucose for one hour. Then 20mM glucose in Ringer's solution were utilized to induce insulin secretion for one hour. The insulin levels before and after glucose induction were assayed utilizing ELISA kit following manufacture's recommendation (Millipore, Billerica, MA).

Supplementary Figure 1. The *Gαo*$^{-}$ (-) allele from *Gαo*^{F} (F)does not produce dominant negative protein product. (A) GTT assay in mouse with *Gαo*$^{-}$ and *Pdx1*^{Cre} (Cre). Note the lack of GTT defects in all genotyped animals. (B) Fold of insulin secretion induction by 20mM glucose in Ins1 cells transfected with a truncated Gαo protein (expected from the *Gαo*$^{-}$ allele). Presented are the ratios of insulin release after and before 20mM glucose induction (assayed from 4 batches of 20,000 FACS sorted cells).



Supplementary Figure 2. Gαo is not required for islet cell differentiation. (A) Insulin content in newly born *Gαo*$^{F/F}$ (F/F) and *Gαo*$^{F/F}$; *Pdx1*^{Cre} (F/F; Cre) mice. (B) Islet morphology in control (*F/F*) and mutant (*F/F; Cre)* pancreata. The darker-colored cells are results of hormone staining, developed as HRP reactions. Scale bar=20 μm.