

PHYSIOLOGY

Activation of G₁₂-type G proteins in pancreatic β cells protects against impaired glucose homeostasis

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Impaired insulin secretion represents a key feature of type 2 diabetes (T2D), a major global health burden. Insulin release from pancreatic β cells is regulated by G protein–coupled receptors linked to different functional classes of G proteins. The potential metabolic role of β cell G_{12/13} (G₁₂ hereafter) signaling remains unexplored. Thus, we studied the potential metabolic outcomes of β cell G₁₂ activation by using a combination of mouse gene knockout technology and β cell–specific chemogenetic approaches. We found that selective activation of β cell G₁₂ signaling strongly promoted insulin secretion, thus protecting mice against obesity-associated metabolic deficits and improving glycemic control in a mouse model of T2D. Mechanistic studies with perfused pancreatic mouse islets identified ROCK as a key downstream effector of G₁₂ signaling and potent trigger of insulin secretion. Collectively, these findings suggest that G protein–coupled receptors linked to G₁₂ in β cells represent potential targets for antidiabetic drugs.

INTRODUCTION

Type 2 diabetes (T2D) has emerged as a major health crisis in most parts of the world. According to the latest International Diabetes Federation (IDF) Diabetes Atlas (2025) (<https://diabetesatlas.org/resources/idf-diabetes-atlas-2025/>), about 10% of the adult population is living with T2D. In T2D, pancreatic β cells are no longer able to release sufficient amounts of insulin to overcome peripheral insulin resistance to maintain euglycemia (1–3). As a result, therapeutic strategies aimed at enhancing insulin release have proven useful in the management of T2D (3). However, currently only a relatively small number of drugs acts via this mechanism, including sulfonylureas and various incretin receptor mimetics (4, 5). Although these drugs are widely used, unwanted side effects often limit the use of these agents (6, 7). Thus, there is a need to develop additional classes of antidiabetic drugs that can stimulate insulin release with high efficacy but cause few side effects.

Like most other cell types, pancreatic β cells express dozens of G protein–coupled receptors (GPCRs) on their cell surface (8–10). GPCRs form the largest class of plasma membrane receptors and are the target of about one-third of all Food and Drug Administration–approved drugs (11, 12), testifying to their extraordinary clinical relevance. GPCRs are activated following the binding of extracellular ligands which are chemically very diverse (11). Ligand-activated GPCRs can bind to and activate one or more functional classes of heterotrimeric G proteins, the G_s, G_{i/o}, G_{q/11}, and G_{12/13} families (13). Numerous studies have shown that GPCR-mediated activation of β cell G_s and G_q-type G proteins promotes glucose-stimulated insulin secretion (GSIS) (8–10). For example, the two major incretins, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP), are highly efficacious in promoting insulin secretion via activation of β cell GLP-1 and GIP receptors, respectively (G protein preference: G_s) (8–10). On the other hand, receptor-mediated stimulation

of G proteins of the G_i family exerts an inhibitory effect on insulin release (8–10).

Although the effects of β cell G_s, G_{i/o}, and G_{q/11} signaling have been studied in considerable detail, little is known about the potential physiological and pathophysiological roles of β cell G_{12/13} signaling. G α_{12} and G α_{13} are coexpressed in most cell types (14), including mouse and human β cells (15, 16). At the molecular level, the best characterized effect following activation of G_{12/13} signaling is the activation of RhoA caused by G $\alpha_{12/13}$ -mediated activation of Rho guanine nucleotide exchange factors (RhoGEFs) (14, 17).

The lack of GPCRs capable of selectively activating G_{12/13} signaling has been a major obstacle in elucidating the *in vivo* functional roles of this class of G proteins in β cells and other cell types. Research in this field has been further hampered by the lack of compounds that can selectively interfere with G_{12/13} signaling and the fact that G α_{12} and G α_{13} share a high degree of sequence homology and functional redundancy (14, 17).

To shed light on the potential metabolic outcomes of selectively stimulating G_{12/13} signaling in β cells *in vivo*, we generated several new mouse strains, involving the use of chemogenetic and G α knockout (KO) strategies. Systematic phenotyping studies indicated that selective activation of G_{12/13} signaling in β cells *in vivo* led to a pronounced increase in insulin release, resulting in potent blood glucose-lowering effects. Stimulation of this pathway greatly improved glucose homeostasis in obese mice and in a mouse model of T2D. We also identified a GPCR (GPR183) that is endogenously expressed by mouse and human β cells that stimulates insulin release in a G_{12/13}-dependent and glucose-dependent manner. These observations suggest that strategies aimed at enhancing G_{12/13} signaling in β cells may lead to the development of drugs useful for the treatment of T2D and related metabolic disorders.

RESULTS

Generation of mice selectively expressing the G12D designer receptor in β cells (beta-G12D mice)

We first generated a mouse line that expressed a G_{12/13}-coupled designer GPCR (18–20) selectively in β cells. As reported previously (21–24), this designer GPCR, also known as G12 DREADD (designer receptor

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exclusively activated by a designer drug; short name used hereafter: G12D) (20, 24), can be selectively activated by certain small synthetic compounds such as clozapine-*N*-oxide (CNO) (23) or deschloro-clozapine (DCZ) (22). We recently demonstrated (20) that agonist stimulation of G12D activates both G₁₂ and G₁₃ but does not lead to the activation of G_s and G_{q/11}.

Specifically, we crossed *Pdx1-Cre-ER* transgenic mice (25, 26) with *Rosa26-LSL-G12D-IRES-GFP* mice (short name: LSL-G12D mice) (19). Tamoxifen treatment of LSL-G12D mice harboring the *Cre-ER* transgene yielded mice selectively expressing G12D in β cells (short name: beta-G12D mice). LSL-G12D littermates lacking the *Cre-ER* transgene served as control mice (fig. S1). For consistency, control mice were also treated with tamoxifen. Western blotting studies showed that G12D was selectively expressed in pancreatic islets of beta-G12D mice (Fig. 1A). Moreover, immunostaining studies demonstrated that G12D was selectively expressed in pancreatic β cells of beta-G12D mice but not in control littermates (Fig. 1B). As expected, glucagon-expressing pancreatic α cells did not express G12D (fig. S2).

Activation of β cell G12D signaling promotes insulin release and improves glucose tolerance

Initially, we carried out a series of *in vivo* metabolic studies with male mice maintained on regular chow. In the absence of a DREADD agonist, beta-G12D mice and control littermates did not differ in body weight and fed and fasting blood glucose and plasma insulin levels (fig. S3). We first injected control and beta-G12D mice intraperitoneally with a single dose of CNO (3 mg/kg) or saline (Fig. 1C), followed by the measurement of blood glucose levels. CNO treatment of the three control groups (saline-injected and CNO-injected control mice and saline-injected beta-G12D mice) resulted in a modest increase in blood glucose levels, most likely caused by the injection stress. This effect was absent in CNO-treated beta-G12D mice (Fig. 1C), indicating that activation of β cell G12D signaling counteracts the stress-induced elevation of blood glucose levels.

An intraperitoneal glucose tolerance test (IGTT) showed that beta-G12D mice acutely injected with CNO (3 mg/kg, intraperitoneally) displayed greatly improved glucose tolerance, as compared to the three control groups (Fig. 1D). Administration of an intraperitoneal glucose bolus (2 g/kg) resulted in a significant augmentation of plasma insulin levels in beta-G12D mice coinjected with CNO, as compared with the three control groups (GSIS; Fig. 1E). This observation suggests that activation of β cell G12D receptors promotes insulin release, which, in turn, leads to improved glucose tolerance.

An intraperitoneal insulin tolerance test (ITT) demonstrated that CNO-treated beta-G12D mice showed a more pronounced reduction in blood glucose levels, as compared with CNO-treated control mice (Fig. 1F). Although both groups of mice received the same dose of insulin (1 U/kg, intraperitoneally), plasma insulin levels were significantly higher in the CNO-treated beta-G12D mice during the course of the ITT (Fig. 1G), explaining why this group of mice showed a more robust decrease in blood glucose levels. Most likely, the increase in plasma insulin levels displayed by the CNO-treated beta-G12D mice during the ITT was caused by the release of endogenous insulin following stimulation of β cell G12D receptors.

As shown in fig. S4, female beta-G12D mice displayed metabolic phenotypes that closely mirrored those observed with male beta-G12D mice (see previous paragraphs).

Acute stimulation of β cell G12D signaling protects mice against obesity-associated metabolic deficits

We next investigated whether acute activation of G_{12/13} signaling in β cells was able to improve impaired glucose homeostasis in diet-induced obese mice (males). We maintained beta-G12D mice and control littermates (age: 2 to 3 months) on a high-fat diet (HFD) for at least 8 weeks and then subjected the mice to a series of metabolic tests. After 8 weeks of HFD feeding, the two groups of mice showed similar body weights (Fig. 2A).

We first subjected obese beta-G12D mice and control littermates to an IGTT. Mice were injected intraperitoneally with either glucose alone (1 g/kg) or a mixture of glucose (1 g/kg) plus CNO (3 mg/kg). As shown in Fig. 2B, CNO-treated beta-G12D mice showed a pronounced improvement in glucose tolerance, as compared to the three control groups (saline-injected and CNO-injected control mice and saline-injected beta-G12D mice). Coinjection of beta-G12D mice with glucose plus CNO also resulted in greatly enhanced plasma insulin levels, as compared to the three control groups (Fig. 2C), providing an explanation for the notable improvement in glucose tolerance displayed by the CNO-treated beta-G12D mice.

Coinjection of obese beta-G12D mice and control littermates with insulin (1.5 U/kg, intraperitoneally) and CNO (3 mg/kg, intraperitoneally) led to a more robust glucose-lowering effect in beta-G12D mice, as compared to the three control groups (ITT; Fig. 2D). During the ITT, plasma insulin levels were significantly elevated in beta-G12D mice, as compared to control littermates (Fig. 2E), explaining why the G12D-expressing mice displayed more pronounced glucose-lowering effects during this test. These data suggest that CNO treatment of beta-G12D mice further augments plasma insulin levels during the ITT by promoting the release of endogenous insulin via activation of β cell G12D receptors.

In sum, these observations indicate that selective activation of β cell G_{12/13} signaling strongly stimulates insulin secretion, thereby reversing glucose intolerance in obese mice.

Chronic stimulation of β cell G12D signaling reduces hyperglycemia in streptozotocin-induced diabetes

We next explored whether chronic activation of β cell G12D signaling was able to reduce elevated blood glucose levels in a mouse model of diabetes. Specifically, we injected beta-G12D mice and control littermates (8-week-old males) with a low dose of streptozotocin (STZ) for 5 consecutive days (50 mg/kg, intraperitoneally, daily) (27–29). Previous works have shown that this STZ injection protocol decreases β cell mass by ~80% (27, 28). A similar decrease in β cell mass has been found in humans with advanced T2D (30). Moreover, Hrovatin *et al.* (31) recently showed that the gene expression pattern of β cells from mice subjected to a low-dose STZ injection protocol was similar to that observed with β cells from humans with T2D.

All beta-G12D mice and control littermates received DCZ, a DREADD agonist that is more potent and metabolically stable than CNO (22), via the drinking water (10 mg/liter), starting from the first day of STZ treatment. We also included an additional group of control mice that consumed DCZ water but were not treated with STZ. We previously demonstrated that DCZ is stable in aqueous solution at room temperature (23°C) for at least 4 weeks (24). Moreover, prolonged consumption of DCZ water does not affect water and food intake in control mice (24). Four weeks after the start of the STZ injection protocol, STZ-treated control mice weighed significantly

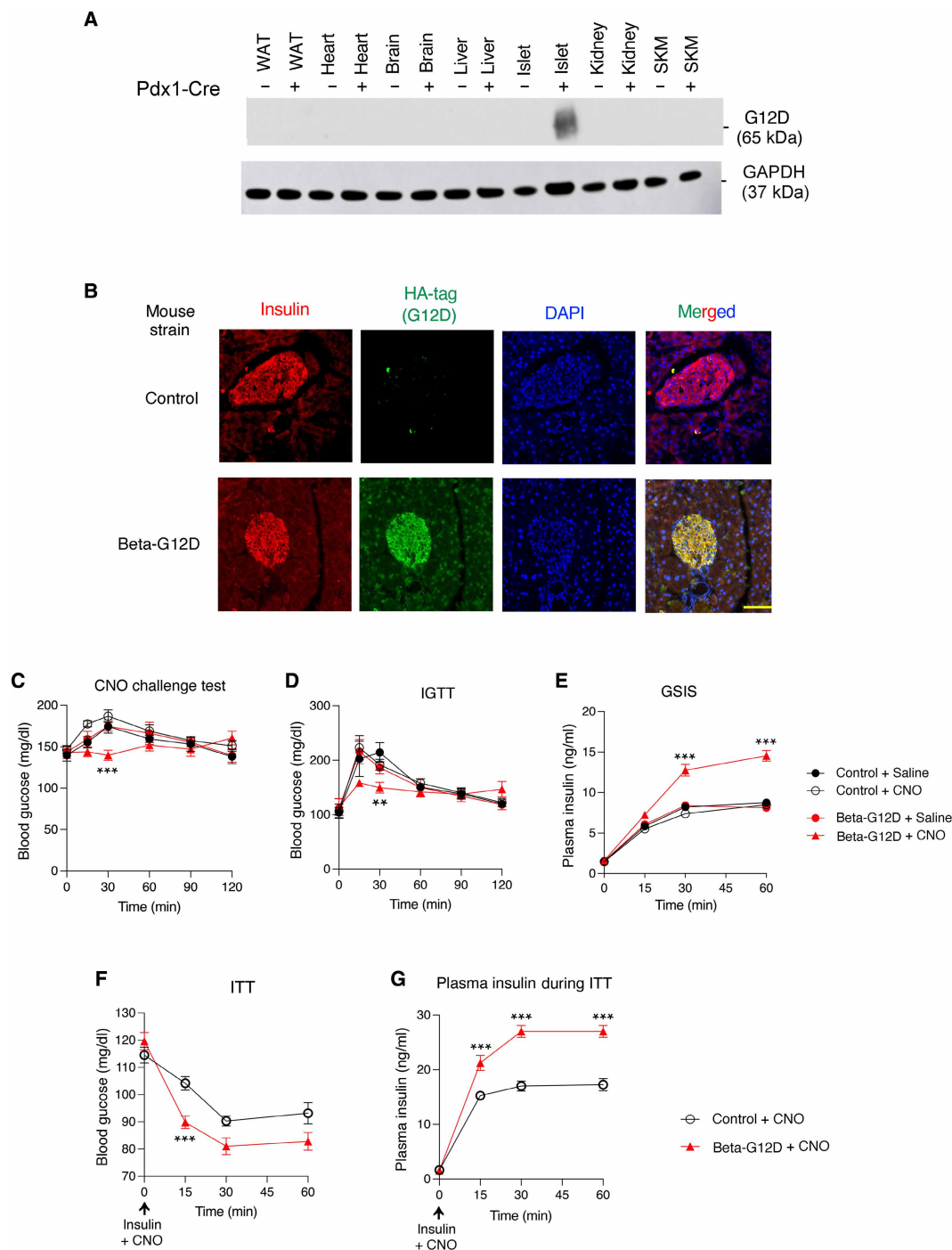


Fig. 1. Activation of β cell G12D receptors increases insulin secretion and improves glucose tolerance in mice consuming regular chow. (A) Western blot indicating the selective expression of G12D in islets of tamoxifen-treated *LSL-G12D Pdx1-Cre-ERtm* mice (beta-G12D mice). G12D was detected with an antibody targeting the N-terminal HA-tag. WAT, white adipose tissue; SKM, skeletal muscle; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. (B) Pancreatic sections from beta-G12D and control mice were stained for G12D (HA-tag), insulin, and 4',6-diamidino-2-phenylindole (DAPI) (staining of nuclei). Note that G12D was expressed in insulin-containing β cells of beta-G12D mice but not in control mice. The images shown are representative of pancreatic sections prepared from three to four mice per strain. Scale bar, 50 μ m. (C to G) In vivo metabolic tests performed with beta-G12D mice and control littermates maintained on regular chow (RC). All studies were performed using 9- to 16-week-old male mice. (C) CNO challenge test. Freely fed mice were injected intraperitoneally with either CNO (3 mg/kg) or saline, followed by the monitoring of blood glucose levels. (D) IGTT. Following an overnight fast (14 hours), mice received glucose (2 g/kg, intraperitoneally), either in the presence or absence of CNO (3 mg/kg intraperitoneally). (E) GSIS. Beta-G12D mice and control littermates were coinjected intraperitoneally with a glucose bolus (2 g/kg) plus CNO (3 mg/kg) after an overnight fast (14 hours). Plasma insulin levels were then measured at the indicated postinjection time points. (F and G) ITT. After a 4-hour fast, mice of the indicated genotypes were treated intraperitoneally with insulin (1 U/kg) plus CNO (3 mg/kg), followed by the monitoring of blood glucose (F) and plasma insulin levels (G). Data represent means $\pm$ SEM ($n = 6$ to 8 mice per group). ** $P < 0.01$ and *** $P < 0.001$ versus the corresponding control group(s) (two-way ANOVA followed by Bonferroni's post hoc test).

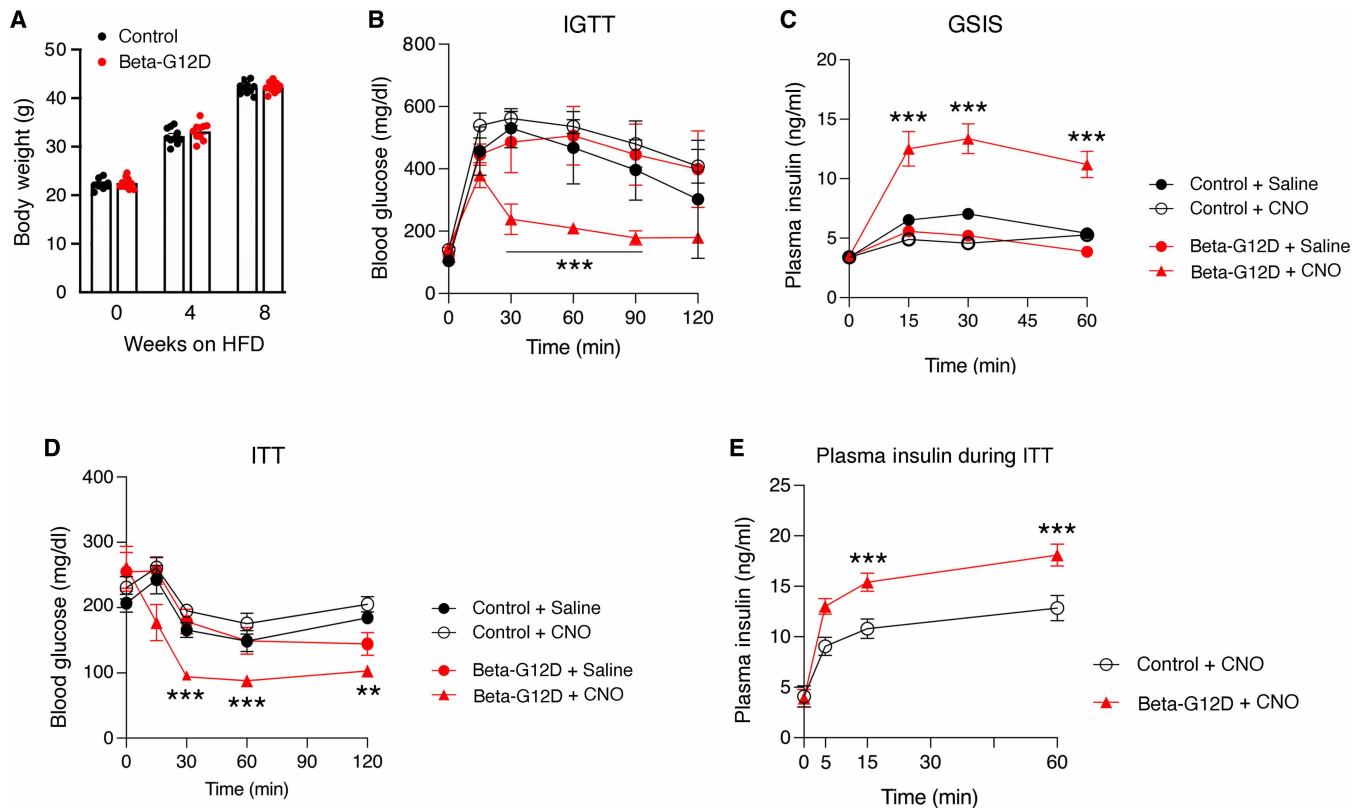


Fig. 2. Activation of β cell G12D receptors greatly improves glucose homeostasis in obese mice. Beta-G12D mice and control littermates (males) were maintained on an HFD for at least 8 weeks and then subjected to several in vivo metabolic tests. **(A)** Body weight measurements. Mice started to consume the HFD when they were ~10 weeks old. **(B)** IGTT. After an overnight fast (14 hours), mice (age: 18 weeks) received glucose (1 g/kg, intraperitoneally) in the presence or absence of CNO (3 mg/kg intraperitoneally), followed by the monitoring of blood glucose levels. **(C)** GSIS. Following an overnight fast (14 hours), mice were injected with glucose (1 g/kg, intraperitoneally) in the presence or absence of CNO (3 mg/kg, intraperitoneally). Plasma insulin levels were then measured at the indicated postinjection time points. **(D)** ITT. Mice were fasted for 4 hours and then injected with insulin (1.5 U/kg, intraperitoneally) in the presence or absence of CNO (3 mg/kg, intraperitoneally), followed by the monitoring of blood glucose levels. **(E)** Plasma insulin levels during the ITT. After a 4-hour fast, mice (age: 18 weeks) were injected intraperitoneally with insulin (1.5 U/kg) plus CNO (3 mg/kg) (see D). Subsequently, plasma insulin levels were measured at the indicated time points. Data represent means $\pm$ SEM ($n = 6$ to 8 mice per group). ** $P < 0.01$ and *** $P < 0.001$ versus the corresponding control group(s). Statistical significance was determined by a two-way ANOVA followed by Bonferroni's post hoc test.

less than control mice that had not received STZ (Fig. 3A). In contrast, at the same time point, the body weight of STZ-treated beta-G12D mice did not differ significantly from that of control mice that had not been injected with STZ (Fig. 3A).

As expected, control mice that had not been treated with STZ showed unchanged blood glucose levels during the 4-week observation period (Fig. 3B). In contrast, STZ-treated control mice developed severe hyperglycemia during this time period (Fig. 3B). In agreement with this finding, plasma insulin levels measured 3 weeks after the start of the STZ injection protocol were greatly reduced in STZ-treated control mice, as compared to non-STZ-treated control littermates (Fig. 3C). Notably, starting during week one, STZ+DCZ-treated beta-G12D mice showed significantly lower blood glucose levels than their STZ+DCZ-treated control littermates (Fig. 3B). Consistent with this observation, STZ+DCZ-treated beta-G12D mice displayed ~2-fold higher plasma insulin levels than STZ+DCZ-treated control mice (Fig. 3C).

Additional in vivo metabolic studies showed that STZ+DCZ-treated beta-G12D mice displayed a marked improvement in glucose tolerance, as compared with STZ+DCZ-treated control mice which exhibited severe glucose intolerance (IGTT; Fig. 3D). Moreover, in

contrast to STZ+DCZ-treated control mice, STZ+DCZ-treated beta-G12D mice showed a marked increase in plasma insulin levels following injection of an intraperitoneal glucose bolus (1 g/kg) (GSIS; Fig. 3E).

As expected, STZ+DCZ-treated control mice showed a pronounced reduction in total pancreatic insulin content, β cell mass, and islet size, as compared to control mice that had not received STZ (Fig. 3, F to H). STZ+DCZ-treated beta-G12D mice showed a significant increase in total pancreatic insulin, β cell mass, and islet size, as compared to STZ+DCZ-treated control mice (Fig. 3, F to H). Representative immunofluorescence images of pancreatic islets from the three groups of mice are shown in Fig. 3I.

Together, these data indicate that chronic activation of β cell $G_{12/13}$ signaling leads to a notable improvement in glucose homeostasis in the STZ mouse model of T2D and partially protects against STZ-induced loss of β cell mass and pancreatic insulin content.

Expression of a constitutively active version of $G\alpha_{12}$ in β cells promotes insulin secretion and lowers blood glucose levels

To further support the concept that chronic stimulation of β cell $G_{12/13}$ signaling leads to improved glycemic control, we expressed a

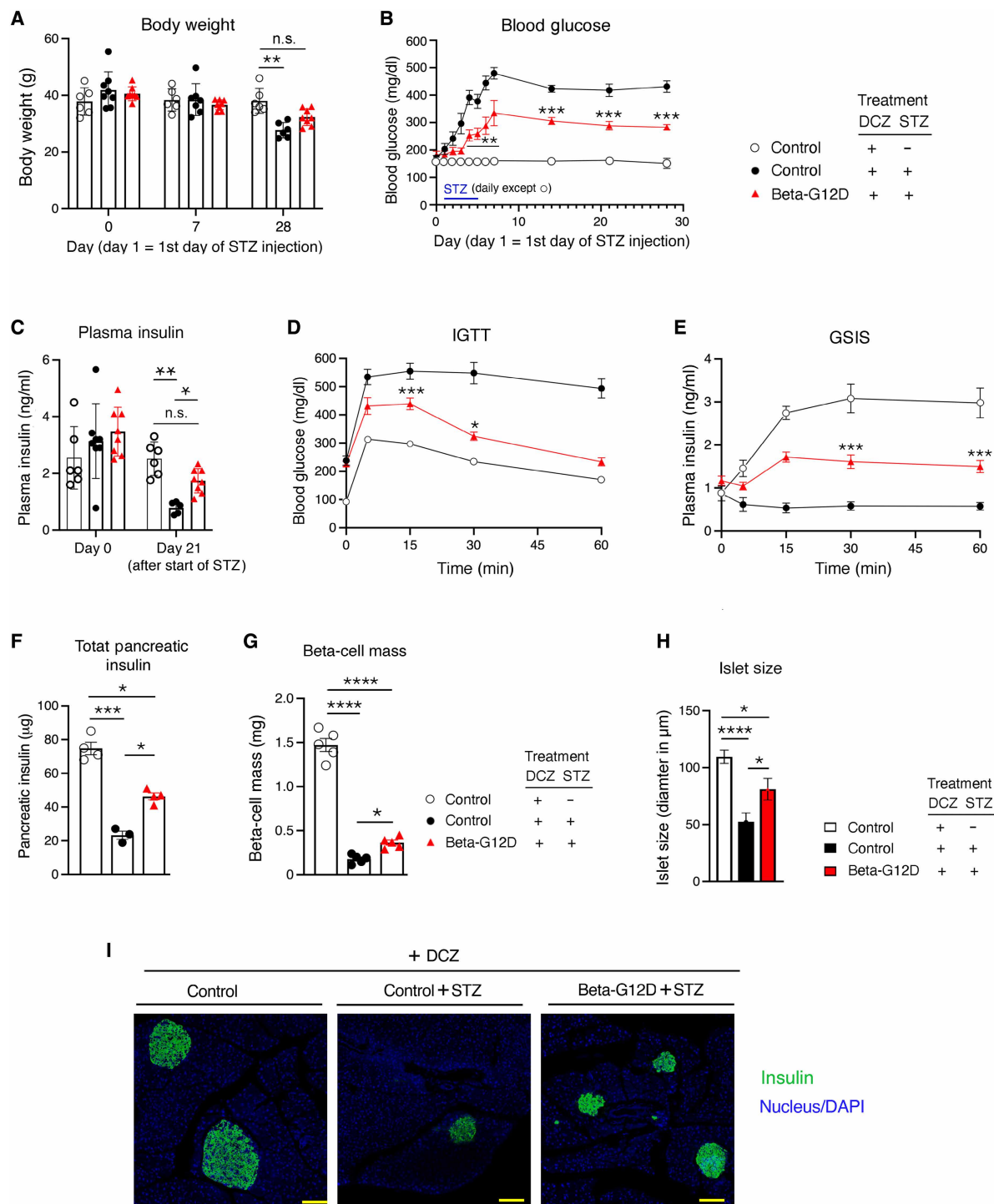


Fig. 3. Chronic activation of β cell G12D signaling alleviates STZ-induced diabetes. Beta-G12D mice and control littermates (males; age: 8 weeks) were injected with a low dose of STZ for 5 consecutive days (50 mg/kg, intraperitoneally, daily). Another set of control mice did not receive STZ. All three groups of mice consumed drinking water containing DCZ (10 mg/liter) throughout the duration of the study. (A) Changes in body weight over time ($n = 6$ to 8 per group). (B) Effect of STZ treatment on blood glucose levels. Mice had free access to food (regular chow) ($n = 7$). (C) Plasma insulin levels before STZ injections (day 0) and 21 days after the first STZ injection. Mice had free access to food ($n = 6$). (D) IGTT. Following a 6-hour fast, mice were injected with glucose (1 g/kg, intraperitoneally), followed by the monitoring of blood glucose levels. (E) GSIS. After an 8-hour fast, mice received an intraperitoneal glucose bolus (1 g/kg). Plasma insulin levels were then determined at the indicated postinjection time points ($n = 5$). (F) Total pancreatic insulin content ($n = 3$). (G) β Cell mass ($n = 5$). (H) Quantification of islet size at the end of the DCZ treatment period (mouse age: 15 weeks; $n = 5$). (I) Representative immunofluorescence images showing insulin staining of islets from the indicated groups of mice. Scale bars, 50 μ m. Data are presented as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ versus the corresponding control value(s) (two-way ANOVA followed by Bonferroni's post hoc test). n.s., no statistically significant difference.

constitutively active version of $G\alpha_{12}$ (CA- $G\alpha_{12}$; short name: CA-G12) selectively in β cells (beta-CA-G12 mice). The CA- $G\alpha_{12}$ protein contained the Gln231Leu (Q231L) point mutation (32). Beta-CA-G12 mice were generated by using an adeno-associated virus (AAV)-based approach previously described by Makhmutova *et al.* (33). Wild-type (WT) mice (males) were injected intraperitoneally with AAVs (serotype 8) containing the rat insulin promoter 2 (RIP2) promoter, thus leading to the β cell-specific expression of G12D or mCherry (control mice) (viruses used: AAV-RIP2-CA-G12-mCherry and AAV-RIP2-mCherry, respectively) (Fig. 4A). As expected, glucagon-expressing pancreatic α cells did not express CA-G12-mCherry (Fig. 4A). Western blotting studies confirmed that CA-G12-mCherry was not expressed by extra-islet tissues (fig. S5A).

Beta-CA-G12 and control mice were subjected to in vivo metabolic studies 1 week after AAV injections. The two groups of mice did not differ in body weight (Fig. 4B). Notably, beta-CA-G12 mice showed significantly reduced blood glucose levels (mice had free access to regular chow), as compared to control littermates (Fig. 4C). During the same time period, plasma insulin levels were significantly increased in beta-CA-G12 mice (Fig. 4D). Beta-CA-G12 mice also displayed a significant improvement in glucose tolerance, as compared to the corresponding control mice (IGTT; Fig. 4E). Moreover, beta-CA-G12 mice showed a more robust increase in plasma insulin levels after treatment with an intraperitoneal glucose bolus (2 g/kg) (GSIS; Fig. 4F). Following an overnight fast (14 hours), blood glucose and plasma insulin levels did not differ significantly between the two groups of mice, indicating that β cell G_{12} signaling promotes insulin secretion in a glucose-dependent manner (Fig. 4, G and H).

Mice lacking both $G\alpha_{12}$ and $G\alpha_{13}$ in β cells show impaired glucose homeostasis

We also carried out metabolic studies with mice that lacked $G\alpha_{12}$ throughout the body and $G\alpha_{13}$ selectively in β cells. Hereafter, we refer to these mice simply as beta-G12/13 KO mice (genetic background: C57BL/6). Initially, we crossed *Pdx1-Cre-ER* mice with *Gna12*^{-/-} *Gna13* *fl/fl* mice (34). In the next step, *Cre*-positive *Gna12*^{-/+} *Gna13* *fl/fl* mice were backcrossed to *Gna12*^{-/-} *Gna13* *fl/fl* mice to obtain *Pdx1-Cre-ER* *Gna12*^{-/-} *Gna13* *fl/fl* mice (beta-G12/13 KO mice) and control littermates lacking the *Cre* transgene (*Gna12*^{-/-} *Gna13* *fl/fl* mice; short name: G12 KO). *Pdx1-Cre-ER* *Gna12*^{-/-} *Gna13* *fl/fl* mice were treated with tamoxifen to induce the selective expression of Cre recombinase in pancreatic β cells (see Materials and Methods for details).

Western blotting studies confirmed that $G\alpha_{12}$ expression was lost in pancreatic islets derived from $G\alpha_{12}$ KO (G12 KO) and beta-G12/13 KO mice (fig. S6). In contrast, $G\alpha_{12}$ could be easily detected in WT pancreatic islets (fig. S6). Use of a $G\alpha_{13}$ -specific antibody demonstrated that $G\alpha_{13}$ was present in WT and G12 KO pancreatic islets but was barely detectable in islets from beta-G12/13 KO mice, as expected (fig. S6).

We next subjected G12 KO and beta-G12/13 KO mice (males), together with age-matched male WT control mice of the same genetic background (C57BL/6), to a series of metabolic tests. The three groups of mice showed similar body weight (Fig. 5A). Mice were given either free access to food (regular chow) or were fasted overnight for 14 hours. We found that blood glucose levels did not differ significantly between the three groups of mice under both fed and fasting conditions (Fig. 5B). Similarly, the three groups of

mice showed similar plasma insulin levels under fasting conditions (Fig. 5C). However, beta-G12/13 KO mice with free access to food showed a significant reduction in plasma insulin levels, as compared to the corresponding WT mice (Fig. 5C).

An IGTT demonstrated that both G12 KO and beta-G12/13 KO mice showed impaired glucose tolerance, as compared to the corresponding WT animals (Fig. 5D). GSIS was moderately reduced in G12 KO mice and more severely impaired in beta-G12/13 KO mice, respectively, as compared to WT control mice (Fig. 5E).

Total pancreatic insulin content (Fig. 5F) and β cell mass (Fig. 5G) did not differ significantly between the three groups of mice. These observations suggest that the deficits in glucose homeostasis and insulin release observed with the $G_{12/13}$ mutant mice were not caused by reduced pancreatic insulin levels but resulted from impaired or absent $G_{12/13}$ signaling.

G12D-mediated improvements in glucose homeostasis require β cell $G_{12/13}$ signaling

To demonstrate in a more direct manner that activation of the G12D receptor expressed by β cells improved glycemic control by stimulating $G_{12/13}$ signaling, we carried out additional in vivo studies with beta-G12/13 KO mice. By using an AAV-based strategy first described by Makhmutova *et al.* (33), we selectively expressed the G12D construct in mouse β cells by injecting WT mice intraperitoneally with an AAV coding for G12D under the transcriptional control of the β cell-specific RIP2 promoter (AAV8-RIP2-G12D virus; see Materials and Methods for details). Four weeks after virus treatment, we confirmed that G12D was selectively expressed in β cells (Fig. 6A). Expectedly, glucagon-expressing pancreatic α cells did not express G12D (Fig. 6A). In addition, immunoblotting studies showed that G12D was not expressed by extra-islet tissues (fig. S5B). WT mice injected intraperitoneally with an AAV coding for channel rhodopsin (AAV8-RIP2-ChR2 virus served as control animals). We then treated the two groups of mice with an intraperitoneal bolus of a mixture of glucose (2 g/kg) and CNO (3 mg/kg). As expected from the data obtained with beta-G12D mice (Figs. 1 and 2), mice expressing G12D in their β cells showed significantly improved glucose tolerance and enhanced GSIS, as compared to control mice lacking G12D (Fig. 6, B and C). Notably, these beneficial metabolic effects were absent in beta-G12/13 KO mice treated with the AAV8-RIP2-G12D virus (Fig. 6, D and E), clearly indicating that activation of β cell G12D receptors improved glucose tolerance and augmented GSIS by stimulating β cell $G_{12/13}$ signaling.

Insulin release studies with isolated pancreatic islets prepared from beta-G12D mice

To gain insight into the cellular mechanisms through which activation of β cell $G_{12/13}$ signaling stimulates insulin release, we first carried out in vitro perfusion studies using pancreatic islets prepared from control and beta-G12D mice. When islets were exposed to a low concentration of glucose (3 mM), CNO (10 μ M) had little or no effect on basal insulin release in control and beta-G12D islets (Fig. 7A). In the absence of CNO, a high concentration of glucose (12 mM)-stimulated insulin secretion in an identical manner in the two groups of islets (Fig. 7B). However, in the presence of CNO (10 μ M), GSIS was significantly augmented in the beta-G12D islets (Fig. 7, A to C), in good agreement with the outcome of the in vivo insulin release studies.

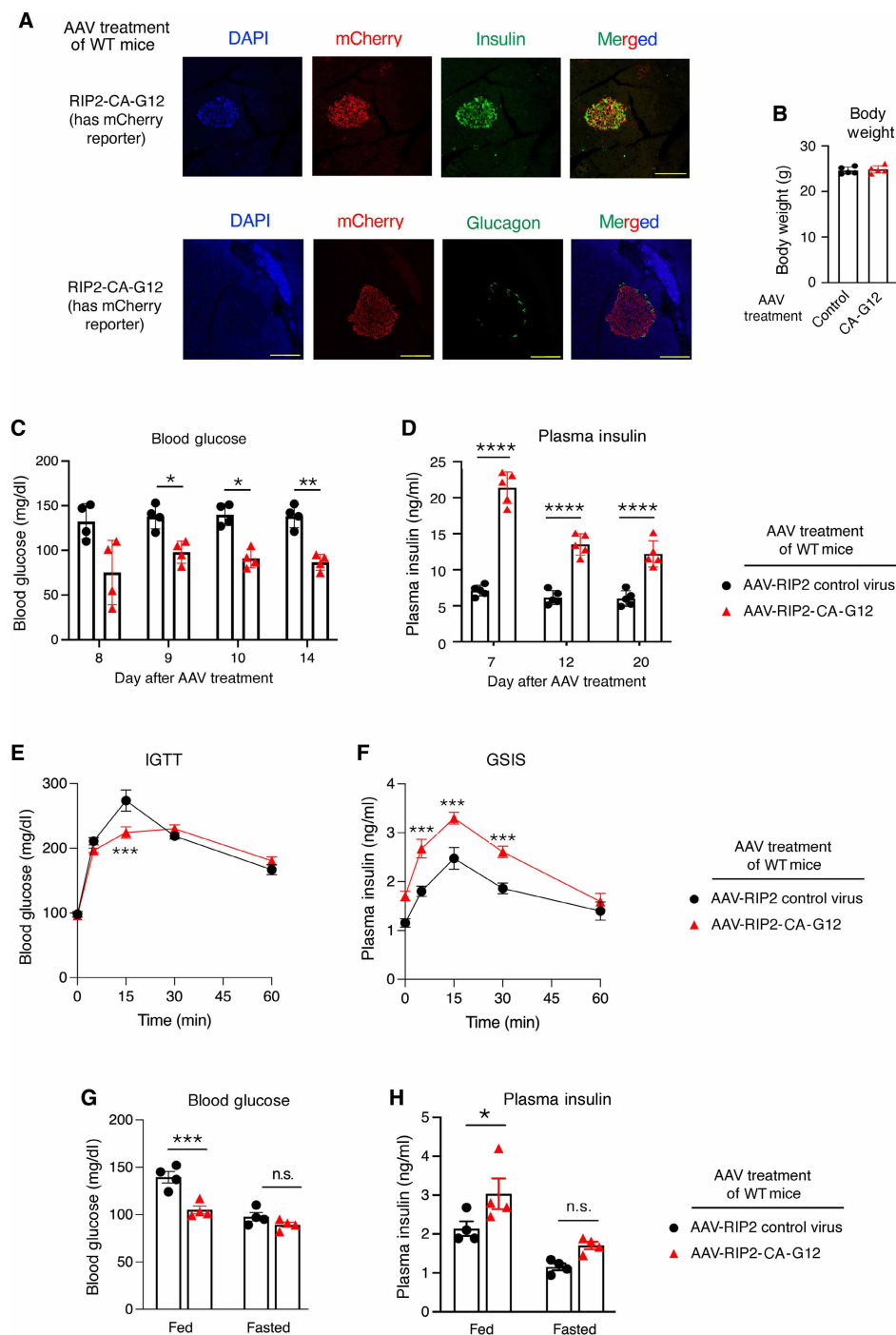


Fig. 4. Selective expression of a constitutively active version of $G\alpha_{12}$ in β cells promotes insulin secretion and lowers blood glucose levels. Mice (males) expressing a constitutively active mutant of $G\alpha_{12}$ (CA- $G\alpha_{12}$; short name: CA-G12) selectively in β cells (beta-CA-G12 mice) were generated by using an AAV-based approach (see Materials and Methods). In vivo metabolic studies were carried out with beta-CA-G12 and control mice 1 week after intraperitoneal AAV injections [AAV-RIP2-CA-G12 and AAV-RIP2-mCherry (control), respectively]. **(A)** Representative images indicating that treatment of WT mice with AAV-RIP2-CA-G12 (note that this construct also codes for an mCherry reporter) results in the selective expression of CA-G12 in β cells. CA-G12D is not expressed in glucagon-expressing α cells. Scale bars, 50 μ m. **(B)** Body weight (8-week-old mice 1 week after AAV injections). **(C)** Blood glucose levels of mice with free access to food (regular chow) at the indicated postinjection days. **(D)** Plasma insulin levels of freely fed mice at the indicated postinjection days. **(E)** IGTT. After a 14-hour fast, mice were injected with glucose (2 g/kg, intraperitoneally), followed by the monitoring of blood glucose levels. **(F)** GSIS. After a 14-hour fast, mice received an intraperitoneal glucose bolus (2 g/kg). Plasma insulin levels were then measured at the indicated postinjection time points. **(G and H)** Fasting blood glucose (G) and fasting plasma insulin (H) levels are not significantly altered in WT mice treated with the AAV-RIP2-CA-G12 virus. Mice were fasted overnight for 14 hours. Data shown in (C) to (H) were performed using 10- to 15-week-old male mice. Data are presented as means $\pm$ SEM ($n = 4$ to 6 mice per group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ versus the corresponding control value. Statistical significance was determined by a two-way ANOVA followed by Bonferroni's post hoc test. n.s., no statistically significant difference.

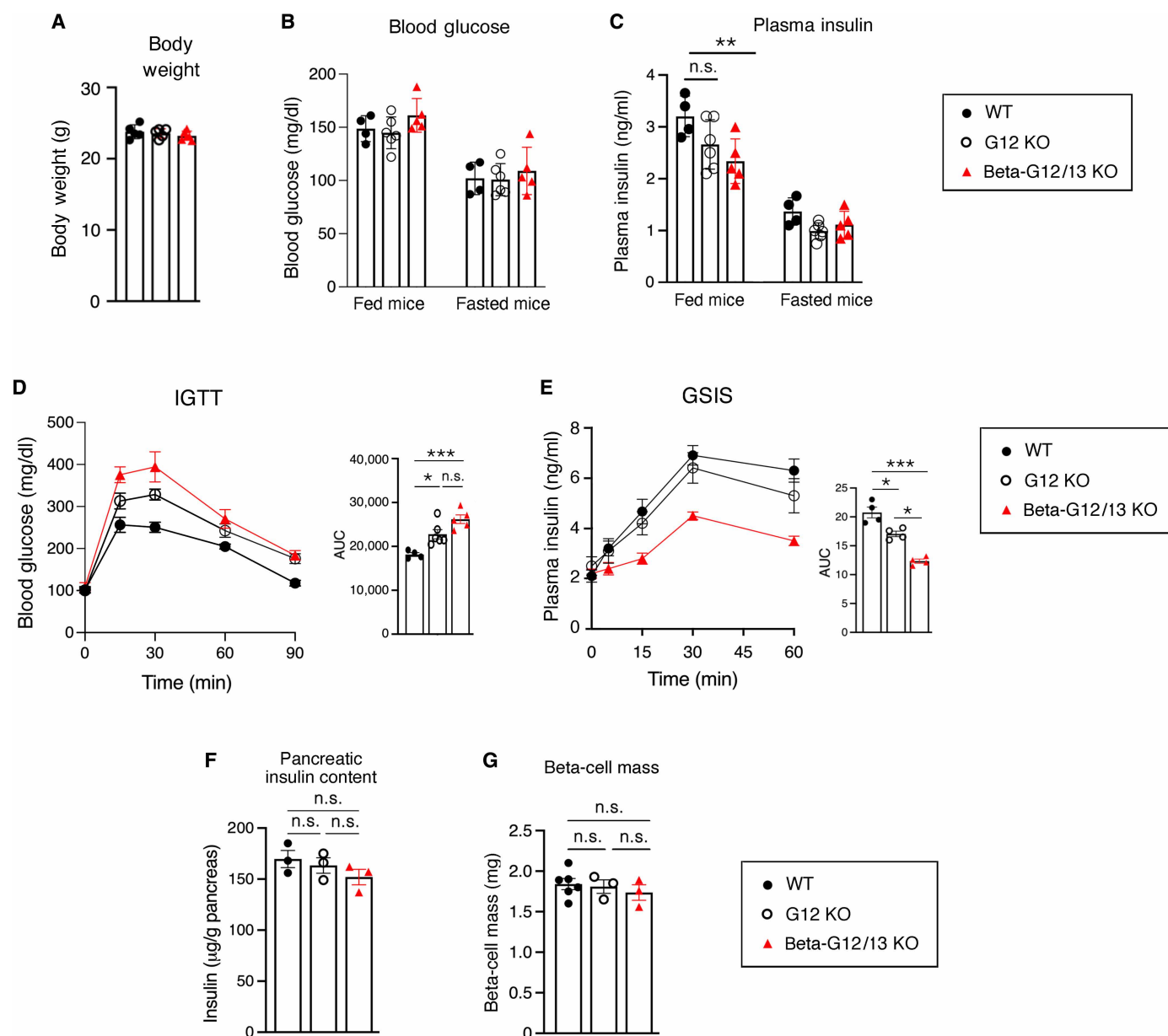


Fig. 5. Mice lacking $G_{12/13}$ signaling in β cells show impaired glucose tolerance and reduced GSIS. WT, G12 KO (mice lacking $G\alpha_{12}$ throughout the body; genotype: *Gna12*^{-/-} *Gna13* fl/fl), and beta-G12/13 KO mice (mice lacking $G\alpha_{12}$ throughout the body and $G\alpha_{13}$ selectively in β cells; genotype: *Pdx1-Cre-ERtm* *Gna12*^{-/-} *Gna13* fl/fl) were obtained as described in Materials and Methods. All mice (males) were treated with tamoxifen when they were 6 to 8 weeks old (see Materials and Methods). One week after the last tamoxifen injection, mice maintained on regular chow were subjected to a series of metabolic tests. **(A)** Body weight measurements (mouse age: 9 weeks). **(B and C)** Blood glucose (B) and plasma insulin (C) levels of mice that had free access to food (fed) or that had been fasted overnight for 14 hours (mouse age: 9 weeks). **(D)** IGTT. Following an overnight (14 hours) fast, mice were injected with glucose (2 g/kg, intraperitoneally), followed by the monitoring of blood glucose levels (mouse age: 12 weeks). **(E)** GSIS. After an overnight fast (14 hours), mice received a glucose bolus (2 g/kg, intraperitoneally). Plasma insulin levels were then measured at the indicated time points (mouse age: 14 weeks). AUC, area under the curve. **(F)** Pancreatic insulin content ($n = 3$ per group; mouse age: 16 weeks). **(G)** β Cell mass ($n = 3$ per group; mouse age: 18 weeks). Data are presented as means $\pm$ SEM (A to E: $n = 4$ to 6 mice per group). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (two-way ANOVA followed by Bonferroni's post hoc test). n.s., no statistically significant difference.

The most thoroughly characterized cellular pathway activated by $G_{12/13}$ signaling is the stimulation of the RhoA-ROCK signaling cascade (14, 17). We therefore hypothesized that G12D-mediated enhancement of GSIS may require the activity of ROCK. In agreement with this hypothesis, coincubation of beta-G12D islets with CNO (10 μ M) and Y27623 (20 μ M), a selective ROCK inhibitor,

prevented the CNO-mediated augmentation of GSIS observed with CNO-treated beta-G12D islets (Fig. 7B).

Previous works have demonstrated that ROCK-mediated remodeling of the actin cytoskeleton plays a critical role in stimulating insulin release from β cells (35, 36). Consistent with this concept, we found that G12D-mediated enhancement of GSIS was prevented by

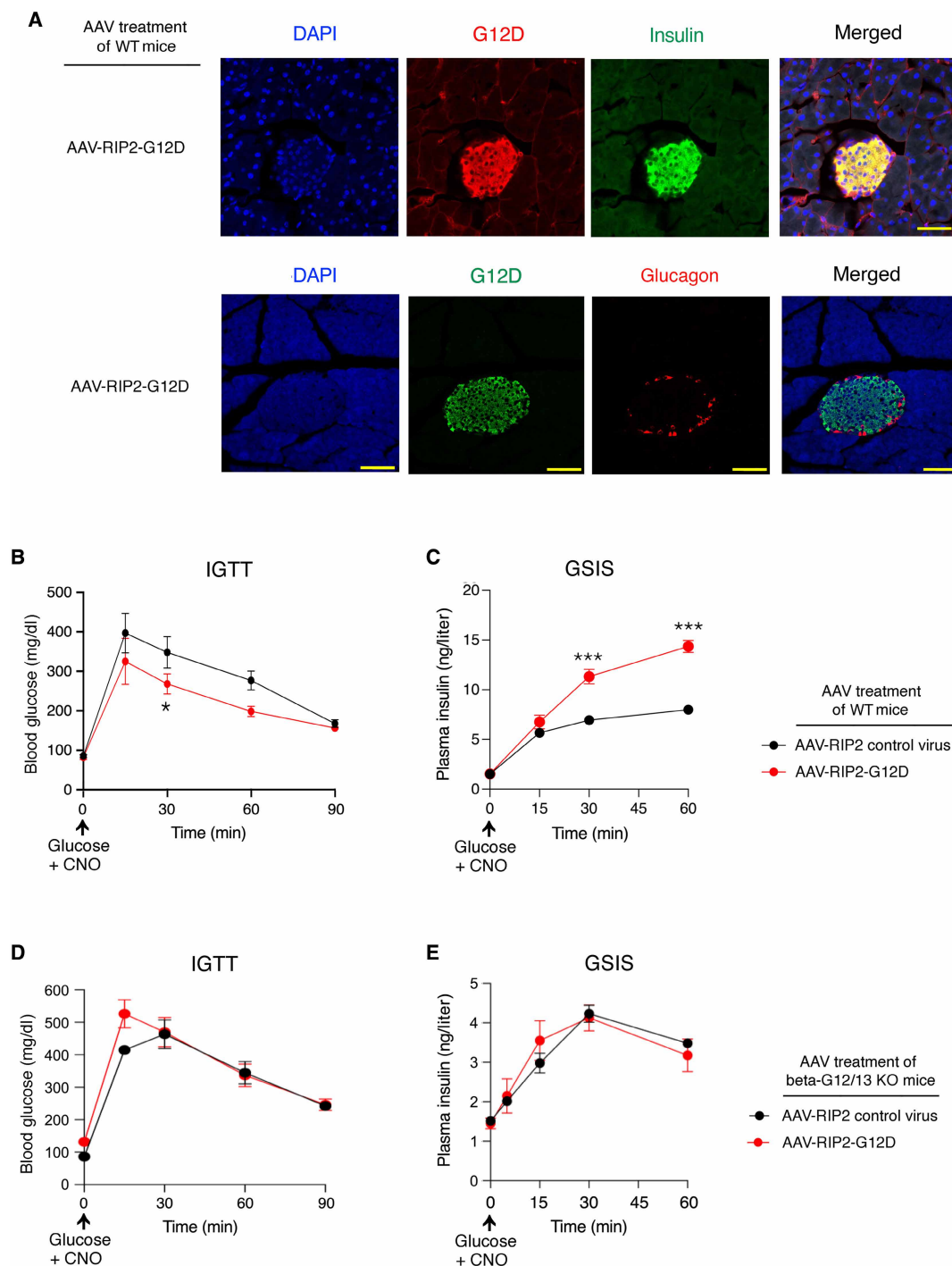


Fig. 6. G12D-mediated improvements in glucose tolerance and GSIS require β cell $G_{12/13}$ signaling. (A) Selective expression of the G12D receptor in β cells of WT mice after intraperitoneal injection of the AAV-RIP2-G12D virus (see Materials and Methods for details). The designer receptor was detected by using an antibody directed against the HA epitope tag present at the N terminus of G12D. Control mice were obtained by injecting WT mice with the AAV-RIP2-ChR2 virus (ChR2, channel rhodopsin). Note that pancreatic glucagon-expressing α cells do not express G12D. Scale bars, 50 μ m. (B to E) In vivo metabolic studies carried out 4 weeks after AAV injections. (B and C) IGTT and GSIS studies with AAV-treated WT mice. After an overnight fast (14 hours), WT mice treated with the indicated AAVs were injected intraperitoneally with a mixture of glucose (2 g/kg) and CNO (3 mg/kg), followed by the monitoring of blood glucose (B) and plasma insulin (C) levels. (D and E) IGTT and GSIS studies with AAV-treated beta-G12/13 KO mice (whole-body $G_{\alpha_{12}}$ KO mice lacking $G_{\alpha_{13}}$ selectively in β cells). Following an overnight fast (14 hours), beta-G12/13 KO mice injected intraperitoneally with the indicated AAVs were treated with a mixture of glucose (2 g/kg) and CNO (3 mg/kg), followed by the monitoring of blood glucose (D) and plasma insulin (E) levels. Data are given as means $\pm$ SEM. All studies were performed using 9- to 12-week-old male mice maintained on regular chow. Data represent means $\pm$ SEM ($n = 5$ or 6 mice per group) * $P < 0.05$ and *** $P < 0.001$ versus the corresponding control value. Statistical significance was determined by a two-way ANOVA followed by Bonferroni's post hoc test.

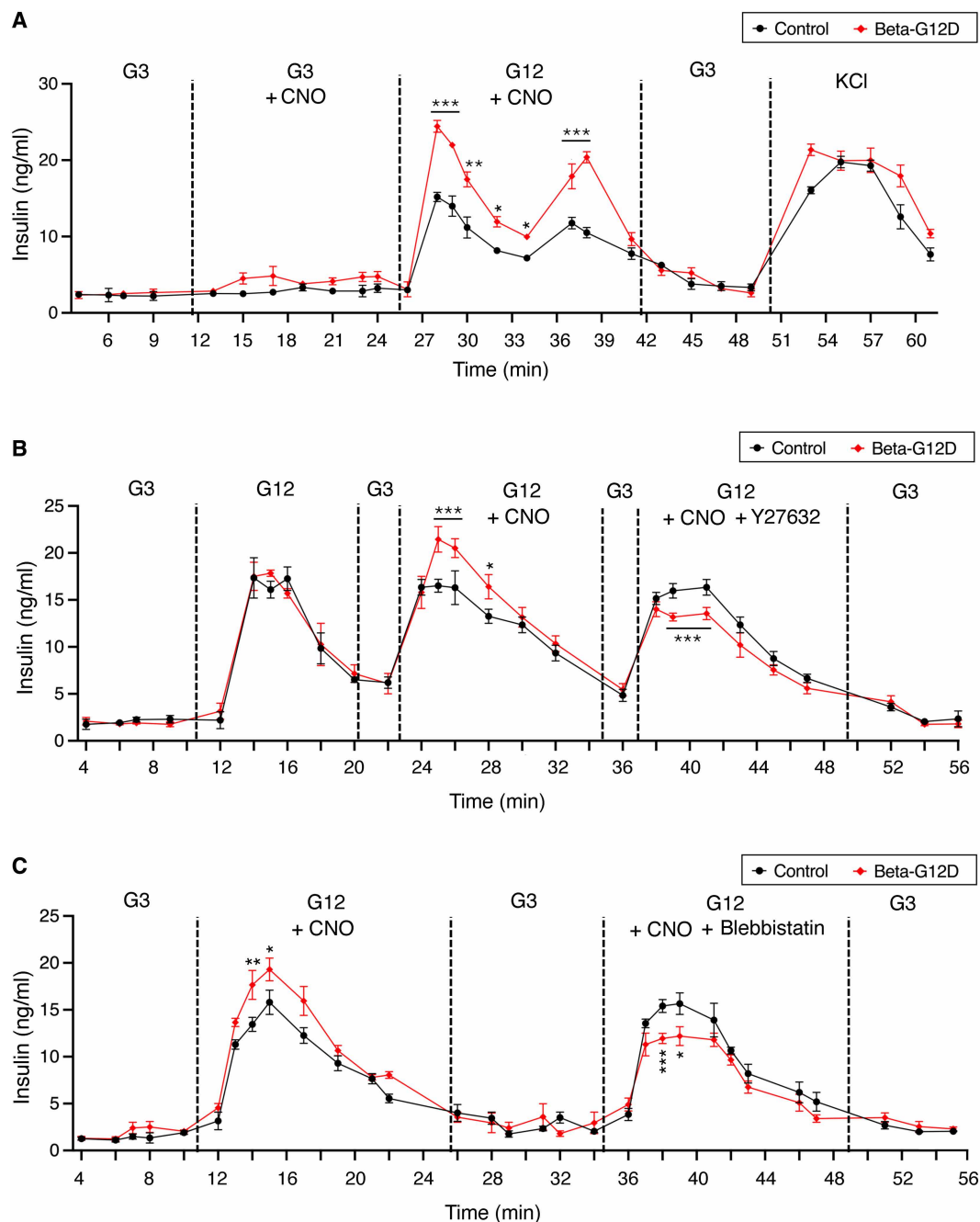


Fig. 7. Activation of β cell G12D receptors stimulates insulin secretion from mouse islets in a glucose-dependent and ROCK-dependent manner. (A to C) Insulin secretion from perfused islets prepared from control and beta-G12D mice. Experiments were carried out at low and high glucose concentrations (3 mM [G3] and 12 mM [G12], respectively). (A) CNO (10 μ M) treatment of beta-G12D islets augments G12-stimulated insulin release, as compared to CNO-treated control islets. KCl (30 mM) treatment caused similar insulin responses in the two groups of islets. (B and C) Y-27632 (20 μ M), a ROCK inhibitor (B), and blebbistatin (10 μ M), an myosin light chain kinase (MLCK) inhibitor (C), abolish the CNO-dependent augmentation of G12-induced insulin release in beta-G12D islets. Islets were obtained from male or female mice (age: 12 to 18 weeks). Data are shown as means $\pm$ SEM (three or four independent perfusions with 50 islets per perfusion chamber). * P < 0.05, ** P < 0.01, and *** P < 0.001 versus the corresponding control value. Data were analyzed by a two-way ANOVA followed by Bonferroni's post hoc test.

incubation of beta-G12D islets with blebbistatin, an agent that prevents the remodeling of the actin cytoskeleton by blocking the adenosine triphosphatase (ATPase) activity of myosin II (37–39) (Fig. 7C).

A GPR183 agonist augments GSIS in a G_{12} -dependent manner in WT pancreatic islets

Our next goal was to target GPCRs that are able to activate $G_{12/13}$ signaling and are endogenously expressed by pancreatic β cells. Recent studies (18, 40) have identified more than 20 GPCRs that can couple to G_{12} -type G proteins with relatively high efficacy. In the present study, we focused on GPR183 because this receptor can interact with G_{12} with rather high selectivity and efficacy, as compared to other functional classes of heterotrimeric G proteins (18, 40). The most potent endogenous GPR183 agonist is 7 α ,25-dihydroxycholesterol (abbreviated as DHC hereafter) (41, 42).

To study whether activation of β cell GPR183 signaling can promote insulin secretion, we carried out in vitro perfusion studies using islets prepared from WT mice. Specifically, we monitored GSIS in the absence and presence of DHC (10 μ M). Treatment of WT islets with DHC significantly augmented GSIS (glucose concentration: 12 mM) (Fig. 8A), indicating that activation of endogenous β cell GPR183 receptors enhances GSIS.

We next wanted to confirm that the stimulatory effect of DHC on GSIS was due to the activation of G_{12} -type G proteins. To address this issue, we carried out additional insulin release studies using islets prepared from WT, G_{12} KO, and beta- $G_{12/13}$ KO mice. As expected, DHC treatment of WT islets led to a significant augmentation in GSIS (glucose concentration: 12 mM; Fig. 8, B and C). Notably, this effect was abolished in islets prepared from G_{12} KO and beta- $G_{12/13}$ KO mice (Fig. 8, B and C), indicating that DHC-stimulated insulin secretion requires the activation of β cell $G_{12(13)}$ signaling.

The presence of β cell G_{12} and G_{13} is required for maximum GSIS in WT mouse islets

When islets prepared from WT, G_{12} KO, and beta- $G_{12/13}$ KO mice were incubated with a low concentration of glucose (3 mM), basal insulin release did not differ significantly between the three groups of islets (Fig. 8B). Subsequent exposure of islets to 12 mM glucose led to a robust increase in insulin secretion in all three groups of islets (Fig. 8, B and D). However, under these conditions, beta- $G_{12/13}$ KO islets released significantly less insulin than WT and G_{12} KO islets (Fig. 8, B and D), suggesting that the presence of both β cell G_{12} and G_{13} is required for maximum insulin release when glucose levels are elevated.

Activation of GPR183 augments GSIS in a human β cell line in a G_{12} -dependent manner

Last, we investigated whether activation of GPCRs that are endogenously expressed by human β cells and that can couple to G proteins of the G_{12} family are able to affect insulin release in a similar manner as observed with mouse β cells. To address this question, we used human EndoC- β H1 β cells, which closely mimic the functional properties of native human β cells as an in vitro model system (43, 44). Like human β cells, EndoC- β H1 cells express both $G_{\alpha_{12}}$ and $G_{\alpha_{13}}$ (43).

Moreover, human β cells, like their mouse counterparts, express GPR183, as supported by single-cell RNA sequencing (scRNA-seq) and functional data (15, 45). In agreement with the results obtained with perfused mouse islets, DHC (1 μ M) treatment of EndoC- β H1

cells led to a significant enhancement of GSIS (Fig. 9A). At a low glucose concentration (1 mM), DHC did not affect basal insulin release (Fig. 9A). The DHC-mediated augmentation of GSIS in EndoC- β H1 cells was completely blocked by NIBR189 (1 μ M), a selective GPR183 antagonist (46), confirming that DHC promotes insulin secretion via activation of GPR183.

Recent reports indicate that GPR183 couples to G_{12} with rather high selectivity and efficacy, as compared to G_{13} and other functional classes of G proteins (40). To explore whether the stimulatory effect of DHC on insulin release was mediated by activation of G_{12} signaling, we treated EndoC- β H cells with *GNA12*-specific small interfering RNA (siRNA), which selectively prevented the expression of $G_{\alpha_{12}}$ (Fig. 9B). Notably, in the absence of $G_{\alpha_{12}}$, DHC failed to augment GSIS (Fig. 9C), indicating that GPR183-mediated activation of G_{12} -type G proteins enhances GSIS in both mouse and human β cells.

DISCUSSION

G proteins of the G_{12} family (G_{12}/G_{13}) are expressed in most tissues and cell types, including pancreatic β cells (15, 16). Other functional classes of heterotrimeric G proteins (G_s , $G_{q/11}$, and $G_{i/o}$) are known to exert major effects on glucose homeostasis by modulating insulin release from pancreatic β cells (8–10). In contrast, the metabolic outcomes of activating $G_{12/13}$ signaling in β cells remains unexplored. In the present study, we used a chemogenetic strategy, together with loss-of-function and gain-of-function $G_{12/13}$ mouse models, to investigate the potential role of β cell $G_{12/13}$ signaling in regulating insulin release and whole-body glucose homeostasis.

By using a chemogenetic approach, we generated a mouse line (beta-G12D) that enabled us to selectively activate G_{12} -type G proteins in a drug (CNO or DCZ)-dependent manner. In addition, we analyzed mouse strains with inactivating mutations in the genes coding for $G_{\alpha_{12}}$ and $G_{\alpha_{13}}$ (*Gna12* and *Gna13*, respectively) in β cells. We made the unexpected observation that drug-induced stimulation of $G_{12/13}$ signaling in β cells greatly augmented GSIS. Activation of this pathway restored normal glucose tolerance in obese mice (Fig. 2). Moreover, in a mouse model of T2D (treatment of mice with low-dose STZ), chronic stimulation of β cell $G_{12/13}$ signaling in β cells greatly improved glycemic control by augmenting insulin secretion (Fig. 3, B to E). STZ treatment of beta-G12D mice maintained on DCZ water showed a significantly less pronounced loss in β cell mass and total pancreatic insulin content, as compared to STZ+DCZ-treated control mice lacking the G_{12D} transgene (Fig. 3, F and G). It is well known that stimulation of $G_{12/13}$ signaling promotes the proliferation of many different cell types (17, 47). Thus, one possibility is that G_{12D} -mediated stimulation of β cell proliferation counteracts the STZ-induced destruction of β cells. We are planning to explore this possibility and the pathways mediating this effect in a detailed follow-up study.

STZ+DCZ-treated beta-G12D mice showed a significant increase in total pancreatic insulin and β cell mass, as compared to STZ+DCZ-treated control mice lacking the G_{12D} transgene (Fig. 3, F and G). It is well known that stimulation of $G_{12/13}$ signaling promotes the proliferation of many different cell types (17, 47). Moreover, G_{12} -type G proteins have been implicated in the regulation of apoptotic signaling pathways (48). Thus, detailed follow-up studies are needed to explore the cellular mechanisms through which activation of $G_{12/13}$ signaling in β cells can increase β cell mass under severe hyperglycemic conditions.

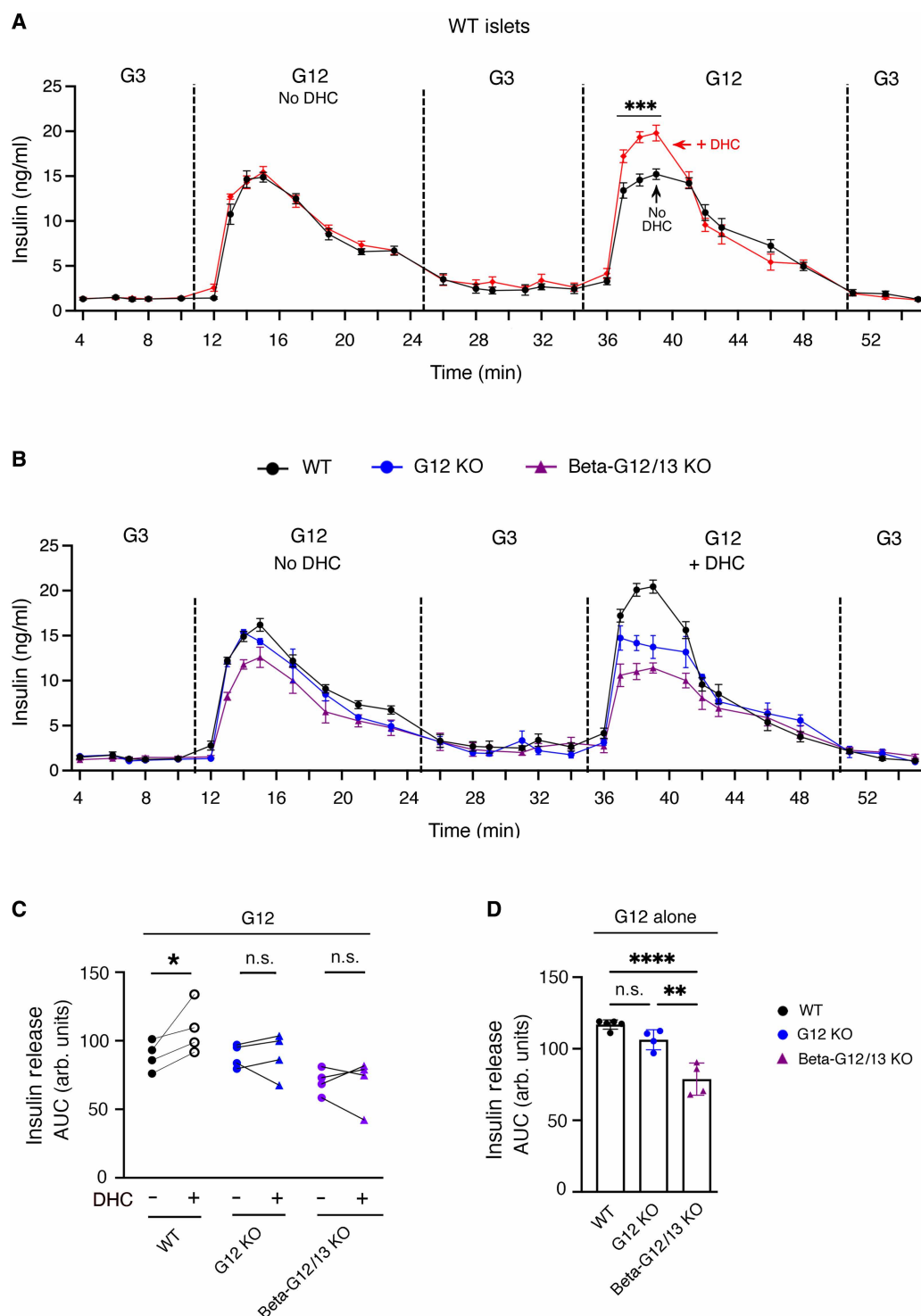


Fig. 8. Stimulation of β cell GPR183 receptors promotes insulin secretion from mouse islets in a $G_{12/13}$ -dependent manner. (A) and (C) A GPR183 agonist (DHC) (10 μ M) augments G_{12} -induced insulin release from perfused WT islets. (B) and (C) DHC-dependent augmentation of G_{12} -induced insulin secretion is absent in islets prepared from whole-body $G\alpha_{12}$ KO mice (G12 KO) and beta- $G_{12/13}$ KO mice (whole-body $G\alpha_{12}$ KO mice lacking $G\alpha_{13}$ selectively in β cells). Islets were obtained from male or female mice (age: ~20 weeks). (C) Summary of insulin release in the absence or presence of DHC at 12 mM glucose (G_{12}) derived from the data shown in (B). arb. units, arbitrary units. (D) Summary of the insulin release data (12 mM glucose, G_{12}) shown in (B). Data are given as means $\pm$ SEM (four independent perfusions with 50 islets per perfusion chamber). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ versus the corresponding control value. Data were analyzed by a two-way ANOVA followed by Bonferroni's post hoc test. n.s., no statistically significant difference.

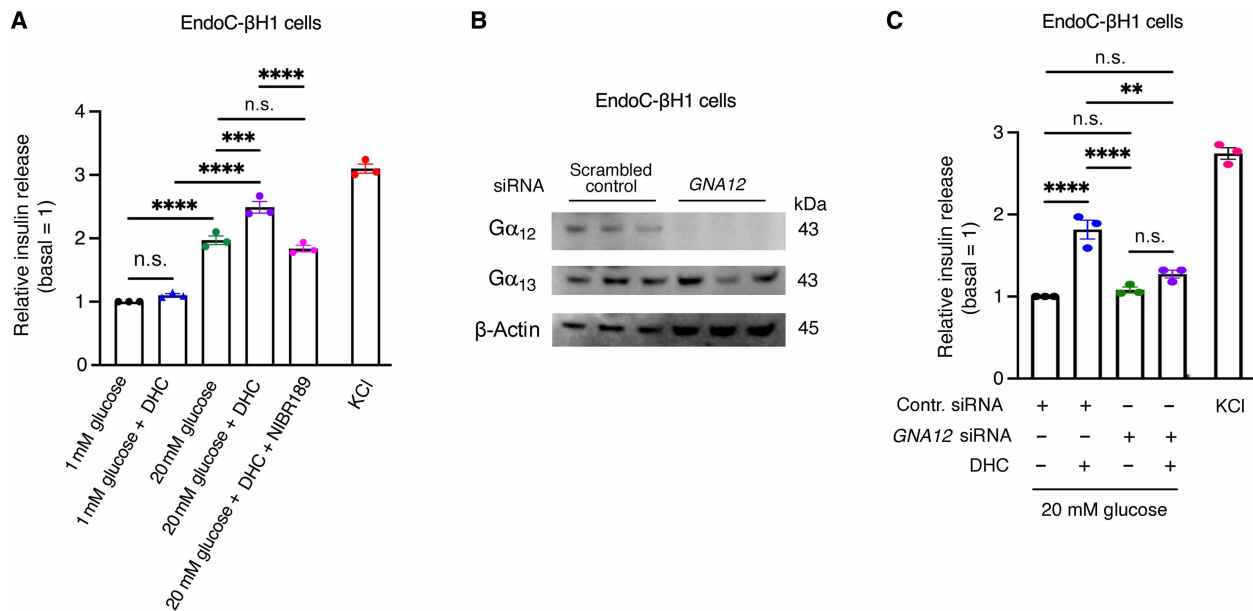


Fig. 9. A GPR183 agonist stimulates insulin secretion from human EndoC-βH1 β cells in a glucose-dependent and G₁₂-dependent manner. (A) Stimulatory effect of a GPR183 agonist (DHC) on insulin release from cultured human EndoC-βH1 β cells. Glucose (20 mM)-induced insulin secretion is further augmented in the presence of DHC (1 μM). This DHC effect is completely blocked by NIBR189 (1 μM), a selective GPR183 antagonist. KCl (30 mM) served as positive control. (B) Western blot showing the selective loss of Gα₁₂ expression after treatment of EndoC-βH1 cells with siRNA selective for *GNA12*. (C) Treatment of EndoC-βH1 cells with *GNA12* siRNA greatly reduces the DHC (1 μM)-induced enhancement of GSIS (20 mM glucose). KCl (30 mM) served as positive control. Data are given as means ± SEM from three independent experiments. ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001. Data were analyzed by a two-way ANOVA followed by Bonferroni's post hoc test. n.s., no statistically significant difference.

The G12D-mediated improvements in glucose tolerance and GSIS were absent in beta-G12/13 KO mice, clearly indicating that these effects were mediated by G₁₂-type G proteins (Fig. 6). Moreover, metabolic studies with mice expressing a constitutively active version of Gα₁₂ selectively in β cells showed that chronic activation of β cell G₁₂ signaling mimicked the metabolic improvements observed after acute CNO treatment of beta-G12D mice (Fig. 4). Together, these studies convincingly support the concept that activation of β cell G_{12/13} signaling strongly promotes insulin release.

Studies with isolated mouse beta-G12D islets showed that activation of β cell G_{12/13} signaling had little or no effect on insulin release in the presence of a low concentration of glucose (3 mM) (Fig. 7A). In agreement with these in vitro studies, CNO treatment of beta-G12D mice had no or only minor effects on blood glucose levels in freely fed mice (Fig. 1C). These observations suggest that agents capable of activating β cell G_{12/13} signaling exert their beneficial metabolic effects primarily when blood glucose levels are high, similar to drugs that stimulate β cell G_s and G_{q/11} signaling (8–10).

Both human and mouse β cells express several GPCRs that are able to couple to G_{12/13}, including the ghrelin, LPA₁, and S1P₁ receptors as well as GPR183 (EBI2) (15, 16). Among these receptors, we focused on the functional role of β cell GPR183, primarily due to two reasons. First, activation of GPR183 stimulates G₁₂ with relatively high efficacy, as compared to other functional classes of G proteins (40). Second, studies with human islets indicated that *GPR183* is overexpressed in islets from diabetic human donors (45), suggesting that GPR183 represents a potential pharmacological target.

We found that DHC-mediated activation of GPR183 strongly promoted insulin release in a glucose-dependent manner in both

mouse islets (Fig. 8, A to C) and human EndoC-βH1 cells (Fig. 9). Two lines of evidence indicate that this effect depends on GPR183-mediated activation of G₁₂ signaling. First, studies with isolated mouse islets showed that DHC failed to augment GSIS in islets prepared from G12 KO and beta-G12/13 KO mice (Fig. 8, A to C). Second, knock-down of Gα₁₂ expression in human EndoC-βH1 prevented the DHC-mediated increase in GSIS observed with control EndoC-βH1 cells (Fig. 9, A to C). In agreement with these data, Taneera *et al.* (45) recently reported that DHC treatment also augmented GSIS in human islets. However, the molecular mechanisms underlying this effect remained unexplored in this latter study.

Taneera *et al.* (45) also showed that pancreatic islets from hyperglycemic donors displayed increased *GPR183* levels, as compared to islets from euglycemic donors. At the same time, *GPR183* expression and HbA_{1c} levels showed a highly significant negative correlation (45). Thus, it is possible that the increase in *GPR183* expression observed with islets from hyperglycemic donors represents a compensatory response aimed at improving impaired β cell function.

GPR183 is expressed at particularly high levels by many cell types of the immune system, thus regulating many important immunological functions (49, 50). As a result, GPR183 agonists and antagonists are currently being developed for the treatment of autoimmune disorders, infectious diseases, and several other pathophysiological conditions. It should be of interest to explore to which extent GPR183-dependent G_{12/13} signaling (activation or inhibition) contributes to the beneficial effects caused by these agents.

GPR183 is also expressed by many other peripheral tissues and most parts of the brain (49, 51). The broad pattern of GPR183 distribution raises the question whether the potential therapeutic use of

GPR183 agonists as insulinotropic agents might cause significant off-target effects. Previous studies have shown that it is feasible to selectively deliver biologically active agents covalently linked to a GLP-1 receptor agonist (GLP-1RA) to GLP-1 receptor-expressing cells (52–54). Because GLP-1 receptors are expressed at very high levels by pancreatic β cells (55), the potential use of GPR183 agonist/GLP-1RA conjugates may allow the selective targeting of GPR183 agonists to β cells, thus avoiding major off-target effects.

Accumulating evidence also suggests that structurally different agonists acting on the same GPCR can induce multiple distinct active GPCR conformations that differ in their G protein coupling profiles (56–59). This finding suggests that it should be possible to design GPCR agonists that lead to the activation of multiple G proteins capable of augmenting GSIS, including β cell $G_{12/13}$. This approach may lead to the development of therapeutic agents that can stimulate insulin release with enhanced efficacy, as compared to insulin secretagogues in current clinical use.

Previous studies have shown that remodeling of the actin cytoskeleton plays a key role in promoting insulin release from β cells (35, 36). Moreover, it has been reported that insulin-containing granules use actin filaments as “rails” to get transported toward their site of release at the plasma and that perturbation of β cell actin cytoskeleton remodeling leads to impaired insulin release (35). Consistent with this model, we found that G_{12D} -mediated augmentation of GSIS was prevented by a selective ROCK inhibitor (Y27632) or by blebbistatin, which blocks the ATPase activity of myosin II (37, 38), thus preventing the remodeling of the actin cytoskeleton triggered by glucose stimulation (39). In agreement with these findings, metabolic studies with mutant mice selectively lacking ROCK1 in pancreatic β cells showed that β cell ROCK1 is essential for efficient GSIS and the maintenance of euglycemia (60).

In this context, it should be noted that Y27632 at the concentration used in this study does not only block ROCK activation but can also interfere with the activity of several other kinases (61). Moreover, blebbistatin has strong effects on cell morphology in general (62) that can inhibit cellular signaling pathways in an indirect manner. For this reason, the detailed cellular mechanisms through which activation of $G_{12/13}$ in β cells promotes insulin secretion remain to be explored. Defining the precise cellular events downstream of G_{12} activation, including potential effects on glucose-induced Ca^{2+} transients and insulin granule dynamics, will require complementary experimental strategies including the use of electron microscopic and electrophysiologic techniques.

Beta- $G_{12/13}$ KO mice showed impaired glucose tolerance, combined with a significant reduction in GSIS, as compared to the corresponding control mice (Fig. 5, D and E). Because total pancreatic insulin content and β cell mass remained unaffected in beta- $G_{12/13}$ KO mice (Fig. 5, F and G), the most likely scenario is that the metabolic deficits displayed by beta- $G_{12/13}$ KO mice resulted from impaired β cell $G_{12/13}$ signaling. Taken together, these observations indicate that β cell $G_{12/13}$ signaling is a key regulator of β cell function and whole-body glucose homeostasis.

To explore the potential translational relevance of our findings, it remains to be determined whether the beneficial metabolic effects of β cell $G_{12/13}$ signaling are retained long term (over months). However, we found that STZ-treated beta- G_{12D} mice consuming DCZ-containing drinking water for 4 weeks displayed greatly reduced blood glucose levels, as compared to STZ-treated control mice. We obtained similar results when we studied beta-CA- G_{12} mice over a

period of 2 to 3 weeks. These data indicate that activation of β cell $G_{12/13}$ signaling can enhance β cell function for at least several weeks.

In summary, we found that activation of $G_{12/13}$ signaling in pancreatic β cells strongly augments GSIS. This pathway is operative in both mouse and human β cells. Activation of β cell $G_{12/13}$ signaling greatly improves glucose homeostasis in obese mice and in a mouse model of T2D. These findings provide an experimental rationale for the development of therapeutic agents capable of enhancing β cell $G_{12/13}$ signaling for the treatment of T2D and related metabolic disorders.

Limitations of the study

As already discussed above, the detailed cellular mechanisms through which activation of G_{12} -type G proteins in β cells augments GSIS remain to be explored in an in-depth follow-up study. Because essentially all GPCRs are expressed in multiple tissues and cell types (51), preclinical studies need to be carried out to determine whether GPCR agonists capable of stimulating β cell $G_{12/13}$ signaling are devoid of major unwanted side effects. As mentioned earlier, β cells express many GPCRs that are able to couple to G_{12} -type G proteins, besides other functional classes of heterotrimeric G proteins (15, 16, 18, 40). In vitro functional studies with human islets using agonists targeting these receptors should prove useful for determining which of these GPCRs are particularly efficacious in augmenting GSIS.

MATERIALS AND METHODS

Study approval

All animal studies were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committees of the NIDDK, NIH, Bethesda, MD (approval number: ASP K005-LBC-24).

Drugs, reagents, commercial kits, and antibodies

The sources of all drugs, reagents, antibodies, mouse strains, etc. are listed in table S1.

Mouse maintenance and diet

All mice were housed in a pathogen-free barrier facility at 23°C with a 12-hour light/12-hour dark cycle (light period: 6:00 a.m. to 6:00 p.m.). Mice consumed either regular mouse chow (LabDiet, #5018, 15% kcal fat, 3.05 kcal/g) or an HFD (F3282, 60% kcal fat, 5.5 kcal/g, Bio-Serv) with unlimited access to water.

Generation of mutant mouse strains

All mutant mice used in this study were maintained on a C57BL/6 genetic background. To generate mice that expressed the G_{12D} DREADD (G_{12D}) selectively in β cells, we crossed *Pdx1-Cre-ER* transgenic mice that carried one allele of the *Cre* transgene (25, 26) with *Rosa26-LSL-G12D-IRES-GFP* mice that were homozygous for the *LSL-G12D-IRES-GFP* insertion (abbreviated name: LSL- G_{12D} mice) (19). The resulting offspring harboring the *Cre-ER* transgene were treated with tamoxifen for 5 consecutive days (80 mg/kg per day, intraperitoneally) (mouse age: 8 weeks), yielding mice selectively expressing G_{12D} in β cells (abbreviated name: beta- G_{12D} mice). *LSL-G12D-IRES-GFP* littermates lacking the *Cre-ER* transgene, which served as control littermates, were injected with tamoxifen in the same manner (fig. S1).

In addition, we used a virus-based approach to selectively express G_{12D} in mouse β cells (33). In this case, 8-week-old male WT mice (C57BL/6 mice, Taconic) were injected intraperitoneally with

AAV8-RIP2-G12D (1×10^{11} viral particles per mouse suspended in 100 μ l of saline). Using the same approach, we also generated mice that expressed a constitutively active version of $G\alpha_{12}$ (short name: CA-G12) mice in β cells of WT mice (virus used: AAV8-RIP2-CA-G12). The CA-G12 $G\alpha$ subunit contained the Q231L point mutation (32). To generate a set of corresponding control mice, we treated WT mice in the same manner with a pharmacologically inert AAV (AAV8-RIP2-mCherry). Metabolic experiments were initiated 4 weeks after AAV injections.

We also generated mice that lacked $G\alpha_{12}$ throughout the body and $G\alpha_{13}$ selectively in β cells. For simplicity, we refer to these mice as beta-G12/13 KO mice (genetic background: C57BL/6). To obtain this mouse strain, we crossed *Pdx1-Cre-ER* mice with *Gna12*^{−/−} *Gna13* *fl/fl* mice (34). In the next step, *Cre*-positive *Gna12*^{−/+} *Gna13* *fl/+* mice were backcrossed to *Gna12*^{−/−} *Gna13* *fl/fl* mice to obtain *Pdx1-Cre-ER* *Gna12*^{−/−} *Gna13* *fl/fl* mice (short name: beta-G12/13 KO mice) and control littermates lacking the *Cre* transgene (*Gna12*^{−/−} *Gna13* *fl/fl* mice; short name: G12 KO mice). *Pdx1-Cre-ER* *Gna12*^{−/−} *Gna13* *fl/fl* mice were treated with tamoxifen for 5 consecutive days (80 mg/kg per day, intraperitoneally) (mouse age: 8 weeks) to ensure the selective inactivation of *Gna13* in β cells. Control littermates lacking the *Cre* transgene were treated with tamoxifen in the same manner.

Mouse genotyping

Mouse tail DNA was used to detect the presence of the *Pdx1-Cre-ER*, *Rosa26-LSL-G12D-IRES-GFP*, *Gna12*^{−/−}, and *Gna13* *fl/fl* alleles. Polymerase chain reaction (PCR) primers used for genotyping studies are listed in table S2. PCR reactions were carried out using standard procedures (63).

Recombinant viruses

AAVs (serotype 8) coding for G12D (AAV8-RIP2-G12D) or channel rhodopsin (ChR2) (AAV8-RIP2-ChR2) were obtained from VectorBuilder. The expression of G12D and ChR2 was under the transcriptional control of the β cell-specific RIP2. By using the same approach, we also generated AAVs coding for mCherry (AAV8-RIP2-mCherry; control virus) and CA-G12 (see previous paragraphs; AAV8-RIP2-CA-G12). The C terminus of the coding sequences of G12D, ChR2, and CA-G12 was fused to mCherry via a short linker (GCGGCCGCC).

In vivo metabolic studies

All metabolic tests were performed with adult mice that were at least 10 weeks old (age range: 10 to 30 weeks) using standard protocols (64). Unless stated otherwise, male littermates were used. Blood glucose and plasma insulin levels were measured in both freely fed mice at 9:00 a.m. or in mice that had been fasted overnight for 14 hours (time of measurement: 9:00 a.m.). To assess the in vivo effects of acute activation of G12D signaling in β cells, freely fed beta-G12D mice and their control littermates were injected intraperitoneally with either CNO (3 mg/kg) or vehicle (saline). For IGTs, mice that had been fasted for 14 hours were injected with either 2 g glucose/kg (mice consuming regular chow) or 1 g glucose/kg (HFD mice), respectively. For ITTs, mice that had been fasted for 4 hours were injected intraperitoneally with insulin with either 1 IU/kg (regular chow mice) or 1.5 IU/kg (HFD mice), respectively. To study GSIS, mice were fasted overnight for 14 hours and then injected intraperitoneally with either 1 or 2 g/kg glucose, followed by the measurement of plasma insulin levels. Blood was collected from the tail vein. Blood

glucose levels were determined before injections and at defined postinjection time points using the Contour Blue glucometer (Bayer). For plasma insulin measurements, blood was collected in EDTA-coated tubes at specific time points, and plasma insulin was detected using insulin-specific enzyme-linked immunosorbent assay (ELISA) kits (Crystal Chem or Mercodia), according to the manufacturer's instructions.

Isolation of mouse pancreatic islets

Pancreatic islets were prepared from male or female mice (mouse age: 14 to 24 weeks) as described previously (65). Islets were collected in culture medium [RPMI 1640 medium with 1% penicillin/streptomycin, 10% fetal bovine serum (FBS), and 11 mM glucose].

Measurement of pancreatic insulin content

To quantify pancreatic insulin content, whole mouse pancreata were excised and weighed. Tissues were then homogenized in 3 ml of an acid-ethanol extraction buffer (1.5% HCl in 70% ethanol) by using a Precellys Evolution Touch Homogenizer (Bertin Instruments). Subsequently, samples were incubated on ice for 1 hour, followed by centrifugation at 10,000g for 20 min at 4°C. The supernatants were then collected, and insulin concentrations were determined using an ELISA kit (see table S1).

Islet perfusion studies

Islet perfusion studies were performed as described by Barella *et al.* (65). Handpicked islets that had been incubated overnight in culture medium (RPMI 1640, 10% FBS, and 1% penicillin/streptomycin) were placed into perfusion chambers (50 islets per chamber) with Bio-Gel P-4 media (Bio-Rad) to immobilize islets in an automated perfusion system (Biorep Perfusion System). All compounds used were dissolved in perfusion buffer [125 mM NaCl, 5.9 mM KCl, 2.56 mM CaCl₂, 1.2 mM MgCl₂, 25 mM Hepes, and 0.1% bovine serum albumin (BSA) (pH 7.4)]. A peristaltic pump pushed reagents continuously into the islet-containing chambers. Perfusates were collected in ice-cold 96-well plates for further analysis. Insulin concentrations in the perfusates were determined with mouse insulin-specific ELISA kits (see table S1).

Islet morphometric studies

Pancreatic sections were prepared as described by Jain *et al.* (27). To measure β cell mass, areas staining positive for insulin and the total area of each section were quantified with a Keyence digital microscope (BZ-9000) with a CFI Plan Apo λ $\times$ 4 lens. The ratio of the insulin-stained area to the area of the total pancreatic section was averaged for each mouse and then multiplied by pancreas weight. For these studies, three or four mice (males) per genotype were used (27).

Western blotting

For Western blotting studies (64), different mouse tissues (~20 mg) were homogenized in 400 μ l of ice-cold radioimmunoprecipitation assay buffer containing a protease inhibitor cocktail (cOmplete Protease Inhibitor Cocktail, MilliporeSigma). Protein concentrations were determined by a BCA protein assay kit (Thermo Fisher Scientific). Protein extracts (20 μ g) were first incubated at 37°C for 10 min in NuPAGE LDS sample buffer (Thermo Fisher Scientific). Subsequently, proteins were separated using 3 to 8% Tris-acetate SDS-polyacrylamide gel electrophoresis gels (Thermo Fisher

Scientific) and then blotted onto nitrocellulose membranes (Bio-Rad). Membranes were blocked for 1 hour at room temperature in blocking buffer (5% BSA and 0.1% Tween 20 in PBS) and then incubated overnight at 4°C with primary antibody in blocking buffer. Horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies and SuperSignal West Dura Chemiluminescent Substrate (Pierce) were used to visualize immunoreactive bands via an Azure 300 Imaging System Imager (Azure Biosystems). Detailed information regarding the antibodies used is given in table S1.

Immunofluorescence studies

To detect the expression of the HA (hemagglutinin)-tag fused to the N terminus of G12D and the expression of insulin in mouse pancreatic β cells, pancreata were fixed overnight with 4% paraformaldehyde, embedded in paraffin, and then sectioned (section thickness: 5 μ m). After deparaffinization and rehydration, sections were subjected to heat-induced antigen retrieval (low pH, eBioscience) and then treated with blocking buffer (5% goat serum and 0.1% Tween 20 in PBS) for 1 hour at room temperature. In the first step, sections were incubated in blocking buffer for 1 hour at room temperature. Subsequently, all sections were stained with primary antibody (overnight incubation at 4°C) and the corresponding secondary antibodies carrying conjugated fluorophores (2-hour incubation at room temperature). Fluorescence images were acquired using a confocal microscope (Zeiss LSM 700). Details regarding the antibodies used are provided in table S1.

STZ-induced diabetes

To generate a mouse model of diabetes, 8-week-old male beta-G12D mice and control littermates received daily intraperitoneal injections of STZ (MilliporeSigma) for 5 consecutive days (50 mg/kg), as described previously (27–29). All beta-G12D mice and control littermates consumed DCZ drinking water (10 mg/liter) starting from the first day of STZ treatment. We also included an additional group of control mice (WT mice) that were maintained on DCZ drinking water but did not receive STZ.

Culture of human EndoC- β H1 cells and knockdown of *GNA12* expression

Human EndoC- β H1 cells (44) were cultured in a humidified incubator with 95% air and 5% CO₂ at 37°C with Dulbecco's modified Eagle's medium (DMEM) containing 1 g glucose/liter, 2% albumin from bovine serum fraction V (Equitech), 50 μ M 2-mercaptoethanol (Sigma-Aldrich), 10 mM nicotinamide (Millipore), transferrin (5.5 μ g/ml; Sigma-Aldrich), sodium selenite (6.7 ng/ml; Sigma-Aldrich), and penicillin (100 U/ml)/streptomycin (100 μ g/ml). The culture flasks were precoated with medium containing 1% ECM (extracellular matrix; Sigma-Aldrich) and fibronectin (2 μ g/ml; Sigma-Aldrich). For gene silencing studies, $\sim 1.5 \times 10^6$ cells were electroporated with 100 nM human *GNA12* siRNA or scrambled control siRNA (ON-TARGETplus siRNA; Horizon Discovery). After electroporation, cells were maintained in 6-well plates (Corning) in Opti-MEM Reduced Serum Medium (Thermo Fisher Scientific) for 6 hours. Subsequently, this medium was replaced with standard DMEM. After an additional 24-hour culture period, cells were used for insulin secretion studies or harvested and stored at –80°C for gene expression and Western blotting studies.

Insulin secretion studies with EndoC- β H1 β cells

To measure GSIS in EndoC- β H1 cells, cells grown in 6-well plates (Corning) were first preincubated for 1 hour in Krebs-Ringer buffer (115 mM NaCl, 24 mM NaHCO₃, 5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 10 mM Hepes, 0.2% BSA, and 1 mM glucose). After this equilibration period, the buffer was replaced with fresh Krebs-Ringer buffer containing either 1 mM glucose (low glucose), 20 mM glucose (high glucose), or 30 mM KCl (positive control). Cells were then incubated for 1 hour at 37°C in the presence or absence of a selective GPR183 agonist (1 μ M DHC; MedChemExpress). After this incubation step, insulin concentrations in the medium were quantified using a human insulin ELISA kit (Crystal Chem) according to the manufacturer's protocol.

Statistical analyses

Data are expressed as means $\pm$ SEM for the indicated number of observations. Before performing specific statistical tests, we performed tests for normality and homogeneity of variance. Data were then tested for statistical significance by one-way or two-way analysis of variance (ANOVA), followed by the indicated post hoc tests, or by using a two-tailed unpaired Student's *t* test, as appropriate. A *P* value of <0.05 was considered statistically significant. The specific statistical tests that were used are indicated in the figure legends.

Supplementary Materials

This PDF file includes:

Figs. S1 to S6

Tables S1 and S2

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Activation of G₁₂-type G proteins in pancreatic β cells protects against impaired glucose homeostasis

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