Pro-α-cell-derived β-cells contribute to β-cell neogenesis induced by antagonistic glucagon receptor antibody in type 2 diabetic mice

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Highlights
Blockage of α-cell-derived glucagon promotes β-cell regeneration in situ in type 2 diabetic (T2D) mice
Glucagon receptor (GCGR) mAb induces the trans-differentiation of α-cells to β-cells
GCGR mAb promotes α-cell regression to pancreatic endocrine progenitors
GCGR mAb induces Ngn3⁺ progenitor reactivation and differentiation toward β-cells
Pro-α-cell-derived β-cells contribute to β-cell neogenesis induced by antagonistic glucagon receptor antibody in type 2 diabetic mice

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SUMMARY
The deficiency of pancreatic β-cells is the key pathogenesis of diabetes, while glucagon-secreting α-cells are another player in the development of diabetes. Here, we aimed to investigate the effects of glucagon receptor (GCGR) antagonism on β-cell neogenesis in type 2 diabetic (T2D) mice and explore the origins of the neogenic β-cells. We showed that GCGR monoclonal antibody (mAb) elevated plasma insulin level and increased β-cell mass in T2D mice. By using α-cell lineage-tracing (glucagon-cre-β-gal) mice and inducible Ngn3+ pancreatic endocrine progenitor lineage-tracing (Ngn3-CreERT2-tdTomato) mice, we found that GCGR mAb treatment promoted α-cell regression to progenitors, and induced Ngn3+ progenitor reactivation and differentiation toward β-cells. Besides, GCGR mAb upregulated the expression levels of β-cell regeneration-associated genes and promoted insulin secretion in primary mouse islets, indicative of a direct effect on β-cell identity. Our findings suggest that GCGR antagonism not only increases insulin secretion but also promotes pro-α-cell-derived β-cell neogenesis in T2D mice.

INTRODUCTION
Global prevalence of diabetes has been rising in adults, and 90-95% of diabetes is type 2 diabetes (Li et al., 2020). The deficiency of pancreatic insulin-producing β-cells is an essential factor for the pathophysiology of type 2 diabetes (Kahn, 2003; Weyer et al., 1999). Restoration of functional β-cell mass is a promising therapeutic strategy. So far, however, there is almost no clinically available anti-diabetic agent that can achieve β-cell recovery. Moreover, only a few agents have shown a clinical potential to achieve β-cell regeneration. In patients with type 2 diabetes, treatment with anti-diabetic agents is difficult to maintain the long-term glycemic control on target as they cannot prevent the progressive failure of β-cell function (Turner et al., 1999). Therefore, it is urgent to develop an anti-diabetic agent which can effectively promote β-cell regeneration.

Recently, the role of glucagon-producing α-cells in glucose homeostasis regulation and diabetes development has become increasingly emphasized (Lee et al., 2014). Blockage of glucagon receptor (GCGR) lowers blood glucose level and improves glucose tolerance in type 1 diabetic (T1D) and type 2 diabetic (T2D) animals and humans (Conarello et al., 2007; Lee et al., 2011; Okamoto et al., 2017), which indicates the therapeutic potential in developing GCGR antagonists. Our recent studies have shown that REMD 2.59, an antagonist GCGR monoclonal antibody (mAb), has a strong hypoglycemic effect in T1D and T2D mice (Lang et al., 2020b; Wei et al., 2019). Interestingly, we and other groups found that GCGR mAb promoted β-cell regeneration by inducing the trans-differentiation of α-cells to β-cells in T1D mice (Wang et al., 2021b; Wei et al., 2019). Whether GCGR mAb also has such beneficial effects on β-cell regeneration in T2D animal models has not been revealed, and the potential mechanisms remain unclear.

In this study, we investigated the effects of GCGR mAb on β-cell mass and function in two mouse models of T2D and traced the source of β-cell regeneration by using several tracing methods. We also determined whether GCGR mAb had a direct effect on islet cell phenotype conversion and β-cell function in cultured primary mouse islets. Our research reveals a novel pharmacological function of GCGR mAb on diabetes
control and suggests that blockage of α-cell-derived glucagon can promote pro-α-cell-derived β-cell neogenesis, a new approach to β-cell regeneration, in T2D mice.

RESULTS

Glucagon receptor monoclonal antibody lowers the blood glucose level and improves glucose tolerance in type 2 diabetic mice

During the 4-week treatment, no significant difference was identified between GCGR mAb and IgG control groups in terms of body weight in db/db mice and high-fat diet + streptozotocin (HFD + STZ)-induced T2D mice (Figures 1A and 1G). Compared with baseline or IgG treatment, blood glucose levels at random or fasting state were significantly declined after treatment with GCGR mAb in these two T2D mouse models (Figures 1B and 1H). GCGR mAb significantly decreased the post-load glucose levels during the intraperitoneal glucose tolerance test (IPGTT) in db/db mice (Figures 1C and 1D) and HFD + STZ-induced T2D mice (Figures 1I and 1J). After treatment with GCGR mAb, fasting plasma glucagon levels were significantly increased in these two T2D mouse models, compared with control groups (Figures 1E and 1K). Fasting plasma insulin level in GCGR mAb-treated db/db mice was higher than that in the control group (Figure 1F). Similarly, GCGR mAb had a tendency to increase fasting plasma insulin and C-peptide levels in HFD + STZ-induced T2D mice (p = 0.204 and 0.125, respectively) (Figures 1La and 1n). To further evaluate the β-cell function, we detected the insulin level during IPGTT. Results showed that GCGR mAb increased the glucose-challenged insulin levels, especially 30 min after the glucose loading (Figure S1B).

Glucagon receptor monoclonal antibody increases islet number and area, and α-cell and β-cell numbers in type 2 diabetic mice

The islet number and islet area were significantly increased by GCGR mAb in db/db mice and HFD + STZ-induced T2D mice as indicated by histological analysis of the entire pancreata (Figure S2). Notably, GCGR mAb induced a striking expansion in the number of glucagon+ α-cells and insulin+ β-cells in db/db mice compared with IgG control group and db/m mice (Figures 2A and 2B). Similarly, glucagon+ α-cell and insulin+ β-cell numbers were significantly increased in HFD + STZ-induced T2D mice after treatment with GCGR mAb (Figures 2Ca and 2D).

Glucagon receptor monoclonal antibody accelerates progenitor-derived β-cell neogenesis in type 2 diabetic mice

As GCGR mAb treatment increased insulin+ β-cell number and plasma insulin level, we tried to explore the possible origin of the increased β-cells. Interestingly, histological analysis in the pancreata of db/db mice and HFD/STZ-induced T2D mice showed that there were some glucagon+ α-cells and insulin+ β-cells located in the ductal region in GCGR mAb treatment groups, but they were rare in IgG control groups (Figures 3A and 3B). These findings implied that GCGR mAb might induce pancreatic endocrine progenitor reactivation and differentiation toward β-cells in T2D mice.

Glucagon receptor monoclonal antibody induced α-cell regression to the progenitor state in type 2 diabetic mice

Subsequently, we explored the source of progenitors which contributed to β-cell neogenesis. Progenitor harbored in pancreatic ducts is one source of β-cell regeneration (Bonner-Weir et al., 2008; Xu et al., 2008). In this study, we found that GCGR mAb induced the appearance of insulin+ cells located in the ductal region (Figures 3A and 3B), but the insulin+ cells were not co-immunostained with cytokeratin 19, a marker of mature duct cells (Figure S3). The progenitor lineage-tracing marker RFP+ cells were rarely located within or near pancreatic ducts in the Ngn3+ cell lineage-tracing T2D mice despite treatment with GCGR mAb or IgG
Figure 1. Metabolic parameters and hormone levels in two T2D mouse models treated with GCGR mAb or IgG control for 4 weeks

(A–F) Parameters in db/db mice. Age-matched db/m mice treated with IgG were included as a normal control.

(G–L) Parameters in HFD + STZ-induced T2D mice.

(A and G) Body weight. (B and H) Random or fasting blood glucose. (C, I) Blood glucose during the intraperitoneal glucose tolerance test (IPGTT). Black triangle indicated 33.3 mmol/L (the upper detection limit of the glucometer). (D, J) The areas under curve (AUC) of blood glucose during the IPGTT. (E, K) Fasting plasma glucagon. (F, L) Fasting plasma insulin.

n = 6 in db/m mice and n = 10 per group in db/db mice. n = 6 in control group and n = 9 in GCGR mAb group in HFD + STZ-induced T2D mice. Data represent the mean ± SEM. Statistical analysis was conducted by ANOVA followed by the post hoc Tukey-Kramer test or by Student’s t-test, as appropriate. *p < 0.05, **p < 0.01, ***p < 0.001 vs. control; #p < 0.05, ##p < 0.01, ###p < 0.001 vs. pretreatment in the same group. See also Figure S1.
**db/db**

A

| d/b/m | Control | mAb |
| --- | --- | --- |
| ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |
| ![Image](image7.png) | ![Image](image8.png) | ![Image](image9.png) |

B

| Cell number per islet | Cell number per islet | Ratio of β or α cells |
| --- | --- | --- |
| ![Image](image10.png) | ![Image](image11.png) | ![Image](image12.png) |

**HFD+STZ**

C

| Control | mAb |
| --- | --- |
| ![Image](image13.png) | ![Image](image14.png) |
| ![Image](image15.png) | ![Image](image16.png) |
| ![Image](image17.png) | ![Image](image18.png) |

D

| Cell number per islet | Cell number per islet | Ratio of β or α cells |
| --- | --- | --- |
| ![Image](image19.png) | ![Image](image20.png) | ![Image](image21.png) |
Glucagon receptor monoclonal antibody enhances insulin secretion and regulates islet cell phenotype in cultured primary mouse islets

Subsequently, we tried to clarify whether GCGR mAb treatment could directly affect pancreatic β-cell function and islet cell phenotype. We first detected GCGR expression in β-cells. Results showed that GCGR was expressed in mouse β-cells at both the mRNA and protein levels (Figure S8A, S8B, and S8C), suggesting that GCGR mAb might have direct effects on β-cells. Besides, data from RNA Chip and RNA sequencing analyses indicated that GCGR expression in human and mouse β-cells were comparable between T2D and control individuals (Figures S8D and S8E). We then incubated primary mouse islets with GCGR mAb or IgG for 24 h in high glucose (25 mmol/L) medium. As expected, the intracellular glucagon content and supernatant glucagon level were higher in the islets treated with GCGR mAb than those with IgG.
Ngn3+ progenitors are considered to be required for β-cell neogenesis (Wang et al., 2009). Our previous study showed that GCGR mAb induced duct-derived neogenesis in T1D mouse (Wei et al., 2019). Here, we conducted a more comprehensive exploration by using Ngn3+ cell lineage-tracing mice to confirm progenitor-derived β-cell neogenesis in T2D mice. Moreover, we demonstrated that the main source of progenitors which contributed to β-cell neogenesis might be α-cell regression to the progenitor state. Notably, human islets could also regenerate from progenitors (Qadir et al., 2020; Yoneda et al., 2013). Therefore, the promoting effects of GCGR mAb on progenitor-derived β-cell neogenesis as shown in this study suggest the potential clinical application of GCGR mAb to regenerate human islets.

Cell trans-differentiation, also known as lineage reprogramming, is a new path for β-cell regeneration (Wei and Hong, 2016). Several strategies were reported to promote the trans-differentiation of α-cells to β-cells (Ben-Othman et al., 2017; Collombat et al., 2009; Courtney et al., 2013; Furuyama et al., 2019; Thorel et al., 2010). However, the extreme β-cell loss conditions and gene modification methods are difficult to be translated into clinical treatment. GABA and artemisinins were reported to induce cell trans-differentiation, also known as lineage reprogramming, is a new path for β-cell regeneration (Wei and Hong, 2016). Several strategies were reported to promote the trans-differentiation of α-cells to β-cells (Ben-Othman et al., 2017; Collombat et al., 2009; Courtney et al., 2013; Furuyama et al., 2019; Thorel et al., 2010). However, the extreme β-cell loss conditions and gene modification methods are difficult to be translated into clinical treatment. GABA and artemisinins were reported to induce
neogenesis in Ngn3+ cell lineage-tracing T2D mice. Taken together, these results suggested that GCGR before the initiation of islet hormone expression (Serafimidis et al., 2008; Vuguin and Charron, 2011).

expression is early step for initiating the synthesis of Glut2 and Ngn3 and then is turned off by Ngn3 embryonic development, pancreatic islet cells differentiate from endocrine progenitors in which Pdx1 found that after treatment with GCGR mAb, whether a cell conversion is not yet clear. In this study, we investigated whether α-to-β cell conversion induced by GCGR mAb was direct or indirect trans-differentiation. We found that after treatment with GCGR mAb, α-cells could regress to the immature state, and express the progenitor’s markers (Ngn3, Glut2, and Pdx1) in pancreatic α-cell lineage-tracing T2D mice. During embryonic development, pancreatic islet cells differentiate from endocrine progenitors in which Pdx1 expression is early step for initiating the synthesis of Glut2 and Ngn3 and then is turned off by Ngn3 before the initiation of islet hormone expression (Serafimidis et al., 2008; Vuguin and Charron, 2011).

It has been shown that the global deletion of Gcgr inhibits the progression of α-cells to a mature state (Vuguin et al., 2006). In addition, we also revealed that GCGR mAb promoted progenitor-derived β-cell neogenesis in Ngn3+ cell lineage-tracing T2D mice. Taken together, these results suggested that GCGR antagonism not only could directly promote α-to-β cell conversion but also induced α-cell regression to progenitors which were responsible for β-cell neogenesis, thereby contributing to β-cell regeneration in T2D mice.

However, the exact process of α-to-β cell conversion is not yet clear. In this study, we investigated whether α-to-β cell conversion induced by GCGR mAb was direct or indirect trans-differentiation. We found that after treatment with GCGR mAb, α-cells could regress to the immature state, and express the progenitor’s markers (Ngn3, Glut2, and Pdx1) in pancreatic α-cell lineage-tracing T2D mice. During embryonic development, pancreatic islet cells differentiate from endocrine progenitors in which Pdx1 expression is early step for initiating the synthesis of Glut2 and Ngn3 and then is turned off by Ngn3 before the initiation of islet hormone expression (Serafimidis et al., 2008; Vuguin and Charron, 2011). It has been shown that the global deletion of Gcgr inhibits the progression of α-cells to a mature state (Vuguin et al., 2006). In addition, we also revealed that GCGR mAb promoted progenitor-derived β-cell neogenesis in Ngn3+ cell lineage-tracing T2D mice. Taken together, these results suggested that GCGR antagonism not only could directly promote α-to-β cell conversion but also induced α-cell regression to progenitors which were responsible for β-cell neogenesis, thereby contributing to β-cell regeneration in T2D mice.

In conclusion, our study demonstrates that treatment with GCGR mAb in T2D mice ameliorates hyperglycemia and increases functional β-cell mass via inducing pro-α-cell-mediated progenitor-derived β-cell neogenesis. Our study suggests that blockage of α-cell-derived glucagon promotes β-cell regeneration, and provides new insight into the clinical development of GCGR mAb in treating T2D.

Limitation of the study
It has been demonstrated that GCGR mAb displays an improved glycemic control with less need for exogenous insulin in T1D rodents and humans (Pettus et al., 2018; Wang et al., 2021b; Wei et al., 2019). This effect might be accounted for by the enhanced insulin action in both liver and skeletal muscle (Sharma et al., 2018), or by the upregulated circulating glucagon-like peptide-1 (GLP-1) level (Lang et al., 2020a, 2020b). In this study, we found that GCGR mAb increased β-cell mass and elevated plasma insulin levels in T2D mice. Whether this elevation in plasma insulin is owing to altered insulin clearance or other mediators induced by GCGR mAb needs to be clarified.

The levels of endogenous glucagon are highest in the venous drainage of pancreas and hepatic portal vein, and its principal target is liver. GCGR is predominantly expressed in liver, and also lowly expressed in other tissues, including pancreas, kidney, adipose tissue, and brain (Muller et al., 2017). Therefore, the possible mechanism of the GCGR mAb-induced β-cell regeneration may be driven by the loss of GCGR signaling in pancreas per se, or because of the upregulation of the proglucagon-related peptide production that results from loss of GCGR activity in hepatocytes. The exact mechanism deserves further in-depth investigation in the future.

The incretins GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) are gut hormones that potentiate insulin secretion in a glucose-dependent manner. GLP-1 and GIP exert their insulinotropic actions through distinct G-protein-coupled receptors highly expressed on pancreatic β-cells (Campbell and Drucker, 2013). Moreover, it has been reported that GLP-1 and GIP improve β-cell survival in rodents (Drucker, 2013). In our previous studies, we demonstrated that treatment with GCGR mAb could significantly upregulate the GLP-1 production and its circulating level in T2D mice (Lang et al., 2020a, 2020b). Therefore, GLP-1 might participate in the process of β-cell regeneration induced by GCGR

α-to-β cell conversion (Ben-Othman et al., 2017; Li et al., 2017), albeit there were some controversial results (van der Meulen et al., 2018). Our previous studies indicated that GCGR mAb induced α-to-β cell conversion in two T1D mouse models (Wei et al., 2019). Similarly, this study showed that GCGR mAb also promoted the trans-differentiation of α-cells to β-cells in T2D mice. As α-to-β cell conversion can also occur in human islets (Ben-Othman et al., 2017), we believe that GCGR mAb may have a similar effect in T2D humans.

However, the exact process of α-to-β cell conversion is not yet clear. In this study, we investigated whether α-to-β cell conversion induced by GCGR mAb was direct or indirect trans-differentiation. We found that after treatment with GCGR mAb, α-cells could regress to the immature state, and express the progenitor’s markers (Ngn3, Glut2, and Pdx1) in pancreatic α-cell lineage-tracing T2D mice. During embryonic development, pancreatic islet cells differentiate from endocrine progenitors in which Pdx1 expression is early step for initiating the synthesis of Glut2 and Ngn3 and then is turned off by Ngn3 before the initiation of islet hormone expression (Serafimidis et al., 2008; Vuguin and Charron, 2011). It has been shown that the global deletion of Gcgr inhibits the progression of α-cells to a mature state (Vuguin et al., 2006). In addition, we also revealed that GCGR mAb promoted progenitor-derived β-cell neogenesis in Ngn3+ cell lineage-tracing T2D mice. Taken together, these results suggested that GCGR antagonism not only could directly promote α-to-β cell conversion but also induced α-cell regression to progenitors which were responsible for β-cell neogenesis, thereby contributing to β-cell regeneration in T2D mice.

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STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Animals
  - Genetically modified mice
  - Primary mouse islet isolation and culture
- METHOD DETAILS
  - Animals’ intervention
  - Glucose monitoring

mAb. Genetic and pharmacological blockage of the GLP-1 receptor would help to answer the question.

Figure 5. Immunofluorescent analysis of α-to-β cell conversion in the pancreatic tissues of T2D mouse models treated with GCGR mAb or IgG control for 4 weeks

(A and B) Quantification of glucagon+/insulin+ cells in db/db mice (A) and HFD + STZ-induced T2D mice (B) as shown in Figures 2A and 2C.

(C and D) Representative image of an islet immunostained with β-gal (α-cell lineage-tracing marker) and insulin (C), and quantification of β-gal+/insulin+ cells (D) in α-cell lineage-tracing T2D mice that were induced by HFD + STZ. The arrows indicate co-labeled cells. The cells in the small box are enlarged at the right of the image. Scale bar = 50 μm. n = 5 sections/mouse multiplied by 6 mice/group in db/db mice, n = 3 sections/mouse multiplied by 9 mice/group in HFD + STZ-induced T2D mice, and n = 3 sections/mouse multiplied by 5 mice/group in the α-cell lineage-tracing T2D mice. Data represent the mean ± SEM. Statistical analysis was conducted by Student’s t-test. ***p < 0.001 vs. control. See also Figures S5, S6, and S7.

mAb. Genetic and pharmacological blockage of the GLP-1 receptor would help to answer the question.
Primary mouse islet

(A–D) Intracellular glucagon content (A), supernatant glucagon level (B), intracellular insulin content (C), and supernatant insulin level (D) were detected by ELISA. The hormone levels were normalized to total protein content.

(E) Glucose-stimulated insulin secretion assay in the islets after treated with GCGR mAb or IgG. The insulin levels were normalized to total protein content.

(F) Gene expression was determined by quantitative RT-PCR. The experiments were repeated 3 times. Data represent the mean ± SEM. Statistical analysis was conducted by Student’s t-test or two-way ANOVA as appropriate. *p < 0.05, **p < 0.01, ***p < 0.001 vs. control. See also Figure S8.

Supplemental Information

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104567.

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AUTHOR CONTRIBUTIONS

Conceptualization: X.C., R.W., and T.H.; methodology: X.C., J.F., T.W., L.G., D.W., S.L., and K.Y.; software: T.W.; validation: J.F. and T.W.; formal analysis: X.C., J.F., R.W., and T.H.; investigation: X.C., J.F., D.W., S.L., K.Y., and J.Y.; resources: H.Y.; writing – original draft: X.C. and J.F.; writing – review & editing: R.W. and T.H.; visualization: X.C., J.F. and L.G.; supervision: J.Y., H.Y., R.W., and T.H.; project administration: R.W. and T.H.; funding acquisition: X.C., L.G., R.W., and T.H.

DECLARATION OF INTERESTS

Hai Yan is a shareholder of REMD Biotherapeutics. The authors have no additional financial interests.

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### Key Resources Table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Rabbit polyclonal anti-glucagon | Cell Signaling Technology | Cat#2760; RRID: AB_659831 |
| Mouse monoclonal anti-glucagon | Sigma-Aldrich | Cat#G2654; RRID: AB_259852 |
| Mouse monoclonal anti-insulin | Sigma-Aldrich | Cat#I2018; RRID: AB_260137 |
| Rabbit monoclonal anti-cytokeratin 19 | Abcam | Cat#ab52625; RRID: AB_2281020 |
| Rabbit polyclonal anti-RFP | Abcam | Cat#ab62341; RRID: AB_945213 |
| Rabbit polyclonal anti-J-gal | Abcam | Cat#ab203749; RRID: AB_2920785 |
| Mouse monoclonal anti-Ngn3 | Santa Cruz | Cat# sc-374442; RRID: AB_10988579 |
| Mouse monoclonal anti-Glut2 | Santa Cruz | Cat#sc-518022; RRID: AB_2890905 |
| Rabbit polyclonal anti-Pdx1 | Abcam | Cat#ab47267; RRID: AB_777179 |
| Rabbit monoclonal anti-Nkx6.1 | Abcam | Cat#ab221549; RRID: AB_2754979 |
| Rabbit polyclonal anti-PC1/3 | Millipore | Cat#AB10553; RRID: AB_1977441 |
| Rabbit polyclonal anti-C-peptide | Cell Signaling Technology | Cat#4593; RRID: AB_10691857 |
| Rabbit polyclonal anti-GCGR | Proteintech | Cat#26784-1-AP; RRID: AB_2880634 |
| Mouse monoclonal anti-GAPDH | Zhongshan Biotechnology | Cat#TA-08; RRID: AB_2107448 |
| Alexa Fluor 488-conjugated AffiniPure goat polyclonal anti-rabbit IgG (H + L) | Jackson ImmunoResearch Laboratories | Cat#115-545-003; RRID: AB_2338046 |
| Alexa Fluor 594-conjugated AffiniPure goat polyclonal anti-mouse IgG (H + L) | Jackson ImmunoResearch Laboratories | Cat#115-585-003; RRID: AB_2338871 |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| REMD 2.59 (a human GCGR mAb and competitive antagonist) | REMD Biotherapeutics | N/A |
| High-fat diet (HFD) | Research Diets | Cat#D12492 |
| Streptozocin (STZ) | Sigma-Aldrich | Cat#S0130 |
| Tamoxifen | Sigma-Aldrich | Cat#T5648 |
| Goat serum | Zhongshan Biotechnology | Cat#ZLI-9056 |
| DAPI | Sigma-Aldrich | Cat#D9542 |
| Trizol reagent | Thermo Fisher Scientific | Cat#15596018 |
| SYBR qPCR Mix | TOYOBO | Cat#QPS-201 |
| RIPA lysis buffer | Applygen Technologies Inc. | Cat#C1053 |
| Protease inhibitor | Applygen Technologies Inc. | Cat#P1265 |
| Phosphatase inhibitor | Applygen Technologies Inc. | Cat#P1260 |
| **Critical commercial assays** |        |            |
| Insulin ELISA kit | Millipore | Cat#EZRMI-13K |
| Glucagon ELISA kit | R&D Systems | Cat#DGC0 |
| C-peptide ELISA kit | Millipore | Cat#EZRMC2-21K |
| Opal 7 color manual kit | Akoya Bioscience | Cat#NEL811001KT |
| RevertAid First Strand cDNA Synthesis kit | Thermo Fisher Scientific | Cat#K1622 |
| **Experimental models: Organisms/strains** |        |            |
| Mouse: C57BL/6N | Vital River Animal Center | Cat#213 |
| Mouse: db/db (BKS.Cg-Dock7m+/+Lepr<sup>db</sup>/Nju) mice | Nanjing Biomedical Research Institution of Nanjing University | Cat#T001463 |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact Tianpei Hong (tpho66@bjmu.edu.cn).

Materials availability
This study did not generate new unique reagents.

Data and code availability
Any additional information required to reanalyze the data reported in this paper is available from the lead contact Tianpei Hong (tpho66@bjmu.edu.cn) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals
All animal experimental procedures were conducted at Peking University Health Science Center and approved by the Institutional Animal Care and Use Committee. All the animals were group-housed conventionally and maintained on a 12-h light/dark cycle with free access to food and water. Eight-week-old male db/db (BKS.Cg-Dock7m+/+Leprb/Nju) mice (Nanjing Biomedical Research Institution of Nanjing University, Nanjing, China) were used as a typical T2D model. The littermate male db/m mice served as normal controls. n = 10 per group in db/db mice, and n = 6 in db/m mice.

To generate high-fat diet + streptozotocin (HFD + STZ)-induced T2D model, 5-week-old male C57BL/6N (Vital River Animal Center, Beijing, China) or genetically modified mice were fed with HFD (fat 60%, carbohydrate 20% and protein 20%; Research Diets, New Brunswick, NJ, USA) for 3–4 months, and then were given 75 mg/kg STZ (Sigma-Aldrich, St. Louis, MO, USA) intraperitoneally. Diabetic condition was confirmed if the fasting blood glucose level was ≥11.1 mmol/L. Mice were sorted into groups having similar distributions based on body weight and blood glucose levels.

Genetically modified mice
Tg(Ngn3-cre/ERT2)1Able/J mice (expressing a tamoxifen-inducible cre recombinase directed to Ngn3-expressing cells; The Jackson Laboratory, Barr Harbor, ME, USA) and B6/JGpt-Rosa26tm1(CAG-LSLCas9-tdTomato)/Gpt mice (when crossed to a cre recombinase-expressing strain, tdTomato expression is observed in the cre-expressing tissues; GemPharmatech, Nanjing, China) were crossed to generate pancreatic endocrine progenitor lineage-tracing mice, namely, Ngn3-CreERT2-tdTomato mice. Male mice were selected for subsequent experiments. Tamoxifen (Sigma-Aldrich) was dissolved in corn oil (Sigma-Aldrich), and intraperitoneal injection (20 mg/day) was given every 5 days since 1 week before being sacrificed. n = three to four per group.

B6.Cg-Tg(Gcg-cre)1Herr/Mmnc mice (cre expression in pancreatic α-cell lineage; Mutant Mouse Resource & Research Centers, Columbia, MO, USA) and B6; 129-Gt(Rosa26Sor+tm1Sor/J mice (when crossed to a cre recombinase-expressing strain, lacZ (which encodes β-gal) expression is observed in the cre-expressing tissues; The Jackson Laboratory) were crossed to generate pancreatic α-cell lineage-tracing mice, namely, glucagon-cre-β-gal mice. Male mice were selected for subsequent experiments. n = five to eight per group.
Primary mouse islet isolation and culture

Primary islets were isolated from male C57BL/6N mice aged 8–10 weeks as previously reported (Wang et al., 2014; Wei et al., 2020; Zmuda et al., 2011). Briefly, the pancreas was perfused by collagenase V (Sigma-Aldrich), and individual islets were handpicked to near 100% purity under a dissecting microscope. The islets were cultured for 24 h before treatment in the RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; HyClone, Logan, UT, USA), 2 mmol/L GlutMax, 1 mmol/L sodium pyruvate, and 1% (v/v) penicillin-streptomycin (Thermo Fisher Scientific, Waltham, MA, USA).

METHOD DETAILS

Animals’ intervention

All mice were treated for 4 weeks via weekly intraperitoneal administration of REMD 2.59 (5 mg/kg; REMD Biotherapeutics, Camarillo, CA, USA), a human antagonistic GCGR mAb, or IgG (5 mg/kg, as control).

Glucose monitoring

Blood samples for glucose detection were collected from the tail vein, and measured by the glucose oxidase method using a hand-held OneTouch Ultra glucometer (LifeScan, Milpitas, CA, USA).

Glucose tolerance test and blood sample collection

To perform IPGTT, basal blood glucose levels were first measured after overnight fasting. A 40% (w/v) of glucose solution was administrated by intraperitoneal injection at 2 g/kg, and blood glucose levels were measured at 30, 60 and 120 min after the glucose loading. If the glucose level was higher than 33.3 mmol/L (upper detection limit of the glucometer), the value of 33.3 mmol/L was recorded.

Blood samples for hormone detection were collected from the orbital vein. Aprotinin (1 μg/mL; Sigma-Aldrich) and heparin sodium (1,000 IU/mL; Qinhong Bio-pharma, Changzhou, China) were added to each blood sample.

Immunofluorescent staining and quantification

Pancreata were fixed with 10% (v/v) neutral-buffered formalin and embedded in paraffin, and 5-μm-thick sections were prepared. For immunofluorescence, sections were incubated with primary antibodies at 4 °C overnight and secondary antibodies for 1 h at room temperature, followed by staining with DAPI. Images were captured under Leica TCS SP8 confocal fluorescence microscope (Leica Microsystems, Wetzlar, Germany) or an automatic digital slide scanner (Pannoramic MIDI, 3D HISTECH, Budapest, Hungary).

The primary antibodies were as follows: rabbit polyclonal anti-glucagon (1:800; Cell Signaling Technology, Boston, MA, USA; RRID: AB_659831), mouse monoclonal anti-glucagon (1:400; Sigma-Aldrich; RRID: AB_259852), mouse monoclonal anti-insulin (1:800; Sigma-Aldrich; RRID: AB_260137), rabbit monoclonal anti-cyto-keratin 19 (1:200; Abcam, Cambridge, UK; RRID: AB_2281020), rabbit polyclonal anti-RFP (1:200; Abcam; RRID: AB_945213), rabbit polyclonal anti-Iβ-gal (1:100; Abcam; RRID: AB_2920785), mouse monoclonal anti-Ngn3 (1:50; Santa Cruz, CA, USA; RRID: AB_10988579), mouse monoclonal anti-Glut2 (1:100; Santa Cruz; RRID: AB_2890905), rabbit polyclonal anti-Pdx1 (1:200; Abcam; RRID: AB_777179), rabbit monoclonal anti-Nkx6.1 (1:400; Abcam; RRID: AB_2754979), rabbit polyclonal anti-PC1/3 (1:400; Millipore, Darmstadt, Germany; RRID: AB_1977441), rabbit polyclonal anti-C-peptide (1:400; Cell Signaling Technology; RRID: AB_10691857), rabbit anti-GCGR (Proteintech, Rosemont, IL, USA; RRID: AB_2880634). The secondary antibodies were as follows: Alexa Fluor 488-conjugated AffiniPure goat polyclonal anti-rabbit IgG (H + L) (RRID: AB_2338046) and Alexa Fluor 594-conjugated AffiniPure goat polyclonal anti-mouse IgG (H + L) (RRID: AB_2338871) (both at 1:800; Jackson ImmunoResearch Laboratories, Philadelphia, PA, USA).

Opal seven color manual kit (Akoya Bioscience, Marlborough, MA, USA) was used for co-immunostaining of Nkx6.1, PC1/3 and C-peptide with glucagon, which was detected by Vectra Polaris (Akoya Bioscience).

For cell quantification in the immunofluorescent staining, three to five equally spaced sections (which covered the entire pancreas) per pancreas were imaged, and the total numbers of positive staining cells from three to nine mice per group were counted manually.
Primary mouse islet culture and intervention
Primary mouse islets were incubated with 1,000 nmol/L REMD 2.59 or human IgG for 24 h in high glucose (25 mmol/L) condition. Islets and supernatants were collected for quantitative RT-PCR analysis and hormone measurement, respectively.

Glucose-stimulated insulin secretion test
After cultured with 1,000 nmol/L REMD 2.59 or IgG for 24 h, five similarly-sized islets were selected from each group. The islets were preincubated in Krebs-Ringer bicarbonate buffer (KRBB) supplemented with 1 g/L BSA and 2.8 mmol/L glucose for 1 h at 37 °C, and then were transferred to another dish with the same buffer. After 1 h of incubation, the buffer was sampled for insulin measurement, and the islets were exposed to a 1-h 16.7 mmol/L glucose challenge followed by another sample for insulin measurement. The insulin secretion in each group was normalized to the total protein content.

Hormone measurement
Blood samples, culture supernatants and islet lysates, and KRBB buffer samples were evaluated with specific ELISA kits for detecting insulin (Millipore), C-peptide (Millipore) and glucagon (R&D System, Minneapolis, MN, USA) according to the manufacturer’s instructions.

Protein extraction and Western blot analysis
The mouse pancreatic β-cell line Min6 cells, kindly gifted by Prof. Yiming Mu from the General Hospital of the People’s Liberation Army (Beijing, China), were cultured in DMEM (25 mmol/L glucose; Invitrogen) supplemented with 15% (v/v) FBS, 2 mmol/L GlutMax and 55 μmol/L β-mercaptoethanol (Thermo Fisher Scientific). Total proteins from Min6 cells were obtained using radioimmunoprecipitation assay (RIPA) lysis buffer, which contained protease inhibitor and phosphatase inhibitor. The denatured proteins (approximately 30 μg) were separated by 12% (w/v) SDS-PAGE electrophoresis and transferred to a nitrocellulose membrane. The membranes were incubated overnight at 4 °C with the primary antibodies (both at 1:1,000 dilution): rabbit anti-GCGR (Proteintech; RRID: AB_2880634) and mouse anti-GAPDH (Zhongshan Biotechnology, Beijing, China; RRID: AB_2107448). After three washes, the blots were incubated for 1 h with RDye 800CW-conjugated goat anti-rabbit IgG or goat anti-mouse IgG (both at 1:10,000 dilutions; LICOR Biosciences, Lincoln, NE, USA). Protein bands were visualized with an Odyssey 290 infrared imaging system (LICOR Biosciences). GAPDH was used as a loading control.

RNA extraction and reverse transcription
Total RNA was extracted with Trizol reagent (Thermo Fisher Scientific) and reversely transcribed to cDNA using a RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific).

Conventional and quantitative RT-PCR
The cDNA was subjected to either the conventional RT-PCR analysis using Taq PCR Mastermix (Tiangen biotechnology, Beijing, China) on an Applied Biosystems Veriti 96 well Thermal Cycler PCR detection system (Thermo Fisher Scientific) or the quantitative RT-PCR analysis using iQ SYBR Green supermix (BioRad Laboratories, Hercules, CA, USA) on a QuantStudio five Real-Time PCR System (Thermo Fisher Scientific). Relative quantification for gene expression was calculated using the 2^(-ΔΔCT) method, which was normalized to the internal reference, β-actin. The primer sequences were summarized in Table S1.

QUANTIFICATION AND STATISTICAL ANALYSIS
Data are presented as mean ± SEM or median (interquartile range). All statistical analyses were performed using GraphPad Prism v.7.0 (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was defined as p < 0.05 and determined by Student’s t tests, one-way or two-way ANOVA followed by the post hoc Tukey-Kramer test, or Mann-Whitney test, when appropriate.