Supplementary online-only materials

Animals

Eleven-week-old male C57BL/6J mice were purchased from SPF Biotechnology Co., Ltd (Beijing, China). Seven-week-old male BKS-*Lepr*^{em2Cd479}/Gpt (*db/db*, Strain No. T002407) mice were obtained from GemPharmatech Co. Ltd. (Nanjing, China). All the animals were used after one-week adaption.

Global *Glp1r*-knockout (*Glp1r*$^{-/-}$) mice, which were generated by View-Solid Biotech, Inc. (Beijing, China), were kindly gifted by Prof. Yingmei Feng from Beijing Youan Hospital (Beijing, China). A targeted sequence within the sixth exon of *Glp1r* gene was chosen. Transcription activator-like effector nuclease (TALEN)-Left was designed against the sequence (5′-AGACACTTGCACTGCACC-3′) for the sense strand, and TALEN-Right was designed against the sequence (5′-AACCTGTTTGCATCCTTC-3′) for the anti-sense strand. The pair was separated by a spacer region of 18 bp (5′-AGGAACTACATCCACCTG-3′) and sequence analysis was applied to confirm frameshift mutations (Supplementary Fig. 1*A* and *B*). The heterozygotes were intercrossed to generate *Glp1r*$^{-/-}$ mice and wild-type (WT) *Glp1r*$^{+/+}$ littermates.

B6.FVB-Tg(Pdx1-cre)6Tuv/J (Pdx1-Cre) mice, which were purchased from the Jackson laboratory (Stock No. 014647; Barr Harbor, ME), were kindly gifted by Prof. Jingjing Zhang from the Second Xiangya Hospital of Central South University (Changsha, China) (1). These Pdx1-Cre transgenic mice express cre recombinase under the control of *Pdx1* promoter and present pancreas-specific conditional mutations in the islet cells, ductal cells, and acinar cells. B6/JGpt-Glp1r^{em1Cflox}/Gpt (*Glp1r*-flox) mice (Strain No. T005818; GemPharmatech) were generated by the clustered regularly interspaced short palindromic repeats (CRISPR) – CRISPR associated protein 9

(Cas9)–mediated genome editing. Exon2–exon3 of transcript *Glp1r*-201 (ENSMUST00000114574.2) was selected to conduct the targeting strategy. In brief, Cas9 mRNA, single guide RNAs (sgRNAs), and donors were coinjected into the zygotes of C57BL/6JGpt mice, directing Cas9 endonuclease cleavage and LoxP site insertion in intron 1 and intron 3 of mouse *Glp1r* (Supplementary Fig. 1*C* and *D*). Subsequently, the zygotes were transferred into the uterus of pseudopregnant mice to form positive F0 mice. A stable F1 generation was obtained by mating positive F0 generation mice with WT C57BL/6JGpt mice. To obtain pancreas-specific *Glp1r*-knockout ($Glp1r^{pan-/-}$) mice, Pdx1-Cre mice were crossed with *Glp1r*-flox mice.

Mice were housed in a temperature- and humidity-controlled room on a regular 12 h light/dark cycle with free access to water and standard rodent chow.

Animal Intervention

Male diabetic *db/db* mice (8 weeks old) were divided into four groups, according to their body weight and blood glucose level, and treated for 4 weeks with IgG, glucagon receptor (GCGR) monoclonal antibody (mAb), exendin 9-39 (Ex9), or GCGR mAb combined with Ex9, respectively. There were 4–5 mice per group. The antagonistic GCGR mAb REMD 2.59 (REMD Biotherapeutics, Camarillo, CA), which has been proved to specifically bind to GCGR while shows no binding to glucagon-like peptide 1 receptor (GLP-1R) (2), and IgG (as control) were intraperitoneally injected weekly at the dose of 5 mg/kg body weight. Ex9 (Bachem, Bubendorf, Switzerland) or saline was administered by micro-osmotic pumps (ALZET, Cupertino, CA), which was implanted subcutaneously and replaced weekly, at the dose of 50 nmol/kg/day to release Ex9 continuously. The dose has been reported to impair glucose homeostasis but not affect islet histology or induce toxic effect in mice (3,4).

To induce T1D model, male and female *Glp1r*$^{-/-}$ mice and WT littermates, and male *Glp1r*$^{pan-/-}$ mice and Flox/cre littermates were fasted for 8 h, and injected with streptozotocin (STZ, 125 mg/kg; MilliporeSigma, St. Louis, MO) to ablate β-cells at the age of 8–12 weeks. Diabetic condition was defined if the fasting blood glucose was ≥11.1 mmol/L or random blood glucose was ≥16.7 mmol/L for twice at an interval of more than 24 h. The diabetic mice were assigned to two groups having comparable body weight and blood glucose level, and then treated weekly with 5 mg/kg GCGR mAb or IgG for 4 weeks. There were 6–7 mice per group.

Male C57BL/6J mice (12 weeks old) were given with STZ to induce a T1D model. Based on the body weight and blood glucose level, the diabetic mice were sorted into four groups: *1)* control group, injected with IgG and A-TNP; *2)* GCGR mAb group, received injection of GCGR mAb and A-TNP; *3)* GCGR mAb + glucagon-neutralizing antibody (nAb) group, injected with GCGR mAb and glucagon nAb; 4) glucagon nAb group, received injection of IgG and glucagon nAb. There were 4–5 mice per group. GCGR mAb (5 mg/kg) or IgG (5 mg/kg, as control of GCGR mAb) was injected intraperitoneally once a week. Glucagon nAb GLU-001 (which shows no binding to GLP-1, gastric inhibitory polypeptide, glicentin-related pancreatic peptide, vasoactive intestinal polypeptide or pancreatic polypeptide) and A-TNP (the inert monoclonal control IgG, as control of glucagon nAb) were kindly gifted by Dr. Christian L. Brand (Diabetes Research Unit, Novo Nordisk A/S, Bagsvaerd, Denmark), and given to mice via daily intraperitoneal injection (5). The dose (4 mg/kg) of glucagon nAb has been demonstrated to have the ability to immunoneutralize >90% of circulating glucagon (the binding capacity of nAb was 40 μmol/L glucagon) for several hours (6).

For cell proliferation detection, all mice were given with 1 g/L 5-bromo-2'-deoxyuridine (BrdU, MilliporeSigma) via drinking water for one week before sacrifice.

Blood Sample and Pancreatic Tissue Collection

Mice received general anesthesia with avertin (0.4 g/kg, MilliporeSigma). Blood samples were collected from the orbital sinus. Heparin sodium (1000 IU/mL; Changzhou Qianhong Bio-pharma Co. Ltd, Changzhou, China), aprotinin (1 μg/mL; Roche, Basel, Switzerland), and dipeptidyl peptidase 4 inhibitor (50 μmol/L; Merck Millipore, Darmstadt, Germany) were added to blood sample to prevent protein and active GLP-1 degradation.

Pancreatic tissues were obtained and homogenized in radioimmunoprecipitation assay (RIPA) lysis buffer (Applygen Technologies Inc., Beijing, China) containing protease inhibitor (Applygen Technologies Inc.), phosphatase inhibitor (Applygen Technologies Inc.), dipeptidyl peptidase 4 inhibitor, and aprotinin. After centrifugation, supernatants were collected for hormone detection as pancreatic hormone content.

Pancreatic tissues were obtained and fixed with 10% (v/v) neutral-buffered formalin for 24 h, and then embedded in paraffin for immunofluorescent staining.

Primary Mouse Islet Isolation and Intervention

Primary mouse islets were isolated from eight-week-old male normal C57BL/6J mice, *db/db* mice, and *Glp1r*$^{pan-/-}$ mice as previously reported (7). Briefly, mouse pancreas was perfused with 0.5 mg/mL collagenase-V (MilliporeSigma) and then digested for 10 min at 37ºC. Islets were purified using Histopaque 1077 (MilliporeSigma) and then handpicked. Islets were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (HyClone, Logan, UT), 2 mmol/L GlutMax (Gibco, Grand Island, NY), 1 mmol/L sodium pyruvate (Gibco) and 1% (v/v) penicillin-streptomycin (Gibco).

After 12-h recovery, islets were treated with 1,000 nmol/L GCGR mAb or IgG in the absence or presence of Ex9 (200 nmol/L) or glucagon nAb (10 mg/L) for 24 h in high glucose (30 mmol/L) condition. For hormone measurement, dipeptidyl peptidase 4 inhibitor (50 μmol/L) was added to culture medium to prevent active GLP-1 degradation. Hormones in islet culture supernatants were considered as hormone release.

Hormone Measurements

To detect the hormone level in mouse plasma, pancreatic lysates and islet culture supernatants, ELISA kits specific for insulin, C-peptide, glucagon, and active GLP-1 listed in Supplementary Table 1 were used according to the manufacturer's instruction. The hormone levels in pancreatic lysates and islet culture supernatants were normalized to the protein content of pancreatic lysates and cultured islets, respectively. The protein content was measured by BCA protein assay (Thermo Fisher Scientific, Waltham, MA).

As for glucagon ELISA kit, no significant cross-reactivity or interference is observed with gastric inhibitory polypeptide, GLP-1, GLP-2, or glicentin-related pancreatic peptide. Oxyntomodulin cross-reacts < 12%. As for active GLP-1 ELISA kit, the cross-reactivity of GLP-1 (7-36 amide) and GLP-1 (7-37) is 100%, and the cross-reactivity of GLP-1 (9-36 amide), GLP-2 or glucagon is not detectable.

References

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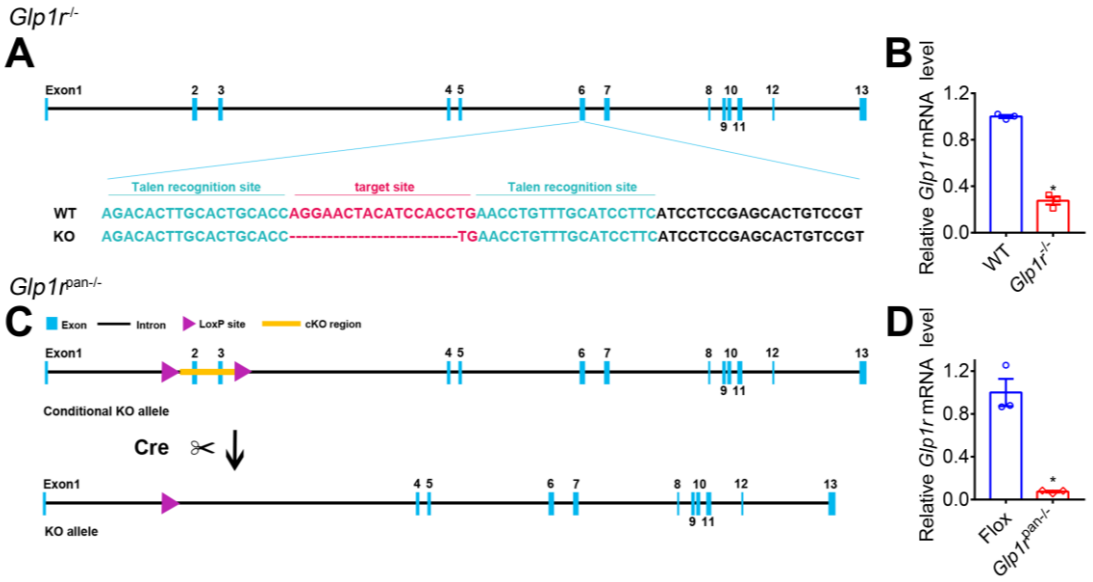
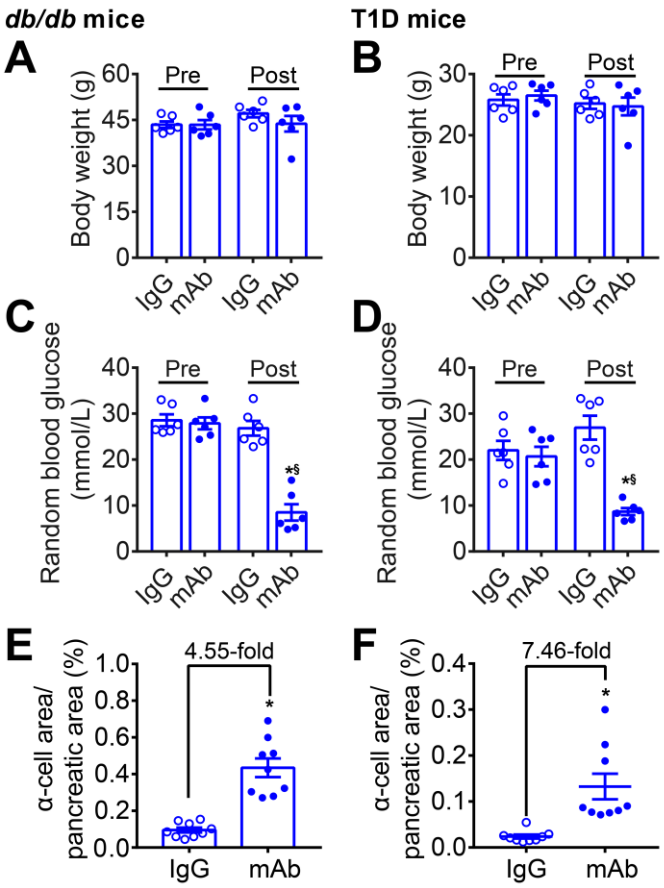
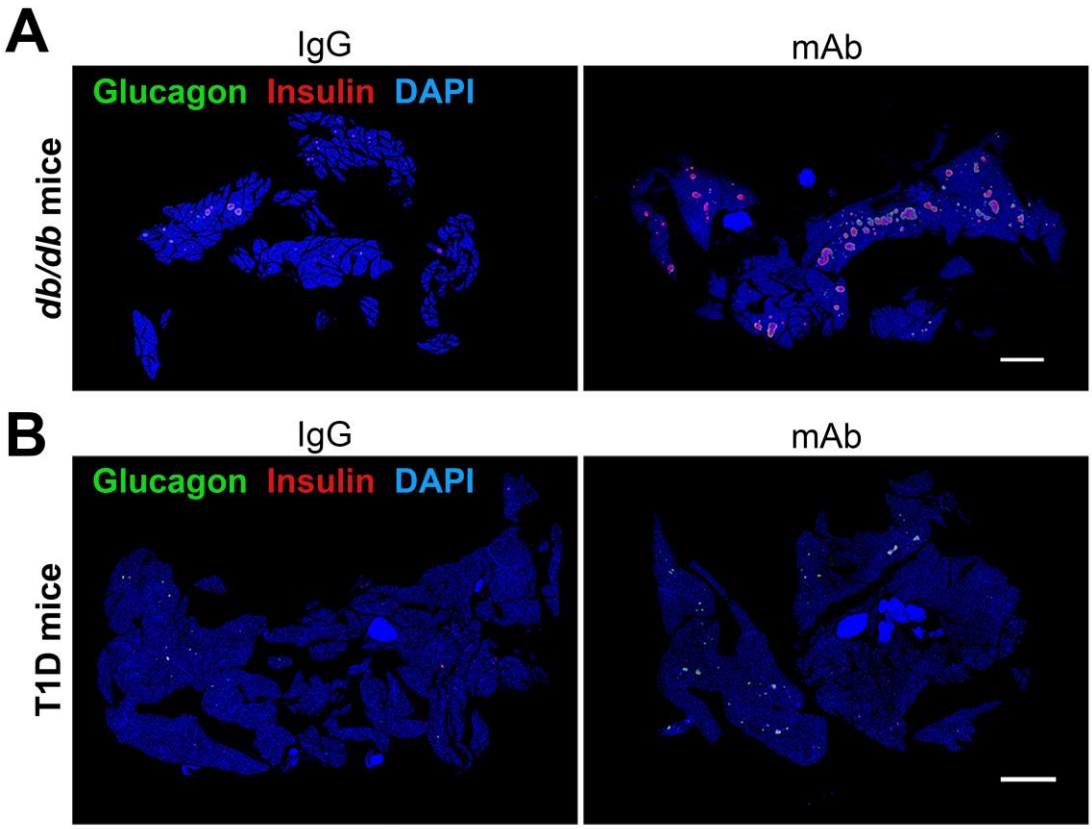
Supplementary figures



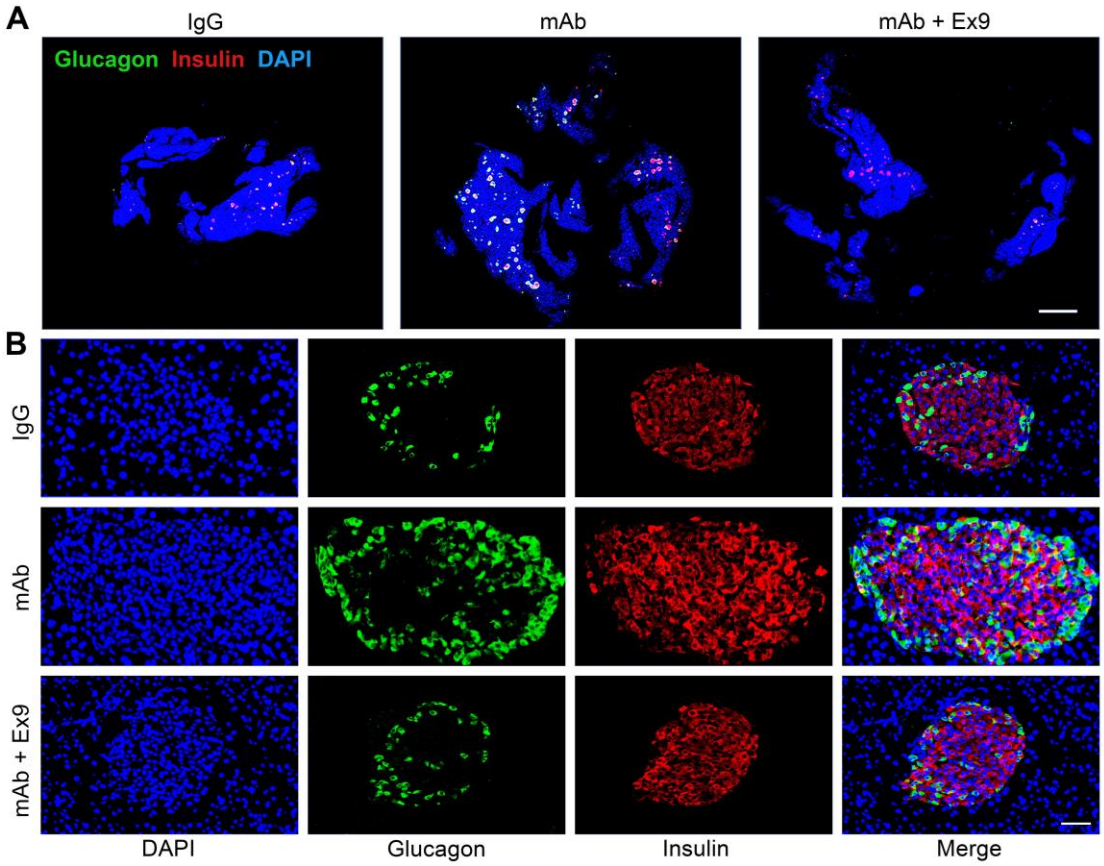
Supplementary Figure 1–Generation and verification of *Glp1r*$^{-/-}$ mice and *Glp1r*$^{pan-/-}$ mice. *A*: Schematic depicting the strategy for generating *Glp1r*$^{-/-}$ mice. *B*: *Glp1r* mRNA level in the isolated islets of *Glp1r*$^{-/-}$ and WT (*Glp1r*$^{+/+}$) mice. *C*: Schematic depicting the strategy for generating *Glp1r*$^{pan-/-}$ mice. *D*: *Glp1r* mRNA level in the isolated islets of *Glp1r*$^{pan-/-}$ and Flox littermate mice (n = 3). Data are expressed as the mean ± SEM. Statistical analysis was performed by unpaired Student t test. *$P < 0.05$ vs. WT or Flox littermates.



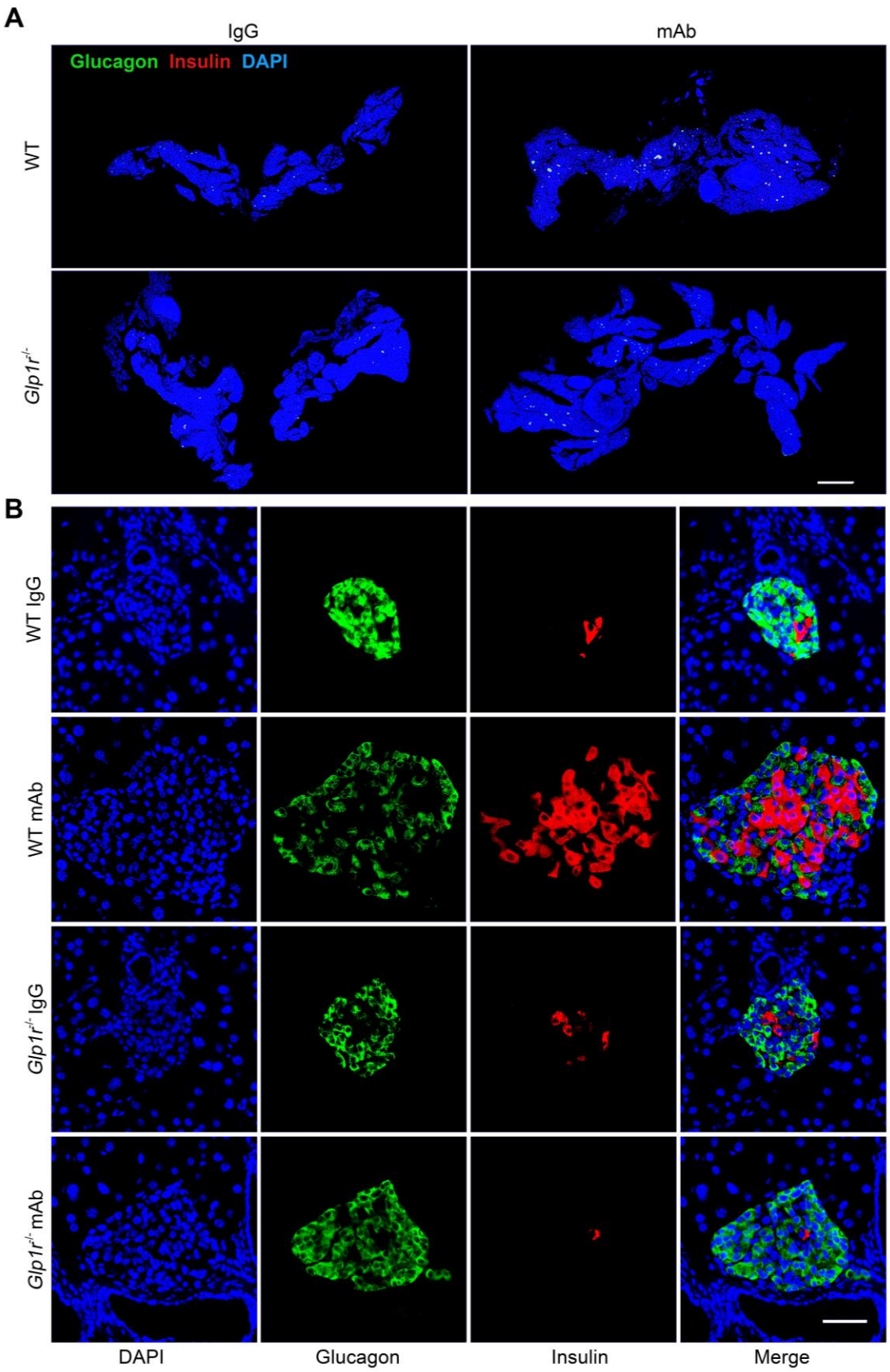
Supplementary Figure 2–GCGR mAb ameliorates hyperglycemia and promotes α-cell hyperplasia in T2D and T1D mice. Male *db/db* mice (8 weeks) were used as a T2D model. Male C57BL/6J mice (12 weeks) were injected with STZ to induce a T1D model. Mice were treated weekly with IgG (5 mg/kg, as control) or GCGR mAb (5 mg/kg) for 4 weeks. *A*, *C* and *E*: Parameters in *db/db* mice. *B*, *D* and *F*: Parameters in T1D mice. *A* and *B*: Body weight. *C* and *D*: Random blood glucose (n = 6 mice/ group). *E* and *F*: Quantification of the α-cell area per pancreatic section (n = 3 section/mouse × by 3 mice/group). Data are expressed as the mean ± SEM or median (interquartile range). Statistical analysis was performed by two-way ANOVA, followed by Bonferroni multiple comparisons test in *A–D*, or by unpaired student *t* test in *E*, or by Mann-Whitney test in *F*. *$P < 0.05$ vs. IgG control; §$P < 0.05$ vs. pretreatment in the same group.



Supplementary Figure 3–GCGR mAb increases α-cell and β-cell areas in T2D and T1D mice. Male *db/db* mice (8 weeks) and male STZ-induced T1D C57BL/6J mice (12 weeks) were treated weekly with IgG (5 mg/kg, as control) or GCGR mAb (5 mg/kg) for 4 weeks. Representative photographs of the entire pancreatic section of *db/db* mice (*A*) and T1D mice (*B*). Pancreatic sections were stained for insulin, glucagon and DAPI. Scale bar = 2,000 μm.

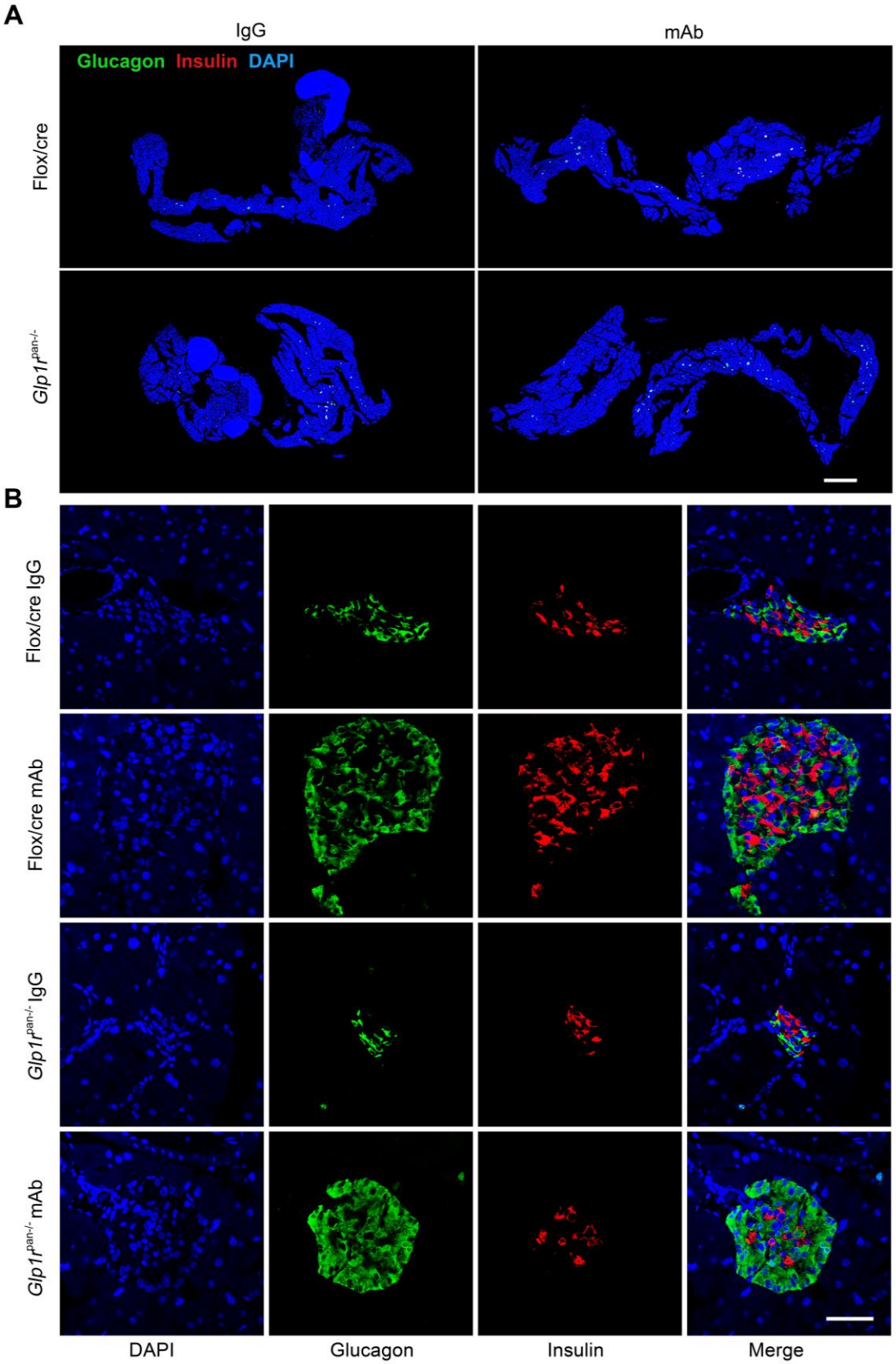


Supplementary Figure 4–GCGR mAb increases the islet number, islet area, α-cell area, and β-cell area in *db/db* mice, while GLP-1R antagonist Ex9 attenuated these effects. Male *db/db* mice (8 week) were treated with IgG (5 mg/kg/week, as control), or GCGR mAb (5 mg/kg/week), or GCGR mAb combined with Ex9 (50 nmol/kg/day) for 4 weeks. Pancreatic sections were stained for insulin, glucagon and DAPI. *A*: Representative images of the whole pancreatic section. Scale bar = 2,000 μm. *B*: Representative image of an islet. Scale bar = 50 μm.



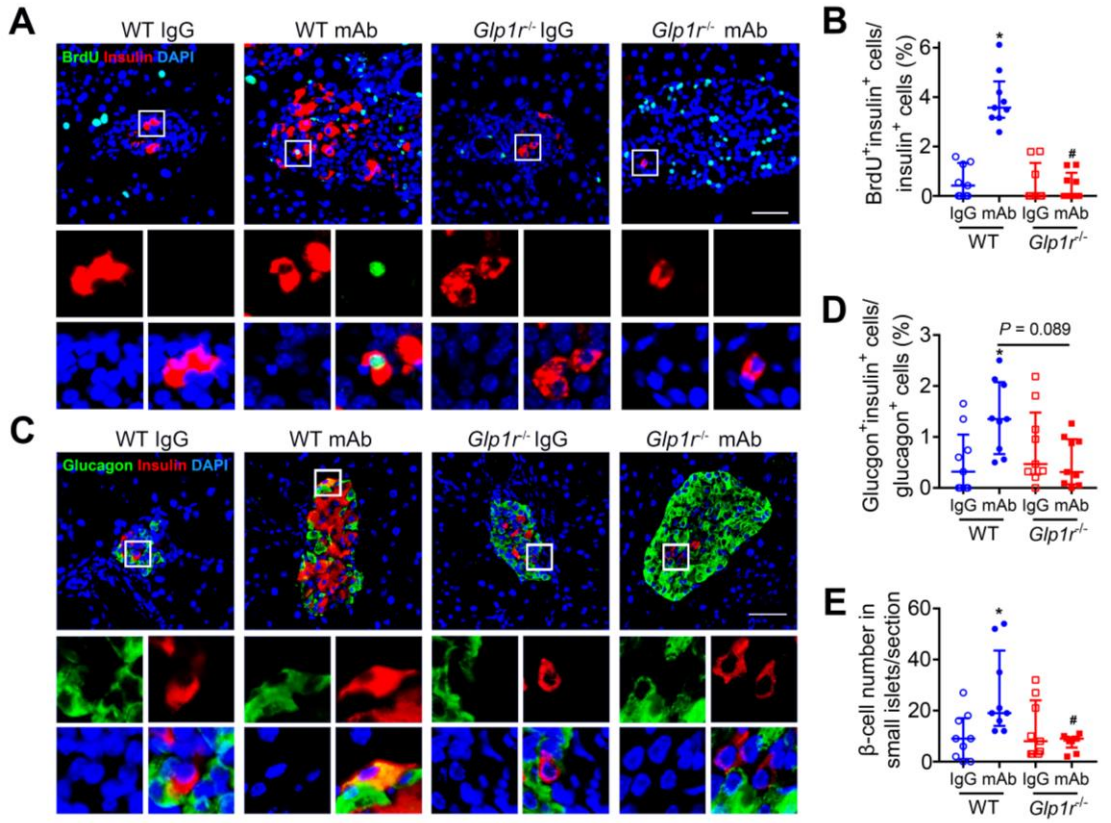
Supplementary Figure 5–GCGR mAb increases the islet number, islet area, α-cell area, and β-cell area in T1D WT littermates, while did not increase β-cell area in T1D *Glp1r*-

$^{/-}$ mice. Male and female *Glp1r*$^{-/-}$ mice and WT (*Glp1r*$^{+/+}$) littermates were injected with STZ to induce T1D models at the age of 8−12 weeks and treated weekly with IgG (5 mg/kg, as control) or GCGR mAb (5 mg/kg) for 4 weeks. Pancreatic sections were stained for insulin, glucagon and DAPI. *A*: Representative images of the whole pancreatic section. Scale bar = 2,000 μm. *B*: Representative image of an islet. Scale bar = 50 μm.

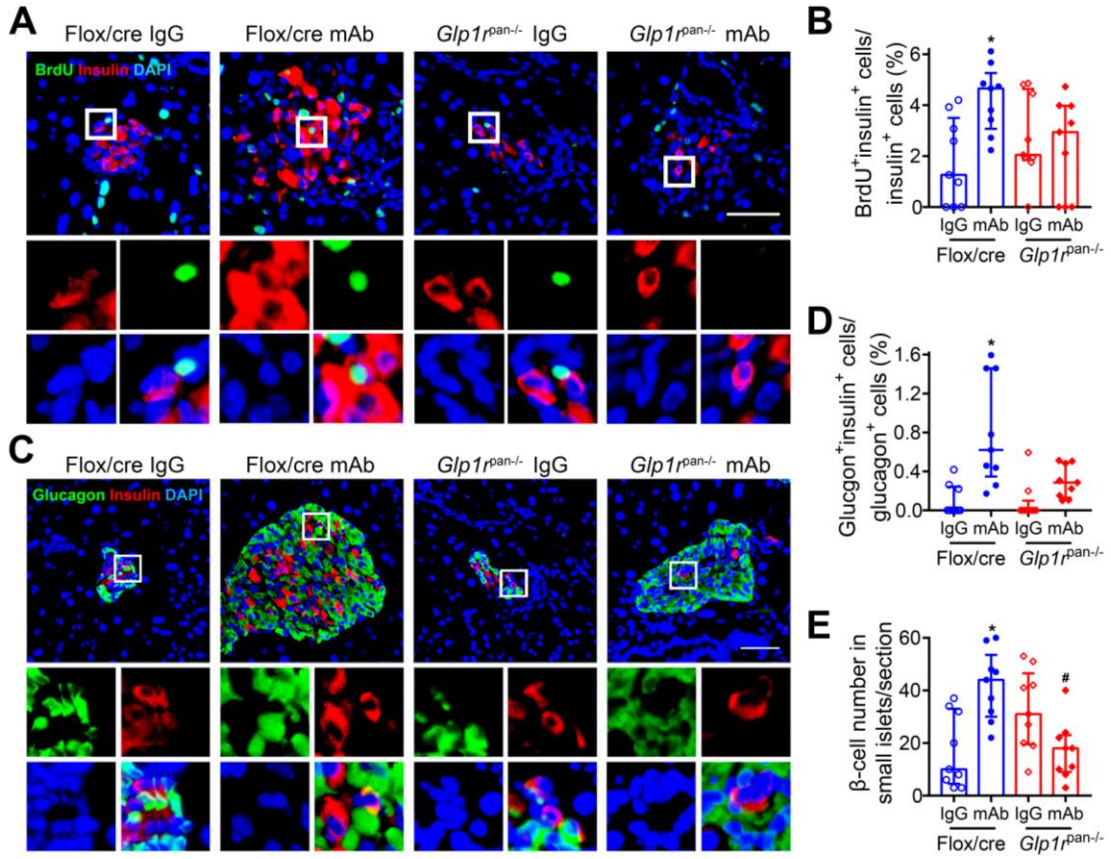


Supplementary Figure 6–GCGR mAb increases the islet number, islet area, α-cell area, and β-cell area in T1D Flox/cre littermates, while did not increase the β-cell area in

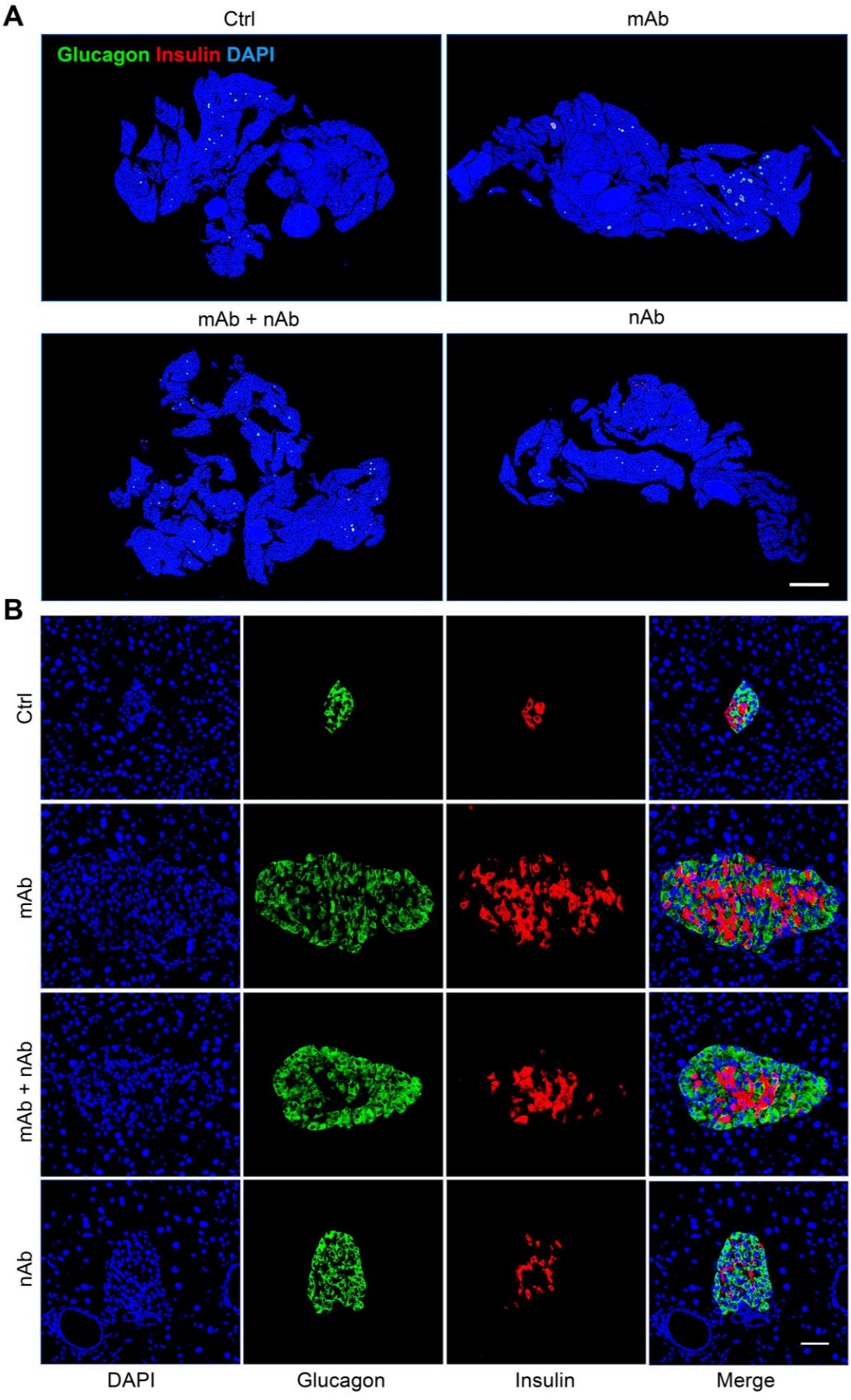
T1D *Glp1r*$^{pan-/-}$ mice. Male *Glp1r*$^{pan-/-}$ mice and Flox/cre littermates were injected with STZ to induce T1D models at the age of 8−12 weeks and treated weekly with IgG (5 mg/kg, as control) or GCGR mAb (5 mg/kg) for 4 weeks. Pancreatic sections were stained for insulin, glucagon and DAPI. *A*: Representative images of the whole pancreatic section. Scale bar = 2,000 μm. *B*: Representative image of an islet. Scale bar = 50 μm.



Supplementary Figure 7–Systemic GLP-1R signaling is involved in β-cell self-replication, α- to β-cell transdifferentiation and β-cell neogenesis triggered by GCGR antagonism in T1D mice. Male and female *Glp1r*$^{-/-}$ mice and WT (*Glp1r*$^{+/+}$) littermates were injected with STZ to induce T1D models at the age of 8–12 weeks and treated weekly with IgG (5 mg/kg, as control) or GCGR mAb (5 mg/kg) for 4 weeks. *A* and *C*: Representative images of an islet immunostained for BrdU, insulin, and DAPI (*A*); representative images of an islet immunostained for glucagon, insulin, and DAPI (*C*). Scale bar = 50 μm. The pictures in the small boxes are enlarged in the bottom. *B* and *D*: Quantification of the BrdU^{+}insulin^{+} cells (*B*) and glucagon^{+}insulin^{+} cells (*D*). *E*: Quantification of β-cell number in small islet per section (n = 3 section/mouse × by 3 mice/group). Data are expressed as the median (interquartile range). Statistical analysis was performed by Kruskal-Wallis test, followed by Dunn multiple comparisons test. **P* < 0.05 vs. IgG control in the same genotype of mice; #*P* < 0.05 vs. WT littermates on the same treatment.



Supplementary Figure 8–Pancreatic GLP-1R signaling is involved in β-cell self-replication, α- to β-cell transdifferentiation and β-cell neogenesis triggered by GCGR antagonism in T1D mice. Male *Glp1r*$^{pan-/-}$ mice and Flox/cre littermates were injected with STZ to induce T1D models at the age of 8–12 weeks and treated weekly with IgG (5 mg/kg, as control) or GCGR mAb (5 mg/kg) for 4 weeks. *A* and *C*: Representative images of an islet immunostained for BrdU, insulin, and DAPI (*A*); representative images of an islet immunostained for glucagon, insulin, and DAPI (*C*). Scale bar = 50 μm. The pictures in the small boxes are enlarged in the bottom. *B* and *D*: Quantification of the BrdU^{+}insulin^{+} cells (*B*) and glucagon^{+}insulin^{+} cells (*D*). *E*: Quantification of β-cell number in small islet per section (n = 3 section/mouse × by 3 mice/group). Data are expressed as the mean ± SEM or median (interquartile range). Statistical analysis was performed by one-way ANOVA, followed by Tukey multiple comparisons test in *B* and *E*, or by Kruskal-Wallis test, followed by Dunn multiple comparisons test in *D*. **P* < 0.05 vs. IgG control in the same genotype of mice; #*P* < 0.05 vs. Flox/cre littermates on the same treatment.


A
Ctrl
mAb
Glucagon Insulin DAPI
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B
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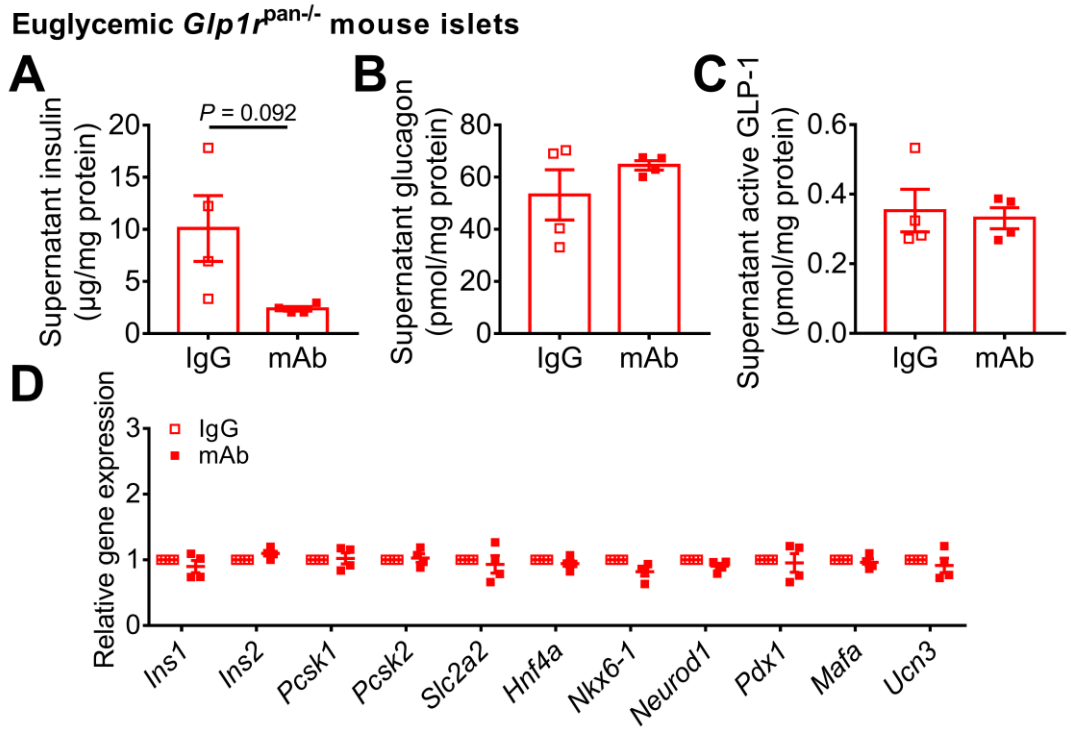
mAb
mAb + nAb
nAb
DAPI
Glucagon
Insulin
Merge

Supplementary Figure 9–GCGR mAb increases the islet number, islet area, α-cell area, and β-cell area in T1D C57BL/6J mice, while glucagon nAb addition attenuated the GCGR mAb-induced increment of β-cell area. Male C57BL6/J (12 weeks) mice were injected with STZ to induce a T1D model. The diabetic mice were divided into four groups and given the 4-week treatments as followings: *1)* control (Ctrl) group, injected with IgG (5 mg/kg/week, as control of GCGR mAb) and A-TNP (4 mg/kg/day, as control of glucagon nAb); *2)* GCGR mAb group, received injection of GCGR mAb (5 mg/kg/week) and A-TNP; *3)* GCGR mAb + glucagon nAb group, injected with GCGR mAb and glucagon nAb (4 mg/kg/day); *4)* glucagon nAb group, received injection of IgG and glucagon nAb. Pancreatic sections were stained for insulin, glucagon and DAPI. *A*: Representative images of the whole pancreatic section. Scale bar = 2,000 μm. *B*: Representative image of an islet. Scale bar = 50 μm.



Supplementary Figure 10–Pancreatic GLP-1R signaling takes part in regulation of β-cell identity by GCGR antagonism in vitro. Primary mouse islets were isolated from 8-week-old male euglycemic *Glp1r*$^{pan-/-}$ mice, and cultured with 1,000 nmol/L GCGR mAb or IgG for 24 h in high glucose (30 mmol/L) condition. *A*–*C*: Supernatant insulin (*A*), glucagon (*B*) and active GPL-1 (*C*) levels in *Glp1r*$^{pan-/-}$ mouse islets. *D*: Relative mRNA levels in *Glp1r*$^{pan-/-}$ mouse islets ($n = 4$). Data are expressed as the mean ± SEM. Statistical analysis was performed by unpaired student *t* test.

Supplementary Table 1. Antibodies and ELISA kits used in this study

Reagent	Source	Identifier
Insulin ELISA kit	Millipore	Cat#EZRMI-13K
C-peptide ELISA kit	Millipore	Cat#EZRMCP2-21K
Glucagon ELISA kit	R&D Systems	Cat#DGCG0
Active GLP-1 ELISA kit	Millipore	Cat#EGLP-35K
Guinea pig polyclonal anti-insulin antibody (1:1,000)	Bio-Rad Laboratories	Cat#5330-0104G RRID: AB_1605150
Rabbit polyclonal anti-glucagon antibody (1:200)	Cell Signaling Technology	Cat#2760 RRID: AB_259852
Mouse monoclonal anti-BrdU antibody (1:200)	Abcam	Cat#ab136650
Alexa Fluor 488-conjugated AffiniPure goat polyclonal anti-rabbit IgG (H+L) antibody (1:800)	Jackson ImmunoResearch Laboratories	Cat#111-545-003 RRID: AB_2338046
Alexa Fluor 594-conjugated AffiniPure goat polyclonal anti-mouse IgG (H+L) antibody (1:800)	Jackson ImmunoResearch Laboratories	Cat#111-585-003 RRID: AB_2338871
Alexa Fluor 647-conjugated AffiniPure donkey polyclonal anti-guinea pig IgG (H+L) antibody (1:800)	Jackson ImmunoResearch Laboratories	Cat#706-605-148 RRID: AB_2340476

Supplementary Table 2. Primers for quantitative PCR used in this study

Gene ID	Gene symbol	Gene name	Primer sequences (5'-3')	Tm (°C)	Product length (bp)
11461	*Actb*	actin, beta	F: TGTACCCAGGCATTGCTGAC R: CTGCTGGAAGGTGGACAGTG	60	149
15378	*Hnf4a*	hepatic nuclear factor 4, alpha	F: GAGAGGGTCAGAAGCAGACG R: TTGCACAACCACAGGAAGGT	60	171
16333	*Ins1*	insulin 1	F: TAGTGACCAGCTATAATCAGAG R: ACGCCAAGGTCTGAAGGTCC	62	289
16334	*Ins2*	insulin 2	F: CCCTGCTGGCCCTGCTCTT R: AGGTCTGAAGGTCACCTGCT	60	213
18012	*Neurod1*	neurogenic differentiation 1	F: GCCCAGCTTAATGCCATCTTT R: CAAAAGGGCTGCCTTCTGTAA	59	113
18096	*Nkx6-1*	NK6 homeobox 1	F: AACACACCAGACCCACGTTCT R: ATCCCCAGAGAATAGGCCAAG	60	115
18548	*Pcsk1*	proprotein convertase subtilisin/kexin type 1	F: AGTTGGAGGCATAAGAATGCTG R: GCCTTCTGGGCTAGTCTGC	60	159
18549	*Pcsk2*	proprotein convertase subtilisin/kexin type 2	F: GTGTGATGGTTTTTGCGTCTG R: GGGAGCTTTCGGACTCCAA	59	130
18609	*Pdx1*	pancreatic and duodenal homeobox 1	F: GAAATCCACCAAAGCTCACG R: CGGGTTCCGCTGTGTAAG	58	65
20526	*Slc2a2*	solute carrier family 2 (facilitated glucose transporter), member 2	F: GTGCTGCTGGATAAATTCGCC R: ATTGCAGACCCAGTTGCTGA	60	180
83428	*Ucn3*	urocortin 3	F: GCTGTGCCCCTCGACCT R: TGGGCATCAGCATCGCT	60	71
378435	*Mafa*	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein A (avian)	F: AGGAGGAGGTCATCCGACTG R: CTTCTCGCTCTCCAGAATGTG	59	113

Supplementary Table 3. The list of nonstandard abbreviations used in this study

Abbreviation	Spelled-out version
Ex9	exendin 9-39
GCGR	glucagon receptor
GLP-1	glucagon-like peptide 1
GLP-1R	glucagon-like peptide 1 receptor
IPGTT	intraperitoneal glucose tolerance test
mAb	monoclonal antibody
nAb	neutralizing antibody
STZ	streptozotocin
T1D	type 1 diabetes
T2D	type 2 diabetes
WT	wild-type