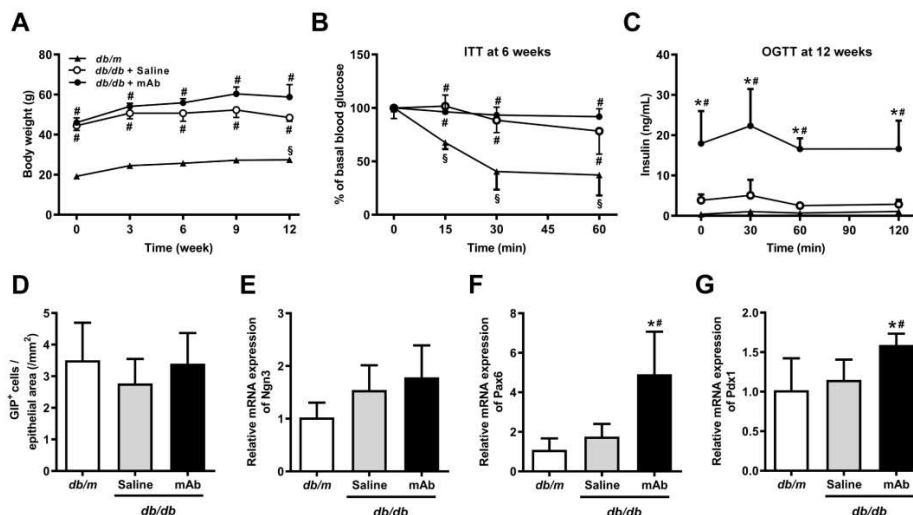
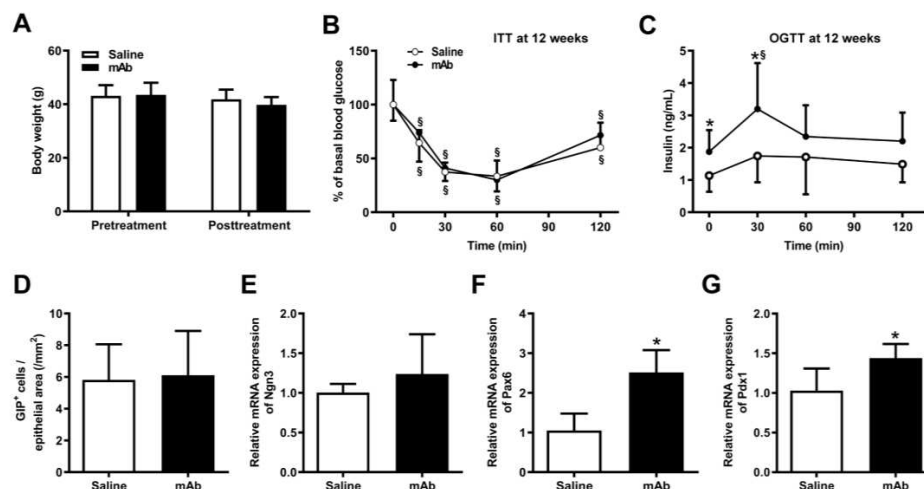


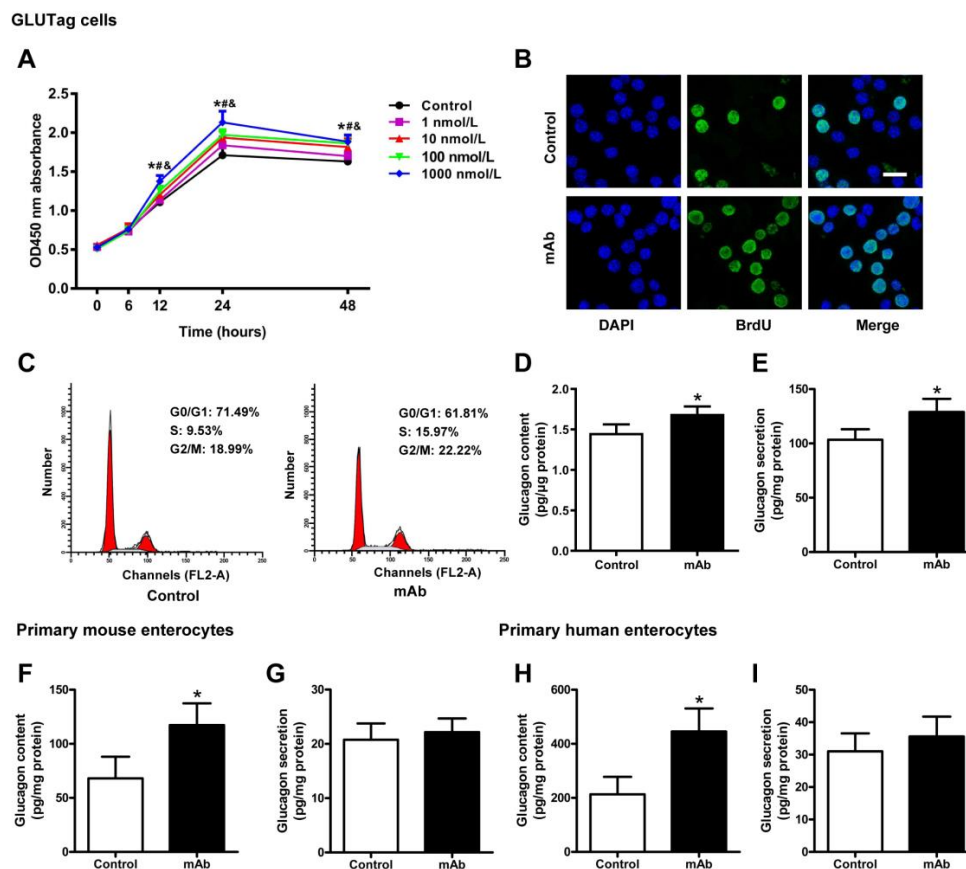
Supplementary figures and tables



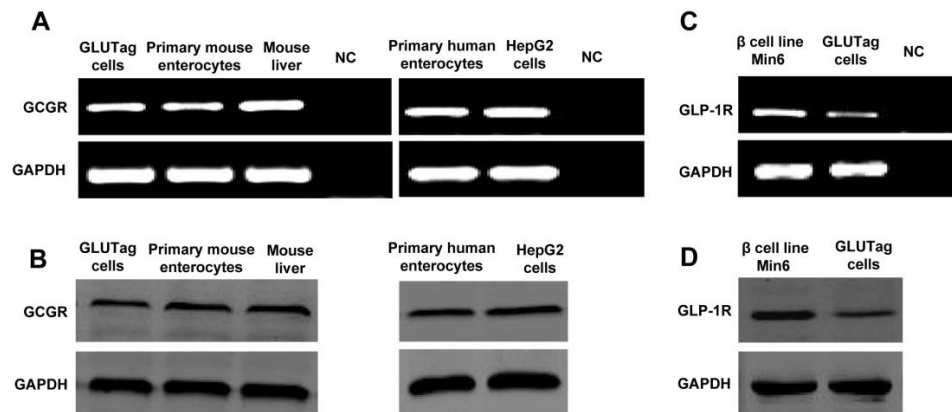
Supplementary figure S1. Metabolic parameters and histocytological markers of *db/db* mice treated with GCGR mAb or vehicle for 12 weeks. A GCGR mAb, REMD 2.59 (5 mg/kg), or saline was intraperitoneally administered once a week in male diabetic *db/db* mice, and age-matched male *db/m* mice were included as normal controls. (A-C) Metabolic parameters including body weight (A), blood glucose levels during ITT (B), and plasma insulin levels during OGTT (C) after the treatment. $n=4-5$ mice per group. (D) Quantification of GIP-positive K-cells per epithelial area. $n=3-5$ sections per mouse multiplied by 4-5 mice per group. (E-G) Relative mRNA levels of Ngn3 (E), Pax6 (F) and Pdx1 (G) in ileum tissues were detected by qRT-PCR. $n=4-5$ mice per group. Data are shown as mean \pm SD. Statistical analysis was conducted using one-way analysis of variance followed by the post hoc Tukey-Kramer test. * $p<0.05$ versus saline; # $p<0.05$ versus *db/m*; § $p<0.05$ versus pretreatment in the same group. GCGR, glucagon receptor; GIP, glucose-dependent insulinotropic polypeptide; ITT, insulin tolerance test; mAb, monoclonal antibody; mRNA, messenger RNA; Ngn3, neurogenin3; OGTT, oral glucose tolerance test; Pax6, paired box 6; Pdx1, pancreatic and duodenal homeobox-1; qRT-PCR, quantitative reverse transcription PCR.



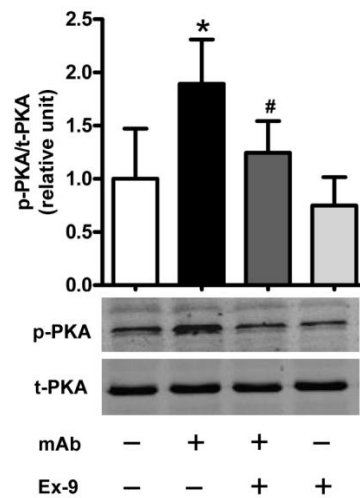
Supplementary figure S2. Metabolic parameters and histocytological markers of high-fat diet + streptozotocin-induced type 2 diabetic male mice treated with the GCGR mAb or vehicle for 12 weeks. The GCGR mAb (5 mg/kg) or saline was intraperitoneally administered once a week. (A-C) Metabolic parameters including body weight (A), blood glucose levels during ITT (B), and plasma insulin levels during OGTT (C) after the treatment. $n=7-9$ mice per group. (D) Quantification of GIP-positive K-cells per epithelial area. $n=3-5$ sections per mouse multiplied by 7-9 mice per group. (E-G) Relative mRNA levels of Ngn3 (E), Pax6 (F) and Pdx1 (G) in ileum tissues were detected by qRT-PCR. $n=7-9$ mice per group. Data are expressed as the mean \pm SD. Statistical analysis was carried out using Student's t test or one-way analysis of variance followed by the post hoc Tukey-Kramer test when appropriate. * $p<0.05$ versus saline; § $p<0.05$ versus pretreatment in the same group. GCGR, glucagon receptor; GIP, glucose-dependent insulinotropic polypeptide; ITT, insulin tolerance test; mAb, monoclonal antibody; mRNA, messenger RNA; Ngn3, neurogenin3; OGTT, oral glucose tolerance test; Pax6, paired box 6; Pdx1, pancreatic and duodenal homeobox-1; qRT-PCR, quantitative reverse transcription PCR.



Supplementary figure S3. Cell proliferation and glucagon production in cultured GLUTag cells, primary mouse and human intestinal cells. (A) GLUTag cells were incubated with GCGR mAb (1, 10, 100 and 1000 nmol/L) for different time period (6, 12, 24 and 48 hours). Cell proliferation was measured using the cell counting kit-8 assay. (B) Representative fluorescence images showing BrdU positive cells by BrdU incorporation assay after a 24-hour GCGR mAb (1000 nmol/L) or vehicle treatment and subsequent 12-hour BrdU incorporation. Scale bar = 25 μ m. (C) Representative cell cycle distributions images determined by flow cytometry after treatment with GCGR mAb (1000 nmol/L) or vehicle for 24 hours. (D-I) GLUTag cells (D-E), primary mouse enterocytes (F-G) and primary human enterocytes (H-I) were cultured with GCGR mAb (1000 nmol/L) or vehicle for 24 hours. Intracellular (D, F, H) and supernatant (E, G, I) glucagon protein levels were measured by ELISA. Data were obtained from at least 3 independent experiments and are represented as mean \pm SD. Statistical analysis was conducted by one-way analysis of variance followed by the post hoc Tukey-Kramer test or Student's t test when appropriate. Data in A, * p <0.05 (1000 nmol/L versus Control); # p <0.05 (100 nmol/L versus Control); & p <0.05 (10 nmol/L versus Control). Data in D-I * p <0.05 versus Control. BrdU, 5-bromo-2'-deoxyuridine; DAPI, 4',6-diamidino-2-phenylindole; GCGR, glucagon receptor; mAb, monoclonal antibody.



Supplementary figure S4. Expression of the GCGR and GLP-1R in GLUTag cells, and cultured primary mouse and human enterocytes. (A-D) The mRNA and protein expression of GCGR (A, B) and GLP-1R (C, D) was determined by RT-PCR and western blot, respectively. Mouse liver tissue or human hepatic cell line HepG2 served as a positive control for GCGR expression, and mouse pancreatic β -cell line Min6 was used as a positive control for GLP-1R expression. NC: no template control. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GCGR, glucagon receptor; GLP-1R, glucagon-like peptide-1 receptor; mRNA, messenger RNA; RT-PCR, reverse transcription PCR.



Supplementary figure S5. The effects of GCGR mAb on PKA phosphorylation in GLUTag cells. Cells were preincubated with the GLP-1R antagonist exendin (9–39) (Ex-9; 200 nmol/L) for 30 min and then coincubated with GCGR mAb for additional 23.5 hours. The protein levels of p-PKA and t-PKA were detected by western blot. Data are represented as mean \pm SD. Statistical analysis was conducted using one-way analysis of variance followed by the post hoc Tukey-Kramer test. * $p < 0.05$ versus control; # $p < 0.05$ versus GCGR mAb. GCGR, glucagon receptor; GLP-1R, glucagon-like peptide-1 receptor; mAb, monoclonal antibody; PKA, protein kinase A; p-PKA, phosphorylated PKA; t-PKA, total PKA.

Supplementary table S1. Key resource table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-GLP-1 (1:1000)	Abcam	Catalog # ab23472; RRID: AB_447455
Rabbit polyclonal anti-GLP-1 (1:2000)	Abcam	Catalog # ab22625; RRID: AB_447206
Rabbit polyclonal anti-BrdU (1:150 for mouse tissues; 1:500 for GLUTag cells)	Abcam	Catalog # ab152095; RRID: AB_2813902
Rabbit monoclonal anti-GIP (1:1000)	Abcam	Catalog # ab209792; RRID: AB_2813903
Mouse monoclonal anti-Ki-67 (1:1000)	Santa Cruz Biotechnology	Catalog # sc-23900; RRID: AB_627859
Mouse monoclonal anti-PCNA (1:1000)	Cell Signaling Technology	Catalog # 2586; RRID: AB_2160343
Rabbit polyclonal anti-GCGR (1:1000)	Sigma-Aldrich	Catalog # SAB4501138; RRID: AB_10746075
Goat polyclonal anti-GLP-1 receptor (1:1000)	OriGene Technologies, Inc.	Catalog # TA326758; RRID: AB_2813904
Rabbit polyclonal anti-PKA (1:1000)	Cell Signaling Technology	Catalog # 4782; RRID: AB_2170170
Rabbit polyclonal anti-phospho-PKA C (Thr197) (1:1000)	Cell Signaling Technology	Catalog # 4781; RRID: AB_2300165
IRDye 800CW-conjugated goat anti-rabbit IgG (1:10,000)	Rockland	Catalog # 611-131-002 RRID: AB_1660973
IRDye 800CW-conjugated goat anti-mouse IgG (1:10,000)	Rockland	Catalog # 610-131-003 RRID: AB_220122
Alexa Fluor 594-conjugated AffiniPure goat polyclonal anti-mouse IgG (H+L) (1:800)	Jackson ImmunoResearch Labs	Catalog #115-585-003; RRID: AB_2338871
Alexa Fluor 488-conjugated AffiniPure goat polyclonal anti-rabbit IgG (H+L) (1:800)	Jackson ImmunoResearch Labs	Catalog #115-545-003; RRID: AB_2338046
Chemicals, Peptides, and Recombinant Proteins		
REMD 2.59 (a human GCGR mAb and competitive antagonist)	REMD Biotherapeutics	N/A
High-fat diet (HFD)	Beijing Keao Xieli Feed Co.,Ltd., Beijing, China	Catalog # D12451
Streptozocin (STZ)	Sigma-Aldrich	Catalog # S0130
Dipeptidyl peptidase-4 inhibitor	Millipore	Catalog # DPP4

Aprotinin	Sigma-Aldrich	Catalog # ROAPRO
DAPI	Sigma-Aldrich	Catalog # D9542
DMEM basic (1 g/L D-Glucose)	Thermo Fisher Scientific	Catalog # C11885500BT
DMEM basic (4.5 g/L D-Glucose)	Thermo Fisher Scientific	Catalog # C11995500BT
L-15 Medium (Leibovitz)	Sigma-Aldrich	Catalog # L1518
Fetal bovine serum	HyClone	Catalog # SH30084.03
Penicillin Streptomycin	Thermo Fisher Scientific	Catalog # 15140-122
GlutaMax	Thermo Fisher Scientific	Catalog # 35050-061
Matrigel	Corning	Catalog # 354234
Y-27632 dihydrochloride	APEXBIO	Catalog # A3008
Collagenase type XI	Sigma-Aldrich	Catalog # C9407
BrdU	Sigma-Aldrich	Catalog # B9285
Propidium iodide	Beyotime	Catalog # C1052-2
RNase	Beyotime	Catalog # C1052-3
Trizol reagent	Thermo Fisher Scientific	Catalog # 15596018
SYBR qPCR Mix	TOYOBO	Catalog # QPS-201
RIPA lysis buffer	Applygen Technologies Inc.	Catalog # C1053
Protease inhibitor	Applygen Technologies Inc.	Catalog # P1265
Phosphatase inhibitor	Applygen Technologies Inc.	Catalog # P1260
Critical Commercial Assays		
Active GLP-1 ELISA kit	Millipore	Catalog # EGLP-35K
Total GLP-1 ELISA kit	Millipore	Catalog # EZGLP1T-36K
GLP-2 ELISA kit	Biovendor	Catalog # RSCYK142R
Glucagon ELISA kit	R&D Systems	Catalog # DGCG0
Insulin ELISA kit	Millipore	Catalog # EZRMI-13K
CCK-8 kit	Dojindo Laboratories	Catalog # CK04
RevertAid First Strand cDNA Synthesis kit	Thermo Fisher Scientific	Catalog # K1622
Experimental Models and Cells		
Mouse: C57BL/6N	Vital River Animal Center, Beijing, China	Catalog # 213
Mouse: <i>db/db</i>	Nanjing Biomedical Research Institute of Nanjing University, China	Catalog # T001463
Cell: Murine GLUTag cells	A gift from Prof. Daniel J Drucker (Mt. Sinai Hospital, University of Toronto, Canada)	N/A

Supplementary table S2. Primers used in PCR

Gene symbol	Gene name	Species	Gene ID	Primer sequences (5'-3')	Tm (°C)	Product size (bp)
Gcg	proglucagon	Mus musculus	14526	F: GCTTATAATGCTGGTGCAAG R: TTCATCTCATCAGGGTCCTC	60	114
Gcgr	glucagon receptor	Mus musculus	14527	F: ATTTCTGCCCTGGTACCT R: CGGGCCCACACCTCTTG	60	73
Glp-1r	glucagon-like peptide-1 receptor	Mus musculus	14652	F: AGCACTGTCCGTCTTCATCA R: AGAAGGCCAGCAGTGTGTAT	60	203
Ngn3	neurogenin 3	Mus musculus	11925	F: GCATGCACAACCTCAACTC R: TTTGTAAGTTTGGCGTCATC	60	79
Pdx1	pancreatic and duodenal homeobox 1	Mus musculus	18609	F: GAAATCCACAAAGCTCACG R: CGGGTTCCGCTGTGTAAG	62	65
Pax6	paired box 6	Mus musculus	18508	F: TAACGGAGAAGACTCGGATGAAGC R: CGGGCAAACACATCTGGATAATGG	62	144
Ki67	proliferation marker protein Ki-67	Mus musculus	17345	F: GACAGCTTCCAAAGCTCACCC R: TGTGTCCTTAGCTGCCTCCT	60	230
Pena	proliferating cell nuclear antigen	Mus musculus	18538	F: CCACATTGGAGATGCTGTTG R: CAGTGGAGTGGCTTTTGTGA	60	208
Gapdh	glyceraldehyde-3-phosphate dehydrogenase	Mus musculus	14433	F: TGCACCACCAACTGCTTAGC R: GGCATGGACTGTGGTCATGAG	60	87
GCG	proglucagon	Homo sapiens	2641	F: AATAACATTGCCAAACGTCACG R: TCTGGGAAATCTCGCCTTCCT	60	146
GcGR	glucagon receptor	Homo sapiens	2642	F: CATCCACGCGAATCTGTTTGC R: CGTCGCCAATTTTCTGGCTG	60	101
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Homo sapiens	2597	F: CATGAGAAGTATGACAACAGCCT R: AGTCCTTCCACGATACCAAAGT	60	113