Supplementary online-only materials

Supplementary methods

Animals and intervention

All animal experiments were approved by the Animal Care and Use Committee of Peking University. Six-week-old male *db/db* mice (BKS-*Lepr*^{em2Cd479}/Gpt, Strain no. T002407) and *db/m* mice, purchased from GemPharmatech (Nanjing, China), were maintained in a 12-h light/dark cycle with free access to food and water. After one-week adaption, diabetes mellitus was verified if fasting blood glucose was ≥ 11.1 mmol/L or random blood glucose was ≥ 16.7 mmol/L. Twelve diabetic *db/db* mice and 12 *db/m* mice were respectively divided into two groups, and were daily administered with dapagliflozin by gavage (1 mg/kg per day; AstraZeneca Pharmaceutical Co. Ltd., London, UK; n = 6) or vehicle (water; n = 6) for five weeks. These mice were used for plasmatic non-targeted metabolomics, cecal shotgun metagenomics and cecal metabolomics.

Twenty male *db/db* mice were treated with dapagliflozin or vehicle (n = 10 per group) for five weeks to collected feces. After sacrifice, mouse cecal feces were sampled and were resuspended in a concentration of 50 mg/mL in pre-cooled phosphate buffered saline (PBS) with 20% glycerin. The samples were centrifuged with 1000 rpm for 10 min at 4°C, and the supernatants were stored at −80°C. Another patch of male *db/db* mice (n = 28) were designated for fecal microbiota transplantation (FMT) interventions. After one-week adaptation, the diabetic mice

were depleted the host microbiota by antibiotic cocktail administration: unpalatable antibiotics (100 μL of vacomycin 5 mg/mL and metronidazole 10 mg/mL) were given by gavage, while other antibiotics (ampicillin 1 g/L and neomycin 0.5 g/L) were provided in drinking water for 3 days [1]. Subsequently, these mice were randomly assigned into four groups (n = 7 per group) and were intragastrically administrated with fecal microbiota (100 μL for per mouse) from dapagliflozin- or vehicle-treated mice at 1-day interval for five weeks, combined with or without GLP-1 receptor (GLP-1R) antagonist exendin 9-39 (50 nmol/kg per day; Bachem, Bubendorf, Switzerland) treatment. Exendin 9-39 or vehicle (saline with 1% bovine serum albumin) was administered by micro-osmotic pumps (Alzet model 1007D; Durect, Cupertino, CA). The micro-osmotic pump was implanted subcutaneously and replaced weekly. The dose of exendin 9-39 was referred to the previous studies [2-4]. To rule out the effect of different blood glucose levels, glycemic control was maintained at the level similar to dapagliflozin treatment by using insulin implants (LinBit; Linshin, Scarborough, Canada). After 1-week administration of FMT, the insulin implants were inserted in the subcutaneous tissue of the back under the short-acting anesthetic conditions (2,2,2-Tribromoethanol; 0.3 g/kg; Sigma-Aldrich, St. Louis, MO) as previously described [5]. The releasing rate of the insulin implants was about 0.1 units/24 h per implant for > 30 days.

To investigate the effect of L-tryptophan on islet regeneration, 21 male *db/db* mice were randomly divided into three groups (n = 7 per group) and administrated with

vehicle control, L-tryptophan (200 mg/kg per day), or L-tryptophan combined with exendin 9-39 for five weeks. L-tryptophan or vehicle was daily administered by gavage. Similarly, insulin implants were used for glycemic control. Age-matched *db/m* mice were included as normal control (n = 7).

In addition, 28 *db/db* mice were randomly divided into four groups, and treated for five weeks with vehicle control (n = 9), dapagliflozin (1 mg/kg; n = 9), exendin 9-39 (50 nmol/kg per day n = 5), or dapagliflozin combined with exendin 9-39 (n = 5). Age-matched *db/m* mice were included as normal control (n = 6).

B6.FVB-Tg(Pdx1-cre)6Tuv/J (*Pdx1*-Cre) mice (strain No. 014647; Cre expression in the pancreatic epithelium, antral stomach and duodenum in neonates and in pancreatic β-cells in adults) were purchased from the Jackson Laboratory (Barr Harbor, ME). B6/JGpt-*Glp1r*^{em1Cflox}/Gpt (*Glp1r*-flox) mice (strain No. T005818) were constructed by GemPharmatech. In this study, CRISPR/Cas9 technology was used to modify *Glp1r* gene. According to the structure of *Glp1r* gene, exon2-exon3 of *Glp1r*-201 (ENSMUST00000114574.2) transcript is recommended as the knockout region. The brief process is as follows: CRISPR/Cas9 system and donor were microinjected into the fertilized eggs of C57BL/6J mice. Fertilized eggs were transplanted to obtain positive F0 mice, which were confirmed by PCR and sequencing analyses. A stable F1 mice were obtained by mating positive F0 mice with C57BL/6J mice. F1 heterozygous mice self-crossed to obtain F2 homozygous mice. *Glp1r*-flox mice were crossed with *Pdx1*-Cre mice to generate pancreas-specific

Glp1r knockout (*Glp1r*$^{pan-/-}$) mice (Supplementary Fig. 1*A*). Ablation of *Glp1r* expression was assessed by real-time quantitative RT-PCR using islet RNA samples (Supplementary Fig. 1*B*). *Pdx1*-Cre and *Glp1r*-flox littermates were used as the controls. Six-week-old male *Glp1r*$^{pan-/-}$ mice and *Pdx1*-Cre or *Glp1r*-flox littermates were fed with high-fat diet (HFD; fat 60%, carbohydrate 20% and protein 20%; Research Diets, New Brunswick, NJ) for 16 weeks. Subsequently, a single intraperitoneal dose of 50 mg/kg streptozotocin (STZ; Sigma-Aldrich) was given to induce type 2 diabetes. After one-week injection, diabetic condition was defined if the fasting blood glucose was ≥ 11.1 mmol/L or random blood glucose was ≥ 16.7 mmol/L. The diabetic *Glp1r*$^{pan-/-}$ mice and their littermates were randomly assigned to two groups respectively, and treated for six weeks by daily intragastric administration of 1 mg/kg dapagliflozin or vehicle (n = 8, dapagliflozin in the littermates; n = 7, vehicle in the littermates; and n = 6 per group in *Glp1r*$^{pan-/-}$ mice). Dapagliflozin or vehicle (water) was administered by gavage once a day. In addition, age-matched wild-type, *Pdx1*-Cre or *Glp1r*-flox mice (n = 6 per group) were used to exclude the effect of gene manipulation on β-cell mass.

Primary mouse islet isolation for verification of *Glp1r* knockout efficiency

Mouse islets were isolated from *Glp1r*$^{pan-/-}$ mice and their littermates by collagenase-V (Sigma-Aldrich) digestion. Islets were handpicked under a dissecting microscope as reported previously [6]. Islets were cultured for 24 h in the RPMI 1640 medium supplemented with 10% (*vol/vol*) fetal bovine serum (Hyclone, Logan, UT),

2 mmol/L GlutMax, 1 mmol/L sodium pyruvate and 1% (*vol/vol*) penicillin-streptomycin. Islets were collected for mRNA detection to verify knockout efficiency.

Glucose measurement, and blood sample and intestinal tissue collection

Blood glucose was measured by the glucose oxidase method using a hand-held OneTouch Ultra glucometer (LifeScan, Milpitas, CA). The value of 33.3 mmol/L was recorded if exceeding this upper detection limit of the glucometer. Oral glucose tolerance test (OGTT) was performed as follows. FMT- or L-tryptophan-treated mice could not be fasted for a long time because of the insulin implants. Hence, the fasting blood glucose in these mice was measured after 3-h starvation, and blood glucose levels were determined at 30 min after the glucose loading (1 g/kg). Blood samples from FMT- or L-tryptophan-treated mice were collected from the inner canthus during OGTT. In diabetic *Glp1r*$^{pan-/-}$ mice or *db/db* mice without the insulin implants, the fasting blood glucose was measured after 12-h overnight starvation, and blood glucose levels were determined at 30, 60 and 120 min after the glucose loading (1 g/kg). Blood samples from these mice were collected after an 8-h fast before sacrifice.

Blood samples were added with aprotinin (1 μg/mL), heparin sodium (1000 IU/mL) and dipeptidyl peptidase-4 (DPP-4) inhibitor (Gliptins; 50 μmol/L; Millipore, Billica, MA) for hormone detection.

Intestinal tissues were homogenized in radioimmunoprecipitation assay (RIPA) lysis buffer (Applygen Technologies Inc., Beijing, China) containing protease

inhibitor (Applygen Technologies Inc.), phosphatase inhibitor (Applygen Technologies Inc.), aprotinin and DPP-4 inhibitor. After centrifugation, supernatants were collected for GLP-1 detection. Quantities of active GLP-1 level were normalized to protein contents in the tissue lysates.

Immunofluorescent staining

Pancreatic tissues were fixed with 10% (*vol/vol*) neutral-buffered formalin at 4°C overnight and prepared into 5-μm-thick paraffin sections. The sections were pre-incubated in a permeabilization buffer (0.1 mmol/L PBS, pH 7.3, 0.2% Triton X-100), heated in an autoclave in a citrate buffer (12 mmol/L, pH 6.0), and blocked for 30 min with 10% (*vol/vol*) goat serum (Zhongshan Biotechnology, Beijing, China). Subsequently, sections were incubated with primary antibodies at 4°C overnight and secondary antibodies for 1 h at room temperature. Finally, sections were stained with 4',6-diamidino-2-phenylindole (DAPI; 1 μg/mL) to label nuclei. Images were captured under an automatic digital slide scanner (Pannoramic MIDI, 3D HISTECH, Budapest, Hungary). The primary antibodies were as follows: rabbit anti-glucagon antibody (1:200; Servicebio Technology Co. Ltd., Wuhan, China), mouse anti-insulin antibody (1:500; Servicebio Technology Co. Ltd.), rabbit anti-insulin antibody (1:500; Servicebio Technology Co. Ltd.), mouse anti-BrdU antibody (1:1000; Servicebio Technology Co. Ltd.) and mouse anti-proliferating cell nuclear antigen (PCNA) antibody (1:400; Cell Signaling technology, Danvers, MA). The secondary antibodies were as follows: Alexa Fluor 594-conjugated AffiniPure goat polyclonal anti-mouse

or anti-rabbit IgG (H + L), Alexa Fluor 488-conjugated AffiniPure goat polyclonal anti-rabbit or anti-mouse IgG (H + L) (all 1:800; Jackson ImmunoResearch Laboratories, Philadelphia, PA).

For cell quantification, three to four independent sections (which covered the whole pancreas) per pancreas were imaged from three to four mice per group. The total area of positive staining cells was analyzed by Fiji software (National Institutes of Health, Bethesda, MD). Insulin- or glucagon-positive cell clusters less than 8 cells were defined as small islets to indicate islet cell neogenesis [7].

Non-targeted metabolomic analysis

Blood samples were mixed with heparin sodium and centrifuged to obtain the supernatants. Cecal contents were homogenized with a ball mill. After centrifugation, 400 μL of 70% methanol water internal standard extractant was added. The sample was shaken and then placed on ice for 15 min. After centrifugation (12,000 rpm, 4°C, 10 min), supernatant was transferred to –20°C for 30 min. Finally, the sample were centrifuged (12,000 rpm, 4°C) for 3 min and the supernatant was used for analysis. All samples were acquired by the LC–MS system following machine orders. The analytical conditions were as follows: UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm×100 mm); column temperature, 40°C; flow rate, 0.4 mL/min; injection volume, 2 μL; solvent system, water (0.1% formic acid): acetonitrile (0.1% formic acid); gradient program, 95:5 (*vol/vol*) at 0 min, 10:90 (*vol/vol*) at 11.0 min, 10:90 (*vol/vol*) at 12.0 min, 95:5 (*vol/vol*) at 12.1 min, and 95:5

(*vol/vol*) at 14.0 min.

Shotgun metagenomic sequencing and analysis

Total DNA of cecal contents was extracted. Precise quantification of DNA concentrations was measured using Qubit® dsDNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA). A total amount of 1 μg DNA per sample was used as input material. Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs Inc., Ipswich, MA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample. DNA samples were fragmented, and then were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing with further PCR amplification. After being constructed, the library was diluted. Subsequently, libraries were analyzed for size distribution by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and quantified using real-time PCR. After the index-coded sample clusters were generated on a cBot Cluster Generation System, the library preparations were sequenced on an Illumina NovaSeq platform (Illumina, San Diego, CA) and paired-end reads were generated.

Readfq (version 8, https://github.com/cjfields/readfq) was used to obtain the clean data. Host sequences were then discarded by mapping the sequences against the reference genome (hg19) using BowTie2.2.4 (http://bowtiebio.sourceforge.net/bowtie2/index.shtml). The assembled scaftigs (> 500 bp) were predicted as open reading frames (ORFs) by MetaGeneMark software

(version 2.10, http://topaz.gatech.edu/GeneMark/), and the length information shorter than 100 nt was filtered. CD-HIT software (version 4.5.8, http://www.bioinformatics.org/cd-hit) was used to acquire the unique initial gene catalog and ORF prediction. The sequences of bacteria, fungi, archaea, and viruses were extracted from the NR database (version 2018-01-02, https://www.ncbi.nlm.nih.gov/), and the unigenes were blasted to these sequences by DIAMOND software (version 0.9.9, https://github.com/bbuchfink/diamond/). DIAMOND software (version 0.9.9) was used to blast unigenes to the functional database, including the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (version 2018-01-01, http://www.kegg.jp/kegg/).

Mouse intestinal L-cell culture and intervention

Mouse intestinal L-cell line STC1 cells (CRL-3254; ATCC) were cultured in DMEM medium (4.5 g/L glucose) supplemented with 10% (*vol/vol*) fetal bovine serum (Hyclone) and 1% (*vol/vol*) penicillin-streptomycin.

STC1 cells were incubated with different concentrations of L-tryptophan (1, 5, 10 and 20 mmol/L; MedChemExpress, Monmouth Junction, NJ), indoleacetate (1, 10, 100 and 1000 μmol/L; MedChemExpress), N-acetylserotonin (1, 10, 100 and 1000 μmol/L; MedChemExpress), or vehicle for 24 h. In the other experiments, STC1 cells were cultured with L-tryptophan (10 mmol/L) or vehicle in the presence or absence of GPR142 antagonist CLP-3094 (10 μmol/L; MedChemExpress) for 24 h, or incubated with dapagliflozin (25 μmol/L) or vehicle with or without palmitate (0.5

mmol/L) for 8 h. Cells were collected for mRNA detection by real-time quantitative RT-PCR.

Two days before the experiments, STC1 cells were seeded on 24-well plates in the amount of 3×10^5 per well. Confluent cells were washed twice and pre-incubated for 60 min with warm Hanks' balanced salt solution (HBSS). Subsequently, cells were incubated in HBSS supplemented with different concentrations of L-tryptophan (1, 5, 10 and 20 mmol/L), indoleacetate (1, 10, 100 and 1000 μmol/L), N-acetylserotonin (1, 10, 100 and 1000 μmol/L), or vehicle for 60 min at 37°C. In another experiment, STC1 cells were incubated in HBSS supplemented with L-tryptophan (10 mmol/L) or vehicle in the presence or absence of CLP-3094 (10 μmol/L) for 60 min at 37°C. During the treatment, DPP-4 inhibitor (50 μmol/L) was added in the medium to prevent active GLP-1 degradation. The buffer supernatants were collected for GLP-1 detection, and cells were lysed with lysis buffer. Quantities of active GLP-1 were normalized to protein contents in the cell lysates.

Primary human islet culture and intervention

Human islets isolated from two nondiabetic donors were kindly gifted by Prof. Shusen Wang (Organ Transplant Center, Tianjin First Central Hospital, Tianjin, China). This study was approved by the Medical Science Research Ethics Committee of Peking University Third Hospital. Donor information was listed in Supplementary Table 1. Human islets were cultured for 13–14 h in CMRL-1066 medium (Corning, Manassas, VA) supplemented with 10% (*vol/vol*) human serum albumin (Baxter, Vienna, Austria),

100 U/mL penicillin, and 100 μg/mL streptomycin. Thereafter, the islets were incubated for 24 h with L-tryptophan (1 mmol/L) or vehicle in the presence or absence of CLP-3094 (1 μmol/L) with or without palmitate (0.5 mmol/L). Culture supernatants were sampled for insulin detection, and islets were lysed with lysis buffer. Quantities of secreted insulin were normalized to protein contents in the islet lysates.

Real-time quantitative RT-PCR

Total RNA from cultured islets or STC1 cells was extracted by Trizol reagent (Thermo Fisher Scientific, Waltham, MA), and reversely transcribed to cDNA with a RevertAid First Strand cDNA Synthesis kit (Fermentas, Vilnius, Lithuania). Quantitative RT-PCR was performed using THUNDERBIRD SYBR qPCR Mix (Toyobo Co. Ltd., Osaka, Japan) on a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific). The relative expression of target genes to internal control (β-actin) was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences synthesized by Aoke Biotechnology (Beijing, China) were listed in Supplementary Table 2.

Hormone and L-tryptophan measurements

Commercial ELISA kits specific for mouse insulin (Cat#80-INSMSU-E01; Alpco, Salem, NH), C-peptide (Cat#80-CPTMS-E01; Alpco), glucagon (Cat#DGCG0; R&D System, Minneapolis, MN), active GLP-1 (Cat#80-GLP1A-CH01; Alpco), L-tryptophan (Cat#BA E-2700; Labor Diagnostika Nord, GmbH, Nordhorn, Germany), and human insulin (Cat#10-1113-01; Mercodia, Uppsala, Sweden) were used to detect the hormone and L-tryptophan concentration in blood samples, culture

supernatants, or intestinal tissue lysates according to the manufacturer's instructions.

Statistical analysis

Data are expressed as the mean ± SEM or median (interquartile range). Shapiro-Wilk test was used to check whether data were normally distributed. If data were normally distributed, they were analyzed by unpaired Student's *t*-test between two groups, or by one-way or two-way ANOVA followed by Dunnett's T3 multiple comparisons test, Dunnett's multiple comparisons test, Tukey's multiple comparisons test or Bonferroni's multiple comparisons test between multiple groups, as appropriate. If data were not normally distributed, they were analyzed by Kruskal-Wallis test followed by Dunn's multiple comparisons test between multiple groups. Spearman correlation analysis was used to estimate the correlation between gut microbiota and microbiotic metabolites. A P value < 0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA).

References

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Supplementary tables

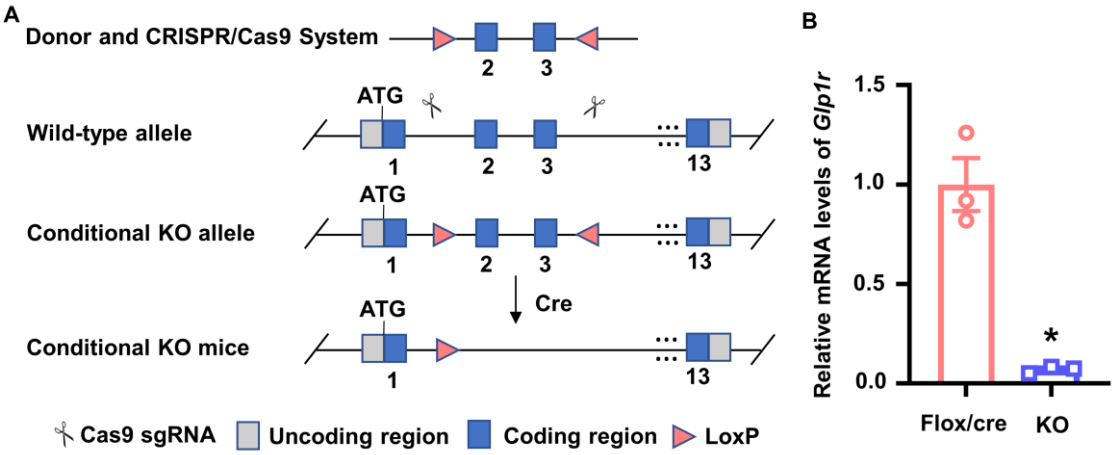
Supplementary Table 1. Donor information of human islets

Islet preparation	**1**	**2**
MANDATORY INFORMATION		
Unique identifier	H212	H214
Donor age (years)	50	53
Donor sex (M/F)	M	M
Donor BMI (kg/m^2)	22.22	24.22
Donor HbA_{1C}	Unavailable	Unavailable
Origin/source of islets	Tianjin First Central Hospital	Tianjin First Central Hospital
Islet isolation center	Tianjin First Central Hospital	Tianjin First Central Hospital
Donor history of diabetes? Please select yes/no	No	No
If Yes, complete the next two lines if this information is available		
Diabetes duration (years)	/	/
Glucose-lowering therapy at time of death	/	/
RECOMMENDED INFORMATION		
Donor cause of death	Thalamus hemorrhage	Brainstem hemorrhage
Warm ischaemia time (h)	0	0
Cold ischaemia time (h)	6	6
Estimated purity (%)	80	90
Estimated viability (%)	95	95
Total culture time (h)	37	38
Glucose-stimulated insulin secretion or other functional measurement	/	/
Handpicked to purity? Please select yes/no	No	No
Additional notes	/	/

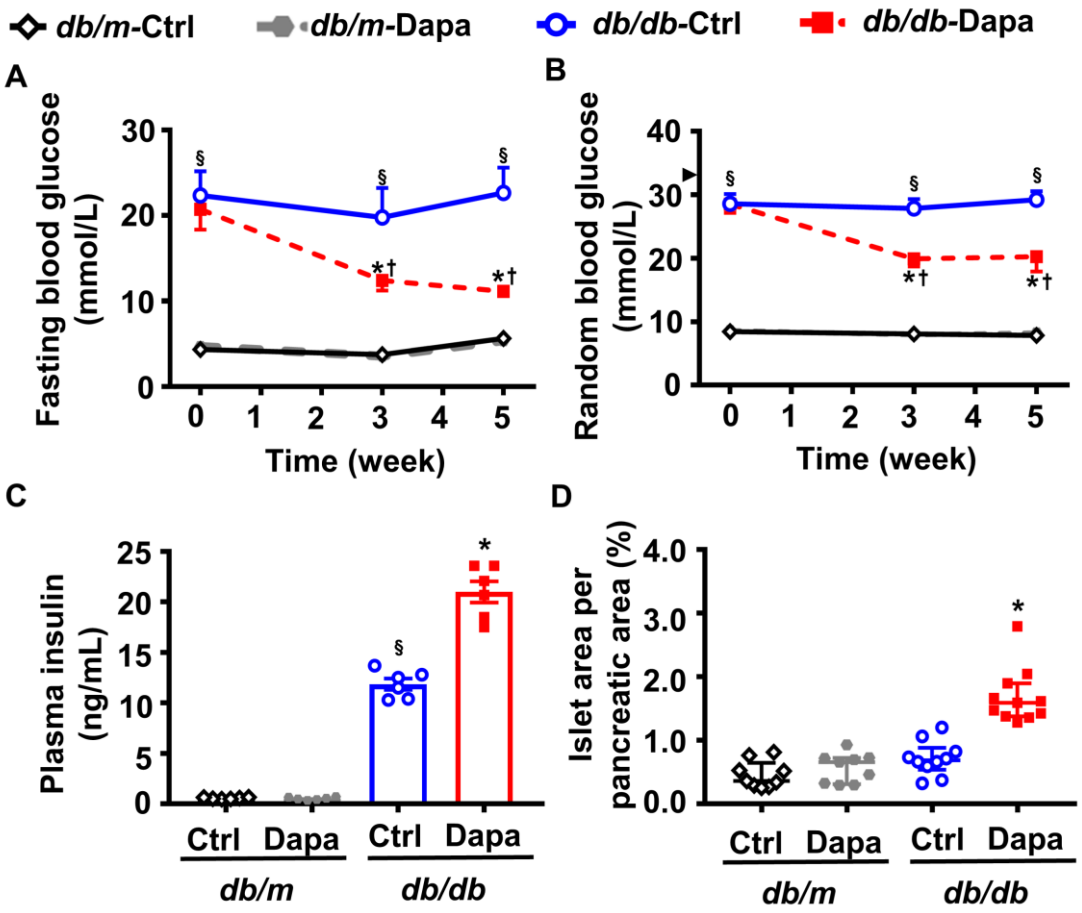
Supplementary Table 2. Primers for quantitative RT-PCR

Gene ID	Gene symbol	Gene Name	Species	Primer sequences (5'-3')	Tm (˚C)	Product length (bp)
11461	*Actb*	actin, beta	Mouse	F: TGTACCCAGGCATTGCTGAC R: CTGCTGGAAGGTGGACAGTG	60	149
14526	*Gcg*	glucagon	Mouse	F: GAAGACAAACGCCACTCACA R: CAGCATGCCTCTCAAATTCA	59	154
14652	*Glp1r*	glucagon-like peptide-1 receptor	Mouse	F: ACGGTGTCCCTCTCAGAGAC R: ATCAAAGGTCCGGTTGCAGAA	60	117
18548	*Pcsk1*	proprotein convertase subtilisin/kexin type 1	Mouse	F: AGTTGGAGGCATAAGAATGCTG R: GCCTTCTGGGCTAGTCTGC	60	159

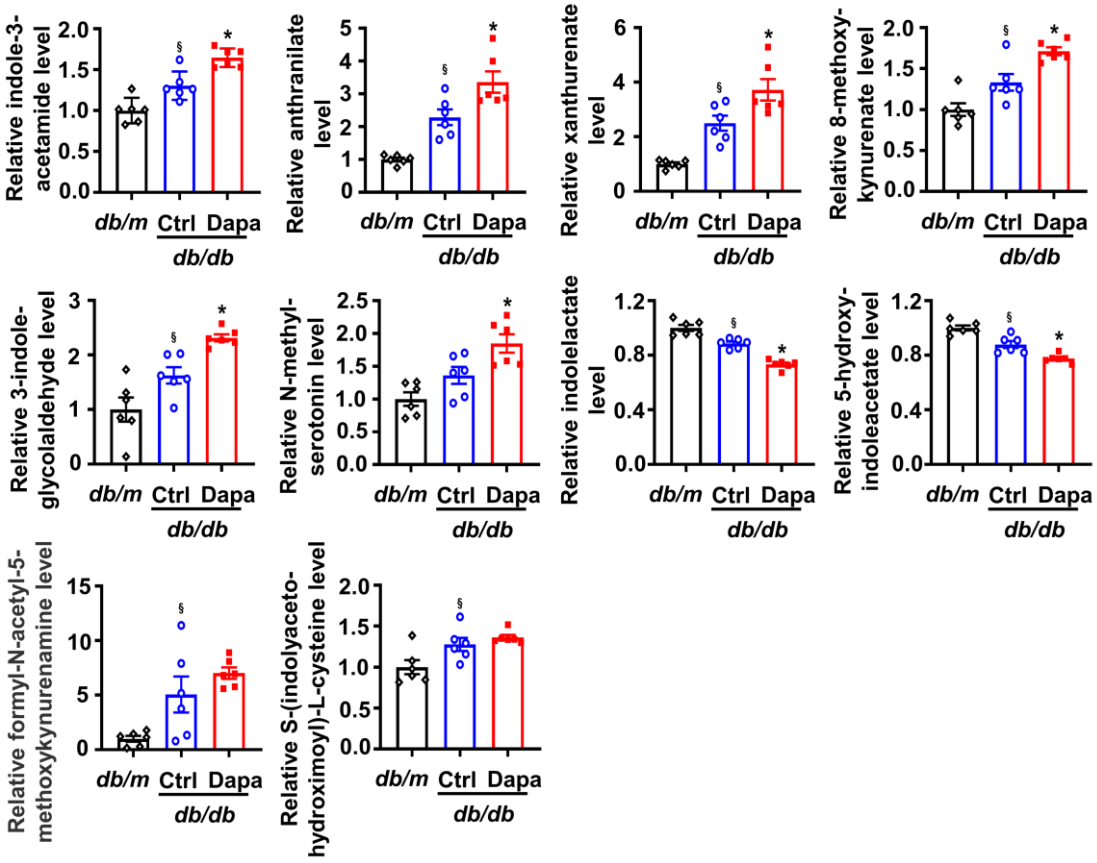
Supplementary figures



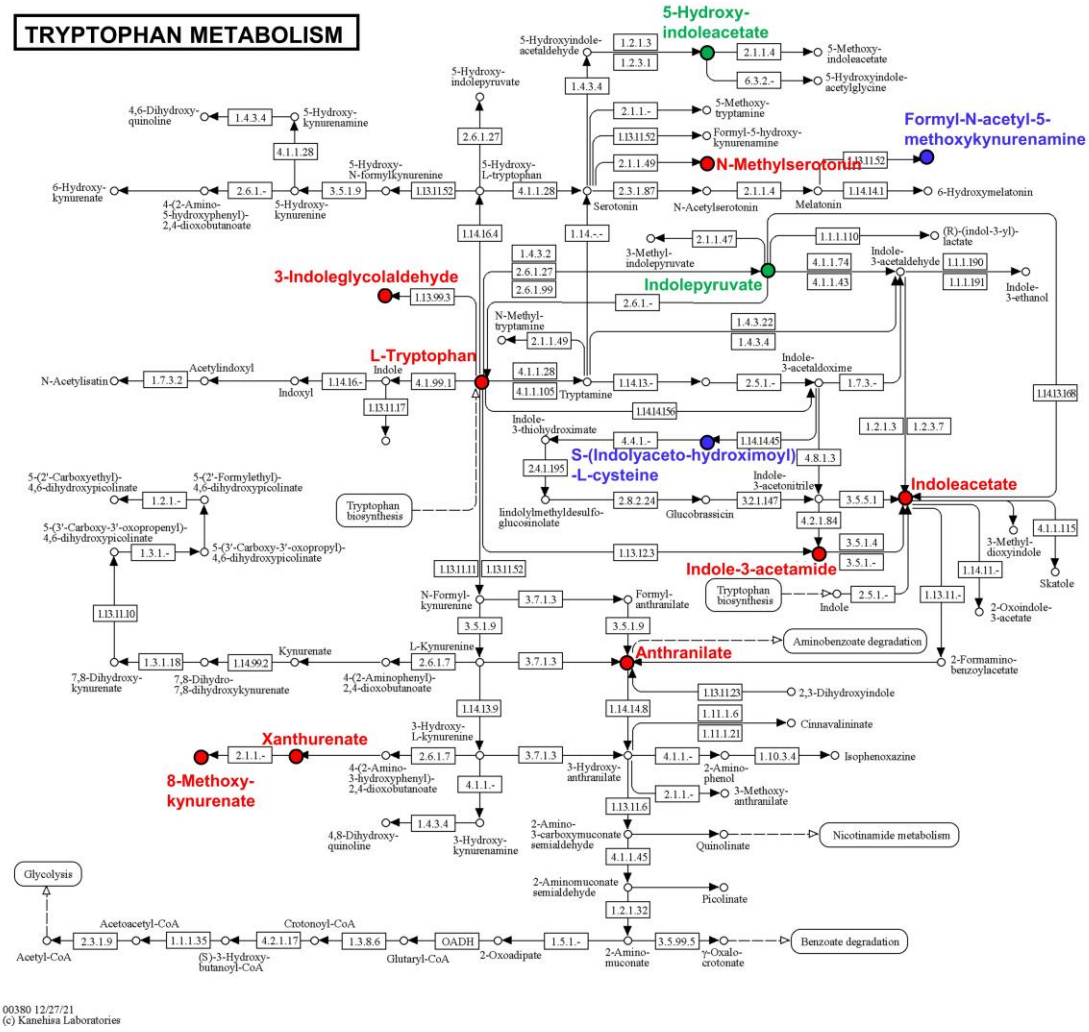
Supplementary Figure 1 – Generation and verification of *Glp1r*$^{pan-/-}$ mice. *A*: Schematic depicting the strategy for generating pancreas-specific *Glp1r* knockout (*Glp1r*$^{pan-/-}$) mice. *B*: *Glucagon-like peptide-1 receptor* (*Glp1r*) expression in islets isolated from *Glp1r*$^{pan-/-}$ mice and Flox/cre littermates. n = 3. Data are expressed as the means ± SEM. Statistical analysis was performed by unpaired Student's *t*-test in *B*. $^{*}P < 0.05$ (*vs* Flox/cre).



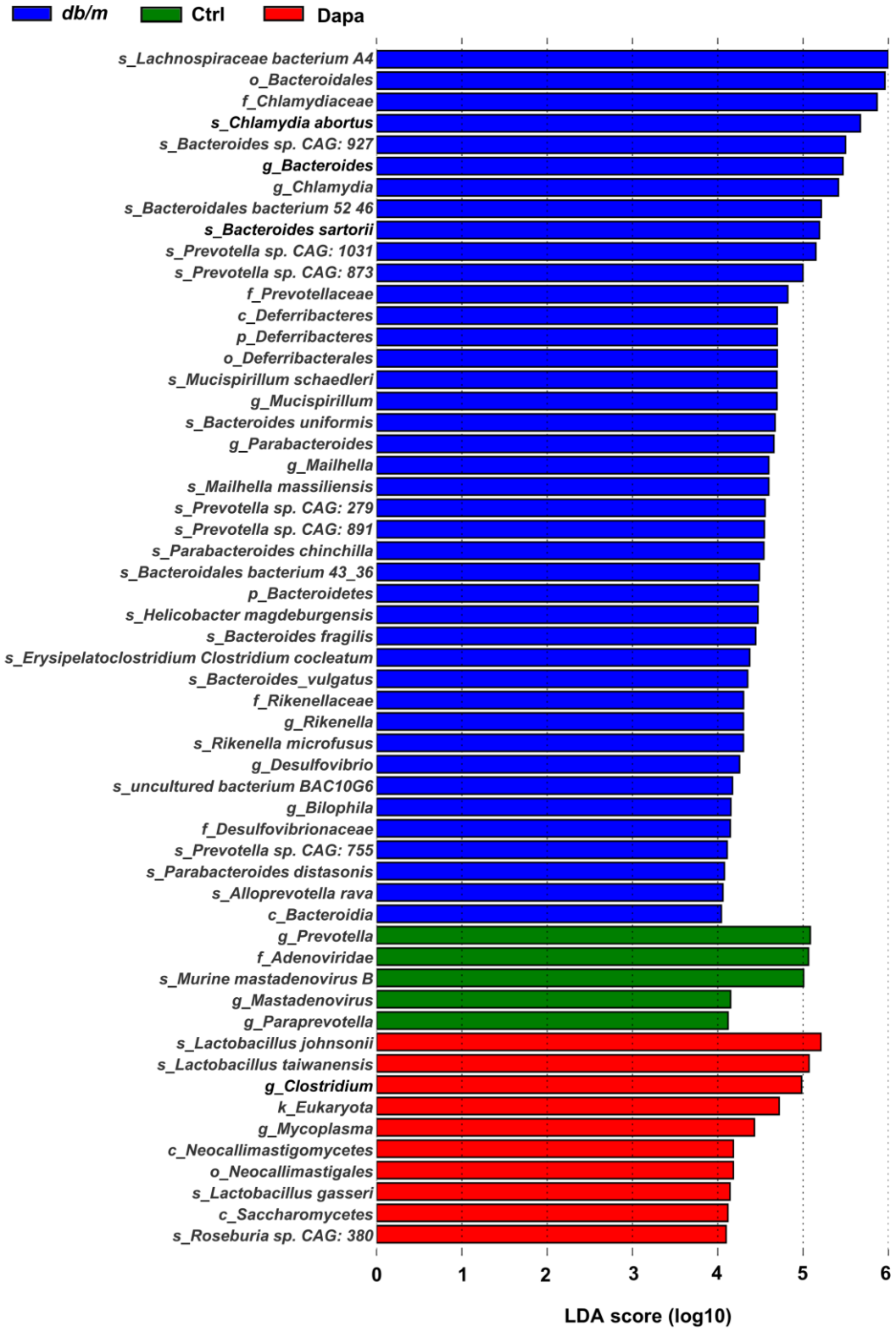
Supplementary Figure 2 – Metabolic parameters in *db/db* mice and *db/m* mice treated with dapagliflozin or vehicle control for five weeks. n = 6 mice per group. *A*: Fasting blood glucose. *B*: Random blood glucose. The arrowheads indicate the upper detection limit (33.3 mmol/L) of the glucometer. *C*: Fasting plasma insulin. *D*: Quantification of the islet area per pancreatic area. n = 3–4 sections/mouse and 3 mice/group. Data are expressed as the means ± SEM or median (interquartile range). Statistical analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test in *A* and *B*, or by one-way ANOVA followed by Dunnett's T3 multiple comparisons test in *C*, or by Kruskal-Wallis test followed by Dunn's multiple comparisons test in *D*. $^{\S}P < 0.05$ (*vs db/m*); $^{*}P < 0.05$ (*vs* control); $^{\dagger}P < 0.05$ (post-treatment *vs* pre-treatment in the same group). Ctrl, control; Dapa, dapagliflozin.



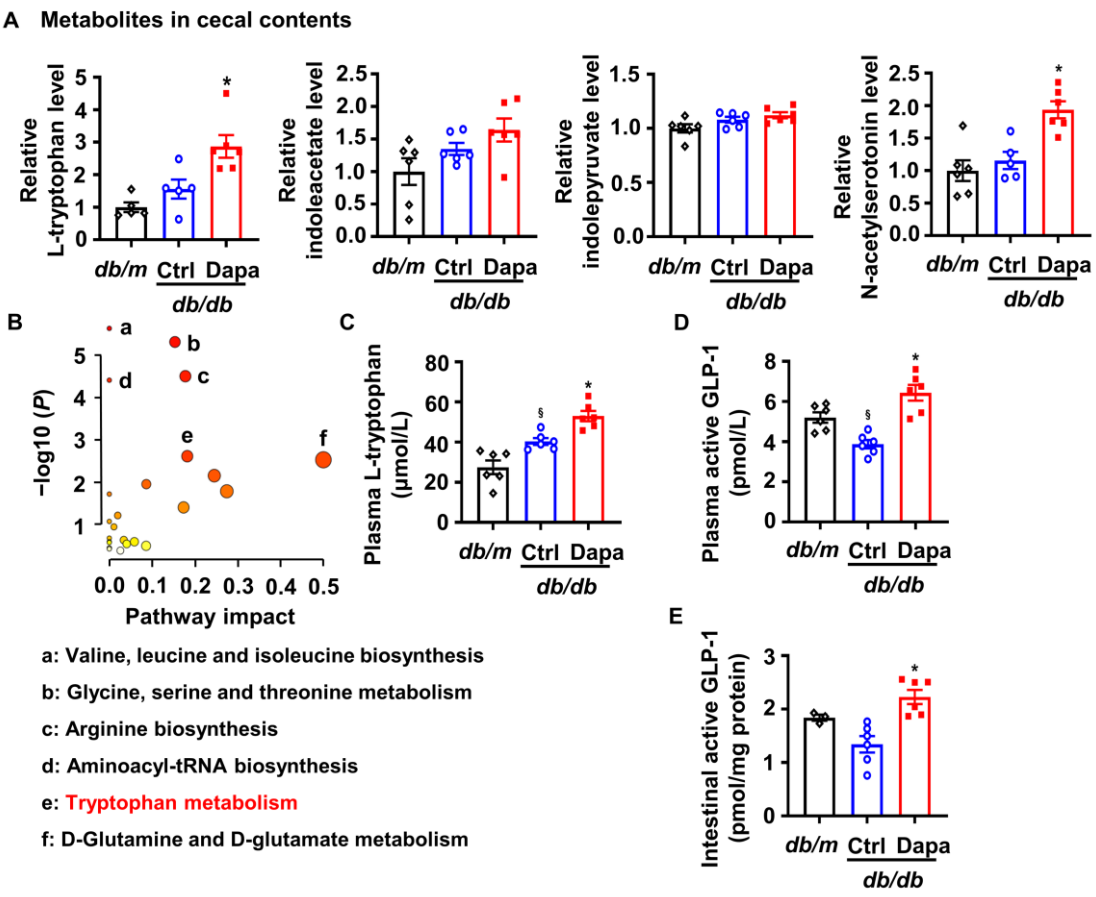
Supplementary Figure 3 – Relative levels of differential metabolites related to tryptophan metabolism in the plasma of *db/db* mice treated with dapagliflozin or vehicle control for five weeks. Age-matched *db/m* mice treated with vehicle were included as normal control. n = 6. Data are expressed as the means ± SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnett's T3 multiple comparisons test. $^{\S}P < 0.05$ (*vs db/m*); $^{*}P < 0.05$ (*vs* control). Ctrl, control; Dapa, dapagliflozin.



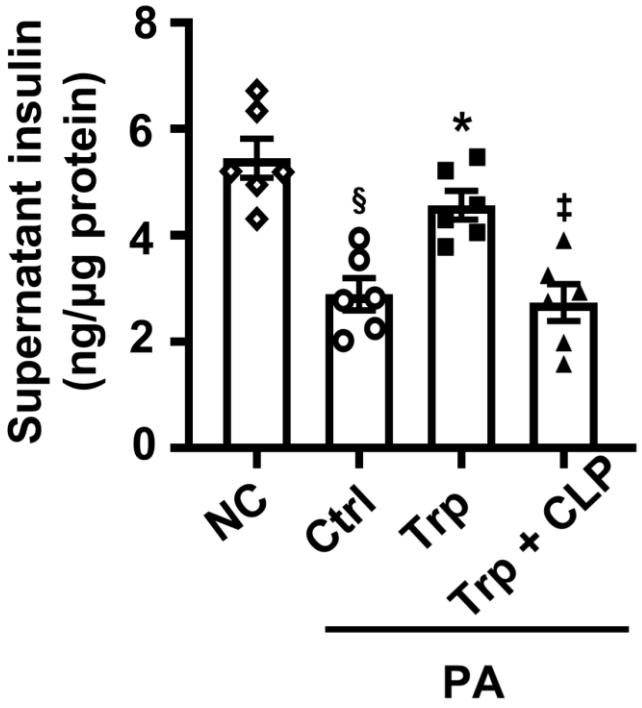
Supplementary Figure 4 – The changes of differential plasmatic metabolites related to tryptophan metabolism in KEGG pathway map. In the plasma of *db/db* mice receiving a five-week treatment with dapagliflozin versus vehicle control, upregulated metabolites were shown in red and downregulated metabolites were shown in green. The metabolites in *db/db* mice that were different from those in *db/m* mice but were unchanged by dapagliflozin were shown in blue.



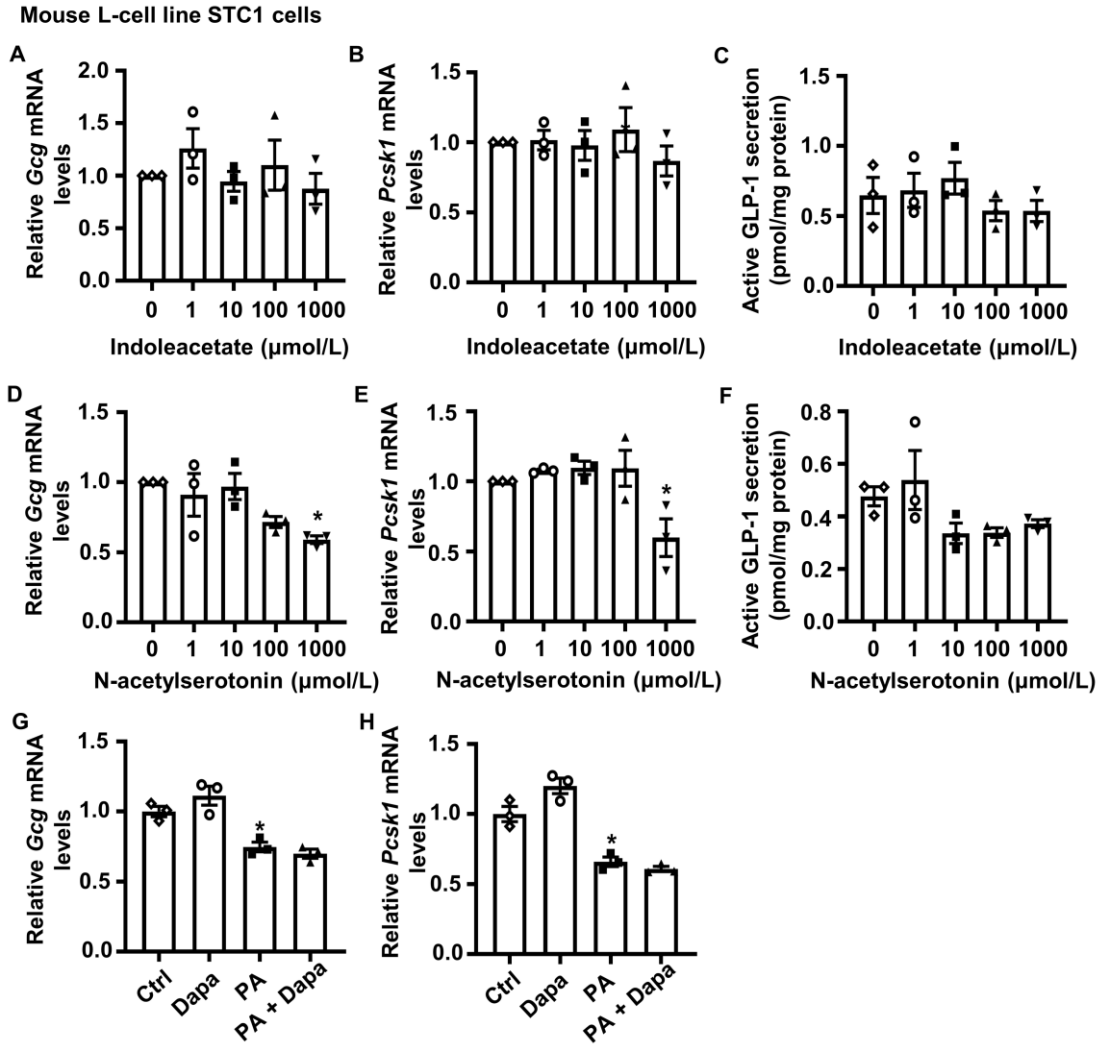
Supplementary Figure 5 – Differential gut microbiota revealed by the linear discriminant analysis (LDA) effect size (LEfSe) method, with the microbiota of $P < 0.05$ and LDA score > 4.



Supplementary Figure 6 – The microbial metabolomic profile, plasma L-tryptophan level and active GLP-1 level in *db/db* mice treated with dapagliflozin or vehicle control for five weeks. Age-matched *db/m* mice treated with vehicle were used as normal control. *A*: Relative levels of differential metabolites related to tryptophan metabolism in cecal contents. *B*: Metabolic pathway analysis (MetPA) of differential microbiotic metabolites. *C*: Plasma L-tryptophan level. *D*: Plasma active GLP-1 level. *E*: Intestinal active GLP-1 level. n = 3–6 mice per group. Data are expressed as the means ± SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnett's T3 multiple comparisons test in *A* and *C–E*. $^{§}P < 0.05$ (*vs db/m*); $^{*}P < 0.05$ (*vs* control). Ctrl, control; Dapa, dapagliflozin.

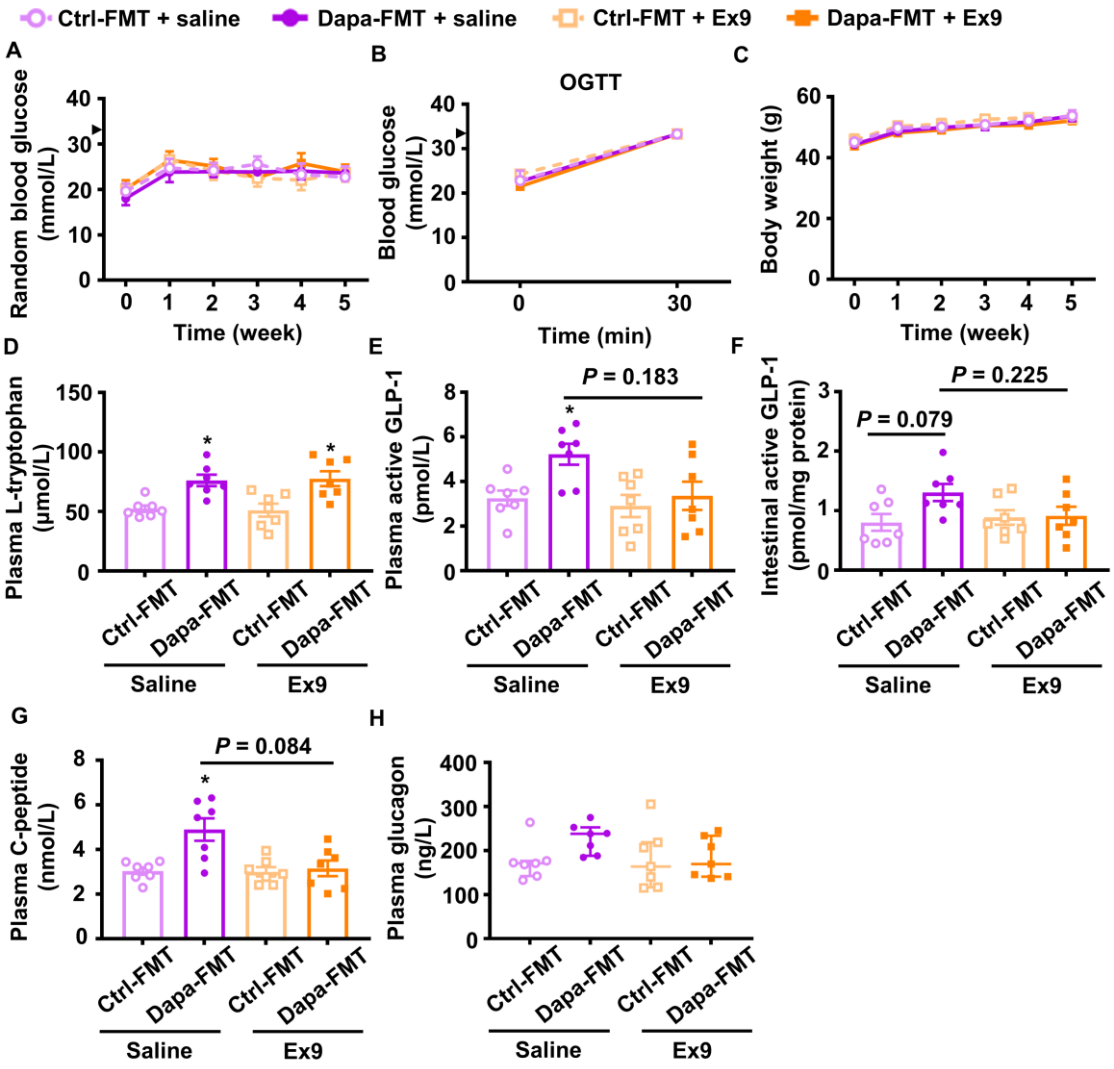


Supplementary Figure 7 – Insulin secretion in primary human islets exposed to L-tryptophan with or without CLP-3094, a GPR142 antagonist. Human islets were incubated for 24 h with L-tryptophan (1 mmol/L) or vehicle in the presence or absence of CLP-3094 (1 μmol/L) with or without palmitate (0.5 mmol/L). Supernatant insulin level was measured by using ELISA kits. n = 6 (three independent duplications per islet preparation isolated from two nondiabetic donors). Data are expressed as the means ± SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnett's T3 multiple comparisons test. $^{\S}P < 0.05$ (*vs* normal control); $^{*}P < 0.05$ (*vs* palmitate); $^{\ddagger}P < 0.05$ (*vs* palmitate + L-tryptophan). CLP, CLP-3094; Ctrl, control; Dapa, dapagliflozin; NC, normal control; PA, palmitate; Trp, L-tryptophan.



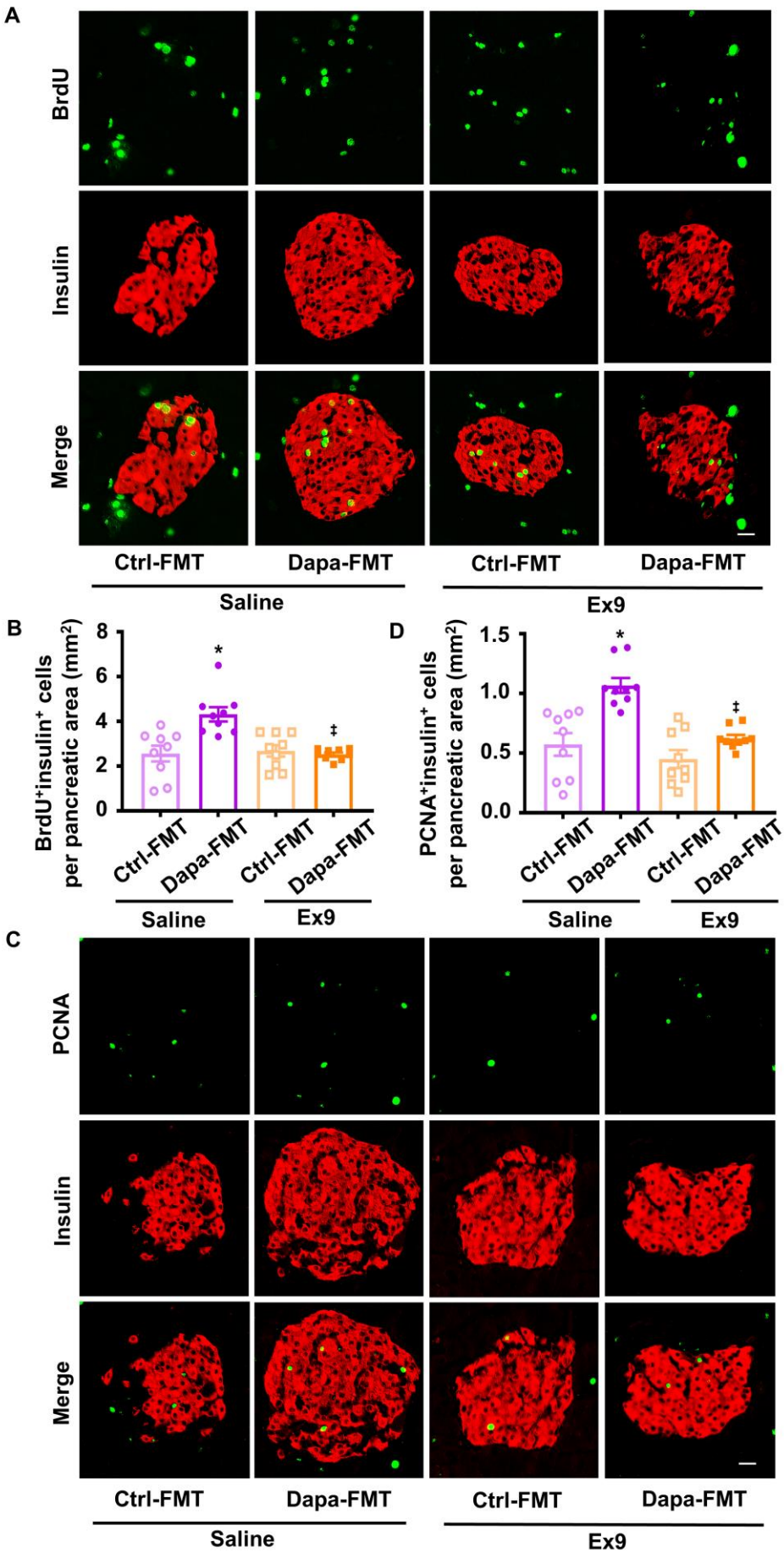
Supplementary Figure 8 – The effects of microbiotic metabolites or dapagliflozin on GLP-1 production-related gene expression and GLP-1 secretion in L-cells. *A*, *B*, *D*, and *E*: Relative *Gcg* and *Pcsk1* mRNA levels in the intestinal L-cell line STC1 cells incubated with different concentrations of indoleacetate or N-acetylserotonin for 24 h. *C* and *F*: GLP-1 secretion in STC1 cells cultured with different concentrations of indoleacetate or N-acetylserotonin for 60 min. *G* and *H*: Relative *Gcg* and *Pcsk1* mRNA levels in STC1 cells incubated for 8 h with dapagliflozin (25 μmol/L) or vehicle with or without palmitate (0.5 mmol/L). n = 3. Data are expressed as the means ± SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnett's multiple comparisons test in *A*–*F*, or by one-way ANOVA followed by Dunnett's T3 multiple comparisons test in *G* and *H*. $^{*}P < 0.05$ (*vs* control). Ctrl,

control; Dapa, dapagliflozin; PA, palmitate.

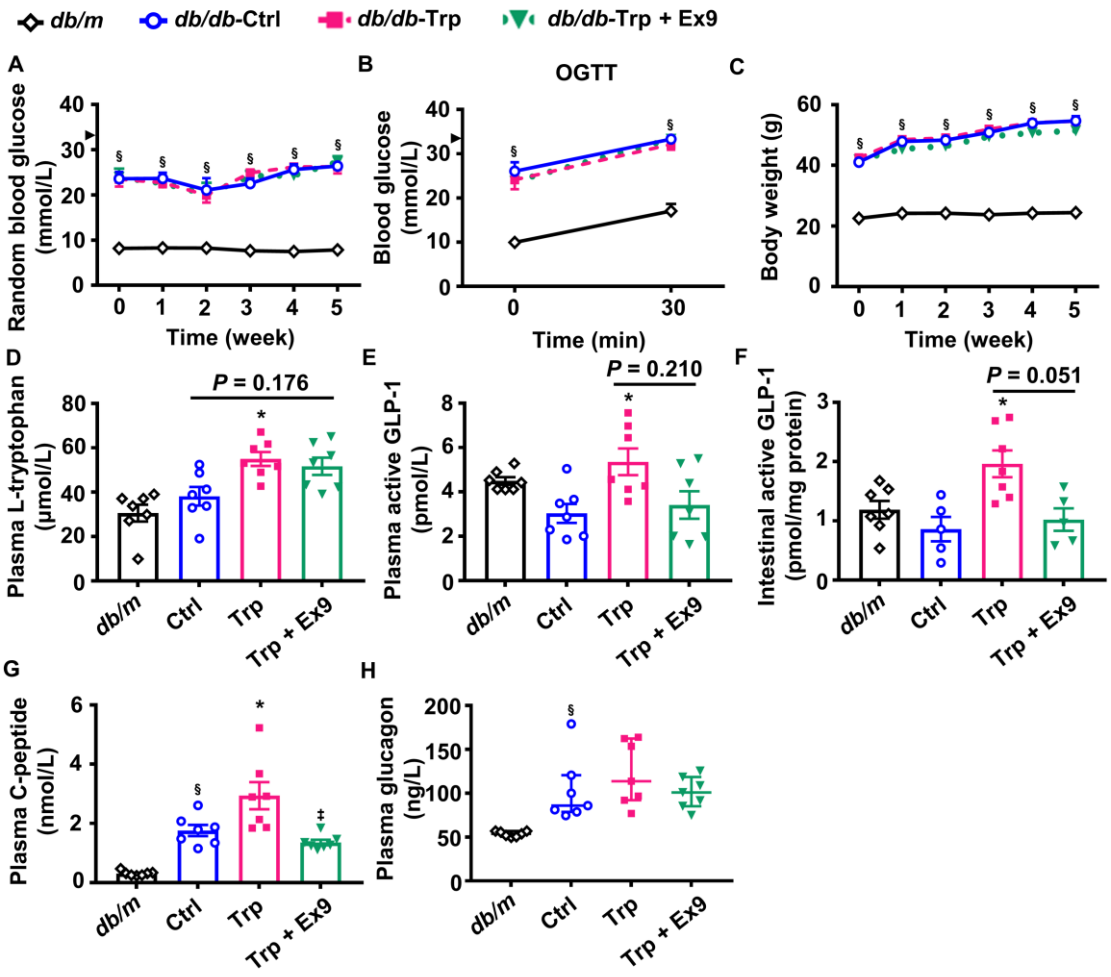


Supplementary Figure 9 – Metabolic parameters and hormone levels in *db/db* mice receiving fecal microbiota transplantation (FMT) from dapagliflozin- or vehicle-treated mice, combined with or without GLP-1 receptor (GLP-1R) antagonist exendin 9-39 for five weeks. n = 7 mice per group. *A*: Random blood glucose. *B*: Blood glucose levels during the oral glucose tolerance test (OGTT). The arrowhead in *A* and *B* indicates the upper detection limit (33.3 mmol/L) of the glucometer. *C*: Body weight. *D*: Plasma L-tryptophan. *E*: Plasma active GLP-1 at 30 min after the glucose loading during the OGTT. *F*: Intestinal active GLP-1. *G*: Plasma C-peptide at 30 min after the glucose loading during the OGTT. *H*: Fasting plasma glucagon. Data are expressed as the means ± SEM or median (interquartile range). Statistical analysis

was performed by one-way or two-way ANOVA followed by Tukey's multiple comparisons test as appropriate in *A*–*C*, and *F*, or by one-way ANOVA followed by Dunnett's T3 multiple comparisons test in *D*, *E* and *G*, or by Kruskal-Wallis test followed by Dunn's multiple comparisons test in *H*. $^{*}P < 0.05$ (*vs* control-FMT + saline). Ctrl, control; Dapa, dapagliflozin; Ex9, exendin 9-39.

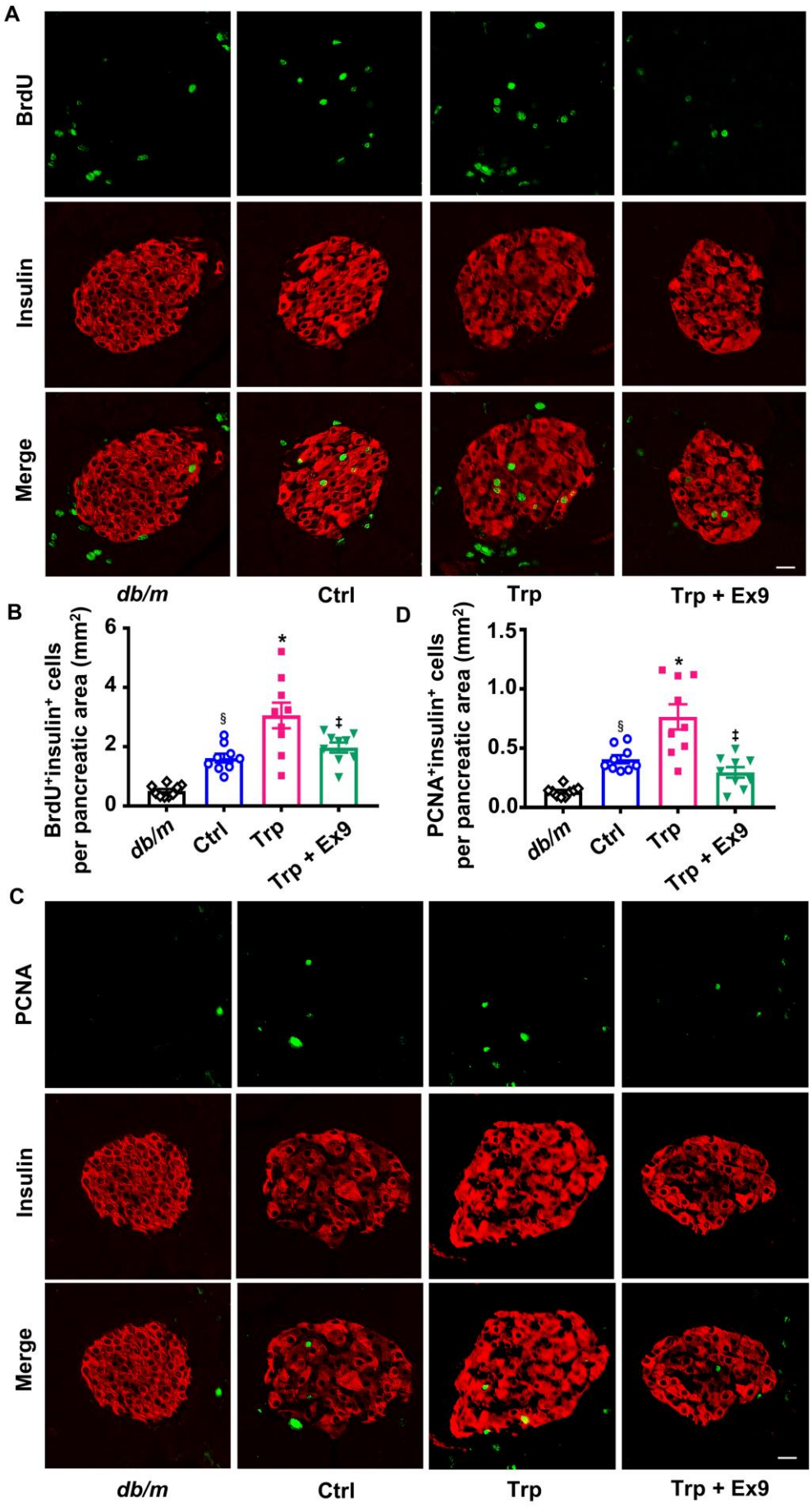

A
BrdU
Insulin
Merge
Ctrl-FMT
Dapa-FMT
Ctrl-FMT
Dapa-FMT
Saline
Ex9
B
BrdU+insulin+ cells per pancreatic area (mm2)
8
6
4
2
0
*
‡
Ctrl-FMT
Dapa-FMT
Ctrl-FMT
Dapa-FMT
Saline
Ex9
D
PCNA+insulin+ cells per pancreatic area (mm2)
1.5
1.0
0.5
0.0
*
‡
Ctrl-FMT
Dapa-FMT
Ctrl-FMT
Dapa-FMT
Saline
Ex9
C
PCNA
Insulin
Merge
Ctrl-FMT
Dapa-FMT
Ctrl-FMT
Dapa-FMT
Saline
Ex9

Supplementary Figure 10 – Histological analysis of β-cell proliferation in the pancreatic tissues of *db/db* mice receiving fecal microbiota transplantation (FMT) from dapagliflozin- or vehicle-treated mice, combined with or without exendin 9-39 for five weeks. *A*: Representative photograph showing immunostaining of BrdU and insulin. Scale bar = 20 μm. *B*: Quantification of BrdU and insulin double-positive cells. *C*: Representative photograph showing immunostaining of PCNA and insulin. Scale bar = 20 μm. *D*: Quantification of PCNA and insulin double-positive cells. n = 3 sections/mouse multiplied by 3 mice/group. Data are expressed as the mean ± SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnett's T3 multiple comparisons test in *B* and *D*. $^{*}P < 0.05$ (*vs* control-FMT + saline); $^{‡}P < 0.05$ (*vs* dapagliflozin-FMT + saline). Ctrl, control; Dapa, dapagliflozin; Ex9, exendin 9-39.

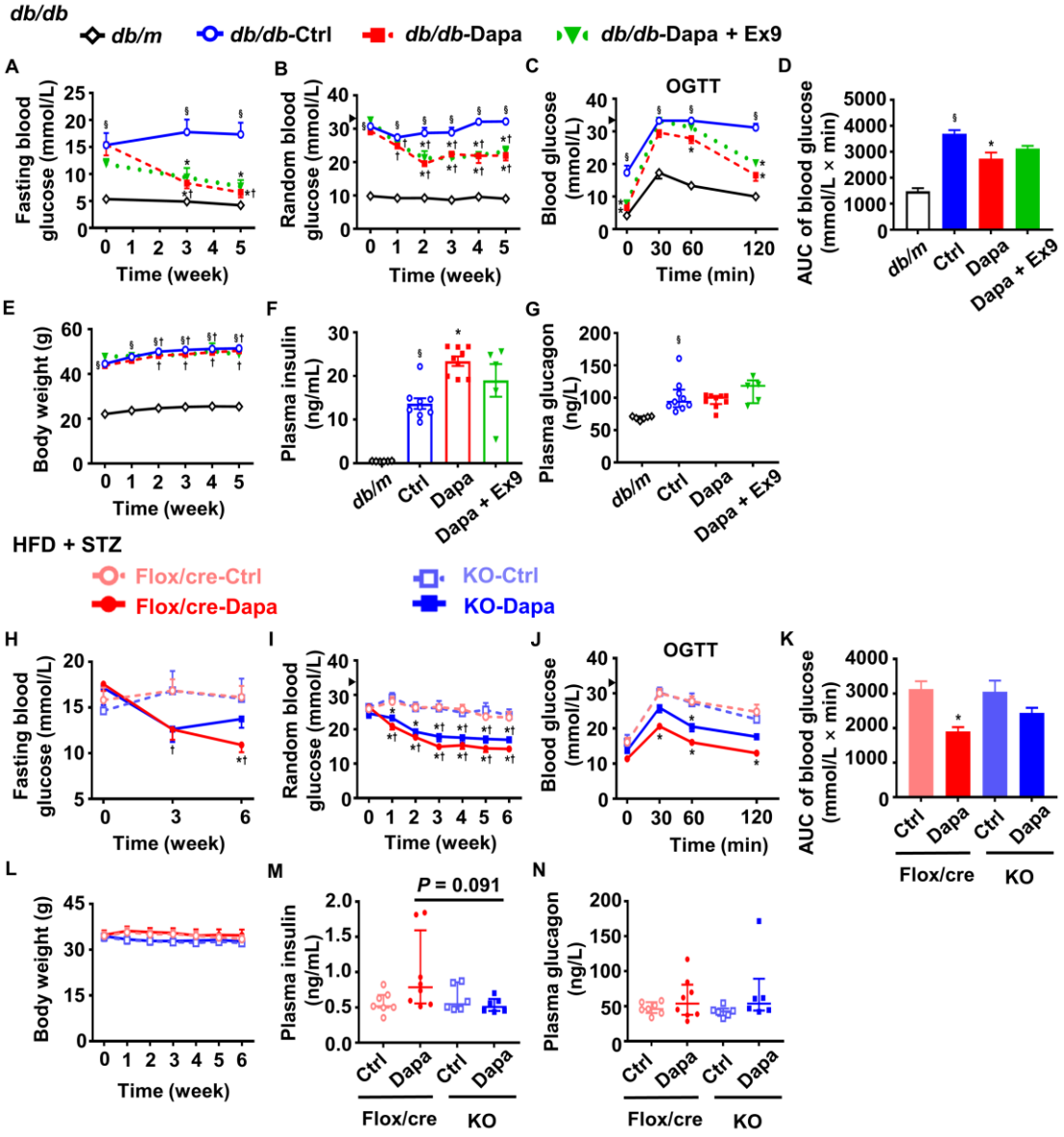


Supplementary Figure 11 – Metabolic parameters and hormone levels in *db/db* mice treated with L-tryptophan with or without exendin 9-39 for five weeks. Age-matched *db/m* mice treated with vehicle were included as normal control. *A*: Random blood glucose. *B*: Blood glucose levels during the oral glucose tolerance test (OGTT). The arrowhead in *A* and *B* indicates the upper detection limit (33.3 mmol/L) of the glucometer. *C*: Body weight. *D*: Plasma L-tryptophan. *E*: Plasma active GLP-1 at 30 min after the glucose loading during the OGTT. *F*: Intestinal active GLP-1. *G*: Plasma C-peptide at 30 min after the glucose loading during the OGTT. *H*: Fasting plasma glucagon. n = 5–7 mice per group. Data are expressed as the means ± SEM or median (interquartile range). Statistical analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test in *A*–*C*, or by one-way ANOVA followed by

Dunnett's T3 multiple comparisons test in *D–G*, or by Kruskal-Wallis test followed by Dunn's multiple comparisons test in *H*. $^{\S}P < 0.05$ (*vs db/m*); $^{*}P < 0.05$ (*vs* control); $^{\ddagger}P < 0.05$ (*vs* L-tryptophan). Ctrl, control; Ex9, exendin 9-39; Trp, L-tryptophan.

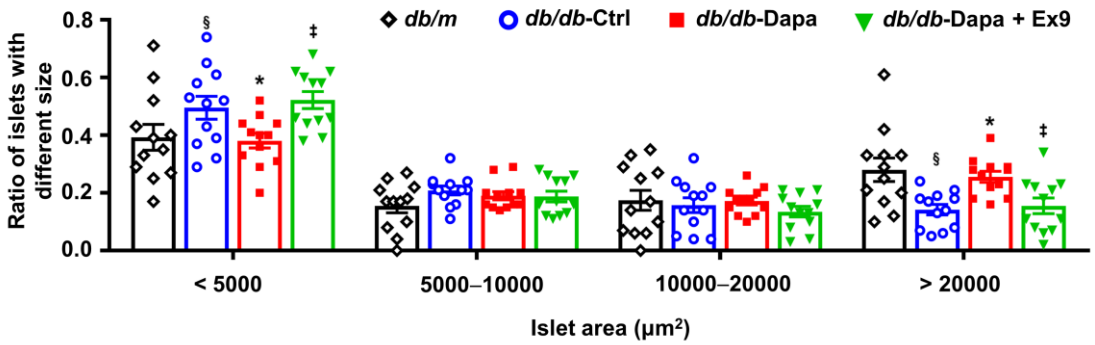

A
BrdU
Insulin
Merge
db/m
Ctrl
Trp
Trp + Ex9
B
BrdU+insulin+ cells per pancreatic area (mm2)
6
4
2
0
§
*
‡
db/m
Ctrl
Trp
Trp + Ex9
D
PCNA+insulin+ cells per pancreatic area (mm2)
1.5
1.0
0.5
0.0
§
*
‡
db/m
Ctrl
Trp
Trp + Ex9
C
PCNA
Insulin
Merge
db/m
Ctrl
Trp
Trp + Ex9

Supplementary Figure 12 – Histological analysis of β-cell proliferation in the pancreatic tissues of *db/db* mice treated with L-tryptophan with or without exendin 9-39 for five weeks. Age-matched *db/m* mice treated with vehicle were included as normal control. *A*: Representative photograph showing immunostaining of BrdU and insulin. Scale bar = 20 μm. *B*: Quantification of BrdU and insulin double-positive cells. *C*: Representative photograph showing immunostaining of PCNA and insulin. Scale bar = 20 μm. *D*: Quantification of PCNA and insulin double-positive cells. n = 3 sections/mouse multiplied by 3 mice/group. Data are expressed as the mean ± SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnett's T3 multiple comparisons test in *B* and *D*. $^{\S}P < 0.05$ (*vs db/m*); $^{*}P < 0.05$ (*vs* control); $^{\ddagger}P < 0.05$ (*vs* L-tryptophan). Ctrl, control; Ex9, exendin 9-39; Trp, L-tryptophan.

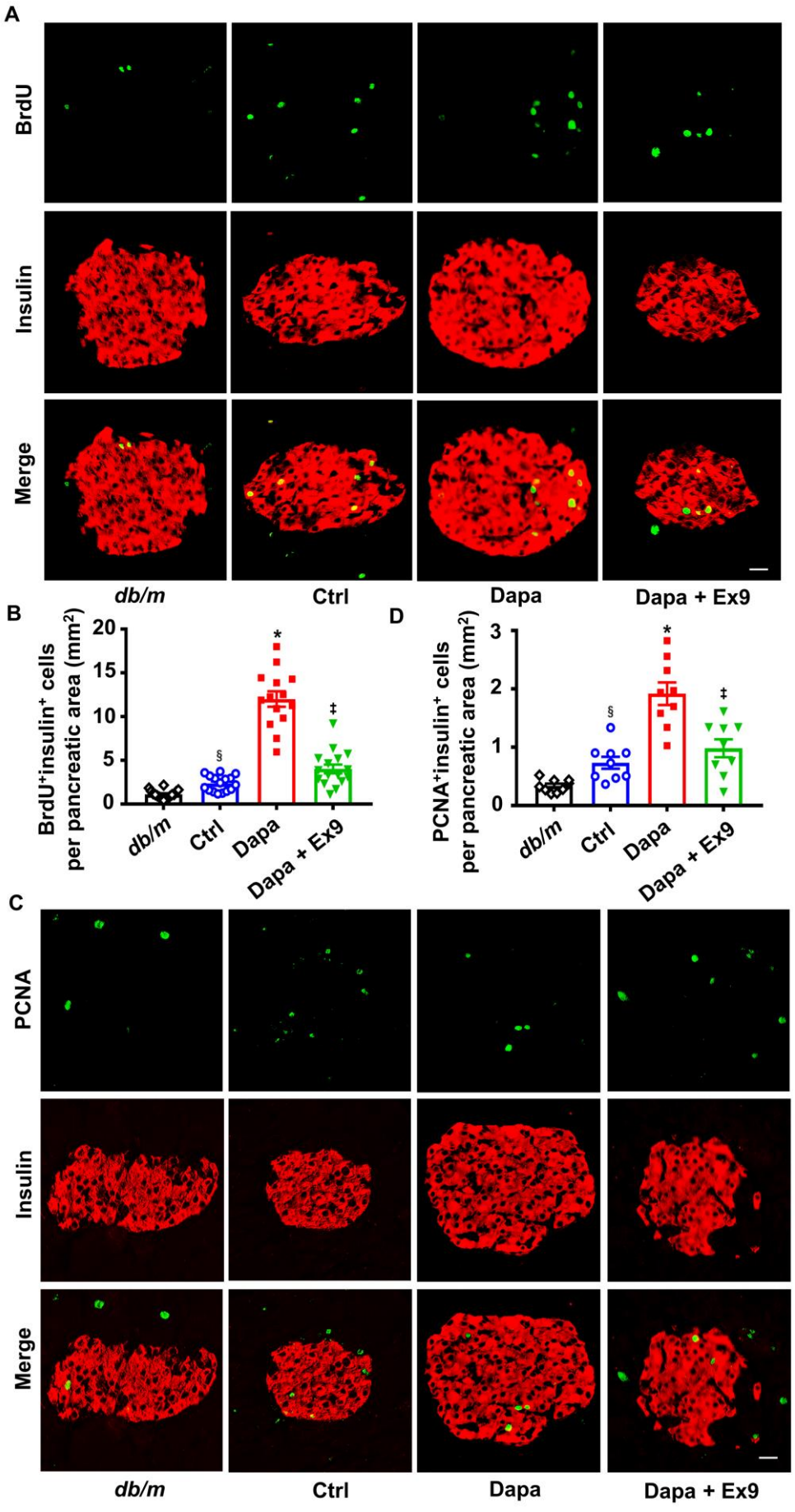


Supplementary Figure 13 – Metabolic parameters and hormone levels in *db/db* mice treated with dapagliflozin with or without exendin 9-39 for five weeks, and in diabetic pancreas-specific *Glp1r* knockout (*Glp1r*$^{pan-/-}$) mice treated with dapagliflozin or vehicle control for six weeks. *A*–*G*: Parameters in *db/db* mice. Age-matched *db/m* mice treated with vehicle were included as normal control. n = 5–9 mice per group. *H*–*N*: Parameters in *Glp1r*$^{pan-/-}$ mice and Flox/cre littermates whose diabetes was induced by high-fat diet (HFD) + streptozotocin (STZ). n = 6–8 mice per group. *A* and *H*: Fasting blood glucose. *B* and *I*: Random blood glucose. *C* and *J*: Blood

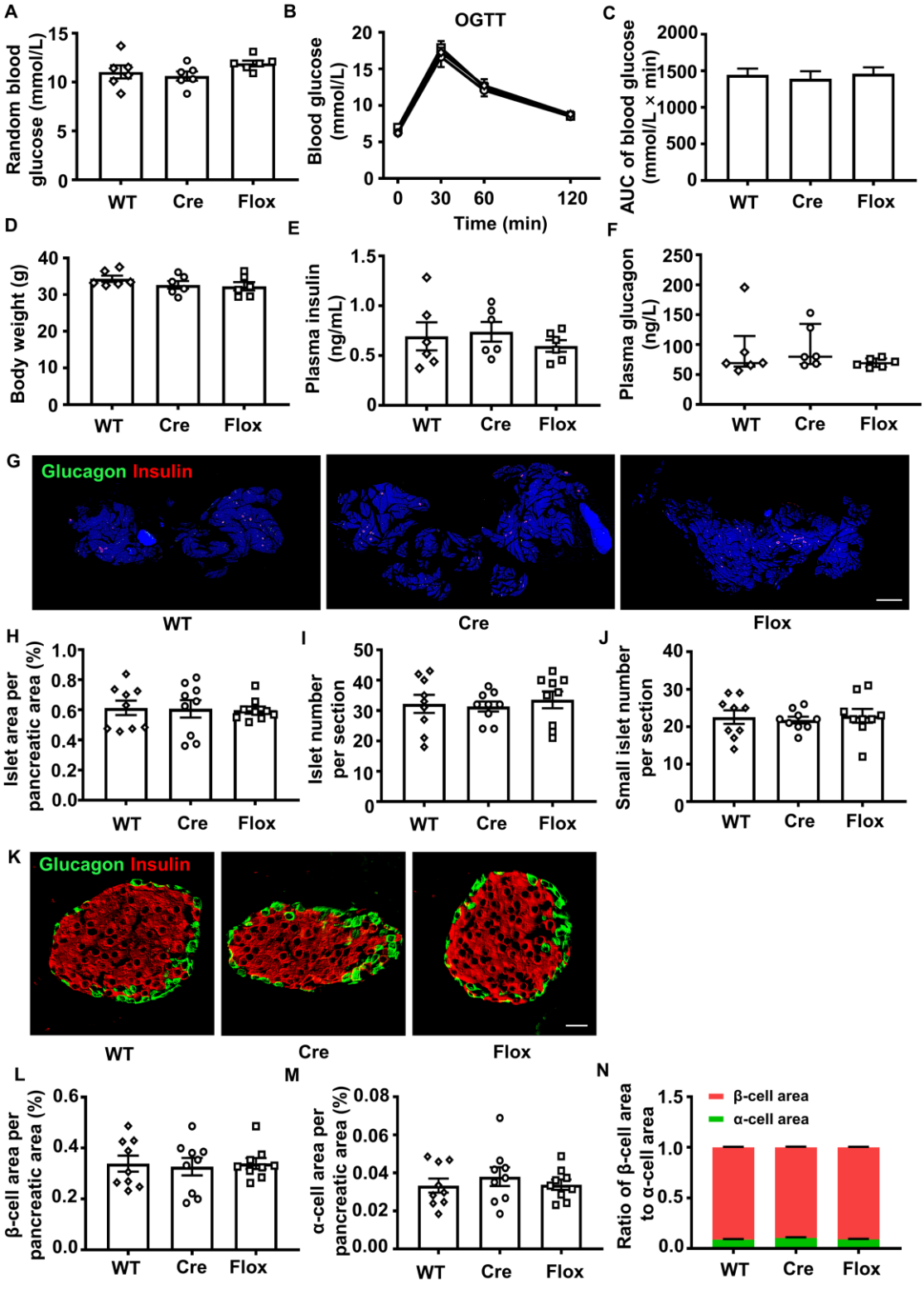
glucose levels during the oral glucose tolerance test (OGTT). The arrowheads in *B*, *C*, *I*, and *J* indicate the upper detection limit (33.3 mmol/L) of the glucometer. *D* and *K*: The area under curve (AUC) of blood glucose during the OGTT. *E* and *L*: Body weight. *F* and *M*: Fasting plasma insulin. *G* and *N*: Fasting plasma glucagon. Data are expressed as the means ± SEM or median (interquartile range). Statistical analysis was performed by two-way ANOVA followed by Bonferroni's multiple comparisons test in *A–C*, *E*, *H–J*, and *L*, or by one-way ANOVA followed by Dunnett's T3 multiple comparisons test in *D*, *F*, and *K*, or by Kruskal-Wallis test followed by Dunn's multiple comparisons test in *G*, *M*, and *N*. $^{\S}P < 0.05$ (*vs db/m*); $^{*}P < 0.05$ (*vs* control in the same genotype); $^{\dagger}P < 0.05$ (post-treatment *vs* pre-treatment in the same group). Ctrl, control; Dapa, dapagliflozin; Ex9, exendin 9-39; KO, knockout.



Supplementary Figure 14 – Histological analysis of the entire pancreata of *db/db* mice treated with vehicle control, dapagliflozin, or dapagliflozin combined with exendin 9-39 for five weeks. Age-matched *db/m* mice treated with vehicle served as normal control. Quantification of the ratios of islets with different size. n = 4 sections/mouse and 3 mice/group. Data are expressed as the means ± SEM. Statistical analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test. $^{§}P < 0.05$ (*vs db/m*); $^{*}P < 0.05$ (*vs* control); $^{‡}P < 0.05$ (*vs* dapagliflozin). Ctrl, control; Dapa, dapagliflozin; Ex9, exendin 9-39.


A
BrdU
Insulin
Merge
db/m
Ctrl
Dapa
Dapa + Ex9
B
BrdU+insulin+ cells per pancreatic area (mm2)
20
15
10
5
0
§
*
‡
db/m
Ctrl
Dapa
Dapa + Ex9
D
PCNA+insulin+ cells per pancreatic area (mm2)
3
2
1
0
§
*
‡
db/m
Ctrl
Dapa
Dapa + Ex9
C
PCNA
Insulin
Merge
db/m
Ctrl
Dapa
Dapa + Ex9

Supplementary Figure 15 – Histological analysis of β-cell proliferation in the pancreatic tissues of *db/db* mice treated with dapagliflozin with or without exendin 9-39 for five weeks. Age-matched *db/m* mice treated with vehicle were included as normal control. *A*: Representative photograph showing immunostaining of BrdU and insulin. Scale bar = 20 μm. *B*: Quantification of BrdU and insulin double-positive cells. n = 3–4 sections/mouse multiplied by 4 mice/group. *C*: Representative photograph showing immunostaining of PCNA and insulin. Scale bar = 20 μm. *D*: Quantification of PCNA and insulin double-positive cells. n = 3 sections/mouse multiplied by 3 mice/group. Data are expressed as the mean ± SEM. Statistical analysis was performed by one-way ANOVA followed by Dunnett's T3 multiple comparisons test in *B* and *D*. $^{\S}P < 0.05$ (*vs db/m*); $^{*}P < 0.05$ (*vs* control); $^{\ddagger}P < 0.05$ (*vs* dapagliflozin). Ctrl, control; Dapa, dapagliflozin; Ex9, exendin 9-39.



Supplementary Figure 16 – Metabolic parameters, hormone levels, and histological analysis of the pancreata in age-matched wild-type, *Pdx1*-Cre, and *Glp1r*-flox mice. *A*: Random blood glucose. *B*: Blood glucose levels during the oral glucose tolerance test (OGTT). *C*: The area under curve (AUC) of blood glucose during the OGTT. *D*: Body

weight. *E*: Fasting plasma insulin. *F*: Fasting plasma glucagon. n = 6 mice per group. *G*: Representative photographs of the entire pancreata immunostained for glucagon and insulin. Scale bar = 2000 μm. *H*: Quantification of the islet area per pancreatic area. *I*: Quantification of the islet number per section. *J*: Quantification of the small islet number per section. An islet with a cell number < 8 is defined as a small islet. *K*: Representative photographs of an islet immunostained for glucagon and insulin. Scale bar = 20 μm. *L*: Quantification of the β-cell area per pancreatic area. *M*: Quantification of the α-cell area per pancreatic area. *N*: The ratios of β-cell area to α-cell area. n = 3 sections/mouse multiplied by 3 mice/group. Data are expressed as the means ± SEM or median (interquartile range). Statistical analysis was performed by one-way ANOVA or two-way ANOVA followed by Tukey's multiple comparisons test as appropriate in *A–E*, *H–J*, and *L–N*, or by Kruskal-Wallis test followed by Dunn's multiple comparisons test in *F*. WT, wild-type.