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Microbiota-Targeted Intervention in Pancreatic Islet Transplant Recipients: Remodeling Glucose-Metabolism Related Microbial Communities Restores Gut Microbiota Balance and Glucose Stability in Type 2 Diabetes

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ABSTRACT: Islet cell transplantation (ICT) effectively treats type 2 diabetes mellitus (T2DM), but the use of adjunct immunosuppressants/antibiotics disrupts gut microbiota, thereby impairing glucose homeostasis. This study revealed how ICT-induced dysbiosis in functional microbes modulates islet function and glucose metabolism. T2DM patients exhibited marked gut microbiota dysbiosis after ICT, characterized by a significant enrichment of pro-inflammatory microbiota such as *Streptococcus* and *Enterococcus*. Conversely, glucose metabolism-related commensals, including *Akkermansia*, *Faecalibacterium*, *Bacteroides*, *Fusobacterium*, and *Bifidobacterium*, were significantly reduced. Using a humanized gut microbiota-associated T2DM-ICT mouse model (HMA-T2DM-ICT), intervention with the defined MicroAFB consortium (*Akkermansia*, *Faecalibacterium*, *Bacteroides*) was tested. MicroAFB intervention significantly improved postoperative antibiotic/medication-induced deficiencies in insulin (INS) and C-peptide (C-P) secretion and elevated glycated hemoglobin (HbA1c). It also significantly reduced serum levels of inflammation markers (IP-10, MCP-1) and upregulated intestinal barrier tight junction proteins (MUC2, Occludin, ZO-1). Importantly, microbiota-derived metabolites (bile acids, vitamins B/K) were not only involved in the improvement of glucose homeostasis but also significantly correlated with key biomarkers, including INS, C-P, HbA1c, and GLP-1. Mechanistically, MicroAFB activated insulin signaling via the IRS/PI3K/AKT and GSK-3 β /FOXO1 pathways and regulated metabolites such as bile acids and B vitamins/vitamin K, thus suppressing gluconeogenesis and ultimately enhancing insulin sensitivity and glucose homeostasis. The gut microbiota-derived bile acid 3-sulfodeoxycholic acid restored host homeostasis by rebalancing the gut microbiota and activating the TGR5 receptor and its downstream PI3K/AKT/GSK3 β pathway. This work pioneered microbial consortium therapy to rectify post-ICT dysbiosis, directly linking microbiota restoration to improved islet function for durable diabetes remission.

Keywords: Islet cell transplantation; Islet function; Gut microbiota; Gut barrier; Gut metabolites

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1. Introduction

Type 2 diabetes mellitus (T2DM) poses a significant global health challenge due to its high prevalence, severe complications, and substantial costs. Projections indicate T2DM cases will reach 783 million by 2045 [1,2]. Despite significant progress in pharmacological interventions, including the development of insulin analogs, GLP-1 receptor agonists, and SGLT2 inhibitors, patients with end-stage T2DM, marked by irreversible β -cell failure, remain at high risk. They often experience life-threatening glycemic variability, including unpredictable hypoglycemic episodes and sudden death. Existing therapeutic approaches are insufficient for effectively mitigating these critical glucose fluctuations [3,4]. Islet cell transplantation (ICT) provides an advanced and minimally invasive treatment. Islet cells, isolated from allogeneic or autologous pancreatic, are purified and infused into the hepatic portal vein. After transplantation, the engrafted islet cells resume physiological insulin secretion. This process is crucial for maintaining glucose control and reducing the incidence of hypoglycemic events. Notably, a proportion of patients can achieve sustained insulin independence following ICT. This eliminates the need for exogenous insulin administration and significantly improves their overall quality of life [5]. Clinical trials have shown that ICT significantly reduces hypoglycemic events in 80% of patients with brittle diabetes, with 50%-70% achieving sustained insulin independence within a five-year postoperative period [6].

However, challenges persist, including transplant rejection, islet loss, and immunosuppressant adverse reactions, limiting ICT success and long-term efficacy. In addition, post-transplantation complications, including graft dysfunction, immune rejection, and blood glucose fluctuations, are potentially linked to the intestinal dysbiosis caused by prolonged immunosuppressant and antibiotic use [7]. However, the mechanisms by which these drugs induce gut microbiota dysbiosis, alter microbial metabolic profiles, and impair islet function recovery and glycemic balance, remain unclear. This lack of clarity hinders the development of effective prevention and intervention strategies for gut microbial dysregulation in patients after ICT surgery.

Currently, immunosuppressive drugs, such as mycophenolic acid, tacrolimus, and cyclosporine A, are essential for treating post-ICT patients. Substantial evidence shows that these medications significantly reshape the gut microbiota structure and indirectly influence transplant outcomes by modulating the host immune system. Diabetic research has shown that tacrolimus induces hyperglycemia in mice by reducing the butyrate-producing bacterial abundance in the gut [8]. Immunosuppressive agents, such as tacrolimus, glucocorticoids, and mycophenolate mofetil, can induce gut microbiota dysbiosis. This condition is characterized by an increase in potentially pathogenic bacteria such as *Proteobacteria* and *Streptococcus*, and a decrease in beneficial taxa, including *Roseburia*, *Lactobacillus*, and *Verrucomicrobiae* [9,10]. Therefore, developing effective dietary and pharmaceutical intervention strategies are essential for preventing and reshaping the disrupted gut microbiota ecology and metabolic functions induced by immunosuppressive agents during ICT.

Gut microbiota dysbiosis is firmly linked to insulin resistance, inflammation, and metabolic dysfunction. For instance, *Parabacteroides distasonis* from T2DM patients mechanistically ameliorate insulin resistance via the nicotinic acid biosynthesis-mediated activation of gut GPR109a receptors, which enhances intestinal barrier integrity and restores metabolic homeostasis ^[11]. Additionally, *Alistipes indistinctus* can reduce host inflammation and lipid accumulation by consuming monosaccharides in the gut, which significantly improves insulin resistance ^[12]. Therefore, it is hypothesized that alterations in the gut microbiota composition, particularly the core functional microbes or microbial communities, may influence ICT efficacy and the duration of insulin independence. These modifications may represent potential targets for improving islet function after ICT.

This study aimed to elucidate the mechanistic links between gut dysbiosis, gut barrier impairment, and pancreatic β -cell glucose metabolism in post-ICT T2DM patients. It examined the therapeutic potential of the MicroAFB consortium, comprising *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, and *Bacteroides fragilis*, in ameliorating postoperative inflammation, maintaining intestinal homeostasis, and restoring β -cell functionality, thereby clarifying how glucose metabolism-associated gut microbiota orchestrate functional pancreatic β -cell restitution. These findings established microbiota-targeted strategies to enhance glycemic control post-ICT and inform patient stratification paradigms.

2. Materials and methods

2.1 Subject recruitment and clinical sample collection

This study enrolled 46 volunteers aged 18-75 years old, including 16 patients who underwent ICT and 30 patients with T2DM who served as controls. Among the 46 volunteers recruited, 33 were male and 15 were female. All participants were recruited from the department of endocrinology and the department of organ transplant center at Shanghai Changzheng hospital and provided informed written consent. The study was conducted in strict accordance with the principles outlined in the Declaration of Helsinki and complied with the ethical standards approved by the institutional review board (Ethics Approval Number: ECUST-2022-104), and the privacy rights of human subjects must always be observed.

Stringent inclusion and exclusion criteria ensured cohort robustness and representativeness. Enrollment of the patients was contingent upon compliance with the WHO diagnostic criteria for T2DM, which included a fasting blood glucose (FBG) level ≥ 7 mmol L⁻¹, glycated hemoglobin (HbA1c) $\geq 6.5\%$, a blood glucose level ≥ 11.1 mmol L⁻¹ 2 h after the oral glucose tolerance test (OGTT), or a random blood glucose level ≥ 11.1 mmol L⁻¹ in the presence of diabetic symptoms. Additional inclusion criteria required the T2DM patients who underwent ICT (1) to be within three months post-ICT, (2) to be aged between 18 and 75 years, (3) to receive ongoing immunosuppressive therapy, (4) to be INS dependent, and (5) to have suboptimal glycemic control (HbA1c $> 8\%$ or time in range [TIR] $< 70\%$). The exclusion criteria included (1) recent use of probiotics, prebiotics, or specialized dietary regimens (e.g., yogurt consumption), (2) failure to provide scheduled biospecimens, (3) active viral hepatitis B/C infection, hepatic cirrhosis, cholecystitis ^[13], or cholelithiasis, pregnancy, lactation, or menstruation in female participants, and (4) inflammatory

bowel disease or other gastrointestinal pathologies. The multi-time point sampling of the ICT group was conducted at 1 week, 1 month, and 3 months after transplantation. Finally, a total of 34 fecal samples from ICT participants and 29 samples from T2DM control subjects met the quality control criteria, and were included in the study as the discovery set. Eight T1DM samples and seven ICTV samples were used as the validation set for supplementary research. Supplementary Table S2 presented the comprehensive clinical metadata.

2.2 Microbiota analysis

Here, 0.5 g fecal samples from the post-ICT and T2DM cohorts were homogenized in 2 mL microcentrifuge tubes. Genomic DNA was extracted using the PowerSoil® DNA Isolation kit (MO BIO, Carlsbad, USA), followed by quantitative assessment and quality control (QC) via spectrophotometric analysis. Full-length bacterial 16S rDNA was amplified using the 27F (5'-AGRGTTTGATYNTGGCTCAG-3') and 1492R (5'-TASGGHTACCTTGTTASGACTT-3') universal primers, with the PCR conditions strictly adhering to the manufacturer specifications. PCR amplicons were purified with VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified via the Qubit dsDNA HS Assay Kit and Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Oregon, USA). Amplicons were normalized to equimolar concentrations, pooled and sequenced on the PacBio Sequel II platform (Beijing Biomarker Technologies Co., Ltd., Beijing, China)^[14]. Original subreads were calibrated to generate CCS sequences (SMRT Link, v8.0), and Lima (v1.7.0) software was used to identify sample-specific CCS sequences by barcodes and remove chimeras for high-quality CCS sequences^[15]. Taxonomic annotation was performed against the SILVA 16S rRNA reference database (Release 132; <http://www.arb-silva.de>)^[16], and bioinformatics analyses were conducted on the Omic Studio cloud platform (LC-Bio, Hangzhou, China). Detailed methodologies are available in Supplementary Materials 1.1.

2.3 Fecal metabolomics analysis

Fecal samples (60 mg) were precisely weighed and homogenized in 600 µL methanol-acetonitrile (1:1, v/v, 4 µg mL⁻¹ L-2-chlorophenylalanine). After 10 min ultrasonic extraction and centrifugation (12,000 ×g, 4 °C, 10 min), the supernatant was lyophilized. The residue was reconstituted in 400 µL methanol-water (1:1, v/v), sonicated for 5 min, and centrifuged again (12,000 ×g, 4 °C, 10 min). The final supernatant was collected for analysis. QC samples were prepared by mixing equal volumes of all sample extracts to ensure analytical reproducibility^[17].

Fecal metabolites were analyzed using a Shimadzu Nexera UHPLC LC-30A system (Shimadzu Corporation, Kyoto, Japan) coupled with a Q-Exactive quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). MS data were acquired in both positive and negative ion modes over the range of 200-1200 m/z at a resolution of 70,000 (at m/z 200)^[18]. Raw MS data were cross-referenced against HMDB and LIPID MAPS databases for compound identification, followed by qualitative analysis and peak area normalization to generate a metabolite abundance matrix. MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca>) and OmicStudio (<https://www.omicstudio.cn/tool>) were applied for

multivariate statistical analyses (PLS-DA, OPLS-DA) to delineate metabolic patterns among experimental cohorts. Volcano plots for metabolite screening were generated using R v4.0.3 (ggplot2 v3.3.3).

2.4 Animal models and experimental design

The ARRIVE guidelines 2.0 were followed for the experimental protocols, and all animal treatments and experiments were strictly conducted in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The procedures were approved by the East China University of Science and Technology Bioethics Committee (Ethics Approval number: ECUST-2022-104; Shanghai, China). Six-week-old male C57BL/6J mice weighing 16–20 g (Qualification NO.: 20230004006021) were obtained from Shanghai Jiesijie Experimental Animal Co., Ltd. and raised in specific pathogen-free (SPF) conditions, with the temperature maintained at 22 °C, a 12-hour light/dark cycle and humidity levels of 55%–60%.

After one-week acclimatization, mice were randomly divided into two groups: blank control (CON, $n = 6$) and type 2 diabetes (T2DM, $n = 24$). Fig. 1 showed the experimental design. The CON group received a standard chow diet and sterile water. The T2DM group received a 60% high-fat diet (D12492, Research Diets, New Brunswick, USA) and a single intraperitoneal injection of streptozotocin (STZ) (Sigma, Missouri, USA) at a dose of 110 mg kg^{-1} , dissolved in 0.1 M citrate buffer (pH 4.5) (Servicebio, Wuhan, China). The random blood glucose levels were measured at 3 days and 7 days after the STZ injection. Sustained hyperglycemia $\geq 16.7 \text{ mmol L}^{-1}$ confirmed the successful establishment of the diabetic model^[19]. Male mice were selected for the STZ-induced diabetic model due to their heightened sensitivity to pancreatic islet cytotoxins compared to their female counterparts^[20].

Following rigorous humanized microbiota transplantation, mice were administered a combined antibiotic cocktail via drinking water to deplete gut microbiota. The sterile aqueous antibiotic solution containing 1 g L^{-1} ampicillin, 1 g L^{-1} metronidazole, 1 g L^{-1} neomycin sulfate, and 0.5 g L^{-1} vancomycin was prepared in autoclaved drinking water and filtered through a $0.22 \mu\text{m}$ membrane for sterilization and complete microbial exclusion^[13,21]. To establish pseudo-sterile mouse models, antibiotic water was administered for three weeks and replaced twice weekly. Fresh fecal samples were collected in sterile centrifuge tubes, serially diluted with phosphate-buffered saline (PBS, Servicebio, Wuhan, China), and inoculated onto Columbia agar plates. After 18 h to 24 h of incubation, the bacterial growth was examined to determine the fecal bacterial concentration. A 90% reduction in the viable fecal bacteria confirmed the successful establishment of the antibiotic-treated mouse model^[22,23]. Human microbiota-associated mouse models were established via the oral gavage of the fecal bacterial suspensions from the T2DM patients at a volume of $100 \mu\text{L}$, with an OD_{600} of approximately 1. Each mouse received gut microbiota from a single T2DM patient. This procedure was repeated every two weeks, with three consecutive daily administrations per cycle, for a total of four cycles^[24].

HMA-T2DM mice ($n = 6/\text{group}$, Fig. 1) were divided into various groups. Group (a) HMA received 0.1 mL solvent via oral gavage every other day. Group (b) SHAME underwent ICT surgery with Hank's solution as the graft substitute and received 0.1 mL solvent (without rapamycin) every other day. Group (c) ICT received 5 mg kg^{-1} postoperative rapamycin (RAPA) via oral gavage every other day^[25,26]. Group (d)

ICT+MicroAFB received 5 mg kg⁻¹ postoperative RAPA and 2×10⁸ CFU/0.1 mL MicroAFB consortium via oral gavage every other day [27–29]. Groups (c) and (d) underwent ICT surgical intervention. After experimental intervention and a 12 h fast for serum isolation, blood was collected from the retro-orbital venous plexus. The mice were then euthanized via cervical dislocation, after which the liver, kidneys, pancreas, and colon were collected for analysis.

Stringent control of the cage effect (co-housing controls, randomized caging, and housing three genotype-matched mice per cage per experimental group) is essential in experimental design [30]. This practice guards against the conflation of cage-driven microbial variations with genotypic or treatment outcomes and necessitates a critical interpretation of human microbiota-associated (HMA) animal model data [31].

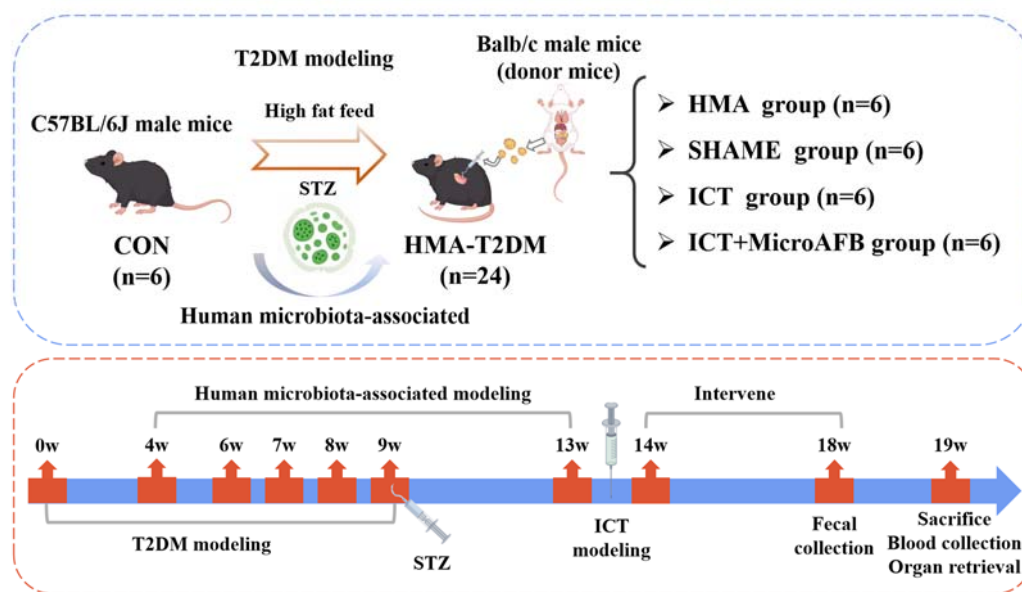


Fig. 1 Establishment of the HMA-T2DM mouse model and experimental group design. C57BL/6J mice were randomly divided into two groups: a blank control group (CON group, n = 6) and a type 2 diabetes group (T2DM group, n = 24). The T2DM group was transformed into the HMA-T2DM group after humanized microbiota modeling. The HMA-T2DM mice (n = 6/group) were divided into various groups. After intervention, fecal, blood, and tissue samples were collected from mice.

2.5 MicroAFB consortium culture and treatments

Faecalibacterium prausnitzii (DSM 107838), *Akkermansia muciniphila* (ATCC BAA-835) and *Bacteroides fragilis* (ATCC 25285), purchased from Mingzhou Biotechnology Co., Ltd (Ningbo, China), were anaerobically cultured at 37 °C for 48–72 h. *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* were cultivated in CMC medium, while *Bacteroides fragilis* was cultured in GAM medium [32]. The workstation maintained constant conditions at 37 °C with 75% humidity and an atmospheric composition of 80% CO₂, 10% N₂, and 10% H₂. The bacterial stock solutions were centrifuged at 6,000 ×g for 5 min, after which the supernatant was removed, and the pellets were resuspended in sterile PBS until reaching a concentration of 2×10⁸ CFU mL⁻¹. The MicroAFB consortium was formulated according to the absolute quantitative ratio of three bacterial species in healthy populations: *Akkermansia muciniphila*: *Faecalibacterium prausnitzii*: *Bacteroides fragilis* = 2:4:1 [18,33]. Aliquots of the diluted bacterial suspension

were aseptically transferred into sterilized centrifuge tubes, sealed, and administered to the mice via oral gavage.

2.6 Animal modeling of the ICT

Male Balb/c mice (6-8 weeks old; Qualification NO.: 20240418Abzz0105000483) were used as islet donors. After euthanasia by cervical dislocation, the abdominal cavity was aseptically exposed following disinfection with 75% ethanol. Pre-chilled collagenase solution (2 mg mL⁻¹, 4 °C) was infused into the common bile duct. The distended pancreas was excised and digested at 37 °C for 10 min. After termination with HBSS buffer and washing, the pellet was filtered through a 35-μm mesh. Density gradient centrifugation was performed using Histopaque 1119, Histopaque 1077, and Hank's solution, and islets were collected manually [34].

The HMA-T2DM recipient mice were anesthetized with 1% pentobarbital sodium (50 mg/kg; Sigma, Missouri, USA). The transplantation site was shaved and disinfected. A 2-cm dermal incision and a 1-cm peritoneal incision were made to expose the left kidney. A 2-mm incision was created on the renal capsule using a needle. Approximately 100 μL of islet cells was slowly infused into the subcapsular space. Hemostasis was achieved by gentle compression with a cotton swab for 10 s. The peritoneum and skin were then sutured and closed with wound clips, respectively. Postoperatively, mice were kept under a heat lamp until full recovery from anesthesia [35].

2.7 Enzyme-linked immunosorbent assay (ELISA)

The mouse blood samples were centrifuged at 4 °C and 3,000 ×g for 15 min to obtain the serum. Then, the serum cytokine levels were measured, including INS (ml001983), C-P (ml063022), GLP-1 (ml201801), HbA1c (ml401824), monocyte chemoattractant protein-1 (MCP-1) (ml037840), and interferon-γ-inducible protein 10 (IP-10) (ml101834). To prepare the mouse pancreatic tissue samples, accurately weighed pancreatic tissue was homogenized at 4 °C and centrifuged at 3,000 ×g and 4 °C for 15 min. The supernatant was collected to determine the IRS (ml058259), protein kinase B (AKT) (ml058485), phosphoinositide 3-kinase (PI3K) (ml001929), forkhead box protein O1 (FOXO1) (ml037860), and glycogen synthase kinase 3β (GSK3β) (ml058082) levels [36]. The above reagent kits were purchased from Shanghai Enzyme linked Biotechnology Co., Ltd (Shanghai, China).

2.8 RNA extraction and quantitative real-time PCR

A TransZol Up Plus RNA Kit (Transgen, ER501, Beijing, China) was used for total RNA extraction from tissues and feces, after which the concentration and quality were assessed using a multimode microplate reader (Bio TeK, Vermont, USA). A TransScript All-in-One First Strand cDNA Synthesis Kit (Transgen, AT341, Beijing, China) was employed to reverse-transcribe the RNA to cDNA for qPCR. Real-time fluorescence PCR was performed using the AceQ qPCR SYBR Green Master Mix and the primers listed in Table S1. Primers for the target genes were designed using PrimerBank (<https://pga.mgh.harvard.edu/primerbank>). GAPDH (rodent tissues) and 16S (bacteria) [37-39] served as stable

reference genes. Relative mRNA expression was quantified by the $2^{-\Delta\Delta C_t}$ method with normalization to the corresponding reference gene.

2.9 Histopathological evaluation and immunofluorescence staining

Tissue specimens were fixed in 4% paraformaldehyde (Servicebio, Wuhan, China), dehydrated, and embedded in paraffin. After trimming, 4- μ m sections were prepared, flattened at 42 °C, and baked for 20 min. Pancreatic and colonic tissues were stained with hematoxylin and eosin (H&E) using standard protocols, and histopathological changes were observed under an optical microscope (Nikon, Tokyo, Japan). For immunofluorescence, dewaxed sections underwent antigen retrieval and were blocked with BSA for 30 min. After incubation with primary antibodies overnight at 4 °C, sections were incubated with corresponding secondary antibodies (INS: GB15336; Glucagon: GB113496; Mucin2: GB11344; Occludin: GB15149) for 50 min at room temperature. Nuclei were counterstained with DAPI for 10 min. All reagents were purchased from Sevier Biotechnology Co., Ltd (Wuhan, China). Images were captured using a confocal fluorescence microscope (Nikon, Tokyo, Japan) ^[40].

2.10 Molecular docking simulation

The molecular structure of 3-sulfodeoxycholic acid (3-SDCA) was obtained from PubChem, and the 3D structures of target proteins (AKT2, FOXO1, GSK3B, IRS1, PIK3C2G, TGR5) were acquired from the RCSB Protein Data Bank (www.rcsb.org). Protein structures were preprocessed in PyMOL v2.3.0 to remove water and original ligands, then prepared in AutoDockTools v1.5.6 by hydrogen addition, charge calculation and atom typing, and exported as pdbqt files. Molecular docking was performed with AutoDock Vina v1.1.2. The complexes were analyzed and visualized in PyMOL v2.3.0 to characterize molecular interactions.

2.11 Statistical analysis

All data were presented as the mean $\pm$ standard deviation (SD), with no fewer than five samples per group. The chi-square test was used to compare categorical variables between the groups, while Student's t-test was employed for pairwise comparisons. One-way ANOVA and Tukey's multiple comparisons test were utilized to compare multiple groups. The statistical analyses were performed using GraphPad Prism 10.0 (GraphPad Software, San Diego, CA, USA), and a $P < 0.05$ was considered statistically significant.

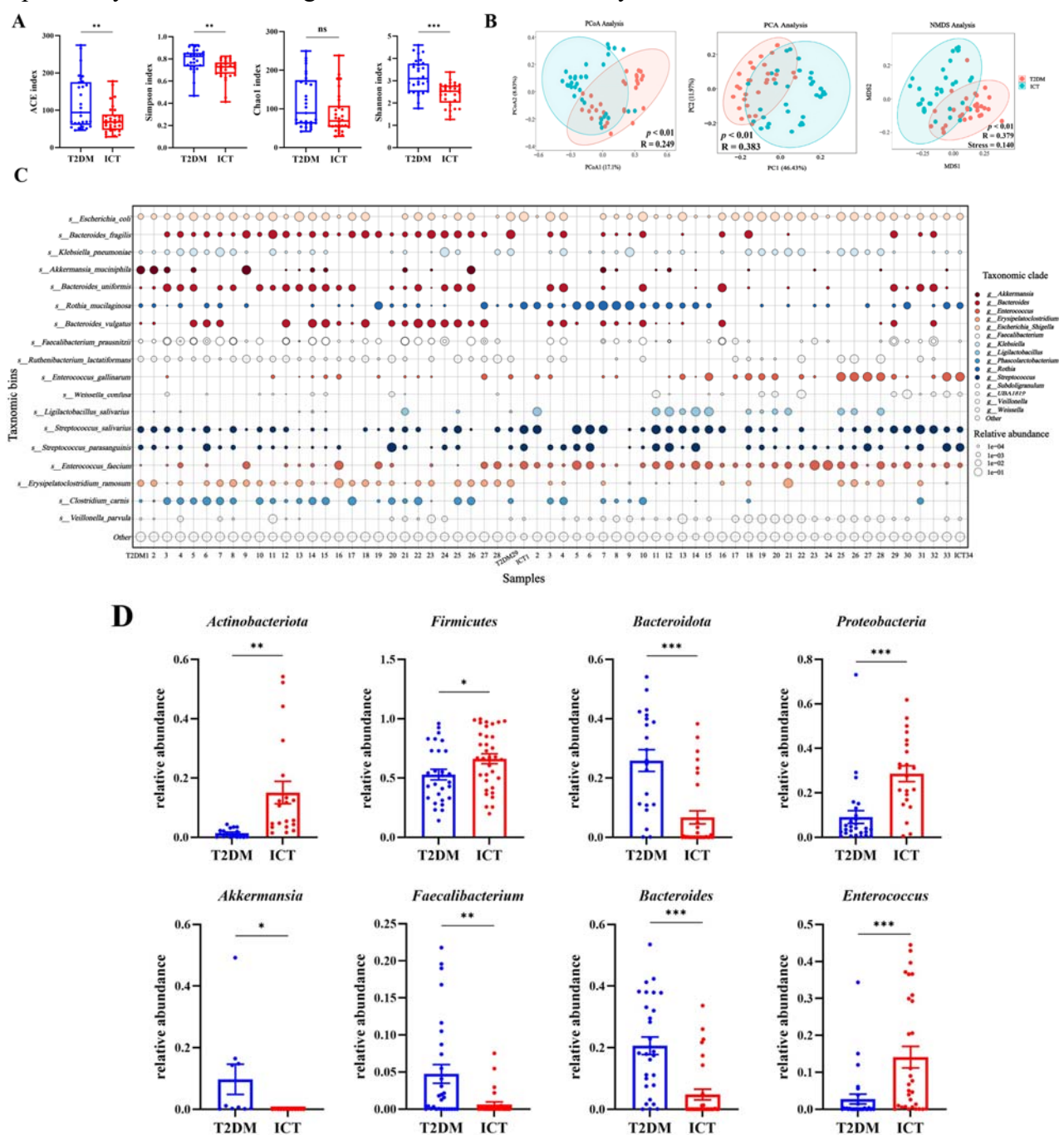
3. Results

3.1 ICT surgery significantly reduced gut bacterial diversity and richness

Comparative α -diversity analysis revealed significant differences between the microbial community richness and diversity in the pre- and post-transplantation states (Fig. 2A). The ICT cohort exhibited statistically significant reductions in the Simpson ($P < 0.01$), ACE ($P < 0.01$), and Shannon indices ($P < 0.001$), while Chao1 did not show significant changes compared to the T2DM group ($P > 0.05$). These findings indicated a decrease in species diversity and abundance of gut microbiota after ICT. Postoperative clinical monitoring demonstrated that the ICT recipients developed gastrointestinal complications, mainly

manifesting as constipation or diarrhea. Bristol Stool Scale assessments revealed predominant Type 6 stool characteristics (Table S2), suggesting underlying intestinal microecological dysbiosis in the transplant cohort.

Furthermore, β -diversity analysis was performed to examine the impact of ICT on the intestinal bacterial community structures in the patients. Both groups displayed dispersion in the PCoA plot with no significant differences (Fig. 2B). The T2DM and ICT groups exhibited clear gut microbiota clustering patterns in the PCA analysis plot, showing intra-group similarities and significant inter-group differences between the microbial community structures ($P < 0.05$). The NMDS plot demonstrated significant microbial community structure differences. The intestinal bacterial diversity and richness decreased after ICT, accompanied by substantial changes in the bacterial community structures.



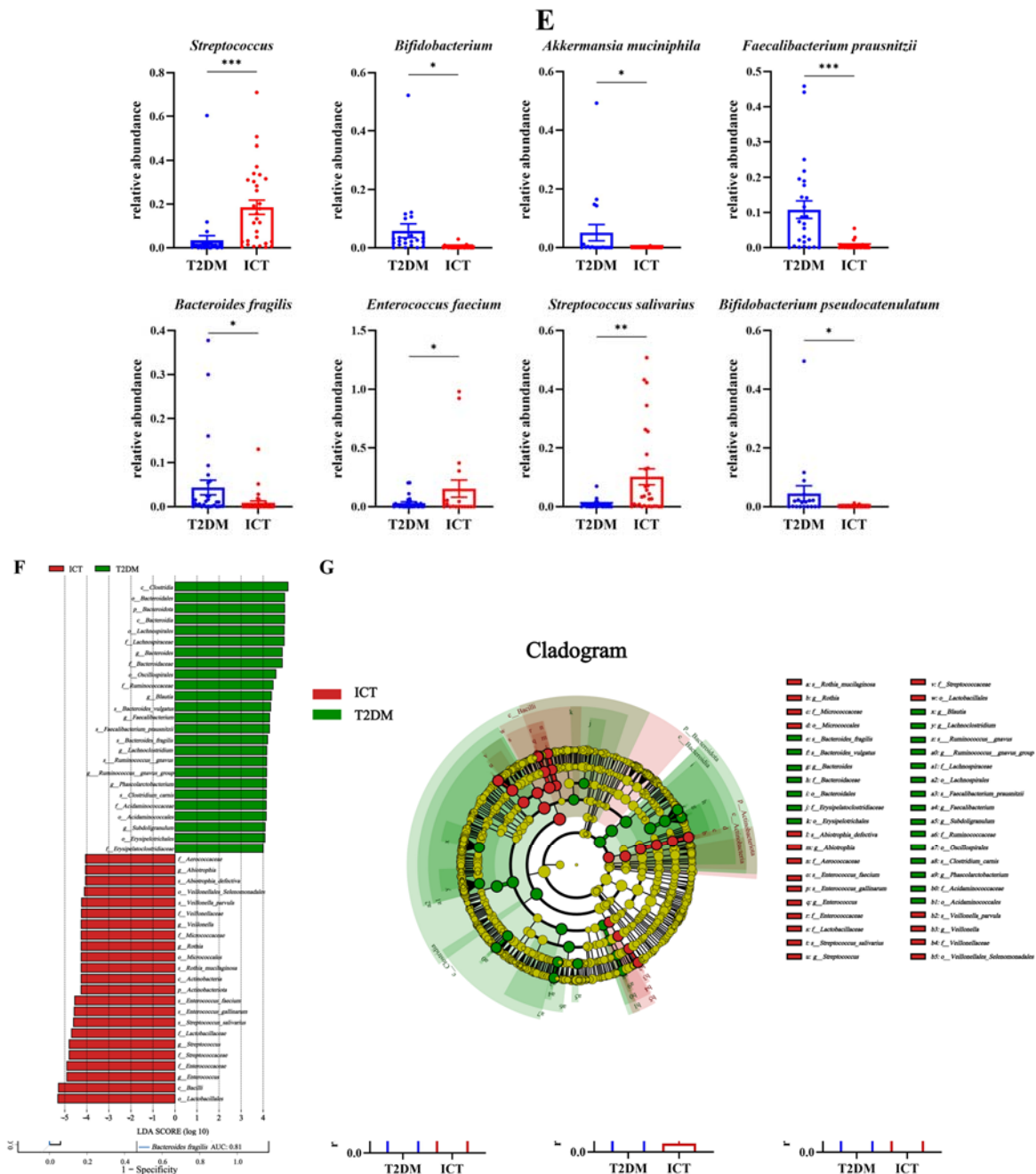


Fig. 2 The microbial composition and differential features between the T2DM and ICT groups. **A** The α -diversity analysis evaluated the diversity and richness of the microbial communities in the T2DM and ICT samples. **B** The β -diversity analysis assessed differences between the microbial structures of the T2DM and ICT samples. **C** The Microbial distribution OTU bubble plot of samples at the genus to species level. **D-E** The significance analysis of the gut bacteria. **F-G** Linear discriminant analysis effect size (LEfSe) was employed to identify the differential microorganisms between the T2DM and ICT groups, with LDA scores > 4 and $P < 0.05$ serving as threshold criteria for screening. **H** The ROC curve analysis of *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Bacteroides fragilis*. **I** The relative mRNA expression levels of *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Bacteroides fragilis* in the T2DM and ICT groups. The Simpson diversity index was calculated using the 1-D method. * Represents $P < 0.05$. ** Represents $P < 0.01$. *** Represents $P < 0.001$.

3.2 *Faecalibacterium*, *Akkermansia*, and *Bacteroides* were identified as core characteristic microbial communities associated with glucose metabolism after ICT

The fecal microbial compositions were analyzed at the phylum, genus, and species levels in the pre- and post-ICT populations (Figs. 2C, S11). As depicted in Fig. 2D-E, at the genus level, the relative abundance of *Akkermansia* (T2DM: ICT, 9.7%:0%; $P < 0.05$), *Bifidobacterium* (T2DM: ICT, 5.8%:0.3%; P

< 0.05), *Faecalibacterium* (T2DM: ICT, 10.8%:0.4%; $P < 0.01$), and *Bacteroides* (T2DM: ICT, 20%:4.7%; $P < 0.001$) decreased significantly in the ICT group, while that of *Streptococcus* (T2DM: ICT, 3.5%:19%) and *Enterococcus* (T2DM: ICT, 2.8%:14%; $P < 0.001$) increased substantially. At the species level, *Akkermansia muciniphila* (T2DM: ICT, 5.1%:0%; $P < 0.05$) was significantly lower in the ICT group than in the T2DM group, while *Streptococcus salivarius* (T2DM: ICT, 0.7%:10%; $P < 0.01$), *Faecalibacterium prausnitzii* (T2DM: ICT, 10.8%:0.5%; $P < 0.001$), *Bacteroides fragilis* (T2DM: ICT, 4.3%:0.9%; $P < 0.05$), *Enterococcus faecium* (T2DM: ICT, 15%:3.1%; $P < 0.05$), and *Bifidobacterium pseudocatenulatum* (T2DM: ICT, 4.5%:0%; $P < 0.05$) showed significant changes. The microbial community structure exhibited a substantial alteration after ICT, with markedly reduced *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, and *Bacteroides fragilis* abundance in the ICT group.

The LEfSe analysis was employed to analyze the differentially significant gut bacterial biomarkers between the T2DM and ICT groups. Fig. 2F-G presents the cladograms and LDA score distribution histograms spanning the phylum to the species levels. The microbial enrichment in the ICT group included *Rothia mucilaginosa* (associated with pulmonary infections) ^[41], *Micrococcaceae* (linked to cutaneous infections) ^[42], *Veillonella* (related to periodontitis and respiratory infections) ^[43], *Actinobacteriota* (associated with pulmonary infections) ^[44], *Streptococcus* (related to inflammation) ^[45], and *Enterococcus* (linked to urinary tract infections) ^[46]. The enriched microbiota in the T2DM group included *Bacteroidia*, *Ruminococcus*, *Faecalibacterium*, *Lachnospiraceae*, *Erysipelatoclostridiaceae*, and *Acidaminococcaceae*.

Analysis of the species composition distribution showed that the ICT group displayed significantly lower *Faecalibacterium prausnitzii* (associated with butyrate production) ^[32], *Bacteroides fragilis* (linked to reduced inflammation) ^[47], and *Ruminococcus gnavus* (which produces short-chain fatty acids (SCFAs)) levels than the T2DM group ^[48]. Conversely, the ICT group exhibited significantly increased abundance of *Streptococcus salivarius* (opportunistic pathogen) ^[49], *Enterococcus gallinarum* (associated with wound infections) ^[50], and *Veillonella parvula* (related to inflammation) ^[51]. These findings indicated that ICT reduced the SCFAs-producing bacteria and increased the inflammation-linked microbiota. The ROC curve analysis of these three bacterial species (Fig. 2H) yielded area under the curve values of 0.60, 0.72, and 0.81, respectively, indicating that they were accurately identified by the model as signature functional microorganisms that distinguished between the pre- and post-ICT states. These results demonstrated that ICT induced structural gut bacterial community reorganization and microecological imbalance. The functional bacterial mRNA expression in the ICT human fecal samples was validated via qPCR, revealing significantly lower relative *Akkermansia muciniphila* ($P < 0.05$), *Faecalibacterium prausnitzii* ($P < 0.001$), and *Bacteroides fragilis* ($P < 0.01$) levels than in the T2DM group (Fig. 2I). The findings also identified *Faecalibacterium*, *Akkermansia*, and *Bacteroides* as core functional microbial communities potentially critical for post-transplant glucose metabolic recovery.

The validation cohort comprised 8 fecal samples from individuals with type 1 diabetes (T1DM) and 7 samples from islet cell transplantation (ICTV) recipients. The 16S rRNA gene sequencing data are available

in Fig. S1 A-H. The results of the validation set were similar to those mentioned above (Supplementary Material 1.2).

3.3 The *MicroAFB* consortium improved glucose homeostasis in HMA-T2DM-ICT mice

The HMA-T2DM mouse model was established to simulate the human gut microbiota. Random blood glucose was measured two weeks after STZ injection, and levels exceeding 16.7 mmol L⁻¹ indicated successful modeling (Fig. 3A). The HMA group showed significantly higher FBG ($P < 0.01$) and random blood glucose ($P < 0.01$) than the CON group (Fig. 3B). OGTT revealed elevated blood glucose at all time points in the model group compared with controls. The HMA model group also had significantly higher AUC values ($P < 0.05$, Fig. 3C), confirming successful establishment of the diabetic model.

Following HMA model establishment and ICT surgery, body weight and blood glucose were monitored. As shown in Figs. 3D and 3E, the mice in the HMA group exhibited higher body weights than the other groups. The postoperative pancreatic islet to body weight ratio in the ICT group was significantly higher than in the HMA and SHAME groups ($P < 0.01$), which promoted functional recovery. The random blood glucose levels in the surgical mice were significantly lower than in the HMA model group ($P < 0.001$). At one-week post-ICT, the surgical group demonstrated a marked blood glucose decline, while no significant changes were evident in the HMA group ($P < 0.05$) (Fig. 3H). After four weeks, the AUC increased significantly in the HMA and SHAME groups compared to the CON group ($P < 0.001$). Additionally, the blood glucose levels in the surgical group were considerably lower than in the T2DM group ($P < 0.001$), indicating that ICT modeling effectively reversed T2DM-associated hyperglycemia.

The blood glucose fluctuations were evaluated using the variance (Var), standard deviation (SD), mean amplitude of glycemic excursions (MAGE), and coefficient of variation (CV). According to postoperative monitoring, compared to the CON group, although ICT reduced blood glucose, its Var, SD, and MAGE values were higher than those of other groups, indicating more severe fluctuations. The CV value of the ICT group reached 23.47%, showing the most significant relative fluctuations when considering average blood glucose levels. Although the blood glucose level of the ICT group fluctuated during the four postoperative weeks, it remained higher than normal levels.

The *MicroAFB* consortium intervention significantly elevated the pancreatic weight ratio in the ICT+*MicroAFB* group ($P < 0.05$), improving T2DM-induced pancreatic weight loss. During the four-week postoperative intervention, the ICT+*MicroAFB* group exhibited significantly lower blood glucose levels than the ICT group ($P < 0.05$), while postoperative hyperglycemia was markedly alleviated. This group also showed the lowest CV value (12.57%), indicating minimal relative glucose fluctuations (Figs. 3F, G). After four weeks of *MicroAFB* consortium intervention, the AUC values of postoperative mice were significantly lower than in the ICT group ($P < 0.05$), demonstrating improved islet function and enhanced glucose tolerance after microbiota modulation.

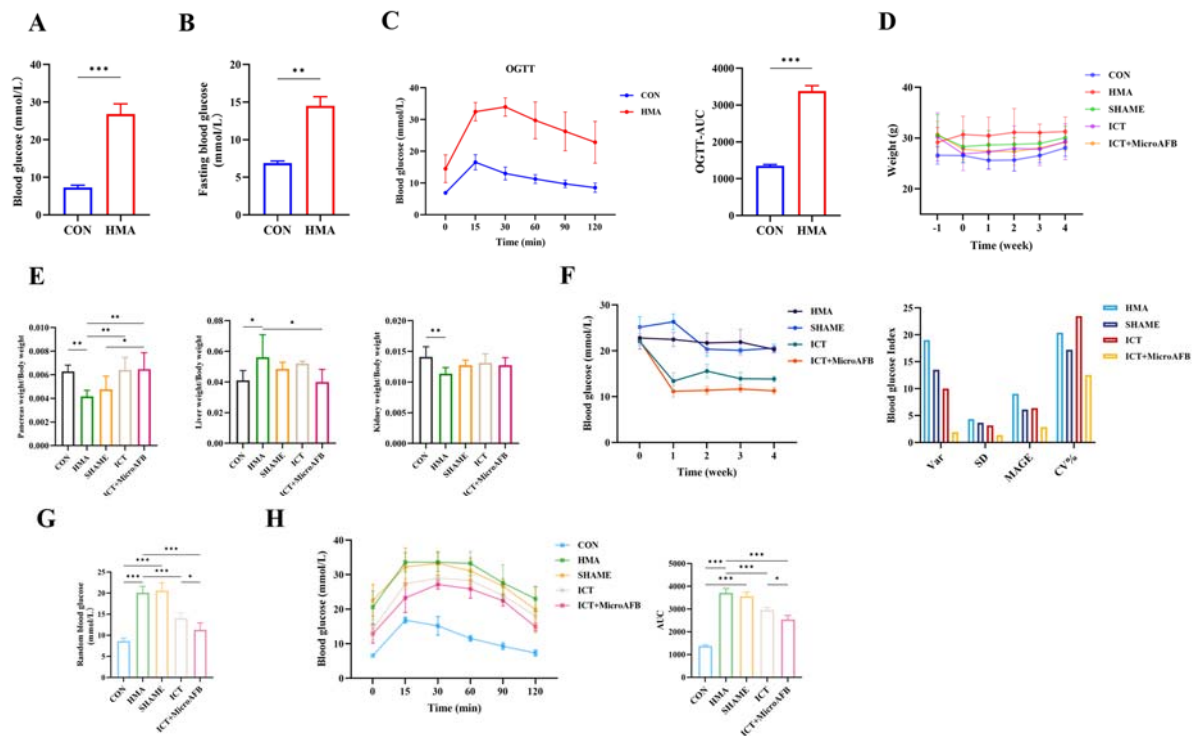


Fig. 3 The MicroAFB consortium improves glucose homeostasis after ICT. **A** The random blood glucose levels in the HMA-T2DM mouse models. **B** The FBG in the T2DM model. **C** The glucose tolerance test and AUC after T2DM modeling. **D** The weekly postoperative weight monitoring after ICT. **E** The ratios of the pancreas, kidneys, and liver to the body weight. **F** The weekly postoperative blood glucose monitoring and fluctuation parameter analysis after ICT. **G** The random blood glucose levels across groups after MicroAFB consortium intervention for four weeks. **H** The glucose tolerance test and AUC across the groups after MicroAFB consortium intervention for four weeks. * Represents $P < 0.05$. ** Represents $P < 0.01$. *** Represents $P < 0.001$.

3.4 The MicroAFB consortium improved islet function and inflammatory levels in the HMA-T2DM-ICT mice

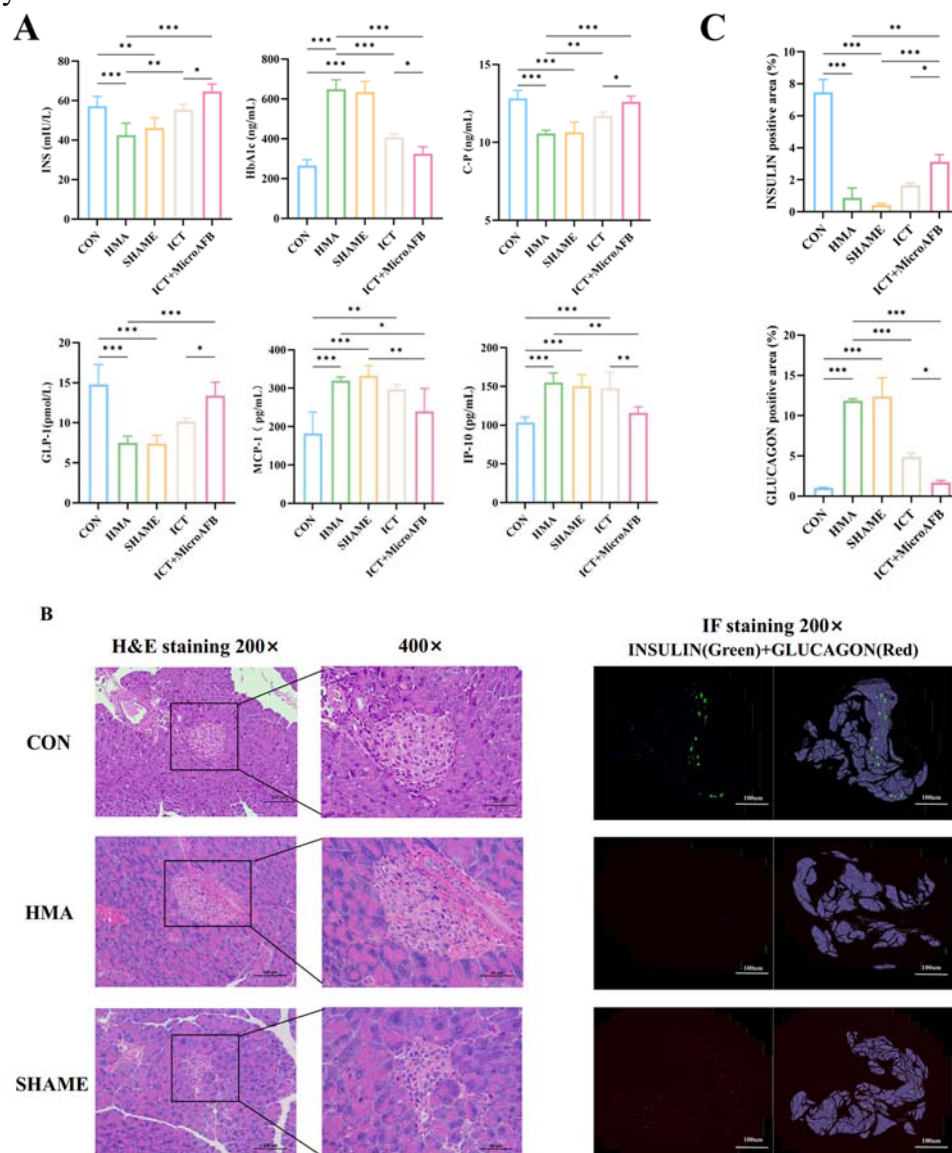
Serum levels of INS, C-P, HbA1c, GLP-1, and inflammatory cytokines (IP-10, MCP-1) were quantified to assess the impact of the MicroAFB consortium on islet function and inflammation (Figs. 4A, B). Compared to the CON group, the model group exhibited markedly reduced INS, C-P, and GLP-1 levels ($P < 0.01$), which increased significantly after ICT ($P < 0.05$), while the HbA1c levels displayed the opposite trend. ICT-induced systemic inflammatory responses, with the HMA, SHAME, and ICT groups demonstrating significantly elevated IP-10 and MCP-1 levels compared to the CON group ($P < 0.01$).

Compared to the ICT mice, the ICT+MicroAFB group exhibited significantly increased INS ($P < 0.05$) and C-P levels ($P < 0.05$), reduced HbA1c content ($P < 0.05$), and enhanced GLP-1 secretion ($P < 0.05$), indicating that MicroAFB consortium intervention effectively improved post-transplant islet function and glycemic regulation. Contrarily, the inflammatory cytokine levels in the ICT+MicroAFB group were significantly lower than in the ICT group ($P < 0.01$) after microbiota intervention. These results demonstrated that the MicroAFB consortium suppressed inflammatory factor production to alleviate postoperative inflammatory responses.

H&E staining was performed to evaluate pancreatic histopathology (Fig. 4B). The CON group showed intact pancreatic morphology with well-organized, compact islets. In contrast, the HMA and SHAME groups exhibited obvious pancreatic injury, including inflammatory infiltration, interstitial fibrosis, acinar disruption and stromal expansion. The SHAME group displayed severe lesions with disorganized islets,

partial fragmentation and marked atrophy, and islet injury was similar between the SHAME and HMA groups. Pancreatic histopathological damage was reversed in the ICT and MicroAFB intervention groups. Islets exhibited orderly arrangement and reduced vacuolation, indicating both treatments alleviated islet injury and preserved physiological function. ICT surgery exacerbated pancreatic inflammation and macrophage infiltration around islets in T2DM mice, suggesting inflammation-mediated β -cell damage, which was significantly attenuated by MicroAFB intervention.

IF staining for insulin (red) and glucagon (green) assessed islet cell function (Fig. 4B). HMA and SHAME groups showed markedly reduced insulin-positive β -cells and an elevated glucagon-positive α -cell ratio, indicating impaired β -cell function and compensatory α -cell activation, consistent with progressive islet dysfunction. ICT and ICT+MicroAFB groups exhibited significantly increased insulin secretion ($P < 0.01$). MicroAFB intervention significantly decreased glucagon levels ($P < 0.05$) and increased insulin levels ($P < 0.05$). These findings suggest that MicroAFB modulates islet α/β -cell proportions, elevates β -cell quantity, and improves islet function and glucose homeostasis. IF revealed that the MicroAFB consortium significantly restored β -cell function, reduced inflammatory infiltration, and alleviated STZ-induced pancreatic injury.



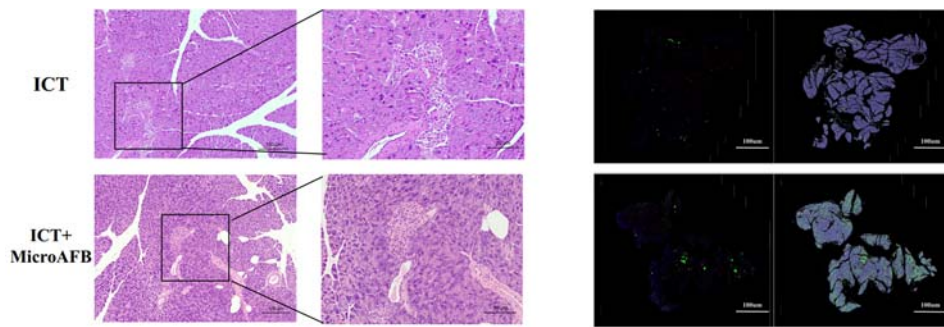


Fig. 4 The pancreatic islet function and inflammation in the model mice after four weeks of MicroAFB consortium intervention. A The serum levels of the inflammation-associated factors and islet function-related biomarkers in the mice. B The pancreatic H&E staining (scale bars: 100 μ m and 50 μ m). IF staining of insulin and glucagon in the pancreatic tissues (observed at 200 $\times$ magnification), with a quantitative area percentage of insulin across the treatment groups. C The quantitative area percentage of the glucagon. * Represents $P < 0.05$. ** Represents $P < 0.01$. *** Represents $P < 0.001$.

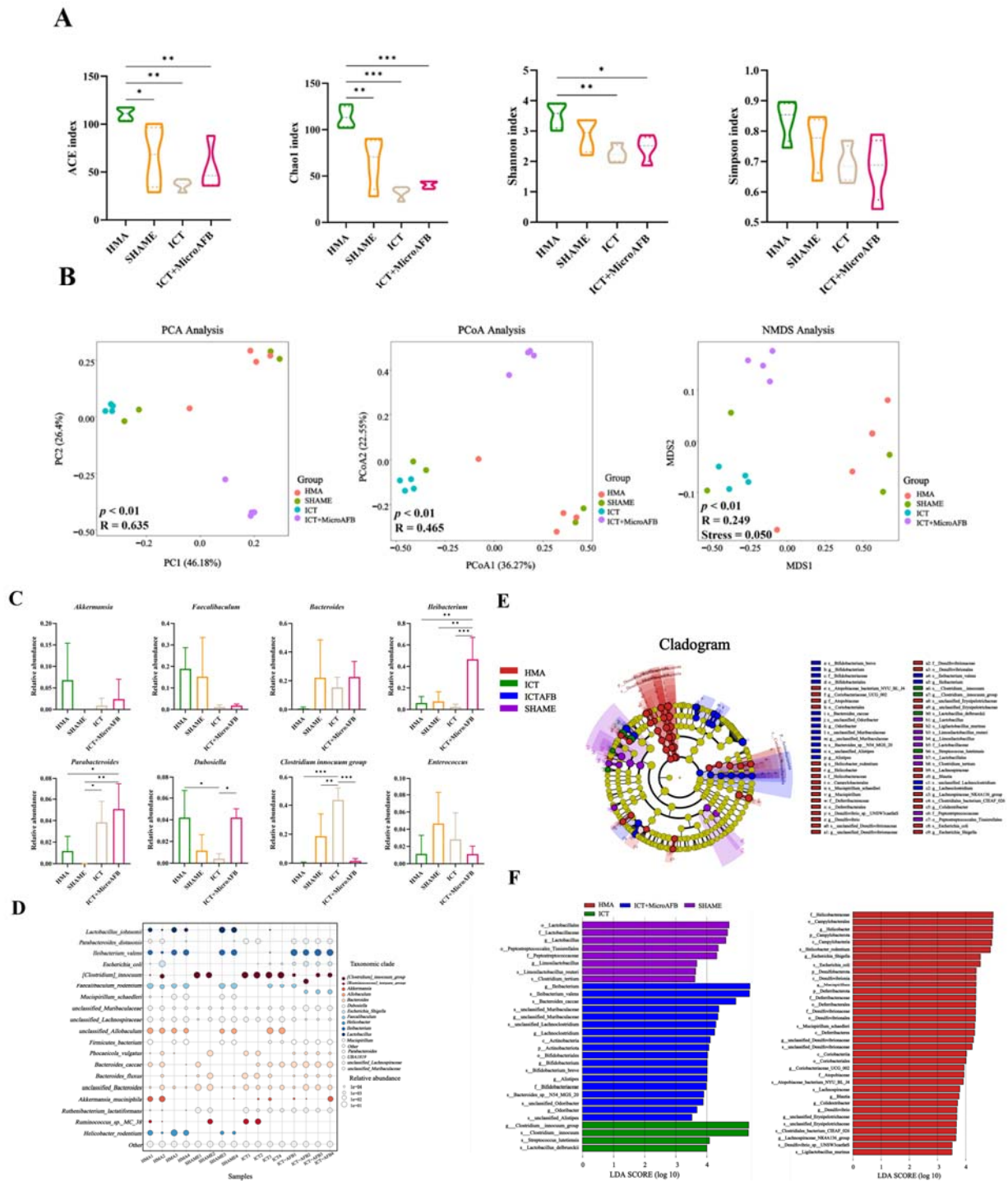
3.5 The MicroAFB consortium enhanced gut bacterial diversity and restored gut microbial homeostasis in HMA-T2DM-ICT mice

This study investigated the effects of ICT and the MicroAFB consortium on gut bacteria in HMA-T2DM mice. α -Diversity analysis demonstrated significantly lower ACE, Chao1 and Shannon indices in the SHAME and ICT groups ($P < 0.05$, Fig. 5A), which were notably increased by MicroAFB intervention ($P > 0.05$). β -Diversity (Fig. 5B) and NMDS, PCA, PCoA analyses demonstrated distinct microbial community structure differences among groups ($P < 0.05$). Compared with the HMA group, ICT reduced the relative abundance of *Faecalibaculum* and *Akkermansia* but elevated *Enterococcus* (Figs. 5C, D), suggesting decreased microbial diversity and potential postoperative infection. MicroAFB consortium treatment increased Chao1 and ACE indices, indicating gut microbiota restoration.

LEfSe analysis was employed to identify the differentially abundant bacterial taxa between the ICT and ICT+MicroAFB groups. As shown in Figs. 5E and 5F, the ICT group exhibited a significantly increased relative abundance of *Clostridium innocuum* group, *Streptococcus lutetiensis*, and *Lactobacillus delbrueckii* ($P < 0.05$). The ICT+MicroAFB group showed substantially elevated levels of *Bacteroidia*, *Ileibacterium*, *Actinobacteria*, *Lachnospiraceae*, *Muribaculaceae*, and *Bifidobacteriota* ($P < 0.05$).

Using the HMA group as control, Spearman correlation and Mantel tests analyzed associations between gut bacteria and disease indicators in the ICT and ICT+MicroAFB groups (Figs. 5G, H). Gut bacteria closely correlated with islet function markers (INS, C-P, GLP-1, HbA1c, GC), suggesting microbiota alterations affect post-ICT progression and recovery. *Faecalibaculum* significantly correlated with INS ($P < 0.01$, $r = 0.828$), C-P ($P < 0.01$, $r = 0.612$), GLP-1 ($P < 0.01$, $r = 0.599$) and HbA1c ($P < 0.05$, $r = 0.568$), linking it to islet dysfunction in post-ICT recovery. *Bacteroides* correlated with INS ($P < 0.05$, $r = 0.469$), HbA1c ($P < 0.05$, $r = 0.671$), C-P ($p < 0.05$, $r = 0.579$) and GLP-1 ($p < 0.05$, $r = 0.599$). Most enriched bacteria (*Clostridioides*, *Lactobacillus*, *Dubosiella*, *Lachnospiraceae*, *Enterococcus*) were strongly associated with C-P, MCP-1, INS and GC. *Ileibacterium*, *Bacteroides*, *Faecalibaculum*, *Allobaculum* and *Lachnospiraceae* *NK4A136* group dominated the ICT+MicroAFB group and were linked to inflammatory indicators, with *Ileibacterium* correlating significantly with MCP-1 and IP-10.

Functional gene profiles were predicted from 16S rRNA sequencing data using Tax4Fun (Figs. 5I, J). Compared with the HMA and SHAME groups, the ICT and ICT+MicroAFB groups showed enrichment in key metabolic pathways, including Metabolic pathways, Biosynthesis of secondary metabolites, and Glycolysis/Gluconeogenesis. MicroAFB intervention modulated the post-transplantation metabolic profile, affecting carbohydrate metabolism, amino acid homeostasis, and microbial functional adaptation. These findings provide a functional genomic basis for exploring the mechanism of MicroAFB consortium.



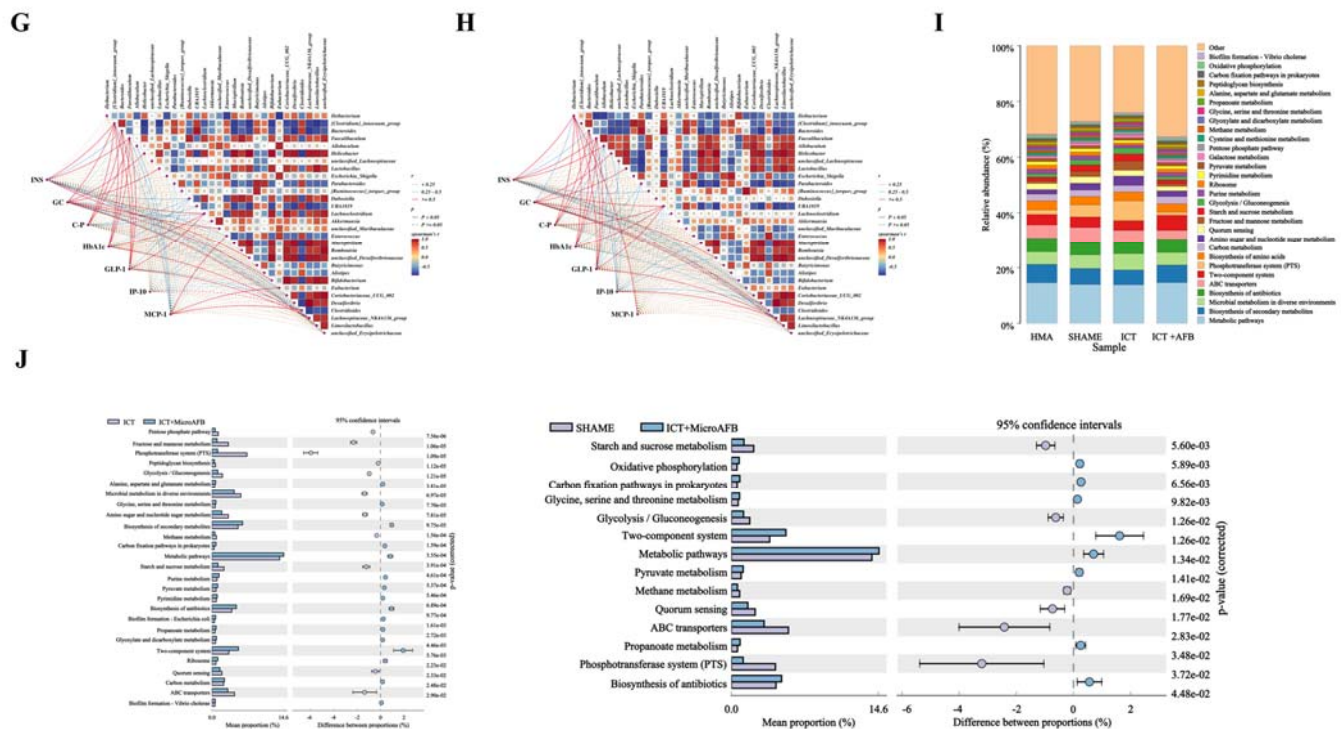


Fig. 5 The analysis of the gut bacterial communities in the different treatment groups and their correlations with disease indicators. A The α -diversity analysis of the gut bacterial communities in the different treatment groups. B The β -diversity analysis of the gut bacterial communities in the different treatment groups. C The significance analysis of gut bacteria at the genus level. D A bar plot comparing the distribution of the gut bacteria in the different treatment groups. E-F The LEfSe analysis of the differences between the gut bacterial community levels in the treatment groups. G The Spearman correlation and Mantel test analyses demonstrating the overall associations between differential microbiota and serum indicators in the HMA and ICT groups. H The overall association analysis between the differential microbiota and serum indicators in the HMA and ICT+MicroAFB groups. I Analysis of functional predictive composition via Tax4Fun between the four sample groups. J Differential analysis of Tax4Fun-predicted functions between the two sample groups. In the Mantel test results, $P < 0.05$ indicates significant differences, while r -values > 0.25 suggest the presence of correlations. The HMA group was regarded as the control group, and other groups were constructed with a humanized microbiota model. * Represents $P < 0.05$. ** Represents $P < 0.01$. *** Represents $P < 0.001$.

3.6 The MicroAFB consortium ameliorated intestinal metabolic homeostasis in the mouse models

Untargeted metabolomic profiling of fecal samples from intervention groups was conducted to elucidate gut microbial changes in ICT model mice. The PLS-DA and OPLS-DA analyses revealed statistically distinct metabolic profiles across treatment groups (Fig. 6A). The OPLS-DA model showed superior clustering performance and was used for differential metabolite screening and identification. Fecal metabolomic profiles differed distinctly among SHAME, HMA and ICT groups, and significantly between ICT and ICT+MicroAFB groups. These results indicate that coordinated changes in fecal metabolites and microbial function may critically determine islet functional recovery.

OPLS-DA was used to screen differential metabolite biomarkers. Secondary spectral analysis and manual curation identified 44 and 46 differential fecal metabolites in the ICT and ICT+MicroAFB groups, respectively (Figs. 6B-D). These metabolites mainly included lipids, fatty acids (mostly upregulated unsaturated FAs), carbohydrate derivatives, glycerophosphates, bile acids (predominantly secondary bile acids), tryptophan metabolites (indoles), organic acids and immunomodulatory compounds. ICT markedly altered mouse gut metabolites, mainly involving bile acid metabolism, xenobiotic metabolism, immunometabolic pathways, and vitamin B/K biosynthesis. Using model groups as controls, Mantel tests

and Spearman correlation analyses were performed between intestinal metabolites and bacterial communities in the ICT and ICT+MicroAFB groups (Fig. S2).

Combined with KEGG pathways from gut bacterial functional prediction, bile acid metabolites differed significantly across groups (Fig. 6E). Differential metabolite screening showed that 3-sulfodeoxycholic acid (3-SDCA) was markedly decreased after islet cell transplantation ($P < 0.001$) and elevated following MicroAFB intervention ($P > 0.05$). As a key sulfonated derivative of the secondary bile acid deoxycholic acid (DCA), 3-SDCA is produced via host hepatic metabolism and gut microbial synergy [52].

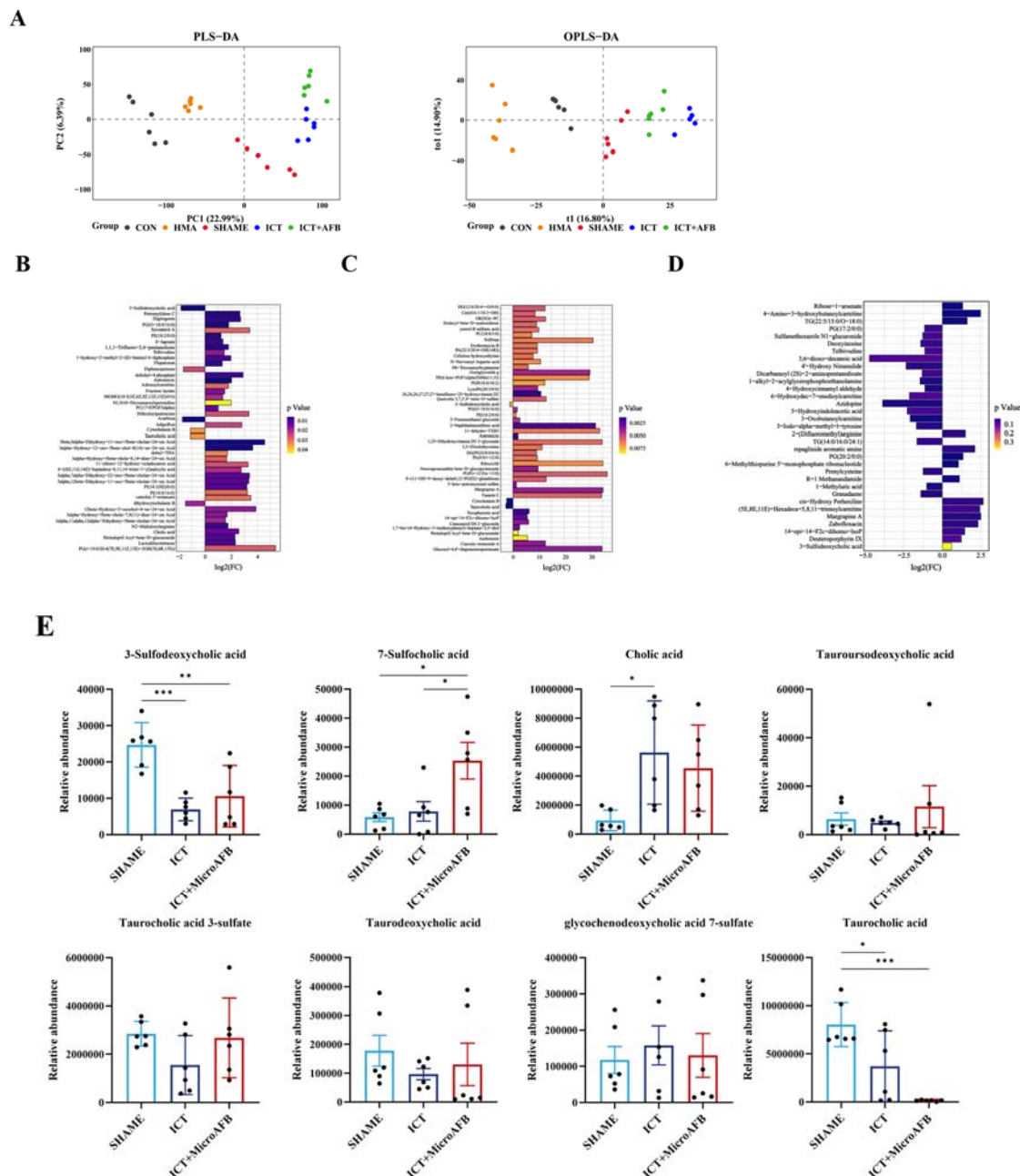


Fig. 6 The analysis of the differential metabolites, enriched pathways, and microbiota-metabolite correlations in the ICT mice treated with the MicroAFB consortium. A The clustering OPLS-DA of the untargeted fecal metabolomics in the experimental groups. B-D The FC value and p value of the differential metabolites in the intervention groups were analyzed by histogram, which were ordered as follows (left to right): ICT vs SHAME, ICT+MicroAFB vs SHAME, and ICT vs ICT+MicroAFB. E Relative abundance of bile acid metabolites in fecal metabolites of mice in different groups. The selection criteria included a fold change > 2 or < 0.5 , a VIP > 1 , and a $P < 0.05$. * Represents $P < 0.05$. ** Represents $P < 0.01$. *** Represents $P < 0.001$.

3.7 The MicroAFB consortium modulated hepatic glucose metabolism and inflammatory responses

To elucidate how the MicroAFB consortium improves glucose homeostasis, we assessed hepatic glycogen content and expression of genes related to glucose and lipid metabolism in mice across treatment groups. As shown in Figs. 7A and 7B, the mRNA expression levels of the key gluconeogenic enzymes glucose-6-phosphatase (*G6pase*) ($P > 0.05$), fructose-1,6-bisphosphatase (*Fbpase*) ($P < 0.05$), and phosphoenolpyruvate carboxykinase (*Pepck*) ($P < 0.05$) were significantly lower in the livers of the mice in the ICT+MicroAFB group than those in the HMA group. Compared with the SHAME group, ICT+MicroAFB significantly upregulated *Cyp7a1* expression ($P < 0.05$). Compared with the T2DM group, ICT and ICT+MicroAFB markedly increased hepatic *Glut2* mRNA expression ($P < 0.05$) and decreased hepatic *Tnf- α* and *Il-1 β* levels ($P > 0.05$). These results suggest that MicroAFB alleviates hyperglycemia and inflammation, promoting hepatic glucose metabolism and cholesterol excretion in diabetic mice.

Impaired insulin secretion or resistance disrupts insulin-mediated hepatic glycogen homeostasis, leading to hepatic glycogen depletion [53]. As shown in Fig. 7C, hepatic glycogen levels were markedly lower in the HMA and SHAME groups than in the CON group ($P < 0.001$). ICT and ICT+MicroAFB both significantly restored glycogen content versus the HMA group ($P < 0.001$), with ICT+MicroAFB showing a further increase ($P > 0.05$). These improvements were consistent with the ability of the MicroAFB consortium to enhance postoperative islet function, as reflected by the reduced OGTT glucose AUC.

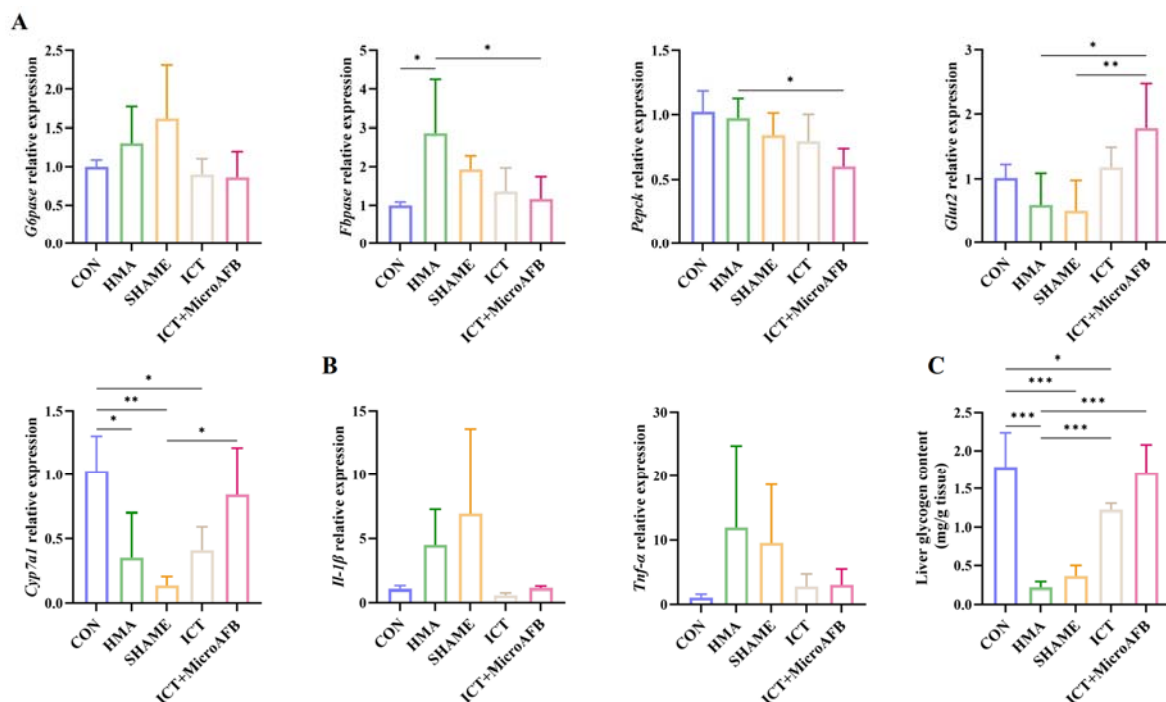


Fig. 7 The effect of the MicroAFB consortium on hepatic glucose metabolism and inflammation-related mRNA expression. **A** The expression of the gluconeogenesis-associated mRNAs and proteins in the mouse liver tissue. **B** The hepatic inflammatory cytokine mRNA expression profiles. **C** The quantification of the hepatic glycogen content. * Represents $P < 0.05$. ** Represents $P < 0.01$. *** Represents $P < 0.001$.

3.8 The MicroAFB consortium ameliorated islet function via the IRS/PI3K/AKT Islet signaling pathway

This study also examined pancreatic islet function and inflammatory gene expression in mice (Figs. 8A, B). The SHAME and HMA groups exhibited higher *Mcp-1* and *Il-1 β* ($P > 0.05$) expression levels than the

CON group, indicating progressive pancreatic inflammation in T2DM. Although the transplantation procedures decreased the inflammatory factor levels in the ICT group, they remained higher than in the CON group.

Glut2 expression was significantly lower in the HMA and SHAME groups than in the CON group ($P > 0.05$), suggesting impaired pancreatic β -cell function reduced *Glut2* expression and insulin (INS) secretion. Compared with the CON group, the T2DM model group showed significantly lower glucokinase (*Gck*) expression ($P < 0.05$). As a key glucose metabolism enzyme, reduced *Gck* indicates impaired β -cell glucose sensing and insulin secretion. Conversely, the ICT+MicroAFB group exhibited a substantial decrease in inflammatory factor expression ($P > 0.05$), which attenuated pancreatic inflammation. MicroAFB consortium intervention significantly increased *Gck* mRNA expression ($P > 0.05$). TGR5 is a G protein-coupled bile acid receptor pivotal for regulating glucose and energy metabolism [54]. The intervention of MicroAFB significantly increased the mRNA expression level in pancreatic tissue of HMA mice ($P < 0.05$).

ICT+MicroAFB significantly upregulated mRNA levels of *Irs* ($P > 0.05$), *PI3k* ($P > 0.05$) and *Akt* ($P < 0.001$), while markedly reducing *Foxo1* ($P < 0.001$) and *Gsk3 β* ($P > 0.05$) expression. The insulin signaling pathway was impaired in the HMA model, significantly reducing the *Akt* activity and *Foxo1* phosphorylation. Excessive *Foxo1* nuclear accumulation promotes gluconeogenesis-related gene expression (e.g., *G6pase* and *Pepck*), resulting in hyperglycemia, which is consistent with the results in this study. Aberrant *Foxo1* activation also promotes cell apoptosis and further impairs pancreatic β -cell function.

Fig. 8B further verified protein levels of insulin signaling pathways in pancreatic tissues. The ICT and ICT+MicroAFB groups exhibited substantially higher IRS ($P < 0.001$), AKT ($P < 0.05$), and PI3K ($P < 0.001$) protein levels than the HMA and SHAME groups, while the GSK3 β ($P < 0.001$) and FOXO1 ($P < 0.05$) protein levels displayed a significant decline. The MicroAFB consortium intervention enhanced blood glucose regulation, reduced gluconeogenesis, and promoted postoperative glycemic stability and islet functional recovery.

Molecular docking was performed to analyze intermolecular interactions between 3-SDCA and receptor proteins, and the simulation results were compared. Lower binding energy and more hydrogen bonds indicated more stable binding between 3-SDCA and the receptor proteins [55]. Molecular docking simulations were performed between 3-SDCA and each pathway receptor protein, and the specific results are shown in Fig. S3. For AKT2, the binding energy of 3-SDCA was -8.4 kcal/mol. 3-SDCA formed hydrogen bonds with TYR-231, LEU-215, TYR-475, and ARG-208 in the active pocket of AKT2, with hydrogen bond lengths of 2.2 Å, 2.7 Å, 2.4 Å, 2.4 Å, and 2.2 Å, respectively, as shown in Fig. 8C. These results verified that 3-SDCA binds tightly to the AKT2 active pocket and activates AKT2 kinase activity. 3-SDCA also targeted the ATP-binding pocket of GSK3 β , suppressed GSK3 β protein levels, and further regulated the insulin signaling pathway.

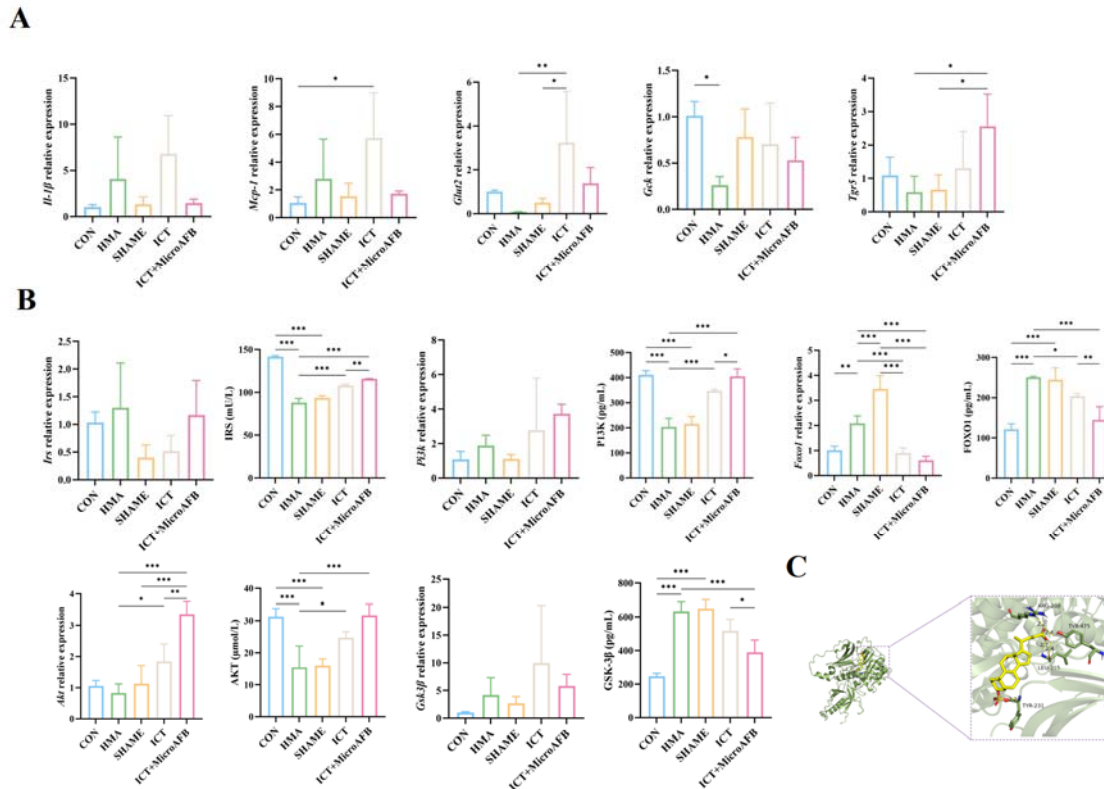


Fig. 8 The impact of the MicroAFB consortium on the pancreatic islet function and inflammation-related molecular profiles. **A** The statistical evaluation of the differential mRNA expression of the pancreatic inflammatory cytokine genes across the experimental cohorts. **B** The quantitative assessment of the pancreatic functional gene transcripts and associated signaling protein levels in the pancreatic tissue specimens from distinct intervention groups. **C** Schematic diagram of molecular docking between 3-SDCA and receptor protein AKT2. * Represents $P < 0.05$. ** Represents $P < 0.01$. *** Represents $P < 0.001$.

3.9 The MicroAFB consortium improved intestinal barrier homeostasis in the mouse models after ICT

H&E staining was used for histopathological evaluation of colonic tissues to assess intestinal injury and the effects of T2DM and ICT on intestinal structure and inflammation. The CON group exhibited long, intact colonic villi with orderly arrangement and abundant goblet cells in the mucosa (Fig. 9B). In contrast, the HMA and SHAME groups exhibited markedly shortened and discontinuous villi with submucosal edema, and the SHAME group showed obvious inflammatory cell infiltration. The ICT group presented severe inflammatory infiltration in the lamina propria, including lymphocytes, monocytes and neutrophils. Compared with the ICT group, the MicroAFB consortium intervention group showed reduced inflammatory infiltration, increased goblet cells and restored colonic villus morphology. These results demonstrated that the MicroAFB consortium protected the intestinal barrier function in mice after ICT.

As shown in Fig. 9A, the HMA and SHAME mice showed decreased mRNA levels of *ZO-1*, *Occludin* and *Muc2* ($P > 0.05$), indicating impaired intestinal barrier function in diabetic mice. MicroAFB intervention protected the colonic barrier by upregulating *ZO-1*, *Occludin*, and *Muc2* expression ($P < 0.05$), alleviating colonic pathological injury, epithelial damage and excessive inflammation. These effects restored tight junction integrity, normalized mucin layer synthesis and secretion, and repaired intestinal barrier function. This restoration prevented bacterial translocation, reduced harmful stimulation to pancreatic islets, and exerted pancreatic protective effects [56].

Further validation was performed by IF staining of MUC2 (red) and Occludin (green) in colonic tissues. The T2DM mice showed significantly decreased MUC2 and Occludin positive cell ratios ($P < 0.001$), indicating impaired intestinal barrier integrity (Figs. 9B, C). MicroAFB consortium intervention markedly elevated these ratios ($P < 0.05$), confirming improved barrier function, reduced inflammation, preserved permeability and enhanced epithelial tight junctions.

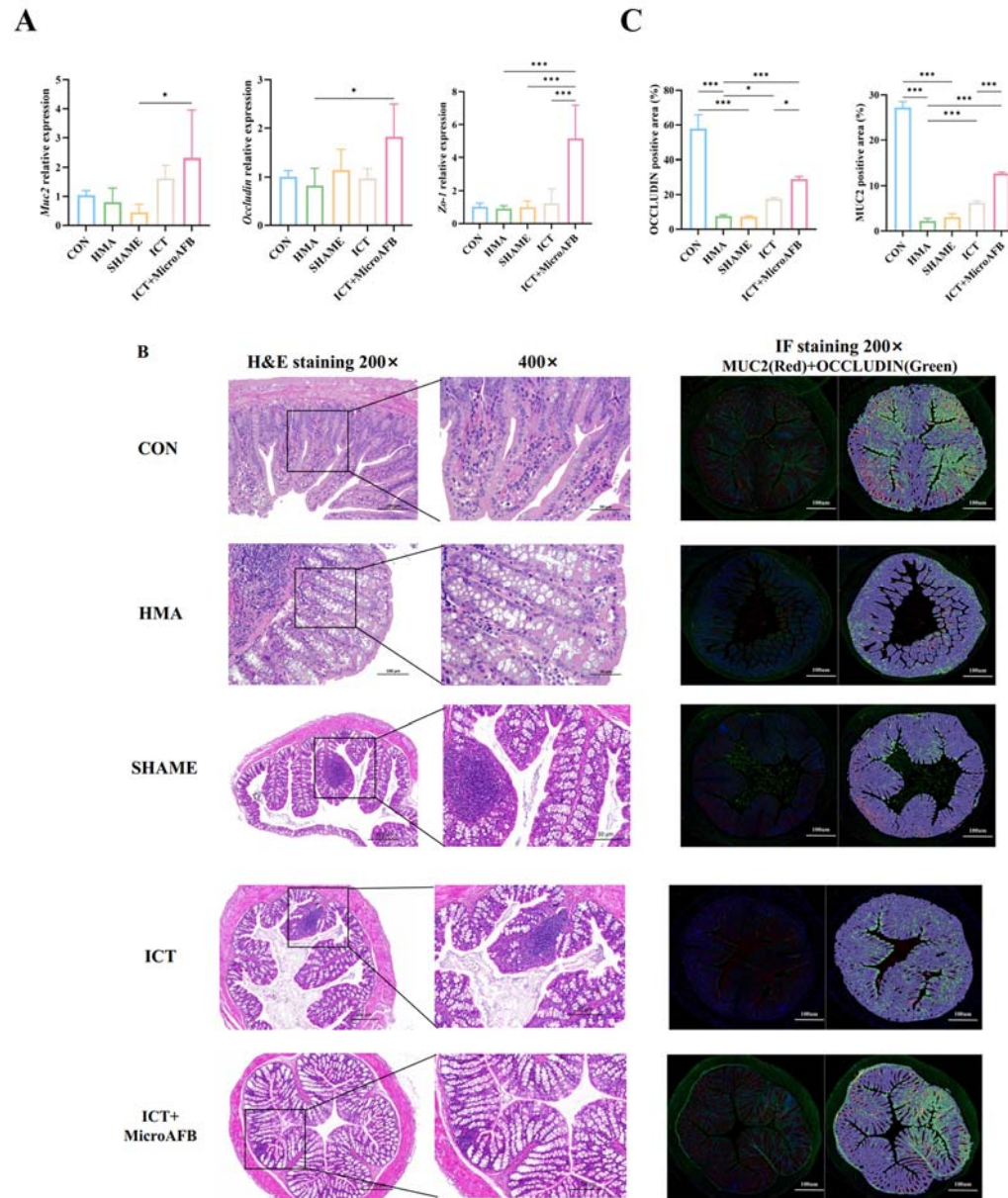


Fig. 9 The improvement of the colonic architecture and intestinal barrier in the ICT model mice by the MicroAFB consortium. **A** The expression of the intestinal barrier-associated genes in the colonic tissues of the treatment groups. **B** The representative H&E staining of the colonic sections from different treatment groups (scale bars: 100 μ m and 50 μ m) and the immunofluorescence staining of the MUC2 and Occludin in the colonic tissues (observed at 200 $\times$ magnification). **C** The quantitative area percentage of the MUC2 and Occludin in the colonic tissues of the intervention groups and treatment groups. * Represents $P < 0.05$. ** Represents $P < 0.01$. *** Represents $P < 0.001$.

4. Discussion

Post-ICT complications, including rejection, inflammatory responses, and opportunistic infections, are strongly linked to gut ecological dysregulation characterized by reduced microbial diversity and increased pathogenic taxa abundance. In hematological malignancies, higher baseline gut microbiome diversity

predicts better prognosis after hematopoietic stem cell transplantation (HSCT) [57,58]. Graft-versus-host disease after allogeneic HSCT has been mechanistically linked to both microbial dysbiosis and impaired bile acid metabolism [59]. Post-ICT antibiotic and immunosuppressive regimens, known drivers of gut dysbiosis, may impair the pharmacodynamics of immunosuppressive agents and exacerbate risks of rejection, infection, diarrhea and graft failure [10,60]. Antibiotic-induced ecological shifts affect beneficial taxa such as *Bifidobacterium* and *Lactobacillus*, while modulating host metabolism via microbiome-dependent pathways [61]. Liver transplantation studies further show that immunosuppressants and perioperative antibiotics reduce total microbial diversity. They promote the proliferation of *Enterobacteriaceae* and *Enterococcaceae* while depleting *Faecalibacterium prausnitzii* and *Bacteroides*, which are directly associated with postoperative morbidity and mortality [62]. These findings position targeted microbiome restoration as a critical determinant of post-ICT glycemic stabilization and recovery.

Previous investigations have revealed diminished gut fungal diversity after ICT, predominantly characterized by abnormal *Candida albicans* proliferation and islet inflammation [26]. Furthermore, gut-derived bacterial flagellin induces β -cell inflammation and dysfunction, which reduces insulin secretion [63]. Research has also shown that T2DM patients exhibit reduced levels of butyrate-producing taxa, including *Faecalibacterium*, *Clostridium*, and *Akkermansia* [64]. Post-ICT gut microbiota exhibit significantly reduced α -diversity, depletion of core functional taxa (*Faecalibacterium*, *Akkermansia*, *Bacteroides*) and enrichment of opportunistic pathogens (*Streptococcus*, *Enterococcus*), indicating that distinct microbial communities critically affect post-transplant graft survival. Loss of gut barrier integrity facilitates pathogen and toxin translocation to hepatic and pancreatic tissues through the portal vein, eliciting inflammatory and immune cascades [65]. Consequently, post-ICT microbial shifts promote enteric leakage into systemic circulation, exacerbating inflammation and glycemic instability. Microbiome-targeted interventions enhance beneficial metabolite production and mitigate dysbiosis [66].

Supplementation with characteristic functional gut microbiota substantially ameliorates diabetic neuropathy, gut barrier integrity, and systemic inflammation by enriching butyrogenic taxa in the gut ecosystem [67]. *Akkermansia muciniphila* effectively ameliorates STZ-induced hyperglycemia and muscle wasting in diabetic mice by increasing intestinal INS-like growth factor 2 secretion and activating skeletal muscle PI3K/Akt/mTOR signaling [29]. The transplantation of insulin sensitivity-associated bacteria, such as *Alistipes indistinctus*, reduces blood glucose and fecal monosaccharide levels, mitigates adiposity, and alleviates INS resistance [12]. This study hypothesizes that engraftment with the glycometabolism-associated core MicroAFB consortium can enhance graft fitness and survival, reduce transplant rejection, and promote islet function recovery. Specifically, *Faecalibaculum* and *Akkermansia* balance microbial consortia, producing SCFAs that attenuate systemic inflammation by reducing lipopolysaccharide permeation to improve insulin sensitivity and enhance microbial diversity. Consequently, the MicroAFB consortium was employed to mechanistically elucidate core microbiota modulation in post-ICT outcomes.

Targeted supplementation with the MicroAFB consortium effectively modulated the gut microbiota structure and homeostasis in a mouse model to ameliorate metabolic disorders and immune dysregulation. For instance, *Faecalibacterium prausnitzii* supplementation alleviates renal dysfunction and inflammation via the renal GPR43 axis and reduces serum uremic toxin levels in mice with chronic kidney disease [68]. The oral administration of *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* alleviates DNCB-induced atopic dermatitis symptoms [28]. Similarly, *Akkermansia muciniphila* supplementation attenuates high-fat diet-induced metabolic-associated fatty liver disease by regulating gut microbiota and bile acid metabolism [69]. Consistent with previous findings, the MicroAFB consortium intervention in ICT mice significantly improved glucose tolerance and islet function, evidenced by elevated C-P and INS levels, reduced HbA1c, and suppressed IP-10 and MCP-1 levels. Furthermore, it enhanced hepatic glucose metabolism by downregulating gluconeogenic enzymes, upregulating glucose transporters, and increasing hepatic glycogen storage.

The MicroAFB consortium intervention post-ICT significantly improved islet function, enhanced hepatic glucose absorption and utilization, and suppressed hepatic gluconeogenesis through IRS phosphorylation and subsequent PI3K/AKT pathway activation. AKT promoted glycogen synthesis via GSK-3 β phosphorylation and inhibition, while suppressing FOXO1 transcriptional activity. Previous studies have demonstrated that diosmetin [70], ceramides [71], and baicalin ameliorate glucose metabolic disorders and insulin resistance in diabetic mice by modulating the IRS/PI3K/AKT signaling pathways [72]. Therefore, the microbial modulation of the IRS/PI3K/AKT pathway by the MicroAFB consortium represented the principal mechanism underlying pancreatic functional enhancement. Additionally, ICT combined with the MicroAFB consortium improved intestinal barrier integrity and attenuated inflammatory responses. Analysis indicated that post-intervention reinforced tight junctions between the intestinal epithelial cells, ameliorated histoarchitectural disorganization, and reduced inflammatory infiltration.

Gut microbial metabolites play well-established roles in modulating host metabolic and immune functions. Both ICT and the MicroAFB consortium intervention induced structural microbiota alterations with targeted enrichment of specific metabolites. The results indicated that the accumulation of bile acids, vitamins B and K, SCFAs, and choline normalized the gut microenvironment. The MicroAFB consortium enhanced microbial diversity and systemically influenced host metabolism. Vitamin K2 supplementation activated intestinal bile acid receptors by elevating secondary bile acids, which increased the circulating GLP-1 levels and improved glycemic control and insulin sensitivity [73]. These findings suggested that the MicroAFB intervention facilitated the entry of gut bacteria and their metabolites (e.g., bile acids and derivatives) into systemic circulation via the hepatic portal vein, thereby enhancing glucose metabolic recovery in pancreatic islets and hepatic tissues.

Recent studies have demonstrated that secondary bile acids exert critical protective effects in metabolic diseases. As key signaling molecules, they activate nuclear receptors, including farnesoid X receptor and G protein-coupled bile acid receptor 1 (TGR5), thereby playing pivotal roles in regulating glucose metabolism,

energy balance and immune homeostasis. Specifically, TGR5 activation stimulates intestinal L cells to secrete GLP-1, and this process is essential for maintaining glucose homeostasis.

Notably, 3-succinylcholic acid alleviates metabolic dysfunction-associated steatohepatitis (MASH) and ameliorates metabolic disorders by promoting the growth of the beneficial gut bacterium *Akkermansia muciniphila* [74]. Patients with T2DM show significantly lower fecal levels of tryptophan-cholic acid (Trp-CA) than healthy controls. Unlike traditional bile acid receptors, Trp-CA activates the orphan receptor MRGPRe in the intestine to promote GLP-1 secretion, thereby improving glucose homeostasis [75]. Patients with T1DM commonly exhibit gut microbiota dysbiosis and impaired secondary bile acid metabolism. Washed microbiota transplantation restores gut microbiota and secondary bile acids (lithocholic acid, isolithocholic acid), accompanied by improved islet function and reduced inflammation in some patients [76].

In ICT mouse models, MicroAFB intervention modulated gut microbiota abundance and community structure, reshaping intestinal metabolite profiles and notably enriching 3-SDCA. This microbial remodeling and elevated 3-SDCA further upregulated *Tgr5* mRNA expression in target organs. Specifically, 3-SDCA bound to the active site of the target protein and regulated the phosphorylation and activity of the insulin signaling pathway (IRS/PI3K/AKT/GSK3 β /FOXO1), enhanced key glycolytic processes and suppressed gluconeogenesis. These changes synergistically improved systemic glucose homeostasis, as shown by reduced fasting blood glucose, increased GLP-1 secretion and decreased HbA1c, thereby promoting functional recovery and favorable prognosis in ICT mice.

Conclusions

In conclusion, this study elucidated the alterations in gut microbial ecosystems after ICT and the regulatory mechanisms governing host glucose metabolism and islet function recovery. In T2DM patients after ICT, key functional taxa (*Faecalibacterium*, *Akkermansia*, *Bacteroides*) were significantly reduced, while opportunistic pathogens (*Streptococcus*, *Enterococcus*) were enriched. These dysbiotic patterns strongly correlated with postoperative glycemic instability, suggesting that microbial ecological disruption might impair graft survival and function via metabolic and inflammatory cascades. The MicroAFB consortium, consisting of *Akkermansia*, *Faecalibacterium*, and *Bacteroides*, was validated to ameliorate post-ICT metabolic dysregulation. The MicroAFB consortium restored gut microbial diversity and improved glycemic homeostasis through multiple mechanisms. It repaired the gut barrier by upregulating tight junction proteins (*Muc2*, *Occludin*, *ZO-1*), suppressed inflammatory mediators (*Ip-10*, *Mcp-1*), enhanced insulin sensitivity, and activated the IRS/PI3K/AKT and GSK3 β /FOXO1 signaling pathways. These mechanisms promoted hepatic glucose metabolism and inhibited gluconeogenesis. Moreover, enriched microbial metabolites, including bile acids and vitamins B and K, improved host metabolic function by regulating insulin secretion and GLP-1 levels. This modulation altered the gut metabolomic profile and significantly enriched 3-SDCA. The remodeling of gut microbiota composition and the elevation of 3-SDCA levels further upregulated the mRNA expression level of TGR5 in target organs. 3-SDCA regulated insulin signaling

phosphorylation and activity by binding to protein active sites. These results indicated that the MicroAFB consortium-centered gut microbiota regulated post-ICT islet glucose metabolism and represented a potential therapeutic target for clinical intervention.

However, this study presented certain limitations, including a relatively small ICT cohort size, suboptimal precision in microbial consortium optimization, and the need for clinical validation across heterogeneous transplant populations (e.g., T1DM and T2DM). Additionally, this study employed 16S rRNA gene full-length sequencing for gut microbiota analysis, which enables reliable species-level taxonomic annotation but still cannot match the resolution of metagenomic sequencing. Notably, the MicroAFB consortium intervention improved glucose metabolism in islet transplant recipients and provided new insights into reducing long-term reliance on immunosuppressants and antibiotics. Future efforts should focus on developing microbiome-mediated host-microbiota co-regulatory therapies to support sustained recovery in diabetic patients following ICT.

Declaration of competing interest

The authors declare no competing interests.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials, and that all such data are additionally available from the corresponding author upon reasonable request.

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