

Effects of Gut Microbiota on The Recovery of Intestinal Function After Colorectal Surgery: A Prospective Cohort Study

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Abstract

Purpose

This study aimed to identify risk factors and gut microbiome signatures linked to postoperative gastrointestinal recovery following colorectal surgery.

Methods

Patients who underwent colorectal surgery from January to October 2023 were categorized into two groups according to their first postoperative defecation time (≤ 5 days) and delayed recovery (> 5 days). Comprehensive clinical variables collected to screen for independent predictors of postponed bowel recovery. Postoperative fresh fecal samples were subjected to metagenomic sequencing to explore associations between gut microbial composition and postoperative gut functional recovery.

Results

A total of 35 patients were included in the final analysis. Multivariate regression revealed that the initiation time of early postoperative enteral feeding ($p < 0.01$) was an independent risk factor for delayed defecation. No intergroup disparities in gut microbial alpha diversity were observed. Relative abundances of beneficial commensal genera including *Bifidobacterium* and *Lactobacillus* were significantly depleted, whereas pathogenic bacterial populations were enriched in patients with delayed bowel recovery. Functional metagenomic annotation indicated notable suppression of core microbial metabolic pathways in the delayed-recovery cohort, such as carbohydrate metabolism (glycolysis/gluconeogenesis) and amino acid metabolism (selenocysteine and taurine biosynthesis).

Conclusions

Early initiation of postoperative enteral nutrition alongside probiotic supplementation may facilitate the restoration of postoperative intestinal function. Downregulation of microbe-mediated glycolysis/gluconeogenesis as well as selenocysteine and taurine biosynthesis likely disrupts intestinal mucosal repair and gut homeostasis, ultimately leading to protracted postoperative gastrointestinal recovery.

Introduction

The human gastrointestinal tract hosts roughly 38 trillion bacterial cells, forming an extraordinarily diverse microbial ecosystem[1]. Dysbiosis is typified by a compositional shift away from dominant obligate anaerobes toward facultative anaerobes, a shift that fosters overgrowth of pathobionts implicated in inflammatory bowel disease (IBD) and colorectal tumorigenesis[2]. The human

gastrointestinal tract hosts roughly 38 trillion bacterial cells, forming an extraordinarily diverse microbial ecosystem[3]. Inside the tumor microenvironment, innate immune cells release cytokines, chemokines, matrix metalloproteinases, growth factors and reactive oxygen species. These mediators trigger genomic damage, fuel uncontrolled epithelial proliferation and boost invasive and metastatic capacities of malignant cells[4]. Colorectal cancer (CRC) ranks as the third most frequently diagnosed malignancy globally, responsible for 10% of all new cancer cases and 9.4% of cancer-associated fatalities worldwide; accordingly, it represents the second leading contributor to cancer-related mortality across populations[5]. Surgical resection constitutes the definitive curative backbone for localized CRC, yet postoperative restoration of bowel function is governed by a complex array of clinical variables. Both domestic and international investigations into post-surgical gastrointestinal rehabilitation have pinpointed actionable modifiable interventions, encompassing minimally invasive operative techniques, early enteral feeding and targeted probiotic administration[6–8]. Implementation of Enhanced Recovery After Surgery (ERAS) pathways has further improved postoperative gut functional recovery among CRC patients[9]. Nevertheless, routine perioperative measures including mechanical bowel preparation, prophylactic antimicrobial prophylaxis, surgical tissue injury and perioperative fluid therapy perturb commensal gut communities, and such microbial perturbation correlates with adverse postoperative sequelae such as anastomotic dehiscence and surgical site infection[10]. Therefore, understanding the relationship between gut microbiota dynamics and bowel function recovery in colorectal surgery patients is a critical research priority.

Methods

Study design and ethics

This prospective study explored the association between gut microbiota composition and postoperative bowel function recovery after colorectal resection. The study protocol obtained ethical approval from the Ethics Committee of Peking University People's Hospital (approval reference: 2022PHB053-001). Written informed consent was obtained from all enrolled subjects before study entry.

Participants

Eligible candidates were adult patients receiving elective colorectal surgery at Peking University People's Hospital from 1 January, 2023 to 31 October, 2023. All patients underwent preoperative mechanical bowel preparation with oral laxatives. General anaesthesia was induced and maintained using intravenous propofol, remifentanyl and dexmedetomidine alongside inhaled sevoflurane. Perioperative prophylactic antibiotics were standardised to cefoperazone-sulbactam, and postoperative pain control was achieved with a multimodal regimen combining opioids and non-steroidal anti-inflammatory drugs. Patients fulfilling any of the following criteria were excluded:

- Prior history of gastrointestinal reconstructive surgery.

- Oral probiotic exposure within three months before surgery
- Age younger than 18 years
- Voluntary withdrawal of informed consent during follow-up
- Incomplete clinical documentation unavailable for final analysis

Existing literature reports a median postoperative first defecation interval of around five days following colorectal surgery[11]. Accordingly, enrolled patients were split into two subgroups according to their first defecation time: Group A (early recovery, ≤ 5 days) and Group B (delayed recovery, > 5 days).

Data collection

Baseline demographic information including age, sex, and body mass index (BMI) was prospectively documented. All pre-existing comorbidities before hospital admission were recorded on standard case report forms (CRFs), covering hypertension, coronary heart disease (CHD), arrhythmia, cerebral infarction, intracranial hemorrhage, hypothyroidism, diabetes mellitus, chronic obstructive pulmonary disease (COPD), renal insufficiency, hyperlipidaemia, hepatic insufficiency, and hematological diseases. Preoperative clinical indicators collected comprised prior neoadjuvant chemotherapy, anemia status, preoperative anemia, preoperative paralytic ileus, American Society of Anesthesiologists (ASA) physical status classification. Intraoperative parameters documented on CRFs included operative procedure (right hemicolectomy, transverse colectomy, left hemicolectomy, sigmoidectomy or proctectomy), surgical access (open laparotomy versus laparoscopic resection), total operative duration and estimated intraoperative blood loss. Postoperative variables consisted of timing of initial enteral feeding, length of postoperative antibiotic and analgesic courses, and postoperative hypoproteinaemia. All clinical data were extracted from institutional electronic medical records and archived paper charts.

Stool sample collection

Fresh fecal samples (minimum 6 g per specimen) were collected at the patients' first postoperative defecation and immediately stored at -80°C for subsequent metagenomic sequencing.

Materials and Methods for Metagenomics

DNA extractions

Genomic DNA was extracted from samples using the E.Z.N.A.® Stool DNA Kit (Omega Bio-tek, D4015-02) per manufacturer's protocol. This method effectively isolates bacterial DNA from low-biomass specimens. Negative controls (sterile swabs processed identically) confirmed absence of contamination. Eluted DNA (50 μl) was stored at -80°C prior to downstream analysis.

DNA Library Construction

Libraries were prepared using Illumina's TruSeq Nano DNA Kit. DNA fragmentation employed dsDNA Fragmentase (NEB) at 37°C for 30 min. Fragments were end-repaired, size-selected with AMPure XP beads, and adenylated for adapter ligation. Indexing utilized T-overhang adapters compatible with paired-end sequencing. PCR amplification conditions: 95°C for 3 min; 8 cycles of (98°C, 15s; 60°C, 15s; 72°C, 30s); final extension at 72°C for 5 min.

Data analysis

Raw sequencing data were processed through adapter removal (Cutadapt), quality trimming (Fqtrim), and host DNA filtering (Bowtie2), followed by *de novo* metagenome assembly (IDBA-UD). Coding sequences were predicted (MetaGeneMark), clustered into unigenes (CD-HIT), and quantified as TPM. Taxonomic assignment used DIAMOND (v2.1.8) against NCBI NR. Functional annotation employed GO, KEGG, eggNOG, CAZy, CARD, PHI, MGEs, and VFDB databases. Differential abundance analysis (Fisher's exact and Kruskal-Wallis tests) was performed on taxonomic, functional, and gene-level features (TPM-normalized) (Additional file 1: Figure S1).

Statistical analysis

All statistical analyses were performed with SPSS version 26.0 (IBM, Armonk, NY, USA). Univariate analyses were first implemented to screen candidate risk factors associated with delayed postoperative first defecation. Continuous variables conforming to a normal distribution were expressed as mean $\pm$ standard deviation (SD), whereas non-normally distributed data were reported as median and interquartile range (IQR). Intergroup comparisons of normally distributed continuous variables were conducted via one-way analysis of variance (ANOVA). Categorical data were summarised as counts and corresponding percentages and compared using the χ^2 test or Fisher's exact test where indicated. Variables yielding $p < 0.05$ on univariate screening were entered into a backward stepwise multivariate logistic regression model to determine independent predictors of delayed defecation. Effect sizes were presented as odds ratios (OR) alongside their 95% confidence intervals (CIs). All statistical tests were two-tailed, and a two-tailed p -value < 0.05 was set as the threshold for statistical significance.

Results

Baseline characteristics

Between January 1 and October 31, 2023, a total of 93 patients were screened for eligibility, among whom 35 were finally enrolled after exclusions (Fig. 1). Seven patients (20.0%) experienced delayed postoperative defecation, defined as the first defecation occurring more than 5 days after surgery. The overall cohort had a mean age of 62.49 ± 11.74 years (range: 35–87 years), with detailed baseline demographic and clinical characteristics summarized in Table 1. Significant intergroup differences were

observed in transverse colectomy ($p = 0.04$), malignancy ($p = 0.04$), operative duration ($p = 0.01$), and the timing of postoperative enteral feeding ($p < 0.01$).

Table 1

Baseline and characteristics of postoperative defecation for patients undergoing colon surgery

variables	GroupA(≤ 5 day) n = 28	GroupB(> 5day) n = 7	χ^2/ t	P value
Female	15(53.6%)	3(42.9%)	0.26	0.61
Age(years)	61.43 $\pm$ 12.47	66.71 $\pm$ 7.39	1.26	0.29
BMI ^a (kg/m ²)	24.34 $\pm$ 3.99	23.21 $\pm$ 3.02	0.92	0.18
ASA ^b classification			1.64	0.44
☐	1(3.6%)	0		
☐	24(85.7)	5(71.4%)		
☐	3(10.7%)	2(28.6%)		
Probiotics	8(28.6%)	2(28.6%)	0	1.00
Right colectomy	13(46.4%)	2(28.6%)	0.73	0.39
Transverse colectomy	1(3.6%)	2(28.6%)	4.47	0.04
Left colectomy	2(7.1%)	0	5.3	0.47
Sigmoid colectomy	13(46.4%)	3(42.9%)	0.29	0.87
Preoperative ileus	5(17.9%)	1(14.3%)	0.05	0.82
Hypertension	6(21.4%)	2(28.6%)	0.16	0.69
CHD ^c	2(7.1%)	0	0.53	0.47
Diabetes mellitus	7(25.0%)	1(14.3%)	0.37	0.55
COPD ^d	1(3.6%)	1(14.3%)	1.19	0.28
Malignancy	28(100%)	6(85.7%)	4.12	0.04
Laparotomy	4(14.3%)	2(28.6%)	0.81	0.37
Duration of operative (min)	158.04 $\pm$ 49.30	215.00 $\pm$ 39.37	0.1	0.01
Intraoperative bleeding	53.93 $\pm$ 38.43	94.26 $\pm$ 135.26	9.31	0.18
Time of postoperative feeding(day)	3.36 $\pm$ 0.83	5.86 $\pm$ 1.68	5.69	< 0.01
Duration of analgesia	3.04 $\pm$ 0.43	3.29 $\pm$ 0.95	7.53	0.30
^a BMI: Body mass index, ^b ASA American society of Anesthesiologists, ^c CHD: Coronary atherosclerotic heart disease, ^d COPD: Chronic obstructive pulmonary disease				

variables	GroupA(≤ 5 day) n = 28	GroupB(> 5day) n = 7	χ^2/ t	P value
Duration of antibiotics	25(89.3%)	7(100%)	0.82	0.37
Hypoproteinemia	10(35.7%)	3(42.9%)	0.12	0.73
^a BMI: Body mass index, ^b ASA American society of Anesthesiologists, ^c CHD: Coronary atherosclerotic heart disease, ^d COPD: Chronic obstructive pulmonary disease				

Primary outcome

Multivariate logistic regression analysis confirmed that delayed postoperative enteral feeding was the sole independent risk factor for prolonged first defecation time (OR = 19.53, 95% CI 1.80-211.60; p = 0.01).

Microbiome analysis

Coding sequences (CDS) of contigs (≥ 500 bp) were predicted and microbial biodiversity was assessed using MetaGeneMark (v3.38). Sequences with CDS shorter than 100 nt were excluded from subsequent analysis. All qualified sequences were clustered at 95% sequence identity and 90% coverage to generate a non-redundant unigene dataset, with the longest sequence of each cluster selected as the representative. Patients with delayed defecation (> 5 days) exhibited significantly fewer unigenes in both nucleic acid and protein annotation compared with patients with early recovery (≤ 5 days) (Additional file 1: Figure S2).

Alpha diversity indices, including Chao1, observed species, Shannon and Simpson indices, revealed no significant differences in microbial richness and evenness between the two groups (Table 2). Rarefaction curves verified sufficient sequencing depth and further confirmed the absence of intergroup differences in alpha diversity (Fig. 2).

Table 2
Analysis of Alpha diversity index of intestinal microbiome for patients undergoing colon surgery

Chao1	GroupA(≤ 5 day)	GroupB(> 5day)	P value
	2482.90 $\pm$ 804.45	2552.80 $\pm$ 1021.13	0.65
Observed_species	1914.60 $\pm$ 662.93	1985.50 $\pm$ 795.87	0.68
Shannon	4.88 $\pm$ 1.02	4.86 $\pm$ 0.90	0.96
Simpson	0.88 $\pm$ 0.07	0.89 $\pm$ 0.09	0.84

Microbiome composition

Unigene protein sequences were aligned against the NR_meta database (a microbial-specific subset of NCBI NR containing 52,375,954 sequences) using DIAMOND (BLASTp; $e\text{-value} \leq 1 \times 10^{-5}$). Taxonomic assignment was based on the highest-scoring alignment for each unigene. Abundance was calculated hierarchically, with the top 20 species visualized; remaining taxa were aggregated as "Other".

Species richness analysis

Comparative taxonomic profiling demonstrated distinct microbial compositional signatures between the two groups. Specifically, patients with delayed defecation harbored reduced abundances of beneficial probiotic genera (e.g., *Lactobacillus* and *Bifidobacterium*) and enriched pathogenic bacteria relative to the early recovery cohort. At the phylum level, the ≤ 5 -day group presented higher abundance of Bacteroidetes and Archaea, alongside lower levels of Proteobacteria, Firmicutes, Actinobacteriota, and Chytridiomycota (Additional file 1: Fig. S3). The dominant microbial classes across all samples included Bacteroidia, Proteobacteria, Clostridia, Bacilli and Actinobacteria. At the class level, the delayed recovery group showed increased Bacteroidia and α -Proteobacteria, but decreased Proteobacteria, Bacilli and Actinobacteria (Additional file 1: Figure S4).

At the order level, significant depletion of Lactobacillales and Enterobacterales was detected in the delayed recovery cohort (Additional file 1: Figure S5). At the family level, patients with delayed defecation exhibited lower abundances of Enterobacteriaceae, Enterococcaceae and Coriobacteriaceae, whereas the abundances of Klebsiellaceae and Mucoraceae were elevated (Additional file 1: Figure S6). Genus-level analysis further revealed depleted Enterococcus, Escherichia, Ruminococcus and Bifidobacterium, and enriched Klebsiella in the delayed recovery group (Additional file 1: Figure S7). At the species level, beneficial probiotic species including Escherichia coli, Enterococcus faecalis, Bacteroides multivorans, Bifidobacterium spp. and Clostridium prasicolum were markedly reduced, while pathogenic species such as Bacteroides fragilis, Klebsiella pneumoniae and Bacteroides faecalis were significantly enriched in patients with delayed bowel recovery (Additional file 1: Figure S8).

Analysis of species richness differences

Differential abundance analysis was performed with thresholds of $|\log\text{FC}| > 1$ and $p < 0.05$. The most prominent differential taxa belonged to Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and viral taxa (Additional file 1: Figure S9). The early recovery cohort (≤ 5 days) had significantly lower abundances of Ruminococcus ($p < 0.01$), Lachnospiraceae ($p < 0.01$), Escherichia albertii ($p < 0.01$) and Escherichia sp. 4_140B ($p < 0.01$), but higher abundances of Ruminococcaceae ($p < 0.01$), Subdoligranulum ($p < 0.01$), Bacteroidetes ($p < 0.01$) and Ruminococcaceae bacterium ($p < 0.01$) (Fig. 3A).

Random Forest analysis based on Gini impurity was applied to identify key microbial taxa associated with delayed postoperative defecation. The core discriminatory taxa included Alistipes AF48.12, Clostridium sp. AF12.41, Hyphomicrobiaceae bacterium, Paenibacillus sp. PY1325, Bacteroides spp. and Yersinia enterocolitica (Fig. 3B).

Functional annotation of gut microbiome metabolism

The top 30 Gene Ontology (GO) terms with the lowest p -values were selected to compare functional profiles of unigenes protein sequences between the two groups (Additional file 1: Figure S10). The delayed recovery group (Group B) showed significant downregulation of multiple biological processes, including peptidase activity ($p < 0.01$), DNA replication initiation ($p < 0.01$), pyrimidine nucleobase metabolic process ($p < 0.01$), phosphate acetyltransferase activity ($p < 0.01$), glutamate biosynthetic process ($p < 0.01$), pyrimidine nucleoside salvage ($p < 0.01$), and enzyme-directed rRNA 2'-O-methylation ($p < 0.01$) (Fig. 4A).

KEGG pathway annotation further demonstrated that patients with delayed defecation exhibited suppressed functions in carbohydrate metabolism, membrane transport and amino acid metabolism [12]. Multiple core microbial metabolic pathways were significantly impaired, including biosynthesis of secondary metabolites, microbial metabolism in diverse environments, cofactor biosynthesis, amino acid biosynthesis, two-component regulatory systems, ABC transporters, carbon metabolism, ribosomal function, purine metabolism, amino sugar and nucleotide sugar metabolism, quorum sensing, glycolysis/gluconeogenesis, starch and sucrose metabolism, pyruvate metabolism, pyrimidine metabolism, galactose metabolism, cysteine and methionine metabolism, fructose and mannose metabolism, and oxidative phosphorylation (Additional file 1: Figure S11).

Further KEGG Pathway enrichment analysis confirmed significant reductions in multiple critical pathways in the delayed recovery group, including Flagellar assembly ($p < 0.01$), Glycolysis/Gluconeogenesis ($p < 0.01$), D-glutamine and D-glutamate metabolism ($p < 0.01$), Cysteine and methionine metabolism ($p < 0.01$), selenocompound metabolism ($p < 0.01$), Aminoacyl-tRNA biosynthesis ($p < 0.01$), protein export ($p < 0.01$), Peptidoglycan biosynthesis ($p < 0.01$), D-Alanine metabolism ($p < 0.01$), and pyrimidine metabolism ($p < 0.01$) (Fig. 4B).

Discussion

This study performed metagenomic sequencing on fecal specimens collected from the first postoperative bowel movement after colorectal surgery to characterize gut microbiota composition. The microbiota was predominantly composed of Bacteroidetes, Proteobacteria, Firmicutes, and Actinobacteria. Patients with delayed postoperative bowel recovery showed reduced proportions of probiotic taxa and increased prevalence of pathogenic bacteria. However, no statistically significant intergroup differences in microbial biodiversity were observed after surgery. Functional annotation and comparative analysis of Unigene protein sequences across multiple databases revealed that delayed defecation was associated with impaired intestinal digestion and absorption, reduced mucosal barrier integrity, and compromised epithelial repair mechanisms.

Research on the gut microbiota–colorectal cancer axis has established that colorectal cancer patients exhibit reduced microbial diversity. Studies on postoperative anastomotic leakage further demonstrated

higher microbial diversity in patients without leakage, whereas leakage correlated with reduced diversity and increased abundances of Bacteroidaceae and Lachnospiraceae (particularly Blautia)[13]. In our cohort, prolonged postoperative defecation likewise corresponded to lower microbial diversity. However, non-parametric intergroup comparisons of biodiversity indices showed no statistical significance—likely due to the very small sample size of the > 5-day group (n = 7). Contemporary implementation of Enhanced Recovery After Surgery (ERAS) protocols and standardized perioperative care has shortened key recovery milestones, including time to first flatus and first defecation. Alternatively, partial restoration of microbiota diversity and metabolic function may begin during bowel recovery, potentially obscuring any strong correlations between microbial diversity and defecation timing.

Healthy human gut microbiota typically includes one archaeal phylum alongside five dominant bacterial phyla: Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia. High-sugar/low-fiber diets correlate with increased Bacteroidetes abundance, while fiber-rich diets favor Firmicutes proliferation[14]. In colorectal cancer (CRC), specific pathogens—including *Fusobacterium nucleatum*, *Escherichia coli*, *Bacteroides fragilis*, and *Campylobacter jejuni*—drive tumorigenesis by creating a pro-oncogenic microenvironment[15]. *Bacteroides fragilis*, a common gut symbiont essential for digestion and mucosal health, exists as two subtypes: non-toxigenic *B. fragilis* and enterotoxigenic *B. fragilis* (ETBF). ETBF secretes *B. fragilis* toxin (BFT), which cleaves E-cadherin to disrupt epithelial integrity, activates Wnt/ β -catenin signaling, induces chronic inflammation, and promotes carcinogenesis. ETBF also upregulates spermine oxidase in colonocytes, enhancing reactive oxygen species (ROS) production, DNA damage, and CRC progression[16].

In this study, we compared gut microbiota abundance across six taxonomic levels (from phylum to species) between patients with postoperative defecation times ≤ 5 days and those with > 5 days. Key findings included: elevated levels of pathogenic bacteria—*Bacteroides fragilis*, *Ruminococcus* spp., and *Klebsiella pneumoniae*—in the > 5-day group, and reduced levels of beneficial taxa—*Enterococcus faecalis*, *Bifidobacterium* spp., and *Bacteroides thetaiotaomicron*—in patients with delayed recovery. *Ruminococcus* (a Gram-positive anaerobe in the Lachnospiraceae family) degrades cellulose and carbohydrates to produce acetate and formate. Although historically linked to Crohn's disease and irritable bowel syndrome via butyrate metabolism[17], its unexpectedly higher abundance in the ≤ 5 -day group—despite reduced carbohydrate metabolism in delayed-recovery patients—suggests strain-specific functional heterogeneity and warrants further investigation. *Bacteroides*, a dominant anaerobic genus in the human gut, hydrolyzes complex plant polysaccharides into absorbable sugars. *Bacteroides thetaiotaomicron*, which encodes glycoside hydrolases and regulates glutamate metabolism, is crucial for ileal and colonic mucosal health. Its depletion in the > 5-day group may therefore impair carbohydrate processing and epithelial repair [18]. Reduced levels of *Bifidobacterium* and *Lactobacillus* (common immunomodulatory probiotics) are likely compromised gut barrier integrity and anti-inflammatory capacity[19]. Similarly, diminished *Enterococcus faecalis* (a Gram-positive facultative anaerobe used in dysbiosis therapy) may indicate impaired microbial resilience[20].

It is well-established that gut microbiota primarily interact with intestinal epithelial and stromal cells to maintain microenvironmental homeostasis. For example, *Bifidobacterium* and *Lactobacillus* ferment fructooligosaccharides to produce lactate and acetate, which are then metabolized by commensal bacteria such as *Faecalibacterium* into butyrate—the main energy source for colonic enterocytes[21]. Butyrate enhances epithelial integrity by upregulating *MUC2* (which encodes mucin 2) and modulating tight junction protein expression, thereby reinforcing the intestinal barrier. Additionally, it lowers luminal pH, sustains microbial equilibrium, activates AMP-activated protein kinase (AMPK) to stimulate enterocyte differentiation, and strengthens mucosal defenses[22]. Beyond metabolic functions, butyrate has anti-inflammatory and anti-neoplastic properties through direct modulation of colonic cell metabolism and mucosal immunity[23]. Functional annotation of differentially expressed genes revealed reduced carbohydrate metabolism in patients with delayed postoperative bowel recovery. This impairment likely stems from probiotic depletion, which compromises dietary fiber processing, induces epithelial barrier dysfunction, and contributes to prolonged intestinal hypomotility.

Dietary fiber degradation—carried out by symbiotic bacteria such as *Bacteroides* using glycan-sensing enzymes—generates short-chain fatty acids (SCFAs). SCFAs bind G protein-coupled receptors, inducing a tolerogenic dendritic cell phenotypes, promoting regulatory T-cell (Treg) differentiation, polarizing macrophages toward an anti-inflammatory M2 state, and stabilizing gut homeostasis[24]. In this cohort, reduced glycolysis/gluconeogenesis and decreased fructose/mannose metabolism in delayed-recovery patients correlated with attenuated immunomodulatory capacity in intestinal epithelia.

Probiotics enhance bile salt hydrolase (BSH) activity, catalyzing the dissociation of taurine from tauro-conjugated bile acids[25]. The observed deficit in taurine metabolism among delayed-recovery patients likely reflects probiotic deficiency. Selenium, an essential trace element incorporated as selenocysteine (the 21st proteogenic amino acid), is critical for intestinal redox homeostasis. Selenoproteins—including glutathione peroxidase, selenoprotein S, and selenoprotein P—coordinate antioxidant defenses, mitigate oxidative stress, and suppress chronic inflammation[26]. Preclinical evidence indicates that gut microbiota composition modulates host selenium bioavailability, thereby influencing selenoprotein expression and inflammatory responses[27]. The reduced selenocysteine incorporation observed here suggests impaired mucosal protection mechanisms in delayed-recovery patients.

This study investigated the role of gut microbiota in postoperative gut recovery after colorectal surgery by metagenomic sequencing of fecal samples obtained at the first postoperative bowel movement. Key limitations include:

(1) Sample Size Constraints: a) Increasingly prevalent rapid-discharge protocols have shortened hospital stays to ≤ 7 days, complicating the collection of preoperative, intraoperative, and immediate postoperative fecal specimens. b) Suboptimal patient adherence—particularly delayed reporting of bowel movements—often prevented timely sample acquisition. c) the high cost of metagenomic sequencing limited cohort expansion. (2) Despite advances in 16S rRNA and next-generation sequencing (NGS) technologies—which have accelerated discoveries in microbial diversity and host-microbe interactions—

the precise mechanisms by which gut microbiota influence bowel function remain incompletely understood. The gut microbiome comprises numerous species with multifunctional roles, ranging from direct mucosal crosstalk to systemic signaling via inflammatory cytokines and microbial metabolites. Although the microbiome has been implicated in conditions such as chronic inflammation, oncogenesis, neuropsychiatric disorders, and metabolic diseases, current evidence is insufficient to establish causal mechanisms or identify key molecular mediators. These gaps highlight the need for hypothesis-driven, multi-omics studies (e.g., metabolomics, transcriptomics) to elucidate microbiota-host dynamics in postoperative recovery.

Conclusion

Timely resumption of enteral nutrition facilitates the restoration of postoperative bowel motility in patients undergoing colorectal surgery. A reduced proportion of probiotics and an increased abundance of pathogenic bacteria may delay the time of postoperative defecation for patients undergoing colorectal surgery. The reduction of metabolism in the bowel flora, namely the decrease of butyrate, taurine and selenocysteine, may prolong the recovery of postoperative bowel function for patients undergoing colorectal surgery.

Abbreviations

IBD

Inflammatory bowel disease, CRC:Colorectal cancer, ERAS:Enhanced recovery after surgery, BMI:Body mass index

CHD

Coronary heart disease

COPD

Chronic obstructive pulmonary disease

ASA

American Society of Anesthesiologists

NSAIDs

Non-steroidal anti-inflammatory drugs

CRF

case report form

CDS

Coding regions, SD:Standard deviations, OR:Odds ratios

CI

Confidence intervals

GO

Gene Ontology Consortium, KEGG:Kyoto Encyclopedia of Genes and Genomes, ETBF:Enterotoxigenic B. fragilis, ROS:Reactive oxygen species, MUC2:Mucin 2, Oligomeric Mucus/Gel-Forming, AMPK:Adenosine 5'-monophosphate-activated protein kinase, SCFAs:Short-chain fatty acids, BSH:Bile salt hydrolase.

Declarations

Ethics approval and consent to participate

This study was approved by medical ethics committee of Peking University People's Hospital(2022PHB053-001). Conformed with the provisions of the Declaration of Helsinki. Written, informed consent was obtained from all patients

Consent publication

Not applicable.

Competing interests

The authors declared no potential conflict of interest concerning the research, authorship, and/or publication of this article.

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Author Contribution

SY and ZG designed the study. SY and YS participated in the literature search, analysis of data, as well as manuscript writing. YY participated in the literature search and data analysis revised the manuscript. HZ participated in the data analysis and revised the manuscript. HZ and TW had made contributions to the acquisition, analysis of data. SY and YS are equal the first author. YY and ZG are corresponding authors and are responsible for ensuring that all listed authors have approved the manuscript before submission. All authors approved the final manuscript for publication.

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Data Availability

PRO-Seq data were deposited into the Gene Expression Omnibus database under accession number PRJNA1516131 and are available at the following URL:

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Figures

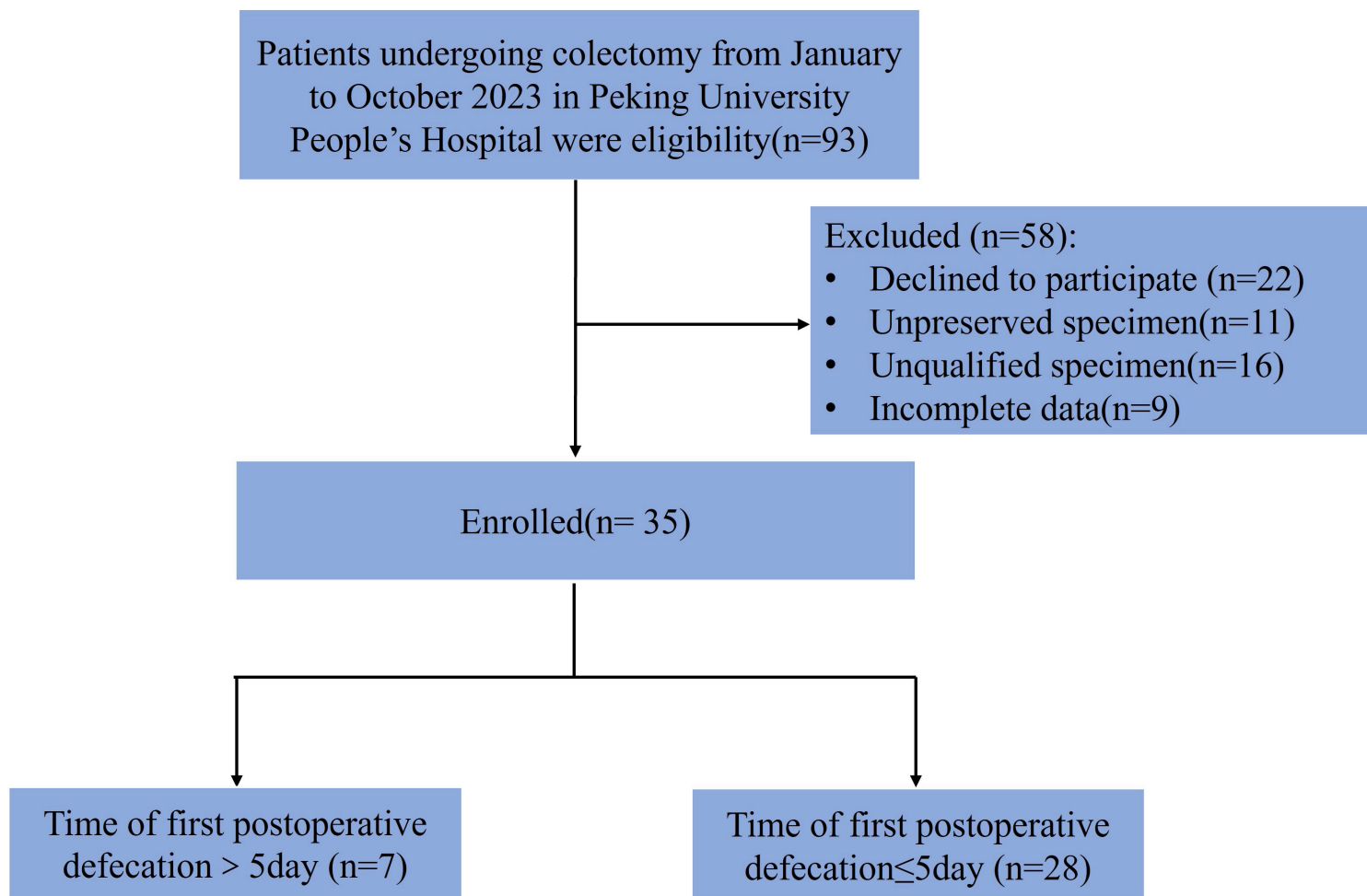


Figure 1

Flowchart of participants enrolled in this study

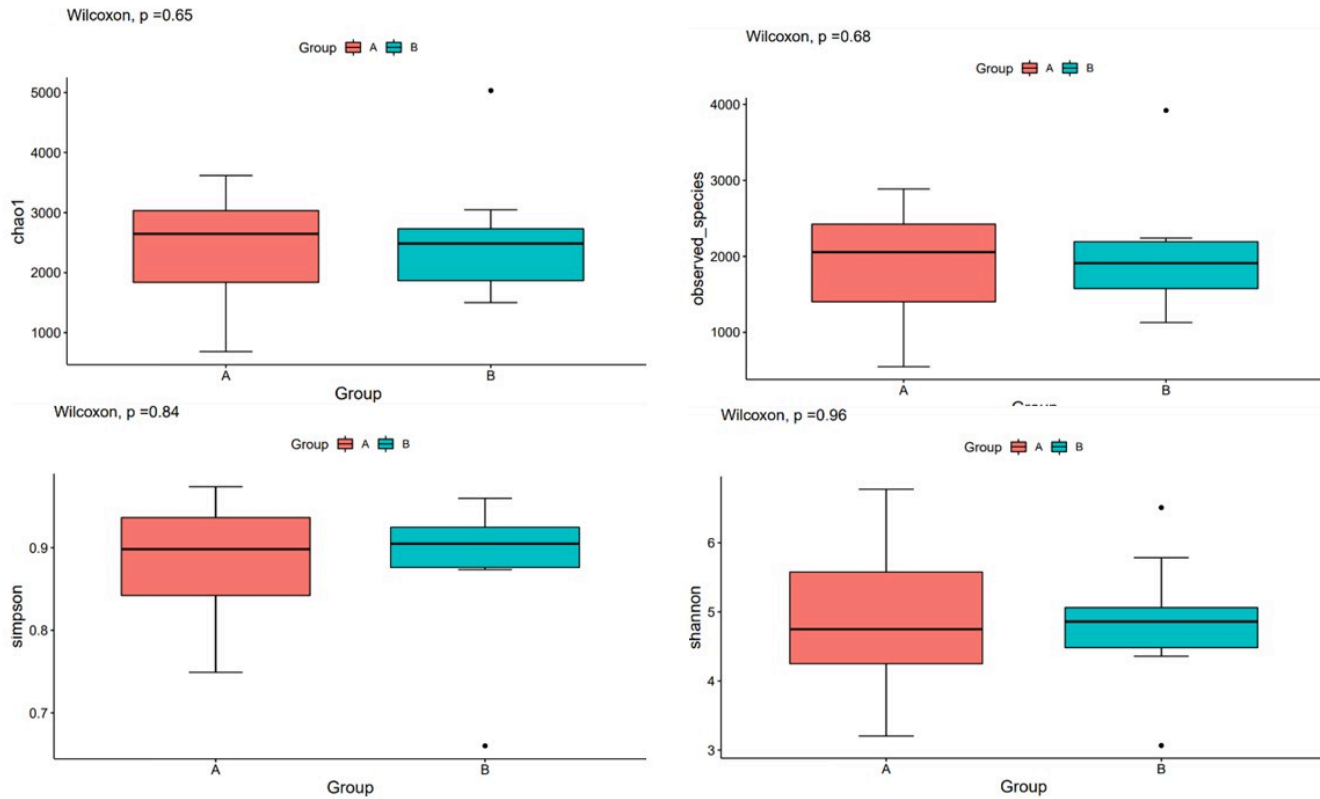


Figure 2

Box plot of postoperative bowel flora diversity for patients undergoing colorectal surgery. The left side of the graph shows different diversity indices, and the upper left corner shows the p value. A: Postoperative defecation time ≤ 5 days, B: Postoperative defecation time > 5 days

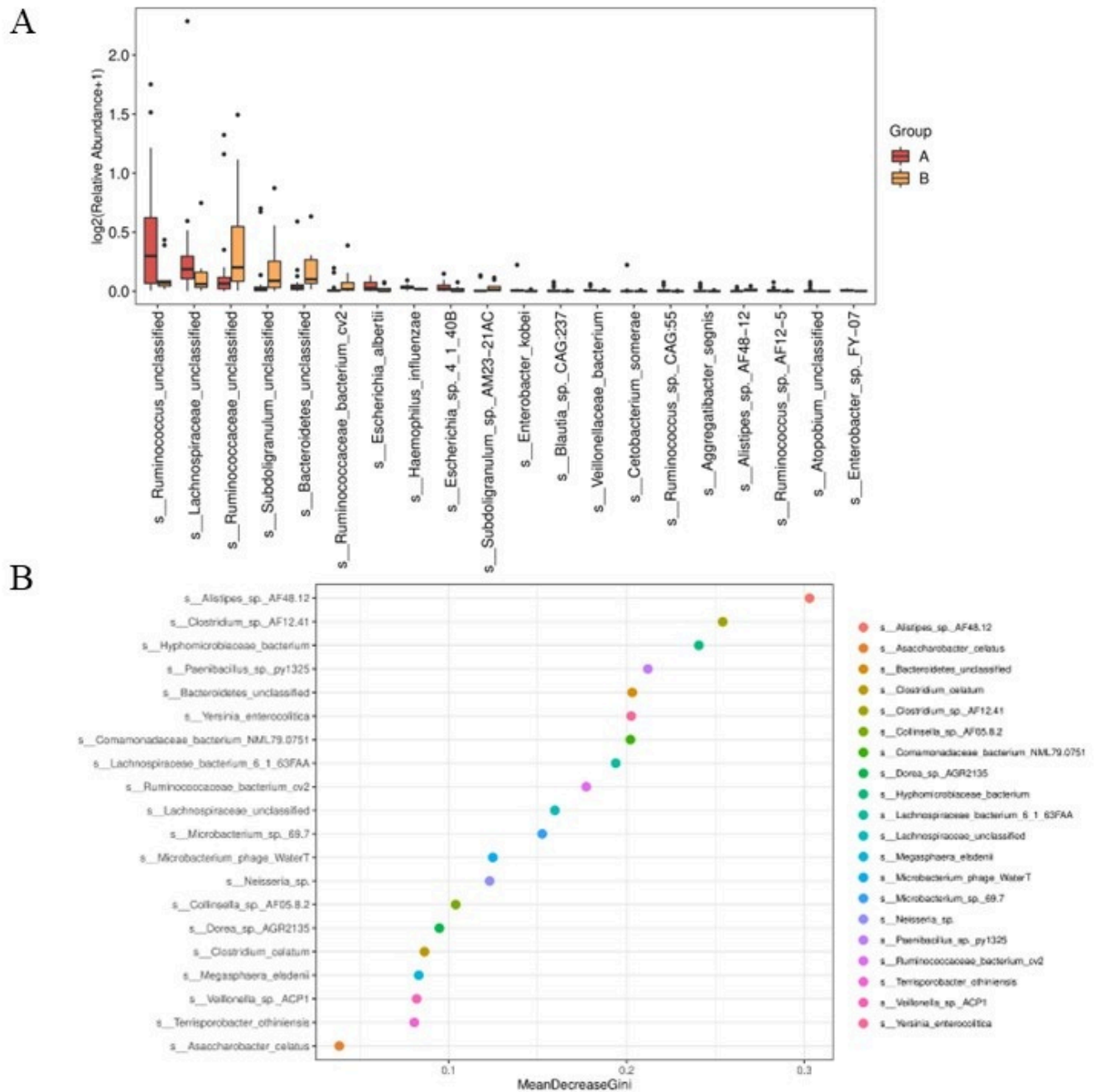


Figure 3

Differences of Species richness between two groups, A: Box plot of species differences in bowel flora for patients undergoing colorectal surgery (Species). B: Random forest plot of the difference in bowel species abundance for patients undergoing colorectal surgery. Different colors represent different species. The closer the color is to red, the smaller the p value. The larger the Gini Index, the greater the importance of the species. A: Postoperative defecation time ≤ 5 days, B: Postoperative defecation time > 5 days.

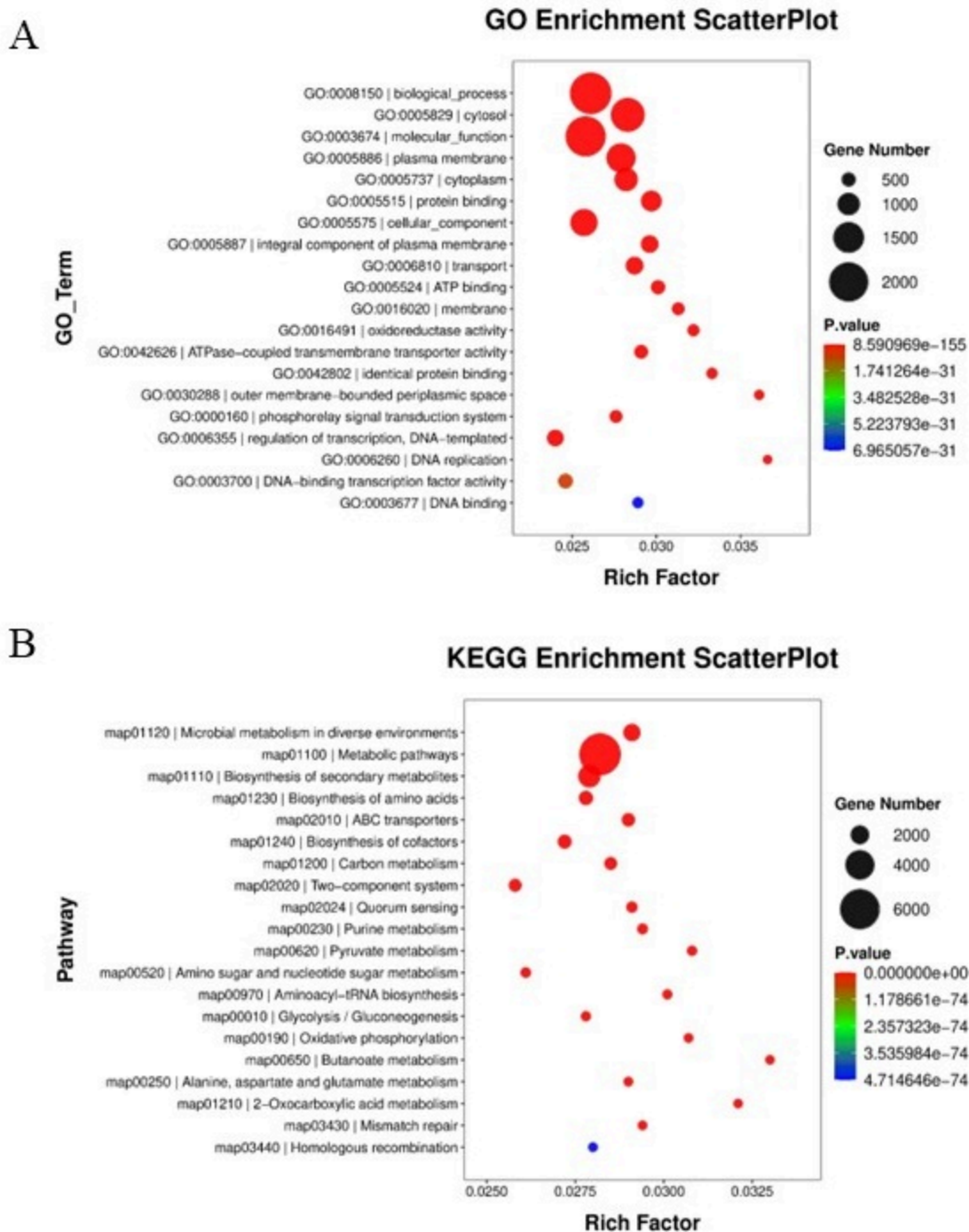


Figure 4

The differential GO and KEGG enrichment map of bowel Unigene for patients undergoing colorectal surgery. **A:** The differential GO enrichment map. Genes in GO to the total number of genes in GO (Rich Factor). The larger the Rich Factor, the higher the enrichment degree of GO. The vertical coordinate represents the functional annotation of GO, the number of Unigene groups that meet the difference in bubble size. **B:** Enrichment map of the differential KEGG pathway of Unigene in the bowel of patients undergoing colorectal surgery. the horizontal coordinate Rich Factor represents the ratio of the number of differential Unigene genes located in the KEGG database pathway to the total number of genes in

this pathway. The larger the Rich Factor, the higher the degree of pathway enrichment. The vertical coordinate is the KEGG path; The size of the bubble represents the number of Unigene differences. The color of the bubble represents the *p*-value of the enrichment analysis. The closer the bubble color is to red, the smaller the *p*-value. A: Postoperative defecation time ≤ 5 days, B: Postoperative defecation time > 5 days. KEGG enrichment analysis of differentially expressed genes.

Supplementary Files

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