

Faecal microbiota transplantation in patients with irritable bowel syndrome modulates the gut microbiome but lacks clinical effects: results of a randomised, double-blind crossover clinical trial

Ondrej Cinek

Ondrej.Cinek@lfmotol.cuni.cz

Charles University in Prague, University Hospital Motol and Homolka <https://orcid.org/0000-0002-0526-8714>

Jiri Vejmelka

Third Faculty of Medicine, Charles University and Thomayer University Hospital

Jakub Hurych

Second Faculty of Medicine, Charles University, and University Hospital Motol and Homolka

Klara Hubackova

Second Faculty of Medicine, Charles University, and University Hospital Motol and Homolka

Iliya Dauda Kwoji

Second Faculty of Medicine, Charles University, and University Hospital Motol and Homolka

<https://orcid.org/0000-0003-3454-1190>

Matej Bezdicek

Department of Laboratory Medicine, University Hospital Brno

Karolina Litosova

Second Faculty of Medicine, Charles University, and University Hospital Motol and Homolka

Lucie Hlinakova

Second Faculty of Medicine, Charles University, and University Hospital Motol and Homolka

Lenka Kramna

Second Faculty of Medicine, Charles University, and University Hospital Motol and Homolka

Martina Kollerova

Third Faculty of Medicine, Charles University and Thomayer University Hospital

Ivana Prazanova

Third Faculty of Medicine, Charles University and Thomayer University Hospital

Michal Kulich

Faculty of Mathematics and Physics, Charles University

Pavel Kohout

Article

Keywords: FMT, IBS, gut microbiota, gastrointestinal disorders, dysbiosis

Posted Date: October 23rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11289849/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: There is **NO** Competing Interest.

**Faecal microbiota transplantation in patients with irritable bowel syndrome
modulates the gut microbiome but lacks clinical effects: results of a randomised,
double-blind crossover clinical trial**

Jiri Vejmelka¹, Jakub Hurych², Klara Hubackova², Iliya Dauda Kwoji², Matěj Bezdíček³, Karolina Litosova²,
Lucie Hlinakova², Lenka Kramna², Martina Kollerova⁵, Ivana Prazanova⁶, Michal Kulich⁷, Ondrej Cinek^{2*},
Pavel Kohout^{1,4,*}

(*) shared last authorship

Affiliations:

¹Department of Internal Medicine, Third Faculty of Medicine, Charles University and Thomayer
University Hospital, Prague, Czechia

²Department of Medical Microbiology, Second Faculty of Medicine, Charles University, and University
Hospital Motol and Homolka, Prague, Czechia

³Division of Clinical Microbiology and Immunology, Department of Laboratory Medicine, University
Hospital Brno, Brno, Czechia

⁴Division of Clinical Microbiology and Immunology, Department of Laboratory Medicine, Faculty of
Medicine, Masaryk University, Brno, Czechia

⁵Nutrition Centre of Thomayer University Hospital, Prague, Czechia

⁶Department of Hygiene, Third Faculty of Medicine, Charles University, Prague, Czech Republic

⁷Department of Probability and Statistics, Faculty of Mathematics and Physics, Charles University,
Prague, Czechia

24

25 **Correspondence to:**

26 Prof. Ondrej Cinek, Ph.D.

27 Department of Medical Microbiology

28 University Hospital Motol and Homolka, V Uvalu 84, Prague; CZ-150 06, Czechia

29 ondrej.cinek@Lfmotol.cuni.cz

30 Phone +420 2 2443 2026

Abstract

We investigated whether symptom severity of irritable bowel syndrome (IBS) can be ameliorated by faecal microbiota transplantation (FMT) of high-diversity mixed faecal material from donors preselected by their bacteriome composition. In this three-arm, randomised, double-blind, placebo-controlled crossover study, 61 adults with diarrhoea-predominant or mixed IBS were randomised to multi-donor live FMT followed by autoclaved placebo FMT, a reverse sequence, or placebo FMT twice; 50 were included in the current efficacy analysis. The prespecified primary endpoint, change in IBS Symptom Severity Score four weeks after intervention, was not met (active versus autoclaved FMT effect, 2.5 points; 95% confidence interval, -33.6 to 38.6; $P=0.893$). Live FMT dramatically increased bacterial diversity and shifted the composition of recipients' bacterial communities towards that of the donors. It also resulted in acquisition of donors-derived strains. No grade 3 or higher adverse events occurred. This finding, alongside those of previous, often less rigorous studies, suggests the futility of FMT in treating IBS.

Keywords: FMT, IBS, gut microbiota, gastrointestinal disorders, dysbiosis

1. INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most prevalent disorders of gut-brain interaction, characterised by recurrent abdominal pain associated with defecation and altered bowel habits and capable of substantially impairing quality of life (Lacy, Mearin et al. 2016; Sperber, Bangdiwala et al. 2021; Drossman, Chang et al. 2026). Its distressing symptoms impair patients' quality of life: diarrhoea, constipation or their endless alternation, fatigue, bloating, abdominal pain, and the most troublesome symptom, faecal urgency (Rangan, Mitsuhashi et al. 2018). Treatment is multifaceted, with limited evidence for the clinical effectiveness of either diets, medication or cognitive behavioural therapy, which underscores the urgent need for innovative therapeutic approaches.

The aetiology of IBS remains unclear (Hartikainen, Jalanka et al. 2024); however, genetic predisposition, diet, lifestyle, psychological stress, and post-infectious events are thought to contribute to its development (Enck, Aziz et al. 2016). An imbalance in gut microbiota has been observed in some IBS patients, yet its precise role in the disease pathophysiology remains undetermined (Borody, George et al. 1989).

As there are indications that the composition of the faecal microbiota is impaired in IBS, faecal microbiota transplantation (FMT), a therapy expected to ameliorate the gut microbiome composition, seemed a logical treatment option (Iribarren, Maasfeh et al. 2022). This therapeutic strategy, which has already proven highly effective in the treatment of *Clostridioides difficile* infection (Kachrimanidou, Sarmourli et al. 2016), has been repeatedly tested in IBS using various designs: case studies and open-label trials have shown improvement in symptom severity, as summarised in (Elhusein and Fadlalmola 2022; Korner and Lorentz 2023), but results from randomised controlled trials (RCTs) have been inconsistent (Halkjaer, Christensen et al. 2018; Johnsen, Hilpusch et al. 2018; Aroniadis, Brandt et al.

2019; Holster, Lindqvist et al. 2019; El-Salhy, Hatlebakk et al. 2020; Lahtinen, Jalanka et al. 2020; Holvoet, Joossens et al. 2021; Singh, Alm et al. 2022; El-Salhy, Gilja et al. 2024). The therapeutic effect of FMT, if any, is certainly not universal: rather, important evidence points to efficacy only in specific subgroups (of important meta-analyses, e.g., (Halkjaer, Lo et al. 2023; Korner and Lorentz 2023)). Namely, the variations in the origin of the faecal material, the handling of the faeces before transplantation, the dose and the route of administration may all influence the success of the therapy (El-Salhy, Gilja et al. 2024; Lo, Hung et al. 2024).

Few interventional IBS studies have reported data on the composition of the gut microbiota before and after FMT, and, to our knowledge, none of these used FMT with stool material pooled from multiple preselected donors. Theoretically, such pooling should increase the probability of including a super-donor (a donor with a readily engrafting microbiota) and may also create a novel quality of transplanted material by broadening the repertoire of microbes. Selection of donors based on the microbial profile of their stool may also increase the likelihood of success by reducing the dependence on the microbial characteristics of any single donor.

The aim of this study was to test the effect of FMT in alleviating IBS symptoms. The transferred material was either live microbiota mixed from several healthy donors preselected based on their stool bacteriome composition, or placebo - the same material inactivated by autoclaving. We hypothesised that transferring a high-diversity microbiota would lead to changes in the patient's gut microbiome composition and/or function, and the new microbiota would subsequently alleviate IBS symptoms.

2. METHODS

2.1. Trial Design

The MISCEAT study was a three-arm, randomised, double-blind, placebo-controlled, crossover trial. It was conducted at the Department of Internal Medicine, Third faculty of Medicine and Charles University and Thomayer University Hospital, Prague, Czechia. From July 2021 to September 2023, we enrolled adult IBS patients diagnosed with diarrhoea-predominant or mixed subtypes. Follow-up was completed in March 2024. All participants underwent an initial screening and baseline assessment and provided written consent. Eligible consenting participants were randomised to one of three treatment groups: group A received live FMT in the first intervention period, followed by placebo FMT in the second period; group B received placebo FMT in the first period, followed by live FMT in the second period; group C received placebo FMT in both periods (**Figure 1**).

2.2. Participants and randomisation

Eligible participants were adults (aged 18 to 65 years) diagnosed with diarrhoea-predominant or mixed IBS according to the Rome IV criteria (Drossman 2016) . The exclusion criteria were the use of antibiotics or probiotics within one month before enrolment, renal or hepatic failure, inflammatory bowel disease, gastrointestinal malignancy, systemic autoimmune disease, HIV infection, diabetes mellitus, uncontrolled thyroid dysfunction, pregnancy, a history of major abdominal surgery, moderate to severe anxiety, bipolar disorder, and schizophrenia. All eligible participants provided written informed consent before randomisation.

Participants were then randomised by block randomisation (blocks of nine) in a 3:3:3 ratio using codes generated via a dedicated randomisation website. The personnel who generated the codes were not

involved in patient assessment, enrolment, or FMT allocation. The allocated treatment group remained concealed from the participants, caregivers, and study personnel throughout the trial.

2.3. The FMT interventions and their timing

The cross-over design was implemented as a consecutive application of two FMTs to each participant. Two weeks after randomisation, the first intervention period started with the administration of FMT twice in two consecutive days (either live FMT or placebo, depending on the randomisation group). The first period was concluded four weeks later by measuring the primary and secondary outcomes. After a four-week washout period, the second intervention period started with another double administration of live or placebo FMT in two consecutive days. Four weeks later, the primary and secondary outcomes for the second period were assessed. The final part of the trial was a long-term follow-up until study week 32.

2.4. The mixed stool material for FMT, autoclaved placebo, and the FMT procedure

Faecal material for all study interventions was the same for all participants and their FMTs, having been prepared in one batch as described in the published protocol (Hurych, Vejmelka et al. 2022). Briefly, stool donor candidates for the preparation of faecal microbiota were recruited among blood donors at Thomayer University Hospital and preclinical medical students from the Second Faculty of Medicine, Charles University, Prague. A total of 58 individuals were screened as potential donors. The inclusion criteria included a normal BMI ($18.7 - 27 \text{ kg/m}^2$), not being on restrictive diets, and having a Bristol stool scale between 3 and 4 (Cammarota, Ianiro et al. 2019).

Eight donors were identified based on the maximum distance (i.e. dissimilarity) of their faecal microbiota from that of IBS patients, and their high alpha diversity. After proper screening (Cammarota,

laniro et al. 2019), donors were asked to provide repeated stool doses, which were processed and frozen at -80°C. Each donor contributed multiple doses of stool with totals ranging from 0.054 kg to 5.7 kg and a median of 2.3 kg. Once a sufficient amount of stool had been collected for the entire study, the faecal donations were thawed on melting ice and mixed into one 19 kg bulk in a large stainless steel vessel, still on ice and under a nitrogen atmosphere. This mixed bulk was then aliquoted into identical vials containing an equivalent of 13-14 g of stool mass. Three such aliquots were then used as one dose for FMT (~40 g of stool in ~130 ml buffer).

The vials were labelled with non-revealing codes. The placebo aliquots were first autoclaved and then frozen at -80°C, whereas the live microbiota aliquots were frozen immediately after preparation. The vials for non-autoclaved live microbiota aliquots were first autoclaved empty to gain an appearance identical to that of the autoclaved placebo. All clinical investigators were blinded to the allocation of patients and the contents of the vials. Laboratory staff were also blinded to the contents of the vials they distributed, and the distribution was based on a predefined automated algorithm running on the central server.

Each intervention consisted of two FMTs by colonic enema, administered one day apart, using the same assigned substance (live FMT or placebo): on the first day, the enema was preceded by bowel preparation, and on the second day, it was administered without bowel preparation. Bowel preparation consisted of one sachet of Fortrans (Beaufour Ipsen Industrie, Dreux, France) in 1 L of water, taken the afternoon before the enema, with adequate fluid intake and a low-fibre, puréed diet. The reduction in Fortrans dose to approximately one quarter of the standard colonoscopy preparation dose was intended to facilitate enema retention and promote proximal colonic distribution.

Immediately before administration, the study microbiota mixture was thawed in a water bath (at 37°C), with the tubes being inverted intermittently to mix. The FMT procedure was performed with the patient in the left lateral (“Sims”) position. Using a standard enema kit, a lubricated catheter was gently inserted per rectum to deliver the allocated intervention (live FMT or placebo). The time of enema instillation was recorded by the physician. After administration, patients remained in the left lateral position for 2 minutes, then were repositioned to supine with the pelvis elevated for 2 minutes, followed by 2 minutes in the right lateral position to facilitate intraluminal distribution. Patients then remained lying in bed for at least 20 minutes to promote enema retention and later self-recorded the time of the first bowel movement.

2.5. Trial outcomes

The primary outcome was the change in IBS-SSS during a four-week intervention period, depending on the intervention received in that period (live FMT or placebo). Secondary outcomes included (a) short-term changes in IBS-SSS (two weeks post-FMT) and (b) long-term changes in IBS-SSS and IBS-QoL scores (from baseline until week 32). In the long-term effects analyses, participants who received live FMT in any intervention period (Groups A and B) were compared with those who received placebos only (Group C). Additional secondary outcomes included changes in anthropometric characteristics (weight, BMI, waist circumference, body fat measured at baseline and weeks 2, 5, 10, 13, and 32), visceral and subcutaneous fat (measured at baseline and weeks 5, 13, and 32), daily loose stool frequency and pain intensity (measured at baseline and week 32), and gut microbiome composition.

2.6. Assessment of Adverse Events

The participants were regularly monitored for adverse events (AEs), and the research team recorded all occurrences. We provided each participant with contact details for the study coordinators to report any

safety concerns that required prompt clinical evaluation. An independent Data Monitoring and Safety Committee (DMSC) oversaw safety throughout the trial and had the authority to pause or terminate the trial if the rate of severe AEs exceeded the predefined 5% threshold. The severity of AEs was graded based on the guideline outlined in the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (Institute 2017).

2.7. Stool sample collection and gut microbiome profiling

Participants provided stool samples for microbiome profiling before and after intervention at pre-specified time points (**Figure 1** and **Supplementary Figure 1**). Faecal samples were collected by patients at home using a paper strip placed over the toilet seat and a sampling kit. The samples were immediately frozen in a pre-chilled transport container in their home freezers and brought in these containers to the nearest study visit. There, the samples were transferred to a -80°C freezer. DNA was extracted from approximately 100 mg of stool using PowerSoil kits on a QiaCube instrument (both Qiagen, Hilden, Germany).

In all samples, the bacteriome profiling was performed using next-generation amplicon sequencing of a PCR product spanning the V3 and V4 regions of the 16S rRNA gene. The tagged specific primer sequences, 341-F (Muyzer, de Waal et al. 1993) and 806-R (Caporaso, Lauber et al. 2011) were synthesised with additional bases that increase the sequencing signal heterogeneity (Cinek, Hubackova et al. 2026). Each sample was amplified in a duplicate of PCR reactions using high-fidelity DNA-dependent Phusion Plus DNA polymerase (Thermo Scientific, US). After reaction clean-up with SPRIselect Beads (Beckman Coulter, US) at a ratio of 1:0.8 between the PCR product and the bead solution, samples were indexed using a limited count of PCR cycles with Illumina Nextera index primers. Indexed PCR products were purified again using the SPRIselect Beads, equalised, and sequenced on the

NextSeq 2000 platform (Illumina, USA) using a P2 sequencing kit run at 2x260 cycles. The samples were sequenced along with several replicates of DNA from the Microbial Mock Community (HM-276D, BEI Resources, USA) and with negative controls (PCR-grade water instead of DNA).

The raw data were downloaded as demultiplexed *fastq* files with trimmed adapters; the heterogeneity spacers were trimmed, and the orientation of amplicons was unified using a custom Python script (as detailed in (Cinek, Hubackova et al. 2026), script available at github.com/ondrejcinsek/primer_spacers). Reads of the same fragment were merged using USEARCH (Edgar 2010). After quality inspection, data were processed in the DADA2 pipeline (Callahan, McMurdie et al. 2016). Reads were filtered with the maximum allowed number of expected errors equal to 2, demultiplexed, chimerae removed, and the size distribution of amplicons checked. The amplicon sequence variants (ASV) were counted and taxonomically classified using the Silva taxonomic database, version 138. A phylogenetic tree of ASVs was constructed using FastTree2 (Price, Dehal et al. 2010) with the generalised time-reversible model of evolution and gamma20 likelihood. Negative controls were checked for the absence of a significant signal, and the mock community positions were assessed for agreement with their declared content. To verify agreement between replicates, an ordination plot was created by principal coordinate analysis of the Bray–Curtis dissimilarity among individual reactions; after its inspection, duplicates were merged by summing counts. The taxonomic and frequency tables were converted into a *phyloseq* object and analysed in the R programming language with the *phyloseq* (McMurdie and Holmes 2013) and *vegan* (Oksanen, Guillaume Blanchet et al. 2019) packages.

2.8. Metagenomic substudy on the acquisition or replacement of new bacterial strains and on antibiotic resistance genes

A subset of 100 samples was also investigated using shotgun metagenomic sequencing. These were samples from 25 subjects who belonged to the study arms that received one pair of interventions with live microbiota (i.e. not the placebo-only Arm C). Two samples flanking each intervention were subjected to shotgun metagenomic sequencing (taken before and four weeks after the intervention), i.e. four samples per patient, as each patient underwent two interventions in the cross-over design. We also sequenced the contents of the live microbiota used for FMT.

Total faecal DNA was subjected to metagenomic library construction using the Illumina DNA Prep kit (Illumina, Foster City, CA, U.S.A.) according to the manufacturer's instructions. This involved tagmentation of genomic DNA, post-tagmentation clean-up, indexing and cleaning the libraries. The resulting libraries were then sequenced on an Illumina NextSeq 2000 instrument using NextSeq 2000 P3 Reagents (bidirectional, 2x150 bases sequencing).

The demultiplexed sequencing data were downloaded, filtered and trimmed by quality using Trimmomatic (Bolger, Lohse et al. 2014), human reads were eliminated, and the microbial metagenome communities were profiled using MetaPhlan 4 (Blanco-Miguez, Beghini et al. 2023). Reads were classified into the Species-level Genome Bins (SGBs) (Pasolli, Asnicar et al. 2019). Individual strains were identified, and the phylogenetic trees plotted using StrainPhlAn (Blanco-Miguez, Beghini et al. 2023), run with somehow relaxed stringency criteria in order to increase the sensitivity to capture a strain acquisition or replacement following the live FMT (parameters *marker_in_n_samples*=50, *sample_with_n_markers*=50, *breadth_thresh*=50, similar to what was used to be set by the '*--relaxed_parameters*' option in the previous version of StrainPhlAn).

Antibiotic resistance genes were detected in the metagenomes using ResFinder 4.3.3 (Florensa, Kaas et al. 2022). This software generates a score for each of the tested antibiotics or their groups: the score is zero if no antibiotic resistance (AR) gene is found for a given antibiotic, 1 if there is incomplete identity and incomplete length of the match between the metagenome and the database of AR genes, 2 for complete identity but incomplete length of the match, or 3 for a complete match that covers the entire length of at least one resistance gene to the respective antibiotic. We then used the McNemar test to assess whether the change in the AR score was discordant between live microbiota FMT and placebo FMT. In addition, changes in the scores were tested using mixed-effects linear regression, similar to the models described below for the alpha diversity indices; in the latter test, the same weight was given to an increase in the AR gene score after live FMT as to a decrease in the score after placebo FMT.

2.9. Statistical analysis

The analysis set included all participants who successfully completed both intervention periods and adhered to the prescribed visit schedule. Dropouts and participants who seriously violated the visit schedule were removed. Baseline data were summarised by counts and percentages or means and standard deviations. Differences between treatment groups at baseline were tested by the chi-square test (categorical outcomes) or one-way ANOVA (quantitative outcomes) without adjustment for multiple comparisons. Changes since baseline were analysed using longitudinal generalised estimating equation (GEE) models, with outcome measures at different visits considered as a multivariate response. The visit was modelled as a factor variable. The effect of live FMT was included at the end of the period that started with the administration of live FMT. The analysis accounted for potential carry-over effects by including them in the model at the second-period visits, following the earlier administration of live FMT. The working variance function was identity; the working correlation matrix was first-order autoregressive.

282
283 Bacterial alpha diversity was quantified as the observed count of ASVs, Chao1, ACE, Shannon and
284 Simpson metrics, using the *phyloseq* package. Effects of FMT interventions on alpha diversity were
285 analysed using a mixed-effects regression model (MLM), with alpha diversity metrics as outcome
286 variables. Random intercepts were the subjects receiving the FMT at one level and the FMT intervention
287 (the first or second period) at the second level. The fixed effects were the terms for the type of
288 microbiota transferred (live FMT or placebo), time (before or after FMT), and their interaction: the
289 interaction term of the sample after the live FMT then showed the effect of live FMT. Eight time points
290 per subject were used, one being the last sample before the intervention, and three were collected at
291 weeks 1, 2, and 4 after the intervention. Long-term effects were assessed using samples at baseline
292 (week -2 to -1) and those brought to the last follow-up visit (taken at weeks 26 to 36) - here the model
293 was constructed with a random intercept for the patient, whereas fixed effects were timing of the
294 sample (before baseline or sample brought to the last visit), whether the patient received live FMT
295 (groups A and B) or placebo only (group C), and their interaction which showed the long-term effect of
296 live FMT. Both models were also used in a version with adjustment for the composition of the
297 bacteriome by adding terms for the sample scores of the first three components from principal
298 component analysis of Hellinger-transformed bacterial abundances at the genus level.

299
300 The data were then filtered to eliminate low-abundance taxa, defined as those with less than 20 reads in
301 no more than two samples, and agglomerated at taxonomic ranks of phylum, class, order, family, and
302 genus for downstream analyses. Beta diversity, reflecting between-sample differences, was estimated
303 using Bray-Curtis and Sørensen distance matrices, calculated with the *vegan* package at the level of ASVs
304 and genera.

305

306 The impact of FMT on the abundance of individual bacterial taxa was assessed using models identical to
307 those for alpha diversity measures. Here, the outcome variables were the individual taxa abundances
308 from various taxonomic ranks, transformed using centred log-ratio (CLR). The model coefficients for the
309 interaction term between intervention and time point (before and after) represented the log-fold
310 change in taxon abundance as a measure of live FMT effect. A taxon abundance was considered to have
311 changed with live FMT if increased at least 2-fold or decreased to at least 0.5 at a statistical significance
312 level greater than 0.05, following Bonferroni correction for the number of tested taxa. To avoid
313 stochastic noise, only taxa with a mean abundance exceeding 0.1% were considered, and each
314 association signal was visually examined on an abundance plot for outliers.

3. RESULTS

3.1. Trial enrolment and characteristics of the study participants

Of 114 screened patients, 61 were randomised into three groups as previously described: Group A - live FMT first, followed by placebo (n = 21); Group B - placebo first, then live FMT (n = 20), or Group C - placebo only (n = 20). Out of the 61 randomised participants, an additional 11 had to be excluded for the following reasons: failure to show up for the treatment (n = 2), loss to follow-up (n = 4), and protocol violations (n = 5). See **Figure 2** for more details.

The patients' characteristics are listed in **Table 1**. Almost two-thirds of the participants were males (32 of 50, 64%), and the mean age was 36.7 (SD 10.0). The average IBS symptom severity score at baseline was 265.1 (SD 97.2). None of the reported baseline factors were significantly different across the three treatment groups.

3.2. The primary outcome: a lack of effect of live FMT on the IBS symptom severity score

A decreasing temporal trend in the mean IBS symptom severity score was observed in all three groups (**Figure 3, Table 2, Supplementary Figure 2**), without any detectable effect of the live FMT.

Administering live FMT was associated with a decrease of 27.5 points in the mean IBS-SSS in Group A during the first period and a decrease of 39.1 points in Group B during the second period, whereas placebo FMT was associated with a decrease of approximately 50 points in groups B and C during the first period and a decrease of 14.7 and 5.3 in groups A and C during the second period. The estimated effect of live FMT on the IBS-SSS was +2.5 points (95% CI -33.6 to +38.6) with a p-value of 0.893 (**Table 3**). Thus, live FMT did not affect the mean IBS-SSS at all.

3.3. Secondary clinical outcomes: almost no significant changes

We studied possible early effects of live FMT on IBS-SSS at two weeks after the intervention: there was no such effect (-9.4 points, $P=0.568$). Weight, visceral fat, and waist circumference were measured at 5 weeks and were also unaffected (**Table 3**). There was a weakly significant effect at 5 weeks on body fat mass (an increase by 5.63 kg with live FMT, 95% CI 0.75 to 10.5, $P=0.024$), paralleled by borderline-significant increases in body fat percentage (an increase by 4.19%, 95% CI -0.01 to 8.38, $P=0.051$), subcutaneous fat measured by caliper (an increase by 3.68%, 95% CI -0.16 to 7.51, $P=0.060$), and in BMI (an increase by 2.22 kg/m², 95% CI -0.28 to 4.71, $P=0.082$).

No significant long-term improvement was observed in any of the clinical outcomes measured 32 weeks after baseline, irrespective of the period when live FMT was administered (**Table 4**).

3.4. Safety: no serious adverse events were observed

All randomised participants ($n = 59$) who presented for treatment, and received at least one dose of the allocated intervention (live FMT or placebo) were assessed for AEs. All reported and observed AEs were between grade 1 and 2 of the CTCAE guideline, i.e., mild, self-limiting, or mild requiring non-invasive treatment. We did not have any occurrence of AEs in Grade 3 or higher, i.e. requiring invasive medication or hospitalization (**Table 5**). One participant in the placebo-only arm C discontinued the study because of intolerance of the enema. Overall, 27 adverse events were recorded, of which six were classified as treatment-emergent: these included flatulence, loss of appetite, enema intolerance and diarrhoea.

3.5. Administration of live FMT profoundly affected the composition of gut microbiota

Administration of the mixed live microbiota in FMT was associated with a prominent increase in the gut bacteriome alpha diversity; a strong effect was apparent in the observed count of ASVs, Chao1, ACE and Shannon indices, whereas the effect on the Simpson index was only borderline-significant (**Figure 4, Supplementary Table 1-A**). There was no net effect of the timing of post-intervention samples, indicating that the effect occurred immediately and persisted over the 4 weeks follow-up post-intervention. The estimates did not materially change upon the adjustment for the first three components of PCA of the bacteriome.

The baseline alpha diversity before placebo interventions was higher than that before live FMT interventions (e.g. 16.9, CI95% 2.2 to 31.5, $P=0.025$ for the Chao1 index): as this was a cross-over study, this indicates that the effect of live FMT persisted over the washout period and was carried forward to the second intervention. There was no net effect of FMT using placebo (P for all indices > 0.5 for the term of FMT itself).

In the evaluation of the long-term follow-up samples (~ week 32), no effects of live FMT versus placebo could be observed on either of the alpha diversity indices, unless the models were adjusted for the composition of the bacteriome by including the first three principal component scores from Hellinger-transformation-based PCA on genus abundance (**Supplementary Table 1-B**). After this adjustment, an increase in Observed species, Chao1, and ACE indices was observable after live FMT. This together indicates that the long-term effect is limited to certain taxonomic compositions only.

3.6. The patients' bacteriome composition became more similar to the transferred live FMT material

The acquisition of novel taxonomic entities after live FMT was also reflected by an increase in similarity (i.e. a decrease in distance) between patients' gut microbiomes and the administered mixed stool

material (**Figure 5-A** projects these vectors on two dimensions of a principal coordinate analysis). Vectors after live microbiota were mostly oriented towards the composition of the material used for live FMTs, whereas the vectors after placebo FMT were oriented randomly. This was reflected in the prominent rise in Sørensen similarity of the samples after live FMT to the transferred material ($P=1.6 \times 10^{-14}$ for the ASV level and $P=1.4 \times 10^{-9}$ for the genus level, **Figure 5-B**) than by Bray-Curtis similarity ($P=0.012$ for the ASV level, but non-significant for the genus-based distance, $P=0.48$). The effect of live FMT applied as the first intervention was carried over to the baseline of the second intervention period (Sørensen similarity, $P=8.7 \times 10^{-4}$). In the analysis of long-term effects (baseline vs samples collected at week ~ 32), patients who received any live FMT had a higher Sørensen similarity to this material at the ASV level ($P=0.023$) as compared to the placebo-only group C, but no effect was noted at the genus level, or in the Bray-Curtis similarity measure.

The impetus for change provided by administering live FMT material was also reflected in the length of bacteriome trajectories in the multi-dimensional ASV space. These trajectories were assessed as vectors from the sample taken before the intervention to the sample taken 4 weeks after the intervention. After live FMT, the vectors were significantly longer than after placebo FMT: at the level of ASV, for the Sørensen metric the trajectories were longer by 0.19 (95%CI 0.16-0.22, $P<10^{-10}$), and for the Bray-Curtis metric by 0.17 (95%CI 0.13-0.21, $P<10^{-10}$), **Figure 5-C**.

In the analysis of long-term effects (baseline vs sample at week ~32), the similarity to live FMT significantly increased in those who received live FMT vs the placebo-only group when measured by Sørensen index at the level of ASV ($P=0.023$) but not at the level of genus, and not by the Bray-Curtis measure. The lengths of total trajectories travelled over the study in the multi-dimensional microbiome space also differed: the Sørensen distance between the baseline and the sample at ~32 weeks was

longer for patients who received live FMT than for those on placebo only ($P=0.0084$), and a borderline longer trajectory was also observed in the Bray-Curtis metric ($P=0.061$).

3.7. Bacterial taxa changing their abundance with live FMT, strain acquisition and strain replacement

The administration of live FMT influenced the abundance of bacterial taxa, of which several significantly increased in quantity (**Table 6**, and the illustration of taxa abundance over time in **Supplementary Table 2**). With the exception of *Collinsella* and Prevotellaceae, these were mostly taxa of initially minor abundance (**Figure 6-B**).

Because the observed changes in bacterial abundances are likely consistent with acquisitions of the bacterial strain from the live FMT, we used the metagenomic substudy data to work with individual strain sequences. Strain analyses were performed on two types of taxa - the ones significantly associated with live FMT, and the 50 most frequently observed SGBs. Firstly, we examined strains of taxa whose abundance changed significantly after live FMT: of these, only three had meaningful coverage and a sufficient number of strain-informative markers for the StrainPhlAn analysis. In *Prevotella copri* clade A (now *Segatella copri*), 14 patients newly acquired the strain from the live FMT material and retained it (patients No. 2, 6-8, 10, 15, 19, 22, 29, 35, 36, 39, 42, 44 in **Figure 7**). Two other patients (No. 16, 21) had their original strain replaced by that from live FMT material. Of the patients carrying *P. copri* prior to FMT, only two retained their original strain (patients 31 and 45, **Figure 7**). In the case of *Paraprevotella clara*, the low abundance of the microorganism did not allow any definite conclusions to be drawn about strain transfer. Secondly, strain analysis was attempted to trace individual strains among the 50 most abundant SGBs whose overall abundance did not change with live FMT. Of these 50, in 25 cases the tree was not generated or was too small, or the FMT material did not contain the SGB. Examination of the remaining trees indicated a probable replacement of an original

strain with the FMT strain in 6 patients: one had 4 strain replacements, one 2 replacements and the remaining four had one documented replacement. The replacement was noted for *Bacteroides uniformis*, *Alistipes putredinis*, *Phocaeicola vulgatus*, *Barnesiella intestinihominis*, *Faecalibacterium prausnitzii*, *Roseburia faecis* and *Parabacteroides distasonis*.

3.8. The magnitude of the microbiome reaction to live FMT, and the clinical response

Our study was underpowered for formal analyses of the clinical response by the magnitude of microbiome response. We, however, performed an unplanned post-hoc analysis, inspecting the clinical response in the patients whose microbiome was resilient to the live FMT: **Supplementary Figure 3** displays the quartile of patients with the least microbiome response on the background of the whole study. It is apparent that the clinical response to live FMT was in line with other patients, with a decrease in IBS-SSS in six out of nine individuals. It is therefore unlikely that the microbiome-unresponsive individuals would delineate any special group within the study.

3.9. *Prevotella* analysis

An unplanned analysis was performed to verify the recent observation made by Hartikainen et al (Hartikainen, Jalanka et al. 2024) who had found an increase in the *Prevotella*/*Bacteroides* ratio after FMT. In our study, we noted two prominent increases among Bacteroidota: one was lowly prevalent *Paraprevotella*, another - orthogonal in the ordination plot - was *Prevotella* (*Prevotella copri* clade A from the more accurate metagenomic sequencing). To follow the paper by Hartikainen et al, we calculated the *Prevotella*/*Bacteroides* ratio from our metagenomic data using a formula of *Prevotella* / (*Bacteroides* + *Phocaeicola*), as the genus *Bacteroides* has been split since 2020 (Garcia-Lopez, Meier-Kolthoff et al. 2019). The ratio (with 0.001 for those samples with zero in the numerator, and 1000 imputed for those with zero in the denominator) was then entered as the logarithm-transformed

dependent variable into the mixed-effects model specified above. The index increased significantly after FMT with live microbiota (coefficient estimating a 36-fold increase, $P = 6.7 \times 10^{-5}$).

3.10. Antibiotic resistance genes

There were 46 antibiotics whose resistance genes occurred at least once in the metagenomic substudy. Of these, only one antibiotic had its AR score significantly associated with live FMT vs placebo FMT. This nominally significant gain in ampicillin resistance ($P_{\text{uncorrected}} = 0.023$ in McNemar test) would not survive correction for the number of independent tests performed. In the mixed effects model, we observed a similar finding: administration of live microbiota FMT was associated with a mean increase in the AR gene match score for ampicillin of 1.04 ($P_{\text{corrected}} = 0.011$, $P_{\text{raw}} = 2.9 \times 10^{-4}$) compared to placebo FMT, **Supplementary Figure 4**. There was no change in AR gene occurrence for other antibiotics (all corrected P values > 0.80), **Supplementary Figure 5**.

4. DISCUSSION

In this randomised trial, we observed no appreciable effects of mixed-donor live FMT as compared to autoclaved placebo on the primary outcome, the IBS symptom severity score. Although the enemas using live microbiota dramatically changed the patients' faecal bacteriomes, these compositional shifts, donor-derived strain engraftment, and marked increases in faecal bacterial richness did not translate into relief of IBS symptom severity.

4.1. The effect of FMT on IBS-SSS, or lack thereof, in previous studies

The contrast we observed between the shift in microbiota following FMT, and a complete lack of clinical effect, is consistent with a pattern gradually emerging in the literature over the past decade. Several randomised trials have demonstrated that FMT can modify the gut microbiome without any parallel clinical benefit. In a Danish study (Halkjaer, Christensen et al. 2018), administration of FMT capsules was associated with restoration of alpha diversity and with modification of the bacteriome composition, but placebo was significantly superior to active treatment in the reduction of symptoms measured by IBS-SSS. In a US multicentre study prematurely terminated for futility upon interim analysis, the similarity between donors' and patients' bacteriomes increased after active FMT, yet no clinical effect followed (Aroniadis, Brandt et al. 2019). In a Finnish RCT (Lahtinen, Jalanka et al. 2020), the FMT was associated with sustained changes in microbiota composition and its functional potential, but again these changes were not accompanied by symptom resolution (Hartikainen, Jalanka et al. 2024). The degree of donor microbiota engraftment varied, but clinical effects were entirely absent in a study testing two antibiotic pretreatment regimens before FMT in IBS (Singh, Alm et al. 2022). Finally, a recent very large multicentre study from Norway (with 299 subjects in the intervention and 151 in the placebo group)

provided no support for therapeutic efficacy of FMT in IBS, despite profound reaction of the gut microbiome to the transferred stool material (Johnsen, Juul et al. 2026).

Shortly before Johnsen et al published the latter mentioned negative interventional study (Johnsen, Juul et al. 2026), a metaanalysis by Aumpan et al (Aumpan, Watanabe et al. 2026) summarised then available 13 interventional studies with a total of almost 700 subjects, finding no benefit of FMT in the intention-to-treat analysis, but a weak improvement of symptom in the per-protocol analysis. An editorial to this meta-analysis recommended that future studies incorporate serial microbiome shotgun sequencing, donor-recipient matching analyses and assessment of long-term microbial survival (Kupcinkas and Ianiro 2026), analyses similar to what we performed.

The absence of clinical effects in our and the above-cited studies may point against a true participation of microbiome imbalance in the aetiology of IBS. Microbiome imbalance (if present) may not be a cause, but rather a mere marker of another undisclosed truly etiological factor. The Bradford-Hill criteria (Hill 1965), traditionally used for assessing causality when observing association, are indeed not fulfilled: the association between bacteriome and IBS is inconsistent between populations, not specific, without proven temporal sequence, and if the imbalance is removed in an experiment, the condition often remains unaffected.

4.2. Placebo effects

The parallel reduction in IBS-SSS across allocation groups (**Figure 3**) highlights the importance of placebo effects in IBS trials. Amelioration of symptoms following placebo has been observed in studies of FMT in IBS since the very first randomised clinical trial (Johnsen, Hilpusch et al. 2018), including the largest study so far (Johnsen, Juul et al. 2026). In our study, the decrease in severity score spanned longer time

interval than elsewhere. This may be related to multiple visits required by our cross-over design. The only other available cross-over study in IBS is the one by Aroniadis et al. using FMT capsules (Aroniadis, Brandt et al. 2019), and also there a decrease in IBS-SSS was apparent after both interventions.

Strong placebo effects are also often seen in IBS trials testing other treatment modalities than FMT (Dorn, Kaptchuk et al. 2007; Bosman, Elsenbruch et al. 2021). It is conceivable that frequent observations, patient interaction and sampling may *per se* remind the patients of earlier taught regimen recommendations, thus causing an improvement in subjective symptoms. However, a large meta-analysis by Patel et al considers frequent visits as a remedy to, rather than a cause of, the placebo effect (Patel, Stason et al. 2005). The effect of visits may be dependent on their type, namely on the type of contact between the patient and health care professional: a carefully designed trial aiming at disentangling the placebo effect indicated that IBS significantly improves with frequent patient-practitioner contact with specific encouraging behavioural interventions, as compared to limited contact (Kaptchuk, Kelley et al. 2008). Complicating the pattern further, it should be noted that only a small proportion of patients with IBS seek gastroenterology specialist care, and the decision to seek healthcare is influenced not only by the severity of pain, faecal urgency and other symptoms, but also by psychosocial disturbances that are not intrinsic to IBS (Drossman 1999).

This all however does not exclude a remote possibility that the administration of autoclaved FMT substance was actually instrumental in symptom relief. In such a case, the design of many studies using faecal material as placebo would be inadequate. For instance, Holster et al (Holster, Lindqvist et al. 2019) speculated whether bowel cleansing on its own might be beneficial and ameliorate the symptoms, or whether placebo handling might render the substance novel therapeutic qualities: the latter is relevant for our study where placebo was subjected to autoclaving that not only killed microbes, but

also might have altered the composition of the metabolome. The faecal metabolome was not assessed in the present study.

4.3. The faecal material and its application route

We transferred the same mixed live faecal material in every live FMT. It was prepared as a high-diversity microbial suspension, pooled from multiple preselected donors. This strategy resulted in creating an 'artificial super-donor' whose microbiota was documented to engraft in the recipients' colon. The concept of a 'super-donor' has been proposed to explain donor-dependent efficacy of FMT, but clear defining criteria remain lacking. El-Salhy and colleagues speculated that certain clinical and microbiota features of their superdonor - such as being vaginally born, antibiotic-sparing, physically active, and normobiotic with high microbial diversity - might have contributed to the success of FMT (El-Salhy, Casen et al. 2021). While our donors did not meet all of these criteria, they were rigorously screened according to international guidelines valid in 2020 (Camarota, Ianiro et al. 2019), and selected for their bacteriomes being distant from those of IBS patients (Hurych, Vejmelka et al. 2022).

The FMT was delivered via retention rectal enema with subsequent positioning, primarily because of its ease and relative non-invasiveness, which facilitated repeated interventions. Route of administration was not significant determinant of symptom improvement in a recent meta-analysis (Aumpan, Watanabe et al. 2026), and one later study, the largest so far, used the same route as we did (Johnsen, Juul et al. 2026).

4.4. Adverse events and secondary clinical effects of FMT intervention

The FMT intervention was generally very safe as to reported adverse events, in line with previous studies (Halkjaer, Lo et al. 2023; Aumpan, Watanabe et al. 2026). We also systematically assessed

anthropometric parameters, which, to our knowledge, have not been reported in prior FMT trials in IBS. We identified a borderline-significant increase in body fat mass, BMI, body fat percentage, and subcutaneous fat at 4 weeks since intervention. In contrast, there was no long-term effect (at 32 weeks) on any of the anthropometric parameters. The findings warrant caution regarding potential metabolic consequences of FMT in IBS.

4.5. Changes in gut microbiome after FMT

Live FMT induced profound changes in the gut bacteriome of IBS patients. The intervention led to a marked increase in richness-based alpha diversity indices (observed number of taxa, Chao1, Shannon), while the Simpson index reflecting bacteriome evenness remained unchanged. This indicates that donor-derived taxa predominantly expanded within the low-abundance fraction without disturbing the evenness of the recipients' bacteriome. Beta-diversity analyses showed that recipients' microbiomes shifted closer to the composition of the donor material. Among the engrafting taxa, *Prevotella* species stood out, expanding robustly in multiple recipients, consistent with its ecological adaptability. These findings are highly concordant with those of previous randomised FMT trials in IBS. Many of them observed similar changes in alpha diversity and a shift towards the donor's composition (Halkjaer, Christensen et al. 2018; Goll, Johnsen et al. 2020; Guo, Lin et al. 2021; Holvoet, Joossens et al. 2021; Hartikainen, Jalanka et al. 2024; Johnsen, Juul et al. 2026). Among others, the follow-up paper of a Finnish RCT (Lahtinen, Jalanka et al. 2020) identified *Prevotella* among the most consistent engrafting genera, similarly to our result (Hartikainen, Jalanka et al. 2024).

4.6. Strengths and limitations

The strengths of our study can be seen in: (1) the cross-over design, where participants in arms A and B serve as their own control. This rather robust design has not been widely utilised in IBS trials; the only

such similar study used oral capsules as the microbiota delivery route (Aroniadis, Brandt et al. 2019). (2) We included a placebo-only group to account for placebo effects frequently observed in IBS trials, and minimise the likely limitations of a simple cross-over design in assessing long-term intervention effect. Also our scheme with a high visit frequency should have helped against placebo effect (Patel, Stason et al. 2005). (3) The enema route for FMT delivery is simple, well-tolerated and less invasive than colonoscopy or upper endoscopy; therefore, each intervention consisted of two microbiota transfers performed two days apart. Only the first of each pair of enemas was preceded by bowel preparation, which was achieved using a less stringent bowel cleansing regimen than required for colonoscopy, with one-quarter of the usual dosage. This cautious approach may be relevant in light of a study showing that the effects of bowel cleansing on colonic microbiota are dose-dependent (Jalanka, Salonen et al. 2015). (4) Finally, we used pooled microbiota to ensure engraftment of a wide spectrum of microbes from donors with microbiome profiles distant from those of IBS patients.

Our trial also has several limitations; firstly, we did not perform colonoscopy and thus did not perform biopsy that would allow assessing mucosa-associated gut microbiota. Secondly, the limited study size did not allow performing subgroup analyses. We did not assess the faecal metabolome. Finally, despite this is a chronic disease, the treatment was not repeated and our trial thus did not test a real durable therapeutic program scheduled to reflect the long-term character of ailments of subjects with IBS.

4. 7. Conclusions

In conclusion, we have demonstrated that FMT of material mixed from carefully preselected donors profoundly alters the faecal bacteriome of patients with diarrhoea-predominant or mixed-type IBS. However, the complete lack of clinical effects renders no support for FMT as a treatment for IBS.

613 Perhaps it is time to stop conducting similar studies and reconsider designs that address other
614 therapeutic options.

Technical information:

Author Contributions: OC, PK, JH, and JV contributed to the conception and design of the clinical trial. JH, LH, LK, and OC contributed to the microbiome analysis for donor selection. JH, OC, and JV contributed to the donor screening. JH, and OC contributed to preparing the microbiota mixture for the study. MK contributed to the power size calculations and statistical analysis. OC, KL, KH and IDK contributed to the analysis of the microbiome data. MB: antibiotic resistance gene analysis. MK and IP: body composition monitoring and anthropometric measurements. IDK, MK, OC, MB and JV contributed to writing the manuscript draft.

Acknowledgements: The authors gratefully acknowledge Peter Holger Johnsen and Rasmus Goll for their invaluable consultations at the design phase of this study. We thank the study nurses for their assistance with study procedures and participant care. Michaela Čermáková is acknowledged for her technical assistance in preparing samples for bacteriome profiling. Special thanks go to Jakub Beránek, Simona Tomšů, Kateřina Poláčková, and Kateřina Chudá for their help in mixing stool samples for FMT preparation. Lastly, we sincerely thank Vladyslav Larionov, a former member of our laboratory at Charles University, Prague, and now with Dr Ing. h.c. F. Porsche Aktiengesellschaft (Porsche 365 AG) for maintaining the study database and allocation engine in his free time.

Competing interests: None declared.

Patient consent for publication: Not required

638 **Ethics approval:** This clinical trial was approved by the Ethics Committee of the Institute for Clinical and
639 Experimental Medicine and Thomayer University Hospital, Czechia (G-18-26), and was registered at
640 clinicaltrials.gov (NCT04899869).

641

642 **Funding:** The study was supported by the Ministry of Health of the Czech Republic through the Czech
643 Health Research Council grant NV19-01-00127 and the *National Institute of Virology and Bacteriology*
644 (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union—Next Generation
645 EU.

646

647 BEI Resources (Manassas, VA) are gratefully acknowledged for providing us with the Microbial Mock
648 Community DNA (HM-276D).

649

650 **Data availability:**

651 The sequencing data and with relevant study metadata have been deposited at the NCBI Sequencing Read
652 Archive: PRJNA1367098 (the 16S rDNA sequencing, and metadata), PRJNA1214038 (the metagenomic
653 substudy - strain tracing and antibody resistance genes).

654

655

References

- Aroniadis, O. C., L. J. Brandt, et al. (2019). "Faecal microbiota transplantation for diarrhoea-predominant irritable bowel syndrome: a double-blind, randomised, placebo-controlled trial." Lancet Gastroenterol Hepatol **4**(9): 675-685.
- Aumpan, N., J. Watanabe, et al. (2026). "Fecal Microbiota Transplantation for Symptom Improvement in Patients With Irritable Bowel Syndrome: Systematic Review and Meta-Analysis of Randomized Controlled Trials." Gastroenterology **171**(4): 667-681.
- Blanco-Miguez, A., F. Beghini, et al. (2023). "Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4." Nat Biotechnol.
- Bolger, A. M., M. Lohse, et al. (2014). "Trimmomatic: a flexible trimmer for Illumina sequence data." Bioinformatics **30**(15): 2114-20.
- Borody, T. J., L. George, et al. (1989). "Bowel-flora alteration: a potential cure for inflammatory bowel disease and irritable bowel syndrome?" Med J Aust **150**(10): 604.
- Bosman, M., S. Elsenbruch, et al. (2021). "The placebo response rate in pharmacological trials in patients with irritable bowel syndrome: a systematic review and meta-analysis." Lancet Gastroenterol Hepatol **6**(6): 459-473.
- Callahan, B. J., P. J. McMurdie, et al. (2016). "DADA2: High-resolution sample inference from Illumina amplicon data." Nat Methods **13**(7): 581-3.
- Camarota, G., G. Ianaro, et al. (2019). "International consensus conference on stool banking for faecal microbiota transplantation in clinical practice." Gut **68**(12): 2111-2121.
- Caporaso, J. G., C. L. Lauber, et al. (2011). "Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample." Proc Natl Acad Sci U S A **108** Suppl 1(Suppl 1): 4516-22.
- Cinek, O., K. Hubackova, et al. (2026). "Heterogeneity Primer Spacers Improve the Performance of Massively Parallel Amplicon Sequencing of the V3-V4 Region of the 16 S rDNA as well as the 18 S Region for Blastocystis Subtyping." Microb Ecol **89**(1).
- Dorn, S. D., T. J. Kaptchuk, et al. (2007). "A meta-analysis of the placebo response in complementary and alternative medicine trials of irritable bowel syndrome." Neurogastroenterol Motil **19**(8): 630-7.
- Drossman, D. A. (1999). "Do psychosocial factors define symptom severity and patient status in irritable bowel syndrome?" Am J Med **107**(5A): 41S-50S.
- Drossman, D. A. (2016). "Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features and Rome IV." Gastroenterology.
- Drossman, D. A., L. Chang, et al. (2026). "Disorders of Gut-Brain Interaction and the Rome V Process." Gastroenterology **170**(6): 1083-1098.
- Edgar, R. C. (2010). "Search and clustering orders of magnitude faster than BLAST." Bioinformatics **26**(19): 2460-1.
- El-Salhy, M., C. Casen, et al. (2021). "Responses to faecal microbiota transplantation in female and male patients with irritable bowel syndrome." World J Gastroenterol **27**(18): 2219-2237.
- El-Salhy, M., O. H. Gilja, et al. (2024). "Factors affecting the outcome of fecal microbiota transplantation for patients with irritable bowel syndrome." Neurogastroenterol Motil **36**(1): e14641.
- El-Salhy, M., J. G. Hatlebakk, et al. (2020). "Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study." Gut **69**(5): 859-867.
- Elhusein, A. M. and H. A. Fadlalmola (2022). "Efficacy of Fecal Microbiota Transplantation in Irritable Bowel Syndrome Patients: An Updated Systematic Review and Meta-Analysis." Gastroenterol Nurs **45**(1): 11-20.
- Enck, P., Q. Aziz, et al. (2016). "Irritable bowel syndrome." Nat Rev Dis Primers **2**: 16014.

Florensa, A. F., R. S. Kaas, et al. (2022). "ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes." *Microb Genom* **8**(1).

Garcia-Lopez, M., J. P. Meier-Kolthoff, et al. (2019). "Analysis of 1,000 Type-Strain Genomes Improves Taxonomic Classification of Bacteroidetes." *Front Microbiol* **10**: 2083.

Goll, R., P. H. Johnsen, et al. (2020). "Effects of fecal microbiota transplantation in subjects with irritable bowel syndrome are mirrored by changes in gut microbiome." *Gut Microbes* **12**(1): 1794263.

Guo, Q., H. Lin, et al. (2021). "Dynamic changes of intestinal flora in patients with irritable bowel syndrome combined with anxiety and depression after oral administration of enterobacteria capsules." *Bioengineered* **12**(2): 11885-11897.

Halkjaer, S. I., A. H. Christensen, et al. (2018). "Faecal microbiota transplantation alters gut microbiota in patients with irritable bowel syndrome: results from a randomised, double-blind placebo-controlled study." *Gut* **67**(12): 2107-2115.

Halkjaer, S. I., B. Lo, et al. (2023). "Fecal microbiota transplantation for the treatment of irritable bowel syndrome: A systematic review and meta-analysis." *World J Gastroenterol* **29**(20): 3185-3202.

Hartikainen, A. K., J. Jalanka, et al. (2024). "Fecal microbiota transplantation influences microbiota without connection to symptom relief in irritable bowel syndrome patients." *NPJ Biofilms Microbiomes* **10**(1): 73.

Hill, A. B. (1965). "The Environment and Disease: Association or Causation?" *Proc R Soc Med* **58**: 295-300.

Holster, S., C. M. Lindqvist, et al. (2019). "The Effect of Allogenic Versus Autologous Fecal Microbiota Transfer on Symptoms, Visceral Perception and Fecal and Mucosal Microbiota in Irritable Bowel Syndrome: A Randomized Controlled Study." *Clin Transl Gastroenterol* **10**(4): e00034.

Holvoet, T., M. Joossens, et al. (2021). "Fecal Microbiota Transplantation Reduces Symptoms in Some Patients With Irritable Bowel Syndrome With Predominant Abdominal Bloating: Short- and Long-term Results From a Placebo-Controlled Randomized Trial." *Gastroenterology* **160**(1): 145-157 e8.

Hurych, J., J. Vejmelka, et al. (2022). "Protocol for faecal microbiota transplantation in irritable bowel syndrome: the MISCEAT study - a randomised, double-blind cross-over study using mixed microbiota from healthy donors." *BMJ Open* **12**(6): e056594.

Institute, N. C. (2017). Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0. D. o. H. a. H. Services. Bethesda, MD: U.S., National Institutes of Health.

Iribarren, C., L. Maasfeh, et al. (2022). "Modulating the gut microenvironment as a treatment strategy for irritable bowel syndrome: a narrative review." *Gut Microbiome (Camb)* **3**: e7.

Jalanka, J., A. Salonen, et al. (2015). "Effects of bowel cleansing on the intestinal microbiota." *Gut* **64**(10): 1562-8.

Johnsen, P. H., F. Hilpusch, et al. (2018). "Faecal microbiota transplantation versus placebo for moderate-to-severe irritable bowel syndrome: a double-blind, randomised, placebo-controlled, parallel-group, single-centre trial." *Lancet Gastroenterol Hepatol* **3**(1): 17-24.

Johnsen, P. H., F. E. Juul, et al. (2026). "Faecal microbiota transplantation in irritable bowel syndrome (REFIT2): a randomised, double-blind, placebo-controlled, phase 3 trial." *Lancet*.

Kachrimanidou, M., T. Sarmourli, et al. (2016). "Clostridium difficile infection: New insights into therapeutic options." *Crit Rev Microbiol* **42**(5): 773-9.

Kaptchuk, T. J., J. M. Kelley, et al. (2008). "Components of placebo effect: randomised controlled trial in patients with irritable bowel syndrome." *BMJ* **336**(7651): 999-1003.

Korner, E. and A. Lorentz (2023). "Fecal microbiota transplantation in patients with irritable bowel syndrome: an overview of current studies." *J Appl Microbiol* **134**(3).

Kupcinskis, J. and G. Ianiro (2026). "Finding the Signal in the Noise: Can Fecal Microbiota Transplantation Deliver for Irritable Bowel Syndrome?" *Gastroenterology* **171**(4): 593-595.

Lacy, B. E., F. Mearin, et al. (2016). "Bowel Disorders." *Gastroenterology* **150**(6): 1393-1407.

- Lahtinen, P., J. Jalanka, et al. (2020). "Randomised clinical trial: faecal microbiota transplantation versus autologous placebo administered via colonoscopy in irritable bowel syndrome." Aliment Pharmacol Ther **51**(12): 1321-1331.
- Lo, S. W., T. H. Hung, et al. (2024). "Clinical efficacy and safety of faecal microbiota transplantation in the treatment of irritable bowel syndrome: a systematic review, meta-analysis and trial sequential analysis." European Journal of Medical Research **29**(1).
- McMurdie, P. J. and S. Holmes (2013). "phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data." PLoS One **8**(4): e61217.
- Muyzer, G., E. C. de Waal, et al. (1993). "Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA." Appl Environ Microbiol **59**(3): 695-700.
- Oksanen, J., F. Guillaume Blanchet, et al. (2019). vegan: Community Ecology Package. R package version 2.5-6.
- Pasolli, E., F. Asnicar, et al. (2019). "Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle." Cell **176**(3): 649-662 e20.
- Patel, S. M., W. B. Stason, et al. (2005). "The placebo effect in irritable bowel syndrome trials: a meta-analysis." Neurogastroenterol Motil **17**(3): 332-40.
- Price, M. N., P. S. Dehal, et al. (2010). "FastTree 2--approximately maximum-likelihood trees for large alignments." PLoS One **5**(3): e9490.
- Rangan, V., S. Mitsuhashi, et al. (2018). "Risk Factors for Fecal Urgency Among Individuals With and Without Diarrhea, Based on Data From the National Health and Nutrition Examination Survey." Clin Gastroenterol Hepatol **16**(9): 1450-1458 e2.
- Singh, P., E. J. Alm, et al. (2022). "Effect of antibiotic pretreatment on bacterial engraftment after Fecal Microbiota Transplant (FMT) in IBS-D." Gut Microbes **14**(1): 2020067.
- Sperber, A. D., S. I. Bangdiwala, et al. (2021). "Worldwide Prevalence and Burden of Functional Gastrointestinal Disorders, Results of Rome Foundation Global Study." Gastroenterology **160**(1): 99-114 e3.

781 Table 1: Baseline characteristics of study participants.

Variable	All participants <i>n</i> =50	Group A (live FMT, then placebo) <i>n</i> =18	Group B (placebo, then live FMT) <i>n</i> =16	Group C (both interventions with placebo) <i>n</i> =16	P- value
Male gender	32 (64.0%)	10 (55.6%)	11 (68.8%)	11 (68.8%)	0.64
Age at baseline [years]	36.66 (10.03)	37.60 (10.09)	37.27 (10.98)	35.00 (9.38)	0.73
IBS symptom severity score	265.10 (97.15)	268.33 (122.16)	278.44 (71.29)	248.12 (91.25)	0.67
IBS types: diarrhoeal	41 (82%)	15	12	14	0.73
mixed	9 (18%)	3	4	2	
Height [cm]	178.00 (9.61)	176.83 (10.72)	178.03 (6.14)	179.28 (11.41)	0.77
Weight [kg]	75.86 (20.74)	79.45 (27.77)	76.26 (12.87)	71.44 (18.12)	0.54
BMI [kg/m ²]	23.82 (5.63)	25.12 (7.81)	24.04 (3.84)	22.12 (3.84)	0.30
Daily number of loose stools	2.26 (1.63)	2.44 (1.42)	2.12 (1.75)	2.19 (1.80)	0.84
Pain intensity scale	4.10 (2.50)	4.28 (2.78)	4.38 (2.06)	3.62 (2.66)	0.66
IBS Quality of Life Score	46.75 (16.71)	44.49 (16.81)	45.96 (18.70)	50.09 (14.95)	0.61
Number of stool samples with bacteriome profiles	11.1 (2.4)	11.5 (2.6)	10.4 (2.1)	11.2 * (2.5)	0.42

782

783 Means (counts) are shown in the first line and standard deviations (percentages) in the second line.

784 The P-values test the differences in the means (percentages) between the three groups.

785

786 *) Stool samples from one subject in Group C were collected but inadvertently omitted from the

787 sequencing run due to a clerical error. Bacteriome data are therefore from 49 subjects.

788 **Table 2: Changes in average IBS-SSS scores by group and period.**

<i>First period: baseline to week 5</i>					<i>Second period: week 9 to week 13</i>			
Group	Treatment	Beginning	End	Change	Treatment	Beginning	End	Change
A	Live FMT	268.3	240.8	-27.5	Placebo FMT	231.9	217.2	-14.7
B	Placebo FMT	278.4	228.4	-50.0	Live FMT	225.9	186.9	-39.1
C	Placebo FMT	248.1	197.8	-50.3	Placebo FMT	199.4	194.1	-5.3

789

790

791 The first period started at baseline and finished at week five. Only group A received live FMT at the start of this period. The second period started

792 at week 8 and finished at week 13. Only group B received live FMT at the start of this period.

793 **Table 3. Estimated effects of live FMT on the study outcomes.**

Outcome	Effect of live FMT	95% CI	P value
IBS symptom severity score at 4 weeks after FMT	2.48	-33.61 to 38.57	0.893
IBS symptom severity score at 2 weeks after FMT	-9.40	-41.67 to 22.87	0.568
Weight [kg]	6.57	-2.99 to 16.12	0.178
BMI [kg/m ²]	2.22	-0.28 to 4.71	0.082
Body fat [kg]	5.63	0.75 to 10.5	0.024
Body fat [%]	4.19	-0.01 to 8.38	0.051
Visceral fat [L]	0.59	-0.14 to 1.32	0.114
Subcutaneous fat (calliper) [%]	3.68	-0.16 to 7.51	0.060
Waist circumference [cm]	4.21	-2.27 to 10.7	0.203

794

795

796 **Table 4. Estimated long-term clinical effects of live FMT on the expected outcomes at 32 weeks.**

Outcome	Mean change from baseline till week 32		Live FMT effect	95% conf. interval	P value
	<i>Groups A+B</i>	Group C			
	(had live FMT)	(had only placebo)			
IBS symptom severity score	-46.21	-66.56	32.93	(-24.89 to 90.75)	0.258
Weight [kg]	-0.67	0.20	-0.57	(-3.6 to 2.45)	0.703
BMI [kg/m ²]	-0.24	0.06	-0.07	(-0.95 to 0.8)	0.864
Body fat [kg]	-0.79	-0.16	0.03	(-2.14 to 2.19)	0.981
Body fat [%]	-0.55	-0.23	-0.30	(-2.2 to 1.61)	0.756
Visceral fat [L]	-0.23	-0.07	-0.02	(-0.32 to 0.27)	0.872
Subcutaneous fat (calliper) [%]	0.32	-0.34	1.18	(-0.14 to 2.51)	0.079
Waist circumference [cm]	-0.68	-0.50	0.28	(-2.4 to 2.97)	0.833
IBS QoL	11.12	9.05	1.04	(-7.29 to 9.36)	0.803
Daily number of loose stools	-0.88	-0.56	-0.28	(-1.26 to 0.71)	0.576
Pain intensity scale	-1.48	-1.25	0.16	(-1.13 to 1.45)	0.808

797

798 **Table 5: Adverse events on the course of the trial**

	Group A, live FMT, then placebo	Group B, placebo, then live FMT	Group C, placebo only	Total
All adverse events, n (%)	12 (44.4 %)	8 (29.6 %)	7 (25.9 %)	27 (100 %)
Treatment-emergent AEs, n (%)	2 (7.4 %)	2 (7.4 %)	2 (7.4 %)	6 (22.2 %)
Flatulence	2 (7.4 %)	0	0	2 (7.4 %)
Loss of appetite	0	0	1 (3.7 %)	1 (3.7 %)
Enema intolerance	0	0	1 (3.7 %)	1 (3.7 %)
Diarrhea	0	2 (7.4 %)	0	2 (7.4 %)
Non-treatment emergent AEs, n (%)	10 (37.0 %)	6 (22.2 %)	5 (18.5 %)	21 (77.8 %)
Upper respiratory tract infection	4 (14.8 %)	2 (7.4 %)	1 (3.7 %)	7 (25.9 %)
COVID-19 related	2 (7.4 %)	1 (3.7 %)	2 (7.4 %)	5 (18.5 %)
Urinary tract infection	1 (3.7 %)	0	0	1 (3.7 %)
Insomnia/constipation	1 (3.7 %)	1 (3.7 %)	0	2 (7.4 %)
Eczema	0	1 (3.7 %)	0	1 (3.7 %)
Vaginosis	0	0	1 (3.7 %)	1 (3.7 %)
Oral aphthous lesion	0	0	1 (3.7 %)	1 (3.7 %)
Mild uveitis	1 (3.7 %)	0	0	1 (3.7 %)
Other adverse events	1 (3.7 %)	1 (3.7 %)	0	2 (7.4 %)

799

800 The reported adverse events (AEs) reported were all Grade ≤ 2 – Mild self-limiting and/or mild requiring

801 noninvasive treatment.

802 The AEs are reported as counts of events (n) and percentages of grand total (%). All participants were

803 assessed (59 subjects).

804 **Table 6. The effect of live FMT on the relative bacterial abundance**

805

Bacterial taxon	Median taxon abundance in patients	Abundance in live FMT material	Fold change associated with live FMT [95% CI]	Corrected P value	Notes
p. Actinobacteriota └ c. Coriobacteriia └ o. Coriobacteriales └ f. Coriobacteriaceae └ g. <i>Collinsella</i>	6.7%	0.66%	2.3 [1.5, 3.5]	0.014	
└ f. Eggerthellaceae └ g. <i>Enterorhabdus</i> └ g. <i>Senegalimassilia</i> └ g. <i>Slackia</i>	0.29% 0.26% 0.22%	0.14% 0.10% 0.08%	2.7 [1.7, 4.1] 4.2 [2.8, 6.3] 2.6 [1.7, 3.8]	0.0014 2.8 x 10 ⁻⁹ 5.9 x 10 ⁻⁴	
p. Bacteroidota └ c. Bacteroidia └ o. Bacteroidales └ f. Prevotellaceae └ g. <i>Paraprevotella</i> └ g. <i>Prevotella_9</i>	1.0% 0.01% 0.11%	1.5% 0.81% 0.63%	7.4 [3.6, 15] 2.9 [1.7, 4.8] 23 [11, 47]	5.3 x 10 ⁻⁶ 0.0098 < 10 ⁻¹⁰	(1) (2)
└ f. Rikenellaceae └ g. <i>Rikenellaceae RC9</i>	0	0.60%	16 [9.6, 27]	< 10 ⁻¹⁰	(3,4)
p. Desulfobacterota └ c. Desulfovibrionia └ o. Desulfovibrionales └ f. Desulfovibrionaceae └ g. <i>Desulfovibrio</i>	0.06% 0.06% 0.06% 0.05% 0.00%	0.24% 0.27% 0.24% 0.27% 0.12%	1.8 [1.2, 2.7] 2.0 [1.3, 3.0] 2.3 [1.5, 3.4] 2.1 [1.4, 3.2] 3.2 [2.1, 5.0]	0.038 0.010 0.0061 0.021 1.4 x 10 ⁻⁵	

p. Firmicutes					
└ c. Bacilli					
└ o. Lactobacillales	0.43%	0.14%	0.33 [0.19, 0.59]	0.0049	
└ f. Streptococcaceae	0.38%	0.19%	0.30 [0.17, 0.53]	0.0018	
└ g. <i>Streptococcus</i>	0.39%	0.16%	0.36 [0.21, 0.63]	0.048	
└ o. Erysipelotrichales					
└ f. Erysipelotrichaceae	0.24%	0.12%	2.9 [1.7, 4.9]	0.0029	
└ g. <i>Holdemanella</i>	0.03%	0	2.7 [1.7, 4.3]	0.0029	
└ c. Clostridia					
└ o. Christensenellales	0.33%	2.6%	2.6 [1.6, 4.0]	0.0010	
└ f. Christensenellaceae	0.31%	2.7%	2.6 [1.7, 4.0]	6.4 x 10 ⁻⁴	
└ g. Christensenellaceae	0.31%	2.7%	2.8 [1.8, 4.4]	0.0012	
R-7 group					
└ o. Lachnospirales					
└ f. Lachnospiraceae					
└ g. <i>Marvinbryantia</i>	0.20%	0.10%	2.9 [1.6, 5.1]	0.030	
└ g. [<i>Eubacterium</i>]	0.09%	0.23%	0.28 [0.16, 0.50]	0.0022	
ventriosum group					
└ o. Oscillospirales					
└ f. Oscillospiraceae					
└ g. <i>UCG-005</i>	0.15%	0.19%	2.6 [1.7, 4.1]	0.0052	
└ c. Negativicutes					
└ o. Veillonellales-Selenomonadales	0.78%	2.2%	2.6 [1.6, 4.3]	0.0029	
└ f. Veillonellaceae	0.78%	2.3%	3.4 [2.1, 5.6]	1.0 x 10 ⁻⁴	
└ g. <i>Dialister</i>	0.36%	1.1%	3.3 [2.0, 5.4]	8.7 x 10 ⁻⁴	

806

807

808 p., phylum; c. class; o., order; f. family; g., genus

809 The fold-change is the exponentiated regression coefficient of the interaction term between the timing after intervention (vs before), and
810 intervention with live FMT (vs placebo). As the bacterial abundance is CLR-transformed, the coefficient is log-fold change.

811 P-values have been corrected for the number of tested taxa using Bonferroni procedure.

812

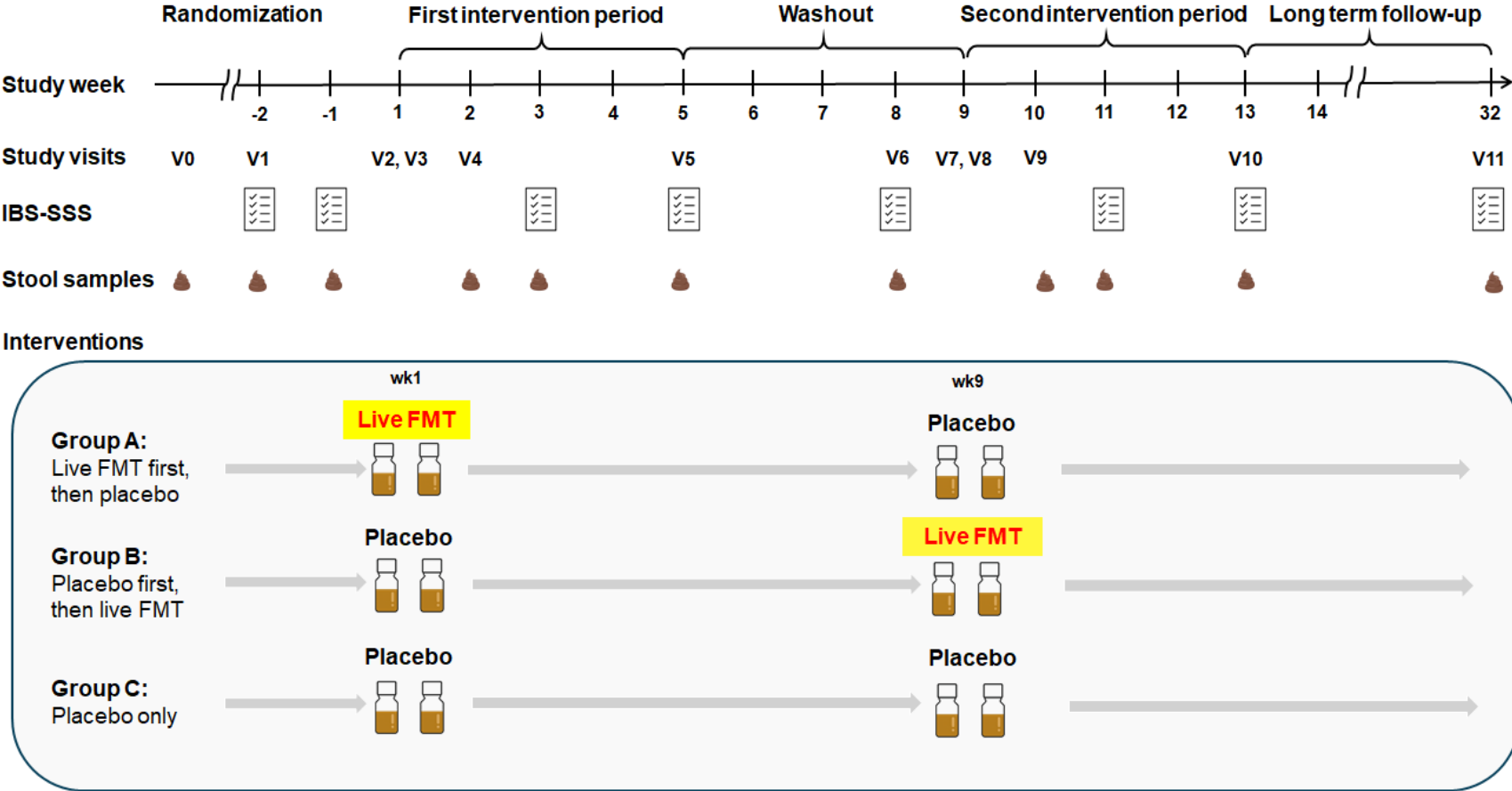
813 (1) In the long-term model (samples at ~ wk 32 vs baseline; patients who received live FMT vs those who had placebo only), the taxon fold
814 change with live FMT was 1.5, $P_{\text{corrected}} = 0.043$

815 (2) The fold change in the long-term model was 3.2, $P_{\text{corrected}} = 0.0089$

816 (3) The fold change in the long-term model was 2.8, $P_{\text{corrected}} = 1.4 \times 10^{-8}$

817 (4) Taxonomically classified as *Rikenellaceae RC9 gut group*

818 Figure 1: The trial scheme.



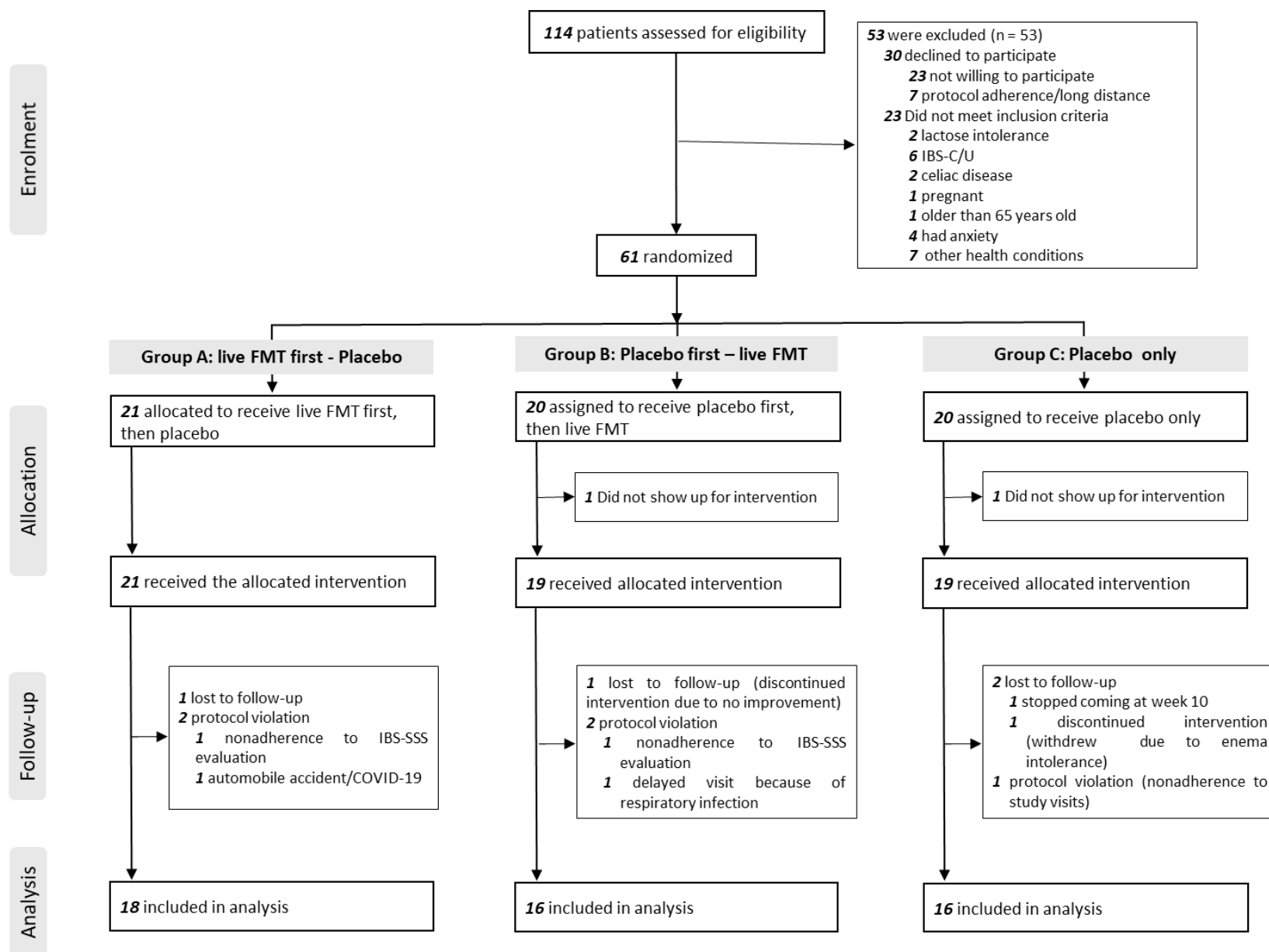
819

820 Timing is expressed in study weeks, with interventions at weeks 1 and 9..

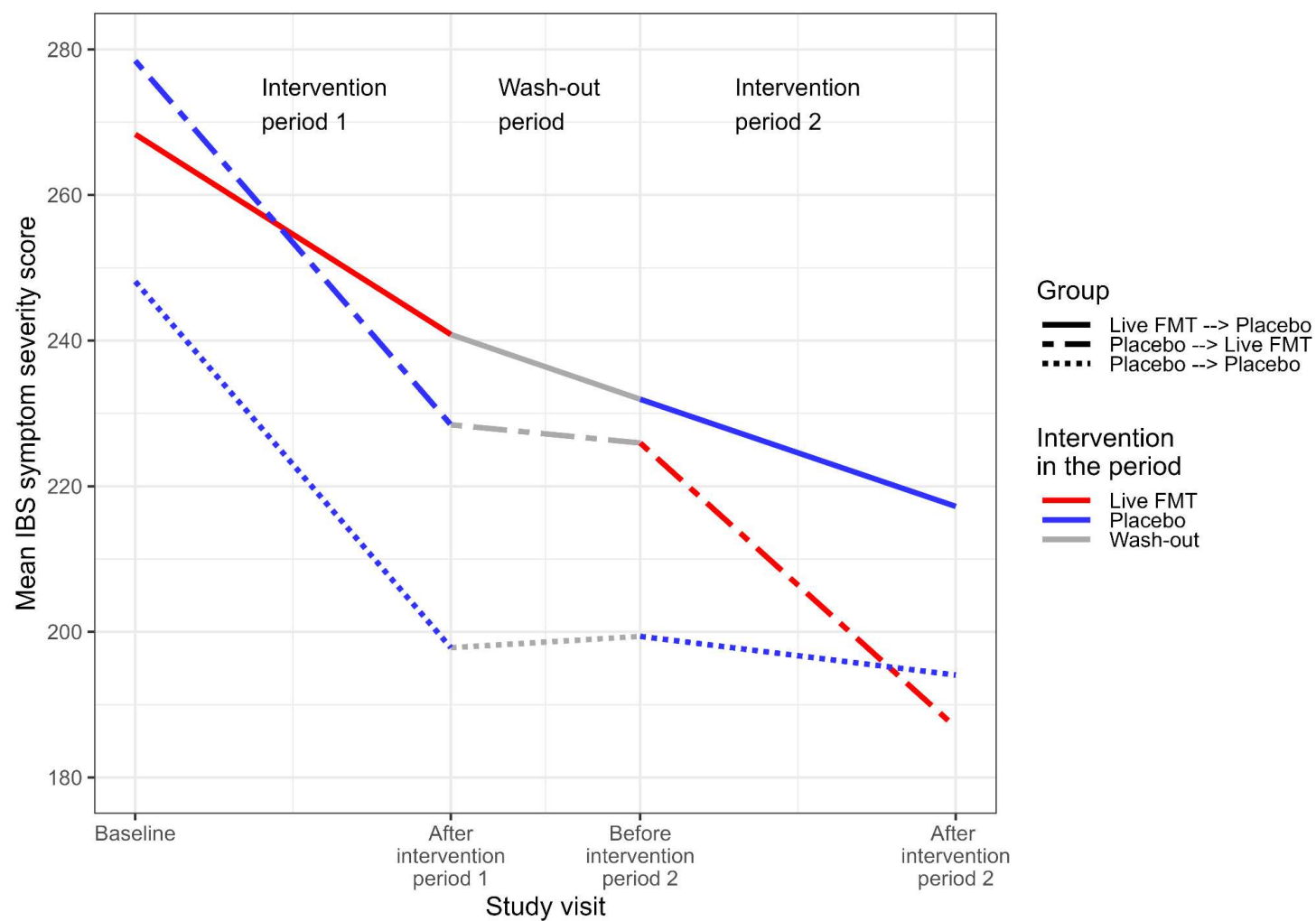
821

822 **Figure 2: CONSORT diagram of participants' flow through each stage of the clinical trial.**

823

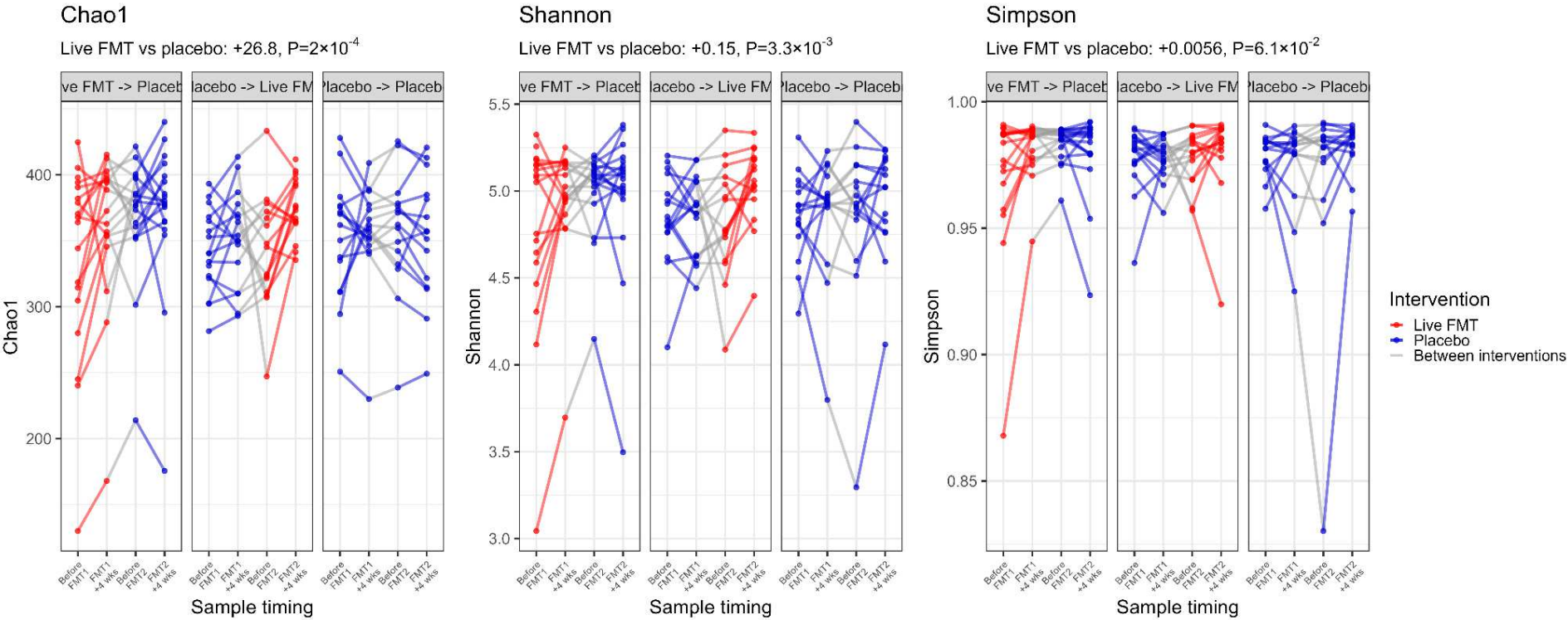


824 **Figure 3: The mean IBS-SSS scores by group and visits.**



825 Group A (solid line) received live FMT during period 1. Group B (dashed line) received live FMT during period 2. Group C (dotted line) received
 826 placebo FMT in both periods.

827 **Figure 4: Bacterial alpha diversity.**



828

829

830 **Figure 5: The trajectories of the bacteriome profiles after the FMT**

831

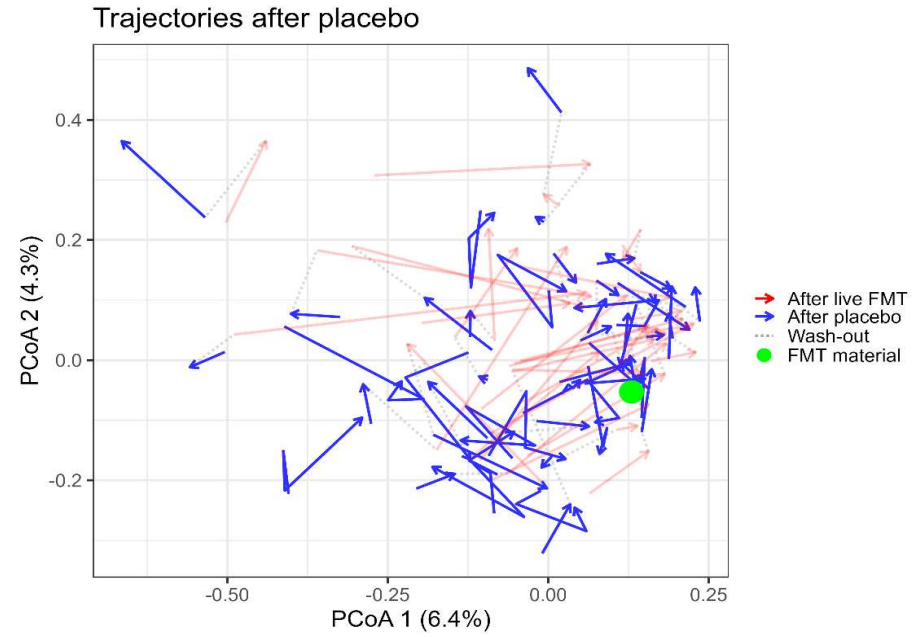
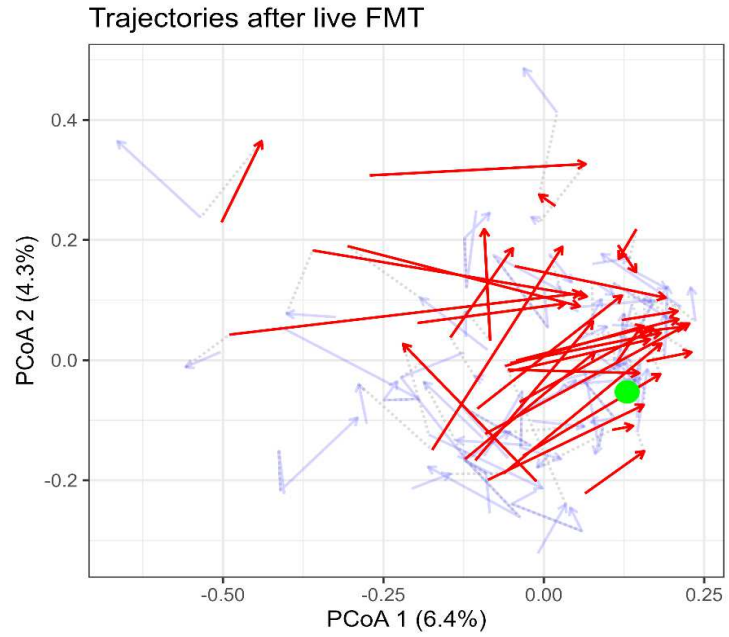
832 **(A) Principal coordinates analysis (PCoA) plot based on Sørensen distance at the genus level**

833 Each patient's trajectory consists of three segments connecting four important sampling time points: the red segment represents changes

834 following live FMT, the blue segments indicate changes after placebo FMT, and the grey dotted segments correspond to the washout period

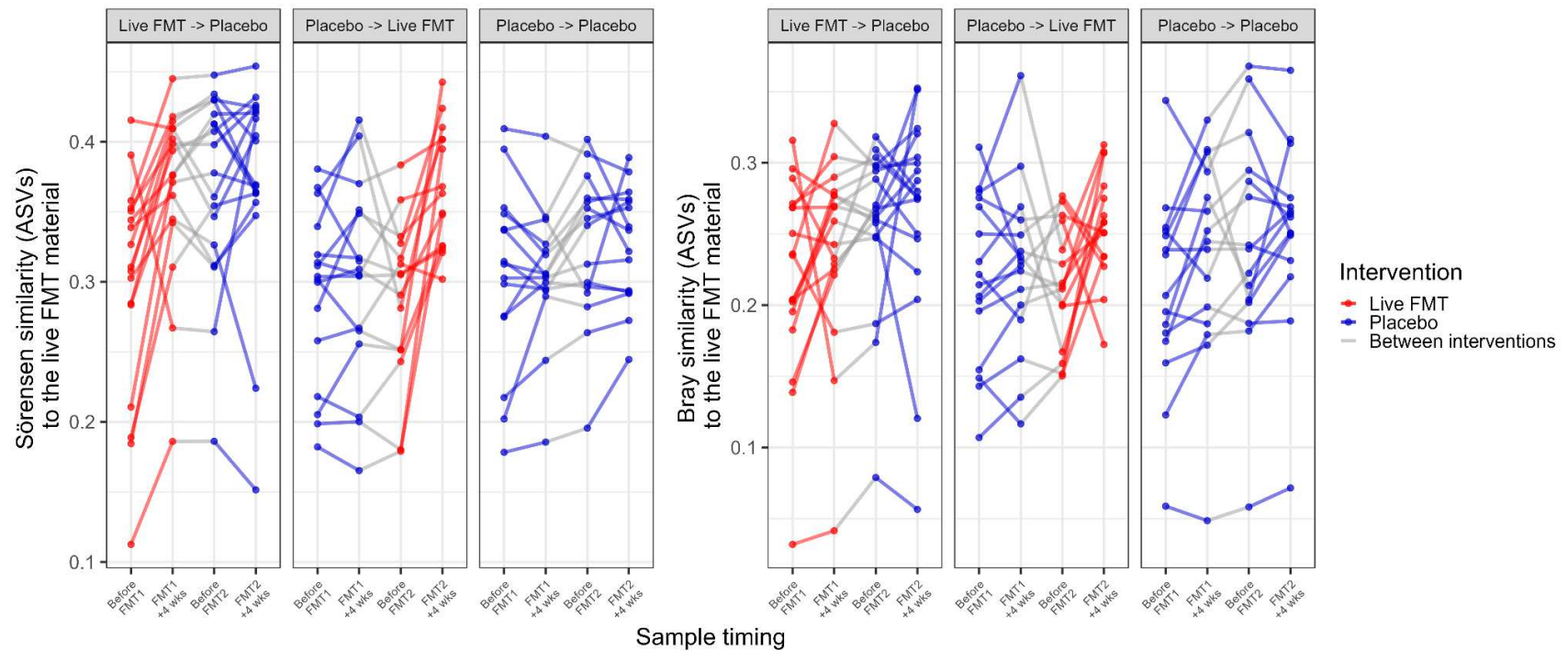
835 between interventions. The green circle represents the live FMT material. The vectors after live FMT are mostly parallel and longer than the

836 randomly oriented blue vectors after placebo.



837

838 **(B) The similarity to the FMT material increases after live FMT.** Administration of live FMT (before vs 1 month after, red lines) significantly
839 increased the similarity of patients' samples to the live FMT material as compared to placebo (blue lines). This is apparent both in Sørensen
840 distance ($P=1.6 \times 10^{-14}$) and in Bray-Curtis distance ($P=0.012$).

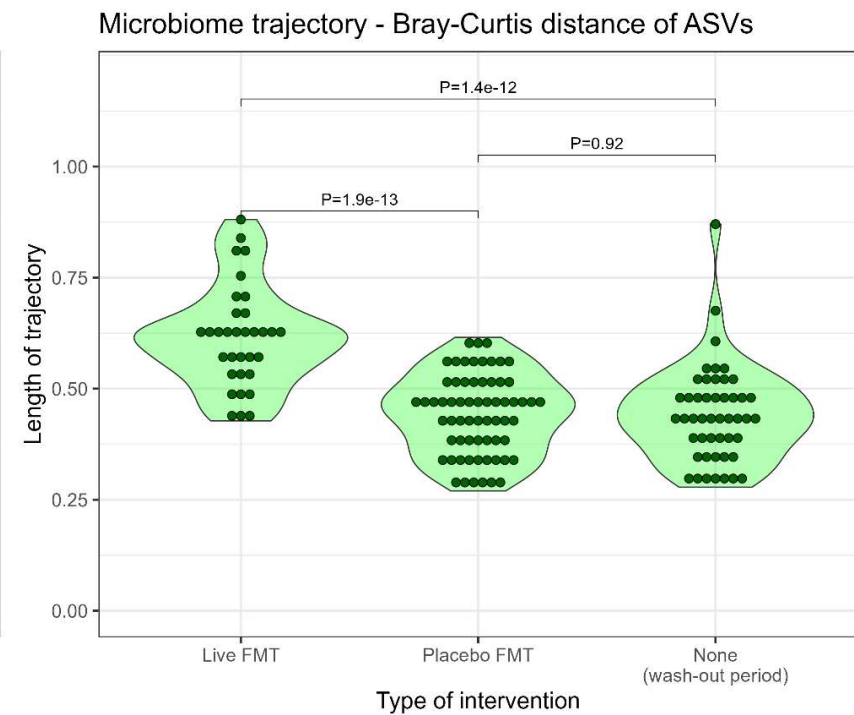
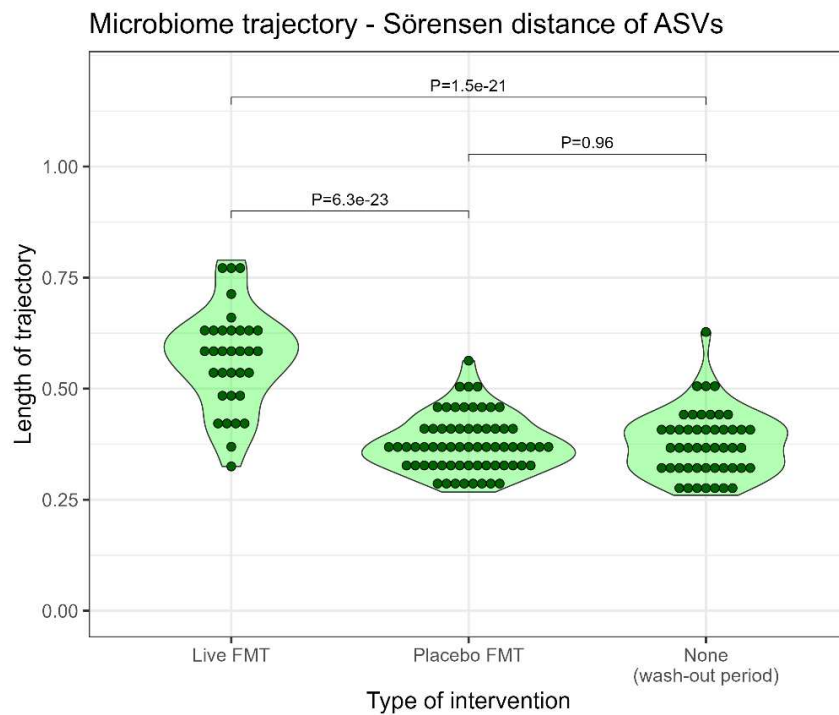


841

842 **(C) Live FMT is associated with longer trajectories than the placebo.**

843 The trajectories after live FMT were significantly longer than the trajectories after placebo or during the washout period. There was no difference
844 between the distance travelled by the bacteriome after the placebo FMT and during the washout period. Plotted are distances at the ASV level of
845 the 16S rDNA profile.

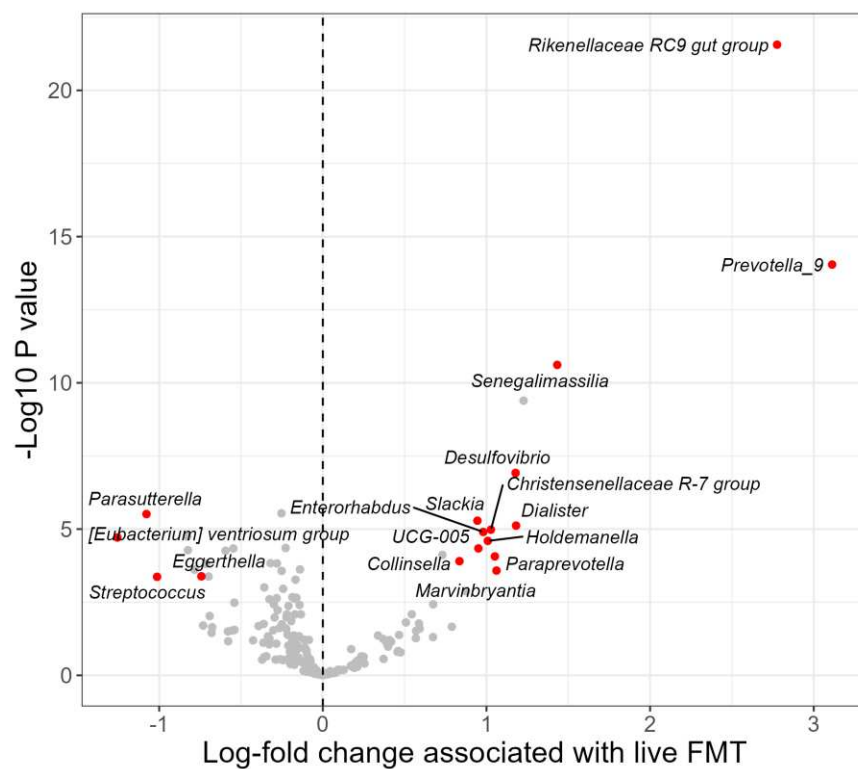
846



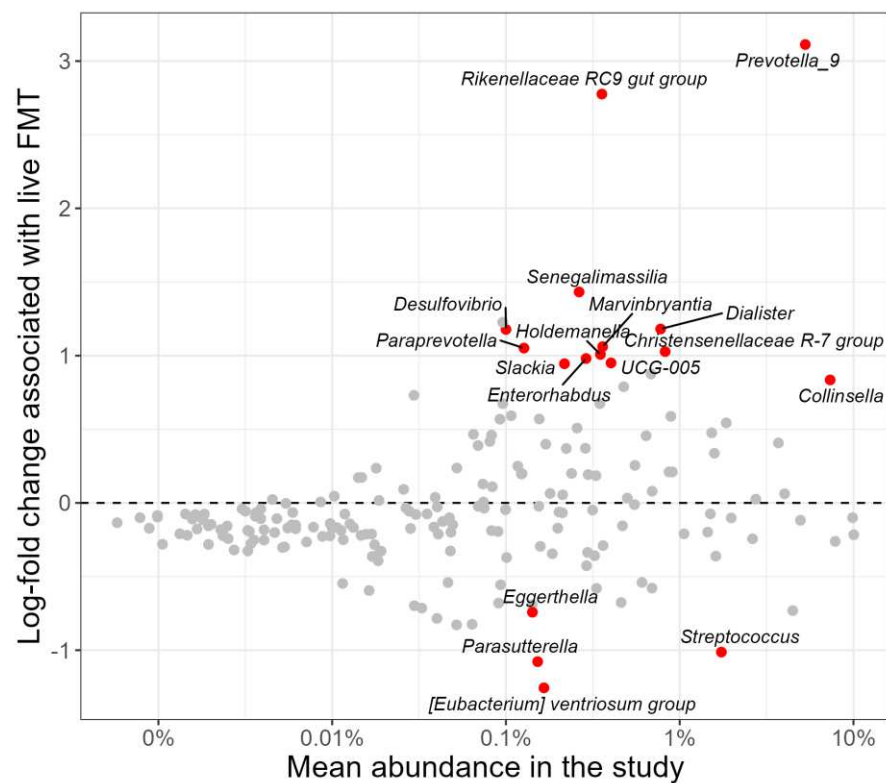
847

848 **Figure 6: Genera whose abundance changed after live FMT**

849 **(A) Volcano plot.** Each point represents a genus. The vertical axis represents the negative logarithms of the respective P-value for the log-fold
850 change in abundance (horizontal axis), associated with the administration of live FMT.



853 **(B) MA-plot.** Each point represents a genus; its mean abundance in the study is plotted on the x-axis, while the effect of live FMT on the genera is
 854 shown on the y-axis as a log-fold change.



855
 856
 857 Both plots also show two genera whose nominal significance was due to the presence of outliers: *Eggerthella*, *Parasutterella*.

858 **Figure 7. Strain transfer and replacement in *Prevotella copri* clade A after FMT with live microbiota**

Leaf labels show sample number (s.), patient anonymisation code (p.; both as deposited in NCBI SRA), the time point 1-4, and whether it was taken before or after intervention with placebo (P) or live microbiota (A).

The strain in the live FMT (highlighted in dark red) clusters with samples taken after live microbiota was applied as FMT (samples at time points 2, 3, 4 in those having live microbiota first, and sample at time point 4 in those having placebo first). Two patients, 45 (orange) and 31 (sandy colour), retained their own strain throughout the study. Two other patients, 16 (green) and 21 (blue), replaced their original strain with the new strain acquired from the FMT.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementarymaterialVejmelkaetal.pdf](#)
- [PROTOCOLoffprinte056594.full.pdf](#)