

## Supplementary methods

**Baseline assessment:** All patients underwent a uniform baseline evaluation including clinical, laboratory and endoscopic assessment. The laboratory assessment included blood for hemogram, liver and renal function tests, blood sugar, serologies for human immunodeficiency virus, hepatitis B and C, serum inflammatory markers (C-reactive protein, erythrocyte sedimentation rate), and stool examination for ova, cysts, parasite and *C. difficile* infection (tested by toxin assay). Stool samples were also collected in a sterile airtight container for fecal calprotectin (FCP) measurement and stored at -80°C. All patients underwent a baseline colonoscopy for assessment of endoscopic activity using UCEIS (at the area of maximum endoscopic inflammation).

**Donor screening during the COVID-19 pandemic-** When the trial was restarted in Aug 2021, in addition to the screening measures described above, donors were also screened to rule out COVID-19 infection. The screening was done through a questionnaire (Supplementary table 2), and RT-PCR for SARS-CoV-2 in nasopharyngeal swab before every donation. Stool testing for SARS-CoV-2 could not be done due to the unavailability of fecal SARS-CoV-2 testing platform. Patients undergoing FMT were informed about the risk of COVID-19 transmission, and informed consent was obtained (supplementary table 2).

**Dietary analysis:** Nutritional composition and the nutrient analysis of the dietary data was computed using a validated software 'DietCal' version 10.0 (Profound Tech Solutions, New Delhi)[1], which is based on values from Indian Food composition tables 2017, National Institute of Nutrition, ICMR (IFCT 2017) [2]. Patients were asked to record their diet in a diary for a week at each time-point (8 week, 24 week, 48 week) which was then converted into raw amount and entered into the 'DietCal software'. Food frequency with diet recall was taken from the patients who did not maintain the diet diary. Patients were regularly followed up telephonically.

**Assessment of dietary adherence:** The dietary adherence was assessed quantitatively as well as qualitatively:

**Qualitative adherence:** The diet adherence as per the seven day food diary was calculated as follows- For recommended food groups (AhR ligand rich foods and curd) intake for 6-7 days/week was given a score of 5, 4-5 days/ week score of 3, 2-3 days/ week score of 1, and 0-1 days/ week score of 0. For prohibited food groups (milk and milk products, non-vegetarian food, processed food, and wheat) reverse scoring was done- intake of 0 – 1 days/ week score of 5, 2-3 days/ week score of 3, 4 – 5 days/ week score of 1, and 6 – 7 days/ week score of 0. The score of all six food groups was added, then percentage adherence was calculated with 30 as maximum dietary adherence. Patients with >80%

adherence were considered highly adherent, 60-80% intake as moderately adherent, 30 – 60% as poorly adherent, and < 30% as non-adherent.

**Quantitative adherence:** Adherence to prohibited food groups (processed food, milk and milk product except curd, non-vegetarian food, and wheat) was estimated by calculating the percentage difference between baseline and 8 or 48 weeks intake. For recommended food groups (AhR ligand rich foods and curd), adherence was estimated by calculating percentage intake with respect to their recommended intake (200 grams for AhR ligand rich foods and 500 grams for curd). Average adherence of these 6 food groups was then calculated. Patients with >80% adherence were considered highly adherent, 60-80% intake as moderately adherent, 30 – 60% as poorly adherent, and < 30% as non-adherent.

**Assessment of adverse events:** Adverse events were assessed according to common criteria for adverse events (CTCEA) These were graded as grade 1—mild symptoms (no intervention required); grade 2—moderate symptoms (non-operative intervention required); grade 3—(operative intervention required); grade 4—(life-threatening consequences; urgent intervention required); and grade 5—death.

### Donor Microbiome Analysis

#### DNA Extraction from the faecal samples

DNA extraction was carried out using the Translational Health Science and Technology Institute protocol[3], with slight modification. Briefly, the frozen samples were thawed, precisely weighed to 200 mg and homogenized using glass beads-2.3mm (Biospec, USA). This was followed by enzymatic cell lysis using lysozyme (10mg/ml) (Sigma Aldrich), mutanolysin (25KU/ml) (Sigma Aldrich) and lysostaphin (4KU/ml) (Sigma Aldrich), at 37°C for 1 hr. Post-incubation samples were subjected to treatment with 4M Guanidine thiocyanate (Sigma-Aldrich) and 10% N-lauryl sarcosine (Sigma Aldrich), followed by incubation at 37°C for 10 min and 70°C for 1 hr. This was followed by mechanical lysis of cells by bead beating cycles using zirconia beads-0.1mm (Biospec, USA), after which polyvinylpyrrolidone mol wt. 40,000 (Sigma-Aldrich) was added to the lysate. Beads and PVPP were removed by centrifugation of tubes at 14,000 rcf for 5 min. The supernatants were transferred to a fresh tube and subjected to protein removal by phenol: chloroform: isoamyl alcohol (25:24:1) extraction. Nucleic acids were pelleted out using ice-cold ethanol (96%) by centrifugation at 14000 rcf for 10 min at 4°C. For RNA removal, these pellets were resuspended in sodium phosphate buffer (pH 8.0) and 3M potassium acetate and suspensions were incubated in ice for 90 min, followed by which RNase (10mg/ml) was added to the solution followed by incubation at 37°C for 30 min. The final precipitation of DNA was carried out by adding 3M sodium phosphate and 1ml of 96% ethanol and subjecting the pellet to centrifugation at 14,000 rcf for 10 min at 4°C. The DNA pellet was resuspended in 200 µL of Tris (10mM)- EDTA (1mM) buffer (pH 8.0). DNA concentration and purity were controlled spectrophotometrically using a NanoDrop apparatus (Thermo Fisher Scientific). Extracted DNA was sequenced using Illumina MiSeq platform, followed by which high-quality reads were obtained by

using Trimmomatic v0.38 to remove adapter sequences, ambiguous reads and low-quality reads (reads with more than 10% quality threshold (QV) < 20 Phred score) along with a sliding window of 10 bp and a minimum length of 100 bp. Stitching the PE data into single-end reads was then carried out by FLASH (v1.2.11).

#### **Sequencing data analysis and statistical analysis**

Raw sequence data were processed using Quantitative Insights Into Microbial Ecology (QIIME2) version 2022.22. All the following procedures were conducted in the QIIME2-2020.2 environment using QIIME plug-ins. Raw reads were subjected to demultiplexing, denoising and chimera removal using Deblur pipeline. The Amplicon sequence variants (ASV) feature table was then annotated via a taxonomy classifier built using reference sequence annotation and curation pipeline (RESRIPT), based on the NCBI bio projects 33175 and 33317 (NCBI 16S rRNA gene RefSeq database). For subsequent analysis through R packages, the QIIME outputs and associated metadata were imported into R as a phyloseq object using QIIME2R (version 0.99.6) (<https://github.com/jbisanz/qiime2R.git>). Beta diversity analysis was performed using QIIME2 plugin DEICODE version 0.2.3 (<https://github.com/biocore/DEICODE>). For differential abundance analysis of the microbial data between the effective and ineffective donor groups, DeSeq2 package was used with FDR correction of 0.01 and adjustment for confounders like donor Age, Gender and BMI.

#### **Donor metabolite analysis:**

Fecal short chain fatty acids (SCFAs) were analyzed using gas chromatography/ flame ionization detection (GC-FID). An aliquot of fecal content (250 mg) was extracted with 1 mL of extraction buffer [0.1% (w/v) HgCl<sub>2</sub> and 1% (v/v) H<sub>3</sub>PO<sub>4</sub>] supplemented with 0.045 mg/mL 2,2-dimethylbutyrate (as internal standard). The resulting slurry was centrifuged for 30 min at 5000 g at 4 °C, and the supernatant was filtered through a 0.2-µm filter. SCFAs in the supernatant collected were analyzed using a GC (Shimadzu-2010) equipped with FID and a stabilwax column (Restek, United States) of 30 m length, 530 µm diameter and 1 µm film thickness. The system was run with nitrogen as carrier gas at an inlet constant pressure of 18.1 kPa. Samples were run at an initial temperature of 120 °C for 0.5 min, and then with 8 °C/min change in temperature till it reached 220 °C and were held at 220 °C for 8 min for a total program time of 20.5 min. SCFAs were identified using external standards consisting of acetate, iso-butyrate, n-butyrate (Sigma, India) and the concentration were calculated using the area percentage method.

## References

1. “DietCal – A Tool for Dietary Assessment and Planning” Version 10.0 (Profound Tech Solutions; <http://dietcal.in/>)
2. Longvah T, Ananthan R, Bhaskarachary T, Venkaiah K. Indian food composition tables. National Institute of Nutrition, Indian Council of Medical Research Hyderabad, Telengana, India; 2017.
3. Das B, Ghosh TS, Kedia S, *et al.* Analysis of the Gut Microbiome of Rural and Urban Healthy Indians Living in Sea Level and High Altitude Areas. *Sci Rep* 2018;**8**:10104. doi:10.1038/s41598-018-28550-3