**SUPPLEMENTARY 3**

**Microbiota assessment**

**Sample preparation**

Total DNA was extracted from all samples using QIAamp PowerFecal DNA Kit (QIAGEN, Copenhagen, Denmark). Samples processed with the QIAamp PowerFecal Pro DNA Kit are added to a bead beating tube. Rapid and thorough homogenization occurs using mechanical and chemical methods. Once cells are lysed, our patented IRT is used to remove inhibitors. Total genomic DNA is captured on a silica membrane in a spin-column format. DNA is then washed and eluted, ready for downstream applications.

Isolated DNA from the fecal samples were prepared for sequencing by a two-step PCR amplification. The first PCR amplification were prepared as 25 µL reactions using PCRBIO Ultra Mix (PCR Biosystems, lokalitet) with 10 ng of isolated DNA as template and 400 nM primer-mix (515F : GTGYCAGCMGCCGCGGTAA (1) and 806R: GGACTACNVGGGTWTCTAAT (2)). Thermocycler settings for the first PCR: Initial denaturation at 95°C for 2 min, 30 cycles of 95°C for 15 s, 55°C for 15 s, 72°C for 50 s, and a final elongation for 5 min. Both a negative and a positive control were included in the PCR setup. The negative control consisted of nuclease-free water. The positive control contained template DNA from an anaerobic digester sample known to amplify PCR product with the selected primer-set. The first PCR amplification were performed with duplicate reactions for each sample and pooled after amplification. Incorporation of barcodes was carried out in a second PCR amplification. The reactions (25 µL) were performed with 2 µl cleaned amplicon PCR product (diluted to 5 ng/µL) as template as well as X5 PCRBIO reaction buffer (x1), PCRBIO Hifi polymerase (1U) and 1 µM Illumina adaptor mix. Thermocycler settings for the second PCR: Initial denaturation at 95°C for 2 min, 8 cycles of 95°C for 20 s, 55°C for 30 s, 72°C for 60 s and a final elongation at 72°C for 5 min. The second PCR was performed in single reactions. PCR product from both PCR runs were purified using 0.8x CleanNGS beads (CleanNA) and eluted in nuclease-free water. DNA concentrations were measured with Quant-iT HS DNA Assay (Thermo Fisher Scientific) and the purified PCR amplicon products were visualized on D1K ScreenTapes using a TapeStation 2200 Analyzer (Agilent). Prior to sequencing, all samples were pooled into one tube in equimolar concentrations and barcoded with the Nextera indexing kit. Sequencing of the library pools was performed on the Illumina MiSeq platform with v3 chemistry and 2x301bp paired-end setting.

**Data analysis**

The raw sequencing data was demultiplexed with bcl2fastq v2.17.1.14 then processed with AmpProc v5.1beta1.0 (<http://www.github.com/eyashiro/AmpProc/>), which is primarily based on the USEARCH v10.0.240 workflow (3). Data was summarised into amplicon sequencing variants (ASVs), avoiding clustering by nucleotide identity to maximize taxonomic resolution and reduce clustering biases (4). After filtering out phix contamination, the paired-end reads were merged using –fastq\_mergepairs with settings “-fastq\_maxdiffs 15” (5). Merged reads were filtered to determine quality using –fastq\_filter with settings “-fastq\_maxee 1 –fastq\_minlen 200”, dereplicated using –fastx\_uniques and clustered into ASVs by –unoise3. Clustered reads were further curated by filtering out reads more distantly related to known sequences than 60% identity using –userarch\_global with settings “-db gg\_13\_8\_otus97/97\_otus-fasta –id 0.6 –maxaccepts 1 –maxrejects 8” (6). The ASV table was generated using the function –otutab with settings “–zotus and –id 0.97” (7), and taxonomy was assigned to each ASV using –sintax with setting “-sintax\_cutoff 0.8” along with the SILVA LTP vers. 132 as the reference database (8) (<https://www.arb-silva.de/>).

**References**

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