Supplemental Digital Content 2:

Changes in the abundance of *Faecalibacterium prausnitzii* phylogroups I and II in the intestinal mucosa of inflammatory bowel disease and colorectal cancer patients.

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Supplementary text

Bacterial strains, growth conditions and DNA extraction from pure cultures.

F. prausnitzii strains were from stocks held by the authors (Rowett Institute of Nutrition and Health, Aberdeen, United Kingdom) and several came from previous studies (Barcenilla et al., 2000; Cato, 1974; Duncan et al., 2002; Lopez-Siles et al., 2012; Louis et al., 2004). Additional bacterial strains were either available in our laboratory collection or were otherwise obtained from several biological resource centers specified in Table S2. When possible, bacteria were cultured aerobically or anaerobically on the recommended medium. DNA was extracted and purified by using the Wizard™ Genomic Purification Kit (Promega Corporation, USA) following the manufacturer's guidelines.

Optimisation and characterization of the multiplex qPCR assay for *F. prausnitzii* phylogroups

To determine the best reagent concentrations for the qPCR assay, experiments were performed using different primer and probe concentrations ranging from 50 to 900 nM. Those reagents concentrations that yield the maximum fluorescent signal and the lowest quantification cycle (C_q) value for 10^6 copies/reaction of the target DNA were chosen as optimal, and have therefore been used for further quantification in samples (as described in the main text section Quantitative PCR conditions).

Inclusivity and exclusivity tests

For the multiplex quantification of *F. prausnitzii* phylogroups using a qPCR assay, specificity was also tested *in vitro* by comparing the quantification of pure *F. prausnitzii* DNA (10 ng) recovered from nine isolates, representative of both phylogroups. DNA from 80 additional representative

bacterial species (see list in Table S2) which are either close relatives of *F. prausnitzii* or belong to the major groups of bacteria present in the colon were also included.

F. prausnitzii strains were from stocks held by the authors (Rowett Institute of Nutrition and Health, Aberdeen, United Kingdom) and several came from previous studies (1-5). Additional bacterial strains were either available in our laboratory collection or were otherwise obtained from several biological resource centers specified in Table S2. When possible, bacteria were cultured aerobically or anaerobically on the recommended medium. DNA was extracted and purified by using the Wizard™ Genomic Purification Kit (Promega Corporation, USA) following the manufacturer's guidelines.

The qPCR was carried out as described in the section Quantitative PCR conditions of the main text. Negative results were cross checked by alternative amplification by end-point, conventional PCR with universal bacterial primers Bac27F and Uni1492R as previously reported (6, 7). Results from the specificity test are also shown in Table S2. The assay was totally specific. All the *F. prausnitzii* isolates were only detected for the phylogroup they belong to, and no statistically significant differences in C_q values between isolates were observed. There was no cross-reaction with any of the non-target microorganisms, and negative results were validated by a positive amplification by conventional PCR.

Linear quantification range and efficiency of the qPCR

To determine the confident quantification range of the assay, decaplicate ten-fold dilutions (ranging from 2×10^8 to 2 target gene copies per reaction) of a linearized plasmid containing either a single copy of the 16S rRNA gene of *F. prausnitzii* S3L/3 (phylogroup I) or *F. prausnitzii* DSM 17677 (phylogroup II) were used. The linear range for quantification was considered for those concentrations having a SD value lower than 0.34 between replicates. Regression analysis plotting the obtained Cq against the logarithm of the number of target genes in the reaction was also performed. The efficiency of the qPCR assay was calculated using the formula: Efficiency= $[10^{(-1/slope)}]-1$.

Detection limit of the assay.

A calibration curve of two-fold serial dilutions between 1 and 100 target copies of *F. prausnitzii* 16S rRNA gene was performed. Eight replicas of each dilution were assayed. Data was analyzed by a Probit test (Minitab® 14 Statistical Software, Pennsylvania, US), in which the ratio of

positive/negative amplification events was plotted against the amount of target genes present per reaction.

References

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