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☐ Initial submission ☒ Revised version ☐ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

No sample size calculation was performed. Cohort sizes were determined based on previous findings (Falony et al., Science 2016).

2. Data exclusions

Describe any data exclusions.

To assess microbiome feature and metadata associations, taxa unclassified at the genus level or present in less than 20% of samples were excluded from the statistical analyses. Taxa unclassified at the genus level or present in less than 50% of samples were excluded from co-abundance network reconstruction. The same 50% cut-off was handled in the simulated datasets.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All findings were replicated. All replications were successfull and this is reported in the manuscript.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

No experimental groups were defined. The study/longitudinal and validation cohorts were recruited among the staff of the KU Leuven research facilities located at university hospital (Leuven, Belgium) and staff and students of the Hogeschool PXL (Hasselt, Belgium), respectively. No inclusion or exclusion criteria were imposed. For a description of the disease cohort reanalyzed in the study, we refer to the original publication (doi: 10.1136/gutjnl-2015-311004).

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Investigators were not blinded during data collection and analyses.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	An open source QMP R-script is available on http://www.raeslab.org/software/QMP and the Github repository (https://github.com/raeslab/QMP) with public access.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	n.a.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	n.a.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	n.a.
b. Describe the method of cell line authentication used.	n.a.
c. Report whether the cell lines were tested for mycoplasma contamination.	n.a.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	n.a.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	n.a.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	Study cohort: 20 male/20female. Median age: 29; BMI: 22.3; BSS: 4. Longitudinal cohort: 10 male/10 female. Median age: 27, BMI: 23, BSS: 4. Validation cohort: 10 male/44 female. Median age: 38, BMI: 23, BSS: 3.5. Disease cohort: 29 Crohn's disease patients/66 healthy controls (doi: 10.1136/gutjnl-2015-311004).

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Reported in method section.
6. Identify the instrument used for data collection.	Reported in method section.
7. Describe the software used to collect and analyze the flow cytometry data.	Reported in method section.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	n. a.
9. Describe the gating strategy used.	Reported in method section.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒