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Title	Investigation of the gut microbiome, bile acid composition and host immunoinflammatory response in a model of azoxymethane-induced colon cancer at discrete timepoints
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Reporting Summary

Springer Nature wishes to improve the reproducibility of the work that we publish. This checklist is used to ensure good reporting standards and to improve the reproducibility. Please respond completely to all questions relevant to your manuscript. For more information, please read the journal's Guide to Authors.

☑ **Check here to confirm that the following information is available in the Material & Methods section:**

- **The exact sample size (*n*)** for each experimental group/condition, given as a number, not a range
- **A description of the sample collection** allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, culture, etc.)
- **A statement of how many times the experiment shown was replicated in the laboratory**
- **Definitions of statistical methods and measures**: For small sample sizes (n<5) descriptive statistics are not appropriate, instead plot individual data points
    - Very common tests, such as *t*-test, simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section
    - Are tests one-sided or two-sided?
    - Are there adjustments for multiple comparisons?
    - **Statistical test results**, e.g., ***P* values**
    - Definition of **'center values'** as **median or mean**;
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Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents and animal models. Below, provide the page number or section and paragraph number.

Statistics and general methods	**Reported in section/paragraph or page #**
1. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size? (Give section/paragraph or page #)	Sample size was calculated using g*power, with the standard deviation and magnitude of difference calculated from previous studies quantifying medium to large ACF development in response to AOM as an endpoint
For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A (see above)
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? (Give section/paragraph or page #)	No criteria were set for including or excluding animals. Outlier data points were detected using Grubbs' Test. (Line 181) In the PCR analysis, where samples were below the detection limit, they were excluded from the final analysis
3. If a method of randomization was used to determine how samples/animals were allocated to experimental groups and processed, describe it. (Give section/paragraph or page #)	After acclimatisation, equal numbers of mice were randomly assigned to two groups and were administered an intraperitoneal (i.p.) injection of 10mg/kg AOM to induce tumorigenesis (n = 32) each week for five consecutive weeks while control mice received phosphate
For animal studies, include a statement about randomization even if no randomization was used.	See above

4. If the investigator was blinded to the group allocation during the experiment and/or when assessing the outcome, state the extent of blinding. (Give section/paragraph or page #)	The investigators administering the AOM or PBS treatment groups were aware of the group allocations. Following necropsy, each sample was allocated a random number, to which the subsequent investigators were blinded.
For animal studies, include a statement about blinding even if no blinding was done.	See above
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)?	Normality was determined by the Shapiro-Wilk test. Lines 181 - 182
Is there an estimate of variation within each group of data?	Where appropriate, SEM and IQR are denoted in the Figure legends, and scatter plots included in Figure 4.
Is the variance similar between the groups that are being statistically compared? (Give section/paragraph or page #)	The variance between the groups was determined, and where the F value was statistically significant, data was analysed using the Welch t-test.

Reagents

	Reported in section/paragraph or page #
6. Report the source of antibodies (vendor and catalog number)	N/A
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination	HT29 cells were obtained from the ATCC, and are routinely tested for mycoplasma contamination. Cells tested negative. Line 161

Animal Models

	Reported in section/paragraph or page #
8. Report species, strain, sex and age of animals	Reported in lines 120 - 122
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Reported in lines 118 - 120

10. We recommend consulting the ARRIVE guidelines (*PLoS Biol.* **8**(6), e1000412,2010) to ensure that other relevant aspects of animal studies are adequately reported.

Human subjects

	Reported in section/paragraph or page #
11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent).	N/A

15. For phase II and III randomized controlled trials, please refer to the CONSORT statement and submit the CONSORT checklist with your submission.

16. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines.

Data deposition

	Reported in section/paragraph or page #
17. Provide accession codes for deposited data. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Microarray data	N/A

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available in the Guide to Authors. We encourage the provision of other source data in supplementary information or in unstructured repositories such as Figshare and Dryad. We encourage publication of Data Descriptors (see Scientific Data) to maximize data reuse.

18. If computer code was used to generate results that are central to the paper's conclusions, include a statement in the Methods section under "**Code availability"** to indicate whether and how the code can be accessed. Include version information as necessary and any restrictions on availability.	N/A