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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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The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Materials & Methods, lines 121-124 Materials & Methods, lines 124-125
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Materials & Methods, lines 121-124 and line 118. Sample size was calculated using g*power, with the standard deviation and magnitude of difference calculated from previous studies quantifying medium to large ACF
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	No criteria were set for including or excluding animals. Following randomisation one mouse assigned to the AOM group (week 12) died before treatment began. A second mouse was humanely culled due a to a skin Materials & Methods, line 160; n numbers are included in each figure legend.
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	All animals were randomly allocated into their groups by staff in the animal unit. Subsequent to that, there was a random selection of animal cages into the respective treatment groups; Material & Methods, line 120. Mice were randomly assigned to different groups, and were group housed based on treatment (PBS or AOM). Within each cage, mice were then randomly assigned to particular time points and euthanised accordingly by cervical dislocation.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	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.
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	All outcomes measured are clearly in the figures and figure legends. See section 2(b). Following consultation with our co-author, Prof. Paul Cotter, n=8 was deemed appropriate to sufficiently power the microbiome studies.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Materials & Methods, lines 156-167. Normality was determined by the Shapiro-Wilk test, lines 161-162
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Materials & Methods, line 118. NA.
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Materials & Methods, lines 120-125. Materials & Methods, lines 120-125. Materials & Methods, lines 118-120. Materials & Methods, lines 121-122.
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Figures & Figure legends. NA.