nature portfolio

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Last updated by author(s):	Nov 2, 2023

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Confirmed
	\square The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
	A description of all covariates tested
	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about <u>availability of computer code</u>

Data collection

Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.

Data analysis

Provide a description of all commercial, open source and custom code used to analyse the data in this study, specifying the version used OR state that no software was used.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The sequencing data for 16S rRNA have been deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the accession number SUB13309160. The raw mass spectrometry data of fecal metabolites has been uploaded to Metabolights with the identifier MTBLS7836.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.

Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).

Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)

Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one belo	w that is the best fit for your research	. If you a	are not sure, read the appropriate sections before making your selection.
X Life sciences	Behavioural & social sciences	ПЕ	cological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see $\underline{\text{nature.com/documents/nr-reporting-summary-flat.pdf}}$

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size Number of samples determined based on previous studies and bi

Number of samples determined based on previous studies and biological replicates. In the subcutaneous tumor model, the initial number of mice per group was five. In the AOM/DSS model, the initial number of mice per group was 10. For fecal metabolite sequencing, sample replicates were six. For 16s rRNA sequencing, samples were repeated five times.

Data exclusions No data were excluded.

Replication All experiments were performed in triplicate at least.

Randomization All experimental groups were randomly assigned.

Blinding Double-blind experiments were performed

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experime	ental s	ystems Methods				
n/a Involved in the study		n/a Involved in the study				
Antibodies	ChIP-seq					
Eukaryotic cell lines	i	Flow cytometry				
Palaeontology and a	d archaeology MRI-based neuroimaging					
Animals and other of	organism	is and the second secon				
Clinical data						
Dual use research o	f concer	n				
Plants						
Antibodies						
Antibodies used	Specific rabbit primary antibody against His-Tag (66005-1, proteintech); Second antibody (HRP-conjugated Affinipure Goat Anti-Mouse IgG (H+L), SA00001-1, proteintech)					
Validation	The Specific rabbit primary antibody against His-Tag (66005-1, proteintech) has been verified to specifically recognize His-Tag.					
Eukaryotic cell lin	ies					
•		and Sex and Gender in Research				
Cell line source(s)		CT-26 mouse colon cancer cell, RKO human colon adenocarcinoma cell, and SW480 human colon cancer cell were purchased from Procell (Wuhan, China).				
Authentication	All cell lines were identified through SRT and confirmed to be correct.					
Mycoplasma contamination All cell lines were tested for mycoplasma and confirmed to be free of mycoplasma contamination.		All cell lines were tested for mycoplasma and confirmed to be free of mycoplasma contamination.				
Commonly misidentified (See <u>ICLAC</u> register)	Commonly misidentified lines (See ICLAC register) No misidentified cell lines were used in this study.					
Animals and othe	er res	earch organisms				
Policy information about <u>st</u> <u>Research</u>	<u>cudies ir</u>	nvolving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in				
Laboratory animals	Female Balb/c mice (6 weeks old, 18-22g, used for the subcutaneous tumor model) and C57BL-6J mice (6 weeks old, 18-22g, used for the AOM/DSS-induced CRC model) were utilized in the study.					
Wild animals	We did not use wild animals.					
Reporting on sex	To avoid the influence of sex, only female Balb/c or C57BL-6J mice were used in this study.					
Field-collected samples	This study did not involve samples collected from the field.					
Ethics oversight	The animal experimental proposal was approved and supervised by the Animal Ethics Committee of School of Life Sciences, Lanzhou University and carried out in accordance with ethical guidelines of the 1975 Declaration of Helsinki (permission number: EAF2022053).					
Note that full information on t	the appro	oval of the study protocol must also be provided in the manuscript.				
Plants						
Seed stocks		on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If pecimens were collected from the field, describe the collection location, date and sampling procedures.				
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches,					

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

was applied.

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.