# nature portfolio

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## **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)
$\boxtimes$	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
$\boxtimes$	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 <u>guidelines for submitting code & software</u> for further information.

#### Data

Policy information about availability of data

All manuscripts must include a data availability statement. 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 of the 16S rRNA gene have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB59736

(https://www.ebi.ac.uk/ena/browser/view/PRJEB59736).	
Supporting Information is available from the author.	

## 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 below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

X Life sciences

Behavioural & social sciences

| Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

## Life sciences study design

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

Sample size

For an experimentation of carcinogenesis at preneoplastic stage in rats, the number of rats is defined to evidence differences in the number of MDF per colon. When the difference is huge (to test a chemical carcinogen), N=6 or N=8 rats per group can be enough. However, to test the effect of a food, N>10 per experimental groups is necessary to get enough power. The team's previous studies with commercial or model processed meats has shown that a number of 12 rats per group is necessary and sufficient to demonstrate an effect on the promotion of MDF.

Data exclusions

Outliers were identified and removed by a ROUT Test.

More generally, for the animal experiment, once outliers were identified/removed by a ROUT test, and normality was checked, ANOVA was performed using GraphPad Prism software (version 9.5.0), followed by a Dunnett's or a Tukey's mean comparison test, in case of comparison to a control group (sodium nitrite effect), or comparison of all means (alternative effect), respectively. In case of heteroscedasticity (tested by Bartlett's and Brown-Forsythe tests), data were log transformed before ANOVA (DHN-MA and NOCs). In case of non-normality of residuals, a nonparametric test (Kruskal-Wallis) was performed followed by Dunn's mean comparison test (some RT-qPCR and bacterial taxa). Finally, if equal SD cannot be assumed despite data transformation, a Welch's ANOVA was performed (cytotoxicity test).

Replication

For L. monocytogenes groth assay, three specific and independent batches of sliced cooked ham model products were used for this assay. For animal study, the unit of analysis wasthe individual rats:

-for microbiota and for carcinogenesis endpoint (MDF), n=12

-for fecal water and urinary samples; each sample was measured in triplicate after three individual preparation

Randomization

At reception, rats were placed randomly, one by one, in each cage. When each cage contained one rat, a second rat was placed in it, sequentially. After acclimation to the animal colony, the experimental groups were decided at random, by placing labels on the cage, in a "random" order, avoiding to place two label of same group on next cages, or in the same "raw" or the same column of cages. Rats were then weighted, and some changes were made so that no statistical difference could be seen in mean body weights at the beginning of the study.

Blinding

Each sample (feces, urine, colon) was given a two-letter code randomly generated by a computer. Codes were kept in a file that was known

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only to senior investigators, who did not participate to laboratory analyses or microscopic scoring. Code was broken only before statistical testing.

All analytical techniques (biochemical assay of fecal water and urine) were done by a unique investigator, blinded for the origin of the sample. Carcinogenesis end-points (ACF and MDF) were counted under light microscope in duplicate by two independent readers, blinded for the origin of the colon.

# 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.

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Materials & experime	ental systems	Methods	
n/a   Involved in the study		n/a   Involved in the study	
Antibodies		ChIP-seq	
Eukaryotic cell lines		Flow cytometry	
Palaeontology and archaeology		MRI-based neuroimaging	
Animals and other organisms			
Clinical data	Clinical data		
Plants			
Animals and other	r receased ergan	isms	
Animals and othe	r research organ	ISMIS	
	udies involving animals; A	RRIVE guidelines recommended for reporting animal research, and Sex and Gender in	
Research			
Laboratory animals	Male Fischer 344 (F344/DuCrl) rats were purchased from Charles River Laboratories, 12 rats/group, aged 5-6 weeks.		
Wild animals	The study did not include wild animals		
Reporting on sex			
	· ·	trong impact on the response to pro-inflammatory stimuli and the antioxidant response, so we decided to d this additional bias in the interpretation of our results.	
Field-collected samples	The study did not involve field collected samples		
Ethics oversight	Animal experiment was approved by the local Ethical Committee (CE n°86), authorized by the French Ministry of Research (APAFIS 27180_2020091017209910_v2) and conducted in accordance with the European Council on Animals used in Experimental Studies		

Note that full information on the approval of the study protocol must also be provided in the manuscript.  $\frac{1}{2}$ 

and ARRIVE guidelines.