nature portfolio

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Last updated by author(s): Oct 5, 2024

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 Editorial Policies and the Editorial Policy Checklist.

Statistics

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	
☐	☒	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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	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 *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	We did not use any commercial, open source or custom code to collect the data in this study.
Data analysis	GraphPad Prism versions 8.4 and 10.0, Sigmaplot 13.0, and R4.1 were used for statistical analysis of the data

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 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

All data supporting the findings of this study are available within the paper and its Supplementary Information. Source data are provided with this paper. A data availability statement is included in the manuscript.

Research involving human participants, their data, or biological material

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

Reporting on sex and gender	Not applicable since this study does not involve human participants, their data, or biological materials.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable since this study does not involve human participants, their data, or biological materials.
Population characteristics	Not applicable since this study does not involve human participants, their data, or biological materials.
Recruitment	Not applicable since this study does not involve human participants, their data, or biological materials.
Ethics oversight	Not applicable since this study does not involve human participants, their data, or biological materials.

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.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see 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	A sample size of three or more independent replicates was chosen to allow statistical analysis when comparing different treatment groups. A sample size of five mice in each group was chosen due to prior experience with similar study design. No additional sample size calculation was performed due to the lack of information on data variability prior to the study.
Data exclusions	No data were excluded from the analyses.
Replication	All attempts of replication were successful and included in the data analyses.
Randomization	Mice were assigned to each study group randomly.
Blinding	Investigators were not blinded to the group allocation during mouse data collection and data analysis.

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 & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Polyclonal antisera of CYP5122A1 and CYP51 were raised against the purified recombinant proteins in rats and provided as gifts from Dr. Jianming Qiu of the University of Kansas Medical Center. Antibody to alpha-tubulin was purchased from Thermo Fisher Scientific without further validation.
Validation	Polyclonal antisera of CYP5122A1 and CYP51 were validated by Wester blot analysis of recombinant antigen proteins to confirm

specificity.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Female BALB/c mice (7-8 weeks old) were purchased from Charles River Laboratories International (Wilmington, MA). Female CD-1 mice (6-7 weeks old at the time of purchase) were obtained from Envigo.
Wild animals	Studies did not involve wild animals.
Reporting on sex	Only female mice were used in the Leishmania infection model and hence results apply to the female sex, although sex was not known to play a role in Leishmania donovani infection of mice. Female mice were used to obtain peritoneal macrophage host cells for experiments involving intracellular Leishmania donovani, hence results apply to the female sex. Female CD-1 mice have frequently been employed as a source of peritoneal macrophages for drug susceptibility assays against intracellular Leishmania (DOI: 10.1128/AAC.01772-19, DOI: 10.1093/jac/dkad162). Macrophages from female mice of other strains have also been commonly used for such assays (DOI: 10.1371/journal.pntd.0007885, DOI:10.1093/jac/dky014, DOI: 10.1007/s00436-018-6059-4).
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal procedures were performed as per approved protocol by Animal Care and Use Committees at Texas Tech University (PHS Approved Animal Welfare Assurance No A3629-01) and The Ohio State University (PHS Approved Animal Welfare Assurance No A3261-01)

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

Flow Cytometry

Plots

Confirm that:

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

☒ 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).

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

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

Methodology

Sample preparation	L. donovani WT, CYP5122A1+/- +pXNG4-22A1, and Cyp5122A1¯+ pXNG4-22A1 promastigotes were inoculated in complete M199 media in the presence or absence of GCV (the negative selection agent) or nourseothricin (the positive selection agent) and then analyzed by flow cytometry based on GFP fluorescence signal.
Instrument	Attune NxT Acoustic Flow Cytometer
Software	Attune NxT Software v 3.1
Cell population abundance	Both GFP-high and GFP-low populations after sorting were at 1 x 10^5 cells/ml-6 x 10^5 cells/ml. The GFP fluorescence levels of these sorted populations were verified as we recently described (PMID: 36712124)
Gating strategy	Only single cells were used in GFP flow cytometry. Single cells were gated in FSC/SSC plot using log phase L. donovani wild type cells as the reference. For gating in GFP histogram, L. donovani wild type cells were used as GFP-negative control and L. donovani with pXNG4-Ld22A1 were used as GFP-positive control.

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