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 Editorial Policies and the Editorial Policy Checklist.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

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	Next-Generation Sequencing: NextSeq 550 (Illumina); NovaSeq 6000 (Illumina); MiSeq (Illumina) Widefield Microscope: DeltaVision Ultra (GE HealthCare) Multiomodal Imager: Odyssey M (LI-COR)
Data analysis	Data were analyzed using 1) cellranger-7.0.1 and cellranger-atac-2.1.0; 2) R (version 4.2.2) with Seurat R (version 4.3.0), MyEllipsefit R, and dtwclust R packages; 3) bedtools, BWA-mem (version 0.7.17), and samtools (version 1.4.1); 4) Python with scvelo package; 5) ToxoDB.org (version 64); 6) BAMM motif; 7) MEME-Suite package; 8) ImageJ FIJI (version 1.53t)

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

Code Availability:
The analysis R code is available on GitHub: https://github.com/umbibio/scToxoplasmaCDC (DOI: 10.5281/zenodo.8219739).

Data Availability:
An interactive web-application for visualization and exploration of our data set can be found here: https://umbibio.math.umb.edu/toxosc/.
scRNA-seq, scATAC-seq and CUT&RUN data (fastq) have been deposited to the Sequence Read Archive (SRA) under the accession number PRJNA1002574.

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	
Reporting on race, ethnicity, or other socially relevant groupings	
Population characteristics	
Recruitment	
Ethics oversight	

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	1. All single-cell sequencing experiments were only performed once. The number of cells was detected by 10x Genomics Cell Ranger pipeline. The cell number was pre-determined before experiments according to the optimal cell recovery yield listed in the 10x Genomics protocol. Here are the number of cells collected for each run: a) RH scRNA-seq: 12,735 cells. b) RH scATAC-seq: 3,506 cells. c) RH-H3K4me3-CUT&RUN-seq: 8,981 cells. 2. For CUT&RUN experiments, four negative controls were included. All the CUT&RUNs that were used for the final analysis except RH-neg and H3K4me3 used 200 million intracellular parasites. The RH-neg and H3K4me3 used 500 million intracellular parasites. The number of parasites was optimized to be 200 million for CUT&RUN. For detailed rationale, please refer to the main text. 3. For the microscopy-based assays, three biological replicates were performed for each experiment, and for each case, a minimum of 100 vacuoles were considered.100 vacuoles are the standard choices in Toxoplasma gondii studies when performing quantification analyses like these.
Data exclusions	No data were excluded.
Replication	Two or three biological replicates were performed for each indicated experiments. All attempts at replicatoin were successful.
Randomization	In our study, randomization was not employed because the experimental design and the nature of the experiments did not necessitate it. Randomization is typically crucial clinical studies or experiments involving multiple treatment groups to eliminate bias. However, our study's focus and methodology did not involve conditions where randomization would impact the results or conclusions.
Blinding	We did not perform any blinding techniques as the quantitative analysis of the microscopy-based assays should not be affected by personal perception.

Behavioural & social sciences study design

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

Study description	
Research sample	
Sampling strategy	
Data collection	
Timing	
Data exclusions	
Non-participation	
Randomization	

Ecological, evolutionary & environmental sciences study design

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

Study description	
Research sample	
Sampling strategy	
Data collection	
Timing and spatial scale	
Data exclusions	
Reproducibility	
Randomization	
Blinding	

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	
Location	
Access & import/export	
Disturbance	

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	1. anti-IMC3 (N: 1-120 aa), rabbit (in-house) [used in 1:2,000 dilution for IFA] 2. anti-Centrin (clone 20H5), mouse (Millipore) [used in 1:1,000 dilution for IFA] 3. anti-Ty1 Tag Monoclonal Antibody (BB2), mouse (Thermo Fisher Scientific) [used in 1:5,000 dilution for western blot; 0.04ng, 0.2ng, 1ng, 5ng, 25ng for IFA; 0.5µg, 1µg, or 2µg for CUT&RUN] 4. anti-Ty1 Antibody (monoclonal), mouse (Diagenode) [0.04ng, 0.2ng, 1ng, 5ng, 25ng for IFA; 0.625µg, 1.25µg, or 2.5µg for CUT&RUN] 5. anti-IgG1 Isotype Control, mouse (Invitrogen) [0.5µg or 2µg for CUT&RUN] 6. anti-Tubulin (12G10), mouse (DSHB) [used in 1:2,000 dilution for IFA] 7. anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594, goat (Thermo Fisher Scientific) [used in 1:400 dilution for IFA] 8. anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 488-5 nm colloidal gold, goat (Thermo Fisher Scientific) [used in 1:400 dilution for IFA] 9. anti-Mouse Immunoglobulins/HRP, goat (Agilent) [used in 1:10,000 dilution for western blot] 10. anti-H3K4me3, rabbit (EpiCypher) [0.5µg for CUT&RUN]
Validation	1. anti-IMC3 (N: 1-120 aa), rabbit [Anderson-White, B. R. et al. A family of intermediate filament-like proteins is sequentially assembled into the cytoskeleton of Toxoplasma gondii. Cell Microbiol 13, 18-31 (2011).] 2. anti-Centrin (clone 20H5), mouse [Millipore, Cat# 04-1624; RRID: AB_10563501] 3. anti-Ty1 Tag Monoclonal Antibody (BB2), mouse [Thermo Fisher Scientific, Cat# MA5-23513; RRID: AB_2610644] 4. anti-Ty1 Antibody (monoclonal), mouse [Diagenode, Cat# C15200054-10; https://www.diagenode.com/en/p/ty1-monoclonal-antibody-classic-10-ug] 5. anti-IgG1 Isotype Control, mouse [Invitrogen, Cat# 02-6100; https://www.thermofisher.com/antibody/product/Mouse-IgG1-Monoclonal/02-6100] 6. anti-Tubulin (12G10), mouse [DSHB, Cat# 12G10 anti-alpha-tubulin; RRID: AB_1157911] 7. anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594, goat [Thermo Fisher Scientific, Cat# A-11032; https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11032] 8. anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 488-5 nm colloidal gold, goat [Thermo Fisher Scientific, Cat# A-31565; RRID: AB_253617] 9. anti-Mouse Immunoglobulins/HRP, goat [Agilent, Cat# P0447; RRID: AB_2617137] 10. anti-H3K4me3, rabbit [EpiCypher, Cat# 13-0041k; https://www.epicypher.com/products/antibodies/snap-chip-certified-antibodies/histone-h3k4me3-antibody-snap-chip-certified-cutana-cut-run-compatible]

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	1. BJ-1 human telomerase reverse transcriptase (hTERT)-immortalized human foreskin fibroblasts (HFFs) were used for parasite maintainance and all the other experiments except the immunofluorescence assays (IFAs) [ATCC, Cat# CRL-4001; sex of this cell line is male] 2. HFFs were only used for the IFAs [ATCC, Cat# SCRC-1041; sex of this cell line is male] 3. T. gondii RH was obtained from published work [PMID: 19218426] 4. All the other T. gondii lines were created in this study
Authentication	The hTERT-immortalized HFFs, HFFs, and T. gondii RH were authenticated by the providers and had published records. All the T. gondii mutants created in this study were validated through PCR assays with specific primers.
Mycoplasma contamination	No mycoplasma contamination was observed in any cell lines used for experiments at any time.
Commonly misidentified lines (See ICLAC register)	We did not use any commonly misidentified lines in this study.

Palaeontology and Archaeology

Specimen provenance	
Specimen deposition	
Dating methods	

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	

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

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	
Wild animals	
Reporting on sex	
Field-collected samples	
Ethics oversight	

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	
Study protocol	
Data collection	
Outcomes	

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks

Novel plant genotypes

Authentication

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.

Files in database submission

Genome browser session
(e.g. UCSC)

Methodology

Replicates

Sequencing depth

Antibodies

Peak calling parameters

Data quality

Software

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	
Instrument	
Software	
Cell population abundance	
Gating strategy	

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

Magnetic resonance imaging

Experimental design

Design type	
Design specifications	
Behavioral performance measures	

Imaging type(s)	
Field strength	
Sequence & imaging parameters	
Area of acquisition	

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	
Normalization	
Normalization template	
Noise and artifact removal	
Volume censoring	

Statistical modeling & inference

Model type and settings	
Effect(s) tested	

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

(See Eklund et al. 2016)

Correction

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis