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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	FACS data was collected using FACSDiva Software Version 6.1.3, StepOne™ and StepOnePlus™ Real-Time PCR System software v. 2.1 was used to collect qPCR data
Data analysis	FlowJo (v. 10.3), Samtools (v. 1.3.1), HISAT2 (v 2.0), Integrated Genome Viewer(v. 2.3.91), R studio (v. 1.0.136), Dreme (v. 4.10.0)

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

RNA-seq data generated in this study is available through GEO database repository (study accession number GSE110201).

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	For qualitative experiments (i.e genotyping, Western blots) one mouse per strain was used in each of the experiments. For quantitative experiments, the numbers were adjusted based on the variability of the studied trait and the effect size of interest. Thus for quantification of gametocyte production, the means and standard deviations for male and female gametocyte conversion rates were assumed to be 0.028 ±0.011 and 0.033±0.14 respectively based on previous experiments. Therefore (based on the two-sided t-test power calculations in R) n=5 biological replicates were used, as they allow to detect a major (>2 fold) difference in gametocyte production with the significance of < 0.05 with a probability of 0.93 and 0.9 for males and females respectively. Similarly, for the ookinete conversion rate (typically 0.849±0.096), n=3 was chosen as it allows to detect the major difference in the conversion rates (>0.4) with significance of < 0.05 with probability of 0.96. For transcriptome analysis, 6 hour intervals for RNAseq analysis were determined to sufficiently cover the time of gametocyte development without unnecessarily increasing rodent use.
Data exclusions	No Data was excluded from the study.
Replication	Key findings of the manuscript were replicated independently multiple times by two different research teams. Exact number of replicates of each experiment included in figure legends
Randomization	Animals were assigned to experimental groups at random and processed in parallel.
Blinding	All animals/samples were assigned a number and all data was harvested without the knowledge of the infection type or conditions/treatment to ensure minimal bias.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	Ter119 PEcy7 (1:500) – Thermofisher, Catalog # 25-5921-81, clone TER-119, lot 4302206, FKBP12 (1:1000) – abcam, Catalog # ab2918, polyclonal, lot GR237896-1 Cre (1:1000) - abcam, Catalog # ab190177, lot GR245386-2 Enolase (1:1000) – rabbit polyclonal antibody generated against Plasmodium berghei α-enolase. Goat Anti-Rabbit IgG-HRP secondary antibody (1:2000)- abcam, Catalog # ab205718, polyclonal, various lots.
Validation	Ter119 PEcy7 (1:500) – Thermofisher, Catalog # 25-5921-81, clone TER-119, lot 4302206, validated by manufacturer for use in flow cytometry in mouse RBC and used in >30 previous publications (https://www.thermofisher.com/antibody/product/TER-119-Antibody-clone-TER-119-Monoclonal/25-5921-81) FKBP12 (1:1000) – abcam, Catalog # ab2918, polyclonal, lot GR237896-1, validated by manufacturer for use in Western blot, can

be blocked with FKBP12 peptide (ab4935) http://www.abcam.com/fkbp12-antibody-ab2918.html, previously used for detection of FKBP12 in closely related apicomplexan parasites: toxoplasma (PMID: 23263690) and Plasmodium falciparum (PMID: 23489321)

Cre (1:1000) - abcam, Catalog # ab190177, lot GR245386-2, validated by manufacturer for use in Western blot, http://www.abcam.com/cre-recombinase-antibody-ab190177.html, previously successfully used for detection of of Cre recombinase in rodents (PMID: 26631266)

Enolase (1:1000) – rabbit polyclonal antibody generated against Plasmodium berghei α-enolase (peptide: KTYDLDFKTPNNDK) by at Proteintech group (Chicago, USA). Used in multiple previous P.berghei publications (PMID: 26118994, PMID: 25275500).

Goat Anti-Rabbit IgG-HRP secondary antibody (1:2000)- abcam, Catalog # ab205718, polyclonal, various lots, validated by manufacturer for use in Western blot, http://www.abcam.com/goat-rabbit-igg-hl-hrp-ab205718.html, used in >50 publications.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Female Theiler's Original (TO) (Envigo) 8 -12 weeks of age.
Wild animals	Study did not involve wild animals
Field-collected samples	Study did not involve field samples.

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	Red blood cells for analysis were collected from tail drops, cardiac puncture or parasite cultures. Cells were suspended in pre-warmed rich PBS with Hoechst 33342 dye and relevant antibodies, and stained for 30 min at 37oC. Stained samples were washed and resuspended in flow cytometry buffer (10% rich PBS, 1 mM EDTA in PBS).
Instrument	SR-II flow cytometer (Becton Dickinson)
Software	FACSDiva Software Version 6.1.3 and FlowJo (v. 10.3),
Cell population abundance	Parasites were typically constituting 1-10% of the total cell population. Gametocytes were between 0.1 and 70% of total parasite population.
Gating strategy	Illustrated and explained in Supplementary Figure S4A

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