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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work we publish. This form is published with all life science papers and is intended to promote consistency and transparency in reporting. All life sciences submissions use this form; while some list items might not apply to an individual manuscript, all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   For the analysis of phenotypes 3- 5 animals per treatment were routinely used for analysis. Our previous analyses (e.g. Mony, B.M., et al., Genome-wide dissection of the quorum sensing signalling pathway in Trypanosoma brucei. Nature, 2014. 505(7485): p. 681-5) indicate that this sample size is sufficient to detect differences between cell lines and treatment groups (for example where gene silencing is activated by provision of doxycycline). Using that data as an exemplar, we tested 5 genes for effects with and without doxycycline mediated gene-silencing in vivo. Using cell cycle status as the measured parameter, the effect size ranged from 0.637 to 1.804. Those values were then used to calculate the power for different samples sizes. This showed that a sample size of 3-5 per group (+ or - DOX), or total of 6 to 10 allowed us to achieve 80% power for all test genes except one. In the current manuscript, the visual analytical assays applied (manual scoring by microscope) to the different treatments and groups (cell cycle scoring, analysis of PAD1 staining, scoring of flagellar labelling for parasite species, morphological analysis) required analyses to be limited to 3 animals per group. Data were examined before analysis to ensure normality and that no transformations were required. P values of less than 0.05 were considered statistically significant.

2. Data exclusions

   Describe any data exclusions.

   No data were excluded from the analyses.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   All figures include information on the replicate number for each experiment. Experiments were also validated in additional pilot experiments or independent replicates.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Animals were allocated at random into treatment groups from a group of female, age matched MF1 mice.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   No blinding was done.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *p* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Most statistical analyses were carried out in GraphPad Prism version 6 (GraphPad Software, La Jolla, California, USA, www.graphpad.com). A General Linear Model was used to analyse T. congolense parasitaemias for cell cycle arrest using Minitab®. This model tested the significance of the effect of parasitaemia on % 2K1N, 2K2N cells and incorporated mouse as a random factor, which was not significant (p=0.55)

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* guidance for providing algorithms and software for publication may be useful for any submission.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

The materials and datasets generated during and/or analysed during the current study are available either within the manuscript or from the corresponding author on reasonable request.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

2-5x10^6 cells were washed twice in PBS prior to fixing in 500µl 2% formaldehyde/0.05% glutaraldehyde >1h at 4°C. Cells were then washed 3x in PBS and resuspended in 2%BSA:PBS for 30 min. Cells were then resuspended in primary antibody diluted in 2%BSA:PBS (αPAD1 was diluted 1:200, αEP procyclin (Cedar Lane laboratories) was diluted 1:500) and were incubated overnight at 4°C. The cells were washed twice in PBS and were resuspended in secondary antibody diluted in 2%BSA:PBS (α-rabbit CY5 and α-mouse FITC were each diluted 1:1000). The cells were washed twice in PBS and were resuspended in 500µl PBS containing 0.02µg/ml DAPI. Samples were then processed on an LSRII flow cytometer (BD Biosciences). Positive controls and secondary antibody only controls were included. Analysis was performed using FlowJo software (Tree Star).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

T. congolense parasites of the IL3000 strain were used both for infections and in vitro experiments. This strain was derived from the ILC-49 strain that was isolated from a cow in the Trans Mara, Kenya. The T. congolense IL3000 parasites used for in vivo experiments were provided by Dr Annette MacLeod (University of Glasgow) in a blood straw. The T. congolense IL3000 parasites used for in vitro experiments were supplied as culture-adapted bloodstream forms by Dr Liam Morrison (Roslin Institute, Edinburgh), who had received them from Professor Théo Baltz (University of Bordeaux).

b. Describe the method of cell line authentication used.

Morphological distinction from T brucei in vitro and in vivo.
In vivo competition experiments used labelled PFR staining to unambiguously distinguish T brucei form T. congolense.

c. Report whether the cell lines were tested for mycoplasma contamination.

No

d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Female, age matched MF1 mice were used

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Not applicable