nature research

Corresponding author(s): Alfred Cortés

☐ Initial submission ☐ Revised version ☒ Final submission

# Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but 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.

This manuscript does not include any population-based or epidemiological research. For our lab-based experiments, we selected the number of independent biological replicates based on feasibility and variability between replicates. When differences between conditions were obvious and the patterns were consistently observed in all replicates and also in similar experiments under slightly different conditions (which could not be counted as replicates), typically we performed 2 to 4 independent biological replicates.

2. Data exclusions

Describe any data exclusions.

As described in the Methods and in the legend of Supplementary Table 1, in our plaque assays we excluded clusters that contained more than one free hemozoin pigment (residual body), because such clusters likely originated from multiply infected schizonts in the overlaid culture This criteria was adopted during the optimization of the assays.

The 10-15h samples from one of the replicate experiments in Figure 1 d-e were lost. This is the reason why, as stated in the figure legend, data from these samples could not be included in the analysis (N=2 instead of N=3 for these time points).

3. Replication

Describe whether the experimental findings were reliably reproduced.

All findings reported could be reproduced in multiple independent experiments. The number of independent replicates is indicated in the figure legends. Findings were considered reproducible if the results of independent experiments supported the same conclusions.

4. Randomization

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

N/A. This study did not involve animals or human research. All experiments were laboratory-based. None of the experiments were susceptible to randomization.

5. Blinding

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

N/A. This study did not involve animals or human research. All experiments were laboratory-based. Blinding was not suitable for any of the experiments.

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 in 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 clear description of 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.

*Graph Pad Prism v5 and v7 were used to generate the majority of graphics.
*Dropseq toolbox (including Picard tools and STAR aligner) and R were used for single cell RNA-seq analysis, including the following R packages: Seurat, Monocle, igraph, ggplot2, and rgl.
*SDS 2.4 (Applied Biosystems) was used for the analysis of real-time PCR data.
*BD Cell Quest (Becton Dickinson) was used for flow cytometry determination of parasitemia or proportion of multiply-infected erythrocytes.
*FlowJo 10.2 was used for the analysis of cell sorting data.
*CellSens Standard 1.11, LAS-AF Lite and Zen 2012 software were used for microscopy image acquisition, and ImageJ was used for image processing.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

## 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.

All unique materials are readily available from the authors.

9. Antibodies

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

All primary antibodies used in this study had been previously described (source and references provided in the Methods section) or are commercial antibodies:

-rat anti-HA (1:100; Roche #11867423001; lot number not available);
-rabbit anti-HA (1:100; Life technologies #71-5500, lot #936618A1);
-rabbit anti-GFP (1:1,000; Invitrogen #A11122, lot #1828014; this antibody also reacts with eYFP);
-mouse anti-Pfs16 (1:400-1:2,000, 32F717:B02, a gift from Robert Sauerwein, Radboud University). Reference 51 of the article;
-mouse anti-Pfg27 (1:2,000, 4B2, a gift from Richard Carter, University of Edinburgh). Reference 23 of the article;
-rabbit anti-H3K4me3 (1:10,000; Merck-Millipore #04-745, lot #DAM1606783);
-rabbit anti-H3K9me3 (1:1,000; Merck-Millipore #07-442, lot #DAM1810831).

The secondary antibodies used were:
-goat-anti-rat IgG conjugated with Alexa Fluor 488 (1:1,000, Thermo Fisher #A11006, different lots);
-goat-anti-mouse IgG - Alexa Fluor 594 (1:1,000, Thermo Fisher #A11007, different lots);
-goat-anti-mouse IgG - Alexa Fluor 488 (1:1,000, Thermo Fisher #A11029, different lots);
-donkey-anti-mouse IgG - Alexa Fluor 546 (1:1,000, Thermo Fisher #A10036, different lots);
-goat-anti-rabbit IgG - Alexa Fluor 594 (1:1,000, Thermo Fisher #A11012, different lots).

As stated in the Methods section, our validation of the specificity of the antibodies included absence of signal at parasite stages in which the protein is not expressed, and in the case of antibodies against an artificial 3xHA or eYFP tag comparison with the level of signal in wild type parasites that do not carry the tag (analyzed at the same stage of the life cycle). For anti-Pfs16 and anti-Pfg27 antibodies, additional characterization of the temporal dynamics and specificity of the signal is presented in Supplementary Fig. 4.

For commercial antibodies against the histone post-translational modifications H3K9me3 and H3K4me3, validation includes coincidence with the previously reported distribution of heterochromatin and euchromatin within P. falciparum nuclei, and in the case of the antibody against H3K9me3 also previous reports of ChIP analysis with these antibodies that gave the expected distribution of the signal (e.g. Crowley et al. 2011, PMID: 21306446; López-Rubio et al. PMID: 19218088). Additionally, the company (Merck-Millipore) provides the following statements:
-Rabbit anti-H3K9me3 #07-442: Peptide Inhibition: Specificity was confirmed by the ability of 10 mM of the immunizing peptide to completely abolish detection of histone H3 in immunoblot analysis of HeLa acid extracts. No inhibition of detection was observed by preabsorption of the antibody with 10 mM histone H3 peptide containing mono- or dimethyl-lysine 9, or mono-, di- or trimethyl-lysine 27 modifications (Data not shown). Western Blot Analysis: 1:500 dilution of this lot detected trimethyl Histone H3 on 10 µg of HeLa acid extract but not on recombinant Histone H3.
-Rabbit anti-H3K4me3: routinely evaluated by immunoblot on acid extracted proteins from HeLa cells, but not recombinant unmethylated Histone H3.

10. Eukaryotic cell lines

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

All Plasmodium falciparum lines used in this study are described in the first section of the Methods.
Their sources are:
-3D7 subclone E5 (derived from the 3D7-B stock), E5-PfAP2-G–HAx3 transgenic line expressing 3xHA-tagged endogenous PfAP2-G (clone 9A, here named E5-HA), and E5-eYFP transgenic line expressing eYFP-tagged endogenous PfAP2-G: generated in our laboratory;
-ligand-regulatable transgenic line E5-PfAP2-G–ddFKBP expressing PfAP2-G fused to a 3xHA-tag and the FKBP-derived destabilizing domain (clone C2, here named E5-HA-DD): obtained from Manuel Llinás (Pennsylvannia State University, USA);
-3D7 stock at Imperial College (3D7-Imp.): obtained from Michael J. Delves (Imperial College, UK);
-gametocyte-deficient 3D7-subclone F12: obtained from Pietro Alano (Istituto Superiore di Sanità, Italy).

b. Describe the method of cell line authentication used.

The parasite lines used were not directly authenticated, but their sexual conversion rates, pfap2-g transcript levels and expression of an exogenous 3xHA/eYFP tag or a destabilization domain were always consistent between experiments and with previous published reports. This somehow serves as a "phenotypic" authentication. Additionally, the E5-eYFP line was regularly monitored by PCR analysis to validate presence of the sequence encoding the tag and absence of wild type parasites.

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

The parasite line E5-HA-DD (C2 clone) tested positive for mycoplasma contamination in a PCR-based test, whereas other parasite lines tested negative. However, mycoplasma contamination is not believed to affect any of the parameters analyzed in this study.

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

N/A. None of the cell lines used in this study (all P. falciparum) are listed in the database of commonly misidentified cell lines.

## 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.

No animals 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.

This study did not involve human research participants.