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Sample-size estimation

- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

Sample replicate numbers were determined based on prior experience with the experiments that were performed and on practical considerations for carrying out these experiments. N numbers and statistical tests are described in the manuscript text, methods section, and figure legends.

Replicates

- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:



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We defined biological and technical replicates following the conventions of Blainey et al. (2014) *Nature Methods*, 11: 879-880. Biological replicates consisted of distinct parasite sample wells set-up and monitored in parallel for the duration of a given growth assay. Numbers of replicates for each experiment are given in the Methods section and in figure legends.

- <u>1. Growth assays (Figures 1E, 3D, and 5A/B):</u> Individual data points in plots are the average \pm standard deviation of 2-3 biological replicates. No outlying data points have been excluded in plots.
- 2. Mass spectrometry experiments (Figure 2C and Figure 2- figure supplement 4): three biological replicates (distinct experiments and samples) were performed. Spectral counts observed for bait proteins (mACP and aACP) and Nfs1 are reported for each experiment. No outlying data have been excluded.
- 3. Microscopy analysis (Figure 5 C/D): two independent experiments (biological replicate samples) were conducted and 20-30 parasite images per replicate experiment were analyzed for each condition (40-50 total parasites counted). This information is stated in the legend for Figure 5 and in the methods section. No outlying data was excluded. Raw parasite counts are given in Figure 5- source data 1.

Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

Descriptions of statistical tests are given in Methods and in figure legends. For experiments involving repeat measurements (microscopy analysis in Figure 5C/D), an average ±standard error of the mean is reported. Differences in averages from repeat measurements were analyzed for significance by two-tailed unpaired t-test. P values for all such tests are explicitly stated in Figure 5D.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

Group allocation

 Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied



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 Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

For the microscopy analysis in Figure 5D, mitochondrial staining based on MitoTracker Red signal was scored as focal or dispersed based on size similarity to nuclear stain (focal) or parasite cytoplasm (dispersed). This information is stated in the legend for Figure 5.

Additional data files ("source data")

- We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
- Where provided, these should be in the most useful format, and they can be uploaded as "Source data" files linked to a main figure or table
- Include model definition files including the full list of parameters used
- Include code used for data analysis (e.g., R, MatLab)
- Avoid stating that data files are "available upon request"

Please indicate the figures or tables for which source data files have been provided:

All data is shown in figures and supplemental figures in our manuscript. Uncropped western blot images for mACP knockdown conditions in Figures 1D and 4A/B are provided in Figure 1- source data 1. Parasite counts for the microscopy analysis in Figure 5D is provided in Figure 5- source data 1.