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JOURNAL

Microtubule number and length determine cellular shape and function in *Plasmodium*

Benjamin Spreng, Hannah Fleckenstein, Patrick Kübler, Claudia Di Biagio, Madlen Benz, Pintu Patra, Ulrich S. Schwarz, Marek Cyrklaff and Friedrich Frischknecht

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 26th Nov 2018

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by three referees whose comments are shown below. As you can see, all referees express interest in your study investigating how microtubule number and length regulate cellular shape and function in Plasmodium. However, they also raise concerns that need to be addressed in full before we can consider publication of the manuscript here.

Given the referees' positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the comments of all three reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version.

REFEREE REPORTS:

Referee #1:

In this manuscript, Spreng, Frischknecht and colleagues studied the role of microtubules in the formation and infectivity of sporozoites, which correspond to the form adopted by Plasmodium as they travel between the oocytes and the salivary gland of the mosquito.
The deletion of the gene encoding for alpha1-tubulin induced severe morphological defects during sporozoites budding and prevented the infection of the midgut and salivary gland of the mosquito. The authors performed an extensive study of the microtubule network architecture based on both fluorescence imaging and electronic microscopy. Both techniques have intrinsic limitations and their associations gave string support to all authors observations and conclusions. Microtubules appeared essential for bud formation on the sporoblast and the displacement of the nucleus in it. The absence of microtubules led to the formation of small sporozoites with highly covoluted shapes. Surprisingly nuclear divisions could proceed in the sporoblast, in the absence of microtubules.
Intermediate levels of alpha1-tubulin deletion, or complete deletion of a1-tubulin compensated by

the expression of full or truncated form of a2-tubulin, were used to characterize the specific morphologies and intra-cellular architectures of sporozoites containing 1 to 16 microtubules. Interestingly, the various approaches concur with the identification of a sharp threshold of 11 microtubules as the lower possible values for the proper budding of the sporozoite. Surprisingly all microtubules had similar length whatever their number. This observation contrasts with most classical conditions in which microtubule dynamics impose a broad distribution of length. This unusual architecture could be explained by a model based on a difficult nucleation step followed by an easy polymerization. Considering that microtubules apparently have to apply well balanced pushing forces on the sporoblast membrane to extrude properly the future sporozoite, this tight length regulation is likely central for the budding process.
This is overall an excellent study based on solid observations and thoughtful analyses which led to original conclusions about the role of microtubule in the regulation of parasite morphology. I enjoyed very much reading it and found it thought provoking. I have only one concern relative to the model: only the « correct » set of parameters, capable to reproduce the observed length and numbers of microtubules, is shown. It would have been more informative to show the analysis of the entire range of ratio between the efficiency of elongation to nucleation that allows this conformation to be established. In addition, why is it necessary to distinguish the « bound » and the « nucleating » state? Is it because elongation can only occur when all protofilaments have been initiated? Couldn't a simpler model, taking into account nucleation rate and elongate rate only, be capable to reproduce the observed microtubule patterns?

Minor issue

- Although unexplained, it is known that all cells are not equally permeant to SiR-tubulin. I am wondering whether the non-tensed shape of the mutants could prevent the entry of SiR-tubulin at specific stages of sporozoites formation.

- could microtubules formed with alpha2-tubulin be more dynamic (and thus compatible with mitosis) than those formed with alpha1-tubulin?

Referee #2:

This is an elegant study from the Frischknecht lab that offers fundamental new insight into the role of microtubules in malaria sporozoite formation and infectivity, with implications for cell biology more generally. The study is elaborate and technically challenging, but well conducted and written. The results support the conclusions and are convincing. I have no hesitation in recommending that the paper be accepted for publication.

The authors have not mentioned an important structure of the pellicle, the subpelicular network, that links the subpellicular microtubules and inner membrane complex. Alveolin and other protein components of the SPN contribute to sporozoite shape, size, tensile strength, motility and infectivity, and can affect the distribution of the microtubules. Changing the size and number of microtubules might impact on SPN formation or function, and thus I feel it would be appropriate that a section is devoted to this in the Discussion.

Referee #3:

Plasmodium species encode two α-tubulins (-1 and 2). PlasmoDB data indicate that at least in P. falciparum, they are both expressed at all stages of development, with particularly high expression in oocysts.
This manuscript by Spreng et al describes an analysis of the consequences of deletion of the α-tubulin genes. The authors find that α-tubulin-2 is essential, as reported previously (Kooij et al 2005). The authors are successful in their attempt to delete α-tubulin-1. Previous attempts to knock

out the gene for α-tubulin-1 in P. berghei have been unsuccessful (Kooij et al 2005). The authors should comment on why they have had success where previous attempts have failed.
The authors found that the α-tubulin-1(-) parasites showed no defect in blood stage development nor in the initial stages of mosquito infection. They report that in the α-tubulin-1(-) parasites, microtubules are not present in sporozoites developing within the oocysts and that sporozoites do not develop correctly.
It is not clear what is happening with α-tubulin-2 in these parasites. Previous work shows that alpha-tubulin-2 is transcribed in the asexual blood stages, gametocytes, ookinetes and oocysts (Kooij et al 2005). The authors undertook qRT-PCR analysis of α-tubulin-2 in oocysts and found a low level of expression, which was unchanged in the α-tubulin-1(-) parasites.
Which α-tubulin isoforms are expressed in asexual blood stages and in gametocytes? Is α-tubulin-2 expressed? Does it perform the tubulin functions in these stages? Why is it not observed by SiR-tubulin in early stage the α-tubulin-1(-) oocysts where it is expressed? Can it be confirmed that alpha-tubulin-2 binds SiR-tubulin (cf yeast tubulin which does not).
Based on their studies with the early stages of nuclear division in oocysts the authors conclude that the chromosome segregation needed for nuclear division occurs even in the absence of microtubules. What is driving chromosomal segregation during asexual division? The authors need to present Western and qRT-PCR analysis of alpha and beta tubulins in early in the early oocyst stage as well as SiR-tubulin labelling and EM data in the asexual stage before it can be concluded that chromosome segregation can occur in the absence of microtubules. It appears more likely that α-tubulin-2 performs the various functions of microtubules at all stages of development until the later stages of nuclear division in oocysts, where the levels become insufficient. A careful analysis of α-tubulin-1 and -2 at the protein level would be needed to determine whether α-tubulin-2 is contributing to the division process.
The authors examined the effect of replacing wildtype α-tubulin-1 with a codon-modified α-tubulin-1 that is expressed at a lower level. Interestingly, they found that the lower level of expression was associated with the formation of fewer microtubules that exhibit a similar length to the MTs in wildtype parasites. Similarly, other modifications to α-tubulin-1, such as adding the C-terminal residues from α-tubulin-2 resulting in lower levels of expression and fewer (and in some cases shorter) microtubules. The defects in MT production led to defects in ability of transfer to the salivary glands and to transmit to recipient mice. It is perhaps not surprising that alterations in the levels of expression of tubulins leads to aberrant biology.
The authors developed a mathematical model that supports the conclusion that the concentration of α-tubulin protomers severely limits the rate of nucleation leading to fewer MTs that have a similar length.
In summary, this is a very interesting study but additional work is needed to support the authors conclusions

1st Revision - authors' response 19th Feb 19

Referee #1:

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Intermediate levels of alpha1-tubulin deletion, or complete deletion of a1-tubulin compensated by the expression of full or truncated form of a2-tubulin, were used to characterize the specific morphologies and intra-cellular architectures of sporozoites containing 1 to 16 microtubules.
Interestingly, the various approaches concur with the identification of a sharp threshold of 11 microtubules as the lower possible values for the proper budding of the sporozoite. Surprisingly all microtubules had similar length whatever their number. This observation contrasts with most

classical conditions in which microtubule dynamics impose a broad distribution of length. This unusual architecture could be explained by a model based on a difficult nucleation step followed by an easy polymerization. Considering that microtubules apparently have to apply well balanced pushing forces on the sporoblast membrane to extrude properly the future sporozoite, this tight length regulation is likely central for the budding process.
This is overall an excellent study based on solid observations and thoughtful analyses which led to original conclusions about the role of microtubule in the regulation of parasite morphology. I enjoyed very much reading it and found it thought provoking. I have only one concern relative to the model: only the « correct » set of parameters, capable to reproduce the observed length and numbers of microtubules, is shown. It would have been more informative to show the analysis of the entire range of ratio between the efficiency of elongation to nucleation that allows this conformation to be established.

A: Thanks for the enthusiastic support and interesting questions on the model. Our choice of model parameters is the result of an optimization process that previously has been described in the supplementary subsection "Estimation of model parameters". We agree with the referee that it is appropriate to explain this in more detail in the main text and we, therefore, have added subpanels B and C to Figure 6 that includes a set of additional “non-optimal” parameters. The accompanying text can be found in lines 330 to 341 and is highlighted in yellow.

This analysis shows the variation of mean squared error in MT number and length between the model outcome and experimental result for a wide range of binding rates and binding cooperativity to nucleation site (with a fixed value of the microtubule elongation rate taken from the literature, supplementary text ref. 1). The final set of parameters are chosen such that the MSE is MT number is minimal for a given cooperativity value. The cooperativity value is chosen such that the MSE in MT length is considerably low is close to the threshold in binding rate where it starts to rise.

In addition, why is it necessary to distinguish the « bound » and the « nucleating » state? Is it because elongation can only occur when all protofilaments have been initiated? Couldn't a simpler model, taking into account nucleation rate and elongate rate only, be capable to reproduce the observed microtubule patterns?

A: We make the nucleation a two-step process (naming them as binding to nucleation site and nucleation step) to connect our model to existing models from the literature that suggests the existence of a critical nucleus (of 12 dimers) that forms via multi-step process before the nucleation begins. In principle, one could write a simpler model with a single step nucleation reaction only, if the assumption of a critical nucleus of size 12 dimers is relaxed. For example, if we assume critical nucleus requires only 3 (p=3) dimers, then the second equation describing nucleus formation can be discarded (then S_b will be N_{nuc}). However, we decided to keep the critical nucleus size to 12 to be in agreement with the existing models and, hence, use a two-step process for nucleation.
This choice also makes sense biologically because it is reasonable to expect that the size of the nucleus should be similar to a full ring at the base of the microtubule. The supplementary text on mathematical modelling describes these assumptions and the rationale behind different terms in the model.

Minor issue

- Although unexplained, it is known that all cells are not equally permeant to SiR-tubulin. I am wondering whether the non-tensed shape of the mutants could prevent the entry of SiR-tubulin at specific stages of sporozoites formation.

A: This is indeed a possibility. However, the observation that we can correlate the number of microtubules as found by EM with the staining intensity obtained by SiR tubulin would argue that permeability to SiR tubulin is mostly unaffected.

- could microtubules formed with alpha2-tubulin be more dynamic (and thus compatible with mitosis) than those formed with alpha1-tubulin?

A: This is a possibility indeed. In addition to the parasite lines described in this paper, we have also made one where alpha2-tubulin is replaced by alpha1-tubulin. Like the reverse line, this has had no impact on the blood stages, suggesting that at least for this stage there is no difference in their capacity to undergo mitosis. This line, together with others will be described in a follow-up paper.

Referee #2:

This is an elegant study from the Frischknecht lab that offers fundamental new insight into the role of microtubules in malaria sporozoite formation and infectivity, with implications for cell biology more generally. The study is elaborate and technically challenging, but well conducted and written. The results support the conclusions and are convincing. I have no hesitation in recommending that the paper be accepted for publication.

A: Many thanks for the enthusiastic support.

The authors have not mentioned an important structure of the pellicle, the subpelicular network, that links the subpellicular microtubules and inner membrane complex. Alveolin and other protein components of the SPN contribute to sporozoite shape, size, tensile strength, motility and infectivity, and can affect the distribution of the microtubules. Changing the size and number of microtubules might impact on SPN formation or function, and thus I feel it would be appropriate that a section is devoted to this in the Discussion.

A: Thanks for pointing out this oversight. Indeed the involvement of the SPN in sporozoite formation is one topic we would like to address experimentally in the future. We added a paragraph in the discussion (lines 507-516) including some new references. The text reads as follows:

Another possibility contributing to decreased infectivity of the parasites showing fewer microtubules could be a change in the subpellicular network, a cytoskeletal system underlying the inner membrane complex and being linked to microtubules (Gould *et al,* 2008; Kudryashev *et al,* 2012; Harding & Meissner 2014, Frischknecht & Matuschewski 2017). It is not clear how this network is established but perturbation by genetic deletion of proteins shows that a destabilization of the network influences sporozoite shape and tensile strength (Khater *et al,* 2004). It is possible that the subpellicular network interconnects with the microtubules during parasite formation and is affected by a perturbation of microtubules. Indeed the opposite has been shown recently through deletion of the subpellicular network protein G2 in *P. berghei* sporozoites (Tremp *et al,* 2013) and GAPM1a in *T. gondii* (Harding *et al,* 2019).

Referee #3:

Plasmodium species encode two α-tubulins (-1 and 2). PlasmoDB data indicate that at least in P. falciparum, they are both expressed at all stages of development, with particularly high expression in oocysts. This manuscript by Spreng et al describes an analysis of the consequences of deletion of the α-tubulin genes. The authors find that α-tubulin-2 is essential, as reported previously (Kooij et al 2005). The authors are successful in their attempt to delete α-tubulin-1. Previous attempts to knock out the gene for α-tubulin-1 in P. berghei have been unsuccessful (Kooij et al 2005). The authors should comment on why they have had success where previous attempts have failed.

A: It is correct that both isoforms are expressed at all stages. Expression of proteins across the life cycle is not completely conserved between species. A similar situation has been reported by us and our collaborators before for the actin binding protein coronin, which is expressed in blood stages in P. falciaprum but not in P. berghei (Olshina et al., Malaria J 2015 and Bane et al. PLoS Pathogens 2016). In a sense we were lucky to study P. berghei to address the role of microtubules in sporogony. Our success is most likely being rooted in improved transfection methods and we now state this in line 124 - 125.

The authors found that the α-tubulin-1(-) parasites showed no defect in blood stage development nor in the initial stages of mosquito infection. They report that in the α-tubulin-1(-) parasites, microtubules are not present in sporozoites developing within the oocysts and that sporozoites do not develop correctly.

It is not clear what is happening with α-tubulin-2 in these parasites. Previous work shows that alpha-tubulin-2 is transcribed in the asexual blood stages, gametocytes, ookinetes and oocysts (Kooij et al 2005). The authors undertook qRT-PCR analysis of α-tubulin-2 in oocysts and found a low level of expression, which was unchanged in the α-tubulin-1(-) parasites.
Which α-tubulin isoforms are expressed in asexual blood stages and in gametocytes?

A: Both α-tubulin isoforms are expressed in asexual blood stages, although from data by Otto et al., 2014 alpha2-tubulin is much stronger expressed. This is now stated in lines 118-120.

Is α-tubulin-2 expressed? Does it perform the tubulin functions in these stages?

A: Yes, the the absense of α-tubulin-1 fully functioning gametes and ookinetes are formed

Why is it not observed by SiR-tubulin in early stage the α-tubulin-1(-) oocysts where it is expressed?

A: We do report a SiR-tubulin stainging in early oocysts, see Figure 3B. We have now also included EM images to show that these SiR tubulin foci likely correspond to spindles and hemispindels in early oocysts (see new EV Figure 4).

Can it be confirmed that alpha-tubulin-2 binds SiR-tubulin (cf yeast tubulin which does not).

A: Yes, if we force a2 expression in sporozoites we readily detect MTs by SiR tubulin, see figure 5 and supplementary figure 6.

Based on their studies with the early stages of nuclear division in oocysts the authors conclude that the chromosome segregation needed for nuclear division occurs even in the absence of microtubules. What is driving chromosomal segregation during asexual division?

A: As discussed in line 424-447 we think that it might be possible, like in fission yeast, that actin plays a role. Naturally this needs much more work to be understood.

The authors need to present Western and qRT-PCR analysis of alpha and beta tubulins in early in the early oocyst stage as well as SiR-tubulin labelling and EM data in the asexual stage before it can be concluded that chromosome segregation can occur in the absence of microtubules.

A: As we discussed (lines 314-317) Western analysis is impossible due to tubulins from mosquito tissue. We did perform qRT from as early as day 5 onwards (EV Figure 3). To perform analysis earlier makes less sense as the earliest oocysts are only formed around day 3. We reformulated that chromosome segregation in oocysts can occur without MTs, such that it becomes clearer that during early oocyst formation they appear to play a role (as suggested by Figure 3B and the new EV Figure 4) but not in later divisions. To this end we added statement (marked yellow) in lines 316, 424, 443 and 450-451. We also included images of SiR tubulin staining as well as electron micrographs showing both subpellicular and nuclear microtubules of blood stages in EV Figure 4A. These show similar staining (SiR tubulin) and microtubules (EM) in WT and alpha1 knock-out parasites suggesting that alpha2 tubulin is sufficient for proper segregation in blood stages. We also expanded the materials to accommodate this new work (lines 700-706 and 717).

It appears more likely that α-tubulin-2 performs the various functions of microtubules at all stages of development until the later stages of nuclear division in oocysts, where the levels become insufficient. A careful analysis of α-tubulin-1 and -2 at the protein level would be needed to determine whether α-tubulin-2 is contributing to the division process.

A: We agree but as discussed (lines 314-317) this is technically not possible. We attempted both Western blotting and proteomic analysis. We added “too much contamination from mosquito midgut tubulin” to the discussion. One way to do this would be to engineer a mutant that expresses after a skip peptide mCherry after a2 tub and GFP after a1 tub. We might include this for our follow up study on the role of a2.

The authors examined the effect of replacing wildtype α-tubulin-1 with a codon-modified α-tubulin-1 that is expressed at a lower level. Interestingly, they found that the lower level of expression was

associated with the formation of fewer microtubules that exhibit a similar length to the MTs in wildtype parasites. Similarly, other modifications to α-tubulin-1, such as adding the C-terminal residues from α-tubulin-2 resulting in lower levels of expression and fewer (and in some cases shorter) microtubules. The defects in MT production led to defects in ability of transfer to the salivary glands and to transmit to recipient mice. It is perhaps not surprising that alterations in the levels of expression of tubulins leads to aberrant biology.

A: Indeed it is not, but what amazed us is that we could titrate the numbers of MTs by the levels of expression, a first not just in Plasmodium but in any system. Hence, this shows the value of protozoans to study fundamental questions in cell biology.

The authors developed a mathematical model that supports the conclusion that the concentration of α-tubulin protomers severely limits the rate of nucleation leading to fewer MTs that have a similar length.
In summary, this is a very interesting study but additional work is needed to support the authors conclusions.

A: We thank the reviewer for his constructive critique and hope that our answers and the newly included EV Figure 4 suffice to proceed with publication of our manuscript.

2nd Editorial Decision 28th Mar 2019

Thank you for submitting a revised version of your manuscript. It has now been seen by all of the original referees whose comments are shown below.

As you will see, the referees find that all criticisms have been sufficiently addressed and recommend the manuscript for publication. However, before I can send the official acceptance letter, there are a few editorial issues concerning text and figures that I need you to address.

REFEREE REPORTS:

Referee #1:

the authors have properly respond to my question and made the appropriated changes to their manuscript which I look forward seeing published in EMBO Journal.

Referee #2:

The authors have adequately addressed my comments from the first round of revision and I am happy for this manuscript to be accepted for publication.

Referee #3:

The authors have addressed my queries.

2nd Revision - authors' response 12th Apr 2019

The authors performed the requested editorial changes.

3rd Editorial Decision 26th Apr 2019

Thank you for submitting your revised manuscript. I have now looked at everything and all looks fine. Therefore I am very pleased to accept your manuscript for publication in The EMBO Journal.

Congratulations on the very nice work!

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Friedrich Frischknecht
Journal Submitted to: EMBO Journal
Manuscript Number: 100984

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	We used different sample sizes for different experiments. For analysis of microtubule length we initially aimed at investigating at least 50 parasites, while for determination of microtubule number by electron microscopy we aimed at 20 parasites. To determine parasite numbers in mosquitoes we aimed at investigating at least 50 mosquitos and for determination of motility parameters to investigate at least 100 sporozoites. Depending on the phenotype of the mutant not all these numbers could be matched, while usually they were exceeded. As we used outbred mice in this study to investigate mosquito to mouse transmission we aimed to investigate at least 8 animals in transmission experiments, although we used only 4 when there was no phenotype detectable prior to salivary glands infection and within motility analysis, strongly suggesting that there would be no phenotype during transmission.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	We used animals (mice) here for infection study by mosquito bites and by injection of P. berghei sporozoites. For the use of inbred mice the lowest possible size is generally accepted to be 4 mice in a single experiment, while for outbred mice this is 8.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	In this study no animals were excluded from the analysis. One criteria to do so would have been a notification from the animal facility about potential secondary infections detected during the course of the study or a problem during injection of the animal with parasites.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	In critical experiments the data were blinded and counted by a separate student. These included the measurements of microtubule length and the determination of microtubule numbers in EM images.
For animal studies, include a statement about randomization even if no randomization was used.	Randomization was used in the evaluation of the blood smears at critical days to avoid bias. Critical days include those around the day of patency.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Yes, critically important data (microtubule number and length evaluation) was performed partly in paralell by a blinded investigator.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Determination of prepatency, which was a key readout in this study to assess transmission efficiency, was blinded.
5. For every figure, are statistical tests justified as appropriate?	yes

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Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. To test for gaussian distribution, we used the D'Agostino-Pearson omnibus normality test. However, our data was not normal distributed. Therefore, we only used the non-parametric Kruskal-Wallis-Test which does not require normal distribution.
Is there an estimate of variation within each group of data?	No, we used standard deviation or the blotting of individual datapoints where appropriate but refrained from filling the table with standard deviation statement in order to not drown the trends in too many numbers. This is an intentional deviation from the practise in the field, where often parasite numbers are given to ridiculous detail.
Is the variance similar between the groups that are being statistically compared?	Not in all groups, e.g. as the numbers of parasites decrease in mosquitoes the variance becomes larger between individual mosquitoes.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	No antibodies were used in the study.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	No cell lines were used, details to the used parasite lines are stated.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	All this info is stated in the materials and methods. Mice are housed in cages of 4 initially and upon increasing weight get split into cages housing 3 mice. Food and water are provided continuously as are some enrichments.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	We included this statement. We naturally complied to the ethical regulations as our animal experiments are approved by the state authorities according to German federal and European law. The state authority involved is the 'Regierungspräsidium Karlsruhe'. Prior to this approval the university animal facility checks our application.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirme compliance.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	We did not generate any data that needs to be deposited.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	We generated a source file with the raw data for potential use in later meta analysis.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	not applicaple
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	The code for simulating the mathematical model is not written in a standardised format (such as SBML, CellML) or as a Matlab script. The model simulation is done in custom written codes in C programming language, which can be provided upon request

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	no