Protein Phosphatase 1 regulates atypical chromosome segregation and cell polarity during mitotic and meiotic division in *Plasmodium* sexual stages

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Short title: Role of PP1 during mitosis and meiosis in *Plasmodium*

Abstract

PP1 is a conserved eukaryotic serine/threonine phosphatase that regulates many aspects of mitosis and meiosis, often working in concert with other phosphatases, such as CDC14 and CDC25. The proliferative stages of the parasite life cycle include sexual development within the mosquito vector, with male gamete formation characterized by an atypical rapid mitosis, consisting of three rounds of DNA synthesis, successive spindle formation with clustered kinetochores, and a meiotic stage during zygote to ookinete development following fertilization. It is unclear how PP1 is involved in these unusual processes. Using real-time live-cell and ultrastructural imaging, conditional gene knockdown, RNA-seq and proteomic approaches, we show that *Plasmodium* PP1 is involved in both chromosome segregation during mitotic exit, and establishment of cell polarity during zygote development in the mosquito midgut, suggesting that small molecule inhibitors of PP1 should be explored for blocking parasite transmission.

Introduction

Cell cycle progression involves sequential and highly ordered DNA replication and chromosome segregation in eukaryotes[1, 2], which is tightly controlled and coordinated by reversible protein phosphorylation catalysed by protein kinases (PKs) and protein phosphatases (PPs)[3]. Numerous studies have highlighted the importance of the phosphoprotein phosphatase (PPP) family in regulating mitosis in model eukaryotic organisms, often working in conjunction with CDC25 and CDC14 phosphatases, as key regulators of mitotic entry and exit, respectively[4, 5, 6, 7].

Protein Phosphatase 1 (PP1) is a member of the PPP family and is expressed in all eukaryotic cells. It plays a key role in the progression of mitosis through dephosphorylation of a large variety of proteins, including mitotic kinases (such as cyclin-dependent kinase 1 (CDK1)[8, 9, 10, 11], and regulators of chromosome segregation[12]. Chromosome segregation is orchestrated at the kinetochore, a protein complex that assembles on the centromeres, located at the constriction point of sister chromatids to facilitate and monitor attachment of the sister chromatids to spindle microtubules[13, 14]. Correct attachment of the kinetochore to spindle microtubules is regulated by the KMN (KNL1, MIS12 and NDC80) protein network[15], which in mammalian cells integrates the activities of at least five protein kinases (including MPS1, Aurora B, BUB1, PLK1, and CDK1) and two protein phosphatases (PP1 and PP2A-B56). The KMN network mediates kinetochore-microtubule attachments and scaffolds the spindle assembly checkpoint (SAC) to prevent chromosome segregation until all sister chromatids are properly connected to the spindle[16]. The orchestration of reversible protein phosphorylation is crucial to control the spatial-temporal progression of the cell cycle and PP1 has a key role in this

process, in particular during mitotic exit. Throughout mitosis, PP1 is inhibited by the cyclin-dependent kinase (CDK1)/cyclin B complex and Inhibitor 1[17, 18]; however, during mitotic exit concomitant destruction of cyclin B and reduced activity of CDK1 through dephosphorylation by CDC14 results in subsequent reactivation of PP1 via autophosphorylation and completion of mitotic exit[10, 17, 19, 20, 21].

In *Plasmodium*, the causative organism of malaria, there is a single PP1 orthologue, which is expressed throughout the parasite's complex life-cycle[22] and located in both nucleus and cytoplasm[23]. Previous studies determined that *P. falciparum* PP1 functionally complements the *Saccharomyces cerevisiae* glc7 (PP1) homologue[24], and subsequent phylogenetic analyses revealed that homologues of the phosphatases CDC14 and CDC25 are absent from *Plasmodium*[23]. Genetic screens and inhibitor studies have shown that PP1 is essential for asexual blood stage development[23, 25, 26, 27] (also reviewed[28]), in particular for parasite egress from the host erythrocyte[29]. Most studies in *Plasmodium* have been focused on these asexual blood stages of parasite proliferation, and very little is known about the importance of PP1 for transmission stages within the mosquito vector. Recent phosphoproteomic studies and chemical genetics analysis have identified a number of potential *Plasmodium falciparum* (Pf) PP1 substrates modified during egress, including a HECT E3 protein-ubiquitin ligase and GCα, a guanylyl cyclase with a phospholipid transporter domain[29]. Other biochemical studies in *Plasmodium* have identified numerous PP1-interacting partners (PIPs) that are structurally conserved and regulate PP1 activity, including LRR1 (a human SDS22 orthologue), Inhibitor 2 and Inhibitor 3. Other PIPs including eif2β, and GEXP15 have also been identified[9, 28, 30,

[31]. However, little is known regarding how PP1 is involved in regulating mitosis and meiosis in the absence of CDC14 and CDC25.

The *Plasmodium* life-cycle is characterised by several mitotic stages and a single meiotic stage (reviewed in[32, 33, 34]). However, in this organism kinetochore dynamics and chromosome segregation are poorly understood, especially in mitosis during male gametogenesis. This is a very rapid process with three rounds of spindle formation and genome replication from 1N to 8N within 12 minutes. In addition, little is known about the first stage of meiosis that occurs during the zygote to ookinete transition. We have followed recently the spatio-temporal dynamics of NDC80 throughout mitosis in schizogony, sporogony and male gametogenesis, and during meiosis in ookinete development[35], using approaches that offer the opportunity to study the key molecular players in these crucial stages of the life cycle. Although PP1 has been partially characterised and shown to have an essential role during asexual blood stage development in the vertebrate host[23], the role and importance of PP1 during sexual stage development in the mosquito is completely unknown.

Here, using the *Plasmodium berghei* (Pb) mouse model of malaria, we determined the importance of PP1 during the sexual stages within the mosquito vector. PP1 expression and location were studied using the endogenous, GFP-tagged protein and co-localisation with the kinetochore marker, NDC80, to follow progression through chromosome segregation during male gamete formation and zygote differentiation. Using a conditional gene knockdown approach we examined how PP1 orchestrates atypical mitosis and meiosis, and investigated the ultrastructural consequences of PP1 gene knockdown for cell morphology, nuclear pole

multiplication and flagella formation during male gamete formation. RNA-Seq analysis was used to determine the consequence of PP1 gene knockdown on global transcription, which disclosed a marked differential expression of genes involved in reversible phosphorylation, motor activity and the regulation of cell polarity. Proteomics studies identified motor protein kinesins as interacting partners of PP1 in the gametocyte. The knockdown of PP1 gene expression blocks parasite transmission by the mosquito, showing that this protein has a crucial function in *Plasmodium* sexual development during both mitosis in male gamete formation and meiosis during zygote to ookinete differentiation.

Results

PP1-GFP has a diffuse subcellular location during asexual blood stage schizogony and forms discrete foci during endomitosis.

To examine the spatio-temporal expression of *Plasmodium* PP1 in real-time during cell division, we generated a transgenic *P. berghei* line expressing endogenous PP1 with a C-terminal GFP tag. An in-frame *gfp* coding sequence was inserted at the 3' end of the endogenous *pp1* locus using single crossover homologous recombination (**Fig S1A**), and successful insertion was confirmed by diagnostic PCR (**Fig S1B**). Western blot analysis of schizont protein extracts using an anti-GFP antibody revealed a major 62-kDa band, the expected size for PP1-GFP protein, compared to the 29 kDa WT-GFP (**Fig S1C**).

During asexual blood stage proliferation, schizogony is characterised by multiple rounds of asynchronous nuclear division without chromosome condensation or cytokinesis. Nuclear division is a closed mitotic process without dissolution-

reformation of the nuclear envelope and with the spindle-pole body (SPB)/microtubule-organising centre (MTOC) embedded within the nuclear membrane[36]. It results in a multinucleated coenocyte termed a schizont, which is resolved by cytokinesis at the end of schizogony into individual merozoites. Live cell imaging of *P. berghei* asexual blood stages revealed a diffuse cytoplasmic and nuclear distribution of PP1-GFP, together with a distinct single focus of concentrated PP1-GFP in the nucleus of early trophozoite stage (**Fig 1A**), representing the early S-phase of the cell cycle when DNA synthesis starts. As schizogony proceeds, the diffuse distribution of PP1-GFP remained; however, in early schizonts each cell displayed two distinct PP1-GFP foci in close association with the stained nuclear DNA. These pairs of PP1-GFP foci became clearer during the middle and late schizont stages but following merozoite maturation and egress the intensity of the foci diminished (**Fig 1A**).

To study further the location of the PP1-GFP foci throughout mitosis, we generated by genetic cross parasite lines expressing either PP1-GFP and the kinetochore marker NDC80-mCherry[35] or PP1-GFP and the inner membrane complex (IMC)-associated myosin A (MyoA)-mCherry. Live cell imaging of these lines revealed co-localisation of PP1-GFP and NDC80-mCherry foci close to the DNA of the nucleus through blood stage development, and especially during late schizogony and segmentation (**Fig 1B**); whereas the PP1-GFP foci at the outer periphery of the cell showed a partial co-location with MyoA-mCherry, and only during mid- to late schizogony (**Fig 1C**). Thus, in schizonts there is a concentration of PP1-GFP at the kinetochore and at a transient peripheral location during specific stages of schizogony, as well as the diffuse distribution throughout the cytoplasm (**Fig.1**).

PP1-GFP is enriched on kinetochores during chromosome segregation associated with putative mitotic exit in male gametogony

To study PP1 expression and location through the three rounds of DNA replication and chromosome segregation prior to nuclear division during male gametogony, we examined PP1-GFP by live-cell imaging over a 15-minute period following male gametocyte activation. Following activation with xanthurenic acid and decreased temperature, male gametocytes undergo three rounds of DNA replication and mitotic division followed by chromosome condensation and exflagellation, resulting in eight gametes[37]. Before activation (0 min), PP1-GFP was detected with a diffuse location throughout gametocytes (**Fig 2A**). At one minute post-activation, PP1-GFP accumulated at one end of the nucleus at a single focal point (**Fig 2A**). After 2-3 min two distinct foci were observed on one side of the nucleus, concurrent with the first round of chromosome replication/segregation (**Fig 2A**). Subsequently, four and eight PP1-GFP foci were observed at 6 to 8 min and 8 to 12 min post-activation, respectively, corresponding to the second and third rounds of chromosome replication/segregation. These discrete PP1-GFP foci dispersed prior to karyokinesis and exflagellation of the mature male gamete 15 min post-activation, leaving residual protein remaining diffusely distributed throughout the remnant gametocyte and flagellum (**Fig 2A**).

To investigate further the location of PP1-GFP during spindle formation and chromosome segregation, the parasite line expressing both PP1-GFP and NDC80-mCherry was examined by live cell imaging to establish the spatio-temporal relationship of the two proteins. We found that the discrete PP1-GFP foci colocalized

with NDC80-mCherry at different stages of male gametogony, including when up to eight kinetochores were visible **(Fig 2B)**, but PP1-GFP was not associated with the arc-like bridges of NDC80-mCherry representing the spindle[35] at 2 min and 4 to 5 min post activation, suggesting that PP1-GFP is only increased at the kinetochore during initiation and termination of spindle division (**Fig 2B**).

PP1-GFP may determine apical polarity during zygote-ookinete development and has a nuclear location on kinetochores during meiosis

After fertilisation in the mosquito midgut, the diploid zygote differentiates into an ookinete within which the first stage of meiosis occurs. During this process DNA is duplicated to produce a tetraploid cell with four haploid genomes within a single nucleus in the mature ookinete. PP1 is known to be crucial during meiotic chromosome segregation[12], and therefore we analysed the spatio-temporal expression of PP1-GFP during ookinete development using live cell imaging. PP1-GFP was expressed in both male and female gametes with a diffuse distribution, along with a single focus of intense fluorescence at one end (potentially the basal body) of each male gamete (**Fig. 3A**). Initially, the zygote also had a diffuse PP1-GFP distribution, but after two hours (stage I) an enriched focus developed at the periphery of the zygote, marking the point that subsequently protruded out from the cell body and developed into the apical end of the ookinete. A strong PP1-GFP fluorescence signal remained at this apical end throughout ookinete differentiation (**Fig 3A**). In addition, PP1-GFP was enriched in the nucleus at four discrete foci in mature ookinetes (**Fig 3A**). Analysis of the parasite line expressing both PP1-GFP and NDC80-mCherry showed that these four foci correspond to the kinetochores of meiotic development in the ookinete (**Fig 3B**).

PP1-GFP has a diffuse distribution with multiple nuclear foci during oocyst development, representing endomitosis

Upon maturation the ookinete penetrates the mosquito midgut wall and embeds in the basal lamina to form an oocyst. Over the course of 21 days sporogony occurs in which several rounds of closed endomitotic division produce a multiploid cell termed a sporoblast[38]; with subsequent nuclear division resulting in thousands of haploid nuclei and concomitant sporozoite formation. We observed multiple foci of PP1GFP along with diffused localization during oocyst development (**Fig 3C**). Dual colour imaging showed a partial co-localisation of nuclear PP1-GFP and NDC80-mCherry foci throughout oocyst development and in sporozoites (**Fig 3D**), suggesting that PP1-GFP is diffusely distributed during oocyst development but also recruited to kinetochores, as highlighted by multiple nuclear foci during oocyst development.

Generation of transgenic parasites with conditional knockdown of PP1

Our previous systematic analysis of the *P. berghei* protein phosphatases suggested that PP1 is likely essential for erythrocytic development[23], a result which was further substantiated in a recent study of *P. falciparum*[29]. Since little is known about the role of PP1 in cell division during the sexual stages of parasite development in the mosquito vector, we attempted to generate transgenic, conditional knockdown lines using either the auxin inducible degron (AID) or the promoter trap double homologous recombination (PTD) systems. Despite successful generation of a parasite line expressing PP1-AID, addition of indole-3-acetic acid (IAA) did not result in PP1 depletion (**Fig S2C**) and male gametogony was not affected (**Fig S2C**). Therefore, we used a promoter trap method that had been used previously for

functional analysis of the condensin core subunits SMC2/4 and the APC/C complex component, APC3[39, 40]. Double homologous recombination was used to insert the *ama* promoter upstream of *pp1* in a parasite line that constitutively expresses GFP[41]. The expected outcome was a parasite expressing PP1 during asexual blood stage development, but not at high levels during the sexual stages (**Fig S2D**). This strategy resulted in the successful generation of two independent PP1PTD parasite clones (clones 3 and 5) produced in independent transfections, and integration confirmed by diagnostic PCR (**Fig S2E**). The PP1PTD clones had the same phenotype and data presented here are combined results from both clones. Quantitative real time PCR (qRT-PCR) confirmed a downregulation of *pp1* mRNA in gametocytes by approximately 90% (**Fig 4A**).

Knockdown of PP1 gene expression during the sexual stages blocks parasite transmission by affecting parasite growth and development within the mosquito vector

The PP1PTD parasites grew slower than the WT-GFP controls in the asexual blood stage (**Fig S2E**) but produced normal numbers of gametocytes in mice injected with phenylhydrazine before infection. Gametocyte activation with xanthurenic acid in ookinete medium resulted in significantly fewer exflagellating PP1PTD parasites per field in comparison to WT-GFP parasites, suggesting that male gamete formation was severely retarded (**Fig. 4B**). For those few PP1PTD gametes that were released and viable, zygote-ookinete differentiation following fertilization was severely affected (**Fig 4C)**, with significantly reduced numbers of fully formed, banana-shaped ookinetes. In all PP1PTD samples analysed 24 hours post-fertilisation, the vast majority of zygotes were still round, and there was a significant number of abnormal

retort-shaped cells with a long thin protrusion attached to the main cell body (**Fig 4C, D**).

To investigate further the function of PP1 during parasite development in the mosquito, *Anopheles stephensi* mosquitoes were fed on mice infected with PP1PTD and WT-GFP parasites as a control. The number of GFP-positive oocysts on the mosquito gut wall was counted on days 7, 14 and 21 post-infection. No oocysts were detected from PP1PTD parasites; whereas WT-GFP lines produced normal oocysts (**Fig 4E, F**), all of which had undergone sporogony (**Fig 4G**). Furthermore, no sporozoites were observed in the salivary glands of PP1PTD parasite-infected mosquitoes, in contrast to WTGFP parasite-infected mosquitoes (**Fig 4H**). This lack of viable sporozoites was confirmed by bite back experiments that showed no further transmission of PP1PTD parasites, in contrast to the WT-GFP lines, which showed positive blood stage infection four days after mosquito feed (**Fig S2F**).

Ultrastructure analysis of PP1PTD gametocytes shows defects in nuclear pole and axoneme assembly

To determine the ultrastructural consequences of reduced PP1 expression, WTGFP and PP1PTD gametocytes were examined at 6- and 30-min post activation (pa) by electron microscopy. In mutant PP1PTD lines, at 6-min pa microgametocytes contained a large central nucleus with a few nuclear poles (**Fig 5a**). Basal bodies and axonemes were often associated with the nuclear poles (**Fig 5a, b**). In cross section, certain, but not all, axonemes displayed the 9+2 microtubular arrangement (**Fig 5c**). These appearances were similar to those observed in the WTGFP line at 6-

min pa (**Fig 5a-c and Fig 5g-i**). However, when counts were made of the relative number of nuclear poles, basal bodies and axonemes, quantitative differences were observed with, PP1PTD gametocytes appearing less developed with fewer nuclear poles (0.86 WT v 0.56 Mutant), basal bodies (1.03 WT v 0.56 Mutant) and axonemes (2.57 WT v 1.27 Mutant); the relative numbers were based on random sections through 30 microgametocytes in each group.

At 30-min pa, the majority of PP1PTD microgametocytes still appear similar to those at 6-min pa, exhibiting an open nucleus with a few nuclear poles, basal bodies and associated axonemes (**Fig 5a-c and Fig 5d-f**). The main morphological difference was a marked increase in the number and length of the axonemes (**Fig 5a and Fig 5d**). In contrast, the majority of WTGFP microgametocytes, at 30-min pa, were at an advanced stage of development with a number displaying nuclei with chromatin condensation (**Fig 5j**) and others exhibiting examples of active exflagellation with the flagellum protruding from the surface (**Fig 5k**). This was in addition to the presence of free microgametes (**Fig 5j, l**). In summary the development of the PP1PTD microgametocytes was severely retarded although there was an increase in axoneme growth.

PP1PTD parasites have altered expression of genes involved in cell cycle progression, cell motility, and apical cell polarity

To determine the consequences of PP1 knockdown on global mRNA transcription, we performed RNA-seq in duplicate on PP1PTD and WT-GFP gametocytes, 0 min and 30 min post-activation (**Fig 6A and B**). All replicates were clustered together based on condition (**Fig S3A**) and totals of 13 to 32 million RNA-seq reads were generated per sample for the global transcriptome **(Fig S3B).** In both 0 min and 30

min activated gametocytes, *pp1* was significantly down-regulated (by more than 16-fold, q value < 0.05) in PP1PTD parasites compared to WT-GFP parasites (**Fig 6A**), thus confirming the qPCR result (**Fig 4A**). We observed significant transcriptional perturbation in both 0 min and 30 min activated gametocytes, affecting the expression of 530 and 829 genes respectively (representing ~ 10% and 16% of the total gene complement) (**Table S1**). Of the total perturbed genes, 344 and 581 were significantly down-regulated and 186 and 248 genes were significantly up-regulated in the PP1PTD parasites compared to WT-GFP controls in 0 min and 30 min activated gametocytes, respectively (**Table S1**).

To explore the biological roles of the genes down-regulated following reduced *pp1* expression, we performed gene-ontology (GO) based enrichment analysis. We observed that many genes encoding kinases, phosphatases and motor proteins were differentially expressed in either or in both 0 min or 30 mins activated gametocytes (**Fig 6C**), complementing the observations from our phenotypic analysis (**Fig 4C, D**). In addition, we also observed that many genes encoding proteins involved in cell motility, cell-cycle progression, host-cell entry and ookinete development were significantly affected in activated PP1PTD gametocytes. Transcript levels measured by RNA-seq were further validated by qRT-PCR for a few selected genes modulated in gametocytes at 0 min and 30 min, and involved in regulation of cell cycle, cell motility, and ookinete invasion (**Fig 6D**).

PP1–GFP interacts with similar proteins in schizonts and gametocytes, but with a predominance of microtubule motor kinesins in gametocytes

Previous studies have analysed the PP1 interactome in *P. falciparum* schizonts, revealing several interacting partners[31, 42]. Here, we analysed the PP1 interactome in *P. berghei* schizonts and gametocytes to establish whether there were differences that might reflect distinct functions in the two stages. We immunoprecipitated PP1-GFP from lysates of schizonts following parasite culture *in vitro* for 10 hours and 24 hours and from lysates of gametocytes 10 to 11 min post activation because of high PP1-GFP abundance at these stages (**Fig 7A, Table S2**). Mass spectrometric analysis of these pulldowns identified several proteins common to both schizont and gametocyte stages, suggesting similar protein complexes in both stages (**Fig 7A, B**). In addition, we also identified in the pulldown from gametocyte lysates a number of microtubule proteins that are associated with the spindle or axoneme, including kinesin-8B, kinesin-15, kinesin-13 and PF16 (**Fig 7A, B)**. These proteins are specific to male gametocytes and may have important roles in axoneme assembly and male gametogony[37, 43].

Discussion

Reversible protein phosphorylation is crucial for cell cycle progression in eukaryotes and is tightly controlled by a variety of protein kinases and phosphatases[44, 45]. *Plasmodium* possesses a set of divergent protein kinases and protein phosphatases, which regulate many processes during cell division and parasite development throughout different stages of the life-cycle[23, 46], and PP1 is quantitatively one of the most important protein phosphatases that hydrolyse serine/threonine linked phosphate ester bonds[47]. It is expressed in all cells and highly conserved in organisms throughout the Eukaryota including *Plasmodium*[23, 48]. It forms stable complexes with several PP1-interacting proteins (PIPs) that are

diverse and differ in various organisms, control its location and function, and assist in processes throughout the cell cycle[8, 49, 50, 51].

The cell cycle in *Plasmodium* differs from that of many other eukaryotes with atypical mitotic and meiotic divisions throughout its life cycle[35, 37, 52]. It lacks several classical cell cycle regulators including the protein phosphatases CDC14 and CDC25[23], and key protein kinases including polo-like kinases[46, 53]. Therefore, the role of PP1, or indeed other PPPs in the absence of these protein phosphatases and kinases may be more crucial during cell division in *Plasmodium*. In the present study, we focused on the location and functional role of PP1 in mitotic division, particularly during male gametogony, and in meiosis during the zygote to ookinete transition. Both processes are essential for the sexual stage of the *Plasmodium* life cycle and are required for transmission by the mosquito vector. Our previous systematic functional analysis of the *Plasmodium* protein phosphatome showed that PP1 has an essential role during asexual blood stage development; this finding was supported by a recent study, which showed its role in merozoite egress from the host erythrocyte[29]. Our findings here reveal that PP1 is also expressed constitutively and co-localises with the kinetochore protein NDC80 during different stages of *Plasmodium* asexual and sexual development, hinting at a role during atypical chromosome segregation. Our conditional PP1 gene knockdown suggests that it plays a crucial role in mitotic division during male gametogony; and regulates cell polarity in meiosis during zygote to ookinete transformation.

Male gametogony in *Plasmodium* is a rapid process with DNA replication and three mitotic divisions followed by karyokinesis and cytokinesis to form eight flagellated

397 male gametes within 15 min of gametocyte activation[35, 52]. The expression and
398 localization profiles show that although the protein is diffusely distributed throughout
399 the cytoplasm and the nucleus, there is a cyclic enrichment of PP1-GFP at the
400 kinetochore, associating with NDC80-mCherry during all three successive genome
401 replication and closed mitotic divisions without nuclear division[35]. The accumulation
402 of PP1-GFP at the kinetochore at the start of nuclear division and subsequent
403 decrease upon completion is similar to the situation in other eukaryotes where the
404 activity of PP1 increases in G2 phase, reduces during prophase and metaphase, and
405 increases again during anaphase[45]. This behaviour suggests a role for PP1 in
406 regulating rapid mitotic entry and exit during male gametogony. Further analysis of
407 PP1 gene knockdown showed a significant decrease in male gamete formation (i.e.
408 during male gametogony), and ultrastructural analysis revealed fewer nuclear poles
409 and basal bodies associated with axonemes, with a concomitant absence of
410 chromosome condensation in male gametocytes of PP1PTD parasites, suggesting a
411 role for PP1 during chromosome segregation and gamete formation (i.e. flagella
412 formation). A similar phenotype was observed in our recent study of a divergent
413 *Plasmodium* cdc2-related kinase (CRK5)[54]. CRK5 deletion resulted in fewer nuclear
414 poles, and no chromatin condensation, cytokinesis or flagellum formation,
415 suggesting that there may be a coordinated activity of PP1 and CRK5 in reversible
416 phosphorylation. A recent phosphoregulation study across the short time period of
417 male gametogony showed tightly controlled phosphorylation events mediated by
418 several protein kinases including ARK2, CRK5, and NEK1[55]; however, the reciprocal
419 protein phosphatases that reverse these events are unknown. The expression
420 pattern and cyclic enrichment of PP1 at the kinetochores suggest a key reciprocal
421 role of PP1 in reversing the protein phosphorylation mediated by these kinases. This

interpretation is supported by our global transcriptome analysis of the PP1PTD parasite, which showed a modulated expression of several serine/threonine protein kinases, protein phosphatases and motor proteins. The similar pattern of reduced expression of some of these protein phosphatases and kinases suggests they have a coordinated role in reversible phosphorylation. Of note, phosphoregulation of motor proteins in *Plasmodium* has been described previously[55] and it plays an important role in spindle assembly and axoneme formation during male gametogony[35, 37].

The proteomics analysis showed that PP1 interacts with a conserved set of proteins in both asexual and sexual stages of development, and with additional proteins in gametocytes, implicating major motor proteins that may be required for spindle and axoneme assembly during male gametogenesis. Several of the conserved interacting partners also form complexes with PP1 in other eukaryotes but the gametocyte specific proteins such as kinesin-8B, kinesin-15, kinesin-13, and PF16 are unique PIPs in *Plasmodium*[9, 42]. These findings are consistent with our transcriptomic analysis of PP1PTD showing modulated expression of motor proteins in male gametocytes.

Analysis of PP1 during the zygote to ookinete transformation showed an additional location that defines the cell's polarity: at the apical end during the early stages of ookinete development. These data suggest that PP1 may also regulate cell shape during ookinete differentiation. This suggestion is supported by the consequence of PP1PTD gene knock down in which numerous underdeveloped ookinetes with long, thin protrusions attached to the main cell body were observed. This is substantiated by a recent study that showed PP1-mediated and SDS22 dependent dephosphorylation of LGL, an actomyosin-associated protein, controls apical-basal polarity in *Drosophila*[56]. Our transcriptomic analysis of PP1PTD-gametocytes

showed the modulation of several proteins involved in gliding motility including IMC proteins, MyoA, GAPs, GAPMs and ookinete specific proteins. These proteins are important for maintenance of cell shape, gliding motility, and mosquito gut wall invasion by *Plasmodium* ookinetes. This suggests that PP1 is involved in the phospho-regulation of proteins involved in defining polarity and maintenance of ookinete shape.

In conclusion, PP1 is a constitutively expressed phosphatase, distributed throughout the cell but enriched in the nucleus, and associated with the kinetochore during mitosis and meiosis, with a role in the regulation of mitosis and meiosis throughout the *Plasmodium* life-cycle.

Material and Methods

Ethics statement

The animal work performed in the UK passed an ethical review process and was approved by the United Kingdom Home Office. Work was carried out under UK Home Office Project Licenses (40/3344 and 30/3248) in accordance with the United Kingdom 'Animals (Scientific Procedures) Act 1986'. Six- to eight-week-old female CD1 outbred mice from Charles River laboratories were used for all experiments in the UK.

Generation of transgenic parasites

GFP-tagging vectors were designed using the p277 plasmid vector and transfected as described previously[23]. A schematic representation of the endogenous *pp1* locus (PBANKA_1028300), the constructs and the recombined *pp1*

locus can be found in **Fig S1A**. For GFP-tagging of PP1 by single crossover homologous recombination, a region of *pp1* downstream of the ATG start codon was used to generate the construct. For the genotypic analyses, a diagnostic PCR reaction was performed as outlined in **Fig. S1A**. Primer 1 (intP6tg) and primer 2 (ol492) were used to determine correct integration of the *gfp* sequence at the targeted locus. For western blotting, purified gametocytes were lysed using lysis buffer (10 mM TrisHCl pH 7.5, 150 mM NaCl, 0.5 mM EDTA and 1% NP-40). The lysed samples were boiled for 10 min at 95 ºC after adding Laemmli buffer and were centrifuged at maximum speed (13000g) for 5 min. The samples were electrophoresed on a 4–12% SDS-polyacrylamide gel. Subsequently, resolved proteins were transferred to nitrocellulose membrane (Amersham Biosciences). Immunoblotting was performed using the Western Breeze Chemiluminescence Anti-Rabbit kit (Invitrogen) and anti-GFP polyclonal antibody (Invitrogen) at a dilution of 1:1250, according to the manufacturer's instructions.

To study the function of PP1, we used two conditional knock down systems; a promoter exchange/trap using double homologous recombination (PP1PTD) and an auxin inducible degron (PP1AID) system. The PP1AID construct was derived from the p277 plasmid, where the GFP sequence was excised following digestion with *Age*I and *Not*I restriction enzymes and replaced with an AID/HA coding sequence. The AID-HA sequence was PCR amplified (using primers: 5'-CCCCAGACGTCGGATCCAATGATGGGCAGTGTCGAGCT-3' and 5'-ATATAAGTAAGAAAAACGGCTTAAGCGTAATCTGGA-3') from the GW-AID/HA plasmid (http://plasmogem.sanger.ac.uk/). Fragments were assembled following the Gibson assembly protocol to generate the PP1-AID/HA transfection plasmid that was transfected in the 615 line. Conditional degradation of PP1-AID/HA was performed

as described previously[57]. A schematic representation of the endogenous *pp1* locus (PBANKA_1028300), the constructs and the recombined *pp1* locus can be found in **Fig S2A.** A diagnostic PCR was performed for *pp1* gene knockdown parasites as outlined in **Fig. S2A**. Primer 1 and Primer 3 were used to determine successful integration of the targeting construct at the 3' gene locus **(Fig S2B)**. Primer 1 and Primer 2 were used as controls **(Fig S2B)**.

The conditional knockdown construct PP1-PTD was derived from P_{ama1} (pSS368)) where *pp1* was placed under the control of the *ama1* promoter, as described previously[58]. A schematic representation of the endogenous *pp1* locus, the constructs and the recombined *pp1* locus can be found in **Fig S2D**. A diagnostic PCR was performed for *pp1* gene knockdown parasites as outlined in **Fig. S2D**. Primer 1 (5'-intPTD36) and Primer 2 (5'-intPTD) were used to determine successful integration of the targeting construct at the 5' gene locus. Primer 3 (3'-intPTD) and Primer 4 (3'-intPTama1) were used to determine successful integration for the 3' end of the gene locus **(Fig. S2E)**. All the primer sequences can be found in **Table S3**. *P. berghei* ANKA line 2.34 (for GFP-tagging) or ANKA line 507cl1 expressing GFP (for the knockdown construct) parasites were transfected by electroporation[41].

Purification of schizonts and gametocytes

Blood cells obtained from infected mice (day 4 post infection) were cultured for 11h and 24 h at 37˚C (with rotation at 100 rpm) and schizonts were purified the following day on a 60% v/v NycoDenz (in PBS) gradient, (NycoDenz stock solution: 27.6% w/v NycoDenz in 5 mM Tris-HCl, pH 7.20, 3 mM KCl, 0.3 mM EDTA).

The purification of gametocytes was achieved using a protocol described previously[59] with some modifications. Briefly, parasites were injected into

phenylhydrazine treated mice and enriched by sulfadiazine treatment after 2 days of infection. The blood was collected on day 4 after infection and gametocyte-infected cells were purified on a 48% v/v NycoDenz (in PBS) gradient (NycoDenz stock solution: 27.6% w/v NycoDenz in 5 mM Tris-HCl, pH 7.20, 3 mM KCl, 0.3 mM EDTA). The gametocytes were harvested from the interface and activated.

Live cell imaging

To examine PP1-GFP expression during erythrocyte stages, parasites growing in schizont culture medium were used for imaging at different stages (ring, trophozoite, schizont and merozoite) of development. Purified gametocytes were examined for GFP expression and localization at different time points (0, 1-15 min) after activation in ookinete medium[37]. Zygote and ookinete stages were analyzed throughout 24 h of culture. Images were captured using a 63x oil immersion objective on a Zeiss Axio Imager M2 microscope fitted with an AxioCam ICc1 digital camera (Carl Zeiss, Inc).

Generation of dual tagged parasite lines

The PP1-GFP parasites were mixed with NDC80-cherry and MyoA-cherry parasites in equal numbers and injected into mice. Mosquitoes were fed on mice 4 to 5 days after infection when gametocyte parasitaemia was high. These mosquitoes were checked for oocyst development and sporozoite formation at day 14 and day 21 after feeding. Infected mosquitoes were then allowed to feed on naïve mice and after 4 - 5 days, and the mice were examined for blood stage parasitaemia by microscopy with Giemsa-stained blood smears. In this way, some parasites expressed both PP1-GFP and NDC80-cherry; and PP1-GFP and MyoA-cherry in the resultant gametocytes, and these were purified and fluorescence microscopy images were collected as described above.

Parasite phenotype analyses

Blood containing approximately 50,000 parasites of the PP1PTD line was injected intraperitoneally (i.p.) into mice to initiate infections. Asexual stages and gametocyte production were monitored by microscopy on Giemsa-stained thin smears. Four to five days post infection, exflagellation and ookinete conversion were examined as described previously [60] with a Zeiss AxioImager M2 microscope (Carl Zeiss, Inc) fitted with an AxioCam ICc1 digital camera. To analyse mosquito transmission, 30–50 *Anopheles stephensi* SD 500 mosquitoes were allowed to feed for 20 min on anaesthetized, infected mice with an asexual parasitaemia of 15% and a comparable number of gametocytes as determined on Giemsa-stained blood films. To assess mid-gut infection, approximately 15 guts were dissected from mosquitoes on day 14 post feeding, and oocysts were counted on an AxioCam ICc1 digital camera fitted to a Zeiss AxioImager M2 microscope using a 63x oil immersion objective. On day 21 post-feeding, another 20 mosquitoes were dissected, and their guts crushed in a loosely fitting homogenizer to release sporozoites, which were then quantified using a haemocytometer or used for imaging. Mosquito bite back experiments were performed 21 days post-feeding using naive mice, and blood smears were examined after 3-4 days.

Electron microscopy

Gametocytes activated for 6 min and 30 min were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer and processed for electron microscopy as previously described[61]. Briefly, samples were post fixed in osmium tetroxide, treated *en bloc* with uranyl acetate, dehydrated and embedded in Spurr's epoxy resin. Thin sections

were stained with uranyl acetate and lead citrate prior to examination in a JEOL JEM-1400 electron microscope (JEOL Ltd, UK)

Quantitative Real Time PCR (qRT-PCR) analyses

RNA was isolated from gametocytes using an RNA purification kit (Stratagene). cDNA was synthesised using an RNA-to-cDNA kit (Applied Biosystems). Gene expression was quantified from 80 ng of total RNA using a SYBR green fast master mix kit (Applied Biosystems). All the primers were designed using the primer3 software (https://primer3.ut.ee/). Analysis was conducted using an Applied Biosystems 7500 fast machine with the following cycling conditions: 95˚C for 20 s followed by 40 cycles of 95˚C for 3 s; 60˚C for 30 s. Three technical replicates and three biological replicates were performed for each assayed gene. The *hsp70* (PBANKA_081890) and *arginyl-t RNA synthetase* (PBANKA_143420) genes were used as endogenous control reference genes. The primers used for qPCR can be found in **Table S1.**

Transcriptome study using RNA-seq

For RNA extraction, parasite samples were passed through a plasmodipur column to remove host DNA contamination prior to RNA isolation. Total RNA was extracted from activated gametocytes and schizonts of WT-GFP and PP1PTD parasites (two biological replicates each) using an RNeasy purification kit (Qiagen). RNA was vacuum concentrated (SpeedVac) and transported using RNA-stable tubes (Biomatrica). Strand-specific 354mRNA sequencing was performed on total RNA and using TruSeq stranded mRNA sample prep 355kit LT (Illumina), as previously described[62]. Libraries were sequenced using an Illumina Hiseq 4000 sequencing platform with paired-end 150 bp read chemistry. The quality of the raw reads was

assessed using FATSQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Low-quality reads and Illumina adaptor sequences from the read ends were removed using Trimmomatic R[63]. Processed reads were mapped to the *P. beghei ANKA* reference genome (release 40 in PlasmoDB - http://www.plasmoddb.org) using Hisat2[64] (V 2.1.0) with parameter "—rna-strandness FR". Counts per feature were estimated using FeatureCounts[65]. Raw read counts data were converted to counts per million (cpm) and genes were excluded if they failed to achieve a cpm value of 1 in at least one of the three replicates performed. Library sizes were scale-normalized by the TMM method using EdgeR software[66] and further subjected to linear model analysis using the voom function in the limma package[67]. Differential expression analysis was performed using DeSeq2[68]. Genes with a fold-change greater than two and a false discovery rate corrected p-value (Benjamini-Hochberg procedure) < 0.05 were considered to be differentially expressed. Functional groups shown in Figure 6C were inferred from annotations available in PlasmoDB: Release 49 (https://plasmodb.org/plasmo/app).

Immunoprecipitation and Mass Spectrometry

Schizonts, following 11 hours and 24 hours, respectively in *in vitro* culture, and male gametocytes 11 min post activation were used to prepare cell lysates. Purified parasite pellets were crosslinked using formaldehyde (10 min incubation with 1% formaldehyde, followed by 5 min incubation in 0.125M glycine solution and 3 washes with phosphate buffered saline (PBS) (pH, 7.5). Immunoprecipitation was performed using crosslinked protein and a GFP-Trap®_A Kit (Chromotek) following the manufacturer's instructions. Proteins bound to the GFP-Trap®_A beads were digested using trypsin and the peptides were analysed

by LC-MS/MS. Briefly, to prepare samples for LC-MS/MS, wash buffer was removed, and ammonium bicarbonate (ABC) was added to beads at room temperature. We added 10 mM TCEP (Tris-(2-carboxyethyl) phosphine hydrochloride) and 40 mM 2-chloroacetamide (CAA) and incubation was performed for 5 min at 70° C. Samples were digested using 1 µg Trypsin per 100 µg protein at room temperature overnight followed by 1% TFA addition to bring the pH into the range of 3-4 before mass spectrometry.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 8 (GraphPad Software). An unpaired t-test and two-way anova test were used to examine significant differences between wild-type and mutant strains for qRT-PCR and phenotypic analysis accordingly.

Data Availability. RNA Sequence reads have been deposited in the NCBI Sequence gene expression omnibus with the accession number GSE164175.
"The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium with the dataset identifier PXD023571 and 10.6019/PXD023571.

Author contributions.

RT and MZ conceived and designed all experiments. RT, MZ, RP, DB, DSG, GK performed the GFP tagging and conditional knockdown with promoter trap experiments. RR and MBr generated and characterised the PP1-AID/HA line. MZ, RP, GK, DB and RT performed protein pull-down experiments. ARB performed mass

spectrometry. AS, RN and AP performed RNA sequencing (RNA-seq). DJPF and SV performed electron microscopy. RT, AAH, MZ, DSG, AS, RN, AP and DJPF analyzed the data. MZ, DSG and RT wrote the original draft. RT, AAH, AP, DJPF, MB edited and reviewed the manuscript and all other contributed to it.

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Figure legends

Fig. 1. Location of PP1 during asexual blood stage schizogony and its association with kinetochore (Ndc80) and glideosome (MyosinA)

(A) Live cell imaging of PP1-GFP (Green) showing its location during different stages of intraerythrocytic development and in free merozoites. DIC: Differential

interference contrast; Hoechst: stained DNA (blue); Merge: green and blue images merged. A schematic guide showing the locations of PP1GFP foci during segmentation of merozoites is depicted in right hand panel. **(B)** Live cell imaging showing location of PP1–GFP (green) in relation to the kinetochore marker NDC80-mCherry (red) and DNA (Hoechst, blue). Merge: green, red and blue images merged. A schematic guide showing the locations of PP1GFP foci with NDC80-mCherry during segmentation of merozoites is depicted in right hand panel. **(C)** Live imaging showing the location of PP1–GFP (green) in relation to inner membrane complex marker MyoA-mCherry (red) and DNA (Hoechst, blue) during different stages of intraerythrocytic development and in extracellular merozoites. A schematic guide showing the locations of PP1GFP foci with NDC80-mCherry and MyoA-mCherry during segmentation of merozoites is depicted in the three right-hand panels. Merge: green, red and blue images merged. Sch-E (Early schizont), Sch-M (Middle schizont), Sch-L (Late schizont) Sch-S (Segmented schizont). In all panels, scale bar = 5 µm.

Fig. 2. The location of PP1 and its association with the kinetochore during chromosome segregation in male gametogony.

(A) Live-cell imaging of PP1-GFP during male gametogony showing an initial diffused localization before activation and focal points after activation in the later stages (shown as minutes post activation). Panels are DIC, Hoechst (blue, DNA), PP1-GFP (green) and Merge (green and blue channels). **(B)** Live-cell imaging of parasite line expressing both PP1-GFP and NDC80-mCherry showing location of PP1 (green) and NDC80 (red) in male gametocytes at different time points after activation**.** A schematic guide showing the locations of PP1GFP foci with DNA and

NDC80-mCherry during male gametogony is depicted in the right panel. Merge/DNA is green, red and blue (Hoechst, DNA) channels. In both panels, scale bar = 5 µm.

Fig. 3. PP1-GFP localization during zygote formation, ookinete development, and sporogony inside the mosquito gut

(A) Live cell imaging showing PP1-GFP location in male and female gametes, zygote and during ookinete development (stages I to V and mature ookinete). A cy3-conjugated antibody, 13.1, which recognises the P28 protein on the surface of zygotes and ookinetes, was used to mark these stages. Panels: DIC (differential interference contrast), PP1-GFP (green, GFP), 13.1 (red), Merged: Hoechst (blue, DNA), PP1-GFP (green, GFP) and P28 (red). Scale bar = 5 µm. Insets show, at higher magnification, the PP1-GFP signal on the zygote and developing apical end of early stage 'retorts', and in the nucleus of late retorts and ookinete stage. **(B)** Live cell imaging of PP1-GFP (green) in relation to NDC80-mCherry (red) and Hoechst staining (blue, DNA) in zygote and ookinete stages. **(C)** Live cell imaging of PP1-GFP in developing oocysts in mosquito guts at 7-, 14- and 21-days post-infection and in a sporozoite. Panels: DIC, Hoechst (blue, DNA), PP1-GFP (green), Merged (blue and green channels). **(D)** Live cell imaging of PP1-GFP in relation to NDC80 in developing oocysts and in a sporozoite. Panels: DIC (differential interference contrast), PP1-GFP (green), NDC80-mCherry (red), Merge (Hoechst, blue, DNA; PP1-GFP, green; NDC80-mCherry, red). Scale bar = 5 µm

Fig. 4. PP1 has an important role during male gamete formation and zygote - ookinete development (A) qRT-PCR analysis of *pp1* transcription in PP1PTD and WT-GFP parasites, showing the downregulation of *pp1*. Each bar is the mean of

three biological replicates ± SD. **(B)** Male gametogony (exflagellation) of PP1PTD line (black bar) and WT-GFP line (white bar) measured as the number of exflagellation centres per field. Mean ± SD; n=3 independent experiments. **(C)** Ookinete conversion as a percentage for PP1PTD and WT-GFP parasites. Ookinetes were identified using P28 antibody as a surface marker and defined as those cells that differentiated successfully into elongated 'banana shaped' ookinetes. Round cells show zygotes that did not start to transform and 'retorts' could not differentiate successfully in ookinetes. Mean ± SD; n=3 independent experiments. **(D)** Representative images of round cells, retorts and fully differentiated ookinetes. **(E)** Total number of GFP-positive oocysts per infected mosquito in PP1PTD and WT-GFP parasites at 7-, 14- and 21-days post infection (dpi). Mean ± SD; n=3 independent experiments. **(F)** Representative images of mosquito midguts on day 14 showing them full of oocysts in WTGFP and no oocyst in PP1PTD **(G)** Total number of sporozoites in oocysts of PP1PTD and WT-GFP parasites at 14 and 21 dpi. Mean ± SD; n=3 independent experiments. **(H)** Total number of sporozoites in salivary glands of PP1PTD and WT-GFP parasites. Bar diagram shows mean ± SD; n=3 independent experiments. Unpaired t-test was performed for statistical analysis. *p<0.05, **p<0.01, ***p<0.001.

Figure 5: Ultrastructure analysis of PP1PTD gametocytes shows defects in nuclear pole and axoneme assembly during male gametogony

Electron micrographs of PP1PTD (a-f) and WTGFP (g-l) male gametocytes at 6 min (a-c, g-i) and 30 min (d-f, j-l) post activation. Bars represent 1 µm (a, d, g, j) and 100 nm in all other micrographs. **(a)** Low power of mutant showing the central nucleus (N) with a nuclear pole and associated basal body (enclosed). **(b)** Enlargement of

the enclosed area showing the nuclear pole (NP) with adjacent basal body (B) and associated axoneme (A). **(c)** Cross section through an axoneme showing the 9+2 arrangement of microtubules. **(d)** Low power of a mutant at 30 min showing the central nucleus (N) with a nuclear pole and an increased number of axoneme profiles (A) within the cytoplasm. **(e)** Detail of the periphery of a nucleus showing the nuclear pole (NP), basal body (B) and associated axoneme (A). **(f)** Cross section of an axoneme showing the 9+2 arrangement. **(g)** Low power of a WT microgametocyte illustrating 2 nuclear poles (NP) associated with the nucleus (N). **(h)** Enlargement of the enclosed area showing the nuclear pole (NP) with adjacent basal body (B) and associated axoneme (A). **(i)** Cross section of an axoneme showing the 9+2 arrangement. **(j)** Low power of a late microgametocyte showing the nucleus with areas of condensed chromatin. Note the cross sectioned free microgametes (Mg). A – axoneme. **(k)** Periphery of a microgametocyte undergoing exflagellation with the nucleus (N) associated with flagellum (F) protruding from the surface. **(l)** Cross section of a free microgamete showing the 9+2 flagellum (F) and electron dense nucleus (N). These observations are based on random sections through 30 microgametocytes.

Figure 6: Transcriptome of PP1PTD mutant reveals important roles of PP1 in parasite cell cycle, motor protein function, and cell polarity during gametocyte biology

Volcano plots showing significantly down-regulated (blue Log_2 fold ≤ -1, q value < 0.05) and up-regulated genes (brown Log_2 fold ≥ 1, q value < 0.05) in PP1PTD compared to wild-type lines in non (0 min)-activated gametocytes **(A)** and 30 min activated gametocytes **(B).** Non-differentially regulated genes are represented as

black dots. **(C)** Expression heat maps showing affected genes from specific functional classes of proteins such as kinases, phosphatases, motor proteins and proteins associated with parasite motility, entry into the host cell, ookinete surface and cell cycle. Genes are ordered based on their differential expression pattern in non-activated gametocytes. **(D)** Validation by qRT-PCR of a few genes randomly selected based on the RNA-seq data. All the experiments were performed three times in duplicate with two biological replicates. *p ≤05.

Fig. 7. Interacting partners of PP1 during asexual schizont and sexual gametocyte stages

(A) List of proteins interacting with PP1 during schizont and gametocyte stages. **(B)** Venn diagram showing common interacting partners in schizonts and gametocytes with some additional proteins specific to gametocytes.

Supplementary figures

Fig. S1. Generation and genotypic analysis of PP1GFP parasites

(A) Schematic representation for 3'-tagging of *pp1* gene with green fluorescent protein (GFP) sequence via single homologous recombination. **(B)** Integration PCR showing correct integration of tagging construct. **(C)** Western blot showing expected size of PP1-GFP protein.

Fig. S2. Generation and genotype analysis of conditional knockdown PP1 parasites

(A) Schematic representation of auxin inducible degron (AID) strategy to generate PP1AID parasites. **(B)** Integration PCR of the PP1AID construct in the *pp1* locus.

Primer 1 and Primer 2 were used for control PCR while primer 1 and primer 3 were used to determine successful integration of AID-HA sequence and selectable marker at 3'-end of *pp1* locus **(C)** PP1AID protein expression level as measured by western blotting upon addition of auxin to mature purified gametocytes; α-tubulin serves as a loading control. Auxin treatment of PP1AID showed no defect in exflagellation (error bars show standard deviation from the mean; technical replicates from three independent infections. **(D)** Schematic representation of the promoter swap strategy (PP1PTD, placing *pp1* under the control of the *ama1* promoter) by double homologous recombination. Arrows 1 and 2 indicate the primer positions used to confirm 5' integration and arrows 3 and 4 indicate the primers used for 3' integration. **(E)** Integration PCR of the promotor swap construct into the *pp1* locus. Primer 1 (5'-IntPTD36) with primer 2 (5'-IntPTD) were used to determine successful integration of the selectable marker. Primer 3 (3'-intPTama1) and primer 4 (3'-IntPTD36) were used to determine the successful integration of ama1 promoter. Primer 1 (5'-IntPTD36) and primer 4 (3'-IntPTD36) were used to show complete knock-in of the construct and the absence of a band at 2.1 kb (endogenous) resulting in complete knock-in of the construct. **(F)** Parasitaemia during blood stage schizogony showing a significant slow growth of PP1PTD compared to WTGFP parasites. Experiment was done with three mice each with 1000 parasites per mice injected intraperitonially. ***P<0.001 **(G)** Bite back experiments show no transmission of PP1PTD parasites (black bar) from mosquito to mouse, while successful transmission was shown by WT-GFP parasites. Mean ± SD; n= 3 independent experiments.

Fig. S3. RNA seq analysis:

(A) Clustered dendrogram of two biological replicates of WTGFP and PP1PTD mutant parasite lines during gametocyte stage using hierarchical clustering algorithm. Analysis was performed on normalized count data**. (B)** RNA-seq read statistics.

Supplementary Tables

Table S1. Differentially expressed genes in PP1PTD parasites

Table S2. List of proteins pulled down by PP1GFP

Table S3. Primers used in this study

References

1. Marston AL, Amon A. Meiosis: cell-cycle controls shuffle and deal. *Nat Rev Mol Cell Biol* **5**, 983-997 (2004).

2. Sullivan M, Morgan DO. Finishing mitosis, one step at a time. *Nat Rev Mol Cell Biol* **8**, 894-903 (2007).

3. Novak B, Kapuy O, Domingo-Sananes MR, Tyson JJ. Regulated protein kinases and phosphatases in cell cycle decisions. *Curr Opin Cell Biol* **22**, 801-808 (2010).

4. Afshar K, Werner ME, Tse YC, Glotzer M, Gonczy P. Regulation of cortical contractility and spindle positioning by the protein phosphatase 6 PPH-6 in one-cell stage C. elegans embryos. *Development* **137**, 237-247 (2010).

5. Boutros R, Dozier C, Ducommun B. The when and wheres of CDC25 phosphatases. *Curr Opin Cell Biol* **18**, 185-191 (2006).

6. Chen F*, et al.* Multiple protein phosphatases are required for mitosis in Drosophila. *Curr Biol* **17**, 293-303 (2007).

7. Stegmeier F, Amon A. Closing mitosis: the functions of the Cdc14 phosphatase and its regulation. *Annu Rev Genet* **38**, 203-232 (2004).

8. Bollen M, Gerlich DW, Lesage B. Mitotic phosphatases: from entry guards to exit guides. *Trends Cell Biol* **19**, 531-541 (2009).

9. Heroes E, Lesage B, Gornemann J, Beullens M, Van Meervelt L, Bollen M. The PP1 binding code: a molecular-lego strategy that governs specificity. *FEBS J* **280**, 584-595 (2013).

10. Holder J, Poser E, Barr FA. Getting out of mitosis: spatial and temporal control of mitotic exit and cytokinesis by PP1 and PP2A. *FEBS Lett* **593**, 2908-2924 (2019).

11. Nilsson J. Protein phosphatases in the regulation of mitosis. *J Cell Biol* **218**, 395-409 (2019).

12. Hattersley N*, et al.* A Nucleoporin Docks Protein Phosphatase 1 to Direct Meiotic Chromosome Segregation and Nuclear Assembly. *Dev Cell* **38**, 463-477 (2016).

13. Cheeseman IM. The kinetochore. *Cold Spring Harb Perspect Biol* **6**, a015826 (2014).

14. Nagpal H, Fukagawa T. Kinetochore assembly and function through the cell cycle. *Chromosoma* **125**, 645-659 (2016).

15. Varma D, Salmon ED. The KMN protein network--chief conductors of the kinetochore orchestra. *J Cell Sci* **125**, 5927-5936 (2012).

16. Lampert F, Westermann S. A blueprint for kinetochores - new insights into the molecular mechanics of cell division. *Nat Rev Mol Cell Biol* **12**, 407-412 (2011).

17. Wu JQ*, et al.* PP1-mediated dephosphorylation of phosphoproteins at mitotic exit is controlled by inhibitor-1 and PP1 phosphorylation. *Nat Cell Biol* **11**, 644-651 (2009).

18. Dohadwala M*, et al.* Phosphorylation and inactivation of protein phosphatase 1 by cyclin-dependent kinases. *Proc Natl Acad Sci U S A* **91**, 6408-6412 (1994).

19. Bouchoux C, Uhlmann F. A quantitative model for ordered Cdk substrate dephosphorylation during mitotic exit. *Cell* **147**, 803-814 (2011).

20. Grallert A*, et al.* A PP1-PP2A phosphatase relay controls mitotic progression. *Nature* **517**, 94-98 (2015).

21. Mochida S, Hunt T. Protein phosphatases and their regulation in the control of mitosis. *EMBO Rep* **13**, 197-203 (2012).

22. Howick VM*, et al.* The Malaria Cell Atlas: Single parasite transcriptomes across the complete Plasmodium life cycle. *Science* **365**, (2019).

23. Guttery David S*, et al.* Genome-wide Functional Analysis of Plasmodium Protein Phosphatases Reveals Key Regulators of Parasite Development and Differentiation. *Cell Host & Microbe* **16**, 128-140 (2014).

24. Bhattacharyya MK, Hong Z, Kongkasuriyachai D, Kumar N. Plasmodium falciparum protein phosphatase type 1 functionally complements a glc7 mutant in Saccharomyces cerevisiae. *Int J Parasitol* **32**, 739-747 (2002).

25. Zhang M*, et al.* Uncovering the essential genes of the human malaria parasite Plasmodium falciparum by saturation mutagenesis. *Science* **360**, (2018).

26. Bushell E*, et al.* Functional Profiling of a Plasmodium Genome Reveals an Abundance of Essential Genes. *Cell* **170**, 260-272 e268 (2017).

27. Yokoyama D, Saito-Ito A, Asao N, Tanabe K, Yamamoto M, Matsumura T. Modulation of the growth of Plasmodium falciparum in vitro by protein serine/threonine phosphatase inhibitors. *Biochem Biophys Res Commun* **247**, 18-23 (1998).

28. Khalife J, Freville A, Gnangnon B, Pierrot C. The Multifaceted Role of Protein Phosphatase 1 in Plasmodium. *Trends Parasitol*, (2020).

29. Paul AS*, et al.* Co-option of Plasmodium falciparum PP1 for egress from host erythrocytes. *Nat Commun* **11**, 3532 (2020).

30. Fardilha M, Esteves SL, Korrodi-Gregorio L, da Cruz e Silva OA, da Cruz e Silva FF. The physiological relevance of protein phosphatase 1 and its interacting proteins to health and disease. *Curr Med Chem* **17**, 3996-4017 (2010).

31. Hollin T*, et al.* Essential role of GEXP15, a specific Protein Phosphatase type 1 partner, in Plasmodium berghei in asexual erythrocytic proliferation and transmission. *PLoS Pathog* **15**, e1007973 (2019).

32. Gerald N, Mahajan B, Kumar S. Mitosis in the human malaria parasite Plasmodium falciparum. *Eukaryot Cell* **10**, 474-482 (2011).

33. Guttery DS, Roques M, Holder AA, Tewari R. Commit and Transmit: Molecular Players in Plasmodium Sexual Development and Zygote Differentiation. *Trends Parasitol* **31**, 676-685 (2015).

34. Matthews H, Duffy CW, Merrick CJ. Checks and balances? DNA replication and the cell cycle in Plasmodium. *Parasit Vectors* **11**, 216 (2018).

35. Zeeshan M*, et al.* Real-time dynamics of Plasmodium NDC80 reveals unusual modes of chromosome segregation during parasite proliferation. *J Cell Sci* **134**, (2020).

36. Arnot DE, Ronander E, Bengtsson DC. The progression of the intra-erythrocytic cell cycle of Plasmodium falciparum and the role of the centriolar plaques in asynchronous mitotic division during schizogony. *Int J Parasitol* **41**, 71-80 (2011).

37. Zeeshan M*, et al.* Plasmodium kinesin-8X associates with mitotic spindles and is essential for oocyst development during parasite proliferation and transmission. *PLoS Pathog* **15**, e1008048 (2019).

38. Vaughan JA. Population dynamics of Plasmodium sporogony. *Trends Parasitol* **23**, 63-70 (2007).

39. Pandey R*, et al.* Plasmodium Condensin Core Subunits SMC2/SMC4 Mediate Atypical Mitosis and Are Essential for Parasite Proliferation and Transmission. *Cell Rep* **30**, 1883-1897 e1886 (2020).

40. Wall RJ*, et al.* Plasmodium APC3 mediates chromosome condensation and cytokinesis during atypical mitosis in male gametogenesis. *Sci Rep* **8**, 5610 (2018).

41. Janse CJ*, et al.* High efficiency transfection of Plasmodium berghei facilitates novel selection procedures. *Molecular and biochemical parasitology* **145**, 60-70 (2006).

42. Hollin T, De Witte C, Lenne A, Pierrot C, Khalife J. Analysis of the interactome of the Ser/Thr Protein Phosphatase type 1 in Plasmodium falciparum. *BMC Genomics* **17**, 246 (2016).

43. Straschil U*, et al.* The Armadillo repeat protein PF16 is essential for flagellar structure and function in Plasmodium male gametes. *PloS one* **5**, e12901 (2010).

44. Gelens L, Qian J, Bollen M, Saurin AT. The Importance of Kinase-Phosphatase Integration: Lessons from Mitosis. *Trends Cell Biol* **28**, 6-21 (2018).

45. Nasa I, Rusin SF, Kettenbach AN, Moorhead GB. Aurora B opposes PP1 function in mitosis by phosphorylating the conserved PP1-binding RVxF motif in PP1 regulatory proteins. *Sci Signal* **11**, (2018).

46. Tewari R*, et al.* The Systematic Functional Analysis of Plasmodium Protein Kinases Identifies Essential Regulators of Mosquito Transmission. *Cell Host & Microbe* **8**, 377-387 (2010).

47. Ceulemans H, Bollen M. Functional diversity of protein phosphatase-1, a cellular economizer and reset button. *Physiol Rev* **84**, 1-39 (2004).

48. Lesage B, Qian J, Bollen M. Spindle checkpoint silencing: PP1 tips the balance. *Curr Biol* **21**, R898-903 (2011).

49. Peggie MW, MacKelvie SH, Bloecher A, Knatko EV, Tatchell K, Stark MJ. Essential functions of Sds22p in chromosome stability and nuclear localization of PP1. *J Cell Sci* **115**, 195-206 (2002).

50. Rodrigues NT, Lekomtsev S, Jananji S, Kriston-Vizi J, Hickson GR, Baum B. Kinetochore-localized PP1-Sds22 couples chromosome segregation to polar relaxation. *Nature* **524**, 489-492 (2015).

51. Verbinnen I, Ferreira M, Bollen M. Biogenesis and activity regulation of protein phosphatase 1. *Biochem Soc Trans* **45**, 89-99 (2017).

52. Sinden RE. Mitosis and meiosis in malarial parasites. *Acta Leidensia* **60**, 19-27 (1991).

53. Solyakov L*, et al.* Global kinomic and phospho-proteomic analyses of the human malaria parasite Plasmodium falciparum. *Nat Commun* **2**, 565 (2011).

54. Balestra AC*, et al.* A divergent cyclin/cyclin-dependent kinase complex controls the atypical replication of a malaria parasite during gametogony and transmission. *Elife* **9**, (2020).

55. Invergo BM, Brochet M, Yu L, Choudhary J, Beltrao P, Billker O. Sub-minute Phosphoregulation of Cell Cycle Systems during Plasmodium Gamete Formation. *Cell Rep* **21**, 2017-2029 (2017).

56. Moreira S, Osswald M, Ventura G, Goncalves M, Sunkel CE, Morais-de-Sa E. PP1-Mediated Dephosphorylation of Lgl Controls Apical-basal Polarity. *Cell Rep* **26**, 293-301 e297 (2019).

57. Philip N, Waters AP. Conditional Degradation of Plasmodium Calcineurin Reveals Functions in Parasite Colonization of both Host and Vector. *Cell Host Microbe* **18**, 122-131 (2015).

58. Sebastian S*, et al.* A Plasmodium calcium-dependent protein kinase controls zygote development and transmission by translationally activating repressed mRNAs. *Cell Host Microbe* **12**, 9-19 (2012).

59. Beetsma AL, van de Wiel TJ, Sauerwein RW, Eling WM. Plasmodium berghei ANKA: purification of large numbers of infectious gametocytes. *Exp Parasitol* **88**, 69-72 (1998).

60. Guttery DS*, et al.* A putative homologue of CDC20/CDH1 in the malaria parasite is essential for male gamete development. *PLoS Pathog* **8**, e1002554 (2012).

61. Ferguson DJ*, et al.* Maternal inheritance and stage-specific variation of the apicoplast in Toxoplasma gondii during development in the intermediate and definitive host. *Eukaryot Cell* **4**, 814-826 (2005).

62. Roques M*, et al.* Plasmodium centrin PbCEN-4 localizes to the putative MTOC and is dispensable for malaria parasite proliferation. *Biol Open* **8**, (2019).

63. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120 (2014).

64. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. *Nature methods* **12**, 357-360 (2015).

65. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**, 923-930 (2014).

66. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139-140 (2010).

67. Ritchie ME*, et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic acids research* **43**, e47 (2015).

68. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**, 550 (2014).

1067

1068

Fig 1

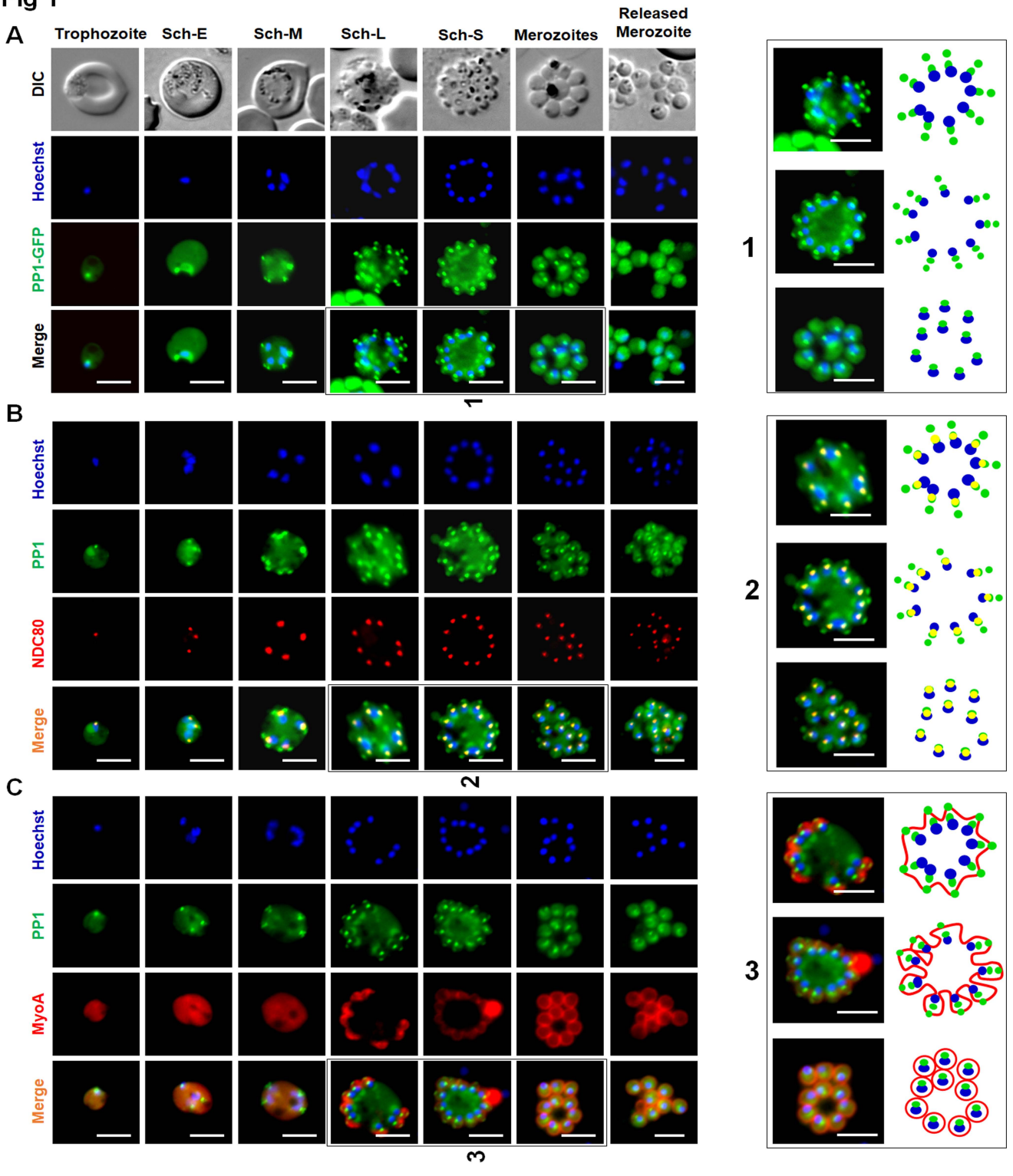

A
Trophozoite
Sch-E
Sch-M
Sch-L
Sch-S
Merozoites
Released Merozoite
DIC
Hoechst
PP1-GFP
Merge
1
B
Hoechst
PP1
NDC80
Merge
2
C
Hoechst
PP1
MyoA
Merge
3

Fig 2

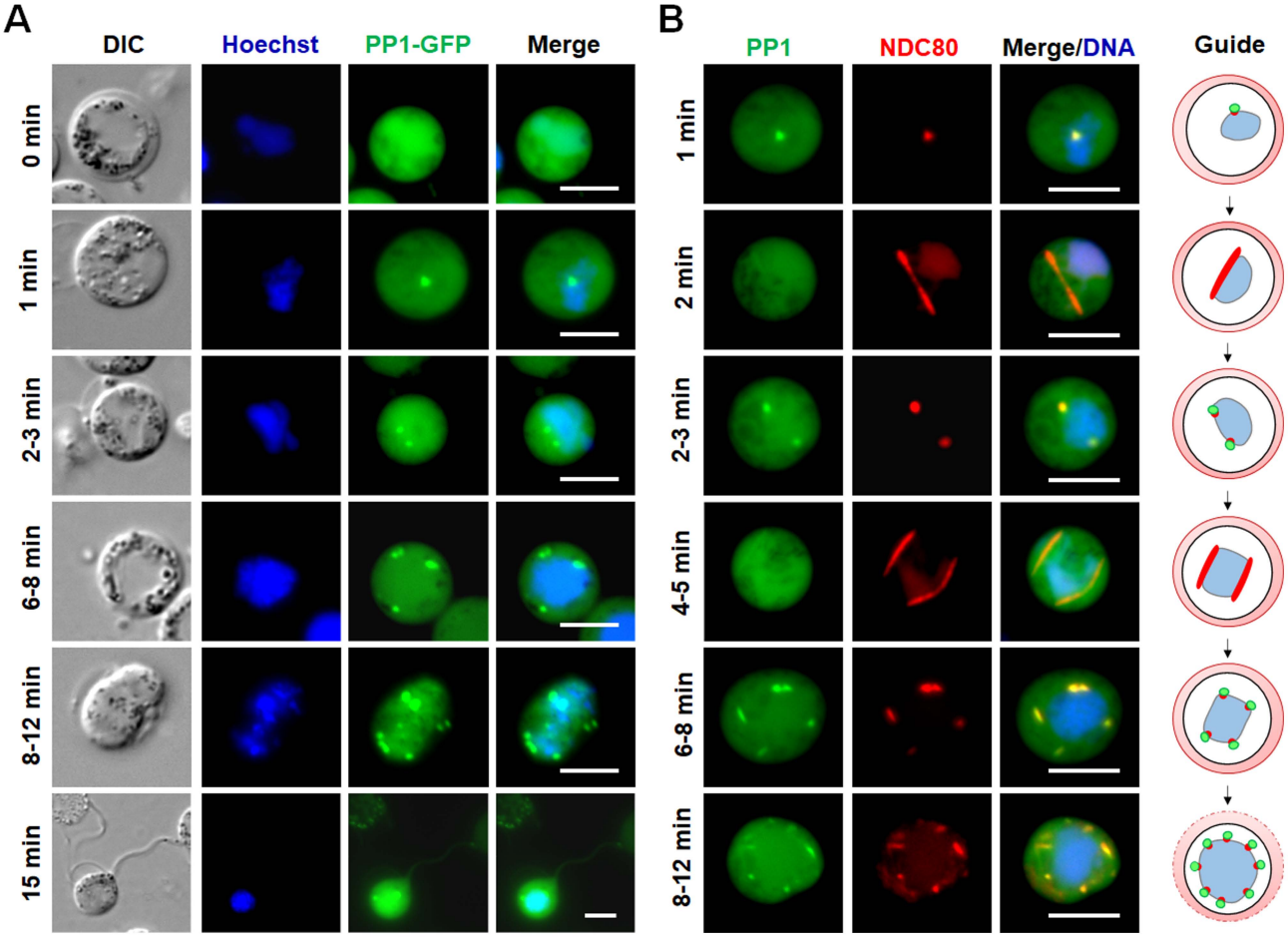

A
DIC
Hoechst
PP1-GFP
Merge
0 min
1 min
2-3 min
6-8 min
8-12 min
15 min
B
PP1
NDC80
Merge/DNA
Guide
1 min
2 min
2-3 min
4-5 min
6-8 min
8-12 min

Fig 3

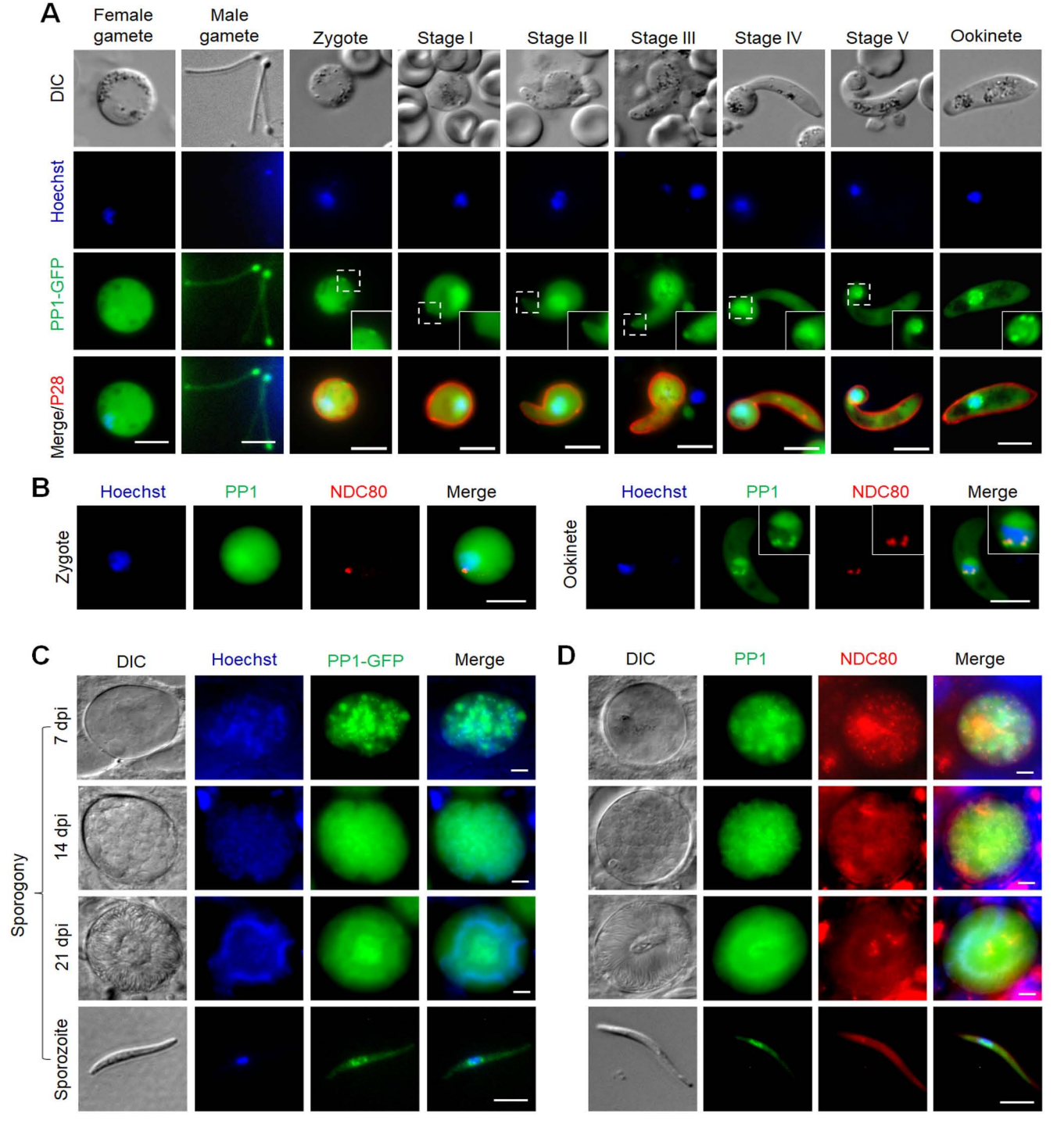

A
Female gamete
Male gamete
Zygote
Stage I
Stage II
Stage III
Stage IV
Stage V
Ookinete
DIC
Hoechst
PP1-GFP
Merge/P28
B
Hoechst
PP1
NDC80
Merge
Zygote
Ookinete
C
DIC
Hoechst
PP1-GFP
Merge
7 dpi
14 dpi
21 dpi
Sporozoite
Sporogony
D
DIC
PP1
NDC80
Merge

Fig 4

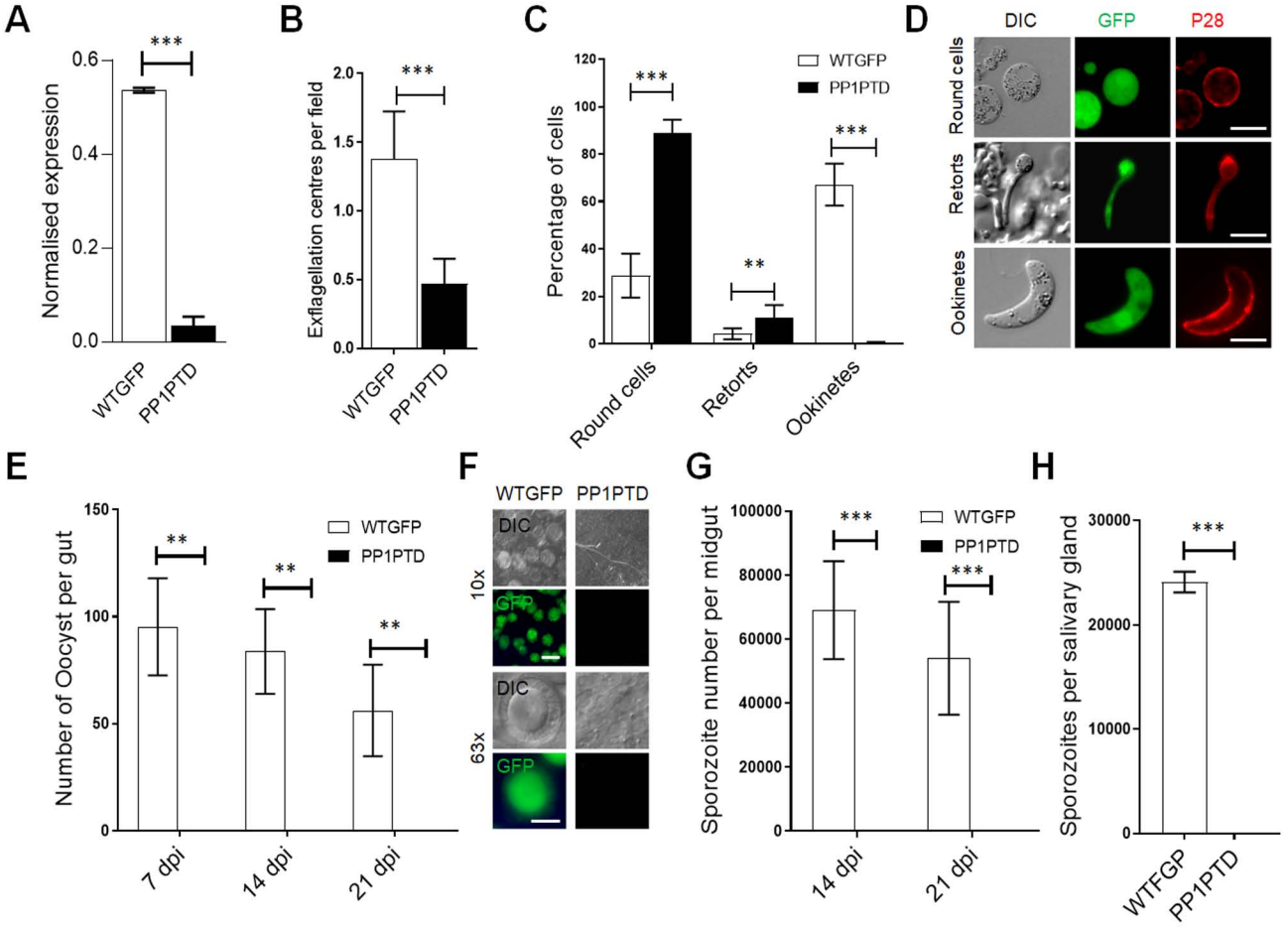

A
Normalised expression
0.6
0.4
0.2
0.0
***
WTGFP
PP1PTD
B
Exflagellation centres per field
2.0
1.5
1.0
0.5
0.0
***
WTGFP
PP1PTD
C
Percentage of cells
120
100
80
60
40
20
0
WTGFP
PP1PTD
***
**
***
Round cells
Retorts
Ookinetes
D
DIC
GFP
P28
Round cells
Retorts
Ookinetes
E
Number of Oocyst per gut
150
100
50
0
WTGFP
PP1PTD
**
**
**
7 dpi
14 dpi
21 dpi
F
WTGFP
PP1PTD
10x
63x
DIC
GFP
DIC
GFP
G
Sporozoite number per midgut
100000
80000
60000
40000
20000
0
WTGFP
PP1PTD
***
***
14 dpi
21 dpi
H
Sporozoites per salivary gland
30000
20000
10000
0
***
WTFGP
PP1PTD

Fig 5

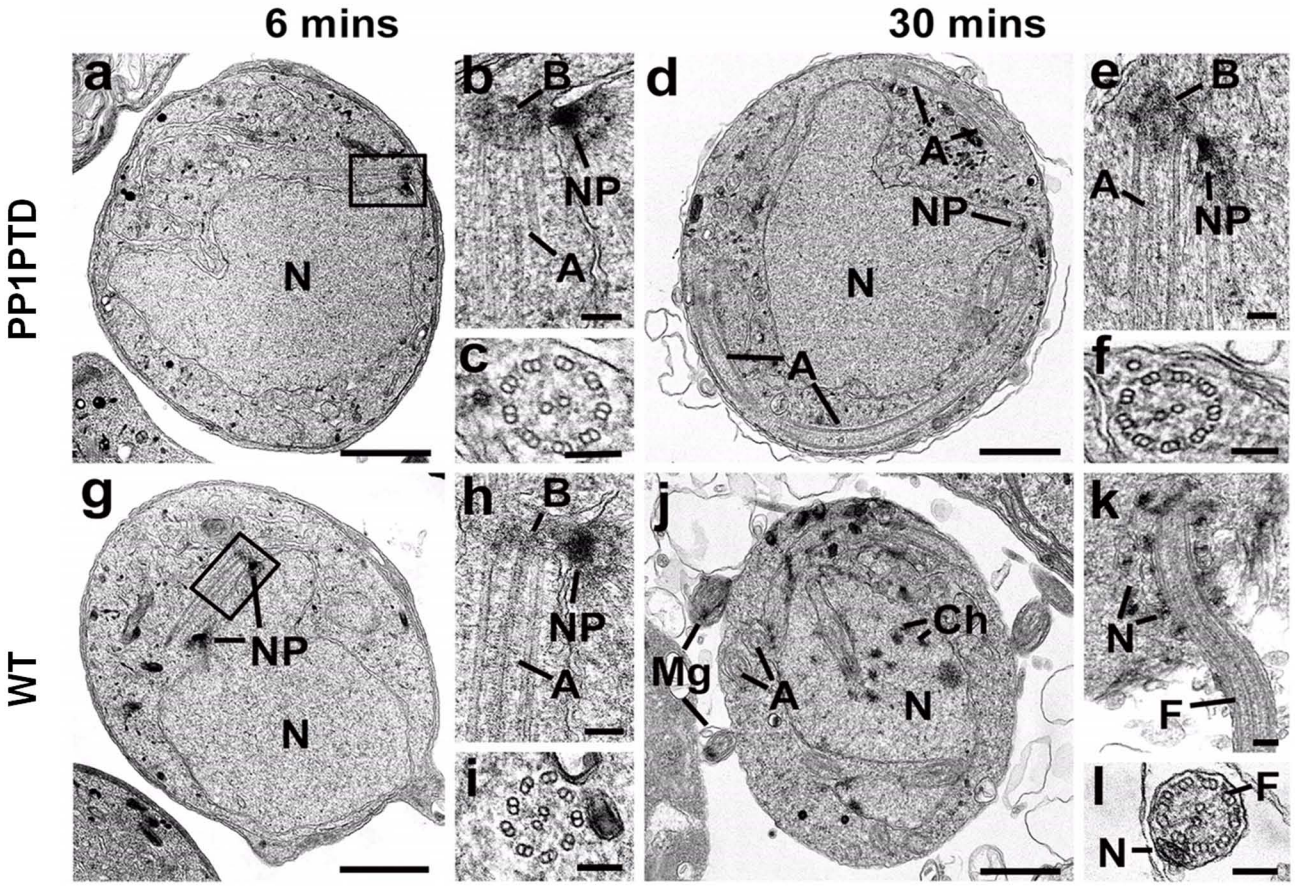


Fig 6

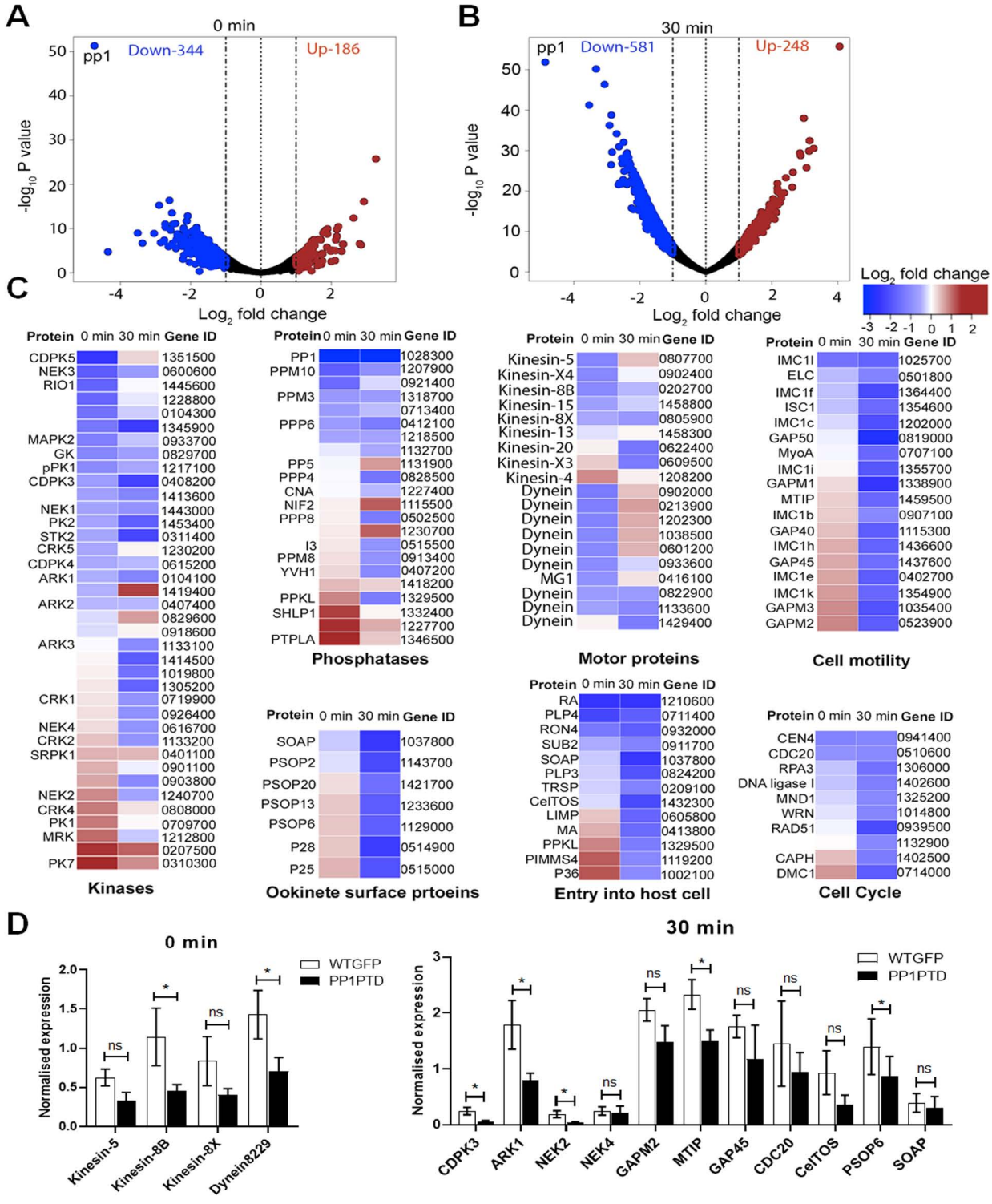

A
0 min
pp1
Down-344
Up-186
-log10 P value
Log2 fold change
B
30 min
pp1
Down-581
Up-248
-log10 P value
Log2 fold change
Log2 fold change
C
Protein 0 min 30 min Gene ID
CDPK5 1351500
NEK3 0600600
RIO1 1445600
1228800
0104300
1345900
MAPK2 0933700
GK 0829700
pPK1 1217100
CDPK3 0408200
1413600
NEK1 1443000
PK2 1453400
STK2 0311400
CRK5 1230200
CDPK4 0615200
ARK1 0104100
1419400
ARK2 0407400
0829600
0918600
ARK3 1133100
1414500
1019800
1305200
CRK1 0719900
0926400
NEK4 0616700
CRK2 1133200
SRPK1 0401100
0901100
0903800
NEK2 1240700
CRK4 0808000
PK1 0709700
MRK 1212800
0207500
PK7 0310300
Kinases
Protein 0 min 30 min Gene ID
PP1 1028300
PPM10 1207900
0921400
PPM3 1318700
0713400
PPP6 0412100
1218500
1132700
PP5 1131900
PPP4 0828500
CNA 1227400
NIF2 1115500
PPP8 0502500
1230700
I3 0515500
PPM8 0913400
YVH1 0407200
1418200
PPKL 1329500
SHLP1 1332400
1227700
PTPLA 1346500
Phosphatases
Protein 0 min 30 min Gene ID
SOAP 1037800
PSOP2 1143700
PSOP20 1421700
PSOP13 1233600
PSOP6 1129000
P28 0514900
P25 0515000
Ookinete surface prtoeins
Protein 0 min 30 min Gene ID
Kinesin-5 0807700
Kinesin-X4 0902400
Kinesin-8B 0202700
Kinesin-15 1458800
Kinesin-8X 0805900
Kinesin-13 1458300
Kinesin-20 0622400
Kinesin-X3 0609500
Kinesin-4 1208200
Dynein 0902000
Dynein 0213900
Dynein 1202300
Dynein 1038500
Dynein 0601200
Dynein 0933600
MG1 0416100
Dynein 0822900
Dynein 1133600
Dynein 1429400
Motor proteins
Protein 0 min 30 min Gene ID
IMC1l 1025700
ELC 0501800
IMC1f 1364400
ISC1 1354600
IMC1c 1202000
GAP50 0819000
MyoA 0707100
IMC1i 1355700
GAPM1 1338900
MTIP 1459500
IMC1b 0907100
GAP40 1115300
IMC1h 1436600
GAP45 1437600
IMC1e 0402700
IMC1k 1354900
GAPM3 1035400
GAPM2 0523900
Cell motility
Protein 0 min 30 min Gene ID
RA 1210600
PLP4 0711400
RON4 0932000
SUB2 0911700
SOAP 1037800
PLP3 0824200
TRSP 0209100
CelTOS 1432300
LIMP 0605800
MA 0413800
PPKL 1329500
PIMMS4 1119200
P36 1002100
Entry into host cell
Protein 0 min 30 min Gene ID
CEN4 0941400
CDC20 0510600
RPA3 1306000
DNA ligase I 1402600
MND1 1325200
WRN 1014800
RAD51 0939500
1132900
CAPH 1402500
DMC1 0714000
Cell Cycle
D
0 min
WTGFP
PP1PTD
Normalised expression
Kinesin-5
Kinesin-8B
Kinesin-8X
Dynein8229
30 min
WTGFP
PP1PTD
Normalised expression
CDPK3
ARK1
NEK2
NEK4
GAPM2
MTIP
GAP45
CDC20
CelTOS
PSOP6
SOAP

Fig 7

A

P. berghei Gene ID	Product Description	Gene names	MW (kDa)	Schizonts- 24 h				Schizonts-10 h				Gametocytes-10 min			
				PP1-GFP			GFP	PP1-GFP			GFP	PP1-GFP			GFP
PBANKA_1028300	serine/threonine protein phosphatase PP1	PP1	34963	31	26	23	0	37	26	25	0	23	33	26	0
PBANKA_1218500	protein phosphatase inhibitor 2, putative	PPI2	16664	11	7	9	0	9	8	7	0	10	9	9	0
PBANKA_0516600	leucine-rich repeat protein	LRR1	36735	33	26	26	0	22	29	29	0	11	22	25	0
PBANKA_0601600	conserved protein, unknown function	NEMF-like	201925	36	17	35	0	21	37	21	0	12	22	24	0
PBANKA_0515500	protein phosphatase inhibitor 3, putative	PPI3	13065	3	3	3	0	0	4	2	0	2	2	3	0
PBANKA_0515400	conserved Plasmodium protein	CD2BP2	77377	17	10	18	0	7	22	12	0	3	2	9	0
PBANKA_0310800	RTR1 domain-containing protein, putative	RTR1-like	97344	15	8	11	0	19	32	19	0	0	0	7	0
PBANKA_1458800	kinesin, putative	kinesin-15	164999	0	0	0	0	0	0	0	0	2	7	2	0
PBANKA_0202700	kinesin-8, putative	kinesin-8B	168815	0	0	0	0	0	0	0	0	2	4	2	0
PBANKA_1458300	kinesin-13, putative	kinesin-13	117181	0	0	0	0	0	0	0	0	0	3	3	0
PBANKA_0917400	armadillo repeat protein PF16	PF16	57748	0	0	0	0	0	0	0	0	0	2	2	0

B

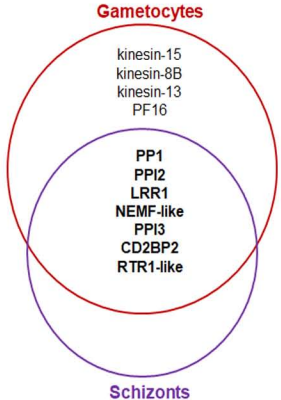


Fig S1

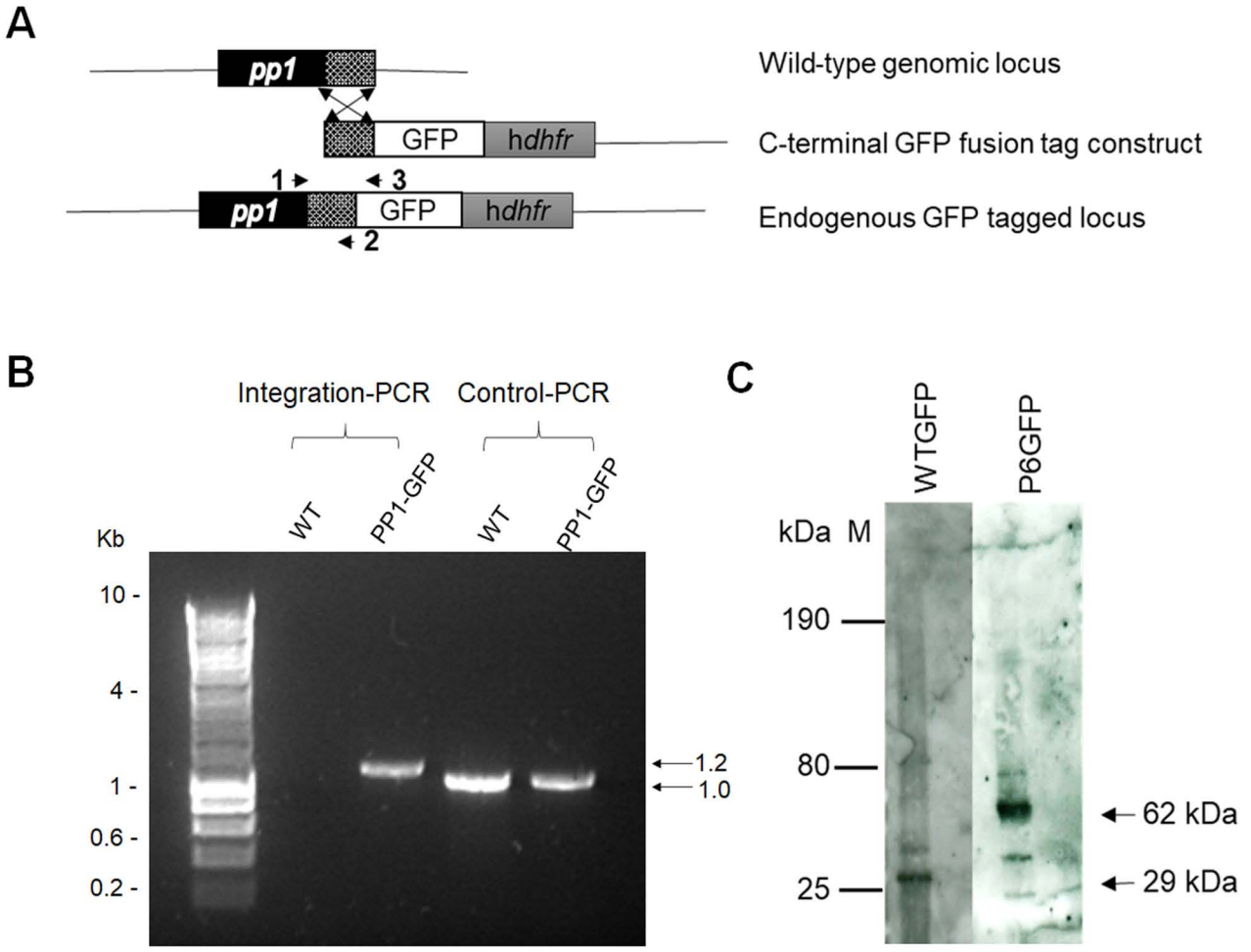

A
pp1
Wild-type genomic locus
GFP
hdhfr
C-terminal GFP fusion tag construct
1
3
pp1
GFP
hdhfr
2
Endogenous GFP tagged locus
B
Integration-PCR
Control-PCR
WT
PP1-GFP
WT
PP1-GFP
Kb
10 -
4 -
1 -
0.6 -
0.2 -
1.2
1.0
C
WTGFP
P6GFP
kDa M
190
80
25
62 kDa
29 kDa

Fig S2

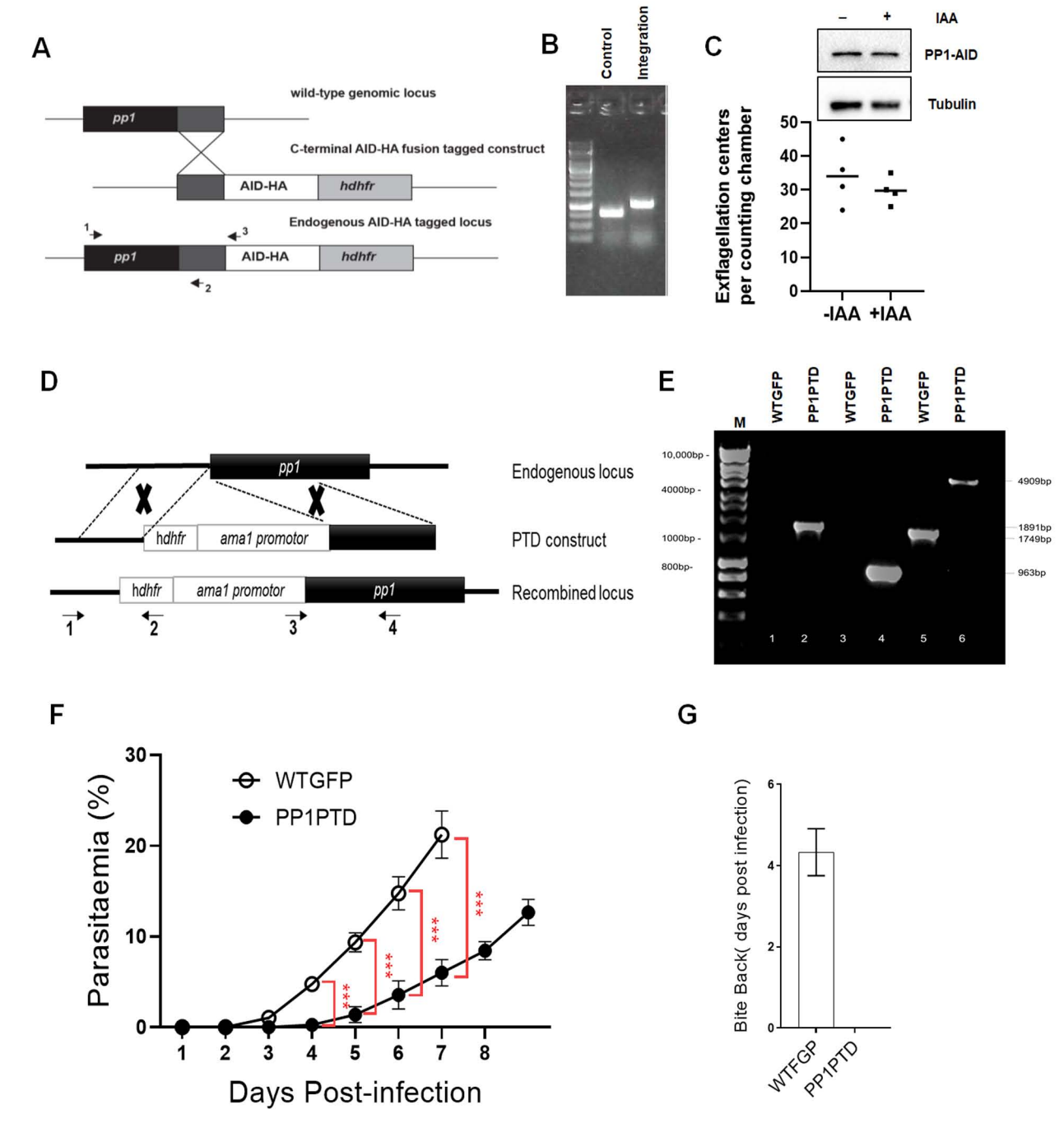
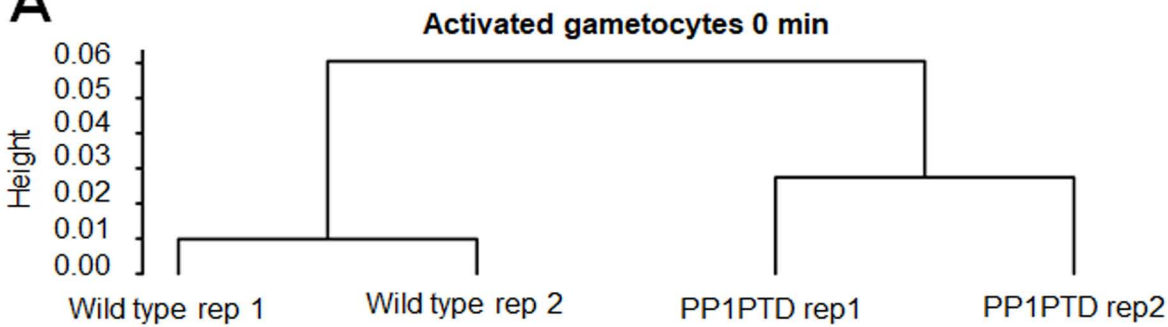
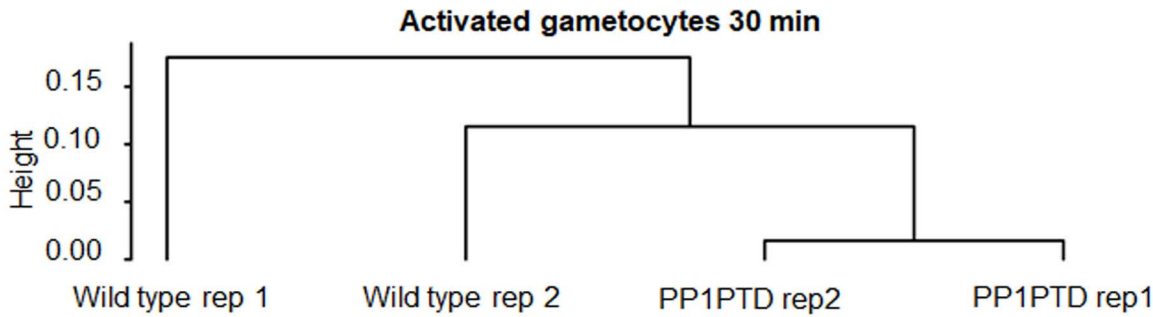

A
wild-type genomic locus
pp1
C-terminal AID-HA fusion tagged construct
AID-HA
hdhfr
Endogenous AID-HA tagged locus
1
2
3
B
Control
Integration
C
IAA
–
+
PP1-AID
Tubulin
Exflagellation centers per counting chamber
50
40
30
20
10
0
-IAA
+IAA
D
pp1
Endogenous locus
hdhfr
ama1 promotor
PTD construct
Recombined locus
1
2
3
4
E
M
WTGFP
PP1PTD
WTGFP
PP1PTD
WTGFP
PP1PTD
10,000bp -
4000bp -
1000bp -
800bp-
4909bp
1891bp
1749bp
963bp
1
2
3
4
5
6
F
Parasitaemia (%)
WTGFP
PP1PTD
30
20
10
0
***
1
2
3
4
5
6
7
8
Days Post-infection
G
Bite Back(days post infection)
6
4
2
0
WTFGP
PP1PTD

Fig S3

A





B

	Wild type (in millions)		PP1PTD (in millions)	
Gametocytes	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0 min	45.99	25.5	58.6	19.8
30 min	22.57	31.57	16.33	20.65