SI Appendix

Materials and Methods

Reference *P. berghei* ANKA lines. Six reference *P. berghei* ANKA parasite lines were used. Details can be found in the RMgm database (www.pberghei.eu). Line 683cl1 (DOZI::GFP; RMgm-133) (1) expressing a C-terminally GFP-tagged version of *dozi* (PBANKA_121770); line 909cl1 (CITH::GFP; RMgm-358) (2) expressing a C-terminally GFP-tagged version of *cith* (PBANKA_130130); line HPE, a non-gametocyte producer clone (3); line 820cl1m1cl1 (Fluo-frmg; RMgm-164) (2) expressing RFP under the control of a female gametocyte specific promoter and GFP under the control of a male gametocyte specific promoter; line 676m1cl1 (PbGFP-LUCcon; RMgm-29) (4) expressing the fusion protein GFP-Luciferase under the control of the constitutive *eef1a* promoter; and line cl15cy1, which is the reference parent line of *P. berghei* ANKA (4). Lines Fluo-frmg and PbGFP-LUCcon contain the transgenes integrated into the silent *230p* gene *locus* (PBANKA_0306000) and do not contain a drug-selectable marker.

Generation of *dhhc10* gene deletion mutants. To disrupt *dhhc10* (PBANKA_0512000) we constructed a standard replacement constructs (5) using plasmid pL0001 (www.mr4.com) which contains the pyrimethamine resistant *Toxoplasma gondii* (*tg*) *dhfr/ts* as a selectable marker cassette. See **SI Appendix, Table S1** and **SI Appendix, Figure S2A** for the name and details of the final construct. Target sequences for homologous recombination were PCR-amplified from *P. berghei* WT genomic DNA using primers specific for the 5' or 3' flanking regions of the *dhhc10* gene (see **SI Appendix, Table S4** for the sequence of the different primers). The PCR-amplified target sequences were cloned in plasmid pL0001 either upstream or downstream of the selectable marker to allow for integration of the construct into the genomic target sequence by homologous recombination. DNA construct used for transfection was obtained after digestion of the replacement construct with the appropriate restriction enzymes (**SI Appendix, Table S1**). Transfection, selection and cloning of mutant parasite lines were performed as described (5). Correct deletion of the *dhhc10* gene was confirmed by diagnostic PCR (for primers see **SI Appendix, Table S5**) and Southern analysis of FIGE-separated chromosomes (**SI Appendix, Figure S2B**). Chromosomes were hybridized with a probe recognizing the 3' UTR of *pbdhfr/ts*. Absence of *dhhc10* mRNA was determined by RT-PCR analysis (**SI Appendix, Figure S2C**) using RNA collected from infected blood containing asexual blood stages and gametocytes (see **SI Appendix, Table S5** for primers used for RT-PCR). Two cloned lines were used for further phenotype analyses: 2097cl1 (Δ*dhhc10*-a, in the Fluo-frmg background) and 2365cl2 (Δ*dhhc10*-b, in the PbGFP-LUCcon background).

Generation of transgenic line expressing GFP-tagged DHHC10. *In situ* C-terminal GFP tagging of *dhhc10* was performed by single cross-over homologous recombination into the corresponding *locus*. See **SI Appendix, Table S1** and **SI Appendix, Figure S3** for the name and details of the final construct. The construct contains the *tgdhfr/ts* selectable marker. Primers used to amplify the targeting region of *dhhc10*, corresponding to the 3' end of the open reading frame (ORF) excluding the stop codon are listed in **SI Appendix, Table S4**. The targeting region was cloned in frame with *gfp*. Linearised plasmid was transfected into cl15cy1 parasites using standard methods. Transfection, selection and cloning of mutant parasite line was performed as described (5), resulting in the following transgenic line: 2187cl1m1 (*dhhc10*::*gfp*). See **SI Appendix, Table S1** for details of the transfection experiment performed. Correct integration of the construct was confirmed by diagnostic PCR (for primers see **SI Appendix, Table S5**) and Southern analysis of FIGE-separated chromosomes using a probe for the 3' UTR of *pbdhfr/ts* (**SI Appendix, Figure S3B**). Transcription and processing (splicing) of the *gfp* fusion was confirmed by RT-PCR using RNA from mixed blood stage forms (**SI Appendix, Figure S3C**). Primers used for RT-PCR are listed in **SI Appendix, Table S5**.

Generation of GFP-tagged DHHC10 mutant expressing mCherry-tagged PbLAP3 (PBANKA_0204500). The coding sequence of *mCherry* plus 3' UTR of *pbdhfr/ts* were PCR-amplified from plasmid pDNR-mCherry (6) with primers mCherryswap-F and mCherryswap-R (see **SI Appendix, Table S4** for primer sequences) and introduced into *Apa*I-digested pLP-PbLAP3/EGFP (7) by In-Fusion® cloning system (Clontech® Laboratories, Inc.) to generate pLP-PbLAP3/mCherry. This construct contains the entire *lap3* coding sequence plus 0.6 kb of its upstream sequence as well as the human *dhfr/ts* selectable marker. Circular plasmid was transfected into *dhhc10*::*gfp* parasites using standard methods. Transfection was performed as described (5), and selection of mutant parasites was performed with the drug WR99210 (8), resulting in the following transgenic line: 2492 (*dhhc10*::*gfp*;*lap3*::*mCherry*). See **SI Appendix, Table S1** and **SI Appendix, Figure S6** for details of the construct and transfection experiment performed. Success of transfection was confirmed by Southern analysis of FIGE-separated chromosomes using a probe for the human *dhfr/ts* together with a chromosome 5 control probe for the gene *p25* (PBANKA_0515000) (**SI Appendix, Figure S6B**).

Generation of *dhhc10* gene deletion parasites expressing GFP-tagged PbLAP2 (PBANKA_1300700). *In situ* C-terminal GFP-tagging of *lap2* was performed by single cross-over homologous recombination into the endogenous *locus* using a previously published construct (7). The construct used contains the human *dhfr/ts* selectable marker. Linearised plasmid was transfected into Δ*dhhc10*-a parasites using standard methods. Transfection was

performed as described (5), and selection of mutant parasites was performed with the drug WR99210 (8), resulting in the following transgenic line: 2433 (Δ*dhhc10*;*lap2*::*gfp*). See **SI Appendix, Table S1** and **SI Appendix, Figure S8** for details of the construct and transfection experiment performed. Correct integration of the construct was confirmed by Southern analysis of FIGE-separated chromosomes using a probe for the human *dhfr/ts* (**SI Appendix, Figure S8B**).

Generation of GFP-tagged DHHC10 complementation line. Complementation of the Δ*dhhc10-a* line was performed by double cross-over homologous recombination of construct pLIS0486 into the deleted *dhhc10 locus*. See **SI Appendix, Table S1** and **SI Appendix, Figure S10** for the name and details of the final construct. The construct contains the *human dhfr/ts* selectable marker. Primers used to amplify the targeting region of *dhhc10* (*dhhc10* ORF plus an additional 1151 bp of the 5' flanking region) are listed in **SI Appendix, Table S4**. The targeting region was cloned in frame with *gfp*. Linearised plasmid was transfected into Δ*dhhc10-a* parasites using standard methods. Transfection and parasite cloning was performed as described (5), and selection of mutant parasites was performed with the drug WR99210 (8), resulting in the following transgenic line: 486.3 (Δ*dhhc10*;*dhhc10*::*gfp*). See **SI Appendix, Table S1** for details of the transfection experiment performed. Correct integration of the construct was confirmed by diagnostic PCR (**SI Appendix, Figure S10**; for primers see **SI Appendix, Table S5**).

Reverse Transcriptase-PCR (RT-PCR). Immunoprecipitation (IP) of DOZI::GFP and CITH::GFP parasite lysates, and subsequent RNA extraction and RT-PCR were performed as described (2). Transcription patterns of *dhhc* genes by RT-PCR were performed with RNA from different life cycle stages obtained using TRIzol® Reagent. RT was performed with random primers and oligo-d(T) using SuperScript® II Reverse Transcriptase. RNA sample origins were: asexual blood stages from line HPE (a non-gametocyte producer line) and mixed blood stages (asexuals & gametocytes) from line Fluo-frmg. Primers used in RT-PCRs are shown in **SI Appendix, Table S3**.

Transmission electron microscopy of ookinetes. Wildtype (Fluo-frmg) and Δ*dhhc10*-b *in vitro* ookinete production followed by transmission electron microscopy was performed as previously described (9) with an additional post-staining step: prior to specimen imaging, 100 nm-thick sections were post-stained for 10 minutes at RT with 7% uranyl acetate in ultrapure water and 5 minutes at RT with lead citrate prepared according to Reynolds' method (10).

Western analysis of CSP expression in Δ*dhhc10* oocysts. To determine circumsporozoite protein (CSP) expression, Fluo-frmg- and Δ*dhhc10*-a-infected midguts were dissected at day 13 p.i. and resuspended in 1X Laemmli buffer. Samples were adjusted to 200 mM DTT, boiled

and volumes equivalent to 2 midguts were loaded per SDS-PAGE gel lane for each parasite line. Nitrocellulose membranes were blocked for 1 h at RT with 5% skim milk/PBS-Tween 20 (0.05%), probed overnight at 4 °C with 3D11 mouse anti-CSP (11), 0.17 µg/mL in blocking solution) or parasite-specific 2E6 mouse monoclonal anti-PbHSP70 (12), 7.5 µg/mL in blocking solution) as primary antibodies, and 1h at RT with goat anti-mouse IgG-HRP [Santa Cruz Biotechnology, Inc.®, #sc-2005, 1:5000-1:10000 in PBS-Tween 20 (0.05%)] as secondary antibody. Westerns were developed with Immobilon™ Western Chemiluminescent HRP Substrate (Millipore, #P36599). Staining with the antibody recognizing *P. berghei* HSP70 was used as loading control.

***In vivo* multiplication rate of asexual blood stages.** The multiplication rate of asexual blood stages in mice was determined during the cloning procedure of gene-deletion/transgenic parasites (13). The percentage of infected erythrocytes in OF-1 mice injected with a single parasite is quantified at days 8–11 on Giemsa-stained blood films. The mean asexual multiplication rate per 24 h is then calculated assuming a total of 1.2×10^{10} erythrocytes per mouse and a blood volume of 2 mL. The percentage of infected erythrocytes in mice infected with reference lines of the *P. berghei* ANKA strain consistently ranges between 0.5 and 2% at day 8 after infection, resulting in a mean multiplication rate of 10 per 24 h (13, 14).

Gametocyte production and ookinete formation assays. Gametocyte production of the different parasite lines was determined as described (15). The gametocyte conversion rate is defined as the percentage of ring-forms that develop into gametocytes in standard synchronized *in vivo* infections in mice. Ookinete formation assays were performed following published methods using gametocyte-enriched blood collected from mice treated with phenylhydrazine/NaCl (16). Briefly, infected blood containing gametocytes was mixed in standard ookinete culture medium in 24-well plates and cultures were incubated for 18-24 h at 21-22°C. The ookinete conversion rate is defined as the percentage of female gametes that develop into mature ookinetes and is determined by counting female gametes and mature ookinetes in Giemsa-stained blood smears 16-18 h after *in vitro* induction of gamete formation.

Oocyst production, sporozoite production and transmission experiments. Oocyst and sporozoite production of mutant parasites was analysed by performing standard mosquito infections. Naïve female Balb/c ByJ mice were infected intraperitoneally (IP) with 10^6 infected red blood cells (iRBCs) of each line. On days 4-5 post-infection (p.i.), these mice were anesthetised and *Anopheles stephensi* female mosquitoes allowed to feed for 30 min. Twenty-four hours after feeding, mosquitoes were anesthetised by cold shock and unfed mosquitoes were removed. Oocyst and sporozoite numbers were counted at days 12-13 and

20-22 after mosquito infection, respectively. Oocysts were counted after mercurochrome staining and measured using ImageJ 1.47n software (imagej.nih.gov/ij). Sporozoites were counted in pools of 5 to 50 mosquitoes. To test the infectivity of sporozoites, 10 infected mosquitoes were allowed to feed for 30 min on anesthetised naïve female Balb/c ByJ mice on days 20-21 p.i. Successful feeding was confirmed by the presence of blood in the abdomen of mosquitoes. Alternatively, 10000 salivary gland sporozoites were injected intravenously into mouse. Blood stage parasitaemia in these mice were followed up to 32 days post-bite or sporozoite injection.

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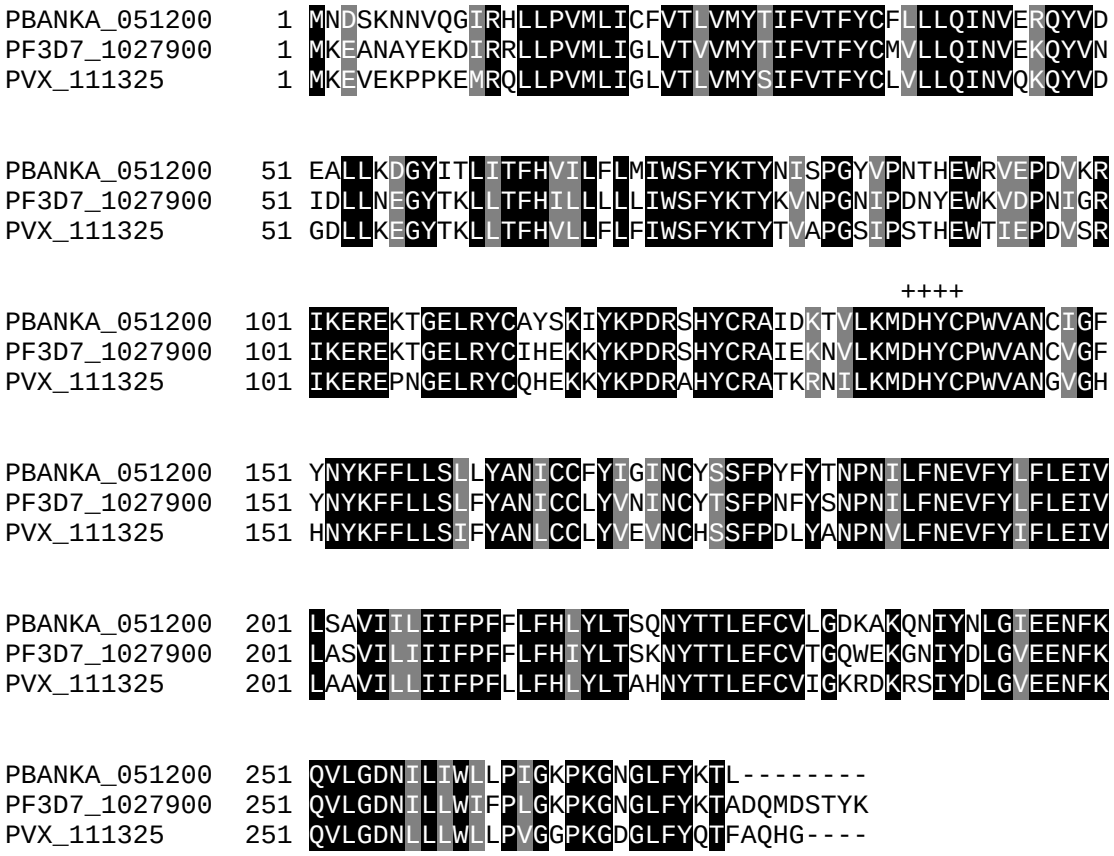


Fig. S1. DHHC10 proteins from rodent and human malaria species are conserved. Boxshaded (www.ch.embnet.org/software/BOX_form.html) ClustalW protein alignment (http://www.ebi.ac.uk/Tools/msa/clustalw2/) of DHHC10 from *Plasmodium berghei* (the rodent malaria model), and the two major human malaria species *P. falciparum* and *P. vivax*. Protein ID's are from www.plasmodb.org. The DHYC motif is indicated with +.

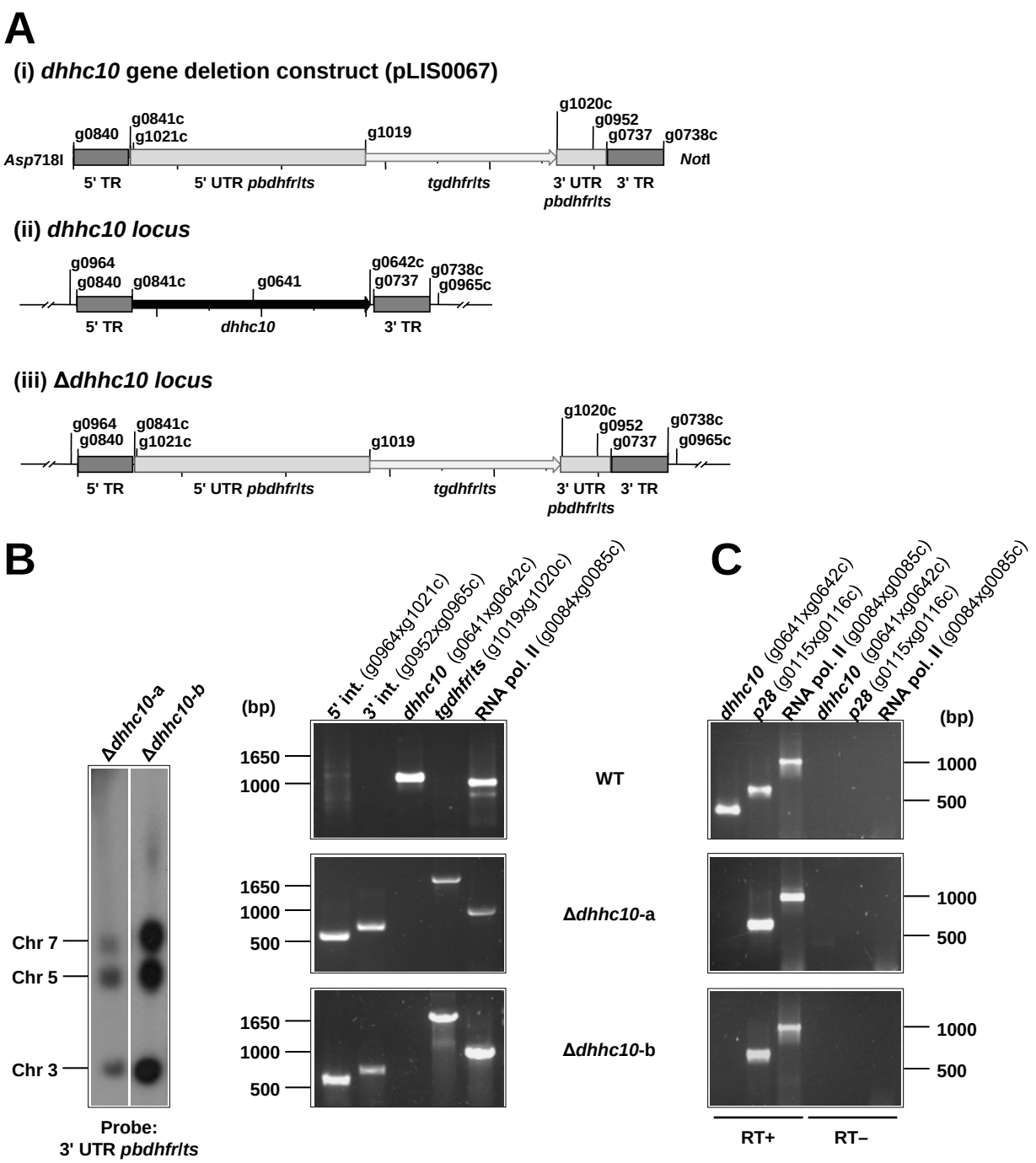


Fig. S2. Generation and genotyping of Δ*dhhc10* parasite lines. (A) *dhhc10* gene deletion construct pLIS0067 (i) was obtained by cloning *dhhc10* 5' and 3' targeting regions (TR) upstream and downstream of the *Toxoplasma gondii dhfr/ts* selectable marker cassette. The construct was integrated into the *dhhc10 locus* (ii) of Fluo-frmg and PbGFP-LUCcon WT lines by double homologous recombination, resulting in the complete deletion of *dhhc10* ORF in Δ*dhhc10* parasites (iii). **(B)** Correct deletion of *dhhc10* was shown by Southern analysis of separated chromosomes (left) and PCR analyses (right). Hybridisation of separated chromosomes with a probe against the 3' UTR of *pbdhfr/ts* recognised integrated pLIS0067 into chromosome 5, the endogenous *pbdhfr/ts locus* in chromosome 7 and reporter gene constructs (GFP/RFP or GFP-Luciferase) in chromosome 3. PCR analyses confirm 5' and 3' integration (int.) of pLIS0067, absence of *dhhc10* ORF and presence of *tgdhfr/ts* gene. **(C)** Absence of *dhhc10* mRNA was confirmed in Δ*dhhc10* mixed blood stages by RT-PCR. *p28* and RNA polymerase II serve as control genes.

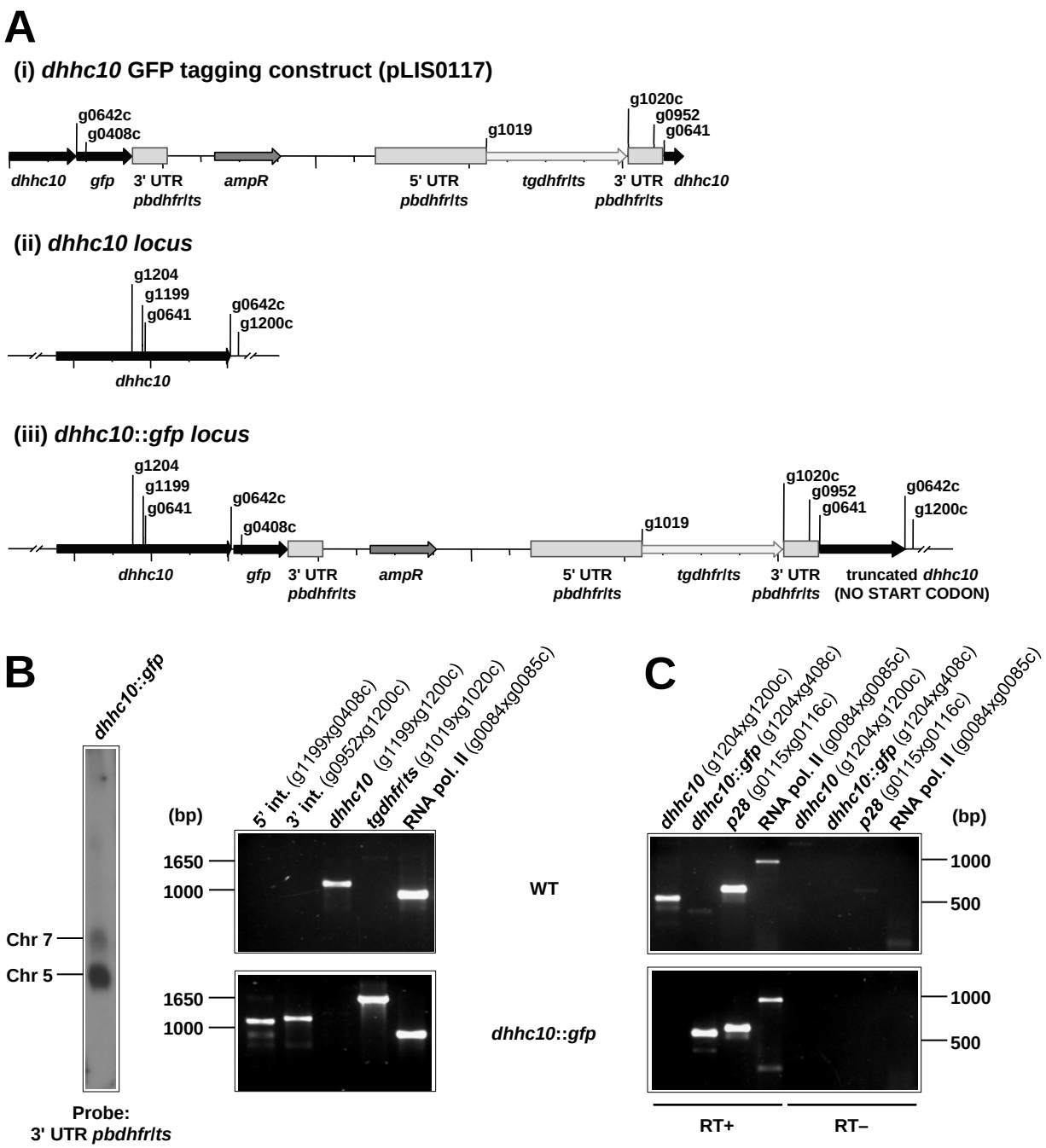


Fig. S3. Generation and genotyping of *dhhc10*::*gfp* parasite line. (A) *dhhc10* GFP tagging construct pLIS0117 (i) was obtained by cloning the last 1116 bp of *dhhc10* ORF excluding the stop codon upstream and in frame with the *gfp* gene. This construct includes the *Toxoplasma gondii dhfr/ts* selectable marker cassette under the control of *P. berghei dhfr/ts* 5' and 3' UTRs. The construct was integrated into the *dhhc10 locus* (ii) of cl15cy1 by single homologous recombination, resulting in the fusion of *dhhc10* to *gfp* in *dhhc10*::*gfp* parasites (iii). **(B)** Correct tagging of *dhhc10* was shown by Southern analysis of separated chromosomes (left) and diagnostic PCR analyses (right). Hybridisation of separated chromosomes with a probe against the 3' UTR of *pbdhfr/ts* recognised integrated pLIS0117 into chromosome 5 and the endogenous *pbdhfr/ts locus* in chromosome 7. PCR analyses confirm 5' and 3' integration (int.) of pLIS0117, absence of WT *dhhc10* ORF and presence of *tgdhfr/ts* gene. **(C)** Absence of WT *dhhc10* and presence of *dhhc10*::*gfp* mRNA was confirmed in cloned *dhhc10*::*gfp* mixed blood stages by RT-PCR. *p28* and RNA polymerase II serve as control genes.

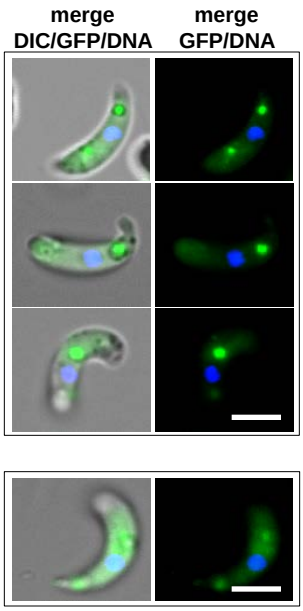


Fig. S4. DHHC10::GFP localisation in ookinetes. Live imaging of *in vitro*-cultured *dhhc10*::*gfp* ookinetes shows DHHC10::GFP localisation (in green) in discrete foci in approximately 75% of the ookinetes (top panel). Remaining parasites showed diffuse cytoplasmic GFP signal (lower panel) (*n*=60). DNA was stained with Hoechst-33342 (in blue). Scale bars = 5 µm.

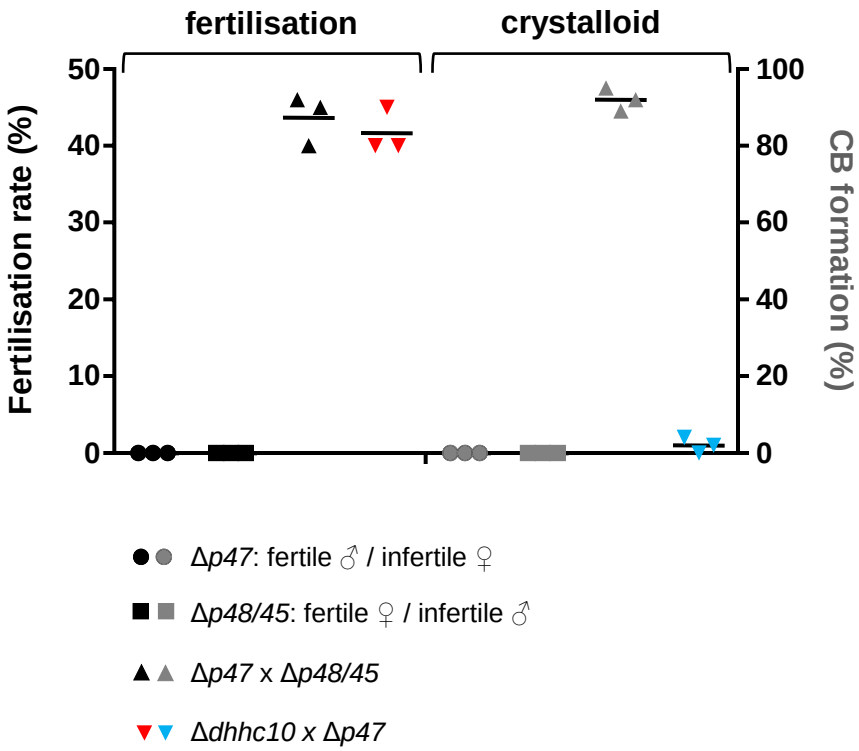


Fig. S5. Genetic crosses show that the male *dhhc10* allele does not rescue the crystalloid formation defect caused by Δ*dhhc10* females. Fertile males (Δ*p47;* females from this line are sterile) and fertile females (Δ*p48/45;* males from this line are sterile) mate to form mature ookinetes with distinctive crystalloid bodies; the fertilisation rate is 44% in these cultures. The Δ*dhhc10* x Δ*p47* cross shows a similar fertilisation rate, yet fails to establish crystalloids, demonstrating that the DHHC10 protein necessary for crystalloid biogenesis is exclusively of female origin and cannot be provided by the male copy of *dhhc10*. Fertlisation rate, defined as the precentage of female gametes developing in ookinetes, was determined in triplicate in two independent cultures.

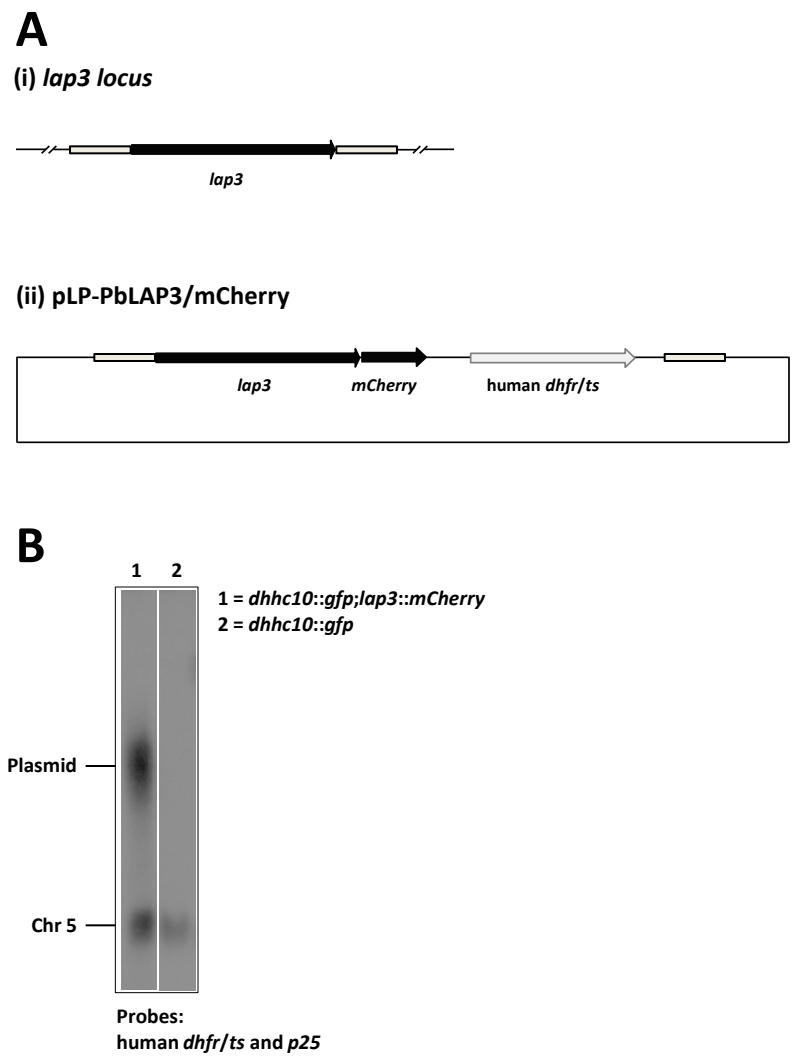


Fig. S6. Generation and genotyping of *dhhc10*::*gfp*;*lap3*::*mCherry* parasite line. (A) *lap3* mCherry tagging construct pLP-PbLAP3/mCherry (ii) was obtained by cloning *mCherry* into the published plasmid pLP-PbLAP3/EGFP (Saeed et al., *Mol Biochem Parasitol* 2010 170(1): p. 49-53), substituting eGFP with mCherry. This construct includes the human *dhfr*/*ts* selectable marker cassette. **(B)** Successful transfection of circular pLP-PbLAP3/mCherry construct into *dhhc10*::*gfp* parasites was confirmed by Field-Inversion Gel Electrophoresis (FIGE) of separated whole chromosomes in the uncloned *dhhc10*::*gfp*;*lap3*::*mCherry* parasite line. FIGE was developed using a probe for the human *dhfr*/*ts* together with a control probe for *p25* (chromosome 5).

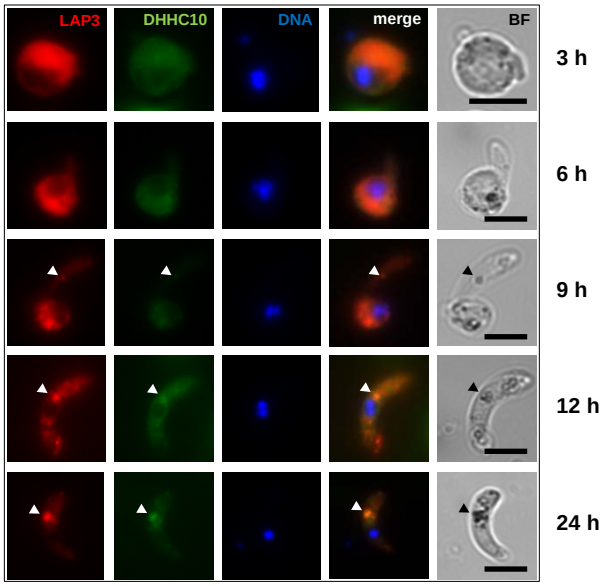
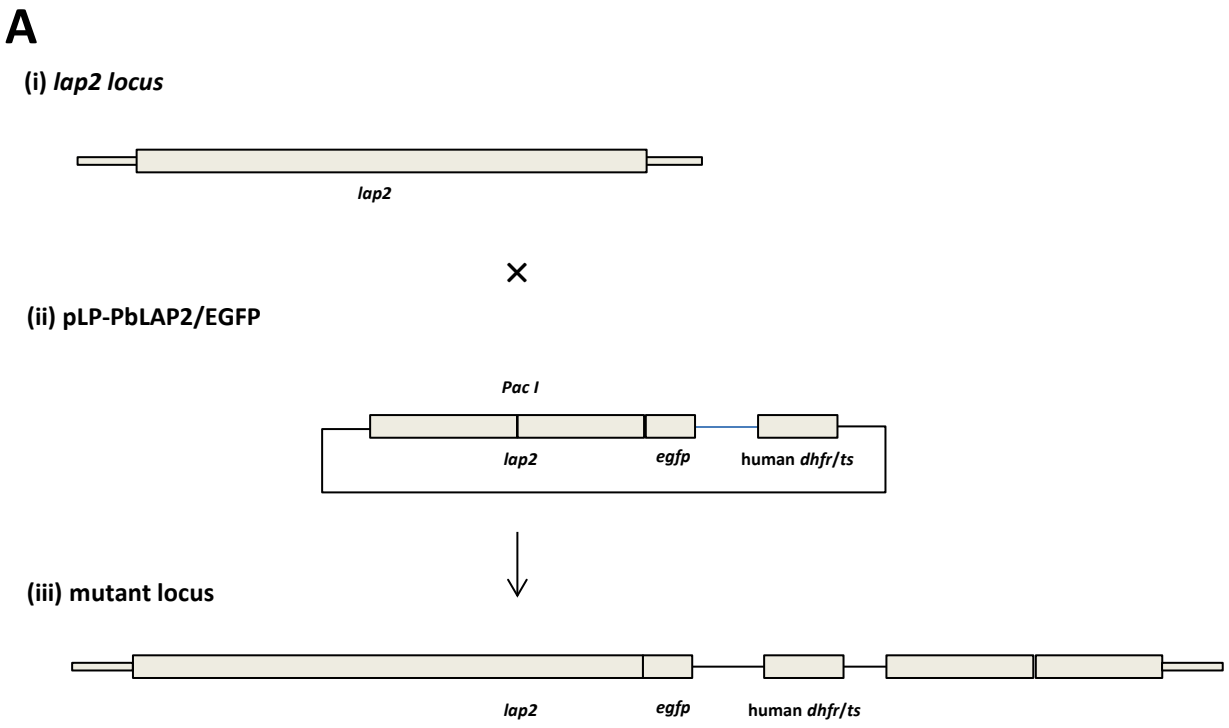


Fig. S7. DHHC10::GFP expression and subcellular localisation follows that of LAP3::mCherry throughout zygote to ookinete transformation. Live imaging of *in vitro*-cultured *dhhc10*::*gfp*;*lap3*::*mcherry* parasites at different time points shows DHHC10 expression (in green) as early as 3 h post gametocyte activation, with first signs of clustering (arrowheads) at 9 h post-fertilisation. At 12 h, accumulation of DHHC10, as well as LAP3 (in red) in the crystalloid body (arrowheads) becomes evident, and by 24 h, the proteins perfectly co-localise in these organelles. Overall, no differences exist in the timing of DHHC10 and LAP3 shuttling to the crystalloids as established in two independent cultures (>50 zygotes/ookinetes counted per time point). DNA was stained with Hoechst-33342 (in blue). BF: bright field microscopy. Scale bars = 5 µm.



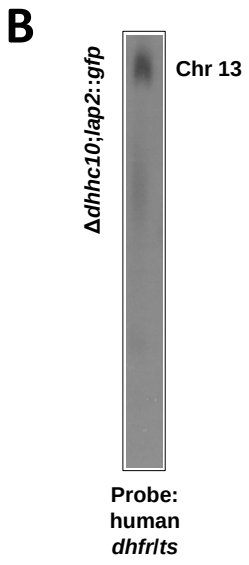


Fig. S8. Generation and genotyping of Δ*dhhc10*;*lap2*::*gfp* parasite line. (A) *lap2* GFP tagging construct pLP-PbLAP2/EGFP (ii) is from Saeed et al., *Mol Biochem Parasitol* 2010 170(1): p. 49-53. This construct includes the human *dhfr*/*ts* selectable marker cassette. **(B)** Correct genomic integration of pLP-PbLAP2/EGFP into chromosome 13 of Δ*dhhc10*-a parasites was confirmed by Field-Inversion Gel Electrophoresis (FIGE) of separated whole chromosomes in the uncloned Δ*dhhc10*;*lap2*::*gfp* parasite line. FIGE was developed using a probe for the human *dhfr*/*ts*.

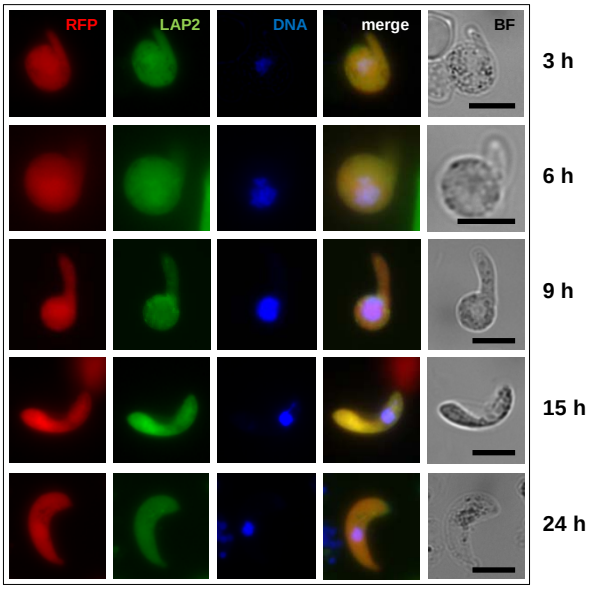


Fig. S9. LAP2::GFP remains cytoplasmic throughout zygote to ookinete transformation. Live imaging of *in vitro*-cultured Δ*dhhc10*;*lap2*::*gfp* parasites at different time points after gametocyte activation shows that LAP2 (in green) remains cytoplasmic in zygotes, retorts and mature ookinetes and fails to accumulate in discrete foci typical for crystalloid body-resident proteins. No haemozoin clusters are seen under bright field (BF) microscopy in any developmental stage. Haemozoin crystals remain scattered throughout the parasite cytoplasm. Zygotes/ookinetes were analysed in two independent cultures (>50 per time point). Red fluorescence signal in these parasite line originates from RFP expression of the background parental line used for genetic modification (Fluo-frmg). DNA was stained with Hoechst-33342 (in blue). Scale bars = 5 µm.

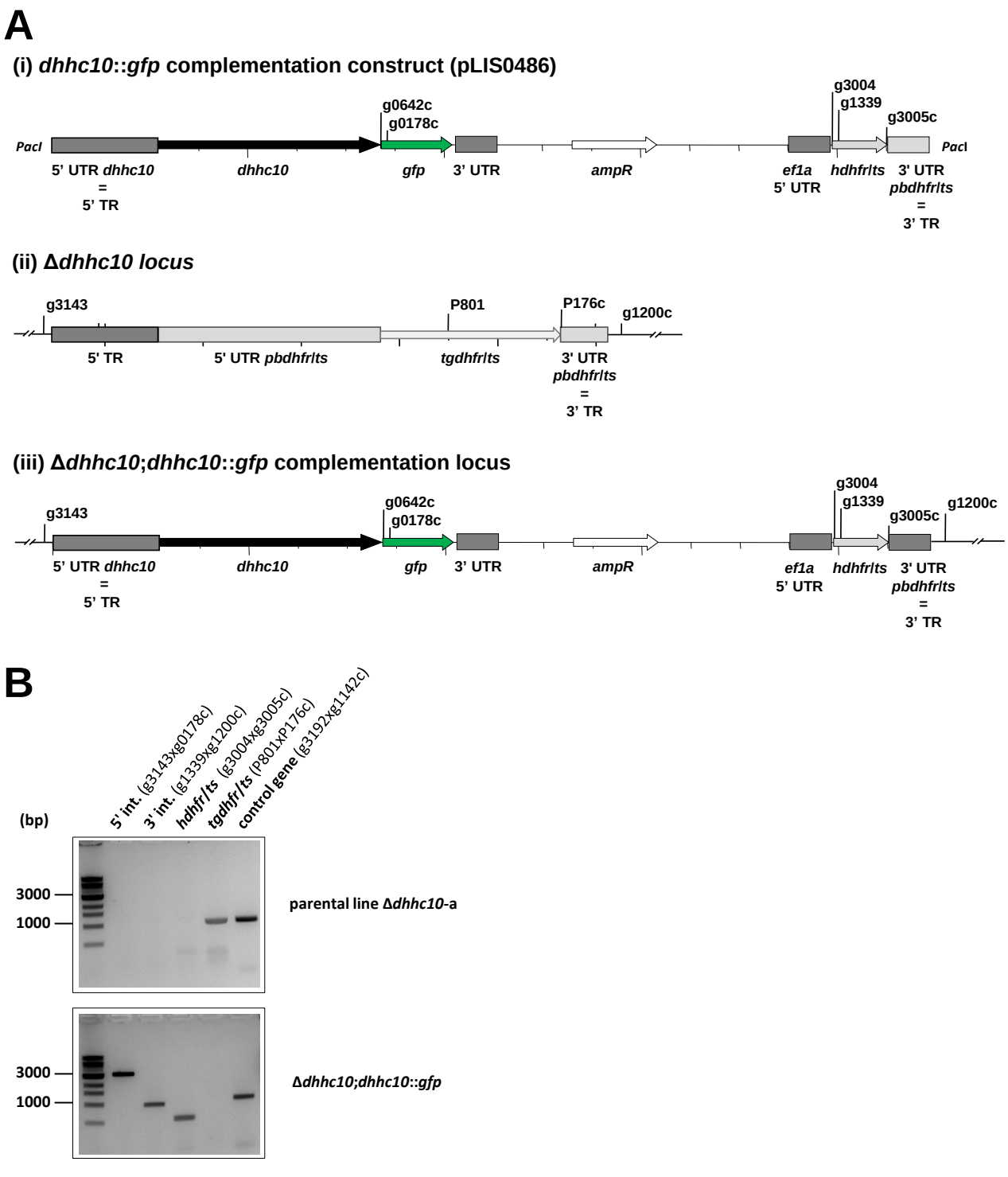


Fig. S10. Generation and genotyping of Δ*dhhc10*;*dhhc10*::*gfp* complementation parasite line. (A) *dhhc10*::*gfp* complementation construct pLIS0486 (i) was obtained by cloning the 5' UTR and entire ORF of *dhhc10* upstream and in frame with the *gfp* gene. This construct includes the human *dhfr*/*ts* selectable marker cassette under the control of *P. berghei ef1a* 5' UTR and *dhfr*/*ts* 3' UTR. The construct was integrated into the Δ*dhhc10 locus* (ii) of Δ*dhhc10*-a by double homologous recombination, resulting in the reintroduction of *dhhc10* into its original *locus* in Δ*dhhc10*;*dhhc10*::*gfp* parasites (iii). **(B)** PCR analyses confirm correct 5' and 3' integration (int.) of pLIS0486 as well as presence of *hdhfr*/*ts* and absence of *tgdhfr*/*ts* genes.

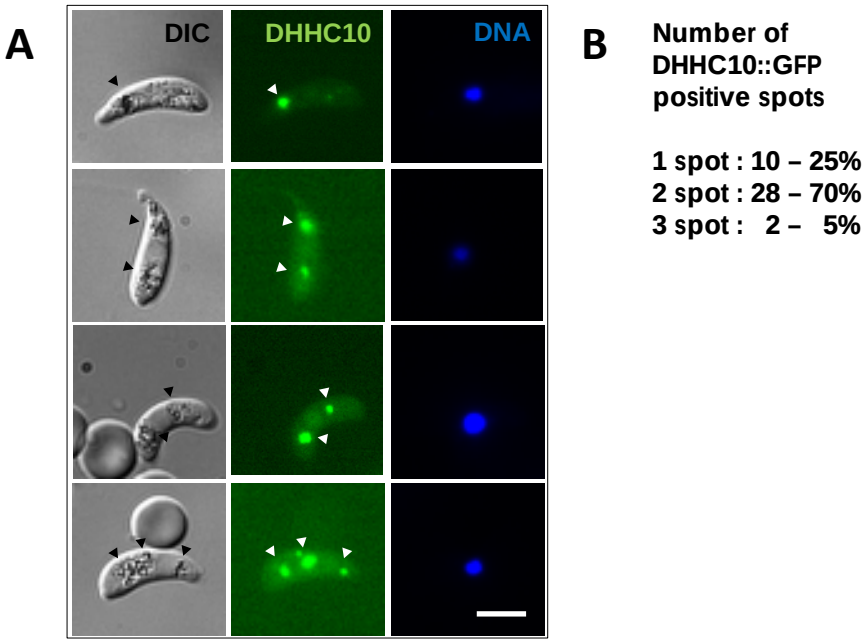


Fig. S11. Complementation of Δ*dhhc10* parasites with *dhhc10*::*gfp* gene restores crystalloid biogenesis. (A) Live imaging of *in vitro*-cultured Δ*dhhc10*;*dhhc10*::*gfp* ookinetes shows DHHC10::GFP expression (in green) in 1 to 3 discrete foci co-localising with haemozoin clusters (arrowheads). DNA was stained with Hoechst-33342 (in blue). DIC: differential interference contrast microscopy. Scale bar = 5 µm. **(B)** DHHC10::GFP spot-per-ookinete quantification; n=40

Table S1. Parasite transfection experiments

Gene name/ mutant name	Gene ID	DNA construct name	Restriction enzymes[1]	Experiment #/ mutant clone ID[2]	Parental line[3]
Gene deletion mutants					
Δ*dhhc10*-a	PBANKA_0512000	pLIS0067	*Asp*718I and *Not*I	2097cl1	820cl1m1cl1
Δ*dhhc10*-b				2365cl2	676m1cl1
GFP-tagged mutants					
dhhc10::*gfp*	PBANKA_0512000	pLIS0117	*Sna*BI	2187cl1m1	cl15cy1
Δ*dhhc10*;*lap2*::*gfp*	PBANKA_1300700	pLP-PbLAP2/EGFP [1]	*Pac*I	2433	Δ*dhhc10*-a
mCherry-tagged mutants					
dhhc10::*gfp*;*lap3*::*mCherry*	PBANKA_0204500	pLP-PbLAP3/mCherry	n.a.	2492	*dhhc10*::*gfp*
Complementation mutants					
Δ*dhhc10*;*dhhc10*::*gfp*	PBANKA_0512000	pLIS0486	*Pac*I	486.3	Δ*dhhc10*-a

[1] Restriction enzymes used for plasmid linearisation before transfection

[2] Experiment number for independent transfection experiments: the unsuccessful attempts and the experiment number/ID of the mutants clones

[3] Parental *P. berghei* ANKA line in which the transfection experiment was performed

[1] Saeed et al., *Mol Biochem Parasitol* 2010 170(1): p. 49-53

n.a.: not applicable

Table S2. Developmental figures for *P. berghei* mutants

Mutant	Asexual multiplication rate[1] (s.d.)	Gametocyte production[2] % (s.d.)	Ookinete production[3] % (s.d.)	Oocyst production[4] (s.d.)	MG Spz production[5] X10^4 (s.d.)	SG Spz production[6] X10^4 (s.d.)	Prepatent period[7]
Δ*dhhc10*-a	10 (0) *n*=2	19.7 (1.2) *n*=3	69.0 (5.4) *n*=4	161.4 (112.5) *n*=5	0 (0) *n*=4	0 (0) *n*=4	n.a.
Δ*dhhc10*-b	10 (0) *n*=4	17.7 (1.5) *n*=3	62.5 (6.6) *n*=4	306.7 (83.1) *n*=1	0 (0) *n*=1	0 (0) *n*=2	n.d.
dhhc10::*gfp*	10 (0) *n*=4	18.3 (2.5) *n*=3	82.0 (8.8) *n*=4	337.5 (47.9) *n*=1	n.d.	1.9 (0.6) *n*=1	5-7 *n*=4
WT[8]	10 (0) *n*>10	15-25 *n*>10	50-90 *n*>10	112-377.2 *n*=6	2.6-22 *n*=5	2.2-7.2 *n*=6	4-6 *n*=8

[1] The multiplication rate per 24 hours of blood stage parasites in mice infected with a single parasite

[2] The mean percentage of blood stage parasites developing into gametocytes in vivo

[3] The mean percentage of female gametes developing into mature ookinetes in vitro

[4] The mean number of oocysts per mosquito (days 12–13)

[5] The mean number of midgut sporozoites (MG Spz) per mosquito (days 20–22)

[6] The mean number of salivary gland sporozoites (SG Spz) per mosquito (days 20–22)

[7] The prepatent period (measured in days post bite of 10 infected females or intravenous injection of 10000 SG Spz per mouse) is defined as the day when parasites are detected in Giemsa-stained blood smears of mice

[8] The developmental data for wild type (WT) parasites are shown as the range of mean values. s.d.: standard deviation; n.d.: not determined; n.a.: not applicable

Table S3. Oligonucleotide primers used in RT-PCR

"c" or "Rev" at the end of primer names means they are antisense primers; all others are sense primers. Nucleotide stretches in capital letter correspond to the complementary sequence to the respective gene. n.a.: not applicable; ORF: open reading frame.

Gene name	Gene ID	Primer name	Sequence	Description
dhhc3	PBANKA_0927300	g1256	TGGGTTAATAATTGCATAGG	*dhhc3* ORF
		g1257c	ATATTTTATAGACCTTTCAGCTTC	*dhhc3* ORF
dhhc10	PBANKA_0512000	g0641	aaagaattcAAAACTGTTTTAAAGATG	*dhhc10* ORF
		g0642c	aaagcggccgcATAATGTTTTATAAAATAGCC	*dhhc10* ORF
***18S* rRNA**	n.a.	PbA18SFw	AAGCATTAAATAAAGCGAATACATCCTTAC	*18S* rRNA
		PbA18SRev	GGAGATTGGTTTTGACGTTTATGTG	*18S* rRNA
hsp70	PBANKA_0711900	g0258	AAAAGCAAAGCCAAACTTACC	*hsp70* ORF
		g0259c	GGATGGGGTTGTTCTATTACC	*hsp70* ORF
p25	PBANKA_0515000	g0385	CCGGAATTCATAAACAAATATACCTGG	*p25* 3' UTR
		g0476c	CGGGATCCTCATACGAATTTTATTG	*p25* 3' UTR
p28	PBANKA_0514900	g0115	TTCGATATCATGAATTTTAAATACAG	*p28* ORF
		g0116c	tccgcggccgcGCATTACTATCACGTAAATAAC	*p28* ORF
dozi	PBANKA_1217700	g0546	TAATTGTGTCGCTTCAAATG	*dozi* ORF
		g0548c	TAATTCTTTTATCATAGCAG	*dozi* ORF
cith	PBANKA_1301300	g0549	GAAAAAAGCAAAGATGTATTATCTG	*cith* ORF
		g0550c	ATAGGCTGGGTATCTGTTAAATG	*cith* ORF
alba3	PBANKA_1204400	g0003	aaacccggggaattcCAAGAAAGAGCTGAAAAC	*alba3* ORF
		g0004c	aaagcggccgctATTAGCAACAAAGTTTG	*alba3* ORF

Table S4. Oligonucleotide primers used in the generation of gene deletion, tagging and complementation constructs.

"c" at the end of primer names means they are antisense primers; all others are sense primers. Nucleotide stretches in capital letter correspond to the complementary sequence to the respective gene. Underlined are restriction site sequences. ORF: open reading frame.

Gene name	Gene ID	plasmid name	Primer name	Sequence	Restriction sites	Description
Gene deletion constructs						
dhhc10	PBANKA_0512000	pLIS0067	g0735	aaaggtaccTTTTTCTCCAAATTTTTG	*Asp*718I	*dhhc10* 5' targeting region
			g0736c	aaaaagcttCGTTAATATATAATAATAG	*Hind*III	*dhhc10* 5' targeting region
			g0737	aaagaattcGAAATATTTATTCTATTTG	*Eco*RI	*dhhc10* 3' targeting region
			g0738c	aaagcggccgcTTAATCTATATGCATTTC	*Not*I	*dhhc10* 3' targeting region
GFP-tagging constructs						
dhhc10	PBANKA_0512000	pLIS0117	g0641	aaagaattcAAAACTGTTTTAAAGATG	*Eco*RI	*dhhc10* ORF
			g0642c	aaagcggccgcATAATGTTTTATAAAATAGCC	*Not*I	*dhhc10* ORF
mCherry tagging of LAP3						
mCherry	na	na	mCherryswap-F	TAAAAATAGCTTAGGTGCCCTCATGAGTAAAGGAGAA	na	*mCherry ORF*
			mCherryswap-R	ATGACCACTCACCTGGCCCGCGCGCCAAAC	na	*mCherry ORF*
Complementation construct						
dhhc10	PBANKA_0512000	pLIS0486	g3143	ACACAAGATTGCCCAAGC	*Bst*Z17I downstream of g3143 within PCR product	*dhhc10* 5' targeting region
			g0642c	aaagcggccgcATAATGTTTTATAAAATAGCC	*Not*I	*dhhc10* ORF

Table S5. Primers used in genotyping and RT-PCR of mutant parasite lines.

"c" at the end of primer names means they are antisense primers; all others are sense primers. Nucleotide stretches in capital letter correspond to the complementary sequence to the respective gene. n.a.: not applicable; pb: Plasmodium berghei; tg: Toxoplasma gondii; dhfr/ts: dihydrofolate reductase/thymidylate synthase; ORF: open reading frame; UTR: untranslated region.

Gene name/ mutant name	Gene ID	Primer name	Sequence	Description
Primers for genotyping				
Δ*dhhc10*-a and Δ*dhhc10*-b		g0964	AACGAATTTGACTTGCATTC	*dhhc10* 5' integration
		g0965c	GGTATGAACTCATACATGTC	*dhhc10* 3' integration
dhhc10::*gfp*		g1199	ATTTTTGGGGGTTTTCAG	*dhhc10* 5' integration
		g1200c	GTTTTCAACACAAGTGTG	*dhhc10* 3' integration
dhhc10	PBANKA_0512000	g0641	aaagaattcAAAACTGTTTTAAAGATG	*dhhc10* ORF
		g0642c	aaagcggccgcATAATGTTTTATAAAATAGCC	*dhhc10* ORF
		g1199	ATTTTTGGGGGTTTTCAG	*dhhc10* ORF
		g1200c	GTTTTCAACACAAGTGTG	dhhc10 3' UTR
Δ*dhhc10*;*dhhc10*::*gfp*		g3143	ACACAAGATTGCCCAAGC	*dhhc10* 5' integration
		g1200c	GTTTTCAACACAAGTGTG	*dhhc10* 3' integration
Primers for RT-PCR				
dhhc10	PBANKA_0512000	g0641	aaagaattcAAAACTGTTTTAAAGATG	*dhhc10* ORF
		g0642c	aaagcggccgcATAATGTTTTATAAAATAGCC	*dhhc10* ORF
dhhc10::*gfp*		g1204	ATACAAACCAGACAGATC	*dhhc10* ORF
		g1200c	GTTTTCAACACAAGTGTG	*dhhc10* 3' UTR
		g1204	ATACAAACCAGACAGATC	*dhhc10* ORF
General primers				
pbdhfr/ts	PBANKA_0719300	g0952	GATTCATAAATAGTTGGACTTG	3' UTR *pbdhfr/ts*
		g1021c	ATTGTTGACCTGCAGGCATG	5' UTR *pbdhfr/ts*
tgdhfr/ts	n.a.	g1019	ATGCATAAACCGGTGTGTC	*tgdhfr/ts* ORF
		g1020c	AGCTTCTGTATTTCCGC	*tgdhfr/ts* ORF
		P801	aaaCTCGAGAAGAGAAGGAAGAC	*tgdhfr/ts* ORF
		P176c	CTAGACAGCCATCTCCATCTGG	*tgdhfr/ts* ORF
hdhfr/ts	n.a.	g1339	ACGAATTTAGATATTTCC	*hdhfr/ts* ORF
		g3004	aaaaGATCTATGGTTGGTTCGCTAAACTG	*hdhfr/ts* ORF
		g3005c	aaaaCAATTGTTAATCATTCTTCTCATATAC	*hdhfr/ts* ORF
RNA polymerase II	PBANKA_0807000	g0084	aaagaattcTGATGGTTTACAATCACC	RNA pol II ORF
		g0085c	aaagcggccgctTTCTTCCTGCATCTCCTC	RNA pol II ORF
p28	PBANKA_0514900	g0115	TTCGATATCATGAATTTTAAATACAG	*p28* ORF
		g0116c	tccgcggccgcGCATTACTATCACGTAAATAAC	*p28* ORF
gfp	n.a.	g0408c	GTATGTTGCATCACCTTC	*gfp* ORF
	n.a.	g0178c	CCGTATGTTGCATCACCTTCACCC	*gfp* ORF