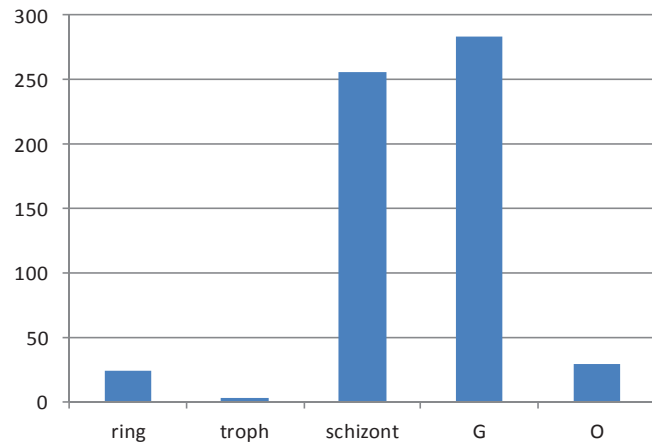


The *Plasmodium* palmitoyl-S-acyl-transferase DHHC2 is essential for ookinete morphogenesis and malaria transmission

Jorge M. Santos, Jessica Kehrer, Blandine Franke-Fayard, Friedrich Frischknecht, Chris J. Janse, Gunnar R. Mair



Supplementary Figure S1. PBANKA_010830 transcript levels as reads per kilobase of exon model per million mapped reads (RPKM). Ring 4-hour ring stage; troph 16-hour trophozoite; schizont 22-hour schizont; G gametocytes; O 24-hour ookinete; Otto et al. 2014

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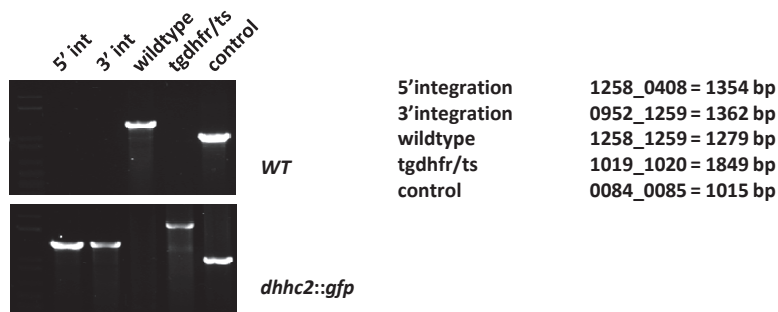
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Supplementary Figure S2. CLUSTAL W (1.83) multiple sequence alignment of DHHC2 from 7 *Plasmodium* species: PBANKA_010830 *Plasmodium berghei* ANKA; PCHAS_010890 *Plasmodium chabaudi chabaudi*; PY17X_0109900 *Plasmodium yoelii yoelii* 17X; PKNH_1140400 *Plasmodium knowlesi* strain H; PVX_113640 *Plasmodium vivax* Sal-1; PF3D7_0609800 *Plasmodium falciparum* 3D7; PRCDC_0608500 *Plasmodium reichenowi* CDC. Asterisk indicate position of the signature DHHC motif.

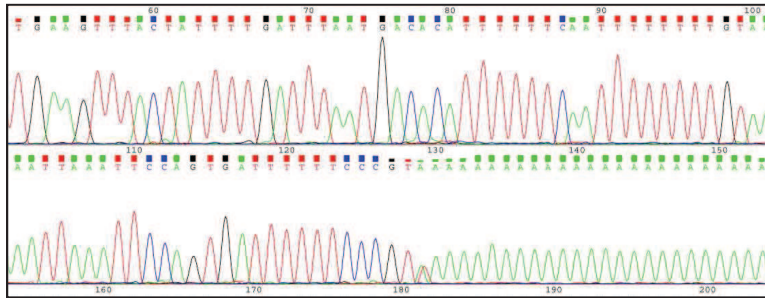
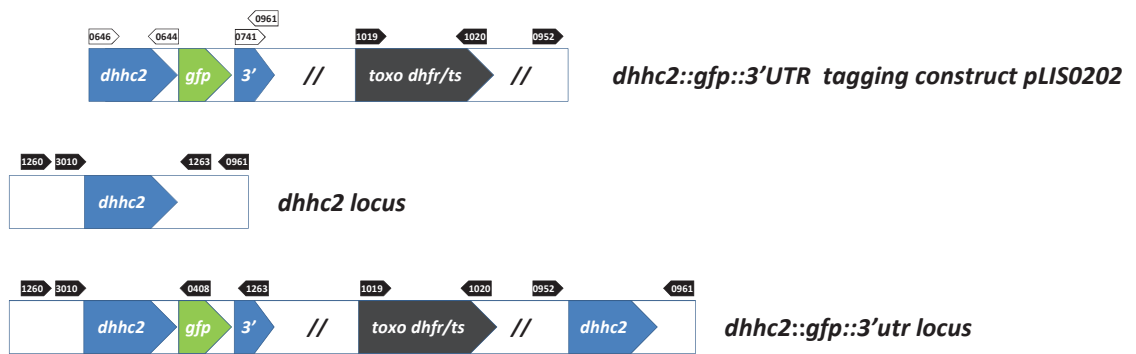
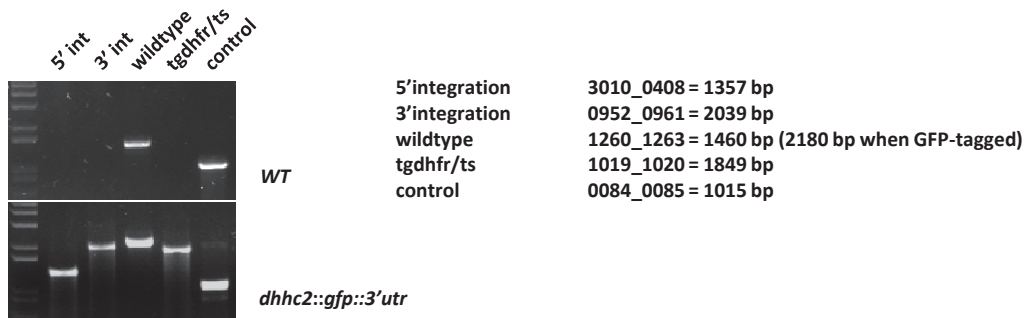
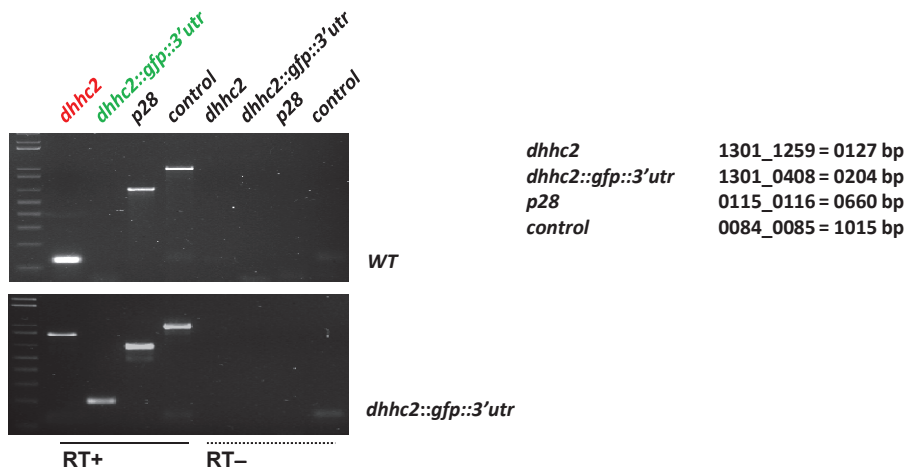
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NCLIV_067160	335	DGVRWNHMYVADEQV-
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PBANKA_010830	270	DGVRWRVSMSSHCSNI--

Supplementary Figure S3. CLUSTAL W (1.83) multiple sequence alignment of *Plasmodium berghei* PBANKA_010830 and proteins from three related apicomplexan species: TGGT1_278850 *Toxoplasma gondii* GT1; NCLIV_067160 *Neospora caninum* Liverpool; EfaB_MINUS_7900.g824 *Eimeria falciformis* Bayer Haberkorn 1970. Note the high sequence conservation surrounding the central DHHC motif which is located within the loop region between transmembrane domains 2 and 3. Asterisk indicate position of the signature DHHC motif.

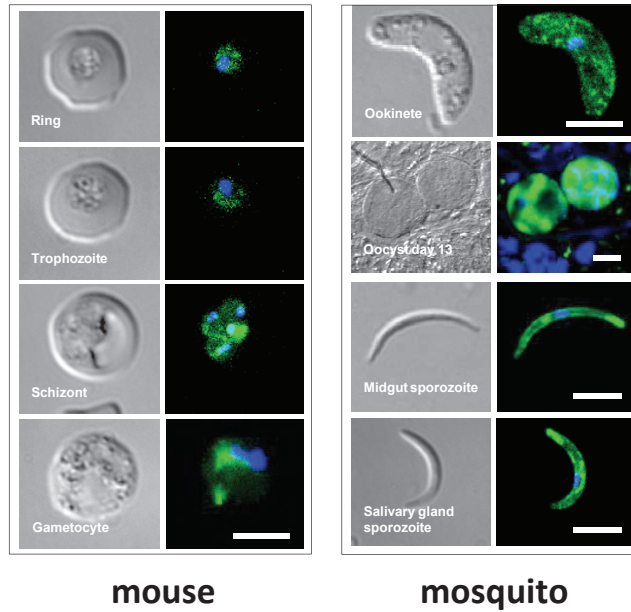
A**B**

Supplementary Figure S4. Generation and genotyping of *dhhc2::gfp* parasite line. (A) *dhhc2* GFP tagging construct pLIS0086 was obtained by cloning the last 1167 bp of *dhhc2* 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 *dhhc2* locus of cl15cy1 by single homologous recombination, resulting in the fusion of *dhhc2* to *gfp* in *dhhc2::gfp* parasites. (B) Correct tagging of *dhhc2* was shown by PCR genotyping analyses. PCR analyses confirm 5' and 3' integration (int.) of pLIS0086, absence of WT *dhhc2* ORF and presence of *tgdhfr/ts* gene. Primer combinations and expected amplicon sizes in basepairs (bp) are shown on the right.

A**B****C****D**

Supplementary Figure S5. Generation and genotyping of *dhhc2::gfp::3'utr* parasite line. (A) The chromatogram shows the sequencing result of a 3' RACE product allowing determination of the length of the 3'UTR of *dhhc2*. (B) *dhhc2* GFP tagging construct pLIS0202 was obtained by cloning the last 1167 bp of *dhhc2* ORF excluding the stop codon upstream and in frame with the GFP gene and nucleotide positions 36 to 541 of the *dhhc2* 3' UTR downstream of *gfp*. 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 *dhhc2* locus of *P. berghei* cl15cy1 by single homologous recombination, resulting in the fusion of *dhhc2* to *gfp* and maintaining the endogenous *dhhc2* 3' utr in *dhhc2::gfp-3'utr* parasites. (C) Correct tagging of *dhhc2* was shown by PCR genotyping analyses, confirming 5' and 3' integration (int.) of pLIS0202, absence of WT *dhhc2* ORF and presence of the *tgdhfr/ts* gene. (D) Absence of WT *dhhc2* and exclusive presence of *dhhc2::gfp* mRNA was confirmed in cloned *dhhc2::gfp-3'utr* mixed blood stages by RT-PCR.

DIC DHC2::GFP Nucleus

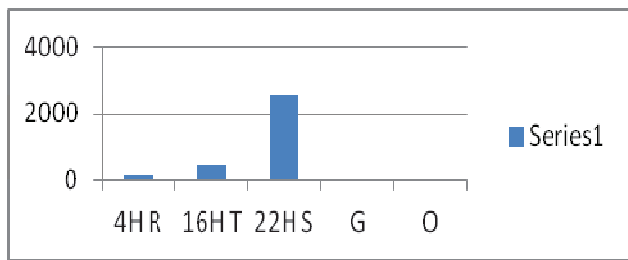


Supplementary Figure S6. Expression of DHC2::GFP in *dhhc2::gfp::3'utr* parasites. Scale bars = 5 μ m (blood stages, ookinetes and sporozoites) or 20 μ m (oocysts).

Supplementary Figure S7. Transcript levels as reads per kilobase of exon model per million mapped reads (RPKM). Ring 4-hour ring stage; troph 16-hour trophozoite; schizont 22-hour schizont; G gametocytes; O 24-hour ookinete; Otto et al. 2014

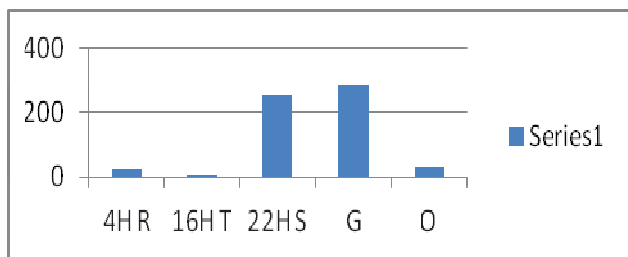
PBANKA_140060 cytoadherence linked asexual protein, putative

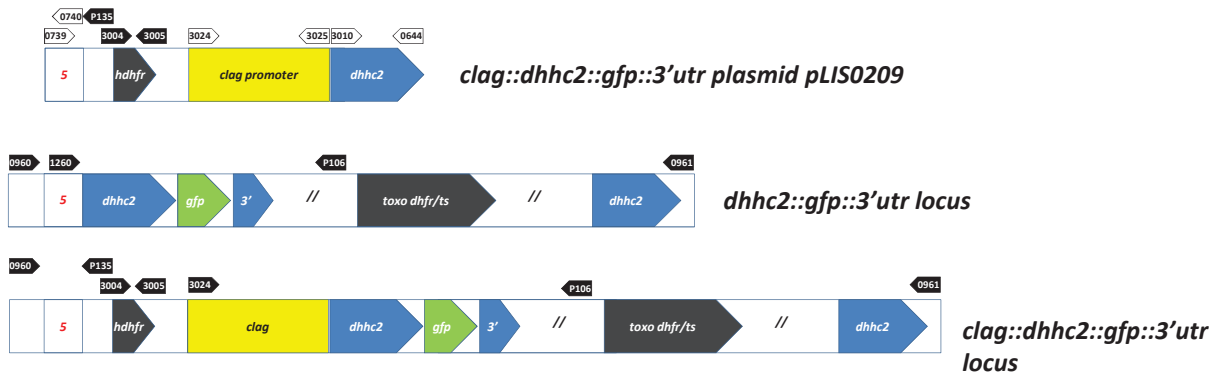
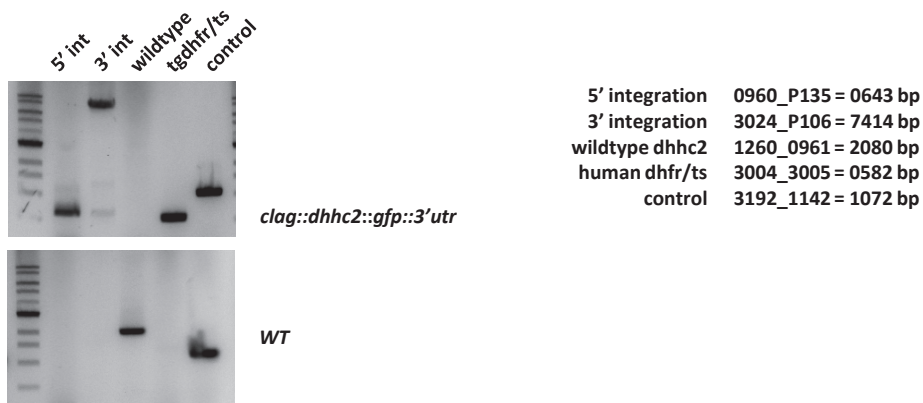
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16H T	479.428
22H S	2563.504
G	26.995
O	25.725



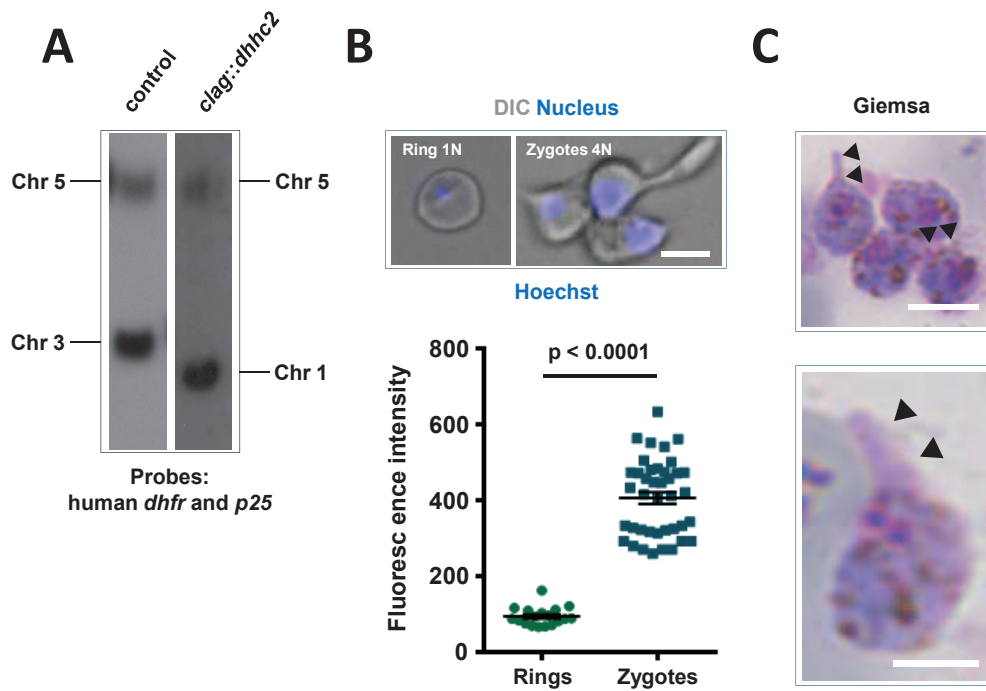
PBANKA_010830 palmitoyltransferase, putative (DHHC2)

4H R	24.294
16H T	3.636
22H S	255.355
G	283.007
O	29.628



A**B**

Supplementary Figure S8. Generation and genotyping of the *clag::dhhc2::gfp::3'utr* parasite line. (A) The *dhhc2* GFP promoter swap construct pLIS0209 was obtained by cloning the *clag* promoter in front of the *dhhc2* ORF. The human *dhfr* selectable marker cassette under the control of *P. berghei ef1a* 5' and 3' UTRs is further flanked upstream by *dhhc2* upstream region and is thus integrated into the *dhhc2* locus of *dhhc2::gfp::3'utr* by double homologous recombination, resulting in the exchange of the endogenous *dhhc2* promoter for the *clag* promoter. **(B)** Correct promoter swap of *dhhc2* was shown by PCR genotyping analyses, confirming 5' and 3' integrations (int.) of pLIS0209, absence of WT *dhhc2* gene, and presence of the human *dhfr* gene.



Supplementary Figure S9. *clag::dhhc2* parasites (parental line: wildtype) are capable of normal fertilization but fail to form ookinetes, replicating the phenotype of *clag::dhhc2::gfp::3'utr* mutants. (A) Construct pLIS0209 was integrated into the *dhhc2* locus of parental wildtype reference line 676m1c1 by double homologous recombination, resulting in the exchange of the endogenous *dhhc2* promoter for the *clag* promoter. Correct promoter swapping was shown by Southern analysis of separated chromosomes. Hybridisation of separated chromosomes was done with a mixture of two probes: one against the human *dhfr*, recognising integrated pLIS0209 into chromosome 1, and another against the gene *p25* on chromosome 5. As control, a transgenic parasite line containing the human *dhfr* in the *p230p* locus on chromosome 3 was used. **(B)** Live imaging of Hoechst-stained ring stage and zygotes of the *dhhc2* promoter swap line (scale bar=5 μ m), and quantification of DNA content. Nuclear DNA content of asexual blood stage rings (haploid DNA content, 1N) and zygotes (tetraploid DNA content, 4N) was determined by Hoechst-fluorescence intensity measurements. The mean fluorescence intensity of ring-form nuclei (1N) is 94 (n=18) and of zygotes (4N) is 406 (n=40). Mean \pm SEM values and p-value for Student's t-test are shown. **(C)** Giemsa staining of overnight ookinete cultures reveals 2-BMP-like morphological defects in mutant zygotes displaying thin protrusions (arrowheads). Scale bars=5 μ m.

Table S1a - Primers used in the present work.

a) Primers used in life-cycle RT-PCRs, RT-PCRs of IP samples and 3'RACE

“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

Gene name	Gene ID	Primer name	Sequence	Description
<i>dhhc2</i>	PBANKA_010830	g0646	aaagaattcTCGATATTCATATTTATTG	<i>dhhc2</i> ORF
		g0644c	aaagcggccgcATATATTACTTCCATGTG	<i>dhhc2</i> ORF
18S rRNA	n.a.	PbA18SFw	AAGCATTAAATAAAGCGAATACATCCTTAC	18S rRNA
		PbA18SRev	GGAGATTGGTTTTGACGTTTATGTG	18S rRNA
<i>hsp70</i>	PBANKA_071190	g0258	AAAAGCAAAGCCAACTTACC	<i>hsp70</i> ORF
		g0259c	GGATGGGGTTGTTCTATTACC	<i>hsp70</i> ORF
<i>p25</i>	PBANKA_051500	g0385	CCGGAATTCATAAACAATATACCTGG	<i>p25</i> 3' UTR
		g0476c	CGGGATCCTCATACGAATTTTATTG	<i>p25</i> 3' UTR
<i>p28</i>	PBANKA_051490	g0115	TTCGATATCATGAATTTTAAATACAG	<i>p28</i> ORF
		g0116c	tccgcgccgcGCATTACTATCACGTAATAAC	<i>p28</i> ORF
<i>dozi</i>	PBANKA_121770	g0546	TAATTGTGTCGCTTCAAATG	<i>dozi</i> ORF
		g0548c	TAATTCTTTTATCATAGCAG	<i>dozi</i> ORF
<i>cith</i>	PBANKA_130130	g0549	GAAAAAAGCAAAGATGTATTATCTG	<i>cith</i> ORF
		g0550c	ATAGGCTGGGTATCTGTAAATG	<i>cith</i> ORF
<i>alba3</i>	PBANKA_120440	g0003	aaaccggggaattcCAAGAAAGAGCTGAAAAC	<i>alba3</i> ORF
		g0004c	aaagcggccgctATTAGCAACAAAGTTTG	<i>alba3</i> ORF
<i>dhhc2</i>	PBANKA_010830	g3315	TAATGCAAAACAGGTTTTTGG	<i>dhhc2</i> ORF
		g1301	CATTGTATTTCAAATAGACC	<i>dhhc2</i> ORF
		g3318	CAATACTTTTCAGTAATAAAAATG	<i>dhhc2</i> 3'utr
		g3319	AAATCACTGGAATTTAATTTTAC	<i>dhhc2</i> 3'utr
n/a	oligo d(T)	g0357c	GCTGCAAGTGAACATCTGTTTTTTTTTTTTTTTTT	<i>poly(A)</i>

Table S1b - Primers used in the present work.

b) Primers used in the generation of gene deletion and GFP-tagging constructs.

“c” at the end of primer names means they are antisense primers. All others are sense primers.

ORF: open reading frame; UTR: untranslated region.

Gene name	Gene ID	Construct name	Primer name	Sequence	Restriction sites
Gene deletion constructs					
<i>dhhc2</i>	PBANKA_010830	pLIS0065	g0739	aaaggtaccTTTATTATTTGAGTGTTG	<i>Asp718I</i>
			g0740c	aaaaagcttTTTATATTGATTTTGATTG	<i>HindIII</i>
			g0741	aaagaattcTTGAGTTTATAAATATGTC	<i>EcoRI</i>
			g0742c	aaagcgccgcATATCCTAAAACTATTG	<i>NotI</i>
GFP-tagging constructs					
<i>dhhc2</i>	PBANKA_010830	pLIS0086	g0646	aaagaattcTCGATATTCATATTTATTG	<i>EcoRI</i>
			g0644c	aaagcgccgcATATATTACTTCCATGTG	<i>NotI</i>
		pLIS0202	g0646	aaagaattcTCGATATTCATATTTATTG	<i>EcoRI</i>
			g0644c	aaagcgccgcATATATTACTTCCATGTG	<i>NotI</i>
			g0741	aaagaattcTTGAGTTTATAAATATGTC	<i>EcoRI</i>
			g0742c	aaagcgccgcATATCCTAAAACTATTG	<i>NotI</i>
Promoter swap construct					
<i>dhhc2</i>	PBANKA_010830	pLIS209	g3010	aaaactagtATGACACATAAATATATGCAGATAT	<i>SpeI</i>
			g0644c	aaagcgccgcATATATTACTTCCATGTG	<i>NotI</i>
<i>clag</i>	PBANKA_140060		g3024	aaacgatcgCTGATATTTATGAGTATTCC	<i>PvuI</i>
			g3025c	aaatctagaTATATTACGAATTGACACC	<i>XbaI</i>

Table S1c - Primers used in the present work.

c) 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; ORF: open reading frame; UTR: untranslated region.

Gene name/ mutant name	Gene ID	Primer name	Sequence
Primers for genotyping			
<i>dhhc2::gfp</i>		g1258	ACACATAAATATATGCAG
		g1259c	ATGACATATTATAAACTC
		3010	ATGACACATAAATATATGCAGATAT
<i>dhhc2::gfp</i> -3'UTR	PBANKA_010830	g0961c	GAGAATTAAGCCTATATATAC
		g1260	TGAGGAGAATATTATAAG
		g1263c	AATGCTATTGCATATTTG
		g1258	ACACATAAATATATGCAG
<i>dhhc2</i>		g1259c	ATGACATATTATAAACTC
		g1260	TGAGGAGAATATTATAAG
		g1263c	AATGCTATTGCATATTTG
Primers for RT-PCR			
<i>dhhc2</i>		g1261	AATATATGCAGATATCAC
		g1262c	AAAAAATATACTTGTGC
		g1301	CATTGTATTTCAAATAGACC
<i>dhhc2::gfp</i>	PBANKA_010830	g1259c	ATGACATATTATAAACTC
		g1258	ACACATAAATATATGCAG
		g1301	CATTGTATTTCAAATAGACC
<i>dhhc2::gfp</i> -3'UTR		g1301	CATTGTATTTCAAATAGACC
		g1259c	ATGACATATTATAAACTC
General primers			
<i>pbdhfr/ts</i>	PBANKA_071930	g0952	GATTCATAAATAGTTGGACTTG
		P106	GTCCATACAACTATATCCGAAC
<i>tgdhfr/ts</i>	n.a.	g1019	ATGCATAAACCGGTGTGTC
		g1020c	AGCTTCTGTATTTCCGC
RNA polymerase II subunit RPB1	PBANKA_080700	g0084	aaagaattcTGATGGTTTACAATCACC
		g0085c	aaagcgccgctTTCTTCCTGCATCTCCTC
<i>p28</i>	PBANKA_051490	g0115	TTCGATATCATGAATTTTAAATACAG
		g0116c	tccgcgccgcGCATTACTATCACGTAATAAC
<i>gfp</i>	n.a.	g0408c	GTATGTTGCATCACCTTC
<i>ef1a</i>	PBANKA_113330	P135	GTAAACTTAAGCATAAAGAGCTCG
<i>human dhfr</i>	n.a.	g3004	AAAAGATCTATGGTTGGTTCGCTAAACTG
<i>human dhfr</i>	n.a.	g3005	AAACAATTGTTAATCATTCTTCTCATATAC

Supplementary Table S2. Parasite transfection experiments overview

Gene name/ mutant name	Gene ID	DNA construct name	Restriction enzymes ¹	Experiment #/ mutant clone ID ²	Parental line ³
Unsuccessful gene deletion attempts					
<i>dhhc2</i>	PBANKA_010830	pLIS0065	<i>Asp718I</i> and <i>NotI</i>	2095	820cl1m1cl1
				2362, 2363	676m1cl1
GFP-tagged mutants					
<i>dhhc2::gfp</i>	PBANKA_010830	pLIS0086	<i>FspAI</i>	2185cl1m1	cl15cy1
<i>dhhc2::gfp-3'utr</i>	PBANKA_010830	pLIS0202	<i>BsmI</i>	202cl1m1	cl15cy1
Promoter swap					
<i>clag::dhhc2::gfp::3'utr</i>	PBANKA_010830	pLIS209	<i>KpnI</i> , <i>BglI</i>	209cl2	202cl1m1
<i>clag::dhhc2</i>	PBANKA_010830	pLIS209	<i>KpnI</i> , <i>BamHI</i>	2593	676cl1m1

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