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

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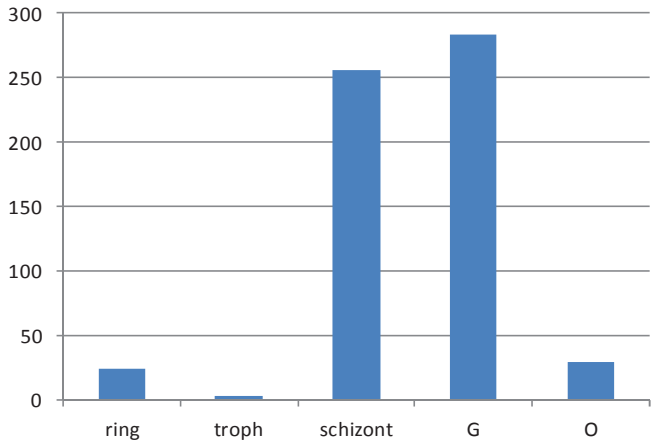
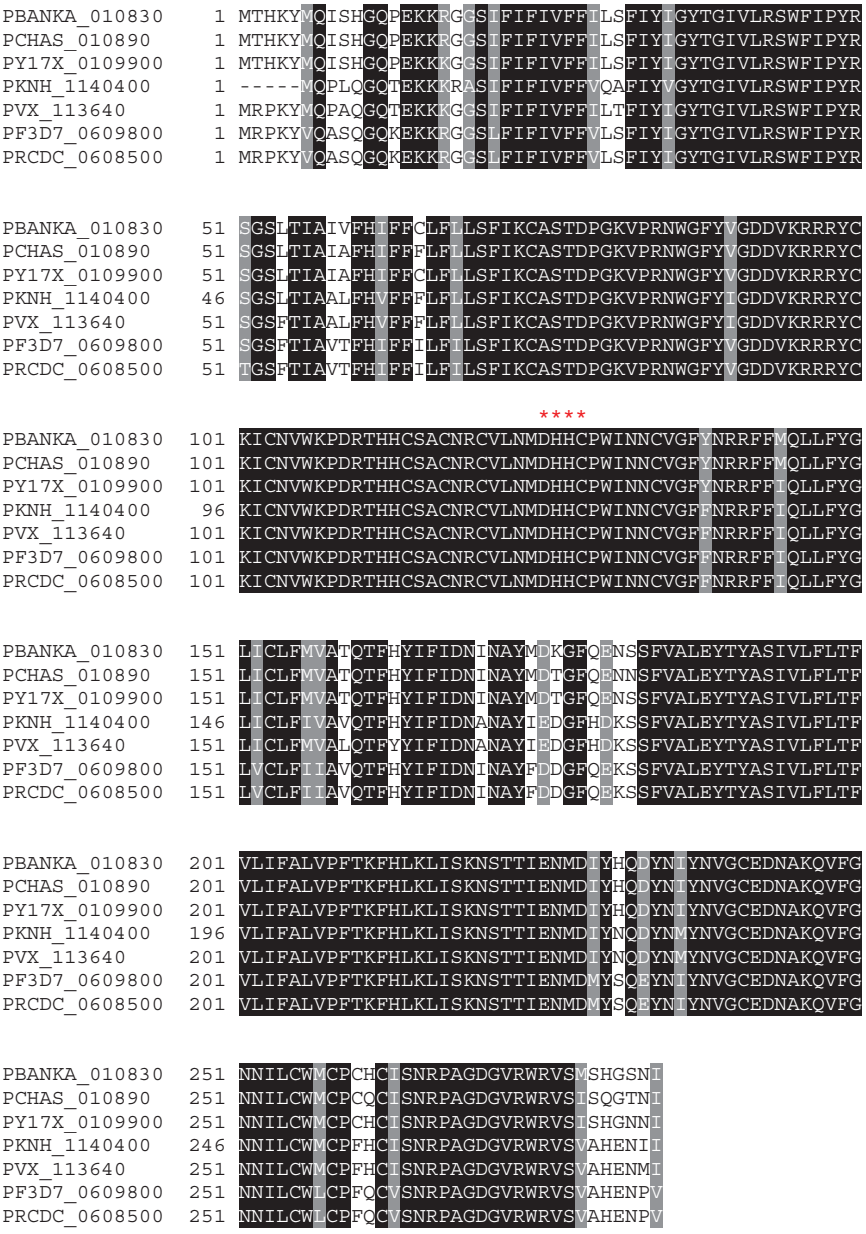
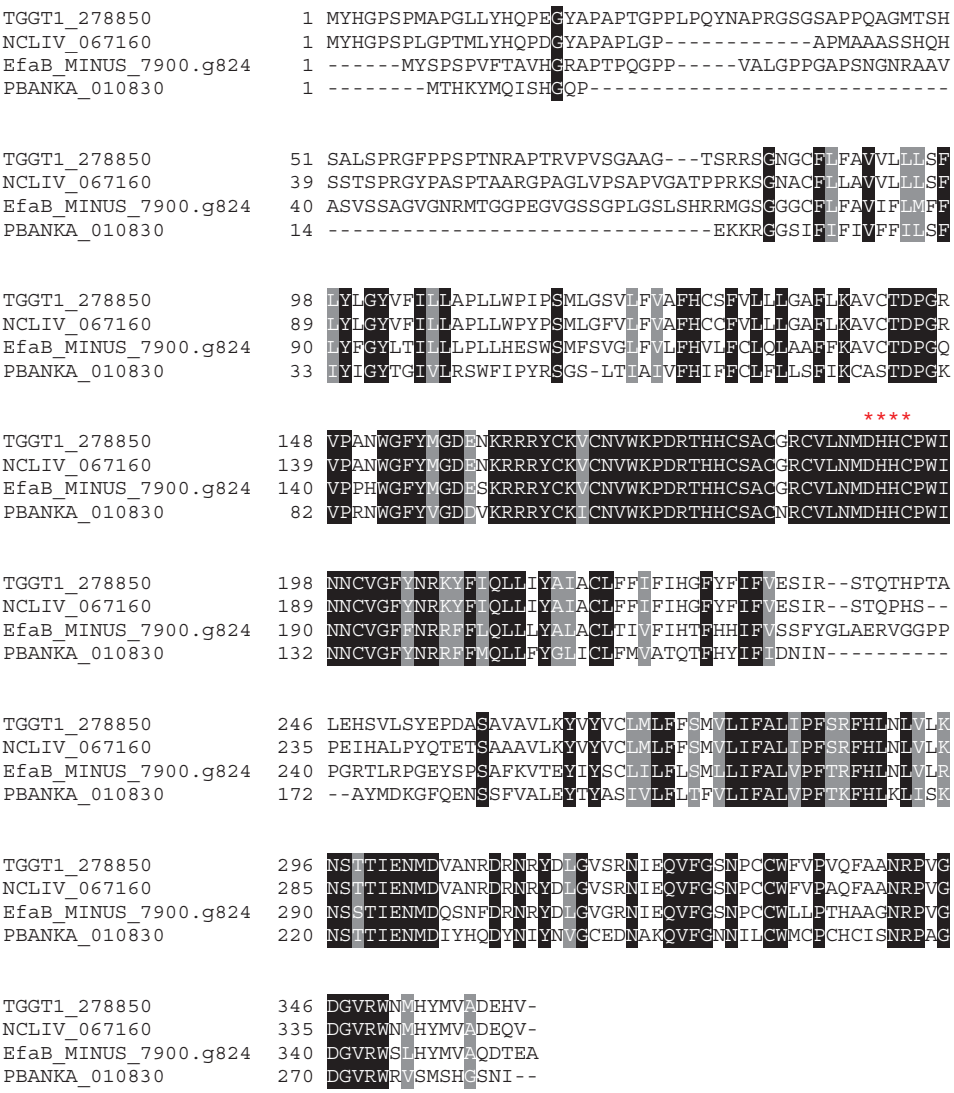


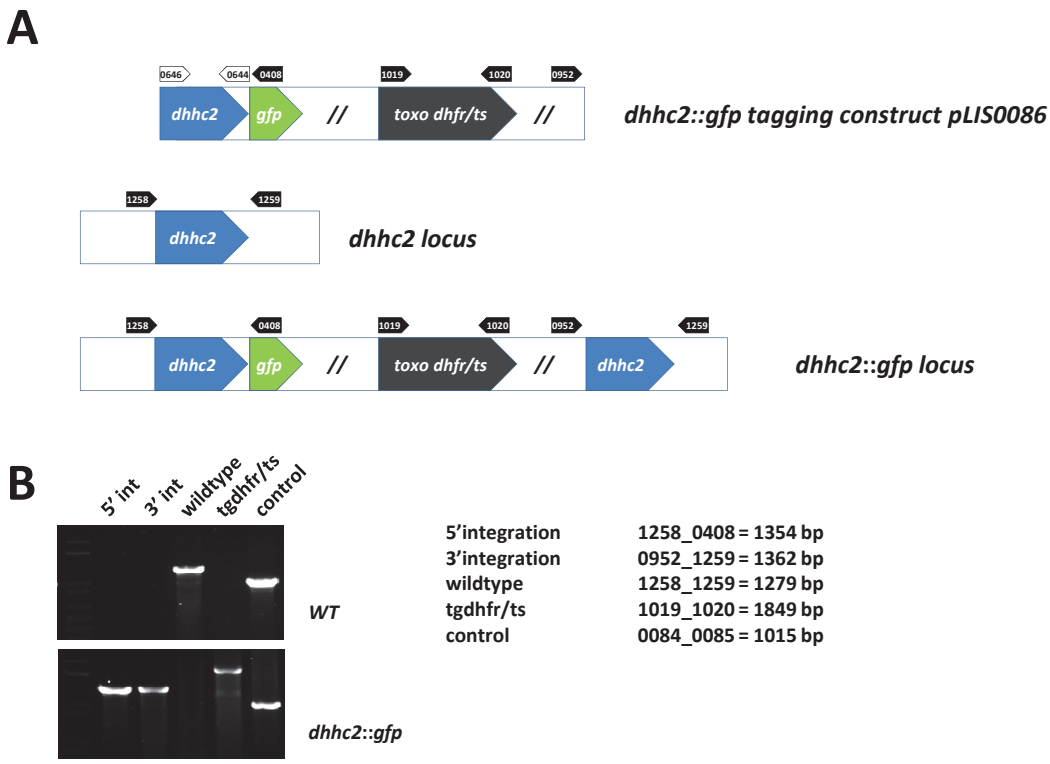
Supplementary Figure S1. PBANKA_010830 transcript levels as reads per kilobase of exon model per million mapped reads (RPKM). Ring 4-hour ring stage; troph 16-hour trophozoite; schizont 22-hour schizont; G gametocytes; O 24-hour ookinete; Otto et al. 2014



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.

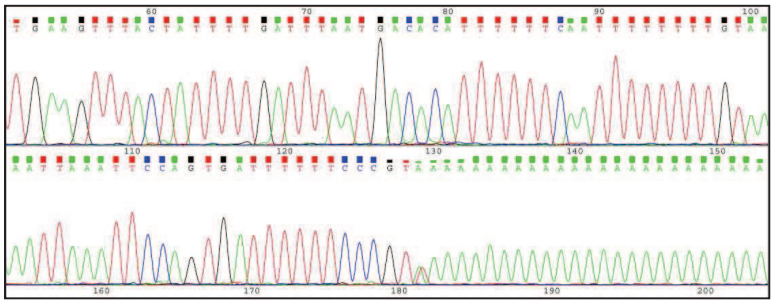


Supplementary Figure S3. CLUSTAL W (1.83) multiple sequence alignment of *Plasmodium berghei* PBANKA_010830 and proteins from three related apicomplexan species: TGGT1_278850 *Toxoplasma gondii* GT1; NCLIV_067160 *Neospora caninum* Liverpool; EfaB_MINUS_7900.g824 *Eimeria falciformis* Bayer Haberkorn 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.

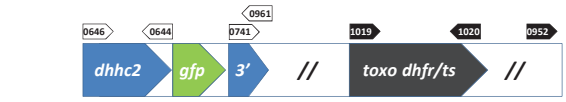


Supplementary Figure S4. Generation and genotyping of *dhhc2*::*gfp* parasite line. (A) *dhhc2* GFP tagging construct pLIS0086 was obtained by cloning the last 1167 bp of *dhhc2* ORF excluding the stop codon upstream and in frame with the *gfp* gene. This construct includes the *Toxoplasma gondii dhfr*/*ts* 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

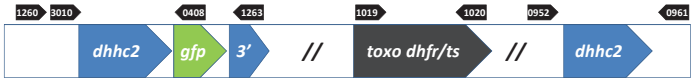


B



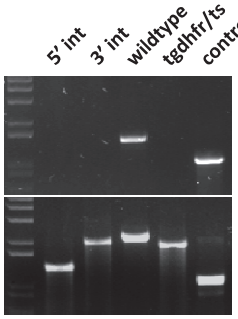
dhhc2::gfp::3'UTR tagging construct pLIS0202

dhhc2 locus



dhhc2::gfp::3'utr locus

C

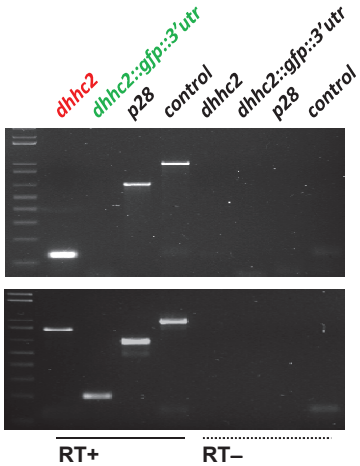


WT

dhhc2::gfp::3'utr

5'integration	3010_0408 = 1357 bp
3'integration	0952_0961 = 2039 bp
wildtype	1260_1263 = 1460 bp (2180 bp when GFP-tagged)
tgdhfr/ts	1019_1020 = 1849 bp
control	0084_0085 = 1015 bp

D

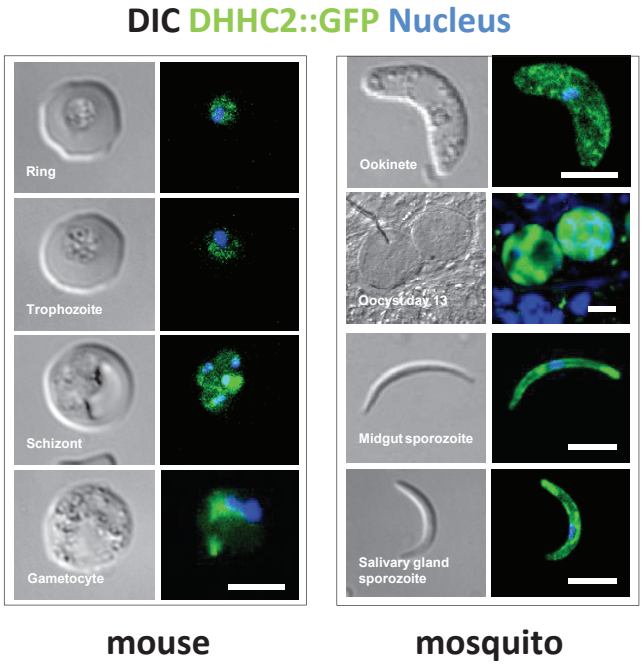


WT

dhhc2::gfp::3'utr

dhhc2	1301_1259 = 0127 bp
dhhc2::gfp::3'utr	1301_0408 = 0204 bp
p28	0115_0116 = 0660 bp
control	0084_0085 = 1015 bp

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.

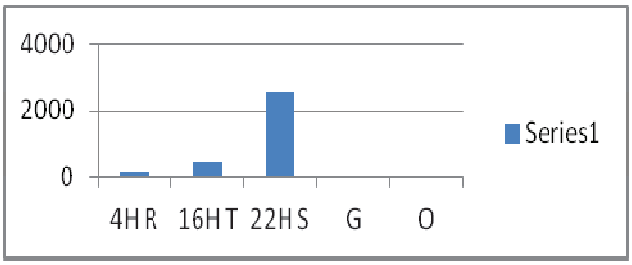


Supplementary Figure S6. Expression of DHHC2::GFP in *dhhc2::gfp::3'utr* parasites. Scale bars = 5 µm (blood stages, ookinetes and sporozoites) or 20 µm (oocysts).

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

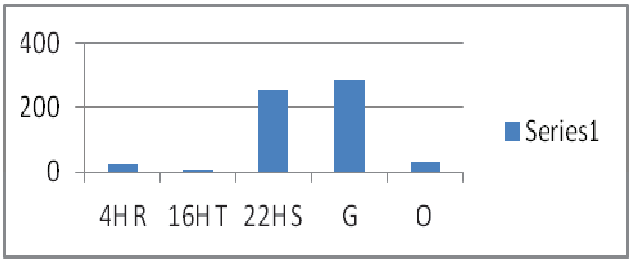
PBANKA_140060 cytoadherence linked asexual protein, putative

4H R	209.626
16H T	479.428
22H S	2563.504
G	26.995
O	25.725

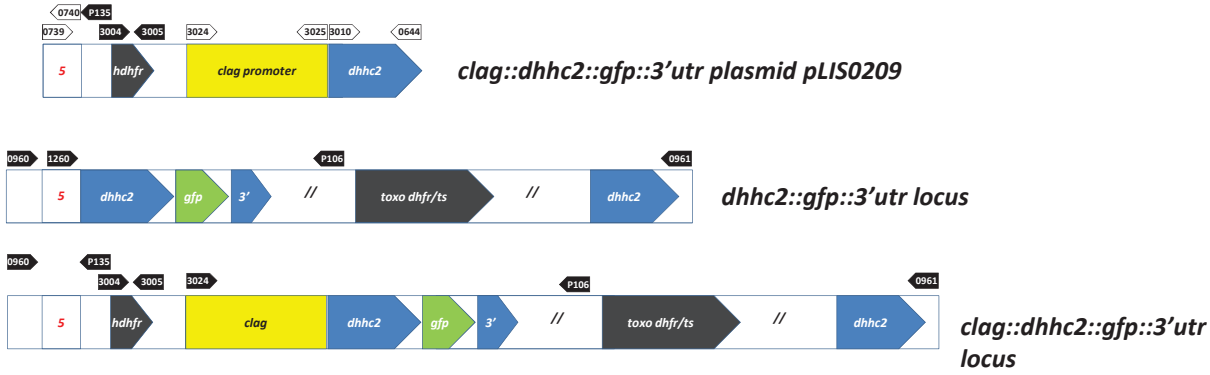


PBANKA_010830 palmitoyltransferase, putative (DHHC2)

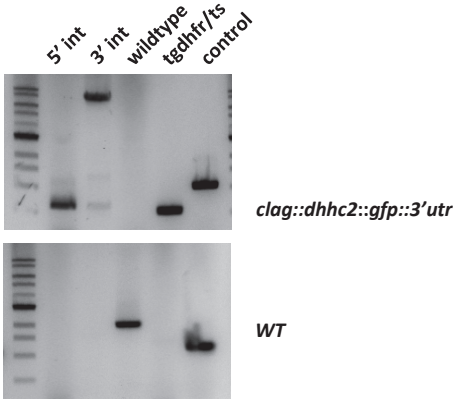
4H R	24.294
16H T	3.636
22H S	255.355
G	283.007
O	29.628



A

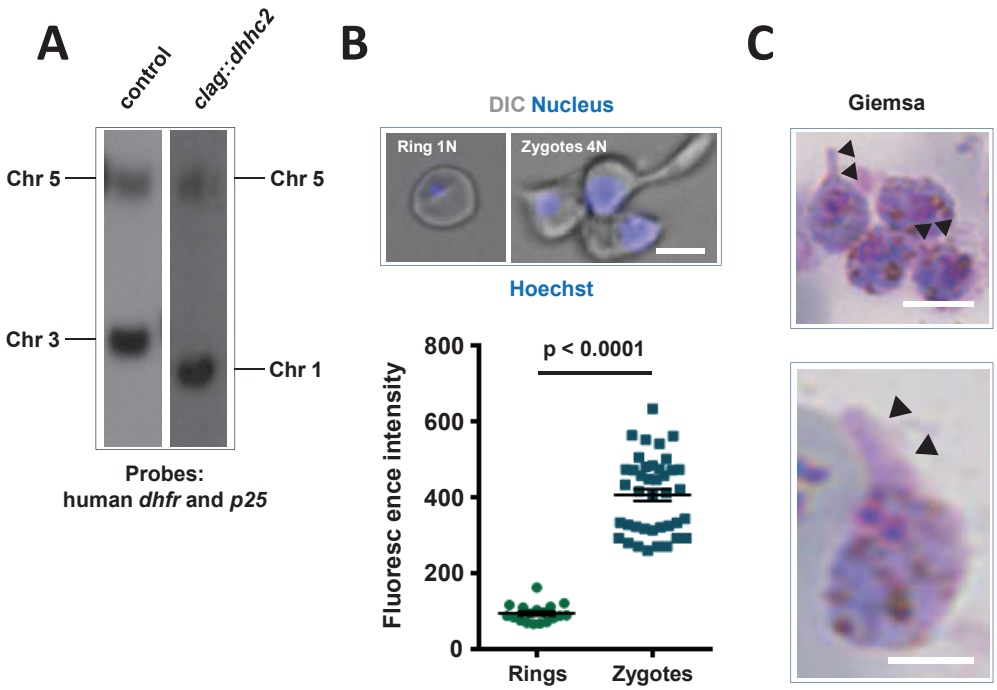


B



5' integration	**0960_P135 = 0643 bp**
3' integration	**3024_P106 = 7414 bp**
wildtype dhhc2	**1260_0961 = 2080 bp**
human dhfr/ts	**3004_3005 = 0582 bp**
control	**3192_1142 = 1072 bp**

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 676m1cl1 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 µm), 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 ± 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 µm.

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
dhhc2	PBANKA_010830	g0646	aaagaattcTCGATATTCATATTTATTG	*dhhc2* ORF
		g0644c	aaagcggccgcATATATTACTTCCATGTG	*dhhc2* ORF
***18S* rRNA**	n.a.	PbA18SFw	AAGCATTAAATAAAGCGAATACATCCTTAC	*18S* rRNA
		PbA18SRev	GGAGATTGGTTTTGACGTTTATGTG	*18S* rRNA
hsp70	PBANKA_071190	g0258	AAAAGCAAAGCCAAACTTACC	*hsp70* ORF
		g0259c	GGATGGGGTTGTTCTATTACC	*hsp70* ORF
p25	PBANKA_051500	g0385	CCGGAATTCATAAACAAATATACCTGG	*p25* 3' UTR
		g0476c	CGGGATCCTCATACGAATTTTATTG	*p25* 3' UTR
p28	PBANKA_051490	g0115	TTCGATATCATGAATTTTAAATACAG	*p28* ORF
		g0116c	tccgcggccgcGCATTACTATCACGTAAATAAC	*p28* ORF
dozi	PBANKA_121770	g0546	TAATTGTGTCGCTTCAAATG	*dozi* ORF
		g0548c	TAATTCTTTTATCATAGCAG	*dozi* ORF
cith	PBANKA_130130	g0549	GAAAAAAGCAAAGATGTATTATCTG	*cith* ORF
		g0550c	ATAGGCTGGGTATCTGTTAAATG	*cith* ORF
alba3	PBANKA_120440	g0003	aaacccggggaattcCAAGAAAGAGCTGAAAAC	*alba3* ORF
		g0004c	aaagcggccgctATTAGCAACAAAGTTTG	*alba3 ORF*
dhhc2	PBANKA_010830	g3315	TAATGCAAAACAGGTTTTTGG	*dhhc2 ORF*
		g1301	CATTGTATTTCAAATAGACC	*dhhc2 ORF*
		g3318	CAATACTTTTCAGTAATAAAATG	*dhhc2 3'utr*
		g3319	AAATCACTGGAATTTAATTTTAC	*dhhc2 3'utr*
n/a	oligo d(T)	g0357c	GCTGCAAGTGAACTATCTGTTTTTTTTTTTTTTTT	*poly(A)*

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					
dhhc2	PBANKA_010830	pLIS0065	g0739	aaaggtaccTTTATTATTTGAGTGTTG	*Asp718I*
			g0740c	aaaaagcttTTTATATTGATTTTGATTG	*HindIII*
			g0741	aaagaattcTTGAGTTTATAATATGTC	*EcoRI*
			g0742c	aaagcggccgcATATCCTAAAAACTATTG	*NotI*
GFP-tagging constructs					
dhhc2	PBANKA_010830	pLIS0086	g0646	aaagaattcTCGATATTCATATTTATTG	*EcoRI*
			g0644c	aaagcggccgcATATATTACTTCCATGTG	*NotI*
		pLIS0202	g0646	aaagaattcTCGATATTCATATTTATTG	*EcoRI*
			g0644c	aaagcggccgcATATATTACTTCCATGTG	*NotI*
			g0741	aaagaattcTTGAGTTTATAATATGTC	*EcoRI*
			g0742c	aaagcggccgcATATCCTAAAAACTATTG	*NotI*
Promoter swap construct					
dhhc2	**PBANKA_010830**	pLIS209	g3010	aaaactagtATGACACATAAATATATGCAGATAT	*SpeI*
			g0644c	aaagcggccgcATATATTACTTCCATGTG	*NotI*
clag	**PBANKA_140060**		g3024	aaacgatcgCTGATATTTATGAGTATTCC	*PvuI*
			g3025c	aaatctagaTATATTACGAATTGACACC	*XbaI*

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 aplicable; ORF: open reading frame; UTR: untranslated region.

Gene name/ mutant name	Gene ID	Primer name	Sequence
Primers for genotyping			
dhhc2::*gfp*	PBANKA_010830	g1258	ACACATAAATATATGCAG
		g1259c	ATGACATATTATAAACTC
dhhc2::*gfp*-3'UTR		3010	ATGACACATAAATATATGCAGATAT
		g0961c	GAGAATTAAGCCTATATATAC
		g1260	TGAGGAGAATATTATAAG
		g1263c	AATGCTATTGCATATTTG
dhhc2		g1258	ACACATAAATATATGCAG
		g1259c	ATGACATATTATAAACTC
		g1260	TGAGGAGAATATTATAAG
		g1263c	AATGCTATTGCATATTTG
Primers for RT-PCR			
dhhc2	PBANKA_010830	g1261	AATATATGCAGATATCAC
		g1262c	AAAAAAATATACTTGTGC
dhhc2::*gfp*		g1301	CATTGTATTTCAAATAGACC
		g1259c	ATGACATATTATAAACTC
		g1258	ACACATAAATATATGCAG
		g1301	CATTGTATTTCAAATAGACC
dhhc2::*gfp*-3'UTR		g1301	CATTGTATTTCAAATAGACC
		g1259c	ATGACATATTATAAACTC
General primers			
pbdhfr/*ts*	PBANKA_071930	g0952	GATTCATAAATAGTTGGACTTG
		P106	GTCCATACAACTATATCCGAAC
tgdhfr/*ts*	n.a.	g1019	ATGCATAAACCGGTGTGTC
		g1020c	AGCTTCTGTATTTCCGC
RNA polymerase II subunit RPB1	PBANKA_080700	g0084	aaagaattcTGATGGTTTACAATCACC
		g0085c	aaagcggccgctTTCTTCCTGCATCTCCTC
p28	PBANKA_051490	g0115	TTCGATATCATGAATTTTAAATACAG
		g0116c	tccgcggccgcGCATTACTATCACGTAAATAAC
gfp	n.a.	g0408c	GTATGTTGCATCACCTTC
ef1a	PBANKA_113330	P135	GTAAACTTAAGCATAAAGAGCTCG
human dhfr	n.a.	g3004	AAAAGATCTATGGTTGGTTCGCTAAACTG
human dhfr	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]
Unsussessful gene deletion attempts					
dhhc2	PBANKA_010830	pLIS0065	*Asp*718I and *Not*I	2095 2362, 2363	820cl1m1cl1 676m1cl1
GFP-tagged mutants					
dhhc2::*gfp*	PBANKA_010830	pLIS0086	*Fsp*AI	2185cl1m1	cl15cy1
dhhc2::*gfp*-3'utr	PBANKA_010830	pLIS0202	*BsmI*	202cl1m1	cl15cy1
Promoter swap					
clag::dhhc2::gfp::3'utr	PBANKA_010830	pLIS209	*KpnI, BglI*	209cl2	202cl1m1
clag::dhhc2	PBANKA_010830	pLIS209	*KpnI, BamHI*	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