SUPPLEMENTAL INFORMATION

Type II fatty acid biosynthesis is essential for *Plasmodium falciparum* sporozoite development in the midgut of *Anopheles* mosquitoes

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Table S1. Primers used in this study

Primers	Sequence (5'-3')	Restriction site[a]	Target[b]
P1	ATTAACCCTCACTAAAGGGA	N/A	T3
P2	GATAGCGATTTTTTTTACTGTCTG	N/A	*hrp*2-3'
P3	AGAAAGAAGAACAAGAT	N/A	*fabI*-F
P4	TTGATTTAAGTGGTCCTGCACTG	N/A	*fabI* -R
P5	GCGGCGGCCGCTAATGATTTGTTTTATTGCTGGTATAG	NotI	*fabI* -F[c]
P6	CGGGATCCTTAGGCTAAAACTCTGGTATCAG	BamHI	*fabI* -R[d]
P7	ATTGTGTATAATAAACATTTACGC	N/A	U *fabB/F*[e]
P8	CTCTAAATTATTATCGTCCCTTG	N/A	D *fabB/F*[e]
P9	CACTGCGCGCGTGTACACCTGCATGAGC	BssHII	*fabB/F* -F
P10	GATCGTACGCAGGAGCATTATTTTAAGGAACAACC	BsiWI	*fabB/F* -R
P11	ATGCCCGGGATTTGGAGGCCATAACACAGC	XmaI	*fabB/F* -F
P12	GATGCTAGCGAGTGTTATGTAAAAGGGATAATGATC	NheI	*fabB/F* -R

[a] Restriction sites are underlined. N/A, not applicable

[b] F= forward primer, R= reverse primer

[c] The NotI site is followed by a TAA stop codon and TG to change the frame of the disrupted downstream sequence

[d] The BamHI site is followed by a TAG stop codon and a G to change the frame of the disrupted downstream sequence

[e] Upstream (U) and Downstream (D) primers used for diagnostic long-range PCR

Fig. S1. Genetic deletion of *fabB/F* in *P. falciparum* NF54 parasites

(A) Schematic representation of the genetic deletion of *P. falciparum* (*Pf*) *fabB/F* by double crossover homologous recombination on chromosome 6. The targeting construct p-*PffabB/F* carries the resistance marker cassette human *dhfr* fused to *gfp* (*hdhfr-gfp*). This fusion is flanked by two FRT sites (white triangles), which serve as the recognition sites for the enhanced recombinase enzyme FLPe. The schematic shows the disrupted locus (*Pf*Δ*fabB/F*), as well as the locus from which the *dhfr-gfp* marker cassette was removed (*Pf*Δ*fabB/F* *FLPe), following transfection with the plasmid pMV-FLPe. p7, p8: primer pairs for long range PCR analysis. HindIII (H) was used for further characterization of the long range PCR products by restriction enzyme analysis; EcoRI (E) restriction sites were used for Southern blot analysis. *cam:* calmodulin; *hrp2:* histidine rich protein 2; *hsp86:* heatshock protein 86; *fcu:* cytosine deaminase/uracil phosphoribosyl-transferase; *pbdt: P. berghei dhfr-ts* 3' untranslated region. (B) Long range PCR analysis of genomic DNA from WT and *Pf*Δ*fabB/F* before and after pMV-FLPe transfection (*Pf*Δ*fabB/F* *FLPe), confirmed the integration and removal of the *hdhfr-gfp* marker, respectively. (C) Southern analysis of genomic DNA from WT and mutant asexual blood stage parasites confirmed double crossover-based deletion of the *fabB/F* gene and the subsequent excision of the *hdhfr-gfp* marker in the FLPe-transfected *PfΔfabB/F* parasites. DNA was digested with EcoRI and probed with the *fabB/F* 3' untranslated region (shown as D-*fabb/f).* The expected product sizes were 10.6 kb for the plasmid Δ*fab b/f*, 6.0 kb for the wild type NF54 locus; 1.4 kb for the *Pf*Δ*fabB/F* locus (containing *dhfr-gfp* in the place of the original *fab B/F* gene); and 4.3 kb for the *Pf*Δ*fabB/F* *FLPe locus (no longer carrying *dhfr-gfp*).

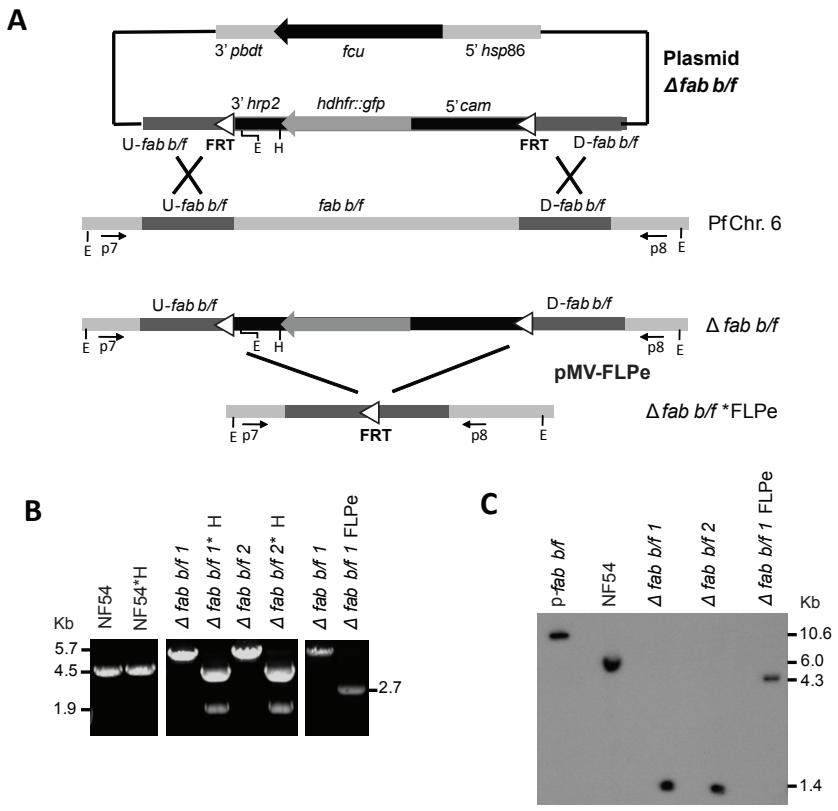

Fig. S2. Genetic disruption of *fabI* in *P. falciparum* NF54 parasites

(A) Representation of genetic disruption of *P. falciparum* (*Pf*) *fabI* by homologous recombination and single site crossover between the endogenous full-length 1.3 kb *PffabI* coding sequence and a truncated 0.6 kb *PffabI* fragment present on the transfection plasmid pcam-*bsd*-*Pf*Δ*fabI*. This resulted in an upstream truncated fragment lacking the 3' end of the coding sequence corresponding to the C-terminal 136 amino acids, followed by one plasmid copy, then a downstream truncated fragment lacking the *PffabI* promoter and 5' coding sequence corresponding to the N-terminal 98 amino acids. The schematic indicates the locations of the primers used to detect integration, as well as the HindIII (H) restriction sites and the *PffabI* probe (black bar) used for Southern blot analysis. (B) PCR results showing *PffabI* disruption via homologous recombination in the *Pf*Δ*fabI* B1 and C10 knockout clones. Lanes 1-4: *PfΔfabI* B1; Lanes 5-8: *Pf*Δ*fabI* C10; Lanes 9-12: wild type NF54. p1+p2 were specific for plasmid sequences and detected a 0.9 kb band in the knockout clones but not the wild type. p2+p3 produced a 1.1 kb band specific for the upstream truncated *PffabI* fragment only in the knockout clones. Similarly, p1+p4 produced a 0.7 kb band specific for the downstream-truncated *PffabI* fragment only in the knockout clones. p3+p4 detected a 0.8 kb band only in parasites harboring the endogenous full-length *PffabI* allele. The absence of p3+p4 bands in the knockout clones was consistent with complete genetic disruption of *PffabI* in these lines. Sizes of markers are indicated beside the gel. M, Gene Ruler (1 kb plus) marker. (C) Southern blot hybridization of HindIII-digested genomic DNA hybridized with a *PffabI* probe. Lanes are the same as for panel B. For the HindIII digests, the 8.4 kb and 2.1 kb bands in *Pf*Δ*fabI* B1 and *Pf*Δ*fabI* C10 were consistent with genetic disruption of the endogenous *PffabI* allele with a single copy insertion of the targeting plasmid. This contrasts with the unique 5.4 kb band in wild type NF54. Linearization of the plasmid with HindIII yielded a 5.1 kb band.