Supplementary information Tran et al.

"Human dihydrofolate reductase influences the sensitivity of the malaria parasite *Plasmodium falciparum* to ketotifen"

Figure S1

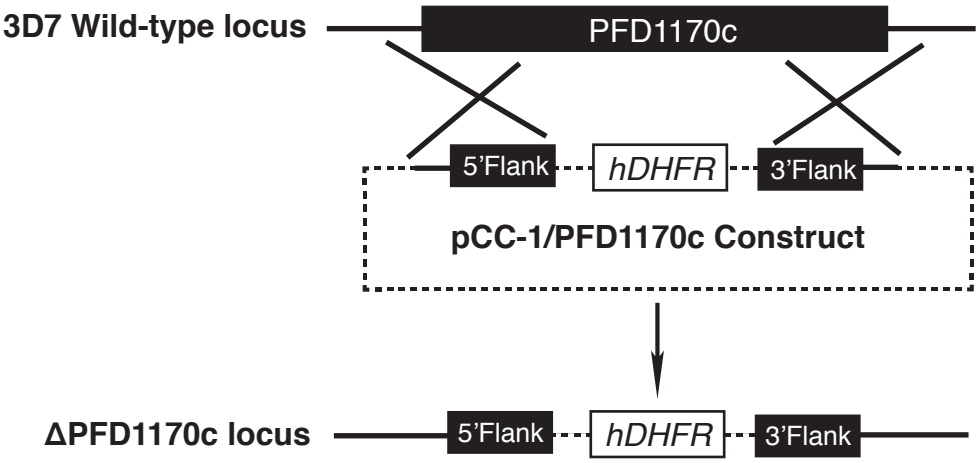
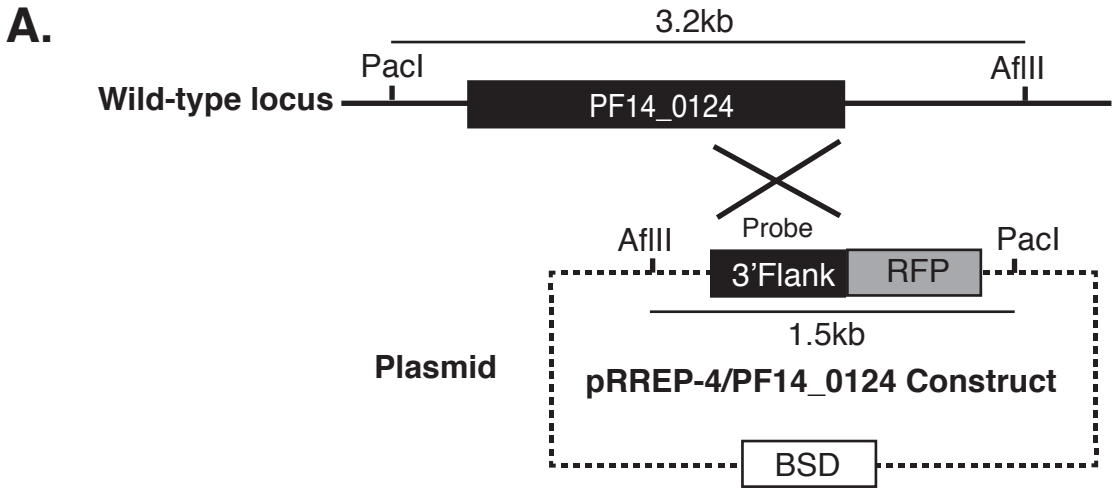


Figure S1. Schematic representation of the replacement vector used to disrupt PFD1170c and the genomic loci before and after insertion of the vector. The vector contains the *hDHFR* selection cassette flanked by *PFD1170c* targeting sequences for replacement. Black box, *PFD1170c* gene sequence; open box, *hDHFR* selection cassette.

Figure S2



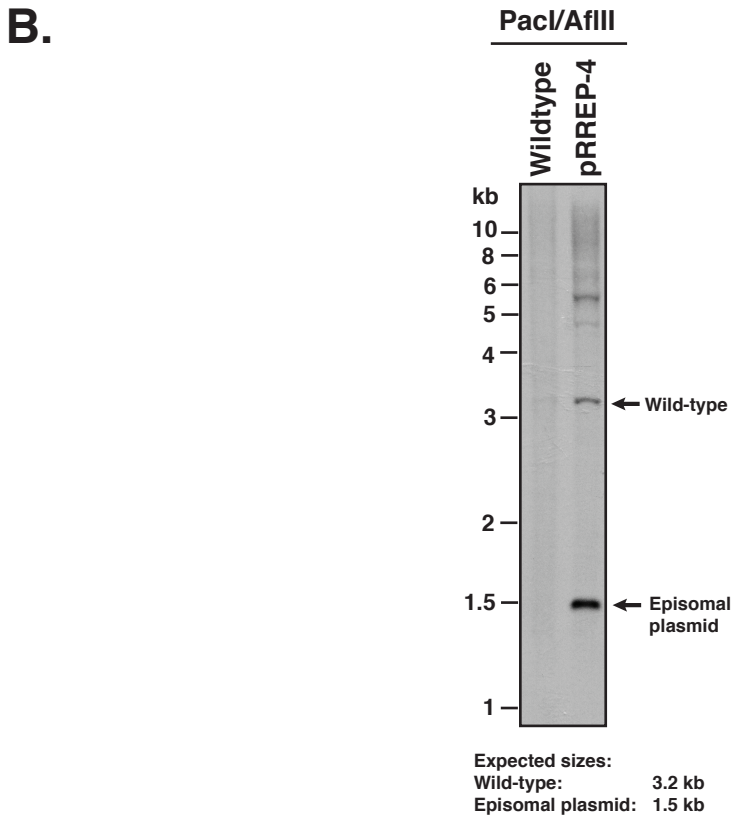


Figure S2. Generation and analysis of transgenic *P. falciparum* 3D7 expressing BSD.
(A) Schematic representation of the plasmid used to generate the BSD expressing parasites. The plasmid used contains the RFP gene following the 3' coding region of PF14_0124 gene and BSD selection cassette. Black box, PF14_0124 coding region; open box, BSD selection cassette; grey box, RFP. **(B)** Southern blot analysis of digested genomic DNA from wild-type and the transgenic parasites demonstrates the episomal presence of the plasmid construct.

Figure S3

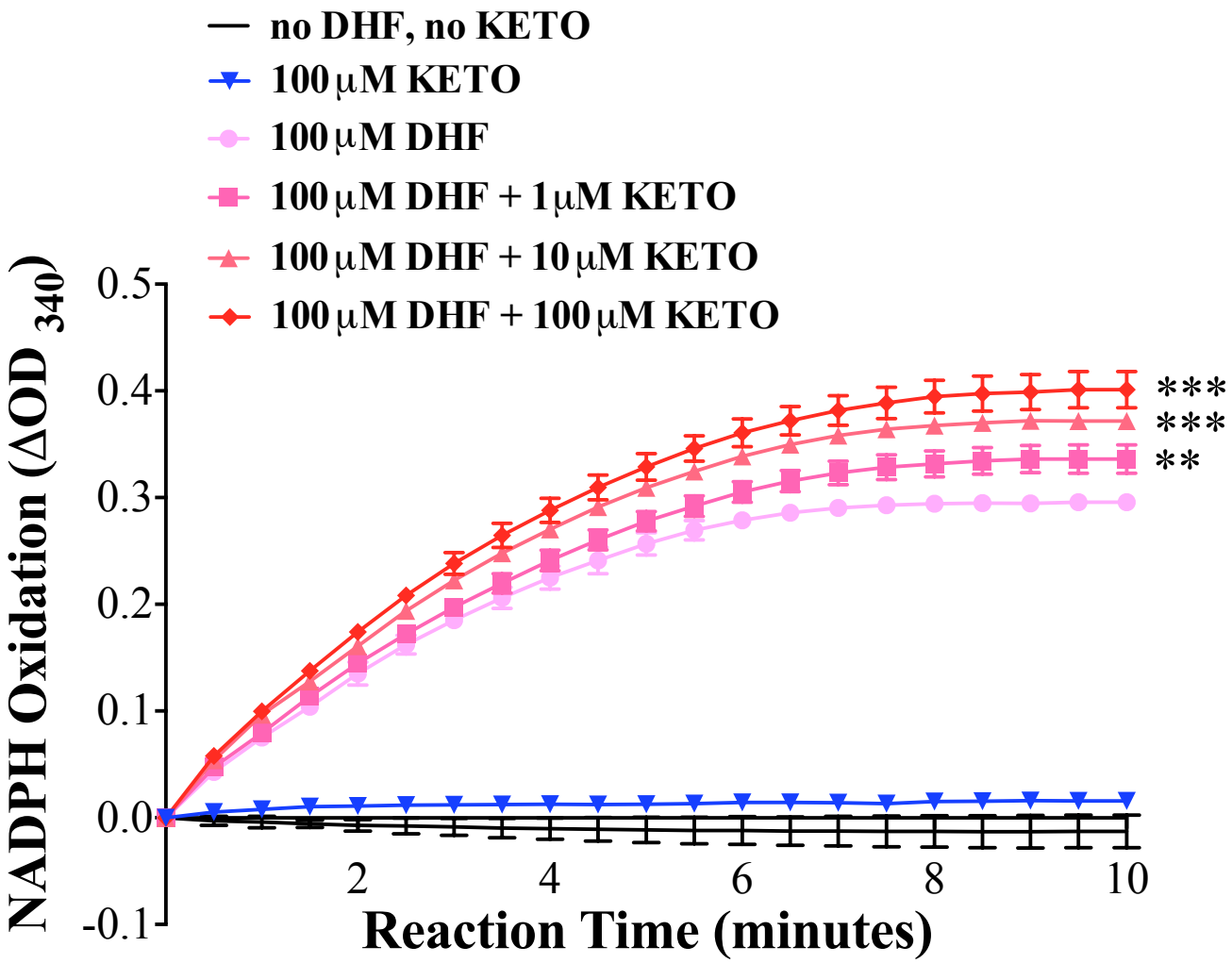


Figure S3. hDHFR activity increased with increasing amounts of ketotifen - Time course. Increasing amounts of ketotifen (KETO) led to increased reduction of the substrate DHF and increased oxidation of NADPH by recombinant hDHFR. Mean and standard deviation (SD) values of biological triplicate were plotted. *** ($p < 0.001$) and ** ($p < 0.01$) were shown in comparison to the 100 µM DHF condition at 10 min (Student's unpaired t-test).

Figure S4

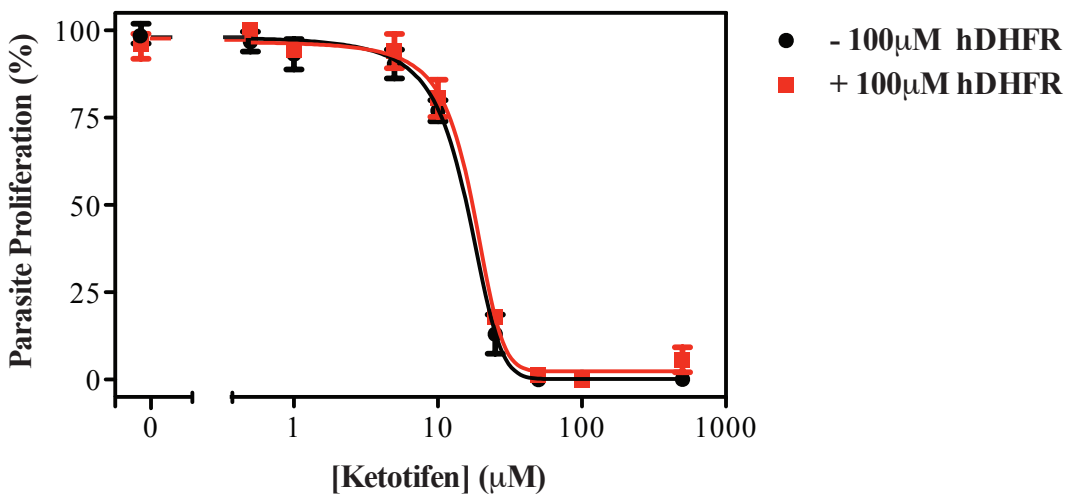


Figure S4. Extracellular hDHFR does not interfere with the inhibition of *P. falciparum* parasite proliferation by ketotifen. The proliferation of wild-type 3D7 asexual blood-stage parasites in the presence of a range of ketotifen concentrations was assessed over 72 h with or without the addition of 100 μM hDHFR to the culture medium. Mean and standard deviation values of three technical replicates are shown.

Table S1:

Cell line	Descriptor	gABCG2		hDHFR		BSD	
		integ.	episom.	integ.	episom.	integ.	episom.
Wild-type	3D7 wild-type	+	-	-	-	-	-
ΔPfgABCG2-hDHFR (i)	ΔPfgABCG2 knock-out	-	-	+	(-)	-	-
ΔPfgABCG2-hDHFR (i) /gPfABCG2-BSD (e)	gABCG2 knockout comple-mented	-	+	+	(-)	-	+
ΔPFD1170c-hDHFR (i)	PFD1170c knock-out	+	-	+	(-)	-	-
BSD (e)	PF14_0124-RFP-BSD episomal	+	-	-	-	-	+
hDHFR (e)	ΔPfgABCG2 episomal	+	-	-	+	-	-

Table S1: Cell lines used in this study. The presence or absence of the genes for gABCG2, hDHFR and BSD is indicated with "+" or "-", respectively. The location of these genes is specified as being either integrated (i) ("integ."; i.e. integrated in the chromosomal DNA) or episomal (e) ("episom."; i.e. located on an extra-chromosomal plasmid) "(-)" indicates, that some cells might harbour additional copies of free or integrated plasmid (see (Rug and Maier, 2013)).

Table S2:

Cell line		IC_{50} ± SD (µM)
Wild-type		2.8 ± 0.2
ΔPfgABCG2-hDHFR(i)		26.8 ± 1.5
ΔPFD1170c-hDHFR(i)		28.6 ± 0.7
ΔPfgABCG2(i)-PfgABCG2(e)		28.1 ± 0.4
BSD(e)		3.1 ± 0.2
hDHFR(e)		40.7 ± 9.1

Table S2: Half maximum inhibitory concentration (IC50) of ketotifen for *P. falciparum* cell lines used in this study. Selectable marker cassettes integrated into the genome are indicated by (i), selectable marker cassettes located on episomal plasmids are indicated by (e).