Supporting Information

CRISPR-Cas9-modified *pfmdr1* protects *Plasmodium falciparum* asexual blood stages and gametocytes against a class of piperazine-containing compounds but potentiates artemisinin-based combination therapy partner drugs

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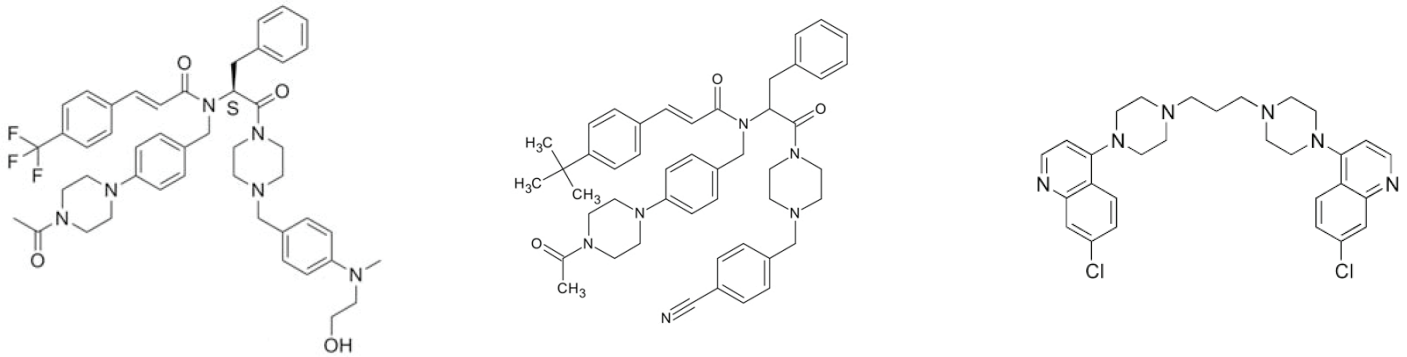


Fig. S1. Chemical structures of the piperazine-containing compounds ACT-213615, ACT-451840, and piperaquine. ACT-213615 (Brunner *et al.*, 2012) and ACT-451840 (Bruderer *et al.*, 2015) show structural similarity. A piperazine ring is a cyclohexane with two nitrogen atoms at opposite ends of the ring.

A

gRNA	Sequence	Sequence with binding site mutations	Distance (#nt) away from: nt 2523	nt 2772	Doensch score	Editing success
1	GCTTATAAAGACTCAGATACAGG	GCGTACAAGGATAGCGACACGGG	(+) 354	(+) 603	0.256	0 of 2
2	GATCAAGATAAAAATACCCCAGG	GACCAGGACAAGAACACTCCGGG	(-) 114	(+) 111	0.383	1 of 2
3	GATGTACATTTATTAAAAACGGG	GACGTGCACTTGTTGAAGACTGG	(-) 162	(+) 63	0.280	0 of 2
4	GTTAATACAGCTGCAACAATTGG	GTCAACACGGCCGCGACGATAGG	(-) 264	(-) 15	0.097	0 of 2
5	GCTTCCTGTATTAAAAAACTTGG	GCCTCTTGGATCAAGAACGAGGG	(-) 420	(-) 171	0.238	0 of 2
6	GCTATTGATTATAAAAATAAAGG	GCGATCGACTACAAGAACAAGGG	(-) 510	(-) 261	0.094	0 of 2
7	GGATCCTTCTTAATTAAAAGAGG	GGGAGTTTTTTGATCAAGAGGGG	(-) 621	(-) 372	0.779	0 of 2
8	GGAAAATTAATGTCCTTAAAAGG	GGCAAGTTGATGAGTTTGAAGGG	(-) 708	(-) 459	0.215	3 of 4

Initial G of $GN_{19}GG$ shown in red; protospacer adjacent motif shown in blue. Silent binding site substitutions shown in green. Substitutions in nt 2523 result in mutations in aa 841; substitutions in nt 2772 result in mutations in aa 921. (-) indicates mutation is upstream of gRNA; (+) indicates mutation is downstream of gRNA.

B

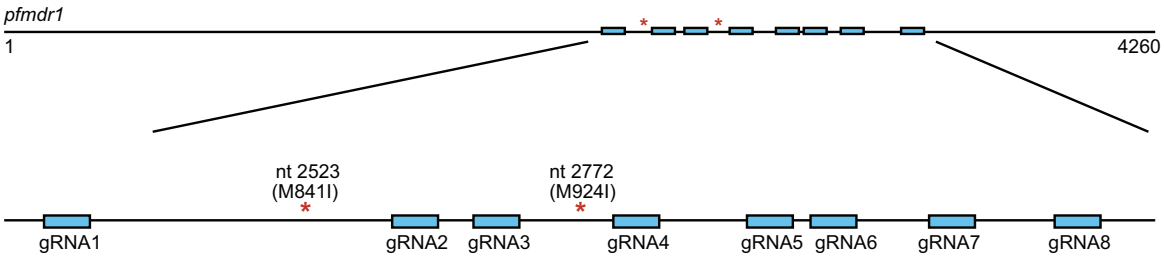


Fig. S2. Identities and positions of guide RNAs (gRNAs) directed to *pfmdr1*. (**A**) Tabulated information about *pfmdr1*-specific gRNAs tested in this study, including the distances of each gRNA from the targeted mutations at nucleotide positions 2523 and 2772, the gRNA Doensch scores (Doench *et al.*, 2014), and the editing success (proportion of transfections that resulted in successful *pfmdr1*-edited events). (**B**) Position of gRNA and mutations M841I and M924I within the *pfmdr1* gene.

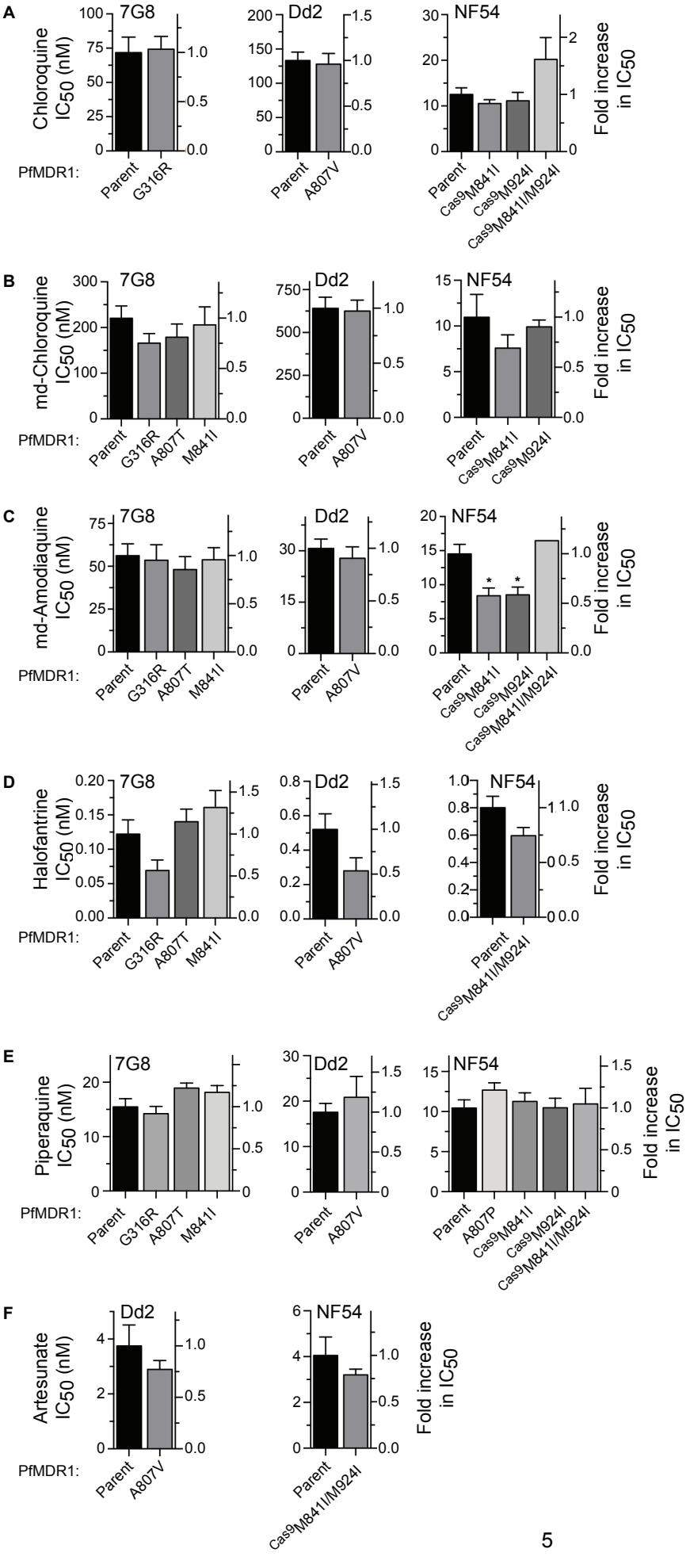
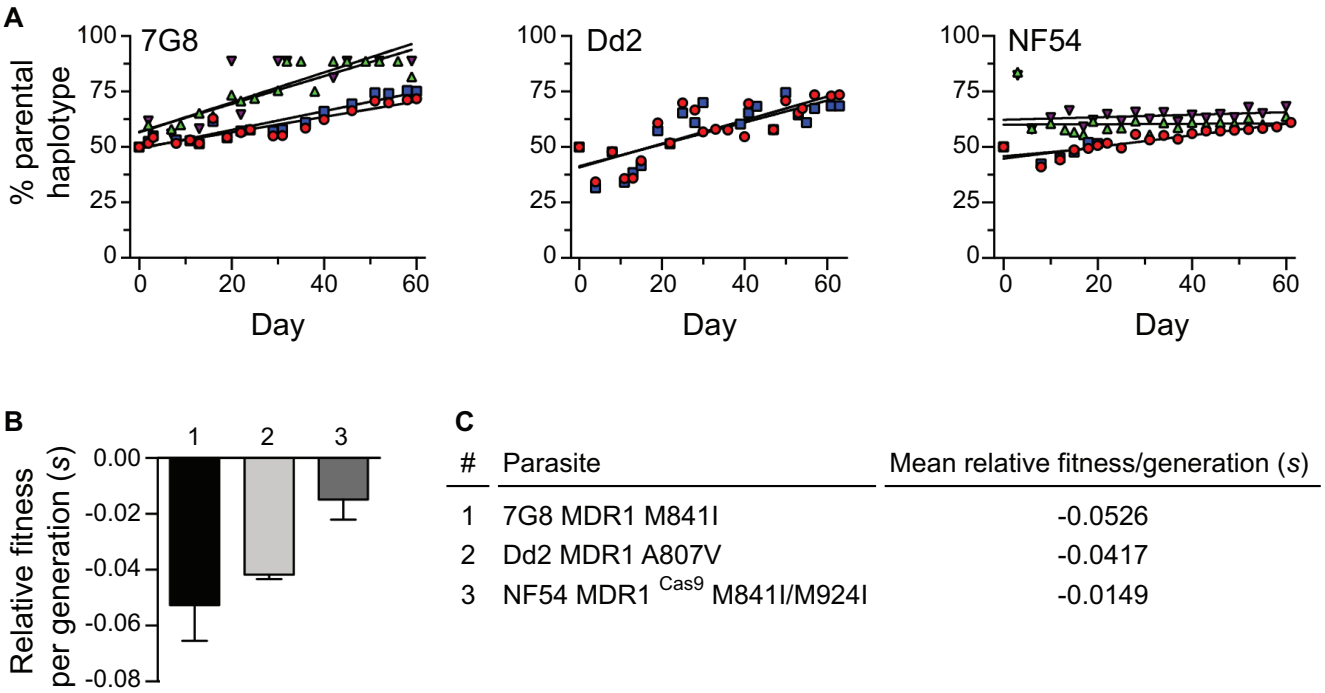


Fig. S3. Sensitivity of ACT-451840-resistant lines to antimalarial drugs. IC_{50} levels of parental and ACT-451840-resistant lines in 7G8, Dd2, and NF54 backgrounds to (**A**) chloroquine, (**B**) monodesethyl (md)-chloroquine, (**C**) md-amodiaquine, (**D**) halofantrine, (**E**) piperaquine, and (**F**) artesunate. Bar graphs display mean IC_{50} values ± S.E.M. Student's t-test was performed comparing resistant to parental lines. **P* < 0.05. Numerical data and numbers of independent repeats are listed in **Table S6**.



C

#	Parasite	Mean relative fitness/generation (*s*)
1	7G8 MDR1 M841I	-0.0526
2	Dd2 MDR1 A807V	-0.0417
3	NF54 MDR1 ^{Cas9} M841I/M924I	-0.0149

Fig. S4. Growth rates measured in parental and mutant PfMDR1 parasites. (**A**) The allelic frequency of co-cultured parental and mutant parasites was monitored over a 60-day period by pyrosequencing. The relative nucleotide proportions of parental vs. mutant alleles in 7G8, Dd2, and NF54 parasites are shown as the proportion of parental parasites in the culture. Each symbol indicates a separate experiment (n=4 for 7G8 and NF54, n=2 for Dd2). (**B**) Quantification of results from (**A**) expressed as the relative fitness per generation (*s*; (Hartl *et al.*, 1985; Gabryszewski *et al.*, 2016)) of the mutant parasites. Bar graphs display mean *s* values ± S.E.M. (**C**) Numerical depiction of (**B**).

Table S1. Frequency of recrudescence in ACT-451840-pressured *P. falciparum* lines.

Parasite	Selection pressure (nM)	Initial inoculum	Number of positive flasks (day positive)
7G8	1.7 (6 x IC_{50})	2 x 10^9	3 of 3 (20, 20, 21)
		2 x 10^8	2 of 3 (21, 61)
		2 x 10^7	2 of 3 (17, 45)
		2 x 10^6	0 of 3
Dd2	2.7 (4 x IC_{50})	2 x 10^9	3 of 3 (19, 19, 19)
		2 x 10^8	2 of 3 (25, 34)
		2 x 10^7	0 of 3
		2 x 10^6	1 of 6 (29)
		2 x 10^5	0 of 3
		2 x 10^4	0 of 3
NF54	2.7 (5 x IC_{50})	2 x 10^9	2 of 2 (18, 19)
		2 x 10^8	3 of 3 (19, 49, 50)
		2 x 10^7	2 of 3 (22, 24)
		2 x 10^6	0 of 3

Table S2. PfMDR1 variants identified in ACT-451480-selected lines.

Name	PfMDR1 position and amino acid																				ACT-451840 IC_{50} tested
	74	86	184	290	297	316	321	326	379	806	807	841	921	924	1034	1042	1066	1069	1076	1088	1246	
Canonical WT	F	N	Y	Y	G	G	I	V	G	F	A	M	R	M	S	N	S	T	Y	E	D	-
7G8 MDR1 parent	F	N	**F**	Y	G	G	I	V	G	F	A	M	R	M	**C**	**D**	S	T	Y	E	**Y**	Yes
7G8 MDR1 G316R	F	N	**F**	Y	G	**R**	I	V	G	F	A	M	R	M	**C**	**D**	S	T	Y	E	**Y**	Yes
7G8 MDR1 A807T	F	N	**F**	Y	G	G	I	V	G	F	**T**	M	R	M	**C**	**D**	S	T	Y	E	**Y**	Yes
7G8 MDR1 M841I	F	N	**F**	Y	G	G	I	V	G	F	A	**I**	R	M	**C**	**D**	S	T	Y	E	**Y**	Yes
Dd2 MDR1 parent	F	**Y**	Y	Y	G	G	I	V	G	F	A	M	R	M	S	N	S	T	Y	E	D	Yes
Dd2 MDR1 A807V	F	**Y**	Y	Y	G	G	I	V	G	F	**V**	M	R	M	S	N	S	T	Y	E	D	Yes
Dd2 MDR1 M841I	F	**Y**	Y	Y	G	G	I	V	G	F	A	**I**	R	M	S	N	S	T	Y	E	D	Yes
Dd2 MDR1 Y1076F	F	**Y**	Y	Y	G	G	I	V	G	F	A	M	R	M	S	N	S	T	**F**	E	D	Yes
NF54 MDR1 parent	F	N	Y	Y	G	G	I	V	G	F	A	M	R	M	S	N	S	T	Y	E	D	Yes
NF54 MDR1 F74S	**S**	N	Y	Y	G	G	I	V	G	F	A	M	R	M	S	N	S	T	Y	E	D	No
NF54 MDR1 Y290F	F	N	Y	**F**	G	G	I	V	G	F	A	M	R	M	S	N	S	T	Y	E	D	Yes
NF54 MDR1 G297V	F	N	Y	Y	**V**	G	I	V	G	F	A	M	R	M	S	N	S	T	Y	E	D	Yes
NF54 MDR1 I321F	F	N	Y	Y	G	G	**F**	V	G	F	A	M	R	M	S	N	S	T	Y	E	D	Yes
NF54 MDR1 V326E	F	N	Y	Y	G	G	I	**E**	G	F	A	M	R	M	S	N	S	T	Y	E	D	No
NF54 MDR1 G379D	F	N	Y	Y	G	G	I	V	**D**	F	A	M	R	M	S	N	S	T	Y	E	D	No
NF54 MDR1 F806L	F	N	Y	Y	G	G	I	V	G	**L**	A	M	R	M	S	N	S	T	Y	E	D	No
NF54 MDR1 A807P	F	N	Y	Y	G	G	I	V	G	F	**P**	M	R	M	S	N	S	T	Y	E	D	Yes
NF54 MDR1 A807V	F	N	Y	Y	G	G	I	V	G	F	**V**	M	R	M	S	N	S	T	Y	E	D	Yes
NF54 MDR1 M841I+M924I	F	N	Y	Y	G	G	I	V	G	F	A	**I**	R	**I**	S	N	S	T	Y	E	D	Yes
NF54 MDR1 R921G	F	N	Y	Y	G	G	I	V	G	F	A	M	**G**	M	S	N	S	T	Y	E	D	No
NF54 MDR1 S1066P	F	N	Y	Y	G	G	I	V	G	F	A	M	R	M	S	N	**P**	T	Y	E	D	No
NF54 MDR1 T1069I	F	N	Y	Y	G	G	I	V	G	F	A	M	R	M	S	N	S	**I**	Y	E	D	No
NF54 MDR1 Y1076H	F	N	Y	Y	G	G	I	V	G	F	A	M	R	M	S	N	S	T	**H**	E	D	Yes
NF54 MDR1 E1088K	F	N	Y	Y	G	G	I	V	G	F	A	M	R	M	S	N	S	T	Y	**K**	D	No

Blue letters indicate residues that differ from 3D7, the canonical wild-type *P. falciparum* parasite. Red letters indicate residues that differ from parental line.

Table S3. Whole-genome sequencing.

Parasite line	7G8 cl.1	7G8 Act3-1cl.1	7G8 Act3-2 cl.2	7G8 Act3-5 cl.1	7G8 Act3-6 cl.2
Name	7G8 MDR1 Parent	7G8 MDR1 M841I	7G8 MDR1 A807T	7G8 MDR1 A807T	7G8 MDR1 G316R
Genome coverage (x)	*21.47*	38.61	47.85	22.95	17.27
% covered by 5 or more reads	*76.6*	82.5	95.2	75.9	74.5
SNPs identified					
raw[a]	*44914*	54149	62616	46008	41596
quality[b]	*4660*	5081	6220	3207	1933
unique prior to IGV[c]		916	2196	270	154
unique post IGV[c]		5	5	3	4
intergenic		1	2	1	1
intronic		1	2	0	1
synonymous		0	0	0	0
total nonsynonymous		3	1	2	2
genes mutated in all samples (SNP mutation)		Pf3D7_0523000: MDR1 (M481I)	Pf3D7_0523000: MDR1 (A807T)	Pf3D7_0523000: MDR1 (A807T)	Pf3D7_0523000: MDR1 (G316R)

[a]After alignment to P. falciparum 3D7 reference genome. [b]Quality filters based on parameters defined in Methods. [c]Compared to 7G8cl1 parent.

Table S4. ACT-451840 resistance in 7G8, Dd2, and NF54 parental and selected lines.

Parasite	Mean IC_{50} values ± SEM (nM)	Number of assays	p-value	Clones	IC_{50} fold change
7G8 MDR1 parent	0.3 ± 0.02	10	-	Yes	1
7G8 MDR1 G316R	9.4 ± 1.4	15	< 0.0001	Yes	31
7G8 MDR1 A807T	8.0 ± 0.6	18	< 0.0001	Yes	27
7G8 MDR1 M841I	9.6 ± 0.8	16	< 0.0001	Yes	32
Dd2 MDR1 parent	0.7 ± 0.08	13	-	Yes	1
Dd2 MDR1 A807V	8.4 ± 0.5	20	< 0.0001	Yes	12
Dd2 MDR1 M841I	8.6 ± 1.2	3	< 0.0001	Yes	12
Dd2 MDR1 Y1076F	10.6 ± 2.1	5	< 0.0001	Yes	15
NF54 MDR1 parent	0.6 ± 0.04	14	-	Yes	1
NF54 MDR1 Y290F	22.7 ± 9.1	2	< 0.0001	Yes	38
NF54 MDR1 G297V	50.7 ± 5.0	4	< 0.0001	No	85
NF54 MDR1 I322F	6.7 ± 0.3	3	< 0.0001	No	11
NF54 MDR1 A807P	65.0 ± 1.7	3	< 0.0001	Yes	108
NF54 MDR1 A807V	20.2 ± 1.4	4	< 0.0001	No	34
NF54 MDR1 ^{Cas9}M841I	7.7 ± 1.6	2	< 0.0001	No	13
NF54 MDR1 ^{Cas9}M924I	1.8 ± 0.3	3	< 0.0001	Yes	3
NF54 MDR1 M841I/M924I	45.4 ± 5.9	4	0.0005	Yes	76
NF54 MDR1 ^{Cas9}M841I/M924I	38.5 ± 4.2	4	< 0.0001	Yes	64
NF54 MDR1 Y1076H	57.1 ± 4.1	8	< 0.0001	No	95

7G8 carries PfMDR1 mutations Y184F, S1034C, N1042D, and D1246Y; Dd2 carries PfMDR1 mutation N86Y; NF54 has no mutations in PfMDR1 compared to the standard reference strain 3D7. 7G8 MDR1 G316R, A807T, M841I, and Dd2 A807V were found in multiple clones, and each clone was profiled at least three times. For simplicity, parasites with the same genotype at PfMDR1 were grouped together. Parental lines were included in each assay of mutant lines. IC_{50} values were calculated from 72 h dose-response data measured by flow cytometry of parasites stained with SYBR Green and Mitotracker Deep Red. Values indicate mean ± SEM, shown in nM. Significance determined by Student's t-test.

Table S5. CRISPR-Cas9 engineered lines.

Name	PfMDR1 amino acid 841	PfMDR1 amino acid 924	Cas9	Selection	Binding site silent mutations	Day positive	Cloned
NF54 MDR1 parent	M	M	N/A	N/A	N/A	N/A	Yes
NF54 MDR1 ^{Cas9} M841I	I	M	Yes	ACT-451840	Yes	22	No
NF54 MDR1 ^{Cas9} M924I	M	I	Yes	WR/BSD 6 days	Yes	21	Yes
NF54 MDR1 ^{Cas9} M841I/M924I	I	I	Yes	ACT-451840	Yes	18, 17	Yes

Red letters indicate residues that differ from parental line. N/A Not applicable. Selection: 2.7 nM ACT-451840; 2.5 nM WR99210; 2μg ml^{-1} blasticidin.

Table S6. Cross-resistance to licensed antimalarials in asexual parasites.

Parasite	ACT-213615				Halofantrine			
	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value
7G8 MDR1 parent	1.6 ± 0.3	1	3	-	0.12 ± 0.02	1	6	-
7G8 MDR1 G316R	9.1 ± 1.0	6	3	<0.01	0.07 ± 0.02	0.6	4	0.10
7G8 MDR1 A807T	N.D.	-	-	-	0.14 ± 0.02	1.2	6	0.53
7G8 MDR1 M841I	N.D.	-	-	-	0.16 ± 0.02	1.3	3	0.29
Dd2 MDR1 parent	1.2 ± 0.2	1	3	-	0.52 ± 0.09	1	5	-
Dd2 MDR1 A807V	25.2 ± 1.6	21	3	0.0001	0.28 ± 0.08	0.5	3	0.12
NF54 MDR1 parent	0.9 ± 0.1	1	8	-	0.8 ± 0.08	1	7	-
NF54 MDR1 A807P	33.5 ± 3.5	37	3	<0.0001	N.D.	-	-	-
NF54 MDR1 ^{Cas9} M841I	16.5 ± 4.4	18	2	<0.0001	N.D.	-	-	-
NF54 MDR1 ^{Cas9} M924I	7.1 ± 0.8	8	3	<0.0001	N.D.	-	-	-
NF54 MDR1 ^{Cas9} M841I/M924I	43.2 ± 7.8	6	4	<0.0001	0.6 ± 0.06	0.8	7	0.07

Parasite	Mefloquine				Lumefantrine			
	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value
7G8 MDR1 parent	1.6 ± 0.3	1	8	-	0.6 ± 0.08	1	7	-
7G8 MDR1 G316R	0.5 ± 0.1	0.3	5	0.02	0.3 ± 0.06	0.5	5	0.01
7G8 MDR1 A807T	0.9 ± 0.1	0.6	6	0.08	0.6 ± 0.04	1.0	6	0.80
7G8 MDR1 M841I	0.8 ± 0.2	0.5	3	0.16	0.6 ± 0.1	1.0	3	0.83
Dd2 MDR1 parent	12.0 ± 1.8	-	7	-	2.9 ± 0.5	1	6	-
Dd2 MDR1 A807V	7.6 ± 1.7	0.6	7	0.09	1.7 ± 0.3	0.6	6	<0.05
NF54 MDR1 parent	9.0 ± 1.0	-	10	-	2.5 ± 0.3	1	13	-
NF54 MDR1 A807P	7.2 ± 1.3	0.8	6	0.30	2.1 ± 0.2	0.8	8	0.27
NF54 MDR1 ^{Cas9} M841I	7.6 ± 1.7	0.8	6	0.46	1.8 ± 0.2	0.7	6	0.13
NF54 MDR1 ^{Cas9} M924I	9.9 ± 0.8	1.1	7	0.52	3.0 ± 0.4	1.2	7	0.33
NF54 MDR1 ^{Cas9} M841I/M924I	7.6 ± 0.8	0.8	10	0.29	1.3 ± 0.1	0.5	3	0.06

Parasite	Quinine				Artesunate			
	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value
7G8 MDR1 parent	48.0 ± 8.6	1	4	-	N.D.	-	-	-
7G8 MDR1 G316R	20.0 ± 2.4	0.4	4	0.02	N.D.	-	-	-
7G8 MDR1 A807T	40.8 ± 3.4	0.9	6	0.40	N.D.	-	-	-
7G8 MDR1 M841I	41.7 ± 1.7	0.9	3	0.57	N.D.	-	-	-
Dd2 MDR1 parent	115.3 ± 13.8	1	6	-	3.7 ± 0.8	1	2	-
Dd2 MDR1 A807V	54.4 ± 9.1	0.5	6	0.004	2.9 ± 0.3	0.8	2	0.41
NF54 MDR1 parent	19.5 ± 2.8	1	10	-	4.1 ± 0.8	1	3	-
NF54 MDR1 A807P	N.D.	-	-	-	N.D.	-	-	-
NF54 MDR1 ^{Cas9} M841I	14.0 ± 2.0	0.7	4	0.26	N.D.	-	-	-
NF54 MDR1 ^{Cas9} M924I	16.5 ± 3.3	0.8	4	0.56	N.D.	-	-	-
NF54 MDR1 ^{Cas9} M841I/M924I	18.8 ± 3.3	1	6	0.88	3.2 ± 0.3	0.8	3	0.37

Table S6 (continued). Cross-resistance to licensed antimalarials in asexual parasites.

Parasite	Chloroquine				Monodesethyl chloroquine			
	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value
7G8 MDR1 parent	71.9 ± 11.2	1	5	-	220.6 ± 26.7	1	7	-
7G8 MDR1 G316R	74.3 ± 9.3	1.0	5	0.87	166.1 ± 20.7	0.8	7	0.13
7G8 MDR1 A807T	N.D.	-	-	-	179.2 ± 28.5	0.8	6	0.31
7G8 MDR1 M841I	N.D.	-	-	-	206.0 ± 39.5	0.9	3	0.77
Dd2 MDR1 parent	133.2 ± 12.3	1	3	-	641.7 ± 64.2	1	3	-
Dd2 MDR1 A807V	127.8 ± 15.7	1.0	3	0.80	625.1 ± 63.3	1.0	3	0.86
NF54 MDR1 parent	12.5 ± 1.4	1	8	-	11.0 ± 2.5	1	4	-
NF54 MDR1 A807P	N.D.	-	-	-	N.D.	-	-	-
NF54 MDR1 ^{Cas9} M841I	10.5 ± 0.9	0.8	4	0.38	7.6 ± 1.5	0.7	4	0.29
NF54 MDR1 ^{Cas9} M924I	11.1 ± 1.9	0.9	4	0.58	9.9 ± 0.7	0.9	4	0.70
NF54 MDR1 ^{Cas9} M841I/M924I	20.2 ± 4.8	1.6	4	0.07	N.D.	-	N.D.	-

Parasite	Monodesethyl amodiaquine				Piperaquine			
	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value
7G8 MDR1 parent	56.3 ± 7.0	1	7	-	15.5 ± 1.5	1	4	-
7G8 MDR1 G316R	53.6 ± 9.0	1.0	5	0.82	14.2 ± 1.3	0.9	5	0.55
7G8 MDR1 A807T	48.2 ± 7.5	0.9	6	0.45	18.9 ± 1.0	1.2	5	0.08
7G8 MDR1 M841I	53.8 ± 7.1	1.0	4	0.83	18.1 ± 1.3	1.2	5	0.22
Dd2 MDR1 parent	30.7 ± 2.7	1	6	-	17.6 ± 1.9	1	6	-
Dd2 MDR1 A807V	27.8 ± 3.3	0.9	6	0.52	20.9 ± 4.5	1.2	6	0.52
NF54 MDR1 parent	14.5 ± 1.4	1	6	-	10.5 ± 1.0	1	7	-
NF54 MDR1 A807P	N.D.	-	-	-	12.7 ± 0.9	1.2	4	0.19
NF54 MDR1 ^{Cas9} M841I	8.4 ± 1.1	0.6	4	0.01	11.3 ± 1.0	1.1	4	0.63
NF54 MDR1 ^{Cas9} M924I	8.5 ± 1.2	0.6	4	0.02	11.0 ± 2.0	1.0	3	0.80
NF54 MDR1 ^{Cas9} M841I/M924I	16.5 ± 0.01	1.1	3	0.37	10.5 ± 1.2	1.0	4	0.98

IC_{50} values were calculated from 72 h dose-response data measured by flow cytometry of parasites stained with SYBR Green and Mitotracker Deep Red. Values indicate mean ± SEM, shown in nM. Significance determined by Student's t-test against parental lines.

Table S7. Sensitivity to ACT-451840 in parental and resistant early and mature gametocytes.

Parasite	Stage II				Stage V			
	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value	IC_{50} (nM)	IC_{50} fold change	Number of assays	p-value
Dd2 MDR1 parent	1.8 ± 0.7	1	4	-	2.1 ± 0.3	1	2	-
Dd2 MDR1 A807V	208.9 ± 108.6	116	4	0.11	383.0 ± 175.4	182	2	0.16
NF54 MDR1 parent	1.8 ± 0.4	1	4	-	3.9 ± 1.0	-	2	-
NF54 MDR1 ^{Cas9} M841I/M924I	883.6 ± 382.1	491	4	0.06	973.6 ± 15.4	249.6	2	0.0003

Parasites are incubated with various concentrations of ACT-451840 for 48 h, then drug is washed out. 72 h later, cell viability was measured by a parasite lactate dehydrogenase assay. Significance determined by Student's t-test comparing mutant to parental line.

Table S8. List of primers used in this study.

Primer	Sequence	Lab identification	Assay
1	ATGGGTAAAGAGCAGAAAGAG	p3283	*pfmdr1* genotyping
2	TATCTGCATCATTACCTGTATC	p314	*pfmdr1* genotyping
3	TTCTAATTCTTGATGAAGCTACATC	p3341	*pfmdr1* genotyping
4	GCTCTAGCTATAGCTATTCTCTG	p4307	*pfmdr1* genotyping
5	TTCAAACCAATCTGGATCTGCA	p238	*pfmdr1* genotyping
6	ACGGACAAGAGTTGATACTGTTCAT	p395	*pfmdr1* genotyping
7	ATTTATGTTTGTGGTGTCATATG	p3289	*pfmdr1* genotyping
8	TTCTATAATGGACATGGTATTGT	p231	*pfmdr1* genotyping
9	TAAACTATTTGTTGCTTCTAAAGCTTTCAT	p285	*pfmdr1* genotyping
10	TAAATAGTGCAACGAATCAATACC	ALmdr1seqF2	*pfmdr1* genotyping
11	TCTCATAATTGCTCTTGCAATGG	p3298	*pfmdr1* genotyping
12	AATTTTCCAGCATAACTACCAGT	p290	*pfmdr1* genotyping
13	ATTATGGTTTAGAAGATTATTTCTGTAATTTGA	p425	*pfmdr1* genotyping
14	ATGATCACATTATATTAAAAAATGATATGACAA	p426	*pfmdr1* genotyping
15	TGATCAAAGAAACTCATTTCTTGA	p233	*pfmdr1* genotyping
16	GTATTTTAGTAGCTGGAGGATTATATCCC	7G8 FA5'	*pfmdr1* pyrosequencing - M841I detection
17	5'Biotin-AATATGTGCAGATAAAACACCTGGGG	7G8 FA3'	*pfmdr1* pyrosequencing - M841I detection
18	CTATATATATTCTACTTATTGCTATTGCTAT	7G8 pyro	*pfmdr1* pyrosequencing - M841I detection
19	5'Biotin- GACTCAGATACAGGTAATGATGCAG	p4063	*pfmdr1* pyrosequencing - A807V detection
20	TGAACATAGCAATAGCAATAAGTAG	p4064	*pfmdr1* pyrosequencing - A807V detection
21	GATACATATCTAGCATATAATAAA	p4065	*pfmdr1* pyrosequencing - A807V detection
22	TTATAACAACAAAATAGGAGAAAAAGTCG	PCR_FG2772AMDR	*pfmdr1* pyrosequencing - M921I detection
23	5'Biotin- CTCAATTTCTTTACTTTTGGTTAATCTAGC	PCR_RG2772AMDR	*pfmdr1* pyrosequencing - M921I detection
24	CATAATGCTCTTTCTGGTTAGCATGGTTAT	p4085	*pfmdr1* pyrosequencing - M921I detection

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