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Supplemental information

The *Plasmodium falciparum* ABC transporter ABCI3 confers parasite strain-dependent pleiotropic antimalarial drug resistance

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Supplementary Materials for

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Supplementary Figures and Tables

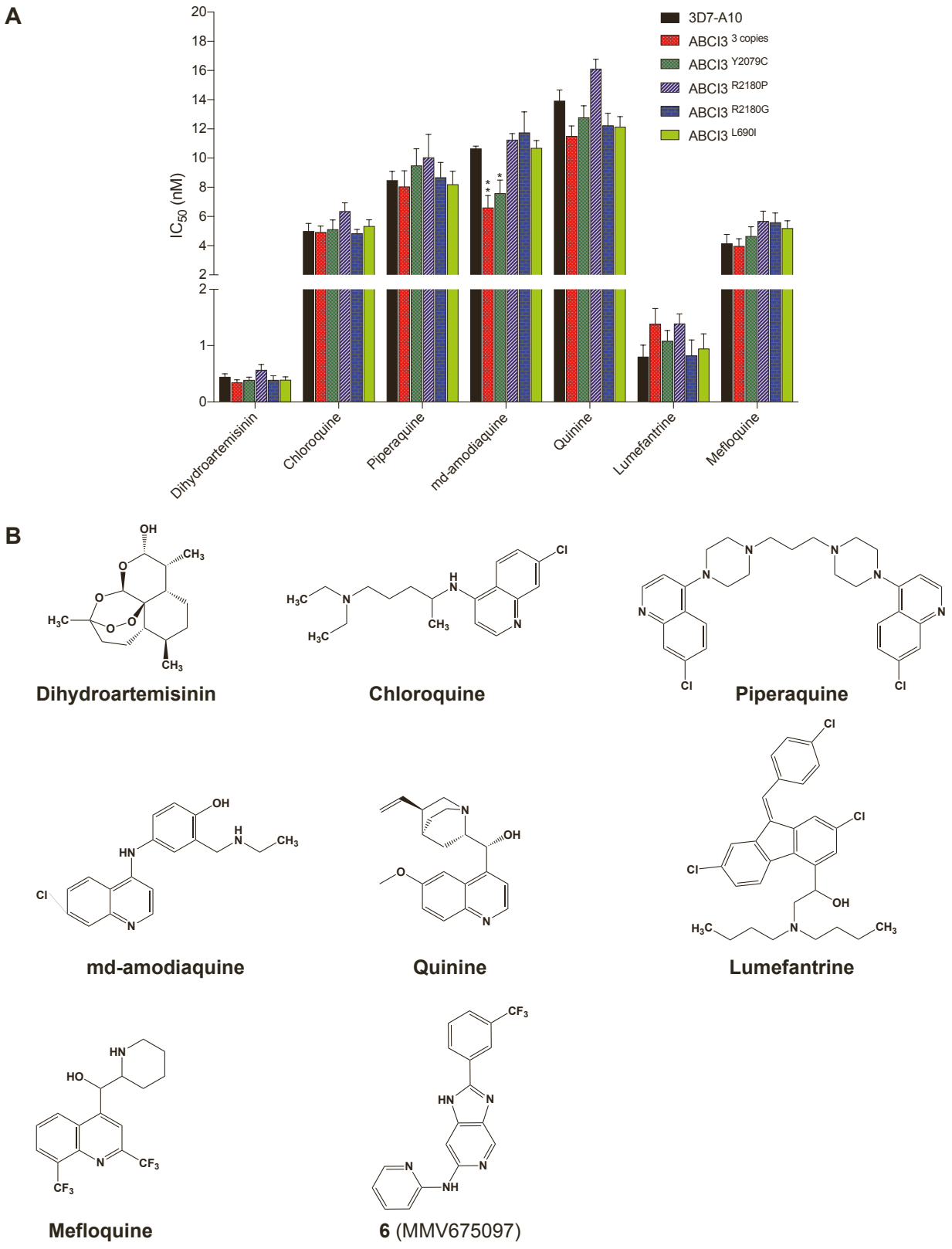

Figure S1 (related to Fig. 1, Table S1). Genetic modifications of ABCI3 do not confer cross resistance to first-line antimalarials. (**A**) Dose-response assays of ABCI3 CNV and SNP lines showed no cross-resistance against a panel of clinical antimalarials. Mean ± SEM; N≥4, n=2. Mann-Whitney *U* tests vs. 3D7-A10. (**B**) Chemical structure of first-line antimalarials and MMV compound **6**, which is identical to compound **1** apart from the absence of a –CF3 group in the pyridyl ring.

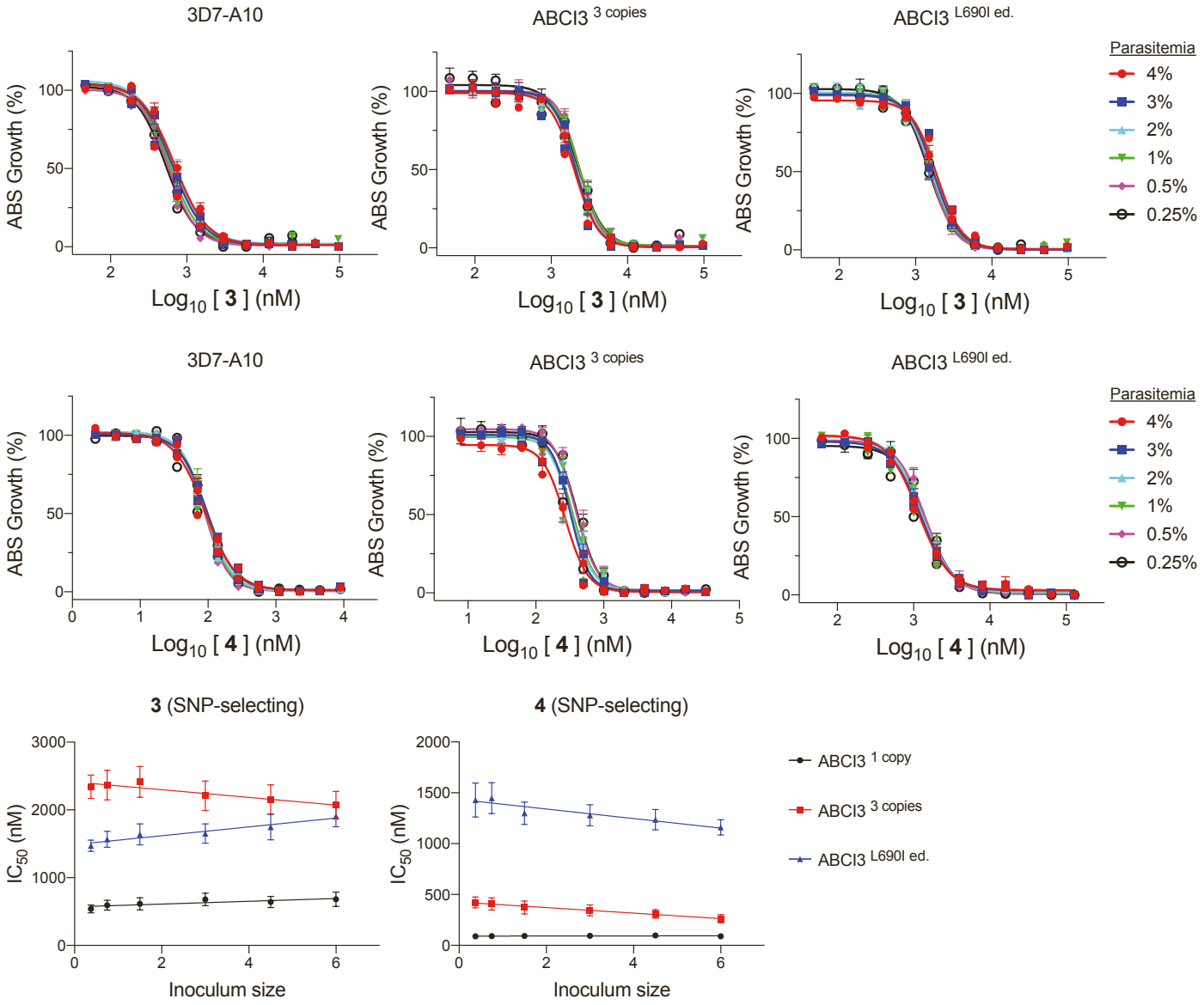

Figure S2 (related to Fig. 5). ABCI3 mutations and amplifications do not show parasitemia-dependent dose responses against SNP-selecting compounds. Compounds **3** and **4** had similar dose-response across all the three tested lines regardless of the starting parasite inoculum size. The absolute IC_{50} could therefore not be calculated from extrapolating the linear relationship between starting inoculum size and the measured IC_{50}. Mean ± SEM; N, n = 5, 2.

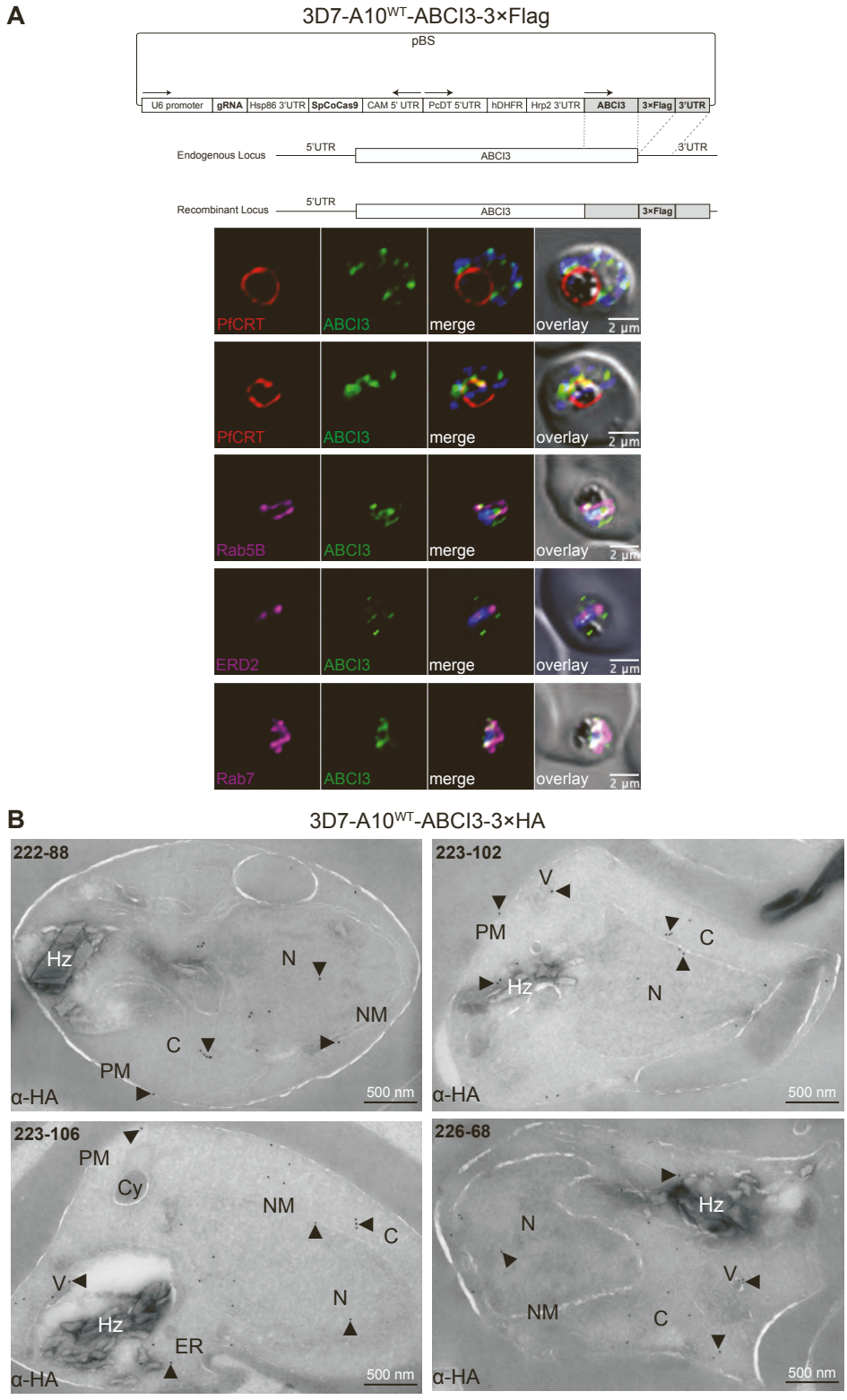

Figure S3 (related to Fig. 5D, E). ABCI3 foci localize to vesicles and various cellular organelles. (**A**) In the fluorescent image, ABCI3 Flag-tagged parasites were stained with anti-Flag (green), DAPI (nuclear, blue), anti-PfCRT (DV membrane, red), anti-ERD2 (cis-Golgi), or anti-Rab5B or anti-Rab7 (markers of vesicular transport) antibodies. The plasmid used to generate the tagged lines is illustrated. Scale bars: 2 µm. (**B**) Immuno-EM images of HA-tagged ABCI3 parasites stained with anti-HA antibodies. ER, endoplasmic reticulum; N, nucleus; NM, nuclear membrane; Hz, hemozoin crystals (digestive vacuole); V, vacuole; PM, plasma membrane and C, cytosol. Cy, cytostome. Scale bar: 500 nm.

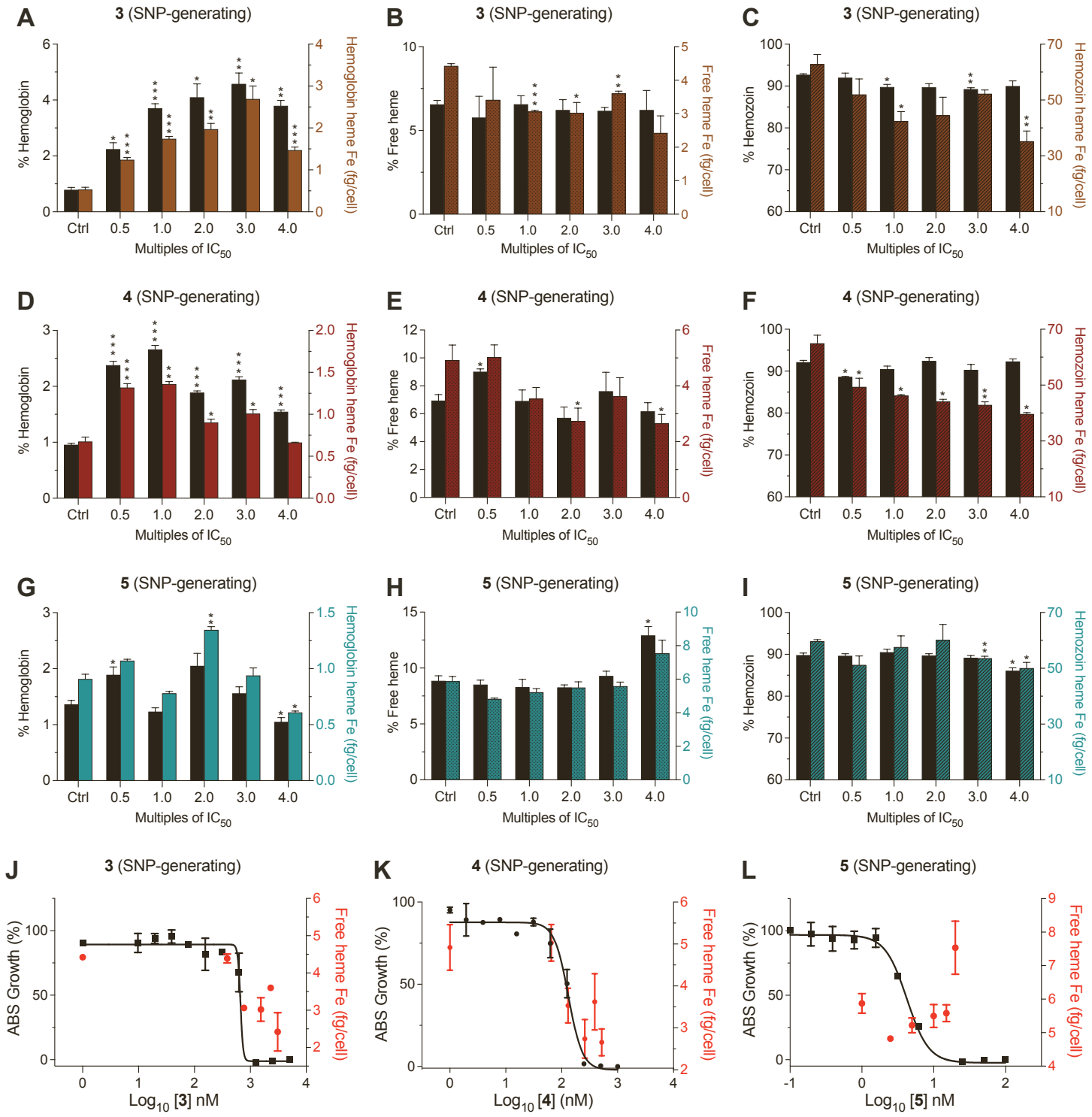

Figure S4 (related to Fig. 6). Parasites treated with compounds 3-5 do not display a heme fractionation profile similar to CQ. (**A-I**) Treatment of parasites with compounds **3-5** did not interfere with heme or Hz accumulation. (**J-L**) Concentration-dependent inhibition of parasite growth obtained with compounds **3-5** was independent of free heme levels. Statistical comparisons of the drug-treated lines to their untreated controls were performed using two-tailed Student's tests (with Welch's correction). N, n = 3, 2. *p<0.05, **p<0.01, ***p<0.001.

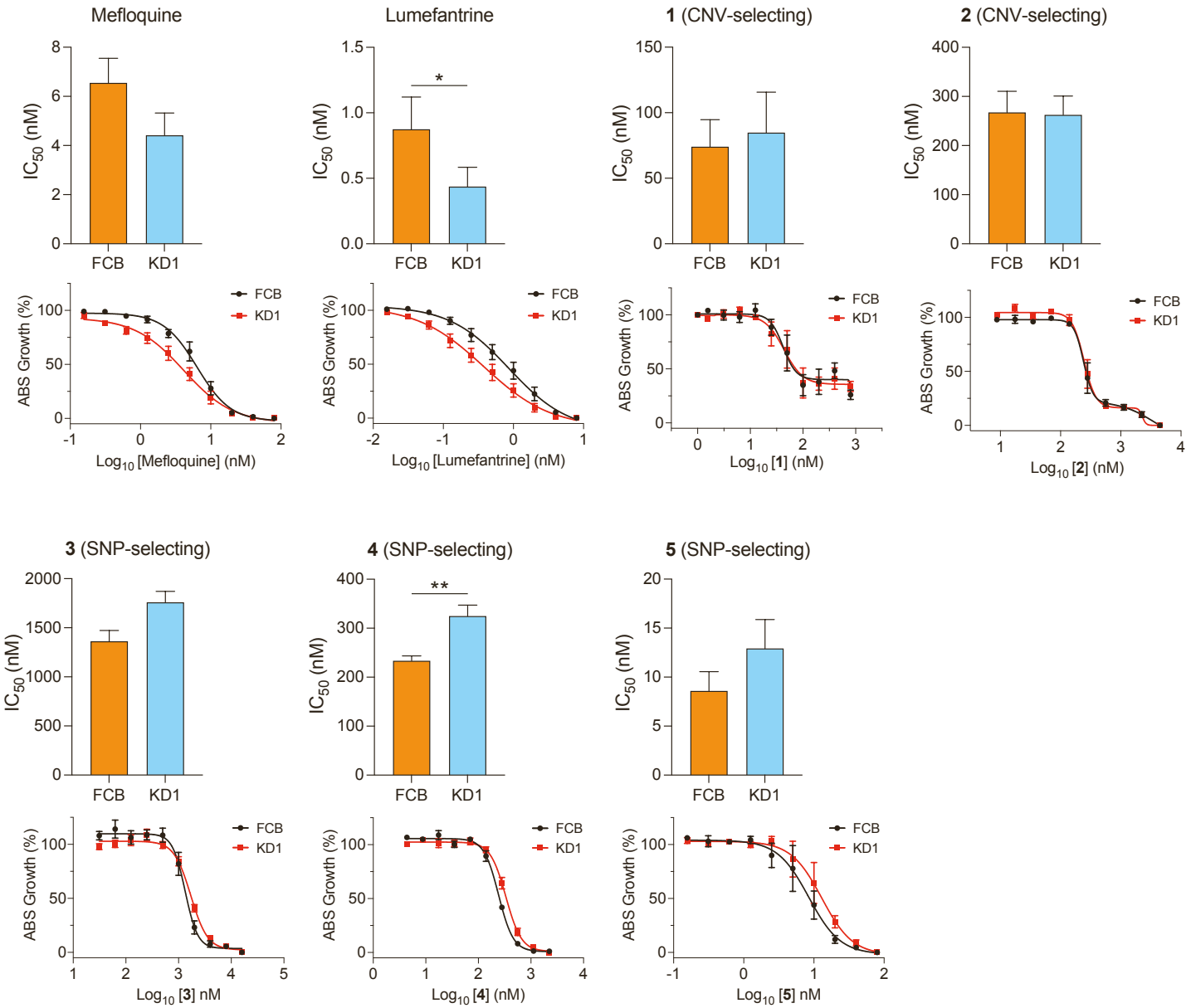

Figure S5 (related to Fig. 7, Table S7). PfMDR1 amplifications do not affect parasite susceptibility to ABCI3-associated compounds. Isogenic parasite lines expressing 1 (KD1) or 2 (FCB) copies of *pfmdr1* were equally susceptible to all five ABCI3-associated inhibitors. Mefloquine and lumefantrine were used as positive controls. *P* values were determined by comparison between the KD and parental FCB lines using Mann-Whitney *U* tests. N, n = 3-5, 2; *p<0.05, **p<0.01.

Table S1 (related to Fig. 3, S1). *Plasmodium falciparum* asexual blood stage IC_{50} data in nM for the tested antimalarials.

Antimalarials	3D7-A10			ABCI3 $^{3\ copies}$				ABCI3 ^{Y2079C}				ABCI3 ^{R2180P}				ABCI3 ^{R2180G}				ABCI3 ^{L690I}			
	Mean IC_{50}	SEM	N	Mean IC_{50}	SEM	N	*P* value	Mean IC_{50}	SEM	N	*P* value	Mean IC_{50}	SEM	N	*P* value	Mean IC_{50}	SEM	N	*P* value	Mean IC_{50}	SEM	N	*P* value
1	47.0	0.8	8	106 (1249)	8.0 (79.0)	15	<0.001 (<0.0001)	48.0	0.8	14	ns	58.0	2.0	14	<0.001	56.0	2.0	5	<0.01	45.0	1.0	7	ns
2	281	19.0	6	265 (4054)	34.0 (69.0)	4	ns (0.0095)	252	13.0	6	ns	275	12.0	6	ns	260	14.0	6	ns	208	11.0	6	<0.01
3	1012	64.0	11	2890	246	5	<0.001	2746	89.0	15	<0.0001	3029	141	15	<0.0001	2784	196	8	<0.0001	2511	225	8	<0.0001
4	140	14.0	10	500	47.0	15	<0.0001	1241	34.0	8	<0.0001	1918	61.0	7	<0.001	1268	55.0	4	<0.01	2300	217	5	<0.001
5*	2.0	0.1	10	25.0	0.9	11	<0.0001	8.0	0.7	6	<0.001	29.0	1.7	6	<0.001	21.0	1.0	6	<0.001	2.0	0.3	9	ns
6	16.0	2.0	11	32.0	3.0	15	<0.001	20.0	1.0	10	ns	25.0	1.0	10	<0.01	23.0	1.0	8	<0.05	17.0	0.7	8	ns
Dihydroartemisinin	0.4	0.1	6	0.3	0.0	6	ns	0.4	0.1	6	ns	0.6	0.1	6	ns	0.4	0.1	6	ns	0.4	0.1	6	ns
Chloroquine	5.0	0.5	4	4.9	0.4	5	ns	5.1	0.6	5	ns	6.4	0.6	6	ns	4.8	0.3	5	ns	5.3	0.4	5	ns
Piperaquine	8.5	0.6	5	8.0	1.1	5	ns	9.5	1.2	5	ns	10.0	1.6	5	ns	8.7	1.0	5	ns	8.2	0.9	5	ns
md-amodiaquine	10.6	0.2	6	6.6	0.8	6	<0.01	7.6	0.9	6	<0.05	11.3	0.4	5	ns	11.7	1.4	4	ns	10.7	0.5	5	ns
Quinine	13.9	0.7	6	11.5	0.7	4	ns	12.8	0.8	5	ns	16.1	0.7	6	ns	12.2	0.8	4	ns	12.1	0.7	4	ns
Lumefantrine	0.8	0.2	4	1.4	0.3	4	ns	1.1	0.2	4	ns	1.4	0.2	5	ns	0.8	0.3	5	ns	0.9	0.3	5	ns
Mefloquine	4.2	0.6	5	4.0	0.5	5	ns	4.6	0.6	6	ns	5.7	0.7	6	ns	5.6	0.7	5	ns	5.2	0.5	5	ns

SEM: standard error of the mean; N: number of biological repeats (with technical duplicates); () refers to the IC_{50} and SEM of the second shift of the biphasic curve. * Selections with compound **5** were run on a Dd2-B2 parental background (IC_{50} = 8 nM). *P* values were determined by comparison between the variant lines and parental 3D7-A10 using Mann-Whitney *U* tests. ns: not significant (p>0.05).

Table S2 (related to Fig. 3). *Plasmodium falciparum* asexual blood stage IC_{50} data in nM for the antiplasmodial compounds assayed against the Dd2-B2 parent as well as the selected and edited ABCI3 F689C and S696Y parasite lines.

	Dd2-B2			ABCI3 $^{F689C\ ed.}$				ABCI3 $^{S696Y\ ed.}$			
Antimalarials	Mean IC_{50}	SEM	N	Mean IC_{50}	SEM	N	*P* value	Mean IC_{50}	SEM	N	*P* value
1	27.0 (1404)	3.0 (343)	4	30.0	3.0	6	--	34.0	2.0	6	--
2	265 (3542)	14.0 (465)	5	257	26.0	7	--	279	26.0	7	--
3	1546	95.0	7	956	112	7	<0.01	>5 mM		4	<0.01
4	246	20.0	7	36.0	4.0	7	<0.001	>10 mM		4	<0.01
5*	8.0	0.9	6	94.0 (89.0)	10.0 (4.0)	6	<0.01	1626 (1433)	56.0 (24.0)	7	<0.01

SEM: standard error of the mean; N: number of biological repeats (with technical duplicates); For compounds **1** and **2**, the brackets denote the mean IC_{50} and SEM of the second shift of the biphasic curve. For compound **5** where selections were run on a Dd2-B2 parental background, the brackets denote the mean IC_{50} and SEM values of the selected clones. ABCI3 $^{F689C\ ed.}$/ ABCI3 $^{S696Y\ ed.}$: *P. falciparum* lines generated by introducing ABCI3 F689C and S696Y mutations into parental Dd2-B2 using CRISPR/Cas9. p values were determined by comparison between the variant lines and parental Dd2-B2 using Mann-Whitney *U* tests. -- not determined.

Table S3 (related to Fig. 2C). *Plasmodium falciparum* asexual blood stage IC_{50} data in nM for compounds 1, 3 and 4 against 3D7-A10 parent, drug-selected, and gene-edited L690I parasite lines.

	3D7-A10			ABCI3 ^{L690I}				ABCI3 $^{L690I\ ed.}$			
Compounds	Mean IC_{50}	SEM	N	Mean IC_{50}	SEM	N	*P* value	Mean IC_{50}	SEM	N	*P* value
1	23.0	2.6	6	16.0	2.4	4	ns	16.0	0.8	4	ns
3	1548	161	4	3616	299	4	<0.05	3327	436	4	<0.05
4	188	30.0	4	4168	286	4	<0.05	3805	585	4	<0.05

SEM: standard error of the mean; N: number of biological repeats (with technical duplicates). ABCI3 ^{L690I}: *P. falciparum* line generated from selections with compound **4**. ABCI3 $^{L690I\ ed.}$: *P. falciparum* line generated by introducing the ABCI3 L690I mutation into parental 3D7-A10 using CRISPR/Cas9. *P* values were determined by comparison between the variant lines and parental 3D7-A10 using Mann-Whitney *U* tests. ns: p>0.05.

Table S4 (related to Fig. 4). *Plasmodium falciparum* asexual blood stage IC_{50} data in nM for ABCI3-linked antiplasmodial inhibitors tested in the presence or absence of aTc in an ABCI3 conditional knockdown parasite line.

	50 nM aTc			3 nM aTc				0 nM aTc			
Antimalarials	Mean IC_{50}	SEM	N	Mean IC_{50}	SEM	N	*P* value	Mean IC_{50}	SEM	N	*P* value
Chloroquine	6.4	0.2	4	6.4	0.1	3	ns	6.0	0.4	4	ns
1	60.2	12.0	7	28.7	4.6	8	<0.05	19.4	3.4	7	<0.01
2	236	13.5	8	160	6.2	7	<0.001	128	8.7	8	<0.001
3	804	35.2	7	153	4.2	8	<0.001	74.9	9.6	7	<0.001
4	117	5.5	8	24.5	0.9	7	<0.001	14.1	0.3	8	<0.001
5	10.0	2.1	7	2.4	0.6	7	<0.01	1.4	0.4	8	<0.001
6	28.3	5.4	7	18.0	2.6	8	ns	12.9	2.7	7	<0.05

SEM: standard error of the mean; N: number of biological repeats (with technical duplicates). *P* values were determined by comparing the IC_{50} values of parasites cultured with 3 or 0 nM aTc with those cultured at 50 nM, using Mann-Whitney *U* tests. ns: p>0.05.

Table S5 (related to Fig. 5C). Cellular accumulation ratio of chloroquine and compound 1 in *Plasmodium falciparum* asexual blood stage parasites.

	3D7-A10			ABCI3 $^{3\ copies}$				ABCI3 $^{L690I\ ed.}$			
Antimalarials	Mean IC_{50}	SEM	N	Mean IC_{50}	SEM	N	*P* value	Mean IC_{50}	SEM	N	*P* value
Chloroquine	36553	2385	6	17658	414	6	<0.01	28916	729	6	<0.01
1	59915	2678	6	2082	426	6	<0.01	25744	1288	6	<0.01

SEM: standard error of the mean; N: number of biological repeats (with technical duplicates). *P* values were determined by comparing accumulation levels between the variant lines and parental 3D7-A10, using Mann-Whitney *U* tests.

Table S6 (related to Fig. 6, S4): Mean ± SEM amount of hemoglobin, free heme and hemozoin in drug-treated parasites represented percent proportion or absolute amount of heme iron per cell in fg/cell and the *in vitro* β-hematin inhibition assay IC_{50} data in µM for the tested antimalarials.

Compound	Drug concentration	% Heme species			Heme Fe (fg/cell)			β-hematin inhibition assay		
		Hemoglobin	Free heme	Hemozoin	Hemoglobin	Free heme	Hemozoin	Mean IC_{50}	SEM	N
Chloroquine	No drug control	1.08 ± 0.11	6.12 ± 0.34	92.79 ± 0.31	0.80 ± 0.09	4.49 ± 0.14	68.30 ± 1.79	20.0	1.2	3
	0.5×IC_{50} (6 nM)	0.65 ± 0.14	6.72 ± 0.40	92.62 ± 0.32	0.47 ± 0.10	4.78 ± 0.19	66.04 ± 2.95			
	1.0×IC_{50} (12 nM)	0.65 ± 0.08*	7.63 ± 0.42	91.71 ± 0.38	0.47 ± 0.05*	5.48 ± 0.24*	65.91 ± 2.22			
	2.0×IC_{50} (24 nM)	1.12 ± 0.02	11.09 ± 0.35***	87.79 ± 0.35**	0.70 ± 0.01	6.92 ± 0.14**	54.83 ± 1.45*			
	3.0×IC_{50} (36 nM)	1.82 ± 0.03*	10.23 ± 0.28***	87.95 ± 0.24***	1.20 ± 0.06*	6.78 ± 0.31**	58.30 ± 2.51*			
	4.0×IC_{50} (48 nM)	0.78 ± 0.10	12.23 ± 0.41***	86.99 ± 0.32***	0.46 ± 0.06*	7.17 ± 0.27**	51.05 ± 1.15**			
Pyrimethamine	No drug control	2.12 ± 0.11	5.85 ± 0.28	92.04 ± 0.48	1.43 ± 0.13	3.94 ± 0.18	62.11 ± 0.54	>500	>500	3
	0.5×IC_{50} (12.5 nM	2.56 ± 0.14	5.53 ± 0.20	91.91 ± 0.29	1.61 ± 0.12	3.51 ± 0.21	58.13 ± 1.54			
	1.0×IC_{50} (25 nM)	2.61 ± 0.08	5.51 ± 0.21	91.88 ± 0.29	1.64 ± 0.16	3.58 ± 0.19	59.77 ± 3.21			
	2.0×IC_{50} (50 nM)	2.48 ± 0.14	5.47 ± 0.13	92.05 ± 0.26	1.57 ± 0.09	3.47 ± 0.13	58.27 ± 1.26*			
	3.0×IC_{50} (75 nM)	3.15 ± 0.12**	5.76 ± 0.13	91.09 ± 0.11	1.89 ± 0.11*	3.46 ± 0.11	54.62 ± 1.02**			
	4.0×IC_{50} (100 nM)	2.76 ± 0.08	5.53 ± 0.22	91.71 ± 0.27	1.72 ± 0.14	3.40 ± 0.13	56.49 ± 1.56*			
1	No drug control	3.12 ± 0.36	4.76 ± 0.50	92.12 ± 0.72	1.80 ± 0.21	2.73 ± 0.29	53.09 ± 0.32	29.3	2.2	3
	0.5×IC_{50} (24 nM)	3.81 ± 0.67	4.98 ± 0.43	91.21 ± 0.81	1.87 ± 0.37	2.84 ± 0.33	51.65 ± 0.74			
	1.0×IC_{50} (48 nM)	3.73 ± 0.48	5.45 ± 0.21	90.82 ± 0.54	1.84 ± 0.07	3.02 ± 0.21	50.28 ± 3.01			
	2.0×IC_{50} (96 nM)	3.48 ± 0.46	8.24 ± 0.31**	88.28 ± 0.45*	1.98 ± 0.28	4.67 ± 0.11*	50.18 ± 1.81			
	3.0×IC_{50} (144 nM)	3.71 ± 0.52	10.41 ± 0.18**	85.87 ± 0.64**	1.97 ± 0.31	5.48 ± 0.18**	45.17 ± 0.76***			
	4.0×IC_{50} (192 nM)	4.97 ± 0.83	13.32 ± 0.29***	81.71 ± 0.62***	2.83 ± 0.33	6.40 ± 0.29***	39.28 ± 1.55**			
3	No drug control	0.78 ± 0.09	6.55 ± 0.25	92.67 ± 0.27	0.53 ± 0.05	4.42 ± 0.07	62.83 ± 3.52	>500	>500	3
	0.5×IC_{50} (385 nM)	2.24 ± 0.24*	5.77 ± 1.27	92.00 ± 1.13	1.24 ± 0.06***	3.41 ± 0.98	52.01 ± 5.63			
	1.0×IC_{50} (770 nM)	3.07 ± 0.17***	6.55 ± 0.52	89.75 ± 0.68*	1.74 ± 0.06***	3.06 ± 0.04***	42.47 ± 3.47*			
	2.0×IC_{50} (1540 nM	4.09 ± 0.48*	6.22 ± 0.62	89.69 ± 0.88	1.97 ± 0.14**	3.02 ± 0.32*	44.52 ± 6.56			
	3.0×IC_{50} (2310 nM	4.57 ± 0.40**	6.16 ± 0.21	89.27 ± 0.34**	2.69 ± 0.31*	3.60 ± 0.07**	52.27 ± 1.42			
	4.0×IC_{50} (3080 nM	3.79 ± 0.20**	6.21 ± 1.17	90.0 ± 1.24	1.47 ± 0.08***	2.42 ± 0.51	35.27 ± 3.72**			
4	No drug control	0.95 ± 0.03	6.94 ± 0.44	92.11 ± 0.46	0.67 ± 0.05	4.92 ± 0.54	64.91 ± 2.97	>500	>500	3
	0.5×IC_{50} (65 nM)	2.38 ± 0.07***	9.00 ± 0.22*	88.62 ± 0.14*	1.32 ± 0.05***	5.03 ± 0.44	49.34 ± 3.13*			
	1.0×IC_{50} (130 nM)	2.66 ± 0.07***	6.91 ± 0.80	90.43 ± 0.78	1.36 ± 0.03**	3.53 ± 0.41	46.21 ± 0.37*			
	2.0×IC_{50} (260 nM)	1.89 ± 0.03***	5.70 ± 0.79	92.42 ± 0.82	0.90 ± 0.04*	2.74 ± 0.46*	44.10 ± 0.82*			
	3.0×IC_{50} (390 nM)	2.12 ± 0.05***	7.60 ± 1.37	90.28 ± 1.35	1.01 ± 0.05*	3.62 ± 0.67	42.89 ± 1.14**			
	4.0×IC_{50} (520 nM)	1.54 ± 0.04***	6.16 ± 0.63	92.29 ± 0.61	0.66 ± 0.01	2.65 ± 0.32*	39.60 ± 0.59*			
5	No drug control	1.36 ± 0.07	8.84 ± 0.47	89.80 ± 0.50	0.91 ± 0.04	5.87 ± 0.29	59.68 ± 0.57	>500	>500	3
	0.5×IC_{50} (2.5 nM)	1.89 ± 0.14*	8.50 ± 0.42	89.61 ± 0.56	1.07 ± 0.01	4.82 ± 0.06	51.16 ± 3.26			
	1.0×IC_{50} (5 nM)	1.23 ± 0.07	8.29 ± 0.71	90.48 ± 0.77	0.78 ± 0.02	5.22 ± 0.22	57.60 ± 4.04			
	2.0×IC_{50} (10 nM)	2.05 ± 0.22	8.26 ± 0.24	89.69 ± 0.46	1.35 ± 0.03**	5.50 ± 0.34	60.09 ± 5.64			
	3.0×IC_{50} (15 nM)	1.56 ± 0.12	9.29 ± 0.44	89.15 ± 0.55	0.94 ± 0.07	5.58 ± 0.25	53.59 ± 0.76**			
	4.0×IC_{50} (20 nM)	1.05 ± 0.07*	12.90 ± 0.81*	86.04 ± 0.75*	0.61 ± 0.01*	7.54 ± 0.79	50.00 ± 2.10*			

Mean±SEM amount of hemoglobin, free heme and hemozoin represented as percent and fg/cell. The amounts of heme in different parasite lines were determined by the heme fractionation assay (see methods). Parasites were treated with increasing concentrations of chloroquine, pyrimethamine or compounds **1,3-5** at different multiples of their IC_{50} values. Hemoglobin, free heme and hemozoin amounts were measured 30 h later. N, n = 1, >3. Statistical comparisons of the drug-treated lines to their untreated controls were performed using two-tailed Student's tests (with Welch's correction). *p<0.05; **p<0.01; ***p<0.001. Amodiaquine and doxycycline were also tested in biological triplicate in the *in vitro* β-hematin inhibition assays. The mean IC_{50} in µM and SEM were 9.4 and 1.3, respectively for amodiaquine and >500 and >500, respectively for doxycycline. Compound 2 was not tested because of lack of material.

Table S7 (related to Fig. 7, S5). *Plasmodium falciparum* asexual blood stage IC_{50} data in nM for the antiplasmodial compounds tested against PfCRT and PfMDR1 isoforms.

	PfCRT isoforms							PfMDR1 isoforms						
	Dd2 ^{3D7}			Dd2 ^{Dd2}				FCB			KD1			
Antimalarials	Mean IC_{50}	SEM	N	Mean IC_{50}	SEM	N	*P* value	Mean IC_{50}	SEM	N	Mean IC_{50}	SEM	N	*P* value
1	47.0	6.7	4	29.0(1234)	5.8(162)	4	ns (0.0286)	74.12	20.55	3	84.86	30.87	3	--
2	203	24.0	4	236(2780)	23.0(504)	4	ns (0.0286)	278.4	34.92	4	270	33.05	4	ns
3	563	18.0	4	1268	58.0	4	<0.05	1361	108.8	5	1760	111	5	ns
4	100	4.9	4	236	23.0	4	<0.05	233.5	10.05	5	324.8	22.4	5	<0.01
5	2.2	0.2	4	4.9	0.4	4	<0.05	8.59	1.96	4	12.93	2.94	4	ns
Chloroquine	11.0	2.2	4	101	12	4	<0.05	--			--			
Mefloquine	--			--				6.55	1.00	4	4.42	0.91	4	ns
Lumefantrine	--			--				0.64	0.10	4	0.29	0.04999	4	<0.05

SEM: standard error of the mean; N: number of biological repeats (with technical duplicates); () refers to the IC_{50} and SEM of the second shift of the biphasic curve. *P* values were determined by comparing the IC_{50} shift of the Dd2 ^{Dd2} parasite line with Dd2 ^{3D7} (PfCRT), or the FCB *pfmdr1* two-copy parasite line with its isogenic *pfmdr1* single-copy KD1 (PfMDR1) using Mann-Whitney *U* tests. ns: p>0.05. '--: not determined.

Table S8 (related to Fig. 5, S3B). Transmission electron microscopy image scoring of *Plasmodium falciparum* asexual blood stage parasite subcellular localization of anti-HA stained ABCI3-3×HA.

Sample number	Image number	Plasma membrane	Edoplasmic reticulum	Digestive vacuole	Vesicles	Nucleus	Nuclear membrane	Cytosol	Total ABCI3-3×HA label per sample
222	79	2	0	4	1	1	0	5	13
222	81	2	ND	1	ND	2	1	6	12
222	82	0	ND	1	0	2	0	1	4
222	83	1	0	1	0	4	0	3	9
222	85	3	ND	0	5	2	0	15	26
222	86	0	0	1	2	0	1	10	15
222	88	1	ND	0	0	2	7	6	17
222	89	1	2	2	0	5	0	4	15
222	90	0	3	0	0	5	3	2	13
222	91	1	3	0	1	0	2	5	13
223	95	0	ND	0	ND	2	1	6	9
223	96	0	ND	2	ND	3	0	8	13
223	97	0	ND	0	ND	2	1	4	7
223	99	1	ND	0	1	2	0	8	12
223	100	0	ND	2	0	4	1	3	10
223	102	1	0	3	1	4	1	8	18
223	104	2	2	0	ND	2	0	5	11
223	106	3	1	1	2	5	2	9	23
226	64	0	ND	0	0	0	0	3	3
226	65	0	0	0	0	1	3	7	11
226	66	0	2	1	0	3	0	4	11
226	68	0	0	2	3	3	1	11	20
226	70	0	ND	5	0	2	0	16	23
226	72	1	1	0	0	1	0	14	18
226	73	2	0	1	0	0	0	1	5
226	74	0	1	1	0	3	1	2	8
226	75	0	ND	0	0	0	1	8	9
226	77	1	0	0	1	2	0	4	8
226	78	0	7	3	4	1	1	2	19
226	79	1	ND	0	0	1	1	10	13
226	80	2	0	2	0	1	4	6	15
Total ABCI3-3×HA label per organelle		25	22	33	21	65	32	196	403
% ABCI3-3×HA label per organelle		**6**	**5**	**8**	**5**	**16**	**8**	**49**	

Results were collated from parasites obtained on three separate occasions for electron microscopy processing and imaging. ND: not determined.

Table S9 (related to Fig. 6). Association constants with Fe(III)PPIX in 40% DMSO and either 0.02 M HEPES (pH 7.4) or MES (pH 5.6).

	log *K*	
Compound	pH 5.6	pH 7.4
Chloroquine	5.16 ± 0.03	5.32 ± 0.03
1	3.64 ± 0.03	4.00 ± 0.08
3	2.54 ± 0.02	2.82 ± 0.03
4	2.53 ± 0.11	2.59 ± 0.02
5	2.58 ± 0.02	2.42 ± 0.04

Higher log *K* values indicate higher heme-binding constants, with chloroquine showing strong binding, **1** being intermediate and **3-5** yielding low values. Mean±SEM.

Table S10 (Related to STAR Methods). Oligonucleotides used in this study.

Experiment	Nucleotide Sequence (5' to 3')	Description	Lab name
L690I validation in 3D7-A10 (related to **Fig. 2C**)	GGGAAATAACTATGGAATATAAAAAAACAG	ABCI3 L690I donor fragment fwd	p6417
	GTTGTGTCGAAGAGGTATCATGGG	ABCI3 L690I donor fragment rev	p6418
	GTTTCGATATAAATAAAGAG	ABCI3 L690I guide RNA	p6387/p6388
F689C and S696Y validation in Dd2-B2 (related to **Fig. S1A**)	GACAAACAAAATGACGAATG	ABCI3 F689C guide RNA (1)	p8159/p8160
	GTTTCGATATAAATAAAGAG	ABCI3 F689C guide RNA (2)	p8165/p8166
	GAGGTACCGAGCTCGaattcCAGATGAAAAGGAGTATCAGG	ABCI3 F689C/S696Y donor fragment fwd (In-Fusion)	p8161
	GAAAAGTGCCACCTGacgtcCAATCCTTAAACACATTTGAC	ABCI3 F689C/S696Y donor fragment rev (In-Fusion)	p8162
cKD in NF54^{pCRISPR} line (related to **Fig. S3**)	GTACGGTACAAACCCGGAATTCGAGCTCGGAGAAATTGCTTTAATGAGTTACATGGG	ABCI3 RHR forward	--
	GGGTATTAGACCTAGGGATAACAGGGTAATGGAAAAATATAAAAAATGAAACTACACC	ABCI3 RHR reverse	--
	GTTTAACGACAAAGATATCG	sgRNA target site	--
ABCI3 3×Flag and 3×HA tagging in 3D7-A10 (related to **Fig. S5**)	ATTGCTTTAATGAGTTACAT	ABCI3 3' tagging guide RNA	p7421/p7422
	AGAGGTACCGAGCTCGaattcCTCATCTCACCAGAAGATATG	ABCI3 3' donor fragment fwd	p7423
	CGAAAAGTGCCACCTGacgtcTCTACAACTATATAAGAAACTCC	ABCI3 3' donor fragment rev	p7424
	GCAGAAAATTTATATTTTCAAAGTGGAGATTATAAAGATCATGATGGAGATTATAAAGATCATGATATAGATTATAAAGATGATGATGATAAATaa	TEV + 3×Flag tag fragment	--
	TACCCATACGATGTTCCTGACTATGCTGGTTATCCTTATGACGTGCCTGACTATGCAGGATCCTATCCATATGACGTTCCAGATTACGCT	3×HA tag fragment	--

--: No oligonucleotides