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Table S1. Characterization of *P. falciparum* isolates and clones used in this study. *In vitro* susceptibility was assessed in the T_0 assay for artemisinin drugs and by using standard methods for mefloquine. Results represent ≥ 2 biological replicates for the drug susceptibility assays.

TF = Treatment Failure
Thailand
Cambodia

Parasite Information				IC50, nM (±SD)					SNPs Associated with Resistance				
									Takala-Harrison et al, 2013		Cheeseman et al, 2012		
Parasite ID	Collection site	Date of Collection	In vivo clearance 1/2 life (hr)	Artemisinin	Artelinic Acid	Artesunate	Dihydro-artemisinin	Mefloquine	MAL10 (688956)	MAL13 (1718319)	MAL13-220 (660)	MAL13-239 (1646)	MAL13-405 (3142)
W2				10.4 (±3.2)	22.6 (±8.6)	4.9 (±1.7)	3.7 (±2.2)	8.2 (±3.9)	T	T	A	T	G
D6				3.3 (±0.4)	6.7 (±1.6)	1.0 (±0.3)	0.5 (±0.1)	2.5 (±0.5)	T	T	A	T	G
ARC08-19	Tasan	9-Sep-08	4.09	14.6 (±5.5)	33.5 (±10.7)	3.8 (±0.6)	2.7 (±1.4)	38.2 (±3.3)			A	T	G
ARC08-22	Tasan		6.92	27.9 (±11.6)	50.6 (±9.4)	6.8 (±2.1)	6.6 (±1.9)	49.0 (±22.3)	A	A	A	T	G
ARC08-32	Tasan		2.58	35.5 (±12.2)	59.6 (±14.3)	10.6 (±1.7)	13.3 (±11.9)	52.8 (±18.2)	T	A	A	T	G
ARC08-34	Tasan	27-Oct-08	3.60	15.4 (±2.1)	22.2 (±4.1)	4.0 (±0.6)	3.2 (±0.5)	23.6 (±19.2)			A	T	G
ARC08-36	Tasan		7.21	9.9 (±1.1)	24.3 (±3.1)	6.1 (±0.5)	4.0 (±0.4)	9.4 (±1.8)					
ARC08-39	Tasan	10-Nov-08	1.48	14.1 (±1.1)	28.7 (±1.0)	4.1 (±0.4)	3.4 (±0.2)	53.4 (±6.6)	T	A	A	T	G
ARC08-63	Tasan		7.60	28.1 (±19.9)	42.6 (±24.2)	7.6 (±1.1)	6.5 (±1.5)	44.9 (±24.4)		A	A	T	G
ARC08-88	Tasan		TF*	18.8 (±8.0)	43.7 (±16.6)	6.2 (±2.1)	5.7 (±3.1)	44.5 (±29.8)		A	A	T	G
PL08-004	Pailin	18-Apr-08	4.04	20.2 (±8.4)	25.4 (±4.4)	6.4 (±0.9)	6.0 (±1.8)	49.7 (±37.8)			A	T	G
PL08-009	Pailin		5.30	38.7 (±11.9)	74.0 (±9.1)	12.0 (±4.0)	8.0 (±4.8)	67.1 (±20.4)	A	A	A	T	G
PL08-015	Pailin	21-Apr-08	5.15	16.7 (±6.7)	15.9 (±8.7)	4.7 (±1.5)	4.7 (±4.0)	43.6 (±20.5)			A	T	G
PL08-025	Pailin		13.07	17.0 (±4.8)	34.9 (±6.0)	9.0 (±2.8)	7.0 (±2.4)	9.3 (±1.3)	T	A	A	T	G
Cam5 (SC031)	Pailin	1-Aug-07	N/A	9.7 (±4.1)	14.6 (±7.2)	3.4 (±1.3)	3.1 (±1.2)	36.3 (±20.5)	T	T	A	T	G
NHP2065	Mae Sot		6.67	19.6 (±9.8)	27.8 (±16.8)	10.0 (±6.0)	11.2 (±6.1)	61.2 (±13.7)	T	T	G	T	G
NHP4197	Mae Sot		2.18	39.9 (±8.8)	57.5 (±12.8)	6.9 (±0.7)	5.0 (±1.3)	70.7 (±13.3)	T	T			
NHP4306	Mae Sot		1.83	17.3 (±10.4)	33.5 (±8.1)	5.1 (±2.7)	3.2 (±1.5)	65.1 (±21.6)	T	T	A	T	G
Thai 13 (FARS012)	Mae Sot	1-Feb-08	3.18	23.5 (±3.7)	43.6 (±8.8)	4.6 (±1.9)	3.8 (±0.6)	125.2 (±27.6)			A	T	G
Thai 14 (ARS40)	Mae Sot	1-Apr-08	1.90	22.7 (±8.6)	30.0 (±1.0)	4.3 (±0.8)	3.9 (±0.1)	113.3 (±25.8)			A	T	G
Thai 22 (ARS014)	Mae Sot	1-Feb-08	3.63	20.5 (±7.4)	34.8 (±1.3)	4.9 (±1.4)	4.2 (±1.6)	100.1 (±18.6)	A	T	A	T	G

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Table S2. Description of primers and conditions used in this study.

Gene	SNP	Forward Primer	Reverse Primer	Sequencing Primer	T_M (^oC)
PF3D7_1343700	C580Y	GTCAACAATGCYGGCGTAT	TCACCATTAGTTCCACCAATGACA (BIO)	CCAATGACATAAATTTTATT	63.0
PF3D7_1343700	R539T	TTAGTCAACAATGCTGGCGTAT (BIO)	CTCCCATTAGTTCCACCAATG	AGTGGAAGACATCATGTAA	58.0
PF3D7_1343700	Y493H	TTAGTCAACAATGCTGGCGTAT (BIO)	CTCCCATTAGTTCCACCAATG	GTGCTGTATTGAATAATTTC	58.0
PF3D7_101700	MAL10 (688956)	TGGATTGGTGAAAAGGAAAATGTA	GGAAACATTTATGCCATCAACATT (BIO)	TTTTTATAAAAGAACTATGC	61.8
PF3D7_1343400	MAL13 (1718319)	AAGACAACGGTGACGATCTAAAA (BIO)	CGTCTGCACATTTTTTACACATAA	ATTATTTCCTATATTATTCT	58.6
PF3D7_1344600	MAL13 (660)	CCGTATGTGAAGAAGCACAATGT (BIO)	CTAGCTCCACCATCTGGTAAATCA	GGTAAATCATCTCTATCAAC	63.4
PF3D7_1344700	MAL13 (1646)	TTGATAAACCTGAATGGGGACTTA (BIO)	TTGATCACTCATGGGATGAAAATA	ACTCTAATGTTTGTTTCAAT	61.6
PF3D7_1344300	MAL13 (3142)	TTTTTCATCGTGCATCTCAAAC (BIO)	TTCCTAGTTGGCGTATCCTTAAAT	TTTGATTAGGGTACACATAA	59.0

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Table S3. Compilation of Pf3D7_1343700 (K13) sequence results for *P. falciparum* isolates and clones used in this study.

Parasite	In vivo clearance 1/2 life	**TAC-->CAC Y493H**	GAT-->GTT D584V	**TGT-->TAT C580Y**	ATT-->ACT I543T	**AGA-->ACA R539T**	AAT-->ATT N537I	GGT-->AGT G533S
W2		**WT**	WT	**WT**	WT	**WT**	WT	WT
D6		**WT**	WT	**WT**	WT	**WT**	WT	WT
CB132		**WT**	WT	**WT**	WT	**WT**	WT	WT
CB137		**WT**	WT	**WT**	WT	**WT**	WT	WT
ARC08-19	4.09	**WT**	WT	**WT**				
ARC08-22	6.92	**WT**	WT	**Mut**	WT	**WT**	WT	WT
ARC08-22 (4G)	6.92	**WT**	WT	**Mut**	WT	**WT**	WT	WT
ARC08-22 (5G)	6.92	**WT**	WT	**Mut**	WT	**WT**	WT	WT
ARC08-22 (7C)	6.92	**WT**	WT	**Mut**				
ARC08-32	2.58	**WT**	WT	**WT**	WT	**WT**	WT	WT
ARC08-34	3.6	**WT**	WT	**WT**	WT	**WT**	WT	WT
ARC08-63	7.6	**WT**	WT	**Mut**	WT	**WT**	WT	WT
ARC08-88	TF	**WT**	WT	**Mut**	WT	**WT**	WT	WT
ARC08-88 (5A)	TF	**WT**	WT	**Mut**				
ARC08-88 (8A)	TF	**WT**	WT	**Mut**				
ARC08-88 (9C)	TF	**WT**	WT	**Mut**				
ARC08-88 (9D)	TF	**WT**	WT	**Mut**				
ARC08-88 (11G)	TF	**WT**	WT	**Mut**				
PL08-004	4.04	**Mut**	WT	**WT**	WT	**WT**	WT	WT
PL08-009	5.3	**WT**	WT	**Mut**	WT	**WT**	WT	WT
PL08-009 (5C)	5.3	**WT**	WT	**Mut**				
PL08-009 (6B)	5.3	**WT**	WT	**Mut**				
PL08-009 (9H)	5.3	**WT**	WT	**Mut**				
PL08-015	5.15	**WT**	WT	**WT**	WT	**Mut**	WT	WT
PL08-025	13.07	**WT**	WT	**Mut**	WT	**WT**	WT	WT
Cam5 (SC031)	N/A	**WT**	WT	**WT**	WT	**Mut**	WT	WT
NHP2065	6.67	**WT**	WT	**WT**	WT	**WT**	WT	WT
NHP2065 (4A)	6.67	**WT**	WT	**WT**	WT	**WT**	WT	WT
NHP2065 (4F)	6.67	**WT**	WT	**WT**				
NHP2065 (6H)	6.67	**WT**	WT	**WT**				
NHP2065 (7C)	6.67	**WT**	WT	**WT**	WT	**WT**	WT	WT
NHP4197	2.18	**WT**	WT	**WT**				
NHP4197 (2A)	2.18	**WT**	WT	**WT**				
NHP4306	1.83	**WT**	WT	**WT**	WT	**WT**	WT	WT
Thai 13 (FARS012)	3.18	**WT**	WT	**WT**	WT	**WT**	WT	WT
Thai 14 (ARS40)	1.9	**WT**	WT	**WT**	WT	**WT**	WT	WT
Thai 22 (ARS014)	3.63	**WT**	WT	**WT**	WT	**WT**	WT	WT

Note: NHP2065 contained the A675V mutation in K13 (Francois Nosten, personal communication).

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Figure S1. Parasite development was monitored following exposure to DHA. Parasites (n=100) were categorized as ring, trophozoite/schizont, or dormant/dead for each time point. In the delayed parasite clearance (DCA) assay *P. falciparum* clones were exposed to three, six-hour pulses of 700 nM DHA.

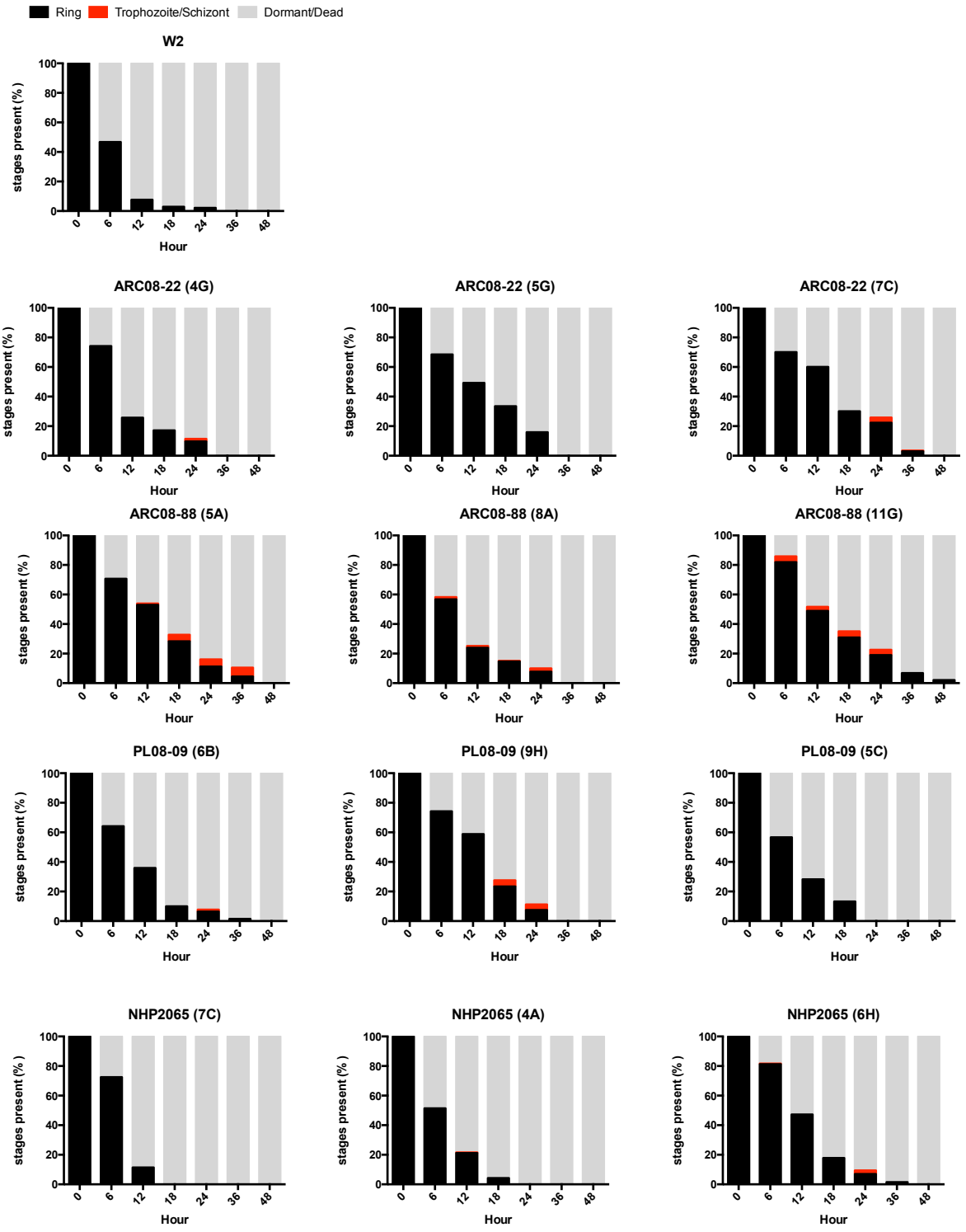

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Figure S2. The in vitro response (IC_{50}s) as determined in the T_0 ^{3}H-hypoxanthine assay were not correlated directly to the in vitro parasite reduction ratio (PRR). The PRR data were determined from the delayed clearance assay as outlined in the methods. The presence of mutations in Pf3D7_1343700 are noted by the symbol color (**WT**; **C580Y**; **R539T**; **Y493H**; **A675V**) (DHA = dihydroartemisinin; QHS = artemisinin; AS = artesunate; AL = artelinic acid).

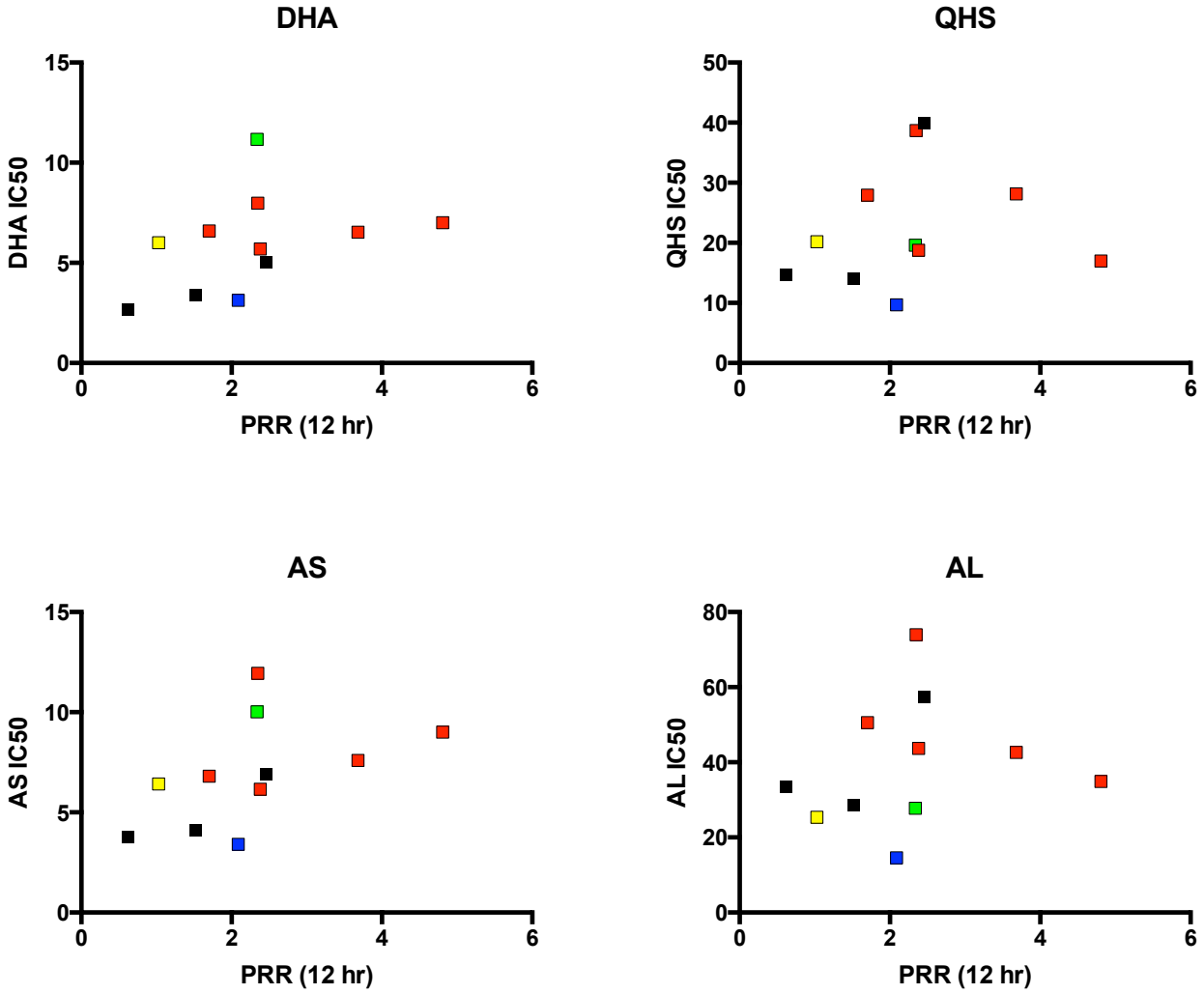
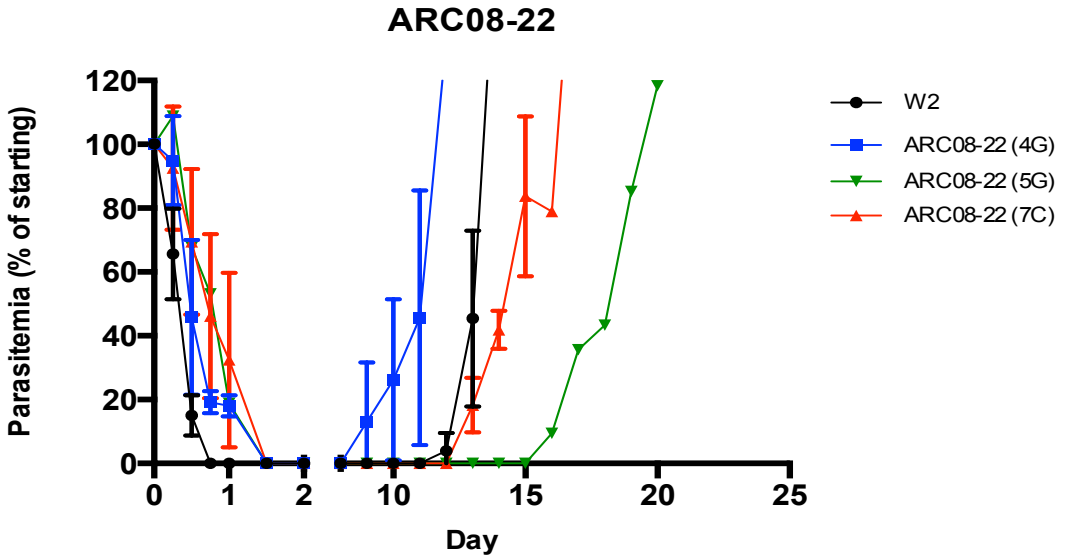


Figure S3. **Clearance, recrudescence, and susceptibility of ARC08-22 clones compared to W2.** ARC08-22 (4G) recrudesced before W2 reaching initial parasitemia 2 days before W2. ARC08-22 (5G) and (7C) recrudesced after W2 and grew to initial parasitemia 3 and 6 days after W2, respectively. In vitro susceptibility to artemisinin derivatives was determined in the T_0 assay (n $\geq$ 2 biological replicates). The time to reach starting parasitemia did not correlate with IC_{50} values to artemisinin derivative or mefloquine.



IC_{50}, nM (±SD)

	QHS	AL	ART	DHA	MFQ
W2	10.4 (±3.2)	22.6 (±8.6)	4.9 (±1.7)	3.7 (±2.2)	8.2 (±3.9)
ARC08-22	27.9 (±11.6)	50.6 (±9.4)	6.8 (±2.1)	6.6 (±1.9)	49.0 (±22.3)
ARC08-22 4G	33.6 (±14.0)	48.3 (±19.8)	8.3 (±2.5)	7.3 (±1.6)	25.8 (±13.9)
ARC08-22 5G	42.2 (±9.0)	60.4 (±7.2)	10.7 (±1.7)	10.5 (±3.6)	48.5 (±11.2)
ARC08-22 7C	34.2 (±11.4)	58.8 (±16.2)	10.2 (±2.7)	8.2 (±3.6)	51.5 (±32.9)

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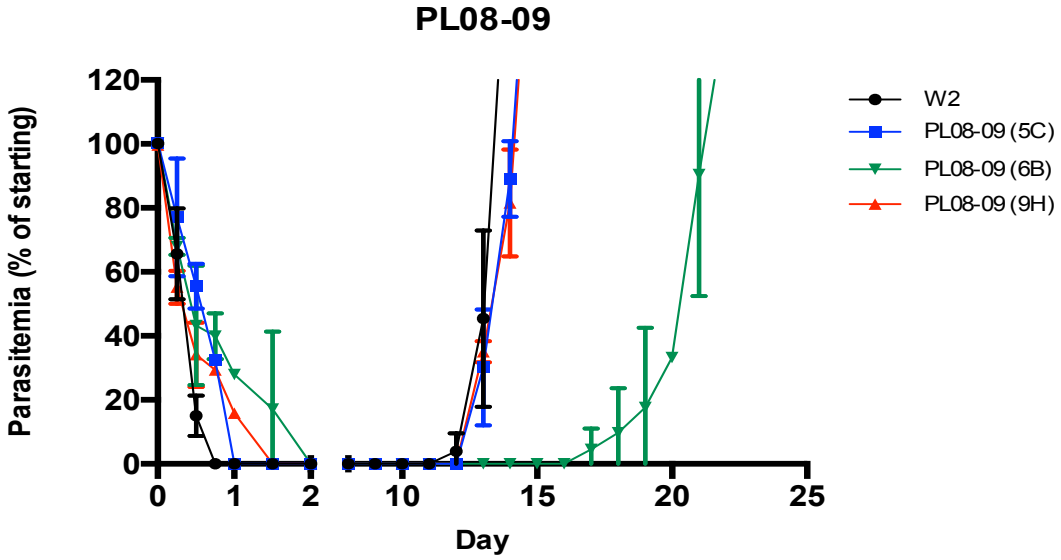
Figure S4. Clearance, recrudescence, and susceptibility of PL08-09 clones compared to W2. PL08-09 (6B) cleared up to 24 hours slower than PL08-09 (5C) and (9H). PL08-09 (5C) and (9H) recrudescence following dormancy was not significantly delayed as compared to W2. PL08-09 (6B) took 7 days longer than W2 to reach initial parasitemia. In vitro susceptibility to artemisinin derivatives was determined in the T_0 assay ($n \geq 2$ biological replicates). The time to reach starting parasitemia did not correlate with IC_{50} values to artemisinin derivative or mefloquine.



IC_{50}, nM (±SD)

	QHS	AL	ART	DHA	MFQ
W2	10.4 (±3.2)	22.6 (±8.6)	4.9 (±1.7)	3.7 (±2.2)	8.2 (±3.9)
PL08-09	38.7 (±11.9)	74.0 (±9.1)	12.0 (±4.0)	8.0 (±4.8)	67.1 (±20.4)
PL08-09 6B	25.3 (±5.5)	51.4 (±10.5)	6.9 (±1.3)	5.3 (±1.5)	70.4 (±6.6)
PL08-09 9H	38.7 (±15.6)	70.5 (±24.2)	11.4 (±4.4)	7.1 (±1.1)	89.0 (±48.2)
PL08-09 5C	72.9 (0.02)	116.1 (±10.1)	12.6 (±2.2)	8.0 (±2.5)	53.5 (±3.1)

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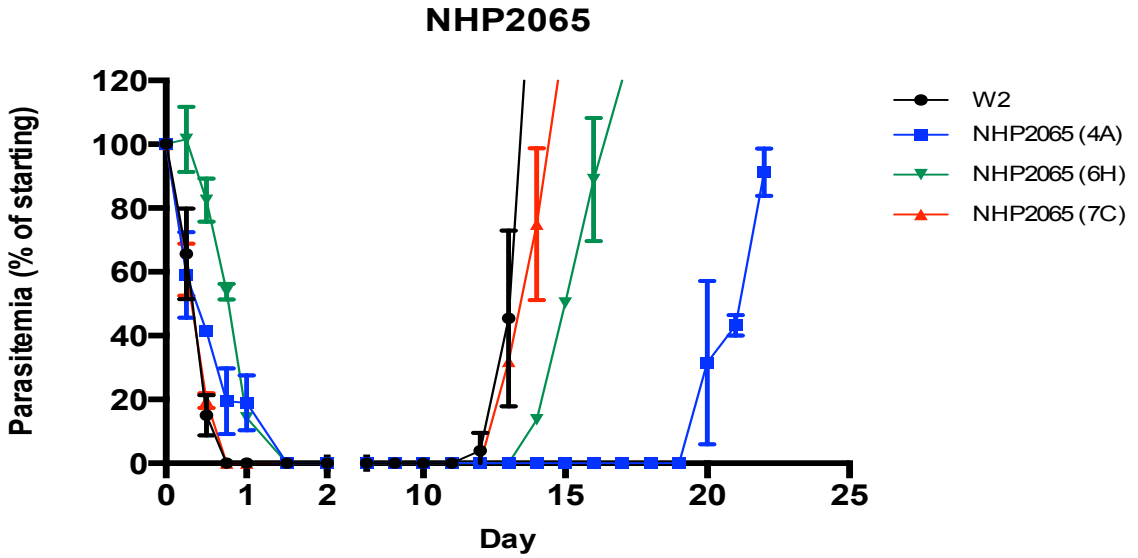
Figure S5. Clearance, recrudescence, and susceptibility of NHP2065 clones compared to W2. NHP2065 (7C) cleared at a similar rate as W2 and began to recrudesce around the same time as W2. NHP2065 (6H) and (4A) cleared 18 hours after W2 and recrudesced 2 and 7 days after W2 respectively. In vitro susceptibility to artemisinin derivatives was determined in the T_0 assay ($n \geq 2$ biological replicates). All NHP2065 clones tested are more susceptible to artemisinin derivatives compared to other patient clones tested but are resistant to mefloquine. NHP2065 is the only isolate in this study with the A675V mutation in K13 (Francois Nosten, personal communication).



IC_{50}, nM (±SD)

	QHS	AL	ART	DHA	MFQ
W2	10.4 (±3.2)	22.6 (±8.6)	4.9 (±1.7)	3.7 (±2.2)	8.2 (±3.9)
NHP2065	19.6 (±9.8)	27.8 (±16.8)	10.0 (±6.0)	11.2 (±6.1)	61.2 (±13.7)
NHP2065 4A	15.2 (±4.0)	25.1 (±7.7)	3.4 (±1.3)	5.1 (±1.0)	48.1 (±5.3)
NHP2065 7C	14.6 (±1.0)	19.0 (±3.7)	3.4 (±0.7)	5.4 (±1.5)	61.4 (±18.2)
NHP2065 6H	16.6 (±8.2)	18.4 (±5.5)	2.7 (±0.9)	3.7 (±0.8)	33.9 (±21.8)