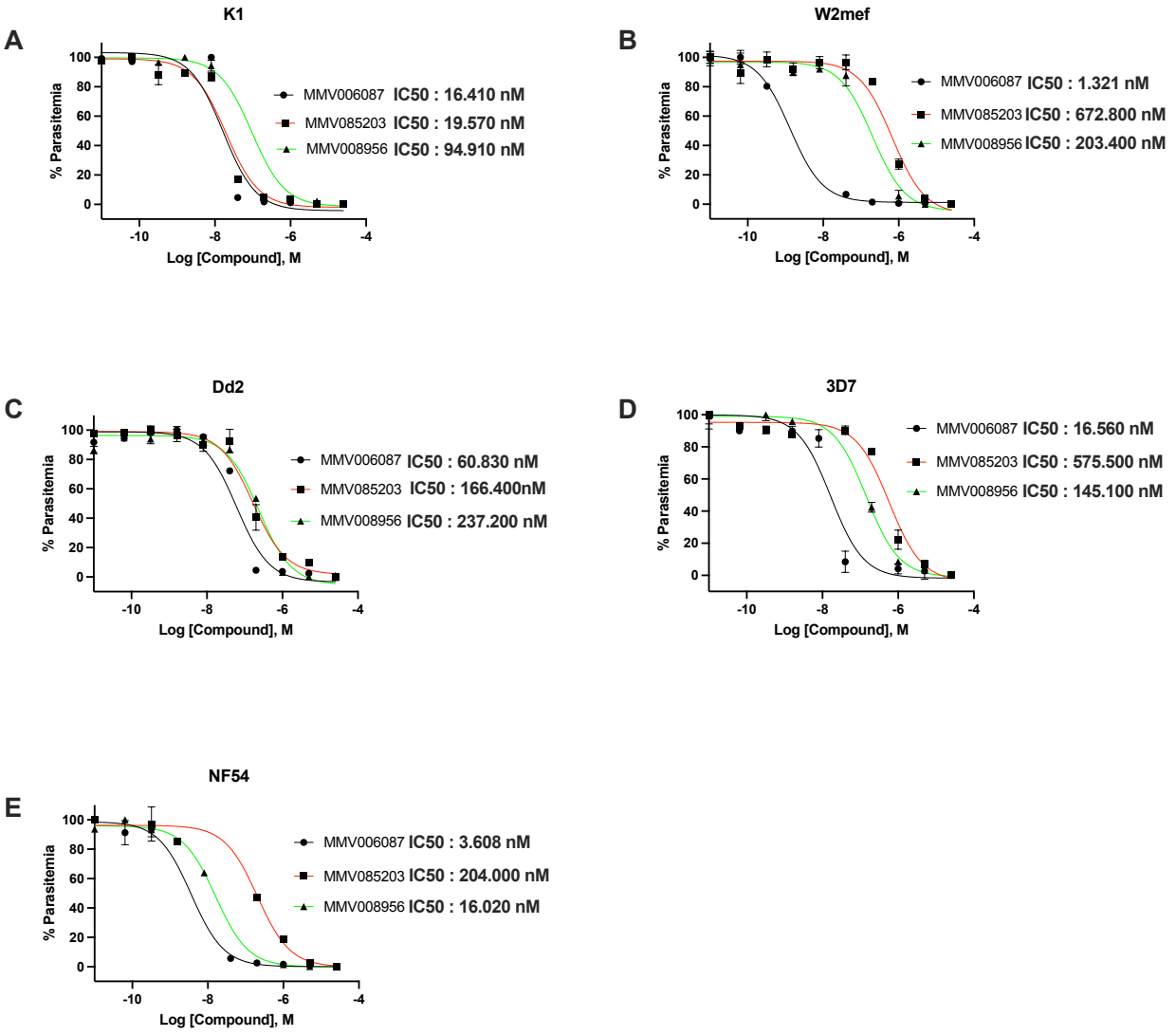
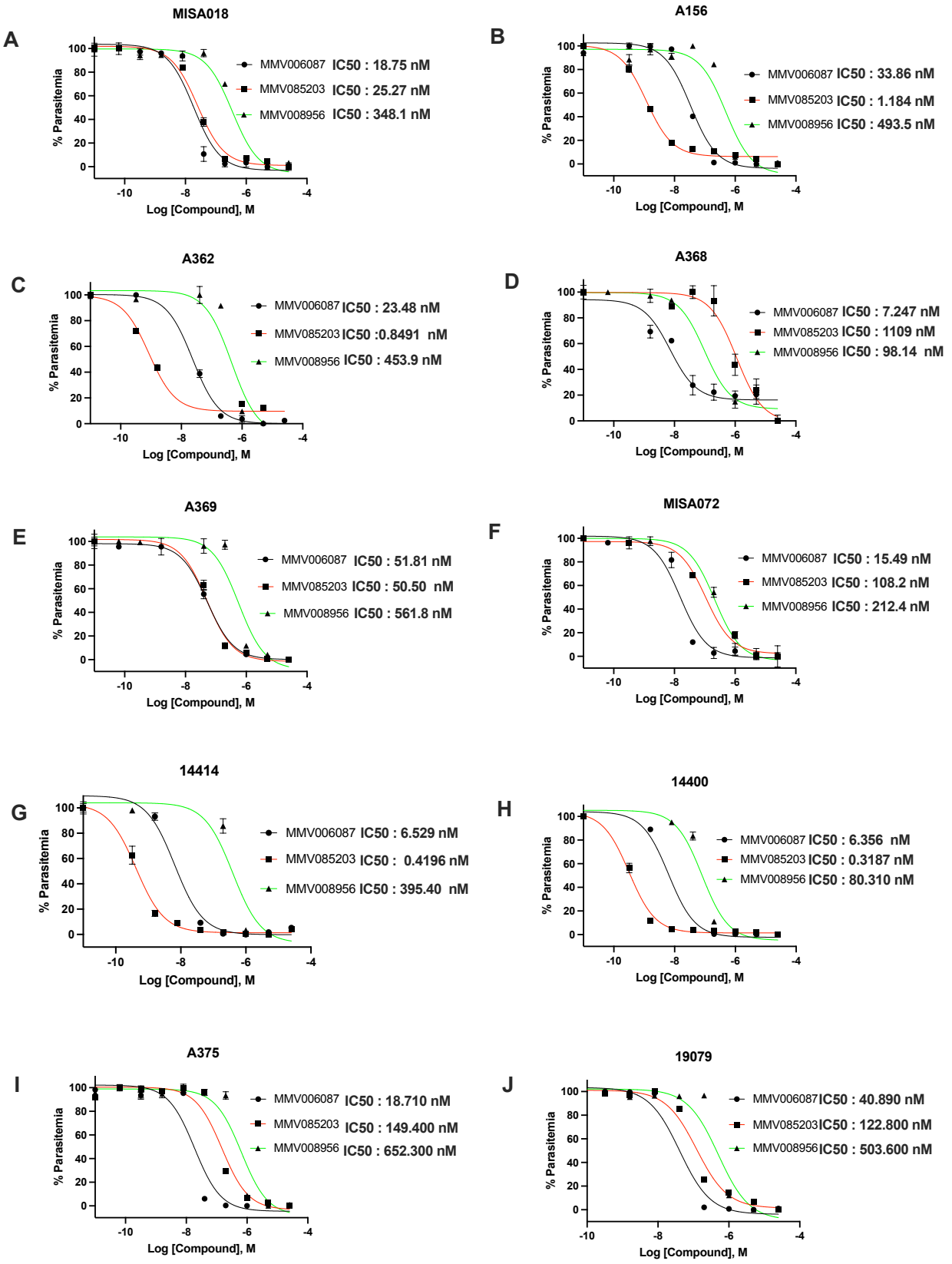
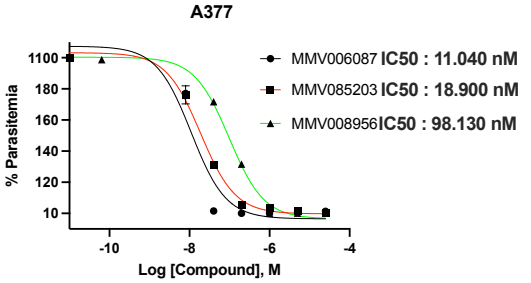
Supplementary Material



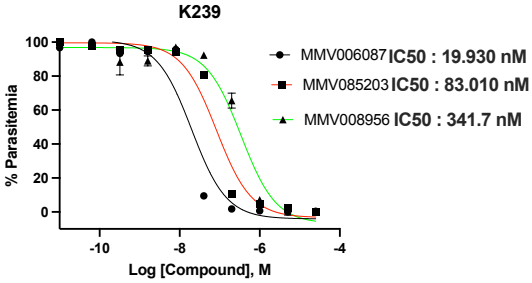
Supplementary Figure S1. The potency of MMV006087, MMV085203 and MMV008956 against laboratory strains of *P. falciparum.* Panel A–E are dose-response curves showing the response of 5 clinical isolates to the three Malaria Box compounds at concentrations from 0.064 nM to 25 µM. Each data point represents the mean ± SEM (n=3). The plot shows percentage parasitemia against the log of the concentration of the compound.


A
MISA018
MMV006087 IC50 : 18.75 nM
MMV085203 IC50 : 25.27 nM
MMV008956 IC50 : 348.1 nM
B
A156
MMV006087 IC50 : 33.86 nM
MMV085203 IC50 : 1.184 nM
MMV008956 IC50 : 493.5 nM
C
A362
MMV006087 IC50 : 23.48 nM
MMV085203 IC50 :0.8491 nM
MMV008956 IC50 : 453.9 nM
D
A368
MMV006087 IC50 : 7.247 nM
MMV085203 IC50 : 1109 nM
MMV008956 IC50 : 98.14 nM
E
A369
MMV006087 IC50 : 51.81 nM
MMV085203 IC50 : 50.50 nM
MMV008956 IC50 : 561.8 nM
F
MISA072
MMV006087 IC50 : 15.49 nM
MMV085203 IC50 : 108.2 nM
MMV008956 IC50 : 212.4 nM
G
14414
MMV006087 IC50 : 6.529 nM
MMV085203 IC50 : 0.4196 nM
MMV008956 IC50 : 395.40 nM
H
14400
MMV006087 IC50 : 6.356 nM
MMV085203 IC50 : 0.3187 nM
MMV008956 IC50 : 80.310 nM
I
A375
MMV006087 IC50 : 18.710 nM
MMV085203 IC50 : 149.400 nM
MMV008956 IC50 : 652.300 nM
J
19079
MMV006087 IC50 : 40.890 nM
MMV085203 IC50 : 122.800 nM
MMV008956 IC50 : 503.600 nM
% Parasitemia
Log [Compound], M

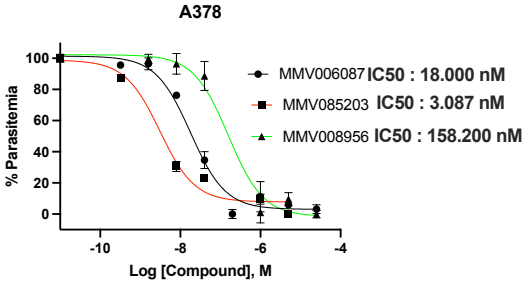
K


A377
MMV006087 IC50 : 11.040 nM
MMV085203 IC50 : 18.900 nM
MMV008956 IC50 : 98.130 nM
% Parasitemia
Log [Compound], M

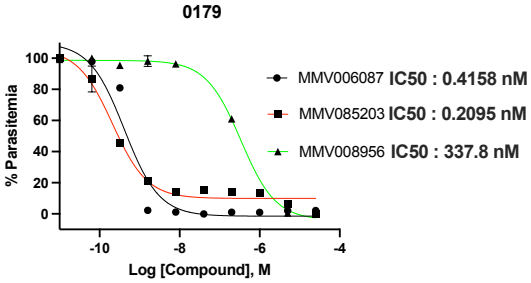
L


K239
MMV006087 IC50 : 19.930 nM
MMV085203 IC50 : 83.010 nM
MMV008956 IC50 : 341.7 nM
% Parasitemia
Log [Compound], M

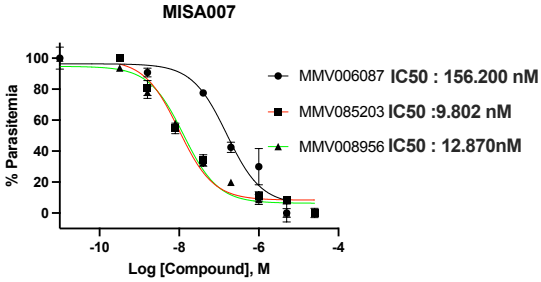
M


A378
MMV006087 IC50 : 18.000 nM
MMV085203 IC50 : 3.087 nM
MMV008956 IC50 : 158.200 nM
% Parasitemia
Log [Compound], M

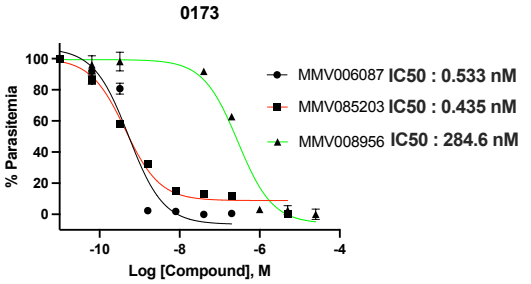
N


0179
MMV006087 IC50 : 0.4158 nM
MMV085203 IC50 : 0.2095 nM
MMV008956 IC50 : 337.8 nM
% Parasitemia
Log [Compound], M

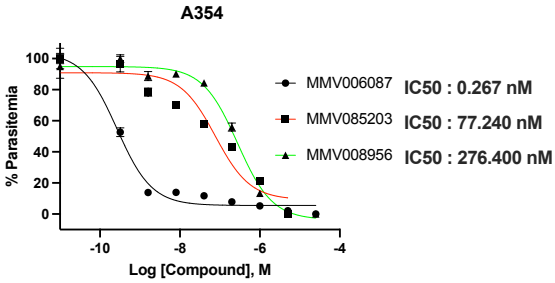
O


MISA007
MMV006087 IC50 : 156.200 nM
MMV085203 IC50 :9.802 nM
MMV008956 IC50 : 12.870nM
% Parasitemia
Log [Compound], M

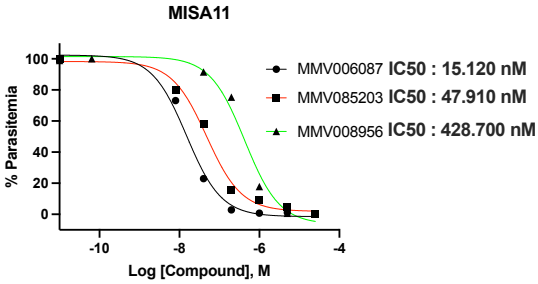
P


0173
MMV006087 IC50 : 0.533 nM
MMV085203 IC50 : 0.435 nM
MMV008956 IC50 : 284.6 nM
% Parasitemia
Log [Compound], M

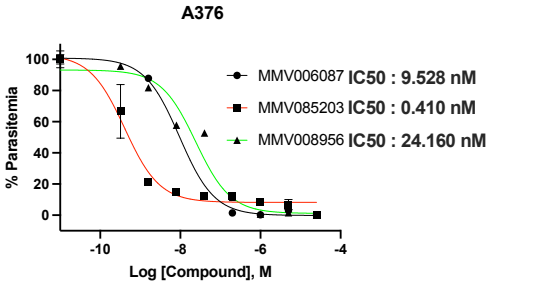
Q


A354
MMV006087 IC50 : 0.267 nM
MMV085203 IC50 : 77.240 nM
MMV008956 IC50 : 276.400 nM
% Parasitemia
Log [Compound], M

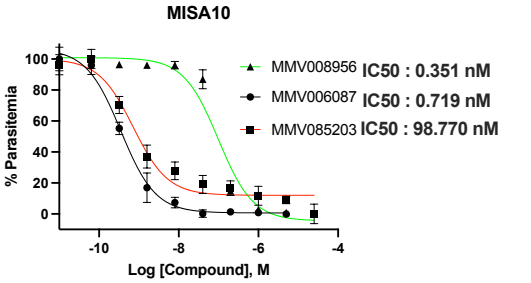
R


MISA11
MMV006087 IC50 : 15.120 nM
MMV085203 IC50 : 47.910 nM
MMV008956 IC50 : 428.700 nM
% Parasitemia
Log [Compound], M

S


A376
MMV006087 IC50 : 9.528 nM
MMV085203 IC50 : 0.410 nM
MMV008956 IC50 : 24.160 nM
% Parasitemia
Log [Compound], M

T


MISA10
MMV008956 IC50 : 0.351 nM
MMV006087 IC50 : 0.719 nM
MMV085203 IC50 : 98.770 nM
% Parasitemia
Log [Compound], M

Supplementary Figure S2. The potency of MMV006087, MMV085203 and MMV008956 against clinical isolates of *P. falciparum*. Panel A–T are dose-response curves showing the response of 20 clinical isolates to the three Malaria Box compounds at concentrations from 0.064 nM to 25 µM. Each data point represents the mean ± SEM (n=3). The plot shows percentage parasitemia against the log of the concentration of the compound.

Supplementary Table S2. A summary of the IC_{50} values of the Malaria Box compounds against the laboratory strains of *P. falciparum.*

Isolate	Compound		
	MMV006087	**MMV085203**	**MMV008956**
	IC_{50} Values (nM) (95% CI)	**IC_{50} Values (nM) (95% CI)**	**IC_{50} Values (nM) (95% CI)**
K1	16.41 (9.15-28.85)	19.57 (13.78- 27.79)	94.91 (60.71- 152.20)
NF54	3.61 (2.13- 5.96)	204.00 (123.00-327.70)	16.02 (12.33- 21.43)
W2mef	1.32 (0.84- 2.54)	672.80 (464.40-974.50)	203.40 (116.40-358.20)
3D7	16.56 (9.48- 29.05)	575.50 (416.00-802.40)	145.10 (96.86-207.80)
Dd2	60.83 (46.36- 79.30)	166.40 (115.60-239.10)	237.20 (190.90- 294.30)

CI=Confidence Interval

Supplementary Table S2. A summary of the IC_{50} values of the Malaria Box compounds against the clinical isolates of *P. falciparum.*

Isolate	Compound		
	MMV006087	**MMV085203**	**MMV008956**
	IC_{50} (nM) (95% CI)	**IC_{50} (nM) (95% CI)**	**IC_{50} (nM) (95% CI)**
EIMA375	18.71 (13.94- 25.11)	149.40 (117.0-191.10)	652.30 (458.5- 936.80)
EIMA377	11.04 (8.03-15.28)	18.90 (15.46-23.22)	98.13 (85.54-112.70)
EIMA368	7.25 (2.73-20.01)	1109 (566.90-2297)	98.14 (15.49- 407.30)
EIMA369	51.81 (35.25-77.59)	50.50 (35.00-72.48)	561.80 (332.90- 954.10)
MISA018	18.75 (13.50- 26.07)	25.27 (19.44- 32.91)	348.10 (268.40- 451.30)
14414	6.53 (4.52- 9.50)	0.42 (0.33- 5.43)	395.40 (207.30-753.60)
14400	6.36 (4.705-8.672)	0.32 (0.26- 0.38)	80.31 (54.19- 119.4)

MISA072	15.49 (9.90- 24.21)	108.20 (69.35-194.00)	212.40 (129.40- 337.90)
MISA010	0.35 (0.25-0.49)	0.72 (0.39-1.38)	98.77 (70.60-138.00)
K239	19.93 (13.26- 30.00)	83.01 (60.92-112.90)	341.7 (241.80- 481.40)
A156	33.86 (25.00-45.94)	1.18 (0.98-1.43)	493.50 (313.00-778.50)
A362	23.48 (18.98- 28.63)	0.85 (0.58-1.26)	453.90 (264.40- 771.40)
A378	18.00 (10.89- 28.62)	3.09 (1.45-5.51)	158.20 (85.41- 306.30)
A376	9.53 (7.032-13.47)	0.41 (0.26- 0.63)	24.16 (12.98- 45.01)
MISA007	156.20 (72.77-350.80)	9.80 (5.82-16.59)	12.87 (8.238- 20.28)
A354	0.27 (0.21- 0.34)	77.24 (18.14-291.90)	276.40 (214.90-355.20)
0179	0.42 (0.27- 0.62)	0.21 (0.15- 0.30)	337.80 (282.70- 406.00)
0173	0.53	0.44	284.60

	(0.31- 9.12)	(0.31-0.61)	(184.00-437.60)
19079	40.89 (27.45- 60.78)	122.80 (89.38-170.40)	503.60 (291.60-858.10)
MISA011	15.12 (11.84-19.34)	47.91 (36.58-62.97)	428.70 (309.30-595.20)

Supplementary Table S3. Summary of IC_{50} values of standard antimalarials against laboratory strains of *P. falciparum.*

Isolate	Artesunate IC_{50} (nM) (95% CI)	Chloroquine IC_{50} (nM) (95% CI)	Mefloquine IC_{50} (nM) (95% CI)	Halofantrine IC_{50} (nM) (95% CI)	MMV 006087 IC_{50} (nM) (95% CI)	MMV 085203 IC_{50} (nM) (95% CI)	MMV 008956 IC_{50} (nM) (95% CI)
3D7	6.00 (3.63- 9.55)	7.43 (5.22- 10.51)	2.95 (1.83- 5.05)	54.13 (35.15- 82.87)	16.56 (9.48- 29.05)	575.50 (416.00-802.40)	145.10 (96.86-207.80)
Dd2	16.23 (11.74-22.70)	156.90 (115.00- 215.10)	22.59 (16.41-31.10)	112.30 (65.09- 247.80)	60.83 (46.36- 79.30)	166.40 (115.60-239.10)	237.20 (190.90- 294.30)
NF54	2.41 (1.71-3.39)	13.46 (7.86-22.86)	19.31 (13.72-27.18)	16.19 (10.91- 23.92)	3.61 (2.13- 5.96)	204.00 (123.00-327.70)	16.02 (12.33- 21.43)

Supplementary Table S4. Summary of IC_{50} values of standard antimalarials against clinical isolate.

Isolate	Artesunate	Chloroquine	Mefloquine	Halofantrine	MMV 006087	MMV 085203	MMV 008956
	IC_{50}(95% CI)	IC_{50} (95% CI)	IC_{50}(95% CI)	IC_{50} (95% CI)	IC_{50} (95% CI)	IC_{50} (95% CI)	IC_{50} (95% CI)
A369	5.32 (4.03 -7.06)	10.51 (7.17 -15.32)	38.43 (22.10- 68.12)	15.21 (9.058-26.09)	51.81 (35.25-77.59)	50.50 (35.00-72.48)	561.80 (332.90- 954.10)
A368	2.49 (1.71-3.80)	26.40 (8.93-57.98)	81.23 (30.52- 394.7)	4.09 (0.63- 21.70)	7.25 (2.73-20.01)	1109 (566.90-2297)	98.14 (15.49- 407.30)
A377	2.30 (1.63-3.24)	0.11 (0.07- 0.17)	1.07 (0.84- 1.36)	146.70 (107.60- 199.00)	11.04 (8.03-15.28)	18.90 (15.46-23.22)	98.13 (85.54-112.70)
14414	15.11 (11.29-20.21)	14.39 (10.34- 20.13)	6.69 (5.68-7.88)	33.69 (26.52- 42.84)	6.53 (4.52- 9.50)	0.42 (0.33- 5.43)	395.40 (207.30-753.60)
MISA11	11.15 (7.45-16.37)	30.17 (24.59- 36.95)	2.58 (2.04-3.24)	0.68 (0.52- 0.88)	15.12 (11.84-19.34)	47.91 (36.58-62.97)	428.70 (309.30-595.20)

14400	16.63	237.00	5.34	68.11	6.36	0.32	80.31
	(12.13-22.66)	(190.90-293.30)	(4.18-6.86)	(43.90-103.30)	(4.705-8.672)	(0.26- 0.38)	(54.19- 119.4)
A375	4.65	16.59	3.70	22.03	18.71	149.40	652.30
	(3.65-5.93)	(12.06-22.76)	(2.65-5.17)	(14.01- 35.20)	(13.94- 25.11)	(117.0-191.10)	(458.5- 936.80)

Supplementary Table S5. The PCR cycling conditions, controls and primer sequence for the MSP genotyping

Gene	PCR round	Primer name	Sequence (5'-3')	Cycling conditions	Allelic family (Annealing temperature)	Positive control
msp1	Primary	M1-OF:	CTAGAAGCTTTAGAAGATGCAGTATTG	Initial denaturation: 95 °C for 5 min; PCR: 25 cycles of 94 °C for 1 min, 61 °C for 45 s, 72 °C for 1.5 min; final elongation:72 °C for 5 min	61 °C	
		M1-OR:	CTTAAATAGTATTCTAATTCAAGTGGATCA			
	Secondary	M1-KF:	AATGAAGAAGAAATTACTACAAAAGGTGC	Initial denaturation: 95 °C for 5 min; PCR: 30 cycles of 94 °C for 45 s, allele-specific °C for 30s, 72 °C for 1 min; final elongation: 72 °C for 5 min	K1(61 °C)	3D7
		M1-KR:	GCTTGCATCAGCTGGAGGGCTTGCACCAGA			
		M1-MF:	AAATGAAGGAACAAGTGGAACAGCTGTTAC		MAD20 (61°C)	HB3
		M1-MR:	ATCTGAAGGATTTGTACGTCTTGAATTACC			

		M1-RF:	TAAAGGATGGAGCAAATACTCAAGTTGTTG		R033 (63 °C)	R033
		M1-RR:	CATCTGAAGGATTTGCAGCACCTGGAGATC			
msp2	Primary	MSP2-1	ATGAAGGTAATTAAAACATTGTCTATTATA	Initial denaturation: 94 °C for 5 min; PCR: 40 cycles of 94 °C for 1.5 min, 55 °C for 45 s, 72 °C for 1.5 min; final elongation: 72 °C for 10 min	55 °C	
		MSP2-4	ATATGGCAAAAGATAAAACAAGTGTTGCTG			
	secondary	A1:	GCAGAAAGTAAGCCTTCTACTGGTGCT	Initial denaturation: 94 °C for 2 min; PCR: 30 cycles of 94 °C for 1.5 min, 55 °C for 45 s, 72 °C for 1.5 min; final elongation: 72 °C for 10 min	IC/3D7 (55 °C)	3D7
		3D7 A2:	GATTTGTTTCGGCATTATTATGA			
		FC27 B1:	GCAAATGAAGGTTCTAATACTAATAG		FC27 (55 °C)	HB3
		FC27 B2:	GCTTTGGGTCCTTCTTCAGTTGATTC			