Supporting Information

A high-throughput luciferase-based assay for the discovery of therapeutics that prevent malaria

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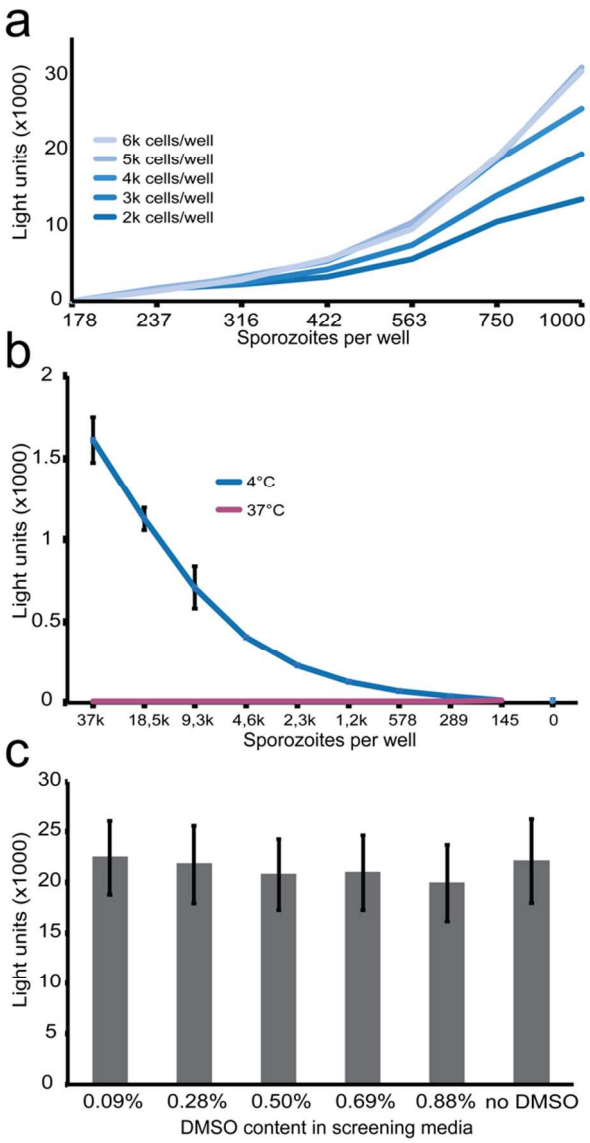

Figure S1. Luciferase-based high-throughput screening assay optimization
a) Optimization of Pb-Luc sporozoite to host HepG2-A16-CD81^{EGFP} cell ratio. Bioluminescence light output was measured for varying numbers of sporozoites in relation to cells per well. Higher cell numbers resulted in diminishing increases in light output. A ratio of 750 sporozoites to 3,000 cells was determined to be optimal for our assay. b) Luciferase light signal from sporozoites after 24 hours at 4°C and 37°C. In order to determine the contribution of extracellular sporozoites to the background signal intensity, we incubated sporozoites in cell culture media for 24 hours. After 24 hours at 37°C, there was negligible luminescent signal from even the highest concentration of sporozoites tested (37,000 per well), and thus extracellular sporozoites do not significantly contribute to background signal in our assay. c) Effect of different DMSO concentrations in the screening media on the malaria EEFs. The final DMSO concentration in the assay is 0.5%. DMSO concentrations up to 0.88% do not significantly alter EEF development d) Light output of QuantiLum Recombinant Luciferase in 1536-well assay conditions. We chose 0.02 µl per well to replicate the typical light outputfrom a Pb-Luc infected 1536 well. This was used to measure direct luciferase inhibition by the tested compound.

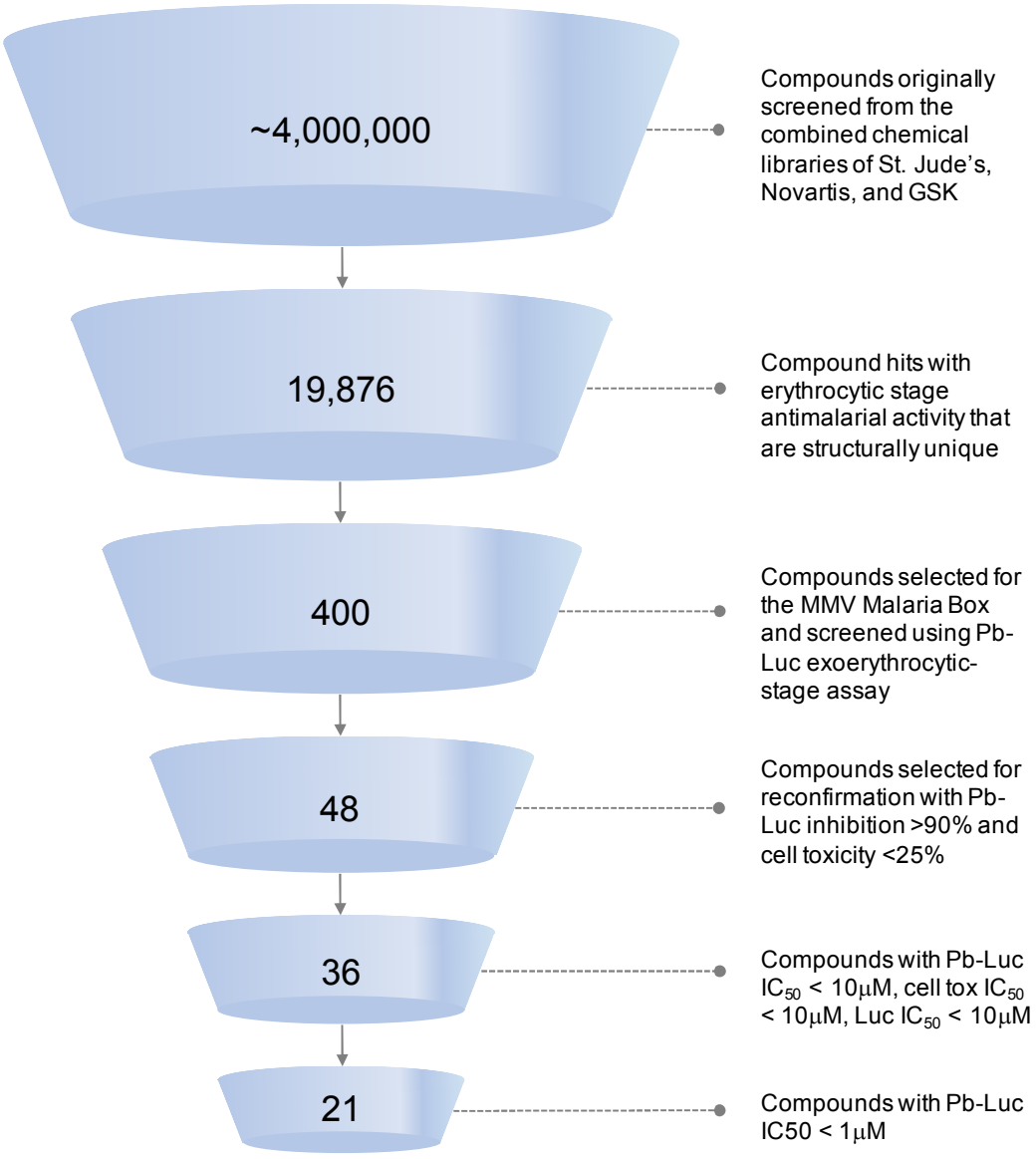

Figure S2. The selection process for the MMV Malaria Box compounds and exoerythrocytic-stage active hits.
Diagram illustrating the flow of the selection process for the inclusion of MMV Malaria Box compounds and the resulting malaria exoerythrocytic-stage active screening hits. The selection process begins with the creation of the MMV Malaria Box, a collection of 400 diverse compounds with erythrocytic-stage antimalarial activity derived from the combined screening of roughly 4 million compounds. Our high-throughput exoerythrocytic-stage screen identified 36 compounds with exoerythrocytic-stage activity of less than 10 μM, and 21 compounds with exoerythrocytic-stage activity of less than 1 μM.

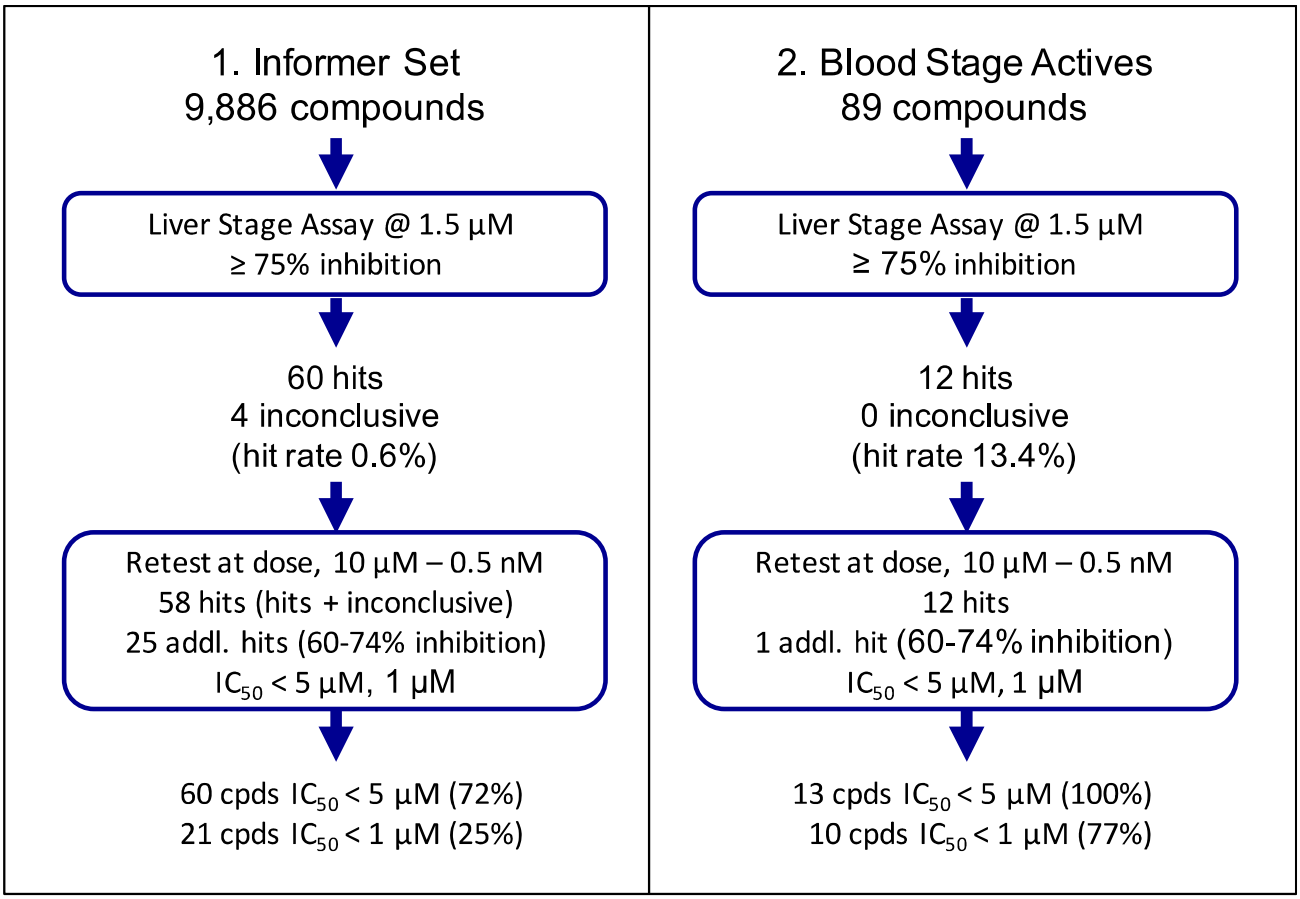

Figure S3. Overview of the Broad Diversity-Oriented Synthesis Library Screen
Screening pipeline for naïve informer set compounds and pre-selected erythrocytic-stage compounds. The hit rate for the naïve compounds set was 0.6%, while the hit rate for the pre-selected compounds was 13.4%.

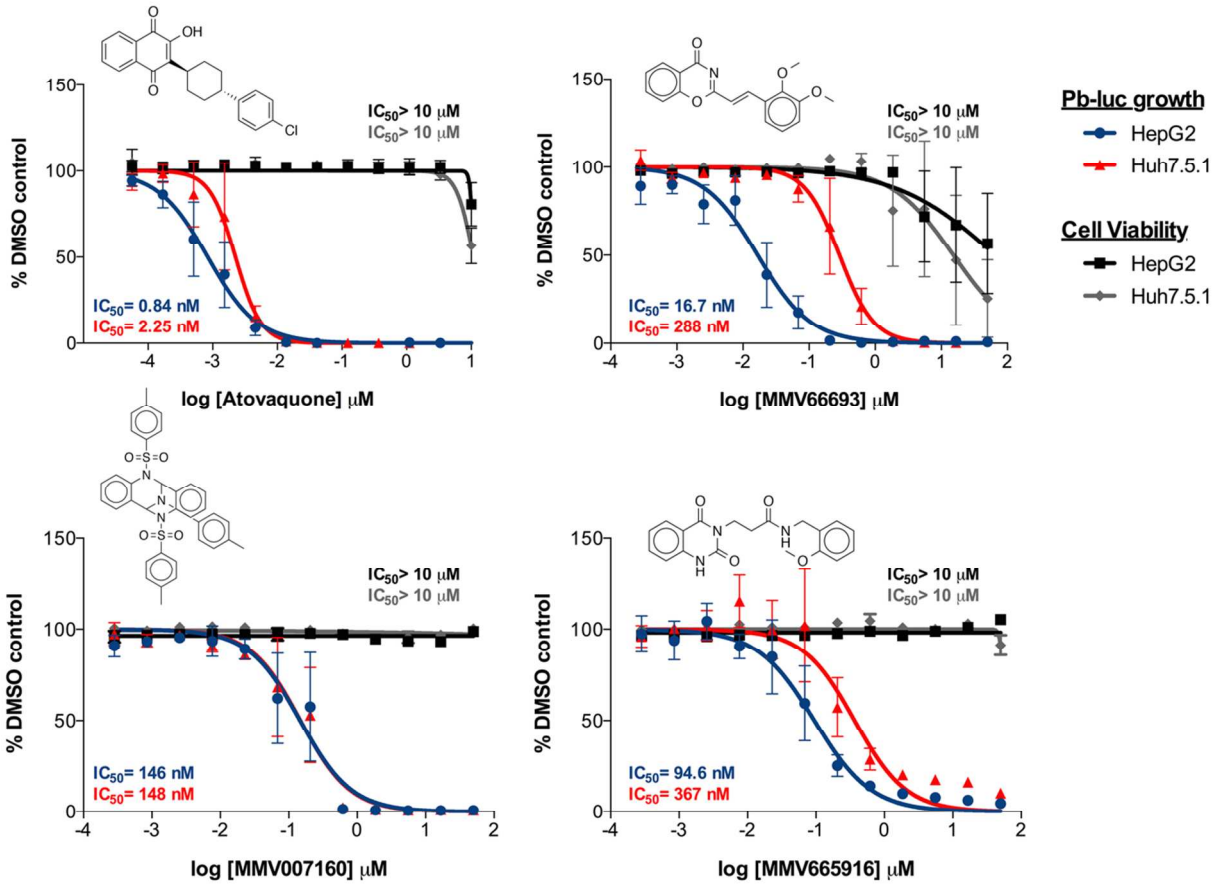

Figure S4. Contribution of different host cell lines to compound potency.
Serial dilutions of MMV666693, MMV007160, and MMV665916 were performed to calculate Pb-Luc growth in both HepG2 and Huh7.5.1 cell lines using the high-throughput luciferase-based assay. Cell viability was determined by monitoring HepG2 growth using a bioluminescent assay (Cell Titer Glo, Promega). Chemical structures for selected compounds are displayed.

Table S1. IC_{50} data for validation set of 50 antimalarials

Compound Class	Common Name	*P. berghei* (liver)		*P. falciparum* (blood)	
		IC_{50} (nM)	95% CI (nM)	IC_{50} (nM)	95% CI (nM)
4-aminoquinolines	***Naphthoquine****	***391***	214 to 714	***11.4***	***7.6 to 17.2***
	AQ-13	3160	1067 to 9355	8.7	ND
	Amodiaquine	1590	433 to 5842	>62500	ND
	Pyronaridine	6791	3037 to 12500	9.6	ND
	Piperaquine	>12500	ND	72	ND
	Hydroxychloroquine	>12500	ND	13.9	ND
	Chloroquine	>12500	ND	2.1	1.7 to 2.7
8-aminoquinolines	***Primaquine****	***705***	***321 to 1546***	***695***	***398 to 1212***
	NPC-1161B	3551	2141 to 5888	1320	855 to 2040
	Pamaquine	>12500	ND	>10000	8435 to 36010
	Tafenoquine	5860	3120 to 11000	2818	1992 to 3986
Amino alcohols	Halofantrine	2843	974 to 8294	1.5	1.2 to 1.9
	Mefloquine (+ RS)	2334	1348 to 4042	16.6	13.1 to 21.1
	Mefloquine (Racemic)	3059	1.520 to 6.159	<0.4	ND
	Quinidine	>12500	ND	2.9	2.3 to 3.8
	Quinine sulfate dihydrate	>12500	ND	12.3	4.2 to 35.8
	Lumefantrine	>12500	ND	7.3	5.9 to 9.1
Antibiotics	***Trimethoprim****	***132***	53 to 324	***5640***	***3396 to 9367***
	Thiostrepton	3127	175 to 12500	1908	950 to 3833
	Doxycyclin	11580	3258 to 12500	8822	5336 to 14590
	Cis-Mirincamycin	>12500	ND	7560	4457 to 12820
	Trans-Mirincamycin	>12500	ND	>10000	10250 to 36050
	Fosmidomycin	>12500	ND	>10000	5784 to 18320
	Clindamycin	>12500	ND	198	157 to 251
	Tetracycline	>12500	ND	>10000	13340 to 33130
	Azithromycin	>12500	ND	257	199 to 331
Antifolates	***P218.HCl****	***0.9162***	***0.6 to 1.4***	***0.4***	***0.3 to 0.4***
	Pyrimethamine*	***5.1***	***(Very wide)***	***19.9***	***12.1 to 32.7***
	Cycloguanil*	***7***	***3.8 to 12.9***	***3.1***	***2.4 to 3.9***
	Dapsone*	***2435***	***389 to 12500***	***>62500***	***ND***
	Chlorproguanil HCL	499.7	160.8 to 1553	4716	2697 to 8248
	Proguanil HCL	4560	2173 to 9569	6239	3426 to 11360
Endoperoxidases	***Artemisone****	***178.2***	***50.4 to 630***	***0,7***	***0.6to 0.8***
	Artemisinin*	***1716***	***379.9 to 7752***	***4.7***	***3.6 to 6.1***
	Artesunate*	***246***	***131 to 462***	***0.9***	***0.6 to 1.3***
	OZ277*	***1639***	***540.4 to 4972***	***2.3***	***1.9 to 2.7***
	Artemether*	***3144***	***1186 to 8334***	***<0.4***	***ND***
	Artenimol	1564	981.2 to 2494	<0.4	ND
	OZ439	5236	2774 to 9883	2.8	2.5 to 3.2
Others	***Atovaquone****	***0.25***	***0.2 to 0.3***	***<0.4***	***ND***
	Cycloheximide*	***20.7***	***10.6 to 40.4***	***1.8***	***1.2 to 2.7***
	Methylene Blue*	***95.6***	***47.3 to 193.2***	***7984***	***1308 to 48740***
	Pentamidine*	***286.1***	***130.8 to 626***	***0.5***	***0.2 to 1.7***
	Deferoxamine	>12500	ND	>10000	11830 to 33830
	Dehydroepiandroster-one sulphate	>12500	ND	>62500	ND
	N-acetyl-D-penicillamine	>12500	ND	>62500	ND
	Riboflavin	>12500	ND	>62500	ND

Sulfonamides	***Sulfadiazine****	***16***	***2.0 to 126***	>62500	***ND***
	Sulfadoxine*	***74***	***103 to 538***	>62500	***ND***
	Sulfamethoxazole*	***216***	***75 to 625***	***>10000***	***ND***

* Antimalarial compounds with exoerythrocytic-stage activity (Pb-Luc IC_{50} < 1000 nM)

Table S2. Anti-*Plasmodium cynomolgi* exoerythrocytic-stage activity in primary hepatocytes for selected MMV Malaria Box Compounds.

Small and large parasites are representative for hypnozoites and liver stage schizonts, respectively.

Compound	***P. cynomolgi*, primary hepatocytes (% inhibition normalized to untreated control)**					
	Small 0.1 µM	Small 1 µM	Small 10 µM	Large 0.1 µM	Large 1 µM	Large 10 µM
KAI407	-1	92	100	23	94	100
Primaquine	4	19	24	1	3	13
MMV006962	-8	11	17	34	25	10
MMV007160	17	-25	-11	17	14	0
MMV007224	30	20	67	23	43	96
MMV019066	10	11	3	3	-2	5
MMV665807	17	34	24	6	10	12
MMV665916	20	-13	-15	6	2	-13
MMV666101	4	19	11	7	11	17
MMV666693	29	16	30	22	-3	15