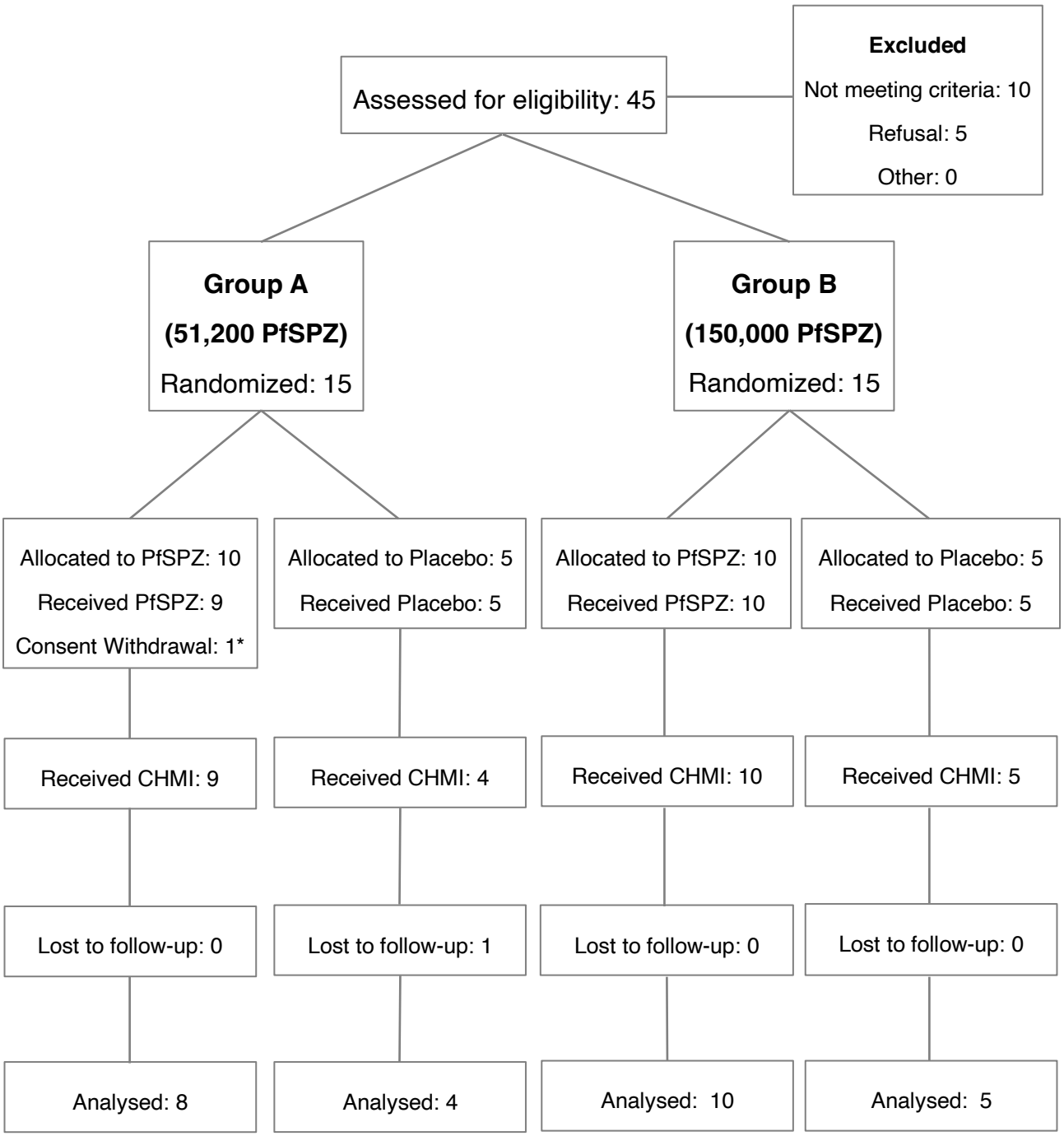
Appendix Borrmann *et al.*

Table of Contents:

Appendix Figure S1



* Participant received only the first PfSPZ injection

Appendix Table S1: Baseline characteristics of study population.

	Vaccinees		Placebo
	$5.12x10^4$ PfSPZ	**$1.5x10^5$ PfSPZ**	
	n=10	n=10	n=10
Sex			
Female	7	6	5
Male	3	4	5
Age (years)			
Mean (± S.D.)	26.9 (± 6.8)	28.1 (± 2.2)	24.7 (± 4.6)
Median	24	28	24
Min, Max	19, 36	26, 32	19, 32
BMI			
under 18.5	1	1	1
18.5-24.9	7	5	6
25.0-29.9	2	4	3
30.0 or over	0	0	0
Mean (± SD)	22.4 (± 3.5)	22.2 (± 3.2)	24 (± 3.4)
Min, Max	18, 28	18, 27	18, 29

Appendix Table S2: Most frequent related adverse events.

	Placebo	Vaccinees	
		$5.12x10^4$ PfSPZ	**$1.5x10^5$ PfSPZ**
	n=10	n=10	n=10
AEs related to immunization	**33**	**19**	**13**
	Headache 21% (7) • grade 1 (6) • grade 2 (1) Dizziness 12% (4) • grade 1 (4) Fatigue 5% (3) • grade 1(3) Tachycardia 5% (3) • grade 1 (3) Miscellaneous (48%)	Hypertension 31% (6) • grade 1 (3) • grade 2 (2) • grade 3 (1) Dizziness 21% (4) • grade 1 (4) Pruritus at injection site 10.5% (2) • grade 1 (2) Headache 10.5% (2) • grade 1 (1) • grade 2(1) Diarrhoea 10.5% (2) • grade 1 (2) Miscellaneous (16%)	Fatigue 23% (3) • grade 1 (2) • grade 2 (1) Neutropenia 30% (4) • grade 1 (3) • grade 2 (1) Miscellaneous (46%)

AEs related to CHMI	**75**	**44**	**37**
	Lymphopenia 13% (10) • grade 1 (5) • grade 2 (4) • grade 3 (1) Fever/Feverish 9% (7) • grade 1 (2) • grade 2 (4) • grade 3 (1) Headache 12% (9) • grade 1 (6) • grade 2 (3) Fatigue 9% (7) • grade 1 (5) • grade 2 (2) Nausea 5% (4) • grade 1 (4) Sweating 5% (4) • grade 1 (3) • grade 2 (1) Miscellaneous (54.6%)	Headache 16% (7) • grade 1 (5) • grade 2 (2) Lymphopenia 9% (4) • grade 1 (2) • grade 2 (2) Fatigue 9% (4) • grade 1 (3) • grade 2 (1) Diarrhoea 7% (3) • grade 1 (3) Miscellaneous (59%)	Headache 16% (6) • grade 1 (4) • grade 2 (2) Neutropenia 16% (6) • grade 1 (4) • grade 2 (2) Fatigue 16% (6) • grade 1 (5) • grade 2 (1) Lymphopenia 13% (5) • grade 1 (3) • grade 2 (2) Miscellaneous (38%)

Appendix Table S3: Differentially recognized antigens, expression profiles and inclusion into subunit vaccine strategies

				mRNA expression [1-6]			Protein expression [4-8]			
Protein	Gene ID	Description	Surface	SPZ	LS	BS	SPZ	LS	BS	Subunit vaccine
Liver stage antigen 1 (LSA1)	PF3D7_1036400	liver stage antigen	-	-	-	-	-	+ [9]	-	yes [10]
Liver specific protein 2 (LISP2)	PF3D7_0405300	exported to hepatocyte	-	-	+	-	-	+	-	-
Merozoite surface protein 5 (MSP5)	PF3D7_0206900	duplicated antigen	yes	+	-	+	+	-	+	yes [11]
Circumsporozoite protein (CSP)	PF3D7_0304600	sporozoite surface, yes dominant antigen	+	-	-		+	-	-	yes [12]
Repetitive interspersed families of polypeptides (Rifin; RIF)	PF3D7_1040800	variant antigen	?	+	-	-	+	-	-	-
Erythrocyte binding antigen 181 (EBA181)	PF3D7_0102500	parasite invasion	yes	+	+	+	+	+	+	-
Parasitophorous vacuole membrane protein S16 (Pfs16)	PF3D7_0406200	sexual stage/sporozoite-specific antigen	yes	+	-	+	+	-	+	yes [13]
Dynactin subunit 2 (DCTN2/p50)	PF3D7_1346000	dynein anchor protein	-	+	-	-	+	-	-	-
Liver stage antigen 3 (LSA3)	PF3D7_0220000	ubiquitous antigen	-	+	+	+	+	+	+	yes [14]
Endoplasmin (GRP94/HSP90)	PF3D7_1222300	ER-resident chaperone	-	+	+	+	+	+	+	-
Reticulocyte binding protein 2 homologue b (Rh2b)	PF3D7_1335300	merozoite invasion yes	-	+	+	+	-	+	+	yes [15]
hypothetical protein	PF3D7_0706100	-	?	+	-	+	+	-	-	-

Blue and red denote antigens enriched in PfSPZ-CVac (CQ) and PfSPZ-CVac (AP) immune sera, respectively.

SPZ, salivary gland sporozoites; LS, liver stages; BS, asexual blood stages.

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Appendix Table S4: Numbers

Figure 1A
Images of liver stages in cultured hepatoma cells

group	untreated
n	2

one experiment, technical replicates, 2 wells per group

Figure 1B
% blood infection-negative mice

group	control
n	5

Figure 1C
relative levels *Pb*18s rRNA

group	atovaquone
n	5

one set of experiments

Figure 1D
relative level *Pb*18s rRNA

group	atovaquone-proguanil
n	6

one set of experiments

Figure 2A
% blood infection-negative mice

group	control
n	14

Figure 2B
relative level *Pb*18s rRNA

group	control
n	6

one set of experiments

Figure 2C
% $IFN\gamma^{+}$ $CD11a^{hi}$ $CD8^{+}$ T cells

group	control
n	6

biological replicates; one set of experiments;
this experiment also generated data for EV4 A-E

Figure 2D
Reciprocal antibody titers

group	control
n	5

biological replicates; one set of experiments

Figure EV1A

Images of liver stages in cultured hepatoma cells

group	WT-irradiated
n	3

one set of experiments, technical replicates (3 wells per group)

Figure EV1B

Liver stage volume in % of untreated

group	untreated
n	41

one set of experiments, same as in 1A, technical replicates (2 wells per group)

Figure EV1C

Parasite numbers

group	untreated
n	2

one set of experiments, same as in 1A, technical replicates (2 wells per group)

Figure EV2A

% blood infection-negative mice

group	atovaquone-proguanil *ex vivo*
n	5

biological replicates; one set of experiments

Figure EV2B

% blood infection-negative mice

group	atovaquone-proguanil treated
n	5

biological replicates; one set of experiments

Figure EV2C

Parasitemia

group	atovaquone-proguanil treated
n	5

biological replicates; one set of experiments

Figure EV4A

No. of $CD8^+$ $CD62L^-$ T cells

group	control
n	6 (18)

one set of experiment, same as EV4 B-E and Fig 2C; biological, (technical) replicates

Figure EV4B

TRAP No. of IFNy in $CD8^+CD11a^{hi}$ cells

group	control
n	6

biological replicates; one set of experiments

Figure EV4C

S20 No. of IFNy in $CD8^{+}CD11a^{hi}$ cells

group	control
n	6

biological replicates; one set of experiments

Figure EV4D

S20 % of IFNy in $CD8^{+}CD11a^{hi}$ cells

group	control
n	6

biological replicates; one set of experiments

Figure EV 4 E

no peptide control % of IFNy in $CD8^{+}CD11a^{hi}$cells

group	control
n	6

biological replicates; one set of experiments

Appendix Table S5: Exact levels of all *p* values

Figure	Groups		Significant?	P-value		Test
Figure 1B						
	Kaplan Meier estimator	control *vs.* atovaquone	Yes	****	<0,0001	Log rank (Mantel-Cox) test
	Kaplan Meier estimator	control *vs.* atovaquone-proguanil	Yes	****	<0,0001	Log rank (Mantel-Cox) test
Figure 1C						
	Relative levels of *Pb*18s rRNA	control *vs.* atovaquone	Yes	**	0,0079	Mann-Whitney U test
Figure 1D						
	Relative levels of *Pb*18s rRNA	control *vs.* atovaquone-proguanil	Yes	**	0,0022	Mann-Whitney U test
Figure 2A						
	Kaplan Meyer estimator	control *vs.* atovaquone 3x	Yes	****	<0,0001	Log rank (Mantel-Cox) test
		control *vs.* atovaquone 2x	Yes	****	<0,0001	Log rank (Mantel-Cox) test
		control *vs.* atovaquone-proguanil	Yes	****	<0,0001	Log rank (Mantel-Cox) test
Figure 2B						
	Relative levels of *Pb*18s rRNA	control *vs.* atovaquone-proguanil	Yes	**	0,0043	Mann-Whitney U test
	Relative levels of *Pb*18s rRNA	control *vs.* atovaquone 3x	Yes	**	0,0022	Mann-Whitney U test
	Relative levels of *Pb*18s rRNA	control *vs.* atovaquone 2x	Yes	*	0,0159	Mann-Whitney U test
Figure 2C						
	% $IFN\gamma^{+}$ $CD11a^{hi}$ $CD8^{+}$ T-cells	control *vs.* atovaquone 3x	Yes	**	0,0022	Mann-Whitney U test
		control *vs.* atovaquone-proguanil 2x	Yes	**	0,0043	Mann-Whitney U test
Figure 2D						
	Reciprocal antibody titers	control *vs.* atovaquone 3x	Yes	**	0,0043	Mann-Whitney U test
		control *vs.* atovaquone-proguanil 2x	Yes	**	0,0079	Mann-Whitney U test
Figure EV1B						
	Liver stage volume in % of untreated	control *vs.* atovaquone	Yes	****	<0,0001	Mann-Whitney U test
		control *vs.* atovaquone-proguanil	Yes	****	<0,0001	Mann-Whitney U test

Figure EV1C						
	Parasite numbers	control *vs.* atovaquone	No	n.s.	0,6667	Mann-Whitney U test
		control *vs.* atovaquone-proguanil	No	n.s.	0,3333	Mann-Whitney U test
Figure EV2A						
	Kaplan Meyer estimator					
		control *vs.* atovaquone-*proguanil ex vivo*	No	n.s.	0.1967	Log rank (Mantel-Cox) test
Figure EV2B						
	Kaplan Meyer estimator					
		control vs. atovaquone-proguanil treated	No	n.s.	0.9999	Log rank (Mantel-Cox) test
Figure EV4A						
	No. of $CD8^+$ $CD62L^-$ T cells	control *vs.* atovaquone 3x	Yes	****	<0,0001	Mann-Whitney U test
		control *vs.* atovaquone-proguanil 2x	Yes	****	<0,0001	Mann-Whitney U test
Figure EV4B						
	TRAP No. of IFNy in $CD8^+CD11a^{hi}$ cells	control *vs.* atovaquone 3x	Yes	**	0,0022	Mann-Whitney U test
		control *vs.* atovaquone-proguanil 2x	Yes	**	0,0043	Mann-Whitney U test
Figure EV4C						
	S20 No. of IFNy in $CD8^+CD11a^{hi}$ cells	control *vs.* atovaquone 3x	Yes	**	0,0043	Mann-Whitney U test
		control *vs.* atovaquone-proguanil 2x	Yes	**	0,0043	Mann-Whitney U test
Figure EV4D						
	S 20 % of IFNy in $CD8^+CD11a^{hi}$ cells	control *vs.* atovaquone 3x	Yes	**	0,0022	Mann-Whitney U test
		control *vs.* atovaquone-proguanil 2x	Yes	**	0,0043	Mann-Whitney U test
Figure EV4E						
	No peptide control % of IFNy in $CD8^+CD11a^{hi}$ cells	control *vs.* atovaquone 3x	No	n.s.	0,6991	Mann-Whitney U test
		control *vs.* atovaquone-proguanil 2x	No	n.s.	0,5346	Mann-Whitney U test