Supplementary Materials and Methods:

Study procedures and monitoring

Study participants were observed for adverse events (AEs) for at least 60 minutes after each PfGAP3KO administration. Solicited local and systemic AEs were recorded on a memory aid beginning on the day of first vaccine administration and continuing through 28 days after the last administration. During the vaccination phase, scheduled clinical laboratory evaluations for safety (white blood cell count [WBC], hemoglobin, platelet count, creatinine, and alanine aminotransferase [ALT]) were performed on venous blood collected at 3, 7, and 14 days after the first vaccination, and at 14 days after each subsequent vaccination, and as clinically indicated. Unsolicited AEs were collected from the day of first vaccination through 28 days after last vaccination, and serious adverse events (SAEs) were collected from the day of first PfGAP3KO vaccination through the end of study follow-up.

Study participants were monitored for possible breakthrough peripheral parasitemia with qRT-PCR testing of samples collected at 7, 8, 10, 12, 14, and 16 days after the first vaccination, at 10 and 14 days after each subsequent vaccination, and additionally at 7 days after the last vaccination, and as clinically indicated from the time of first vaccination through 28 days after the last vaccination. The study was designed so that if a study participant had signs or symptoms consistent with malaria but was qRT-PCR negative, a thick blood smear (TBS) would be performed from the sample collected for the qRT-PCR assay. Positive qRT-PCR tests were also to be followed by repeat testing as soon as possible. The study protocol denoted that any study participants with confirmed parasitemia following vaccination (defined as a positive TBS or two

positive qRT-PCR assays with parasite densities of ≥20 estimated parasites/mL from blood samples obtained at least six hours apart) would be treated for malaria and followed for safety.

Four weeks after the last PfGAP3KO administration, all study participants who completed the immunization phase and a group of six malaria-naïve infectivity controls were challenged on the same day (defined as Day 1) with wild-type Pf NF54 sporozoites. On the day of challenge, study participants received five infectious *A. stephensi* mosquito bites using standard CHMI procedures. Approximately twenty-six weeks (six months) after the first challenge, all previously-protected study participants from Study Arms 1 and 2 plus a further six malaria-naïve infectivity controls received five additional infectious *A. stephensi* mosquito bites as well by standard CHMI procedures.

After each CHMI, study participants were followed as outpatients and were closely monitored for the onset of malaria infection through Study Day 29 after CHMI by clinical assessment and by same day qRT-PCR testing. For all study participants, qRT-PCR testing was performed daily starting six days after CHMI (thus defined as Study Day 7) and continuing for the next nine days (through Study Day 16) and then on Study Days 19, 22, 25, and 29 and as clinically indicated. Documented parasitemia, defined as a positive qRT-PCR assay with an estimated parasite density of ≥ 20 parasites/mL, or a positive TBS, on a blood sample obtained within 28 days following CHMI, resulted in antimalarial treatment at the next day's study visit. Such infections were defined as resolved after treatment when a negative qRT-PCR on a blood sample was obtained on Day T+2 or later. Study participants without documented parasitemia through Study

Day 29 were presumptively treated for malaria starting on Day 30 and were considered to be protected from the CHMI.

For study participants in Study Arms 1 and 2 without documented parasitemia, additional post-CHMI follow-up occurred on Day 90 after the first CHMI and on Days 90 and 180 after the second CHMI. For study participants in Study Arms 1 and 2 with documented parasitemia after the first CHMI or who were discontinued for other reasons after the first CHMI, and for the first CHMI infectivity controls, additional follow-up occurred on Days 90 and 180. SAEs were recorded from the day of CHMI through the end of study follow up and clinical laboratory evaluations for safety were performed on Day 29 and as clinically indicated (all study participants) and, for study participants with documented parasitemia, on the day malaria treatment was initiated and three days after malaria treatment was initiated.

Study participants and eligibility criteria

Study participants were eligible to participate in the trial if they were healthy, malaria-naïve men or non-pregnant women aged 18-50 years in good general health as demonstrated by medical history, physical examination, ECG, and laboratory assessment within 90 days of enrollment. Complete criteria are listed in **Table S3**. Study participants were required to have a low risk of coronary heart disease, with evaluation of cardiac risk based on NHANES 1 screening criteria and a non-significant ECG as read by an independent cardiologist. Laboratory values for eligibility included hemoglobin, WBC, platelets, and creatinine within normal laboratory ranges, and ALT, AST, bilirubin and alkaline phosphate within 1.25 times the upper limit of normal defined by the diagnostic laboratory normal ranges. Normal urine values were defined as

negative urine glucose, negative or trace protein and negative or trace hemoglobin. Study participants required negative HIV-1 and 2, HBsAg and anti-HCV results. A negative pregnancy test was required for women study participants.

Study participants were not eligible to participate in the trial if they recently traveled or plan to travel to a malaria-endemic area, had a positive diagnosis of malaria, received malaria chemoprophylaxis prior to challenge, and/or recently received investigational malaria vaccine. Study participants were excluded if they received antibiotics with an anti-malarial effect, medications that would interact with Malarone® (atovaquone/proguanil) and/or chloroquine, and certain immunosuppressive medications. History of anaphylaxis, severe allergic reactions to mosquito bites, and history of psoriasis or porphyria were also exclusion criteria for the trial. Study participants with a psychiatric condition that may have precluded compliance to the protocol including psychosis, ongoing risk for suicide or history of suicide attempt within the past three years prior to enrollment, were also excluded.

Objectives and endpoint measures

The primary study objectives were to monitor safety, tolerability and efficacy of PfGAP3KO in healthy malaria-naïve adults following vaccination and CHMI. Secondary objectives included assessment of humoral immune responses and inhibition of sporozoite infection after PfGAP3KO administration. Primary endpoint measures were frequency of solicited and unsolicited adverse events, frequency of serious adverse events, and patent parasitemia via TBS. Secondary endpoint measures included CSP antibody titer by ELISA and percent inhibition of in vitro sporozoite cell invasion and traversal by immune sera after PfGAP3KO administration.

Unsolicited adverse events, solicited adverse events, and serious adverse events were tabulated by relationship, severity, and visit day for all MedDRA body systems and preferred terms. For these summaries, each extant AE or SAE was counted once per study participant at the maximum occurring severity or strongest causal relationship to the study treatment. An independent, chartered Safety Monitoring Committee and a separate Medical Monitor provided safety oversight.

Screening and safety laboratory assays were conducted by a local CLIA/CAP-certified Clinical Laboratory licensed in the State of Washington (Quest). Immunology endpoint assays were performed at Seattle Children's Research Institute (formerly CID Research) and Fred Hutchinson Cancer Research Center under established SOPs. Electrocardiograms (ECG) performed for cardiac risk assessments were performed by clinical trial staff and reviewed by a board-certified cardiologist contracted from the Department of Medicine, Division of Cardiology at the University of Washington.

Production of the PfGAP3KO parasite

The PfGAP3KO parasite was engineered to remove three genes necessary for early liver stage development, *P52, P36* and *SAP1* from the haploid parasite genome. Although essential for the liver stage, there genes are not essential for any other life cycle and thus infectious mosquitoes can be produced harboring PfGAP3KO. The gene deletion procedure and plasmids used for gene deletion were described previously (*53, 54*). The parent strain was Pf NF54 supplied by WRAIR.

The PfGAP3KO Master Cell Bank (MCB) and Working Cell Bank (WCB) was produced following Good Manufacturing Practices (cGMP) and quality systems appropriate for Phase 1 manufacturing and filed under an FDA Investigational New Drug application. The MCB underwent full product release testing, including safety and identity testing, testing for adventitious agents and testing to demonstrate sensitivity to atovaquone, chloroquine, quinine and doxycycline. For the experimental exposure, PfGAP3KO were thawed from the WCB and expanded in normal human erythrocytes using standard culture medium, containing 6% human erythrocytes and 10% normal human serum. All blood products used for malaria and mosquito culturing were obtained from FDA-approved commercial blood suppliers and were commercially tested for HIV, HBV, HCV, HTLV I/II and syphilis. Laboratory-born and reared *A. stephensi* mosquitoes were infected by allowing them to feed through membranes on cultures derived from the PfGAP3KO WCB containing a large proportion of gametocytes. Following mosquito infections, midgut and salivary gland infection rates were assessed. Infected mosquitoes were released for experimental infection/challenge following evaluation of the salivary gland sporozoite load from a representative sampling of the infected mosquitoes 1-2 days prior to administration.

Supplementary Figures

Figure S1. Study participant-specific qRT-PCR results and systemic AEs during vaccination phase. Solicited systemic AEs are displayed by maximum grade in the timeline below each study participant's qRT-PCR results. qRT-PCR results show a complete absence of breakthrough parasitemia in all subjects at all vaccination time points as indicated by zero data points above the baseline. Arrows denote vaccination days (Arm 1: Days 1, 29, 57, 85, and 141; Arm 2: Days 1, 29, 85).

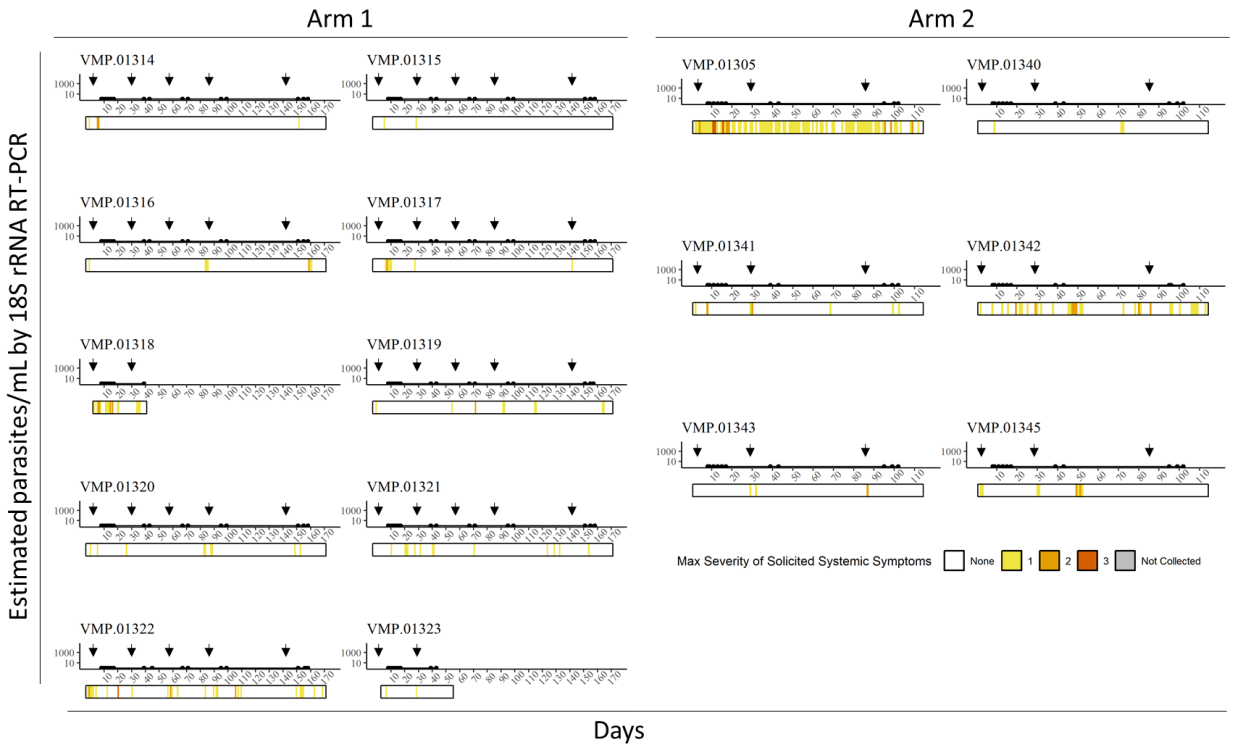


Figure S2. Trends in chemistry laboratory parameters by treatment group across all vaccination and CHMI time points. Laboratory abnormalities (high ALT, high creatinine) are shown for study participants in Arm 1, Arm 2, or the CHMI-only infectivity control groups. Grading is shown as in the key (green normal, yellow Grade 1, orange Grade 2).

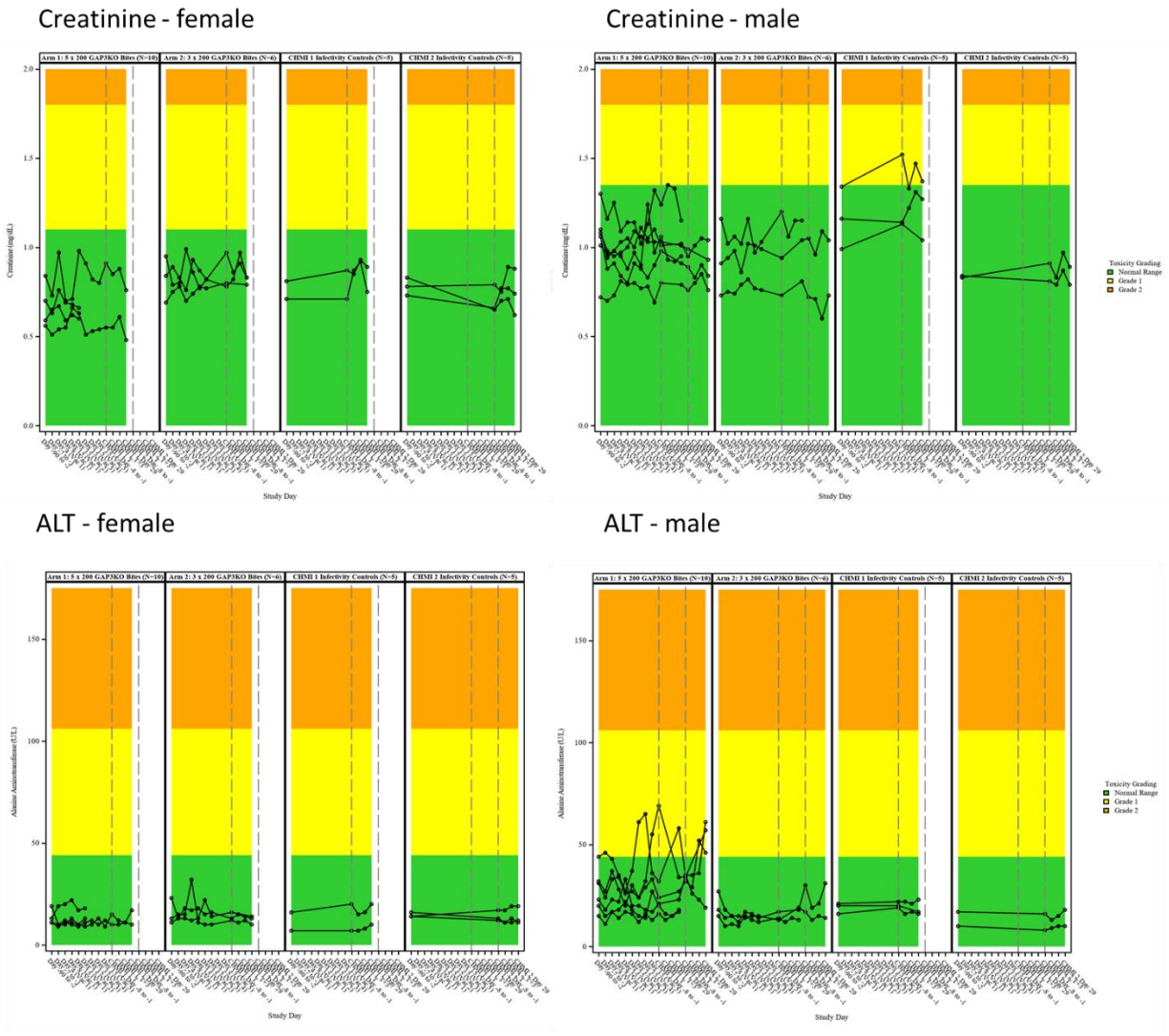


Figure S3. Trends in hematology laboratory parameters by treatment group across all vaccination and CHMI time points. Laboratory abnormalities (low hemoglobin, low or high white blood cells, low or high platelets) are shown for study participants in Arm 1, Arm 2, or the CHMI-only infectivity control groups. Grading is shown as in the key (green normal, yellow Grade 1, orange Grade 2).

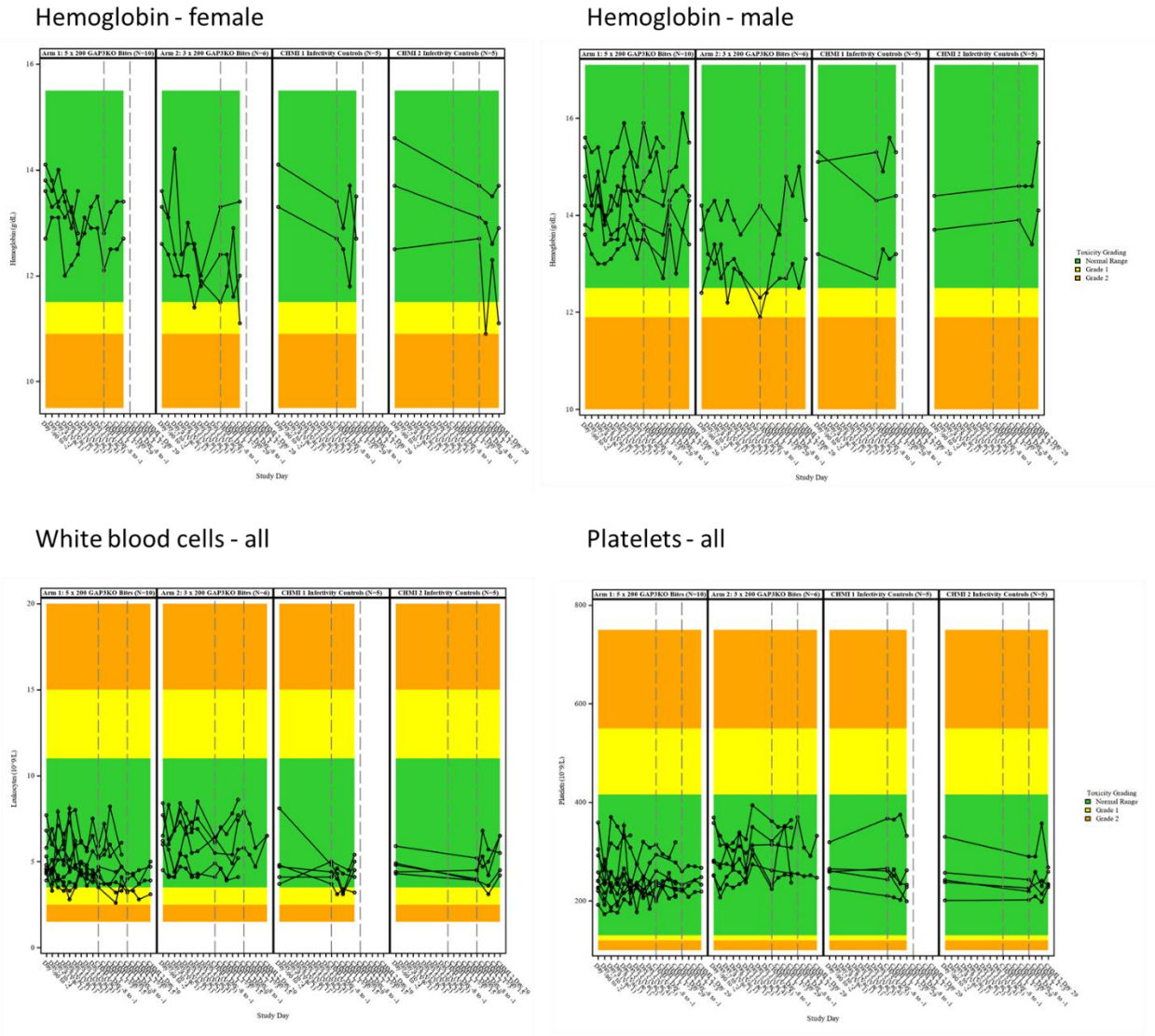
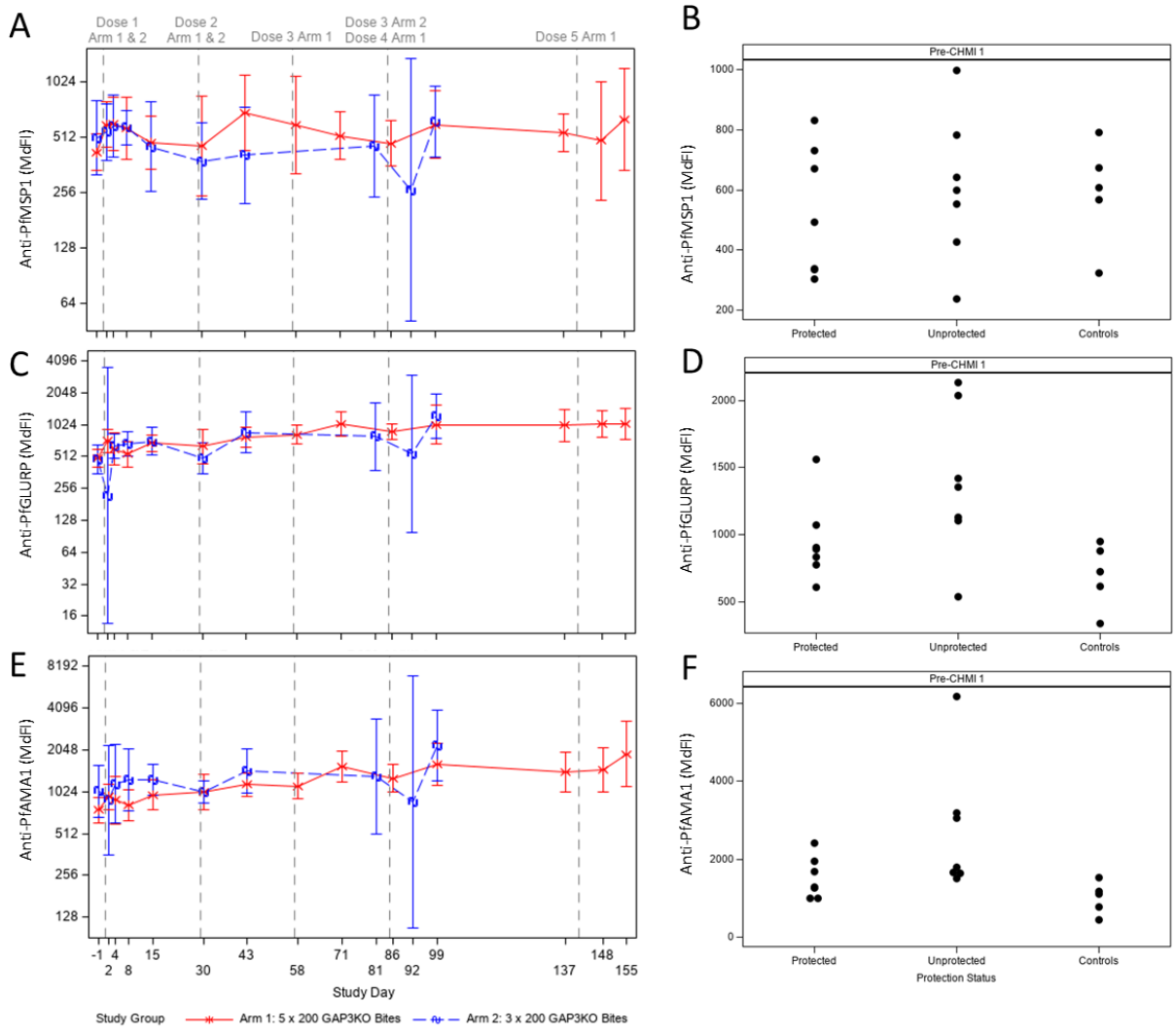


Figure S4. Antibody immunogenicity to blood stage *P. falciparum* antigens. Vaccine-induced antibodies recognizing full-length Pf MSP1 (A), Pf GLURP (C), and Pf AMA1 (E) were quantified by ELISA at each time point as shown. Arm 1 is shown in the solid red line, and Arm 2 is shown in the dashed blue line. Antibody levels are expressed in median fluorescence intensity (MdFI). Vertical dashed lines correspond to immunization procedures as noted at the top. Each data point represents the geometric mean for each Study Arm at each time point; error bars represent 95% confidence intervals. When pre-CHMI antibody levels were compared for protected vs. unprotected vs. naïve controls for Pf MSP1 (B), Pf GLURP (D), and Pf AMA1 (F), there was no significant difference between protected, unprotected, and control groups for responses to any of the three antigens.


A
Dose 1 Arm 1 & 2
Dose 2 Arm 1 & 2
Dose 3 Arm 1
Dose 3 Arm 2 Dose 4 Arm 1
Dose 5 Arm 1
Anti-PfMSP1 (MdFI)
1024
512
256
128
64
B
Pre-CHMI 1
1000
800
600
400
200
Protected
Unprotected
Controls
C
Anti-PfGLURP (MdFI)
4096
2048
1024
512
256
128
64
32
16
D
Pre-CHMI 1
2000
1500
1000
500
Protected
Unprotected
Controls
E
Anti-PfAMA1 (MdFI)
8192
4096
2048
1024
512
256
128
-1
2
4
8
15
30
43
58
71
81
86
92
99
137
148
155
Study Day
Study Group
Arm 1: 5 x 200 GAP3KO Bites
Arm 2: 3 x 200 GAP3KO Bites
F
Pre-CHMI 1
6000
4000
2000
0
Protected
Unprotected
Controls
Protection Status

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Supplementary Tables

Table S1. Study participant demographics.

		Arm 1: 5 x 200 GAP3KO Bites (N=10)		Arm 2: 3 x 200 GAP3KO Bites (N=6)		CHMI 1 Infectivity Controls (N=5)		CHMI 2 Infectivity Controls (N=5)		All Subjects (N=26)	
Variable	**Characteristic**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Sex	Male	6	60	3	50	3	60	2	40	14	54
	Female	4	40	3	50	2	40	3	60	12	46
Ethnicity	Not Hispanic or Latino	10	100	6	100	4	80	5	100	25	96
	Hispanic or Latino	-	-	-	-	1	20	-	-	1	4
	Not Reported	-	-	-	-	-	-	-	-	-	-
	Unknown	-	-	-	-	-	-	-	-	-	-
Race	American Indian or Alaska Native	-	-	-	-	-	-	-	-	-	-
	Asian	1	10	-	-	-	-	-	-	1	4
	Native Hawaiian or Other Pacific Islander	-	-	-	-	-	-	-	-	-	-
	Black or African American	2	20	-	-	-	-	-	-	2	8

		Arm 1: 5 x 200 GAP3KO Bites (N=10)		Arm 2: 3 x 200 GAP3KO Bites (N=6)		CHMI 1 Infectivity Controls (N=5)		CHMI 2 Infectivity Controls (N=5)		All Subjects (N=26)	
Variable	**Characteristic**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
	White	7	70	6	100	5	100	5	100	23	88
	Multi-Racial	-	-	-	-	-	-	-	-	-	-
	Unknown	-	-	-	-	-	-	-	-	-	-

Note: N=Number of subjects enrolled in the study.

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Table S2. Number and percentage of subjects experiencing solicited AEs

		Arm 1: 5 x 200 GAP3KO Bites (N=10)			Arm 2: 3 x 200 GAP3KO Bites (N=6)		
Symptom	**Severity**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Any Symptoms							
Any Symptom	None	0	0	0, 28	0	0	0, 39
	Mild	0	0	0, 28	0	0	0, 39
	Moderate	7	70	40, 89	4	67	30, 90
	Severe	3	30	11, 60	2	33	10, 70
Systemic Symptoms							
Any Systemic Symptom	None	0	0	0, 28	0	0	0, 39
	Mild	4	40	17, 69	1	17	3, 56
	Moderate	5	50	24, 76	4	67	30, 90
	Severe	1	10	2, 40	1	17	3, 56
Arthralgia	None	6	60	31, 83	6	100	61, 100
	Mild	2	20	6, 51	0	0	0, 39
	Moderate	2	20	6, 51	0	0	0, 39
	Severe	0	0	0, 28	0	0	0, 39
Chills	None	6	60	31, 83	3	50	19, 81
	Mild	2	20	6, 51	2	33	10, 70
	Moderate	2	20	6, 51	1	17	3, 56
	Severe	0	0	0, 28	0	0	0, 39

		Arm 1: 5 x 200 GAP3KO Bites (N=10)			Arm 2: 3 x 200 GAP3KO Bites (N=6)		
Symptom	**Severity**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Diarrhea	None	6	60	31, 83	5	83	44, 97
	Mild	4	40	17, 69	0	0	0, 39
	Moderate	0	0	0, 28	0	0	0, 39
	Severe	0	0	0, 28	1	17	3, 56
Elevated Oral Temperature	None	10	100	72, 100	5	83	44, 97
	Mild	0	0	0, 28	0	0	0, 39
	Moderate	0	0	0, 28	1	17	3, 56
	Severe	0	0	0, 28	0	0	0, 39
Fatigue	None	2	20	6, 51	1	17	3, 56
	Mild	6	60	31, 83	0	0	0, 39
	Moderate	2	20	6, 51	4	67	30, 90
	Severe	0	0	0, 28	1	17	3, 56
Headache	None	1	10	2, 40	0	0	0, 39
	Mild	7	70	40, 89	3	50	19, 81
	Moderate	2	20	6, 51	2	33	10, 70
	Severe	0	0	0, 28	1	17	3, 56
Malaise	None	2	20	6, 51	1	17	3, 56
	Mild	4	40	17, 69	4	67	30, 90
	Moderate	4	40	17, 69	1	17	3, 56

		Arm 1: 5 x 200 GAP3KO Bites (N=10)			Arm 2: 3 x 200 GAP3KO Bites (N=6)		
Symptom	**Severity**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
	Severe	0	0	0, 28	0	0	0, 39
Myalgia	None	3	30	11, 60	4	67	30, 90
	Mild	5	50	24, 76	1	17	3, 56
	Moderate	2	20	6, 51	1	17	3, 56
	Severe	0	0	0, 28	0	0	0, 39
Nausea/Vomiting	None	6	60	31, 83	2	33	10, 70
	Mild	2	20	6, 51	2	33	10, 70
	Moderate	1	10	2, 40	1	17	3, 56
	Severe	1	10	2, 40	1	17	3, 56
Local Symptoms							
Any Local Symptom	None	0	0	0, 28	0	0	0, 39
	Mild	0	0	0, 28	0	0	0, 39
	Moderate	8	80	49, 94	5	83	44, 97
	Severe	2	20	6, 51	1	17	3, 56
Edema (Qualitative)	None	0	0	0, 28	0	0	0, 39
	Mild	9	90	60, 98	6	100	61, 100
	Moderate	1	10	2, 40	0	0	0, 39
	Severe	0	0	0, 28	0	0	0, 39

		Arm 1: 5 x 200 GAP3KO Bites (N=10)			Arm 2: 3 x 200 GAP3KO Bites (N=6)		
Symptom	**Severity**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Edema (Quantitative)	None	0	0	0, 28	0	0	0, 39
	Mild	0	0	0, 28	0	0	0, 39
	Moderate	8	80	49, 94	6	100	61, 100
	Severe	2	20	6, 51	0	0	0, 39
Erythema (Qualitative)	None	0	0	0, 28	0	0	0, 39
	Mild	10	100	72, 100	6	100	61, 100
	Moderate	0	0	0, 28	0	0	0, 39
	Severe	0	0	0, 28	0	0	0, 39
Erythema (Quantitative)	None	0	0	0, 28	0	0	0, 39
	Mild	0	0	0, 28	0	0	0, 39
	Moderate	8	80	49, 94	5	83	44, 97
	Severe	2	20	6, 51	1	17	3, 56
Induration (Qualitative)	None	0	0	0, 28	1	17	3, 56
	Mild	10	100	72, 100	5	83	44, 97
	Moderate	0	0	0, 28	0	0	0, 39
	Severe	0	0	0, 28	0	0	0, 39
Induration (Quantitative)	None	0	0	0, 28	1	17	3, 56
	Mild	2	20	6, 51	0	0	0, 39
	Moderate	8	80	49, 94	5	83	44, 97

		Arm 1: 5 x 200 GAP3KO Bites (N=10)			Arm 2: 3 x 200 GAP3KO Bites (N=6)		
Symptom	**Severity**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
	Severe	0	0	0, 28	0	0	0, 39
Pain	None	4	40	17, 69	3	50	19, 81
	Mild	5	50	24, 76	2	33	10, 70
	Moderate	1	10	2, 40	1	17	3, 56
	Severe	0	0	0, 28	0	0	0, 39
Pruritus	None	0	0	0, 28	0	0	0, 39
	Mild	6	60	31, 83	4	67	30, 90
	Moderate	4	40	17, 69	2	33	10, 70
	Severe	0	0	0, 28	0	0	0, 39
Tenderness	None	2	20	6, 51	2	33	10, 70
	Mild	8	80	49, 94	4	67	30, 90
	Moderate	0	0	0, 28	0	0	0, 39
	Severe	0	0	0, 28	0	0	0, 39

Notes: N=Number of subjects in the safety population who received the specified dose.

Local quantitative symptoms that were present, reported not to extend to the upper arm, but for which a measurement was not provided, are reported as moderate severity.

Table S3. Eligibility criteria for study participants

Inclusion Criteria Subjects must have met all of the following inclusion criteria to be eligible for enrollment in this study:
1. 18 through 50 years of age, inclusive.
2. Able and willing to participate for the duration of the study and able to understand and comply with planned study procedures.
3. Able and willing to provide written (not proxy) informed consent.
4. Provided informed consent before initiation of any study procedure, correctly answered ≥ 80% of questions* on the post consent quiz and was available for all study visits. *Subjects who scored less than 80% may have retaken the quiz one time and were excluded if the second test was also less than 80%.
5. Was in good health, as judged by the investigator, and determined by medical history and physical examination. * Existing medical diagnoses or conditions (except those in the Subject Exclusion Criteria) must have been deemed as stable. A stable medical condition was defined as no change in prescription medication, dose, or frequency of medication in the last three months (90 days) and health outcomes of the specific disease were considered to be within acceptable limits in the last six months (180 days). Any changes due to change of health care

provider, insurance company, or that were done for financial reasons, as long as in the same class of medication, were not considered a violation of this inclusion criterion. Any change in prescription medication due to improvement of a disease outcome, as determined by the site principal investigator (PI) or appropriate sub-investigator, was not considered a violation of this inclusion criterion. Subjects were allowed to be on chronic or as needed (prn) medications if, in the opinion of the site PI or appropriate sub-investigator, they posed no additional risk to subject safety or assessment of solicited events and immunogenicity.
6. Women of childbearing potential* must have had a negative serum pregnancy test at screening and a negative urine pregnancy test prior to each mosquito exposure.** *Not sterilized via bilateral tubal ligation, bilateral oophorectomy, or hysterectomy, or, if menopausal, still menstruating or < 1 year of the last menses. **Study vaccination or CHMI.
7. Women of childbearing potential must have used a highly effective form of contraception* in the 30 days prior to their first mosquito exposure.** *Highly effective single forms of contraception included abstinence from sexual activity that could lead to pregnancy, monogamous relationship with vasectomized partner who had been vasectomized for six months or more prior to enrollment, successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented confirmation test at least three months after the procedure or use of effective intrauterine devices or the contraceptive implant (Nexplanon). If none of the highly effective single

forms of contraception were used, a combination of an acceptable barrier method and an acceptable hormonal method was required to be used. Acceptable barrier methods included condom (male or female) and a spermicide (cream, film, foam, or gel), diaphragm or cervical cap with spermicide, and the birth control sponge. Acceptable hormonal methods included birth control patch, shot (Depo-Provera), and pills, and the vaginal ring (NuvaRing). ** Study vaccination or CHMI.
8. Women of childbearing potential must have agreed to continue use of a highly effective form of contraception through 90 days after their last mosquito exposure.
9. Women must not have been breastfeeding or have had any plans to start breastfeeding at any time before the end of study follow up.
10. At low (≤10%) 5-year cardiovascular risk.* *Per the risk prediction method of Gaziano (PMID 18342687). Risk for persons <35 years of age was based on the age 35-44 group.
11. No history of malaria infection or vaccination, residence in a malaria-endemic area for ≥5 years, or participation in a malaria research study.* *Participation without exposure to malaria infection or to a malaria vaccine was not exclusionary.
12. No receipt of malaria prophylaxis or travel to a malaria-endemic area in the six months prior to first mosquito exposure.

13. No receipt of blood products or immunoglobulin within six months prior to, or donation of a unit of blood within two months prior to, enrollment.
14. Weight ≥50 kg and body mass index (BMI) <35 kg/m^2.
15. Negative serology for human immunodeficiency virus (HIV)1/2.* *If the enzyme-linked immunosorbent assay (ELISA) was positive, HIV confirmation was performed. If the HIV Western Blot was not consistent with HIV infection, the subject may have been enrolled. A past subject in an HIV vaccine trial who had a positive antibody ELISA may have participated if the Western Blot was not consistent with pending seroconversion or positive or an HIV PCR assay result was below the level of detection of HIV.
16. Negative hepatitis B surface antigen and hepatitis C virus antibody.
17. No Grade 1 or higher screening clinical lab value.* *Screening clinical labs included blood tests (white blood count [WBC], hemoglobin, platelet count, creatinine, and alanine aminotransferase [ALT]) and urine dipstick tests (protein and hemoglobin). Any Grade 1 or higher value for any screening test excluded the subject from enrollment with the exception of hematuria ≥1+ detected concurrent with endometrial bleeding for females. In this situation, the test could have been repeated if clinically warranted but was not considered an indicator of poor health status or increased risk and so was not a contraindication to enrollment.
18. Screening ECG with no clinically significant abnormalities.*

*Pathologic Q waves and significant ST-T wave changes; left ventricular hypertrophy; any non-sinus rhythm excluding isolated premature atrial or ventricular contractions; right or left bundle branch block; QT/QTc interval >450 ms; or advanced (secondary or tertiary) A-V heart block.
19. No known allergy to mosquito bites, chloroquine, hydroxychloroquine, amodiaquine, atovaquone, proguanil, non-steroidal anti-inflammatory drugs, or acetaminophen.
20. No known sickle cell trait or other hemoglobinopathy.
21. Negative sickle cell screening laboratory test.
22. Did not plan to undergo surgery (elective or otherwise) between screening and the end of the study.
23. No dermatologic abnormalities in either forearm that could have impaired assessment of local reactions.
24. No history of psoriasis or porphyria.
25. No history of glucose-6-phosphate dehydrogenase (G6PD) deficiency.
26. No contraindication to repeated phlebotomy.* *Such as minimal venous access or recent history of anemia.
27. Reachable (24/7) by mobile phone during the duration of the study period and willing to provide two close contacts to assist with making contact.

28. Lived in the greater Seattle area and within an approximately one hour commute to the study research clinic.

29. Willing to avoid non-study related blood donation for the duration required by the blood bank* following last mosquito exposure.

    *The local blood bank prohibits donation from persons who have had malaria.

30. Agreed not to travel to a malaria endemic region during the entire course of the trial.

31. Agreed not to travel away from the greater Seattle area in the 14 days after a study immunization*, and from the day of CHMI until the end of malaria treatment visits.

    *Subjects in Study Arms 1 and 2.

Exclusion Criteria

A subject meeting any of the following exclusion criteria was not eligible for enrollment:

1. Use of any antibiotic or drug with antimalarial properties within 28 days prior to first mosquito exposure or planned use during the study period.

2. Any clinically significant acute or chronic medical condition* or need for chronic medications** that, in the opinion of the investigator, would interfere with immunity or affect safety.

   *Included, but was not limited to, disorders of the liver, kidney, lung, heart, or nervous system, or other metabolic or autoimmune/inflammatory conditions.

**Receipt of systemic, prescription medications for the treatment of chronic medical conditions or variations of normal physiologic functions were permissible if, in the opinion of the investigator, they were used for conditions that were not clinically significant and would not impact the effectiveness of the vaccine or the safety of the subject or the safety and immunogenicity outcomes of the protocol. Use of systemic, over-the-counter medications and PRN systemic, prescription medications were allowed if, in the opinion of the investigator, they posed no additional risk to subject safety, vaccine efficacy or assessment of immunogenicity/reactogenicity. Topical (except corticosteroid) medications, nasal (including corticosteroid) medications, vitamins, and supplements were permissible. Following enrollment, use of topical corticosteroid medications for treatment of PfGAP3KO administration reactions was permissible. Any drug with antimalarial properties was not permissible.
3. Asthma, other than mild, well-controlled asthma.* *Cold or exercise-induced asthma controlled with inhaled medications other than inhaled corticosteroids was permissible. Subjects were excluded if they required daily bronchodilator use or have had an asthma exacerbation requiring oral/parenteral steroid use or have used theophylline or inhaled corticosteroids in the past year.
4. Known atherosclerotic cardiovascular disease or history of myocardial infarction, pericarditis, or myocarditis.
5. Diabetes mellitus.

6. History of a psychiatric condition that may make study compliance difficult, such as schizophrenia or bipolar disorder.* *Included persons with psychoses or history of suicide attempt or gesture in the 3 years before study entry or an ongoing risk for suicide.
7. Chronic or active neurologic condition (including seizures and migraine headaches).
8. Autoimmune disease (autoimmune thyroid disease was permissible and vitiligo or mild eczema not requiring chronic therapy was permissible)
9. Known or suspected congenital or acquired immunodeficiency including anatomic or functional asplenia* or immunosuppression as a result of underlying illness or treatment. *Any splenectomy was exclusionary.
10. Abuse of alcohol or drugs that, in the opinion of the investigator, may have interfered with ability to comply with the protocol or increased risk to subject's health during the study period.
11. Active neoplastic disease.* *Subjects with a history of malignancy may have been included if treated by surgical excision or if treated by chemotherapy or radiation therapy and has been observed for a period that in the investigator's estimation provides a reasonable assurance of sustained cure (not less than 36 months). Cervical neoplasia under surveillance was acceptable.

12. Chronic topical or systemic corticosteroid use.*

    *Corticosteroid nasal sprays for allergic rhinitis were permissible. Persons using a topical corticosteroid for a limited duration for mild uncomplicated dermatitis such as poison ivy or contact dermatitis prior to enrollment may have been enrolled the day after their therapy was completed. Oral or parenteral (intravenous, intramuscular, subcutaneous) corticosteroids given for non-chronic conditions not expected to recur were permissible if, within the year prior to enrollment, the longest course of therapy was no more than 14 days, and no oral or parenteral corticosteroids were given within 30 days prior to enrollment. Intraarticular, bursal, tendon, or epidural injections of corticosteroids were permissible if the most recent injection was at least 30 days prior to enrollment. Topical or systemic corticosteroid use for study related AEs was not exclusionary.

13. Receipt or planned receipt of inactivated vaccine or allergy desensitization injection within 14 days before or after a mosquito exposure.

14. Receipt or planned receipt of live attenuated vaccine within 28 days before or after a mosquito exposure.

15. Current use of tenofovir/emtricitabine (Truvada).

16. Receipt of any experimental agent* within 30 days prior to screening or planned receipt prior to the end of the study.

    *Vaccine, drug, biologic, device, blood product, or medication.

17. Planned to enroll in another clinical trial* that could have interfered with safety assessment of the investigational product at any time during the study period. *Included trials that have a study intervention such as a drug, biologic, or device.
18. Systolic blood pressure ≥161 mm Hg or diastolic blood pressure ≥96 mm Hg.
19. Resting heart rate ≤49 or ≥101 beats per minute.
20. Oral temperature ≥38°C (100.4° F).
21. Acute febrile illness (oral temperature ≥38°C [100.4°F]) or other acute illness within three days prior to mosquito exposure.* *Note for afebrile, acute illness only: If a subject was afebrile, his/her acute illness was nearly resolved with only minor residual symptoms remaining, and, in the opinion of the site PI or appropriate sub-investigator, the residual symptoms would not have interfered with the ability to assess safety parameters as required by the protocol, the subject could receive vaccination or CHMI without further approval from the DMID Medical Officer.
22. Was using or intended to use within 28 days after a mosquito exposure a medication with a known interaction with atovaquone-proguanil* or chloroquine**. *Included, for example, tetracycline (may have reduced atovaquone concentrations), or metoclopramide (may have reduced bioavailability of atovaquone). ** Included, for example, cimetidine, metoclopramide, carbamazepine, phenytoin, St. John's wort, and antidepressants. Antacids and kaolin may have reduced absorption of

chloroquine but could have been administered if separated by at least 4 hours from intake of chloroquine.
23. Had any condition that would have, in the opinion of the site investigator, placed the subject at an unacceptable risk of injury or rendered the subject unable to meet the requirements of the protocol.

Table S4. Rating scale for sporozoite salivary gland load

Salivary gland score	Approximate number of sporozoites
0	No sporozoites observed
+1	1-10 sporozoites observed
+2	11-100 sporozoites observed
+3	101-1,000 sporozoites observed
+4	>1,000 sporozoites observed

Scale based on previously described methods (*52*)