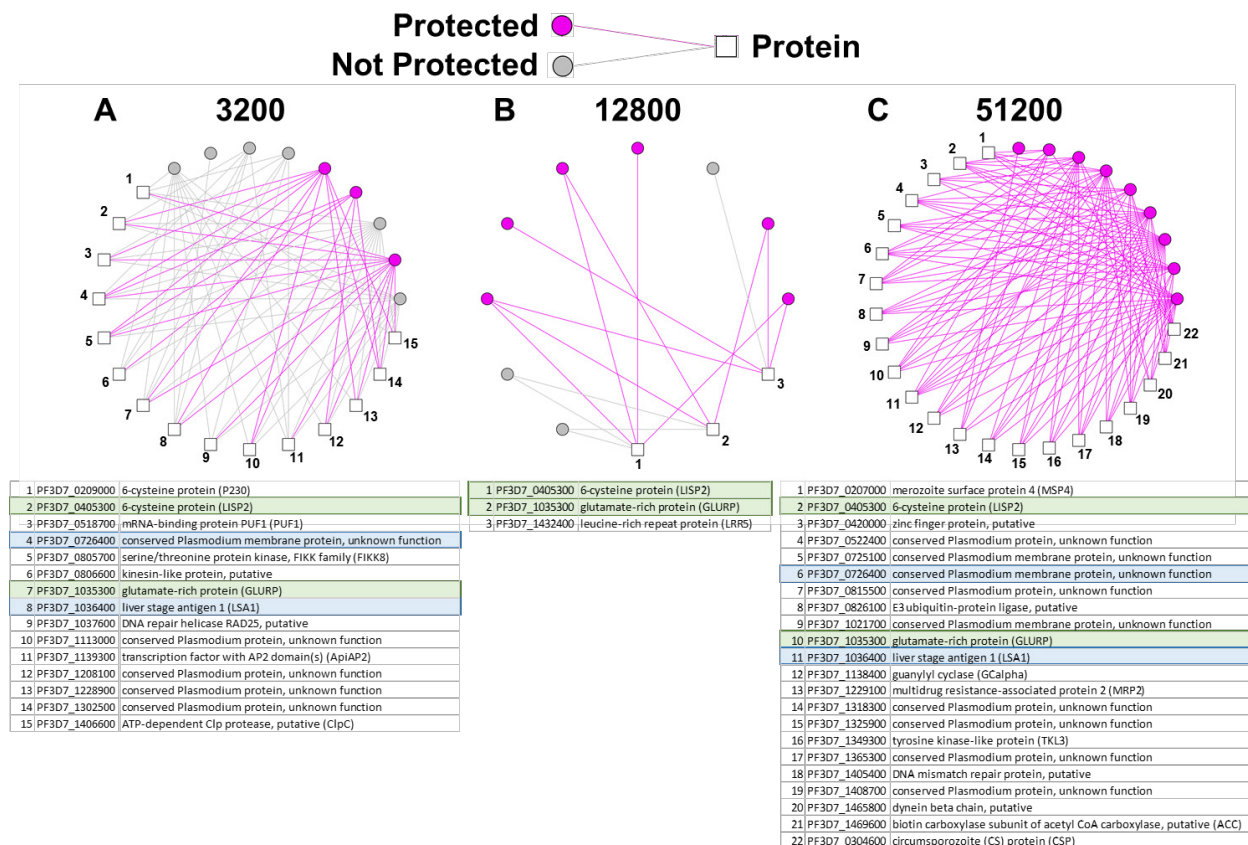


SUPPLEMENTARY INFORMATION

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Supplementary Figure S1 | Bipartite network of antibody binding from serum of protected and non-protected volunteers in the groups that received doses of 3.2×10^3 PfSPZ-CVac (A), 1.28×10^4 PfSPZ-CVac (B), and 5.12×10^4 PfSPZ-CVac (C). Purple circles represent protected volunteers, grey circles represent non-protected volunteers, and white squares represent proteins. Lines represent positive responses from individuals for connected proteins. The tables below each network show the proteins labeled numerically in the networks for which at least 5/9 volunteers responded. Blue cells represent proteins recognized in two dose groups, and green cells represent proteins recognized in three dose groups.



Supplementary Table S1 | Type, grade and number of adverse events during the trial.

Clinical adverse events during immunization. Number of adverse events

Group	Placebo		3.2x10 ³ PfSPZ		Placebo		1.28x10 ⁴ PfSPZ		Placebo			5.12x10 ⁴ PfSPZ	
	1	2	1	2	1	2	1	3	1	2	3	1	2
Abdominal cramps	0	0	0	0	0	0	0	0	0	0	0	2	0
Accident automobile	0	0	0	0	0	0	0	1	0	0	0	0	0
Blurry vision	1	0	0	0	0	0	0	0	1	0	0	1	0
Bronchitis	0	0	0	0	0	0	0	0	0	1	0	0	0
Contusion of foot	0	0	0	0	0	0	0	0	0	0	0	0	1
Diarrhea	0	0	1	0	0	0	1	0	0	0	0	2	0
Dizziness	0	0	1	0	0	0	1	0	2	0	0	1	0
Drowsiness	0	0	0	0	0	0	0	0	0	0	0	1	0
Dysmenorrhoea	1	0	0	0	0	0	0	0	0	0	0	0	0
EBV infection	0	0	0	0	0	1	0	0	0	0	0	0	0
Epigastric pain	0	0	1	0	0	0	0	0	0	0	0	0	0
Epistaxis	0	0	1	0	0	0	0	0	0	0	0	0	0
Erythema	0	0	0	0	0	0	1	0	0	0	0	0	0
Euphoria	1	0	0	0	0	0	0	0	0	0	0	0	0
Fatigue	0	0	0	0	0	0	1	0	0	0	0	0	1
Feeling hot	0	0	0	0	1	0	0	0	0	0	0	0	0
Fever	0	0	0	0	0	0	0	0	0	1	0	0	2
Flatulence	0	0	0	0	0	0	0	0	0	0	0	2	0
Flu	1	0	0	0	1	0	0	0	0	0	0	0	1
Head injury	0	0	0	0	0	0	0	0	0	0	1	0	0
Headache	1	0	4	1	0	0	3	0	1	0	0	5	0
Hypertension diastolic	0	0	0	0	0	0	1	0	0	0	0	0	1
Laryngitis viral	0	0	0	0	1	0	0	0	0	0	0	0	0
Myalgia	0	0	0	0	0	0	0	0	0	0	0	1	0
Nausea	0	0	0	0	0	0	0	0	3	0	0	3	0
Pain ear	0	0	1	0	0	0	0	0	0	1	0	0	0
Rash	0	0	0	1	0	0	0	0	0	0	0	0	0
Rhinitis	0	0	0	0	0	0	0	0	0	0	0	0	1
Sensation of pressure in the eyes	1	0	0	0	0	0	0	0	1	0	0	0	0
Sinusitis	0	0	0	0	0	0	0	0	0	1	0	0	1
Splinter	0	0	0	0	0	0	0	0	0	1	0	0	0
Sprained ankle	0	1	0	0	0	0	0	0	0	0	0	0	0
Stomach cramps	0	0	0	0	0	0	1	0	0	0	0	0	0
Sweating	0	0	0	0	0	0	1	0	0	0	0	0	0
Systolic Hypertension	0	0	0	0	0	0	1	0	0	0	0	1	0
Tachycardia	0	0	0	0	0	0	0	0	0	0	0	0	1
Taste metallic	0	0	0	0	0	0	0	0	0	0	0	1	0
Urinary tract infection	0	0	0	0	0	1	0	0	0	0	0	0	2
Vertigo	0	0	0	0	0	0	0	0	1	0	0	1	0

Laboratory adverse events during immunization. Number of adverse events

Group	Placebo			3.2x10 ³ PfSPZ			Placebo			1.28x10 ⁴ PfSPZ			Placebo			5.12x10 ⁴ PfSPZ		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ALT increased	0	0	0	0	0	0	2	1	0	4	1	0	1	0	1	0	0	0
Anemia	2	0	0	0	0	0	2	0	0	4	0	0	1	0	1	1	0	0
Basophilia	1	0	0	0	0	0	3	1	0	4	0	0	1	0	5	0	0	0
Bilirubinemia	1	0	0	1	0	0	1	0	0	3	0	0	0	0	0	0	0	0
Blood basophils decreased	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0
Blood creatinine decreased	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood eosinophils decreased	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0
Blood eosinophils increased	1	0	0	2	1	0	2	0	0	5	0	0	0	0	1	0	0	0
Blood neutrophils increased	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Creatinine blood increased	0	0	0	1	0	0	1	0	0	0	0	0	1	0	3	0	0	0
Erythrocytes decreased	0	0	0	0	0	0	0	0	0	1	0	0	3	0	2	0	0	0
GOT increased	5	0	0	0	0	0	3	0	0	4	0	0	0	0	0	0	0	0
Hematocrit decreased	2	0	0	5	0	0	1	0	0	5	0	0	1	0	2	1	0	0
Hyperglycemia	0	0	0	0	1	0	0	1	0	2	0	0	2	0	0	0	0	0
Hyperkalemia	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hyponatremia	0	1	0	1	0	0	0	0	0	2	0	0	1	0	1	0	0	0
Hypoglycaemia	1	0	0	2	0	0	0	0	0	1	2	2	0	1	1	0	0	0
Hypokalemia	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
LDH	3	0	0	1	0	0	3	0	0	5	0	0	0	0	7	0	0	0
Leucocytosis NOS	1	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0
Leucopenia	3	0	0	1	0	0	0	0	0	2	0	0	2	0	3	1	0	0
Lymphocytosis	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Lymphopenia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1*
Monocytopenia	0	0	0	3	0	0	0	0	0	2	0	0	1	0	3	0	0	0
Monocytosis	0	0	0	0	0	0	1	0	0	0	0	0	3	0	3	0	0	0
Neutropenia	1	0	0	1	0	0	0	0	0	0	0	0	1	1	2	0	0	0
Thrombocytosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Thrombopenia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

* Lymphopenia was 470 per µL (threshold for Grade 3 was 490 per µL).

Clinical adverse events during CHMI. Number of adverse events

Group	Placebo			3.2x10 ³ PfSPZ			Placebo			1.28x10 ⁴ PfSPZ			Placebo			5.12x10 ⁴ PfSPZ	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2
Cavernoma	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Chills	0	0	0	0	0	0	0	2	0	0	3	0	0	1	0	1	0
Cough	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Diarrhea	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	1	0
Dizziness	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	0
Ear infection	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Faint	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Fall	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Fatigue	1	2	1	1	4	1	2	1	0	0	3	0	0	3	1	0	1
Fever	1	2	1	2	2	0	0	2	0	0	0	0	1	1	0	0	1
Flu	1	0	0	1	1	0	1	1	0	2	1	0	0	0	0	0	1

Gastritis	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Gastroenteritis	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Headache	1	2	1	3	4	1	2	2	1	0	2	1	0	4	0	2	1
Hematuria	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Hypothyroidism	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Leg pain	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Myalgia	0	2	1	1	4	1	0	2	0	0	3	0	0	3	1	0	1
Nausea	0	1	1	1	3	1	0	3	0	0	2	0	0	3	0	1	0
Rhinitis	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Rigor	0	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Skin lesion excision	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Sore throat	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
Sweating	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Tachycardia	1	0	0	2	0	0	2	0	0	0	0	0	0	0	0	1	0
Toe injury	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Tonsillitis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Tooth infection	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urinary tract infection	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Vomiting	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Laboratory adverse events during CHMI. Number of adverse events

Group	Placebo			3.2x10 ³ PfSPZ			Placebo			1.28x10 ⁴ PfSPZ			Placebo		5.12x10 ⁴ PfSPZ	
	1	2	3	1	2	3	1	2	1	2	3	1	2	1	2	
ALT increased	1	0	0	0	0	0	1	0	3	1	0	1	0	2	0	
Anemia	2	0	0	1	0	0	2	1	7	0	0	1	3	1	0	
Basophilia	0	0	0	2	0	0	2	0	2	0	0	1	0	0	0	
Blood basophils decreased	0	0	0	2	0	0	0	0	3	0	0	1	0	3	0	
Blood creatinine decreased	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	
Blood eosinophils decreased	0	0	0	2	0	0	0	0	1	0	0	0	0	3	0	
Blood eosinophils increased	1	0	0	5	0	0	3	0	3	0	0	0	0	2	0	
Blood neutrophils increased	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	
Creatinine blood increased	0	0	0	1	0	0	2	0	0	0	0	1	0	2	0	
Erythrocytes decreased	0	0	0	0	0	0	0	0	2	0	0	2	0	4	0	
GOT increased	2	0	0	1	0	0	1	0	3	1	0	1	1	1	0	
Hematocrit decreased	4	0	0	7	0	0	5	0	7	0	0	2	0	8	0	
Hemoglobin increased	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
Hyperglycemia	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
LDH	2	0	0	4	0	0	5	0	5	0	0	2	0	5	0	
Leucocytosis NOS	1	0	0	2	0	0	3	0	1	0	0	1	0	1	0	
Leucopenia	3	1	0	4	0	0	1	0	3	0	0	2	0	0	0	
Lymphadenopathy	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
Lymphocytosis	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	
Lymphopenia	1	1	0	2	1	2	0	1	0	1	1	1	1	0	0	
Monocytopenia	0	0	0	1	0	0	0	0	1	0	0	2	0	0	0	
Monocytosis	4	0	0	1	0	0	3	1	2	0	0	3	0	1	0	
Neutropenia	1	1	0	1	0	0	0	0	0	0	0	1	0	1	0	

Thrombocytosis	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Thrombopenia	0	1	1	0	0	0	0	0	0	2	0	0	0	0	1
Vitamin D deficiency	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Supplementary Table S2 | PfCSP ELISA, aIFA, and ISI (75% inhibition) results at 2 weeks and 8 weeks after the third dose of vaccine.

PfSPZ/ Dose	Volunteer ID	Protected Against CHMI	ISI (75% inhibition) Pre- Immune	2 Weeks Post 3 rd Dose			8 Weeks Post 3 rd Dose		
				PfCSP Net OD 1.0	aIFA (PfSPZ)	ISI (75% inhibition)	PfCSP Net OD 1.0	aIFA (PfSPZ)	ISI (75% inhibition)
3.2x10 ³	002	Yes	15.59	427	76.04	15.67	215	65.29	10.19
	050	No	15.34	362	265.00	27.39	138	73.15	33.16
	007	No	23.84	150	111.50	16.75	106	80.11	3.61
	034	No	21.22	149	0	29.91	124	6.26	18.91
	046	No	2.77	126	93.67	16.88	19	71.44	15.70
	006	Yes	23.52	38	33.12	43.74	40	0	25.23
	030	No	6.14	11	0	9.33	-2	0	3.26
	012	No	2.47	-9	0	1.00	-12	0	1.00
	015	Yes	1.00	-1425	73.96	1.00	-5551	50.44	10.78
	Median			15.34	126	73.96	16.75	40	50.44
1.28x10 ⁴	049	Yes	12.74	10835	5592.38	58.78	7216	1083.53	47.98
	018	Yes	1.00	802	120.40	13.57	1289	78.28	38.56
	038	Yes	16.32	696	164.44	50.08	1091	82.47	44.63
	028	No	1.00	498	76.04	12.08	797	76.20	20.37
	022	No	18.20	397	789.41	47.61	735	483.74	12.41
	039	Yes	1.00	299	72.86	4.64	110	74.53	9.87
	064	Yes	1.00	276	400.16	4.98	236	176.81	10.64
	067	Yes	3.28	155	171.22	16.58	163	104.28	5.41
	010	No	17.82	109	121.89	2.71	128	73.10	8.24
	Median			3.28	397	164.44	13.57	735	82.47
5.12x10 ⁴	072	Yes	5.87	67088	14496.24	158.49	34651	7693.12	52.38
	071	Yes	17.70	30518	15009.49	152.26	11503	5573.98	141.47
	051	Yes	4.09	15914	8817.87	79.06	9056	2127.35	77.25
	073	Yes	1.00	12964	4025.73	94.27	5493	1514.38	112.75
	042	Yes	4.50	3844	4630.11	35.19	1536	1756.16	26.61
	052	Yes	16.70	3180	239.82	19.79	2025	183.93	27.12
	017	Yes	27.18	1621	599.48	43.64	849	259.55	15.76
	035	Yes	23.01	1529	669.49	21.46	632	236.94	29.91
	026	Yes	6.65	1357	480.70	20.31	466	241.21	22.97
	Median			6.65	3844	4025.73	43.64	2025	1514.38

There was a dose response for antibodies at 2 weeks after the third dose of PfSPZ Challenge to PfCSP by ELISA (Spearman rho = 0.84, $P < 0.0001$), PfSPZ by aIFA (Spearman's rho = 0.80, $P < 0.0001$), and PfSPZ by ISI (rho = 0.51, $P = 0.007$). There was also a dose response at 8 weeks after the third dose of PfSPZ Challenge to PfCSP (rho = 0.82, $P < 0.0001$), aIFA (rho = 0.87, $P < 0.0001$), and ISI (rho = 0.59, $P = 0.0013$). The results of these three assays were highly correlated with each other at 2 weeks (PfCSP versus aIFA, rho = 0.85, $P < 0.0001$; PfCSP vs ISI, rho = 0.73, $P < 0.0001$; aIFA vs ISI, rho = 0.71, $P < 0.0001$) and at 8 weeks (PfCSP vs aIFA, rho = 0.88, $P < 0.0001$; PfCSP vs ISI, rho = 0.8028, $P < 0.0001$; aIFA vs ISI, rho = 0.64, $P = 0.0003$) after the third dose of PfSPZ Challenge. Volunteer IDs in red designate protected subjects and those in black designate unprotected subjects.

Supplementary Table S3 | PfCSP ELISA levels 7-14 days after the first, second, and third doses of PfSPZ-CVac.

PfSPZ/ Dose	Vol. ID	Protected Against CHMI	11 Days Post 1 st Dose		# Subjects Positive for Antibodies (%)	7 Days Post 2 nd Dose		# Subjects Positive for Antibodies (%)	14 Days Post 3 rd Dose		# Subjects Positive for Antibodies (%)
			OD 1.0			OD 1.0			OD 1.0		
			Net	Ratio		Net	Ratio		Net	Ratio	
3.2x10 ³	002	Yes	-23	0.8	0/9 (0%)	25	1.2	0/9 (0%)	238	3.1	4/9 (44%)
	006	Yes	-28	0.7		-7	0.9		37	1.4	
	007	No	-14	0.8		7	1.1		150	2.6	
	012	No	0	1.0		8	9.0		0	1.0	
	015	Yes	-3245	0.7		-1084	0.9		29,931	4.2	
	030	No	-6	0.8		-3	0.9		-13	0.6	
	034	No	38	1.6		-2	1.0		195	4.2	
	046	No	14	1.1		-8	1.0		64	1.4	
	050	No	-17	0.8		-25	0.8		289	3.8	
	Median		-14	0.8		-3	1.0		150	2.6	
1.28x10 ⁴	010	No	1	1.0	0/9 (0%)	-3	0.9	2/9 (22%)	134	4.9	6/9 (67%)
	018	Yes	-197	0.8		11	1.0		181	1.2	
	022	No	-4	0.9		23	1.7		394	12.9	
	028	No	22	1.1		-4	1.0		229	2.5	
	038	Yes	-25	0.7		78	2.1		610	9.4	
	039	Yes	-186	0.7		-426	0.3		9	1.0	
	049	Yes	6	1.2		-34	0.0		8074	231.7	
	064	Yes	-50	0.2		178	3.9		159	3.6	
	067	Yes	4	1.2		1,084	50.3		114	6.2	
	Median		-4	0.9		11	1.0		181	4.9	
5.12x10 ⁴	017	Yes	-20	0.8	1/9 (11%)	184	2.6	5/9 (56%)	706	7.0	9/9 (100%)
	026	Yes	1	1.0		523	6.9		850	10.6	
	035	Yes	-50	0.8		78	1.4		854	5.3	
	042	Yes	-1	1.0		143	4.6		2787	70.7	
	051	Yes	5	1.0		-122	0.1		13,249	99.1	
	052	Yes	10	1.1		-78	0.1		2235	25.6	
	071	Yes	20	1.7		193	8.1		13,139	487.6	
	072	Yes	60	61.0		72	73.0		33,340	33,341.0	
	073	Yes	-11	0.9		454	4.8		5580	48.3	
	Median		1	1.0		143	4.6		2787	48.3	

Values are reported as net OD 1.0, which is the OD 1.0 at the specific time point after immunization minus the OD 1.0 prior to immunization, and as OD 1.0 ratio, which is the ratio of the OD 1.0 at the post immunization time point to the OD 1.0 prior to immunization. A value was arbitrarily considered positive if the net OD 1.0 was ≥ 50 and the OD 1.0 ratio was ≥ 3 . Serum was not available for all time points; therefore, the results in this table are from plasma only. Volunteer IDs in red designate protected subjects and those in black designate unprotected subjects.

Supplementary Table S4 | PfCSP ELISA levels 2, 8 and 12-13 weeks after the third dose of vaccine.

PfSPZ/ Dose	Vol. ID	Protected Against CHMI	2 Weeks Post 3 rd Dose		# Subjects Positive for Antibodies (%)	8 Weeks Post 3 rd Dose (Pre-CHMI)		# Subjects Positive for Antibodies (%)	12-13 Weeks Post 3 rd Dose (4 Weeks Post CHMI)		# Subjects Positive for Antibodies (%)
			OD 1.0			OD 1.0			OD 1.0		
			Net	Ratio		Net	Ratio		Net	Ratio	
3.2x10 ³	012	No	-9	0.8	3/9 (33%)	-12	0.7	0/9 (0%)	-11	0.7	1/9 (22%)
	015	Yes	-1425	0.9		-5551	0.5		-6669	0.4	
	006	Yes	38	1.3		40	1.3		-33	0.8	
	030	No	11	1.3		-2	1.0		77	2.8	
	046	No	126	1.5		19	1.1		277	2.2	
	007	No	150	2		106	1.7		84	1.6	
	034*	No	149	3.0		124	2.7		78	2.0	
	002*	Yes	427	3.5		215	2.3		39	1.2	
	050*	No	362	4.3		138	2.2		338	4.0	
	Median		126	1.5		40	1.3		77	1.6	
1.28x10 ⁴	039	Yes	299	1.4	7/9 (78%)	110	1.1	7/9 (78%)	-17	1.0	5/9 (78%)
	018	Yes	802	1.5		1289	1.8		510	1.3	
	010*#	No	109	3.4		128	3.8		404	10.0	
	028*#	No	498	4.2		797	6.1		584	4.7	
	067*#	Yes	155	4.9		163	5.1		65	2.6	
	064*#	Yes	276	5.5		236	4.8		105	2.7	
	038*#	Yes	696	7.5		1091	11.2		545	6.1	
	022*#	No	397	8.1		735	14.1		593	11.6	
	049*#	Yes	10835	178.6		7216	119.3		4955	82.2	
	Median		397	4.9		735	5.1		510	4.7	
5.12x10 ⁴	035*#	Yes	1529	6.8	9/9 (100%)	632	3.4	9/9 (100%)	964	4.7	9/9 (100%)
	017*#	Yes	1621	10.6		849	6.1		1035	7.2	
	026*#	Yes	1357	12.2		466	4.9		569	5.7	
	052*#	Yes	3180	25.7		2025	16.7		1939	16.0	
	051*#	Yes	15914	73		9056	42.0		11547	53.2	
	042*#	Yes	3844	84.6		1536	34.4		2596	57.4	
	073*#	Yes	12964	84.6		5493	36.4		6476	42.8	
	071*#	Yes	30518	463.4		11503	175.3		12444	189.5	
	072*#	Yes	67088	1917.8		34651	991.0		49600	1418.1	
	Median		3844	73		2025	34.4		2596	42.8	

Values are reported as net OD 1.0, which is the OD 1.0 at the specific time point after immunization minus the OD 1.0 prior to immunization, and as OD 1.0 ratio, which is the ratio of the OD 1.0 at the post immunization time point to the OD 1.0 prior to immunization. A value was arbitrarily considered positive if the net OD 1.0 was ≥ 50 and the OD 1.0 ratio was ≥ 3 . (*) next to volunteer ID denote volunteers positive for both criteria at 2 weeks. (#) next to volunteer ID denote volunteers positive for both criteria at 8 weeks. Volunteer IDs in red designate protected subjects and those in black designate unprotected subjects.

Supplementary Table S5 | Antibodies to well-defined Pf proteins 2 weeks after the third dose of PfSPZ-CVac. Volunteers were considered seropositive with a net OD 1.0 \geq 50 and a ratio \geq 3 when compared to readings from pre-immune sera. Values shown are medians.

Protein	Dose of PfSPZ Challenge								
	3.2x10 ³			1.28x10 ⁴			5.12x10 ⁴		
	No. Positive/No. Tested (%)	Net OD 1.0 of Positives	OD 1.0 Ratio of Positives	No. Positive/No. Tested (%)	Net OD 1.0 of Positives	OD 1.0 Ratio of Positives	No. Positive/No. Tested (%)	Net OD 1.0 of Positives	OD 1.0 Ratio of Positives
1st Expressed in Sporozoites									
PfCSP	3/9 (33%)	362	3.48	7/9 (78%)	397	5.45	9/9 (100%)	3844	73.01
PfSSP2/TRAP	0/9	.	.	0/9	.	.	1/9 (11%)	273*	6.81*
PfCelTOS	0/9	.	.	0/9	.	.	0/9	.	.
PfMSP5	1/9 (11%)	65*	14.00*	2/9 (22%)	325	33.78	5/9 (56%)	452	28.86
PfAMA1	0/9	.	.	1/9 (11%)	192*	5.57*	3/9 (33%)	556	43.77
1st Expressed in Early Liver Stages									
PfEXP1	0/9	.	.	1/9 (11%)	725*	6.80*	1/9 (11%)	6317*	7.41*
PfLSA1	0/9	.	.	0/9	.	.	3/9 (33%)	1090	12.00
1st Expressed in Late Liver Stages									
PfMSP1	0/9	.	.	0/9	.	.	4/9 (44%)	1670	863.00
PfEBA175	0/9	.	.	0/9	.	.	0/9	.	.

*For these values, the single value is recorded, not the median.

Supplementary Table S6 | Logistic regression of peak antibody levels (2 weeks after final immunization) and baseline antibody levels on probability of sterile protection against CHMI, adjusted by dose of PfSPZ-CVac. (See attached PDF)

Supplementary Table S7 | ELISA measuring antibodies against proteins expressed in sporozoites (PfCSP, PfSSP2/TRAP, PfCeITOS, PfMSP5, PfAMA1), early liver stages (PfEXP1 and PfLSA1) and late liver/blood stages (PfMSP1 and PfEBA175).

Recombinant Protein Details	
PfCSP	The recombinant <i>P. falciparum</i> (Pf) circumsporozoite protein (rPfCSP, 3D7 strain) is a 48 kDa protein with an N-terminal sequence SLGEND, approximately 299 amino acids in length, without the signal sequence. The 1 st amino acid is residue 50 at the N-terminus and comprising both the N- and C-portions with 22 NANP and 4 NVDP repeats, without the last 13 amino acids of the C-terminal portion. GenBank Accession No.: ADF48458.1 (Protein Potential).
PfSSP2/TRAP	The recombinant Pf sporozoite surface protein 2 (recombinant fragment of PfSSP2, 3D7) is a 27 kDa protein. The rPfSSP2 lacks the hydrophobic regions at the amino terminus (22 aa) and the C-terminus (63 aa). The sequence selected for expression is the entire extracellular domain of PfSSP2, which includes the A-type domain (of von Willebrand factor) and the type 1 repeat of thrombospondin (TSR). GenBank Accession No.: AAC18657.1 (Protein Potential).
PfMSP5	The recombinant Pf merozoite surface protein-5 is a 40-kDa protein that is located on the merozoite surface and is non-covalently associated with merozoite surface protein 1 (MSP1) complex shed from the surface at erythrocyte invasion. GenBank Accession No: AAF12722.1 (Ross Coppel, Monash University, Australia).
PfCeITOS	The recombinant Pf cell-traversal protein for ookinetes and sporozoites (rPfCeITOS) is an 18 kDa protein with an N-terminal sequence FRGNNG. It is approximately 151 amino acids in length, with the first amino acid being residue 25 at the N-terminus without the hydrophobic signal sequence. GenBank Accession No.: BAD97684.1 (Protein Potential).
PfMSP1	PfMSP142 (EcMSP142-3D7, Lot# WRAIR11150, 0.99 mg/mL) is a recombinant of the C-terminal 42-kDa portion of the merozoite surface protein-1 (PfMSP1) from the 3D7 strain of Pf expressed, refolded, and purified at the Walter Reed Army Institute of Research (WRAIR) Pilot Bioproduction Facility. GenBank Accession Number: ABS84655.1 (WRAIR).
PfAMA1	The recombinant Pf apical membrane antigen-1 PfAMA1 (rPfAMA1-3D7, Lot# MV-1187 Final Bulk Protein, 1.00 mg/mL). The rPfAMA1-3D7 represents the ectodomain of the protein. GenBank Accession No: AAN35928.1 (Carole Long, NIAID, NIH).
PfEBA175	The recombinant Pf erythrocyte binding antigen-175 (rPfEBA175 RII, Lot# 321-0904-007, 2.38 mg/mL) region II with an N-terminal sequence GRQTSS, approximately 616 amino acids in length. GenBank Accession No.: AAF72186.1 (Protein Potential).
PfLSA1	Recombinant PfLSA1 (recombinant 22-kDa Lot# 061907, 0.50 mg/mL). The recombinant Pf liver stage antigen-1, rPfLSA1 is a 22-kDa protein with an N-terminal sequence KENKLN, approximately 259 amino acids in length, the first amino acid being residue 28 at the N-terminus and truncated at residue 286 at the C-terminus. GenBank Accession No.: AAW78332.1 (Protein Potential).
PfEXP1	Pf exported protein-1 (rPfEXP1, 23-kDa Lot# 010507, 1.00 mg/mL). The recombinant Pf exported protein-1 is a 23 kDa protein with an N-terminal sequence SLAEKT and is approximately 143 amino acids in length, with the hydrophobic signal sequence removed. GenBank Accession No.: CAA28735.1 (Protein Potential).

Assay details

Pf antigens	CSP	SSP2	MSP5	CeITOS	MSP1	AMA1	EBA175	LSA1	EXP1
Coating antigen concentration in 50 µL per well	2.0 µg /mL	1.5 µg /mL	2.0 µg /mL	1.5 µg /mL	1.5 µg /mL	2.0 µg /mL	1.0 µg /mL	1.0 µg /mL	0.5 µg /mL
Washing Buffer	1X Imidazole-based wash solution containing 2 mM imidazole, 160 mM NaCl, 0.02% Tween 20, 0.5 mM EDTA (wash 3 times after each incubation)								
Blocking buffer composition (diluent)	1% Bovine Serum Albumin (BSA) blocking buffer (KPL)								
Serum	1% milk	5% milk	1% milk	5% milk	5% milk	1% milk	5% milk	1% milk	1% milk
Secondary ab	1:100 starting dilution and three fold serial dilution in triplicate								
Secondary ab	Peroxidase labeled goat anti-human IgG (KPL)								
Secondary ab	0.1	0.1	0.1	0.1	0.1	0.1	0.05	0.2	0.1

concentration $\mu\text{g/mL}$		ABTS Peroxidase substrate								
Substrate		75 mins	60 mins	75 mins	60 mins	45 mins	75 mins	45 mins	60 mins	50 mins
Substrate incubation period (Room temperature)										

Supplementary Table S8 | Flow cytometry staining panels.

#	Detector	Fluorophore	Antibody specificity		
			PfSPZ Panel 1	PfRBC Panel 2	PfRBC Panel 3
1	B515	FITC	Ki-67		TCR-V δ 1
2	B710	Cy5.5PerCP	TCR-V α 7.2		
3	V440	BV421	PD-1	CD127	IL-4
4	V510	Aqua/BV510 [†]	Viability/CD14 [†]	Viability/CD14 [†]	Viability/CD14 [†]
5	V570	BV570			
6	V605	BV605	IL-2	IL-2	
7	V650	BV650	TNF- α	TNF- α	
8	V710	BV711/QD705 [#]	CD56	CD57 [#]	
9	V750	BV750			
10	V800	BV785	CD4	CD4	CD4
11	R660	APC	IFN- γ	IFN- γ	TCR-V γ 9
12	R710	Ax680	CCR7	CCR7	CCR7
13	R780	Cy7APC	CD3	CD3	CD3
14	G560	PE	CXCR6	Perforin	IL-10
15	G610	CF594PE	TCR- $\gamma\delta$	TCR- $\gamma\delta$	TCR- $\gamma\delta$
16	G660	Cy5PE	CD161	CD38	TCR-V δ 2
17	G710	Cy5.5PE	CD45RA	CD45RA	CD45RA
18	G780	Cy7PE	CD8	CD8	CD8

Three flow cytometry staining panels were developed to assess the magnitude, quality, and phenotype of Pf-specific lymphocytes. Each of the three panels allow for the identification of $\gamma\delta$, CD4, and CD8 T cell lineages, each of which have been shown to contribute to immunity against liver stage malaria in animal models with SPZ vaccines. Within each lineage, naïve, central memory (T_{CM}), effector memory (T_{EM}), and terminal effector memory (TE) subsets can be identified on the basis of CD45RA and CCR7 expression.

Panels 1 and 2 allow for identification of Pf-specific cells by intracellular cytokine staining of IFN- γ , IL-2, or TNF- α following antigen stimulation.

Panel 1 includes the following phenotypic markers:

CXCR6, a chemokine receptor expressed by tissue-resident T cells trafficking in the liver

PD-1, a marker of cellular activation that can influence T cell function

Ki-67, a transcription factor expressed in cells that have recently undergone cell division

Panel 1 stains for NK and MAIT cell subsets. MAIT cells were identified by CD161⁺⁺CD4⁻CD8⁺CD45RA⁻CCR7⁻CXCR6⁺ (Wingender G & Kronenberg M, *Cytometry Part A*, 2015).

Panel 2 includes the following phenotypic markers:

CD38, a marker of T cell activation

Perforin, an effector molecule

CD127, differentiation markers

Panel 3 allows for identification of Pf-specific cells by intracellular cytokine staining of IL-4 and IL-10 following antigen stimulation.

Panel 3 allows for the identification of the $\gamma\delta$ T cell subfamilies defined by the expression of V γ 9, V δ 1, or V δ 2 TCRs.

PBMCs were stained following stimulation with:

Panel 1

1. PfSPZ Vaccine (1.5×10^5 PfSPZ)

2. Vaccine diluent (1% HSA)

Panel 2 and 3

3. Pf-infected erythrocytes (2.0×10^5 PfRBC)

4. Uninfected erythrocytes (2.0×10^5 uRBC)

Supplementary Table S9 | Flow cytometry antibody clones and manufacturers.

Specificity	Fluorophore	Clone	Manufacturer	Catalog
CD3	Cy7APC	SP34.2	BD	557757
CD4	BV785	OKT4	BioLegend	317441
CD8	Cy7PE	RPA-T8	BioLegend	301012
CD14	BV510	M5E2	BioLegend	301842
CD38	Cy5PE	HIT2	BD	555461
CD45RA	Cy5.5PE	MEM-56	Invitrogen	MHCD45RA18
CD56	BV711	NCAM16.2	BD	563169
TCR-V α 7.2	Cy5.5PerCP	3C10	BioLegend	351710
CD161	Cy5PE	DX12	BD	551138
TCR- $\gamma\delta$	CF594PE	B1	BD	562511
TCR-V δ 1	FITC	TS8.2	ThermoScientific	TCR2730
TCR-V δ 2	Cy5PE	B6	VRC	--
TCR-V γ 9	APC	B3	BioLegend	331310
CXCR6	PE	K041E5	BioLegend	356004
CCR7	Ax680	150503	VRC	--
PD-1	BV421	EH12.2H7	BioLegend	329920
IFN- γ	APC	4S.B3	BioLegend	502512
IL-2	BV605	MQ1-17H12	BioLegend	500331
TNF- α	BV650	MAb11	BioLegend	502937
Perforin	PE	B-D48	BioLegend	353303
Ki-67	FITC	B56	BD	556026
CD127	BV421	A019D5	BioLegend	351310
CD57	QD705	NK-1	VRC	--
IL-4	BV421	MP4-25D2	BD	564110
IL-10	PE	JES3-9D7	BioLegend	501404

Fluorophore-conjugated antibodies were used to stain PBMCs. Different combinations of antibodies were used in different staining panels, as described elsewhere in the Supplementary information.