Supplementary Appendix for

Protection against *Plasmodium falciparum* malaria by PfSPZ Vaccine

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1. Supplementary Tables and Figures

Table S1. Metadata and SNP count relative to 3D7 for 19 clinical isolates and 2 culture-adapted strains of *P. falciparum*. Data obtained from Genbank's Short Read Archive (http://www.ncbi.nlm.nih.gov/sra/), and pulled by Experiment Accession ID.

Sample Accession ID	Experiment Accession ID	Source	Read Count	Bases (Gpb)	Genome Coverage[a]	Total SNPs[b]	SNP Panel[c]
SAMN02374945	SRX364110	Mali	13,746,800	1.4	60	17,544	4,330
SAMN02373818	SRX363947	Mali	17,199,480	1.7	75	18,834	4,322
SAMN02373653	SRX363851	Mali	16,876,880	1.7	73	23,824	4,576
SAMN02373819	SRX363957	Mali	21,376,140	2.2	93	23,181	4,767
SAMN02373656	SRX363863	Mali	17,426,280	1.8	76	24,033	4,632
SAMN02373820	SRX363963	Mali	19,739,780	2.0	86	23,800	4,552
SAMN02373821	SRX363972	Mali	14,439,220	1.5	63	20,673	4,502
SAMN02373822	SRX363979	Mali	22,572,540	2.3	98	20,845	4,462
SAMN02374901	SRX364033	Mali	22,217,920	2.2	96	20,380	4,420
SAMN02374900	SRX364020	Mali	34,062,020	3.4	148	21,210	4,556
SAMN02374902	SRX364039	Mali	14,143,340	1.4	61	19,580	4,426
SAMN02374907	SRX364050	Mali	17,353,440	1.8	75	21,190	4,571
SAMEA679437	ERX005736, ERX007433	Ghana	129,305,774	9.8	422	23,515	4,906
SAMEA679680	ERX004689, ERX005050	Uganda	40,801,074	3.1	133	22,416	4,918
SAMEA678966	ERX007459, ERX008950	Kenya	58,056,936	4.8	189	22,909	4,927
IGS-MLW-001	Awaiting SRA #	Malawi	30,179,568	3.0	131	22,436	4,670
IGS-MLW-002		Malawi	36,578,884	3.7	159	21,852	4,590
IGS-MLW-003		Malawi	28,665,742	2.9	124	21,817	4,774
IGS-MLW-004		Malawi	36,874,068	3.7	160	23,199	4,666
SAMN01737343 (NF54)[d]	SRX113472, SRX111834	Africa	62,292,174	6.2	270	55	0
SAMN00765682 (7G8)[d]	SRX208839, SRX113476, SRX111831	Brazil	65,542,020	6.6	284	22,056	4,925

[a] Genome coverage calculated as: (read count x read length) / length of Pf reference genome.
[b] Total number of SNPs that pass the high-stringency filter used (see methods in Fig. S1).
[c] Subset of all SNPs that fall within the ~1M SNP panel validated by the Sanger Institute (see methods in Fig. S1).
[d] Culture-adapted strain.

Table S2 Homologous *vs.* heterologous challenge: 7G8 *vs*. 3D7. Of the differences identified genome-wide in 22.1 K nucleotide positions, many correspond to AA differences in key pre-erythrocytic loci. The number of synonymous and non-synonymous differences between the two isolates in 13 pre-erythocytic antigens is shown.

Gene	PfNF54 *vs*. Pf7G8	
	Synonymous	**Non-synonymous**
Circumspozoite protein**, CSP**	1	8
Sporozoite invasion-associated protein 1**, SIAP1**	0	4
Sporozoite invasion-associated protein-2, **SIAP-2**	0	4
Sporozoite threonine and asparagine-rich protein, **STARP**	2	7
Thrombospondin-related anonymous protein, **TRAP**	1	26
Exported protein 1, **EXP1**	0	1
Cell traversal protein for ookinetes & sporozoites, **CelTos**	0	10
Sporozoite microneme protein essential for cell traversal, **SPECT1**	0	2
Sporozoite microneme protein essential for cell traversal, **SPECT2**	1	2
Liver stage antigen 1, **LSA1**	16	22
Liver stage antigen 3, **LSA3**	9	11
Merozoite surface protein 1, **MSP1**	11	55
Merozoite surface protein 1, **MSP2**	5	26

Table S3. Results of CHMI done 3 weeks and 24 weeks after the last dose of PfSPZ Vaccine for the challenged population

Group	Total Number of PfSPZ	Pf3D7 or Pf7G8 for CHMI	Challenged population			
			No. Protected/ Number Undergoing CHMI	Protective Efficacy		Median [range] Pre-patent Period (days)[b]
				%	95% CI[a]	
CHMI 3wks						
1 $2.7x10^5$ PfSPZ	$1.35x10^6$	3D7	12/14	85.7	35.9, 98.2	14.2 [13.9, 14.4]
3 $4.5x10^5$ PfSPZ	$1.35x10^6$	3D7	13/15	86.7	35.9, 98.3	15.4 [13.9, 16.9]
Controls	-	3D7	0/6	0.0	-	11.6 [10.9, 13.7]
2 $2.7x10^5$ PfSPZ	$1.35x10^6$	7G8	4/5	80.0	10.4, 99.5	11.9*
Controls	-	7G8	0/4	0.0	-	11.9 [9.8, 12.9]
CHMI 24wks						
1 $2.7x10^5$ PfSPZ	$1.35x10^6$	3D7	7/11	63.6	7.9, 89.1	15.2 [12.0, 17.0]
3 $4.5x10^5$ PfSPZ	$1.35x10^6$	3D7	8/14	57.1	8.7, 82.3	14.0 [11.9, 15.0]
Controls	-	3D7	0/6	0.0	-	11.6 [10.9, 14.0]
2 $2.7x10^5$ PfSPZ	$1.35x10^6$	7G8	1/11	9.1	-41.9, 35.6	11.9 [10.9, 13.5]
Controls	-	7G8	0/6	0.0	-	10.9 [9.8, 11.7]

a Confidence intervals are calculated by inverting two one-sided tests for the score statistic.
b Includes only subjects who developed parasitemia. Groups with a single subject who developed parasitemia are indicated by an asterisk (*).

Table S4. Protection and parasitemia status (a) and protection, antibody, and cellular immune response status for all subjects in the trial at the time of CHMI 1 (b) and CHMI 2 (c). Protection and parasitemia status of controls are listed in (d).

a.

PfSPZ/ Dose	ID	Protection		Prepatent Period (days)				Parasite Density (parasites/μL)					
		CHMI		CHMI 1		CHMI 2		CHMI 1			CHMI 2		
		1	2	PCR	TBS	PCR	TBS	By PCR at PCR+	By PCR at TBS+	By TBS at TBS+	By PCR at PCR+	By PCR at TBS+	By TBS at TBS+
Group 1: 5 doses of $2.7x10^5$ PfSPZ (3D7 CHMI)	1001	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1002	Yes	No	.	.	14.98	16.95	.	.	.	4.94	35.97	17.3
	1004	Yes	No	.	.	13.08	14.95	.	.	.	12.28	111.98	30.3
	1005	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1006*	No	No	10.95	14.43	7.00	11.98	2.33	324.50	36.36	0.33	241.87	54.5
	1007	No	.	10.91	13.92	.	.	3.59	301.22	25.97	.	.	.
	1015	Yes	.	.	.	.	.	.	.	.	.	.	.
	1018	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1019	Yes	No	.	.	11.99	15.36	.	.	.	0.80	25.53	30.3
	1020	Yes	.	.	.	.	.	.	.	.	.	.	.
	1025	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1026	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1032	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1034	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	Median			**10.93**	**14.17**	**12.53**	**15.16**						
	MedianA			**10.91**	**13.92**	**13.08**	**15.36**						
Group 2: 5 doses of $2.7x10^5$ PfSPZ (7G8 CHMI)	1009*	.	No	.	.	6.86	11.89	.	.	.	5.74	6768.50	4398.3
	1010	Yes	No	.	.	8.03	11.45	.	.	.	5.04	67.80	72.7
	1012	Yes	No	.	.	7.99	10.90	.	.	.	2.45	67.80	17.3
	1014**	NC	NC	.	.	.	.	.	.	.	.	.	.
	1016	.	No	.	.	9.93	13.45	.	.	.	6.27	7.04	2350.6
	1017	.	No	.	.	8.92	11.92	.	.	.	1.68	998.87	145.5
	1022	.	No	.	.	6.88	12.06	.	.	.	0.60	7599.72	6748.9
	1023	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1024	Yes	No	.	.	8.99	10.97	.	.	.	2.65	11.13	36.4
	1029	.	No	.	.	8.91	12.02	.	.	.	1.78	971.97	72.7
	1030	No	No	8.90	11.95	8.95	12.02	2.32	793.08	200.00	1.49	1296.79	745.5
	1033	.	No	.	.	9.87	11.90	.	.	.	5.77	341.01	181.8
	Median			**8.90**	**11.95**	**8.91**	**11.91**						
	MedianA			**8.90**	**11.95**	**8.92**	**11.92**						
Group 3: 3 doses of $4.5x10^5$ PfSPZ (3D7 CHMI)	1031	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1035	Yes	No	.	.	7.07	13.98	.	.	.	0.45	5322.37	3610.4
	1036	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1037	Yes	No	.	.	10.97	13.99	.	.	.	5.63	339.03	86.6
	1038	Yes	No	.	.	9.01	14.01	.	.	.	0.01	392.23	90.9
	1039	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1040	Yes	No	.	.	11.00	14.99	.	.	.	2.18	95.68	17.3
	1041	No	Yes	13.87	16.88	.	.	0.70	596.19	90.91	.	.	.
	1042	Yes	No	.	.	8.87	11.93	.	.	.	0.17	99.95	54.5
	1043	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1045	Yes	No	.	.	8.93	13.99	.	.	.	0.55	453.12	54.5
	1046	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1047	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	1048	No	.	6.90	13.88	.	.	0.90	1138.26	290.91	.	.	.
	1049	Yes	Yes	.	.	.	.	.	.	.	.	.	.
	Median			**10.39**	**15.38**	**8.97**	**13.99**						

MedianA = median for analysis population. Median = median for the challenged population.
*Subject did not receive the full dosage according to their respective group.
**Subject was excluded from the analysis population due to non-compliance (NC= non-complaint).

b.

PfSPZ/ Dose	ID	Protection		2 weeks post final immunization									
		CHMI		PfCSP OD 1.0		aIFA	ISI (% Inhibition)	IFN-γ		IL2		IFN-γ/IL2	
		1	2	Net	Ratio			Net	Ratio	Net	Ratio	Net	Ratio
Group 1: 5 doses of $2.7x10^5$ PfSPZ (3D7 CHMI)	1001	Yes	Yes	2627	21	7244	17.88	27	3	148	148***	23	23***
	1002	Yes	No	21576	720	34440	75.39	316	48	331	93	118	118***
	1004	Yes	No	2142	62	3357	50.84	40	30	123	64	17	17***
	1005	Yes	Yes	882	16	1676	0	-1	1	41	8	2	2
	1006*	No	No	11894	57	17252	88.10	143	10	286	20	65	21
	1007	No	.	9895	762	6360	94.02	73	5	169	14	58	10
	1015	Yes	.	219	5	583	2.43	72	72***	97	176	24	24***
	1018	Yes	Yes	12051	247	7404	58.72	196	30	192	116	46	34
	1019	Yes	No	12042	118	5699	69.97	113	103	334	151	64	64***
	1020	Yes	.	20824	249	6881	47.59	169	4	278	7	82	4
	1025	Yes	Yes	38363	198	25625	83.31	62	10	68	7	21	9
	1026	Yes	Yes	2578	75	1881	36.31	60	8	65	34	10	8
	1032	Yes	Yes	10741	251	5765	77.17	108	3	247	8	70	5
	1034	Yes	Yes	8262	8263	3237	66.83	54	3	267	10	37	5
	Median			**10318**	**158**	**6063**	**62.78**	**73**	**9**	**181**	**27**	**42**	**14**
	Geometric Mean**			**5971**	**136**	**5505**	**.**	**.**	**10**	**158**	**31**	**32**	**14**
Group 2: 5 doses of $2.7x10^5$ PfSPZ (7G8 CHMI)	1010	Yes	No	18889	226	13212	78.28	36	8	57	10	11	11
	1012	Yes	No	13326	231	7429	73.40	149	16	229	83	46	17
	1014	.	.	3422	99	2292	71.33	18	5	54	2	13	2
	1023	Yes	Yes	14243	1188	12402	65.74	0	1	90	9	10	5
	1024	Yes	No	21045	490	9733	75.09	64	7	137	22	41	80
	1030	No	No	12486	1562	19914	81.28	109	14	142	134	35	36***
	1009*	.	No	.	.	.	.	.	.	.	.	.	.
	1011	.	.	.	.	.	.	.	.	.	.	.	.
	1013	.	.	.	.	.	.	.	.	.	.	.	.
	1016	.	No	8963	73	8903	53.51	11	11	38	69	5	11
	1017	.	No	399	3****	570	16.21	37	28	58	53	14	26
	1022	.	No	3809	3810	5806	53.86	0	1	27	33	0	1
	1027	.	.	16546	20	11341	84.15	10	8	37	35	6	6***
	1028	.	.	11617	1937	9269	74.71	38	7	57	8	20	38
	1029	.	No	5453	29	2773	80.47	8	2	72	8	3	2
	1033	.	No	25333	3168	6093	88.77	194	8	285	25	84	29
	Median			**12486**	**231**	**8903**	**74.71**	**36**	**8**	**58**	**25**	**13**	**11**
	Geometric Mean**			**8476**	**249**	**6368**	**64.66**	**.**	**6**	**77**	**22**	**.**	**11**
Group 3: 3 doses of $4.5x10^5$ PfSPZ (3D7 CHMI)	1031	Yes	Yes	31283	293	8243	84.91	172	14	278	29	83	51
	1035	Yes	No	14005	68	6415	83.93	65	17	156	282	43	43***
	1036	Yes	Yes	32759	391	13543	71.96	169	9	195	9	41	6
	1037	Yes	No	7551	211	5999	63.51	12	2	68	10	8	6
	1038	Yes	No	17026	124	9742	71.59	8	2	61	10	14	26
	1039	Yes	Yes	10272	264	12018	66.72	-4	1	59	15	2	5
	1040	Yes	No	10480	257	9296	80.87	91	48	116	10	30	23
	1041	No	Yes	2732	17	5912	81.77	7	4	55	14	1	4
	1042	Yes	No	15711	394	9366	89.02	6	2	44	12	7	5
	1043	Yes	Yes	41405	171	15134	74.58	58	4	189	22	43	18
	1045	Yes	No	6906	6	4822	84.11	8	4	113	69	9	9***
	1046	Yes	Yes	24713	853	12948	87.97	34	8	143	44	19	15
	1047	Yes	Yes	26696	723	18756	84.45	6	12	44	9	7	7***
	1048	No	.	3496	30	7558	41.89	9	1	60	6	10	3
	1049	Yes	Yes	34457	734	28489	74.09	127	9	266	16	42	26
	Median			**15711**	**257**	**9366**	**80.87**	**12**	**4**	**113**	**14**	**14**	**9**
	Geometric Mean**			**14206**	**161**	**9992**	**74.96**	**.**	**5**	**101**	**19**	**14**	**11**

*These subjects did not receive the full dosage according to their respective group.

**Geometric mean was only calculated when the data set included only positive numbers.

***Since the denominator was zero, this number was calculated by changing the denominator to 1.

****This value, unrounded, was 2.7, rendering this volunteer negative for seroconversion.

C.

PfSPZ/ Dose	ID	Protection		23 weeks post final immunization									
		CHMI		PfCSP OD 1.0		aIFA	ISI (% inhibition)	IFN-γ		IL2		IFN-γ/IL2	
		1	2	Net	Ratio			Net	Ratio	Net	Ratio	Net	Ratio
Group 1: 5 doses of $2.7x10^5$ PfSPZ (3D7 CHMI)	1001	Yes	Yes	1309	11	14878	21.75	-14	<1	11	11***	1	1***
	1002	Yes	No	9472	317	24630	64.28	105	17	114	33	52	52***
	1004	Yes	No	869	26	2738	58.66	1	2	31	17	1	1***
	1005	Yes	Yes	473	9	2189	0	-6	<1	11	3	-1	1
	1006*	No	No	7682	37	48503	84.4	-8	1	7	1	-1	1
	1007	No	.	.	.	.	.	.	.	.	.	.	.
	1015	Yes	.	.	.	.	.	.	.	.	.	.	.
	1018	Yes	Yes	2332	49	4168	81.65	14	5	32	20	8	7
	1019	Yes	No	1035	11	2582	0	4	5	10	6	3	3***
	1020	Yes	.	.	.	.	.	.	.	.	.	.	.
	1025	Yes	Yes	14010	73	68191	89.67	1	1	0	1	-1	2
	1026	Yes	Yes	1061	31	8320	22.4	2	1	21	12	1	3
	1032	Yes	Yes	3514	83	2624	69.22	-18	1	44	2	3	22
	1034	Yes	Yes	1694	1695	2557	54.87	-28	<1	-16	0	-6	2
	Median			**1694**	**37**	**4168**	**58.66**	**1**	**1**	**11**	**6**	**1**	**2**
	Geometric Mean**			**2273**	**50**	**7522**	**.**	**.**	**.**	**.**	**.**	**.**	**3**
Group 2: 5 doses of $2.7x10^5$ PfSPZ (7G8 CHMI)	1010	Yes	No	3470	42	4453	58.93	0	1	7	2	0	1
	1012	Yes	No	3940	69	2834	60.1	9	2	9	4	0	1
	1014	.	.	599	18	1715	52.16	-3	<1		17	1	1***
	1023	YES.	Yes	7088	592	11939	81.58	-41	<1	-13	1	-5	<1
	1024	Yes	No	4177	98	5679	71.67	-3	1	5	1	-1	1
	1030	No	No	7384	924	26519	77.98	20	3	6	2	1	2
	1009*	.	No	1048	4	1336	18.97	24	4	18	9	11	11
	1011	.	.	.	.	.	.	.	.	.	.	.	.
	1013	.	.	1668	140	8238	47.68	15	8	26	25	7	7***
	1016	.	No	1236	11	1248	57.09	-1	<1	1	2	-1	<1
	1017	.	No	314	2	253	28.28	4	4	11	10	0	1
	1022	.	No	1645	1646	1068	50.5	-1	1	0	<1	0	1
	1027	.	.	.	.	.	.	.	.	.	.	.	.
	1028	.	.	.	.	.	.	.	.	.	.	.	.
	1029	.	No	1921	11	3350	62.39	-1	1	10	2	-1	1
	1033	.	No	8379	1048	.	67.37	3	1	29	4	1	1
	Median			**1921**	**69**	**3092**	**58.93**	**0**	**1**	**8**	**2**	**0**	**1**
	Geometric Mean**			**2222**	**68**	**2976**	**53.01**	**.**	**.**	**.**	**.**	**.**	**.**
Group 3: 3 doses of $4.5x10^5$ PfSPZ (3D7 CHMI)	1031	Yes	Yes	22399	210	70064	96.01	13	2	44	5	4	4
	1035	Yes	No	6107	30	4915	81.27	3	2	34	62	4	4***
	1036	Yes	Yes	15274	183	7064	55.83	.	.	.	.	.	.
	1037	Yes	No	2571	72	1423	52.91	-1	1	9	2	1	1
	1038	Yes	No	8817	65	3863	70.19	-8	1	9	2	2	5
	1039	Yes	Yes	1423	37	1175	60.95	-5	1	21	6	4	8
	1040	Yes	No	5128	126	5396	57.4	12	7	19	2	4	4
	1041	No	Yes	168	2	1609	0	-2	<1	8	3	0	1
	1042	Yes	No	1920	49	6069	77.53	0	1	14	4	-1	1
	1043	Yes	Yes	5040	22	7132	55.59	-14	<1	-3	1	-1	<1
	1045	Yes	No	7501	7	6397	54.21	0	1	20	13	2	2***
	1046	Yes	Yes	13754	475	10596	89.62	-1	1	23	8	-1	<1
	1047	Yes	Yes	9930	269	17324	84.45	3	6	19	4	1	1***
	1048	No	.	.	.	.	.	.	.	.	.	.	.
	1049	Yes	Yes	10126	216	12355	62.47	6	1	35	3	6	5
	Median			**6804**	**69**	**6233**	**61.71**	**0**	**1**	**19**	**4**	**2**	**2**
	Geometric Mean**			**4984**	**61**	**6038**	**.**	**.**	**.**	**.**	**4**	**.**	**.**

*These subjects did not receive the full dosage according to their respective group.

**Geometric mean was only calculated when the data set included only positive numbers.

***Since the denominator was zero, this number was calculated by changing the denominator to 1.

d.

PfSPZ/ Dose	ID	Protection		Prepatent Period (days)				Parasite Density (parasites/μL)					
		CHMI		CHMI 1		CHMI 2		CHMI 1			CHMI 2		
		1	2	PCR	TBS	PCR	TBS	By PCR at PCR+	By PCR at TBS+	By TBS at TBS+	By PCR at PCR+	By PCR at TBS+	By TBS at TBS+
Controls (3D7 CHMI)	1050	No	.	6.99	10.97	.	.	0.26	136.14	51.95	.	.	.
	1052	No	.	8.81	12.25	.	.	2.84	234.56	34.63	.	.	.
	1056	No	.	8.90	13.70	.	.	1.11	298.33	43.29	.	.	.
	1058	No	.	6.91	10.95	.	.	0.23	166.99	69.26	.	.	.
	1061	No	.	9.28	13.03	.	.	0.95	102.79	54.55	.	.	.
	1063	No	.	8.85	10.93	.	.	0.14	25.22	3.25	.	.	.
	1065	.	No	.	.	7.11	10.92	.	.	.	3.37	145.96	36.4
	1067	.	No	.	.	7.05	11.92	.	.	.	0.49	431.42	72.7
	1071	.	No	.	.	9.00	12.84	.	.	.	1.56	129.81	56.3
	1072	.	No	.	.	7.00	10.98	.	.	.	0.33	116.97	26.0
	1073	.	No	.	.	6.95	11.23	.	.	.	0.54	37.15	8.7
	1075	.	No	.	.	10.97	14.03	.	.	.	1.50	145.91	13.0
	Median			**8.83**	**11.61**	**7.08**	**11.57**						
Controls (7G8 CHMI)	1051	No	.	6.76	9.76	.	.	1.69	53.16	54.55	.	.	.
	1054	No	.	6.92	12.82	.	.	2.47	170.67	47.62	.	.	.
	1055	No	.	8.99	10.99	.	.	4.85	25.78	36.36	.	.	.
	1060	No	.	10.74	12.87	.	.	13.78	103.71	17.32	.	.	.
	1064	.	No	.	.	7.95	11.74	.	.	.	5.61	17,074.05	10,757.6
	1066	.	No	.	.	6.86	9.81	.	.	.	7.80	346.07	72.7
	1068	.	No	.	.	6.91	10.87	.	.	.	0.75	25.15	8.7
	1069	.	No	.	.	6.93	10.91	.	.	.	2.12	446.48	54.5
	1070	.	No	.	.	7.07	9.93	.	.	.	0.93	241.17	13.0
	1074	.	No	.	.	6.96	10.90	.	.	.	1.09	138.77	36.4
	Median			**7.95**	**11.90**	**6.95**	**10.88**						

Table S5. Inhibition of antibodies to rPfCSP and $(NANP)_6$ by $(NANP)_6$. We assessed antibodies to rPfCSP and $(NANP)_6$ by ELISA in sera taken 2 weeks after the third dose of PfSPZ Vaccine from 14 subjects in Group 3 who underwent CHMI #1. We then incubated the sera with 500 ng $(NANP)_6$ for 120 minutes and reassessed the sera in the same ELISA. The same concentration of $(NANP)_6$ blocked 80% of the binding to $(NANP)_6$ and 60-62% of the binding to the nearly full-length recombinant PfCSP. Approximately 77% of the antibodies to rPfCSP were directed against the central repeat region peptide $(NANP)_6$.

Volunteer ID	OD 1.0 rPfCSP Pre-Absorption	OD 1.0 $(NANP)_6$ Pre-Absorption	OD 1.0 rPfCSP Post- Absorption	OD 1.0 $(NANP)_6$ Post- Absorption
2080-1031	35,990	18,242	13588	3935
2080-1035	17,846	7,910	6318	1398
2080-1036	41,638	20,285	16027	1449
2080-1037	10,926	4,241	3588	805
2080-1038	18,616	10,550	7216	2,234
2080-1039	10,792	6,467	3846	1,526
2080-1040	10,075	4,463	4302	1,253
2080-1041	2,575	1,082	1097	314
2080-1042	13,314	4,854	5764	1,110
2080-1045	8,413	2,032	4658	544
2080-1046	32,372	9,143	10892	1,842
2080-1047	24,689	12,218	8819	2,708
2080-1048	3,524	1,320	1657	324
2080-1049	39,612	17,740	14871	4,112
Mean	**19,313**	**8,610**	**7,332 (-62%)**	**1,682 (-80%)**

Table S6. Antibodies to well-defined Pf proteins 2 weeks after the last dose of PfSPZ Vaccine

Protein	Group								
	Group 1 (5 doses of $2.7x10^5$ PfSPZ, CHMI with Pf3D7)			Group 2 (5 doses of $2.7x10^5$ PfSPZ, CHMI with Pf7G8)			Group 3 (3 doses of $4.5x10^5$ PfSPZ, CHMI with Pf3D7)		
	No. Positive * /No. Tested (%)	Net OD 1.0 of Positives (GM)	Net OD 1.0 Ratio of Positives (GM)	No. Positive /No. Tested (%)	Net OD 1.0 of Positives (GM)	Net OD 1.0 Ratio of Positives (GM)	No. Positive /No. Tested (%)	Net OD 1.0 of Positives (GM)	Net OD 1.0 Ratio of Positives (GM)
1st Expressed in Sporozoites									
PfCSP	13/14 (93%)	5971	135.98	12/13 (92%)	10,935	359.50	15/15 (100%)	14,206	161.61
PfSSP2/ TRAP	5/14 (36%)	232	58.55	3/13 (23%)	876	160.74	2/15 (13%)	175	25.76
PfCelTOS	0/14			0/13			0/15		
PfMSP5	3/14 (21%)	176	5.11	7/13 (54%)	317	5.61	11/15 (73%)	386	6.26
PfAMA1	6/14 (43%)	342	8.62	6/13 (46%)	256	6.03	1/15 (7%)	51**	52.00**
1st Expressed in Early Liver Stages									
PfEXP1	0/14			1/13 (8%)	24*	24.00*	0/15		
PfLSA1	0/14			0/13			0/15		
1st Expressed in Late Liver Stages									
PfMSP1	0/14			0/13			0/15		
PfEBA175	0/14			0/13			0/15		

*To be considered positive the Net OD 1.0 had to be greater than 50 and the ratio of the OD 1.0 post immunization to the OD 1.0 pre-immunization had to be >3.0.

**For these values, the single value is recorded, not the geometric mean (GM).

Table S7. Injection pain, efficiency, and speed

	Group 1 and 2	*Group 3*	*Total*
Pain assessment, n (%)			
Number of pain questionnaire responses	145 (100)	45 (100)	190 (100)
Immunizations with subject assessment of:			
No pain	109 (75)	29 (64)	138 (73)
Mild pain	31 (21)	15 (33)	46 (24)
Moderate pain	5 (3)	1 (2)	6 (3)
Severe pain	0	0	0
Comparison to previous injections received[a]			
Less painful	66 (46)	13 (29)	79 (42)
Same	65 (45)	32 (71)	97 (51)
More painful	14 (10)	0	14 (7)
Injector assessment, n (%)			
Number of stick injections per immunization			
1 stick	139 (96)	43 (96)	182 (96)
2 sticks	6 (4)	2(4)	8 (4)
$\geq$3 sticks	0	0	0
Unsuccessful	0	0	0
Difficulty of 1-stick injections[b]			
Simple	137/139 (99)	39/43(91)	176/182 (97)
Moderately difficult	2 (1)	4 (9)	6 (3)
Very difficult	0	0	0
Elapsed time (minutes)			
Vaccine thawing to syringe receipt by injector			
N	145	45	190
Mean (SD)	4.6 (1.6)	6.1 (1.0)	4.9 (1.6)
Range (min, max)	3, 20	5, 8	3, 20
Quartiles (25^{th}, median, 75^{th})	4, 4, 5	5, 6, 7	4, 5, 6
Syringe receipt by injector to injectioncompletion			
N	145	45	190
Mean (SD)	1.3 (1.8)	2.0 (1.5)	1.5 (1.7)
Range (min, max)	0, 20	0, 8	0, 20
Quartiles (25^{th}, median, 75^{th})	1, 1, 1	1, 2, 2	1, 1, 2

Column header counts and denominators are the total number of immunizations administered, unless indicated otherwise.

a Comparison is to other injections received in the subject's lifetime.

b Denominators are the number of immunizations with 1-stick injections.

Table S8. Global summary of possibly, probably, or definitely related adverse events

	Group 1 and 2 *N = 30* *# imm = 5*	*Group 3* *N = 15* *# imm = 3*	*Total* *N = 45*
Solicited AEs[a]			
Volunteers with at least one event, n (%)	16 (53)	8 (53)	24 (53)
Volunteers with at least one Grade 3 event, n (%)	0	0	0
Number of events	43	23	66
Mean number of events per volunteer with events	2.7	2.9	2.8
Maximum grade reported	2	2	2
Solicited local AEs[a]			
Volunteers with at least one event, n (%)	4 (13)	2 (13)	6 (13)
Volunteers with at least one Grade 3 event, n (%)	0	0	0
Number of events	6	5	11
Mean number of events per volunteer with events	1.5	2.5	1.8
Maximum grade reported	1	1	1
Solicited systemic AEs[a]			
Volunteers with at least one event, n (%)	13 (43)	8 (53)	21 (47)
Volunteers with at least one Grade 3 event, n (%)	0	0	0
Number of events	37	18	55
Mean number of events per volunteer with events	2.8	2.3	2.6
Maximum grade reported	2	2	2
Unsolicited AEs[b]			
Volunteers with at least one event, n (%)	14 (47)	4 (27)	18 (40)
Volunteers with at least one Grade 3 event, n (%)	0	0	0
Number of events	18	6	24
Mean number of events per volunteer with events	1.3	1.5	1.3
Maximum grade reported	1	1	1
Serious AEs[c]			
Volunteers with at least one event, n (%)	0	0	0
Number of events	0	0	0

Column header counts and denominators are the number of volunteers who received at least one immunization.
a Solicited AEs are from the day of immunization through 7 days after immunization.
b Unsolicited AEs are from the day of first immunization through the day of first CHMI or study discontinuation, whichever occurs first.
c Serious AEs are from the the day of first immunization through the day of first CHMI or study discontinuation, whichever occurs first.

Table S9. Summary of possibly, probably, or definitely related solicited adverse events for each group, by immunization.

	Imm 1	*Imm 2*	*Imm 3*	*Imm 4*	*Imm 5*
Group 1 and 2					
Subjects receiving immunization	30	30	29	29	27
Local solicited AEs					
Subjects with at least one event, n (%)[a]	2 (7)	0	1 (3)	0	1 (4)
Number of events	3	0	2	0	1
Systemic solicited AEs					
Subjects with at least one event, n (%)[a]	5 (17)	9 (30)	2 (7)	4 (14)	1 (4)
Number of events	7	19	5	5	1
Group 3					
Subjects receiving immunization	15	15	15	n/a	n/a
Local solicited AEs					
Subjects with at least one event, n (%)[a]	1 (7)	1 (7)	2 (13)		
Number of events	1	2	2		
Systemic solicited AEs					
Subjects with at least one event, n (%)[a]	5 (33)	4 (27)	3 (20)		
Number of events	7	6	5		

[a]Denominators are the number of subjects receiving the specified immunization.

Table S10a. Possibly, probably, or definitely related solicited adverse events for each immunization, by group (all immunizations)

	Group 1 and 2 N=30 # imm = 5		*Group 3 N=15 # imm = 3*		*Total N=45*	
	Vol n (%)	*Max grade*	*Vol n (%)*	*Max grade*	*Vol n (%)*	*Max grade*
Any immunization	**16/30(53)**	**2**	**8/15(53)**	**2**	**24/45 (53)**	**2**
Any local reaction at injection site	**4(13)**	**1**	**2(13)**	**1**	**6(13)**	**1**
Pain	2(7)	1	2(13)	1	4(9)	1
Tenderness	1(3)	1	1(7)	1	2(4)	1
Erythema	1(3)	1	0(0)		1(2)	1
Induration	1(3)	1	0(0)		1(2)	1
Swelling	0(0)		1(7)	1	1(2)	1
Any systemic reaction	**13(43)**	**2**	**8(53)**	**2**	**21(47)**	**2**
Headache	10(33)	1	7(47)	2	17(38)	2
Fatigue	4(13)	1	3(20)	2	7(16)	2
Malaise	5(17)	1	0(0)		5(11)	1
Myalgia	3(10)	1	1(7)	1	4(9)	1
Arthralgia	3(10)	2	0(0)		3(7)	2
Chills	1(3)	1	1(7)	1	2(4)	1
Nausea	1(3)	1	1(7)	1	2(4)	1
Diarrhoea	1(3)	1	0(0)		1(2)	1
Allergic reaction	0		0		0	
Pyrexia	0		0		0	
Vomiting	0		0		0	

Column header counts are the number of subjects receiving at least one immunization. Denominators are the number of subjects receiving the specified immunization. Max grade is the maximum grade experienced by a subject for the specified event and immunization.

Table S10b. Possibly, probably, or definitely related solicited adverse events for each immunization, by group (Immunization 1).

	Group 1 and 2 N=30 # imm = 5		*Group 3 N=15 # imm = 3*		*Total N=45*	
	Vol n (%)	*Max grade*	*Vol n (%)*	*Max grade*	*Vol n (%)*	*Max grade*
Immunization 1	**7/30(23)**	**1**	**6/15(40)**	**2**	**13/45(29)**	**2**
Any local reaction at injection site	**2(7)**	**1**	**1(7)**	**1**	**3(7)**	**1**
Tenderness	1(3)	1	1(7)	1	2(4)	1
Erythema	1(3)	1	0(0)		1(2)	1
Induration	1(3)	1	0(0)		1(2)	1
Pain	0		0		0	
Swelling	0		0		0	
Any systemic reaction	**5(17)**	**1**	**5(33)**	**2**	**10(22)**	**2**
Headache	4(13)	1	5(33)	1	9(20)	1
Chills	1(3)	1	1(7)	1	2(4)	1
Fatigue	0(0)		1(7)	2	1(2)	2
Malaise	1(3)	1	0(0)		1(2)	1
Myalgia	1(3)	1	0(0)		1(2)	1
Allergic reaction	0		0		0	
Arthralgia	0		0		0	
Diarrhoea	0		0		0	
Nausea	0		0		0	
Pyrexia	0		0		0	
Vomiting	0		0		0	

Column header counts are the number of subjects receiving at least one immunization. Denominators are the number of subjects receiving the specified immunization. Max grade is the maximum grade experienced by a subject for the specified event and immunization.

Table S10c. Possibly, probably, or definitely related solicited adverse events for each immunization, by group (Immunization 2).

	Group 1 and 2 N=30 # imm = 5		*Group 3 N=15 # imm = 3*		*Total N=45*	
	Vol n (%)	*Max grade*	*Vol n (%)*	*Max grade*	*Vol n (%)*	*Max grade*
Immunization 2	**9/30(30)**	**2**	**4/15(27)**	**2**	**13/45(29)**	**2**
Any local reaction at injection site	**0(0)**		**1(7)**	**1**	**1(2)**	**1**
Pain	0(0)		1(7)	1	1(2)	1
Swelling	0(0)		1(7)	1	1(2)	1
Erythema	0		0		0	
Induration	0		0		0	
Tenderness	0		0		0	
Any systemic reaction	**9(30)**	**2**	**4(27)**	**2**	**13(29)**	**2**
Headache	6(20)	1	3(20)	2	9(20)	2
Fatigue	4(13)	1	1(7)	1	5(11)	1
Malaise	3(10)	1	0(0)		3(7)	1
Arthralgia	2(7)	2	0(0)		2(4)	2
Myalgia	2(7)	1	0(0)		2(4)	1
Nausea	1(3)	1	1(7)	1	2(4)	1
Allergic reaction	0		0		0	
Chills	0		0		0	
Diarrhoea	0		0		0	
Pyrexia	0		0		0	
Vomiting	0		0		0	

Column header counts are the number of subjects receiving at least one immunization. Denominators are the number of subjects receiving the specified immunization. Max grade is the maximum grade experienced by a subject for the specified event and immunization.

Table S10d. Possibly, probably, or definitely related solicited adverse events for each immunization, by group (Immunization 3).

	Group 1 and 2 N=30 # imm = 5		*Group 3 N=15 # imm = 3*		*Total N=45*	
	Vol n (%)	*Max grade*	*Vol n (%)*	*Max grade*	*Vol n (%)*	*Max grade*
Immunization 3	**3/29(10)**	**1**	**4/15(27)**	**2**	**7/44(16)**	**2**
Any local reaction at injection site	**1(3)**	**1**	**2(13)**	**1**	**3(7)**	**1**
Pain	1(3)	1	2(13)	1	3(7)	1
Erythema	0		0		0	
Induration	0		0		0	
Swelling	0		0		0	
Tenderness	0		0		0	
Any systemic reaction	**2(7)**	**1**	**3(20)**	**2**	**5(11)**	**2**
Headache	1(3)	1	3(20)	2	4(9)	2
Fatigue	1(3)	1	1(7)	1	2(5)	1
Malaise	2(7)	1	0(0)		2(5)	1
Diarrhoea	1(3)	1	0(0)		1(2)	1
Myalgia	0(0)		1(7)	1	1(2)	1
Allergic reaction	0		0		0	
Arthralgia	0		0		0	
Chills	0		0		0	
Nausea	0		0		0	
Pyrexia	0		0		0	
Vomiting	0		0		0	

Column header counts are the number of subjects receiving at least one immunization. Denominators are the number of subjects receiving the specified immunization. Max grade is the maximum grade experienced by a subject for the specified event and immunization.

Table S10e. Possibly, probably, or definitely related solicited adverse events for each immunization, by group (Immunization 4).

	Group 1 and 2 N=30 # imm = 5		*Group 3 N=15 # imm = 3*		*Total N=45*	
	Vol n (%)	*Max grade*	*Vol n (%)*	*Max grade*	*Vol n (%)*	*Max grade*
Immunization 4	**4/29(14)**	**1**	**n/a**	**n/a**	**4/29(14)**	**1**
Any local reaction at injection site	**0**				**0**	
Erythema	0				0	
Induration	0				0	
Pain	0				0	
Swelling	0				0	
Tenderness	0				0	
Any systemic reaction	**4(14)**	**1**			**4(14)**	**1**
Headache	2(7)	1			2(7)	1
Fatigue	1(3)	1			1(3)	1
Malaise	1(3)	1			1(3)	1
Nausea	1(3)	1			1(3)	1
Allergic reaction	0				0	
Arthralgia	0				0	
Chills	0				0	
Diarrhoea	0				0	
Myalgia	0				0	
Pyrexia	0				0	
Vomiting	0				0	

Column header counts are the number of subjects receiving at least one immunization. Denominators are the number of subjects receiving the specified immunization. Max grade is the maximum grade experienced by a subject for the specified event and immunization.

Table S10f. Possibly, probably, or definitely related solicited adverse events for each immunization, by group (Immunization 5).

	Group 1 and 2 N=30 # imm = 5		*Group 3 N=15 # imm = 3*		*Total N=45*	
	Vol n (%)	*Max grade*	*Vol n (%)*	*Max grade*	*Vol n (%)*	*Max grade*
Immunization 5	**2/27(7)**	**2**	**n/a**	**n/a**	**2/27(7)**	**2**
Any local reaction at injection site	**1(4)**	**1**			**1(4)**	**1**
Pain	1(4)	1			1(4)	1
Erythema	0				0	
Induration	0				0	
Swelling	0				0	
Tenderness	0				0	
Any systemic reaction	**1(4)**	**2**			**1(4)**	**2**
Arthralgia	1(4)	2			1(4)	2
Allergic reaction	0				0	
Chills	0				0	
Diarrhoea	0				0	
Fatigue	0				0	
Headache	0				0	
Malaise	0				0	
Myalgia	0				0	
Nausea	0				0	
Pyrexia	0				0	
Vomiting	0				0	

Column header counts are the number of subjects receiving at least one immunization. Denominators are the number of subjects receiving the specified immunization. Max grade is the maximum grade experienced by a subject for the specified event and immunization.

Table S11a – Laboratory abnormalities pre- and post-immunization

Parameter/Grade	*Baseline[a] (Day of immunization 1) n/N (%)*	*Day of immunization 2, 3, 4, or 5[b] n/N (%)*	*Day 2 post-imm n/N (%)*	*Day 7 post-imm n/N (%)*	*Day 21 post-imm[c] n/N (%)*	*Max severity post-baseline[d] n/N (%)*
Any parameter						
Grade 0	35/45 (78)	25/45 (56)	17/45 (38)	16/45 (36)	28/42 (67)	11/45 (24)
Grade 1	7 (16)	15 (33)	20 (44)	23 (51)	10 (24)	22 (49)
Grade 2	3 (7)	5 (11)	7 (16)	6 (13)	4 (10)	11 (24)
Grade 3	0	0	1 (2)	0	0	1 (2)
ALP						
Grade 0	45/45 (100)	45/45 (100)	45/45 (100)	45/45 (100)	41/41 (100)	45/45 (100)
Grade 1	0	0	0	0	0	0
Grade 2	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0
ALT						
Grade 0	44/45 (98)	38/45 (84)	39/45 (87)	39/45 (87)	40/42 (95)	35/45 (78)
Grade 1	1 (2)	7 (16)	5 (11)	6 (13)	2 (5)	9 (20)
Grade 2	0	0	1 (2)	0	0	1 (2)
Grade 3	0	0	0	0	0	0
AST						
Grade 0	45/45 (100)	39/45 (87)	37/45 (82)	41/45 (91)	41/42 (98)	32/45 (71)
Grade 1	0	6 (13)	7 (16)	3 (7)	1 (2)	11 (24)
Grade 2	0	0	1 (2)	1 (2)	0	2 (4)
Grade 3	0	0	0	0	0	0
Total bilirubin						
Grade 0	43/45 (96)	44/45 (98)	43/45 (96)	41/45 (91)	42/42 (100)	41/45 (91)
Grade 1	2 (4)	1 (2)	2 (4)	4 (9)	0	4 (9)
Grade 2	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0

Subjects in Group 1 and 2 received up to five immunizations and subjects in Group 3 received up to three immunizations. Subjects are counted at most once under a column according to the highest grade reported across all immunizations received. Denominators are the number of subjects with at least one laboratory record available at the specified visit across all immunizations. Grading is based upon the U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research guidelines titled 'Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials' 2007.

a Baseline is defined as the measurement taken just prior to immunization on the day of first immunization.

b The day of immunization measurement for immunizations 2, 3, 4, or 5 was taken prior to the administration of vaccine on that day.

c Laboratory results for Day 21 were only scheduled for the final immunization (i.e., Immunization 5 for Groups 1 and 2 or Immunization 3 for Group 3).

d The maximum severity post-baseline is the highest grade reported by a subject after the day of first immunization.

e Subject 1035 had unscheduled neutrophil, platelet, and WBC lab abnormalities within 28 days of an immunization that exceeded the maximum grade reported at one of the scheduled visits included in this table. See the accompanying listing for more details on Subject 1035's lab abnormalities.

Parameter/Grade	*Baseline[a] (Day of immunization 1) n/N (%)*	*Day of immunization 2, 3, 4, or 5[b] n/N (%)*	*Day 2 post-imm n/N (%)*	*Day 7 post-imm n/N (%)*	*Day 21 post-imm[c] n/N (%)*	*Max severity post-baseline[d] n/N (%)*
BUN						
Grade 0	44/44 (100)	45/45 (100)	43/45 (96)	44/45 (98)	42/42 (100)	42/45 (93)
Grade 1	0	0	2 (4)	1 (2)	0	3 (7)
Grade 2	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0
Creatinine						
Grade 0	45/45 (100)	42/45 (93)	40/45 (89)	40/45 (89)	40/42 (95)	38/45 (84)
Grade 1	0	3 (7)	5 (11)	5 (11)	2 (5)	7 (16)
Grade 2	0	0	0	0	0	0
Grade 3	0	0	0	0	0	0
Hemoglobin						
Grade 0	39/45 (87)	38/45 (84)	35/45 (78)	33/45 (73)	34/42 (81)	30/45 (67)
Grade 1	4 (9)	5 (11)	7 (16)	10 (22)	5 (12)	11 (24)
Grade 2	2 (4)	2 (4)	2 (4)	2 (4)	3 (7)	3 (7)
Grade 3	0	0	1 (2)	0	0	1 (2)
Neutrophils						
Grade 0	44/45 (98)	43/45 (96)	39/45 (87)	42/45 (93)	38/42 (90)	39/45 (87)
Grade 1	0	1 (2)	3 (7)	1 (2)	3 (7)	2 (4)
Grade 2	1 (2)	1 (2)	3 (7)	2 (4)	1 (2)	4 (9)[e]
Grade 3	0	0	0	0	0	0
Platelets						
Grade 0	45/45 (100)	43/45 (96)	42/45 (93)	43/45 (96)	42/42 (100)	42/45 (93)[e]
Grade 1	0	1 (2)	2 (4)	2 (4)	0	2 (4)
Grade 2	0	1 (2)	1 (2)	0	0	1 (2)
Grade 3	0	0	0	0	0	0

Subjects in Group 1 and 2 received up to five immunizations and subjects in Group 3 received up to three immunizations. Subjects are counted at most once under a column according to the highest grade reported across all immunizations received. Denominators are the number of subjects with at least one laboratory record available at the specified visit across all immunizations. Grading is based upon the U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research guidelines titled 'Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials' 2007.

a Baseline is defined as the measurement taken just prior to immunization on the day of first immunization.

b The day of immunization measurement for immunizations 2, 3, 4, or 5 was taken prior to the administration of vaccine on that day.

c Laboratory results for Day 21 were only scheduled for the final immunization (i.e., Immunization 5 for Groups 1 and 2 or Immunization 3 for Group 3).

d The maximum severity post-baseline is the highest grade reported by a subject after the day of first immunization.

e Subject 1035 had unscheduled neutrophil, platelet, and WBC lab abnormalities within 28 days of an immunization that exceeded the maximum grade reported at one of the scheduled visits included in this table. See the accompanying listing for more details on Subject 1035's lab abnormalities.

Parameter/Grade	*Baseline[a] (Day of immunization 1) n/N (%)*	*Day of immunization 2, 3, 4, or 5[b] n/N (%)*	*Day 2 post-imm n/N (%)*	*Day 7 post-imm n/N (%)*	*Day 21 post-imm[c] n/N (%)*	*Max severity post-baseline[d] n/N (%)*
WBC						
Grade 0	43/45 (96)	42/45 (93)	37/45 (82)	41/45 (91)	38/42 (90)	36/45 (80)
Grade 1 (low)	2 (4)	2 (4)	7 (16)	3 (7)	4 (10)	6 (13)
Grade 2 (low)	0	0	1 (2)	0	0	1 (2)
Grade 3 (low)	0	0	0	0	0	0
Grade 1 (high)	0	0	0	0	0	0
Grade 2 (high)	0	1 (2)	0	1 (2)	0	2 (4)[e]
Grade 3 (high)	0	0	0	0	0	0

Subjects in Group 1 and 2 received up to five immunizations and subjects in Group 3 received up to three immunizations. Subjects are counted at most once under a column according to the highest grade reported across all immunizations received. Denominators are the number of subjects with at least one laboratory record available at the specified visit across all immunizations. Grading is based upon the U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research guidelines titled 'Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials' 2007.

a Baseline is defined as the measurement taken just prior to immunization on the day of first immunization.

b The day of immunization measurement for immunizations 2, 3, 4, or 5 was taken prior to the administration of vaccine on that day.

c Laboratory results for Day 21 were only scheduled for the final immunization (i.e., Immunization 5 for Groups 1 and 2 or Immunization 3 for Group 3).

d The maximum severity post-baseline is the highest grade reported by a subject after the day of first immunization.

e Subject 1035 had unscheduled neutrophil, platelet, and WBC lab abnormalities within 28 days of an immunization that exceeded the maximum grade reported at one of the scheduled visits included in this table. See the accompanying listing for more details on Subject 1035's lab abnormalities.

Parameter/Grade	*Day of immunization n/N (%)*	*Day 2 n/N (%)*	*Day 7 n/N (%)*	*Day 21[a] n/N (%)*	*Max severity post-imm[b] n/N (%)*
Creatinine					
Grade 0	185/ 190 (97)	184/ 189 (97)	180/ 190 (95)	40/ 42 (95)	176/ 190 (93)
Grade 1	5 (3)	5 (3)	10 (5)	2 (5)	14 (7)
Grade 2	0	0	0	0	0
Grade 3	0	0	0	0	0
Hemoglobin					
Grade 0	166/ 190 (87)	162/ 189 (86)	167/ 190 (88)	34/ 42 (81)	153/ 190 (81)
Grade 1	16 (8)	20 (11)	18 (9)	5 (12)	27 (14)
Grade 2	8 (4)	6 (3)	5 (3)	3 (7)	9 (5)
Grade 3	0	1 (1)	0	0	1 (1)
Neutrophils					
Grade 0	186/ 190 (98)	181/ 189 (96)	185/ 190 (97)	38/ 42 (90)	180/ 190 (95)
Grade 1	2 (1)	5 (3)	3 (2)	3 (7)	4 (2)
Grade 2	2 (1)	3 (2)	2 (1)	1 (2)	6 (3)
Grade 3	0	0	0	0	0
Platelets					
Grade 0	187/ 190 (98)	183/ 189 (97)	186/ 190 (98)	42/ 42 (100)	184/ 190 (97)
Grade 1	2 (1)	4 (2)	4 (2)	0	4 (2)
Grade 2	1 (1)	2 (1)	0	0	2 (1)
Grade 3	0	0	0	0	0
WBC					
Grade 0	185/ 190 (97)	178/ 189 (94)	185/ 190 (97)	38/ 42 (90)	177/ 190 (93)
Grade 1 (high)	0	0	0	0	0
Grade 1 (low)	4 (2)	10 (5)	4 (2)	4 (10)	11 (6)
Grade 2 (high)	1 (1)	0	1 (1)	0	1 (1)
Grade 2 (low)	0	1 (1)	0	0	1 (1)
Grade 3 (high)	0	0	0	0	0
Grade 3 (low)	0	0	0	0	0

Subjects are counted multiple times under each column according to available laboratory data and the number of immunizations received (e.g., a subject who received three immunizations and has no missing data for the scheduled timepoints is counted three times under each column). Denominators are the number of laboratory records available for the specified timepoint across all subjects and immunizations.

a Laboratory results for Day 21 were only scheduled for the final immunization (i.e., immunization 5 for Groups 1 and 2 or immunization 3 for Group 3).

b The maximum severity post-immunization is the highest grade reported by a subject across all parameters within a given immunization.

Table S11b – Laboratory abnormalities: Subject #1035 (Group 3)

			Platelets 140-400 x10^3/uL		*Neutrophils 1500-7800 cells/uL*		*WBC 3.8-10.8 x10^3/uL*	
Group	*Subject ID*	*Study visit*	*Val*	*Gr*[a]	*Val*	*Gr*[a]	*Val*	*Gr*[a]
Group 3	1035	Screening	243	0	1776	0	4.0	0
		Immunization 1	232	0	2148	0	4.7	0
		Immunization 1, Day 2	233	0	2244	0	5.5	0
		Immunization 1, Day 7	217	0	16135	0	19.3	2
		Unscheduled (Imm 1, Day 10)	216	0	18360	0	23.3	3*
		Unscheduled (Imm 1, Day 15)	154	0	2024	0	4.4	0
		Unscheduled (Imm 1, Day 16)	138	1*	672	3*	2.8	-1
		Unscheduled (Imm 1, Day 18)	140	0	858	3*	3.3	-1
		Unscheduled (Imm 1, Day 21)	207	0	577	3*	3.7	-1
		Unscheduled (Imm 1, Day 25)	305	0	1312	1	4.1	0
		Immunization 2	254	0	2336	0	6.1	0
		Immunization 2, Day 2	249	0	1642	0	4.2	0
		Immunization 2, Day 7	244	0	1982	0	5.4	0
		Unscheduled (Imm 2, Day 9)	260	0	2795	0	6.5	0
		Unscheduled (Imm 2, Day 20)	237	0	2507	0	6.1	0
		Immunization 3	225	0	1593	0	4.4	0
		Immunization 3, Day 2	199	0	1148	2	3.5	-1
		Immunization 3, Day 7	230	0	1512	0	4.5	0
		Unscheduled (Imm 3, Day 10)	232	0	1758	0	3.7	-1
		Unscheduled (Imm 3, Day 14)	272	0	1488	1	3.5	-1
		Immunization 3, Day 21	275	0	1261	1	3.3	-1

All available laboratory data within 28 days of an immunization are displayed for subjects that had at least one unscheduled measurement within 28 days of an immunization that had a grade higher than the maximum grade reported at one of the scheduled visits. The grade for an unscheduled visit meeting these criteria are indicted by an asterisk (*). The normal range for each parameter is provided under the parameter name.

a Grade: 1=mild, 2=moderate, 3=severe, 4=life-threatening. Grading is based upon the U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research guidelines titled 'Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials' 2007. Per the guidelines, platelets and neutrophils are not graded for abnormalities above the normal range. WBCs are graded for abnormalities both below and above the normal range. WBC graded abnormalities with values below the normal range are shown with a negative grade.

Table S12. Results of potency and sporozoite membrane integrity assays on two lots of PfSPZ Vacine used in the WRAIR 2080 clinical rial

Lot #	*Time Point*	*Potency (# of parasites expressing PfLSA-1/well)*	*% Viability (Sporozoite Membrane Integrity Assay)*
1	**Fresh (Nov. 2013)**	466 ± 17	98.6%
	Release (Nov. 2013)	422 ± 41	88.5% ± 2.3%
	3 Months	439 ± 18	84.4% ± 0.7%
	6 Months (Pre-Use)	347 ± 27	86.4% ± 5.2%
	9 Months (Post-Use)	432 ± 67	82.5% ± 1.0%
2	**Fresh (May 2014)**	408 ± 11	98.2%
	Release (May 2014)	368 ± 3	89.9% ± 1.4%
	3 Months (Pre-Use)	413 ± 38	89.0% ± 1.3%
	6 Months	377 ± 41	85.5% ± 1.5%
	9 Months (Post-Use)	409 ± 3	84.3% ± 0.6%

For the 3-day hepatocyte potency assay, 4.0×10^4 HC-04 (1F9) cells/well in triplicate were infected with 2.5×10^4 PfSPZ for 3 hours, washed, and incubated for 3 days with daily media change. Liver stage parasites expressing PfLSA-1 were counted after staining the slides with a polyclonal antibody against PfLSA-1 and fluorescently labeled secondary antibody. For the sporozoite membrane integrity assay, propidium iodide and SYBR® Green were added to 1.5×10^4 PfSPZ. PfSPZ were applied to a hemocytometer and incubated in a dark humidity chamber for 20 min, at which point the red PfSPZ (those with compromised membranes) and green PfSPZ (those with intact membranes) in 16 quadrants were counted under a fluorescent microscope. Those with intact membranes were considered viable, and viability is expressed as the total green PfSPZ over the total number of PfSPZ counted × 100. Fresh PfSPZ were assessed prior to cryopreservation. Cryopreserved PfSPZ were assessed several days later for release of each lot, and stability was assessed at defined time points after cryopreservation.

Table S13: ELISA measuring antibodies against proteins expressed in sporozoites (PfCSP, PfSSP2/TRAP, PfCelTOS, PfMSP5, PfAMA1), early liver stages (PfEXP1 and PfSLSA1) and late liver/blood stages (PfMSP1and PfEBA175)

Recombinant Protein Details									
PfCSP	The recombinant *P. falciparum* (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 1st amino acid is residue 50 at the N-terminus and comprising both the 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) (1).								
PfCelTOS	The recombinant Pf cell-traversal protein for ookinetes and sporozoites (rPfCelTOS) 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 precursor of AMA1 is processed proteolytically, to cleave away the pro-sequence, converting the protein into a 66 kDa form, which allows the merozoite relocalization. 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	PfLSA1 (recombinant 52-kDa Lot# 061907, 0.50 mg/mL). The recombinant Pf liver stage antigen-1, PfLSA1 is a 20-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-kDaLot# 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, without the hydrophobic signal sequence removed. GenBank Accession No.: CAA28735.1 (Protein Potential).								
Assay Details									
Pf Proteins	**CSP**	**SSP2**	**MSP5**	**CelTOS**	**MSP1**	**AMA1**	**EB175**	**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 Imidizole-based wash solution containing 2 mM imidazole, 160 mM NaCl, 0.02% Tween 20, 0.5 mM EDTA (3 times after each incubations)								
Blocking buffer composition (diluent)	1% Bovine Serum Albumin (BSA) blocking buffer (KPL)								
	1% milk	5% milk	1% milk	5% milk	5% milk	1% milk	5% milk	1% milk	1% milk
Serum	1:100 starting dilution and three fold serial dilution in triplicate								
Secondary ab	Peroxidase labeled goat anti-human IgG (KPL)								

Secondary ab µg/mL	0.1	0.1	0.1	0.1	0.05	0.1	0.05	0.2	0.1
Substrate	ABTS Peroxidase substrate								
Substrate incubation period (room temp)	75 mins	60 mins	75 mins	60 mins	60 mins	75 mins	60 mins	60 mins	50 mins

Figure. S1. Genetic distance between *P. falciparum* isolates. The first two coordinates of a principal coordinates analysis (PCoA) are shown, reflecting genetic distance among PfNF54 (black), Pf7G8 (green), and 19 clinical isolates from Mali (dark red), Ghana (red), Malawi (orange), Uganda (goldenrod), and Kenya (yellow). Whole genome sequence data from each sample (Table S1) were aligned to the Pf3D7 genome assembly (PlasmoDB v24) with bowtie2 (2). Alignments were converted to BAM files (3) and processed for duplicate read removal using picard tools (http://picard.sourceforge.net/). SNP calls were then generated using GATK and according to GATK's Best Practices documentation, including indel realignment and quality score recalibration steps (4, 5). An additional high-stringency filter was applied to the initial SNP set to remove false positives based on quality scores (QUAL), depth (DP), strand bias (FS), and mapping quality (MQ0). The filter used is defined as follows: (DP < 12 || QUAL < 50 || FS > 14.5 || (MQ0 >= 2 && (MQ0/(1.0 * DP)) > 0.1)). This filter is conservative, and may result in an underestimation of the number of true SNPs. The total number of SNPs in each sample relative to the reference Pf 3D7 genome assembly was identified with this approach. In order to determine the relative distance between all isolates and strains and Pf3D7, SNPs for all samples were filtered according to a panel of 944,270 bi-allelic positions identified in protein-coding regions of the Pf genome, a SNP panel validated by the Sanger Institute (6). Pairwise distances were estimated based only on the high-confidence SNPs in this panel, and for positions called in all samples. Custom Python and R scripts were used to create a pairwise distance matrix based on the SNP calls, and to format data for the PCoA. Coordinate 1 separates African samples by longitude, from West to East Africa; in addition, Pf7G8, from South America, clusters with samples from West Africa, as expected, since the introduction of Pf to South America is believed to be associated with the slave trade from West Africa (7). The second coordinate separates Pf7G8 from the African samples. The first and second coordinates explain 8% and 7% of the variation in the data, respectively.

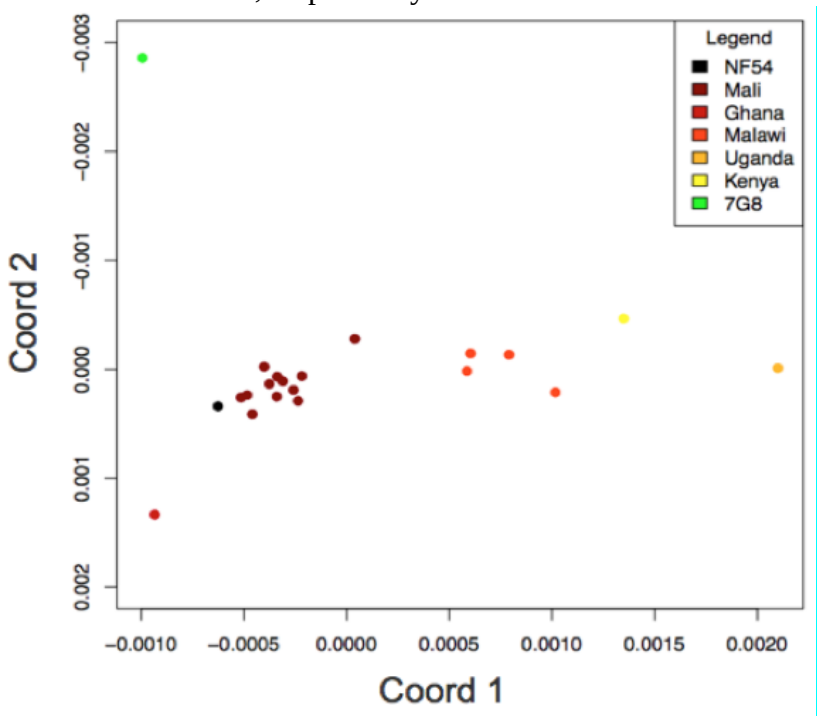


Figure S2. IgM and IgG antibodies against PfSPZ for all 33 volunteers in Groups 1-3 who received the full dosage regimen and underwent CHMI #1 in sera taken two weeks after the last dose of PfSPZ Vaccine. The median AFU $2x10^5$ levels are delineated. Median IgG antibody levels were 11.2-fold higher than median IgM antibody levels.

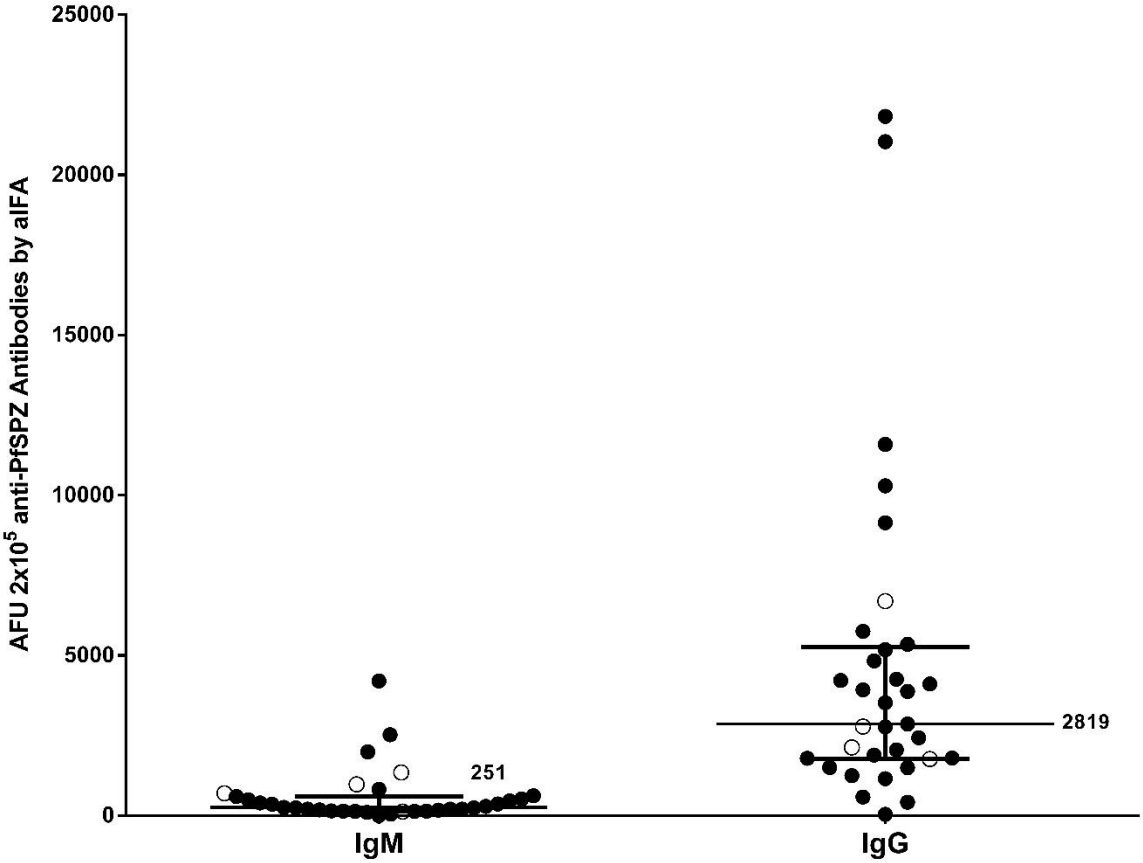
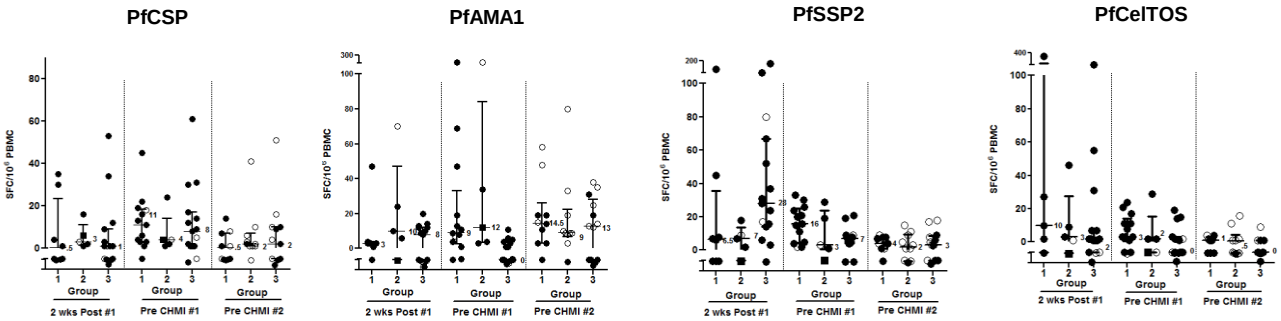


Figure. S3. Cellular immune responses to stimulation with synthetic peptides from multiple Pf proteins. Peptide-specific circulating peripheral blood mononuclear cells (PBMC) secreting single cytokines; IFNγ only, IL2 only, or IFNγ+IL2, were evaluated in the FluoroSpot assay (see Methods) after stimulation with pools of synthetic peptides [PfCSP (A), PfAMA1 (B), PfSSP2/TRAP (C), PfCelTOS (D)]. For each of the 3 study groups, Group 1, Group 2, and Group 3, individual volunteer responses are recorded as spot forming cells per 10^6 PBMC (SFC/10^6 PBMC). Pre-immunization responses were subtracted from the post immunization peptide responses shown which include 2 weeks post dose 1, pre-CHMI #1, and pre-CHMI #2. Protected subjects are shown as filled symbols and not protected subjects are shown as unfilled symbols. For each of the 3 groups, the interquartile ranges and the median values of responses of subjects against each test antigen in each group are shown. There is no statistically significant association between any response and protection.



2. Supplementary Materials and Methods:

PfSPZ Vaccine

PfSPZ Vaccine, composed of aseptic, purified, cryopreserved, metabolically active, radiation-attenuated PfSPZ, was manufactured as described using Pf NF54 parasites (8-11) and met all quality control release and stability assay specifications (Table S12). Two different lots of PfSPZ Vaccine, manufactured in November 2013 and May 2014, were used in this trial.

Study Design

The primary study endpoints were (1) incidence and type of adverse events (AEs), including breakthrough infections or abnormal vital signs, clinical laboratory assessments, physical examination findings, and (2) evidence of vaccine-mediated protection against CHMI 3 weeks and 24 weeks after last immunization in Groups 1, 2, and 3, by preventing blood stage infection for 28 days (as detected by thick blood smear analysis and confirmed by PCR) following CHMI.

Assessment of Tolerability and Safety

PfSPZ Vaccine. All subjects receiving at least one dose of vaccine were included in the immunization phase tolerability and safety analyses. Grading of AEs was based upon the CBER guidelines entitled "Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" 2007 (http://www.fda.gov/cber/guidelines.htm). In general, grading was as follows: Grade 1(mild): Does not interfere with routine activities, minimal level of discomfort; Grade 2 (moderate): Interferes with routine activities, moderate level of discomfort; Grade 3(severe): Unable to

perform routine activities, significant level of discomfort; Grade 4 (potentially life-threatening): Hospitalization or ER visit for potentially life-threatening event. Solicited adverse events (AEs) were collected for 7 days following each immunization. Unsolicited AEs for the immunization phase were collected for 28 days following each immunization except the final immunization; unsolicited AEs for the final immunization were collected until the day of first CHMI.

CHMI. Solicited AEs were collected from the day of exposure to PfSPZ-infected mosquitoes through day 28 after exposure. AEs occurring during the first 6 days after initiation of CHMI were potentially attributed to exposure to PfSPZ-infected mosquitoes. Those occurring from days 7-28 were considered potentially attributable to malaria.

Metrics for injection pain were calculated as a proportion of the total number of immunizations administered. Safety was assessed by calculating the proportion of immunized subjects in each group who experienced vaccine-, mosquito bite-, or malaria-related (definitely, probably, or possibly) mild, moderate, severe, or serious AEs as defined by symptoms, clinical signs, breakthrough infections, or laboratory values. To further define risk factors for AEs, proportions were compared within groups (by dose installment) and across groups (by dose installment and overall). The Safety Monitoring Committee (SMC) assessed safety data after first immunization with $4.5x10^5$ PfSPZ of PfSPZ Vaccine.

Safety was assessed by calculating the percentage of subjects in each group who experienced vaccine-related (definitely, probably, or possibly) AEs and laboratory abnormalities. AEs are presented within groups (by dose) and across groups (by dose and overall). Subjects are presented by group according to the vaccine dose received, with Groups 1 and 2 combined (identical 5 dose regimens, 2.7×10^5 PfSPZ per dose). Where safety data are presented by grade,

subjects were counted under the maximum grade experienced for the specified event and immunization.

Assessment of Protective Efficacy Against CHMI

All immunized subjects who received at least three scheduled immunizations were eligible to participate in CHMI. Real time quantitative DNA polymerase chain reaction (qPCR) was done retrospectively on blood collected at the time of each TBS in those who developed parasitemia, and on days 11, 18, and 28 after CHMI in those who did not develop parasitemia as previously described (12, 13). Subjects who became parasitemic post-CHMI were treated with a 3-day course of atovaquone/proguanil (Malarone) and considered cured of Pf after two sequential daily negative TBSs.

Assessment of Immunological Responses

Antibody Assays: Sera were assessed for vaccine-induced antibodies by enzyme linked immunosorbent assay (ELISA), immunofluorescence assay (IFA), and inhibition of sporozoite invasion (ISI) assay (10, 11).

Enzyme Linked Immunosorbent Assay (ELISA): ELISAs were performed for antigens first expressed in 1) sporozoites (PfCSP, PfSSP2/TRAP, PfCelTOS, PfMSP5, PfAMA1), 2) early liver stages (PfEXP1 and PfSLSA1) and 3) late liver stages (PfMSP1and PfEBA175). The ELISA methods for each antigen are described below.

ELISA Procedure: Recombinant (r) proteins used in ELISA assays are listed in Table S13. 96-well plates (Nunc Maxisorp Immuno Plate) were coated overnight at 4°C with 2.0 μg

recombinant proteins in 50 μL per well in coating buffer (KPL). Plates were washed three times with 1X Imidazole-based wash solution containing 2 mM imidazole, 160 mM NaCl, 0.02% Tween 20, 0.5 mM EDTA and blocked with 1% Bovine Serum Albumin (BSA) blocking buffer (KPL) containing 1% or 5% non-fat dry milk (Table S13) for 1 h at 37 °C. Plates were washed three times and serially diluted serum samples (in triplicates) were added and incubated at 37 °C for 1 h. After three washes, peroxidase labeled goat anti-human IgG (KPL) was added at a dilution of 0.1 μg/mL and incubated at 37°C for 1 hour. Plates were washed three times, ABTS Peroxidase substrate was added for plate development, and the plates were incubated for designated periods (Table S13) at 22°C room temperature. The plates were read with a Spectramax Plus384 microplate reader (Molecular Devices) at 405 nm. The data were collected using Softmax Pro GXP v5 and fit to a 4-parameter logistic curve, to calculate the serum dilution at OD 1.0. To serve as an assay control a negative control (pooled serum from non-immune individuals from malaria free area) was always included. Positive controls varied for each antigen as follows:

a. PfCSP : Serum from an individual with anti-PfCSP antibodies
b. PfLSA-1: Pooled serum from individuals immunized against PfLSA-1 (DMID)
c. PfEBA-175: Pooled serum from individuals immunized against PfLSA-1 (DMID)
d. PfAMA1, PfEXP1, PfMSP-1: Pooled serum of volunteers from a malaria-endemic area (Kenya blood bank)

Samples were considered positive if the difference between the post-immunization OD 1.0 and the pre-immunization OD 1.0 (net OD 1.0) was > 50 and the ratio of post-immunization OD 1.0 to pre-immunization OD 1.0 (ratio) was > 3.

The half-life of anti-PfCSP antibodies was calculated using the following formula:

$t_{1/2} = \frac{(t_1 - t_0)\ln 2}{\ln\left(\frac{N_0}{N_1}\right)}$, where $t_{1/2}$ is the half-life of anti-PfCSP antibodies, $t_1 - t_0$ is the elapsed time in weeks between time points, N_0 is the net OD 1.0 anti-PfCSP ELISA at time t_0, and N_1 is the net OD 1.0 anti-PfCSP ELISA at time t_1.

Inhibition of antibodies to rPfCSP by $(NANP)_6$: We assessed antibodies to rPfCSP and $(NANP)_6$ by ELISA in sera taken 2 weeks after the third dose of PfSPZ Vaccine from 14 subjects in Group 3 who underwent CHMI #1. To assess inhibition by $(NANP)_6$, the sera was pre-incubated with 500 ng $(NANP)_6$ for 120 minutes and reactivity against $(NANP)_6$ or rPfCSP was assessed by ELISA.

Automated Immunofluorescence Assay (aIFA):

Preparation of slides with air-dried PfSPZ: Purified PfSPZ (NF54 strain) from aseptic *A. stephensi* mosquitoes produced by Sanaria were resuspended in phosphate buffered saline (PBS, pH 7.4) to obtain 0.5 x 10^4 PfSPZ per 40 µL. 40 µL (0.5 x 10^4 PfSPZ) were added to each well of Granier cellstar clear bottom black 96 well plates (Sigma-Aldrich). After addition of the suspension, plates were left at room temperature for 12-18 h for air-drying.

IFA procedure: 50 µL of sera diluted in PBS with 2% BSA were added to each well of the 96-well plate containing air-dried PfSPZ. Sera samples were added at 2-fold dilutions starting at 1:50. After adding samples, plates were incubated at 37 °C for 1 h. Plates were washed in PBS three times on an Aquamax Microplate washer. Alexa Fluor 488 conjugated goat anti-human IgG (Molecular Probes) was diluted to 1:250 in PBS with 2 % BSA and 50 µL was added to each well. For assessment of IgM titers, Alexa Fluor 488 conjugated goat anti-human IgM (Molecular Probes) was diluted to 1:250 in PBS with 2 % BSA and 50 µL was added to each well. The plates were then incubated for 1 h at 37 °C. Plates were washed three times with PBS on an Aquamax Microplate washer. 100 µL PBS was added to each well. The plates were sealed using

a plate sealer and stored in the dark at 4 °C until data acquisition. Samples were assessed by scanning the plates using an Acumen eX3 laser scanning imaging cytometer. The positive control was pooled human serum taken two weeks after the last immunization from 12 protected volunteers immunized 4 or 5 times with PfSPZ Vaccine in the VRC 312 clinical trial (11). The Acumen image cytometer scans the entire surface area of each well in a 96-well plate and the fluorescence intensity values (arbitrary units) therefore represent the signal from all 0.5×10^4 PfSPZ that were seeded in each well.

Analysis and readout: The data obtained from Acumen image cytometer was plotted to fit a 4-parameter sigmoidal curve in GraphPad Prism software using serum dilution (log transformed) as the x-axis variable and arbitrary fluorescence units (AFU) on the y axis. Over many iterations during development of this assay we have determined that sera from naïve volunteers in the USA and Europe, including pre-immune sera, always register an arbitrary fluorescence value less than 2.0×10^5 even at the highest concentration (1:50 dilution, see above) used in this assay. Moreover, for sera that do bind to PfSPZ, 2.0×10^5 AFU falls in the exponential portion of their sigmoidal curves. Therefore 2.0×10^5 has been chosen as a threshold in the aIFA assay and the results for each volunteer for antibodies to PfSPZ are reported as the reciprocal serum dilution at which fluorescence intensity was equal to 2.0×10^5 AFU.

Inhibition of Sporozoite Invasion Assay (ISI): HC-04 (1F9) cells (hepatocytes) (14) were cultured in complete medium (10% FBS in DMEM/F12 with 100 units/mL penicillin and 100 µg/mL streptomycin; Gibco by Life Technologies) in (Entactic-Collagen IV-Laminin) ECL-coated 96-well clear bottom black well plates (Greiner Bio-One North America) at a density of 2.5×10^4 cells per well, and incubated for 24 h at 37°C, 5% CO2 with 85% relative humidity.

Twenty-four h later cells were infected with 10^4 aseptic, purified, cryopreserved PfSPZ per well, with or without sera diluted 1:5 from subjects immunized with PfSPZ Vaccine. The assay control included PfSPZ added with media alone. All subjects were assessed at pre-immunization (baseline) and pre-CHMI (post immunization with PfSPZ Vaccine-prior to CHMI) time points. Three hours later PfSPZ that had not invaded the HC-04 cells were removed by washing three times with Dulbecco's phosphate-buffered saline (DPBS), and the cultures were fixed using 4% paraformaldehyde for 15 min at room temperature. Differential immunostaining was performed to differentiate between PfSPZ inside the hepatocytes versus PfSPZ outside the hepatocytes. PfSPZ outside the hepatocytes were stained with an anti-PfCSP mAb (2A10, 6.86 µg/mL) (Protein Potential LLC, with permission from New York University School of Medicine) conjugated with Alexa Fluor 633 (far-red) (1 µg/mL; custom-conjugated at GenScript®), a 1:500X dilution. The hepatocytes were then permeabilized with 0.1% Triton X-100 for 15 min at room temperature, and the PfSPZ inside the hepatocytes were stained with the anti-PfCSP mAb (2A10, 6.86 µg/mL) conjugated with Alexa Fluor 488 (green; 1 µg/mL, conjugated from Genscript), a 1:500X dilution. The numbers of infected hepatocytes (green only) were counted by scanning the plates using an Acumen eX3 laser scanning imaging cytometer. Percent inhibition was calculated by the following formula,

(No. PfSPZ invaded in presence of pre-immune serum)-(No. PfSPZ invaded in presence of pre-CHMI serum)/ (No. of PfSPZ invaded in presence of pre-immune serum) x 100. The number of invaded PfSPZ scored in this assay in the absence of sera ranges from 400 – 600. Pre-immune sera at a 1:5 dilution can reduce this to a range of 300 – 500 invaded PfSPZ. Typically, the difference in invasion inhibition activity is highest between the pre and post-immune sera at a dilution of 1:5 therefore this dilution was chosen to report the assay results.

Cellular Immunology Assays: Antigen-specific circulating peripheral blood mononuclear cells (PBMC) secreting single or multiple cytokines were evaluated using pre-coated FluoroSpot plates and kits purchased from Mabtech (Mabtech AB, Nacka Strand, Sweden). Plates and kits were used according to the manufacturer's instructions. A modification of previously described methods used in ELISpot was followed (15). Briefly, PBMCs were isolated by Ficoll density centrifugation of fresh, anticoagulated (collected in EDTA tubes) whole blood. 4×10^5 PBMC were suspended in 100 µL complete medium (RPMI-1640 supplemented with 1% penicillin/streptomycin, 1% glutamine, 1% MEM NEAA [all from GIBCO, Grand Island, NY], and 10% human AB serum [SIGMA]) and incubated in the FluoroSpot plates with 2.5×10^4 irradiated (150 Gy), aseptic, purified, cryopreserved PfSPZ suspended in 100 µL of complete medium. The PfSPZ were manufactured, cryopreserved and thawed by the identical procedures used to produce PfSPZ Vaccine. Additional wells were stimulated with synthetic peptides purchased from Chiron Technologies, Clayton, Victoria, Australia. Full-length PfCSP, PfAMAI, PfSSP2/TRAP, and PfCelTOS amino acid sequences were covered by a series of 15 amino acid (aa) peptides overlapping by 11 aa. These were combined into a single pool for each antigen, which ranged in number from 43 to 153 peptides. The total number of 15 aa peptides pooled into a single pool for PfCSP, PfAMA1, PfSSP2/TRAP, and PfCelTOS were 65,153,132, and 43 respectively.

CEF-Class I Peptide Pool Plus (CTL, Ohio, USA) consisting of 32 peptides corresponding to defined HLA class I-restricted T cell epitopes from cytomegalovirus, Epstein-Barr virus and influenza virus was used as an internal control for each subject. PHA, a mitogen, was used as a

positive control for cell viability. Negative control unstimulated PBMCs wells received medium only.

Cultures were incubated for 36 h at 37°C in 5% CO_2. Each PBMC sample was assayed in triplicate and the number of single-staining IFNγ- and IL-2-secreting cells and double-staining IFNγ and IL-2-secreting cells were recognized as spot-forming cells (SFC) and enumerated using an automated FluoroSpot reader (AID iSpot, GmbH, Germany). For each triplicate, outliers were rejected if any single value contributed more than 50% of the standard deviation of the triplicate and if its value was three-fold greater or less than the average of the remaining two values. After removing outliers, the mean number of SFCs obtained in negative control wells (no antigen) was subtracted from the value of each test well. Negative counts generated by this background subtraction were converted to zero. The mean SFCs of the test sample was then calculated and expressed as SFCs/10^6 PBMCs.

3. Supplementary Results

Ease of Administration, Tolerability and Safety:

Of the 190 total immunizations received, 73% were associated with no pain, 24% were associated with mild pain, and 3% were associated with moderate pain. The subjects assessed 42% of the immunizations to be less painful than other injections received in the subject's lifetime. All but 8 of the 190 immunizations were performed with 1 stick (Table S7). Preparing each syringe (which is done in a biological safety cabinet) took an average of 5 minutes, and injecting the prepared syringe by DVI an average of 1.5 minutes including prepping the arm for the injection. Actual needle insertion and injection generally took 10 to 15 seconds (data not shown).

There were 66 solicited AEs considered to be possibly, probably, or definitely related (designated "related") to immunization. Of the 66 AEs, 11 were local to the site of injection, and 55 were systemic. Of the 45 immunized subjects, 24 subjects (53%) experienced at least one solicited AE considered related to the vaccine (Table S8). Overall, 13% of subjects experienced a local AE, 4/30 (13%) in Groups 1 and 2 and 2/15 (13%) in Group 3. Likewise, 47% of subjects experienced a systemic AE, 13/30 (43%) in Groups 1 and 2 and 8/15 (53%) in Group 3. There was no difference in the overall rates between lower dose Groups 1 and 2 (16/30 (53%) immunized subjects) and higher dose Group 3 (8/15 (53%) immunized subjects) (Table S8).

Eighteen (40%) of subjects, 14 subjects in Groups 1 and 2 (47%) and 4 in Group 3 (27%), reported a total of 24 possibly, probably, or definitely related unsolicited adverse events. As with solicited AEs, there were no dose-related effects. No Grade 3 or serious adverse event that was considered possibly, probably, or definitely related was reported (Table S8).

When AE's were broken down by immunization, there were no trends of increasing or decreasing AE frequencies as immunizations progressed, for either Groups 1 and 2 (percent of subjects experiencing local AEs over the five immunizations: 7%, 0%, 3%, 0% and 4%; percent of subjects experiencing systemic AEs over the five immunizations: 17%, 30%, 7%, 14%, and 4%), or Group 3 (percent of subjects experiencing local AEs over the three immunizations: 7%, 7% and 13%; percent of subjects experiencing systemic AEs over the three immunizations: 33%, 27%, 20%) (Table S9).

The most frequently reported related solicited AE was headache, occurring in 38% of subjects, followed by fatigue (16%), malaise (11%), myalgia (9%), arthralgia (7%), chills (4%), nausea (4%) and diarrhea (2%) (Table S10a). The most frequent local AE was pain, occurring in 9% of subjects, followed by tenderness (4%), erythema (2%), induration (2%) and swelling (2%). The majority of related solicited AEs resolved within a day. All AEs except 5 were mild (Grade 1). The only Grade 2 AEs were arthralgia (2 events in 2 subjects), headache (2 events in 2 subjects), and fatigue. When specific AEs were broken down by immunizations (Table S10b-f), there were no trends of increasing or decreasing rates for a given AE evident.

No breakthrough malaria infections occurred during immunization. No subjects became pregnant during the trial.

Laboratory abnormalities are listed separately from "adverse events". Six subjects (20%) in Groups 1 and 2 and 4 (27%) in Group 3 had at least one grade 2 laboratory abnormality within 7 days of any immunization (Table S11a). One additional subject who received 2.7×10^5 PfSPZ had a grade 3 decrease in hemoglobin, which occurred 2 days after fourth immunization. This subject had low hemoglobin prior to the first immunization, which persisted throughout the trial. One subject in Group 3 (Subject #1035) had an asymptomatic grade 3 leukocytosis (with

neutrophilia) detected 8 days after the first immunization determined to be possibly related to immunization), followed by leukopenia, neutropenia and thrombocytopenia (Table S11b). The subject remained asymptomatic and continued with immunizations uneventfully and without a recurrence. The most common hematology and chemistry abnormalities were decreased hemoglobin and decreased white blood cell count and elevated AST and ALT.

Adverse events occurring in the 28 days after each CHMI were consistent with what was expected after exposure to mosquito bites and acquisition of malaria infection.

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Table S1. Metadata and SNP count relative to 3D7 for 19 clinical isolates and 2 culture-adapted strains of *P. falciparum*. Data obtained from Genbank's Short Read Archive (http://www.ncbi.nlm.nih.gov/sra/), and pulled by Experiment Accession ID.

Sample Accession ID	Experiment Accession ID	Source	Read Count	Bases (Gpb)	Genome Coverage[a]	Total SNPs[b]	SNP Panel[c]
SAMN02374945	SRX364110	Mali	13,746,800	1.4	60	17,544	4,330
SAMN02373818	SRX363947	Mali	17,199,480	1.7	75	18,834	4,322
SAMN02373653	SRX363851	Mali	16,876,880	1.7	73	23,824	4,576
SAMN02373819	SRX363957	Mali	21,376,140	2.2	93	23,181	4,767
SAMN02373656	SRX363863	Mali	17,426,280	1.8	76	24,033	4,632
SAMN02373820	SRX363963	Mali	19,739,780	2.0	86	23,800	4,552
SAMN02373821	SRX363972	Mali	14,439,220	1.5	63	20,673	4,502
SAMN02373822	SRX363979	Mali	22,572,540	2.3	98	20,845	4,462
SAMN02374901	SRX364033	Mali	22,217,920	2.2	96	20,380	4,420
SAMN02374900	SRX364020	Mali	34,062,020	3.4	148	21,210	4,556
SAMN02374902	SRX364039	Mali	14,143,340	1.4	61	19,580	4,426
SAMN02374907	SRX364050	Mali	17,353,440	1.8	75	21,190	4,571
SAMEA679437	ERX005736, ERX007433	Ghana	129,305,774	9.8	422	23,515	4,906
SAMEA679680	ERX004689, ERX005050	Uganda	40,801,074	3.1	133	22,416	4,918
SAMEA678966	ERX007459, ERX008950	Kenya	58,056,936	4.8	189	22,909	4,927
IGS-MLW-001	Awaiting SRA #	Malawi	30,179,568	3.0	131	22,436	4,670
IGS-MLW-002		Malawi	36,578,884	3.7	159	21,852	4,590
IGS-MLW-003		Malawi	28,665,742	2.9	124	21,817	4,774
IGS-MLW-004		Malawi	36,874,068	3.7	160	23,199	4,666
SAMN01737343 (NF54)[d]	SRX113472, SRX111834	Africa	62,292,174	6.2	270	55	0
SAMN00765682 (7G8)[d]	SRX208839, SRX113476, SRX111831	Brazil	65,542,020	6.6	284	22,056	4,925

[a] Genome coverage calculated as: (read count x read length) / length of Pf reference genome.
[b] Total number of SNPs that pass the high-stringency filter used (see methods in Fig. S1).
[c] Subset of all SNPs that fall within the ~1M SNP panel validated by the Sanger Institute (see methods in Fig. S1).
[d] Culture-adapted strain.