Supplementary information

Protection against malaria at 1 year and immune correlates following PfSPZ vaccination

Andrew S Ishizuka[1,13], Kirsten E Lyke[2,13], Adam DeZure[1], Andrea A. Berry[2], Thomas L Richie[3], Floreliz H Mendoza[1], Mary E Enama[1], Ingelise J Gordon[1], Lee-Jah Chang[1,12], Uzma N Sarwar[1,12], Kathryn L Zephir[1], LaSonji A Holman[1], Eric R James[3], Peter F Billingsley[3], Anusha Gunasekera[3], Sumana Chakravarty[3], Anita Manoj[3], MingLin Li[3,4], Adam J Ruben[3], Tao Li[3], Abraham G Eappen[3], Richard E Stafford[3,4], Natasha KC[3,4], Tooba Murshedkar[3], Hope DeCederfelt[5], Sarah H Plummer[1], Cynthia S Hendel[1], Laura Novik[1], Pamela JM Costner[1], Jamie G Saunders[1], Matthew B Laurens[2], Christopher V Plowe[2], Barbara Flynn[1], William R Whalen[1], JP Todd[1], Jay Noor[1], Srinivas Rao[1,12], Kailan Sierra-Davidson[1], Geoffrey M Lynn[1], Judith E Epstein[6], Margaret A. Kemp[7], Gary A Fahle[7], Sebastian A Mikolajczak[8], Matthew Fishbaugher[8], Brandon K Sack[8], Stefan HI Kappe[8], Silas A Davidson[9], Lindsey S Garver[9], Niklas K Björkström[10], Martha C Nason[11], Barney S Graham[1], Mario Roederer[1], B Kim Lee Sim[3,4], Stephen L Hoffman[3,14], Julie E Ledgerwood[1,14] & Robert A Seder[1,14], for the VRC 312 and VRC 314 Study Teams[13]

[1]Vaccine Research Center, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, USA.
[2]Institute for Global Health, Center for Vaccine Development and Division of Malaria Research, University of Maryland School of Medicine, Baltimore, Maryland, USA.
[3]Sanaria Inc., Rockville, Maryland, USA.
[4]Protein Potential, LLC, Rockville, Maryland, USA.
[5]Pharmaceutical Development Section, Clinical Center, National Institutes of Health, Bethesda, Maryland, USA.
[6]Naval Medical Research Center, Malaria Department, Silver Spring, Maryland, USA.
[7]Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland, USA.
[8]Center for Infectious Disease Research, Seattle, Washington, USA.
[9]Entomology Branch, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA.
[10]Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.
[11]Biostatistics Research Branch, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.
[12]Present addresses: Sanofi, Swiftwater, Pennsylvania, USA (L-J.C.); Albert Einstein College of Medicine, Montefiore Medical Center, New York, New York, USA (U.N.S.); Sanofi, Cambridge, Massachusetts, USA (S.R.).
[13]These authors contributed equally to this work.
[14]These authors jointly directed this work.
Correspondence should be addressed to R.A.S. (rseder@mail.nih.gov).

Supplementary Text
Supplementary Figures 1–11
Supplementary Tables 1–7

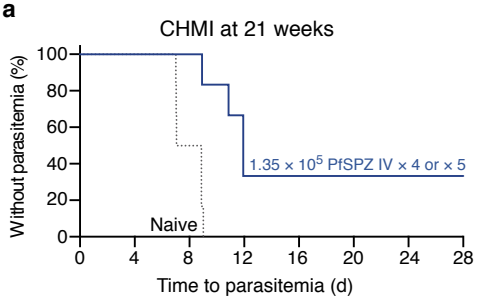
Supplementary Text

Members of the VRC 312 Study Team have been previously reported[11].

The members of the VRC 314 Study Team are as follows:

EMMES Corporation, Rockville, Maryland, USA: L. Diep, M. Kunchai, H. Loblein, P. Renehan. Entomology Branch, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA: C. Brando, M. Dowler, K. Kobylinski, D. Patel, T. Savransky, and K. Walker. Institute for Global Health, Center for Vaccine Development and Division of Malaria Research, University of Maryland School of Medicine, Baltimore, Maryland, USA: M. Adams, M. Billington, L. Chrisley, J. Courneya, T. Crawford, B. Dorsey, N. Johnson, N. Greenberg, D. Ingram, S. Joshi, P. Komninou, A. Kwon, K. Wilhelmi, T. Robinson, B. Sreshtha, K. Strauss, M. Travassos, and X. Wang. U.S. National Institutes of Health (NIH) Clinical Center (CC) Pharmacy, Bethesda, Maryland, USA: J. Starling. NIH CC Department of Laboratory Medicine, Bethesda, Maryland, USA: S. Antonara, J. Brocious, A. Lau, E. Williams. Sanaria Inc., Rockville, Maryland, USA, and Protein Potential LLC, Rockville, Maryland, USA: Y. Abebe, A. Awe, F. Beams, E. Fomumbod, L. Gao, A. Hoffman, H. Huang, J. Jackson, M. Laskowski, B. Jiang, M. King, S. Matheny, K. Nelson, M. Orozco, J. Overby, D. Padilla, A. Patil, V. Pich, E. Saverino, R. Thompson, T.T. Wai, Y. Wen, and R. Xu. Vaccine Research Center, National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, Maryland, USA: R. Bailer, N. Berkowitz, J. Casazza, E. Coates, B. Larkin, I. Pittman, S. Sitar, O. Vasilenko, P. Williams, B. Wilson, and G. Yamshchikov.

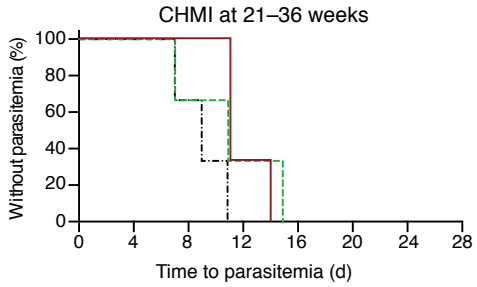
a



b

Vaccination Dose	Inj. #	21 week CHMI Outcome at 3 week CHMI	# of subjects	Parasite free	Subgroup VE	Cumulative VE
1.35 × 10⁵	**4 or 5**	Not parasitemic	6	2	33%	25%
Naive			6	0		

c



d

Vaccination Dose	Inj. #	Outcome at 3 week CHMI	21–36 week CHMI # of subjects	Parasite free
3.00 × 10⁴	**4 or 6**	**Parasitemic**	3	0
1.35 × 10⁵	**4 or 5**	**Parasitemic**	3	0
Naive		**2 of 3 Parasitemic**	3	0

Supplementary Figure 1 Preliminary assessment of PfSPZ Vaccine efficacy at 21–36 weeks after vaccination (VRC 312).

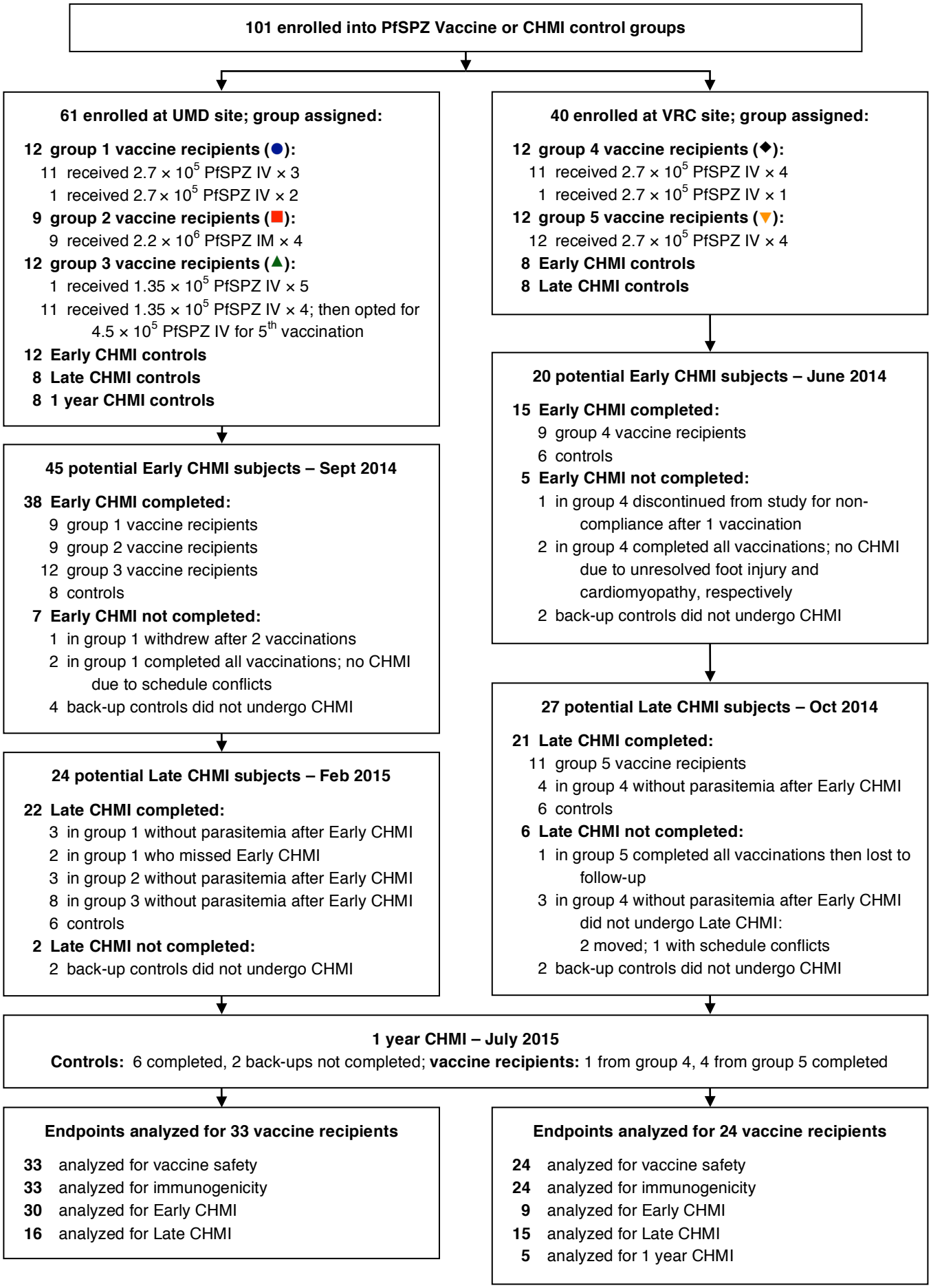
Following demonstration of vaccine efficacy 3 weeks after 4 or 5 intravenous immunizations with 1.35×10^5 PfSPZ in the VRC 312 study, subjects were re-enrolled to undergo repeat CHMI 21–36 weeks after final immunization.

(**a**) Kaplan-Meier curve for six subjects who received 1.35×10^5 PfSPZ administered intravenously (IV) 4 or 5 times ($n = 3$ per group) and who were not parasitemic at CHMI 3 weeks after final immunization (October 2012) underwent repeat CHMI 21 weeks after final immunization (February 2013). Kaplan-Meier curve shows the percent of individuals who did not develop parasitemia after CHMI.

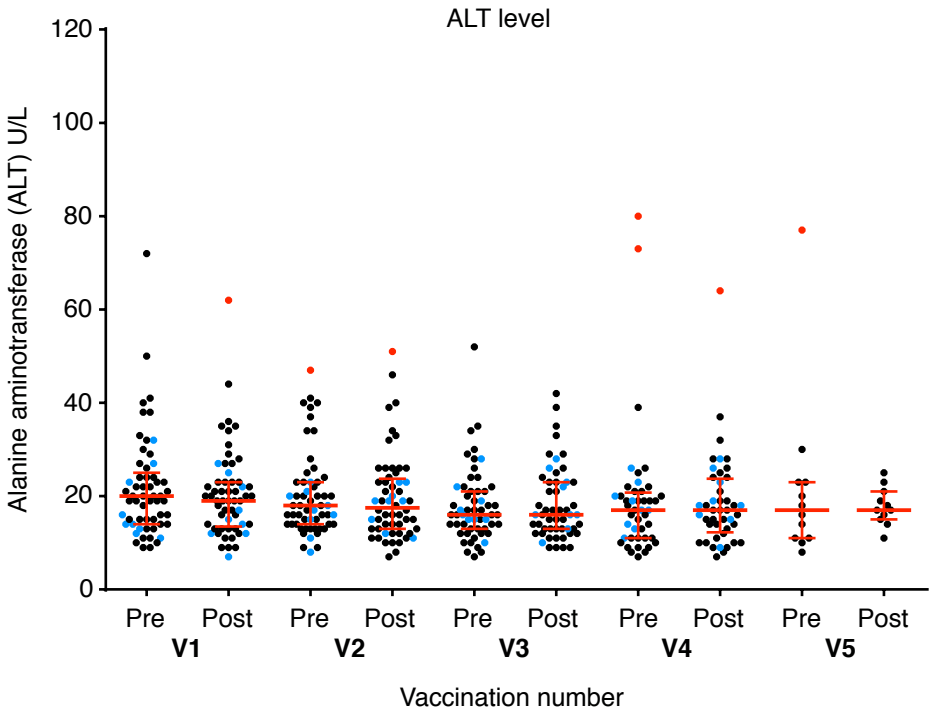
(**b**) Vaccine efficacy. Of the 6 vaccine recipients who were previously not parasitemic at 3 week CHMI, 4/6 developed parasitemia after CHMI 21 weeks after vaccination. Subgroup VE was 33% ($P = 0.23$, one-sided Fisher's exact test) and cumulative VE for the entire 1.35×10^5 PfSPZ dose group was 25%.

(**c**) Kaplan-Meier curve for additional subjects that underwent CHMI 21–36 weeks after final immunization (February 2013). Vaccine recipients who were parasitemic at 3 week CHMI in July 2012 and October 2012 ($n = 3$ from 3.0×10^4 PfSPZ/dose group and $n = 3$ from 1.35×10^5 PfSPZ/dose group) and unvaccinated controls ($n = 3$) from October 2012 CHMI also underwent repeat CHMI in February 2013. All developed parasitemia. One additional subject from the 3.0×10^4 PfSPZ/dose group had an incomplete CHMI and is excluded from further analysis.

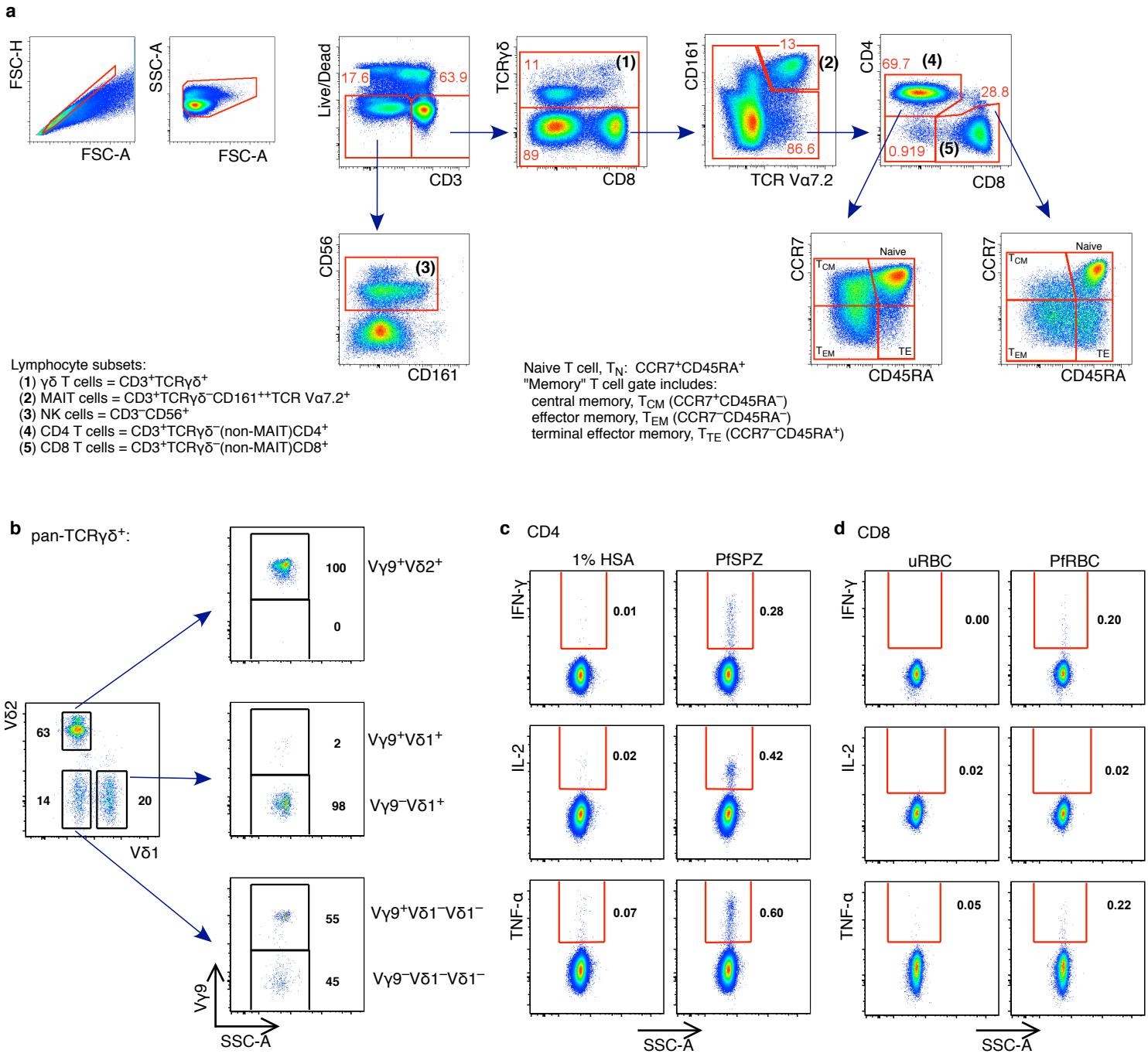
(**d**) Summary of outcomes for additional subjects from February 2013 CHMI.



Supplementary Figure 2 Enrollment, vaccinations, CHMIs, and study endpoints (VRC 314).
"Early CHMI" occurred 3 weeks after final vaccination, "Late CHMI" occurred 21–25 weeks after final vaccination, and "1 year CHMI" occurred 59 weeks after final vaccination.



Supplementary Figure 3 Liver enzyme (ALT) levels pre- and post-vaccination. Alanine aminotransferase (ALT) levels were measured immediately before (Pre) and 14 days after (Post) each vaccination (V1 through V5) for all vaccine groups. There was no difference ($P > 0.05$) between pre- and post-vaccination ALT levels after any vaccination (comparisons between pre- and post- values for each vaccination by Wilcoxon signed rank test with Bonferroni correction for multiple comparisons). A grade 1 ALT of 60 U/L was measured in one vaccine recipient 28 days after vaccination #3. Red bars, median ± interquartile range; black dots, IV administration; blue dots, IM administration; red dots, grade 1 adverse events (1.1–2.5x the upper limit of normal). Normal ALT range: 0–41 U/L males, 0–33 U/L female (VRC site); 21–72 U/L males, 9–52 U/L females (UMD site).



Supplementary Figure 4 Flow cytometry gating tree for analysis of human PBMCs.
Three flow cytometry staining panels were developed to assess the magnitude, quality, and phenotype of Pf-specific lymphocytes. The rationale for the cellular subsets and the phenotypic markers are described in **Supplementary Table 6**.

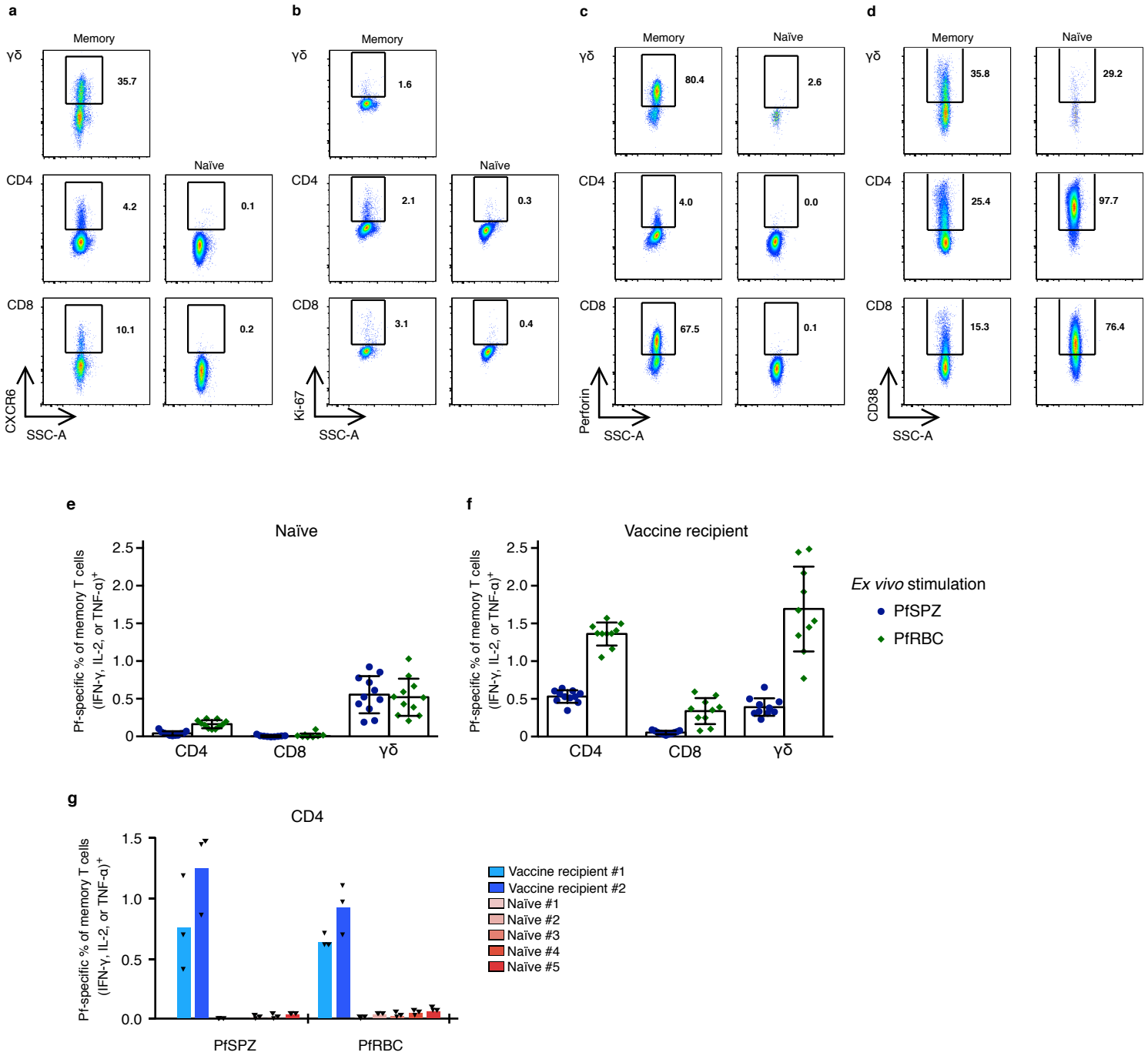
(**a**) Gating tree for staining Panels 1, 2, and 3 (**Supplementary Table 6**), with the following exceptions:

Panel 2, CD4 vs. CD8 T cell gate directly follows TCRγδ$^-$ gate.

Panel 3, TCRγδ$^+$ cells are subdivided into Vδ2$^+$ vs. Vδ1$^+$ vs. Vδ2$^-$Vδ1$^-$ cells, and then further subdivided into Vγ9$^+$ vs. Vγ9$^-$ subsets (**b**).

(**c**) PBMCs were stimulated with PfSPZ or vaccine diluent (1% HSA) and assessed for intracellular cytokine expression by flow cytometry. PfSPZ-specific CD4 T cells are identified by expression of any combination of IFN-γ, IL-2, or TNF-α.

(**d**) PBMCs were stimulated with Pf-infected erythrocyte lysate (PfRBC) or uninfected control lymphocytes (uRBC) and assessed for intracellular cytokine expression by flow cytometry. PfRBC-specific CD8 T cells are identified by expression of any combination of IFN-γ, IL-2, or TNF-α.



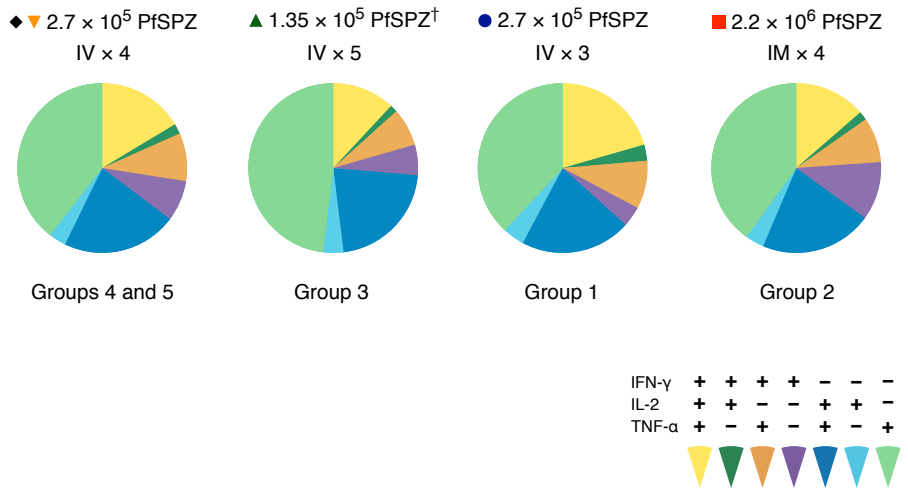
Supplementary Figure 5 Flow cytometry gating of phenotypic markers and ICS assay reproducibility.

(**a**–**b**) Phenotype gates from Panel 1. Expression of CXCR6 (**a**) or Ki-67 (**b**) on memory (left) or naïve (right) cells from the γδ, CD4, or CD8 T cell lineages.

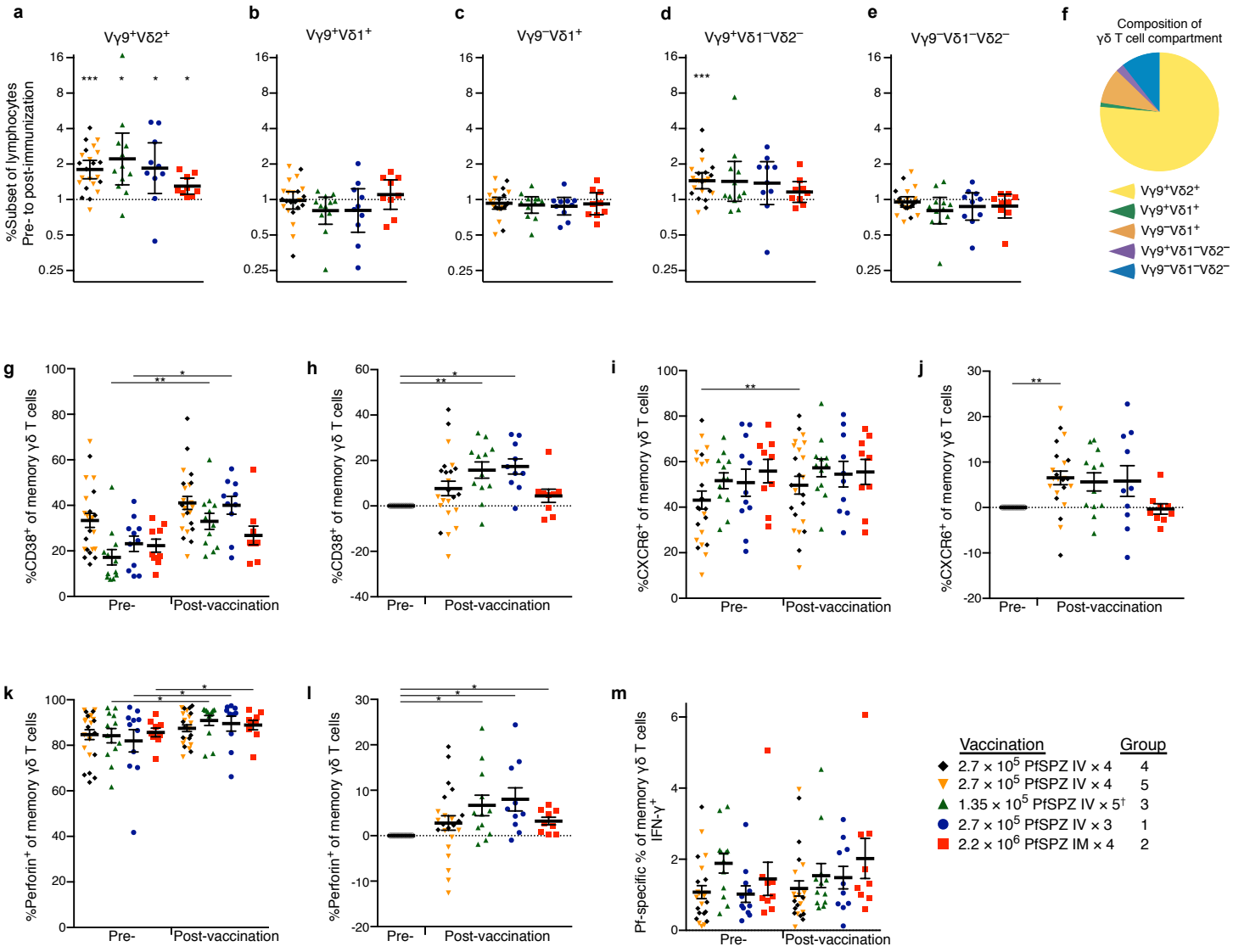
(**c**–**d**) Phenotype gates from Panel 2. Perforin (**c**) or CD38 (**d**) on memory (left) or naïve (right) cells from the γδ, CD4, or CD8 T cell lineages.

(**e**–**g**) Reproducibility of intracellular cytokine staining (ICS) assay.

For **e**–**f**, frozen PBMCs from one malaria-naive donor and one PfSPZ Vaccine recipient were included in 10 consecutive assays on separate days analyzing PfSPZ or PfRBC responses from vaccinated and control subjects. The measured cytokine responses are reported for each lineage as mean ± s.d. For **g**, three different operators stimulated and stained PBMCs from two vaccine recipients and five naïve controls, using two different staining panels and two different LSR IIs to further determine the variability in stimulation and staining. Bar denotes mean.



Supplementary Figure 6 Quality of PfSPZ-specific cytokine-producing CD4 T cells by vaccine regimen.
PBMCs from subjects taken 2 weeks after final vaccination were stimulated with PfSPZ or vaccine diluent (1% HSA) and stained for intracellular cytokine expression. The pie charts show the proportion of cells expressing any combination of IFN-γ, IL-2, or TNF-α for each vaccine regimen. †Group 3 received four doses of 1.35×10^5 PfSPZ and a fifth dose of 4.5×10^5 PfSPZ.



Supplementary Figure 7 Sub-family and phenotypic analysis of γδ T cells following vaccination with PfSPZ.

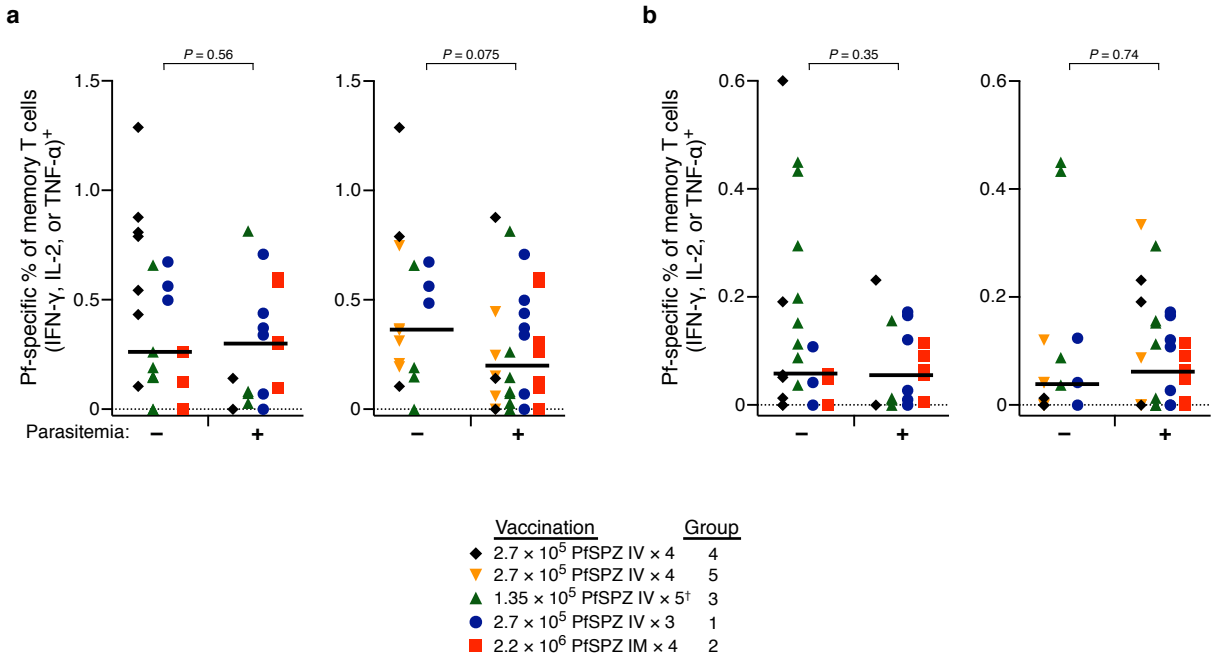
(**a**–**f**) The frequency of the circulating γδ T cell subsets as a percentage of total lymphocytes was assessed in unstimulated PBMCs before the first immunization (pre-immunization) and 2 weeks after final immunization (post-immunization). Fold change from pre-vaccination to post-vaccination for (**a**) $Vγ9^+Vδ2^+$ (**b**) $Vγ9^+Vδ1^+$ (**c**) $Vγ9^-Vδ1^+$ (**d**) $Vγ9^+Vδ1^-Vδ2^-$ or (**e**) $Vγ9^-Vδ1^-Vδ2^-$ subsets. The frequency of $Vγ9^-Vδ2^+$ subset is low to undetectable.

(**f**) Relative frequencies of γδ T cell subsets in unstimulated PBMCs from 55 vaccine recipients from the pre-vaccination time point.

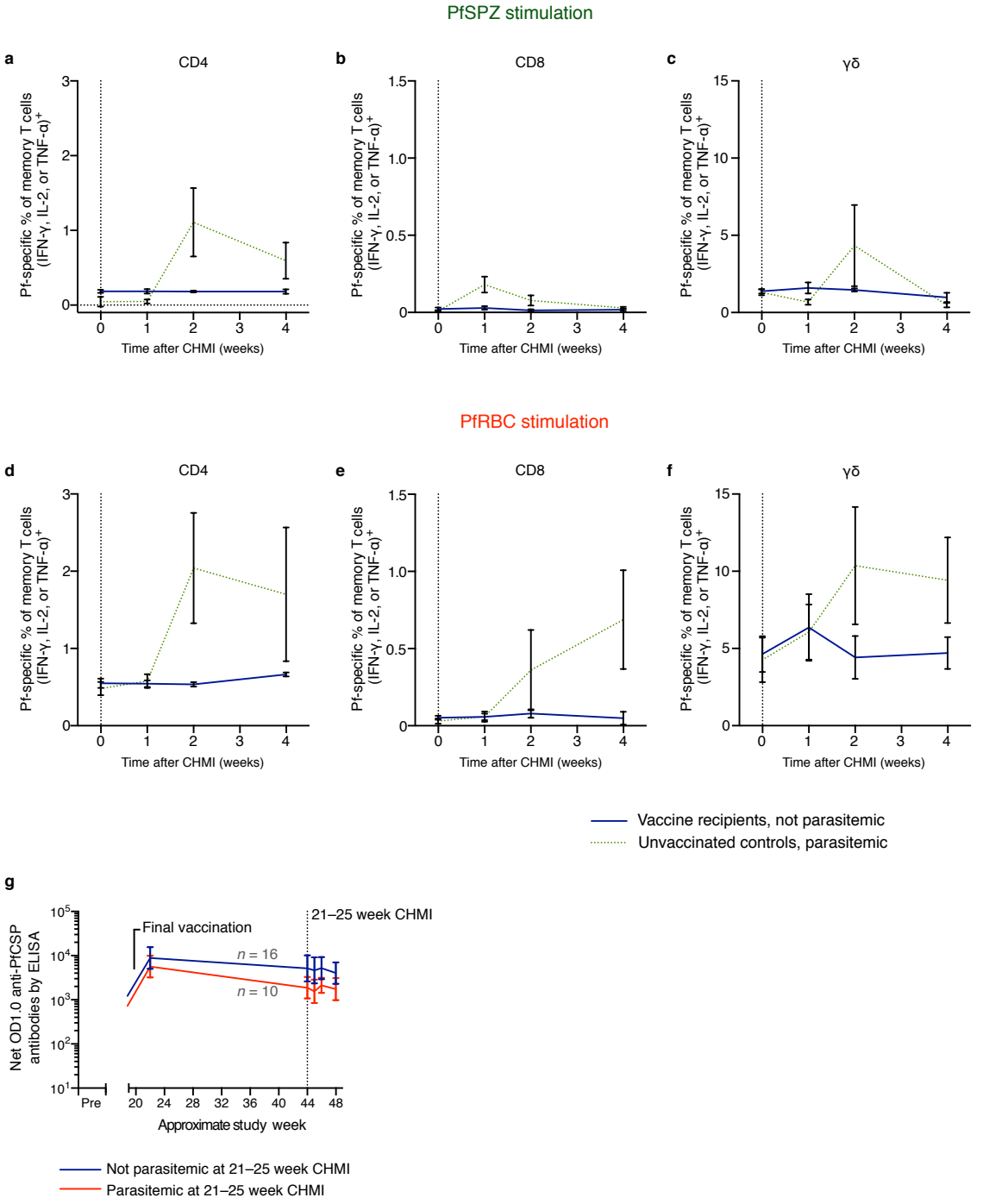
(**g**–**l**) Total memory γδ T cells were assessed pre- and post-immunization for the percent of cells expressing the activation marker CD38 (**g**,**h**), the liver-homing chemokine receptor CXCR6 (**i**,**j**), or the effector molecule perforin (**k**,**l**). The absolute frequencies are shown in **g**, **i**, and **k** and the change from pre- to post-vaccination is shown in **h**,**j**, and **l**.

(**m**) Pf-specific memory γδ T cells secreting IFN-γ by intracellular cytokine staining (ICS) pre- and post-vaccination. Results are the percent of cytokine producing cells after incubation with PfSPZ minus percent of cells after incubation with vaccine diluent (medium with 1% HSA) as control.

For **a**–**l**, difference from pre-vaccine was assessed by Wilcoxon signed rank test. *P*-values were corrected for multiple comparisons by the Bonferroni method. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For **a**–**e**, data are geometric mean ± 95% c.i. For **g**–**m**, data are mean ± s.e.m. †Group 3 received four doses of 1.35×10^5 PfSPZ and a fifth dose of 4.5×10^5 PfSPZ.



Supplementary Figure 8 Comparison of cellular immune responses with CHMI outcome. (**a**) PfSPZ-specific CD4 T cell cytokine responses were assessed 2 weeks after final immunization in subjects who did (+) and did not (–) develop parasitemia at 3 (left) and 21–25 week (right) CHMIs. Since we expected subjects who were parasitemic at 3 weeks to be parasitemic at 21–25 weeks (**Supplementary Fig. 1**), the immune data from individuals who were parasitemic at 3 weeks were included in the analysis at 21–25 weeks. (**b**) PfRBC-specific CD8 T cell cytokine responses 2 weeks after final immunization in subjects who did (+) and did not (–) develop parasitemia at 3 (left) and 21–25 week (right) CHMIs. Subject inclusion was the same as in **a** above. †Group 3 received four doses of 1.35×10^5 PfSPZ and a fifth dose of 4.5×10^5 PfSPZ.



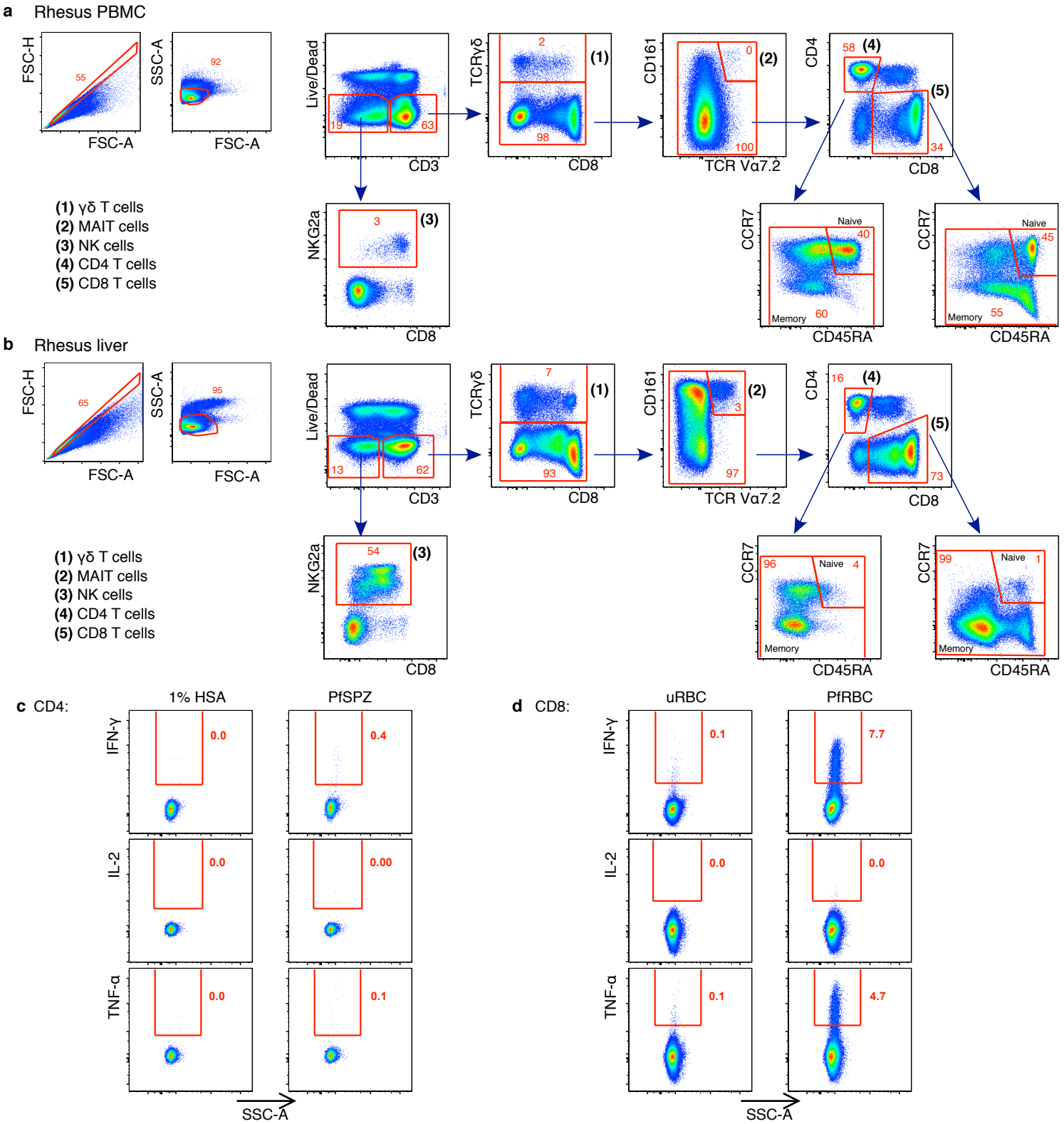
Supplementary Figure 9 Magnitude of Pf-specific T cell and antibody responses after CHMI. Samples were taken at the time of CHMI, and 1, 2, and 4 weeks following CHMI 59 weeks after immunization in $n = 5$ vaccine recipients who did not develop parasitemia and $n = 5$ unvaccinated controls who developed parasitemia.

(**a–c**) Magnitude of PfSPZ-specific CD4 (**a**), CD8 (**b**), and γδ (**c**) T cell response measured following stimulation of PBMCs with PfSPZ (background subtracted from vaccine diluent, 1% HSA).

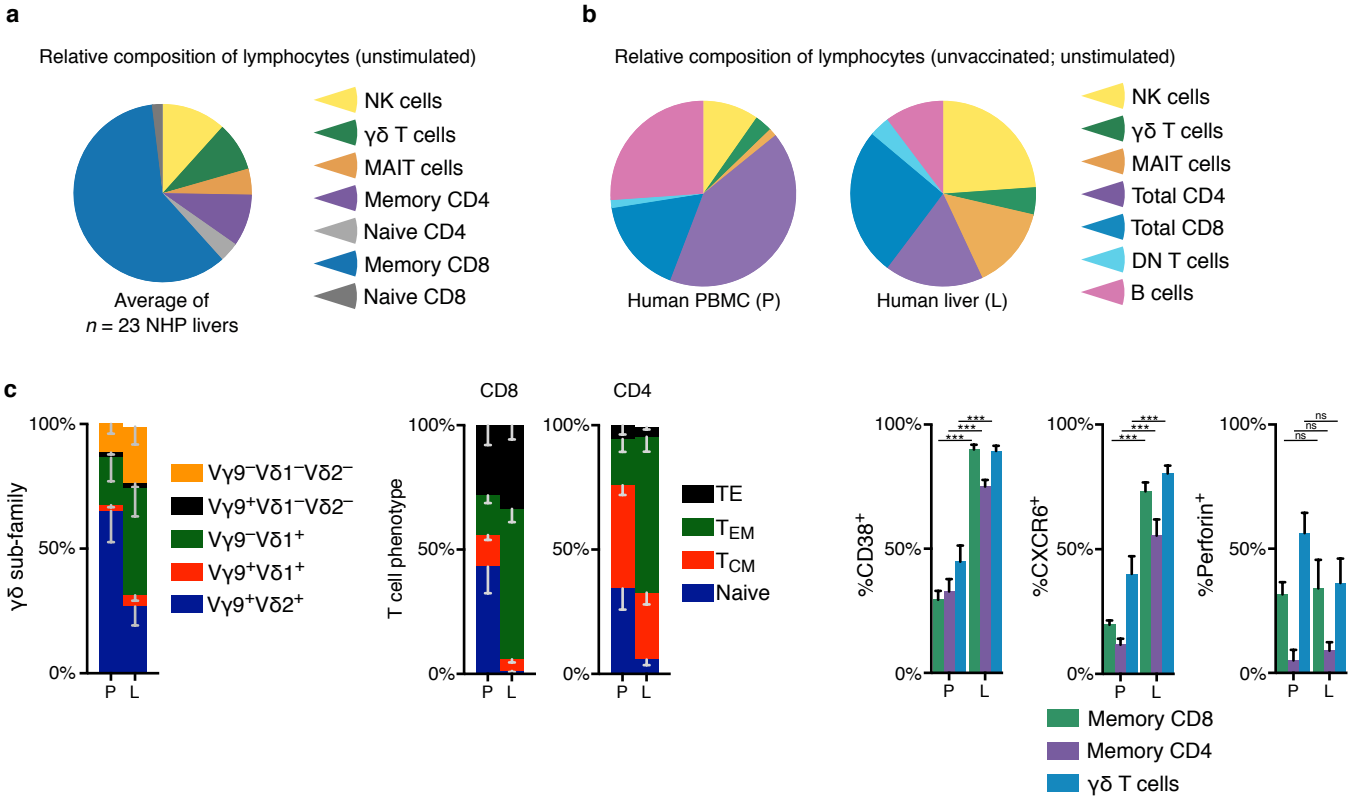
(**d–f**) Magnitude of PfRBC-specific CD4 (**d**), CD8 (**e**), and γδ (**f**) T cell response measured following stimulation of PBMCs with Pf-infected erythrocyte lysate (background subtracted from PBMCs stimulated with uninfected erythrocytes).

(**g**) Antibodies to PfCSP by ELISA (net OD1.0) two weeks after final vaccination, and at the time of CHMI at 21–25 weeks, and 1, 2, and 4 weeks later. Pre-vaccination value is defined as 0 when calculating net OD1.0. Vaccine recipients from all groups (1–5) are included and divided into parasitemic vs. not parasitemic following 21–25 week CHMI.

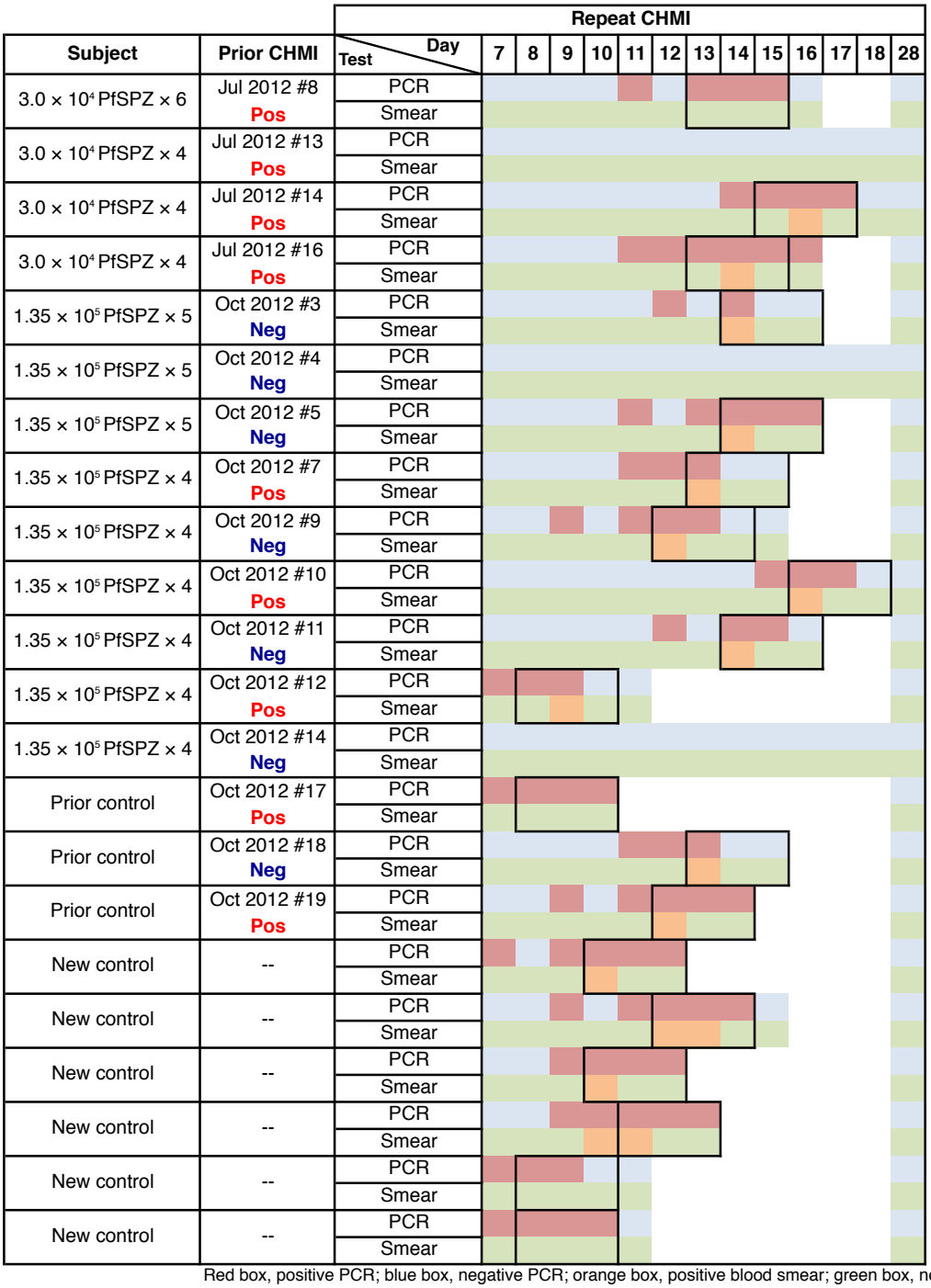
For **a–f**, data are mean ± s.e.m. For **g**, data are geometric mean ± 95% c.i.



Supplementary Figure 10 Gating tree for rhesus PBMCs and liver lymphocytes.
(**a**) Gating tree for NHP PBMCs identifies the same lymphocyte populations as in human PBMCs (**Supplementary Fig. 4**).
(**b**) Gating tree for NHP liver lymphocytes identifies the same lymphocyte populations as in human PBMCs (**Supplementary Fig. 4**) and rhesus PBMCs (**a**).
(**c**) Liver lymphocytes were stimulated with PfSPZ or vaccine diluent (1% HSA) and assessed for intracellular cytokine expression by flow cytometry. PfSPZ-specific CD4 T cells are identified by expression of any combination of IFN-γ, IL-2, or TNF-α.
(**d**) Liver lymphocytes were stimulated with Pf-infected erythrocyte lysate (PfRBC) or uninfected control lymphocytes (uRBC) and assessed for intracellular cytokine expression by flow cytometry. PfRBC-specific CD8 T cells are identified by expression of any combination of IFN-γ, IL-2, or TNF-α.



Supplementary Figure 11 Lymphocyte lineages in rhesus and human liver.
(**a**) Average relative proportions of lymphocyte populations (NK cells, γδ, MAIT, naive and memory CD4 and CD8 T cells) are shown for 23 rhesus livers.
(**b**–**e**) Human PBMC ($n = 7$) and human intrahepatic mononuclear cells (liver; $n = 5$) were stained in parallel with the panels listed in **Supplementary Table 6**.
(**b**) Major lymphocyte subsets found in PBMC (P) and liver (L). DN = $CD3^+TCR\gamma\delta^-$(MAIT marker)$^-CD4^-CD8^-$.
(**c**) Proportions of γδ sub-families in PBMC and liver.
(**d**) Memory phenotype of total CD8 (left) and CD4 (right) T cells in PBMC and liver.
(**e**) Expression of CD38, CXCR6, and perforin by memory CD8, CD4, and γδ T cells in PBMC and liver.
For **c**–**e**, data are mean ± s.e.m. For **e**, comparisons between PBMC and liver was by two-way ANOVA with Bonferroni correction. ns, not significant ($P > 0.05$); *** $P < 0.001$.



Red box, positive PCR; blue box, negative PCR; orange box, positive blood smear; green box, negative blood smear.
Black border surrounds days of atovaquone/proguanil treatment.

Supplementary Table 1 PCR and blood smear following repeat CHMI (VRC 312).

In the VRC 312 study, a repeat CHMI was conducted February 2013 at 21–36 weeks after final vaccination to assess the durability of vaccine efficacy. The participants included 3 prior unvaccinated controls and 13 vaccine recipients that underwent CHMI 3 weeks after their final immunization from across dose groups and 6 new unvaccinated controls. “Pos” (red) and “Neg” (blue) refers to the outcome after the prior CHMI (parasitemia or no parasitemia, respectively). Supplementary Table 1 shows daily PCR and blood smear results following CHMI for all volunteers.

For this CHMI, the study protocol allowed for malaria diagnosis under any the following criteria: a positive blood smear; two consecutive positive PCRs; or one positive PCR with signs or symptoms of malaria. Both blood smear and PCR were performed daily starting on day 7 after CHMI. Post-hoc analysis of smear vs. PCR diagnostic assays revealed that using 2 positive PCR (even if not on consecutive days) as the criteria for treatment resulted in 10 of 19 subjects with parasitemia beginning treatment 1 day earlier, while none would have started treatment later. Based on these data, two positive PCR (even if not on consecutive days) were used as the primary diagnostic criteria in the VRC 314 clinical study.

Supplementary Table 2 Baseline characteristics of participants.

Characteristic	Site 1 – UMD				Site 2 – VRC		
	Grp 1 (●) 2.7×10^5 PfSPZ IV × 3 (*n* = 12)	Grp 2 (■) 2.2×10^6 PfSPZ IM × 4 (*n* = 9)	Grp 3 (▲) 1.35×10^5 PfSPZ IV** (*n* = 12)	UMD CHMI controls (**n* = 20)	Grp 4 (◆) 2.7×10^5 PfSPZ IV × 4 (*n* = 12)	Grp 5 (▼) 2.7×10^5 PfSPZ IV × 4 (*n* = 12)	VRC CHMI controls (**n* = 16)
Sex – no. (%)							
Female	5 (41.7)	5 (55.6)	2 (16.7)	9 (45.0)	6 (50)	2 (16.7)	6 (37.5)
Male	7 (58.3)	4 (44.4)	10 (83.3)	11 (55.0)	6 (50)	10 (83.3)	10 (62.5)
Age – years							
Mean	28.5	28.9	34.2	30.3	30.6	32.3	29.6
Range	[22, 38]	[23, 45]	[22, 45]	[21, 45]	[22, 42]	[23, 45]	[22, 41]
Race – no. (%)							
Asian	1 (8.3)	1 (11.1)	1 (8.3)	2 (10.0)	1 (8.3)	0 (0)	2 (12.5)
Black/African American	2 (16.7)	1 (11.1)	7 (58.3)	9 (45.0)	3 (25.0)	1 (8.3)	2 (12.5)
White	9 (75.0)	7 (77.8)	3 (25.0)	8 (40.0)	7 (58.3)	10 (83.3)	10 (62.5)
Other races combined	0 (0)	0 (0)	1 (8.3)	1 (5.0)	1 (8.3)	1 (8.3)	2 (12.5)
Ethnicity – no (%)							
Non-Hispanic/Latino	12 (100)	8 (88.9)	12 (100)	19 (95.0)	12 (100)	11 (91.7)	16 (100)
Hispanic/Latino	0 (0)	1 (11.1)	0 (0)	1 (5.0)	0 (0)	1 (8.3)	0 (0)
Body mass index (BMI)							
Mean (s.d.)	23.2 (4.9)	23.2 (2.7)	26.2 (4.1)	26.8 (5.2)	25.2 (3.4)	27.1 (4.1)	26.1 (4.4)
Range	[16, 35]	[20, 27]	[20, 33]	[19, 37]	[20, 33]	[23, 34]	[20, 35]
Education – no. (%)							
<High school graduate	1 (8.3)	0 (0)	2 (16.7)	2 (10.0)	0 (0)	0 (0)	0 (0)
High school/GED	0 (0)	0 (0)	3 (25.0)	5 (25.0)	0 (0)	2 (16.7)	3 (18.8)
College graduate	6 (50.0)	8 (88.9)	3 (25.0)	5 (25.0)	7 (58.3)	7 (58.3)	8 (50.0)
Advanced degree	5 (41.7)	1 (11.1)	4 (33.3)	8 (40.0)	5 (41.7)	3 (25.0)	5 (31.3)

*Includes back-up control subjects who did not participate in a controlled human malaria infection (CHMI).

**Group 3 was enrolled to receive 5 vaccinations at 1.35×10^5 PfSPZ IV. By protocol amendment, these subjects were offered the option of the 4.5×10^5 PfSPZ dose for the 5^{th} vaccination; 11 opted for this dose.

Group	Dose (PfSPZ)	Route	Inj. #	Regimen (weeks)	Subject ID	CHMI #1 (days)	CHMI #2 (days)	CHMI #3 (days)
1 ●	2.7×10^5	IV	3	0, 4, 20	102	21	.	.
					103	23	175	.
					104	.	175	.
					105	22	.	.
					106	22	175	.
					107	.	175	.
					108	22	.	.
					109	22	175	.
					110	22	.	.
					111	22	.	.
					112	22	.	.
2 ■	2.2×10^6	IM	4	0, 4, 8, 20	201	22	.	.
					202	22	.	.
					203	22	.	.
					204	22	175	.
					205	22	174	.
					206	21**	.	.
					207	22	174	.
					208	21	.	.
					209	22	.	.
3 ▲	1.35×10^5 (dose #1-4), 4.5×10^5 (dose #5)	IV	5	0, 4, 8, 12, 20	301	21	.	.
					302	22	174	.
					303	22	174	.
					304	22	.	.
					305	21	.	.
					306	22	174	.
					307*	22	.	.
					308	21	174**	.
					309	22	174	.
					310	22	174	.
					311	22	174	.
					312	21	174	.
4 ◆	2.7×10^5	IV	4	0, 4, 8, 20	401	27	167	.
					402	27	167	.
					403	27	.	.
					405	21	.	.
					406	22	.	.
					407	21	161	.
					408	22	.	427
					411	15	.	.
					412	26	166	.
5 ▼	2.7×10^5	IV	4	0, 4, 8, 20	501	.	147	.
					502	.	147	412
					504	.	159	.
					505	.	145	.
					506	.	141	406
					507	.	145	.
					508	.	145	410
					509	.	146	.
					510	.	147	.
					511	.	147	412
					512	.	146	.

Supplementary Table 3 Groups, vaccinations, and days of CHMIs.

*Received 5 doses of 1.35×10^5 PfSPZ

**Did not complete CHMI follow-up and excluded from further analysis

Red indicates that the subject developed parasitemia following CHMI.

Blue indicates that the subject did not develop parasitemia following CHMI

Supplementary Table 4 Local reactogenicity of PfSPZ Vaccine administration by group.

	Grp 1 (●) 2.7×10^5 PfSPZ IV × 3 (*n* = 12)	Grp 2 (■) 2.2×10^6 PfSPZ IM × 4 (*n* = 9)	*Grp 3 (▲) 1.35×10^5 PfSPZ IV (*n* = 12)	*Grp 3 (◭) 4.5×10^5 PfSPZ IV (*n* = 11)	Grp 4 (◆) 2.7×10^5 PfSPZ IV × 4 (*n* = 12)	Grp 5 (▼) 2.7×10^5 PfSPZ IV × 4 (*n* = 12)
	no. (%)					
Pain/tenderness						
None	8 (66.7)	4 (44.4)	12 (100)	11 (100)	8 (66.7)	11(91.7)
Mild	3 (25.0)	5 (55.6)	0 (0)	0 (0)	4 (33.3)	1 (8.3)
Moderate	1 (8.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Swelling						
None	12 (100)	9 (100)	12 (100)	11 (100)	12 (100)	12 (100)
Mild	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Moderate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Redness						
None	12 (100)	9 (100)	11 (91.7)	11 (100)	11 (91.7)	12 (100)
Mild	0 (0)	0 (0)	1 (8.3)	0 (0)	1 (8.3)	0 (0)
Moderate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Any local symptom						
None	8 (66.7)	4 (44.4)	11 (91.7)	11 (100)	7(58.3)	11 (91.7)
Mild	3 (25.0)	5 (55.6)	1 (8.3)	0 (0)	5 (41.7)	1 (8.3)
Moderate	1 (8.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Local and systemic reactogenicity for each group is reported as the "worst severity" reported at any time in the 3 days after any vaccination administered at the indicated dose.

*Group 3 was enrolled to receive 5 vaccinations at 1.35×10^5 PfSPZ IV. By protocol amendment, these subjects were offered the option of the 4.5×10^5 PfSPZ dose for the 5^{th} vaccination; 11 opted for this dose.

Supplementary Table 5 Systemic reactogenicity of PfSPZ Vaccine administration by group.

	Grp 1 (●) 2.7×10^5 PfSPZ IV × 3 (*n* = 12)	Grp 2 (■) 2.2×10^6 PfSPZ IM × 4 (*n* = 9)	*Grp 3 (▲) 1.35×10^5 PfSPZ IV (*n* = 12)	*Grp 3 (◭) 4.5×10^5 PfSPZ IV (*n* = 11)	Grp 4 (◆) 2.7×10^5 PfSPZ IV × 4 (*n* = 12)	Grp 5 (▼) 2.7×10^5 PfSPZ IV × 4 (*n* = 12)
	no. (%)					
Malaise						
None	8 (66.7)	7 (77.8)	9 (75.0)	11 (100)	5 (41.7)	9 (75.0)
Mild	3 (25.0)	1 (11.1)	3 (25.0)	0 (0)	5 (41.7)	2 (16.7)
Moderate	1 (8.3)	1 (11.1)	0 (0)	0 (0)	2 (16.7)	1 (8.3)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Myalgia						
None	7 (58.3)	7 (77.8)	10 (83)	11 (100)	7 (58.3)	11 (91.7)
Mild	3 (25.0)	2 (22.2)	2 (16.7)	0 (0)	4 (33.3)	1 (8.3)
Moderate	2 (16.7)	0 (0)	0 (0)	0 (0)	1 (8.3)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Headache						
None	9 (75.0)	9 (100)	11 (91.7)	11 (100)	7 (58.3)	11 (91.7)
Mild	3 (25.0)	0 (0)	1 (8.3)	0 (0)	3 (25.0)	1 (8.3)
Moderate	0 (0)	0 (0)	0 (0)	0 (0)	2 (16.7)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chills						
None	11 (91.7)	8 (88.9)	12 (100)	11 (100)	10 (83.3)	12 (100)
Mild	0 (0)	1 (11.1)	0 (0)	0 (0)	1 (8.3)	0 (0)
Moderate	1 (8.3)	0 (0)	0 (0)	0 (0)	1 (8.3)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nausea						
None	8 (66.7)	9 (100)	11 (91.7)	10 (90.9)	8 (66.7)	10 (83.3)
Mild	3 (25.0)	0 (0)	1 (8.3)	1 (9.1)	4 (33.3)	1 (8.3)
Moderate	1 (8.3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (8.3)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Temperature						
None	12 (100)	9 (100)	12 (100)	11 (100)	11 (91.7)	12 (100)
Mild	0 (0)	0 (0)	0 (0)	0 (0)	1 (8.3)	0 (0)
Moderate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Joint pain						
None	10 (83.3)	8 (88.9)	10 (83)	11 (100)	12 (100)	11 (91.7)
Mild	0 (0)	1 (11.1)	2 (16.7)	0 (0)	0 (0)	1 (8.3)
Moderate	2 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Any systemic						
None	6 (50)	6 (66.7)	9 (75.0)	10 (90.9)	4 (33.3)	7 (58.3)
Mild	4 (33.3)	2 (22.2)	3 (25.0)	1 (9.1)	6 (50)	4 (33.3)
Moderate	2 (16.7)	1 (11.1)	0 (0)	0 (0)	2 (16.7)	1 (8.3)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*See Supplementary Table 4.

			Panel 1	Panel 2	Panel 3	Panel 4	Panel 5
			Human ICS (PfSPZ stim)	Human ICS (PfRBC stim)	Human ICS (PfRBC stim)		NHP ICS (PfSPZ / PfRBC stim)
#	Detector	Fluorophore	Human liver (unstim)	Human liver (unstim)	Human liver (unstim)	Human liver (unstim)	NHP liver (unstim)
1	B515	FITC	Ki-67		TCR Vδ1		
2	B710	Cy5.5PerCP	TCR Vα7.2			TCR Vα7.2	
3	V440	BV421					IFN-γ
4	V510	Aqua/BV510*	Viability/CD14*				
5	V570	BV570					CD8
6	V605	BV605	IL-2				IL-2
7	V650	BV650	TNF-α				TNF-α
8	V710	BV711	CD56			CD56	
9	V750	BV750					
10	V800	BV785	CD4				
11	R660	APC	IFNγ		TCR Vγ9		NKG2a
12	R710	Ax680	CCR7				
13	R780	Cy7APC	CD3				
14	G560	PE	CXCR6	Perforin			TCR Vα7.2
15	G610	CF594PE	TCRγδ				
16	G660	Cy5PE	CD161	CD38	TCR Vδ2	CD161	
17	G710	Cy5.5PE/Ax700PE#	CD45RA			CD20#	CD45RA
18	G780	Cy7PE	CD8				CD161

Manufacturer	Specificity	Fluorophore	Clone	Catalog
BD	CD3	Cy7APC	SP34.2	557757
BioLegend	CD4	BV785	OKT4	317441
BioLegend	CD8	Cy7PE	RPA-T8	301012
BioLegend	CD14	BV510	M5E2	301842
BD	CD38	Cy5PE	HIT2	555461
Invitrogen	CD45RA	Cy5.5PE	MEM-56	MHCD45RA18
BD	CD56	BV711	NCAM16.2	563169
BioLegend	TCR Vα7.2	Cy5.5PerCP	3C10	351710
BD	CD161	Cy5PE	DX12	551138
BD	TCR γδ	CF594PE	B1	562511
ThermoScientific	TCR Vδ1	FITC	TS8.2	TCR2730
VRC	TCR Vδ2	Cy5PE	B6	--
BioLegend	TCR Vγ9	APC	B3	331310
BioLegend	CXCR6	PE	K041E5	356004
VRC	CCR7	Ax680	150503	--
BioLegend	IFN-γ	APC	4S.B3	502512
BioLegend	IL-2	BV605	MQ1-17H12	500331
BioLegend	TNF-α	BV650	MAb11	502937
BioLegend	Perforin	PE	B-D48	353303
BD	Ki-67	FITC	B56	556026
BioLegend	CD8	BV570	RPA-T8	301038
BioLegend	CD161	Cy7PE	HP-3G10	339918
Beckman Coulter	NKG2a	APC	Z199	A60797
BioLegend	TCR Vα7.2	PE	3C10	351706
BioLegend	IFN-γ	BV421	4S.B3	502532
VRC	CD20	Ax700PE	2H7	--

Supplementary Table 6 Flow cytometry staining panels.

Supplementary Table 6 Flow cytometry staining panels.

Three flow cytometry staining panels were developed to assess the magnitude, quality, and phenotype of Pf-specific lymphocytes. All three panels allow for the identification of γδ, 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, naive, central memory (T_{CM}), effector memory (T_{EM}), and terminal effector memory (TE) subsets are identified.

Panels 1 and *2* allow for identification of Pf-specific cells by intracellular cytokine staining of IFN-γ, IL-2, or TNF-α following stimulation with PfSPZ or PfRBC, respectively. Stimulation with PfRBC is used to increase the sensitivity of detected Pf-specific CD8 T cell responses.

Panel 1 includes the following phenotypic markers:

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

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

Panel 1 stains for NK and MAIT cell subsets.

Panel 2 includes the following phenotypic markers:

CD38, a marker of T cell activation

Perforin, an effector molecule

Panel 3 allows for the identification of the γδ T cell sub-families defined by the expression of Vγ9, Vδ1, and Vδ2 TCRs.

PBMCs (1.5×10^6 cells per well) were stained with the 3 different panels 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)

Group	Subject ID	Net OD1.0 anti-PfCSP antibodies by ELISA	AFU 2 × 10^5 anti-PfSPZ antibodies by aIFA
1 ●	102	8711	4950
	103	5227	5235
	104	9066	10799
	105	1040	396
	106	36163	25800
	107	no sample	no sample
	108	8601	2984
	109	3893	3519
	110	4902	2085
	111	22050	12907
	112	3463	2184
2 ■	201	1368	301
	202	1371	294
	203	2189	820
	204	979	271
	205	1155	235
	206	4257	1030
	207	3566	818
	208	763	148
	209	279	100
3 ▲	301	3476	1784
	302	10986	4922
	303	14309	4240
	304	25735	17659
	305	4550	380
	306	8046	2567
	307*	2458	735
	308	3603	2489
	309	10385	10537
	310	18871	18141
	311	3379	973
	312	11731	6309
4 ◆	401	11249	4910
	402	2829	1065
	403	5114	2376
	405	15083	3746
	406	480	621
	407	22754	32215
	408	35589	15840
	411	11411	17571
	412	15468	13769
5 ▼	501	14369	9980
	502	792	597
	504	10171	539
	505	1196	7534
	506	1004	435
	507	2924	1355
	508	11361	8592
	509	17921	7863
	510	7090	6389
	511	12102	1076
	512	9094	23101

Supplementary Table 7 Individual antibody responses two weeks after final vaccination.

*Received 5 doses of 1.35 × 10^5 PfSPZ