

## SUPPLEMENTARY TEXT 1

**Operating procedures for ELISA.** The standardized dried blood spots (1 cm diameter containing  $\approx$ 40  $\mu$ L of blood) were eluted by incubation in 400 mL of phosphate-buffered saline (PBS-Tween 0.1%) at 4°C for 24 hours. Maxisorp plates (Nunc, Roskilde, Denmark) were coated with the Pf merozoite surface protein 119 (MSP1-19)/Pf CSP peptide (Vaximax, France) at a concentration of 1  $\mu$ g/mL in coating buffer (PBS + Phenol Red 1%) at 4°C overnight. After washing (with a solution of PBS-Tween 0.1% + NaCl), the plates were blocked for 1 hour under stirring at room temperature with 150 mL of saturation buffer (PBS-Tween 0.1% + milk powder 3%). Thereafter, each eluted sample was incubated in duplicate for 2 hours under stirring at room temperature at 1/20 dilution in sample dilution buffer (PBS-Tween 0.1% + milk powder 1% + sodium azide 0.02%). Antihuman IgG coupled to the peroxidase (Invitrogen) was incubated at a 1/3,000 dilution for 1 hour under stirring at room temperature. Substrate TMB one (3,3',5,5'-tetramethylbenzidine) (Promega) was added for 30 minutes at room temperature protected from light then stopping the reaction with 0.2 M H<sub>2</sub>SO<sub>4</sub>, and absorbance was measured at 450 nm. In parallel, each test sample was assessed in a blank well containing no Pf MSP1-19/Pf CSP peptide (ODn) to measure nonspecific reactions. Individual results were expressed as the  $\Delta$ OD value:  $\Delta$ OD = ODx - ODn. ODx and ODn represent the mean of individual optical density (OD) in two antigen wells and one blank well containing no Pf MSP1-19/Pf CSP peptide, respectively. Specific anti-Pf MSP1-19/Pf CSP IgG responses were also assayed in nonmalaria-exposed individuals (negative samples from France:  $n$  = 18) to quantify the nonspecific background Ab level and to calculate the cut-off value (mean [ $\Delta$ OD<sub>neg</sub>] + 3SD). Based on our findings, a participant was classified as an immune responder to Pf MSP1-19 and Pf CSP if  $\Delta$ OD was > 0.162 and > 0.115, respectively.

All peptide batches were shipped in lyophilized form and then suspended in ultrafiltered water and frozen in aliquots at -20°C until use.

SUPPLEMENTAL TABLE 1  
Seroprevalence in antibody response to CSP per age group and surveys

Survey of blood collection	Total population	Blood collection date	Characteristic of blood spot				Gender				Age (years)			
			Number of dry blood spot		Male	Female	Male		Female	Total	0–4		5–15	16–59
	Village		Htoo Pyin Nyar TPN											
M0	373	May 20, 2013	257	145	112	27.4 (1–66)	%	5.5	3.6	4.7	16.7	3.9	4.8	0.0
M3	406	August 23, 2013	267	143	124	25.1 (1–66)	(95% CI) (n/N)	(2.6–11.0) (8/145)	(1.2–9.4) (4/112)	(2.6–8.2) (12/257)	(0.9–63.5) (1/6)	(1.0–11.7) (3/77)	(2.2–9.6) (8/167)	(0.0–43.9) (0/7)
M6	434	November 7, 2013	300	159	141	24.6 (0–66)	(95% CI) (n/N)	(15.4–29.5) (31/143)	(14.4–29.4) (26/124)	(16.7–26.9) (57/267)	(0.0–15.5) (0/27)	(8.9–26.7) (12/75)	(21.9–36.5) (45/157)	(0.0–40.2) (0–8)
M9	464	January 28, 2014	307	160	147	24.0 (0–66)	(95% CI) (n/N)	(1.2–7.5) (5/160)	(1.3–8.2) (5/147)	(1.7–6.1) (10/307)	(0.0–10.0) (0/46)	(0.1–7.2) (1/86)	(2.7–10.4) (9/166)	(0.0–37.1) (0/9)
M12	473	April 23, 2014	264	138	126	22.2 (0–66)	(95% CI) (n/N)	(3.6) (5/138)	(2.4) (3/126)	(3.0) (8/264)	(0.0) (0/45)	(1.2) (1/85)	(5.4) (7/130)	(0.0–43.9) (0/7)
M15	492	July 14, 2014	234	127	107	24.4 (0–66)	(95% CI) (n/N)	(2.4) (0.6–7.3) (3/127)	(0.9) (1/107)	(1.7) (4/234)	(0.0) (0/33)	(1.5) (1/66)	(2.4) (3/127)	(57.1) (20.2–38.2) (4/7)
M18	501	October 8, 2014	266	139	127	22.8 (0–66)	(95% CI) (n/N)	(5.0) (6.6–7.3) (3/139)	(2.4) (0.6–7.3) (3/127)	(3.8) (1.9–7.0) (10/266)	(0.0) (0/45)	(2.5) (0.4–9.7) (2/79)	(5.2) (2.3–10.7) (7/136)	(16.7) (0.9–63.5) (1/6)
Village														
Tar Au Ta														
M0	740	June 14, 2013	402	192	210	26.7 (0–80)	%	17.7	9.1	13.2	7.1	0.7	18.5	40.0
M3	785	September 12, 2013	308	145	163	22.5 (0–70)	(95% CI) (n/N)	(2.7–24.0) (34/192)	(5.7–14.0) (19/210)	(10.1–17.0) (53/402)	(3.7–35.8) (1/14)	(0.0–4.7) (1/135)	(13.6–24.2) (43/233)	(20.0–63.6) (8/20)
M6	789	November 26, 2013	205	102	103	23.7 (1–70)	(95% CI) (n/N)	(20.0–35.0) (39/145)	(23.9) (17.8–31.4) (39/163)	(20.6–30.6) (78/308)	(4.7) (0.8–17.1) (2/43)	(11.0–25.9) (19/110)	(35.5) (27.7–44.0) (50/141)	(24.0–76.0) (7/14)
M9	814	February 18, 2014	292	143	149	21.5 (0–80)	(95% CI) (n/N)	(10.3–25.6) (17/102)	(16.7) (10.3–17.5) (10/103)	(9.0–18.8) (27/205)	(3.6) (0.2–20.2) (1/205)	(7.1) (2.7–16.6) (5/70)	(16.0) (9.5–25.3) (15/94)	(46.2) (20.4–73.9) (6/13)
M12	833	May 15, 2014	250	114	136	19.2 (0–70)	(95% CI) (n/N)	(7.1–20.1) (27/143)	(18.9) (2.3–10.7) (22/149)	(14.8) (5.4–12.7) (49/292)	(5.4) (1.4–15.8) (3/56)	(6.8) (2.8–14.8) (6/88)	(24.8) (18.0–33.1) (34/137)	(54.6) (24.6–81.9) (6/11)
M15	861	August 6, 2014	305	150	155	21.5 (0–80)	(95% CI) (n/N)	(9.5–21.6) (22/150)	(14.7) (6.2–16.5) (16/155)	(10.3) (9.1–16.8) (38/305)	(1.6) (0.1–10.8) (1/56)	(0.0) (0.4–7.9) (2/98)	(16.5) (14.4–28.7) (28/135)	(22.2) (20.8–69.5) (7/16)
M18	879	October 30, 2014	196	94	102	18.9 (0–70)	(95% CI) (n/N)	(6.3–20.4) (11/94)	(11.7) (3.0–14.1) (7/102)	(6.9) (5.7–14.3) (18/196)	(2.0) (0.1–12.2) (1/49)	(1.8) (0.1–10.6) (1/57)	(16.9) (9.9–27.0) (14/83)	(28.6) (5.1–69.7) (2/77)

(continued)

SUPPLEMENTAL TABLE 1  
Continued

Survey of blood collection	Total population	Blood collection date	Characteristic of blood spot				Pf/CSP antibody prevalence %								
							Gender			Age (years)					
			Village	Number of dry blood spot	Male	Female	Median age (range) (years)	Male	Female	Total	0–4	5–15	16–59	60 up	
Ka Nu Hta															
M0	348	June 14, 2013	323	178	145	29.0 (1–73)	% (95% CI)	7.9	4.1	6.2	0.0	1.2	8.0	15.4	
M3	417	September 12, 2013	275	148	127	27.9 (1–73)	(95% CI)	(4.5–13.1)	(1.7–9.2)	(3.9–9.6)	(0.0–34.5)	(0.1–7.1)	(4.9–12.7)	(2.7–46.3)	
M6	435	December 11, 2013	261	140	121	28.0 (0–73)	(95% CI)	26.4	26.8	(20/323)	(0/10)	(1/87)	(17/213)	(2/13)	
M9	449	March 5, 2014	303	155	148	25.9 (0–73)	(95% CI)	(19.6–34.3)	(19.5–35.5)	(21.5–32.3)	(3.4–34.7)	(10.1–28.5)	(25.8–40.5)	(3.5–55.8)	
M12	469	May 26, 2014	301	160	141	24.9 (0–73)	(95% CI)	(n/N)	(18/140)	(5/121)	(73/275)	(3/23)	(13/74)	(55/168)	
M15	485	August 21, 2014	303	164	139	25.8 (0–70)	(95% CI)	(n/N)	(0.8–6.9)	(1.5–9.9)	(5.8–13.1)	(0/19.2)	(0.5–10.7)	(7.5–18.3)	
M18	495	November 13, 2014	285	155	130	24.6 (0.70)	(95% CI)	(n/N)	(4.1–13.0)	(0.6–6.6)	(2.9–8.3)	(0.0–10.7)	(0.1–7.3)	(4.8–13.8)	
Htee Kaw Taw															
M0	899	July 4, 2013	487	238	249	26.2 (0–94)	% (95% CI)	8.8	6.4	7.6	0.0	3.0	10.1	22.2	
M3	979	October 7, 2013	524	263	261	24.1 (0–89)	(n/N)	(5.7–13.4)	(3.8–10.4)	(5.5–10.4)	(0/16.7)	(1.1–7.2)	(6.9–14.4)	(7.4–48.1)	
M6	1,029	January 8, 2014	493	241	252	21.9 (0–78)	(95% CI)	19.0	23.4	(21/249)	(37/487)	(0/25)	(5/167)	(28/277)	(4/18)
M9	1,070	May 1, 2014	478	241	237	22.6 (0–78)	(95% CI)	(4.6–24.4)	(18.5–29.1)	(17.8–25.0)	(3.8–23.0)	(7.9–17.8)	(23.4–34.4)	(12.1–58.5)	
M12	1,192	June 24, 2014	512	257	255	21.8 (0–89)	(95% CI)	(2.1–7.7)	(1.6–6.8)	(2.3–6.0)	(0/6.8)	(0.2–4.7)	(3.4–10.0)	(4.0–59.8)	
M15	1,217	September 16, 2014	504	264	240	21.8 (0–78)	(95% CI)	5.3	4.2	(1/478)	(0/67)	(2/166)	(14/236)	(2/9)	
M18	1,236	December 8, 2014	471	233	238	19.9 (0–78)	(95% CI)	(1.1–5.8)	(0.5–4.5)	(1.6–4.7)	(0/6.8)	(0.2–4.4)	(2.5–8.5)	(0.4–3.8)	

CI = confidence interval.



SUPPLEMENTAL TABLE 2  
Continued

Survey of blood collection	Total population	Village	Characteristic of blood spot						Pf Msp1-19 antibody prevalence %						
			Number of dry blood spot			Male Female Median age (years)			Male Female Total			0-4 5-15 5-59 60+ up			
			Htoo Pyin Nyar			Ka Nu Hta			Gender			Age (years)			
Village															
M0	348	June 14, 2013	321	178	143	29.0 (1-73)	% (95% CI)	75.9 (68.8-81.8)	65.7 (57.3-73.3)	71.3 (66.0-76.2)	20.0 (3.5-55.8)	53.5 (42.5-64.2)	79.3 (73.7-84.4)	100.0 (71.7-100.0)	
M3	417	September 12, 2013	272	146	126	28.1 (1-73)	% (95% CI)	76.7 (73.6-87.2)	69.8 (61.2-78.0)	73.5 (57.4-81.1)	18.2 (12.1-67.3)	55.6 (43.4-67.1)	86.9 (80.6-91.4)	100.0 (65.6-100.0)	
M6	435	December 11, 2013	260	139	121	28.3 (0-73)	% (95% CI)	81.3 (113/139)	70.3 (85/121)	76.2 (198/260)	45.5 (200/272)	60.9 (4/22)	82.4 (40/72)	85.5 (146/168)	100.0 (10/10)
M9	449	March 5, 2014	304	156	148	25.9 (0-73)	% (95% CI)	90.4 (141/156)	84.5 (125/148)	87.5 (266/304)	68.4 (26/38)	60.9 (72.2-82.0)	82.4 (70/85)	93.5 (88.4-96.6)	100.0 (67.9-100.0)
M12	469	May 26, 2014	300	159	141	24.7 (0-73)	% (95% CI)	84.3 (134/159)	80.9 (114/141)	82.7 (248/300)	46.3 (19/41)	51.2 (72.1-89.5)	82.4 (70/85)	90.5 (159/170)	100.0 (67.9-100.0)
M15	485	August 21, 2014	302	163	139	25.7 (0-70)	% (95% CI)	76.1 (124/163)	74.8 (104/139)	75.5 (228/302)	39.5 (17/43)	51.2 (72.1-89.5)	82.4 (84.8-94.3)	90.5 (153/169)	100.0 (66.6-100.0)
M18	495	November 13, 2014	285	155	130	24.6 (0-70)	% (95% CI)	79.4 (123/155)	76.2 (99/130)	77.9 (222/285)	42.9 (21/49)	51.2 (72.5-82.5)	77.3 (65.0-86.3)	87.6 (51/66)	100.0 (62.9-100.0)
Village															
M0	899	July 4, 2013	488	237	251	26.3 (0-94)	% (95% CI)	65.0 (58.5-71.0)	57.0 (50.6-63.1)	60.9 (56.4-65.2)	16.7 (5.5-38.2)	48.5 (40.8-56.3)	70.8 (65.0-76.0)	83.3 (57.7-95.6)	
M3	979	October 7, 2013	533	267	266	24.3 (0-89)	% (95% CI)	59.6 (154/237)	56.0 (143/251)	57.8 (297/488)	20.0 (4/24)	43.7 (82/169)	72.5 (196/277)	75.0 (15/18)	
M6	1,029	January 8, 2014	496	240	256	22.0 (0-78)	% (95% CI)	64.6 (155/240)	61.2 (147/256)	63.3 (308/533)	20.0 (10/50)	36.5 (80/183)	43.7 (206/284)	47.4-91.7 (12/16)	
M9	1,070	May 1, 2014	479	243	236	22.4 (0-78)	% (95% CI)	67.5 (151/258)	58.9 (130/255)	63.3 (13/65)	20.0 (1365)	56.0 (104/193)	53.9 (176/227)	77.8 (9/11)	
M12	1,192	June 24, 2014	513	258	255	21.8 (0-89)	% (95% CI)	68.6 (164/243)	67.4 (139/236)	68.0 (303/479)	20.5 (13/68)	49.7 (94/168)	71.2 (189/234)	81.8 (79/91)	
M15	1,217	September 16, 2014	506	264	242	21.7 (0-78)	% (95% CI)	69.5 (181/264)	64.7 (163/242)	54.8 (344/506)	20.5 (28/83)	49.7 (108/175)	71.2 (198/236)	81.5 (10/12)	
M18	1,236	December 8, 2014	469	231	238	19.9 (0-78)	% (95% CI)	67.5 (156/231)	60.9 (145/238)	64.2 (301/469)	29.5 (23/78)	64.0 (126/197)	78.9 (142/180)	71.4 (42.0-90.4) (10/14)	
Village															
Hee Kaw Taw															

CI = confidence interval.

**SUPPLEMENTAL TABLE 3**  
**Seroprevalence in antibody response to gSGG6-P1 per age group and surveys**

SUPPLEMENTAL TABLE 3  
Continued

Survey blood collection Village	Total population	Blood collection date	Characteristic of blood spot						Antibody prevalence %						
			Number of dry blood spot			Median age (range) (years)			Male			Female			
			Male	Female	Median age (range) (years)	Male	Female	Median age (range) (years)	Total	0-4	5-15	15-59	60 up	Age (years)	
Village															
M0	348	June 14, 2013	325	180	145	29.1 (2–73)	%	55.6	51.7	53.8	33.3	40.2	59.3	69.2	
M3	417	September 12, 2013	277	147	130	28.1 (1–73)	(95% CI) (n/N)	(48.0–62.9) (100/180)	(43.3–60.0) (75/145)	(48.3–59.3) (175/325)	(9.0–69.1) (3/9)	(30.0–51.3) (35/87)	(52.4–65.8) (128/216)	(38.9–89.7) (9/13)	
M6	435	December 11, 2013	263	141	122	28.1 (0–73)	(95% CI) (n/N)	(39.4–56.0) (70/147)	(42.7–60.3) (67/130)	(43.4–55.5) (137/277)	(14.1–53.0) (7/23)	(27.4–50.5) (28/73)	(49.0–64.2) (97/171)	(20.1–79.9) (5/10)	
M9	449	March 5, 2014	301	155	146	25.9 (0–73)	(95% CI) (n/N)	(53.1–69.7) (87/141)	(43.3–61.5) (64/122)	(51.2–63.4) (151/263)	(36.7–78.5) (13/22)	(55.7) (39/70)	(43.4–67.4) (91/161)	(48.5–64.2) (8/10)	
M12	469	May 26, 2014	298	160	138	25.1 (0–73)	(95% CI) (n/N)	(39.7–55.9) (74/155)	(41.0–57.7) (72/146)	(48.5) (146/301)	(42.8–54.3) (16/37)	(27.5–60.4) (38/84)	(34.5–56.4) (88/169)	(12.4–68.4) (4/11)	
M15	485	August 21, 2014	300	162	138	26.0 (0–70)	(95% CI) (n/N)	(91.3–97.3) (153/162)	(91.3–98.7) (133/138)	(65.4–90.9) (286/300)	(89.1–99.5) (34/42)	(34.5–56.4) (68/70)	(44.3–59.8) (88/169)	(12.4–68.4) (4/11)	
M18	495	November 13, 2014	282	154	128	24.6 (0–70)	(95% CI) (n/N)	(86.4) (79.7–91.2)	(86.4) (75.8–89.3)	(85.1) (80.3–89.0)	(83.3) (73.1–92.0)	(31.7) (240/282)	(47.6) (31/49)	(36.7–58.7) (55/65)	(31.0–46.4) (63/164)
Village															
Ka Nu Ha															
M0	899	July 4, 2013	490	241	249	26.4 (0–94)	%	92.9	94.8	93.9	91.7	92.2	95.0	94.4	
M3	979	October 7, 2013	535	269	266	24.5 (0–89)	(95% CI) (n/N)	(88.8–95.7) (224/241)	(91.0–97.1) (236/249)	(71.3–95.8) (460/490)	(71.5–98.6) (22/24)	(86.8–95.6) (154/167)	(91.7–97.2) (267/281)	(70.6–99.7) (17/18)	
M6	1,029	January 8, 2014	500	243	257	22.1 (0–78)	(95% CI) (n/N)	(80.6–89.4) (230/269)	(82.0–90.5) (190/243)	(83.9–89.0) (220/257)	(67.5–90.8) (410/500)	(85.7) (40/49)	(85.7) (158/193)	(94.1) (195/230)	
M9	1,070	May 1, 2014	478	240	238	22.4 (0–78)	(95% CI) (n/N)	(83.0–91.6) (211/240)	(81.4–90.5) (206/238)	(83.8–90.0) (417/478)	(75.8) (53/68)	(75.8) (156/182)	(86.8) (249/287)	(69.2–99.7) (16/17)	
M12	1,192	June 24, 2014	527	269	258	21.7 (0–89)	(95% CI) (n/N)	(80.2–89.0) (229/269)	(84.6–92.6) (230/258)	(83.9–89.8) (459/527)	(77.9) (78/89)	(86.9) (166/187)	(84.8) (146/168)	(63.6) (209/233)	
M15	1,217	September 16, 2014	506	266	240	21.6 (0–78)	(95% CI) (n/N)	(81.2) (216/240)	(85.0) (204/240)	(83.0–90.0) (420/506)	(80.6–91.4) (53/68)	(84.9–93.2) (151/176)	(84.8) (146/168)	(31.6–87.6) (7/11)	
M18	1,236	December 8, 2014	467	233	234	20.0 (0–78)	(95% CI) (n/N)	(82.8) (77.2–87.3)	(87.2) (193/233)	(84.0) (204/234)	(83.1–92.8) (397/467)	(80.5–89.8) (63/75)	(84.9) (170/199)	(53.7–97.3) (5/14)	
Htee Kaw Taw															
M0															

CI = confidence interval; MSP-1<sub>19</sub> = merozoite surface protein 119.