

**Table S1.** Associations between IgG subclass antibodies against PfRh2 proteins and age, parasite status, or reactivity to schizont protein extract.

Antigen	IgG subclass	All	Age <sup>3</sup>		P <sup>5</sup>	Enrolment <i>P. falciparum</i> parasitemic status <sup>4</sup>		
			≤9.0yrs n=91	>9.0yrs n=115		PCR- n=67	PCR+ n=139	P <sup>5</sup>
<b>PfRh2-2030</b>								
Seropositive <sup>1</sup> %	IgG1	175 85%	71 78.0%	104 90.4%	0.013	45 67.2%	130 93.5%	<0.001
Median OD [IQR] <sup>2</sup>		0.172 [0.062-0.359]	0.183 [0.04-0.335]	0.172 [0.065-0.365]	0.430	0.084 [0.026-0.315]	0.2 [0.086-0.41]	0.001
Seropositive <sup>1</sup> %		IgG2	30 14.56%	11 12.1%	19 16.5%	0.370	11 16.4%	19 13.7%
Median OD [IQR] <sup>2</sup>	0.004 [0-0.01]		0.004 [0-0.01]	0.004 [0-0.011]	0.413	0.002 [0-0.01]	0.004 [0-0.01]	0.312
Seropositive <sup>1</sup> %	IgG3		130 63.1%	49 53.9%	81 70.4%	0.014	31 46.3%	99 71.2%
Median OD [IQR] <sup>2</sup>		0.052 [0.018-0.113]	0.042 [0.01-0.092]	0.061 [0.026-0.149]	0.013	0.029 [0.01-0.084]	0.061 [0.026-0.131]	0.006
Seropositive <sup>1</sup> %		IgG4	29 14.08%	11 12.1%	18 15.7%	0.465	11 16.4%	18 13.0%
Median OD [IQR] <sup>2</sup>	0.0035 [0-0.016]		0.005 [0-0.017]	0.002 [0-0.016]	0.122	0.004 [0-0.017]	0.003 [0-0.016]	0.534
<b>PfRh2-2530</b>								
Seropositive <sup>1</sup> %	IgG1	175 85.0%	69 75.8	106 92.2%	0.001	48 71.6%	127 91.4%	<0.001
Median OD [IQR] <sup>2</sup>		0.296 (0.093-0.609]	0.292 [0.042-0.647]	0.298 [0.108-0.578]	0.606	0.203 [0.022-0.449]	0.376 [0.164-0.649]	<0.001
Seropositive <sup>1</sup> %		IgG2	119 57.77%	48 52.8%	71 61.7%	0.194	31 46.3%	88 63.3%
Median OD [IQR] <sup>2</sup>	0.022 [0.006-0.067]		0.018 [0.004-0.052]	0.031 [0.008-0.101]	0.023	0.014 [0.004-0.055]	0.029 [0.008-0.081]	0.043
Seropositive <sup>1</sup> %	IgG3		162 78.6%	67 73.6%	95 82.6%	0.118	44 65.7%	118 84.9%
Median OD [IQR] <sup>2</sup>		0.085 [0.019-0.483]	0.104 [0.014-0.372]	0.063 [0.022-0.578]	0.800	0.033 [0.008-0.317]	0.104 [0.031-0.657]	0.005
Seropositive <sup>1</sup> %		IgG4	66 32.04%	26 28.6%	40 34.8%	0.343	17 25.4%	49 35.3%
Median OD [IQR] <sup>2</sup>	0.0115 [0-0.04]		0.012 [0-0.039]	0.011 [0-0.04]	0.765	0.005 [0-0.035]	0.015 [0-0.043]	0.131

**Notes:**

1. Seropositive (number of seropositive individuals) and percentage (%) of seropositive individuals were defined by IgG reactivity that was higher than the mean plus three standard deviations of control sera (unexposed donors) measured by ELISA.
2. Median optical densities (OD) are displayed; [IQR] - interquartile range.
3.  $\leq 9.0$  yrs indicates individuals younger than 9 years of age;  $> 9.0$  yrs indicates individuals older than 9 years of age.
4. PCR- indicates *P. falciparum* was not detected by PCR; PCR+ indicates *P. falciparum* was detected by PCR
5. p values were calculated using the chi squared test for comparison of proportions or Kruskal-Wallis test for comparison of medians.

**Table S2.** Correlation between IgG subclass responses to PfRh2-2030 and PfRh2-2530.

		PfRh2-2030				PfRh2-2530			
		IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
<b>PfRh2-2030</b>	<b>IgG1</b>								
	<b>IgG2</b>	0.221*							
	<b>IgG3</b>	0.460	0.138*						
	<b>IgG4</b>	0.284	0.353	0.129#					
<b>PfRh2-2530</b>	<b>IgG1</b>	0.846	0.237	0.446	0.229				
	<b>IgG2</b>	0.426	0.429	0.368	0.291	0.486			
	<b>IgG3</b>	0.542	0.156*	0.725	0.167*	0.570	0.457		
	<b>IgG4</b>	0.417	0.347	0.199*	0.487	0.451	0.552	0.311	
<b>Schizont extract</b>		0.579	0.266	0.561	0.127	0.582	0.429	0.575	0.245

**Notes:**

Correlation coefficients are Spearman's rho. All correlations are  $p \leq 0.001$  unless otherwise indicated: \*  $p < 0.05$ , #  $p > 0.05$ .

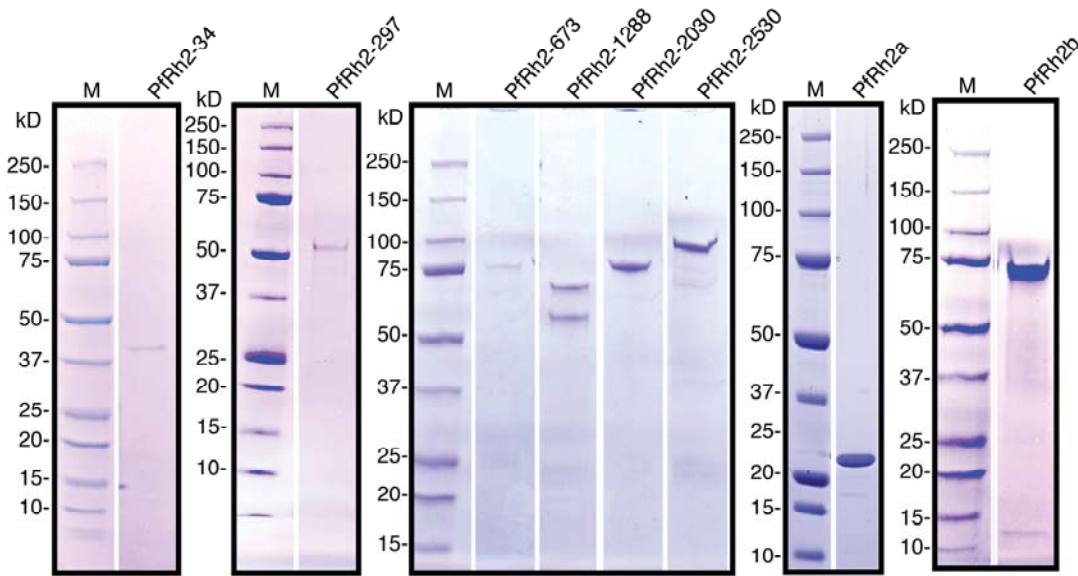
**Table S3.** IgG subclass levels against PfRh2-2030 and PfRh2-2530 by number of malaria episodes.

protein	IgG subclass	Group <sup>1</sup>	Antibody level <sup>2</sup>	p value <sup>3</sup>
PfRh2-2030	IgG1	protected	0.45 [0.20-1.2]	0.0713
		susceptible 1	0.38 [0.17-0.91]	
		susceptible 2	0.25 [0.09-1.26]	
	IgG2	protected	0.02 [0.004-0.08]	0.2711
		susceptible 1	0.01 [0.002-0.02]	
		susceptible 2	0.01 [0.003-0.11]	
	IgG3	protected	0.12 [0.049-1.91]	0.0192
		susceptible 1	0.11 [0.06-0.63]	
		susceptible 2	0.07 [0.03-0.75]	
	IgG4	protected	0.02 [0.002-0.10]	0.7761
		susceptible 1	0.02 [0.01-0.14]	
		susceptible 2	0.01 [0.004-0.36]	
PfRh2-2530	IgG1	protected	0.67 [0.38-1.39]	0.011
		susceptible 1	0.46 [0.26-1.47]	
		susceptible 2	0.41 [0.12-1.60]	
	IgG2	protected	0.13 [0.03-2.30]	0.0843
		susceptible 1	0.06 [0.02-1.88]	
		susceptible 2	0.05 [0.01-0.68]	
	IgG3	protected	0.45 [0.1-2.42]	0.0009
		susceptible 1	0.40 [0.12-1.8]	
		susceptible 2	0.12 [0.03-2.31]	
	IgG4	protected	0.04 [0.01-0.60]	0.7373
		susceptible 1	0.04 [0.01-0.23]	
		susceptible 2	0.04 [0.01-0.44]	

Notes:

1. The cohort was divided into three groups according to whether individuals experienced episodes of symptomatic malaria. Protected - no recorded symptomatic *P. falciparum* malaria and low-grade parasitemia only (<5000 parasites/ $\mu$ l); Susceptible 1 - one episode of symptomatic malaria; Susceptible 2 - two or more episodes of symptomatic malaria. Children who had no detected parasitemia during follow-up (5%) were regarded as not exposed and therefore were excluded from this analysis.
2. Values represent median OD [interquartile range]
3. p values calculated using the Kruskal-Wallis test

Supplementary Figure S1  
Recombinant PfRh2 proteins on SDS PAGE



**Figure S1:** Coomassie stained SDS PAGE of recombinant PfRh2 proteins.

All recombinant proteins were run in SDS PAGE gels prior to performing ELISA assays. The Coomassie stained gels show proteins in reducing sample buffer. Size standards are shown on the left for each gel. M: Marker lane. Coomassie stained bands were cut out and analysed by mass spectrometry to verify PfRh2 identity (not shown). The two bands seen for PfRh2-1288 were both recognized by anti-His tag antibodies in Western blots and were conformed to be PfRh2 proteins by mass spectrometry (not shown).

**Figure S2:** Alignment of Pfrh2 polymorphic region.

Alignment of sequences was performed using Clustal W. Numbers above the aligned sequences represent the position of bases within the analysed fragment. Origin of sequences: XHA, XIE were from PNG; HCS3 from Thailand; Pf2004 and Pf2006 were from West Africa. FVO, Dd2, 7G8, and 3D7 sequences were obtained from the public database. The Ghana and IT line sequences were obtained from the Sanger Institute, the sequences for HB3, FCC-2, IGH-CR14, K1, RAJ116, RO-33, Senegal, SL and VS/1 from the Broad Institute's database.











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1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
AAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XIE010.seq(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc5.3.seq(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc5.4.seq(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc5.6.seq(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc5.7.seq(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc226(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc26(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc30(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc32(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc47 (1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc101(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc102(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc107(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc404(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
XHAc434(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
HCS3.seq(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
Pf2004.seq(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
3D7(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
HB3(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
FV0(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
d4z(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
7G8(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
SL(1>1522) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
K1(1>978) → -----
FCC-2(1>1581) → AAACAACAGGGTAAATGAACTTCAACAAATTTAAATACATCAAAATATGAATGTGCTAAATTTAAATTTTATGACATCT-----
Senegal(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
RAJ116(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
RO-33(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
VS/1(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
IGH-CR14(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
IT(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
Ghana(1>1600) → AAAAAAAAAAGTGAAA-TGAACCTTCAACAAATTTAAAT-ACATCAAAATATG-AATGTGCTAAATTTAAA-TTTTATGAAATCTGATAATAATAATAATA
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