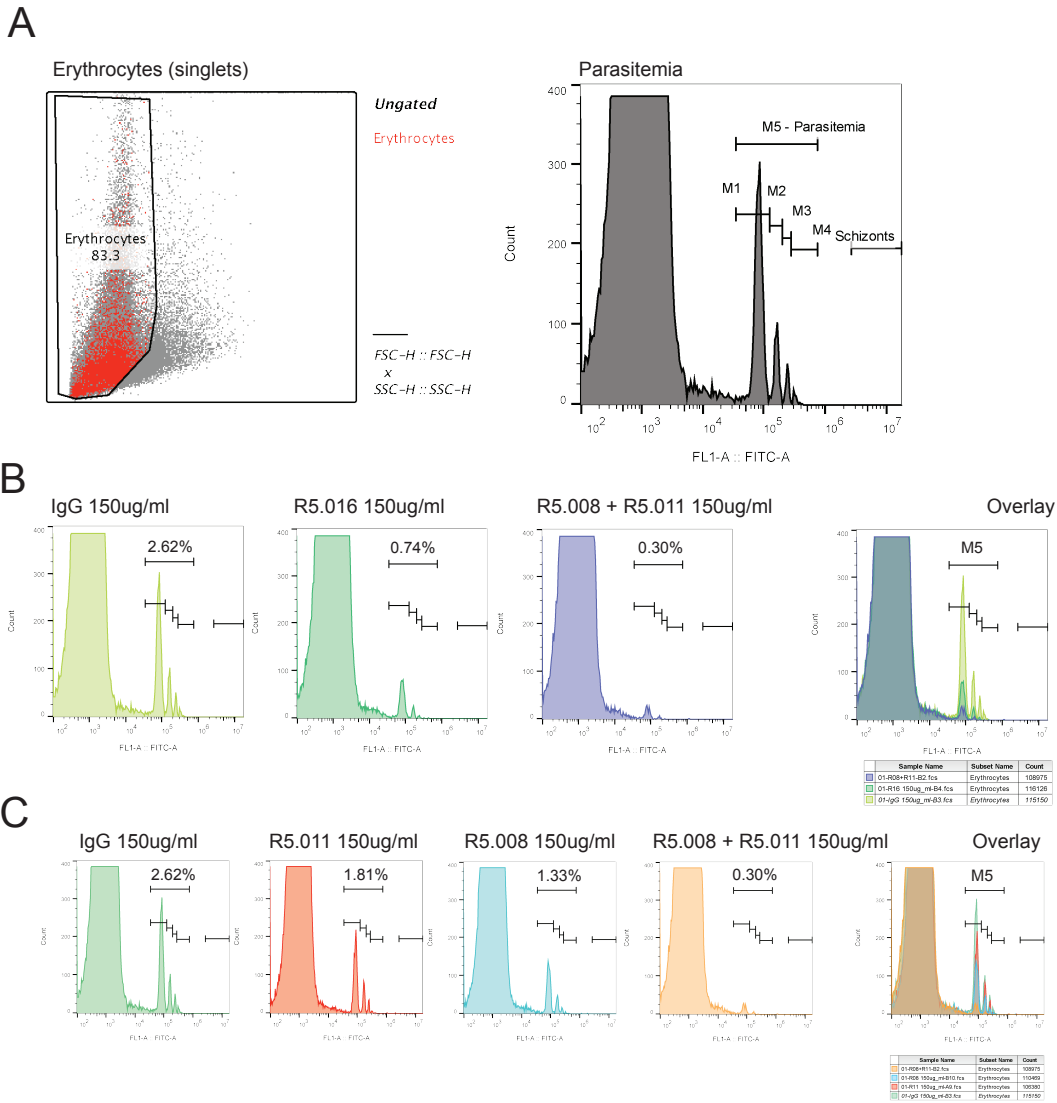
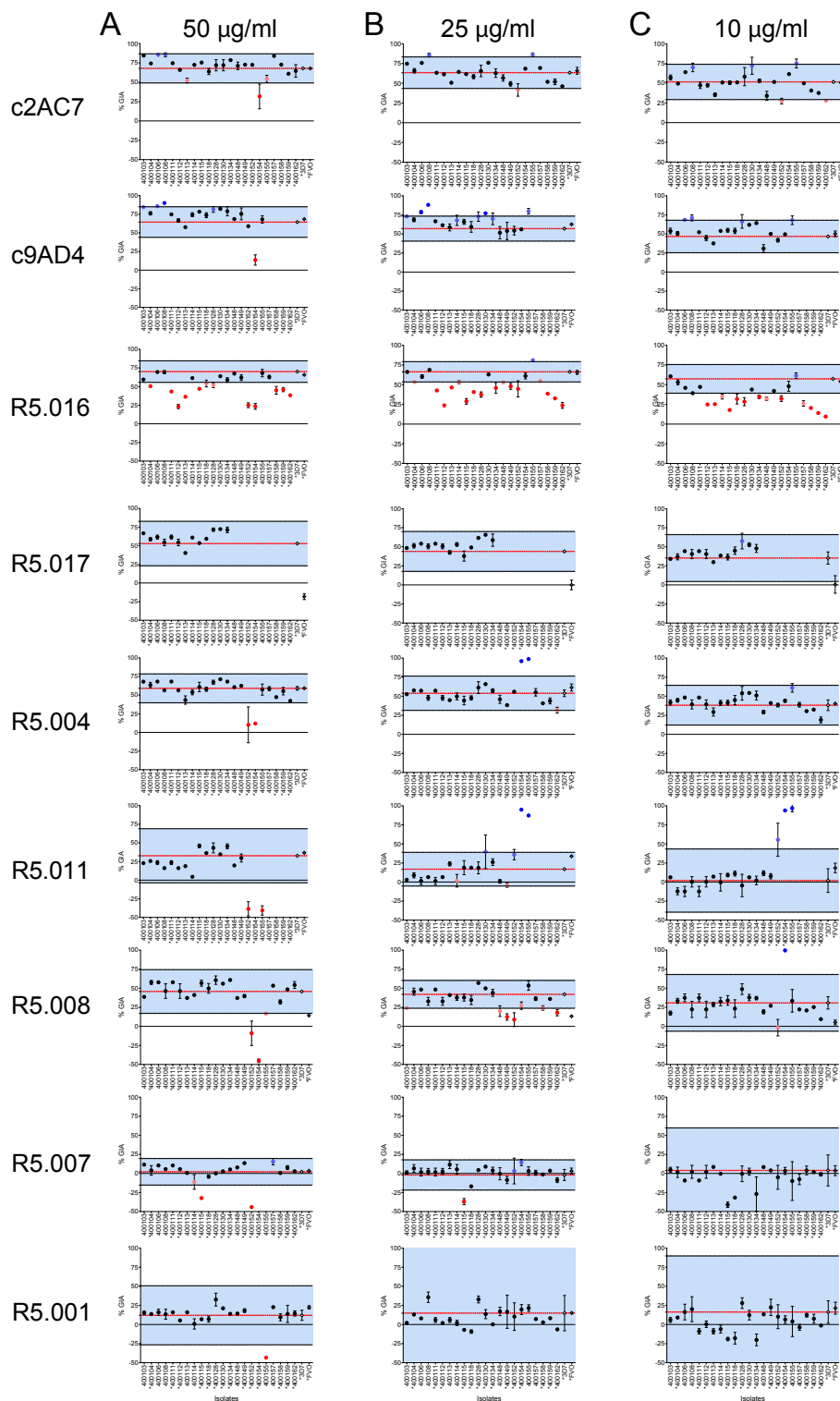


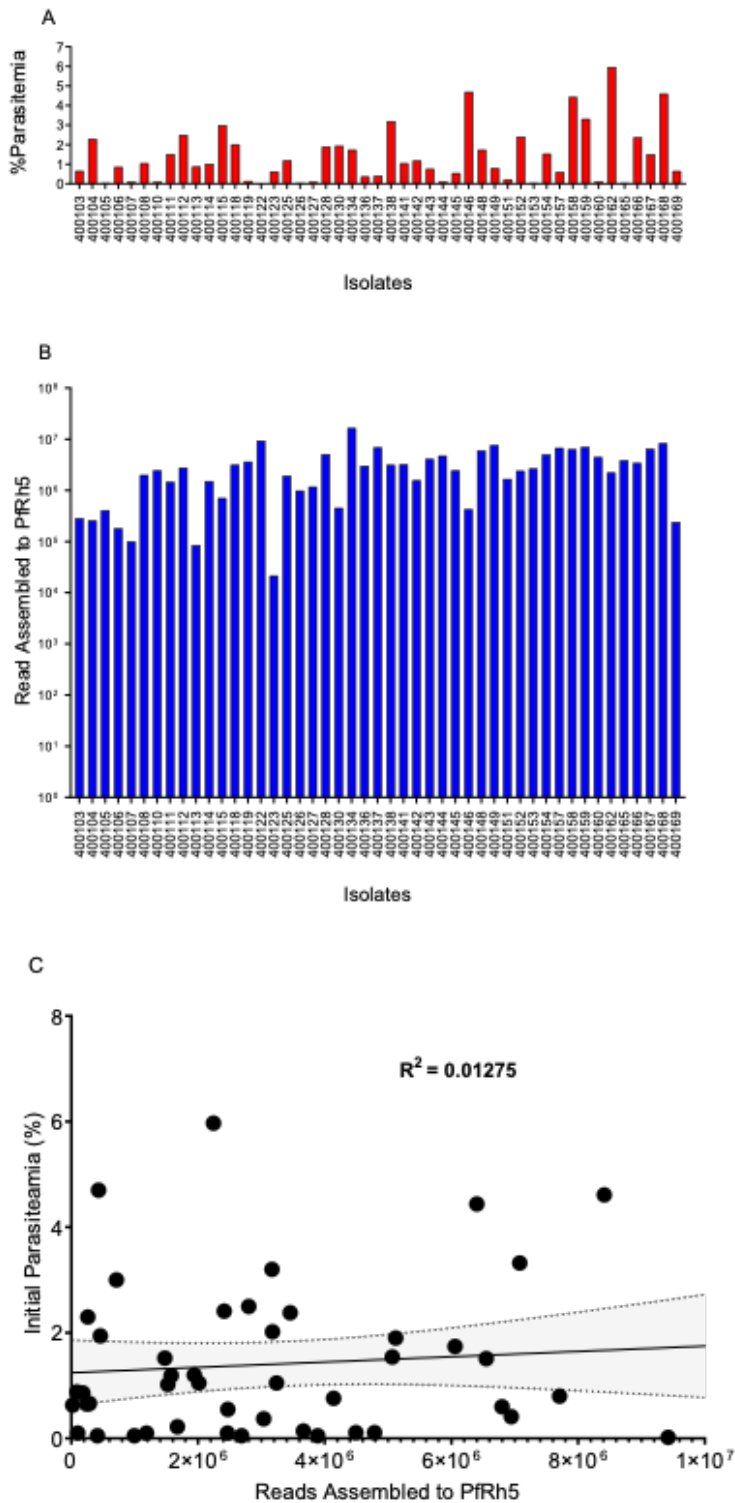
Supplementary Figure 1: Relationship between the clinical isolates parasitemia and the parasite multiplication rates (PMR) in the positive control wells (RMPI only). (A) Initial parasitemia of *P. falciparum* clinical isolates. (B) PMR of *P. falciparum* clinical isolates used in GIA assays (N = 23). The PMR is defined as the ratio of the final parasitemia by that of the initial parasitemia in the control RPMI wells and assays were only considered successful and included in the final analysis if PRM was greater than 1. (C-D) Relationships between the clinical isolates initial parasitemia / GIA starting parasitemia and the PMR computed using the simple linear regression analysis and the goodness of fit (R^2) in GraphPad prism 10.2.3. Blue dots in (D) represent isolates for which the assays were diluted with fresh O+ erythrocytes from a single donor.



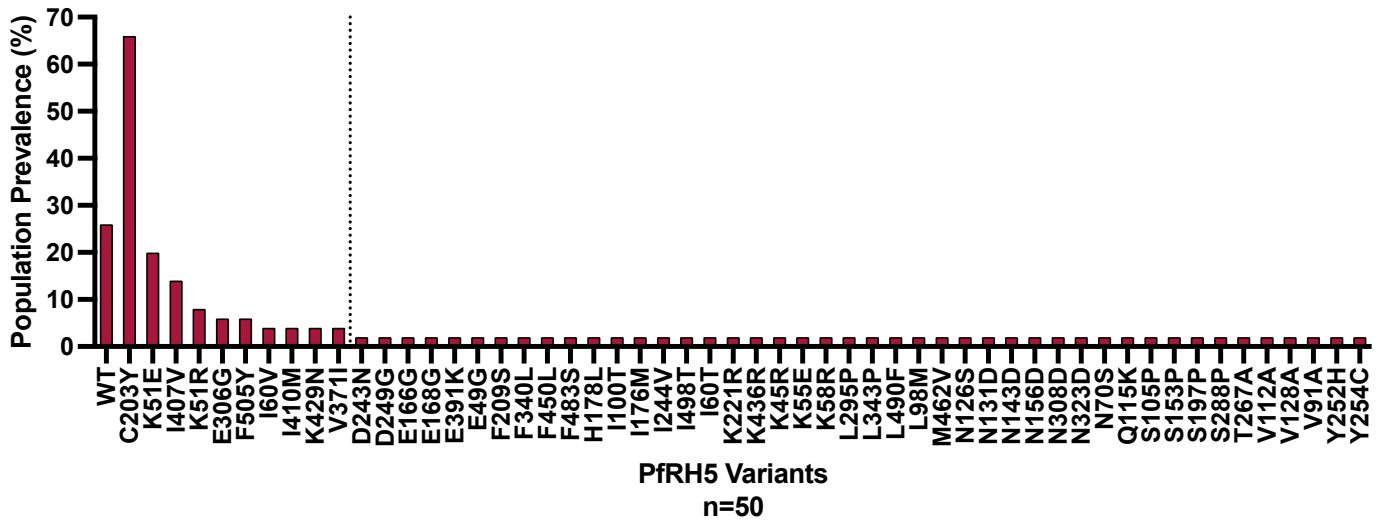
Supplementary Figure 2. Gating strategy and examples of data generated by SYBR Green Flow Cytometry. A. Gating strategy. Total number of events recorded and the total number of erythrocytes gated in the forward scatter (FSC) by side scatter (SSC) dot plot. Erythrocyte singlets are gated as “Erythrocytes”. Histogram plot showing the erythrocyte counts (y axis) and the fluorescence intensity of SYBR Green, FL1 (FITC) positive infected erythrocytes (x axis). Parasitemia is quantified as percentage of infected erythrocytes relative to the total erythrocytes. Infected erythrocytes are gated as M1-M5, with M1 representing singly infected, M2 representing doubly, M3 representing triply, M4 representing quadruple infected erythrocytes, and M5 representing total parasitemia. Any residual schizonts are not included in the re-invasion parasitemia. C-D. Example parasitemia data generated in the histogram view for growth inhibition activities by IgG and potent single mAb treatments (R5.016) compared to combination (R5.008 + R5.011), and (C) treatment with individual and mAb combinations (D).



Supplementary Figure 3: Variation of percent GIA ranges and immune susceptibility profiles of *P. falciparum* clinical isolates to PfRH5-vaccine-induced mAbs. Dot plots of %GIA to anti-PfRH5 mAbs (Y-axis) of *P. falciparum* clinical isolates (X-axis) at different concentrations (50 µg/ml (A), 25 µg/ml (B) or 10 µg/ml (C)). The %GIA susceptibility ranges are defined as 3D7 mean %GIA (red dotted lines) \pm 3SD 3D7 %GIA (black dotted lines). Black dots represent isolates lying within the defined susceptibility ranges. Red dots represent isolates reflecting a reduced GIA susceptibility phenotype (bright red) or laying at the borderline (dim red) of the lower limit of the defined susceptibility threshold. Blue dots represent isolates showing an increased GIA susceptibility (bright blue) or laying at the borderline (dim blue) of the upper limit of the defined susceptibility threshold. Isolates where parasitemia was diluted with O+ blood from a single donor are indicated with a star (*).



Supplementary Figure 4: Relationship between the clinical samples' initial parasitemia and the number of sequencing reads assembled to the Pfrh5 reference sequence. (A) Percent parasitemia of *P. falciparum* clinical isolates (N = 50) defined as the percentage of infected erythrocytes over the total number of counted erythrocytes using a thin blood smear. (B) Total number of sequencing reads assembled to the *pfrh5* reference sequence. The Pfrh5 sequencing was performed using the Novaseq 6000 sequencing platform and het data were analyzed using the Geneious Prime software version 23.1.1. and the graphs plotted using GraphPad Prism version 10.2.3. (C) Relationship between initial parasitemia and number of sequencing reads assembled to Pfrh5 computed using the simple linear regression analysis and the goodness of fit (R^2) in GraphPad prism 10.2.3.



Supplementary Figure 5: PfrH5-associated genetic diversity in *P. falciparum* clinical isolates from Kédougou (N = 50). The prevalence of PfrH5-associated SNPs was calculated as the percentage of SNPs detected within the total sample population (N= 50). PfrH5 sequencing was performed from *pfprh5* amplicons using the Illumina NovaSeq 6000 sequencing platform and variant analysis was performed using the Geneious Prime software version 23.1.1. The graphs were plotted using the GraphPad Prism version 1.0.2 software. SNPs are ordered by prevalence, with higher prevalence SNPs to the left and rare SNPs to the left. SNPs displayed to the left of the dotted line represent SNPs present in a single sample (and at a prevalence of 2%). “WT” represents the *pfprh5* 3D7 reference allele.

mAb	Concentration	Isolate	Genotype	p-value	
c2AC7	50	400106	C203Y	0.034	
		400162	C203Y	0.048	
c9AD4	50	400128	C203Y; E306G	0.046	
	25	400108	WT	0.038	
		400130	WT	0.024	
10	400113	D243N	0.004		
R5.016	50	400111	K51E; K51R; C203Y	0.011	
		400112	K51E; C203Y	0.026	
		400113	D243N	0.047	
		400152	C203Y	0.039	
	25	400162	C203Y	0.012	
		400112	K51E; C203Y	0.005	
		400113	D243N	0.027	
		400118	K51E; C203Y	0.015	
	10	400158	C203Y; V371I	0.027	
		400159	K51E; K51R; I60V	0.017	
		400106	C203Y	0.022	
		400108	WT	0.009	
		400112	K51E; C203Y	0.017	
		400113	D243N	0.004	
		400115	WT	0.0007	
		400149	C203Y; I407V; F505Y	0.037	
		400158	C203Y; V371I	0.002	
400159	K51E; K51R; I60V	0.027			
400162	C203Y	0.042			
R5.004	50	400154	C203Y	0.013	
R5.017**	150	400113	D243N	<0.0001	
		400130	WT	0.005	
		FVO	S197Y	<0.0001	
	50	400103	D249G; Y254C	0.02	
		400113	D243N	0.03	
		400128	C203Y; E306G	0.0006	
		400130	WT	0.0003	
		400134	WT	0.0008	
		FVO	S197Y	<0.0001	
	25	400128	C203Y; E306G	0.001	
		400130	WT	<0.0001	
		400134	WT	0.01	
		FVO	S197Y	<0.0001	
		10	400128	C203Y; E306G	<0.0001
			400130	WT	0.001
400134	WT		0.042		
FVO	S197Y	<0.0001			
R5.008	150	400114	C203Y	0.024	
		400155	WT	0.036	
		400158	C203Y; V371I	0.024	
		400162	C203Y	0.012	
	50	400154	C203Y	0.002	
		FVO	S197Y	0.025	
	25	400128	C203Y; E306G	0.038	
		400128	C203Y; E306G	0.007	
R5.011	50	400108	WT	0.049	
		400112	K51E; C203Y	0.049	
		400154	C203Y	0.002	
	25	400103	D249G; Y254C	0.029	
400154		C203Y	0.001		
400155	WT	0.014			
R5.007	50	400103	D249G; Y254C	0.002	
		400115	WT	0.008	
		400152	C203Y	0.034	
R5.001	150	400103	D249G; Y254C	0.03	
		400108	WT	0.033	
		400155	WT	0.041	
		FVO	S197Y	0.046	
	50	400154	C203Y	0.026	
		400115	WT	0.024	
10	400162	C203Y	0.033		

Supplementary Table 1: Statistic table showing clinical isolates with a significant difference of %GIA relative to the 3D7 reference clone. P-values were computed using the Dunnett's multiple comparison test following the Two-way ANOVA analysis and parasite genotypes were obtained using the Illumina NovaSeq 6000 next-generation sequencing platform.

PDB ID	Complex	Mutation	ddG (kcal/mol)	Rh5 stability (kcal/mol)
4U0Q	RH5/Basigin	Y203C	2.4296	0.753
4U0Q	RH5/Basigin	E306G	0	1.8518
4U0Q	RH5/Basigin	F505Y	0	0.1749
4U0Q	RH5/Basigin	I407V	0	1.1062
4U0Q	RH5/Basigin	K221R	0	-0.2696
4U0Q	RH5/Basigin	V371I	0	-0.124
4U0R	RH5/9AD4	Y203C	-0.0134	0.8367
4U0R	RH5/9AD4	E306G	0	1.3719
4U0R	RH5/9AD4	F505Y	0	-0.0289
4U0R	RH5/9AD4	I407V	0	1.3516
4U0R	RH5/9AD4	K221R	0	0.6477
4U0R	RH5/9AD4	V371I	0	-0.5554
4U1G	RH5/QA1	Y203C	0	0.868
4U1G	RH5/QA1	E306G	0	1.2399
4U1G	RH5/QA1	F505Y	0	-0.018
4U1G	RH5/QA1	I407V	0	0.7001
4U1G	RH5/QA1	K221R	0	1.9648
4U1G	RH5/QA1	V371I	0	-0.646
6MPV	Rh5/CyPRA/Ripr	C203Y	0	-0.298
6MPV	Rh5/CyPRA/Ripr	E306G	0	1.144
6MPV	Rh5/CyPRA/Ripr	F505Y	1.3743	-0.424
6MPV	Rh5/CyPRA/Ripr	I407V	0	0.801
6MPV	Rh5/CyPRA/Ripr	K221R	0	-0.384
6MPV	Rh5/CyPRA/Ripr	V371I	0	0.482
6RCU	Rh5/R5.004	C203Y	0	-0.84124
6RCU	Rh5/R5.004	E306G	0	2.09274
6RCU	Rh5/R5.004	F505Y	0	0.06114
6RCU	Rh5/R5.004	I407V	0	0.89026
6RCU	Rh5/R5.004	K221R	0	0.03218
6RCU	Rh5/R5.004	V371I	0	0.7566
6RCV	Rh5/R5.011	C203Y	0	1.811
6RCV	Rh5/R5.011	E306G	-0.0436	1.1167
6RCV	Rh5/R5.011	I407V	0	0.7807
6RCV	Rh5/R5.011	K221R	-0.027	-1.006
6RCV	Rh5/R5.011	V371I	0	1.4678
7PHU	Rh5/R5.015	Y203C	0	0.8163
7PHU	Rh5/R5.015	E306G	0	2.763
7PHU	Rh5/R5.015	F505Y	0.5901	-0.5333
7PHU	Rh5/R5.015	I407V	0	1.4872
7PHU	Rh5/R5.015	K221R	0	0.2871
7PHU	Rh5/R5.015	V371I	0	0.4805
6RCU	Rh5/R5.016	C203Y	-0.099	-1.0154
6RCU	Rh5/R5.016	E306G	0	1.7303
6RCU	Rh5/R5.016	F505Y	0	0.0265
6RCU	Rh5/R5.016	I407V	0	0.8954
6RCU	Rh5/R5.016	K221R	-0.0415	0.0724
6RCU	Rh5/R5.016	V371I	0	0.7306

Supplementary Table 2. Predicted binding energy changes for BSG and RH5 variant proteins:

Individual FASTA files with PfRH5 and individual SNP were threaded through the crystal structure and the impact of the mutant versions of the protein was evaluated for predicted binding affinity of PfRH5 to its binding partner PfCyRPA, its erythrocyte receptor, Basigin, or the test human mAbs. The structural effect of the PfRH5-associated SNPs was evaluated for predicted binding affinity between the mutant version of the RH5 protein and, binding partner PfCyRPA, the Basigin receptor or test mAbs. The binding energy alternation for SNPs and BSG were predicted by FoldX version 5.0. Predicted binding energies are shown for reference and mutant alleles of the protein in Kcal/mol for each SNP. Changes between the two are shown as ddG (Kcal/mol). A negative ddG indicates a predicted increase in binding and a positive ddG indicates a predicted decrease in binding.