

Supplementary Table 1. Recombinant PfEMP1 proteins used in multiplex immunoassay

Protein identifier	Domain	Group	Associated DC/ gene	Predicted binding receptor	Expression system	Source
SM1	CIDR α 2.4	B			WGCFS	<i>a</i>
SM2	CIDR β 1	B			WGCFS	<i>a</i>
SM3	DBL β 12	B/A	DC8		WGCFS	<i>a</i>
SM4	DBL β 3	A			WGCFS	<i>a</i>
SM5	DBL β 3	A	DC4		WGCFS	<i>a</i>
SM6	DBL δ 1	B			WGCFS	<i>a</i>
SM8	DBL δ 1	B			WGCFS	<i>a</i>
SM9	DBL δ 1	B	DC8		WGCFS	<i>a</i>
SM11	DBL ϵ 3	B	DC11		WGCFS	<i>a</i>
SM12	DBL ϵ 9	B			WGCFS	<i>a</i>
SM14	DBL γ 3	B	DC9?		WGCFS	<i>a</i>
SM15	DBL ζ 4	B	DC9?		WGCFS	<i>a</i>
SM17	DBL α 1.5	A	DC4		WGCFS	<i>a</i>
SM18	CIDR α 1.1	B/A	DC8	EPCR	WGCFS	<i>a</i>
SM19	CIDR α 1.6	A	DC4	EPCR	WGCFS	<i>a</i>
SM22	DBL ϵ 5	A	<i>var1</i>		WGCFS	<i>a</i>
SM24	DBL ζ 3	B/A			WGCFS	<i>a</i>
SM25	DBL β 13	B			WGCFS	<i>a</i>
SM26	CIDR γ 12	B			WGCFS	<i>a</i>
SM27	DBL δ 7	A			WGCFS	<i>a</i>
SM28	CIDR α 2.6DBL β 5	B			WGCFS	<i>a</i>
UM1	DBL α 0.13	B			WGCFS	<i>a</i>
UM2	DBL δ 1	B			WGCFS	<i>a</i>
UM8	DBL γ 9	B/A			WGCFS	<i>a</i>
UM14	DBL δ 1	B			WGCFS	<i>a</i>

UM19	DBL δ 1	B			WGCFs	<i>a</i>
UM20	CIDR α 3.1	B/C			WGCFs	<i>a</i>
UM45	CIDR α 1.7	A			WGCFs	<i>a</i>
M1	DBL β 3*	A	PF11_0521_D4	ICAM-1	<i>Escherichia coli</i>	<i>b</i>
M6	DBL β 3*	A	3D7_Pfd1235w_D4	ICAM-1	<i>Escherichia coli</i>	<i>b</i>
M9	DBL β 1*	A	Dd2VAR32_D4	ICAM-1	<i>Escherichia coli</i>	<i>b</i>
CIDR–DBL β 3*	CIDR α 1.6–DBL β 3*	A	3D7_Pfd1235w_D3-4	EPCR-ICAM-1	<i>Escherichia coli</i>	<i>b</i>
N27	DBL β 3	B	IT4VAR13_D4	ICAM-1	<i>Escherichia coli</i>	<i>b</i>
P1021	DBL β 3	B	KOB58843/HB3var34	ICAM-1	<i>Escherichia coli</i>	<i>b</i>
P1014	DBL β 5	B	KOB63129/HB3var21	ICAM-1	<i>Escherichia coli</i>	<i>b</i>
P1011	DBL β 5	B	AA75396/Dd2var01a	ICAM-1	<i>Escherichia coli</i>	<i>b</i>
N30	DBL β 7	A	Dd2VAR52_D4		<i>Escherichia coli</i>	<i>b</i>
N31	DBL β 7	A	Hb3vAR01_D4		<i>Escherichia coli</i>	<i>b</i>
N32	DBL β 6	A	BT1983_D4		<i>Escherichia coli</i>	<i>b</i>

Table 1: (DC) Domain Cassette. (*) contains DBL β _{motif} associated with dual EPCR-ICAM-1 binding IE. (WGCFs) wheat germ cell free system. (a) Proteins labelled SM and UM were derived from sequences of Papuan field isolates (1) and were expressed by Professor Takafumi Tsuboi and Dr. Eizo Takashima, Proteo-Science Center, Ehime University, Matsuyama, Ehime, Japan (2). (b) DBL β proteins were supplied by Prof Anja Jensen, University of Copenhagen, Copenhagen, Denmark (3–5).

Supplementary Table 2. Recombinant merozoite and sporozoite proteins used in multiplex immunoassay

Protein	Expression system
AMA1	HEK293
MSP2 (FC27 allele)	<i>Escherichia coli</i>
MSP3	WGCFS
EBA175-RIII-V	<i>Escherichia coli</i>
CSP	HEK293

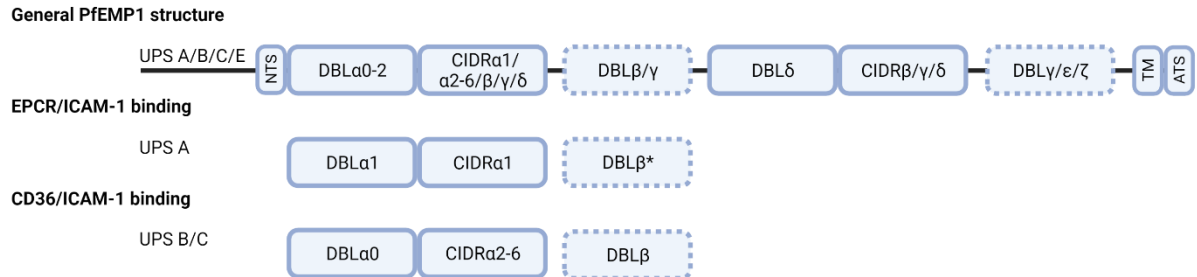
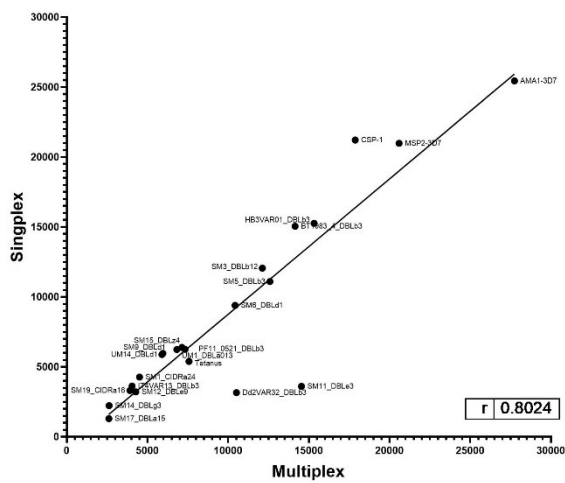


Figure S1. **General structure of PfEMP1.** PfEMP1 are classified based on the up stream promotor sequence (Groups A/B/C/E). The NTS is followed by a DBL α and CIDR domain head structure. Head structure CIDR α 1 domains bind to EPCR and CIDR α 2-6 bind to CD36. The head structure is followed by at least 2 domains, shown with solid borders, and longer PfEMP1 have additional domains, shown with dashed borders. Group A EPCR binding head structures can sometimes be followed by DBL β with the ICAM-1 binding motif (*). Group B CD36 binding head structures can sometimes be followed by DBL β that also bind ICAM-1 (but cannot be predicted by the motif). DBL: Duffy Binding Like; CIDR: Cysteine-rich interdomain Region; NTS: N-terminal sequence, TM: Transmembrane region; ATS: A-terminal sequence. Figure adapted from Smith *et al.* 2013, created with BioRender.com.

A



B

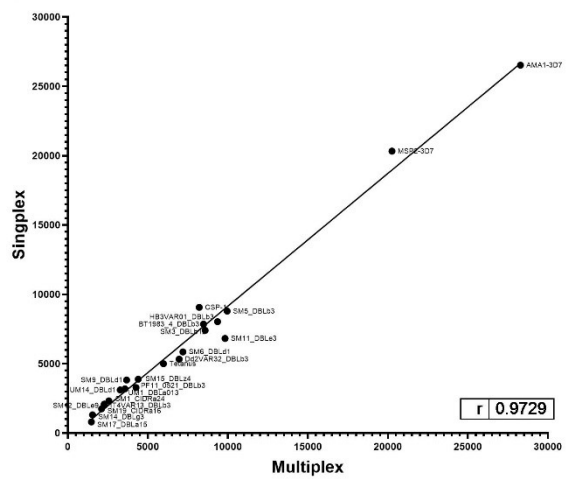


Figure S2. IgG responses for array of 17 antigens (one of two arrays) compared in single and multiplex formats, using serum pooled from 10 Malawian children, diluted by A) 1:50 and B) 1:100. r = Spearman's correlation coefficient. DBL = Duffy Binding Like domain. CIDR = Cysteine-Rich Interdomain Region. a = alpha, b = beta, d = delta, e = epsilon, g = gamma, z = zeta.

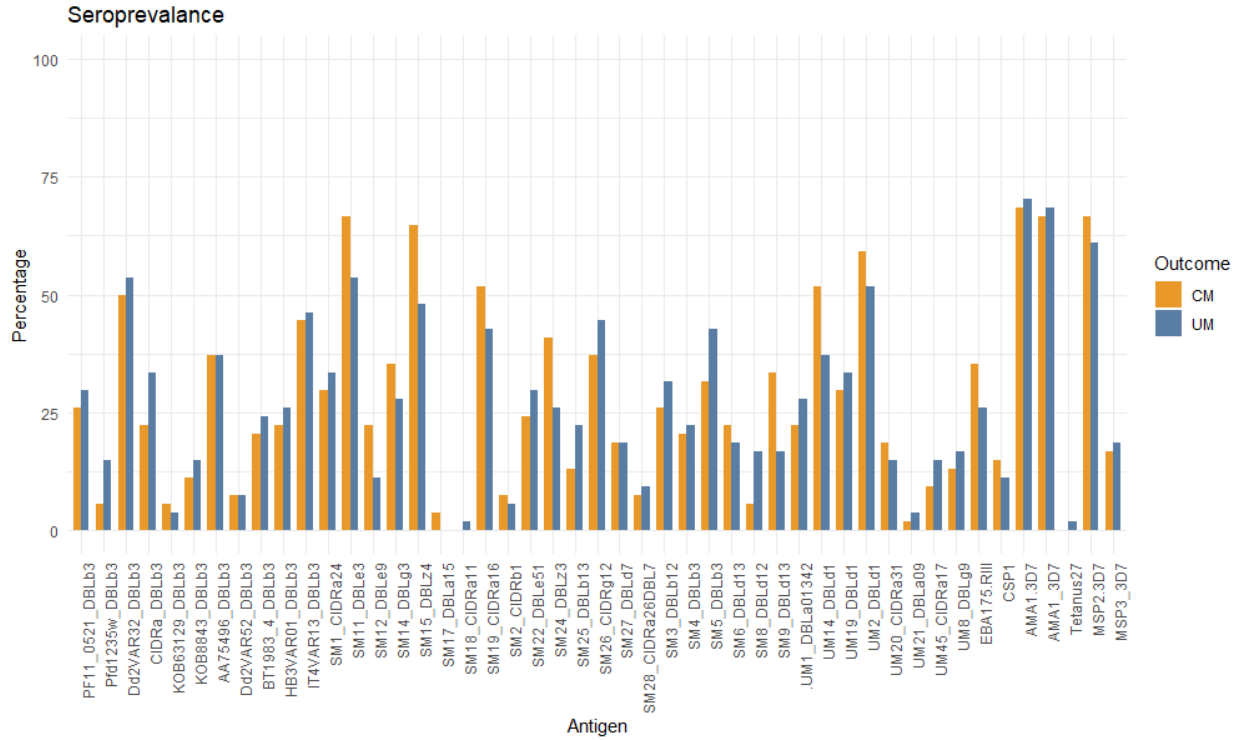
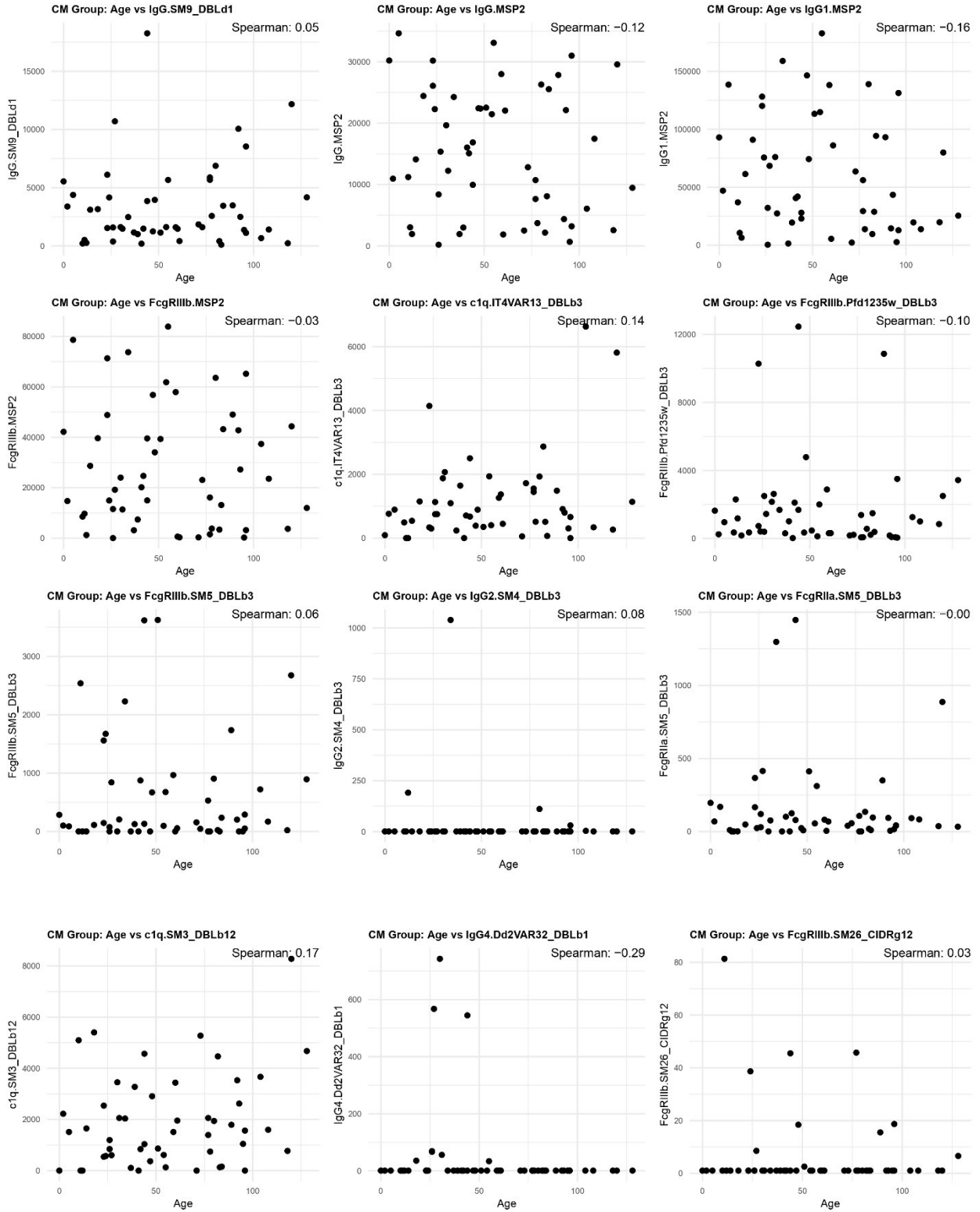


Figure S3. Percentage children with cerebral malaria (orange) or uncomplicated malaria (blue) who are seropositive to PfEMP1 antigens. Seropositivity was defined as IgG titers greater than 2 SD above the mean of malaria naïve individuals from Melbourne, Australia. DBL = Duffy Binding Like domain. CIDR = Cysteine-Rich Interdomain Region. a = alpha, b = beta, d = delta, e = epsilon, g = gamma, z = zeta.



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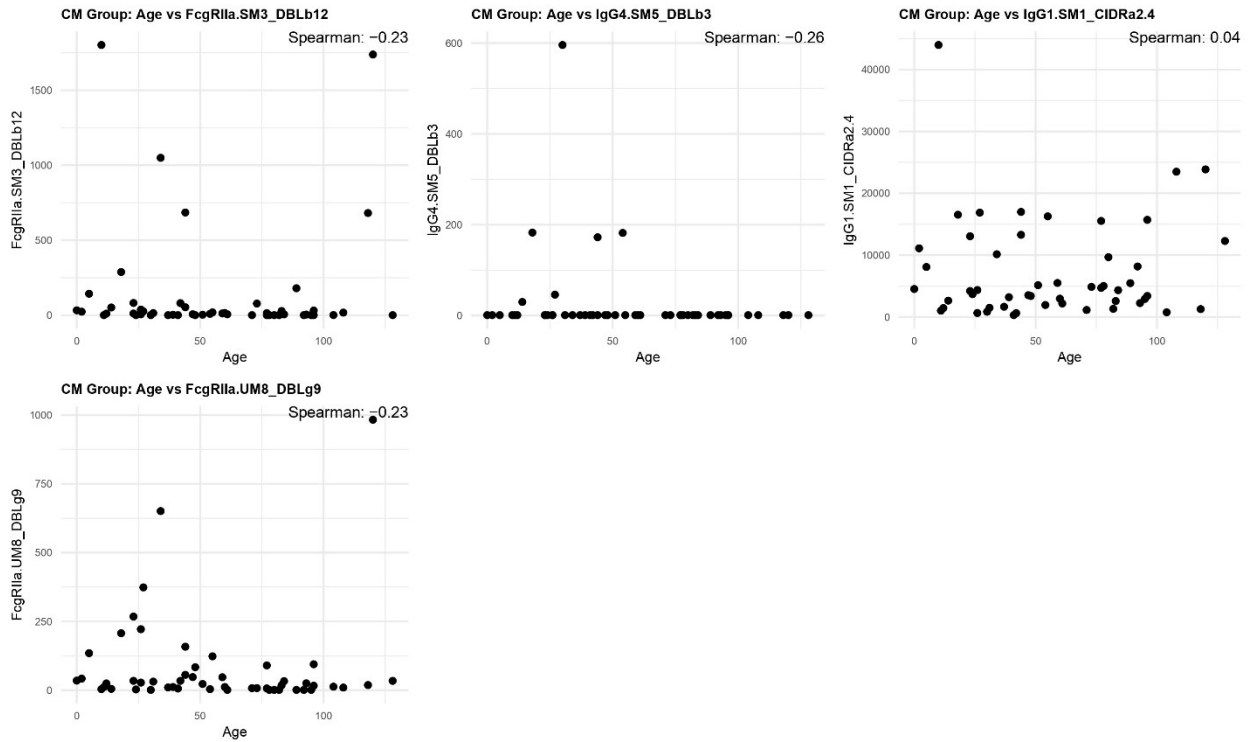
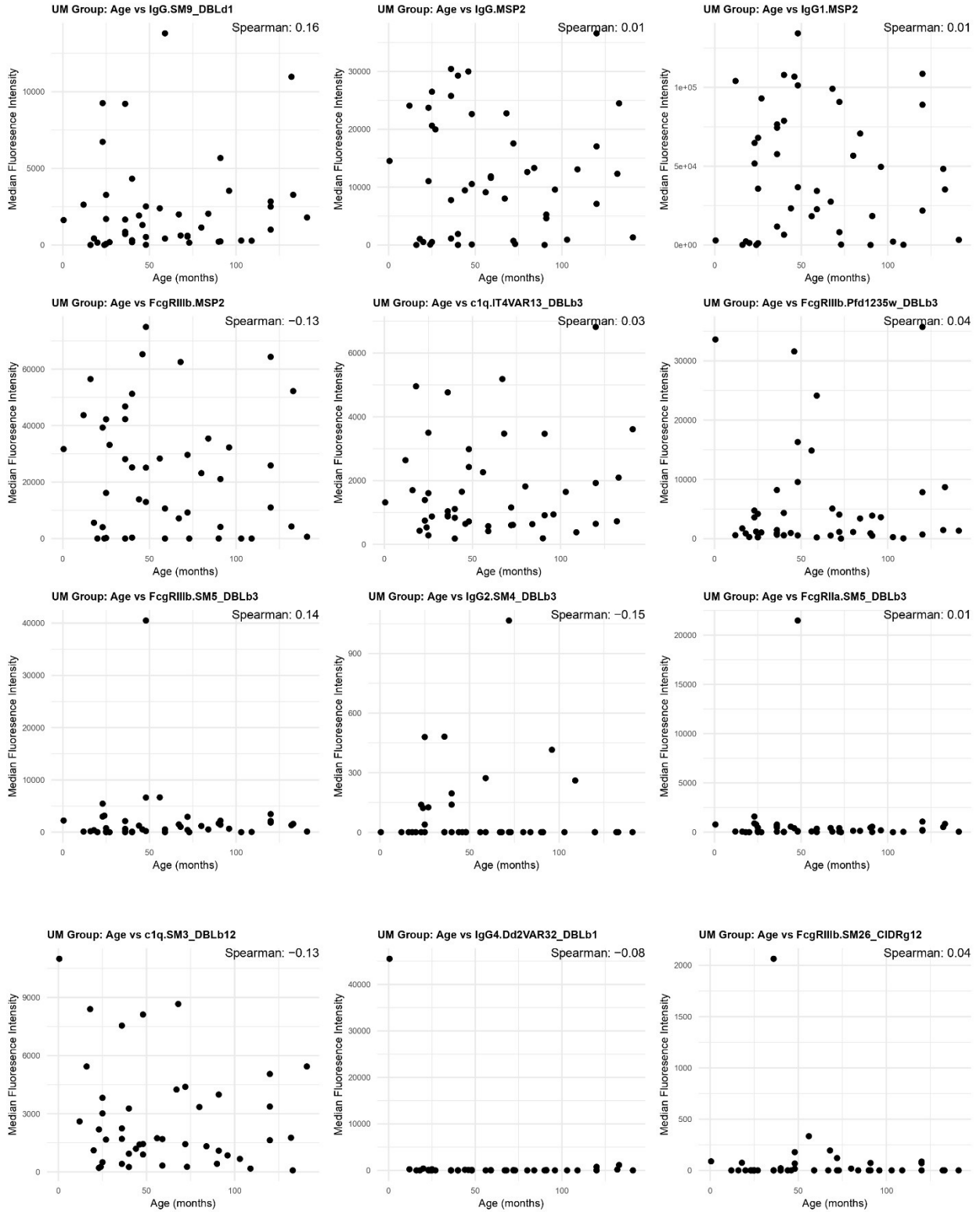


Figure S4: Correlation between age (months) and antibody response (median fluorescence intensity, measured by multiplex immunoassay) amongst children with cerebral malaria (CM), for antibody features found to be significantly and by greater than 2 folds different in children with cerebral and uncomplicated malaria. Strength of correlation represented by Spearman correlation coefficient, R (Spearman). DBL = Duffy Binding Like domain. CIDR = Cysteine-Rich Interdomain Region. a = alpha, b = beta, d = delta, e = epsilon, g = gamma, z = zeta.



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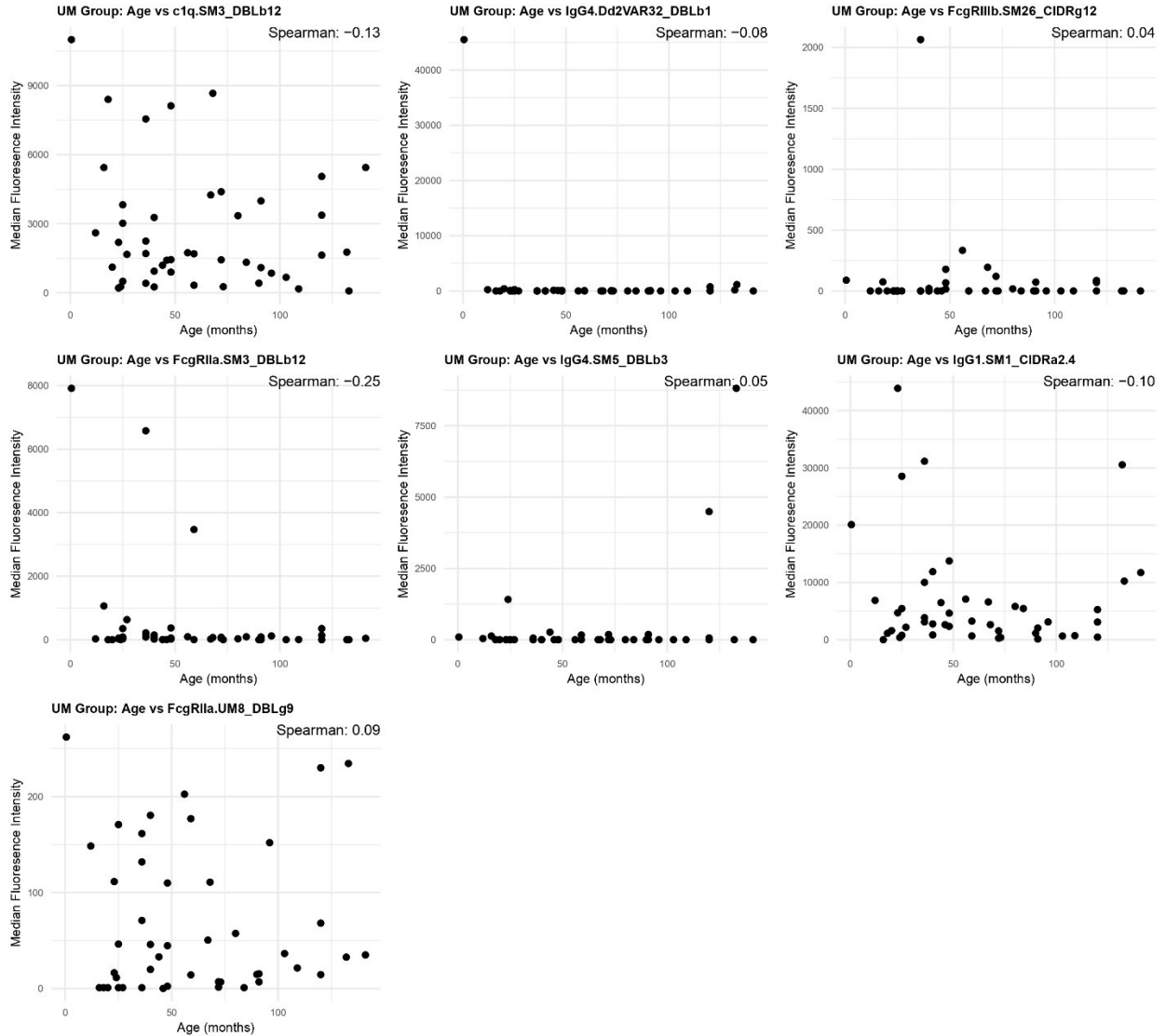


Figure S5: Correlation between age (months) and antibody response (median fluorescence intensity, measured by multiplex immunoassay) amongst children with uncomplicated malaria (UM), for antibody features found to be significantly and by greater than 2 folds different in children with cerebral and uncomplicated malaria. Strength of correlation represented by Spearman correlation coefficient, R (Spearman). DBL = Duffy Binding Like domain. CIDR = Cysteine-Rich Interdomain Region. a = alpha, b = beta, d = delta, e = epsilon, g = gamma, z = zeta.

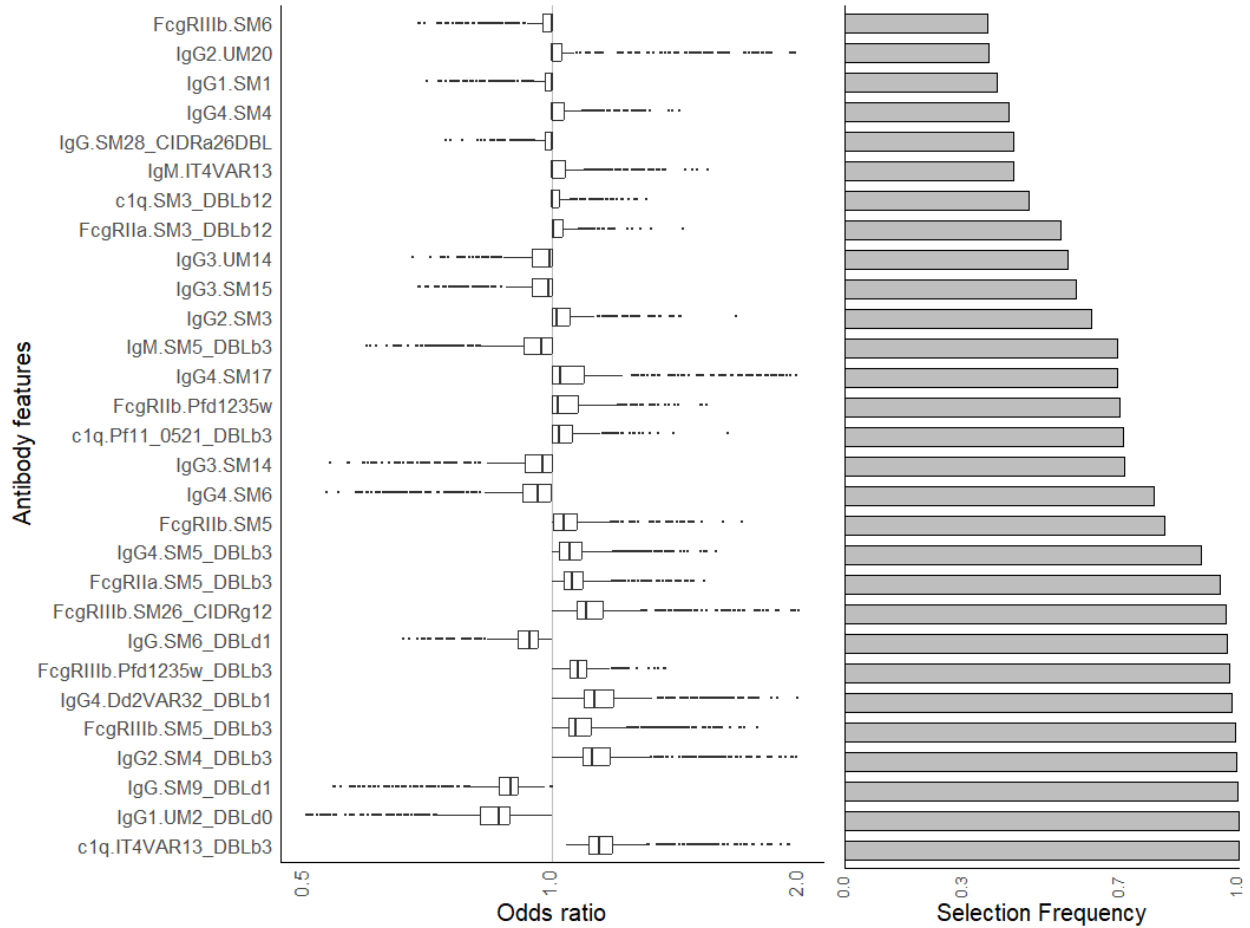


Figure S6. Odds ratio of antibody features from 5,000 repeats of 10-fold cross validated ENET regression models using alpha tuning parameter of 0.25. Variables shown in order of selection frequency (top 29 most selected variables are shown). Features with mean odds ratio greater than 1 represent responses associated with increased odds of uncomplicated malaria and features with mean odds ratio less than 1 are associated with increased odds of cerebral malaria. DBL = Duffy Binding Like domain. CIDR = Cysteine-Rich Interdomain Region. a = alpha, b = beta, d = delta, e = epsilon, g = gamma, z = zeta.

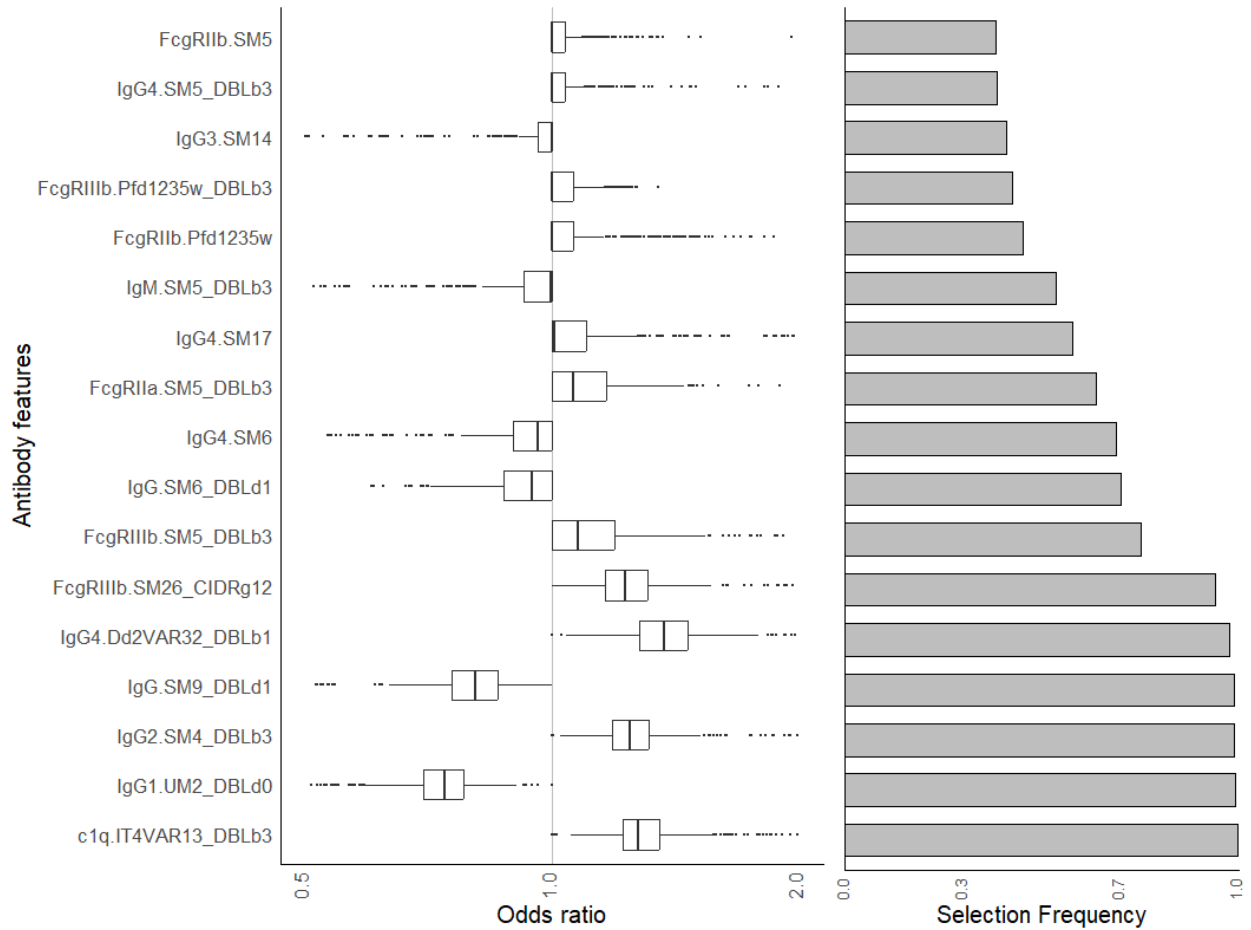


Figure S7. Odds ratio of antibody features from 5,000 repeats of 10-fold cross validated ENET regression models using alpha tuning parameter of 1. Variables shown in order of selection frequency (top 17 most selected variables are shown). Features with mean odds ratio greater than 1 represents responses associated with increased odds of uncomplicated malaria and features with mean odds ratio less than 1 are associated with increased odds of cerebral malaria. DBL = Duffy Binding Like domain. CIDR = Cysteine-Rich Interdomain Region. a = alpha, b = beta, d = delta, e = epsilon, g = gamma, z = zeta.

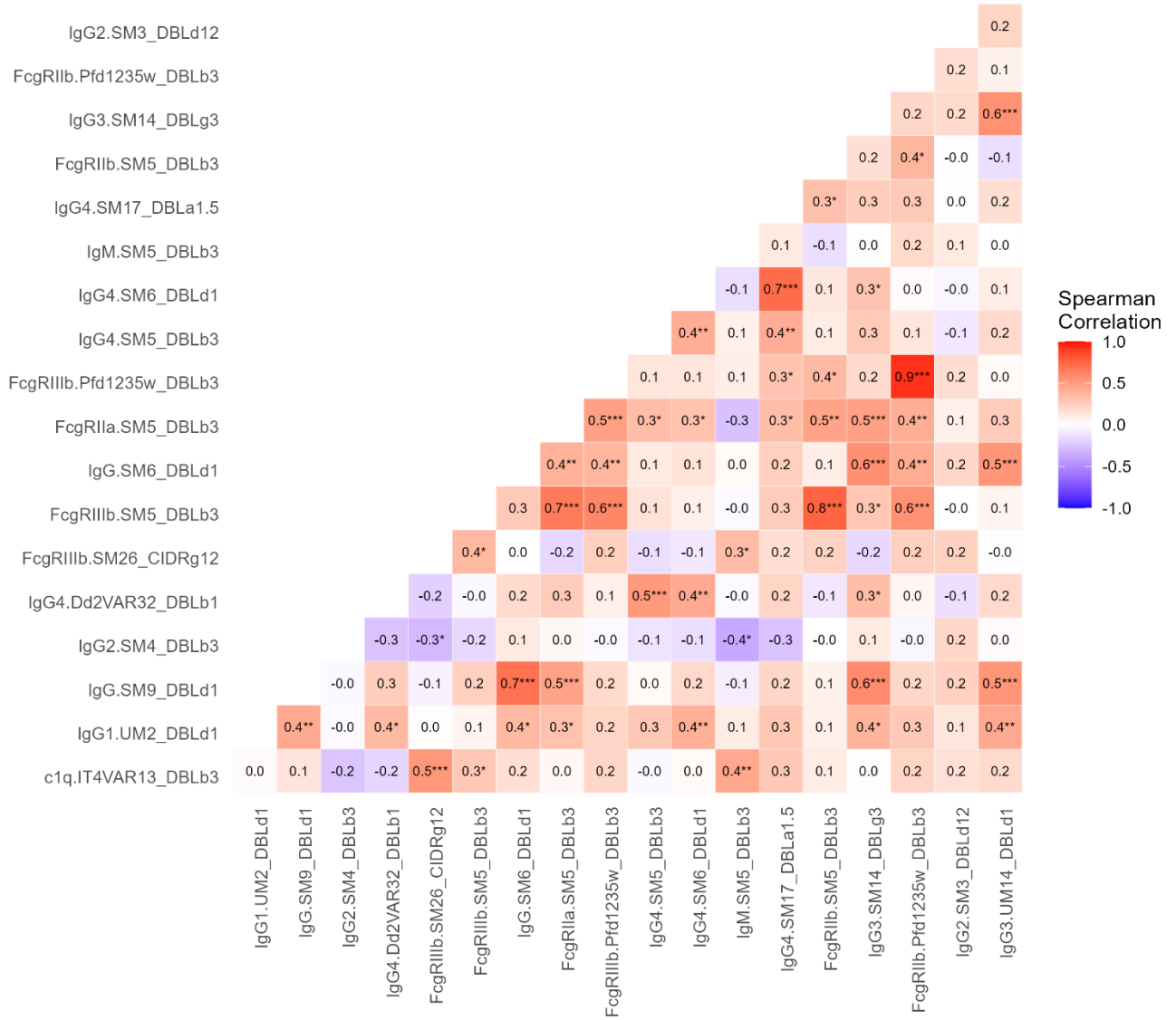


Figure S8. Spearman correlation of features that appeared in >70% of ENLR model iterations amongst children with uncomplicated malaria. Negative correlations are shaded blue and positive correlations in red. DBL = Duffy Binding Like domain. CIDR = Cysteine-Rich Interdomain Region. a = alpha, b = beta, d = delta, e = epsilon, g = gamma, z = zeta. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001.

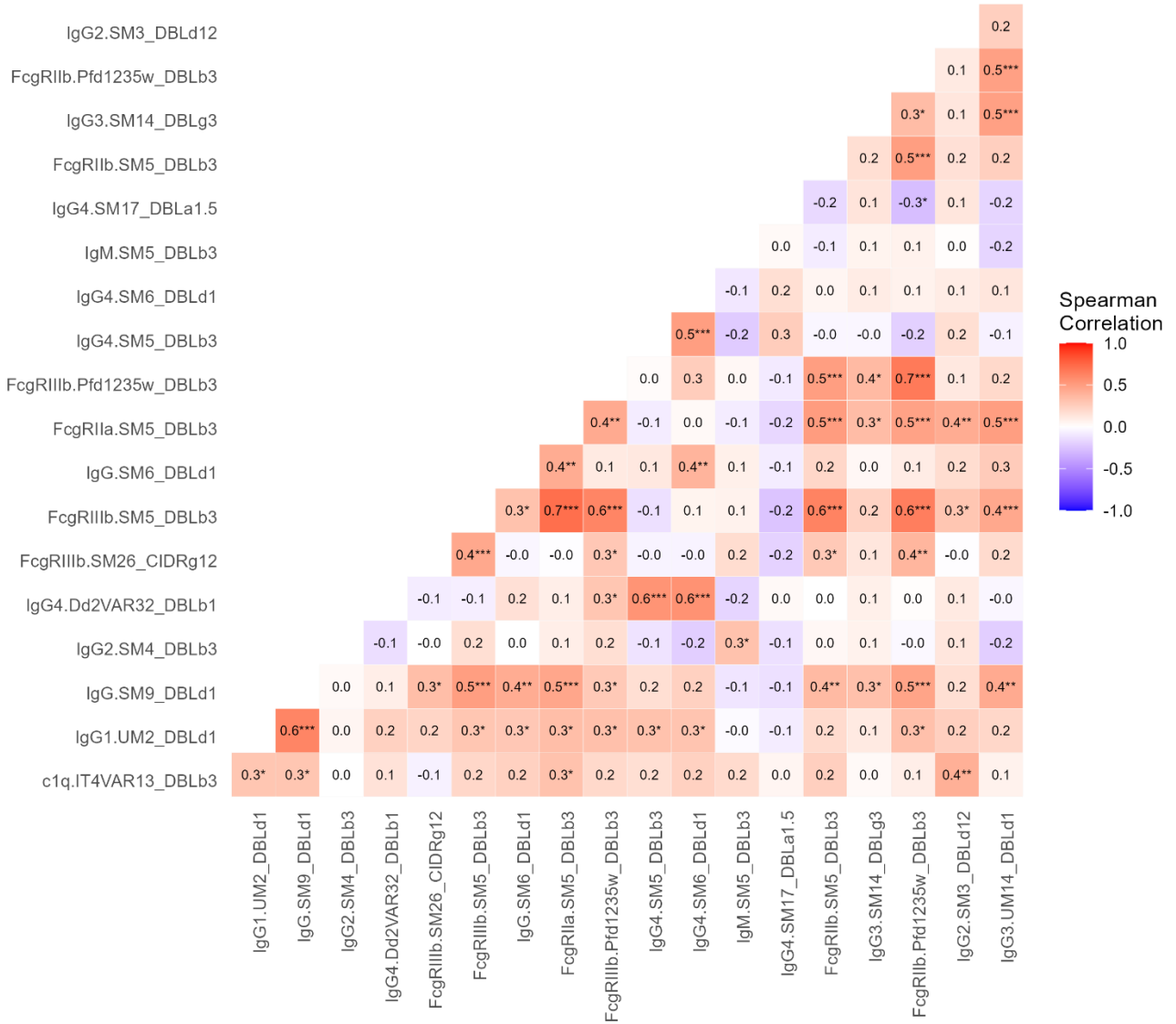


Figure S9. Spearman correlation of features that appeared in >70% of ENLR model iterations amongst children with cerebral malaria. Negative correlations are shaded blue and positive correlations in red. DBL = Duffy Binding Like domain. CIDR = Cysteine-Rich Interdomain Region. a = alpha, b = beta, d = delta, e = epsilon, g = gamma, z = zeta. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001.

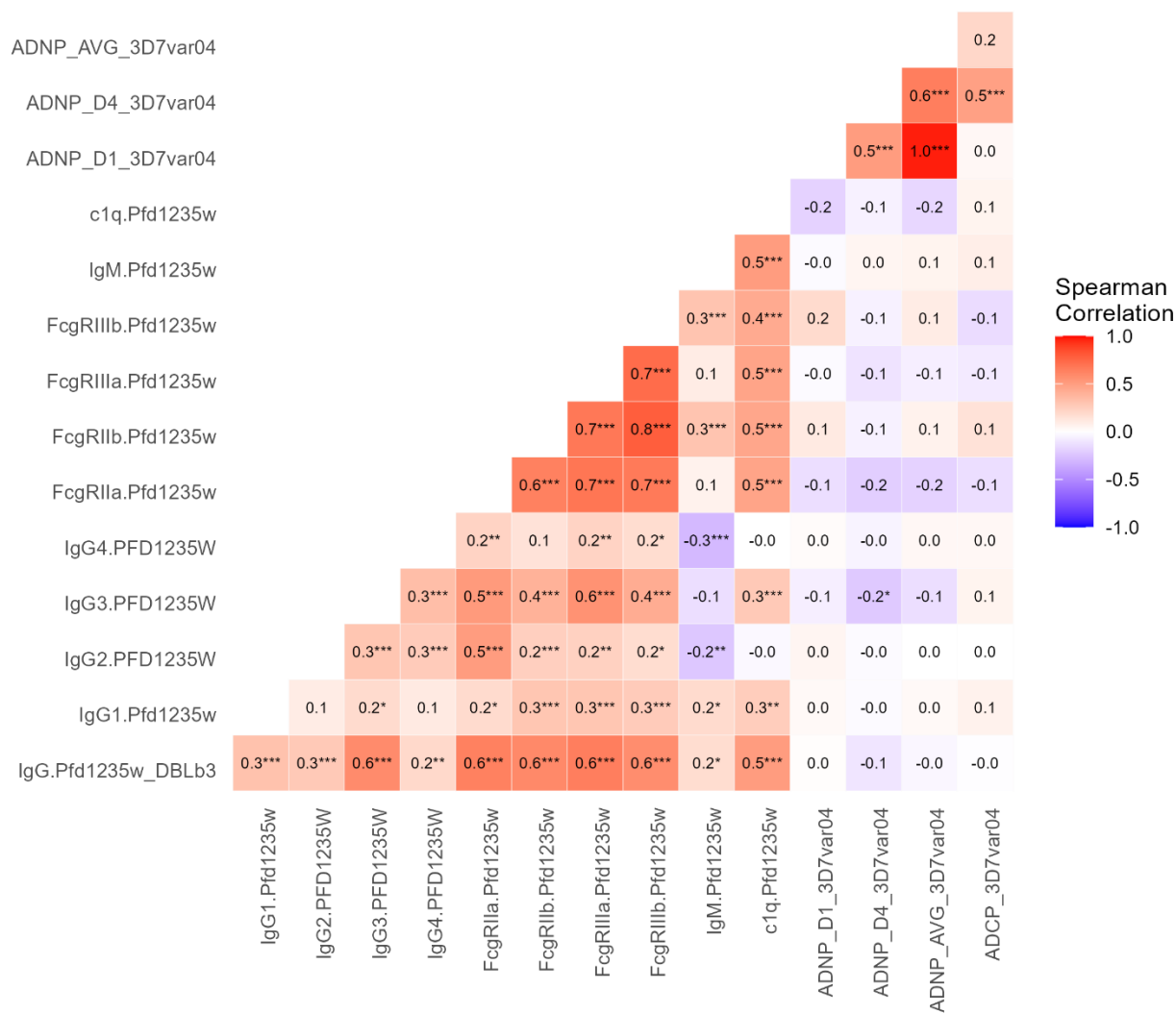


Figure S10. Correlations between antibody features targeting DBL β 3_Pfd1235w and antibody dependent phagocytosis (ADNP and ADCP) of IE expressing the corresponding PfEMP1, 3D7VAR04. Circle size and shade represents Spearman's correlation co-efficient, r . Negative correlations are shaded blue and positive correlations in red. ADNP_D1 and ADNP_D4 show ADNP from two individual neutrophil donors and ADNP_AVG shows the average ADNP of the two donors. ADCP: antibody dependent THP-1 cell phagocytosis. DBL = Duffy Binding Like domain. CIDR = Cysteine-Rich Interdomain Region. a = alpha, b = beta, d = delta, e = epsilon, g = gamma, z = zeta. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001.

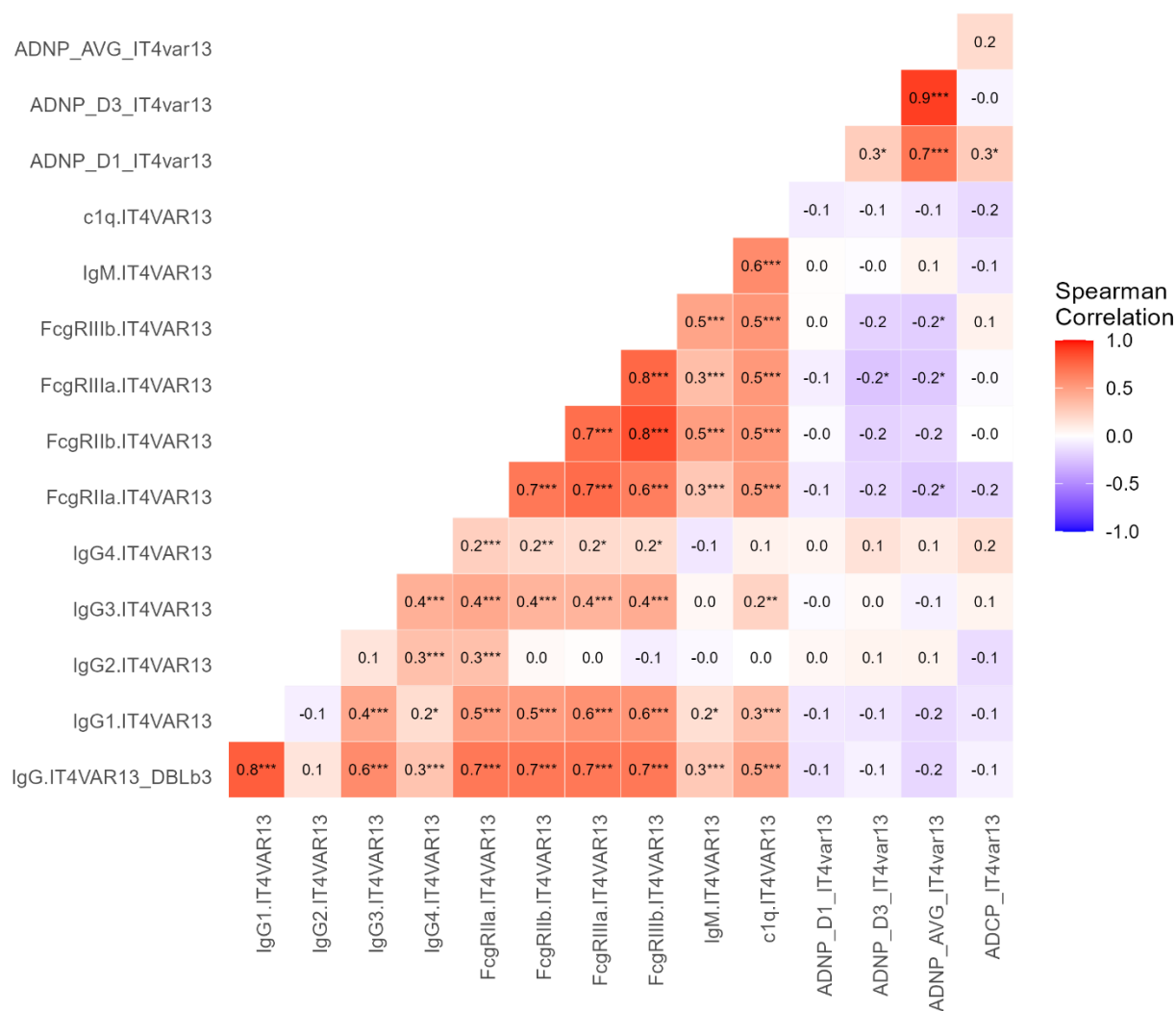


Figure S11. Correlations between antibody features targeting DBL β 3_IT4VAR13 and antibody dependent neutrophil phagocytosis (ADNP and ADCP) of IE the corresponding PfEMP1, IT4VAR13. Circle size and shade represents Spearman's correlation co-efficient, r . Negative correlations are shaded blue and positive correlations in red. ADNP_D1 and ADNP_D3 show ADNP from two individual neutrophil donors and ADNP_AVG shows the average ADNP of the two donors. ADCP: antibody dependent THP-1 cell phagocytosis. DBL = Duffy Binding Like domain. CIDR = Cysteine-Rich Interdomain Region. a = alpha, b = beta, d = delta, e = epsilon, g = gamma, z = zeta. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001.

References:

1. Tonkin-Hill GQ, Trianty L, Noviyanti R, Nguyen HHT, Sebayang BF, Lampah DA, et al. The *Plasmodium falciparum* transcriptome in severe malaria reveals altered expression of genes involved in important processes including surface antigen–encoding var genes. *PLoS Biol.* 2018;16:e2004328.
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4. Olsen RW, Ecklu-Mensah G, Bengtsson A, Ofori MF, Kusi KA, Koram KA, et al. Acquisition of IGG to ICAM-1-Binding DBLB Domains in the *plasmodium falciparum* erythrocyte membrane protein 1 antigen family varies between groups A, B, and C. *Infect Immun.* 2019;87:e00224-19.
5. Bengtsson A, Joergensen L, Rask TS, Olsen RW, Andersen MA, Turner L, et al. A Novel Domain Cassette Identifies *Plasmodium falciparum* PfEMP1 Proteins Binding ICAM-1 and Is a Target of Cross-Reactive, Adhesion-Inhibitory Antibodies. *J Immunol.* 2013;190:240–9.