

# Analysis of the Diverse Antigenic Landscape of the Malaria Invasion Protein RH5 Identifies a Potent Vaccine-Induced Human Public Antibody Clonotype

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## 31 SUMMARY

32 The highly conserved and essential *Plasmodium falciparum* reticulocyte-binding protein homolog 5 (PfRH5) has  
33 emerged as the leading target for vaccines that seek to protect against the disease-causing blood-stage of  
34 malaria. However, the features of the human vaccine-induced antibody response that confer highly potent  
35 inhibition of malaria parasite invasion into red blood cells are not well defined. Here we characterize over 200  
36 human IgG monoclonal antibodies induced by the most advanced PfRH5 vaccine. We define the antigenic  
37 landscape of this molecule, and establish epitope specificity, antibody association rate and intra-PfRH5  
38 antibody interactions are key determinants of functional anti-parasitic potency. In addition, we identify a  
39 germline gene combination that results in an exceptionally potent class of antibody and demonstrate its  
40 prophylactic potential to protect against *P. falciparum* parasite challenge *in vivo*. This comprehensive dataset  
41 provides a framework to guide rational design of next-generation vaccines and prophylactic antibodies to  
42 protect against blood-stage malaria.

## 43 INTRODUCTION

44 The exponential growth of *Plasmodium falciparum* parasites in the blood of infected individuals drives the  
45 disease state known as malaria. Preventative measures including the use of insecticides, bednets and anti-  
46 malarial drugs have proven effective in reducing the global malaria burden since the turn of the millennium,  
47 however recent evidence suggests progress has stalled. Current estimates indicate clinical cases rose in 2021  
48 to 247 million, leading to ~619,000 deaths, primarily in young African infants and children<sup>1</sup>. Immune-based  
49 interventions, including prophylactic monoclonal antibodies (mAbs) and vaccines, offer highly promising and  
50 alternative strategies to complement the current public health tools for malaria<sup>2,3</sup>. Indeed, substantial  
51 progress has been made recently with the clinical development of the RTS,S/AS01 and R21/Matrix-M™ subunit  
52 vaccines and the L9LS mAb, all of which target the *P. falciparum* circumsporozoite protein (PfCSP) thereby  
53 blocking infection at the pre-erythrocytic stage<sup>4-6</sup>. Nevertheless, when these interventions fail or immunity  
54 wanes, sporozoites eventually establish liver infection from where they develop into merozoites that emerge  
55 to initiate their continual and disease-causing cycle of growth in the blood. Blockade of merozoite invasion into  
56 the host red blood cell (RBC) thus provides a second and highly complementary opportunity for immune-  
57 intervention. Vaccines or mAbs against blood-stage antigens could provide standalone immunity, but also offer  
58 a leading strategy to achieve very high and more durable efficacy against *P. falciparum* via combination with  
59 the anti-PfCSP interventions in a multi-stage approach.

60 Merozoite invasion of the human RBC is a rapid and complex process, mediated by numerous host receptor –  
61 parasite ligand interactions. Indeed, the polymorphic and redundant nature of these parasitic targets thwarted  
62 blood-stage vaccine development for many years<sup>2</sup>, however, the discovery of the *P. falciparum* reticulocyte-  
63 binding protein homolog 5 (PfRH5) as a target that overcame these historical challenges has offered new  
64 promise<sup>7,8</sup>. PfRH5 is highly conserved and presented on the parasite's apical surface within a pentameric  
65 invasion complex<sup>9,10</sup>; here it forms an essential interaction with host basigin on the RBC<sup>8</sup> that also defines the  
66 human host tropism of this parasite<sup>11</sup>. Vaccination studies with PfRH5 in *Aotus* monkeys and healthy UK adults  
67 have shown significant efficacy against blood-stage challenge with *P. falciparum*, with the degree of *in vivo*  
68 inhibition of parasite growth strongly correlating with *in vitro* growth inhibition activity (GIA) as measured  
69 using purified total IgG in a standardized assay<sup>12,13</sup>. This ability of vaccine-induced anti-PfRH5 growth  
70 inhibitory antibodies to protect against blood-stage *P. falciparum* was subsequently validated by passive

71 transfer of mAb in both *Aotus* monkeys<sup>14</sup> and humanized mice<sup>15</sup>. Nonetheless, the protection outcomes in the  
72 UK adult clinical trial, of a protein-in-adjuvant vaccine called RH5.1/AS01<sub>B</sub>, were relatively modest<sup>13</sup>. These  
73 data thus indicated a clear need to increase the quantitative magnitude and/or qualitative potency of the  
74 vaccine-induced anti-PfRH5 polyclonal IgG response by next-generation vaccines in order to reach the same  
75 high levels of protection observed in the *Aotus* monkey model<sup>12</sup>.

76 The RH5.1 soluble protein vaccine<sup>16</sup>, as well as another PfRH5 viral-vectorized vaccine<sup>17,18</sup>, are in Phase 1/2  
77 clinical trials and deliver the full-length PfRH5 molecule (526 amino acids; ~60 kDa in size). *In silico* analyses  
78 initially indicated regions of disorder within full-length PfRH5 including a long N-terminal region, an intrinsic  
79 loop and a small C-terminus. Thereafter, a crystal structure was first reported using a protein known as  
80 RH5ΔNL that included the small C-terminus but lacked the disordered N-terminus and intrinsic disordered loop  
81 (IDL); this showed an α-helical diamond-like architecture forms the core of the PfRH5 protein, with basigin  
82 binding across the top of the diamond-like molecule<sup>19</sup>. At the bottom of the diamond, PfRH5 forms an  
83 interaction with the *P. falciparum* cysteine-rich protective antigen (PfCyRPA), thereby joining it to the hetero-  
84 pentameric invasion complex that displays PfRH5 towards the RBC membrane<sup>9,20</sup>.

85 Further studies have since investigated individual or small panels of anti-PfRH5 mAbs raised in mice<sup>21-23</sup> or  
86 from humans vaccinated with the first-generation viral-vectorized vaccine<sup>24-26</sup>. These studies have provided  
87 valuable insights but lacked sufficient power to understand the relationships that underlie human antibody  
88 recognition of PfRH5 and functional growth inhibition of *P. falciparum*. We therefore conducted a high-  
89 throughput campaign to isolate over 200 novel anti-PfRH5 human mAbs from vaccinees in the RH5.1/AS01<sub>B</sub>  
90 vaccine trial who showed reduced growth of blood-stage *P. falciparum* following experimental challenge<sup>13</sup>.  
91 Characterization of this large panel of new clones defines the determinants of antibody functional potency  
92 across a varied epitope landscape, thereby providing the high-resolution data needed for next-generation  
93 PfRH5 vaccine immunogen design. In addition, we identify a germline gene combination that results in an  
94 exceptionally potent class of anti-PfRH5 antibody and demonstrate its prophylactic potential to protect against  
95 *P. falciparum* parasite burden *in vivo*.

## 96 RESULTS

### 97 The functional epitope landscape of PfRH5

98 Peripheral blood mononuclear cells (PBMC) were collected from UK adult volunteers vaccinated with a full-  
99 length PfRH5 soluble protein vaccine, called RH5.1, formulated in AS01<sub>B</sub> adjuvant<sup>13,16</sup>. PfRH5-specific IgG+ B  
100 cells were sorted by fluorescence assisted cell sorting (FACS) using a probe bound to streptavidin labelled with  
101 two different fluorophores (**Figure S1A**). This probe was composed of a biotinylated form of PfRH5 lacking the  
102 disordered N- and C-termini (called “RH5 $\Delta$ NC”), given we have reported that these regions do not contribute  
103 growth-inhibitory antibodies in the vaccinees’ sera. Cells that were double-positive for both probes were  
104 sorted and lysed. Matched heavy and light chain variable antibody gene sequences were obtained through  
105 reverse transcriptase PCR (RT-PCR). Antibody genes were cloned into vectors encoding the human IgG1  
106 backbone for expression in HEK293T cells and purification of recombinant mAbs. Purified mAbs were screened  
107 by ELISA for binding to RH5.1, resulting in the isolation of 236 novel anti-PfRH5 mAb clones. Of these, nine  
108 (3.8%) were capable of binding heat-denatured RH5.1, indicating that they bind a linear epitope (**Figure S1B**).  
109 To map these linear epitopes, the mAbs were then tested for binding to a panel of 62 previously reported 20-  
110 mer overlapping peptides spanning the length of PfRH5<sup>17</sup> (**Figure S1C**); 7/9 mAbs bound at least one peptide in  
111 the panel. The majority of these (6/7) bound peptides 27-34, corresponding to the IDL region of PfRH5; the  
112 remaining mAb bound peptide 35 (clone R5.246). This peptide borders the IDL and is the only linear epitope  
113 mapped that also lies within the conformational core of PfRH5 (RH5 $\Delta$ NL).

114

115 To further resolve the epitopes of all antibodies, the 236 mAb panel was subjected to competition binning on  
116 RH5.1 using high-throughput surface plasmon resonance (HT-SPR). These studies used seven human “sentinel  
117 mAbs” from a previous study<sup>24</sup> to bridge this work to the epitope communities identified here in this much  
118 larger mAb panel. Thirty mAbs were excluded from this analysis due to behaviour incompatible with the assay  
119 (**Data S1A**). The remaining 206 mAbs (+7 sentinels) were sorted into epitope communities using Carterra  
120 Epitope software. Data for seven mAbs required manual processing, otherwise normalized response unit (RU)  
121 values for every other mAb pair were automatically sorted into a heatmap readout (**Figure S1D, Data S1B**).  
122 These values were then used to plot a dendrogram (**Figure S1E**) to cluster antibodies, and a cut-off height was

123 set to classify mAbs into monophyletic communities. These designations were overlaid onto a community  
124 network plot, to visualize the mAb communities and their competitive binding interactions (**Figure 1A**). This  
125 analysis resulted in the definition of 12 epitope communities: 1a (Blue, N=75, sentinel R5.004); 1b (Cyan, N=6);  
126 1c (Grey, N=1); 2 (Red, N=43, sentinel R5.016); 3a (Violet, N=14); 3b (Pink, N=17, sentinel R5.008); 4a (Teal,  
127 N=10); 4b (Green; N=3, sentinel R5.011); 4c (Turquoise, N=2); 5a (Orange, N=25, sentinel R5.015); and 5b  
128 (Yellow, N=4, sentinel R5.001). The IDL binders were pooled together in community 6 (the group that required  
129 manual processing); these span a series of adjacent linear epitopes as defined above (Purple, N=6, sentinel  
130 R5.007). Several of these communities (identified by subletters a-c) are further grouped into  
131 supercommunities 1, 3, 4 and 5, given they share overlapping competition profiles, but differ in their  
132 competition with external communities (**Figure 1A, Data S1C**). Notably, the human anti-PfRH5 mAbs isolated in  
133 this study were predominantly from epitope communities 1a (Blue = 75/206) and 2 (Red = 43/206), together  
134 comprising over half of the panel. Moreover, communities 1b (Cyan), 1c (Grey), 3a (Violet), 4a (Teal) and 4c  
135 (Turquoise) represent novel epitope regions of the PfRH5 molecule that are recognized by human mAbs that  
136 were not identified by the sentinel mAbs.

137

138 To understand the functionality of these epitope communities, we next determined the ability of each mAb to  
139 block binding of PfRH5 to basigin and PfCyRPA by bio-layer interferometry (BLI). These binary blocking results  
140 were then overlaid onto the community network plot (**Figure 1B**). Antibodies in supercommunity 1 and  
141 community 3a blocked basigin binding, along with a fraction of those in communities 2 (13/43) and 3b (11/17).  
142 These latter two communities likely lie on the edge of the basigin binding site on PfRH5, explaining their  
143 bimodal functionality. PfCyRPA-blocking mAbs were entirely confined to supercommunity 5.

144

145 We next measured the ability of mAbs to block 3D7 clone *P. falciparum* parasite invasion *in vitro* (at a high  
146 concentration of 0.8-2 mg/mL) using the assay of GIA, and also overlaid these data onto the community  
147 network plot (**Figure 1C**). The distribution of GIA-positive mAbs largely followed that of the basigin-blocking  
148 mAbs, although community 2 showed a subset of growth inhibitory antibodies that did not block basigin  
149 binding as measured by BLI. Conversely, community 1c (clone R5.149) blocked basigin binding but did not  
150 show evidence of GIA. Comparison of the GIA of basigin-blocking and non-blocking clones demonstrated a  
151 mutually exclusive and significant relationship ( $P<0.0001$ , Mann-Whitney test) in supercommunities 1 and 3

152 (with only two exceptions, R5.149 and R5.030) (**Figure S1F**). In contrast, the same analysis with community 2  
153 mAbs revealed no significant relationship between GIA and basigin-blocking as measured by BLI (**Figure S1G**).  
154 To measure relative GIA potency, mAbs were subsequently tested by GIA assay using a dilution series. Non-  
155 linear regression was fitted to the resultant log-transformed data to interpolate the effective concentrations  
156 needed to reach 30 % ( $EC_{30}$ ), 50 % ( $EC_{50}$ ) and 80 % ( $EC_{80}$ ) GIA (**Figure 1D, Data S1D**). The most potent mAbs  
157 were found in communities 1a, 2 and 3a, however, the spread of GIA potency within these differed. Notably,  
158 most members of community 1a had a similar potency, whilst community 2 showed a much wider distribution,  
159 ranging from multiple GIA-negative antibodies through to the most potent in the entire panel. Indeed, the  
160 most potent mAb in community 2, R5.034, showed an  $EC_{50}$  of 2.5  $\mu$ g/mL, 8.3-fold lower than the previously  
161 reported best-in-class (mAb R5.016, also the sentinel for community 2)<sup>24</sup>.

162

### 163 Antibody on-rate correlates with growth inhibitory activity

164 To further investigate contributing factors to GIA potency, the binding kinetics of all 206 mAbs (+7 sentinels) to  
165 full-length RH5.1 protein were determined using HT-SPR (**Data S2**). An iso-affinity plot of mAb association ( $K_{on}$ )  
166 and dissociation ( $K_{off}$ ) rates revealed a range of antibody affinity constants ( $K_D$ ) between 30 pM – 10 nM and an  
167 average  $K_D$  of approximately 1 nM (**Figure 2A**). All epitope communities spanned a similar range of affinity  
168 constants (**Figure S2A**). Fifty-eight mAbs (27%) had  $K_{off}$  rates too slow to reliably determine within the  
169 parameters of the assay ( $<6 \times 10^{-5} \text{ s}^{-1}$ ). R5.034 in community 2, the most potent mAb identified in the assay of  
170 GIA, had one of the fastest  $K_{on}$  values ( $1.69 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ) and had a dissociation rate constant too slow to be  
171 reported under the experimental conditions used. We also analysed the kinetic data according to the  
172 RH5.1/AS01<sub>B</sub> vaccine dosing regimen in the Phase 1/2a clinical trial. These mAbs were isolated from vaccinees  
173 after their final immunization, either given in a monthly dosing schedule, or following a delayed (4-5 month)  
174 final booster dose; we previously reported the latter groups showed higher anti-RH5.1 polyclonal IgG avidity  
175 by ELISA<sup>13</sup>. Notably, mAbs derived from vaccinees receiving the delayed booster doses had highly significantly  
176 different  $K_{off}$  and  $K_D$  values as compared to those receiving a monthly boost (**Figure S2B-D**), suggesting that  
177 delayed boosting in a human vaccination regimen can substantially impact the affinity of the resultant  
178 antibody response and this is largely driven by slower dissociation rates.

179

180 We next focused exclusively on community 2 and supercommunities 1 and 3 (together these contained nearly  
181 every growth inhibitory mAb in the entire panel) and assessed for correlation between the kinetic parameters  
182 and GIA EC<sub>50</sub> as a measure of functional potency. The  $K_{on}$  and  $K_D$  parameters were highly correlated with  
183 antibody potency, whereas no correlation was observed with  $K_{off}$  (Figure 2B-D). The correlation between  $K_{on}$   
184 and mAb GIA EC<sub>50</sub> was also highly significant and comparable across all three (super)communities (Figure 2E-  
185 G). In contrast, the correlation between  $K_D$  and mAb GIA EC<sub>50</sub> was weaker and only significant for  
186 (super)communities 1 and 2 (Figure 2H-J), and likely driven by the underlying correlation with  $K_{on}$ . Two obvious  
187 exceptions to this trend were observed – R5.129 in community 1a and R5.036 in community 2 (circled, Figure  
188 2E-F). These mAbs may bind to distal regions of their community's epitope footprint on PfRH5; indeed, neither  
189 of these block basigin (Figure 1B), and both display competition interactions outside of their  
190 (super)community (Figure 1A). Overall, these data suggest speed of PfRH5 binding is a major determinant of  
191 growth inhibitory mAb potency, regardless of epitope binding site (within or close to the basigin binding site  
192 on PfRH5), and that a slow rate of binding is likely sufficient to render an antibody ineffective.

193

#### 194 Sequence analysis of anti-PfRH5 mAbs reveals a potent public clonotype

195 To complement the high-resolution epitope and functionality mapping of anti-PfRH5 antibodies, we next  
196 conducted a sequence analysis of all 206 mAbs. The variable heavy and light chain gene segment usage of each  
197 mAb was annotated using IMGT V-quest (Data S3A). The range of somatic hypermutation (SHM) in the variable  
198 heavy chain was comparable across the epitope communities, with a trend towards greater SHM in  
199 communities 3a, 4c, 5b and 6, and the converse in communities 1b, 4a and 4b (Figure S3A). Levels of SHM also  
200 differed by dosing regimen in the RH5.1/AS01<sub>B</sub> clinical trial. Antibodies isolated from the delayed boosting  
201 groups showing significantly higher levels of SHM in their heavy and light chain gene segments as compared to  
202 the monthly boost group (Figure S3B-C); in line with these groups showing slower dissociation rates and  
203 improved affinity constants (Figure S2C-D). The median CDRH3 length of the N=206 mAbs was 14 amino acids,  
204 similar to the average length reported for the human IgG repertoire<sup>27</sup>. However, individual epitope  
205 communities diverged from this median, with supercommunity 1 and community 6 using marginally shorter  
206 median CDRH3 lengths (Figure S3D). Antibodies with exceptionally long CDRH3 sequences, >20 amino acids,

207 occurred in 8/12 communities. Communities 3a and 4b were noted for their bias towards these longer CDRH3  
208 sequences, and had median lengths of 21.5 and 21 amino acids, respectively.

209

210 Analysis of heavy and light chain gene family usage across the whole anti-PfRH5 panel (**Figure 3A, Data S3B**)  
211 revealed a diverse repertoire of N=5 heavy chain and N=10 light chain gene families, although with a notable  
212 predominance of HV3 (N=85), HV4 (N=86), KV1 (N=88), KV3 (N=47) and LV3 (N=42) gene family usage. The HV4  
213 gene family was used by most antibodies in community 1a (61/75, 81%), whereas the HV3 gene family was  
214 frequently used by community 2 (32/43, 74%), 3b (13/17, 76%) and 4a (10/10, 100%) antibodies. No single  
215 light chain gene family was used by more than 50 % of mAbs within a community, with the exception of LV3  
216 which was used by 9/10 and 3/3 community 4a and 4b mAbs, respectively. Across these gene families, N=27  
217 possible combinations of pairings were observed, with the HV4/KV3 pairing most frequently identified  
218 (N=38/206). Notably, some pairings were commonly associated with specific epitope communities – HV4/KV3  
219 and HV4/KV1 in community 1a and HV3/LV3 in community 4a (**Figure S3E**), otherwise other common gene  
220 family pairings were often present in antibodies from different epitope communities. Finally, we analysed the  
221 pairings of individual genes, which similarly revealed a diverse repertoire; here the highest frequency gene  
222 pairing included only 8 mAbs (HV4-39/KV3-11) which were all community 1a antibodies, whilst 97/206 mAbs  
223 used a unique gene pairing (**Figure 3B, Data S3B**).

224

225 To assess for association of antibody gene pairing with mAb GIA EC<sub>50</sub> potency, we initially analysed all gene  
226 pairs with N≥4 representative mAbs, which revealed a specific gene combination, HV3-7/LV1-36, of  
227 exceptional potency (**Figure 3C**). This same combination of heavy and light chain variable gene segment usage  
228 was also independently identified as predictive of high GIA by an unbiased computational modelling analysis of  
229 all available antibody gene sequence data across the mAb panel (**Figure S3F, Data S3C**). Notably all four of the  
230 HV3-7/LV1-36 mAbs were in community 2, were independently isolated from four separate vaccinees, had the  
231 same CDRH3 length (**Figure S3G**), and had GIA EC<sub>50</sub>s below 5 µg/mL up to 8-fold more potent than the previous  
232 best-in-class human mAb R5.016<sup>24</sup> (**Figure 3D**). This grouping encompassed 4/5 of the highest potency mAbs  
233 identified across the entire panel, including the most potent clone R5.034 (**Figure 1D**) along with R5.102,  
234 R5.237 and R5.270; the single exception in this high potency cluster was mAb R5.268 which utilized HV3-  
235 48/LV3-21. We further explored the contribution of gene sequence to potency by producing a panel of

236 germline revertants. Although SHM of the light chain gene contributed to GIA potency for some of the mAbs,  
237 all were highly dependent on their CDRH3s for mediating parasite growth inhibition (**Figure 3E**). The HV3-  
238 7/LV1-36 gene combination thus, in summary, defined a highly potent anti-PfRH5 public clonotype.

239

## 240 Structural definition of the PfRH5 antigenic landscape

241 We next mapped the epitope (super)communities onto the structure of PfRH5. Structural information existed  
242 for sentinel mAbs across most super(communities), however, supercommunity 3 was undefined. To address  
243 this, we obtained a 3.3 Å structure of R5.251 (community 3a) in complex with RH5ΔNL (**Table S1**). This clone  
244 was the most potent identified in supercommunity 3, with a GIA EC<sub>50</sub> of 29 µg/mL – similar to the previously  
245 reported best-in-class antibody R5.016 (community 2) and 3-fold more potent than the sentinel R5.008 (GIA  
246 EC<sub>50</sub> 90 µg/mL, community 3b)<sup>24</sup>. R5.251 bound around the tip of PfRH5 (**Figure 4A**). Analysis of the binding  
247 interface using PDBePISA predicted involvement of 18 residues on R5.251 (**Data S4**) primarily through the  
248 CDRH3, CDRL1 and CDRL3 loops, with no direct CDRH1 or CDRH2 involvement (**Figure 4B**). Docking of basigin  
249 to this structure showed steric clashes between basigin<sup>19</sup> and the light chain of R5.251 (**Figure 4C**), consistent  
250 with its ability to block basigin in the direct blocking assay (**Figure 1B**).

251

252 We next docked into this model the basigin ectodomain and transmembrane helix<sup>28</sup> and the other available  
253 structures of growth inhibitory anti-PfRH5 human antibodies – sentinel mAbs R5.004 and R5.016 from  
254 (super)communities 1 and 2, respectively<sup>24</sup>. We also supplemented these with the available structural data on  
255 two mouse mAb clones, QA1 and 9AD4<sup>19</sup>, which we placed into communities 1a and 2, respectively, using our  
256 new mAb panel (**Figure S4A**). These data revealed a “crown” of growth inhibitory mAbs, with varied footprints  
257 and binding angles, that decorate around the region of the basigin binding site on PfRH5 (**Figure 4D**). We next  
258 determined the interfacing residues of all anti-PfRH5 antibody clones on the surface of PfRH5<sup>29</sup> further  
259 supplementing the above analysis with the available structural data on sentinel mAbs R5.011 and R5.015 from  
260 supercommunities 4 and 5, respectively<sup>24,25</sup> (**Figure S4B**). This provided a complete map of all five epitope  
261 (super)communities as recognized by these Fabs on the alpha-helical diamond core of PfRH5 (**Figure 4E**).

262

263 Multiple intra-PfRH5 mAb interactions modulate parasite growth inhibition

264 Having characterized the functional, biophysical and structural properties of the mAb panel, we next sought to  
265 assess for functional interactions between the various epitope super(communities). We previously reported  
266 that a non-inhibitory antibody (R5.011, community 4b) was able to potentiate or synergize with the growth  
267 inhibitory sentinel antibodies R5.004, R5.016 and R5.008 (communities 1a, 2 and 3b, respectively) in the GIA  
268 assay<sup>24</sup>. We thus systematically screened for this phenotype of functional intra-PfRH5 antibody interaction on  
269 a large scale and also sought to assess whether the high GIA EC<sub>50</sub> potency of the HV3-7/LV1-36 public  
270 clonotype could be outperformed via a combination of anti-PfRH5 mAbs. Initially we devised a screening  
271 strategy to test growth inhibitory or “neutralizing” antibodies (nAbs) in combination with non-neutralizing  
272 antibodies (non-nAbs). Nine representative nAbs (of high potency wherever possible and spanning  
273 communities 1a, 1b, 2, 3a and 3b and the HV3-7/LV1-36 public clonotype) were selected; all blocked basigin in  
274 the BLI assay, with the exception of R5.034 and R5.102 in community 2. These were subsequently screened for  
275 GIA in pair-wise combinations with a further 23 non-nAbs that spanned all applicable epitope communities,  
276 i.e., those with at least one non-nAb (1a, 1c, 2, 3b, 5a, 5b and 6) and also all clones in supercommunity 4  
277 (which includes R5.011). All nAbs were tested in the GIA assay at their EC<sub>50</sub> concentration, with and without  
278 addition of each non-nAb held at 0.3 mg/mL (**Figure S5A**). The predicted Bliss additivity GIA value<sup>30,31</sup>,  
279 calculated from the % GIA of the nAb and non-nAb tested alone, was subtracted from the % GIA of the test  
280 combination, with thresholds defined to categorize pairings as synergistic, additive, or antagonistic (**Figure 5A**).  
281

282 Non-nAbs from communities 1a, 1c, 2, 5a, 5b and 6 showed no obvious interactions with any nAb in the test  
283 panel, neither reducing nor potentiating the overall level of GIA. In contrast, the non-nAbs from community 4b  
284 (including R5.011) were consistently synergistic with mAbs from supercommunities 1 and 3, but did not affect  
285 the GIA of nAbs from community 2. Interestingly non-nAbs that comprise community 4a showed the same  
286 potentiating effect with nAbs from communities 1a, 1b and 3a. However, they were antagonistic with nAb  
287 clones from community 2 (despite no competitive binding for PfRH5) and from community 3b, although this  
288 was likely due to some competitive binding. Community 4c showed a less consistent profile, although overall  
289 these clones were able to synergize with some nAbs from each community, albeit weakly. Notably, the  
290 representative non-nAb from community 3b (R5.028) could synergize with nAbs from communities 1a, 1b and

291 2; whilst exhibiting antagonism with the two nAb clones from supercommunity 3 (R5.021 and R5.251) with  
292 which it competes for PfRH5 binding. Further testing using a range of concentrations of representative nAbs  
293 from communities 1a (R5.077), 2 (R5.034, R5.268) and 3a (R5.251) in combination with a fixed concentration  
294 of representative modulatory non-nAbs from communities 3b, 4a, 4b and 4c, confirmed these results (**Figure**  
295 **S5B-C**).

296

297 Having shown a range of non-nAb specificities could modulate the GIA of nAb clones, we also assessed  
298 whether the potentiating non-nAb clones from epitope communities 3b, 4a, 4b and 4c could synergize with  
299 antibody clones that exhibit very low or no GIA. Here we selected clones from communities 1a, 1b, 2 and 3b  
300 that originally showed poor GIA assay EC<sub>50</sub> outcome (**Figure 1D**) in contrast to the majority of other nAbs in  
301 each of these communities. In this case, although a number of antibody combinations continued to show  
302 minimal or no GIA, over half of the pairs of clones tested now showed improved GIA and these spanned across  
303 the range of epitope communities (**Figure 5B**). Moreover, the non-nAbs from community 3b also showed  
304 synergy with the clones from community 4b, including the potentiating clone R5.028. Further analysis of the  
305 combination of R5.028 (community 3b) and R5.246 (community 4b) showed how these two GIA-negative and  
306 non-basigin blocking mAbs could combine to give high-level synergy resulting in growth inhibition of parasite  
307 invasion (EC<sub>50</sub> 105 µg/mL) when tested in a 1:1 mixture (**Figure 5C**).

308

309 Having defined non-nAbs from two non-competing epitope communities (3b and 4b) that both potentiate  
310 nAbs and each other, we next tested them in triple combination with the best nAb from community 1a  
311 (R5.077) and the most potent representative of the public clonotype from community 2 (R5.034). In the case  
312 of R5.077, a 45-fold increase in EC<sub>80</sub> potency was observed under the GIA assay test conditions when both  
313 R5.028 and R5.246 were added (1.9 µg/mL versus 83.4 µg/mL for predicted additivity). This was far greater  
314 than that observed when combining with R5.028 (2.6 fold-change) or R5.246 (6.9 fold-change) alone (**Figure**  
315 **5D**), suggesting that the synergistic effect of either or both antibody clones is enhanced in the presence of the  
316 other. A similar observation was seen with R5.034, despite the fact that mAbs from community 2 do not  
317 synergize with R5.246 alone. Here, the combination of R5.028 and R5.246 with R5.034 produced a greater fold  
318 change in EC<sub>80</sub> (3.9-fold) than the predicted additive effect of the triple combination under the GIA assay test  
319 conditions (**Figure 5E**). Finally, we also replicated this phenomenon with a pool of polyclonal anti-PfRH5 IgG

320 from the RH5.1/AS01<sub>B</sub> vaccine trial (**Figure S5D**), suggesting there remains substantial room for improvement  
321 in terms of the qualitative potency of the vaccine-induced antibody response. However, notably, most of these  
322 previous experiments involved testing a titrated concentration of one test antibody with another held at fixed  
323 concentration. We thus finally sought to identify the most potent anti-PfRH5 mAb or combination on a per µg  
324 basis for assessment as a novel blood-stage anti-malarial intervention. A series of single mAbs and  
325 combinations were down-selected based on the previous analysis of synergistic interactions. Each antibody  
326 mixture was compared head-to-head in a titration analysis in the assay of GIA against 3D7 clone *P. falciparum*  
327 parasites. Although some of these combinations could almost match R5.034 in potency, none could ultimately  
328 outperform the EC<sub>50</sub> of the single most potent clone R5.034 from community 2 (**Figure S5E-G**).

329

330 The public clonotype antibody R5.034 protects against *P. falciparum*  
331 sporozoite challenge

332 Given the R5.034 public clonotype mAb demonstrated the most potent GIA EC<sub>50</sub> across all of our analyses, we  
333 investigated the merits of this clone as a prophylactic intervention against *P. falciparum*. To better understand  
334 the structural and binding characteristics of this candidate, we obtained a crystal structure of R5.034 (resolved  
335 to show its Fv region only) in complex with RH5ΔNL to 4 Å (**Figure 6A, Table S1**). Analysis of the binding  
336 interface using PDBePISA showed that R5.034 bound an upper facet, close to the tip of PfRH5. Within this  
337 interface, only 5/13 residues used by the heavy and light chains of R5.034 (excluding those in the CDRH3) were  
338 mutated from germline HV3-7/LV1-36 sequence (**Data S4**), in line with this mAb's tolerance of germline  
339 reversion mutation outside the CDRH3 (**Figure 3E**). In the case of PfRH5, the binding interface was centred on a  
340 3-helical bundle of the PfRH5-fold, with the CDRH3 loop projecting towards a cleft created by the outermost  
341 two α-helices. The interfacing area on PfRH5 spanned 19 amino acids (**Data S4**) and contained none of the few  
342 commonly observed polymorphisms<sup>32</sup>, suggesting that the epitope of R5.034 is conserved. Comparison of  
343 R5.034 with the other structurally characterized mAbs from community 2 (R5.016 and 9AD4) demonstrated  
344 that R5.034 shared a similar overlapping epitope as predicted by the competition data. All three antibodies  
345 bound close to the basigin binding site, with their Fab constant regions (modelled for R5.034) projecting into  
346 the space predicted to be occupied by the erythrocyte membrane (**Figure 6B**).

347 We subsequently generated a second version of this antibody with the “LS” mutation in the IgG1 Fc domain  
348 (R5.034LS) used in the clinical development of candidates mAbs to extend serum half-life via increased  
349 antibody recirculation<sup>33</sup>. R5.034 and R5.034LS demonstrated comparable binding kinetic rate constants and  
350 high affinities for PfRH5 binding of 30-40 pM (**Figure S6A**). To further assess the LS half-life extension  
351 mutation, we determined binding to the human neonatal Fc receptor (FcRn). Here, as expected, neither  
352 R5.034 or R5.034LS bound to FcRn at pH7.4 (**Figure S6B**), but at lower pH6.0 (to mirror endosomal recycling of  
353 plasma IgG), R5.034LS showed an ~7-fold higher affinity for FcRn as compared to the wild-type R5.034 IgG1  
354 molecule (**Figure 6C, S6B**). Further screening in the GIA assay confirmed both R5.034 and R5.034LS exhibited  
355 identical functional potencies against *P. falciparum* *in vitro* (**Figure 6D**). Finally, we assessed protective efficacy  
356 of R5.034 passive transfer prior to *P. falciparum* mosquito-bite challenge in a humanized mouse model (**Figure**  
357 **6E**). Control animals developed very high-level blood-stage parasitemia following parasite emergence from the  
358 liver, starting from a median level of  $3.2 \times 10^5$  parasites per mL of blood (p/mL) on day 7 and peaking on day 9 at  
359  $7.9 \times 10^5$  p/mL. This declined over time, but all animals remained parasitemic on day 13 when the experiment  
360 was ended. In contrast, animals receiving R5.034 peaked on day 7 at a median level of  $2.5 \times 10^3$  p/mL (100-fold  
361 lower than controls) with this difference widening by day 9, whereby all animals showed decreased parasite  
362 burden to a median level of  $1.4 \times 10^2$  p/mL (5600-fold lower than controls). All animals receiving R5.034 were  
363 subsequently parasite negative, as measured by qRT-PCR, on day 11. Serum antibody levels in the R5.034-  
364 treated animals reached a median of 93 µg/mL (range: 48-98 µg/mL) on day 6 and were maintained at 83  
365 µg/mL (range: 30-122 µg/mL) on day 13 (**Figure S6C**). At these antibody levels, R5.034 thus showed high-level  
366 efficacy against blood-stage *P. falciparum*.

## 367 DISCUSSION

368 Here we provide the first high-resolution analysis of the antigenic landscape of the most advanced blood-stage  
369 malaria vaccine candidate antigen, PfRH5, and characterize in detail the biophysical, structural and  
370 combinatorial features of the human IgG antibody response that associate with functional blood-stage growth  
371 inhibition of *P. falciparum*. In doing so, we also identified an anti-PfRH5 public clonotype that shows the  
372 highest levels of *in vitro* growth inhibition reported to-date and confirmed its prophylactic ability to control  
373 and clear high-level blood-stage *P. falciparum* parasitemia in humanized mice. To perform our analyses, we  
374 isolated and characterized over 200 anti-PfRH5 mAbs from healthy UK adults immunized with the full-length  
375 protein-in-adjuvant vaccine RH5.1/AS01<sub>B</sub><sup>13,16</sup>; the RH5.1 vaccine has since entered Phase 2b field efficacy  
376 testing in infants in Burkina Faso reformulated in Matrix-M™ adjuvant (ClinicalTrials.gov NCT05790889).  
377 Previous analyses of RH5.1/AS01<sub>B</sub> vaccinee sera have shown the disordered N- and C-termini of the PfRH5  
378 molecule do not contribute growth-inhibitory antibodies, in line with recent data showing that N-terminal  
379 cleavage of PfRH5 occurs in the micronemes prior to movement of PfRH5 to the merozoite's surface<sup>34</sup>. We  
380 therefore focussed our analysis on the structured core of the PfRH5 molecule ("RH5ΔNC"; amino acids K140-  
381 N506)<sup>19</sup>, where we determined antigenic sites in line with the network of competitive binding interactions  
382 across the mAb panel. We thus defined 12 communities of human antibodies that bind PfRH5, and 10 of these  
383 clustered into four supercommunities.

384

385 Over 95 % of the mAb panel recognized conformational epitopes; of the few that could bind denatured RH5.1  
386 protein, most bound to linear peptide epitopes within the IDL region (amino acids N248-M296) and we  
387 grouped these mAbs into community 6. We could not identify any functional outcomes for these antibodies;  
388 they neither mediated parasite growth inhibition nor showed any detectable interaction with PfRH5 binding  
389 partners or other anti-PfRH5 mAbs. Serological analyses of the anti-PfRH5 polyclonal IgG response in human  
390 vaccinees suggest responses against the IDL are common<sup>17</sup>; these data would suggest this antigenic region  
391 should be removed in a next-generation PfRH5 vaccine immunogen aiming to improve the qualitative potency  
392 of vaccine-induced antibody responses.

393

394 PfRH5 is delivered to the merozoite surface at the end of an elongated hetero-pentameric invasion complex,  
395 within which it forms an interaction with PfCyRPA<sup>9,20</sup>. Antibodies capable of blocking the PfRH5-PfCyRPA  
396 interaction were exclusively contained within supercommunity 5. Conflicting reports in the literature, using  
397 individual or very small panels of antibodies<sup>23-25,35</sup>, have debated whether blockade of the PfRH5-PfCyRPA  
398 interaction has functional consequence for inhibition of parasite invasion. Here, like for community 6  
399 antibodies, we could ascribe no other functional outcomes to these clones, neither with regard to *in vitro*  
400 parasite growth inhibition nor modulatory interactions with anti-PfRH5 mAbs from other communities. Our  
401 analysis of ~30 mAbs thus strongly suggests that blockade of this interaction cannot block parasite invasion of  
402 the RBC, and that the antigenic surface of PfRH5 ascribed to supercommunity 5 is covered by PfCyRPA and  
403 therefore buried within the invasion complex at the point of exposure to the host immune system.

404

405 Notably, our data show growth inhibitory anti-PfRH5 antibodies span five epitope communities: 1a, 1b, 2, 3a  
406 and 3b. Although structural data existed for exemplar antibodies spanning communities 1a, 1b and 2<sup>19,24</sup>,  
407 there were none from supercommunity 3. We therefore resolved the structure of mAb R5.251, the best-in-  
408 class from this supercommunity. Mapping of these structural data onto the  $\alpha$ -helical core of PfRH5 shows the  
409 footprints of these epitope communities form a “crown” on the top half of the diamond-like architecture,  
410 overlapping with or adjacent to the basigin-bind site. Communities 1a, 1b and 3a were composed of mAbs that  
411 i) blocked basigin-binding as assessed by BLI, and ii) with only one exception showed functional GIA. Combined  
412 these accounted for almost half of the antibodies analysed in this study. In contrast, community 2 antibodies  
413 (the second largest cluster in the panel after community 1a) bind close or adjacent to the PfRH5-basigin  
414 interaction site, explaining why ~70% of these mAbs failed to block basigin binding as measured here by BLI.  
415 Notably, the range of GIA potency in this community was the largest (ranging from GIA negative to the most  
416 potent clones identified) and GIA positivity did not associate with measurement of direct basigin-binding  
417 blockade. Other data now suggest basigin is present within macromolecular complexes in the RBC membrane,  
418 and that community 2 antibodies may in fact inhibit growth by sterically blocking the interaction of PfRH5 with  
419 basigin in its physiological context<sup>26</sup>. Whether this kind of blocking assay could better predict GIA positivity  
420 across a large panel of community 2 mAb clones remains to be determined. In contrast, a key finding from our  
421 analysis was the highly significant correlation between mAb potency in the GIA assay and the association rate  
422 ( $K_{on}$ ) of PfRH5 binding but not the dissociation rate ( $K_{off}$ ), in line with the theorized kinetic constraints of an

423 anti-merozoite vaccine<sup>36</sup>. Notably, this relationship transcended (super)communities 1, 2 and 3, suggesting  
424 the speed with which an antibody engages PfRH5 at any of these three major antigenic sites is a central driver  
425 of its growth inhibitory potency against the rapidly invading *P. falciparum* merozoite. Indeed, this analysis  
426 builds on previous data reported for small panels of mAbs targeting both *P. falciparum* and *P. vivax* merozoites  
427<sup>21,24,37</sup>, and now strongly suggests that combining these antibodies with other anti-malarial antibodies or drugs  
428 that slow merozoite invasion could offer novel strategies to improve functional potency of anti-PfRH5 IgG.

429

430 The community 3b epitope region is next to the site of supercommunity 4, which itself descends down one  
431 side of the PfRH5 diamond-like structure, on the opposite side to the PfCyRPA-binding site recognized by mAbs  
432 in supercommunity 5. Over half of the community 3b mAbs blocked basigin-binding as assessed by BLI and  
433 showed a similar range of GIA potencies to community 1b, whilst the remaining clones in community 3b and all  
434 of those in supercommunity 4 were otherwise GIA negative. However, we previously reported mAb R5.011  
435 (the sentinel clone for community 4b) was able to potentiate or synergize with growth inhibitory antibodies in  
436 the GIA assay, via a 3-fold lengthening of *P. falciparum* merozoite invasion time<sup>24</sup>. Our analysis with this much  
437 larger panel of mAbs now defines this phenomenon in far greater resolution, indicating that multiple intra-  
438 PfRH5 mAb interactions can modulate anti-parasitic growth inhibition. We found one main example of  
439 consistent antagonism in the GIA assay between antibodies from community 4a and those from community 2;  
440 this warrants further investigation given the apparent physical separation of their binding sites on PfRH5 and  
441 lack of competitive binding. Notably, otherwise, our data show the antigenic sites spanning community 3b and  
442 all of supercommunity 4 can reproducibly elicit human antibody clones with the potentiating phenotype. Here,  
443 when combined, these GIA-negative clones synergize with or potentiate the basigin-blocking and growth  
444 inhibitory antibodies from communities 1a, 1b, 3a and 3b. Moreover, we provide the first evidence that two  
445 non-neutralizing and non-basigin-blocking anti-PfRH5 mAbs can combine to give high-level synergy resulting in  
446 GIA, as exemplified with the combination of clones from communities 3b and 4b. These data have important  
447 implications for how intra-PfRH5 antibody interactions are likely occurring within polyclonal anti-PfRH5 IgG to  
448 inhibit growth of *P. falciparum* parasites, and indeed offer a possible explanation for the impressive qualitative  
449 potency (i.e., low GIA EC<sub>50</sub>) of vaccine-induced PfRH5-specific antibody reported across the Phase 1 vaccine  
450 trials<sup>18</sup>. Further studies are now underway to investigate the composition of the plasma anti-PfRH5 polyclonal  
451 IgG response in human vaccinees with respect to these phenomena.

452

453 These anti-PfRH5 antibody data also provide the high-resolution insight required to guide the rational design  
454 of next-generation PfRH5 vaccine immunogens that seek to induce responses that are qualitatively superior to  
455 the current clinical vaccine RH5.1. Indeed these results suggest focusing on immunogen designs that  
456 incorporate (super)communities 1-3, 4b and 4c with removal or masking of antagonizing community 4a and  
457 non-functional (super)communities 5-6. Importantly, antibodies recognizing these functional epitope sites can  
458 arise from a diverse range of human antibody gene usage, however, human vaccine delivery technologies,  
459 adjuvants and/or regimens that can also drive improved  $K_{on}$  rates need to be investigated. Previous serological  
460 data from the RH5.1/AS01<sub>B</sub> vaccine trial suggested that delayed, as opposed to monthly, vaccine boosting in  
461 humans could improve memory B cell responses as well as serum antibody response longevity and avidity;  
462 however, the purified total IgG with more avid anti-PfRH5 IgG failed to show improved GIA potency<sup>13,38</sup>. Our  
463 data now explain this result, because although the antibody clones isolated from the delayed booster  
464 vaccinees showed more SHM and higher RH5.1 binding affinity ( $K_D$ ), the underlying driver for this was  
465 significantly slower dissociation rates, as opposed to faster association rates, which we would not predict to  
466 improve GIA.

467

468 Finally, our data identified that an exceptionally potent class of anti-PfRH5 antibody can derive from the  
469 antibody gene combination HV3-7/LV1-36. This public clonotype identified a small subset of antibodies within  
470 community 2, all with GIA EC<sub>50</sub>s below 5 µg/mL against 3D7 clone *P. falciparum*. Structural and mutational  
471 analyses of the most potent clone, R5.034, showed PfRH5 binding at this conserved epitope is driven by its  
472 CDRH3. Moreover, passive transfer of R5.034 into humanized mice challenged with *P. falciparum* sporozoites  
473 showed reductions in blood-stage parasitemia, as compared to controls, in the range of 2-3 orders of  
474 magnitude following parasite emergence from the liver, and then absence of detectable parasites by qRT-PCR  
475 within 4 days whilst all controls remained parasitemic. Given the recent exciting clinical advances with the L9LS  
476 and TB31F candidate mAbs against the sporozoite- and transmission-stages of *P. falciparum*, respectively<sup>6,39</sup>,  
477 the data here identify a new blood-stage mAb candidate, R5.034LS, that could be considered for clinical  
478 development as part of a multi-stage multi-mAb approach to achieve high-level single-shot prophylaxis against  
479 *P. falciparum* malaria.

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489

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504

## 505 AUTHOR CONTRIBUTIONS

506 Conceived and performed experiments and/or analysed the data: JRB, DP, NDW, AJRC, GG, DQ, AML, HD, CR,  
507 MA, BGW, WJB, NGP, TM, PK, LP, CDW, FRD, LDWK, LTW, JFP, SES, JdRS, BKW, LK, JT, CMN, KMc, SJD.  
508 Performed project management: KS, VK, ARN, RSM, CRK, AJB, LAS.  
509 Contributed reagents, materials, and analysis tools: AJRC, AMM, DAL, KMi, CAL, JT.  
510 Wrote the paper: JRB, KMc, SJD.

511

## 512 DECLARATION OF INTERESTS

513 • JRB, AJRC, GG, BGW, LDWK, LTW, JT, KMc and SJD are inventors on patent applications relating to RH5  
514 malaria vaccines and/or antibodies.  
515 • AMM and SJD have consulted to GSK on malaria vaccines.  
516 • AMM has an immediate family member who is an inventor on patent applications relating to RH5 malaria  
517 vaccines and antibodies.  
518 • All other authors have declared that no conflict of interest exists.

519

## 520 INCLUSION AND DIVERSITY

521 We support inclusive, diverse, and equitable conduct of research.

## 522 METHODS

### 523 CONTACT FOR REAGENT AND RESOURCE SHARING

524 Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact,  
525 Simon J. Draper ([simon.draper@bioch.ox.ac.uk](mailto:simon.draper@bioch.ox.ac.uk)).

526

### 527 Data and Code Availability

528

- 529 Requests for monoclonal antibodies (mAbs) generated in the study should be directed to the Lead  
Contact, Simon J. Draper ([simon.draper@bioch.ox.ac.uk](mailto:simon.draper@bioch.ox.ac.uk)).
- 530 Crystal structures of RH5ΔNL bound to R5.034 and R5.251 are deposited into the Protein Data Bank  
(PDB) under ID codes: 8QKS and 8QKR, respectively.
- 532 The R markdown file for the prediction of GIA from germline gene usage (**Figure S3F**) has been  
533 included as a supplemental file (see **Data S3C**).

534

## 535 EXPERIMENTAL MODEL AND SUBJECT DETAILS

536

### 537 Human Blood Sample Collection

538 All mAbs were obtained from samples of adult volunteers immunized with the RH5.1/AS01<sub>B</sub> vaccine as part of  
539 the VAC063 clinical trial <sup>13</sup>. VAC063 was an open-label, multi-center, dose-finding Phase I/IIa trial, including a  
540 controlled human malaria infection (CHMI) component, to assess the safety, immunogenicity and efficacy of  
541 the candidate *P. falciparum* blood-stage malaria vaccine RH5.1/AS01<sub>B</sub>. Volunteers were healthy, malaria-naïve  
542 UK adults ranging from 18-45 years of age. The study was conducted in the UK at the Centre for Clinical  
543 Vaccinology and Tropical Medicine, University of Oxford, Oxford, Guys and St Thomas' NIHR CRF, London and  
544 the NIHR Wellcome Trust Clinical Research Facility in Southampton. The study received ethical approval from  
545 the UK NHS Research Ethics Service (Oxfordshire Research Ethics Committee A, Ref 16/SC/0345), and was  
546 approved by the UK Medicines and Healthcare products Regulatory Agency (Ref 21584/0362/001-0011).  
547 Volunteers signed written consent forms and consent was verified before each vaccination. The trial was  
548 registered on ClinicalTrials.gov (NCT02927145) and was conducted according to the principles of the current  
549 revision of the Declaration of Helsinki 2008 and in full conformity with the ICH guidelines for Good Clinical  
550 Practice (GCP).

551

552 Donations of human RBC from healthy adult volunteers for use in assays received ethical approval from the UK  
553 NHS Research Ethics Service (London – City & East Research Ethics Committee, Ref 18/LO/0415).

554

555 The RH5.1 vaccine was based on the full-length PfRH5 antigen (amino acids E26 - Q526) with 3D7 clone *P.*  
556 *falciparum* sequence, as reported in detail elsewhere <sup>16</sup>. In brief, the vaccine was manufactured as a secreted

557 soluble product from a stable *Drosophila melanogaster* Schneider 2 (S2) cell line and affinity purified via a C-  
558 terminal four amino acid (E-P-E-A) “C-tag” <sup>42</sup>. All four putative N-linked glycosylation sequons (N-X-S/T) were  
559 mutated Thr to Ala. Volunteer samples from VAC063 Groups 1, 2, 3, 5 and 7 were used in this study <sup>13</sup>.  
560 Participants in Groups 1, 2 and 5 received three “monthly” vaccinations of RH5.1/AS01<sub>B</sub> at days 0, 28 and 56,  
561 with a dose escalation of RH5.1 from 2 µg (Group 1), to 10 µg (Groups 2 and 5). Volunteers in Group 3 received  
562 two 50 µg doses of RH5.1 followed by a final dose of 10 µg RH5.1 given at day 182 (a “delayed fractional  
563 dosing” (DFx) regimen). All vaccines were formulated in 0.5 mL AS01<sub>B</sub> adjuvant (GSK) regardless of RH5.1  
564 protein dose. Group 5 underwent blood-stage CHMI with 3D7 clone *P. falciparum* 14 days after their third  
565 vaccination. Group 7 was composed of a subset of Group 5 vaccinees who went on to receive a final, delayed  
566 and fourth booster vaccination (D4thB) with 10 µg RH5.1/AS01<sub>B</sub> approximately four months after their third  
567 vaccination followed by a second round of CHMI. PBMC samples were isolated and cryopreserved at  
568 approximately 2-4 weeks post-final vaccination for each group. Human blood samples were collected into  
569 lithium heparin-treated vacutainer blood collection systems (Becton Dickinson). PBMC were isolated and used  
570 within 6 h in fresh assays, otherwise excess cells were frozen in fetal calf serum (FCS) containing 10 % dimethyl  
571 sulfoxide and stored in liquid nitrogen. Plasma samples were stored at -80 °C. For serum preparation,  
572 untreated blood samples were stored at room temperature (RT) and then the clotted blood was centrifuged  
573 for 5 min at 1800 rpm. Serum was stored at -80°C.  
574

## 575 Experimental Animal Models

576 The study using liver-humanized mice was carried out at the Oregon Health and Sciences University (OHSU)  
577 which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care  
578 International (AAALACi) and is a Category I facility with an approved Assurance (#A3304-01) on file with the  
579 Office for Laboratory Animal Welfare (OLAW), NIH, USA. The protocol was approved by the OHSU Institutional  
580 Animal Care and Use Committee (IACUC) under protocol IP00002077. FRG huHep mice on the NOD  
581 background were purchased from Yecuris, Inc. (Beaverton, OR, USA).  
582

## 583 Cell Lines

584 Expi293F HEK cells were cultured in suspension in Expi293 expression medium (Thermo Fisher Scientific) at  
585 37°C, 8 % CO<sub>2</sub>, on an orbital shaker set at 125 RPM. *Drosophila* S2 cells were cultured in suspension in EX-CELL  
586 420 medium (Sigma-Aldrich) supplemented with 100 U/mL penicillin, 0.1 mg/mL streptomycin and 10 % foetal  
587 bovine serum (FBS) at 25 °C. Stable S2 cell lines expressing PfRH5 proteins were generated using the ExpreS<sup>2</sup>  
588 platform (ExpreS<sup>2</sup>ion Biotechnologies) as previously described <sup>16,43</sup>.  
589

## 590 METHOD DETAILS

### 591 Isolation of PfRH5-specific B cells

592 PfRH5-specific B cells were single cell sorted from the majority of cryopreserved PBMC samples as previously  
593 described <sup>38</sup> but with minor modification as follows. In brief, samples were thawed into R10 media (RPMI

594 [R0883, MilliporeSigma] supplemented with 10 % heat-inactivated FCS [60923, Biosera], 100 U/mL penicillin /  
595 0.1 mg/mL streptomycin [P0781, MilliporeSigma], 2 mM L-glutamine [G7513, Millipore Sigma]) and were then  
596 washed and rested in R10 for 1 h. B cells were enriched (Human Pan-B cell Enrichment Kit [19554, StemCell  
597 Technologies]) and then stained with viability dye FVS780 (565388, BD Biosciences). Next, B cells were stained  
598 with anti-human CD19-BV786 (Clone: SJ25C1, 563325, BD Biosciences) and anti-human IgG-BB515 (Clone: G18-  
599 145, 564581, BD Biosciences), as well as two fluorophore-conjugated PfRH5 probes. To prepare the probes,  
600 monobiotinylated PfRH5 was produced by transient co-transfection of HEK293F cells with a plasmid encoding  
601 BirA biotin ligase and a plasmid encoding a modified PfRH5. The PfRH5 plasmid was based on 'RH5-bio' (a gift  
602 from Gavin Wright; University of York, York, United Kingdom; Addgene plasmid #47780)<sup>44</sup>. RH5-bio was  
603 modified prior to transfection to incorporate a C-terminal C-tag for subsequent protein purification, as well as  
604 a 15 amino acid deletion to remove the disordered C-terminus of PfRH5 and a 115 amino acid deletion from  
605 the linear N-terminus to produce a protein known as "RH5 $\Delta$ NC"<sup>38,45</sup>. Probes were freshly prepared for each  
606 experiment by incubation of mono-biotinylated RH5 $\Delta$ NC with streptavidin-PE (S866, Invitrogen) or  
607 streptavidin-APC (405207, eBioscience) at an approximately 4:1 molar ratio to facilitate tetramer generation  
608 and subsequent centrifugation to remove aggregates (13,000–14,000 rpm [max microcentrifuge speed] at RT  
609 for 10 min). Following surface staining, cells were washed and kept on ice until acquisition on the MoFlo (Dako  
610 cytometry). RH5 $\Delta$ NC-specific B cells were identified as live CD19+ IgG+ RH5 $\Delta$ NC-APC+ RH5 $\Delta$ NC-PE+ cells and  
611 single cell sorted into 96-well plates containing 10 $\mu$ L/well lysis buffer (10mM Tris [T3038, Merck], 1 unit/mL  
612 RNasin Ribonuclease Inhibitor [N2515, Promega]) and frozen at -80 °C.

613

614 A subset of eight mAbs (R5.242, R5.243, R5.244, R5.246, R5.247, R5.248, R5.249 and R5.250) were derived  
615 from a cell sort that included an additional cell hashing step (TotalSeq-C0251/C0252/C0253/C0254/C0255 anti-  
616 human Hashtag 1/2/3/4/5 antibodies, cat # 394661/394663/394665/394667/394669, Biolegend) prior to B cell  
617 enrichment and staining. These hashed samples were then either single-cell sorted into 96-well plates (as  
618 above), or pooled for B cell receptor (BCR) sequencing analysis using the 10X chromium platform; performed  
619 at the Oxford Genomics Center, University of Oxford, UK.

620

## 621 Monoclonal Antibody Cloning

622 Reverse transcription and nested PCR of antibody heavy (VH) and light (VL) chains was carried out as  
623 previously described and using previously reported primers<sup>24,46</sup>. PCR products were purified using a PCR  
624 purification kit (Qiagen, 28006) and then cloned into the AbVec expression plasmids to produce recombinant  
625 human IgG1 mAbs (a gift from Patrick C. Wilson, University of Chicago, USA). In brief, plasmids and PCR  
626 products were 5' digested using BshT1 and 3'digested using Sall (AbVec-HC IgG1), Xhol (AbVec-LLC) and Pfl23II  
627 (AbVec-KLC) before ligation using QuickLigase (NEB). Ligation products were then used to transform  
628 MultiShot™ StripWell TOP10 chemically competent *Escherichia coli* (Life Technologies) following the  
629 manufacturer's instructions. Transformed cells were plated on LB agar 8-well Petri plates (Teknova) containing  
630 100  $\mu$ g/mL carbenicillin and grown at 37 °C overnight in a static incubator. Colonies were picked and sent to  
631 Source BioScience for sequencing, and those with productive antibody VH and VL sequences (analysed with

632 Geneious® software) were inoculated and plasmids extracted using a QIAgen MINIprep kit. Sequence analysis  
633 was carried out using Geneious Prime®. Germline identity and gene usage parameters were determined using  
634 IMGT/V-QUEST. Heavy chain CDR3 (CDRH3) lengths were identified using IMGT numbering. Somatic  
635 hypermutation (SHM) % was calculated from the outputted identity % for each V, D and J region from IMGT, by  
636 subtracting the value from 100 %.

637

638 To clone R5.034LS, the R5.034 IgG1 heavy chain constant region was replaced with a gene string encoding the  
639 same human IgG1 constant heavy region containing the two LS mutations (M451L/N457S)<sup>33,47</sup> via the Sall and  
640 HindIII restriction sites. Recombinant R5.034 was then produced as previously described.

641

## 642 Expression and Purification of Antibodies

643 Transfections of HEK293F cells using a 1:1 ratio of HC and light chain (LC) plasmids were set up in 10 mL culture  
644 volumes in 50 mL vented cap reaction tubes using Expifectamine™ transfection kit (Life Technologies) as per  
645 the manufacturer's instructions. IgG purification was carried out using Econo-PAC® chromatography columns  
646 (Bio-Rad) and Protein A Sepharose (Sigma-Aldrich), and purified mAbs were buffer-exchanged into phosphate  
647 buffered saline (PBS).

648

## 649 Expression and Purification of Proteins

650 Unless stated otherwise, all PfRH5 and PfCyRPA soluble proteins and reagents were designed based on the  
651 3D7 clone *P. falciparum* reference sequence with all the N-glycan sequons (N-X-S/T) mutated from a serine or  
652 threonine to alanine.

653

654 RH5.1 and RH5ΔNL: The design, production and purification of RH5.1 and RH5ΔNL have previously been  
655 described<sup>16,19,24</sup>. In brief, stable *Drosophila* S2 polyclonal cell lines expressing full-length RH5.1 (residues E26-  
656 Q526) or RH5ΔNL (residues K140-K247 and N297-Q526 of PfRH5 with 3D7 or 7G8 *P. falciparum* sequence,  
657 differing only by the C203Y polymorphism) were cultured in EX-CELL 420 media and expanded in shake flasks  
658 to the desired scale. Cell supernatant was harvested and loaded onto a 10 mL column packed with  
659 CaptureSelect affinity C-Tag XL resin, washed in 10 column volumes of TBS pH 7.4 and eluted in 2M MgCl<sub>2</sub>.  
660 Fractions were then pooled, concentrated using a 10 kDa Amicon ultra centrifugal filter and run on an HiLoad  
661 16/600 Superdex 200 pg size exclusion chromatography (SEC) column into TBS pH 7.4 (20 mM TRIS-HCl, 150  
662 mM NaCl).

663

664 RH5ΔNC-Biotin: The production of the RH5ΔNC-Biotin has been described<sup>38</sup>. Mono-biotinylated RH5ΔNC-  
665 Biotin, previously referred to as 'RH5-Bio', was generated through co-transfection of Expi293 cells with a  
666 plasmid encoding RH5ΔNC-Biotin and another plasmid encoding BirA biotin ligase. RH5ΔNC-Biotin was then  
667 purified from the supernatant by C-tag affinity chromatography follow by SEC into TBS pH 7.4 (20 mM TRIS-  
668 HCl, 150 mM NaCl).

669  
670 PfCyRPA: Full-length PfCyRPA (residues 29-362) was expressed in Expi293 cells and purified through C-tag  
671 affinity chromatography follow by SEC into TBS pH 7.4 as previously described<sup>25,48</sup>.  
672  
673 Basigin: Native human basigin sequence, encoding residues M1-L206, followed by rat CD4 domains 3 and 4 and  
674 a C-terminal hexa-histidine tag was expressed through transient transfection of Expi293 cells. Protein was then  
675 purified from the supernatant by immobilized metal affinity chromatography (IMAC) using a Ni<sup>2+</sup> resin followed  
676 by SEC into TBS pH 7.4 (20 mM TRIS-HCl, 150 mM NaCl) as previously described<sup>8,24,49</sup>.  
677

### 678 ELISA

679 For assessment of antibody binding by ELISA, Nunc Maxisorp plates were coated overnight (>16 h) with either  
680 RH5.1, heat-denatured RH5.1 (held at 90 °C for 10 min), or RH5ΔNL at 2 µg/mL. Plates were washed in wash  
681 buffer (PBS with 0.05 % Tween 20 [PBST]) and blocked with 100 µL/well of Blocker™ Casein (Thermo Fisher  
682 Scientific) for 1 h. Plates were washed and antibodies at 10 µg/mL diluted in casein were added. Following a 2  
683 h incubation, plates were washed and a 1:1000 dilution of goat anti-human IgG (γ-chain specific) alkaline  
684 phosphate conjugate antibody (A3187, Thermo Fisher) was added and incubated for 1 h. Plates were washed  
685 in washing buffer and 100 µL development buffer (*p*-nitrophenyl phosphate substrate diluted in  
686 diethanolamine buffer) was added per well and developed according to internal controls. All mAbs were tested  
687 in duplicate against each coating antigen. Unless otherwise stated, 50 µL was added per well and all steps were  
688 carried out at RT. A given mAb was tested against all antigens within the same plate.

689  
690 For the peptide array ELISAs, steps were identical to the above except streptavidin-coated plates (Pierce) were  
691 used and were coated with an array of 62 x 20-mer PfRH5 peptides overlapping by 12 amino acids as  
692 previously reported<sup>17</sup>.  
693

### 694 Antibody Kinetics

695 High-throughput SPR binding experiments in **Figure 2** and **Figure S2** were performed on a Carterra LSA  
696 instrument equipped with a 2D planar carboxymethyldextran surface (CMDP) chip type (Carterra) using a 384-  
697 ligand array format. The CMDP chip was first conditioned with 60 s injections of 50 mM NaOH, 1 M NaCl and  
698 10 mM glycine (pH 2.0) before activation with a freshly prepared 1:1:1 mixture of 100 mM MES (pH 5.5), 100  
699 mM sulfo-N-hydroxysuccinimide, and 400 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride.  
700 A coupled lawn of goat anti-human IgG Fc (hFc; 50 µg/mL in 10 mM sodium acetate, pH 4.5) (Jackson  
701 ImmunoResearch) was then prepared before quenching with 0.5 M ethanolamine (pH 8.5) and washing with  
702 10 mM glycine (pH 2.0). mAbs prepared at 100 ng/mL in Tris-buffered saline with 0.01 % Tween-20 (TBST)  
703 were then captured onto the surface in a 384-array format via a multi-channel device, capturing 96 ligands at a  
704 time. For binding kinetics and affinity measurements, an eight-point threefold dilution series of RH5.1 protein  
705 ending at 100 nM in TBST was sequentially injected onto the chip from lowest to highest concentration. For

706 each concentration, the antigen was injected for 5 min (association phase), followed by TBST injection for 15  
707 min (dissociation phase). Two regeneration cycles of 30 s were performed between each dilution series by  
708 injecting 10 mM glycine (pH 2.0) on the chip surface. The SPR results were exported to Kinetics Software  
709 (Carterra) and analyzed as nonregenerative kinetics data to calculate association rate constant ( $K_{on}$ ),  
710 dissociation rate constant ( $K_{off}$ ) and equilibrium dissociation constant ( $K_D$ ) values via fitting to the Langmuir 1:1  
711 model. Prior to fitting, the data were referenced to the anti-hFc surface then double referenced using the final  
712 stabilizing blank injection.

713

## 714 Epitope Binning by Surface Plasmon Resonance

715 High-throughput epitope binning experiments shown in **Figure 1**, **Figure S1** and **Figure S3** were performed in a  
716 classical sandwich assay format using the Carterra LSA and an HC30M chip. The chip was conditioned as  
717 described above before antibodies prepared at 10  $\mu$ g/mL in 10 mM sodium acetate (pH 4.5) with 0.05 %  
718 Tween were coupled to the surface: the chip surface was first activated with a freshly prepared 1:1:1  
719 activation mix of 100 mM MES (pH 5.5), 100 mM sulfo-N-hydroxysuccinimide, and 400 mM 1-ethyl-3-(3-  
720 dimethylaminopropyl) carbodiimide hydrochloride, and antibodies were injected and immobilized onto the  
721 chip surface by direct coupling. The chip surface was then quenched with 1 M ethanolamine (pH 8.5), followed  
722 by washing with 10 mM glycine (pH 2.0). Sequential injections of 50 nM RH5.1 protein (5 min) followed  
723 immediately by the 10  $\mu$ g/mL sandwiching antibody (5 min), both diluted in HEPES-buffered saline Tween-  
724 EDTA (HBSTE) with 0.5 mg/mL bovine serum albumin (BSA), were added to the coupled array and the surfaces  
725 regenerated with 10 mM glycine (pH 2.0) using two 30 s regeneration cycles. Data were analyzed using the  
726 Carterra Epitope software.

727

728 The epitope binning experiment for mouse mAbs QA1 and 9AD4<sup>21</sup> shown in **Figure S4** was performed as above  
729 but using a HC200M chip and TBST as the running buffer and diluent.

730

## 731 Determination of Protein Blockade by Bio-layer Interferometry (BLI)

732 All BLI for data shown in **Figure 1** and **Figure S1** was carried out on an OctetRED384 (ForteBio) using anti-  
733 human Fc-capture sensors (Sartorius, 18-5060) to immobilize anti-PfRH5 mAbs. Assays were carried out in a  
734 384-well format in black plates (Greiner). For assaying the ability of each mAb to block RH5.1 binding to basigin  
735 and PfCyRPA, the experiment followed a sequential assay: mAb immobilization (15  $\mu$ g/mL, 300 s), RH5.1  
736 binding (1  $\mu$ M, 300 s), protein ligand binding (3  $\mu$ M PfCyRPA or basigin, 300 s) with a 30 s dissociation phase in  
737 TBST between each step. Finally a 120 s dissociation step was carried out in TBST before a 10 s pulsed  
738 regeneration of biosensors with 10 mM glycine (pH 2). Within each plate, PfCyRPA blocking was first assessed  
739 for a set of mAbs immediately followed by regeneration and then basigin blocking activity assessed for the  
740 same set of mAbs. As internal or “sentinel mAb” controls, each plate included a PfCyRPA-blocking mAb  
741 (R5.015), a basigin blocking mAb (R5.004) and a non-blocking mAb (R5.011)<sup>24,25</sup>. In addition, in each assay, a

742 reference baseline set of biosensors was run in parallel using the same format but replacing the protein ligand  
743 binding step with a TBST step.

744

745 Data were analyzed in the Octet Data Analysis HT software (ForteBio). The reference biosensors were assigned  
746 as references in the software and subtracted from the test biosensors. Steps were aligned to the start of each  
747 association step and the association and dissociation was fitted. Response value report points were set at 20 s  
748 (start of association) and 290 s (end of association) and exported. For the majority of mAbs, the 290 s report  
749 point was used. For a subset of mAbs with very fast dissociation, the 20 s timepoint was used (because report  
750 points at 290 s could be erroneously reported as blocking due to dissociation of the underlying RH5.1 surface  
751 from the captured mAb). For the RH5.1 report points, the data were unreferenced. For each mAb, the basigin  
752 and PfCyRPA responses were normalized by dividing by the RH5.1 response. Any mAb with a normalized  
753 response value of <0.04 nm for basigin or PfCyRPA was categorized as “blocking” for that protein ligand. Data  
754 were discarded if R5.015 had a response >0.04 nm for PfCyRPA; R5.004 had a response >0.04 nm for basigin;  
755 or if R5.011 had a response <0.04 nm for either protein ligand.

756

## 757 Assay of Growth Inhibition Activity (GIA)

758 Single concentration *in vitro* GIA assays were carried as previously described according to the methods of the  
759 GIA International Reference Centre at NIAID/NIH, USA<sup>50</sup>. All assays used 3D7 clone *P. falciparum* parasites  
760 cultured in human RBC from in-house volunteer donations or supplied by the UK NHS Blood and Transplant  
761 service for non-clinical issue. Briefly, mAbs were buffer exchanged into incomplete parasite growth media (ICM  
762 = RPMI, 2 mM L-glutamine, 0.05 g/L hypoxanthine, 5.94 g/L HEPES) before performing the GIA assay and  
763 allowing parasites to go through a single cycle of growth. To ensure consistency between experiments, in each  
764 case the activity of a negative control human mAb, EBL040<sup>51</sup> which binds to the Ebola virus glycoprotein, and  
765 three anti-PfRH5 mAbs with well-characterized GIA (2AC7, QA5, and 9AD4; or 2AC7, R5.016, and R5.034<sup>21,24</sup>)  
766 were run alongside the test mAbs, and were all tested in triplicates. mAbs showing >30 % GIA were  
767 subsequently tested in an eight step, five-fold dilution series with a final assay start concentration of 2 mg/mL  
768 to determine interpolated EC values. The resultant data were transformed according to  $x=\log(x)$  and the  
769 transformed data were fitted by four-parameter non-linear regression. GIA values were interpolated from the  
770 resultant curve. If a mAb did not reach a sufficiently high GIA (i.e. the mAb did not reach 30 %, 50 % or 80 % at  
771 any test concentration), then it was assigned a “negative” value of 10,000 µg/mL for that particular EC  
772 readout.

773

774 For screening of intra-PfRH5 mAb interactions, shown in **Figure 5A and Figure S5A**, single concentration assays  
775 were carried out as above, with neutralizing antibodies added at a concentration equivalent to their  
776 interpolated EC<sub>50</sub> value and non-neutralizing antibodies held at 0.3 mg/mL. For testing of non-neutralizing  
777 antibodies in combination, shown in **Figure 5B**, both mAbs in a pair were held at 0.2 mg/mL. For dilution  
778 curves, titrated mAbs were set up in a 7-step, five-fold dilution series starting at 0.5 mg/mL per mAb. To each  
779 titrated mAb dilution, a held concentration of a second mAb (or a premixed combination of two mAbs) was

780 added at a final concentration of 0.2 mg/mL per held mAb. For each curve, a well containing the second mAb  
781 (or premixed combination) at the test concentration was set up alone within the same assay plate. Curve  
782 fitting and data processing was carried out as above.

783

784 For mixed titration curves, shown in **Figure 5C** and **Figure S5E-G**, two or more mAbs were premixed and set up  
785 in an 8-step, five-fold dilution series starting at 0.5 mg/mL per test mAb. Curve fitting and data processing was  
786 carried out as above.

787

788 For synergy curves with polyclonal antibody shown in **Figure S5D**, total IgG (purified from the serum of VAC063  
789 vaccinees<sup>13</sup>) was set up as above at a starting concentration of 14 mg/mL total IgG in a 7-step 2-fold dilution  
790 curve.

791

792 For analysis of synergistic or antagonistic interactions, the Bliss additivity<sup>52</sup> was determined based on the  
793 measured activity from each antibody alone (1 and 2) using the following formula:

$$GIA_{1+2} = \left[ 1 - \left( 1 - \frac{GIA_1}{100} \right) \times \left( 1 - \frac{GIA_2}{100} \right) \right] \times 100$$

794

795

## 796 Purification of IgG

797 For the polyclonal antibody (pAb) pool used in **Figure S5D**, a pool of human serum from the VAC063 clinical  
798 trial<sup>13</sup> was filtered through a 0.22 µM syringe filter (Milipore) and diluted 1:1 in PBS. Total IgG was then  
799 purified using a HiTrap Protein G column (Cytiva) on an ÄKTA Pure chromatography system (Cytiva), and the  
800 eluted total IgG was buffer exchanged into ICM using a centrifugal concentrator with a 30K MWCO (Cytiva).  
801 Total IgG concentration was determined by reading absorbance at 280 nm on a Thermo Scientific™  
802 NanoDrop™ OneC Spectrophotometer. IgG was then depleted for anti-RBC antibodies by the addition of 1 µg  
803 packed 100 % hematocrit RBC per 1 µg IgG and incubated at RT with agitation for 1 h. RBC were then pelleted  
804 by centrifugation at 1,000 xg for 5 min and the supernatant removed. Anti-RH5.1 antibody titers or  
805 concentrations were determined by standardized quantitative ELISA methodology as previously reported<sup>17</sup>.  
806

## 807 Antibody Sequence Annotation

808 All annotation of antibody heavy and light chain gene sequences was performed using IMGT V-Quest program  
809 version 3.5.31 using default parameters. The IGH locus was selected for heavy chain sequences, IGL for λ light  
810 chains and IGK for κ light chains. F+ORF+ in-frame P was used as the IMGT/V-QUEST reference directory set  
811 and the option to search for insertions and deletions was selected.

812

## 813 Design of Public Clonotype Germline Revertant mAbs

814 Synthetic antibodies shown in **Figure 3E** were designed based on the HV3-7/LV1-36 gene combination. For the  
815 germline heavy chains (“HC GL”), amino acids E1 to R98 of the V-region of each public clonotype mAb (R5.034,

816 R5.102, R5.237 and R5.270) were replaced with amino acids E1 to R98 of the germline HV3-7\*01 F gene (IMGT  
817 accession number: M99649) V-region obtained from IMGT. The sequence for each mAb from R98 was  
818 unchanged from the wildtype (WT) mAb, resulting in four germline HV3-7 heavy chains, each biased with the  
819 CDRH3 J- and D- regions of the respective public clonotype mAb.

820

821 For the germline LV1-36 light chain (“LC GL”), amino acids Q1 to G98 of the germline LV1-36\*01 F gene (IMGT  
822 accession number Z73653) V-region were concatenated with amino acids VVFGGGTKLTVL of the germline  
823 LJ2\*01 F gene (IMGT accession number M15641). Note that R5.034, R5.102 and R5.270 were equally likely to  
824 use the LJ2\*01 F or LJ3\*01 F (IMGT accession number M15642) gene segments, only R5.237 had a greater  
825 identity to the LJ3\*02 (IMGT accession number D87023) gene segment.

826

827 Synthetic genes were cloned into AbVec expression vectors as described above. For the production of heavy  
828 chain revertant antibodies (HC GL), HEK293F cells were transfected with expression plasmids containing one of  
829 the four germline HV3-7 heavy chains, and the cognate WT light chain of the respective public clonotype mAb.  
830 For the production of light chain revertant antibodies (LC GL), cells were transfected with expression plasmids  
831 containing one of the four WT public clonotype mAb heavy chains and their respective germline LV1-36 light  
832 chain. For the production of full revertants (“Full GL”), cells were transfected with expression plasmids  
833 containing one of the four germline HV3-7 heavy chains and the respective germline LV1-36 light chain.

834

835 To generate the public clonotype CDRH3 knockout antibodies (“CDRH3\_KO”), the 9 amino acids from positions  
836 99 to 107 in the CDRH3 of each antibody (IMGT numbering) were replaced with a ‘randomized’ sequence of  
837 amino acids with a matched length – HQSGKLVNMN. No amino acid in this sequence was conserved with  
838 respect to the equivalent position in any of the public clonotype CDRH3 sequences. The ‘randomized’ region  
839 was chosen to test the effects of altering amino acids derived from somatic hypermutation of rearrangement,  
840 whilst preserving the largely conserved and germline templated sequences at the N- (positions 97-98) and C-  
841 (positions 108-109) termini of the 13 amino acid CDRH3. CDRH3 knockout heavy chains were cloned into  
842 expression vectors as described above. Cells were transfected with expression plasmids containing the  
843 respective CDRH3 knockout heavy chain for each public clonotype antibody and its cognate WT light chain.

844

## 845 Fab Cloning and Expression

846 To express recombinant Fabs, heavy chain plasmids were generated using primers to amplify the heavy chain  
847 VH and CH1 domain only (5’ – GAG GAT GGT CAT GTA TCA TC and 5’ – CGC AAG CTT CTA AGT TTT GTC ACA  
848 AGA TTT GGG C) and then used to transfect Expi293F cells in combination with the corresponding light chain  
849 plasmids. Fab-containing supernatants were purified by affinity chromatography with either a HiTrap  
850 LambdaFabSelect column (17548211, Cytiva) or a HiTrap Protein G column (17040501, Cytiva) on an ÄKTA  
851 Pure chromatography system as per manufacturer’s instructions.

852

853 Production of RH5ΔNL:Fab Complexes for X-ray Crystallography

854 Fabs and RH5ΔNL protein (7G8 sequence with Y203) were produced as described above, and buffer exchanged  
855 into HEPES-buffered saline (HBS, 25 mM HEPES, 150 mM NaCl, pH 7.5) using a 10K MWCO centrifugal  
856 concentrator device (Cytiva). RH5ΔNL was subjected to limited proteolysis at a concentration of 1-2 mg/mL,  
857 with the addition of 1  $\mu$ L GluC protease (P8100S, NEB) at 1 mg/mL per mg of RH5ΔNL (1:1000 wt/wt). Protein  
858 was incubated at RT for 4-16 h. Fabs were then added at a 5 % molar excess. The complex was incubated for 1  
859 h at RT. The complex was then subjected to surface lysine methylation by the addition of 20  $\mu$ L per mL of  
860 complex of 1 M dimethylamine borane complex (ABC) (Santa Cruz Biotechnology, sc-252506) and 40  $\mu$ L per mL  
861 of complex of 1 M formaldehyde prepared from 37 % stock (Thermo Fisher, 10630813). The complex was  
862 incubated for 1 h at RT, followed by a further addition of 20  $\mu$ L ABC and 20  $\mu$ L formaldehyde per mL of  
863 complex. After a further 1 h incubation, 20  $\mu$ L ABC per mL of complex was added and the solution was  
864 incubated at 4 °C overnight. Aggregates were removed by spinning the sample in a centrifugal filter device  
865 (UFC40GV00, Milipore) at 4000 xg for 10 min. The flow-through was concentrated to <2 mL if necessary and  
866 run onto a Superdex 16/600 200 pg SEC column (Cytiva) that had been pre-equilibrated in Tris-buffered saline  
867 (25 mM Trizma, 150 mM NaCl, pH 7.4). Complex containing fractions were pooled and concentrated to 8-12  
868 mg/mL using a centrifugal concentrator with a 10K MWCO (Cytiva) for crystal screening.  
869

870 Structure Determination by X-ray Crystallography

871 All crystallization was conducted using vapour diffusion in MRC 3 Lens sitting drop crystallization plates  
872 (SwisSci, High Wycombe, UK) and 150 nL drops (ratio 100 nL protein solution (8 mg/ $\mu$ L:50 nL screen condition)  
873 dispensed using a Mosquito nano-pipetting robot (STP Labtech, Melbourn, UK). Crystallization plates were  
874 incubated at 20 °C with crystals appearing between 4 and 28 days. The crystals were mounted with LithoLoops  
875 (Molecular Dimension, Rotherham, UK) using the CrystalShifter crystal harvesting robot<sup>53</sup> (Oxford LabTech,  
876 UK) and cryo-protected in a solution of 25% Ethylene Glycol.  
877

878 Diffraction quality crystals of complex of RH5ΔNL:R5.034:R5.028 Fabs were obtained from PEG/Ion Screen  
879 (Hampton Research, Aliso Viejo, CA) condition H6 (0.02 M Calcium chloride dihydrate, 0.02 M Cadmium  
880 chloride hydrate, 0.02 M Cobalt(II) chloride hexahydrate, 20 % w/v Polyethylene glycol 3,350). All diffraction  
881 data were collected at Diamond Light Source (Proposal ID: mx28172). Initially samples were sent to i03 for  
882 Unattended Data Collection (Native Experiment; 12.7 keV, 1.7 Å, 2 x 360° sweeps, 1st at chi=0 and 2nd at  
883 chi=30), before being transferred to i24 for manual data collection (4.00 Å, 360°).  
884

885 Diffraction quality crystals of complex RH5ΔNL:R5.251 Fab were obtained from the PurePEGs (Anatrace,  
886 Maumee, OH) condition B8 (0.3M Calcium chloride, and 0.1M Magnesium formate HCl 6, 22.5% (v/v)  
887 PurePEGs Cocktail). Data were collected at i03 (Diamond Light Source (Proposal ID: mx28172)) using  
888 Unattended Data Collection (Native Experiment; 12.7 keV, 1.7 Å, 2 x 360° sweeps, 1st at chi=0 and 2nd at  
889 chi=30).

## 890 Data Processing and Model Refinement

891 Datasets for RH5ΔNL:R5.251 and RH5ΔNL:R5.034:R5.028 were manually processed on the Diamond Cluster  
892 using DIALSs<sup>54</sup>, confirming the auto-processed xia2.DIALS<sup>55</sup> output from SynchWeb/iSpyB<sup>56,57</sup>. All subsequent  
893 processing was done on local computers using the CCP4i GUI<sup>58</sup> or on CCP4 Cloud<sup>59</sup>. RH5ΔNL:R5.251 and  
894 RH5ΔNL:R5.034 data were truncated at 3.2 Å and 4 Å, respectively. Although the apparent crystal symmetry  
895 was orthorhombic, refinement failed to reduce R-free as would usually be expected. Data for the putative  
896 complex of RH5ΔNL:R5.034:R5.028 were reprocessed in P21 with a beta angle of ~90° and pseudomerohedral  
897 twinning is suspected; however, there was no evidence of R5.028 Fab in the data, suggesting it was lost during  
898 crystal formation with just RH5ΔNL:R5.034 remaining. Molecular replacement was done with Phaser<sup>60</sup> using  
899 homology model coordinates downloaded from the Protein Data Bank (PDB) based on a sequence similarity  
900 search<sup>61</sup>. The following homology models were used for: i) RH5ΔNL (PDB: 4WAT); and ii) R5.251 Variable,  
901 Heavy (PDB: 6OC7); Constant, Heavy (PDB: 6OC7); Variable, Light (PDB: 5XKU); Constant, Light (PDB: 5XKU);  
902 and iii) R5.034 Variable, Heavy (PDB: 5X8M); Constant, Heavy (PDB: 1BJ1); Variable, Light (PDB: 5WL2);  
903 Constant, Light (PDB: 3HOT). Subsequent model refinement and building was performed in Coot<sup>62</sup> and  
904 REFMAC<sup>63</sup>. Once stable models had been built they were put through the PDB-REDO<sup>64</sup> pipeline to be  
905 optimized, and LORESTR<sup>65</sup> for further low-resolution refinement.

906

## 907 Computational Prediction of GIA from mAb Gene Usage

908 Subject gene usage was one-hot encoded using the R package mltools (v0.3.5). To more robustly assess the  
909 relationship between gene usage and GIA %, only pairs of genes that occurred in at least 4 individuals were  
910 considered. Further filtering was applied to select only gene pairs whose mean associated GIA % was outside a  
911 conservatively selected 33 % deviation from the dataset mean (below 42 % GIA %, or above 82 % GIA %). An  
912 additional requirement was added that GIA % values associated with gene pairs must be either entirely above  
913 or below the dataset mean and not spanning. To assess statistical significance, 1000 rounds of permutation  
914 were run, where gene use was scrambled across the dataset and the same filtering was performed to search  
915 for significant gene pairs. A P value ( $P < 0.001$ ) was calculated from the tail probability of the generated null  
916 distribution. An R markdown for the analysis is available.

917

## 918 R5.034 and R5.034LS Binding Kinetics to RH5.1

919 For **Figure S6A**, SPR was carried out using the Biacore™ X100 machine and software. Purified recombinant  
920 R5.034 or R5.034LS was immobilized on Sensor Chip Protein G (Cytiva) through a 30 s injection of 16 nM  
921 antibody. RH5.1 protein was diluted in PBS + P20 running buffer (137 mM NaCl, 2.7 mM KCl, 10mM Na<sub>2</sub>HPO<sub>4</sub>,  
922 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 0.005 % surfactant P20 (Cytiva)) to yield a final concentration of 15.6 nM. Samples were  
923 injected for 180 s at 30 µL/min before dissociation for 800 s. The chip was then regenerated with a 45 s  
924 injection of 10 mM glycine pH 1.5. Antibody kinetics were determined through a two-fold, five-step dilution  
925 curve. Data were analyzed using the Biacore X100 Evaluation software v2.0.2. A global Langmuir 1:1  
926 interaction model was used to determine antibody kinetics.

927

## 928 R5.034 and R5.034LS Binding Kinetics to Human FcRn

929 For **Figure 6C**, SPR was carried out using the Biacore™ X100 machine and software. Recombinant human FcRn  
930 protein (Acro Biosystems) was immobilized onto a CM5 Sensor Chip (Cytiva) using the standard amine coupling  
931 protocol yielding ~200 response units (RU). R5.034 and R5.034LS were diluted to a final concentration of 6.4  
932  $\mu$ M and 1.6  $\mu$ M, respectively, in either MES pH 6.0 (20 mM MES, 150 mM NaCl) or TBS pH 7.4 (20 mM TRIS HCl,  
933 150 mM NaCl) running buffer. Samples were injected for 180 s at 30  $\mu$ L/min before dissociation for 600 s. The  
934 chip was regenerated with a 30 s injection of PBS pH 7.4. Affinity was determined using a two-fold, nine-step  
935 dilution curve. Data were analyzed using the Biacore X100 Evaluation software v2.0.2, and the equilibrium  
936 dissociation constant was determined from a plot of steady state binding levels.

937

## 938 *P. falciparum* Sporozoite Challenge in Liver-Chimeric Humanized Mice

939 The FRG huHep mouse studies in **Figure 6E** were conducted similar to studies previously published<sup>66</sup> with  
940 modifications. FRG huHep mice on the NOD background were purchased from Yecuris, Inc. (Beaverton, OR,  
941 USA). Mice were pre-screened to have a serum human albumin level indicative of >90 % humanization of  
942 hepatocytes. Mice were then infected with *P. falciparum* NF54 strain via mosquito bite. Mosquitos were  
943 purchased from the Johns Hopkins Malaria Research Institute Insectary Core and used only if >50 % of  
944 mosquitos were infected with a mean of >10 oocysts per midgut. Based on this midgut prevalence and/or  
945 salivary gland “smash test” (dissection of individual mosquito salivary glands followed by microscopic  
946 observation of sporozoites), mosquitos were apportioned to cages equivalent to 5 infectious mosquitos per  
947 mouse. Mosquitos were then allowed to feed on mice anesthetized under isoflurane for 10 min, with lifting of  
948 mice every minute to encourage probing as opposed to blood feeding.

949

950 On day 5 post-infection, mice were intravenously injected with both mAb and human RBC. Monoclonal  
951 antibodies (either 625  $\mu$ g anti-PfRH5 human IgG1 mAb R5.034 or 675  $\mu$ g human IgG1 negative control mAb  
952 1245 against the sexual-stage malaria antigen Pfs25<sup>67</sup>) were delivered via the retro-orbital route diluted to  
953 100  $\mu$ L total volume in sterile PBS. Human RBC were obtained from a commercial vendor (BloodWorks  
954 Northwest, Seattle, WA, USA) and washed three times with sterile RPMI to remove serum and white blood  
955 cells. Human RBC were injected via the tail vein in a total volume of 400  $\mu$ L containing 50  $\mu$ L clodronate  
956 liposomes (Formumax Cat #F70101C-AH), 5  $\mu$ L penicillin/streptomycin and 345  $\mu$ L 70 % hematocrit human RBC  
957 (packed RBC diluted to 70 % hematocrit with sterile RPMI).

958

959 On day 6 post-infection, mice were bled via the retro-orbital plexus using non-heparinized capillary tubes.  
960 Blood was transferred to 1.5 mL Eppendorf tubes and allowed to clot for 30 min at room temperature (RT).  
961 Serum was separated by centrifugation in a table top centrifuge at 9600 rpm for 10 min and stored at -80 °C  
962 until use. Mice were then injected with 700  $\mu$ L 70 % hematocrit human RBC. Injection of this volume of human  
963 RBC was repeated on days 9 and 11.

964

965 On days 7, 9, 11 and 13 post-infection, mice were bled via the retro-orbital plexus using heparinized capillary  
966 tubes and 100  $\mu$ L whole blood was transferred to 1.9 mL nucliSENS Lysis Buffer (Biomerieux Inc. Cat# 200292).  
967 Blood was allowed to lyse at RT for at least 30 min prior to storage at -80 °C before qRT-PCR analysis. Terminal  
968 serum was also collected on day 13 via cardiac puncture into a 1 mL syringe with no anticoagulant with  
969 separation performed as above. Blood samples were then blinded and sent to the lab of Dr. Sean Murphy at  
970 the University of Washington for quantification of *Plasmodium* 18s rRNA following published methods<sup>68</sup>.

971

972 Serum levels of R5.034 antibody were determined by testing dilutions of test sera on a RH5.1 protein capture  
973 ELISA including use of a standard curve of purified recombinant R5.034 mAb. In brief, ELISA plates were coated  
974 with RH5.1 protein at 2  $\mu$ g/mL and then blocked with Blocker™ Casein solution. Test sera were diluted and  
975 then titrated using a 12-point dilution curve with 1:2 dilutions, and read off an R5.034 standard curve to  
976 determine concentration.

977

## 978 STATISTICAL ANALYSIS

979 Analysis was performed using GraphPad Prism version 10.0.2 (GraphPad Software, LLC). Tests and statistics are  
980 described in Figure Legends. Non-parametric tests were chosen for non-normally distributed data. To  
981 determine EC values, mAb dilution curves were transformed according to  $x=\log(x)$  and the transformed data  
982 were fitted to a curve by four-parameter non-linear regression. GIA values were interpolated from the  
983 resultant curve with upper and lower 95 % confidence intervals. If a mAb did not reach a sufficiently high GIA  
984 (i.e. the mAb did not reach 30 %, 50 % or 80 % GIA at any test concentration), then it was assigned a “GIA-  
985 negative” value of 10,000  $\mu$ g/mL for the purposes of data visualization and statistical testing. In all statistical  
986 tests, reported  $P$  values are two-tailed and  $P < 0.05$  considered significant.

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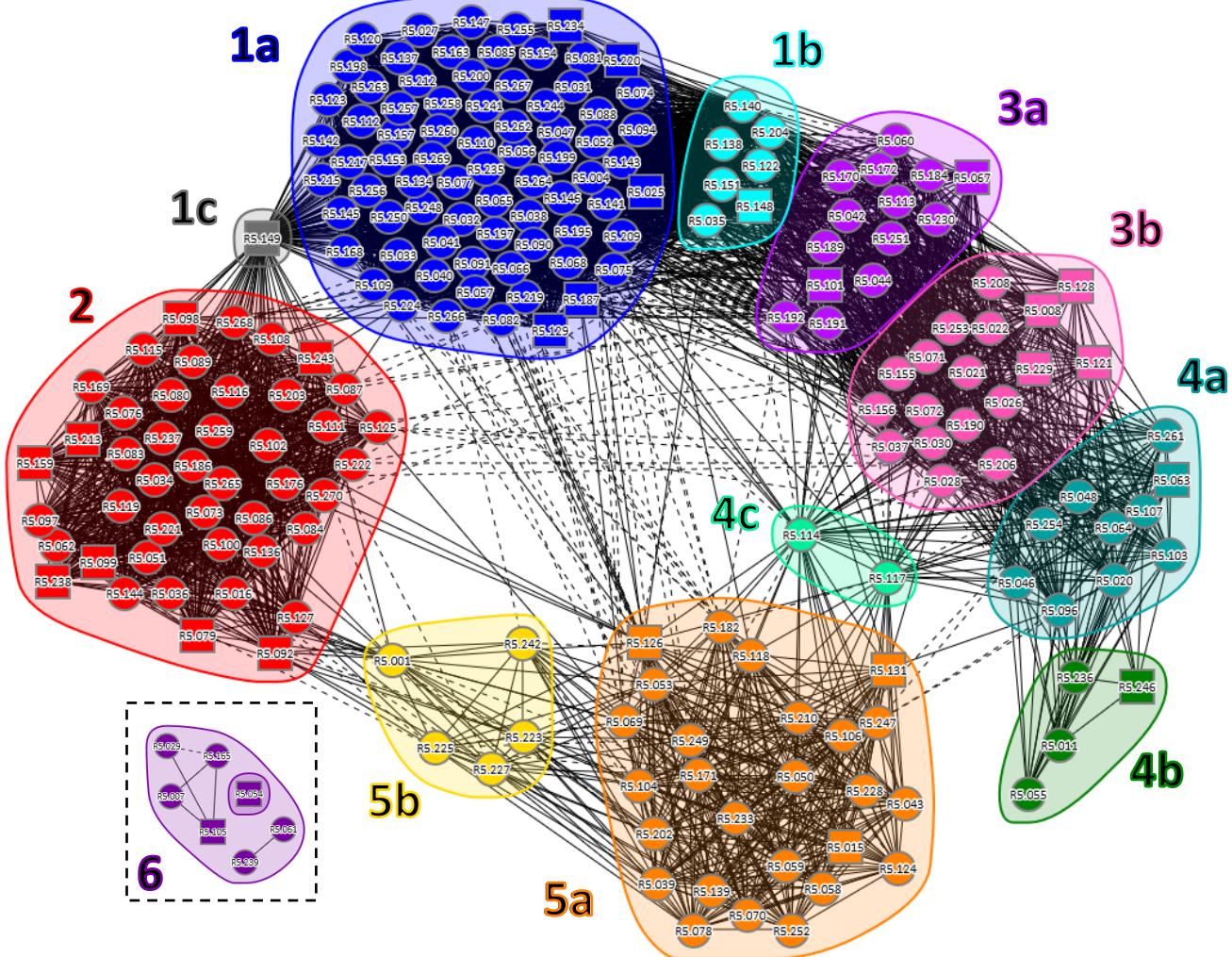
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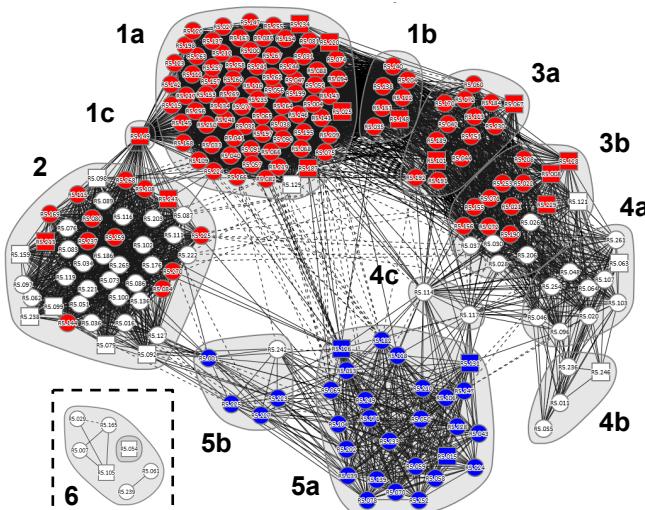
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Figure 3.11

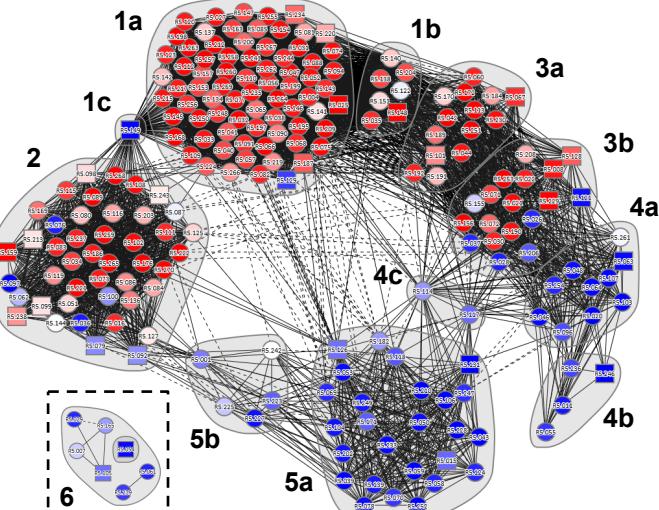
A



B

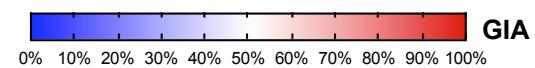


C



■ Basigin Blocking ■ PfCyRPA Blocking

## No Blocking



D

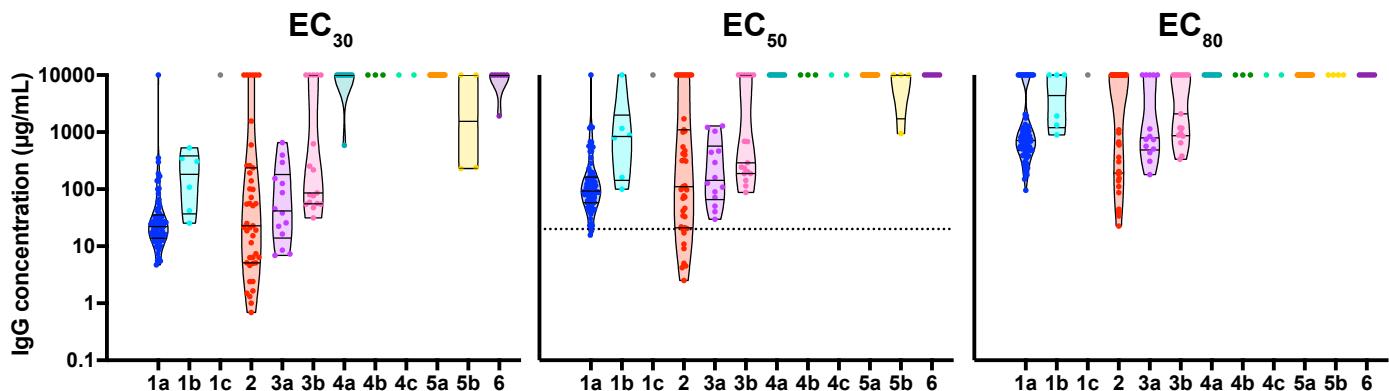


Figure 1: The functional epitope landscape of PfRH5.

**(A)** Community network plot illustrating the competitive relationship between 206 vaccine-induced anti-PfRH5 human mAbs. Supercommunities and communities are defined by number code and color. Individual mAbs are represented as nodes. Solid lines between nodes indicate bidirectional competition. Dashed lines between nodes indicate unidirectional competition. Square nodes indicate mAbs that were excluded as either a ligand or an analyte. Community 6 (representing the IDL binders; N=7) was analysed separately and is shown here as an inset. **(B)** Community network plot overlaid with blocking category for PfRH5 binding to basigin or PfCyRPA as defined by BLI, or **(C)** GIA % as tested using a high concentration (0.8-2 mg/mL) of each mAb. **(D)** Violin plots showing the GIA potency of each epitope community as measured by the effective concentration (EC) needed to reach 30 %, 50 % or 80 % GIA. Data are log transformed and lines indicate the median and quartiles. Dashed line indicates the previously reported best-in-class sentinel mAb, R5.016<sup>24</sup>, with GIA EC<sub>50</sub> against 3D7 clone *P. falciparum* = 20.7 µg/mL (**Data S1C**). Weak or non-active mAbs, for which EC values could not be determined, were assigned values of 10 mg/mL for the purpose of analysis.

## Figure 2

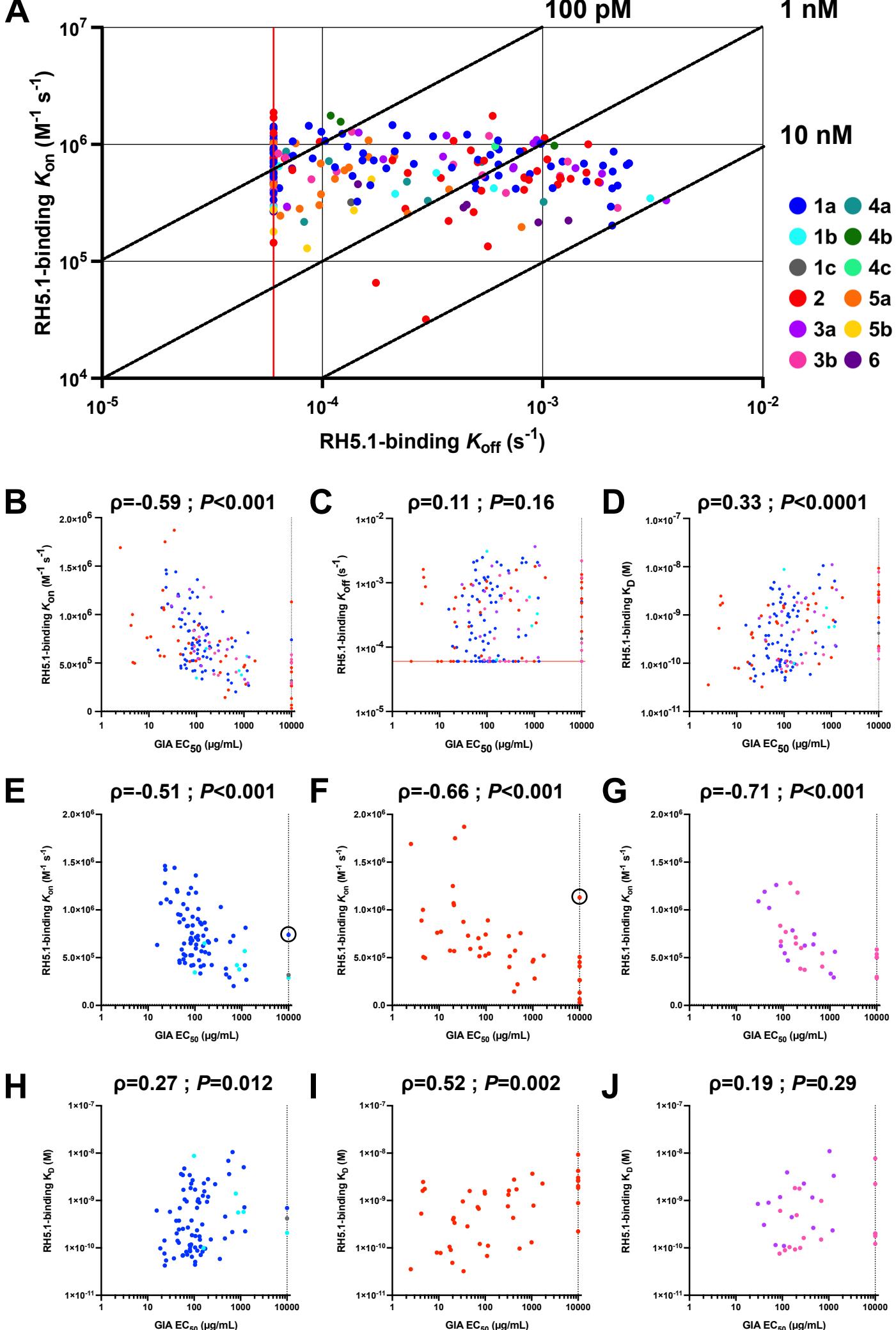


Figure 2: Binding kinetics of anti-PfRH5 mAbs.

**(A)** Iso-affinity plot showing kinetic rate constants for binding of mAbs to RH5.1 (full-length PfRH5 protein) as determined by HT-SPR. Diagonal lines represent equal affinity ( $K_D = K_{off} / K_{on}$ ). Red vertical line indicates lowest limit of  $K_{off}$  measurement ( $6 \times 10^{-5} \text{ s}^{-1}$ ). mAbs colored by epitope community (N=213 in total). **(B)** The RH5.1-binding parameters of  $K_{on}$ , **(C)**  $K_{off}$  and **(D)**  $K_D$  were correlated with GIA EC<sub>50</sub> for all antibodies in the growth inhibitory antibody epitope (super)communities 1, 2 and 3 (N=159). **(E)** The RH5.1-binding parameter of  $K_{on}$  or **(H)**  $K_D$  was correlated with GIA EC<sub>50</sub> for all antibodies in the growth inhibitory antibody epitope supercommunity 1 (N=83), **(F,I)** community 2 (N=44) and **(G,J)** supercommunity 3 (N=32). Anti-PfRH5 clones R5.129 and R5.036 are circled in panels **E** and **F**, respectively. Spearman's rank correlation coefficient ( $\rho$ ) and two-tailed  $P$  value are shown.

A

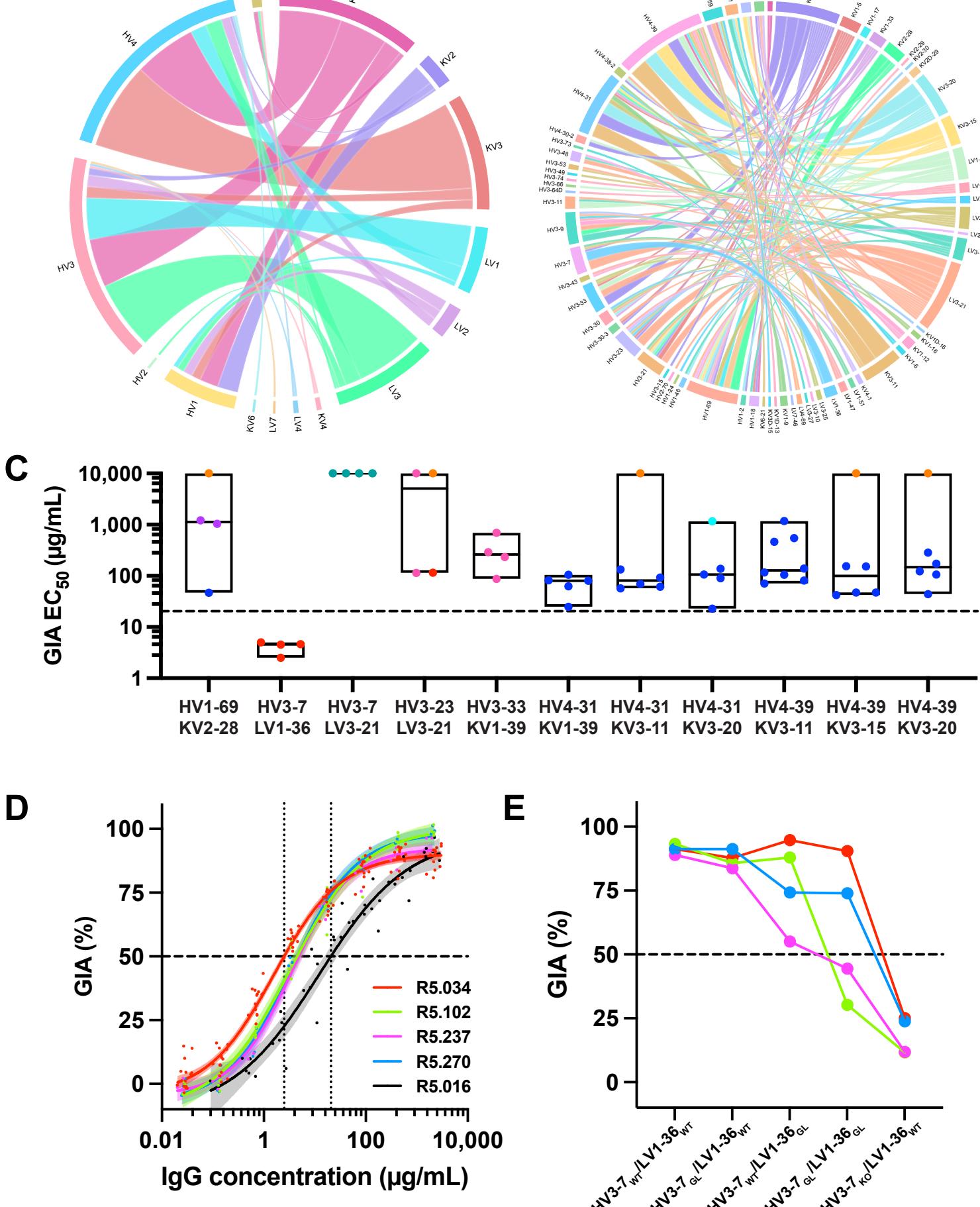


Figure 3: Sequence analysis of anti-PfRH5 mAbs.

**(A)** Chord plot representing pairings of immunoglobulin HV and KV/LV gene families and **(B)** genes used by N=206 anti-PfRH5 human mAbs. The width of the cord is proportional to the number of mAbs which utilize that pairing. **(C)** Gene pairs in the anti-PfRH5 panel with  $N \geq 4$  representative mAbs plotted in groups along with their GIA  $EC_{50}$  value. Each mAb is colored by its epitope community. Boxes show the mean with minimum to maximum. Dashed line shows the R5.016 bench mark  $EC_{50}$  of 20.7  $\mu$ g/mL. **(D)** GIA assay titration curves of mAbs utilizing the HV3-7/LV1-36 gene combination and sentinel mAb R5.016 for comparison. Data were combined from repeat assays: N=14 for R5.034; N=4 for R5.237; N=3 for R5.237; N=3 for R5.270; and N=6 for R5.016. Data were log transformed and a four parameter nonlinear regression was fitted. Individual points are from the all replicate titrations. The shaded regions show the 95% confidence limits of the curves. Dashed line shown at 50 % GIA and dotted lines at  $EC_{50}$  readouts for the R5.034 and R5.016 curves. **(E)** For each mAb utilizing the HV3-7/LV1-36 public clonotype gene combination (color-coded as in **D**) a panel of four germline revertant antibodies was designed. Each mAb was tested at a concentration of 0.5 mg/mL in the GIA assay against 3D7 clone *P. falciparum* parasites. Points shown the mean of triplicate test wells and connecting lines are shown for clarity. WT = wild-type mAb sequence; HV3-7<sub>GL</sub> has all mutations up to the beginning of the CDRH3 sequence mutated to germline combined with WT light chain; LV1-36<sub>GL</sub> has all mutations reverted to germline, including the CDRL3 and J-region, combined with WT heavy chain; HV3-7<sub>GL</sub>/LV1-36<sub>GL</sub> is mAb with both of these heavy and light chain sequences; HV3-7<sub>KO</sub> is the HV3-7 WT sequence for each respective mAb but with the CDRH3 mutated to a random sequence of 13 amino acids, combined with WT light chain.

# Figure 4

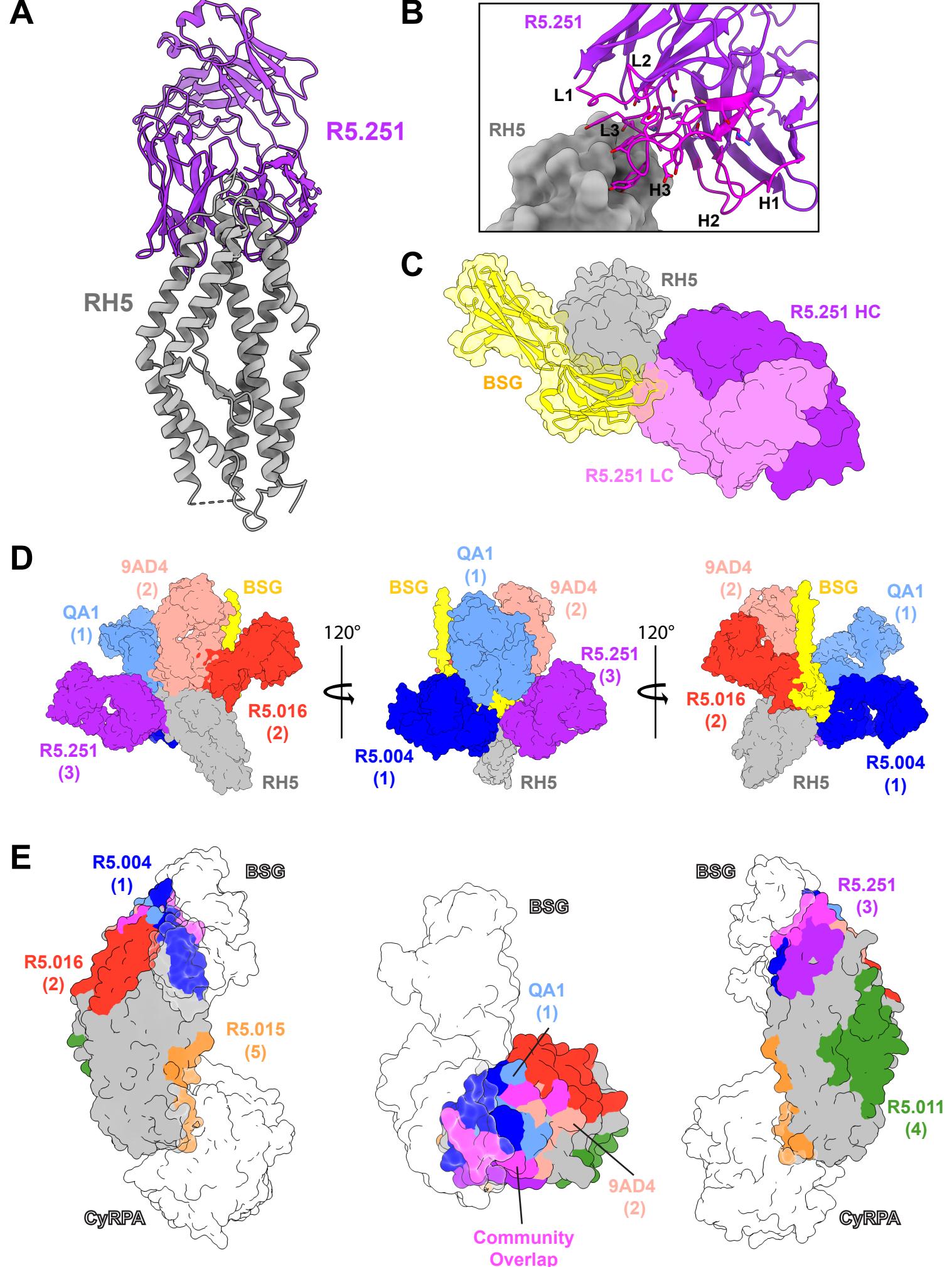


Figure 4: The antigenic landscape of PfRH5.

**(A)** Crystal structure of PfRH5 using RH5 $\Delta$ NL protein (grey) bound to R5.251 Fab fragment (violet). **(B)** Close-up view of the PfRH5 (grey) and R5.251 (violet) binding interface. Complementarity determining regions (CDRs) are highlighted in magenta, and labelled with their identifier, according to IMGT annotation. **(C)** Structure of human basigin (CD147, yellow) (PDB: 4UOQ)<sup>19</sup> aligned to the structure of PfRH5 (grey) in complex with the heavy (violet) and light (pink) chains of R5.251 Fab. **(D)** Structure of PfRH5 (grey) and R5.251 Fab (violet, community 3a) aligned to the structures of Fabs R5.004 (blue, community 1a, PDB: 6RCU); R5.016 (red, community 2, PDB: 6RCU)<sup>24</sup>; QA1 (pale blue, community 1a, PDB: 4U1G) and 9AD4 (pale red, community 2, PDB: 4U0R)<sup>19</sup>; and to basigin (yellow, PDB: 7CKR)<sup>28</sup>. **(E)** Structure of PfRH5 (grey, PDB: 4WAT)<sup>29</sup> colored by the interface residues with Fabs R5.251 (violet, community 3a); R5.004 (blue, community 1a, PDB: 6RCU); R5.011 (green, community 4b, PDB: 6RCV)<sup>24</sup>; R5.015 (orange, community 5b, PDB: 7PHU)<sup>25</sup>; R5.016 (red, community 2, PDB: 6RCU); QA1 (pale blue, community 1a, PDB: 4U1G) and 9AD4 (pale red, community 2, PDB: 4U0R). Interfacing residues used by two or more different communities are highlighted in magenta. Basigin (PDB: 4U0R)<sup>19</sup> and CyRPA (PDB: 6MPV)<sup>20</sup> are shown as silhouettes. The leftmost and rightmost images are flipped 180° relative to one another. The centre images is a top-down view, centred on the apex of PfRH5; PfCyRPA has been omitted from this view.

# Figure 5.

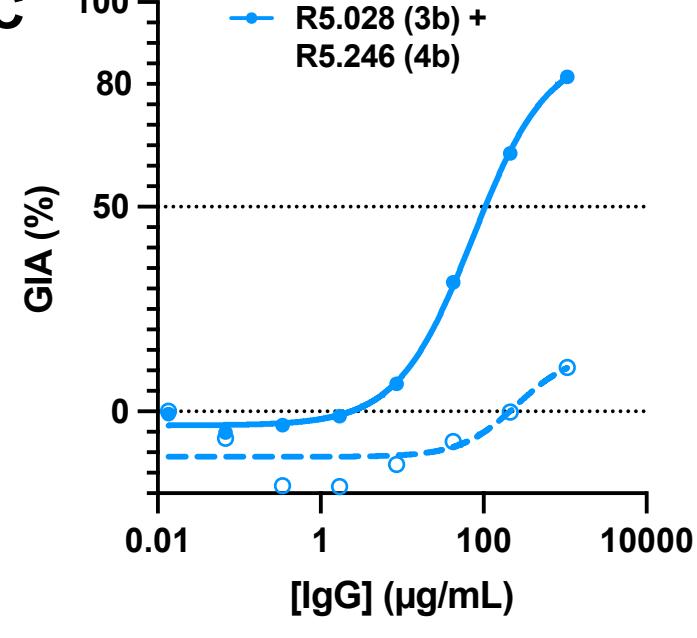
**A**

<b>1a</b> R5.077	5	3	7	36	35	37	11	34	15	36	35	38	-1	17	32	40	29	41	12	4	-1	8	8	
<b>1b</b> R5.204	0	2	7	23	25	28	21	24	23	31	24	29	1	21	42	36	23	36	4	12	2	4	9	
<b>R5.034</b>	4	1	7	10	-28	-22	-29	-22	-13	-23	-27	-21	-12	-6	4	10	-1	9	5	2	8	-7	-5	
<b>R5.102</b>	3	3	7	7	-19	-11	-24	-18	-7	-18	-26	-18	-1	3	-3	0	-9	-1	14	5	10	-4	2	
<b>2</b>	<b>R5.237</b>	7	4	1	8	-22	-20	-25	-19	-4	-18	-24	-22	-4	6	1	0	-8	9	9	5	7	4	7
<b>R5.268</b>	4	5	10	15	-14	-11	-25	-15	-6	-18	-22	-18	-9	1	-5	-5	-7	-6	13	6	8	2	-1	
<b>R5.270</b>	2	-1	-5	28	-22	-16	-21	-15	-15	-13	-16	-7	-5	-2	3	-1	-6	-4	10	11	8	-5	6	
<b>3a</b> R5.251	4	-1	2	-53	26	30	23	19	22	28	27	19	3	21	37	39	22	38	22	18	8	-3	-9	
<b>3b</b> R5.021	3	4	1	-50	-23	-18	9	-58	-43	-37	-35	-37	7	-13	42	39	26	41	19	13	6	-2	7	
<b>nAb</b>			<b>R5.129</b>	<b>R5.149</b>	<b>R5.036</b>	<b>R5.028</b>	<b>R5.020</b>	<b>R5.046</b>	<b>R5.048</b>	<b>R5.063</b>	<b>R5.064</b>	<b>R5.096</b>	<b>R5.103</b>	<b>R5.107</b>	<b>R5.254</b>	<b>R5.261</b>	<b>R5.011</b>	<b>R5.055</b>	<b>R5.236</b>	<b>R5.114</b>	<b>R5.117</b>	<b>R5.058</b>	<b>R5.227</b>	<b>IDL Ab</b>
<b>non-nAb</b>			<b>1a</b>	<b>1c</b>	<b>2</b>	<b>3b</b>	<b>4a</b>	<b>4b</b>	<b>4c</b>						<b>4b</b>	<b>4c</b>	<b>5a</b>	<b>5b</b>	<b>6</b>					

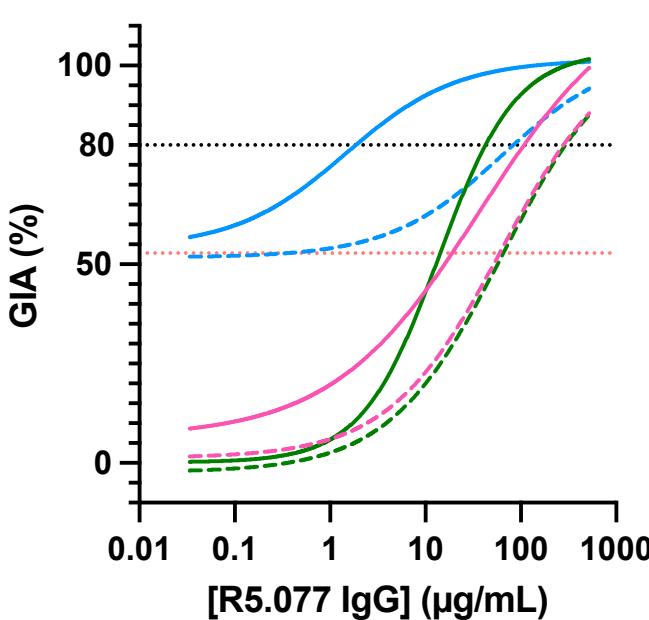
**B**

<b>1a</b> R5.066	41	8	31	31	5																		
<b>1a</b> R5.129	23	2	13	16	4																		
<b>R5.137</b>	49	38	58	64	24																		
<b>1b</b> R5.122	1	40	55	72	44																		
<b>R5.036</b>	13	0	0	0	13																		
<b>2</b> R5.092	58	19	33	31	19																		
R5.097	9	0	9	7	1																		
<b>3b</b> R5.028	X	0	49	66	-7																		
R5.037	-2	4	30	44	2																		
<b>low-nAb</b>			<b>R5.028</b>	<b>R5.020</b>	<b>R5.011</b>	<b>R5.246</b>	<b>R5.117</b>																
<b>non-nAb</b>			<b>3b</b>	<b>4a</b>	<b>4b</b>	<b>4c</b>																	

**C**



**D**



**E**

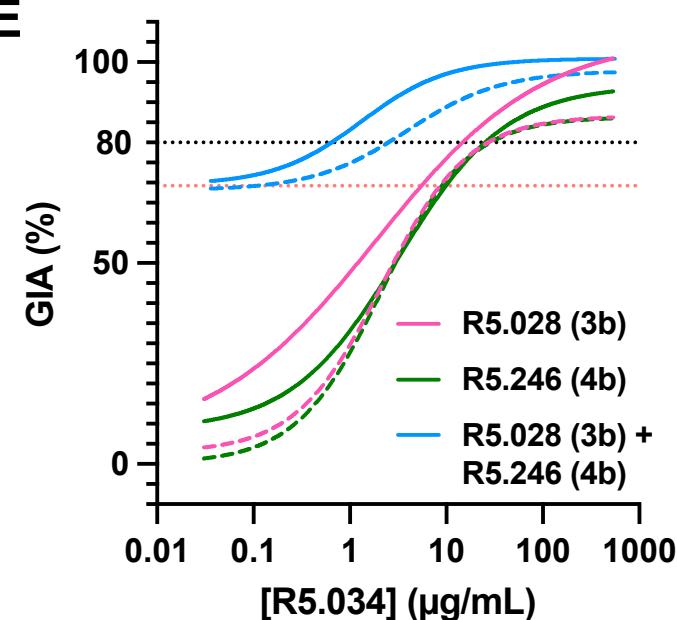


Figure 5: Assessment of intra-PfRH5 antibody synergy.

**(A)** Growth-inhibitory/neutralizing antibodies (nAbs) were tested at a final concentration equivalent to their EC<sub>50</sub> value and non-nAbs were tested at a final concentration of 0.3 mg/mL. The predicted Bliss additivity % GIA was subtracted from the measured % GIA of the test antibody combination, and the difference is plotted as a percentage in the heatmap. Thresholds were used to categorize combinations as synergistic ( $\geq 10\%$ ; red), additive (grey), or antagonistic ( $\geq -10\%$ ; blue). Test mAbs are annotated with their epitope community assignment. **(B)** As for panel **A** except pairs of non-nAbs or mAbs with minimal GIA were combined. Antibodies were tested at a final concentration of 0.2 mg/mL each. **(C)** GIA assay dilution curve of R5.028 (community 3b) in combination with R5.246 (community 4b). Antibodies were combined in an equal ratio and run in a 5-fold dilution curve starting from  $\sim 1$  mg/mL. Data were log transformed and a four-parameter non-linear regression was plotted. The predicted Bliss additivity % GIA of the mixture is shown as a dashed curve. A dotted line is shown at 50 % GIA for reference. **(D)** GIA assay dilution curves of the community 1a nAb R5.077 run in a 5-fold dilution starting from  $\sim 0.5$  mg/mL under various test conditions. Data were log transformed and a four-parameter non-linear regression was plotted. For each curve, a non-nAb (R5.028 from community 3b or R5.246 from community 4b), or a combination of both non-nAbs was added at a fixed concentration of 0.2 mg/mL each. Predicted Bliss additivity GIA curves for each combination are shown as a dashed line. The black dotted line indicates 80 % GIA. The red dotted line indicates the level of GIA measured alone for the fixed concentration combination of the two non-nAbs (R5.028+R5.246). **(E)** Same assay set-up as in **D** except using R5.034 (community 2) in place of R5.077.

# Figure 6.

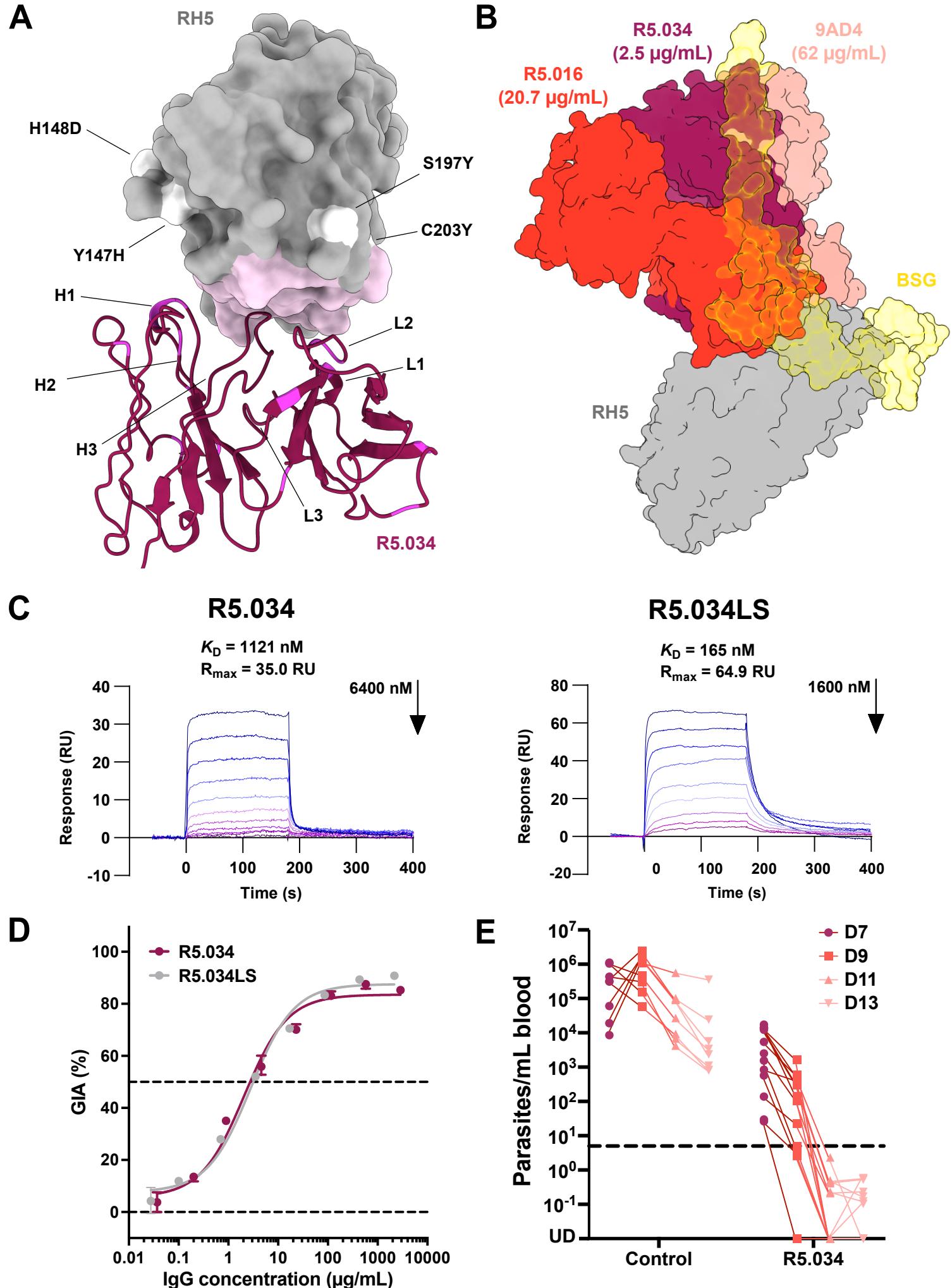


Figure 6: Structure of R5.034 and efficacy against *P. falciparum* challenge.

**(A)** Crystal structure of PfRH5 using RH5 $\Delta$ NL protein (grey) in complex with R5.034 Fv region (maroon). The image is of a top-down view, tilted 10° along the x-axis to view the binding interface on PfRH5 as predicted by PDBePISA, which is coloured in pink. Common PfRH5 polymorphisms are colored white and annotated. CDR loops in the R5.034 structure are labelled with their IMGT identifier. Residues in the HV and LV regions which are mutated from germline are highlighted in magenta. **(B)** Structure of PfRH5 (grey) and R5.034 modelled Fab (maroon) aligned with the community 2 Fabs of 9AD4 (pale red, PDB: 4U0R)<sup>19</sup> and R5.016 (red, PDB: 6RCU)<sup>24</sup> and the basigin ectodomain with transmembrane helix (yellow, PDB: 7CKR)<sup>28</sup>. An AlphaFold predictive model<sup>40,41</sup> of R5.034 Fab was aligned with the experimentally observed R5.034 Fv structure to hypothesize the spatial arrangement of the three antibodies. **(C)** Steady-state affinity, as assessed using SPR, of the R5.034 and R5.034LS mAb binding to human FcRn at pH 6.0. Sensorgrams are shown of a 9-step dilution curve beginning at the indicated concentration of mAb. Calculation of steady-state affinity ( $K_D$ ) at pH 6.0 is shown in **Figure S6B**. **(D)** Titration of the R5.034 and R5.034LS mAbs in the assay of GIA against 3D7 clone *P. falciparum* parasites. Dots show the mean and error bars the range of N=3 triplicate test wells per test mAb concentration. Non-linear regression curve is shown. **(E)** FRG huHep mice were each exposed to the equivalent of five bites using mosquitos infected with N54 strain *P. falciparum*. On day 5 post-infection, mice were administered 675  $\mu$ g control mAb or 625  $\mu$ g R5.034, as well as human RBC, via the intravenous route. Administration of more human RBC was repeated on days 6, 9 and 11. Parasitemia in the blood was monitored on days 7, 9, 11 and 13 by quantitative RT-PCR. Data from individual mice are shown combined from two independent experiments (Control N=8 ; R5.034-treated N=13). Dashed line is the lower limit of detection of the qRT-PCR assay at 5 parasites per mL blood.