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# A high affinity human monoclonal antibody against Pfs230 binds multiple parasite stages and blocks oocyst formation in mosquitoes

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## ABSTRACT

Malaria elimination requires tools that interrupt parasite transmission. Here, we characterized B cell receptor responses among Malian adults vaccinated against the first domain of the cysteine-rich 230kDa gamete surface protein Pfs230[1-3] to neutralize sexual stage *P. falciparum* parasites and halt their further spread. We generated nine Pfs230 human monoclonal antibodies (mAbs). One mAb potently blocked transmission to mosquitoes in a complement-dependent manner and reacted strongly to gamete surface while eight mAbs showed only low or no blocking activity. This study provides a rational basis to improve malaria vaccines and develop therapeutic antibodies for malaria elimination.

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67 **MAIN TEXT**

68 Malaria eradication is a global priority and will require innovative strategies that,
69 in addition to preventing or controlling human infection, might block parasite
70 transmission through mosquitoes. Sequences of matched heavy and light chain variable
71 regions from single human B cells have been used to identify antibodies generated in
72 response to infection or vaccination and inform vaccinology[4-7]. In this study, we apply
73 this approach to examine human antibodies elicited in response to a transmission
74 blocking vaccine (TBV), that used a Pfs230 fragment as antigen. Pfs230 is present on
75 the surface of *P. falciparum* gametocytes and gametes and mediates binding of
76 exflagellating microgametes to red blood cells, thus parasites lacking this protein cannot
77 bind to red blood cells or further develop into oocysts.[1] We collected Pfs230 domain 1
78 (D1)-specific single memory B cells **(Extended Data Fig. 1, Extended Data Fig. 2a)**
79 from eight Malian adults immunized with four doses of Pfs230D1-EPA/Alhydrogel®
80 vaccine (Clinicaltrials.gov NCT02334462) to identify functional monoclonal antibodies
81 elicited in response to a TBV. This vaccine aims to neutralize sexual stage *P. falciparum*
82 parasites by targeting Pfs230, a 230kDa gamete surface protein comprised of fourteen
83 6-cysteine (6-Cys) domains[1-3]. All samples were chosen from subjects presenting high
84 serum Transmission-Reducing Activity (TRA), measured by the capacity of serum
85 antibodies from immunized subjects to reduce the number of oocysts that develop in
86 mosquitoes fed on in vitro cultured *P. falciparum* gametocytes **(Extended Data Table**
87 **1)**.

88 We obtained 272 VH and 351 VL sequences of B cell receptor (BCR) from
89 Pfs230D1-specific single memory B cells from the vaccinees via amplification and

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90 sequencing of the V(D)J region **(Extended Data Fig. 3).** When analysing V gene usage
91 of the BCR sequences, 87.5% of the subjects presented Pfs230D1-specific memory B
92 cells using kappa chains derived from IGKV4-1 **(Extended Data Fig. 2e).** This light
93 chain gene has also been identified in sequences of functional human mAbs obtained in
94 response to other *Plasmodium* antigens[4-6,8]. For the heavy chain, IGHV1-69 was the
95 most commonly expressed gene and detected in 100% (8/8) of vaccinees **(Extended**
96 **Data Fig. 2f)**. IGHV1-69 is one of the most polymorphic loci of the IGHV gene cluster[9]
97 and is frequently found in broadly neutralizing antibodies generated in response to
98 influenza haemagluttinin[10,11].

99 Nine pairs of BCR sequences were chosen for expression of fully human
100 Pfs230D1 IgG1 antibodies by assessing whether the CDR3 sequences were shared
101 between sorted B cells. This approach identifies identical sequences in multiple B cells
102 from the same subject, indicating that they have been activated in response to
103 vaccination. These nine pairs **(Fig. 1a)** represented distinct heavy and light chain
104 germline genes with an overabundance of IGHV1-18 (N=6), IGHV1-69 (N=3), and
105 IGKV4-1 (N=7). The resulting recombinant antibodies bound to Pfs230D1 antigen
106 **(Figure 1d,e, Extended Data Figure 4).** Competitive epitope binning of the nine mAbs
107 suggested they bind three non-overlapping epitopes in Pfs230D1 **(Fig. 1b)**. LMIV230-01
108 forms a distinct group (Group 1) and has potent neutralizing activity **(Fig. 1b, c)**. The
109 remaining mAbs do not compete with LMIV230-01 and may form two additional epitope
110 groups. Group 2 and 3 mAbs possess low or no neutralizing activity **(Fig. 1c)**. We

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111 therefore focused most of our subsequent analyses on LMIV230-01 and to a lesser
112 extent on LMIV230-02, which demonstrated low functional activity.

113 LMIV230-01 and 02 bound to Pfs230D1 recombinant protein **(Fig. 1d)** with
114 strong and similar binding affinities **(Fig. 1e, Extended Data Fig. 4, Extended Data**
115 **Table 2).** We confirmed the two mAbs bind distinct epitopes using competition ELISA
116 **(Extended data Figure 5d)** consistent with the epitope binning results **(Fig. 1b)**.
117 Despite their shared use of IGHV1-69, LMIV230-01 and LMIV230-02 displayed
118 numerous differences in their heavy chain CDRs, consistent with their recognition of
119 distinct epitopes **(Extended Data Figure 12)**.

120 Although presenting similar affinity to Pfs230D1, the mAbs differed in their
121 functional activity as measured by SMFA. LMIV230-01 ablated *P. falciparum* oocyst
122 burden in mosquitoes in a dose-dependent manner with 91.7% neutralization (TRA) at
123 1000 µg/mL **(Fig. 1f).** Importantly, 80.3% neutralization was retained at 60 µg/mL. On
124 the other hand, LMIV230-02 reduced oocyst burden by only 58.7% at the maximum
125 concentration of 1000 µg/mL and activity was lost at 250 µg/mL. As previously reported,
126 TRA values higher than 80% are highly reproducible across independent
127 experiments[12,13].Combining the two antibodies did not increase their overall activity:
128 TRA values were not statistically different when 500µg of LMIV230-02 was combined
129 with 10µg of LMIV230-01 (TRA= 58.7%) versus 10µg of LMIV230-01 alone (TRA=
130 52.5%) in mosquito feeding assays **(Extended Data Figure 5e).**

131 To understand the differences in functional activity of the two mAbs, we
132 assessed binding to the native protein. Both mAbs reacted to the protein extract of

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133 parasites and were sensitive to reduction of the two disulfide-bonds, suggesting the
134 presence of conformational epitopes **(Fig. 1g, Extended Data Fig. 5c**). Interestingly,
135 LMIV230-01 strongly labelled the surface of live *P. falciparum* gametes purified 2 hours
136 post-exflagellation, while LMIV230-02 did not **(Fig. 1h)**. This suggests that the
137 LMIV230-02 epitope is not completely accessible on the surface-displayed native
138 protein, possibly due to structural limitations imposed by the multi-domain protein
139 Pfs230, as has been seen for other proteins[14,15] including another 6-Cys TBV
140 candidate[16].

141

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**a**

| mAb | Heavy chain | Light chain |
|---|---|---|
| **LMIV230-01** | IGHV1-69 | IGKV1-5 |
| **LMIV230-02** | IGHV1-69 | IGKV4-1 |
| **GKV16** | IGHV1-69 | IGKV4-1 |
| **GKV08** | IGHV1-18 | IGKV4-1 |
| **GKV01** | IGHV1-18 | IGKV4-1 |
| **GKV07** | IGHV1-18 | IGKV4-1 |
| **GKV05** | IGHV1-18 | IGKV4-1 |
| **GKV06** | IGHV1-18 | IGKV4-1 |
| **GKV22** | IGHV1-18 | IGKV2-28 |

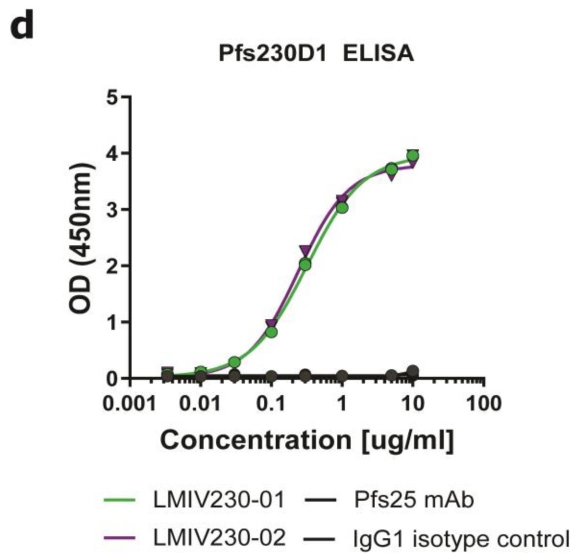
**b**

| | Group 1 | Group 2 | | | | | | Group 3 | |
|---|---|---|---|---|---|---|---|---|---|
| | LMIV230-01 | LMIV230-02 | GKV01 | GKV05 | GKV06 | GKV07 | GKV22 | GKV08 | GKV16 |
| LMIV230-01 | | 95 | 100 | 100 | 99 | 100 | 100 | 100 | 100 |
| LMIV230-02 | 85 | | 10 | 13 | 14 | 18 | 4 | 51 | 60 |
| GKV01 | 100 | 0 | | 1 | 1 | 1 | 3 | 41 | 50 |
| GKV05 | 100 | 0 | 0 | | 0 | 0 | 0 | 40 | 49 |
| GKV06 | 100 | 0 | 0 | 0 | | 1 | 0 | 40 | 51 |
| GKV07 | 100 | 0 | 0 | 0 | 1 | | 0 | 41 | 53 |
| GKV22 | 89 | 0 | 0 | 4 | 3 | 7 | | 38 | 41 |
| GKV08 | 71 | 0 | 0 | 0 | 0 | 0 | 0 | | 18 |
| GKV16 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |

**c**

| TRA% | LMIV230-01 | LMIV230-02 | GKV01 | GKV05 | GKV06 | GKV07 | GKV22 | GKV08 | GKV16 |
|---|---|---|---|---|---|---|---|---|---|
| 375 µg/mL | --- | --- | 20% | 0% | 10% | 10% | 0% | 0% | 0% |
| 250 µg/mL | 85% | 51% | --- | --- | --- | --- | --- | --- | --- |

**d**

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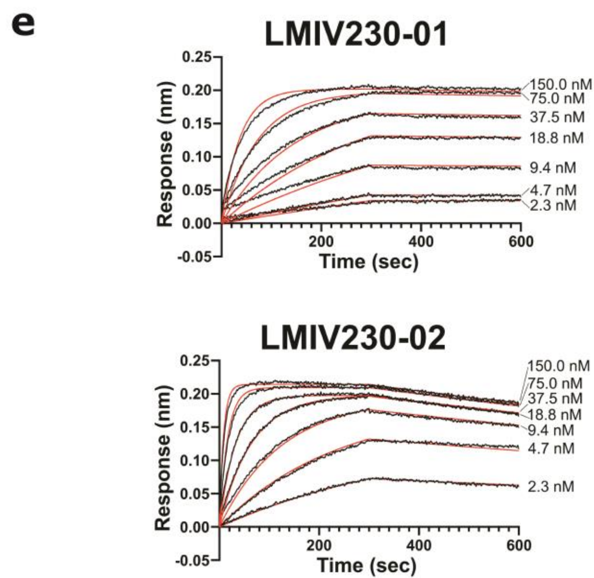
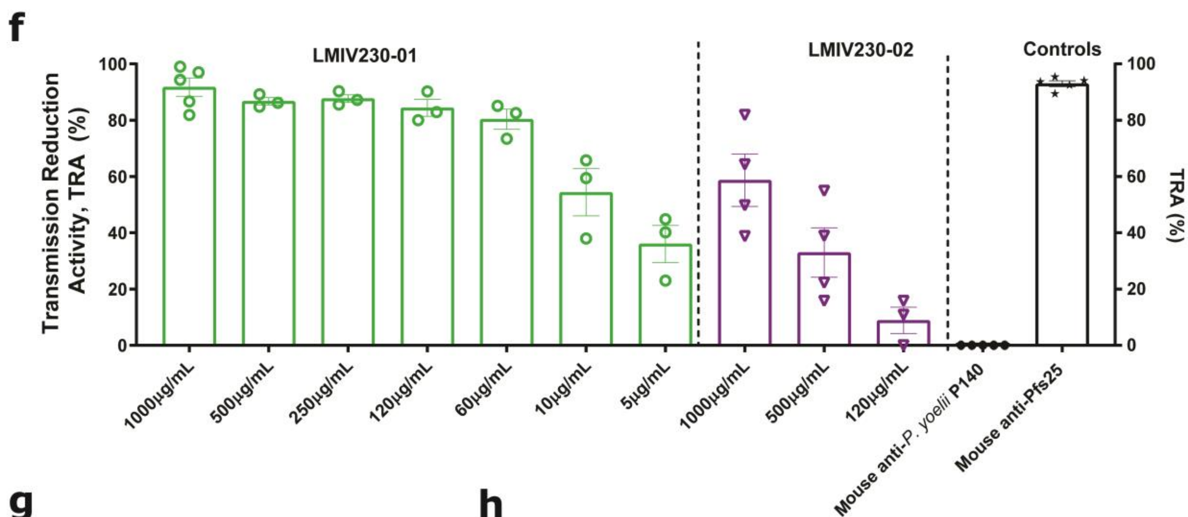
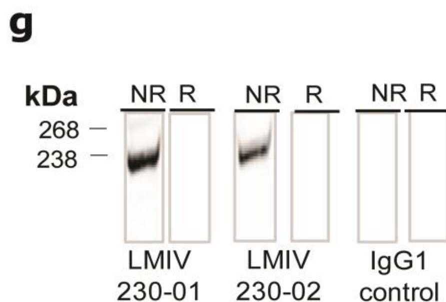



**f**

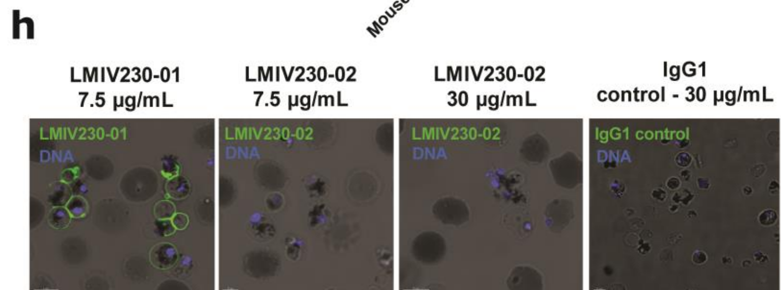



**g**

142




**h**



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143 **Fig. 1| Human recombinant mAbs were generated from Pfs230D1-specific single memory B cells**
144 **of Malian adults vaccinated with the Pfs230D1-EPA/Alhydrogel® TBV. a,** VH and VL genes
145 corresponding to each mAb. LMIV230-01 and LMIV230-02 sequences originate from the IGHV1-69 heavy
146 chain gene but utilize different kappa chain genes. Complete V gene usage determined in Pfs230-specific
147 memory B cells is described in **Extended Data Figures 2e,f**. **b,** Epitope binning of human anti-
148 Pfs230D1scFvs. The primary binding scFv is listed on the left and the competing scFv are listed on the
149 top. Reported scores are a percentage of total binding of that antibody in the absence of a competitor
150 scFv. Values greater that 50% display low amounts of competition, while values lower than 50% exhibit
151 greater competition. Any experiment with >100% binding was given a score of 100, while negative values
152 were given a score of 0. Potential epitope bins are grouped and labelled above the table. **c,** Functional
153 activity of each mAb, assessed by Standard Membrane Feeding Assay (SMFA) and measured as the %
154 reduction (versus control mAb) in the number of *P. falciparum* NF54 oocysts in midguts of *Anopheles*
155 mosquitoes ("TRA"). **d,** LMIV230-01 and LMIV230-02 mAbs bound similarly to Pfs230D1 and **e,** show
156 high affinity to recombinant Pfs230D1 **(Extended Data Fig. 4, Extended Data Table 2)** **f**, LMIV230-01
157 reduces *P. falciparum* NF54 oocyst numbers by 91.7% at 1000 µg/mL, 86.7% at 500 µg/mL, 84.4% at
158 250 µg/mL and 80.3% at 60 µg/mL, while LMIV230-02 displays only modest activity with 58.7% reduction
159 at the maximum concentration of 1000 µg/mL, in SMFA. Data from eleven independent SMFA and each
160 concentration was evaluated in at least three biological replicates for each mAb. N ≥ 20 mosquitos per
161 assay. Average oocyst numbers in the control mosquitoes (fed with mouse IgG1 mAb targeting *P. yoelii*
162 P140 protein) for each experiment were: exp. 1 = 29.73; exp.2= 7.18; exp. 3= 57.86; exp. 4= 36.41; exp.
163 5= 51.71, exp. 6= 4.55; exp. 7= 62.35; exp. 8= 20.50, exp.9= 8.71, exp 10= 18.05, exp. 11= 5.86.
164 Negative oocyst reduction values were set to zero. Human isotype IgG1 and US human serum pool were
165 used as additional negative controls **(Extended Data Fig. 5b)**. Values are shown as mean ± s.e.m. **g,**
166 LMIV230-01 and LMIV230-02 bind to non-reduced (NR) protein extract of *P. falciparum* NF54 gametes
167 purified on Nycodenz after 2 hours in exflagellation medium. **h**, LMIV230-01 strongly binds to gametes at
168 7.5µg/mL while LMIV230-02 does not bind at 7.5µg/mL. or 30µg/mL. Both mAbs were labelled with Alexa
169 Fluor 488. Scale bars: 5µM.

170

171 LMIV230-01 bound strongly to fixed parasites in numerous developmental stages
172 including gametocytes, exflagellation centers, microgametes, macrogametes and round
173 forms (zygotes) collected 4 hours after mosquito feeding. As expected, the mAb did not
174 bind to the post-fertilization stage ookinetes, obtained 24 hours after the mosquito
175 bloodmeal **(Fig. 2a)**.

176 Pfs230 antibody activity depends on complement fixation to lyse *P. falciparum*[17].
177 To test whether the activity of LMIV230-01 was dependent on activation of the
178 complement system, we incubated parasites with LMIV230-01 in the presence of intact
179 or heat-inactivated sera from US donors then assessed lysis of gametes **(Fig. 2b)** as
180 well as transmission of parasites fed to mosquitoes after treatment using the same

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181 conditions **(Fig. 2c)**. Functional activity of LMIV230-01 to lyse gametes and block oocyst
182 formation in mosquitoes was substantially reduced in the heat-inactivated sera **(Figs.
183 2b and 2c)**, demonstrating complement-dependency. Activation of complement leads to
184 the formation of the membrane attack complex (MAC), an assembly of the complement
185 molecules C5b, C6, C7, C8, and C9[18,19] on the parasite surface. Using an antibody that
186 recognizes assembled MAC, we demonstrated complement fixation on the surface of
187 live *P. falciparum* gametes that were incubated with LMIV230-01 in the presence of
188 intact but not heat-inactivated serum **(Fig. 2d and Extended Data Fig. 7).**

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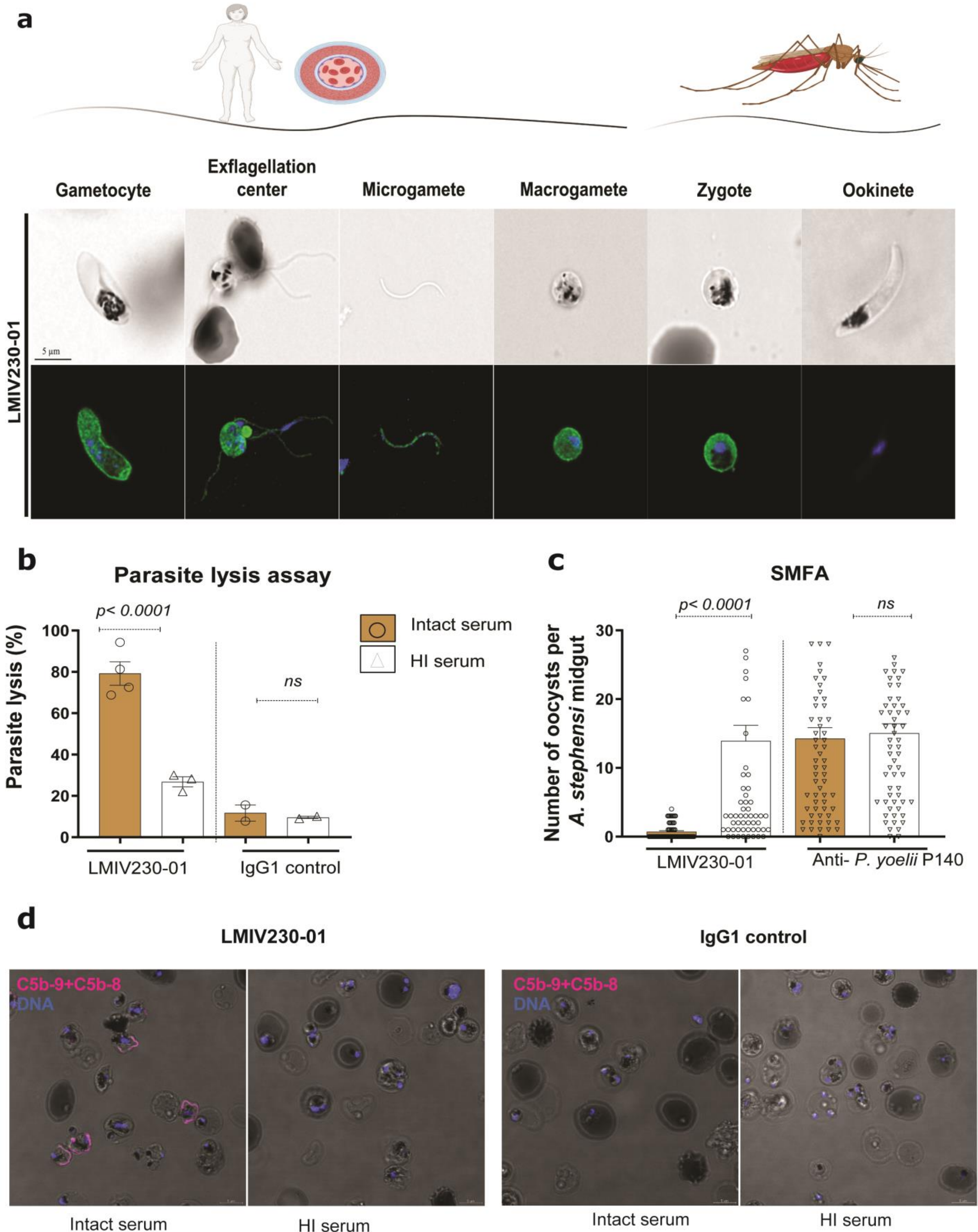

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190 **Fig. 2| LMIV230-01 binds to multiple parasite stages and its activity is complement-dependent. a,**
191 LMIV230-01 strongly binds to permeabilized gametocytes, gametes and zygotes and does not bind to
192 ookinetes. Parasites were fixed and permeabilized, and 7.5 µg/mL of antibody was used to stain the
193 different parasite stages. Scale bars: 5µM. **b,** *In vitro* parasite lysis by LMIV230-01 is complement-
194 dependent. Samples were tested in two independent assays, using two different parasite cultures **c,**
195 Functional activity of LMIV230-01 is also complement-dependent *in vivo* (SMFA with mosquitoes). Data
196 from three independent SMFA assays. N ≥ 20 mosquitos per assay. Oocyst averages in the control
197 mosquitoes (fed with IgG1 targeting *P. yoelli* P140) for each of the experiments were: exp. 1= 4.55; exp.
198 2= 20.50, exp. 3=5.86. Data obtained from mosquitoes fed with LMIV230-01 at 1000 µg/mL with intact
199 sera were also used to generate figure 1f. Values are shown as mean ± s.e.m**.** One-Way ANOVA and
200 Turkey's multiple comparisons test were used to compare the different groups **d,** Live imaging of *P.*
201 *falciparum* NF54 female gametes incubated with LMIV230-01 in the presence of intact serum from a
202 healthy donor revealed surface-deposited MAC (membrane attack complex) using anti-C5b-9+C5b-8
203 antibody (magenta color). MAC deposition was not detected in the presence of heat-inactivated (HI)
204 serum. Scale bars: 5µM.

205

206 To assess whether LMIV230-01 would also bind to other *P. falciparum* strains,
207 we prepared gametocytes from a culture-adapted Malian isolate [20] and from St. Lucia
208 strain (originally from El Salvador) [21]. LMIV230-01 labelled in vitro-induced gametes
209 from both strains **(Fig. 3a and b)**. Induction of gamete stage from the newly
210 characterized Malian isolate was confirmed using a murine anti-Pfs48/45 mAb **(Fig. 3c)**.
211 LMIV230-01 fixed complement on the gamete surface of both strains, confirming that
212 the antibody is functional against heterologous parasites **(Figs. 3d and e).**

213 To assess the abundance of antibodies that share paratope specificity with
214 LMIV230-01, we developed an ELISA assay to demonstrate the competition between
215 post-vaccination sera (tested at a 1:2500 dilution) and LMIV230-01 for binding the
216 vaccine antigen **(Figure 3f)**. Among subjects who received the vaccine, levels of
217 competing antibody ranged from ~10-70% displacement of Pfs230D1 binding to
218 LMIV230-01, with a normal distribution confirmed by Shapiro-Wilk test (p= 0.52) **(Figure**
219 **3g)**. Levels of competition strongly correlated with total Pfs230D1 IgG titers in sera
220 (Spearman's rho= 0.604, p<0.0001) **(Figure 3h**). Increasing levels of competing

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221 antibody also corresponded to serum functional activity measured by SMFA. Because
222 serum TRA levels of vaccines were high with minimal variability ranging from 95-100%
223 **(Extended Data Figure 14)**, our primary correlation analysis used TBA (Transmission
224 Blocking Activity) which indicates the % reduction in the proportion of infected
225 mosquitoes, a high bar for TBV activity generally seen only when TRA is very high.
226 Correlation analyses showed that % serum competition was related to TBA
227 (Spearman's rho= 0.324, p= 0.05) **(Figure 3i)**, suggesting that antibodies that compete
228 for the LMIV230-01 epitope play an important role in serum functional activity. This
229 result, however, does not exclude the possible role of antibodies that do not compete
230 with LMIV230-01 in mediating vaccine activity, and notably some sera with high TBA
231 demonstrated low levels of LMIV230-01 competing antibodies.

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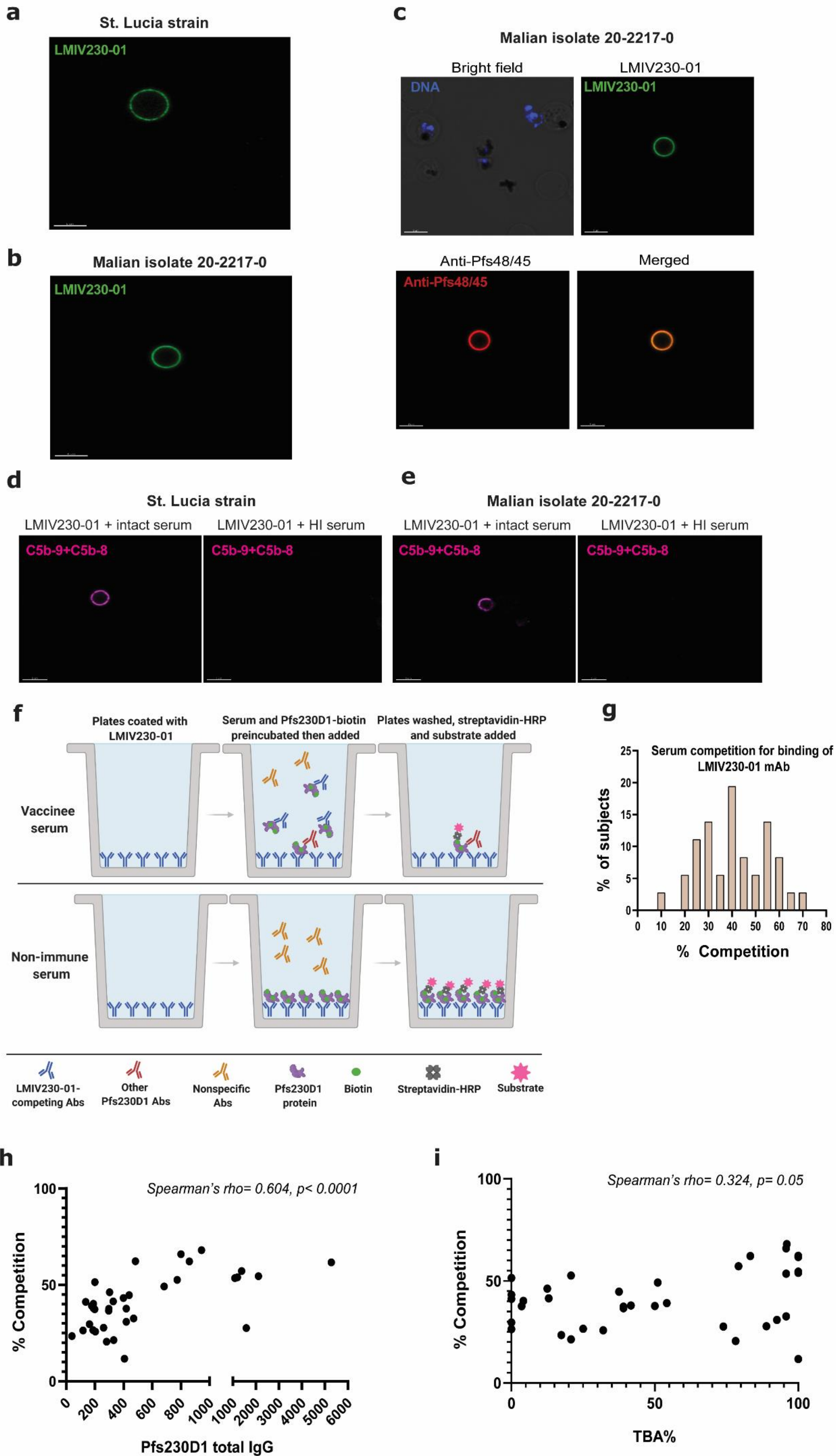




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233 **Figure 3| LMIV230-01 binds to heterologous *P. falciparum* strains and antisera from Pfs230D1**
234 **vaccinees vary widely in levels of antibody that compete with LMIV230-01 for binding. a,** LMIV230-
235 01 bound to gametes of *St. Lucia* parasite strain and **b,** of an isolate obtained from a Malian adult and
236 adapted to culture. **c,** Murine anti-48/45 mAb confirms formation of gametes by Malian isolate and its
237 signal colocalizes with LMIV230-01. “Merged” refers to combination of green and red channels. **d,**
238 Membrane attack complex forms on gametes of St. Lucia strain and **e,** of a Malian isolate incubated with
239 LMIV230-01 in the presence of intact but not heat-inactivated serum**.** Scale bars for all images in this
240 panel: 5μM. **f,** Cartoon schematizing LMIV230-01 competition ELISA assay. **g,** Distribution of serum
241 antibody levels that compete with LMIV230-01 for binding to Pfs230D1 in 36 subjects who received
242 Pfs230D1-EPA vaccine. Values displayed represent mean from three independent experiments. **h,**
243 Relationship of LMIV230-01-competing antibody levels to total Pfs230D1 antibody titers, or **i**, to serum
244 functional activity (TBA, transmission blocking activity) measured by SMFA.

245

246 Altogether, our data confirm that vaccination with TBV can elicit strong
247 neutralizing antibodies, capable of binding to gametocytes, gametes, and zygotes, and
248 of impairing fertilization in the mosquito. Due to its complex domains and repeating
249 motifs with numerous disulfide bonds, expression of full length Pfs230 has been
250 difficult[22,23]. Preclinical studies of Pfs230 fragments have shown that immunization with
251 recombinant domain 1 of Pfs230 (Pfs230D1), but not other domains, induces strong
252 functional TRA in SMFA[3,22,24].

253 Our data support further development of TBV strategies to induce potent
254 antibody responses against mosquito sexual stage parasites.

255

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267

268

269 **AUTHOR CONTRIBUTIONS**

270

271 C.H.C. and P.E.D. conceived the single B cell sorting of Pfs230D1-specific B cells, V
272 gene repertoire analyses, antibody generation, conventional and competitive ELISAs,
273 Western blot, microscopy-based binding assays and in vitro and in vivo functional
274 characterization of mAbs. W.K.T. and N.H.T. conceived the epitope binning and
275 biophysical studies. C.H.C, W.K.T., N.H.T and P.E.D conceived the analysis of
276 polymorphisms. C.H.C., W.K.T., M.B., J.R., A.S., T.A.S., W.P., X.H., B.B., O.M., B. J,
277 M.S. and N.D.S. performed the experiments. M.E., C.H.C., and J.D.G. performed
278 bioinformatic analyses. N.J.M., K.R., V.N., R.H., R.S. and D.N. generated recombinant
279 Pfs230D1. I.S., J.J.T., J.V.R., J.T., J.H., M.B.S, J.R., N.H.T. and P.E.D. supervised the

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experiments and interpreted the data. C.H.C., W.K.T., N.H.T. and P.E.D. wrote the manuscript, with input from all authors.

## COMPETING INTERESTS

M.B.S, W.P., X.H., B.B., and M.E. declare competing financial interests as all are employees of iRepertoire Inc., and J.H. is co-founder and CEO. J.D.G. is an employee of Alchemab Therapeutics Limited.

## CODE AVAILABILITY

Code is available on request from the corresponding author.

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304 ## EXTENDED DATA - FIGURES

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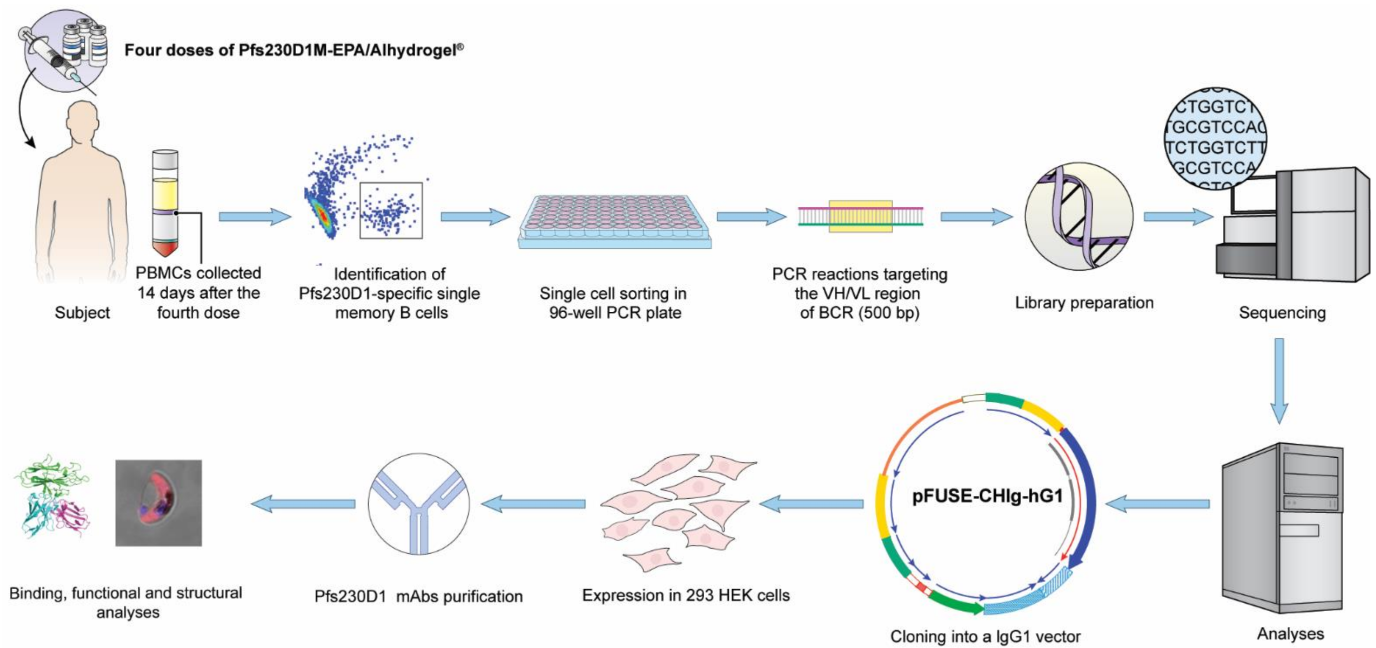


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307 **Extended Data Fig. 1|** Experimental pipeline. Pfs230D1-specific single B cells were sorted from PBMCs
308 of eight Malian adults who had been immunized with four doses of 40µg of Pfs230D1-EPA/Alhydrogel®.
309 After extraction of single B cells, a 500 bp fragment of the BCR variable regions of VH/VL were amplified
310 and sequenced. Matched VH/VL pairs that were identified in more than one B cell were preferentially
311 selected for cloning in an IgG1 vector for expression in 293 HEK cells and subsequent analyses.

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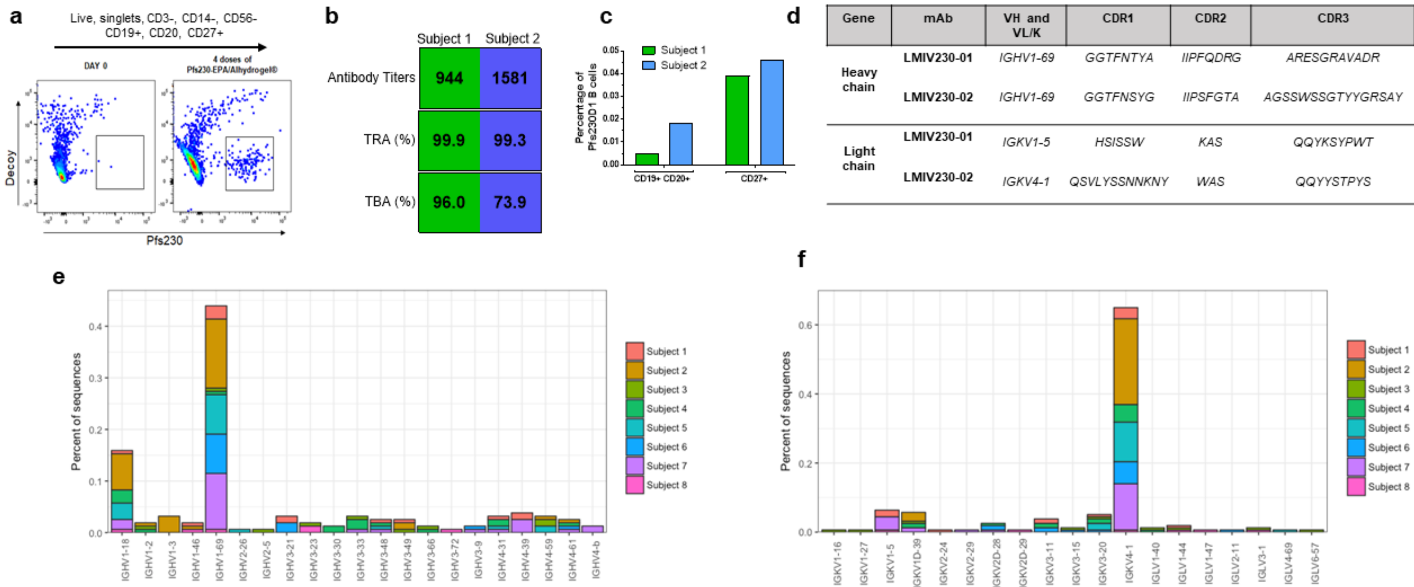


**Extended Data Fig. 2| Pfs230D1-specific mAbs belong to the same heavy chain germline subgroup but differ for kappa chain. a,** Sorted memory B cells were gated as live, single cells, excluded for CD3, CD14 and CD56, and gated on CD19+, CD20+, CD27+ cells. Then, a tetramer approach was used to select antigen-specific cells and reduce nonspecific binding. Cells binding to the decoy tetramer (BSA) were excluded and only those binding to Pfs230D1 were selected for sorting. **b,** Serum from each subject was used to measured antibody titers against Pfs230D1 and functional activity to reduce oocyst burden in Standard Membrane Feeding Assays (SMFA). TRA= Transmission Reducing Activity measured as the reduction in average oocyst count; TBA= Transmission Blocking Activity measured as the reduction in the proportion of infected mosquitoes. **c,** Proportion of memory B cells for each subject that are Pfs230D1-specific. **d,** Complementarity-determining regions (CDRs) of each sequence selected for mAb expression. **e,** IGKV4-1 germline (gene sequence in LMIV230-02) was the most frequent for the kappa chain genes. IGKV1-5 germline (gene sequence in LMIV230-01) was found in only three subjects **f,** Sequences related to germline 1-69 of the IGHV gene were the most frequently elicited in response to the vaccination.

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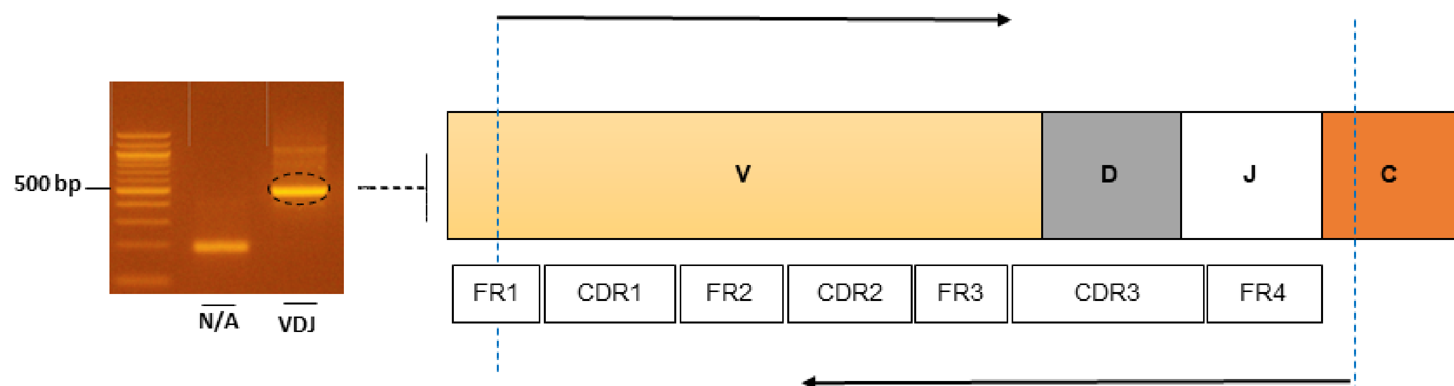


331 **Extended Data Fig. 3| Amplification of V(D)J region.** 500 bp fragment amplified from cDNA of sorted
332 Pfs230D1-specific single B cell. This fragment was obtained using primers targeting the V(D)J region
333 (iRepertoire Inc.).

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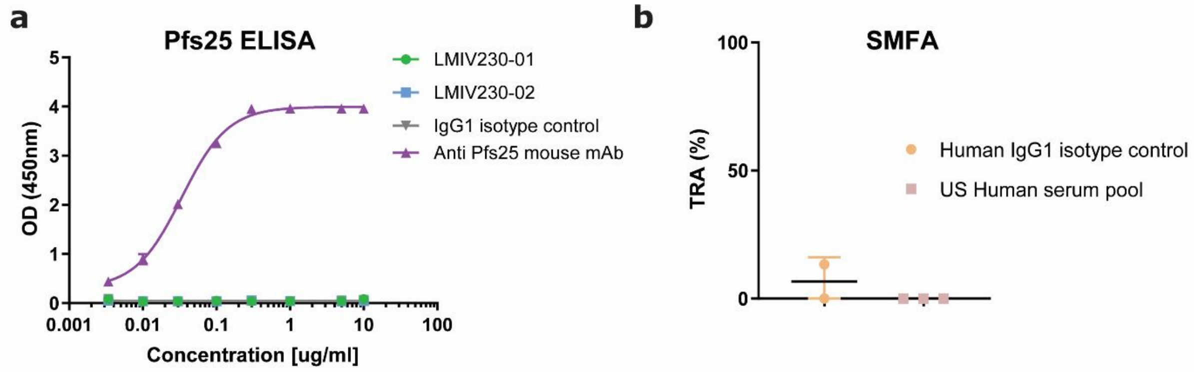


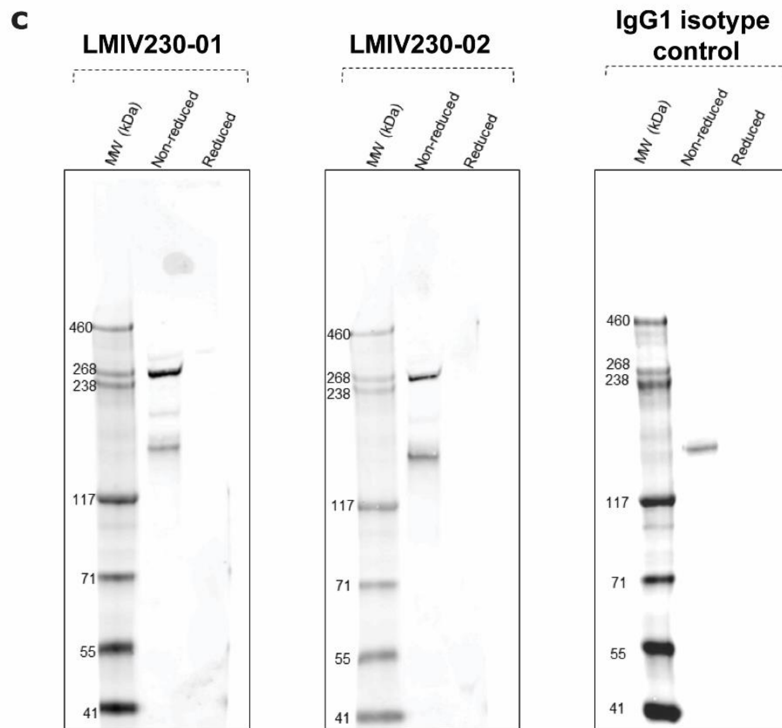


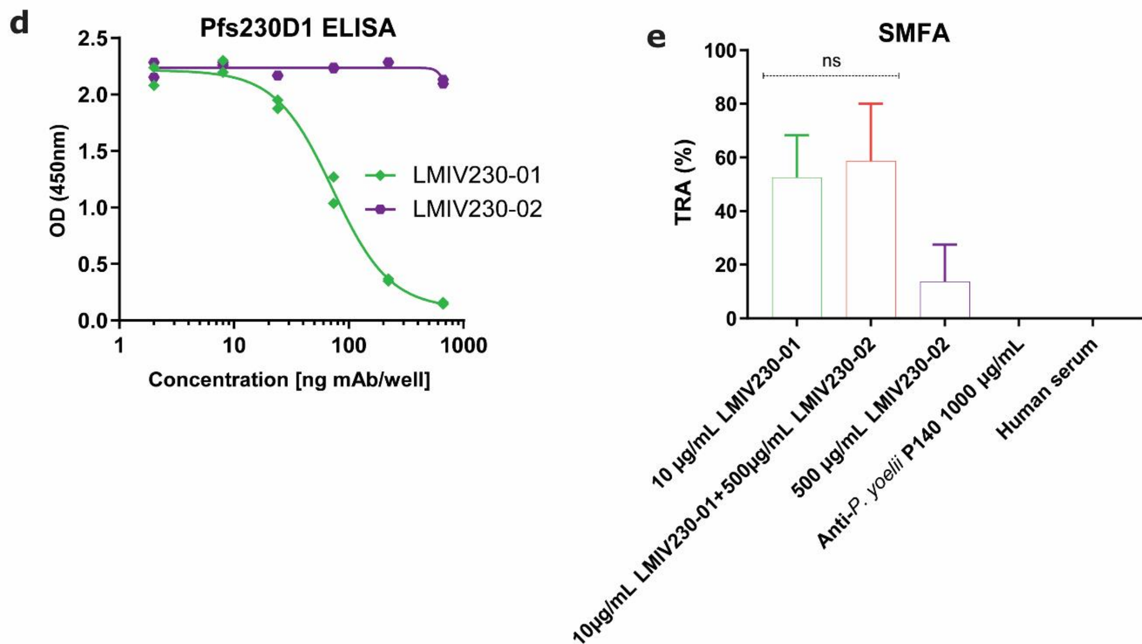

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340 **Extended Data Fig.5| Additional binding and functional characterization of LMIV230-01 and -02. a,**
341 Both mAbs failed to bind to the ookinete protein Pfs25. **b,** Additional controls for the Standard Membrane
342 Feeding Assay (SMFA). Human IgG1 isotype control was expressed using the same conditions as
343 LMIV230-01 and -02 and was used in this assay at 1000µg/mL. Sixty microliters of undiluted human
344 pooled serum obtained from US healthy donors were used as additional control. Values are shown as
345 mean ± s.e.m. **c,** Full depiction of the Western blot gel displayed in Fig. 1g **.d,** The two mAbs do not
346 compete for the same epitope in the recombinant Pfs230D1 protein, since unlabelled LMIV230-01 blocks
347 binding of LMIV-230-01-HRP to immobilized Pfs230D1 but LMIV230-02 does not. **e,** Combination of
348 LMIV230-01 and LMIV230-02 did not increase functional activity over LMIV230-01 alone. Control
349 mosquitoes were fed with mouse IgG1 mAb targeting *P. yoelii* P140 protein, or with non-immune human
350 serum.

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356 *[EXT. DATA FIGURE 6 WILL BE AVAILABLE IN THE PUBLISHED VERSION]*

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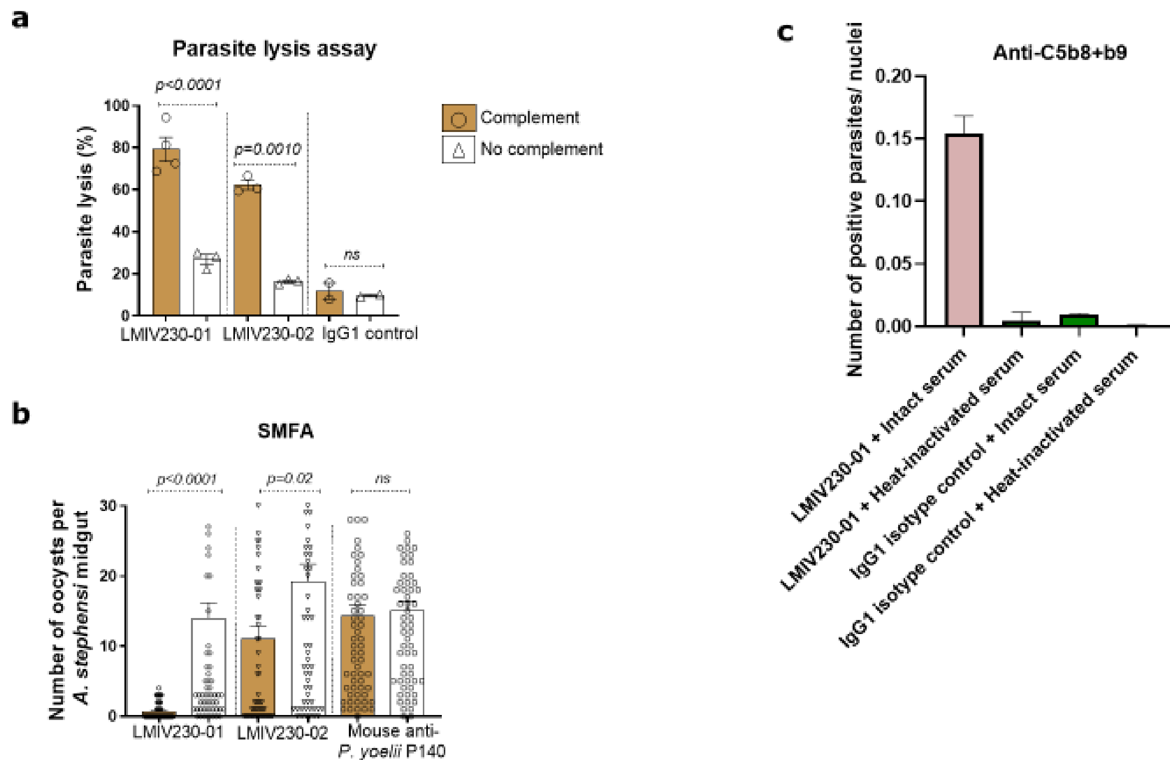


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363 **Extended Data Fig.7| Pfs230 mAbs activity is complement-dependent and LMIV230-01 competing**
364 **antibodies are acquired at varying levels by vaccinees. a,** Activity of LMIV230-01 and LMIV230-02 is
365 complement-dependent in the vitro lysis assay and **b,** in the vivo mosquito feeding assay. **c,** Membrane
366 attack complexes (MAC) on parasites were detected using an Alexa 488-labeled antibody that recognizes
367 the assembled MAC complex (anti C5b-9+ C5b-8). Gametes incubated with LMIV230-01 and intact
368 serum produced MAC-positive parasites. Heat-inactivating serum to degrade the heat-labile components
369 of the complement pathway eliminated deposition of MAC on gametes. MAC-positive *P. falciparum* strain
370 NF54 gametes were enumerated in a large, tiled confocal image and normalized to the number of
371 Hoechst-stained nuclei.

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381 *[EXT. DATA FIGURES 8- 13 WILL BE AVAILABLE IN THE PUBLISHED VERSION*

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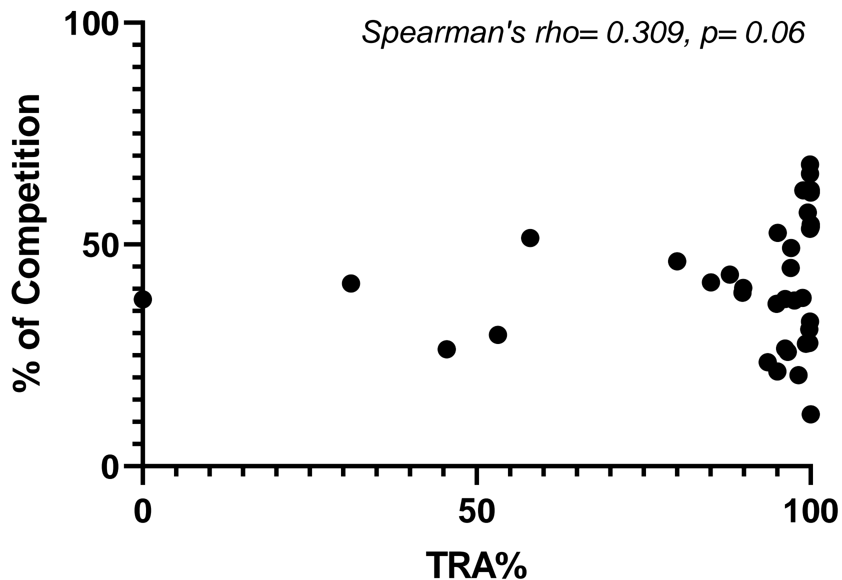


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386 **Extended Data Fig. 14| Correlation between levels of LMIV230-01 competing antibodies and**
387 **Transmission-Reducing Activity (TRA) measured in SMFA.**

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399 **EXTENDED DATA – TABLES**

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| Subject ID | Antibody titers | TRA (%) | TBA (%) |
|---|---|---|---|
| 1 | 944 | 99.9 | 96 |
| 2 | 1581 | 99.3 | 73.9 |
| 3 | 2115 | 100 | 100 |
| 4 | 1382 | 99.6 | 79.2 |
| 5 | 2100 | 100 | 100 |
| 6 | 5277 | 100 | 100 |
| 7 | 800 | 99.9 | 95.8 |
| 8 | 774 | 95.1 | 20.8 |

401

402 **Extended Data Table 1-** Antibody titers and functional activity of sera from the eight subjects whose
403 sequences were analyzed in this study. TRA= Transmission-reducing activity. TBA=Transmission
404 blocking activity.

405

406

| | $K_D$ (x $10^{-10}$ ± SEM M) | $k_a$ (x $10^5$ ± SEM 1/Ms) | $k_{dis}$ (x $10^{-4}$ ± SEM 1/s) | N |
|---|---|---|---|---|
| **LMIV230-01** | | | | |
| **Biological Replicate 1** | 1.58 ± 0.77 | 1.71 ± 0.06 | 0.28 ± 0.15 | 3 |
| **Biological Replicate 2** | 2.06 ± 0.99 | 1.80 ± 0.04 | 0.37 ± 0.18 | 3 |
| **LMIV230-02** | | | | |
| **Biological Replicate 1** | 6.36 ± 0.24 | 7.67 ± 0.21 | 4.87 ± 0.06 | 3 |
| **Biological Replicate 2** | 4.27 ± 0.22 | 6.37 ± 0.13 | 2.71 ± 0.10 | 3 |

407

408 **Extended Data Table 2-** Binding of mAbs LMIV230-01 and LMIV230-02 to Pfs230D1 using Biolayer
409 Interferometry. Binding data for each mAb was fitted using a 1:1 binding model. The averages for two
410 biological replicates, composed of three technical replicates each, are shown for both mAbs.

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413 *[EXT. DATA TABLES 3-7 WILL BE AVAILABLE IN THE PUBLISHED VERSION]*

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