1 **High-throughput profiling of natural acquired humoral immune response to a large** 2 **panel of known and novel full length ectodomain *P. falciparum* merozoite vaccine** 3 **candidates under reduced malaria transmission.**

4 Duncan Ndegwa Ndungu^{1,2}, James³ Tuju, Emily Chepsat³, Rinter Mwai³, Kennedy Mwai³,
5 Lydia Nyamako³, Moses Mosobo³, Awa B. Deme⁴, Baba Dieye⁴, Ibrahima Mbaye Ndiaye⁴,
6 Mouhamad Sy⁴, Mamadou Alpha Diallo⁴, Younous Diedhiou⁴, Amadou Moctar Mbaye⁴, Dyann
7 Wirth⁴, Daouda Ndiaye^{4*}, Faith Osier^{3,6*}, Amy K. Bei^{1, 4, 5*}

8 **Author affiliations**

9 1. Yale School of Public Health, of Epidemiology of Microbial Diseases.

10 2. Department of Biological Sciences, University of Embu, Embu, Kenya

11 3. KEMRI-Wellcome Trust Research Programme, Centre for Geographic Medicine Research
12 —Coast, Kilifi, Kenya

13 4. Université Cheikh Anta Diop de Dakar, Hôpital Aristide Le Dantec, Laboratoire de
14 Parasitologie -Mycologie, Dakar, Senegal

15 5. Harvard TH Chan School of Public Health, Department of Immunology & Infectious
16 Diseases.

17 6. International AIDS Vaccine Initiative (IAVI)-Human Immunology Laboratory,
18 Imperial College, UK

19 * Last authors

20 *Correspondence:*

21 Amy K. Bei

22 amy.bei@yale.edu

23 **ABSTRACT**

24 Despite recent progress in the fight against malaria, it still remains a global health challenge
 25 necessitating development of intervention strategies. However, the search for malaria
 26 vaccine(s) has so far been very challenging. Multiple targets have been tested and so far only
 27 a few show promise with one having been endorsed by the WHO. In this study we explore the
 28 development of immunity in a low transmission setting, with very few documented re-
 29 infections, in order to understand the kinetics of the development and waning of immunity to
 30 current and novel blood-stage vaccine candidate antigens. To do this we performed a high-
 31 throughput measurement of natural acquired immunity against *P. falciparum* antigens utilizing
 32 a well-established micro-array platform based on the mammalian protein expression system.
 33 This large panel of known and novel recombinant full length ectodomain *P. falciparum*
 34 merozoite vaccine candidates were differently recognized by the immune system. Based on
 35 the overall spread of the data, some of these antigens induced the acquisition of high levels
 36 (1st tertile) of antibodies, among which included novel antigens such as PF3D7_1025300,
 37 PF3D7_1105800, PF3D7_1334400, PF3D7_0911300, PF3D7_1252300, PF3D7_1460600,
 38 PF3D7_1453100, PF3D7_0831400 and some induced low levels of antibodies (3rd tertile)
 39 while others induced moderate levels (4th tertile). In this longitudinal cohort with low level of
 40 malaria endemicity, acquisition of humoral immunity to these full length ectodomains *P.*
 41 *falciparum* antigens demonstrate different dynamics over-time, whereby it was either not
 42 acquired or if it was acquired it was either maintained or lost at different rates. These various
 43 identified novel antigens are potentially ideal candidates to be prioritized for further functional
 44 and or serological studies.

45 INTRODUCTION

46 Malaria is a disease caused by parasites of the species *Plasmodium*. Among the five
 47 *Plasmodium* species that can cause malaria in humans, the majority of clinical cases are
 48 caused by *P. falciparum* and *P. vivax* with the former mainly being the major cause of the
 49 malaria cases in Africa. Despite recent progress in the fight against malaria, progress has
 50 plateaued, and malaria remains a global health challenge (World Malaria Report 2019). As of
 51 2019, malaria caused over 200 million cases and more than 400,000 deaths (World Malaria
 52 Report 2020), most of which were African children, therefore necessitating rapid development
 53 of intervention strategies. The complex life-cycle of the human malaria parasites present
 54 multiple potential targets for these intervention strategies. These include targeting the liver,
 55 blood and mosquito stages of the parasite as well as the mosquito vector to prevent disease
 56 and the transmission of the parasite from one infected individual to another. However, this
 57 vector of the malaria parasite continues to develop resistance to pesticides while the complex
 58 biology of this parasite has so far been an obstacle in the development of a permanent cure
 59 for complete elimination of this disease. The malaria parasite continues to develop resistance
 60 to anti-malarial drugs. Despite huge efforts to develop malaria vaccine(s) with multiple targets
 61 having been tested (Reviewed in (Barry and Arnott 2014; Duffy and Patrick Gorres 2020))
 62 only a few show promise: the malaria pre-erythrocytic vaccine RTS,S which was recently
 63 approved for use by the WHO, and its improved version R21 (Datoo et al. 2021) .

64 In malaria endemic countries, repeated exposure to malaria infection from childhood to
 65 adulthood results in natural acquired immunity to malaria disease, but does not result in
 66 sterilizing immunity from infection and antibodies purified from serum obtained from malaria
 67 immune adults has been used to treat non-immune malaria patients (Cohen, McGREGOR,
 68 and Carrington 1961; Sabchareon et al., 1991), providing compelling evidence of the

importance of antibodies in the immune response to malaria that can control parasitemia (Cohen, McGREGOR, and Carrington 1961). Protective immunity to malaria is associated with the recognition of a combination of antigens (Proietti et al. 2020; Bustamante et al. 2017; Daou et al. 2015; Osier et al. 2014; Yman et al. 2020) and development of antibodies to *P. falciparum* antigens play a role in protection from malaria disease (Cohen, McGREGOR, and Carrington 1961). But identifying the dominant protective candidates among these potential 5400 (Gardner et al. 2002) proteins in the *P. falciparum* proteome has remained a challenge. In spite of this and even though during pre-genomic era the number of candidates of interest was always few, various studies managed to identify malarial vaccine candidates (Sirima et al. 2016; Chitnis et al. 2015; Ogutu et al. 2009; Sheehy et al. 2011; 2012; Blard et al. 2011; Thera et al. 2011; Srinivasan et al. 2017; Duncan et al. 2011; Ellis et al. 2012; Biswas et al. 2014; Koram et al. 2016; El Sahly et al. 2010; Lawrence et al. 2000; Genton et al. 2002, 2003; Salamanca et al. 2019; Beeson et al. 2016). But because the focus was on antigens that are generally immunodominant and didn't represent the global parasite population, these candidates didn't succeed mainly due to antigenic diversity and strain specific immunity (Ouattara et al. 2013; Genton et al. 2002, 2003; Thera et al. 2011; Barry et al. 2009; E. et al. 2012; Takala et al. 2009; Barry and Arnott 2014; Bailey et al. 2020). Now with the completion of the sequencing of the malaria parasite genome (Gardner et al. 2002) coupled with the mining of genomics, transcriptomics or proteomic data, its now possible to screen the whole proteome of the malaria parasite to prioritise vaccine candidates (reviewed in (Tuju et al. 2017; Proietti and Doolan 2015)). Indeed, using microarray, various studies have probed the malarial proteome for antigens that could be used for vaccine candidate development, malaria diagnosis, malaria surveillance or to generally understand the immunology of the malaria parasite (Bailey et al. 2020; Camponovo et al. 2020; Liang and Felgner 2015; Yman et al. 2020; Proietti et al. 2020; Zhou et al. 2019; Obiero et al. 2019; Kobayashi et al. 2019; van den

94 Hoogen et al. 2019; Jaenisch et al. 2019; Taghavian et al. 2018; Kamuyu et al. 2018; Uplekar
95 et al. 2017; Helb et al. 2015; Dent et al. 2015; Finney et al. 2014; Baum et al. 2013; Fan et al.
96 2013; Crompton et al. 2010; Doolan et al. 2008; Proietti et al. 2020; Kobayashi et al. 2019).

97 As opposed to the whole malaria parasite antigen which induces rapid and long-lasting
98 antibody, humoral immunity to single antigens develops slowly (Corran et al. 2007). This
99 humoral immune responses to individual malaria antigens display different dynamics of
100 acquisition and decay depending on the intensity of transmission (Ondigo et al., 2014). This
101 immunity can persist over months or years (Corran et al. 2007) followed with decrease over
102 time in an antigen specific manner, making it possible to select biomarkers that can be used to
103 predict recent and distant malaria exposure (Helb et al. 2015). As malaria transmission goes
104 down as we are achieving the goal of regional malaria elimination, more malaria endemic
105 settings will look like the situation in Senegal – low sporadic transmission. Thus this study can
106 teach us about what happens to immunity in a low transmission setting.

107 Previous malaria seroepidemiology utilised cell free protein expression systems such as *E.*
108 *coli* and wheat germ to express *Plasmodium* antigens. But with recent technological
109 advancements the mammalian protein expression system has now been adapted making it
110 possible to overcome some of the previous challenges such as incorrect folding post-
111 translation modification and potential missing of immunodominant epitopes (Crosnier et al.
112 2013; Zenonos, Rayner, and Wright 2014) which could therefore interfere with epitope
113 integrity, antigen presentation and consequently their recognition by the host immune system.
114 In this study our main objective is to study natural immune responses in a low-transmission
115 setting to determine the kinetics of antibody acquisition and decline, without frequent re-
116 exposure. To achieve this we performed a high-throughput measurement of natural acquired

immunity against *P. falciparum*, utilizing the well-established Kilchip micro-array platform (Kamuyu et al. 2018), which is a multiplex large panel of mostly full length protein ectodomains (about 70% in total) of novel and known malaria vaccine candidates that were selected based on the *P. falciparum* merozoite surface localized, secreted and invasion proteome, and then expressed mainly using the mammalian protein expression system. We show that natural immunity to this large panel of known and novel recombinant full length ectodomain *P. falciparum* merozoite vaccine candidates is acquired, maintained, and lost with unique kinetics to what has been described in other settings. Understanding the unique kinetics of antibody acquisition, maintenance, and reversion can inform frameworks for development of the next generation of malaria vaccine candidates.

RESULTS

Study Sites and Patient Demographics

Study participants consisted of a total of 70 male patients with a median age of 11 (5-16) years, median parasitemia on the day of recruitment of 0.43(0.02-4.89)% and with a mean of 1 (0-5) number of past malaria infections (Table 1), described previously (Sy et al. 2020). These participants were recruited from six neighborhoods of the Thiès region of Senegal. These neighborhoods include Diakhao, Nasrou, Takhikao, Thially, Cite Senghor and Escale. The participants were followed for a total period of two years with scheduled follow-up and sample collection done on Days 0, 14, 28, 92, 183, 365, 543 and 730 (Supplementary Table 1). Only 4 participants had malaria re-infections over the 2 year follow-up period, defined based on symptoms and confirmed with RDT and microscopy. These unscheduled visits occurred on days 326, 374 and 397 (Supplementary Table 1).

139 **A large panel of known and novel recombinant full length ectodomain *P. falciparum*** 140 **merozoite vaccine candidates are recognized by the humoral immune system.**

141 To determine the kinetics of antibody acquisition and decline, without frequent re-exposure we
142 utilised the well-established Kilchip micro-array platform (Kamuyu et al. 2018) to perform a
143 high-throughput measurement of antibody recognition of a large panel of mostly full-length
144 protein ectodomains of novel and known malaria vaccine candidates expressed mainly using
145 the mammalian protein expression system against serum from the above cohort. We focused
146 on full length ectodomains selected from the merozoite proteome (Table 2). These selected
147 antigens are localized on different regions of the merozoite (Figure 1) which include: 1.
148 Secreted proteins: PF3D7_0323400 (RIPR), PF3D7_0207900 (SERA2), PF3D7_1334400
149 (MSRP4), PF3D7_0902800 (SERA9), PF3D7_0911300 (MSP3.6), PF3D7_0207700
150 (SERA4), PF3D7_0207400 (SERA7), PF3D7_0207800 (SERA3), PF3D7_1017100
151 (100166), PF3D7_0911300 (MSP3.5), PF3D7_0104200 (PF210C), PF3D7_0424400 (B29),
152 PF3D7_0424400 (B27), PF3D7_0424400 (B30), PF3D7_0424400 (B31) (Zhu et al. 2017;
153 Zenonos, Rayner, and Wright 2014; van Ooij et al. 2013); 2. Surface or GPI-Anchored
154 proteins: PF3D7_0502400 (MSP8), PF3D7_0707300 (RAMA), PF3D7_0102700 (0135W),
155 PF3D7_0206800 (MSP2), PF3D7_0612700 (P12), PF3D7_0930300 (MSP1),
156 PF3D7_1420700 (P113), PF3D7_0620400 (MSP10), PF3D7_0207000 (MSP4),
157 PF3D7_0508000 (P38), PF3D7_1136200 (1136200), PF3D7_0206900 (MSP5),
158 PF3D7_1364100 (PF92), PF3D7_1401600 (1401600) (Crosnier et al. 2013; Zenonos,
159 Rayner, and Wright 2014; Ntumngia et al. 2004); 3. Microneme proteins: PF3D7_1133400
160 (AMA1), PF3D7_0731500 (EBA175), PF3D7_1301600 (EBA140), PF3D7_0102500
161 (EBA181), PF3D7_0405900 (ASP), PF3D7_1028700 (MTRAMP), PF3D7_0828800 (GAMA)
162 (13); 4. Peripheral proteins: PF3D7_1035400 (MSP3), PF3D7_0404900 (P41),
163 PF3D7_1036000 (MSP11), PF3D7_1035500 (MSP6), PF3D7_1228600 (MSP9),

164 PF3D7_1334600 (MSRP3), PF3D7_1335100 (MSP7) (Crosnier et al. 2013); 5. Rhoptry
165 proteins: PF3D7_0424100 (RH5), PF3D7_0402300 (RH1), PF3D7_0212600 (SPATR),
166 PF3D7_0423400 (AARP), PF3D7_0905400 (RHOP3), PF3D7_0302200 (CLAG3.2)
167 (Crosnier et al. 2013; Kalra et al. 2016; Holder et al. 1985); 6. Membrane proteins:
168 PF3D7_1462300 (1462300), PF3D7_1460600 (1460600) (Morse et al. 2016; Poulin et al.
169 2013; Wang et al. 2020); 7. Parasitophorous vacuole membrane: PF3D7_1021800 (PFSEA),
170 PF3D7_1033200 (ETRAMP) (Raj et al. 2014; Spielmann, Ferguson, and Beck 2003); 8.
171 Dense granules: PTEX150 (Bullen et al. 2012) and others with no localization data:
172 PF3D7_0606800 (606800), PF3D7_0616500 (TLP), PF3D7_1345100 (1345100),
173 PF3D7_1453100 (145300), PF3D7_1105800 (1105200), PF3D7_0925900 (925900),
174 PF3D7_0525800 (525800), PF3D7_0830500 (83500), PF3D7_1237900 (1237900),
175 PF3D7_0206200 (206200), PF3D7_1229300 (1229300), PF3D7_1025300 (1025300),
176 PF3D7_1252300 (1252300), PF3D7_0419700 (PF34), PF3D7_0831400 (831400),
177 PF3D7_1334300 (MSRP5), PF3D7_1137300 (113700), PF3D7_0730800 (730800.2). These
178 antigens were expressed using the mammalian protein expression system then they were
179 printed on micro-array slides and detected as described in (Kamuyu et al. 2018) against a
180 panel of whole serum donated by malaria infected children with low previous malaria infection
181 history resident in a lowly malarial endemic region of Senegal (Table 1). The resulting
182 reactivity measured as median fluorescent intensity (MFI) relative to mean MFI plus 2
183 standard deviations of non-immune control serum varied from strong to weak with an average
184 of 0.666104 (range -0.88-2.34) with the respective antigens being lowly, averagely or highly
185 recognized by the test serum (Figure 2A and 2B).

186 **Full length ectodomains *P. falciparum* merozoite vaccine candidates induce three** 187 **distinct patterns of antibodies acquisition.**

188 To measure antibody acquisition to *P. falciparum* antigens, the MFI of each test protein
189 (Figure 2A) as recognized by test serum per each contact day was divided by mean MFI plus
190 2 standard deviations of non-immune control serum. Samples with values above 1 were
191 considered as seropositive while samples with values below 1 were considered as
192 seronegative. This indicates that some antigens are able to induce antibody acquisition to
193 malaria infections while some are not. To measure the levels of these acquired antibody
194 against *P. falciparum* antigens in the sampled population and in order to determine the most
195 immunogenic antigen, the total number of sero-positive serum samples per each protein over
196 the whole study period was determined. These values were then ranked from highest to
197 lowest, divided into three groups based on the MFI tertiles and labeled respectively as 1st, 2nd
198 and 3rd tertile (Figure 3A and supplementary table 2). This analysis was done in the absence
199 of the individuals with re-infection, thus all data is from a single infection or boosting alone.

200 With a mean cumulative percent sero-positivity of 44.40% (range 16.82-81.25%) the proteins
201 in the 1st tertile were the most reactive, followed with those in the 2nd tertile with a mean
202 cumulative percent sero-positivity of 7.79%(range 3.00-16.13%) while those in the 3rd tertile
203 were least reactive with a mean cumulative percent sero-positivity of 1.06%(range 0.00-
204 2.50%) (supplementary table 2). Antigens in the highest tertile included known dominant
205 serodominant antigens (AMA-1, MSP-1, other MSPs, RHOP3, SERAs) any of which are
206 merozoite surface antigens, in addition to previously un-explored targets (1105200, MSRP4,
207 MSP3.5, 1252300, SERA2, 1025300). Proteins in the second tertile included invasion ligands
208 (RIPR, RH5), components of a translocon (PTEX150), PVM components (PFSEA, ETRAMP),
209 and previously unexplored targets (145300, 1460600, 831400, 206200, 925900, B29).

Proteins in the third tertile included previously unexplored targets (1229300, 1462300, 1237900, 730800.2, 113700, 525800, B30, 1345100, 83500, B31 and B27), redundant/dispensable antigens (P12, RH1, 1401600) and others (0135W, MSRP5, MSRP3, MTRAMP, PF92, MSP7, TLP, GAMA).

To measure change in the levels of these acquired antibodies in this cohort we determined the percentage of serum samples that were sero-positive per test proteins per each contact day during the study period. This was done separately using a) samples without malaria reinfection and b) samples without malaria re-infection and with malaria re-infection. The percent seropositive test serums per each contact day over the whole study period varied in an antigen specific manner that could be generalized into three groups based on the above observed tertiles (Figure 3B). Antigens falling under the 1st tertiles (Figure 3A) generally had the highest percent sero-positivity (Figure 3B and supplementary figure 1A) and antigens falling under the 3rd tertiles (Figure 3A) generally had the lowest percent sero-positivity (Figure 3B and supplementary figure 1A) while those in the 2nd tertile (Figure 3A) generally had moderate percent sero-positivity (Figure 3B and supplementary figure 1A). Confirmed re-infection with *P. falciparum* did not have a major influence on percent sero-positivity (supplementary figure 1B). Only in 28% of the antigens did boosting of percent sero-positivity occur (supplementary figure 1C). These antigens include 1RH1, 4MSP2, P38, 5MSP3, ETRAMP, 606800, SPATR, PF210C, 1025300, 1SERA2, AMA1, 1460600, 8MSP4, P113, 5SERA9, 4SERA, P41, 1PF34, 3MSP11, 3SERA4, RHOP3. The boosting in majority of these antigens occurred between day 300 and 400. In the remaining few, boosting had a different pattern. In 8MSP4 it occurred through-out the study period, in RHOP3 it occurred from the beginning of the study period to day 400, in AMA1 it occurred between day 100 and around

233 day 325, in ETRAMP P41 and P113 boosting occurred at multiple time-points between day 0
234 and day 400.

235 **Kinetics of naturally acquired antibodies to next generation malaria vaccine targets**

236 Longevity of antibodies to *P. falciparum* antigen is antigen specific (Akpogheneta et al. 2008).
237 Therefore, to determine how this natural acquired humoral immunity to malarial antigens
238 lasts, we determined antigen specific sero-conversion by comparing antigen specific sero-
239 status (Figure 4A) during Day0 and Day14, Day0 and Day365, Day0 and Day730 as
240 indicators of short and long-term antigen specific immunity respectively. Test serum exhibited
241 three different dynamics towards these antigens, some serum samples did not change their
242 serostatus while others changed their serostatus from either seropositive to seronegative or
243 from seronegative to seropositive after 14, 365 or 730 of follow-up days relative to day 0
244 (Figure 4A and 4B). This change was directly proportional to the total cumulative seropositivity
245 as observed for the protein tertiles (Figure 3B). Proteins in the 1st tertile changed the most;
246 with a mean percent sero-positivity of 43.45% (range 17.19-81.25%), 46.55% (range 18.25-
247 82.48%), 38.25% (range 11.76-75.47%), 32.83% (range 12.12-74.19) on day0, day14,
248 day365 and day730 respectively, and with a mean seroconversion of 3.10% (range -8.00-
249 21.50), -5.20% (range 6.7-7), -10.62% (range -31.25-15.625) from day0 to day14, day0 to
250 day365 and day0 to day730 respectively (supplementary table 2). While proteins in the 2nd
251 tertile changed moderately; with a mean percent sero-positivity of 7.62% (range 0.00-
252 20.31%), 7.58% (range 3.00-17.83), 7.20% (range 0.00-21.15%), 7.04% (range 0.00-35.48%)
253 on day0, day14, day365 and day730 respectively, and with a mean sero-conversion of -
254 0.037%(range -5.09-5.31%), -0.42%(range -6.37-7.09), -0.57%(range -18.75-24.54%) from
255 day0 to day14, day0 to day365 and day0 to day730 respectively (supplementary table 2).
256 Proteins in the 3rd tertile changed the least; with a mean percent sero-positivity of 0.56%

(range 0.00-3.13%), 0.775194%(range 0.00-3.10%), 1.92%(range 0.00-6.00), 2.23%(range 0.00-9.38%) on day0, day14, day365 and day730 respectively, and with a mean sero-conversion of 0.59%(range -1.38-2.94%), 1.36%(range -1.56-6.00%), 1.67%(range -3.13-9.38) from day0 to day14, day0 to day365 and day0 to day730 respectively (supplementary table 2). This shows that humoral immune response against *P. falciparum* antigens change over time in an antigen specific manner that can be generalized into 3 patterns (Figure 4B); immunity to some malaria antigens is acquired quickly and maintained (high percent sero-positivity on day0, day14, day365 and day730 with low or no sero-reversion during day0-day14, day0-day365, day0-day730), or is acquired slowly and not maintained (low percent sero-positivity on day0, day14, day365 and day730 with sero-reversion during day0-day14, day0-day365, day0-day730) or is acquired moderately and maintained or lost (moderate percent sero-positivity on day0, day14, day365 and day730 with moderate or no sero-reversion during day0-day14, day0-day365, day0-day730) (Figure 4B). Antigens that quickly induced immunity included 8MSP4,AMA1, 5MSP3, 606800, 1EBA140, 5SERA9, 1MSP1, P113, PF210C, P41, 13MSP9, 3MSP11, 10MSP6, 2EBA175. Among these, immunity to 8MSP4, AMA1, 1EBA140, 1MSP1, P113, PF210C, P41, 13MSP9, 3MSP11, 10MSP6, 2EBA175 was lost to varying degrees while immunity towards 5MSP3, 606800, 5SERA9 was mostly maintained.

DISCUSSION

Using a low transmission setting with few or no malaria re-exposure we set out to study natural immune response in the absence of immune exposure by determining how long immunity to various malarial antigen takes to develop and get lost. Our main goal was to reduce the complications resulting from the influence of potential prevailing immunity from past malaria exposure in a high malaria transmission setting that limit the study of antigen

specific dynamics. Furthermore, because cell free protein expression systems, such as *E. coli* and wheat germ are likely to result in incorrect folding and post-translation modification of proteins expressed this way and if coupled with the usage of protein constructs covering protein fragments as opposed to whole proteins, these approaches are highly likely to miss other immunodominant epitopes. All together, these limitations could therefore interfere with epitope integrity, antigen presentation and consequently their recognition by the host immune system. In this study we set out to over-come these limitations by using serum samples obtained from a longitudinal cohort in low malaria transmission setting to perform a high-throughput measurement of natural acquired immunity against *P. falciparum* by utilizing the well established Kilchip micro-array plat-form (Kamuyu et al. 2018), which is a multiplex large panel of mostly full length protein ectodomains (about 70% in total) of novel and known malaria vaccine candidates that were selected based on the *P. falciparum* merozoite surface localised, secreted and invasion proteome (Crosnier et al. 2013; Zenonos, Rayner, and Wright 2014), and then expressed mainly using the mammalian protein expression system. Acquisition of humoral immunity to these full length ectodomains *P. falciparum* antigens demonstrate different dynamics over-time, falling into three categories of the reponse: Highly reactive, moderately reactive and least reactive.

The most reactive antigens inducing high levels of antibodies towards them included 1105200, MSRP4, MSP3.5, 1252300, SERA2, 1025300, EBA140, EBA175, EBA181, MSP3, PF210C, AARP, P113, AMA1, SERA4, MSP2, RHOP3, 606800, SERA9, MSP1, MSP4, MSP10, MSP11, P41, MSP6 and MSP9. There was no sero-reversion towards SERA2, MSP3, SERA4, MSP2, 606800 and SERA9, but only by day 730 for 606800 and SERA9, suggesting that immunity to these antigens is acquired quickly and maintained. Indeed, SERA4 (Zenonos, Rayner, and Wright 2014), 606800 (Osier et al. 2014), SERA2 (Zenonos,

Rayner, and Wright 2014), SERA9(Bustamante et al. 2017; Zenonos, Rayner, and Wright 2014), MSP2 (Osier et al. 2014; Richards et al. 2013) and MSP3 (Osier et al. 2014; Sirima et al. 2016) have all been associated with strong protection against malaria. However, MSP3 (Mills et al. 2002), SERA2 (Miller et al. 2002), SERA4 (McCoubrie et al. 2007) and SERA9 (Bustamante et al. 2013) are dispensable during blood-stage. There was sero-reversion towards MSP3.5, 1025300, EBA175, EBA181, P113, RHOP3, MSP6 and MSP9 after day 14 suggesting that immunity to these antigens is acquired quickly and lost slowly. For AMA1, EBA140, 1252300, 1105200, MSRP4, PF210C, AARP, MSP1, MSP4, MSP10, MSP11 and P41 sero-reversion occurred by day 14 suggesting that immunity to these antigens is acquired quickly and lost quickly. While RHOP3 is associated with strong protection against malaria (Osier et al. 2014), MSP1, MSP4, MSP10, MSP6 and MSP9 (Osier et al. 2014; Richards et al. 2013) have all been associated with weak protection against malaria. The rest have a mixed association with protection against malaria, especially the erythrocyte binding antigens (EBAs): Moderate to strong association: EBA140 RIII-V, AMA1 and P113 (Osier et al. 2014; Richards et al. 2013). Weak to moderate: EBA175 RIII-V EBA175 RII, EBA181 RIII-V (Osier et al. 2014; Richards et al. 2013). Weak to strong: MSP11 and P41 (Osier et al. 2014; Richards et al. 2013). Infact P41 is also dispensable during blood-stage (Taechalertpaisarn et al. 2012). AARP has low invasion inhibitory activity as part of a Triple Chimeric Antigen of *Plasmodium falciparum* AARP, MSP-3 11 and MSP-1 19 (Kalra et al. 2016) .

Moderately reactive antigens inducing moderate levels of antibodies towards them included 145300, 1460600, 831400, PFSEA, 100166, RIPR, MSP8, RH5, SPATR, P38, 1136200, PF34, PTEX150, MSP5, ETRAMP, ASP, RAMA, 206200, MSP3.6, 925900, B29, SERA7 and SERA3. Among these antigens RH5, PFSEA, 1460600, P38, 1136200, SERA7, SERA3, MSP3.6, PF34, ASP, 925900 and B29 had no sero-reversion towards them, but only by day

730 for SERA3, MSP3.6, PF34, ASP, 925900 and day 14 for B29, suggesting that immunity to these antigens is acquired moderately and maintained. Sero-reversion occurred by day 14 towards 145300, 831400, 100166, RIPR, MSP8, SPATR, PTEX150, MSP5, ETRAMP, RAMA and 206200 suggesting that immunity to these antigens is acquired moderately and lost quickly. While 1136200 is strongly correlated with protection against malaria (Osier et al. 2014), P38, RH5 (Osier et al. 2014; Richards et al. 2013) and RIPR (Richards et al. 2013; Bustamante et al. 2013; Reddy et al. 2015) are all weakly to moderately correlated with protection against malaria. PF34 (Osier et al. 2014; Richards et al. 2013), PTEX150, MSP5, ETRAMP, ASP (Osier et al. 2014) and RAMA (Reddy et al. 2015) are all weakly correlated with protection against malaria. PFSEA is associated with protection to *P. falciparum* infection, antibodies against it inhibit egress of schizonts (Raj et al. 2014). 100166 is immuno-reactive and its antibodies inhibit merozoite invasion into erythrocytes (Ito et al. 2019). MSP8, SERA7, SERA3 (Zenonos, Rayner, and Wright 2014) and SPATR (Crosnier et al. 2013), are immuno-reactive towards serum from malaria infected individual. However, SERA7, SERA3 (Miller et al. 2002) and 100166 (Knuepfer et al. 2014) are all dispensable during blood-stage.

The least reactive antigens inducing the lowest levels of antibodies towards them included 1229300, 1462300, 1237900, 730800.2, 113700, 525800, 0135W, MSRP5, MSRP3, RH1, MTRAMP, PF92, MSP7, TLP, P12, GAMA, B30, 1401600, 1345100, 83500, B31 and B27. Among these antigens, 1237900, 113700, 525800, 0135W, MSRP5, MSRP3, RH1, MTRAMP, MSP7, TLP, P12, GAMA, 1401600, 1345100, 83500, B27 and B31 had no sero-reversion towards them suggesting that immunity to these antigens is acquired slowly and maintained at low levels while for 1462300, 730800.2, PF92 and B30 sero-reversion occurred by day 14 suggesting that immunity to these antigens is acquired slowly and lost quickly. While 1229300 is strongly associated with protection against malaria infection (Venkatesh et al. 2019) most

353 of these antigens indeed are weakly correlated with protection against malaria: MTRAMP,
 354 PF92, TLP (Osier et al. 2014), MSP7, GAMA, P12 (Osier et al. 2014; Richards et al. 2013)
 355 and MSRP3 (Osier et al. 2014; Crosnier et al. 2013). Infact P12 (Taechalerpaisarn et al.
 356 2012), RH1 (Triglia et al. 2004) and 1401600 (Knuepfer et al. 2014) are actually dispensable
 357 during blood-stage. 0135W is immunogenic but lowly recognised (Ntumngia et al. 2004).
 358 MSRP5 is immunoreactive towards serum from malaria infected individuals (Zenonos,
 359 Rayner, and Wright 2014).

360 Among the above three categories of these antigens, some don't have longitudinal/serological
 361 data and association about them with malaria protection is not known but some have been
 362 demonstrated to perform or have been implicated with certain biological processes. These
 363 are: 1025300 present in a complex of multiple apicoplast proteins (Mallari et al. 2014),
 364 83500, its synthetic peptides have been shown to inhibit merozoite invasion suggesting that it
 365 functions in merozoite invasion (Curtidor et al. 2006), B27/B29/B30/B31, bind to the RBC
 366 membrane skeleton, important for the translocation from Maurer's clefts to the iRBC surface
 367 (Zhu et al. 2017), 206200, is a pantothenate transporter and it is essential in parasite
 368 development (Augagneur et al. 2013), 925900, is essential for hemozoin formation (Joachim
 369 M. Matz et al. 2020; Joachim Michael Matz and Matuschewski 2018), 1345100, is an auxiliary
 370 subunit of PTEX (the Plasmodium translocon of exported proteins), it functions in protein
 371 export (Egea 2020; Peng, Cascio, and Egea 2015), 1105200, is associated with mRNA-
 372 binding in trophozoite and schizont-stage parasites as well as detergent-resistant membrane
 373 microdomains in trophozoite blood stage (Bunnik et al. 2016; Yam et al. 2013), MSRP4, it is
 374 predicted to be involved in invasion (Ngwa et al. 2017), 1460600, is apart of the IMC sub-
 375 compartment proteins (ISPs) together with ISP1 and it is also associated with cytoskeleton in
 376 *P. falciparum* female gametocyte (Miao et al. 2017; Poulin et al. 2013; Wang et al. 2020),

145300, is associated with Cytoskeleton in *P. falciparum* female gametocyte (Miao et al. 2017), 1237900, it's transcription changes in absence of SEMP1 (Dietz et al. 2014), 525800, it's *P. vivax* ortholog (PVP01_1008000), is among the highly abundant proteins identified in *P. vivax* salivary gland sporozoites (Swearingen et al. 2017) and 1462300, associates with PfRab1 bodies likely being translocated to rhoptries (Morse et al. 2016). Others have not been demonstrated to be involved in or implicated with specific biological processes, these are: MSP3.5, 1252300, 831400, 113700 and 730800.2. Out of these MSP3.5, 1252300, 831400 were among the most reactive proteins, making them high priority candidates ideal for further functional investigation to understand their role in merozoite biology.

Malaria blood stage vaccine candidates that have been studied before individually or in combination with other proteins as part of pre/clinical vaccine trials, such as MSP2 (Lawrence et al. 2000; Genton et al. 2002, 2003), PFSEA (Raj et al. 2014), MSP1 (Ogutu et al. 2009; Chitnis et al. 2015; Sheehy et al. 2011; 2012), MSP3 (Bélard et al. 2011; Sirima et al. 2016), AMA1 (Sheehy et al. 2011; Thera et al. 2011; Srinivasan et al. 2017; Duncan et al. 2011; Ellis et al. 2012; Biswas et al. 2014), EBA175 (Koram et al. 2016; El Sahly et al. 2010) and RH5 (Payne et al. 2017; Douglas et al. 2015) all induced higher levels of antibodies, generally falling under the moderate to most reactive antigens indicating that they all were able to induce high levels of humoral immunity. This acquired immunity changed differently: There was high sero-reversion by day 14 towards EBA175, AMA1 and MSP1 indicating that their long-term immunity was lost quickly while there was no sero-reversion towards RH5, PFSEA, MSP2 and MSP3 indicating that their immunity is long-lasting. While this trend is not similar to other malaria endemic areas, there is variation in the time it takes for antimalaria antibody to decay. While generally levels of antibodies to malaria antigens decline within 6 months (Crompton et al. 2010), antibodies to PfAMA1, PfMSP2, PfMSP1 and EBA175 have a half-life

401 of 10-50 days in the Gambia (White et al. 2014; Akpogheneta et al. 2008), in Ghana there
402 half-life is 10-20 days (White et al. 2014) while in Kenya their half-life is reported to be 10-20
403 days (Kinyanjui et al. 2007) and $>20 \leq 100$ years (Ondigo et al. 2014). Classical well known
404 and studied antigens such as MSPs and AMA1 have been moderately to highly correlated
405 with protection against malaria and demonstrated to be highly immune-reactive (Osier et al.
406 2014; Boyle et al. 2014; Richards et al. 2013; Sirima et al. 2016) but generated strain-specific
407 immunity (Sirima et al. 2016; Ogutu et al. 2009; Chitnis et al. 2015; Sheehy et al. 2011; 2012;
408 Blard et al. 2011; Thera et al. 2011; Srinivasan et al. 2017; Duncan et al. 2011; Ellis et al.
409 2012; Biswas et al. 2014; Koram et al. 2016; El Sahly et al. 2010; Ouattara et al. 2013;
410 Genton et al. 2002, 2003; Thera et al. 2011; Barry et al. 2009; E. et al. 2012; Takala et al.
411 2009; Barry and Arnott 2014; Bailey et al. 2020). On the contrary, novel antigens like RH5,
412 which is currently a promising blood-stage malaria vaccine candidate antigen in Phase 1b
413 clinical trials (Payne et al. 2017; Douglas et al. 2015, 5), has been shown to rarely induce
414 significant levels of anti-PfRh5 antibodies by natural infection, however those produced do
415 associate with protection to varying degrees *in vitro* or in clinical cohorts (Patel et al. 2013;
416 Tran et al. 2014; Richards et al. 2013; Bustamante et al. 2013; Reddy et al. 2015; Douglas et
417 al. 2015) while we here demonstrated that it induces moderate levels of antibodies and
418 immunity against it last-long suggests that it would be ideal to revise the criteria of choosing
419 blood-stage malaria vaccine candidates. We propose that while a potential future malaria
420 blood-stage vaccine candidate(s) besides being essential, having a conserved genetic
421 diversity and the antibodies it induces should have no sero-reversion, it doesn't necessarily
422 need to have a strong association with protection to malaria nor to have a high immune-
423 reactivity profile.

By utilizing a large panel of full length protein ectodomains of novel and known malaria vaccine candidates selected from the *P. falciparum* merozoite proteome (Crosnier et al. 2013; Zenonos, Rayner, and Wright 2014) and expressed using the mammalian protein expression system (Kamuyu et al. 2018), we have demonstrated that these antigens can be recognized by the humoral immune system inducing acquisition of different levels of antibodies and humoral immunity against them, whereby it was either not acquired or if it was acquired, it was either maintained or lost at different rates. Some of the limitations of this study are: absence of a non-exposed baseline in the cohort, Day 0 starts as they present with malaria; the study focused only on blood stage antigens and only less than 100 of them; the composition of the cohort is not broadly representative of the population structure, it didn't include individuals less than 5 years or greater than 16 years old. We have never-the-less managed to identify several antigens with ability to induce high levels of antibodies after minimal exposure in a low transmission setting making them ideal candidates to be prioritized for further functional or serological studies. These include novel antigens such as PF3D7_1025300, PF3D7_1105800, PF3D7_1334400, PF3D7_0911300, PF3D7_1252300, PF3D7_1460600, PF3D7_1453100, PF3D7_0831400.

MATERIAL AND METHODS

Protein microarray probing

P. falciparum full length ectodomains were expressed and printed on micro-array slides as described in (Kamuyu et al. 2018) with a slight modification regarding the number of replicate protein spots per each array. Proteins spots were printed in duplicates per each array as opposed to triplicates as done in (Kamuyu et al. 2018). Antigen detection was carried out as well as described in (Kamuyu et al. 2018). Briefly, before the assay samples were diluted at 1:400 in blocking solution (prepared as 2% BSA in Hepes Buffer Saline with 0.1% Tween 20)

448 and arranged in a 96 well plate format. On the day of the assay, printed micro-array slides
449 were pre-scanned using a microarray scanner (GenePix) before starting the assay with PMT
450 set to 400 and power to 100. Four of these pre-scanned slides were set up in 96 well plate
451 format using slide holders. Using a multichannel, 200 µl of washing buffer one (prepared as
452 1X Hepes Buffer Saline with 0.1% Tween 20) was added to each well, the plates were then
453 sealed and incubated for 5 min at room temperature while shaking at 300 rpm in a
454 hybridization station. After this incubation period the buffer was discarded the and these
455 washing steps repeated two more times. After these washings with wash buffer one, three
456 more washings were done but using wash buffer two (WB2, prepared as 1X Hepes Buffer
457 Saline). 200 µl of blocking solution per well was then added using a multichannel, the plates
458 were then sealed with a plate sealer, covered with a foil paper and incubated for 2 hrs at room
459 temperature while shaking at 300 rpm. The slides were then again washed three times as
460 above with wash buffer one followed with washing buffer two. Diluted samples span at 4000
461 RPM for 2 minutes s at 4 degrees Celsius, were added at 250 µl of sample per well. The
462 plates were then sealed with a plate sealer and covered with foil paper then incubated
463 overnight at +4 degrees Celsius while shaking at 300 rpm. The slides were then again
464 washed three times as above with wash buffer one followed with washing buffer two.
465 Secondary antibody, diluted at 1:800 in blocking buffer was done using a multichannel, at 150
466 µl per well. The plates were then sealed with a plate sealer and covered with foil paper then
467 incubated for 3 hours at room temperature while shaking at 300 rpm. The slides were then
468 again washed three times as above with wash buffer one followed with washing buffer two.
469 After these washing steps, the plates were dismantled and then while working on one slide at
470 a time, the slides were dipped several times in distilled water in a Giemsa tray. Eventually,
471 these slides were dry span by putting them upright on a cassette then centrifuging at 1000

472 rpm for 2 mins at room temperature scanned using a microarray scanner (GenePix) with PMT
473 set to 400 and power to 100.

474 **Data processing and analysis**

475 Data preprocessing was carried out as described in (Kamuyu et al. 2018). Briefly, data quality
476 check was performed using R v3.6.1 and included evaluation of background intensities, buffer
477 signals, replicate correlation, negative and positive controls. The median fluorescent
478 intensities (MFI) of the local spot background surrounding each spot was subtracted from the
479 MFI of each antigen spot. The extent of within sample variation was then analysed using
480 coefficient of variation (CV). To account for technical slide-to-slide, batch-to-batch variation
481 and mean-variance dependence (MVD) a two-step normalisation process was applied. First,
482 to handle batch-to-batch variation we utilize a wrapper to SVA's function *ComBat()*. Secondly,
483 data normalization was done using variance stabilization normalization (VSN) .

484 **Figure Legends**

485 **Figure 1: Localization of selected antigens on different regions of the merozoite.**

486 Selected antigens included those localised on the surface, micronemes, rhoptries, dense
487 granules, parasitophorous vacuole and others of unknown localisation.

488 **Figure 2: Serological response to merozoite antigens (known and novel vaccine 489 candidates) in a lowly endemic region of Senegal (response and dynamics with time).**

490 **A). Antibody reactivity against a large panel of known and novel *P. falciparum*
491 merozoite vaccine candidates.** A heat-map of normalized back-ground corrected median
492 fluorescent intensity(MFI) relative to mean MFI plus 2 standard deviations of 8 non-immune
493 control serum representing recognition of test protein (Vertical axis) by patient serum from the

494 day of contact (horizontal axis) clustered using hierarchical clustering. Red represents low
 495 MFI while blue represents high MFI. **B). Change in recognition of *P. falciparum* proteins**
 496 **over time of follow-up without re-infection.** MFI of each test protein as recognized by test
 497 serum per each contact day (Y-axis) is plotted relative to mean MFI plus 2 standard deviations
 498 of non-immune control serum (red line) over the whole study period (X-axis).

499 **Figure 3: Antibody acquisition to a large panel of known and novel *P. falciparum***
 500 **merozoite full length ectodomains vaccine candidates.**

501 **A). Levels of acquired antibody against *P. falciparum* antigens in the sampled**
 502 **population.** Y-axis is the total number of sero-positive serum samples per each protein on the
 503 X-axis over the whole study period. These values were then ranked from highest to smallest
 504 and divided into three groups. The proteins in the highest recognized category are indicated
 505 as 1st tertile while the least recognized are indicated as 2nd tertile. The moderately recognized
 506 proteins are indicated as 3rd tertile. **B). Change in the levels of acquired antibodies against**
 507 ***P. falciparum* antigens.** Line plots of percent sero-positive test serums per each contact day
 508 (Y-axis) over the whole study period (X-axis) against representative test proteins from each of
 509 the three tertiles.

510 **Figure 4. Dynamics in the acquisition and maintenance or loss of humoral immunity**
 511 **towards full length ectodomains *P. falciparum* antigens over time. A). Antibody**
 512 **acquisition to *P. falciparum* antigens.** A heat-map of sero-status of patient serum from the
 513 day of contact (horizontal axis) clustered using hierarchical clustering. White represents sero-
 514 positive while gray represents sero-negative. MFI of each test protein (vertical axis) as
 515 recognized by test serum per each contact day was divided by mean MFI plus 2 standard
 516 deviations of non-immune control serum. Samples with values above 1 were considered as

517 sero-positive while samples with values below 1 were considered as sero-negative. **B).**
 518 **Cumulative prevalence of acquired antibody against *P. falciparum* antigens is antigen**
 519 **specific.** A bar plot of sero-conversion (Y-axis) which represents the number of test serums
 520 that either maintained their sero-status (no change) or seroreverted (from sero-positive to
 521 sero-negative: negative bar-plot) or seroconverted (from sero-negative to sero-positive:
 522 positive bar-plot) per each test protein (X-axis) after 14 (blue), 365 (orange) or 730 (green) of
 523 follow-up days relative to day 0, as indicated by the color coding.

524 **Supplementary information**

525 Please use the any of the following links to access the supplementary information
 526 (Supplementary table 1, Supplementary table 2 and Supplementary figure 1A-C):
 527 https://datadryad.org/stash/share/xiZ10dUp6j4C4MKrLWxJIFJmw4BtFmBt_720l3k27mQ or
 528 <https://doi.org/10.5061/dryad.kh189327h>

529 **Supplementary figure 1.** Line plots of percent sero-positive test serums per each contact
 530 day over the whole study period against representative test proteins. **A.** Analysis was done
 531 including re-infection samples. **B.** Analysis was done excluding re-infection samples. **C.**
 532 Analysis was done including samples both with re-infections (blue) and without re-infections
 533 (orange) but plotted differently.

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Table 1

	Diakhao	Nasrou	Takhikao	Thially	Cite Senghor	Escale
Number of participants	47	11	5	5	1	1
Median age	10(5-15)	11(8-16)	11	12(8-14)	13	10
Gender	M	M	M	M	M	M
Median percent parasitemia on Day0	0.45(0.03-4.27)	0.43(0.03-3.01)	0.015(0.06-2.28)	1.01(0.02-4.89)	NA	0.03
Median number of past malaria infections	1(0-5)	0(0-1)	0(0-2)	0	0	2

Table 2

	PlasmoDB ID	Gene Name	Abbreviation 1	Abbreviation 2
1	PF3D7_0502400	merozoite surface protein 8	MSP8	12MSP8
2	PF3D7_0707300	rhostry-associated membrane antigen	RAMA	RAMA
3	PF3D7_1133400	apical membrane antigen 1 (AMA1)	AMA1	AMA1
4	PF3D7_1301600	erythrocyte binding antigen-140	EBA140	1EBA140
5	PF3D7_0731500	erythrocyte binding antigen-175	EBA175	2EBA175
6	PF3D7_0102500	erythrocyte binding antigen-181	EBA181	3EBA181
7	PF3D7_0405900	apical sushi protein	ASP	ASP
8	PF3D7_0828800	GPI-anchored micronemal antigen	GAMA	GAMA
9	PF3D7_1028700	merozoite TRAP-like protein	MTRAMP	MTRAMP
10	PF3D7_0606800	VFT1	606800	606800
11	PF3D7_1033200	early transcribed membrane protein 10.2	ETRAPM	ETRAPM
12	PF3D7_1021800	schizont egress antigen-1	PFSEA	3PFSEA
13	PF3D7_0616500	TRAP-like protein	TLP	TLP
14	PF3D7_1036000	merozoite surface protein 11	MSP11	3MSP11
15	PF3D7_1035400	merozoite surface protein 3	MSP3	5MSP3
16	PF3D7_1035500	merozoite surface protein 6	MSP6	10MSP6
17	PF3D7_1228600	merozoite surface protein 9	MSP9	13MSP9
18	PF3D7_0404900	6-cysteine protein P41	P41	P41
19	PF3D7_1335100	merozoite surface protein 7	MSP7	11MSP7
20	PF3D7_1334600	MSP7-like protein	MSRP3	1MSRP3
21	PF3D7_0423400	apical asparagine-rich protein AARP	AARP	AARP
22	PF3D7_0905400	high molecular weight rhostry protein 3	RHOP3	RHOP3
23	PF3D7_0424100	reticulocyte binding protein homologue 5	RH5	2RH5
24	PF3D7_0212600	secreted protein with altered thrombospondin repeat domain	SPATR	SPATR
25	PF3D7_0402300	reticulocyte binding protein homologue 1	RH1	1RH1
26	PF3D7_0911300	cysteine repeat modular protein 1	MSP3.5	6MSP3.5
27	PF3D7_1334400	MSP7-like protein	MSRP4	2MSRP4
28	PF3D7_0104200	StAR-related lipid transfer protein	PF210C	PF210C
29	PF3D7_0207900	serine repeat antigen 2	SERA2	1SERA2
30	PF3D7_0207700	serine repeat antigen 4	SERA4	3SERA4
31	PF3D7_0902800	serine repeat antigen 9	SERA9	5SERA9
32	PF3D7_1017100	rhostry neck protein 12	100166	100166
33	PF3D7_0911300	cysteine repeat modular protein 1	MSP3.6	7MSP3.6
34	PF3D7_0323400	Rh5 interacting protein	RIPR	RIPR
35	PF3D7_0207800	serine repeat antigen 3	SERA3	2SERA3
36	PF3D7_0207400	serine repeat antigen 7	SERA7	4SERA7

	PlasmoDB ID	Gene Name	Abbreviation 1	Abbreviation 2
37	PF3D7_0102700	MaTrA	0135W	0135W
38	PF3D7_0620400	merozoite surface protein 10	MSP10	2MSP10
39	PF3D7_0930300	merozoite surface protein 1	MSP1	1MSP1
40	PF3D7_0206800	merozoite surface protein 2	MSP2	4MSP2
41	PF3D7_0207000	merozoite surface protein 4	MSP4	8MSP4
42	PF3D7_1420700	surface protein P113	P113	P113
43	PF3D7_1136200	PF3D7_1136200	1136200	1136200
44	PF3D7_0206900	merozoite surface protein 5	MSP5	9MSP5
45	PF3D7_0508000	6-cysteine protein	P38	P38
46	PF3D7_0612700	6-cysteine protein P12	P12	P12
47	PF3D7_1364100	6-cysteine protein P92	PF92	2PF92
48	PF3D7_1025300	PF3D7_1025300	1025300	1025300
49	PF3D7_1105800	PF3D7_1105800	1105200	1105200
50	PF3D7_1252300	PF3D7_1252300	1252300	1252300
51	PF3D7_0206200	pantothenate transporter	206200	206200
52	PF3D7_0831400	Plasmodium exported protein	831400	831400
53	PF3D7_0925900	lipocalin(LCN), parasitophorous vacuolar protein 5(PV5)	925900	925900
54	PF3D7_1453100	dynactin subunit 4	145300	145300
55	PF3D7_1460600	inner membrane complex sub-compartment protein 3	1460600	1460600
56	PF3D7_0424400	surface-associated interspersed protein 4.2 (SURFIN 4.2)	B29	20B29
57	PF3D7_0419700	apical merozoite protein	PF34	1PF34
58	PF3D7_1436300	translocon component PTEX150	PTEX150	PTEX150
59	PF3D7_0525800	inner membrane complex protein 1g (IMC1g)	525800	525800
60	PF3D7_0730800	Plasmodium exported protein	730800.2	730800.2
61	PF3D7_0830500	TryThrA	83500	83500
62	PF3D7_1137300	CLPTM1 domain-containing protein	113700	113700
63	PF3D7_1229300	PF3D7_1229300	1229300	1229300
64	PF3D7_1237900	PF3D7_1237900	1237900	1237900
65	PF3D7_1345100	Thioredoxin 2	1345100	1345100
66	PF3D7_1401600	Plasmodium exported protein (PHISTb)	1401600	1401600
67	PF3D7_1462300	GTP-binding protein	1462300	1462300
68	PF3D7_0424400	surface-associated interspersed protein 4.2 (SURFIN 4.2)	B27	18B27
69	PF3D7_0424400	surface-associated interspersed protein 4.2 (SURFIN 4.2)	B30	22B30
70	PF3D7_0424400	surface-associated interspersed protein 4.2 (SURFIN 4.2)	B31	23B31
71	PF3D7_1334300	MSP7-like protein	MSRP5	3MSRP5
72	PF3D7_0302200	cytoadherence linked asexual protein 3.2	CLAG3.2	CLAG3.2

Figure 1

Figure 2A

Figure 2B

Figure 3A

Figure 3B

Figure 4A

Figure 4B