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# 1 *Plasmodium falciparum* increased time in circulation underlies persistent asymptomatic 2 infection in the dry season

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33

34 The dry season is a major challenge for *Plasmodium falciparum* parasites in many malaria  
35 endemic regions, where water availability limits mosquitoes to only part of the year. How *P.*  
36 *falciparum* bridges two transmission seasons months apart, without being cleared by the host or  
37 compromising host survival is poorly understood. Here we show that low levels of *P. falciparum*  
38 parasites persist in the blood of asymptomatic Malian individuals during the 5- to 6-month dry  
39 season, rarely causing symptoms and minimally affecting the host immune response. Parasites  
40 isolated during the dry season are transcriptionally distinct from those of subjects with febrile  
41 malaria in the transmission season, reflecting longer circulation within each replicative cycle of  
42 parasitized erythrocytes without adhering to the vascular endothelium. Low parasite levels  
43 during the dry season are not due to impaired replication, but rather increased splenic clearance  
44 of longer-circulating infected erythrocytes. We propose that *P. falciparum* virulence in areas of  
45 seasonal malaria transmission is regulated so that the parasite decreases its endothelial binding  
46 capacity, allowing increased splenic clearance and enabling several months of subclinical  
47 parasite persistence.

48 **(Introduction)** The mosquito-borne *Plasmodium falciparum* parasite is responsible for over 200

49 million malaria cases yearly, and killed nearly 400,000 African children in 2018<sup>1</sup>. *P. falciparum*  
50 causes disease while multiplying asexually within red blood cells (RBCs) and exporting its  
51 variant surface antigens to the RBC surface. Variant surface antigens mediate adhesion to  
52 vascular endothelium, thereby helping the parasite avoid splenic clearance<sup>2,3</sup>. During each ~48h  
53 replicative cycle in RBCs, *P. falciparum* follows a regulated transcriptional pattern from the  
54 invading merozoite, through the ring- and trophozoite-stages, and to the multinucleated  
55 schizont<sup>4,5</sup>, which yields 16-32 new merozoites. In parallel with a predictable transcriptional  
56 pattern, the parasite develops a network of membrane structures<sup>6</sup> in the infected RBC (iRBC)  
57 and at the trophozoite stage the host cell membrane presents knobs<sup>7</sup> exposing parasite-derived  
58 *P. falciparum* erythrocyte membrane protein 1 (PfEMP1)<sup>8</sup>. The multigene family *var* is  
59 expressed in a monoallelic fashion, coding for PfEMP1s that bind host endothelial cell  
60 receptors, with different binding phenotypes associating with varying virulence and pathological  
61 outcomes<sup>9</sup>. In Mali and many African regions, malaria cases are restricted to the rainy season  
62 when the mosquitoes transmitting *P. falciparum* are present<sup>10</sup>, while subclinical *P. falciparum*  
63 infections can persist throughout the dry season, enabling the parasite to bridge transmission  
64 seasons several months apart<sup>11-13</sup>. We have recently shown that although *P. falciparum*-specific  
65 humoral immunity is higher in subclinical *P. falciparum* carriers in the dry season, it decreases  
66 similarly during this time in carriers and non-carriers<sup>13</sup>, suggesting that chronic low parasitaemia  
67 in endemic settings may not maintain nor boost malaria immunity. While much is known about  
68 immune responses to clinical malaria, and to some extent to subclinical infections during the  
69 transmission season<sup>14</sup>, the impact on immunity of subclinical *P. falciparum* persistence in the  
70 dry season has not been extensively studied. Host survival during the dry months is essential  
71 for resuming *P. falciparum* transmission in the ensuing rainy season, thus the parasite has likely  
72 evolved strategies to prevent potentially fatal host pathology, and assure persistence during  
73 mosquito-free periods. In this study we address the host and parasite features that associate  
74 with parasite persistence between two transmission seasons, and provide insights into the  
75 complex interaction between *P. falciparum*, its human hosts and the surrounding environment.  
76 By comparing parasites from the dry season to malaria-causing parasites in the transmission  
77 season, we show that despite inducing a minimal immune response and conserving its  
78 replication ability, *P. falciparum* dry season parasitaemias can be maintained low by splenic  
79 clearance of a large proportion of iRBCs that circulate longer than observed in malaria cases.

80

## 81 RESULTS

82 ***P. falciparum* persists during the dry season.** In a cohort study in Kalifabougou, Mali, we  
83 followed ~600 individuals from 3 months to 45 years of age during 2017 and 2018. As reported  
84 earlier<sup>13</sup>, clinical episodes of malaria (temperature  $\geq 37.5^{\circ}\text{C}$ ,  $\geq 2500$  asexual parasites/ $\mu\text{L}$ , and no  
85 other clinically discernible cause of fever) were largely restricted to the transmission seasons  
86 (June - December), whereas nearly all subjects remained free of symptomatic malaria during  
87 the intervening dry seasons (January - May). Specifically, there were 386 and 347 febrile  
88 malaria cases diagnosed during the 2017 and 2018 rainy seasons, respectively, while only 12  
89 and 5 febrile malaria episodes occurred during the dry seasons (Fig.1a and Table 1). Despite  
90 the very low incidence of clinical malaria in the dry season, we consistently observed 10-20%  
91 prevalence of subclinical *P. falciparum* infections during this time. Analysing over 400  
92 individuals with paired data at the beginning (January) and end (May) of each dry season, we  
93 found that 20% of individuals were *P. falciparum* PCR+ both in January 2017 and January 2018,  
94 and 15% and 12% of individuals were *P. falciparum* PCR+ at the end of the 2017 and 2018 dry  
95 seasons, respectively (Fig. 1b). Older children and young adults carried subclinical *P.*  
96 *falciparum* more frequently than young children at the end of the dry season (Fig. 1c and Table  
97 1), as reported earlier for this cohort<sup>13</sup>. Also, as previously reported, we observed that  
98 subclinical *P. falciparum* carriers at the end of the dry season were very likely to have been  
99 infected since its beginning (January 2017 or January 2018), while uninfected subjects at the  
100 beginning of dry season remained uninfected until its end (Fig. 1b). The odds ratios (OR) of  
101 maintaining the same infection status through the entire dry seasons were OR 90.9 (95% CI  
102 (38.6 , 213.8)  $P < 0.0001$ ) in 2017, and OR Odds ratio 43.5(95% CI (17.5 , 107.5)  $P < 0.0001$ )  
103 in 2018 (Supplementary Table 1). Consistent with the continued absence of clinical malaria  
104 during the dry season, parasitaemias of subclinical carriers determined by RT-qPCR and flow  
105 cytometry were found to remain low, or in some individuals decline as the dry season  
106 progressed, while clinical cases of malaria in the wet season presented with high parasite  
107 burdens (Fig.1d). Interestingly, the very few clinical malaria cases occurring during the dry  
108 seasons had significantly lower parasitaemias than those presented by the same individuals in  
109 clinical cases during the ensuing rainy seasons (Extended Data Fig. 1).

110

111 ***P. falciparum* induces a minimal immune response during the dry season.** To test the  
112 hypothesis that host immunity may contribute to the suppression of parasitaemia during the dry  
113 season, we compared the immune responses of subclinical carriers of *P. falciparum* (May+)   
114 versus non-infected children (May-). We profiled serological markers of inflammation and  
115 cytokines, circulating immune cells, and humoral responses to *P. falciparum* variant surface

116 antigens (VSAs) of age- and gender-matched children who did or did not carry *P. falciparum*  
117 during the dry season, detected retrospectively by PCR. Inflammation markers previously  
118 reported to be elevated in clinical cases of malaria, such as C-reactive protein (CRP)<sup>15</sup>, von  
119 Willebrand factor (vWF)<sup>16</sup>, and hepcidin<sup>17</sup> were quantified in plasma samples obtained at the  
120 beginning (Jan) and end (May) of the dry season. None of the three markers were significantly  
121 different in subclinical carriers compared to uninfected children at either timepoint (Fig. 2a). We  
122 complemented these serological analyses with a multiplex bead array to detect 32 cytokines  
123 and chemokines and observed no differences between children with or without *P. falciparum* at  
124 the end of the dry season (May) in all but one of the quantified analytes (Supplementary Table  
125 2). Only CXCL1, a pro-inflammatory chemokine known to recruit neutrophils, that has thus far  
126 not been associated with malaria in the clinical setting, was significantly increased in children  
127 with *P. falciparum* persistent parasitaemias at the end of the dry season (Fig. 2b). In contrast,  
128 CCL3, IL-10, IL-6 and IL-1 $\beta$ , previously associated with clinical malaria<sup>18-20</sup> were comparable in  
129 the plasma of infected versus uninfected children at the end of the dry season (Fig. 2b and  
130 Supplementary Table 2). We next quantified the proportions of major leucocyte populations from  
131 thawed PBMCs collected at the end of the dry season from children with or without subclinical  
132 *P. falciparum* (gating strategy in Extended Data Fig. 2). We observed that monocytes, T cells, B  
133 cells and NK cell subpopulations were not significantly different between children who carried  
134 (May+) or not (May-) *P. falciparum* (Fig. 2c and Extended Data Fig. 3). To interrogate  
135 differences in cell function, we quantified intracellular cytokines, activation or cytotoxicity  
136 markers, transcription factors or exhaustion markers of freshly collected PBMCs from *P.*  
137 *falciparum* subclinical infected and non-infected children at the end of the dry season. The  
138 levels of the activation marker CD25, transcription factor T-bet or cytokine IL-2 of CD4 T cells;  
139 granzyme B of CD8 T and NK cells, and exhaustion marker FCRL5 of atypical memory B cells  
140 were comparable between children who carried or not *P. falciparum* (Fig. 2d and Supplementary  
141 Table 3). We further questioned whether memory B cells (MBCs defined as CD19+, CD10-,  
142 CD21- and CD27+ or -, gating strategy in Extended Data Fig. 2) specific for *P. falciparum* were  
143 affected in subclinical carriers compared to non-infected individuals at the end of the dry  
144 season. Using biotinylated AMA-1 and MSP1<sup>21</sup>, we quantified AMA-1- or MSP1-specific MBCs  
145 in children who carried *P. falciparum* parasites, and non-infected children at the end of the dry  
146 season. We found that the proportion of class-switched *P. falciparum*-specific MBCs (AMA1+ or  
147 MSP1+ IgG+ IGM- MBCs) was significantly increased in subclinical carriers at the end of the dry  
148 season, while no such difference was found in non-class-switched MBC population (AMA1+ or  
149 MSP1+ IgG- IGM+ MBCs) (Fig. 2e). Within the IgG+ MBC subpopulations, we failed to observe

150 differences between *P. falciparum* carriers and uninfected individuals at the end of the dry  
151 season in *P. falciparum*-specific classical and atypical MBCs, but detected increased *P.*  
152 *falciparum*-specific activated MBCs in subclinical carriers (Extended Data Fig. 3). Using another  
153 multiplex bead array, we quantified humoral responses of *P. falciparum* subclinical carriers and  
154 uninfected individuals at the beginning and end of the dry season, to 35 domain types of the  
155 VSA multigene family *var*, which were grouped according to their endothelial-receptor affinity  
156 (CD36, EPCR or unknown receptor) and PfEMP1 UPS type (A, B or B/A types)<sup>22</sup>  
157 (Supplementary Table 4). We observed that more subclinical carriers (May<sub>+</sub>) than non-infected  
158 individuals (May<sub>-</sub>) were reactive against PfEMP1 domains binding to CD36, EPCR or to  
159 unknown receptors at both time-points, and also that the proportion of individuals reactive to the  
160 different PfEMP1 domains decreased over the dry season independently of individual infection  
161 status (Fig. 2f and Extended Data Fig. 3), these differences parallel our previously published  
162 data on *P. falciparum*-specific humoral responses to non-VSA<sub>13</sub>, suggesting comparable  
163 humoral dynamics for PfEMP1s and non-VSA antigens. Additionally, we observed that the  
164 magnitude of IgG reactivity to A, B or B/A types of PfEMP1 declined similarly from the beginning  
165 to end of the dry season in children who carried subclinical infection (May<sub>+</sub>) or were uninfected  
166 (May<sub>-</sub>) during the dry season (Fig. 2g and Extended Data Fig. 3). Antibodies against PfEMP1  
167 domains (Fig. 2f-g), against a large set of *P. falciparum* non- VSA<sub>13</sub>, and also particularly  
168 against RBC invasion-related proteins<sup>23</sup> (Extended Data Fig. 3) were consistently higher in  
169 subclinical carriers compared to non-infected children at the end of the dry season, so we  
170 questioned if the difference in humoral response at the end of the dry season could impose  
171 variance in inhibition of merozoite invasion in vitro. We tested merozoite invasion of a  
172 laboratory-adapted *P. falciparum* strain in the presence of plasma from Malian children who  
173 carried parasites or not during the dry season, and evaluated the antibodies' ability to block  
174 RBC invasion. Testing complete and antibody-depleted plasma, we observed that antibody-  
175 depleted Malian plasma allowed for ~5-fold increase in invasion of merozoites compared to  
176 complete Malian plasma, while antibody depletion had no differential effect on the control  
177 German plasma used (Fig. 2h and Extended Data Fig. 3). Notably, however, plasma from  
178 Malian children carrying subclinical infections (May<sub>+</sub>) or not carrying parasites (May<sub>-</sub>), had  
179 similar ability to inhibit merozoite invasion, suggesting that the antibodies remaining elevated at  
180 the end of the dry season have no significant effect on inhibiting merozoites invasion of RBCs,  
181 and are unlikely to contribute to the maintenance of low parasitaemias through this mechanism.  
182

183 ***P. falciparum* genetic diversity is maintained throughout the year.** Next, we asked whether  
184 *P. falciparum* parasites persisting through the dry season are genetically distinct from those  
185 causing acute malaria during the transmission season. To that end, we measured the size of the  
186 merozoite surface protein 2 gene (*mSP2*), which is highly polymorphic and discriminates  
187 different *P. falciparum* genotypes<sup>24,25</sup>. Through nested PCR followed by fragment analysis using  
188 capillary electrophoresis, we compared paired samples from 93 subclinical carriers at the  
189 beginning (Jan) and end (May) of the dry season, with 136 samples from clinical cases of  
190 malaria in the ensuing transmission season (MAL). The number of clones detected per  
191 individual did not significantly differ between parasites isolated during the dry season or  
192 transmission season, nor did the percentage of individuals with different numbers of clones (Fig.  
193 3a-b). Furthermore, the size and distribution of *mSP2* clones identified during the dry season  
194 were similar to those isolated from clinical malaria cases during the transmission season (Fig.  
195 3c-d), with the most frequent clone sizes being the same at any of the time-points analysed.

196

197 **Transcriptome of circulating subclinical *P. falciparum* at the end of the dry season differs**  
198 **from that of *P. falciparum* during clinical malaria.** We performed RNA sequencing of  
199 leucocyte-depleted blood from 12 children with persistent subclinical *P. falciparum* at the end of  
200 the dry season (May), and from 12 age- and gender- matched children presenting with their first  
201 clinical malaria case in the ensuing transmission season (MAL) (Supplementary Table 5).  
202 Principal component (Fig. 4a) and unsupervised clustering (Fig. 4b) analyses showed  
203 segregation of transcription profiles based on seasonality. Differentially expressed genes  
204 (DEGs) determined at a false discovery rate threshold of 5% resulted in 1607 DEGs, 1131  
205 transcripts up-regulated, and 476 transcripts down-regulated in the dry season compared to  
206 clinical malaria samples (Fig. 4c and Supplementary Table 6). Validation of the RNA-Seq data  
207 was performed by RT-qPCR of eight DEGs and the correlation between the two methods  
208 resulted in highly significant  $r_2=0.929$  (Fig. 4d and Supplementary Table 7). Furthermore,  
209 samples from additional children (6 end of the dry season and 12 malaria cases during the  
210 transmission season) were used to quantify expression of three DEGs in parallel with the initial  
211 24 samples, and revealed similar fold changes by RT-qPCR (Fig. 4e). We investigated  
212 similarities in the DEGs obtained in this study with those of previous reports comparing parasite  
213 physiological states and transcriptomes from a range of clinical malaria severities<sup>26</sup>, or parasites  
214 causing malaria in high versus low transmission areas<sup>27</sup>, but no enrichment was found  
215 (Extended Data Fig. 4), suggesting that singular mechanisms may be at play during the dry  
216 season. Functional and Gene Ontology analysis of the dry season DEGs revealed a significant

217 enrichment of transcripts involved in cellular processes related with several metabolic pathways  
218 and also *Phagosome*, *DNA replication* or *Homologous recombination* (Fig. 4f). Indeed, DEGs  
219 involved in metabolic pathways suggest that *Glycolysis*, *Glycerophospholipid*, *Purine and*  
220 *Pyrimidine* pathways were increased in parasites from the end of the dry season (May), while  
221 *Fatty acid biosynthesis* appeared downregulated compared to parasites from clinical malaria  
222 (MAL) in the wet season (Fig. 4g). Interestingly, apparent exceptions to the trend of up- or  
223 down-regulation in each of the pathways, highlight transcripts which also have exceptional  
224 patterns of expression within the 48-hour intraerythrocytic developmental cycle<sup>4</sup> thus promoting  
225 different relative expressions (Extended Data Fig. 5). To follow-up on possible metabolic  
226 differences between parasites persisting through the dry season and parasites causing malaria  
227 in the transmission season we used liquid chromatography–mass spectrometry to profile both  
228 hydrophilic and hydrophobic metabolites from the plasma of 12 subclinical children with *P.*  
229 *falciparum* infections at the end of the dry season (May), and of 12 children presenting with their  
230 first clinical malaria case (MAL) in the rainy season. We found significant separation between  
231 metabolites present in the two groups of samples (Fig. 4h and Supplementary Table 8),  
232 however the difficulty of normalization of measured metabolite levels to parasite burden, plus  
233 the strong parasitaemia differences at the time of the blood draw, hinder conclusive  
234 interpretation of what may be seasonal or parasite induced metabolic alterations  
235 (Supplementary Table 9).

236

237 ***P. falciparum* replication is not impaired in the dry season.** We then tested the hypothesis  
238 that dry season parasitaemias were maintained low and subclinical due to decreased parasite  
239 replication capacity during this period. We cultured *P. falciparum* in vitro directly after blood  
240 draw for 36 to 48 h, from rapid diagnostic test positive (RDT+) samples of asymptomatic  
241 individuals at three time points of the dry season (January, March and May), and from samples  
242 of children presenting with their first clinical malaria episode of the ensuing transmission season  
243 (MAL). By flow cytometry, we measured the increase in parasitaemia and parasite development  
244 at 0, 16, 24, 30, 36 and 48 h after in vitro culture. Parasite in vitro growth rates of samples from  
245 >40 subclinical donors during the dry season, and ~30 malaria cases in the transmission  
246 season indicated that the highest growth determined between any two time-points of the short-  
247 term culture was similar throughout the year. Parasitaemias increased in vitro between 2 and 5-  
248 fold at any point in the year, 2.8-fold in January, 95% CI (2.3 , 3.3), 4.1-fold in March, 95% CI  
249 (3.4 , 4.7), 3.6-fold in May, 95% CI (2.6 , 4.6), and 2.8-fold in malaria cases, 95% CI (2.1 , 3.5).  
250 However, the number of hours in culture needed to increase parasitaemia was shorter in the dry



251 season samples than in samples from malaria-causing parasites in the transmission season  
252 (Fig. 5a). In accordance with an earlier increase in parasitaemia in vitro during the dry season,  
253 we could frequently identify on Giemsa smears mature schizonts after 16 and 24 h of culture,  
254 and young ring-stages after 30 or 36 h in the dry season samples; while mature schizonts of  
255 malaria-causing parasite samples were mostly observed after 36 h in culture and young ring-  
256 stages were largely found after 48 h in vitro (Fig. 5b). When we calculated the number of hours  
257 in culture at which the highest increase of parasitaemia could be detected for each sample, we  
258 observed that it decreased from the beginning to the end of the dry season, Jan 26.4 h, 95% CI  
259 (24.5 , 28.3), Mar 24.9 h, 95% CI (23.9 , 25.9), May 22.7 h, 95% CI (20.8 , 24.6), and was  
260 maximum during malaria cases in the transmission season, MAL 44.0 h , 95% CI (41.5 , 46.6)  
261 (Fig. 5c). Nevertheless, when we measured the number of merozoites per multinucleated  
262 schizont prior to or at the time of the highest increase in parasitaemia in vitro, we obtained  
263 comparable values at the end of dry season and in clinical malaria cases in the wet season (Fig.  
264 5d). Finding later developmental parasite stages at earlier times in this short-term in vitro  
265 experiment during the dry season could indicate a faster than 48 h intraerythrocytic replicative  
266 cycle, or alternatively, that dry season parasites circulate longer without adhering to the host  
267 vascular endothelium, and were more developed than circulating parasites in clinical malaria  
268 cases at the time of the blood draw. To test the latter, we used the RNA-seq data described in  
269 Fig. 4 to estimate, with a likelihood-based statistical method previously described<sup>28</sup>, the age in  
270 hours post-invasion (hpi) of circulating parasites from subclinical children at the end of the dry  
271 season (May) and from clinical cases during the wet season (MAL). We determined that  
272 parasites circulating in the dry season had a transcriptional signature of ~17 hpi, 95%CI (14.05 ,  
273 20.8), while parasites circulating in malaria cases during the wet season had a transcription  
274 profile comparable to parasites with ~7 hpi, 95% CI (6.5 , 7.7) (Fig. 5e). Accordingly, imaging  
275 the thick blood films made in the field at the time of the blood draw, we confirmed that  
276 trophozoites were present on the end dry season samples, while clinical malaria samples in the  
277 transmission season had much smaller ring-stages (Fig. 5f-g).

278

279 **Infected erythrocytes in circulation at the end of the dry season are at higher risk of**

280 **splenic clearance.** To investigate if longer circulation of iRBCs in the dry season would impact  
281 host RBC deformability and potentiate splenic clearance, we used a microspherultration system  
282 mimicking the narrow and short inter-endothelial slits of the human spleen with different sized  
283 microspheres<sup>29</sup>. Using freshly collected blood samples from asymptotically infected children  
284 at the end of the dry season and from children presenting with febrile malaria during the

285 transmission season, we assessed retention in the microspheres and flow-through of circulating  
286 iRBCs at time 0, and after 6, 18 and 30 h in vitro. We observed that iRBCs collected from  
287 malaria (MAL) cases were not significantly retained in the spleen-like system at 0, 6 or 18 h  
288 post-culture, and that only after 30 h was the percentage of iRBCs in the flow-through reduced,  
289 indicating splenic retention of iRBCs (Fig. 6a). Conversely, iRBCs in RDT+ blood collected at the  
290 end of the dry season (May) had significantly reduced flow-through immediately after the blood  
291 draw (~25% retention of 0 h iRBCs in the microsphere-system), and over 50% retention of  
292 iRBCs after 6 h or 18 h in culture (Fig. 6a). Accordingly, we observed that trophozoites or  
293 schizonts which fail to flow through the microsphere system were circulating (at 0 h) only in the  
294 dry season samples (Fig. 6b).

295 We then investigated whether differences in cytoadhesion, affecting the length of time that  
296 parasitized cells remain in circulation, could explain the observed age distributions and  
297 microfiltration results. For this we used a mathematical model to describe the within-host  
298 growth and removal of iRBCs from circulation through cytoadhesion in the vasculature and  
299 through splenic retention (see Methods). Both processes were assumed to be dependent on the  
300 parasite's developmental stage, increasing as the parasite starts to express adherence-  
301 mediating surface antigens and RBC modification leads to cell rigidity. Whereas cytoadhering  
302 parasites still replicate, those filtered out by the spleen were assumed to be removed. As shown  
303 in Fig. 6c, effective growth rates and population sizes of low-cytoadhering parasites are  
304 significantly lower than those of high-cytoadhering parasites, which can avoid splenic clearance  
305 before parasitized cells become too rigid to pass through the spleen. We then obtained  
306 estimated parasite age distributions for both scenarios (Fig. 6d) by sampling from the modelled  
307 parasite population at random points over the simulated infection time course, akin to blood  
308 sampling from a population. As low-adhesion parasites are predominantly removed by the  
309 spleen towards the end of their life-cycle, they show a much broader age-range than high-  
310 cytoadhesion parasites, which are removed from circulation earlier through cytoadhesion and  
311 therefore show a narrower and younger age range, in agreement with the observed age  
312 distribution from thick blood smears (Fig. 5e-f). Next, we simulated a microfiltration  
313 experiment by "growing" our sampled model parasites older and evaluating their projected  
314 average flow-through based on our assumed, age-dependent splenic retention function (see  
315 Methods and [Extended Data Fig. 6.](#)). The throughput of high-cytoadhering parasites is high for  
316 the first 6-10h before dropping off gradually as parasites grow older (Fig. 6e). In contrast,  
317 samples from low-cytoadhering parasites with their more uniformly distributed age range  
318 already have a much-reduced flow-through at 0 h, which, however, remained more stable as

319 parasites mature over the next 30h; again in line with the empirical observations (Fig. 6a).  
320 These mathematical results suggest that cytoadhesion alone can explain the differences  
321 between parasites sampled during the dry season (low-adhesion) and parasites sampled from  
322 malaria cases (high-adhesion).  
323 To investigate if the expression of cytoadhesion-mediating PfEMP1 proteins differed in  
324 abundance or quality in parasites found subclinical in the dry season and parasites found in  
325 malaria cases during the wet season, we assembled the *var* genes from the RNA-Seq reads of  
326 the 24 samples from end of the dry season and malaria cases (see Methods). Expression of  
327 several *var* genes has been shown to remain fairly stable between ~10 and 20h post invasion<sup>30</sup>  
328 which should be close to the average ages estimated for parasites in malaria cases and dry  
329 season samples respectively. Using a recently developed analytical pipeline<sup>31</sup>, we could detect  
330 LARSFADIG motifs identifying PfEMP1 coding genes<sup>32</sup> in 8 out of 12 samples from the dry  
331 season, and in 10 out of 12 malaria cases (Table 2). We were able to annotate full length *var*  
332 genes, including both the start N-terminal sequence (NTS) and the acidic terminal sequence  
333 (ATS) domains, and also many isolated fragments, and we observed more contigs with  
334 LARSFADIG motifs and *var* gene fragments in the wet season samples (Supplementary Table  
335 10). We used different methods to assess enrichment of higher expressed *var* genes in the wet  
336 versus the dry season samples. Although we did not see statistically significant enrichment, we  
337 observed a trend that the top expressed *var* genes in each individual in the wet season are  
338 higher expressed (Fig. 6f), and anticipate that further studies with larger samples size will  
339 confirm this trend. Furthermore, we identified known *var* gene domains such as Duffy Binding  
340 Like (DBL) and Cysteine Rich Interdomain Regions (CIDR) as well as the NTS and ATS, and  
341 searched for typical 5' upstream (UPS) sequences associating with different pathological  
342 outcomes<sup>22</sup> in the dry season and malaria cases' assembled *vars*. Although we were unable to  
343 determine the UPS type of expressed *vars* due to the short assembly of the 5' UTR region, we  
344 did find more *var* genes with a DBLz domains in the malaria samples (13 out of 61 in *var*  
345 fragments longer than 3.5 kb) compared to the dry season (1 out of 11 in *var* fragments longer  
346 than 3.5 kb) (Supplementary Table 10).

347

## 348 Discussion

349 Asymptomatic individuals carrying *P. falciparum* at the end of the dry season in areas of  
350 seasonal malaria have been broadly described<sup>13,33-37</sup>, but how the parasite bridges two rainy  
351 seasons without promoting malaria symptoms or being cleared remained elusive. In this study,  
352 with samples from Malians exposed to alternating six-month dry and transmission seasons, we

353 show that within each 48h replicative cycle, *P. falciparum* iRBCs circulate longer in the  
354 bloodstream during the dry season, allowing increased clearance in the spleen, and thus  
355 preventing high parasitaemias which could lead to immune activation or malaria symptoms<sup>38,39</sup>.  
356 Although asymptomatic parasitaemia at the end of the dry season associates with a lower risk  
357 of clinical malaria in the ensuing wet season<sup>13,33-35</sup>, clearance of parasitaemia with anti-malarials  
358 prior to the transmission season does not increase subsequent malaria risk, and the persistence  
359 of infection during the dry season does not prevent nor slow the decline of *P. falciparum*-specific  
360 antibodies<sup>13</sup> (Fig. 2f-g). Consistent with these observations, low parasitaemia during the dry  
361 season did not elicit detectable inflammation, nor affect immune cell function (Fig. 2), indicating  
362 that chronic low parasitaemia in seasonal endemic settings might differ from Controlled Human  
363 Malaria Infections (CHMI) in naïve individuals, where low parasitaemias appear to induce  
364 immunity<sup>40</sup>; and also pointing that slow and continuous stimulation of the immune system is less  
365 effective than sudden changes in antigenic stimulation<sup>41</sup>. Nevertheless, cumulative immunity  
366 may be determinant to sustain the dry season reservoir of *P. falciparum*. Dry season subclinical  
367 carriers have higher anti-*P. falciparum* humoral immunity (<sup>13</sup> and Fig. 2f-g), and higher *P.*  
368 *falciparum*-specific MBCs (Fig. 2e) than non-infected individuals, suggesting that a certain  
369 cumulative exposure is necessary to carry dry season subclinical infections. Additionally, we  
370 and others have shown that end of dry season parasitaemias are more frequent in older than  
371 younger children<sup>13,42</sup>, which is consistent with an age-dependent decrease in parasitaemia and  
372 increase in anti-parasitic immunity<sup>43,44</sup>. It is possible that within each *P. falciparum* infection,  
373 sequential presentation of different VSAs on the surface of iRBCs, and its corresponding  
374 ordered acquisition of antibodies<sup>45,46</sup> favours progressively less virulent parasites. Accordingly, a  
375 recent study of CHMI including naïve and semi-immune individuals observed clinical cases in  
376 naïve individuals, while chronic infections appeared in semi-immune with intermediate antibody  
377 levels<sup>47</sup>.

378 Reports from the transmission season show that increasing malaria severity associates with  
379 different parasite transcriptional profiles<sup>26,48-50</sup>, but the persisting dry season reservoir had not  
380 been investigated. Our data shows that, while *P. falciparum* causing malaria in the transmission  
381 season has a ring-stage transcriptional signature, parasites persisting at the end of the dry  
382 season resemble more developed intraerythrocytic stages, which we confirmed both visually  
383 and through differential growth kinetics in vitro (Fig. 5). Future single-cell transcript analysis of  
384 iRBCs<sup>5</sup> will allow comparing stage-matched pools of parasites to better understand how *P.*  
385 *falciparum* achieves low cytoadhesion in the dry season. Also of interest will be to revisit earlier  
386 reports of transcriptional differences between parasites inducing varying degrees of malaria

387 severity<sup>26,48,49</sup>, and question if these could be partially imposed by the hpi of circulating  
388 parasites. In fact, Tonkin-Hill and colleagues found a bias towards early trophozoite transcription  
389 in the non-severe malaria samples compared to the ring-stage transcriptional profile of the  
390 severe malaria cases<sup>48</sup>, which could be due to differing adhesion efficiencies in vivo.  
391 Interestingly, in vitro replication rates of severe and uncomplicated malaria causing parasites<sup>51,52</sup>  
392 have not consistently explained the higher parasitaemias observed in severe malaria cases,  
393 suggesting that adhesion efficiency differences may also contribute. Continued asexual  
394 replication (independently or coupled with immunity) may lead to progressively less adhesive  
395 iRBCs, as observed in parasites collected during the dry season. In a rodent-malaria model,  
396 uninterrupted asexual-stage growth led to bias in gene expression of VSA and parasite  
397 virulence<sup>53</sup>; and the transition between acute and chronic phases is suggested to be  
398 independent of adaptive immunity<sup>54</sup>. Also, in humans it has been suggested that continued  
399 asexual replication can skew the PfEMP1 expression profile<sup>47,55</sup>, which is consistent with our *var*  
400 genes RNA-Seq data (Fig. 6f and Table 2). The mechanisms by which the parasite adapts to  
401 the dry season, and how transmission is assured as the rainy season ensues remain to be  
402 investigated. In a varying or unpredictable environment, organisms can overcome unfavourable  
403 conditions by sensing environmental changes and adapting their individual developmental  
404 program to increase survival; or alternatively, stochastic population heterogeneity increases the  
405 probability of survival under changing conditions<sup>56</sup>. *P. falciparum* may sense and respond to  
406 environmental cues of transmissibility opportunity, as has been described for detection of  
407 nutrient availability<sup>57</sup>, sexual commitment<sup>58</sup>, or appropriate environment for gametogenesis<sup>59</sup>.  
408 Such a mechanism could act through epigenetic modulation of VSAs, or be seasonally imposed  
409 by different metabolic states of the host, driving a shift on the parasite from a fast- to a slow-  
410 growing program as the transmission season ends and persistence is required, and return to  
411 fast growth as transmission resumes. In an avian-malaria model, chronic *P. relictum* has been  
412 shown to respond to bites from uninfected mosquitoes and increase its replication promoting  
413 transmission<sup>60</sup>. Parasite survival during the dry season is imperative, but will only be efficient in  
414 resuming transmission if these retain the ability to produce gametocytes that mosquitoes can  
415 uptake, thus investigating potential adaptive changes in the sexual stages of *P. falciparum*  
416 during the dry season will likely also reveal seasonal adjustments. Also, investigating the  
417 transcriptional profile of the few malaria cases diagnosed in the dry season (Fig. 1a, Extended  
418 Data Fig. 1) should be the focus of future studies, which we could not do because study protocol  
419 did not include venipuncture blood draws from clinical cases during the dry season.

420 The survival of *P. falciparum*-infected individuals during the dry season is advantageous for the  
421 parasite, and low adhesion of infected erythrocytes is likely a central feature to the subclinical  
422 carriage of *P. falciparum* demonstrating the adaptability of *P. falciparum* parasites to the vector-  
423 free period.

424

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438

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440 CMA, HF, RTL, NFL, CA, JH, CSH, SL, MN, HC, DS, CM, SR, KM, MVH, ELA and SP  
441 performed experiments and analysed data; SD, DD, KK, AO, BB and PDC designed, conducted  
442 and supervised field work generating the clinical data and samples; JM, NOS and TDO  
443 performed bioinformatic analysis; MEN, CL, TL, AM, and AF, ML provided technical expertise  
444 and TL and LT provided essential reagents; MR performed mathematical models; TMT, JS and  
445 VW provided statistics expertise; CMA prepared figures and CMA, HF, RTL, TMT, NSO and  
446 TDO and helped prepare the manuscript; TMT, AF, ML, TL, TDO, MR and PDC provided  
447 insightful comments to the manuscript. PDC discussed initial field and study designs. SP  
448 designed the study and wrote the manuscript. All authors read and approved the final  
449 manuscript.

#### 450 **Competing interests**

451 The authors declare no competing interests.



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670  
671

## 672 Figure Legends

673 **Fig. 1 | *P. falciparum* persists during the dry season.** **a**, Frequency of clinical malaria episodes  
674 every 2 days over 2 years in a cohort of ~600 subjects aged 3 months-45 years. Clinical malaria  
675 defined as axillary temperature  $\geq 37.5^{\circ}\text{C}$ ,  $\geq 2500$  asexual parasites/ $\mu\text{L}$  of blood, and no other  
676 discernible cause of fever. **b**, Prevalence of subclinical *P. falciparum* detected by PCR in paired  
677 individuals (rows) at the beginning (Jan), middle (Mar), and end (May) of two consecutive dry  
678 seasons (2017 left, 2018 right). Columns are sorted such that the same individual is represented  
679 by each row at the three timepoints in each dry season. **c**, Age-stratified point prevalence of  
680 subclinical *P. falciparum* infection detected by PCR at the end of the dry season in May 2017 (left)  
681 and May 2018 (right). **d**, Parasite load detected by qRT-PCR (left) and flow cytometry (right) of  
682 RDT+ subclinical children at the beginning (Jan), mid (Mar) and end (May) of the dry season and  
683 children with their first clinical malaria episode (MAL) in the wet season. Parasitaemia data  
684 represented as median  $\pm$  IQR; Kruskal-Wallis test with multiple comparisons.

685 **Fig. 2 | *P. falciparum* induces a minimal immune response during the dry season.** **a**, C-  
686 reactive protein (CRP) (n=71 May<sup>-</sup>, 117 May<sup>+</sup>), von Willebrand factor (vWF) (n=33 May<sup>-</sup>, 51 May<sup>+</sup>)  
687 and Hcpidin (n=41 May<sup>-</sup>, 37 May<sup>+</sup>) of paired plasma samples at the beginning (Jan) and end  
688 (May) of the dry season carrying *P. falciparum* (May<sup>+</sup>) or not (May<sup>-</sup>). **b**, Plasma cytokines from  
689 children carrying or not (n= 21 May<sup>+</sup>, 12 May<sup>-</sup>) *P. falciparum* at the end of the dry season. **c**,  
690 Surface markers of frozen PBMCs from children that carried (May<sup>+</sup>) or not (May<sup>-</sup>) *P. falciparum*  
691 at the end of the dry season. **d**, Intracellular markers of fresh PBMCs from children carrying (May<sup>+</sup>)  
692 or not (May<sup>-</sup>) *P. falciparum* at the end of the dry season, (Supplementary Table 3). **e**, *P.*  
693 *falciparum*-specific AMA1<sup>+</sup> or MSP1<sup>+</sup> memory B cells (MBCs) in *P. falciparum* carriers or non-  
694 carriers (n=23 May<sup>+</sup>, 28 May<sup>-</sup>) at the end of the dry season for class-switched (IgG<sup>+</sup> IGM<sup>-</sup>) or non-  
695 class switched (IgG<sup>-</sup> IGM<sup>+</sup>) MBCs. **f**, Proportion of children with antibodies specific to PfEMP1  
696 domains at the beginning and end of the dry season (n=106 Jan and May<sup>-</sup>, 112 Jan and May<sup>+</sup>).  
697 **g**, Magnitude of anti-PfEMP1 domains between the beginning and end of the dry season in  
698 children carrying or not subclinical *P. falciparum* (n=112 Jan and May<sup>+</sup>, 106 Jan and May<sup>-</sup>). **h**,  
699 Parasitaemia after invasion in complete or antibody-depleted plasma from children who carried  
700 or not (n= 29 May<sup>+</sup>, 24 May<sup>-</sup>) *P. falciparum* subclinical infections during the dry season. All data  
701 indicate median  $\pm$  IQR; a, and f, One-way ANOVA with multiple comparisons correction b, ANOVA  
702 with Sidak multiple comparisons test. c, d, and h, Kruskal-Wallis test with multiple comparisons.  
703 e, Mann-Whitney test. g, slopes compared with a linear non-interaction model.

704 **Fig. 3 | *P. falciparum* genetic diversity is maintained throughout the year.** **a**, Number of *P.*  
705 *falciparum* clones in subclinical PCR+ children in beginning (Jan) and end (May) of the dry season,  
706 and first clinical malaria case (MAL) during the transmission season, determined by size  
707 differences in the polymorphic region of *msp2*. **b**, Proportion of individuals with different number  
708 of *P. falciparum* clones in the beginning (Jan) and end (May) of the dry season and clinical malaria  
709 cases (MAL). **c**, Size of the *msp2* alleles FC27 (left) and IC/3D7 (right) in beginning (Jan) and end  
710 (May) of the dry season, and clinical malaria cases (MAL). **d**, Prevalence of different *msp2* clone  
711 sizes of FC27 (left) and IC/3D7 (right) allelic families in Jan (n= 93), May (n= 93) and clinical  
712 malaria (MAL, n= 136); Mood's median test.

713 **Fig. 4 | Transcriptome of circulating *P. falciparum* at the end of the dry season differs from**  
714 **malaria-causing *P. falciparum* during the transmission season.** **a**, Principal components  
715 analysis and **b**, Unsupervised clustering analyses of RNA-Seq data of *P. falciparum* parasites  
716 collected at the end of the dry season and from clinical malaria cases (n= 12 May, 12MAL). **c**,  
717 Heatmap showing normalized reads of differentially expressed genes (DEGs) (rows) for each  
718 subject (columns) from *P. falciparum* collected at the end of the dry season (May) and at the first  
719 clinical malaria case (MAL) in the ensuing transmission season. **d**, RT-qPCR validation of RNA-  
720 Seq data for eight labelled DEGs (n= 24). *P* and *r*<sub>2</sub> determined by Pearson correlation. **e**, RT-  
721 qPCR validation of initial 24 RNA-Seq samples (left) and 18 additional samples (right) for three  
722 DEGs. Data indicate mean ± SD, Mann-Whitney test. **f**, Summary of KEGG pathways significantly  
723 enriched with DEGs. Blue bars indicate *P*<sub>adj</sub> for enrichment of each pathway. Grey dashed line  
724 indicates the threshold *P* = 0.05. Orange line indicates the percentage of genes in each pathway  
725 present in the DEG list. **g**, Heatmap showing normalized reads of DEGs involved in different  
726 metabolic pathways (rows) for each subject (columns) from *P. falciparum* at the end of the dry  
727 season and at the first clinical malaria (n= 12 May, 12MAL). **h**, Principal components analysis of  
728 target metabolites' data of plasmas from subclinical *P. falciparum* carriers at the end of the dry  
729 season and from clinical malaria cases (n= 12 May, 12MAL).

730 **Fig. 5. | Replication of persistent dry season *P. falciparum* is not impaired.** **a**, Parasitaemia  
731 fold change at 16, 24, 30 and 36 h post-culture of parasites collected from children at different  
732 times during the dry season (Jan, Mar and May) and clinical malaria case (MAL). Fold change is  
733 defined as %iRBC t(n)/ %iRBC t(n-1). Data indicate median ± IQR. Dashed line at fold change of  
734 1. **b**, Giemsa-stained thin blood smears 16, 24, 36 or 48 h post-culture of *P. falciparum* parasites  
735 collected from children during the dry season (Jan, Mar and May) and clinical malaria case (MAL).  
736 Scale bar= 2 µm **c**, Time of highest increase in parasitaemia detected during in vitro culture of *P.*

737 *falciparum* parasites from children in Jan, Mar and May during the dry season, and clinical malaria  
738 case (MAL). Data indicate mean  $\pm$  SD. Kruskal-Wallis with multiple comparisons test. **d**, Number  
739 of merozoites inside multinucleated schizonts determined by flow cytometry at the end of the dry  
740 season (May) and clinical malaria (MAL) samples. **e**, Maximum likelihood estimation (MLE) of the  
741 hours post-invasion of dry season (May) and clinical malaria (MAL) parasites. Data indicate mean  
742  $\pm$  SD; Mann-Whitney test. **f**, Giemsa-stained thick blood films of *P. falciparum* parasites collected  
743 straight from the arm of children, at the end of the dry season (May) and at their first clinical  
744 malaria (MAL). Scale bar= 5  $\mu$ m. **g**, *P. falciparum* area measured from Giemsa-stained thick  
745 smears in subclinical infections at the end of the dry season (May) and in malaria cases (MAL) in  
746 the wet season. Data indicate median  $\pm$  IQR; Mann-Whitney test.

747 **Fig. 6 | Infected erythrocytes in circulation at the end of the dry season are at higher risk**  
748 **of splenic clearance.** **a**, *P. falciparum* iRBCs filtration through beads mimicking the human  
749 spleen at 0, 18 and 30 h post culture at the end of the dry season and during clinical malaria (n=  
750 8 May, 8 MAL) relative to the non-filtered same-time control. Flow-through % is defined as  
751 downstream %iRBCs / upstream %iRBCs) x 100. Data indicate mean  $\pm$  SD. Dunn's multiple  
752 comparison test of the mean rank of each condition to 0h MAL, \* shows  $P < 0.001$ . **b**, Percentage  
753 of circulating non-ring stage iRBCs at the end of the dry season and during malaria cases (n= 50  
754 May, 39 MAL) determined by flow cytometry. Mean  $\pm$  SD; Mann-Whitney test. **c**, Within-host  
755 dynamics simulation of growth rates and population sizes over five replication cycles of low-  
756 cytoadhering (left), and high-cytoadhering (right) parasites, stratified as circulating (red lines),  
757 cytoadhering (orange dashed lines) and total biomass (black lines). **d**, Simulation of circulating  
758 parasites' age distribution over two replication cycles after repeated sampling of low-cytoadhering  
759 parasites (Low), and high-cytoadhering parasites (High). **e**, Simulation of circulation and passage  
760 through the spleen of parasites aging over time, with low-cytoadhering (Low), and high-  
761 cytoadhering (High) parasites. **f**, Expression level of the highest expressed *var* gene at the end  
762 of the dry season and during a clinical malaria case (n= 8May, 10 MAL).

763

764

## 765 **Extended Data Figure Legends**

766 **Extended Data Fig. 1 |** Parasite density detected by thick blood smear of 17 individuals that  
767 presented malaria cases both in the dry season (January to May, MALdry) and in the ensuing

768 wet or malaria transmission season (June to December, MALwet) in the years on 2017 and  
769 2018. Parasitaemia data indicates median  $\pm$  IQR, Mann-Whitney test.

770 **Extended Data Fig. 2** | Flow cytometry gating strategy to identify **a**, major leucocyte  
771 populations from frozen and fresh PBMCs (Surface staining) and fresh PBMCs intracellular  
772 content (Intracellular staining). **b**, *P. falciparum*-specific memory B cells in frozen PBMCs at the  
773 end of the dry season of children with or without subclinical *P. falciparum* infection.

774 **Extended Data Fig. 3** | **a**, Proportions of NK subpopulations defined by CD56 and CD16 in  
775 children carrying (May+) or not (May-) *P. falciparum* at the end of the dry season. **b**, Proportion  
776 of *P. falciparum*-specific IgG+ MBCs in classical, activated or atypical MBCs of *P. falciparum*  
777 carriers (May+) or uninfected individuals (May-) at the end of the dry season. **c**, Proportion of  
778 children with antibodies specific to PfEMP1 domains binding to unknown receptors at the  
779 beginning (Jan) and end (May) of the dry season (n=106 Jan and May-, 112 Jan and May+). **d**,  
780 Magnitude of IgG antibodies against PfEMP1 domains of B/A subtype between the beginning  
781 and end of the dry season in children carrying (May+) or not (May-) subclinical *P. falciparum*  
782 infection at the end of the dry season (n=106 Jan and May-, 112 Jan and May+) **e**, Breadth (left)  
783 and magnitude (right) of IgG reactivity to invasion-related antigens (Supplementary Table 11) at  
784 the end of the dry season in non-infected and subclinical children carrying *P. falciparum* (n=143  
785 May-, 139 May+). Individual antigen reactivity (detailed in Portugal et al.<sup>13</sup>) of invasion-related  
786 antigens based on Cowman et al.<sup>23</sup>. Breadth is the number of antigens to which the level of IgG  
787 reactivity exceeds 2 SDs above the no DNA control. Magnitude is the sum of log<sub>2</sub> -IgG intensity  
788 values for all antigens per sample. Boxes indicate median IQR, Tukey method. Mann-Whitney  
789 test. **f**, *P. falciparum* invasion ratio between merozoite invasion in antibody depleted plasma and  
790 merozoite invasion in paired complete plasma from subclinical carriers and non-infected  
791 children (n=28 May+, 23 May-), and malaria-naïve control (German adults' plasma, n=9).

792 **Extended Data Fig. 4** | Volcano plots comparing the DEGs found in this study with the DEGs  
793 reported in two other studies <sup>26,27</sup> highlighted in yellow in the figure. Dots in blue represent the  
794 transcripts up-regulated (1131) and in red the transcripts down-regulated (476) in the dry  
795 season compared to clinical malaria samples. **a**, 141 DEGs previous reported in the comparison  
796 of transcriptomes of severe vs moderate malaria parasite physiological states were matched to  
797 the present study. 69 were not DEG in the dry season, 67 were upregulated and 5 were  
798 downregulated. **b**, 306 DEGs previous reported in the comparison of high vs low transmission

799 clinical malaria causing parasites were matched to the present study. 182 were not DEGs in the 800 dry season, 103 were upregulated and 21 were downregulated.

801 **Extended Data Fig. 5** | Expression patterns in 3D7 parasites along the ~48h intraerythrocytic  
 802 developmental cycle (defined by Bozdech et al. PLoS Biology, 2003) of DEGs between *P.*  
 803 *falciparum* of asymptomatic carriers at the end of the dry season (May) and at the first clinical  
 804 malaria case (MAL) in the ensuing transmission season, assigned to **a.** Glycerophospholipid  
 805 metabolism and **b.** Purine and Pyrimidine metabolism pathways by KEGG enrichment analysis  
 806 using the DAVID tool.

807 **Extended Data Fig. 6** | Modelled removal of infected RBCs from circulation by means of  
 808 cytoadhesion and splenic clearance over the 48h replicative cycle, assuming the rate of removal  
 809 increases with increasing expression of cytoadhering variant surface antigens, and where RBC  
 810 modification by the parasite gradually increases the cell's rigidity and hence splenic retention.

## 811 Tables

812 **Table 1** | Characteristics of study participants, clinical cases, and dry season asymptomatic  
 813 infections stratified by year.

	all			MAL		May	
	n	female %	Age, y, mean (95% CI)	Malaria cases n	Age malaria case (y, 95% CI)	% Pf+ in May n	Age %Pf+ in May (y, 95% CI)
2017	604	46.7	11.2 (10.5 - 11.8)	398	9.4 (8.8 - 10.1)	13.4	13.3 (11.8 - 14.9)
2018	603	46.3	10.8 (10.1 - 11.5)	352	9.2 (8.5 - 10.0)	10.9	11.8 (10.0 - 13.6)

814

815 **Table 2** | LARSFADIG motifs that identify PfEMP1 coding genes of 12 subclinical individuals at  
 816 the end of the dry season (May) and 12 first clinical malaria episodes in the ensuing wet season  
 817 (MAL).

SampleID	de novo assembly					Mapping		
	total length in bp	# contigs	#LARSFADIG	Max expression	longest contig in bp	# varDB hits	Id of var gene with highest hit	
May 9307	18,920	10	1	50	6,763	2,693	PF0159-C.g637	NTS-D
May 9308	9,624	5	1	37	5,348	14	PF0145-C.g513	NTS-D



May 9309	1,296	2	0	NA	670	13	PF0084-C.g173	NTS-D
May 9310	18,769	10	3	87	6,227	216	PF0043-C.g402	DBLd-C
May 9311	2,292	1	0	NA	2,292	14	PT0294-C.g1327	DBLa-C
May 9312	20,556	18	1	30	3,170	64	PF0051-C.g887	DBLa-C
May 9313	4,721	8	0	NA	2,292	6	PT0122-C.g1412	NTS-D
May 9314	39,049	28	2	66	9,011	248	PF0262-C.g611	DBLb-I
May 9315	5,824	3	1	307	4,176	193	PF0062-C.g430	NTS-D
May 9316	9,185	13	0	NA	3,400	121	PA0182-C.g826	NTS-D
May 9317	19,689	20	2	176	5,234	915	PM0098-C.g1086	NTS-D
May 9318	10,131	10	1	55	6,004	51	PF0417-C.g422	NTS-D
MAL 9319	173,674	133	24	159	10,423	1,299	PA0146-C.g1029	DBLd-C
MAL 9320	8,666	20	0	NA	2,208	66	PA0224-C.g312	DBLa-C
MAL 9321	23,668	12	2	1,263	9,564	1,149	PF0097-C.g294	NTS-D
MAL 9322	138,491	170	16	104	10,046	846	PA0092-C.g461	DBLd-C
MAL 9323	35,909	43	5	48	3,571	103	PA0253-C.g804	NTS-D
MAL 9324	43,517	56	5	27	4,626	136	PF0189-C.g397	DBLd-C
MAL 9325	3,849	1	0	NA	3,849	391	PF0801-C.g1202	DBLg-I
MAL 9326	113,437	87	17	71	8,812	785	QG0212-C.g1592	NTS-D
MAL 9327	11,330	14	2	109	4,225	82	PA0237-C.g706	NTS-D
MAL 9328	12,501	16	1	163	2,688	63	PF0228-C.g1045	CIDRa
MAL 9329	122,076	120	17	1,140	9,161	1,288	PA0167-C.g886	NTS-D
MAL 9330	106,347	87	13	85	11,752	420	PT0211-C.g738	NTS-D

818

819

## 820 **Methods**

### 821 **Study subjects and Ethical approval**

822 The Ethics Committee of Heidelberg University Hospital, the Faculty of Medicine, Pharmacy and  
823 Odontostomatology (FMPOS) at the University of Bamako, and the National Institute of Allergy  
824 and Infectious Diseases of the National Institutes of Health Institutional Review Board approved  
825 this study. Samples and clinical data were obtained in a cohort study conducted between 2011  
826 and 2019 in Kalifabougou, Mali, a rural village where malaria transmission occurs from June  
827 through December. A single clinic and pharmacy provided the only access to antimalarial drugs.  
828 A detailed description of the study site and cohort design has been published elsewhere<sup>43</sup>. From  
829 an age-stratified, random sample of the village population, individuals aged 3 months to 45  
830 years were enrolled. Written informed consent was obtained from all subjects and the  
831 parents/guardians of participating children. Exclusion criteria at enrolment consisted of  
832 haemoglobin concentration  $<7$  g/dL, axillary temperature  $\geq 37.5^{\circ}\text{C}$ , acute systemic illness, or use  
833 of antimalarial or immunosuppressive medications in the preceding 30 days. Clinical malaria  
834 episodes were detected prospectively by passive surveillance and were defined by axillary  
835 temperature  $\geq 37.5^{\circ}\text{C}$ ,  $\geq 2500$  asexual parasites/ $\mu\text{L}$  of blood, and no other cause of fever upon  
836 physical examination. Malaria episodes were treated with a standard 3-day course of  
837 artemether/lumefantrine according to national guidelines. Cross-sectional clinical visits and  
838 blood draws were performed at the beginning (January), mid (March) and end (May) of each dry  
839 season. The study is registered at ClinicalTrials.gov (identifier NCT01322581).

840

### 841 **Sample collection**

842 Dried blood spots on filter paper (Protein Saver 903, Whatman), thick blood smear and venous  
843 blood (4 or 8 mL depending if donor age was below or above 4 years old) were collected on dry  
844 season cross-sectional visits, and at their first malaria episode of the transmission season.  
845 Blood samples drawn by venepuncture were collected in sodium citrate-containing cell  
846 preparation tubes (Vacutainer CPT Tubes, BD) and transported to the laboratory where PBMCs,  
847 plasma and RBC pellet were separated by centrifugation and used freshly or stored at  $-80^{\circ}\text{C}$   
848 within three hours. Additional 2 mL of venous blood from RDT+ individuals who tested at the end  
849 of the dry season (May 2012) cross-sectional<sup>13</sup>, and from individuals at their first malaria  
850 episode of the ensuing transmission season, was collected into EDTA tubes (Vacutainer  
851 K3EDTA Tubes, BD) and processed directly at the field site. Plasma (used for metabolomic  
852 analysis) was separated by centrifugation and immediately frozen in liquid  $\text{N}_2$ . The buffy coat  
853 was discarded and leucocytes were removed from the RBC pellet in a two-step procedure; first

854 by density gradient on Lymphoprep solution (Fresenius Kabi), followed by Plasmodipur  
855 (EuroProxima) filtration, all according to the manufacturer instructions and as previously  
856 described<sup>61</sup>. RBCs pellets were then frozen in liquid N<sub>2</sub> and were later used for the RNA-Seq  
857 and RT-qPCR validation.

858

#### 859 **Detection of clinical malaria and subclinical *P. falciparum* infection**

860 Thick blood smears were stained with Giemsa and *Plasmodium* parasites were counted against  
861 300 leukocytes of all symptomatic participants presenting to the study clinic; parasite densities  
862 were recorded as the number of parasites/ $\mu$ L of whole blood based on a mean leukocyte count  
863 of 7500 cells/ $\mu$ L. Two expert microscopists evaluated each smear separately, and a third  
864 resolved discrepancies Subclinical infections during the dry season were detected at cross-  
865 sectional timepoints by RDT (SD BIOLINE Malaria Ag P.f test of histidine-rich protein II) with a  
866 sensitivity of  $\sim$ 100 parasites/ $\mu$ L<sup>62</sup>) once the blood was in the laboratory, and by Giemsa stained  
867 smear, and nested PCR amplification of *P. falciparum* DNA retrospectively as previously  
868 described<sup>43</sup> from filter papers (2017) and frozen red blood cell pellets (2018). The sensitivity of  
869 the nested PCR is  $\sim$ 0.5 - 1 parasites/ $\mu$ L<sup>43</sup>.

870

#### 871 ***P. falciparum* quantification**

872 By flow cytometry, RBC pellets removed from CPT tubes after leucocyte isolation were washed  
873 and stained with 5x SYBR Green II (Invitrogen) and 7,5 $\mu$ M MitoTracker (Applied Biosystems) for  
874 30 min at 37 °C. Cells were acquired using an LSR II and analysed using FlowJo software (Tree  
875 Star). By RT-qPCR, RNA was extracted immediately after thawing RBC pellets using TRIzol LS  
876 (Ambion) according to manufacturer instructions, and cDNA was synthesized using SuperScript  
877 IV VILO Master Mix with ezDNase (Invitrogen). RT-qPCR was run on a qTower (Analytik Jena)  
878 using Power SYBR Green PCR Master Mix (Applied Biosystems) with primers for the *P.*

879 *falciparum* reference gene glycine-tRNA ligase (PF14\_0198) (F:

880 5'TGAGTGATATGGATAATATAAAGGAACAAA3'; R: 5'GGATGATATTTTCAAAAC GTATCT  
881 TTCT3') and for human GAPDH (F: 5'ACAACTTTGGTATCGTGAAGG3';

882 R:5'GCCATCAGCCA CAGTTTC3').

883

#### 884 **Serologic analyses**

885 C reactive protein, vWF and Hcpidin in human plasmas were detected through CRP human  
886 ELISA kit (Invitrogen), human vWF A2 Domain Sandwich ELISA (R&D Systems) and Hcpidin  
887 25 ELISA kit (DRG Diagnostics) respectively, all according to the manufacturer instructions.

888 Results were measured using Cytation3 plate reader (BioTek). Milliplex Human  
889 Cytokine/Chemokine Magnetic Bead Panel 41-Plex kit (Merck) was used to measure circulating  
890 analytes in human plasma according to the manufacturer instructions, and read on a Bio-Plex  
891 200 System (Bio-Rad). PfEMP1 domain antibody reactivity was measured using PfEMP1  
892 domains covalently coupled beads following the method described by Cham *et al.*, 2008.  
893 PfEMP1 coupled beads were incubated with diluted plasma samples for 30 min, followed by  
894 incubation with human secondary F(ab')<sub>2</sub> Goat Anti-Human IgG detection antibody for 30 min.  
895 The results were read on a Bio-Plex 200 System (Bio-Rad). The threshold for reactivity of each  
896 PfEMP1 domain was calculated with the average value of 21 German controls plus 3 standard  
897 deviations. Reactivity to PfEMP1 A, B and B/A subtypes was by determined by the fluorescence  
898 intensity of reactive individuals in the beginning (Jan) and end (May) of the dry season of  
899 subclinical carriers and non-infected individuals. Slopes of subclinical carriers and non-infected  
900 individuals were compared using a linear no interaction model.

901

#### 902 **Processing of PBMCs**

903 PBMCs were isolated from the Vacutainer CPT Tubes (DB) according to the manufacturer  
904 instructions, and were either stained and analysed immediately, or frozen within 3 hours of the  
905 blood draw in FBS containing 7.5% DMSO (FBS: Gibco, DMSO: Sigma-Aldrich) The cells were  
906 first frozen at -80°C for 24 h, and then subsequently transferred to liquid N<sub>2</sub> for long term  
907 storage. For experiments where PBMCs were used from frozen vials, PBMCs were rapidly  
908 thawed in a 37°C water bath, washed with 10% heat-inactivated FBS in PBS followed by  
909 complete RPMI (RPMI 1640 supplemented with 10% heat-inactivated FBS, 1% penicillin/  
910 streptomycin and 0,5% β-Mercaptoethanol). For each experiment, PBMCs from all individuals  
911 were thawed and assayed at the same time. The trypan blue dye exclusion assay consistently  
912 demonstrated >80% viability of PBMCs after thawing. Immediately after thawing or after  
913 washing if used fresh, 1 - 2x10<sup>6</sup> PBMCs were incubated for 30 min at 4 °C with different  
914 combinations of the following fluorescently labelled surface antibodies: CD14-PE/BV711, CD16-  
915 BV421/BV605/BV711, CD19-PETexasRed/ BV421/PerCPcy5.5, CD21-  
916 FITC/PETexasRed/BV421/APC, CD27- FITC/PEcy7/AlexaFluor 700/BV421/BV650/APCcy7,  
917 CD3-BV510/BV711/PerCPcy5.5, CD4-FITC/PEcy5/APC, CD8-PEcy7/APCcy7, TCRγδ-PE,  
918 CD56-BV510/BV605. After fixing and permeabilizing the cells with FoxP3 Staining Buffer Set  
919 (eBioscience) according to the manufacturer instructions, PBMCs were stained for 30 min with  
920 fluorescently labelled intracellular antibodies: CD25-PEcy7, T-bet-PE Texas Red, IL-2-FITC,

921 Granzyme B-PE Texas Red, FCRL5-PE, Granzyme A-APC. Cells were acquired using a BD  
922 LSR II or LSR Fortessa flow cytometers (BD) and analysed using FlowJo software (Tree Star).  
923

#### 924 **Flow cytometry with *P. falciparum*-specific B cell probes**

925 Recently thawed PBMCs were stained with biotinylated recombinant *Pf*MSP1 and *Pf*AMA1 and  
926 then coupled to fluorescently labelled streptavidin (Hopp *et al. in preparation*), together with the  
927 following labelled monoclonal antibodies: CD3-BV510 (clone UCHT1), CD4-BV510 (clone SK3),  
928 CD8-BV510 (RPA-T8), CD14-BV510 (clone M5E2), CD16-BV510 (clone 3G8), CD56-BV510  
929 (clone HCD56), CD10-BUV737 (clone Hi10a), IgD-BUV737 (clone IA-2), CD19-ECD (clone J3-  
930 119), CD21-PE-Cy7 (clone B-ly4), CD27-BV605 (clone M-T271), IgM-PerCP-Cy5.5 (clone G20-  
931 127) and IgG-AlexaFluor700 (clone G18-145). Aqua dead cell stain was added for live/dead  
932 discrimination (Thermo Fisher Scientific). Stained samples were run on a LSR Fortessa X20  
933 (BD) and data were analysed using FlowJo (TreeStar). *Pf*MSP1 or *Pf*AMA1-specific MBCs were  
934 identified after exclusion of CD3<sup>+</sup> CD4<sup>+</sup> CD8<sup>+</sup> CD14<sup>+</sup> CD16<sup>+</sup> CD56<sup>+</sup> non-B cells, CD10<sup>+</sup>  
935 immature and IgD<sup>-</sup> B cells. In order to increase the frequency of specific B cells detected in any  
936 given sample, PBMCs were stained simultaneously with *Pf*AMA1 and *Pf*MSP1 probes.  
937 Therefore, *Pf*AMA1 and *Pf*MSP1 probe-binding cells are indistinguishable by flow cytometry  
938 analysis and are referred to together as “Pf-specific” cells. Influenza hemagglutinin (HA) antigen  
939 was used as a non Pf-specific cells control.

940

#### 941 ***P. falciparum* culture**

942 3D7 *P. falciparum* parasites were maintained in fresh human O<sub>Rh</sub><sup>+</sup> erythrocytes at 5%  
943 haematocrit in RPMI 1640 complete medium (with L-glutamine and HEPES 7.4% Sodium  
944 Bicarbonate, 100µM Hypoxhantine (cc pro) and 25 mg/mL gentamycin (all Gibco)) added with  
945 0.25% Albumax II (Gibco), at 37°C either in the presence of a gas mixture containing 5% O<sub>2</sub>,  
946 5% CO<sub>2</sub> and 90% N<sub>2</sub> or using the candle jar system described by Trager and Jensen 1976  
947 method.

948

#### 949 ***P. falciparum* invasion assay**

950 3D7 *P. falciparum* parasites were cultured and regularly synchronized by the use of 5%  
951 sorbitol<sup>63</sup>, and heparin to prevent re-invasion. At early schizont stage E64 compound (Sigma-  
952 Aldrich) was use to prevent merozoites egress, and later merozoites were purified through  
953 filtration as previously described<sup>64</sup>. Merozoites were cultured with non-infected RBCs and RPMI  
954 medium supplemented with 25% human plasma from different donors for 30 min to allow

955 invasion, and then RPMI medium supplemented with Albumax-II was provided to all parasites  
956 for 30 h. iRBCs were fixed as previously described<sup>65</sup>, stained with 5% SybrGreen and  
957 successful invasion by merozoites was measured 30 h after invasion using FACS Canto II (BD)  
958 and analysed using FlowJo software (Tree Star). Antibody depletion from human plasma was  
959 achieved using Protein G and Protein L Plus Agarose beads (Pierce, Thermo Fisher Scientific).  
960 Successful depletion was obtained after 3 incubations of plasma with the beads, and was  
961 verified with a Ready-SET-Go! Elisa kit (eBioscience) measuring total IgG and IgM quantities in  
962 the plasma before and after depletion read. Results were read on a Cytation3 plate reader  
963 (BioTek).

964

### 965 ***Msp2* fragment analysis by capillary electrophoresis**

966 DNA was extracted from two 3-mm circular punches of blood spots on filter papers (Whatman  
967 3M), using the DNeasy blood & tissue kit (Qiagen). Genotyping of *msp2* was performed using a  
968 previously described nested PCR reaction<sup>25</sup>. The first PCR amplified the outer *msp2* domain (F:  
969 5'ATGAAGGTAATTAACATTGTCTATTATA3' R: 5'CTTGTACACG GTACATTCTT3'),,and the  
970 second PCR used fluorescently labelled primers to identify two *msp2* allelic families: IC/3D7  
971 (F: 5'AGAAGATGCAGAAAGAAKCCTYCTACT3' and R:5'GA  
972 TGTAATCGGGGATCAGTTTGTTCG 3'VIC) and FC27 (FC27 F: 5'AATACAAGAGGTGGG  
973 CRATGC TCCA3' and R: 5'TTTTATTTGGTGCATTGCCAGAACTTGAAC3' 6-FAM). Fragment  
974 analysis was performed by capillary electrophoresis using a DNA sequencer (3730, Applied  
975 Biosystems) and analysed using GeneMapper 5 software (Thermo Fisher Scientific), where a  
976 cut-off of 300 relative fluorescent units was set. Fragments were considered to be the same  
977 allele within each allelic type if the size difference between them was less than 3 base pairs.

978

### 979 **Transcriptome analysis**

980 RNA was extracted from frozen RBC pellets using TRIzol (Ambion) as previously described<sup>4</sup>  
981 was performed from RBC pellets that were frozen in liquid N<sub>2</sub> immediately after blood draw.  
982 RNA quality was tested using a Bioanalyzer (Agilent). The average RIN value was 5.4 for the  
983 dry season samples and 5.3 for the clinical malaria cases. The average Bioanalyzer yield was  
984 27.4 ng on the dry season samples, while the clinical malaria cases average Bioanalyzer yield  
985 was 25.0 ng. Twenty-four samples were selected based on parasitaemia, the highest 12 titres  
986 for the wet and dry seasons. The RNA input going into NGS sample preparation ranged from  
987 100 pg up to 100 ng. The samples were prepared for transcriptome analysis following a  
988 previously described protocol<sup>66</sup> with minor modifications. Initially, samples were treated with

989 TURBO DNase as described. Following DNase treatment, the Agencourt RNAClean SPRI  
990 beads (Beckman Coulter) were resuspended in 19  $\mu$ L to modify the protocol for a low input  
991 ribosomal RNA depletion step. Ribosomal RNA was depleted following the Clontech Modified  
992 Protocol for Removal of rRNA from Small Amounts of Total RNA (100 ng) using  
993 Human/Mouse/Rat Ribo-Zero Magnetic Kit (Epicentre). The purification beads were  
994 resuspended in 21  $\mu$ L to proceed with the fragmentation step as described<sup>66</sup>. The first and  
995 second strand cDNA synthesis steps were followed without modification except for the final  
996 bead purification was eluted in 55  $\mu$ L. Aliquots from the DNase, Ribo-zero treatment, and  
997 fragmentation were analysed on Agilent BioAnalyzer Pico RNA chips, along with the final cDNA  
998 constructs. The purified cDNA was below detectable levels of the BioAnalyzer Pico chips. 50  $\mu$ L  
999 of purified cDNA were prepared for NGS sequencing using the KAPA Hyper Prep Kit (KAPA  
1000 Biosystems). The adaptor stock concentration was 300 nM for the ligation with 4 h incubation at  
1001 20°C. The USER digestion step was omitted as the Hi-Fi polymerase does not amplify uracil  
1002 containing products. The number of amplification cycles for PCR was determined to be 14  
1003 based on quantification of the amount of post-ligation product with a KAPA Quant Kit for Illumina  
1004 Sequencing (KAPA Biosystems). The purified amplified libraries were visualized on Agilent DNA  
1005 1000 chips. Libraries were quantified using the KAPA Quant Kit for Illumina Sequencing (KAPA  
1006 Biosystems) and normalized to 2 nM. The samples were pooled based on parasitaemias,  
1007 denatured and diluted to 11 pM for cluster generation and paired-end 100 cycle sequencing on  
1008 a HiSeq 2500 Rapid flow cell (Illumina) producing ~13 million reads per sample. Raw reads  
1009 were trimmed of adapter sequence and low-quality bases and filtered for low quality reads using  
1010 the FASTX-Toolkit. Remaining reads were mapped to the *P. falciparum* genome, build  
1011 ASM276v1 using Hisat2<sup>67</sup>. Reads mapping to genes were counted using htseq-count<sup>68</sup>. For  
1012 each sample, parasite age (hours post-invasion, hpi) was estimated based on gene expression  
1013 using a previously described maximum likelihood method<sup>28</sup>. Differential expression analysis was  
1014 performed using the Bioconductor package DESeq2<sup>69</sup>, with adjusted *P*-values (*P*<sub>adj</sub>) < 0.05  
1015 considered significant. Differentially expressed genes (DEGs) were analysed for enrichment in  
1016 gene ontology categories using DAVID<sup>70</sup>. RNA-Seq data (normalized counts data and raw  
1017 sequencing reads) is available on NCBI GEO (project ID no. GSE148125). Validating RT-qPCR  
1018 was performed on the 24 above-mentioned samples and on an extra set of 18 samples, 6 dry  
1019 season and 12 clinical cases during the wet season. VILO cDNAs were synthesized using the  
1020 SuperScript VILO cDNA synthesis kit (Invitrogen) and purified according to QIAquick 96-well  
1021 protocol (Qiagen) with a modified centrifugation protocol<sup>71</sup>. Expression levels of 8 transcripts  
1022 (sir2, rex3, Pfsec23, PFB0100c/KARHP, PF07\_0006/STARP antigen, PFB0900c/PHISTc

1023 GEX20, PF08\_0020/UBE4B, MIF) were determined by RT-qPCR using Invitrogen Express  
1024 qPCR SuperMix with premixed ROX reference dye (Invitrogen) in 20  $\mu$ L reactions. All gene  
1025 specific oligo sequences were designed using Beacon Designer software (Premier Biosoft) and  
1026 purchased from LGC Biosearch technologies with double quencher BHQnova fluorescent  
1027 probes due to AT-rich *P. falciparum* sequences (Supplementary Table 7). In a multiplex format  
1028 we used reference gene glycine-tRNA ligase%2C putative (PF14\_0198) and a standard made  
1029 from pooled SPIA cDNA. RT-qPCR reactions were carried out at 95°C for 2 min, and 55 cycles  
1030 of 95°C for 15 secs and 60°C for 1 min. Data was analysed using the 7900HT version 2.4  
1031 sequence detection system software per the manufacturer's recommendations.

1032

### 1033 **Metabolite profiling**

1034 Plasma metabolomics was performed using both targeted and untargeted approaches, across  
1035 several LC-MS platforms, for small molecules and lipids, in order to obtain full metabolite  
1036 coverage. Each plasma sample was split into two independent samples for metabolite extraction.  
1037 For hydrophilic metabolites, 50  $\mu$ L of plasma was extracted by the addition of 9X volumes of ice-  
1038 cold methanol. Samples were briefly vortexed before centrifuging for 10 min to remove  
1039 precipitated protein. The clarified supernatants were dried under N<sub>2</sub> gas and resuspended in 100  
1040  $\mu$ L (1:2 dilution). Sample groups were pooled to create a group QA and all samples were pooled  
1041 to create a batch QC, which were injected periodically throughout each run. The hydrophilic  
1042 extracts were randomized and analysed using reversed-phase high-performance LC-MS by  
1043 injecting 10  $\mu$ L onto an ABSciex 5600 (QTOF) TripleTOF in positive ESI mode prior to injection  
1044 on a Thermo Exactive Plus Orbitrap in negative ESI mode. Samples were separated on the  
1045 ABSciex 5600 by reverse phase HPLC using a Prominence 20 UFLCXR system (Shimadzu) with  
1046 a Waters (Milford) BEH C18 column (100 mm x 2.1 mm 1.7  $\mu$ m particle size) maintained at 55°C  
1047 and a 20 min aqueous acetonitrile gradient, at a flow rate of 250  $\mu$ L/min. Solvent A was HPLC  
1048 grade water with 0.1% formic acid and Solvent B was HPLC grade acetonitrile with 0.1% formic  
1049 acid. The initial condition were 97% A and 3 % B, increasing to 45% B at 10 min, 75% B at 12  
1050 min where it was held at 75% B until 17.5 min before returning to the initial conditions. The eluate  
1051 was delivered into a 5600 TripleTOF using a Duospray ion source (all AB Sciex). The capillary  
1052 voltage was set at 4.5 kV in negative ion mode, with a declustering potential of 80 V. The mass  
1053 spectrometer was operated in Information Dependent Acquisition mode with a 100 ms survey  
1054 scan from 100 to 1200 m/z, and up to 20 MS/MS product ion scans (100 ms) per duty cycle using  
1055 a collision energy of 50 V with a 20 V spread. Metabolite separation was performed on the Thermo  
1056 Exactive Plus Orbitrap as previously described<sup>72</sup> using a Waters XSelect HSS T3 column (2.1 x



1057 100 mm; 2.5 µm). For hydrophobic metabolites, 25 µL of plasma was extracted by the addition of  
1058 3X volumes of isopropanol. Samples were briefly vortexed and allowed to sit at room temperature  
1059 for 10 min. Samples were then placed at -20°C to precipitate overnight. Precipitated samples  
1060 were centrifuged for 20 min and the clarified supernatant was diluted to 50% water in a glass  
1061 LCMS sample vial (1:6 dilution). Sample groups were pooled to create a group QA and all samples  
1062 were pooled to create a batch QC, which were injected periodically throughout each run. The  
1063 hydrophobic extracts were randomized and analysed using reversed-phase high-performance  
1064 LC-MS by injecting 10 µL of sample onto an ABSciex 5600 TripleTOF in positive and negative  
1065 ESI modes. Metabolite separation was performed as previously<sup>73</sup> using a Waters Acquity UPLC  
1066 CSH C18 column (100 × 2.1 mm; 1.7 µm). Ammonium formate and formic acid were added to the  
1067 positive ESI solvents and ammonium acetate was used for negative ESI. Targeted analysis of the  
1068 Orbitrap data was performed as previously described<sup>74</sup>. Untargeted analysis from the 5600  
1069 TripleTOF was performed using the default settings in MS-DIAL<sup>75</sup>. The built-in databases were  
1070 used for putative identification of metabolites at the MS/MS level. QA/QC samples were evaluated  
1071 to minimize systematic/ technical issues. All data were normalized to the TIC and blank  
1072 subtracted, to remove background noise, prior to statistical analysis.

1073

#### 1074 ***P. falciparum* field isolates short term culture**

1075 RBC pellets isolated from CPT tubes of RDT+ samples in the dry season and of malaria cases'  
1076 samples in the transmission season were cultured in fresh human O<sub>Rh+</sub> erythrocytes at 7%  
1077 haematocrit in complete RPMI supplemented with 0.25% Albumax II (Gibco) at 37°C in a candle  
1078 jar for 36 or 48 h. Malaria cases' samples were cultured undiluted and diluted 1:10, 1:25 and  
1079 1:50 with non-infected blood, to assure that initial parasitaemia was low (0.5 - 1%) and that all  
1080 cultures parasites could grow to their maximum potential. Parasitaemia and parasite  
1081 development were assessed at 0, 16, 24, 30, 36 and 48 h in culture by thin blood smears and  
1082 flow cytometry. Parasitaemia fold change was determined for each sample (ratio of %iRBCs at  
1083 each timepoint over its preceding one). The time of highest increase of parasitaemia was the  
1084 timepoint at which the ratio of %iRBCs at given timepoint over its preceding one was the highest  
1085 for each sample. Progeny number was determined dividing SybrGreen fluorescence of  
1086 multinucleated schizonts prior or at the time of the highest increase in parasitaemia in vitro, by  
1087 the fluorescence of the smallest ring-stage population that sample presented. Fiji software was  
1088 used to measure *P. falciparum* area.

1089

#### 1090 **Microfiltration of *P. falciparum* iRBCs**

1091 RBC pellets isolated from CPT tubes of RDT+ samples in the dry season and of malaria cases'  
1092 samples in the transmission season were leucocyte depleted using EasySep CD45 depletion kit  
1093 (STEMcell Technologies), according to manufacturer instructions. iRBCs were cultured in  
1094 complete RPMI medium supplemented with Albumax, and microsphere filtration was performed in  
1095 triplicates at 0, 6, 18 and 30 h in culture as previously described<sup>29</sup>. Briefly, calibrated  
1096 microspheres of 5-15 µm and 15-25 µm in diameter (Sn96.5% tin, 3% silver, 0.5% copper,  
1097 Industrie des Poudres Sphériques) were mixed at 4 g each in 12 mL complete medium. Filter  
1098 tips (ref 732-0534, VWR) were cut diagonally on the tip and wet by pushing 200 µL of complete  
1099 medium through the filter. The bead suspension was vortexed and 400 µL were loaded onto the  
1100 filter tips, yielding a 1.5 mm layer of microsphere beads. The tips were then filled up with  
1101 medium and connected to a three-way stopcock. Microsphere filtration tips were used within 12 h of  
1102 preparation. At each timepoint, 600 µL of the 2% haematocrit culture was loaded onto the  
1103 microbead layer and perfused with 5 mL complete medium at 1 mL/min using a syringe pump  
1104 (AL-4000, World Precision Instruments). The upstream and downstream samples were  
1105 collected at the different timepoints and stained for *P. falciparum* quantification by flow  
1106 cytometry. Samples which parasitaemia increased (fold change > 1) between 0 and 30 h (May)  
1107 or 0 and 48 h (MAL) were included in the analysis.

1108

### 1109 **Simulation of *P. falciparum* cytoadhesion and splenic clearance**

1110 We developed a discrete-time within-host infection model to monitor parasite replication,  
1111 cytoadhesion and splenic clearance in the absence of host immune responses. The parasite's  
1112 48 h life-cycle was divided into eight 6 h time steps and we assumed that the parasite  
1113 population was fully synchronised. The dynamics of circulating,  $B_i$ , and cytoadhering parasites,  
1114  $V_i$ , are described through the following iterative scheme:

$$1115 \quad B_0^{t+1} = \gamma(B_8^t + V_8^t)$$

$$1116 \quad B_1^{t+1} = (1 - \sigma_1)(1 - \eta_1)B_0^{t+1}$$

$$1117 \quad V_1^{t+1} = \eta_1 B_0^{t+1}$$

$$1118 \quad B_i^{t+1} = (1 - \sigma_i)(1 - \eta_i)B_{i-1}^{t+1}, \quad \forall i \in [2,3, \dots, 8]$$

$$1119 \quad V_i^{t+1} = \eta_i B_{i-1}^{t+1} + V_{i-1}^{t+1}, \quad \forall i \in [2,3, \dots, 8],$$

1120

1121 where  $B_i^t$  and  $V_i^t$  are the numbers of circulating and cytoadhering parasites of age  $i$  and at time  
1122  $t$ , respectively.  $\gamma$  is the intrinsic parasite growth rate, i.e. the average number of newly infected  
1123 red blood cells arising from a single infected cell. As seen from the above equations, only  
1124 cytoadhering and freely circulating iRBC replicate and contribute to parasite population growth,

1125 whereas the spleen is assumed to remove retained parasites. Removal of infected red blood  
 1126 cells from circulation by means of cytoadhesion,  $\eta_i$ , and splenic clearance,  $\sigma_i$ , is assumed to be  
 1127 dependent on the age of the parasite,  $i$ , where the rate of removal increases as the parasite  
 1128 starts to express cytoadhering variant surface antigens, PfEMP1, shortly after invasion, and  
 1129 where RBC modification by the parasite gradually increases the cell's rigidity and hence splenic  
 1130 retention. Both removal functions are given by the following sigmoidal forms

$$1131 \quad \sigma_i = \sigma(i) = \frac{\sigma_{max}}{1 + e^{p_{\sigma} \times (T_{\sigma} - i)}}$$

$$1132 \quad \eta_i = \eta(i) = \kappa \frac{\eta_{max}}{1 + e^{p_{\eta} \times (T_{\eta} - i)}}$$

1133

1134 and are visualised in [Extended Data Fig. 6](#).  $\sigma_{max}$  and  $\eta_{max}$  are the maximum removal rates by  
 1135 the spleen and cytoadhesion, respectively.  $p_{\sigma, \eta}$  and  $T_{\sigma, \eta}$  are the shape and location parameters  
 1136 of the sigmoidal functions, where  $T$  determines the age at which 50% of the parasites are  
 1137 removed. The factor  $\kappa \in [0,1]$  is included to investigate the effect of cytoadhesion on the within-  
 1138 host growth dynamics. All parameters and values used are listed in Supplementary Table 12.

1139

#### 1140 **PfEMP1 genes expression**

1141 To exclude human reads, all reads were mapped against the human genome (hg19), using bwa  
 1142 mem<sub>76</sub> (-k 32). Reads and their pair that did not map the human genome were used further.  
 1143 Reads were correct with quorum<sub>77</sub>, parameter -k 35. Adapters were trimmed with cutadapt<sub>78</sub>,  
 1144 using the TruSeq LT adapter sequences. To assemble the *var* genes, the RNA-Seq sequencing  
 1145 reads (fastq files) were first assembled with the pipeline recently published in Otto *et al*<sub>31</sub>. Due  
 1146 to the variable coverage of the sequence reads, the results were not satisfactory. Therefore, an  
 1147 older approach was used to assemble all the non-human sequencing reads with velvet<sub>79</sub>,  
 1148 following parameters: Kmer 41, exp\_cov 999999999, ins\_length 420, cov\_cutoff 5,  
 1149 ins\_length\_sd 30, min\_pair\_count 5. Different k-mer were tested and k-mer of 41 returned the  
 1150 best results. The obtained contigs were annotated and the domains were called as previously  
 1151 described<sub>31</sub>. For each *var* genes domains and subdomains were annotated as defined by Rask  
 1152 *et al* (*vardom*<sub>80</sub>). The expression per *var* gene was calculated with two methods. In both  
 1153 methods the reads back with bwa mem were mapped against the assemblies. In the first  
 1154 method, the number of mapped reads was counted on *var* genes larger than 3.5k and  
 1155 normalised by the length of the *var* genes and the number of reads mapped against all contigs,  
 1156 similar to RPKM. In the second method the coverage over the middle of the LARSFADIG motifs

1157 (samtools depth) was addressed. These values were divided by the number of reads mapped  
1158 on each assembly and multiplied with 10 million. As a complementary validation, a mapping  
1159 approach was used: the sequencing reads were mapped using bwa mem, parameter -k 31 -a  
1160 (to allow multiple hits), against a combined database of *var* genes from Otto *et al*<sup>81</sup>  
1161 (varDB.fulldataset.1kb.nt.fasta.gz) and the *var* genes from Otto *et al*<sup>82</sup>, limited to *var* genes of  
1162 the length of at least 3kb. Next, the number of reads mapped with an AS score of at least 95  
1163 against the *var* genes from this combined database were counted.

1164

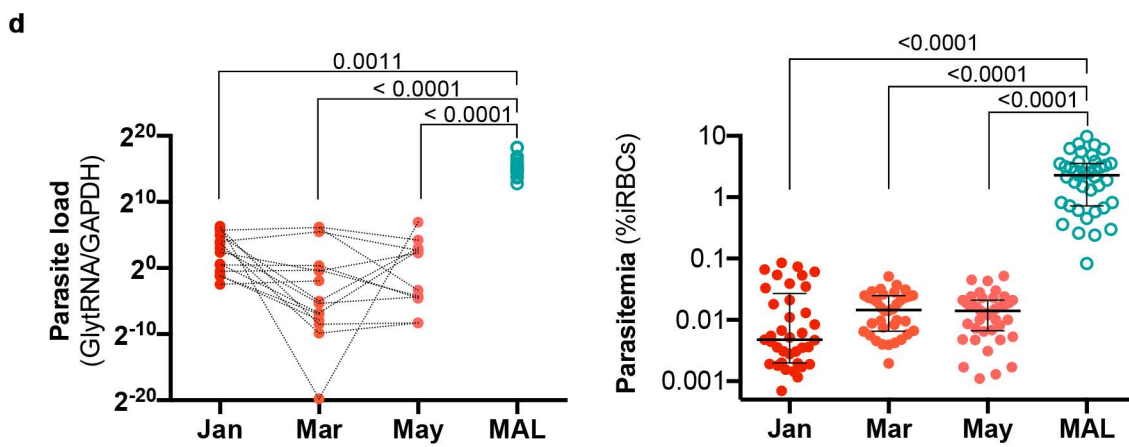
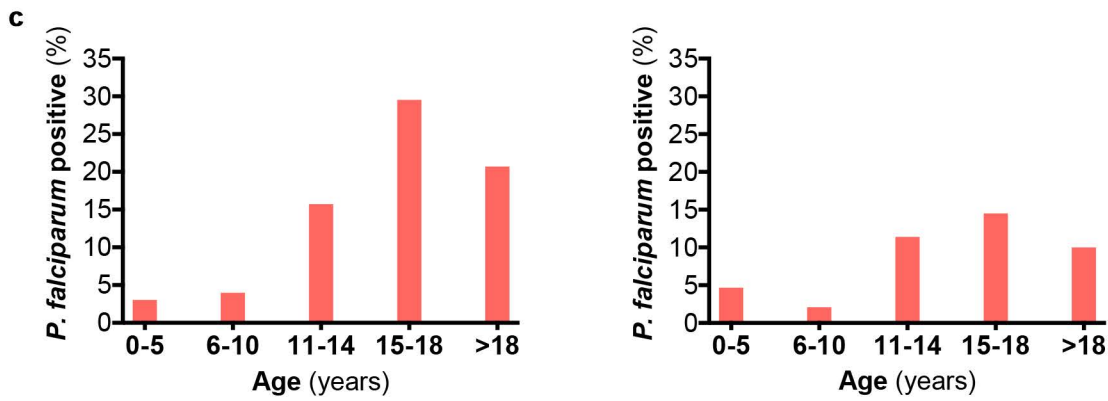
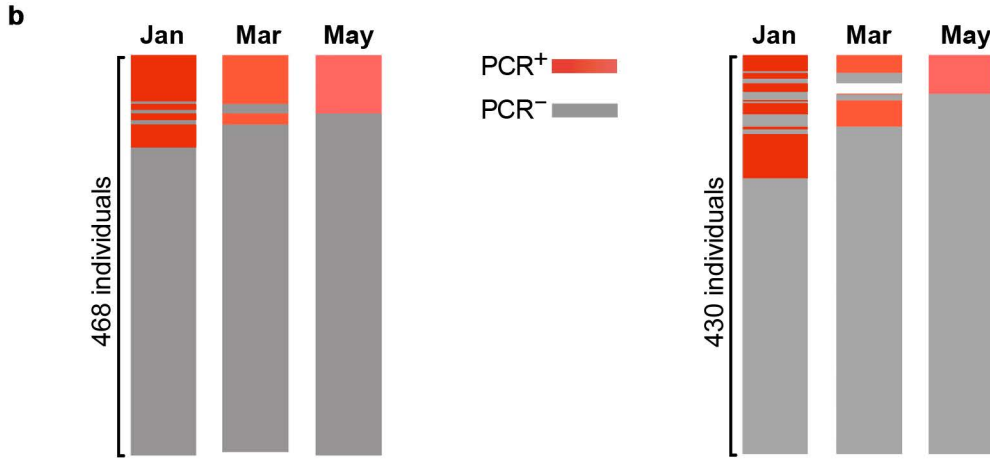
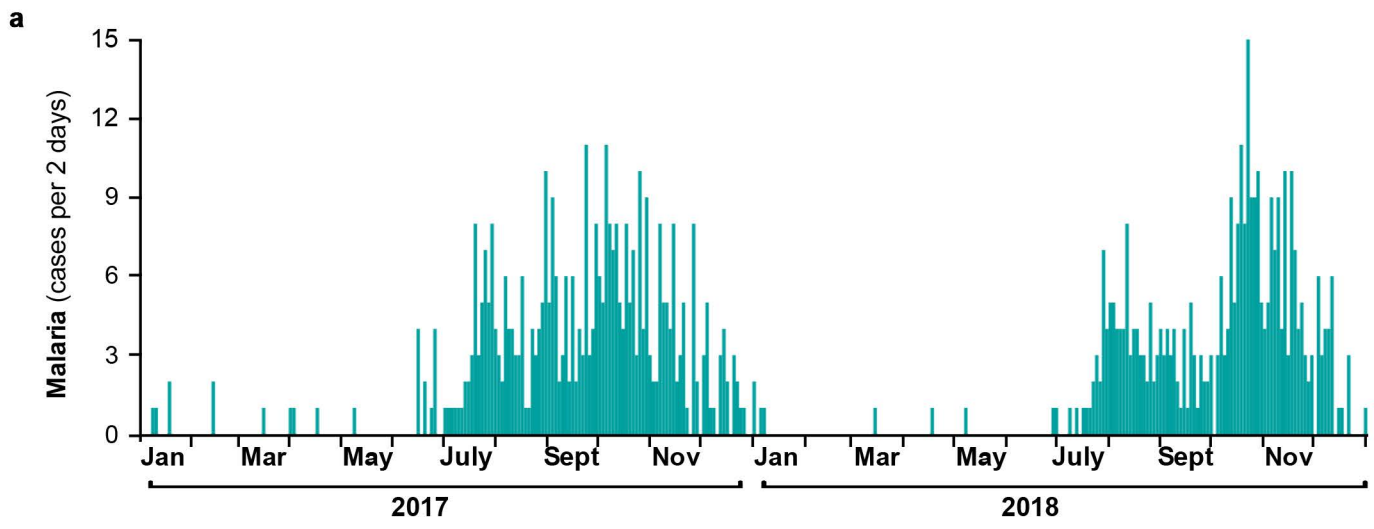
#### 1165 **Statistical analysis**

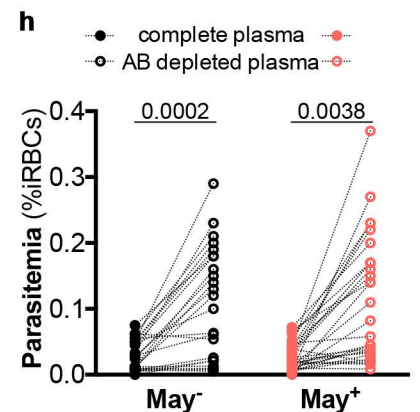
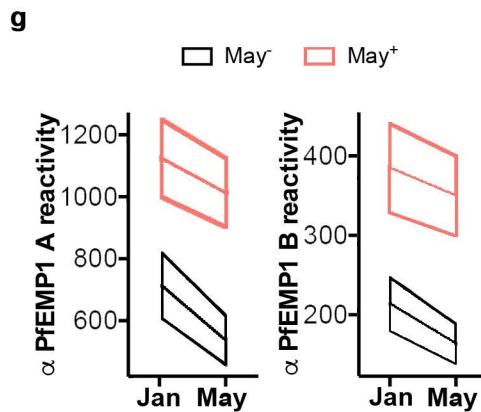
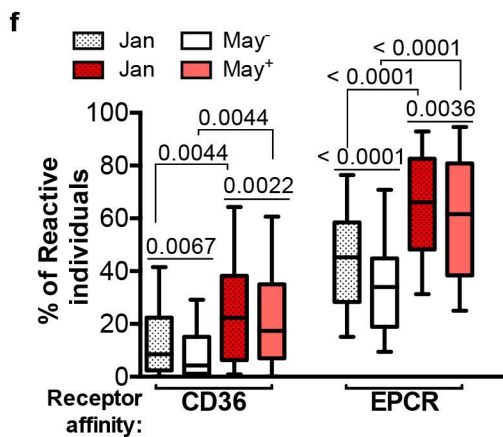
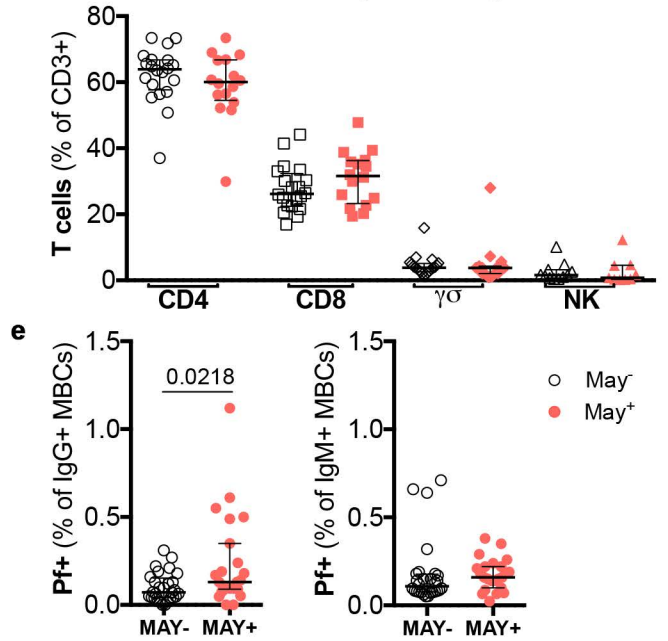
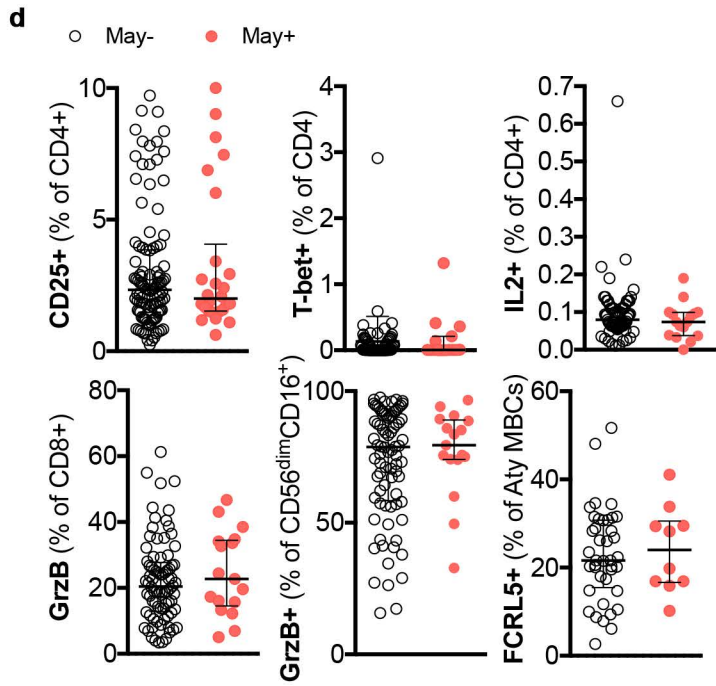
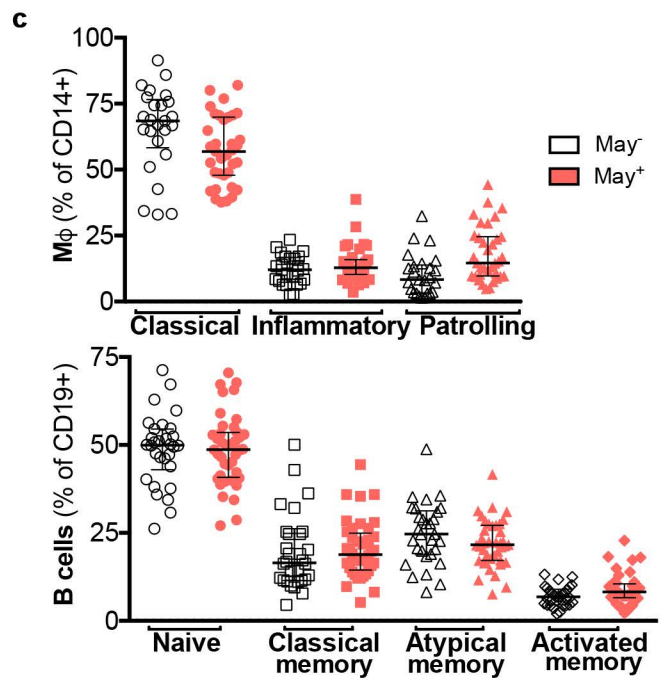
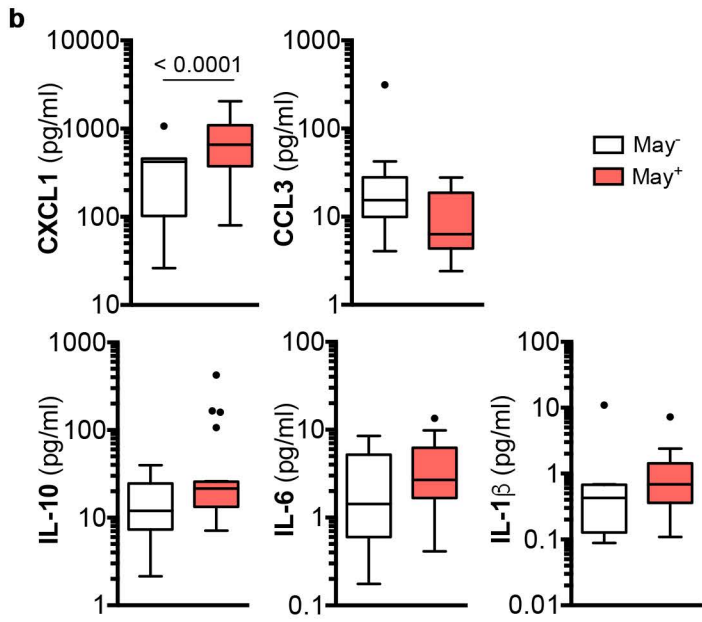
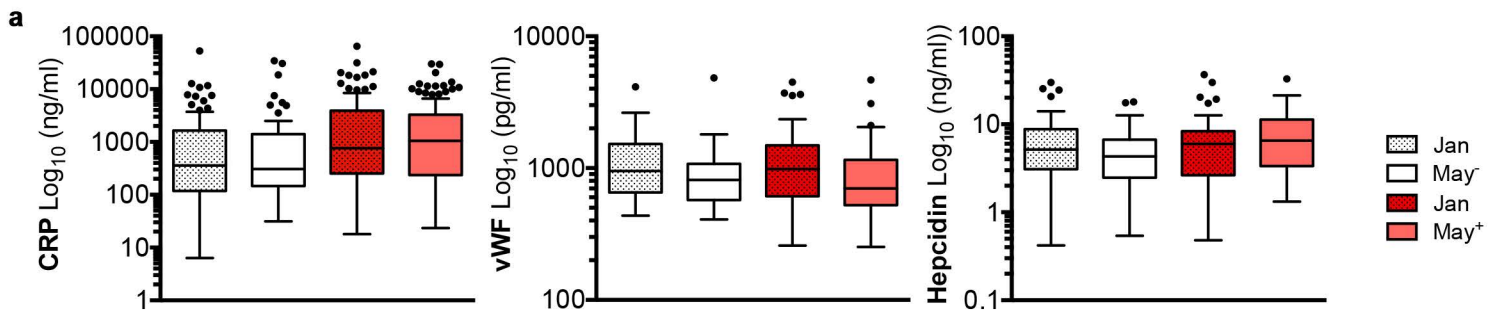
1166 Mann-Witney or Kruskal-Wallis were used to test for differences in between two or more groups  
1167 respectively. Differences between No. of clones, and clones' sizes were tested by Mood's  
1168 median test. PfEMP1 slopes between the beginning and the end of the dry season of subclinical  
1169 carriers and non-infected individuals were compared using a linear non -interaction model. A  
1170 spearman rank correlation between linear RT-qPCR normalized data and linear RNA-Seq  
1171 normalized data of each gene was obtained along with p-values using GraphPad Prism 8.0  
1172 software. Metabolites significant differences were determined by a 2-way ANOVA corrected for  
1173 multiple comparison controlling for an FDR of 0.05%. Statistical significance was defined as a 2-  
1174 tailed *P*-value of  $\leq 0.05$ . All analyses were performed with GraphPad Prism versions 6.0 or 8.0,  
1175 JMP 14.0.1, or R.

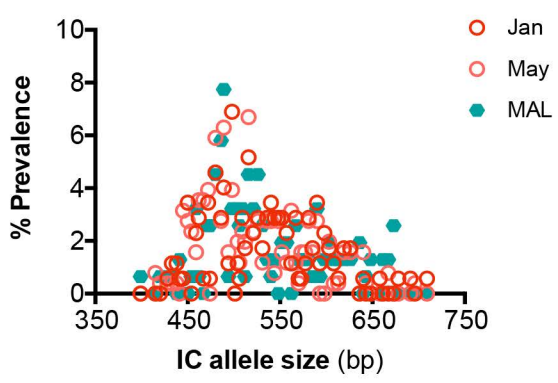
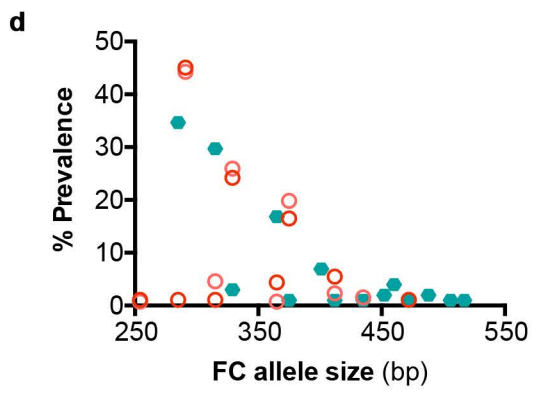
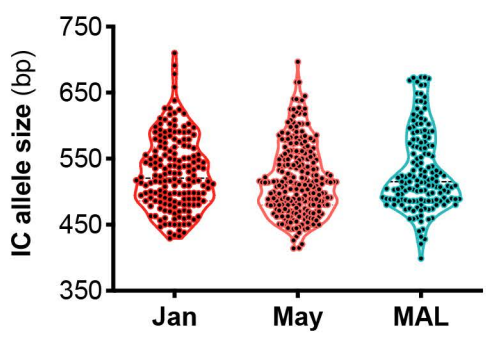
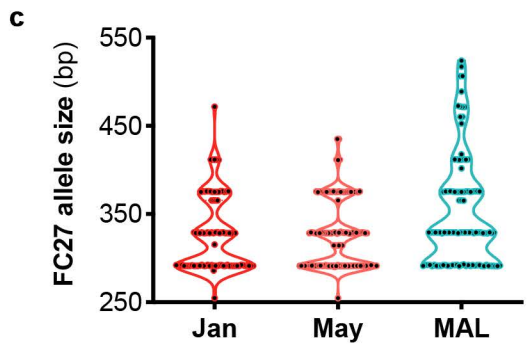
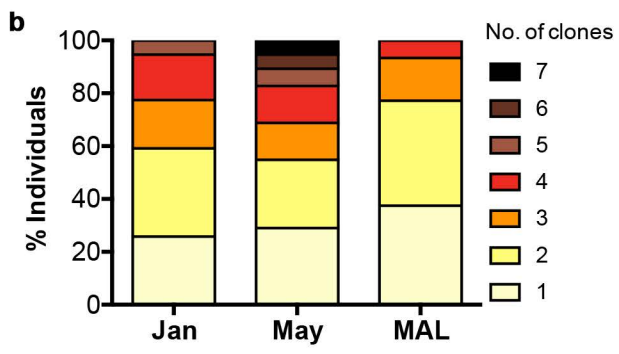
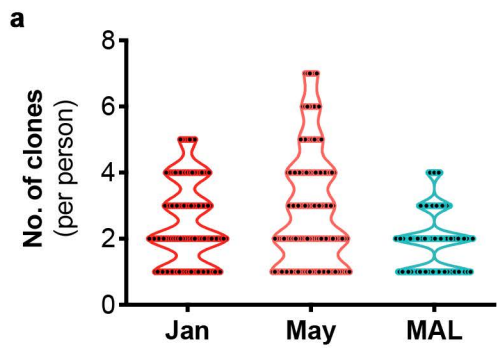
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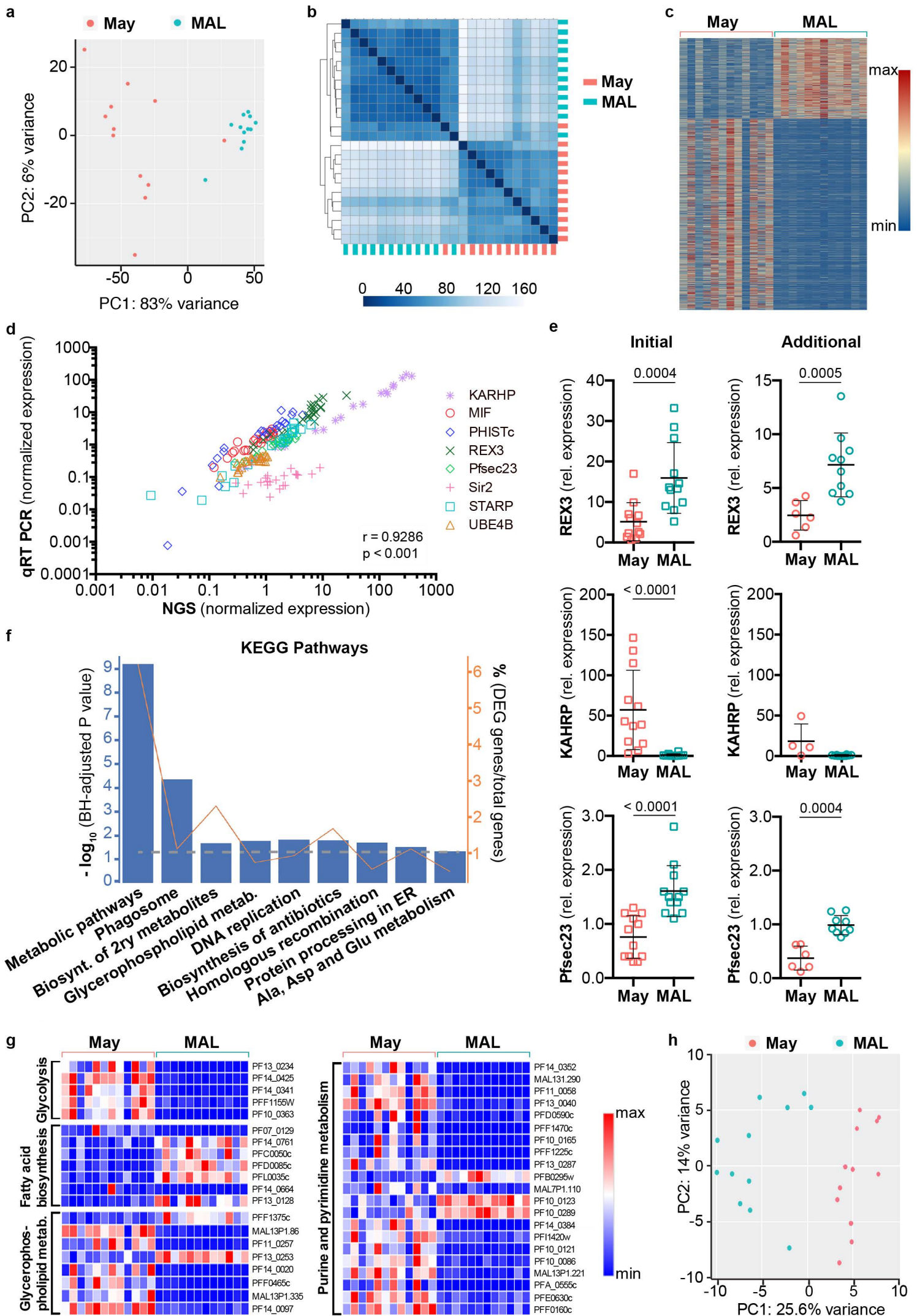
#### 1177 **Data availability**

1178 RNA-Seq data (normalized counts data and raw sequencing reads) have been deposited in  
1179 NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number  
1180 GSE148125 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE148125>)  
1181 All processed metabolomics spectral data and analytical metadata from this study have been  
1182 deposited into the international repository for metabolomics data and metadata NIH  
1183 Metabolomics Workbench (Study number from Metabolomics Workbench: ST001384).  
1184 The data file of assembled *var* gene fragments of all isolates are available at:  
1185 [https://github.com/ThomasDOtto/varDB/tree/master/Otherdatasets/Andrade\\_DryWet2020](https://github.com/ThomasDOtto/varDB/tree/master/Otherdatasets/Andrade_DryWet2020)

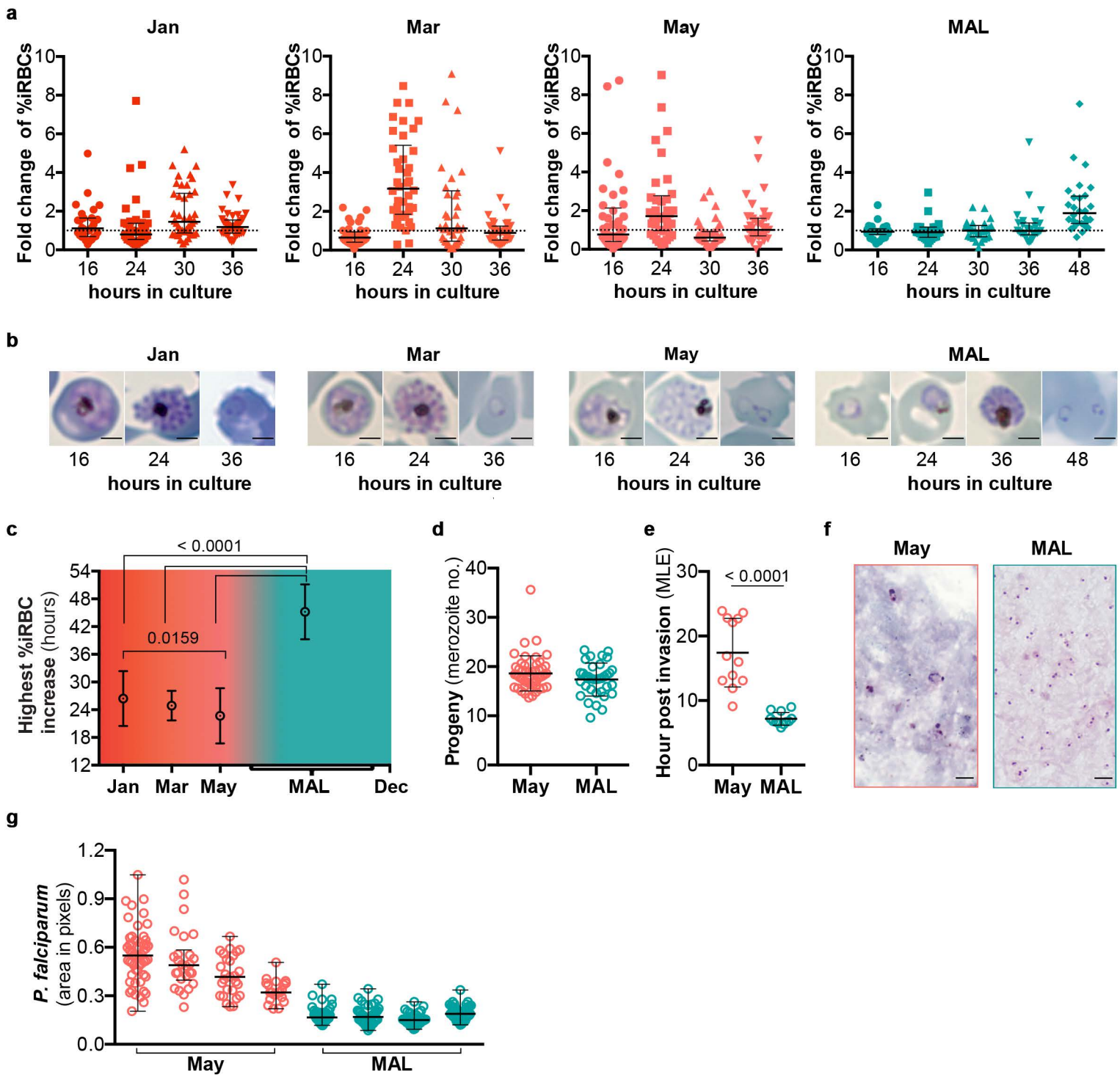


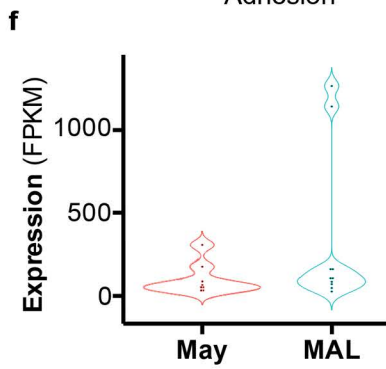
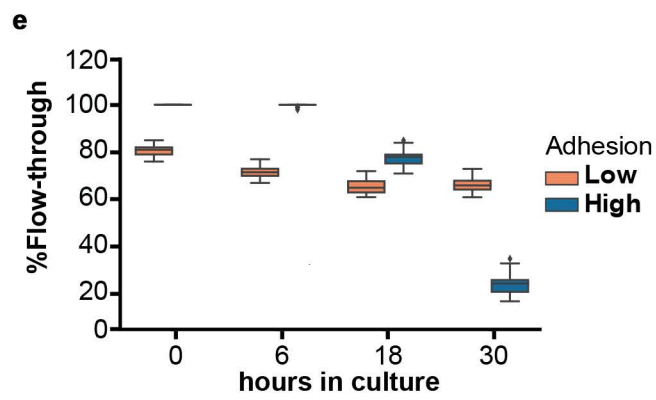
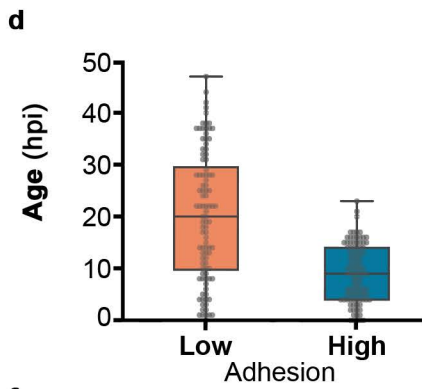
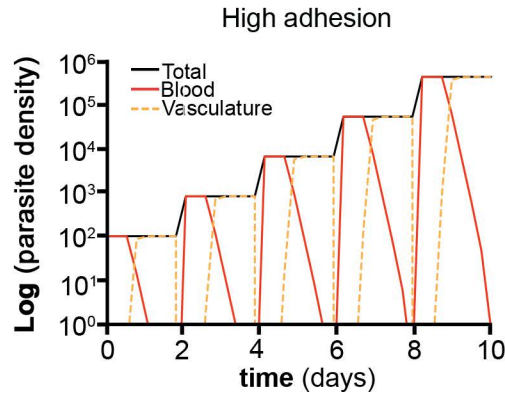
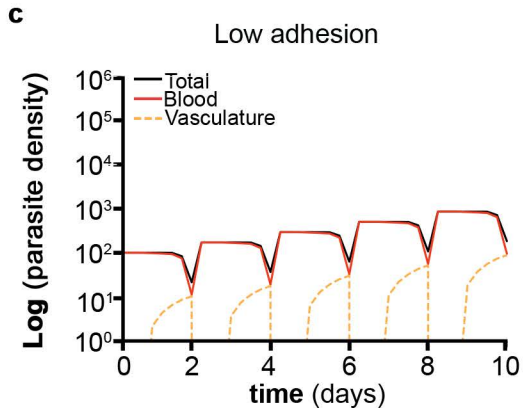
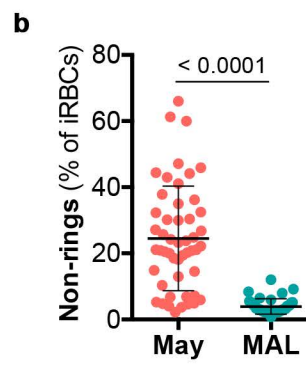
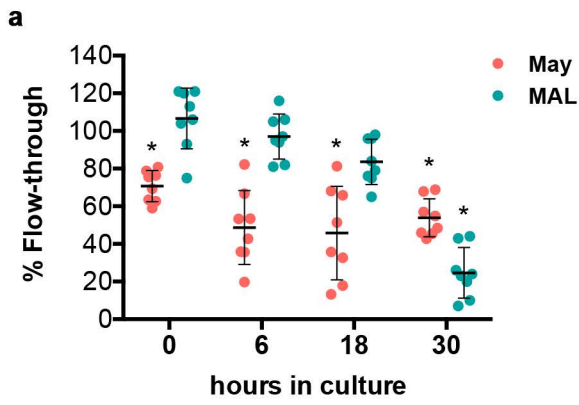


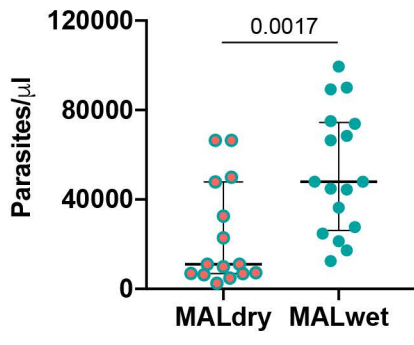




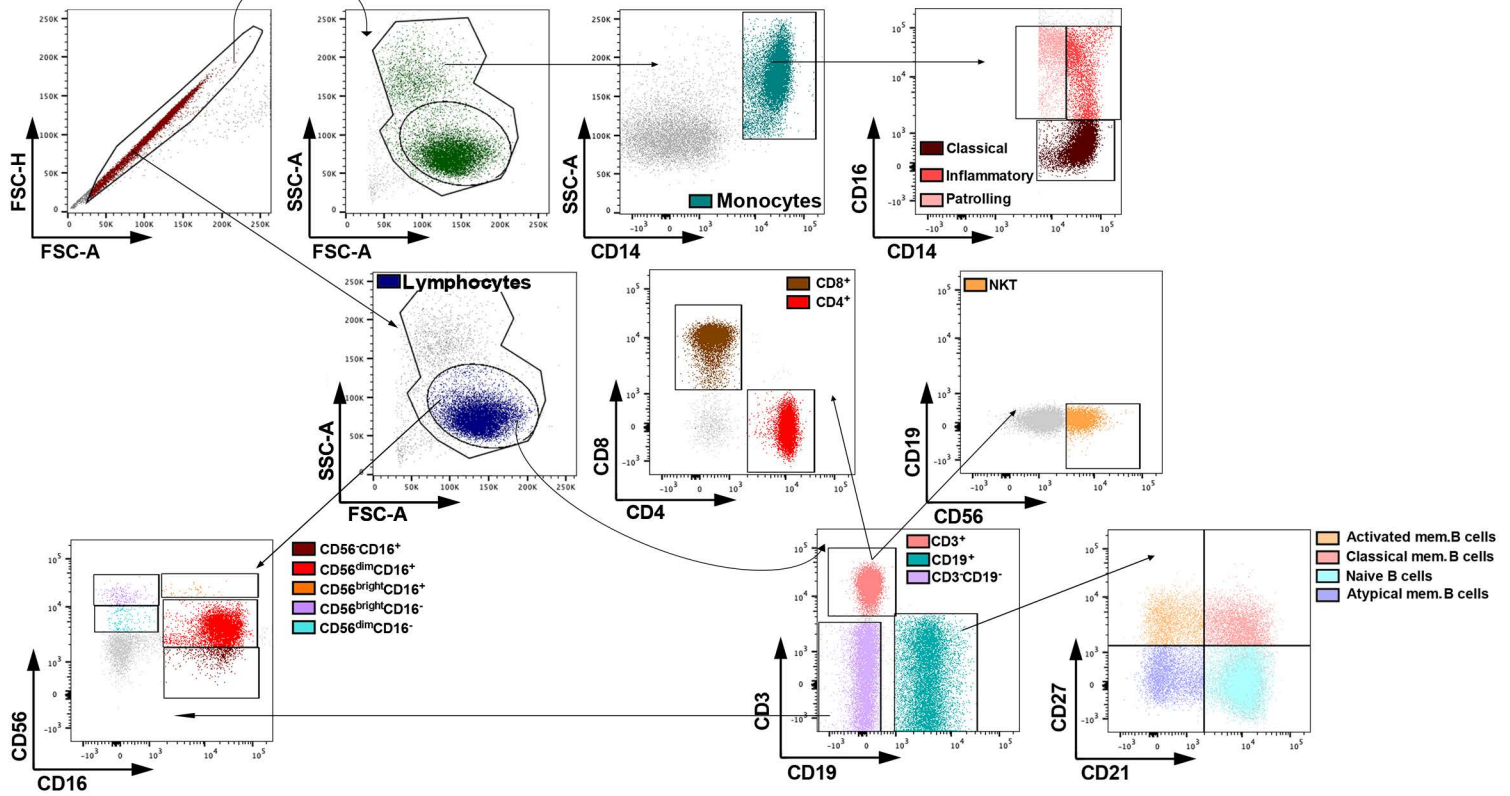




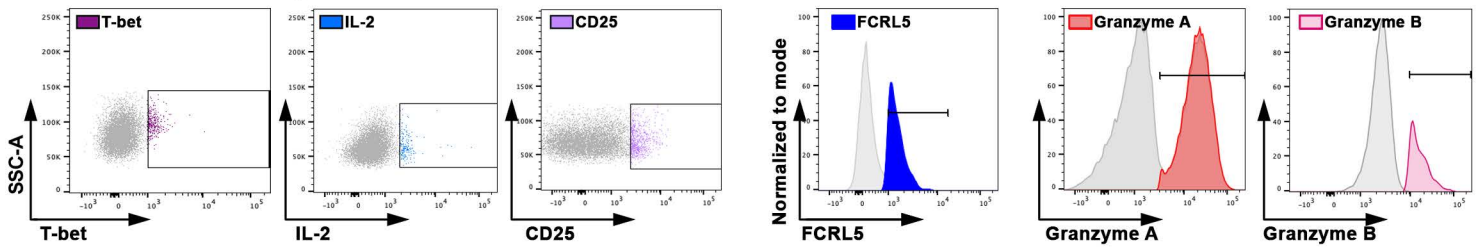




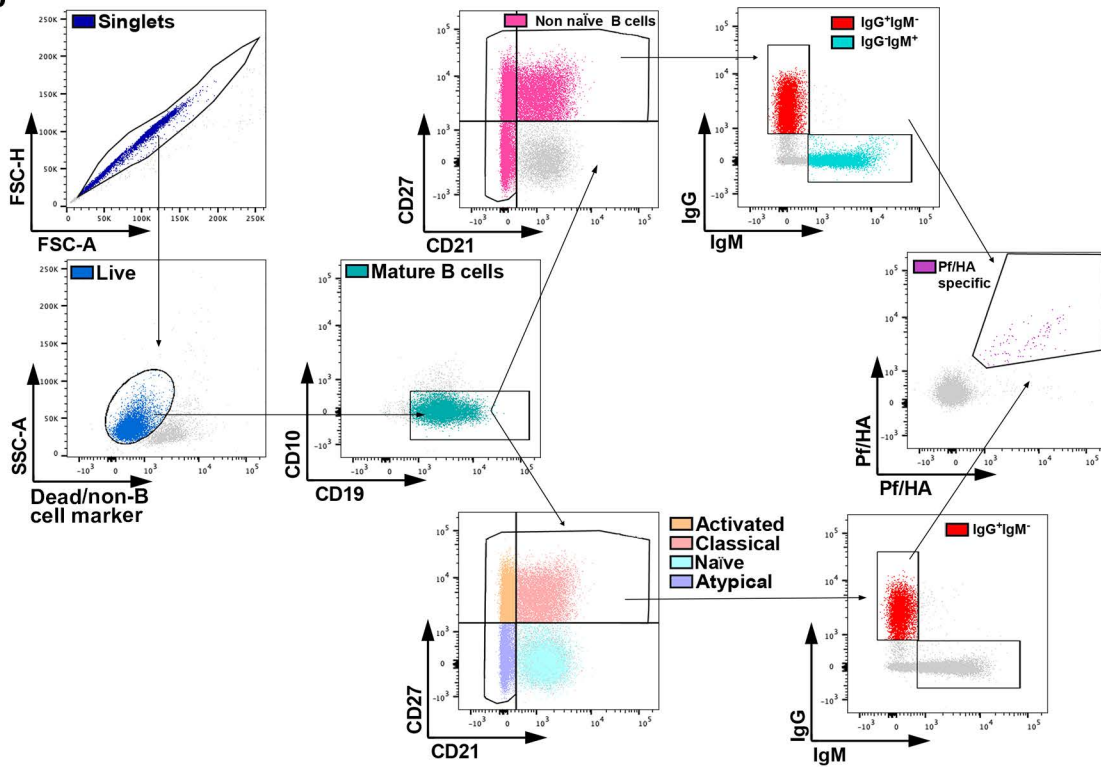
**a Surface staining**

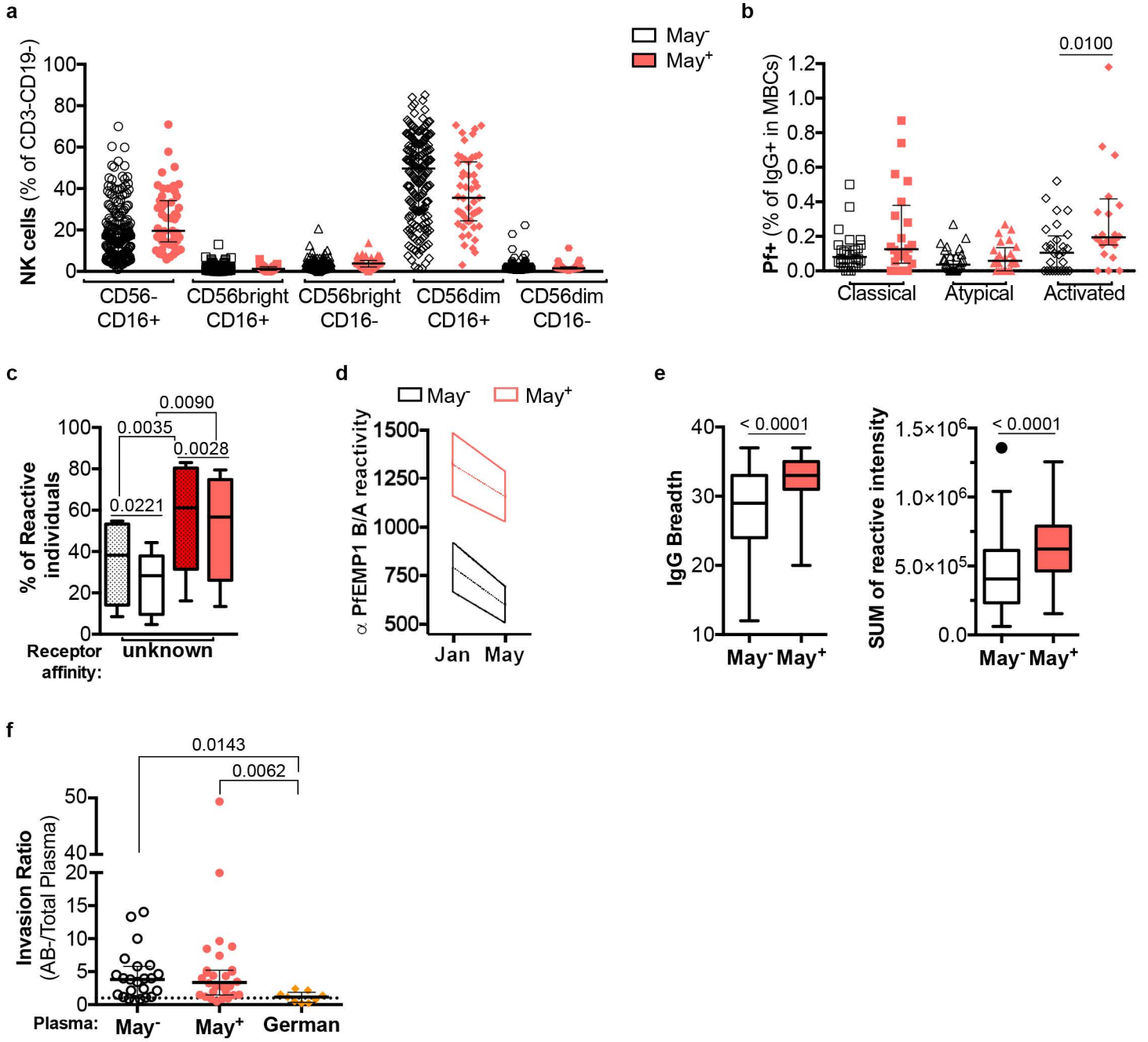


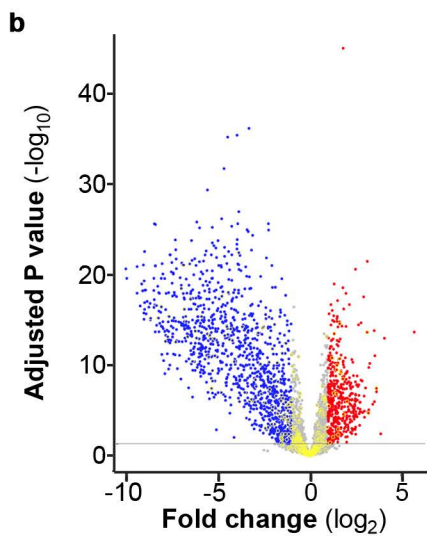
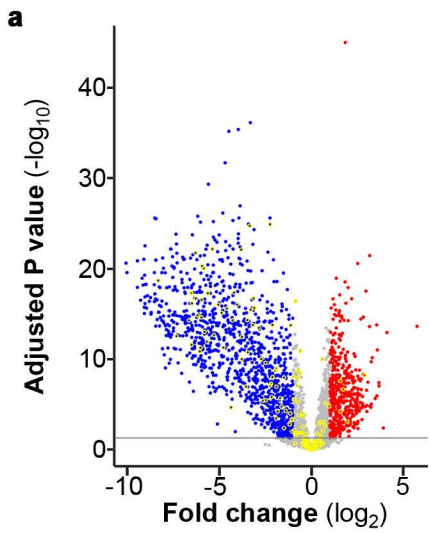
**Intracellular staining**

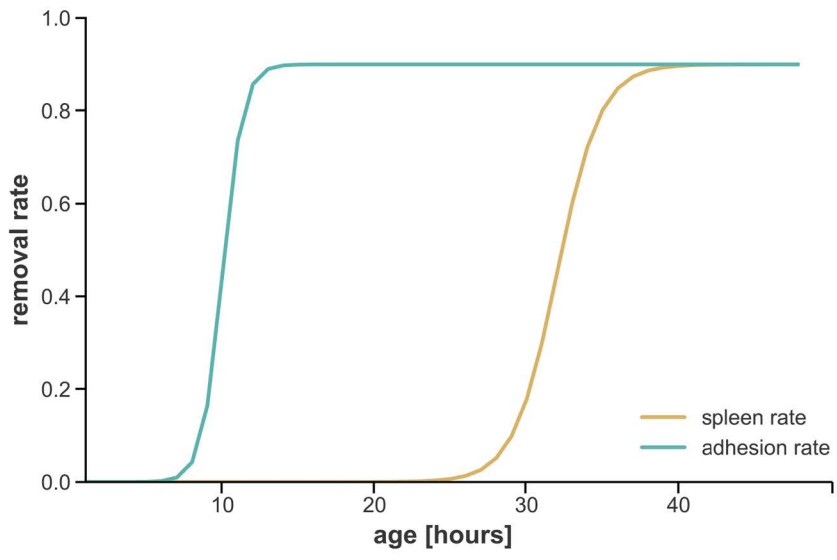


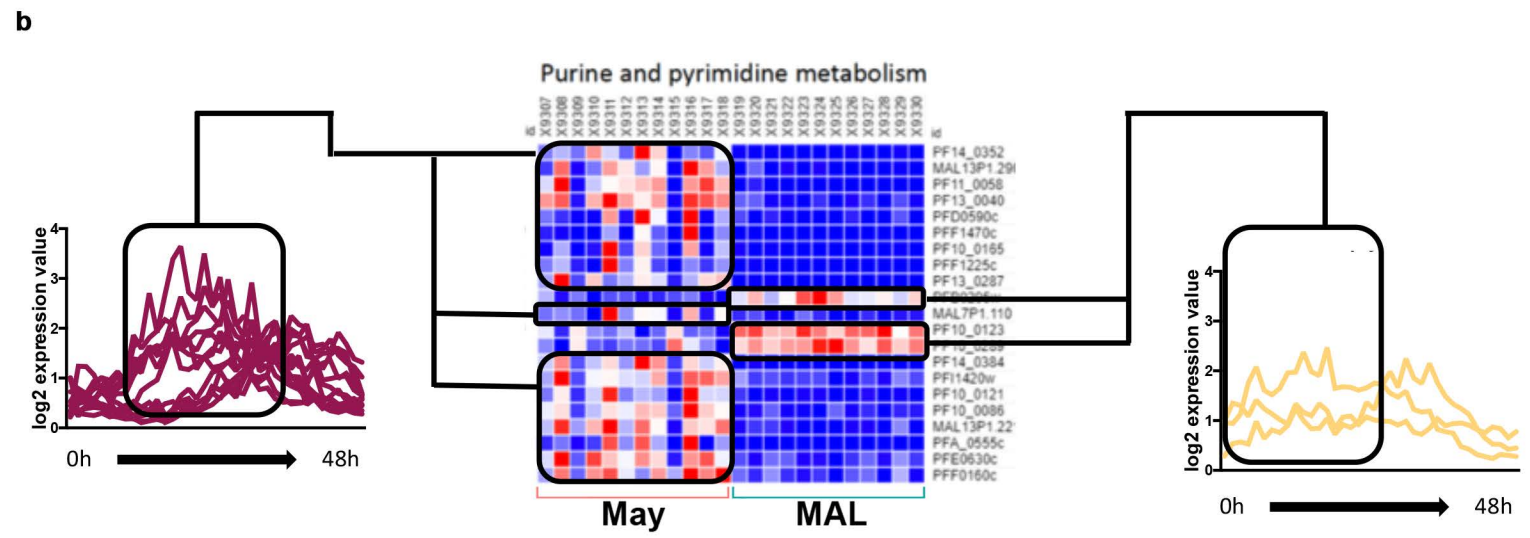
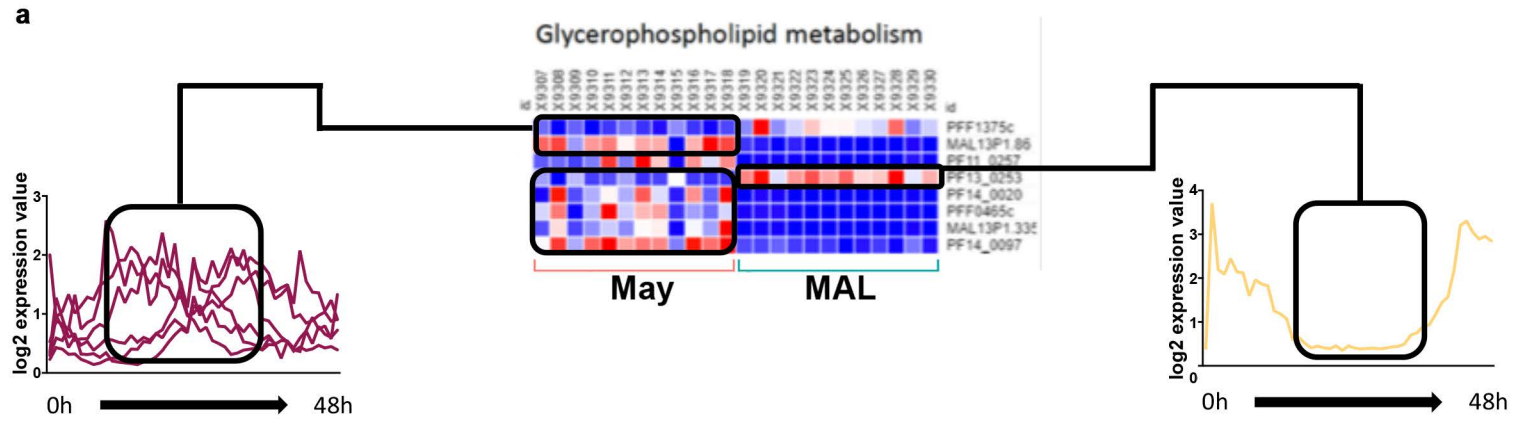
**b**













**Supplementary table 1:** Contingency tables with PCR data from beginning and end of the dry season of 2017 and 2018 used to calculate the odds ratio of maintaining the same infection status through the entire dry season

	Neg May17	Pos May17	
Neg Jan17	365	7	372
Pos Jan17	35	61	96
	400	68	468

Odds ratio 2017: 90.88 (CI: 38.63;213.82)

	Neg May18	Pos May18	
Neg Jan18	331	6	337
Pos Jan18	52	41	93
	383	47	430

Odds ratio 2018: 43.5 (CI: 17.46 , 107.54)

**Supplementary Table 2.** Multiplex bead array quantification of cytokines and chemokines in the plasma children with (May+) or without (May-) persisting *P. falciparum* at the end of the dry season. (n=12 May- and n=21 May+).

Cytokine	May- Median (Interquartile range)	May+ Median (Interquartile range)	Adjusted P Value
<b>CXCL1 (GRO)</b>	421.4 (102.2 - 455.8)	656.3 (374.8 - 1095)	< 0,0001
<b>CXCL10 (IP10)</b>	202.4 (71.52 - 245.2)	137.3 (107.4 - 383.8)	0,2705
<b>CCL2 (MCP1)</b>	134.1 (103.1 - 280.8)	252.4 (154.1 - 356.8)	0,9973
<b>CCL22 (MDC)</b>	465.6 (220.9 - 817.7)	400.1 (288.5 - 624.5)	0,9991
<b>sCD40L</b>	166.9 (91.06 - 439.7)	181.3 (77.97 - 514.1)	0,9997
<b>CCL3 (MIPa)</b>	15.41 (9.91 - 28)	6.33 (4.35 - 18.69)	> 0,9999
<b>CCL4 (MIP1b)</b>	14.37 (11.49 - 25.77)	16.47 (14.08 - 25.65)	> 0,9999
<b>CCL7 (MCP3)</b>	3.86 (2.45 - 6.95)	2.65 (1.98 - 3.91)	> 0,9999
<b>EGF</b>	7.72 (3.26 - 18.96)	11.35 (3.99 - 21.3)	> 0,9999
<b>Eotaxin</b>	12.59 (11.63 - 15.99)	15.45 (11.9 - 26.76)	> 0,9999
<b>FGFa</b>	7.82 (4.53 - 10.26)	2.74 (1.99 - 4.24)	> 0,9999
<b>Fractaline</b>	5.09 (4.10 - 7.78)	5.58 (4.75 - 12.16)	> 0,9999
<b>GCFS</b>	178.9 (42.32 - 755.2)	185.9 (65.12 - 542.7)	> 0,9999
<b>GMCSF</b>	0.53 (0.34 - 1.26)	1.5 (0.73 - 2.65)	> 0,9999
<b>IFNa2</b>	2.72 (0.91 - 6.64)	5.73 (3.80 - 14.1)	> 0,9999
<b>IFNy</b>	0.84 (0.51 - 2.84)	2.07 (0.64 - 3.21)	> 0,9999
<b>IL1a</b>	10.26 (6.18 - 30.8)	18.16 (5.39 - 34.62)	> 0,9999
<b>IL1b</b>	0.43 (0.13 - 0.68)	0.69 (0.36 - 1.43)	> 0,9999
<b>IL-2</b>	1.86 (1.32 - 2.60)	1.92 (1.53 - 2.59)	> 0,9999
<b>IL-3</b>	0.36 (0.23 - 0.45)	0.41 (0.25 - 0.69)	> 0,9999
<b>IL-6</b>	1.66 (0.81 - 15.63)	2.68 (1.67 - 6.24)	> 0,9999
<b>IL-7</b>	2.42 (1.18 - 49.67)	3.47 (1.25 - 8.30)	> 0,9999
<b>IL-8</b>	18.7 (14.5 - 76.06)	44.99 (22.13 - 122.6)	> 0,9999
<b>IL-9</b>	2.64 (0.73 - 7.38)	2.80 (0.97 - 11.84)	> 0,9999
<b>IL12P40</b>	5.32 (0.40 - 15.76)	9.972 (3.62 - 17.51)	> 0,9999
<b>IL13</b>	1.11 (0.17 - 9.57)	0.57 (0.23 - 97)	> 0,9999
<b>IL15</b>	1.61 (1.33 - 4.87)	1.76 (1.47 - 2.57)	> 0,9999
<b>IL-10</b>	10.23 (7.23 - 24.33)	21.53 (13.28 - 25.77)	> 0,9999
<b>IL-17</b>	1.59 (1.57 - 1.62)	1.61 (1.58 - 1.76)	> 0,9999
<b>IL1RA</b>	1.02 (0.58 - 6.65)	2.52 (1.05 - 8.07)	> 0,9999
<b>TGFb</b>	1.18 (0.86 - 1.66)	1.58 (1.23 - 3.09)	> 0,9999
<b>VEGF</b>	3.46 (1.29 - 8.54)	3.46 (1.20 - 13.14)	> 0,9999

(shown are Adjusted P values calculated through Sidak's multiple comparisons test)

**Supplementary Table 3.** Expression of surface and intracellular markers of freshly collected PBMCs from children that carried *P. falciparum* parasites (May+) or remained uninfected (May-) during the dry season.

Parameter	n	n May-	May- Mean (SD)	n May+	May+ Mean (SD)	p value	Significant?	test
CD3 (% of Lymphocytes)	252	201	71.72 (8.54)	51	73.75 (7.61)	0,074	No	Mann-Whitney
CD4 (% of CD3 T cells)	235	188	61.9 (6.54)	47	63.63 (5.62)	> 0,9999	No	Kruskal-Wallis
CD8 (% of CD3 T cells)	252	201	29.52 (5.61)	51	28.68 (5.4)	> 0,9999	No	Kruskal-Wallis
NKT cells (% of CD3 T cells)	252	201	2.659 (1.81)	51	2.15 (1.21)	> 0,9999	No	Kruskal-Wallis
Granzyme B (% of CD8 T cells)	97	80	22.2 (12.66)	17	24.51 (12.69)	> 0,9999	No	Kruskal-Wallis
Granzyme B (% of NKT cells)	97	80	43.02 (21.75)	17	44.92 (22.59)	> 0,9999	No	Kruskal-Wallis
Granzyme B (Geo Mean of CD8 T cells)	97	80	3399 (3169)	17	3294 (2149)	> 0,9999	No	Kruskal-Wallis
Granzyme B (Geo Mean of NKT cells)	97	80	18733 (19005)	17	13471 (14620)	> 0,9999	No	Kruskal-Wallis
T-bet (% of CD4 T cells)	76	61	0.13 (0.38)	15	0.1656 (0.3477)	> 0,9999	No	Kruskal-Wallis
T-bet (% of CD8 T cells)	80	62	3.13 (4.64)	18	2.31 (3.795)	> 0,9999	No	Kruskal-Wallis
T-bet (% of NKT cells)	80	62	4.86 (7.85)	18	3.65 (6.19)	> 0,9999	No	Kruskal-Wallis
T-bet (Geo Mean of CD4 T cells)	74	59	2577 (6708)	15	1879 (1293)	> 0,9999	No	Kruskal-Wallis
T-bet (Geo Mean of CD8 T cells)	77	59	1327 (212.6)	18	1571 (677.4)	0,141	No	Kruskal-Wallis
T-bet (Geo Mean of NKT cells)	74	57	1424 (327.4)	17	1533 (411.2)	0,453	No	Kruskal-Wallis
IL2 (% of CD4 T cells)	84	68	0.094(0.084)	16	0.076 (0.047)	> 0,9999	No	Kruskal-Wallis
IL2 (% of CD8 T cells)	92	72	0.081(0.067)	20	0.082 (0.061)	> 0,9999	No	Kruskal-Wallis
IL2 (% of NKT cells)	92	72	3.01(3.58)	20	3.03 (5.40)	> 0,9999	No	Kruskal-Wallis
CD25 (% of CD4 T cells)	133	107	3.0 (2.30)	26	3.27 (2.74)	> 0,9999	No	Kruskal-Wallis
CD25 (% of CD8 T cells)	144	114	0.03 (0.034)	30	0.026 (0.020)	> 0,9999	No	Kruskal-Wallis
CD25 (% of NKT cells)	144	114	0.52 (0.51)	30	0.48 (0.44)	> 0,9999	No	Kruskal-Wallis
CD25 (Geo Mean of CD4 T cells)	133	107	2846 (424.2)	26	2769 (438.6)	> 0,9999	No	Kruskal-Wallis
CD25 (Geo Mean of CD8 T cells)	119	94	3615 (6454)	25	2981 (1753)	0,604	No	Kruskal-Wallis
CD25 (Geo Mean of NKT cells)	137	109	4710 (4167)	28	4378 (2489)	> 0,9999	No	Kruskal-Wallis
IL2 (Geo Mean of CD4 T cells)	82	67	5534 (1913)	15	5374 (1551)	> 0,9999	No	Kruskal-Wallis
IL2 (Geo Mean of CD8 T cells)	91	71	5262 (1705)	20	5118 (1355)	> 0,9999	No	Kruskal-Wallis
IL2 (Geo Mean of NKT cells)	91	72	6113 (5387)	19	5407 (2237)	> 0,9999	No	Kruskal-Wallis
CD19 (% of Lymphocytes)	249	198	15.85 (3.98)	51	16.84 (4.45)	0,241	No	Mann-Whitney
Activated MBCs (% of CD19 B cells)	225	177	3.52 (2.0)	48	4.86 (2.87)	0,704	No	Kruskal-Wallis
Classical MBCs (% of CD19 B cells)	225	177	14.47 (6.09)	48	12.92 (5.11)	> 0,9999	No	Kruskal-Wallis
Naive (% of CD19 B cells)	225	177	70.7 (9.2)	48	68.1 (9.8)	> 0,9999	No	Kruskal-Wallis
Atypical MBCs (% of CD19 B cells)	225	177	11.34 (5.51)	48	14.1 (6.41)	0,423	No	Kruskal-Wallis
FCRL5 (% of Activated MBCs)	50	40	19.32 (8.31)	10	22.36 (10.62)	> 0,9999	No	Kruskal-Wallis
FCRL5 (% of Classical MBCs)	50	40	2.06 (1.45)	10	2.61 (2.29)	> 0,9999	No	Kruskal-Wallis
FCRL5 (% of Naive MBCs)	50	40	0.46 (0.61)	10	0.63 (0.57)	> 0,9999	No	Kruskal-Wallis
FCRL5 (% of Atypical MBCs)	50	40	22.94 (10.59)	10	24.17(9.66)	> 0,9999	No	Kruskal-Wallis
Granzyme B (% of Activated MBCs)	97	80	24.05 (20.02)	17	22.79 (19.61)	> 0,9999	No	Kruskal-Wallis
Granzyme B (% of Classical MBCs)	97	80	6.13 (10.71)	17	9.4 (14.57)	> 0,9999	No	Kruskal-Wallis
Granzyme B (% of Naive MBCs)	97	80	2.43 (5.52)	17	3.73 (8.18)	> 0,9999	No	Kruskal-Wallis
Granzyme B (% of Atypical MBCs)	97	80	6.84 (11.36)	17	7.62 (11.67)	> 0,9999	No	Kruskal-Wallis
IL2 (Geo Mean of Activated MBCs)	91	72	841.4 (281)	19	954 (281.6)	0,649	No	Kruskal-Wallis
IL2 (Geo Mean of Classical MBCs)	91	72	754.6 (249.7)	19	864 (243.3)	0,348	No	Kruskal-Wallis
IL2 (Geo Mean of Naive MBCs)	91	72	557.8 (175.5)	19	632.5 (174.4)	0,580	No	Kruskal-Wallis
IL2 (Geo Mean of Atypical MBCs)	91	72	648.7 (202.6)	19	775.2 (230.9)	0,148	No	Kruskal-Wallis
Granzyme B (Geo Mean of Activated MBCs)	96	80	4121 (3791)	16	3773 (3121)	> 0,9999	No	Kruskal-Wallis
Granzyme B (Geo Mean of Classical MBCs)	97	80	2359 (2015)	17	2564 (2019)	> 0,9999	No	Kruskal-Wallis
Granzyme B (Geo Mean of Naive MBCs)	97	80	1851 (1500)	17	1899 (1565)	> 0,9999	No	Kruskal-Wallis
Granzyme B (Geo Mean of Atypical MBCs)	97	80	2231 (2087)	17	2207 (1771)	> 0,9999	No	Kruskal-Wallis
CD25 (Geo Mean of Activated MBCs)	91	72	88.82 (34.49)	19	100.6 (25.79)	> 0,9999	No	Kruskal-Wallis
CD25 (Geo Mean of Classical MBCs)	91	72	163.4 (29.63)	19	168.5 (21.83)	> 0,9999	No	Kruskal-Wallis
CD25 (Geo Mean of Naive MBCs)	91	72	57.71 (16.91)	19	50.82 (10.1)	> 0,9999	No	Kruskal-Wallis
CD25 (Geo Mean of Atypical MBCs)	91	72	38.51 (10.56)	19	42.23 (10.08)	> 0,9999	No	Kruskal-Wallis
CD56-CD16+ (% of CD3-CD19-)	249	198	18.83 (13.22)	51	24.77 (14.38)	> 0,9999	No	Kruskal-Wallis
CD56dimCD16- (% of CD3-CD19-)	249	198	1.95 (2.24)	51	1.99 (1.72)	> 0,9999	No	Kruskal-Wallis
CD56dimCD16+ (% of CD3-CD19-)	249	198	45.73 (19.98)	51	37.86 (17.53)	> 0,9999	No	Kruskal-Wallis
CD56brightCD16- (% of CD3-CD19-)	249	198	3.03 (2.26)	51	4.00 (2.47)	0,766	No	Kruskal-Wallis
CD56brightCD16+ (% of CD3-CD19-)	249	198	1.4 (1.55)	51	1.52 (1.14)	> 0,9999	No	Kruskal-Wallis
Granzyme A (% of CD56-CD16+)	50	40	32.05 (28.37)	10	12.63 (12.04)	0,256	No	Kruskal-Wallis
Granzyme A (% of CD56dimCD16-)	50	40	17.02 (15.51)	10	8.92 (5.98)	> 0,9999	No	Kruskal-Wallis
Granzyme A (% of CD56dimCD16+)	50	40	35.02 (25.13)	10	18.71 (17.21)	0,780	No	Kruskal-Wallis
Granzyme A (% of CD56brightCD16-)	50	40	7.71 (9.69)	10	3.61 (5.98)	> 0,9999	No	Kruskal-Wallis
Granzyme A (% of CD56brightCD16+)	50	40	19.05 (18.66)	10	10.6 (15.33)	0,444	No	Kruskal-Wallis
Granzyme B (% of CD56-CD16+)	97	80	54.99 (21.82)	17	54.35 (21.6)	> 0,9999	No	Kruskal-Wallis
Granzyme B (% of CD56dimCD16-)	97	80	16.45 (11.94)	17	15.72 (11.77)	> 0,9999	No	Kruskal-Wallis
Granzyme B (% of CD56dimCD16+)	97	80	72.86 (21.37)	17	77.09 (16.52)	> 0,9999	No	Kruskal-Wallis
Granzyme B (% of CD56brightCD16-)	97	80	6.038 (7.042)	17	7.82 (7.589)	> 0,9999	No	Kruskal-Wallis
Granzyme B (% of CD56brightCD16+)	97	80	24.72 (25.01)	17	20.92 (16.2)	> 0,9999	No	Kruskal-Wallis
MΦ (CD14+) (% of PBMCs)	234	187	6.356(4.591)	47	5.68 (3.97)	0,464	No	Mann-Whitney
Classical MΦ (% of CD14+)	234	187	73.92(13.3)	47	69 (19.78)	> 0,9999	No	Kruskal-Wallis
Inflammatory MΦ (% of CD14+)	234	187	12.83 (7.75)	47	13.15 (9.171)	> 0,9999	No	Kruskal-Wallis
Patrolling MΦ (% of CD14+)	234	187	12.21 (8.95)	47	17.01 (14.87)	0,763	No	Kruskal-Wallis
Activated (% of B cells)	52	28	3.827 (2.126)	24	8.807 (6.443)	0,1209	No	Kruskal-Wallis
Classical (% of B cells)	52	28	19.5 (6.028)	24	17.75 (4.915)	> 0,9999	No	Kruskal-Wallis
Atypical (% of B cells)	52	28	9.866 (3.702)	24	14.4 (6.849)	0,5455	No	Kruskal-Wallis
Naive (% of B cells)	52	28	66.8 (7.496)	24	59.05 (14.19)	> 0,9999	No	Kruskal-Wallis
Pf specific (IgM+ of non-naive mature B cells)	52	28	0.1765 (0.1833)	24	0.1753 (0.09002)	0,1046	No	Mann-Whitney
HA specific (IgM+ of non-naive mature B cells)	52	28	0.05646 (0.05032)	24	0.04124 (0.02893)	0,5074	No	Mann-Whitney
Pf specific (IgG+ of non-naive mature B cells)	52	28	0.1005 (0.08167)	24	0.237 (0.2624)	0,0218	Yes	Mann-Whitney
HA specific (IgG+ of non-naive mature B cells)	52	28	0.163 (0.1773)	24	0.09068 (0.06453)	0,1151	No	Mann-Whitney
Pf specific (% IgG+ of Classical)	52	28	0.1098 (0.1107)	24	0.2724 (0.3818)	0,6448	No	Kruskal-Wallis
Pf specific (% IgG+ of Atypical)	52	28	0.0615 (0.06772)	24	0.08229 (0.08235)	> 0,9999	No	Kruskal-Wallis
Pf specific (% IgG+ of Activated)	52	28	0.1297 (0.1439)	24	0.3593 (0.3929)	0,01	Yes	Kruskal-Wallis
HA specific (% IgG+ of Classical)	52	28	0.1306 (0.1008)	24	0.0773 (0.0575)	0,3174	No	Kruskal-Wallis
HA specific (% IgG+ of Activated)	52	28	0.313 (0.779)	24	0.08079 (0.09381)	> 0,9999	No	Kruskal-Wallis
HA specific (% IgG+ of Atypical)	52	28	0.1906 (0.1914)	24	0.1117 (0.12)	0,3054	No	Kruskal-Wallis

**Supplementary Table 4.** Different domain types of the VSA multigene family var, which were grouped according to their endothelial-receptor affinity (CD36, EPCR or unknown receptor) and PfEMP1 UPS type (A, B or B/A) and non-VSA controls.

Protein name	CIDR domain class	PfEMP1 group	Binding phenotype	Genome/ Isolate	PfEMP1
CIDRa6_IT4var12_IT4	α6	B	CD36	IT4	IT4var12
CIDRa5_IT4var14_IT4	α5	B	CD36	IT4	IT4var14
CIDRa2.2_IT4var24_IT4	α2.2	B	CD36	IT4	IT4var24
CIDRa3.3_IT4var26_IT4	α3.3	B	CD36	IT4	IT4var26
CIDRa2.10_IT4var30_IT4	α2.10	B	CD36	IT4	IT4var30
CIDRa2.4_IT4var33_IT4	α2.4	B	CD36	IT4	IT4var33
CIDRa2.9_IT4var45_IT4	α2.9	B	CD36	IT4	IT4var45
CIDRa2.7_IT4var61_IT4	α2.7	B	CD36	IT4	IT4var61
CIDRa3.1_DD2var01_DD2	α3.1	B	CD36	DD2	DD2var01
CIDRa3.1_HB3var27_HB3	α3.1	B	CD36	HB3	HB3var27
CIDRa3.1_IT4var21_IT4	α3.1	B	CD36	IT4	IT4var21
CIDRa3.5_IT4var15_IT4	α3.5	B	CD36	IT4	IT4var15
CIDRa1.4_HB3var03_HB3	α1.4	A	EPCR	HB3	HB3var03
CIDRa1.1_IT4var20_IT4	α1.1	B/A	EPCR	IT4	IT4var20
CIDRa1.4_IT4var7_IT4	α1.4	A	EPCR	IT4	IT4var7
CIDRa1.1_igh_var19_IGH	α1.1	B/A	EPCR	IGH	igh_var19
CIDRa1.1_raj116_var8_raj116	α1.1	B/A	EPCR	raj116	raj116_var8
CIDRa1.5a_1965_2_1965	α1.5a	A	EPCR	1965	1965_2
CIDRa1.5a_GA013_ERS010323	α1.5a	A	EPCR	ERS010323	GA013
CIDRa1.5a_GA014_ERS010022	α1.5a	A	EPCR	ERS010022	GA014
CIDRa1.5b_1918_5_1918	α1.5b	A	unknown	1918	1918_5
CIDRa1.5b_1983_13_1983	α1.5b	A	unknown	1983	1983_13
CIDRa1.6a_HB3var02_HB3	α1.6a	A	EPCR	HB3	HB3var02
CIDRa1.6b_GA018_ERS010570	α1.6b	A	EPCR	ERS010570	GA018
CIDRa1.6b_GA019_ERS010031	α1.6b	A	EPCR	ERS010031	GA019
CIDRa1.7_1965_8_1965	α1.7	A	EPCR	1965	1965_8
CIDRa1.7_1918_3_1918	α1.7	A	EPCR	1918	1918_3
CIDRa1.7_GA024_ERS010438	α1.7	A	EPCR	ERS010438	GA024
CIDRa1.8a_Ga026_ERS010178	α1.8a	B/A	EPCR	ERS010178	Ga026
CIDRa1.8b_Ga027_2053	α1.8b	B/A	EPCR	2053	Ga027
CIDRa1.8b_Ga029_ERS010532	α1.8b	B/A	EPCR	ERS010532	Ga029
CIDRδ_HB3var05_HB3	δ	A	unknown	HB3	HB3var05
CIDRδ_HB3var35_HB3	δ	A	unknown	HB3	HB3var35
CIDRδ_IT4var02_IT4	δ	A	unknown	IT4	IT4var02
CIDRγ_IT4var08_IT4	γ	A	unknown	IT4	IT4var08
CSP-1	N/A	N/A	N/A	N/A	N/A
AMA-1	N/A	N/A	N/A	N/A	N/A
MSP-1	N/A	N/A	N/A	N/A	N/A
TET TOX	N/A	N/A	N/A	N/A	N/A
BSA	N/A	N/A	N/A	N/A	N/A

**Supplementary Table 5.** RNAseq of RDT+ individuals at the end of the dry season (May) and first clinical malaria cases (MAL).

	n	<i>P. falciparum</i> smear+ (n)	<i>P. malariae</i> PCR+ (n)	age mean (95% CI)	parasites/ul mean (95% CI)	Million reads mapped mean (95% CI)	Million reads mapped to genes mean (95% CI)
May	12	12	3	7.8 (7.5 - 8.2)	1067 (542 - 1591)	0.67 (0.46 - .75)	0.35 (0.22 - 0.48)
MAL	12	12	0	7.9 (7.2 - 8.6)	104231 (62193 - 146270)	2.21 (1.66 - 2.75)	1.34 (1.00 - 1.67)



**Supplementary Table 7.** Oligo sequences and probes for RTq-PCR expression data of eight selected and one housekeeping *P. falciparum* transcripts.

Gene	GeneBank#	Oligo	5'-3' sequence
GlyRS	XM_001348335.2	FOR	TGAGTGATATGGATAATATAAAGGAACAAA
		REV	GGATGATATTTACAAAACGTATCTTTCT
		CalFluorGold540_BHQNova	TAAGCTCTTCCAACTACCACATCCATCATTCTCT
REX3	XM_001352191.1	FOR	TTTCCTTTGCTTATATCTTAATACGTTCAACT
		REV	CTTGTGAAGTTGTTGCTTCTATATGTGAT
		6FAM_BHQNova	ACCACCTCATACGAAGGGAGCAGTTTC
MIF	XM_001350654.1	FOR	GGTAGCAACGAAGCTTATTGTTTTGTA
		REV	CAAGTTGCTTACAAGGAGTTTCGGTTAT
		6FAM_BHQNova	ATCAGCAAGAGCAGAATTATTGACCTATTAATTCCTCCA
Pfsec23	XM_001349263.1	FOR	CGGTGTAGCATTGAGTTTGCT
		REV	CTTAAAGGCGTATCTACAATTTACCAG
		6FAM_BHQNova	TGCTCCACCTATAACATCATAACACGACCCTTAATT
Sir2	XM_001349975.1	FOR	AAGAGATATATCATCCGATTATGAAATAGAAAT
		REV	AATCCATCTACATTTGTGTTACTACTGATT
		6FAM_BHQNova	CCAAACTTCTAAAGTTGATAAAGCTACATGACCATTGTT
UBE4B	XM_002808834.1	FOR	TATGCCTGTAAATACGACCGATTCT
		REV	TTGCATTATTATTATTAGAGTTGGTGGTTG
		6FAM_BHQNova	CCTCTTGTGGAACATCATTATTACCATTGCTGT
PHISTc	XM_001349662.1	FOR	TTTGTTATGGTTATACGAACAAGG
		REV	TTTTCTACATTATTCGAAGATTTCTTTCTCT
		6FAM_BHQNova	ATCCTCATACAGATTAATTTCAAGTTGAATCAAAAACGT
KAHRP	XM_001349498.2	FOR	GTGAGAACCATCGTGTGCACTT
		REV	TGTGGACCTGCCGCTATAGATT
		6FAM_BHQNova	TGTTCCAGCAGATGCACCAATGGCT
STARP	XM_001348922.1	FOR	CCGTGGTTGTTGTAATATTATTGTCT
		REV	TAAATACAAAACCCATTGATAATAATAACACA
		6FAM_BHQNova	TGCTGTGTTCTTATTATCTGTTTCCTTTGTGCCTG





**Supplementary Table 9.** Metabolites significantly different by LC-MS with a FDR 0.05% in the plasma of 12 subclinical individuals at the end of the dry season (May) and 12 first clinical malaria episodes in the ensuing wet season (MAL), and their correlation with parasitaemia (X) at the time of blood draw.

	Mean Diff. MAL-May	Discovery?	q value	Individual P Value	Spearman r x parasitaemia	95% confidence interval	P (two-tailed)	P value summary	Significant? (alpha = 0.05)
X vs. acetoacetate	269575000	Yes	<0.0001	<0.0001	0.2475	-0.1854 to 0.6000	0.2436	ns	No
X vs. aspartate	411666667	Yes	<0.0001	<0.0001	<b>0.5635</b>	0.1950 to 0.7925	0.0041	**	Yes
X vs. EtherPC 38:4e; PC(14:0e/24:4); [M+H] <sup>+</sup>	-2248333	Yes	<0.0001	<0.0001	<b>-0.4566</b>	-0.7321 to -0.05259	0.0249	*	Yes
X vs. EtherPC 38:5e; PE(16:1e/22:4); [M+H] <sup>+</sup>	-3989167	Yes	<0.0001	<0.0001	<b>-0.4446</b>	-0.7251 to -0.03764	0.0295	*	Yes
X vs. FA 16:0; [M+H] <sup>-</sup>	14593333	Yes	<0.0001	<0.0001	0.2962	-0.1342 to 0.6326	0.1599	ns	No
X vs. FA 18:1; [M+H] <sup>-</sup>	33825833	Yes	<0.0001	<0.0001	0.2784	-0.1532 to 0.6208	0.1878	ns	No
X vs. FA 18:2; [M+H] <sup>-</sup>	13220833	Yes	<0.0001	<0.0001	0.2418	-0.1912 to 0.5961	0.2549	ns	No
X vs. glutamate	-381666667	Yes	<0.0001	<0.0001	<b>-0.4923</b>	-0.7528 to -0.09840	0.0145	*	Yes
X vs. glutamine	-380416667	Yes	<0.0001	<0.0001	<b>-0.6804</b>	-0.8538 to -0.3709	0.0003	***	Yes
X vs. Hexose	876666667	Yes	<0.0001	<0.0001	<b>0.6042</b>	0.2537 to 0.8144	0.0018	**	Yes
X vs. Ketoleucine	244416667	Yes	0.0001	<0.0001	0.2358	-0.1974 to 0.5919	0.2674	ns	No
X vs. leucine	184666667	Yes	0.0102	0.0006	0.2663	-0.1660 to 0.6127	0.2085	ns	No
X vs. LysoPC 16:0; [M+H] <sup>+</sup>	-33613333	Yes	<0.0001	<0.0001	<b>-0.7564</b>	-0.8913 to -0.4986	<0.0001	****	Yes
X vs. LysoPC 18:1; [M+H] <sup>+</sup>	-8505250	Yes	<0.0001	<0.0001	<b>-0.789</b>	-0.9068 to -0.5570	<0.0001	****	Yes
X vs. LysoPC 20:4; [M+H] <sup>+</sup>	-10976750	Yes	<0.0001	<0.0001	<b>-0.7705</b>	-0.8980 to -0.5236	<0.0001	****	Yes
X vs. phenylalanine	-3494750	Yes	<0.0001	<0.0001	<b>-0.777</b>	-0.9011 to -0.5353	<0.0001	****	Yes
X vs. pyruvate	1399166667	Yes	<0.0001	<0.0001	<b>0.6976</b>	0.3989 to 0.8625	0.0002	***	Yes
X vs. pyruvate	15933333	Yes	0.0462	0.0032	-0.06092	-0.4632 to 0.3621	0.7773	ns	No
X vs. TG 50:3; TG(16:0/16:1/18:2); [M+NH4] <sup>+</sup>	-1688167	Yes	0.005	<0.0001	-0.2201	-0.5811 to 0.2133	0.3014	ns	No
X vs. TG 52:2; TG(16:0/18:1/18:1); [M+NH4] <sup>+</sup>	3079167	Yes	<0.0001	<0.0001	0.3654	-0.05718 to 0.6769	0.0791	ns	No
X vs. TG 52:3; TG(16:0/18:1/18:2); [M+NH4] <sup>+</sup>	4867500	Yes	<0.0001	<0.0001	<b>0.5766</b>	0.2137 to 0.7997	0.0032	**	Yes
X vs. Unknown 190.0861	-501567	Yes	0.0445	0.001	<b>-0.6751</b>	-0.8512 to -0.3624	0.0003	***	Yes
X vs. Unknown 203.0527	504167	Yes	0.0433	0.001	<b>0.4747</b>	0.07558 to 0.7427	0.0191	*	Yes
X vs. Unknown 218.1301	6368459	Yes	<0.0001	<0.0001	<b>0.4591</b>	0.05580 to 0.7336	0.024	*	Yes
X vs. Unknown 230.0128	3706116	Yes	0.0001	<0.0001	<b>0.4655</b>	0.06389 to 0.7374	0.0219	*	Yes
X vs. Unknown 240.1101	1569890	Yes	0.0131	0.0003	0.3743	-0.04688 to 0.6825	0.0715	ns	No
X vs. Unknown 263.2371	1022750	Yes	<0.0001	<0.0001	0.2845	-0.1467 to 0.6249	0.1778	ns	No
X vs. Unknown 281.2479	563000	Yes	0.0114	0.0002	0.3019	-0.1281 to 0.6363	0.1517	ns	No
X vs. Unknown 291.0827	-4316667	Yes	<0.0001	<0.0001	-0.3272	-0.6527 to 0.1003	0.1186	ns	No
X vs. Unknown 293.0988	1783333	Yes	<0.0001	<0.0001	-0.04986	-0.4544 to 0.3717	0.817	ns	No
X vs. Unknown 299.1394	1484000	Yes	0.0254	0.0006	<b>0.4176</b>	0.004405 to 0.7090	0.0423	*	Yes
X vs. Unknown 315.0831	2277758	Yes	<0.0001	<0.0001	0.3045	-0.1252 to 0.6380	0.1479	ns	No
X vs. Unknown 337.1052	-1773333	Yes	0.0023	<0.0001	<b>-0.5024</b>	-0.7586 to -0.1117	0.0124	*	Yes
X vs. Unknown 369.3516	-15250000	Yes	<0.0001	<0.0001	<b>-0.4899</b>	-0.7514 to -0.09528	0.0151	*	Yes
X vs. Unknown 369.352	-1483333	Yes	0.0254	0.0006	0.122	-0.3074 to 0.5102	0.57	ns	No
X vs. Unknown 376.2602	2654167	Yes	<0.0001	<0.0001	0.2712	-0.1608 to 0.6160	0.1999	ns	No
X vs. Unknown 381.299	1118083	Yes	<0.0001	<0.0001	0.3065	-0.1231 to 0.6393	0.1453	ns	No
X vs. Unknown 412.285	830175	Yes	<0.0001	<0.0001	0.3933	-0.02464 to 0.6942	0.0573	ns	No
X vs. Unknown 414.2155	-551167	Yes	0.0148	0.0003	-0.2921	-0.6299 to 0.1386	0.1661	ns	No
X vs. Unknown 414.3008	1659250	Yes	<0.0001	<0.0001	0.2127	-0.2207 to 0.5759	0.3183	ns	No
X vs. Unknown 415.2119	9286167	Yes	<0.0001	<0.0001	0.3661	-0.05637 to 0.6774	0.0785	ns	No
X vs. Unknown 415.2123	601667	Yes	0.0042	<0.0001	0.2515	-0.1813 to 0.6027	0.2358	ns	No
X vs. Unknown 416.317	668059	Yes	0.0008	<0.0001	0.2636	-0.1687 to 0.6109	0.2133	ns	No
X vs. Unknown 460.27	-2532917	Yes	<0.0001	<0.0001	-0.2052	-0.5706 to 0.2281	0.3362	ns	No
X vs. Unknown 480.3077	-4261500	Yes	<0.0001	<0.0001	<b>-0.7451</b>	-0.8858 to -0.4789	<0.0001	****	Yes
X vs. Unknown 494.3241	-1262000	Yes	<0.0001	<0.0001	<b>-0.7068</b>	-0.8671 to -0.4140	0.0001	***	Yes
X vs. Unknown 496.3408	-1594583	Yes	<0.0001	<0.0001	<b>-0.7031</b>	-0.8652 to -0.4078	0.0001	***	Yes
X vs. Unknown 496.3409	-12285833	Yes	<0.0001	<0.0001	<b>-0.7209</b>	-0.8740 to -0.4375	<0.0001	****	Yes
X vs. Unknown 518.322	-2000833	Yes	0.0002	<0.0001	<b>-0.7255</b>	-0.8763 to -0.4453	<0.0001	****	Yes
X vs. Unknown 520.341	-4395000	Yes	<0.0001	<0.0001	<b>-0.5476</b>	-0.7839 to -0.1729	0.0056	**	Yes
X vs. Unknown 522.3561	-5231667	Yes	<0.0001	<0.0001	<b>-0.6056</b>	-0.8152 to -0.2558	0.0017	**	Yes
X vs. Unknown 524.3711	-927333	Yes	<0.0001	<0.0001	<b>-0.5523</b>	-0.7864 to -0.1794	0.0051	**	Yes
X vs. Unknown 524.3712	-15630250	Yes	<0.0001	<0.0001	<b>-0.7776</b>	-0.9014 to -0.5363	<0.0001	****	Yes
X vs. Unknown 524.3714	-1526358	Yes	0.0186	0.0004	<b>-0.7656</b>	-0.8957 to -0.5147	<0.0001	****	Yes
X vs. Unknown 524.3723	-8718333	Yes	<0.0001	<0.0001	<b>-0.6221</b>	-0.8239 to -0.2803	0.0012	**	Yes
X vs. Unknown 542.3224	-1412500	Yes	0.0448	0.001	<b>-0.7648</b>	-0.8953 to -0.5134	<0.0001	****	Yes
X vs. Unknown 544.3409	-2318692	Yes	<0.0001	<0.0001	<b>-0.4941</b>	-0.7539 to -0.1008	0.0141	*	Yes
X vs. Unknown 546.3528	-907083	Yes	<0.0001	<0.0001	<b>-0.7566</b>	-0.8914 to -0.4989	<0.0001	****	Yes
X vs. Unknown 546.3536	-1720333	Yes	0.0038	<0.0001	<b>-0.7825</b>	-0.9037 to -0.5452	<0.0001	****	Yes
X vs. Unknown 546.356	-827917	Yes	<0.0001	<0.0001	<b>-0.563</b>	-0.7923 to -0.1943	0.0042	**	Yes
X vs. Unknown 549.4878	-1617417	Yes	0.0091	0.0002	-0.1931	-0.5621 to 0.2400	0.3659	ns	No
X vs. Unknown 549.4885	-3514100	Yes	<0.0001	<0.0001	-0.3046	-0.6381 to 0.1251	0.1478	ns	No
X vs. Unknown 554.3441	-6504750	Yes	<0.0001	<0.0001	<b>-0.702</b>	-0.8647 to -0.4061	0.0001	***	Yes
X vs. Unknown 562.3274	-620917	Yes	0.0028	<0.0001	<b>-0.6456</b>	-0.8361 to -0.3162	0.0007	**	Yes
X vs. Unknown 562.3284	-1586583	Yes	0.0116	0.0002	<b>-0.7982</b>	-0.9111 to -0.5738	<0.0001	****	Yes
X vs. Unknown 577.5192	6582500	Yes	<0.0001	<0.0001	0.3976	-0.01959 to 0.6968	0.0544	ns	No
X vs. Unknown 585.2725	1576957	Yes	<0.0001	<0.0001	<b>0.6664</b>	0.3486 to 0.8467	0.0004	***	Yes
X vs. Unknown 603.5349	1811667	Yes	0.0017	<0.0001	0.1953	-0.2379 to 0.5637	0.3604	ns	No
X vs. Unknown 605.5502	2558317	Yes	<0.0001	<0.0001	<b>0.4367</b>	0.02781 to 0.7204	0.0329	*	Yes
X vs. Unknown 637.3054	506083	Yes	0.0429	0.0009	0.2488	-0.1841 to 0.6009	0.2411	ns	No
X vs. Unknown 732.5544	2539542	Yes	<0.0001	<0.0001	<b>0.5007</b>	0.1094 to 0.7576	0.0127	*	Yes
X vs. Unknown 79.0212	-1381500	Yes	<0.0001	<0.0001	-0.2392	-0.5943 to 0.1939	0.2603	ns	No
X vs. Unknown 812.6735	-2508450	Yes	<0.0001	<0.0001	<b>-0.428</b>	-0.7152 to -0.01712	0.0369	*	Yes
X vs. Unknown 813.6857	-2590833	Yes	<0.0001	<0.0001	0.07482	-0.3500 to 0.4741	0.7283	ns	No
X vs. Unknown 814.6336	-1678500	Yes	0.0053	<0.0001	-0.3933	-0.6942 to 0.02464	0.0573	ns	No
X vs. Unknown 818.5896	4249417	Yes	<0.0001	<0.0001	<b>0.5457</b>	0.1702 to 0.7828	0.0058	**	Yes
X vs. Unknown 885.3681	620333	Yes	0.0028	<0.0001	<b>0.4317</b>	0.02163 to 0.7174	0.0352	*	Yes
X vs. Unknown 922.7857	-1874104	Yes	0.0009	<0.0001	-0.03567	-0.4431 to 0.3839	0.8686	ns	No
X vs. Unknown 991.6738	-1587592	Yes	<0.0001	<0.0001	<b>-0.6929</b>	-0.8601 to -0.3912	0.0002	***	Yes
X vs. w/o MS2:LysoPE 19:0; [M+H] <sup>+</sup>	231750000	Yes	0.0003	<0.0001	<b>-0.7242</b>	-0.8757 to -0.4431	<0.0001	****	Yes
X vs. w/o MS2:ML5001165756-01flexofenadine M <sub>2</sub>	-2934667	Yes	<0.0001	<0.0001	<b>-0.7525</b>	-0.8894 to -0.4917	<0.0001	****	Yes
X vs. valine	-606531	Yes	0.0039	<0.0001	<b>0.4152</b>	0.001531 to 0.7075	0.0436	*	Yes

Supplementary Table 10. var gene overview of 12 subclinical individuals at the end of the dry season (May) and 12 first clinical malaria episodes in the ensuing wet season (MAL).

Sample ID	LAHS FADJ contain contigs			All fragments			500 bp contigs			length in bp of assembly of RNA-Seq	ratio var / genes	#reads non human	mapped reads	% back onto whole transcriptome assembly	ratio # var versus mapped	# hits against VarDB	# reads mapped (multi hits possible)	Id of highest hit	Domain structure of highest hit
	total length	#	longest	total length	#	longest	total length	#	longest										
9307	2492	1	6763	18920	10	6763	18301	4	6763	742606	2.5	15975868.0	8895625	55.7	7.03	2693	197714	PF0159-C-g637	NTS-DBL-a-CIDRa-DBL-b-DBL-g-DBL-d-CIDRb-DBL-b-DBL-g-
9308	5533	1	5348	9624	5	5348	9455	4	5348	1445482	0.7	11848168.0	6148180	51.9	687.43	14	628	PF0145-C-g513	NTS-DBL-a-CIDRa-DBL-b-DBL-g-DBL-d-CIDRb-
9309	0	0	670	1296	2	670	1296	2	670	1046246	0.1	11998236.0	6188146	51.5	99.69	13	246	PF0084-C-g173	NTS-DBL-a-CIDRa-DBL-d-CIDRb-
9310	14655	3	6227	18769	10	6227	17668	6	6227	1941324	1.4	13326412.0	7123920	53.5	86.89	216	8458	PF0043-C-g402	DBL-d-CIDRb-
9311	0	0	2292	2292	1	2292	2292	1	2292	611982	0.4	14767424.0	8843839	59.9	163.71	14	266	PT0294-C-g1327	DBL-a-CIDRa-DBL-b-DBL-g-DBL-d-CIDRb-DBL-b-DBL-g-
9312	2706	1	3170	20556	18	3170	18992	12	3170	2975794	0.7	20051744.0	11653423	58.1	321.19	64	2097	PF0051-C-g887	DBL-a-CIDRa-DBL-d-CIDRb-
9313	0	0	2292	4721	8	2292	3948	3	2292	752927	0.6	8928980.0	4343024	48.6	786.83	6	125	PT0122-C-g1412	NTS-DBL-a-CIDRa-DBL-b-DBL-g-DBL-d-CIDRb-DBL-b-DBL-g-
9314	10604	2	9011	39049	28	9011	35930	16	9011	2114337	1.8	14321116.0	7415290	51.8	157.46	248	6516	PF0262-C-g611	DBL-b-DBL-b-DBL-d-CIDRg-
9315	6708	0	4176	5824	3	4176	5685	2	4176	2541773	0.2	26000276.0	13663141	52.5	30.18	193	12370	PF0062-C-g430	NTS-DBL-a-CIDRa-DBL-d-CIDRb-
9316	0	0	3400	9185	13	3400	6840	5	3400	1478168	0.6	14287136.0	7202926	50.4	75.91	121	2046	PA0182-C-g826	NTS-DBL-a-CIDRa-
9317	12594	1	5234	19689	20	5234	17610	10	5234	2407641	0.8	14286292.0	6838626	47.9	21.52	915	31564	PM0098-C-g1086	NTS-DBL-a-CIDRa-DBL-d-CIDRg-
9318	6244	1	6004	10131	10	6004	9313	3	6004	2285506	0.5	17728064.0	8526775	48.1	198.65	51	2534	PF0417-C-g422	NTS-DBL-a-CIDRa-DBL-d-CIDRb-
9319	78542	23	10423	173674	133	10423	158685	68	10423	3537833	4.9	7132196.0	3328961	46.7	133.70	1299	39212	PA0146-C-g1029	DBL-d-CIDRb-
9320	0	0	2208	8666	20	2208	4740	4	2208	3381312	0.3	9539044.0	4380531	45.9	131.30	66	1224	PA0224-C-g312	DBL-a-CIDRa-DBL-d-CIDRb-
9321	20294	2	9564	23668	12	9564	22883	5	9564	3822936	0.6	9449748.0	4344423	46.0	20.60	1149	79918	PF0097-C-g294	NTS-DBL-a-CIDRa-DBL-b-DBL-g-DBL-d-CIDRb-
9322	55784	16	10046	138491	170	10046	107548	47	10046	3729370	3.7	14093972.0	7193001	51.0	163.70	846	33778	PA0092-C-g461	DBL-d-CIDRg-DBL-g-DBL-z-
9323	7436	5	3571	35909	43	3571	30853	23	3571	3066791	1.2	11050240.0	5527362	50.0	348.63	103	2823	PA0253-C-g804	NTS-DBL-a-CIDRa-DBL-d-CIDRb-
9324	4363	5	4626	43517	56	4626	36282	28	4626	3237633	1.3	10679044.0	5404733	50.6	319.98	136	3264	PF0189-C-g397	DBL-d-CIDRb-
9325	0	0	3849	3849	1	3849	3849	1	3849	2549504	0.2	10567928.0	5285348	50.0	9.84	391	9861	PF0801-C-g1202	DBLg-DBLz-DBLe-DBLe-
9326	76081	16	8812	113437	87	8812	101455	36	8812	3969051	2.9	16488982.0	8496227	51.7	144.51	785	23150	OG0212-C-g1592	NTS-DBL-a-CIDRa-DBL-b-DBL-g-DBL-d-CIDRb-
9327	5170	2	4225	11330	14	4225	8242	4	4225	2543923	0.4	5085208.0	2190678	43.1	138.17	82	1893	PA0237-C-g706	NTS-DBL-a-CIDRa-DBL-d-CIDRb-
9328	2752	1	2688	12501	16	2688	10623	6	2688	2862046	0.4	8045316.0	3659940	45.5	198.43	63	2197	PF0228-C-g1045	CIDRa-DBL-d-CIDRb-
9329	49030	17	9161	122076	120	9161	105911	48	9161	4088463	3.0	13708444.0	6617359	48.3	94.78	1288	64690	PA0167-C-g886	NTS-DBL-a-CIDRa-DBL-b-DBL-g-DBL-d-CIDRg-
9330	52218	13	11752	106347	87	11752	98474	49	11752	3878236	2.7	15329884.0	7832231	51.1	253.21	420	14671	PT0211-C-g738	NTS-DBL-a-CIDRa-DBL-b-DBL-d-CIDRb-

PM - Mali, PF - Ghana, PT - Malawi, PA - The Gambia/Guinea, QG - Congo

**Supplementary Table 11.** List of *P. falciparum* invasion-specific proteins included in the analysis of the protein microarray.

Previous ID	ID	Name	Description
MAL13P1.174	PF3D7_1334800	MSRP2	MSP7-like protein (MSRP2)
MAL13P1.176	PF3D7_1335300	RH2B	reticulocyte binding protein 2 homologue b (RH2b)
MAL7P1.176	PF3D7_0731500	EBA175	erythrocyte binding antigen-175 (EBA175)
PF10_0281	PF3D7_1028700	MTRAP	merozoite TRAP-like protein (MTRAP)
PF10_0348	PF3D7_1035700	DBLMSP	duffy binding-like merozoite surface protein (DBLMSP)
PF10_0351	PF3D7_1035900	M566	probable protein, unknown function
PF10_0352	PF3D7_1036000	MSP11	merozoite surface protein (MSP11)
PF10_0355	PF3D7_1036300	DBLMSP2	merozoite surface protein (MSP3.8)
PF11_0344	PF3D7_1133400	AMA1	apical membrane antigen 1 (AMA1)
PF11_0381	PF3D7_1136900	SUB2	subtilisin-like protease 2 (SUB2)
PF13_0197	PF3D7_1335100	MSP7	merozoite surface protein 7 (MSP7)
PF14_0201	PF3D7_1420700	P113	surface protein, Pf113 (Pf113)
PF14_0495	PF3D7_1452000	RON2	rhostry neck protein 2 (RON2)
PFA0125c	PF3D7_0102500	EBA181	erythrocyte binding antigen-181 (EBA181)
PFB0300c	PF3D7_0206800	MSP2	merozoite surface protein 2 (MSP2)
PFB0305c	PF3D7_0206900	MSP5	merozoite surface protein 5 (MSP5)
PFB0310c	PF3D7_0207000	MSP4	merozoite surface protein 4 (MSP4)
PFB0335c	PF3D7_0207500	SERA6	serine repeat antigen 6 (SERA6)
PFB0340c	PF3D7_0207600	SERA5	serine repeat antigen 5 (SERA5)
PFB0345c	PF3D7_0207700	SERA4	serine repeat antigen 4 (SERA4)
PFB0350c	PF3D7_0207800	SERA3	serine repeat antigen 3 (SERA3)
PFD1145c	PF3D7_0424100	RH5	reticulocyte binding protein homologue 5 (RH5)
PFE0340c	PF3D7_0506900	ROM4	rhomboid protease ROM4 (ROM4)
FFF0995c	PF3D7_0620400	MSP10	merozoite surface protein 10 (MSP10)
FFI1475w	PF3D7_0930300	MSP1	merozoite surface protein 1 (MSP1)
PFL1385c	PF3D7_1228600	MSP9	merozoite surface protein 9 (MSP9)
PFL2505c	PF3D7_1252100	RON3	rhostry neck protein 3 (RON3)

**Supplementary Table 12.** Model parameters and values.

Parameter	Description	Value
$\gamma$	parasite growth rate	8
$\sigma$	maximal proportion of parasites removed by spleen [6h-1]	0,9
$\eta$	maximal proportion of parasites removed through cytoadhesion [6h-1]	0,9
$\rho_s$	shape parameter, spleen	0,7
$\rho_n$	shape parameter, cytoadhesion	1,5
$T_s$	location parameter, spleen	32
$T_n$	location parameter, cytoadhesion	10
$\kappa$	scale parameter (relative cytoadhesion)	[0,1]