Comparative analysis of IgG Responses to recombinant Qβ phage displayed MSP3 and UB05 in Dual HIV-malaria infected adults living in areas differing in Malaria transmission intensities.

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66 **Abstract**

67 Immunoglobulin G specific responses against *Plasmodium falciparum* merozoite antigens such
68 as the merozoite surface protein 3 (MSP3) and UB05 are known to play critical roles in
69 parasitemia control and protection from symptomatic illness. However when there is intense
70 perennial malaria transmission coupled with concurrent infection with the human
71 immunodeficiency virus type 1 (HIV), knowledge of IgG antibody response profiles is limited.
72 In this study we assessed the impact of dual HIV-Malaria infections on IgG subclass responses to
73 MSP3 (QβMSP3) and UB05 (QβUB05) in individuals living in two areas of Cameroon differing
74 in transmission intensity. We observed differences in antigen specific IgG and IgG subclass
75 responses which was dependent upon the antigen type, malaria transmission intensity, HIV
76 infection, malaria infection and dual HIV-malaria infections. Individuals living in high malaria
77 transmission areas irrespective of HIV or malaria status had significantly higher IgG responses to
78 both antigens (P=0.0001 for QβMSP3, P=0.0001 for QβUB05) than their counterpart from low
79 transmission areas. When dual HIV-Malaria infection is considered significantly higher
80 QβMSP3 specific IgG1 (P=0.0001) and IgG3 (P=0.04) responses in double negative individuals
81 was associated with protection against malaria in low transmission areas. Superior QβUBO5
82 specific IgG1 responses (P=0.0001) in double negative individuals were associated with
83 protection in high transmission areas in contrast to significantly higher IgG3 responses to

QβUB05 (P=0.0001) which were more relevant to protection in low malaria transmission areas in the same population. Thus, understanding immune responses to QβUB05 and QβMSP3 could facilitate the development of immunotherapeutic strategies suitable for areas differing in malaria transmission intensity.

Key words: Dual HIV-Malaria, QβUB05, QβMSP3, IgG subclass, antibodies, natural immunity

Introduction

In Cameroon like in most sub Saharan African countries people living in low or high malaria transmission areas are exposed to different frequencies of *Plasmodium falciparum*. Within such regions long term inhabitants suffer repeated exposure to varying strains of malaria parasite during the course of several years eventually developing protection from infection and symptomatic illness irrespective of malaria transmission intensity (1). A critical component of this naturally acquired immunity is *Plasmodium falciparum* induced IgG and IgG subclass antibody responses targeting a number of parasite derived antigens. However little is known about the IgG antibody subclass profile that mediates protective immunity to malaria in both low and high malaria transmission areas. Also, when there is concurrent infection with the human Immunodeficiency virus type 1(HIV), knowledge of the precise nature of parasite antigen directed IgG subclass antibody responses functional in the afflicted individuals is limited. Given that HIV infection depletes the immune system there is need to understand the role of promising malaria target antigens and the profile of IgG subclass responses driving protective immunity to malaria in both low and high transmission areas.

Antibodies to several asexual blood stage antigens including apical membrane antigen 1 (AMA-1), erythrocyte binding antigen (EBA-175), the merozoite surface proteins (MSPs), reticulocyte-binding protein homologue (Rh5), Glutamate-rich protein (GLURP), UB05 and

107 circumsporozoite protein (CSP) have been demonstrated to be an essential component of
108 naturally acquired immunity reducing parasite multiplication thereby preventing infection and
109 clinical disease in long term inhabitants of endemic regions (2-8). In this regards high levels of
110 IgG antibody subclass responses and diversity of the target antigens have been associated with
111 naturally acquired immunity to malaria (6-9). However, due to inherent polymorphism in the
112 asexual blood stage antigens (10) some elements of antibody-mediated immunity to *P.*
113 *falciparum* have been reported to be strain specific (11, 12) thereby limiting their utility as global
114 malaria vaccine candidates.
115 In low and high transmission areas the attainment of clinical immunity against malaria or
116 protection from infection is largely dependent upon continuous exposure to multiple parasite
117 variants (13) leading to an accumulation of a broad range of antibody specificities responsible for
118 the naturally acquired immunity. In areas differing in transmission intensities it is uncertain
119 which IgG subclass respond profiles are relevant to naturally acquired immunity. The scenario
120 becomes even more challenging when there is attendant co-infection with HIV which
121 dysregulates antibody responses and depletes the immune system.
122 In this study we have determined in low and high malaria transmission areas the impact of dual
123 HIV-malaria infection on IgG subclass responses to two conserved *P. falciparum* derived
124 asexual blood stage antigens displayed separately upon a recombinant RNA coliphage Qβ as
125 previously described by our group (14, 15). The recombinant phage QβMSP3 displays the
126 conserved C-terminal 88 aa of the merozoite surface protein 3 (16, 17) whilst QβUB05 bears the
127 previously described malaria antigen UBO5 (18). Surface display upon the recombinant RNA
128 coliphage Qβ as previously demonstrated by our group improves the antigenicity of inserted
129 antigens (14, 15).

Antibodies specific to *Plasmodium falciparum* MSP3 are known to mediate parasite killing in association with monocytes in a process referred to as (19-21) antibody-dependent cellular inhibition (ADCI). MSP3 specific antibodies therefore contribute in preventing symptomatic disease through the inhibition of blood parasite invasion cycles ultimately leading to a reduction in parasite burden and episodes of malaria (22, 23). A number of malaria vaccine candidates incorporating this highly conserved C-terminal end of MSP3 have been assessed in clinical trials with promising outcomes (19, 24-26). On other hand UB05 specific antibodies have also been associated with protection in exposed populations (18). We compared between dual HIV-malaria infected and double negative individuals the IgG subclass responses specific to the malaria vaccine antigens in both low and high malaria transmission area of Cameroon. Our study can facilitate the identification of surrogate markers of malaria immunity useful in the design of novel highly efficacious vaccines and the development of immunotherapeutic strategies to enhance immunity to malaria in people living in areas differing in transmission intensity.

Materials and Methods

Study site

The study was carried out in two areas of Cameroon (Yaounde and Bikop) differing in malaria transmission intensity. As the Capital city of Cameroon Yaounde (3°52′N11°31′E) is a multi-ethnic city situated at an average elevation of 750 m. Bikop on the other hand is a remote rural area located 48 KM away from Yaounde with year round intense malaria transmission. Both Yaoundé and Bikop are holendemic for malaria however with differing transmission intensity. This is mainly because unlike Yaounde, Bikop is located in the heart of the rain forest with a large number of mosquitoe breedings sites and poorly constructed houses favoring sustained high malaria transmission. The temperature in both areas is around 23.7°C with a similar average

153 annual rain fall of 1643 mm. There also have similar rainy (March to June, September to
154 November) and dry seasons (December to February, July–August) (27).

155 **Ethical clearance**

156 This study received ethical approval from the Cameroon National Ethics Committee for Human
157 Health Research (Reference numbers 2015/03/561/CE/CNERSH/SP and
158 2018/01969/CE/CNERSH/SP) and the CIRCB institutional review board (protocol number 14-
159 11). All participants provided written informed consent. Data were processed using specific
160 identifiers for privacy and confidentiality purposes. Clinical data generated during the course of
161 this study was provided free of charge to all participants.

162 **Study design**

163 This was a cross-sectional study which enrolled HIV-1 infected and non-infected people who
164 were 21 years or older. Participants with other infection (including microfilaria, dengue, TB, and
165 hepatitis B and C) and pregnant women were excluded from the study. All participants were
166 members of the CIRCB AFRODEC cohort (28-30)

167 **Study area**

168 This study was carried out in two areas of Cameroon (Yaounde and Bikop) differing in malaria
169 transmission intensity. As the Capital city of Cameroon Yaounde (3°52′N11°31′E) is a multi-
170 ethnicity situated at an average elevation of 750 m. Bikop is malaria hotspot with a high
171 incidence of malaria located 48 KM away from Yaounde with year round intense malaria
172 transmission. Both Yaoundé and Bikop are holoendemic for malaria however with differing
173 transmission intensities. This is mainly because unlike Yaounde, Bikop is located in the heart of
174 the rain forest with a high density of mosquitoes and poorly constructed houses favoring
175 sustained high malaria transmission. The temperature in both areas is around 23.7°C with similar

176 average annual rain fall of 1643 mm. There also have similar rainy (March to June, September to
177 November) and dry seasons (December to February, July–August).

178 **Study Population**

179 A total of 124 participants were recruited for this study. All participants were adults participants
180 of the CIRCB AFRODEC cohort 23-25. Participants were constituted in for groups consisting of
181 dual HIV-malaria infected (HIV+/Mal+), HIV mono-infected (HIV+/Mal-), malaria mono-
182 infected (HIV-/Mal+) and double negative (HIV-/Mal-) people.

183 **Plasma sample collection and Processing**

184 About 4 ml of blood was collected into plastic Vacuum blood spray-coated K2EDTA tubes
185 called Vacutest (Vacutestkirma, Italy). Subsequently, samples were transported to the
186 Vaccinology laboratory of Chantal BIYA International Reference Centre (CIRCB) for storage
187 and analysis. All samples were stored at room temperature and processed within 4 hours of
188 collection. To obtain plasma, samples were centrifuged at 2,000 rpm for 10 min at 4°C. The
189 plasma fraction was harvested sterile under the hood, aliquoted in small single-use volumes and
190 stored at -20°C until use. The plasma obtained from participants was heat inactivated for 30
191 minutes at 56°C prior to ELISA assay.

192 **HIV infection and CD4 T cell Enumeration**

193 Confirmation of HIV status was done as described for the CIRCB AFRODEC cohort using the
194 Cameroon's national algorithm for the diagnosis of HIV infection as previously reported for the
195 CIRCB AFRODEC cohort (31)

196 Absolute numbers of helper CD4+ T cells for HIV+ participants were determined in fresh
197 whole blood using BD multitest CD3/CD8/CD45/CD4 and TruCount tubes (BD biosciences,
198 USA) according to the manufacturer's instructions.

199 **Malaria Diagnosis and microscopy**

200 A malaria rapid diagnostic test was done on the blood samples according to the manufacturer's
201 instructions (SD Bioline, USA). In addition, thick peripheral blood films were stained with
202 Giemsa and examined using a microscope following standard quality-controlled procedures, for
203 the presence of malaria parasites.

204 **Study antigens**

205 The antigens consisted of recombinant Qβ displaying Plasmodium falciparum 3D7 strain
206 sequence derived C-terminal part of MSP3 (QβMSP3) and UB05 (QβUB05) generated in our
207 group as previously described (14, 15).

208 **Determination of IgG and IgG subclasses antibody responses specific to QβUB05 and**
209 **QβMSP3**

210 The plasma levels of antibodies specific to the malaria antigens QβUB05 and QβMSP3 were
211 determined through ELISA assay. Briefly high binding ELISA plates were coated with
212 10^7particles/well of each recombinant phage and incubated overnight at 4˚C. The following day,
213 Plates were washed 3x with PBST (PBS containing 0.05% Tween 20) and blocked with 3% BSA
214 in PBS for one hour at 37 ˚C. Heat inactivated plasma samples were diluted in PBS at 1:300 (for
215 IgG detection) or 1:100 (for IgG subclasses detection), then 100 µl/well added in triplicate and
216 incubated for two hours at 37 ˚C. The plates were washed four times with PBST after which the
217 bound antibody was probed with the peroxidase-conjugated mouse anti-human IgG and IgG
218 subclasses (IgG1, IgG2, IgG3 and IgG4) diluted 1:4000 in 1X PBS. Bound conjugate was
219 detected using ABTS substrate and stop solution according to the manufacturer's protocol
220 (southern biotech, Birmingham USA). The colorimetric signal was measured at 405 nm using a
221 multiscan FC microplate reader (Thermo Fisher Scientific, USA).

Statistical analysis

Data analysis was performed with Graphpad Prism Software version 6.1. The data were expressed as median (25th percentile-75th percentile). Comparisons of medians among two groups were performed by the U-Mann- Whitney test. Statistical significance was confirmed when $P < 0.05$.

Results

High malaria transmission intensity is associated with superior QβMSP3 and QβUB05 specific IgG antibodies

Individuals in areas of high perennial malaria transmission intensity developed significantly higher (P=0.0001) levels of QβMSP3 and QβUB05 specific IgG responses than those living in low transmission zones (compare Fig 1A with B). In high malaria transmission regions QβMSP-3 specific IgG responses were comparatively higher but not significant different than responses specific to QβUB05 in both positive (P=0.09) and negative (P=0.08) individuals (Fig. S1). In addition no significant difference is observed in dual HIV-Malaria infected people in their plasma reactivity with the two recombinant antigens both in low and high transmission areas (Fig 1A&C). Similarly when people negative for both malaria and HIV (double negative individuals) were considered no significant difference is observed in the IgG responses specific to the two malaria vaccine antigens. The effect of HIV-1 infection was a significant reduction in IgG responses specific to the two antigens in both low (P=0.0001 for QβMSP3, P=0.04 for QβUB05) and high (P=0.0001 for QβMSP3) malaria transmission areas. Surprisingly, there was no difference in these values for UB05 in the high malaria transmission region (Fig. 1 C&D).

On the other hand, the overall effect of *Plasmodium falciparum* infection in both low and high malaria transmission regions was a significant increase in IgG responses specific to both antigens

irrespective of dual HIV-malaria infection (Fig. 1E&F). Thus whereas HIV infection resulted to a significant a reduction in antigen specific IgG antibody levels *Plasmodium falciparum* infection resulted into a significant increase in the IgG antibody levels.

IgG1 subclass response in relation to Dual HIV-malaria infection and the intensity of malaria transmission

Whereas double negative individuals in low malaria transmission areas showed significantly higher (P=0.0001) IgG1 responses specific to QβMSP3 than to QβUB05; in high malaria transmission areas the converse was true (Fig. 2B) with IgG1 responses specific to QβUB05 being superior (P=0.0001). The effect of dual HIV-malaria infection in low transmission area was a significant reduction in the QβMSP3 specific IgG1 responses (P=0.0001) in contrast to individuals living in high malaria transmission areas where QβMSP3 specific IgG1 responses were similar to those of the double negative participants. On the other hand IgG1 responses specific to QβUB05 in high transmission areas remain comparatively higher than those to QβMSP3 in both dual HIV-malaria positive (P=0.004) and double negative (P=0.0001) individuals (Fig. 2A&B). This probably indicates the relevance of QβUB05 specific IgG1 in predicting malaria immunity in high transmission areas even under the challenging circumstances of HIV infection. The impact of dual HIV-malaria infection in low transmission areas was therefore a significant reduction (P=0.0001) in IgG1 responses specific to QβMSP3 (Fig 1C) in contrast to QβUB05 where IgG1 responses in this group remain higher than the double negative participants (P=0.0001). Again in a high transmission area the IgG1 responses to the two antigens were comparatively higher than values for the low transmission area. In addition IgG1 responses to QβUB05 in double negative individuals were also significantly higher (P=0.004) than dual HIV-malaria infected people. Thus in addition to malaria

transmission intensity differences in IgG1 subclass antibody levels might also be dependent upon co-infection with HIV-1 and the antigen of choice.

Thus in a low malaria transmission area, significantly high IgG1 responses specific to QβMSP3 is associated with resistance to malaria in contrast to superior QβUB05 specific IgG1 antibodies which were instead relevant to protection in high a transmission area.

IgG2 subclass response in relation to Dual HIV-malaria infection and the intensity of malaria transmission

In low malaria transmission areas significantly higher IgG2 subclass responses (P=0.0001 for QβMSP3 and P=0.0001 for QβUB05) were observed in double negative individuals relative to dual HIV-malaria positive participants (Fig. 3A&B). The converse was the case for high malaria transmission areas where dual HIV-malaria infection resulted to significantly higher IgG2 responses to both QβMSP3 (P=0.0001) and QβUB05 (P=0.0001). Overall the IgG2 responses specific to both antigen was comparatively superior in low relative to high transmission areas (Fig. 3A&B). The effect of an infection with HIV was a general increase in IgG2 responses specific to both antigens in low and high transmission areas (Fig. 3A&B). Several studies from other endemic areas of Africa have indicated that high circulating levels of malaria parasite antigen specific IgG2 could be a marker of severity of malaria infection (32-34). Except for MSP3 specific IgG2 an infection with plasmodium falciparum was associated with an increased in IgG2 responses to both antigens in all transmission areas. In a low malaria transmission area the impact of dual HIV-malaria infection was therefore a significant reduction in IgG2 responses to both antigens. In contrast in a high malaria transmission area dual HIV-malaria infection resulted to superior IgG2 responses to both QβMSP3 (P=0.0001) and QβUB05 (p=0.0001)

relative to the double negative participants. Thus with respect to both antigens there is a differential IgG2 response between high and low malaria transmission areas.

IgG3 subclass response in relation to Dual HIV-malaria infection and the intensity of malaria transmission

In low malaria transmission areas the IgG3 subclass antibody responses were significantly higher in double negative (p=0.0001 for QβMSP3 and p=0.0001 for QβUB05) compared to dual HIV-malaria infected participants. On the other hand in high malaria transmission areas no difference is observed between dual HIV-malaria infected and double negative individuals with respect to IgG3 subclass responses to both antigens. The effect of HIV infection was a significant reduction of IgG3 responses specific to QβMSP3 in both low (p=0.0003) and high (p=0.0001) malaria transmission areas. Similarly IgG3 specific responses to QβUB05 were significantly reduced in low (P=0.01) and high (P=0.0001) transmission areas after HIV infection. In low transmission areas *Plasmodium falciparum* infection resulted to a significant increase in IgG3 responses specific to both QβMSP3 (P=0.0003) and QβUB05 (P=0.0004) respectively. In contrast in high malaria transmission we observed significantly lower IgG3 responses specific to QβMSP3 (P=0.0001) and QβUB05 (P=0.0001) after Plasmodium falciparum infection. Thus there is differential effect of HIV and *Plasmodium falciparum* infections on IgG3 specific responses to both antigens which was also associated with the malaria transmission intensity.

IgG4 subclass response in relation to Dual HIV-malaria infection and the intensity of malaria transmission

There was a differential expression of antigen specific IgG4 subclass responses between low and high malaria transmission areas (Fig. 5A&B). Overall for both antigens in low compared to high transmission areas dual HIV-malaria positive and double negative individuals showed

313 significantly higher IgG4 responses. However in low transmission areas whereas QβMSP3
314 specific IgG4 responses in dual HIV-malaria infected individuals were superior to those of
315 double negative individual (P=0.0001); for QβUB05 IgG4 responses the converse was true. On
316 the other hand in high transmission areas no difference was observed between dual HIV-malaria
317 infected and double negative individuals with respect to antigen specific IgG4 responses. The
318 effect of HIV infection in all the malaria transmission areas was a significant reduction in IgG4
319 responses specific to both antigens (Fig.5 C&D). Plasmodium falciparum infection in low
320 transmission areas resulted to a significant increase in IgG4 responses specific to QβMSP3
321 (P=0.0001) and QβUB05 (P=0.0001) respectively (Fig.5E&F). In contrast in high transmission
322 areas the converse is true as IgG4 responses specific to both QβMSP3 (P=0.0001) and QβUB05
323 (P=0.0001) decreased significantly after Plasmodium falciparum infection (compare Fig. 5E with
324 F). Thus variation in IgG4 specific responses was dependent upon several factors including
325 transmission intensity, malaria parasite antigen, HIV infection, *Plasmodium falciparum* infection
326 and dual HIV-malaria infection.

327 **Discussion**

328 In this population based cross sectional study we profiled IgG and IgG subclass immune
329 responses to QβUB05 and QβMSP3 in dual HIV-malaria infected people living in two areas of
330 Cameroon differing in malaria transmission intensity. MSP3 and UB05 are asexual blood stage
331 antigens known to be associated with naturally acquired immunity in individuals living in
332 malaria endemic regions (18, 21, 23). However in populations living in malaria endemic regions
333 parasite prevalence rate differ significantly between areas of low and high transmission (35).
334 Since naturally acquired immunity to malaria is dependent upon the cumulative exposure in
335 endemic regions low malaria transmission could limit exposure to parasites which has been

linked to a waning antimalarial immunity and an increase in clinical episodes of malaria in adults (36-38). This in effect can modulate the profiles of target antigen specific IgG and IgG subclass responses that can be achieved especially when the individuals are co-infected with HIV which depletes the immune system. The impact of a high malaria transmission intensity in both dual HIV-malaria infected and double negative individuals was a significant increase in antigen specific IgG irrespective of the targeted antigen type. This might be in line with previous reports suggesting that high levels of antibodies to several blood stage antigens (4-7, 36) were a necessary component of protective immunity to malaria. Whereas this is probably correct for IgG antibody levels specific to QβUB05 and QβMSP3 in high malaria transmission areas the comparatively lower antibody levels in low transmission areas indicates that partial immunity to malaria could be waning in these areas due to a reduction in parasite antigen challenge (39)

HIV infection resulted to a significant reduction in IgG antibody responses specific to QβMSP3 in both low (P=0.0001) and high (P=0.0001) malaria transmission areas. In contrast, IgG antibody responses specific to QβUB05 were not significantly affected by HIV infection (compares S1A&B). Infection with *Plasmodium falciparum* was associated with a significant increase in IgG antibodies specific for both antigens in the two transmission areas. This is especially true when dual HIV-malaria infected individuals are compared with participants infected with HIV alone. Here we demonstrated that even when there was HIV infection the impact of *Plasmodium falciparum* infection was a significant increase in IgG antibodies specific to MSP3 in both low (P=0.0001) and high (P=0.0001) malaria transmission areas (S1E&F).This indicates that asexual blood stage antigens are capable of modulating parasite antigen specific IgG antibody levels in both low and high malaria transmission areas. Such antibody levels are influenced by other factors including malaria and HIV infection.

When responses are examined qualitatively with respect to IgG subclasses and dual HIV-malaria infections differential outcomes are observed. In comparing IgG1 responses to the two antigens QβMSP3 specific IgG1 was associated with protection against malaria in low transmission areas in contrast to QβUB05 specific IgG1 responses which were relevant to protection in high malaria transmission areas. In this regards IgG1 responses specific to QβUB05 in high malaria transmission areas were significantly higher than responses to QβMSP3 both for dual HIV-malaria infected (P=0.004) and double negative (P=0.0001) individuals. This is probably in line with previous reports which associated increase UB05 specific antibody responses with recovery from clinical malaria (18). Similarly significantly higher IgG2 and IgG3 responses specific to both antigens were associated with protection in low transmission areas. This is in line with previous findings indicating that high levels of malaria parasite antigen specific IgG3 and IgG2 were relevant to protection against all forms of malaria (23, 40). This probably indicates that in malaria endemic regions novel recombinant phages such as QβUB05 could be more useful for detecting protective levels of antibodies implicated in the control of malaria in both low and high transmission areas. On the other hand the recombinant phage QβMSP3 would be more relevant for profiling IgG subclass responses in low malaria transmission areas.

The effect of dual HIV-malaria infection was a significant reduction in QβMSP3 specific IgG1 (P=0.0001), IgG2 (P=0.0001) and IgG3 (P=0.04) responses in low transmission areas. In low transmission areas this significant reduction of QβMSP3 specific IgG2 together with the cytophilic antibodies IgG1 and IgG3 in dual HIV-malaria infected people could diminish protection from *Plasmodium falciparum* infection and symptomatic illness. In the case of IgG subclass antibodies specific to QβUB05 such a reduction in dual HIV-malaria infected people might lead to increase morbidity and mortality to malaria in both low and high transmission

382 areas. This is mainly because parasite antigen specific IgG1 and IgG3 are critical in monocyte
383 mediated antibody-dependent cellular inhibition (ADCI) of *Plasmodium falciparum* which is
384 responsible for killing asexual blood stages. Other reports have also shown that dual HIV-
385 malaria infections escalate episodes of symptomatic malaria (41)or severe disease in both
386 children and adults (42, 43). In endemic regions such individuals due to persistent parasitaemia
387 could serve as reservoirs of malaria parasite thereby sustaining infection in both low and high
388 transmission areas.
389 In low transmission areas dual HIV-malaria infection resulted to significantly higher QβMSP3
390 specific IgG4 responses which are probably the reason why IgG1 and IgG3 responses to
391 QβMSP3 were significantly lower in this group. On the other hand, IgG4 responses to QβUB05
392 were significantly higher in double negative participants relative to dual HIV-malaria infected
393 individuals. In high transmission areas IgG4 responses to both antigens were low which could
394 explain why IgG antibody responses were comparatively high in this area. Since IgG4 is a
395 noncytophilic IgG subclass which may block antibody mediated natural immunity to malaria (23,
396 32, 44-46) high IgG4 levels tended to be associated with lower IgG1 or IgG3 antibody levels.
397 Overall the antibody responses to both antigens are relatively heterogeneous being certainly
398 influenced by a number of factors including transmission area, ongoing *plasmodium falciparum*
399 infection and coinfection with HIV. Never the less there was a clear indication that MSP3
400 specific IgG subclass responses were associated with protective immunity to malaria mainly in
401 low transmission areas whilst similar responses to QβUB05 were related to protection in both
402 low and high malaria transmission areas. This implies that QβMSP3 might not be suitable as a
403 standalone vaccine in areas differing in transmission intensity. On the other hand antigenicity of
404 UB05 most likely predicts immunity in both low and high transmission areas and could be used

405 either alone or in combination with other antigens for vaccine studies in areas differing in
406 transmission intensities. Thus understanding immune responses to QβUB05 and QβMSP3 could
407 enable the development of efficacious vaccines or commensurate immunotherapeutic strategies
408 suitable for areas differing in malaria transmission intensity.

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426 **References**

427 1. Doolan DL, Dobano C, Baird JK. 2009. Acquired immunity to malaria. Clinical
428 microbiology reviews, 22(1): 13-36, Table of Contents.
429 2. Chan JA, Howell KB, Reiling L, Ataide R, Mackintosh CL, Fowkes FJ, Petter M,
430 Chesson JM, Langer C, Warimwe GM, Duffy MF, Rogerson SJ, Bull PC, Cowman AF,
431 Marsh K, Beeson JG. 2012. Targets of antibodies against Plasmodium falciparum-
432 infected erythrocytes in malaria immunity. The Journal of clinical investigation, 122(9):
433 3227-3238.
434 3. Fowkes FJ, Richards JS, Simpson JA, Beeson JG. 2010. The relationship between anti-
435 merozoite antibodies and incidence of Plasmodium falciparum malaria: A systematic
436 review and meta-analysis. PLoS medicine, 7(1): e1000218.
437 4. Richards JS, Stanisic DI, Fowkes FJ, Tavul L, Dabod E, Thompson JK, Kumar S, Chitnis
438 CE, Narum DL, Michon P, Siba PM, Cowman AF, Mueller I, Beeson JG. 2010.
439 Association between naturally acquired antibodies to erythrocyte-binding antigens of
440 Plasmodium falciparum and protection from malaria and high-density parasitemia.
441 Clinical infectious diseases : an official publication of the Infectious Diseases Society of
442 America, 51(8): e50-60.
443 5. McCarra MB, Ayodo G, Sumba PO, Kazura JW, Moormann AM, Narum DL, John CC.
444 2011. Antibodies to Plasmodium falciparum erythrocyte-binding antigen-175 are
445 associated with protection from clinical malaria. The Pediatric infectious disease journal,
446 30(12): 1037-1042.
447 6. Daou M, Kouriba B, Ouedraogo N, Diarra I, Arama C, Keita Y, Sissoko S, Ouologuem
448 B, Arama S, Bousema T, Doumbo OK, Sauerwein RW, Scholzen A. 2015. Protection of

449 Malian children from clinical malaria is associated with recognition of multiple antigens.
450 Malaria journal, 14: 56.
451 7. Trieu A, Kayala MA, Burk C, Molina DM, Freilich DA, Richie TL, Baldi P, Felgner PL,
452 Doolan DL. 2011. Sterile protective immunity to malaria is associated with a panel of
453 novel P. falciparum antigens. Molecular and cellular proteomics : MCP, 10(9): M111
454 007948.
455 8. Gray JC, Corran PH, Mangia E, Gaunt MW, Li Q, Tetteh KK, Polley SD, Conway DJ,
456 Holder AA, Bacarese-Hamilton T, Riley EM, Crisanti A. 2007. Profiling the antibody
457 immune response against blood stage malaria vaccine candidates. Clinical chemistry,
458 53(7): 1244-1253.
459 9. Dent AE, Nakajima R, Liang L, Baum E, Moormann AM, Sumba PO, Vulule J,
460 Babineau D, Randall A, Davies DH, Felgner PL, Kazura JW. 2015. Plasmodium
461 falciparum Protein Microarray Antibody Profiles Correlate With Protection From
462 Symptomatic Malaria in Kenya. The Journal of infectious diseases, 212(9): 1429-1438.
463 10. Bai T, Becker M, Gupta A, Strike P, Murphy VJ, Anders RF, Batchelor AH. 2005.
464 Structure of AMA1 from Plasmodium falciparum reveals a clustering of polymorphisms
465 that surround a conserved hydrophobic pocket. Proceedings of the National Academy of
466 Sciences of the United States of America, 102(36): 12736-12741.
467 11. Hodder AN, Crewther PE, Anders RF. 2001. Specificity of the protective antibody
468 response to apical membrane antigen 1. Infection and immunity, 69(5): 3286-3294.
469 12. Miura K, Zhou H, Muratova OV, Orcutt AC, Giersing B, Miller LH, Long CA. 2007. In
470 immunization with Plasmodium falciparum apical membrane antigen 1, the specificity of

antibodies depends on the species immunized. Infection and immunity, 75(12): 5827-5836.

13. Jones TR, Obaldia N, 3rd, Gramzinski RA, Hoffman SL. 2000. Repeated infection of Aotus monkeys with Plasmodium falciparum induces protection against subsequent challenge with homologous and heterologous strains of parasite. The American journal of tropical medicine and hygiene, 62(6): 675-680.

14. Skamel C, Aller SG, Bopda Waffo A. 2014. In vitro evolution and affinity-maturation with Coliphage qbeta display. PloS one, 9(11): e113069.

15. Waffo AB, Ngu LN, Singleton RL, Egbo T, Simo JL, Sanders CA, Ambada GE, Nji NN, Ngoh AA, Matthews QL, Robertson BK, Tchouangueu TF, Lissom A, Bawage S, Tchadji C, Okoli AS, Esimone CO, Chukwuanukwu R, Mbacham W, Kaptue L, Nchinda GW. 2017. Surface Engineering of Recombinant RNA Coliphage Qβ to Display gp41 MembraneProximal External-Region Epitopes from HIV-1. J Clin Exp Immunol, 2(1): 1-15.

16. Singh S, Soe S, Mejia JP, Roussilhon C, Theisen M, Corradin G, Druilhe P. 2004. Identification of a conserved region of Plasmodium falciparum MSP3 targeted by biologically active antibodies to improve vaccine design. The Journal of infectious diseases, 190(5): 1010-1018.

17. Daher LJ, Demanga CG, Prieur E, Perignon JL, Bouharoun-Tayoun H, Druilhe P. 2010. Toward the rational design of a malaria vaccine construct using the MSP3 family as an example: contribution of immunogenicity studies in models. Infection and immunity, 78(1): 477-485.

493 18. Titanji VP, Amambua-Ngwa A, Anong DN, Mbandi SK, Tangie E, Tening I, Yengo R.
494 2009. Isolation and expression of UB05, a Plasmodium falciparum antigen recognised by
495 antibodies from semi-immune adults in a high transmission endemic area of the
496 Cameroonian rainforest. Clinical chemistry and laboratory medicine, 47(9): 1147-1158.
497 19. Jepsen MP, Jogdand PS, Singh SK, Esen M, Christiansen M, Issifou S, Hounkpatin AB,
498 Ateba-Ngoa U, Kremsner PG, Dziegiel MH, Olesen-Larsen S, Jepsen S, Mordmuller B,
499 Theisen M. 2013. The malaria vaccine candidate GMZ2 elicits functional antibodies in
500 individuals from malaria endemic and non-endemic areas. The Journal of infectious
501 diseases, 208(3): 479-488.
502 20. Bouharoun-Tayoun H, Attanath P, Sabchareon A, Chongsuphajaisiddhi T, Druilhe P.
503 1990. Antibodies that protect humans against Plasmodium falciparum blood stages do not
504 on their own inhibit parasite growth and invasion in vitro, but act in cooperation with
505 monocytes. The Journal of experimental medicine, 172(6): 1633-1641.
506 21. Oeuvray C, Bouharoun-Tayoun H, Gras-Masse H, Bottius E, Kaidoh T, Aikawa M,
507 Filgueira MC, Tartar A, Druilhe P. 1994. Merozoite surface protein-3: a malaria protein
508 inducing antibodies that promote Plasmodium falciparum killing by cooperation with
509 blood monocytes. Blood, 84(5): 1594-1602.
510 22. Good MF, Doolan DL. 2010. Malaria vaccine design: immunological considerations.
511 Immunity, 33(4): 555-566.
512 23. Roussilhon C, Oeuvray C, Muller-Graf C, Tall A, Rogier C, Trape JF, Theisen M, Balde
513 A, Perignon JL, Druilhe P. 2007. Long-term clinical protection from falciparum malaria
514 is strongly associated with IgG3 antibodies to merozoite surface protein 3. PLoS
515 medicine, 4(11): e320.

516 24. Thera MA, Doumbo OK, Coulibaly D, Laurens MB, Ouattara A, Kone AK, Guindo AB,
517 Traore K, Traore I, Kouriba B, Diallo DA, Diarra I, Daou M, Dolo A, Tolo Y, Sissoko
518 MS, Niangaly A, Sissoko M, Takala-Harrison S, Lyke KE, Wu Y, Blackwelder WC,
519 Godeaux O, Vekemans J, Dubois MC, Ballou WR, Cohen J, Thompson D, Dube T,
520 Soisson L, Diggs CL, House B, Lanar DE, Dutta S, Heppner DG, Jr., Plowe CV. 2011. A
521 field trial to assess a blood-stage malaria vaccine. The New England journal of medicine,
522 365(11): 1004-1013.
523 25. Ouattara A, Laurens MB. 2015. Vaccines against malaria. Clinical infectious diseases :
524 an official publication of the Infectious Diseases Society of America, 60(6): 930-936.
525 26. Lusingu JP, Gesase S, Msham S, Francis F, Lemnge M, Seth M, Sembuche S, Rutta A,
526 Minja D, Segeja MD, Bosomprah S, Cousens S, Noor R, Chilengi R, Druilhe P. 2009.
527 Satisfactory safety and immunogenicity of MSP3 malaria vaccine candidate in Tanzanian
528 children aged 12-24 months. Malaria journal, 8: 163.
529 27. Njunda AL, Njumkeng C, Nsagha SD, Assob JC, Kwenti TE. 2016. The prevalence of
530 malaria in people living with HIV in Yaounde, Cameroon. BMC public health, 16: 964.
531 28. Njambe Priso GD, Lissom A, Ngu LN, Nji NN, Tchadji JC, Tchouangueu TF, Ambada
532 GE, Ngane CSS, Dafeu BL, Djukouo L, Nyebe I, Magagoum S, Ngoh AA, Herve OF,
533 Garcia R, Gutierrez A, Okoli AS, Esimone CO, Njiokou F, Park CG, Waffo AB, Nchinda
534 GW. 2018. Filaria specific antibody response profiling in plasma from anti-retroviral
535 naive Loa loa microfilaraemic HIV-1 infected people. BMC infectious diseases, 18(1):
536 160.
537 29. Ambada GN, Ntsama CE, Nji NN, Ngu LN, Sake CN, Lissom A, Tchouangeu FT,
538 Tchadji J, Sosso M, Etoa FX, Nchinda GW. 2017. Phenotypic characterization of

539 regulatory T cells from antiretroviral-naive HIV-1-infected people. Immunology, 151(4):
540 405-416.
541 30. Sake CS, Ngu L, Ambada G, Chedjou JP, Nji N, Tchadji JC, Lissom A, Tchouangueu
542 TF, Djukouo L, Njambe G, Garcia R, Gutierrez A, Waffo AB, Park CG, Mbacham W,
543 Etoa F-X, Nchinda GW. 2017. The Effect of Antiretroviral Naïve HIV-1 Infection on the
544 Ability of Natural Killer Cells to Produce IFN-γupon Exposure to Plasmodium
545 falciparum -Infected Erythrocytes Biomed Hub, 2.
546 31. Domkam IK, Sonela N, Kamgaing N, Takam PS, Gwom L-C, Michel T, Betilene A,
547 Fokam J, Billong SC, Moukam LV, Etounou TM, Minka CSM, Ndjolo A. 2018.
548 Prevalence and risk factors to HIV-infection amongst health care workers within public
549 and private health facilities in Cameroon. Pan African Medical Journal, 29(158).
550 32. Aucan C, Traore Y, Tall F, Nacro B, Traore-Leroux T, Fumoux F, & Rihet P. 2000. High
551 immunoglobulin G2 (IgG2) and low IgG4 levels are associated with human resistance to
552 Plasmodium falciparum malaria. Infection and immunity, 68(3): 1252-1258.
553 33. Nasr A, Iriemenam NC, Troye-Blomberg M, Giha HA, Balogun HA, Osman OF,
554 Montgomery SM, ElGhazali G, Berzins K. 2007. Fc gamma receptor IIa (CD32)
555 polymorphism and antibody responses to asexual blood-stage antigens of Plasmodium
556 falciparum malaria in Sudanese patients. Scandinavian journal of immunology, 66(1): 87-
557 96.
558 34. Ntoumi F, Ekala MT, Makuwa M, Lekoulou F, Mercereau-Puijalon O, Deloron P. 2002.
559 Sickle cell trait carriage: imbalanced distribution of IgG subclass antibodies reactive to
560 Plasmodium falciparum family-specific MSP2 peptides in serum samples from Gabonese
561 children. Immunology letters, 84(1): 9-16.

562 35. Nkumama IN, O'Meara WP, Osier FHA. 2017. Changes in Malaria Epidemiology in
563 Africa and New Challenges for Elimination. Trends in parasitology, 33(2): 128-140.
564 36. Murungi LM, Kamuyu G, Lowe B, Bejon P, Theisen M, Kinyanjui SM, Marsh K, Osier
565 FH. 2013. A threshold concentration of anti-merozoite antibodies is required for
566 protection from clinical episodes of malaria. Vaccine, 31(37): 3936-3942.
567 37. Bouyou-Akotet MK, Offouga CL, Mawili-Mboumba DP, Essola L, Madoungou B,
568 Kombila M. 2014. Falciparum malaria as an emerging cause of fever in adults living in
569 Gabon, Central Africa. BioMed research international, 2014: 351281.
570 38. Kipanga PN, Omondi D, Mireji PO, Sawa P, Masiga DK, Villinger J. 2014. High-
571 resolution melting analysis reveals low Plasmodium parasitaemia infections among
572 microscopically negative febrile patients in western Kenya. Malaria journal, 13: 429.
573 39. Pinkevych M, Petravic J, Chelimo K, Kazura JW, Moormann AM, Davenport MP. 2012.
574 The dynamics of naturally acquired immunity to Plasmodium falciparum infection. PLoS
575 computational biology, 8(10): e1002729.
576 40. Giha HA, Nasr A, Iriemenam NC, Balogun HA, Arnot D, Theander TG, Troye-Blomberg
577 M, Berzins K, ElGhazali G. 2010. Age-dependent association between IgG2 and IgG3
578 subclasses to Pf332-C231 antigen and protection from malaria, and induction of
579 protective antibodies by sub-patent malaria infections, in Daraweesh. Vaccine, 28(7):
580 1732-1739.
581 41. Kamya MR, Gasasira AF, Yeka A, Bakyaita N, Nsobya SL, Francis D, Rosenthal PJ,
582 Dorsey G, Havlir D. 2006. Effect of HIV-1 infection on antimalarial treatment outcomes
583 in Uganda: a population-based study. The Journal of infectious diseases, 193(1): 9-15.

584 42. Saracino A, Nacarapa EA, da Costa Massinga EA, Martinelli D, Scacchetti M, de
585 Oliveira C, Antonich A, Galloni D, Ferro JJ, Macome CA. 2012. Prevalence and clinical
586 features of HIV and malaria co-infection in hospitalized adults in Beira, Mozambique.
587 Malaria journal, 11: 241.
588 43. Tay SC, Badu K, Mensah AA, Gbedema SY. 2015. The prevalence of malaria among
589 HIV seropositive individuals and the impact of the co- infection on their hemoglobin
590 levels. Annals of clinical microbiology and antimicrobials, 14: 10.
591 44. Stanisic DI, Richards JS, McCallum FJ, Michon P, King CL, Schoepflin S, Gilson PR,
592 Murphy VJ, Anders RF, Mueller I, Beeson JG. 2009. Immunoglobulin G subclass-
593 specific responses against Plasmodium falciparum merozoite antigens are associated with
594 control of parasitemia and protection from symptomatic illness. Infection and immunity,
595 77(3): 1165-1174.
596 45. Mewono L, Matondo Maya DW, Matsiegui PB, Agnandji ST, Kendjo E, Barondi F,
597 Issifou S, Kremsner PG, Mavoungou E. 2008. Interleukin-21 is associated with IgG1 and
598 IgG3 antibodies to erythrocyte-binding antigen-175 peptide 4 of Plasmodium falciparum
599 in Gabonese children with acute falciparum malaria. European cytokine network, 19(1):
600 30-36.
601 46. Bouharoun-Tayoun H, Oeuvray C, Lunel F, Druilhe P. 1995. Mechanisms underlying the
602 monocyte-mediated antibody-dependent killing of Plasmodium falciparum asexual blood
603 stages. The Journal of experimental medicine, 182(2): 409-418.

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606 **Figure Legends**

607 **Figure 1: specific IgG responses to recombinant phages QβMSP3 and QβUB05 with respect**
608 **to dual HIV-Malaria infections in individuals living in low and high malaria transmission**
609 **areas**

610 Comparison of IgG antibody responses between dual HIV-malaria infected and double negative
611 individuals in low and high malaria transmission areas. IgG specific responses to the
612 recombinant phages QβMSP3 **(A)** and QβUB05 **(B)** in low and high transmission areas. Effect
613 of HIV infection on QβMSP3 **(C)** and QβUB05 **(D)** specific IgG responses in the two
614 transmission areas. IgG responses specific to QβMSP3 (E) and QβUB05 (F) in individuals
615 positive for *Plasmodium falciparum* in both transmission areas.

616

617 **Figure 2: IgG1 specific response to recombinant phages QβMSP3 and QβUB05 with to**
618 **dual HIV-Malaria infections in individuals living in low and high malaria transmission**
619 **areas**

620 Comparison of IgG1 antibody responses between dual HIV-malaria infected and double negative
621 individuals in low and high malaria transmission areas. IgG1 responses specific to the
622 recombinant phages QβMSP3 (A) and QβUB05 (B) in low and high transmission areas. Effect
623 of HIV infection on QβMSP3 (C) and QβUB05 (D) specific IgG1 responses in the two
624 transmission areas. IgG1 responses specific to QβMSP3 (E) and QβUB05 (F) in individuals
625 positive for *Plasmodium falciparum* in both transmission areas.

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Figure 3: IgG2 responses specific to the recombinant phages QβMSP3 and QβUB05 with respect to dual HIV-Malaria infection in individuals living in low and high malaria transmission areas

Comparison of IgG2 antibody responses between dual HIV-malaria infected and double negative individuals in low and high malaria transmission areas. IgG2 responses specific to the recombinant phages QβMSP3 (A) and QβUB05 (B) in low and high transmission areas. Effect of HIV infection on QβMSP3 (C) and QβUB05 (D) specific IgG2 responses in the two transmission areas. IgG2 responses specific to QβMSP3 (E) and QβUB05 (F) in individuals positive for *Plasmodium falciparum* in both transmission areas.

Figure 4: IgG3 specific response to the recombinant phages QβMSP3 and QβUB05 with respect to dual HIV-Malaria infection in individuals living in low and high malaria transmission areas

Comparison of IgG3 antibody responses between dual HIV-malaria infected and double negative individuals in low and high malaria transmission areas. IgG3 responses specific to the recombinant phages QβMSP3 (A) and QβUB05 (B) in low and high transmission areas. Effect of HIV infection on QβMSP3 (C) and QβUB05 (D) specific IgG3 responses in the two transmission areas. IgG3 responses specific to QβMSP3 (E) and QβUB05 (F) in individuals positive for *Plasmodium falciparum* in both transmission areas.

Figure 5: IgG4 response specific to the recombinant phages QβMSP3 and QβUB05 with respect to dual HIV-Malaria infection in individuals living in low and high malaria transmission areas

Comparison of IgG4 antibody responses between dual HIV-malaria infected and double negative individuals in low and high malaria transmission areas. IgG4 responses specific to the

651 recombinant phages QβMSP3 (A) and QβUB05 (B) in low and high transmission areas. Effect
652 of HIV infection on QβMSP3 (C) and QβUB05 (D) specific IgG4 responses in the two
653 transmission areas. IgG responses specific to QβMSP3 (E) and QβUB05 (F) in individuals
654 positive for *Plasmodium falciparum* in both transmission areas.

655 **Table 1: Study population characteristics**

Variables	**HIV-/Mal- (n=62)**		**HIV-/Mal+ (n= 59)**		**HIV+/Mal- (n= 62)**		**HIV+/Mal+ (n= 62)**	
Gender	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Participants (%)	30 (48)	32 (52)	30 (51)	29 (49)	44 (71)	18 (29)	44 (71)	18 (29)
Median Age (IQR)	30 (22- 34.5)	35.5 (22.75-48.75)	32 (23–46.25)	29 (26-45)	46 (36.25-58.25)	36 (30-46.25)	38 (31 - 46)	42 (33-52)
Median CD4 count (cell/mm3)	N/A		N/A		480.6 (IQR : 270.5-635)		459.6 (IQR : 241.0–594.8)	

656 **N/A =** Not Applicable**, IQR =** interquartile range**, Mal+=** malaria positive

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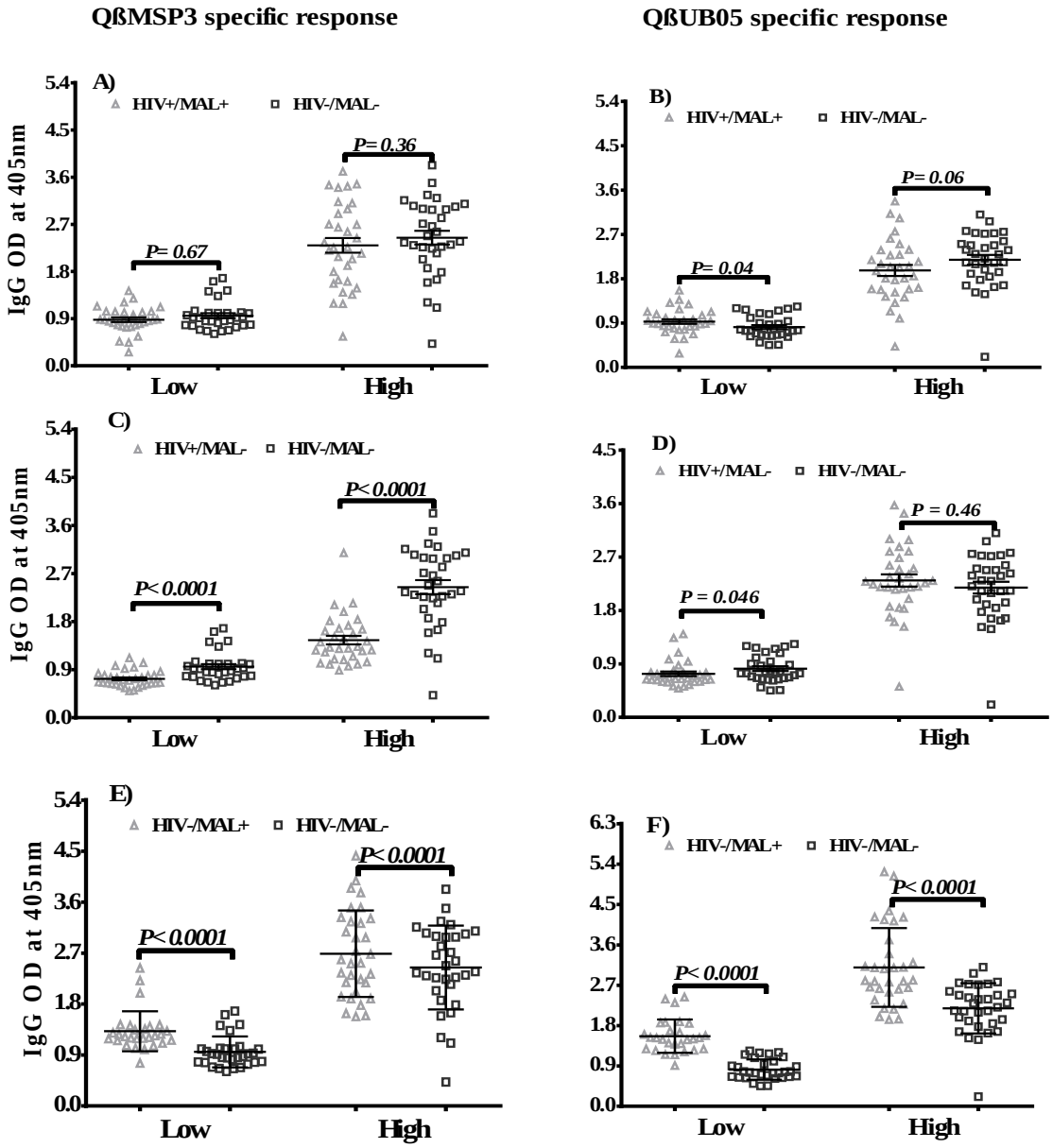
665 **Figures**

666 **Figure 1**



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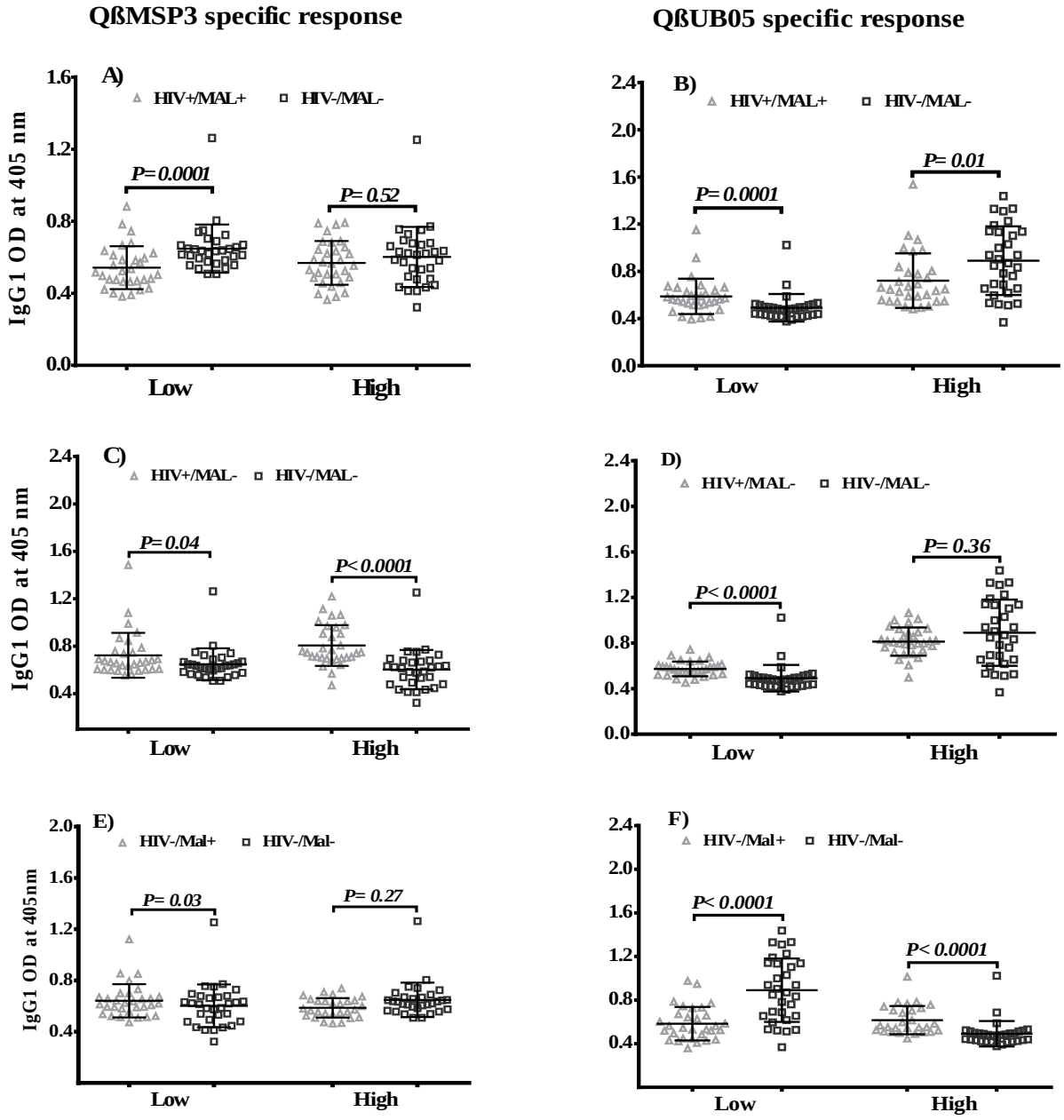
672 **Figure 2**



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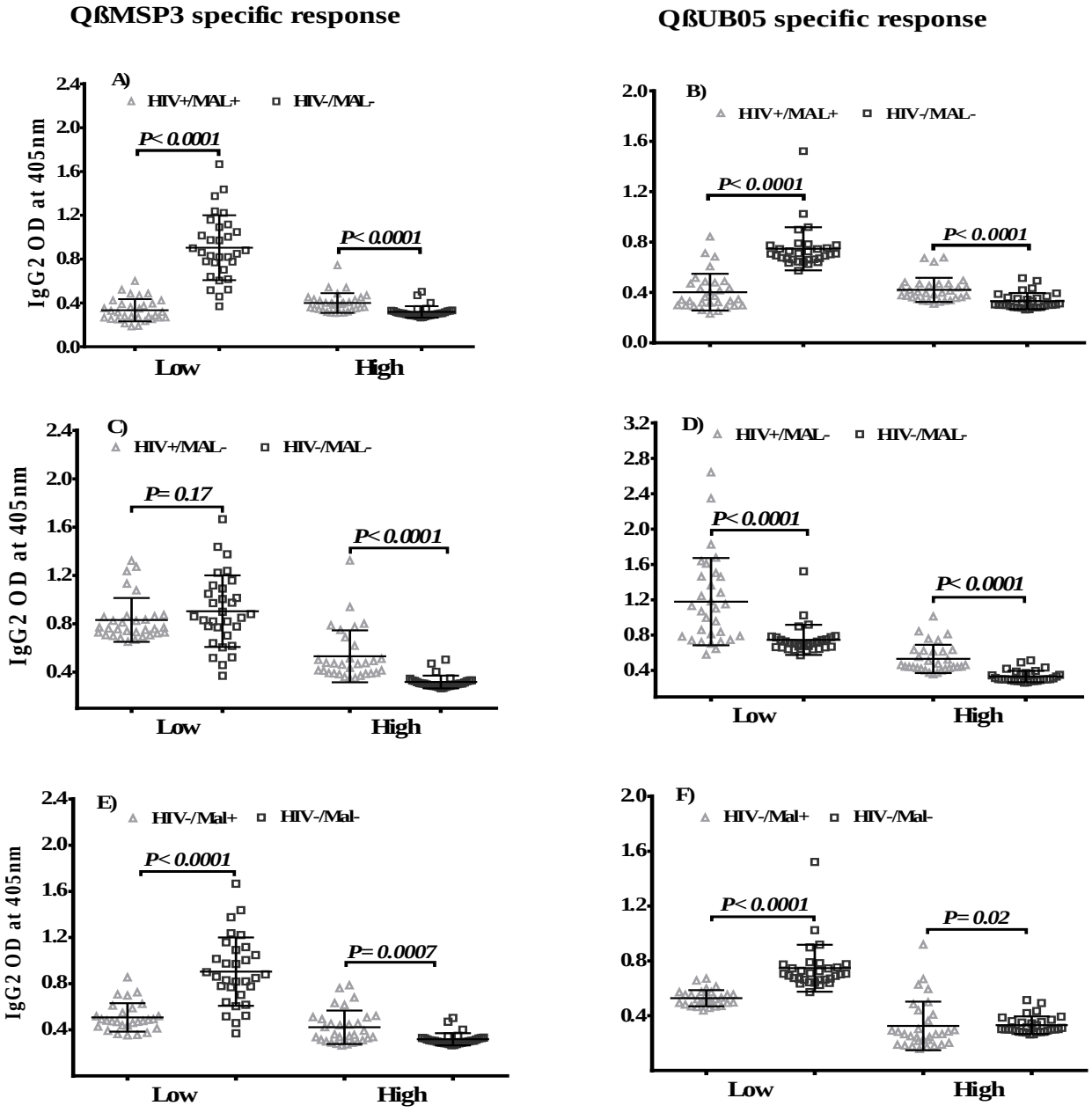
679 **Figure 3**



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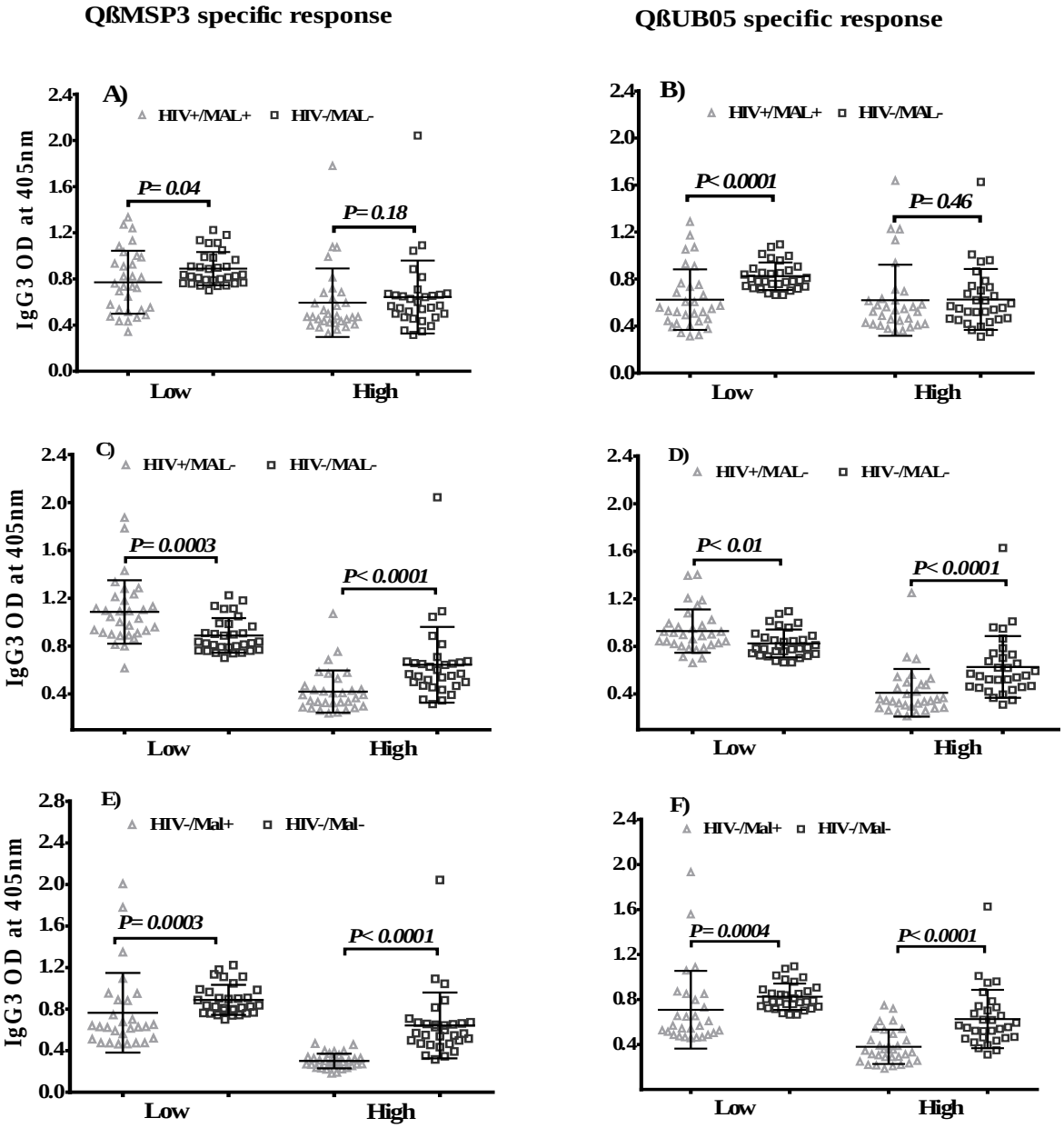
687 **Figure 4**



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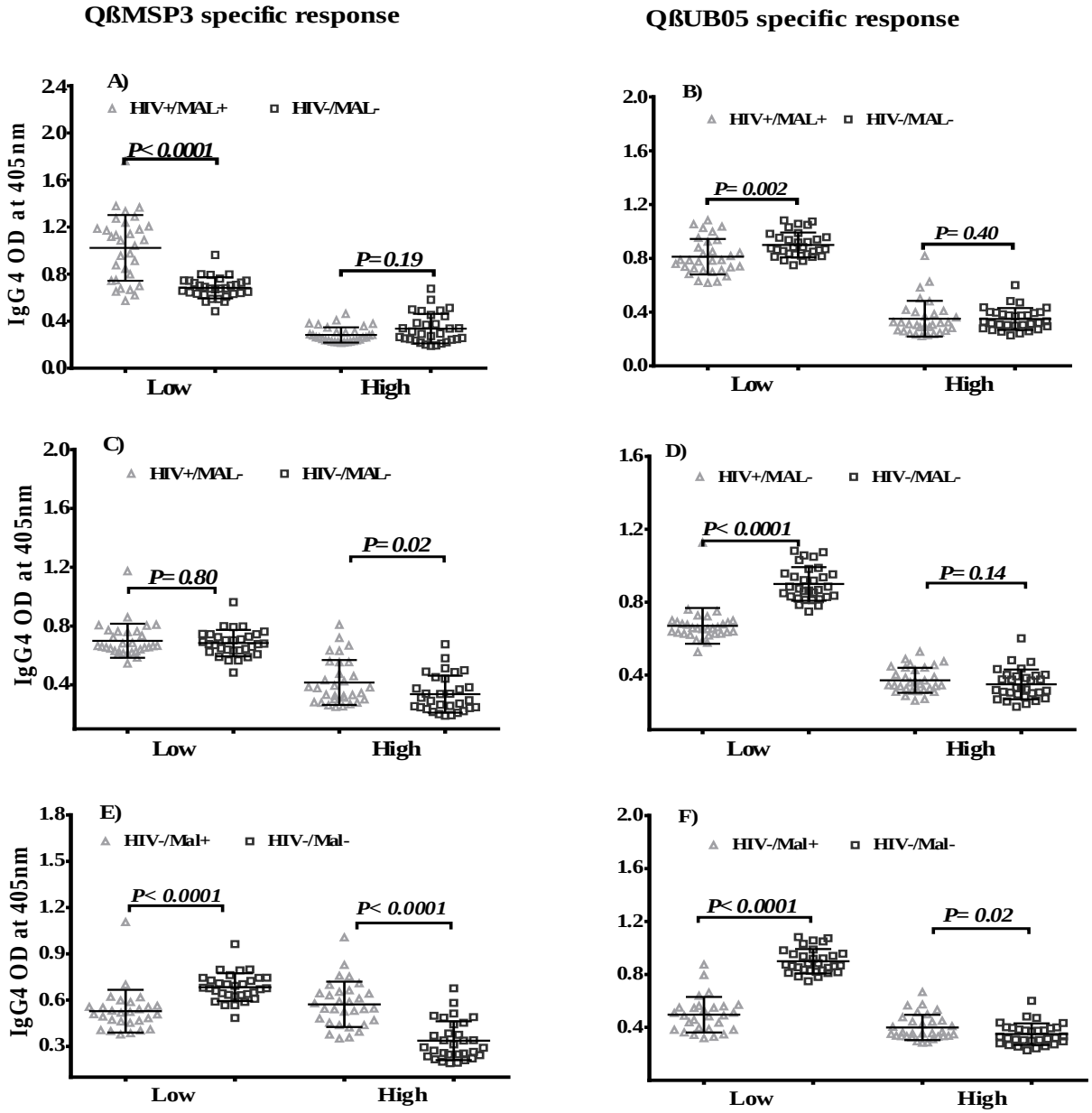
694 **Figure 5**



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