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2 **UB05 in Dual HIV-malaria infected adults living in areas differing in Malaria transmission**
3 **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 (Q β MSP3) and UB05 (Q β UB05) 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 Q β MSP3, P=0.0001 for Q β UB05) than their counterpart from low
79 transmission areas. When dual HIV-Malaria infection is considered significantly higher
80 Q β MSP3 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 Q β UBO5
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

84 Q β UB05 (P=0.0001) which were more relevant to protection in low malaria transmission areas
85 in the same population. Thus, understanding immune responses to Q β UB05 and Q β MSP3 could
86 facilitate the development of immunotherapeutic strategies suitable for areas differing in malaria
87 transmission intensity.

88 **Key words:** Dual HIV-Malaria, Q β UB05, Q β MSP3, IgG subclass, antibodies, natural immunity

89 **Introduction**

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

104 Antibodies to several asexual blood stage antigens including apical membrane antigen 1 (AMA-
105 1), erythrocyte binding antigen (EBA-175), the merozoite surface proteins (MSPs), reticulocyte-
106 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 Q β as
125 previously described by our group (14, 15). The recombinant phage Q β MSP3 displays the
126 conserved C-terminal 88 aa of the merozoite surface protein 3 (16, 17) whilst Q β UB05 bears the
127 previously described malaria antigen UBO5 (18). Surface display upon the recombinant RNA
128 coliphage Q β as previously demonstrated by our group improves the antigenicity of inserted
129 antigens (14, 15).

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

143 **Materials and Methods**

144 **Study site**

145 The study was carried out in two areas of Cameroon (Yaounde and Bikop) differing in malaria
146 transmission intensity. As the Capital city of Cameroon Yaounde (3°52'N11°31'E) is a multi-
147 ethnic city situated at an average elevation of 750 m. Bikop on the other hand is a remote rural
148 area located 48 KM away from Yaounde with year round intense malaria transmission. Both
149 Yaoundé and Bikop are holendemic for malaria however with differing transmission intensity.
150 This is mainly because unlike Yaounde, Bikop is located in the heart of the rain forest with a
151 large number of mosquito breedings sites and poorly constructed houses favoring sustained high
152 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 Q β displaying Plasmodium falciparum 3D7 strain
206 sequence derived C-terminal part of MSP3 (Q β MSP3) and UB05 (Q β UB05) generated in our
207 group as previously described (14, 15).

208 **Determination of IgG and IgG subclasses antibody responses specific to Q β UB05 and**
209 **Q β MSP3**

210 The plasma levels of antibodies specific to the malaria antigens Q β UB05 and Q β MSP3 were
211 determined through ELISA assay. Briefly high binding ELISA plates were coated with
212 10^7 particles/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 μ l/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).

222 **Statistical analysis**

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

227 **Results**

228 **High malaria transmission intensity is associated with superior Q β MSP3 and Q β UB05** 229 **specific IgG antibodies**

230 Individuals in areas of high perennial malaria transmission intensity developed significantly
231 higher ($P=0.0001$) levels of Q β MSP3 and Q β UB05 specific IgG responses than those living in
232 low transmission zones (compare Fig 1A with B). In high malaria transmission regions Q β MSP-
233 3 specific IgG responses were comparatively higher but not significantly different than responses
234 specific to Q β UB05 in both positive ($P=0.09$) and negative ($P=0.08$) individuals (Fig. S1). In
235 addition no significant difference is observed in dual HIV-Malaria infected people in their
236 plasma reactivity with the two recombinant antigens both in low and high transmission areas (Fig
237 1A&C). Similarly when people negative for both malaria and HIV (double negative individuals)
238 were considered no significant difference is observed in the IgG responses specific to the two
239 malaria vaccine antigens. The effect of HIV-1 infection was a significant reduction in IgG
240 responses specific to the two antigens in both low ($P=0.0001$ for Q β MSP3, $P=0.04$ for Q β UB05)
241 and high ($P=0.0001$ for Q β MSP3) malaria transmission areas. Surprisingly, there was no
242 difference in these values for UB05 in the high malaria transmission region (Fig. 1 C&D).
243 On the other hand, the overall effect of *Plasmodium falciparum* infection in both low and high
244 malaria transmission regions was a significant increase in IgG responses specific to both antigens

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

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

250 Whereas double negative individuals in low malaria transmission areas showed significantly
251 higher (P=0.0001) IgG1 responses specific to Q β MSP3 than to Q β UB05; in high malaria
252 transmission areas the converse was true (Fig. 2B) with IgG1 responses specific to Q β UB05
253 being superior (P=0.0001). The effect of dual HIV-malaria infection in low transmission area
254 was a significant reduction in the Q β MSP3 specific IgG1 responses (P=0.0001) in contrast to
255 individuals living in high malaria transmission areas where Q β MSP3 specific IgG1 responses
256 were similar to those of the double negative participants. On the other hand IgG1 responses
257 specific to Q β UB05 in high transmission areas remain comparatively higher than those to
258 Q β MSP3 in both dual HIV-malaria positive (P=0.004) and double negative (P=0.0001)
259 individuals (Fig. 2A&B). This probably indicates the relevance of Q β UB05 specific IgG1 in
260 predicting malaria immunity in high transmission areas even under the challenging
261 circumstances of HIV infection. The impact of dual HIV-malaria infection in low transmission
262 areas was therefore a significant reduction (P=0.0001) in IgG1 responses specific to Q β MSP3
263 (Fig 1C) in contrast to Q β UB05 where IgG1 responses in this group remain higher than the
264 double negative participants (P=0.0001). Again in a high transmission area the IgG1 responses to
265 the two antigens were comparatively higher than values for the low transmission area. In
266 addition IgG1 responses to Q β UB05 in double negative individuals were also significantly
267 higher (P=0.004) than dual HIV-malaria infected people. Thus in addition to malaria

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

270 Thus in a low malaria transmission area, significantly high IgG1 responses specific to Q β MSP3
271 is associated with resistance to malaria in contrast to superior Q β UB05 specific IgG1 antibodies
272 which were instead relevant to protection in high a transmission area.

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

275 In low malaria transmission areas significantly higher IgG2 subclass responses (P=0.0001 for
276 Q β MSP3 and P=0.0001 for Q β UB05) were observed in double negative individuals relative to
277 dual HIV-malaria positive participants (Fig. 3A&B). The converse was the case for high malaria
278 transmission areas where dual HIV-malaria infection resulted to significantly higher IgG2
279 responses to both Q β MSP3 (P=0.0001) and Q β UB05 (P=0.0001). Overall the IgG2 responses
280 specific to both antigen was comparatively superior in low relative to high transmission areas
281 (Fig. 3A&B). The effect of an infection with HIV was a general increase in IgG2 responses
282 specific to both antigens in low and high transmission areas (Fig. 3A&B). Several studies from
283 other endemic areas of Africa have indicated that high circulating levels of malaria parasite
284 antigen specific IgG2 could be a marker of severity of malaria infection (32-34). Except for
285 MSP3 specific IgG2 an infection with plasmodium falciparum was associated with an increased
286 in IgG2 responses to both antigens in all transmission areas. In a low malaria transmission area
287 the impact of dual HIV-malaria infection was therefore a significant reduction in IgG2 responses
288 to both antigens. In contrast in a high malaria transmission area dual HIV-malaria infection
289 resulted to superior IgG2 responses to both Q β MSP3 (P=0.0001) and Q β UB05 (p=0.0001)

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

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

294 In low malaria transmission areas the IgG3 subclass antibody responses were significantly higher
295 in double negative ($p=0.0001$ for Q β MSP3 and $p=0.0001$ for Q β UB05) compared to dual HIV-
296 malaria infected participants. On the other hand in high malaria transmission areas no difference
297 is observed between dual HIV-malaria infected and double negative individuals with respect to
298 IgG3 subclass responses to both antigens. The effect of HIV infection was a significant reduction
299 of IgG3 responses specific to Q β MSP3 in both low ($p=0.0003$) and high ($p=0.0001$) malaria
300 transmission areas. Similarly IgG3 specific responses to Q β UB05 were significantly reduced in
301 low ($P=0.01$) and high ($P=0.0001$) transmission areas after HIV infection. In low transmission
302 areas *Plasmodium falciparum* infection resulted to a significant increase in IgG3 responses
303 specific to both Q β MSP3 ($P=0.0003$) and Q β UB05 ($P=0.0004$) respectively. In contrast in high
304 malaria transmission we observed significantly lower IgG3 responses specific to Q β MSP3
305 ($P=0.0001$) and Q β UB05 ($P=0.0001$) after *Plasmodium falciparum* infection. Thus there is
306 differential effect of HIV and *Plasmodium falciparum* infections on IgG3 specific responses to
307 both antigens which was also associated with the malaria transmission intensity.

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

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

313 significantly higher IgG4 responses. However in low transmission areas whereas Q β MSP3
314 specific IgG4 responses in dual HIV-malaria infected individuals were superior to those of
315 double negative individual (P=0.0001); for Q β UB05 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 Q β MSP3
321 (P=0.0001) and Q β UB05 (P=0.0001) respectively (Fig.5E&F). In contrast in high transmission
322 areas the converse is true as IgG4 responses specific to both Q β MSP3 (P=0.0001) and Q β UB05
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 Q β UB05 and Q β MSP3 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

336 linked to a waning antimalarial immunity and an increase in clinical episodes of malaria in adults
337 (36-38). This in effect can modulate the profiles of target antigen specific IgG and IgG subclass
338 responses that can be achieved especially when the individuals are co-infected with HIV which
339 depletes the immune system. The impact of a high malaria transmission intensity in both dual
340 HIV-malaria infected and double negative individuals was a significant increase in antigen
341 specific IgG irrespective of the targeted antigen type. This might be in line with previous reports
342 suggesting that high levels of antibodies to several blood stage antigens (4-7, 36) were a
343 necessary component of protective immunity to malaria. Whereas this is probably correct for
344 IgG antibody levels specific to Q β UB05 and Q β MSP3 in high malaria transmission areas the
345 comparatively lower antibody levels in low transmission areas indicates that partial immunity to
346 malaria could be waning in these areas due to a reduction in parasite antigen challenge (39)
347 HIV infection resulted to a significant reduction in IgG antibody responses specific to Q β MSP3
348 in both low (P=0.0001) and high (P=0.0001) malaria transmission areas. In contrast, IgG
349 antibody responses specific to Q β UB05 were not significantly affected by HIV infection
350 (compares S1A&B). Infection with *Plasmodium falciparum* was associated with a significant
351 increase in IgG antibodies specific for both antigens in the two transmission areas. This is
352 especially true when dual HIV-malaria infected individuals are compared with participants
353 infected with HIV alone. Here we demonstrated that even when there was HIV infection the
354 impact of *Plasmodium falciparum* infection was a significant increase in IgG antibodies specific
355 to MSP3 in both low (P=0.0001) and high (P=0.0001) malaria transmission areas (S1E&F). This
356 indicates that asexual blood stage antigens are capable of modulating parasite antigen specific
357 IgG antibody levels in both low and high malaria transmission areas. Such antibody levels are
358 influenced by other factors including malaria and HIV infection.

359 When responses are examined qualitatively with respect to IgG subclasses and dual HIV-malaria
360 infections differential outcomes are observed. In comparing IgG1 responses to the two antigens
361 Q β MSP3 specific IgG1 was associated with protection against malaria in low transmission areas
362 in contrast to Q β UB05 specific IgG1 responses which were relevant to protection in high malaria
363 transmission areas. In this regards IgG1 responses specific to Q β UB05 in high malaria
364 transmission areas were significantly higher than responses to Q β MSP3 both for dual HIV-
365 malaria infected (P=0.004) and double negative (P=0.0001) individuals. This is probably in line
366 with previous reports which associated increase UB05 specific antibody responses with recovery
367 from clinical malaria (18). Similarly significantly higher IgG2 and IgG3 responses specific to
368 both antigens were associated with protection in low transmission areas. This is in line with
369 previous findings indicating that high levels of malaria parasite antigen specific IgG3 and IgG2
370 were relevant to protection against all forms of malaria (23, 40). This probably indicates that in
371 malaria endemic regions novel recombinant phages such as Q β UB05 could be more useful for
372 detecting protective levels of antibodies implicated in the control of malaria in both low and high
373 transmission areas. On the other hand the recombinant phage Q β MSP3 would be more relevant
374 for profiling IgG subclass responses in low malaria transmission areas.

375 The effect of dual HIV-malaria infection was a significant reduction in Q β MSP3 specific IgG1
376 (P=0.0001), IgG2 (P=0.0001) and IgG3 (P=0.04) responses in low transmission areas. In low
377 transmission areas this significant reduction of Q β MSP3 specific IgG2 together with the
378 cytophilic antibodies IgG1 and IgG3 in dual HIV-malaria infected people could diminish
379 protection from *Plasmodium falciparum* infection and symptomatic illness. In the case of IgG
380 subclass antibodies specific to Q β UB05 such a reduction in dual HIV-malaria infected people
381 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 Q β MSP3
390 specific IgG4 responses which are probably the reason why IgG1 and IgG3 responses to
391 Q β MSP3 were significantly lower in this group. On the other hand, IgG4 responses to Q β UB05
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 noncytotoxic 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 Q β UB05 were related to protection in both
402 low and high malaria transmission areas. This implies that Q β MSP3 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 Q β UB05 and Q β MSP3 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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424

425

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602 monocyte-mediated antibody-dependent killing of *Plasmodium falciparum* asexual blood
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606 **Figure Legends**

607 **Figure 1: specific IgG responses to recombinant phages Q β MSP3 and Q β UB05 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 Q β MSP3 (A) and Q β UB05 (B) in low and high transmission areas. Effect
613 of HIV infection on Q β MSP3 (C) and Q β UB05 (D) specific IgG responses in the two
614 transmission areas. IgG responses specific to Q β MSP3 (E) and Q β UB05 (F) in individuals
615 positive for *Plasmodium falciparum* in both transmission areas.

616

617 **Figure 2: IgG1 specific response to recombinant phages Q β MSP3 and Q β UB05 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 Q β MSP3 (A) and Q β UB05 (B) in low and high transmission areas. Effect
623 of HIV infection on Q β MSP3 (C) and Q β UB05 (D) specific IgG1 responses in the two
624 transmission areas. IgG1 responses specific to Q β MSP3 (E) and Q β UB05 (F) in individuals
625 positive for *Plasmodium falciparum* in both transmission areas.

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627

628 **Figure 3: IgG2 responses specific to the recombinant phages Q β MSP3 and Q β UB05 with**
629 **respect to dual HIV-Malaria infection in individuals living in low and high malaria**
630 **transmission areas**

631 Comparison of IgG2 antibody responses between dual HIV-malaria infected and double negative
632 individuals in low and high malaria transmission areas. IgG2 responses specific to the
633 recombinant phages Q β MSP3 (A) and Q β UB05 (B) in low and high transmission areas. Effect
634 of HIV infection on Q β MSP3 (C) and Q β UB05 (D) specific IgG2 responses in the two
635 transmission areas. IgG2 responses specific to Q β MSP3 (E) and Q β UB05 (F) in individuals
636 positive for *Plasmodium falciparum* in both transmission areas.

637 **Figure 4: IgG3 specific response to the recombinant phages Q β MSP3 and Q β UB05 with**
638 **respect to dual HIV-Malaria infection in individuals living in low and high malaria**
639 **transmission areas**

640 Comparison of IgG3 antibody responses between dual HIV-malaria infected and double negative
641 individuals in low and high malaria transmission areas. IgG3 responses specific to the
642 recombinant phages Q β MSP3 (A) and Q β UB05 (B) in low and high transmission areas. Effect
643 of HIV infection on Q β MSP3 (C) and Q β UB05 (D) specific IgG3 responses in the two
644 transmission areas. IgG3 responses specific to Q β MSP3 (E) and Q β UB05 (F) in individuals
645 positive for *Plasmodium falciparum* in both transmission areas.

646 **Figure 5: IgG4 response specific to the recombinant phages Q β MSP3 and Q β UB05 with**
647 **respect to dual HIV-Malaria infection in individuals living in low and high malaria**
648 **transmission areas**

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

651 recombinant phages Q β MSP3 (A) and Q β UB05 (B) in low and high transmission areas. Effect
 652 of HIV infection on Q β MSP3 (C) and Q β UB05 (D) specific IgG4 responses in the two
 653 transmission areas. IgG responses specific to Q β MSP3 (E) and Q β UB05 (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)	
	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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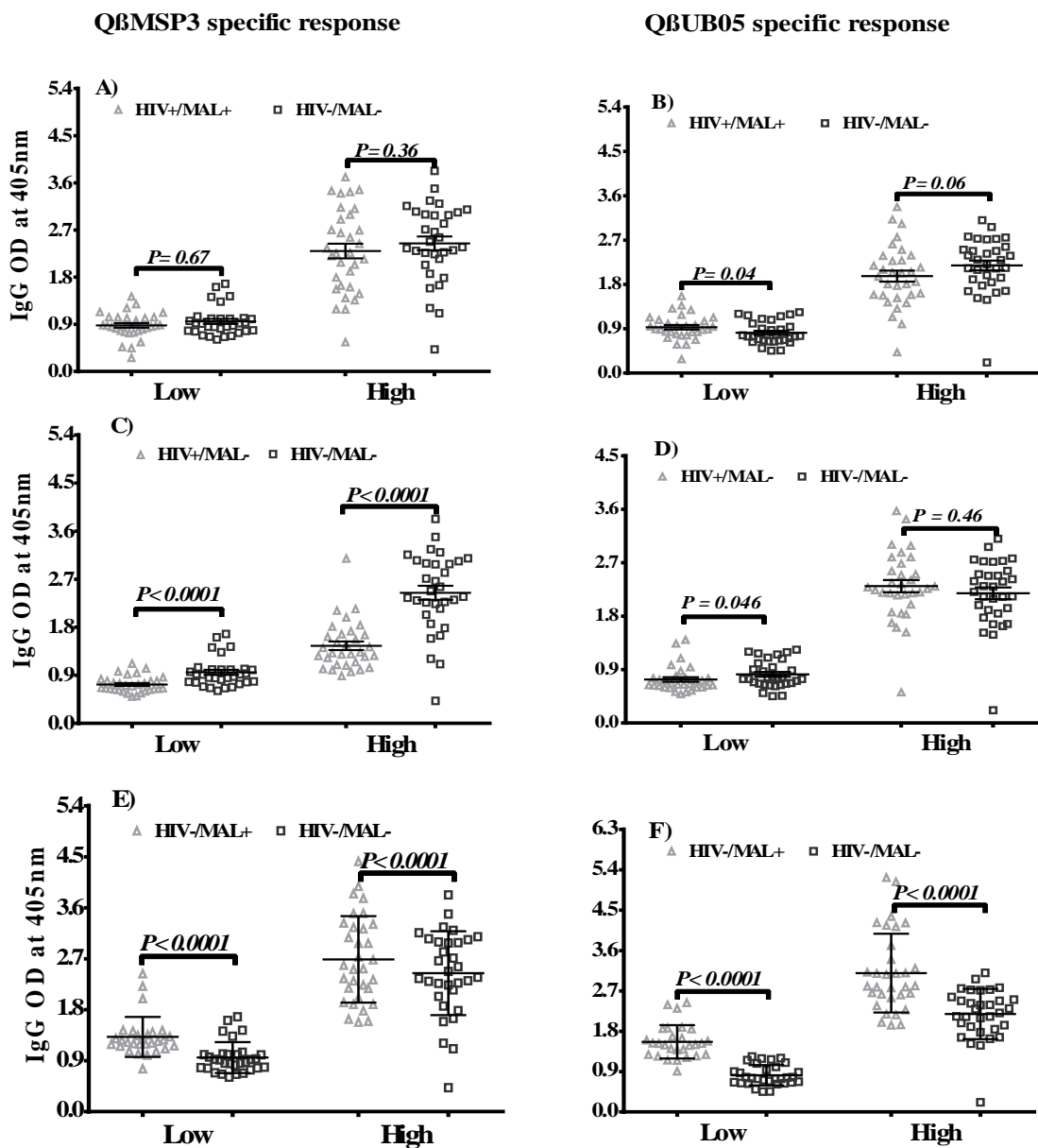
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665 **Figures**

666 **Figure 1**



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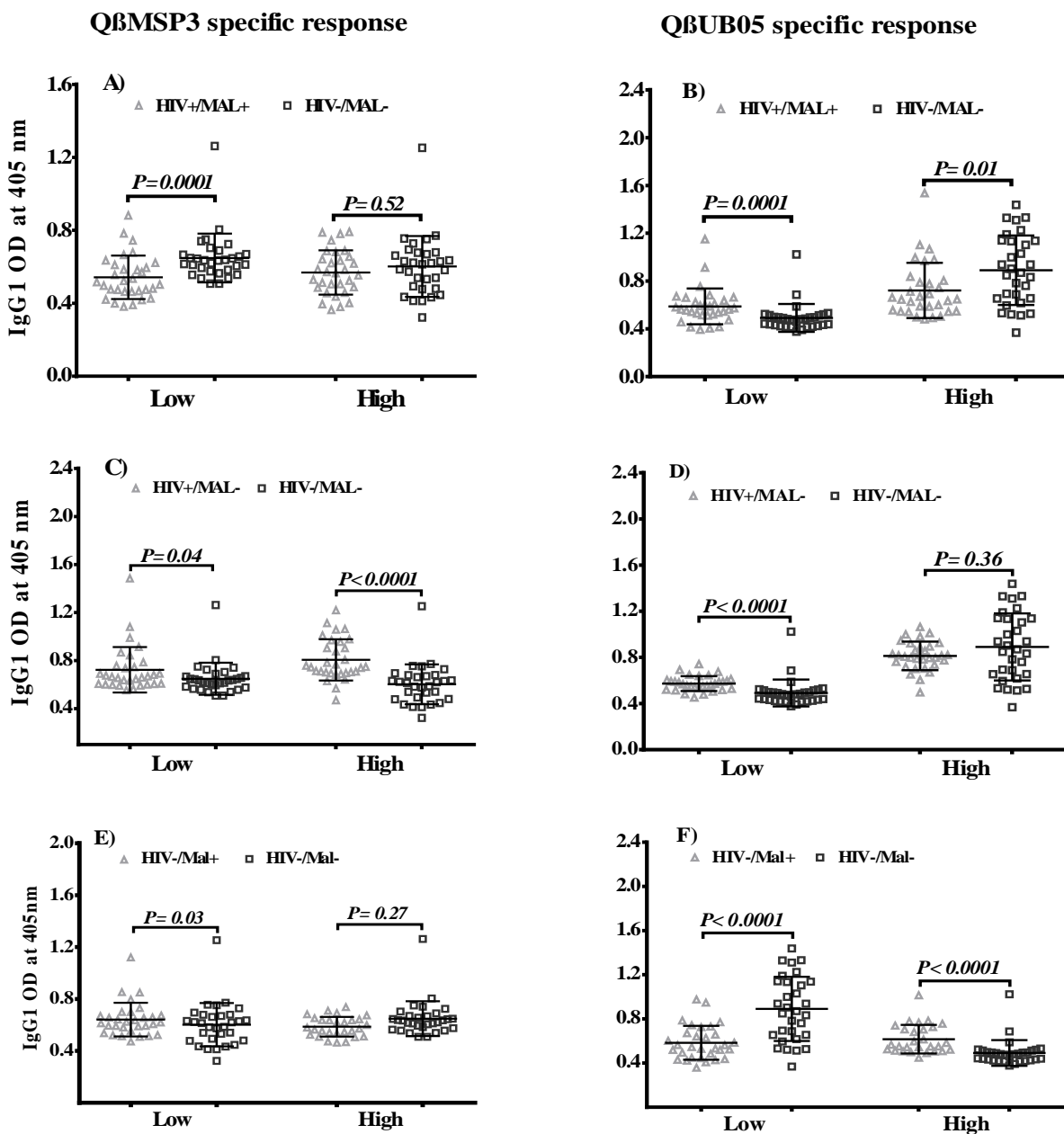
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672 **Figure 2**



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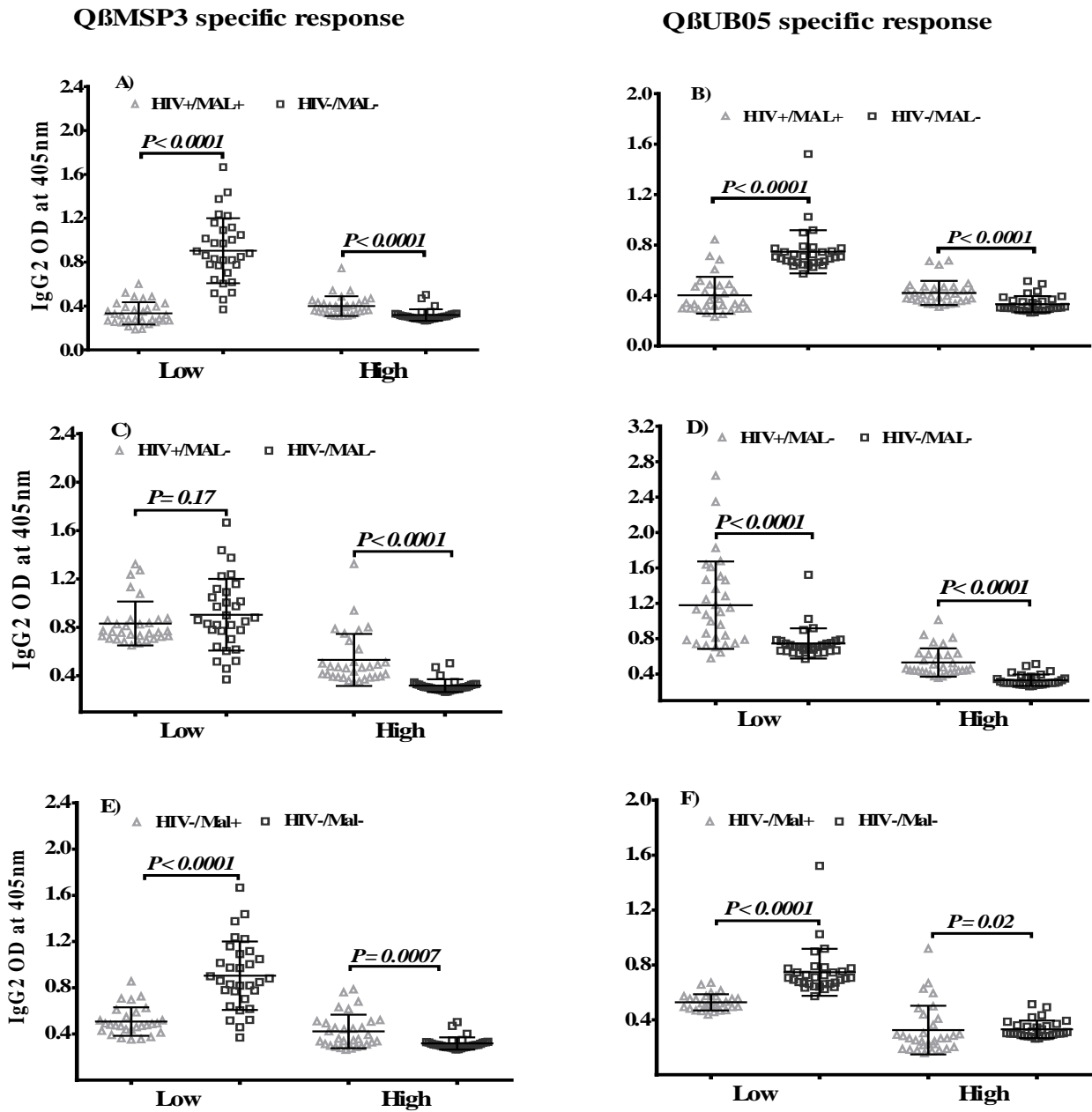
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679 **Figure 3**



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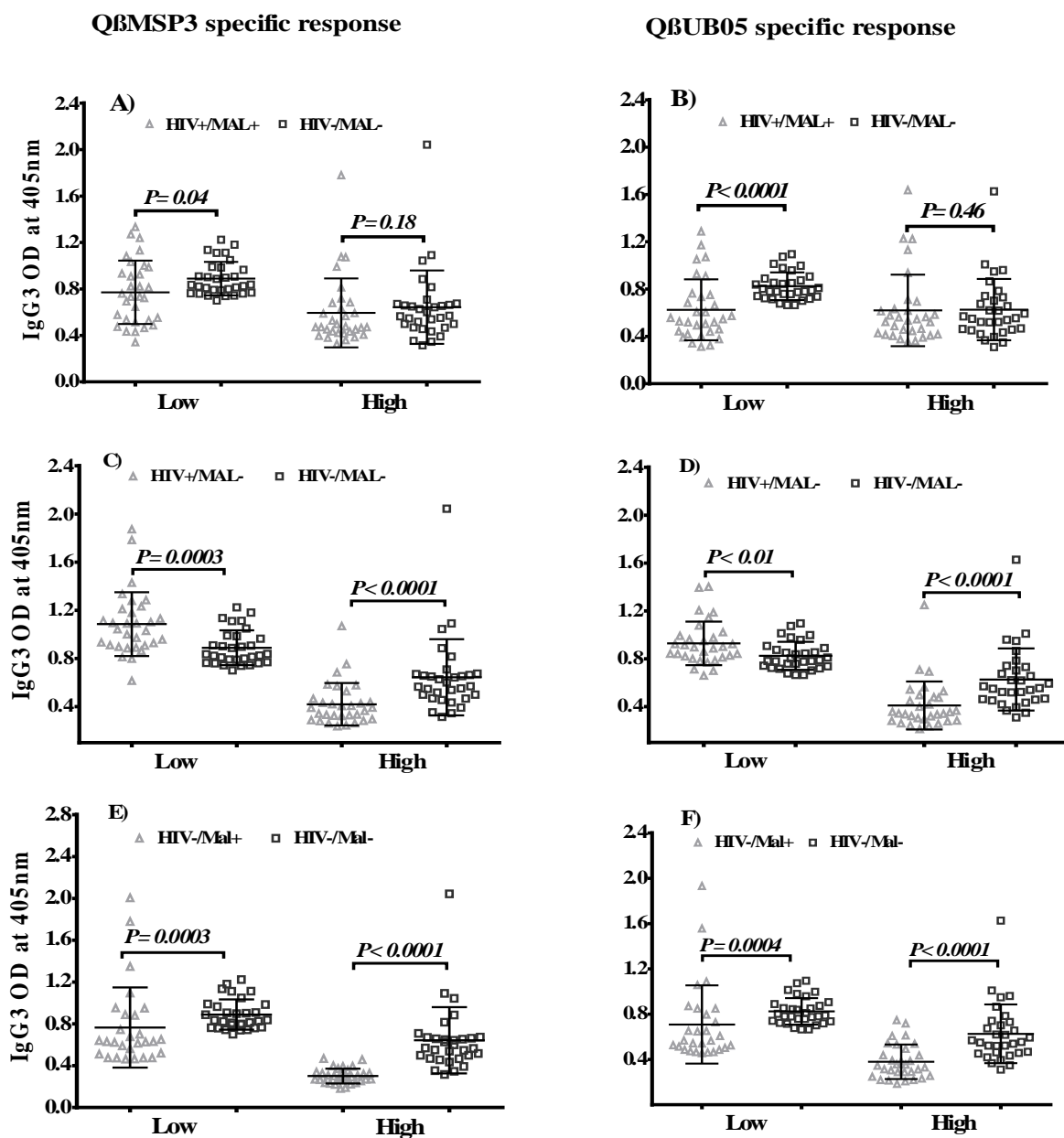
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687 **Figure 4**



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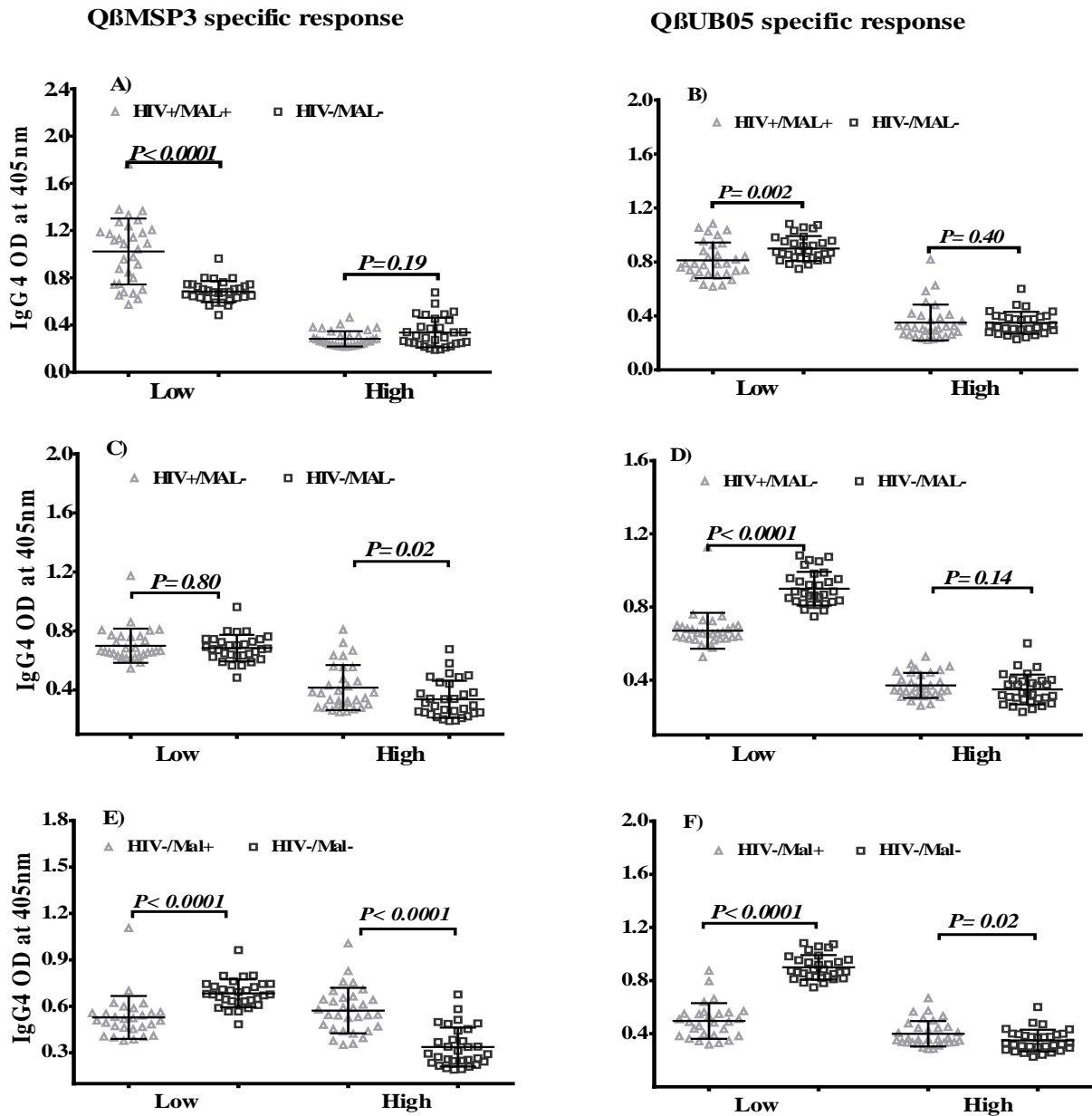
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694 **Figure 5**



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