1 **TITLE**

2 ***Plasmodium falciparum*** **and helminth coinfections increase IgE and parasite-specific IgG**
3 **responses**

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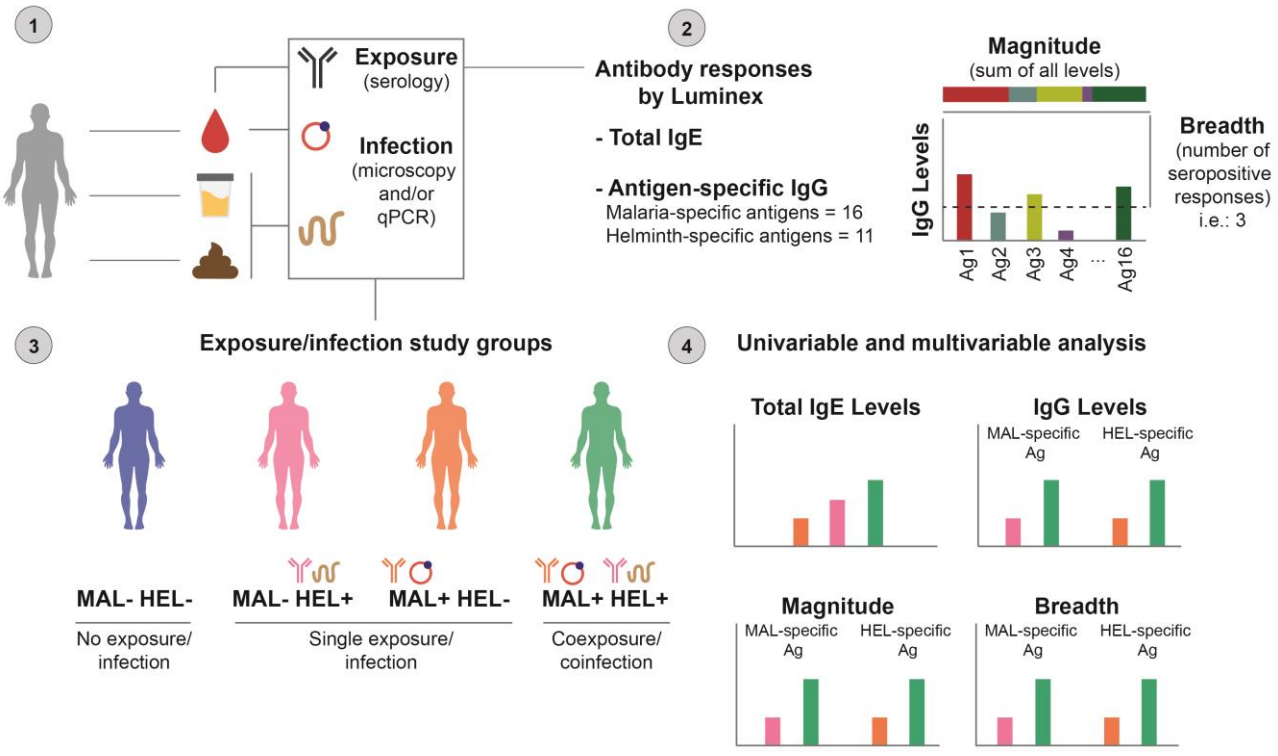
ABSTRACT

Coinfection with *Plasmodium falciparum* and helminths may impact the immune response to these parasites since they induce different immune profiles. We studied the effects of coinfections on the antibody profile in a cohort of 715 Mozambican children and adults using the Luminex technology with a panel of 16 antigens from *P. falciparum* and 11 antigens from helminths (*Ascaris lumbricoides*, hookworm, *Trichuris trichiura*, *Strongyloides stercoralis* and *Schistosoma* spp.) and measured antigen-specific IgG and total IgE responses. We compared the antibody profile between groups defined by *P. falciparum* and helminth previous exposure (based on serology) and/or current infection (determined by microscopy and/or qPCR). In multivariable regression models adjusted by demographic, socioeconomic, water and sanitation variables, individuals exposed/infected with *P. falciparum* and helminths had significantly higher total IgE and antigen-specific IgG levels, magnitude (sum of all levels) and breadth of response to both types of parasites compared to individuals exposed/infected with only one type of parasite ($p \leq 0.05$). There was a positive association between exposure/infection to *P. falciparum* and exposure/infection to helminths or the number of helminth species, and *vice versa* ($p \leq 0.001$). In addition, children coexposed/coinfected tended ($p = 0.062$) to have higher *P. falciparum* parasitemia than those single exposed/infected. Our results suggest that an increase in the antibody responses in coexposed/coinfected individuals may reflect higher exposure and be due to a more permissive immune environment to infection in the host.

GRAPHICAL ABSTRACT



INTRODUCTION

Malaria and helminthiasis are endemic parasitic diseases in tropical and subtropical areas, especially in impoverished countries with poor water and sanitation access. Their overlapping spatial distribution makes coinfections with these pathogens a frequent event (Brooker et al., 2006). Both cause high burden of morbidity, and malaria is a leading cause of mortality, particularly in children (World Health Organization, 2020a, 2020b, 2020c). The highest burden of malaria cases (82%) and malaria deaths (94%) occurs in sub-Saharan Africa (World Health Organization, 2020a), where *Plasmodium falciparum* is the most prevalent species causing malaria (World Health Organization, 2021). Helminths such as *Schistosoma* spp. and soil-transmitted helminths (STH) (*Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale, Necator americanus and Strongyloides stercoralis*) are also prevalent in Sub-Saharan Africa affecting more than 1.5 billion people worldwide (World Health Organization, 2020b, 2020c).

P. falciparum and helminths induce different types of immune responses. On one hand, the clearance of *P. falciparum* infection is achieved by an initial T helper (Th)1 response with the production of IgG antibodies, mainly from the cytophilic IgG1 and IgG3 subclasses (Bouharoun-Tayoun and Druilhe, 1992; Cohen et al., 1961). Evidence from animal models and humans suggest that later, a Th2/T regulatory (Treg) response is required to counteract severe immunopathological consequences of an excessive proinflammatory Th1 milieu (Hartgers and Yazdanbakhsh, 2006; Langhorne et al., 1998; Malaguarnera and Musumeci, 2002). On the other hand, helminths generally induce a Th2 polarization with the production of IgE and IgG4 antibodies (Hartgers and Yazdanbakhsh, 2006; Maizels et al., 2004; Maizels and Yazdanbakhsh, 2003). However, helminths are a heterogeneous group of parasites, each with a complex life cycle; therefore, differences exist by species and life-cycle stage. For example, *Schistosoma* spp. infection induces an acute proinflammatory Th1 response while there is a shift towards a Th2 response upon deposition of eggs (Pearce and MacDonald, 2002). Adding another layer of complexity, helminths are well known for their immunomodulating effects that deviate the cellular response towards a regulatory profile. This allows helminths to survive in the host for years and this may affect not only helminth antigens

but also bystander antigens (Hartgers and Yazdanbakhsh, 2006; Maizels et al., 2004; Maizels and Yazdanbakhsh, 2003; McSorley and Maizels, 2012).

By altering the immunological balance, coinfections can impact immunity and course of infections. A paradox exists for *P. falciparum*: a Th2 and Treg response could protect against severe disease, but it may render subjects more susceptible to *P. falciparum* infection. This may explain the conflicting results existing in the literature (Degarege and Erko, 2016). In some cases, helminths have been associated with detrimental effects on *P. falciparum* infection, complications and parasite load (Babamale et al., 2018; Degarege et al., 2012, 2009; Le Hesran et al., 2004; Nacher et al., 2002b, 2001a; Ntonifor et al., 2021; Sokhna et al., 2004; Spiegel et al., 2003; Tshikuka et al., 1996) with protective effects in other cases (Briand et al., 2005; Brutus et al., 2007, 2006; Efunshile et al., 2015; Lemaitre et al., 2014; Lyke et al., 2005; Murray et al., 1978; Nacher et al., 2002a, 2001b, 2000) or no effect (Shapiro et al., 2005). The outcome may be critically influenced by the species, timing (Salazar-Castañón et al., 2018), parasite load, (Briand et al., 2005; Lemaitre et al., 2014; Lyke et al., 2005), and endpoint analyzed (infection or disease) (Degarege et al., 2016). Additionally, the limited information available regarding the effect of *P. falciparum* on helminth infections in humans suggests that *P. falciparum* could also affect the frequency and course of helminth infections, perhaps by delaying or dampening the production of required Th2 cytokines. In fact, a study showed that coinfection with malaria reduced the Th2 cytokine IL-4 in response to hookworm antigens (Quinnell et al., 2004). Lessons learned from other pathogens that induce Th1 responses support these findings, where the human T cell lymphotropic virus type 1 (HTLV-1) in coinfection with *S. stercoralis* also reduced the Th2 cytokine IL-5 and IgE (Porto et al., 2001), and previous leishmaniasis increased the probability to subsequently be infected with *S. mansoni* (Miranda et al., 2021). This is relevant since a Th1 polarization, depending on the species and life-stage, has been associated with persistence of helminth infection (Artis and Grencis, 2008).

Based on the different immune responses triggered by *P. falciparum* and helminths and the immunomodulating effect of helminths, we hypothesized that prior exposure to *P. falciparum* and helminths or their coinfection, would have an impact on the immune response to these parasites.

Thus, our objective was to describe the effects of *P. falciparum* and helminth coinfections on the antibody profiles in an endemic cohort from Southern Mozambique. We measured antigen-specific IgG and total IgE responses with a multiplex suspension array technology (Luminex) including a panel of 16 antigens from different life stages of *P. falciparum* and 11 antigens from helminths (STH and *Schistosoma* spp.). The antibody profile was compared between different exposure/infection groups defined by previous exposure (based on serology) and/or the presence of current infection (determined by microscopy and/or qPCR).

RESULTS

Characteristics of the study population

The study included 715 subjects (363 children and 352 adults) from six different locations of Manhiça district, in the Maputo province, Southern Mozambique (**Table 1**). From all the participants, 9.93% were asymptomatically infected with *P. falciparum* as diagnosed by qPCR, and 53.57% carried a helminth infection (diagnosed by either qPCR and/or microscopy) (**Table S1**). Twenty-eight percent were infected by hookworm (*A. duodenale* and/or *N. americanus*), 14.41% by *T. trichiura*, 12.03% by *Schistosoma* spp., 10.35% by *S. stercoralis* and 7.41% by *A. lumbricoides.* From those infected with helminths, 29.24% were infected with more than one helminth species, with the coinfection of hookworm with *S. stercoralis* being the most common (**Table S2** and **Figure S1A**). Assessed by serology, 91.05% of individuals had been exposed to *P. falciparum* and 79.16% to any helminth (69.51% to hookworm, 52.73% to *T. trichiura,* 43.92% to *Schistosoma* spp. 27.27% to *S. stercoralis* and 20.70% to *A. lumbricoides*) (**Table S1**). Most of the helminth exposed individuals (77.21%) had also been exposed to multiple helminth species, mainly to hookworm and *T. trichiura* (**Table S2** and **Figure S1B**).

For subsequent analyses, we considered only helminth infection regardless of the species. Combining the diagnosis of exposure and/or infections, 91.05% and 87.69% of individuals had been exposed to and/or were infected with *P. falciparum* and helminths, respectively (**Table S1**), with the following breakdown into exposure/infection groups: no exposure/infection (MAL- HEL- [N= 21,

2.94%]), single exposure/infection (MAL- HEL+ [N= 43, 6.01%] or MAL+ HEL- [N= 67, 9.37%]) and coexposure/coinfection (MAL+ HEL+ [N= 584, 82.68%]) (**Table 1**). The median antibody levels for each group defined by exposure/infection followed a similar pattern but were lower compared to the same groups defined by current infection only (**Figure S2**). Using a combination of past exposure and current infection, we were able to capture a large proportion of exposed individuals not detected by current infection diagnosis (**Table S3**).

Females (N= 404) and males (N= 311) were equally distributed across exposure/infection study groups but there were significant differences in terms of age (**Table 1**). As expected, the MAL- HEL- group had the lowest age median (7.7 years), while the MAL+ HEL+ group had the highest age median (23.9 years) (**Table 1**). Further, the breakdown by age group revealed a very unbalanced distribution of children (5-14 years old) and adults (≥ 15 years old) across study groups, with the vast majority of adults in the MAL+ HEL+ group (**Table 1**).

The percentage of individuals with asymptomatic malaria and infection by any helminth was calculated for each of the 6 locations. Then, these were classified into “high” and “low” prevalence locations based on higher or lower percentage of infected individuals compared to the median percentage of infections from all locations (**Tables 1** and **S4**). No statistically significant differences were found by high or low location between study groups (**Table 1**).

Regarding socioeconomic, water and sanitation metrics, such as socioeconomic score (SES), ownership of latrine, accessibility to piped water inside the household and payment for water, there were no statistically significant differences between exposure/infection groups (**Table 1**).

Association of demographic, SES, water and sanitation variables with antibody levels

Sex had a different impact depending on the immunoglobulin isotype. For antigen-specific IgG, there was a clear and consistent trend for higher antibody levels in females than males, which was statistically significant ($p \leq 0.05$) for IgG to EXP1, AMA1, As37, AyCp2, NaAPR1, NaGST1, NaSAA2 and MEA (**Figures 1** and **S3A**). For total IgE, there was a trend in the opposite direction, with males exhibiting higher levels ($p = 0.087$) (**Figures 1** and **S3A**). When differences by sex were assessed

stratifying by age group, there were no differences for antigen-specific IgG (data not shown), whereas total IgE was statistically significantly higher in male compared to female children (**Figure S3B**).

There was a positive correlation between age and all antigen-specific IgG (rho= 0.31 – 0.64) and total IgE levels (rho= 0.2) (**Figures 1** and **S4**). IgG and IgE antibody levels rose until 15−24 years old and then remained relatively stable during the rest of adulthood (**Figure 2**).

Being from a location with a high prevalence of malaria was associated with higher total IgE levels ($p \leq 0.001$) and *P. falciparum*-specific IgG levels, specifically for EXP1, AMA1, $MSP1_{42}$, MSP2 and MSP5 but not with helminth-specific IgG levels (**Figures 1** and **S5**). Living in a location with a higher burden of helminth infection was associated with higher total IgE levels ($p \leq 0.05$), but not with helminth-specific nor *P. falciparum*-specific IgG levels (**Figures 1** and **S6**).

SES was negatively association with IgG anti-EXP1, AMA1, MSP1 block 2, $MSP1_{42}$, and NIE and total IgE (**Figure 1**). Overall, there was a weak but significant negative correlation (rho= -0.17 to -0.1) between antigen-specific IgG levels and SES, whereas the negative correlation with total IgE levels was somewhat stronger (rho= -0.21) (**Figure S7**).

Regarding water and sanitation metrics, individuals with a latrine at home had significantly lower total IgE levels than individuals without a latrine at home ($p \leq 0.001$), while specific-IgG levels were not significantly associated (**Figures 1** and **S8**). Additionally, living in a household with piped water inside and paying for water access were also significantly associated with lower total IgE levels compared to living in a household with piped water in the yard ($p = 0.002$) or a household that did not pay for water ($p \leq 0.001$) (**Figures 1**). Piped water accessibility outside the household was associated with higher antigen-specific IgG levels against AMA1, EBA175, MSP1 block 2, MSP3 and MSP5 and no payment for water with IgG levels against α-gal, SSP2, LSA1, EXP1, MSP1 block 2, $MSP1_{42}$, MSP3, Rh1 and NIE ($p \leq 0.05$) (**Figures 1, S9** and **S10**).

Association of coexposure/coinfection with antibody levels

The MAL+ HEL+ group consistently showed higher IgG levels against all *P. falciparum* and helminth antigens and higher total IgE levels (**Figures 3A, 3B** and **S11**), higher magnitude (defined as the sum of antibody levels against *P. falciparum* or helminth antigens) (**Figure 3C**) and breadth of response (defined as the number of *P. falciparum* or helminth seropositive responses) (**Figure 3D**), compared to the MAL+ HEL- or MAL- HEL+ groups ($p \leq 0.001$ except for IgE levels between MAL+ HEL+ and MAL-HEL+ where $p= 0.033$). Since all antibody levels increased with age, and there was a biased distribution of age groups across infection groups, this analysis was also performed stratified by age, where only the children had enough sample size in all the groups. The results in children confirmed the results in the whole cohort, with statistically significant differences in all comparisons except for IgG against highly immunogenic antigens such as AMA1, EBA175 and $MSP1_{42}$, where after adjusting for multiple comparisons statistical significance disappeared, although a clear trend remained (**Figure S12**).

In multivariable analysis, linear regression models adjusted by their corresponding covariables revealed that the MAL+ HEL+ group had significantly higher antibody levels against most of the antigens, magnitude and breadth of response compared to single infection/exposure groups (MAL+ HEL- or MAL- HEL+) (**Figure 4**).

For most *P. falciparum* antigens, IgG levels increased between 5% and 15% in the MAL+ HEL+ group compared to the MAL+ HEL- group, although it reached a 17% increase for CelTOS (95% CI = 7.750 – 26.932) and a 30% for α-gal (95% CI = 18.438 - 42.570) (**Figure 4A**). The only antigens without a significant increase associated with coinfection were EBA175, MSP1 block 2 and Rh5 (**Figure 4A**). The MAL+ HEL+ group resulted in an increase of up to a 10% (95% CI = 5.462 - 14.889) in the magnitude of response, and an increase of 2 in the total number of seropositive responses (95% CI = 1.254 - 3.001) (**Figure 4A**).

Regarding helminth antigens, the increase in IgG levels in the MAL+ HEL+ group compared to the MAL- HEL+ group ranged from 6% for Sm25 (95% CI = 0.624 - 12.463) to 22% for As16 (95% CI =

213 10.213 - 35.249) (**Figure 4B**). The magnitude increased up to a 13% (95% CI = 7.995 - 18.684)
214 while the breadth was not significantly affected (0.852, 95% CI = -0.030 - 1.734) (**Figure 4B**).

215 For total IgE levels, MAL- HEL+ or MAL+ HEL+ groups were significantly associated with a 74%
216 (95% CI = 6.193 – 185.409) or 106% (95% CI = 46.453 – 191.300) increase in total IgE levels,
217 respectively, compared to the MAL+ HEL- group (**Figure 4C**).

218 **Association of magnitude of response and total IgE with antibody levels**

219 We also performed additional multivariable models in the whole cohort using total IgE or the
220 magnitude of response against malaria and helminth antigens as predictor exposure variables
221 instead of exposure/infection groups (**Figure S13** and **S14**). Total IgE was significantly positively
222 associated with the magnitude and breadth of response and IgG levels against *P. falciparum* and
223 helminth antigens (**Figure S13**). The magnitude of response against helminth antigens was also
224 positively associated with IgG levels against *P. falciparum* antigens. Magnitude of response against
225 *P. falciparum* antigens was also positively associated with IgG levels against helminth antigens, and
226 both magnitudes with total IgE levels (**Figure S14**). Lastly, as expected, the magnitude of response
227 against helminth antigens was more associated with an increase in total IgE levels (31%, 95% CI =
228 22.314 - 39.504) than the magnitude of response against *P. falciparum* antigens (9%, 95% CI =
229 2.778 – 15.956) (**Figure S14**).

230 **Association of coexposure/coinfection with susceptibility to infection**

231 Logistic regression analysis adjusted by their corresponding covariables showed a consistent trend
232 for higher odds of having malaria or helminth infections if exposed to or currently having the other
233 parasite type (**Table S5**). In support of this trend, a higher magnitude of antibody response to
234 helminth or *P. falciparum* antigens was significantly associated to having *P. falciparum* or helminth
235 infection, respectively (**Figure 5**). In addition, there was a positive association between being
236 exposed/infected with *P. falciparum* and the number of helminth species exposed/infected with
237 (**Table 2**). Furthermore, there was a statistically significant higher proportion of individuals

238 exposed/infected with multiple helminth species in the MAL+ HEL+ group than in the MAL- HEL+
239 group (p≤ 0.001) (**Table S2**).

240 **Association of coexposure/coinfection with parasite burden**

241 Next, we evaluated whether being exposed and/or having a coinfection had an impact on parasite
242 burden. In the case of malaria, 93% of the people with current *P. falciparum* infection were
243 coexposed/coinfected with helminths (66/71), leaving the single infection group with a much-
244 reduced sample size. In this case, the coexposure/coinfection group was not significantly
245 associated (p= 0.34) with *P. falciparum* parasitemia (parasites/µL) (**Figure 6A**). However, since
246 immunity develops over time and most of the coexposed/coinfected individuals were adults, we
247 stratified by age group, which resulted in a trend (p= 0.062) for higher parasitemia in the
248 coexposure/coinfection group compared to the malaria only group in children (**Figure 6A**). In the
249 case of helminths, coexposure/coinfection had a significant protective effect on parasite burden
250 (expressed as qPCR Ct values) of *T. trichiura* (p≤ 0.01), also after stratification by age group (p≤
251 0.05) (**Figure 6B**). For the remainder of the helminth species there was no significant association
252 between coexposure/coinfection and parasite burden (**Figure S15**).

253 **Association of antibody levels with parasite burden**

254 To investigate the associations of antibody responses with parasite burden and their possible
255 involvement in protection, we performed linear regression models adjusted by age. For *P.*
256 *falciparum* parasitemia, there was a significant (raw p≤ 0.05) positive association of specific IgG
257 responses to CelTOS (17.793, 95% CI = 2.99 – 35.634), SSP2 (22.229, 95% CI = 1.38 – 47.361),
258 AMA1 (34.277, 95% CI = 2.410 – 76.060), MSP5 (26.824, 95% CI = 5.440 – 52.545) and the
259 breadth of response to *P. falciparum* antigens (20.838, 95% CI = 1.111 – 44.415) only before
260 adjustment for multiple comparisons (**Figure 7**).

261 As for helminths, we found significant negative associations of qPCR Ct values with total IgE (-
262 0.085, 95% CI = -0.141 – -0.028) for hookworm, specific IgG to Sm25 (-0.963, 95% CI = -1.573 – -
263 0.353) and MEA (-1.065, 95% CI = -1.862 – -0.268) for *Schistosoma* spp. and NIE for *S. stercoralis*

(-0.542, 95% CI = -0.828 – -0.256) (**Figure 7**). These negative associations with Ct values are indicative of a positive association with parasite burden. We found no association between *A. lumbricoides* and *T. trichiura* antibody levels and Ct values (**Figure S16**).

DISCUSSION

In this study we found that 83% of a cohort of children and adults from Southern Mozambique was infected or had been exposed to *P. falciparum* and helminths, mainly hookworm. Our main finding was that coexposure/coinfection was associated with increased antibody levels, magnitude and breadth of response to both types of parasites compared to the single exposure/infection groups.

We initially expected a deviation in the coexposure/coinfection group towards one of the branches of the immune response, which would result in reduced antibody production of the specificity and type required for one or other type of infection. On the contrary, we found that antigen-specific IgG and total IgE responses were higher in the coexposure/coinfection group compared to the single exposure/infection groups. For *P. falciparum*, this is consistent with previous studies that reported increased *P. falciparum*-specific IgG1, IgG2 and IgG3 responses in coinfection with *S. haematobium* (Diallo et al., 2010; Tokplonou et al., 2020). In contrast, other studies have reported a detrimental effect of coinfection with helminths in the humoral response to malaria with a reduction in *P. falciparum*-specific IgG (Ateba-Ngoa et al., 2016), IgG1 and IgG3 (Courtin et al., 2011; Roussilhon et al., 2010), and an increase in the non-cytophilic IgG4 (Roussilhon et al., 2010). As for helminths, the effect of *P. falciparum* infection on antibody levels has been mainly restricted to *Schistosoma* spp. and, in line with our results, coinfection with *P. falciparum* has been associated with an increase in both antigen-specific IgG and IgE responses to *Schistosoma* spp. antigens compared to the single infection group (Remoue et al., 2003). Does this increase mean there is a synergy at the immune humoral compartment or does it simply reflect an increased susceptibility, and therefore exposure to infections in the coinfection group?

In support of the first option, antibody production requires the aid of classical Th2-like cytokines such as IL-4 (Kotowicz and Callard, 1993; Troye-Blomberg et al., 1990). The fact that both types of

parasites are able to induce Th2 responses, at least at some point during the course of infection, could favor higher levels of antibodies. However, whether these antibodies are protective or not needs to be evaluated in longitudinal studies and by looking at the subclass composition of the IgG responses. A Th2 milieu could favor the production of antibodies, but without the appropriate Th1 balance, production would be expected to be skewed towards the non-cytophilic IgG2 and IgG4 (Druilhe et al., 2005) and IgE, which are suggested to increase the risk to *P. falciparum* infection or severe disease (Bouharoun-Tayoun and Druilhe, 1992; Perlmann et al., 1994). In fact, low levels of IL-4 are required for induction of IgG1, IgG2 and IgG3. In contrast, high levels of IL-4 had no effects or even suppressed those IgG subclasses while IgE and IgG4 were enhanced (Kotowicz and Callard, 1993). This hypothesis, however, is not supported by data of increase in *P. falciparum*-specific IgG1 and IgG3 during coinfection with *S. haematobium* (Diallo et al., 2010). Similarly, *P. falciparum* coinfection with hookworm increased IgG1 and IgG3 levels to the GMZ2 vaccine and related antigens, while deworming treatment reduced these IgG1 and IgG3 levels (Amoani et al., 2021). On the other hand, following the synergy hypothesis, our results could be beneficial for helminth immunity assuming that IgE has a protective effect. However, in our study, total IgE levels had a significant positive association with hookworm burden and with all antigen-specific IgG levels, indirectly suggesting total IgE as a marker of exposure/infection rather than protection. Other studies have also reported a positive correlation between parasite burden or helminth-specific IgG levels and total IgE levels (Cooper et al., 2008; Mangano et al., 2020; Mulu et al., 2014).

In addition to higher antibody levels, coexposed/coinfected individuals had higher magnitude and breadth of response to *P. falciparum* and helminth antigens compared to single exposure/infection groups. A possible explanation is the ability of *P. falciparum* and helminths to induce polyclonal B cell responses (Donati et al., 2004; Harris and Gause, 2011), which is particularly evident for IgE during helminth infections (Maizels and Yazdanbakhsh, 2003). This phenomenon consists in the induction and differentiation of antibody-secreting cells from different B cell clones, regardless of their antigen specificity. While this may play an important role in defense against infections by enhancing natural antibody production, it could also be a strategy to avoid host-specific immune

317 responses by “diluting” antibodies against important epitopes for protection (Montes et al., 2007).
318 Either way, bystander antigens in coinfections could be affected by this spillover effect (Maizels and
319 Yazdanbakhsh, 2003).

320 Nonetheless, our results point towards another more plausible option, which is that antibody
321 responses are a consequence of an increased exposure due to higher susceptibility to infection in
322 the coexposure/coinfection group. Firstly, antibody levels were positively associated with parasite
323 burden, indicating that they reflect exposure rather than protection. These antibody responses that
324 reflect exposure were increased in the coexposure/coinfection compared to single
325 exposure/infection groups. We checked whether differences in the proportion of current infections
326 between groups could be the cause due to boosting of the immune response, but there were no
327 differences between groups except for *T. trichiura*. In fact, the proportion of *T. trichiura* current
328 infection was higher in the helminth single exposure/infection group, but even so *T. trichiura* specific
329 IgG levels were higher in the coexposure/coinfection group.

330 Secondly, there was a trend for higher odds of *P. falciparum* or helminths infection if coinfected or
331 exposed to the other type of parasite. In fact, when using the magnitude of response to *P.*
332 *falciparum* or helminth antigens as a surrogate of exposure, we found that *P. falciparum*
333 exposed/infected individuals had a significantly higher exposure to helminths and *vice versa*. In line
334 with this, others were able to confirm that infection with helminths increases the odds of *P.*
335 *falciparum* infection (Afolabi et al., 2021; Babamale et al., 2018; Degarege et al., 2016; Nacher et
336 al., 2002b). This is consistent with our finding that exposure/infection to an increasing number of
337 helminth species augmented the odds of being infected or exposed to *P. falciparum*. Several
338 studies have revealed similar results in terms of higher *P. falciparum* infection risk or density with
339 increasing number of helminth species (Babamale et al., 2018; Degarege et al., 2012, 2009; Nacher
340 et al., 2002b). In relation to this, one study indicated that helminth polyparasitism is more important
341 than the effects of individual species when driving immunoregulatory effects (Turner et al., 2008).
342 This supports the idea of higher susceptibility to *P. falciparum* infection, and in fact to any infection,
343 with increasing number of helminths and aligns with our observation of higher proportion of

exposure/infection with multiple helminth species in the coexposure/coinfection group compared to the only helminths group. This observation is equally consistent with the concept of hyper-susceptible hosts, who are individuals infected to a greater degree as a result of exposure to pathogens or underlying conditions.

Thirdly, there was a trend for higher *P. falciparum* burden in the coexposure/coinfection group compared to the *P. falciparum* single exposure/infection group in children, which is consistent with other reports for *P. falciparum* (Degarege et al., 2016) and *P. vivax* (Easton et al., 2020), but contrary to others (Amoani et al., 2019; Tokplonou et al., 2020). The only significant association for helminths with regards to parasite burden was for *T. trichiura*, where having been exposed to or currently infected with *P. falciparum* seemed to have a protective effect.

Therefore, if higher antibody levels simply reflect higher exposure, is there any underlying factor that renders these individuals in the coexposure/coinfection group more prone to multiple infections? It has been suggested that factors favoring coinfections may be common socioeconomic or environmental factors rather than true immunological interactions (Brooker et al., 2007; Mwangi et al., 2006). One of the strengths of this study is that we accounted for socioeconomical and water and sanitation factors, besides demographic variables, that could influence the exposure to the parasites. Among all variables, age had the strongest association with antibody levels. We clearly showed that the acquisition of immunity is similar across all antibody responses, with levels rising during childhood and stabilizing during adulthood. This is consistent with a cumulative exposure over time, which resulted in a clear age bias whereby most of the adults were in the coexposure/coinfection group. A study that detected IgG against some of the helminth antigens measured in this study (NIE, Sm25) and other *P. falciparum* antigens also observed similar age trends in Kenya (Njenga et al., 2020). Socioeconomic and water and sanitation metrics had the strongest associations with total IgE, with higher SES and improved water and sanitation conditions showing the lowest IgE levels. This is consistent with a higher exposure to helminths, potent inducers of IgE, under poor sanitation and hygiene conditions, considering that transmission of STH and *Schistosoma* spp. relies on fecal spread of eggs and larvae. It is noteworthy that also specific

IgG levels against some *P. falciparum* and helminth antigens were affected by these variables, suggesting shared transmission risks, such as fecal contaminated water in the household surroundings that can serve as a reservoir of helminth eggs and mosquito larvae. However, only age was significantly higher in the coexposure/coinfection group compared to the rest. Therefore, immunological permissiveness seems to be a likely cause for the observed increase in antibody responses. Nonetheless, we cannot rule out other underlying conditions, such as malnutrition, that could make this group of individuals more susceptible to infections.

Total IgE levels were, as expected, associated with locations with high prevalence of helminths but surprisingly also with locations with high prevalence of *P. falciparum*. However, this could be because the location with the highest percentage of helminth infected individuals also had a high percentage of *P. falciparum* infected individuals. Nonetheless, it is remarkable that in the absence of infection and exposure to helminths, *P. falciparum* seems to be an inducer of total IgE. Additionally, in the coexposure/coinfection group, total IgE levels were also higher than in the helminth single exposure/infection group. This suggests that *P. falciparum* also contributes to these IgE levels, since exposure to or coinfection with *P. falciparum* does not seem to increase helminth burden. Interestingly, another study has also found elevated total IgE levels in malaria patients regardless of helminth coinfection (Mulu et al., 2014). IgE is a neglected isotype in the field of malaria, with limited studies focusing on it. However, there is evidence that *P. falciparum* indeed induces IgE but the role it plays in protection or susceptibility remains to be elucidated. Some have argued it has a protective role (Bereczky et al., 2004; Duarte et al., 2012, 2007; Farouk et al., 2005) while others indicate that it is involved in malaria pathogenesis (Calissano et al., 2003; Maeno et al., 2000; Perlmann et al., 1994, 2000, 1997; Seka-Seka et al., 2004; Tangteerawatana et al., 2007). Total IgE seems to be a good proxy of a Th2 polarization and, due to its short half-life when free in serum, it also serves as a proxy of recent infection with Th2 inducers such as helminths (Geha, 1984). In our linear models, IgE was positively associated with *P. falciparum*-specific IgG levels that have been shown to reflect exposure rather than protection. This indirectly supports the idea of a non-protective role of IgE in terms of *P. falciparum* infection, as it would be associated with increased exposure. However, we

could not asses its implication in clinical outcomes or associations for *P. falciparum* antigen-specific IgE, which might be different than those of total IgE (Duarte et al., 2007) probably due to the polyclonal, non-specific nature of total IgE responses (Wang et al., 2011). A possible explanation for high IgE levels in malaria comes from the observation that establishment of antigen-specific IgE responses during the early years of life primes the immune system to produce IgE responses upon antigen contact without the need of cytokines controlling class switch to IgE (Kotowicz and Callard, 1993). This might be the case of people in the coexposure/coinfection group infected with *P. falciparum* and previously primed into a predominant Th2 profile by helminths.

A caveat of this study is the inability to determine causality due to its cross-sectional nature. A longitudinal design, on the other hand, would be more appropriate to determine whether people with *P. falciparum* or helminths are more susceptible to subsequent infections. Furthermore, this study only included asymptomatic malaria cases, which may be hindering the detection of important associations with disease severity and masking the true burden of coinfections. Nonetheless, even with only asymptomatic cases, we were able to identify significant associations that suggest a higher susceptibility to infection. Therefore, we expect that the inclusion of symptomatic cases would only accentuate these associations. An additional limitation is the pooling of all helminth species together due to small sample size of the groups at species level, possibly resulting in masked effects of individual species (Abbate et al., 2018).

In conclusion, this study shows a significant increase in antibody responses in individuals coexposed to or coinfected with *P. falciparum* and helminths in comparison with individuals infected and/or exposed to only one of these parasites. Although we cannot rule out a possible synergistic effect of these parasites at the immunological level, our results suggest that the increase in the antibody responses is due to a more permissive immune environment to infection in the host. Additional studies are needed for a better understanding of the mechanisms involved during coinfections at the immunological level, but also that lead to coinfections in first place. We also highlight the importance of taking previous exposure into account, especially in endemic areas where continuous infections imprint and shape the immune system. For future studies, we suggest

425 including the measurement of antigen-specific IgE and IgG subclasses, cytokines and cell
426 populations. Deciphering the implications of coinfections deserves attention since accounting for the
427 real interactions that occur in nature could improve the design of integrated disease control
428 strategies.

429 **MATERIALS AND METHODS**

430 **Study design**

431 This immunological analysis was done in the context of a community-based, case-cohort study
432 (MARS) that recruited 819 individuals. MARS aimed to design and implement a surveillance
433 platform to identify and characterize genotypically and phenotypically anthelminthic resistance in
434 Manhiça district, Maputo province, Southern Mozambique. MARS included inhabitants aged 5 years
435 or above, censed in the Manhiça Health Research Centre (CISM) Demographic Surveillance
436 System, who had not taken anthelmintics at any time during the previous 30 days. The locations
437 included were: 3 Fevereiro, Calanga, Ilha Josina, Maluana, Manhiça-sede and Xinavane. The study
438 protocol was approved by the National Bioethics Committee for Health in Mozambique with the
439 approval code number Ref.:517/CNBS/17. An informed consent from individuals who were willing to
440 participate was obtained and, in the case of children, from their parents or guardians. Additionally,
441 participants aged 15 to 17 also gave inform assent.

442 The final sample size for this analysis was 715. One hundred and three samples were excluded
443 from the analysis because they were either not shipped to Barcelona (Spain) (N= 5) or due to study
444 withdrawal (N= 25), sample insufficiency (N= 4) or incorrect serum elution (N= 69).

445 **Detection and quantification of *P. falciparum* and helminth infections**

446 Stool, urine, and blood samples were collected from participants for laboratory analyses.
447 Microscopic diagnosis of *N. americanus*, *A. duodenale*, *T. trichiura, A. lumbricoides* and *S. mansoni*
448 was performed at the CISM by search of eggs in two stool samples using duplicate Kato-Katz thick
449 smear technique. *S. stercoralis* was detected in two stool samples using the Telemann technique.

S. haematobium infection was detected by urine filtration. In addition, molecular diagnosis of STH infections and schistosomiasis was performed at the Leiden University Medical Center (The Netherlands). In brief, the presence of parasite specific DNA was determined in one stool sample per participant by multiplex semi-quantitative real-time Polymerase Chain Reactions (qPCR), using sequences of primers and probes and a general PCR set-up as described before (Kaisar et al., 2017). Individuals were considered to be positive for helminth infection if they had a positive result in at least one of the techniques (microscopy or qPCR) and in at least one of the stool samples.

Capillary blood samples were collected through finger pricking on Whatman 903 filter paper to obtain dried blood spots (DBS). *P. falciparum* infection was determined at the Barcelona Institute for Global Health (ISGlobal) by 18S ribosomal RNA gene detection through qPCR from DBS samples. Briefly, six DBS punches of 3 mm in diameter were used for DNA extraction by the Chelex method (Taylor et al., 2014) along with negative controls (NC) consisting of DBS from non-infected erythrocytes (Banc de Sang i Teixits [BST], Barcelona, Spain) and positive controls (PC) of DBS with *P. falciparum* (3D7 isolate) ring-infected red blood cells diluted in whole blood. Purified DNA templates were amplified following a previously described method (Mayor et al., 2009). A NC with ddH2O instead of DNA template as well as the punch controls were included in each plate. Each specimen was run in duplicate and the standard curve in triplicate for each plate. Parasite density was quantified by Ct interpolation with a standard curve ranging from 18,000 to 1.8 parasites/µL.

Measurement of antibody responses

Antigen panel: A total of 27 antigens, 16 from *P. falciparum* (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1 FVO, EBA175 reg2 PfF2, MSP1 block 2 MAD20, $MSP1_{42}$ 3D7, MSP2 full length CH150, MSP3 3D7, MSP5, P41, Rh1, Rh5 and PTRAMP) and 11 from helminths (STH [NaGST1, NaAPR1, NaSAA2, AyCp2, TmWAP, Tm16, As16, As37, NIE] and *Schistosoma* spp. [MEA, Sm25]), were selected based on their important role as vaccine candidates and markers of exposure (**Table S6**). They are representative of the different stages of *P. falciparum* lifecycle and of the most common

intestinal helminth infections. In addition, glutathione S-transferase (GST) was added to the panel as control for unspecific binding to this tag fused to MSP1 and MSP2 antigens.

As16, As37, AyCp2, NaAPR1, NaGST1, NaSAA2, Tm16 and TmWAP were expressed and purified from yeast *Pichia pastoris* X-33 culture at Baylor University (USA) (**Table S6**). NIE was kindly provided by Thomas Nutman (National Institute of Health and National Institute of Allergy and Infectious Diseases, USA) and Sukwan Handali (Centers for Disease Control and Prevention, USA); MEA and Sm25 were purchased from MyBioSource (USA); α-gal from Dextra Laboratories (UK); and CelTOS, SSP2, LSA1 and EXP1 from Sanaria (USA). AMA1 FVO, $MSP1_{42}$ 3D7 and GST were produced at Walter Reed Army Institute of Research (USA); MSP5 at Monash University (Australia); MSP1 block 2 MAD20 and MSP2 full length CH150 at University of Edinburgh (UK) and EBA175 R2 F2, Rh1, Rh5, PTRAMP and P41 at International Centre for Genetic Engineering and Biotechnology (India).

Serum elution from DBS: Antibodies were obtained from DBS samples through an adaptation of a described method (Fonseca et al., 2017). Briefly, serum was eluted from 4 DBS punches, collected as described before, and incubated with 100µl of PBS-BN [filtrated PBS with 1% BSA and 0.05% sodium azide (ref. S8032, MilliporeSigma, St. Louis, USA)] and 0.05% Tween20 (ref. P1379, MilliporeSigma, St. Louis, USA) at 4ºC overnight (ON) with gentle mixing (~600 rpm). Next day, the tubes were centrifuged at 10,000 rpm for 10 min and the supernatant containing the eluted serum was transferred into a new tube, ready for serological measurement. Assuming a haematocrit of 50%, the eluted blood proteins concentration resulted equivalent to a 1:25 plasma dilution. Samples with reddish-brown spots against a pale background were discarded due to incorrect elution. To assure the validity of the punch and the serum elution procedure, a NC of filter paper without DBS and three elution control (EC) samples with known antibody levels (volunteer donors) were included in each elution batch.

Luminex: Quantitative suspension array technology (qSAT) assays were used to measure *P. falciparum* and helminth specific-IgG and total IgE as described previously (Vidal et al., 2018).

Briefly, the antigens and a capture anti-human IgE (Mouse anti-Human IgE Fc) (ref. ab99834, Abcam PLC, Cambridge, UK) were coupled to magnetic MagPlex® 6.5µm COOH-microspheres (Luminex Corporation, Austin, USA) at the optimal coupling conditions (**Table S7**). Coupled beads were validated (in multiplex in the case of antigen-specific IgG) by measuring antigen-specific IgG and total IgE in serial dilutions of a PC. The antigen-coupled beads were used in multiplex at 1,000 beads per region per sample, while the anti-human IgE-coupled beads were used in singleplex at 3,000 beads per sample.

Coupled beads were incubated with eluted serum samples, eluted controls, a PC curve, and blanks in 96-well µClear® flat bottom plates (ref. 655096, Greiner Bio-One, Frickenhausen, Germany) at 4ºC ON in a shaker at 600 rpm. For the antigen-specific IgG quantification we assayed the samples at 1:100 and 1:2000 dilutions, and included a WHO malaria pool was used as PC [First WHO Reference Reagent for Anti-malaria (*P. falciparum*) human serum, NIBSC code: 10/198 (NIBSC, Herts, UK)] at 12 serial dilutions (3-fold) starting at 1:100. For total IgE quantification we assayed the samples at 1:50 dilution and, included a WHO IgE PC [Third WHO International Standard Immunoglobulin E, human serum, NIBSC code: 11/234 (NIBSC, Herts, UK)] at 12 serial dilutions (2-fold) starting at 1/50. After the incubation of coupled beads with samples, beads were washed three times with PBS + 0.05% Tween20, using a manual magnetic washer platform (ref. 40-285, Bio-Rad, Hercules, USA), and the biotinylated secondary anti-human IgG (ref. B1140-1ML, MilliporeSigma, St. Louis, USA)] and the anti-human IgE (ref. A18803, Invitrogen, Waltham, USA) antibodies were added at 1:1250 and 1:100 dilutions, respectively, and incubated for 45 min at RT shaking at 600 rpm. Then, beads were washed three times and streptavidin-R-phycoerythrin (ref. 42250-1ML, MilliporeSigma, St. Louis, USA) was added at 1:1000 dilution and incubated at RT for 30 min at 600 rpm. Finally, beads were washed, resuspended, and at least 50 beads per analyte and sample were acquired in a FlexMap 3D xMAP® instrument (Luminex Corporation, Austin, USA). Crude median fluorescent intensities (MFI) and background fluorescence from blank wells were exported using the xPONENT software v.4.3 (Luminex Corporation, Austin, USA).

Determination of seropositivity

Gaussian Mixture Models were used to estimate a seropositivity threshold for each antigen-specific IgG and total IgE response and classify the samples into positive and negative by serology. The models were estimated by the Expectation-Maximization (EM) algorithm. For each antigen, two models were fitted assuming either equal or unequal variances, and each of those two models were built with two clusters for classification. The two clusters of classification are meant to represent the distribution of seropositive and seronegative samples. For each antigen, classification of samples was done using the model showing the optimal Bayesian Information Criterion (BIC). Briefly, the EM algorithm is an unsupervised clustering algorithm that works in two steps. The first step is the Expectation step, where the data are assigned to the closest centroid of the two clusters. The second step is the Maximization step, where the centroids of the clusters are recalculated with the newly assigned data. These two steps are repeated until the model converges. The models were fitted using the R CRAN package mclust (Scrucca et al., 2016).

Definition of study groups

Study participants were classified into study groups based on diagnosis of current infection (defined by microscopy and/or qPCR) and prior exposure (defined by seropositivity to any of the antigens for each type of parasite). The resulting groups were: a) no exposure/infection (MAL- HEL-), which included people that have not been exposed to or currently infected with *P. falciparum* nor helminths; b) single exposure/infections (MAL- HEL+ or MAL+ HEL-), which included people that have been exposed to and/or currently infected with only one type of parasite; and c) coexposure/coinfection (MAL+ HEL+), which included people that have been exposed to and/or currently infected with both types of parasites.

Definition of study variables

In this study we included demographic variables such as sex, age, age group (children [5-15 years old] or adults [≥15 years old]) and percentage of infection by location. The latter was calculated based on the *P. falciparum* or helminth percentage of infection in each location of the study. Then,

participants were classified into *P. falciparum* or helminth "high" and "low" groups of locations defined according to higher or lower percentage of *P. falciparum* or helminths compared to the overall median by type of parasite (**Table S4**). Additionally, we also included socioeconomic (socioeconomic score), water (accessibility to piped water, payment for water) and sanitation (ownership of latrine) variables (Grau-Pujol et al., 2021, in preparation). Finally, we calculated the magnitude and breadth of response to *P. falciparum* and helminth antigens as previously described (Tran et al., 2019). The magnitude of response corresponds to the sum of all specific IgG levels (MFI) to the different antigens belonging to *P. falciparum* or helminths, and the breadth of response is defined as the number of antigens with seropositive responses.

Statistical analysis

To determine the seropositivity and perform the statistical analysis, normalized and dilution corrected data (see Supplementary Material) were $\log_{10}$-transformed. The Shapiro-Wilk test was used to check the normality of the data and since most of the data did not follow a normal distribution, non-parametric tests were used. Then, we explored differences in the distribution of study variables across study groups. For continuous variables, the median and first and third quartiles were calculated and differences in the median between more than two groups were assessed by Kruskall-Wallis test followed by pair-wise comparisons by Wilcoxon rank sum test and Benjamini-Hochberg (BH) adjustment for multiple testing. For categorical variables, differences in proportions between groups were calculated by Chi-square test or Fisher's exact test when applicable. This was automatically determined by the R CRAN package compareGroups (Subirana et al., 2014). For comparisons between two groups we applied the Wilcoxon rank sum test and, in the case of antigen-specific IgG responses, p-values were adjusted for multiple testing by the BH. Correlations between variables were assessed with the Spearman's rank correlation coefficient ρ (rho). For Spearman's test, p-values were computed via the asymptotic *t* approximation.

For regression models, assumptions on the data were checked by exploratory plots. For linear regression models, we checked linearity of the data, normality of residuals, homogeneity of

residuals variance and independence of residuals error terms. For logistic regression models, we checked the linearity between the logit of the outcome and each predictor variable, the presence of influential values and multicollinearity among the predictor variables. All the assumptions were met without the presence of extreme patterns, except for normality of the residuals in most cases.

The first linear regression models were performed to assess the association between antibody responses (including antigen-specific IgG levels, total IgE levels, magnitude of response and breadth of response) as outcome variables and sex, $\log_{10}$ age, percentage of infected individuals by location, socioeconomic score, accessibility to piped water, payment for water and ownership of latrine in univariable models. The second linear regression models were multivariable models fitted to assess the association between antibody responses as outcome variables and three predictor variables: a) the exposure/infection status as categorical variable (coexposed/coinfected or single exposed/infected), b) $\log_{10}$ total IgE levels as continuous variable or c) the magnitude of response to *P. falciparum* or helminth antigens as continuous variable. We evaluated the association of these three predictor variables independently by building three different base models. To select the adjusting covariables, we first selected those variables significantly associated with each block of antigens by univariable models. Then, we explored all possible combinations of the predictor covariables with the base models and chose per block of antigens those combinations that provided the simplest models (based on BIC) without a significant compromise of the Akaike information criterion (AIC) and adjusted r-square. The third linear regression models explored the association between *P. falciparum* exposure/infection (predictor variable) and the number of helminth species (outcome variable) adjusted by the corresponding covariables selected as explained above. The fourth linear regression models were performed to assess the association between parasite burden as outcome variable and antibody responses as predictor variables adjusted by age. We adjusted for multiple comparisons by BH, where the different antibody responses penalized.

The logistic regression models evaluated the association of exposure/infection with either *P. falciparum* or helminths (predictor variable) with being exposed/infected with the counterpart parasite (outcome variable). For *P. falciparum*, we also evaluated by logistic regression the

association of the number of helminth species (predictor variable) with exposure/infection with *P. falciparum* (outcome variable). The selection of adjusting covariables was done based on significant associations by univariable models.

The resulting betas and 95% confidence intervals (CI) obtained in each linear regression model were transformed when appropriate for interpretation if the outcome (log-linear model), predictor (linear-log model), or both (log-log model) variables were $\log_{10}$-transormed. For log-log models, the transformed values (%) were calculated with the formula ((10^(beta*$\log_{10}$(1.1)))-1)*100. This represents the effect (in percentage) on the outcome variable of a 10% increase in the corresponding predictor variable. For log-linear models, the transformed values (%) were calculated with the formula ((10^beta)-1)*100. This represents the effect (in percentage) on the outcome variable of an increase in one unit of the predictor variable, or with respect to the reference category in cases of categorical variables. Finally, for linear-log models, the transformed value was calculated with the formula beta*$\log_{10}$(1.1). This represents the additive effect on the outcome variable of a 10% increase in the corresponding predictor variable.

Adjusted and raw (if not adjusted for multiple testing) p-values of ≤0.05 were considered statistically significant. All data processing and statistical analyses were performed using the statistical software R version 4.0.3 and R Studio Version 1.1.463. Other R CRAN packages not mentioned above but used to manage data, generate tables and plots were tidyverse (Wickham et al., 2019), ggiraphExtra (Moon, 2020), ggpubr (Kassambara, 2020), ggbeeswarm (Clarke and Sherrill-Mix, 2017).

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636 **COMPETING INTERESTS**

637 All authors listed in the manuscript certify that they have no financial or non-financial competing
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Table 1. Demographic, socioeconomic, water and sanitation characteristics of the study population by exposure/infection group.

	MAL- HEL- (N= 21, 2.94%)	MAL- HEL+ (N= 43, 6.01%)	MAL+ HEL- (N= 67, 9.37%)	MAL+ HEL+ (N= 584, 82.68%)	p-value	Groups with significant differences
Sex						
Female (N= 404)	9 (42.9%)	23 (53.5%)	40 (59.7%)	332 (56.8%)	0.561	None
Male (N= 311)	12 (57.1%)	20 (46.5%)	27 (40.3%)	252 (43.2%)		
Age (years)	7.7 [6.0;11.5]	8.6 [6.7;9.9]	9.4 [6.9;11.8]	23.9 [10.7;50.8]	**≤0.001**	MAL- HEL- vs MAL+ HEL+, MAL- HEL+ vs MAL+ HEL+, MAL+ HEL- vs MAL+ HEL+
Age group						
Children (N= 363)	21 (100.0%)	42 (97.7%)	62 (92.5%)	238 (40.8%)	**≤0.001**	MAL- HEL- vs MAL+ HEL+, MAL- HEL+ vs MAL+ HEL+, MAL+ HEL- vs MAL+ HEL+
Adults (N= 352)	0 (0.0%)	1 (2.3%)	5 (7.5%)	346 (59.2%)		
Malaria % in location						
High (N= 222)	5 (23.8%)	10 (23.3%)	25 (37.3%)	182 (31.2%)	0.397	None
Low (N= 493)	16 (76.2%)	33 (76.7%)	42 (62.7%)	402 (68.8%)		
Helminth % in location						
High (N= 445)	10 (47.6%)	27 (62.8%)	38 (56.7%)	370 (63.4%)	0.377	None
Low (N= 270)	11 (52.4%)	16 (37.2%)	29 (43.3%)	214 (36.6%)		
Socioeconomic score	-0.16 [-0.22;-0.04]	-0.18 [-0.26;-0.08]	-0.14 [-0.26;-0.05]	-0.21 [-0.29;-0.10]	**0.011**	None
Ownership of latrine						
No (N= 328)	10 (47.6%)	18 (41.9%)	23 (34.3%)	277 (47.5%)	0.21	None
Yes (N= 386)	11 (52.4%)	25 (58.1%)	44 (65.7%)	306 (52.5%)		
Piped water access						
Outside (N= 535)	13 (61.9%)	31 (72.1%)	47 (70.1%)	444 (76.2%)	0.339	None
Inside (N= 179)	8 (38.1%)	12 (27.9%)	20 (29.9%)	139 (23.8%)		
Payment for water						
No (N= 472)	12 (57.1%)	29 (67.4%)	43 (64.2%)	388 (66.6%)	0.813	None
Yes (N= 242)	9 (42.9%)	14 (32.6%)	24 (35.8%)	195 (33.4%)		

Comparisons were made between study groups: no exposure/infection (MAL- HEL-), only exposure/infection with helminths (MAL- HEL+), only exposure/infection with *P. falciparum* (MAL+ HEL-) or coexposure/coinfection with *P. falciparum* and helminths (MAL+ HEL+). Malaria and helminth percentage of infection in each location of the study were calculated and participants were classified into groups of locations with higher or lower percentage of infection compared to the overall median by type of parasite. Total sample size was N= 715, except for the socioeconomic score, ownership of latrine, piped water access and payment for water that was N= 714.

Table 2. Association between exposure/infection with *Plasmodium falciparum* and number of helminth species defined by exposure/infection.

Outcome		Predictor	Estimate	95% CI	p-value
Mutivariable logistic regression					
MAL+ (N)	MAL- (N)	Number of helminth species			
67	21	0			
109	26	1	OR*= 1.607	[1.214 – 2.167]	**≤ 0.001**
144	12	2			
148	4	3			
114	0	4			
69	0	5			
Multivariable linear regression					
Number of helminth species		MAL+	β***= 0.637	[0.316 – 0.958]	**≤ 0.001**

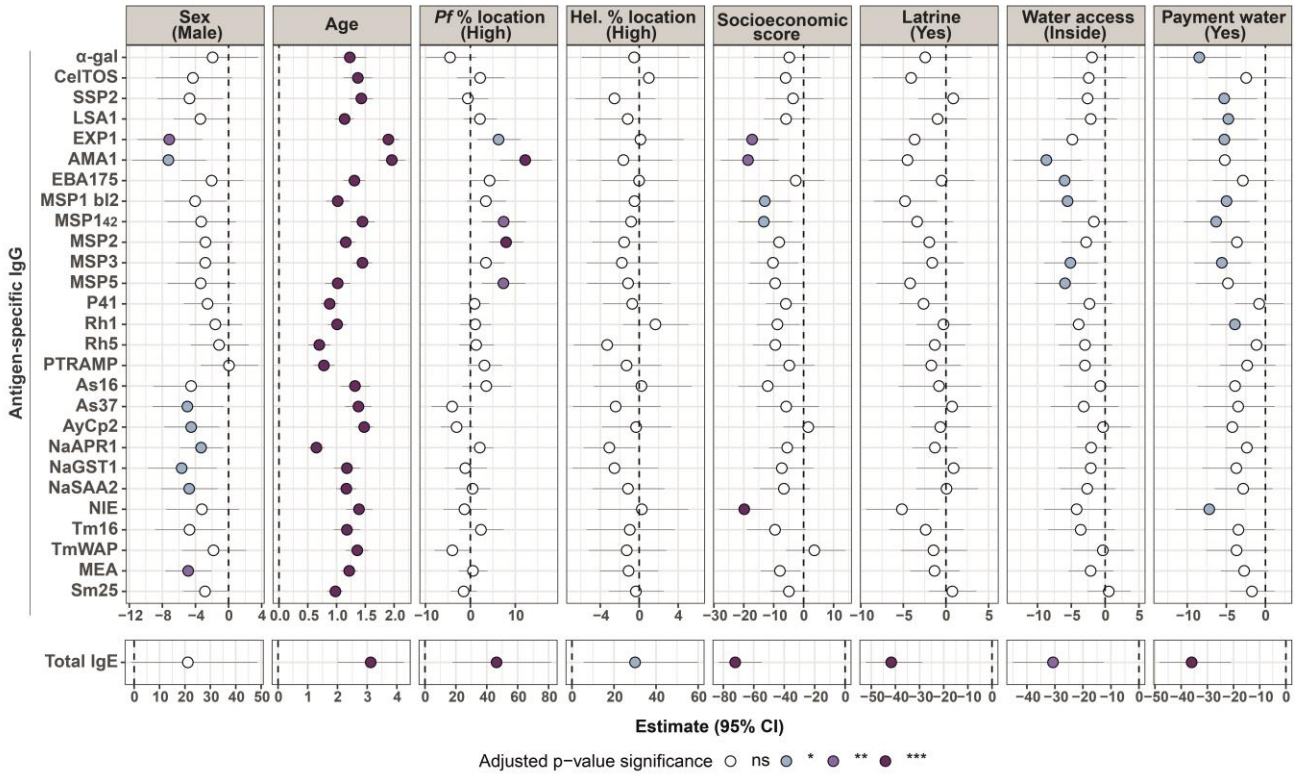
In the linear regression model, the reference category was MAL-.

The models were adjusted by covariables selected based on their significant association with the outcome variable in univariable models:

* $\log_{10}$ age

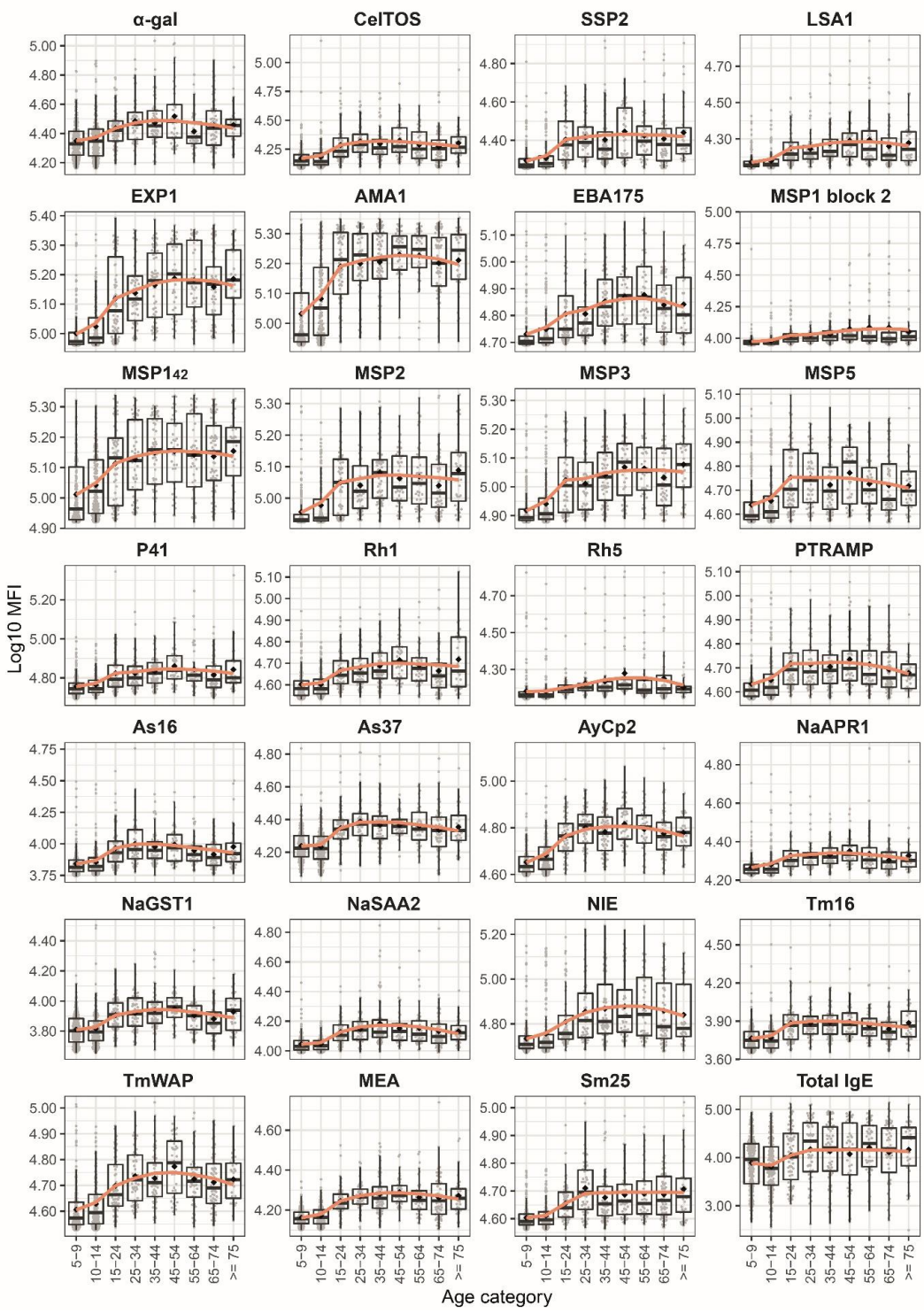
*** $\log_{10}$ age, sex and socioeconomic score

N: number of individuals, OR: odds ratio, CI: Confidence interval, MAL+: *Plasmodium falciparum* positive infection or exposure/infection, MAL-: *P. falciparum* negative infection or exposure/infection.



672

673 **Figure 1. Association of demographic, socioeconomic, water and sanitation metrics with antibody**
674 **levels in univariable linear regression models.** Forest plots show the association of sex (reference:
675 female), age, percentage of *P. falciparum* infected individuals by location (reference: locations with
676 percentage lower than the median of all locations), percentage of helminth infected individuals by location
677 (reference: locations with percentage lower than the median of all locations), socioeconomic score, owning a
678 latrine (reference: no), piped water accessibility (reference: outside the household), and payment for water
679 (reference: no) with specific IgG levels to *P. falciparum* and helminth antigens and total IgE levels. Univariable
680 linear regression models were fitted to calculate the estimates (dots) and 95% confidence intervals (CI)
681 (lines). The represented values are the transformed betas and CI in percentage. The color of the dots
682 represents the p-value significance after adjustment for multiple testing by Benjamini-Hochberg, where ns=
683 not significant, * = p-value ≤ 0.05, ** = p-value ≤ 0.01 and *** = p-value ≤ 0.001. Pf: *P. falciparum*; Hel.:
684 Helminth.



685

686 **Figure 2. Antibody levels by age groups.** Antigen-specific IgG levels against *Plasmodium falciparum* and
687 helminth antigens and total IgE levels are expressed as $\log_{10}$-transformed median fluorescence levels (MFI).
688 Age categories are grouped in 5 years for children and 10 years for adults and the boxplots represent the
689 median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest
690 values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers.
691 The kinetics curves in orange were calculated using the locally estimated scatterplot smoothing (LOESS)

692 method. *P. falciparum* antigens: α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$,
693 MSP2, MSP3, MSP5, P41, Rh5, PTRAMP. Helminth antigens: As16 and As37 (*Ascaris lumbricoides*), AyCp2,
694 NaAPR1, NaGST1, NaSAA2 (Hookworm), NIE (*Strongyloides stercoralis*), Tm16 and TmWAP (*Trichuris*
695 *trichiura*), MEA and Sm25 (*Schistosoma* spp).

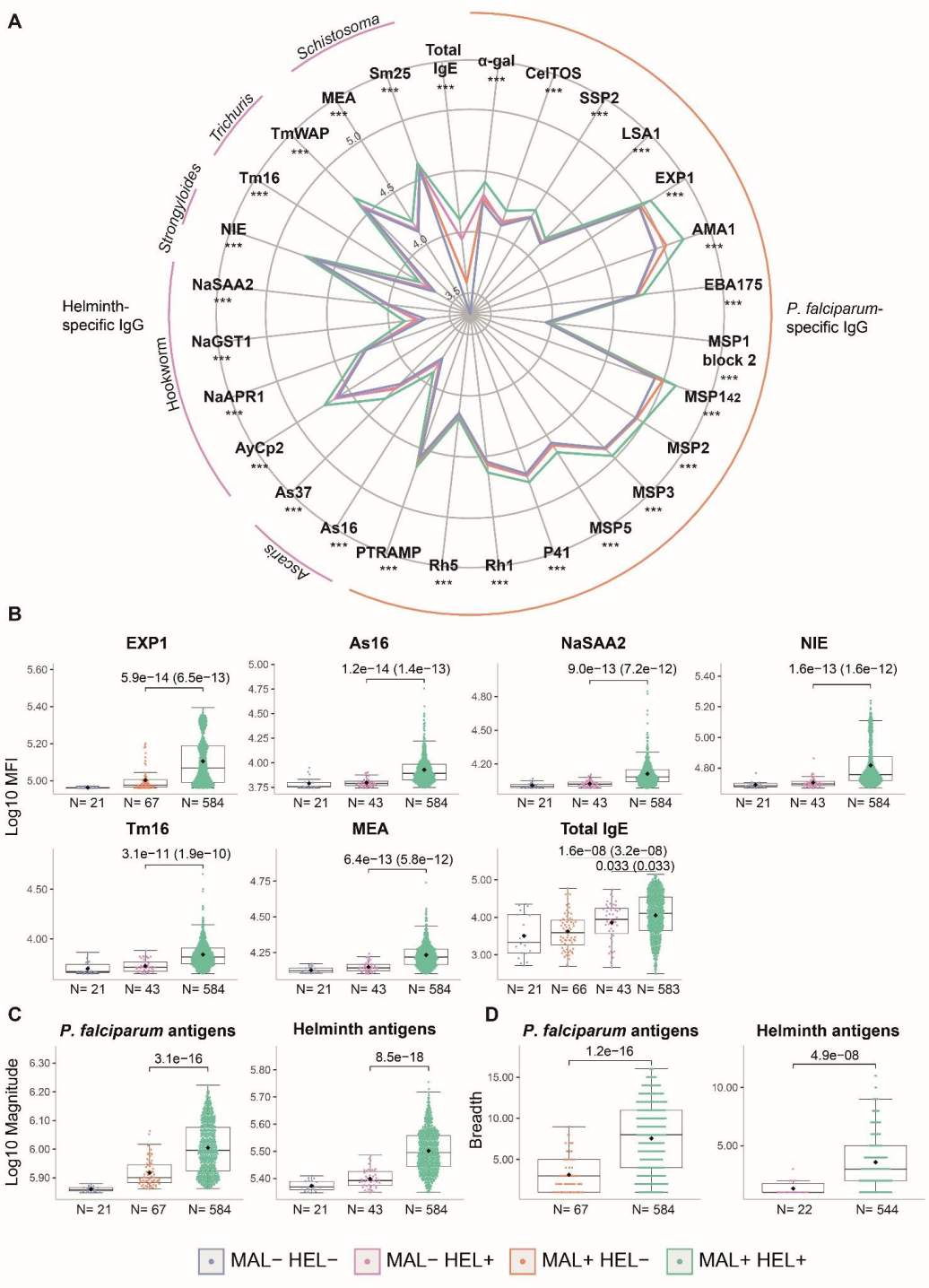


Figure 3. Comparison of antibody responses between exposure/infection groups. (A) Radarplot summarizing the antibody responses per exposure/infection group. In the vertices, the antibody responses are grouped by specific IgG against *Plasmodium falciparum* antigens, helminth antigens or total IgE. The colored lines represent the median of antibody levels expressed as $\log_{10}$-transformed median fluorescence levels (MFI). For *P. falciparum* and helminth antigen-specific IgG, the medians of the MAL+ HEL+ group were compared with the median of the MAL+ HEL- or MAL- HEL+ groups, respectively, by Wilcoxon rank sum test.

For total IgE, the median of the MAL+ HEL+, MAL+ HEL- and MAL- HEL+ groups were compared by Kruskall-Wallis test. Statistically significant differences are indicated with asterisks (*** $p \leq 0.001$). The boxplots compare infection groups based on **(B)** the $\log_{10}$-transformed MFI specific IgG levels against representative antigens per parasite species and total IgE, **(C)** the $\log_{10}$-transformed magnitude of response (sum of MFI), and **(D)** the breadth of response (number of seropositive antigen-specific IgG responses). The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown. Adjusted p-values by the Benjamini-Hochberg approach are shown in brackets. Study groups are shown in colors: no exposure/infection (violet) (MAL- HEL-), only exposure/infection with helminths (pink) (MAL- HEL+), only exposure/infection with *P. falciparum* (orange) (MAL+ HEL-) and coexposure/coinfection with *P. falciparum* and helminths (green) (MAL+ HEL+). The rest of the antigens are showed in Supplementary Figure 10.

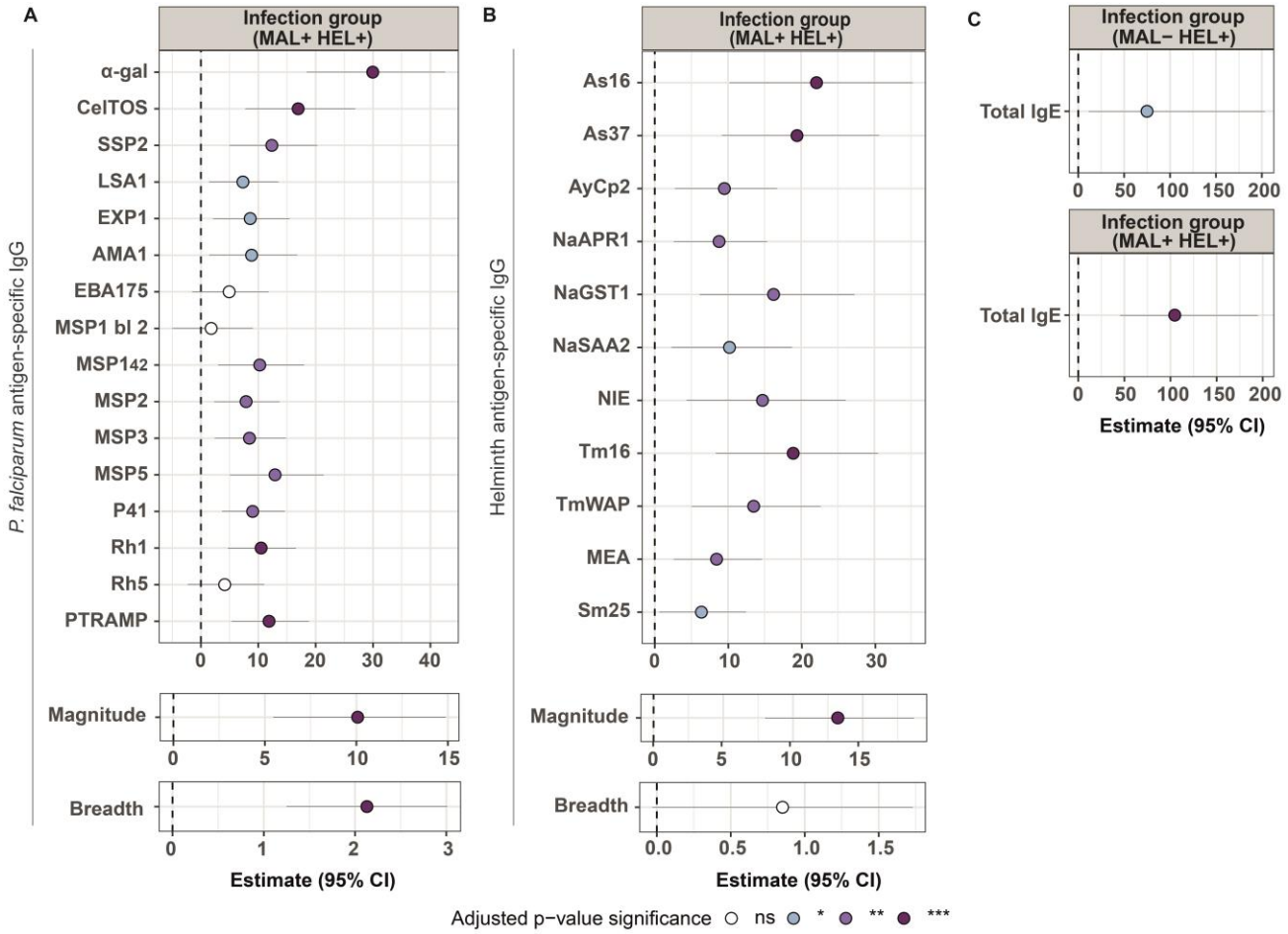


Figure 4. Association of exposure/infection group with antibody levels in multivariable linear regression models. Forest plots show the association of the MAL+ HEL+ group with **(A)** IgG levels to *P. falciparum* antigens, magnitude and breadth of response (reference: MAL+ HEL- group) or **(B)** IgG levels to helminth antigens, magnitude and breadth of response (reference: MAL- HEL+ group). In **(C)**, the association of MAL+ HEL+ group or the MAL- HEL+ group with IgE levels was assessed (reference: MAL+ HEL- group). The magnitude of response was calculated as the sum of all specific IgG levels to the different antigens belonging to *P. falciparum* or helminths. The breadth was defined as the number of seropositive antigen-specific IgG responses. Multivariable linear regression models were fitted to calculate the estimates (dots) and 95% confidence intervals (CI) (lines). The represented values are the transformed betas and CI in percentage for IgG levels to antigens, total IgE and magnitude of response (log-linear models), and the untransformed betas and CI for the breadth of response (linear-linear models). The color of the dots represents the p-value significance after adjustment for multiple testing by Benjamini-Hochberg, where ns= not significant, * = p-value ≤ 0.05, ** = p-value ≤ 0.01 and *** = p-value ≤ 0.001. Models were adjusted by age and percentage of *P. falciparum* infected individuals by location for *P. falciparum* antigens, age for helminth antigens, and sex,

731 percentage of helminth infected individuals by location, ownership of latrine, piped water accessibility,
732 payment for water and socioeconomic score for total IgE. MSP1 bl 2: MSP1 block 2. MAL+ HEL+:
733 coexposure/coinfection with *P. falciparum* and helminths; MAL+ HEL-: only exposure/infection with *P.*
734 *falciparum;* MAL- HEL+: only exposure/infection with helminths.

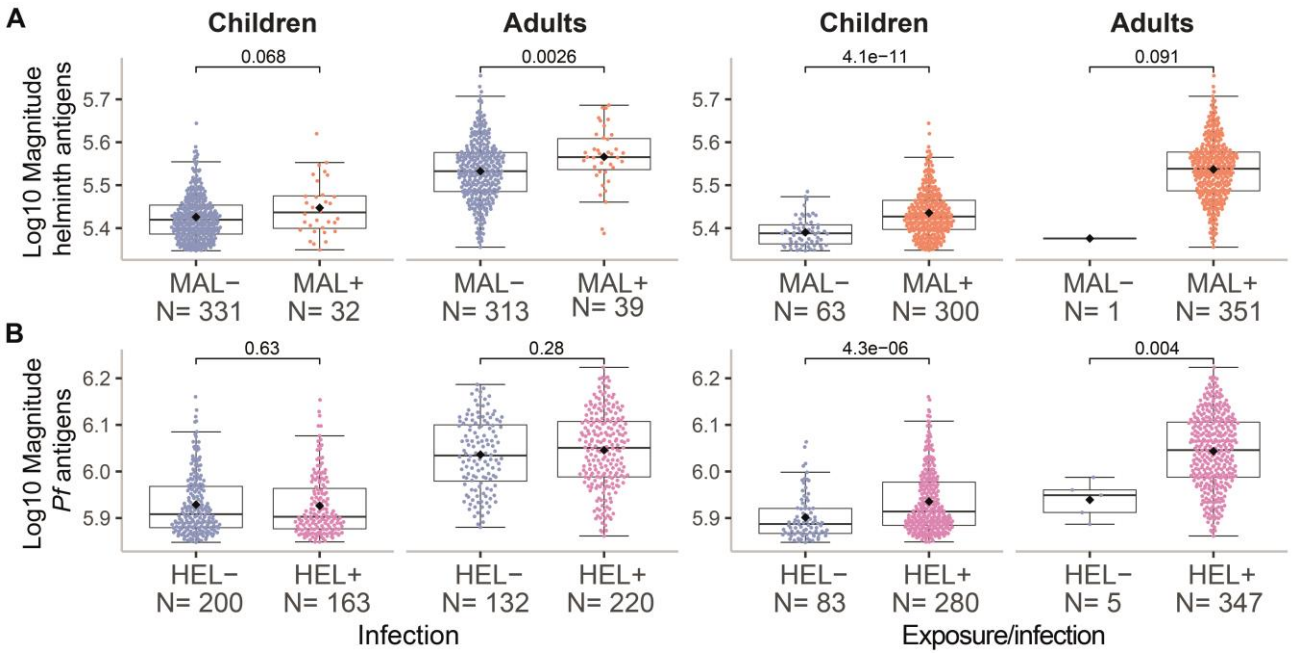


Figure 5. Magnitude of response by infection or exposure/infection to malaria or helminths. The magnitude of response represents the sum of all specific IgG responses against **(A)** helminth or **(B)** *P. falciparum* antigens. The X-axis shows the classification of samples into negative (-) or positive (+) based on either past exposure and/or present infection (detected by serology and molecular and/or microscopic diagnosis) or present infection (detected by molecular and/or microscopic diagnosis) for **(A)** malaria (MAL) or **(B)** helminths (HEL). The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown.

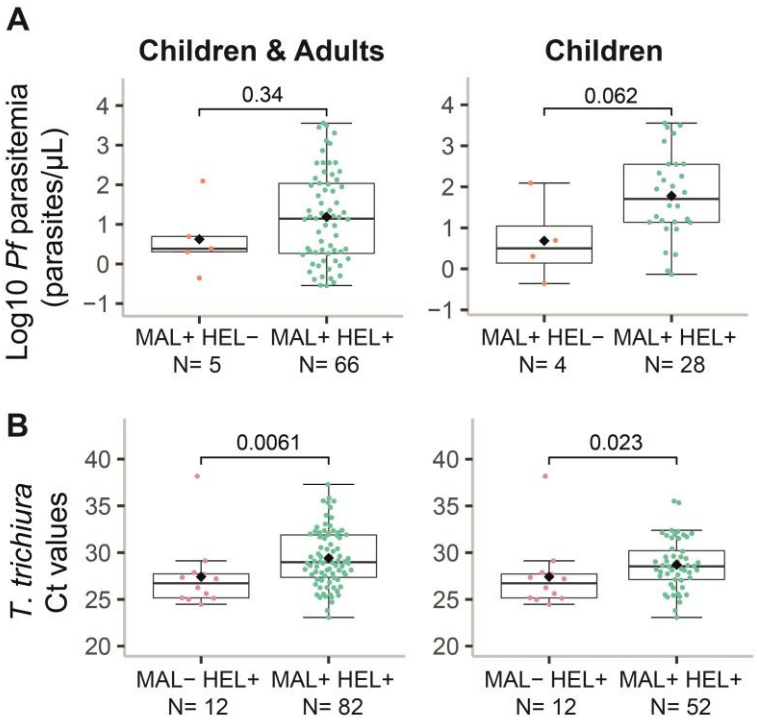


Figure 6. Parasite burden by exposure/infection groups. The levels of parasite burden are represented as **(A)** the $\log_{10}$-transformed parasitemia of *Plasmodium falciparum* (parasites/µL) or **(B)** the *Trichuris trichiura* Ct values, and are compared between the coinfection group (MAL+ HEL+) and the only malaria group (MAL+ HEL-) for *P. falciparum,* or the only helminths group (MAL- HEL+) for *T. trichiura*. For each parasite, data for all individuals with parasite burden information or only children are displayed. The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown. See supplementary Figure S15 for the plots of the rest of parasites.

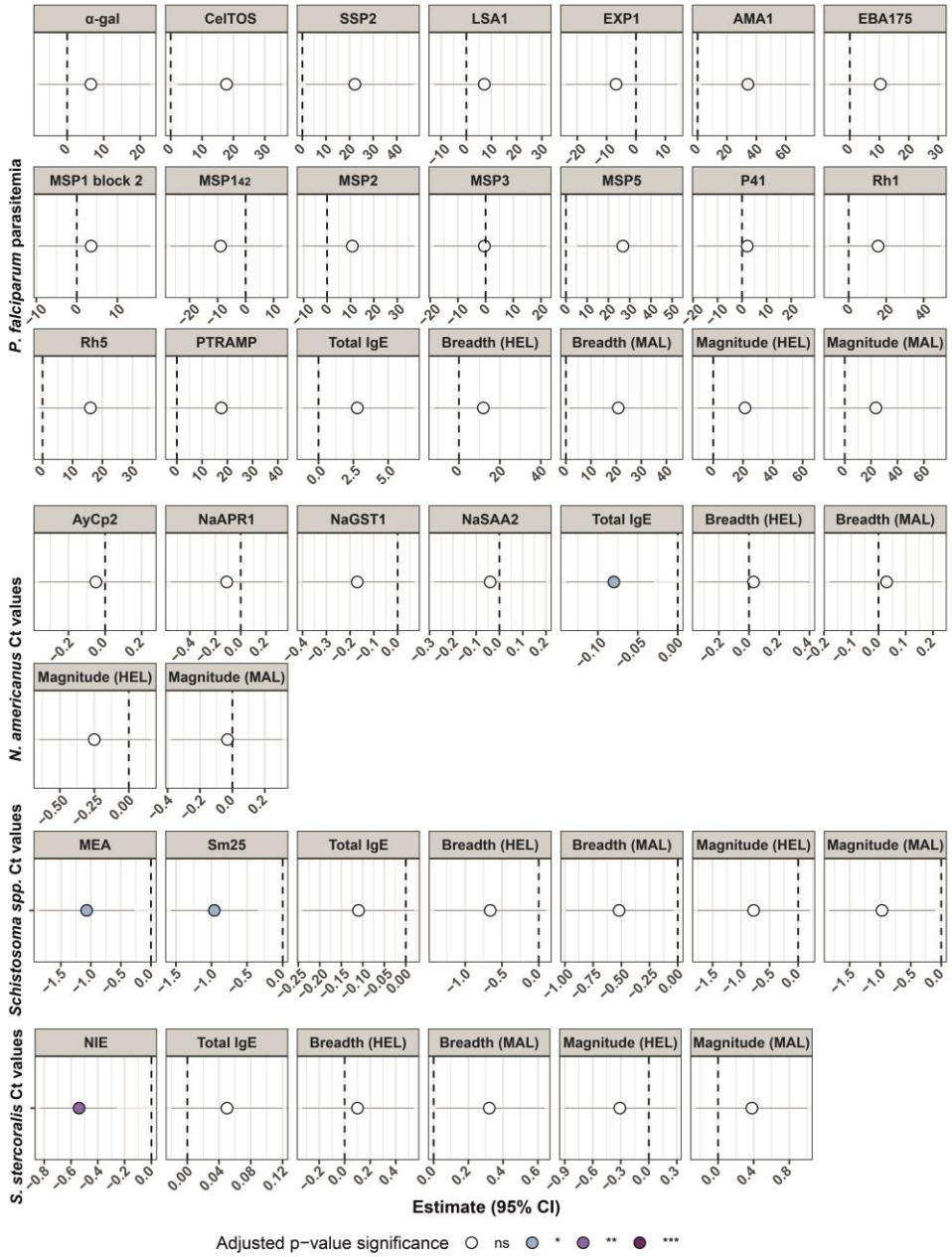


Figure 7. Association of antibody responses with parasite burden in multivariable linear regression models. Forest plots show the association of antibody responses on parasite burden represented as the log_{10}-transformed parasitemia (parasites/μL) of *Plasmodium falciparum* or the Ct values of *Schistosoma* spp., *Necator americanus* and *Strongyloides stercoralis*. For each parasite, linear regression models adjusted by age were fitted, where specific IgG levels against the antigens from the corresponding parasite, total IgE levels and magnitude (sum of antigen-specific IgG levels) and breadth (number of seropositive antigen-specific IgG responses) of response were the predictor variables, and the parasite burden was the outcome variable. The estimates (dots) and 95% confidence intervals (CI) (lines) of the models were transformed into percentages for *P. falciparum* parasitemia models (log-log or log-linear models). For helminths models, when

765 the predictor variables were antibody levels or magnitude of response (linear-log models), the betas and 95%
766 CI were transformed to represent the additive effect on the Ct values of a 10% increase in the predictor
767 variable. For the breadth of response in helminths models (linear-linear models), the untransformed betas
768 were used. The color of the dots represents the adjusted p-value significance, where ns= not significant, * = p-
769 value ≤ 0.05, ** = p-value ≤ 0.01 and *** = p-value ≤ 0.001. See supplementary Figure S16 for *Ascaris*
770 *lumbricoides* and *Trichuris trichiura* models.

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SUPPLEMENTARY TABLES

Table S1. Percentage of exposure, infection or both by parasite species and groups.

	Total	MAL- HEL- (N=21)	MAL- HEL+ (N=43)	MAL+ HEL- (N=67)	MAL+ HEL+ (N=584)	p-value
Present infection						
Hookworm	201 (28.11%)	0 (0.00%)	17 (39.5%)	0 (0.00%)	184 (31.5%)	0.358
T. trichiura	103 (14.41%)	0 (0.00%)	14 (32.6%)	0 (0.00%)	89 (15.2%)	**0.008**
A. lumbricoides	53 (7.41%)	0 (0.00%)	5 (11.6%)	0 (0.00%)	48 (8.22%)	0.399
S. stercoralis	74 (10.35%)	0 (0.00%)	1 (2.33%)	0 (0.00%)	73 (12.5%)	0.158
Schistosoma spp.	86 (12.03%)	0 (0.00%)	1 (2.33%)	0 (0.00%)	85 (14.6%)	0.097
Any helminth	383 (53.57%)	0 (0.00%)	31 (72.1%)	0 (0.00%)	352 (60.3%)	0.170
P. falciparum	71 (9.93%)	0 (0.00%)	0 (0.00%)	5 (7.46%)	66 (11.3%)	0.455
Positive serology						
Hookworm	497 (69.51%)	0 (0.00%)	17 (39.5%)	0 (0.00%)	480 (82.2%)	**≤0.001**
T. trichiura	377 (52.73%)	0 (0.00%)	7 (16.3%)	0 (0.00%)	370 (63.4%)	**≤0.001**
A. lumbricoides	148 (20.7%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	148 (25.3%)	**≤0.001**
S. stercoralis	195 (27.27%)	0 (0.00%)	1 (2.33%)	0 (0.00%)	194 (33.2%)	**≤0.001**
Schistosoma spp.	314 (43.92%)	0 (0.00%)	4 (9.30%)	0 (0.00%)	310 (53.1%)	**≤0.001**
Any helminth	566 (79.16%)	0 (0.00%)	22 (51.2%)	0 (0.00%)	544 (93.2%)	**≤0.001**
P. falciparum	651 (91.05%)	0 (0.00%)	0 (0.00%)	67(100%)	584 (100%)	NA
Exposure/infection						
Hookworm	540 (75.52%)	0 (0.00%)	31 (72.1%)	0 (0.00%)	509 (87.2%)	**0.011**
T. trichiura	426 (59.58%)	0 (0.00%)	20 (46.5%)	0 (0.00%)	406 (69.5%)	**0.003**
A. lumbricoides	190 (26.57%)	0 (0.00%)	5 (11.6%)	0 (0.00%)	185 (31.7%)	**0.012**
S. stercoralis	210 (29.37%)	0 (0.00%)	2 (4.65%)	0 (0.00%)	208 (35.6%)	**≤0.001**
Schistosoma spp.	339 (47.41%)	0 (0.00%)	5 (11.6%)	0 (0.00%)	334 (57.2%)	**≤0.001**
Any helminth	627 (87.69%)	0 (0.00%)	43 (100%)	0 (0.00%)	584 (100%)	NA
P. falciparum	651 (91.05%)	0 (0.00%)	0 (0.00%)	67(100%)	584 (100%)	NA

1043 **Table S2. Percentage of single or multiple helminth species exposure, infection or both by**
1044 **helminth positive groups**

Helminth species	Total	MAL- HEL+	MAL+ HEL+	p-value
Present infection				
Single	271 (70.76%)	25 (80.6%)	246 (69.9%)	0.291
Multiple	112 (29.24%)	6 (19.4%)	106 (30.1%)	
Total	383 (100%)	31 (100%)	352 (100%)	
Positive serology				
Single	129 (22.79%)	16 (72.7%)	113 (20.8%)	**≤0.001**
Multiple	437 (77.21%)	6 (27.3%)	431 (79.2%)	
Total	566 (100%)	22 (100%)	544 (100%)	
Exposure/Infection				
Single	136 (21.69%)	27 (62.8%)	109 (18.7%)	**≤0.001**
Multiple	491 (78.31%)	16 (37.2%)	475 (81.3%)	
Total	627 (100%)	41 (100%)	584 (100%)	

1045

Table S3. Comparison of results by diagnostic methods and serology.

			Serology	
Species	**Diagnostic method**	**Diagnostic method result**	-	+
P. falciparum	**qPCR**	-	64	580
		+	0	71
A. lumbricoides	**Microscopy**	-	541	141
		+	26	7
	qPCR	-	517	126
		+	36	11
	Microscopy and/or PCR	-	525	137
		+	42	11
S. stercoralis	**Microscopy**	-	519	188
		+	1	7
	qPCR	-	489	130
		+	14	57
	Microscopy and/or qPCR	-	505	136
		+	15	59
Hookworm	**Microscopy**	-	196	401
		+	22	96
	qPCR	-	173	333
		+	38	146
	Microscopy and/or PCR	-	175	339
		+	43	158
T. trichiura	**Microscopy**	-	318	342
		+	20	35
	qPCR	-	283	313
		+	45	49
	Microscopy and/or qPCR	-	289	323
		+	49	54
Schistosoma spp.	**Microscopy**	-	393	290
		+	8	24
	qPCR	-	371	248
		+	19	52
	Microscopy and/or qPCR	-	376	253
		+	25	61

Gain in positive results by qPCR and/or microscopy in dark grey. Gain in positive results by serology in light grey.

Table S4. Classification of locations based on percentage of individuals infected with *Plasmodium falciparum* or helminths.

Location	MAL-	MAL+	Total	MAL + (%)	Median % of infection	Q1	Q3	Classification with respect to the median % of infection
3 Fevereiro	101	15	116	12.931	13.203	6.823	21.569	Low
Calanga	122	19	141	13.475				High
Ilha Josina	22	8	30	26.667				High
Maluana	179	9	188	4.787				Low
Manhiça-sede	180	9	189	4.762				Low
Xinavane	40	11	51	21.569				High
	HEL-	**HEL+**		**HEL + (%)**				
3 Fevereiro	51	65	116	56.034	48.814	45.331	51.596	High
Calanga	36	105	141	74.468				High
Ilha Josina	24	6	30	20				Low
Maluana	91	97	188	51.596				High
Manhiça-sede	102	87	189	46.032				Low
Xinavane	28	23	51	45.098				Low

Malaria (MAL) and helminth (HEL) percentage of infection in each location of the study were calculated and these were classified into “high” and “low” prevalence locations based on higher or lower percentage of infected individuals compared to the median percentage of infections from all locations by type of parasite.

Table S5. Risk of exposure to or infection with *Plasmodium falciparum* or helminths.

		Outcome (Exposure/ infection)			Outcome (Exposure/ infection)		
		MAL +	**MAL -**	**OR [95% CI]**	**HEL+**	**HEL-**	**OR [95% CI]**
Predictor (Exposure/ infection)	**MAL +**				584	64	1.620* [0.858 – 2.992]
	MAL -				43	21	
	HEL+	584	42	1.745** [0.923 - 3.221]			
	HEL-	67	21				

The models were adjusted by covariables selected based on their significant association with the outcome variable in univariable models:

* Log_{10} age and socioeconomic score

** Log_{10} age.

OR: odds ratio, CI: Confidence interval.

SUPPLEMENTARY FIGURES

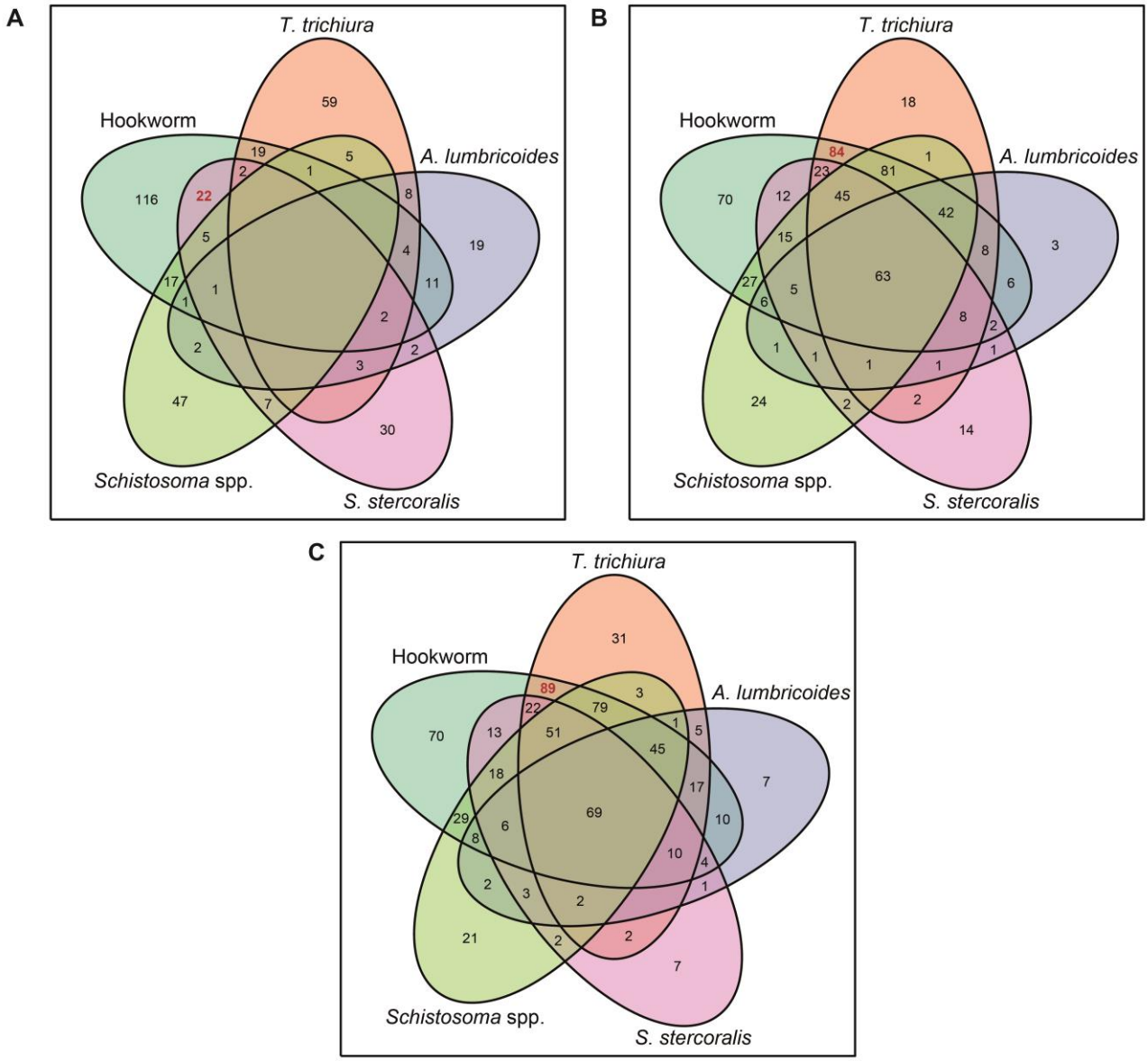

Figure S1. Venn diagrams showing the combinations of (A) coinfections, (B) coexposure and (C) coexposure/coinfection of helminth species. The most common combination of species in each classification is highlighted in red.

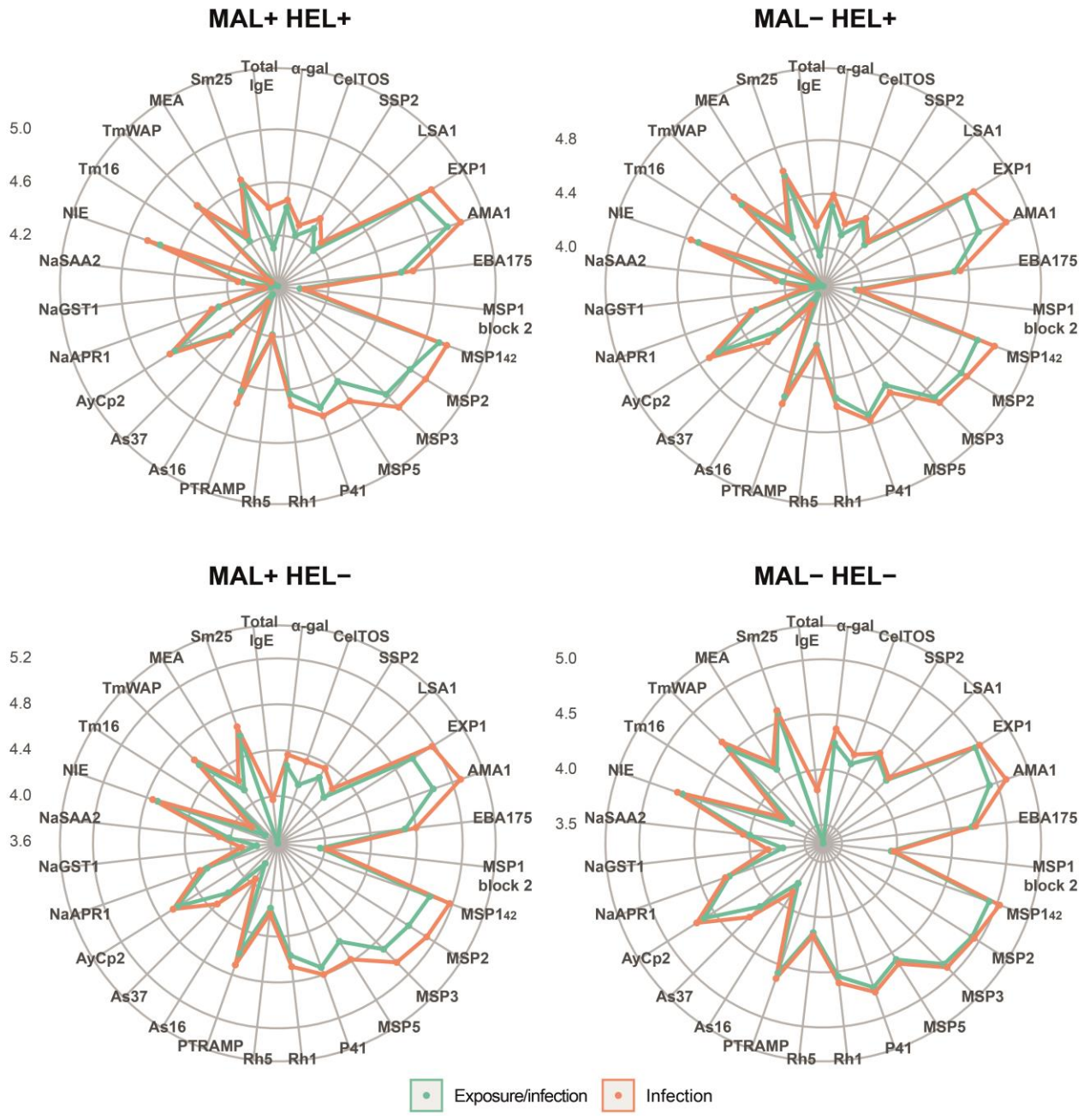

Figure S2. Comparison of antibody responses between groups classified by exposure/infection and only infection. In the vertices, the antibody responses are grouped by specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As16 and As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1, NaSAA2 [Hookworm], NIE [*Strongyloides stercoralis*], Tm16 and TmWAP [*Trichuris trichiura*], MEA and Sm25 [*Schistosoma* spp.] or total IgE. The colored lines represent the median of antibody levels expressed as log_{10}-transformed median fluorescence levels (MFI). Comparisons were made within study groups: no exposure/infection (green) or no infection (orange) (MAL- HEL-), only exposure/infection (green) or only infection (orange) with helminths (MAL- HEL+), only exposure/infection (green) or only infection (orange) with *P. falciparum* (MAL+ HEL-) and coexposure/coinfection (green) or coinfection (orange) with *P. falciparum* and helminths (MAL+ HEL+).

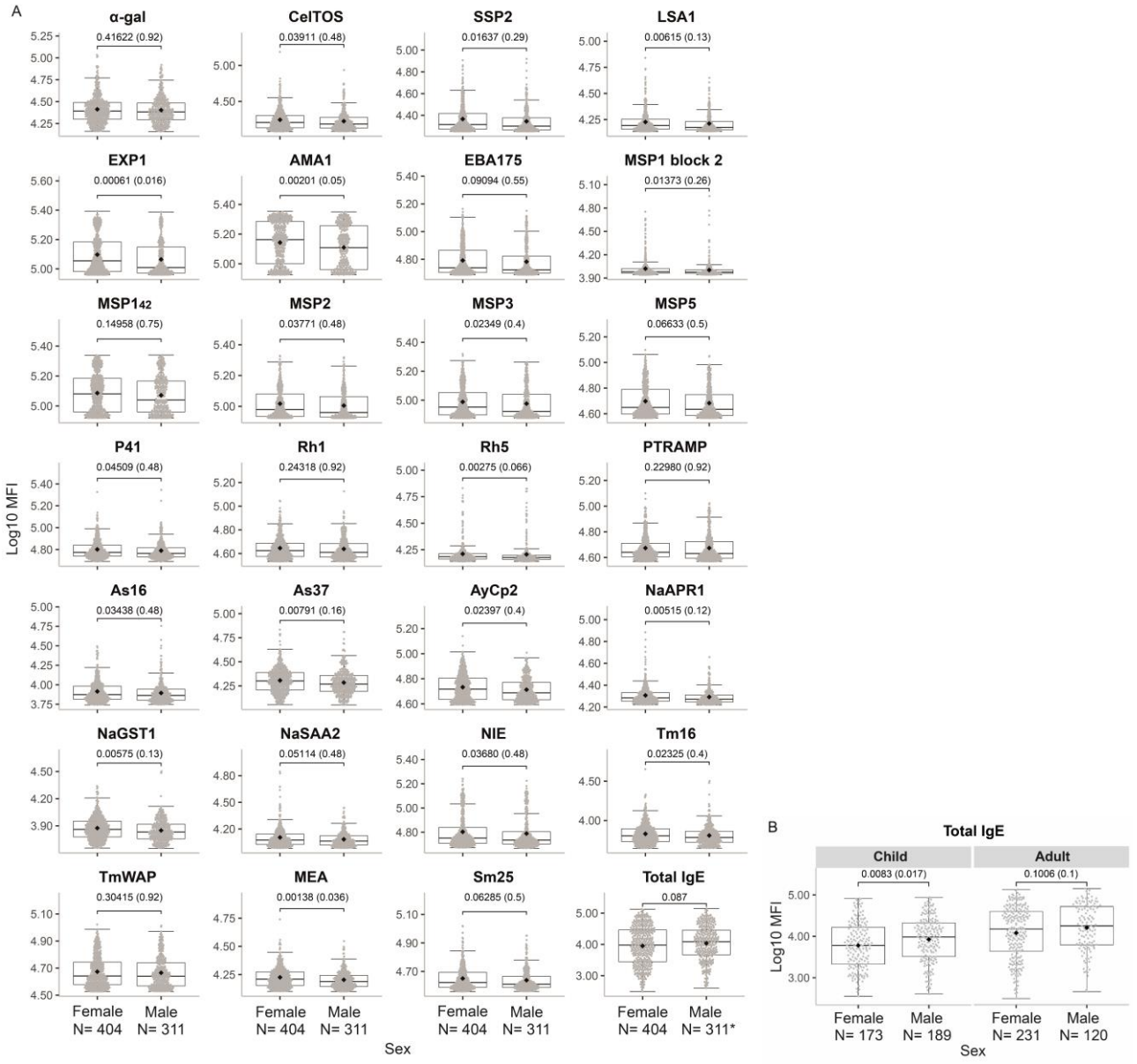

Figure S3. Comparison of antibody responses by sex. (A) Antibody levels expressed as the log_{10}-transformed median fluorescence (MFI) of specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As16 and As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1, NaSAA2 [Hookworm], NIE [*Strongyloides stercoralis*], Tm16 and TmWAP [*Trichuris trichiura*], MEA and Sm25 [*Schistosoma* spp.] or total IgE. **(B)** Log_{10}-transformed MFI levels of total IgE stratified by age. The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown. Adjusted p-values by the Benjamini-Hochberg approach are shown in brackets Wilcoxon rank sum test. *N= 309.

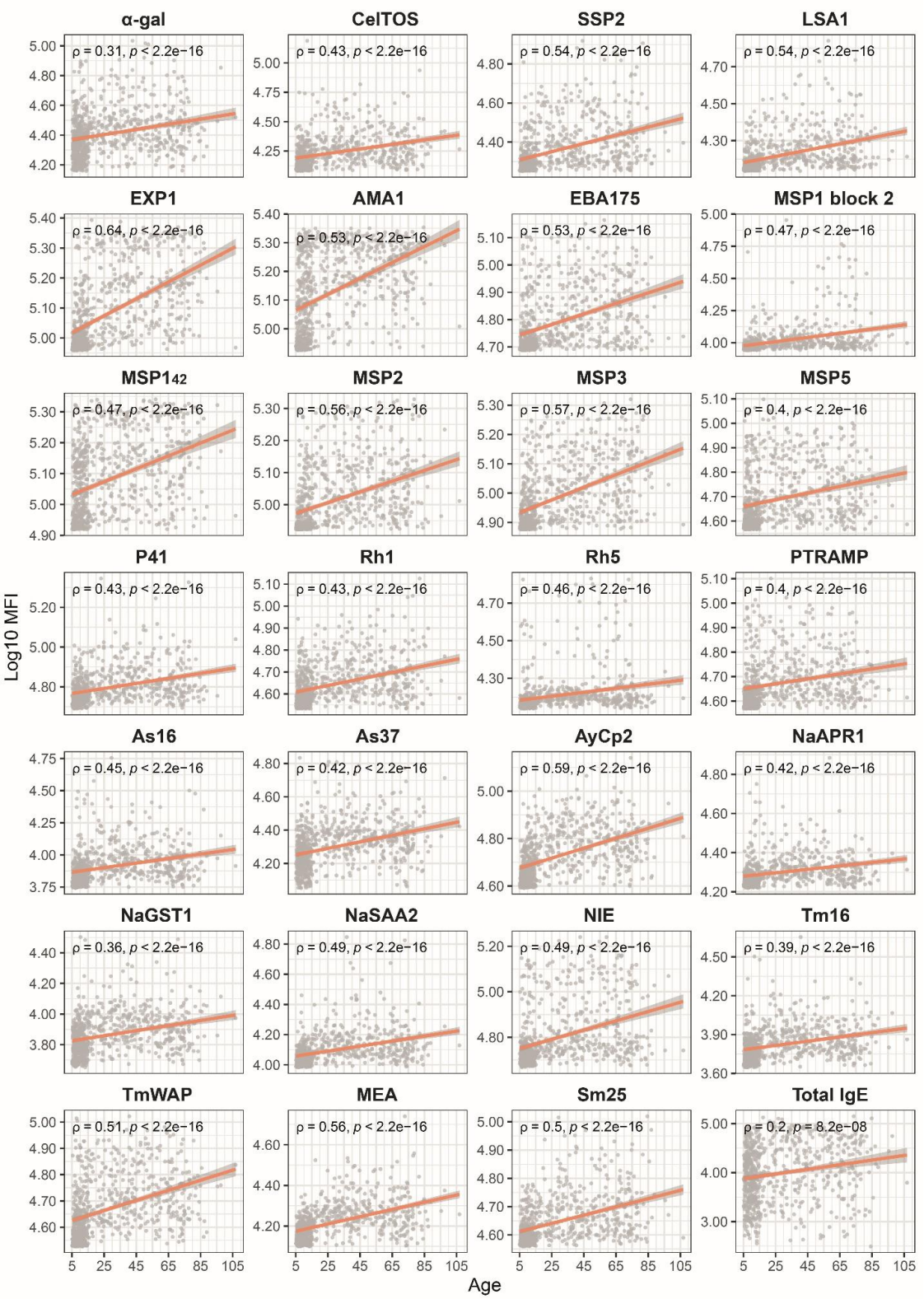

Figure S4. Antibody levels correlation with age. Antibody levels expressed as the $\log_{10}$-transformed median fluorescence (MFI) of specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As16 and As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1, NaSAA2 [Hookworm], NIE [*Strongyloides stercoralis*], Tm16 and TmWAP [*Trichuris trichiura*], MEA and Sm25 [*Schistosoma* spp.] or total IgE. Age is expressed in years. ρ (rho) and p-values were calculated by Spearman, shaded areas represent 0.95 confidence intervals.

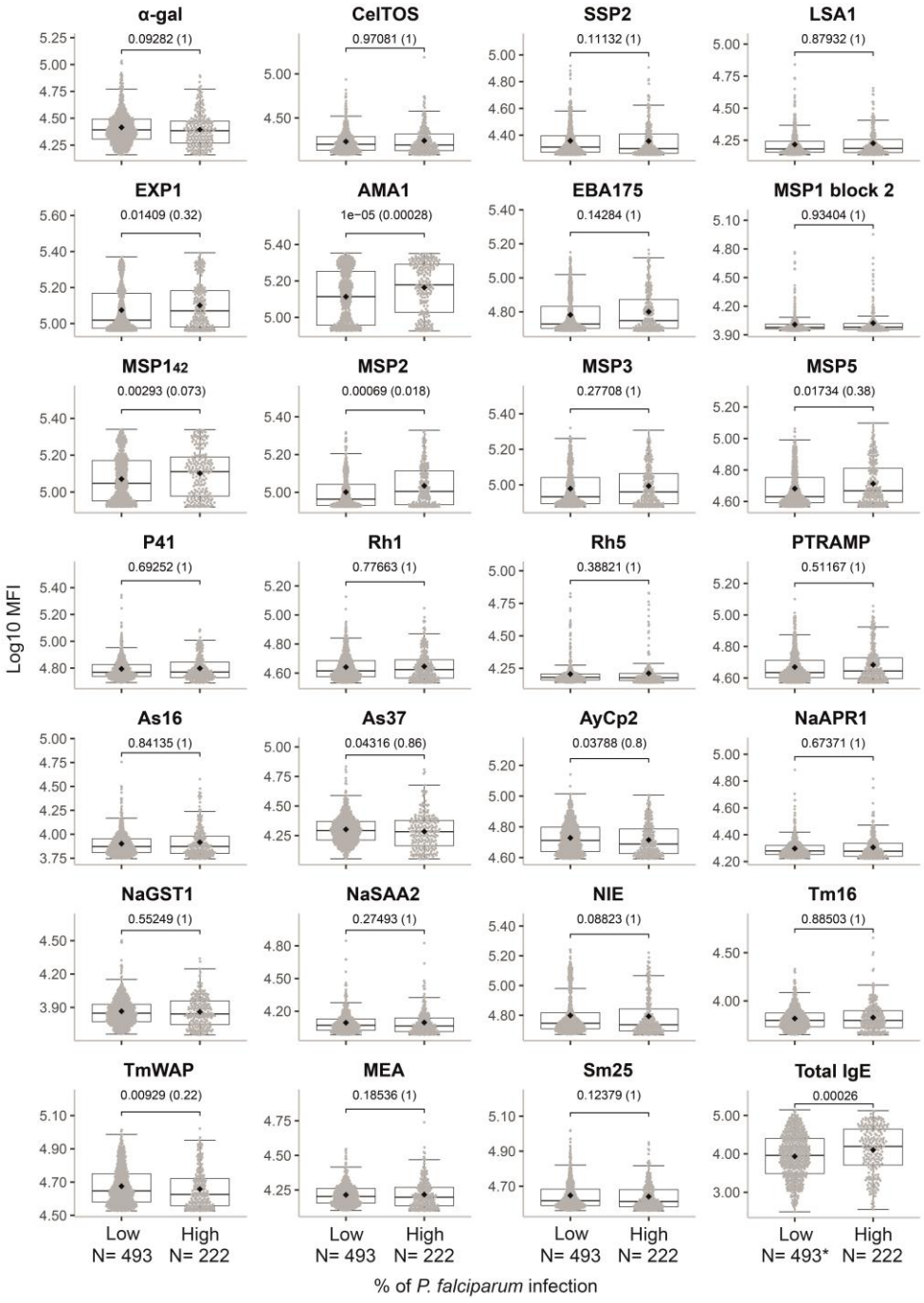

Figure S5. Comparison of antibody responses by percentage of *P. falciparum* infection by location. Antibody levels expressed as the $\log_{10}$-transformed median fluorescence (MFI) of specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As16 and As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1, NaSAA2 [Hookworm], NIE [*Strongyloides stercoralis*], Tm16 and TmWAP [*Trichuris trichiura*], MEA and Sm25 [*Schistosoma* spp.] or total IgE. In the X-axis, locations were classified into "high" and "low" prevalence based on whether the percentage of *P. falciparum* infection was lower or higher than the median percentage of *P. falciparum* infections in all locations. The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown. Adjusted p-values by the Benjamini-Hochberg approach are shown in brackets. *N= 491.

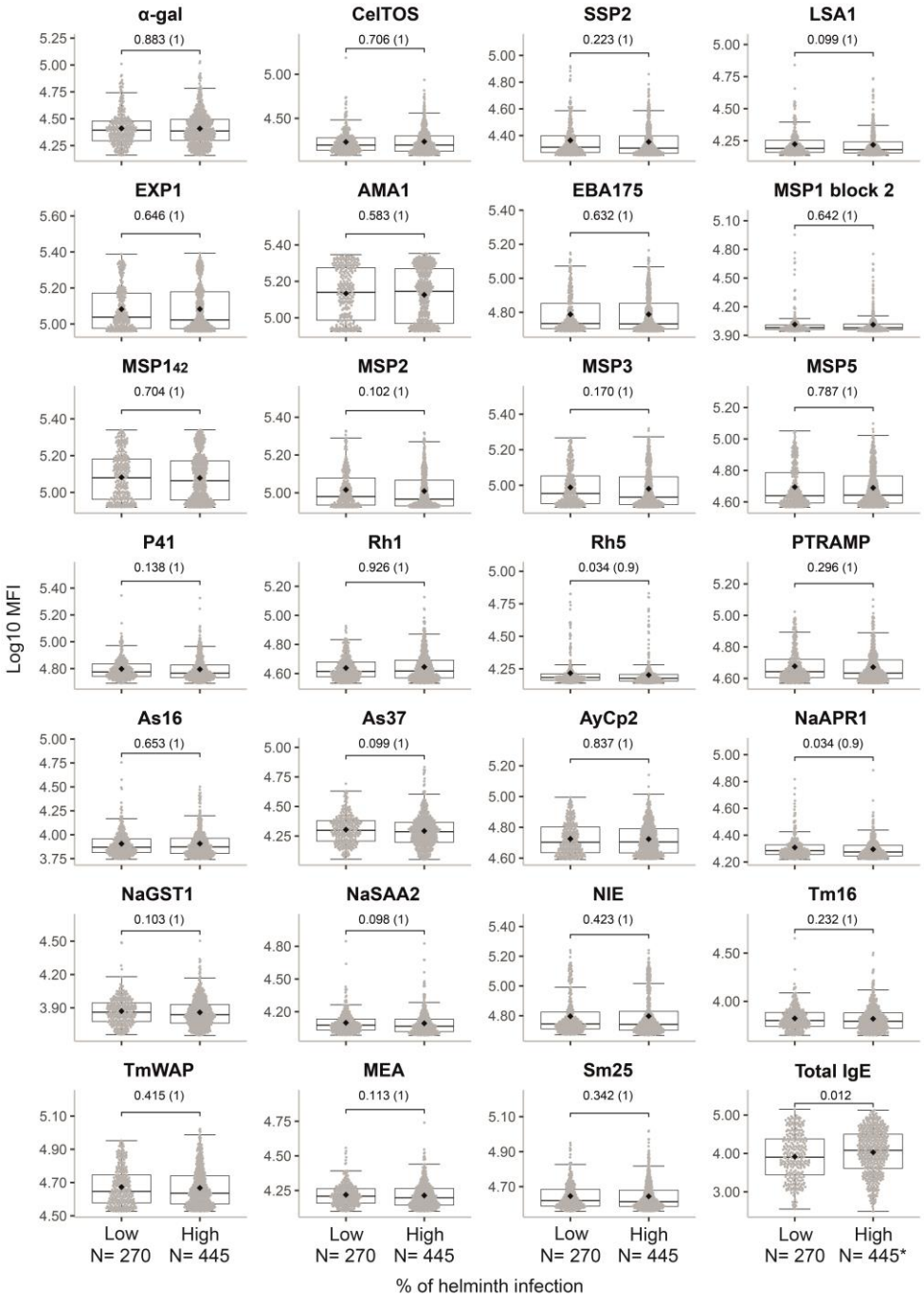

Figure S6. Comparison of antibody responses by percentage of helminth infection by location. Antibody levels expressed as the log_{10}-transformed median fluorescence (MFI) of specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As16 and As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1, NaSAA2 [Hookworm], NIE [*Strongyloides stercoralis*], Tm16 and TmWAP [*Trichuris trichiura*], MEA and Sm25 [*Schistosoma* spp.] or total IgE. . In the X-axis, locations were classified into "high" and "low" prevalence based on whether the percentage of helminth infection was lower or higher than the median percentage of helminth infections in all locations. The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown. Adjusted p-values by the Benjamini-Hochberg approach are shown in brackets. *N= 443.

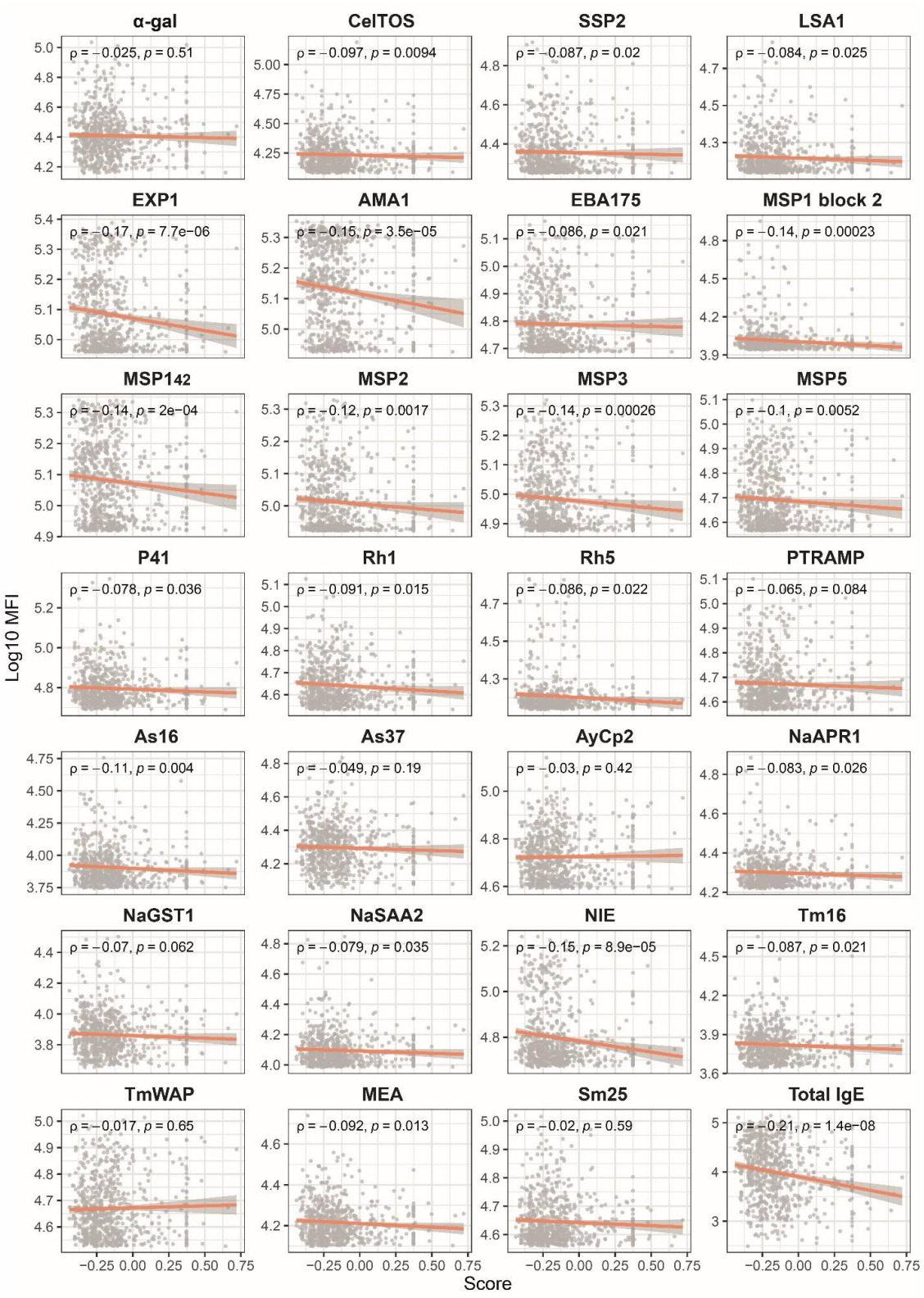

Figure S7. Antibody levels correlation with socioeconomic score. Antibody levels expressed as the log_{10}-transformed median fluorescence (MFI) of specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As16 and As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1, NaSAA2 [Hookworm], NIE [*Strongyloides stercoralis*], Tm16 and TmWAP [*Trichuris trichiura*], MEA and Sm25 [*Schistosoma* spp.] or total IgE. ρ (rho) and p-values were calculated by Spearman, shaded areas represent 0.95 confidence intervals.

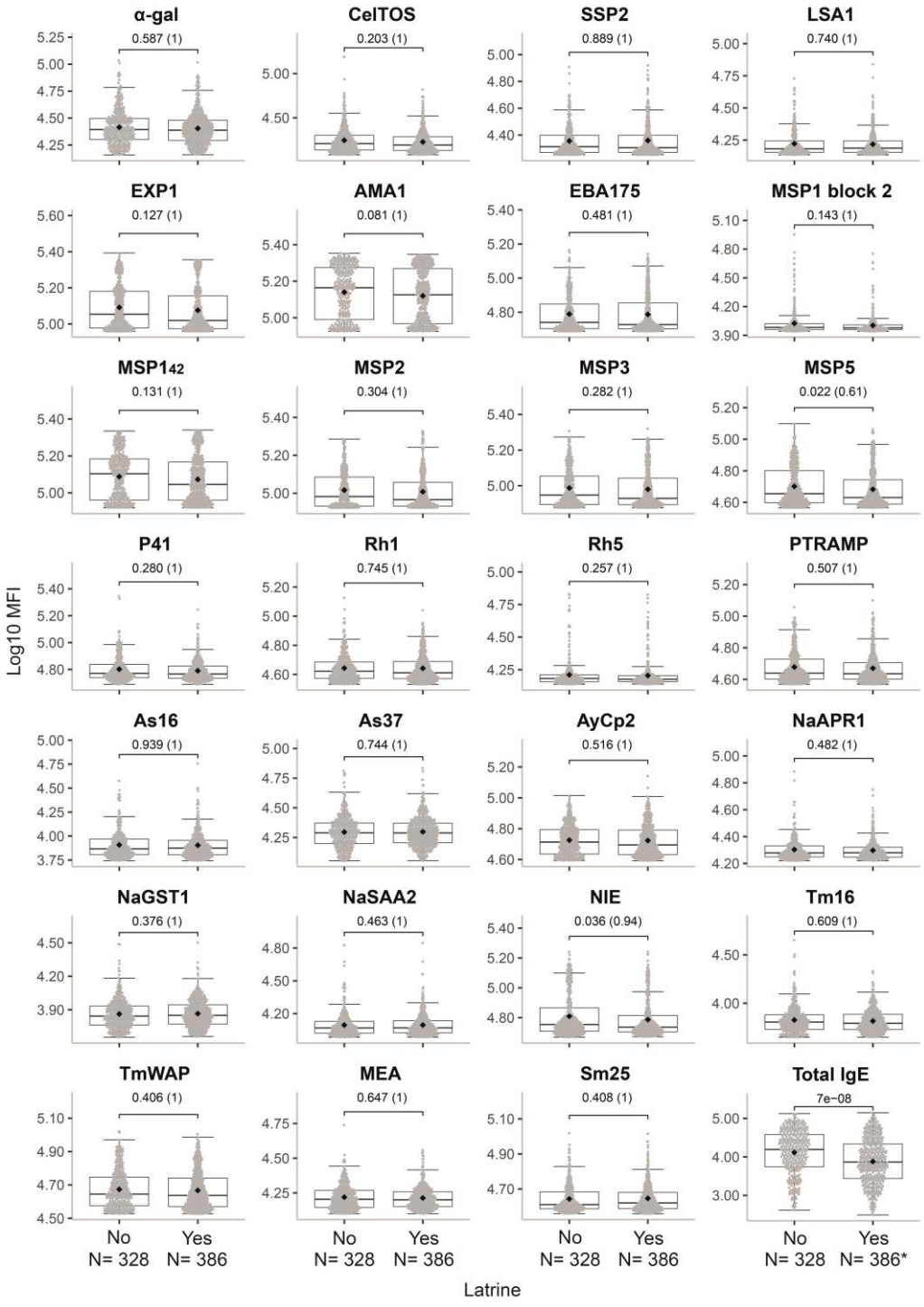

Figure S8. Comparison of antibody responses by ownership of latrine. Antibody levels expressed as the log_{10}-transformed median fluorescence (MFI) of specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As16 and As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1, NaSAA2 [Hookworm], NIE [*Strongyloides stercoralis*], Tm16 and TmWAP [*Trichuris trichiura*], MEA and Sm25 [*Schistosoma* spp.] or total IgE. The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown. Adjusted p-values by the Benjamini-Hochberg approach are shown in brackets. *N= 384.

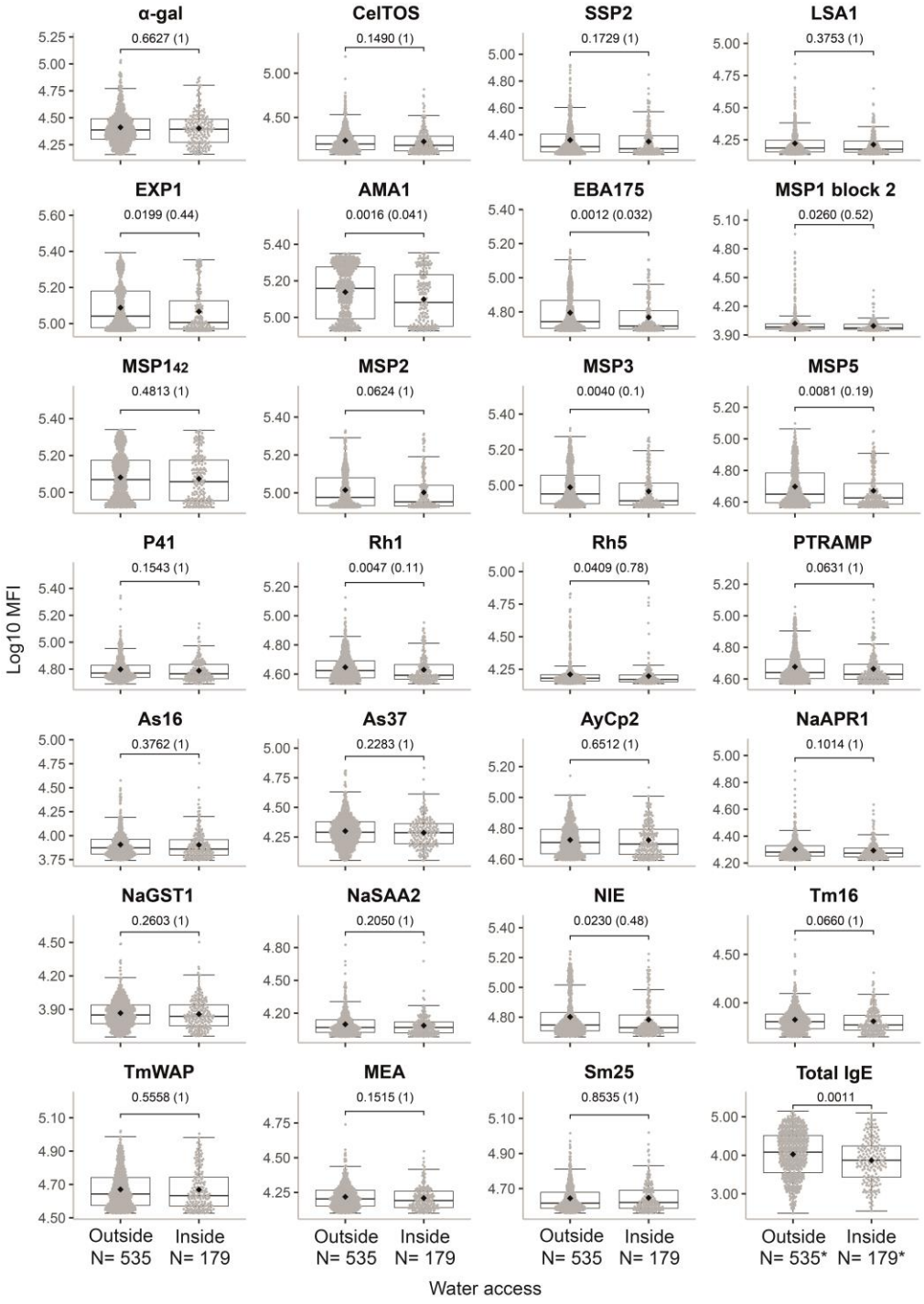

Figure S9. Comparison of antibody responses by accessibility to piped water in the household. Antibody levels expressed as the log_{10}-transformed median fluorescence (MFI) of specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As16 and As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1, NaSAA2 [Hookworm], NIE [*Strongyloides stercoralis*], Tm16 and TmWAP [*Trichuris trichiura*], MEA and Sm25 [*Schistosoma* spp.] or total IgE. The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown. Adjusted p-values by the Benjamini-Hochberg approach are shown in brackets. *****N= 535 for Outside and N=179 for Inside.

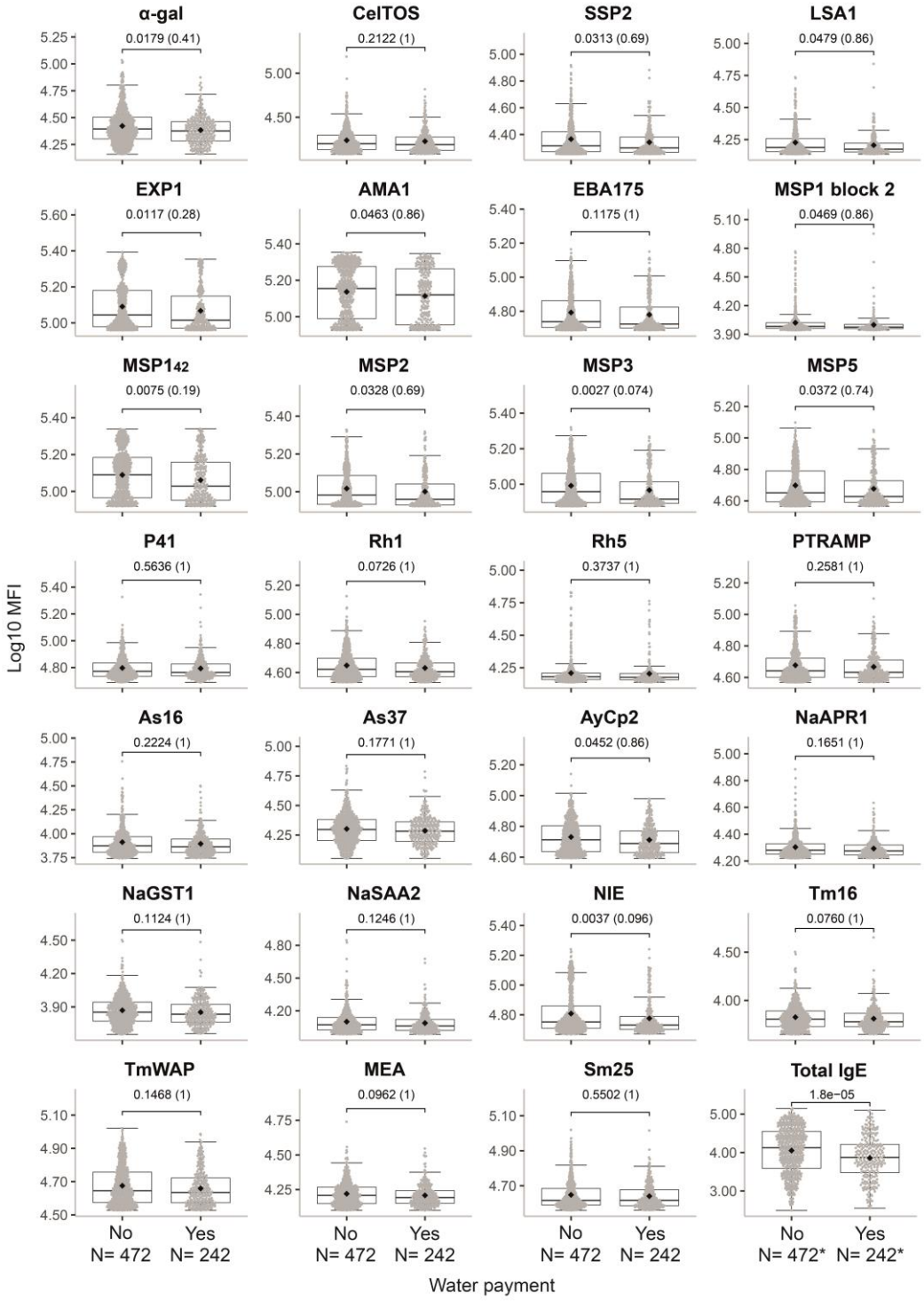

Figure S10. Comparison of antibody responses by payment of piped water. Antibody levels expressed as the $\log_{10}$-transformed median fluorescence (MFI) of specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As16 and As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1, NaSAA2 [Hookworm], NIE [*Strongyloides stercoralis*], Tm16 and TmWAP [*Trichuris trichiura*], MEA and Sm25 [*Schistosoma* spp.] or total IgE. The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown. Adjusted p-values by the Benjamini-Hochberg approach are shown in brackets. *****N= 471 for No and N= 241 for Yes.

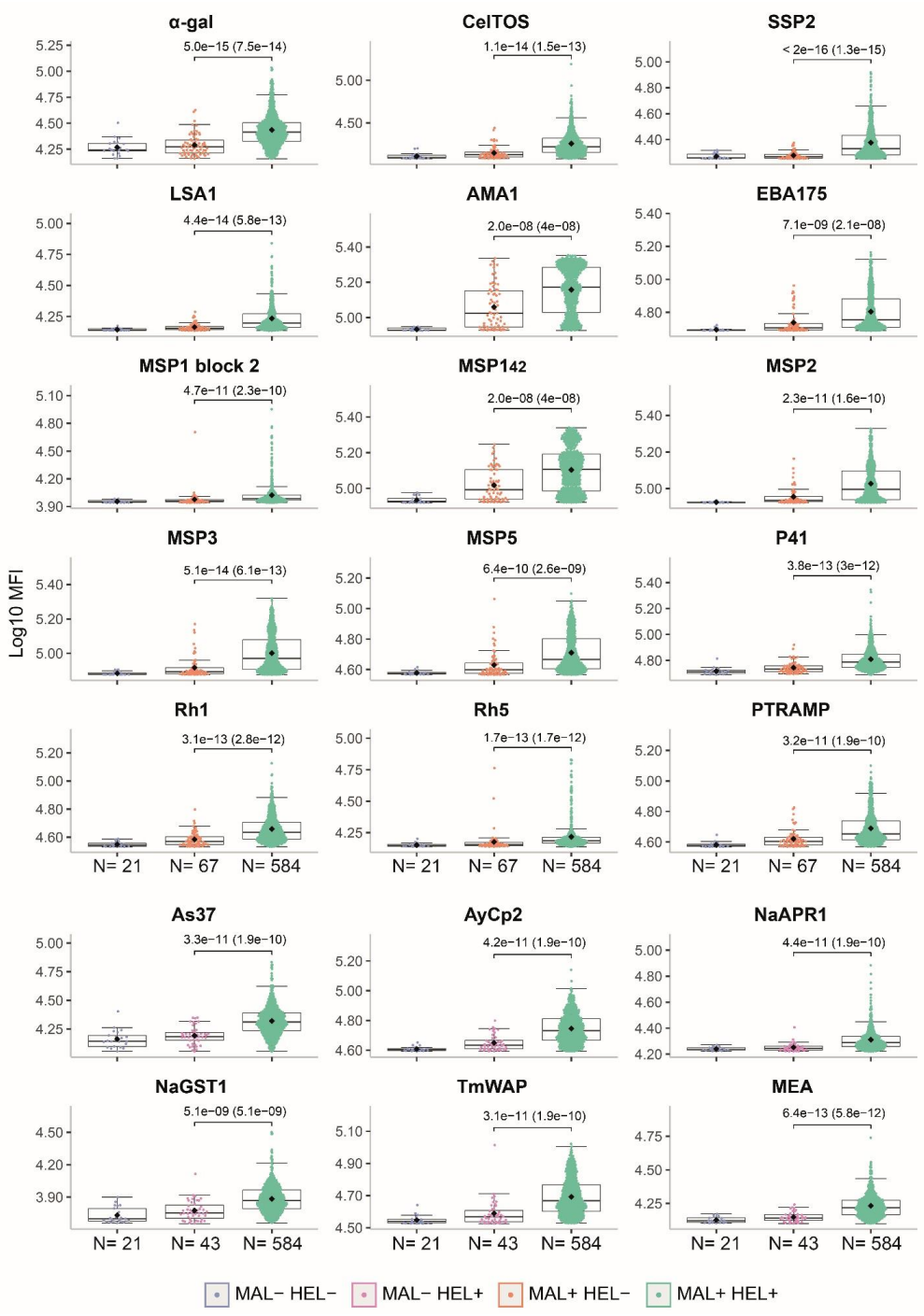

Figure S11. Comparison of antibody responses between exposure/infection groups. Antibody levels expressed as the $\log_{10}$-transformed median fluorescence (MFI) of specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1 [Hookworm], TmWAP [*Trichuris trichiura*], Sm25 [*Schistosoma* spp.]. The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown. Adjusted p-values by the Benjamini-Hochberg approach are shown in brackets. Study groups are shown in colors: no exposure/infection (violet) (MAL- HEL-), only exposure/infection with helminths (pink) (MAL- HEL+), only exposure/infection with *P. falciparum* (orange) (MAL+ HEL-) and coexposure/coinfection with *P. falciparum* and helminths (green) (MAL+ HEL+).

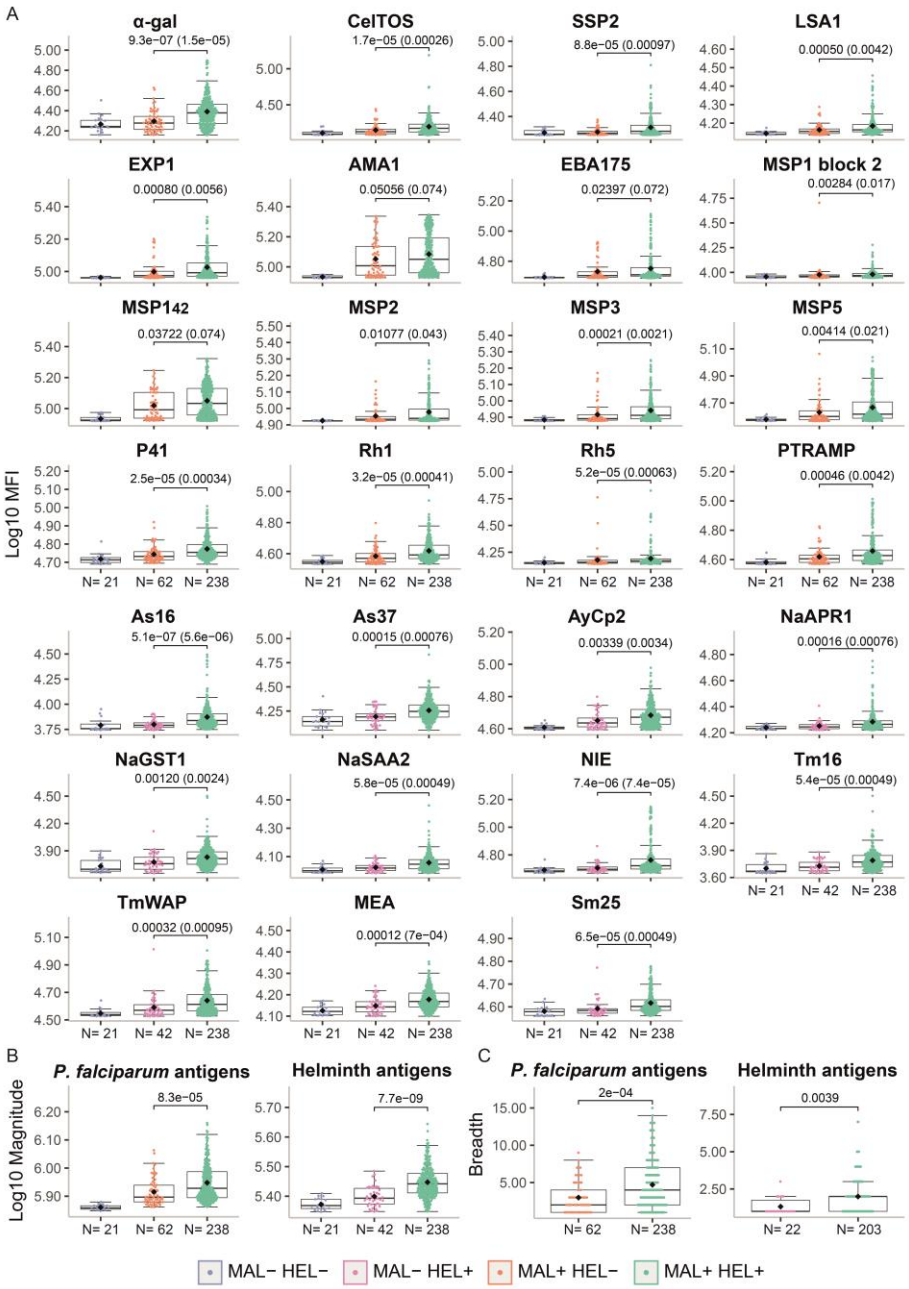

Figure S12. Comparison of antibody responses between exposure/infection groups in children. Antibody responses are expressed as **(A)** the $\log_{10}$-transformed median fluorescence (MFI) of specific IgG to *P. falciparum* antigens (α-gal, CelTOS, SSP2, LSA1, EXP1, AMA1, EBA175, MSP1 block 2, $MSP1_{42}$, MSP2, MSP3, MSP5, P41, Rh5, PTRAMP), helminth antigens (As16 and As37 [*Ascaris lumbricoides*], AyCp2, NaAPR1, NaGST1, NaSAA2 [Hookworm], NIE [*Strongyloides stercoralis*], Tm16 and TmWAP [*Trichuris trichiura*], MEA and Sm25 [*Schistosoma* spp.] or total IgE, **(B)** the $\log_{10}$-transformed magnitude of response (sum of MFI), and **(C)** the breadth of response (number of seropositive antigen-specific IgG responses). The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown. Adjusted p-values by the Benjamini-Hochberg approach are shown in brackets. Study groups are shown in colors: no exposure/infection (violet) (MAL- HEL-), only exposure/infection with helminths (pink) (MAL- HEL+), only exposure/infection with *P. falciparum* (orange) (MAL+ HEL-) and coexposure/coinfection with *P. falciparum* and helminths (green) (MAL+ HEL+).

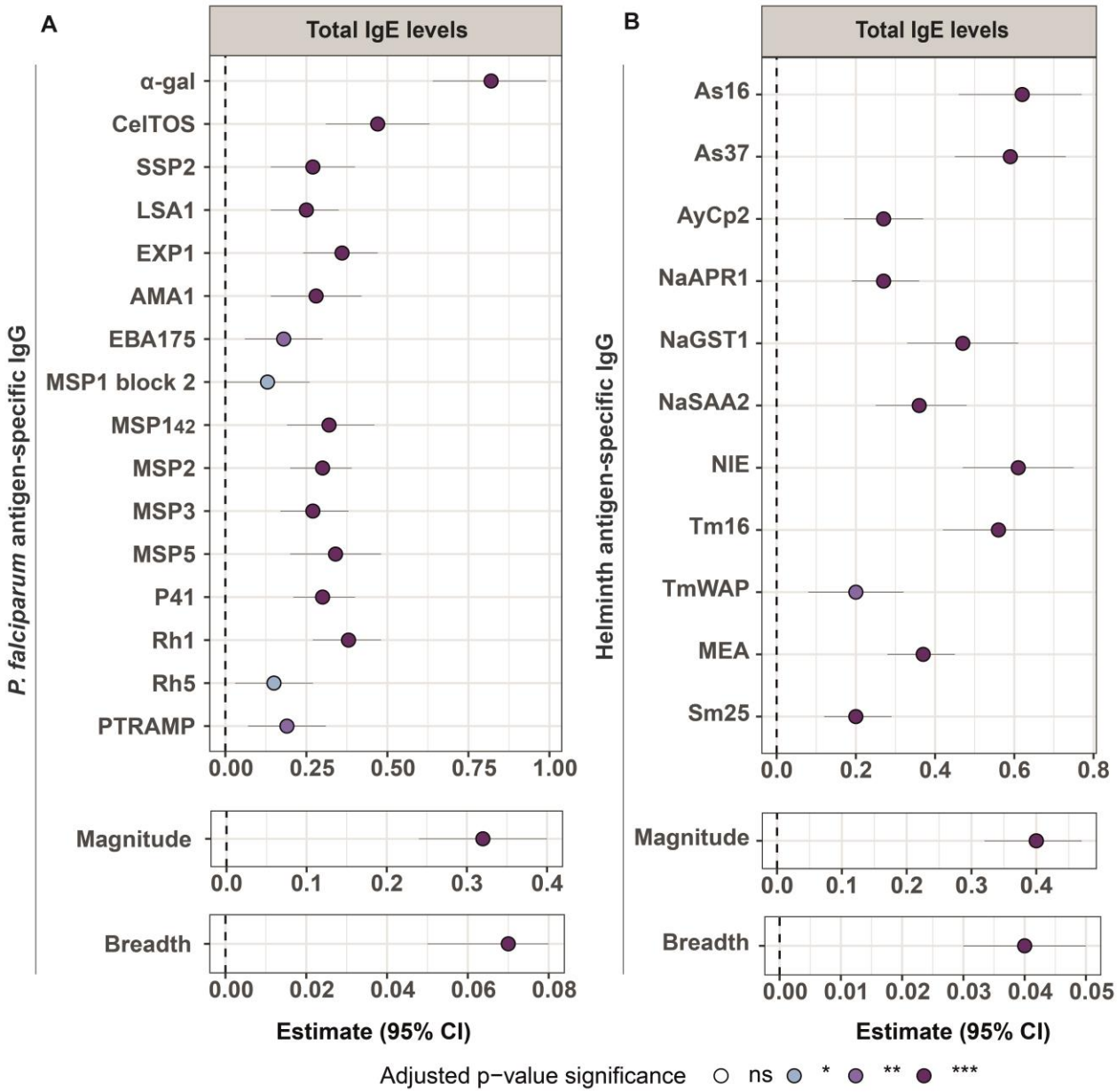

Figure S13. Association of total IgE levels with antibody levels in multivariable linear regression models. Forest plots show the association of total IgE levels with **(A)** *P. falciparum* antigens, magnitude and breadth of response or **(B)** helminth antigens, magnitude (sum of antigen-specific IgG levels) and breadth (number of seropositive antigen-specific IgG responses) of response. Multivariable linear regression models were fitted to calculate the estimates (dots) and 95% confidence intervals (CI) (lines). The represented values are the transformed betas and CI in percentage for antigen and magnitude of response (log-log models). For breadth of response (linear-log models), the betas and 95% CI were transformed to represent the additive effect on the breadth of response of a 10% increase in total IgE levels. The color of the dots represents the p-value significance after adjustment for multiple testing by Benjamini-Hochberg, where ns= not significant, * = p-value ≤ 0.05, ** = p-value ≤ 0.01 and *** = p-value ≤ 0.001. Models were adjusted by age and percentage of *P. falciparum* infection by location for *P. falciparum* antigens and age for helminth antigens.

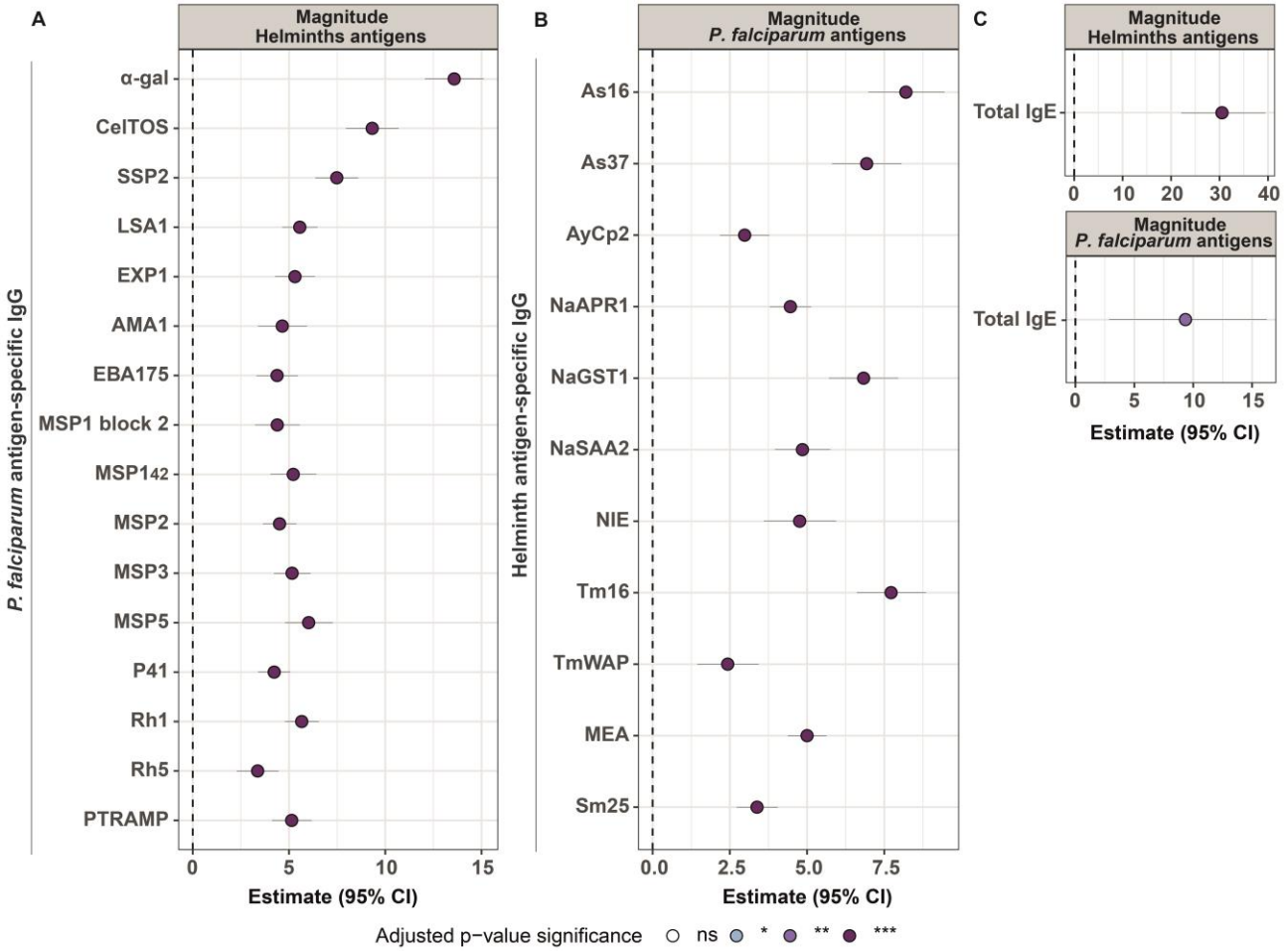

Figure S14. Association of magnitude of response with antibody levels in multivariable linear regression models. Forest plots show the association of **(A)** magnitude of response to helminth antigens with *P. falciparum* antigens, **(B)** magnitude of response to *P. falciparum* antigens with helminth antigens and **(C)** magnitude of response to helminth and *P. falciparum* antigens with total IgE levels. The magnitude of response was calculated as the sum of all specific IgG levels (MFI) to the different antigens belonging to *P. falciparum* or helminths. Multivariable linear regression models were fitted to calculate the estimates (dots) and 95% confidence intervals (CI) (lines). The represented values are the transformed betas and CI in percentage (log-log models). The color of the dots represents the p-value significance after adjustment for multiple testing by Benjamini-Hochberg, where ns= not significant, * = p-value ≤ 0.05, ** = p-value ≤ 0.01 and *** = p-value ≤ 0.001. Models were adjusted by age and percentage of *P. falciparum* infection by location for *P. falciparum* antigens, age for helminth antigens and sex, percentage of helminth infection by location, ownership of latrine, piped water accessibility, payment for piped water and socioeconomic score for total IgE.

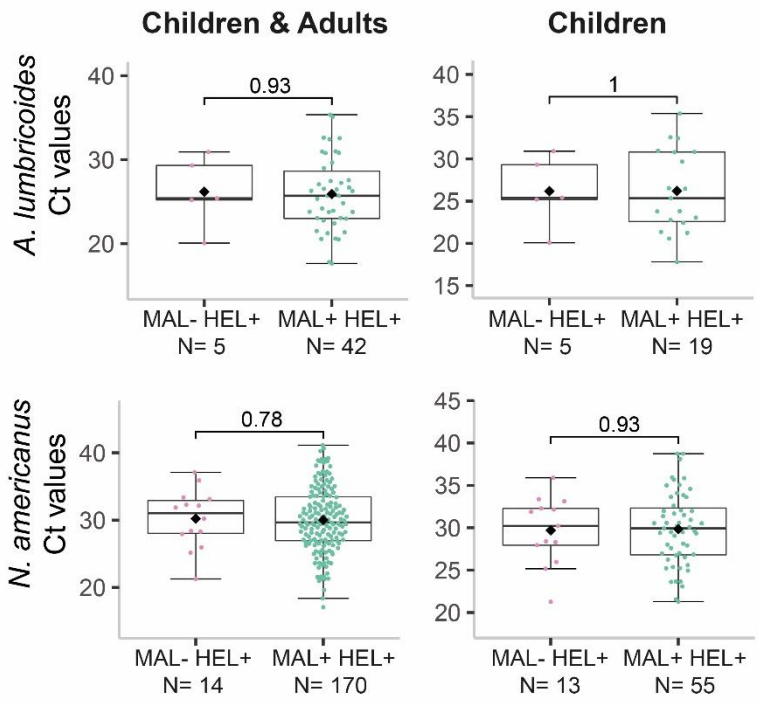

Figure S15. Parasite burden by infection groups. The levels of parasite burden are represented as the Ct values and are compared between the coexposure/coinfection group (MAL+ HEL+) and the single helminth exposure/infection group (MAL- HEL+) for *Ascaris lumbricoides and Necator americanus*. For each parasite, data for all individuals with parasite burden information or only children are displayed. The boxplots represent the median (bold line), the mean (black diamond), the 1st and 3rd quartiles (box) and the largest and smallest values within 1.5 times the inter-quartile range (whiskers). Data beyond the end of the whiskers are outliers. Statistical comparison between groups was performed by Wilcoxon rank sum test and the exact p-values are shown.

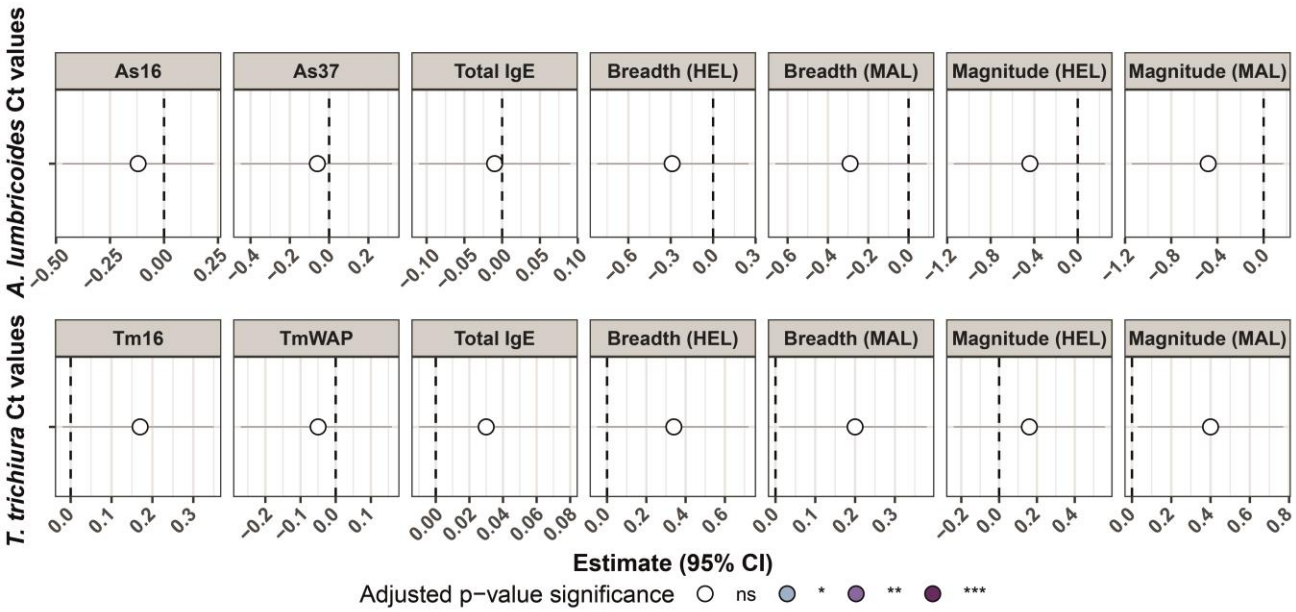

Figure S16. Association of antibody responses with parasite burden in multivariable linear regression models. Forest plots show the association of antibody responses with parasite burden represented as the Ct values of *Ascaris lumbricoides* and *Trichuris trichiura*. For each parasite, linear regression models adjusted by age were fitted, where specific IgG against the antigens from the corresponding parasite, total IgE and magnitude (sum of antigen-specific IgG levels) and breadth (number of seropositive antigen-specific IgG responses) of response were the predictor variables, and the parasite burden was the outcome variable. When the predictor variables were antibody levels or magnitude of response (linear-log models), the estimates (dots) and 95% confidence intervals (CI) (lines) of the models were transformed to represent the additive effect on the Ct values of a 10% increase in the predictor variable. For the breadth of response (linear-linear models), the untransformed betas were used. The color of the dots represents the adjusted p-value significance, where ns= not significant, * = p-value ≤ 0.05, ** = p-value ≤ 0.01 and *** = p-value ≤ 0.001.

SUMPPLEMENTARY METHODS

Data pre-processing

For antigen-specific IgG quantification results, we selected the dilution that was within the quantitative range of the PC curve, ideally in the linear part of it. Briefly, the point of the PC curve with the maximum slope was calculated per plate and antigen. The dilution of the sample with the MFI value more similar to this maximum slope point was chosen, as the maximum slope is the most reliable part of the PC curve for quantification. The selection of the dilution was performed after checking for hook effect, since it could disturb the results.

Then, we performed a double normalization to correct a batch effect caused by the use of two different streptavidin-R-phycoerythrin lots. The first normalization was intra-batch to correct for technical artefacts between plates within the same streptavidin-R-phycoerythrin lot. The second normalization was inter-batch to correct for the differences due to the different lots of streptavidin-R-phycoerythrin. First, MFI values after the selection of sample dilution were normalized by multiplying each MFI by the first normalization factor estimated by plate and antigen within each batch. To do so, first an average curve per antigen and batch was calculated by performing the mean of each of the dilution points of the PC curves (i.e.: mean of dilution 1 of all plates within batch 1 for AMA1). Next, the dilution point closest to the maximum slope of the average curve per antigen and batch was identified and denominated as the “normalization dilution point”. This normalization dilution point was then used to calculate the first normalization factor, which was the ratio of the normalization dilution point of each plate curve between the normalization point of the average curve per antigen, plate, and batch. The second normalization factor was obtained by calculating the ratio of the normalization dilution point per antigen between the two batches. Given that the batch 1 included 15 plates and the batch 2 only 6, the reference was batch 1 because its sample size was bigger. Therefore, the second normalization factor was applied to the batch 2 by multiplying the MFI values by the second normalization factor. In this step, the hook effect was also considered. Finally, a correction for the dilution factor was applied to be able to compare samples tested at different dilutions for IgG. The correction factor was obtained with a regression method based on the maximum slope of the batch 1 average curve for each antigen. The regression line has the next formula: $\boldsymbol{y}=\boldsymbol{mx}+\boldsymbol{b}$, where $\boldsymbol{y}$ is the final corrected MFI value, $\boldsymbol{m}$ is the maximum slope, $\boldsymbol{x}$ the log10-transformed dilution factor and $\boldsymbol{b}$ the normalized MFI.

For total IgE, neither selection of dilution, nor a correction by dilution was needed for the data pre-processing. However, normalization was also required due to the batch effect caused by the different streptavidin-R-phycoerythrin lots. MFI values were normalized by calculating the normalization factor of each plate. This factor was obtained by calculating the ratio of the MFI values from the elution control (EC) 1 per plate between the average of all plates. The EC 1 was selected for normalization since the performance of the PC curve was not good enough to use as a reference. Among the ECs, EC 1 had the lowest CV% (EC1 = 6.28%, EC2 = 23.47% and EC3 = 20.86%).

Data from blanks were excluded since they were negligible.

1293 **Table S6. Antigens included in the multiplex panel**

Parasite	Antigen	Life stage	Abbreviation and/or information	Rationale	Ref.
STH					
Necator americanus	NaGST1	A	Glutathione S-transferase involved in haemoglobin digestion	Candidate vaccine antigen	(Adegnika et al., 2021)
Necator americanus	NaAPR1	A	Aspartic protease involved in haemoglobin digestion	Candidate vaccine antigen	(Adegnika et al., 2021)
Necator americanus	NaSAA2	L	Surface associated antigen	Candidate vaccine antigen	(Asojo et al., 2010)
Ancylostoma ceylanicum	AyCp2	A	Cysteine protease involved in haemoglobin digestion	Candidate vaccine antigen	(Wei et al., 2016)
Trichuris muris	TmWAP	A	Whey acidic protein, secreted immunodominant protein	Candidate vaccine antigen	(Briggs et al., 2018)
Trichuris muris	Tm16	A	Secreted immunodominant protein	Candidate vaccine antigen	(Liu et al., 2017)
Ascaris suum	As16	L / A	Secreted immunodominant protein	Candidate vaccine antigen	(Wei et al., 2017)
Ascaris suum	As37	L / A	Secreted immunodominant protein	Candidate vaccine antigen	(Versteeg et al., 2020)
Strongyloides stercoralis	NIE	L / A	Recombinant antigen used for immunodiagnostic	Exposure to larvae infection	(Rascoe et al., 2015)
Schistosoma					
Schistosoma spp.	MEA	Egg	Major component of schistosome eggs	Immunogenic antigen	(Silva-Moraes et al., 2019)
Schistosoma mansoni	Sm25	A	Tegumental glycoprotein	Involved in protection	(Petzke et al., 2000)
Plasmodium falciparum *The antigen fragments and variants included are: AMA1 FVO, EBA175 reg2 PfF2, MSP1 Block 2 MAD20, $MSP1_{42}$ 3D7, MSP2 full length CH150 and MSP3 3D7	α-gal	PE	Alpha-galactosidase also expressed in bacteria	Involved in malaria protection	(Aguilar et al., 2018; Yilmaz et al., 2014)
	CelTOS	PE	Cell-traversal protein	Exposure to SPZ infection	(Bergmann-Leitner et al., 2013; Kusi et al., 2014)
	SSP2(TRAP)	PE	Thrombospondin adhesive protein	Exposure to SPZ infection	(Khusmith et al., 1991; Robson et al., 1988)
	LSA1	LS	Liver stage antigen	Expressed in infected hepatocytes	(Guerin-Marchand et al., 1987; Zhu and Hollingdale, 1991)
	EXP1	LS/BS	Exported protein	Exposure to asexual BS	(Doolan et al., 1996)
	AMA1*	BS	Apical membrane antigen	Exposure to asexual BS	(Kocken et al., 2002)
	EBA175*	BS	Erythrocyte binding antigen	Exposure to asexual BS	(Sim et al., 1994)
	MSP1 bl 2*	BS	Merozoite surface protein	Exposure to asexual BS	(Cavanagh and McBride, 1997)
	$MSP1_{42}$*	BS	Merozoite surface protein	Exposure to asexual BS	(Angov et al., 2003)
	MSP2*	BS	Merozoite surface protein	Exposure to asexual BS	(Metzger et al., 2003)
	MSP3*	BS	Merozoite surface protein	Exposure to asexual BS	(Imam et al., 2014)
	MSP5	BS	Merozoite surface protein	Exposure to asexual BS	(Black et al., 2002)
	P41	BS	Unknown function	Exposure to asexual BS	(Taechalertpaisarn et al., 2012)
	Rh1	BS	Reticulocyte binding protein	Exposure to asexual BS	(Gaur et al., 2004)
	Rh5	BS	Reticulocyte binding protein	Exposure to asexual BS	(Reddy et al., 2014)
	PTRAMP	BS	Thrombospondin apical protein	Exposure to asexual BS	(Siddiqui et al., 2013)
Others	GST		Glutathione S-transferase	Control fusion protein	

1294 STH: Soil-transmitted helminths, A: adult, L: larvae, PE: pre-erythrocytic stage, LS: liver-stage, BS: blood-stage, SPZ: sporozoite, Ref.: References

1295 **Table S7. Panel of antigens with their corresponding optimal coupling conditions**

Antigen	Coupling concentration	Buffer
NaGST1	100 µg/ml	MES
NaAPR1	30 µg/ml	MES
NaSAA2	100 µg/ml	MES
AyCp2	50 µg/ml	MES
TmWAP	30 µg/ml	MES
Tm16	50 µg/ml	MES
As16	50 µg/ml	MES
As37	75 µg/ml	MES
NIE	50 µg/ml	MES
MEA	30 µg/ml	MES
Sm25	10 µg/ml	MES
α-gal	30 µg/ml	MES
CelTOS	50 µg/ml	PBS
SSP2 or TRAP	30 µg/ml	PBS
LSA1	30 µg/ml	PBS
EXP1	30 µg/ml	MES
AMA1 FVO	30 µg/ml	MES
EBA175 reg2 PfF2	30 µg/ml	MES
MSP1 Block2 MAD20	50 µg/ml	MES
MSP1 42 3D7	30 µg/ml	MES
MSP2 full length CH150	30 µg/ml	MES
MSP3 3D7	30 µg/ml	MES
MSP5	30 µg/ml	MES
P41	50 µg/ml	MES
PfRh1	30 µg/ml	MES
PfRh5	30 µg/ml	MES
PTRAMP	30 µg/ml	MES
GST	50 µg/ml	MES
anti-IgE	25 µg/ml	MES

1296

1297 MES: Morpholineethane sulfonic acid

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