Reviewed Preprint
v1 • October 23, 2025
Not revised

Reviewed Preprint
v2 • May 21, 2026
Revised by authors

A natural experiment in Kenya reveals durable immunosuppressive effects of early childhood malaria: a longitudinal cohort study

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Funding: See page 17

Reviewing editor: Marcelo U Ferreira, University of São Paulo, Brazil

eLife Assessment

This **important** study provides **solid** evidence that early childhood malaria exposure affects the development of antibody responses to unrelated pathogens and vaccine-derived antigens in Kenyan children. The findings are of major public health importance and limitations of the observational study design are properly acknowledged.

https://doi.org/10.7554/eLife.107820.2.sa2

Abstract

Background Chronic malaria exposure has been proposed to modulate immune function, but its long-term effects on antibody-mediated responses to unrelated pathogens remain poorly defined. Whether these effects persist beyond periods of active infection, and how early-life exposure shapes humoral immunity over time, is not well understood.

Methods We leveraged a natural experiment in coastal Kenya - where two regions (Junju and Ngerenya) diverged sharply in malaria transmission from around 2004 - to evaluate the long-term immunological consequences of malaria exposure in childhood. Using a protein microarray platform, we measured IgG responses to vaccine and pathogen antigens in 123 children sampled longitudinally over a 15-year period. Active weekly malaria surveillance enabled precise reconstruction of individual exposure histories.

Results IgG responses to *Plasmodium falciparum* apical membrane antigen 1 (AMA1) tracked closely with clinical malaria episodes, confirming the ability of the microarray platform to detect biologically meaningful variation in antigen-specific immunity. Despite comparable vaccination histories, children from the high malaria transmission setting (Junju) exhibited persistently lower measles-specific IgG levels than children from the low-transmission setting (Ngerenya), a pattern validated by ELISA. In longitudinal analyses, children from Junju exhibited lower antibody responses to a range of unrelated antigens, including Bordetella pertussis, CMV, rubella, and measles, with similar differences evident in cross sectional analyses at 10 years of age. Within the Ngerenya cohort, children with documented early-life malaria had broadly lower IgG responses at

age 10 compared to malaria-naïve peers, despite identical geography, vaccines, and follow-up duration.

Conclusions These findings suggest that malaria exposure during early childhood is linked with durable suppression of antibody responses to unrelated pathogens and vaccines. This effect persists long after infection and may partially explain the overall diminished long-term vaccine effectiveness in malaria-endemic settings.

Introduction

Malaria remains a major cause of childhood morbidity and mortality in sub-Saharan Africa. In 2023, Africa accounted for 95% of global malaria deaths, with over three-quarters occurring in children under five years old[1]. Alongside malaria, children in these settings face high exposure to a wide array of pathogens - including respiratory, enteric, and helminth infections [2], making immune competence critical for survival and long-term health. Despite widespread vaccination efforts, several studies report reduced vaccine efficacy in malaria-endemic regions compared to malaria-naïve populations [3–5]. Although multiple factors may contribute[6], growing evidence suggests that malaria itself can impair host immunity[5,7,8]. Repeated P. falciparum infection has been associated with immunomodulatory changes, including expansion of regulatory T cells [9,10], regulatory B cells[11], and atypical memory B cells with limited effector function[12,13]. These alterations promote host tolerance for persistent parasitaemia, resulting from recurrent exposure in endemic settings, but they also suppress effector immune responses[14], potentially to unrelated antigens, including vaccines. While malaria-induced immunosuppression has been described in both experimental and observational studies[7,8,15,16], its duration and broader impact on the developing immune system remain poorly understood. Most prior work has focused on short-term or antigen-specific outcomes, leaving open the question of whether early-life malaria exposure durably attenuates antibody responses over the long term. Conflicting findings across settings highlight the need for context-specific, and longitudinal data studies[16,17].

Here, we address this gap using two longitudinal cohorts from coastal Kenya with contrasting malaria transmission histories. One region (Junju) has experienced a sustained malaria burden, while the other (Ngerenya) underwent a rapid decline in transmission after 2004[18]. Intensive weekly malaria surveillance was conducted over more than a decade, enabling precise classification of individual exposure histories. We combined these data with serial serological measurements using a high-throughput in-house protein microarray. This design enabled us to investigate whether clinical malaria in early life leaves a lasting immunological imprint that compromises antibody responses to common childhood pathogens and vaccines. Our findings reveal a durable suppression of humoral immunity linked to malaria exposure in early childhood, with implications for vaccine effectiveness, serosurveillance interpretation, and immune recovery in endemic regions.

Materials and Methods

Study setting and cohort design

This study was conducted in Kilifi County, a rural region on the northern coast of Kenya, within the catchment area of the Kilifi Health and Demographic Surveillance System (KHDSS), a long-term population-based platform maintained by the KEMRI-Wellcome Trust Research Programme[19]. Serum samples were obtained retrospectively from two well-characterised paediatric cohorts - Ngerenya and Junju - enrolled between 1998 and 2017 as part of annual malaria cross-sectional surveys[20]. Ngerenya and Junju were selected for their contrasting malaria transmission intensities; Ngerenya experienced a sharp decline in malaria transmission beginning in the early 2000s[18], whereas Junju maintained moderate malaria endemicity throughout the study period, with *P.falciparum* prevalence approximating 30% during the rainy seasons[21]. All children were visited weekly at home for the detection of febrile episodes, and any child with an axillary temperature ≥37.5°C was tested for P. falciparum parasitaemia using a rapid diagnostic test, with confirmation by microscopic examination of Giemsa-stained thick and thin blood

smears. A clinical malaria episode was defined as fever in the presence of ≥2,500 parasites/μL. In addition to active malaria surveillance, serum samples were collected annually from each child and for future serological analysis. The vaccination status of each child for routine childhood vaccines was assessed using digitised immunisation records stored at the KEMRI-Wellcome Trust Research Programme.

Protein microarray antibody profiling

Antibody responses were measured using an in-house protein microarray platform. Recombinant and whole-virus lysate antigens (Supplementary table 1) were reconstituted in a glycerol-based buffer containing 1% Triton X-100 and printed in duplicate onto epoxy-coated glass slides using a non-contact microarrayer (Marathon Argus, Arrayjet, Scotland). In addition to antigen spots, each miniarray contained a series of internal controls. These included anti-human IgG and anti-human IgA capture antibodies to confirm the presence and isotype of immunoglobulin in each sample, fluorophore-conjugated IgG and IgA (Alexa Fluor 647 and Alexa Fluor 555) to assess scanner performance independently of antigen binding, and printing buffer-only spots to quantify non-specific background signal. Slides were fitted into hybridisation cassettes, washed with PBST (0.05% Tween-20 in PBS), and blocked for 1 hour at 37°C using PBST containing 5% BSA. Serum samples were diluted 1:30 in PBST with 5% BSA and incubated on the slides for 3 hours at room temperature. For each slide, one miniarray was incubated with PBS in place of serum as a negative control, and one miniarray with pooled adult serum, comprising sera from multiple healthy adults, to provide a consistent positive reference for antigen recognition across slides. Following incubation, slides were washed and probed with secondary antibodies: goat anti-human IgG conjugated to Alexa Fluor 647 and goat anti-human IgA conjugated to Alexa Fluor 555, enabling simultaneous detection of IgG and IgA binding. Slides were scanned using a GenePix 4300A scanner with dual-wavelength acquisition (635 nm and 532 nm) to capture isotype-specific signals. To improve measurement robustness and account for spatial variation, each sample was assayed on two independent miniarrays per slide, yielding four spatially separated replicate measurements per antigen. Mean fluorescence intensities (MFIs) were extracted and background-corrected using printing buffer and negative control spots to account for non-specific signal. Technical variation was assessed by calculating the coefficient of variation (CV) across the four replicate spots for each antigen. Measurements with CV >20% were excluded, and retained values were averaged to generate a single antigen-specific response per sample. Pooled adult serum controls were used to monitor inter-slide consistency over time. All data processing and quality control steps were implemented in R (version 4.4.2).

Enzyme-linked immunosorbent assay (ELISA)

Measles-specific IgG was quantified using a conventional direct ELISA. Plates were coated overnight at 4°C with 2.30 μg/mL measles antigen diluted in PBS. After blocking with 5% skimmed milk for 1 hour at 37°C, serum samples (1:100 dilution) were added and incubated for 1.5 hours at 37°C. Plates were washed with PBST and incubated with HRP-conjugated secondary antibody (1:100 dilution) for 1 hour. Following additional washes, 100 μL of OPD substrate solution (30 mg OPD in 30 mL PBS with 30 μL H_2O_2) was added and incubated in the dark for 10 minutes. Reactions were stopped with 50 μL of 2.5 M sulfuric acid, and absorbance was measured at 490 nm.

Statistical analysis

All statistical analyses were performed using R (version 4.4.2). Antibody responses between cohorts were compared using the Wilcoxon rank-sum test. P-values <0.05 were considered statistically significant. To compare antibody responses between the Junju and Ngerenya cohorts, longitudinal analyses were performed using linear mixed-effects models, which accommodate unbalanced data and allow inclusion of all available observations without requiring imputation. Antibody responses were modelled as a function of cohort, with age included as a non-linear term and a random intercept for each child to account for repeated measurements. From these models, we estimated the average age-adjusted difference in antibody responses between cohorts across

the full follow-up period. P-values were adjusted for multiple antigen testing using the false discovery rate (FDR) method. To examine the relationship between malaria exposure and heterologous antibody responses, we used cumulative febrile malaria episode count derived from longitudinal surveillance data as a measure of long term exposure. Antibody measurements were log transformed prior to analysis, and values for each antigen were standardised to z scores to enable comparison of responses to different antigens with differing dynamic ranges. Associations between malaria exposure and antibody responses were assessed using linear mixed-effects models, with malaria episode count as the primary exposure, age modelled as a non-linear term, and random intercepts for both child and antigen to account for repeated measurements and between-antigen variability. For visualisation, unadjusted scatterplots with fitted linear trends were used to illustrate the relationship between malaria episode burden and antibody responses, stratified by antigen. To assess potential population-level differences in nutritional status between regions, we analysed contemporaneous hospital-based surveillance data from the same geographic populations. Anthropometric measures (mid-upper arm circumference (muac), weight-for-age, and height-for-age) were modelled using linear mixed-effects regression, with location (Junju vs Ngerenya) as the primary exposure. Age and calendar year were modelled using natural cubic splines to account for non-linear effects, and models were adjusted for concurrent infections (RSV, parainfluenza, influenza A, human metapneumovirus, OC43, and malaria). Data were anonymised and delinked from all personally identifiable information.

Ethics statement

The study was approved by the Scientific and Ethics Review Unit (SERU) of the Kenya Medical Research Institute. All procedures were conducted in accordance with the principles of Good Clinical Laboratory Practice (GCLP).

Results

A natural experiment in coastal Kenya reveals sharply divergent malaria exposure trajectories in early childhood

Between 1998 and 2017, two rural communities in coastal Kenya - Junju and Ngerenya - underwent markedly different transitions in malaria transmission. Both regions experienced a high malaria burden in the 1990s and early 2000s. However, beginning in 2004, transmission in Ngerenya declined sharply and remained near zero for more than a decade, while Junju continued to experience sustained endemicity well into the mid-2010s. To quantify the scale and timing of this transition, we analysed surveillance data collected from August 1998 to April 2017. A total of 1,243 children were followed in Ngerenya and 659 in Junju. In Ngerenya, the proportion of febrile surveillance visits with confirmed P. falciparum parasitaemia declined from 22.4% before 2004 (1,378 of 6,148 visits) to just 1.1% after 2004 (48 of 4,389 visits). In contrast, Junju children continued to experience high rates of febrile malaria, with 17% of visits testing positive between 2007 and 2017 (869 of 5,130 visits) - Fig. 1. Baseline cohort characteristics are shown in Table 1.

To assess whether the protein microarray platform accurately captured longitudinal P. falciparum-specific immune responses, we examined IgG levels against the plasmodium apical membrane antigen 1 (AMA1) in individual children with known malaria exposure histories. Among children with multiple confirmed episodes of febrile malaria detected via weekly active surveillance, AMA1-specific IgG levels increased sharply over time, tracking closely with the timing of clinical infections (example shown in Fig. 2a), while children from Ngerenya who remained malaria-free throughout follow-up exhibited consistently low IgG levels against AMA1 over more than a decade of surveillance (example shown in Fig. 2b). These patterns mirrored expected immunological trajectories of repeated exposure versus non-exposure, confirming that the microarray platform is capable of detecting meaningful variation in P. falciparum-specific humoral responses. We then extended this analysis to assess longitudinal IgG responses to AMA1 in a subset of 123 children drawn from the two surveillance cohorts. These children contributed a

Figure 1. **Divergent malaria exposure histories in coastal Kenya**

Monthly malaria case counts from active surveillance in two adjacent regions of Kilifi County, Kenya, between 1998 and 2017. Junju (red) maintained sustained malaria transmission throughout the study period, while Ngerenya (blue) experienced a rapid collapse in transmission beginning in 2004. Points represent total cases per month; lines show smoothed trends generated using locally weighted regression (loess) in R (span = 0.8).

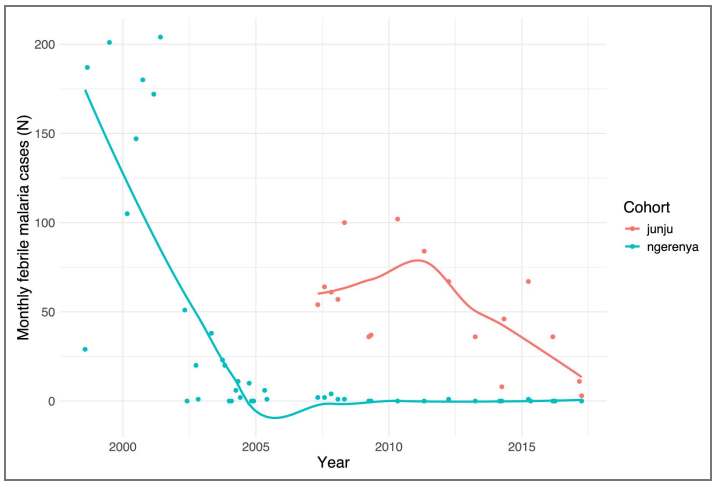


Table 1. **Baseline characteristics of the longitudinal cohorts from Junju and Ngerenya**

Characteristic	Overall	Ngerenya	Junju
Participants, n	123	65	58
Male, n (%)	68 (55.3%)	35 (53.8%)	33 (56.9%)
Female, n (%)	55 (44.7%)	30 (46.2%)	25 (43.1%)
Visits per participant, median (IQR)	10 (9–11)	11 (10–12)	9 (8–9)
Total serum samples, n	1222	717	505
Age at entry, years (median, IQR)	3 (1–3)	2 (1–3)	3 (2–4)
Age at exit, years (median, IQR)	14(13–15)	15 (14–15)	13 (12–13)
Follow-up Years	2002-2017	2002-2017	2007-2017

total of 1222 serum samples, with a median of 10 samples per child, collected between 2002 and 2017 (Supplementary Fig. 1). This subset was selected on the basis of the relative completeness of their longitudinal follow-up serum sampling and included 58 children from Junju (505 samples) and 65 from Ngerenya (717 samples). AMA1-specific IgG levels diverged sharply between cohorts early in life and remained distinct throughout follow-up. In Ngerenya, levels declined rapidly after 2003, stabilising at lower levels by mid-childhood. In contrast, Junju children maintained elevated levels across all time points (Fig. 2c).

Malaria-exposed children exhibit lower antibody levels to non-malarial antigens

To examine the impact of differential malaria endemicity on the antibody response to non-malarial antigens, we first compared IgG responses to the measles virus among Junju and Ngerenya children. We started by validating the ability of the in-house protein microarray platform to detect biologically meaningful measles-specific IgG responses by examining longitudinal measles levels in individual children with known vaccination histories. Among children with a documented history of receiving all three recommended doses of measles vaccine, we observed a sharp rise in IgG levels following immunisation, followed by sustained levels into later childhood (example shown in Fig. 3a). In contrast, unvaccinated children exhibited consistently low IgG trajectories across all time points (example shown in Fig. 3b). These patterns were reproducible across the cohort and recapitulated expected vaccine-induced versus naïve antibody dynamics, supporting the use of this platform for population-level serological inference. Using this platform, we then compared IgG responses to measles virus among children from Junju and Ngerenya. This analysis was restricted only to children with a documented record of measles vaccination. Despite matched vaccination histories, children from Junju - where malaria transmission remained high - consistently exhibited lower measles-specific IgG titres than children from Ngerenya, where malaria transmission had declined. This difference was evident from the earliest timepoints and persisted throughout childhood. Annual antibody measurements showed that mean measles-specific IgG levels were higher in Ngerenya than in Junju in every sampling year (Fig. 3c). To validate these findings, we measured measles-specific IgG levels by ELISA in a subset of 3-year-old children who had completed measles vaccination at least one year prior. The assay included a negative control (PBS) and pooled adult serum as a positive reference. Consistent with the microarray results, Junju children again displayed significantly lower IgG levels than their Ngerenya counterparts (Fig. 3d).

Early-life malaria exposure is associated with long-term suppression of antibody responses

To assess whether the attenuation of antibody responses extended beyond measles, we compared IgG levels to a broader panel of antigens, including vaccine-preventable pathogens (*Bordetella pertussis*, H1N1 influenza virus, rubella virus, and measles virus) and common childhood infections (cytomegalovirus (CMV), Epstein–Barr virus (EBV), herpes simplex virus 1 (HSV-1), and coxsackievirus B1). Using mixed-effects models incorporating all available longitudinal measurements, children from Ngerenya exhibited higher antibody responses than those from Junju after adjustment for age and repeated measurements within individuals (Fig. 4a,b). Effect estimates were consistent in direction across most antigens, with particularly marked differences for HSV-1, EBV and measles. Differences were also observed for coxsackievirus B1 and *Bordetella pertussis*, while smaller or non-significant differences were seen for CMV, rubella, and H1N1 influenza. Several of these associations remained statistically signifcant after correction for multiple testing (Fig 4b). To complement these longitudinal analyses, we performed cross-sectional comparisons of antibody responses at 10 years of age. This showed a similar pattern, with children from Junju exhibiting lower IgG levels for most antigens compared to their Ngerenya counterparts (Fig. 4c). Differences were particularly marked for coxsackievirus, EBV, HSV-1, and measles. Antibody responses to the 2009 pandemic strain of H1N1 were the least differentiated between cohorts.

Figure 2. **AMA1-specific IgG trajectories mirror individual and regional malaria exposure**

(A–B) Longitudinal AMA1 IgG levels in individual children measured by protein microarray. Vertical red lines denote confirmed febrile malaria episodes. Panel A shows a Junju child with multiple documented infections; Panel B shows a Ngerenya child who remained malaria-free throughout follow-up. Each blue spot is a single antibody measurement. Each time point was measured in quadruplicate **(C)** Mean AMA1-specific IgG levels with 95% confidence intervals for all children in the microarray subset plotted by year of sampling. Junju children showed persistently elevated antibodies, while AMA1 antibody levels in Ngerenya declined sharply after 2004.

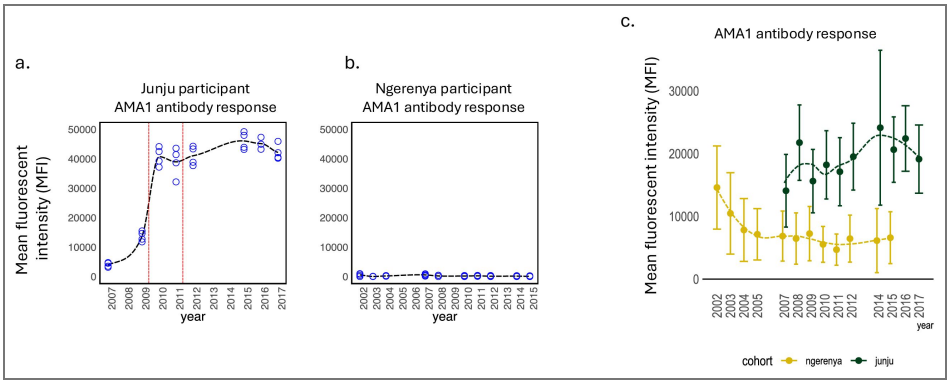
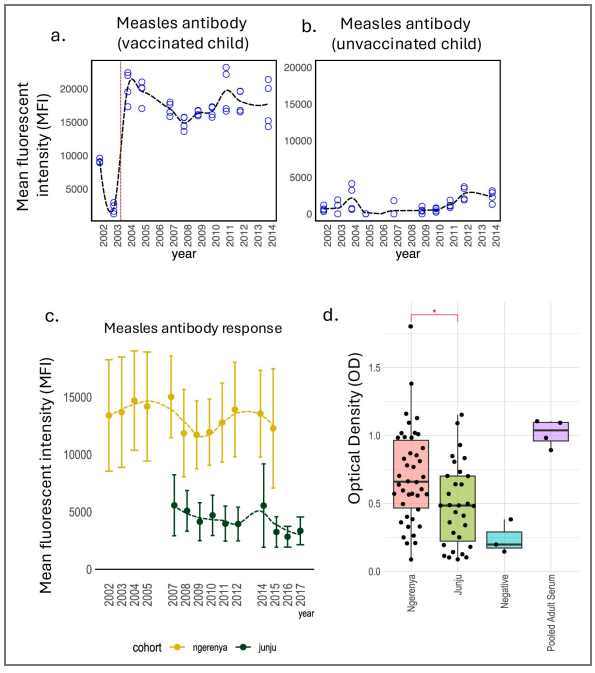


Figure 3. **Malaria exposure is associated with reduced measles-specific antibody levels.**

Example plots of temporal changes in measles-specific antibody in vaccinated and unvaccinated children are shown **(A)** Longitudinal IgG levels in an individual child measured by microarray. The dashed vertical red line indicates the timing of the last dose of the routine measles vaccine. **(B)** Shows a similar temporal trend for a child with no history of measles vaccination. **(C)** Longitudinal IgG responses to measles virus by cohort, measured by protein microarray in vaccinated children. Junju children exhibited consistently lower levels of measles-specific antibody than Ngerenya counterparts. The circles indicate means, and the whiskers denote 95% confidence intervals. **(D)** Measles-specific IgG levels in 3-year-old children from Junju and Ngerenya, measured by ELISA in children with a documented history of measles vaccination, including a negative control (PBS) and pooled adult serum as a positive reference Each dot represents an individual participant.



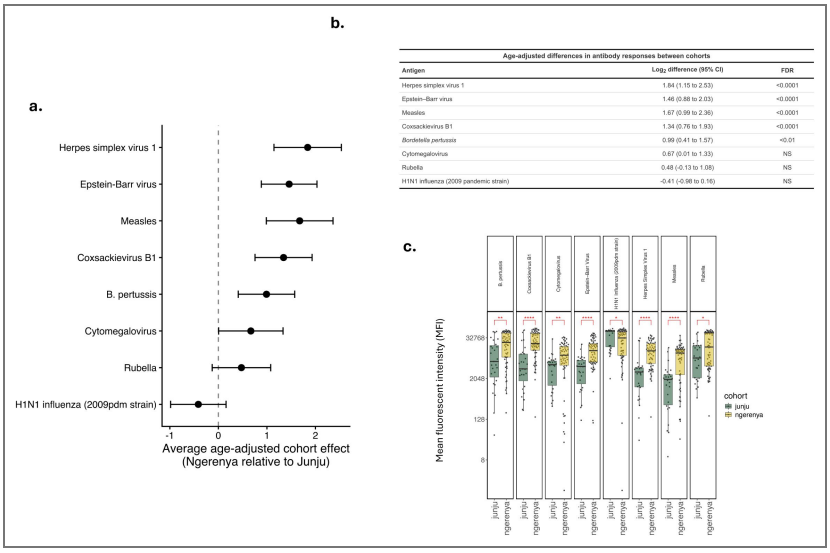


Figure 4. Early-life malaria exposure is associated with reduced antibody responses to multiple antigens.

(A) Forest plot showing the average age-adjusted difference in $\log_2$ antibody responses between children from Ngerenya and Junju, estimated using mixed-effects models incorporating all available longitudinal measurements. Points represent model estimates and horizontal bars indicate 95% confidence intervals. **(B)** Summary table of model-derived estimates, including $\log_2$ differences with 95% confidence intervals and false discovery rate (FDR)-adjusted significance across antigens. **(C)** Cross-sectional comparison of antibody responses at 10 years of age, shown for reference. Boxes indicate interquartile ranges, centre lines denote medians, and whiskers represent 1.5× the interquartile range. Asterisks indicate significance from Wilcoxon rank-sum tests (* P < 0.05, ** P < 0.01, *** P < 0.001, ****. P < 0.0001).

To determine whether the attenuation of antibody responses could still be attributed to early-life malaria exposure independent of geographic or environmental differences, we conducted a stratified analysis within the Ngerenya cohort, which experienced a sharp decline in malaria transmission beginning in 2004. Due to stochastic differences in malaria infection around the time of this inflection, children in Ngerenya had different malaria exposure histories despite living in the same area. At the 10-year-of-age time point, sera were available for 62 out of the 65 children that were originally selected in the Ngerenya cohort subset for serological analysis. Of these, 20 experienced one or more episodes of febrile malaria during early childhood prior to the decline in malaria transmission, while 42 remained entirely malaria-free throughout follow-up (Fig. 5a). At 10 years of age, Ngerenya children who had experienced early-life malaria exhibited significantly lower IgG levels to a wide range of antigens compared to their malaria-naïve peers (Fig. 5b). These included responses to Coxsackievirus B1, CMV, H1N1 influenza, HSV-1 and rubella. Differences in antibody level to *B. pertussis* and EBV did not reach statistical significance.

Population-level comparison of anthropometric and infection profiles between regions

Because anthropometric measurements were not collected routinely within the longitudinal malaria cohorts, we assessed potential population-level differences in nutritional status using contemporaneous hospital-based surveillance data from the same geographic regions. This dataset comprised repeated measurements of mid-upper arm circumference (MUAC), weight-for-age, and height-for-age across early childhood, alongside virological and malaria diagnostics, providing an independent view of the underlying populations from which the longitudinal cohort was drawn. Across early childhood, age-specific distributions of MUAC, weight-for-age, and height-for-age were broadly similar between children from Junju and Ngerenya, with overlapping distributions at all ages (Fig. 6a). To formally assess these differences, we fitted regression models adjusting for age, calendar year, and concurrent infections (RSV, parainfluenza, influenza A, human metapneumovirus, OC43, and malaria). Across all three anthropometric indices, there was no evidence of systematic differences between the two populations (Fig. 6b,c). Adjusted differences between Junju and Ngerenya were small and centred around zero (MUAC: −0.12, 95% CI −0.38 to 0.15; weight-for-age: −0.05, −0.28 to 0.19; height-for-age: 0.08, −0.17 to 0.33). Notably, effect estimates were small and confidence intervals spanned zero in all cases.

Higher malaria episode burden is associated with a graded reduction in heterologous antibody responses

To further examine whether the attenuation of antibody responses varied in relation to the intensity of malaria exposure, we analysed the association between cumulative febrile malaria episode count and antibody responses in the longitudinal cohort. Antibody responses were standardised within antigen to enable comparison for the panel of heterologous antigens. In mixed-effects models incorporating all available longitudinal measurements and adjusting for age and repeated measures, higher malaria episode burden was associated with lower heterologous antibody responses ($\beta = -0.086$, 95% CI −0.142 to −0.029, $p = 0.003$). When examined separately by antigen, the direction of association was consistent for the majority of antigens, including Bordetella pertussis, coxsackievirus B1, Epstein–Barr virus, herpes simplex virus 1, measles virus, and rubella virus, with no evidence of opposing trends. Associations for cytomegalovirus and H1N1 influenza were weaker, but did not contradict the overall pattern (Fig. 7).

Discussion

This study demonstrates that early-life exposure to malaria is associated with broad and durable impairments in antibody-mediated immunity to unrelated pathogens and vaccines. By leveraging a natural experiment in coastal Kenya - where malaria transmission diverged sharply between adjacent communities during the early 2000s - we were able to disentangle the immunological effects of malaria exposure from confounding geographic and temporal factors. Our findings reveal that children exposed to malaria in early childhood not only generate lower antibody titres

Figure 5. Early-life malaria exposure predicts long-term suppression of antibody responses within the same geographic region

(A) Active malaria surveillance records for children in the Ngerenya cohort. Each row represents an individual child, and each column represents a surveillance timepoint. Dark red boxes indicate one or more confirmed febrile malaria episodes; light grey boxes indicate surveillance visits without malaria detection. Children are grouped by early-life exposure status (top: malaria-naïve; bottom: previously exposed). **(B)** IgG levels at 10 years of age among Ngerenya children, stratified by early-life malaria exposure. Children with ≥1 confirmed febrile malaria episode during early childhood (n = 20) show significantly lower titres to multiple unrelated pathogens compared to malaria-naïve peers (n = 42). All children lived in the same geographic area and received identical vaccines and follow-up. The black dots are means and error bars are 95% confidence intervals.

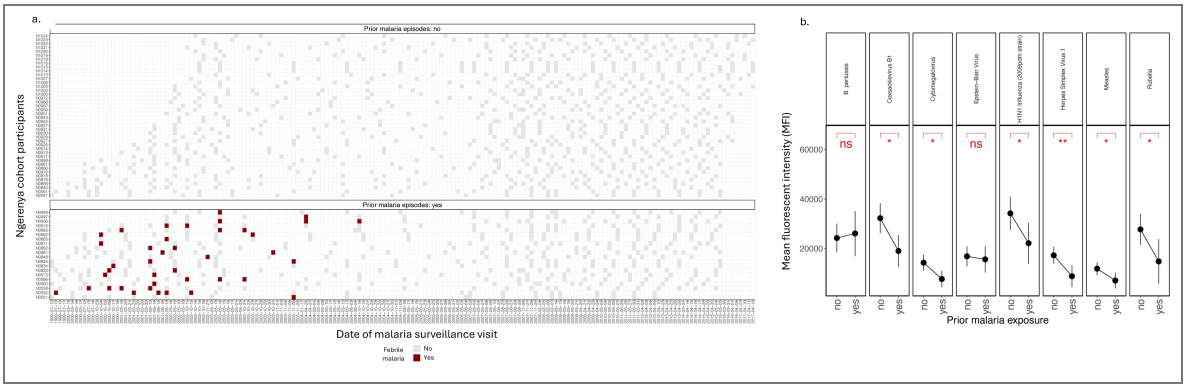
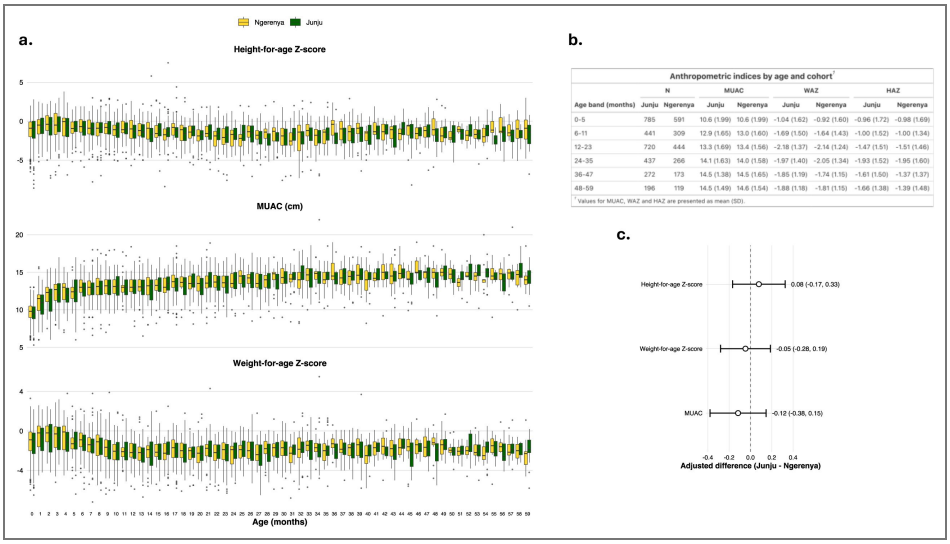


Figure 6. Population-level comparison of anthropometric profiles between Junju and Ngerenya.

(A) Age-specific distributions of height-for-age (HAZ), mid-upper arm circumference (MUAC), and weight-for-age (WAZ) among children aged 0–59 months, derived from contemporaneous hospital-based surveillance data. Boxplots show median and interquartile range, with whiskers extending to 1.5× the interquartile range; points represent individual observations. Distributions are shown separately for children from Junju and Ngerenya. **(B)** Summary of anthropometric indices by age band and location. Values are presented as mean (standard deviation) for MUAC and mean (95% confidence interval) for WAZ and HAZ. **(C)** Adjusted differences in anthropometric indices between children from Junju and Ngerenya. Points represent model-derived estimates for the difference (Junju − Ngerenya), and horizontal lines indicate 95% confidence intervals. Estimates were obtained from regression models adjusting for age (modelled using splines), calendar year, and concurrent infections (RSV, parainfluenza, influenza A, human metapneumovirus, OC43, and malaria)



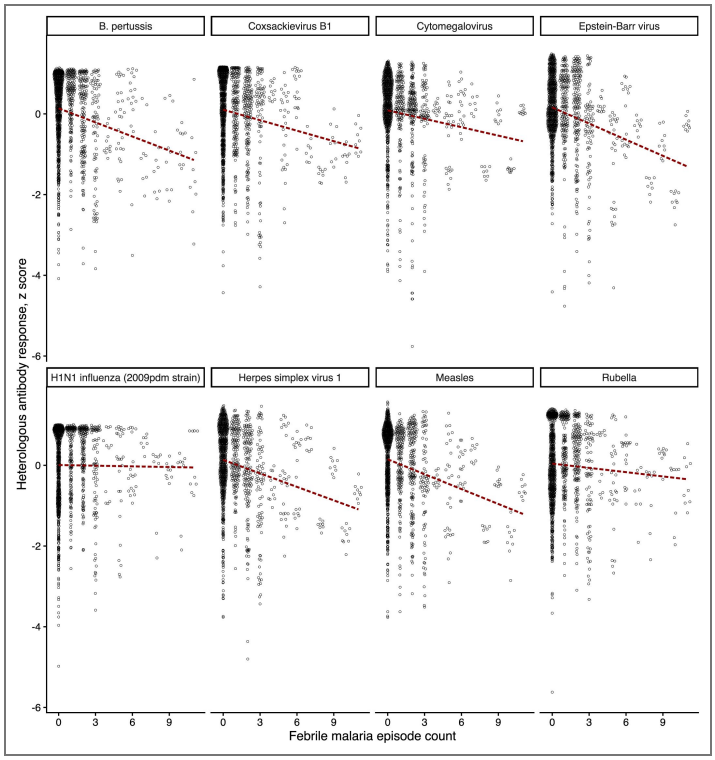


Figure 7. Febrile malaria episode burden is inversely associated with heterologous antibody responses.

Scatterplots show the relationship between cumulative febrile malaria episode count and standardised antibody responses (z scores) to eight heterologous antigens. Each point represents an individual observation, and dashed lines indicate fitted linear trends. For most antigens, higher malaria episode burden was associated with lower antibody responses, with no evidence of opposing trends.

to non-malarial antigens but maintain these attenuated responses well into adolescence, long after malaria transmission has ceased. The attenuating effect of malaria on antibody responses has long been suspected [15] but difficult to quantify. Prior studies have shown reduced vaccine efficacy in malaria-endemic settings [3,16], and experimental models have implicated regulatory T cells, atypical memory B cells, and B cell exhaustion as potential mediators of malaria-induced immune suppression[9,12,13,22]. However, most human studies to date have focused on short-term immune responses or outcomes in the setting of concurrent parasitaemia. Our data extend this work by demonstrating that transient exposure to malaria in early childhood is sufficient to imprint long-lasting changes on the humoral immune repertoire.

The strength of this study lies in its integration of long-term active malaria surveillance with serial antibody profiling. Children were visited weekly for febrile illness surveillance, allowing for precise documentation of clinical malaria episodes. In parallel, our use of a validated protein microarray platform enabled us to track IgG responses to a wide panel of pathogen and vaccine antigens at multiple timepoints. We first confirmed that the microarray platform captured biologically relevant responses by comparing antibody trajectories in individual children with known measles vaccination and malaria exposure histories. The platform reliably detected both vaccine-induced and infection-associated rises in IgG, providing confidence in the longitudinal patterns observed. We found that children from Junju, a region of persistent transmission, had significantly lower antibody levels to multiple pathogens compared to children from Ngerenya, where malaria transmission declined in the mid-2000s. To ensure that these differences were not driven by cross-sectional comparisons at a single timepoint, we additionally analysed antibody responses using mixed-effects models incorporating all available longitudinal measurements. Across multiple antigens, children from Ngerenya exhibited higher antibody responses than those from Junju after adjustment for age and repeated measurements within individuals. Effect estimates were consistent in direction across most antigens and remained evident after correction for multiple testing, indicating that the observed differences reflect a generalised, age-adjusted cohort effect rather than a feature of a specific age or sampling timepoint. Importantly, all children had comparable vaccination histories and were followed through the same longitudinal infrastructure, minimising the likelihood of differential healthcare access or vaccine uptake.

To test whether early-life malaria exposure specifically contributed to long-term immune suppression, we examined antibody responses within the Ngerenya cohort. Because the transmission decline occurred rapidly, children born just before and after the inflection point experienced markedly different levels of malaria exposure while living in the same geographic area. Among children followed longitudinally, those with even limited early-life exposure to malaria had significantly lower antibody titres at 10 years of age than their malaria-naïve peers. This within-cohort contrast strongly implicates early-life infection as the critical window for immune programming. Taken together, these findings support a model in which malaria exposure during critical developmental windows modulates long-term maintenance of immune memory. This may occur through direct effects on B cell maturation, altered antigen presentation[23], or long-lived changes in lymphoid microenvironments[24–26]. While this study was not designed to resolve the mechanistic basis of these observations, the pattern we describe is consistent with a growing body of evidence highlighted above, that malaria infection can induce sustained perturbations in both B cell and T cell compartments. These studies have demonstrated expansion of atypical memory B cells, disruption of germinal centre responses, and increased regulatory immune activity following malaria exposure, all of which may impair the generation and maintenance of effective humoral immunity. In this context, our findings provide population-level evidence of a durable alteration in antibody profiles associated with early-life malaria exposure. The extent to which these changes reflect persistent alterations in immune cell function or the cumulative effects of repeated infection remains to be determined and will require targeted mechanistic studies. Consistent with this, analyses using cumulative febrile malaria episode count demonstrated a graded inverse association between malaria burden and antibody responses across a broad panel of heterologous antigens, with effects evident across childhood. This supports a dose-dependent relationship between malaria exposure and long-term attenuation of humoral immunity.

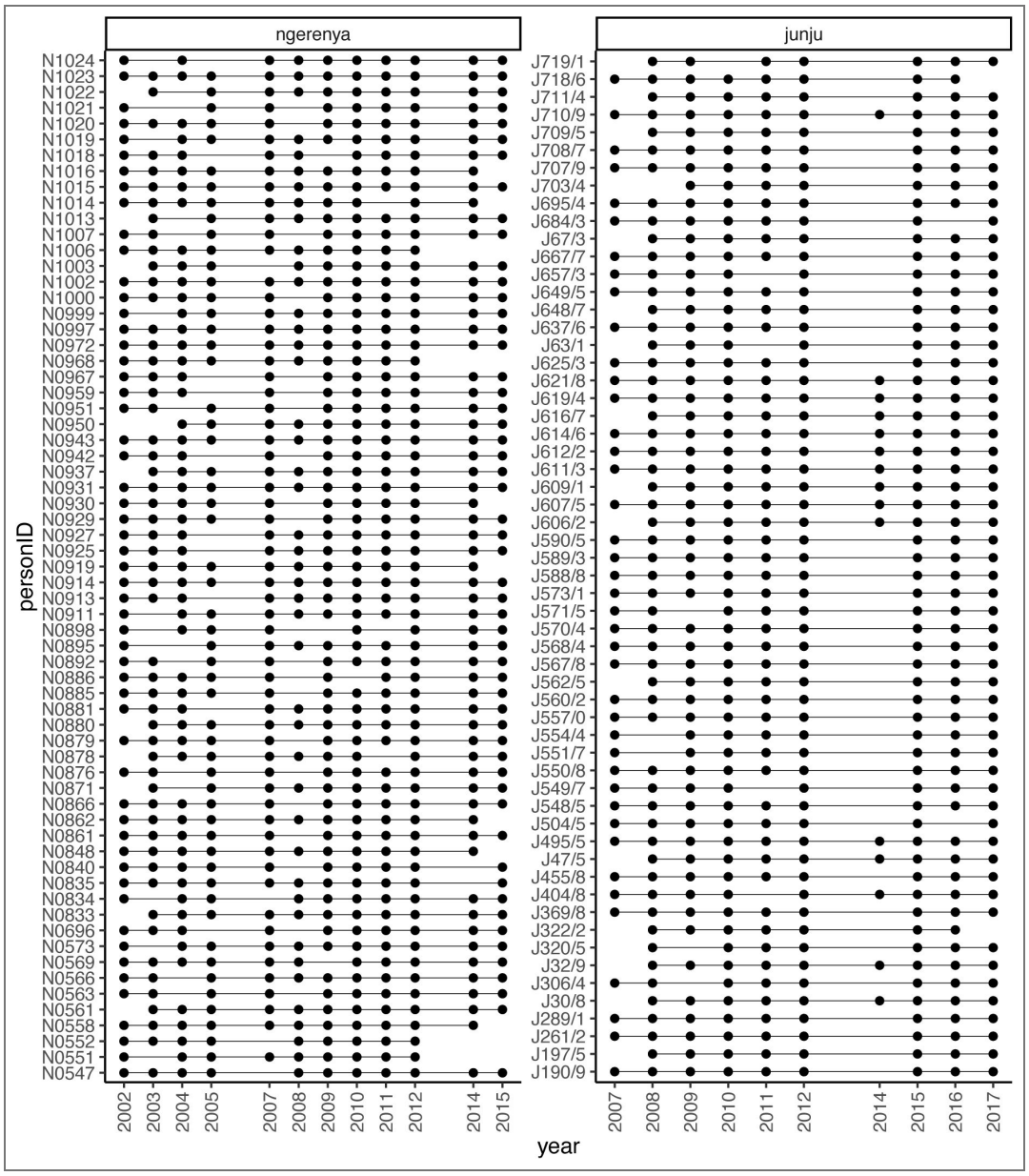
This work has important implications for vaccine policy and infection risk in malaria-endemic regions. Reduced antibody titres to vaccine-preventable diseases may translate into diminished long-term protection, even when vaccine coverage is high. Our findings raise the possibility that children in high-transmission settings may require different immunisation strategies, such as delayed dosing and boosting, to improve vaccine-induced immunity. Moreover, as malaria control efforts continue to shift disease epidemiology, these data highlight the value of longitudinal serological surveillance for understanding the broader immunological legacy of malaria exposure. Our study has several limitations. Although the microarray platform was validated internally and against ELISA, quantitative comparisons across platforms are inherently constrained.

Additionally, while the sample size for antibody profiling was modest, the inclusion of dense longitudinal sampling with weekly malaria surveillance and repeated serological measurements provides high-resolution insight into exposure and immune response trends, and the consistency of findings across multiple antigens, analytical approaches, and assay platforms supports the robustness of the observed effects. Finally, we cannot fully exclude the influence of other infections or environmental exposures that may have differed subtly between subgroups, although the within-Ngerenya analysis provides strong evidence for malaria as a primary driver of long-term immune attenuation. Because the longitudinal cohort was originally designed to characterise the acquisition of naturally acquired immunity to malaria, anthropometric measurements were not collected systematically within that dataset, precluding direct adjustment for nutritional status in the primary analyses. To address this, we analysed contemporaneous hospital-based surveillance data from the same geographic regions, comprising measurements of anthropometry and infection status in early childhood. Across three independent indices of nutritional status (MUAC, weight-for-age, and height-for-age), we found no evidence of systematic differences between children from Junju and Ngerenya after adjustment for age, calendar year, and concurrent infections. Effect estimates were small, crossed zero. As the longitudinal cohorts in Junju and Ngerenya were drawn from these underlying populations, these findings suggest that the two groups were broadly comparable with respect to early-life growth and nutritional status, and make it unlikely that nutritional differences are a major driver of the observed immunological patterns.

While this study demonstrates consistent and durable differences in antibody levels across a wide range of antigens, it does not include functional immunological assays to determine the downstream consequences of these differences. As such, we are unable to directly assess whether the lower antibody titres observed in malaria-exposed children translate into reduced neutralising capacity or diminished clinical protection. The primary aim of this study was to identify long-term alterations in humoral immune profiles associated with early-life malaria exposure, rather than to resolve their functional significance. Future studies incorporating functional assays and clinical outcome data will be important to determine the extent to which these serological differences translate into altered susceptibility to infection.

In summary, our findings reveal that early-life malaria exposure is associated with long-term suppression of antibody responses to unrelated pathogens and vaccines. This effect is detectable many years after infection and appears to persist even in the absence of ongoing transmission. As global malaria control efforts continue, understanding the immunological legacy of childhood malaria may be critical for improving vaccination strategies and mitigating susceptibility to other infections.

Supplementary Materials



Supplementary Figure 1. A summary of the sampling frame for the study cohorts (Junju and Ngerenya). Each vertical line represents the longitudinal time series for a single individual, and each dot represents a timepoint where a serum sample was collected.

Pathogen	Strain/subtype	Antigen
Measles virus	Edmonston	Whole virus antigen
Coxsackie B virus	B1	VP1
Cytomegalovirus	TB40E	PP150
Epstein-Barr virus	Type 1	Nuclear antigen 1
Herpes simplex virus	Type 1	Extract
H1N1 Influenza A	A/California/07/2009	Haemagluttinin
Plasmodium falciparum	3D7	AMA-1
Rubella virus	HPV-77	Whole virus antigen

Supplementary Table 1. Summary of pathogens, strains or subtypes, and corresponding antigens included in the immunoassay panel.

Data availability

De-identified microarray antibody data underlying the main analyses are provided in the supplementary files. The full datasets generated and analysed during this study are not publicly available due to ethical and legal restrictions governing the use of individual-level data collected within the Kilifi Health and Demographic Surveillance System (KHDSS). These data contain sensitive participant information and are subject to local data protection regulations and institutional policies. Additional de-identified data may be made available to qualified researchers upon reasonable request, subject to approval by the KEMRI-Wellcome Trust Research Programme Data Governance Committee and in accordance with applicable ethical and regulatory requirements.

Additional information

Funding

Funder	Grant reference number	Author
Wellcome	https://doi.org/10.35802/105882	Charles J Sande

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James O Tuju, Charles J Sande: Contributed equally
Competing interests: No competing interests declared
Funding statement: This study was supported by fellowship funding to C.J.S. from the Wellcome Trust (WT105882MA). The funder played no role in the conceptualization, design, data collection, analysis, decision to publish, or preparation of the manuscript. M.S.S was funded in whole by Science for Africa Foundation to the Developing Excellence in Leadership, Training, and Science in Africa (DELTAS Africa) program [DEL-22-012] with support from Wellcome Trust and the UK Foreign, Commonwealth & Development Office and is part of the EDCPT2 programme supported by the European Union. For purposes of open access, the author has applied a CC BY public copyright license to any Author Accepted Manuscript version arising from this submission.

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Peer reviews

Reviewer #2 (Public review):

Summary:

The authors investigated whether early-life malaria exposure has long-term effects on immune responses to unrelated antigens. They leveraged a natural experiment in coastal Kenya where two adjacent communities (Junju and Ngerenya) experienced divergent malaria transmission patterns after 2004. Using 15 years of longitudinal data from 123 children with weekly malaria surveillance and annual serological sampling, they measured antibody responses to multiple pathogens using a protein microarray technology and ELISA.

Strengths:

(1) Extensive longitudinal data collection with weekly malaria surveillance, enabling precise exposure classification.

(2) Use of a natural experiment design that allows for causal inference about malaria's immunological effects.

(3) Broad panel of antigens tested, demonstrating generalized rather than antigen-specific effects.

(4) Within-cohort analysis in Ngerenya controls for geographic and environmental factors.

(5) Validation of key findings using both serologic microarray and ELISA.

(6) Important public health implications for vaccine strategies in malaria-endemic regions.

Weaknesses:

(1) Due to its nature, the study lacks the ability to determine the direction of the associations found between malaria exposure and other IgG levels to unrelated pathogens.

(2) No evaluation of the clinical Implications of the reduced IgG levels observed in the area with high malaria exposure.

Assessment of Claims:

The data appear to support the authors' primary claims. The strength of the evidence is limited by the observational nature of the study and the results should be interpreted in that light. Together with the currently available evidence of P. falciparum's impact on the host's immune function, this natural experiment design provides further evidence for a relationship between early malaria exposure and reduced antibody responses to other pathogens and vaccine-derived antigens. The within-Ngerenya analysis controls for geographic factors and thus enhances the quality of the evidence; there is limited physical, nutritional, and socio-economic information on factors that may have driven the observed changes.

Impact and Utility:

This work has fundamental implications for understanding vaccine effectiveness in malaria-endemic regions and may contribute to inform vaccination strategies. The findings, if confirmed, would suggest that children in areas of high malaria transmission may require modified immunization approaches. The dataset provides a valuable resource for future studies of malaria's immunological legacy.

Context:

This study builds on prior work showing acute immunosuppressive effects of malaria but uniquely attempts to demonstrate the durability of these effects years after exposure. The natural experiment design addresses limitations of previous observational studies by providing a more controlled comparison.

https://doi.org/10.7554/eLife.107820.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***eLife Assessment***
>
> *This important study sought to investigate the role that early childhood malaria exposure plays in the development of antibody responses to unrelated pathogens and vaccine-derived antigens in Kenyan children. In this natural experiment, the authors compare antibody levels among children who have been exposed to different levels of malaria transmission by using protein microarray technology. Although the findings are of importance, the evidence remains incomplete, and the analysis would benefit from a more in-depth evaluation of potential confounders. With the appropriate analysis, the findings will be of great interest for global health, immunology, and vaccine development.*

We thank the editors for highlighting the need for a more comprehensive evaluation of potential confounding. We agree that this is a critical aspect of the study and have now undertaken additional analyses to address this directly.

The original longitudinal cohort was designed to investigate the acquisition of naturally acquired immunity to malaria and did not include systematic collection of anthropometric/nutritional, environmental or socioeconomic data, precluding direct adjustment for these factors within the primary dataset. However, to assess whether there were population-level differences in these factors, we leveraged contemporaneous hospital-

based surveillance data from the same geographic regions, which includes measurements of anthropometry and nutritional status (muac, weight-for-age, and height-for-age) and detailed infection diagnostics.

Using this independent dataset, we fitted mixed-effects regression models adjusting for age, calendar year, and concurrent infections (RSV, parainfluenza, influenza A, human metapneumovirus, OC43). For all three anthropometric indices, we found no evidence of systematic differences between children from Junju and Ngerenya. Adjusted differences were small and centred around zero (muac: −0.12, 95% CI −0.38 to 0.15, weight-for-age: −0.05, −0.28 to 0.19, height-for-age: 0.08, −0.17 to 0.33), with no consistent directional effect.

As the longitudinal cohort was randomly selected from these underlying populations, these findings suggest that the groups were broadly comparable with respect to nutritional status and there were no differences in their exposure to the infections that were included in the analysis. We have incorporated these analyses into the revised manuscript, added a new figure focussed on this analysis -fig. 6, updated the statistical analysis and discussion sections), and believe they substantially strengthen the evidence by addressing a key source of potential confounding.

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *The study shows that childhood malaria can weaken the antibody response to other vaccines and infections. This suggests that early exposure to P. falciparum may have a long-lasting effect on immunity, with implications for vaccine efficacy in endemic areas.*
>
> *Strengths:*
>
> *This study stands out for its longitudinal design, the use of robust immunological techniques, and the comparison between areas with different levels of malaria exposure. Its findings reveal that early malaria can weaken the response to childhood vaccines, with important implications for public health in endemic regions.*

We thank the reviewer for this comment

> *Weaknesses:*
>
> *One of the study's main limitations is the lack of functional data confirming the clinical impact of the low antibody levels. Furthermore, although multiple immune responses were measured, other important components, such as cellular immunity, were not assessed. Furthermore, the results may not be generalizable to other regions.*

We thank the reviewer for this important comment and agree that the absence of functional immunological assays is a limitation of the current study. Our analysis was designed to determine whether early-life malaria exposure is associated with durable alterations in antibody responses to unrelated pathogens and vaccine antigens, rather than to establish the downstream functional consequences of these differences. As such, the study is able to demonstrate a broad and persistent attenuation of humoral responses but cannot directly determine whether the lower antibody levels observed translate into reduced neutralising capacity or diminished protection at the individual level.

We have revised the manuscript to make this distinction more explicit. In the revised discussion, we now state that although reduced antibody titres to vaccine-preventable pathogens may have implications for long-term protection, the clinical significance of these

differences remains to be established in future studies incorporating functional assays and clinical outcome data.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *The authors investigated whether early-life malaria exposure has long-term effects on immune responses to unrelated antigens. They leveraged a natural experiment in coastal Kenya where two adjacent communities (Junju and Ngerenya) experienced divergent malaria transmission patterns after 2004. Using 15 years of longitudinal data from 123 children with weekly malaria surveillance and annual serological sampling, they measured antibody responses to multiple pathogens using a protein microarray technology and ELISA.*
>
> *Strengths:*
>
> *(1) Extensive longitudinal data collection with weekly malaria surveillance, enabling precise exposure classification.*
>
> *(2) Use of a natural experiment design that allows for causal inference about malaria's immunological effects.*
>
> *(3) Broad panel of antigens tested, demonstrating generalized rather than antigen-specific effects.*
>
> *(4) Within-cohort analysis in Ngerenya controls for geographic and environmental factors.*
>
> *(5) Validation of key findings using both serologic microarray and ELISA.*
>
> *(6) Important public health implications for vaccine strategies in malaria-endemic regions.*

We thank the reviewer for these comments

> *Weaknesses:*
>
> *(1) Lack of participants' characteristics (socio-economic, nutritional, physical).*

We thank the reviewer for this important comment. We have now included a detailed summary of participant characteristics in Table 1to provide context for the study population. This includes key demographic and longitudinal variables stratified by cohort (Junju and Ngerenya), including sex distribution, age at study entry and exit, duration of follow-up, number of visits per participant, and total serum samples analysed. Detailed data on socio-economic status, nutritional status, and other environmental or physical characteristics were not consistently available across all participants and time points, and therefore could not be included. This has now been explicitly stated as a limitation in the discussion.

> *(2) Somewhat limited sample size (longitudinal analysis of 123 children total), with further subdivision reducing statistical power for some analyses.*

We thank the reviewer for this important observation. The study is based on an intensively followed cohort with weekly malaria surveillance and repeated serological measurements throughout childhood, allowing detailed characterisation of early-life exposure and subsequent immune trajectories. This depth of longitudinal sampling provides resolution that is not achievable in larger cross-sectional studies. We acknowledge that subdivision of the cohort reduces statistical power for some analyses. Nevertheless, the key findings were consistent in several independent comparisons, including a reduction in antibody levels for

broad panel of antigens in the malaria endemic setting, within-cohort analyses in Ngerenya that replicated this observation, and the confirmation of results generated on the protein microarray on the ELISA platform. The consistency of these findings across analytical approaches and measurement platforms reduces the likelihood that the observed effects are driven by small-sample variability. We have clarified this point in the revised discussion to emphasise that the strength of the study lies in the depth and longitudinal resolution of the data rather than the absolute sample size.

> *(3) Potential confounding by unmeasured socioeconomic, nutritional, or environmental factors between communities.*

We thank the reviewer for this important point and agree that residual confounding between communities must be considered. As outlined in reponse to the editorial assesment, we have undertaken additional analyses using contemporaneous population-level data from the same regions and found no evidence of systematic differences in anthropometric indices between children from Junju and Ngerenya after accounting for age, calendar year, and concurrent infections, with effect estimates small and crossing zer. In addition, the within-Ngerenya analysis provides an internal comparison within a shared geographic and environmental setting, reducing the likelihood that unmeasured socioeconomic or environmental differences between communities account for the observed associations. The new analyses suggest that major population-level differences in nutritional status or infection burden are unlikely to explain the observed patterns. We have clarified this point in the revised discussion and explicitly acknowledge the possibility of residual confounding.

> *(4) Lack of ability to determine the direction of the associations found between malaria exposure and other IgG levels to unrelated pathogens.*

We agree that, as an observational study, our analysis cannot definitively establish the direction of the association between malaria exposure and antibody responses to unrelated antigens. However, several features of the study design strengthen the inference that early-life malaria exposure contributes to the observed differences. First, malaria exposure was characterised prospectively through intensive weekly surveillance, allowing precise temporal definition of exposure in early childhood. Second, within the Ngerenya cohort, where children were exposed to different levels of malaria due to a rapid decline in transmission, those with even limited early-life exposure exhibited lower antibody responses at 10 years of age than malaria-naïve peers, despite residing in the same geographic and environmental context. In addition, we now show that these differences are not confined to a single timepoint but are evident across the full longitudinal follow-up after adjustment for age and repeated measurements. While we cannot exclude the possibility of residual confounding or bidirectional relationships, the convergence of evidence from the natural experiment design, within-cohort contrasts, and age-adjusted longitudinal analyses supports early-life malaria exposure as a key contributor to long-term differences in antibody responses. We have clarified this in the discussion.

> *(5) Despite good longitudinal data, the main analysis was conducted as a cross-sectional analysis at age 10 for many comparisons, which limits the understanding of temporal dynamics.*

We thank the reviewer for highlighting this point. While age 10 was initially used as a standardised reference point for cross-sectional comparisons, the underlying dataset is longitudinal, with repeated antibody measurements across childhood. To address this more directly, we have now complemented these analyses with antigen-specific mixed-effects regression models incorporating all available longitudinal data, with adjustment for age and a random intercept for repeated measurements within individuals. These models demonstrate that the differences between cohorts are not confined to the age-10 cross-section but are evident in an age-adjusted longitudinal framework for multiple antigens. We have

retained the age-10 comparisons for reference, but the primary inference is now based on the longitudinal mixed-effects analyses. These changes are reflected in the revised results and statistical analysis sections. We thank the reviewer for this astute point, which we think has substantially improved the manuscript.

> *(6) Statistical analysis is limited to univariable comparisons without consideration for confounders or adjusting for multiple comparisons.*

We agree that the original analyses relied primarily on univariable comparisons. In the revised manuscript, we have extended the analytical framework to include mixed-effects regression models that account for age effects and repeated measurements within individuals. These models estimate the average age-adjusted difference in antibody responses between cohorts across the full follow-up period. We have also applied false discovery rate (FDR) correction to account for multiple antigen testing. For multiple antigens, the direction and magnitude of cohort differences remain consistent under this approach, strengthening the robustness of the findings beyond the original univariable comparisons. These analyses have been incorporated into the revised results and statistical analysis sections.

> *(7) No mechanistic understanding of how early malaria exposure creates lasting immunosuppression.*

We agree that this study does not directly resolve the mechanistic basis underlying the observed long-term differences in antibody responses. The primary aim of this work was to identify and characterise durable alterations in humoral immune profiles associated with early-life malaria exposure, rather than to define the cellular or molecular pathways involved. However, our findings are consistent with a growing body of experimental and clinical literature suggesting that malaria infection can induce sustained perturbations in B cell and T cell compartments, including the expansion of atypical memory B cells, altered germinal centre responses, and increased regulatory immune activity. These mechanisms have been proposed to impair the generation and maintenance of effective humoral immunity. In the revised discussion, we have clarified that the mechanistic basis of this phenomenon remains to be fully defined and have expanded the discussion of plausible pathways in light of existing literature. We now explicitly position our findings as providing population-level evidence of a durable immunological phenotype that warrants further mechanistic investigation.

> *(8) No understanding of the clinical Implications of the reduced IgG levels observed in the area with high malaria exposure.*

We agree that this study does not directly establish the clinical consequences of the reduced antibody levels observed in malaria-exposed children. The primary objective of this study was to characterise long-term differences in humoral immune profiles associated with early-life malaria exposure, rather than to assess downstream clinical outcomes or functional antibody activity. We have clarified this limitation in the revised discussion. Nevertheless, the breadth and consistency of the observed differences for multiple vaccine-preventable and infectious antigens raise the possibility that early-life malaria exposure may have implications for long-term immune protection. We now emphasise in the revised discussion that future studies incorporating functional assays and clinical outcome data will be required to determine whether these serological differences translate into altered susceptibility to infection or reduced vaccine effectiveness.

> *Assessment of Claims:*
>
> *The data appear to support the authors' primary claims, but the strength of the evidence is limited, and the results should be interpreted with caution. Together with the currently available evidence of P. falciparum's impact on the host's immune function, this natural*

> *experiment design provides further evidence for a relationship between early malaria exposure and reduced antibody responses. The within-Ngerenya analysis controls for geographic factors and thus enhances the quality of the evidence, however, it still fails to account for the physical, nutritional, and socio-economic factors that may have driven the observed changes. Additionally, the mechanism underlying this effect remains unclear, and the clinical significance of reduced antibody levels is not established.*

We thank the reviewer for this assessment and for recognising the strengths of the natural experiment design and within-cohort analyses. We agree that, as an observational study, our findings should be interpreted appropriately. In the revised manuscript, we have undertaken additional analyses and clarifications to strengthen the evidential basis of our conclusions and to address the points raised. To address potential confounding by nutritional and related factors, we analysed contemporaneous hospital-based surveillance data from the same geographic populations since nutritional and socioeconomic variables were not consistently collected during the course of longitudinal follow up. For three independent anthropometric indices of nutrition status (muac, weight-for-age, and height-for-age), we found no evidence of systematic differences between children from Junju and Ngerenya after adjustment for age, calendar year, and concurrent infections. As the longitudinal cohort subjects were randomly drawn from these populations, these findings suggest that the two groups were broadly comparable with respect to early-life growth and nutritional status.

We agree that the mechanistic basis of the observed differences is not resolved in this observational study. We have clarified this point in the revised discussion and expanded our consideration of plausible biological pathways based on existing literature, including perturbations in B cell and T cell compartments. Similarly, we now explicitly state that the clinical implications of reduced antibody levels remain to be determined and will require studies incorporating functional assays and clinical outcomes. We believe these revisions strengthen the manuscript by providing a more comprehensive interpretation of the data.

> *Impact and Utility:*
>
> *This work has fundamental implications for understanding vaccine effectiveness in malaria-endemic regions and may contribute to informing vaccination strategies. The findings, if strengthened, would suggest that children in areas of high malaria transmission may require modified immunization approaches. The dataset provides a valuable resource for future studies of malaria's immunological legacy.*

We thank the reviewer for this comment

> *Context:*
>
> *This study builds on prior work showing acute immunosuppressive effects of malaria but uniquely attempts to demonstrate the durability of these effects years after exposure. The natural experiment design addresses limitations of previous observational studies by providing a more controlled comparison.*

We thank the reviewer for this comment

> ***Recommendations for the authors:***
>
> ***Reviewing Editor Comments:***
>
> *We suggest that further analyses of potential confounders such as anthropometric indices, socioeconomic status, and comorbidities would render the evidence more robust.*

We thank the Reviewing Editor for this important suggestion. We agree that careful consideration of potential confounding factors is critical to the interpretation of these findings, and have undertaken additional analyses to address this.

Because anthropometric and related socioeconomic measurements were not collected systematically within the original longitudinal malaria cohort, we assessed potential population-level differences using hospital-based surveillance data from the same geographic regions. This dataset includes measurements of anthropometry (mid-upper arm circumference, weight-for-age, and height-for-age) as well as detailed infection diagnostics in childhood. Using these data, we fitted regression models adjusting for age, calendar year, and concurrent, clinically diagnosed infections. For all three anthropometric indices, we found no evidence of systematic differences between children from Junju and Ngerenya, with effect estimates small and crossing zero (fig. 6). As the longitudinal cohorts were randomly selected from these populations, these findings suggest that the groups were broadly comparable with respect to nutritional status and infection exposure. With respect to socioeconomic status and comorbidities, detailed individual-level data were not available within the longitudinal cohort. However, the within-Ngerenya analysis, where children with differing early-life malaria exposure were compared within the same geographic and environmental setting, provides a complementary control for these factors. We have incorporated these additional analyses and clarifications into the revised manuscript statistical analysis, discussion lines and believe they strengthen the robustness of the findings by addressing key sources of potential confounding.

> ***Reviewer #1 (Recommendations for the authors):***
>
> *The manuscript is well written, with clear and informative figures that effectively support the findings.*

We thank the reviewer for this comment

> *Suggestions:*
>
> *(1) Although the study well controlled for malaria exposure, other environmental or infectious factors that influence immunity could be considered:*
>
> *Nutritional status in childhood (malnutrition impacts immune response), co-infections (helminths, respiratory viruses), socioeconomic differences, or differences in access to health services, even minimal, between Junju and Ngerenya.*

We thank the reviewer for highlighting the potential influence of environmental, infectious, and socioeconomic factors on immune responses. We agree that these are important considerations in the interpretation of observational data. To address nutritional status and concurrent infectious exposures, we analysed contemporaneous hospital-based surveillance data from the same geographic populations. This dataset includes measurements of anthropometric indices (mid-upper arm circumference, weight-for-age, and height-for-age) alongside detailed clinical diagnostics for common childhood infections. Using regression models adjusting for age, calendar year, and concurrent infections, we found no evidence of systematic differences in anthropometric profiles between children from Junju and Ngerenya (fig. 6). These findings suggest that the populations from which the longitudinal cohorts were randomly selected were comparable with regard to early-life growth and nutritional status. We agree that we cannot fully exclude the influence of unmeasured factors such as helminth infections, socioeconomic variation, or subtle differences in healthcare access. However, the within-Ngerenya analysis, where children with differing early-life malaria exposure were compared within the same geographic, environmental, and healthcare setting, provides an internal control for many of these factors. The persistence of similar patterns within this setting supports malaria exposure as a key contributor of the observed differences. We have

clarified these considerations in the revised discussion and believe that, the additional analyses and within-cohort comparisons strengthen the robustness of our conclusions while acknowledging the limitations inherent to observational studies.

> *(2) Measurement of other immunological markers:*
>
> *In addition to IgG, include: B cell subpopulations (naive, memory, atypical), cytokine levels (IL-10, IFN-γ) to characterize the immunological microenvironment.*
>
> *You could include these recommendations in the text for future studies.*

We thank the reviewer for this thoughtful suggestion. We agree that detailed immunophenotyping, including characterisation of B cell subpopulations and cytokine profiles, would provide important insight into the mechanisms underlying the observed differences in antibody responses. In the revised manuscript, we have expanded the discussion to highlight these important avenues for future work, including the potential role of altered B cell subsets (and regulatory or inflammatory cytokine environments in shaping long-term humoral responses).

> ***Reviewer #2 (Recommendations for the authors):***
>
> *The manuscript is well-written.*

We thank the reviewer for this comment

> *(1) Methodological Clarifications:*
>
> *Do the authors have any information regarding the characteristics of these children that could be of use in understanding their immune responses better? (e.g., weight, height, BMI, socioeconomic status, HB level, access to health care, etc.).*

We thank the reviewer for highlighting the importance of participant characteristics in interpreting immune responses. Anthropometric and related clinical measures were not collected systematically within the original longitudinal malaria cohort, as the study was designed to investigate the acquisition of naturally acquired immunity to malaria.

To address this, we analysed contemporaneous hospital-based surveillance data from the same geographic populations, which include measurements of anthropometric indices (mid-upper arm circumference, weight-for-age, and height-for-age) alongside detailed infection diagnostics. Using regression models adjusting for age, calendar year, and concurrent infections, we found no evidence of systematic differences in anthropometric profiles between children from Junju and Ngerenya (fig. 6) Detailed individual-level data on socioeconomic status, haemoglobin levels, and healthcare access were not available within the longitudinal cohort impeding direct adjustment in the longitudinal cohorts. However, the within-Ngerenya analysis, where children with differing early-life malaria exposure were compared within the same geographic and healthcare setting, provides an internal control for many of these factors. These considerations are now clarified in the revised discussion.

> *Could the authors provide more detailed statistical analysis, including power calculations and multiple comparison corrections?*

In the revised manuscript, we have extended the statistical analysis and now include antigen-specific mixed-effects regression models incorporating all available longitudinal measurements, which is comprehensively described in the statistical analysis section. We have also applied false discovery rate (FDR) correction to account for multiple testing across antigens, and report both unadjusted and FDR-adjusted significance in the revised results. With respect to power, the sample size was determined by the number of children meeting

inclusion criteria within the long-term surveillance cohorts in terms of availability of a sufficient number of longitudinal samples. We have clarified this in the revised manuscript.

> *Clarify the criteria for selecting the 123-child subset from the larger surveillance cohorts.*

We thank the reviewer for this comment. The 123 children included in this analysis were selected from the larger surveillance cohorts based on the availability of sufficiently dense longitudinal serum sampling as described above. Specifically, children were required to have at least eight longitudinal samples available in the archive, enabling robust assessment of within-individual antibody trends over time. This criterion was applied to ensure adequate temporal resolution to examine the long-term stability of malaria-associated effects on antibody responses. Children with fewer available samples were therefore excluded, as limited sampling would not allow reliable characterisation of longitudinal patterns. We have clarified these inclusion criteria in the revised manuscript.

> *(2) Additional Analyses and Data Presentation:*
>
> *The authors could consider dose-response analyses relating malaria episode frequency/timing to degree of immunosuppression or even AMA-1 IgG levels and degree of immunosuppression. How do they associate over time?*

We thank the reviewer for this suggestion. To address this, we examined the relationship between malaria exposure (using cumulative febrile malaria episode count derived from longitudinal surveillance data) and the magnitude of heterologous antibody responses. In mixed-effects models adjusting for age and repeated antibody measurements, higher malaria episode burden was associated with lower antibody responses against multiple antigens (fig 7).

> *Analyze whether the effects vary by specific age at malaria exposure.*

We agree that age at exposure is an important consideration. We have now assessed how the relationship between malaria burden and antibody responses varies with age by including age as a non-linear term and modelling interactions between malaria exposure and age as described above. These analyses did not suggest substantial heterogeneity in the association over age, and therefore we have retained the simpler presentation for clarity.

> *Provide correlation analyses between different antibody responses to assess whether suppression is generalized.*

We have addressed this by modelling responses jointly across a panel of heterologous antigens and by examining antigen-specific associations. The direction of effect was consistent for the majority of antigens, with no evidence of opposing trends, supporting a broad rather than antigen-specific effect.

> *The authors could consider moving Figures 2a and b to the supplementary material.*

We thank the reviewer for this suggestion. We carefully considered whether panels 2a and 2b could be moved to the supplementary material. However, we have retained them in the main text because they provide a simple, intuitive illustration of how AMA1 antibody responses track with malaria exposure at the individual level, complementing the population-level analysis shown in fig. 2c. We feel that this helps establish the biological validity of the microarray platform in a way that is immediately interpretable to the reader, and therefore supports the interpretation of subsequent analyses.

> *The authors could consider replacing Figures 3a and b with IgG levels from ALL vaccinated children and ALL non-vaccinated children.*

We thank the reviewer for this suggestion. We would like to retain these figures for the same reasons that have been articulated above for figures 2a and b.

> *(3) Discussion Enhancements:*
>
> *The authors should consider expanding the discussion to address the limitations of the data more thoroughly, particularly regarding the potential differences between cohorts that could have contributed to the results.*

We have expanded the discussion to more explicitly address potential differences between cohorts that could contribute to the observed findings, including nutritional, socioeconomic, and environmental factors.

> *The discussion needs to acknowledge the lack of directionality for the associations observed. As stated above, although I agree in general terms with the observations that the authors have made, it is not possible to distinguish between a suppressive effect of malaria on immune responses to infection-derived pathogens or a protective effect of malaria that leads to less exposure to infection-derived pathogens (and consequently lower IgG levels). The mechanisms behind these could include things like different health-seeking behaviors or social interactions from kids who have malaria versus those who don't, for example.*

We agree that, as an observational study, we cannot definitively establish the direction of the association between malaria exposure and antibody responses to unrelated antigens. We have now clarified this limitation explicitly in the discussion. We acknowledge the alternative interpretations raised by the reviewer, including the possibility that differences in exposure to other pathogens, potentially driven by behavioural, environmental or healthcare-related factors, could contribute to the observed patterns. At the same time, we note that the natural experiment design, prospective malaria exposure classification, and within-Ngerenya comparisons support early-life malaria exposure as a key contributing factor. We have revised the discussion to reflect this balance.

> *Extend the discussion of potential biological mechanisms underlying durable immunosuppression.*

We thank the reviewer for this suggestion. We have expanded the discussion to more fully consider potential biological mechanisms that could underlie the observed long-term differences in antibody responses. Specifically, we now discuss evidence from prior studies indicating that malaria infection can induce sustained alterations in B cell and T cell compartments, including expansion of atypical memory B cells, disruption of germinal centre responses, and increased regulatory immune activity. We position our findings as providing population-level evidence of a durable immunological phenotype, while noting that targeted mechanistic studies will be required to define the underlying pathways.

> *Extend the discussion around the clinical implications of the observed antibody level differences.*

In the revised discussion, we highlight that studies incorporating functional assays and clinical outcome data will be required to determine whether these serological differences translate into altered susceptibility to infection or reduced vaccine effectiveness.

> *(4) Technical Issues:*
>
> *Could the authors please:*
>
> *(1) Clarify microarray data processing and quality control procedures.*

We thank the reviewer for this request. We have expanded the methods section to provide additional detail on microarray data processing and quality control procedures.

> *(2) Provide information on inter-assay variability and batch effects.*

We have expanded the methods section to clarify how these were evaluated and addressed. Inter-assay variability was monitored using pooled adult serum included on every slide as a consistent positive control. This allowed us to assess slide-to-slide consistency in signal detection across the full antigen panel. In addition, fluorophore-conjugated IgG and IgA controls were printed directly onto each miniarray to confirm scanner performance independently of antigen–antibody interactions. At the sample level, each specimen was assayed on two independent miniarrays per slide, generating four spatially separated replicate measurements per antigen. Technical variability was quantified using the coefficient of variation (CV), and measurements with CV >20% were excluded from downstream analyses.

> *(3) Include details on how missing data were handled in longitudinal analyses.*

We thank the reviewer for highlighting this point. We have added clarification in the statistical analysis section describing how missing data were handled. Specifically, mixed-effects models were used, which accommodate unbalanced longitudinal data without requiring imputation, allowing all available observations to contribute to the analysis.

> *(4) Include details of the parameters of the LOWESS analysis shown in Figure 1.*

We have expanded the figure 1 legend to include the parameters used for the loess smoothing shown, including the smoothing span.

> *(5) Include details of the samples used for Figure 3d (Negative and Pooled Adult Serum).*

We have clarified in the methods the nature and purpose of the samples used in Figure 3d. The negative control consisted of phosphate-buffered saline applied to a full miniarray in place of serum, allowing assessment of background and non-specific signal in the absence of antibody binding. The pooled adult serum comprised a composite of sera from multiple healthy adults from the same setting and was included as a positive reference sample, expected to contain a broad repertoire of antigen-specific antibodies. These controls were included on each slide to enable interpretation of assay performance, with the negative control defining baseline signal and the pooled adult serum providing a consistent reference for antigen recognition across the microarray.

https://doi.org/10.7554/eLife.107820.2.sa0