The immune divide: factors influencing immune variation and differences in vaccine responses

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Chapter 5

Differences in the myeloid compartment at baseline are associated with variation in antibody response to yellow fever vaccination in urban and rural Tanzanians

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To be submitted

Abstract

Introduction: Variation in vaccine response is observed between populations living in distinct geographical areas. This is thought to be associated with an altered immune state at baseline.

Aim: Here, we examine the association between the baseline innate immune characteristics and the response to yellow fever (YF) vaccination in healthy Dutch as well as urban and rural Tanzanian volunteers.

Methods: Response to YF vaccination was assessed by measuring plasma levels of IgG specific to non-structural protein 1 (NS1) of the YF virus at days 0 and 178 post-vaccination using ELISA. Using spectral flow cytometry, peripheral blood mononuclear cells were profiled with a panel directed at the innate immune system. PBMC were also analyzed after stimulation with the YF vaccine, ssRNA40 and Staphylococcus aureus enterotoxin B (SEB) for 24 hours.

Results: Post vaccination, a greater proportion of Dutch compared to Tanzanians had a high antibody response to NS1, but no difference was seen between urban and rural Tanzanians, in spite of the heterogeneity in the endpoint anti-NS1 IgG levels. When comparing the high and low responders within the Tanzanian cohort, high responders had an increased frequency of classical monocytes compared to low responders, both *ex vivo* and upon stimulation. Low responding Tanzanians were characterized by decreased frequency of cDC2s and IFN-alpha+ CD1c- CD141- DCs, whereas IL-10 production by cDC2s in unstimulated cultures was increased compared to high responding Tanzanians.

Conclusion: Differences in the frequency of subsets belonging to the myeloid compartment and their cytokine production associated with the antibody response to YF vaccination in Tanzanians. These data might help the understanding of the immunological mechanisms underlying vaccine response variation, necessary to develop interventions to overcome vaccine hyporesponsiveness.

Introduction

Vaccination against infectious diseases saves millions of lives each year [1]. However vaccines are not yet used to their full potential due to low and variable efficacy of some across populations and geographical areas [2-6]. Differences in vaccine efficacy are most clearly be observed for new vaccines evaluated in controlled human infection studies, such as the live *Plasmodium falciparum* sporozoite (PfSPZ) vaccine that demonstrated an protective efficacy of 92.3% in malaria-naïve subjects in the US, whereas in a malaria-endemic area in Tanzania it only protected 20% of the recipients [4, 5]. Moreover, reduced responses upon have been reported for well-established vaccines such as the yellow fever (YF), as Ugandans have been reported to have significantly lower neutralizing antibody titers upon vaccination in comparison to Swiss vaccinees [6].

An important determinant of the response to vaccination is the immune state at baseline, as altered immune baseline profiles have previously been associated with reduced vaccine responses [6-8]. A study among children from Kenya and Gabon vaccinated with the malaria vaccine RTS,S/AS01, showed that the monocyte-to-lymphocyte (ML) ratio at baseline is a good predictor for the efficacy of this vaccine, with reduced vaccine efficacy in those with high ML ratio [7]. This aligns with a study into YF response in Swiss and Ugandans, showing that increased baseline monocyte counts and the frequency of intermediate monocytes were associated with reduced antibody responses [6]. Moreover, a higher frequency of activated B and CD8+ T cells at baseline was found in Ugandans than in Swiss, which negatively correlated with neutralizing antibodies upon vaccination. Together, these results indicate that heightened immune activation at baseline, as observed in Ugandans, may drive the reduced response to YF vaccination [6]. More recent studies have focused on identifying a universal baseline signature that is predictive across several vaccines [8, 9]. A study combining pre-vaccination transcriptome data of multiple studies to predict the immune response across 13 different vaccines, including YF and malaria, identified three endotypes that are defined by multiple sets of genes, including pro-inflammatory and interferon-stimulated genes [8]. Comparison of the vaccine response among these endotypes, showed that among these young American individuals with limited pre-exposure to infections with the most pro-inflammatory endotype demonstrated highest vaccine responses [8]. However, how well these signatures hold up across populations with different environmental exposures remains an open question, as populations from LMICs have not been included in such studies.

Besides variation in the immune system between countries, differences in immune profiles within countries also have been observed [10, 11]. A study employing a multi-omics approach to study the immune profiles of rural and urban Senegal and urban Dutch subjects, revealed a continuous trajectory of immune remodeling along the rural-urban gradient [10]. The immune trajectory of rural individuals in this study was more pro-inflammatory compared to urban Senegalese and Dutch as rural individuals had increased frequency of CD11c+ B cells, monocytes producing TNF-alpha and IL-1β and T helper 1 cells (Th1). Moreover, comparing the cellular immune profiles of individuals from two rural and two urban populations in Northern Tanzania has shown that individuals with a rural lifestyle (low lifestyle score) had a more activated immune system as their immune profile was characterized by expansion of atypical B cells, T helper 2 cells, regulatory T cells as well as activated CD4+ T cells expressing CD38, HLA-DR and CTLA-4 [11]. Those with an urbanized lifestyle (high lifestyle score), however, showed a less activated immune state illustrated by higher frequencies of naïve CD8+ T cells [11].

Although heterogeneity in immune profiles within countries has been reported, the extent to which they affect the response to vaccines remains largely unexplored. The current work examines the differences of the immune system at baseline among rural and urban Tanzanians compared to Dutch and studies their association with the response to YF vaccine. Given the prominent role of the innate immune system in the initiation of the vaccine response and the observed associations between baseline inflammation and reduced vaccine response, the preliminary analysis presented here focuses on the innate immune compartment. Moreover, to assess the immune response to YF vaccine we use IgG specific to non-structural protein 1 of the YF virus (anti-NS1 IgG), an accurate continuous parameter [12], rather than the neutralizing antibody titer, which is often used in a dichotomous or semi-quantitative manner to confirm seroconversion. By studying the innate immune state at baseline among different populations and their relation to vaccine response, we will deepen our understanding of the immunological mechanisms that underly variation in vaccine responses, which is needed to develop interventions to overcome vaccine hyporesponsiveness.

Material and Methods

Study design

The current study is part of a prospective longitudinal cohort study (CapTan) in a healthy, 18-35-year-old Tanzanian population from rural and urban Moshi, Northern Tanzania. Volunteers were randomized into a vaccine group, receiving a single dose of yellow fever vaccine (YF-17D, Sanofi-Pasteur France) via intramuscular injection, and a control group who did not receive any intervention or placebo. Data and samples were collected at baseline and thereafter over a period of six months. The study took place from February to August 2023. The study protocol was approved by the Ethical Board of the Kilimanjaro Christian Medical University College (No. 2588) and by the Tanzania National Ethical Committee Board (NIMR/HQ/R.8a/Vol.IX/4089). The study was registered under The Pan African Clinical Trial Registry (PACTR) with trial number PACTR202405738173023. In addition, samples were collected from 15 Dutch participants who were not vaccinated with YF before. All individuals received the YF vaccine (YF-17D, Sanofi-Pasteur, France) via subcutaneous injection and were followed up for six months. This study took place from March to September 2023 in the Leiden University Medical Centre in Leiden. The study was approved by the Medical Ethical Committee Leiden The Hague Delft (NL70951.058.19) and is registered as clinical trial (ClinicalTrials.gov, NCT05901454).

Study population

Description of the study areas from which the Tanzanian participants were enrolled were published before [11]. In short, the study was conducted in rural and urban Moshi location in the Kilimanjaro region in northern Tanzania. The district of Moshi city (urban Moshi) is the administrative, commercial and educational center of the region and most inhabitants practice a Western lifestyle with generally good quality sanitation. Rural Moshi is an area north of urban Moshi, higher up the slopes of the Kilimanjaro and therefore has an elevation of 2,000-2,100 meters above sea level (compared to 700-950 meters in urban Moshi). Most inhabitants of rural Moshi are involved in farming activities.

Participant enrollment and data collection

Inhabitants of the two regions were informed about the study through community leaders, gatherings and leaflets and all eligible participants (age 18-35 years and permanent residence of the study location) were asked to enroll. Following informed consent, 233 participants were

voluntarily screened for exclusion criteria, including previous YF vaccination, comorbidities, HIV infection and use of medication possibly affecting the immune system; methods have previously been described in detail [11]. Based on the in- and exclusion criteria, 48 were excluded, therefore 185 participants were included and randomized over the vaccine and control group, resulting in 155 vaccinated individuals and 30 individuals that served as control group. Data were collected in REDCap, a cloud-based electronic data collection system, with a server hosted at the Kilimanjaro Clinical Research Institute (KCRI) in Tanzania.

Sample collection and processing

Before vaccination (Day 0), stool, urine and blood samples were collected from all included participants. Urine samples were used to test for pregnancy and determine infections with *Schistosoma* species using point-of-care test for the circulating cathodic antigen (POC-CCA). Kato-Katz was performed on stool samples to detect *Schistosoma* and soil-transmitted helminth eggs. From blood collected in sodium heparin tubes peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved as described previously [13]. Blood collected in EDTA tubes, were centrifuged upon arrival to the KCRI laboratory and plasma was collected and stored at -80°C within two hours. To assess the pre- and post-vaccination antibody response to YF vaccination, plasma was collected both at Day 0 and Day 178 post-vaccination. For the Dutch study EDTA plasma and PBMCs were collected and processed at similar timepoints and using identical protocols.

Enzyme-linked immunosorbent assay (ELISA)

To assess the YF virus antibody response, IgG specific to Yellow Fever Virus NS1 Protein was measured in plasma samples using enzyme-linked immunosorbent assay (ELISA). High-binding half area 96-well ELISA plates (Corning) were coated with 12.5 ng/well Yellow Fever Virus NS1 Protein (NAC-YFV-NS1-100, The Native Antigen Company) in 0.1 M sodium carbonate buffer (pH 9.6) overnight at 4°C. Plates were washed thrice with washing buffer (0.05% Tween-20 in PBS) and blocked for 2h with 5% skimmed milk in PBS containing 0.1% Tween-20. Plates were incubated with 25 µL/well of 4-step 1:2.5 serially diluted sera with a starting dilution of 1:250 for 2h at RT. After washing 5 times, NS1-specific IgG were measured using goat anti-human IgG-HRP (109-035-098, Jackson Immuno Research) at 1:5000 in 0.5% skimmed milk in PBS with 0.1% Tween-20 and 1-step TMB substrate solutions (34021,

ThermoFischer Scientific). Measured absorbance values were normalized using standard curve obtained from IgG (I5154, Sigma Aldrich) with the known concentration on each plate.

Innate baseline profiling

Sample selection for innate baseline profiling

From the 155 vaccinated participants, 50 individuals (25 urban and 25 rural) were randomly selected for innate baseline profiling. Samples were randomly selected after excluding samples with incomplete datasets due to loss-to-follow up (n=17), positive pregnancy test at day 178 (n=6) or insufficient PBMCs (n=6). From the Dutch cohort all participants were selected, except for one that had insufficient PBMCs, which resulted in the inclusion of n=14 Dutch individuals. In total 64 individuals were selected for innate immune profiling that were measured in three batches. Each of these batches were matched for geographical location, age and sex and included a reference control to allow batch correction after acquiring.

***Ex vivo* assays spectral flow cytometry**

Cells were thawed and washed in RPMI 1640 supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 1 mM pyruvate, 2 mM L-glutamine, 2mM Mg2+, 50 U/ml benzonase, and 20% Heat-inactivated Fetal Calf Serum (hiFCS). Thereafter, cells were resuspended in IMDM supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 1 mM pyruvate, 2 mM L-glutamine, and 10% hiFCS, and adjusted to a concentration of 5 x 10^6 cells/mL. Then, cells were plated as 0.5 x 10^6 cells per well in a 96-well V bottom plate. The cells were washed in phosphate-buffered saline (PBS), stained for viability with LIVE/DEAD™ Fixable Blue (Thermofisher) and Human TruStain FcX™ (Biolegend), washed again twice in FACS buffer (PBS supplemented with 0.5% BSA, Roche) and then stained with 50 µL of surface staining cocktail. The surface staining antibody cocktail, prepared in FACS buffer with Brilliant Stain Buffer Plus (BD Biosciences) and True-Stain Monocyte Blocker™ (Biolegend), was added to the cells and incubated for 30 min at RT. The list of antibodies can be found in **Table S1**. Cells were then washed twice in FACS buffer and afterward fixed with the eBioscience™ FoxP3 Transcription Factor Staining Buffer Set (ThermoFisher) for 30 minutes at 4 ˚ C. Subsequently, cells were washed twice with FACS buffer and resuspended in 120 µL of FACS buffer until acquisition. All centrifugation steps before fixation were performed at 450 g at RT and after fixation at 800 g at 4 °C.

***In vitro* stimulation assay spectral flow cytometry**

After thawing the cells as described above, 0.5 x 10^6 cells per well were plated in a 96-well U-bottom plate and rested for 1 hour at 37 ˚ C under 5% CO2. After resting, cells were stimulated with either YF vaccine (YF-17D, Sanofi Pasteur, Lot nr: X3E042V) at a concentration of 50.000 IU/mL (0.01 MOI) dissolved in 10% hiFCS/IMDM, 2 µg/mL ssRNA40 (Invitrogen), 0.2 µg/mL staphylococcal enterotoxin B (SEB; Sigma-Aldrich), or 10% hiFCS/IMDM for 23 h at 37 ˚ C under 5% CO_2. During the last four hours of stimulation, 10 µg/mL Brefeldin A (Sigma-Aldrich) was added.

After stimulation, cells were resuspended in PBS containing 2 mM EDTA and left on ice for 15 minutes, before transferring the cells to a 96-well V-bottom plate for staining. Thereafter, cells were washed in PBS, stained for viability with LIVE/DEAD™ Fixable Blue (Thermofisher) and Human TruStain FcX™ (Biolegend), washed again twice in FACS buffer (PBS supplemented with 0.5% BSA, Roche) and then stained with 50 µL of surface staining antibody cocktail. The surface staining antibody cocktail, prepared in FACS buffer with Brilliant Stain Buffer Plus (BD Biosciences) and True-Stain Monocyte Blocker™ (Biolegend), was added to the cells and incubated for 30 min at RT. The list of antibodies can be found in **Table S1**. Cells were then washed twice in FACS buffer and afterward fixed and permeabilized with the eBioscience™ FoxP3 Transcription Factor Staining Buffer Set (ThermoFisher) for 30 minutes at 4 ˚ C. Subsequently, cells were washes twice with the Permeabilization buffer from the eBioscience™ FoxP3 Transcription Factor Staining Buffer Set and then stained with intracellular cocktail. The intracellular cocktail, prepared in the Permeabilization buffer with Brilliant Stain Buffer Plus (BD Biosciences), True-Stain Monocyte BlockerTM (Biolegend) and Human TruStain FcX™ (BioLegend), was added to the cells and incubated overnight at 4 ˚ C. Next day, cells were washed twice with eBioscience™ Permeabilization buffer and were then resuspended in 120 µL of FACS buffer until acquisition. All centrifugation steps before fixation were performed at 450 g at RT and after fixation at 800 g at 4 °C.

Cell acquisition

Cells were acquired on a 5L-Cytek Aurora instrument at the Leiden University Medical Center Flow Cytometry Core Facility (https://www.lumc.nl/research/facilities/fcf/) with the SpectroFlo® v2.2.0.3 software (Cytek Biosciences). As controls, an unstained cell sample (pooled from all donors of the batch) and a single-stain sample for each antibody were used,

which were either 0.5 x 10^6 million PBMC or 50 µl UltraComp eBeads™ (Invitrogen). All reference controls underwent the same protocol as the fully stained samples, including washes, buffers used, and fixation and permeabilization steps.

Data analysis and statistical analysis

Transformation of spectral flow cytometry data

To obtain live single cells, manual gating of live, single CD45+ cells was performed using the OMIQ software (www.omiq.ai). Thereafter, cell subsets were identified based on the expression of surface markers and according to a pre-defined gating strategy (**Figure S1-S2**). Data that was clustered was then exported as FCS files. The *ex vivo* samples were batch corrected using CytoNorm (on default settings) and the compensated expression matrices arcsinh transformed, with a cofactor of 6000, before the data was merged into one dataset. After running CytoNorm, one cell had a batch corrected value of negative infinity. This value was changed to be the 1% percentile expression value for that fluorophore, across all cells for that individual. Post CytoNorm, and arcsinh transformation, any values above 10 or below -10 were clipped. The compensated expression matrices for the stimulated samples were also arcsinh transformed, with a cofactor of 6000. No batch correction was carried out for the culture samples.

Dimensionality reduction

For both the *ex vivo* merged datasets, a subsample of 100,000 cells was extracted randomly from the merged datasets and FItSNE ran on the arcsinh transformed batch corrected expression values of the *ex vivo* data subset, and the arcsinh transformed expression values of the culture data subset. For FItSNE, every marker associated with fluorophore was included as part of the feature space, except for CD45 and live/dead. For the principal component analysis, only the *ex vivo* dataset was used. In this analysis the frequency as percentage of total CD45+ cells of all the cell types manually gated were used. In addition, the 95th percentile expression of the activation markers were scaled to a mean of 0 and a standard deviation of 1 and thereafter included in the principal component analysis.

Cytokine gating

Cell type specific created to enable classification cells into cytokine positive and cytokine negative cells. For each of the fifteen gated innate cell types, a threshold was calculated to be:

$$percentile(x, 99) \ + \ (\ percentile(x, 99) \ \times 0.2\ )$$

Where x is the arcsinh transformed expression of a given cytokine in a given innate cell type in the unstimulated condition (upon culturing with only media). For the media only, SEB, ssRNA40 and YF-17D samples, cells were assigned as producers of a particular cytokine if they were above the threshold for their cell type.

Statistical analyses

All pairwise comparisons were carried out using Mann-Whitney U test with Benjamini-Hochberg correction. The number of comparisons to adjust for was based on the number of lineages (i.e. 5), not the number of gated subsets. For multiple comparisons of more than 2 groups, a Kruskal-Wallis test was used, with Dunn's test to carry out post-hoc pairwise comparisons between the groups. The FDR and alpha were set to be 5%. Differences in cytokine producing cells between high and low responders were only considered if the median percentage was greater than 0 across all individuals, and differences in activation marker expression were considered if the median of the 95th percentile expression, across all individuals, was greater than 1. PERMANOVA was performed using the adonis function of the vegan R package with 999 permutations. The input to the function was a Euclidean distance matrix of each individual's position across the first two principal components. All statistical analyses were carried out in R version 4.4.1. The packages used, and their versions, are as follows: rstatix 0.7.2, cytolib 2.16.0, kohonen 3.0.12, CytoNorm 2.0.2, igraph 2.2.1, ggpubr 0.6.0, matrixStats 1.4.1, stringr 1.5.1, flowCore 2.16.0 and vegan 2.6-8.

Results

Characteristics of the study population and antibody responses to YF-17D vaccination

To investigate how the innate immune cell compartment differs in individuals across and within different geographical areas and how it may impact vaccine response, volunteers were recruited from rural and urban areas of Tanzania and an urban area of the Netherlands. In total, 185 Tanzanians and 15 Dutch were recruited for the study, with 93 Tanzanians from urban and

92 from the rural area. All the Dutch and 155 of the Tanzanians (n=78 rural and n=77 urban) were vaccinated with the YF-17D vaccine, the remaining 30 Tanzanians served as a control (no vaccine) group. Dutch vaccinees were on average younger than Tanzanian vaccinees and more females were included in the Dutch compared to the Tanzanian cohort (**Table S2)**. The characteristics of the Tanzanians that received the vaccine and that served as control group did not differ (**Table S3**).

The vaccine immunogenicity was determined by measuring IgG specific to Yellow Fever non-structural protein 1, here referred to as anti-NS1 IgG, at baseline and day 178 post-vaccination (~6 months). Data for both these timepoints was available from 147 of the 155 vaccinated Tanzanians (n=70 rural and n=77 urban) and 12 of the 15 Dutch individuals **(Figure 1A).** The demographics of the individuals for which plasma from both timepoints were available did not differ from that of the total vaccinated subjects (**Table S4-5)**. To examine the innate immune profile at baseline, we profiled the PBMC of 50 individuals (n=25 rural and n=25 urban) that were randomly selected from the 155 vaccinated Tanzanians. From the Dutch cohort all participants with sufficient PBMCs were included, which resulted in innate baseline profiling of 14 Dutch individuals (**Figure 1A**). The age, sex and anti-NS1 IgG levels of the individuals selected for innate baseline profiling did not significantly differ from that of all vaccinated subjects included in the study (**Table S4-5**).

When comparing the anti-NS1 IgG levels between the cohorts, the median antibody levels did not significantly differ between the groups, but within the groups heterogeneity in the antibody response was observed (**Figure S3A**). Visualizing the antibody response at baseline and at Day 178 in all vaccinated individuals together on a histogram showed two noticeable peaks (**Figure S3B**). These peaks were modelled using a two-component Gaussian Mixture Model (GMM) (**Figure 1B**). All but three of the pre-vaccination values and all but one of the post-vaccination values of the control group (no vaccine) were positioned into this lower peak (**Figure S3C-D**).Therefore individuals whose post-vaccination anti-NS1 IgG levels clustered in this peak were designated as “low responders”, while individuals in the higher peak were designated as “high responders”. Using this categorization, in total 70 individuals were designated as high responders, whereas 89 individuals were low responders (**Figure 1B**). Examining the antibody response per group, showed that 83% (n=10) of Dutch individuals were in the high responders group, whereas 38% (n=29) of the urban Tanzanians and 44%

(n=31) of the rural Tanzanians were high responders (**Figure 1C**). The proportion of high responders was significantly greater in Dutch compared to all Tanzanians, whereas the proportion in urban and rural Tanzania was similar (**Table S6**).

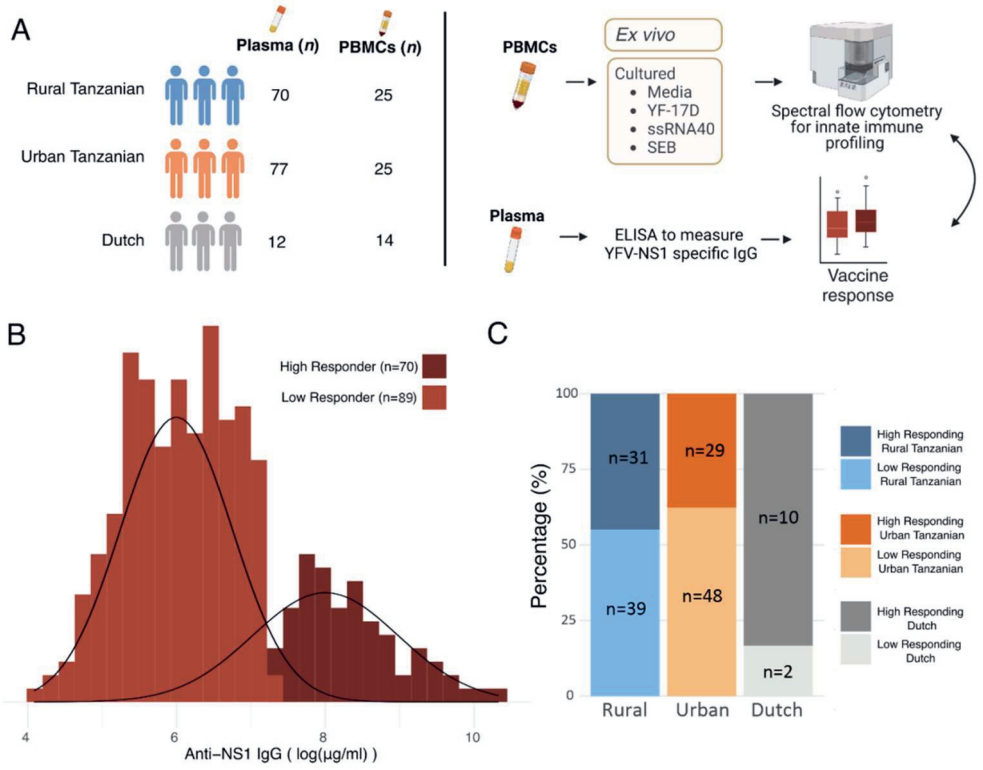

Figure 1. Studying the antibody response to yellow fever vaccination and innate immune baseline profile in rural and urban Tanzanians and Dutch vaccinees

(A) Graphical overview of the study population showing the number of individuals across the three geographical areas (Dutch and rural and urban Tanzanian) who had their PBMCs and plasma sampled (left). Graphical overview of the processing and acquisition steps for the PBMC and plasma samples (right). **(B)** Histogram visualising the anti-NS1 IgG levels of vaccinated individuals, with the individuals coloured by whether they were classified as high or low responders, based on a GMM model. The black lines show the distributions of the two Gaussian models. **(C)** Barplot showing the percentage of vaccinated individuals that belong to either high or low responders across the geographical areas, with each bar also showing the total number of individuals according to geographical area.

Baseline difference in the innate immune profiles of Dutch and Tanzanians

The innate immune profile was characterized before vaccination, at baseline, to assess whether signatures could be discerned that would predict antibody responses after vaccination. PBMCs were profiled from a random selection of 50 Tanzanians (18/32 high vs low responders equally

split between rural and urban, n=25 urban, n=25 rural) and 14 Dutch (10/2 high vs low responders, n=2 no day 178) vaccinees. One individual from the urban Tanzanians was removed due to low recovered cell count following staining. Spectral flow cytometry was performed using a panel to measure 26 markers for the *ex vivo* condition and 32 for the *in vitro* stimulated assay (**Table S1**). Manual gating using OMIQ, allowed us to identify monocytes, ILCs, Natural Killer (NK) cells, Myeloid Dendritic Cells (mDCs), plasmacytoid dendritic cells (pDCs) and Basophils (**Figure 2A**). Moreover, the monocyte population, here defined as CD3- CD19- CD88+ and/or CD14+, was further divided into four groups based on the expression of CD14 and CD16; classical monocytes (CD14+ CD16), intermediate monocytes (CD14+ CD16+), non-classical monocytes (CD14- CD16+) and CD14- CD16- monocytes. Moreover, the ILCs were classified as either ILC1, 2 and 3; the myeloid DCs could be subdivided into cDC1, cDC2 or CD1c-CD141- DC and the NK cells were split into three groups based on expression of CD16 and CD56 (**Figure S4**). To examine the activation status of these subsets, the 95th percentile expression of each activation marker (**Table S1**) for each of the manually gated innate cell types was determined.

To explore the overall innate immune baseline profile of rural and urban Tanzanians and Dutch individuals, a principal component analysis was performed using the frequencies of the manually gated innate cell types and the 95th percentile expression of activation markers in each of these cell types of the *ex vivo* samples. A significant separation (PERMANOVA p = 0.001) was seen across the first two principal components, with the Dutch being distinct from the rural and urban Tanzanians, whereas the rural and urban Tanzanians overlapped greatly **(Figure 2B**). To further examine the cell subsets and characteristics that contribute to the differences in the overall innate baseline profiles across the geographical areas, we compared the individual cell frequencies and expression of activation markers between rural and urban Tanzanians and Dutch (**Figure 2C-D**). With the exception of classical monocytes, which were higher in Dutch compared with Tanzanians, other myeloid cell subsets were significantly lower in the Dutch (**Figure 2C**). Moreover, the frequency of ILC2 and basophils, which indicate expansion of type-2 responses was significantly higher in urban Tanzanians compared to rural Tanzanians or the Dutch (**Figure 2C**). When comparing the activation status using the 95th percentile of the expression of the activation markers in the innate cell types a higher expression of TIM-3, an immunoinhibitory molecule, was observed in classical monocytes and cDC2s in Tanzanians compared to Dutch. Moreover, the expression of TIGIT, a marker of NK

activation and/or exhaustion, was significantly increased in CD16+ and CD16- NK cells of Tanzanians compared to Dutch equivalent cells (**Figure 2D**). Thus, the innate immune profile at baseline *ex vivo* of Dutch is distinct of that of Tanzanians, whereas urban and rural Tanzanian innate baseline profiles show great resemblance.

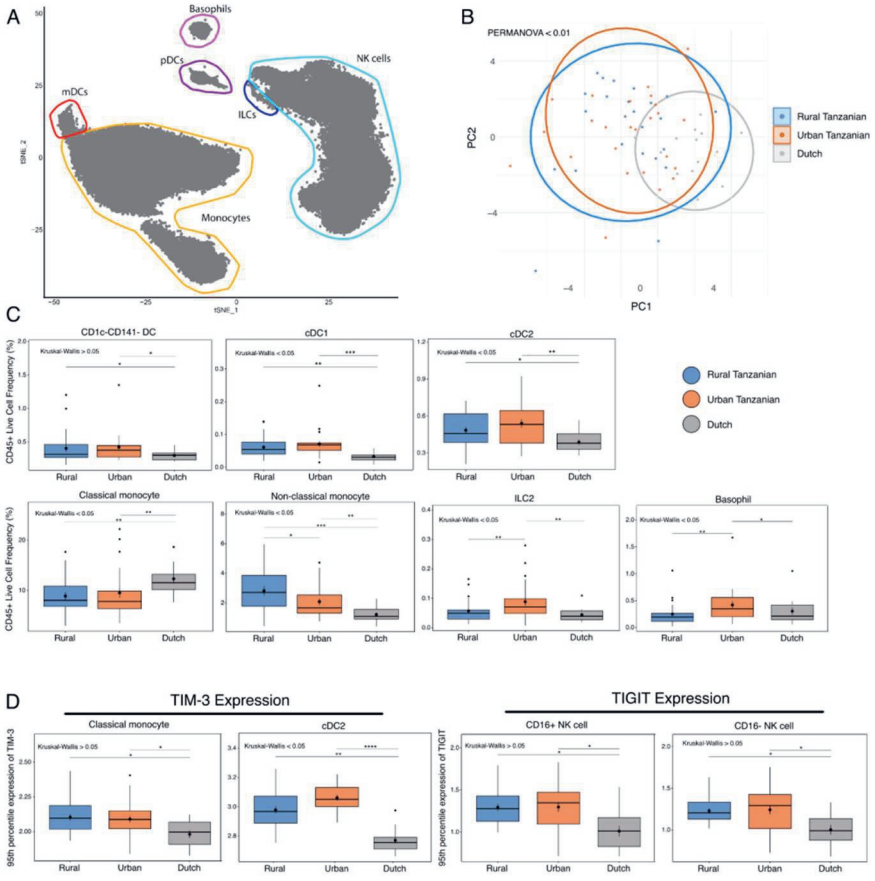

Figure 2. Innate immune profile at baseline of Dutch is distinct from that of Tanzanians

(A) FItSNE projection of a subset of the total innate cells from the *ex vivo* samples. Lineages of cells are outlined and annotated. **(B)** Principal component (PC) analysis of innate immune cells derived *ex vivo* from patients. PCs were generated from the 95th percentile expression of activation markers and frequency of innate cell types (as a percentage of total CD45+ cells). Individuals are coloured by the geographical area where the individual is from, and the coloured outline shows the 95th percentile area for each of the three geographical areas. PERMANOVA comparing the geographical areas is also shown. Boxplots showing the frequency of various innate immune cell types (as a percentage of total CD45+ cells) **(C)** and 95th percentile expression of activation markers TIGIT and TIM-3 **(D)** in rural Tanzanians, urban Tanzanians and Dutch. The boxplots are coloured by geographical area and contains the median (horizontal line), mean (dot) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Differences in innate immune cell frequency and 95th percentile expression of activation marker across the three groups was assessed with Kruskal-Wallis test, with post-hoc pairwise comparisons achieved with Dunn's test, with Benjamini-Hochberg correction to account for multiple testing. ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

Frequency of *ex vivo* classical monocytes at baseline associates with post-vaccination antibody responses in Tanzanians

We next investigated whether differences between the vaccine responder groups, in terms of innate immune cells, could be detected at baseline. As only few Dutch were low responders and the innate immune signature at baseline of the Dutch was distinct from that of Tanzanians (**Figure 1C and 2B**), the high and low responders were compared for the Dutch and Tanzanian cohorts separately. Upon comparison, a significant increase in the frequency of classical monocytes was found in the high compared to low responding Tanzanians and this difference remained significant when Tanzanians were split between urban and rural (**Figure 3A-B**). As the frequency of classical monocytes in both high and low Dutch responders resembled that of high responding Tanzanians, no significant difference was found between high and low responding Dutch, although only few Dutch were low responders (**Figure 3C**). Besides differences in the frequency of classical monocytes, no statistically significant differences were found the frequency of other cell types or in the level of expression of activation markers within cell subsets between high and low responders.

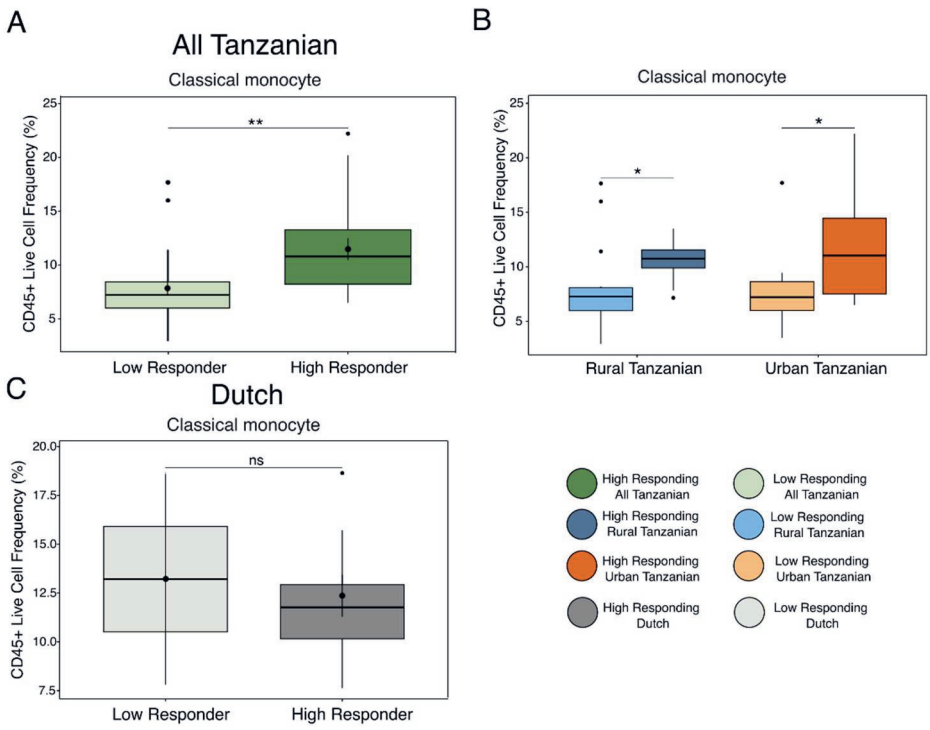

Figure 3. **High responding Tanzanians have increased frequency of *ex vivo* classical monocytes at baseline compared to responding Tanzanians:**
Boxplots showing the frequency of CD16-CD14+ monocytes (as a proportion of the total CD45+ cells) between the high and low responders of all Tanzanian **(A)**, rural and urban Tanzanians **(B) and Dutch individuals (C**). The boxplots are coloured by YF-17D vaccine response and geographical area, and contain the median (horizontal line) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Changes in CD16-CD14+ monocyte frequency was assessed using a Mann-Whitney U test, with multiple test corrections with Benjamini-Hochberg. ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$.

Cultured innate immune cell profiles associated with vaccine response in Tanzanians

We next examined whether responsiveness of innate immune cells to a challenge associates with antibody response to vaccination. To this end, *in vitro* response to either YF-17D, the TLR-8 ligand ssRNA40, the superantigen Staphylococcal Enterotoxin B (SEB) or medium as control (unstimulated culture) was determined. Similar to the *ex vivo* results, in the unstimulated culture, the frequency of classical monocytes were significantly increased in the high responding Tanzanians compared to the low responders (**Figure 4A**). In addition, upon culturing with medium the frequency of cDC2s and CD1c- CD141- DCs was significantly associated with the antibody response to YF in all Tanzanians. The frequency of cDC2s was increased in high compared to low responders, whereas for CD1c- CD141- DCs, the opposite was observed with lower frequencies in the high responding Tanzanians **(Figure 4A).** Interestingly, no such differences were seen in the high and low Dutch responders **(Figure S5).** In these unstimulated cultures, cDC2s producing IL-10 and CD1c- CD141- DCs producing IFN-alpha were different between high and low responding Tanzanians, as high responders had reduced IL-10+, but increased IFN-alpha+ CD1c- CD141- DCs **(Figure 4B).** Again, none of these differences between the high and low Dutch responders reached statistical significance (**Figure S5**).

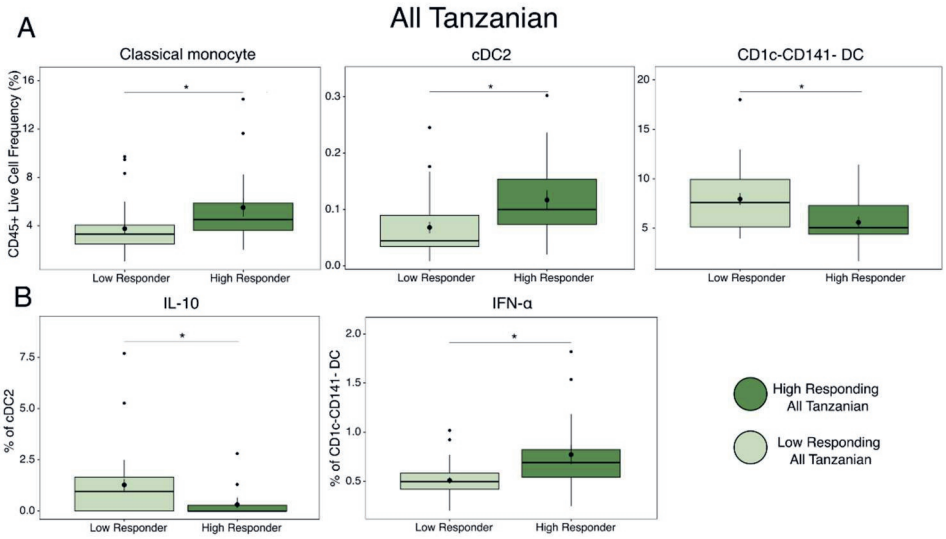

Figure 4. Frequency and cytokine production in dendritic cells in unstimulated culture are associated with the response to yellow fever vaccination in Tanzanians:

Boxplots showing the frequency of various innate immune cell types (as a percentage of total CD45+ cells) **(A)** and frequency of cytokine producing cells (as a percentage of the total parent cell) **(B)** between all Tanzanian high and low responders to the YF-17D vaccine, when the immune cells were cultured in media. The boxplots are coloured by YF-17D vaccine response and contains the median (horizontal line), mean (dot) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Differences in innate immune cell frequency and cytokine producing cell frequency across between high and low responders was assessed with Mann-Whitney U test, with Benjamini-Hochberg correction to account for multiple testing. ns = $p > 0.05$, * = $p < 0.05$.

Upon stimulation with YF-17D, ssRNA40 and SEB, the innate cells responded by increased cytokine production, however when comparing the cytokine production between high and low responding Tanzanians we did not find statistical differences for any of the cytokines in any of the cell types after multiple testing correction (**Figure S6**). Comparison of the cell frequencies upon stimulation between high and low responders from Tanzania revealed that the differences largely reflected what was observed in unstimulated cultures (**Figure 4**). For the YF-17D stimulated culture, the frequency of CD1c-CD141- DCs was higher in the low responders, while that of classical monocytes and cDC2s was higher in the high responders, however these did not reach statistical significance (**Figure 5A**). The same trend, but with statistically significant differences was found in response to ssRNA40 with the frequencies of classical monocytes and cDC2s being higher but CD1c-CD141- DC lower in high responders compared

to low responders (**Figure 5B**). Finally, SEB stimulated cultures showed similar patterns (**Figure 5C**).

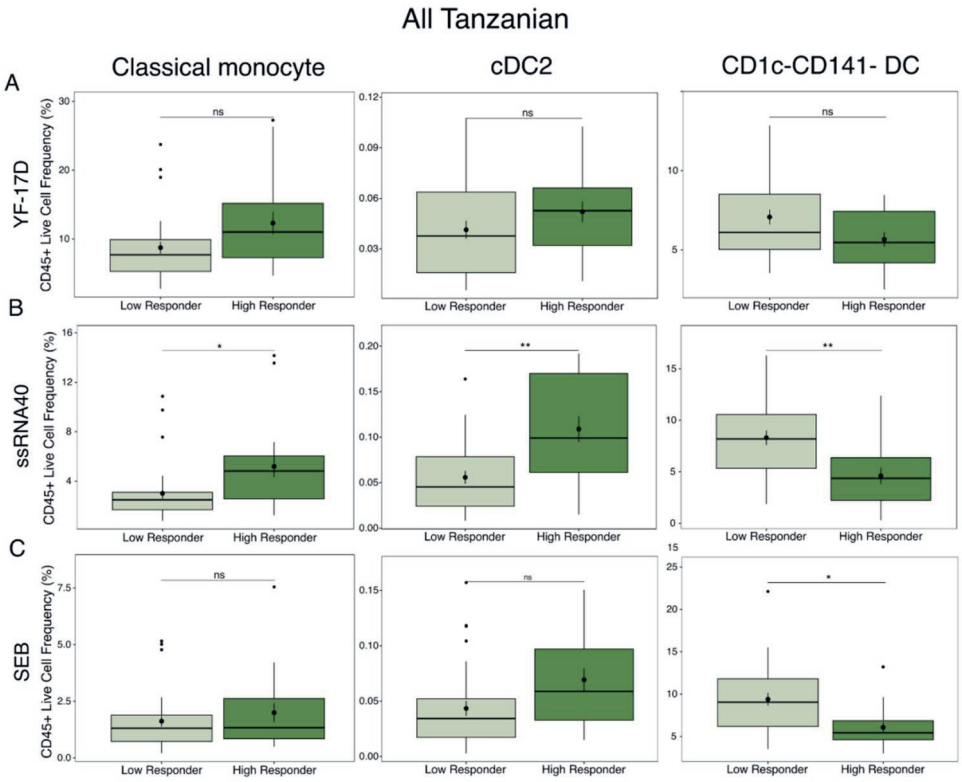

Figure 5. Changes in cell frequency upon culturing differ between stimuli, but cell types associated with antibody response to yellow fever vaccination show overlap across stimulations:
Boxplots showing the frequency of CD16-CD14+ monocytes (left), cDC2 (middle) and CD1c-CD141- DC (right) (as a proportion of the total CD45+ cells) between all Tanzanians who are high or low responders to the YF-17D vaccine. The boxplots are grouped based on whether the innate immune cell cultures were exposed to YF-17D **(A),** ssRNA40 **(B)** or SEB **(C).** The boxplots are coloured by YF-17D vaccine response and contains the median (horizontal line), mean (dot) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Changes in the cell type frequency was assessed using a Mann-Whitney U test, with correction for multiple comparisons being achieved with Benjamini-Hochberg. ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$.

Discussion

In summary, examining the antibody response to YF vaccination across populations revealed that most Dutch were high responders. Although high frequency of low responders were observed among Tanzanian vaccinees, no difference in the proportion of high and low responders between urban and rural Tanzania was observed. Comparing the innate baseline immune profiles across the geographical areas, showed that the overall innate immune profile at baseline of Dutch was distinct from that of Tanzanians and that Dutch had increased frequencies of classical monocytes and decreased frequencies of non-classical monocytes and multiple myeloid dendritic cell subsets. Within the Tanzanian cohort, high responders had a higher frequency of baseline classical monocytes, both *ex vivo* and upon *in vitro* stimulation, in comparison to low responders. When PBMCs were cultured, we found an increased frequency of cDC2s and reduced frequency of CD1c- CD141- DCs in low responding Tanzanians. Moreover, in unstimulated cultures both IL-10 production by cDC2s as well as IFN-alpha concentration in CD1c- CD141- DCs was increased in the high responders.

The increased antibody responses in Dutch compared to Tanzanian found in the current study, aligns with previous reports, as higher YF-17D specific CD8+ T cell and YF neutralizing antibody (nAb) responses have been found in Swiss compared to Ugandans [6]. As the antibody response between urban and rural Tanzanians did not significantly differ, this deviates from the findings of a recent study in Uganda. This study reported significantly higher nAb upon YF vaccination in urban individuals compared to helminth-endemic rural individuals 28 days post-vaccination and to malaria-endemic rural individuals 1 year after vaccination [14]. As helminth and malaria prevalence was low in the current study these discordant results may be explained by different levels of exposure to these pathogens between the Tanzanian and Ugandan cohorts. However, neither preventive treatment for malaria nor intensive praziquantel administration against *Schistosoma mansoni* improved the response to YF vaccine in the same study [15, 16]. As such, either past exposures or differences in the exposure to other environmental factors such as dietary habits should be considered [17].

To further understand the immunological mechanisms underlying variation in the response to YF vaccination, we profiled the innate immune system at baseline from a subset of Dutch and

Tanzanian vaccinees. The frequency of non-classical monocytes were decreased along the rural-urban gradient, while the frequency of classical monocytes showed the opposite trend. This aligns with findings of an earlier study showing increased proportions of intermediate and non-classical monocytes in Africans, while the frequency of classical monocytes followed a rural-urban gradient being highest in Europeans [18]. Within the Tanzanian cohort, the frequency of classical monocytes was significantly increased in high compared to low responders, both *ex vivo* and upon culturing, and when urban and rural were analyzed separately. The study of Muyanja *et al.* (2014) found a negative correlation between monocyte counts at baseline and YF nAb in the Ugandan cohort [6]. Moreover, when combining the data of Swiss and Ugandans vaccinees, the frequency of intermediate monocytes at baseline negatively correlated with YF nAb, although this was clearly driven by the distinct responses between geographical areas [6]. As characterization of the monocyte compartment can be challenging due to downregulation of markers upon culturing and may be affected by the gating strategy and cytometry panel used, the differential findings in our study and the study of Muyanja *et al*. (2014) should be interpreted with caution. A more standardized way of characterizing monocytes [19, 20] would allow comparison of different studies and will facilitate unraveling the role monocytes in response to (YF) vaccination.

Next to monocytes, differences in the proportion and cytokine response of myeloid dendritic cells between high and low responding Tanzanians were observed when PBMCs were cultured. In low responders, we detected an expansion of CD1c- CD141- DCs, a subset known to be enriched for type 1 interferon signaling and to share signatures with CD16+ monocytes [21]. Interestingly, although their frequency was decreased, the IFN-alpha production by these cells in unstimulated cultures was increased in high responders, indicating that in low responders the type 1 interferon production by these DCs might be hampered. Expansion of CD1c- CD141- DCS defective in their IFN signaling has previously been observed in SARS-CoV-2-infected individuals and these DCs were characterized by reduced ability for immune cross-talk and high mitochondrial activity [22]. Efforts should be made to further characterize the CD1c- CD141- DC population identified in the current study as this population is likely to comprise of multiple cell subsets given their high frequency upon culture. In addition to expansion of CD1c- CD141- DCs, increased frequency of IL-10+ cDC2s upon (unstimulated) culturing was observed in low versus high responding Tanzanians. A negative association between high IL-10 levels at baseline and reduced vaccine response has been reported

previously, as the level of IL-10 secreted by baseline PBMCs incubated with YF-17D overnight negatively correlated with YF nAb levels in Ugandans [6]. Given that IL-10 is an anti-inflammatory cytokine that regulates the T cell responses [23], enhanced IL-10 levels at baseline may suppress T cell responses upon vaccination, thereby hampering vaccine response. Indeed, a study in mice showed that increased levels of IL-10 were associated with reduced T cell responses and that blocking IL-10 during vaccination resulted in enhanced effector T cell responses and improved vaccine efficacy [24]. Together, these results indicate that alterations in subsets of myeloid dendritic cells are associated with reduced vaccine response. Therefore, single cell analysis of these dendritic cell subsets would be highly valuable to gain insight into the pathways involved and how these may be modulated to increase vaccine response.

Inherent to the preliminary nature of the analysis presented here, there are some limitations to be accounted for. In the current analysis, the characterization of the innate immune compartment was limited to identification of the main innate immune cell types by manual gating and the expression of the activation markers and cytokine production within these major innate immune subsets. Additional analysis such as sub-clustering of these major cell types which takes into account co-expression would enable us to identify and define smaller subsets and study their role in the response to YF vaccination. Moreover, the innate immune system of a limited number of subjects was profiled at baseline. As differences between the urban and rural populations included in the current study are likely to be relatively small, immune profiling of additional vaccinees from rural and urban Tanzanians might provide more power to detect differences.

In conclusion, upon YF vaccination the Dutch demonstrated increased antibody responses compared to Tanzanians, whereas the proportion of low responders was similar between urban and rural Tanzanians. Within the Tanzanian cohort, low responders were characterized by decreased frequency of classical monocytes and IFN-alpha production by CD1c- CD141- DCs, whereas IL-10 production by cDC2s in unstimulated cultures was increased. These results indicate that differences in the myeloid compartment at baseline may be associated with YF vaccine response, although additional characterization is needed to understand how specific cell subsets contribute to vaccine response variation. The insights obtained by this study contribute to our understanding of the immunological mechanisms underlying vaccine responses, which is essential for the development of interventions needed to overcome vaccine hyporesponsiveness.

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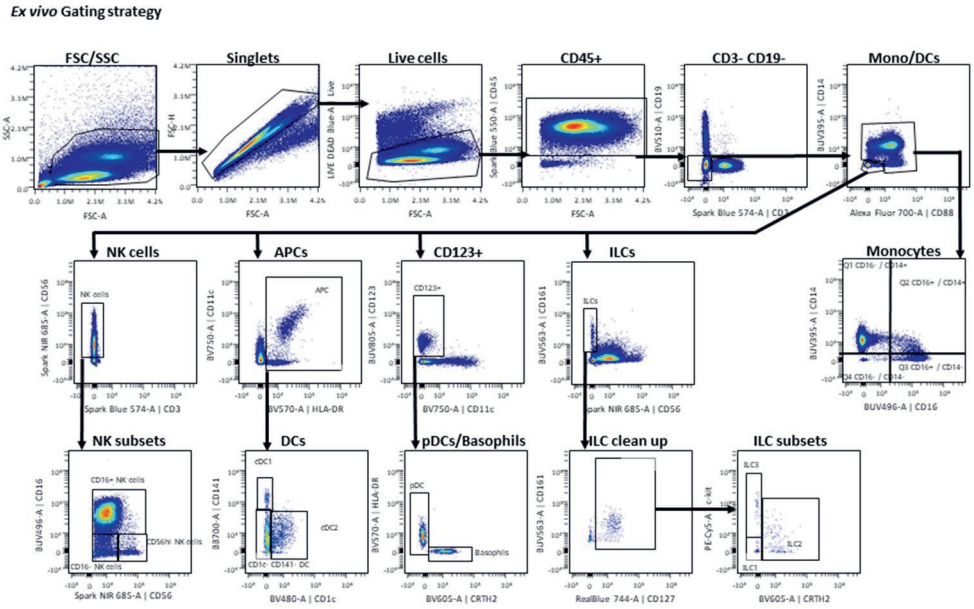
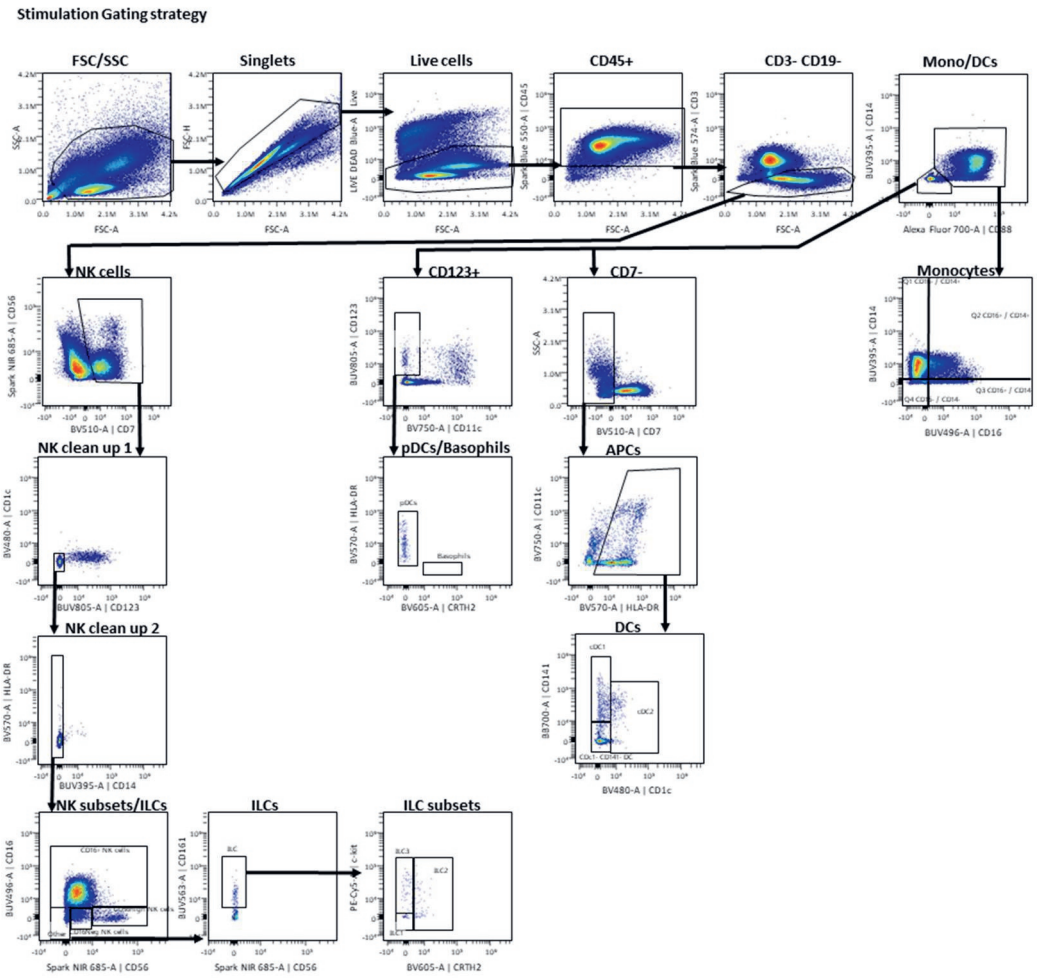
Supplementary material



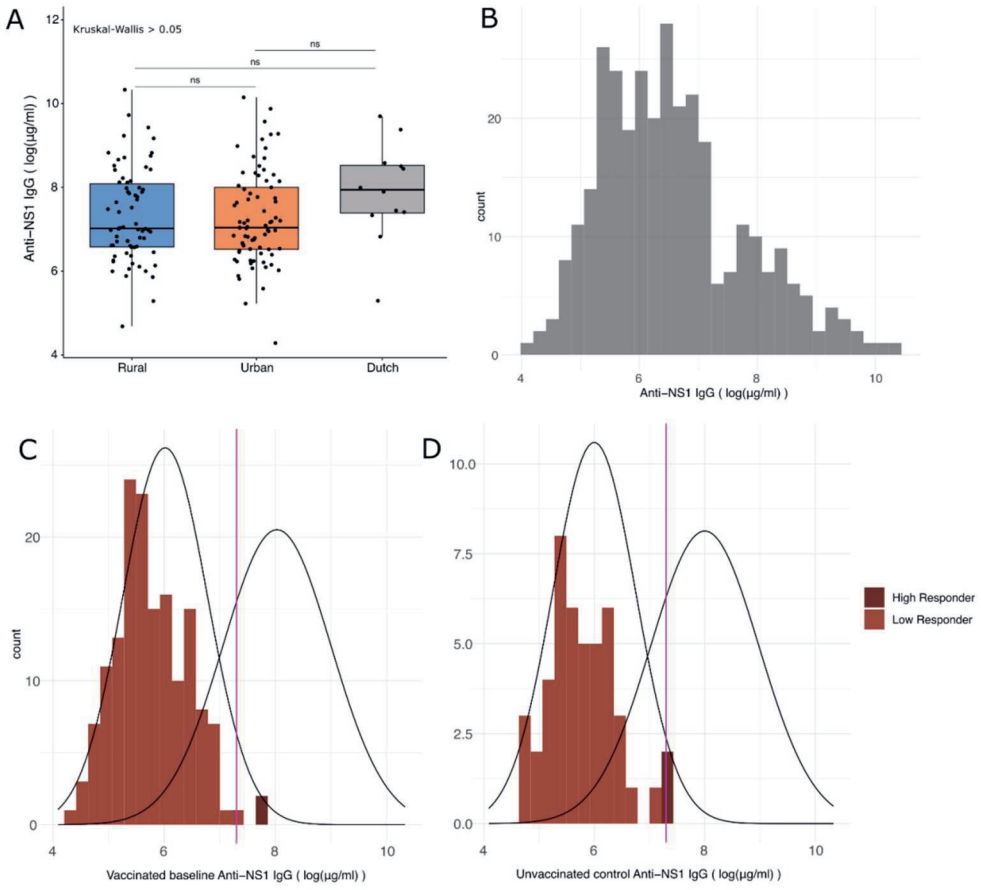
Supp. Figure 1 Gating strategy *ex vivo* spectral flow cytometry

Example of the gating strategies used to identify the cell types present in the *ex vivo* derived PBMCs as captured using spectral flow cytometry. The x and y axes represent the compensated fluorescence values of specific fluorophore/marker combinations or stain, with each dot representing an event and the events coloured by event density. Gating was carried out using OMIQ.



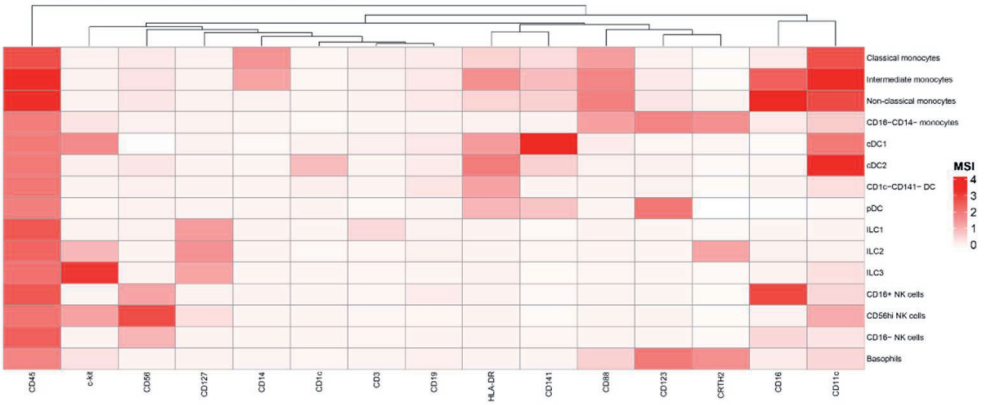
Supp. Figure 2 Gating strategy *in vitro* stimulation spectral flow cytometry

Example of the gating strategies used to identify the cell types present when PBMCs are cultured for 24 hours with either Yellow Fever vaccine, SSRNA40 or SEB as captured using spectral flow cytometry. The x and y axes represent the compensated fluorescence values of specific fluorophore/marker combinations or stain, with each dot representing an event and the events coloured by event density. Gating was carried out using OMIQ.



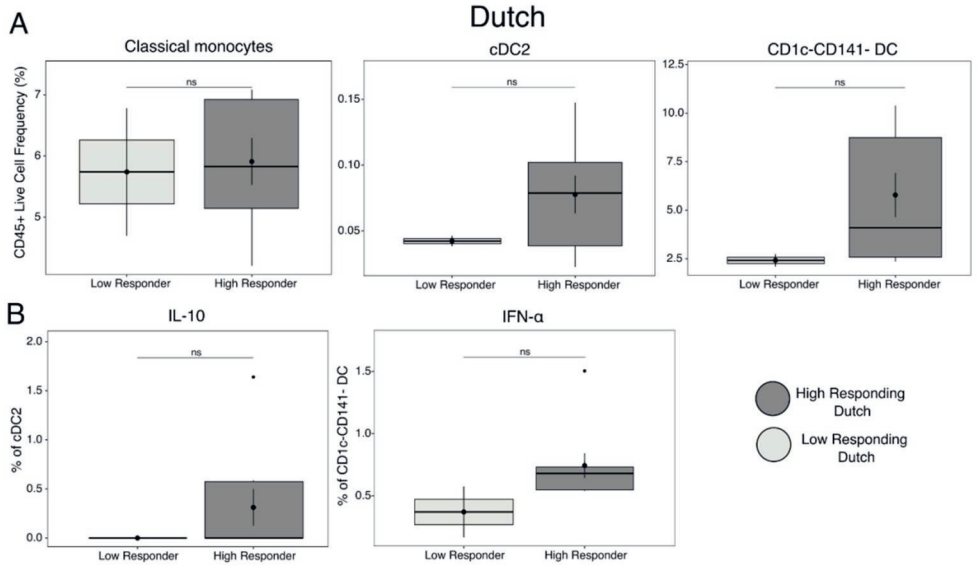
Supp. Figure 3 Yellow fever antibody response across geographical areas and in the control group

(A) Boxplot showing the Log_2 fold change of anti-NS1 IgG levels in individuals from baseline to 178 days post-vaccination with YF-17D. The boxplots are coloured by the geographical area that the individual is from and contains the median (horizontal line), mean (dot) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Differences in log_2FC between the different areas was assessed with Kruskal-Wallis test, with post-hoc multiple comparisons achieved with Dunn's test using Benjamini-Hochberg correction. ns = $p > 0.05$. (B) Histogram visualising the anti-NS1 IgG levels from individuals at baseline and 178 days post-vaccination with YF-17D. Histogram visualising the anti-NS1 IgG levels of **vaccinated individuals at baseline (C)** and unvaccinated control individuals **(D)**, with the individuals coloured by whether they were classified as high or low responders, based on the GMM model shown in Figure 1B. The black lines show the distributions of the two Gaussian models while the purple vertical line denotes the decision boundary between the two clusters.



Supp. Figure 4 Expression of lineage markers in *Ex vivo* dataset cells

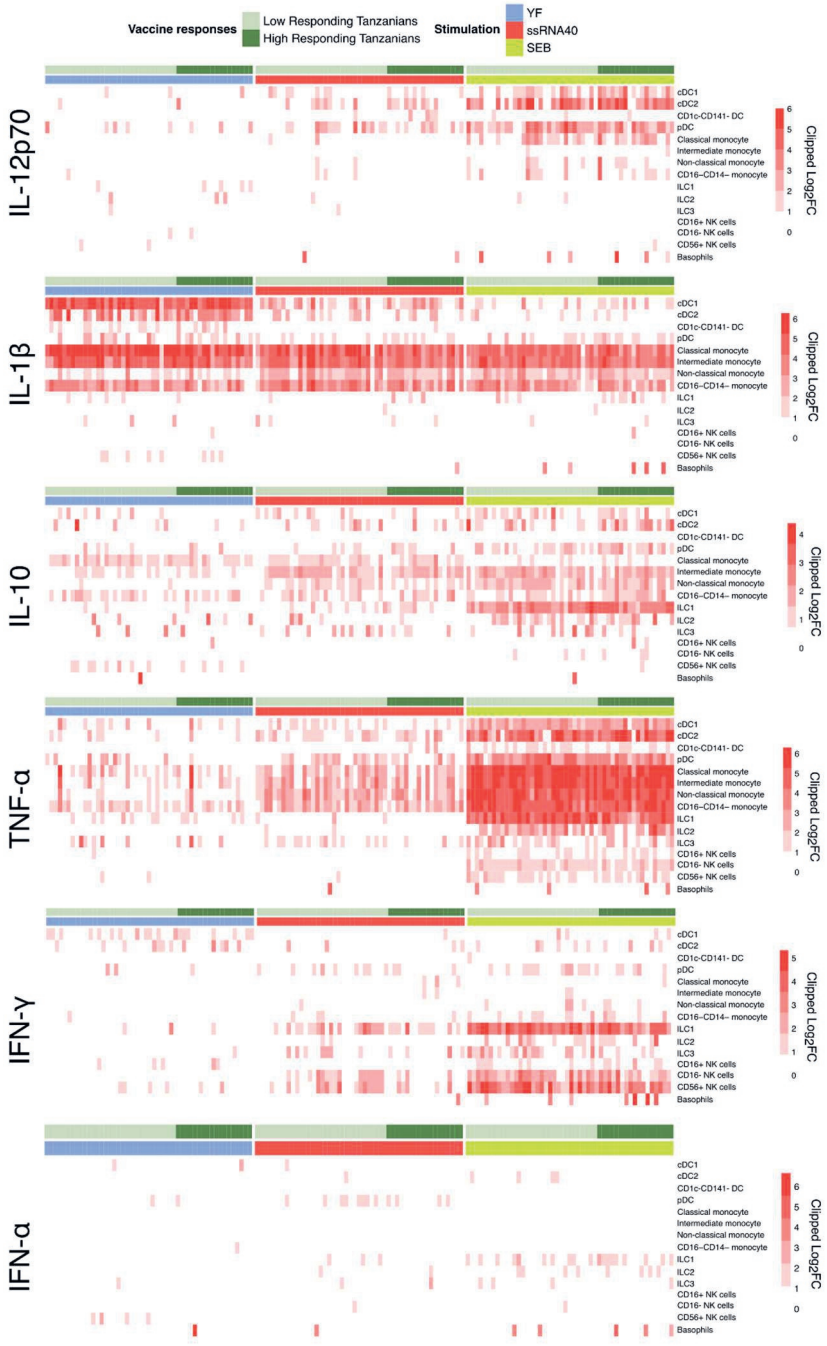
Heatmap showing the median signal intensity (MSI) of lineage markers across the OMIQ defined gates. Data shown is from all *ex vivo* spectral flow cytometry samples collected from Dutch and Tanzanian individuals.



Supp. Figure 5 Difference in frequency and cytokine production in medium condition between low and high responding Dutch vaccines

Boxplots showing the frequency of various innate immune cell types (as a percentage of total CD45+ cells) **(A)** and frequency of cytokine producing cells (as a percentage of the total parent cell) **(B)** between Dutch high and low responders to the YF-17D vaccine, when the immune cells were cultured in media. The boxplots are coloured by YF-17D vaccine response and contains the median (horizontal line), mean (dot) and 25th/75th percentiles with whiskers extending to ±1.5 × IQR. Differences in innate immune cell frequency and cytokine

producing cell frequency across between high and low responders was assessed with Mann-Whitney U test, with Benjamini-Hochberg correction to account for multiple testing. ns = $p > 0.05$.



Supp. Figure 6 Cytokine responses upon culturing with different stimuli across cell subsets
Heatmap showing the Log_2 fold change (Log_2FC) in cytokine+ cell frequency (as a percentage of total parent cell) between the media only culture and the YF-17D, ssRNA40 and SEB stimulated for all Tanzanian derived cultures. Each column represents an individual, with the individuals being grouped by whether they are a high or low responder (top grouping) and which stimuli was used during the culture (bottom grouping). A high Log_2FC indicates that the frequency of cytokine+ cells is higher in the given stimulated condition compared to the media-only. Log_2FC below 0 have been clipped to 0 for the purposes of this plot.

Table S1. Flow cytometry Antibody Panels

No.	Panel	Target	Fluorochrome	Dilution	Clone	Source	Catalogue	Type
1	Both	CD294 (CRTH2)	BV605	50	BM16	Biolegend	350121	Lineage
2	Both	CD279 (PD-1)	PE/Fire 700	50	A17188B	Biolegend	621621	Activation
3	Both	CD11c	BV750	50	B-ly6	BD	747459	Lineage
4	Both	CD159c (NKG2C)	BUV615	50	134591	BD	751059	Activation
5	Both	CD274 (PD-L1)	APC/Fire 810	50	MIH3	Biolegend	374515	Activation
6	Both	CD117 (c-kit)	PE/Cy5	50	104D2	Biolegend	313210	Activation
7	Both	CD178 (FasL)	PE/Cy7	50	NOK-1	Biolegend	306417	Activation
8	Both	CD161	BUV563	100	HP-3G10	BD	749223	Activation
9	Both	TIGIT	BV421	200	741182	BD	747844	Activation
10	Both	KLRG1	APC/Fire 750	200	SA231A2	Biolegend	367717	Activation
11	Both	CD14	BUV395	200	M5E2	BD	740286	Lineage
12	Both	CD45	Spark Blue 550	200	2D1	Biolegend	368549	Lineage
13	Both	CD88	AF700	200	S5/1	Biolegend	344314	Lineage
14	Both	CD366 (TIM-3)	BUV737	400	7D3	BD	568680	Activation
15	Both	CD123	BUV805	400	6H6	BD	751840	Lineage
16	Both	CD40	BV785	400	5C3	Biolegend	334339	Activation
17	Both	CD3	Spark Blue 574	400	UCHT1	Biolegend	300487	Lineage
18	Both	CD56	Spark NIR 685	800	5.1H11	Biolegend	362563	Lineage
19	Both	HLA-DR	BV570	800	L243	Biolegend	307637	Activation
20	Both	CD141	BB700	1600	1A4	BD	742245	Lineage
21	Both	CD16	BUV496	1600	3G8	BD	612944	Lineage
22	Both	CD163	BUV661	1600	MAC2-158	BD	752880	Activation
23	Both	CD1c	BV480	1600	F10/21A3	BD	746677	Lineage
24	Both	CD57	Pacific Blue	3200	HNK-1	Biolegend	359607	Activation
25	Ex vivo	CD127	RB744	50	HIL-7R-M21	BD	570607	Lineage
26	Ex vivo	CD19	BV510	400	HIB19	Biolegend	302241	Lineage
27	Culture	CD19	Spark Blue 574	100	SJ25C1	Biolegend	363048	Lineage
28	Culture	CD7	BV510	200	M-T701	BD	563650	Lineage
29	Culture	IFN-gamma	BV650	1600	4S.B3	Biolegend	502537	Cytokine
30	Culture	TNF-alpha	RealBlue780	12800	Mab11	BD	569091	Cytokine
31	Culture	IL12p70	APC	50	C11.5	BD	554576	Cytokine
32	Culture	IFN-alpha	PE-Vio 615	100	REA1013	BD	560097	Cytokine
33	Culture	IL-10	BV711	100	JES3-9D7	BD	564050	Cytokine
24	Culture	IL1b	FITC	1600	JK1B-1	Biolegend	508206	Cytokine

Ex vivo panel indicated that the antibody was only used in the panel to the *ex vivo* condition Culture panel indicates that the antibody was only used in the panel to measure after *in vitro* stimulation with medium, YF-17D, ssRNA40 or SEB for 24 hours Both panel indicates that the anybody was used for both in vitro stimulation and ex vivo measurements.

Table S2. Baseline characteristics of the vaccinated study population

Variable	N	Vaccinated Dutch N = 15[1]	Vaccinated Tanzanian N = 155[1]	p-value[2]
Sex	170			0.003
Female		13 (87%)	73 (47%)	
Male		2 (13%)	82 (53%)	
Age	170	20 (20, 22)	23 (21, 27)	0.004
Age Category	170			0.003
18-25		15 (100%)	100 (65%)	
26-35		0 (0%)	55 (35%)	

[1] n (%); Median (Q1, Q3)

[2] Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test

N – 170 participants. Values represent the number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables respectively. Comparisons between vaccinated Dutch and all vaccinated Tanzanians were performed using Pearson's Chi-squared test for comparing Sex, Fisher's exact test for Age category and Wilcoxon rank sum test for Age.

Table S3. Baseline characteristics of the vaccinated and unvaccinated Tanzanian study population

Variable	N	Unvaccinated Tanzanian N = 30[1]	Vaccinated Tanzanian N = 155[1]	p-value[2]
Sex	185			0.3
Female		11 (37%)	73 (47%)	
Male		19 (63%)	82 (53%)	
Age	185	22 (20, 25)	23 (21, 27)	0.3
Age Category	185			0.2
18-25		23 (77%)	100 (65%)	
26-35		7 (23%)	55 (35%)	

[1] n (%); Median (Q1, Q3)

[2] Pearson's Chi-squared test; Wilcoxon rank sum test

N – 185 participants. Values represent the number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables respectively. Comparisons between vaccinated Dutch and all vaccinated Tanzanians were performed using Pearson's Chi-squared test for comparing Sex and Age category, and Wilcoxon rank sum test for Age.

Table S4. Comparisons of characteristics between all, plasma anti-NS1 IgG measured and PBMC sampled vaccinated Dutch individuals

Variable	Dutch, All N = 15[1]	Dutch, Plasma N = 12[1]	Dutch, PBMC N = 14[1]	p-value[2]
Sex				>0.9
Female	13 (87%)	11 (92%)	12 (86%)	
Male	2 (13%)	1 (8.3%)	2 (14%)	
Age	20 (20, 22)	21 (20, 22)	20 (20, 22)	>0.9
Anti-NS1, 178 days post-vaccination	7.94 (7.37, 8.54)	7.94 (7.37, 8.54)	7.99 (7.33, 8.58)	>0.9

[1] n (%); Median (Q1, Q3)

[2] Fisher's exact test; Kruskal-Wallis rank sum test

Values represent the number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables respectively. Comparisons between the three populations were performed using Fisher's exact test for comparing Sex and Kruskal-Wallis rank sum test for Age and anti-NS1 levels.

Table S5. Comparisons of characteristics between all, plasma anti-NS1 IgG measured and PBMC sampled vaccinated Tanzanian individuals

Variable	Tanzanian, All N = 155[1]	Tanzanian, Plasma N = 147[1]	Tanzanian, PBMC N = 50[1]	p-value[2]
Sex				>0.9
Female	73 (47%)	69 (47%)	24 (48%)	
Male	82 (53%)	78 (53%)	26 (52%)	
Age	23 (21, 27)	24 (21, 27)	22 (20, 26)	0.2
Anti-NS1, 178 days post-vaccination	7.03 (6.53, 8.04)	7.03 (6.53, 8.04)	6.90 (6.36, 8.15)	0.7

[1] n (%); Median (Q1, Q3)

[2] Pearson's Chi-squared test; Kruskal-Wallis rank sum test

Values represent the number of participants (percentage of total) and median (interquartile range [IQR]) for categorical and continuous variables respectively. Comparisons between the three populations were performed using Pearson's Chi-squared test for comparing Sex and Kruskal-Wallis rank sum test for Age and anti-NS1 levels.

Table S6. Differences in frequencies of vaccine responders between Dutch and Tanzanians, and Rural and Urban Tanzanians

Variable	N	Dutch, Plasma N = 12[1]	Tanzanian, Plasma N = 147[1]	p-value[2]	N	Rural Tanzanian, Plasma N = 70[1]	Urban Tanzanian, Plasma N = 77[1]	p-value[2]
Vaccine Responder	159			0.022	147			0.4
High Responder		9 (75%)	60 (41%)			31 (44%)	29 (38%)	
Low Responder		3 (25%)	87 (59%)			39 (56%)	48 (62%)	

[1] n (%)

[2] Pearson's Chi-squared test

N – 158 participants for Dutch and all Tanzanians, *N* – 146 for all Tanzanians. Values represent the number of participants (percentage of total). Comparisons between the frequency of high and low vaccine responders were performed using Pearson's Chi-squared test.