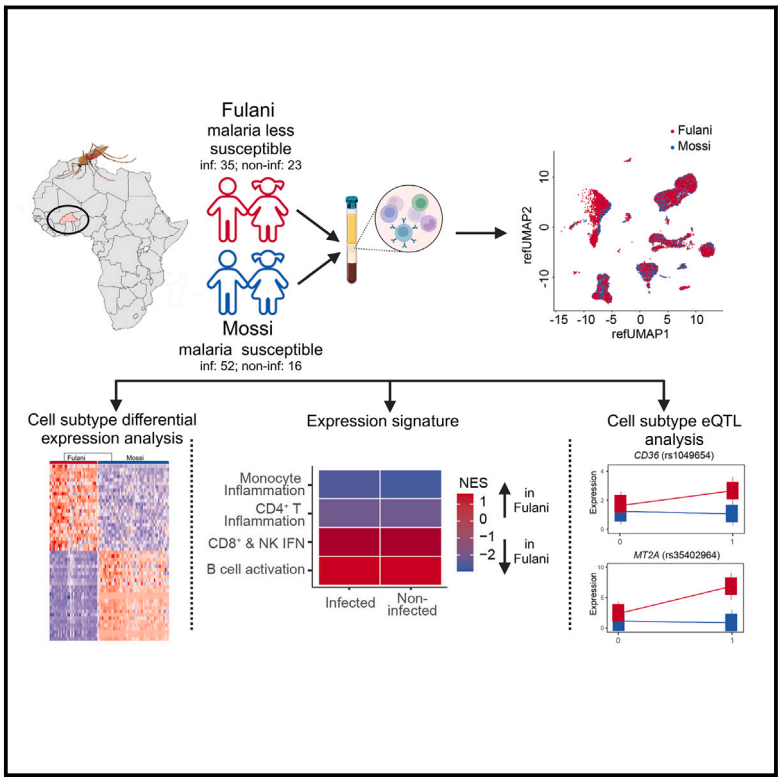
Single-cell transcriptomics reveals inter-ethnic variation in immune response to *Falciparum* malaria

Graphical abstract



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Comparative analysis of the single-cell transcriptomic profiles of peripheral blood immune cells from Fulani and Mossi ethnic groups in Burkina Faso revealed a significant impact of inter-ethnic variation on the immune profiles of children, with varying cell-subtype-specific expression signatures contributing to distinct immune responses to *Plasmodium falciparum* infection.

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Single-cell transcriptomics reveals inter-ethnic variation in immune response to *Falciparum* malaria

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Summary

Africa's environmental, cultural, and genetic diversity can profoundly shape population responses to infectious diseases, including malaria caused by *Plasmodium falciparum*. Differences in malaria susceptibility among populations are documented, but the underlying mechanisms remain poorly understood. Notably, the Fulani ethnic group in Africa is less susceptible to malaria compared to other sympatric groups, such as the Mossi. They exhibit lower disease rates and parasite load as well as enhanced serological protection. However, elucidating the molecular and cellular basis of this protection has been challenging in part due to limited immunological characterization at the cellular level. To address this question, we performed single-cell transcriptomic profiling of peripheral blood mononuclear cells from 126 infected and non-infected Fulani and Mossi children in rural Burkina Faso. This analysis generated over 70,000 single-cell transcriptomes and identified 30 distinct cell subtypes. We report a profound effect of ethnicity on the transcriptional landscape, particularly within monocyte populations. Differential expression analysis across cell subtypes revealed ethnic-specific immune signatures under both infected and non-infected states. Specifically, monocytes and T cell subtypes of the Fulani exhibited reduced pro-inflammatory responses, while their B cell subtypes displayed stronger activation and inflammatory profiles. Furthermore, single-cell expression quantitative trait locus (eQTL) analysis in monocytes of infected children revealed several significant regulatory variants with ethnicity-specific effects on immune-related genes, including *CD36* and *MT2A*. Overall, we identify ethnic, cell-type-specific, and genetic regulatory effects on host immune responses to malaria and provide valuable single-cell eQTL and transcriptomic datasets from under-represented populations.

Africa, the cradle of humankind, is the continent with the largest genetic diversity and extensive population substructure, considering the complex population history and vast variation in climate, diet, modes of subsistence, and pathogen exposure.[1–4] Lifestyle transitions and the constant rise of urbanization have led to variations in susceptibility to communicable and non-communicable diseases as well as to differences in responses to vaccines and pharmacological drugs.[5] However, African populations are significantly under-represented in genomic studies, representing a mere 0.2% of participants in genome-wide association studies to date.[6] This issue is particularly relevant in functional genomic studies, posing serious challenges for interpretation of genotype-phenotype associations and hindering the development of more accurate diagnosis, improved genetic risk prediction, and targeted clinical care for these populations.

Environmental, cultural, and genetic diversity across African populations influence their responses to infectious diseases.[2] Such interplay between genetic variation, environment, and infection has played a pivotal role in shaping disease susceptibility across the continent. Among these infectious diseases, malaria (MIM: 611162) stands out as one of the most significant, having exerted immense selective pressure on African populations throughout history.[7] *Plasmodium falciparum* is one of five malaria-causing species (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*) endemic to many African countries and carries the highest mortality burden (>96%), with nearly 600,000 reported deaths in 2022, most of whom were children under the age of 5 years (https://www.who.int/publications/i/item/9789240086173).[8,9] Clinical manifestations of malaria range from mild/uncomplicated forms to severe disease as well as the poorly defined asymptomatic infections that act as a silent reservoir for disease transmission.[10–13]

In humans, genetic mutations leading to sickle cell disease, hemoglobinopathies, and G6PD deficiency contribute to malaria protection.[14–19] However, beyond these genetic disorders and parasite genetic diversity, the broad spectrum of host clinical manifestations in malaria is largely attributed to the diverse interindividual immune response, dictated by complex genetic factors, environmental and lifestyle determinants, and complex epistatic and gene-by-environment interactions.[20–22] Acquired immunity to recurrent *P. falciparum* infection in African

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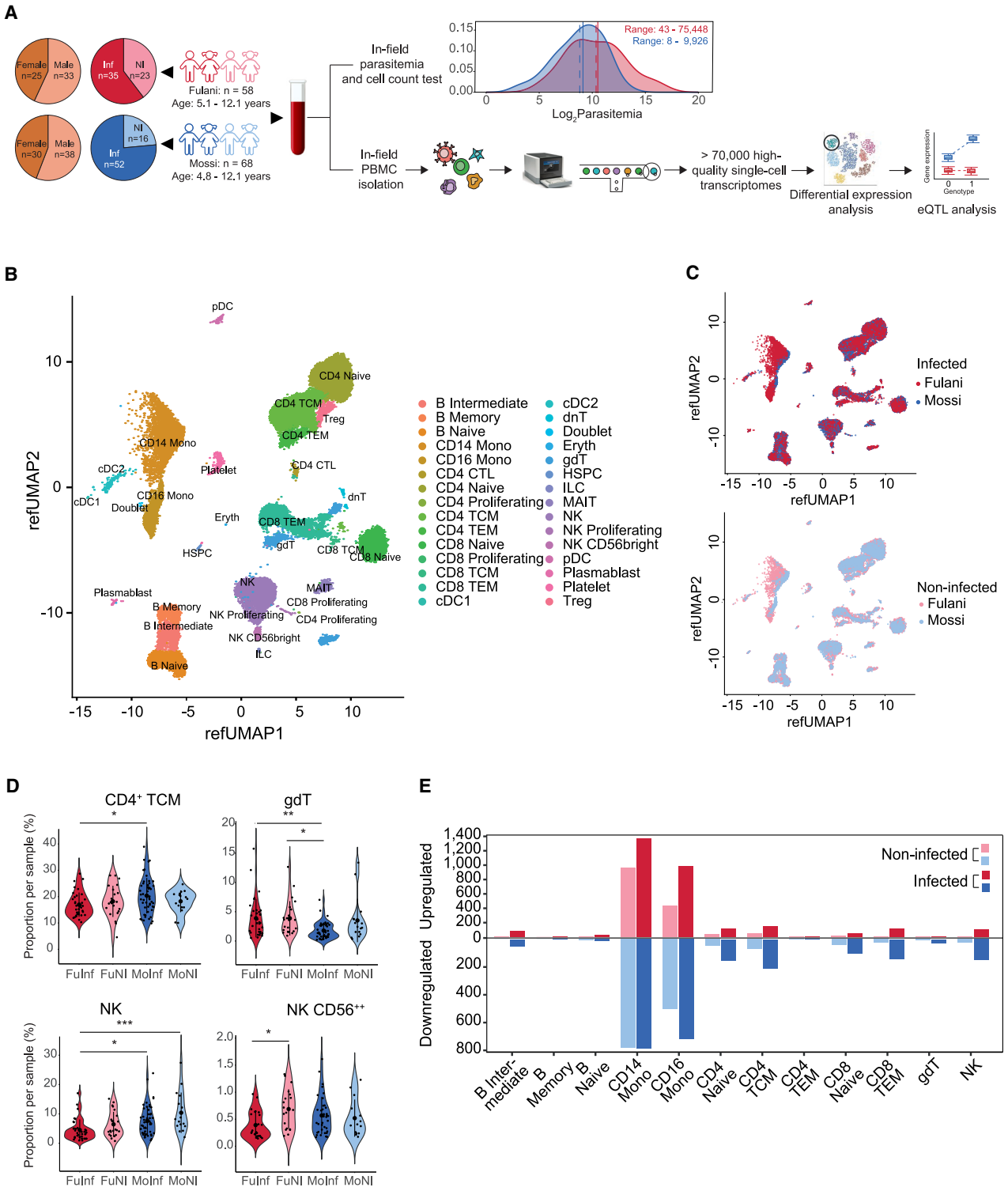

Figure 1. Single-cell RNA sequencing of PBMCs from children of the Fulani and Mossi ethnic groups with and without asymptomatic malaria

(A) Illustration of study participants with 58 (infected [Inf]: 35, non-infected [NI]: 23) Fulani and 68 (Inf: 52, NI: 16) blood samples from Mossi children. Age, sex, infection status, distribution of $\log_2$ parasitemia measured in the samples, and the steps taken for scRNA-seq analysis are shown. Red, infected Fulani (FuInf); pink, non-infected Fulani (FuNI); blue, infected Mossi (MoInf); light blue, non-infected Mossi (MoNI).

(B) Reference UMAP of the combined scRNA-seq dataset comprising 71,784 cells from Fulani and Mossi children colored by level-2 predicted cell subtypes.

(C) Same reference UMAP from (B) split into two plots based on infection status (top: infected; bottom: non-infected) and colored by ethnicity (red, FuInf; pink, FuNI; blue, MoInf; light blue, MoNI).

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children prioritizes host fitness, with the development of anti-parasitic immunity lagging behind. This delay is due to the parasite's ability to induce immunoregulatory and tolerogenic responses across several immune cell subtypes, which prevent immunopathology but hinder protection from mild disease.[23–27] Therefore, in-depth genetic and immunological studies are needed in areas of high malaria endemicity to characterize the immune correlates that establish anti-parasitic immunity while preventing immunopathogenesis.[25,28]

The Fulani ethnic group comprises over 30 million people primarily spread across West and Central Africa, many of whom maintain a nomadic pastoralist lifestyle. Several studies have shown that Fulani individuals are less susceptible to symptomatic *P. falciparum* infection compared to other sympatric ethnic groups, such as the Mossi, despite sharing similar environmental and socioeconomic conditions. Fulani exhibit lower disease rates and parasite densities, coupled with higher serological protection and antibody responses—features that have not been associated with known malaria-resistant genetic variants.[29,30] Interestingly, Fulani lifestyle, characterized by herding practices and a tradition of a milk-based diet, distinguishes them from other sympatric groups.[31] Moreover, Fulani have distinct genetic backgrounds due to ancestral Eurasian admixture.[32] However, the immune mechanisms and gene-regulatory features underlying their reduced susceptibility to malaria at the cell-type-specific level, along with the genetic and environmental interactions involved, remain unresolved.

In this study, we leverage the diversity of sympatric Fulani and Mossi populations to investigate the impact of self-reported ethnicity on the host immune transcriptional response to *P. falciparum* infection at single-cell resolution. Here, self-reported ethnicity is framed as a social construct that reflects lifestyle and cultural traditions of the respective ethnic groups rather than serving as a proxy for ancestry or individual genetic background.[33] At the forefront of our experimental design was the randomization of samples across ethnic groups and infection states in all experimental procedures to avoid batch effects and transcriptional variation due to technical factors. We primarily focused on identifying cell-type-specific transcriptional signatures that differ between the two ethnic groups in both non-infected and infected states. Subsequently, we performed expression quantitative trait locus (eQTL) analysis to uncover potential regulatory genetic variants and assess the dependency of their allelic effect on ethnicity. This effort led to the identification of cell-type-specific molecular cues associated with ethnicity that modulate the host immune response to *P. falciparum* infection.

Following institutional ethical approvals and participant recruitment, informed consent was obtained and whole blood and clinical data were collected from 126 children of the Fulani or Mossi sympatric ethnic groups living in the rural health district of Saponé, Burkina Faso, during the high *P. falciparum* transmission wet season (Figure 1A). Of the 58 Fulani children enrolled, 23 were non-infected (FuNI) and 35 were infected (FuInf, mean $\log_2$ parasitemia 6.486). Of the 68 Mossi children, 16 were non-infected (MoNI) and 52 were infected (MoInf, mean $\log_2$ parasitemia 6.466; Figure 1A and Table S1). A total of 147,458 peripheral blood mononuclear cells (PBMCs) isolated from the blood of the 126 children were subjected to single-cell RNA sequencing (scRNA-seq) and randomized into pools of six samples each across two batches (Table S2; GEO: GSE273781 and GSE273785). Following genotype-based demultiplexing and doublet removal, we applied a reference-mapping-based method to classify the remaining 122,906 cells into cellular subtypes, assigning a computed prediction score to each cell (see supplemental methods; Zenodo: https://doi.org/10.5281/zenodo.14719257; Table S3).[34,35] We then filtered cells based on quality control metrics (mitochondrial content, RNA counts, and unique molecular identifier counts) and applied a stringent prediction score greater than 0.7, leaving a total of 71,784 cells with an average of 570 cells assigned to each sample (Tables S4 and S5). At a more granular level, 30 cell subtypes were identified, across myeloid, T cell, B cell, and natural killer (NK) cell immune lineages, as shown in the reference uniform manifold approximation and projection (refUMAP; Figure 1B). Expression of known marker genes per cell subtype was confirmed (Figure S1). Grouping based on ethnicity, though visually subtle, showed a significant difference in the coordinates of refUMAP1 (x axis) between both ethnic groups limited to the monocyte subsets of the refUMAP (refUMAP1 Fulani mean = −7.6, refUMAP1 Mossi mean = −6.69, p value $<2.2 \times 10^{-16}$, Welch two-sample t test; Figure 1C). Moreover, by performing pseudobulk analysis and grouping cells per individual, unsupervised principal-component analysis (PCA) revealed two major clusters of individuals grouped by ethnicity, further highlighting the impact of ethnicity on the transcriptome

(D) Violin plots showing the proportions of four cell types, $CD4^+$ TCM, γδT, NK, and $CD56^{++}$ NK cells, per individual PBMC sample from children corresponding to one of the four groups (FuInf, FuNI, MoInf, MoNI). Pairwise comparisons were performed by one-way ANOVA with post hoc Tukey honest significant difference (HSD) (*$p \leq 0.05$; **$p \leq 0.01$, ***$p \leq 0.001$).
(E) Barplot of significant upregulated (top; red and pink) and downregulated (bottom; dark and light blue) DEGs ($|\log_2 FC| > 0.263$; $p$adj < 0.05) in the Fulani relative to the Mossi across 12 cell subtypes in the infected (red and blue) and non-infected (pink and light blue) states.
PBMC, peripheral blood mononuclear cell; eQTL, expression quantitative trait locus; refUMAP, reference UMAP; Mono, monocytes; CTL, cytotoxic T; TCM, central memory T; TEM, effector memory T; cDC, conventional dendritic cell; dnT, double-negative T; Eryth, erythroid cell; gdT, γδT; HSPC, hematopoietic stem and progenitor cell; ILC, innate lymphoid cell; MAIT, mucosal associated invariant T; NK, natural killer; pDC, plasmacytoid dendritic cell; Treg, regulatory T.

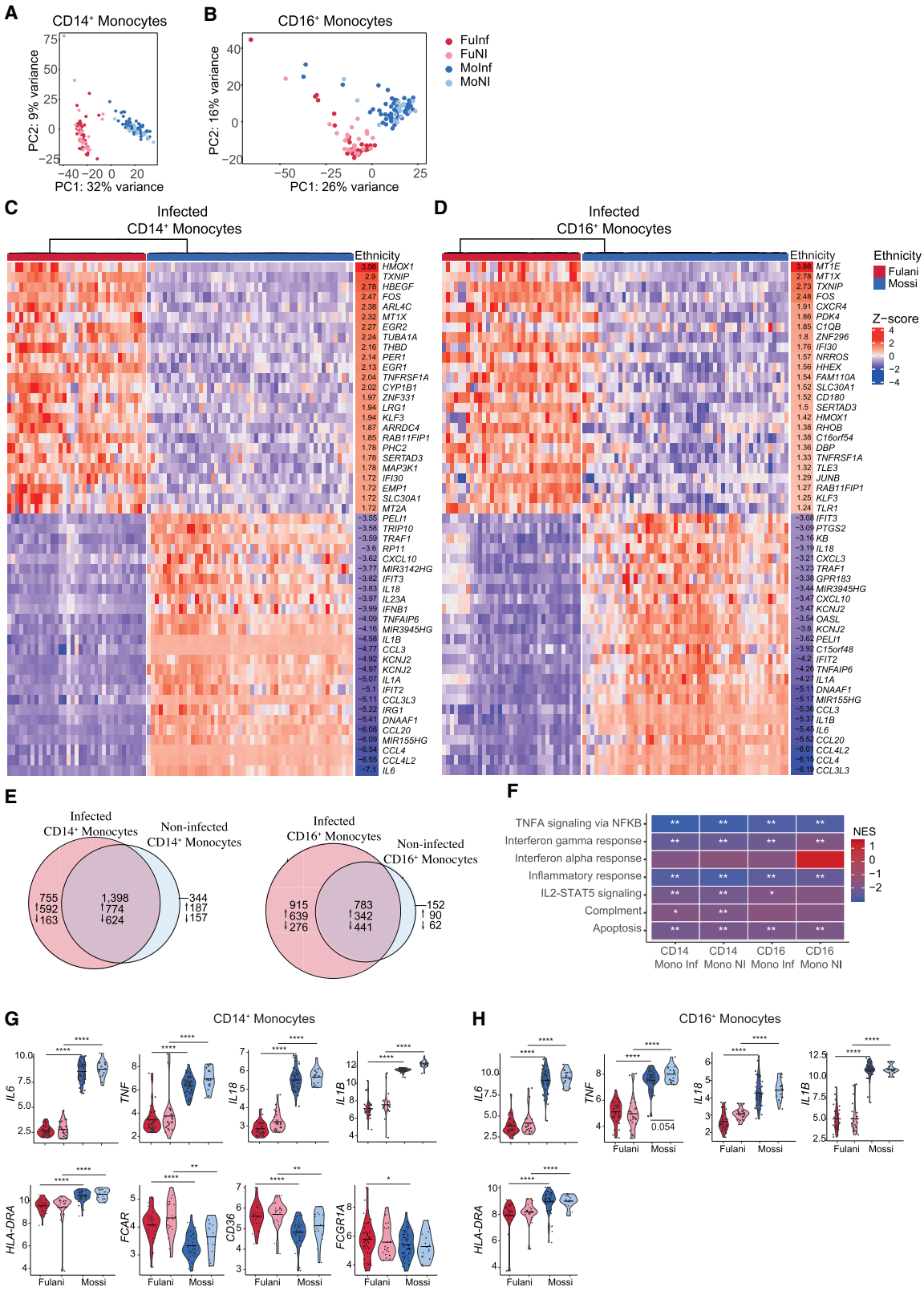

Figure 2. Analysis of transcriptomic changes in infected CD14$^+$ and CD16$^+$ monocytes

(A and B) Principal-component (PC) analysis plots of the pseudobulk transcriptomes of CD14$^+$ and CD16$^+$ monocytes from the four groups (FuInf, FuNI, MoInf, MoNI), with PC1 and PC2 explaining 41% and 42% of the total variance in each cell subtype, respectively. (C and D) One-way hierarchical heatmap of the top 25 upregulated and top 25 downregulated genes in the Fulani infected CD14$^+$ and CD16$^+$ monocytes, respectively, relative to those of the Mossi ($p$adj < 0.05). Log_2FC values for each gene are denoted on the left of the

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(Figure S2). Unsupervised Louvain-based clustering on the filtered cells was also performed, demonstrating high agreement with the reference-based method (see supplemental methods and results; Figures S3 and S4; Tables S6, S7, S8, and S9).

Compositional analysis was then performed to assess changes in cellular frequencies between Mossi and Fulani children in the infected and non-infected states. Twenty-two cellular subsets, each represented by at least three individuals across the four ethnic/infection groups, were analyzed, and their cellular frequencies were calculated per individual (Figure S5 and Table S5). Relative to the infected Mossi, infected Fulani showed a significant decrease in the abundance of $CD4^+$ central memory T ($CD4^+$ TCM; FuInf: mean = 17%, $n = 35$, SD = 4.59; MoInf: mean = 20.4%, $n = 52$, SD = 6.73; $p$adj = 0.041) and NK cells (FuInf: mean = 4.52%, $n = 35$, SD = 3.6; MoInf: mean = 7.66%, $n = 51$, SD = 4.96; $p$adj = 0.018; Figure 1D). However, infected Fulani exhibited a significant increase in the proportion of gamma-delta T (γδT) cells relative to the infected Mossi (FuInf: mean = 3.88%, $n = 35$, SD = 3.27; MoInf: mean = 1.78%, $n = 47$, SD = 1.39; $p$adj = 3.72×10^{-3}; Figure 1D). This observation confirms previous findings in a cohort of Mossi and Fulani adults, which reported higher frequencies of γδT cells in both *P. falciparum*-infected and non-infected Fulani individuals, as measured by flow cytometry ($CD3^+Pan\gamma\delta^+$).[36] Comparisons between infected and non-infected states within each ethnic group revealed only one significant difference: infected Fulani samples had a lower proportion of $CD56^{++}$ NK cells compared to the non-infected counterpart (FuInf: mean = 0.391%, $n = 24$, SD = 0.248; FuNI: mean = 0.688%, $n = 19$, SD = 0.368; $p$adj = 0.021; Figure 1D).

Beyond changes in cell-subtype proportions, gene expression changes at the cell-subtype level in both non-infected and infected states can provide significant insights into differences in immune responses between the two ethnic groups. Therefore, we performed pseudobulk differential gene expression analysis (DGEA) for each cell subtype, comparing Fulani to Mossi children in both the non-infected and infected states, while accounting for age and sex. The highest number of differentially expressed genes (DEGs) were recorded in $CD14^+$ and $CD16^+$ monocytes (2,153 [55.8%] and 1,698 [39.6%] DEGs in the infected state, and 1,742 [42.3%] and 935 [24.8%] DEGs in the non-infected state, respectively; $|\log_2$ fold change (FC)$| > 0.263$, $p$adj < 0.05; Figures 1E and S6A; Table S10). We then conducted the same analysis comparing infected to non-infected children within each ethnic group (Table S11). Interestingly, the number of infection-related gene expression changes was greatly diminished compared to the ethnicity-related counterpart, where the greatest number of DEGs in the comparison of infection states is recorded in $CD16^+$ monocytes of the Mossi (54 [0.014%] DEGs; see supplemental results, Tables S11 and S12, and Figures S7 and S8). As reflected in the pseudobulk PCA (Figure S2), this observation highlights the magnitude of the impact of ethnicity on cell-type-specific transcriptomes.

As the innate immune cellular subtypes with the greatest number of DEGs between Mossi and Fulani samples in both infection states, we hypothesized that $CD14^+$ and $CD16^+$ monocytes would provide novel insights into the *in vivo* immune states and responses to *P. falciparum* infection in both ethnic groups. We primarily performed an unsupervised PCA of the pseudobulk $CD14^+$ and $CD16^+$ monocytes transcriptomic data per individual from each of the four subgroups (FuInf, FuNI, MoInf, and MoNI; Figures 2A and 2B). PCAs of both cell subtypes revealed two distinct clusters, emphasizing ethnicity as having the strongest effect on the correlation structure of the corresponding transcriptomes. Moreover, hierarchical clustering of the significant top 25 upregulated and top 25 downregulated genes clearly illustrates the consistent impact of ethnicity on $CD14^+$ and $CD16^+$ monocyte transcriptomes across children of each group and the underlying gene expression signature (Figures 2C, 2D, S3B, and S3C; Table S10). Interestingly, we noted a large number of overlapping DEGs between infection states in both $CD14^+$ and $CD16^+$ monocytes (Figure 2E). This points to the existence of basal transcriptomic differences prior to infection that retain their differential expression patterns during infection due to the effects of ethnicity.

We then conducted gene set enrichment analysis (GSEA) of the DEGs using pathways derived from hallmark genes to uncover biological insights into the observed patterns. This analysis revealed several pro-inflammatory pathways, including tumor necrosis factor α (TNF-α) signaling via nuclear factor κB (NF-κB), interferon-γ (IFN-γ), and inflammatory responses, to be significantly downregulated in the

heatmaps. Normalized (variant stabilizing transformation [VST]) expression values are represented as Z scores (scale bar on the left) and calculated per gene.
(E) Venn diagrams showing the number of overlapping upregulated and downregulated genes between ethnic group comparisons in the infected and non-infected states in $CD14^+$ (left) and $CD16^+$ (right) monocytes.
(F) GSEA of hallmark pathways from MSigDB, taking into account the fold changes of significant DEGs ($p$adj < 0.05) between Fulani and Mossi children for each monocyte cell type and infection state. Normalized enrichment score (NES) > 0 indicates pathway enrichment in the Fulani relative to the Mossi (*false discovery rate [FDR] < 0.1, **FDR < 0.05).
(G and H) Violin plots showing normalized VST expression values of *IL6*, *TNF*, *IL18*, *IL1β*, and *HLA-DRA* in $CD14^+$ and $CD16^+$ monocytes per group (red, FuInf; pink, FuNI; blue, MoInf; light blue, MoNI), in addition to *FCAR*, *CD36*, and *FCGR1* in $CD14^+$ monocytes. Adjusted p values from multiple correction testing following DGEA are indicated on the bars for the comparison of ethnic groups per infection status and the comparison of infection states per ethnic group, as shown in Tables S10 and S11 (*$p$adj.loc < 0.05, **$p$adj.loc < 0.01, ***$p$adj.loc < 0.001, ****$p$adj.loc < 0.0001). FuInf, infected Fulani; FuNI, non-infected Fulani; MoInf, infected Mossi; MoNI, non-infected Mossi.

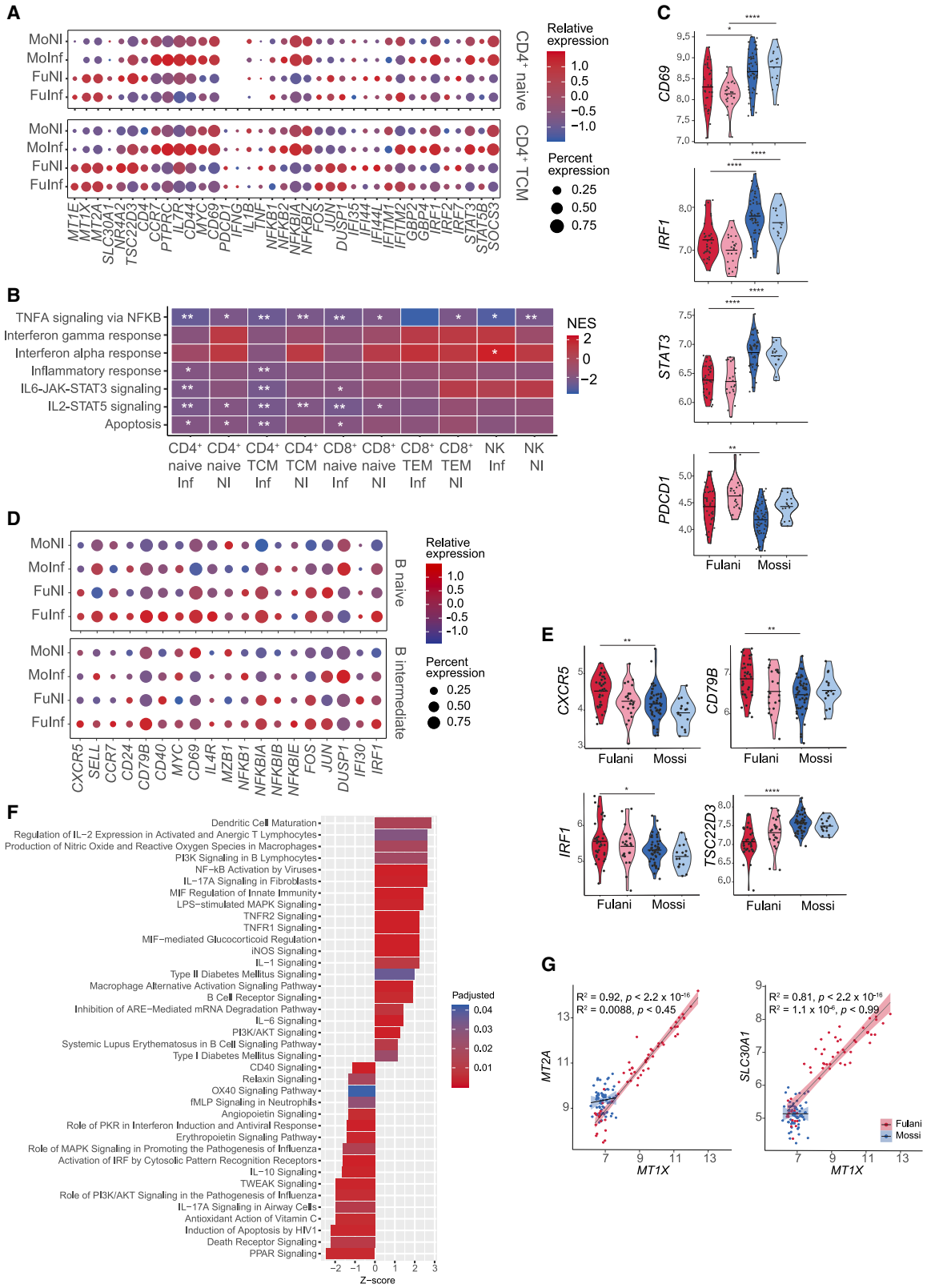

Figure 3. Analysis of transcriptomic changes in T cells and B cells

(A) Dotplot showing the relative (scale bar) and percentage (size of dot) expression of genes involved in T cell activation, cytokines, interferon, NF-κB, AP-1, and JAK-STAT pathway signaling in CD4$^+$ naive (top) and CD4$^+$ TCM (bottom) cells in each of the four groups (FuInf, FuNI, MoInf, MoNI).

(B) GSEA of hallmark pathways from MSigDB, taking into account the fold changes of significant DEGs (*p*adj < 0.05) between Fulani and Mossi children for each NK and T cell subtype and infection state. Normalized enrichment score (NES) > 0 indicates pathway enrichment in the Fulani relative to the Mossi (*FDR < 0.1; **FDR < 0.05).

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infected and non-infected CD14$^+$ and CD16$^+$ monocytes of the Fulani compared to the Mossi counterparts (Figure 2F and Table S13). This observation is further confirmed when comparing the normalized expression levels of the top differentially regulated pro-inflammatory cytokine genes, including *IL6* (MIM: 147620), *TNF* (MIM: 191160), *IL18* (MIM: 600953), and *IL1β* (MIM: 147720), which are significantly downregulated in both infection states and monocyte cell types of the Fulani (Figures 2G, 2H, and S6D). Some of these genes show a non-significant trend toward reduced expression in the infected children of both ethnic groups compared to the non-infected counterpart. This trend may be indicative of the immunoregulatory mechanisms activated in the monocytes upon infection (see supplemental results, Figures S7 and S8, and Tables S11 and S12 for further details on the comparisons of infected and non-infected samples per ethnic group).[23] Interestingly, the classical phagocytic CD14$^+$ monocytes of the Fulani are shown to have lower expression of antigen presentation MHC class II genes, including *HLA-DRA* (MIM: 142860), whereas several phagocytosis genes appear to be more abundant, including *FCAR* (MIM: 147045), *CD36* (MIM: 173510), and *FCGR1A* (MIM: 146760) (Figures 2G and S6D). Overall, the transcriptional signature of the infected monocytes of the Fulani appears to be more immunoregulatory yet maintains effector functions.

Cell-type-specific DGEA of T and NK cell subsets revealed a relatively higher number of genes downregulated in the Fulani in the infected state (Figure 1E). Genes found to be downregulated in T cell subsets of Fulani samples include T cell activation genes such as *CCR7* (MIM: 600242), *CD44* (MIM: 107269), *MYC* (MIM: 190080), and *CD69* (MIM: 107273) as well as NF-κB genes *NFKB2* (MIM: 164012), *NFKBIA* (MIM: 164008), and *NFKBIZ* (MIM: 608004) (Figures 3A and S9A). GSEA across all T cell subsets and NK cells in the infected and non-infected states showed a downregulation of TNF-α via NF-κB signaling, inflammatory, interleukin-6 (IL-6)/STAT3, and IL2/STAT5 signaling as well as apoptosis hallmark pathways in the Fulani, particularly in the infected CD4$^+$ naive and TCM and CD8$^+$ naive T cells (Figure 3B and Table S13). In CD4$^+$ TCM cells, we observed significantly lower expression of the activation marker *CD69*, the interferon response factor *IRF1* (MIM: 147575), and the cytokine signaling transcription factor *STAT3* (MIM: 102582) in Fulani samples relative to Mossi (Figure 3C). Moreover, CD4$^+$ TCM cells of the Fulani exhibited significantly higher expression of the checkpoint inhibitor *PDCD1* (MIM: 600244), encoding PD-1, which was significantly decreased in infected children of both ethnic groups relative to the non-infected counterpart. This observation reflects an immunoregulatory response taking place in CD4$^+$ TCM cells of the Fulani, likely to control overt inflammation. However, a trend toward upregulation of IFN-α and IFN-γ signaling pathways was observed in CD8$^+$ effector memory T (TEM) and NK cells of the infected Fulani children (Figure 3B). Notably, our DGEA revealed an upregulation of both type-I and type-II interferon genes, including *IFNG* (MIM: 147570), *IFI6* (MIM: 147572), *IFITM2* (MIM: 605578), *OASL* (MIM: 603281), *LY6E* (MIM: 601384), and *GBP1* (MIM: 600411) in infected CD8$^+$ TEM of the Fulani, as well as *IFI6*, *IFI35* (MIM: 600735), *IFITM2*, *IFITM3* (MIM: 605579), *LY6E*, and *STAT2* (MIM: 600556) in infected NK cells (Figure S9A and Table S10).

On the other hand, the Fulani children's B cell subsets showed a different signature compared to that of the monocytes and T cells. Based on relative expression, B cell activation and secondary lymphoid organ homing markers, including *CCR7*, *CXCR5* (MIM: 601613), *SELL* (MIM: 153240), *CD40* (MIM: 109535), *CD79B* (MIM: 147245), and *CD69*, are more highly expressed in the naive and intermediate B cells of the Fulani (Figure 3D). More specifically, we observed that the intermediate B cells of the Fulani, which are the effector cells at the time of sampling, have significantly higher expression of *CXCR5*, *CD79B*, *CD40*, and *TNFRSF13B* (encoding TACI; MIM: 604907), as well as IFN-related genes *IRF1* (MIM: 147575) and *IFI16* (MIM: 147586) (Figures 3E and S9B). However, significantly lower expression of the anti-inflammatory mediator *TSC22D3* (MIM: 300506), encoding the glucocorticoid-induced leucine zipper (GILZ), was recorded in B intermediate cells of the Fulani relative to those of the Mossi (Figure 3E). Using Ingenuity Pathway Analysis (IPA), we show that the B intermediate cells' pathways, such as NF-κB activation, TNFR1 and TNFR2 signaling, and IL-1 and IL-6 signaling, are activated in the Fulani cells relative to

(C) Violin plots showing normalized VST expression values of *CD69*, *IRF1*, *STAT3*, and *PDCD1* in CD4$^+$ TCM cells per group (red, FuInf; pink, FuNI; blue, MoInf; light blue, MoNI).
(D) Dotplot showing the relative expression of genes involved in B cell activation and homing, cytokine, interferon, NF-κB, and AP-1 signaling in B naive (top) and B intermediate (bottom) cells in each of the four groups (FuInf, FuNI, MoInf, MoNI).
(E) Violin plots showing normalized VST expression values of *CXCR5*, *CD79B*, *IRF1*, and *TSC22D3* in B intermediate cells per group.
(F) Ingenuity pathway analysis (IPA) taking into account the fold changes of significant DEGs ($p$adj < 0.05) between Fulani and Mossi children for infected B intermediate cells. Z score >0 indicates pathway activation, and Z score <0 indicates pathway inhibition in cells of the Fulani relative to the Mossi ($p$adj < 0.05, shown on the scale bar).
(G) Pearson correlation between normalized expression values of *MT1X* and *MT2A* (left) as well as *MT1X* and *SLC30A1* (right) in Fulani (red) and Mossi (blue) pseudobulk samples. The 95% confidence intervals (CIs) of the line of best fit are shaded.
Adjusted p values from multiple correction testing following DGEA are indicated on the bars of (C) and (E) for the comparison of ethnic groups per infection status and the comparison of infection states per ethnic group, as shown in Tables S10 and S11 (*$p$adj.loc < 0.05, **$p$adj.loc < 0.01, ***$p$adj.loc < 0.001, ****$p$adj.loc < 0.0001). FuInf, infected Fulani; FuNI, non-infected Fulani; MoInf, infected Mossi; MoNI, non-infected Mossi; TCM, T central memory; TEM, T effector memory; NK, natural killer; Inf, infected; NI, non-infected.

the Mossi, whereas IL-10 and death receptor signaling are inhibited (Figure 3F and Table S14). Interestingly, we observed an increased expression of metallothionein genes, which have anti-inflammatory properties, across all cell subtypes in the Fulani children relative to the Mossi (Figure 3G).[37] The B-cell-specific and global transcriptional profiles suggest that the Fulani population likely have a better memory response with overall immune-modulatory mechanisms, which are indeed reflected in their renowned higher seroprotection against malaria antigens and reduced symptomology.[30]

To investigate the magnitude of genetic regulation of gene expression during *P. falciparum* infection, we conducted genome-wide eQTL analysis focusing on capturing regulatory effects impacted by ethnicity. We performed the analysis on the $CD14^+$ and $CD16^+$ monocytes pseudobulk data, as these cell types are home to the highest numbers of expressed genes and levels of differential expression when comparing the two ethnicities (Figure 1E; see supplemental methods; Zenodo: https://doi.org/10.5281/zenodo.14719257). Our analysis was limited to infected samples, as they were greater in number, and to *cis* effects due to power limitations in detecting *trans* effects.[38] To further improve statistical power, we reduced the number of tests by considering only genes detected above the transformed expression value of 1 per gene in at least half of the samples per ethnic group.

The genotyping data of study participants provided 123,721 genetic variants with a minor allele frequency (MAF) of >10% within each ethnic group and located within ±100 kb from the selected genes' start and end positions. A total of 243,293 tests were conducted using a model that accounts for SNP, ethnicity, and gene by ethnicity, with a median of 82 and 80 dominant tests per gene in $CD14^+$ and $CD16^+$ monocytes, respectively. To enhance statistical power and minimize the impact of outliers, a dominant model was used in which two genotypic classes (homozygote individuals vs. individuals carrying at least one alternative allele) were tested against expression levels. Bonferroni correction was applied for all associations in a gene-wise manner. In total, 834 *cis*-eQTL and gene-by-ethnicity effects, implicating 268 genes across both cell types, were identified (Bonferroni corrected p value <0.05, Figure 4A; see Table S15 for the full list of *cis*-eQTLs).

Our analysis identified 120 and 71 *cis*-eQTLs, as well as 54 and 71 gene-by-ethnicity effects for $CD14^+$ and $CD16^+$ monocytes, respectively, that were detected only in one of the two ethnic groups. As we focused on gene-by-ethnicity interactions, the most statistically robust associations implicating DEGs with immune-related functions include *CD36*, *MT2A* (MIM: 156360), *CCR1* (MIM: 601159), *RPL36AL* (MIM: 180469), *GBP2* (MIM: 600412), *RNF145* (MIM: 620640), *EPN1* (MIM: 607262), and *CFD* (MIM: 134350) (Table 1). Notably, we highlight *CD36*, which encodes the cell-surface protein CD36 that facilitates the binding and phagocytosis of *P. falciparum*-infected erythrocytes, and *MT2A*, which encodes the metallothionein MT2A, due to their implication in malaria pathogenesis and immune regulation, as well as their differential expression in our scRNA-seq analysis (Figures 2C, S6D, and S10; Table S10).[37,39] Examining the regulatory regions surrounding the peak *CD36* eQTL (*rs1049654*) revealed its location in an ENCODE-predicted promoter, 190 base pairs upstream of one of the highly expressed *CD36* isoforms in whole blood (Figure S10). The associations implicating *CD36* and *MT2A* underscore the potential role of interaction effects, whereby differential expression between the two ethnic groups takes place in an allele-specific manner (Figure 4B), highlighting the potential impact of gene-by-ethnicity interactions on immunoregulatory mechanisms in malaria.

Mounting a robust anti-parasitic response driven by innate immune cells through the production of pro-inflammatory cytokines, antigen presentation, and maintenance of phagocytic effector mechanisms is required to initiate pathogen clearance, adaptive immunity, and memory responses. However, striking a balance between pro- and anti-inflammatory responses through immunoregulatory mechanisms is essential to establish anti-disease immunity while avoiding immunopathology, particularly in endemic regions where re-exposure is inevitable.[25,28] In this study, we took a single-cell genome-wide association approach and recruited 126 children to interrogate the transcriptomic changes and regulatory variants in two ethnic groups with distinct malaria susceptibility profiles. Our aim was to identify ethnic- and cell-subtype-specific correlates of the host immune response to *Plasmodium* infection. Given the limited biological material collected from children and our study design, which resulted in relatively reduced cellular resolution at the sample level and lower sequencing coverage per cell, we adopted a reference-based mapping method for cell-type classification while using stringent quality control measures and cell-type prediction filters (see supplemental methods).

Our initial analysis of the cell-subtype proportions and pseudobulk transcriptomic profiles has revealed a stronger impact of ethnicity, shaped by distinct lifestyles, on the transcriptional landscape of the various immune cell types compared to that of infection, underscoring the value of integrating ethnicity into studies of the host response to *P. falciparum* infection. We speculate, however, that the relatively low parasitemia levels in the children may account for the less pronounced effect of infection observed. Of interest, and consistent with previous findings, γδT cell levels were higher in infected Fulani children compared to the Mossi.[36] γδT cells play a crucial role in anti-parasitic immunity through their expansion and direct killing mechanisms upon re-exposure to the parasite antigens, potentially contributing to the lower parasitic densities reported in the Fulani population.[43] Regarding regulatory T cells (Tregs), a prior study indicated that adult Fulani Tregs exhibited functional deficits compared to those of sympatric Mossi in Burkina Faso, which may underlie the

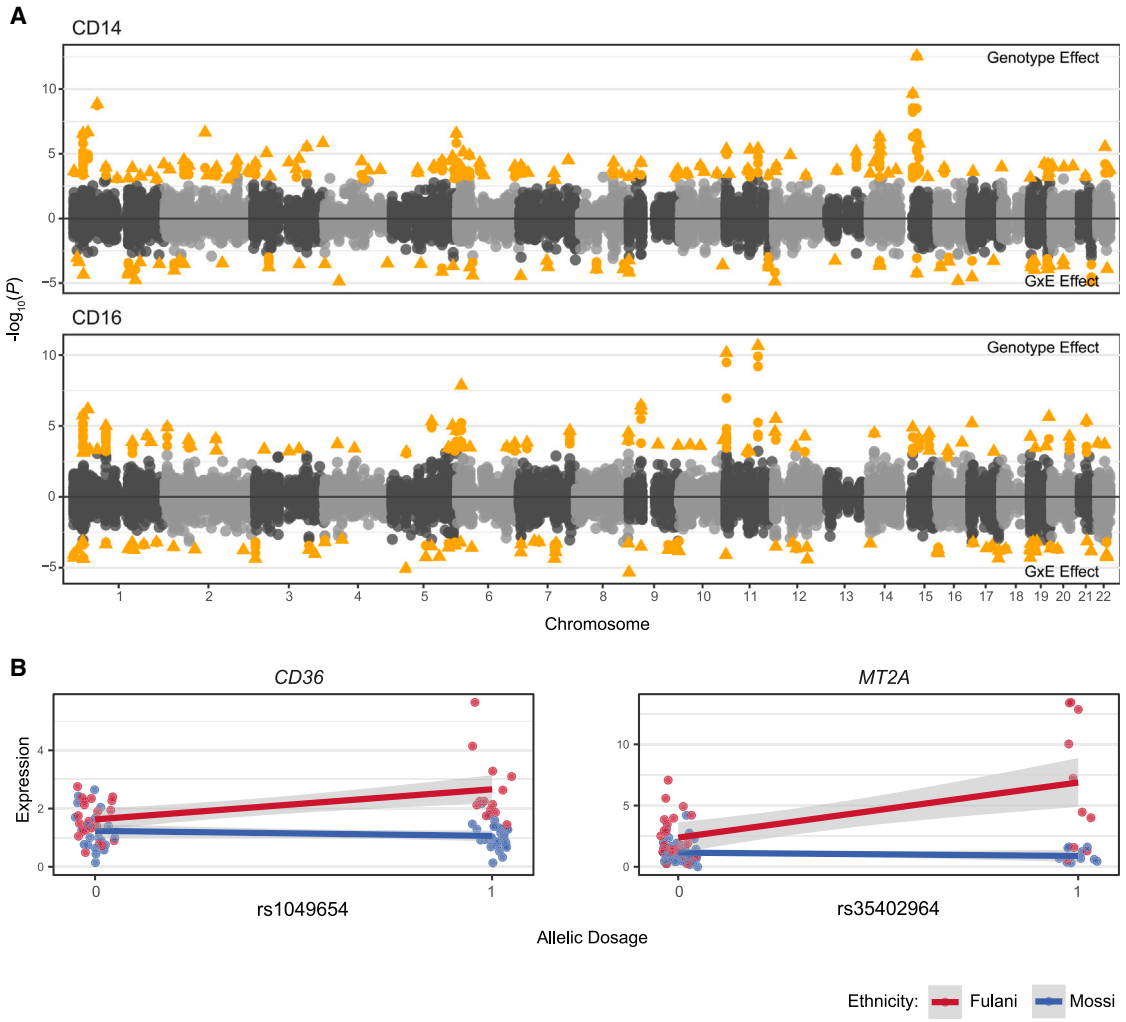

Figure 4. Expressive quantitative trait locus (eQTL) analysis of CD14$^+$ and CD16$^+$ monocytes

(A) Mirrored Manhattan plot depicting the strength of statistical associations of genotype (above 0 line) and genotype-ethnicity interaction (below 0 line) effects between *cis*-localized SNPs and transcript abundance in CD14$^+$ (top) and CD16$^+$ (bottom) monocytes. The highlighted markers in orange indicate a Bonferroni gene-wise corrected p value <0.05 significance, with a triangle denoting the strongest association for a specific gene. G×E, gene by ethnicity.

(B) Fine-mapped eQTL results for *CD36* gene using *rs1049654* SNP marker (left) and *MT2A* gene using *rs1049654* SNP marker (right). Transcript abundance (95% CI of means shown) changes as a function of minor allele dosage and ethnicity. Allelic dosage 0 corresponds to major allele homozygous individuals, while 1 corresponds to individuals carrying at least one minor allele copy.

Fulani's lower susceptibility to malaria.[44] However, as previously reported, while we did not observe any significant difference in Treg frequencies between the ethnic groups in both infection states, the number of Treg single cells was too low to perform differential expression analysis.[36]

Our pseudobulk transcriptome analysis in both infection states identified CD14$^+$ and CD16$^+$ monocytes as the cell subtypes most transcriptionally affected by ethnicity. This finding aligns with a previous transcriptome analysis of isolated monocytes and lymphocytes from Fulani and Mossi individuals in Burkina Faso.[45] We showed that monocytes of the Fulani children display a dampened inflammatory state compared to those of Mossi children, yet exhibit increased expression of phagocytic genes. Similarly, a recent scRNA-seq study on *P. falciparum*-infected individuals in Malaysia reported an immunoregulatory signature in monocytes characterized by reduced expression of inflammatory genes and elevated expression of phagocytic genes relative to non-infected individuals.[23] The similarity in monocyte expression profiles between the Fulani children and those of the aforementioned study suggests shared immunoregulatory mechanisms across populations despite environmental and genetic differences.

Monocytes from Malian individuals have been shown to undergo epigenetic reprogramming toward a regulatory phenotype upon cumulative exposure to *P. falciparum* and aging.[24] This adaptive response entails reduced production of pro-inflammatory cytokines and chemokines along with increased levels of anti-inflammatory IL-10 cytokine while preserving enhanced anti-parasitic mechanisms.[23,25] A similar cytokine profile has been observed in

Table 1. Selected genotype × ethnicity eQTL interaction associations

				External databases annotation					
Gene (MIM #)	**Chr**	**SNP**	**A1**	**Region (Ensembl)**	**Genomic annotation (UCSC)**	**Matching eQTL in GTeX**	**Beta**	***p* value**	***p* Bonferroni**
CD14									
SELL (153240)	1	rs12027035	G	intron	–	–	−1.6020	1.72×10^{-5}	1.70×10^{-3}
CCR1 (601159)	3	rs73833005	T	intron	H3K4Me1 (weak)	–	1.0040	1.72×10^{-4}	8.09×10^{-3}
HMGB2 (163906)	4	rs7655786	T	intron	DNase hypersensitivity cluster, H3K4Me1 (weak), HiC genomic contact	–	−0.6217	3.44×10^{-4}	1.96×10^{-2}
CD36 (173510)	7	rs1049654	C	5′ UTR/promoter	ENCODE promoter	yes	1.2070	1.79×10^{-4}	4.45×10^{-2}
RPS25 (180465)	11	rs60065241	A	non-coding exon	–	yes	−2.8180	1.79×10^{-4}	1.65×10^{-2}
RPL36AL (180469)	14	rs72678065	G	intron	H3K27Ac (weak), ENCODE enhancer (distal)	yes	−1.3460	2.21×10^{-4}	1.48×10^{-2}
MT2A (156360)	16	rs35402964	T	intergenic	proximity to ENCODE cCRE[1]	–	4.7720	1.48×10^{-5}	1.97×10^{-3}
C5AR1 (113995)	19	rs4804038	C	intergenic	–	yes	1.3930	2.49×10^{-4}	1.37×10^{-2}
EEF2 (130610)	19	rs10406174	A	intron, enhancer	H3K4Me1 (weak)	–	−1.6190	1.76×10^{-4}	1.32×10^{-2}
HCK (142370)	20	rs6061136	T	intron	ENCODE enhancer (distal)	–	−0.6621	5.62×10^{-4}	4.04×10^{-2}
CD16									
GBP2 (600412)	1	rs80052123	A	intergenic	–	–	1.4860	4.43×10^{-4}	3.59×10^{-2}
RNF145 (620640)	5	rs270664	T	intergenic	proximity to ENCODE cCREs[1]	–	−0.6208	2.66×10^{-4}	1.52×10^{-2}
CCZ1 (620660)	7	rs1805319	G	CTCF, TF binding	DNase hypersensitivity cluster, HiC genomic contact, ENCODE enhancer (distal)	–	0.6704	1.20×10^{-4}	6.62×10^{-3}
DNAJA1 (602837)	9	rs3758276	A	promoter, 5′ UTR	H3K27Ac + H3K4Me3 (strong)	yes	1.8920	3.02×10^{-4}	2.51×10^{-2}
IFITM3 (605579)	11	rs7479311	A	intergenic	–	–	−7.5760	8.13×10^{-5}	8.94×10^{-3}
GRN (607485)	17	rs7223967	A	intron	–	–	−0.8470	2.33×10^{-4}	1.07×10^{-2}
CDKN2D (600927)	19	rs10402752	C	promoter, intron	HiC genomic contact, H3K4Me1/3 + H3K27Ac (strong), ENCODE enhancer (proximal)	–	−1.2270	3.47×10^{-4}	1.39×10^{-2}
EPN1 (607262)	19	rs8107401	T	intergenic	–	–	−0.4880	2.26×10^{-4}	2.64×10^{-2}
CFD (134350)	19	rs10426534	T	intron	–	–	5.1880	2.10×10^{-4}	2.16×10^{-2}

Gene by ethnicity eQTLs, together with their respective annotations from external databases,[40–42] which exhibited the most statistically robust associations while implicating DEGs with functions that indicate their involvement in malaria pathogenesis were selected and shown for $CD14^+$ and $CD16^+$ monocytes. A1, alternative allele; MAF, minor allele frequency. A dash (–) indicates no current data/information.

individuals with asymptomatic *P. falciparum* infection living in endemic malaria regions, characterized by a lower pro- to anti-inflammatory ratio that aligns with an anti-disease immunity signature.[10,46,47] Cumulatively, malaria research has reinforced a dual consensus of anti-disease immunity coupled with anti-parasite responses.[48]

Yet, the mechanisms underlying the relatively lower malaria susceptibility in Fulani remain unclear. A study in Mali measuring blood plasma cytokine levels from asymptomatic Fulani and sympatric Dogon children found that infected Fulani children exhibited elevated IFN-γ but reduced IL-6 and IL-8 cytokine levels compared to the non-infected state.[49] However, another study in Burkina Faso reported a stronger transcriptional response in Fulani monocytes, highlighting the expression of several histone-modifying genes potentially altering their epigenetic landscape. The same study also observed lower IL-6 and IFN-γ levels in the serum of infected Fulani relative to the sympatric Mossi group.[45] Epigenetic regulation of the host transcriptional response to *P. falciparum* has also been documented in the Gouin ethnic group in Burkina Faso, implicating the master pro-inflammatory gene *TNF* in immune modulation.[50] Our findings indicate that Fulani children's monocytes establish a basal immunoregulatory state, suggesting an inherent priming of their immune system to modulate inflammatory responses and mitigate malarial pathogenesis.[51] We propose that this molecular and cellular state reflects an immune correlate of protection at the clinical level in Fulani children, potentially contributing to their unique malaria susceptibility status. To validate this mechanism at single-cell resolution, functional *in vitro* and *ex vivo* studies, integrating single-cell chromatin accessibility and transcriptomic and immune profiling data are essential to elucidate the multi-omics regulatory states adopted by Fulani immune cell types.

Another group of cell types that underscore the more profound immunoregulatory properties observed in Fulani immune cells are T cells. In both infection states, $CD4^+$ T cell subsets of Fulani children exhibited dampened inflammatory profiles, whereas $CD8^+$ TEM and NK cells retained a stronger IFN-driven transcriptional response. Given the distinct roles of different T helper subsets in *P. falciparum* infection, it remains crucial to examine each subset's contribution to the lower malaria susceptibility in Fulani individuals.[52]

Previous research has demonstrated that Fulani individuals possess more activated memory B cells, elevated circulating immunoglobulin G (IgG) antibody levels, and blood plasma that enhance opsonic phagocytosis.[30,53–55] Our findings revealed that Fulani children's B intermediate cells display a more activated and pro-inflammatory transcriptional state. B cells are central to establishing long-term memory responses against *P. falciparum*.[27] Parasite-specific IgG antibodies, along with opsonization, phagocytosis, and complement activation, are key mechanisms for controlling parasitic load and disease severity. Thus, the enhanced transcriptional immune activation state of Fulani B intermediate cells compared to the Mossi cells likely underpins the distinct clinical features and reduced symptomology associated with the Fulani group.[29,56]

The capability to capture eQTL effects at the cell-subtype-specific level is directly proportional to statistical power, which we enhanced by applying stringent filters.[42,57] In line with our DGEA findings, our analysis confirms the presence of gene-by-ethnicity interaction effects in $CD14^+$ and $CD16^+$ monocytes during *P. falciparum* infection.

Our gene-by-ethnicity interaction analysis revealed potential disease-relevant genes involved in immune modulation. Specifically, we identify that the alternative allele of SNP *rs1049654* is associated with an increase in *CD36* expression in $CD14^+$ monocytes of Fulani children. In the scRNA-seq malaria study by Dooley et al., higher *CD36* expression levels were also observed in both monocyte subsets from infected patients compared to those of non-infected controls.[23] As a monocyte class critical for phagocytosis, the $CD14^+$ monocytes in infected Fulani children carrying one or two alternative alleles of the associated SNP show cell-subtype-specific upregulation of *CD36*. This likely enhances CD36 cell-surface expression, facilitating greater binding to infected erythrocytes and subsequent phagocytosis.[39,58,59] These findings suggest that enhanced monocyte effector mechanisms, as corroborated by transcriptional analysis, could contribute to the Fulani's relatively lower malaria susceptibility.[29,30] While allelic variants leading to CD36 deficiency have been observed at higher frequencies across sub-Saharan populations, their protective role against disease remains unproven, likely due to the receptor's cell-type-specific functions.[60] Thus, further investigations are needed to elucidate how allelic and gene-expression variation in *CD36* influence disease susceptibility at the cellular subtype level.

A similar genotype-by-ethnicity interaction effect is observed in $CD14^+$ monocytes for the metallothionein gene, *MT2A*, chromosomally located near other metallothionein isoform genes. *MT2A* is induced by metals, cytokines, and glucocorticoids in response to stress and/or inflammation, functioning as an antioxidant, homeostatic regulator, and immune modulator.[37,61] While we observed a transcriptional upregulation of multiple metallothionein isoforms across several cellular subtypes in both infection states of the Fulani relative to the Mossi children, cell-type-specific roles of MT2A are expected. Previous studies demonstrated that MT2A in monocytes and macrophages exhibits protective and anti-inflammatory effects in cerebral malaria and inflammatory conditions, such as multiple sclerosis, while also enhancing effector mechanisms such as oxidative burst during infection.[62–64] Thus, we propose that elevated *MT2A* expression in both infected and non-infected states provides an advantage by modulating inflammation and preventing disease pathology. Overall, the transcriptional patterns of both *CD36* and *MT2A* align with the observed lower malarial incidence among the Fulani ethnic group. However, it is important to note

that the statistical power of our eQTL analysis is limited by the sample size and the linkage disequilibrium structure of the populations investigated, particularly when considering genotype-ethnicity interactions. Despite employing analytical strategies to enhance power, such as focusing on *cis*-regulatory effects and adopting an additive genetic model, our ability to detect medium-to-small-effect sizes remains constrained, potentially underestimating significant associations. Importantly, future studies with larger sample sizes and diverse cohorts are essential to validate these findings and uncover additional eQTLs of relevance.

Moreover, while our study design minimizes the differences between the two groups investigated due to geography, environmental exposures, level of access to health care, and *P. falciparum* exposure, a higher-resolution depiction of dietary habits and other socioeconomic factors would have enhanced the resolution of our analysis. Previous studies have demonstrated that milk consumption, a staple in the Fulani diet, can modulate gut microbial communities, which, in turn, influence host immune responses and potentially shape how different populations respond to *P. falciparum* infection.[65,66] We have recently shown striking divergence in the lipidomic profiles of Fulani children compared to Mossi, highlighting the enrichment of diet-derived pentadecanoic acid (C15:0)-containing phospholipids in Fulani with suppressive effects on *P. falciparum* gametocyte production.[67] This underscores the need for further investigations into specific components of the Fulani diet that could be influencing the observed divergent inter-ethnic transcriptional signatures, potentially contributing to variations in malaria susceptibility across populations.

Overall, our study emphasizes the modulation of inflammatory responses as a central mechanism in regulating immune homeostasis. Additionally, our results indicate that ethnic diversity, likely predominantly driven by dietary factors, plays a significant role in shaping these immune regulatory processes. These insights contribute to a deeper understanding of the host immune response to *P. falciparum* and provide comprehensive single-cell transcriptomic and eQTL data from populations under-represented in functional genomic studies.

Data and code availability

Processed RNA-sequencing single-cell data are available via the Gene Expression Omnibus (accession numbers GEO: GSE273781 and GSE273785). All original code, together with the Seurat object, can be found in Zenodo: https://doi.org/10.5281/zenodo.14719257 and GitHub: https://github.com/Yidaghdour/Single-cell-Transcriptomics-Reveals-Inter-ethnic-Variation-in-Immune-Response-to-Falciparum-Malaria.

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Author contributions

Y.I. and T.S. conceptualized the study. I.S. supervised all clinical work and fieldwork in Burkina Faso. M.M.D., W.A., A.D., N.B.H., S.S., D.A., and S.S.S. performed fieldwork including recruitment of children and sample processing in Burkina Faso. M.M.D. and M.A. performed single-cell experiments. B.A., O.B., and J.J. assisted with the establishment and optimization of single-cell analysis pipelines. V.M. and N.D. performed primary bioinformatic analysis of the data. T.S. performed single-cell transcriptomic data analysis. J.J. performed genetic, eQTL, and annotation analyses. T.S. interpreted all the data and wrote the manuscript with input from J.J and supervision from Y.I.

Declaration of interests

The authors declare no competing interests.

Web resources

OMIM, https://omim.org/

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2025.01.020.

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