Repeated *Plasmodium falciparum* infection in humans drives the clonal expansion of an adaptive γδ T cell repertoire

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One Sentence Summary: Malaria drives the adaptive differentiation of the human γδ T cell repertoire.

Abstract

Repeated *Plasmodium falciparum* infections drive the development of clinical immunity to malaria in humans, however, the immunological mechanisms that underpin this response are only partially understood. We investigated the impact of repeated *P. falciparum* infections on human γδ T cells in the context of natural infection in Malian children and adults, as well as serial controlled human malaria infection (CHMI) of U.S. adults, some of whom became clinically immune to malaria. In contrast to the predominant $V\delta2^+$ γδ T cell population in malaria-naive Australian individuals, clonally expanded cytotoxic-$V\delta1_{effector}$ T cells were enriched in the γδ T cell compartment of Malian subjects. Malaria-naïve U.S. adults exposed to four sequential CHMIs defined the precise impact of *P. falciparum* on the γδ T cell repertoire. Specifically, innate-like $V\delta2^+$ γδ T cells exhibited an initial robust polyclonal response to *P. falciparum* infection that was not sustained with repeated infections, whereas $V\delta1^+$ γδ T cells increased in frequency with repeated infections. Moreover, repeated *P. falciparum* infection drove waves of clonal selection in the $V\delta1^+$ TCR repertoire that coincided with the differentiation of $V\delta1_{naive}$ cells into cytotoxic-$V\delta1_{effector}$ cells. Finally, $V\delta1^+$ T cells of malaria-exposed Malian and U.S. individuals were licensed for reactivity to *P. falciparum* parasites *in vitro*. Together, our study indicates that repeated *P. falciparum* infection drives the clonal expansion of an adaptive γδ T cell repertoire and establishes a role for $V\delta1^+$ T cells in the human immune response to malaria.

Introduction

In malaria-endemic regions, non-sterilizing clinical immunity to blood-stage *Plasmodium falciparum* parasites can be acquired, but this typically only occurs after many years of repeated infections (*1*). However, the mechanisms underlying this protection are only partially understood (*2, 3*). Recent observational studies in malaria-endemic areas, as well as clinical trials with the attenuated *P. falciparum* sporozoite vaccine *Pf*SPZ, have suggested that γδ T cells may contribute to protection from malaria (*4-7*).

Human γδ T cells are an unconventional T cell population that are thought to play an important role in immunity to microbial pathogens and cancer (*8*). Unlike conventional αβ T cells, γδ T cells are not restricted by classical MHC or MHC-I-like molecules to recognize antigens (*9-11*), but instead respond directly to non-peptidic metabolite antigens and other diverse ligands (*12, 13*). γδ T cells were present in the first jawed vertebrates and co-evolved with pathogenic organisms for millions of years (*10, 14*). In humans, the major peripheral blood population of γδ T cells (5-10% of total T cells) express a restricted TCR that consists of paired Vδ2 and Vγ9 chains (*15*). The Vγ9/Vδ2$^+$ T cell population directly responds to a prenyl-pyrophosphate metabolite (PAg) (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) produced by the microbial non-mevalonate pathway (*16*). The Vγ9/Vδ2$^+$ TCR repertoire is generated early in gestation and is shaped soon after birth, with a high frequency of public Vγ9 clonotypes (*17-19*). Innate-like Vγ9/Vδ2$^+$ T cells can expand, and they comprise up to 40% of T cells during blood-stage malaria infection, a response thought to be driven by recognition of *P. falciparum*-derived HMB-PP (*20-22*). This subset may participate in limiting parasite replication by targeting *P.*

falciparum blood-stage parasites through granulysin-dependent cytotoxicity (*23*) and phagocytosis of antibody-coated iRBCs (*24*).

In contrast to innate-like Vγ9/Vδ2^{+} γδ T cells, a diverse biology has been established for γδ T cells that predominantly express the Vδ1^{+} TCR chain and circulate in blood at low frequency but are a dominant population in peripheral tissues (*25*). Firstly, Vδ1^{+} T cells that form tissue-associated populations in the intraepithelial lymphocyte (IEL) compartment of gut and breast tissue are thought to provide innate-like immune surveillance through host-encoded Natural Killer-receptors (NKRs) (*26*) and BTN-like (BTNL) 3 proteins (*27*). Secondly, peripheral blood and liver-resident Vδ1^{+} T cells possess hallmarks of adaptive T cells and comprise naïve-like (Vδ1$_{\text{naïve}}$) and effector (Vδ1$_{\text{effector}}$) populations with diverse or highly focused TCR repertoires, respectively (*19, 25, 28*). Acute cytomegalovirus (CMV) infection has been associated with the selection of a limited set of Vδ2$^{\text{neg}}$ γδTCR clonotypes (*19, 29*). Interestingly, expanded populations of Vδ1^{+} T cells have been observed in both children and adults with symptomatic *P. falciparum* infection (*30-32*) and in individuals residing in regions of malaria transmission (*33, 34*). In spite of evidence that γδ T cells contribute to immunity to microbial pathogens, it remains unclear whether *P. falciparum* infection per se or factors associated with malaria transmission in endemic areas are responsible for the expansion of Vδ1^{+} T cells. Moreover, it is also unclear the impact of repeated *P. falciparum* infection on the phenotype, function and clonality of the γδ T cell compartment.

In this study, we investigated the γδ T cell immune repertoire response to *P. falciparum* malaria in a cohort of children and adults residing in a malaria-endemic region of Mali, and in malaria-

naive U.S. adults serially infected with *P. falciparum* via mosquito bite in a controlled setting. We found that repeated *P. falciparum* infections drove the clonal selection and expansion of circulating cytotoxic $V\delta1_{effector}$ T cells that reacted to *P. falciparum* blood-stage parasites.

Results

Heterogeneity in the γδ T cell compartment exists across diverse geographic locations

In general, immune profiles are known to differ between children of high- and low-income countries where the latter typically suffer a disproportionately high burden of infectious disease *(35)*. Here, we compared the circulating γδ T cell repertoire of Malian children (aged 4 – 17 years) who are exposed to intense seasonal malaria transmission (*36*), with that of age and gender matched children from Melbourne, Australia (aged 1 – 17 years) **(Table S1)**. We first analyzed Mali samples collected from uninfected subjects at the end of the dry season when malaria transmission is negligible to assess γδ T cell repertoires in a relatively unperturbed state. We found that γδ T cell and $V\delta1^+$ T cell frequencies were significantly higher in Malian children **(Fig. 1A and S1A)**, whereas the frequency of $V\gamma9/V\delta2^+$ T cells were similar between both groups **(Fig. 1A and B)**. We then analyzed γδ T cells in Malian adults (aged 21 – 26 years) and adults residing in an area of low malaria transmission in Kenya (aged 26 - 49 years) as well as Australian adults with no history of malaria exposure (aged 20 - 71 years). $V\delta1^+$ T cell frequencies were lower in Kenyan and Australian adults compared to Malian children **(Fig. S1B).** Next, we assessed γδ T cell effector subsets in Malian children. From birth, $V\gamma9/V\delta2^+$ T cells typically form a stable innate-like T cell population composed of a $CD27^+$ $CD28^+$ Granzyme (Gzm) A^+ $GzmB^+$ $Perforin^+$ compartment (*18, 19, 37*). In Malian individuals we found that $V\gamma9/V\delta2^+$ T cells had reduced expression of $CD27^+$ $CD28^+$ **(Fig. 1C**) and perforin (**Fig. 1D)**, whereas GzmA increased (**Fig. 1E and F**). In contrast, cord blood $V\delta1^+$ T cell population is typically composed of naïve-like $CD27^{hi}$ CX_3CR1^{neg} $GzmA/B^{neg}$ $Perforin^{neg}$ cells ($V\delta1_{naive}$) (*28*). However, the $V\delta1^+$ compartment in Malian subjects was predominantly composed of $CD27^{lo}$

93 CX_3CR1^+ GzmA/B$^+$ Perforin$^+$ effector-like cells ($V\delta1_{eff}$) **(Fig. 1C-F)**. Interestingly, a CD16$^+$
94 Vγ9/V$\delta2^+$ T cell compartment has recently been implicated in antibody-mediated phagocytosis
95 of iRBCs (*24*). We found that Malian children, when compared to Australian children, tended to
96 have increased frequencies of CD16$^+$ V$\delta1^+$ T cells rather than CD16$^+$ Vγ9/V$\delta2^+$ T cells **(Fig.**
97 **S1C)**. Together, these data suggest that the composition of the $\gamma\delta$ T cell compartment varies
98 across geographic locations. However, it was not clear if high malaria transmission and/or
99 factors associated with malaria transmission drive the proportional expansion of V$\delta1^+$ T cells and
100 skewing towards a $V\delta1_{effector}$ phenotype in the Mali cohort.
101
102 **Episodes of febrile malaria associate with fluctuations in V$\delta1^+$ $\gamma\delta$ T cell frequencies**
103 We directly investigated the potential impact of natural malaria infection on the $\gamma\delta$ T cell
104 compartment by conducting a longitudinal analysis of nine Malian children (aged 8 - 14 years)
105 over three malaria seasons **(Fig. 1G)**. These individuals from Mali were exposed to six-month
106 malaria season annually in which *P. falciparum* transmission is intense and predictable (*36*).
107 Consistent with this, *P. falciparum* parasite density increased during each malaria season in a
108 subset of five children whose blood smears were examined longitudinally **(Fig. S1C and S1D)**.
109 Each subject was selected because they experienced two to three febrile malaria episodes over
110 multiple years, as detected by both passive and active clinical surveillance, allowing for
111 longitudinal analyses of $\gamma\delta$ T cell dynamics in response to acute symptomatic malaria followed
112 by sustained periods without febrile malaria **(Fig. 1G)**. We investigated $\gamma\delta$ T cell and CD8$^+$ $\alpha\beta$ T
113 cell frequencies across consecutive episodes of febrile malaria over three seasons. These
114 analyses pooled T cell frequencies from children who had experienced two or three episodes of
115 febrile malaria **(Fig. 1H)**. $\gamma\delta$ T cell and CD8$^+$ $\alpha\beta$ T cell frequencies were assessed in blood

samples collected on the day febrile malaria was diagnosed and again within 3 - 6 months of diagnosis. We found that Vγ9/Vδ2$^+$ T cell frequencies increased after febrile malaria in year 1 but did not consistently change after febrile malaria in years 2 and 3 **(Fig. 1H)**, although these observations could be due to the different sampling times in each year. CD8$^+$ T cell frequencies were unchanged after each febrile episode **(Fig. 1H)**. In contrast, Vδ1$^+$ T cell frequencies were consistently decreased upon presentation with febrile malaria and increased after each febrile malaria episode across all three years **(Fig. 1H)**. Moreover, across a subset of eight subjects in year 1, we also observed equivalent CD3$^+$ lymphocyte and αβ T cell counts, and all γδ T cell populations expanded in number after febrile malaria **(Fig. S1E)**. We then assessed a subset of children at timepoints without infection before a documented period of asymptomatic *P. falciparum* infection but no febrile malaria episodes (**Fig. 1I;** pooled from data between month 12 – 19 or 24 – 30). Vγ9/Vδ2$^+$ and Vδ1$^+$ T cell frequencies did not change significantly across this six to seven-month period. We noted previously that Vδ1$^+$ T cells in Malian children were predominantly composed of Vδ1$_{effector}$ cells **(Fig. 1C and D)**, however, yearly episodes of febrile malaria had little impact on Vδ1$_{effector}$ frequencies and CD27$^+$ CD28$^+$ Vγ9/Vδ2$^+$ T cell frequencies were reduced in year 3 **(Fig. S1F)**. Together, these data indicate that exposure to seasonal episodes of febrile malaria transiently impacts circulating frequencies of Vδ1$^+$ γδ T cells.

Malian γδTCR repertoires are clonally skewed and change after febrile malaria

We explored the underlying γδTCR repertoires in Malian children and whether febrile malaria impacts individual clonotypes over time. Initially, we conducted a cross-sectional analysis of blood samples collected during periods of no malaria transmission (subjects 066, 521, 766) and

from one subject with febrile malaria (subject 269) and compared these repertoires to those of Australian children (**Fig. 2A and S3A**). We analyzed both Vγ9/Vδ2^{+} (Vδ2^{+}) and non-Vγ9/Vδ2 (Vδ2^{neg}) γδ T cell populations, effectively encompassing the total γδ T cell repertoire (**Fig. S2**). Phenotypically, both Malian and Australian subjects had Vδ2^{+} γδ T cell populations composed of effector-like populations of CD27^{+} CD28^{+} cells, whereas Vδ2^{neg} γδ T cells were composed of CD27^{lo} CX_3CR1^{+} effector cells in Malian subjects and CD27^{hi} CX_3CR1^{neg} naïve cells in Australian subjects **(Fig. S3B)**. The Vγ9/Vδ2^{+} T cell subset displayed γδ TCR repertoires consistent with those seen in children and adults from Europe (*18, 19, 37*) **(Fig. 2A and Fig. S3A),** which are almost exclusively composed of Vδ2–Jδ1 **(Fig. S3C)** paired to Vγ9–JγP **(Fig. S3D)**, with diverse clonotype composition and common CDR3γ9-JγP sequences shared between individuals **(Fig. 2A and Fig. S3C)**. Vδ2^{neg} γδ TCR repertoires were predominantly composed of Vδ1–Jδ1 sequences **(Fig. S3D)** that were paired to various Vγ–Jγ1/2 regions **(Fig. S3D)**. These Vδ2^{neg} γδTCR repertoires from Malian children exhibited expanded clonotypes, indicated by an increase in the accumulated frequency of the top 10 clonotypes in comparison to Vδ2^{neg} γδ TCR repertoires in Australian children **(Fig. 2B)**. In support of the skewing towards expanded Vδ1 clonotypes, Malian Vδ2^{neg} γδ TCR repertoires showed a reduced diversity of clonotype composition **(Fig. 2C)** and a reduced frequency of shared sequences compared to Vδ2^{neg} γδTCR repertoires of Australian individuals **(Fig. 2D)**. These data suggest that the Vγ9/Vδ2^{+} T cell repertoires in Malian subjects are highly similar to those of Australian individuals. In contrast, Vδ2^{neg} γδTCR repertoires of Malian individuals showed evidence of reduced clonotype sharing and diversity because of expanded private clonotypes.

We assessed the impact of episodes of acute febrile malaria on γδTCR clonotype composition within the Vγ9/Vδ2^{+} and Vδ2^{neg} γδ T cell populations in a longitudinal analysis. Vγ9/Vδ2^{+} clonotypes remained remarkably stable during and after acute febrile malaria **(Fig. 2E, Fig. S3E and F)**. We and others have previously reported on the stability of Vδ2^{neg} and Vδ1^{+} γδTCR clonotypes over several years (*18, 19, 28, 29*). Here, Vδ2^{neg} γδTCR repertoires displayed changes after acute febrile malaria, characterized by contraction and expansion of existing clonotypes or emergence of new prevalent sequences **(Fig. 2F, Fig. S3E and G)**. These changes impacted the frequency of Vδ1 sequence usage **(Fig. S3H)**, Vγ2 usage **(Fig. 2G)**, the overall repertoire diversity **(Fig. 2G),** and nucleotide length dynamics **(Fig. S3I)**. To explore the impact of febrile malaria on clonotype composition within Vδ1$_{effector}$ cells, we sorted single cells from the CD27^{lo} CX_3CR1^{+} Vδ1$_{effector}$ cell compartment from samples collected over 32 months and three separate acute febrile malaria episodes from subject 179 **(Fig. 2H)**. We noted by flow cytometry that Vδ1/γδTCR antibody staining intensity changed over time, with distinct Vδ1/γδTCR antibody populations emerging after each episode of febrile malaria **(Fig. 2H and Fig. S3J)**. Underpinning these observations, single cell γδTCR sequencing revealed changes in the frequency and identity of individual Vδ1$_{effector}$ clonotypes over time **(Fig. 2I and Fig. S3K)**. Together, these data suggest that Malian individuals have highly stable Vγ9/Vδ2^{+} T cell repertoires that are retained across episodes of febrile malaria and are shared between individuals. In contrast, γδTCR clonotypes in the Vδ1$_{effector}$ compartment were composed of clonotypes that varied in frequency and identity over time.

Repeated human controlled malaria infection can establish clinical immunity that correlates with increased Vδ1^{+} γδ T cell frequencies

To understand the precise impact of *P. falciparum* infection on the trajectory of γδ T cell development and selection, we assessed γδ T cell subset dynamics in PBMCs collected from five malaria-naïve adults voluntarily exposed to repeated controlled human malaria infection (CHMI). Each volunteer was exposed to the bites of five *Anopheles stephensi* mosquitos infected with *P. falciparum* (strain: NF54) on four separate occasions over 644 days (**Fig. 3A**). Symptomatic malaria occurs during the blood stage of the *P. falciparum* parasite life cycle, which typically develops after an incubation period of nine to fourteen days (*36*). Here, we analyzed samples at baseline (malaria naïve), immediately prior to *P. falciparum* infection (day 1; at CHMI1 and 3), and 21 days after infection for all CHMIs **(Fig 3A)**. We did not observe any noticeable leukopenia measured by white blood cell counts (at day 1 or day 28; **Fig. S4A**) or by clinical tests prior to apheresis (day 21) at the timepoints sampled in this study. Over the course of the four CHMIs, peak parasitemia measured by blood smear did not significantly change **(Fig. S4B)**. We then assessed the instances of febrile malaria and symptomatic disease (ranging from headaches to vomiting; **Table S2**). Fever was observed at CHMI1 or 2 in all but one individual and the number of symptoms observed in each individual decreased after repeated CHMIs **(Fig. 3B)**. Three individuals had asymptomatic *P. falciparum* infections following CHMI4, while two volunteers remained symptomatic **(Fig. 3B)**. Next, we analyzed γδ and αβ T cell frequencies across all CHMIs. Total αβ T cell frequencies within $CD3^+$ T cells showed a non-significant decline with repeated CHMI **(Fig. 3C)**. $CD8^+$ αβ T cells increased in frequency and peaked prior to CHMI3 **(Fig. 3C)**, coinciding with an increase in $CD8^+$ T_{naive} cells and $CD8^+$ T_{CM} on day 21 after CHMI2-4 **(Fig. S4C)**. In contrast, γδ T cells frequencies increased across all CHMIs, and this was largely driven by an increase in $V\gamma9/V\delta2^+$ T cells **(Fig. 3D)**. We also found an increase in $V\delta1^+$ T cell frequencies across repeated CHMIs **(Fig. 3D)**. We analyzed the relationship

between γδ T cell frequencies and the risk of developing symptomatic malaria. Overall, αβ T cell and $CD8^+$ T cell frequencies were similar in asymptomatic and symptomatic individuals **(Fig. S4D**). However, volunteers that progressed to asymptomatic malaria with serial CHMIs displayed robust profiles of increasing $V\delta1^+$ and $V\gamma9/V\delta2^+$ T cells frequencies across CHMIs, while symptomatic volunteers retained frequencies of $V\delta1^+$ and $V\gamma9/V\delta2^+$ T cells that were similar to their baseline samples **(Fig. 3E)**. $V\gamma9/V\delta2^+$ T cells frequencies decreased between CHMIs and were not durably maintained at CHMI4 **(Fig. 3E)**. Repeated measures correlations found a significant inverse association between the number of malaria symptoms and $V\delta1^+$ T cell frequencies (P=0.007) **(Fig. 3F),** but not with $\alpha\beta^+$ (P=0.431), $\gamma\delta^+$ (P=0.109), $CD8^+$ (P=0.391) or $V\gamma9/V\delta2^+$ T cell frequencies (P=0.572) **(Fig. S4E)**. Together, these data from a highly controlled human malaria challenge model confirm that repeated *in vivo P. falciparum* infections drive changes in both $V\delta2^+$ and $V\delta1^+$ T cell frequencies. Increased $V\delta1^+$ T cell frequencies correlated with the development of asymptomatic malaria after CHMI4, while $V\gamma9/V\delta2^+$ T cell frequencies decreased between infections and were not durably maintained after CHMI4 in asymptomatic subjects, suggesting that regulation of $V\gamma9/V\delta2^+$ T cells may contribute to symptom reduction, a hypothesis that is consistent with previous reports in the context of natural infection (*38-40*).

Repeated *P. falciparum* infection initiates $V\delta1_{naive}$ to $V\delta1_{effector}$ T cell differentiation

We investigated the impact of repeated *P. falciparum* infections on the differentiation of γδ T cell subsets. $CD27^{hi}$ $CD28^+$ $V\delta1_{naive}$ T cells were the main population of $V\delta1^+$ γδ T cells in subjects prior to CHMI (malaria naïve), but this cell population decreased after repeated *P. falciparum* infections **(Fig. 4A)**. Conversely, $CD27^{lo}$ CX_3CR1^+ $V\delta1_{effector}$ cells became the dominant population within total $V\delta1^+$ T cells **(Fig. 4B**). The increase in the $CD27^{lo}$ CX_3CR1^+

230 $V\delta1_{effector}$ T cell population also correlated with a reduction in malaria symptoms **(Fig. S4F).** In
231 response to a combination of inflammatory cytokines and HMB-PP stimulation, it has been
232 proposed that $V\gamma9/V\delta2^{+}$ T cells switch phenotype from $CD27^{+}$ $CD28^{+}$ to $CD27^{-}$ $CD28^{-}$ (*41*);
233 however, we found no significant changes in these populations across repeated *P. falciparum*
234 infections **(Fig. 4C)**. As noted earlier, $V\gamma9/V\delta2^{+}$ T cells can control parasite replication through
235 CD16-mediated antibody-dependent cytotoxicity (*24*), we found that CD16 expression was
236 upregulated on $V\delta1^{+}$ T cells, but not $V\gamma9/V\delta2^{+}$ T cells after repeated *P. falciparum* infections
237 **(Fig. 4D)**. Interestingly, subject 17 displayed a major $CD27^{-}$ $CD28^{-}$ $CD16^{+}$ $V\gamma9/V\delta2^{+}$ T cell
238 population that persisted over time **(Fig. 4C and D)**. $V\delta1^{+}$ T cells consistently expressed the T
239 cell activation marker CD38 after each *P. falciparum* infection, while $V\gamma9/V\delta2^{+}$ T cells only
240 significantly upregulated CD38 after CHMI1 and 2 **(Fig. 4E)**. We previously showed that
241 $V\delta1_{effector}$ cells possess significant cytotoxic potential (*19, 28*). Here, we found that repeated *P.*
242 *falciparum* infection drove $V\delta1^{+}$ T cells to express Gzm A, B, perforin, but not Gzm K **(Fig. 4F)**,
243 whereas $CD8^{+}$ T cells had no significant increase in Gzm A, B, perforin, or Gzm K **(Fig. S4G)**.
244 In keeping with their pre-formed cytotoxic potential, $V\gamma9/V\delta2^{+}$ T cells retained robust levels of
245 Gzm A, Gzm B, Gzm K, and perforin after repeated *P. falciparum* infections **(Fig. 4F)**. Our data
246 indicate that *in vivo P. falciparum* infection in humans drives the differentiation of human
247 $V\delta1_{effector}$ γδ T cells.
248
249 **Repeated *P. falciparum* infections drive diverse waves of γδTCR selection**
250 We sought to understand whether repeated CHMIs impacted γδTCR repertoires. We used the
251 approach described above **(Fig. 2)** and sorted $V\delta2^{+}$ and $V\delta2^{neg}$ γδ T cell populations from
252 longitudinal timepoints from all five CHMI subjects. We analyzed the relationship between

253 $CD27^{lo}$ CX_3CR1^+ $V\delta2^{neg}$ $_{effector}$ cells and $V\delta2^{neg}$ TCR repertoires prior to CHMI1 and at CHMI4
254 + 21d in subject 2 **(Fig. 5A)**. At baseline, $V\delta2^{neg}$ γδ T cells were predominantly $CD27^{hi}$
255 CX_3CR1^{neg} and displayed a reasonably diverse γδTCR repertoire **(Fig. 5A),** but we observed a
256 shift toward a $CD27^{lo}$ CX_3CR1^+ effector phenotype after repeated CHMIs (**Fig. 5A**). In addition
257 to these phenotypic changes, clonotypes found prior to CHMI1 remained stable or contracted
258 over time, and new $V\delta1^+$ clonotypes expanded, suggesting the potential recruitment of specific
259 TCR sequences into the γδ T cell immune repertoire after repeated CHMIs (**Fig. 5B**). Analysis of
260 the $V\delta2^{neg}$ γδ T cell repertoires indicated Vγ9 and Vδ1 chain usage to be the most prevalent **(Fig.**
261 **5C).** Overall, diversity within $V\delta2^{neg}$ or $V\delta2^+$ γδ T cell repertoires did not show any significant
262 change **(Fig. S5A)**. We assessed if CDR3 clonotype changes were occurring in $V\delta2^+$ TCR
263 repertoires and found that $V\delta2^+$ clonotypes remained stable over time, despite changes in the
264 frequencies of the total population **(Fig. 5D)**. In subject 17, the $V\delta2^+$ TCR repertoire was
265 dominated by hyperexpanded CDR3γ and δ sequences at baseline **(Fig. S5B)**, which contrasts
266 with other $V\delta2^+$ TCR repertoires in this study. Given the stability of $V\delta2^+$ TCR clonotype
267 repertoires at each CHMI, we assessed the potential for dynamic changes in $V\delta2^{neg}$ γδ T cell
268 repertoires at each CHMI and over time. Analysis of the γδTCR repertoire of subject 4 from
269 baseline and over subsequent CHMI's 1, 3 and 4 indicated dynamic changes in the TCR
270 repertoire, with an increase in low frequency clonotypes at CHMI1 and establishment of a
271 broader immune repertoire over time **(Fig. 5E)**. We analyzed the 20 most prevalent clonotypes at
272 baseline (subject 4, 10, 17) or at CHMI1 (subject 9; **Fig. S5C**). We found that prevalent baseline
273 clonotypes declined with each CHMI and we observed waves of new clonotypes that expanded
274 into the most abundant 20 clonotypes after each CHMI **(Fig. 5F)**. In many cases these clonotypes
275 were found at low frequency in the preceding timepoint, suggesting that each CHMI drove

rounds of γδTCR selection **(Fig. 5F)**. Vδ2^{neg} γδ T cell repertoire clonotypes possessed few overlapping clonotypes between individuals, while there were many inter-individual overlapping TCRγ sequences in Vδ2^{+} TCR repertoires **(Fig. S5D)**. Although subject 10 and 17 were symptomatic at CHMI4 and did not have a dramatic increase in Vδ1^{+} T cell frequencies, the repertoire of their Vδ2^{neg} γδTCRs also displayed waves of clonotype selection **(Fig. 5F)**. Together, the Vγ9/Vδ2^{+} T cell response to *P. falciparum* infection displays a highly stable polyclonal immune repertoire over time and infection. In contrast, Vδ2^{neg} γδ T cell repertoires underwent dramatic remodeling of the γδTCR repertoire and displayed waves of clonal selection after each *P. falciparum* infection.

Previous *P. falciparum* exposure licenses Vδ1^{+} T cells reactivity to blood-stage parasites

Finally, we explored the reactivity of γδ T cell subsets towards *P. falciparum* blood-stage parasites. PBMCs from Australian adults with no history of malaria exposure were co-cultured with *P. falciparum* infected red blood cells (*Pf*RBC) or trophozoite/schizont extracts (*Pf*TSE) or or intact uninfected RBCs (uRBC) or extracts (uRBCE) as controls. Vδ1^{+} T cells from Australian adults were unresponsive to *Pf*RBCs or *Pf*TSE, whereas Vγ9/Vδ2^{+} T cell populations were responsiveness **(Fig. 6A)**, corroborating prior studies (*24, 33, 42*). Our *in vivo* results **(Fig. 3, 4 and 5)** prompted us to re-challenge PBMCs of Australian subjects twice over the 5-day culture period. Upon re-challenge, we found that Vδ1^{+} T cells showed varying levels of proliferation after the second re-stimulation **(Fig. 6B)** but only in response to *Pf*TSE and not *Pf*RBCs. Previous studies have reported that Vδ1^{+} T cells from individuals living in malaria endemic regions of Gambia or Tanzania were unresponsive to *Pf*RBC *in vitro* (*33, 42, 43*). Using PBMCs from two Malian subjects and a malaria-naïve subject after 2 CHMIs (subject 10 at

CHMI3+1d), we found that Vδ1^{+} T cells proliferated in response to *Pf*TSE but not *Pf*RBC after a single stimulation **(Fig. 6C and D)**. This differential responsiveness to *Pf*RBC or *Pf*TSE was not consistently seen in paired Vγ9/Vδ2^{+} T cell populations or in malaria unexposed Australian subjects **(Fig. 6D)**. These data indicate that prior *P. falciparum* infection primes Vδ1^{+} T cells for proliferate upon re-challenge with *P. falciparum* parasites.

Discussion

γδ T cells have been implicated in the immune response to pathogenic microbes, including bacteria, viruses and parasites (*9*). These responses in mice and humans appear to be mediated by innate-like γδ T cell populations, often utilizing semi- or invariant γδTCR repertoires that allow rapid effector responses to be mounted during the acute phases of microbial infection (*9*). Emerging evidence is currently re-shaping our understanding of the immunobiology of human γδ T cell populations and γδ T cells have the potential for both innate and adaptive properties (*44*). However, the adaptive-like features of $V\delta2^{neg}$ γδ T cell subsets are only partially understood (*19, 28, 29, 45*), and the establishment of this arm of the immune response to infectious disease has remained unclear.

Here, we show that repeated *in vivo P. falciparum* infection impacts populations of circulating innate-like $V\delta2^{+}$ and adaptive-like $V\delta1^{+}$ γδ T cells. We found that repeated *P. falciparum* infection triggers the differentiation of $V\delta1^{+}$ T cells from a $V\delta1_{naive}$ phenotype into a distinct $V\delta1_{effector}$ subset, concomitant with dynamic clonotype selection in the γδTCR repertoire with each *P. falciparum* infection. Together, our data indicate that *P. falciparum* infection drives the selection and differentiation of the γδ T cell repertoire.

The association of human γδ T cells and malaria has been largely attributed to the remarkable responsiveness of innate-like $V\gamma9/V\delta2^{+}$ T cells to *P. falciparum* infection (*43, 46, 47*). In line with this, we found that $V\gamma9/V\delta2^{+}$ T cells were retained after natural infection in Malian subjects and increased in frequency upon exposure to repeated CHMI, an observations that is likely due to encounter with blood stage *P. falciparum* merozoite-derived HMB-PP (*15, 24, 48*), and

possibly also be in response to liver stage infection (*4, 7, 49*). However, notwithstanding hypotheses that Vγ9/Vδ2$^+$ T cells mount oligoclonal responses to microbial encounters (*50, 51*), we found that public Vγ9/Vδ2$^+$ TCR repertoires remained highly stable over time despite dramatic changes in cellular frequency. The composition of these repertoires was very similar to those seen in gestation (*52*), in cord blood, and after birth (*17-19*). Moreover, the cellular phenotype of Vγ9/Vδ2$^+$ T cells after repeated *P. falciparum* infection was highly stable. Thus, the γδTCR repertoire of innate-like Vγ9/Vδ2$^+$ T cells appears to allow sustained responsiveness upon *P. falciparum* infection.

In contrast to Vγ9/Vδ2$^+$ T cells, the exact nature of human Vδ2$^-$ γδ T cells, and in particular Vδ1$^+$ T cells, in the immune response to microbial pathogens is poorly defined, with recent studies identifying both innate- (*26*) and adaptive-like potential for these cells (*28*). Moreover, how Vδ1$^+$ T cells participate in the complex immune response to *P. falciparum* is largely unknown (*30, 33, 34, 53, 54*). Current paradigms for conventional memory αβ T cells indicate that T $_{effectors}$ arise from T $_{naïve}$ cells driven by antigen-specific challenge to provide a rapid memory-response upon re-exposure to the same pathogen (*55*). Whether a similar paradigm applies to human γδ T cells is unclear (*56*). Here, we demonstrate that Vδ1$_{effector}$ T cells are a major population in Malian children, and that Vδ1$_{naive}$ cells differentiate into Vδ1$_{effectors}$ after repeated *P. falciparum* infections in malaria-naïve adults. Given that Vδ1$_{effector}$ γδ T cells may infiltrate peripheral tissues (*25*), we speculate that *P. falciparum*-reactive Vδ1$^+$ γδ T cells will subsequently infiltrate the liver (*25*), and spleen (*57*). Therefore, *P. falciparum*-reactive Vδ1$^+$ γδ T cells may exert cytotoxic and/or immunoregulatory functions in peripheral tissues during malaria infection and may be possible to explore under certain clinical circumstances (*58*). Moreover, we also found that

γδTCR repertoires undergo dynamic clonotype selection after each *P. falciparum* infection. Whereas only a handful of the antigenic targets are known for Vδ2$^-$ γδTCRs, nearly all identified ligands to date are endogenous host proteins (*13, 59*). In the case of malaria, it has been proposed that the Vδ1$^+$ T cell response during *P. falciparum* infection is also driven by unknown endogenous host factors, based on the observation that Vδ1$^+$ T cells from malaria-exposed individuals do not respond to *P. falciparum* antigens *in vitro* (*33, 42*). Our findings, that Vδ1$^+$ T cells from malaria-exposed individuals react to *P. falciparum* lysate *in vitro*, suggests that Vδ1$^+$ T cells may also have the potential to recognize parasite-derived antigens.

In malaria endemic regions, non-sterilizing immunity to symptomatic malaria is gradually acquired with repeated *P. falciparum* infections (*60*). It is hypothesized that the acquisition of immunity to malaria in humans involves resistance to severe disease followed by resistance to uncomplicated disease (*3*). Our study provides a window into the dynamic evolution of innate- and adaptive-like γδ T cells in the context of natural *P. falciparum* infection and indicates that these cells may represent an important component of the cellular immune response that contributes to immunity to malaria (*4-6*). However, we cannot conclude from the current study that there is an association between $V\delta1_{effector}$ T cell expansion and protection from febrile malaria in the context of natural infection, as Malian children who still experience febrile malaria show evidence of $V\delta1_{effector}$ T cell expansion. Our previous analysis of the same cohort in Mali shows that the risk of febrile malaria gradually decreases with age over years of repeated malaria exposures (*1*). Subjects in the age range (7-14 years) included in the longitudinal portion of the current study are at lower risk of febrile malaria than younger children in the same cohort, but generally, even 7–14-year-olds have yet to acquire immunity that fully protects against febrile

malaria from year to year, leaving open the possibility that $V\delta1_{effector}$ T cell expansion with repeated infections may contribute to the gradual acquisition of immunity to malaria in endemic areas. Studies with larger sample sizes that encompass a broader age range and include more frequent assessments of γδ T cells relative to incident *P. falciparum* infections (both symptomatic and asymptomatic), will be required to assess the relationship between $V\delta1_{effector}$ T cells and the risk of febrile malaria in the context of natural infection.

The findings from our CHMI study suggest that initial $V\gamma9/V\delta2^+$ T cell activation may contribute to the early priming and activation of naïve $V\delta1^+$ γδ T cells, potentially involving the capacity of $V\gamma9/V\delta2^+$ T cells to phagocytose and present parasite antigens (*22, 24*). Moreover, we noted a reduction in $V\gamma9/V\delta2^+$ T cells at the fourth CHMI, consistent with prior studies showing that loss and dysfunction of $V\delta2^+$ T cells is associated with clinical immunity to malaria (*40*). How the emerging population of $V\delta1^+$ γδ T cells may contribute to protection from symptomatic malaria is unclear. The regulatory functions of γδ T cells in response to infectious diseases remains poorly understood, but mounting evidence suggests that these cells may play a role in regulating inflammation in the context of cancer (*61, 62*). Therefore, it seems plausible that repeated febrile malaria episodes could drive the expansion of a regulatory population of $V\delta1^+$ γδ T cells that dampen inflammation through IL-10 (*31*), TGF-β1 (*63*) or other mechanisms (*64*).

There are several limitations of this study. First, the Mali cohort was conducted in a small rural village where the population is predominantly of a single ethnic group, limiting the generalizability of our findings. Nonetheless, we observed lower frequencies of $CD16^+$ $V\gamma9/V\delta2^+$ T cells in the Mali cohort relative to studies in Uganda and Brazil (*24, 65*). Thus, it

will be of interest to further investigate the impact of genetics and/or environmental factors underlying regional differences. Secondly, the number of subjects included in the CHMI study was relatively small, precluding a rigorous analysis of the factors that underlie the inter-individual variability we observed in γδ T cell responses.

In summary, our study shows that both innate and adaptive-like properties of the human γδ T cell repertoire are driven by *P. falciparum* infection *in vivo*. $V\delta2^+$ γδ T cells mount a rapid innate-like polyclonal immune response to acute *P. falciparum* infection. Alongside these innate-like $V\delta2^+$ γδ T cell responses, repeated *P. falciparum* infection established clonally selected populations of adaptive-like $V\delta1_{effector}$ γδ T cells. Together, our study suggests the importance of future studies exploring the role of the γδ T cell repertoire in contributing to the establishment of clinical immunity to malaria.

Materials and Methods

Study Design

The overall objective of this study was to investigate the impact of repeated *P. falciparum* malaria infection on circulating populations of human γδ T cells and their γδ TCR repertoires. We studied γδ T cell populations from cryopreserved PBMCs from children enrolled in a longitudinal study conducted in a malaria-endemic region of Mali (ClinicalTrials.gov, NCT01322581) and children from Australia with no history of malaria exposure (HREC/16/MonH/253). We also studied γδ T cells and their γδ TCR repertoires in five subjects enrolled in a repeated controlled human malaria infection (CHMI) (ClinicalTrials.gov, NCT03014258). A detailed description of each study cohort is provided in the study populations section of the materials and methods and the number of participants, age and gender are described in **Table S1**. We then established the responsiveness of γδ T cell from subjects with a history of exposure to *P. falciparum* in *in vitro* assays using *P. falciparum* infected red blood cells and their extracts. The sample sizes and experimental replicates were sufficient to permit rigorous statistical analysis as described in the figure legends and Materials and Methods. Primary data are reported in **data file S1**.

Study populations

Malian cohort

We studied peripheral blood mononuclear cells (PBMCs) from a cohort of 23 children (aged 4 – 17 years, 14 females) and five adults (aged 21 – 26, 4 females) who were enrolled in a longitudinal study conducted in a malaria-endemic region of Mali that involved both passive and active surveillance for febrile malaria episodes **(Table S1)**. We followed nine of these children

(aged 8 – 14, five females) longitudinally for up to 32 months **(Fig. 1G)**. A detailed description of this cohort study has been published elsewhere (*1*). Venous blood samples were collected at cross-sectional timepoints from all subjects before (April/May) and after (December/January) each malaria season, and during the first febrile malaria episode of each season when it occurred. The research definition of febrile malaria was ≥2,500 asexual *P. falciparum* parasites/μL, an axillary temperature of ≥37.5°C within 24 hours, and no other cause of fever discernible by physical exam. However, all febrile malaria episodes, irrespective of parasite density, were treated with artemether-lumefantrine according to the Mali National Malaria Control Program guidelines. Subjects were selected for the longitudinal study if they experienced at least two febrile malaria episodes over different seasons and had cryopreserved PBMCs available for analysis. All nine subjects experienced febrile malaria during the first wet season but not all subjects experienced febrile malaria during the second or third wet seasons (**Fig. 1G**). The Ethics Committee of the Faculty of Medicine, Pharmacy and Dentistry at the University of Sciences, Techniques and Technology of Bamako, and the Institutional Review Board of NIAID NIH approved this study (ClinicalTrials.gov, NCT01322581). Written informed consent was obtained from the parents or guardians of participating children and from adult participants.

Australian children cohort

Blood was obtained from a cohort of twenty healthy Australian children (aged 1 – 17 years, 12 females) who were undergoing endoscopy procedures at Monash Children's Hospital, Melbourne. These subjects were being screened for coeliac disease or inflammatory bowel disease, but all subjects included in this study were negative for both conditions **(Table S1)**. Written informed consent was obtained from the parents or guardians of participating children

and this study was approved by Monash Health Research Ethics Committee (HREC/16/MonH/253).

Australian and Kenyan adults

PBMCs were obtained from the buffy packs of fourteen Australian adults (aged 20 - 71, 6 females), procured from the Australian Red Cross (ARC) Lifeblood, Melbourne, and all donors gave written informed consent **(Table S1)**. These samples were approved for use in this study by the Australian Red Cross ethics committee and Monash University Human Research Ethics Committee (19488, 14487). We also studied blood samples from six adults (aged 26 – 49, 3 females) from Kenya **(Table S1)**. The Kenyan adults tested negative for malaria (rapid diagnostic test, RDT) and tuberculosis (interferon γ release assay) and gave written informed consent. The study was approved by the Kenya Medical Research Institute, Scientific and Ethics Review Unit.

Controlled human malaria infection (CHMI) cohort

We studied five CHMI subjects (aged 23 – 44, 5 males) from the greater Baltimore area (Baltimore, MD, U.S.) from whom either leukocytes or venous blood were collected at baseline (60 days before infection) by apheresis, immediately prior to infection (CHMI + 1d) by venipuncture and 21 days (d) after the first infection (CHMI + 21d) by apheresis. Where apheresis was used to obtain leukocytes and plasma, a clinical assessment of each individual was conducted prior to collection, including assessment of lymphopenia. All subjects passed this assessment and proceeded to plasma and leukocyte collection by apheresis. Subjects were infected by the bites of five *Anopheles stephensi* mosquitos carrying *P. falciparum* (strain NF54;

kindly provided by Sanaria). All subjects were then evaluated as part of an inpatient stay to diagnose *P. falciparum* malaria infection and to be treated with Malarone® (Atovaquone/proguanil) and Coartem® (artemether/lumefantrine) as secondary treatment. Daily observations were undertaken from study day 6 until malaria diagnosis based upon the detection of two unquestionable parasites by blood smears. Malaria cure was confirmed by treatment for all subjects after three days of directly observed therapy and two negative blood smears separated by a time interval >12 hours, followed by a third negative smear >12 hours after the previous two daily smears. This procedure was then repeated in each subject on three further occasions. All subjects gave written informed consent and the study was approved by the medical ethics committee of the University of Maryland, Baltimore (ClinicalTrials.gov, NCT03014258).

Peripheral blood mononuclear cell isolation

In the Kenya, Australian and CHMI study cohorts, heparinised venous blood, total leukocytes obtained by apheresis or from buffy packs obtained from the Australian Red Cross Lifeblood, were used to isolate PBMCs. In short, peripheral blood or leukocytes were layered over lymphoprep (Stemcell Technologies) and gradient centrifuged for 20 mins at 600 x g at room temperature (RT) without brake. The resulting PBMC layer was washed twice in Roswell memorial park institute (RPMI)-1640 medium for 10 mins at 400 x g at 4°C. PBMCs were frozen in fetal calf serum (FBS; Sigma Aldrich or Gibco) and 10% dimethyl sulfoxide (Sigma Aldrich) at -80°C for 24 hours and stored until use in liquid nitrogen.

In the Mali study, blood samples drawn by venipuncture were collected in sodium citrate-containing cell preparation tubes (Vacutainer CPT Tubes, BD) and transported to the laboratory in Bamako where PBMCs and plasma were separated by centrifugation. PBMCs were isolated from the Vacutainer CPT Tubes according to manufacturer instructions and were frozen within 3 h of the blood draw in FBS containing 7.5% DMSO (FBS: Gibco; DMSO: Sigma-Aldrich). The cells were first frozen at −80°C for 24 h and subsequently transferred to liquid nitrogen for long-term storage.

Antibodies and flow cytometry

Frozen PBMCs were thawed and washed twice in PBS. For the detection of surface marker expression, cells were stained with Zombie Aqua fixable viability dye (1:500, BioLegend). PBMCs were then stained in FACS buffer (PBS, 2% FBS and 0.04% sodium azide) with antibodies indicated in the cell surface panel **(Table S3)**. To quantify absolute cell numbers, accucheck counting beads (ThermoFisher Scientific) were added to a selection of samples. To detect intracellular cytotoxic molecules PBMCs were fixed and permeabilized using the Foxp3/Transcription factor staining buffer set (eBioscience). Fixed/permeabilized PBMCs were then resuspended in permeabilization buffer containing intracellular antibodies **(Table S3)**. A representative gating strategy is shown in **Fig. S2**. All samples were acquired on the Fortessa X20 (BD Biosciences) flow cytometer and FCS files were analysed using FlowJo v10 (BD Biosciences/Treestar).

Bulk cell sorting and RNA-based TCR repertoire analysis

Frozen PBMCs were thawed and stained with Zombie Aqua dye and then incubated with antibodies indicated in the bulk sort panel **(Table S3)** for 20 mins on ice. Cells were then bulk sorted into RNA*later* (Sigma Aldrich) using the sort strategy described in **Fig. S2** using a FACS ARIA II Fusion (BD Biosciences). Resultant cell numbers sorted for each population is given in **Table S4**. RNA was purified using a RNeasy plus micro kit (Qiagen) following the manufacturers' instructions. The human TCRδ and γ chain iR profile kits (iRepertoire Inc) were used to perform amplicon rescued multiplex (ARM)-PCR to generate complementarity determining region (CDR) 3 libraries for sequencing following the manufacturer's instructions. Sequencing was performed using an Illumina MiSeq (Micromon and Monash Health Translation Precinct Medical Genomics Facility). From raw sequencing data in BioProject Accession Number PRJNA770107, we used iRweb tools (iRepertoire Inc) to assign CDR3 sequences, variable (V), diversity (D), and junction (J) gene usage, calculate diversity indexes (DI; this metric considers the clonal frequency to occupy 50% of the total repertoire (D50) and abundance of unique CDR3 sequences (Shannon entropy)) and plot tree maps.

Single cell sorting and complementarity determining region 3 TCR sequencing

Frozen PBMCs were thawed and stained with Zombie Aqua dye and then incubated with antibodies indicated in the single cell sort panel **(Table S3)**. Single cells were then sorted into 96 wells plates (Axygen) containing 2 µl Superscript VILO cDNA synthesis kit reaction mix (ThermoFisher) containing 0.1% Triton X-100 (ThermoFisher) and incubated according to the manufacturers' instructions. TCRγ and TCRδ cDNAs were amplified by two rounds of nested PCR using GoTaq mastermix (Promega), using external primers for Vδ1 - CAAGCCCAGTCATCAGTATCC, Cδ – GCAGGATCAAACTCTGTTATCTTC, Vγ1-8 –

CTGGTACCTACACCAGGAGGGGAAGG, Vγ9 – AGAGAGACCTGGTGAAGTCATACA, and Cγ – CTGACGATACATCTGTGTTCTTTG, and internal primers for Vδ1 – CAACTTCCCAGCAAAGAGATG and Cδ - TCCTTCACCAGACAAGCGAC, or Vγ1-8 – TGTGTTGGAATCAGGAVTCAG, Vγ9 – GGTGGATAGGATACCTGAAACG, and Cγ – AATCGTGTTGCTCTTCTTTTCTT. PCR products were visualized using the QIAxcel DNA fast analysis kit (Qiagen). products of successful reactions were incubated with ExoSAP-IT PCR cleanup enzyme (Affymetrix) before sequencing with BigDye Terminator v3.1 (Applied Biosystems) following manufacturer's instructions and running on an ABI 3730 capillary sequencer (Micromon, Monash University). Resulting complementarity determining regions 3 (CDR3) nucleotide sequences were identified using the IMGT Junction analysis tool (*75*)

Parasite culture and purification

P. falciparum NF54 asexual blood stages were cultured in RPMI 1640 supplemented with 26mM HEPES, 50μg/mL hypoxanthine, 20μg/mL gentamicin, 2.9% $NaHCO_3$, 5% Albumax II and 5% heat-inactivated human serum (Australian Red Cross). Parasites were maintained with human type O-positive RBCs at 4% haematocrit (Australian Red Cross) in a gaseous mix of 5% CO_2, 1% O_2 in N_2 at 37°C. Synchronous late trophozoite and early schizont parasites (32-40 hr old) were grown to 5% parasitemia before purification. Briefly, cultures were centrifuged at 2000 *g* for 5 min and passed over a MACS column under magnetic force (Miltenyi). Mature parasites retained within the magnet were washed and eluted in the absence of magnetic force with RPMI complete medium before pelleting at 2000 *g* for 5 min. Medium was aspirated and purified infected erythrocytes were used in subsequent experiments. Uninfected erythrocytes were from

the same sources of human type O-positive RBCs. *P. falciparum* trophozoite/schizont extract (*Pf*TSE) was prepared by three freeze-thaw cycles of *Pf*RBCs at -80°C and a 37°C water bath.

PBMC culture and activation

For proliferation of γδ T cells, PBMCs were labelled with Cell Trace violet (ThermoFisher) and cultured with *P. falciparum* infected RBCs (*Pf*RBCs), their extracts (*Pf*TSE) or uninfected RBCs (uRBC) or uRBC extracts (uRBCE) for up to 6 days with 20 U/ml IL-2 (Miltenyi) in RPMI-1640 medium (Invitrogen) supplemented with 2 mM L-glutamine, 1% sodium pyruvate, 50 µg/ml penicillin/streptomycin (Invitrogen) and 10% fetal calf serum (Sigma). Cultured cells were then stained with Zombie UV fixable viability dye (BioLegend) and then stained with the proliferation panel antibodies **(Table S3)**.

Statistical analysis

Tabulated data were analyzed in Graphpad PRISM 9 (Graphpad Software, Inc.). Each data set was assessed for normality using Shapiro–Wilk normality test. Differences between cohorts were analyzed by two-tailed Student's t-tests for normally distributed data, Mann–Whitney for non-parametric data and Wilcoxon signed-rank test for paired data. Differences between groups were analyzed using one-way ANOVA with Dunnett's or Tukey's post tests for normally distributed data or with Kruskal–Wallis test with Tukey's post tests for non-parametric data and RM two-way ANOVA with Tukey's post-hoc test was used when comparing groups with independent variables. Differences between repeated measures and correlations were analyzed via linear mixed effects modelling using the PROC MIXED procedure in SAS software version 9.4 (SAS

591 Institute, Cary, NC, USA). Post-hoc comparisons were performed using Bonferroni method for
592 multiple comparisons. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.
593

Figure Legends

Figure 1. Increased Vδ1$^+$ γδ T cells frequencies in Malian subjects exposed to *P. falciparum* infection. In age and gender matched Malian (n=23) or Australian subjects (n=20): **A.** Frequencies of total γδ, Vγ9/Vδ2$^+$ and Vδ1$^+$ T cells within CD3$^+$ T cells, **B.** Frequencies of Vγ9/Vδ2$^+$ and Vδ1$^+$ T cells in total CD3$^+$ T cells, **C.** Frequencies of CD27^{lo} CX$_3$CR1$^+$ cells within Vδ1$^+$ or CD27$^+$ CD28$^+$ cells within Vγ9/Vδ2$^+$ T cells, **D.** Frequencies of perforin$^+$, **E.** Gzm A$^+$, **F.** Gzm B$^+$ cells within Vδ1$^+$ or Vγ9/Vδ2$^+$ T cells (**D**, **E** and **F**: Malian n=19 and Australian n=15). **G.** Schematic of samples and malaria exposure for Malian subjects included in the longitudinal arm of our study. Subjects are stratified based on presentation with a confirmed febrile malaria episode in all three years (n=4) or two febrile episodes (n=5), with either one episode in year 2 (n=3) or 3 (n=2). **H.** Frequencies of Vδ1$^+$, Vγ9/Vδ2$^+$ and CD8$^+$ T cells in total CD3$^+$ T cells during febrile malaria and 3-6 months following treatment over the 3-year seasonal transmission periods. Year 1 (n=9), Year 2 (n=7) and Year 3 (n=6). **I.** Frequencies of Vδ1$^+$ and Vγ9/Vδ2$^+$ T cells within CD3$^+$ T cells over a 6-month period without a febrile malaria episode (n=5; 12 – 18 months, n=2, or 24 - 30 months, n=3). Bars show the mean and error bars indicate means ± SEM. Normality was tested using the Shapiro-Wilk test.; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; P-values were determined by Mann-Whitney test (**a - f**) and Wilcoxon matched-pairs signed rank test (**h, i**).

Figure 2. γδTCR repertoires in Malian subjects evolve over time. **A**. TCRγ clonotype tree plot analysis of Vδ2^{neg} and Vγ9/Vδ2$^+$ T cell populations from Australian children or Malian children during stable periods without malaria transmission. Tree plots show unique clonotypes (colored segments) and their proportion within the total repertoire (size). In general, colored

clonotypes do not match between plots unless indicated. **B.** Pooled accumulated frequency curves of the top 10 most prevalent clonotypes in $V\delta2^{neg}$ or $V\gamma9/V\delta2^{+}$ TCR repertoires (Australian, n=3; Mali, n=4). **C.** Diversity index of $V\delta2^{neg}$ and $V\delta2^{+}$ γδ T cell repertoires in Malian (n=4) or Australian (n=3) subjects. **D.** Frequency of shared CDR3γ (a.a.) sequences in $V\delta2^{neg}$ and $V\gamma9/V\delta2^{+}$ T cell repertoires (Australian, n=3; Mali, n=4). **E.** Longitudinal tracking of the 20 most abundant TCRγ clonotypes in $V\gamma9/V\delta2^{+}$ and **F.** $V\delta2^{neg}$ T cell repertoires over time in subject 066. (M) indicates acute febrile malaria. **G.** Longitudinal analysis of Vγ chain usage and diversity index for $V\delta2^{neg}$ (red) and $V\gamma9/V\delta2^{+}$ (blue) T cell repertoires from subject 066. **H.** γδTCR expression patterns within ($CD27^{lo}$ CX_3CR1^{+}) $V\delta1^{+}_{effector}$ populations in donor 179. Each flow cytometry plot has two time points overlaid, indicated by an arrow, together covering three febrile *P. falciparum* infections (months 0, 17 and 30). **I.** TCRδ clonotypes sequencing relative to total $CD3^{+}$ T cells from subject 179. Error bars indicate means ± SEM. Normality was tested using the Shapiro-Wilk test.; *P < 0.05; **P < 0.01; ***P < 0.001; P-values were determined by two-way ANOVA with Sidaks post hoc testing (**b**) and one-way ANOVA with Holm-Sidaks post hoc testing (**c, d**).

Figure 3. Repeated controlled *P. falciparum* infections drive clinical immunity to malaria and increased frequencies of γδ T cells. **A**. Controlled human malaria infection (CHMI) study subjects, samples, parasite ultra-sensitive PCR (usPCR) detection curves and diagnosis by blood smear. **B.** Symptomology and fever analysis of each subject during each CHMI. **C-E**. Within total $CD3^{+}$ T cells: **C.** Total $\alpha\beta^{+}$ and $CD8^{+}$ T cell frequencies. **D.** Total $\gamma\delta^{+}$, $V\gamma9/V\delta2^{+}$ (blue), and $V\delta1^{+}$ (red) γδ T cells frequencies and **E.** $V\delta1^{+}$ and $V\gamma9/V\delta2^{+}$ frequencies in individuals that were asymptomatic or symptomatic at CHMI4. **F.** Repeated measure correlation between $V\delta1^{+}$

640 frequencies within total $CD3^+$ T cells and the number of symptoms each individual suffered at
641 each CHMI. Bars show the mean. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; P-values
642 were determined by Kruskall-Wallis test (**b**), linear mixed effects modelling with Bonferroni's
643 correction (**c, d, e**) and repeated measures correlation (**f**).
644
645 **Figure 4. Repeated *P. falciparum* infections drives the differentiation of cytotoxic $V\delta1_{effector}$**
646 **T cells. A.** Representative flow cytometry plot and graph showing the frequencies of $CD27^+$
647 $CD28^+$ cells in $V\delta1^+$ T cells after repeated CHMIs (n=5). **B.** Representative flow cytometry plot
648 and graph showing the differentiation of $CD27^{lo}$ CX_3CR1^+ $V\delta1^+_{effector}$ cells after repeated CHMIs
649 (n=5). **C.** Frequencies of $CD27^+$ $CD28^+$ and $CD27^{neg}$ $CD28^{neg}$ cells within $V\gamma9/V\delta2^+$ T cells. **D-**
650 **F.** Within $V\delta1^+$ (red) and $V\gamma9/V\delta2^+$ (blue) T cells, the frequencies of: **D.** $CD16^+$, **E.** $CD38^+$, **F.**
651 Gzm A^+, B^+, K^+ and $perforin^+$ cells. Bars show the mean. *P < 0.05; **P < 0.01; ***P < 0.001;
652 ****P < 0.0001; P-values were determined by linear mixed effects modelling with Bonferroni's
653 correction.
654
655 **Figure 5. Repeated *P. falciparum* infection drives waves of Vδ1 γδTCR clonotype selection.**
656 **A.** Flow cytometry plots showing frequencies of $CD27^{lo}$ CX_3CR1^+ $V\delta2^{neg}$ γδ T cells after
657 repeated CHMI challenge in subject 2. TCRδ tree plots of the corresponding total $V\delta2^{neg}$ γδ T
658 cells and DI are given for each tree plot. **B.** Increase in new Vδ1 sequences between baseline and
659 CHMI4 within the top 20 clonotypes in TCRγ from subject 2. **C.** Vγ and Vδ usage in $V\delta2^{neg}$ T
660 cell repertoires from baseline to CHMI 4 (n=4-5). **D.** Longitudinal tracking of the top 20 CDR3γ
661 clonotypes in $V\delta2^+$ T cell repertoires as a frequency of total $CD3^+$ T cell populations. **E.** TCRγ
662 tree plots showing $V\delta2^{neg}$ TCR repertoires at baseline and after repeated CHMIs in subject 4.

663 The D75 and DI metrics are indicated. The graphs show the accumulated frequency of the top 20
664 clonotypes for each repertoire. **F.** Longitudinal tracking of the top 20 CDR3γ clonotypes in
665 Vδ2^{neg} TCR repertoires from subject 4, 9, 10, 17; displayed as a proportion of the total TCRγ
666 repertoire (left) or within the total CD3^{+} T cell population (right).
667
668 **Figure 6. Previous *P. falciparum* exposure licenses Vδ1^{+} T cells for parasite reactivity**. Vδ1^{+}
669 and Vγ9/Vδ2^{+} T cells were assessed for proliferation in Australian adult donors with no history
670 of malaria. PBMCs were labelled with Cell Trace and incubated for 6 days with **A.** One or **B.**
671 two stimulations (at day 0 and 3 of culture) with *P. falciparum* trophozoite/early schizont extract
672 (*Pf*TSE) or infected red blood cells (RBCs) and uninfected RBCs (uRBC) or extract (uRBCE)
673 (uRBCE/PfTSE: n=6; uRBC/PfRBC: n=10). **C.** Representative flow cytometry plots show Vδ1^{+}
674 (blue) and Vδ2^{+} (black) T cells assessed for proliferation in the PBMCs from a Malian subject
675 after co-culture with *Pf*TSE. *Pf*RBCs, uRBC or uRBCE controls. **D.** Graphs show the
676 proliferation of Vδ1^{+} and Vγ9/Vδ2^{+} T cells from two Malian subjects with a history of repeated
677 prior exposure to *P. falciparum* malaria; subject 10 at CHMI3 + 1d and three independent
678 Australian donors with no history of malaria exposure. Each data point represents the proportion
679 of proliferating cells in cultures exposed to *Pf*RBCs or *Pf*TSE minus the response to uRBC or
680 uRBCE controls. Bars show the mean ± SEM. Normality was tested using the Shapiro-Wilk
681 test.; **$P < 0.01$; P-values were determined by one-way ANOVA with Holm-Sidak's post hoc
682 testing (**d**).
683

Supplementary Materials

References

1. T. M. Tran, S. Li, S. Doumbo, D. Doumtabe, C. Y. Huang, S. Dia, A. Bathily, J. Sangala, Y. Kone, A. Traore, M. Niangaly, C. Dara, K. Kayentao, A. Ongoiba, O. K. Doumbo, B. Traore, P. D. Crompton, An intensive longitudinal cohort study of Malian children and adults reveals no evidence of acquired immunity to Plasmodium falciparum infection. *Clin Infect Dis* **57**, 40-47 (2013); published online EpubJul (10.1093/cid/cit174).
2. M. F. Good, D. L. Doolan, Immune effector mechanisms in malaria. *Curr Opin Immunol* **11**, 412-419 (1999); published online EpubAug (10.1016/S0952-7915(99)80069-7).
3. P. D. Crompton, J. Moebius, S. Portugal, M. Waisberg, G. Hart, L. S. Garver, L. H. Miller, C. Barillas-Mury, S. K. Pierce, Malaria immunity in man and mosquito: insights into unsolved mysteries of a deadly infectious disease. *Annu Rev Immunol* **32**, 157-187 (2014)10.1146/annurev-immunol-032713-120220).
4. A. S. Ishizuka, K. E. Lyke, A. DeZure, A. A. Berry, T. L. Richie, F. H. Mendoza, M. E. Enama, I. J. Gordon, L. J. Chang, U. N. Sarwar, K. L. Zephir, L. A. Holman, E. R. James, P. F. Billingsley, A. Gunasekera, S. Chakravarty, A. Manoj, M. Li, A. J. Ruben, T. Li, A. G. Eappen, R. E. Stafford, C. N. K, T. Murshedkar, H. DeCederfelt, S. H. Plummer, C. S. Hendel, L. Novik, P. J. Costner, J. G. Saunders, M. B. Laurens, C. V. Plowe, B. Flynn, W. R. Whalen, J. P. Todd, J. Noor, S. Rao, K. Sierra-Davidson, G. M. Lynn, J. E. Epstein, M. A. Kemp, G. A. Fahle, S. A. Mikolajczak, M. Fishbaugher, B. K. Sack, S. H. Kappe, S. A. Davidson, L. S. Garver, N. K. Bjorkstrom, M. C. Nason, B. S. Graham, M. Roederer, B. K. Sim, S. L. Hoffman, J. E. Ledgerwood, R. A. Seder, Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. *Nat Med* **22**, 614-623 (2016); published online EpubJun (10.1038/nm.4110).
5. I. Zaidi, H. Diallo, S. Conteh, Y. Robbins, J. Kolasny, S. Orr-Gonzalez, D. Carter, B. Butler, L. Lambert, E. Brickley, R. Morrison, M. Sissoko, S. A. Healy, B. K. L. Sim, O. K. Doumbo, S. L. Hoffman, P. E. Duffy, gammadelta T Cells Are Required for the Induction of Sterile Immunity during Irradiated Sporozoite Vaccinations. *J Immunol* **199**, 3781-3788 (2017); published online EpubDec 1 (10.4049/jimmunol.1700314).
6. S. E. de Jong, V. van Unen, M. D. Manurung, K. A. Stam, J. J. Goeman, S. P. Jochems, T. Hollt, N. Pezzotti, Y. D. Mouwenda, M. E. Betouke Ongwe, F. R. Lorenz, Y. C. M. Kruize, S. Azimi, M. H. Konig, A. Vilanova, E. Eisemann, B. P. F. Lelieveldt, M. Roestenberg, B. K. L. Sim, M. J. T. Reinders, R. Fendel, S. L. Hoffman, P. G. Kremsner, F. Koning, B. Mordmuller, B. Lell, M. Yazdanbakhsh, Systems analysis and controlled malaria infection in Europeans and Africans elucidate naturally acquired immunity. *Nat Immunol* **22**, 654-665 (2021); published online EpubMay (10.1038/s41590-021-00911-7).
7. A. Mwakingwe-Omari, S. A. Healy, J. Lane, D. M. Cook, S. Kalhori, C. Wyatt, A. Kolluri, O. Marte-Salcedo, A. Imeru, M. Nason, L. K. Ding, H. Decederfelt, J. Duan, J. Neal, J. Raiten, G. Lee, J. C. C. Hume, J. E. Jeon, I. Ikpeama, N. Kc, S. Chakravarty, T. Murshedkar, L. W. P. Church, A. Manoj, A. Gunasekera, C. Anderson, S. C. Murphy, S. March, S. N. Bhatia, E. R. James, P. F. Billingsley, B. K. L. Sim, T. L. Richie, I. Zaidi, S. L. Hoffman, P. E. Duffy, Two chemoattenuated PfSPZ malaria vaccines induce sterile hepatic immunity. *Nature* **595**, 289-294 (2021); published online EpubJul (10.1038/s41586-021-03684-z).
8. M. Bonneville, R. L. O'Brien, W. K. Born, Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat Rev Immunol* **10**, 467-478 (2010); published online EpubJul (10.1038/nri2781).
9. Y. H. Chien, C. Meyer, M. Bonneville, gammadelta T cells: first line of defense and beyond. *Annu Rev Immunol* **32**, 121-155 (2014)10.1146/annurev-immunol-032713-120216).
10. A. C. Hayday, [gamma][delta] cells: a right time and a right place for a conserved third way of protection. *Annu Rev Immunol* **18**, 975-1026 (2000)10.1146/annurev.immunol.18.1.975).
11. J. Rossjohn, S. Gras, J. J. Miles, S. J. Turner, D. I. Godfrey, J. McCluskey, T cell antigen receptor recognition of antigen-presenting molecules. *Annu Rev Immunol* **33**, 169-200 (2015)10.1146/annurev-immunol-032414-112334).
12. Y. Tanaka, S. Sano, E. Nieves, G. De Libero, D. Rosa, R. L. Modlin, M. B. Brenner, B. R. Bloom, C. T. Morita, Nonpeptide ligands for human gamma delta T cells. *Proc Natl Acad Sci U S A* **91**, 8175-8179 (1994); published online EpubAug 16 (10.1073/pnas.91.17.8175).
13. B. E. Willcox, C. R. Willcox, gammadelta TCR ligands: the quest to solve a 500-million-year-old mystery. *Nat Immunol* **20**, 121-128 (2019); published online EpubFeb (10.1038/s41590-018-0304-y).

14. M. Hirano, P. Guo, N. McCurley, M. Schorpp, S. Das, T. Boehm, M. D. Cooper, Evolutionary implications of a third lymphocyte lineage in lampreys. *Nature* **501**, 435-438 (2013); published online EpubSep 19 (10.1038/nature12467).
15. C. T. Morita, C. Jin, G. Sarikonda, H. Wang, Nonpeptide antigens, presentation mechanisms, and immunological memory of human Vgamma2Vdelta2 T cells: discriminating friend from foe through the recognition of prenyl pyrophosphate antigens. *Immunol Rev* **215**, 59-76 (2007); published online EpubFeb (10.1111/j.1600-065X.2006.00479.x).
16. M. Hintz, A. Reichenberg, B. Altincicek, U. Bahr, R. M. Gschwind, A. K. Kollas, E. Beck, J. Wiesner, M. Eberl, H. Jomaa, Identification of (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate as a major activator for human gammadelta T cells in Escherichia coli. *FEBS Lett* **509**, 317-322 (2001); published online EpubDec 7 (10.1016/s0014-5793(01)03191-x).
17. M. Papadopoulou, T. Dimova, M. Shey, L. Briel, H. Veldtsman, N. Khomba, H. Africa, M. Steyn, W. A. Hanekom, T. J. Scriba, E. Nemes, D. Vermijlen, Fetal public Vgamma9Vdelta2 T cells expand and gain potent cytotoxic functions early after birth. *Proceedings of the National Academy of Sciences of the United States of America*, (2020); published online EpubJul 14 (10.1073/pnas.1922595117).
18. S. Ravens, A. S. Fichtner, M. Willers, D. Torkornoo, S. Pirr, J. Schoning, M. Deseke, I. Sandrock, A. Bubke, A. Wilharm, D. Dodoo, B. Egyir, K. L. Flanagan, L. Steinbruck, P. Dickinson, P. Ghazal, B. Adu, D. Viemann, I. Prinz, Microbial exposure drives polyclonal expansion of innate gammadelta T cells immediately after birth. *Proc Natl Acad Sci U S A* **117**, 18649-18660 (2020); published online EpubAug 4 (10.1073/pnas.1922588117).
19. M. S. Davey, C. R. Willcox, S. Hunter, S. A. Kasatskaya, E. B. M. Remmerswaal, M. Salim, F. Mohammed, F. J. Bemelman, D. M. Chudakov, Y. H. Oo, B. E. Willcox, The human Vdelta2(+) T-cell compartment comprises distinct innate-like Vgamma9(+) and adaptive Vgamma9(-) subsets. *Nat Commun* **9**, 1760 (2018); published online EpubMay 2 (10.1038/s41467-018-04076-0).
20. C. Roussilhon, M. Agrapart, P. Guglielmi, A. Bensussan, P. Brasseur, J. J. Ballet, Human TcR gamma delta+ lymphocyte response on primary exposure to Plasmodium falciparum. *Clin Exp Immunol* **95**, 91-97 (1994); published online EpubJan (10.1111/j.1365-2249.1994.tb06020.x).
21. C. Riganti, M. Massaia, M. S. Davey, M. Eberl, Human gammadelta T-cell responses in infection and immunotherapy: common mechanisms, common mediators? *Eur J Immunol* **42**, 1668-1676 (2012); published online EpubJul (10.1002/eji.201242492).
22. J. Howard, S. Loizon, C. J. Tyler, D. Duluc, B. Moser, M. Mechain, A. Duvignaud, D. Malvy, M. Troye-Blomberg, J. F. Moreau, M. Eberl, O. Mercereau-Puijalon, J. Dechanet-Merville, C. Behr, M. Mamani-Matsuda, The Antigen-Presenting Potential of Vgamma9Vdelta2 T Cells During Plasmodium falciparum Blood-Stage Infection. *J Infect Dis* **215**, 1569-1579 (2017); published online EpubMay 15 (10.1093/infdis/jix149).
23. G. Costa, S. Loizon, M. Guenot, I. Mocan, F. Halary, G. de Saint-Basile, V. Pitard, J. Dechanet-Merville, J. F. Moreau, M. Troye-Blomberg, O. Mercereau-Puijalon, C. Behr, Control of Plasmodium falciparum erythrocytic cycle: gammadelta T cells target the red blood cell-invasive merozoites. *Blood* **118**, 6952-6962 (2011); published online EpubDec 22 (10.1182/blood-2011-08-376111).
24. C. Junqueira, R. B. Polidoro, G. Castro, S. Absalon, Z. Liang, S. Sen Santara, A. Crespo, D. B. Pereira, R. T. Gazzinelli, J. D. Dvorin, J. Lieberman, gammadelta T cells suppress Plasmodium falciparum blood-stage infection by direct killing and phagocytosis. *Nat Immunol* **22**, 347-357 (2021); published online EpubMar (10.1038/s41590-020-00847-4).
25. S. Hunter, C. R. Willcox, M. S. Davey, S. A. Kasatskaya, H. C. Jeffery, D. M. Chudakov, Y. H. Oo, B. E. Willcox, Human liver infiltrating gammadelta T cells are composed of clonally expanded circulating and tissue-resident populations. *J Hepatol*, (2018); published online EpubMay 18 (10.1016/j.jhep.2018.05.007).
26. Y. Wu, F. Kyle-Cezar, R. T. Woolf, C. Naceur-Lombardelli, J. Owen, D. Biswas, A. Lorenc, P. Vantourout, P. Gazinska, A. Grigoriadis, A. Tutt, A. Hayday, An innate-like Vdelta1(+) gammadelta T cell compartment in the human breast is associated with remission in triple-negative breast cancer. *Sci Transl Med* **11**, (2019); published online EpubOct 9 (10.1126/scitranslmed.aax9364).
27. R. Di Marco Barros, N. A. Roberts, R. J. Dart, P. Vantourout, A. Jandke, O. Nussbaumer, L. Deban, S. Cipolat, R. Hart, M. L. Iannitto, A. Laing, B. Spencer-Dene, P. East, D. Gibbons, P. M. Irving, P. Pereira, U. Steinhoff, A. Hayday, Epithelia Use Butyrophilin-like Molecules to Shape Organ-Specific gammadelta T Cell Compartments. *Cell* **167**, 203-218 e217 (2016); published online EpubSep 22 (10.1016/j.cell.2016.08.030).

28. M. S. Davey, C. R. Willcox, S. P. Joyce, K. Ladell, S. A. Kasatskaya, J. E. McLaren, S. Hunter, M. Salim, F. Mohammed, D. A. Price, D. M. Chudakov, B. E. Willcox, Clonal selection in the human Vdelta1 T cell repertoire indicates gammadelta TCR-dependent adaptive immune surveillance. *Nat Commun* **8**, 14760 (2017); published online EpubMar 1 (10.1038/ncomms14760).
29. S. Ravens, C. Schultze-Florey, S. Raha, I. Sandrock, M. Drenker, L. Oberdorfer, A. Reinhardt, I. Ravens, M. Beck, R. Geffers, C. von Kaisenberg, M. Heuser, F. Thol, A. Ganser, R. Forster, C. Koenecke, I. Prinz, Human gammadelta T cells are quickly reconstituted after stem-cell transplantation and show adaptive clonal expansion in response to viral infection. *Nat Immunol* **18**, 393-401 (2017); published online EpubApr (10.1038/ni.3686).
30. L. Hviid, J. A. Kurtzhals, V. Adabayeri, S. Loizon, K. Kemp, B. Q. Goka, A. Lim, O. Mercereau-Puijalon, B. D. Akanmori, C. Behr, Perturbation and proinflammatory type activation of V delta 1(+) gamma delta T cells in African children with Plasmodium falciparum malaria. *Infect Immun* **69**, 3190-3196 (2001); published online EpubMay (10.1128/IAI.69.5.3190-3196.2001).
31. T. Taniguchi, K. Md Mannoor, D. Nonaka, H. Toma, C. Li, M. Narita, V. Vanisaveth, S. Kano, M. Takahashi, H. Watanabe, A Unique Subset of gammadelta T Cells Expands and Produces IL-10 in Patients with Naturally Acquired Immunity against Falciparum Malaria. *Front Microbiol* **8**, 1288 (2017)10.3389/fmicb.2017.01288).
32. S. Worku, A. Bjorkman, M. Troye-Blomberg, L. Jemaneh, A. Farnert, B. Christensson, Lymphocyte activation and subset redistribution in the peripheral blood in acute malaria illness: distinct gammadelta+ T cell patterns in Plasmodium falciparum and P. vivax infections. *Clin Exp Immunol* **108**, 34-41 (1997); published online EpubApr (10.1046/j.1365-2249.1997.d01-981.x).
33. M. Goodier, M. Krause-Jauer, A. Sanni, A. Massougbodji, B. C. Sadeler, G. H. Mitchell, M. Modolell, K. Eichmann, J. Langhorne, Gamma delta T cells in the peripheral blood of individuals from an area of holoendemic Plasmodium falciparum transmission. *Trans R Soc Trop Med Hyg* **87**, 692-696 (1993); published online EpubNov-Dec (10.1016/0035-9203(93)90299-6).
34. L. Hviid, C. Smith-Togobo, B. E. Willcox, Human Vdelta1(+) T Cells in the Immune Response to Plasmodium falciparum Infection. *Front Immunol* **10**, 259 (2019)10.3389/fimmu.2019.00259).
35. D. L. Hill, E. J. Carr, T. Rutishauser, G. Moncunill, J. J. Campo, S. Innocentin, M. Mpina, A. Nhabomba, A. Tumbo, C. Jairoce, H. A. Moll, M. C. van Zelm, C. Dobano, C. Daubenberger, M. A. Linterman, Immune system development varies according to age, location, and anemia in African children. *Sci Transl Med* **12**, (2020); published online EpubFeb 5 (10.1126/scitranslmed.aaw9522).
36. S. Portugal, T. M. Tran, A. Ongoiba, A. Bathily, S. Li, S. Doumbo, J. Skinner, D. Doumtabe, Y. Kone, J. Sangala, A. Jain, D. H. Davies, C. Hung, L. Liang, S. Ricklefs, M. V. Homann, P. L. Felgner, S. F. Porcella, A. Farnert, O. K. Doumbo, K. Kayentao, B. M. Greenwood, B. Traore, P. D. Crompton, Treatment of Chronic Asymptomatic Plasmodium falciparum Infection Does Not Increase the Risk of Clinical Malaria Upon Reinfection. *Clin Infect Dis* **64**, 645-653 (2017); published online EpubMar 1 (10.1093/cid/ciw849).
37. M. Papadopoulou, T. Dimova, M. Shey, L. Briel, H. Veldtsman, N. Khomba, H. Africa, M. Steyn, W. A. Hanekom, T. J. Scriba, E. Nemes, D. Vermijlen, Fetal public Vgamma9Vdelta2 T cells expand and gain potent cytotoxic functions early after birth. *Proc Natl Acad Sci U S A* **117**, 18638-18648 (2020); published online EpubAug 4 (10.1073/pnas.1922595117).
38. P. Jagannathan, F. Lutwama, M. J. Boyle, F. Nankya, L. A. Farrington, T. I. McIntyre, K. Bowen, K. Naluwu, M. Nalubega, K. Musinguzi, E. Sikyomu, R. Budker, A. Katureebe, J. Rek, B. Greenhouse, G. Dorsey, M. R. Kamya, M. E. Feeney, Vdelta2+ T cell response to malaria correlates with protection from infection but is attenuated with repeated exposure. *Sci Rep* **7**, 11487 (2017); published online EpubSep 13 (10.1038/s41598-017-10624-3).
39. L. A. Farrington, P. Jagannathan, T. I. McIntyre, H. M. Vance, K. Bowen, M. J. Boyle, F. Nankya, S. Wamala, A. Auma, M. Nalubega, E. Sikyomu, K. Naluwu, V. Bigira, J. Kapisi, G. Dorsey, M. R. Kamya, M. E. Feeney, Frequent Malaria Drives Progressive Vdelta2 T-Cell Loss, Dysfunction, and CD16 Up-regulation During Early Childhood. *J Infect Dis* **213**, 1483-1490 (2016); published online EpubMay 1 (10.1093/infdis/jiv600).
40. P. Jagannathan, C. C. Kim, B. Greenhouse, F. Nankya, K. Bowen, I. Eccles-James, M. K. Muhindo, E. Arinaitwe, J. W. Tappero, M. R. Kamya, G. Dorsey, M. E. Feeney, Loss and dysfunction of Vdelta2(+) gammadelta T cells are associated with clinical tolerance to malaria. *Sci Transl Med* **6**, 251ra117 (2014); published online EpubAug 27 (10.1126/scitranslmed.3009793).

41. K. M. Wragg, H. X. Tan, A. B. Kristensen, C. V. Nguyen-Robertson, A. D. Kelleher, M. S. Parsons, A. K. Wheatley, S. P. Berzins, D. G. Pellicci, S. J. Kent, J. A. Juno, High CD26 and Low CD94 Expression Identifies an IL-23 Responsive Vdelta2(+) T Cell Subset with a MAIT Cell-like Transcriptional Profile. *Cell Rep* **31**, 107773 (2020); published online EpubJun 16 (10.1016/j.celrep.2020.107773).
42. T. Rutishauser, M. Lepore, D. Di Blasi, J. P. Dangy, S. Abdulla, S. Jongo, K. Ramadhani, B. K. L. Sim, S. L. Hoffman, M. Tanner, C. Daubenberger, G. De Libero, Activation of TCR Vdelta1(+) and Vdelta1(-)Vdelta2(-) gammadelta T Cells upon Controlled Infection with Plasmodium falciparum in Tanzanian Volunteers. *J Immunol* **204**, 180-191 (2020); published online EpubJan 1 (10.4049/jimmunol.1900669).
43. M. Goodier, P. Fey, K. Eichmann, J. Langhorne, Human peripheral blood gamma delta T cells respond to antigens of Plasmodium falciparum. *Int Immunol* **4**, 33-41 (1992); published online EpubJan (10.1093/intimm/4.1.33).
44. A. C. Hayday, gammadelta T Cell Update: Adaptate Orchestrators of Immune Surveillance. *J Immunol* **203**, 311-320 (2019); published online EpubJul 15 (10.4049/jimmunol.1800934).
45. H. Kaminski, C. Menard, B. El Hayani, A. N. Adjibabi, G. Marseres, M. Courant, A. Zouine, V. Pitard, I. Garrigue, S. Burrel, J. F. Moreau, L. Couzi, J. Visentin, P. Merville, J. Dechanet-Merville, Characterization of a unique gammadelta T cell subset as a specific marker of CMV infection severity. *J Infect Dis*, (2020); published online EpubJul 5 (10.1093/infdis/jiaa400).
46. M. Schmaler, N. Orlova-Fink, T. Rutishauser, S. Abdulla, C. Daubenberger, Human unconventional T cells in Plasmodium falciparum infection. *Semin Immunopathol* **42**, 265-277 (2020); published online EpubJun (10.1007/s00281-020-00791-3).
47. C. Behr, P. Dubois, Preferential expansion of V gamma 9 V delta 2 T cells following stimulation of peripheral blood lymphocytes with extracts of Plasmodium falciparum. *Int Immunol* **4**, 361-366 (1992); published online EpubMar (10.1093/intimm/4.3.361).
48. M. A. Hernandez-Castaneda, K. Happ, F. Cattalani, A. Wallimann, M. Blanchard, I. Fellay, B. Scolari, N. Lannes, S. Mbagwu, B. Fellay, L. Filgueira, P. Y. Mantel, M. Walch, gammadelta T Cells Kill Plasmodium falciparum in a Granzyme- and Granulysin-Dependent Mechanism during the Late Blood Stage. *J Immunol* **204**, 1798-1809 (2020); published online EpubApr 1 (10.4049/jimmunol.1900725).
49. R. A. Seder, L. J. Chang, M. E. Enama, K. L. Zephir, U. N. Sarwar, I. J. Gordon, L. A. Holman, E. R. James, P. F. Billingsley, A. Gunasekera, A. Richman, S. Chakravarty, A. Manoj, S. Velmurugan, M. Li, A. J. Ruben, T. Li, A. G. Eappen, R. E. Stafford, S. H. Plummer, C. S. Hendel, L. Novik, P. J. Costner, F. H. Mendoza, J. G. Saunders, M. C. Nason, J. H. Richardson, J. Murphy, S. A. Davidson, T. L. Richie, M. Sedegah, A. Sutamihardja, G. A. Fahle, K. E. Lyke, M. B. Laurens, M. Roederer, K. Tewari, J. E. Epstein, B. K. Sim, J. E. Ledgerwood, B. S. Graham, S. L. Hoffman, V. R. C. S. Team, Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science* **341**, 1359-1365 (2013); published online EpubSep 20 (10.1126/science.1241800).
50. C. R. Willcox, M. S. Davey, B. E. Willcox, Development and Selection of the Human Vgamma9Vdelta2(+) T-Cell Repertoire. *Front Immunol* **9**, 1501 (2018)10.3389/fimmu.2018.01501).
51. C. D. Pauza, C. Cairo, Evolution and function of the TCR Vgamma9 chain repertoire: It's good to be public. *Cell Immunol* **296**, 22-30 (2015); published online EpubJul (10.1016/j.cellimm.2015.02.010).
52. T. Dimova, M. Brouwer, F. Gosselin, J. Tassignon, O. Leo, C. Donner, A. Marchant, D. Vermijlen, Effector Vgamma9Vdelta2 T cells dominate the human fetal gammadelta T-cell repertoire. *Proc Natl Acad Sci U S A* **112**, E556-565 (2015); published online EpubFeb 10 (10.1073/pnas.1412058112).
53. L. Hviid, B. D. Akanmori, S. Loizon, J. A. Kurtzhals, C. H. Ricke, A. Lim, K. A. Koram, F. K. Nkrumah, O. Mercereau-Puijalon, C. Behr, High frequency of circulating gamma delta T cells with dominance of the v(delta)1 subset in a healthy population. *Int Immunol* **12**, 797-805 (2000); published online EpubJun (10.1093/intimm/12.6.797).
54. K. Deroost, J. Langhorne, Gamma/Delta T Cells and Their Role in Protection Against Malaria. *Front Immunol* **9**, 2973 (2018)10.3389/fimmu.2018.02973).
55. K. D. Omilusik, A. W. Goldrath, The origins of memory T cells. *Nature* **552**, 337-339 (2017); published online EpubDec 21 (10.1038/d41586-017-08280-8).
56. M. S. Davey, C. R. Willcox, A. T. Baker, S. Hunter, B. E. Willcox, Recasting Human Vdelta1 Lymphocytes in an Adaptive Role. *Trends Immunol* **39**, 446-459 (2018); published online EpubJun (10.1016/j.it.2018.03.003).
57. B. Falini, L. Flenghi, S. Pileri, P. Pelicci, M. Fagioli, M. F. Martelli, L. Moretta, E. Ciccone, Distribution of T cells bearing different forms of the T cell receptor gamma/delta in normal and pathological human tissues. *J Immunol* **143**, 2480-2488 (1989); published online EpubOct 15 (

58. S. Kho, L. Qotrunnada, L. Leonardo, B. Andries, P. A. I. Wardani, A. Fricot, B. Henry, D. Hardy, N. I. Margyaningsih, D. Apriyanti, A. M. Puspitasari, P. Prayoga, L. Trianty, E. Kenangalem, F. Chretien, I. Safeukui, H. A. Del Portillo, C. Fernandez-Becerra, E. Meibalan, M. Marti, R. N. Price, T. Woodberry, P. A. Ndour, B. M. Russell, T. W. Yeo, G. Minigo, R. Noviyanti, J. R. Poespoprodjo, N. C. Siregar, P. A. Buffet, N. M. Anstey, Hidden Biomass of Intact Malaria Parasites in the Human Spleen. *N Engl J Med* **384**, 2067-2069 (2021); published online EpubMay 27 (10.1056/NEJMc2023884).
59. B. E. Willcox, F. Mohammed, C. R. Willcox, gammadelta TCR Recognition of MR1: Adapting to Life on the Flip Side. *Trends Biochem Sci* **45**, 551-553 (2020); published online EpubJul (10.1016/j.tibs.2020.03.012).
60. K. Marsh, S. Kinyanjui, Immune effector mechanisms in malaria. *Parasite Immunol* **28**, 51-60 (2006); published online EpubJan-Feb (10.1111/j.1365-3024.2006.00808.x).
61. B. Silva-Santos, K. Serre, H. Norell, gammadelta T cells in cancer. *Nat Rev Immunol* **15**, 683-691 (2015); published online EpubNov (10.1038/nri3904).
62. G. Peng, H. Y. Wang, W. Peng, Y. Kiniwa, K. H. Seo, R. F. Wang, Tumor-infiltrating gammadelta T cells suppress T and dendritic cell function via mechanisms controlled by a unique toll-like receptor signaling pathway. *Immunity* **27**, 334-348 (2007); published online EpubAug (10.1016/j.immuni.2007.05.020).
63. G. Bhagat, A. J. Naiyer, J. G. Shah, J. Harper, B. Jabri, T. C. Wang, P. H. Green, J. S. Manavalan, Small intestinal CD8+TCRgammadelta+NKG2A+ intraepithelial lymphocytes have attributes of regulatory cells in patients with celiac disease. *J Clin Invest* **118**, 281-293 (2008); published online EpubJan (10.1172/JCI30989).
64. G. M. Siegers, L. S. Lamb, Jr., Cytotoxic and regulatory properties of circulating Vdelta1+ gammadelta T cells: a new player on the cell therapy field? *Mol Ther* **22**, 1416-1422 (2014); published online EpubAug (10.1038/mt.2014.104).
65. L. A. Farrington, P. C. Callaway, H. M. Vance, K. Baskevitch, E. Lutz, L. Warrier, T. I. McIntyre, R. Budker, P. Jagannathan, F. Nankya, K. Musinguzi, M. Nalubega, E. Sikyomu, K. Naluwu, E. Arinaitwe, G. Dorsey, M. R. Kamya, M. E. Feeney, Opsonized antigen activates Vdelta2+ T cells via CD16/FCgammaRIIIa in individuals with chronic malaria exposure. *PLoS Pathog* **16**, e1008997 (2020); published online EpubOct (10.1371/journal.ppat.1008997).

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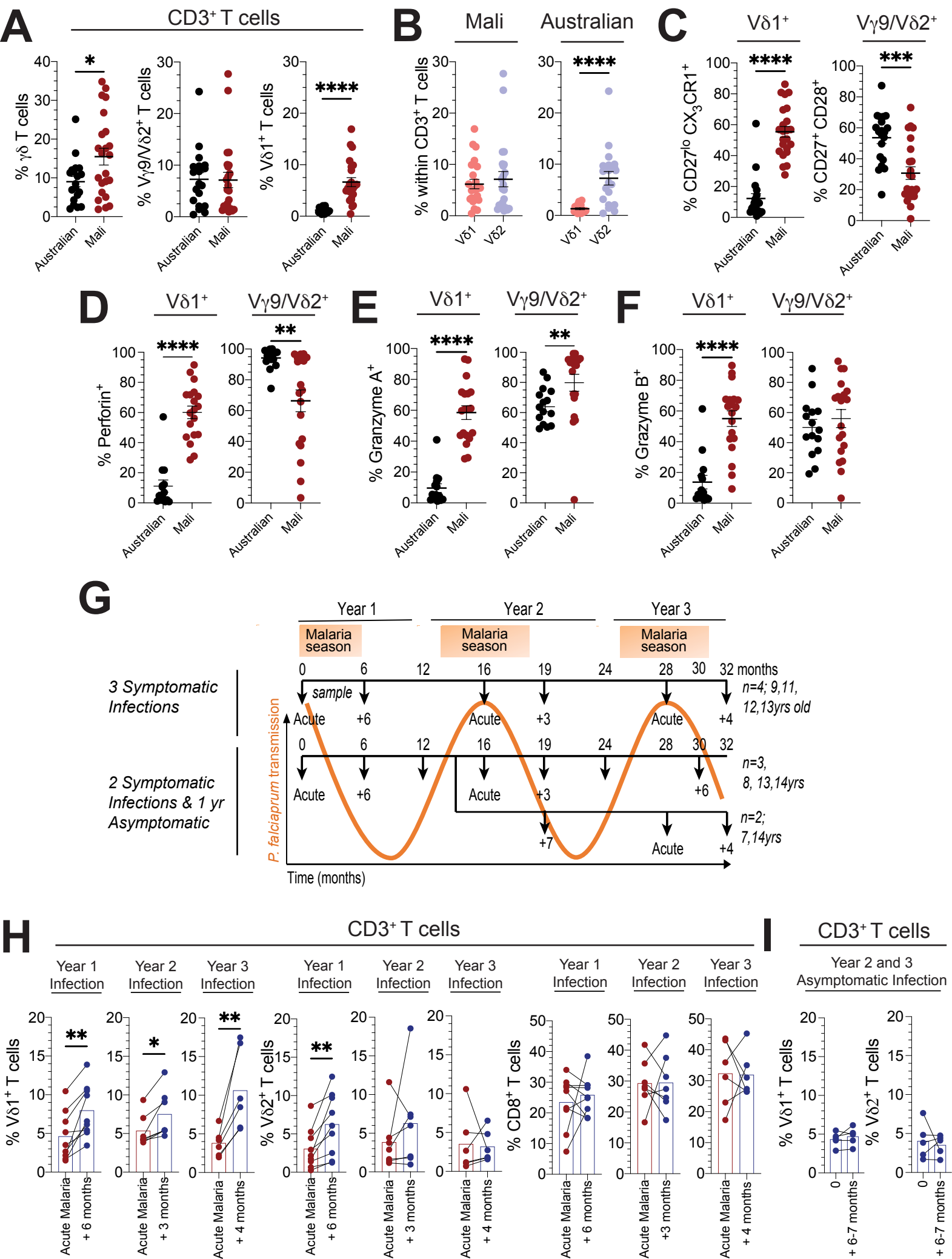
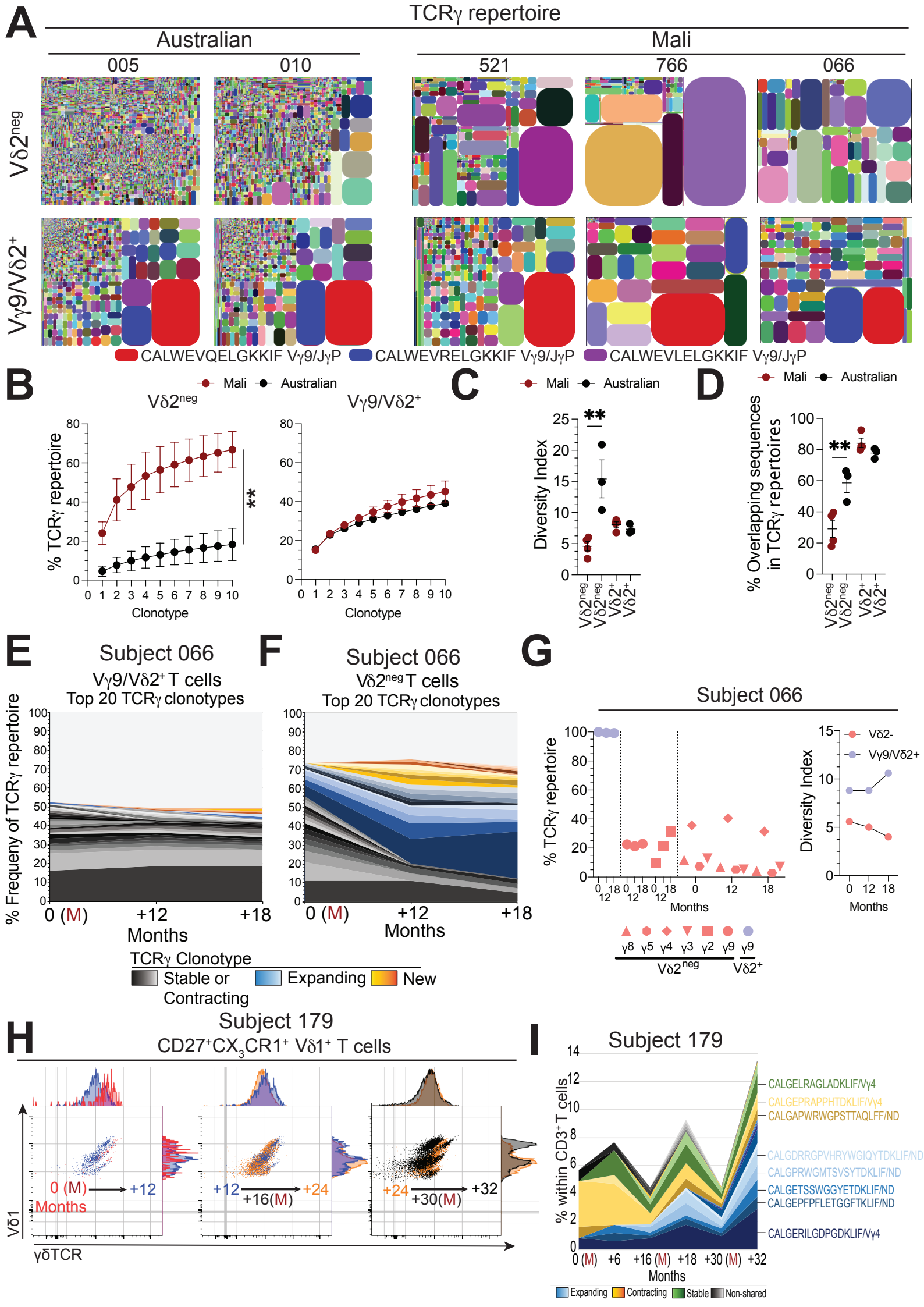
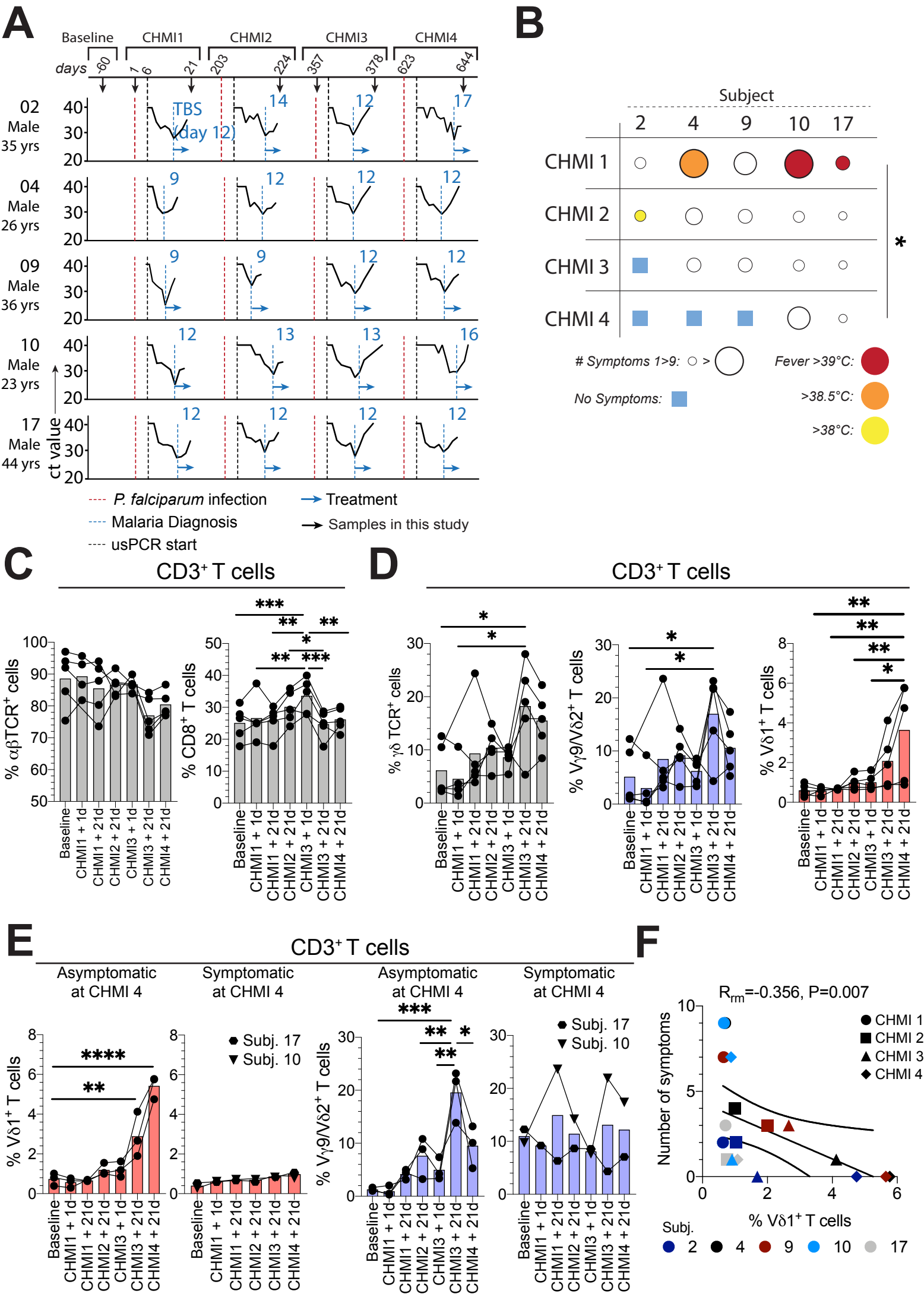
Disclaimers: The opinions and assertions expressed herein are those of the author(s) and do not necessarily reflect the official policy or position of the Uniformed Services University or the Department of Defense.

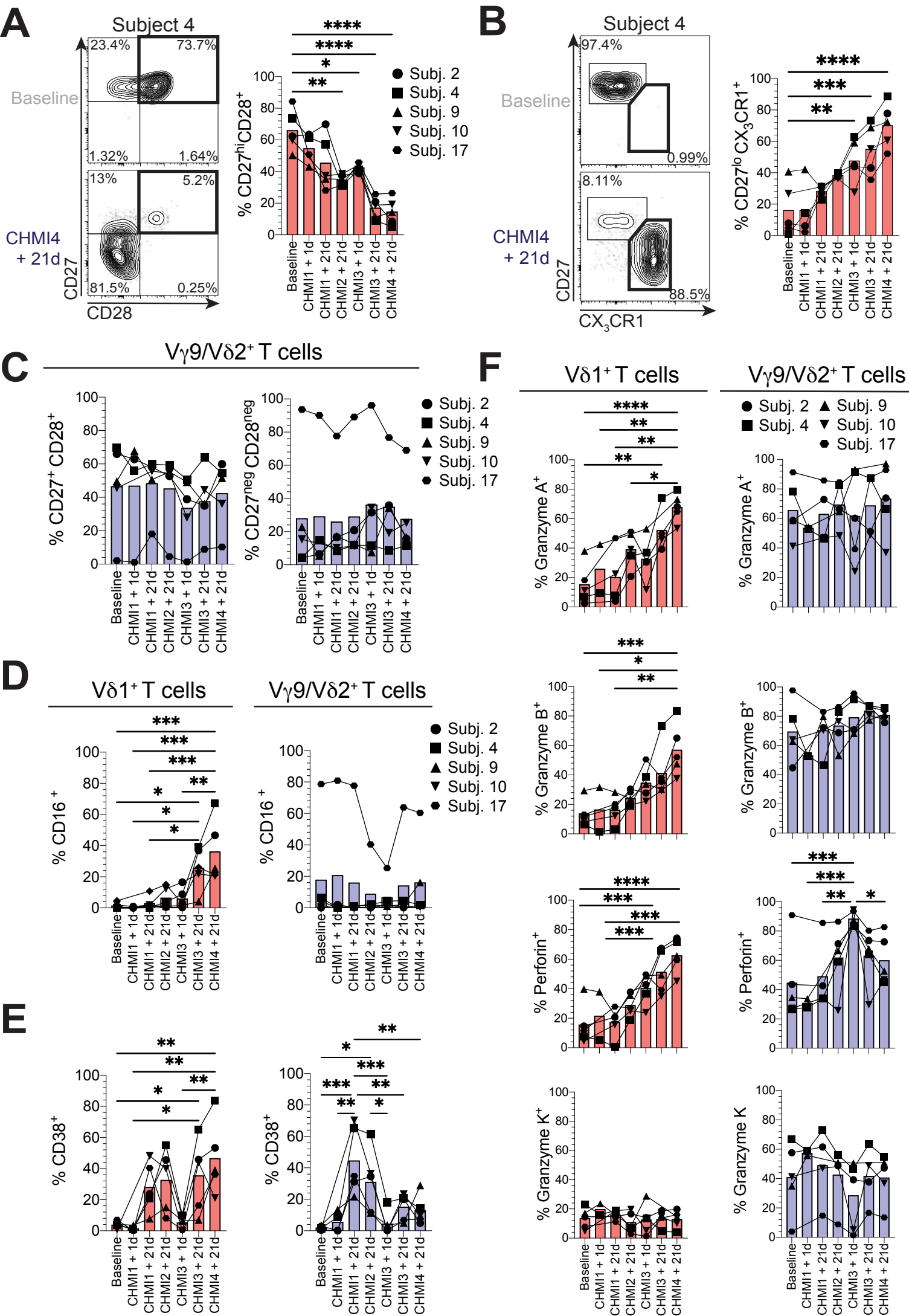
Competing interests: The authors declare no competing interests.

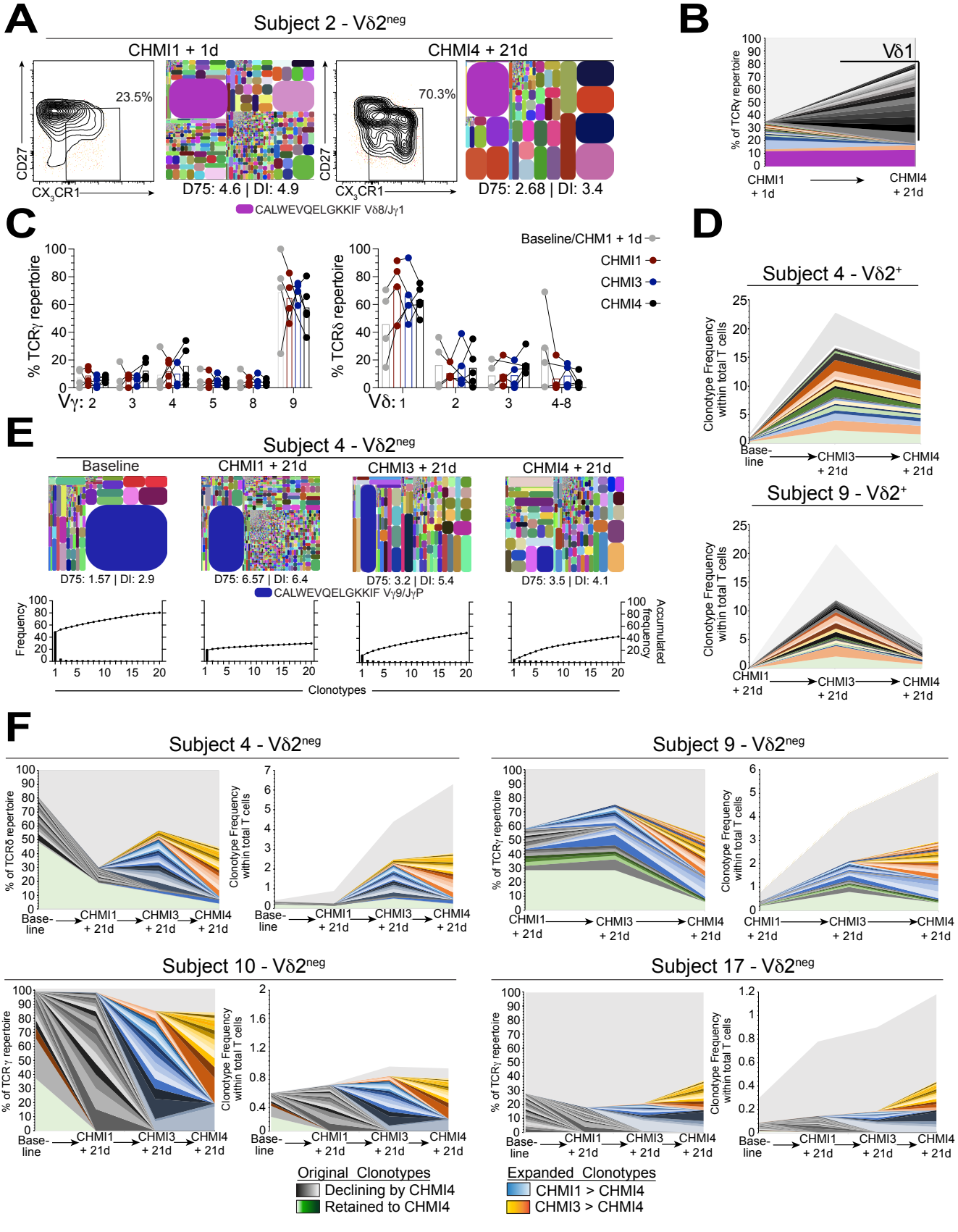
Data and materials availability: All data are available in Data File S1. The γδ T cell receptor (TCR) sequence data that support the findings of this study have been deposited in the sequence read archive (SRA) under the BioProject Accession Number PRJNA770107.

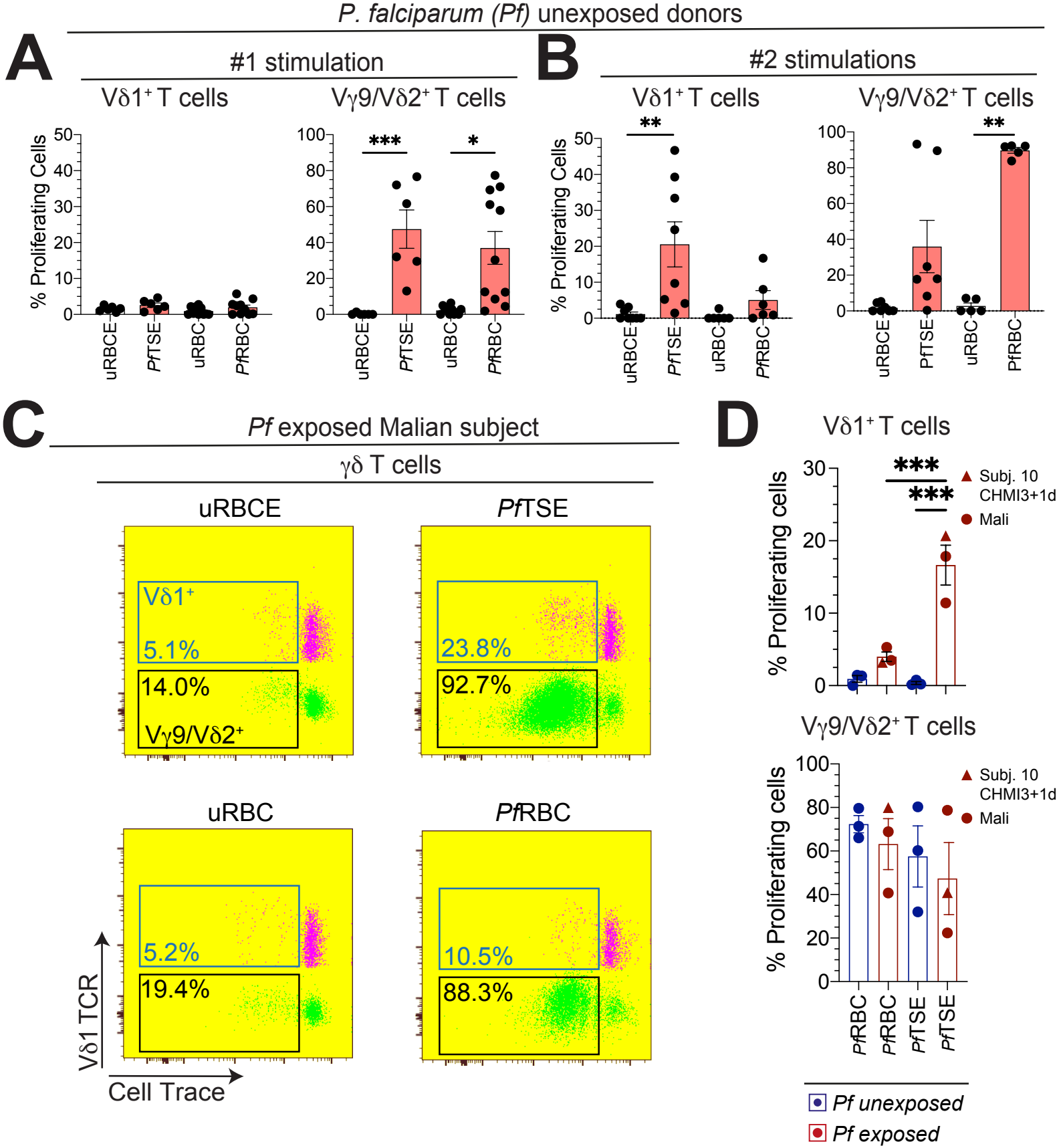

A
CD3+ T cells
% γδ T cells
% Vγ9/Vδ2+ T cells
% Vδ1+ T cells
Australian
Mali
B
Mali
Australian
% within CD3+ T cells
Vδ1
Vδ2
C
Vδ1+
Vγ9/Vδ2+
% CD27lo CX3CR1+
% CD27+ CD28+
D
% Perforin+
E
% Granzyme A+
F
% Grazyme B+
G
Year 1
Year 2
Year 3
Malaria season
3 Symptomatic Infections
2 Symptomatic Infections & 1 yr Asymptomatic
sample
Acute
+6
+3
+4
+7
n=4; 9,11, 12,13yrs old
n=3, 8, 13,14yrs
n=2; 7,14yrs
P. falciaprum transmission
Time (months)
H
CD3+ T cells
Year 1 Infection
Year 2 Infection
Year 3 Infection
% Vδ1+ T cells
% Vδ2+ T cells
% CD8+ T cells
Acute Malaria
+ 6 months
+ 3 months
+ 4 months
I
CD3+ T cells
Year 2 and 3 Asymptomatic Infection
+ 6-7 months


A
TCRγ repertoire
Australian
Mali
005
010
521
766
066
Vδ2neg
Vγ9/Vδ2+
CALWEVQELGKKIF Vγ9/JγP
CALWEVRELGKKIF Vγ9/JγP
CALWEVLELGKKIF Vγ9/JγP
B
Mali
Australian
Vδ2neg
Vγ9/Vδ2+
% TCRγ repertoire
Clonotype
**
C
Diversity Index
D
% Overlapping sequences in TCRγ repertoires
E
Subject 066
Vγ9/Vδ2+ T cells
Top 20 TCRγ clonotypes
% Frequeny of TCRγ repertoire
0 (M)
+12
+18
Months
F
Subject 066
Vδ2neg T cells
Top 20 TCRγ clonotypes
G
Subject 066
% TCRγ repertoire
Diversity Index
Vδ2-
Vγ9/Vδ2+
γ8 γ5 γ4 γ3 γ2 γ9 γ9
Vδ2neg
Vδ2+
TCRγ Clonotype
Stable or Contracting
Expanding
New
H
Subject 179
CD27+CX3CR1+ Vδ1+ T cells
Vδ1
γδTCR
0 (M)
+12
+16(M)
+24
+30(M)
+32
I
Subject 179
% within CD3+ T cells
CALGELRAGLADKLIF/Vγ4
CALGEPRAPPHTDKLIF/Vγ4
CALGAPWRWGPSTTAQLFF/ND
CALGDRRGPVHRYWGIQYTDKLIF/ND
CALGPRWGMTSVSYTDKLIF/ND
CALGETSSWGGYETDKLIF/ND
CALGEPFPFLETGGFTKLIF/ND
CALGERILGDPGDKLIF/Vγ4
Expanding
Contracting
Stable
Non-shared


A
Baseline
CHMI1
CHMI2
CHMI3
CHMI4
days
-60
1
6
21
203
224
357
378
623
644
02
Male
35 yrs
04
Male
26 yrs
09
Male
36 yrs
10
Male
23 yrs
17
Male
44 yrs
ct value
TBS
(day 12)
P. falciparum infection
Malaria Diagnosis
usPCR start
Treatment
Samples in this study
B
Subject
2
4
9
10
17
CHMI 1
CHMI 2
CHMI 3
CHMI 4
*
Symptoms 1>9:
No Symptoms:
Fever >39°C:
>38.5°C:
>38°C:
C
CD3+ T cells
% αβTCR+ cells
% CD8+ T cells
D
CD3+ T cells
% γδ TCR+ cells
% Vγ9/Vδ2+ T cells
% Vδ1+ T cells
E
CD3+ T cells
Asymptomatic at CHMI 4
Symptomatic at CHMI 4
Asymptomatic at CHMI 4
Symptomatic at CHMI 4
Subj. 17
Subj. 10
% Vδ1+ T cells
% Vγ9/Vδ2+ T cells
F
Rrm=-0.356, P=0.007
Number of symptoms
% Vδ1+ T cells
CHMI 1
CHMI 2
CHMI 3
CHMI 4
Subj.
2
4
9
10
17


A
Subject 4
23.4%
73.7%
Baseline
1.32%
1.64%
13%
5.2%
CHMI4
+ 21d
81.5%
0.25%
CD27
CD28
% CD27hiCD28+
Subj. 2
Subj. 4
Subj. 9
Subj. 10
Subj. 17
Baseline
CHMI1 + 1d
CHMI1 + 21d
CHMI2 + 21d
CHMI3 + 1d
CHMI3 + 21d
CHMI4 + 21d
B
Subject 4
97.4%
Baseline
0.99%
8.11%
CHMI4
+ 21d
88.5%
CD27
CX3CR1
% CD27loCX3CR1+
C
Vγ9/Vδ2+ T cells
% CD27+CD28+
% CD27negCD28neg
D
Vδ1+ T cells
Vγ9/Vδ2+ T cells
% CD16+
E
% CD38+
F
Vδ1+ T cells
Vγ9/Vδ2+ T cells
% Granzyme A+
% Granzyme B+
% Perforin+
% Granzyme K+
% Granzyme K


A
Subject 2 - Vδ2neg
CHMI1 + 1d
CHMI4 + 21d
23.5%
70.3%
CD27
CX3CR1
D75: 4.6 | DI: 4.9
D75: 2.68 | DI: 3.4
CALWEVQELGKKIF Vδ8/Jγ1
B
Vδ1
% of TCRγ repertoire
CHMI1 + 1d
CHMI4 + 21d
C
Baseline/CHM1 + 1d
CHMI1
CHMI3
CHMI4
% TCRγ repertoire
% TCRδ repertoire
Vγ: 2 3 4 5 8 9
Vδ: 1 2 3 4-8
D
Subject 4 - Vδ2+
Clonotype Frequency within total T cells
Base-line
CHMI3 + 21d
CHMI4 + 21d
Subject 9 - Vδ2+
CHMI1 + 21d
E
Subject 4 - Vδ2neg
Baseline
CHMI1 + 21d
CHMI3 + 21d
CHMI4 + 21d
D75: 1.57 | DI: 2.9
D75: 6.57 | DI: 6.4
D75: 3.2 | DI: 5.4
D75: 3.5 | DI: 4.1
CALWEVQELGKKIF Vγ9/JγP
Frequency
Accumulated frequency
Clonotypes
F
Subject 4 - Vδ2neg
Subject 9 - Vδ2neg
Subject 10 - Vδ2neg
Subject 17 - Vδ2neg
% of TCRδ repertoire
% of TCRγ repertoire
Clonotype Frequency within total T cells
Original Clonotypes
Declining by CHMI4
Retained to CHMI4
Expanded Clonotypes
CHMI1 > CHMI4
CHMI3 > CHMI4


P. falciparum (Pf) unexposed donors
A
#1 stimulation
Vδ1+ T cells
Vγ9/Vδ2+ T cells
% Proliferating Cells
uRBCE
PfTSE
uRBC
PfRBC
***
*
B
#2 stimulations
**
C
Pf exposed Malian subject
γδ T cells
uRBCE
PfTSE
uRBC
PfRBC
Vδ1+
5.1%
14.0%
Vγ9/Vδ2+
23.8%
92.7%
5.2%
19.4%
10.5%
88.3%
Vδ1 TCR
Cell Trace
D
Vδ1+ T cells
% Proliferating cells
Subj. 10 CHMI3+1d
Mali
Vγ9/Vδ2+ T cells
Pf unexposed
Pf exposed

Supplemental Materials

Repeated *Plasmodium falciparum* infection in humans drives the clonal expansion of an adaptive γδ T cell repertoire

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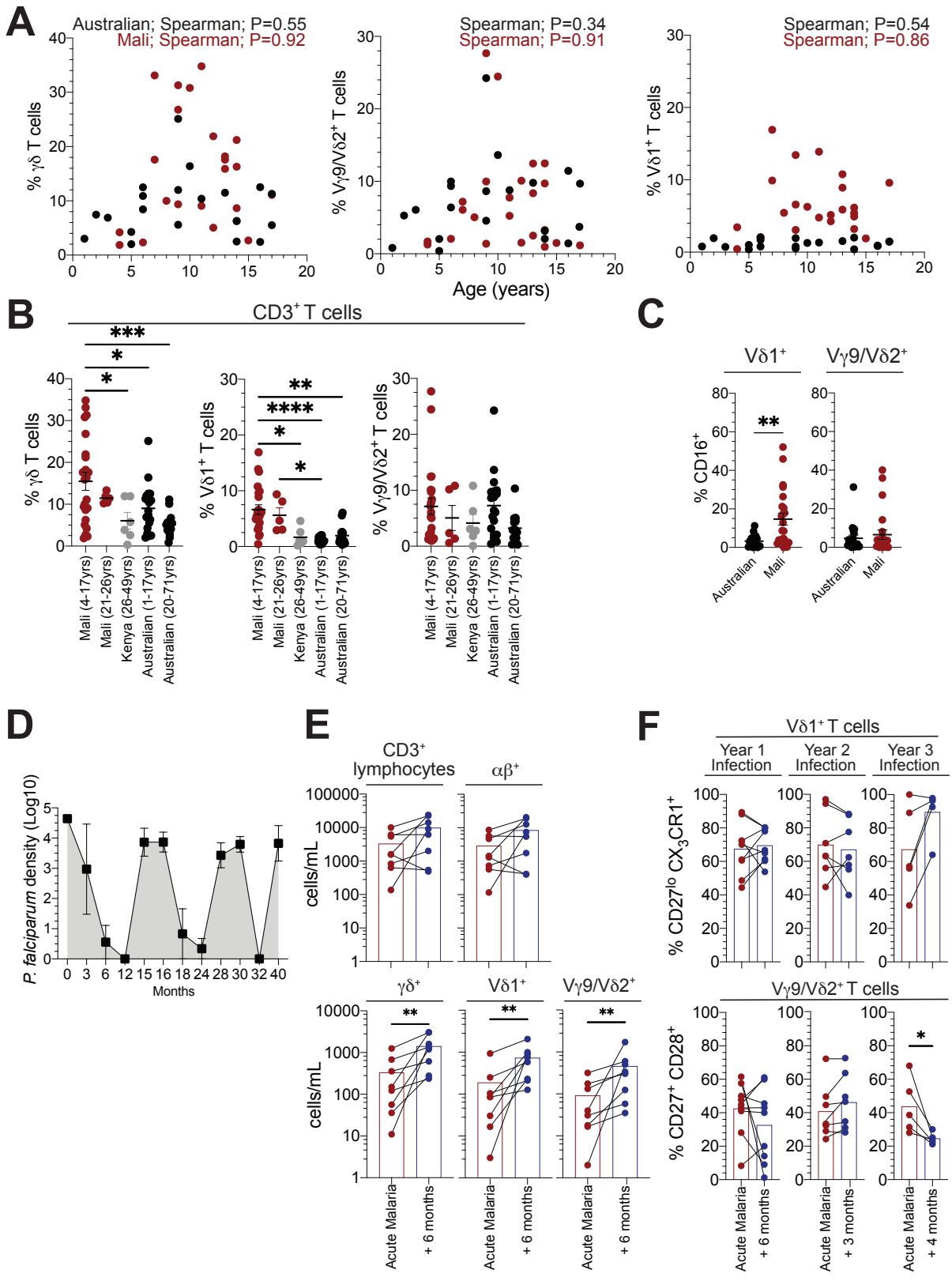
A
Australian; Spearman; P=0.55
Mali; Spearman; P=0.92
% γδ T cells
Spearman; P=0.34
Spearman; P=0.91
% Vγ9/Vδ2+ T cells
Spearman; P=0.54
Spearman; P=0.86
% Vδ1+ T cells
Age (years)
B
CD3+ T cells
% γδ T cells
% Vδ1+ T cells
% Vγ9/Vδ2+ T cells
Mali (4-17yrs)
Mali (21-26yrs)
Kenya (26-49yrs)
Australian (1-17yrs)
Australian (20-71yrs)
C
Vδ1+
Vγ9/Vδ2+
% CD16+
Australian
Mali
D
P. falciparum density (Log10)
Months
E
CD3+ lymphocytes
αβ+
cells/mL
γδ+
Vδ1+
Vγ9/Vδ2+
Acute Malaria
+ 6 months
F
Vδ1+ T cells
Year 1 Infection
Year 2 Infection
Year 3 Infection
% CD27lo CX3CR1+
Vγ9/Vδ2+ T cells
% CD27+ CD28+
+ 3 months
+ 4 months

Figure S1. T cell frequencies in Malian subjects exposed to *P. falciparum* infection. **A.** Frequencies of total γδ, Vγ9/Vδ2$^+$ and Vδ1$^+$ T cells within CD3$^+$ T cells versus age. **B.** Frequencies of total γδ, Vγ9/Vδ2$^+$ and Vδ1$^+$ T cells within CD3$^+$ T cells in children and adults from Mali, Kenya and Australia (details in **Table S1**). **C.** *P. falciparum* density in Malian subjects over 40 months (n=5). **D.** Frequencies of CD16$^+$ cells within Vδ1$^+$ or Vγ9/Vδ2$^+$ T cells in Australian (n=20) or Malian (n=23) children. **E.** Absolute cell counts of CD3$^+$ lymphocytes, αβ$^+$, γδ$^+$, Vγ9/Vδ2$^+$ and Vδ1$^+$ T cells (n=8; from a subset of subjects in year 1). **F.** Frequencies of CD27^{lo} CX$_3$CR1$^+$ cells in Vδ1$^+$ or CD27$^+$ CD28$^+$ cells in Vδ2$^+$ T cells over three years of seasonal malaria transmission and episodes of acute malaria. Normality was tested using the Shapiro-Wilk test. Error bars indicate means ± SEM and bars indicate mean; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; P-values were determined by Spearman correlation (**a**), Kruskal-Wallis test with Dunn's post hoc testing (**b**) and Mann-Whitney test (**d, e, f**).

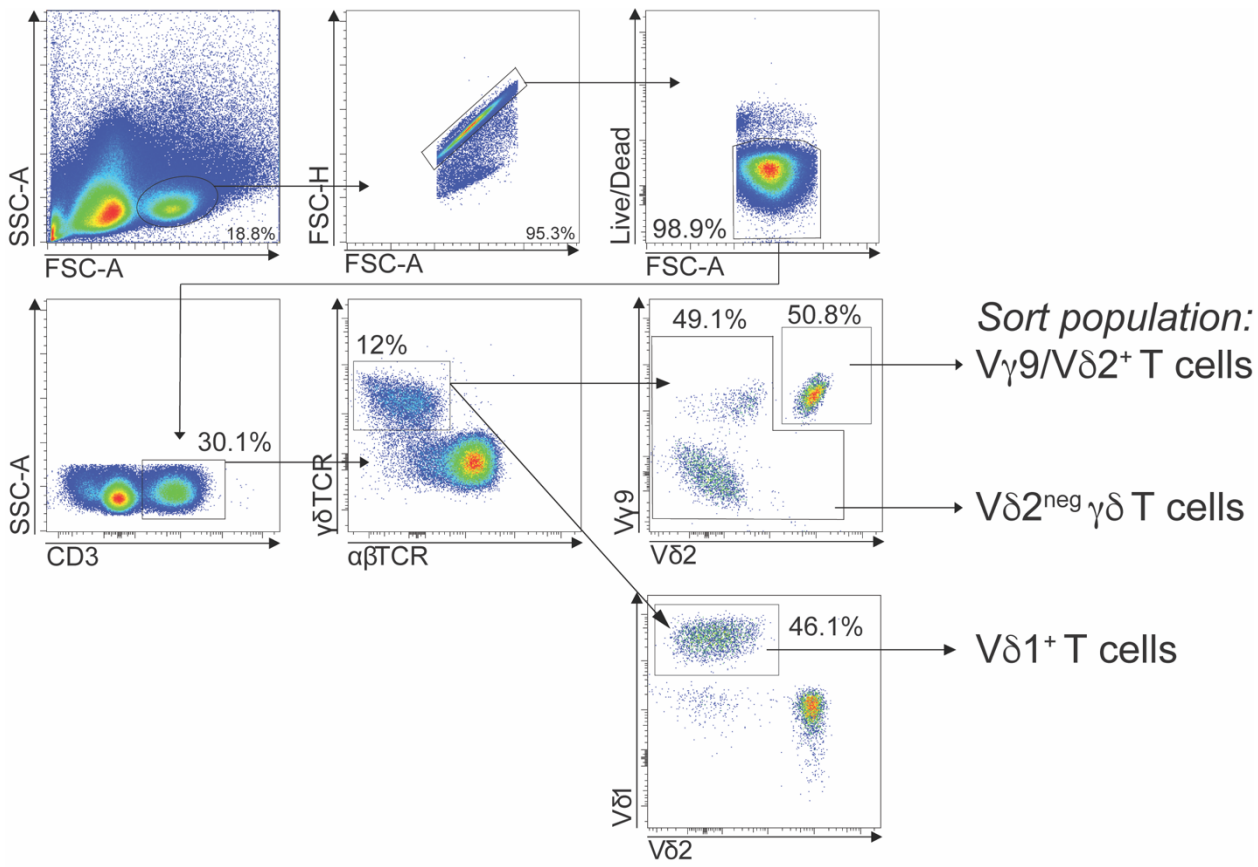

Figure S2. The gating strategy used to sort γδ T cells. The gating strategy used to identify and sort γδ T cell subsets shown in a representative donor. Lymphocytes were gated using the FSC-A/SSC-A, doublets were excluded using FSC-A/FSC-H, viable cells gated in the FSC-A/Live/Dead and then viable $CD3^+$ T cells identified using the CD3/SSC-A plot. To sort γδ T cell populations, viable $CD3^+$ T cells were gated for total γδ T cells using an αβTCR/γδTCR plot and then within the γδ T cell population, we gated on $V\delta1^+$ T cells using Vδ2/Vδ1 and $V\delta2^+$ or $V\delta2^{neg}$ T cells identified using a Vδ2/Vγ9 plot (antibody panels are detailed in **Table S3**).

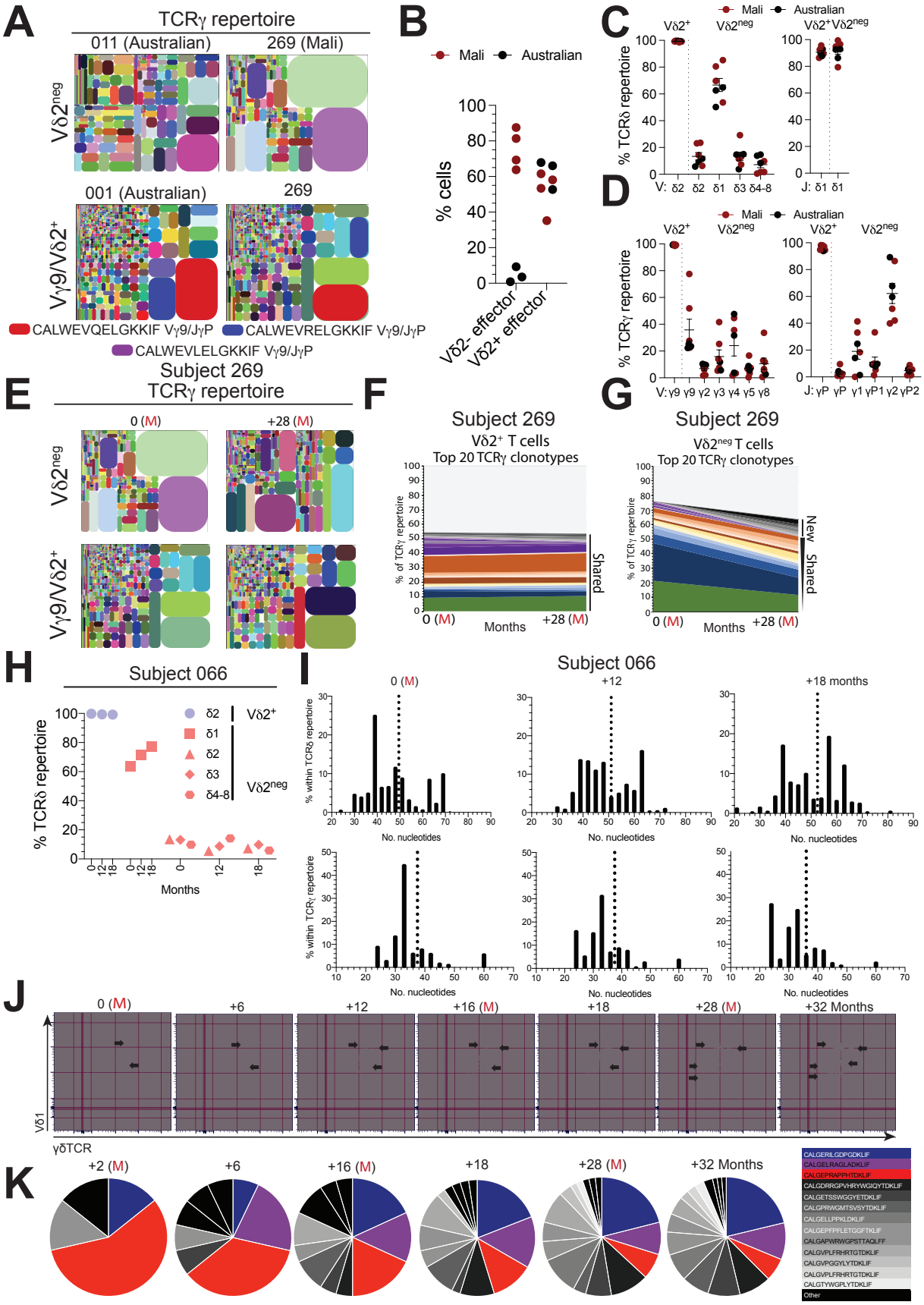
A
TCRγ repertoire
011 (Australian)
269 (Mali)
001 (Australian)
269
Vδ2neg
Vγ9/Vδ2+
CALWEVQELGKKIF Vγ9/JγP
CALWEVRELGKKIF Vγ9/JγP
CALWEVLELGKKIF Vγ9/JγP
B
Mali
Australian
% cells
Vδ2- effector
Vδ2+ effector
C
Mali
Australian
Vδ2+
Vδ2neg
Vδ2+Vδ2neg
% TCRδ repertoire
V: δ2 δ2 δ1 δ3 δ4-8
J: δ1 δ1
D
% TCRγ repertoire
V: γ9 γ9 γ2 γ3 γ4 γ5 γ8
J: γP γP γ1 γP1 γ2 γP2
E
Subject 269
TCRγ repertoire
0 (M)
+28 (M)
F
Subject 269
Vδ2+ T cells
Top 20 TCRγ clonotypes
% of TCRγ repertoire
Shared
Months
G
Subject 269
Vδ2neg T cells
Top 20 TCRγ clonotypes
New
Shared
H
Subject 066
% TCRδ repertoire
δ2
δ1
δ2
δ3
δ4-8
Vδ2+
Vδ2neg
Months
I
Subject 066
0 (M)
+12
+18 months
% within TCRδ repertoire
% within TCRγ repertoire
No. nucleotides
J
0 (M)
+6
+12
+16 (M)
+18
+28 (M)
+32 Months
Vδ1
γδTCR
K
+2 (M)
+6
+16 (M)
+18
+28 (M)
+32 Months
CALGERILGDPGDKLIF
CALGELRAGLADKLIF
CALGEPRAPPHTDKLIF
CALGDRRGPVHRYWGIQYTDKLIF
CALGETSSWGGYETDKLIF
CALGPRWGMTSVSYTDKLIF
CALGELLPPKLDKLIF
CALGEPFPFLETGGFTKLIF
CALGAPWRWGPSTTAQLFF
CALGVPLFRHRTGTDKLIF
CALGVPGGYLYTDKLIF
CALGVPLFRHRTGTDKLIF
CALGTYWGPLYTDKLIF
Other

Figure S3. Longitudinal γδTCR analysis in Malian subjects. A. TCRγ clonotype tree plot analysis of $V\delta2^{neg}$ and $V\delta2^{+}$ T cell populations from Australian and Malian children. **B.** Frequency of $CD27^{lo}$ CX_3CR1^{+} effector cells in $V\delta2^{neg}$ ($V\delta2^{-}$ effector) or $CD27^{+}$ $CD28^{+}$ effector cells in $V\delta2^{+}$ T cells ($V\delta2^{+}$ effector) from Australian subject (1, 5, 10 and 11) and Malian subjects (066, 521, 766 and 269). **C.** Vδ and Jδ usage and **D.** Vγ and Jγ usage in $V\delta2^{neg}$ and $V\delta2^{+}$ T cells from Malian (red dots; n=4) and Australian (black dots; n=3) subjects. **E.** Tree plots of TCRγ repertoires and clonotype analysis from **F.** $V\delta2^{+}$ and **G.** $V\delta2^{neg}$ T cells at 0 or 28 months, from subject 269. **H.** Longitudinal Vδ chain usage in $V\delta2^{+}$ and $V\delta2^{neg}$ T cell repertoires after acute febrile malaria in Malian subject 066. **I.** Non-normalised CDR3 amino acid sequence length in TCRδ and TCRγ repertoires over time in Malian subject 066. **J.** Flow cytometry plots of γδTCR vs Vδ1 antibody staining in total $V\delta1^{+}$ γδ T cells from subject 179. Each flow cytometry plot represents a different timepoint, acute febrile malaria indicated by (M) and arrows indicate distinct populations. **K.** TCRδ single cell sequencing pie charts from $CD27^{lo}CX_3CR1^{+}$ $V\delta1^{+}$ T cells at each timepoint from subject 179.

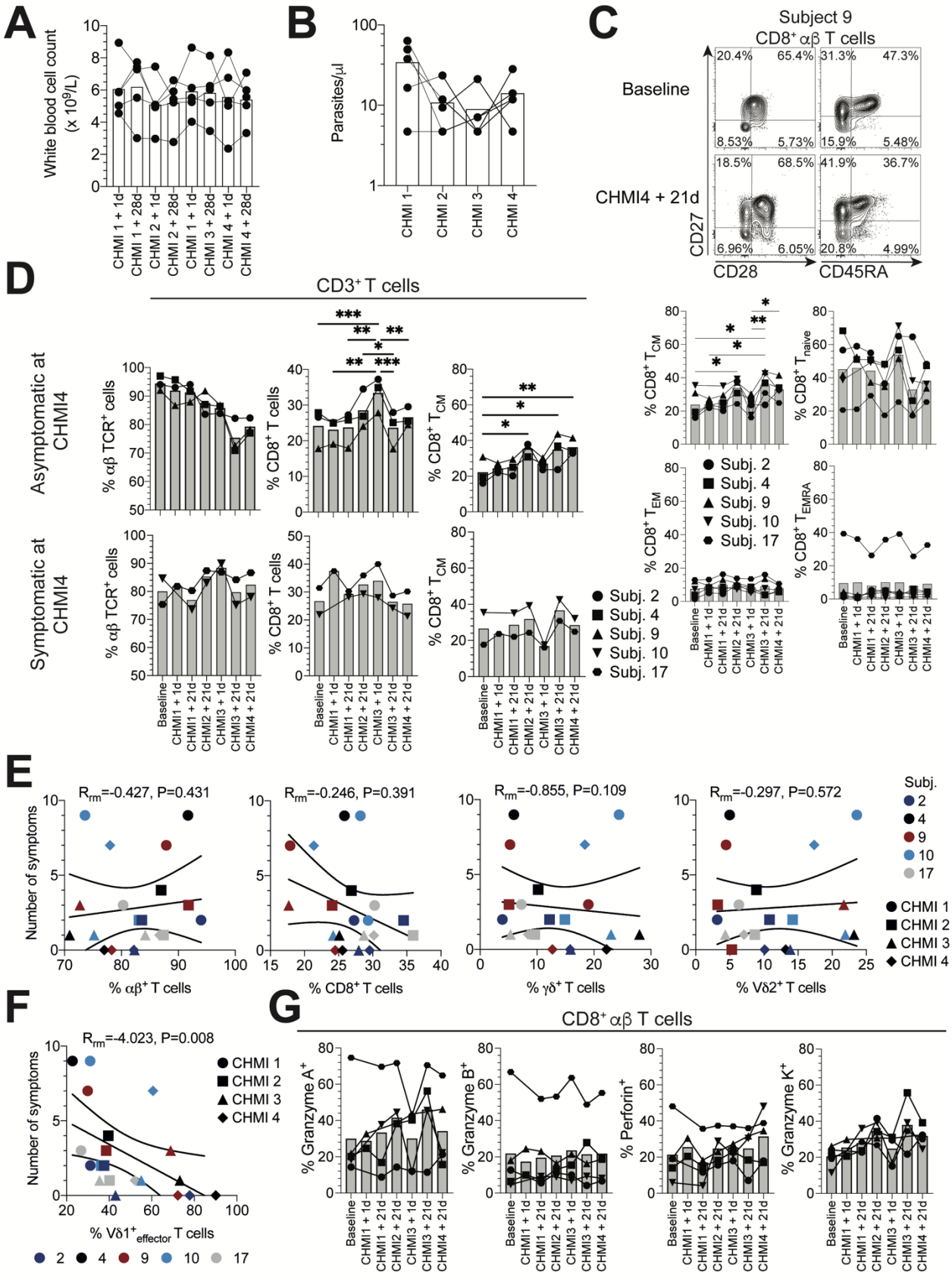
A
White blood cell count (x 10⁹/L)
CHMI 1 + 1d
CHMI 1 + 28d
CHMI 2 + 1d
CHMI 2 + 28d
CHMI 1 + 1d
CHMI 3 + 28d
CHMI 4 + 1d
CHMI 4 + 28d
B
Parasites/µl
CHMI 1
CHMI 2
CHMI 3
CHMI 4
C
Subject 9
CD8⁺ αβ T cells
Baseline
CHMI4 + 21d
20.4%
65.4%
8.53%
5.73%
31.3%
47.3%
15.9%
5.48%
18.5%
68.5%
6.96%
6.05%
41.9%
36.7%
20.8%
4.99%
CD27
CD28
CD45RA
D
CD3⁺ T cells
Asymptomatic at CHMI4
Symptomatic at CHMI4
% αβ TCR⁺ cells
% CD8⁺ T cells
% CD8⁺ TCM
% CD8⁺ Tnaive
% CD8⁺ TEM
% CD8⁺ TEMRA
Subj. 2
Subj. 4
Subj. 9
Subj. 10
Subj. 17
Baseline
CHMI1 + 1d
CHMI1 + 21d
CHMI2 + 21d
CHMI3 + 1d
CHMI3 + 21d
CHMI4 + 21d
E
Rrm=-0.427, P=0.431
Rrm=-0.246, P=0.391
Rrm=-0.855, P=0.109
Rrm=-0.297, P=0.572
Number of symptoms
% αβ⁺ T cells
% CD8⁺ T cells
% γδ⁺ T cells
% Vδ2⁺ T cells
Subj.
2
4
9
10
17
CHMI 1
CHMI 2
CHMI 3
CHMI 4
F
Rrm=-4.023, P=0.008
Number of symptoms
% Vδ1⁺effector T cells
G
CD8⁺ αβ T cells
% Granzyme A⁺
% Granzyme B⁺
% Perforin⁺
% Granzyme K⁺

Figure S4. T cell frequencies in Malian subjects exposed to *P. falciparum* infection. **A**. White blood cell counts (WBC) taken 1 day before or 28 days after each CHMI (n=5). **B.** During each CHMI, the peak parasite density measured by blood smear (n=5). **C.** Representative flow cytometry plots show memory population of $CD8^+$ T cells and graphs show frequencies of $CD8^+$ naive (T_{naive}), central memory (T_{CM}), effector memory (T_{EM}) and effector memory CD45RA-revertants (T_{EMRA}) across repeated CHMIs (n=5). **D.** Frequencies of total $\alpha\beta^+$, $\alpha\beta^+ CD8^+$ and $\alpha\beta^+ CD8^+ T_{CM}$ cells within total $CD3^+$ T cells, separated into individuals that were asymptomatic or symptomatic after CHMI4. Repeated measures correlation between **E.** total $\alpha\beta^+$, $\alpha\beta^+ CD8^+$, $V\gamma9/V\delta2^+$ T cells within $CD3^+$ T cells or **F.** $V\delta1^+ T_{effector}$ frequencies within total $V\delta1^+$ T cells, and the number of symptoms each individual suffered at each CHMI. **G.** Frequencies of $GzmA^+$, B^+, K^+ and $perforin^+$ cells within total $\alpha\beta^+ CD8^+$ T cells before and after repeated CHMIs (n=5). Error bars indicate means ± SEM and bars indicate mean. *P < 0.05; **P < 0.01; ***P < 0.001; P-values were determined by linear mixed effects modelling with Bonferroni's correction (**a, b, c, d, g**) and repeated measures correlation (**e, f**).

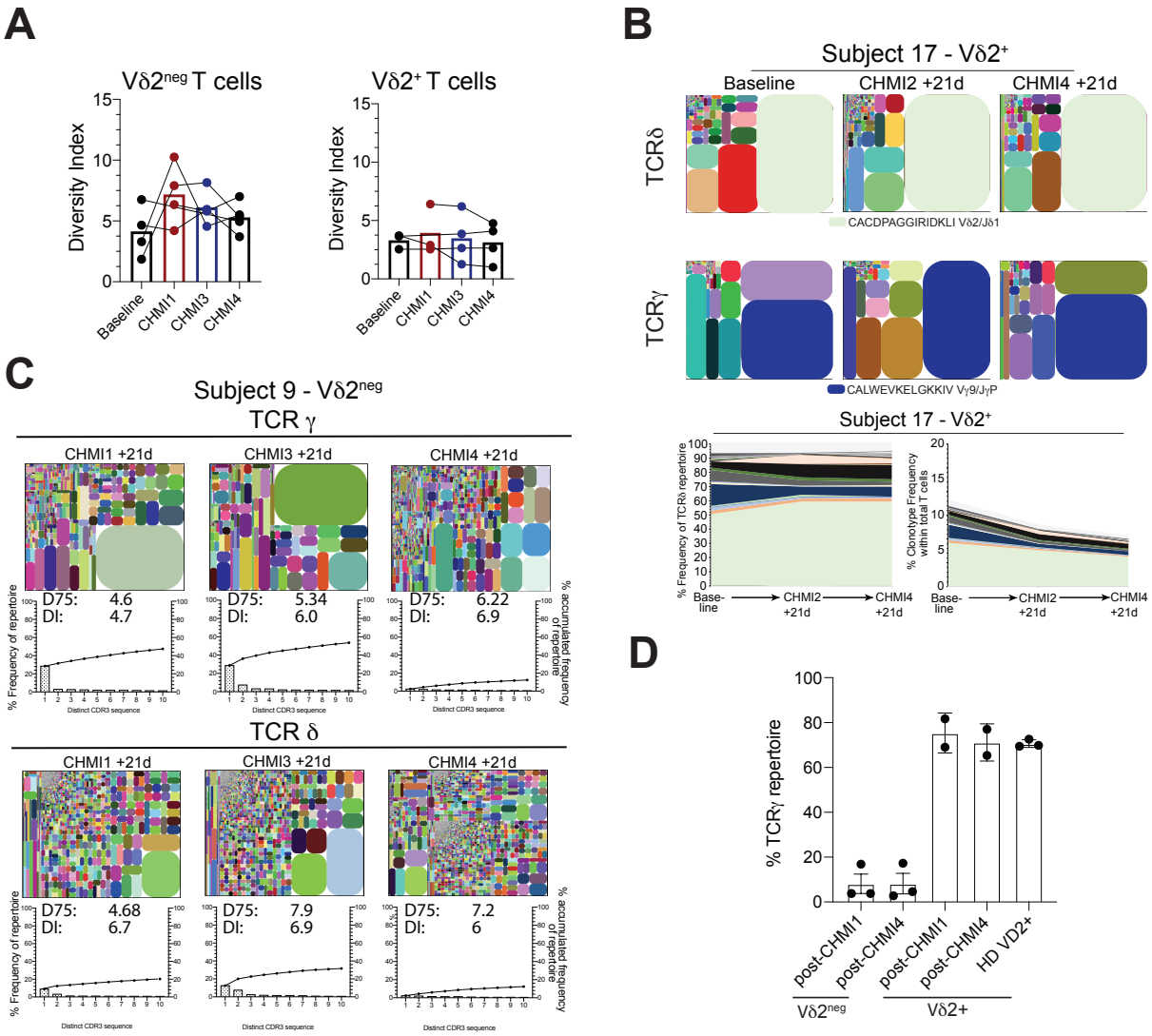
A
Vδ2neg T cells
Vδ2+ T cells
Diversity Index
Baseline
CHMI1
CHMI3
CHMI4
B
Subject 17 - Vδ2+
Baseline
CHMI2 +21d
CHMI4 +21d
TCRδ
CACDPAGGIRIDKLI Vδ2/Jδ1
TCRγ
CALWEVKELGKKIV Vγ9/JγP
% Frequency of TCRδ repertoire
% Clonotype Frequency within total T cells
C
Subject 9 - Vδ2neg
TCR γ
CHMI1 +21d
CHMI3 +21d
CHMI4 +21d
D75: 4.6
DI: 4.7
D75: 5.34
DI: 6.0
D75: 6.22
DI: 6.9
% Frequency of repertoire
% accumulated frequency of repertoire
Distinct CDR3 sequence
TCR δ
D75: 4.68
DI: 6.7
D75: 7.9
DI: 6.9
D75: 7.2
DI: 6
D
% TCRγ repertoire
post-CHMI1
post-CHMI4
post-CHMI1
post-CHMI4
HD VD2+
Vδ2neg
Vδ2+

Figure S5. Longitudinal γδTCR analysis in CHMI subjects. A. Diversity index (DI) in $V\delta2^{neg}$ and $V\delta2^{+}$ γδ T cell repertoires across CHMIs (n=3-5). **B.** Tree plot and tracking of the top 20 clonotypes in $V\gamma9/V\delta2^{+}$ TCRγ repertoires over repeated CHMIs from subject 17. **C.** Tree plot analysis and top 10 clonotype frequency after repeated CHMI from Subject 9. **D.** Sharing of sequences between independent CHMI study subjects, in $V\delta2^{neg}$ (subjects 2, 4 and 9) and $V\delta2^{+}$ T cell repertoires (subjects 4 and 9). For $V\delta2^{+}$ T cell repertoires three healthy UK donor repertoires from Davey *et al* (*24*) were used to compare the shared sequence frequency. Error bars indicate means ± SEM and bars indicate mean.

Table S1. Study cohort.

	Mali - Children	Australian - Children	Mali - Adults	Kenya - Adults	Australian - Adults
N	23	20	5	6	14
Age in years, median (range)	10 (4-17)	9 (1-17)	22 (21-26)	29 (26-49)	44 (20-71)
Gender, no. females	14	12	4	3	6

Table S2. Symptoms recorded for each CHMI.

	CHMI 1					CHMI 2					CHMI 3					CHMI 4				
Subject	2	4	9	10	17	2	4	9	10	17	2	4	9	10	17	2	4	9	10	17
Total symptoms	2	9	7	9	3	2	4	3	2	1	0	1	3	1	1	0	0	0	7	1
Total number times	3	25	12	22	5	2	15	6	5	1	0	2	7	3	2	0	0	0	14	2
Day of onset	12	9	11	8	12	14	7	9	7	12		12	12	13	12				13	8
Total subjects with symptoms			5					5					4					2		
Fever (Temp)		39		39	39	38														

Symptoms identified included: Abdominal Pain, Joint Pain, Chills, Dizziness, Headache, Malaise, Myalgia, Nausea, Vomiting.

Table S3. Flow cytometry antibodies.

Panel	Surface/intracellular staining	Target (anti-human)	Fluorochrome	Provider	Cat. No.
Surface Panel	Surface	CD3	BUV395	BD Biosciences	563546
		CD8	BV786	BD Biosciences	563823
		CD16	AF700	BioLegend	302026
		CD27	PE-Dazzle594	BioLegend	356422
		CD28	PE	BioLegend	302908
		CD38	BV605	BioLegend	303532
		αβTCR	Vioblue	Miltenyi Biotec	130-110-457
		γδTCR	APC-Vio770	Miltenyi Biotec	130-114-035
		Vδ1	FITC	Miltenyi Biotec	130-100-532
		Vδ2	APC	Miltenyi Biotec	130-095-803
		Vγ9	PC5	Beckman Coulter	A63663
		CX3CR1	PE-Cy7	BioLegend	341612
Intracellular panel	Surface	CD3	BUV395	BD Biosciences	563546
		CD8	BV786	BD Biosciences	563823
		γδTCR	PerCp-Vio700	Miltenyi Biotec	130-113-514
		Vδ1	FITC	Miltenyi Biotec	130-100-532
		Vδ2	APC	Miltenyi Biotec	130-095-803
		Vγ9	PC5	Beckman Coulter	A63663
	Intracellular	Granzyme A	PE-Cy7	BioLegend	507221
		Granzyme B	APC-Fire750	BioLegend	372209
		Granzyme K	PE	BioLegend	370512
		Perforin	BV421	BioLegend	353307
Proliferation panel	Surface	αβTCR	APC-Vio770	Miltenyi Biotec	130-114-062
		γδTCR	PerCp-Vio700	Miltenyi Biotec	130-113-514
		Vδ1	PE	Miltenyi Biotec	130-100-535
		Vδ2	APC	Miltenyi Biotec	130-095-803
Bulk sort panel	Surface	CD3	BUV395	BD Biosciences	563546
		αβTCR	Vioblue	Miltenyi Biotec	130-110-457
		γδTCR	APC-Vio770	Miltenyi Biotec	130-114-035
		Vδ1	FITC	Miltenyi Biotec	130-100-532
		Vδ2	APC	Miltenyi Biotec	130-095-803
		Vγ9	PC5	Beckman Coulter	A63663
Single cell sort panel	Surface	CD3	BUV395	BD Biosciences	563546
		CD27	PE-Dazzle594	BioLegend	356422
		αβTCR	Vioblue	Miltenyi Biotec	130-110-457
		γδTCR	APC-Vio770	Miltenyi Biotec	130-114-035
		Vδ1	FITC	Miltenyi Biotec	130-100-532
		CX3CR1	PE-Cy7	BioLegend	341612

Cat. no., catalogue number;

Table S4. Number of cells sorted and reads for TRD and TRG for each sample.

Study ID	Sample	Subset	No. cells sorted	Reads	
				TRD	TRG
Australian 001	NA	Vδ2+	46 148	774 911	843 344
Australian 005	NA	Vδ2neg	6 900	687 651	576 303
	NA	Vδ2+	22 000	1 009 689	687 577
Australian 010	NA	Vδ2neg	29 890	814 200	663 395
	NA	Vδ2+	50 000	1 007 881	649 601
Australian 011	NA	Vδ2neg	8 684	595 627	429 271
Mali Subject 66	0 (M)	Vδ2neg	6 417	93 453	460 062
		Vδ2+	3 527	106 823	1 818 790
	12 months	Vδ2neg	3 100	272 340	280 343
		Vδ2+	2 200	332 651	274 631
	18 months	Vδ2neg	11 600	351 799	1 725 472
		Vδ2+	6 500	226 372	110 770
Mali Subject 269	0 (M)	Vδ2neg	16 000	314 569	538 039
		Vδ2+	16 000	319 494	251 369
	28 months	Vδ2neg	7 800	314 568	620 297
		Vδ2+	7 500	273 338	361 549
Mali Subject 521	NA	Vδ2neg	50 000	715 573	427 974
		Vδ2+	33 759	618 551	426 234
Mali Subject 766	NA	Vδ2neg	50 000	501 491	619 906
		Vδ2+	5 231	589 258	384 494
CHMI Subject 2	CHMI1 + 1d	Vδ2neg	18 707	940 963	612 682
	CHMI4 + 21d	Vδ2neg	21 058	498 717	212 095
CHMI Subject 4	Baseline	Vδ2+	6 329	721 929	523 621
		Vδ2neg	4 395	661 676	190 381
	CHMI1 + 21d	Vδ2neg	11 032	992 581	558 789
	CHMI3 + 21d	Vδ2+	50 581	502 524	645 173
		Vδ2neg	11 747	1 075 848	835 176
	CHMI4 + 21d	Vδ2+	35 000	502 524	645 173
		Vδ2neg	20 000	877 076	429 142
CHMI Subject 9	CHMI1 + 21d	Vδ1+	3 774	511 711	806 350
		Vδ2+	8 294	727 289	1 060 102
	CHMI3 + 21d	Vδ1+	10 000	538 013	930 511
		Vδ2+	34 000	646 519	1 114 960
	CHMI4 + 21d	Vδ2neg	50 000	752 397	936 806
		γδTCR+	22 000	252 552	397 432
CHMI Subject 10	Baseline	Vδ2neg	6 310	11 991	4 628
	CHMI1 + 21d	Vδ2neg	5 139	373 479	30 157
	CHMI3 + 21d	Vδ2neg	10 732	402 445	38 588