Supplemental Materials

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

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Supplemental Materials and Methods

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 cohort

Blood was obtained from a cohort of nineteen 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 MUHREC (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 (Clinical trial 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 (https://osf.io/7rdm9/ and https://osf.io/3qvmh/) 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.

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 (iRBCs), their extracts (PfTSE) 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 Institute, Cary, NC, USA). Post-hoc comparisons were performed using Bonferroni method for multiple comparisons. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

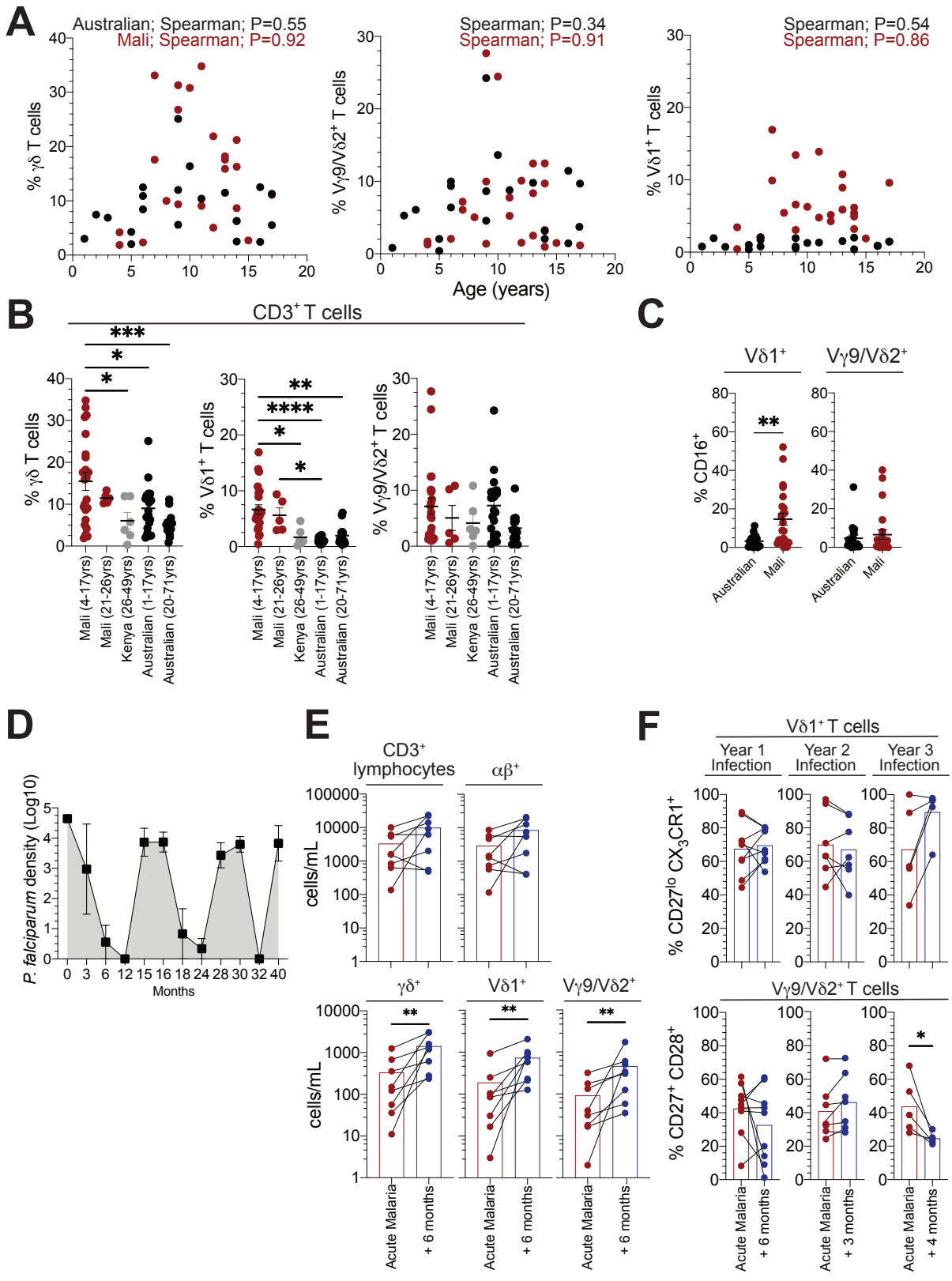
A
Australian; Spearman; P=0.55
Mali; Spearman; P=0.92
% γδ T cells
Spearman; P=0.34
Spearman; P=0.91
% Vγ9/Vδ2+ T cells
Spearman; P=0.54
Spearman; P=0.86
% Vδ1+ T cells
Age (years)
B
CD3+ T cells
% γδ T cells
% Vδ1+ T cells
% Vγ9/Vδ2+ T cells
Mali (4-17yrs)
Mali (21-26yrs)
Kenya (26-49yrs)
Australian (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**).

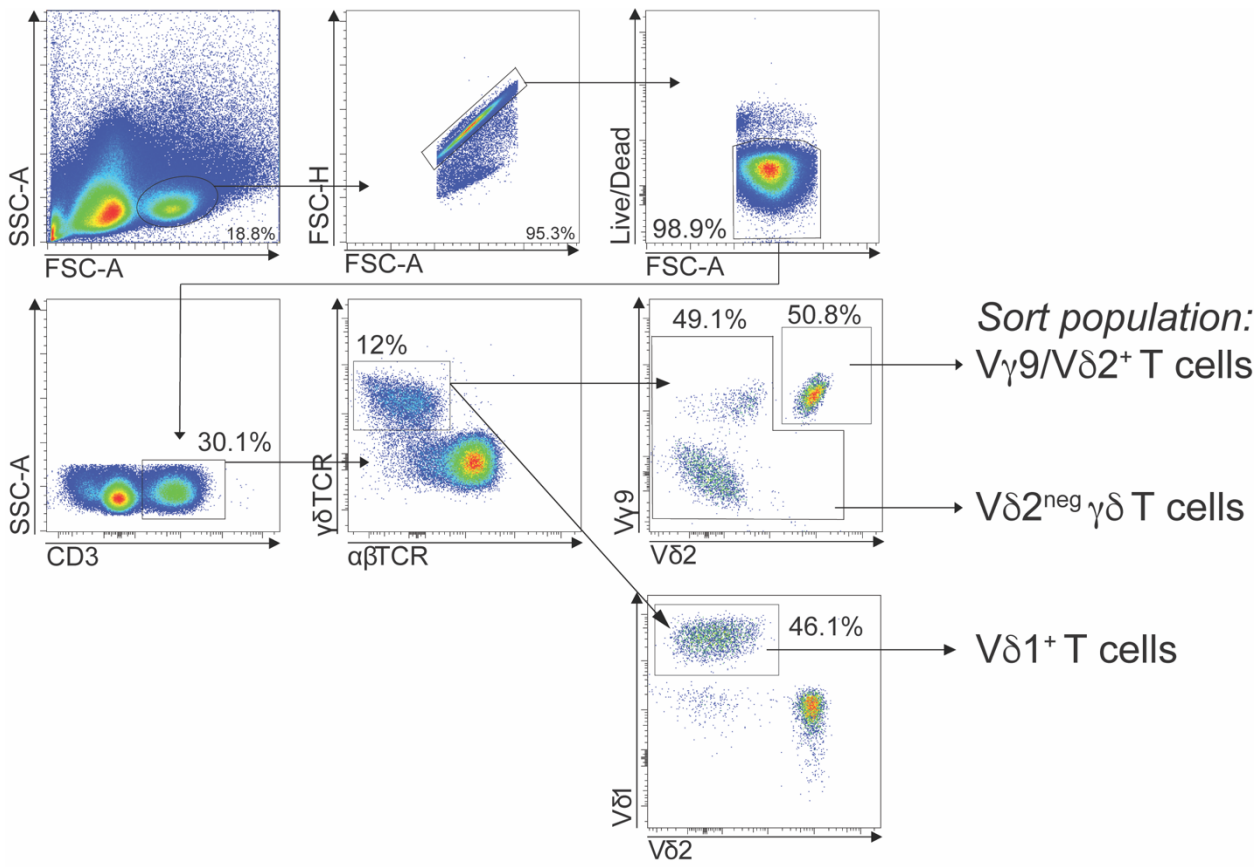

Figure S2. The gating strategy used to sort γδ T cells. The gating strategy used to identify and sort γδ T cell subsets shown in a representative donor. Lymphocytes were gated using the FSC-A/SSC-A, doublets were excluded using FSC-A/FSC-H, viable cells gated in the FSC-A/Live/Dead and then viable $CD3^+$ 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**).

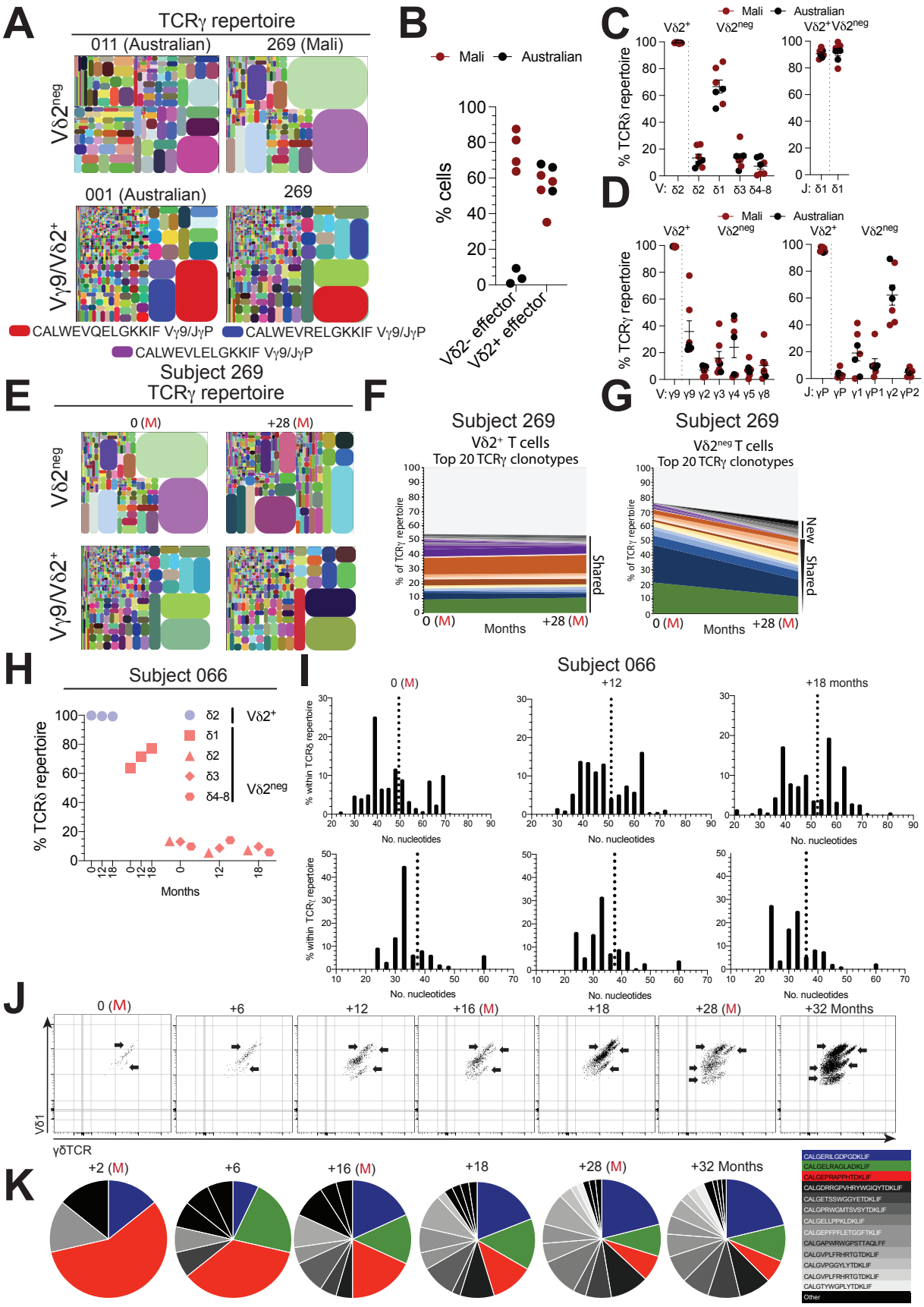
A
TCRγ repertoire
011 (Australian)
269 (Mali)
001 (Australian)
269
Vδ2neg
Vγ9/Vδ2+
CALWEVQELGKKIF Vγ9/JγP
CALWEVRELGKKIF Vγ9/JγP
CALWEVLELGKKIF Vγ9/JγP
B
Mali
Australian
% cells
Vδ2- effector
Vδ2+ effector
C
Mali
Australian
Vδ2+
Vδ2neg
Vδ2+Vδ2neg
% TCRδ repertoire
V: δ2 δ2 δ1 δ3 δ4-8
J: δ1 δ1
D
% TCRγ 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.

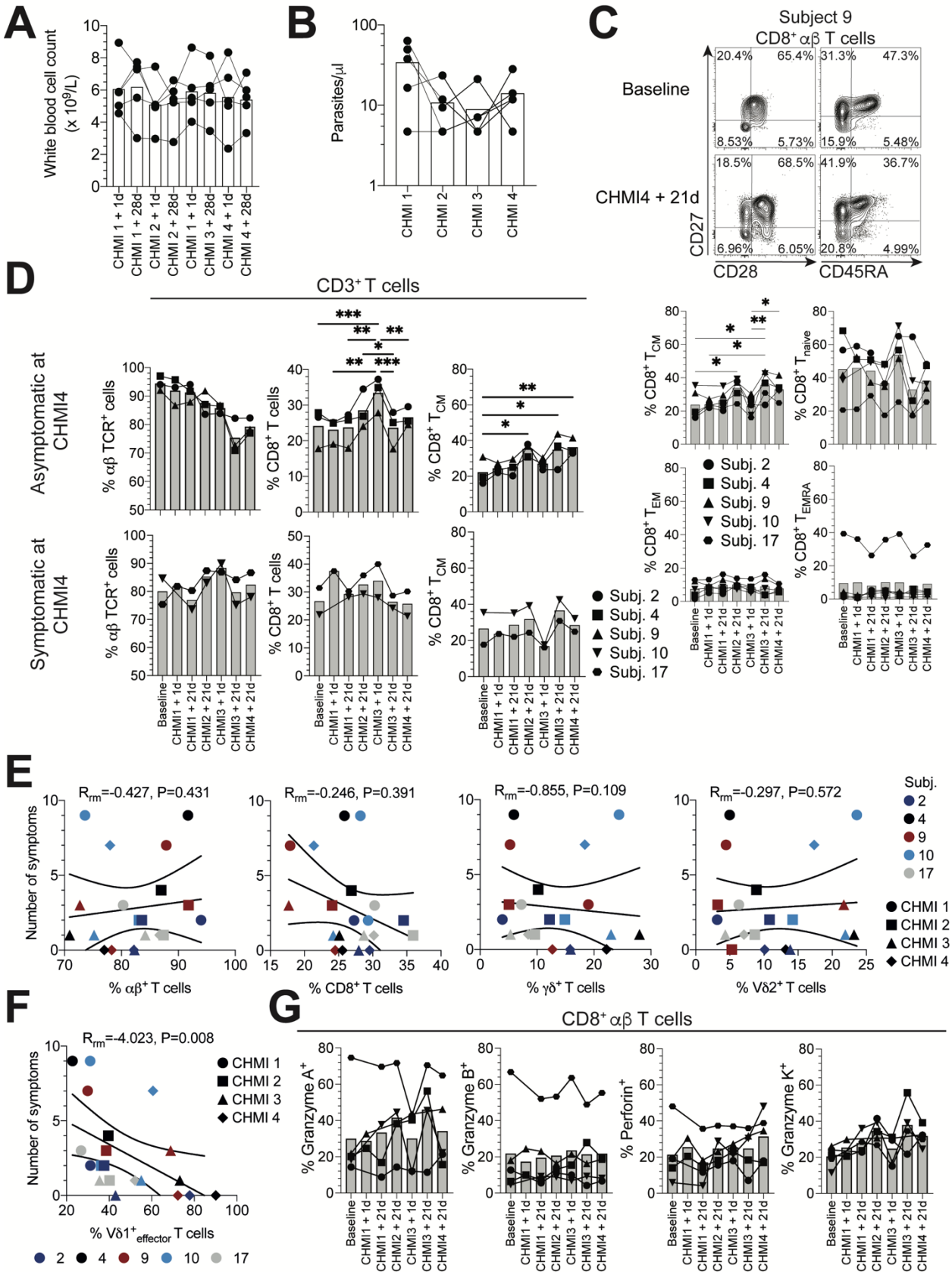
A
White blood cell count (x 10⁹/L)
CHMI 1 + 1d
CHMI 1 + 28d
CHMI 2 + 1d
CHMI 2 + 28d
CHMI 1 + 1d
CHMI 3 + 28d
CHMI 4 + 1d
CHMI 4 + 28d
B
Parasites/µl
CHMI 1
CHMI 2
CHMI 3
CHMI 4
C
Subject 9
CD8⁺ αβ T cells
Baseline
CHMI4 + 21d
20.4%
65.4%
8.53%
5.73%
31.3%
47.3%
15.9%
5.48%
18.5%
68.5%
6.96%
6.05%
41.9%
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**).

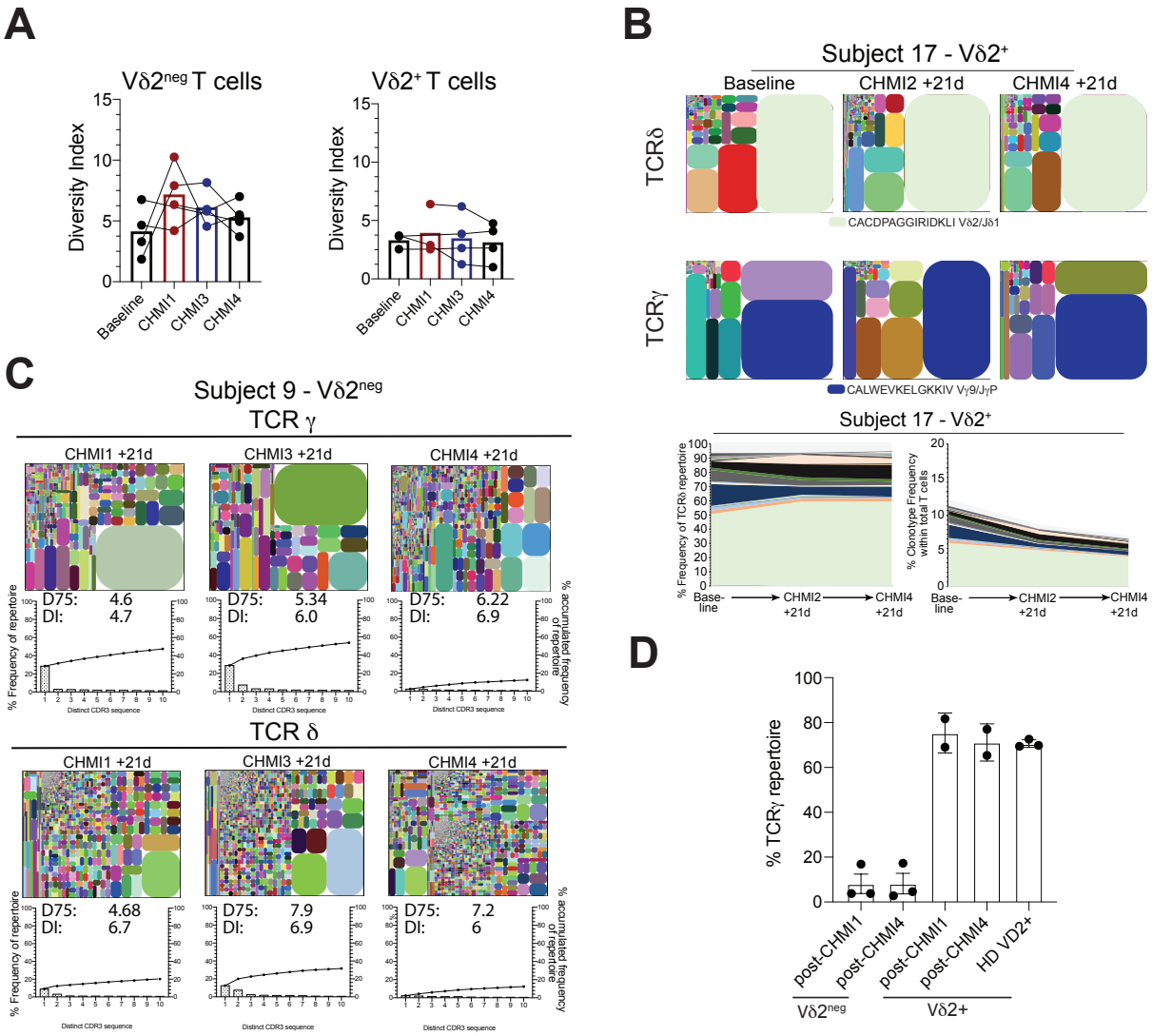
A
Vδ2neg T cells
Vδ2+ T cells
Diversity Index
Baseline
CHMI1
CHMI3
CHMI4
B
Subject 17 - Vδ2+
Baseline
CHMI2 +21d
CHMI4 +21d
TCRδ
CACDPAGGIRIDKLI Vδ2/Jδ1
TCRγ
CALWEVKELGKKIV Vγ9/JγP
% Frequency of TCRδ repertoire
% Clonotype Frequency within total T cells
C
Subject 9 - Vδ2neg
TCR γ
CHMI1 +21d
CHMI3 +21d
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