

## Supplementary Tables and Figures

### Supplementary Tables

#### Supplementary Table S1: Sub-Cohort 1: Demographics and clinical data

Characteristics	Endemic healthy controls (EC), N = 8	<i>P. falciparum</i> (Pf), N = 24	<i>P. vivax</i> (Pv), N = 12
Male (N, %)	7 (88%)	11 (46%)	6 (50%)
Age Median (IQR)	29 (27, 31)	22 (20, 28)	25 (21, 30)
Hb (g/dL)	13.25 (12.25, 14.90)	10.65 (9.33, 12.35)	11.80 (10.05, 12.20)
Parasites (per/ $\mu$ L) <sup>#</sup>	NA	15,860 (6,974, 48,250)	6,895 (4,915, 9,630)

\*Missing age information (N; Pf = 2, Pv = 1)

<sup>#</sup>Blood film diagnosis

## Supplementary Tables and Figures

**Supplementary Table S2: Sub-Cohort 2 demographics and clinical data**

Characteristics	Malaria naive (Naive), N=5	Endemic healthy controls (EC), N = 5	<i>P. falciparum</i> (Pf), N = 8	<i>P. vivax</i> (Pv), N = 8
Male (N, %)	2(40%)	3 (60%)	4 (50%)	4 (50%)
Age, Median (IQR)	43 (36, 43)	22 (20, 29)	26 (23, 27)	25 (20, 28)
Hb (g/dL)	NA	12.50 (10.80, 13.60)	11.90 (11.30, 12.60)	11.40 (10.00, 12.23)
Parasites (per/ $\mu$ L) <sup>#</sup>	NA	NA	186,648 (7,253, 275,528)	4,997 (4,589, 7,282)

\*Missing demographic information (N; Pf = 1)

<sup>#</sup>Blood film diagnosis

## Supplementary Tables and Figures

**Supplementary Table 3: Antibodies for phenotyping of Tfh cells *ex vivo* via flow cytometry for Sub-Cohort 1**

Fluorochrome	Marker	Clone	RRID	Manufacturer	Dilution
PE	PD-1	EH12.1	AB_2033989	BD	1 in 20
PE-CF594	CD25	M-A251	AB_11151919	BD	1 in 80
PerCPCy5.5	CD4	RPA-T4	AB_893322	BD	1 in 200
Pe-Cy7	CXCR5	J252D4	AB_2562355	BioLegend	1 in 80
APC-R700	CCR6	11A9	AB_2739092	BD	1 in 20
APC-Cy7	ICOS	C398.4A	AB_2566128	BioLegend	1 in 60
BV421	CXCR3	1C6	AB_2737653	BD	1 in 10
V500	CD14	M5E2	AB_10611856	BD	1 in 100
V500	CD19	HIB19	AB_10562391	BD	1 in 200
Aqua	Live/dead			Invitrogen	1 in 80
<b>Intracellular staining</b>					
FITC	Ki67	B56	AB_396302	BD	1 in 80
AF647	FoxP3	206D	AB_439754	BioLegend	1 in 80

## Supplementary Tables and Figures

**Supplementary Table 4: Antibodies for phenotyping of Tfh cells *ex vivo* via flow cytometry for Sub-Cohort 2**

Fluorophore	Marker	Clone	Catalogue number	Manufacturer	Dilution
BUV496	CD8	RPA-TS	612942	BD	1/100
BUV563	CD45RA	H100	565702	BD	1/400
BUV737	CD25	2A3	612807	BD	1/200
BUV805	CD3	SK7	612893	BD	1 in 50
BV480	CD38	HIT2	566137	BD	1 in 50
BV510	CD14	M5E2	301842	BioLegend	1 in 100
BV510	CD19	H1B19	30184	BioLegend	1 in 100
BV570	CD127	A019D5	351308	BioLegend	1 in 50
BV605	CCR4	L291HL1	301842	BioLegend	1 in 50
BV650	CCR6	11A9	359418	BioLegend	1 in 50
BV711	CXCR5	J252D4	563922	BioLegend	1 in 50
BV785	CD4	OKT4	317442	BioLegend	1 in 50
PE-CF594	CXCR3	IC6/CXCR3	302906	BD	1 in 50
PE-Cy7	PD-1	EH12.1	562451	BD	1 in 50
AF700	CD161	HP-3G10	563473	BioLegend	1 in 50
APCCy7	ICOS	C398.4A	313530	BioLegend	1 in 25
Live dead Blue			L23105	Invitrogen	1 in 5000
<b>Stain at 37C, 45 minutes</b>					
PerCP-Cy5.5	CCR7	G043H7	353220	BD	1 in 12.5
<b>Intracellular staining</b>					
BUV395	Ki67	B56	584071	BD	1 in 250
PE	GZMB	GB11	561142	BD	1 in 400
AF647	FOXP3	320114	206D	BioLegend	1 in 50

## Supplementary Tables and Figures

**Supplementary Table 5: Antibodies for cytokine measurement after stimulation with PMA/Ionomycin**

<b>Fluorophore</b>	<b>Marker</b>	<b>Clone</b>	<b>Catalogue number</b>	<b>Manufacturer</b>	<b>Dilution</b>
BUV496	CD8	RPA-TS	612942	BD	1 in 100
BUV563	CD45RA	H100	565702	BD	1 in 400
BUV805	CD3	SK7	612893	BD	1 in 50
BV510	CD14	M5E2	301842	BioLegend	1 in 100
BV510	CD19	H1B19	30184	BioLegend	1 in 100
BV605	CCR4	L291HL1	301842	BioLegend	1 in 100
BV650	CCR6	11A9	359418	BioLegend	1 in 50
BV711	CXCR5	J252D4	563922	BioLegend	1 in 50
BV785	CD4	OKT4	317442	BioLegend	1 in 50
PE-CF594	CXCR3	IC6/CXCR3	302906	BD	1 in 50
PE-Cy7	PD-1	EH12.1	562451	BD	1 in 50
APC-Fire	Vd2	B6	331419	BioLegend	1 in 50
<b>Stain at 37C, 45 minutes</b>					
PerCP-Cy5.5	CCR7	G043H7	353220	BD	1 in 25
APC-R700	CD107a	H4A3	565184	BD	1 in 100
<b>Intracellular cytokine staining</b>					
BUV395	IFN $\gamma$	B27	563563	BD	1 in 50
BV421	IL-10	JES3-9D7	564053	BD	1 in 100
BV750	TNF $\alpha$	Mab11	566359	BD	1 in 100
FITC	IL-17a	BL168	B252099	BioLegend	1 in 25
PE	IL-21	3A3-N2.1	560463	BD	1 in 10
APC	IL-4	MP4-25D2	500812	BioLegend	1 in 10

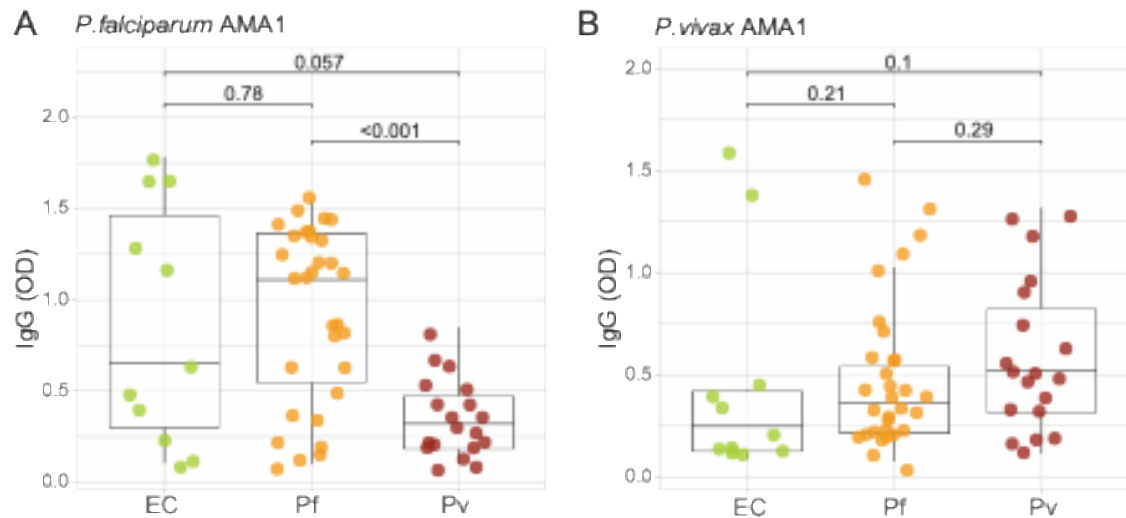
## Supplementary Tables and Figures

Supplementary Table 6: Cell counts used for clustering.

ID	Group	Total cells	Temra	Tfh	Th1	Th1_17	Th17	Th2	Tregs
C1	EHC	13090	310	2853	4451	1018	721	2879	858
C2	EHC	21954	690	4284	7113	2435	2010	3883	1539
C3	EHC	21125	611	6479	5726	830	2263	4054	1162
C4	EHC	16853	529	3323	6642	1188	1735	2066	1370
C5	EHC	20054	106	5011	4470	1563	1958	4386	2560
H1	Naive	33277	13931	2682	7970	1846	1629	3948	1271
H2	Naive	11961	181	1287	3920	959	1716	2933	965
H3	Naive	7690	165	1035	2943	473	666	1802	606
H4	Naive	38971	8798	3145	15350	2131	2362	5566	1619
H5	Naive	17026	202	3414	3749	2626	2968	2835	1232
F1	Pf	13398	458	4678	3176	786	1278	1902	1120
F2	Pf	12527	114	4034	3772	571	964	2096	976
F3	Pf	12121	40	2395	6496	773	783	1381	253
F4	Pf	18969	63	5260	8156	1740	1263	1322	1165
F5	Pf	6444	63	1774	2715	526	377	642	347
F6	Pf	15612	69	5277	3449	1282	1503	2833	1199
F7	Pf	14868	107	4804	4656	989	897	2684	731
F8	Pf	6194	83	1820	1210	377	762	1636	306
V1	Pv	16639	227	2899	7333	1569	1498	2200	913
V2	Pv	10692	65	3288	3246	896	982	1711	504
V3	Pv	18924	76	5830	7754	204	889	3016	1155
V4	Pv	18251	65	4837	7543	1745	1159	2173	729
V5	Pv	6345	78	1234	2726	405	674	769	459
V6	Pv	14891	208	5141	4420	1081	1035	2479	527
V7	Pv	6964	288	1348	2867	360	478	883	740

## Supplementary Tables and Figures

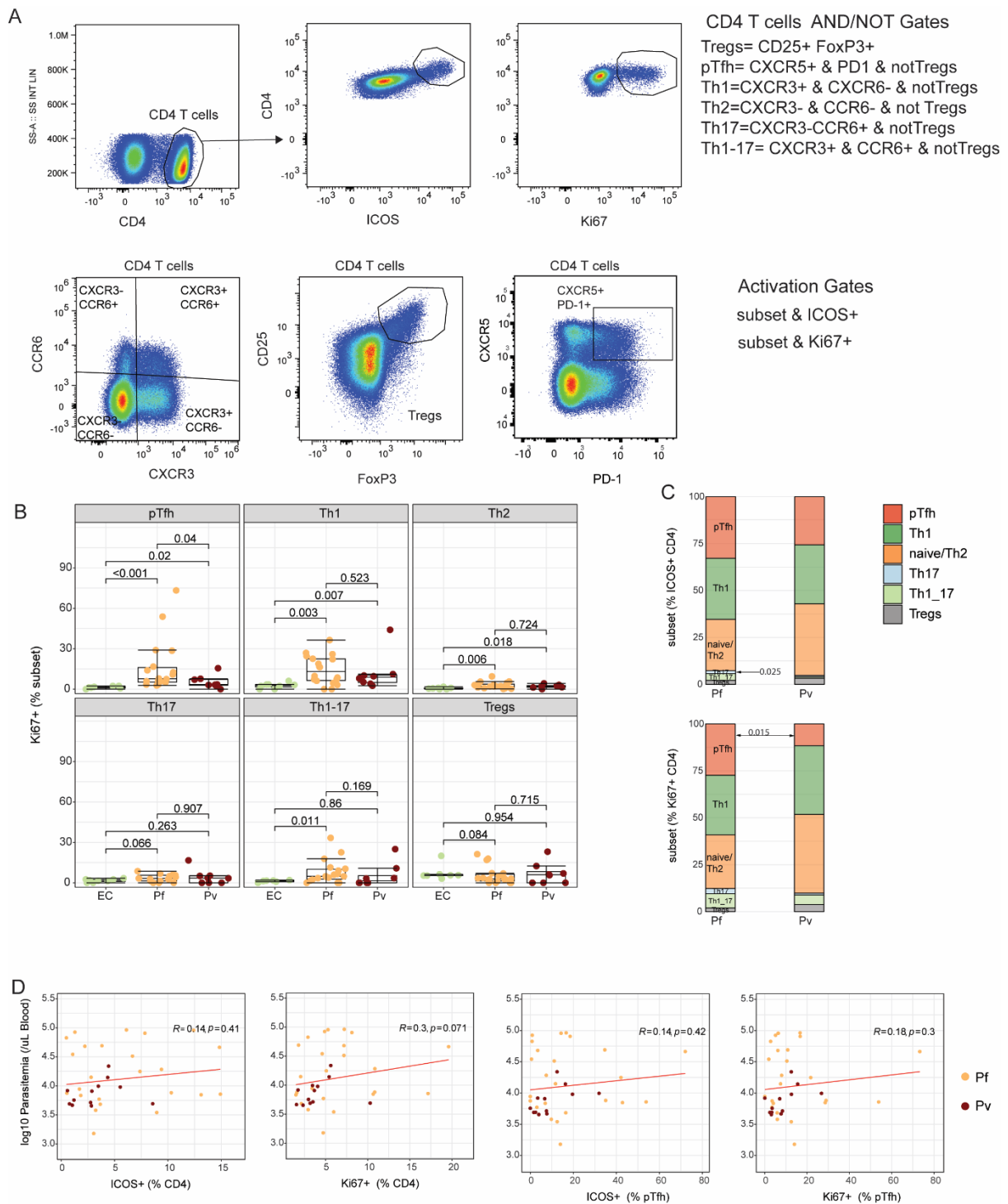
### Supplementary Figures



#### **Supplementary Figure S1: IgG to *P. falciparum* and *P. vivax* AMA1**

IgG to *P. falciparum* AMA1 (A) and *P. vivax* AMA1 (B) was quantified in endemic controls (EC,  $n = 11$ ) and in individuals with current *P. falciparum* (Pf,  $n = 31$ ) or *P. vivax* infection (Pv,  $n = 19$ ). Boxplot box and whisker plots indicate first and third quartiles for the hinges, median line, and lowest and highest values no further than 1.5 interquartile range from the hinges for whisker lines. P-values indicate unpaired Wilcoxon rank-sum test.

## Supplementary Tables and Figures

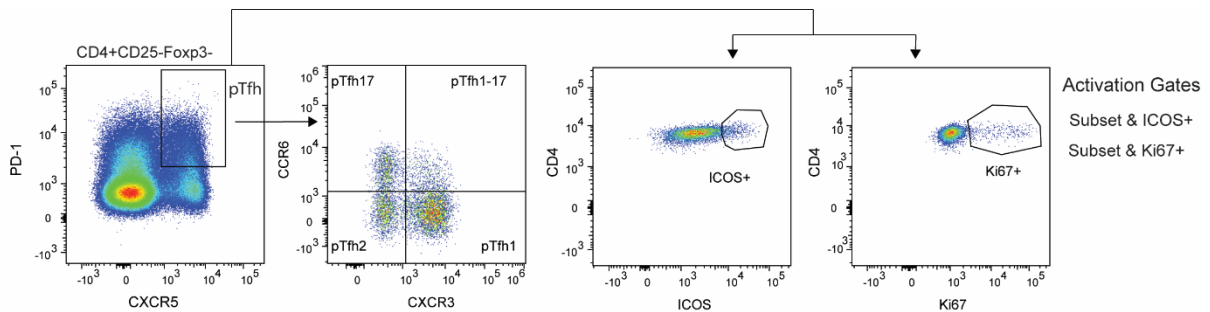


### Supplementary Figure S2: Gating and proliferation of CD4 T cells in *P. falciparum* and *P. vivax* malaria (Sub-Cohort 1)

CD4 T cell responses were assessed in adults with *P. falciparum* (Pf,  $n = 24$ ) and *P. vivax* (Pv,  $n = 12$ ) compared with endemic healthy controls (EC,  $n = 8$ ). **A**) Gating strategy to identify CD4 T cell subsets and activation and proliferation. **B**) Ki67+ as a proportion of each CD4 T

## Supplementary Tables and Figures

cell subset in healthy controls and patients C. Composition of ICOS+ and Ki67+ CD4 T cell in infected groups. D). Correlation between the frequency of ICOS+ and Ki67+ CD4 T cells and pTfh cells with parasitemia.  $R$  and  $p$  are spearman's correlations. Box and whisker plots indicate first and third quartiles for the hinges, median line, and lowest and highest values no further than 1.5 interquartile range from the hinges for whisker lines.  $P$ -values indicate unpaired Wilcoxon rank-sum test.

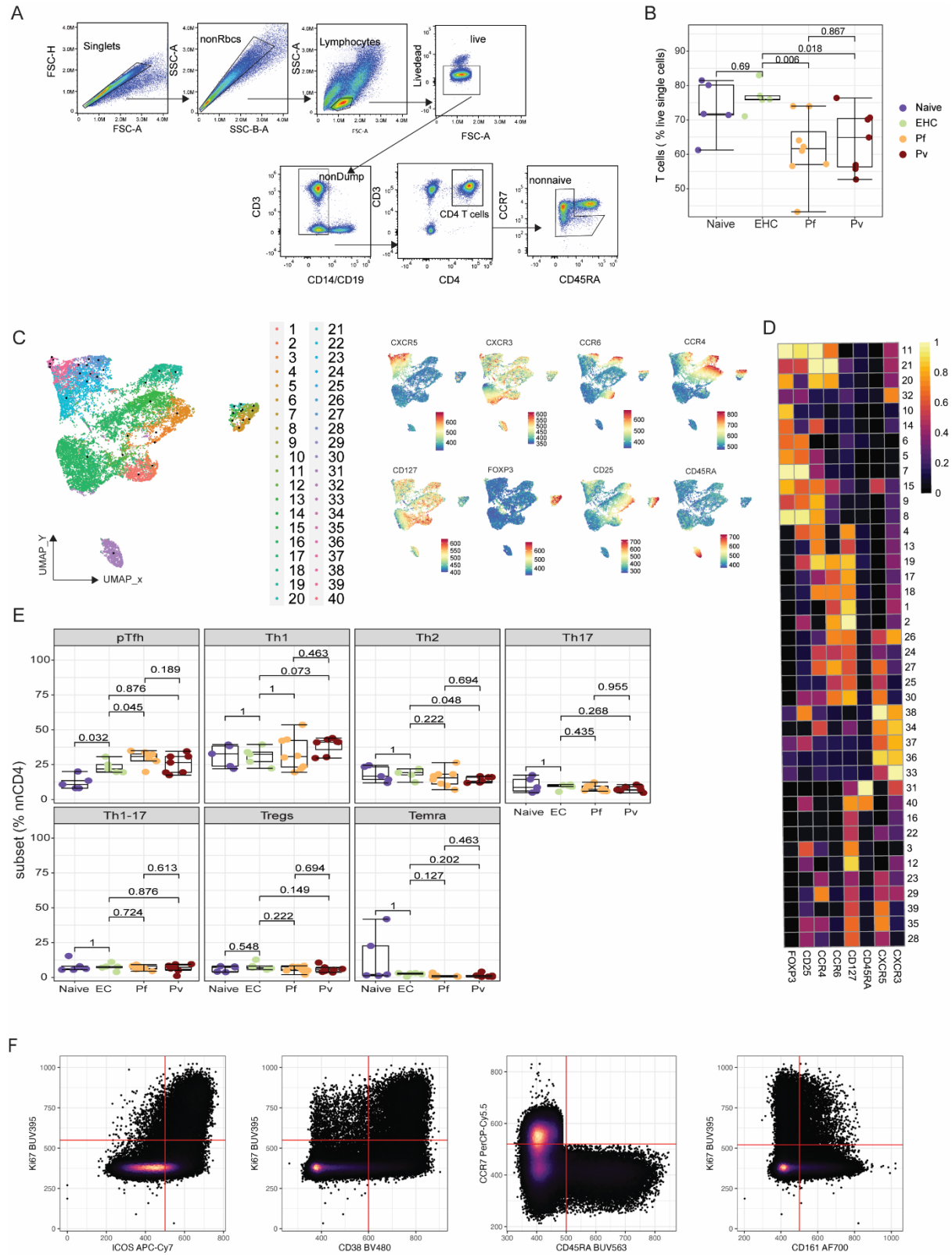


### Supplementary Figure S3: pTfh gating and correlations between variables (Sub-Cohort 1)

Gating strategy to identify pTfh cells and activation (ICOS) and proliferation (Ki67).



# Supplementary Tables and Figures

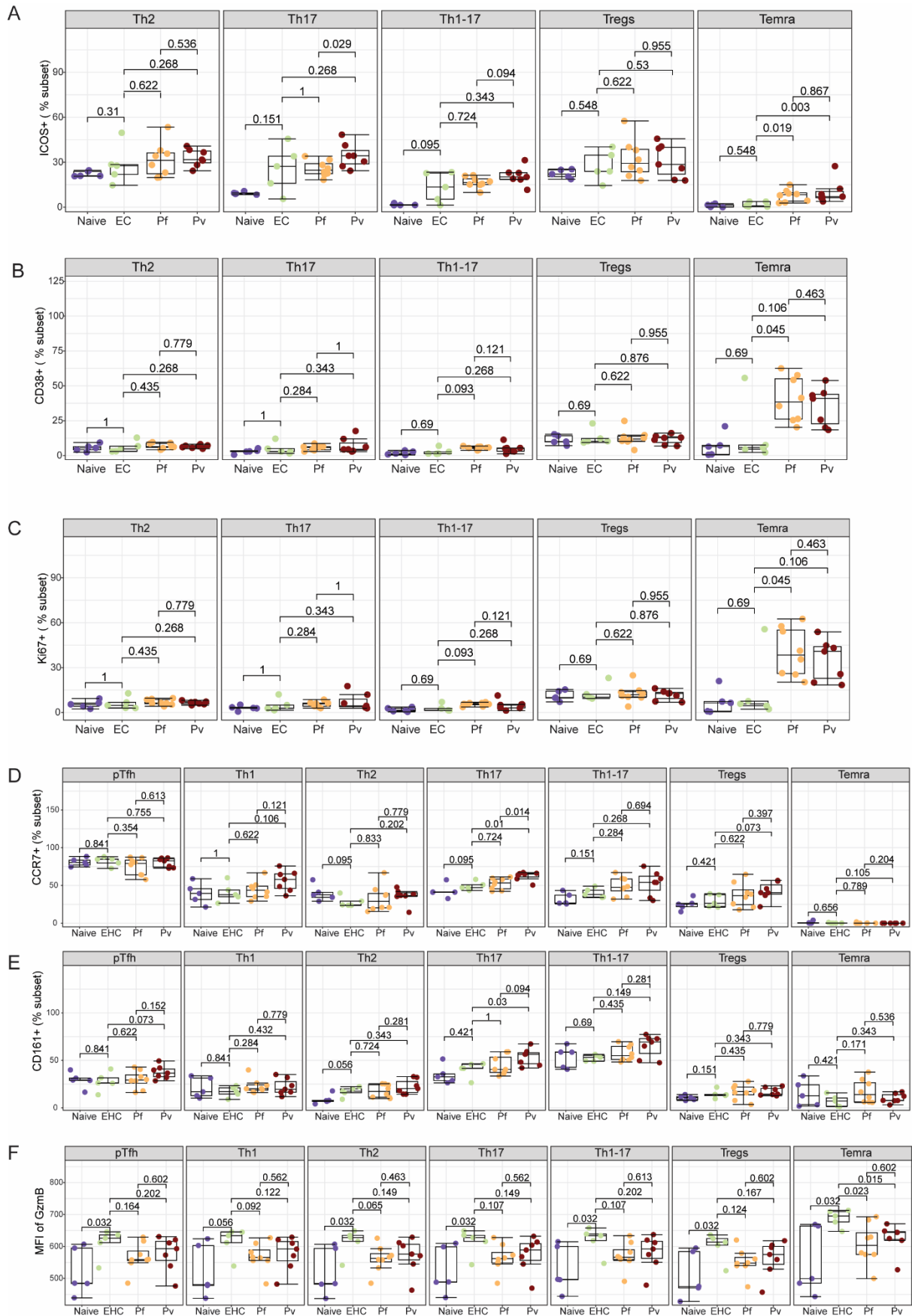


**Supplementary Figure S5: Activation of CD4 T cell subsets (Sub-Cohort 2)**

## Supplementary Tables and Figures

*CD4 T cells were analysed with a comprehensive panel and unbiased approaches in healthy malaria naïve Australians (Naïve, n=5), uninfected endemic controls (EC, n=5), P. falciparum (Pf, n = 8) and P. vivax (Pv, n = 7) patients. A) Gating strategy for identification of non-naïve CD4 T cells. B) Total T cells as proportion of live single cells. C) Unsupervised clustering with cell clusters visualised on a UMAP. D) Expression of markers used to annotate clusters shown on a UMAP and heatmap. E) Frequencies of subsets as proportions of CD4 T cells. F) Gating strategy to identify ICOS+, CD38+, Ki67+, CCR7 and CD161 cells. Data in box plots are individual responses coloured according to the control and malaria groups. Box and whisker plots indicate first and third quartiles for the hinges, median line, and lowest and highest values no further than 1.5 interquartile range from the hinges for whisker lines. P-values indicate unpaired Wilcoxon rank-sum test.*

## Supplementary Tables and Figures

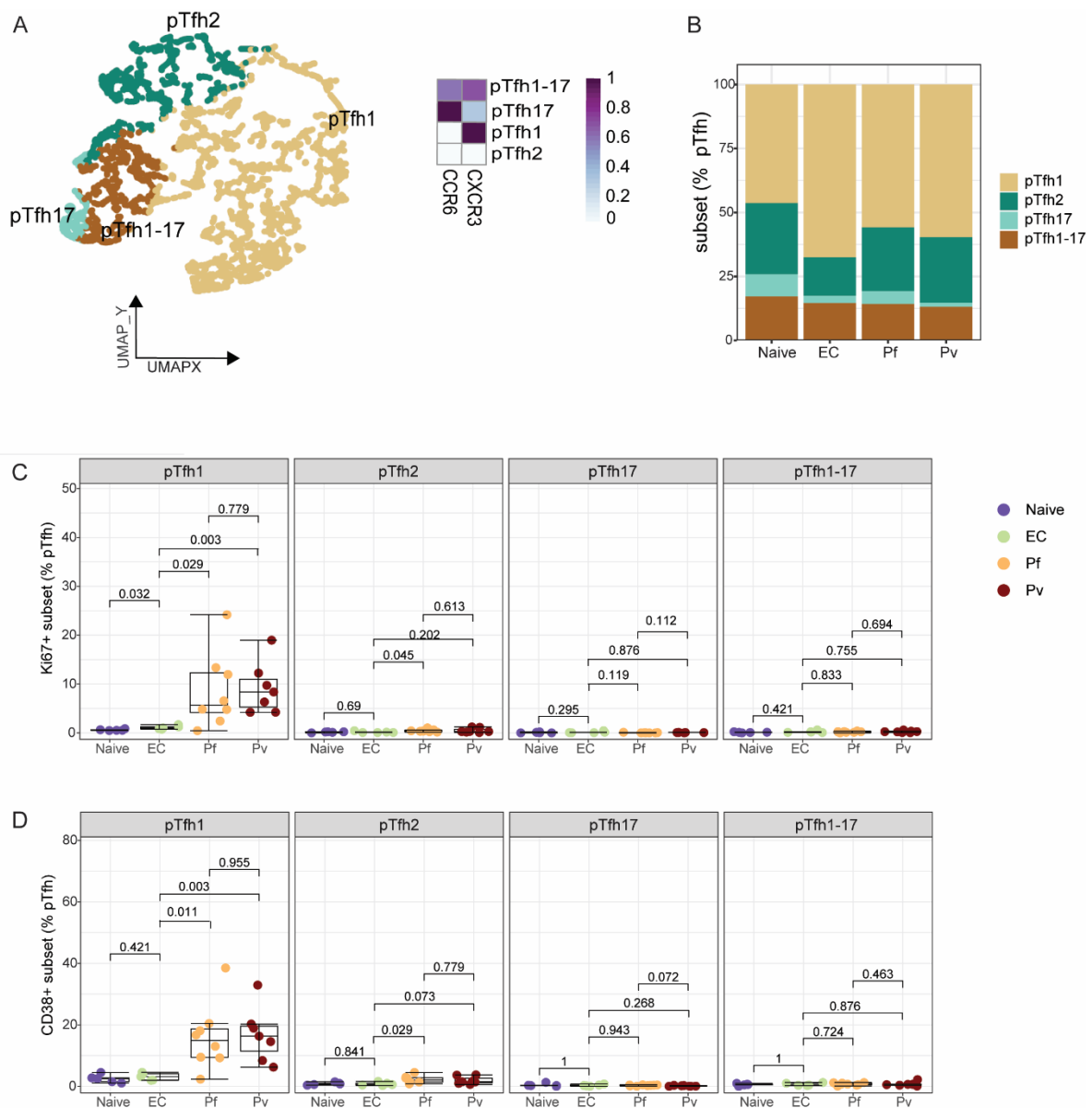


**Supplementary Figure S6: Activation of CD4 T cell subsets (Sub-Cohort 2)**

## Supplementary Tables and Figures

*CD4 T cells were analysed with a comprehensive panel and unbiased approaches in healthy malaria naïve Australians (Naïve, n=5), uninfected endemic controls (EC, n=5), P. falciparum (Pf, n = 8) and P. vivax (Pv, n = 7) patients. Frequency of **A) ICOS+**, **B) CD38+**, **C) Ki67+** **D) CCR7+** and **E) CD161+** cells as proportions of CD4 T cell subsets. **F) MFI of granzyme B** expression on CD4 T cell subsets. Data are individual responses coloured according to the control ad malaria groups. Box and whisker plots indicate first and third quartiles for the hinges, median line, and lowest and highest values no further than 1.5 interquartile range from the hinges for whisker lines. P-values indicate unpaired Wilcoxon rank-sum test.*

## Supplementary Tables and Figures



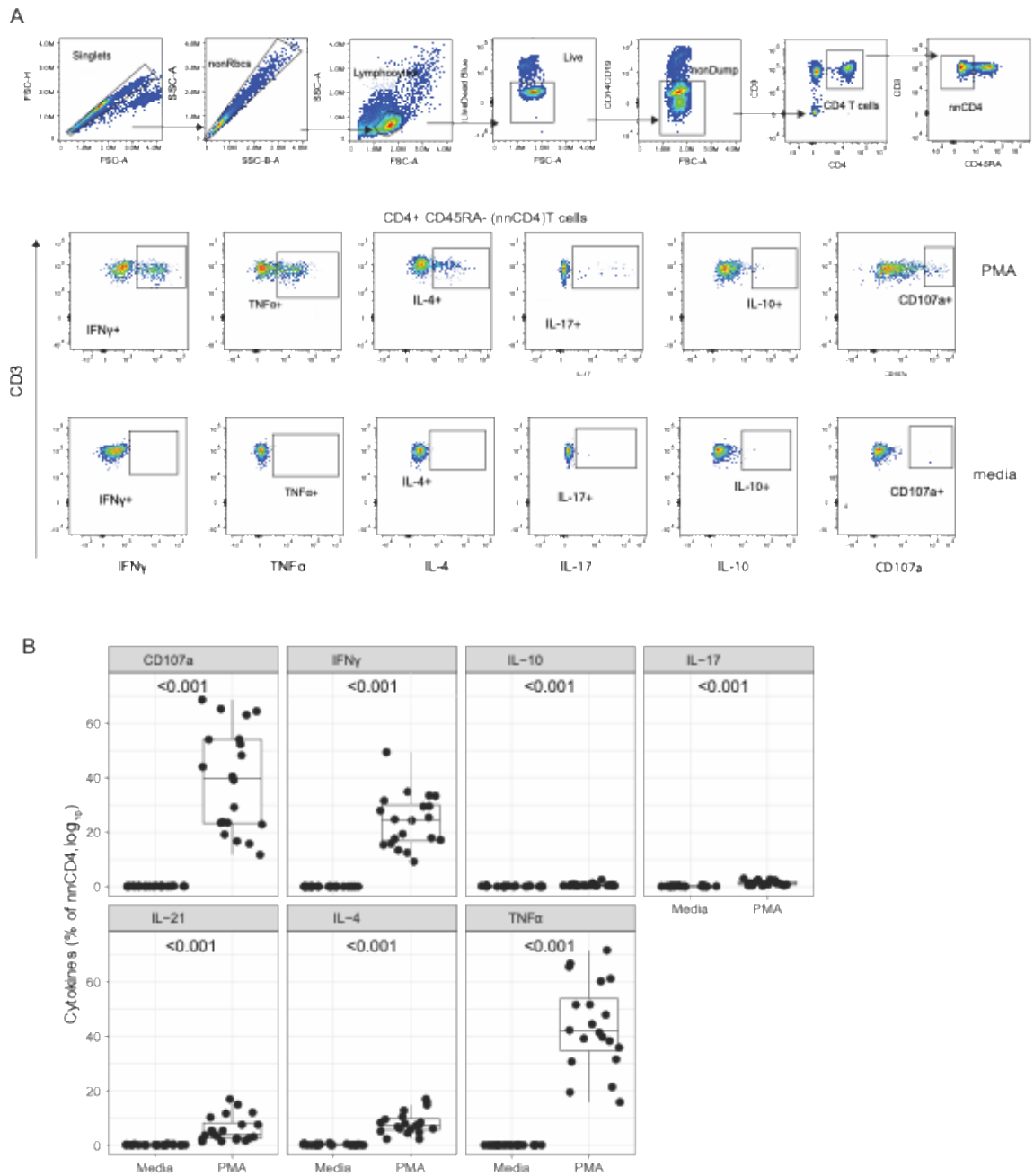
### Supplementary Figure S7: pTfh gating and subset activation (Sub-Cohort 2)

pTfh cell subsets were identified by unsupervised clustering assessed in adults with *P. falciparum* (Pf,  $n = 25$ ) and *P. vivax* (Pv,  $n = 12$ ) compared with uninfected endemic controls (EC,  $n = 8$ ). **A**) UMAP showing clusters of pTfh cell subsets based on the expression of markers in the heatmap. **B**) Ki67<sup>+</sup> cells as proportions of total pTfh in controls and malaria patients. **C**) ICOS<sup>+</sup> and **D**) Ki67<sup>+</sup> cells within pTfh subsets. For all plots, box and whisker plots indicate first and third quartiles for the hinges, median line, and lowest and highest



# Supplementary Tables and Figures

according to the Rho values using hierarchical clustering

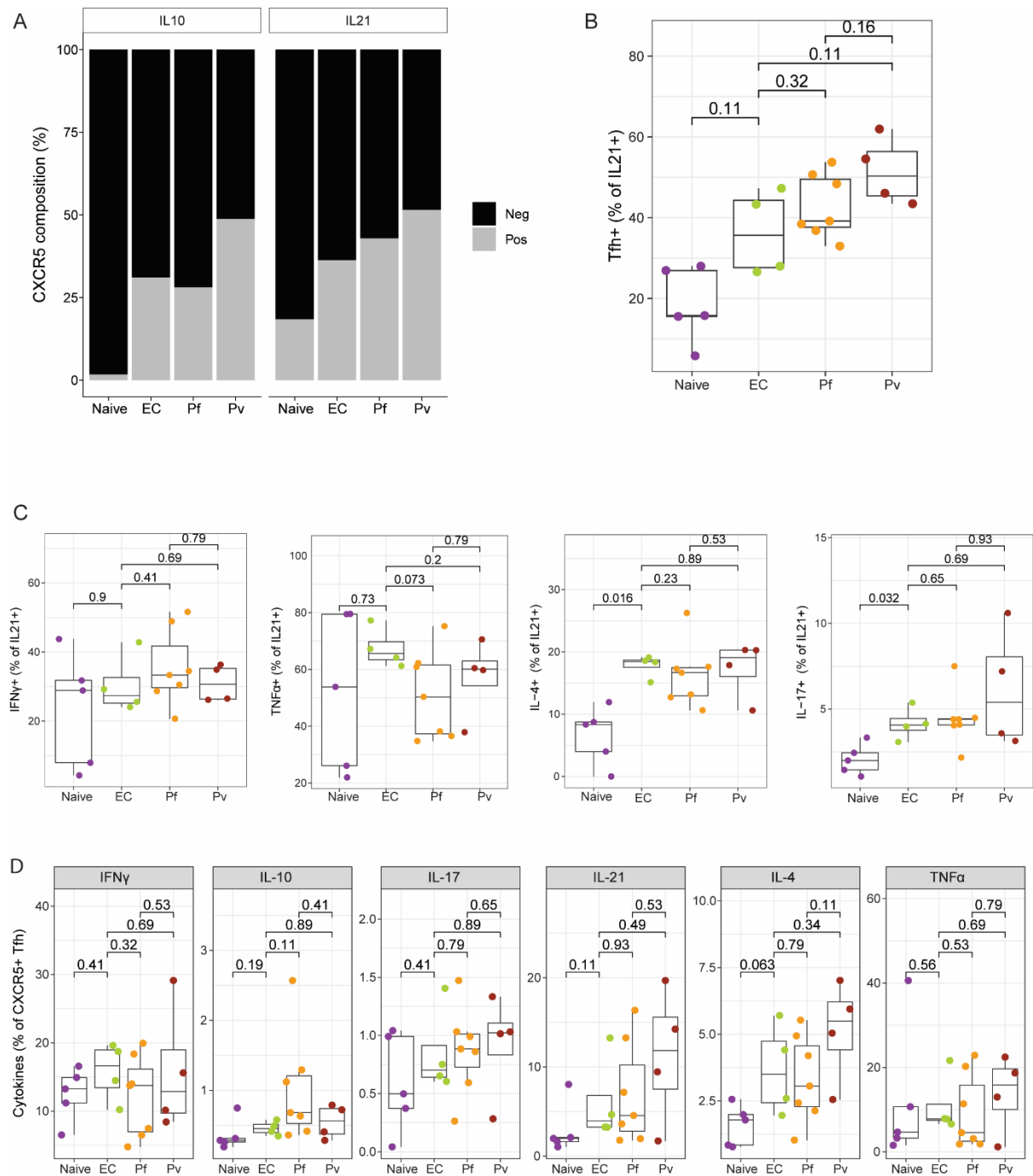


## Supplementary Tables and Figures

### ***Supplementary Figure S9: Gating for PMA cytokine staining and CD4 T cell cytokine expression***

*PBMC from healthy malaria naïve Australians (Naïve, n = 5), uninfected endemic controls (EC, n = 5), P. falciparum (Pf, n = 8) and P. vivax (Pv, n =4) infected adults were stimulated with either PMA/Ionomycin or unstimulated (media), and IFN $\gamma$ , TNF $\alpha$ , IL-4, IL-17, IL-21, IL-10 production and CD107a expression determined by intracellular staining. **A)** Gating strategy to identify non-naïve CD4 T cells (nnCD4, upper panel) and cytokine expression. **B)** Frequency of CD107a<sup>+</sup>, IFN $\gamma$ , IL-10, IL-17, IL-21, IL-4 and TNF $\alpha$  producing nnCD4 T cells between PMA/Ionomycin stimulated (PMA) and unstimulated (media) condition. Box and whisker plots indicate first and third quartiles for the hinges, median line, and lowest and highest values no further than 1.5 interquartile range from the hinges for whisker lines. P-values indicate unpaired Wilcoxon rank-sum test.*

## Supplementary Tables and Figures



### Supplementary Figure S10: Co-expression of IL-21 with other cytokines

**A)** Proportion of CXCR5<sup>+</sup> within IL-10<sup>+</sup> and IL-21<sup>+</sup> CD4 T cells following stimulation with PMA/Ionomycin in malaria naive Australians (Naive,  $n = 5$ ), uninfected endemic controls (EC,  $n = 5$ ), *P. falciparum* (Pf,  $n = 8$ ) and *P. vivax* (Pv,  $n = 4$ ) infected adults. **B)** Frequency of Tfh (CXCR5<sup>+</sup>) within IL-21<sup>+</sup> cells. **C)** Frequency of IFN $\gamma$ , TNF $\alpha$ , IL-4, IL-17, IL-21 and IL-10 within IL-21<sup>+</sup> cells. **D)** Cytokine producing cells as proportion of Tfh (CXCR5<sup>+</sup>) cells.

## **Supplementary Tables and Figures**

*Box and whisker plots indicate first and third quartiles for the hinges, median line, and lowest and highest values no further than 1.5 interquartile range from the hinges for whisker lines. P-values indicate unpaired Wilcoxon rank-sum test.*