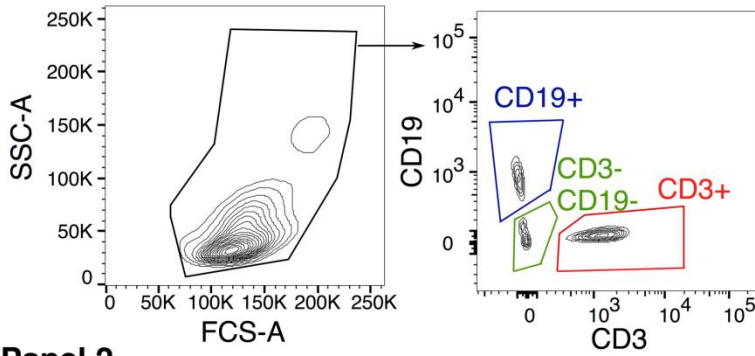
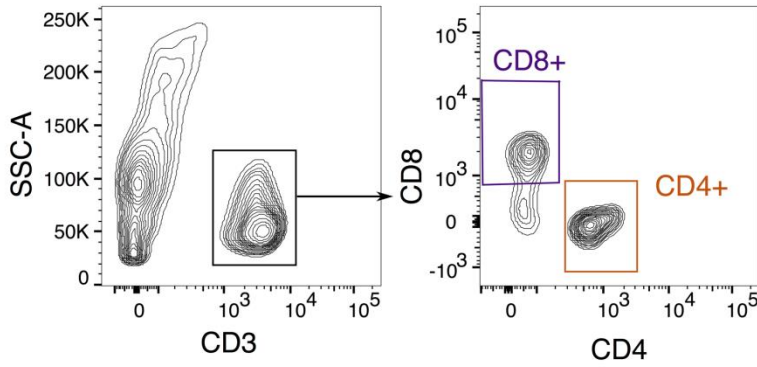


A Panel 1



Panel 2



B

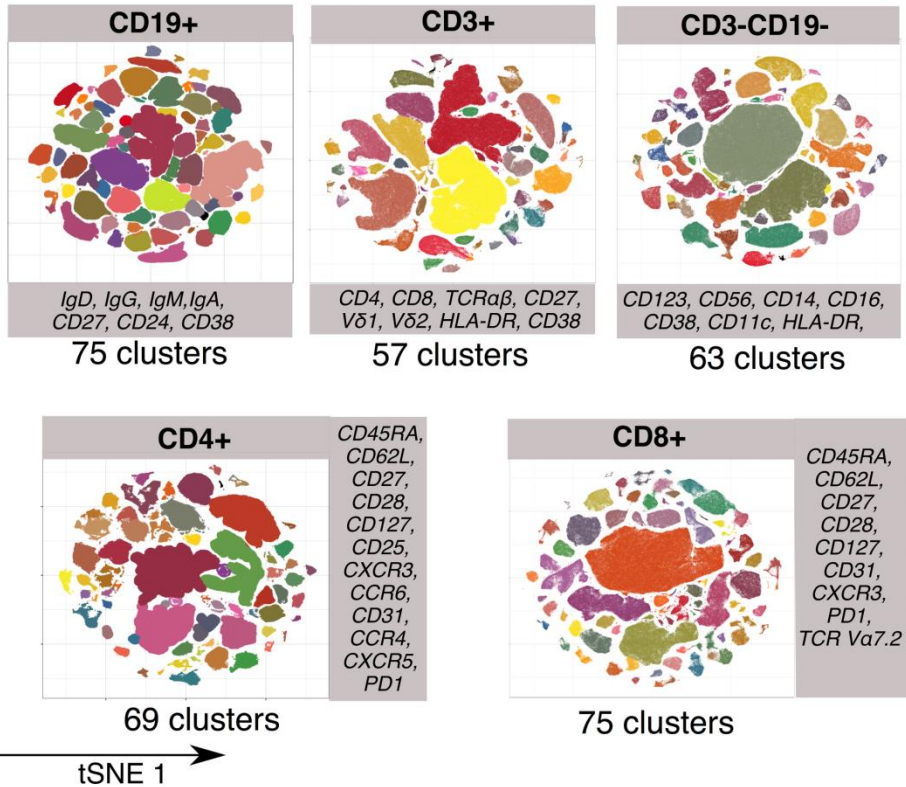


Fig. S1. Pregating of cell types for tSNE.

A) Representative flow cytometry plots for the five bulk populations analyzed by tSNE: CD3⁺ T cells, CD19⁺ B cells, CD4⁺ T cells, CD8⁺ T cells, and CD3⁻CD19⁻ leukocytes. Cells were stained with antibody panels indicated in table S2. From panel 1, CD3⁺, CD19⁺ and CD3⁻CD19⁻ populations were identified from single, live leukocytes on the basis of CD3 and CD19 expression. From panel 2, CD4⁺ and CD8⁺ cells were identified from single, live lymphocytes that were CD3⁺ and side scatter area (SSC-A) low. B) Representative tSNE plots with DBSCAN-defined clusters for 5 bulk populations from immunophenotyping. Colors represent discrete clusters, the markers used for clustering each population are shown in grey boxes, and the number of clusters for each bulk population is shown below each plot (n = 336 total clusters).

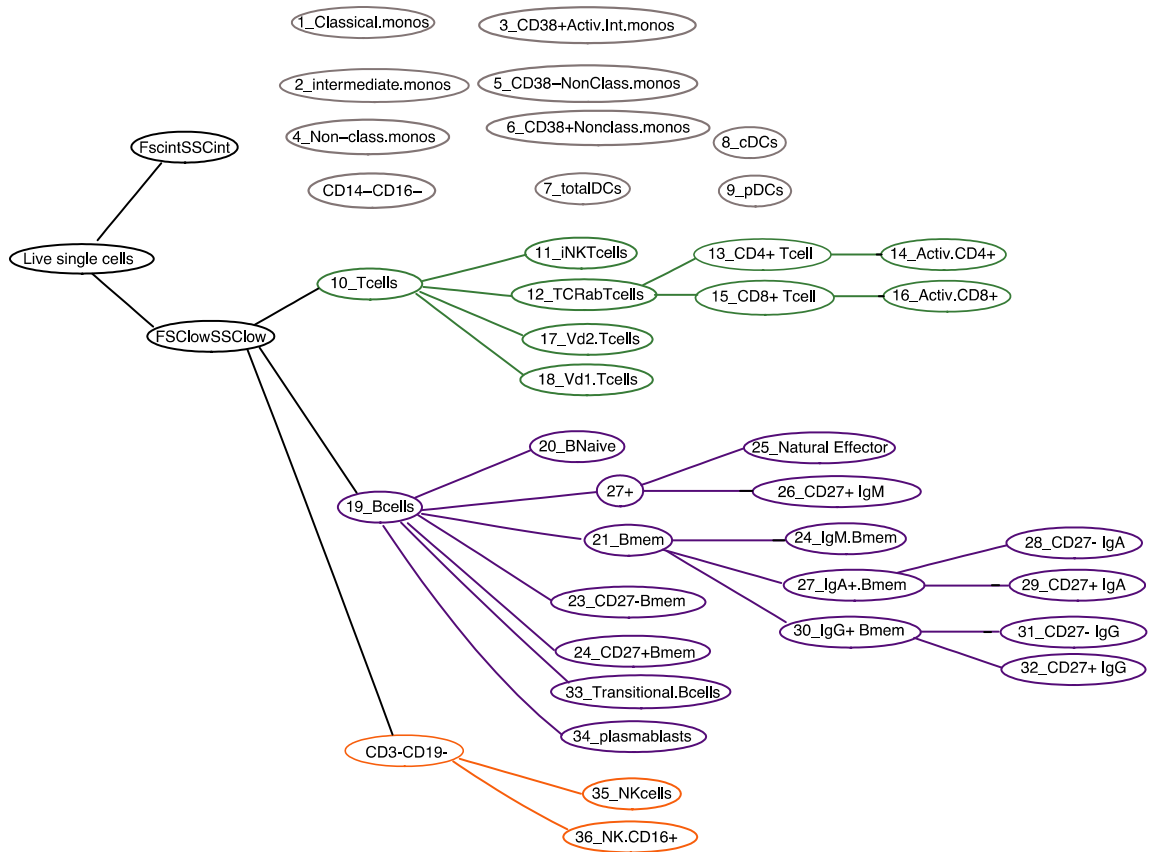


Fig. S2. Gating hierarchy panel 1.

The gating hierarchy for cell subsets defined using Panel 1. ‘Live single cells’ was the starting population. Circles represent the cell subsets, and the numbers correspond to the representative flow cytometry plots (fig. S4) and subset definitions (table S3). The colors correspond to those used in fig. S4.

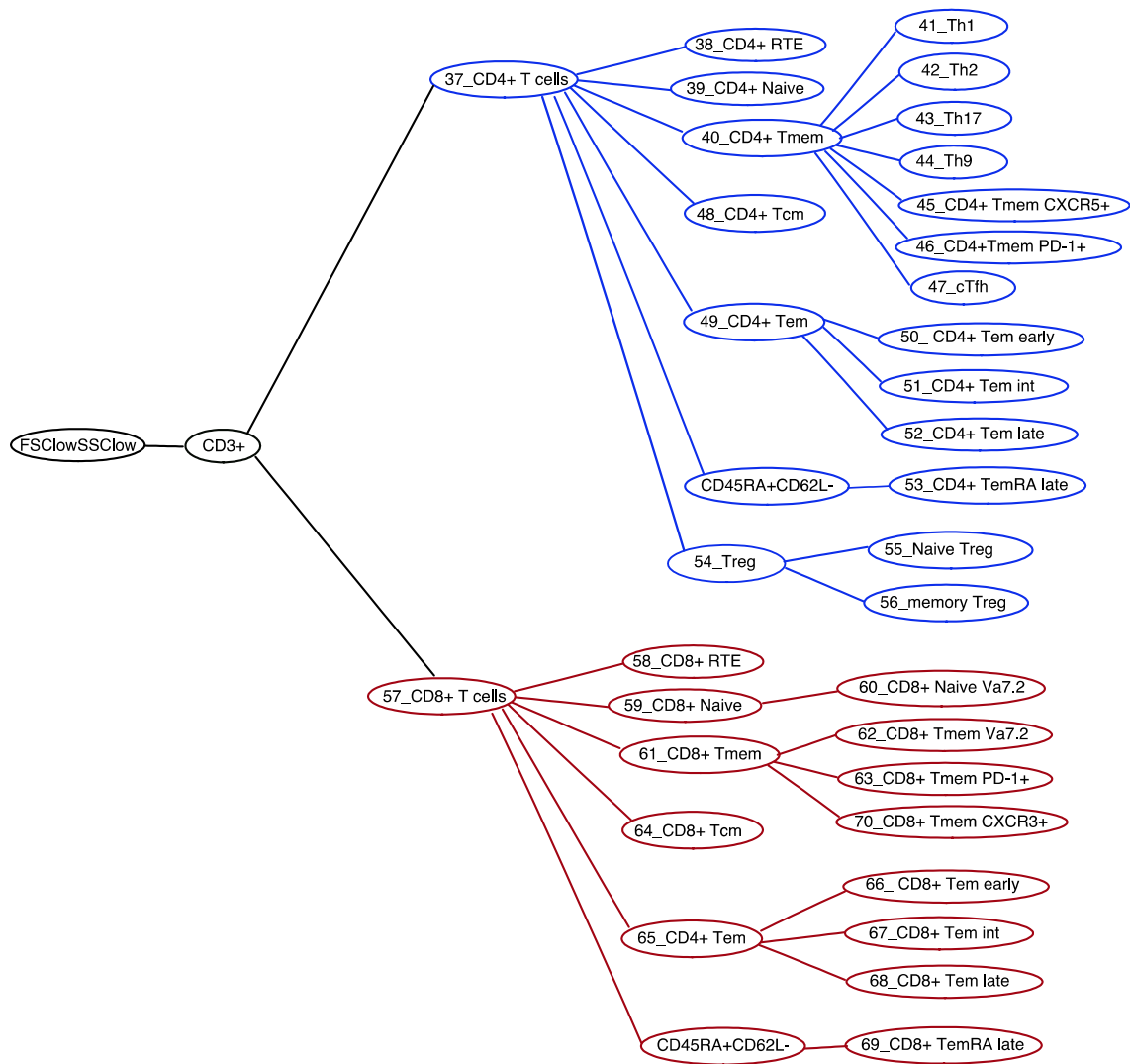


Fig. S3. Gating hierarchy panel 2.

The gating hierarchy for cell subsets defined using Panel 2. ‘Live single cells’ was the starting population. Circles represent the cell subsets, and the numbers correspond to the representative flow cytometry plots (fig. S5) and subset definitions (table S4). The colors correspond to those used in fig. 5.

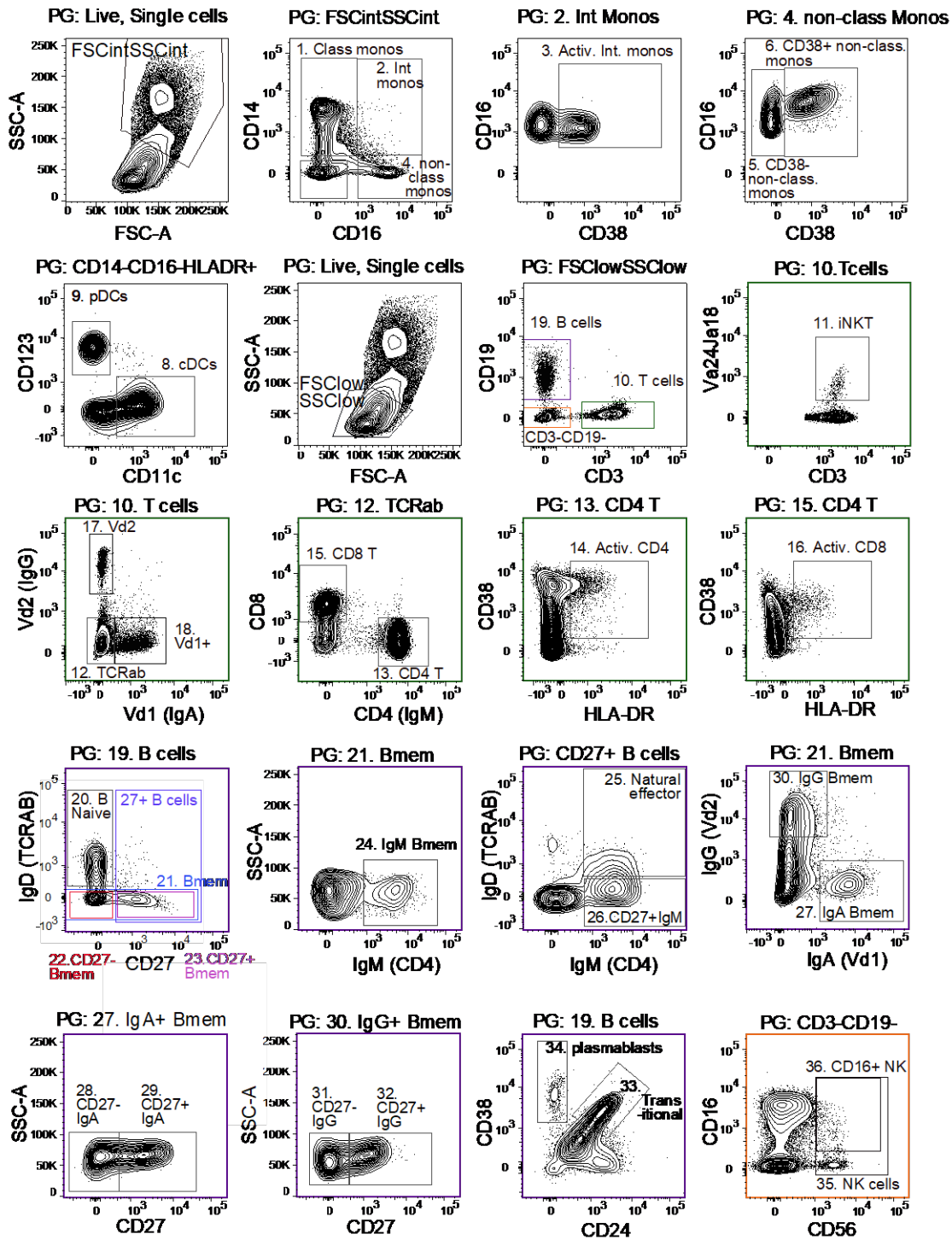


Fig. S4. Representative flow plots panel 1.

Representative flow cytometry plots for manually gated cell subsets from antibody panel 1. Parent gate (PG) listed above each plot describes the parental population (see also fig. S2 and table S3). The plot outline colors correspond to those used in fig. S3.

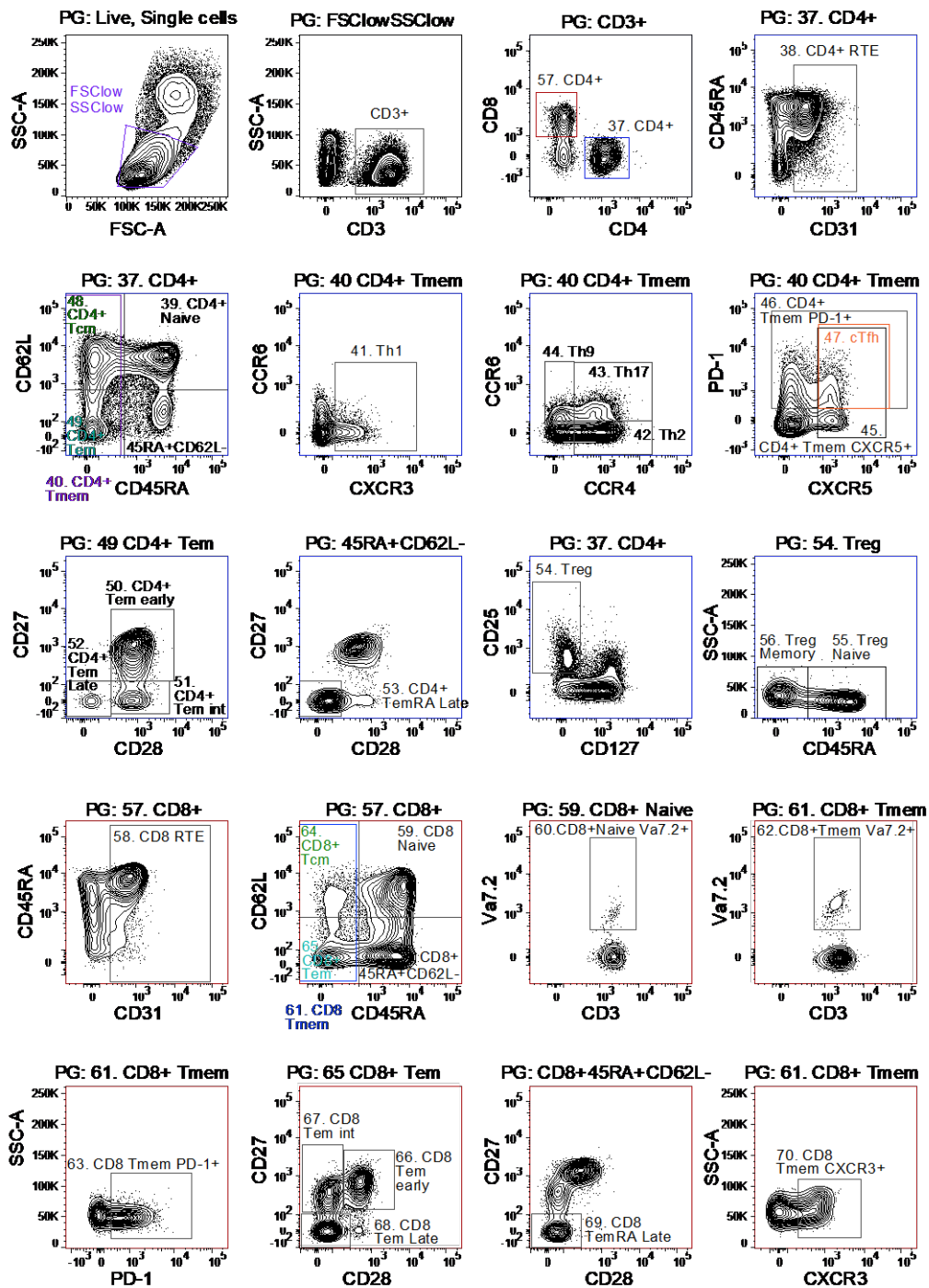


Fig. S5. Representative flow plots panel 2.

Representative flow cytometry plots for manually gated cell subsets from antibody panel 1. Parent gate (PG) listed above each plot describes the parental population (see also fig. S3 and table S4). The plot outline colors correspond to those used in fig. S4.

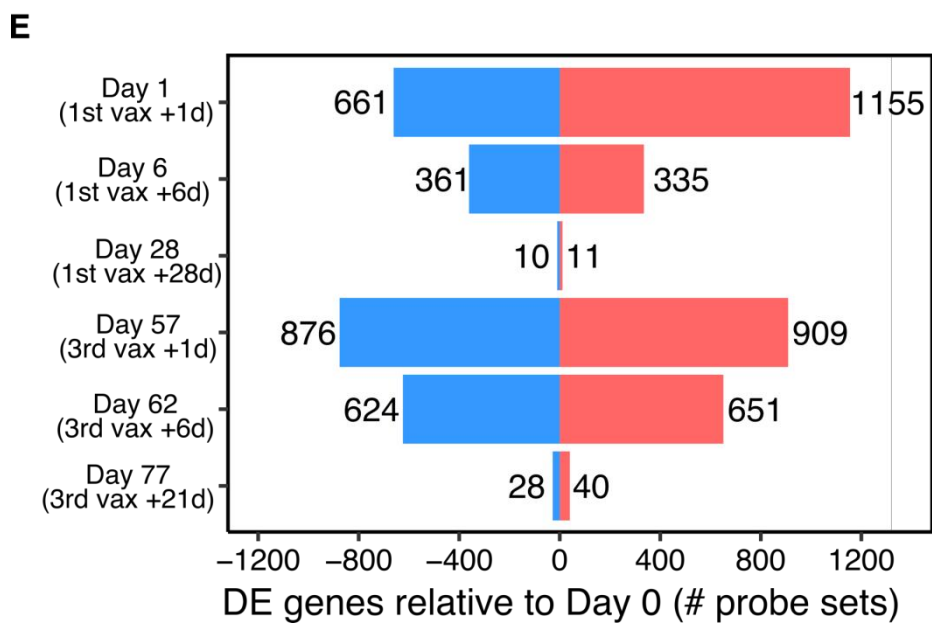
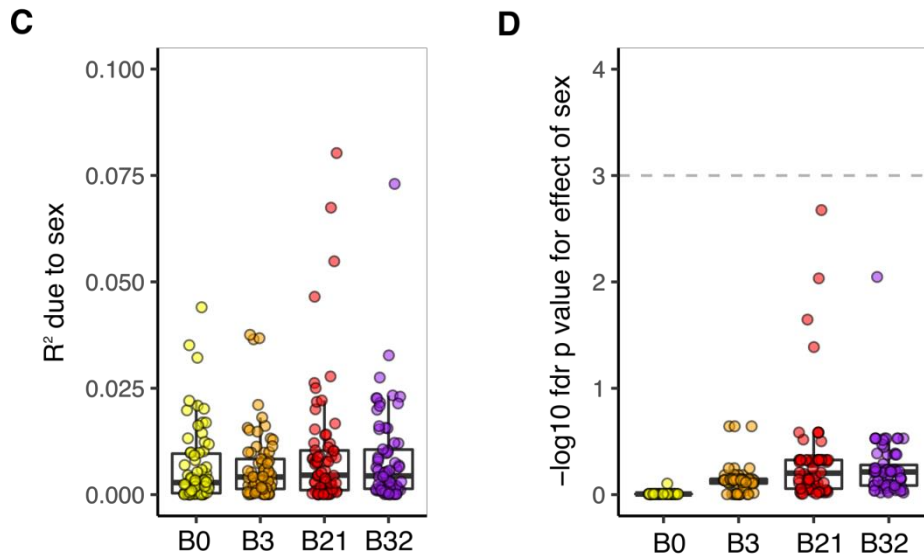
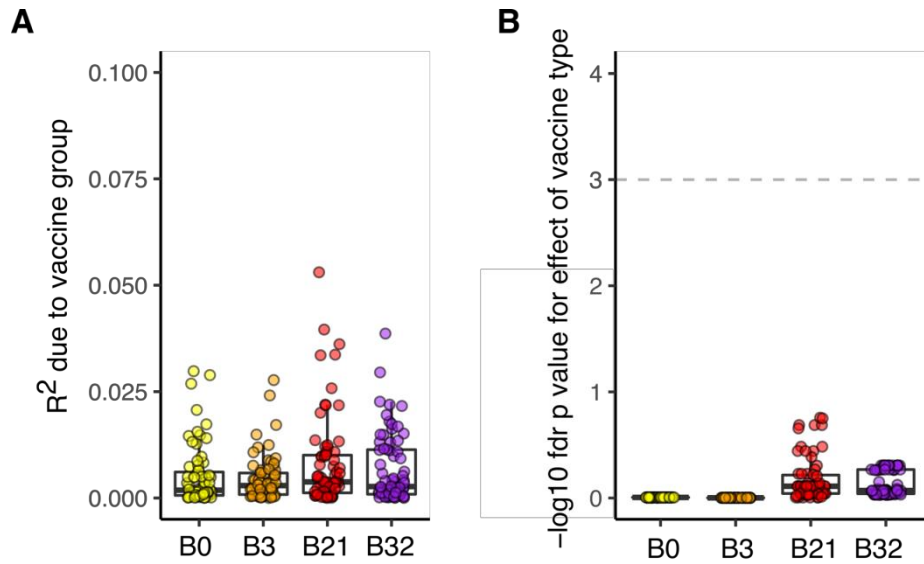


Fig. S6. No vaccine-induced changes are detectable 1 month after vaccination.

The proportion of variance (A) and corresponding p-value (B, $-\log_{10}$ fdr adjusted) attributed to vaccine group (R3R, R3C, C3C) at each time point for each cell type was determined by linear regression (modelled as cell type \sim vaccine group + sex+ subject ID). The proportion of variance (C) and corresponding p-value (D, $-\log_{10}$ fdr adjusted) attributed to sex (male or female) at each time point for each cell type was determined by linear regression (modelled as cell type \sim sex+ subject ID). In B and D any population with an adjusted p-value of less than 0.01 were deemed significantly different, the dotted line indicates $p_{adj}=0.001$. E) Analysis of microarray data from Kazmin et al (13), in which gene expression was measured at day 0, 1, 6 and 28 days after the first RTS,S vaccination, and days 1 (57), 6 (62) and 21 (77) days after the third vaccination (n=14 individuals at each time-point). Differential gene expression was determined relative to pre-vaccination (Day 0 or Day 56) using Limma to fit a linear model to each gene, and probe sets with fdr-adjusted p-values of <0.01 , and a \log_2 fold change of >1 or <-1 are shown. The number of probe sets that were upregulated (red) or downregulated (blue) at each time-point are indicated on the graph.

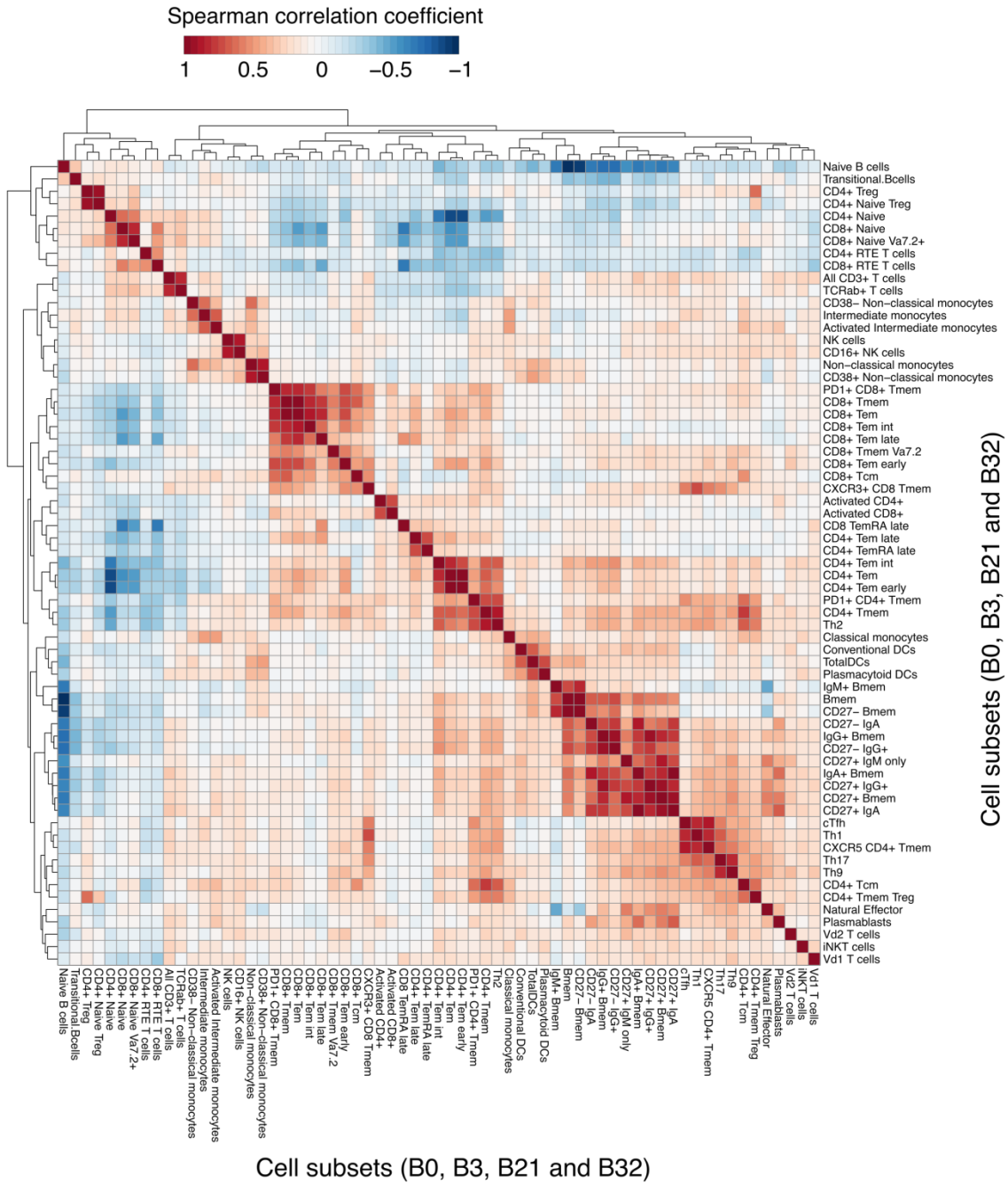


Fig. S7. Strong correlations observed within related cell types.

Spearman correlation coefficients for cell subsets (n=65, as a % of their parent population) including samples from all time-points in children from Tanzanian and Mozambique (n=718). All correlations were statistically significant ($p < 0.001$).

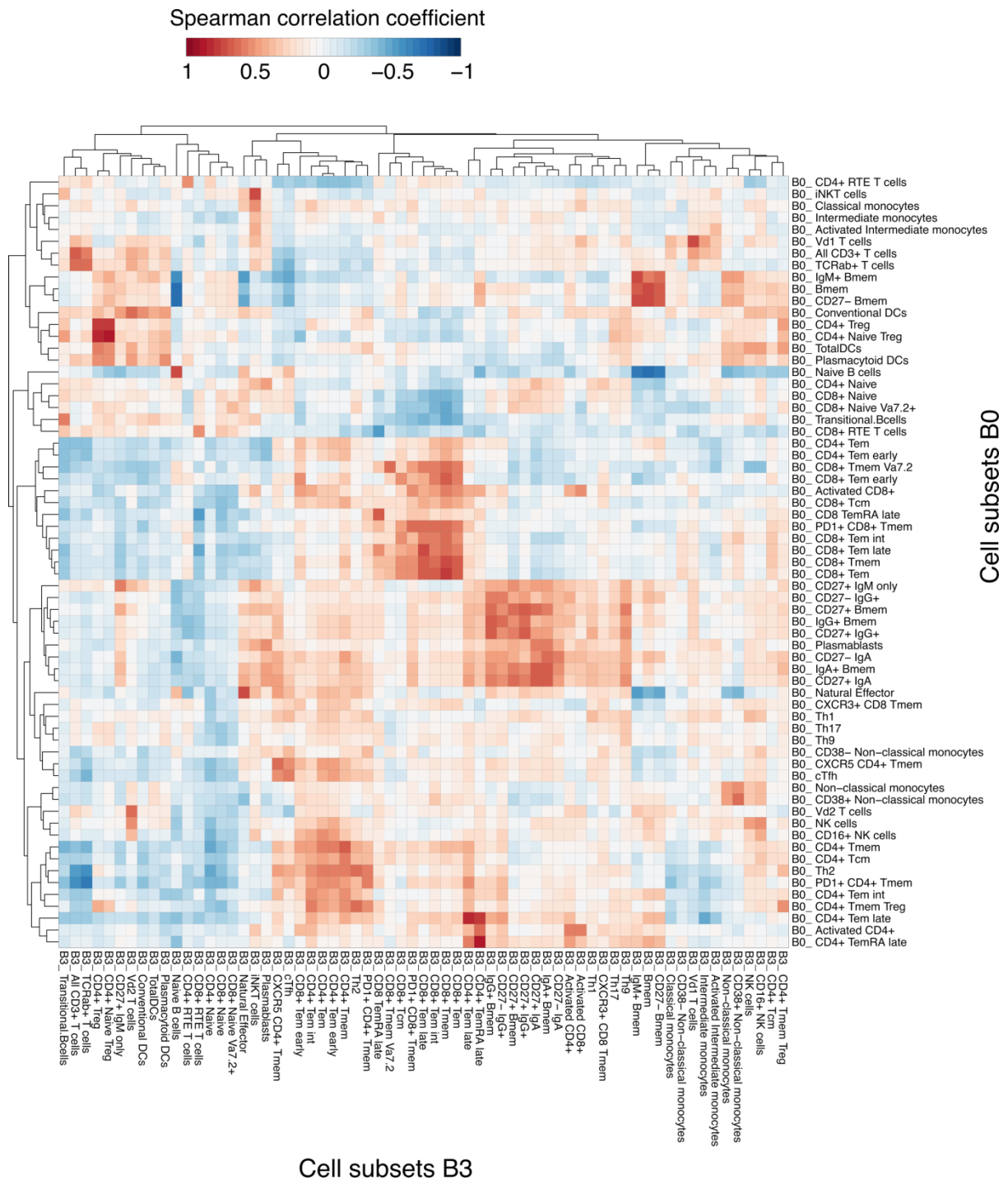


Fig. S8. Cell subset frequency correlations between baseline and B3 time points.

Spearman correlation coefficients for cell subsets ($n=65$, as a % of their parent population) including participants with samples from baseline (B0) and 3 months into the trial (B3) in children from Tanzanian and Mozambique ($n=120$). All correlations were statistically significant ($p < 0.001$).

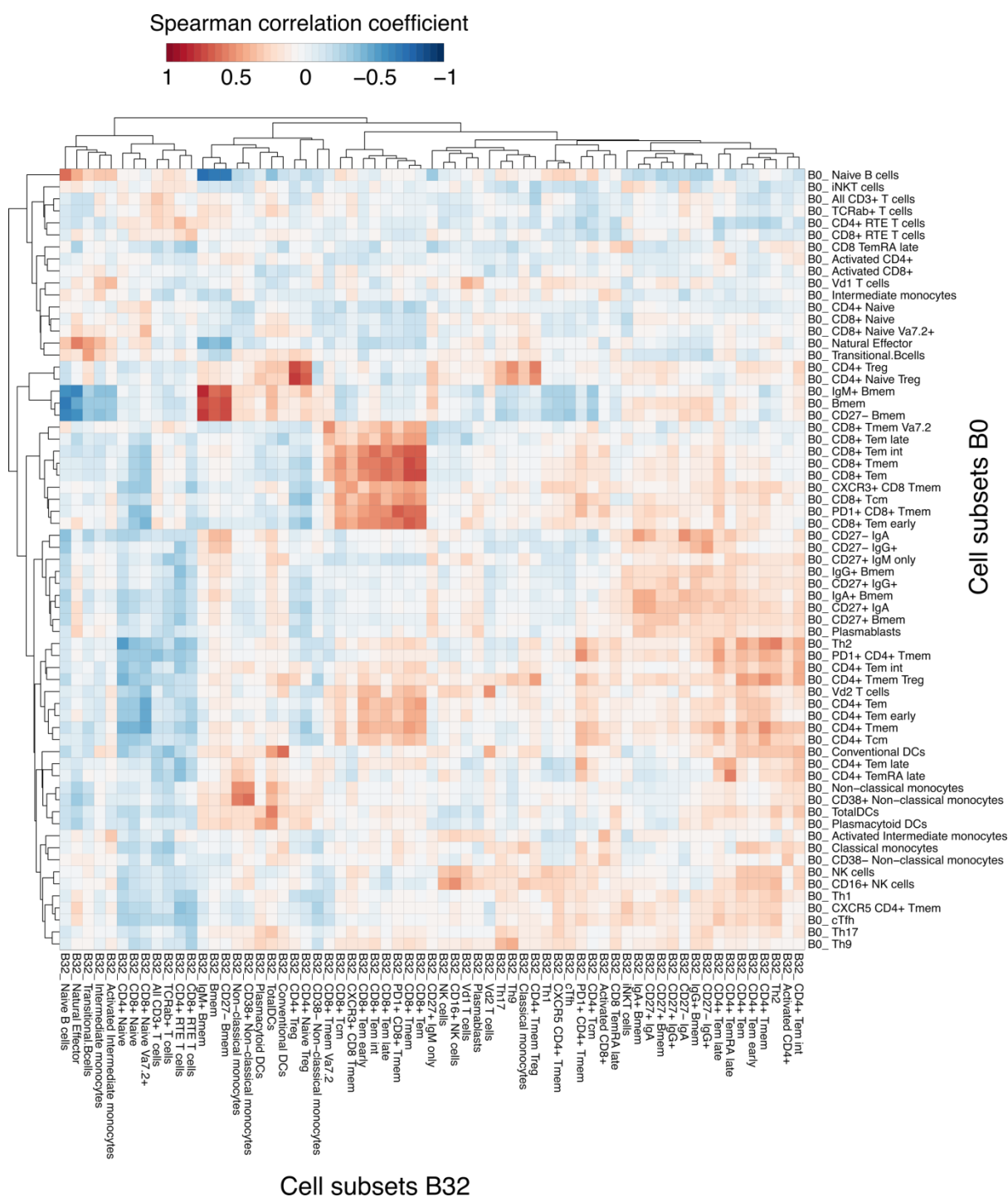


Fig. S9. Cell subset frequency correlations between baseline and B32 time points.

Spearman correlation coefficients for cell subsets (n=65, as a % of their parent population) including samples from baseline (B0) and 32 months into the trial (B32) in children from Tanzanian and Mozambique (n=120). All correlations were statistically significant ($p < 0.001$).

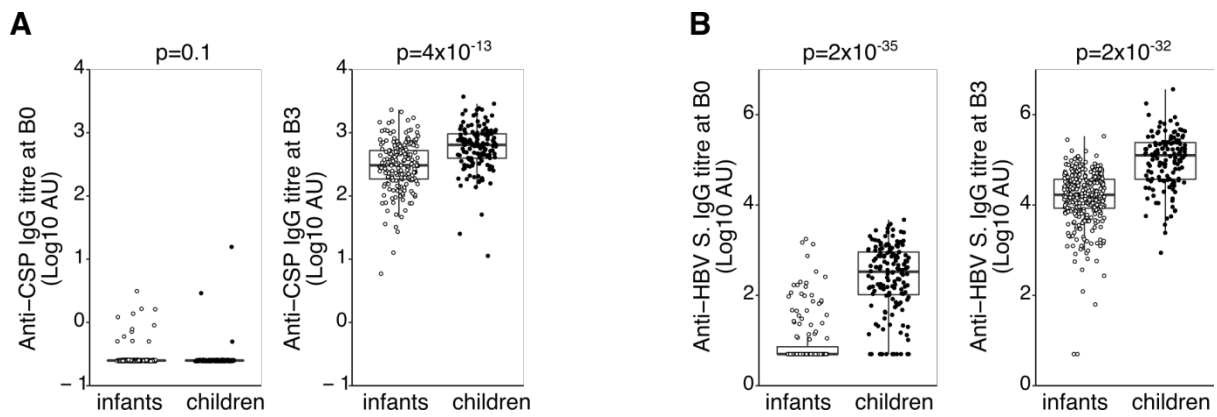


Fig. S10. Anti-CSP and anti-HBV.S IgG titers before and after vaccination in infants and children from Mozambique.

IgG titers for infants and children from Mozambique at B0 and B3. IgG titers measured by ELISA as Log₁₀ of arbitrary units (AU) are shown for (A) CSP and (B) HBV.S antigens. P-values determined using Mann-Whitney test.

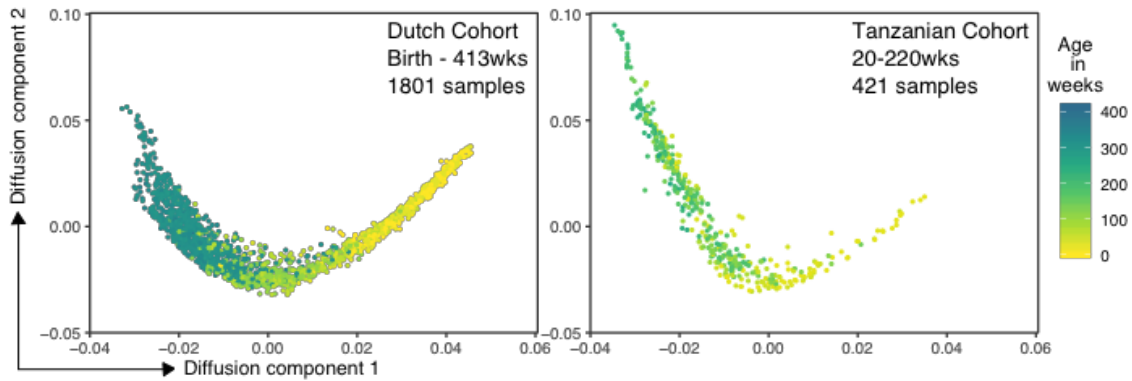


Fig. S11. Distribution of participant age along the diffusion-pseudotime trajectory.

Diffusion-map dimensionality reduction of all Dutch and Tanzanian samples using scaled cellular frequencies and the diffusion-pseudotime algorithm. The trajectory was built with 2222 samples as shown in Fig. 5, and shown here are the diffusion component co-ordinates separated for each study. Each dot represents a sample, color represents the corresponding age in weeks for each sample.

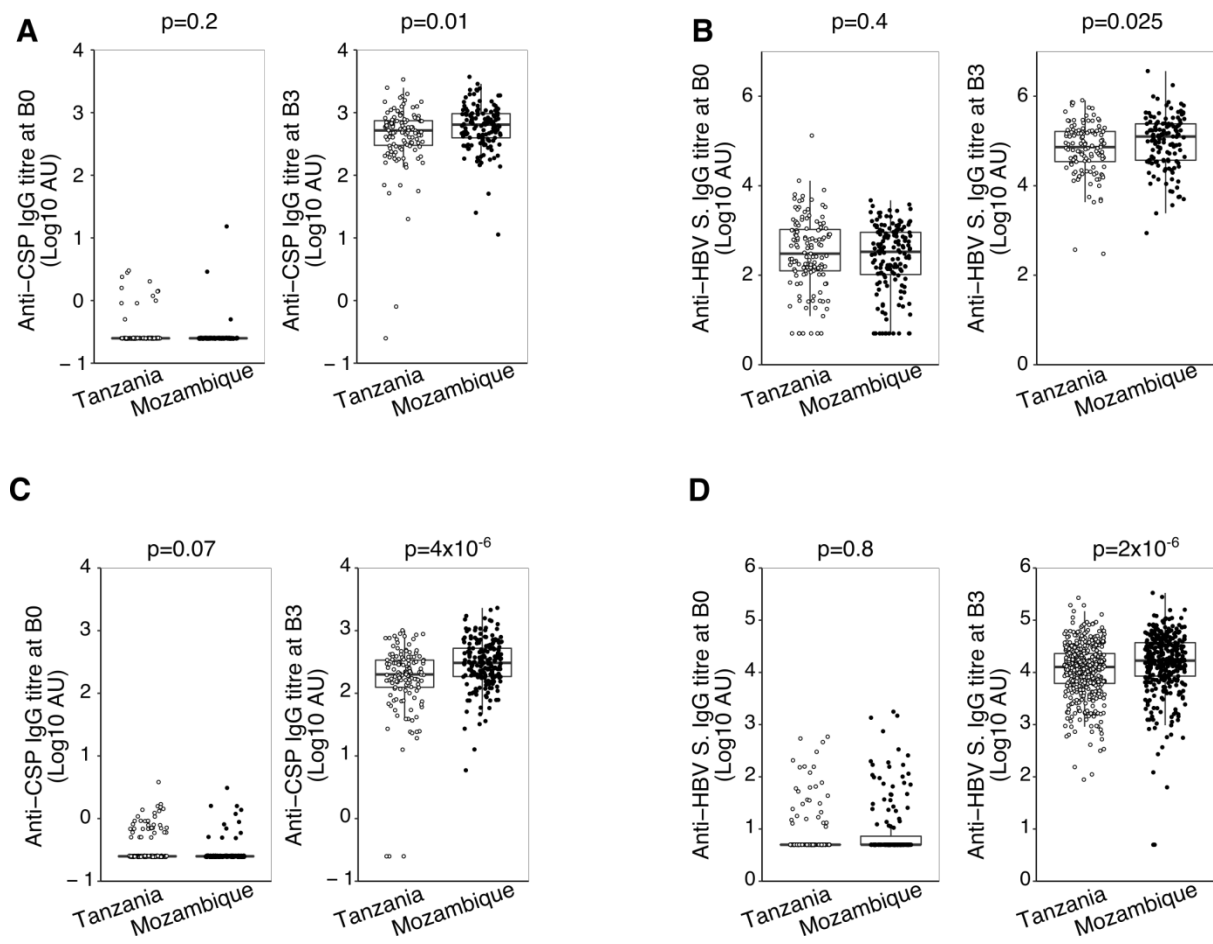


Fig. S12. Anti-CSP and anti-HBV.S IgG titers before and after vaccination in children from Tanzania and Mozambique.

IgG titers measured by ELISA as Log₁₀ of arbitrary units (AU) are shown for (A, C) CSP and (B, D) HBV.S antigens. A-B) IgG titers for children from Tanzania and Mozambique at B0 and B3. C-D) Titers for infants from Tanzania and Mozambique at B0 and B3. P-values determined using Mann-Whitney test.

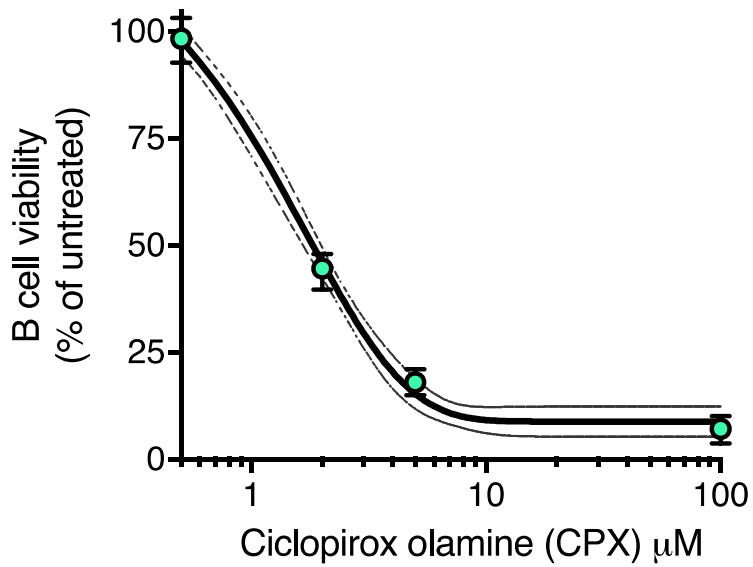


Fig. S13. Dose-dependent toxicity of ciclopirox olamine on B cells in culture.

Purified B cells from adult UK volunteers were stimulated in culture media containing IL-21 and CD40L for 5 days. CPX was added to cultures after 24 hours at the concentrations indicated ranging from 500nM – 100 μM and cell viability assessed by flow cytometry and staining with antibody panels containing a viability dye. Representative of 1 experiment, 6 biological replicates.

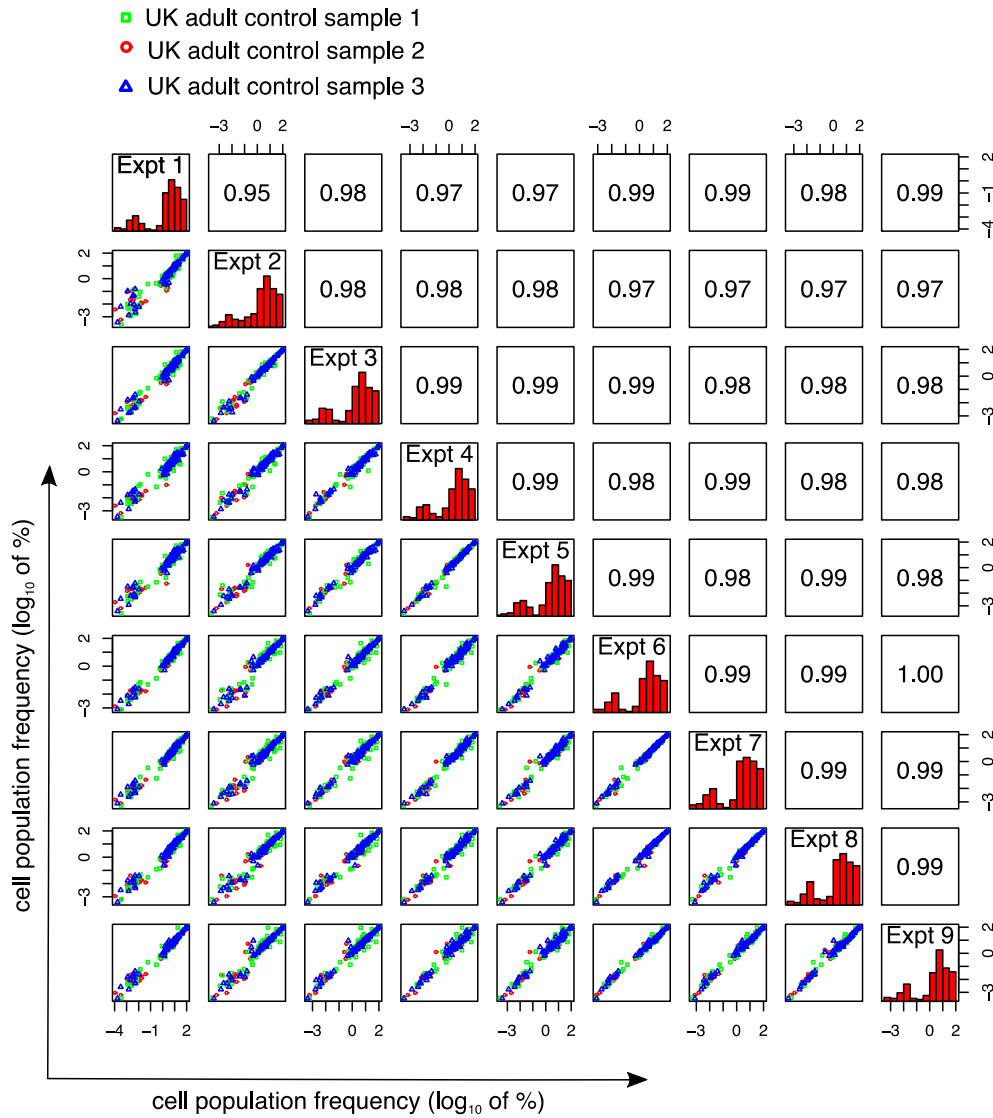


Fig. S14. Highly reproducible flow cytometry data between experiments.

Three UK adult volunteer PBMC samples were included in each experiment to measure batch effects. Shown here are the correlations between the 70 manually gated cell types (% of lymphocytes \log_{10} transformed) between each experiment ($n=270$ per experiment). Symbol color and shape represent the different UK adult samples used. The red histograms represent the distribution of cell subset frequency in each experiment. All experiments were highly correlated ($p<0.0001$) and the numbers in the upper quadrant represent the Pearson correlation coefficient (R^2) for each comparison.

Table S1. Participant characteristics.

	Study participant samples [n]		
RTS,S Immunophenotyping cohort	Total	Infants	Children
Both sites	214	43	169
Mozambique	98	43	55
Mozambique Female	46	20	26
Tanzania	116	0	116
Tanzania Female	52	0	52
RTS,S Serology Cohort			
Both sites	458	198	260
Mozambique	335	198	137
Tanzania	123	0	123

Table S2. Consolidated Standards of Reporting Trials table.

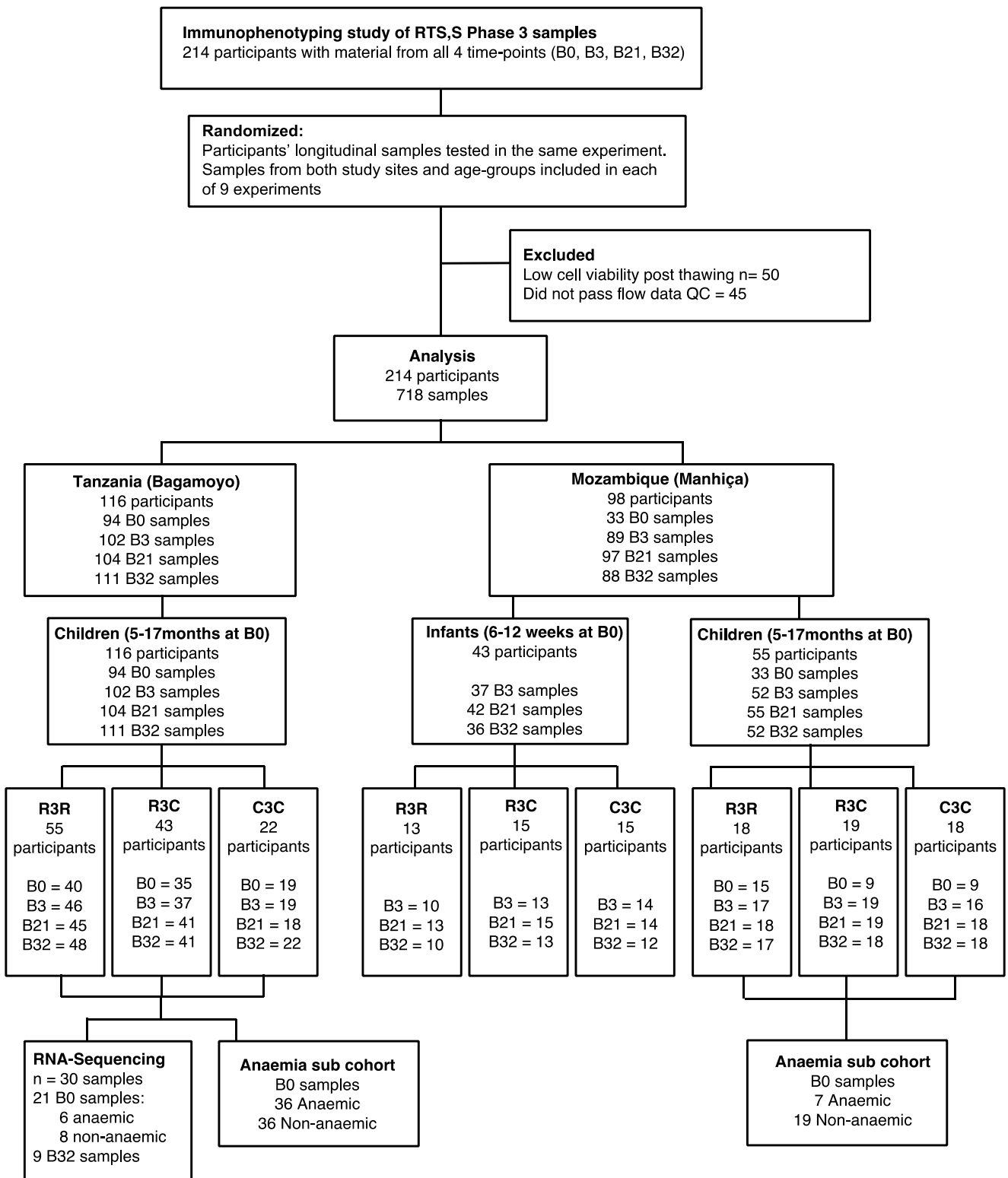


Table S3. Antibody panels.

Panel	marker	clone	fluo	company	Catalogue number
1, 2, 3*	viability dye	n/a	eFluor780	Thermo Fisher	65-0865-18
1, 2, 3*	CD32 (FcR Block)	6C4	n/a	ThermoFisher	16-0329-85
1& 2	CD3	UCHT1	BUV395	BD Biosciences	563546
1	CD19	SJ25C1	BUV496	BD Biosciences	564655
1, 3*	IgD	IA6-2	BUV737	BD Biosciences	564687
1	TCRab	T10B9	BUV737	BD Biosciences	564725
1	vδ1	TS8.2	FITC	Thermo Fisher	TCR2730
1	IgA	goat poly	FITC	Southern biotech	2050-02
1	CD4	SK3	Percpe710	ThermoFisher	46-0047-42
1	IgM	SA-DA4	Percpe710	ThermoFisher	46-9998-42
1	CD38	HIT2	BV421	Biolegend	303526
1	CD16	3G8	BV480	BD Biosciences	566108
1	CD24	ML5	BV605	Biolegend	311124
1, 3*	CD27	O323	BV711	Biolegend	302834
1	CD11C	3.9	BV786	Biolegend	301644
1	vδ2	B6	PE	Biolegend	331408
1	IgG	goat poly	PE	Southern biotech	2040-09
1	CD123	6H6	PE*Dazzle	Biolegend	306034
1	CD56	CMSSB	PeCy5.5	ThermoFisher	35-0567-42
1	CD14	61D3	PeCy7	ThermoFisher	25-0149-42
1	Va24-Ja18	6B11	Pecy7	Biolegend	342912
1	HLADR	LN3	APC	ThermoFisher	17-9956-42
1& 2	CD8	RPA-T8	A700	ThermoFisher	56-0088-42
2	CD4	SK3	BUV496	BD Biosciences	564651
2	CD45RA	HI100	BUV737	BD Biosciences	564442
2	CD28	CD28.2	BB515	BD Biosciences	555726
2	CD31	WM-59	PerCpe710	ThermoFisher	46-0319-42
2	CXCR3	1C6/CXCR3	BV421	BD Biosciences	562558
2	CD62L	DREG-56	BV480	BD Biosciences	566174
2	CCR4	L291H4	BV605	Biolegend	359418
2	CD25	BC96	BV650	Biolegend	302634
2	TCRVa7.2	3C10	BV711	Biolegend	351732
2	CCR6	11A9	BV786	BD Biosciences	563704
2	CXCR5	J252D4	PE	Biolegend	356920
2	CD127	A019D5	PE*Dazzle	Biolegend	351336
2	CD27	O323	PeCy7	ThermoFisher	25-0279-42
2	PD1	eBioJ105	APC	ThermoFisher	17-2799-42
3*	CD19	HIB19	BB515	BD Biosciences	564456
3*	CD20	2H7	PECY7	Biolegend	302312
3*	CD38	HIT2	APC	eBioscience	17-0389-42
3*	IRF4	IRF4.3E4	PE	Biolegend	646403

* Note: Panel 3 was used to stain in vitro cultured B cells, while Panel 1 and 2 were used with African PBMC samples

Table S4. Manually gated cell subset definitions panel 1.

#	Population name	Markers used for manual gating
1	Classical.monos	FSCintSSCint; CD14+; CD16-
2	Intermediate.monos	FSCintSSCint; CD14+; CD16+
3	CD38+Activ.Int.monos	FSCintSSCint; CD14+; CD16+; CD38+
4	Non-class.monos	FSCintSSCint; CD14-; CD16+
5	CD38-NonClass.monos	FSCintSSCint; CD14-; CD16+; CD38-
6	CD38+Nonclass.monos	FSCintSSCint; CD14-; CD16+; CD38+
7	Total DCs	FSCintSSCint; CD14-; CD16-; HLA-DR+
8	cDCs	FSCintSSCint; CD14-; CD16-; HLA-DR+; CD11c+;CD123-
9	pDCs	FSCintSSCint; CD14-; CD16-; HLA-DR+; CD11c- ;CD123+
10	T cells	FSClowSSClow; CD3+ ; CD19-
11	iNKT cells	FSClowSSClow; CD3+ ; CD19- ; Va24-Ja18+
12	TCRab Tcells	FSClowSSClow; CD3+ ; CD19- ; Vd1- ; Vd2-
13	CD4+ Tcell	FSClowSSClow; CD3+ ; CD19- ; Vd1- ; Vd2- ; CD4+ ; CD8-
14	Activ. CD4+	FSClowSSClow; CD3+ ; CD19- ; Vd1- ; Vd2- ; CD4+ ; CD8- ; HLA-DR+ ; CD38+
15	CD8+ Tcell	FSClowSSClow; CD3+ ; CD19- ; Vd1- ; Vd2- ; CD4- ; CD8+
16	Activ. CD8+	FSClowSSClow; CD3+ ; CD19- ; Vd1- ; Vd2- ; CD4- ; CD8+ ; HLA-DR+ ; CD38+
17	Vd2	FSClowSSClow; CD3+ ; CD19- ; Vd1- ; Vd2+
18	Vd1	FSClowSSClow; CD3+ ; CD19- ; Vd1+ ; Vd2-
19	B cells	FSClowSSClow; CD3- ; CD19+
20	B Naïve	FSClowSSClow; CD3- ; CD19+ ; CD27- ; IgD+
21	Bmem	FSClowSSClow; CD3- ; CD19+ ; IgD-
22	CD27- Bmem	FSClowSSClow; CD3- ; CD19+ ; IgD- ; CD27-
23	CD27+ Bmem	FSClowSSClow; CD3- ; CD19+ ; IgD- ; CD27+
24	IgM Bmem	FSClowSSClow; CD3- ; CD19+ ; IgD- ; IgM+
25	Natural effector	FSClowSSClow; CD3- ; CD19+ ; CD27+ ; IgD+ ; IgM+
26	CD27+ IgM	FSClowSSClow; CD3- ; CD19+ ; CD27+ ; IgD- ; IgM+
27	IgA Bmem	FSClowSSClow; CD3- ; CD19+ ; IgD- ; IgA+ ; IgG-
28	CD27-IgA	FSClowSSClow; CD3- ; CD19+ ; IgD- ; IgA+ ; IgG- ; CD27-
29	CD27+IgA	FSClowSSClow; CD3- ; CD19+ ; IgD- ; IgA+ ; IgG- ; CD27+
30	IgG Bmem	FSClowSSClow; CD3- ; CD19+ ; IgD- ; IgA- ; IgG+
31	CD27- Bmem	FSClowSSClow; CD3- ; CD19+ ; IgD- ; IgA- ; IgG+ ; CD27-
32	CD27+ Bmem	FSClowSSClow; CD3- ; CD19+ ; IgD- ; IgA- ; IgG+ ; CD27+
33	Transitional B cells	FSClowSSClow; CD3- ; CD19+ ; CD24+ ; CD38+
34	plasmablasts	FSClowSSClow; CD3- ; CD19+ ; CD24- ; CD38++
35	NK cells	FSClowSSClow; CD3- ; CD19- ; CD56+
36	CD16+ NK	FSClowSSClow; CD3- ; CD19- ; CD56+ ; CD16+

Abbreviations: monos - monocytes; NonClass - non classical; Activ. -Activated; cDCs - conventional dendritic cells; pDCs - plasmacytoid dendritic cell, Vd2 - Vdelta2 expressing gamma-delta cell; Vd1 - Vdelta1 expressing gamma-delta cell.

Table S5. Manually gated cell subset definitions panel 2.

#	Population name	Markers used for manual gating
37	CD4+ T cells	FSClowSSClow; CD3+ ; CD4+ ; CD8-
38	CD4+ RTE	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD31+
39	CD4+ Naïve	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA+ ; CD62L+
40	CD4+ Tmem	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA-
41	Th1	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CXCR3+
42	Th2	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CCR4+ CCR6-
43	Th17	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CCR4+ CCR6+
44	Th9	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CCR4- CCR6+
45	CD4+ Tmem CXCR5+	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CXCR5+
46	CD4+ Tmem PD-1+	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; PD-1+
47	cTfh	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CXCR5+ ; PD-1+
48	CD4+ Tcm	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CD62L+
49	CD4+ Tem	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CD62L-
50	CD4+ Tem early	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CD62L- ; CD27+ ; CD28+
51	CD4+ Tem int	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CD62L- ; CD27- ; CD28+
52	CD4+ Tem late	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CD62L- ; CD27- ; CD28-
53	CD4+ TemRA late	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA+ ; CD62L- ; CD27- ; CD28-
54	Treg	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD25+ ; CD127-
55	Naïve Treg	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD25+ ; CD127- ; CD45RA+
56	memory Treg	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD25+ ; CD127- ; CD45RA-
57	CD8+ T cells	FSClowSSClow; CD3+ ; CD4- ; CD8+
58	CD8+ RTE	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD31+
59	CD8+ Naïve	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA+ ; CD62L+
60	CD8+ Naïve Va7.2+	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA+ ; CD62L+ ; Va7.2+
61	CD8+ Tmem	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA-
62	CD8+ Tmem Va7.2+	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA- ; Va7.2+
63	CD8+ Tmem PD-1+	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA- ; PD-1+
64	CD8+ Tcm	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA- ; CD62L+
65	CD8+ Tem	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA- ; CD62L-
66	CD8+ Tem early	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA- ; CD62L- ; CD27+ ; CD28+
67	CD8+ Tem int	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA- ; CD62L- ; CD27- ; CD28+
68	CD8+ Tem late	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA- ; CD62L- ; CD27- ; CD28-
69	CD8+ TemRA late	FSClowSSClow; CD3+ ; CD4- ; CD8+ ; CD45RA+ ; CD62L- ; CD27- ; CD28-
70	CD8+ Tmem CXCR3+	FSClowSSClow; CD3+ ; CD4+ ; CD8- ; CD45RA- ; CXCR3+

Abbreviations: RTE - recent thymic emigrant ; Tmem - memory T cells ; cTfh - circulating T follicular helper cell ; Tcm - Central memory T cells; Tem - Effector memory T cells; TemRA - CD45RA+ Tem cell; Treg - Regulatory T cell.