

*Supplementary Materials*

**Supplementary Table 1. Composition of the antibody panels**

tube	fluorochrome										
	BV421	BV510	BV605	BV711	FITC/ BB515	PerCP- Cy5.5	PE	PE- Dazzle	PC7 / PE-Cy7	APC	APC-H7 / APC-Ax750
1. TruCount	-	-	-	-	CD3	CD45	CD16 + CD56	-	CD4	CD19	CD8A
2. B-cell	CD27	IgM	CD38	CD21	IgE + IgA	IgD	IgG + IgA	-	CD19	CD5	CD24
3. T-effector	CD27	CD4	CD45RA	CD3	CD45RO	CD28	CD31	CCR7	TCR $\gamma\delta$	HLA-DR	CD8A
4. Th subset	CD25	CD4	CD45RA	CD3	CXCR5	CCR6	CXCR3	CCR7	CCR4	CD127	CD8A

**Supplementary Table 2. Antibody list**

Marker	Fluorochrome	Clone	Source	Cat. number	volume/ test (in $\mu$ l)	tube(s)
CD3	FITC	UCHT1	BD Biosciences	555332	3	1
CD3	BV711	UCHT1	BD Biosciences	563725	2.5	3, 4
CD4	PC7	SFC112T4D11	Beckman Coulter	6607101	0.2	1
CD4	BV510	RPA-T4	Biologend	300546	1.5	3, 4
CD5	APC	UCHT2	Biologend	300612	0.2	2
CD8A	APC-H7	SK1	BD Biosciences	560179	4	1, 3, 4
CD16	PE	B73.1	Biologend	360704	0.2	1
CD19	APC	SJ25C1	Biologend	363006	0.4	1
CD19	PE-CY7	SJ25C1	BD Biosciences	557835	5	2
CD21	BV711	B-ly4	BD Biosciences	563163	5	2
CD24	APC-Alexa750	ALB9	Beckman Coulter	B10738	2.5	2
CD25	BV421	BC96	Biologend	302630	2.5	4
CD27	BV421	M-T271	BD Biosciences	562513	1	2, 3
CD28	PerCP-Cy5.5	CD28.2	Biologend	302922	5	3
CD31	PE	WM59	BD Biosciences	555446	5	3
CD38	BV605	HB7	BD Biosciences	562665	0.2	2
CD45	PerCP-Cy5.5	2D1	BD Biosciences	340953	2	1
CD45RA	BV605	HI100	Biologend	304134	0.2	3, 4
CD45RO	FITC	UCHL1	Biologend	304204	5	3
CD56	PE	B159	BD Biosciences	555516	5	1
CD127	APC	A019D5	Biologend	351316	5	4
CCR4	PE-CY7	L291H4	Biologend	359410	5	4
CCR6	PerCP-CY5.5	G034E3	Biologend	353406	2.5	4
CCR7	PE-Dazzle	G043H7	Biologend	353236	2.5	3, 4
*CCR7	PE-CF594	150503	BD Biosciences	562381	5	3, 4
CXCR3	PE	1C6/CXCR3	BD Biosciences	557185	20	4
CXCR5	BB515	RF8B2	BD Biosciences	564624	5	4
HLA-DR	APC	L243	Biologend	307610	2	3
IgA	VioBright FITC	IS11-8E10	Miltenyi Biotec	130-104-726	0.5	2
IgA	PE	IS11-8E10	Miltenyi Biotec	130-093-128	0.5	2
IgD	PerCP-Cy5.5	IA6-2	Biologend	348208	1.5	2
IgE	FITC	goat polyclonal	Invitrogen	H15701	1	2
IgG	PE	G18-145	BD Biosciences	555787	5	2
IgM	BV510	MHM-88	Biologend	314522	1	2
TCR $\gamma$ $\delta$	PC7	IMMU510	Beckman Coulter	B10247	1	3

\* alternative reagent

**Supplementary Table 3. Definition of leukocyte subsets**

n=55	Population name	Phenotype definition
<b>Tube 1: Trucount</b>		
1	Granulocytes	SSC <sup>high</sup> CD45 <sup>+</sup>
2	Monocytes	SSC <sup>inter</sup> CD45 <sup>+</sup>
3	Lymphocytes	SSC <sup>low</sup> CD45 <sup>+</sup>
4	└ B cells	SSC <sup>low</sup> CD45 <sup>+</sup> CD19 <sup>+</sup>
5	└ NK cells	SSC <sup>low</sup> CD45 <sup>+</sup> CD3 <sup>-</sup> CD16 <sup>+</sup> and/or CD56 <sup>+</sup>
6	└ T cells	SSC <sup>low</sup> CD45 <sup>+</sup> CD3 <sup>+</sup> CD16 <sup>-</sup> or CD56 <sup>-</sup>
	└ CD4 <sup>+</sup> T cells	SSC <sup>low</sup> CD45 <sup>+</sup> CD3 <sup>+</sup> CD16 <sup>-</sup> or CD56 <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup>
	└ CD8 <sup>+</sup> T cells	SSC <sup>low</sup> CD45 <sup>+</sup> CD3 <sup>+</sup> CD16 <sup>-</sup> or CD56 <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup>
<b>Tube 2: B cells</b>		
	B cells	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup>
7	transitional B cells	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>hi</sup> CD27 <sup>-</sup>
8	plasmablasts	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>hi</sup> CD27 <sup>+</sup>
9	CD21 <sup>lo</sup> B cells	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>lo</sup>
	CD21 <sup>+</sup> B cells	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup>
	└ CD21 <sup>+</sup> CD27 <sup>-</sup>	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>-</sup>
10	└ naive B cells	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>-</sup> IgM <sup>+</sup> IgD <sup>+/-</sup>
11	└ pre-naive	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>-</sup> IgM <sup>+</sup> IgD <sup>+/-</sup> CD5 <sup>+</sup>
12	└ naive/mature	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>-</sup> IgM <sup>+</sup> IgD <sup>+/-</sup> CD5 <sup>-</sup>
	└ CD27 <sup>-</sup> Memory	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>-</sup> IgM <sup>+</sup> IgD <sup>-</sup>
13	└ IgG <sup>+</sup> CD27 <sup>-</sup> memory	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>-</sup> IgM <sup>+</sup> IgD <sup>-</sup> IgG <sup>+</sup>
14	└ IgA <sup>+</sup> CD27 <sup>-</sup> memory	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>-</sup> IgM <sup>+</sup> IgD <sup>-</sup> IgA <sup>+</sup>
	└ IgE <sup>+</sup> CD27 <sup>-</sup> memory	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>-</sup> IgM <sup>+</sup> IgD <sup>-</sup> IgE <sup>+</sup>
	└ CD21 <sup>+</sup> CD27 <sup>+</sup>	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>+</sup>
15	└ natural effector	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>+</sup> IgM <sup>+</sup> IgD <sup>+</sup>
16	└ IgM only	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>+</sup> IgM <sup>+</sup> IgD <sup>-</sup>
17	└ IgG <sup>+</sup> CD27 <sup>+</sup> memory	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>+</sup> IgM <sup>+</sup> IgD <sup>-</sup> IgA <sup>+</sup>
18	└ IgA <sup>+</sup> CD27 <sup>+</sup> memory	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>+</sup> IgM <sup>+</sup> IgD <sup>-</sup> IgA <sup>+</sup>
	└ IgE <sup>+</sup> CD27 <sup>+</sup> memory	FSC <sup>low</sup> SSC <sup>low</sup> CD19 <sup>+</sup> CD38 <sup>low</sup> CD21 <sup>+</sup> CD27 <sup>+</sup> IgM <sup>+</sup> IgD <sup>-</sup> IgE <sup>+</sup>
<b>Tube 3: T effector</b>		
	T cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup>
19	γδ <sup>+</sup> T cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>+</sup>
	γδ <sup>-</sup> T cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup>
20	└ CD4 T cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup>
21	└ CD4 Tnaive	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CCR7 <sup>+</sup> CD45RO <sup>-</sup>
22	└ CD4 Tcm	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CCR7 <sup>+</sup> CD45RO <sup>+</sup>
23	└ CD4 TemRO	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CCR7 <sup>-</sup> CD45RO <sup>+</sup>
24	└ CD4 early TemRO	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CCR7 <sup>-</sup> CD45RO <sup>+</sup> CD27 <sup>+</sup> CD28 <sup>+</sup>
25	└ CD4 intermediate TemRO	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CCR7 <sup>-</sup> CD45RO <sup>+</sup> CD27 <sup>-</sup> CD28 <sup>+</sup>
26	└ CD4 late TemRO	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CCR7 <sup>-</sup> CD45RO <sup>+</sup> CD27 <sup>-</sup> CD28 <sup>-</sup>
27	└ CD4 TemRA	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CCR7 <sup>-</sup> CD45RO <sup>-</sup>
28	└ CD4 early TemRA	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CCR7 <sup>-</sup> CD45RO <sup>-</sup> CD27 <sup>+</sup> CD28 <sup>+</sup>
29	└ CD4 intermediate TemRA	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CCR7 <sup>-</sup> CD45RO <sup>-</sup> CD27 <sup>-</sup> CD28 <sup>+</sup>
30	└ CD4 late TemRA	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CCR7 <sup>-</sup> CD45RO <sup>-</sup> CD27 <sup>-</sup> CD28 <sup>-</sup>
31	└ CD8 T cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup>
32	└ CD8 Tnaive	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CCR7 <sup>+</sup> CD45RO <sup>-</sup>
33	└ CD8 Tcm	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CCR7 <sup>+</sup> CD45RO <sup>+</sup>
34	└ CD8 TemRO	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CCR7 <sup>-</sup> CD45RO <sup>+</sup>
35	└ CD8 early TemRO	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CCR7 <sup>-</sup> CD45RO <sup>+</sup> CD27 <sup>+</sup> CD28 <sup>+</sup>
36	└ CD8 intermediate TemRO	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CCR7 <sup>-</sup> CD45RO <sup>+</sup> CD27 <sup>-</sup> CD28 <sup>+</sup>
37	└ CD8 late TemRO	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CCR7 <sup>-</sup> CD45RO <sup>+</sup> CD27 <sup>-</sup> CD28 <sup>-</sup>
38	└ CD8 TemRA	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CCR7 <sup>-</sup> CD45RO <sup>-</sup>
39	└ CD8 early TemRA	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CCR7 <sup>-</sup> CD45RO <sup>-</sup> CD27 <sup>+</sup> CD28 <sup>+</sup>
40	└ CD8 intermediate TemRA	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CCR7 <sup>-</sup> CD45RO <sup>-</sup> CD27 <sup>-</sup> CD28 <sup>+</sup>
41	└ CD8 late TemRA	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> γδ <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CCR7 <sup>-</sup> CD45RO <sup>-</sup> CD27 <sup>-</sup> CD28 <sup>-</sup>
<b>Tube 4: Th subset</b>		
	T Cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup>

	CD8 T Cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>-</sup> CD8 <sup>+</sup>
42	└ CD8 Tfh Cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>-</sup> CD8 <sup>+</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup>
	CD4 T Cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup>
43	└ Treg cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>+</sup> CD127 <sup>lo/-</sup>
44	└ naive Treg	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>+</sup> CD127 <sup>lo</sup> CCR4 <sup>+</sup> CD45RA <sup>+</sup>
45	└ memory Treg	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>+</sup> CD127 <sup>lo</sup> CCR4 <sup>-</sup> CD45RA <sup>-</sup>
46	└ CD4 Tfr	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>+</sup> CD127 <sup>lo</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup>
	└ Non-Treg cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup>
	└ Th Cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>-</sup> CD45RA <sup>-</sup>
	└ CCR6- Th	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>-</sup> CD45RA <sup>-</sup> CCR6 <sup>-</sup>
47	└ Th1	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>-</sup> CD45RA <sup>-</sup> CCR6 <sup>-</sup> CCR4 <sup>-</sup> CXCR3 <sup>+</sup>
48	└ Th2	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>-</sup> CD45RA <sup>-</sup> CCR6 <sup>-</sup> CCR4 <sup>+</sup> CXCR3 <sup>-</sup>
	└ CCR6+ Th	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>-</sup> CD45RA <sup>-</sup> CCR6 <sup>+</sup>
49	└ Th17	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>-</sup> CD45RA <sup>-</sup> CCR6 <sup>+</sup> CCR4 <sup>+</sup> CXCR3 <sup>-</sup>
50	└ Th17.1	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>-</sup> CD45RA <sup>-</sup> CCR6 <sup>+</sup> CCR4 <sup>-</sup> CXCR3 <sup>+</sup>
51	└ Tfh Cells	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup>
	└ CCR6- Tfh	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup> CCR6 <sup>-</sup>
52	└ Tfh1	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup> CCR6 <sup>-</sup> CCR4 <sup>-</sup> CXCR3 <sup>+</sup>
53	└ Tfh2	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup> CCR6 <sup>-</sup> CCR4 <sup>+</sup> CXCR3 <sup>-</sup>
	└ CCR6+ Tfh	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup> CCR6 <sup>+</sup>
54	└ Tfh17	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup> CCR6 <sup>+</sup> CCR4 <sup>+</sup> CXCR3 <sup>-</sup>
55	└ Tfh17.1	FSC <sup>low</sup> SSC <sup>low</sup> CD3 <sup>+</sup> CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>-</sup> CD127 <sup>lo/hi</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup> CCR6 <sup>+</sup> CCR4 <sup>-</sup> CXCR3 <sup>+</sup>

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**Supplementary Table 4. Flowcytometer set-up**

LSRFortessa X-20		LSR-II and LSRFortessa		Fluorochromes used in this study
<b>355 nm</b>		-		-
379/28	No LP	-	-	-
525/50	505 LP	-	-	-
740/35	690 LP	-	-	-
<b>405 nm</b>		<b>405 nm</b>		
450/50	No LP	450/50	No LP	BV421
525/50	505 LP	525/50	505 LP	BV510
-	-	586/15	570 LP	-
610/20	600 LP	610/20	600 LP	BV605
670/30	635 LP	660/20	630 LP	-
710/50	685 LP	710/50	685 LP	BV711
780/60	750 LP	780/60	750 LP	-
<b>488 nm</b>		<b>488 nm</b>		
488/10	No LP	488/10	No LP	SSC
530/30	505 LP	530/30	505 LP	FITC, BB515, VioBright FITC
710/50	685 LP	710/50	630 LP	PerCP-Cy5.5
<b>561 nm</b>		<b>561 nm</b>		
586/15	No LP	582/15	No LP	PE
610/20	600 LP	610/20	600 LP	PE-Dazzle
675/50	635 LP	685/35	635 LP	-
780/60	750 LP	780/60	750 LP	PE-Cy7, PC7
<b>640 nm</b>		<b>633 nm</b>		
670/30	No LP	670/14	No LP	APC
730/45	690 LP	730/45	690 LP	Fixable Viability Stain 700
780/60	750 LP	780/60	750 LP	APC-H7, APC-Alexa750

**Supplementary Table 5. Target values for 7<sup>th</sup> peak of rainbow beads in fluorescent channels**

<b>Fluorochrome</b>	<b>Channel</b>	Lower (-15%)	<b>Target MFI</b>	Upper (+15%)	recommendation
<b>BV421</b>	<b>V450</b>	100,452	<b>118,178</b>	135,905	EuroFlow
<b>BV510</b>	<b>V525</b>	93,871	<b>110,436</b>	127,002	EuroFlow
<b>BV605</b>	<b>V610</b>	47,731	<b>56,154</b>	64,577	In-house
<b>BV711</b>	<b>V710</b>	15,079	<b>17,740</b>	20,401	In-house
<b>FITC, BB515</b>	<b>B530</b>	28,752	<b>33,826</b>	38,900	EuroFlow
<b>PerCP-Cy5.5</b>	<b>B710</b>	66,846	<b>78,642</b>	90,438	EuroFlow
<b>PE</b>	<b>YG586</b>	32,381	<b>38,095</b>	43,809	EuroFlow
<b>PE-Dazzle</b>	<b>YG610</b>	178,500	<b>210,000</b>	241,500	In-house
<b>PE-Cy7</b>	<b>YG780</b>	8,316	<b>9,783</b>	11,250	EuroFlow
<b>APC</b>	<b>R670</b>	158,639	<b>186,634</b>	214,629	EuroFlow
<b>AF700</b>	<b>R730</b>	121,550	<b>143,000</b>	164,450	In-house
<b>APC-H7</b>	<b>R780</b>	64,194	<b>75,522</b>	86,850	EuroFlow

Spherotech Rainbow Calibration particles (8 peaks) 3.41µm; cat nr. RCP-30-5A, Lot No. EAG01

Supplementary Table 6: Clinical and immunological details of all patients

Patient	Age at inclusion (yr)	Age at diagnosis (yr)	Sex	Clinical Diagnosis	IgRT at time of sampling	anti-inflammatory within 4-6 months prior to sampling	B-cells/ $\mu$ l blood	T-cells/ $\mu$ l blood	IgG at inclusion (g/L)	IgA (g/L)	IgM (g/L)	Impaired vaccination responses	Infectious complications	Non-infectious complications	NIC at diagnosis
1	18	2/12	M	XLA	Y	N	<1	<u>3148</u>	N/A	N/A	N/A	N/A	sinusitis	none	N/A
2	21	3/12	M	XLA	Y	N	<1	1652	12*	0.1	0.1	N/A	pneumonia, bronchiectasis	none	N/A
3	22	3/12	M	XLA	Y	N	<1	1770	N/A	<0.1	<0.1	N/A	otitis, sinusitis, pneumonia	none	N/A
4	24	13	M	XLA	Y	N	10	1401	N/A	0.1	0.1	N/A	sinusitis, bronchiectasis, prostatitis	none	N/A
5	24	2	M	XLA	Y	N	<0.1	1497	N/A	<0.1	<0.1	N/A	otitis, sinusitis, pneumonia	leukemia^	N/A
6	26	1	M	XLA	Y	N	<0.1	<u>2478</u>	N/A	<0.1	<0.1	N/A	otitis, sinusitis, pneumonia, bronchiectasis	none	N/A
7	34	12	M	XLA	Y	N	1	625	N/A	<0.1	<0.1	N/A	otitis, sinusitis, pneumonia	none	N/A
8	49	12	M	XLA	Y	N	3	801	N/A	0.1	0.1	N/A	otitis, sinusitis, pneumonia, bronchiectasis	none	N/A
9	59	5	M	XLA	Y	HCT	<0.1	<u>3141</u>	N/A	<0.1	<0.1	N/A	otitis, sinusitis, pneumonia, asthma/COPD, bronchiectasis	none	N/A
10	24	18	F	agamma	N <sup>#</sup>	N	10	998	0.6	0.1	0.1	N/A	otitis, sinusitis, pneumonia	none	N/A
11	23	23	M	CVID	Y	N	446	1422	4.9	0.3	0.3	N/A	sinusitis	ITP/ AIHA/ Neutropenia/ Splenomegaly	Y
12	23	22	F	CVID	Y	N	215	1339	4.4	0.2	0.4	N/A	otitis, sinusitis	enteropathy	Y
13	24	24	M	CVID	N <sup>#</sup>	N	228	954	1.1	<0.1	<0.1	not done	otitis, sinusitis, pneumonia, VZV	none	N/A
14	24	23	F	CVID	Y	N	17	736	<1.4	<0.2	<0.2	not done	pneumonia, sinusitis	arthritis, enteropathy - duodenal and ilea tetralogy of Fallot/ VSD	Y
15	25	12	M	CVID	Y	N	106	<u>2562</u>	1.9	0.1	0.4	N/A	otitis, sinusitis, pneumonia, bronchiectasis	arthritis	Y
16	25	22	M	CVID	Y	N	230	1381	2.2	<0.1	0.1	N/A	sinusitis, pneumonia	none	N/A
17	26	15	M	CVID	Y	N	467	<u>3355</u>	5.6	0.6	0.3	pneumo	sinusitis	vitiligo	Y
18	27	27	F	CVID	Y	N	166	2008	3.9	0.7	0.3	N/A	pneumonia, bronchitis	pulmonary nodules and colitis	N
19	28	28	M	CVID	N <sup>#</sup>	N	56	819	3.3	1	0.4	not done	bronchitis, pneumonia	cytopenia	Y
20	30	28	F	CVID	Y	N	<u>1089</u>	<u>2953</u>	2.2	0.4	0.6	N/A	sinusitis	GLILD	Y
21	34	34	F	CVID	N <sup>#</sup>	N	46	1083	<1	<0.1	<0.1	N/A	otitis, sinusitis, pneumonia, bronchiectasis	enteropathy	N
22	35	30	F	CVID	Y	N	222	646	2	0.1	0.1	pneumo	sinusitis, giardia, asthma/COPD	granuloma, enteropathy	N
23	37	24	M	CVID	Y	N	78	981	4.1	0.1	0.4	pneumo, Hib	otitis, sinusitis, pneumonia, systemic viral infection, giardia, asthma/COPD, bronchiectasis	granuloma, enteropathy	N
24	41	31	M	CVID	Y	-	35	1107	2.6	0.2	0.2	pneumo	sinusitis, pneumonia, systemic viral infection	granuloma, enteropathy	Y
25	43	35	F	CVID	Y	-	101	1242	2	0.3	0.2	pneumo	sinusitis, pneumonia	cytopenia	Y
26	44	36	F	CVID	Y	-	177	614	2.7	0.1	0.1	pneumo	sinusitis, pneumonia, giardia	splenomegaly, enteropathy	N

Patient	Age at inclusion (yr)	Age at diagnosis (yr)	Sex	Clinical Diagnosis	IgRT at time of sampling	anti-inflammatory within 4-6 months prior to sampling	B-cells/ $\mu$ l blood	T-cells/ $\mu$ l blood	IgG at inclusion (g/L)	IgA (g/L)	IgM (g/L)	Impaired vaccination responses	Infectious complications	Non-infectious complications	NIC at diagnosis
27	44	43	M	CVID	Y	–	91	834	0.6	0	0.2	N/A	sinusitis, pneumonia, bronchiectasis	splenomegaly, lymphadenopathy, granuloma, enteropathy, arthritis	Y
28	46	40	F	CVID	Y	–	180	818	3	0.5	0.4	pneumo	otitis, sinusitis, shingles	urticaria	N
29	46	41	M	CVID	Y	–	228	870	0.6	0.1	0.1	pneumo	otitis, sinusitis	none	N/A
30	47	40	F	CVID	Y	–	84	1210	3.1	<0.1	<0.1	N/A	sinusitis, bronchitis, conjunctivitis	autoimmune hypothyroidism, collagenous colitis	N
31	52	52	F	CVID	N <sup>#</sup>	PNL	138	1214	4.8	0.8	1.4	pneumo	otitis, sinusitis, pneumonia	none	N/A
32	53	53	F	CVID	Y	PNL	96	678	3.8	0.9	0.8	N/A	bronchitis, sinusitis	pericarditis	Y
33	54	16	F	CVID	N <sup>#</sup>	–	373	1473	5.4	1.6	0.3	pneumo	bronchiectasis, sinusitis	hypothyroidism	Y
34	54	54	F	CVID	Y	–	95	631	11*	0.2	0.4	N/A	viral pneumonia, otitis	none	N/A
35	54	45	M	CVID	Y	–	245	775	2	<0.1	0.1	N/A	sinusitis, pneumonia, asthma/COPD, bronchiectasis	none	N/A
36	54	46	M	CVID	Y	–	152	1040	4.4	0.2	1	pneumo, diphtheria, tetanus, Hib	sinusitis, pneumonia	solid organ malignancy	N
37	56	44	M	CVID	Y	PNL	86	235	11.7*	<0.1	0.2	N/A	pneumonia, hepatitis, colitis	AIHA, ITP, marked splenomegaly	Y
38	62	59	F	CVID	N	–	204	1229	5.6	1.3	0.3	pneumo	sinusitis	none	N/A
39	66	67	F	CVID	Y	PNL	161	539	3.5	0.6	0.2	N/A	bronchitis, bronchiectasis, asthma	none	N/A
40	73	66	F	CVID	Y	–	154	1345	3.3	0.3	0.3	N/A	sinusitis, asthma/COPD, bronchiectasis	none	N/A
41	77	68	F	CVID	Y	PNL	70	553	4.2	0.8	1.9	diphtheria	systemic viral infection, bronchiectasis, ILD	solid organ malignancy, auto-immunity	Y
42	82	68	M	CVID	Y	–	13	727	9.8*	0.6	0.2	N/A	otitis, sinusitis, pneumonia, bronchiectasis	cytopenia	N
43	27	4	M	HGG	Y	–	148	742	10.8*	1.8	1.3	N/A	pneumonia	none	N/A
44	29	21	F	HGG	Y	–	144	1982	5.2	0.3	0.5	normal	asthma/COPD	none	N/A
45	31	27	F	HGG	Y	–	361	983	5.6	0.3	1.3	normal	sinusitis, systemic viral infection, giardia	enteropathy	Y
46	40	40	F	HGG	N	–	202	1038	5.2	1.5	0.9	normal	sinusitis, pneumonia	none	N/A
47	43	42	F	HGG	Y	–	116	1554	4.2	3.2	0.6	pneumo	sinusitis, pneumonia	inflammatory tracheal stenosis	Y
48	45	45	F	HGG	Y	–	570	2029	11*	3.7	1.6	normal	otitis, sinusitis, pneumonia, asthma/COPD, pulmonary nocardia & aspergillus	none	N/A
49	52	50	F	HGG	N	–	193	1329	5.2	0.8	1.3	pneumo	sinusitis, pneumonia	none	N/A
50	52	47	M	HGG	Y	–	195	473	3.2	0.3	0.2	Hib	sinusitis, pneumonia	none	N/A
51	55	54	F	HGG	N <sup>#</sup>	–	215	1362	4.1	0.4	0.8	normal	pneumonia	inflammatory tenosynovitis	Y



Patient	Age at inclusion (yr)	Age at diagnosis (yr)	Sex	Clinical Diagnosis	IgRT at time of sampling	anti-inflammatories within 4-6 months prior to sampling	B-cells/ $\mu$ l blood	T-cells/ $\mu$ l blood	IgG at inclusion (g/L)	IgA (g/L)	IgM (g/L)	Impaired vaccination responses	Infectious complications	Non-infectious complications	NIC at diagnosis
52	60	59	F	HGG	Y	–	119	1408	<b>5.9</b>	<b>0.7</b>	1.3	not done	sinusitis, otitis, pneumonia	Hashimoto's thyroiditis	Y
53	67	61	F	HGG	Y	–	191	1844	<b>4.6</b>	<b>0.6</b>	2.9	diphtheria	sinusitis, pneumonia, asthma/COPD	enteropathy, autoimmunity	Y
54	68	68	F	HGG	N <sup>#</sup>	–	266	1157	<b>5.6</b>	1.7	0.9	pneumo	pneumonia, sinusitis	none	N/A
55	79	74	F	HGG	Y	–	<b>81</b>	<b>706</b>	<b>4</b>	1.1	1.5	pneumo	sinusitis, bronchiectasis, osteomyelitis, pertussis	breast carcinoma, polymyalgia, asthma	Y
56	42	35	M	IGSCD	N	–	477	1796	6.9	<b>0.6</b>	1.1	normal	bronchitis	none	N/A
57	19	19	F	SpAD	N <sup>#</sup>	–	<b>92</b>	2090	11.1	<b>&lt;0.1</b>	0.9	pneumo	sinusitis, bronchitis, tonsillitis	none	N/A
58	21	20	M	SpAD	N	–	192	1209	9.4	0.9	1.1	pneumo	sinusitis	none	N/A
59	41	35	M	SpAD	Y	CT	236	1894	8.2*	2.1	<b>0.3</b>	pneumo	otitis, sinusitis, asthma/COPD	none	N/A
60	57	58	F	SpAD	N <sup>#</sup>	HCQ	252	1087	<u>32*</u>	3	0.5	pneumo	pneumonia	SLE/Sjögren's	Y
61	66	65	F	SpAD	N <sup>#</sup>	–	323	957	10.3	<b>&lt;0.1</b>	0.9	poor	pneumonia, bronchitis	none	N/A
62	72	72	F	SpAD	N <sup>#</sup>	–	119	1413	12	1.8	0.7	poor	pneumonia, bronchiectasis, MAC, pseudomonas	none	N/A

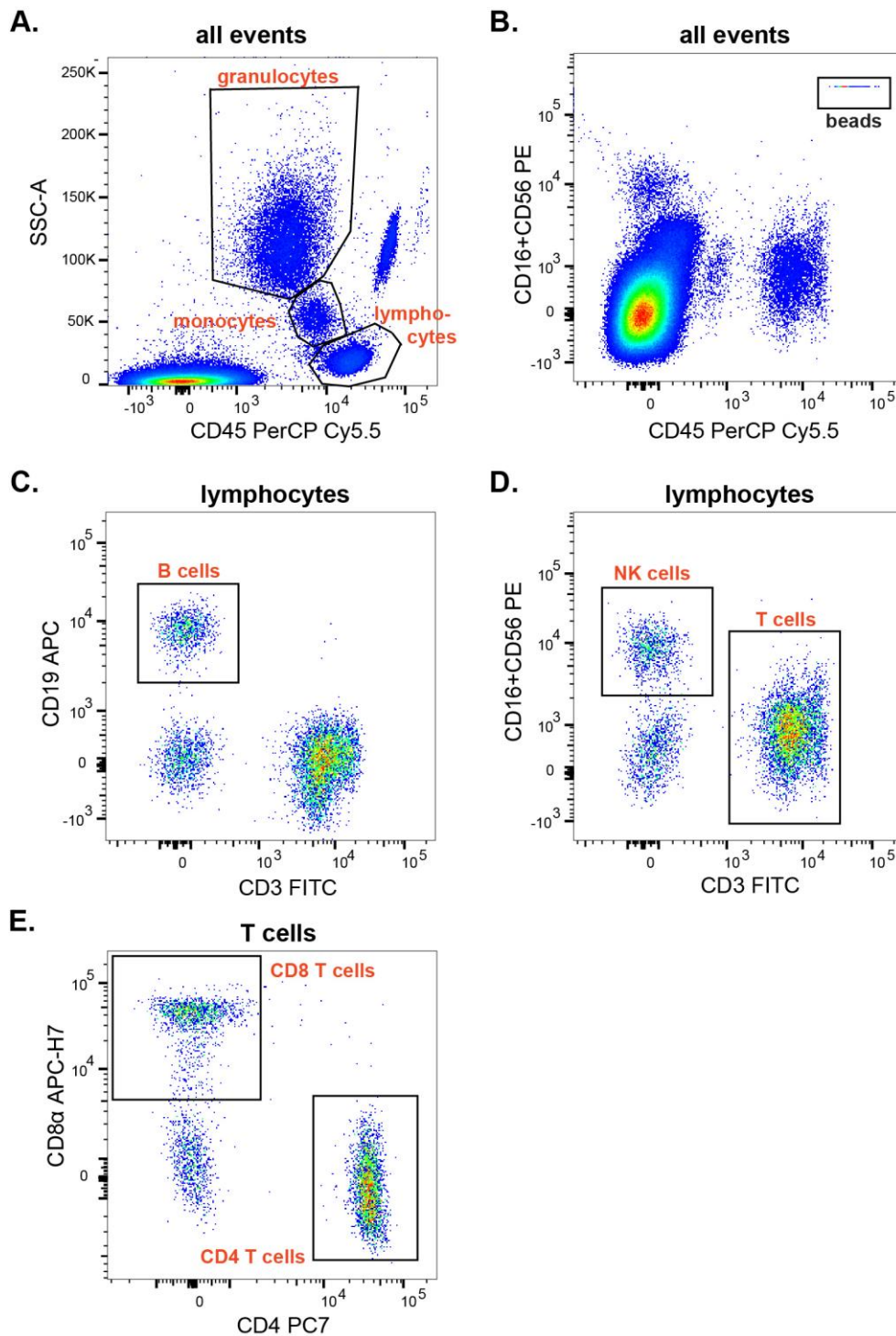
Values below normal range are depicted in **bold** font and above normal range underlined. Reference ranges: IgG, 6.1-16.2g/L; IgA, 0.85-4.99g/L; IgM, 0.35-2.42g/L; B cells, 97-614 cells/ $\mu$ l; T cells, 823-2430 cells/ $\mu$ l. (Reference ranges represent the 5<sup>th</sup> and 95<sup>th</sup> percentiles from our healthy controls).

<sup>#</sup> Ig replacement commenced after inclusion in this study; \* IgG measurement after start of Ig replacement therapy; ^leukemia diagnosed post inclusion (van Zelm et al., 2019); agamma, agammaglobulinemia; CVID, common variable immunodeficiency; HGG, hypogammaglobulinemia; IGSC, IgG subclass deficiency; SpAD, specific antibody deficiency; XLA, X-linked agammaglobulinemia (genetically diagnosed see **Supplementary Table 6**), IgRT, immunoglobulin replacement therapy; CT, cortisone; HCT, hydrocortisone; HCQ, hydroxychloroquine; PNL, prednisolone; MAC, Mycobacterium Avium complex; pneumo, pneumococcal; SLE, systemic lupus erythematosus.

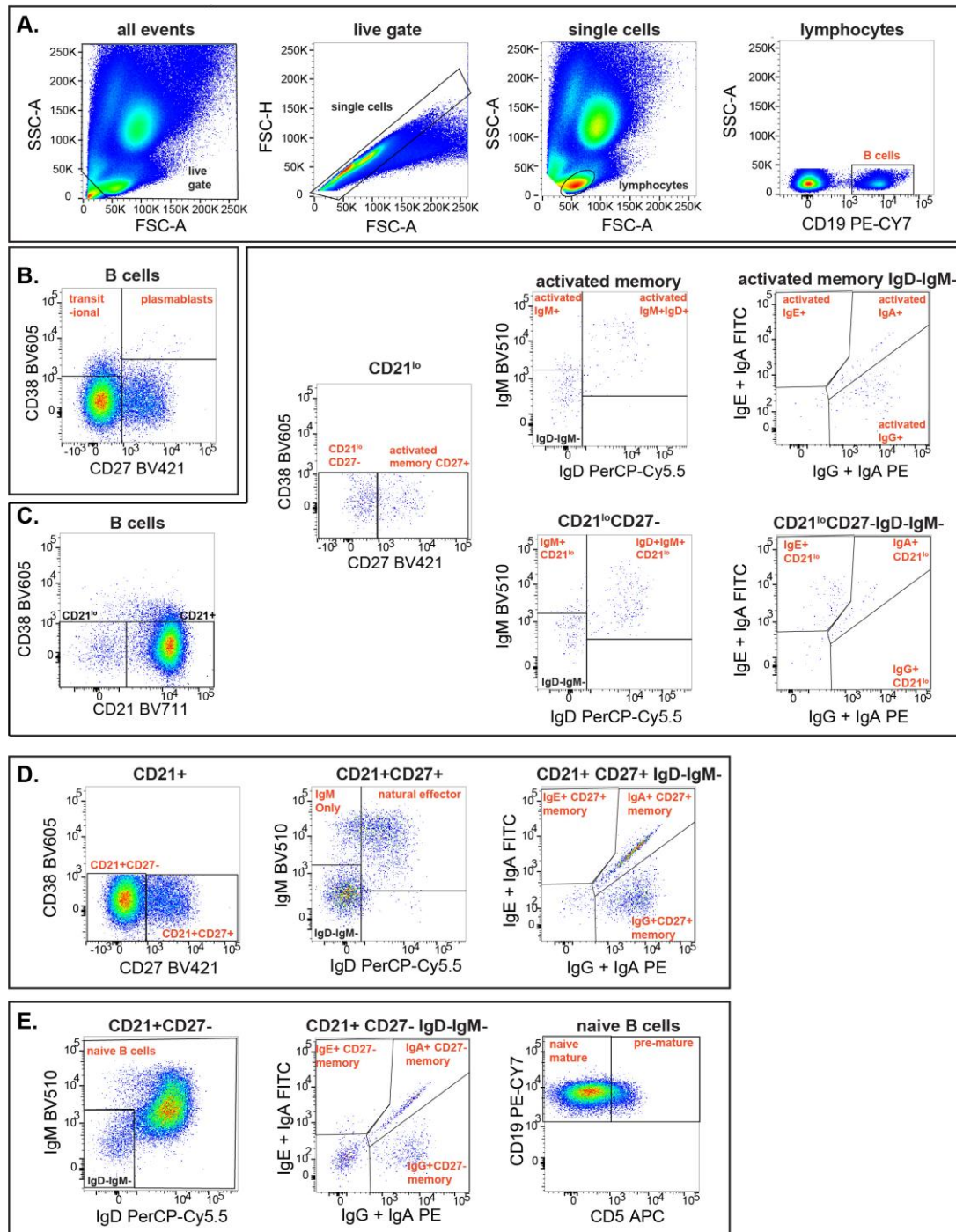
**Supplementary Table 7: Molecular diagnosis of XLA patients.**

Family	Patient	Gene	Mutation (DNA)	location	Effect (protein)
A	1	<i>BTK</i>	c.1257delG	Exon 14	p.G419GfsX11
	2	<i>BTK</i>	c.1257delG	Exon 14	p.G419GfsX11
	3	<i>BTK</i>	c.1257delG	Exon 14	p.G419GfsX11
	4	<i>BTK</i>	c.1257delG	Exon 14	p.G419GfsX11
	6	<i>BTK</i>	c.1257delG	Exon 14	p.G419GfsX11
	B	5 *	<i>BTK</i>	c.1908+1G>C	Exon 18
C	7	<i>BTK</i>	c.862C>T	Exon 10	p.R288W
D	8	<i>BTK</i>	c.1559G>A	Exon 15	p.R520Q
E	9	<i>BTK</i>	c.1787+71C>T	Intron 16	p.A523V fsX5

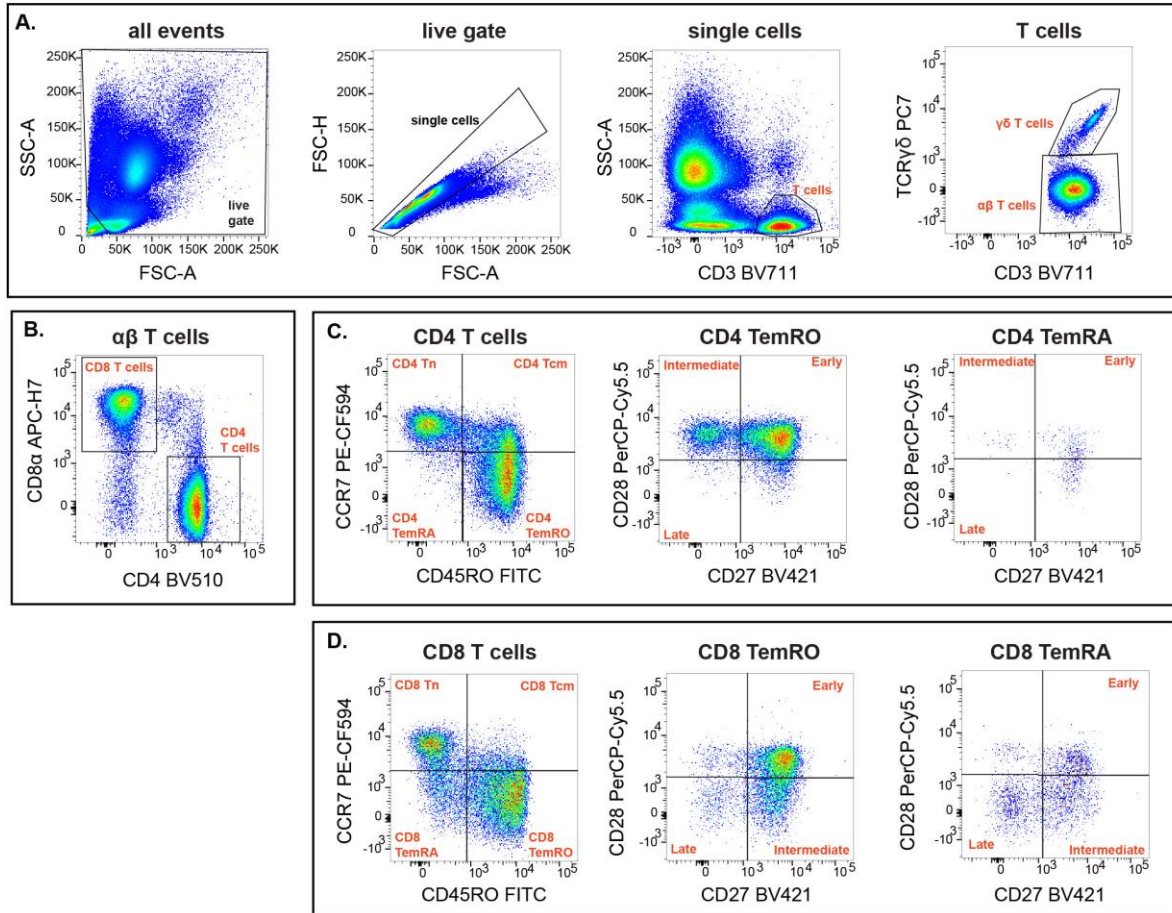
\*Patient presented with B-ALL 6 months after inclusion in this study and was reported in (van Zelm et al., 2019)



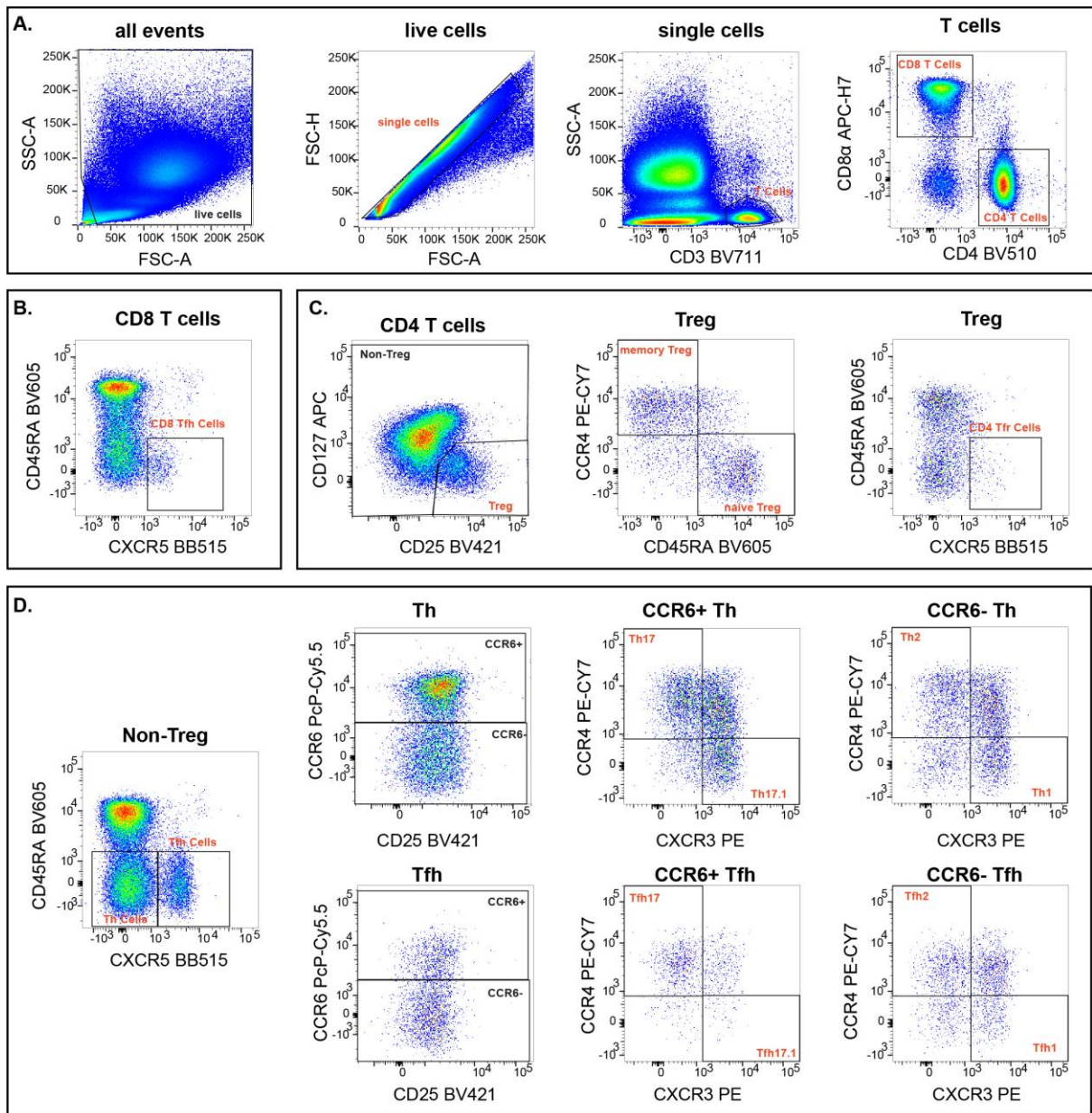
**Supplementary Figure 1. Gating strategy for TruCount tube.** **A.** Gating of major leukocyte subsets, and **B.** beads within all events. **C.** Gating of CD19<sup>+</sup> B cells within the lymphocyte gate. **D.** Gating of CD16/56<sup>+</sup> NK cells and CD3<sup>+</sup> T cells within the lymphocyte gate. **E.** Gating of CD4 T cells and CD8 T cells within the total T cell gate.



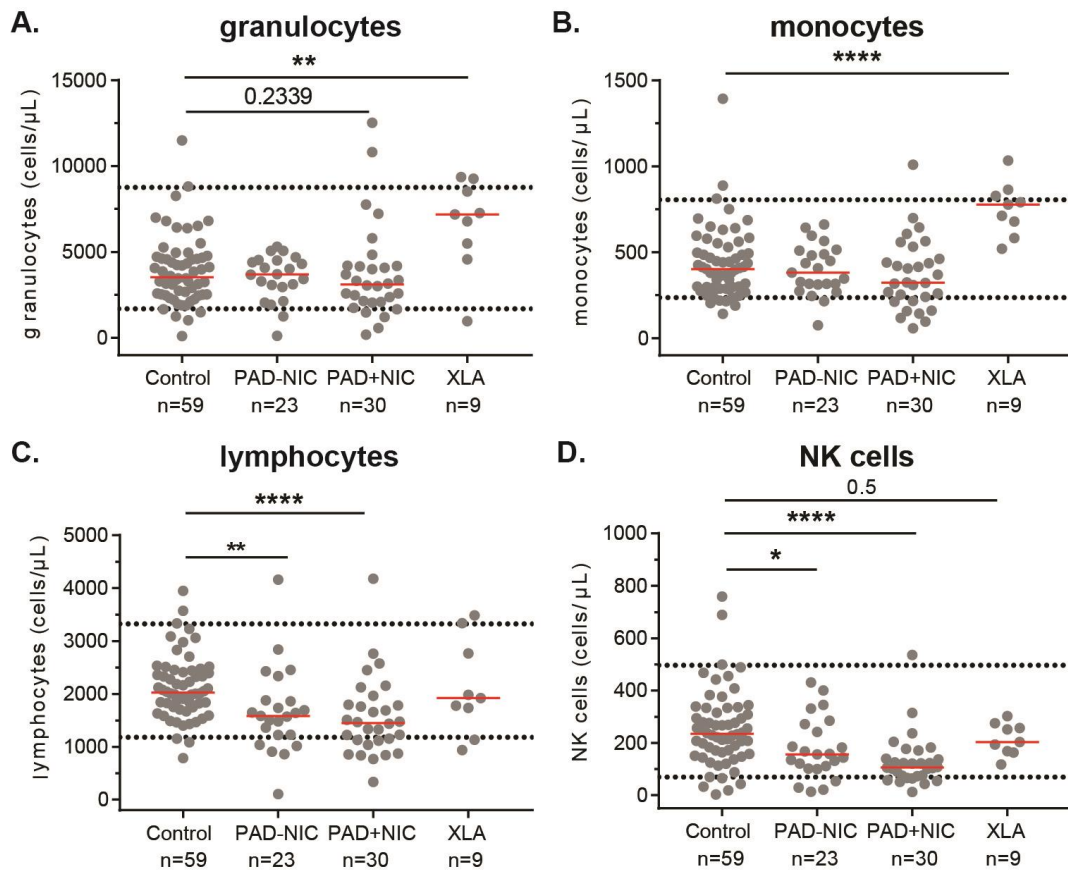
**Supplementary Figure 2. Gating strategy for the B-cell tube.** **A.** Gating strategy to define total CD19<sup>+</sup> B cells. **(B)** Transitional B cells and plasmablasts are delineated from B cells. **C.** In parallel, B cells are gated based on dim expression CD38 and divided into CD21<sup>lo</sup> or CD21<sup>+</sup> B cells. CD21<sup>+</sup> gated in **C.** are subdivided into **D.** CD21<sup>+</sup>CD27<sup>+</sup> and **E.** CD21<sup>+</sup>CD27<sup>-</sup>. **D.** CD21<sup>+</sup>CD27<sup>+</sup> cells are assessed according to their expression of IgD and IgM to delineate IgM only and natural effector cells. The IgD-IgM<sup>-</sup> cells are further characterized according to IgA, IgE and IgG expression into IgE<sup>+</sup>CD27<sup>+</sup> memory, IgA<sup>+</sup>CD27<sup>+</sup> memory and IgG<sup>+</sup>CD27<sup>+</sup> memory. **E.** CD21<sup>+</sup>CD27<sup>-</sup> cells are subdivided according to CD27 expression into naive B cells and IgD-IgM<sup>-</sup>. The IgD-IgM<sup>-</sup> are subsequently assessed for IgA, IgG and IgE expression to delineate IgE<sup>+</sup>CD27<sup>-</sup> memory, IgA<sup>+</sup>CD27<sup>-</sup> memory and IgG<sup>+</sup>CD27<sup>-</sup> memory subsets. Naive B cells are assessed for CD5 expression to delineate pre-naive and naive mature B cells.



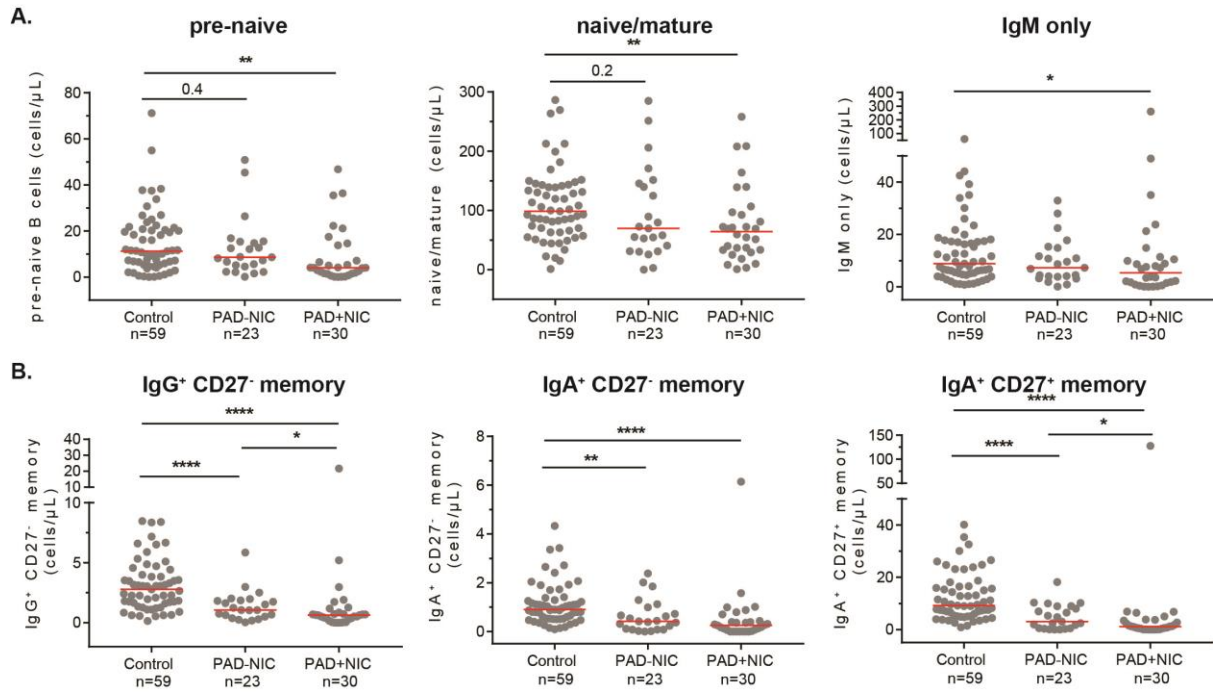
**Supplementary Figure 3. Gating strategy for T Effector tube.** **A.** Gating strategy to define total,  $\gamma\delta$  T cells and  $\alpha\beta$  T cells, **B.**  $\alpha\beta$  CD4 and CD8 T cells. **C.** Gating of CD4 T cells into naive (CD4 Tn) and memory (CD4 Tcm, TemRO and TemRA) T cell subsets, followed by subsetting CD4 TemRO and TemRA into early (CD27<sup>+</sup>CD28<sup>+</sup>), intermediate (CD27<sup>-</sup>CD28<sup>+</sup>) and late (CD27<sup>-</sup>CD28<sup>-</sup>) stage. **D.** Gating of CD8 T cells into naive (CD8 Tn) and memory (CD8 Tcm, TemRO and TemRA) T cell subsets, followed by subsetting CD8 TemRO and TemRA into early (CD27<sup>+</sup>CD28<sup>+</sup>), intermediate (CD27<sup>+</sup>CD28<sup>-</sup>) and late (CD27<sup>-</sup>CD28<sup>-</sup>) stage.



**Supplementary Figure 4. Gating strategy for T helper cell tube.** **A.** Gating strategy to define total CD4 and CD8 T cells. **B.** CD8 Tfh T cells are gated within the CD8 T cell gate defined in A. **C.** Tregs (CD25<sup>hi</sup>CD127<sup>lo</sup>) and non-Tregs are defined within the CD4 T cell gate. Tregs are further separated into naive and memory Tregs and Tfr. **D.** Non-Tregs are subdivided into Th (CXCR5<sup>-</sup>CD45RA<sup>-</sup>) and Tfh (CXCR5<sup>+</sup>CD45RA<sup>-</sup>). Th cells are further split into CCR6<sup>+</sup> and CCR6<sup>-</sup> with each population further delineated using CXCR3 and CCR4 expression, resulting in the gating of four Th subpopulations Th1, Th2, Th17 and Th17.1. Tfh are subdivided into CCR6<sup>+</sup> and CCR6<sup>-</sup> with each of these populations further subdivided to yield Tfh1, Tfh2, Tfh17 and Tfh17.1.

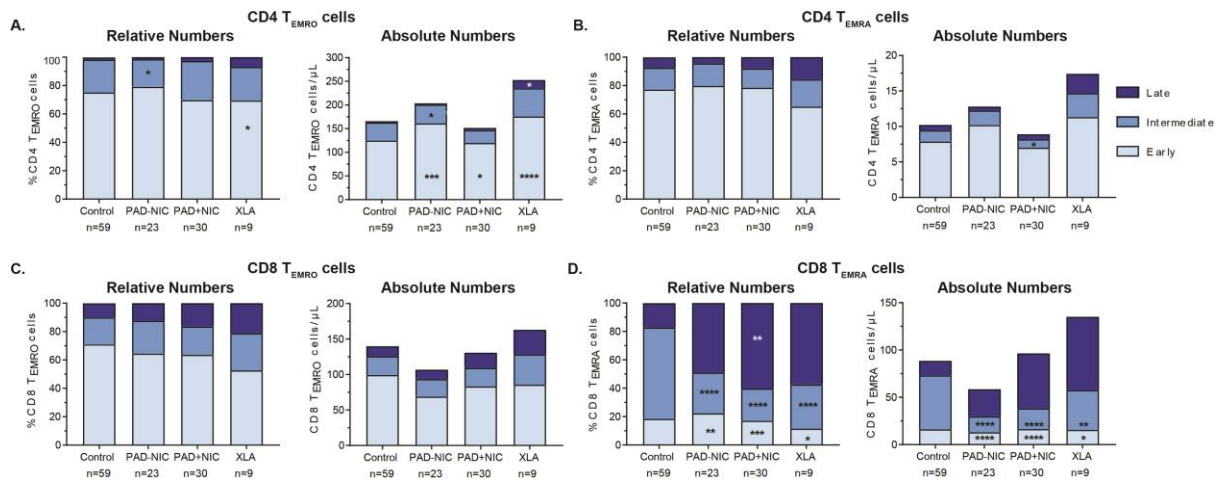


**Supplementary Figure 5. Lymphocyte and NK cell numbers are significantly reduced in PAD patients.** Absolute numbers of **A.** Granulocytes, **B.** Monocytes, **C.** Lymphocytes and **D.** NK cells. Gating strategy is outlined in *Suppl Figure 1* and *Suppl Table 3*. Horizontal dotted lines represent the 5<sup>th</sup> and 95<sup>th</sup> percentiles from the healthy control group. PAD, predominantly antibody deficiency; NIC, non-infectious complications. Statistics were performed using the Kruskal-Wallis test, followed by Mann-Whitney tests for pairwise comparisons. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , and \*\*\*\* $P < 0.0001$ .



**Supplementary Figure 6. Reduced naive and memory B cells in PAD patients.** Absolute numbers of **A.** pre-naive, naive mature and IgM memory, **B.** IgG<sup>+</sup> CD27<sup>-</sup>, IgA<sup>+</sup> CD27<sup>-</sup> and IgA<sup>+</sup> CD27<sup>+</sup> memory B cells. Subsets delineated as in *Suppl Figure 2* and *Suppl Table 3*. PAD, predominantly antibody deficiency; NIC, non-infectious complications. Statistics were performed using a two-way ANOVA, followed by a Mann-Whitney test. \*P<0.5, \*\*P<0.01, \*\*\*P<0.001, and \*\*\*\*P<0.0001.





**Supplementary Figure 7. Altered early, intermediate and late T<sub>EMRO</sub> and T<sub>EMRA</sub> cells in PAD patients.** Relative and absolute numbers of CD4 (A) TemRO and (B) TemRA subsets: early (CD27<sup>+</sup>CD28<sup>+</sup>), intermediate (CD27<sup>-</sup>CD28<sup>+</sup>) and late (CD27<sup>-</sup>CD28<sup>-</sup>) stages. Relative and absolute numbers of CD8 (C) TemRO and (D) TemRA subsets: early (CD27<sup>+</sup>CD28<sup>+</sup>), intermediate (CD27<sup>+</sup>CD28<sup>-</sup>) and late (CD27<sup>-</sup>CD28<sup>-</sup>) stages. Subsets delineated as in *Suppl Figure 3* and *Suppl Table 3*. PAD, predominantly antibody deficiency; NIC, non-infectious complications. Statistics were performed using a two-way ANOVA, followed by a Mann-Whitney test. \*P<0.5, \*\*P<0.01, \*\*\*P<0.001, and \*\*\*\*P<0.0001.