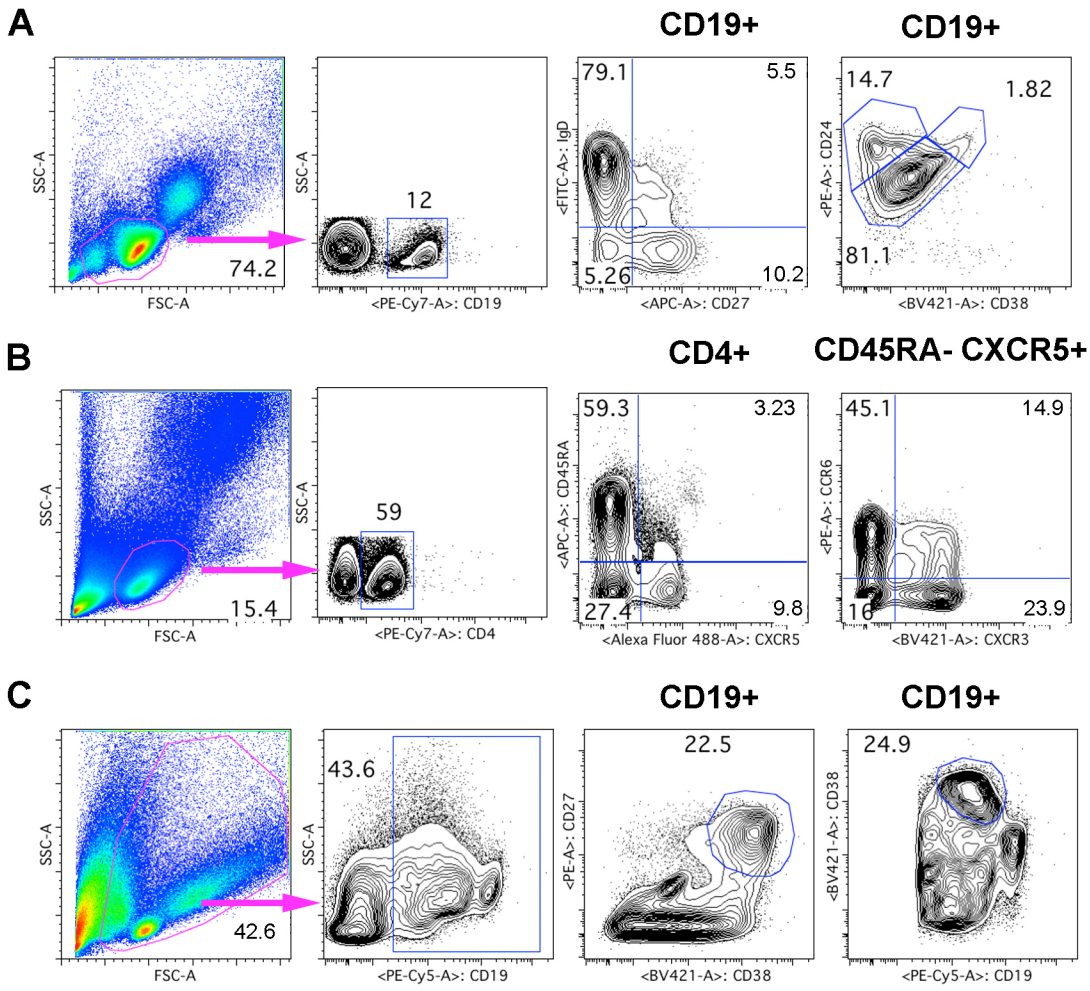
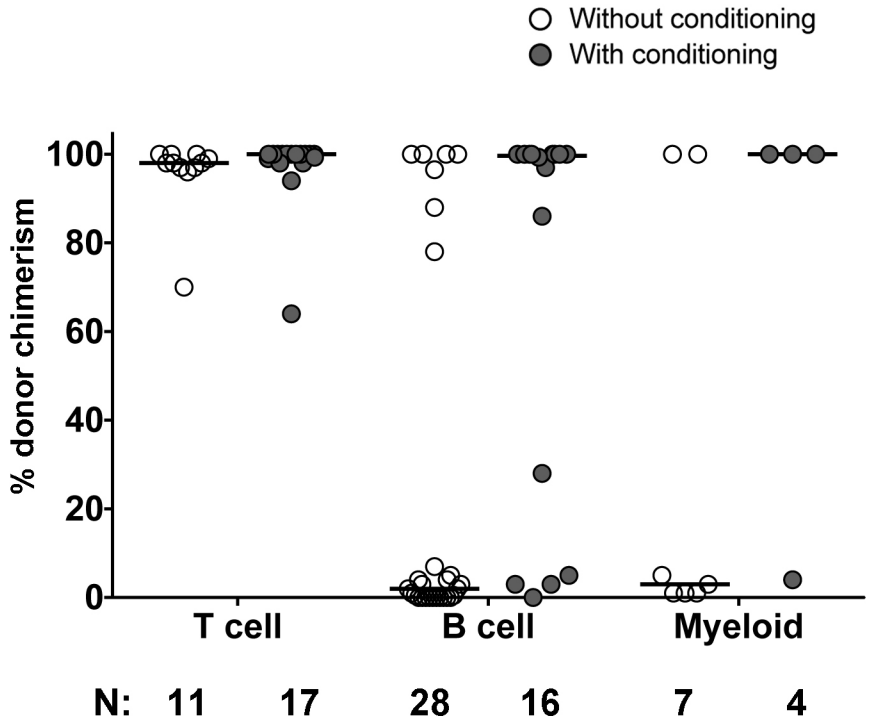
Supplemental Figures

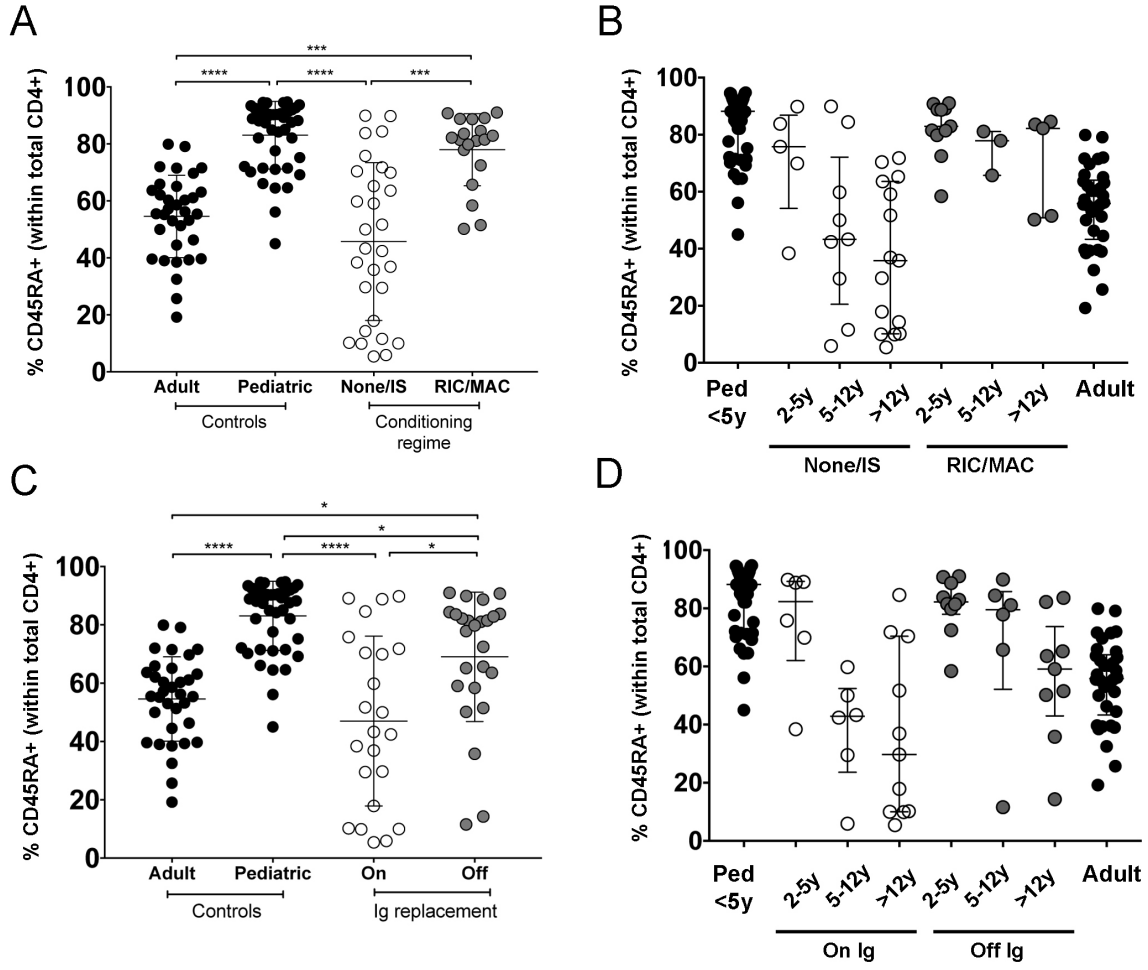
Supplemental Figure 1: Representative flow plots and gating. Gating strategy and identification of A) B cell subsets in peripheral blood: $CD19^{+}$ IgD^{+} $CD27^{+}$ unswitched memory B cells, $CD19^{+}$ IgD^{-} $CD27^{+}$ switched memory B cells, $CD19^{+}$ $CD24^{hi}$ $CD38^{hi}$ transitional B cells, $CD19^{+}$ $CD24^{hi}$ $CD38^{lo}$ marginal-zone-like B cells; B) T follicular helper cells and Tfh subsets in peripheral blood: $CD4^{+}$ $CD45RA^{-}$ $CXCR5^{+}$ T follicular helper (Tfh) cells, $CXCR3^{+}$ $CCR6^{-}$ Th1-like Tfh, $CXCR3^{-}$ $CCR6^{-}$ Th2-like Tfh, $CXCR3^{-}$ $CCR6^{+}$ Th17-like Tfh; C) plasmablasts after in vitro culture with CD40L and IL-21-Fc. For plasmablasts, the percentage of $CD27^{+}$ $CD38^{hi}$ within $CD19^{+}$ cells was recorded and verified with the percentage of $CD19^{med}$ $CD38^{hi}$ cells within $CD19^{+}$ cells.



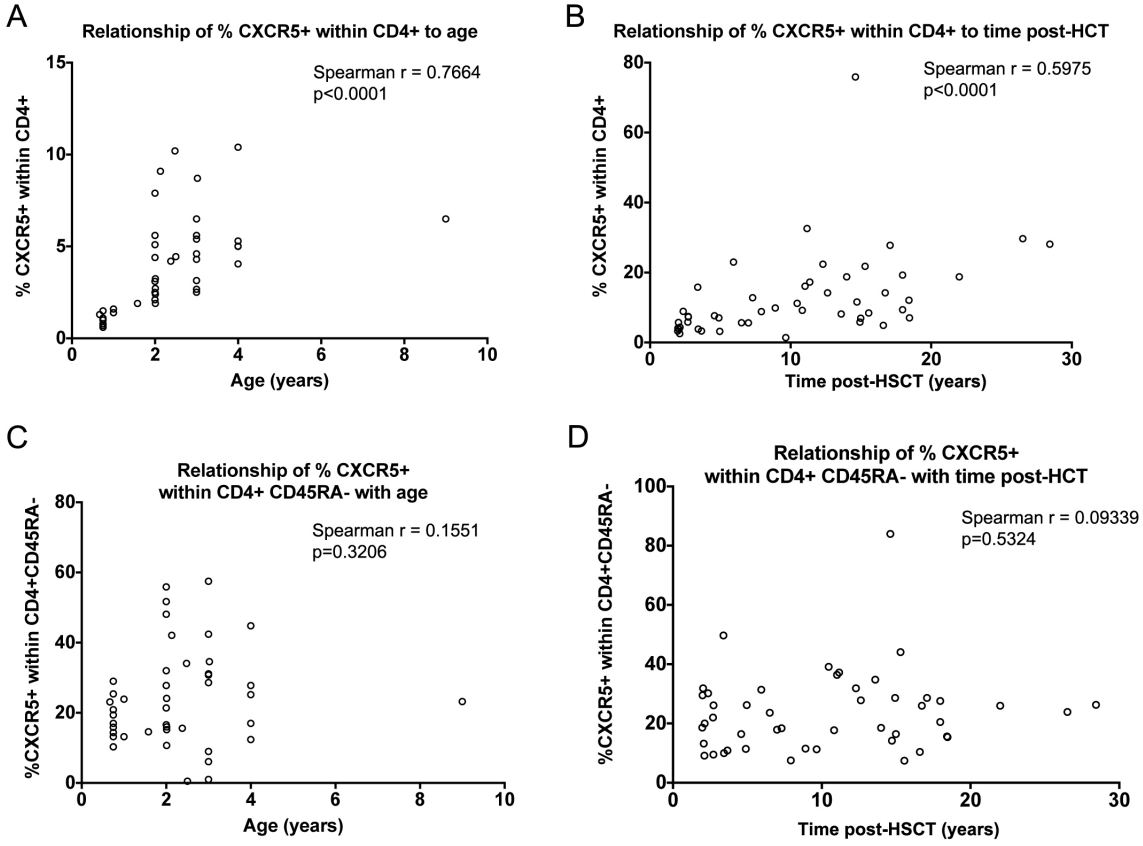
Supplemental Figure 2: Percent donor chimerism in the indicated cell types in patients with IL2RG/JAK3 SCID after allogeneic transplant performed without (open symbols) or with conditioning (closed symbols). Median chimerism is indicated by bars. Comparison according to conditioning was performed by Mann-Whitney test within each cell type, T cell p=0.0530, B cell p=0.0008, myeloid p=0.1273.



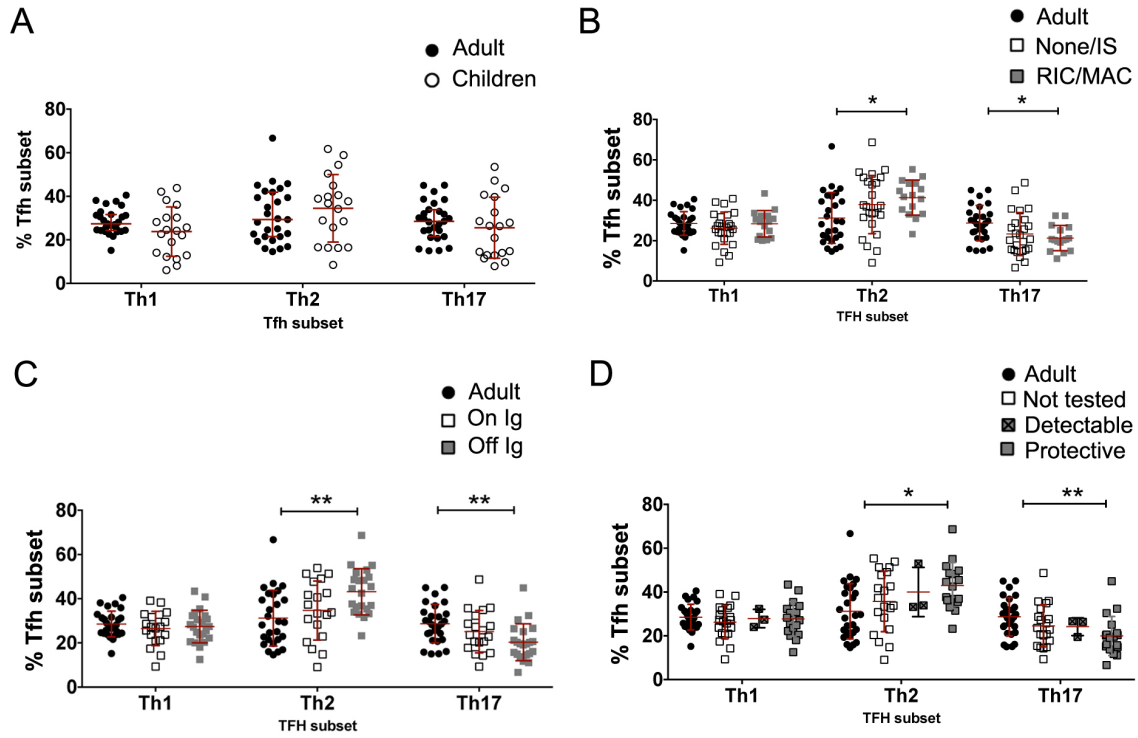
Supplemental Figure 3: Naïve CD4 T cell percentage in healthy adults, healthy children, and patients post-HSCT. Percentage of $CD4^+$ T cells expressing CD45RA (% CD4+ CD45RA+) is depicted for healthy adults, healthy children and for patients. according to conditioning regimen including A) all patients or B) divided in categories by years post-HSCT. Medians and interquartile ranges are shown. Brackets depict comparisons with significant p values, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. In B, comparisons within age groups (2-5y post-HSCT compared to pediatric controls or >12y post-HSCT compared to adult controls) and comparisons between conditioning groups within an age group did not reach statistical significance.



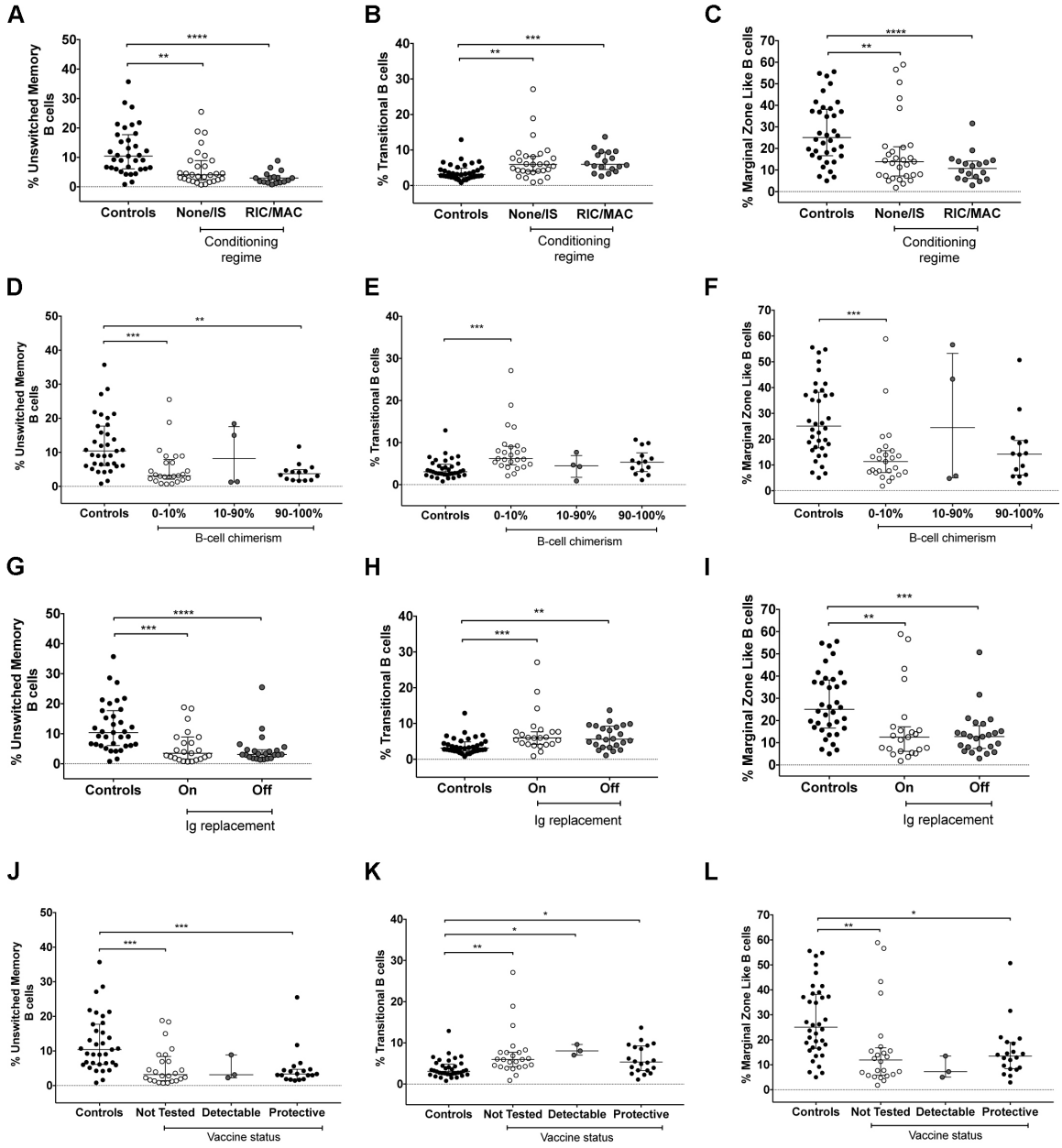
Supplemental Figure 4: Percentage of T_{FH} within non-naïve CD4 T cells is stable with age/time post-HSCT. T_{FH} measured as the percentage of CXCR5+ cells within total CD4 was correlated with age in healthy children (A) or with time post-HSCT in patients (B). T_{FH} measured as the percentage of CXCR5+ cells within non-naïve CD4 T cells (CD4+ CD45RA- T cells) was correlated with age in healthy children (C) or with time post-HSCT in patients (D). Results of Spearman r correlation are shown.



Supplemental Figure 5: T_{FH} subsets in healthy children and adults, and in patients post-HSCT. Subsets of T_{FH} were identified according to the expression of CXCR3 and CCR6. A) Percentages of Th1, Th2 and Th17 T_{FH} in adults (closed circles) and children (open circles). Percentages of Th1, Th2 and Th17 T_{FH} in patients post-HSCT were compared to healthy adults (closed circles) and divided according to B) conditioning, C) Ig replacement status, D) response to tetanus vaccine. Medians and interquartile ranges are in red. Results of Kruskall-Wallis comparisons are shown, * $p<0.05$, ** $p<0.01$. Comparisons between patients (None/IS versus RIC/MAC, On Ig versus Off Ig, vaccine response Not tested versus Detectable versus Protective) were all nonsignificant.



Supplemental Figure 6: Unswitched memory B cells, transitional B cells and marginal zone-like B cells in patients post-HSCT. Percentage of unswitched memory B cells (the percentage of $CD19^+$ B cells that are IgD^+ and $CD27^+$), transitional B cells (the percentage of $CD19^+$ B cells that are $CD24^{hi}$ and CD38hi), and marginal zone-like B cells (the percentage of $CD19^+$ B cells that are $CD24^{hi}$and $CD38^{lo}$) were measured in patients with IL2RG/JAK3 SCID and analyzed according to A-C) conditioning, D-F) donor B cell chimerism, G-I) Ig replacement status, J-L) response to tetanus vaccine. Controls were combined from healthy adults and children. Medians and interquartile ranges are shown. Brackets depict comparisons with significant p values, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.



Supplemental Table 1: Mutations, donor B cell chimerism and immune function of patients studied (n=48)

Pt no	Gene	Nucleotide change	Protein change	Mutation type	% Donor B cell chimerism	Ig replacement	Vaccine response
1	IL2RG	c.270G>A	p.W90X	nonsense	0	On	Not Tested
2	IL2RG	c.975C>G	p.Y325X	nonsense	0	On	Not Tested
3	IL2RG	c.562C>T	p.Q188X	nonsense	0	On	Not Tested
4	IL2RG	c.17T>A	p.L6X	nonsense	3	On	Not Tested
5	IL2RG	c.562C>T	p.Q188X	nonsense	78	On	Not Tested
6	IL2RG	c.775insTTTG	p.A259Vfs*44	frameshift	2	On	Not Tested
7	IL2RG	large deletion promoter to exon 5		large deletion	0	On	Not Tested
8	IL2RG	c.670C>T	p.R224W	missense	0	On	Not Tested
9	IL2RG	c.458T>A	p.I153N	missense	1	On	Not Tested
10	IL2RG	c.676C>T	p.R226C	missense	2	On	Not Tested
11	IL2RG	c.664C>T	p.R222C	missense	3	On	Not tested
12	IL2RG	c.695G>T	p.G232V	missense	4	On	Not Tested
13	IL2RG	c.676C>T	p.R226C	missense	5	On	Not Tested
14	IL2RG	c.676C>T	p.R226C	missense	88	On	Not Tested
15	IL2RG	c.758-1 G>C		splice	0	On	Not Tested
16	IL2RG	c.454+1 G>A		splice	0	On	Not Tested
17	IL2RG	c.454+1 G>A		splice	0	On	Not Tested
18	IL2RG	c.595-1 G>C		splice	3	On	Not Tested
19	IL2RG	c.924+5 G>A		splice	4	On	Not Tested
20	IL2RG	c.854+2T>C		splice	100	On	Not Tested
21	IL2RG	c.924+2 T>G		splice		On	Not Tested
22	IL2RG	c.677G>A	p.R226H	missense	28	Off	Not Tested
23	IL2RG	c.431A>C	p.Q144P	missense	0	Off	Detectable
24	IL2RG	c.437T>C	p.L146P	missense	0.5	Off	Detectable
25	IL2RG	c.737G>A	p.W246X	nonsense	99.3	Off	Protective
26	IL2RG	c.710G>A	p.W237X	nonsense	100	Off	Protective
27	IL2RG	c.908dupA	p.Y303X	nonsense	100	Off	Protective
28	IL2RG	c.865C>T	p.R289X	nonsense	100	Off	Protective
29	IL2RG	c.352C>T	p.Q118X	nonsense		Off	Protective
30	IL2RG	c.737G>A	p.W246X	nonsense		Off	Protective
31	IL2RG	c.865C>T	p.R289X	nonsense		Off	Protective
32	IL2RG	c.151_163delAGTGTTTCCACTC	p.S51Cfs*15	frameshift	97	Off	Protective

33	IL2RG	c.374A>G	p.Y125C	missense	0	Off	Protective
34	IL2RG	c.437T>C	p.L146P	missense	0.5	Off	Protective
35	IL2RG	c.676C>T	p.R226C	missense	5	Off	Protective
36	IL2RG	c.854G>A	p.R285Q	missense	100	Off	Protective
37	IL2RG	c.677G>A	p.R226H	missense	100	Off	Protective
38	IL2RG	c.395T>G	p.L132R	missense	100	Off	Protective
39	IL2RG	c.314A>G	p.Y105C	missense	100	Off	Protective
40	IL2RG	c.455(-1)G>A		splice	96.5	Off	Protective
41	IL2RG	c.115+1 G>T		splice	100	Off	Protective
42	IL2RG	c.758-1G>C		splice	100	Off	Protective
43	JAK3	c.1580_1585delTGGGCC	p.L527_G528del	in-frame deletion	86	On	Not Tested
44	JAK3	c.507C>A, c.1569G>A	p.D169E, p.W523X	missense	0	On	Not Tested
45	JAK3	c.314-1G>C		splice	3	Off	Detectable
46	JAK3	c.1245_1248delCTGT	p.C415Pfs*6	frameshift	100	Off	Protective
47	JAK3	c.578G>A, c.1786+3G	p.C193Y, splice	missense, splice	100	Off	Protective
48	JAK3	unknown		unknown	7	Off	Protective

Supplemental Table 2: Adult and pediatric healthy control B cell phenotyping, plasmablast and Ig secretion data

B cell subset	Adult median (range) N=28 or 29	Pediatric median (range) N=7	P value (Mann Whitney)
Transitional	2.79% (0.78-12.9%)	3.41% (2.27-6.56%)	0.5011
Marginal zone like	28.1% (5.02-55.6%)	19.7% (15.7-27.1%)	0.2058
Unswitched memory	10.7% (0.81-35.7%)	8.92% (5.91-17.70%)	0.4499
Switched memory	17.35% (7.11-31.7%)	14.3% (7.34-34.9%)	0.2268
In vitro plasmablasts	24.35% (13.4-55%)	22.5% (11.3-57%)	0.7549
IgM secretion	17377 (9349-35756)	26945 (13977-36950)	0.2810
IgG secretion	11192 (5110-35912)	20841 (10007-46686)	0.0939