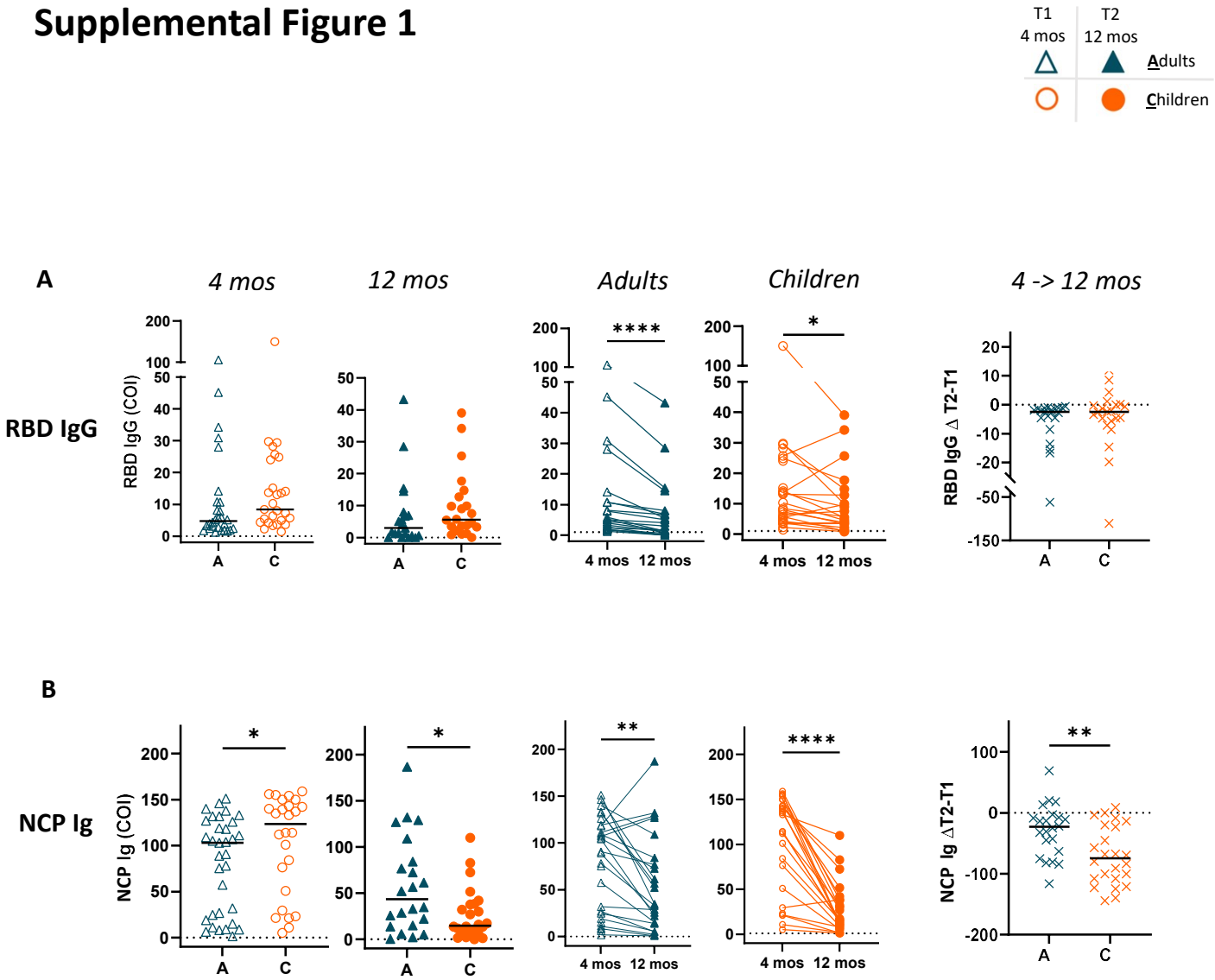
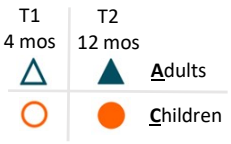
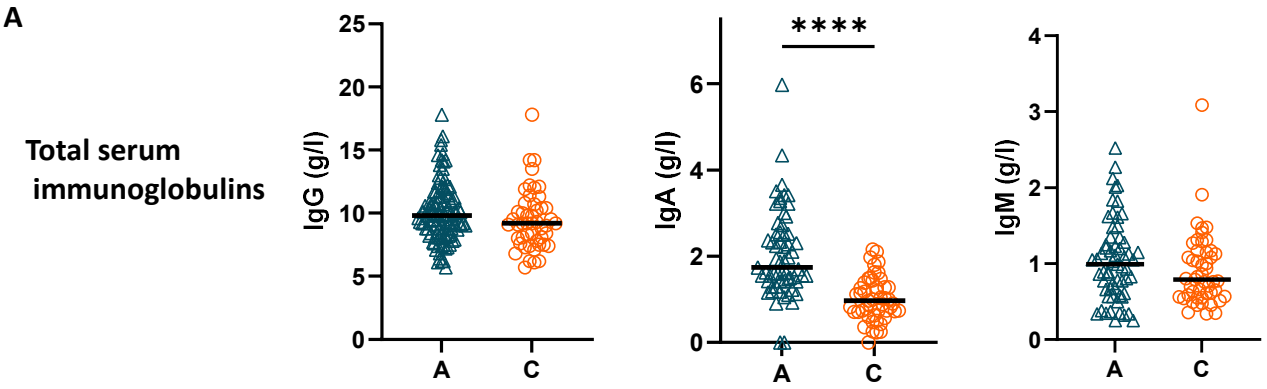
Supplemental Figure 1

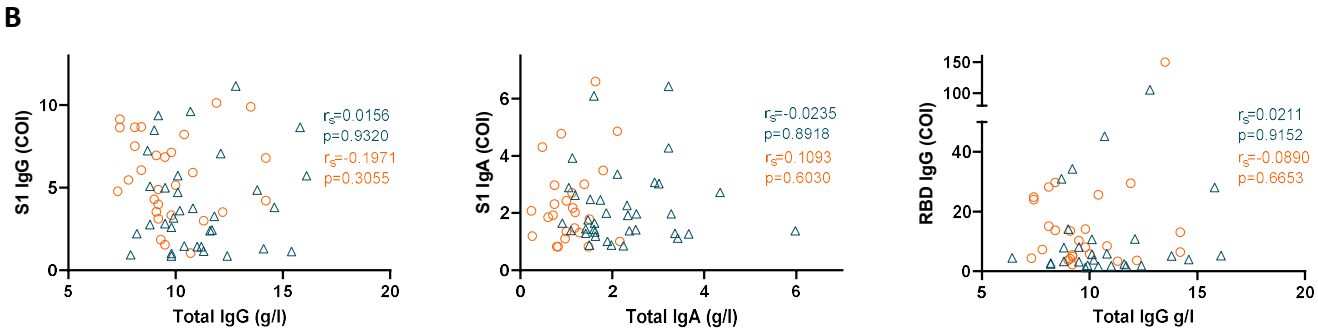


Supplemental Figure 1. Similar decrease of specific antibody levels to RBD of spike protein in children and adults; faster loss of antibodies to nucleocapsid protein (NCP) in children. Only seropositive subjects at T1 are plotted (31 adults, 27 children; for definition of seropositivity see Methods). Antibodies reactive to SARS-CoV-2 RBD IgG (A) and NCP Ig (B) were measured with Siemens Healthineers and Roche Elecsys, respectively at T1 (4 months after diagnosis; empty symbols) and T2 (12 months after diagnosis; filled symbols) in adults (A; blue symbols) and children (C; orange symbols); dotted line represents a cut-off value for reactivity of cut-off index (COI=1). In the far right graphs a difference between values at T1 and T2 is shown (T2 – T1); the dotted line depicts a null difference. Mann-Whitney test and Wilcoxon matched-pairs signed rank test were used for comparing median values (black lines) between adults and children, and between T1 and T2, respectively. Statistical significance was defined as * p≤0.05, **p≤0.01, ***p≤0.001; only statistically significant differences are marked.

Supplemental Figure 2

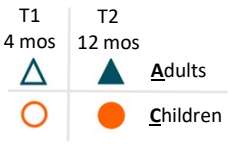




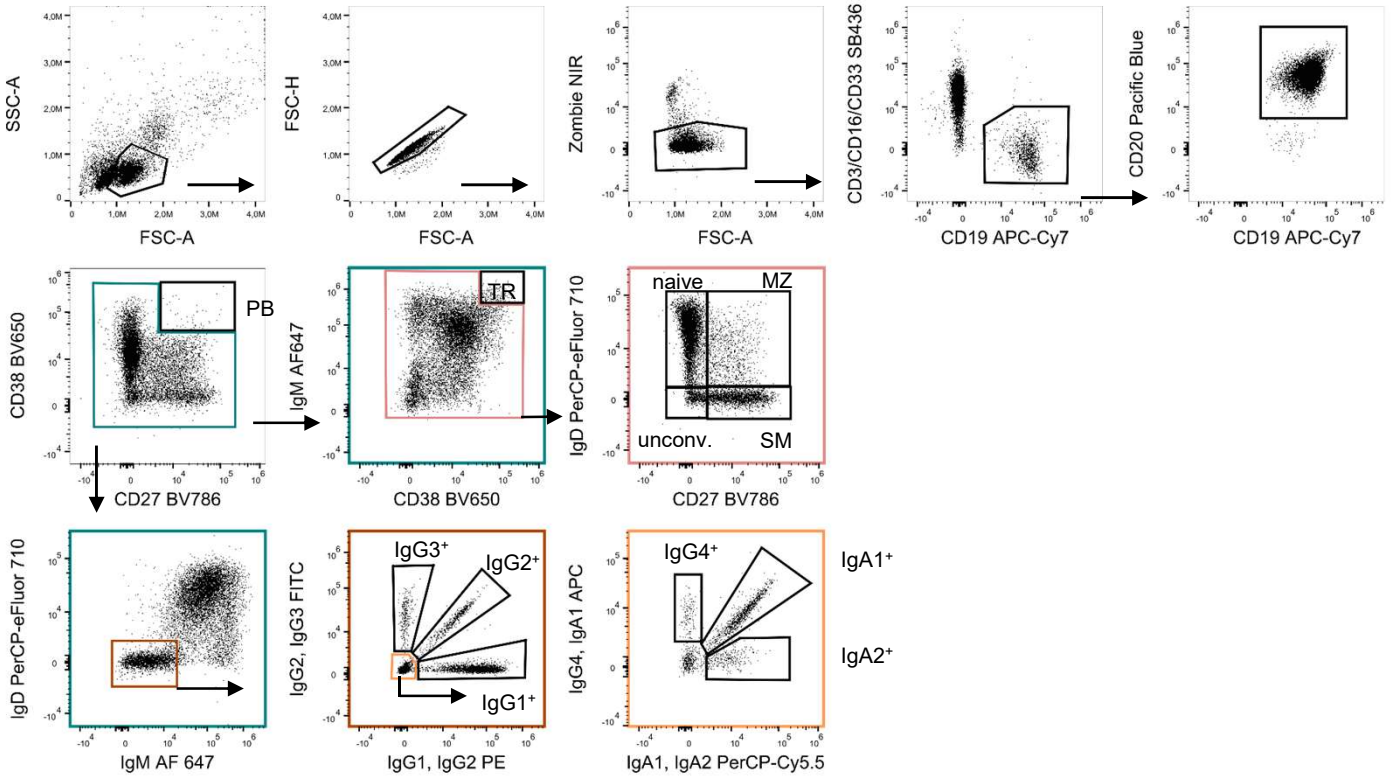


Supplemental Figure 2. Relation between specific antibody levels and total immunoglobulin levels. (A) Total serum antibody levels (IgG, IgA, IgM) in all participants are shown. (B) There is no correlation between the specific SARS-CoV-2 antibodies and total antibody levels in seropositive participants (31 adults,27 children). Mann-Whitney test was used for comparing median values (black lines) between adults and children, statistical significance was defined as ****p≤0.0001; only statistically significant differences are marked. Non-parametric Spearman correlation was applied.

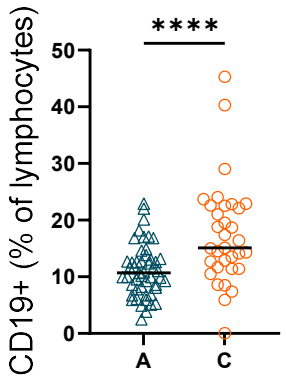
Supplemental Figure 3



A **Gating strategy (extended B-cell phenotype)**

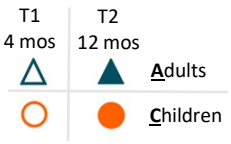


B *All (4 mos)*



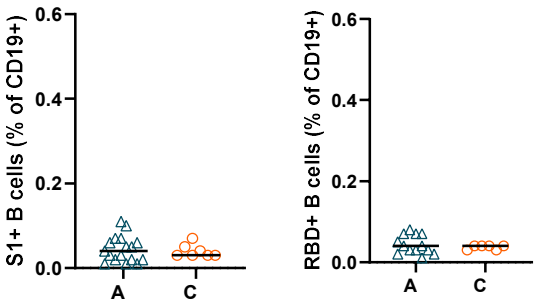
Supplemental Figure 3. Determination of B-cell phenotype in adults and children. (A) Gating strategy for B-cell phenotyping of seropositive participants with flow cytometry (for definition of seropositive and details of flow cytometry measurement see Methods). (B) B-cell count for T1 (4 months) is depicted (adults with blue triangels, children with orange circles) Abbreviation: TR: transitional B cells, PB: plasmablasts; SM: switched memory B cells; Unconv.: unconventional memory B cells; MZ: marginal zone B cells; IgG1-4: subclasses of IgG; IgA1-2: subclasses of IgA. Mann-Whitney test was used for comparing median values (black lines), statistical significance was defined as ****p≤0.0001.

Supplemental Figure 4



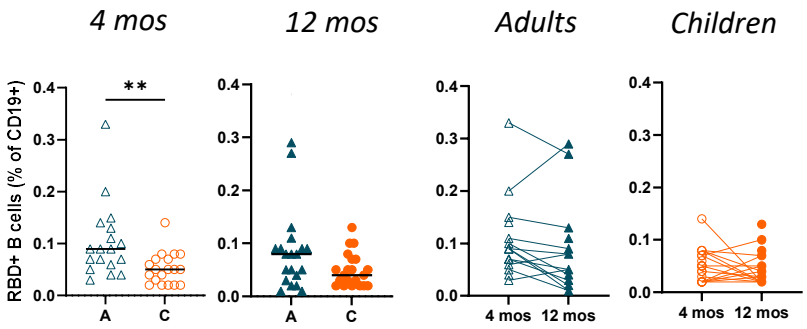
A

S1+ and RBD+ B cells in CoV-2 negative donors



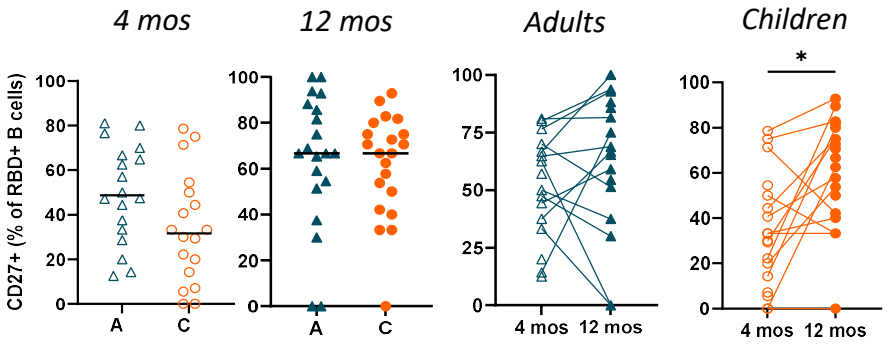
B

RBD+ B cells



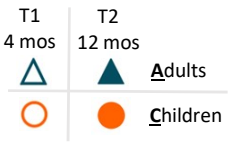
C

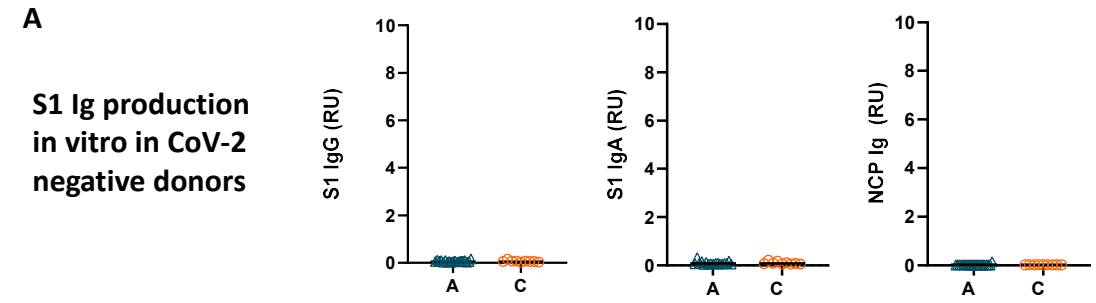
CD27+ RBD+ B cells

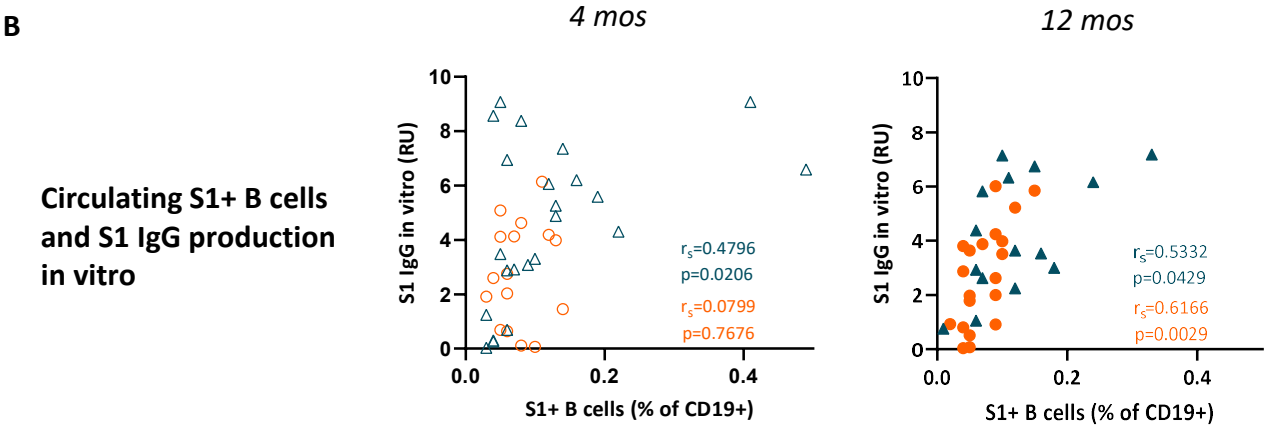


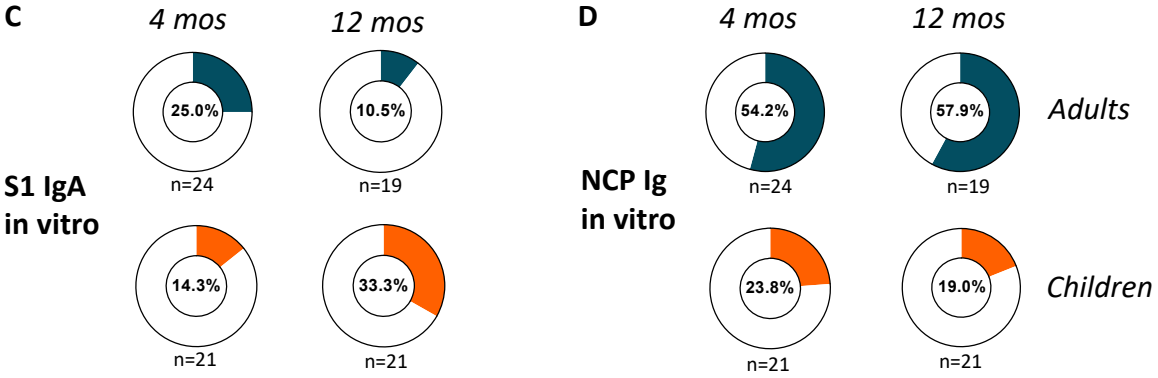
Supplemental Figure 4. RBD specific B cells are low but stable in children and progressively acquire a memory phenotype in children. (A) S1+ and RBD+ B cells were measured in SARS-CoV-2 negative individuals (for definition of seronegativity see Methods) with flow cytometry at T1 (4 months after diagnosis; empty symbols) in adults (A; blue symbols) and children (C; orange symbols). (B) RBD+ B cells for both age groups in SARS-CoV-2 seropositive individuals (for definition of seropositivity see Methods) at T1 and T2 (12 months after diagnosis, filled symbols) are shown. (C) Proportion of CD27+ RBD+ B cells measured with flow cytometry at T1 and T2 in SARS-CoV-2 seropositive individuals. Mann-Whitney test and Wilcoxon matched-pairs signed rank test were used for comparing median values (black lines) between adults and children, and between T1 and T2, respectively. Statistical significance was defined as * p≤0.05, **p≤0.01; only statistically significant differences are marked.

Supplemental Figure 5



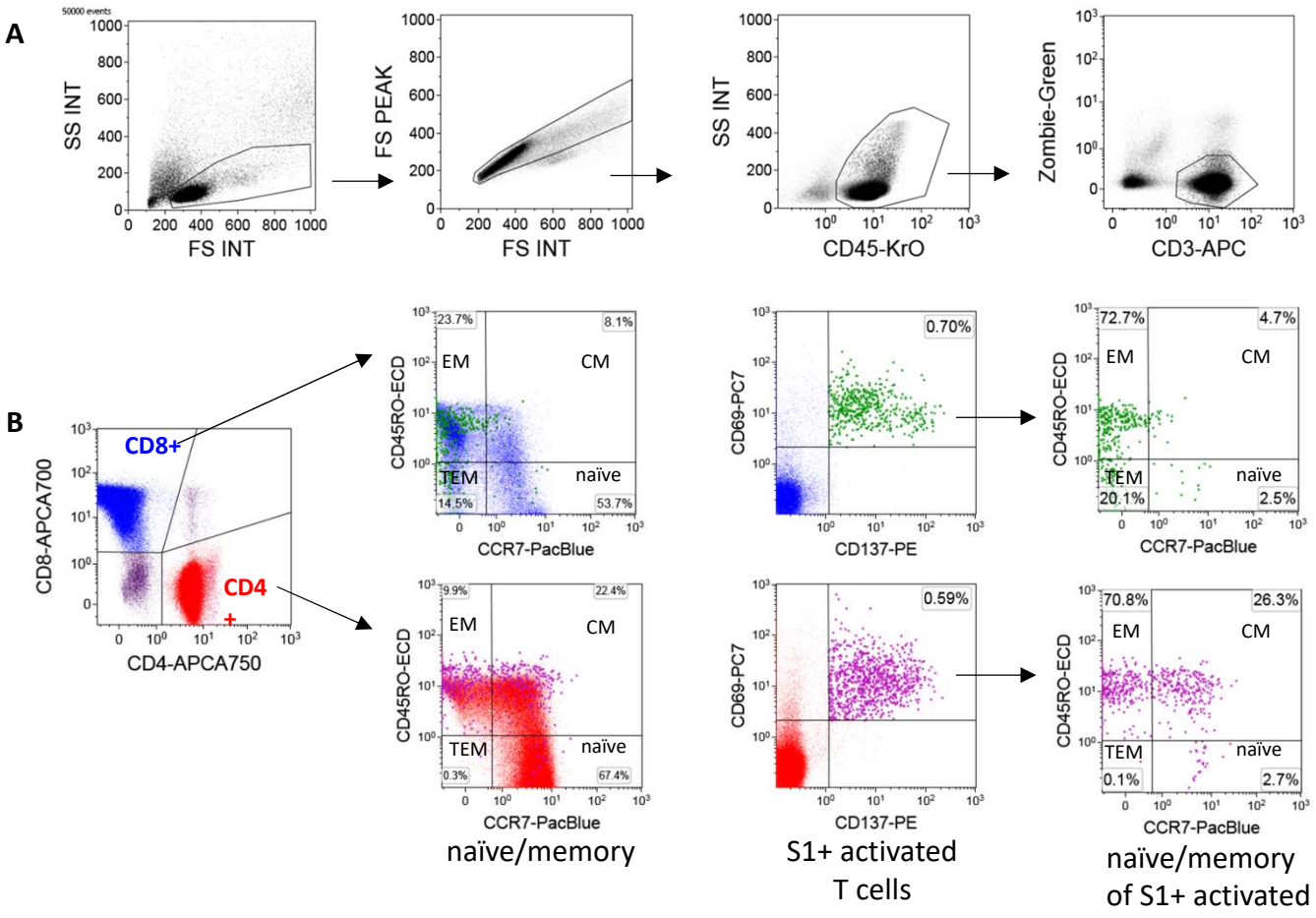


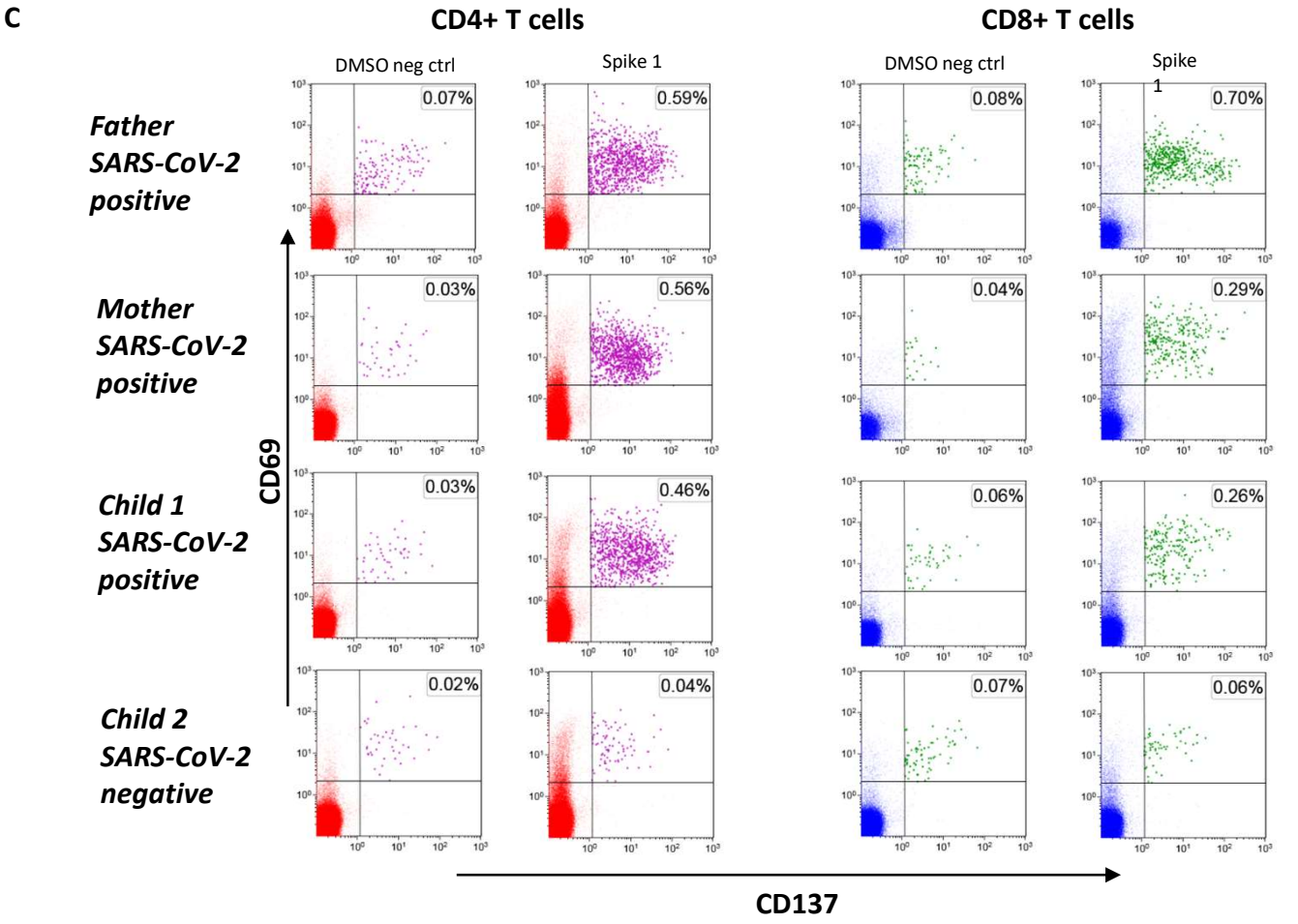




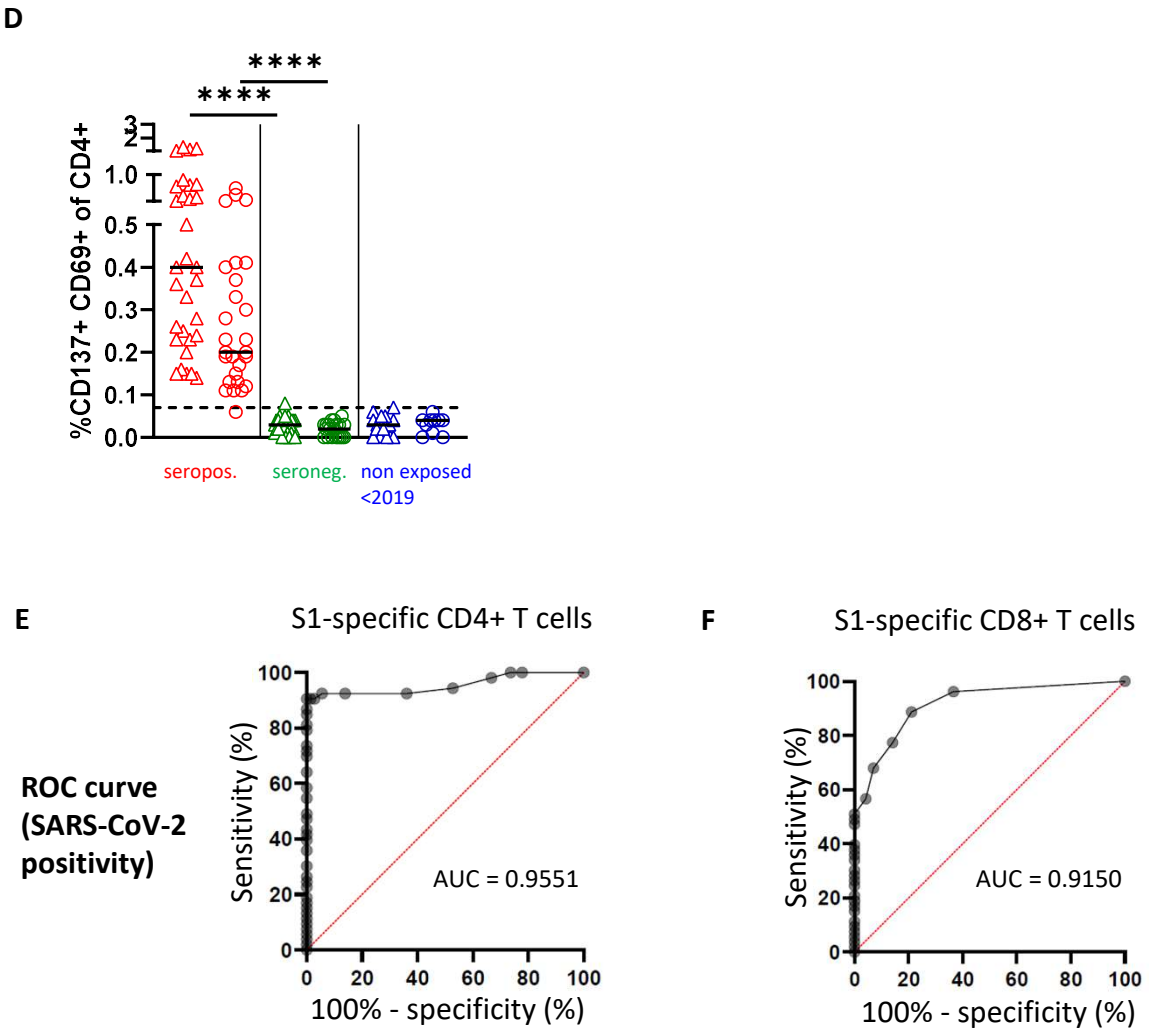
Supplemental Figure 5. Detection of specific antibodies from circulating memory B cells after TLR9 stimulation in vitro. PBMCs were stimulated in vitro with TLR9 agonists and specific antibodies were measured in culture supenatant after 9 days of culture. (A) Control measurement in SARS-CoV-2 seronegative participants in T1 (for definition of seronegativity see Methods). (B) Correlation between circulating S1+ B cells and specific immunoglobulin production *in vitro*. Proportion of participants with proven production of S1 IgA (C) and NCP Ig (D) after stimulation of mononuclear cells (MNC) with TLR9 agonist *in vitro* at T1 and T2 in subjects who were SARS-CoV-2 seropositive in T1 (for definition of seropositivity and methodic details see Methods) were measured (adults in blue, children in orange). Non-parametric Spearman correlation was applied.

Supplemental Figure 6



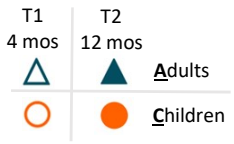


Supplemental Figure 6



Supplemental Figure 6. Detection of S1-specific T cells and subpopulations. SARS-CoV-2 specific T cell response was analyzed after stimulation with SARS-CoV-2 Spike 1 (S1) peptide-mix for 4 days by flow cytometric detection of activation-induced markers (AIM) CD69 and CD137. (A) Common gating strategy: the gates for CD8+ and CD4+ T cells were set after excluding doublets and gating on viable CD3+ T cells (Zombie-green negative). (B) CD8+ (upper row, blue) and CD4+ (lower row, red) T cell subpopulations were determined by using CD45RO as memory cell marker and CCR7 to distinguish central memory (CM, CCR7+) from effector memory (EM, CCR7-) and naïve (CCR7+) from terminal differentiated memory (CCR7-) T cells. S1-specific CD8+ and CD4+ T cells were determined by their co-expression of CD69 and CD137, followed by further differentiation in T-memory subpopulations by CD45RO and CCR7 staining. (C) Representative dot plots of S1-specific CD4+ (red, left) and CD8+ (blue, right) of one family 4 months (T1) after infection. Mother, father and one child (#1) were seropositive for SARS-CoV-2, whereas the second child (#2) remained uninfected. Plots of CD69+CD137+ T cells after DMSO incubation for 4 days are shown as negative/ background controls.(D) High sensitivity and specificity of the AIM test to assess S1-specific CD4+ T cells. To show the functionality of the AIM assay, the results of three different cohort groups are shown, red: SARS-CoV-2 infected, seropositive children (circles) and adults (triangles) 4 months after infection; green: SARS-CoV2 non infected, seronegative family members; blue: non exposed adults (cells were cryopreserved before 2019). The ROC analysis (Graph Pad Prism 9.0.1) illustrate sensitivity and specificity of (E) S1-specific CD4+ T cells (sensitivity 90.6%; 95% CI: 79.8-95.9%, specificity 97.22 %; 95% CI: 90.4-99.5%) and (F) S1-specific CD8+ T cells (sensitivity 67.9%; 95% CI: 54.5-78.9%, specificity 93.0%; 95CI: 84.6-97.0%) in detection of SARS-CoV-2 infection. Mann-Whitney test is used for comparing median values (black lines) between infected and non infected individuals, statistical significance was defined as ****$p \leq 0.0001$.

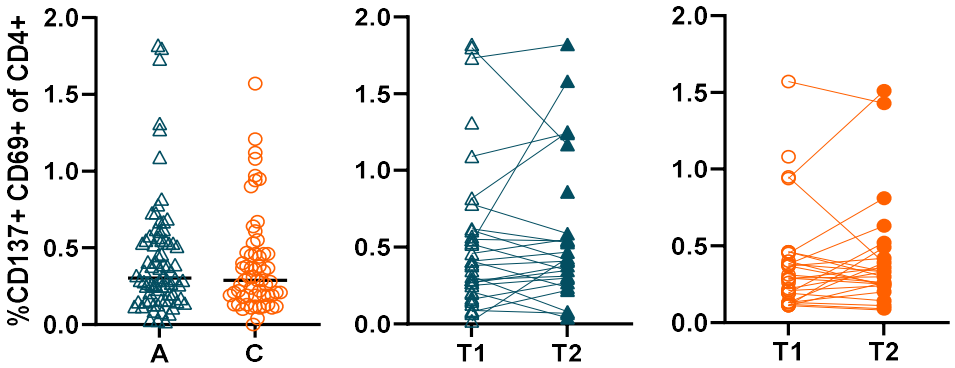
Supplemental Figure 7



A

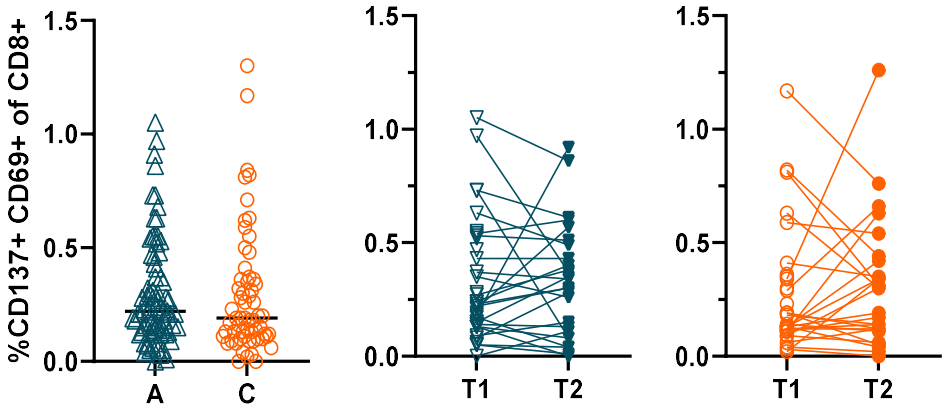
Pan-Corona specific T cells

CD4+



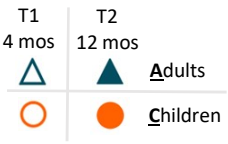
B

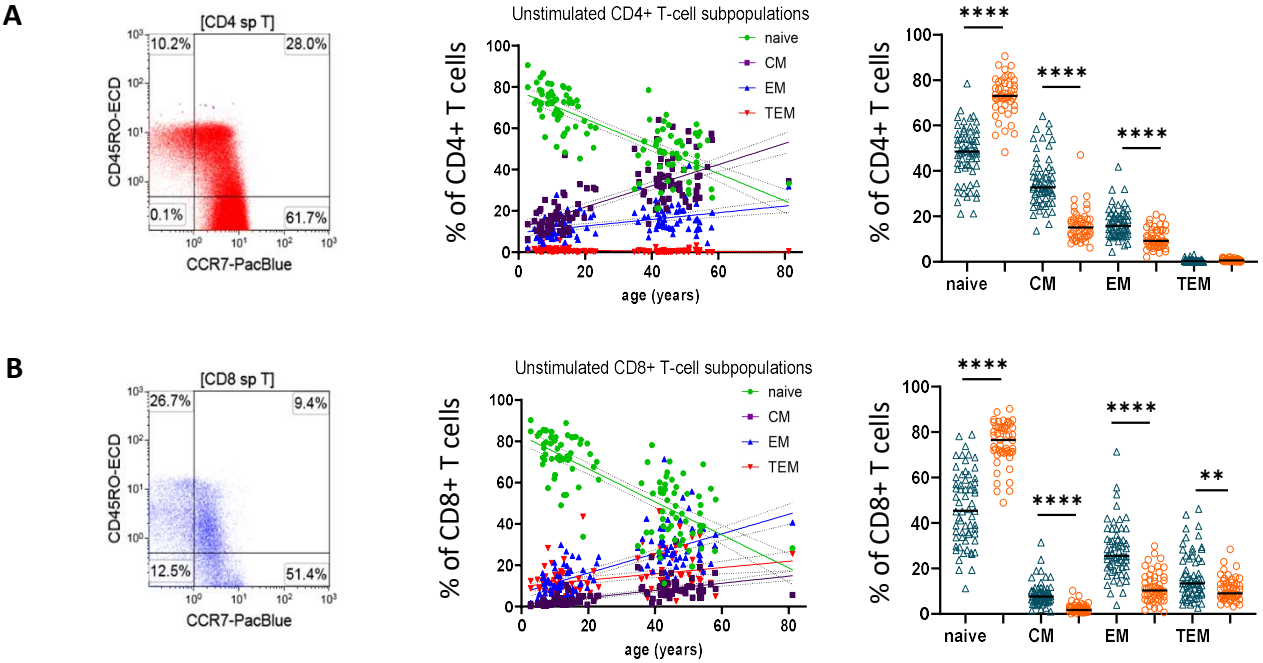
CD8+



Supplemental Figure 7. Similar response to Pan Corona antigens in adults and children. As a control for detection of Spike 1 specific T-cells, Pan-Corona specific T cells were detected after stimulating the cells with pooled Spike protein peptide mixes of common corona viruses HUK1, 229E, OC43 and NL63 ("Pan Corona" peptide mix). No differences in the proportions of Pan Corona reactive CD4+ (A) and CD8+ (B) T cells were found in between adults and children (all family members) and the two timepoints assessed (only SARS-CoV 2 seropositive family-members), respectively. Mann-Whitney test and Wilcoxon matched-pairs signed rank test were used for comparing median values (black lines) between adults and children, and between the two time points; none of the tests detected any statistically significant difference.

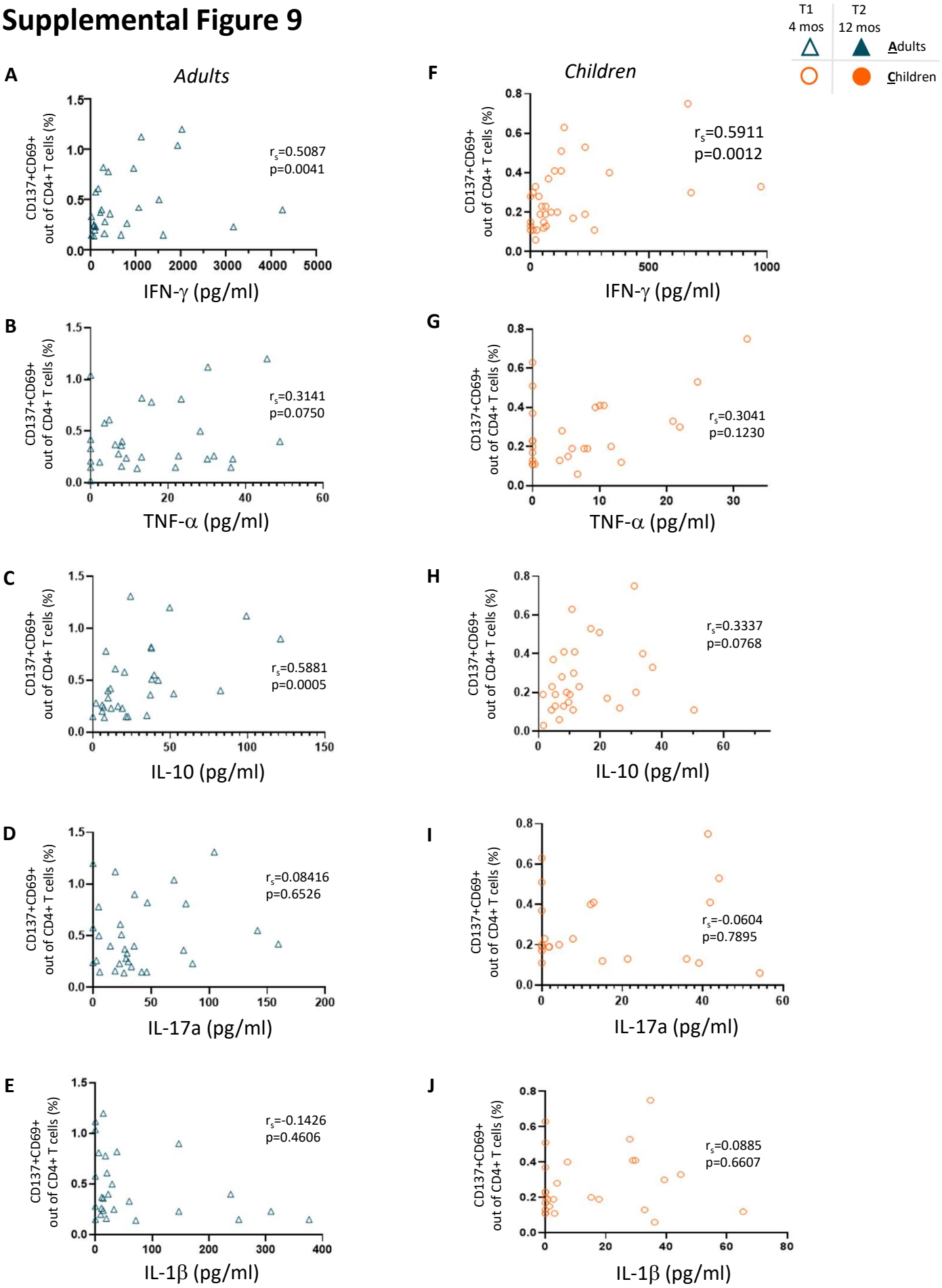
Supplemental Figure 8





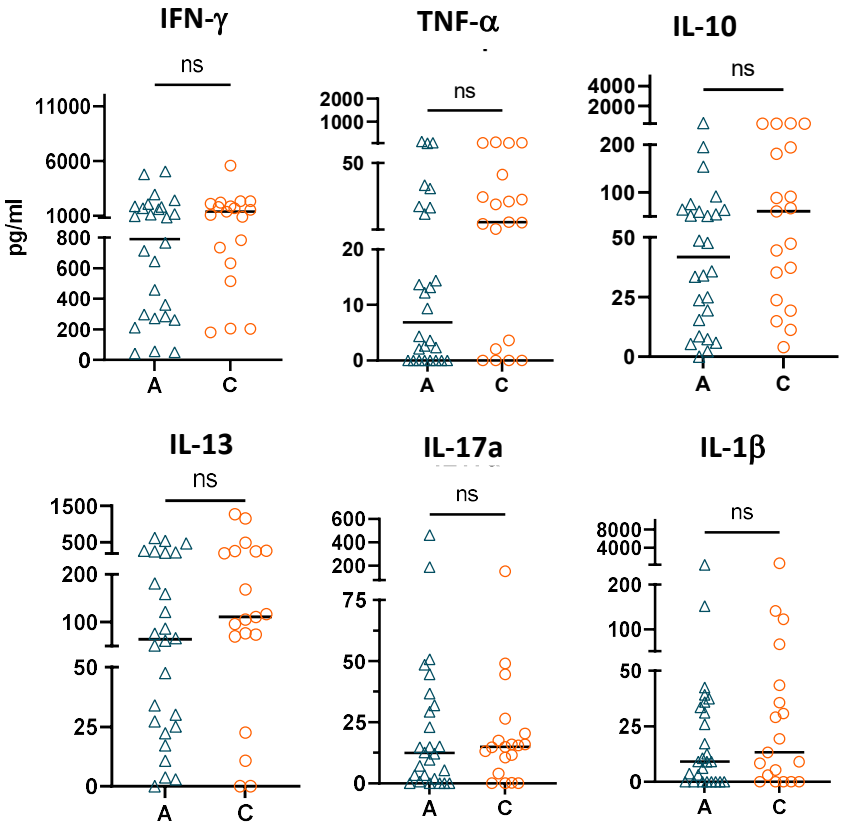
Supplemental Figure 8. Difference in distribution of the T cell subpopulations in unstimulated cells (ex vivo) between children and adults. Age-related distribution of naïve-memory T cell subpopulations of the whole cohort. Representative gating in CCR7+/CD45RO+ dot plots of CD4+ (red) and CD8+ (blue) of one individual are shown, unstimulated CD4+ (A) and CD8+ (B) According to their expression of CD45RO and CCR7, naïve T cells (CD45RO- CCR7+), were discriminated from central memory (CM) T cells (CD45RO+ CCR7+), effector memory (EM) T cells (CD45RO+CCR7-) and terminal effector memory (TEM) T cells (CD45RA- CCR7-). Mann-Whitney test were used for comparing median values (black lines) between adults and children. Statistical significance was defined as * p≤0.05, **p≤0.01, ***p≤0.001, ****p<0,0001, only significant results are marked.

Supplemental Figure 9



Supplemental Figure 9. The amount of the released cytokine in the supernatant of Spike-1 stimulated T cells in adults (A-E) and children (F-J) is plotted against the proportion of CD137+CD69+CD4+ T cells to analyze the correlation. While for IFN-γ (A,F), TNF-α (B,G) and IL-10 (C,F) there is a correlation according to the Spearman r-values (> 0,3 weak correlation, > 0,5 moderate correlation), no correlation was detected for IL-17a (D, I) and IL-1β (E,J).

Supplemental Figure 10



Supplemental Figure 10. Cytokine release in the supernatant of Pan Corona stimulated T cells. As a control for detection of cytokines after Spike-1-specific stimulation, the release of cytokine after Pan-Corona stimulation was analyzed in supernatants of pooled common corona viruses HUK1, 229E, OC43 and NL63 ("Pan Corona" peptide mix) stimulated T-cells by multiplex immunoassay. We found no significant differences in the release of IFN-γ, TNF-α, IL-10, IL-13, IL-17a and IL-1β of children in comparison to adults. Mann-Whitney test is used for comparing median values (black lines) between adults and children. ns = non-significant.