# *Supplementary Material*

## Supplemental Tables

**Table S1. Complete overview of the inclusion and exclusion criteria for this study.**

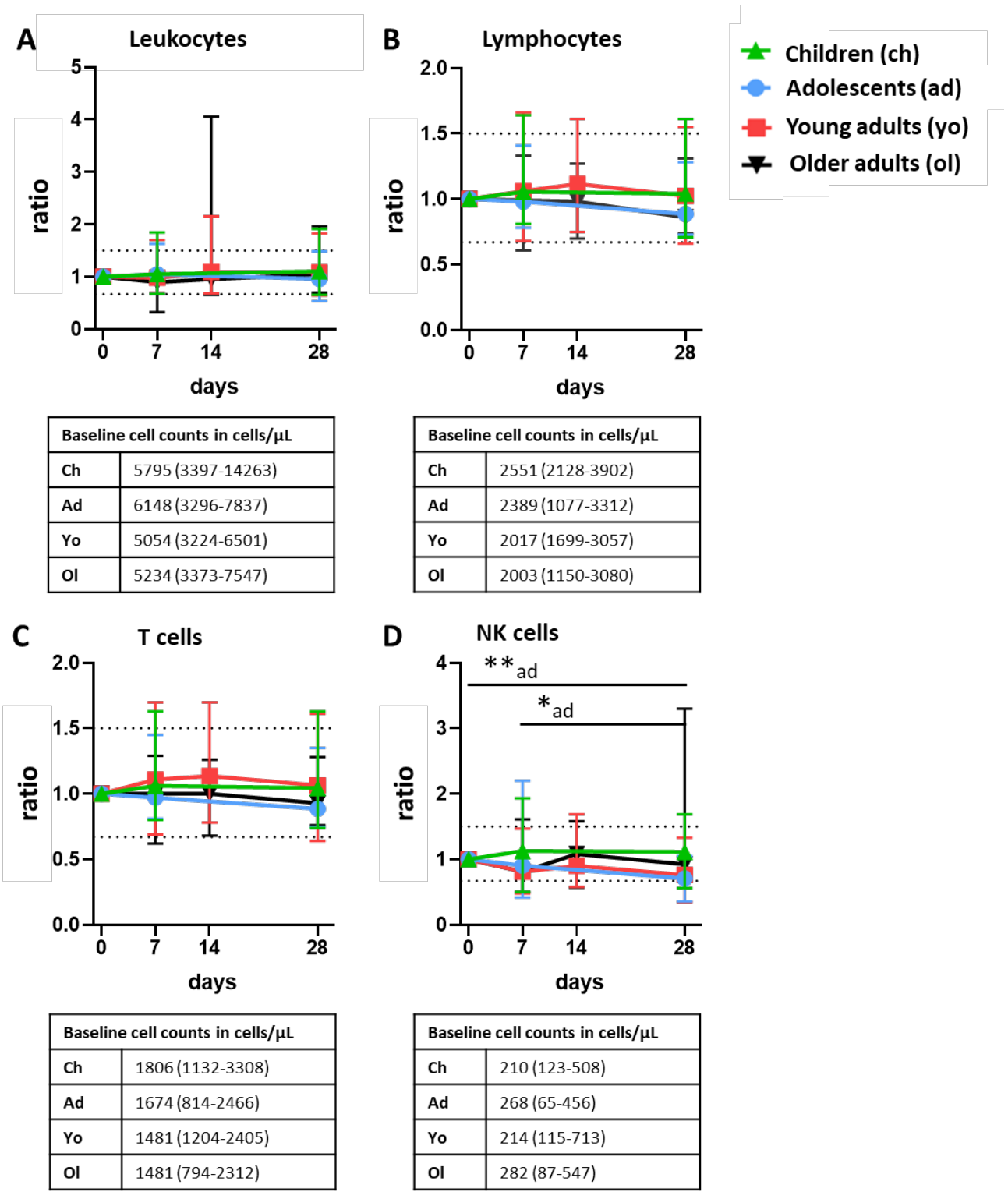
**Table S2. Composition of the EuroFlow B-cell panel and technical information on the reagents for the IMI-2 PERISCOPE BERT study.**

**Table S3. Phenotypic descriptions used to define B-cell subsets stained with the EuroFlow B-cell panel by manual analysis.**

**Table S4. Baseline distribution of leukocytes, lymphocytes, T cells and NK cells in donor groups.**

**Table S5. Spearman Ranking Correlation between IgG1+ plasma cell and memory B-cell kinetics and vaccine component-specific serum IgG.**

**Table S6. Spearman Ranking Correlation between IgA1+ plasma cell and IgA memory B-cell kinetics and vaccine component-specific serum IgA.**




| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 5795 (3397-14263) |
| Ad | 6148 (3296-7837) |
| Yo | 5054 (3224-6501) |
| Ol | 5234 (3373-7547) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 2551 (2128-3902) |
| Ad | 2389 (1077-3312) |
| Yo | 2017 (1699-3057) |
| Ol | 2003 (1150-3080) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 1806 (1132-3308) |
| Ad | 1674 (814-2466) |
| Yo | 1481 (1204-2405) |
| Ol | 1481 (794-2312) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 210 (123-508) |
| Ad | 268 (65-456) |
| Yo | 214 (115-713) |
| Ol | 282 (87-547) |

**Figure S1. No clear over-time post-vaccination changes in major populations in any of the donor groups.** The post-vaccination fluctuations of (**A**) leukocytes, (**B**) lymphocytes, (**C**) T cells, and (**D**) NK cells, presented as ratio over baseline (median, min-max). Dashed lines

indicate a ratio of 0.67 and 1.5 compared to baseline. Underneath each graph, the baseline cell counts per cohort are presented in cells/μL (median, min-max). To assess longitudinal changes within each cohort, Wilcoxon matched pair signed-rank test followed by Bonferroni correction was used. To test differences between cohorts at one timepoint, Kruskal-Wallis followed by Dunn's test was used, with exception of the comparison at day 14. At day 14, only blood samples from the adult cohorts were collected. Here, the Mann-Whitney test followed by Bonferroni correction was used. For longitudinal changes, only significant differences compared to baseline are shown. *, $p < 0.05$; **, $p \leq 0.01$.

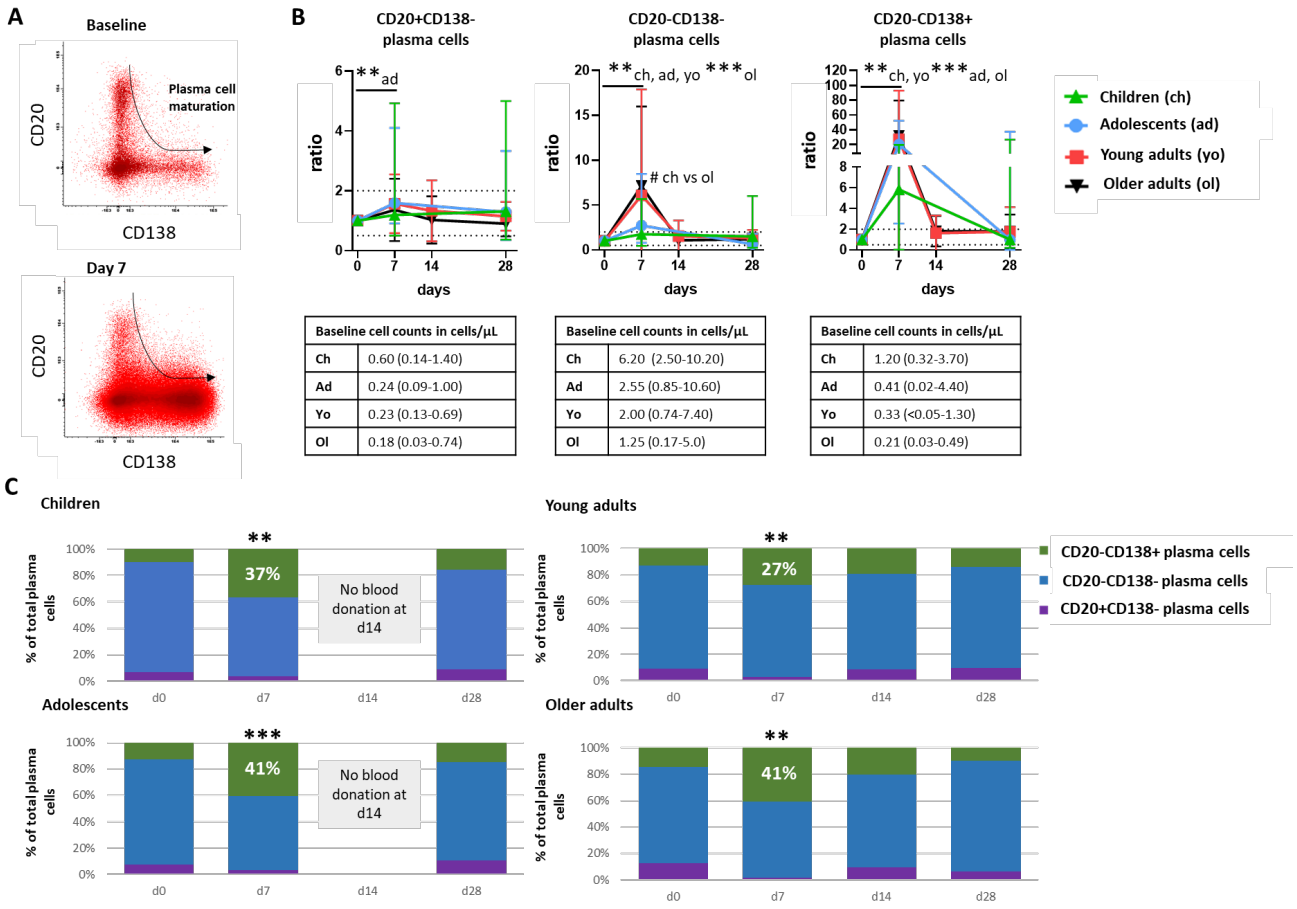
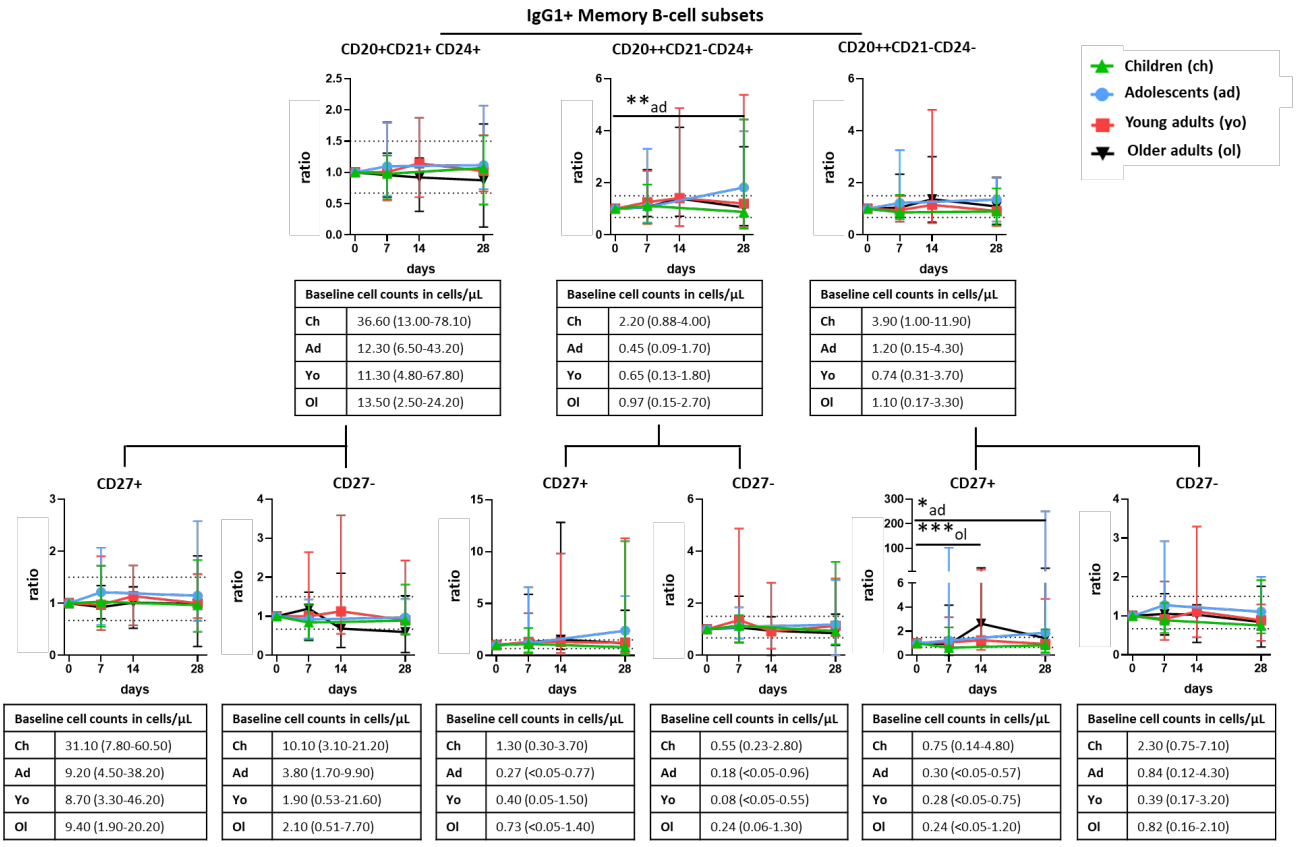



**Figure S2. Over time maturation of total plasma cells. (A)** Representative plots showing the phenotypical changes during plasma cell maturation. Each dot represents an individual cell. The arrow indicates the direction of changes during maturation. **(B)** Over-time quantitative changes in plasma cells belonging to different maturation stages, presented as ratio over baseline (median, min-max). Dashed lines indicate a ratio of 0.5 and 2.0 compared to baseline. Underneath each graph, the baseline cell counts per cohort are indicated in cells/µL (median, min-max). **(C)** Over-time distribution of plasma cells representing different maturation stages within total plasma cells. Median values for each population were used to construct the plots. Wilcoxon matched pair signed-ranked test followed by Bonferroni correction was used to assess longitudinal differences in percentage of CD20-CD138+ cells in total plasma cells within each cohort. Differences in the percentage CD20-CD138+ cells in total plasma cells between cohorts were assessed using Kruskal-Wallis followed by Dunn's test, but did not yield significant differences. At day 14, only blood samples from the adult cohorts were

collected. Here, the Mann-Whitney test followed by Bonferroni correction was used. For longitudinal changes, only significant differences compared to baseline are shown. Significant longitudinal differences within a cohort as indicated with **, $p \leq 0.01$; ***, $p \leq 0.001$. Significant differences between cohorts at the same time point are indicated with #, $p < 0.05$.




| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 36.60 (13.00-78.10) |
| Ad | 12.30 (6.50-43.20) |
| Yo | 11.30 (4.80-67.80) |
| Ol | 13.50 (2.50-24.20) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 2.20 (0.88-4.00) |
| Ad | 0.45 (0.09-1.70) |
| Yo | 0.65 (0.13-1.80) |
| Ol | 0.97 (0.15-2.70) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 3.90 (1.00-11.90) |
| Ad | 1.20 (0.15-4.30) |
| Yo | 0.74 (0.31-3.70) |
| Ol | 1.10 (0.17-3.30) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 31.10 (7.80-60.50) |
| Ad | 9.20 (4.50-38.20) |
| Yo | 8.70 (3.30-46.20) |
| Ol | 9.40 (1.90-20.20) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 10.10 (3.10-21.20) |
| Ad | 3.80 (1.70-9.90) |
| Yo | 1.90 (0.53-21.60) |
| Ol | 2.10 (0.51-7.70) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 1.30 (0.30-3.70) |
| Ad | 0.27 (<0.05-0.77) |
| Yo | 0.40 (0.05-1.50) |
| Ol | 0.73 (<0.05-1.40) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 0.55 (0.23-2.80) |
| Ad | 0.18 (<0.05-0.96) |
| Yo | 0.08 (<0.05-0.55) |
| Ol | 0.24 (0.06-1.30) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 0.75 (0.14-4.80) |
| Ad | 0.30 (<0.05-0.57) |
| Yo | 0.28 (<0.05-0.75) |
| Ol | 0.24 (<0.05-1.20) |

| Baseline cell counts in cells/µL | |
|---|---|
| Ch | 2.30 (0.75-7.10) |
| Ad | 0.84 (0.12-4.30) |
| Yo | 0.39 (0.17-3.20) |
| Ol | 0.82 (0.16-2.10) |

**Figure S3. No significant changes in IgG1+ memory B-cell subsets upon vaccination.** Over-time quantitative changes in IgG1+ memory B-cell subsets, presented as ratio over baseline (median, min-max). Dashed lines indicate a ratio over baseline of 0.67 and 1.5. Underneath each graph, the baseline cell counts per cohort are presented in cells/µL (median, min-max). Wilcoxon matched pair signed-ranked test followed by Bonferroni correction was used to assess differences in ratio compared to baseline over time. For longitudinal changes, only significant differences compared to baseline are shown. *, $p < 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

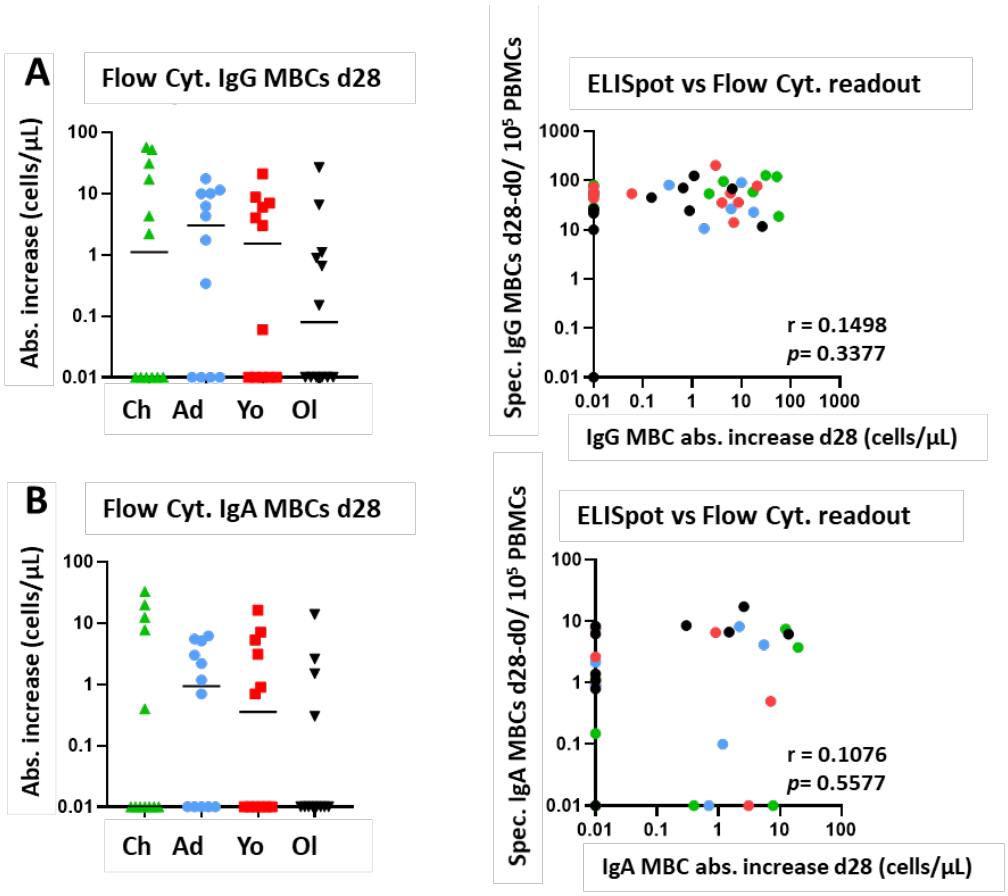



**Figure S4. Correlation between cellular changes as measured by flow cytometry and ELISpot. (A)** Left panel: expansion of IgG+ Memory B cells (day 28) per individual, expressed as absolute increase in cells/µL. Right panel: correlation between the ELISpot readings and the flow cytometry readout for IgG+ Memory B cells. **(B)** Left panel: expansion of IgA+ Memory B cells (day 28) per individual, expressed as absolute increase in cells/µL. Right panel: correlation between the ELISpot readings and the flow cytometry readout for IgA+ Memory B cells. Of note; for visualization purposes, all absolute increases lower than 0.01 were set to 0.01. The original values were used to calculate the Spearman Correlations. Flow Cyt. = flow cytometry; spec.= specific (in this case, specific for the tested vaccine antigens); MBC = Memory B cells; PBMC= peripheral blood mononuclear cells; d= days after vaccination; r= Spearman's Correlation coefficient; abs.= absolute.

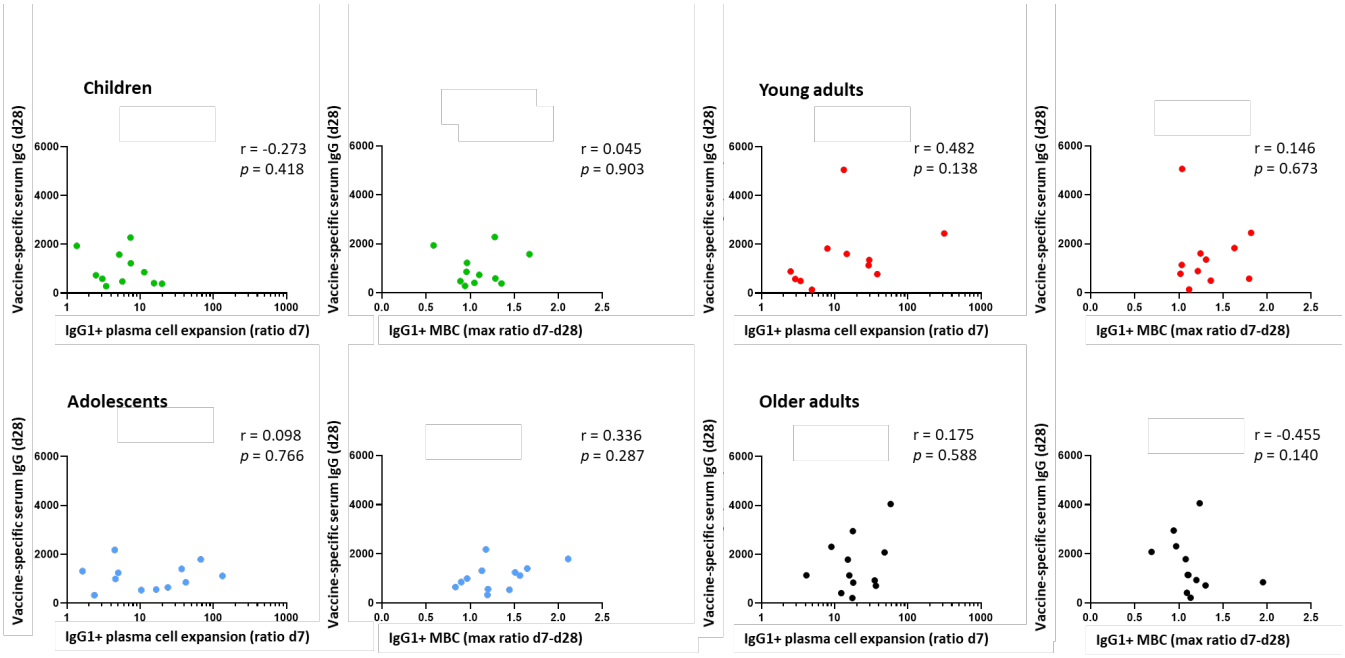



**Figure S5. Correlation between cellular changes and the vaccine-specific serum IgG level post-vaccination as determined by Spearman's Ranking Correlation per age cohort.** Per cohort the left plot shows the correlation between the maximum expansion of IgG1 plasma cells (day 7) and vaccine-specific serum IgG (directed against FHA, Prn, PT and Tet) (day 28). The right plots show the correlation between the maximum expansion of IgG1 memory B cells (day 14 or day 28) and vaccine-specific serum IgG (directed against FHA, Prn, PT and Tet) (day 28). MBC = memory B cell; r= Spearman's correlation coefficient; d= days after vaccination.

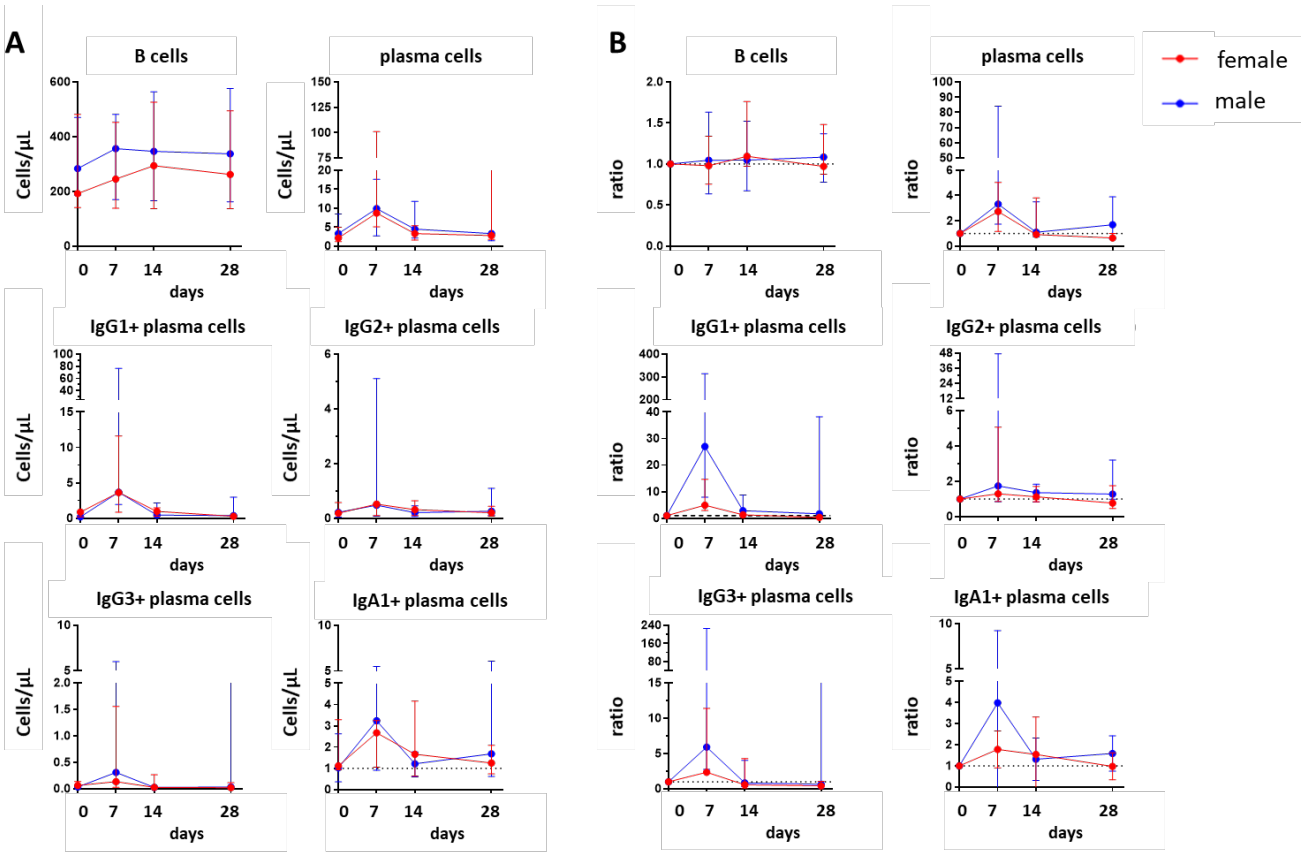



**Figure S6. Impact of sex on cellular responses after vaccination in the young adult cohort (all wP-primed).** Flow cytometry-derived cell numbers (absolute count in cells/µL (**A**) and ratio over baseline (**B**)) and their changes over time in an age-matched, wP-primed male (n= 7) and female (n= 5) cohort. Of note, for one male participant, no baseline B-cell data was available. Therefore, in the graphs showing the ratio over baseline, data of 6 males are shown, whereas absolute counts include the data of 7 males. Graphs indicate median + range. Dashed line indicates ratio of 1.0 (baseline value).

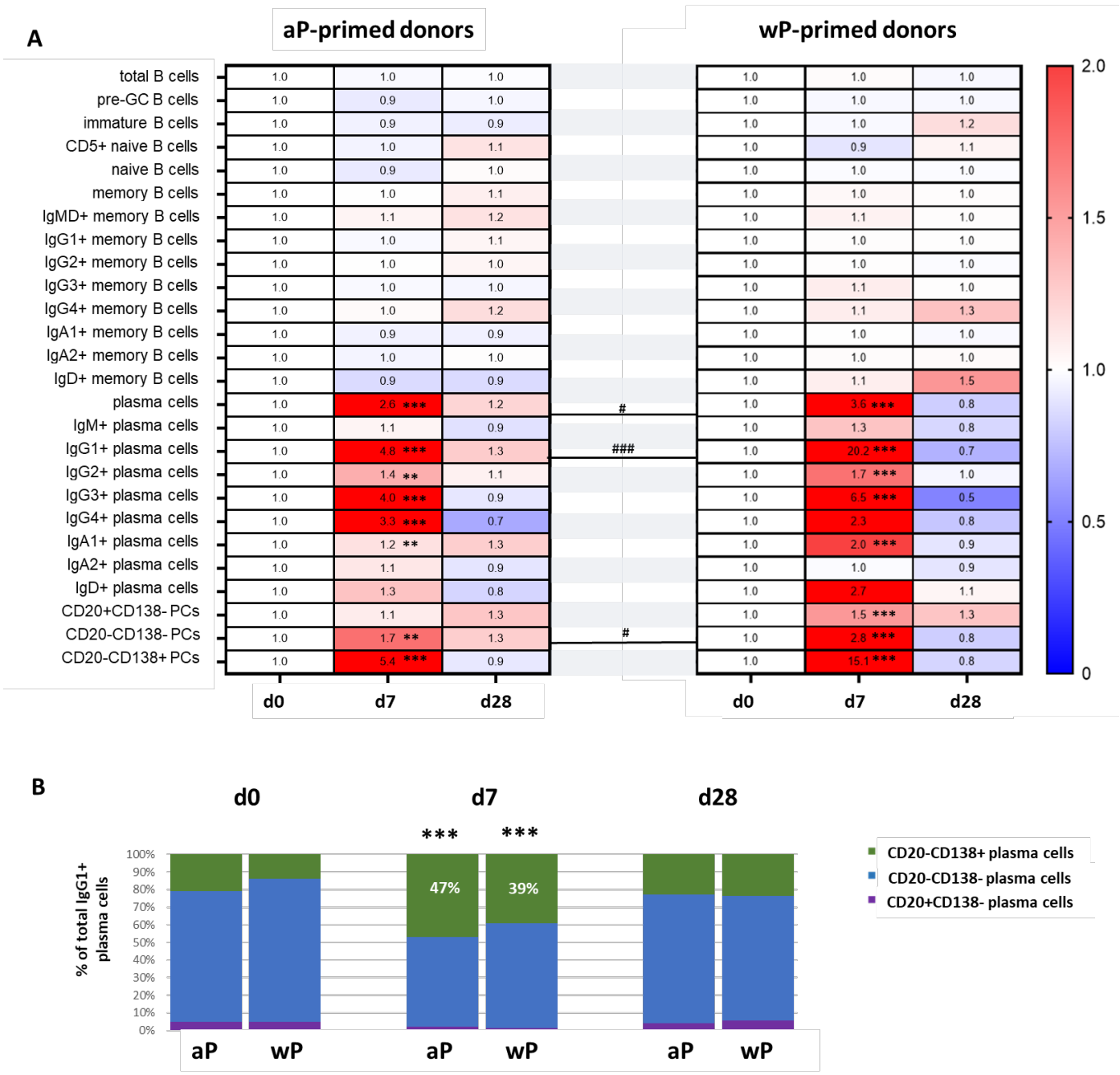



**Figure S7. IgG1+ and total plasma cell expansion is more prominent in non-age-matched donors after wP priming. (A)** Heatmap showing over-time changes in memory B-cell and plasma cell subsets in aP-primed (12 children + 5 adolescents) and wP-primed (7 adolescents and 12 young adults; not the older adults, because of their uncertain vaccination status) donors. **(B)** Over-time distribution of IgG1+ plasma cells representing different maturation stages with total IgG1+ plasma cells. Median values for each population were used to

construct the plots. Wilcoxon matched pair signed-ranked test followed by Bonferroni correction was used to assess longitudinal differences in percentage of CD20-CD138+ cells in total IgG1+ plasma cells within each cohort. Differences in the percentage CD20-CD138+ cells in total IgG1+ plasma cells between cohorts were assessed using Kruskal-Wallis followed by Dunn's test, but did not yield significant differences. For longitudinal changes, only significant differences compared to baseline are shown. Significant longitudinal differences within a cohort as indicated with ***, $p \leq 0.001$. Significant differences between cohorts at the same time point are indicated with #, $p < 0.05$; ###, $p \leq 0.001$. D= days after vaccination; aP = acellular pertussis vaccine; wP = whole cell pertussis vaccine.