Supplementary Material

**Supplemental Tables**

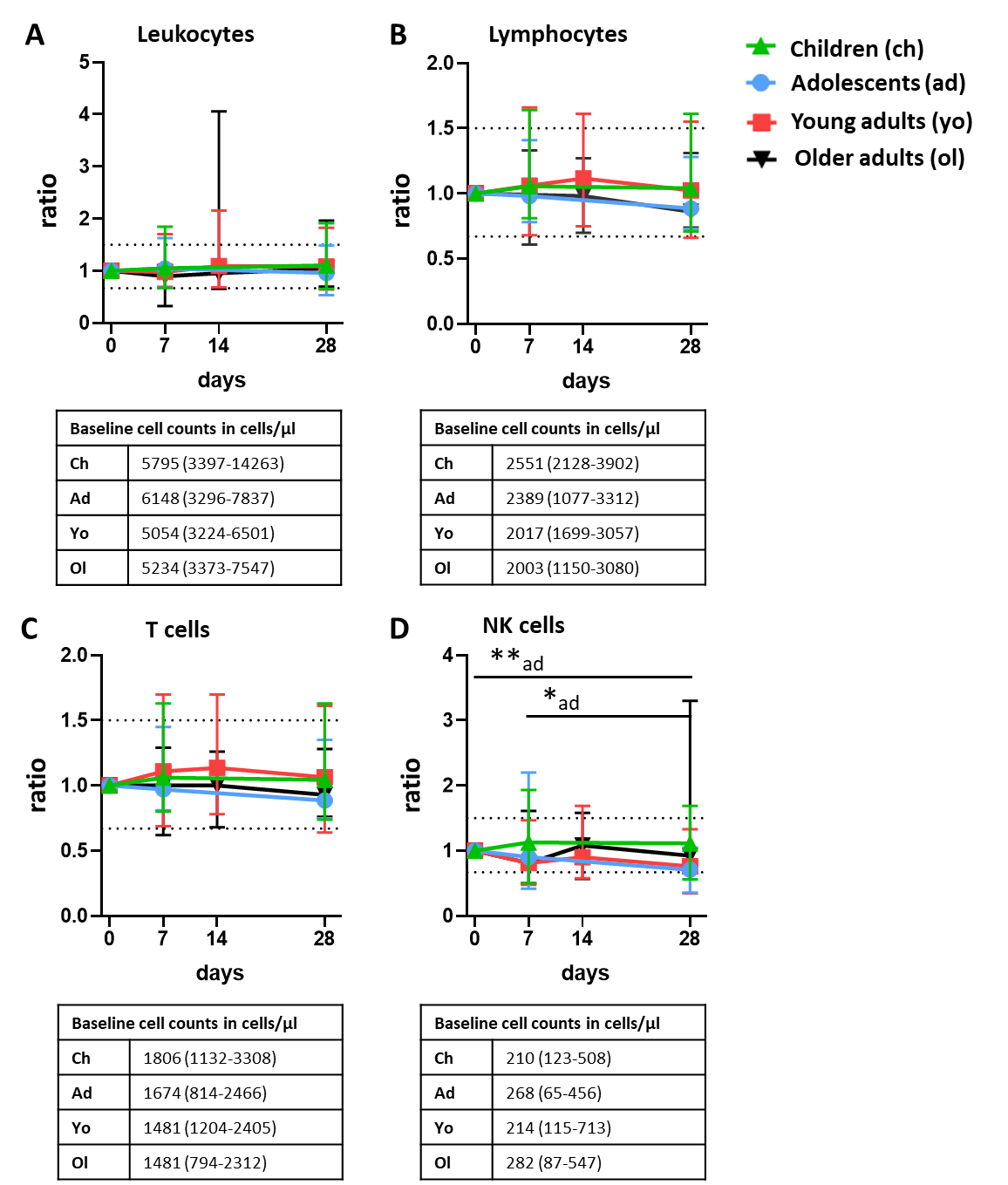
**Supplemental Table 1. Composition of the EuroFlow B-cell panel and technical information on the reagents for the IMI2 PERISCOPE BERT study.**

**Supplemental Table 2. Phenotypic descriptions used to define B-cell subsets stained with the EuroFlow B-cell panel by manual analysis.**

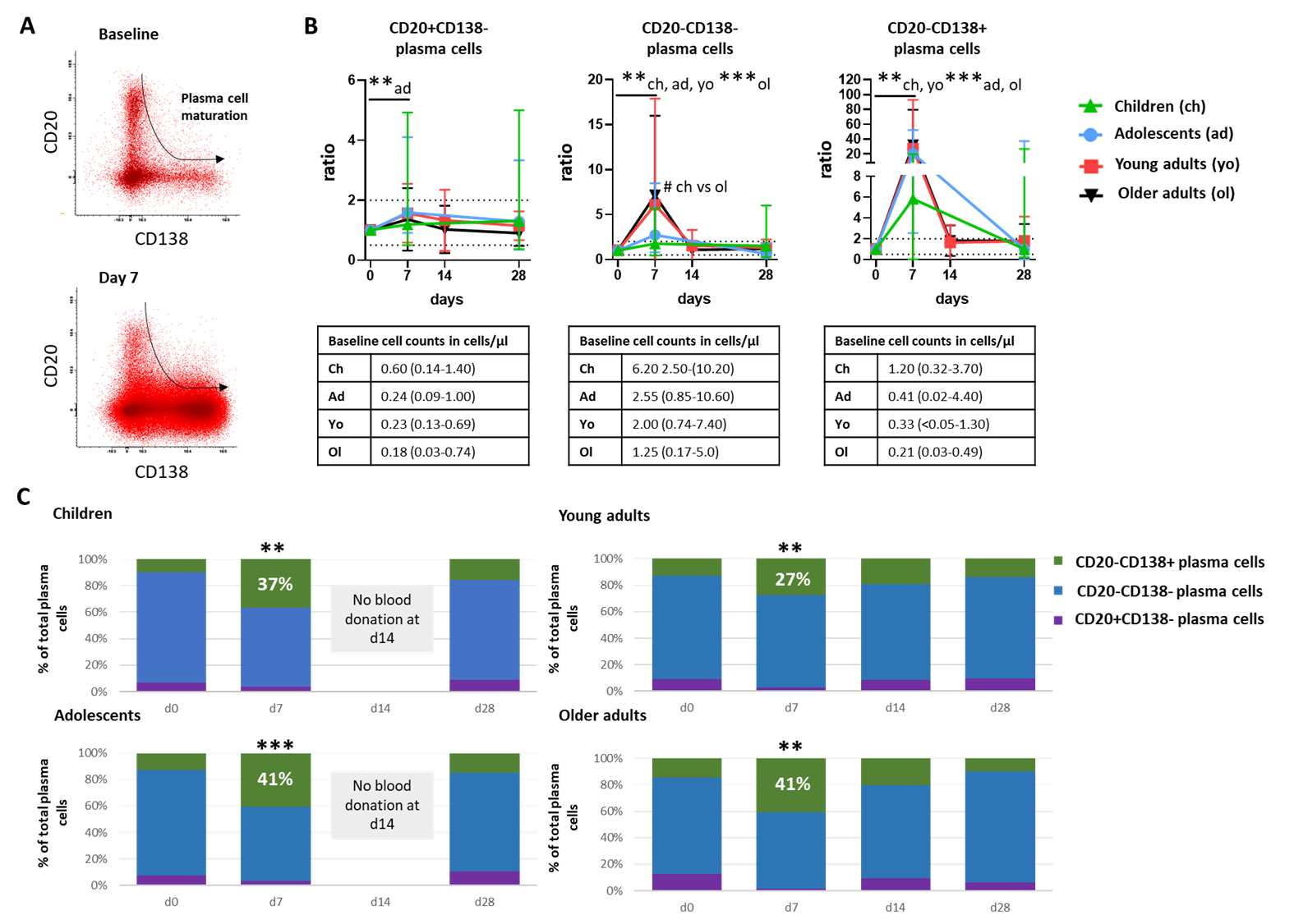
**Supplemental Table 3. Baseline distribution of leukocytes, lymphocytes, T cells and NK cells in donor groups.**

**Supplemental Table 4. Spearman Ranking Correlation between IgG1+ plasma cell and memory B-cell kinetics and vaccine component-specific serum IgG.**

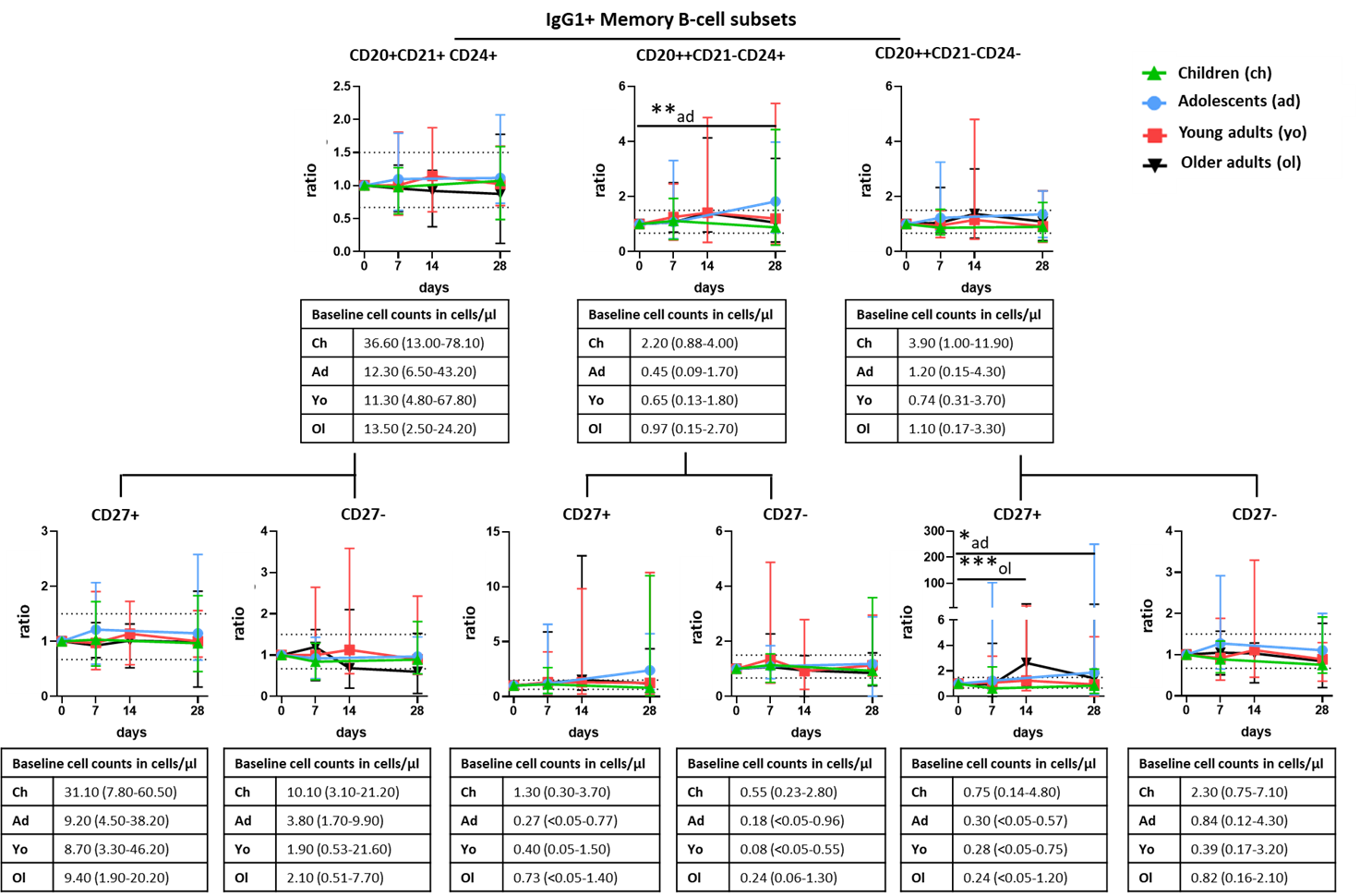
**Supplemental Table 5. Spearman Ranking Correlation between IgA1+ plasma cell and IgA memory B-cell kinetics and vaccine component-specific serum IgA.**



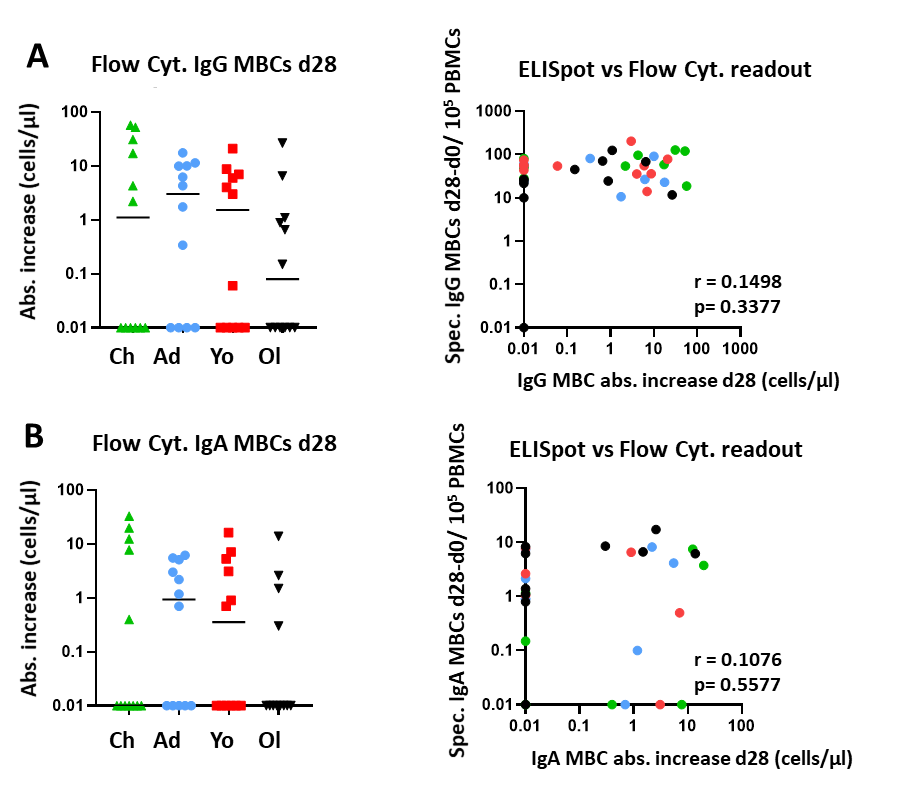
**Supplemental Figure 1. No clear over-time post-vaccination changes in major populations in any of the donor groups.** The post-vaccination fluctuations of (**A**) leukocytes, (**B**) lymphocytes, (**C**) T cells, and (**D**) NK cells, presented as ratio over baseline (median, min-max). Dashed lines 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≤0.01.

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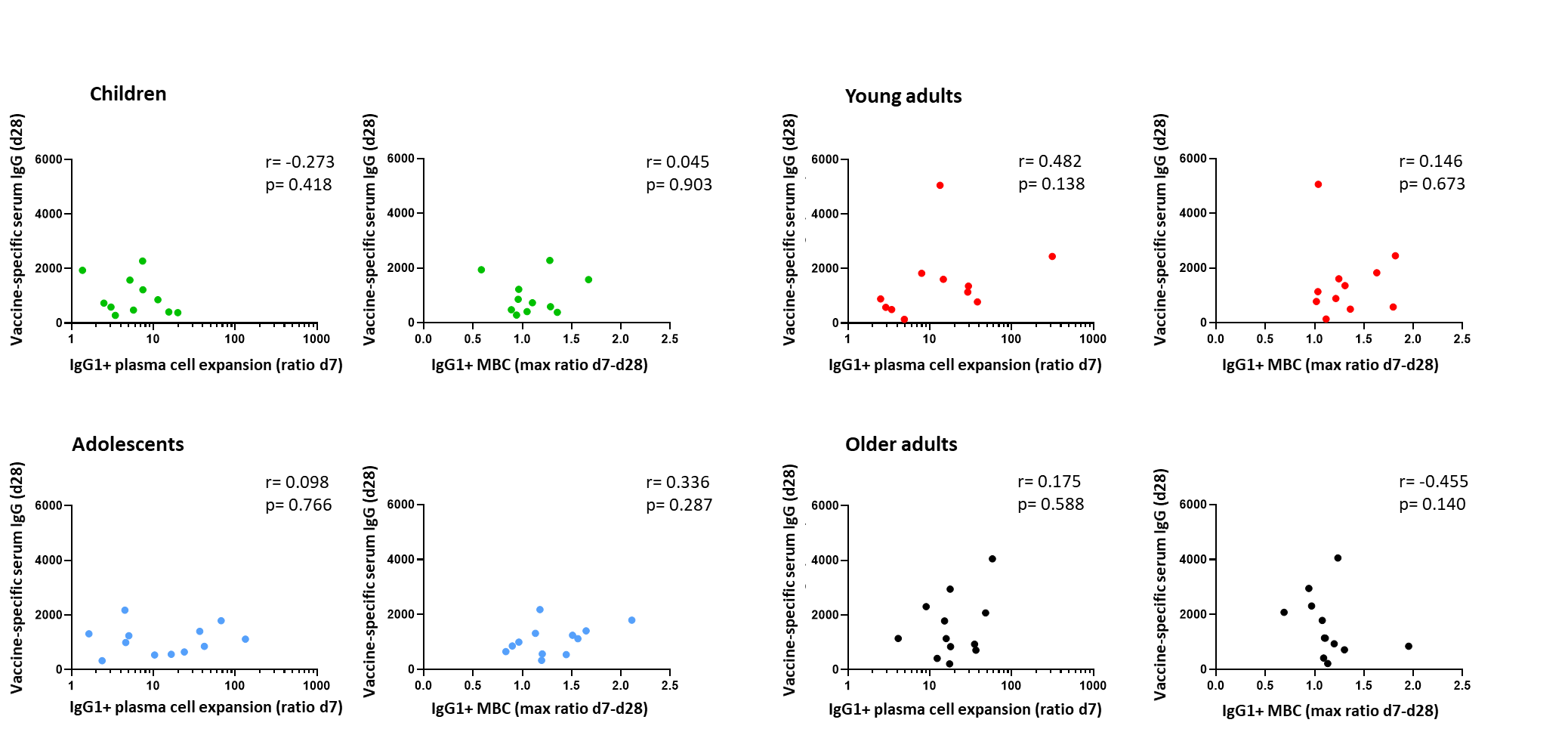
**Supplemental Figure 2. Over time maturation of total plasma cells. (A)** Representative plots showing the phenotypical changes during plasma cell maturation. Each dot represents an individual cell. The arrow indicates the direction of changes during maturation. **(B)** Over-time quantitative changes 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<0.05; \*\*, p≤0.01; \*\*\*, p≤0.001. Significant differences between cohorts at the same time point are indicated with #, p<0.05; ##, p≤0.01; ###, p≤0.001.



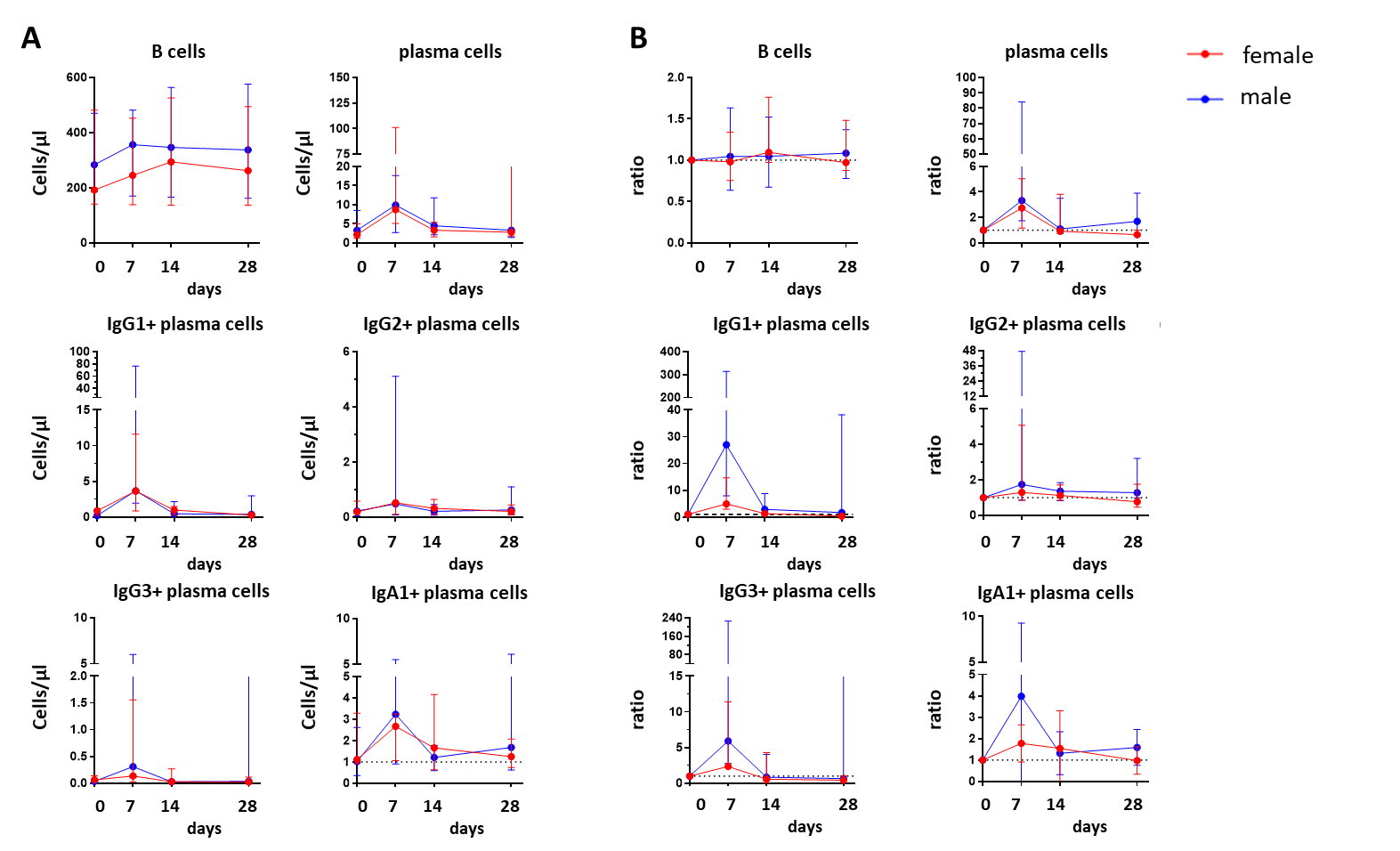
**Supplemental Figure 3. No significant changes in IgG1+ memory B-cell subsets upon vaccination.** Over-time quantitative changes in IgG1+ memory B-cell subsets, presented as ratio over baseline (median, min-max). Dashed lines indicate a ratio over baseline of 0.67 and 1.5. Underneath each graph, the 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≤0.01; \*\*\*, p≤0.001.

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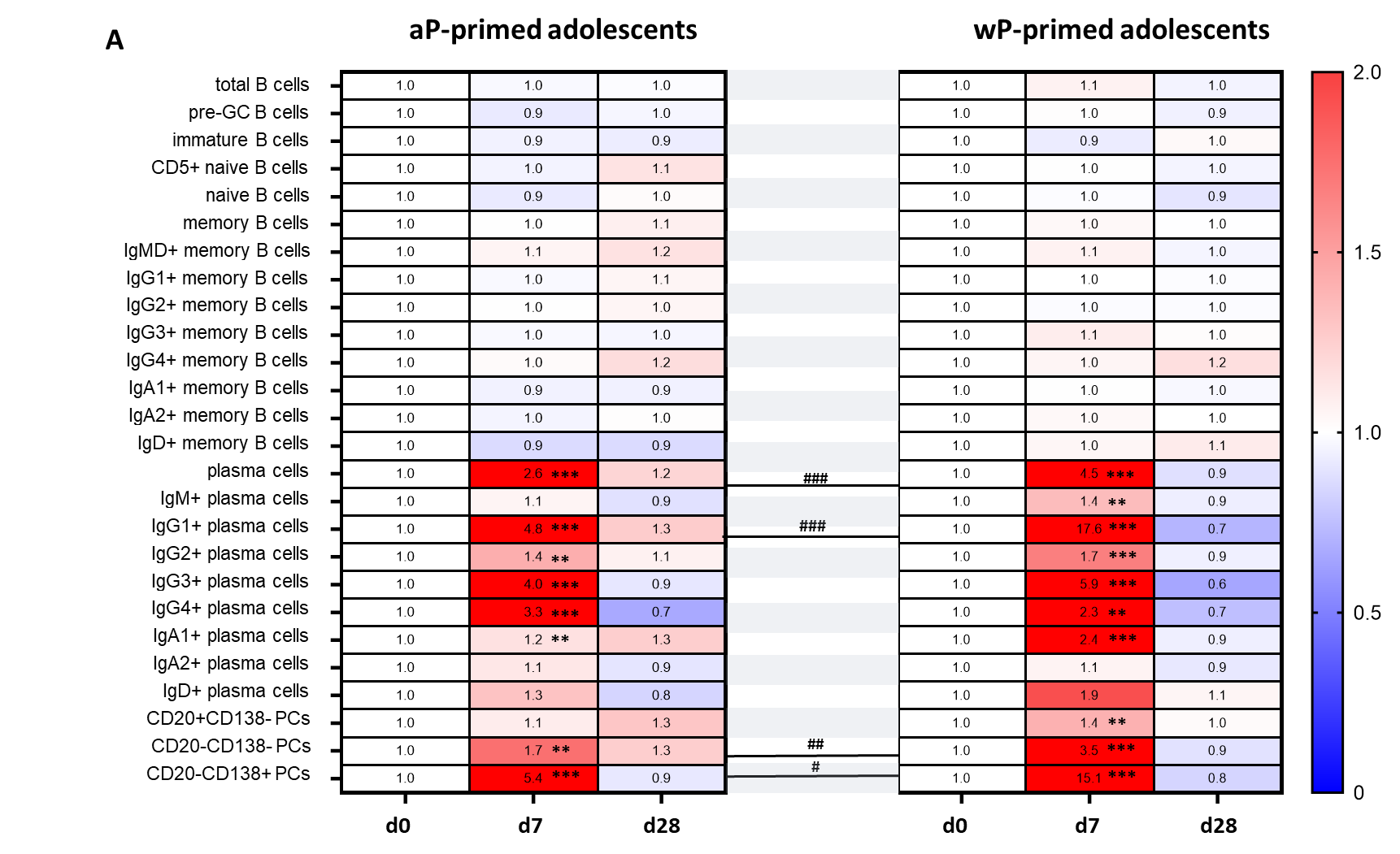
**Supplemental Figure 4. Correlation between cellular changes as measured by flow cytometry and ELISpot.**  **(A)** Left panel: expansion of IgG+ Memory B cells (day 28) per individual, expressed as absolute increase in cells/ul. Right panel: correlation between the ELISpot readings and the flow cytometry 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/ul. 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.

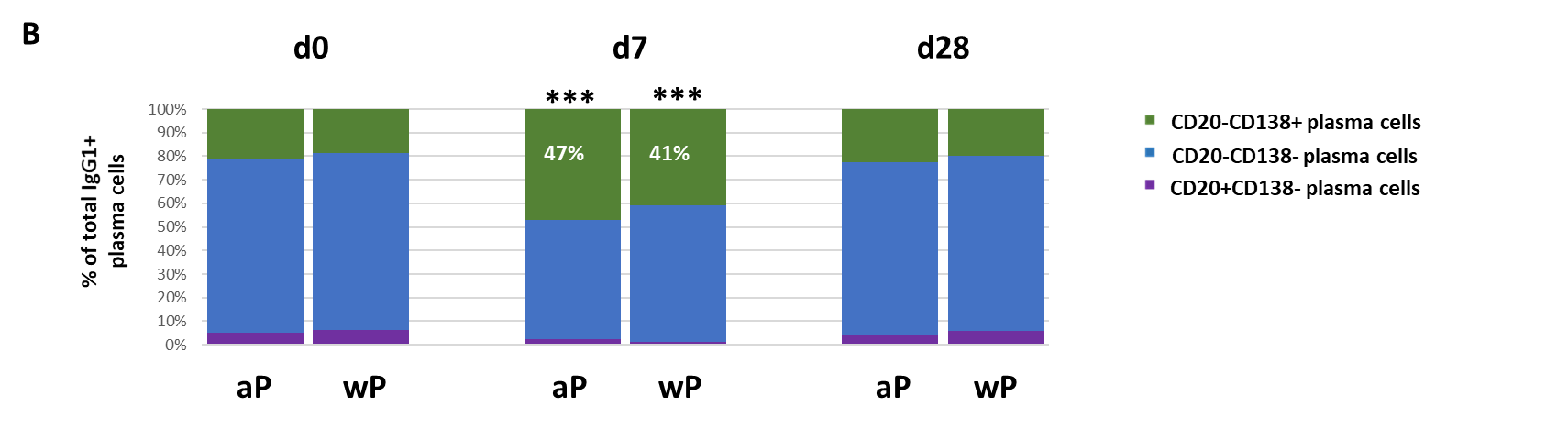


**Supplemental Figure 5.** **Correlation between cellular changes and the vaccine-specific serum IgG level post-vaccination as determined by Spearman’s Ranking Correlation per age cohort.** Per cohort the left plot shows the correlation between the maximum expansion of IgG1 plasma cells (day 7) and vaccine-specific 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.

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**Supplemental Figure 6**. **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/ul (**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).



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**Supplemental Figure 7. 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, 12 young adults and 12 older adults) 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<0.05; \*\*, p≤0.01; \*\*\*, p≤0.001. Significant differences between cohorts at the same time point are indicated with #, p<0.05; ##, p≤0.01; ###, p≤0.001. D= days after vaccination; aP = acellular pertussis vaccine; wP = whole cell pertussis vaccine.