Figure 1

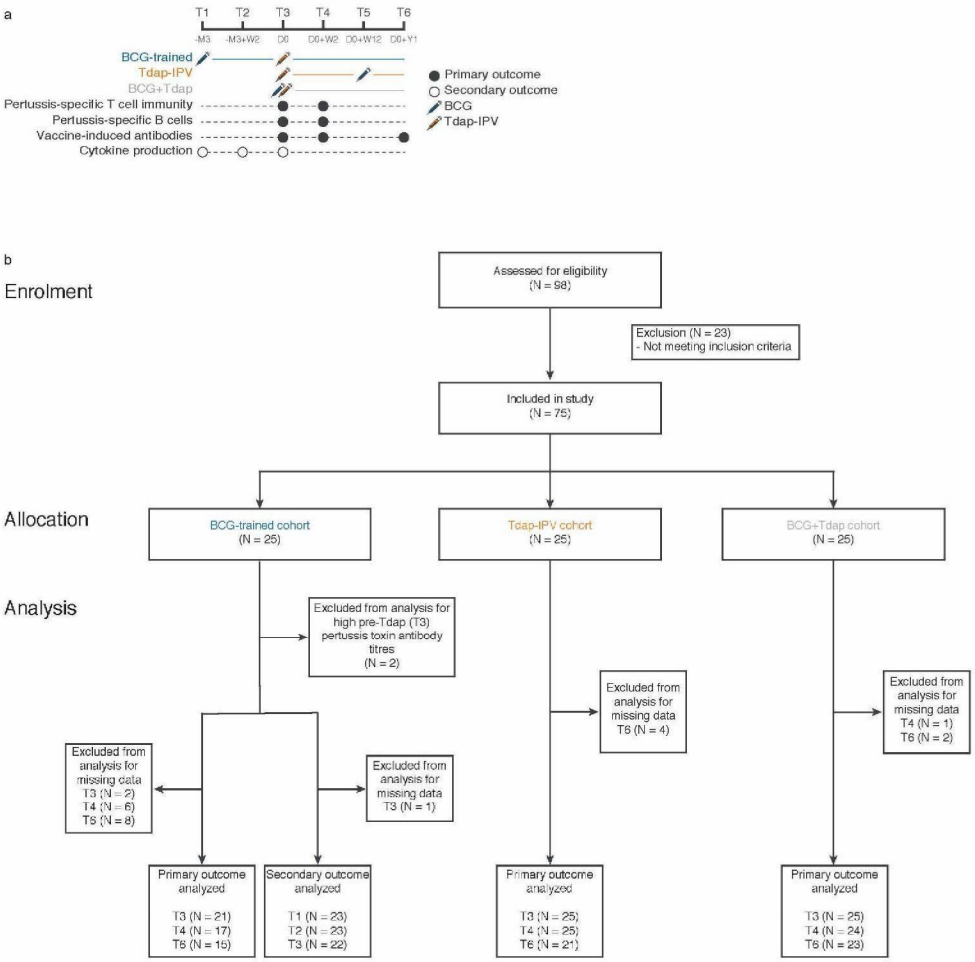

Figure 1. Study procedures and flow diagram of subjects who were included. A) Overview of the study design and measurements. Seventy-five female subjects were randomly assigned to one of three study cohorts to receive Tdap-IPV and/or BCG vaccinations in different sequences. Measurements aimed at quantifying the quality and strength of the immune response to Tdap-IPV vaccination were measured across all three cohorts (primary outcomes, black circles). Additional measurements were taken in the BCG-trained cohort in order to quantify induction of trained immunity. Cytokine production was quantified in peripheral blood mononuclear cells in response to stimulation with heterologous stimuli (secondary outcomes, white circles). Timestamps are defined relative to the baseline of Tdap-IPV vaccination (T1 = three months prior, T2 = three months prior plus two weeks, T3 = baseline of Tdap-IPV or concurrent BCG and Tdap-IPV vaccinations, T4 = two weeks thereafter, T5 = three months plus two weeks thereafter, T6 = one year thereafter), **B)** Flow diagram describing subjects who were enrolled in the study and who were excluded from further analysis.

Figure 2

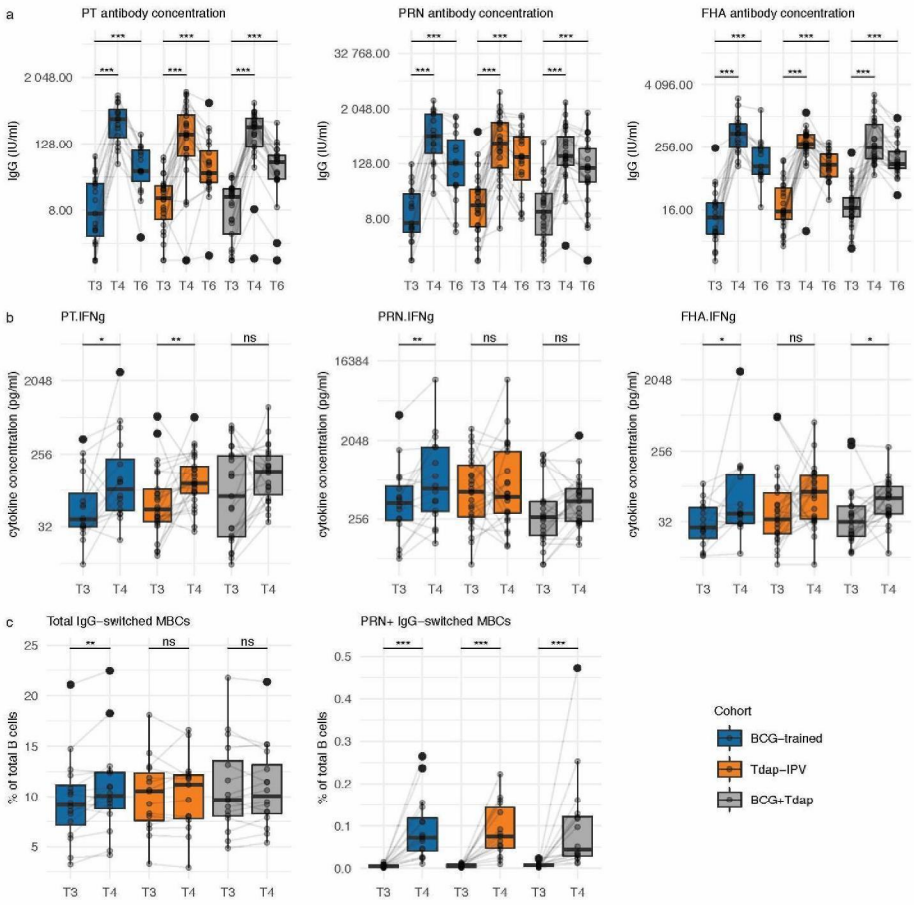

Figure 2. Anti-Tdap antibody concentrations, B-cell responses, and IFNg responses two weeks following Tdap-IPV immunization. A) Pertussis vaccine antigen-specific antibody concentrations, **B)** IFNg produced in response to PT (PT.IFNg), PRN (PRN.IFNg), or FHA (FHA.IFNg) stimulation of peripheral blood mononuclear cells, **C)** frequencies of total and PRN+ IgG-switched memory B cell (MBC) populations. Cytokine and antibody concentration values are shown on the log2 scale. Data are N = 15 - 25 per cohort and are represented as a box-and-whisker plot, with bounds from 25th to 75th percentile, median line, and whiskers, which extend to the largest or smallest value no further than 1.5 * the inter-quartile range (distance between the first and third quartiles). P-values were calculated using a Wilcoxon signed rank test, * p <0.05; ** p<0.01; *** p<0.001.

Figure 3

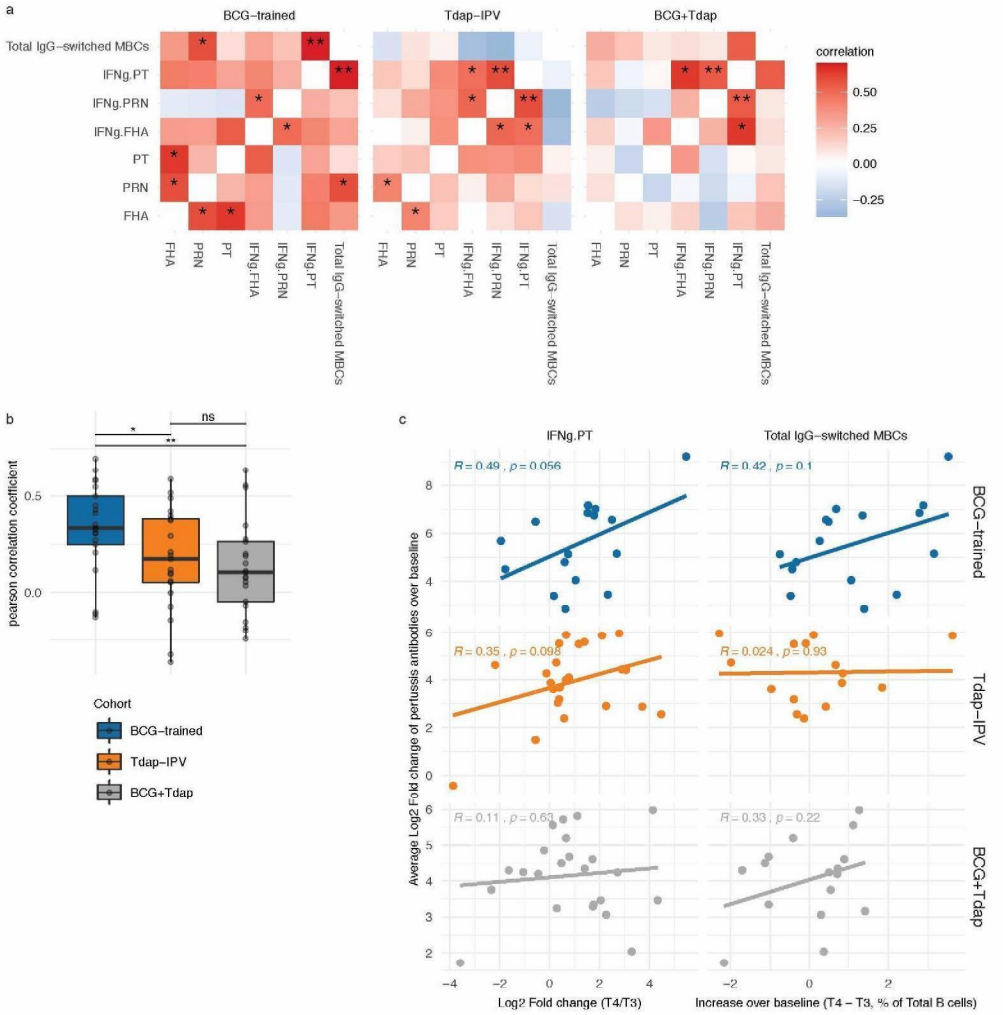

Figure 3. Tdap-IPV induced adaptive immunity is highly correlated in the BCG-trained cohort. A) Pearson correlation heatmap of increases for the specified measurements within each cohort. Increases in pertussis-specific IgG (PT, PRN, FHA) and IFNg production following peripheral blood mononuclear cell stimulation with pertussis antigens (IFNg.PT, IFNg.PRN, IFNg.FHA) were calculated as log2 fold changes (L2FC) (T4 / T3). For total IgG-switched memory B cells (MBCs), increases were calculated as the difference of week two and baseline (T4 – T3). **B)** Pearson correlation coefficients were extracted from A) and plotted per intervention. Data were compared using the Wilcoxon signed rank test. **C)** Scatter plots showing the correlation between Pert_mean and IFNg.PT and total IgG-switched MBCs. Pert_mean was calculated per subject by taking the average of the L2FC (T4 / T3) of the PT, PRN, and FHA IgG responses. Data are from N = 16 – 25 subjects per cohort. A) shows Pearson correlation coefficients and p-values and C) linear regression slopes, Pearson correlation coefficients, and p-values are shown for each cohort, * p <0.05; ** p<0.01; *** p<0.001.

Figure 4

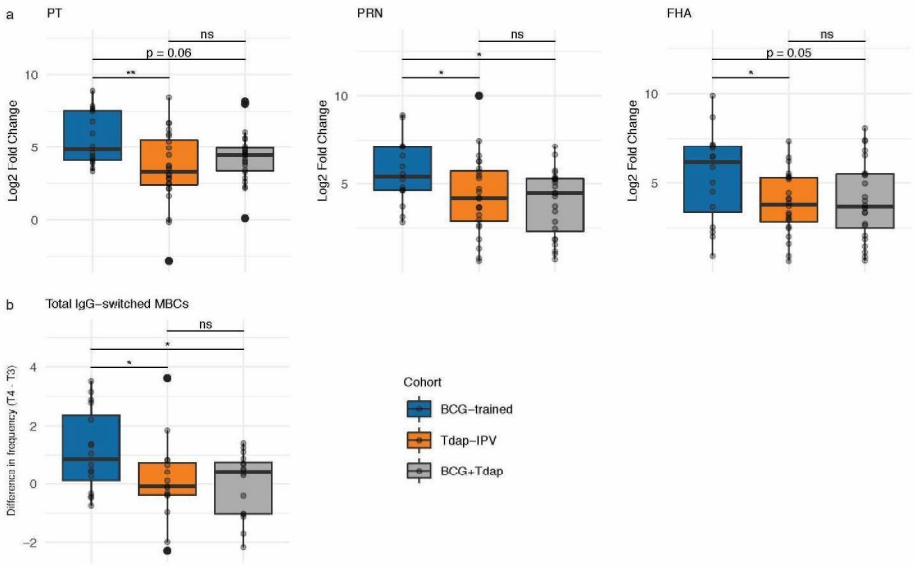

Figure 4. Prior BCG vaccination enhances pertussis-specific IgG and total IgG-switched memory B cell responses to Tdap-IPV vaccination. A) Log2 fold change (T4 / T3) of pertussis antigen-specific IgG responses, data are N = 16 – 25 per cohort. **B)** Differences in total IgG-switched memory B cell (MBC) responses (T4 – T3), data are N = 15 – 16 per cohort. Data are represented as a box-and-whisker plot, with bounds from 25th to 75th percentile, median line, and whiskers, which extend to the largest or smallest value no further than 1.5 * the inter-quartile range (distance between the first and third quartiles). P-values were calculated by 1-way ANOVA with post hoc t test, * p <0.05; ** p<0.01; *** p<0.001.

Figure 5

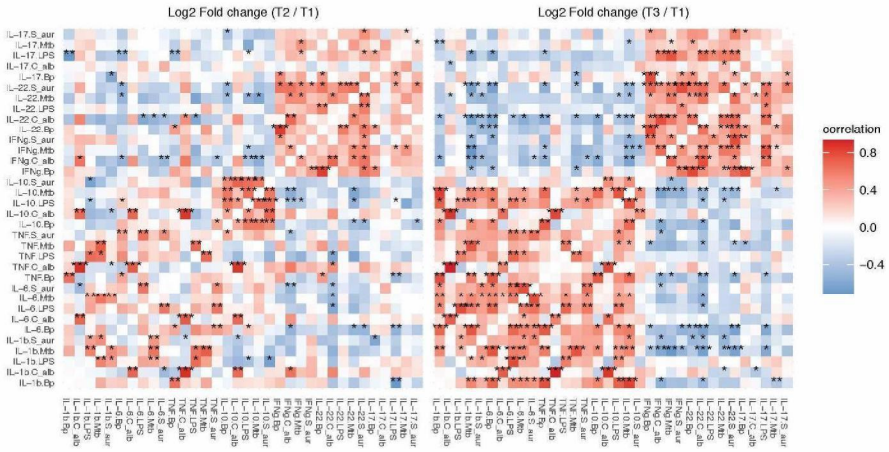

Figure 5. Heterologous effects of BCG vaccination on cytokine production. Within the BCG-trained cohort, log2 fold changes (L2FC) were calculated at two weeks (T2 / T1) and three months post BCG (T3 / T1) for the indicated cytokine:stimulation combinations. Pearson correlations between L2FCs are shown. Data are from N = 23 (left) and N = 22 (right), * p <0.05; ** p<0.01; *** p<0.001.

Figure 5

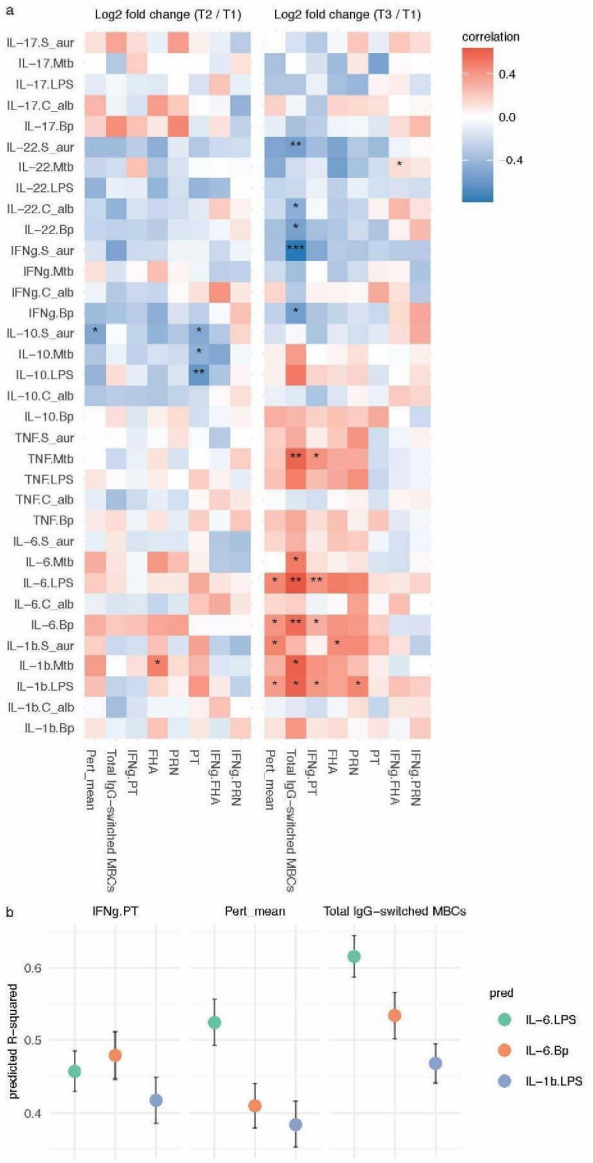

Figure 6. Heterologous effects of BCG vaccination are associated with increases in pertussis vaccination-induced adaptive immune responses. Log2 fold changes (L2FC) were calculated at two weeks post BCG (T2 / T1) and three months post BCG (T3 / T1) for the indicated cytokine:stimulation combinations. **A)** Pearson correlations between the cytokine variables on the y-axis and the L2FC (T4 / T3) responses for the pertussis-specific IgG (PT, PRN, FHA) and pertussis-specific IFNg responses (IFNg.PT, IFNg.PRN, IFNg.FHA), as well as Pert_mean and changes in total IgG-switched memory B cells (MBCs) (T4 – T3) on the x-axis, * p <0.05; ** p<0.01; *** p<0.001. **B)** IL-1b.LPS, IL-6.LPS, and IL-6.Bp responses measured three months post-BCG (T3 / T1) were selected based on their strong correlation with multiple pertussis endpoints. Linear models were constructed with these responses and predicted R-squared was calculated following 50 times repeated, 4-fold cross-validation. The mean predicted R -squared values are shown with 95% confidence intervals. Data are N = 16 subjects in the BCG-trained cohort.