Supplementary Figure 1

A

Viable B cells | Memory B cells | IgD-IgM- mem B cells | IgA+/IgG+ mem B cells

B Serum IgG3 ELISA

C

D

E

F

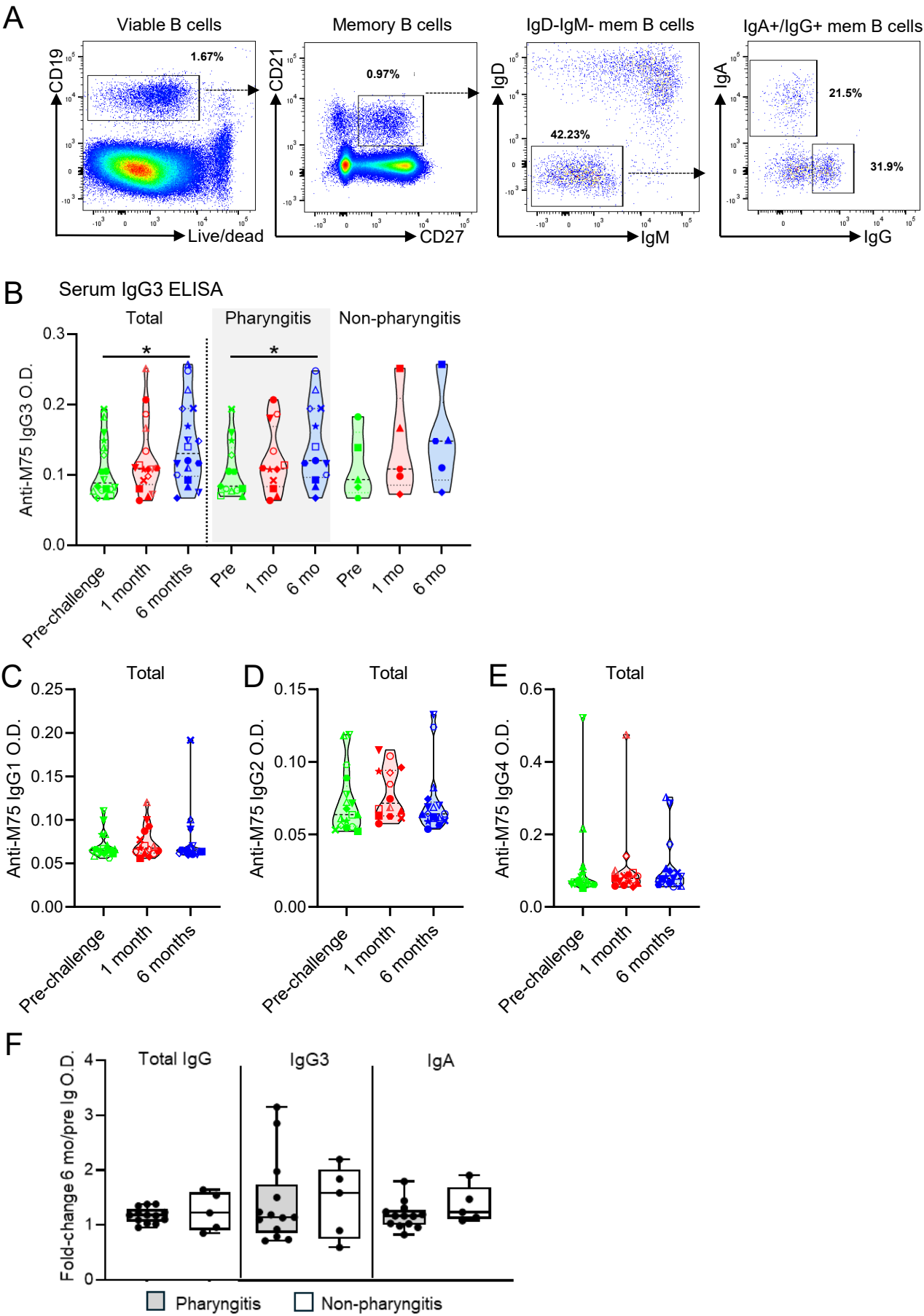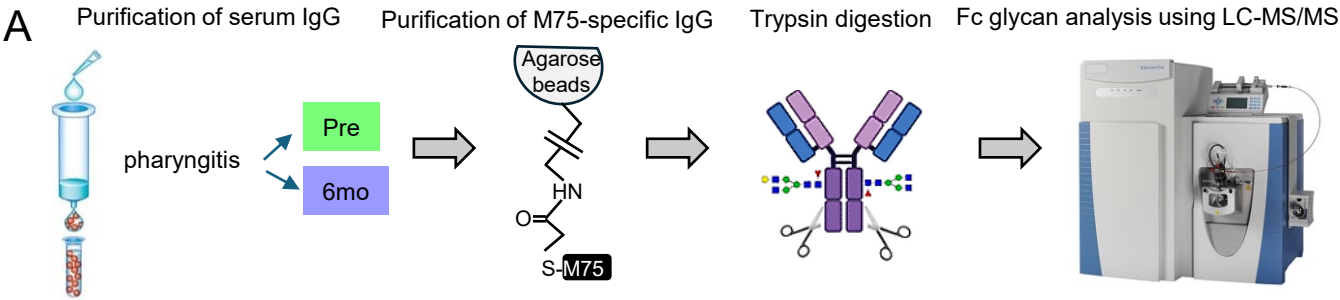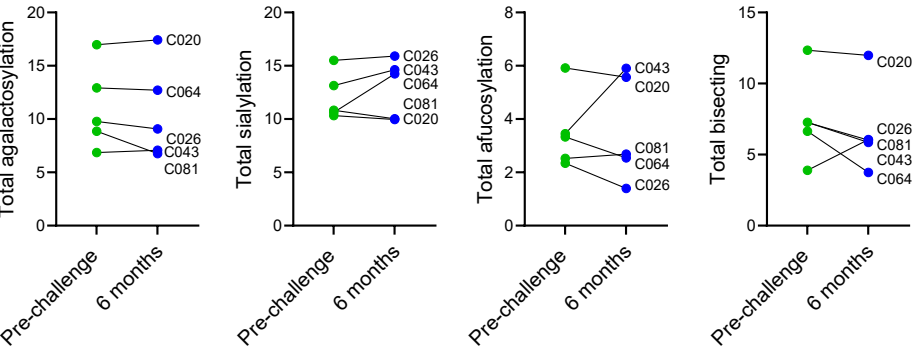**Supplementary Figure 1.** Antigen-specific immune responses to M75-HVR following experimental human Strep A M75 infection. (A) Flow cytometry gating strategy for analysis of IgG$^+$ and IgA$^+$ memory B cells in the peripheral blood. (B,C)) ELISA was used to detect anti-M75-specific IgG antibody subtypes in serum samples. Violin plots show O.D. 450 absorbance values are shown for sera collected at pre-challenge (day -1), 1 month post-challenge and 6 months post-challenge, using a 1:20 dilution. Violine plots show the absorbance values of M75-specific (IgG3 (C) IgG1, (D) IgG2 and (E) IgG4. Violin plots show O.D. 450 absorbance values for sera collected at pre-challenge (day -1) and 6 months post-challenge, using a 1:20 dilution. Data are stratified by participants who developed pharyngitis and those who did not. Each individual is represented by a symbol. One-way ANOVA followed by Bonferroni's comparisons tests were performed in all statistical analyses. *P<0.05, **P<0.01. (F) Box and whiskers plot of the ELISA data showing OD fold-change (6 months/ pre-infection) for M75-specific total IgG, IgG3, and IgA at a 1:20 dilution. Median and 25th, and 75th percentiles (boxes), as well as the minimum and maximum values, are indicated. The fold change represents the difference in OD values of 6 months post-infection compared to pre-infection levels.
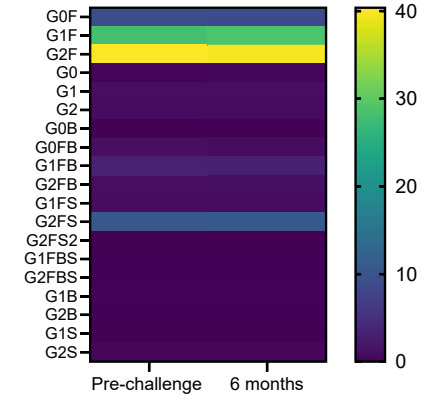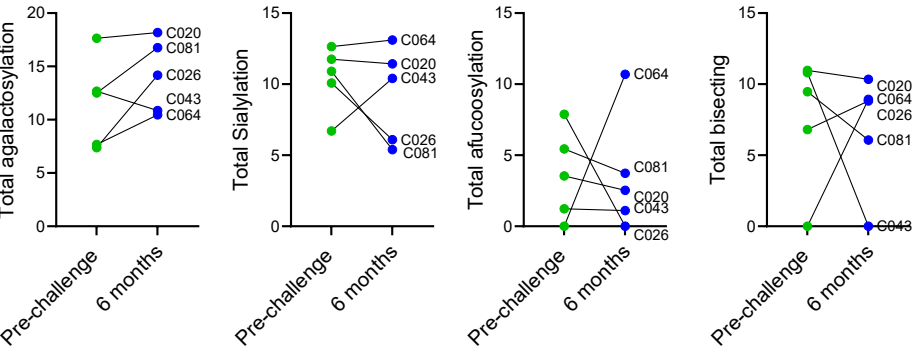
Supplementary Figure 2

A Purification of serum IgG → Purification of M75-specific IgG → Trypsin digestion → Fc glycan analysis using LC-MS/MS

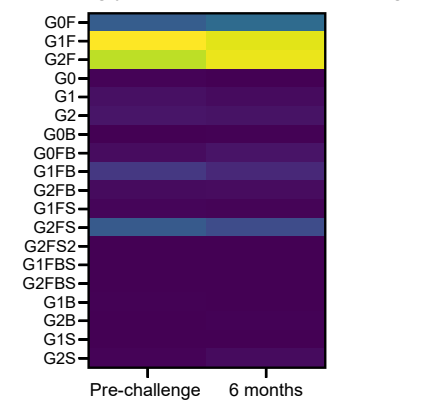**B** Fraction of Fc glycosylation in total (non-specific) IgG1

C Fc glycoforms in total IgG1

D Fraction of Fc glycosylation in M75-specific IgG1

E Fc glycoforms in M75-specific IgG1

Supplementary Figure 2. Fc glycosylation profiling of M75-specific and total serum IgG1 pre- and 6 months post-challenge in participants who developed pharyngitis. (A) Schematic overview of M75-specific IgG purification, digestion and Fc glycan profiling by LC-MS/MS. (B) Line graphs depict the relative abundance of total glycans in total IgG1 antibodies. (C) Heatmaps illustrate the fractional distribution of individual Fc glycan types in total IgG1 antibodies. (D) Line graphs depict the relative abundance of total glycans in M75-specific antibodies in each participant. (E) Heatmaps illustrate the fractional distribution of individual Fc glycan types in M75-specific IgG1 antibodies. Data represent paired samples collected at the time of challenge (green) and 6 months post-challenge (blue) from participants who did not develop pharyngitis (n = 5). Paired t-test between pre- and post-challenge was used for statistical analysis. Statistical analysis revealed no significant differences between the groups.