**Supplementary Information**

# Immune signature of acute pharyngitis in a *Streptococcus pyogenes* human challenge trial

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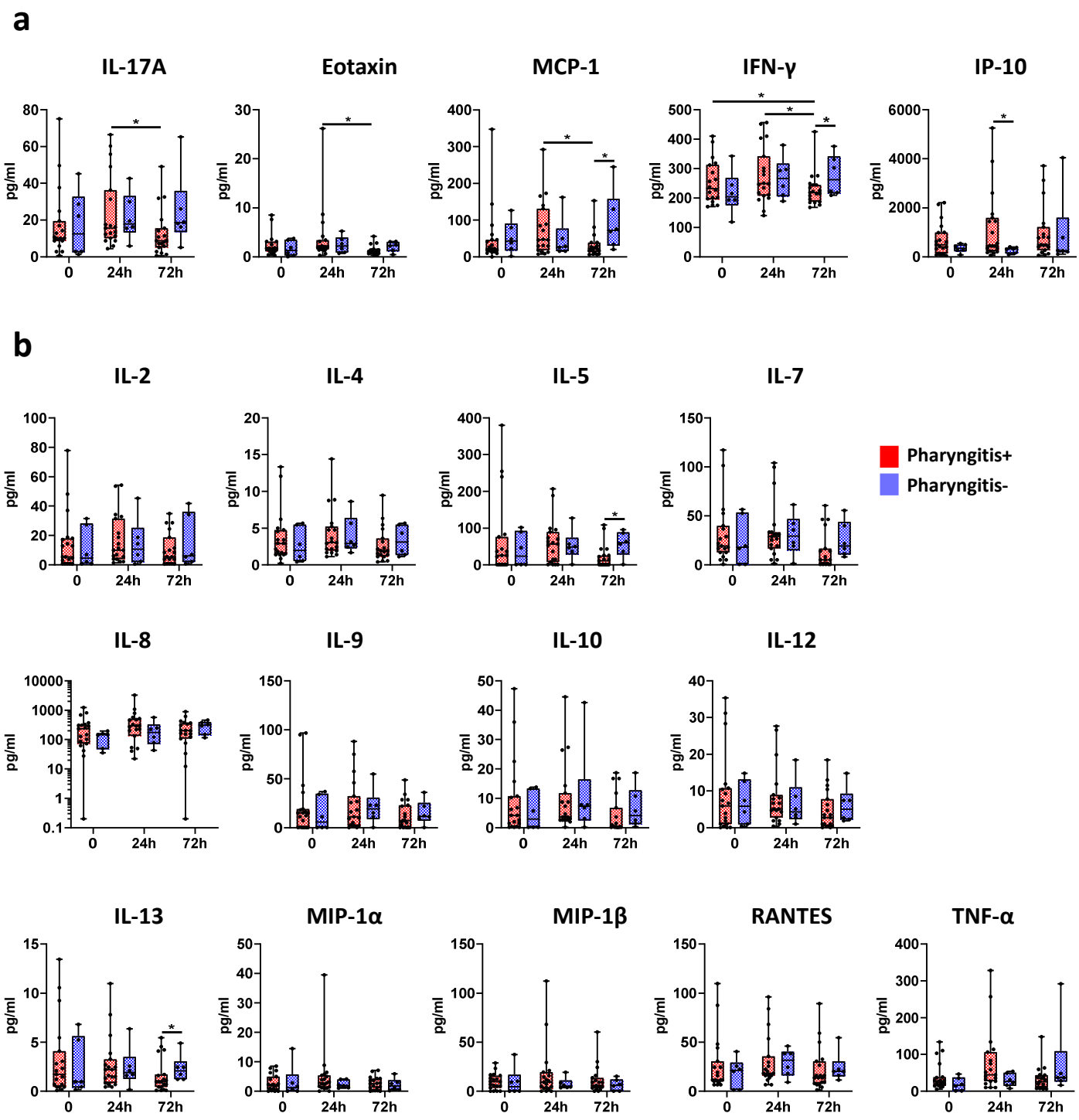
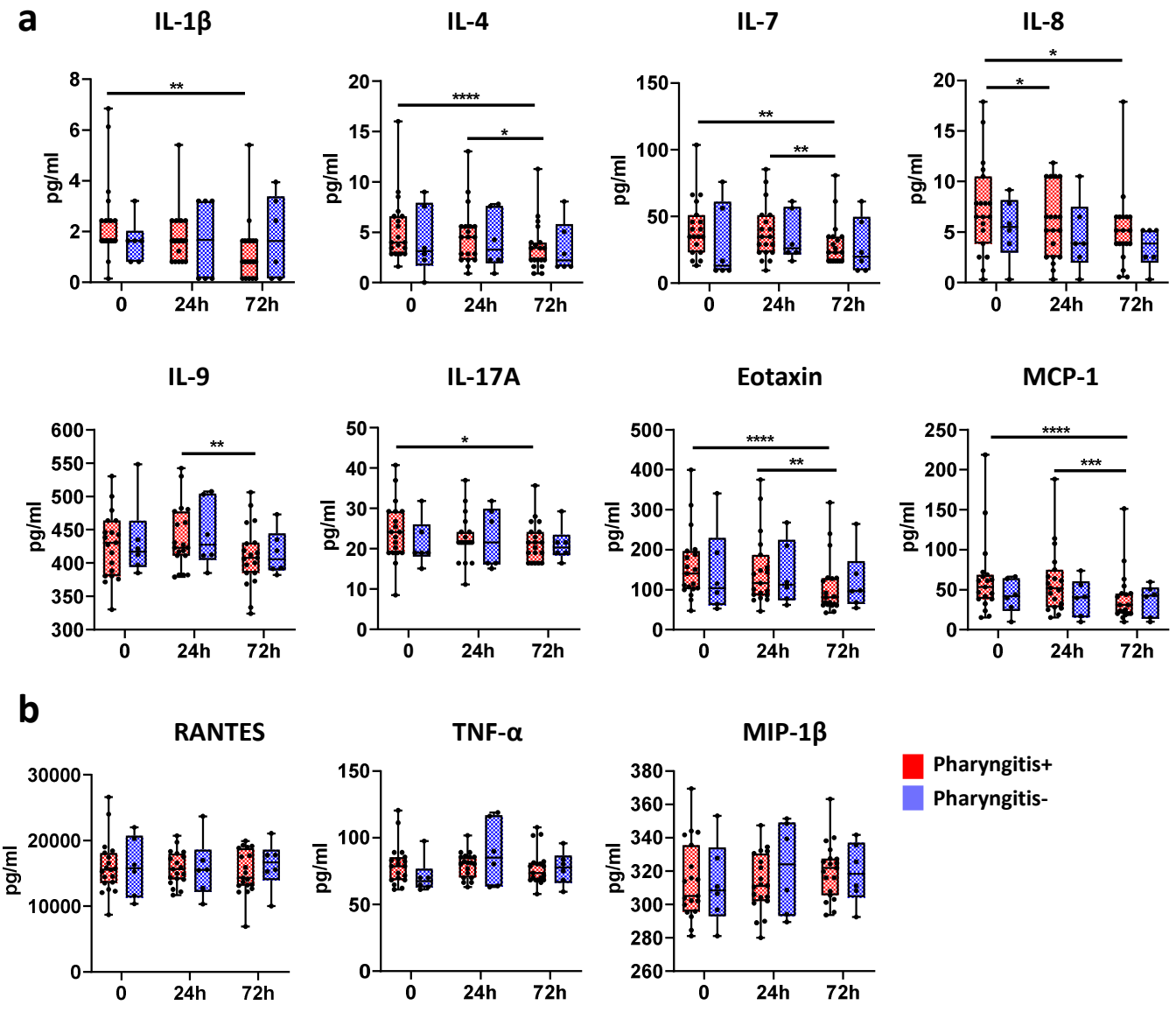
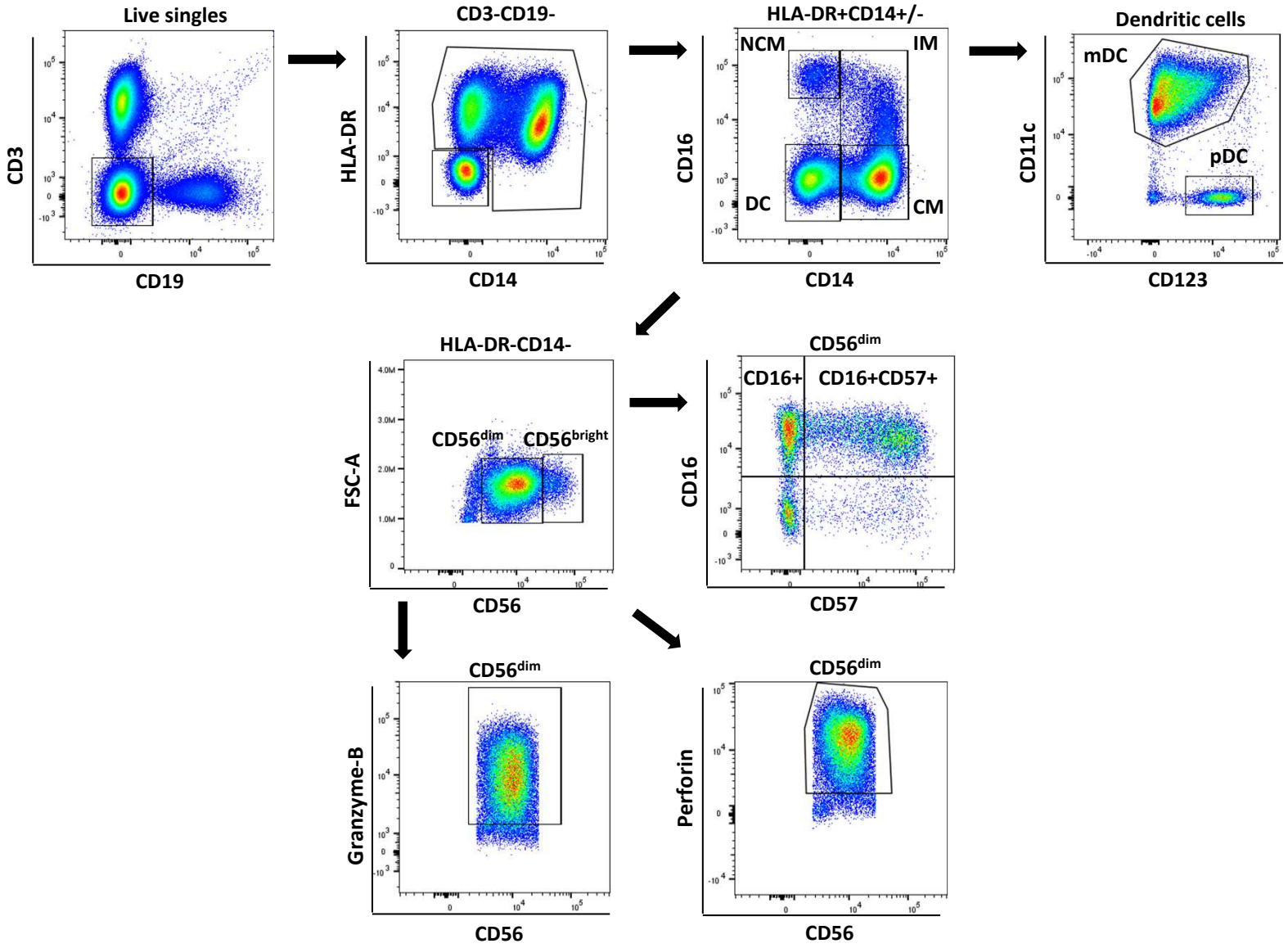


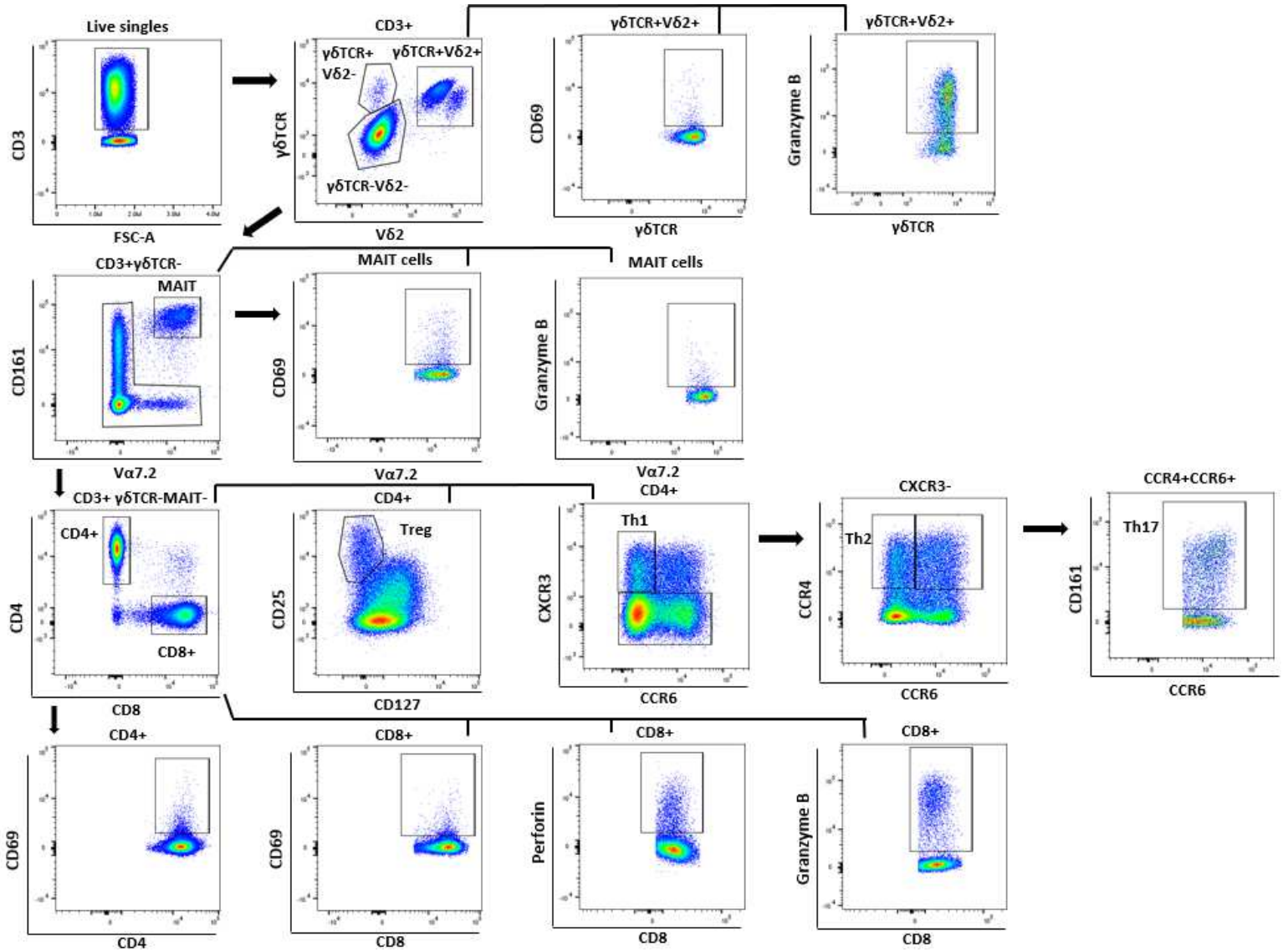

**Supplementary Figure 1: Additional saliva cytokines/chemokines analysed. a,** Cytokines/chemokines that decreased during infection at timepoint 0h (pre-infection) 24h post-infection (24h) and 72h post-infection (72h) between pharyngitis positive (n = 19, red) and negative (n = 6, blue) participants. **b,** Cytokines/chemokines that did not change during infection. Data presented as boxplots shown median ± IQR with min-max whiskers. A Friedman's test was used to compare responses over-time in P+ and P- groups, whilst a Mann-Whitney U-test was performed to compare differences between P+ and P- groups. All tests performed were two-tailed and a p-value <0.05 was considered significant; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



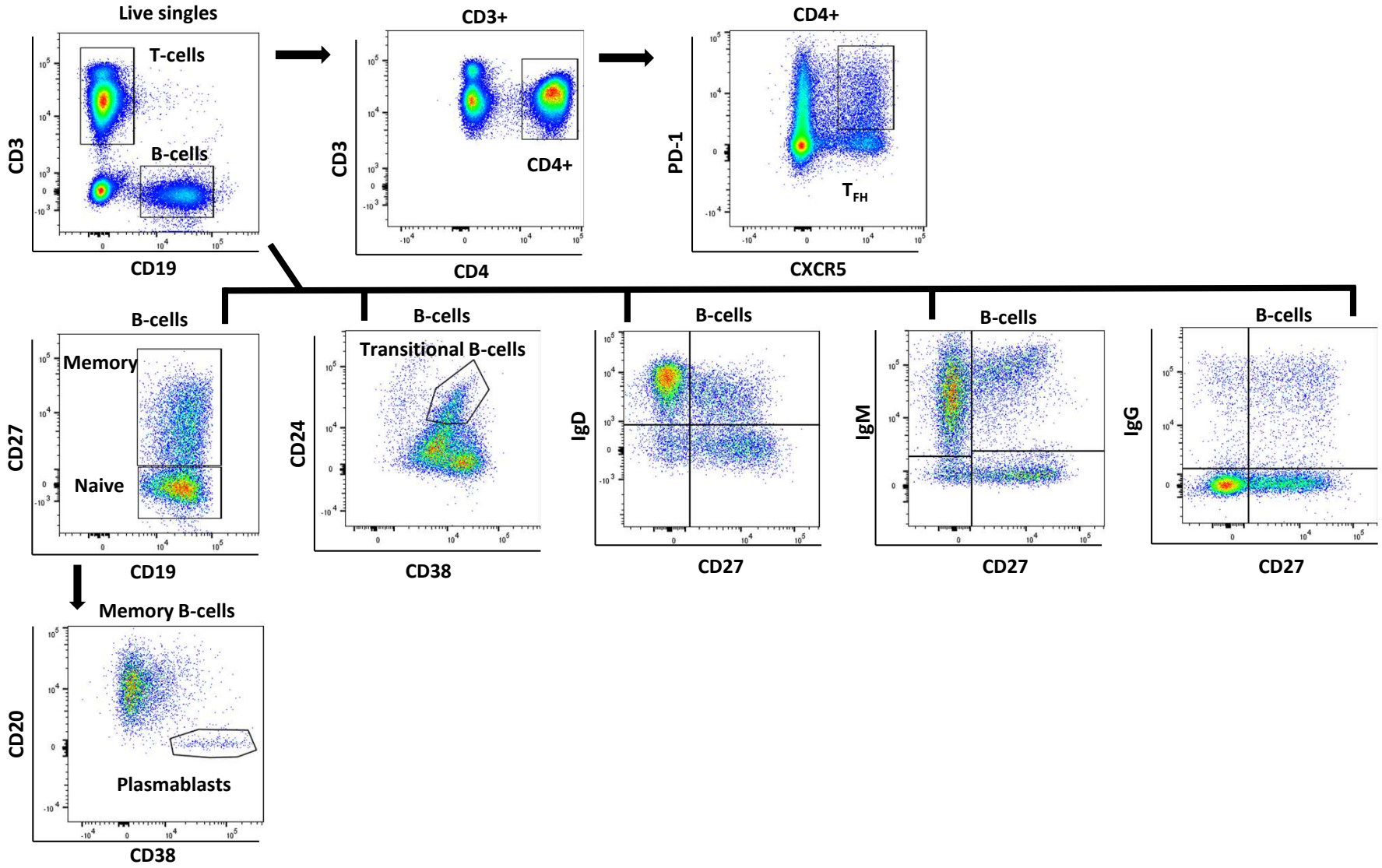

**Supplementary Figure 2: Additional serum cytokines/chemokines analysed. a,** Cytokines/chemokines that decreased during infection timepoint 0h (pre-infection) 24h post-infection (24h) and 72h post-infection (72h) between pharyngitis positive (n = 19, red) and negative (n = 6, blue) participants. **b,** Cytokines/chemokines that did not change during infection. Data presented as boxplots shown median ± IQR with min-max whiskers. A Friedman's test was used to compare responses over-time in P+ and P- groups, whilst a Mann-Whitney U-test was performed to compare differences between P+ and P- groups. All tests performed were two-tailed and a p-value <0.05 was considered significant; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



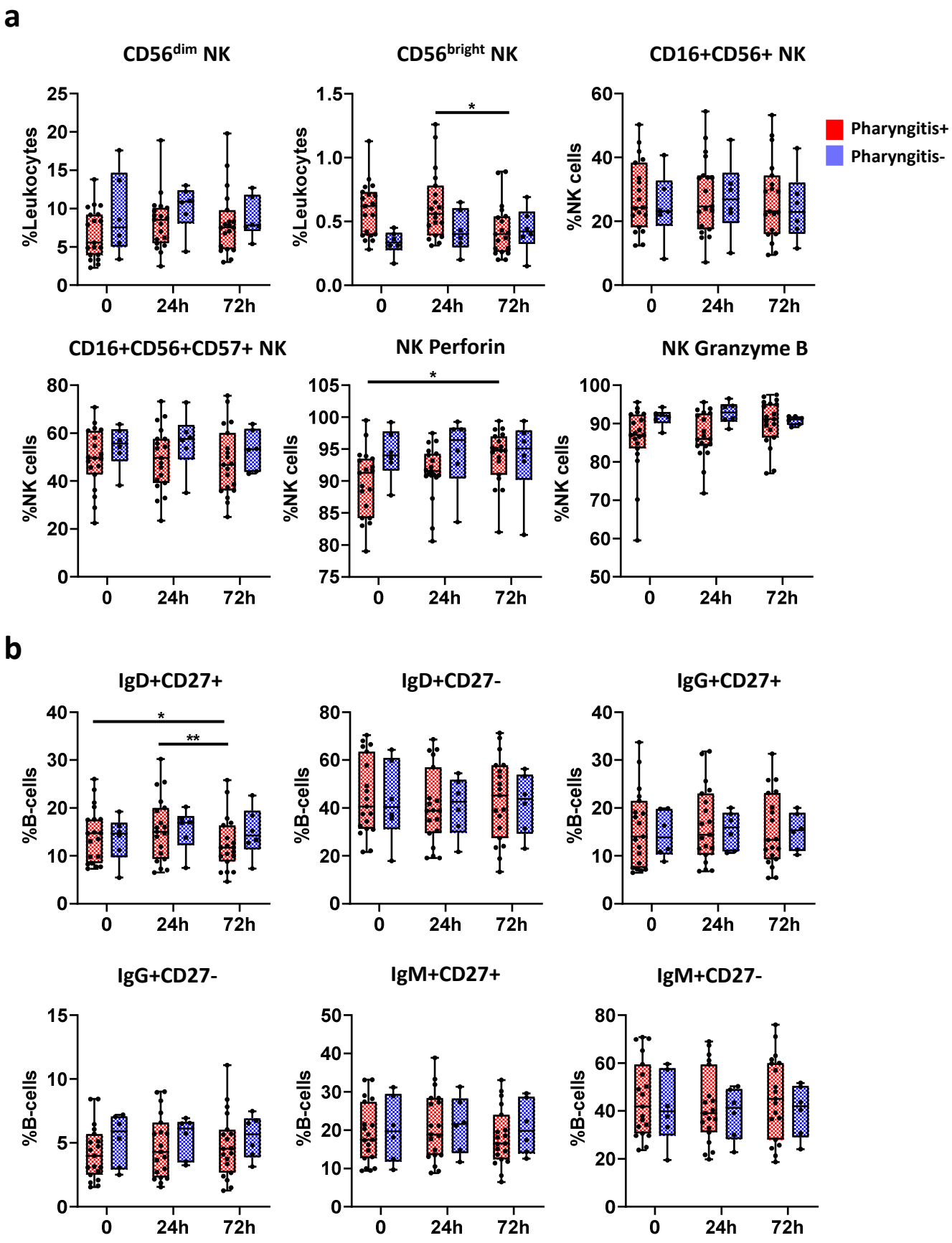

**Supplementary Figure 3: Gating strategy to identify innate cell subsets.** From live single cells, T and B-cells were removed by gating the CD3-CD19- population. HLA-DR and CD14 were used to discriminate between myeloid cells (HLA-DR+CD14+/-) and NK cells (HLA-DR-CD14-). From the myeloid cell populations, CD16 and CD16 were used to discriminate non-classical monocytes (NCM; CD14-CD16+), intermediate monocytes (IM; CD14+CD16+), classical monocytes (CM; CD14+CD16-) and dendritic cells (DC; CD14-CD16-). Dendritic cells were further categorised into myeloid dendritic cells (mDCs, CD11c+CD123-) and plasmacytoid dendritic cells (pDCs; CD11c-CD123+). NK cells were divided into $CD56^{dim}$ and $CD56^{bright}$. From the $CD56^{dim}$ population, CD16, CD57, perforin and granzyme B positive populations were identified.




**Supplementary Figure 4: Gating strategy to identify T-cell subsets.** From live single cells, T-cells were identified by positive CD3 expression. γδTCR+ and γδTCR+Vδ2+ T-cells were identified and CD69+ and granzyme B+ expression was characterised on the γδTCR+Vδ2+ population. From the γδTCR- population, MAIT cells were identified by CD161+Vα7.2+ expression and CD69+ and granzyme B+ expression was characterised on the MAIT cell population. From the MAIT cell negative population, CD4+ and CD8+ T-cells were identified. CD4+ T-cells were characterised into Treg (CD25+CD127$^{lo}$), Th1 (CXCR3+), Th2 (CXCR3-CCR4+CCR6-) and Th17 (CXCR3-CCR4+CCR6+CD161+). CD69+ expression was also explored on CD4+ T-cells. CD8+ T-cells were further characterised into CD69, perforin and granzyme B expression CD8+ T-cells.




**Supplementary Figure 5: Gating strategy to identify B-cell/$T_{FH}$ subsets.** From live single cells, T-cells were identified as CD3+CD19- and B-cells were identified as the CD3-CD19+ population. From the CD3+ population, $T_{FH}$ was characterised as CD4+CXCR5+PD-1+ expressing T-cells. From the B-cell population, memory was identified as CD27+, transitional B-cells as CD24+CD38+ and plasmablasts as CD27+CD20-CD38+. IgD, IgM and IgG expression were also characterised on B-cell populations.




**Supplementary Figure 6: Additional innate and B-cell subsets analysed. a,** NK cell subsets at timepoint 0 (pre-infection) 24h post-infection (24h) and 72h post-infection (72h) between pharyngitis positive (n = 19, red) and negative (n = 6, blue) participants. **b,** B-cells expressing IgD, IgM and IgG. Data presented as boxplots shown median ± IQR with min-max whiskers. A Friedman's test was used to compare responses over-time in P+ and P- groups, whilst a Mann-Whitney U-test was performed to compare differences between P+ and P- groups. All tests performed were two-tailed and a p-value <0.05 was considered significant; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

**Supplementary Table 1: Surface staining antibody cocktails**

| Antibody Cocktail 1 | Supplier | Antibody Cocktail 2 | Supplier | Antibody Cocktail 3 | Supplier |
|---|---|---|---|---|---|
| CXCR3-APC | BD Bioscience, San Diego, CA, USA | CD3-BUV737 | BD Bioscience, San Diego, CA, USA | CD24-BV711 | BioLegend, San Diego, USA |
| CCR6-BUV496 | BD Bioscience, San Diego, CA, USA | CD16-BV605 | BD Bioscience, San Diego, CA, USA | HLA-DR-APC | BD Bioscience, San Diego, CA, USA |
| CCR4-BV605 | BioLegend, San Diego, USA | HLA-DR-BUV395 | BD Bioscience, San Diego, CA, USA | CD20-BV421 | BioLegend, San Diego, USA |
| γδTCR-FITC | BD Bioscience, San Diego, CA, USA | CD11c-AF647 | BioLegend, San Diego, USA | CD27-BUV737 | BD Bioscience, San Diego, CA, USA |
| CD127-APC-R700 | BioLegend, San Diego, USA | CD56-BV510 | BioLegend, San Diego, USA | IgD-BUV395 | BD Bioscience, San Diego, CA, USA |
| CD25-PE-CF594 | BD Bioscience, San Diego, CA, USA | CD14-BUV805 | BD Bioscience, San Diego, CA, USA | CD19-BV785 | BioLegend, San Diego, USA |
| Vδ2-BV480 | BD Bioscience, San Diego, CA, USA | CD123-PEcy7 | BD Bioscience, San Diego, CA, USA | CD38-BUV496 | BD Bioscience, San Diego, CA, USA |
| CD161-PEvio770 | Miltenyi Biotec, New South Wales, Australia | CD19-BV785 | BioLegend, San Diego, USA | CD4-BV510 | BioLegend, San Diego, USA |
| CD3-BUV395 | BD Bioscience, San Diego, CA, USA | CD57-PE-CF594 | BD Bioscience, San Diego, CA, USA | CD3-PerCP/Cy5.5 | BD Bioscience, San Diego, CA, USA |
| CD4-BV421 | BD Bioscience, San Diego, CA, USA | Zombie NIR | BioLegend, San Diego, USA | CXCR5-APCR700 | BD Bioscience, San Diego, CA, USA |
| CD8-BUV805 | BD Bioscience, San Diego, CA, USA | | | PD-1-PEcy7 | BD Bioscience, San Diego, CA, USA |
| CD69-BV650 | BioLegend, San Diego, USA | | | IgG-BV605 | BD Bioscience, San Diego, CA, USA |
| Vα7.2-BV711 | BioLegend, San Diego, USA | | | IgM-FITC | BioLegend, San Diego, USA |
| Zombie NIR | BioLegend, San Diego, USA | | | Zombie NIR | BioLegend, San Diego, USA |

**Supplementary Table 2: Intracellular antibody cocktails**

| Antibody Cocktail 1 | Supplier |
|---|---|
| Granzyme B-Pacific Blue | BioLegend, San Diego, USA |
| Perforin-APCcy7 | BioLegend, San Diego, USA |