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Supplemental information

Protective heterologous T cell immunity in COVID-19 induced by the trivalent MMR and Tdap vaccine antigens

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SUPPLEMENTAL LIST

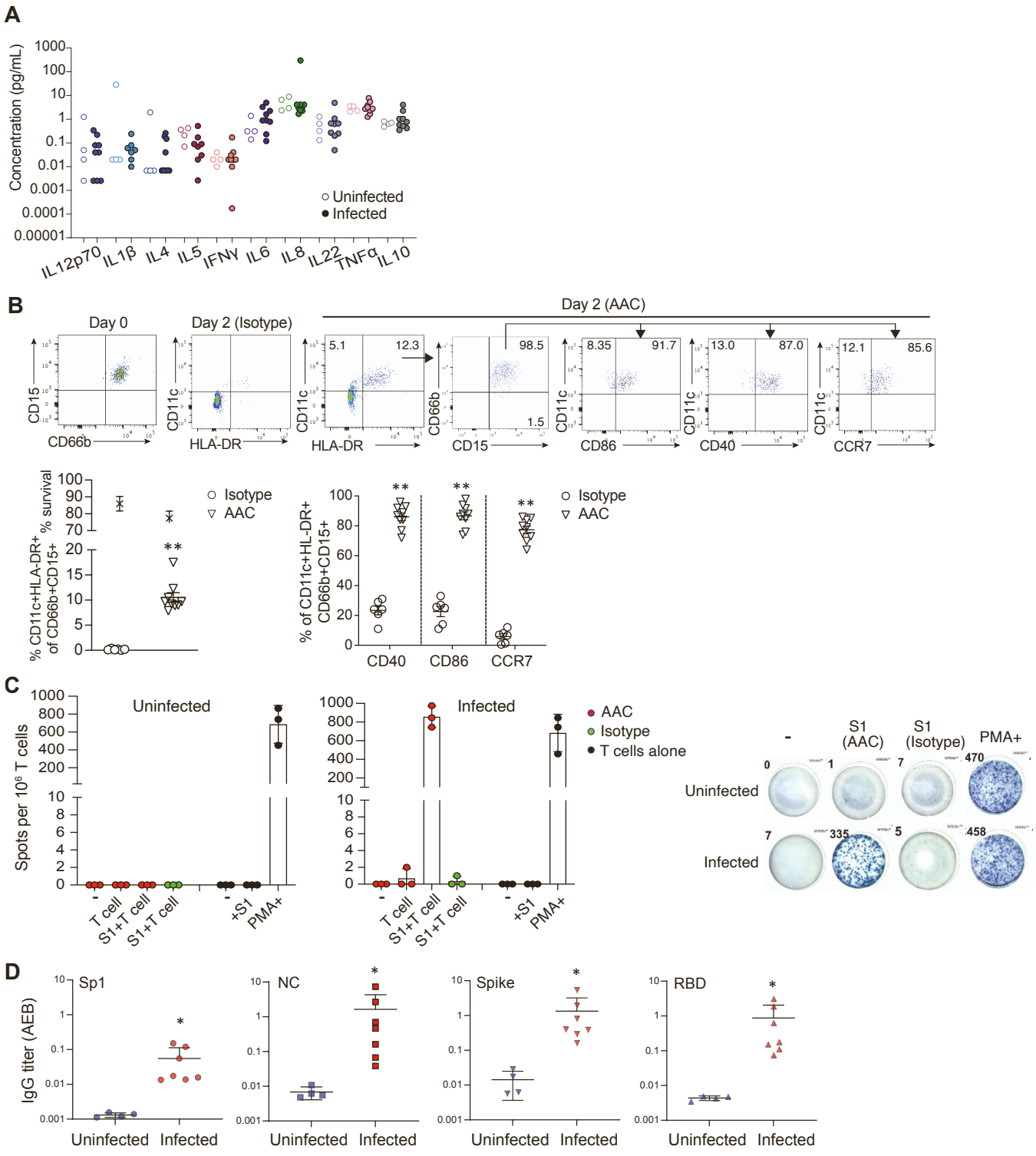
Supplemental Figure 1: Sera cytokine levels, antibody profiles and phenotype of generated nAPCs. Related to Figure 1.

Supplemental Figure 2: Autologous T cell responses to antigen loaded, monocyte-derived dendritic cells (moDC). Related to Figure 1.

Supplemental Figure 3: Gating strategies for T cell subsets, phenotype of T cells co-cultured with SARS-CoV-2 antigen loaded, monocyte-derived dendritic cells (moDC) and unsupervised clustering of T cells co-cultured with antigen loaded nAPC. Related to Figure 2.

Supplemental Figure 4: Autologous T cell responses to antigen loaded, monocyte-derived dendritic cells (moDC) before and after SARS-CoV-2 vaccination. Related to Figure 3.

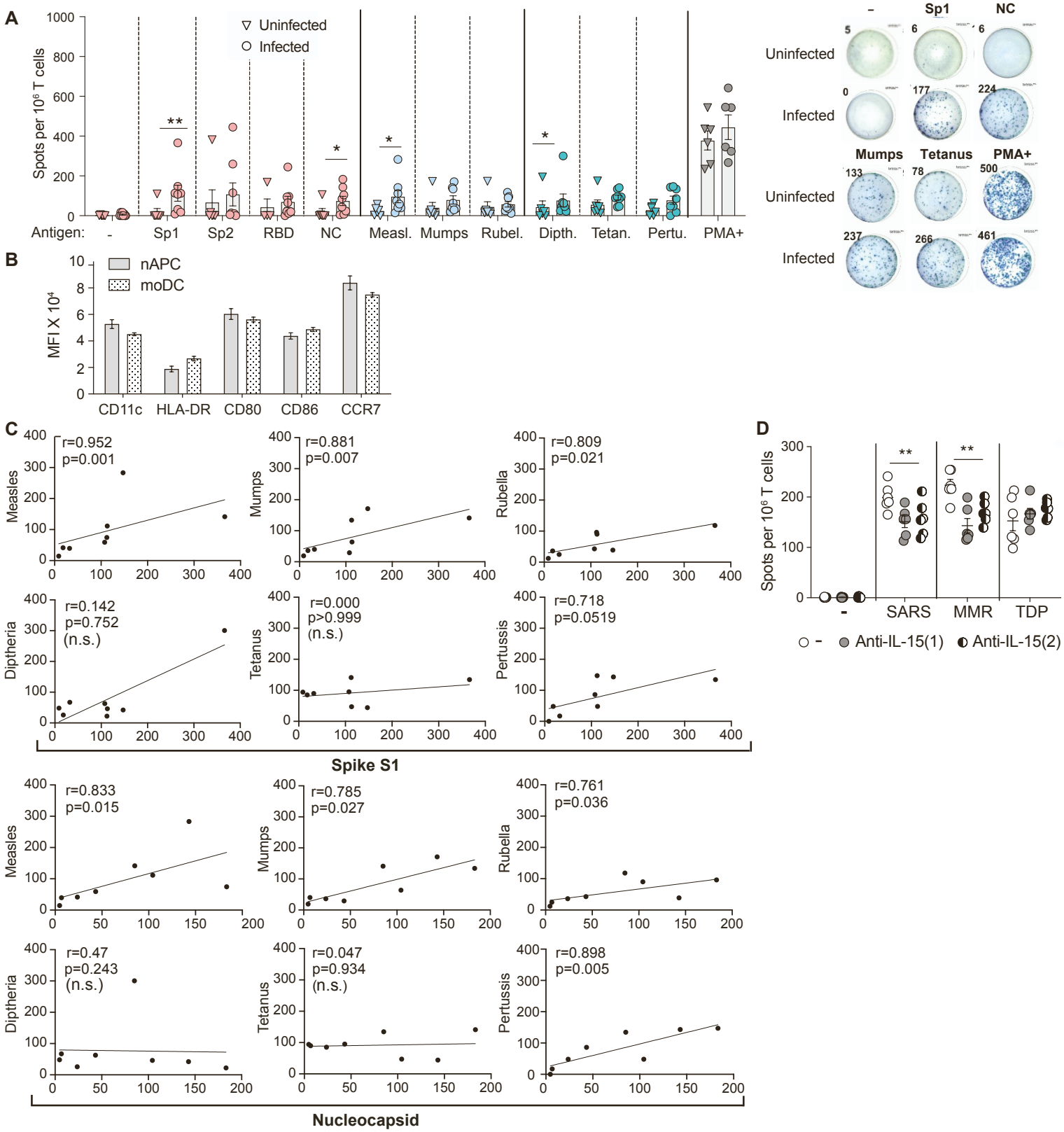
Supplemental Figure 1



Supplemental Figure 1: Sera cytokine and antibody profiles, phenotype of generated nAPCs. Related to Figure 1.

A) Cytokine profile in sera of uninfected and infected individuals. **B)** Representative flow cytometric plots assessing purity of isolated human blood neutrophils at day 0, and gating strategy for CD11c+MHCII+ and CD40/CD86/CCR7 to assess nAPC generation from human neutrophils treated with antibody Isotype or antibody conjugate (AAC) on day 2 after culture. Graphs for frequency of cells with indicated markers are shown below. Percent survival was evaluated by exclusion of Fixable Viability Dye positive cells. **p<0.005 using multiple t-tests between paired samples. **C)** Representative IFN-γ ELISpot assay (samples in triplicate) for an uninfected and infected individual highlighting controls used for the assay. Neutrophils incubated with antibody isotype or AAC to generate nAPCs were used in all assays and co-cultured with T cells at a ratio of 1:5 (nAPC:T cells). Representative images of the ELISpot wells are shown. **D)** IgG, IgA and IgM titers in plasma of uninfected and infected individuals to indicated SARS-CoV-2 antigens were evaluated as in Figure 1C. A significant difference between uninfected and infected individuals was observed for IgG against each of the four viral antigens (Mann Whitney test, p<0.05). For graphs in B)-D), data are average±SEM, and in A)-D) individual values are plotted.

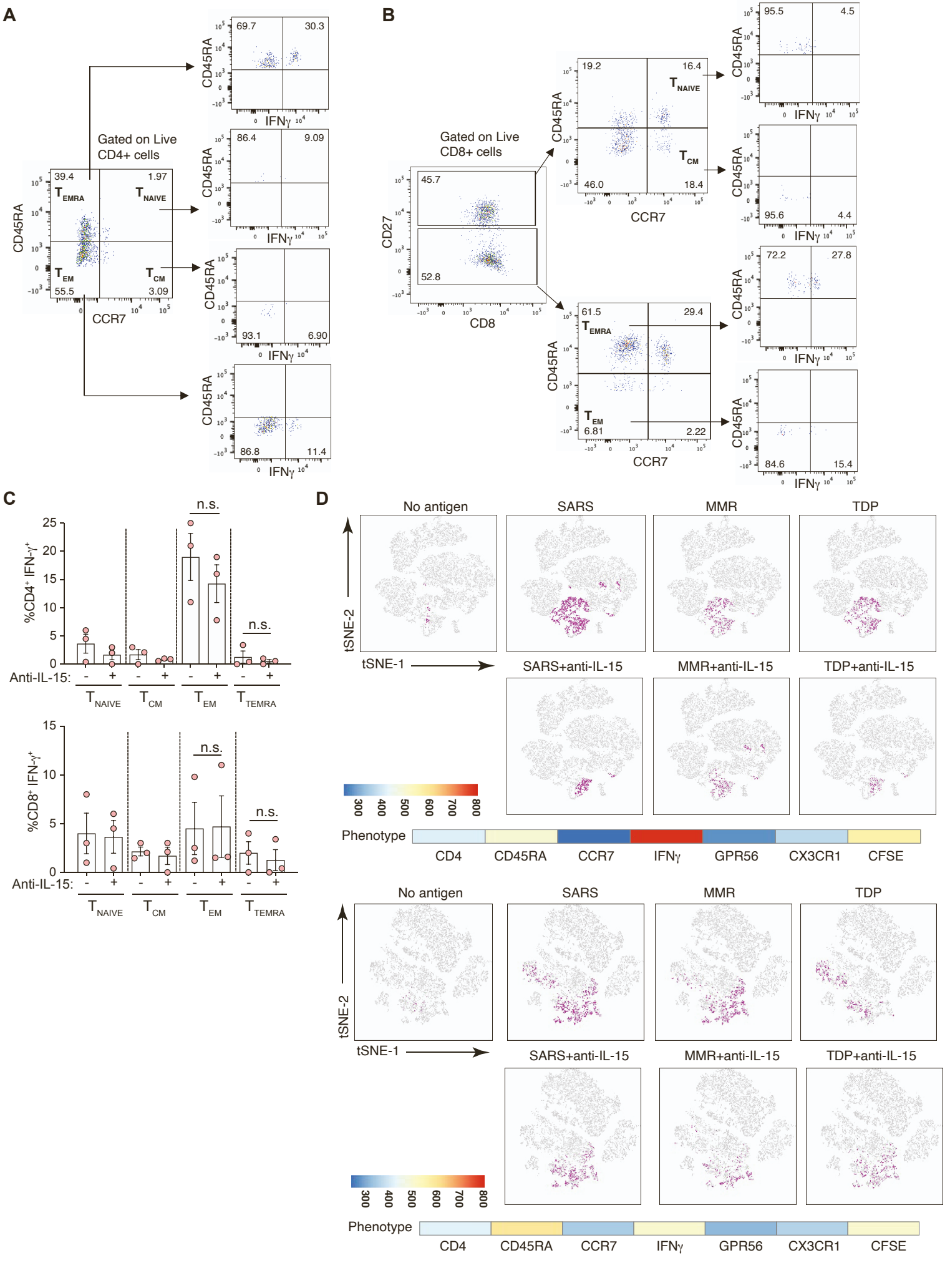
Supplemental Figure 2



Supplemental Figure 2: Autologous T cell responses to antigen loaded, monocyte-derived dendritic cells (moDC). Related to Figure 1.

A) Monocyte-derived DCs (moDC) from a subset of donors were pulsed with indicated antigens, co-cultured with autologous CD3 T cells and analyzed for IFNγ generation using an ELISpot assay as in Figure 1D. *p<0.05; **p<0.005 by two-tailed Mann-Whitney test with Bonferroni correction for multiple comparisons. Representative images of wells with IFN-γ⁺ spots are shown. **B)** Median fluorescence intensity (MFI) for CD11c, HLA-DR, T cell co-stimulatory markers (CD40, CD86) and CCR7, were evaluated on nAPCs and moDCs. No significant differences were observed using multiple t-tests between pair of samples. **C)** Correlations of T cell responses to moDCs pulsed with Spike-S1, nucleocapsid and individual MMR and Tdap vaccine antigens using Spearman's rank correlation coefficients are shown between vaccine antigens (Y axis) and Spike-S1 or Nucleocapsid (X axis). **D)** moDCs pulsed with combined SARS-CoV-2, MMR or Tdap antigens and co-cultured with CD3 T cells for 18h were analyzed for IFN-γ⁺ spots in the presence of two independent anti-IL15 blocking antibodies as in Figure 1G . Two-way analysis of variance and Dunnett's multiple comparison test was used. **p<0.005. For graphs in A)-B) and D), data are average±SEM, and in A) and D) individual values are plotted.

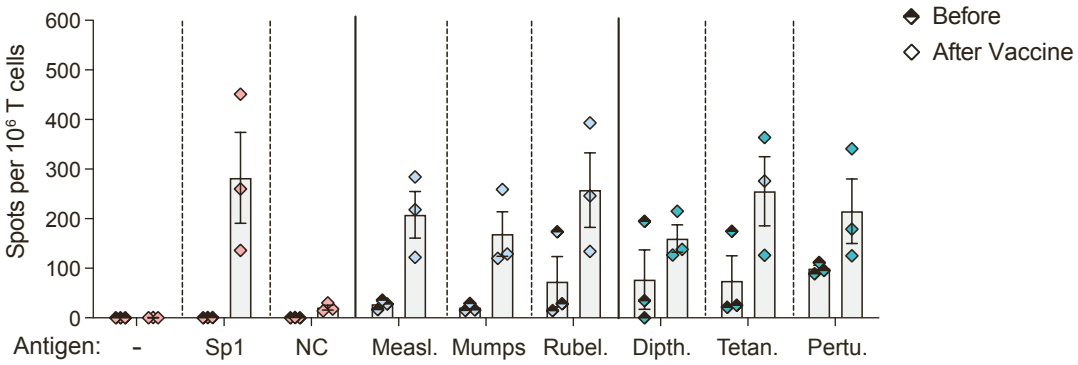
Supplemental Figure 3



Supplemental Figure 3: Gating strategies for T cell subsets, phenotype of T cells co-cultured with SARS-CoV-2 antigen loaded, monocyte-derived dendritic cells (moDC) and unsupervised clustering of T cells co-cultured with antigen loaded nAPC. Related to Figure 2.

A-B) Representative plots of gating strategy to assess for live, naïve, effector and memory $CD4^+$ (A) and $CD8^+$ (B) T cells retrieved after co-culture with SARS-CoV-2 antigen loaded nAPCs. The cells were further gated for IFN-γ. **C)** T cells co-cultured with moDCs loaded with SARS antigen and treated with anti-IL-15 as described in Supplemental Figure 2D were treated with Brefeldin A for 5h and analyzed by flow cytometry to examine IFNγ producing $CD4^+$ and $CD8^+$ T cell subsets (as defined in panel A) and effects after anti-IL-15 treatment. Data are average±SEM, and individual values are plotted. Two-way analysis of variance and Dunnett's multiple comparison test was used. n.s., not significant. **D)** FLOWSOM based visualization on a tSNE plot. tSNE plots of flow cytometry datasets for live $CD4^+$ T cells analyzed as in Figure 2D for two additional infected donors. The phenotypical evaluation of overlapping population between the antigens are shown along with a continuous scale.

Supplemental Figure 4



Supplemental Figure 4: T cell responses to antigen loaded, monocyte-derived dendritic cells (moDC) before and after SARS-CoV-2 vaccination. Related to Figure 3.

Blood was analyzed from three uninfected individuals approximately 3 months before and 2.5 months after receiving the Moderna mRNA-based SARS-CoV-2 vaccine. Monocyte-derived dendritic cells were loaded with individual SARS-CoV-2, MMR or Tdap antigens and IFN-γ secretion by co-cultured autologous T cell responses were evaluated on ELISpot plates as described in Figure 1D). Data is average±SEM and individual values are plotted.