

## T-cell responses to ancestral SARS-CoV-2 and Omicron variant among unvaccinated pregnant and postpartum women living with and without HIV in South Africa

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### SUPPLEMENTARY INFORMATION

#### Supplementary figure legends

##### **Fig. S1. Flow cytometry gating strategy and representative staining profiles.**

The flow cytometry gating strategy and representative staining profiles of two plate controls (unstimulated and PHA-stimulated) using cells from a Covid-19 vaccinated donor are shown. Firstly, a time gate was applied to ensure analysis of data with a consistent flow rate. Doublets were excluded by gating FSC-A against FSC-H, followed by gating on lymphocytes using their FSC-A and SSC-A properties. Approximately 100,000 live CD3<sup>+</sup> cells were acquired for each reaction. CD4<sup>+</sup> and CD8<sup>+</sup> T cells were gated and assessed for surface expression of PD-1 (histogram plots), as well as for intracellular expression of IFN- $\gamma$ , TNF- $\alpha$ , and IL-2 (pseudocolour plots). Percentages indicating responses are presented in each plot.

##### **Fig. S2. Overall median comparisons of SARS-CoV-2-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses in women living with and without HIV.**

Direct median comparisons between CD4<sup>+</sup> and CD8<sup>+</sup> T-cell polyfunctionality profiles in response to the (a) FLS and (b) N proteins on WT SARS-CoV-2 among women living with and without HIV. Each point represents the median T-cell response of positive responders for each group of cytokines produced. CD4<sup>+</sup> T-cell responses are indicated in green and blue. CD8<sup>+</sup> T-cell responses are indicated in red and orange. The Wilcoxon signed-rank test was used for comparing paired CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses. Significant P-values are indicated in bold ( $p < 0.05$ ). Denotations: FLS, full-length spike glycoprotein; IFN- $\gamma$ , interferon gamma; IL-2, interleukin 2; N, nucleocapsid protein; TNF- $\alpha$ , tumor necrosis factor alpha; WLWH, women living with HIV; WT, wild-type (ancestral) SARS-CoV-2.

##### **Fig. S3. Maternal T-cell responses to the FLS and N proteins of WT SARS-CoV-2.**

The proportion of T-cells producing IL-2, IFN- $\gamma$ , or TNF- $\alpha$  in response to the FLS and N proteins of WT SARS-CoV-2, stratified by maternal (pregnant:  $n=17$ ; postpartum:  $n=16$ ; not pregnant:  $n=5$ ) and HIV status (HIV-uninfected:  $n=23$ ; WLWH:  $n=15$ ). SARS-CoV-2-specific CD4<sup>+</sup> T-cell responses to (a) the FLS and (b) N proteins. SARS-CoV-2-specific CD8<sup>+</sup> T-cell responses to (c) the FLS and (d) N proteins. Each participant is represented by an individual point, and medians in each group are indicated by horizontal lines. Statistical analysis was performed using the Mann-Whitney U-test for T-cell response comparisons between unpaired (HIV-uninfected vs. WLWH) responders. Significant P-values are indicated in bold ( $p < 0.05$ ). Denotations: FLS, full-length spike glycoprotein; HIV-, HIV-uninfected women; N, nucleocapsid protein; WLWH, women living with HIV; WT, wild-type (ancestral) SARS-CoV-2.

##### **Fig. S4. Maternal polyfunctionality of CD4<sup>+</sup> T-cells to the FLS and N proteins of WT SARS-CoV-2 in HIV-uninfected women.**

(a) Comparisons of the polyfunctional profiles of CD4<sup>+</sup> T cells to the FLS and N proteins of WT SARS-CoV-2 in HIV-uninfected women, stratified by maternal status. Each participant is represented by an individual point, and medians in each group are indicated by horizontal lines. (b) Each response pattern is colour-coded, and data are summarized in pie charts. The Mann-Whitney U-test was used for comparing unpaired CD4<sup>+</sup> T-cell responses of positive responders between pregnant and postpartum

women. Only significant response differences are indicated ( $p < 0.05$ ). (c) Correlations between FLS- and N-specific CD4<sup>+</sup> T-cell responses according to IL-2, IFN- $\gamma$ , and TNF- $\alpha$  production. Spearman's rank correlation coefficient (two-sided) was used to determine the  $P$  and  $r$  (correlation coefficient) values. Linear regression lines are indicated in red, together with 95% confidence interval lines. Denotations: FLS, full-length spike glycoprotein; HIV-, HIV-uninfected women; IFN- $\gamma$ , interferon gamma; IL-2, interleukin 2; N, nucleocapsid protein; TNF- $\alpha$ , tumor necrosis factor alpha; WT, wild-type (ancestral) SARS-CoV-2.

**Fig. S5. Maternal polyfunctionality of CD4<sup>+</sup> T-cells to the FLS and N proteins of WT SARS-CoV-2 in women living with HIV.**

(a) Comparisons of the polyfunctional profiles of CD4<sup>+</sup> T cells to the FLS and N proteins of WT SARS-CoV-2 in WLWH, stratified by maternal status. Each participant is represented by an individual point, and medians in each group are indicated by horizontal lines. (b) Each response pattern is colour-coded, and data are summarized in pie charts. The Mann-Whitney U-test was used for comparing unpaired CD4<sup>+</sup> T-cell responses of positive responders between pregnant, postpartum, and non-pregnant women. Only significant response differences are indicated ( $p < 0.05$ ). (c) Correlations between FLS- and N-specific CD4<sup>+</sup> T-cell responses according to IL-2, IFN- $\gamma$ , and TNF- $\alpha$  production. Spearman's rank correlation coefficient (two-sided) was used to determine the  $P$  and  $r$  (correlation coefficient) values. Linear regression lines are indicated in red, together with 95% confidence interval lines. Denotations: FLS, full-length spike glycoprotein; IFN- $\gamma$ , interferon gamma; IL-2, interleukin 2; N, nucleocapsid protein; TNF- $\alpha$ , tumor necrosis factor alpha; WLWH, women living with HIV; WT, wild-type (ancestral) SARS-CoV-2.

**Fig. S6. Maternal polyfunctionality of CD8<sup>+</sup> T-cells to the FLS and N proteins of WT SARS-CoV-2 in HIV-uninfected women.**

(a) Comparisons of the polyfunctional profiles of CD8<sup>+</sup> T cells to the FLS and N proteins of WT SARS-CoV-2 in HIV-uninfected women, stratified by maternal status. Each participant is represented by an individual point, and medians in each group are indicated by horizontal lines. (b) Each response pattern is colour-coded, and data summarized in pie charts. The Mann-Whitney U-test was used for comparing unpaired CD8<sup>+</sup> T-cell responses of positive responders between pregnant and postpartum women. Only significant response differences are indicated ( $p < 0.05$ ). (c) Correlations between FLS- and N-specific CD8<sup>+</sup> T-cell responses according to IL-2, IFN- $\gamma$ , and TNF- $\alpha$  production. Spearman's rank correlation coefficient (two-sided) was used to determine the  $P$  and  $r$  (correlation coefficient) values. Linear regression lines are indicated in red, together with 95% confidence interval lines. Denotations: FLS, full-length spike glycoprotein; HIV-, HIV-uninfected women; IFN- $\gamma$ , interferon gamma; IL-2, interleukin 2; N, nucleocapsid protein; TNF- $\alpha$ , tumor necrosis factor alpha; WT, wild-type (ancestral) SARS-CoV-2.

**Fig. S7. Maternal polyfunctionality of CD8<sup>+</sup> T-cells to the FLS and N proteins of WT SARS-CoV-2 in women living with HIV.**

(a) Comparisons of the polyfunctional profiles of CD8<sup>+</sup> T cells to the FLS and N proteins of WT SARS-CoV-2 in WLWH, stratified by maternal status. Each participant is represented by an individual point, and medians in each group are indicated by horizontal lines. (b) Each response pattern is colour-coded, and data summarized in pie charts. The Mann-Whitney U-test was used for comparing unpaired CD8<sup>+</sup> T-cell responses of positive responders between pregnant, postpartum, and non-pregnant women. Only significant response differences are indicated ( $p < 0.05$ ). (c) Correlations between FLS- and N-specific CD4<sup>+</sup> T-cell responses according to IL-2, IFN- $\gamma$ , and TNF- $\alpha$  production. Spearman's rank correlation coefficient (two-sided) was used to determine the  $P$  and  $r$  (correlation coefficient) values. Linear regression lines are indicated in red, together with 95% confidence interval lines. Denotations: FLS, full-length spike glycoprotein; IFN- $\gamma$ , interferon gamma; IL-2, interleukin 2; N, nucleocapsid protein; TNF- $\alpha$ , tumor necrosis factor alpha; WLWH, women living with HIV; WT, wild-type (ancestral) SARS-CoV-2.

**Fig. S8, Cytokine median fluorescence intensities of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells to the FLS and N proteins of WT SARS-CoV-2 in women living with and without HIV.**

Comparisons of individual cytokine median fluorescence intensities (MFIs) of (a) CD4<sup>+</sup> and (b) CD8<sup>+</sup> T cells in response to the FLS- and N-proteins of WT SARS-CoV-2, stratified by HIV status. Each participant is represented by an individual point, and medians in each group are indicated by horizontal lines. The Mann-Whitney U-test was used for comparing unpaired MFIs between women living with and without HIV. The Wilcoxon signed-rank test and Friedman test were used for comparing MFIs between two and three paired groups, respectively. Denotations: FLS, full-length spike glycoprotein; IFN- $\gamma$ , interferon gamma; IL-2, interleukin 2; MFI, median fluorescence intensity; N, nucleocapsid protein; TNF- $\alpha$ , tumor necrosis factor alpha; WLWH, women living with HIV; WT, wild-type (ancestral) SARS-CoV-2.

**Fig. S9, Cytokine median fluorescence intensities of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells to WT SARS-CoV-2 and the Omicron variant in women living with and without HIV.**

Comparisons of individual cytokine median fluorescence intensities (MFIs) of (a) CD4<sup>+</sup> and (b) CD8<sup>+</sup> T cells in response to spike proteins of WT SARS-CoV-2 and the Omicron variant, stratified by HIV status. Each participant is represented by an individual point, and medians in each group are indicated by horizontal lines. The Mann-Whitney U-test was used for comparing unpaired MFIs between women living with and without HIV. The Wilcoxon signed-rank test and Friedman test were used for comparing MFIs between two and three paired groups, respectively. Denotations: FLS, full-length spike glycoprotein; IFN- $\gamma$ , interferon gamma; IL-2, interleukin 2; MFI, median fluorescence intensity; N, nucleocapsid protein; TNF- $\alpha$ , tumor necrosis factor alpha; WLWH, women living with HIV; WT, wild-type (ancestral) SARS-CoV-2.

Supplementary figures

Fig. S1

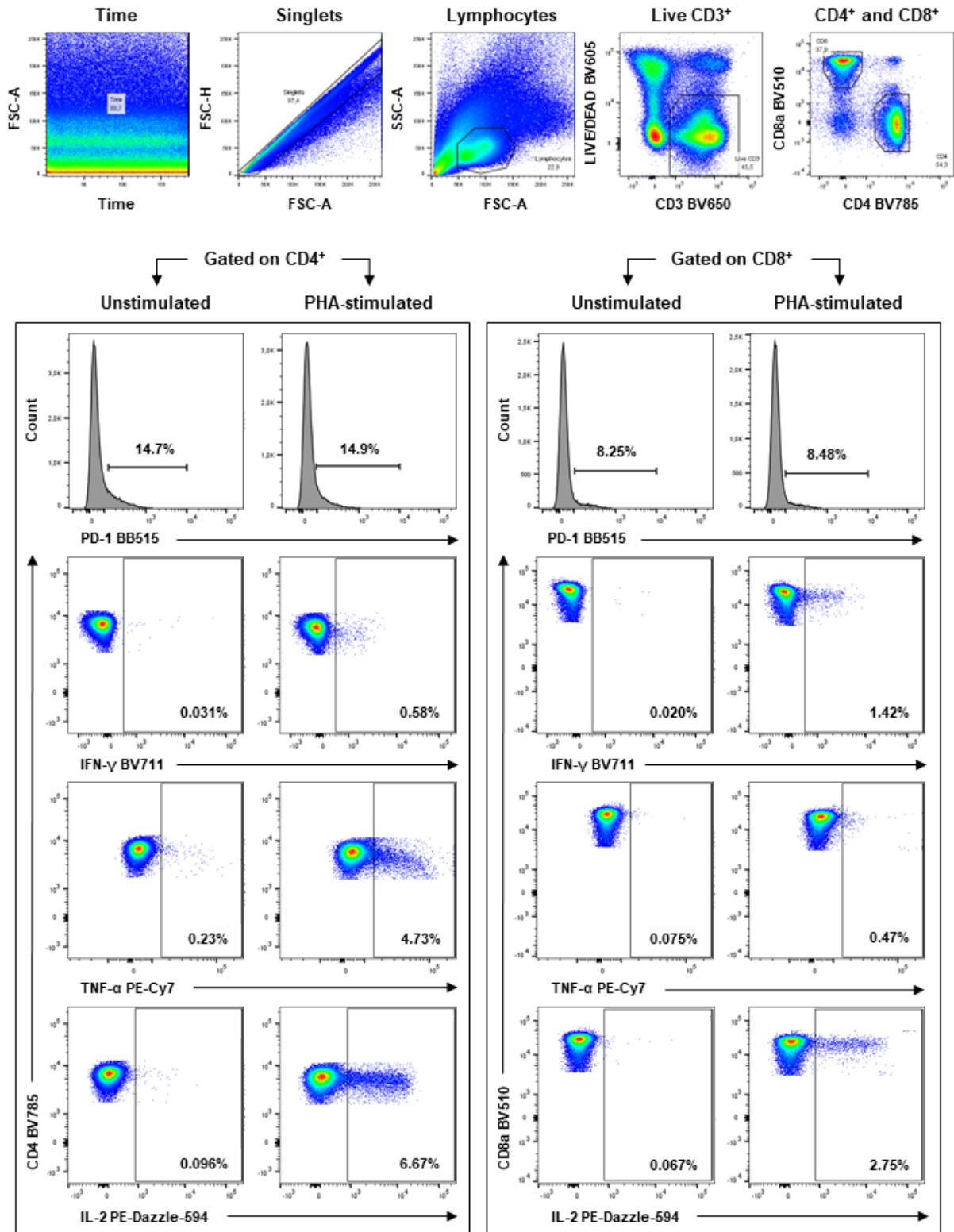


Fig. S2

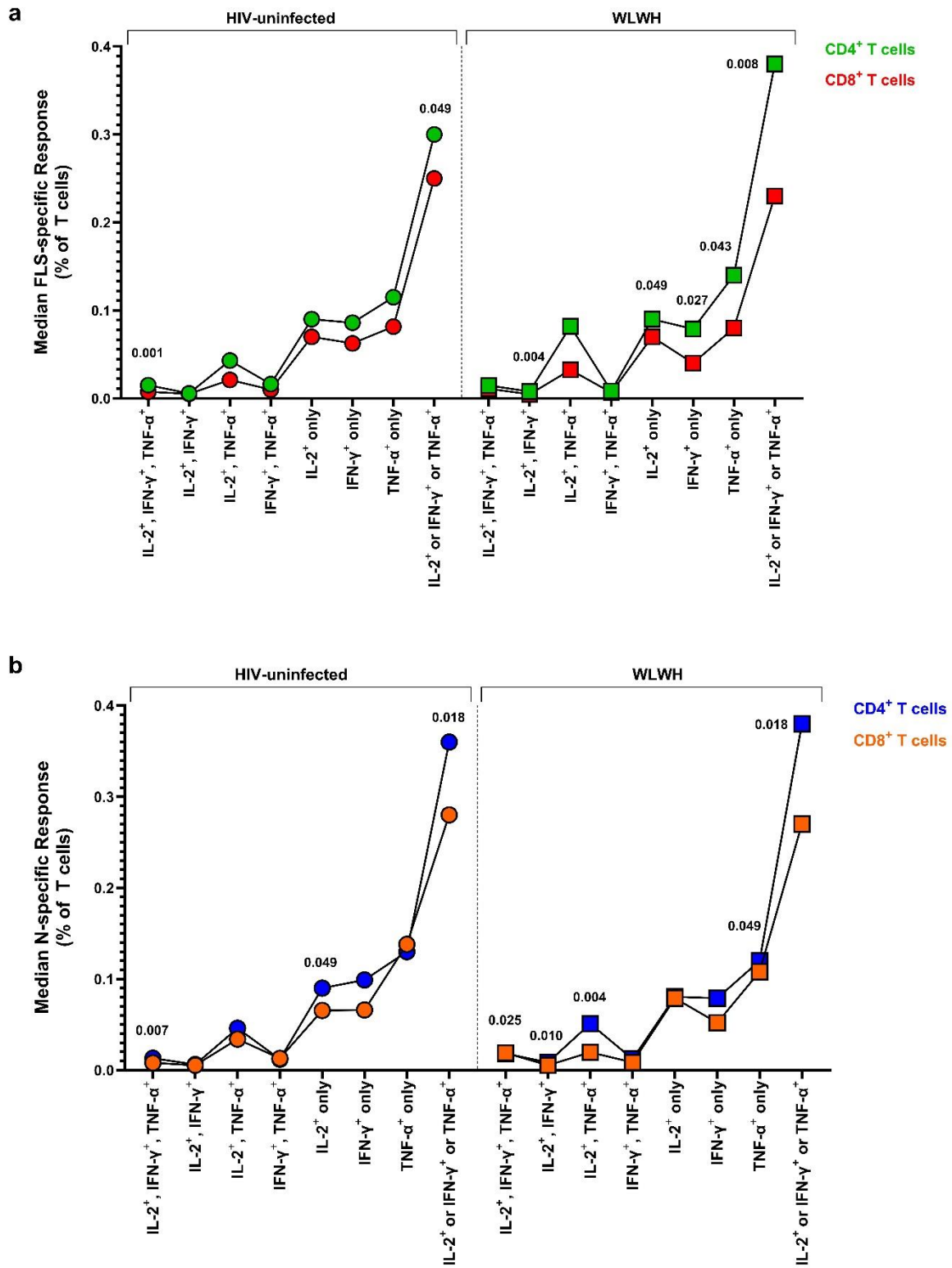


Fig. S3

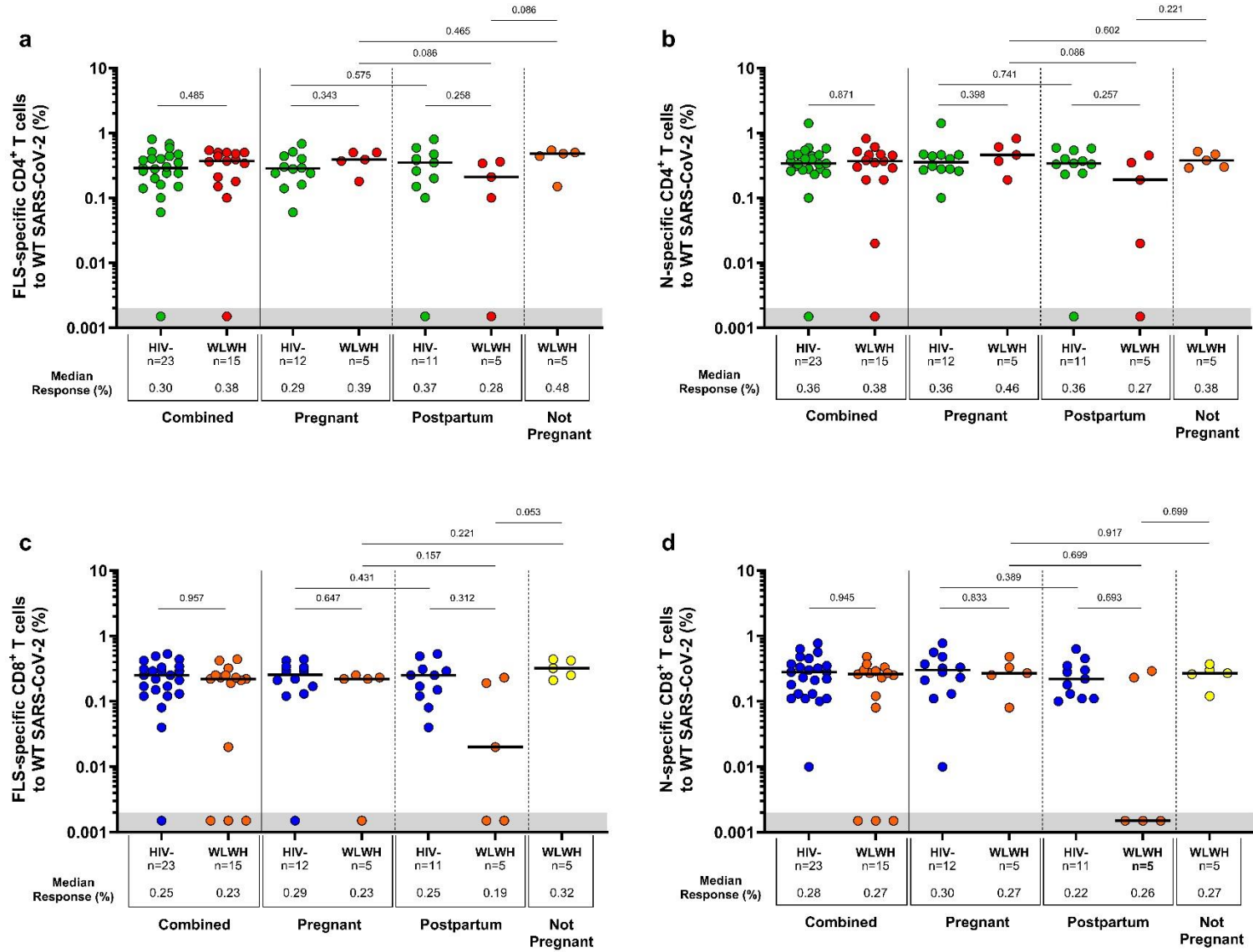


Fig. S4

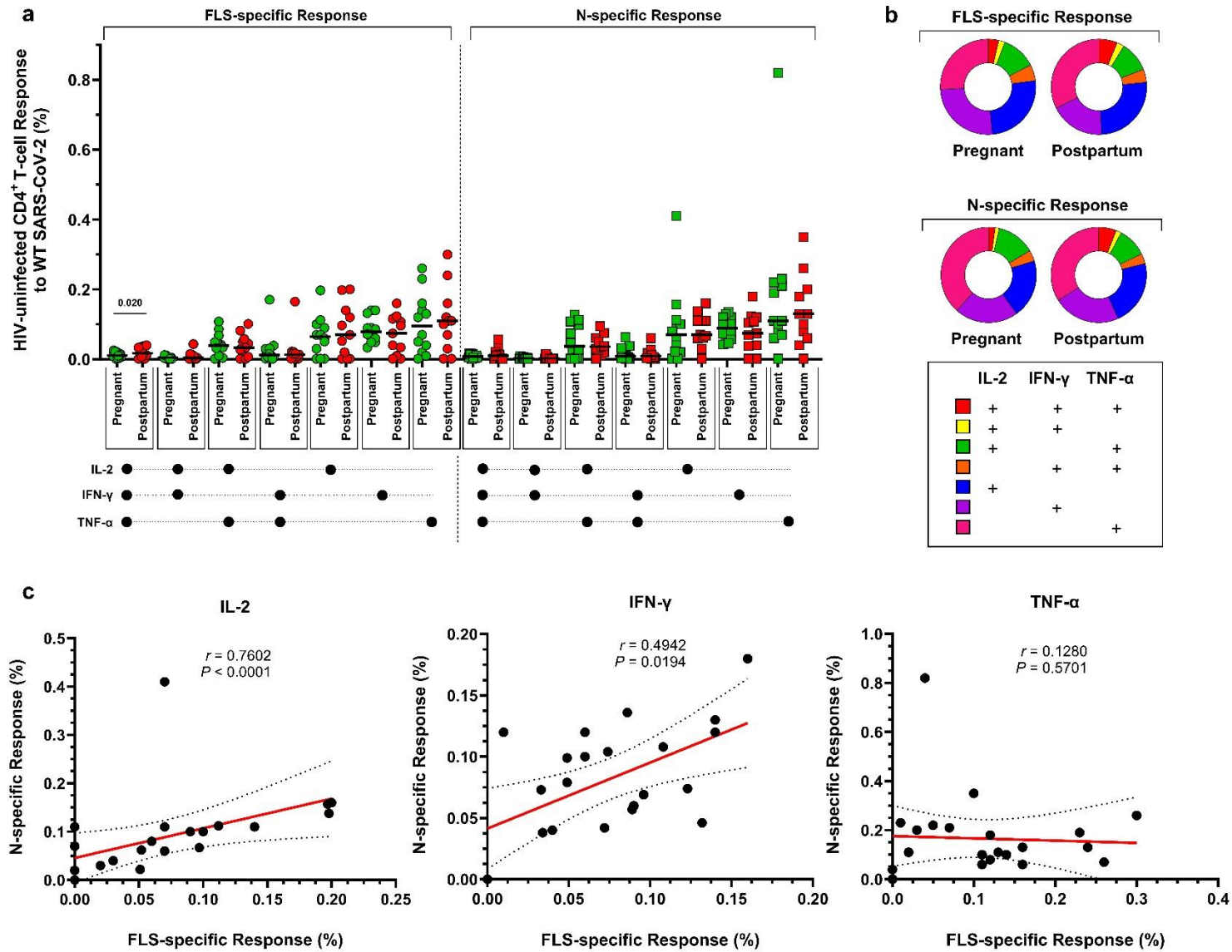


Fig. S5

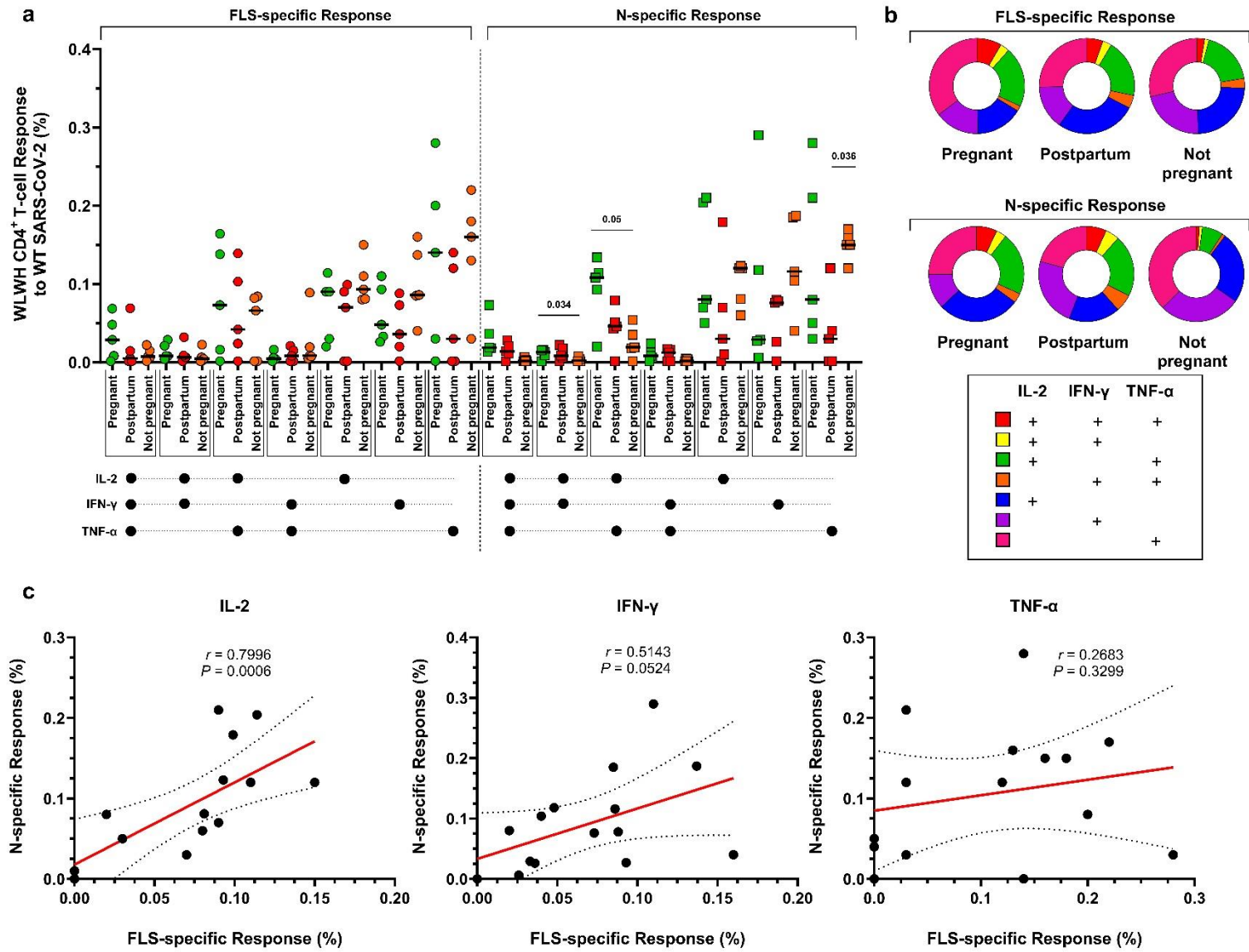


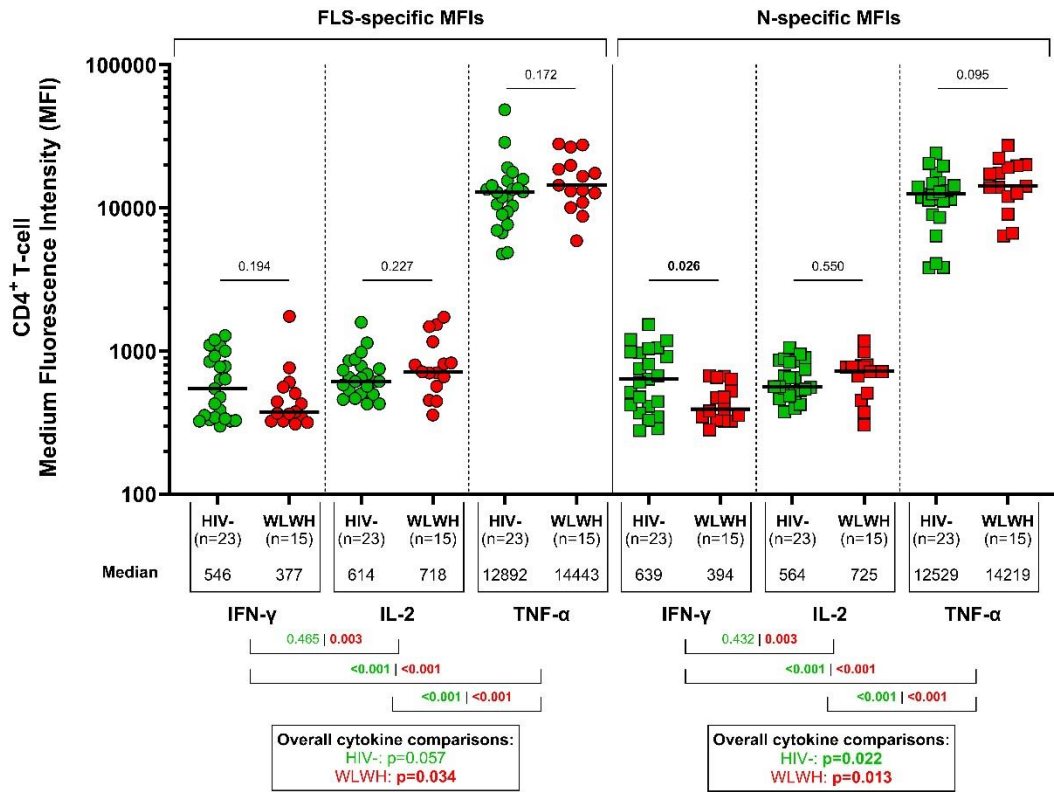






Fig. S8

a



b

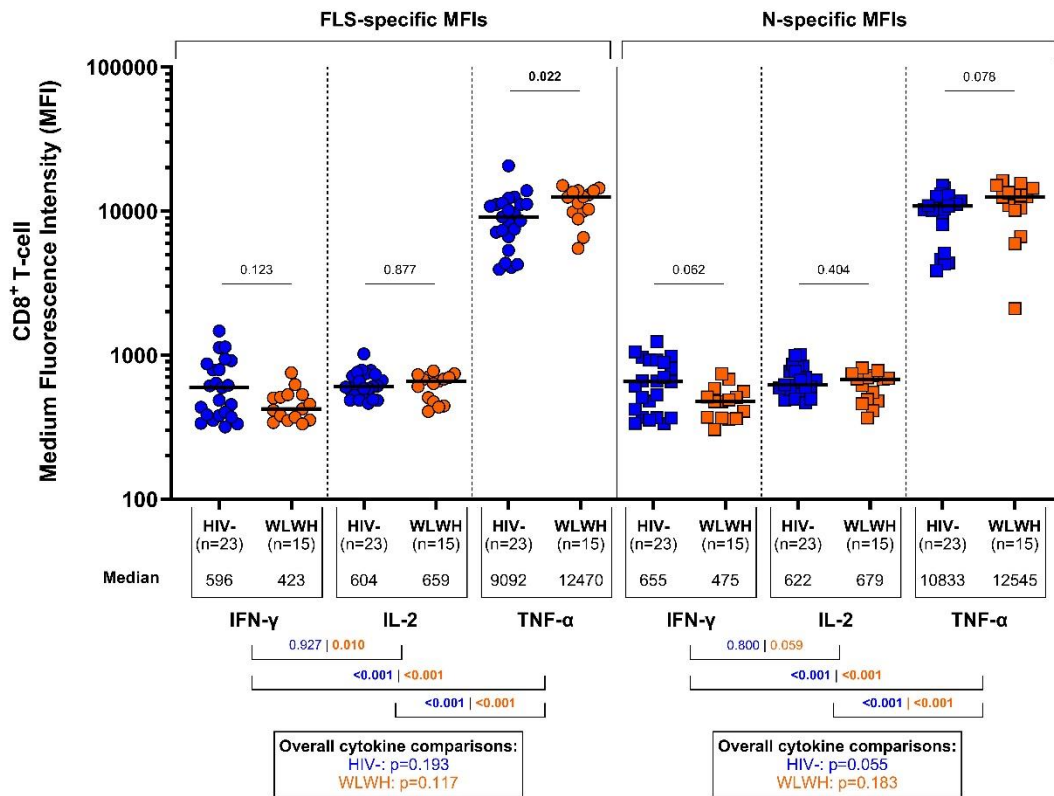
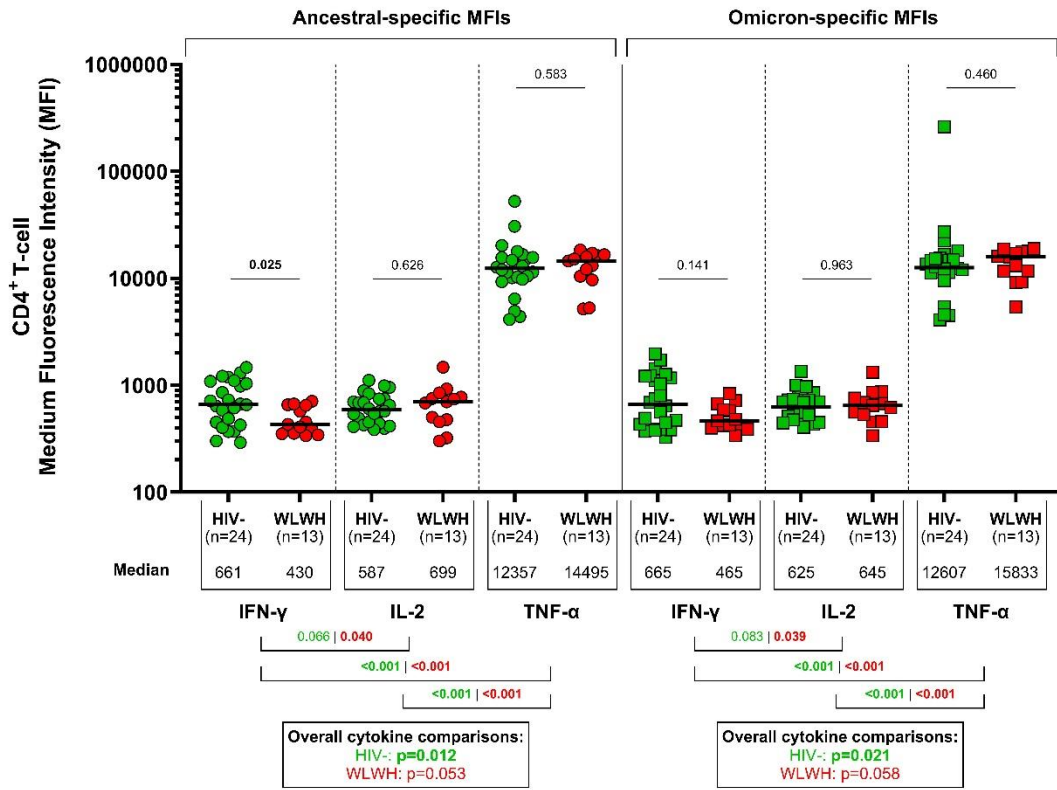


Fig. S9

a



b

