Figures and figure supplements

Unsuppressed HIV infection impairs T cell responses to SARS-CoV-2 infection and abrogates T cell cross-recognition

Thandeka Nkosi *et al*

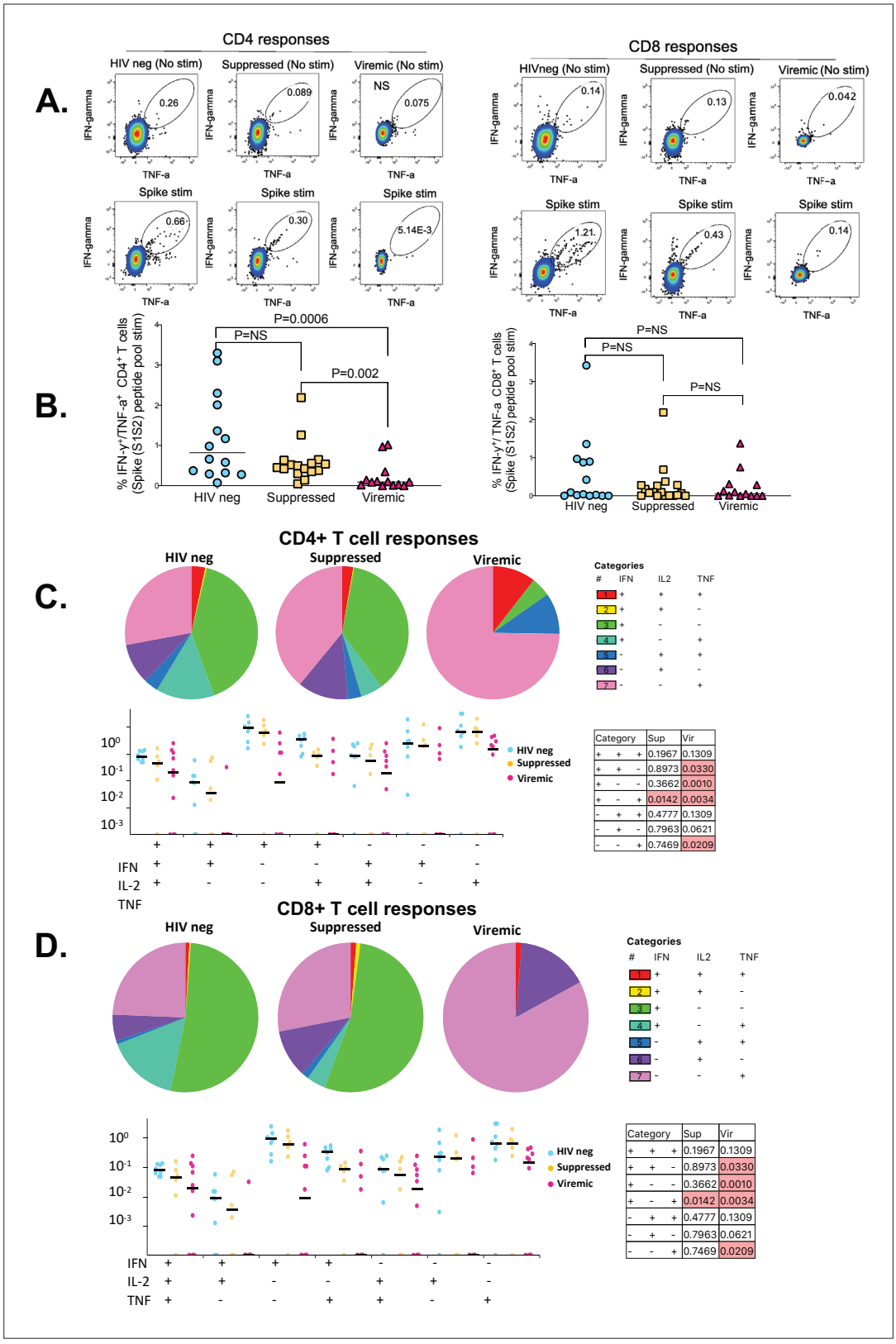

Figure 1. The impact of unsuppressed HIV infection on SARS-CoV-2-specific CD4⁺ and CD8⁺ T cell responses. (**A**) Representative flowplots gated on IFN-γ/TNF-α dual positive CD4⁺ and CD8⁺ T cells. (**B**) Aggregate data for IFN-γ/TNF-α dual positive CD4⁺ and CD8⁺ T cells are shown (HIV-neg, n=14; suppressed, n=16: viremic, n=13). SARS-CoV-2-specific CD4⁺ and CD8⁺ T cells producing IFN-γ, TNF-α, and IL-2 cells in various combinations are

Figure 1 continued on next page

Figure 1 continued

shown. Pie chart and dot plots for (**C**) SARS-CoV-2-specific $CD4^+$ and (**D**) $CD8^+$ T cells. Pie chart represents the mean distribution across subjects of mono-functional, bi-functional, and poly-functional cytokine producing SARS-CoV-2-specific T cells. Size of each pie segment relates to the frequency of a mono-functional, bi-functional, and triple-functional response. Dot plot represents the frequency of combinations of cytokines produced. Wilcoxon test was done among the dot plots using SPICE software (significant p values are highlighted).

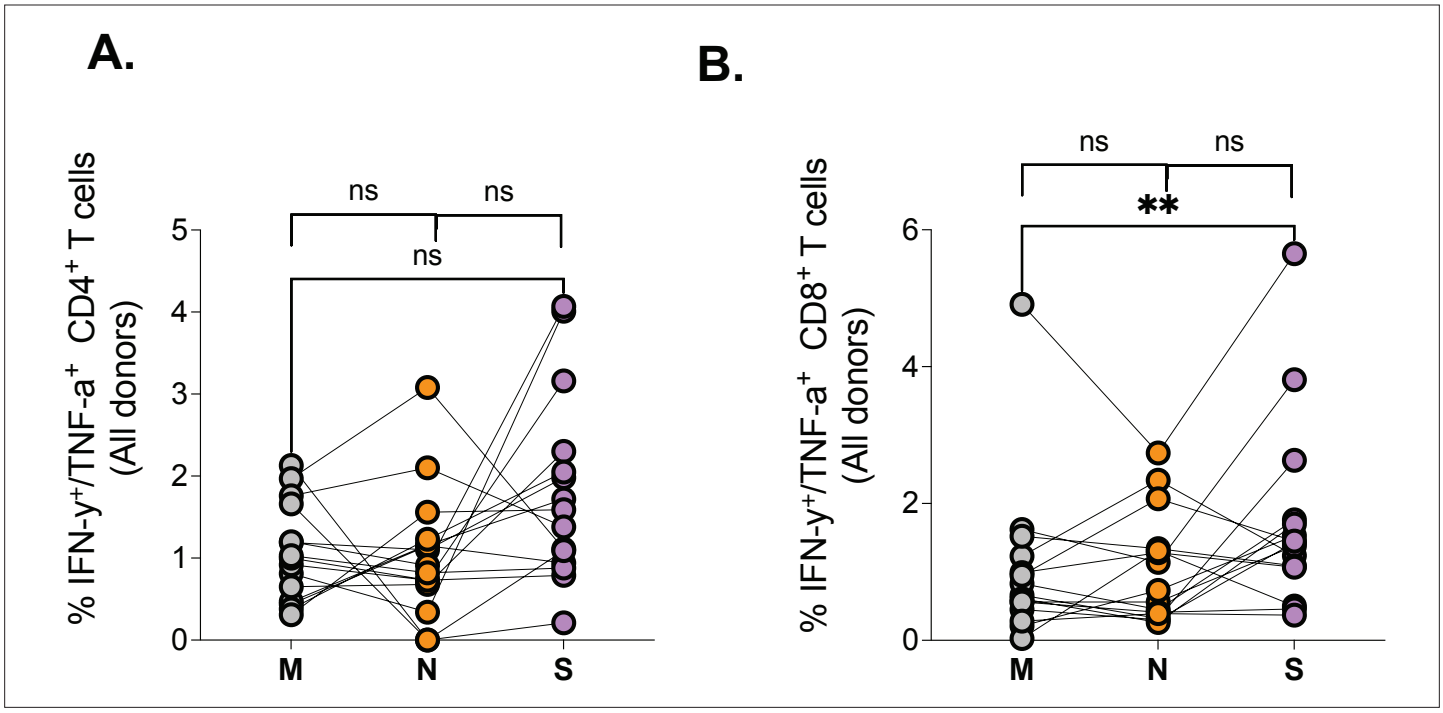

Figure 2. Comparison of SARS-CoV-2 protein targeting by T cell responses among HIV-negatives, suppressed and viremic donors. Magnitude of (**A**) $CD4^+$ T and (**B**) $CD8^+$ T cell responses targeting the Membrane (M), Nucleocapsid (N), and Spike (S) SARS-CoV-2 proteins among study groups. P values for differences among the groups are *<0.05; as determined by the Wilcoxon matched-pairs signed rank test (GraphPad Prism version 9.3.0).

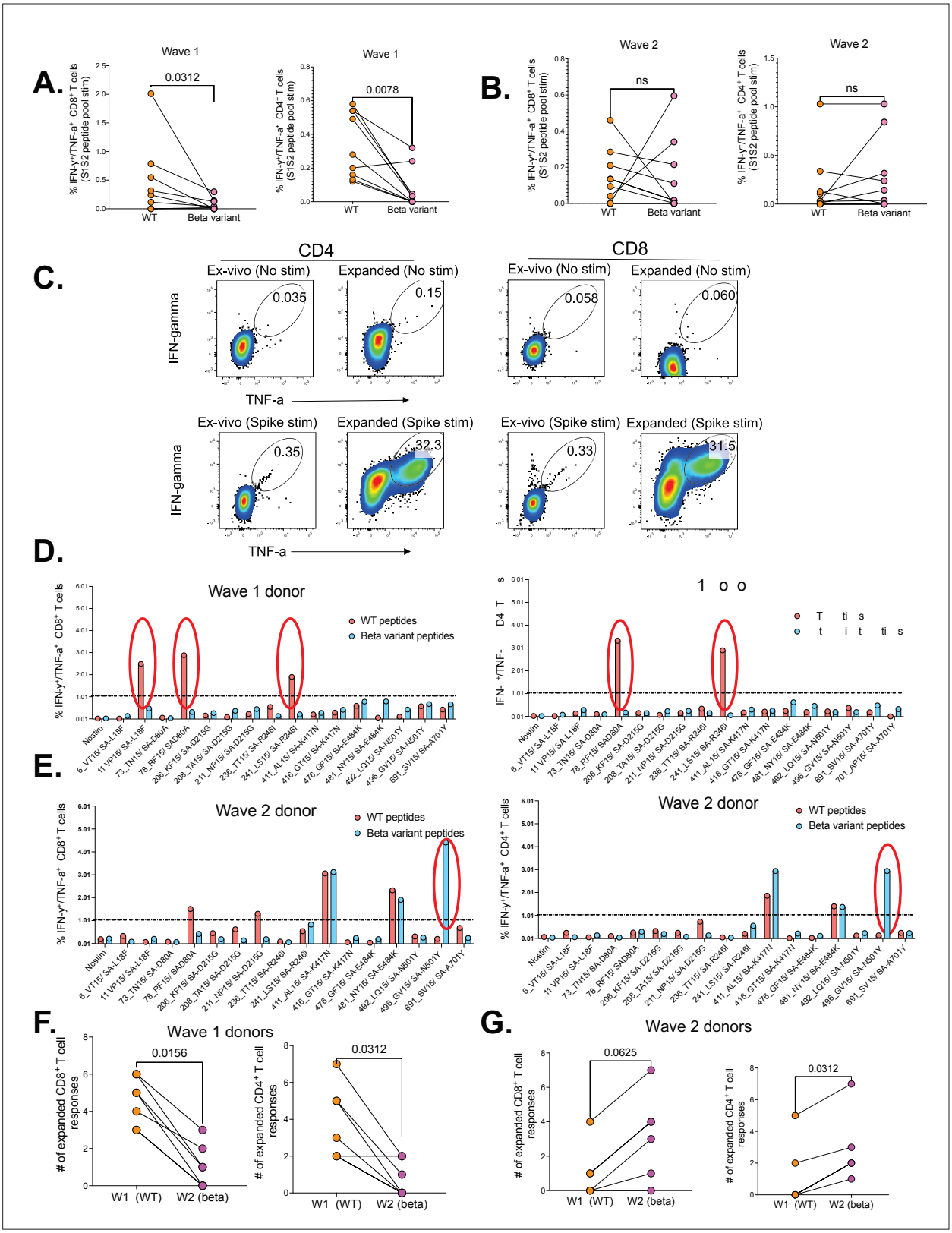

Figure 3. Poor cross-recognition of SARS-CoV-2-specific CD4⁺ and CD8⁺ T cell responses between wt and beta variants in wave 1 and wave 2 COVID-19 participants. Ex vivo assessment of T cell cross-recognition between the two waves. (**A**) Intra-donor SARS-CoV-2-specific T cell responses to wt and corresponding Beta variant peptides by wave 1 participants. (**B**) Intra-donor SARS-CoV-2-specific T cell responses to wt and corresponding Beta variant peptides in wave 2 participants. Next, PBMCs were expanded for 12 days in the presence of S1S2 SARS-CoV-2 peptide pools and tested against wt

Figure 3 continued on next page

Figure 3 continued

and corresponding Beta variants at single peptide level. (**C**) Representative flow plots showing the frequency of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells before and after cultured expansion. (**D**) T cell responses to single wt (red bars) and corresponding Beta (blue bars) peptide stimulation for a representative donor from wave 1. (**E**) T cell responses to single wt and corresponding Beta peptide stimulation for a representative donor from wave 2 (positive responses are circled). A response was deemed positive if ≥1% or higher. (**F**) Number of expanded wt and corresponding Beta responses for each wave 1 donor. (**G**) Number of expanded wt and corresponding Beta responses for each wave 2 donor. P values calculated using Wilcoxin matched-pairs signed rank T test. PBMC, peripheral blood mononuclear cell; wt, wild-type.

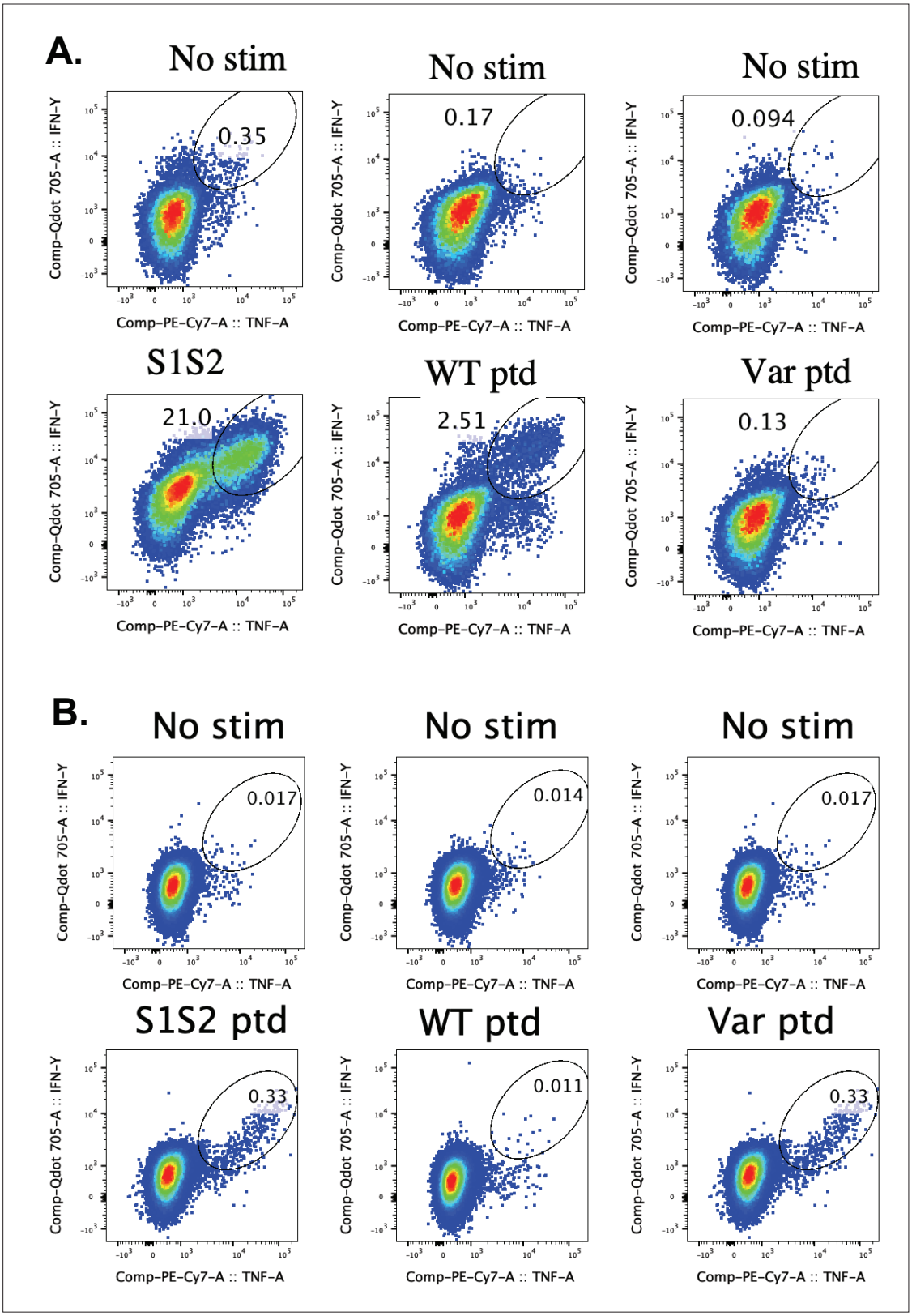

Figure 3—figure supplement 1. Cross-recognition of SARS-CoV-2 CD4⁺ T cell responses between wt and Beta variants in wave 1 and wave 2 COVID-19 donors: PBMCs were expanded for 12 days in the presence of S1S2 SARS-CoV-2 peptide pools. Expanded cells were tested against wt and corresponding Beta variants at single peptide level. (**A**) Intra-donor SARS-CoV-2-specific T cell responses to wt and corresponding Beta variant peptides by wave 1 participants. (**B**) Intra-donor SARS-CoV-2-specific T cell responses to wt and corresponding Beta variant peptides in wave 2 participants. PBMC, peripheral blood mononuclear cell; wt, wild-type.

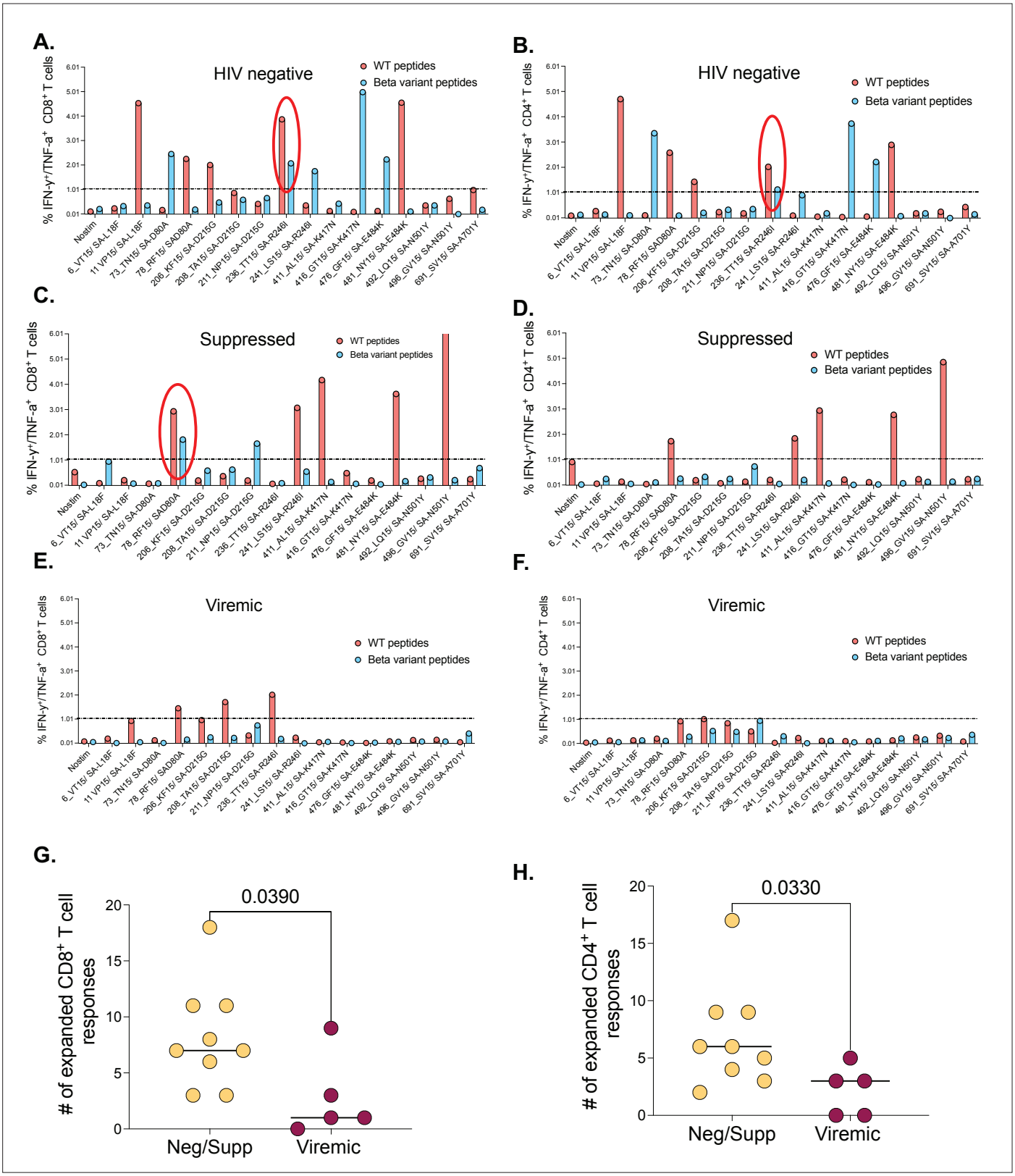

Figure 4. The effects of unsuppressed HIV infection on T cell breadth and ability to cross-recognize the Beta variant. Representative data for a negative donor showing greater, (**A**) CD8⁺ and (**B**) CD4⁺ T cell breadth. A cross-recognized responses between wt and Beta is circled. Representative data for a suppressed donor showing greater, (**C**) CD8⁺ and (**D**) CD4⁺ T cell breadth. A cross-recognized response is circled. Representative data for a viremic donor showing greater, (**E**) CD8⁺ and (**F**) CD4⁺ T cell breadth. (**G**) Aggregate data comparing breath of SARS-CoV-2-specific CD8⁺, and (**H**) CD4⁺ T

Figure 4 continued on next page

Figure 4 continued

cell response between HIV-negative and suppressed versus viremics. Breadth here is simply the number of positive responses among the individual peptides tested.

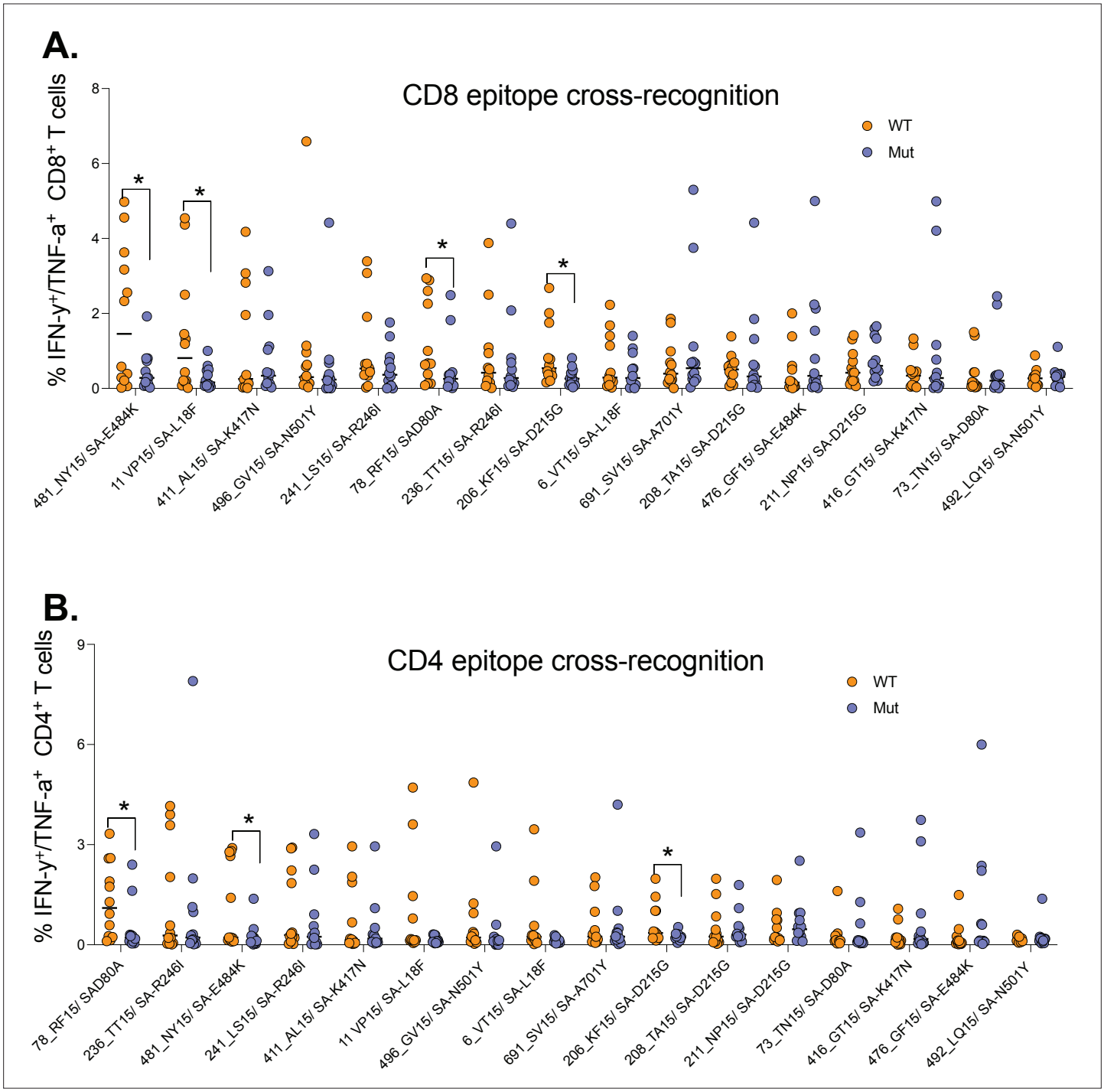

Figure 5. Identification of Beta mutations associated with reduced cross-recognition between wt and Beta variant. (**A**) Side-by-side comparison of SARS-CoV-2-specific CD8⁺ T cell response between wt and Beta. (**B**) Side-by-side comparison of SARS-CoV-2-specific CD4⁺ T cell response between wt. The analysis combined all the 12 participants. P values calculated by Mann-Whitney U-test. wt, wild-type.

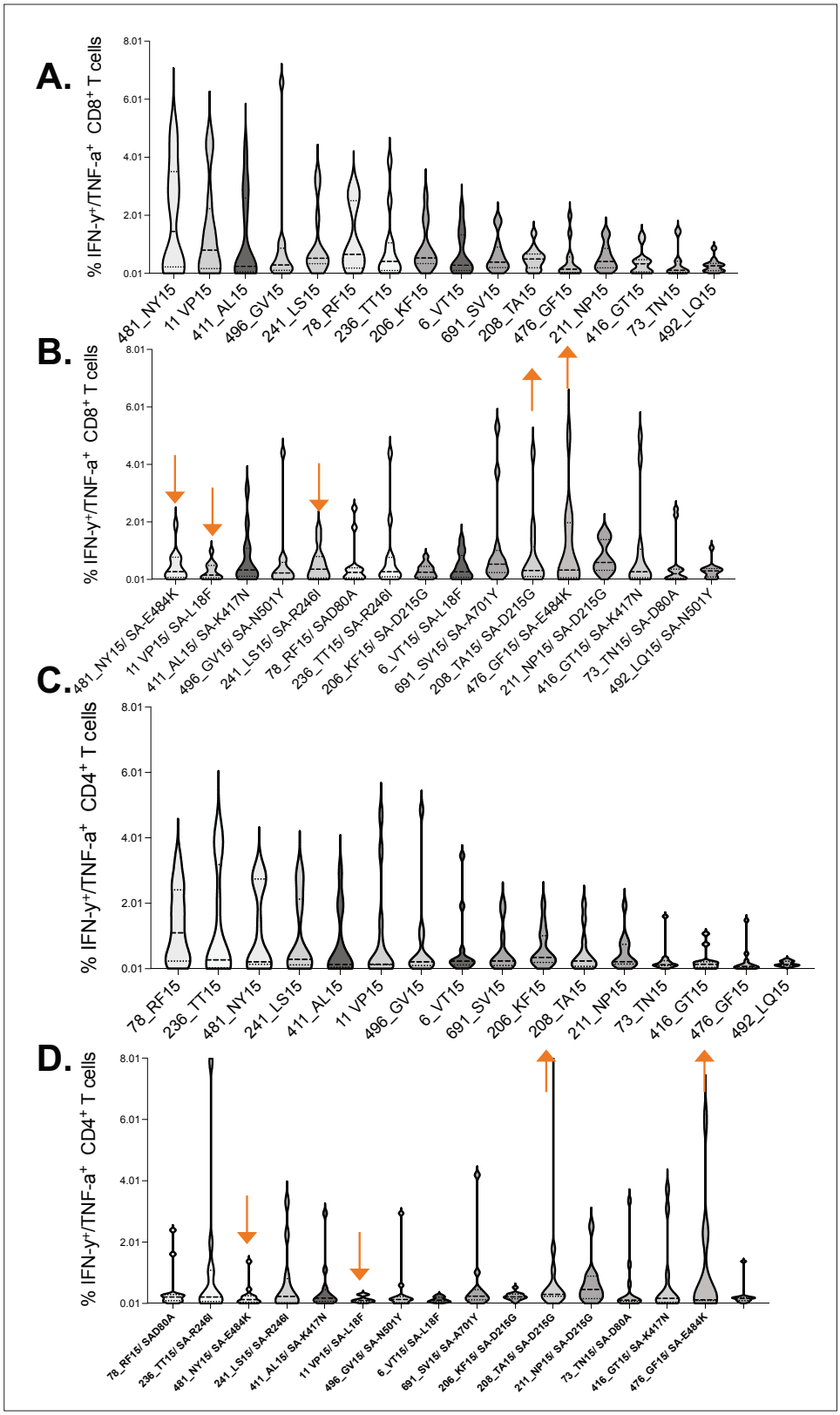

Figure 6. Immunodominance hierarchy of SARS-CoV-2 CD8⁺ and CD4⁺ T cell responses targeting wt and Beta. Immunodominance hierarchy of CD8⁺ T cell responses to, (**A**) wt and (**B**) the corresponding Beta variant peptides. Similarly, Immunodominance hierarchy of CD4⁺ T cell responses to, (**C**) wt and (**D**) the corresponding Beta variant. Arrows indicate responses that changed hierarchical position (among the six most dominant responses) between

Figure 6 continued on next page

Figure 6 continued

the two waves. Data arranged in descending order of magnitude of responses to wt peptide stimulation. wt, wild-type.

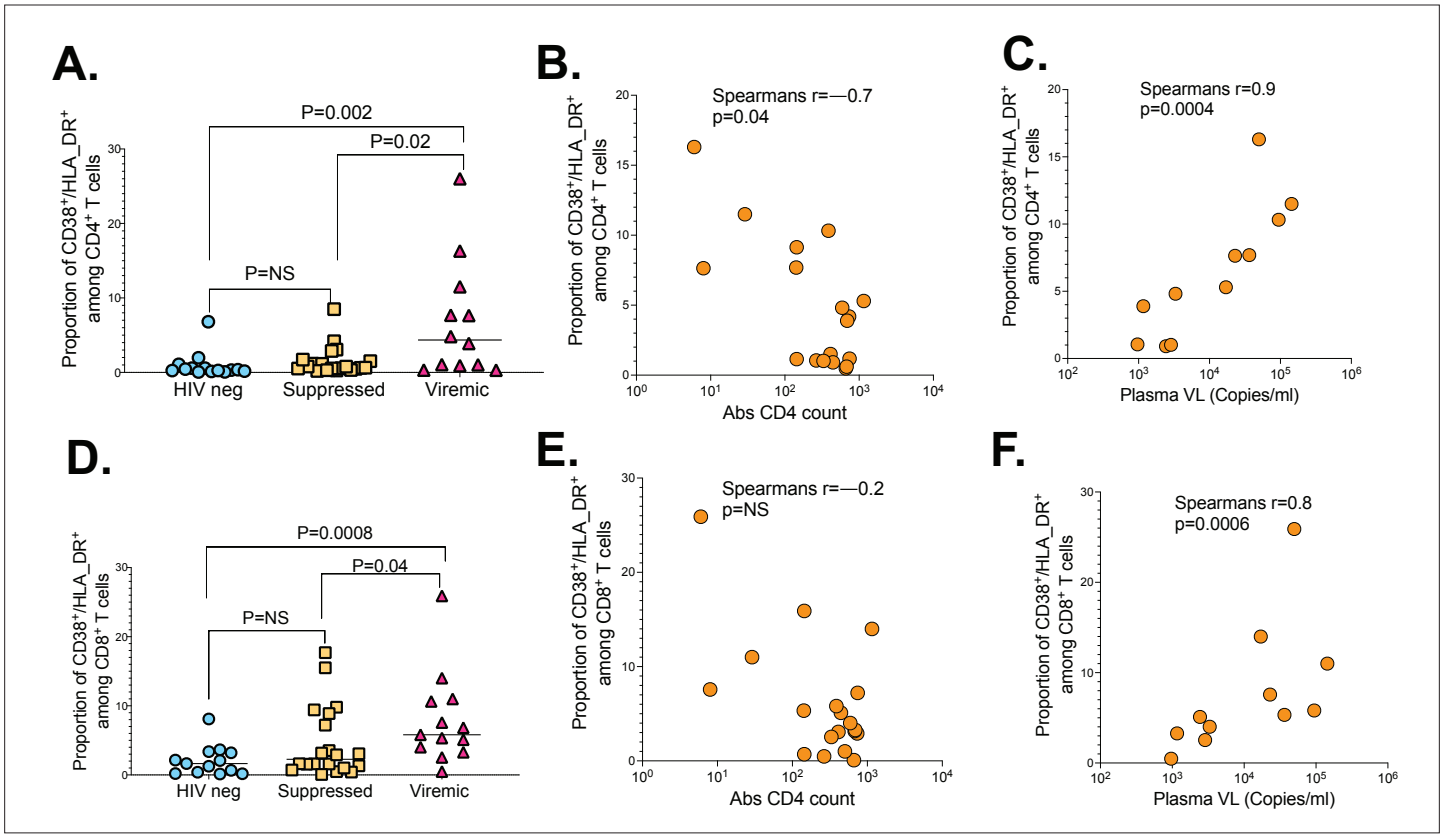

Figure 7. The impact of HIV markers of diseases progression on SARS-CoV-2 T cell immunity. (**A**) CD4⁺ T cell activation graphed based on the frequency of CD38/HLA-DR co-expressing cells. (**B**) Correlation between CD4⁺ T cell activation and absolute CD4 counts of viremic PLWH. (**C**) Correlation between CD4⁺ T cell activation and HIV plasma viral load of viremic PLWH. (**D**) CD8⁺ T cell activation measured by CD38/HLA-DR. (**E**) Correlation between CD8⁺ T cell activation and absolute CD4 counts of viremic PLWH. (**F**) Correlation between CD8⁺ T cell activation and HIV plasma viral load of viremic PLWH. P values calculated by Mann-Whitney U-test and Pearson correlation test. PLWH, people living with HIV.

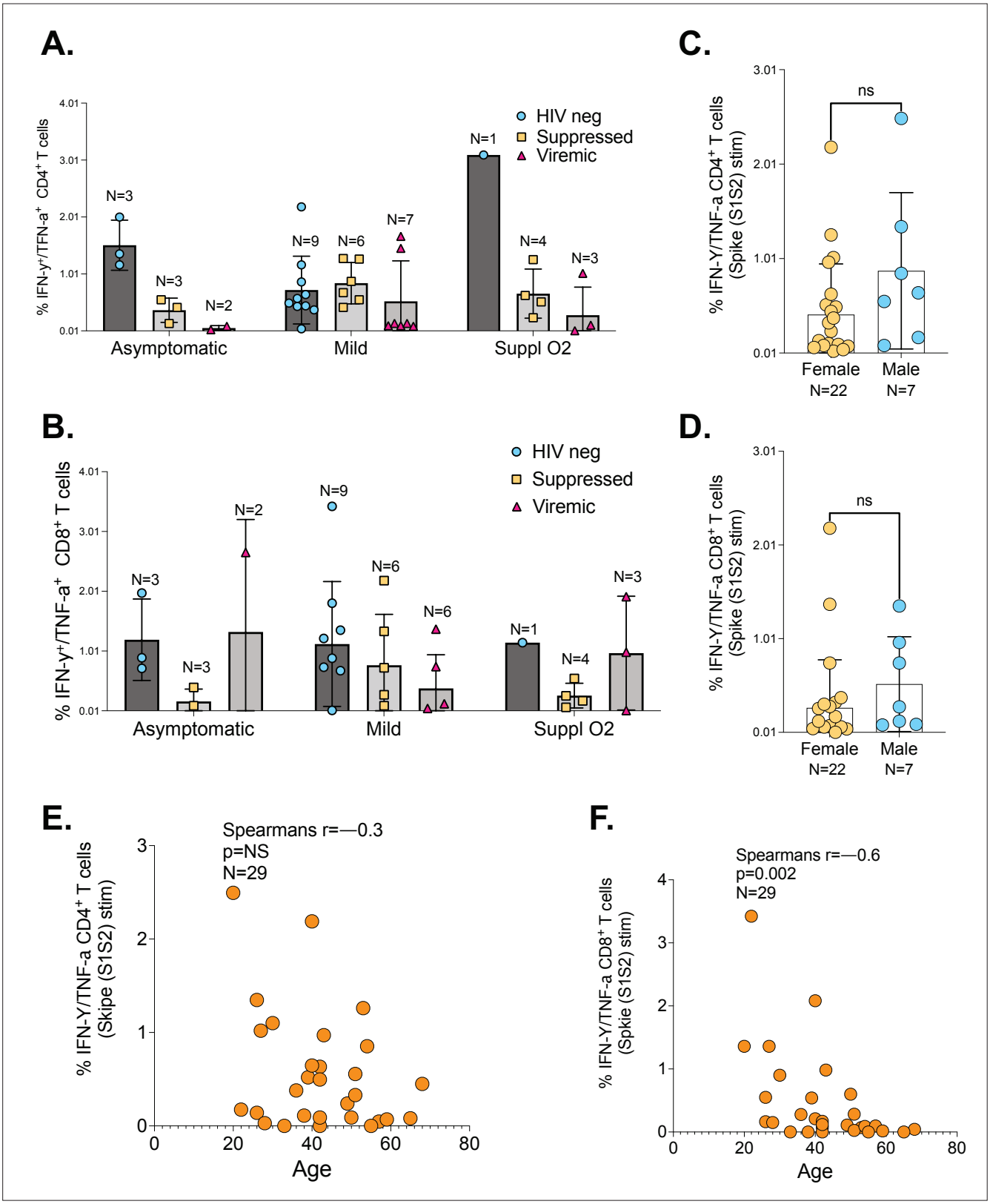

Figure 7—figure supplement 1. Assessment of the effect of COVID-19 disease severity on, (**A**) SARS-CoV-2-specific CD4⁺, and (**B**) CD8⁺ T cell responses. Disease severity categorised as asymptomatic, mild, and on supplemental oxygen or death. (**C, D**) Analysis of SARS-CoV-2 responses based on gender. Correlation between age and SARS-CoV-2-specific, (**E**) CD8⁺T and (**F**) CD4⁺ T cell responses. P values calculated by Mann-Whitney U-test and Pearson correlation test.