Supporting Information:

Supplemental Methods:

Methods: FACS panel design, gating strategy, choice of controls and end-analysis was performed according to guidelines (11). Immunological differentials were done by conventional flow cytometry. Total T-cells, CD4+ T-cells and CD8+ T-cells as well as B-cells were enumerated in EDTA blood after RBC lysis with hypotonic buffer using a single platform lyse/no wash protocol (antibodies: CD45-VioBlue, REA747; CD3-FITC, REA613; CD4-VioGreen, REA623; CD8-APC-Vio770, REA734; CD19-PE-Vio770, REA675; and 7-AAD as vitality marker; all from Miltenyi Biotech, Bergisch Gladbach, Germany). Acquisition and analysis were performed on FACS BD Lyric, using BD FACSSuite software (Becton-Dickinson, Heidelberg, Germany). Normal values were taken from (12).

Enumeration of antigen-specific T-cells was performed using SARS-CoV-2 T-cell Analysis Kit (Whole Blood) as manufacturer-recommended (Miltenyi Biotech, Bergisch Gladbach, Germany). Briefly, 500 µl of heparinized blood were incubated over night with 0.6 nmol/mL of each peptide of S1 peptide library or CytoStim reagent as positive control in the presence of Brefeldin A, followed by fixation-permeabilization and antibody staining as above (antibodies: CD3-APC, REA613; CD4-VioBrightB515, REA623; CD8-VioGreen, REA734; IFN-γ-PE, REA600; TNF-α-PE-Vio770, REA656; CD14-VioBlue, REA599; CD20-VioBlue, REA780; CD40L-APC-Vio770, REA238; IL-2-PE-Vio615, REA689), washing, and analysis by flow cytometry (FACS Canto II, software: DIVA; BD). Frequency and concentration of antigen-specific T-cells (CD4+ response as a sum of IL2+, CD40L+ and IL2+/CD40L+; CD8+ response as a sum of TNF-α+, IFN-γ+ and TNF- α+/IFN-γ+ events) were calculated. A positive response was defined as at least double the frequency of positive events as in the negative control and no fewer than seven positive events to exclude incidental positive results. Gating strategy and examples of negative and positive controls and SARS-CoV-2 specific T-cells are shown in Figure 1C and in Suppl. Figure 1.

The selected positive control, CytoStim, crosslinks TCR and MHC, simulating a canonical APC-T-cell synapse. CytoStim does not test overall ability of T-cells to react to specific antigens. With a mean 10%/27% of CD4+/CD8+ T-cells of HSCT patients responding to CytoStim, vs. 3%/11% in controls, their ability to generate an activation signature is normal despite lymphopenia and skewed CD4:CD8 ratios.

S1 peptide was selected as stimulating peptide because the studies were conceptualized when peptide library covering the whole spike protein was not yet available. Comparison of responses to different libraries in healthy vaccinees informed of their co-occurrence, responses to S1 being the strongest, most discernible. Negative history reliably excluded contact with SARS-CoV2 since inapparent infection is unlikely in our cohort, and given the paucity of available T-cells for testing, N peptide control was therefore omitted.

Statistics were calculated in Excel (Microsoft, Redmond, WA).

Supplemental References:

11. Cossarizza, A., Chang, H. D., Radbruch, A., Abrignani, S., Addo, R., Akdis, M., Andrä, I., et al., Guidelines for the use of flow cytometry and cell sorting in immunological studies (third edition) Eur. J. Immunol. 2021. 51: 2708–3145
12. Jentsch-Ullrich K, Koenigsmann M, Mohren M, Franke A. Lymphocyte subsets' reference ranges in an age- and gender-balanced population of 100 healthy adults--a monocentric German study. Clin Immunol. 2005;116(2):192-197.

Ethics approval statement:

The study was approved by the local ethics committee (#2021-180).

Patient and volunteer consent statement:

Participants gave written informed consent.

Clinical trial registration:

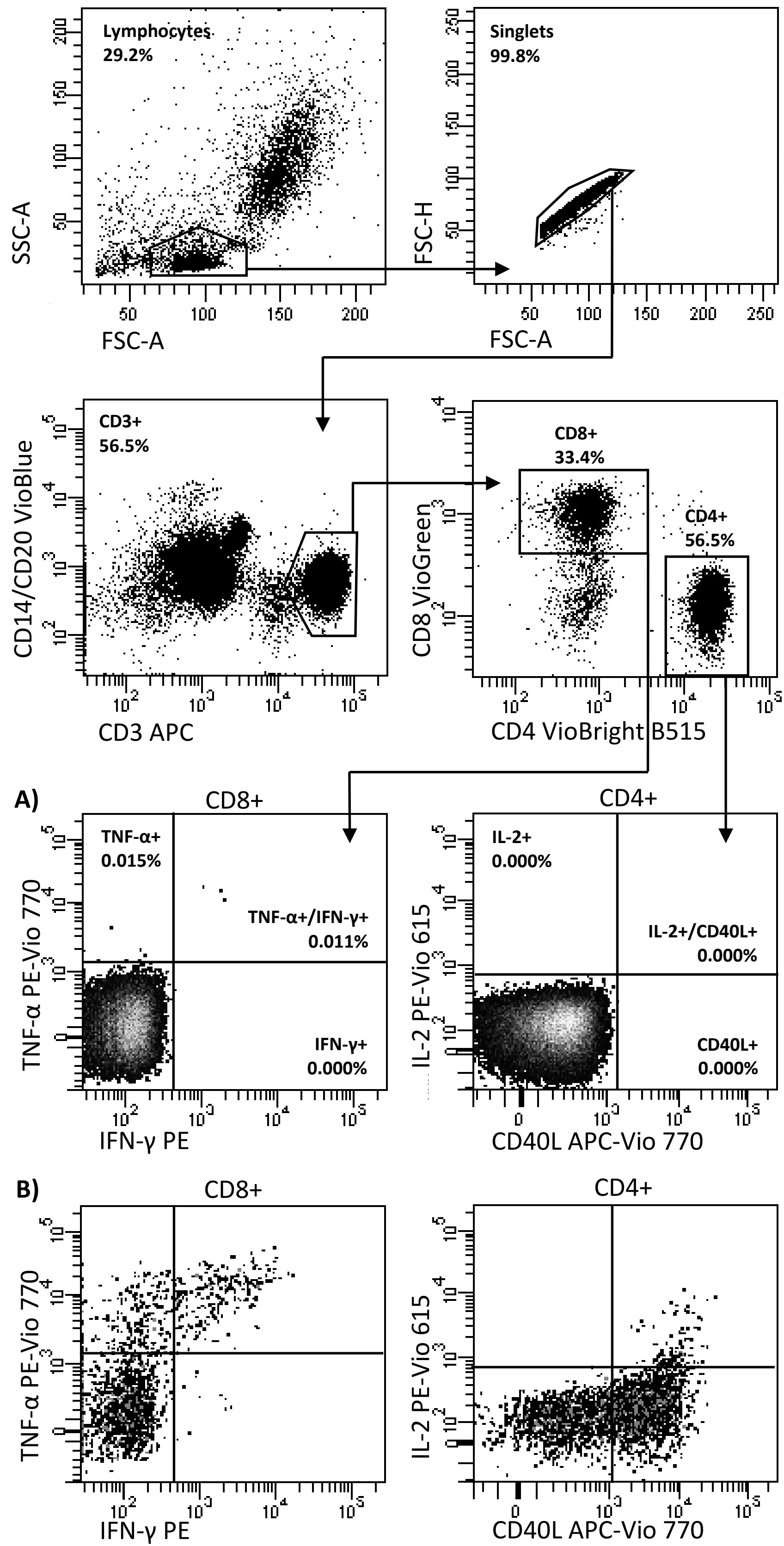
n.a.

Author contributions:

AJ, EW, HB and GB conceived of the studies, interpreted data and wrote the manuscript. EW, SDM, HH and EU performed measurements of T-cell responses, SC of B-cell responses. SA, C, NTTN, AD, KUC provided patient or volunteer blood samples. HS, ES, PB, AJ, EW, HB and GB bear the overall responsibility for the studies. All authors have read and approved of the final manuscript.

Permissions:

n.a.



Suppl. Figure 1:

Gating strategy for enumeration of SARS-CoV-2 specific T-cells. A patient sample was treated with diluent, negative control (A) or with CytoStim, positive control (B). The result of the corresponding SARS-CoV-2 S1 peptide library stimulation is shown in Figure 1C.