

1 **Online supplement for:**

2

3 **Rapid, simplified whole blood-based multiparameter assay to quantify and phenotype**

4 **SARS-CoV-2 specific T cells**

5

6 Catherine Riou^{1,2,*}, Georgia Schäfer^{1,3,4}, Elsa du Bruyn^{1,5}, Rene T. Goliath¹, Cari Stek^{1,5,6}, Huihui

7 Mou⁷, Deli Hung⁸, Katalin A. Wilkinson^{1,5,9}, Robert J. Wilkinson^{1,5,6,9}

8

9 ¹ Wellcome Centre for Infectious Disease Research in Africa and Institute of Infectious Disease
10 and Molecular Medicine, University of Cape Town, Observatory 7925, South Africa

11 ² Division of Immunology, Department of Pathology, University of Cape Town, Observatory
12 7925, South Africa

13 ³ International Centre for Genetic Engineering and Biotechnology (ICGEB) Cape Town,
14 Observatory 7925, South Africa

15 ⁴ Division of Medical Biochemistry and Structural Biology, Department of Integrative
16 Biomedical Sciences, University of Cape Town, Observatory 7925, South Africa

17 ⁵ Department of Medicine, University of Cape Town, Observatory 7925, South Africa

18 ⁶ Department of Infectious Diseases, Imperial College London, W12 0NN, UK

19 ⁷ Department of Immunology and Microbiology, The Scripps Research Institute, Jupiter, FL
20 33458, USA

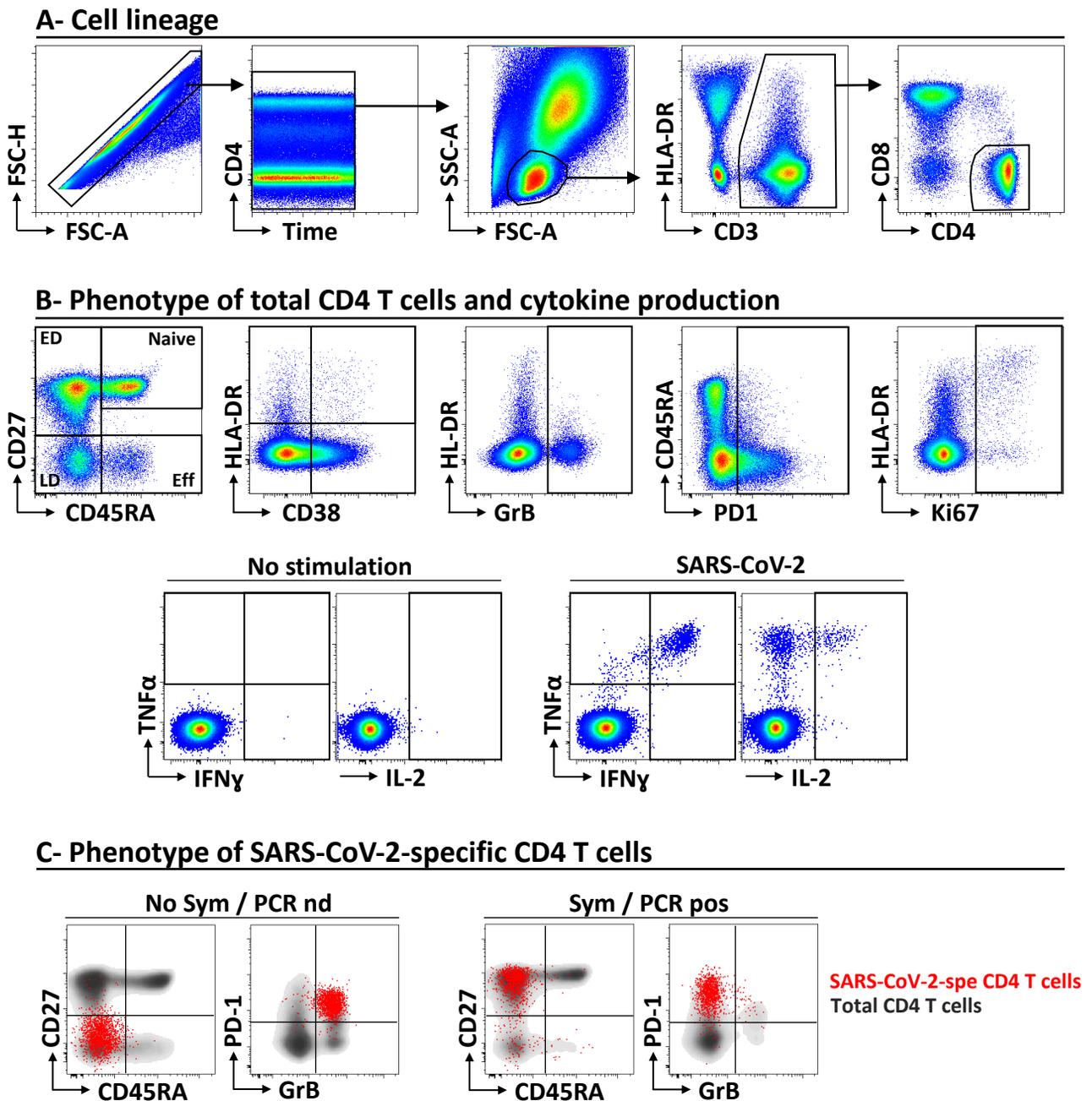
21 ⁸ Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA
22 92037, USA

23 ⁹ The Francis Crick Institute, London, NW1 1AT, UK

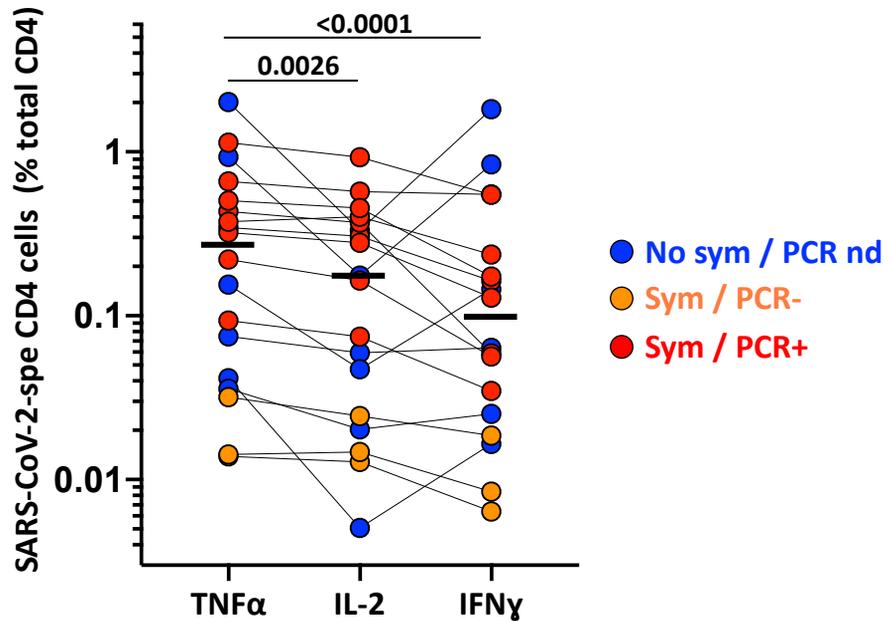
24

Markers	Fluorochrome	Clone	Company	Cat. Number	Role
CD3	BV650	OKT3	BioLegend	317323	Lineage
CD4	BV785	OKT4	BioLegend	317428	
CD8	BV510	RPA-T8	BioLegend	301048	
CD45RA	Alexa 488	HI100	BioLegend	304114	Memory differentiation
CD27	PE-Cy5	1A4CD27	Beckman	6607107	
CD38	APC	HIT2	BD Bioscience	555462	Activation
HLA-DR	BV605	L243	BioLegend	307640	
Ki67	PerCP-Cy5.5	B56	BD Bioscience	561284	
PD-1	PE	EH12.2H7	BioLegend	329906	
GrB	BV421	GB11	BD Bioscience	563388	Cytotoxic potential
IFNγ	BV711	4S.B3	BioLegend	502540	Functions
TNFα	PE-Cy7	MAB11	BioLegend	502930	
IL-2	PE/Dazzle™ 594	MQ1-17H12	BioLegend	500344	

Supp table 1: Description of the antibody panel used in the study.



Supp Figure 1: Gating strategy used to identify SARS-CoV-2 specific CD4 T cells and defined their phenotypic characteristics. **A-** Cell lineage. **B-** Phenotyping of total CD4 T cell and cytokine production in response to SARS-CoV-2 peptides. **C-** Phenotype of SARS-CoV-2 responding CD4 T cells in one participant with no symptom (left) and one COVID-19 confirmed participant (right). SARS-CoV-2-specific CD4 T cells (expressing any measured cytokine) are depicted in red.



Supp Figure 2: Comparison of magnitude of SARS-CoV-2-specific CD4 T cell response expressing TNF α , IL-2 or IFN γ in participants exhibiting a detectable response to SARS-CoV-2 peptide pool. Each dot represent a participant and is color-coded according to clinical characteristics (Blue represents no symptoms, no SARS-CoV-2 PCR performed; Orange: self-reported symptoms, SARS-CoV-2 PCR negative and Red: self-reported symptoms, SARS-CoV-2 PCR positive). Black bars indicate medians. Statistical comparisons were performed using a nonparametric paired Friedman test.