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(2020) Broad and strong memory CD4+ and CD8+ T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. *Nature immunology*. 1336–1345. ISSN 1529-2916

<https://doi.org/10.1038/s41590-020-0782-6>

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1 **Broad and strong memory CD4⁺ and CD8⁺ T cells induced by SARS-CoV-2 in UK**
2 **convalescent COVID-19 patients**

3
4 Yanchun Peng^{1,2†}, Alexander J. Mentzer^{3,4,20†}, Guihai Liu^{2,4,5†}, Xuan Yao^{1,2,4†}, Zixi Yin^{1,2†},
5 Danning Dong^{2,4,6†}, Wanwisa Dejnirattisai^{4†}, Timothy Rostron⁷, Piyada Supasa⁴, Chang Liu^{2,4},
6 César López-Camacho^{3,4}, Jose Slon-campos⁴, Yuguang Zhao⁴, David I. Stuart^{2,3,4,17}, Guido
7 C. Paesen³, Jonathan Grimes^{3,4,17}, Alfred A. Antson⁸, Oliver W. Bayfield⁸, Dorothy EDP.
8 Hawkins⁸, De-Sheng Ker⁸, Beibei Wang^{2,4}, Lance Turtle^{9,13}, Krishanthi Subramaniam¹³, Paul
9 Thomson¹³, Ping Zhang⁴, Christina Dold¹⁰, Jeremy Ratcliff⁴, Peter Simmonds⁴, Thushan de
10 Silva¹¹, Paul Sopp⁷, Dannielle Wellington^{1,2}, Ushani Rajapaksa^{2,4}, Yi-Ling Chen¹, Mariolina
11 Salio¹, Giorgio Napolitani¹, Wayne Paes⁴, Persephone Borrow⁴, Benedikt M. Kessler^{2,4},
12 Jeremy W. Fry¹², Nikolai F. Schwabe¹², Malcolm G Semple^{13,14}, J. Kenneth Baillie¹⁵, Shona
13 C. Moore¹³, Peter JM Openshaw¹⁶, M. Azim Ansari⁴, Susanna Dunachie^{4,20}, Eleanor
14 Barnes^{4,20,21}, John Frater^{4,20}, Georgina Kerr⁴, Philip Goulder^{4,20}, Teresa Lockett²⁰, Robert
15 Levin¹⁸, Yonghong Zhang^{2,5}, Ronghua Jing⁵, Ling-Pei Ho^{1,2,4,21}, Oxford Immunology Network
16 Covid-19 Response T cell Consortium, ISARIC4C Investigators, Richard J. Cornall^{1,4,20},
17 Christopher P. Conlon^{2,4,20}, Paul Klenerman^{4,20,21}, Gavin R. Screaton^{4,20,21}, Juthathip
18 Mongkolsapaya^{2,4,19,21}, Andrew McMichael^{2,4}, Julian C. Knight^{2,3,4,20}, Graham Ogg^{1,2,20,21‡}, Tao
19 Dong^{1,2,4‡*}

20

- 21 1. MRC Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine, Radcliffe
22 Department of Medicine, University of Oxford, Oxford, U.K.
23 2. Chinese Academy of Medical Science (CAMS) Oxford Institute (COI), University of Oxford,
24 Oxford, U.K.
25 3. Wellcome Centre for Human Genetics, University of Oxford, Oxford, U.K.
26 4. Nuffield Department of Medicine, University of Oxford, Oxford, U.K.
27 5. Beijing You'an Hospital, Capital Medical University, Beijing, China

- 28 6. CAMS Key Laboratory of Tumor Immunology and Radiation Therapy, Xinjiang Tumor
29 Hospital, Xinjiang Medical University, China.
- 30 7. Sequencing and Flow cytometry facility, Weatherall Institute of Molecular Medicine,
31 University of Oxford, Oxford, UK
- 32 8. York Structural Biology Laboratory, Department of Chemistry, University of York, York, UK
- 33 9. Tropical and Infectious Disease Unit, Liverpool University Hospitals NHS Foundation
34 Trust
- 35 10. Oxford Vaccine Group, Department of Paediatrics, University of Oxford, and NIHR
36 Oxford Biomedical Research Centre, Centre for Clinical Vaccinology and Tropical Medicine,
37 University of Oxford, UK
- 38 11. The Florey Institute for Host-Pathogen Interactions, Dept. of Infection, Immunity and
39 Cardiovascular Disease, University of Sheffield, Sheffield, UK
- 40 12. ProImmune Limited, Oxford, UK
- 41 13. NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Institute of
42 Infection, Veterinary & Ecological Sciences, University of Liverpool, Liverpool, UK
- 43 14. Respiratory Medicine, Institute in The Park, Alder Hey Children's Hospital, Liverpool,
44 UK
- 45 15. Anaesthesia, Critical Care and Pain Medicine Division of Health Sciences,
46 University of Edinburgh, Edinburgh, UK
- 46 16. National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London,
47 UK
- 48 17. Diamond Light Source, Didcot, UK.
- 49 18. Worthing Hospital, Worthing, UK
- 50 19. Dengue Hemorrhagic Fever Research Unit, Office for Research and Development,
51 Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand
- 52 20. Oxford University Hospitals NHS Foundation Trust, Oxford
- 53 21. NIHR Oxford Biomedical Research Centre
- 54
- 55 † These authors contributed equally to the work.

56 ‡joint senior authors

57 * Correspondence author: Tao Dong (email: tao.dong@imm.ox.ac.uk)

58 **Abstract**

59

60 Development of SARS-CoV-2 vaccines and therapeutics will depend on understanding viral
61 immunity. We studied T-cell memory in 42 patients following recovery from COVID-19 (28
62 mild, 14 severe, 16 unexposed donors), using IFN- γ -based assays with peptides spanning
63 SARS-CoV-2 except ORF1. The breadth and magnitude of T-cell responses were
64 significantly higher in severe compared to mild cases. Total and spike-specific T-cell
65 responses correlated with spike-specific antibody responses. We identified 41 peptides
66 containing CD4⁺ and/or CD8⁺ epitopes, including six immunodominant regions. Six
67 optimised CD8⁺ epitopes were defined, with peptide-MHC-pentamer-positive cells displaying
68 central- and effector-memory phenotype. In mild cases, higher proportions of SARS-CoV-2-
69 specific CD8⁺ T-cells were observed. The identification of T-cell responses associated with
70 milder disease, will support an understanding of protective immunity, and highlights the
71 potential of including non-spike proteins within future COVID-19 vaccine design.

72

73 **Introduction**

74

75 COVID-19 is caused by the recently emerged Severe Acute Respiratory Syndrome
76 coronavirus-2 (SARS-CoV-2). Whilst the majority of COVID-19 infections are relatively mild,
77 with recovery typically within two to three weeks^{1, 2}, a significant number of patients develop
78 severe illness, which is postulated to be related to both an overactive immune response and
79 viral-induced pathology^{3, 4}. The role of T-cell immune responses in disease pathogenesis
80 and longer-term protective immunity is currently poorly defined, but essential to understand
81 in order to inform therapeutic interventions and vaccine design.

82

83 Currently, there are many ongoing vaccine trials, but it is unknown whether they will provide
84 long lasting protective immunity. Most vaccines are designed to induce antibodies to the
85 SARS-CoV-2 spike protein, but it is not yet known if this will be sufficient to induce full
86 protective immunity to SARS-CoV-2^{5,6, 7,8}. Studying natural immunity to the virus, including
87 the role of SARS-CoV-2-specific T-cells is critical to fill the current knowledge gaps for
88 improved vaccine design.

89

90 For many primary virus infections, it typically takes 7-10 days to prime and expand adaptive
91 T-cell immune responses in order to control the virus⁹. This coincides with the typical time it
92 takes for COVID-19 patients to either recover or develop severe illness. There is an
93 incubation time of 4-7 days before symptom onset, and a further 7-10 days before
94 individuals progress to severe disease¹⁰. Such a pattern of progression raises the possibility
95 that a poor T cell response contributes to SARS-CoV-2 viral persistence and COVID-19
96 mortality, whereas strong T cell responses are protective in the majority of individuals.

97

98 Evidence supporting a role for T cells in COVID-19 protection and pathogenesis is currently
99 incomplete and sometimes conflicting^{3,11,12,13,14}. To date there have been few studies
100 analysing SARS-CoV-2-specific T-cell responses and their role in disease progression¹⁵,

101 although virus specific T cells have been shown to be protective in human influenza
102 infection¹⁶. In a study of CD4⁺ and CD8⁺ T-cell responses to SARS-CoV-2 in non-
103 hospitalised convalescent subjects, Grifoni *et al* found that all recovered subjects
104 established CD4⁺ responses and 70% established CD8⁺ memory responses to SARS-CoV-
105 2¹⁷. SARS-CoV-2-specific CD4⁺ T-cell responses were also frequently observed in
106 unexposed subjects in their study, suggesting the possibility of pre-existing cross-reactive
107 immune memory to seasonal coronaviruses. In Singapore, Le Bert *et al*¹⁸ found long lasting
108 T cell immunity to the original SARS coronavirus nucleoprotein (NP) in those that were
109 infected in 2003. These T cells cross-reacted with SARS-CoV-2 NP, and T cells cross
110 reactive with NSP7 and NSP13 of other coronaviruses were also present in those
111 uninfected with either SARS coronaviruses¹⁸.

112

113 In the present study, the overall and immunodominant SARS-CoV-2-specific memory T-cell
114 response in subjects who had recovered from COVID-19 were evaluated *ex vivo* using
115 peptides spanning the full proteome of the SARS-CoV-2, except for ORF-1. Epitopes were
116 identified using two-dimensional matrix peptide pools and CD4⁺ and CD8⁺ T cell responses
117 were distinguished. The epitope specificity and HLA restriction of the dominant CD8⁺ T-cell
118 responses were defined in *ex vivo* assays and using *in vitro* cultured short-term T-cell lines.
119 The *ex vivo* functions of SARS-CoV-2-specific T-cells specific for dominant epitopes were
120 evaluated by their intracellular cytokine production profiles. Broad, and frequently strong,
121 SARS-CoV-2 specific CD4⁺ and CD8⁺ T-cell responses were seen in the majority of
122 convalescent patients, with significantly larger overall T-cell responses in those that had
123 severe compared to mild disease. However, there was a greater proportion of CD8⁺ T-cell
124 compared to CD4⁺ T cell responses in mild cases with higher frequencies of multi-cytokine
125 production by matrix (M) and nucleoprotein (NP)-specific CD8⁺ T-cells.

126 **Results:**

127

128 **Study subjects**

129 42 individuals were recruited following recovery from COVID-19, including 28 mild cases and
130 14 severe cases. In addition, 16 control individuals sampled in 2017-2019, before COVID-19
131 appeared, were studied in parallel. Supplementary Fig. 1 shows the participant
132 characteristics. No significant differences in gender or age were noted between mild and
133 severe groups. The SaO₂/FiO₂ ratio in severe cases ranged from 4.3 (where 4.5 would be
134 the estimate for an individual with mild disease breathing ambient air) to 1.6 with the patients
135 with critical disease having an estimate of 0.8 (median in severe group 3.8).

136

137 ***Ex vivo* assessment of memory T cell responses specific to SARS-CoV-2**

138 PBMCs were tested for responses to a panel of 423 overlapping peptides spanning the
139 SARS-CoV-2 proteome except ORF1, using *ex vivo* IFN- γ ELISpot assays. All overlapping
140 peptides were placed into two 2-dimensional peptide matrices: a total of 61 peptide pools
141 were tested, with 29 peptides in the first-dimension pools, as described in Supplementary
142 Table 1. The majority of the participants exhibited SARS-CoV-2 memory T cell responses to
143 at least one of the peptides. The overall distribution, magnitude and breadth of the IFN- γ
144 responses against all SARS-CoV-2 virus peptides are shown in Fig. 1. There was no
145 correlation between the T cell responses and the time that had elapsed from symptom
146 development (Supplementary Fig. 2). No *ex vivo* IFN- γ -producing SARS-CoV-2-specific T
147 cell responses were observed in healthy volunteers, who were all sampled before any
148 chance of exposure, but in those with appropriate HLA types, T cell responses were
149 observed to influenza virus, EBV, CMV (FEC) using pools of known T cell epitopes as well
150 as PHA as positive controls (Supplementary Fig. 3). The breadth and magnitude of the T
151 cell responses varied considerably between individuals. T cell responses were detected
152 against epitopes distributed across a wide variety of virus proteins. Significantly higher

153 magnitude ($p=0.002$) and broader ($p=0.002$) overall T cell responses were observed in
154 severe cases in comparison with mild cases, in particular for responses to spike
155 (magnitude/breadth, $p=0.021/0.016$), membrane (magnitude/breadth,
156 $p<0.0003/p=0.033$), ORF3 (magnitude/breadth, $p<0.0001/0.001$) and ORF8
157 (magnitude/breadth, $p=0.011/0.014$) proteins (Fig. 2). Overall, we found strong and broad T
158 cell memory responses were induced after recovery from COVID-19, and the breadth and
159 magnitude of T-cell responses were significantly higher in severe compared to mild cases.

160

161 **Correlation with spike specific antibody responses**

162 The relationship between spike-specific, and overall T cell responses in association with
163 spike-specific, receptor binding domain (RBD) and NP-specific antibody endpoint titres
164 (EPTs) was assessed (Fig. 3). There were significant correlations between (a) spike-specific
165 antibody titers and both overall T cell responses ($p=0.0004/R=0.5185$) and spike-specific T
166 cell responses ($p=0.0006/R=0.505$); (b) RBD-specific antibody titers and both overall T cell
167 responses ($p=0.0004/R=0.5198$) and spike-specific T cell responses ($p=0.0004/R=0.5189$);
168 and (c) NP-specific antibody titers and both overall T cell responses ($p=0.0015/R=0.4738$)
169 and spike-specific T cell responses ($p=0.007/R=0.412$). However, there was no significant
170 association between NP-specific antibody titers and NP-specific T cell responses
171 ($p=0.067/R=0.286$); (Fig. 3a-c; and Supplementary Fig. 4). Moreover, significantly higher
172 level of spike, RBD and NP EPTs were observed in severe cases in comparison with mild
173 cases (Fig. 3d). It was noted that some individuals had low RBD-specific antibodies (Fig.
174 3b), yet had detectable spike-specific antibodies (Fig. 3a), suggesting that antibodies were
175 able to target non-RBD regions of spike – these are under further investigation. Thus total
176 and spike-specific T-cell responses were found to be correlated with spike-specific antibody
177 responses.

178

179 **Distribution of SARS-CoV-2-specific CD4⁺ and CD8⁺ memory T cell responses**

180 Having identified overall T cell responses to SARS-CoV-2 peptides, the responses detected
181 against positive peptide pools were characterized by flow cytometry for peptide recognition
182 by CD4⁺ or CD8⁺ T cell subsets and for intracellular production of IFN- γ , TNF- α and IL-2
183 after stimulation (Fig. 4a-b and Supplementary Fig. 5). A greater proportion of the T cell
184 responses to spike (p=0.0268) and M/NP (p=0.02) were contributed to by CD8⁺ T cells in
185 those with mild disease compared to those with severe disease (Fig. 4c, Supplementary Fig.
186 6a). **Differential subsets of SARS-CoV-2-specific T cells therefore associate with clinical**
187 **outcome.**

188

189 **Evaluation of the polyfunctionality of T cells responding to SARS-CoV-2 peptides**

190 Multi-cytokine analysis revealed patterns of IFN- γ , TNF α and IL-2 production by CD4⁺ and
191 CD8⁺ T cells in both mild and severe cases (Fig. 5a), For 22 individuals tested, both CD4⁺
192 and CD8⁺ antigen-specific-T cells produced least one of these three cytokines and others in
193 combination. CD8⁺ but not CD4⁺ T cells targeting different virus proteins showed different
194 cytokine profiles, with the M/NP-specific CD8⁺ T cells showing wider functionality than T cells
195 targeting spike protein (p=0.0231, Fig. 5b and Supplementary Fig. 6b). Furthermore, there
196 were a greater proportion of multifunctional M/NP-specific CD8⁺ T cells compared to spike-
197 specific T cells in those that had mild disease (p=0.0037), but not in those that had severe
198 disease (p=0.3823). In contrast to observations seen in influenza virus infection¹⁹, we did
199 not observe significant differences in the cytotoxic potential (as indicated by expression of
200 the degranulation marker CD107a) in patients with mild and severe disease (Fig. 5c); and
201 we observed very few CD107a⁺ CD4⁺ T cells overall, suggesting cytotoxic CD4⁺ T cells
202 might not be a major contributor to virus clearance.

203

204 **Identification of SARS-CoV-2 specific T cell peptides containing epitopes**

205 IFN- γ ELISpot assays were performed with candidate peptides identified from the 2-
206 dimensional matrix analysis in 34 subjects. A total of 41 peptides containing SARS-CoV-2 T

207 cell epitope regions were recognized by COVID-19 convalescent subjects, 18 from spike, 10
208 from NP, 6 from membrane and 7 from ORF proteins. Strikingly, 6 dominant 18mer peptides
209 were recognised by 6 or more of 34 subjects tested (Table 1). NP-16 was recognised by
210 12/34 (35%) subjects tested and contained at least two epitopes which recognised by either
211 CD4⁺ T cells or CD8⁺ T cells.

212

213 M-24 was recognised by 16/34 subjects (47%) tested and contained one or more CD4⁺ T
214 cell epitopes. Peptide M-20 was recognised by 11/34 subjects tested (32%) and contained
215 one or more CD4⁺ T cell epitopes. 3 dominant spike peptides were also identified, with S-34
216 recognised by 10/34 subjects (29%) containing both CD4⁺ and CD8⁺ T cell epitopes, and a
217 further two spike peptides S-151 and S-174 were recognised by 8/34 and 6/34 subjects (24%
218 and 18%), both containing CD4⁺ T cell epitopes.

219

220 Those dominant responses were further confirmed by *ex-vivo* assays and by using cultured
221 short-term T cell lines. Supplementary Fig. 7 illustrates examples of FACS plots from
222 intracellular cytokine staining (ICS) when short-term T cell lines were stimulated with single
223 peptides containing epitopes. CD4⁺ T cells elicited strong responses against dominant spike
224 peptides and M peptides, whereas cells targeting two NP dominant peptides were CD8⁺ T
225 cells. The optimal epitopes within the long peptides recognized by dominant CD8⁺ T cells
226 and their HLA restriction, matched to the donor's HLA type, were predicted using the IEDB
227 analysis resource (<http://tools.iedb.org/mhci>). The best predicted epitope sequences are
228 shown in supplementary Table 2.

229

230 A set of previously defined SARS epitopes²⁰ with identical sequences to SARS-CoV-2 were
231 also tested by ELISpot assay (Supplementary Table 3), Most of those peptides did not elicit
232 any positive responses in 42 COVID-19 recovered subjects, apart from two NP epitope
233 peptides (N-E-3 MEVTPSGTWL and N-E-11 LLNKHIDAYKTFPTEPK) and one spike
234 epitope peptide (S-E-19 QLIRAAEIRASANLAATK) . N-E-11, which is identical to peptide

235 NP-51, shares the sequence with two other known HLA-A*0201 restricted SARS epitopes
236 (N-E-1 ILLNKHID and N-E-5 ILLNKHIDA). Interestingly, one of the responders to this
237 peptide did not carry the HLA-A*0201 allele (Table 1), indicating this peptide may contain a
238 different SARS-CoV-2 epitope presented by a different HLA molecule. Whereas these NP
239 epitopes are targeted by CD8⁺ T cells, we also detected a CD4⁺ T cell response targeting
240 SARS spike epitope S-E-19 which spans between the overlapping peptides of S-203 and S-
241 204. This peptide is known to be presented by HLA-DRB1*0401 in SARS infection.

242

243 The optimal peptide sequences and their HLA restrictions were confirmed by generating
244 short term T cell lines and clones, which were tested in ELISpot assays by co-culturing with
245 peptide loaded HLA matched and unmatched immortalized B lymphoblastoid cell lines
246 (BCLs) as previously described²¹. In total 6 CD8⁺ T cell epitopes restricted by HLA-A*0101,
247 A*0301, A*1101, B*0702, B*4001 and B*2705 were confirmed (Table 2). HLA-peptide
248 pentamers were synthesized comprising 5 peptides bound to the appropriate HLA class I
249 molecules. T cell staining was verified by flowcytometry (Fig. 6) and their phenotypes were
250 determined (Fig. 7). A pentameric HLA-A*0201 with the spike epitope reported
251 by Shomuradova et al²², was synthesised. Only one out of six HLA-A*0201-positive donors
252 showed detectable staining, but at a very low frequency. The majority of pentamer stained
253 SARS-Cov-2 specific CD8⁺ T cells exhibited central memory (20.7%±8.4%) or effector
254 memory phenotypes (50.3%±13.3%) (Fig. 7) and early (CD27+CD28+, 43.8%±20.9%) or
255 intermediate (CD27+CD28-, 49.3%±21.0%) differentiation phenotypes. Overall, multiple
256 peptides containing epitopes and immunodominant regions were defined from 42 subjects
257 who had recovered from COVID-19. The regions were located in the majority of SARS-CoV-
258 2 structural and non-structural proteins including spike, M, NP and ORF proteins, with CD8⁺
259 T cells exhibiting central memory and effector memory phenotype.

260

261

262 **Discussion**

263 This study demonstrates the presence of robust memory T cell responses specific for SARS-
264 CoV-2 in the blood of donors who have recovered from Covid-19. The broader and stronger
265 SARS-CoV-2 specific T cell responses in patients who had severe disease may be the result
266 of higher viral loads and may reflect a poorly functioning early T cell response that failed to
267 control the virus, in addition to other factors such as direct virus-induced pathology
268 associated with larger viral inoculums or poorer innate immunity. Alternatively, it is possible
269 that the T cell response was itself harmful and contributes to disease severity. Consistent
270 with recent reports from Grifoni *et al* and *Sekine et al*^{17, 23}, a particularly high frequency of
271 spike protein-specific CD4⁺ T cell responses was observed in patients who had recovered
272 from COVID-19. This is very similar to influenza virus infection, where viral surface
273 hemagglutinin (HA) elicited mostly CD4⁺ T cell responses, whereas the majority of CD8⁺ T
274 cell responses were specific to viral internal proteins²⁴. Understanding the roles of different
275 subsets of T cells in protection or pathogenesis is a crucial question for COVID-19. The
276 timing and strength of the first T cell responses, could be critical in determining this balance
277 at an early stage of the infection.

278

279 Among the 41 peptides containing T cell epitopes that were identified in this study, six
280 immunodominant epitope groups (peptides) were frequently targeted by T cells in many
281 donors, including three in spike (29%, 24%, 18%), two in membrane protein (32%, 47%) and
282 one in nucleoprotein (35%). The immunodominant peptide regions identified here may
283 include multiple epitopes restricted by different HLAs (both class I and II, such as S-34 and
284 NP16) with immunodominance preferences imposed by the antigen processing pathways.
285 Whether or not these dominant responses play a role in immune protection merits further
286 investigation in larger prospective cohorts.

287

288 A higher proportion of CD8⁺ T cell responses was observed in mild disease, suggesting the
289 potential protective role of CD8⁺ T cell responses in mild disease or pathogenic role of
290 CD4⁺ T cell responses in severe disease which merits further investigation.

291

292 The majority of pentamer-binding CD8⁺ T cells were effector memory and central memory
293 with early and intermediate differentiation phenotypes, with functional potential on antigen
294 re-exposure. Because the number of donors studied was limited and they would likely show
295 diverse TCRs, peptide/MHC affinities and antigen sensitivities for the different epitopes, it
296 was not possible to make a detailed analysis comparing mild and severe cases. However,
297 the groundwork, including epitope identification, was laid for future studies that can address
298 this important issue.

299

300 Multiple strong dominant T cell responses were seen in study subjects, specific for the M
301 and NP proteins. Dominant epitope regions within NP (NP-16) were detected in 35% of
302 study subjects and M (M-20 and M24) were detected in 32% and 47%. In addition, a higher
303 proportion of multi-cytokine producing M/NP-specific compared to spike-specific CD8⁺ T
304 cells was observed in subjects who had recovered from mild disease. A similar trend was
305 also observed in severe cases, although was not significant possibly due to fewer cases.
306 These data strongly suggest NP and M have potential for inclusion within future vaccines so
307 as to stimulate strong effector T cell responses. Furthermore, T cells responding to these
308 antigens may be more cross-reactive¹⁸.

309

310 IFN- γ producing SARS-CoV-2 specific T cell responses were not observed in 16 healthy
311 unexposed volunteers differing from recently published reports by Grifoni *et al*¹⁷ and Braun
312 *et al*²⁵, both of which used peptide stimulated induction of activation markers (AIM) assays.
313 On the other hand, in a recent immunogenicity study of a recombinant adenovirus type-5
314 (Ad5) vectored COVID-19 vaccine human phase I trial in 108 volunteers without pre-
315 exposure to COVID-19, spike-specific T cell responses, measured IFN- γ ELISpot and
316 intracellular cytokine stimulation (ICS) assays, were not found before vaccination⁶. These
317 differences could result from differences in sensitivity of the detection methods, AIM versus.

318 IFN- γ production assays. IFN- γ -ELISpot and ICS are well-established methods for
319 evaluating antigen specific T cells, used in different virus infections and vaccine studies, that
320 have direct functional relevance^{24, 26, 27, 28}. The AIM assay is more recently developed assay,
321 capable of detecting early responding T cells, that is independent of cytokine production.
322 Both methods are valid but differ in sensitivity and possible functional relevance. However, it
323 is also possible that different circulating coronaviruses have been previously present in the
324 different geographical populations studied, giving cross reactive responses in some regions
325 but not others, as suggested by Le Bert et al¹⁸. These T-cell cross reacting viruses could
326 include not only SARS-CoV-1 and human “common cold” coronaviruses, but also other
327 unknown coronaviruses of animal origin. It is also known that very sensitive assays can
328 detect not only pre-existing naïve antigen specific CD4⁺ T cells but also memory CD4⁺ T
329 cells. The latter are potentially primed by other microbes that cross react with viruses as
330 diverse as CMV, HIV-1 and Ebolavirus in most unexposed humans^{29, 30}. Therefore, similar
331 findings with SARS-CoV-2 peptides do not necessarily mean the T cells were primed by
332 previous infecting coronaviruses. Indeed, the implications of pre-existing cross-reactivity to
333 seasonal coronavirus and other viruses for COVID-19 immunity merits further detailed
334 investigation as nicely highlighted by *Sette A and Crotty S*³¹.

335

336 This study focuses on T cell responses in PBMC. There remains a lack of understanding of
337 memory T cells (T_{rm}) at the site of infection, which is likely providing the most potent
338 protection as observed in influenza virus infection³². It is possible that the hierarchy of
339 immunodominant circulating blood memory T cell pools may not exactly reflect that of T_{rm}
340 in the lung^{17, 33, 34}. Therefore, understanding the features of tissue resident memory T cells
341 and their association with disease severity will be critical and also merits further investigation.

342

343 Taken together, this study has demonstrated strong and broad SARS-CoV-2-specific CD4⁺
344 and CD8⁺ T cell responses in the majority of humans who had recovered from COVID-19.

345 The immunodominant epitope regions and peptides containing T cell epitopes identified in
346 this study will provide critical tools to study the contribution of SARS-CoV-19 specific T cells
347 in protection and immune pathology. Identification of non-spike dominant CD8⁺ T cell
348 epitopes, suggests the potential importance of including of non-spike protein such as NP, M
349 and ORFs into future vaccine designs.

350

351

352 **Acknowledgments**

353 We are grateful to all of the participants for donating their samples and data for these
354 analyses, and the research teams involved in the consenting, recruitment and sampling of
355 these participants.

356

357 This work is supported by UK Medical Research Council (T.D, G.O, Y.P, M.S, G.N, Y-LC);
358 Chinese Academy of Medical Sciences (CAMS) Innovation Fund for Medical
359 Sciences (CIFMS), China (grant number: 2018-I2M-2-002)(T.D, Y.P, X.Y, G.L, D.D, D.I.S,
360 J.M, G.R.S); National Institutes of Health, National Key R&D Program of China
361 (2020YFE0202400) (T.D, Y.Z, R.J); China Scholarship Council (Z.Y, G.L, C.L);
362 The National Institute for Health Research [award CO-CIN-01](M.G.S); the Medical
363 Research Council [grant MC_PC_19059](M.G.S); Wellcome Trust and Department for
364 International Development [215091/Z/18/Z](M.G.S); the Bill and Melinda Gates
365 Foundation [OPP1209135](MGS). The study is also funded by the NIHR Oxford
366 Biomedical Research Centre (L.P.H, G.O, P.K, E.B, G.R.S), Senior Investigator Award (G.O)
367 and Clinical Research Network (G.O) and Schmidt Futures(G.R.S); Health Protection
368 Research Unit in Respiratory Infections NIHR200927 /WHRG_P82523(P.O) ; NIHR Senior
369 Investigator Award, NIHR201385 / WHRR P84026(P.O); Imperial College Biomedical
370 Research Centre , IS-BRC-1215-20013(P.O); National Institute of Allergy and Infectious
371 Disease (Consortium for HIV/AIDS Vaccine Development UM1 AI 144371 (P.B and A.M)
372 and R01 AI 118549 (PB). L.T, P.K and P.S are supported by the National Institute for Health

373 Research Health Protection Research Unit (NIHR HPRU) in Emerging and Zoonotic
374 Infections [NIHR200907] at University of Liverpool in partnership with Public Health England
375 (PHE), in collaboration with Liverpool School of Tropical Medicine and the University
376 of Oxford. L.T is based at the University of Liverpool. P.K and P.S are based at
377 the University of Oxford. L.T , Td.S and A.A.A are supported by the Wellcome Trust
378 [grant numbers 205228/Z/16/Z (L.T) , 110058/Z/15/Z (Td.S) and 206377 (A.A.A). G.R.S is
379 supported as a Wellcome Trust Senior Investigator (grant 095541/A/11/Z). PB and A.M are
380 Jenner Institute Investigators.

381

382 This work uses data provided by patients and collected by the NHS as part of their care and
383 support #DataSavesLives.

384

385 The views expressed are those of the authors and not necessarily those of the Department
386 of Health and Social Care, DID, NIHR, MRC, Wellcome Trust or PHE

387

388 **Author Contribution:** T.D and G.O conceptualized the project, T.D, Y.P designed and
389 supervised T cell experiments, J.M and G.R.S designed antibody experiments; Y.P, G.L, X.Y,
390 Z.Y,D.D performed all T cell experiments; W.D, J.M, P.S, C.L, C.L.C, J.S.C, Y.Z, D.I.S, G.P,
391 J.G, A.A.A,O.B,D.H, B.W and D.S.K performed Spike, RBD and NP EPTs experiments; T.R
392 performed HLA typing; J.K, A.M, T.L, R.L, P.K , L.T, T.D.S, M.G.S, C.P.C, S.C.M, J. K.B,
393 P.O established clinical cohorts and collected clinical sample and data; K.S, P.T, P.Z, C.D,
394 J.R, P.S, P.S, D.W, U.R, Y.L.C, W.P, P.B, J.F, M.A.A, S.D, M.S, E.B, G.K, P.G, Y.Z, R.J,
395 L.P.H provided critical reagents and technical assistance; Y.P, G.L, X.Y, Z.Y,D.D, W.D, P.Z,
396 J.M analysed data, T.D wrote the original draft. G.O, J.K, A.M, P.B, P.K, P.O, L.T., G.R.S,
397 R.C, P.S, M.G.S, B.M.K,C.P.C reviewed and edited the manuscript and Figures.

398 **Oxford Immunology Network Covid-19 response: T cell Immunity Team – additional**
399 **contributors**

400 Barbara Kronsteiner, Anthony Brown , Emily Adland , Patpong Rongkard , Anna Csala ,
401 Helen Brown , Nicola Robinson, Panagiota Zacharopoulou , Vinicius Adriano , Prabhjeet
402 Phalora , Oliver Sampson , Carl-Philipp Hackstein , Nicholas Lim , Matt Edmans , Senthil
403 Chinnakannan , Rachael Brown , Ali Amini , Mathew Jones , Mohammad Ali , Timothy
404 Donnison , Matt Pace , Ane Ogbe , Donal Skelly , Elizabeth Stafford , Helen Fletcher , Lian
405 Lee , Prathiba Kurupati , Rachel Etherington , Nicholas Provine, Hashem Koohy, Chloe
406 Hyun-Jung Lee.

407

408 **ISARIC 4C Investigators**

409 Consortium Lead Investigator: J Kenneth Baillie, Chief Investigator Malcolm G Semple
410 Co-Lead Investigator Peter JM Openshaw. ISARIC Clinical Coordinator Gail Carson.
411 Co-Investigators: Beatrice Alex, Benjamin Bach, Wendy S Barclay, Debby Bogaert, Meera
412 Chand, Graham S Cooke, Annemarie B Docherty, Jake Dunning, Ana da Silva Filipe, Tom
413 Fletcher, Christopher A Green, Ewen M Harrison, Julian A Hiscox, Antonia Ying Wai Ho,
414 Peter W Horby, Samreen Ijaz, Saye Khoo, Paul Klenerman, Andrew Law, Wei Shen Lim,
415 Alexander, J Mentzer, Laura Merson, Alison M Meynert, Mahdad Noursadeghi, Shona C
416 Moore, Massimo Palmarini, William A Paxton, Georgios Pollakis, Nicholas Price, Andrew
417 Rambaut, David L Robertson, Clark D Russell, Vanessa Sancho-Shimizu, Janet T Scott,
418 Louise Sigfrid, Tom Solomon, Shiranee Sriskandan, David Stuart, Charlotte Summers,
419 Richard S Tedder, Emma C Thomson, Ryan S Thwaites, Lance CW Turtle, Maria Zambon.
420 Project Managers Hayley Hardwick, Chloe Donohue, Jane Ewins, Wilna Oosthuyzen, Fiona
421 Griffiths. Data Analysts: Lisa Norman, Riinu Pius, Tom M Drake, Cameron J Fairfield,
422 Stephen Knight, Kenneth A Mclean, Derek Murphy, Catherine A Shaw. Data and Information
423 System Manager: Jo Dalton, Michelle Girvan, Egle Saviciute, Stephanie Roberts Janet
424 Harrison, Laura Marsh, Marie Connor. Data integration and presentation: Gary Leeming,
425 Andrew Law, Ross Hendry. Material Management: William Greenhalf, Victoria Shaw, Sarah

426 McDonald. Outbreak Laboratory Volunteers: Katie A. Ahmed, Jane A Armstrong, Milton
427 Ashworth, Innocent G Asiiimwe, Siddharth Bakshi, Samantha L Barlow, Laura Booth,
428 Benjamin Brennan, Katie Bullock, Benjamin WA Catterall, Jordan J Clark, Emily A Clarke,
429 Sarah Cole, Louise Cooper, Helen Cox, Christopher Davis, Oslem Dincarslan, Chris Dunn,
430 Philip Dyer, Angela Elliott, Anthony Evans, Lewis WS Fisher, Terry Foster, Isabel Garcia-
431 Dorival, Willliam Greenhalf, Philip Gunning, Catherine Hartley, Antonia Ho, Rebecca L
432 Jensen, Christopher B Jones, Trevor R Jones, Shadia Khandaker, Katharine King, Robyn T.
433 Kiy, Chrysa Koukorava, Annette Lake, Suzannah Lant, Diane Latawicz, L Lavelle-Langham,
434 Daniella Lefteri, Lauren Lett, Lucia A Livoti, Maria Mancini, Sarah McDonald, Laurence
435 McEvoy, John McLauchlan, Soeren Metelmann, Nahida S Miah, Joanna Middleton, Joyce
436 Mitchell, Shona C Moore, Ellen G Murphy, Rebekah Penrice-Randal, Jack Pilgrim, Tessa
437 Prince, Will Reynolds, P. Matthew Ridley, Debby Sales, Victoria E Shaw, Rebecca K Shears,
438 Benjamin Small, Krishanthi S Subramaniam, Agnieska Szemiel, Aislynn Taggart, Jolanta
439 Tanianis, Jordan Thomas, Erwan Trochu, Libby van Tonder, Eve Wilcock, J. Eunice Zhang.
440 Local Principal Investigators: Kayode Adeniji, Daniel Agranoff, Ken Agwuh, Dhiraj Ail, Ana
441 Alegria, Brian Angus, Abdul Ashish, Dougal Atkinson, Shahedal Bari, Gavin Barlow, Stella
442 Barnass, Nicholas Barrett, Christopher Bassford, David Baxter, Michael Beadsworth, Jolanta
443 Bernatoniene, John Berridge , Nicola Best , Pieter Bothma, David Brealey, Robin Brittain-
444 Long, Naomi Bulteel, Tom Burden , Andrew Burtenshaw, Vikki Caruth, David Chadwick,
445 Duncan Chambler, Nigel Chee, Jenny Child, Srikanth Chukkambotla, Tom Clark, Paul Collini,
446 Catherine Cosgrove, Jason Cupitt, Maria-Teresa Cutino-Moguel, Paul Dark, Chris Dawson,
447 Samir Dervisevic, Phil Donnison, Sam Douthwaite, Ingrid DuRand, Ahilanadan Dushianthan,
448 Tristan Dyer, Cariad Evans , Chi Eziefula, Chrisopher Fegan, Adam Finn, Duncan Fullerton,
449 Sanjeev Garg, Sanjeev Garg, Atul Garg, Jo Godden, Arthur Goldsmith, Clive Graham,
450 Elaine Hardy, Stuart Hartshorn, Daniel Harvey, Peter Havalda, Daniel B Hawcutt, Maria
451 Hobrok, Luke Hodgson, Anita Holme, Anil Hormis, Michael Jacobs, Susan Jain, Paul
452 Jennings, Agilan Kaliappan, Vidya Kasipandian, Stephen Kegg, Michael Kelsey, Jason
453 Kendall, Caroline Kerrison, Ian Kerslake, Oliver Koch, Gouri Koduri, George Koshy ,

454 Shondipon Laha, Susan Larkin, Tamas Leiner, Patrick Lillie, James Limb, Vanessa Linnett,
455 Jeff Little, Michael MacMahon, Emily MacNaughton, Ravish Mankregod, Huw Masson ,
456 Elijah Matovu, Katherine McCullough, Ruth McEwen , Manjula Meda, Gary Mills , Jane
457 Minton, Mariyam Mirfenderesky, Kavya Mohandas, Quen Mok, James Moon, Elinoor Moore,
458 Patrick Morgan, Craig Morris, Katherine Mortimore, Samuel Moses, Mbiye Mpenge,
459 Rohinton Mulla, Michael Murphy, Megan Nagel, Thapas Nagarajan, Mark Nelson, Igor
460 Otahal, Mark Pais, Selva Panchatsharam, Hassan Paraiso, Brij Patel, Justin Pepperell, Mark
461 Peters, Mandeep Phull , Stefania Pintus, Jagtur Singh Pooni, Frank Post, David Price,
462 Rachel Prout, Nikolas Rae, Henrik Reschreiter, Tim Reynolds, Neil Richardson, Mark
463 Roberts, Devender Roberts, Alistair Rose, Guy Rousseau, Brendan Ryan, Taranprit Saluja,
464 Aarti Shah, Prad Shanmuga, Anil Sharma, Anna Shawcross, Jeremy Sizer, Richard Smith,
465 Catherine Snelson, Nick Spittle, Nikki Staines , Tom Stambach, Richard Stewart, Pradeep
466 Subudhi, Tamas Szakmany, Kate Tatham, Jo Thomas, Chris Thompson, Robert Thompson,
467 Ascanio Tridente, Darell Tupper - Carey, Mary Twagira, Andrew Ustianowski, Nick Vallotton,
468 Lisa Vincent-Smith, Shico Visuvanathan , Alan Vuylsteke, Sam Waddy, Rachel Wake,
469 Andrew Walden, Ingeborg Welters, Tony Whitehouse, Paul Whittaker, Ashley Whittington,
470 Meme Wijesinghe, Martin Williams, Lawrence Wilson, Sarah Wilson, Stephen Winchester,
471 Martin Wiselka, Adam Wolverson, Daniel G Wooton, Andrew Workman, Bryan Yates, Peter
472 Young.

473

474 All authors declare no competing interest

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476

477

478 **References**

479

- 480 1. Fehr A.R., P.S. Coronaviruses: An Overview of Their Replication and Pathogenesis.
481 *In: Maier H., Bickerton E., Britton P. (eds) Coronaviruses. Methods in Molecular*
482 *Biology vol 1282.*
- 483
- 484 2. Perlman, S. & Netland, J. Coronaviruses post-SARS: update on replication and
485 pathogenesis. *Nat Rev Microbiol* **7**, 439-450 (2009).
- 486
- 487 3. Xu, Z. *et al.* Pathological findings of COVID-19 associated with acute respiratory
488 distress syndrome. *Lancet Respir Med* **8**, 420-422 (2020).
- 489
- 490 4. Guan, W.J. *et al.* Clinical Characteristics of Coronavirus Disease 2019 in China. *N*
491 *Engl J Med* (2020).
- 492
- 493 5. Yu, J. *et al.* DNA vaccine protection against SARS-CoV-2 in rhesus macaques.
494 *Science* (2020).
- 495
- 496 6. Zhu, F.C. *et al.* Safety, tolerability, and immunogenicity of a recombinant adenovirus
497 type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised,
498 first-in-human trial. *Lancet* **395**, 1845-1854 (2020).
- 499
- 500 7. van Doremalen, N. *et al.* ChAdOx1 nCoV-19 vaccination prevents SARS-CoV-2
501 pneumonia in rhesus macaques. *bioRxiv*, 2020.2005.2013.093195 (2020).
- 502
- 503 8. Folegatti, P.M. *et al.* Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine
504 against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised
505 controlled trial. *Lancet* (2020).
- 506
- 507 9. St John, A.L. & Rathore, A.P.S. Adaptive immune responses to primary and
508 secondary dengue virus infections. *Nat Rev Immunol* **19**, 218-230 (2019).
- 509
- 510 10. Huang, C. *et al.* Clinical features of patients infected with 2019 novel coronavirus in
511 Wuhan, China. *Lancet* **395**, 497-506 (2020).
- 512
- 513 11. Liao, M. *et al.* Single-cell landscape of bronchoalveolar immune cells in patients with
514 COVID-19. *Nat Med* (2020).
- 515
- 516 12. chen, y. *et al.* The Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-
517 CoV-2) Directly Decimates Human Spleens and Lymph Nodes. *medRxiv*,
518 2020.2003.2027.20045427 (2020).
- 519
- 520 13. Diao, B. *et al.* Reduction and Functional Exhaustion of T Cells in Patients With
521 Coronavirus Disease 2019 (COVID-19). *Front Immunol* **11**, 827 (2020).
- 522
- 523 14. Pereira, B.I. *et al.* Sestrins induce natural killer function in senescent-like CD8(+) T
524 cells. *Nat Immunol* **21**, 684-694 (2020).
- 525
- 526 15. Ni, L. *et al.* Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in
527 COVID-19 Convalescent Individuals. *Immunity* (2020).
- 528

- 529 16. Hayward, A.C. *et al.* Natural T Cell-mediated Protection against Seasonal and
530 Pandemic Influenza. Results of the Flu Watch Cohort Study. *Am J Respir Crit Care*
531 *Med* **191**, 1422-1431 (2015).
532
- 533 17. Grifoni, A. *et al.* Targets of T Cell Responses to SARS-CoV-2 Coronavirus in
534 Humans with COVID-19 Disease and Unexposed Individuals. *Cell* **181**, 1489-1501
535 e1415 (2020).
536
- 537 18. Le Bert, N. *et al.* SARS-CoV-2-specific T cell immunity in cases of COVID-19 and
538 SARS, and uninfected controls. *Nature* (2020).
539
- 540 19. Wilkinson, T.M. *et al.* Preexisting influenza-specific CD4+ T cells correlate with
541 disease protection against influenza challenge in humans. *Nat Med* **18**, 274-280
542 (2012).
543
- 544 20. Ahmed, S.F., Quadeer, A.A. & McKay, M.R. Preliminary Identification of Potential
545 Vaccine Targets for the COVID-19 Coronavirus (SARS-CoV-2) Based on SARS-CoV
546 Immunological Studies. *Viruses* **12** (2020).
547
- 548 21. Ogg, G.S. *et al.* Four novel cytotoxic T-lymphocyte epitopes in the highly conserved
549 major homology region of HIV-1 Gag, restricted through B*4402, B*1801, A*2601,
550 B*70 (B*1509). *AIDS* **12**, 1561-1563 (1998).
551
- 552 22. Shomuradova, A.S. *et al.* SARS-CoV-2 epitopes are recognized by a public and
553 diverse repertoire of human T-cell receptors. *medRxiv*, 2020.2005.2020.20107813
554 (2020).
555
- 556 23. Sekine, T. *et al.* Robust T cell immunity in convalescent individuals with
557 asymptomatic or mild COVID-19. *bioRxiv*, 2020.2006.2029.174888 (2020).
558
- 559 24. Lee, L.Y. *et al.* Memory T cells established by seasonal human influenza A infection
560 cross-react with avian influenza A (H5N1) in healthy individuals. *J Clin Invest* **118**,
561 3478-3490 (2008).
562
- 563 25. Braun, J. *et al.* Presence of SARS-CoV-2 reactive T cells in COVID-19 patients and
564 healthy donors. *medRxiv*, 2020.2004.2017.20061440 (2020).
565
- 566 26. Li, C.K. *et al.* T cell responses to whole SARS coronavirus in humans. *J Immunol*
567 **181**, 5490-5500 (2008).
568
- 569 27. Powell, T.J. *et al.* Identification of H5N1-specific T-cell responses in a high-risk
570 cohort in vietnam indicates the existence of potential asymptomatic infections. *J*
571 *Infect Dis* **205**, 20-27 (2012).
572
- 573 28. Dong, T. *et al.* Extensive HLA-driven viral diversity following a narrow-source HIV-1
574 outbreak in rural China. *Blood* **118**, 98-106 (2011).
575
- 576 29. Su, L.F. & Davis, M.M. Antiviral memory phenotype T cells in unexposed adults.
577 *Immunol Rev* **255**, 95-109 (2013).
578
- 579 30. Champion, S.L. *et al.* Proteome-wide analysis of HIV-specific naive and memory
580 CD4(+) T cells in unexposed blood donors. *J Exp Med* **211**, 1273-1280 (2014).
581
- 582 31. Sette, A. & Crotty, S. Pre-existing immunity to SARS-CoV-2: the knowns and
583 unknowns. *Nat Rev Immunol* (2020).

- 584
585 32. Pizzolla, A. *et al.* Resident memory CD8(+) T cells in the upper respiratory tract
586 prevent pulmonary influenza virus infection. *Sci Immunol* **2** (2017).
587
588 33. Turner, D.L. *et al.* Lung niches for the generation and maintenance of tissue-resident
589 memory T cells. *Mucosal Immunol* **7**, 501-510 (2014).
590
591 34. Yoshizawa, A. *et al.* TCR-pMHC encounter differentially regulates transcriptomes of
592 tissue-resident CD8 T cells. *Eur J Immunol* **48**, 128-150 (2018).
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606 **Table 1 Peptides containing T cell epitopes**

	Peptide	Position	Amino Acid Sequence	CD4/CD8 Response	No of subjects responded
Spike	S-34	166-180	CTFEYVSQPFLMDLE	4/8	<u>10</u>
(n=18)	S-39	191-205	EFVFKNIDGYFKIYS	na	1
	S-42	206-230	KHTPINLVRDL PQGF	na	1
	S-43	211-225	NLVRDLPQGF SALEP	na	1
	S-71	351-365	YAWNKRKISNCVADY	4	1
	S-77	381-395	GVSPTKLNDLCFTNV	4	1
	S-90	446-460	GGNYN YLRLFRKSN	na	1
	S-91	451-465	YLRLFRKSN LKPFE	na	1
	S-103	506-520	VLSFELLHAPATVC	4	1
	S-106	526-540	GPKKSTNLVKNKCVN	8	1
	S-145	721-735	SVTTEILPVSMTKTS	na	1
	S-150	746-760	STEC SNLLQYGSFC	na	1
	S-151	751-765	NLLQYGSFCTQLNR	<u>4</u>	<u>8</u>
	S-161	801-815	NFSQILPDPSPSKR	<u>4</u>	2
	S-174	866-880	TDEMQYTSALLAG	<u>4</u>	<u>6</u>
	S-235	1171-1185	GINASVWNIQKEIDR	na	1
	S-240	1196-1210	LIDLQELGKYEQYI	na	1
	S-242	1206-1220	YEQYIKWPWYIWLGF	na	1
NP (n=10)	NP-1	1-17	MSDNGPQNQRNAPRITF	8	3
	NP-2	8-25	NQRNAPRITF GGPSDSTG	8	3
	NP-12	82-95	DQIGYYRRATRRIR	na	1
	NP-15	101-113	MKDLSRWYFYLL	na	1
	NP-16	104-121	LSPRWYFYLLGTGPEAGL	4/8	<u>12</u>
	NP-46	313-330	AFFGMSRIGMEVTPSGTW	na	1
	NP-47	321-338	GMEVTPSG TWLTYTGAIK	na	1
	NP-48	329-346	TWLTYTGAIK LDDKDPNF	4	2
	NP-50	344-361	PNFKDQVILLNKHIDAYK	4	1
	NP-51	352-369	LLNKHIDAYK TFPTEPK	8	3
M (n=6)	M19	133-150	LLESELVIGAVILRGHLR	na	3
	M-20	141-158	GAVILRGHLLRIAGHHLGR	4	<u>11</u>
	M-21	149-166	LRIAGHHLGR CDIKDLPK	na	3
	M-23	165-181	PKEITVATSRTLSYYKL	na	3
	M-24	172-188	TSRTLSYYKL GASQRVA	4	<u>16</u>
	M-28	201-218	IGNYKLNLDHSSSSDNIA	na	1
ORFs (n=7)	ORF-3a-20	145-160	YFLCWHTNCYDYCIPY	na	1
	ORF-3a-27	198-215	KDCVVLHSYFTSDYYQLY	na	3
	ORF-3a-28	206-225	YFTSDYYQLY STQLSTDTGV	8	4
	ORF-3a-30	224-243	GVEHVTFFIYNKIVDEPEEH	na	1
	ORF-7a-2	9-25	LITLATCELYHYQECVR	na	3
	ORF-7a-7	46-63	FHPLADNKFALTCFSTQF	na	1
	ORF-7a-10	69-86	DGVKHVYQLRARSVSPKL	4	1

607

608 Red highlights the overlaps of two adjacent peptides recognised by same subjects; Bold
609 indicates multiple donor responders; Peptides with underline are the 6 immunodominant
610 peptides. na: not available

611 **Table 2: List of identified optimal CD8 epitopes**

Protein	Position	Epitope sequence	HLA Restriction
NP	9-17	QRNAPRITF	B*2705
	105-113	SPRWYFYYL	B*0702
	322-331	MEVTPSGTWL	B*4001
	362-370	KTFPPTEPK	A*0301
	362-370	KTFPPTEPK	A*1101
ORF3a	207-215	FTSDYYQLY	A*0101

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613 Location , sequence and HLA restriction of six identified SARS-CoV2 CD8 optimal epitopes.

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634 **Figure Legends**

635 **Fig. 1: Memory T cell responses specific to SARS-CoV-2 virus proteins in 42**
636 **convalescent SARS-CoV-2-infected patients.** 28 individuals had mild symptoms while 14
637 showed severe symptoms. PBMC were isolated and IFN- γ production was detected by
638 ELISpot after incubation with SARS-CoV-2 peptides. a) Magnitude of IFN- γ T cell responses
639 from each individual. Each bar shows the total T cell responses of each individual specific to
640 all the SARS-CoV-2 protein peptides tested. Each colored segment represents the source
641 protein corresponding to peptide pools eliciting IFN- γ T cell responses. b) Breadth of T cell
642 responses from each individual. The breadth of T cell responses was calculated by the
643 number of peptide pools in the first-dimension (total 29) cells responded to SFU spot forming
644 units. Experiments were repeated in 35 subjects where sample availability permitted.

645

646 **Fig. 2: Comparison of magnitude and breadth of T cell response specific to each viral**
647 **protein between convalescent patients with mild symptoms and severe symptoms.**

648 PBMCs were isolated and IFN- γ production was detected by ELISpot after incubation with
649 SARS-CoV-2 peptides. a) and b) illustrate the magnitude and the breadth of T cell response
650 against each viral protein between the groups with mild symptoms (n=28) and with severe
651 symptoms (n=14), respectively. Overall, magnitude/breadth: p=0.002/p=0.002; Spike,
652 magnitude/breadth: p=0.021/0p=0.016; M, magnitude/breadth: p=0.0003/p=0.033; ORF3a,
653 magnitude/breadth: p<0.0001/p=0.001; ORF8, magnitude/breadth: p=0.011/p=0.014). Data
654 are presented as median with interquartile range. Mann-Whitney test was used for the
655 analysis and two-tailed p value was calculated. *P<0.05, **P<0.01, ***P<0.001, ****P<0.001.

656 SFU spot forming units;

657

658 **Fig. 3: Correlation of T cell responses against SARS-CoV-2 with Spike, RBD and NP-**
659 **specific antibody responses.** a) EPTs-spike b) EPTs-RBD and c) EPTs-NP in association
660 with overall T cell responses. Red dots represent the patients with severe symptoms

661 whereas the mild cases are shown as black dots. n=42. Spearman's rank correlation
662 coefficient was used for the correlation analysis. d) Comparison of EPT-spike ($p < 0.0001$),
663 EPT-RBD ($p < 0.0001$) and EPT-NP ($p = 0.0004$) with mild symptoms (n=28) and severe
664 symptoms (n=14). Data are presented as median with interquartile range and Mann-Whitney
665 test was used for comparison. Two-tailed p value was calculated. *** $P < 0.001$; **** $P < 0.0001$
666 EPT: Endpoint titer

667

668 **Fig. 4: Distribution of SARS-CoV-2-specific CD4⁺ and CD8⁺ memory T cell responses**

669 Cytokine producing T cells were detected by ICS after incubation with SARS-CoV-2 peptides.
670 a) and b) Flow cytometric plots represent CD4⁺T cell and CD8⁺ T cells expressing IFN- γ (x-
671 axis), TNF (y-axis) and/or IL-2 (y-axis) upon stimulation with respective SARS-CoV-2 peptide
672 pools in examples of mild and severe cases. c) Comparison of relative proportion of SARS-
673 CoV-2 peptide pool-reactive CD8⁺ T cells between mild (Spike, n=11; M/NP, n=14; ORF/Env,
674 n=5; Overall: n=14) and severe cases (Spike, n=7; M/NP, n=7; ORF/Env, n=4; Overall, n=8).
675 Spike, $p = 0.0268$; M/NP, $p = 0.02$; Overall, $p = 0.0159$. The SARS-CoV-2 peptide pool-reactive
676 CD4⁺ or CD8⁺ T cells were identified with at least one of the three cytokines detected: IFN- γ ,
677 TNF and IL-2. Data shown are as median with interquartile range. Mann-Whitney test was
678 used for the analysis. Two-tailed p value was calculated. * $P < 0.05$

679

680 **Fig. 5: Cytokine profile of SARS-Cov-2-specific T cells.** Cytokine production of SARS-
681 Cov-2-specific T cells was assessed by intracellular cytokine staining after incubation with
682 SARS-CoV-2 peptides. a) Pie charts represent the relative proportions of CD4⁺ or CD8⁺ T
683 cells producing, and the relative proportion of T cells producing one, two and three cytokines
684 IFN- γ , TNF and IL-2. Different colored segments represented different pattern of cytokine
685 production. b) Comparison of the frequency of multifunctional CD8⁺ T cells targeting Spike
686 and M/NP. The open circles and squares represent T cell responses in mild cases and
687 severe cases, respectively. Mild, $p = 0.0037$; Severe, $p = 0.3823$; Overall, $p = 0.0231$. c) The
688 relative frequencies of CD4⁺ and CD8⁺ T cells expressing CD107a after antigen-stimulation.

689 Data shown are from 14 subjects with mild symptoms and 8 with severe symptoms. Mann-
690 Whitney test was used for the analysis. Two-tailed p value was calculated. * P<0.05,
691 **P<0.01

692

693 **Fig. 6: Defined SARS-CoV-2-specific CD8 epitopes.** Examples of peptide-MHC Class I
694 pentamers staining ex-vivo with PBMCs (HLA-B0702, B4001, A1101, A0101 and A0201) or
695 with cultured cell lines (A0301), 11 donors were tested with positive Pentamer staining.

696

697 **Fig. 7: Memory phenotype and differentiation status of SARS-CoV-2-specific CD8+ T**
698 **cells.** PBMC were isolated and stained with peptide-MHC class I Pentameric complexes and
699 markers of T cell memory and differentiation. a) Representative FACS plots of gating for
700 different cell subsets b) and c) Expression of memory markers (CCR7 and CD45RA) and
701 differentiation markers (CD27 and CD28) on CD8⁺ Pentamer+ T cells, respectively. n=7
702 donors. Data are presented as mean \pm SEM.

703

704 **Materials and methods**

705

706 **Ethical Statement**

707 Patients were recruited from the John Radcliffe Hospital in Oxford, UK, between March and
708 May 2020 by identification of patients hospitalised during the SARS-COV-2 pandemic and
709 recruited into the Sepsis Immunomics and ISARIC WHO Clinical Characterisation Protocol
710 UK (IRAS 260007 and IRAS126600). Patients were sampled at least 28 days from the start
711 of their symptoms. Unexposed healthy adult donor samples were used from unrelated
712 studies undertaken between 2017-early 2019. Written informed consent was obtained from
713 all patients. Ethical approval was given by the South Central - Oxford C Research Ethics
714 Committee in England (Ref 13/SC/0149), the Scotland A Research Ethics Committee (Ref
715 20/SS/0028), and the WHO Ethics Review Committee (RPC571 and RPC572, 25 April
716 2013).

717

718 **Clinical definitions**

719 All patients were confirmed to have a test positive for SARS-CoV-2 using reverse
720 transcriptase polymerase chain reaction (RT-PCR) from an upper respiratory tract
721 (nose/throat) swab tested in accredited laboratories. The degree of severity was identified as
722 mild, severe or critical infection according to recommendations from the World Health
723 Organisation. Patients were classified as 'mild' if they did not require oxygen (that is, their
724 oxygen saturations were greater than 93% on ambient air) or if their symptoms were
725 managed at home. A large proportion of our mild cases were admitted to hospital for public
726 health reasons during the early phase of the pandemic even though they had no medical
727 reason to be admitted to hospital. Severe infection was defined as COVID-19 confirmed
728 patients with one of the following conditions: respiratory distress with RR>30/min; blood
729 oxygen saturation<93%; arterial oxygen partial pressure (PaO₂) / fraction of inspired O₂
730 (FiO₂) <300mmHg; and critical infection was defined as respiratory
731 failure requiring mechanical ventilation or shock; or other organ failures requiring admission

732 to ICU. Since the Severe classification could potentially include individuals spanning a wide
733 spectrum of disease severity ranging from patients receiving oxygen through a nasal
734 cannula through to non-invasive ventilation we also calculated the SaO₂/FiO₂ ratio at the
735 height of patient illness as a quantitative marker of lung damage. This was calculated by
736 dividing the oxygen saturation (as determined using a bedside pulse oximeter) by the
737 fraction of inspired oxygen (21% for ambient air, 24% for nasal cannulae, 28% for simple
738 face masks and 28, 35, 40 or 60% for Venturi face masks or precise measurements for non-
739 invasive or invasive ventilation settings). Patients not requiring oxygen with oxygen
740 saturations (if measured) greater than 93% on ambient air, or managed at home were
741 classified as mild disease. Viral swab Ct values were not available for all patients. In addition,
742 we have standardised all of our analyses to the days since symptom onset.

743

744 **Synthetic peptides**

745 A total of 423 15- to 18-mer peptides overlapping by 10 amino acid residues and spanning
746 the full proteome of the SARS-CoV-2 except ORF-1 (Supplementary Table 1) were designed
747 using software PeptGen (<http://www.hiv.lanl.gov/content/sequence/PEPTGEN/peptgen.html>)
748 and synthesized (purity >75%; Proimmune).

749 27 previously defined SARS epitopes²⁰ were also synthesised (Supplementary Table
750 2). Pools of Cytomegalovirus (CMV), Epstein-Barr virus (EBV) and influenza virus specific
751 epitope peptides and The human immunodeficiency viruses (HIV) gag were also used as
752 positive and negative controls.

753

754 **2-dimensional peptide matrix system**

755 The overlapping peptides spanning the SARS-CoV-2 were assigned into a 2-dimensional
756 matrix system in which each peptide was represented in 2 different peptide pools. Each
757 peptide pool contains no more than 16 individual peptides. The first dimension of the peptide
758 matrix system was designed so that peptides from different source proteins were separated
759 into different pools. (Supplemental Table 1).

760

761 ***Ex vivo* ELISpot assay**

762 IFN- γ ELISpot assays were performed using either freshly isolated or cryopreserved PBMCs
763 as described previously. No significant difference was observed between responses
764 generated by fresh or cryopreserved PBMCs as described previously^{24, 35}.

765

766 Overlapping peptides were pooled and then added to 200,000 PBMCs per test at the final
767 concentration of 2 μ g/mL for 16–18 h, the positive responses were confirmed by repeat
768 ELISPOT assays. To quantify antigen-specific responses, mean spots of the control wells
769 were subtracted from the positive wells, and the results expressed as spot forming units
770 (SFU)/10⁶ PBMCs. Responses were considered positive if results were at least three times
771 the mean of the negative control wells and >25SFU/10⁶PBMCs. If negative control wells
772 had >30SFU/10⁶ PBMCs or positive control wells (PHA stimulation) were negative, the
773 results were excluded from further analysis.

774

775 **Determination of plasma binding to trimeric spike, RBD and NP by ELISA**

776 MAXISORP immunoplates (442404; NUNC) were coated with 0.125 μ g of StrepMAB-Classic
777 (2-1507-001;iba) , blocked with 2% skimmed milk in PBS for one hour and then incubated
778 with 50 μ L of 5 μ g/mL soluble trimeric Spike 2 μ g/mL or 2% skim milk in PBS. After one hour,
779 50 μ L of serial two-fold dilutions of plasma, from 1:50 to 1:51200 in PBS containing 2%
780 skimmed milk were added followed by ALP-conjugated anti-human IgG (A9544; Sigma) at
781 1:10,000 dilution. The reaction was developed by the addition of PNPP substrate and
782 stopped with NaOH. The absorbance was measured at 405nm. Endpoint titers (EPTs) were
783 defined as reciprocal plasma dilutions that corresponded to two times the average OD
784 values obtained with mock. To determine EPTs to RBD and NP, immunoplates were coated
785 with 0.125 μ g of Tetra-His antibody (34670; QIAGEN) followed by 2 μ g/mL and 5 μ g/mL of
786 soluble RBD and NP, respectively.

787

788 **Intracellular cytokine staining (ICS)**

789 Intracellular cytokine staining was performed as described previously^{36,37}. Briefly, overnight
790 rested PBMCs were stimulated with pooled or individual peptides at a final concentration of
791 10µg/mL for 1 h in the presence of 2µg/mL monoclonal antibodies CD28 and CD49d, and
792 then for an additional 5h with GolgiPlug, GolgiStop and surface stained with PE-anti-CD107a.
793 Dead cells were labelled using LIVE/DEAD™ Fixable Aqua dye from Invitrogen; surface
794 markers including BUV395-anti-CD3, BUV737-anti-CD4, PerCP-Cy5.5-anti-CD8, BV510-
795 anti-CD14 (Biolegend), BV510-anti-CD16 (Biolegend) and BV510-anti-CD19 (Biolegend)
796 were stained. Cells were then washed, fixed with Cytofix/Cytoperm™ and stained with PE-
797 Cy7-anti-IFN γ , APC-anti-TNF α (eBioscience), BV421-anti-IL-2 (Biolegend). Negative
798 controls without peptide-stimulation were run for each sample. All reagents were from BD
799 Bioscience unless otherwise stated. All samples were acquired on BD LSR Fortessa (BD
800 Biosciences) flow cytometer and analyzed using FlowJo™ v.10 software (FlowJo LLC).
801 Peptide pool-reactive CD4⁺ or CD8⁺ T cells with frequency lower than 0.05% of CD4⁺ or
802 CD8⁺ T cells respectively were excluded for analysis. Cytokine responses were background
803 subtracted individually prior to further analysis. To determine the frequency of different
804 response patterns based on all possible combinations, Boolean gates were created using
805 IFN- γ , TNF- α and IL-2. Cytokine responses were background subtracted individually prior to
806 further analysis.

807

808 **Pentamer phenotyping**

809 Cryopreserved PBMCs were thawed as described above. A total of 1×10^6 live PBMCs were
810 labeled with peptide-MHC class I Pentamer-PE (Proimmune, UK) and incubated for 15 min
811 at 37°C. Dead cells were first labelled with LIVE/DEAD™ Fixable Aqua dye (Invitrogen) and
812 then with surface markers CD3-BUV395, CD8-PerCP.Cy5.5, CD14-BV510 (Biolegend UK),
813 CD16-BV510 (Biolegend UK), CD19-BV510 (Biolegend UK), CD28-BV711, CD27-APC-
814 R700, CD45RA-APC-H7 and CCR7-PE-Dazzel 594 (Biolegend UK). All reagents were from

815 BD Bioscience unless otherwise stated. All samples were acquired on BD LSR Fortessa (BD
816 Biosciences) flow cytometer and analyzed using FlowJo™ v.10 software (FlowJo LLC).

817

818 **Generating short-term T cell lines**

819 Short-term SARS-CoV-2-specific T cell lines were established as previously described³⁵.
820 Briefly, 3×10^6 to 5×10^6 PBMCs were pulsed as a pellet for 1 h at 37°C with 10 µM of
821 peptides containing T cell epitope regions and cultured in R10 at 2×10^6 cells per well in a
822 24-well Costar plate. IL-2 was added to a final concentration of 100U/mL on day 3 and
823 cultured for further 10 -14 days.

824

825 **Statistical analysis**

826 Statistical analysis was performed with IBM SPSS Statistics 25 and Fig.s were made with
827 GraphPad Prism 8. Chi-square tests were used to compare ratio difference between two
828 groups. After testing for normality using Kolmogorov-Smirnov test, Independent-samples *t*
829 test or Mann-Whitney U test was employed to compare variables between two groups.
830 Correlations were performed via Spearman's rank correlation coefficient. Statistical
831 significance was set at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$. All the tests were
832 2-tailed.

833

834 **Life Sciences Reporting Summary**

835 Further information on research design is available in the Nature Research Reporting
836 Summary linked to this article.

837 **Data availability**

838 Source data are provided with this paper. The corresponding author can be contacted for
839 further information.

840

841 **Method-Only References:**

842

- 843 35. Peng, Y. *et al.* Boosted Influenza-Specific T Cell Responses after H5N1 Pandemic
844 Live Attenuated Influenza Virus Vaccination. *Front Immunol* **6**, 287 (2015).
845
- 846 36. Lillie, P.J. *et al.* Preliminary assessment of the efficacy of a T-cell-based influenza
847 vaccine, MVA-NP+M1, in humans. *Clin Infect Dis* **55**, 19-25 (2012).
848
- 849 37. de Silva, T.I. *et al.* Correlates of T-cell-mediated viral control and phenotype of
850 CD8(+) T cells in HIV-2, a naturally contained human retroviral infection. *Blood* **121**,
851 4330-4339 (2013).
852

Figure 2

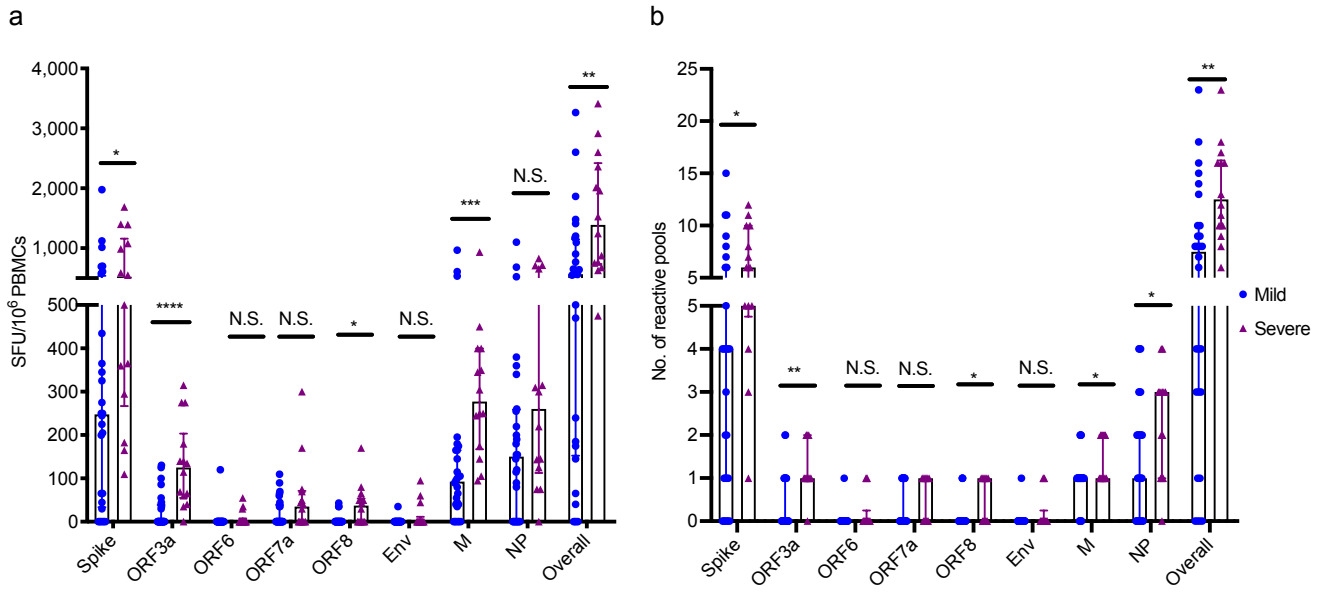


Figure 3

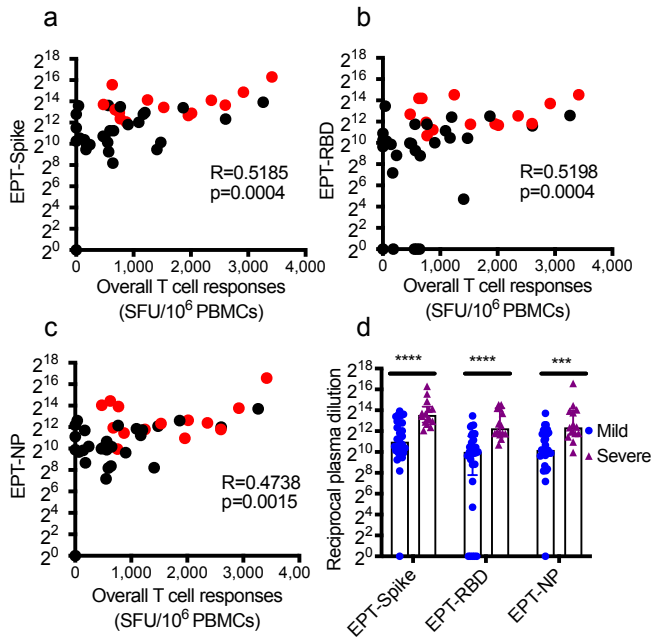


Figure 4

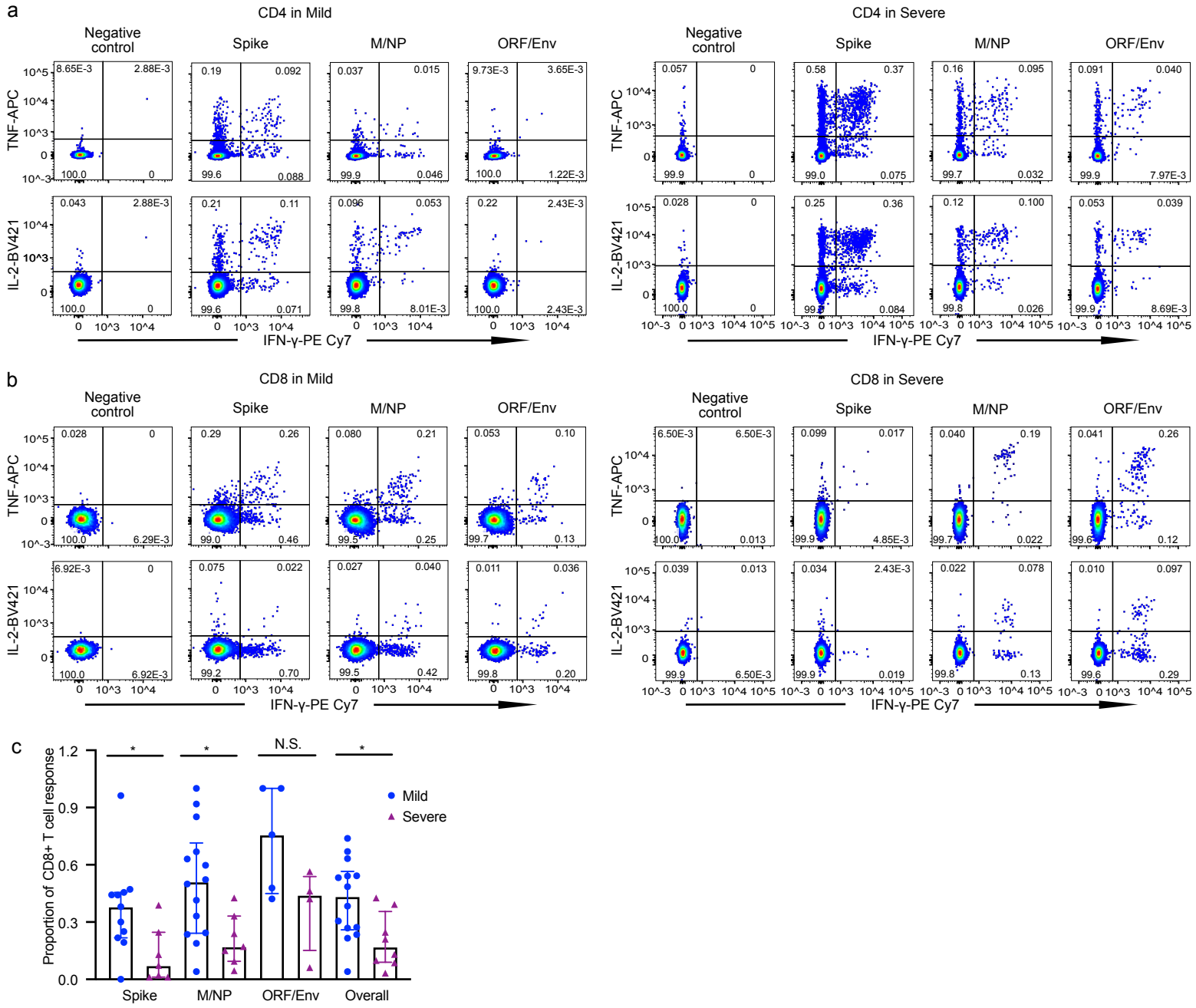


Figure 5

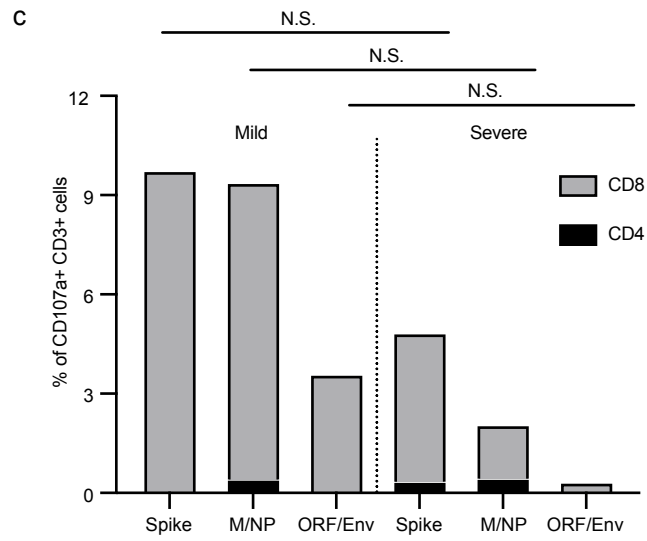
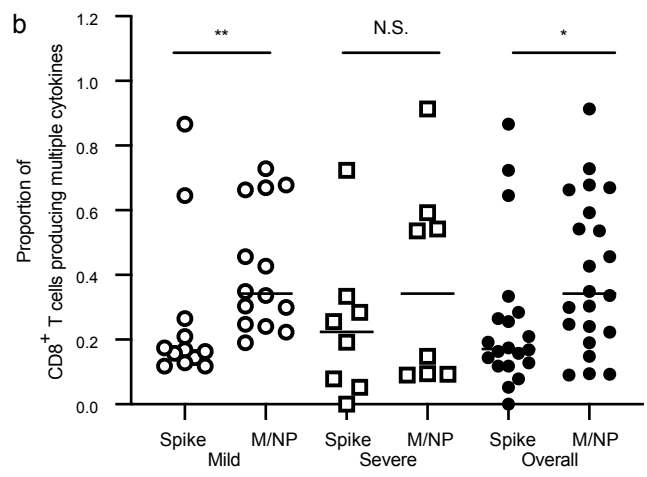
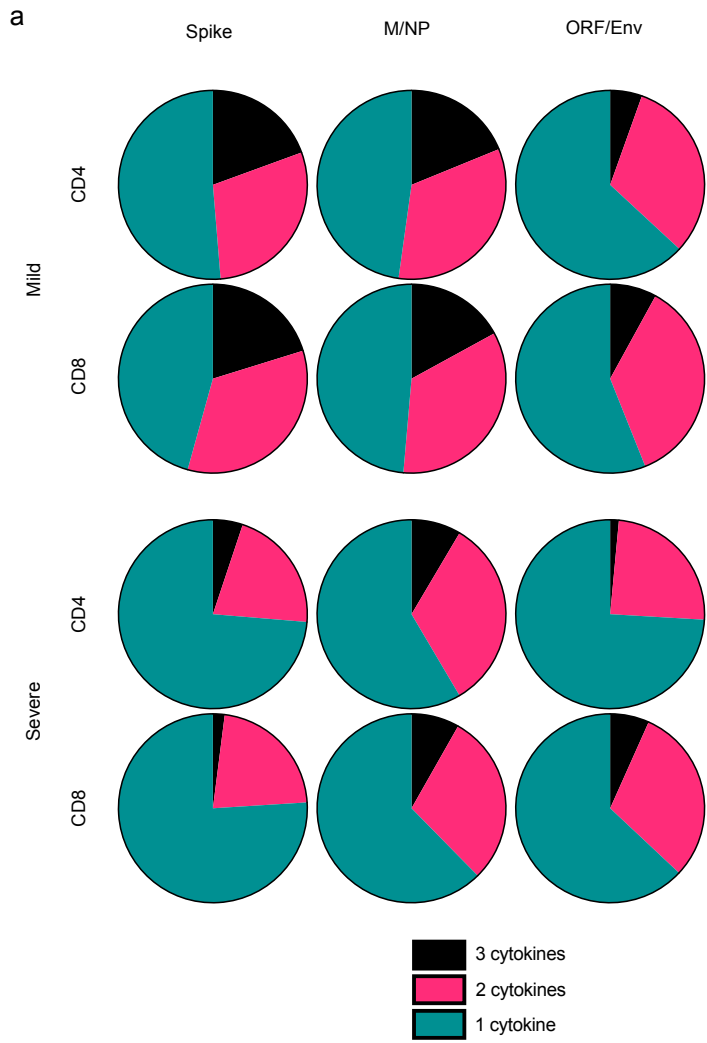


Figure 6

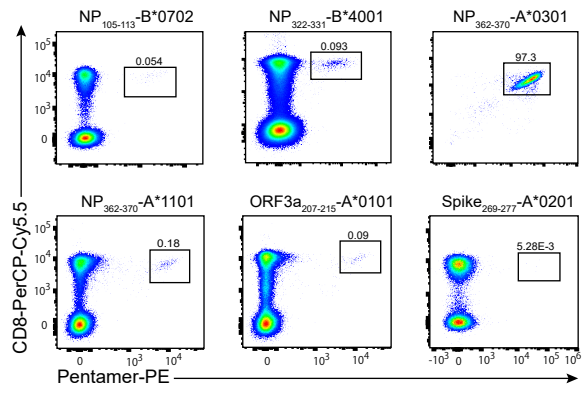
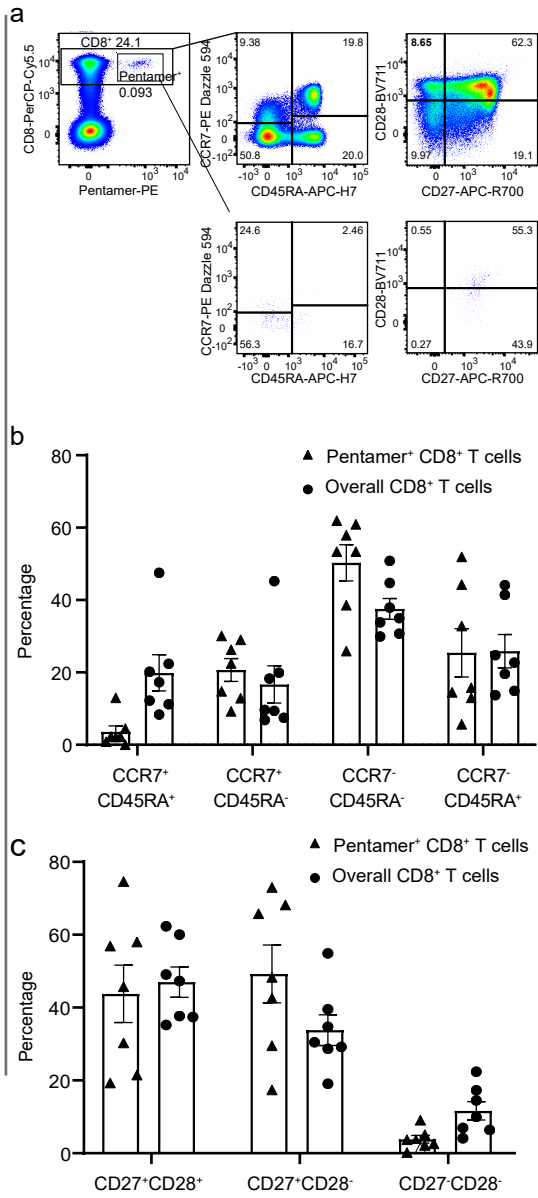


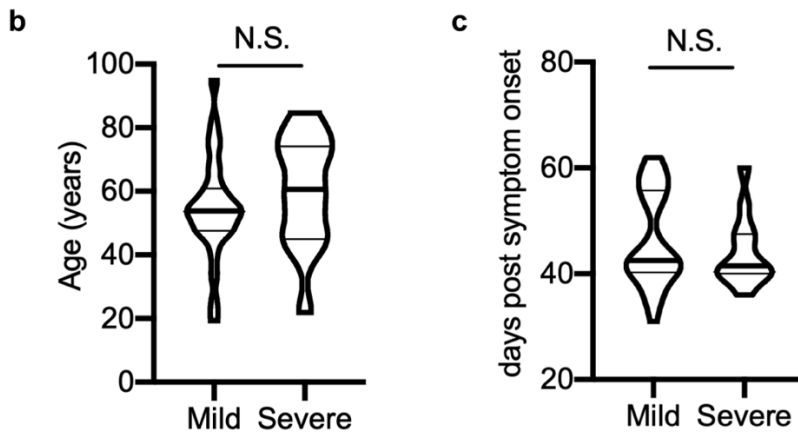
Figure 7



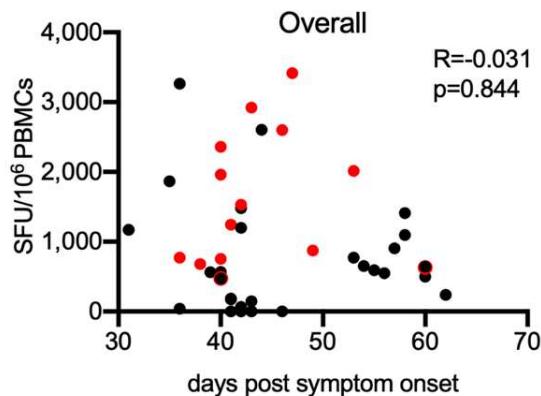
Supplementary Fig. 1: Participant characteristics. a) distribution of age, gender and days post symptom when sampling of the unexposed healthy controls and SSARS-CoV-2 infected patients studied. b) and c) Comparison of age ($p=0.3465$) and days post symptom ($p=0.4075$) when sampling between the patient groups with mild symptoms and severe symptoms. The unpaired t test with Welch's correction and Mann-Whitney test were used for data analysis of b) and c), respectively. Two tailed p value was calculated.

a

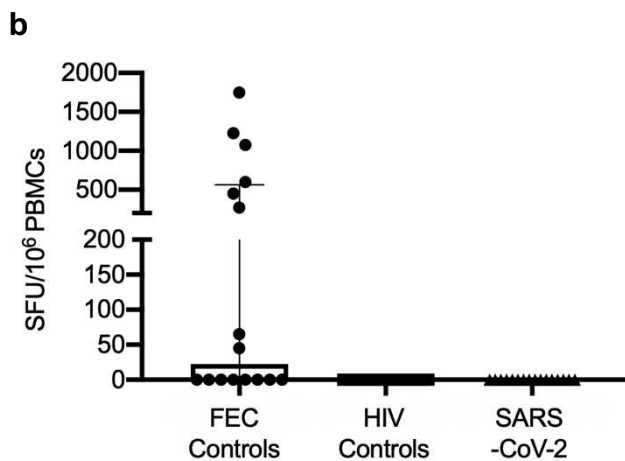
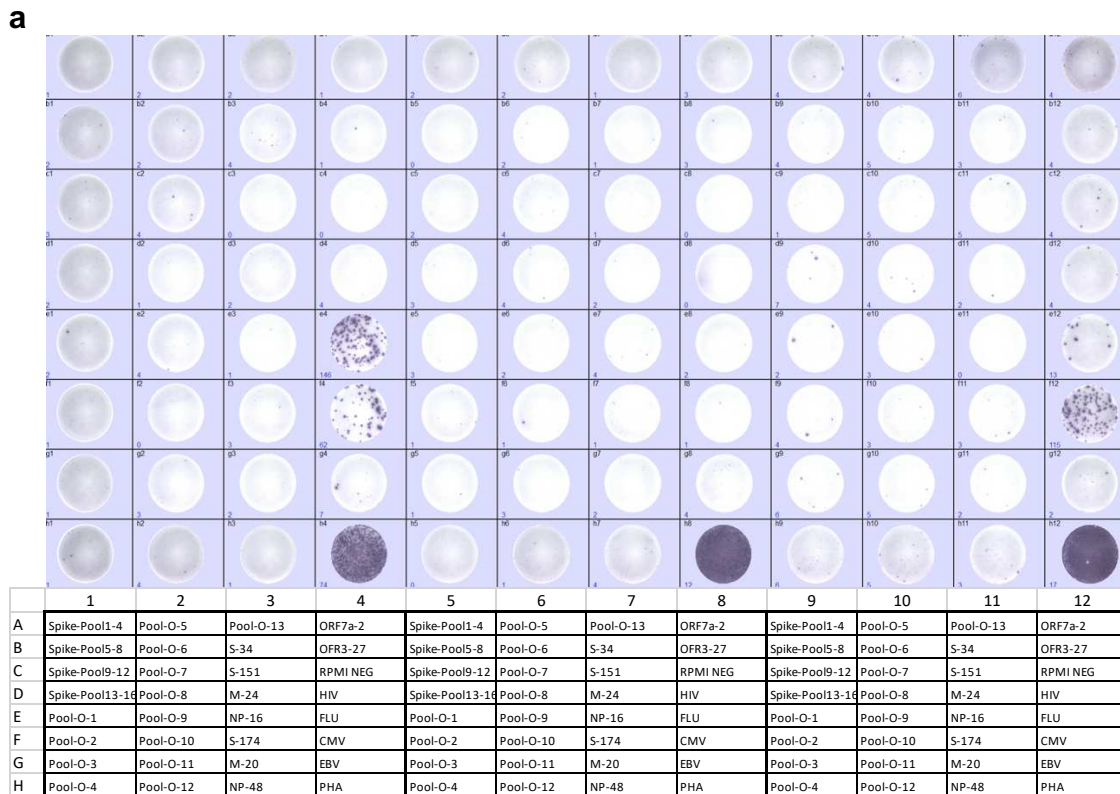
	Unexposed (N=19)	Mild Disease (n=28, 1 asymptomatic)	Severe Disease (n=14, 1 critical)
Age, y, median (IQR)	46.0(31.0-53.0)	53.8(47.6-60.9)	60.6(44.9-74.1)
Male sex	8(53.33)	17(60.71)	9(64.28)
Days post symptom, median (IQR)	NA	42.5(40.2-55.7)	41.5(40.0-47.5)



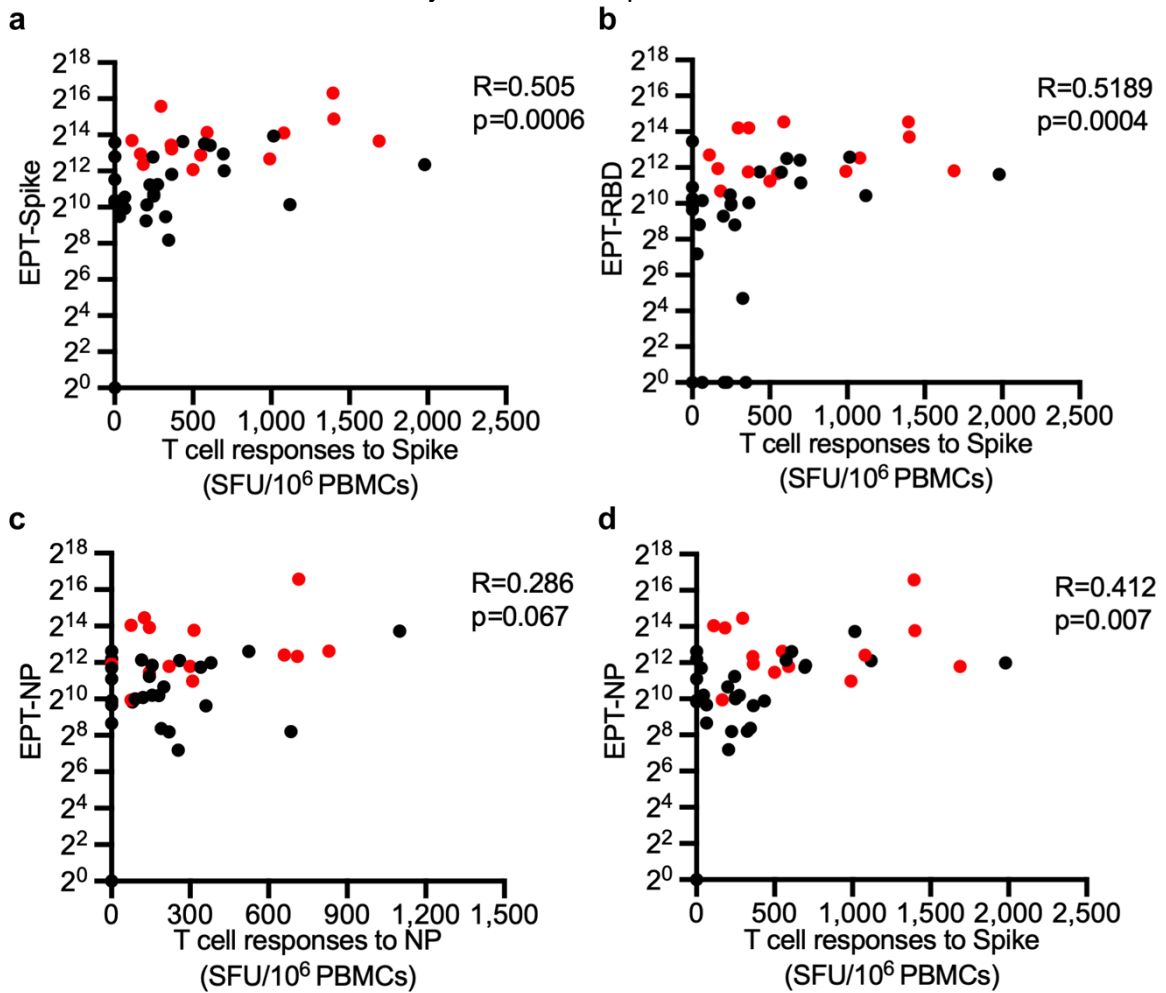
Supplementary Fig. 2: No correlation between overall T cell response of each individual and the days post symptom when blood specimen was taken. $n=42$. Black and red dots represent patients with history of mild symptoms and severe symptoms, respectively. Spearman's rank correlation coefficient was used for the correlation analysis, two tailed p value was calculated.



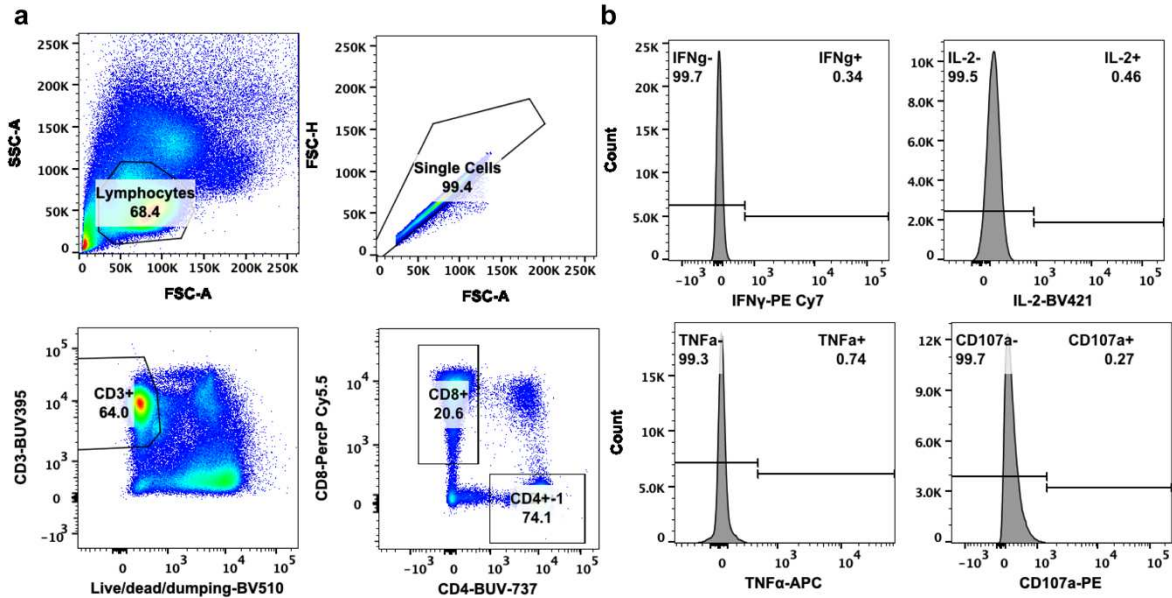
Supplementary Fig. 3: Magnitude of T cell responses of unexposed healthy individuals against SARS-CoV-2 antigens. a) An example of IFN- γ ELISpot plate from three healthy individuals without SARS-CoV-2 infection. Each individual has been tested with four spike pools (Pool 1-4, Pool 5-8, Pool 9-12 and Pool-13-16), 13 first dimension of non-spike pools and nine dominant individual peptides containing epitopes, along with six control wells including: negative controls with no peptide and peptide pools of irrelevant antigens derived from HIV Gag protein; positive controls with PHA and three pools of known CD8⁺ T cell epitopes of human influenza, CMV and EBV viruses (namely FEC controls). b) Magnitude of T cell responses of unexposed healthy individuals against SARS-CoV-2 antigens and control antigens. n=16. Data are presented as median with interquartile range.



Supplementary Fig. 4: Correlation between SARS-CoV-2 antigen-specific T cell responses and SARS-CoV-2 antigen-specific antibody responses. a), b) and c) Correlation of Spike-, RBD-, and NP-specific antibody responses to corresponding antigen-specific T cell responses. d) Correlation between NP-specific antibody response and Spike-specific T cell response. n=42. Black and red dots represent patients with history of mild symptoms and severe symptoms, respectively. Spearman's rank correlation coefficient was used for the correlation analysis, two tailed p value was calculated.

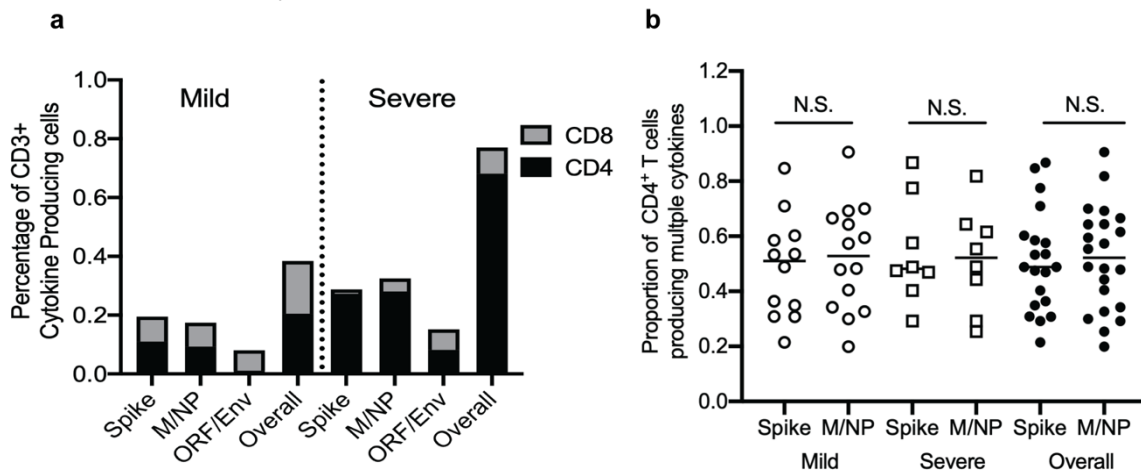


Supplementary Fig. 5: Gating strategy of flow cytometry analysis. a) Gating for CD4⁺/CD8⁺ T cells. Cells were gated on single cell by a forward side scatter gate, followed by CD3/ CD4/CD8 gating excluding dead cells, CD14⁺, CD19⁺, and CD16⁺ cells. This gating strategy was used for Fig. 4-7 and Supplementary Fig. 6. b) Gating for IFN γ ^{+/+}, TNF α ^{+/+}, IL-2^{+/+}, and CD107a^{+/+} population were based on corresponding negative controls. This gating strategy was used for Fig. 4-5.

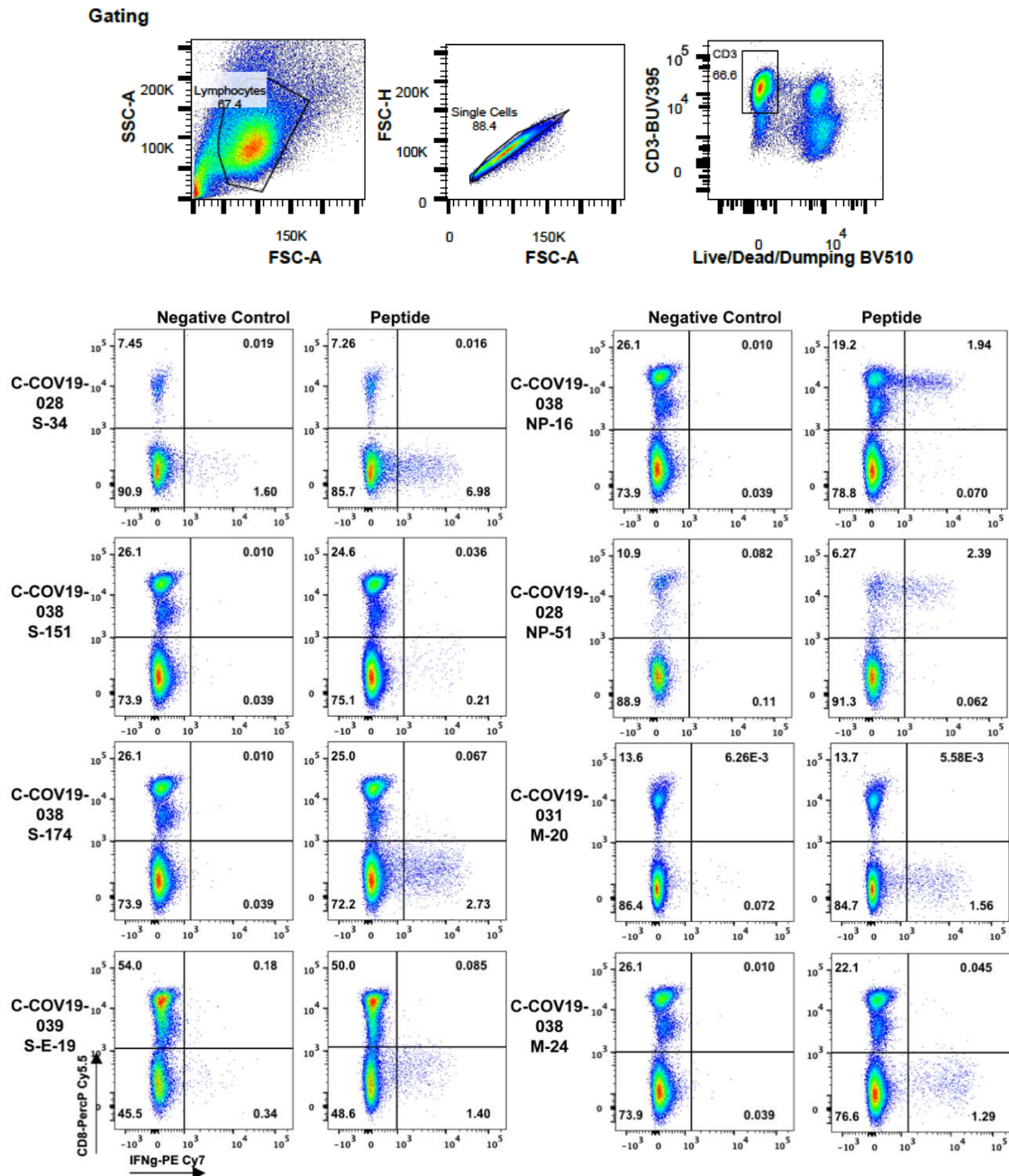


Supplementary Fig. 6: Comparison of Cytokine production of T cells between the patients with different disease severity and T cells targeting different viral proteins.

a) No significant difference in the percentage of CD4⁺ and CD8⁺ T cells producing IFN γ and/or TNF α , and/or IL-2 targeting each viral antigen between mild cases (n=14) and severe cases (n=8). Data are shown in value of median. b) No significant difference in proportion of multifunctional CD4⁺ T cells targeting spike protein (Mild group, n=12; Severe group, n=8) and M/NP protein (Mild group, n=14; Severe group, n=8). Mann-Whitney test was used for the analysis. Two-tailed p value was calculated. N.S. P>0.05



Supplementary Fig. 7: Confirmation of dominant T cell responses with cultured short-term T cell lines. Patient C-COV19-028 showed a CD4 T cell response to peptide S-34 and CD8 T cell response to peptide NP-51. Patient C-COV-19-038 showed CD4 T cell response to three dominant peptides: S-151 (weak), S-174, M24 and a CD8 T cell response to NP-16. Patient C-COV-19-039 showed CD4 T cell response to peptide S-E-19, whereas donor C-COV19-031 had a CD4 T cell response targeting peptide M-20. PBMCs were stimulated with corresponding peptide pools corresponding to the ex vivo ELISpot results and then cultured for 10 days. Cytokine production of the cell lines was then examined by ICS upon the stimulation with single peptides. Cells were gated on the live/singlet/ CD3+ Lymphocyte population.



Supplementary Table 1: Two-dimensional peptide Matrix pools.

a: Spike protein: 253 peptides in total 32 pools including 16 pools in 1st dimension and 16 pools in 2nd dimension

	Pool-17	Pool-18	Pool-19	Pool-20	Pool-21	Pool-22	Pool-23	Pool-24	Pool-25	Pool-26	Pool-27	Pool-28	Pool-29	Pool-30	Pool-31	Pool-32
Pool 1	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-11	S-12	S-13	S-14	S-15	S-16
Pool 2	S-17	S-18	S-19	S-20	S-21	S-22	S-23	S-24	S-25	S-26	S-27	S-28	S-29	S-30	S-31	S-32
Pool 3	S-33	S-34	S-35	S-36	S-37	S-38	S-39	S-40	S-41	S-42	S-43	S-44	S-45	S-46	S-47	S-48
Pool 4	S-49	S-50	S-51	S-52	S-53	S-54	S-55	S-56	S-57	S-58	S-59	S-60	S-61	S-62	S-63	S-64
Pool 5	S-65	S-66	S-67	S-68	S-69	S-70	S-71	S-72	S-73	S-74	S-75	S-76	S-77	S-78	S-79	S-80
Pool 6	S-81	S-82	S-83	S-84	S-85	S-86	S-87	S-88	S-89	S-90	S-91	S-92	S-93	S-94	S-95	S-96
Pool 7	S-97	S-98	S-99	S-100	S-101	S-102	S-103	S-104	S-105	S-106	S-107	S-108	S-109	S-110	S-111	S-112
Pool 8	S-113	S-114	S-115	S-116	S-117	S-118	S-119	S-120	S-121	S-122	S-123	S-124	S-125	S-126	S-127	S-128
Pool 9	S-129	S-130	S-131	S-132	S-133	S-134	S-135	S-136	S-137	S-138	S-139	S-140	S-141	S-142	S-143	S-144
Pool 10	S-145	S-146	S-147	S-148	S-149	S-150	S-151	S-152	S-153	S-154	S-155	S-156	S-157	S-158	S-159	S-160
Pool 11	S-161	S-162	S-163	S-164	S-165	S-166	S-167	S-168	S-169	S-170	S-171	S-172	S-173	S-174	S-175	S-176
Pool 12	S-177	S-178	S-179	S-180	S-181	S-182	S-183	S-184	S-185	S-186	S-187	S-188	S-189	S-190	S-191	S-192
Pool 13	S-193	S-194	S-195	S-196	S-197	S-198	S-199	S-200	S-201	S-202	S-203	S-204	S-205	S-206	S-207	S-208
Pool 14	S-209	S-210	S-211	S-212	S-213	S-214	S-215	S-216	S-217	S-218	S-219	S-220	S-221	S-222	S-223	S-224
Pool 15	S-225	S-226	S-227	S-228	S-229	S-230	S-231	S-232	S-233	S-234	S-235	S-236	S-237	S-238	S-239	S-240
Pool 16	S-241	S-242	S-243	S-244	S-245	S-246	S-247	S-248	S-249	S-250	S-251	S-252	S-253			

b: Non-spike proteins: total 29 pools, 13 pools in 1st dimension including ORF3a (35 peptides in 3 pools), ORF6 (7 peptides in 1 pool), ORF7a(15 peptides in 1 pool), ORF8(16 peptides in 1 pool), Envelope(9 peptides in 1 pool), Membrane Protein(29 peptides in 2 pools) and Nucleoprotein(59 peptides in 4 pools).

	Pool-O-14	Pool-O-15	Pool-O-16	Pool-O-17	Pool-O-18	Pool-O-19	Pool-O-20	Pool-O-21	Pool-O-22	Pool-O-23	Pool-O-24	Pool-O-25	Pool-O-26	Pool-O-27	Pool-O-28	Pool-O-29
Pool-O-1	ORF3a-1	ORF3a-2	ORF3a-3	ORF3a-4	ORF3a-5	ORF3a-6	ORF3a-7	ORF3a-8	ORF3a-9	ORF3a-10	ORF3a-11	ORF3a-12	ORF3a-13	ORF3a-14	ORF3a-15	ORF3a-16
Pool-O-2	ORF3a-17	ORF3a-18	ORF3a-19	ORF3a-20	ORF3a-21	ORF3a-22	ORF3a-23	ORF3a-24	ORF3a-25	ORF3a-26	ORF3a-27	ORF3a-28	ORF3a-29	ORF3a-30	ORF3a-31	ORF3a-32
Pool-O-3	ORF3a-33	ORF3a-34	ORF3a-35													
Pool-O-4	ORF6-1	ORF6-2	ORF6-3	ORF6-4	ORF6-5	ORF6-6	ORF6-7									
Pool-O-5	ORF7a-1	ORF7a-2	ORF7a-3	ORF7a-4	ORF7a-5	ORF7a-6	ORF7a-7	ORF7a-8	ORF7a-9	ORF7a-10	ORF7a-11	ORF7a-12	ORF7a-13	ORF7a-14	ORF7a-15	
Pool-O-6	ORF8-1	ORF8-2	ORF8-3	ORF8-4	ORF8-5	ORF8-6	ORF8-7	ORF8-8	ORF8-9	ORF8-10	ORF8-11	ORF8-12	ORF8-13	ORF8-14	ORF8-15	ORF8-16
Pool-O-7	Env-1	Env-2	Env-3	Env-4	Env-5	Env-6	Env-7	Env-8	Env-9							
Pool-O-8	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8	M-9	M-10	M-11	M-12	M-13	M-14	M-15	M-16
Pool-O-9	M-17	M-18	M-19	M-20	M-21	M-22	M-23	M-24	M-25	M-26	M-27	M-28				
Pool-O-10	NP-1	NP-2	NP-3	NP-4	NP-5	NP-6	NP-7	NP-8	NP-9	NP-10	NP-11	NP-12	NP-13	NP-14	NP-15	NP-16
Pool-O-11	NP-17	NP-18	NP-19	NP-20	NP-21	NP-22	NP-23	NP-24	NP-25	NP-26	NP-27	NP-28	NP-29	NP-30	NP-31	NP-32
Pool-O-12	NP-33	NP-34	NP-35	NP-36	NP-37	NP-38	NP-39	NP-40	NP-41	NP-42	NP-43	NP-44	NP-45	NP-46	NP-47	NP-48
Pool-O-13	NP-49	NP-50	NP-51	NP-52	NP-53	NP-54	NP-55	NP-56	NP-57	NP-58						

Supplementary Table 2: HLA class I typing of CD8⁺ epitope peptides in subjects with confirmed responses. Each patient listed made a CD8 T cell response to the peptides shown. Optimal epitopes and the corresponding HLA-restriction were predicted by IEDB analysis tool (<http://tools.iedb.org/mhci>). Red highlights are the predicted optimal epitope sequences.

Protein	Peptide ID	Peptide sequence	Predicted HLA Restriction	Patients	HLA					
					A1	A2	B1	B2	Cw1	Cw2
NP	NP-1	MSDNGPQN QRNAPRITF	B*2705/06	C-COV19-044	02:07	11:01	27:06	40:01	03:04	07:02
	NP-2	NQRNAPRITF GGPSDSTG		C-COV19-047	24:02	24:02	27:05	27:05	01:02	02:02
				C-COV19-025	02:01	24:02	27:05	44:02	02:02	05:01/03
	NP-16	LSPRWYFY YLTGTGPEAGL		C-COV19-001	02:01	23:01	07:02	49:01	07:01	07:02
				C-COV19-002	03:01	68:02	07:02	49:01	06:02	07:02
				C-COV19-003	02:01	32:01	07:02	44:02	05:01/03	07:02
				C-COV19-004	02:01	02:01	07:02	40:01	03:04	07:02
				C-COV19-005	01:01/04N	02:01	07:02	40:01	01:02	07:02
				C-COV19-006	01:01/04N	29:02	07:02	45:01	07:01	07:02
				C-COV19-007	01:01/04N	01:01/04N	07:02	07:02	07:02	07:02
				C-COV19-035	11:01	11:01	07:02	07:05/06	03:04	07:02
				C-COV19-036	01:01/04N	03:01	07:02	52:01	07:02	12:02
				C-COV19-038	02:01	24:02	07:02	51:01	04:01	07:02
	C-COV19-045	01:01/04N	02:01	07:02	45:01	06:02	07:02			
	C-COV19-046	02:01	03:01	07:02	44:02	05:01/03	07:02			
NP-E-3	MEVTPSGTWL	B*4001	C-COV19-021	02:01	31:01	40:01	40:01	03:04	03:04	
NP-51	LLNKHIDAYKTFPPTEPK	A*0301	C-COV19-044	02:07	11:01	27:06	40:01	03:04	07:02	
			C-COV19-028	02:01	03:01	15:01	44:02	03:03	07:04/11	
C-COV19-036	01:01/04N	03:01	07:02	52:01	07:02	12:02				
NP-51	LLNKHIDAYKTFPPTEPK	A*1101	C-COV19-035	11:01	11:01	07:02	07:05/06	03:04	07:02	
ORF	ORF3a-27	KDCVVLHSY FTSDYYQLY	A*0101	C-COV19-022	01:01/04N	01:01/04N	08:01	08:01	07:01	07:02
	ORF3a-28	YFTSDYYQLY STQLSTDGTV		C-COV19-036	01:01/04N	03:01	07:02	52:01	07:02	12:02
				C-COV19-037	01:01/04N	26:01	08:01	38:01	07:01	12:03
				C-COV19-040	01:04N	03:01	27:05	57:01	01:02	06:02
Spike	S-34	CTF EYVSQPFL MDLE	Cw*0702	C-COV19-035	11:01	11:01	07:02	07:05/06	03:04	07:02
	S-106	GPK KSTNLVKNK CVN	A*3101	C-COV19-021	02:01	31:01	40:01	40:01	03:04	03:04

Supplementary Table 3: Known SARS epitopes with identical sequences to SARS-CoV-2 , and Tetramers/Pentamers. Red highlights the epitope responses detected in the patients who had recovered from COVID-19, whether by tetramer/pentamer staining or ELISpot assay.

Peptide ID	Epitope	Protein	MHC allele	Tetramer/Pentamer
N-E-01	ILLNKHID	NP	HLA-A*02:01	Y
N-E-02	AFFGMSRIGMEVTPSGTW	NP	NA	
N-E-03	MEVTPSGTWL	NP	HLA-B*40:01 I	Y
N-E-04	GMSRIGMEV	NP	HLA-A*02:01 I	Y
N-E-05	ILLNKHIDA	NP	HLA-A*02:01 I	Y
N-E-06	ALNTPKDHI	NP	HLA-A*02:01 I	Y
N-E-07	IRQGTDYKHWPQIAQFA	NP	NA	
N-E-08	KHWPQIAQFAPSASAFF	NP	NA	
N-E-09	LALLLDRL	NP	HLA-A*02:01 I	Y
N-E-10	LLDRLNQL	NP	HLA-A*02:01 I	Y
N-E-11	LLNKHIDAYKTFPPEPK	NP	NA	
N-E-12	LQLPQGTTL	NP	HLA-A*02:01 I	Y
N-E-13	AQFAPSASAFFGMSR	NP	NA	
N-E-14	AQFAPSASAFFGMSRIGM	NP	NA	
N-E-15	RRPQGLPNNTASWFT	NP	NA I	
N-E-16	YKTFPPEPKKDKKKK	NP	NA	
S-E-17	GAALQIPFAMQMAYRF	Spike	HLA-DRA*01:01,HLA-DRB1*07:01	Y
S-E-18	MAYRFNGIGVTQNVLY	Spike	HLA-DRB1*04:01	Y
S-E-19	QLIRAAEIRASANLAATK	Spike	HLA-DRB1*04:01	Y
S-E-20	FIAGLIAIV	Spike	HLA-A*02:01	Y
S-E-21	ALNTLVKQL	Spike	HLA-A*02:01 I	Y
S-E-22	LITGRLQSL	Spike	HLA-A2 I	Y
S-E-23	NLNESLIDL	Spike	HLA-A*02:01 I	Y
S-E-24	QALNTLVKQLSSNFGAI	Spike	HLA-DRB1*04:01	Y
S-E-25	RLNEVAKNL	Spike	HLA-A*02:01 I	Y
S-E-26	VLNDILSRL	Spike	HLA-A*02:01 I	Y
S-E-27	VVFLHVTYV	Spike	HLA-A*02:01 I	Y

Supplementary Table 4:

Oxford Immunology Network Covid-19 response: T cell Immunity Team

Team leader: Graham Ogg,

Barbara Kronsteiner, Anthony Brown, Emily Adland, Patpong Rongkard, Anna Csala, Helen Brown, Nicola Robinson, Panagiota Zacharopoulou, Vinicius Adriano, Prabhjeet Phalora, Oliver Sampson, Carl-Philipp Hackstein, Nicholas Lim, Matt Edmans, Senthil Chinnakannan, Rachael Brown, Ali Amini, Mathew Jones, Mohammad Ali, Timothy Donnison, Matt Pace, Ane Ogbe, Donal Skelly, Lizzie Stafford, Helen Fletcher, Lian Lee, Prathiba Kurupati, Rachel Etherington, Nicholas Provine, Hashem Koohy, Chloe Hyun-Jung Lee, Yanchun Peng, Guihai Liu, Xuan Yao, Zixi Yin, Danning Dong, Mariolina Salio, Giorgio Napolitani, Susanna Dunachie, Eleanor Barnes, John Frater, Georgina Kerr, Philip Goulder, Paul Klenerman, Andrew McMichael, Tao Dong.

Supplementary Table 5: ISARIC 4C Investigators

Consortium Lead Investigator: J Kenneth Baillie,

Chief Investigator: Malcolm G Semple

Co-Lead Investigator: Peter JM Openshaw.

ISARIC Clinical Coordinator: Gail Carson.

Co-Investigators: Beatrice Alex, Benjamin Bach, Wendy S Barclay, Debby Bogaert, Meera Chand, Graham S Cooke, Annemarie B Docherty, Jake Dunning, Ana da Silva Filipe, Tom Fletcher, Christopher A Green, Ewen M Harrison, Julian A Hiscox, Antonia Ying Wai Ho, Peter W Horby, Samreen Ijaz, Saye Khoo, Paul Klenerman, Andrew Law, Wei Shen Lim, Alexander, J Mentzer, Laura Merson, Alison M Meynert, Mahdad Noursadeghi, Shona C Moore, Massimo Palmarini, William A Paxton, Georgios Pollakis, Nicholas Price, Andrew Rambaut, David L Robertson, Clark D Russell, Vanessa Sancho-Shimizu, Janet T Scott, Louise Sigfrid, Tom Solomon, Shiranee Sriskandan, David I Stuart, Charlotte Summers, Richard S Tedder, Emma C Thomson, Ryan S Thwaites, Lance Turtle, Maria Zambon. Project Managers Hayley Hardwick, Chloe Donohue, Jane Ewins, Wilna Oosthuyzen, Fiona Griffiths. Data Analysts: Lisa Norman, Riinu Pius, Tom M Drake, Cameron J Fairfield, Stephen Knight, Kenneth A Mclean, Derek Murphy, Catherine A Shaw. Data and Information System Manager: Jo Dalton, Michelle Girvan, Egle Saviciute, Stephanie Roberts Janet Harrison, Laura Marsh, Marie Connor. Data integration and presentation: Gary Leeming, Andrew Law, Ross Hendry. Material Management: William Greenhalf, Victoria Shaw, Sarah McDonald. Outbreak Laboratory Volunteers: Katie A. Ahmed, Jane A Armstrong, Milton Ashworth, Innocent G Asiiimwe, Siddharth Bakshi, Samantha L Barlow, Laura Booth, Benjamin Brennan, Katie Bullock, Benjamin WA Catterall, Jordan J Clark, Emily A Clarke, Sarah Cole, Louise Cooper, Helen Cox, Christopher Davis, Oslem Dincarslan, Chris Dunn, Philip Dyer, Angela Elliott, Anthony Evans, Lewis WS Fisher, Terry Foster, Isabel Garcia-Dorival, William Greenhalf, Philip Gunning, Catherine Hartley, Antonia Ho, Rebecca L Jensen, Christopher B Jones, Trevor R Jones, Shadia Khandaker, Katharine King, Robyn T. Kiy, Chrysa Koukorava, Annette Lake, Suzannah Lant, Diane Latawiec, L Lavelle-Langham, Daniella Lefteri, Lauren Lett, Lucia A Livoti, Maria Mancini, Sarah McDonald, Laurence McEvoy, John McLauchlan, Soeren Metelmann, Nahida S Miah, Joanna Middleton, Joyce Mitchell, Shona C Moore, Ellen G Murphy, Rebekah Penrice-Randal, Jack Pilgrim, Tessa Prince, Will Reynolds, P. Matthew Ridley, Debby Sales, Victoria E Shaw, Rebecca K Shears, Benjamin Small, Krishanthi S Subramaniam, Agnieska Szemiel, Aislynn Taggart, Jolanta Tanianis, Jordan Thomas, Erwan Trochu, Libby van Tonder, Eve Wilcock, J. Eunice Zhang. Local Principal Investigators: Kayode Adeniji, Daniel Agranoff, Ken Agwuh, Dhiraj Ail, Ana Alegria, Brian Angus, Abdul Ashish, Dougal Atkinson, Shahedal Bari, Gavin Barlow, Stella Barnass, Nicholas Barrett, Christopher Bassford, David Baxter, Michael Beadsworth, Jolanta Bernatoniene, John Berridge, Nicola Best, Pieter Bothma, David Brealey, Robin Brittain-Long, Naomi Bulteel, Tom Burden, Andrew Burtenshaw, Vikki Caruth, David

Chadwick, Duncan Chamblor, Nigel Chee, Jenny Child, Srikanth Chukkambotla, Tom Clark, Paul Collini, Catherine Cosgrove, Jason Cupitt, Maria-Teresa Cutino-Moguel, Paul Dark, Chris Dawson, Samir Dervisevic, Phil Donnison, Sam Douthwaite, Ingrid DuRand, Ahilanadan Dushianthan, Tristan Dyer, Cariad Evans , Chi Eziefula, Chrisopher Fegan, Adam Finn, Duncan Fullerton, Sanjeev Garg, Sanjeev Garg, Atul Garg, Jo Godden, Arthur Goldsmith, Clive Graham, Elaine Hardy, Stuart Hartshorn, Daniel Harvey, Peter Havalda, Daniel B Hawcutt, Maria Hobrok, Luke Hodgson, Anita Holme, Anil Hormis, Michael Jacobs, Susan Jain, Paul Jennings, Agilan Kaliappan, Vidya Kasipandian, Stephen Kegg, Michael Kelsey, Jason Kendall, Caroline Kerrison, Ian Kerslake, Oliver Koch, Gouri Koduri, George Koshy , Shondipon Laha, Susan Larkin, Tamas Leiner, Patrick Lillie, James Limb, Vanessa Linnett, Jeff Little, Michael MacMahon, Emily MacNaughton, Ravish Mankregod, Huw Masson , Elijah Matovu, Katherine McCullough, Ruth McEwen , Manjula Meda, Gary Mills , Jane Minton, Mariyam Mirfenderesky, Kavya Mohandas, Quen Mok, James Moon, Elinoor Moore, Patrick Morgan, Craig Morris, Katherine Mortimore, Samuel Moses, Mbiye Mpenge, Rohinton Mulla, Michael Murphy, Megan Nagel, Thapas Nagarajan, Mark Nelson, Igor Otahal, Mark Pais, Selva Panchatsharam, Hassan Paraiso, Brij Patel, Justin Pepperell, Mark Peters, Mandeep Phull , Stefania Pintus, Jagtur Singh Pooni, Frank Post, David Price, Rachel Prout, Nikolas Rae, Henrik Reschreiter, Tim Reynolds, Neil Richardson, Mark Roberts, Devender Roberts, Alistair Rose, Guy Rousseau, Brendan Ryan, Taranprit Saluja, Aarti Shah, Prad Shanmuga, Anil Sharma, Anna Shawcross, Jeremy Sizer, Richard Smith, Catherine Snelson, Nick Spittle, Nikki Staines , Tom Stambach, Richard Stewart, Pradeep Subudhi, Tamas Szakmany, Kate Tatham, Jo Thomas, Chris Thompson, Robert Thompson, Ascanio Tridente, Darell Tupper - Carey, Mary Twagira, Andrew Ustianowski, Nick Vallotton, Lisa Vincent-Smith, Shico Visuvanathan , Alan Vuylsteke, Sam Waddy, Rachel Wake, Andrew Walden, Ingeborg Welters, Tony Whitehouse, Paul Whittaker, Ashley Whittington, Meme Wijesinghe, Martin Williams, Lawrence Wilson, Sarah Wilson, Stephen Winchester, Martin Wiselka, Adam Wolverson, Daniel G Wooton, Andrew Workman, Bryan Yates, Peter Young.