

SARS-CoV-2-specific T cell memory is long-lasting in the majority of convalescent COVID-19 individuals

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25 ABSTRACT

26 An unaddressed key question in the current *coronavirus disease 2019* (COVID-19)
 27 pandemic is the duration of immunity for which specific T cell responses against the
 28 *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) are an indispensable
 29 element. Being situated in Wuhan where the pandemic initiated enables us to conduct
 30 the longest analyses of memory T cell responses against SARS-CoV-2 in COVID-19
 31 convalescent individuals (CIs). Magnitude and breadth of SARS-CoV-2 memory CD4
 32 and CD8 T cell responses were heterogeneous between patients but robust responses
 33 could be detected up to 9 months post disease onset in most CIs. Loss of memory
 34 CD4 and CD8 T cell responses were observed in only 16.13% and 25.81% of CIs,
 35 respectively. Thus, the overall magnitude and breadth of memory CD4 and CD8 T cell
 36 responses were quite stable and not inversely correlated with the time from disease
 37 onset. Interestingly, the only significant decrease in the response was found for
 38 memory CD4 T cells in the first 6-month post COVID-19 disease onset. Longitudinal
 39 analyses revealed that the kinetics of SARS-CoV-2 memory CD4 and CD8 T cell
 40 responses were quite heterogeneous between patients. Loss of memory CD4 T cell
 41 responses was observed more frequently in asymptomatic cases than after
 42 symptomatic COVID-19. Interestingly, the few CIs in which SARS-CoV-2-specific
 43 IgG responses disappeared showed more durable memory CD4 T cell responses than
 44 CIs who remained IgG-positive for month. Collectively, we provide the first
 45 comprehensive characterization of the long-term memory T cell response in CIs,
 46 suggesting that SARS-CoV-2-specific T cell immunity is long-lasting in the majority
 47 of individuals.

48 **Introduction**

49 Antigen-specific T and B cell responses play fundamental roles in the clearance of
50 most viral infections. Additionally, the establishment of T and B cell memory after
51 recovery is essential for protecting the host against disease upon re-exposure. Faced
52 by the unprecedented medical and socioeconomic crisis caused by severe acute
53 respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated coronavirus
54 disease 2019 (COVID-19), the scientific community has ignited tremendous efforts to
55 map correlates of protection and determinants of immunity against SARS-CoV-2.
56 While antibody-based immunity is relatively well-studied, increasing evidences
57 suggest that T cells may play a fundamental role in the resolution of COVID-19 ^{1,2}.
58 The current dogma is that SARS-CoV-2-specific CD4 and CD8 T cell responses,
59 responding at variably high frequencies recognizing multiple epitopes across the viral
60 proteome, can be detected in most individuals both during acute COVID-19 and
61 convalescence afterwards ³⁻⁸. The magnitude of SARS-CoV-2-specific T cell
62 responses during the early phase is assumed to correlate with the magnitude of
63 antibody responses, and more severe and protracted disease usually drives a more
64 vigorous and, in terms of epitope coverage, broader T cell response ^{5,7,8}. However, it
65 has also been observed that cellular and humoral immune responses can become
66 uncoupled in some SARS-CoV-2-exposed individuals, who showed strong specific T
67 cell immunity but lack detectable antibody responses ⁹. It is assumed that this results
68 from antibody responses waning more quickly than T cell responses ¹⁰ and that
69 SARS-CoV-2-specific antibody responses are rather short-lived, while T cell memory

70 seems to be more durable ^{10,11}. However, all available data on analyzing T cell
 71 memory were mainly generated from individuals recovering from COVID-19 during a
 72 relatively short follow-up period the longest observation duration being less than 60
 73 days post disease onset (dpdo) ⁵. To our knowledge, it is not yet known whether
 74 natural infections with SARS-CoV-2 generate long-lasting memory T cell responses
 75 and how memory T cell responses changes in a long-term post recovery.

76 Wuhan was the very first city hit by SARS-CoV-2. Accordingly, all patients who
 77 experienced the longest phase of convalescence following COVID-19 reside here or
 78 closeby. Wuhan also performed a thorough SARS-CoV-2 RNA test for every resident
 79 in May, 2020 to preclude the possibility of local spread of the virus ever since. This
 80 enabled us to characterize the long-term memory T cell responses in a cohort of
 81 COVID-19 convalescent individuals (CIs) with an unprecedented observation time up
 82 to 274 dpdo. Our results suggest that robust SARS-CoV-2 memory T cell responses
 83 can be detected in the majority of CIs long-term post recovery.

84 **Methods**

85 **Subjects**

86 Thirty-one convalescent individuals who resolved their SARS-CoV-2 infection and 11
87 SARS-CoV-2-unexposed individuals (UIs) were recruited at the Department of
88 Infectious Diseases, Union Hospital, Tongji Medical College, Huazhong University of
89 Science and Technology and the Department of Gastroenterology from April to
90 September 2020. The diagnosis of COVID-19 was based on the Guidelines for
91 Diagnosis and Treatment of Corona Virus Disease 2019 issued by the National Health
92 Commission of China (7th edition). Informed written consent was obtained from each
93 patient and the study protocol was approved by the local medical ethics committee of
94 Union Hospital, Tongji Medical College, Huazhong University of Science and
95 Technology in accordance with the guidelines of the Declaration of Helsinki
96 (2020IEC-J-587).

97

98 **Preparation of PBMCs**

99 Peripheral blood mononuclear cells (PBMCs) of SARS-CoV-2-unexposed individuals
100 and patients were isolated using Ficoll density gradient centrifugation (DAKEWE
101 Biotech, Beijing) and were rapidly assessed by flow cytometry analysis without
102 intermittent cryo-preservation.

103

104 **Analysis of effector T cell responses**

105 Three pools of lyophilized peptides, consisting mainly of 15-mer sequences with 11
106 amino acids (aa) overlap, either covering the immunodominant sequences of the

107 surface glycoprotein (S) or the complete sequences of the nucleocapsid
 108 phosphoprotein (N) or the membrane glycoprotein (M) of SARS-CoV-2 were used for
 109 cell stimulation (PepTivator® Peptide Pools, Miltenyi, Germany). On day 1, PBMCs
 110 were resuspended in complete medium (RPMI 1640 containing 10% [v/v] fetal calf
 111 serum, 100U/ml penicillin, 100µg/ml streptomycin, and 100µM
 112 4-[2-hydroxyethyl]-1-piperazine ethanesulfonic acid [HEPES] buffer), and stimulated
 113 with S, N or M peptide pools (10µg/ml) in the presence of anti-CD28 (1µg/ml; BD
 114 Biosciences, USA) and recombinant interleukin (IL)-2 (20U/ml; Hoffmann-La Roche,
 115 Italy). Cells without peptide stimulation and anti-CD3-stimulated (1µg/ml; BD
 116 Biosciences, USA) cells served as negative and positive controls, respectively. Fresh
 117 medium containing IL-2 was added on day 4 and 7. On day 10, cells were
 118 restimulated for 5 hours with the same peptide pool in the presence of brefeldin A
 119 (BD Biosciences, San Diego, CA). Cells were then tested for IFN-γ, IL-2, and TNF-α
 120 expression by intracellular cytokine staining. Specific cytokine responses were
 121 calculated by subtracting the background activation (the percentage of cytokine
 122 positive cells in the negative control) before further analysis. T cell responses were
 123 defined as detectable if the frequency in the specifically stimulated culture exceeded
 124 the unstimulated control at least twofold (stimulation index > 2). Samples with
 125 responseless positive controls were excluded from further analyses.

126

127 **Flow cytometry**

128 Surface and intracellular staining for flow cytometry analysis were performed as

described previously^{12,13}. For surface staining, cells were incubated with relevant fluorochrome-labeled antibodies for 30 min at 4°C in the dark. For intracellular cytokine staining, cells were fixed and permeabilized using the Intracellular Fixation & Permeabilization Buffer Set (Invitrogen, USA) and stained with FITC-anti-IFN- γ , PE-anti-IL-2 and APC-anti-TNF- α (BD Biosciences, USA). Approximately 100,000 PBMCs were acquired for each sample using a BD FACS Canto II flow cytometer. Data analysis was performed using the FlowJo software V10.0.7 (Tree Star, Ashland, OR, USA). Cell debris and dead cells were excluded from the analysis based on scatter signals and Fixable Viability Dye eFluor 506.

138

139 **Statistical Analysis**

Statistical analyses were performed using the SPSS statistical software package (version 22.0, SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk method was used to test for normality. Mann-Whitney t-test, Pearson product-moment correlation coefficient and Fisher's exact test were used where appropriate. All reported P values were two-sided, and a P value less than 0.05 was considered statistically significant.

145

146 **Results**

147 *Characteristics of the study cohort*

148 To characterize SARS-CoV-2-specific memory CD4 and CD8 T cell responses in
 149 individuals who had recovered from COVID-19, blood samples derived from 31 CIs
 150 together with 11 UIs were assessed. The demographic profiles of all individuals are
 151 shown in Table 1. The median period between disease onset and blood sampling was
 152 169 days (range: 83 to 274 days). Among all COVID-19 cases, 56.67% (17/31) were
 153 hospitalized and 46.67% (14/31) received oxygen inhalation treatment. Leukopenia
 154 and lymphopenia were observed in 52.94% (9/17) and 76.47% (13/17) of tested cases,
 155 respectively. Increased C-reactive protein and IL-6 levels were apparent in 70.59%
 156 (12/17) and 85.71% (12/14) of tested patients, respectively. Abnormal radiological
 157 findings suggesting pneumonia were evident in 74.19% (23/31) CIs by chest
 158 computed tomography scans (CT). Sixteen CIs (51.61%) had positive RT-PCR results
 159 for viral RNA. All patients were confirmed anti-SARS-CoV-2 IgM and IgG
 160 seropositive. At the time of last blood sampling, 45.16% (14/31) were IgG single
 161 positive and 29.03% (9/31) were IgM and IgG double positive. Besides, 8 CIs who
 162 had become IgG seronegative were purposely recruited to study the interdependence
 163 of humoral and cellular immunity. The defining criteria for COVID-19 convalescence
 164 were as follows: being afebrile for more than 3 days, resolution of respiratory
 165 symptoms, substantial improvement of chest CT images, and two consecutive
 166 negative RT-qPCR tests for viral RNA in respiratory tract swab samples obtained at
 167 least 24 h apart. At the time of blood sampling, all CIs were negative for viral RNA

168 and had no medical conditions related to COVID-19.

169

170 *Characterization of the long-term memory T cell response specific to SARS-CoV-2*

171 PBMCs of UIs and CIs were re-stimulated with 3 panels of overlapping peptides
172 spanning the SARS-CoV-2 proteins S, N, and M, respectively, to determine memory T
173 cell responses ex vivo. We used an intracellular cytokine staining flow cytometry
174 assay (Fig. S1), and the magnitude of the overall cytokine responses [interferon
175 (IFN)- γ , interleukin (IL)-2, and tumor necrosis factor (TNF)- α] for CD4 and CD8 T
176 cells of all participants are shown in Fig. 1a. Besides, the magnitude and breadth (to
177 how many peptide pools T cells responded) of the IFN- γ , IL-2 or TNF- α -positive T
178 cells are also shown individually in Fig. 1b and 1c. Consistent with previous reports
179 ^{4,6}, a proportion of T cells weakly responded to SARS-CoV-2 peptides in UIs (both
180 CD4 and CD8 T cells: 27.27%, 3/11), but with a much lower magnitude than those in
181 CIs (Fig. 1a-1c). In general, memory T cell responses considerably varied in breadth
182 and magnitude between individual CIs. The magnitudes of TNF- α responses against S,
183 IFN- γ or TNF- α responses against N, and IFN- γ responses against M of CD4 and
184 CD8 T cells were significantly positively correlated (Fig. 1d and S2). Memory CD4 T
185 cell responses against a single, two or three peptide pools of the different proteins
186 were detected in 6.45% (2/31), 19.35% (6/31), and 58.06% (18/31) of CIs,
187 respectively (Fig. 1e). Memory CD8 T cell responses against a single, two or three
188 peptide pools of the different proteins were detected in 29.03% (9/31), 16.13% (5/31),
189 and 29.03% (9/31) of CIs, respectively (Fig. 1f). Interestingly, 16.13% (5/31 for CD4)

190 and 25.81% (8/31 for CD8) of CIs did not exhibit memory T cell responses against
191 the three viral proteins (Fig. 1e and 1f). There were only 9.68% (3/31) of CIs who
192 showed no any detectable memory T cell responses against the three proteins for both
193 CD4 and CD8 T cells. Taken together, while the vast majority of CIs had clearly
194 measurable T cell responses against SARS-CoV-2, the data also shows substantial
195 individuality in SARS-CoV-2 memory T cell responses.

196

197 Next, we analyzed the correlation between the magnitude and breadth of the overall
198 SARS-CoV-2 memory T cell responses and the time after disease onset. The CIs were
199 studied up to 9 month after disease onset and we combined the data from all patients
200 for the analysis. In addition, we separately analyzed two different time periods after
201 COVID-19, the first 6 month and the following 3 months for changes in memory T
202 cell responses. For CD4 T cells, the magnitude and breadth of SARS-CoV-2 memory
203 responses against S, N or M showed no significant correlation with days post disease
204 onset (dpdo) (Fig. 2a), suggesting that the CD4 T cell response was relatively stable
205 over time. Interestingly, however, during the first 180 dpdo a significant inverse
206 correlation between the magnitude of the memory CD4 T cell response against S and
207 dpdo was observed ($r^2=0.480$, $P=0.003$, Fig. 2b). In contrast, during the late
208 convalescent phase between 6 and 9 month after COVID-19 the magnitude of
209 memory CD4 T cell responses against S ($r^2=0.327$, $P=0.041$) and N ($r^2=0.328$,
210 $P=0.041$) was positively correlated with dpdo (Fig. 2c). For CD8 T cells, the
211 magnitude and breadth of SARS-CoV-2 memory responses against S, N or M did also

not show a significant correlation with dpdo (Fig. 2e). In contrast to CD4 T cells, CD8 T cells did not show a biphasic response during the two different time phases after COVID-19 (Fig. 2d-2f). No significant changes in the magnitude or breadth of the CD8 T cell response to any of the SARS-CoV-2 proteins was observed in the early or late phase, with the only exception that a positive correlation between the breadth of memory CD8 T cell responses and dpdo after 180 days was observed ($r^2=0.311$, $P=0.048$, Fig. 2f).

These results indicated that the overall SARS-CoV-2 memory CD4 and CD8 T cell responses were long-lasting. However, for memory CD4 T cells a decline in the magnitude of the response was observed during the early recovery phase which was reversed in the following months, highlighting the need for long-term follow up studies such as this.

To further characterize the kinetics of SARS-CoV-2 memory T cell responses, the magnitude of T cell responses were longitudinally examined in more detail in 4 individual CIs. Strong and broad CD4 (in all 4 individuals) and CD8 (3 out of 4 individuals) T cell responses against S, N, and M were detected at the first sampling time point (83-127 dpdo, Fig. 3a-3d). In 2 out of 4 individuals, a decrease in the magnitude of both SARS-CoV-2 memory CD4 and CD8 T cell responses was observed on 147 dpdo and 214 dpdo, respectively (Fig. 3a and 3b), which was most pronounced for the response against the S peptide pool. In contrast, one individual showed sustained SARS-CoV-2 memory CD4 and CD8 T cell responses over time

(Fig. 3c), whereas another individual also showed sustained SARS-CoV-2 memory CD4 T cell responses but a strong increase in S-, N-, and M-specific memory CD8 T cell responses, which were undetectable at the early time point in this individual, (Fig. 3d).

Taken together, these results suggested that long-term memory T cell responses to SARS-CoV-2 are quite patient-specific and heterogeneous, and may even fluctuate over time in individuals.

Correlation between the long-term memory T cell response to SARS-CoV-2 and disease severity

Next, we examined the differences in the magnitude and breadth of memory CD4 and CD8 T cell responses in CIs according to their different degrees of COVID-19 severity. CIs were stratified according to the severity of disease into asymptomatic (ACs: 19.35%, 6/31), moderate (MCs: 61.29%, 19/31), and severe COVID-19 cases (SCs: 19.35%, 6/31). No significant difference in the age between the symptomatic and asymptomatic cases was observed. In general, the magnitude of SARS-CoV-2 memory T cell responses against S, N or M, either for the overall or individual cytokine production, were lower in ACs than in MCs and SCs, but the differences were not statistically significant (Fig. 4a, 4b and S3). Also no significant correlations were observed between the magnitude of SARS-CoV-2 memory T cell responses and clinical parameters indicating disease severity, including white blood cell and

lymphocyte numbers, IL-6, C-reactive protein, D-dimer, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, serum creatinine, fibrinogen (FIB), and blood urea nitrogen levels (Fig. S4). However, memory CD4 T cell responses against S, N, and M became undetectable in 50% (3/6) of ACs, but only in 5.26% (1/19) of MCs ($P=0.031$, Fig. 4a). Memory CD8 T cell responses against S, N, and M became undetectable in 50% (3/6) of ACs, but only in 21.05% (4/19) of MCs and 16.67% (1/6) of SCs, respectively (Fig. 4b). No AC showed memory CD8 T cell responses against multiple peptide pools, while 52.63% (10/19) of MCs and 66.67 (4/6) of SCs showed memory CD8 T cell responses to at least 2 different peptide pools (Fig. 4b).

Elderly people are predisposed to develop severe COVID-19 and mortality increases dramatically with age¹⁴. We have previously shown that the cytotoxic CD8 T cell response is impaired in elderly COVID-19 patients¹⁵. Next, we analyzed correlations between the magnitude and breadth of memory CD4 and CD8 T cell responses and age in CIs. We observed that the breadth, but not the magnitude of memory CD4 T cell responses was inversely correlated with the age of CIs ($r^2=0.162$, $P=0.016$, Fig. 5a). No significant correlation between the magnitude and breadth of memory CD8 T cell responses and the age of CIs were observed (Fig. 5b).

CD4 memory T cell responses in individuals who lost their IgG response to SARS-CoV-2

During the acute phase of COVID-19, T cell responses positively correlated with the magnitude of antibody responses^{5,7,8}. However, to our knowledge it is not clear whether this association is maintained during the long-term convalescence. To this end, we compared memory T cell responses and antibody responses in CIs from 83 to 274 dpdo. As shown in Fig. S5, the magnitude of memory CD4 and CD8 T cell responses against S and N showed no significant correlation with the titers of corresponding IgG against S and N. From our large convalescent out-patient cohort very few patients lose their SARS-COV-2-specific IgG responses over time. We were interested if those patients still kept their memory T cells. We therefore selected 8 IgG-seronegative CIs and compared them to 23 seropositive CIs. At the time point of last sampling the age of the IgG-seronegative CIs was significantly lower, and the dpdo was significantly higher, than those of the 23 IgG-seropositive CIs (Fig. S6a). To overcome this bias, we compared the magnitude and breadth of memory T cell responses of IgG-seronegative CIs with 7 selected IgG-seropositive CIs with comparable age and dpdo (Fig. S6b). Interestingly, memory CD4 T cell responses against N and M were significantly higher in IgG-seronegative CIs than those in IgG-seropositive CIs (Fig. 6a). A tendency of increased memory CD4 T cell response against S in IgG-seronegative CIs was also observed, although the difference remained close to the borderline of statistical significance ($P=0.052$, Fig. 6a). All IgG-seronegative CIs showed memory CD4 T cell responses to at least 2 peptide pools, while 28.57% IgG-seropositive CIs showed no memory CD4 T cell responses to S, N or M (Fig. 6b). In contrast to CD4 T cells, no significant differences in the

300 magnitude and breadth of memory CD8 T cell responses between the
301 IgG-seronegative and -seropositive CIs were observed (Fig. 6c and 6d), indicating
302 that CD8 T cells seem to be less correlated with humoral immune responses as
303 compared to CD4 T cells.

304

305 **Discussion**

306 One of the most important and challenging questions facing medicine today concerns
307 the extent to which immunity develops and persists following COVID-19. Previous
308 studies suggest that the persistence of protective immunity against different
309 coronaviruses varies significantly, since those against seasonal coronavirus are
310 short-lived¹⁶ while those against SARS and *middle east respiratory syndrome*
311 *coronavirus* (MERS) are described to last longer^{6,17,18}. Recent studies have
312 demonstrated that macaques infected with SARS-CoV-2 are resistant to reinfection
313 with the same virus isolate following recovery from their initial infection, suggesting
314 the cellular and/or humoral immunity facilitated by the primary infection might have
315 protected the same nonhuman primates against secondary encounters^{19,20}. However,
316 in both studies, reinfections with SARS-CoV-2 were carried out within a relative short
317 time window (4 and 5 weeks after the primary infection). In contrast to the
318 observation in the macaque model, there are some reports demonstrating the principle
319 possibility of reinfections with SARS-CoV-2 in humans²¹⁻²⁴. It has been suggested
320 that the lifespan of the humoral response following SARS-CoV-2 infection is
321 relatively short, especially in mild and asymptomatic cases²⁵. Some believe that

322 although SARS-CoV-2 infection may blunt long-lived antibody responses, immune
 323 memory might still be achieved through virus-specific memory T cell responses ²,
 324 which have been detected in most recently recovered individuals, including
 325 asymptomatic cases and those with undetectable antibody responses ⁹. Here we
 326 provide, to our knowledge, the first characterization of long-term memory T cell
 327 responses in a cohort of COVID-19 convalescent individuals up to 9 months
 328 following primary SARS-CoV-2 infection. We show that the magnitude and breadth
 329 of long-term memory T cell responses to SARS-CoV-2 are heterogeneous. While the
 330 majority of CIs demonstrate strong and broad memory T cell responses up to 9
 331 months post disease onset, some individuals have lost their T cell responses against
 332 the studied antigens within half a year. The magnitude of SARS-CoV-2 memory CD4
 333 T cell response is inversely correlated with the time that had elapsed from disease
 334 onset within 180 days, suggesting SARS-CoV-2 memory CD4 T cell response may
 335 wane over time at the early months following primary SARS-CoV-2 infection.
 336 Intriguingly, half of the asymptomatic cases have lost their memory CD4 and CD8 T
 337 cell responses, suggesting the memory T cell responses might be less durable in
 338 asymptomatic cases than in symptomatic cases. The breadth of memory CD4 T cell
 339 responses were inversely correlated with the age of the patients, suggesting the
 340 memory T cell responses might also be less durable in elderly individuals. Moreover,
 341 the kinetics of memory T cell responses are heterogeneous in the herein examined CIs,
 342 while some show a sharp decline of memory T cell responses over time, others show
 343 rather sustained or even increasing memory T cell responses. Our data document a

durability of cellular immunity against SARS-CoV-2, however, for a fraction of elderly individuals with asymptomatic infections a considerable waning of cellular immunity may occur. Our results also suggest that the intensity of SARS-CoV-2 memory T cell responses detected in peripheral blood may fluctuate over time in CIs, which is unlikely to be caused by reexposure to SARS-CoV-2, since the possibility of local spread of the virus in Wuhan and nearby area has been precluded by the thorough SARS-CoV-2 RNA test conducted in May for every resident. Future studies are needed to closely monitor the SARS-CoV-2 memory T cell responses to address how the intensities of these responses are regulated in CIs.

Different from the observation during and shortly after the acute phase of SARS-CoV-2 infection^{5,7,8}, we observe that the magnitudes of long-term SARS-CoV-2-specific cellular and humoral responses are not positively correlated with each other. In contrast, IgG-seronegative CIs demonstrate even stronger SARS-CoV-2-specific memory CD4 T cell responses than IgG-seropositive CIs. A recent study started to investigate the possible mechanisms of short-lived antibody responses observed in COVID-19 patients and has reported that germinal centers in secondary lymphoid organs were largely absent during the acute phase of COVID-19²⁶. The authors speculate that the absence of germinal centers is a result of abundant Th1 cell responses and aberrant extra-follicular TNF- α accumulation²⁶. Consistently, our current observation, that CIs with short-lived antibody responses demonstrate an increased magnitude of SARS-CoV-2-specific CD4 T cell responses, provides the first

366 evidence that the above-mentioned effect may extent to a far longer period in the
 367 convalescent phase of COVID-19. Although it remains unclear which arms of the
 368 adaptive immune response are responsible for protection against SARS-CoV-2
 369 infection, our data demonstrate that CIs may possess at least one arm of the adaptive
 370 immune response against SARS-CoV-2 long-term post recovery. Further
 371 characterization of the protective roles as well as the interaction of cellular and
 372 humoral immune responses against SARS-CoV-2 has significant implications for
 373 vaccine development and application especially in terms of the need for booster
 374 vaccinations.

375

376 Taken together, we provide the first comprehensive characterization of the long-term
 377 memory T cell responses against SARS-CoV-2, suggesting that the
 378 SARS-CoV-2-specific T cell immunity is sustained in the majority of CIs up to 9
 379 months post infection. The observation that convalescent individuals turning
 380 IgG-seronegative generated robust and sustained memory T cell responses further
 381 suggests that natural infection could prevent recurrent episodes of severe COVID-19.

382

383

384 **Conflict-of-interest disclosure**

385 The authors declare no relevant conflict of interest.

386

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396

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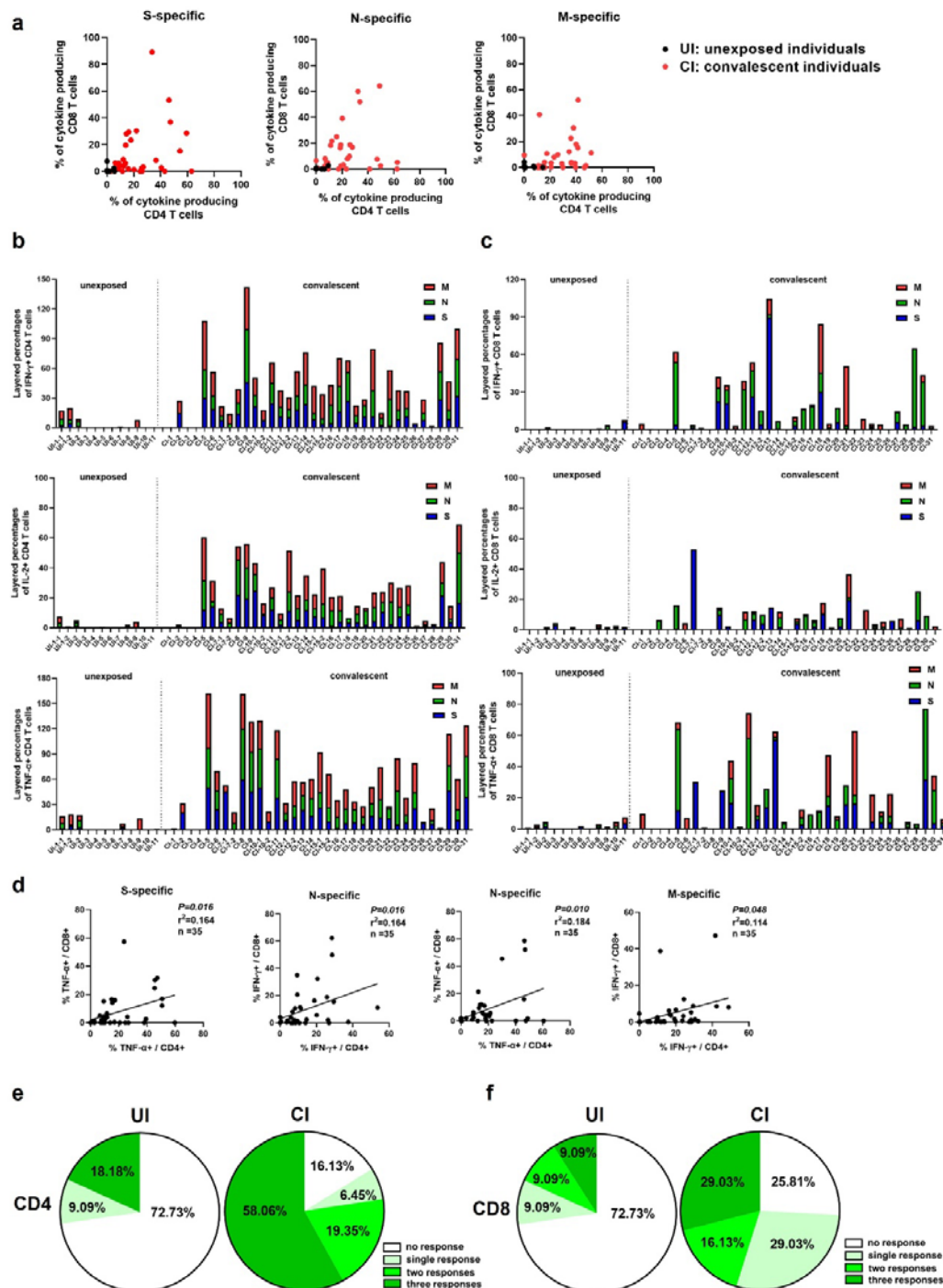
454 **Table 1. Baseline characteristics of the Chinese cohort.**

Parameter	Unexposed individuals	Convalescent Individuals
n	11	31
Gender (M/F)	3/8	3/28
Age	30.5	44.1
Asymptomatic cases %	/	19.35% (6/31)
Mild cases %		61.29% (19/31)
Severe cases %	/	19.35% (6/31)
Days from onset		169 (83-274)
Days from recovery	/	151 (42-249)
Clinical parameters		
Fever %	/	64.52% (20/31)
Respiratory symptoms %	/	58.06% (18/31)
Hospitalized %	/	56.67% (17/31)
Oxygen therapy %	/	46.67% (14/31)
Laboratory parameters		
Leukopenia %	/	52.94% (9/17)
Lymphopenia %	/	76.47% (13/17)
Increased CRP %	/	70.59% (12/17)
Increased ferritin %	/	40.00% (4/10)
Increased LDH %	/	40.00% (6/15)
Abnormal liver function %	/	53.33% (8/15)
Abnormal renal function %	/	0 (0/15)
Increased CK %	/	20.00% (3/15)
Abnormal blood coagulation %	/	6.67% (1/15)
Increased IL-6 %	/	85.71% (12/14)
CT scan		
Normal %	/	25.81% (8/31)
Viral pneumonia %	/	74.19% (23/31)
Virological markers		
RNA positive %	/	51.61% (16/31)
IgG single positive %	/	45.16% (14/31)
IgM & IgG positive %	/	29.03% (9/31)
IgG negative %	/	25.81% (8/31)

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457 **Figures**



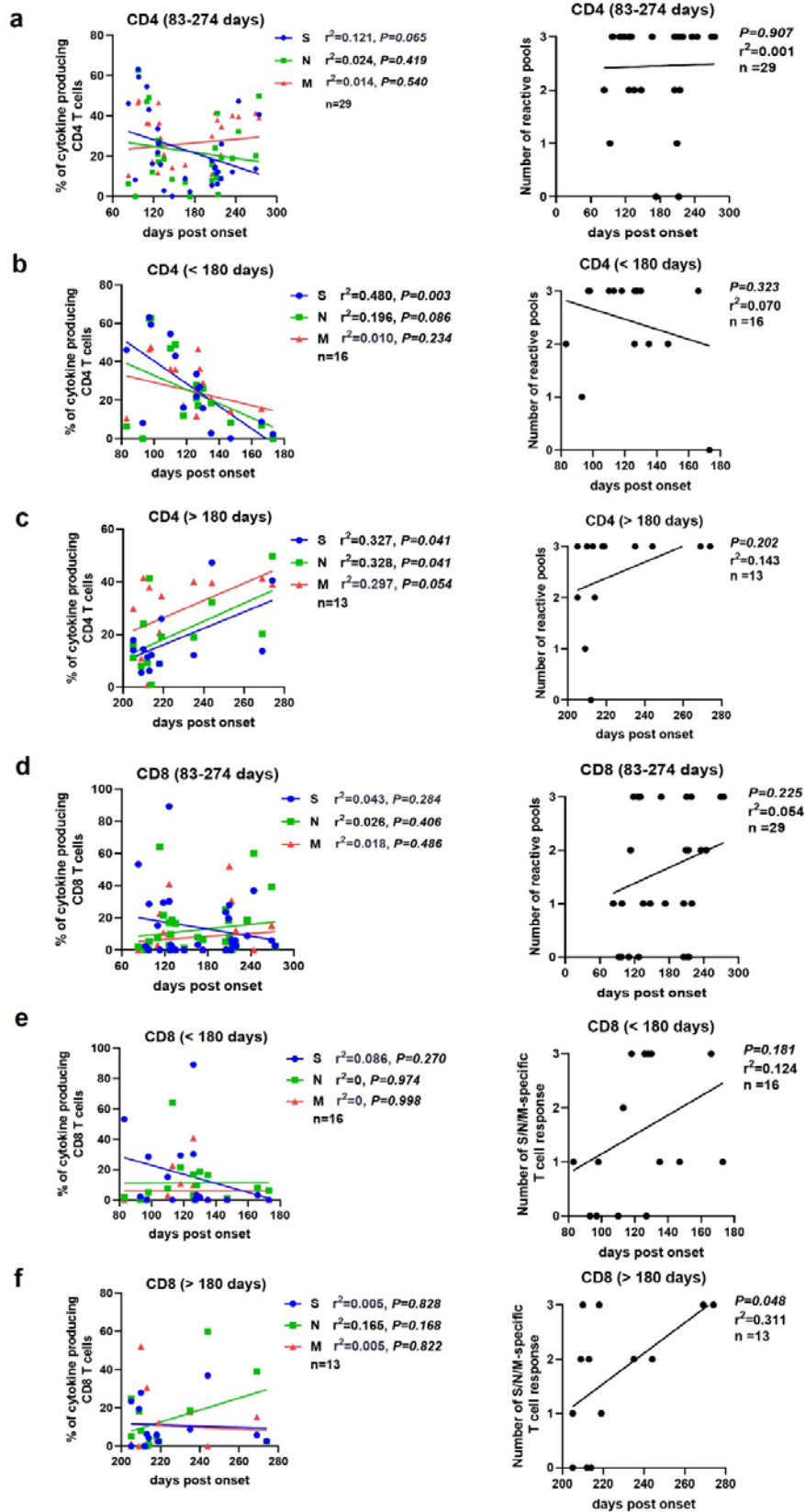
458

459 **Figure 1. The magnitude and breadth of long-term SARS-CoV-2 memory T cell**

460 **responses are heterogeneous in COVID-19 convalescent individuals. PBMCs of**

461 **SARS-CoV-2-unexposed individuals (UI) and COVID-19 convalescent individuals**

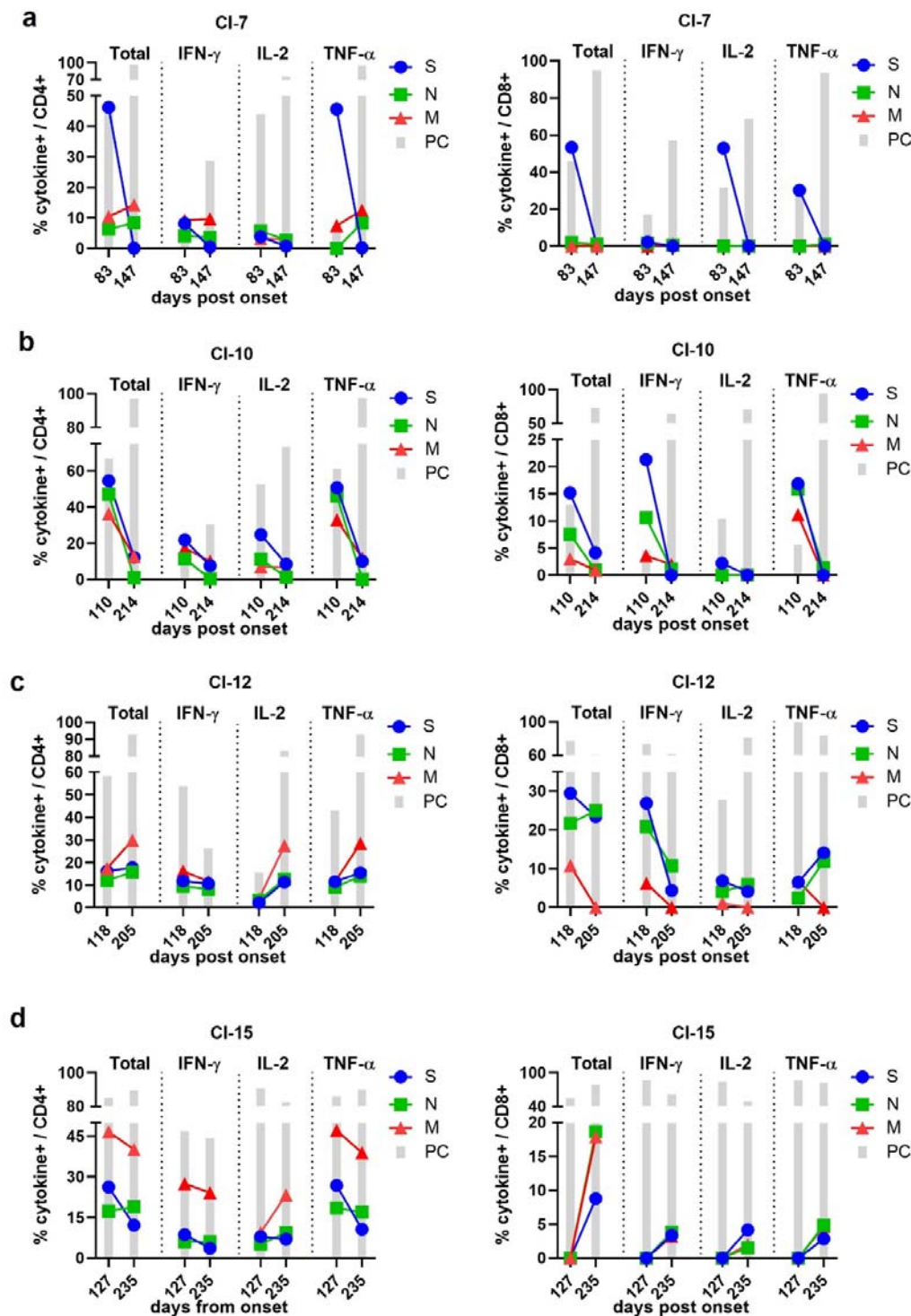
(CI) were tested for responses to 3 panels of overlapping peptides spanning the SARS-CoV-2 S, N, and M, respectively, using intracellular cytokine staining flow cytometry assay. (a) The magnitude of overall cytokine responses of CD4 and CD8 T cells against S, N, and M of SARS-CoV-2 of all participants are shown. (b and c) The magnitude of IFN- γ , IL-2, and TNF- α responses of CD4 and CD8 T cells specific to S, N, and M of SARS-CoV-2 of all participants are also shown individually. Each colored segment represents the source protein corresponding to peptide pools eliciting T cell responses. Bars superimpose percentages of separate T cell culture experiments individually stimulated with indicated antigens. (d) The correlations between the magnitudes of memory CD4 and CD8 T cell responses, as represented by indicated cytokine production, are shown (Pearson product-moment correlation coefficient). (e and f) Breadth of T cell responses of UI and CI. The breadth of T cell responses was calculated by the number of reactive peptide pools of S, N, and M. S: surface glycoprotein; N: nucleocapsid phosphoprotein; M: membrane glycoprotein; IFN: interferon; IL: interleukin; TNF: tumor necrosis factor.



477

478 **Figure 2. Correlation between the magnitude of SARS-CoV-2 memory T cell**

479 **responses and the time that had elapsed from disease onset.** The correlation
 480 between the magnitude of memory CD4 T cell responses specific to S, N and M and
 481 days post disease onset up to 274 days (a), within 180 days (b) and over 180 days (c)
 482 are shown. The correlation between the magnitude of memory CD8 T cell responses
 483 specific to S, N and M and days post disease onset up to 274 days (d), within 180 days
 484 (e) and over 180 days (f) are shown. Pearson product-moment correlation coefficient
 485 test was used to test the significance and P value and r^2 value (correlation coefficient)
 486 are indicated in each panel. S: surface glycoprotein; N: nucleocapsid phosphoprotein;
 487 M: membrane glycoprotein.



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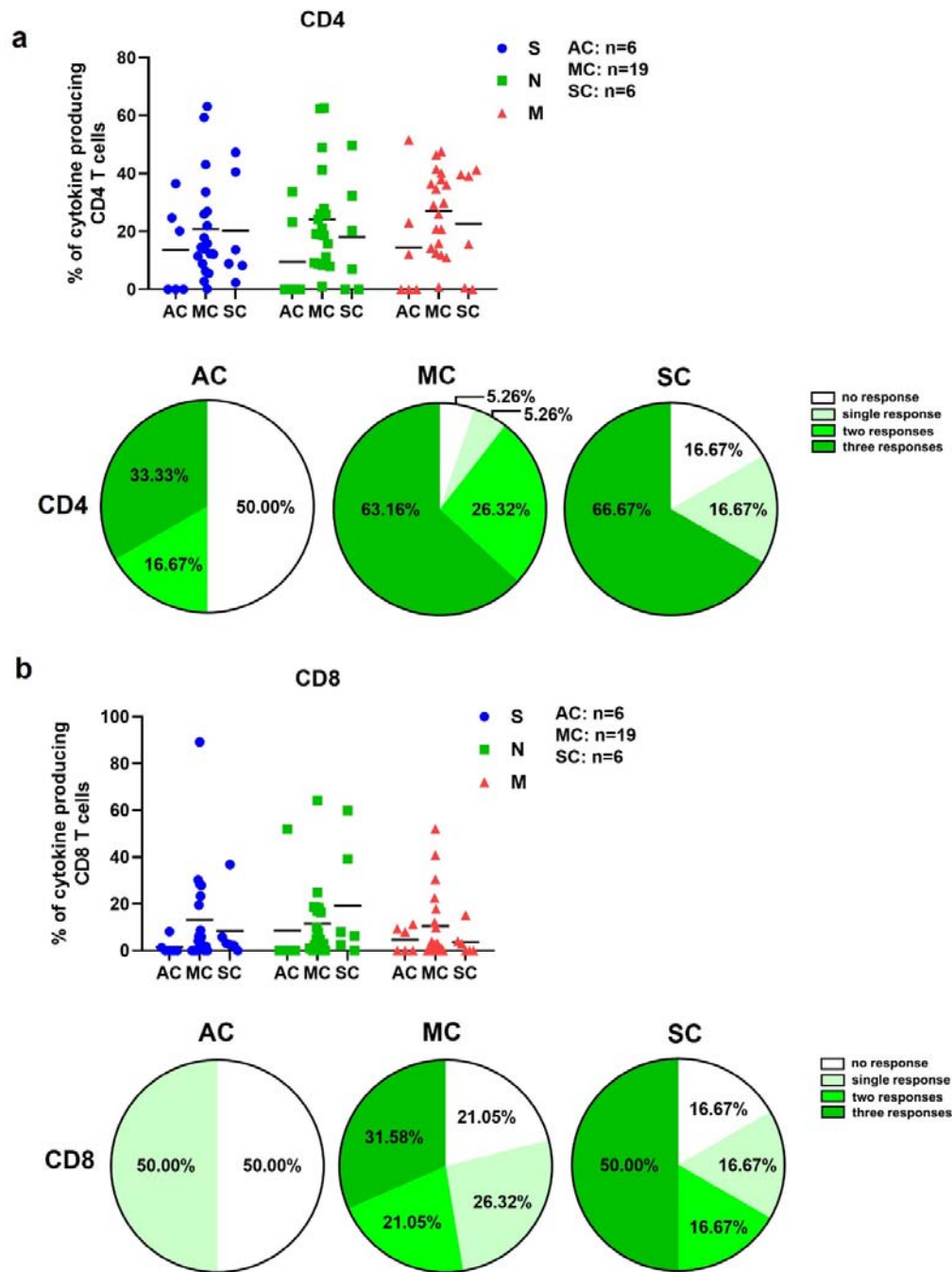
489 **Figure 3. Kinetics of memory T cell responses to SARS-CoV-2 in COVID-19**

490 **convalescent individuals.** PBMCs were longitudinally collected from 4 COVID-19

491 convalescent individuals at indicated time points and were tested for memory T cell

492 responses recognizing SARS-CoV-2 S, N or M by using intracellular cytokine
 493 staining flow cytometry assay. (a) CI-7; (b) CI-10; (c) CI-12; (d) CI-15. S: surface
 494 glycoprotein; N: nucleocapsid phosphoprotein; M: membrane glycoprotein; PC:
 495 positive control stimulation; IFN: interferon; IL: interleukin; TNF: tumor necrosis
 496 factor.
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500 **Figure 4. Loss of SARS-CoV-2 memory CD4 T cell responses is more frequent in**

501 **asymptomatic cases than symptomatic cases.** The magnitude and breadth of

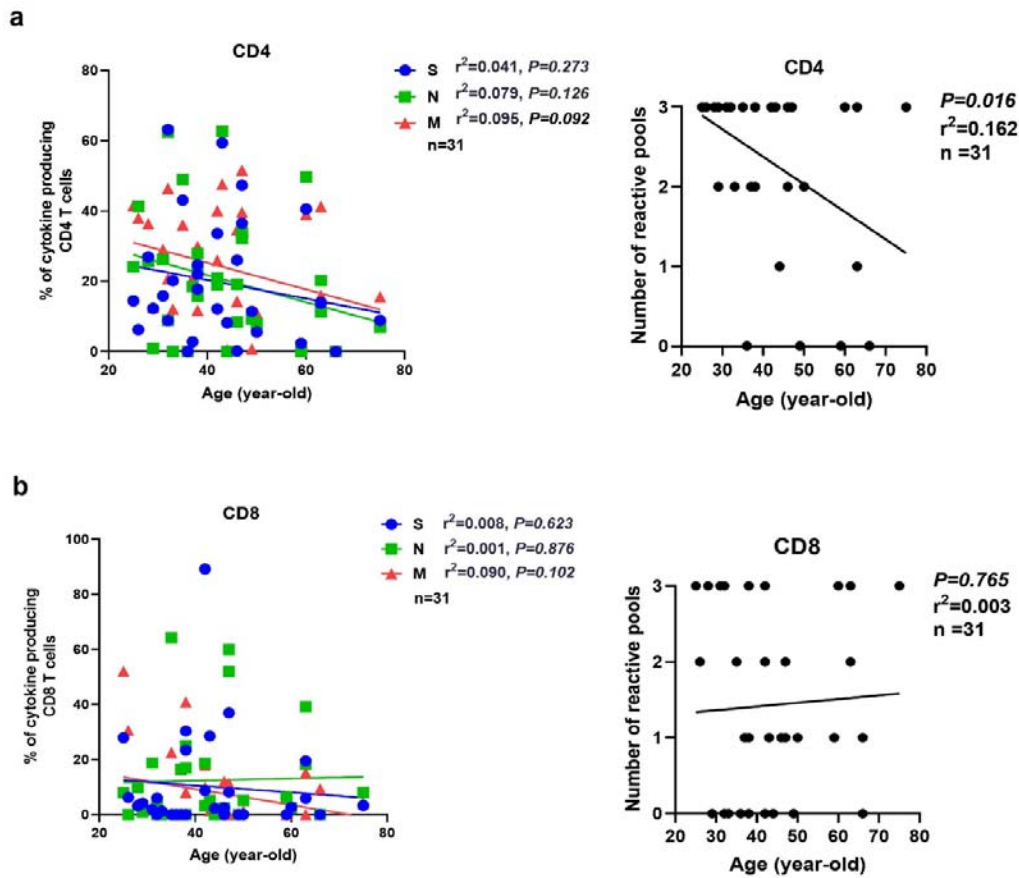
502 memory CD4 (a) and CD8 (b) T cell responses are compared between the

503 asymptomatic (AC, n=6), moderate (MC, n=19) and severe (SC, n=6) cases. S:

504 surface glycoprotein; N: nucleocapsid phosphoprotein; M: membrane glycoprotein.

505

506



507

508 **Figure 5. The breadth of long-term SARS-CoV-2 memory CD4 T cell responses is**
 509 **negatively correlated with the age of COVID-19 convalescent individuals.** The
 510 correlation between the magnitude and breadth of memory CD4 (a) and CD8 (b) T
 511 cell responses specific to S, N and M and age are shown. Pearson product-moment
 512 correlation coefficient test was used to test the significance and P value and r^2 value
 513 (correlation coefficient) are indicated in each panel. S: surface glycoprotein; N:
 514 nucleocapsid phosphoprotein; M: membrane glycoprotein.

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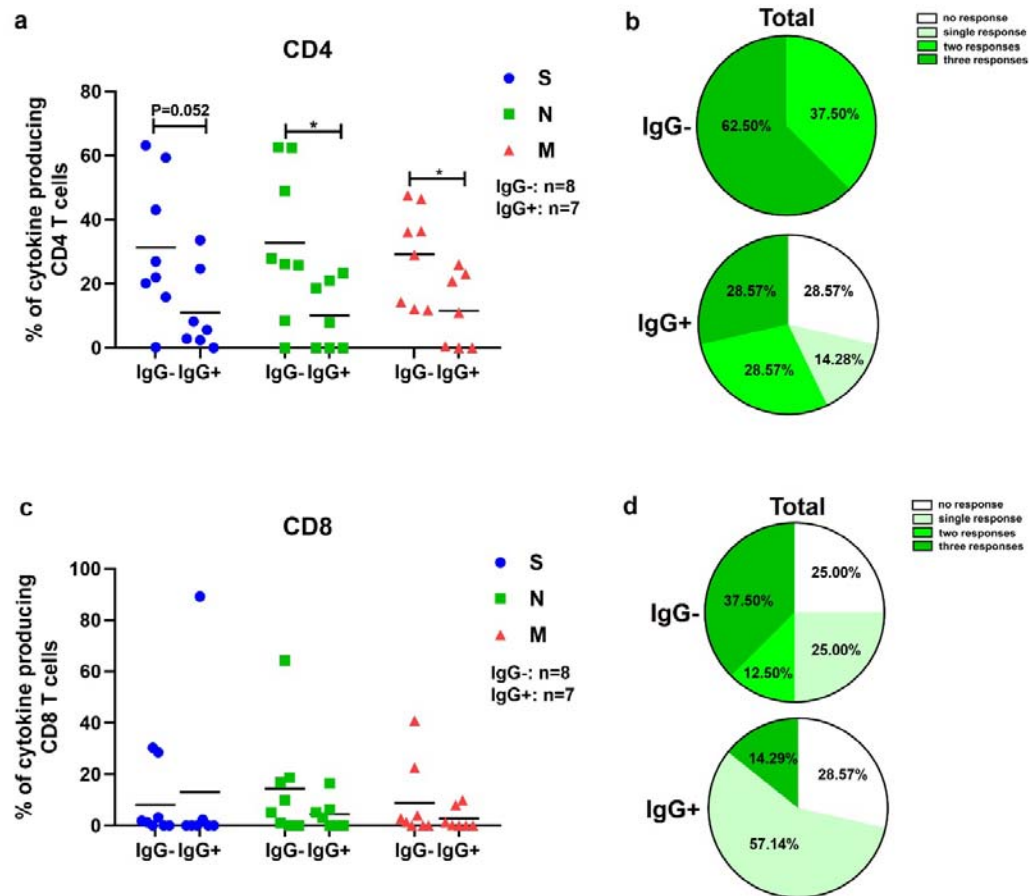


Figure 6. The long-term SARS-CoV-2 memory CD4 T cell responses is robust in IgG-seronegative COVID-19 convalescent individuals. The magnitude (a) and breadth (b) of memory CD4 T cell responses are compared between IgG-seronegative (IgG-, n=8) and IgG-seropositive (IgG+, n=7) CIs. The magnitude (c) and breadth (d) of memory CD8 T cell responses are compared between IgG-seronegative (IgG-, n=8) and IgG-seropositive (IgG+, n=7) CIs. Statistically significant differences are indicated by asterisks (* < 0.05, Non-parametric Mann-Whitney test). S: surface glycoprotein; N: nucleocapsid phosphoprotein; M: membrane glycoprotein.