

1 **Characterization of humoral and SARS-CoV-2 specific T cell responses in people living with**  
2 **HIV**

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20 **One Sentence Summary: Adaptive immune responses to SARS-CoV-2 in the setting of HIV**  
21 **infection**

22

23

24 **Abstract**

25 There is an urgent need to understand the nature of immune responses generated against SARS-  
26 CoV-2, to better inform risk-mitigation strategies for people living with HIV (PLWH). Although  
27 not all PLWH are considered immunosuppressed, residual cellular immune deficiency and  
28 ongoing inflammation could influence COVID-19 disease severity, the evolution and durability  
29 of protective memory responses. Here, we performed an integrated analysis, characterizing the  
30 nature, breadth and magnitude of SARS-CoV-2-specific immune responses in PLWH, controlled  
31 on ART, and HIV negative subjects. Both groups were in the convalescent phase of  
32 predominately mild COVID-19 disease. The majority of PLWH mounted SARS-CoV-2 Spike-  
33 and Nucleoprotein-specific antibodies with neutralizing activity and SARS-CoV-2-specific T  
34 cell responses, as measured by ELISpot, at levels comparable to HIV negative subjects. T cell  
35 responses against Spike, Membrane and Nucleocapsid were the most prominent, with SARS-  
36 CoV-2-specific CD4 T cells outnumbering CD8 T cells. Notably, the overall magnitude of  
37 SARS-CoV-2-specific T cell responses related to the size of the naive CD4 T cell pool and the  
38 CD4:CD8 ratio in PLWH, in whom disparate antibody and T cell responses were observed. Both  
39 humoral and cellular responses to SARS-CoV-2 were detected at 5-7 months post-infection,  
40 providing evidence of medium-term durability of responses irrespective of HIV serostatus.  
41 Incomplete immune reconstitution on ART and a low CD4:CD8 ratio could, however, hamper  
42 the development of immunity to SARS-CoV-2 and serve as a useful tool for risk stratification of  
43 PLWH. These findings have implications for the individual management and potential  
44 effectiveness of vaccination against SARS-CoV-2 in PLWH.

45

46

47 **Introduction**

48 The global outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing  
49 COVID-19 disease, has resulted in an overall 3% case fatality rate, posing unprecedented  
50 healthcare challenges around the world (Guan et al. 2020). With an evolving pandemic, urgent  
51 and efficient strategies are required for optimised interventions especially in patient populations  
52 with underlying chronic diseases. Nearly 40 million people are living with HIV (PLWH)  
53 worldwide and almost half of PLWH in Europe are over the age of 50 (Harris et al. 2018).  
54 However, due to scarcity of data it remains unknown whether antiviral responses to SARS-CoV-  
55 2 are compromised and/or less durable in PLWH following primary infection. Such knowledge is  
56 crucial in the future clinical management of PLWH during the course of the pandemic and for  
57 informing strategies for vaccination programmes.

58  
59 Epidemiological evidence indicates that the risk of severe COVID-19 disease increases with age,  
60 male gender, and in the presence of comorbidities (Williamson et al. 2020; Wu and McGoogan  
61 2020). PLWH, despite efficient virological suppression on antiretroviral treatment (ART),  
62 experience an increased burden of comorbid conditions associated with premature ageing (De  
63 Francesco et al. 2019; Guaraldi et al. 2011). These multi-morbidities are driven by residual  
64 inflammation on ART and ongoing immune dysregulation (Deeks and Phillips 2009) that could  
65 influence COVID-19 disease severity, the durability of protective antiviral responses, which may  
66 prevent future re-infection, and responsiveness to vaccination (Moir and Fauci 2017; Pallikkuth  
67 et al. 2012). Although there is no evidence of increased rates of COVID-19 disease among  
68 PLWH compared to the general population, mortality estimates vary between studies, with  
69 disparities in social health determinants and comorbidities likely having an influence (Bhaskaran

70 et al. 2021; Blanco et al. 2020; Boulle et al. 2020; Cooper et al. 2020; Geretti et al. 2020; Inciarte  
71 et al. 2020; Sigel et al. 2020). More recently, cellular immune deficiency and a lower CD4 T cell  
72 count/low CD4 T cell nadir have been identified as potential risk factors for severe SARS-CoV-2  
73 infection in PLWH, irrespective of HIV virological suppression (Hoffmann et al. 2020).  
74 Burgeoning evidence supports a role for CD4 T cells in the control and resolution of acute  
75 SARS-CoV-2 infection (Oja et al. 2020; Rydyznski Moderbacher et al. 2020; Tan et al. 2021), in  
76 addition to providing CD8 T cell and B cell help for long-term immunity (Crotty 2015; Laidlaw  
77 et al. 2016). Any pre-existing CD4 T cell depletion in PLWH, as described in patients with  
78 haematological malignancy (Annika Fendler 2020), could therefore be a potential driver of  
79 dysregulated immunity to SARS-CoV-2, hampering antiviral responses (Calvet et al. 2020) and  
80 development of immunological memory.

81  
82 Despite the collective efforts to define the correlates of immune protection and evaluate the  
83 durability of protective immune responses elicited post SARS-CoV-2 infection in the general  
84 population, reports in PLWH are limited. Overall, the majority of people infected with SARS-  
85 CoV-2 in the absence of HIV develop durable antibody responses including neutralizing  
86 antibodies and T cell responses (Dan et al. 2020; Ripperger et al. 2020; Rydyznski Moderbacher  
87 et al. 2020; Seow et al. 2020; Wajnberg et al. 2020). In most cases the magnitude of humoral  
88 responses is complemented by multi-specific T cell responses and appears to be dependent on the  
89 severity and protracted course of COVID-19 disease (Robbiani et al. 2020; Rydyznski  
90 Moderbacher et al. 2020; Wajnberg et al. 2020). However, humoral and cellular immune  
91 responses are not always correlative, with T cell immunity being induced even in the absence of  
92 detectable antibodies during mild COVID-19 disease (Reynolds et al. 2020; Rydyznski

93 Moderbacher et al. 2020; Sekine et al. 2020) and predicted to be more enduring from experience  
94 with other coronaviruses (Le Bert et al. 2020; Ng et al. 2020). Notably, older individuals more  
95 often display poorly co-ordinated immune adaptive responses to SARS-CoV-2 associated with  
96 worse disease outcome (Rydyznski Moderbacher et al. 2020; Sattler et al. 2020). This is  
97 particularly pertinent for PLWH, in whom the combined effect of ageing/premature  
98 immunosenescence and residual immune dysfunction in the era of effective of ART could have  
99 important consequences for the development of immune responses to a new pathogen and  
100 vaccination (George et al. 2015). To date, a single case report suggests a longer disease course  
101 and delayed antibody response against SARS-CoV-2 in HIV patients (Wang et al. 2020), but a  
102 simultaneous assessment of antibodies and T cell responses in the convalescent phase of  
103 COVID-19 disease is lacking in PLWH.

104

105 To address this knowledge gap, we performed an integrated cross-sectional analysis of different  
106 branches of adaptive immunity to SARS-CoV-2 in PLWH, controlled on ART, compared to HIV  
107 negative individuals recovered from mainly non-hospitalized mild COVID-19 disease. Our data  
108 reveal an association between the magnitude of SARS-CoV-2 T cell responses and the CD4:CD8  
109 ratio in PLWH, in whom a decreased representation of naïve CD4 T cell subsets could  
110 potentially compromise protective immunity to SARS-CoV-2 infection and/or vaccination.

111

## 112 **Results**

### 113 **COVID-19 cohort**

114 Forty-seven individuals with HIV infection, well controlled on ART (for >2 years) with an  
115 undetectable HIV RNA, were recruited for this study during a defined period of time between

116 July 2020 and November 2020. Of these donors, twenty four previously had laboratory  
117 confirmed SARS-CoV-2 diagnosis (RT-PCR+ and/or Ab positive) with a median days post-  
118 symptom onset (DPSO) of 148 days; twenty three were probable/possible cases with a higher  
119 median DPSO of 181 days. The majority had ambulatory mild COVID-19 disease not requiring  
120 hospitalization (score 1-2 on WHO criteria). Eight subjects out of the laboratory confirmed cases  
121 had moderate disease requiring hospitalization (score 4-5 on WHO criteria). The ages of the  
122 subjects ranged from 30-73 years of age (median 52 years old) and were predominately White  
123 Caucasian males. The cohort included donors capturing a range of CD4 counts (133-1360) and  
124 CD4:CD8 ratios (0.17-2.54), reflective of the different lengths of HIV infection/CD4 T cell nadir  
125 and variable levels of immune reconstitution post treatment. As a comparator group we sampled  
126 thirty five HIV seronegative health care workers (HCW), thirty one with laboratory confirmed  
127 SARS-CoV-2 diagnosis and four suspected/household contacts of a confirmed case. The HCW  
128 group had a mild course of COVID-19 disease sampled at a similar median DPSO, with four  
129 donors recruited in the convalescent phase post moderate disease (score 4-5 on WHO criteria).  
130 HIV negative subjects were younger in age (range 26-65; median 41) with a more equal  
131 female:male distribution (**Table S1**). A group of HIV positive (n=16) donors with samples stored  
132 prior to the pandemic, matched to the HIV cohort recovered from COVID-19 disease, was used  
133 as controls. Inter-experimental variability was minimized by running matched cryopreserved  
134 samples in batches with inter-assay quality controls. Further details on patients' characteristics  
135 and comorbidities are included in **Table S1**.

136

137 **Levels of SARS-CoV-2 antibodies in the study groups**

138 ELISA was used to screen plasma samples for antibodies against the external Spike antigen,  
139 using immobilized recombinant Spike S<sub>1-530</sub> subunit protein (S1), and against immobilized full-  
140 length internal Nucleoprotein (N) antigen to confirm prior infection as previously described (Ng  
141 et al. 2020; O'Nions et al. 2021; Pickering et al. 2020) (**Fig. 1A**). A sample absorbance greater  
142 than 4-fold above the average background of the assay was regarded as positive, using a  
143 threshold established with pre-pandemic samples (**Fig. S1A, B**) and as previously described  
144 (Pickering et al. 2020). The screening assay, followed by titer quantification (based on an in-  
145 assay standard curve) (O'Nions et al. 2021), demonstrated that 95.8% (23/24) of individuals from  
146 the HIV positive group with prior laboratory confirmed COVID-19 and 30.43% (7/23) with  
147 suspected disease, during the first wave of the pandemic, had measurable titers for SARS-CoV-2  
148 S1 and N sampled at a median 146 DPSO (DPSO range 46-232) and 181 DPSO (range 131-228),  
149 respectively (**Fig. 1A-C**). Similarly, in the HIV negative group with laboratory confirmed  
150 COVID-19 disease, 93.5% (29/31) had detectable SARS-CoV-2 antibodies to S1 and N at 146  
151 DPSO (101-220), whereas none of the suspected/household contacts in this group (0/4) had  
152 quantifiable titers (DPSO median 200; range 125-203) (**Fig. 1A-C**). S1 and N titers were found  
153 to be comparable between the HIV positive and negative groups (**Fig. 1B, C**) and correlated with  
154 one another, although levels were heterogenous among donors as previously observed (**Fig. 1D**).

155  
156 To determine whether the SARS-CoV-2 antibodies generated are able to inhibit SARS-CoV-2  
157 infection, we employed a serum neutralisation assay with pseudotyped SARS-CoV-2, to  
158 calculate the 50% inhibitory serum dilution (ID<sub>50</sub>) (Seow et al. 2020). Overall, we detected  
159 similar neutralization levels (**Fig. 1E**) and comparable profiles across the two study groups in  
160 terms of the number of individuals with high potency, low potency or no neutralizing activity

161 (<50 ID<sub>50</sub>) (**Fig. 1F**), which correlated with anti-S1 IgG levels (**Fig. 1G**). A range of  
162 neutralizing antibodies (nAb) was detected in the groups, with some samples exhibiting strong  
163 neutralization despite low S1 titers irrespective of disease severity (**Fig. 1G**).

164

165 No association was observed between S1 binding titers, age and gender in the two groups (**Fig.**  
166 **S1C**). A weak positive correlation was seen between neutralization levels and age according to  
167 male gender in the HIV positive group, where subjects were older and females were notably  
168 under-represented (**Fig. S1D**). Neutralization levels did not correlate with DPSO (**Fig. S1E**) and  
169 were detectable up to 7 months post infection. No clear association was observed according to  
170 ethnicity (**Fig. S1F**). Together these results show no significant differences in the IgG-specific  
171 antibody response to SARS-CoV-2 and neutralization capacity according to HIV status after  
172 recovery from COVID-19 disease. These findings should be considered in the context of this  
173 cohort in which the majority of cases were mild and therefore may not reflect the full burden of  
174 disease associated with SARS-CoV-2 infection.

175

#### 176 **SARS-CoV-2 multi-specific T cell responses**

177 The presence of T helper 1 (Th1) immunity has been described in a number of studies  
178 investigating T cell-specific immune responses to SARS-CoV-2 infection in various phases of  
179 the infection. We therefore initially assessed global SARS-CoV-2 T cell frequencies by IFN- $\gamma$ -  
180 ELISpot using overlapping peptide (OLP) pools to detect T cell responses and cumulative  
181 frequencies directed against defined immunogenic regions, including Spike, Nucleocapsid (N),  
182 membrane (M), Envelope (Env), and open reading frame (ORF)3a, ORF6, ORF7 and ORF8  
183 (**Fig. 2A**). Out of the 30 HIV positive and 30 HIV negative individuals (including previously



184 laboratory confirmed cases and additional subjects found to be SARS-CoV-2 seropositive on  
185 screening), the majority of donors in each group had a demonstrable cellular response directed  
186 predominately against Spike and N/M. Responses to accessory peptide pools (ORFs) and the  
187 structural protein Env were less frequent and significantly lower to other antigens observed,  
188 irrespective of HIV status (**Fig. 2B and Fig. S2A, B**). The overall magnitude of responses  
189 against Spike, M and N did not differ significantly between the groups (**Fig. 2C**). In HIV  
190 positive donors the cumulative SARS-CoV-2 responses across all pools tested were lower in  
191 magnitude compared to that of T cells directed against well-defined CD8 epitopes from  
192 Influenza, Epstein Barr Virus (EBV) and Cytomegalovirus (CMV)-(FEC pools) tested in parallel  
193 within the same donors, but higher compared to HIV-gag responses (**Fig. 2D**). By contrast,  
194 responses to FEC pools were comparable in magnitude to the cumulative SARS-CoV-2-specific  
195 T cell responses detected in the HIV negative donors, likely reflecting the lower CMV  
196 seropositivity in the HIV negative group compared to the HIV positive group (54.28% CMV  
197 seropositive versus 97.87% CMV seropositive, respectively) (**Fig. 2D**). In line with previous  
198 studies we observed a wide breadth and range of cumulative SARS-CoV-2 T cell frequencies,  
199 with over 90% of donors in each group showing a response (**Fig. 2E, F**) (Grifoni et al. 2020;  
200 Peng et al. 2020; Reynolds et al. 2020). However, the proportion of HIV positive and negative  
201 donors with T cell responses to individual SARS-CoV-2 pools within given ranges varied, with a  
202 higher percentage of HIV positive donors having low level responses (**Fig. 2G**).

203

204 Responses to Spike, M and N peptide pools were significantly higher in donors with confirmed  
205 SARS-CoV-2 infection compared to subjects with no evidence of infection who displayed  
206 relatively weak responses; small responses were also noted in a proportion of HIV positive

207 subjects with available pre-pandemic samples (**Fig. S2C**). Additional work is required to  
208 investigate potential cross-reactive components of these responses with other human  
209 coronaviruses, as has been reported in other studies (Braun et al. 2020; Grifoni et al. 2020; Le  
210 Bert et al. 2020; Sekine et al. 2020). These data were derived from cryopreserved samples, which  
211 may underestimate the magnitude of the detected responses (Owen et al. 2007).

212  
213 Given the considerable heterogeneity in the magnitude of the observed responses in both groups,  
214 we related these to HIV parameters, age, gender and DPSO. We detected a positive correlation  
215 between CD4:CD8 ratio and summed total responses to OLP pools against SARS-CoV-2 in HIV  
216 positive subjects ( $r=0.3820$ ,  $p=0.037$ ) (**Fig. 2H**); this relationship was similar for N ( $r=0.4282$ ,  
217  $p=0.018$ ) and stronger for responses against M ( $r=0.4853$ ,  $p=0.007$ ) (**Fig. S2D-E**). These data  
218 suggest that, despite effective ART, incomplete immune reconstitution may potentially impact  
219 on the magnitude of T cell responses to SARS-CoV-2. Previous observations have demonstrated  
220 an association between SARS-CoV-2-specific T cells, age and gender, with T cell immunity to  
221 Spike increasing with age and male gender in some studies (Reynolds et al. 2020). Despite an  
222 older age and male predominance in our HIV cohort we did not detect any association between  
223 ELISpot responses to Spike and donor age (**Fig. S2F-G**). There was no correlation between  
224 DPSO and T cells directed either against Spike or total responses against SARS-CoV-2. These  
225 responses were nonetheless detectable up to 232 DPSO (median 151 range 46-232) in HIV  
226 positive subjects, and similarly in HIV negative donors (median 144; range 101-220) (**Fig. S2H-**  
227 **I**). Given that the majority of the donors, in both groups, experienced mild COVID-19 disease,  
228 any associations between the magnitude of responses and disease severity are limited. No

229 differences were observed in the magnitude of T cell responses according to ethnicity and  
230 gender, irrespective of HIV status (**Fig. SJ-K**).

231

### 232 **T cell and antibody response complementarity**

233 Next, we compared T cell responses, antibody levels and nAb responses in individual donors to  
234 better understand any complementarity between humoral and cellular responses detected by IFN-  
235  $\gamma$ -ELISpot. SARS-CoV-2-specific T cell responses correlated with antibody binding titers in the  
236 HIV negative group (**Fig. 3A, C**). Although the majority of HIV positive subjects had detectable  
237 antibody and T cell responses to SARS-CoV-2, the magnitude of the cellular immune responses  
238 correlated weakly only with N IgG binding titers but not with S1 IgG binding titers (**Fig. 3B, D**).

239 These data suggest that T cell and antibody responses are not always concordant, implying that  
240 the binding titer is not always a good predictor of the magnitude of the T cell response,  
241 especially in PLWH. Nonetheless, in some instances, despite low level or lack of detectable S1-  
242 binding IgG, a strong T cell response to SARS-CoV-2 was demonstrable, suggesting a degree of  
243 compensation between branches of adaptive immunity.

244

245 We subsequently examined neutralization ID50 values for individual donors in relation to T cell  
246 responses to individual SARS-CoV-2 antigen pools and summed responses. In HIV negative  
247 donors a correlation was observed between T cell responses to Spike protein and ID50 ( $r=$   
248  $0.4002$ ,  $p= 0.0315$ ), with donors lacking a response to Spike generally maintaining low-  
249 frequency T cell responses to other specificities. When cumulative responses were ranked by the  
250 magnitude of nAb response, a single HIV negative donor with an  $ID50 > 1000$  had no detectable  
251 SARS-CoV-2-specific T cells (**Fig. 3E**). In HIV positive donors no correlation was detected

252 between neutralization capacity and responses to individual SARS-CoV-2 peptides or pooled  
253 responses. A single HIV positive donor (1/29) with undetectable neutralization activity, and  
254 another donor with potent neutralization (>1000), had no measurable T cell response to any of  
255 the pools tested (**Fig. 3F**).

256

### 257 **SARS-CoV-2 specific T cell responses are dominated by CD4 T cells**

258 Following the initial broad screening of the antiviral responses to SARS-CoV-2, intracellular  
259 cytokine staining (ICS) was used to assess the composition and polyfunctionality of T cell  
260 responses in a group of HIV positive (n=11) and HIV negative (n=12) donors with available  
261 PBMC and detectable responses by IFN- $\gamma$ -ELISpot. To determine the functional capacity of  
262 SARS-CoV-2-specific CD4 and CD8 T cells, we stimulated PBMCs with overlapping Spike, M  
263 and N (non-Spike) peptide pools, in addition to CMV pp65 and HIV gag peptides within the  
264 same individuals. We focused on Spike, M and N as these antigens dominated responses detected  
265 by ELISpot. Expression of the activation marker CD154 and production of IFN- $\gamma$ , IL-2 and TNF-  
266  $\alpha$  were measured as functional readouts (**Fig. 4A**). SARS-CoV-2-specific CD4 T cells directed  
267 against Spike and non-Spike (M/N) predominantly expressed CD154 alone or in combination  
268 with IL-2, TNF- $\alpha$  and IFN- $\gamma$ , consistent with a Th1 profile, and these aggregated responses were  
269 comparable between the groups (**Fig. 4A, B**). SARS-CoV-2-specific CD4 T cells exhibited  
270 polyfunctional responses, with T cells expressing up to three cytokines (**Fig. 4C**). We detected  
271 no significant differences in CD4 T cell responses, according to cytokine profile, to individual  
272 pools directed against Spike, M and N in the two groups (**Fig. 3C and Fig. S3A**). Aggregated  
273 CD4 T cell responses against all SARS-CoV-2 pools tested were higher compared to CMV-  
274 specific responses and HIV-gag responses within the same donors (**Fig. S3D, E**).

275  
276 SARS-CoV-2-specific CD8 T cells largely expressed IFN- $\gamma$  alone or in combination with TNF- $\alpha$ ,  
277 exhibiting a different cytokine profile to CD4 T cells as expected (**Fig. 4D**). A trend toward  
278 lower mean aggregated CD8 T cell responses and polyfunctionality against Spike relative to non-  
279 Spike was observed in HIV negative individuals (**Fig. 4D, E, F**). Although SARS-CoV-2-  
280 specific CD8 T cell responses did not differ significantly between the two groups, mean response  
281 frequency was lower in HIV positive individuals against non-Spike pools (**Fig. 4E**). When we  
282 examined the individual cytokine profile, depending on antigen specificity, IL-2 production was  
283 reduced in CD8 T cells targeting non-Spike pools in HIV positive individuals compared to HIV  
284 negative donors (**Fig. S3F-H**). The proportion of CD8 T cells specific for CMV was higher  
285 compared to SARS-CoV-2-specific CD8 T cells irrespective of HIV status and similar to HIV-  
286 gag responses (**Fig. S3I and J**). Notably, SARS-CoV-2-specific CD8 T cells against Spike and  
287 non-Spike pools were less frequent, with CD4 T cells similarly outnumbering CD8 T cells  
288 regardless of HIV status (**Fig. 4G**). Total SARS-CoV-2-specific CD4 T cell responses correlated  
289 with the magnitude of T cell responses detected by ELISpots and with neutralization titers in the  
290 same individuals when data from HIV positive and negative donors were combined (**Fig. 4H, I**).  
291 This association was also seen between Spike and non-Spike-specific CD4 T cells detected via  
292 ICS and overall T cell responses against Spike/non-Spike detected via ELISpots ( $r=0.5734$ ,  
293  $p=0.0042$  and  $r=0.4852$ ,  $p=0.0189$  respectively), indicating that CD4 T cells are the dominant  
294 population responding to SARS-CoV-2.

295  
296 In line with previous observations, we found that SARS-CoV-2-specific T cells predominately  
297 display an effector memory (EM) and/or a terminally differentiated effector memory (TEMRA)

298 cell phenotype for CD4 and CD8 T cells respectively (**Fig. 5A-D**) (Breton et al. 2020; Dan et al.  
299 2020). Previous studies have suggested that higher expression of programmed cell death-1 (PD-  
300 1) on T cells in COVID-19 patients could signify the presence of exhausted T cells (De Biasi et  
301 al. 2020; Song et al. 2020; Zheng et al. 2020). We therefore examined the expression of PD-1 in  
302 relation to activation and function among SARS-CoV-2-specific CD4 T cells. The proportion of  
303 CD154<sup>+</sup> IFN- $\gamma$  producing cells was significantly higher in PD-1<sup>+</sup> cells compared to PD-1<sup>-</sup> cells  
304 regardless of HIV serostatus, likely reflecting activated functional cells rather than exhausted  
305 populations (**Fig. 5E**) (Rha et al. 2021). An inverse correlation between the expression of PD-1  
306 expressing SARS-CoV-2-specific CD4 T cells and DPSO was observed in HIV negative donors  
307 (**Fig. 5F**).

308

### 309 **Immune profile relationships between convalescent HIV positive and negative individuals**

310 During COVID-19 disease, excessive activation of T cells can lead to lymphopenia, including  
311 altered subset distribution and function, and these alterations can persist into convalescence  
312 (Breton et al. 2020; Chen and John Wherry 2020; De Biasi et al. 2020; Mathew et al. 2020). This  
313 prompted us to further evaluate changes in the T cell compartment and the relationship between  
314 antigen-specific T cells and antibodies with individual T cell parameters and immunological  
315 metrics in our cohort. To this end we utilized a broad immunophenotyping flow cytometry panel  
316 to capture major cell types.

317

318 Global t-distributed stochastic neighbour embedding (t-SNE) high dimensional analysis  
319 demonstrated significant alterations in the T cell compartment between HIV negative and  
320 positive individuals recovered from COVID-19 (**Fig. 6A**). Lower proportions of circulating CD4

321 T cells and higher proportions of CD8 T cells were confirmed by traditional gating in HIV  
322 infected individuals recovered from COVID-19 disease compared to HIV negative individuals  
323 (**Fig. 6B**). Consistent with alterations described in HIV infection (L. Zhang et al 1999, M.D.  
324 Hazenberg et al 2000, D.C. Douek 1998), naïve T cell frequency was reduced in SARS-CoV-2  
325 convalescent HIV positive donors compared to HIV negative subjects. This was accompanied by  
326 higher proportions of terminally differentiated effector memory (TEMRA: CD45RA<sup>+</sup>CCR7<sup>-</sup>)  
327 within the total CD8 T cell population in HIV infected individuals, contributing to an altered  
328 representation of naïve/memory T cells (Roederer et al. 1995) (**Fig. 6C**). Notably, the percentage  
329 of naïve CD4 T cells correlated with the CD4:CD8 ratio and SARS-CoV-2-specific T cell  
330 responses in HIV positive donors (**Fig. 6D, E**), suggesting that the scarce availability of naïve  
331 CD4 T cells could influence the extent/magnitude of the T cell response to SARS-CoV-2  
332 infection. Recent data have demonstrated a link between naïve CD4 T cells, age and COVID-19  
333 disease severity in older individuals (Rydyznski Moderbacher et al 2020). Whereas naïve CD8  
334 and CD4 T cells correlated with age in HIV negative donors, this relationship between age and  
335 naïve T cells was lost in HIV infected donors (**Fig. 6F-I**). Together these observations suggest  
336 that altered T cell homeostasis and likely premature immunosenescence in HIV infection could  
337 compromise T cell mediated responses to a new pathogen (Douek et al. 1998).

338  
339 Previous work focusing on markers of activation/exhaustion to evaluate the T cell compartment  
340 during the acute and convalescent phase of SARS-CoV-2 infection, has demonstrated lasting  
341 changes (Breton et al. 2020). Such information is lacking in the setting of HIV infection,  
342 normally characterised by persistent immune activation together with cell alterations and T cell  
343 exhaustion (Breton et al. 2013; El-Far et al. 2008). The proportion of T cells co-expressing

344 HLADR/CD38 and PD-1/TIGIT (T cell immunoreceptor with Ig and ITIM domains) in HIV  
345 infected individuals was significantly higher when compared with HIV negative donors but did  
346 not correlate with virus-specific parameters (**Fig. S4A-D**).

347

348 Circulating T follicular helper (cTfh) cells represent a substantial proportion of the SARS-CoV-  
349 2-specific T cells in acute and convalescent infection (Rodda et al. 2021; Rydzynski  
350 Moderbacher et al. 2020), and are required for maturation and development of B cell responses  
351 in germinal centres and the induction of IgG production (Crotty 2015). A close association  
352 between cTfh cells and the virus-specific antibody production in the convalescent phase of  
353 COVID-19 disease has been described (Zhang et al. 2021). We detected elevated frequencies of  
354 cTfh (CXCR5+PD-1+) HIV infected subjects compared to HIV infected donors, however, no  
355 correlation was observed with antibody levels (**Fig. S4E, F**). Whether further quantitative and  
356 qualitative differences exist between cTfh cell subsets in HIV positive (Pallikkuth et al. 2017)  
357 and negative individuals that could alter their capacity to instruct B cells and influence responses  
358 to SARS-CoV-2 infection merits further investigation in larger cohorts.

359

## 360 **Discussion**

361 The COVID-19 pandemic is causing much global uncertainty, especially for people with pre-  
362 existing medical conditions such as PLWH. In this study, we aimed to bridge the knowledge  
363 gaps in our understanding of the specificity, magnitude and duration of immunity to SARS-CoV-  
364 2 in this patient group, which is critical for tailoring current and future mitigation measures,  
365 including vaccine strategy. This integrative analysis demonstrates that the majority of PLWH



366 evaluated in the convalescent phase from mild COVID-19 disease can mount a functional  
367 adaptive immune response to SARS-CoV-2.

368

369 Most PLWH developed S1/N-reactive and neutralizing antibody responses similar to HIV  
370 negative donors in our study and similar to observations reported in the general population at  
371 least five months after primary infection (Gaebler et al. 2020; Reynolds et al. 2020) (Seow et al.  
372 2020). Circulating SARS-CoV-2 neutralizing antibody titers were, however, low in a fraction of  
373 recovered COVID-19 cases (Dan et al. 2020; Robbiani et al. 2020; Seow et al. 2020; Wajnberg  
374 et al. 2020), indicating that either the serum concentration/neutralizing antibody potency was  
375 suboptimal or, more likely, that an earlier response could have waned by the time of sampling at  
376 DPSO as previously observed (Seow et al. 2020). Some samples in both groups showed strong  
377 neutralization despite low anti-S1 binding titers, suggesting the possibility of the presence of  
378 neutralizing antibodies directed against other viral epitopes and/or greater production of non-  
379 neutralizing antibodies, or a role of other isotypes in neutralizing responses. It should be noted  
380 that our data reflect those who recovered from mostly mild COVID-19 disease, limiting our  
381 conclusions about disease associations. Whether seroconversion rates and kinetics of antibody  
382 responses differ according to HIV status need to be addressed in longitudinal studies from acute  
383 infection or vaccination into convalescence. While the exact duration of immunity conferred by  
384 natural infection remains unresolved, induction of neutralizing antibodies and presence of  
385 antibodies to SARS-CoV-2 is thought to confer a degree of protection against SARS-CoV-2  
386 (Anderson et al. 2020; Folegatti et al. 2020; Lumley et al. 2020; Polack et al. 2020; V Hall 2020;  
387 Yu et al. 2020).

388

389 In addition to antibodies, CD4 T cells and CD8 T cells can provide protective roles in controlling  
390 SARS-CoV-2 infection (Altmann and Boyton 2020; Kim et al. 2020), with T cell immunity  
391 potentially being more enduring, as in the case of SARS-CoV (Le Bert et al. 2020; Tang et al.  
392 2011). In keeping with the published literature, we detected T cell responses via IFN- $\gamma$ -ELISpot  
393 in the majority of HIV positive and negative donors. In both groups these responses were  
394 variable in magnitude and predominately targeted Spike, M and N, with smaller responses to  
395 regions of the viral proteome tested being detected, consistent with previous studies (Grifoni et  
396 al. 2020; Peng et al. 2020; Reynolds et al. 2020).

397

398 Notably, a positive association was observed between the CD4:CD8 ratio in HIV infected  
399 subjects and the magnitude of T cell responses against SARS-CoV-2. This suggests that some  
400 PLWH with residual immune perturbations, despite effective virological suppression on ART,  
401 may generate suboptimal T cell memory responses. With emerging information on PLWH with  
402 COVID-19 disease, a more pronounced immunodeficiency, defined as a current CD4 count  
403  $<350/\mu\text{L}$  and a low CD4 nadir, has been associated with an increased risk for severe COVID-19  
404 disease and mortality. With modern ART and successful viral suppression, absolute CD4 count  
405 (despite normalisation) may not accurately reflect the extent of immunological alterations that  
406 could persist in HIV infected individuals on treatment (Opportunistic Infections Project Team of  
407 the Collaboration of Observational et al. 2012). A low or inverted CD4/CD8 ratio is considered  
408 an immune risk phenotype associated with altered immune function, immunosenescence and  
409 chronic inflammation in both HIV positive and negative populations (Appay and Sauce 2008;  
410 Serrano-Villar et al. 2014a; Serrano-Villar et al. 2014c). Due to its predictive power for adverse  
411 clinical outcomes in HIV infection and in the ageing general population (Helleberg et al. 2015;

412 Serrano-Villar et al. 2014c; Serrano-Villar et al. 2014b), including its potential role as a  
413 prognostic factor of COVID-19 disease severity (Calvet et al. 2020), the CD4:CD8 ratio could  
414 represent an additional tool for risk stratification of PLWH. The relationship between naïve CD4  
415 T cells, CD4:CD8 ratio and magnitude of SARS-CoV-2-specific responses in our cohort  
416 highlights the dependency between new antigen-specific responses and the available pool of  
417 naïve lymphocytes. Fewer pre-existing naïve CD4 T cells coupled with the relative  
418 overrepresentation of memory CD8 T cells in the context of HIV, independent of age, could  
419 exacerbate the clinical outcome of SARS-CoV-2 infection. These changes in the T cell  
420 compartment can lead to reduced priming and poorly coordinated early and subsequent memory  
421 immune responses to SARS-CoV-2. Our cohort and interim analysis does not represent the entire  
422 spectrum of immune dysfunction, which we will continue to probe through ongoing recruitment  
423 to test these relationships more rigorously. This would be highly relevant in the context of  
424 emergence of specific SARS-CoV-2 variants associated with immunosuppression described in  
425 the UK (SA Kemp 2020), and in parts of the world with high HIV prevalence and suboptimal  
426 HIV suppression levels (Houriiyah Tegally 2020).

427

428 In agreement with other studies, our data show that in individuals with detectable cellular  
429 responses, CD4 T cell responses to SARS-CoV-2 Spike and non-Spike antigens are more  
430 common than CD8 T cell responses (Grifoni et al. 2020; Sekine et al. 2020; Zuo J 2020). This  
431 could reflect a bias, from using peptide pools, towards MHC class II presentation and more  
432 selective recognition by CD4 T cells (Grifoni et al. 2020). Preferential expansion of CD4 T cells  
433 has been associated with control of primary SARS-CoV-2 infection (Rydyznski Moderbacher et  
434 al. 2020) underscoring their relevance in PLWH with persistent alterations in their T cell

435 compartments. In a small group of donors, already found to be responsive via ELISpots, further  
436 evaluation of T cell polyfunctionality in response to SARS-CoV-2 Spike and non-Spike pools  
437 revealed similar profiles in SARS-CoV-2 CD4 T cells irrespective of HIV status. Given that  
438 contributions of T cells specific to any viral protein can be relevant for protective immunity, non-  
439 Spike proteins could also represent valuable components for future vaccine strategies. The  
440 reduced production of IL-2 from SARS-CoV-2-specific CD8 T cells in HIV infected donors  
441 could, however, hinder their proliferative potential and long-term immune memory post natural  
442 infection and/or immunization (Zimmerli et al. 2005). Together, these results provide further  
443 immunological context into the described associations between ongoing immunodeficiency and  
444 worse COVID-19 disease outcome, and the subsequent development of immune memory  
445 responses. Further work is required to comprehensively characterise the epitope repertoire  
446 elicited by SARS-CoV-2 infection in the context of a broad set of HLA alleles to define patterns  
447 of immunodominance.

448  
449 Our data indicate that antibody and T cell responses in convalescent individuals with  
450 predominately mild disease can be uncoupled. The lack of correlation between Spike-specific T  
451 cells and S1 binding IgG titers and neutralization levels in HIV positive individuals could point  
452 toward impairment of Tfh cells (Pallikkuth et al. 2017), which make up a significant proportion  
453 of SARS-CoV-2-specific cells (Boppana et al. 2020; Juno et al. 2020). The observed disparity of  
454 T cell and antibody responses in certain individuals could also reflect differences in early innate  
455 immune responses potentially resulting in dysregulated priming and incongruent T and B cell  
456 responses (Zhou et al. 2020). However, this remains to be determined.

457

458 There are limitations to this study. The observed heterogeneity in the magnitude of cellular and  
459 humoral responses that are not always fully coordinated highlights the need to consider  
460 additional putative factors as they relate to adaptive immunity. This cross-sectional study was not  
461 powered to study age and demographic differences according to the full spectrum of COVID-19  
462 disease by HIV serostatus. Larger studies are required to determine the role of gender, racial and  
463 ethnicity effects, especially in areas of high HIV burden and additional comorbidities, to help  
464 identify individuals who are particularly vulnerable to the impact of SARS-CoV-2 infection and  
465 need targeted vaccination interventions. Nonetheless, the prospective, longitudinal design of this  
466 current study, integrating clinical parameters, antibody and T cell responses, will help address  
467 longer term protective immunity and emerging questions, such as immune responses to new  
468 SARS-CoV-2 variants (C Rees-Spear 2021; Houriiyah Tegally 2020; SA Kemp 2020; Sandile  
469 Cele 2021; Wibmer et al. 2021), and during the subsequent vaccination roll-out.

470

471 Collectively, our results provide benchmark data into the facets of adaptive immunity against  
472 SARS-CoV-2 in the setting of treated HIV infection, providing evidence for medium-term  
473 durable antibody and cellular responses. Although reassuring, our data also have implications for  
474 PLWH with inadequate immune reconstitution, reflected in the low/inverted CD4:CD8 ratio, and  
475 potentially decreased ability to respond to SARS-CoV-2. This subpopulation of PLWH may be  
476 more vulnerable to circulating virus with relevance to vaccine prioritization and potential  
477 effectiveness. In the era of ART, CD4:CD8 ratio, should be considered as a readily accessible  
478 biomarker for assessing individual risks in PLWH, a proportion of whom may require tailored  
479 vaccine strategies to achieve long-term protective immunity.

480

## 481 **Materials and Methods**

### 482 **Ethics statement**

483 The protocols for the human study were approved by the local Research Ethics Committee  
484 (REC) – Berkshire (REC 16/SC/0265). The study conformed to the Helsinki declaration  
485 principles and Good Clinical Practice (GCP) guidelines and all subjects enrolled into the study  
486 provided written informed consent.

487

### 488 **Study Subjects**

489 HIV seronegative adults (>18 years of age, comprising hospital-based healthcare workers) and  
490 chronically HIV infected patients (on antiretroviral treatment for at least 2 years with  
491 undetectable HIV RNA) with prior confirmed or suspected COVID-19 disease were recruited.  
492 All study participants were screen anti-Hepatitis C virus and anti HBsAg negative. Confirmed  
493 SARS-CoV-2 infection by SARS-CoV-2 PCR and/or Roche antibody tests was declared by the  
494 participants, who were asked to provide details on the timing and nature of symptoms.  
495 Additional demographic information and underlying medical conditions were captured on a  
496 health questionnaire. European Centre for Disease Prevention (ECDC) criteria were used for  
497 case definition for COVID-19 disease. Severity of COVID-19 disease was according to the  
498 WHO criteria. This is a cross-sectional analysis of baseline samples collected during the  
499 convalescent phase of SARS-CoV-2 infection as part of a prospective, observational longitudinal  
500 cohort study. A total of n=47 HIV positive and n=35 HIV negative subjects with recovered  
501 confirmed and/or suspected COVID-19 disease were included (**Table. S1 Subject**  
502 **characteristics**). Sixteen demographically age-, sex- and lifestyle-matched HIV-1 seropositive  
503 individuals were included for comparison, from whom sample were collected between (February

504 2017-November 2019; pre-pandemic). All participants were recruited at the Mortimer Market  
505 Centre for Sexual Health and HIV Research and the Royal Free Hospital (London, UK)  
506 following written informed consent as part of a study approved by the local ethics board  
507 committee. Clinical characteristics of participants are summarized in **Table. S1**. Further details  
508 in the exact number of subjects utilized for each assay are indicated in the figure legends and  
509 Results section.

510 Case definition for coronavirus disease 2019 (COVID-19), as of 03 December 2020 European  
511 Centre for Disease Prevention and Control [[https://www.ecdc.europa.eu/en/covid-](https://www.ecdc.europa.eu/en/covid-19/surveillance/case-definition)  
512 [19/surveillance/case-definition](https://www.ecdc.europa.eu/en/covid-19/surveillance/case-definition)]. Severity of COVID-19 was classified according to the WHO  
513 (World Health Organisation) clinical progression scale.

514

#### 515 **Peripheral blood mononuclear cells (PBMC) and Plasma Isolation**

516 Whole blood from all participants was collected in heparin-coated tubes and stored at room  
517 temperature prior to processing. In brief, PBMCs were isolated by density gradient  
518 sedimentation. Whole blood was transferred to conical tubes and then centrifuged at 2000 rpm, at  
519 room temperature (RT) for 5-10 minutes. Plasma was then collected, aliquoted and stored at  
520  $-80^{\circ}\text{C}$  for further use. The remaining blood was diluted with RPMI (Corning, Manassas, VA,  
521 USA), layered over an appropriate volume of room temperature Histopaque (Histopaque-1077  
522 Cell Separation Medium, Sigma-Aldrich, St. Louis, MO, USA), and then centrifuged for 20 min  
523 at 2000 rpm at room temperature without brake. After centrifugation, the PBMC layer was  
524 carefully collected, transferred to a conical tube and washed with RPMI. An aliquot of cells was  
525 stained with trypan blue and counted using Automated Cell Counter (Bio-Rad, Hercules,  
526 California, USA). Isolated PBMCs were then cryopreserved in a cryovial in cell recovery

527 freezing medium containing 10% Dimethyl Sulfoxide (DMSO) (MP Biomedicals, LLC, Irvine,  
528 CA, USA) and 90% heat inactivated fetal bovine serum (FBS) and stored at  $-80^{\circ}\text{C}$  in a Mr.  
529 Frosty freezing container overnight before being transferred to liquid nitrogen for further storage.

530

### 531 **Serum isolation**

532 For serum isolation, whole blood was collected in serum separator tubes and stored briefly at  
533 room temperature prior sample processing. Serum tubes were centrifuged for 5 min at 2000 rpm,  
534 and then serum was collected, aliquoted and stored at  $-80^{\circ}\text{C}$  for further use.

535

### 536 **Semiquantitative ELISA for S1 and N**

537 This assay is based on a previously described assay (O'Nions et al. 2021; Pickering et al. 2020;  
538 Seow et al. 2020). Briefly, 9 columns of a 96-half-well Maxisorp plate (Nalgene, NUNC  
539 International, Hereford, UK) were coated overnight at  $4^{\circ}\text{C}$  with 25  $\mu\text{l}$  of S1 or N (gift from Peter  
540 Cherepanov, Crick Institute) purified protein at 3  $\mu\text{g}/\text{ml}$  in PBS, the remaining 3 columns were  
541 coated with 25  $\mu\text{l}$  goat anti-human F(ab)'<sub>2</sub> (1:1,000) in PBS to generate an internal standard  
542 curve. The next day, plates were washed with PBS-T (0.05% Tween in PBS) and blocked for 1h  
543 at RT with assay buffer (5% milk powder PBS-T). Assay buffer was then removed and 25  $\mu\text{l}$  of  
544 patient sera at dilutions from 1:50 – 1:1000 in assay buffer added to the antigen-coated wells in  
545 duplicate. Serial dilutions of known concentrations of IgG were added to the F(ab)'<sub>2</sub> IgG-coated  
546 wells in triplicate. Following incubation for 2 hours at room temperature, plates were washed  
547 with PBS-T and 25  $\mu\text{l}$  alkaline phosphatase-conjugated goat anti-human IgG (Jackson  
548 ImmunoResearch) at a 1:1000 dilution in assay buffer added to each well and incubated for 1  
549 hour at room temperature. Plates were then washed with PBS-T, and 25 $\mu\text{l}$  of alkaline



550 phosphatase substrate (Sigma Aldrich) added. ODs were measured using a MultiskanFC  
551 (Thermofisher) plate reader at 405nm and S1 & N-specific IgG titers interpolated from the IgG  
552 standard curve using 4PL regression curve-fitting on GraphPad Prism 8.

553

#### 554 **Pseudovirus production and neutralization assays**

555 HIV-1 particles pseudotyped with SARS-Cov-2 spike were produced by seeding  $3 \times 10^6$  HEK-  
556 293T cells in 10ml complete DMEM (DMEM supplemented with 10% FBS, L-Glutamine, 100  
557 IU/ml penicillin and 100  $\mu\text{g/ml}$  streptomycin) in a T-75 culture flask. The following day cells  
558 were transfected with 9.1  $\mu\text{g}$  of HIV p8.91 packaging plasmid (Zufferey et al. 1997) , 9.1  $\mu\text{g}$  of  
559 HIV-1 luciferase reporter vector plasmid (Seow et al. 2020), 1.4  $\mu\text{g}$  of WT-SARS-CoV-2 spike  
560 plasmid (2) and 60  $\mu\text{g}$  of PEI-Max (Polysciences). Supernatants were harvested 48h later,  
561 filtered through a 0.45  $\mu\text{m}$  filter and stored at  $-80^\circ\text{C}$ . Neutralization assays were performed on  
562 96-well plates by incubating serial dilutions of patient serum with pseudovirus for 1h at  $37^\circ\text{C}$  5%  
563  $\text{CO}_2$ . HeLa ACE-2 cells (gift from James E Voss, Scripps Institute) were then added to the assay  
564 (10,000 cells per 100  $\mu\text{L}$  per well). After 48/72h at  $37^\circ\text{C}$  5%  $\text{CO}_2$ , supernatants were removed,  
565 and the cells were lysed; Brightglo luciferase substrate (Promega) was added to the plates and  
566 RLU read on a Glomax luminometer (Promega) as a proxy for infection. Measurements were  
567 performed in duplicate and fifty percent inhibitory dilution (ID50) values were calculated using  
568 GraphPad Prism 8.

569

#### 570 **IgG Purification**

571 For individuals on ART, to avoid off-target neutralization due to the HIV pseudovirus backbone,  
572 we purified IgG from serum using Mini Bio-Spin Chromatography Columns (BioRad) by

573 incubating sera with a resin of protein G Sepharose beads (GE Healthcare) for 2h, eluting with  
574 0.1M Glycine (pH 2.2) into 2M Tris-base, concentrating the IgG-containing fraction in a 50kD  
575 concentrator (Amicon, Merck) and quantifying the amount of IgG by Nanodrop. The purified  
576 IgG was serially diluted from 200 µg/ml in neutralization assays and the resulting ID50  
577 calculated using the total IgG concentration of each serum sample prior to purification.

578

### 579 **Standardized ELISA for measurement of CMV-specific IgG levels in plasma**

580 The levels of CMV-specific IgG were measured using the Abcam Anti-Cytomegalovirus (CMV)  
581 IgG Human ELISA kit following manufacturer's instructions. Assays were run in duplicate and  
582 mean values per participant are reported in International Units (IU) per ml.

583

### 584 **Phenotypic flow cytometric analysis**

585 The fluorochrome-conjugated antibodies used in this study are listed in **Table. S2**. Briefly,  
586 purified cryopreserved PBMCs were thawed and rested for one hour at 37 °C in complete RPMI  
587 medium (RPMI supplemented with Penicillin-Streptomycin, L-Glutamine, HEPES, non-essential  
588 amino acids, 2-Mercaptoethanol, and 10% Fetal bovine serum (FBS)). Cells were then washed,  
589 resuspended in PBS, and surface stained at 4°C for 20 min with different combinations of  
590 antibodies in the presence of fixable live/dead stain (Invitrogen). Cells were then fixed and  
591 permeabilized for detection of intracellular antigens. The Foxp3 intranuclear staining buffer kit  
592 (eBioscience) was used according to the manufacturer's instructions for the detection of  
593 intranuclear markers. Samples were acquired on a BD Fortessa X20 using BD FACSDiva8.0  
594 (BD Bioscience) and subsequent data analysis was performed using FlowJo 10  
595 (TreeStar). Stochastic neighbor embedding (SNE) analysis was undertaken on the mrc.cytobank

596 platform to enable visualization of high-dimensional data in two-dimensional representations,  
597 avoiding the bias that can be introduced by manual gating of specific subsets (Amir el et al.  
598 2013).

599

### 600 **Peptide Pools:**

601 For detection of virus-specific T cell responses, PBMCs were stimulated with the following  
602 peptide pools:

603

- 604 1. SARS-CoV-2 Spike: total of 15 to18-mers overlapping by 10 amino acid residues for  
605 Spike (S) synthesized using Two-dimensional peptide Matrix pools, divided into 16  
606 “minipools” P1-P16 and grouped into pools S1 (P1-8) and S2 (P 9-16) (Peng et al. 2020).
- 607 2. SARS-CoV-2 Structural and accessory proteins: 15-mer peptides overlapping by 10  
608 amino acid residues for Membrane protein (M) (Miltenyibiotec), Nucleocapsid (N)  
609 (Miltenyibiotec), Envelope (Env) protein and open reading frame (ORF) 3, 6, 7 (a kind  
610 gift from Tao Dong) (Peng et al. 2020)
- 611 3. Non-SARS-CoV-2 antigens: Peptide pools of the pp65 protein of human cytomegalovirus  
612 (CMV) (Miltenyibiotec, and NIH AIDS Reagent Repository), or HIVconsv peptide pools  
613 (NIH AIDS Reagent Repository) HIV-1 and Influenza HLA class I-restricted T cell  
614 epitope (ProImmune). CD8 T cell epitopes of human influenza, CMV and EBV viruses  
615 (namely FEC controls, NIH AIDS Reagent Repository) were used as positive controls.

616

### 617 **Ex vivo IFN- $\gamma$ ELISpot Assay**

618 IFN- $\gamma$  ELISpot assays were performed with cryopreserved isolated PBMCs as described  
619 previously (Peng et al. 2020). Briefly, ELISPOT plates (S5EJ044I10; Merck Millipore,  
620 Darmstadt, Germany) pre-wetted with 30  $\mu$ l of 70% ethanol for a maximum of 2 minutes,  
621 washed with sterile PBS and coated overnight at 4 °C with anti-IFN- $\gamma$  antibody (10  $\mu$ g/ml in  
622 PBS; clone 1-D1K; Mabtech, Nacka Strand, Sweden). Prior to use, plates were washed with PBS  
623 and blocked with R10 (RPMI supplemented with Penicillin-Streptomycin, L-Glutamine, and  
624 10% FBS) for 1 hour at 37 °C. The cells were plated at  $2 \times 10^5$  cells/well for most of the  
625 participants or  $1 \times 10^5$  cells/well for participants with lower cell recovery. Cells were cultured  
626 with overlapping peptide pools at 2  $\mu$ g/ml or PHA (Sigma Aldrich, St Louis, MO) at 10  $\mu$ g/ml  
627 as a positive control for 16-18 hours at 37 °C. Cells lacking peptide stimulation were used as a  
628 negative control. Plates were then washed four times with 0.05% Tween/PBS (Sigma-Aldrich)  
629 followed by two washes with PBS and then incubated for 2 hr at RT with biotinylated anti-IFN- $\gamma$   
630 (1  $\mu$ g/mL; clone mAb-7B6-1; Mabtech). After six further washes, cells were incubated with  
631 alkaline phosphatase-conjugated streptavidin (Mabtech) at 1:1000 dilution for 1 hr at RT. Plates  
632 were then washed six times and developed using VECTASTAIN® Elite ABC-HRP according to  
633 the manufacturer's instructions (Mabtech). All assays were performed in duplicate. Spots were  
634 counted using an automated ELISpot Reader System (Autoimmun Diagnostika GmbH). Results  
635 are reported as difference in ( $\Delta$ ) spot forming units (SFU) per  $10^6$  PBMCs between the peptide-  
636 stimulated and negative control conditions. Responses that were found to be lower than two  
637 standard deviations of the sample specific control were excluded. An additional threshold was set  
638 at  $> 5$  SFU/ $10^6$  PBMCs, and results were excluded if positive control wells (PHA, FEC) were  
639 negative.  
640

641 **Intracellular cytokine stimulation (ICS) functional assay**

642 ICS was performed as described previously (Gupta et al. 2019). Briefly, purified PBMCs were  
643 thawed and rested overnight at 37 °C and 5% carbon dioxide in complete RPMI medium. After  
644 overnight rest, PBMCs were stimulated for 6 h with 2µg/mL of SARS-CoV-2 peptide pools,  
645 Influenza, HIV-1 Gag or cytomegalovirus (CMV)-pp65 peptide pools, or with 0.005% dimethyl  
646 sulphoxide (DMSO) as a negative control in the presence of αCD28/αCD49d co-Stim antibodies  
647 (1 µg ml<sup>-1</sup>) GolgiStop (containing Monensin, 2 µmol/L), GolgiPlug (containing brefeldin A, 10  
648 µg ml<sup>-1</sup>) (BD Biosciences) and anti-CD107α BV421 antibody (BD Biosciences). After  
649 stimulation, cells were washed and stained with anti-CCR7 (BioLegend) for 30 min at 37 °C and  
650 then surface stained at 4°C for 20 min with different combinations of surface antibodies in the  
651 presence of fixable live/dead stain (Invitrogen). Cells were then fixed and permeabilised  
652 (CytoFix/CytoPerm; BD Biosciences) followed by intracellular cytokine with IFN-γ APC,  
653 CD154 PE-Cy7 (BioLegend), TNF-α FITC (BD Biosciences) and PerCP-eFluor 710 IL-2  
654 (eBioscience). Samples were acquired on a BD Fortessa X20 using BD FACSDiva8.0 (BD  
655 Bioscience) and data analysed using FlowJo 10 (TreeStar). The gates applied for the  
656 identification of virus-specific CD4 and CD8 T cells were based on the double-positive  
657 populations for interferon-γ (IFN-γ), Tumour necrosis factor (TNF-α), interleukin-2 (IL-2), and  
658 CD40 ligand (CD154). The total population of SARS-CoV-2 CD4 T cells was calculated by  
659 summing the magnitude of CD154<sup>+</sup>IFN-γ<sup>+</sup>, CD154<sup>+</sup>IL-2<sup>+</sup>, and CD154<sup>+</sup> TNF-α<sup>+</sup> responses;  
660 SARS-CoV-2 CD8 T cells were defined as (IFN-γ<sup>+</sup>TNF-α<sup>+</sup>, IFN-γ<sup>+</sup>IL-2<sup>+</sup>). Antibodies used in the  
661 ICS assay are listed in **Table S2**.

662

663 **Statistics:**

664 Prism 8 (GraphPad Software) was used for statistical analysis as follows: the Mann–Whitney *U*-  
665 test was used for single comparisons of independent groups, the Wilcoxon-test paired *t*-test was  
666 used to compare two paired groups. The non-parametric Spearman test was used for correlation  
667 analysis. The statistical significances are indicated in the figures ( $*p < 0.05$ ,  $**p < 0.01$ ,  $***p <$   
668  $0.001$ , and  $****p < 0.0001$ ). Polyfunctionality tests were performed in SPICE version 6.0.

669

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682 drafting of the manuscript; DHB, JK, BC, NFP, LM, AR, CR, CE performed experiments and  
683 contributed to acquisition of data and analysis; PC, PP, LW, FB, SK, TD, LD, SRJ contributed to  
684 study design, data interpretation and critical editing of the manuscript. LEM, DP: conception and  
685 design of study, data analysis and interpretation, critical revision of the manuscript and study  
686 supervision.

687

688 **Competing interests:** The authors have declared that no conflict of interest exists.

689 **Data and materials availability:** Data can be made available by request to the corresponding  
690 authors.

691

692 **Figure Legends:**

693 **Fig. 1. Antibody response in HIV positive and negative donors recovered from COVID-19**

694 **disease. (A)** Seropositivity screen of plasma samples for antibodies against the external Spike

695 antigen, using a recombinant Spike S1<sub>1-530</sub> subunit protein (S1), and against the full-length

696 internal Nucleoprotein (N) antigen to confirm prior infection in HIV negative and positive

697 donors. A sample absorbance greater than 4-fold above the average background of the assay was

698 regarded as positive. Black dots denote laboratory confirmed cases and grey dots

699 suspected/household contacts. **(B)** Comparison of S1 IgG and N IgG antibody titers in HIV

700 negative and **(C)** HIV positive donors. Red dots: hospitalized cases; Black dots: mild (non-

701 hospitalized cases); blue dots: asymptomatic cases. **(D)** Correlation between S1 IgG and N IgG

702 titers in HIV negative and positive donors. **(E)** Neutralization titers in HIV negative and positive

703 donors. Dotted lines indicate detection limit, minimum ID50 and potent levels >1000. **(F)**

704 Proportion of HIV negative and positive donors with neutralizing antibodies within the given

705 ranges. **(G)** Correlation between S1 IgG titers and neutralization titers in HIV negative and

706 positive donors. The non-parametric Spearman test was used for correlation analysis. \* $p < 0.05$ ,

707 \*\* $p < 0.01$

708

709 **Fig. 2. Similar SARS-CoV-2 specific T cell responses by IFN- $\gamma$ -ELISpot in HIV positive**  
710 **and negative donors. (A)** Genome organization of SARS-CoV-2 **(B)** Dominance of the IFN- $\gamma$ -  
711 ELISpot responses. Heatmap depicting the magnitude of the IFN- $\gamma$ -ELISpot responses to the  
712 different SARS-CoV-2 peptide pools in HIV negative and HIV positive individuals. (n=30 in  
713 each group) **(C)** Magnitude of the IFN- $\gamma$ -ELISpot responses. IFN- $\gamma$  SFU/10<sup>6</sup> PBMCs are shown  
714 for SARS-CoV-2 Spike (S), Membrane (M) and Nucleocapsid (N) between HIV negative  
715 (green) and HIV positive (red). (n=30 per group). **(D)** Magnitude of the IFN- $\gamma$ -ELISpot  
716 responses for Total SARS-CoV-2 responses (S, M, N, ORF3a, ORF6, ORF7, ORF8 and Env),  
717 FEC and HIV Gag between HIV negative (green) and HIV positive (red). (n=30 per group). **(E)**  
718 Hierarchy of the IFN $\gamma$ -ELISpot responses. IFN- $\gamma$  SFU/10<sup>6</sup> PBMCs responses in order of  
719 magnitude within each group with the contribution of the responses to a specific pool shown by  
720 color legend. **(F)** Diversity of the IFN- $\gamma$ -ELISpot responses. Number of pools each of the donors  
721 has shown positive responses in the IFN- $\gamma$ -ELISpot assay. The total of SARS-CoV-2 pools  
722 tested was 7. **(G)** Proportion of T cell response magnitude in the HIV negative and HIV positive  
723 individuals. **(H)** Correlation between CD4:CD8 ratio in HIV infected individuals with their total  
724 SARS-CoV-2 responses, depicting disease severity per each donor (Red dots: hospitalized cases;  
725 Black dots: non-hospitalized cases; blue dots: asymptomatic cases.). The non-parametric  
726 Spearman test was used for correlation analysis. Two-way ANOVA was used for groups  
727 comparison. \*p < 0.05, \*\*p<0.01.

728

729 **Fig. 3. Interrelations between T cell and antibody responses in HIV positive and negative**  
730 **donors. (A)** Correlation of total SARS-CoV-2 responses with S1 IgG titers in HIV negative and  
731 **(B)** HIV positive. **(C)** Correlation of total SARS-CoV-2 responses with N IgG titers in HIV



732 negative and (D) HIV positive subjects. Red dots: hospitalized cases; Black dots: non-  
733 hospitalized cases. (E) Hierarchy of the T cell responses ordered by the neutralizing capacity by  
734 their antibody titers for HIV negative and (F) HIV positive donors. The non-parametric  
735 Spearman test was used for correlation analysis. \* $p < 0.05$ , \*\* $p < 0.01$

736

737 **Fig. 4. Composition of SARS-CoV-2-specific T cells in convalescent HIV negative and HIV**  
738 **positive individuals.** Intracellular cytokine staining (ICS) was performed to detect cytokine-  
739 producing T cells to the indicated peptide pools in HIV negative (HIV-, n=12) and HIV positive  
740 individuals (HIV+, n=11). (A) Representative flow cytometric plots for the identification of  
741 antigen-specific CD4 T cells based on double expressions (CD154<sup>+</sup>IFN- $\gamma$ <sup>+</sup>, CD154<sup>+</sup>IL-2<sup>+</sup>, and  
742 CD154<sup>+</sup>TNF- $\alpha$ <sup>+</sup>) following 6-hour stimulation media alone (control) or overlapping SARS-CoV-  
743 2 peptides against Spike pool 1 and 2 (Spike), nucleoprotein (N), and membrane protein (M)  
744 directly *ex vivo*. (B) Frequency of aggregated CD4 T cell responses (CD154<sup>+</sup>IFN- $\gamma$ <sup>+</sup>, CD154<sup>+</sup>IL-  
745 2<sup>+</sup>, and CD154<sup>+</sup>TNF- $\alpha$ <sup>+</sup>) against Spike, M/N or combined (Spike and M/N) peptide pools. (C)  
746 Pie charts representing the relative proportions of Spike, M/N, or total (combined Spike and  
747 M/N) CD4 T cell responses for one (grey), two (green) or three (dark blue) cytokines, and pie  
748 arcs denoting IFN- $\gamma$ , TNF- $\alpha$  and IL-2. (D) Representative flow cytometric plots for the  
749 identification of antigen-specific CD8 T cells based on the expression of (IFN- $\gamma$ <sup>+</sup>, TNF- $\alpha$ <sup>+</sup>, and  
750 IL-2<sup>+</sup>) against the specified peptide pools or media alone (control). (E) Proportion of aggregated  
751 CD8 T cell responses against Spike, M/N or combined (Spike and M/N) responses. (F) Pie charts  
752 representing the relative proportions of Spike, M/N and combined CD8 T cell responses for one  
753 (grey), two (green) or three (dark blue) cytokines, and pie arcs showing IFN- $\gamma$ , TNF- $\alpha$  and IL-2.  
754 (G) Comparison of the frequencies of summed SARS-CoV-2-specific CD4 and CD8 T cell

755 responses against Spike and M/N proteins. **(H)** Correlation between the frequency of total  
756 SARS-CoV-2 specific-CD4 T cells and overall T cell responses detected by IFN- $\gamma$  ELISpot  
757 responses or **(I)** ID50 neutralization titer (log10) in HIV negative and HIV positive individuals.  
758 Error bars represent SEM. The non-parametric Spearman test was used for correlation analysis; p  
759 values for individual correlation analysis within groups, HIV- (green) or HIV+(red) or combined  
760 correlation analysis (black) are presented. Significance determined by Mann-Whitney *U* test or  
761 Wilcoxon matched- pairs signed rank test, \*p<0.05, \*\*p<0.01, \*\*\*p < 0.001. SPICE (simplified  
762 presentation of incredibly complex evaluations) was used for polyfunctional analysis.

763

764 **Fig. 5. Phenotypic characterization of SARS-CoV-2-specific CD4 and CD8 T cells from**  
765 **convalescent HIV negative and HIV positive subjects. (A)** Representative flow plots and **(B)**  
766 pie charts representing proportion of antigen-specific CD4 T cell with a CD45RA<sup>-</sup>/CCR7<sup>+</sup> central  
767 memory (CM), CD45RA<sup>+</sup>/CCR7<sup>+</sup> naïve, CD45RA<sup>+</sup>/CCR7<sup>-</sup> terminally differentiated effector  
768 memory (TEMRA) and CD45RA<sup>-</sup>/CCR7<sup>-</sup> effector memory (EM) phenotype from HIV negative  
769 (HIV-, n=12) and HIV positive individuals (HIV+, n=11) against SARS-CoV-2 Spike, M, N,  
770 CMV pp65 and HIV gag. **(C)** Representative flow plots and **(D)** pie charts representing  
771 proportion of CD45RA<sup>-</sup>/CCR7<sup>+</sup> central memory (CM), CD45RA<sup>+</sup>/CCR7<sup>+</sup> naïve,  
772 CD45RA<sup>+</sup>/CCR7<sup>-</sup> terminally differentiated effector memory (TEMRA) and CD45RA<sup>-</sup>/CCR7<sup>-</sup>  
773 effector memory (EM) antigen-specific CD8 T cell subsets against SARS-CoV-2 Spike, M, N,  
774 CMV pp65 and HIV gag. **(E)** Representative flow plots from an HIV negative donor (HIV-) and  
775 an HIV positive donor (HIV+) showing expression of CD154 and IFN- $\gamma$  production from PD1+  
776 and PD1- SARS-CoV-2-specific CD4 T cells and **(F)** paired analysis of responses in HIV  
777 negative (HIV-, n=12) and HIV positive (HIV+, n=11) individuals. **(F)** Correlation between

778 frequency of (PD-1<sup>+</sup>CD154<sup>+</sup>IFN- $\gamma$ <sup>+</sup> SARS-CoV-2-specific CD4 T cells and DPSO in both  
779 groups. Significance determined by Wilcoxon matched-pairs signed rank test, \*p<0.05,  
780 \*\*p<0.01, \*\*\*p < 0.001. The non-parametric Spearman test was used for correlation analysis; p  
781 values for individual correlation analysis within groups, HIV-, HIV+, or combined correlation  
782 analysis (black) are presented.

783

784 **Fig. 6. Immune profile relationships between convalescent HIV positive and negative**  
785 **individuals. (A)** viSNE analysis of CD3 T cells in HIV negative (top panel) and HIV positive  
786 donors (lower panel). Each point on the high-dimensional mapping represents an individual cell  
787 and colour intensity represents expression of selected markers. **(B)** Frequency of CD4 and CD8 T  
788 cells out of total lymphocytes in SARS-CoV-2 convalescent HIV negative (HIV-, n=26) and HIV  
789 positive individuals (HIV+, n=19) via traditional gating. **(C)** Summary data of the proportion of  
790 CD45RA<sup>-</sup>/CCR7<sup>+</sup> central memory (CM), CD45RA<sup>+</sup>/CCR7<sup>+</sup> naïve, CD45RA<sup>+</sup>/CCR7<sup>-</sup> terminally  
791 differentiated effector memory (TEMRA) and CD45RA<sup>-</sup>/CCR7<sup>-</sup> effector memory (EM) CD4 and  
792 CD8 T cell subsets in the study groups. **(D)** Correlation between CD4:CD8 ratio and frequency of  
793 naïve CD4 T cells in HIV-positive individuals. **(E)** Correlation between frequency of naïve CD4  
794 T cells and total SARS-CoV-2 T cell responses, detected via ELISpot, in HIV positive  
795 individuals. **(F)** Correlation between frequency of naïve CD4 T cells and **(G)** naïve CD8 T cells  
796 and age in HIV negative individuals. **(H)** Correlation between frequency of naïve CD4 T cells  
797 and **(I)** naïve CD8 T cells age in HIV positive donors. Significance determined by Mann-  
798 Whitney test, \*p<0.05, \*\*p<0.01, \*\*\*p < 0.001. The non-parametric Spearman test was used for  
799 correlation analysis.

800

801 **Supplementary Materials:**

802 **Fig. S1. Antigen binding screen and associations between humoral responses and cohort**  
803 **parameters**

804 **Fig. S2. Magnitude of T cell responses and associations with HIV parameters, age, gender**  
805 **and ethnicity**

806 **Fig. S3. Cytokine profile of SARS-CoV-2-, CMV- and Gag- specific T cells**

807 **Fig. S4. Association between T cell immunophenotyping and SARS-CoV-2 adaptive**  
808 **immune responses**

809 **Table S1. Cohort demographics and clinical characteristics**

810 **Table S2. Antibodies used for phenotypic analysis and virus-specific T cell characterization**

811

812 **Fig. S1. Antigen binding screen and associations between humoral responses and cohort**  
813 **parameters. (A)** Antigen binding screen in pre-pandemic samples from n=16 HIV positive  
814 donors and **(B)** the whole cohort with convalescent COVID-19 disease. Dotted lines indicate  
815 negative, low positive and positive threshold for absorbance [450nm]. **(C)** Correlation between  
816 age and S1 IgG titer according to gender in HIV negative donors and HIV positive subjects. **(D)**  
817 Correlation between age and ID50 according to gender in HIV negative donors and HIV positive  
818 subjects and **(E)** between DPSO and ID50 in the two study groups. **(F)** S1 IgG titer and ID50  
819 levels summary dot plots according to ethnicity in HIV positive and negative donors. The non-  
820 parametric Spearman test was used for correlation analysis. \* $p < 0.05$

821

822 **Fig. S2. Magnitude of T cell responses and associations with HIV parameters, age, gender**  
823 **and ethnicity.** (A) Percentage of responders to each peptide pools (B) Magnitude of the INF- $\gamma$ -  
824 ELISpot responses. INF- $\gamma$  SFU/10<sup>6</sup> PBMCs are shown for SARS-CoV-2 ORF3a, ORF6, ORF7,  
825 ORF8 and Env between HIV negative (green) and HIV positive (red). (n=30 per group). (C)  
826 Magnitude of the total SARS-CoV-2 responses analyzed in pre-pandemic samples, confirmed  
827 SARS-CoV-2 and suspected cases with clinical definition but found to be SARS-CoV-2  
828 seronegative on screening. In suspected cases, orange dots depict HIV negative and brown dots  
829 HIV positive donors. Correlation between CD4:CD8 ratio in HIV infected individuals with their  
830 (D) Nucleocapsid and (E) Membrane responses, depicting disease severity per donor.  
831 Correlation of total SARS-CoV-2 responses with age in (F) HIV negative and (G) HIV positive,  
832 depicting disease severity per donor. (H) Correlation of total SARS-CoV-2 responses with  
833 DPSO in HIV negative and (J) HIV positive, depicting disease severity per donor. (J) Magnitude  
834 of the total SARS-CoV-2 responses by ethnicity and (K) gender between HIV negative and HIV  
835 positive. The non-parametric Spearman test was used for correlation analysis. Two-way  
836 ANOVA was used for group comparison. \*p < 0.05, \*\*p < 0.01.

837

838 **Fig. S3. Cytokine profile of SARS-CoV-2-, CMV- and Gag- specific T cells**

839 (A) Frequency of SARS-CoV-2-specific CD154<sup>+</sup> CD4 T cells identified by expression of IFN- $\gamma$ <sup>+</sup>,  
840 IL-2<sup>+</sup>, or TNF- $\alpha$ <sup>+</sup> or overall responses with at least one of the three cytokines (IFN- $\gamma$ , TNF- $\alpha$  and  
841 IL-2) against Spike (S1 and S2 pools) (B) M/N or (C) combined (Spike and M/N) responses in  
842 HIV-negative (HIV-, n=12) and HIV positive individuals (HIV+, n=11) recovering from COVID-  
843 19 disease. (D) Representative flow plots and summary data (E) showing frequencies of overall  
844 (CD154<sup>+</sup>IFN- $\gamma$ <sup>+</sup>, CD154<sup>+</sup>IL-2<sup>+</sup>, or CD154<sup>+</sup>TNF- $\alpha$ <sup>+</sup>) SARS-CoV-2-, CMV-, or Gag-specific CD4

845 T cell responses in the study groups. **(F)** Frequency of SARS-CoV-2-specific CD8 T cells  
846 identified by expression of IFN- $\gamma^+$ , IL-2 $^+$  or TNF- $\alpha^+$ , or overall responses with at least one of the  
847 three cytokines (IFN- $\gamma$ , TNF-  $\alpha$  and IL-2) against Spike (S1 and S2 pools) **(G)** M/N **(H)** or  
848 combined (Spike and M/N) responses in HIV-negative (HIV-, n=12) and HIV-seropositive  
849 individuals (HIV+, n=11). **(I)** Representative flow plots and **(J)** summary data showing  
850 frequencies of overall (IFN- $\gamma^+$ , IL-2 $^+$ , or TNF- $\alpha^+$ ) SARS-CoV-2-, CMV-, or Gag-specific CD8 T  
851 cell responses in the study groups. Error bars represent SEM. The non-parametric Spearman test  
852 was used for correlation analysis; p values for individual correlation analysis within groups,  
853 HIV- (green) or HIV+ (red) or combined correlation analysis (black) are presented. Significance  
854 determined by Mann-Whitney *U* test or Wilcoxon matched- pairs signed rank test, \*p<0.05,  
855 \*\*p<0.01, \*\*\*p < 0.001.

856

857 **Fig. S4. Association between T cell immunophenotyping and SARS-CoV-2 adaptive**  
858 **immune responses**

859 **(A)** Representative flow plots and **(B)** summary data of the frequency of CD38 $^+$  HLADR $^+$  CD4  
860 and CD8 T cells, and correlations between percentage of CD38 $^+$  HLADR $^+$  CD4 and CD8 T cells  
861 and total SARS-CoV-2-specific T cell responses in HIV-seronegative (HIV-, n=26) and HIV  
862 positive individuals (HIV+, n=19). **(C)** Representative flow plots and **(D)** summary data of  
863 frequency of PD-1 $^+$  TIGIT $^+$  CD4 and CD8 T cells, and correlations between percentage of PD-1 $^+$   
864 TIGIT $^+$  CD4 and CD8 T cells and total SARS-CoV-2-specific T cell responses. **(E)**  
865 Representative flow plots showing the gating strategy used to define total circulating and  
866 activated Tfh subsets in the study groups and summary data. **(F)** Correlations between  
867 percentage of activated Tfh and S1 IgG or N IgG titers. Significance determined by Mann-

868 Whitney test, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . The non-parametric Spearman test was used for  
869 correlation analysis; combined correlation analysis is depicted.

870

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Figure 1

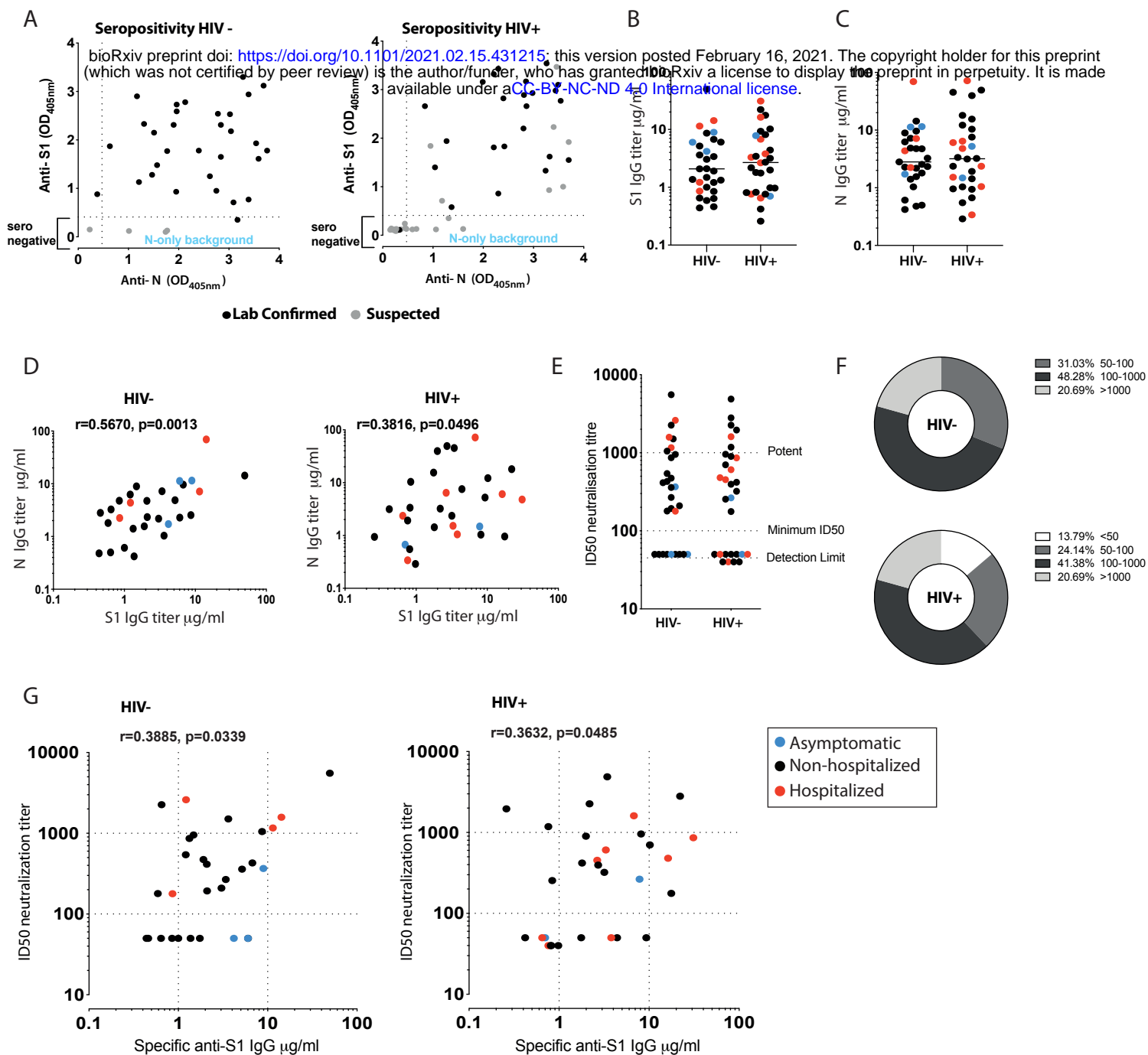


Figure 2

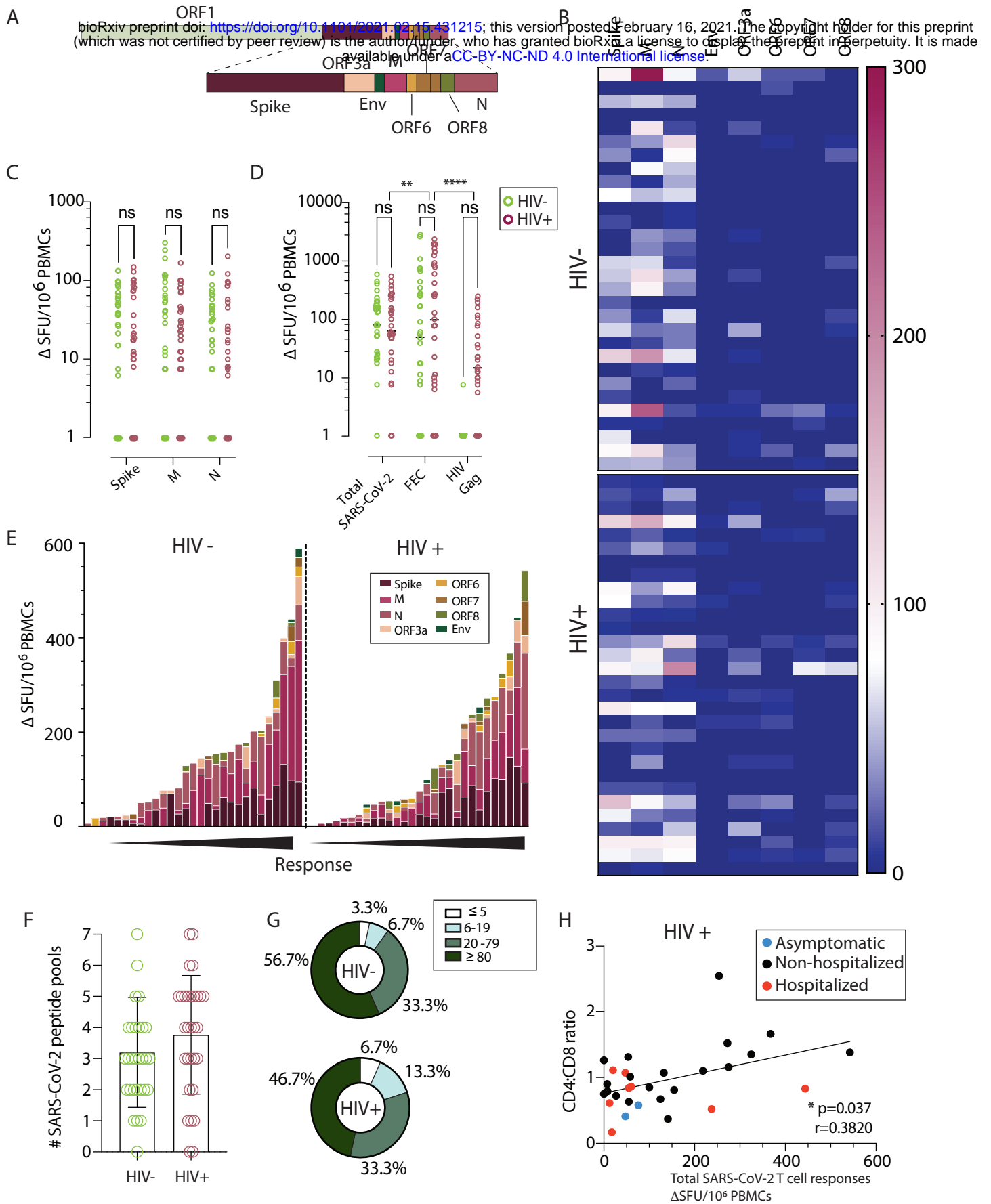
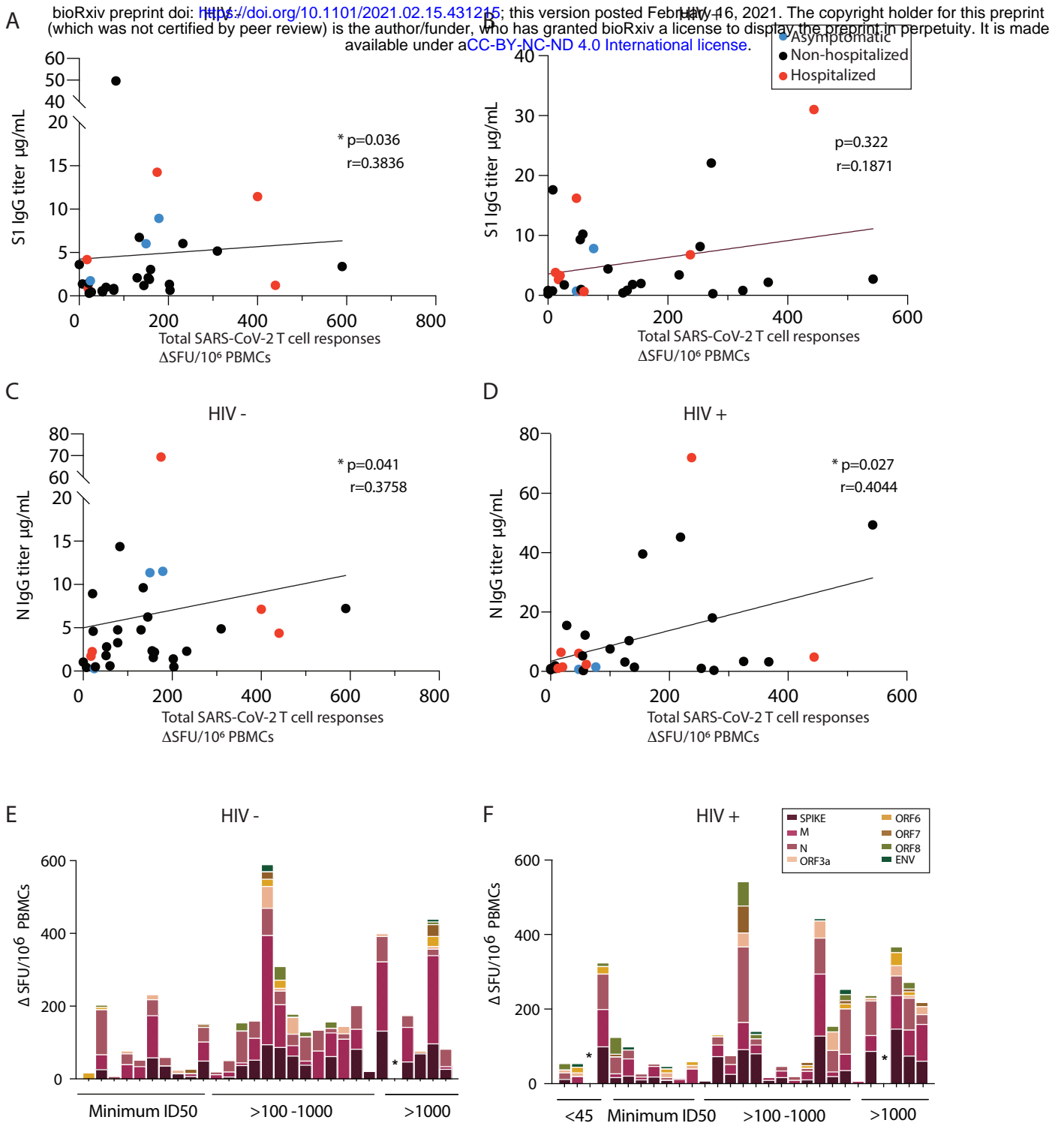
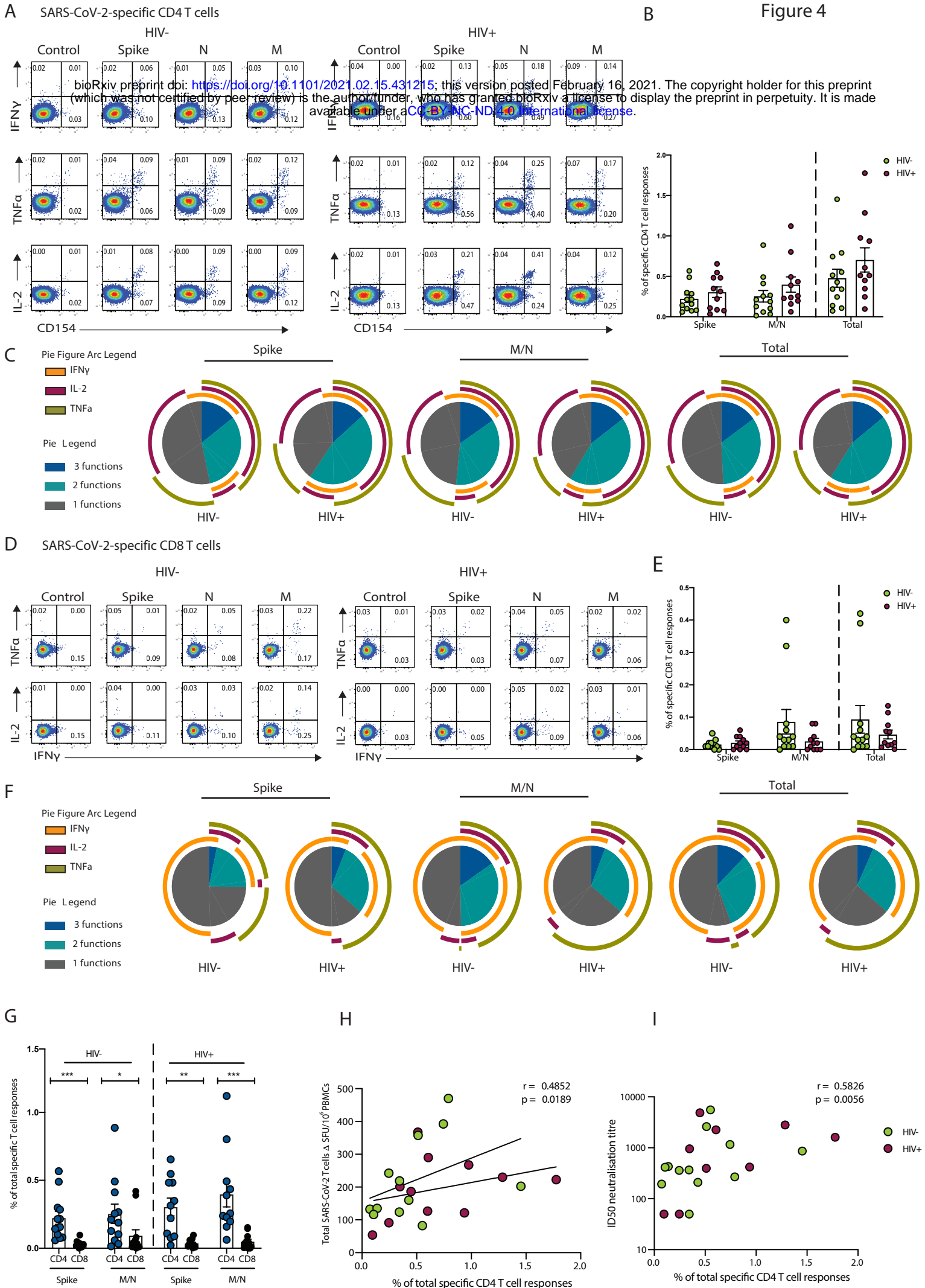


Figure 3





**Figure 5**

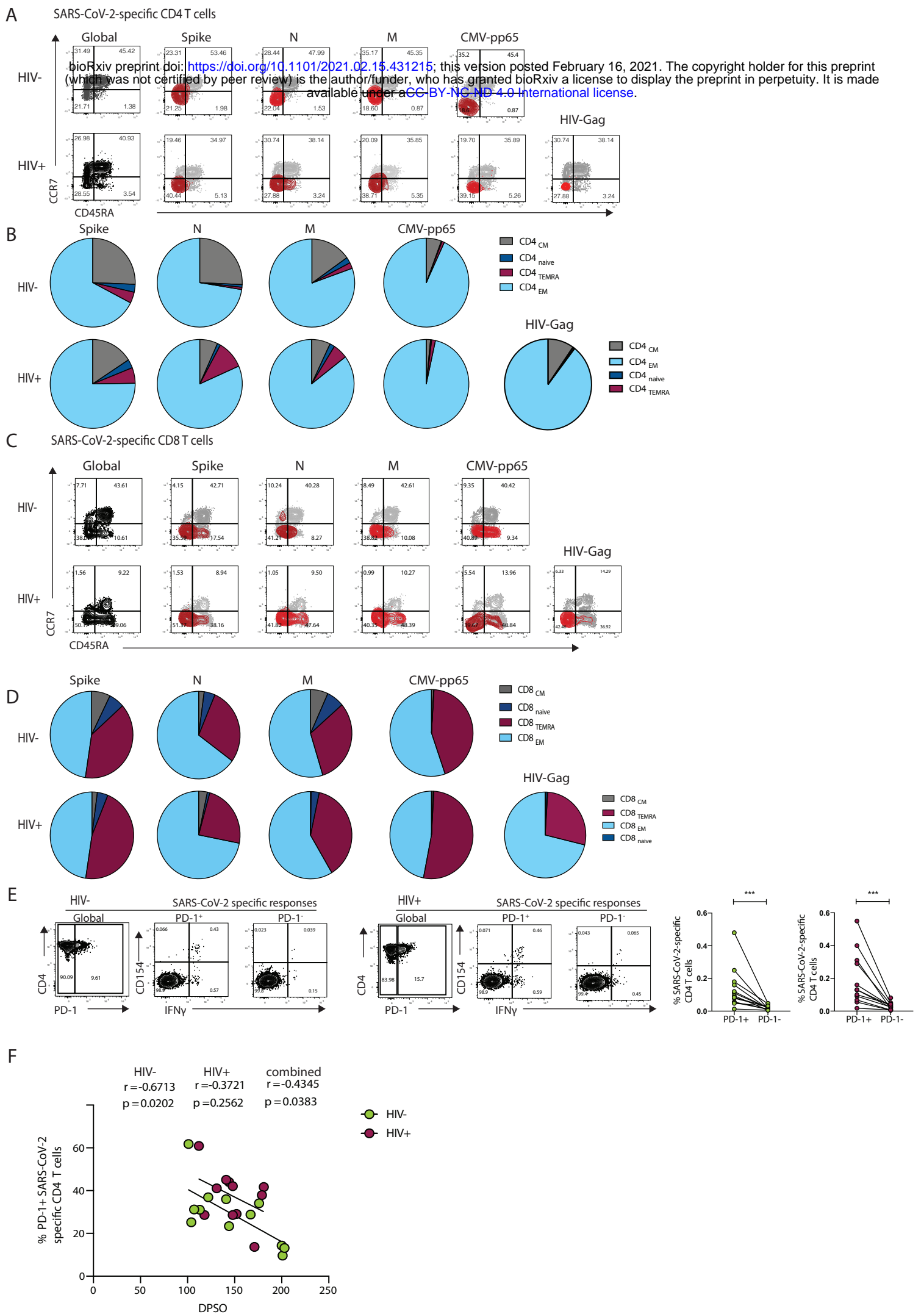
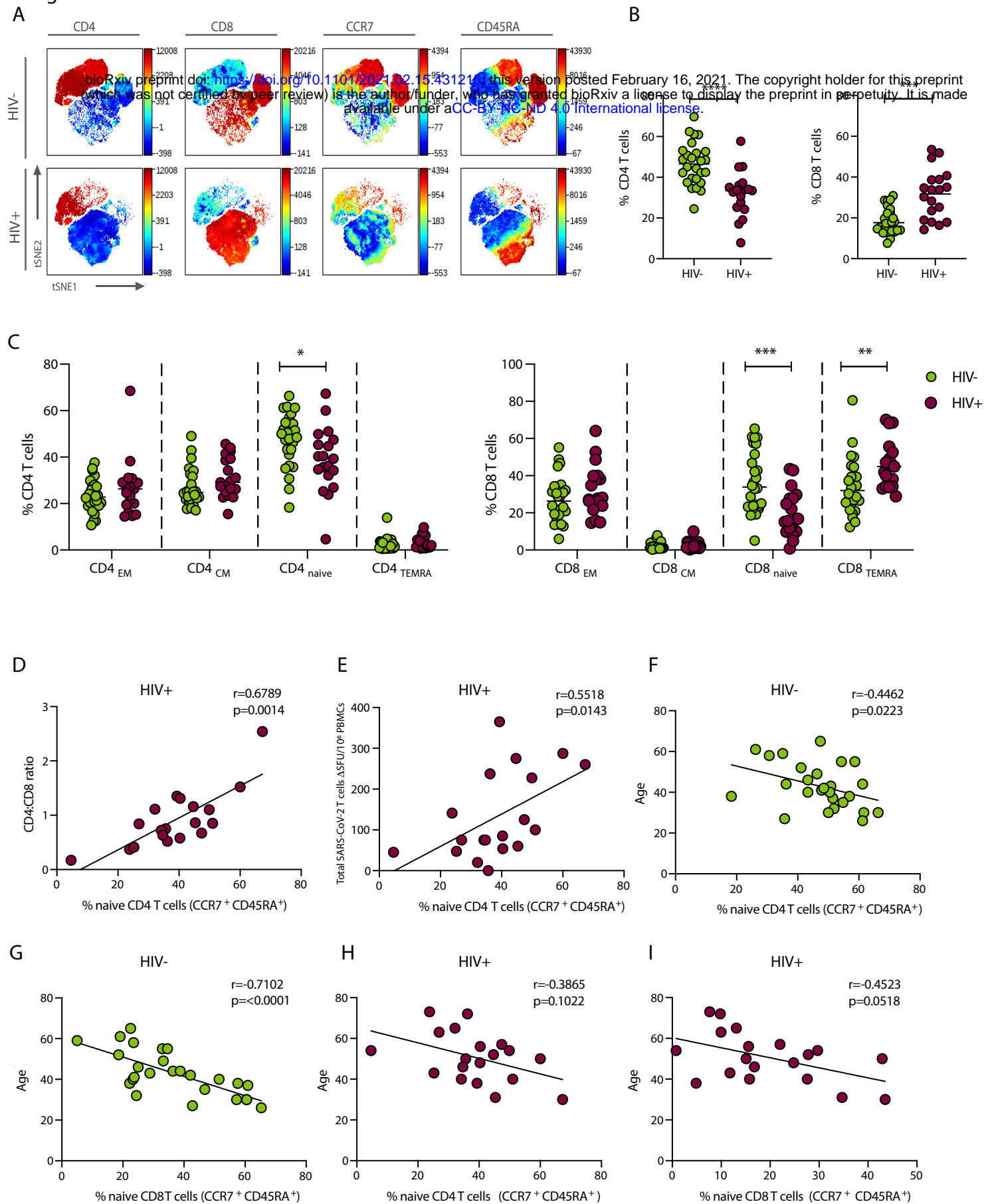


Figure 6



## Table S1. Cohort Demographics and Clinical Characteristics

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		HIV+ Lab confirmed SARS-CoV-2 RT-PCR+ and/or Ab Pos	HIV+ *Suspected SARS-CoV-2	HIV- Lab confirmed SARS-CoV-2 RT-PCR+ and/or Ab Pos	HIV- *Suspected/ Household	HIV+ pre- pandemic
<b>Group size</b>	n	24	23	31	4	16
	SARS-CoV-2 seropositive at date of sampling, n (%)	23 (95.8)	7 (30.43)	29 (93.5)	0 (0.0)	0 (0.0)
<b>COVID-19 severity**</b>	Asymptomatic (score 1), n (%)	2 (8.3)	0 (0.0)	4 (12.9)	1 (25.0)	-
	Non-hospitalized (score 2), n (%)	14 (58.3)	22 (95.7)	23 (74.2)	3 (75.0)	-
	Hospitalized (score 4-5), n (%)	8 (33.3)	1 (4.3)	4 (12.9)	0 (0.0)	-
<b>COVID-19 symptoms</b>	Fever, n (%)	13 (54.2)	18 (78.3)	16 (51.6)	2 (50.0)	-
	Shortness of breath, n (%)	14 (58.3)	14 (60.9)	12 (38.7)	1 (25.0)	-
	Fatigue, n (%)	21 (87.5)	20 (87.0)	20 (64.5)	4 (100.0)	-
	Cough, n (%)	17 (70.8)	18 (78.3)	12 (38.7)	3 (75.0)	-
	Headache, n (%)	10 (41.7)	14 (60.9)	13 (41.9)	2 (50.0)	-
	Altered taste/smell, n (%)	13 (54.2)	10 (43.5)	22 (71.0)	2 (50.0)	-
	Duration of symptoms in days, median (range)	14 (2-48)	21 (3-84)	10 (3-30)	7 (5-7)	-
	Days post-symptom onset (DPSO), median (range)	148 (46-232)	181 (126-273)	144 (101-220)	200 (125-203)	-
<b>Risk factors</b>	Age, median (range)	51 (30-73)	53 (30-67)	42 (26-65)	35.5 (30-41)	48 (30-60)
	Sex, n female:male:other	4:19:1	2:21:0	16:15:0	2:2:0	2:14:0
	BMI, median (range)	27.3 (20.2-33.8)	24.6 (20.3-39.4)	25.7 (19.1-37.7)	21.9 (20.0-27.6)	
<b>Ethnicity</b>	White, n (%)	16 (66.7)	17 (73.9)	19 (61.3)	1 (25.0)	11 (68.8)
	BAME, n (%)	8 (33.3)	6 (26.1)	12 (38.7)	3 (75.0)	5 (31.3)
<b>Smoking</b>	Current, n (%)	3 (12.5)	4 (17.4)	5 (16.1)	0 (0.0)	2 (12.5)
	Ex-smoker, n (%)	1 (4.2)	1 (4.3)	2 (6.5)	0 (0.0)	1 (6.3)
	Non-smoker, n (%)	20 (83.3)	18 (78.3)	24 (77.4)	4 (100.0)	13 (81.3)
<b>HIV parameters</b>	HIV viral load	<50	<50	-	-	<50
	CD4, median (range)	571 (133-1110)	586 (310-1360)	-	-	590 (350-940)
	CD4:CD8, median (range)	0.84 (0.17-2.54)	0.97 (0.37-2.15)	-	-	0.82 (0.43-2.26)
<b>Pre-existing conditions</b>	None, n (%)	10 (41.7)	8 (34.8)	15 (48.4)	4 (100.0)	11 (68.8)
	Diabetes, n (%)	6 (25.0)	1 (4.3)	1 (3.2)	0 (0.0)	1 (6.3)
	Hypertension/CVD, n (%)	7 (29.2)	2 (8.7)	3 (9.7)	0 (0.0)	2 (12.5)
	Renal disease, n (%)	2 (8.3)	2 (8.7)	0 (0.0)	0 (0.0)	0 (0)
	Respiratory disease (asthma and COPD), n (%)	2 (8.3)	4 (17.4)	1 (3.2)	0 (0.0)	0 (0)
	Liver disease, n (%)	1 (4.2)	2 (8.7)	0 (0.0)	0 (0.0)	1 (6.3)
	Other	ITP, psoriasis, pituitary gland failure	Osteoporosis, peripheral neuropathy, hypothyroidism	RA, iron deficiency, psoriasis, gout, SLE, hypothyroidism		

\*Confirmed, possible and probable cases specified by the case definition for COVID-19, as of 29 May 2020, European Centre for Disease Prevention and Control [<https://www.ecdc.europa.eu/en/covid-19/surveillance/case-definition>]

\*\*Severity of COVID-19 was classified according to the WHO (World Health Organisation) clinical progression scale (Aitken et al., 2020).

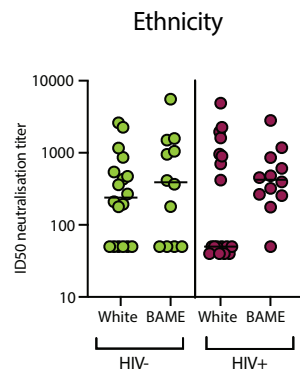
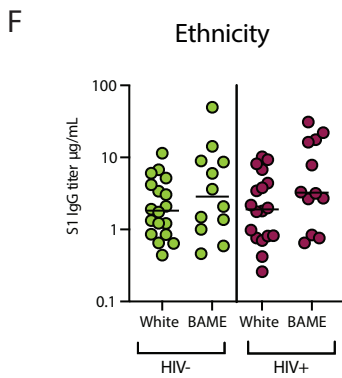
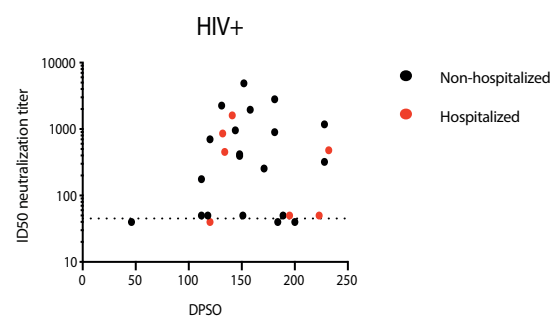
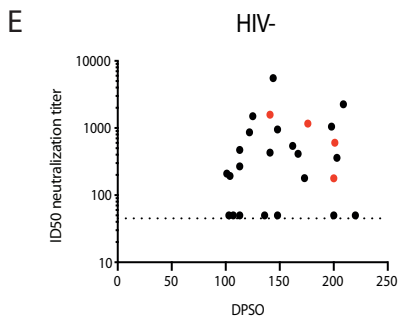
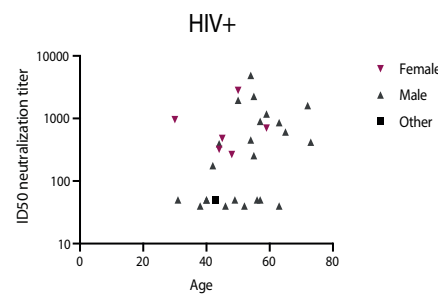
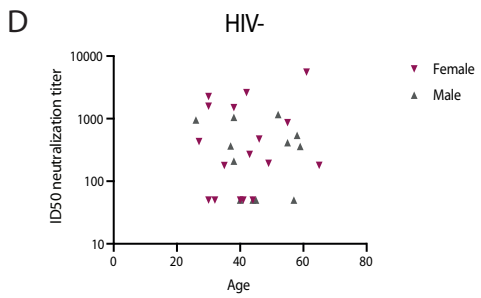
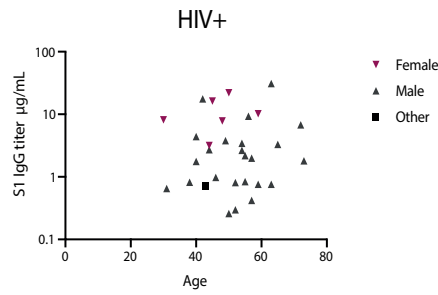
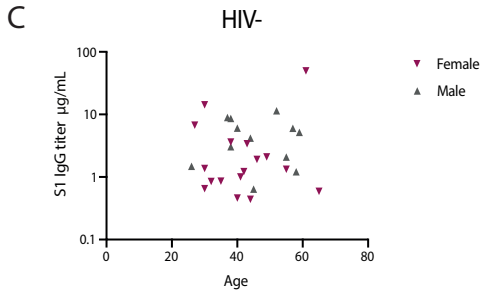
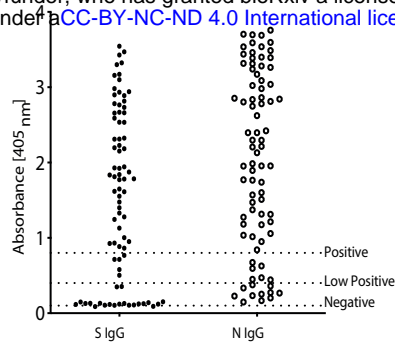
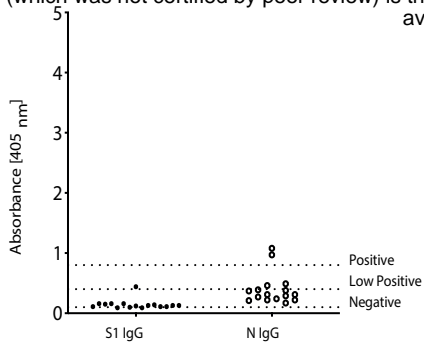


Fig. S1

A Antigen binding screen pre-2020 samples

B Antigen binding screen 2020 samples

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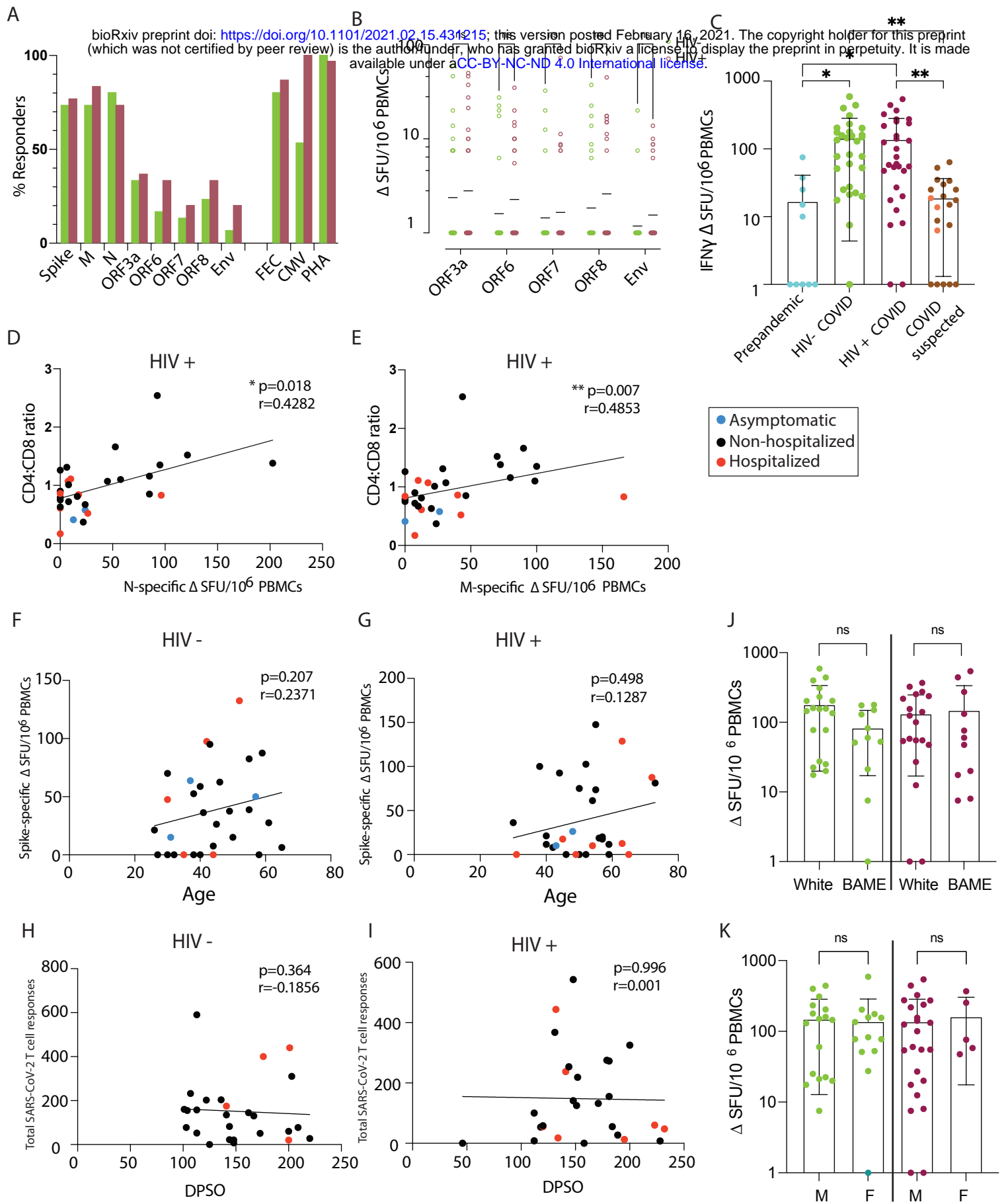
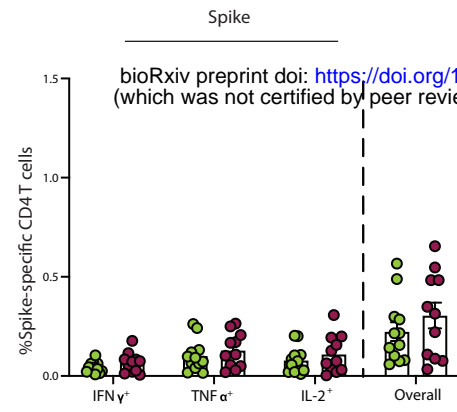
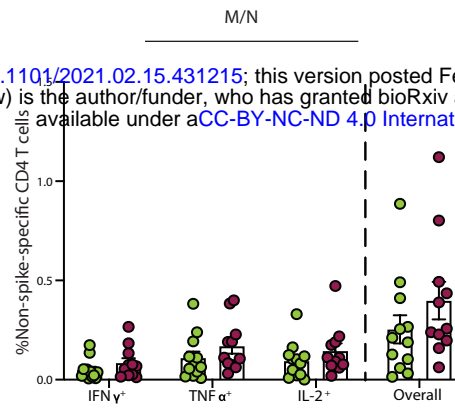


Fig. S3

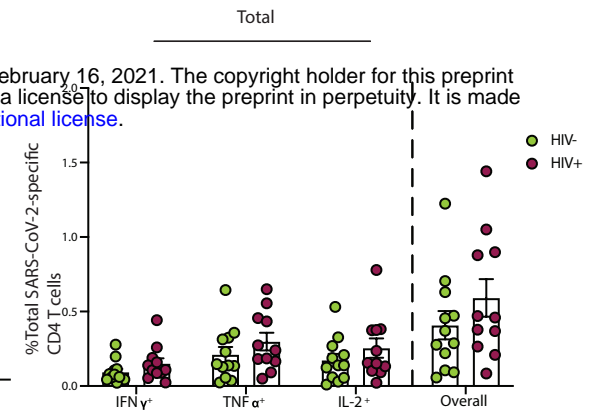
**A** SARS-CoV-2-specific CD4 T cells



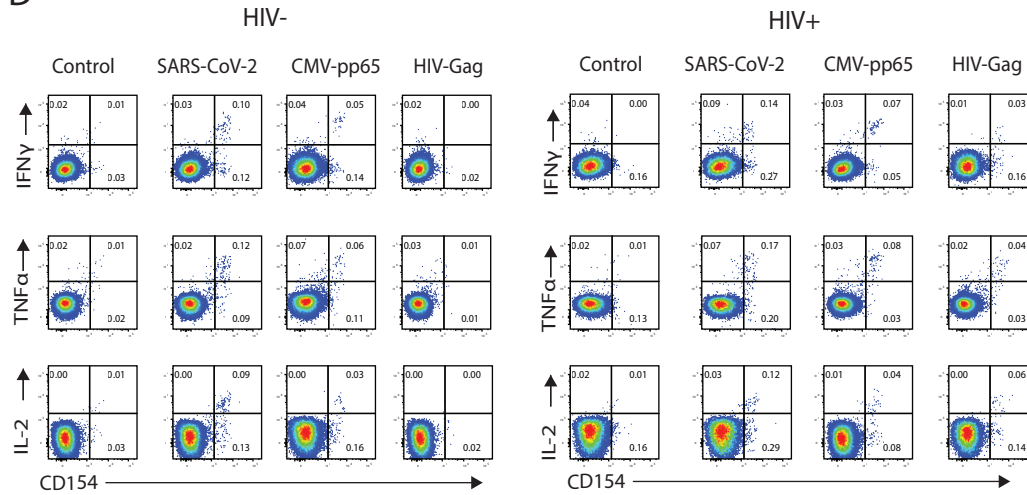
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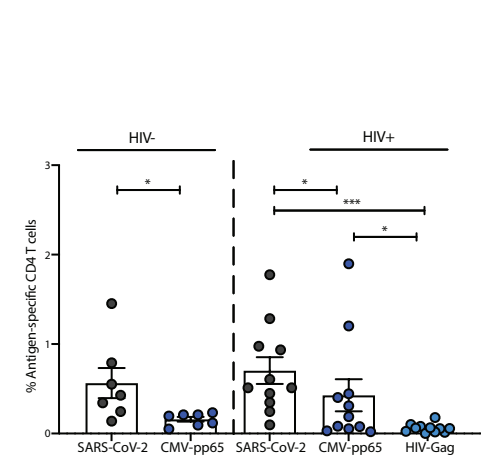
**C**



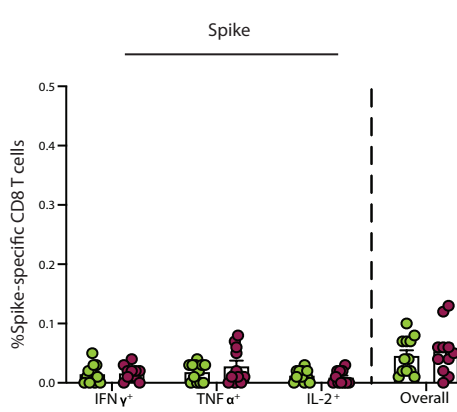
**D** SARS-CoV-2-specific CD4 T cells



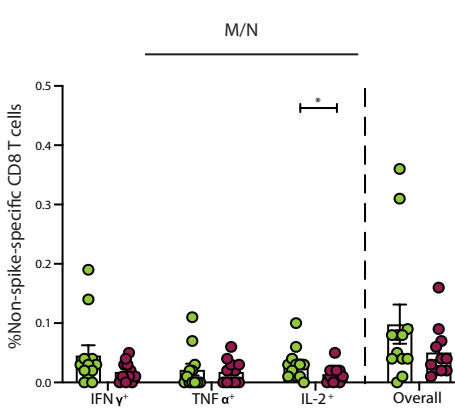
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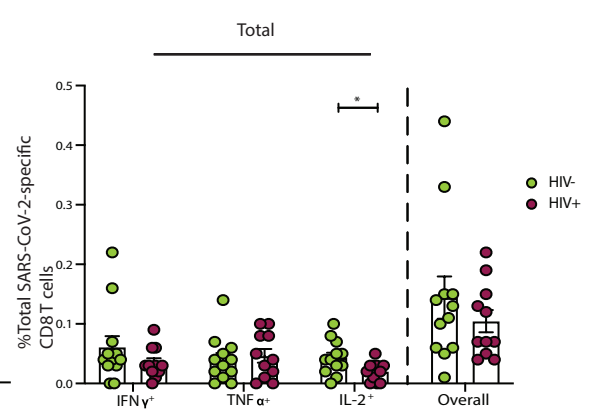
**F** SARS-CoV-2-specific CD8 T cells



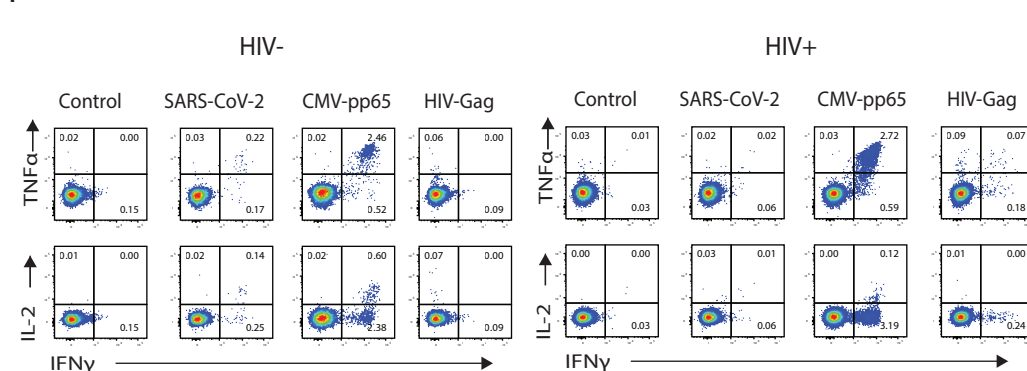
**G**



**H**



**I** SARS-CoV-2-specific CD8 T cells



**J**

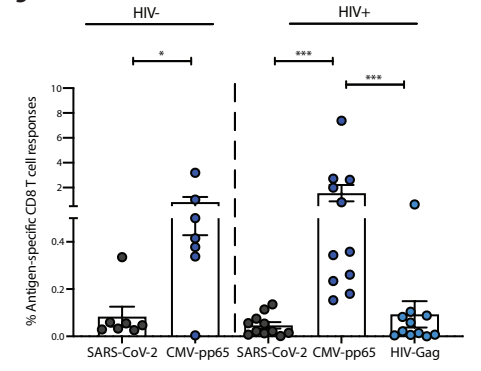


Fig. S4

