Early nucleocapsid-specific T cell responses associate with control of SARS-CoV-2 in the upper airways and reduced systemic inflammation before seroconversion

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Research Article

1   **RESEARCH REPORT**

2

3   **Early nucleocapsid-specific T cell responses associate with control of**

4   **SARS-CoV-2 in the upper airways and reduced systemic inflammation**

5   **before seroconversion**

6

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SUMMARY

Despite intensive research since the emergence of SARS-CoV-2, it has remained unclear precisely which components of the early immune response protect against the development of severe COVID-19. To address this issue, we performed a comprehensive immunogenetic and virologic analysis of nasopharyngeal and peripheral blood samples obtained during the acute phase of infection with SARS-CoV-2. We found that soluble and transcriptional markers of systemic inflammation peaked during the first week after symptom onset and correlated directly with the upper airways viral loads (UA-VLs), whereas the contemporaneous frequencies of circulating viral nucleocapsid (NC)-specific CD4$^+$ and CD8$^+$ T cells correlated inversely with various inflammatory markers and UA-VLs. In addition, we observed high frequencies of activated CD4$^+$ and CD8$^+$ T cells in acutely infected nasopharyngeal tissue, many of which expressed genes encoding various effector molecules, such as cytotoxic proteins and IFN-γ. The presence of functionally active T cells in the infected epithelium was further linked with common patterns of gene expression among virus-susceptible target cells and better local control of SARS-CoV-2. Collectively, these results identified an immune correlate of protection against SARS-CoV-2, which could inform the development of more effective vaccines to combat the acute and chronic illnesses attributable to COVID-19.

INTRODUCTION

SARS-CoV-2 has infected more than 600 million people and caused more than 6 million deaths worldwide (https://www.worldometers.info/coronavirus). Vaccines designed primarily to elicit neutralizing antibodies against the spike (S) protein initially attenuated the course of disease and protected against the development of severe COVID-19 [1, 2, 3, 4, 5]. However, the continual emergence of viral escape variants has undermined this approach, and the ongoing pandemic is now driven largely by strains resistant to antibody-mediated neutralization[6].

Several reports have indicated a likely role for SARS-CoV-2-specific T cells as a key determinant of immune protection against severe COVID-19 [7, 8, 9, 10, 11]. More directly, antigen-specific memory CD4$^+$ T cells in the airways have been shown to protect mice against respiratory coronaviruses after vaccination[12], and depletion studies in rhesus macaques vaccinated with adenoviral-encoded S (Ad26.COV2.S) have implicated CD8$^+$ T cells as important mediators of viral control after intranasal or intratracheal challenge with SARS-CoV-2[13]. It is also notable that antigen-specific memory CD4$^+$ T cells in the circulation have been associated with immune protection in humans after influenza virus challenge [14]. In line with these observations, SARS-CoV-2 has been shown to induce tissue-resident memory T cell immunity[15, 16], but the precise correlates of early viral control and disease mitigation have nonetheless remained elusive[17].

In this study, we investigated the dynamics of adaptive immune responses in relation to markers of disease severity during acute infection with SARS-CoV-2 in previously unexposed, non-vaccinated patients. Our data provided correlative and mechanistic evidence to indicate that viral nucleocapsid (NC)-specific T cells were the central determinants of immune protection, limiting viral replication in the upper airways and suppressing the attendant inflammatory response. Collectively, these observations revealed a cellular and molecular signature of effective antiviral immunity, with potential implications for the development of next-generation vaccines against COVID-19.

4

RESULTS

Viral loads in the upper airways are highly variable during acute infection with SARS-CoV-2

A total of 37 patients with acute COVID-19 were recruited into this study between May and December 2020. All participants had mild symptoms that did not require hospitalization (Table 1)[18]. Twenty-five of these patients were recruited within the first week of symptom onset (median = 5 days, interquartile range [IQR] = 4–6 days). Upper airways viral loads (UA-VLs) were highly variable during the first week of infection (median = 1.7×10^8 RNA copies/ml, range = 1.7×10^2 to 9.8×10^{10} RNA copies/ml) (Figure 1A). IgA and IgG responses against the viral S protein were below the detection threshold in all cases (Supplementary Figure 1), and only 12% of donors (3/25) had detectable neutralization titers at the time of recruitment (Figure 1B). In the second week of infection, all patients had lower UA-VLs (median = 2.1×10^3 RNA copies/ml, range = 4.8×10^0 to 1.1×10^7 RNA copies/ml) (Figure 1B), and SARS-CoV-2 neutralization titers became detectable in 92% of cases (23/25), subsequently peaking during the third week of infection (median IC_{50} = 165, IQR = 66–375) (Figure 1B). Most subjects retained detectable neutralization titers until the last study visit 6 months after symptom onset (Figure 1B). A similar pattern was observed for antibody responses against the viral NC protein (Supplementary Figure 1).

Collectively, these data established that UA-VLs peaked during the first week of infection, before the emergence of detectable antibody responses, and varied considerably among individuals with mild COVID-19.

Nucleocapsid-specific T cell responses correlate inversely with upper airways viral loads during acute infection with SARS-CoV-2

T cell responses against the viral NC and S proteins were measured longitudinally using flow cytometry to detect the intracellular production of IFN-γ. SARS-CoV-2-specific CD4+ T cells were detected more frequently than SARS-CoV-2-specific CD8+ T cells (Figure 2 A–E). Area under the curve (AUC) analyses revealed that the overall frequency of SARS-CoV-2-specific CD4+ T cells was higher than the overall frequency of SARS-CoV-2-specific CD8+ T cells per day across all time points in the study (p < 0.0001) (Figure 2F), and in both lineages, the overall frequency of NC-specific T cells was higher than the overall frequency of S-specific T cells per day across all time points

in the study (p = 0.0102) (Figure 2G). Higher frequencies of NC-specific CD4^{+} T cells and S-specific CD8^{+} T cells were detected in patients versus healthy controls during the first week of infection (p = 0.0005 for NC, p = 0.0085 for S) (Figure 2H and I). SARS-CoV-2-specific CD4^{+} T cell responses typically peaked during the third week after symptom onset for NC (median = 0.045% of CD4^{+} T cells) and S (median = 0.023% of CD4^{+} T cells), whereas SARS-CoV-2-specific CD8^{+} T cell responses typically peaked during the fourth week after symptom onset for NC (median = 0.024% of CD8^{+} T cells) and during the third week after symptom onset for S (median = 0.033% of CD8^{+} T cells) (Figure 2H and I). Of note, 51.1% of patients mounted detectable SARS-CoV-2-specific CD4^{+} T cell responses during the first week of infection, and 37.7% of patients mounted detectable SARS-CoV-2-specific CD8^{+} T cell responses during the first week of infection (Figure 2E).

In total, 21% of healthy controls had detectable NC-specific T cell responses, and 52% of healthy controls had detectable S-specific T cell responses (Figure 2H and I), consistent with previous reports[9, 19, 20, 21]. To investigate this phenomenon, we measured serological reactivity against the four common cold coronaviruses (CCCVs). Strain-specific antibody responses were detected in most patients for NL63 (80%), OC43 (64%), and HKU1 (68%), whereas only 48% of patients were seropositive for 229E (Supplementary Figure 2A). Data from healthy controls are shown in Supplementary Figure 2B. There was no association between the presence of early NC-specific CD4^{+} or CD8^{+} T cell responses and serological reactivity against CCCVs (Supplementary Figure 2C).

In further analyses, we found a strong inverse correlation between the overall frequency of circulating NC-specific T cells during the first week after symptom onset and UA-VLs (r = −0.76, p < 0.0001) (Figure 3A). This association was strongest for NC-specific CD4^{+} T cells (r = −0.70, p < 0.0001) but was also significant for NC-specific CD8^{+} T cells (r = −0.45, p = 0.02) (Figure 3A). In contrast, we found no such correlations for S-specific T cells, irrespective of lineage (Figure 3B). Using a censored linear mixed effects model with random individual effects to control for other potential confounders, we also found that incremental increases in the frequencies of NC-specific but not S-specific CD4^{+} and CD8^{+} T cells reduced individual UA-VLs (Supplementary Figure 3). Age and gender did not play a significant role. Importantly,

190   the model also controlled for time after symptom onset in the regression analysis,
191   ensuring the results were independent of any natural decay in the UA-VLs.
192
193   Collectively, these findings supported a role for early IFN-γ-expressing NC-specific
194   CD4^{+} and CD8^{+} T cells as mediators of viral clearance in the upper airways, which
195   could have important implications for the development of more effective vaccines
196   against SARS-CoV-2.
197

Nucleocapsid-specific T cell responses correlate inversely with markers of
systemic inflammation during acute infection with SARS-CoV-2

200   Excessive production of various chemokines and cytokines, including CXCL10 and
201   CXCL11, has been linked with the severity of COVID-19[22, 23]. Using a 26-plex panel,
202   we found that plasma concentrations of CXCL10 and CXCL11 were significantly
203   elevated during the first week after symptom onset (median = 3,922 pg/ml and 97.5
204   pg/ml, respectively) compared with later time points ($p < 0.001$ or $p < 0.0001$) (Figure
205   3C, Supplementary Figure 4). Moreover, plasma concentrations of CXCL10 during the
206   first week after symptom onset correlated directly with UA-VLs ($r = 0.50$, $p = 0.01$) and
207   inversely with the frequency of circulating NC-specific T cells ($r = -0.43$, $p = 0.03$)
208   (Figure 3C). Similar correlations were found for CXCL11 ($r = 0.65$, $p = 0.0004$ versus
209   UA-VLs; $r = -0.43$, $p = 0.03$ versus NC-specific T cells) (Supplementary Figure 4).
210   Other soluble factors were also upregulated significantly in the first week after symptom
211   onset compared with later time points, including CCL3, CCL19, galectin-9, and MICA
212   (Supplementary Figure 4). Plasma concentrations of CCL2, CCL19, galectin-9, and
213   MICA correlated directly with UA-VLs ($r > 0.4$, $p < 0.05$), and plasma concentrations of
214   CCL19 and MICA correlated inversely with the frequency of circulating NC-specific T
215   cells during the first week after symptom onset ($r < -0.4$, $p < 0.05$) (Supplementary
216   Figure 4). No correlations were identified for S-specific T cells versus any analyte (data
217   not shown).
218

219   To explore the nature of these associations, we profiled the transcriptomes of
220   circulating immune cell subsets, namely CD4^{+} T cells, CD8^{+} T cells, monocytes, and
221   NK cells, isolated during the first week after symptom onset (n = 14 patients with mild
222   COVID-19). We initially focused our analysis on previously reported differentially
223   expressed genes (DEGs), notably *STAT1, OAS1*, and *PKR*, which have been

7

224 implicated in the clearance of SARS-CoV-1 by IFN-γ^{+} NC-specific CD4^{+} T cells after

225 intranasal vaccination[12]. In our cohort, the frequency of circulating NC-specific CD4^{+} T

226 cells correlated inversely with gene expression among circulating immune cell subsets

227 for *STAT1* (CD4^{+} T cells, r = −0.38, p = 0.029; CD8^{+} T cells, r = −0.53, p = 0.001;

228 monocytes, r = −0.34, p = 0.05; NK cells, r = −0.39, p = 0.023), *OAS1* (CD4^{+} T cells, r

229 = −0.21, p = 0.25; CD8^{+} T cells, r = −0.47, p = 0.006; monocytes, r = −0.60, p = 0.0002;

230 NK cells, r = −0.5, p = 0.003), and *PKR* (CD4^{+} T cells, r = −0.42, p = 0.015; CD8^{+} T

231 cells, r = −0.23, p = 0.199; monocytes, r = −0.51, p = 0.003; NK cells, r = −0.43, p =

232 0.012) (Figure 4A). Similar correlation trends were observed among the same immune

233 cell subsets for NC-specific CD8^{+} T cells, and direct correlations were detected for all

234 three markers versus UA-VLs (Figure 4A).

235

236 Next, we conducted mean expression analyses for pathways classified as *Signal*

237 *Transduction*, *Signaling Molecules and Interaction*, *Immune System*, and *Cell Growth*

238 *and Death according to the Kyoto Encyclopedia of Genes and Genomes (KEGG)*

239 *database*. Correlations were performed against the frequency of circulating NC-

240 specific CD4^{+} T cells (Figure 4B), the frequency of circulating NC-specific CD8^{+} T cells

241 (Figure 4C), and UA-VLs (Figure 4D). Signaling pathways involved in the host

242 response and inflammation, including those for NF-□B, RIG-1-like receptors (RLRs),

243 and JAK-STAT, generally correlated inversely with the frequency of NC-specific CD4^{+}

244 T cells and directly with UA-VLs (Figure 4B and D). The frequency of circulating NC-

245 specific CD8^{+} T cells also correlated inversely with the NF-KBpathway but directly with

246 other pathways, including those associated with cytotoxicity (Figure 4C). The pathway

247 scores were then included in the censored linear mixed effect model for further

248 investigation. These analyses confirmed that the pathway scores for NF-KBand RLR

249 signaling, as well as other pathways, including antigen processing and presentation,

250 were influenced by UA-VLs for at least one of the immune cell subsets in each pathway

251 (Supplementary Figure 5).

252

253 Unsupervised hierarchical clustering further revealed three distinct clusters within the

254 overall data set (Figure 4E). One group incorporating NC-specific CD4^{+} T cell

255 responders was characterized predominantly by downregulation of immune system

256 and signaling pathways among circulating immune cell subsets, whereas another

257 cluster incorporating NC-specific CD4^{+} T cell non-responders was characterized

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258 predominantly by upregulation of immune system and signaling pathways among
259 circulating immune cell subsets (Figure 4E). The other cluster incorporated a mixed
260 group of NC-specific CD4$^+$ T cell responders and non-responders, in which immune
261 system and signaling pathways among circulating immune cell subsets were either
262 upregulated, predominantly among T cells, or downregulated, predominantly among
263 monocytes and NK cells (Figure 4E).
264
265 Collectively, these data showed that systemic upregulation of inflammatory pathways
266 during early infection was positively associated with high viral burdens in the upper
267 airways and negatively associated with the frequencies of circulating NC-specific CD4$^+$
268 and CD8$^+$ T cells, which in turn suggested that these immune effectors likely mitigated
269 the inflammatory response via enhanced clearance of SARS-CoV-2.
270

271 **T cells in the upper airways express IFN-γ and cytotoxic effector molecules**
272 **during acute infection with SARS-CoV-2**
273 To pursue this line of investigation, which suggested a potential role for tissue-
274 recirculating and/or tissue-resident NC-specific CD4$^+$ and/or CD8$^+$ T cells as mediators
275 of viral control at the site of infection[12], we interrogated a public single-cell RNA
276 sequencing data set obtained from nasopharyngeal material collected from healthy
277 controls (n = 15), patients in intensive care with no recent history of COVID-19 (n = 6),
278 and patients with mild to severe COVID-19 (n = 37)[24]. A total of 32,587 cells were
279 analyzed in the original study and annotated to 32 clusters spanning distinct identities
280 across the epithelial barrier and the immune system. Initial reclustering and
281 reannotation focused on T cell identity revealed multiple small clusters of CD4$^+$ T cells
282 (n_{cells} = 66, n_{donors} = 16) and a single large cluster of CD8$^+$ T cells (n_{cells} = 310, n_{donors} =
283 23). We then identified T cells expressing effector molecule-encoding mRNAs. The
284 most abundantly expressed transcripts encoded IFN-γ (CD4$^+$ T cells: n_{donors} = 7, f_{cells} =
285 28%; CD8$^+$ T cells: n_{donors} = 17, f_{cells} = 44%), followed by TNF (CD4$^+$ T cells: n_{donors} =
286 6, f_{cells} = 18%; CD8$^+$ T cells: n_{donors} = 15, f_{cells} = 27%), FasL (CD4$^+$ T cells: n_{donors} = 4,
287 f_{cells} = 12%; CD8$^+$ T cells: n_{donors} = 14, f_{cells} = 20%), and CD40L (CD4$^+$ T cells: n_{donors} =
288 7, f_{cells} = 14%), and less frequently by IL-2, IL-10, and IL-21 (Supplementary Figure 6).
289 We also detected transcripts encoding cytotoxic effector molecules, including perforin
290 (CD4$^+$ T cells: n_{donors} = 7, f_{cells} = 31%; CD8$^+$ T cells: n_{donors} = 20, f_{cells} = 39%) and
291 granzyme A (CD4$^+$ T cells: n_{donors} = 7, f_{cells} = 36%; CD8$^+$ T cells: n_{donors} = 15, f_{cells} =

9

292 40%) (Supplementary Figure 6). Of note, *FASL*, *GZMA*, *GZMB*, and *PRF* were often

293 expressed coordinately among nasopharyngeal CD8$^+$ T cells, and overall, *GZMA*,

294 *GZMB*, *IFNG*, and *PRF* were expressed less commonly among nasopharyngeal CD4$^+$

295 and CD8$^+$ T cells from healthy controls versus patients infected with SARS-CoV-2 (p <

296 0.05 for all comparisons, data not shown).

297

298 Collectively, these analyses showed that cytotoxic and other effector molecules were

299 expressed frequently among T cells isolated from the upper airways, especially in

300 patients with mild to severe COVID-19.

301

302 **T cell expression of IFN-γ in the upper airways is linked with antigen**

303 **presentation and viral control during acute infection with SARS-CoV-2**

304 Next, we identified responders (n = 7) and non-responders (n = 9) among the patients

305 with mild to severe COVID-19, defined as those with or without nasopharyngeal IFN-

306 γ$^+$ T cells, respectively. Further interrogation of the original data segregated by

307 responder status revealed that 17 of the 32 initially annotated cell subsets contained

308 DEGs (Figure 5A). The highest numbers of upregulated DEGs were present in

309 developing (n = 291) or IFN-responsive ciliated cells (n = 184) (Figure 5A,

310 Supplementary Tables 1 and 2), which are abundant in the nasopharynx and frequent

311 targets of SARS-CoV-2[24]. In responders, these cells overexpressed master

312 transcription factors involved in antiviral immunity, such as *STAT1* and *IRF1*, and

313 genes associated with antigen processing and presentation, such as *HLA-A*, *HLA-B*,

314 *HLA-C*, *HLA-E*, *HLA-DQB1*, *B2M*, *TAP1*, *TAP2*, *TAPBP*, and the proteasome subunit

315 *PSMD6*, many of which are regulated by *IRF1* (Figure 5B, Supplementary Table 1).

316 Antigen processing and presentation gene sets were also significantly upregulated in

317 both cell types across multiple GO terms (Figure 5C, Supplementary Table 2). Similar

318 enrichments were observed for developing ciliated cells in pathway analyses aligned

319 to KEGG (Figure 5D, Supplementary Table 2). Moreover, these cells exhibited high

320 combined scores for apoptosis, cellular senescence, necroptosis, and signaling via

321 TNF (Figure 5D, Supplementary Table 2). Of further note, developing ciliated cells

322 overexpressed gene sets in responders that associated with the negative regulation of

323 translation under stress, including the PERK-mediated unfolded protein response,

324 consistent with innate suppression of viral replication after entry[25], and IFN-responsive

325 ciliated cells overexpressed *IFITM1*, *IFITM2*, and *IFITM3*, which encode proteins
326 known to modulate viral entry [26, 27, 28, 29].

327 In further analyses, we found that 54 genes were differentially upregulated among
328 nasopharyngeal CD8$^+$ T cells in the presence of site-matched IFN-γ$^+$ CD4$^+$ T cells,
329 most prominently those associated with the induction of apoptosis and cytotoxicity,
330 such as *GZMA*, *GZMB*, and *GNLY* (Figure 5B, Supplementary Tables 1 and 2). Other
331 notable DEGs included *SELL*, which encodes L-selectin, and genes encoding multiple
332 ribosomal subunits (Figure 5B, Supplementary Table 1), which were linked in GO terms
333 with protein translation, RNA processing, and protein export/transport to the cell
334 membrane (Figure 5C, Supplementary Table 2). Some of these genes have been
335 linked previously with CD8$^+$ T cell activation[30]. In addition, we noted that many genes
336 associated with antigen processing and presentation were upregulated
337 contemporaneously, including various *HLA-A*, *HLA-B*, *HLA-C*, *B2M*, *IFI6*, and *TAP1*
338 (Figure 5C, Supplementary Tables 1 and 2). Importantly, we also found that
339 responders exhibited higher fractions of SARS-CoV-2 RNA-free cells and lower
340 abundances of SARS-CoV-2 RNA in infected cells compared with non-responders
341 (responders, n_{cells} = 11,871; non-responders, n_{cells} = 5,386; p = 0.00013), thereby
342 aligning our results with biological efficacy (Supplementary Figure 7).

343

344 Collectively, these findings indicated that the presence of activated T cells in the upper
345 airways was associated with enhanced target cell conditioning for immune recognition,
346 globally upregulated viral clearance mechanisms, and better localized control of
347 SARS-CoV-2.

348

DISCUSSION

In this study, we undertook a comprehensive evaluation of adaptive immune responses, inflammatory cascades, and gene expression profiles among circulating immune cell subsets to define the correlates of viral control during acute infection with SARS-CoV-2. We found that genetic and plasma markers of systemic inflammation peaked during the first week after symptom onset and correlated directly with UA-VLs, whereas the contemporaneous frequencies of circulating viral NC-specific CD4+ and CD8+ T cells correlated inversely with various inflammatory markers and UA-VLs. Moreover, we identified high frequencies of activated CD4+ and CD8+ T cells in acutely infected nasopharyngeal tissue, many of which expressed genes encoding various effector molecules, such as cytotoxic proteins and IFN-γ. The presence of IFN-γ+ T cells in the infected epithelium was further linked with common patterns of gene expression among virus-susceptible target cells and better local control of SARS-CoV-2. Collectively, these results indicated a protective role for viral NC-specific T cells during the acute phase of infection with SARS-CoV-2, thereby providing an immune correlate that could inform the development of more effective vaccines against COVID-19.

T cells have been implicated as mediators of immune protection in some but not all studies of acute infection with SARS-CoV-2[7, 8, 9, 11]. These discrepancies may relate to the exact timing of sample acquisition. In our study, the inverse correlation between circulating viral NC-specific T cell frequencies and UA-VLs was apparent only during the first week after symptom onset, prior to seroconversion. At this time, many of our patients exhibited high plasma concentrations of proinflammatory cytokines, many of which have been linked previously with severe disease, including the CXCR3 ligand CXCL10[8, 31, 32]. In line with an earlier study[8], we detected an inverse correlation between the frequencies of circulating viral NC-specific T cells and plasma concentrations of CXCL10, which in turn correlated directly with UA-VLs. Similar relationships were observed for NF-KB signaling pathway gene expression scores among circulating immune cell subsets, hinting at a potential mechanism. Indeed, many cytokines are transactivated via the NF-κB signaling pathway, including those implicated previously in the inflammatory storm that accompanies severe COVID-19, such as IL-1, IL-6, IL-8, TNF, and CXCL10[33]. These results supported the notion that immune control of early viral replication attenuates the local and systemic inflammation

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383 characteristic of severe COVID-19[34]. Unexposed individuals frequently harbor cross-
384 reactive T cells with functional specificity for SARS-CoV-2, which likely arise in the
385 memory pool as consequence of previous infections with other viruses that exhibit a
386 degree of structural homology, such as CCCVs[19, 20, 21]. In our study, all patients were
387 seropositive for one or more CCCVs before the emergence of detectable antibody
388 responses against SARS-CoV-2, and many healthy controls exhibited T cell cross-
389 reactivity against S (54%) and NC (21%). However, it should be noted that amino acid
390 sequence conservation between CCCVs and SARS-CoV-2 is rather limited across NC
391 (<30%), and that *de novo* priming of antiviral T cells from the naive pool could have
392 occurred before clinical presentation[35].

393

394 Analogous to our finding that viral NC-specific but not viral S-specific T cell frequencies
395 correlated inversely with UA-VLs, previous work has identified broad T cell reactivity
396 against the major viral Gag proteins (matrix, capsid, and NC) but not the viral Env
397 protein as a correlate of immune protection against HIV-1[36, 37]. It is notable here that
398 the corresponding virions are known to contain substantially higher amounts of NC
399 compared with S or Env, respectively, and that target cells infected with SARS-CoV-2
400 *in vitro* have been shown to express approximately fivefold more NC compared with
401 S[38, 39, 40]. High expression of NC has been reported *ex vivo* for upper airways target
402 cells infected with SARS-CoV-2[24]. It is also notable that early viral matrix-specific and
403 NC-specific T cell responses have been associated with protection against disease
404 and reduced viral shedding after influenza virus infection[14]. The abundant expression
405 of internal viral proteins may therefore facilitate early antigen presentation at surface
406 densities sufficient to trigger cognate T cells more rapidly than external viral proteins,
407 leading to greater immune efficacy. This paradigm makes sense in the context of our
408 study and cautions against vaccine strategies that immunize solely against the S
409 protein of SARS-CoV-2.

410

411 IFN-γ^+ T cells were common in acutely infected nasopharyngeal tissue, likely as a
412 consequence of direct specificity for SARS-CoV-2. Moreover, the presence of
413 nasopharyngeal IFN-γ^+ T cells was associated with distinct patterns of gene
414 expression among site-matched target cells, which upregulated pathways associated
415 with antigen processing and presentation, apoptosis regulation, and innate antiviral
416 responses, and also less frequently harbored SARS-CoV-2 RNA. Contemporaneously,

417   the presence of nasopharyngeal IFN-γ⁺ CD4⁺ T cells was associated with the

418   expression of effector genes among site-matched CD8⁺ T cells, especially those

419   associated with cytotoxicity and *SELL*, which encodes L-selectin. It is notable here that

420   L-selectin plays a critical role in transendothelial migration, which is indispensable for

421   viral clearance, at least in mice[41]. Transcripts encoding cytotoxic proteins were also

422   present in nasopharyngeal CD4⁺ T cells, indicating direct lytic activity[14, 42, 43]. In line

423   with these findings, which suggested a coordinated network of viral suppression

424   mechanisms driven by the influx of IFN-γ⁺ T cells during acute infection,

425   nasopharyngeal target cells also expressed lower amounts of SARS-CoV-2 RNA.

426

427   Several preclinical studies have provided support for the notion that next-generation

428   vaccines would benefit from the inclusion of NC antigens to enhance immune efficacy

429   against SARS-CoV-2. For example, IFN-γ production by viral NC-specific T cells in the

430   airways was found to be a key determinant of outcome in mice infected with influenza

431   virus or SARS-CoV-1[12, 43], and local immunization with a single conserved NC epitope

432   recognized by CD4⁺ T cells was sufficient to protect mice from MERS or SARS-CoV-

433   1[12]. Intranasal vaccination of cynomolgus macaques with structural proteins from the

434   inner virion core has also been shown to induce potent NC-specific T cell immunity and

435   reduce peak UA-VLs by almost two orders of magnitude in the absence of neutralizing

436   antibody responses against SARS-CoV-2[44]. Moreover, convalescent patients have

437   been shown to harbor tissue-resident memory T cells targeting the most immunogenic

438   regions of SARS-CoV-2, including epitopes derived from NC[16], consistent with a role

439   in protection against recurrent episodes of COVID-19[45, 46].

440

441   There are several limitations to our study. First, our cohort was relatively small and did

442   not include patients with severe COVID-19. Second, we only report correlations,

443   precluding a definitive assessment of antiviral efficacy. Third, we were unable to define

444   antigen specificity in the single-cell RNA sequencing data set, instead relying on the

445   expression of IFN-γ mRNA as a surrogate marker of T cell activation driven by cognate

446   engagement with epitopes derived from SARS-CoV-2[47]. Fourth, overlapping peptide

447   sets can be suboptimal for the detection of functional CD8⁺ T cell responses, albeit

448   with the concomitant advantage of global antigenic coverage[36, 48]. In spite of these

449   caveats, our results provided clear evidence of a protective role for viral NC-specific T

450   cells in the context of acute infection with SARS-CoV-2, thereby arguing for inclusion

451   of the corresponding antigens in next-generation vaccines designed to combat COVID-
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AUTHOR CONTRIBUTIONS

TME performed most of the experiments and analyzed data supported by MIMA, FD, KH, KP, RRA, NC, and AW; OB, KP, AL, and LN analyzed data; MHu and JH performed computational modeling; KH and LL performed HLA typing; GP performed viral sequencing; MB and DAP provided intellectual input; JR, PK, AM, LO, IK, and MHo contributed samples; KV and FK measured neutralizing antibodies; LO, AW, IK, MHo, and CG conceived the study and wrote the clinical protocol; JH, MHo, and CG acquired funding; TME, OB, and CG wrote the manuscript with input from all contributors.

DECLARATION OF INTERESTS

The authors have no competing interests to declare.

532 **TABLES**

533

534 **Table 1. Patient characteristics.**

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Patients	37
Gender (female)	20 [54.5%]
Median age (years) [IQR]	36 [30/49.5]
WHO score 1	1 [2.7%]
WHO score 2	14 [37.8%]
WHO score 3	22 [59.5%]
Lung involvement	21 [56.75%]
Recruited within first week after symptom onset	25 [67.75%]
Neutralizing antibodies (1–7 days after symptom onset)	4 [16%]
Anti-Ig nucleocapsid (1–7 days after symptom onset)	2 [7.6%]
Anti-IgA spike (1–7 days after symptom onset)	0
Anti-IgG spike (1–7 days after symptom onset)	0
Median log UA-VL (1–7 days after symptom onset) [IQR]	8.2 [6.9/8.8]
Median log UA-VL (8–14 days after symptom onset) [IQR]	3.3 [1.7/5.03]

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537 IQR, interquartile range; UA-VL, upper airways viral load (RNA copies/ml).

MATERIALS AND METHODS

Study participants

A total of 37 patients with acute COVID-19 were recruited into this study between May and December 2020 under the umbrella of the longitudinal KoCo19 Study[49]. All participants tested positive for SARS-CoV-2 via RT-PCR. At the time of recruitment, only the Wuhan strain (lineage A) was circulating in Germany. Clinical presentation was assessed using WHO Clinical Progression Scale. All patients in this study had mild symptoms that did not require hospitalization and therefore scored a maximum of 3[18]. Healthy controls were recruited prior to vaccination and tested negative for SARS-CoV-2 via RT-PCR. Written informed consent was obtained from all participants in accordance with the principles of the Declaration of Helsinki. This study was approved by the Ethics Committee of the Faculty of Medicine at LMU Munich (20–371).

Upper airways viral loads

Nasopharyngeal viral loads were quantified as described previously[49]. Briefly, RT-PCR was performed using a TANBead Maelstrom 9600 (Taiwan Advanced Nanotech Inc.) with an OptiPure Viral Auto Plate Kit (Taiwan Advanced Nanotech Inc.). SARS-CoV-2 RNA was quantified using an Allplex 209-nCov Assay (SeeGene) with a STARlet IVD (SeeGene). UA-VLs were calculated using standardized dilutions of SARS-CoV-2 RNA (INSTAND).

Antibody titers

SARS-CoV-2-specific antibodies were assayed in EDTA plasma as described previously[50, 51] using the following kits: Anti-SARS-CoV-2-ELISA Anti-S1 IgA (EI-S1-IgA, Euroimmun), Anti-SARS-CoV-2-ELISA Anti-S1 IgG (EI-S1-IgG, Euroimmun), and Elecsys Anti-SARS-CoV-2 Anti-N (Ro-N-Ig, Roche).

Neutralization assays

Pseudotyped viral particles were generated via cotransfection of HEK 293T cells with plasmids encoding HIV-1 Tat, HIV-1 Gag/Pol, HIV-1 Rev, luciferase, and the S protein of SARS-CoV-2 (Wu01 S, EPI_ISL_406716 lacking the cytoplasmic domain) using the FuGENE 6 Transfection Reagent (Promega). Culture supernatants were harvested at 48 h and 72 h after transfection, passed through a filter (pore size = 0.45 μm), and

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stored at −80□C. Viral titers were established by infecting ACE2-expressing 293 T cells as described previously [52]. Luciferase activity was revealed after 48 h via the addition of luciferin/lysis buffer (10 mM $MgCl_2$, 0.3 mM ATP, 0.5 mM coenzyme A, 17 mM IGEPAL, and 1 mM D-luciferin in Tris-HCL) and measured using a Tristar Microplate Reader (Berthold Technologies). Neutralization assays were performed using serum samples as described previously [53](Vanshylla et al., 2021). Briefly, serial dilutions of serum were incubated with pseudovirus supernatants for 1 h at 37□C. ACE2-expressing 293 T cells were then added in 15 µg/ml polybrene and incubated for a further 48 h at 37□C. Luciferase activity was determined as above. Results were expressed for each sample as the 50% inhibitory dilution (ID_{50}) after subtraction of background relative light units (RLUs). ID_{50} values were calculated using a non-linear fit model to plot agonist versus normalized dose-response curves with variable slopes in Prism version 7 (GraphPad). Samples that did not achieve 50% neutralization (serum ID_{50} = <10) were assigned a value halfway below the lower limit of quantification (serum ID_{50} = 5).

Common cold coronavirus serology

Antibodies against the common cold coronaviruses 229E, NL63, OC43, and HKU1 were assayed in CPDA plasma using a recomLine SARS-CoV-2 IgG Kit (Mikrogen Diagnostik).

Flow cytometry

PBMCs were isolated within 6 h of blood collection via density gradient centrifugation (Cytiva Sweden AB) and stimulated immediately with peptide pools representing the NC or S proteins of SARS-CoV-2 (1 □g/ml/peptide, Miltenyi Biotec) for 16 h at 37□C in the presence of anti-CD28 (clone L293, 1 □g/ml, BD Biosciences), anti-CD49d (clone L25, 1 □g/ml, BD Biosciences), and brefeldin A (5 □g/ml, Sigma-Aldrich). Negative control wells lacked stimulants (medium alone), and positive control wells contained staphylococcal enterotoxin B (SEB, 0.6 □g/ml, Sigma-Aldrich). Cells were then stained with anti-CD4–ECD (clone SFCI12T4D11, Beckman Coulter), anti-CD8–APC-AF750 (clone B9.11, Beckman Coulter), anti-CD57–APC (clone HNK-1, BioLegend), anti-PD1–PE-Cy5.5 (clone NAT105, BioLegend), and anti-CXCR5–PE-Cy7 (clone J252D4, BioLegend). Labeled cells were fixed/permeabilized using a FoxP3 / Transcription Factor Staining Buffer Set (eBioscience) and further stained

intracellularly with anti-CD3–APC-AF700 (clone UCHT1, Beckman Coulter), anti-IFN-γ–FITC (clone 4S.B3, BioLegend), anti-IL2–PE (clone MQ1-17H12, BioLegend), anti-TNF-α–BV510 (clone mAb11, BioLegend), anti-CTLA-4–BV421 (clone BNI3, BioLegend), anti-Ki-67–BV605 (clone Ki-67, BioLegend), and anti-CD40L–BV785 (clone 24-31, BioLegend). Samples were acquired using a CytoFLEX Flow Cytometer (Beckman Coulter). Data analysis was performed using FlowJo software version 10 (FlowJo LLC). SARS-CoV-2-specific T cell responses were defined on the basis of IFN-γ production and were considered positive at a frequency of ≥0.01% after background subtraction if greater than the corresponding unstimulated values by a factor of ≥2.

Plasma cytokines and proteins

Concentrations of CCL2, CCL3, CCL4, CCL5, CCL17, CCL19, CD23, CXCL1, CXCL4, CXCL5, CXCL10, CXCL11, galectin-1, galectin-3, galectin-9, Gas6, ICAM-1, IL-2, IL-4, IL-10, IL-19, MICA, NCAM-1, PD-L1, syndecan-1, and TFPI were determined in CPDA plasma using a customized 26-plex marker panel (R&D Systems) as described previously[54].

RNA sequencing

RNA isolation and sequencing was performed as described previously (Pekayvaz et al., 2022). Briefly, libraries were prepared from immune cell subsets (n = 500 cells each) using the Prime-seq protocol [55], and quality was determined using a High Sensitivity DNA Kit (Agilent Bioanalyzer). Paired-end sequencing (150 bp) was performed using an S1 or an S4 flow cell on a NovaSeq System (Illumina). An average of □1 × 10^7 reads were acquired per subset per sample. Preprocessing and quantification of the raw data was conducted using zUMIs[56] and referenced against GENCODE V35. Further analyses were performed using non-normalized outputs that mapped to exonic regions only (full data). Raw inputs were normalized using DESeq2 version 1.36.0[57] Analyses were limited to participants in the KoCo19 study enrolled within the first week of symptom onset (n = 14) and healthy controls (n = 8). Initial pathway enrichment analyses were performed using R package gage version 2.46.0[58]. Pathways were included from the KEGG database mapped to BRITE terms in the groups *Signal Transduction* and *Signaling Molecules and Interaction* (environmental information processing), *Immune System* (organismal systems), and *Cell Growth and*

640　*Death* (cellular processing). ENSEMBL IDs were used in the original data set and
641　converted to Entrez IDs using the org.Hs.eg.db R package version 3.15.0[59]. ID
642　mappings for some genes were non-existent or not unique. The relevant genes were
643　discarded in the former case or assigned to the first match in the latter case.
644　Spearman's formula was used to calculate correlations among gene/pathway
645　expression, cell type frequencies, and UA-VLs. Normalized read counts were used for
646　individual genes, and average expression of composite genes was used for pathways.
647　A confidence interval was calculated using bootstrapping of the original data by
648　random resampling with replacement to estimate the range of possible correlations,
649　with subsequent calculation of the mean expression score for each relevant pathway.
650　Reference pathways were generated from 30 (smallest size) or 300 random genes
651　(biggest size). Bootstrapping was performed over 1,000 iterations for each pathway.
652　Correlation coefficients were then ordered and used to pick intervals at quantile values
653　of 2.5% (low) and 97.5% (high).
654
655　**Statistics**
656　Basic statistical analyses were performed using non-parametric tests in Prism version
657　8 (GraphPad).
658
659　**Analysis of single-cell RNA sequencing data**
660　Single-cell data from nasopharyngeal samples were acquired from the Single Cell
661　Portal (https://singlecell.broadinstitute.org/single_cell/study/SCP1289/). Data were
662　normalized using Seurat version 4.1.0[60] with Harmony version 0.1.0[61] and
663　subsequently reclustered using the default settings in FindNeighbors and FindClusters.
664　One patient was excluded due to the presence of abnormally high numbers of
665　macrophages (patient 19). Characterization was performed using scCATCH version
666　3.0[62]. T cells were extracted and reclustered separately. The optimal partition was
667　determined using the silhouette function in Cluster version 2.1.3
668　(https://guix.gnu.org/en/packages/r-cluster-2.1.3/). Clusters were then classified again
669　using scCATCH with subset markers defined according to the Cell Marker Database[63].
670　One cluster was excluded on the basis of annotation failure. T cells with at least one
671　RNA read mapping to a selected function were classified as function-positive.
672　Differentially expressed genes and pathways in the IFN-γ⁺ and IFN-γ⁻ patient groups
673　were identified using the FindMarkers function with default settings in Seurat version

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674   4.1.0. Each previously reported cluster in the original annotation[24]. was interrogated
675   with no initial cutoff for limit fold-change (LFC). All remaining clusters were used as
676   reference. CD8$^+$ T cells were also identified and analyzed independently. In this case,
677   group assignments (IFN-γ$^+$ versus IFN-γ$^-$) were based on CD4$^+$ T cells alone, which
678   were used for reference. Pathway and GO term analyses were based on marker genes
679   with a LFC of 0.25 in either direction and a p-value of <0.01, except for CD8$^+$ T cells,
680   which occurred in low numbers and were analyzed using a p-value of <0.05.
681   Enrichment analyses were performed using enrichR (Kuleshov et al., 2016). Pathway
682   analyses were performed for *Signal Transduction*, *Signaling Molecules and Interaction*,
683   *Immune System*, and *Cell Growth and Death*. The common logarithm of SARS-CoV-2
684   total corrected RNA reported previously [24] was used to quantify host cell VLs. Patient
685   groups were assigned as above. Values from all cells in the IFN-γ$^+$ and IFN-γ$^-$ groups
686   formed the test distribution for the IFN-γ$^+$ and IFN-γ$^-$ groups, and comparisons were
687   performed using a two-sided Man-Whitney U test. Similar results were obtained using
688   uncorrected read counts for SARS-CoV-2 RNA.
689

690   **Interaction models**
691   A univariate linear mixed effects model was established using the default settings in
692   CensReg[64]. Point estimates for the model parameters were obtained by minimizing the
693   negative log-likelihood function using numerical minimization. Standard errors were derived
694   from the inverse of the Hessian matrix evaluated at the point estimates. The likelihood function
695   was constructed using truncated conditional normal distributions based on normality
696   assumptions about individual effects and error terms to account for the limits of viral detection.
697   A mixed effects model was also used to solidify the observed relationship as a correlation
698   between a score for the subset of pathways and cell fractions and/or VLs. A second mixed
699   model equation was added using Julia for joint modeling of subsets and VLs. This model
700   included VL as a mediator of additional confounders to evaluate the influence of the true non-
701   censored VL on each pathway score, despite the censored structure of the observed VLs. The
702   outer marginalization of random effects within the likelihood was approximated using Gauss-
703   Hermite quadrature[65], with weights obtained via the Julia package FastGaussQuadrature
704   across 10 quadrature points
705   (https://juliaapproximation.github.io/FastGaussQuadrature.jl/stable/). Gradients were obtained
706   using automatic differentiation in the Julia package ForwardDiff [66]. Pathways were prefiltered
707   by running ordinary least squares regressions to determine those potentially influenced by the
708   VL. Data preprocessing was conducted in Python using Pandas[67] and Numpy[68]. All code is
709   publicly available at https://github.com/manuhuth/early_t_cell_control.git .

LIST OF SUPPLEMENTARY TABLES

Supplementary Table 1. Differentially expressed genes among nasopharyngeal ciliated cells and CD8+ T cells from patients with COVID-19.

Supplementary Table 2. Gene ontology and pathway analyses for differentially expressed genes among nasopharyngeal ciliated cells and CD8+ T cells from patients with COVID-19.

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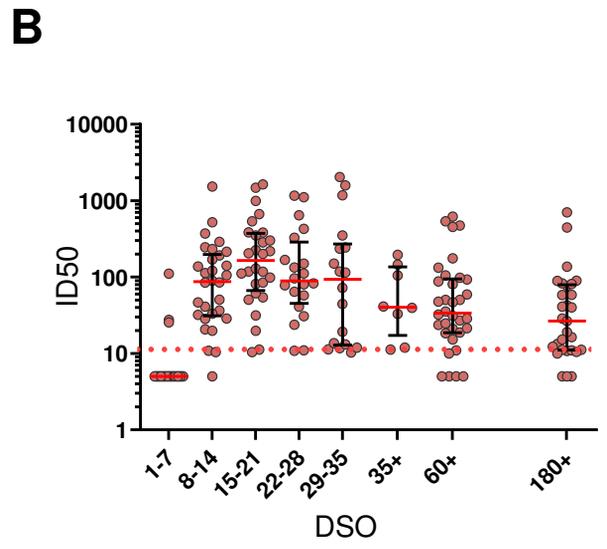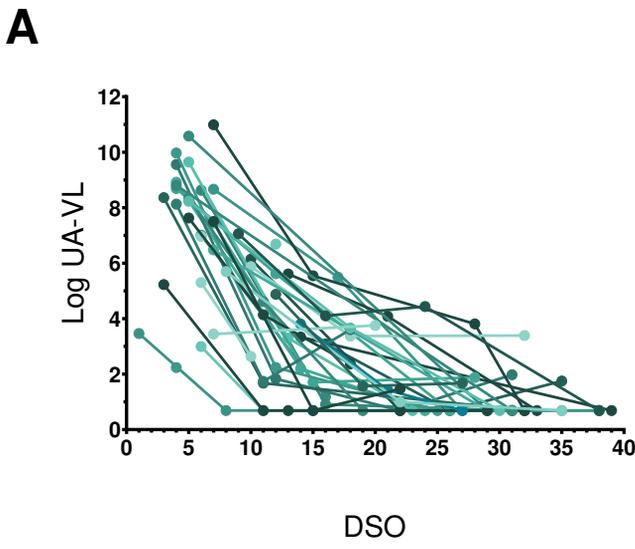
Figure 1 . Study overview, upper airways viral loads, and antibody-mediated neutralization of SARS-CoV-2. (**A**) Longitudinal quantification of upper airways viral loads (UA-VLs) in patients with mild COVID-19 (n = 25) recruited during the first week of symptom onset. Each line represents one donor. DSO, days since symptom onset. (**B**) Pseudovirus neutralization titers (ID_{50}). Each dot represents one donor. The cutoff is indicated by the dotted red line. Serum samples that did not achieve 50% neutralization ($ID_{50} < 10$) were assigned a value halfway below the lower limit of quantification ($ID_{50} = 5$). Data are shown as median ± IQR (**B**).

A Negative control — CD4 vs IFNγ — 0.0003

B NC — 0.18

C S — 0.021

D SEB — 2.23

E

% of responder vs DSO

HC, 1-7, 8-14, 15-21, 22-28, 29-35, 36+, 60+, 180+

Legend:
- IFNγ⁺ CD4⁺
- IFNγ⁺ CD8⁺
- anti-Ig (S/ NC)
- virus neutralization

F AUC per day — CD4⁺, CD8⁺ — SARS-CoV-2 specific T cells — ****

G AUC per day — CD4⁺ NC, CD8⁺ NC, CD4⁺ S, CD8⁺ S — **, ***, ****

H NC

% IFNγ⁺ T cells vs DSO — *

% IFNγ⁺ CD4⁺ T cells — ***

% IFNγ⁺ CD8⁺ T cells

HC, 1-7, 8-14, 15-21, 22-28, 29-35, 35+, 60+, 180+

I S

% IFNγ⁺ T cells

% IFNγ⁺ CD4⁺ T cells

% IFNγ⁺ CD8⁺ T cells — **

HC, 1-7, 8-14, 15-21, 22-28, 29-35, 35+, 60+, 180+

DSO

Figure 2. T cell responses against the nucleocapsid and spike proteins of SARS-CoV-2. (A–D) Representative flow cytometry plots showing the identification of IFN-γ⁺ CD4⁺ T cells in the absence of stimulation (**A**) or in the presence of overlapping nucleocapsid (NC) peptides (**B**), overlapping spike (S) peptides (**C**), or staphylococcal enterotoxin B (SEB) as the positive control (**D**). Plots are gated on CD3. Numbers indicate the percent frequency of CD4⁺ T cells that produced IFN-γ. (**E**) Responder frequencies for IFN-γ⁺ CD4⁺ and IFN-γ⁺ CD8⁺ T cells specific for NC or S, antibody titers against NC or S, and antibody-mediated neutralization of SARS-CoV-2. DSO, days since symptom onset; HC, healthy control. (**F**, **G**) Area under the curve (AUC) per day comparisons of the overall magnitude of SARS-CoV-2-specific CD4⁺ versus CD8⁺ T cells (**F**) and the overall magnitude of SARS-CoV-2-specific CD4+ versus CD8+ T cells broken down by target protein (NC versus S). Each dot represents one donor. (**H**) Frequencies of all NC-specific T cells (left), NC-specific CD4⁺ T cells (middle), and NC-specific CD8⁺ T cells (right). Each dot represents one donor. The cutoff is indicated by the dotted red line. (**I**) Frequencies of all S-specific T cells (left), S-specific CD4⁺ T cells (middle), and S-specific CD8⁺ T cells (right). Each dot represents one donor. The cutoff is indicated by the dotted red line. Data are shown as median □ IQR (**F**, **G**, **H**, and **I**). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ (Mann-Whitney U test or Wilcoxon signed rank test).
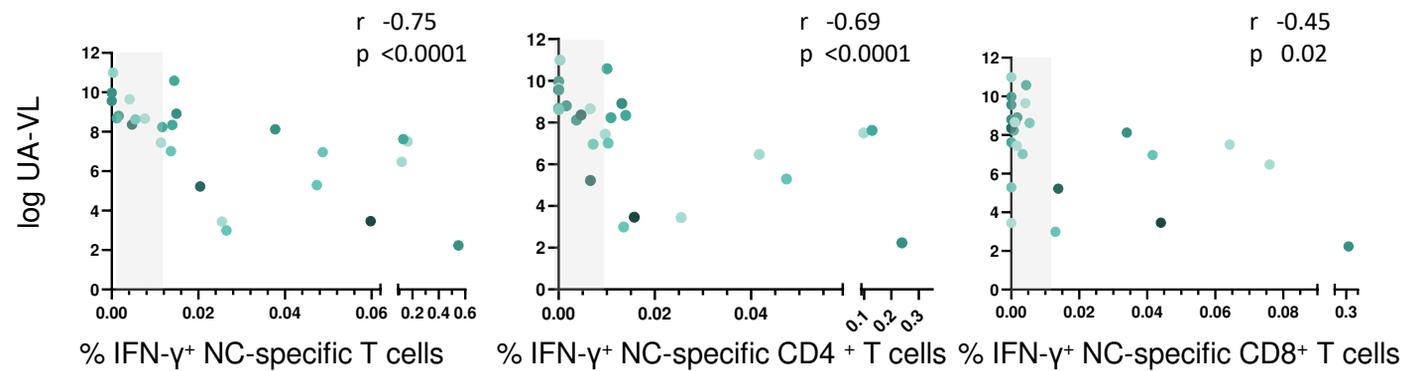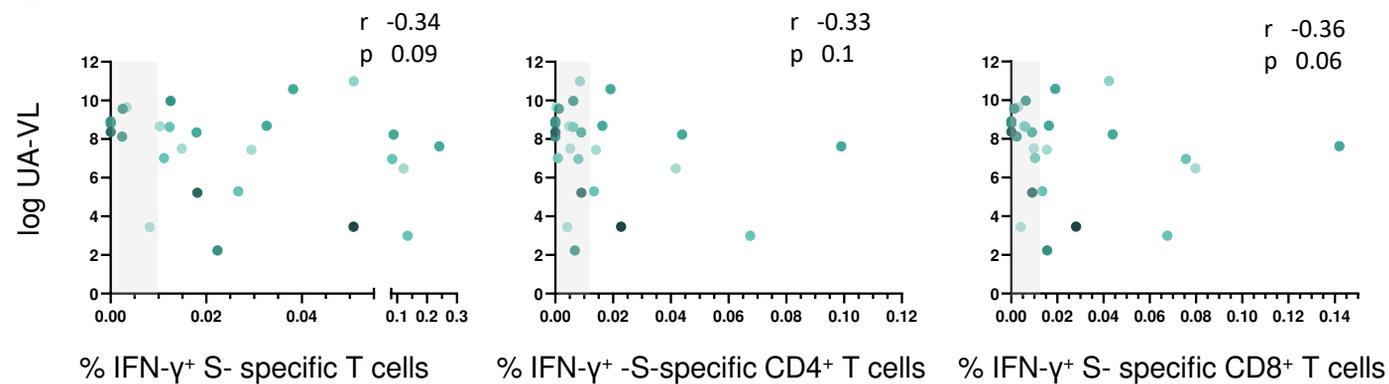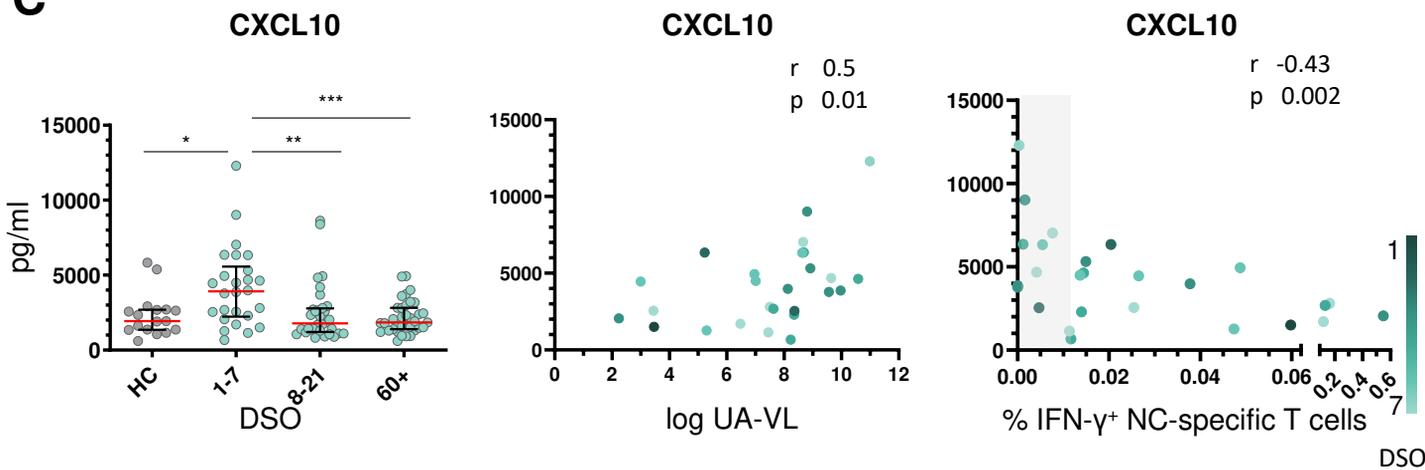
Figure 3. Nucleocapsid-specific T cell responses correlate inversely with upper airways viral loads and systemic markers of inflammation during acute infection with SARS-CoV-2. (**A**, **B**) Spearman rank correlations showing upper airways viral loads (UA-VLs) versus the frequencies of all NC-specific T cells (left), NC-specific CD4+ T cells (middle), or NC-specific CD8+ T cells (right) (**A**) and the frequencies of all S-specific T cells (left), S-specific CD4+ T cells (middle), or S-specific CD8+ T cells (right) (**B**) during the first week after symptom onset. (**C**) Left: plasma concentrations of CXCL10 are shown for healthy controls (HCs) and longitudinally for patients according to the number of days since symptom onset (DSO). Data are shown as median IQR. Middle and right: Spearman rank correlations showing plasma concentrations of CXCL10 during the first week after symptom onset versus UA-VLs (middle) and the frequencies of all NC-specific T cells (right). The gray bar indicates non-responders (right). *p < 0.05, **p < 0.01, ***p < 0.001 (Mann-Whitney U test).

Figure 4

Figure 4. Gene expression profiles in immune cell subsets during acute infection with SARS-CoV-2. RNA sequencing data were obtained from circulating CD4+ T cells (light blue), CD8+ T cells (dark blue), monocytes (light green), and NK cells (dark green) isolated during the first week after symptom onset (n = 14 patients with mild COVID-19). (**A**) Spearman rank correlations showing mean expression scores for *OAS1* (left), *STAT1* (middle), and *PKR* (right) versus IFN-γ+ CD4+ (squares) and IFN-γ+ CD8+ T cell frequencies (triangles) and upper airways viral loads (circles, UA-VLs). Whiskers show 95% confidence intervals calculated using bootstrapping with replacement using sample numbers equal to the original data set. (**B–D**) Spearman rank correlations showing mean pathway expression scores for CD4+ T cells versus IFN-γ+ CD4+ T cell frequencies (**B**), CD8+ T cells versus IFN-γ+ CD8+ T cell frequencies (**C**), and monocytes versus UA-VLs (**D**). Data are shown as r values with 95% confidence intervals. (**E**) Spearman rank correlations were calculated for all KEGG pathways in the categories *Signal Transduction*, *Signaling Molecules and Interaction*, *Immune System*, and *Cell Growth and Death (KEGG database)*. Data are shown as z-normalized mean pathway expression scores restricted to r values above 0.25 or below −0.25. Patients were clustered by expression profile similarity. Pathways are shown for cell subsets with significant gene set enrichment analysis scores (top row, p < 0.05).

Figure 5. The presence of IFN-γ⁺ T cells in the upper airways is linked with antigen presentation and viral control during acute infection with SARS-CoV-2. (**A**) Numbers of differentially expressed genes (DEGs) among cell clusters isolated from the nasopharynx (total n = 16 patients with mild to severe COVID-19). (**B**) Volcano plots showing DEGs (blue) for the indicated cell types (adjusted p < 0.05). (**C**) Gene ontology (GO) terms enrichment plots (top 20) for the indicated cell types based on a log-fold change (LFC) of 0.25 in either direction (adjusted p < 0.01). Dot size represents the number of genes per term per cell type, and adjusted p values are colored according to the key. (**D**) As in (**C**) for developing ciliated cells based on KEGG pathways instead of GO terms. (**E**) Changes in cell type frequencies for patients with (n = 7) or without (n = 9) T cell expression of IFN-γ. Expression of IFN-γ was considered in CD4⁺ T cells only for analyses of CD8⁺ T cells. All cell types were defined according to the original annotation, except for CD8⁺ T cells, which were reclassified in house.
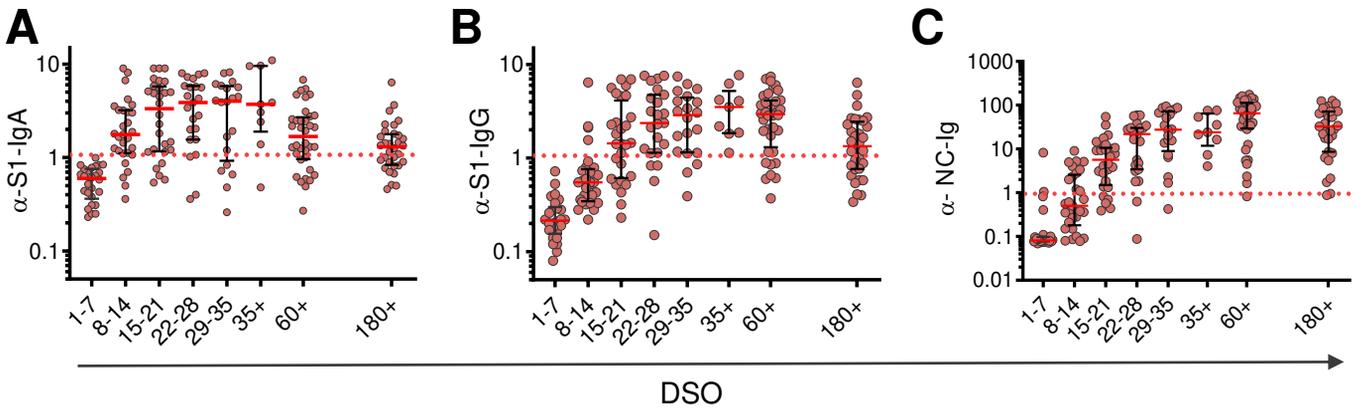
Supp. Figure 1 Supplementary Figure 1. Dynamics of antibody responses against SARS-CoV-2. (**A–C**) Antibody response dynamics are shown for S-specific IgA (ratio versus internal assay standard) (**A**), S-specific IgG (ratio versus internal assay standard) (**B**), and NC-specific total Ig in plasma (arbitrary units) (**C**). The cutoff for each assay is indicated by the dotted red line. Data are shown as median IQR. DSO, days since symptom onset.
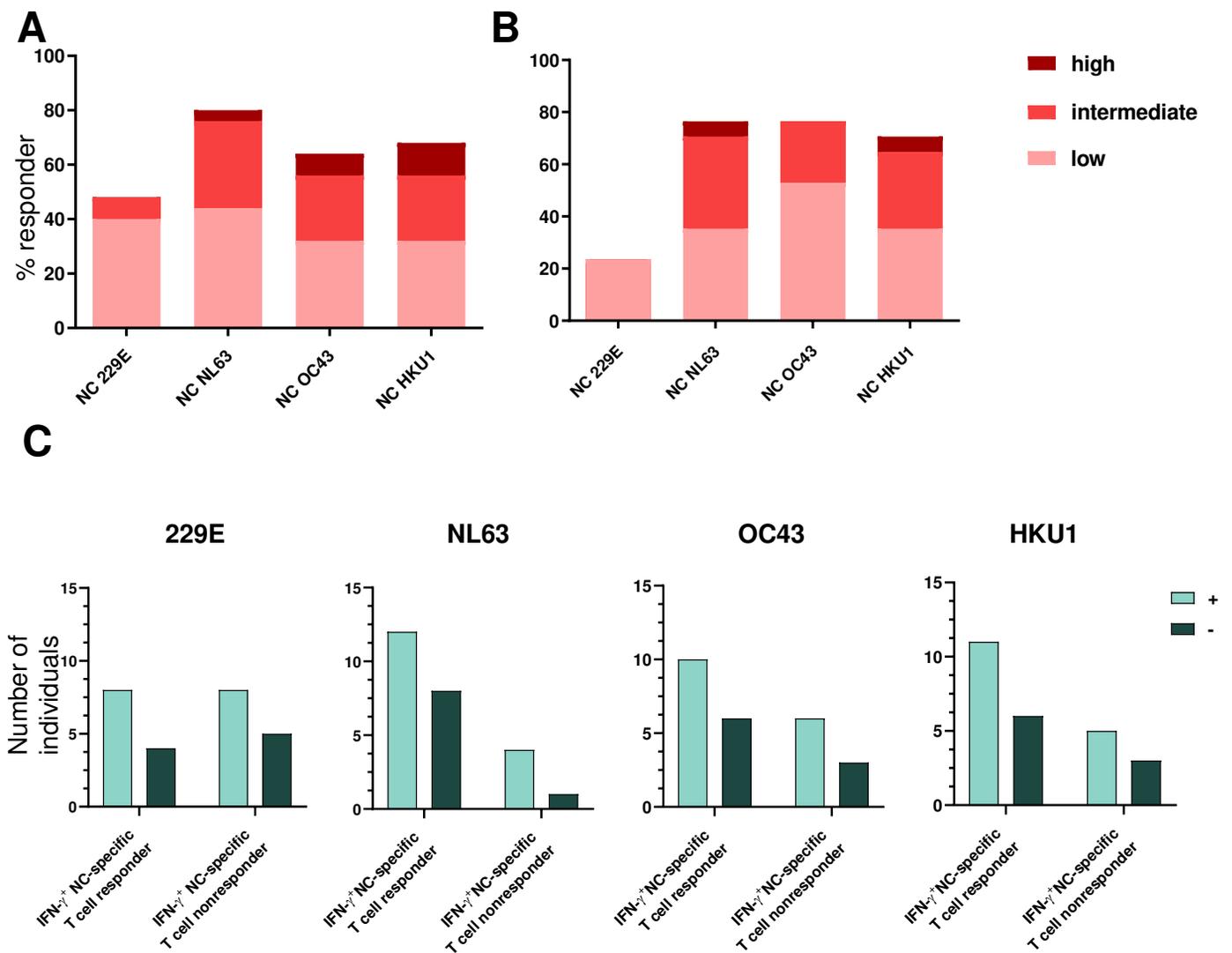
Supp. Figure 2 Supplementary Figure 2. Antibody responses against common cold coronaviruses. (**A**) Percent frequencies of responders (n = 25) with high (dark red), intermediate (red), and low NC-specific antibody responses (pink) against each of the four common cold coronaviruses (CCCVs) during the first week after symptom onset. (**B**) Percent frequencies of non-vaccinated healthy controls (n = 25) with high (dark red), intermediate (red), and low NC-specific antibody responses (pink) against each of the four CCCVs. (**C**) Numbers of responders and non-responders with (light green) or without CCCV seroreactivity (dark green) during the first week after symptom onset
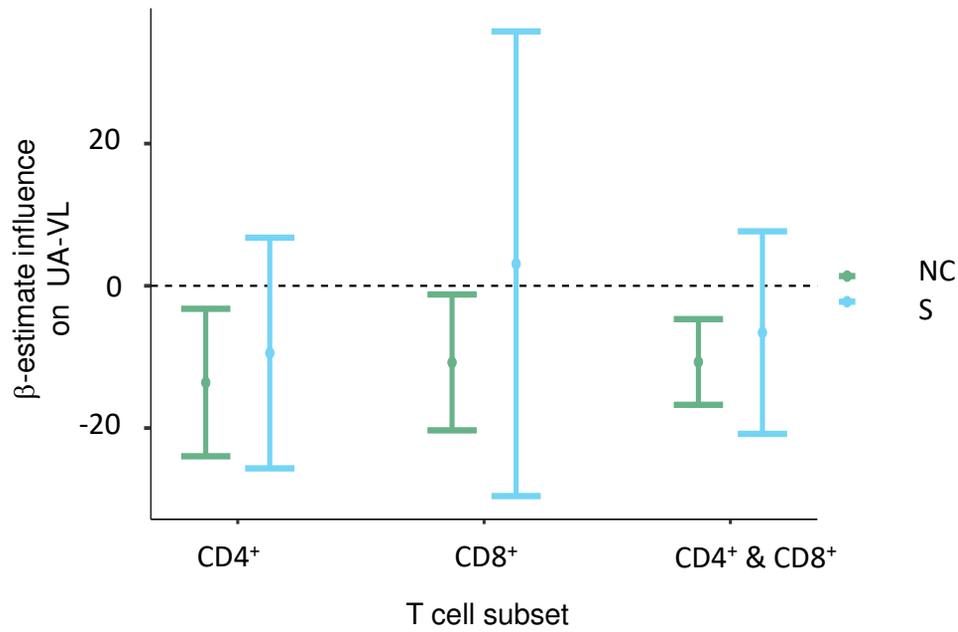
Supp. Figure 3 Supplementary Figure 3. Nucleocapsid-specific CD4+ and CD8+ T cell responses incrementally reduce the upper airways burden of SARS-CoV-2. Summary of a censored linear mixed effects model showing the possible influence of SARS-CoV-2-specific T cells on upper airways viral loads (UA-VLs). Results are shown as β-estimates with confidence intervals for NC-specific (green) and S-specific T cells (blue).
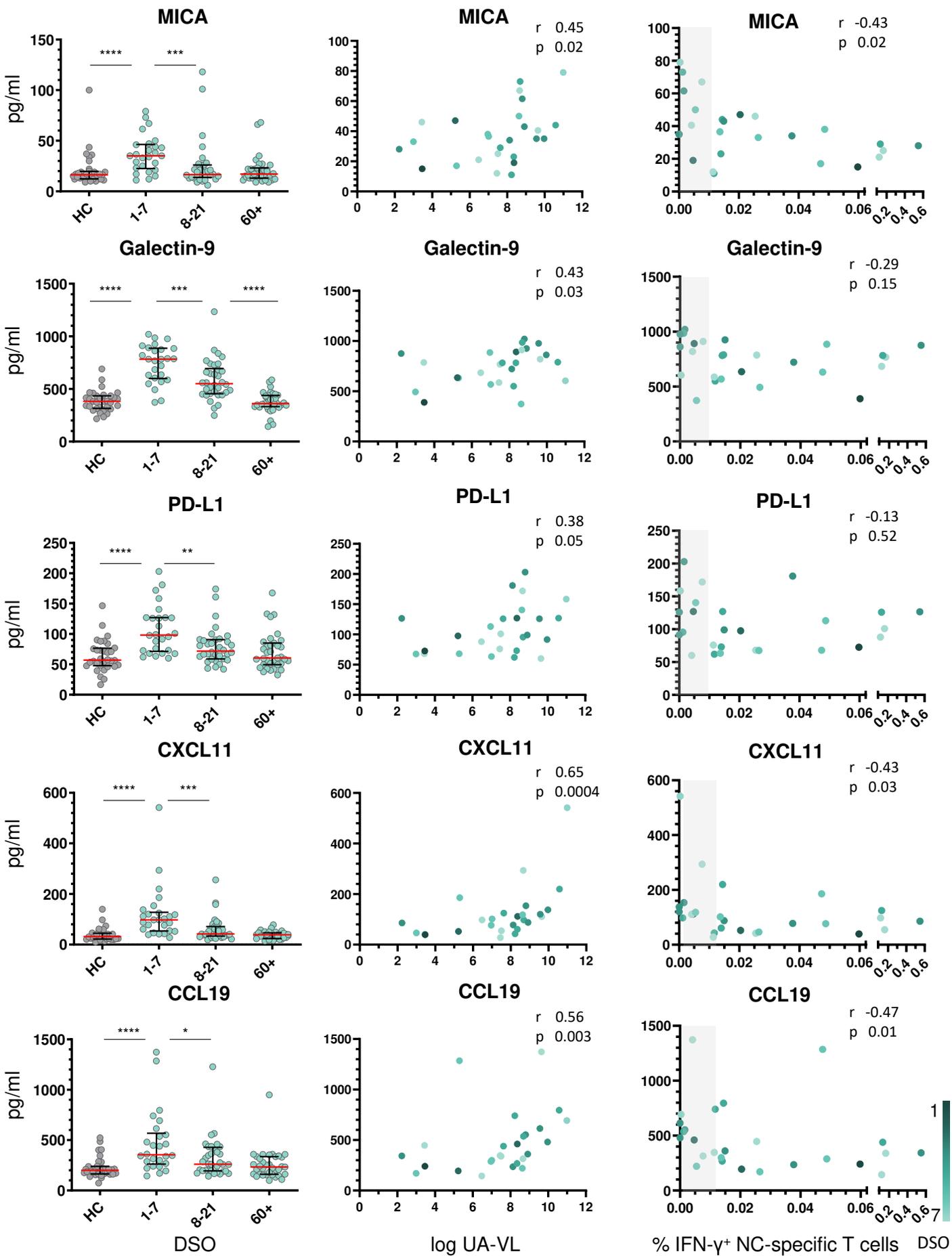
Supplementary Figure 4. Dynamics of plasma soluble factors after infection with SARS-CoV-2. Left: plasma concentrations of MICA, galectin-9, PD-L1, CXCL11, and CCL19 are shown for healthy controls (HC, n = 25) and longitudinally for patients (n = 37) according to the number of days since symptom onset (DSO). Data are shown as median IQR. Middle: Spearman rank correlations showing plasma soluble factors versus upper airways viral loads (UA-VLs) during the first week after symptom onset (key). Right: Spearman rank correlations showing plasma soluble factors versus nucleocapsid (NC)-specific T cell frequencies during the first week after symptom onset (key). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 (Mann-Whitney U test).

A

β estimate influence on UA-VL

NF-kappa B signaling pathway
RIG-I-like receptor signaling pathway
Antigen processing and presentation
C-type lectin receptor signaling pathway
Hedgehog signaling pathway
Necroptosis
RIG-I-like receptor signaling pathway
Th1 and Th2 cell differentiation
Th17 cell differentiation
AMPK signaling pathway
Antigen processing and presentation
C-type lectin receptor signaling pathway
Cell adhesion molecules
Cellular senescence
cGMP-PKG signaling pathway
Chemokine signaling pathway
Cytosolic DNA-sensing pathway
ECM-receptor interaction
Ferroptosis
Hedgehog signaling pathway
Natural killer cell mediated cytotoxicity
Necroptosis
NOD-like receptor signaling pathway
Platelet activation
RIG-I-like receptor signaling pathway
VEGF signaling pathway
Viral protein interaction with cytokine and cytokine receptor
Antigen processing and presentation
HIF-1 signaling pathway
Necroptosis
NOD-like receptor signaling pathway
RIG-I-like receptor signaling pathway

Cell: CD4, CD8, Monocytes, NK

B

Paramater of independent variable

Influence of CD4 on CD8 T cell response
Influence of CD8 on CD4 T cell response

Supp. Figure 5. Modeling interactions between upper airways viral loads and activation of immune pathways in circulating immune cell subsets controlled for the influence of NC-specific T cell responses. (A) beta-estimates for the interaction between upper airways viral loads (UA-VLs) and selected immune pathway scores achieving significance ($p < 0.05$). Dots indicate point estimates, and whiskers indicate 95% confidence intervals, computed using normal approximations. Results are clustered according to immune cell subsets (key). (B) Expected influence of NC-specific CD4+ T cells on NC-specific CD8+ T cell responses (left) and vice versa (right). Estimates were obtained using two separate linear mixed effect
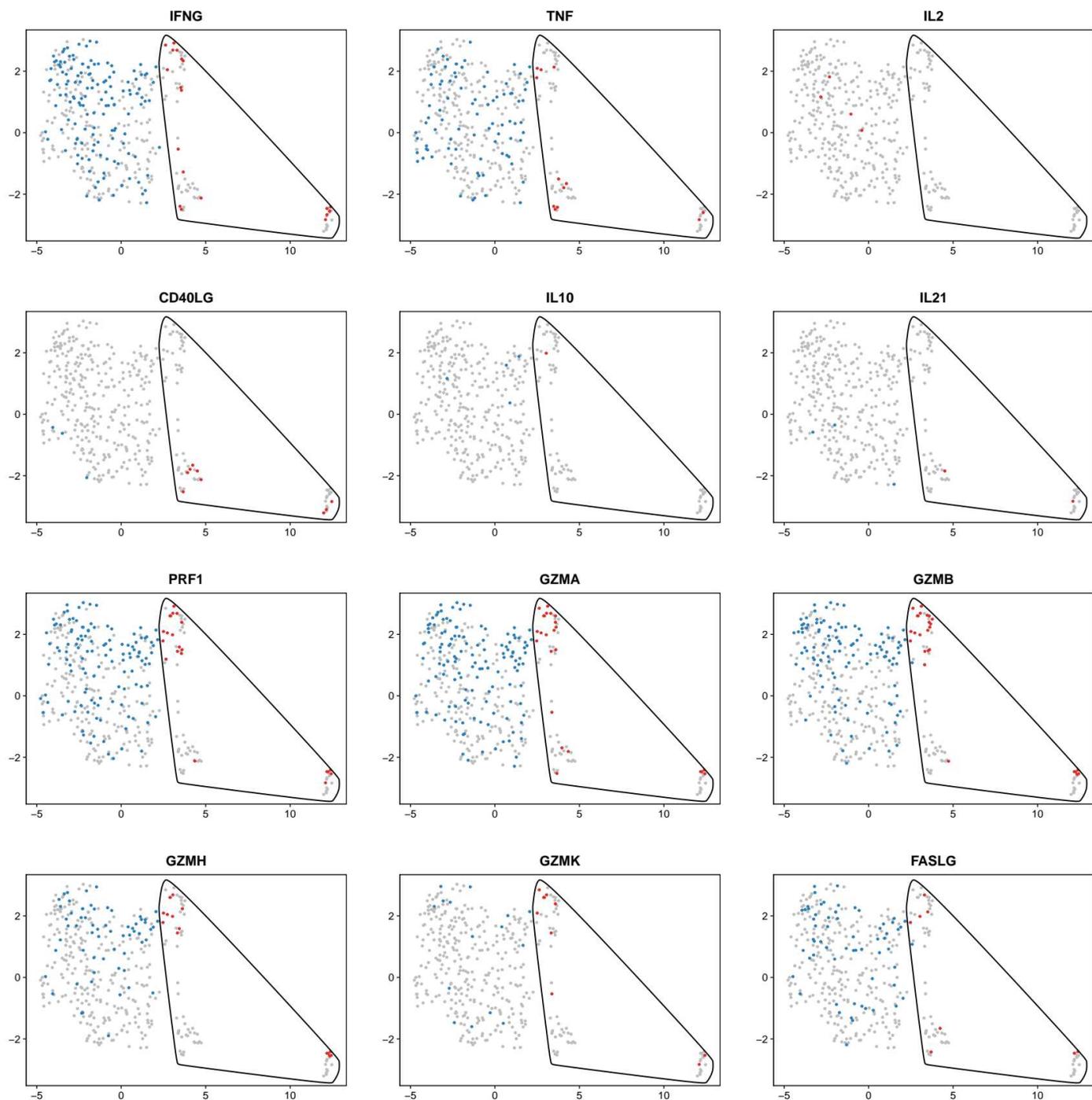
Supp. Figure 6 Supplementary Figure 6. Gene expression of selected cytokines and cytotoxic effector molecules in nasopharyngeal T cells during acute infection with SARS-CoV-2. Expression of the indicated genes is shown for nasopharyngeal CD4^{+} T cells (inside the drawn gate) and nasopharyngeal CD8^{+} T cells (outside the drawn gate). Red dots indicate nasopharyngeal CD4^{+} T cells expressing the indicated gene, and blue dots indicate nasopharyngeal CD8^{+} T cells expressing the indicated gene.
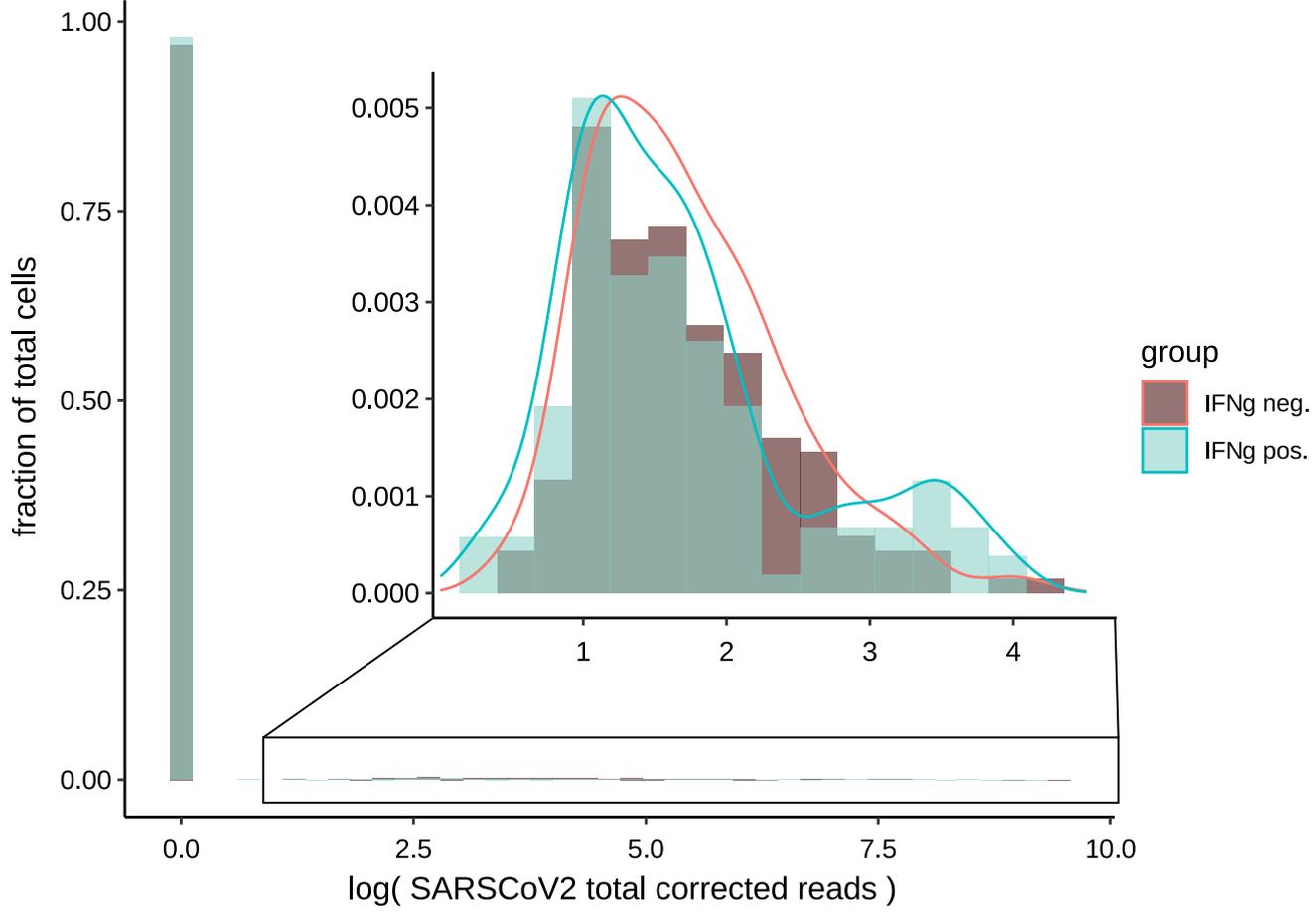
Supp. Figure 7. Influence of IFN-γ on intracellular SARS-CoV-2 RNA. The graph shows the distribution of the logarithm of total corrected read counts of intracellular SARS-CoV-2 RNA in responders (blue) and non-responders (red). Responders were characterized by lower counts and greater numbers of cells without SARS-CoV-2 RNA. The effect was significant (p = 0.00013).