Neuro-COVID long-haulers exhibit broad dysfunction in T cell memory generation and responses to vaccination

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Summary

The high prevalence of post-acute sequelae of SARS-CoV-2 infection (PASC) is a significant health concern. In particular, virus-specific immunity in patients who suffer from chronic neurologic symptoms after mild acute COVID remain poorly understood. Here, we report that neuro-PASC patients have a specific signature composed of humoral and cellular immune responses that are biased towards different structural proteins compared to healthy COVID convalescents. Interestingly, the severity of cognitive deficits or quality of life markers in neuro-PASC patients are associated with reduced effector molecule expressionn in memory T cells. Furthermore, we demonstrate that T cell responses to SARS-CoV-2 mRNA vaccines are aberrantly elevated in longitudinally sampled neuro-PASC patients compared with healthy COVID convalescents. These data provide a framework for the rational design of diagnostics and predictive biomarkers for long-COVID disease, as well as a blueprint for improved therapeutics.

Keywords: COVID-19 immunity, T cell memory, neuro-PASC, long-COVID, vaccine-induced immunity

Introduction

SARS-CoV-2 is the causative agent of a worldwide pandemic that started in Wuhan, China in December, 2019. There have been more than 200 million cases and over 4 million deaths globally attributable to COVID-19 disease (Center, 2021). Although highly effective vaccines are now used to prevent severe disease and death caused by SARS-CoV-2, long-term sequelae of SARS-CoV-2 infection are increasingly becoming an important medical concern.

SARS-CoV-2 infection is associated with presentations ranging from asymptomatic carriage to severe multi-organ dysfunction (Li et al., 2020; Liguori et al., 2020; Syed et al., 2020). Globally, the estimated fatality rate following SARS-CoV-2 infection is approximately 1-2%, but not all patients recover to their baseline states (Higgins et al., 2020). "Long COVID" affects an estimated 10-40% of all infected people and includes symptoms persisting more than 28 days after diagnosis of SARS-CoV-2 infection, termed "post-acute sequelae of SARS-CoV-2 infection" or PASC (Ladds et al., 2020; Vehar et al., 2021). Greater than two-thirds of hospitalized COVID-19 patients experience ongoing fatigue, breathlessness, and psychiatric issues such as post-traumatic stress disorder (PTSD) 4-8 weeks after discharge (Halpin et al., 2021). However, the vast majority of people with COVID experience mild disease not requiring hospitalization, and more than half of these have symptoms persisting more than 4 months after acute infection (Petersen et al., 2020). Many people who survived Middle-East respiratory virus (MERS) and the original SARS-CoV pandemic also experienced PTSD and neurological impairments up to 3.5 years after acute infection (Ahmed et al., 2020; Lam et al., 2009). More recent studies on recovered COVID-19 patients showed significant cognitive deficits in attention, working memory, and emotional processing months after acute infection had resolved (Hampshire, 2021). Here, we focus on a cohort of mostly non-hospitalized long-COVID patients

presenting with neurological symptoms ("neuro-PASC") who exhibit a reduction in quality of life with regards to cognitive and fatigue parameters (Graham et al., 2021).

Numerous studies suggest that a robust T cell response is necessary for viral clearance in infected individuals. In particular, $CD4^+$ T cell responses directed against the Spike protein were found in 100% of healthy COVID convalescents, while 70% displayed Spike-specific $CD8^+$ T cell responses (Grifoni et al., 2020). Another study found that memory T cells cross-reactive to SARS-CoV-2 nucleocapsid (N) protein persisted in people exposed to SARS-CoV up to 17 years after the epidemic of 2003 (Le Bert et al., 2020). Post-mortem autopsies of COVID-19 patients have shown that innate immune cells but not lymphocytes are enriched in lung infiltrate, and that these patients exhibit impaired germinal center formation in lung draining lymph nodes (Duan et al., 2020). Additional studies have shown that $CD8^+$ T cell depletion after SARS-CoV-2 infection of rhesus macaques impairs anamnestic responses, suggesting a role for T cell memory in long-term immunity to SARS-CoV-2 infection (McMahan et al., 2021).

Most studies on T cell responses to SARS-CoV-2 have focused on acutely symptomatic individuals. However, adaptive immune responses in patients who remain chronically symptomatic remain largely unexplored. Our studies indicate that neuro-PASC patients ("CN") had hypo-functional Ag-specific CD8+ T cell memory responses SARS-CoV-2 compared with healthy convalescents ("CC") and enhanced reactivity to N and M proteins compared with CC subjects. The severity of cognitive deficits and quality of life markers were associated with enhanced polarization in cytolytic granule expression in memory T cell subsets. Finally, we demonstrate that T cell responses to SARS-CoV-2 vaccination are more robust and display aberrant kinetics in neuro-PASC patients compared with control groups. Together, these data demonstrate a wide-scale dysfunction in SARS-CoV-2 T cell memory in neuro-PASC long-

hauler patients, with important implications for both appropriate diagnostic and vaccination strategies.

Results

Clinical characteristics of neuro-PASC patients

We enrolled a total of 111 participants prior to SARS-CoV-2 vaccination drawn from the neuro-COVID-19 outpatient clinic at Northwestern Memorial Hospital or from the surrounding Chicago area. This included 56 neuro-PASC "CN" patients (confirmed RT-PCR+ or anti-SARS-CoV-2 Spike IgG+) meeting Infectious Disease Society of America clinical criteria for COVID-19 starting after February 2020 and had neurologic symptoms lasting at least 6 weeks post-infection, as previously reported (Graham et al., 2021). Among those, 48 (86%) were never hospitalized for pneumonia or hypoxemia. We additionally recruited 24 healthy COVID convalescents without lasting symptoms (RT-PCR+ or seropositive for anti- SARS-CoV-2 Spike RBD IgG, "CC"); and 31 healthy controls who were RT-PCR- and seronegative for SARS-CoV-2 Spike-IgG ("HC"; study description in Fig. 1A).

CN patients displayed a constellation of neurological symptoms similar to those previously reported by our group and in other studies (Fig. 1B). In addition, we utilized standardized methods to quantify their quality of life and cognitive disturbances relative to healthy convalescents. Results from the patient reported outcomes information system (PROMIS-57) survey (Tang et al., 2019) showed that CN patients scored significantly lower on physical function and higher on anxiety, depression, pain and other metrics compared with CC subjects or the national average (Fig. 1C). NIH toolbox tests administered to CN patients to assess their cognitive function (Weintraub et al., 2013) showed CN patients had significantly lower T scores in the attention module, which was indicative of cognitive dysfunction relative to the national average (Fig. 1D).

Neuro-PASC is associated with a distinct immunodominance hierarchy.

To determine whether neuro-PASC included alterations in the magnitude and/or specificity of Ag-specific T cell responses in CN patients, we performed ELISPOT for SARS-CoV-2-specific T cells. PBMC from each subject were cultured in the presence of peptide pools derived from spike (S), nucleocapsid (N), or membrane (M) proteins of SARS-CoV-2 (Fig. S1). The magnitude and specificity of both IFN-γ and IL-2 responses to S sub-pools were similar between CN and CC subjects (Fig. 2A-B). However, CN subjects produced high levels of IFN-γ against all sub-pools of N and M proteins, while CC subjects had a specificity for the N1 pool and lower responses against all M pools (Fig. 2C-D), indicative of different immunodominance hierarchies between groups. No significant differences were found in IL-2 production, and healthy controls exhibited some response to N pools likely caused by cross-reactivity with endemic coronaviruses (Fig. 2E-F). Antibody titers against the Spike receptor-binding domain (RBD) did not differ between CN and CC groups (Fig. 2G). Interestingly, high IgG titers did not correlate with the magnitude of the IFN-γ T cell response against S2 or S3 peptide pools comprising the 2 halves of the Spike RBD (Fig. S2).

$CD4^+$ Tfh cells display opposing reactivity to SARS-CoV-2 N- and M-proteins in neuro-PASC vs. healthy COVID convalescents

RNA-Seq analysis of $CD4^+$ T cells from hospitalized and non-hospitalized COVID-19 patients showed that severe disease is associated with elevated $CD4^+$ T follicular helper (Tfh) proportions relative to patients with mild disease (Meckiff et al., 2020). We sought to determine whether Tfh responses (gating in Fig. S3) that prime antibody production from plasma B cells could similarly differentiate neuro-PASC CN patients from CC individuals. Immunophenotyping showed that there were no differences in total percentages of T cell subsets, including Tfh cells,

between groups (Fig. S4). The activation-induced marker (AIM) assay has been previously used to detect TCR-mediated activation of T cells (Grifoni et al., 2020) and we used this method to investigate Tfh cell activation (gating for Tfh cells in Fig. S3). N protein-specific $CD134^+CD137^+$ (AIM^+) CD4 Tfh cells were significantly elevated in CN compared with CC subjects, while the opposite trend was observed in M protein-specific activation (Fig. 3A-B). No differences were seen in Tfh activation between CN and CC groups across Spike peptide pools (Fig. 3B). The magnitude of N- and M-specific Tfh cell activation did not correlate with the time since acute infection in either CN or CC patients (Fig. 3C-D) despite reports showing that antibody titers against N protein decrease rapidly post-infection (Van Elslande et al., 2021) which would presumably lead to decreased N-specific Tfh cell activation over time. N-specific IgG titers were significantly elevated in CN compared with CC subjects (Fig. 3E), consistent with the enhanced N-specific $CD4^+$ Tfh cell activation shown in 3B. Similar to Tfh cells, there was no correlation between anti-N IgG titers and time post-symptom onset for either CN or CC subjects (Fig. S5A). Studies have shown that IgG titers against N protein decline to undetectable levels in 40% of COVID convalescents within 4 months (Muecksch et al., 2021). We largely did not observe this decline in CN patients despite collecting their blood samples an average of 193 days post-symptom onset (Fig. 1B).

$CD4^+$ T cell polyfunctionality to Spike protein differs in neuro-PASC vs. healthy COVID convalescents

Cytotoxic $CD4^+$ T cells acquire the ability to produce cytolytic granules upon activation with cognate viral antigen (Goubard et al., 2015; Sledzinska et al., 2020). Viral infections specifically induce expansion of the memory $CD4^+$ T effector memory cells re-expressing CD45RA (TEMRA) population that secrete copious amounts of cytolytic granules upon antigen

encounter (Tian et al., 2017). We therefore investigated whether Ag-specific production of multiple granzymes and cytokines in $CD4^+$ T cells were altered between groups. While CN and HC groups maintained polyfunctionality and polarization, CC subjects had significantly more polyfunctional $CD4^+$ T cells producing granzymes A, B, and M after Spike pool stimulation (category 2 in yellow, Fig. 3F). A heatmap quantifying this effect showed that CC had a 2-fold elevation in category 2 effector molecules over CN subjects (Fig. 3G). This data suggests that cytotoxic responses to Spike protein in $CD4^+$ T cells from healthy COVID-19 convalescents significantly differ from those experiencing chronic neurologic symptoms.

$CD8^+$ memory T cell functionality in neuro-PASC patients

$CD8^+$ memory T cells are crucial for protection from recurrent viral infections (Mockus et al., 2019). Studies have shown that memory $CD8^+$ cells can persist for several years after patient exposure to SARS-CoV (Chen et al., 2005) and that acute mild disease induces stronger Ag-specific $CD8^+$ memory responses than severe disease (Peng et al., 2020). However, little is known about whether $CD8^+$ memory differs in neuro-PASC long-haulers vs. healthy COVID convalescents months after acute infection resolves. To address this, we probed the dynamics of $CD8^+$ TEM, TCM, and TEMRA activation in CN, CC, and HC subjects. $CD8^+$ TEM were significantly activated by various S, N, and M peptide pools in CC subjects while remaining relatively quiescent after antigen stimulation in CN patients (Fig. 4A-B). $CD8^+$ TEMRA are terminally differentiated memory cells with distinct transcriptional programs that respond quickly to viral infections (Tian et al., 2019). Total percentages of $CD8^+$ TEMRA cells were significantly elevated in CN compared with CC and HC groups (Fig. 4C), but despite their increased numbers, $CD8^+$ TEMRA cells were less activated by the S3/4 peptide pool in CN

patients and showed a trend towards decreased activation after N pool stimulation compared with CC subjects (Fig. 4D-E).

Similarly, antigen stimulation resulted in altered cytokine polarization in $CD8^+$ T cell subsets in CN and CC groups. Effector molecule production in $CD8^+$ T cells was similar across groups in the unstimulated condition, despite showing some statistically significant differences in perforin production (Fig. S6B). However, stimulation with the S3/4 pool induced greater TNF-α production in the CC group while CN patients had enhanced IL-6 production to the same stimulus (Fig. 4F). S pool stimulation also induced $CD8^+$ TEMRA cells from the CC group to produce elevated levels of granzymes, which was not seen in CN or HC groups (Fig. 4G, black lines), demonstrating that $CD8^+$ T cell subsets display decreased functionality in CN vs. CC patients after stimulation.

Impaired cognition and decreased quality of life metrics correlate with distinct patterns of polyfunctionality in memory T cell subsets

Having shown that Tfh and T cell memory responses differed between patient groups, we next sought to probe whether within-group differences in T cell responses correlated with various clinical measures in CN patients. We found a significant positive correlation between the magnitude of IFN-γ production to N protein and higher pain interference scores (Fig. 5A). There was also a trend towards a positive correlation between high scores for depression and the magnitude of the N-specific T cell response (Fig. 5B). To look at associations between clinical parameters and T cell activation, we separated T scores from NIH Toolbox or PROMIS-57 measurements (Fig. 1C-D) into quartiles and only the lowest and highest groups (Q1 vs. Q4) were used for analysis (Fig. S7A, red boxes). CN subjects reporting high (Q4) pain interference scores produced significantly less granzyme A or B and more IL-6 than those with low scores

(Fig. 5C-D) from $CD8^+$ T cells after S pool stimulation. Further, patients reporting low depression scores had highly polyfunctional Spike-specific $CD8^+$ TEM, while those reporting high levels of depression had TEM producing ~3-fold higher granzymes A, B, and M alone (category 2; Fig. 5E, F). The severity of cognitive impairment could also be significantly correlated with T cell responses. Q1 patients scoring low on executive function tests had $CD8^+$ TCM polarized towards granzymes A, B, M and perforin production (category 1), while those in Q4 were biased to produce granzymes A, B, and perforin (Fig. 5G-H). Similar analyses were performed for other $CD8^+$ and $CD4^+$ memory T cell subsets, and significant differences by quartile were also found in correlations with depression, processing speed, working memory, and global pain (Fig. S7B-K). These data show that the severity of cognitive deficits or quality of life measures can be significantly correlated with differences in SARS-CoV-2-specific memory T cell function.

SARS-CoV-2 vaccination induces sustained elevation in Spike-specific IFN-γ responses in neuro-PASC but not healthy COVID convalescents

SARS-CoV-2 mRNA vaccines have been proven to be highly effective against contracting infection in the short-term in both clinical trials and in real-world settings (Haas et al., 2021; Polack et al., 2020). However, neither clinical trials nor field studies specifically characterized the vaccine-induced immune response in long-COVID patients, and it is similarly unknown whether vaccination can ameliorate long-haul symptoms. One prospective study showed that vaccination did not significantly worsen symptoms in 22 patients with long-COVID (Arnold et al., 2021), but also did not provide evidence to show any decrease in symptoms. To address how vaccine-induced immunity evolves over time in neuro-PASC long-haulers, we conducted a longitudinal vaccine study assessing Spike-specific antibody and T cell responses

over the course of 6 months post-vaccination (study design and patient demographics in Fig. 6A-B). While this study is ongoing, we report data from the first 4 months comparing responses in CN, CC, and HC subjects (n for each group per study visit shown in Fig. S8). Vaccination with either Pfizer or Moderna mRNA vaccines induced robust SARS-CoV-2 Spike RBD IgG titers in all groups tested by 3 weeks post-2nd dose, with the highest titers found in CN patients. Antibody titers were at similar levels in all groups by 3 months post-1st dose, but trended higher in CN than in HC subjects at visit (V)4 (Fig. 6C). In parallel, we measured Spike-specific T cell responses in study subjects. The magnitude and kinetics of the T cell response in CC and HC subjects were similar and peaked at V3. However, CN patients had significantly elevated Spike-specific T cell responses compared with either HC or CC groups at V2 after the vaccination boost (Fig. 6D; individual pool data shown in Fig. S9). IFN-γ-specific T cell responses remained high at 4 months post-vaccination in CN patients while not significantly differing from pre-vaccination levels in both CC and HC subjects. To our knowledge, these are the first data that longitudinally compare the T cell response to vaccination in neuro-PASC patients with healthy COVID convalescents and unexposed individuals.

Overall, our study demonstrates that neuro-PASC patients have elevated IFN-γ responses to N and M proteins, enhanced Ag-specific activation of Tfh cells, sustained anti-N IgG production, and deficient activation of multiple CD8$^+$ memory T cell subsets compared with healthy COVID-19 convalescents. There were also correlations between the severity of cognitive deficits or quality of life impairments and Ag-specific cytokine signatures (Fig. 5, S7). Importantly, vaccination resulted in sustained enhancements in the magnitude of Spike-specific T cell responses in neuro-PASC patients vs. all other groups, regardless of prior COVID exposure. Together, we show that CN patients exhibit a large-scale dysfunction in multiple

235 aspects of the memory T cell response which may inform treatment and/or vaccination options

236 down the line.

Discussion

COVID-19 is well-recognized as a multi-organ disease with long-term sequelae associated with neurological disease. PASC has been reported in up to 87% of those hospitalized with SARS-CoV-2 pneumonia and in up to 40% of those with mild disease who do not require hospitalization (Havervall et al., 2021; Hirschtick et al., 2021; Mahase, 2020). SARS-CoV-2 vaccines have decreased the rates of SARS-CoV-2 infection and mortality in unexposed individuals, but it is not clear that vaccines help to ameliorate long-COVID symptoms in previously infected individuals (Arnold et al., 2021) and thus further study is needed to inform treatment options. Long-term sequelae after other coronavirus infections can persist for years (Ahmed et al., 2020); therefore, it is important to specifically characterize SARS-CoV-2-specific immune responses in long-COVID patients. Most studies on effector and memory T cell responses to SARS-CoV-2 have focused on acute infection or healthy convalescents as opposed to those with long-COVID (Rodda et al., 2021; Sekine et al., 2020; Weiskopf et al., 2020). We aimed to fill this knowledge gap and determine whether and how T cell phenotype and function differ in patients with neuro-PASC and healthy COVID-19 convalescents.

Clinically, neuro-PASC resembles myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), which many patients report as a post-viral infectious complication (Rasa et al., 2018). The causes of ME/CFS remain elusive, and the underlying mechanisms of neuro-PASC remain similarly unknown. It is possible that direct infection of the brain is responsible for neuro-PASC symptoms. It is believed that SARS-CoV-2 gains entry into the central nervous system through the olfactory bulb and has been shown to infect neurons in vitro, which is supported by findings of viral protein expression in cortical neurons from post-mortem autopsies (Klingenstein et al., 2020; Song et al., 2021). However, another study was unable to find any evidence of SARS-

CoV-2 in the brains of 4 patients with neurological symptoms during acute infection (Kantonen et al., 2020), suggesting that infection of the nervous system may be transient. Another study of acutely infected neuro-COVID patients did not find any SARS-CoV-2 RNA present in the CSF, though they did identify enhanced presence of exhausted T cells and dedifferentiated monocytes (Heming et al., 2021). However, all of these prior studies were conducted on patients with acute SARS-CoV-2 infection as opposed to neuro-PASC, and thus mechanisms for neurological dysfunction may differ. As lumbar punctures or brain biopsies are not indicated in neuro-PASC outpatients, reproducing the above study results in outpatient populations will not be possible. Additional hypotheses for neuro-PASC pathogenesis include a contribution of autoimmune mechanisms which is suggested by the skewed ratio of females to males affected, similar to that seen in rheumatoid arthritis or systemic lupus erythematosus (Chakravarty et al., 2007; Myasoedova et al., 2010), as well as the possibility of persistent SARS-CoV-2 infection in the periphery (Al-Aly et al., 2021). Much of our findings on the SARS-CoV-2 T cell response in neuro-PASC patients supports this latter hypothesis.

Ag-specific IFN-γ and IL-2 production was similar between CN and CC groups in response to stimulation by different SARS-CoV-2 Spike peptide pools (Fig. 2A-B). However, T cells from CN patients retained high IFN-γ responses to all N- and M-peptide pools tested, while CC subjects had a reactivity limited to N1 and N2 peptide pools with little IFN-γ production in response to M pools (Fig. 2C-D). The presence of an immunodominance hierarchy in CC subjects is the expected outcome of an effective memory response to SARS-CoV-2 infection. Studies have shown that while a primary $CD4^+$ or $CD8^+$ T cell response to influenza A or LCMV infection is highly diverse and contains T cells reactive to many viral proteins and epitopes, the memory response preferentially contains T cells responding to specific immunodominant viral

283 antigens (Sant et al., 2018; Tebo et al., 2005). This effect is more pronounced in the $CD8^+$ T cell
284 compartment where memory responses are dependent on the ability of dendritic cells to present
285 viral antigen (Crowe et al., 2003) or on antigen availability (Henrickson et al., 2013). The
286 evident lack of a narrow and targeted IFN-γ response to N- and M-peptides in CN patients thus
287 suggests that they are unable to effectively generate a memory response to SARS-CoV-2.
288 Though there were no differences observed in the magnitude or specificity of the IFN-γ response
289 to SARS-CoV-2 Spike peptides, this is somewhat expected as memory T cell responses to Spike
290 protein remain diverse after both infection (Grifoni et al., 2020) and vaccination (Alter et al.,
291 2021). It is additionally not surprising that differences in reactivity were observed for IFN-γ and
292 not IL-2 T cell responses between CN and CC groups (Fig. 2B, E-F). Studies have demonstrated
293 that while Ag-specific T cells produce IL-2 as a proliferation factor, IFN-γ production is largely
294 confined to the memory T cell population (Anthony et al., 2012), which is largely where we see
295 differences in activation and reactivity between groups (Fig. 4).

296 Antibody production is a key outcome measure after SARS-CoV-2 infection, Tfh cell
297 activation can inform the effectiveness of an antibody response. Stimulation with SARS-CoV-2
298 N peptides activated Tfh cells in CN but not CC subjects, while the opposite trend was found
299 with M pool stimulation (Fig. 3A-B). Tfh cell activation is crucial to the establishment of
300 germinal centers in secondary lymphoid organs, ultimately resulting in B cell maturation into
301 long-lived plasma cells that can continuously produce class-switched, high-affinity antibodies
302 (Good-Jacobson and Shlomchik, 2010). Studies have demonstrated that the magnitude of the Tfh
303 cell response is dependent on the amount of antigen available and directly correlates with the
304 magnitude of the B cell response (Baumjohann et al., 2013). Indeed, the same CN patients with
305 high N-specific Tfh cell activation also displayed anti-N IgG titers significantly greater than CC

subjects (Fig. 3E) despite the fact that we obtained their samples more than 6 months on average after acute infection. Altogether, these findings are consistent with the presence of a persisting nucleocapsid antigen reservoir resulting in enhanced N-specific Tfh cell activation in CN patients. Clinical reports have identified cases of persistent SARS-CoV-2 infection in the nasopharynx lasting up to 63 days (Bennasrallah et al., 2020) and many patients re-tested RT-PCR+ for SARS-CoV-2 up to 38 days post-discharge (Dao et al., 2021). Though many of our CN patients have not re-tested RT-PCR+ by nasal swab (data not shown), others have yet to be tested. The nasopharynx is not the only possible testing site, however, as there are numerous studies showing that infectious SARS-CoV-2 particles can be detected in fecal matter and can persist for up to 70 days post-symptom onset (Tian et al., 2020; van Doorn et al., 2020). Gastrointestinal symptoms also did not have to be present in order to test RT-PCR+ for SARS-CoV-2 in stool samples (Chen et al., 2020). Thus, it is possible that the gut may also act as a reservoir for persistent SARS-CoV-2 infection which leads to aberrant T cell responses and the development of neuro-PASC.

Effective generation of T cell memory responses is crucial to protect against future infections with the same pathogen. $CD8^+$ TEM cells from CC subjects retained high levels of activation to all S, N, and M pools assayed while CN patients displayed very little TEM activation (Fig. 4A-B), suggestive of aberrant memory T cell function. Studies on convalescents from the original SARS-CoV epidemic of 2002-03 found that the majority of Ag-specific $CD8^+$ memory T cells were TEM, and these persisted up to 4 years after infection (Channappanavar et al., 2014). Sterilizing immunity can be a precondition for the development and maintenance of memory T cells because high levels of foreign antigen favor the generation of short-lived effector cells (Mueller et al., 2013), which is why chronic infections often limit the formation

and/or function of memory T cells (Wherry, 2011). In fact, chronic LCMV infection in mice induced aberrantly functioning Ag-specific memory $CD8^+$ T cells requiring the presence of viral peptide rather than simply the homeostatic cytokines IL-7 and IL-15 to proliferate (Shin et al., 2007; Wherry et al., 2004). The inability of $CD8^+$ TEM to become activated by antigenic stimulation in CN patients is thus suggestive of a chronic infection state wherein viral antigen can persist but limits the formation of $CD8^+$ T cell memory. It is also possible that chronic SARS-CoV-2 infection in CN patients leads to memory T cell exhaustion, as shown in chronic HIV and HBV infections in humans (McLane et al., 2019). However, we did not find any differences in the inhibitory marker PD-1 expression on $CD8^+$ memory T cell subsets between CN and CC patients (data not shown).

Further providing support to the chronic infection hypothesis in neuro-PASC patients, there was a significant elevation in the $CD8^+$ TEMRA cell population in CN patients over CC or HC groups (Fig. 4C). $CD8^+$ TEMRA cells are a terminally differentiated memory subset that do not traffic through secondary lymphoid organs, and their induction during viral infection can be protective (Tian et al., 2019). Yet, they have also been shown to accumulate during persistent viral infections and contribute to immunosenescence (Derhovanessian et al., 2011). $CD8^+$ TEMRA cell reactivity to SARS-CoV-2 peptides was also decreased in CN patients over CC subjects. Functionally, the polarization in granzyme production in S3/4-specific $CD8^+$ TEMRA cells from CC patients (Fig. 4G) suggests higher cytotoxic capacity compared with CN patients and coincides with their higher activation state in Fig. 4E. Ag-specific $CD8^+$ TEMRA cells are expanded and functionally active in people with significant anti-Dengue virus immunity, and this phenotype is seen as a goal for vaccine-induced protection (Tian et al., 2019). We propose that $CD8^+$ TEMRA cells are more expanded but less functionally active in CN subjects over CC as a

352 consequence of inappropriate $CD8^+$ T cell memory formation, which is lacking in multiple T cell
353 subsets for neuro-PASC patients.

354 We also showed that $CD8^+$ T cells from CC subjects skewed towards TNF-α production
355 after S pool stimulation compared with CN patients. Comparatively, CN patients displayed
356 significantly more IL-6 production from S-specific $CD8^+$ T cells (Fig. 4F). Vaccination against
357 the intracellular pathogen *Shigella flexneri* showed that polyfunctional TNF-α-producing $CD8^+$
358 TEM were an important correlate of protection (Toapanta et al., 2018). Despite multiple reports
359 indicating that enhanced TNF-α production is correlated with worse COVID-19 outcomes (Karki
360 et al., 2020, 2021; Pedersen and Ho, 2020), we speculate that Ag-specific TNF-α production is
361 protective in our model system because we are looking at chronically and not acutely infected
362 CN patients. In contrast, IL-6 is known to suppress T_H1 differentiation (Diehl et al., 2000) and
363 can promote pathogen survival and exacerbate clinical disease during the original SARS-CoV
364 infection (Channappanavar and Perlman, 2017). Indeed, studies in severely ill, hospitalized
365 COVID-19 patients demonstrated that high serum levels of IL-6 significantly correlated with
366 poor clinical outcome (Weiskopf et al., 2020). These data suggest a role for enhanced IL-6
367 production by $CD8^+$ T cells in the pathogenesis of neuro-PASC, and open new avenues of
368 research for the treatment of long-COVID through limiting IL-6 activity.

369 Clinically, neuro-PASC patients reported significantly elevated levels of anxiety,
370 depression, fatigue, sleep disturbance and pain as well as decreased physical function compared
371 with healthy convalescents (Fig. 1C). The severity of these deficits was highly correlated with
372 Ag-specific enhancements in polyfunctionality and decreases in polarization of various memory
373 T cell subsets (Fig. 5, S7). It is possible that T cell function contributes to the genesis and
374 persistence of some of these symptoms. Studies in rodents have shown that T cell activation and

function can affect the severity of pain and analgesia (Rosen et al., 2019; Sorge et al., 2015). Indeed, pain is a common hallmark of chronic viral infection (Addis et al., 2020) and recognized among post-COVID sequelae (Kemp et al., 2020); it might follow then that aberrant T cell activation can associate with high pain scores. Additionally, reports have shown that transcriptional programs in immunity and inflammation were differentially regulated in $CD4^+$ T cells from patients with depression compared with healthy controls (Wang et al., 2015). T_{reg} cells may also decrease depressive behavior through negative regulation of inflammation (Miller, 2010), and CN patients do display elevated T_H1-type cytokine production to S pool stimulation (Fig. 2, 6) while not displaying any compensatory upregulation in T_{reg} total numbers or function (data not shown). T cell-derived cytokines can also impact learning and memory. Studies in mouse models of West Nile and Zika viral encephalitis have demonstrated that IFN-γ production from $CD8^+$ T cells in the brain is responsible for neuronal apoptosis and spatial learning deficits (Garber et al., 2019). Thus, there is a precedent for correlating T cell function with cognitive deficits, pain, or depression. Ag-specific cytokine signatures associated with the severity of cognitive and quality of life deficits in neuro-PASC patients may therefore provide some predictive value in terms of clinical outcomes.

Preliminary reports showed that the Pfizer mRNA vaccine could elicit a T cell response 7 days after completion of the full prime-boost protocol (Sahin et al., 2020), but until our studies there has been no data on longitudinal T cell responses primed by vaccination and how these vary between groups with different types of prior SARS-CoV-2 exposure. Our results demonstrate for the first time that vaccine-elicited immune responses are significantly divergent in neuro-PASC versus healthy COVID convalescents (Fig. 6C-D). CN patients consistently had higher antibody titers after receiving the second dose of the vaccine compared with CC and HC

groups, though titers were similar in all groups at 11-15 weeks post-1st dose (Fig. 6C). In contrast, the magnitude of Spike-specific IFN-γ production by T cells remained high in CN patients out to 4 months post-vaccination while not significantly higher than pre-vaccination levels in CC and HC subjects along the same timeline (Fig. 6D). These data suggest that the mRNA vaccines do not induce robust long-term T cell responses in many individuals, regardless of prior COVID exposure, if they are not neuro-PASC patients. Yet, despite vaccines enhancing Spike-specific IFN-γ production in CN T cells, the fact that these responses continue to increase at 15 weeks post-vaccination suggests that CN patients may still have an active SARS-CoV-2 infection or a persistent antigen reservoir rather than developing a robust T cell memory response. These results suggest that alternate SARS-CoV-2 vaccines that induce long-lasting memory T cell responses in previously unexposed individuals as well as healthy COVID convalescents are needed in order to mediate long-term protection from infection. Conversely, vaccination may not be indicated for long-COVID patients who might have a persistent infection as it may be ineffective in the absence of viral clearance. Indeed, current clinical guidance from the CDC recommends that vaccination be delayed until 3 months after acute infection in unvaccinated COVID convalescents. Thus, vaccination strategies that include the memory T cell response as a marker for efficacy should be carefully considered. Together, these data show that irregular Tfh responses, broad scale dysfunction in $CD8^+$ T cell memory generation and aberrant T cell responses to vaccination are hallmarks of neuro-PASC and require further study to inform treatment and vaccination strategies across the population.

Limitations of study

One limitation of our study is the relatively small sample size of unvaccinated neuro-PASC patients. This was due to the wide implementation of SARS-CoV-2 vaccines in the

421 Chicago area soon after beginning study enrollment. Another limitation was not being able to
422 control for time of sample collection with respect to date of COVID-19 symptom onset. As it is
423 possible that neuro-PASC could be the result of a persistent infection, further investigations
424 would require testing of potential cryptic reservoirs, including stool samples from CN patients.

425

Materials and Methods

Ethics Statement

This study was approved by the Northwestern University Institutional Review Board (Koralnik Lab, IRB STU00212583). Informed consent was obtained from all enrolled participants. Samples were de-identified before banking.

Study participants, NIH Toolbox, and PROMIS-57 data collection

We enrolled consenting unvaccinated adult outpatients seen in the Neuro-PASC-19 clinic at Northwestern Memorial Hospital from September 2020-June 2021, including 56 neuro-PASC "long-hauler" patients with documented PCR+ or seropositive IgG results for SARS-CoV-2 (CN). In parallel, we recruited 24 unvaccinated healthy COVID convalescents from the surrounding community who tested PCR+ for SARS-CoV-2 but had no lingering neurological symptoms (CC) and 31 healthy controls who tested PCR- for SARS-CoV-2 and were also seronegative for IgG against SARS-CoV-2 Spike RBD. All study subjects remained living throughout the period of observation. Heparinized blood samples were collected one time from each subject at an average of 155-315 days post-symptom onset (as in Fig. 1B). Other demographic information is contained in Fig. 1B. CN patients completed a cognitive function evaluation in the clinic coincident or near the date of their blood sample acquisition with the National Institutes of Health (NIH) Toolbox v2.1 instrument, including assessments of: processing speed (pattern comparison processing speed test); attention and executive memory (inhibitory control and attention test); executive function (dimensional change card sort test); and working memory (list sorting working memory test) (Lai et al., 2011; Weintraub et al., 2013). PROMIS-57 was administered to CN and CC patients an average of 72 days post-sample

collection. Both PROMIS-57 and NIH Toolbox results are expressed as T-scores adjusted for age, education, gender, and race/ethnicity with a score of 50 representing the normative mean/median of the US reference population with a standard deviation of 10. Lower cognition T-scores indicate worse performance while higher fatigue, depression, anxiety, or pain interference T-scores indicate greater symptom severity.

PBMC and plasma collection

30mL of venous blood from study volunteers was collected in blood collection tubes containing sodium heparin from BD Biosciences. Whole blood was layered on top of 15mL of Histopaque 1077 (Sigma-Aldrich) in 50mL Leucosep blood separation tubes (Greiner Bio-One) and spun at 1000g for 18min at RT. Plasma was collected and stored at -80°C. The PBMC layer was collected and washed 2x in sterile PBS before red blood cell lysis with ACK buffer (Quality Biologicals). PBMCs were used in assays either immediately or frozen down for use in the near term.

SARS-CoV-2 peptide antigens

All S, N and M peptide arrays used in ELISPOT and flow cytometry studies were obtained from BEI Resources, NIAID, NIH: Peptide Array, SARS-Related Coronavirus 2 Spike (S) Protein; NR-52402, Nucleocapsid (N) Protein, NR-52404; Membrane (M) Protein, NR-52403. The S peptide array consisted of 181 peptides of 13-17aa in length and split into 6 sub-pools (S1-S6) containing 30-31 peptides each. The N peptide array consisted of 59 peptides of 13-17aa each split into 3 sub-pools containing 29-30 peptides each. The M peptide array consisted of 31 peptides of 12-17aa and split into 3 sub-pools of 10-11 peptides each (Fig. S1). All peptides were

dissolved in either sterile H_2O or 50% sterile H_2O-DMSO up to 1mL for a universal 1mg/mL stock concentration. Peptides were used at a final concentration at 2µg/mL in all assays.

IgG Spike RBD and Nucleocapsid ELISA

Antigen-specific total antibody titers were measured by ELISA as described previously (Dangi et al., 2020; Palacio et al., 2020). In brief, 96-well flat-bottom MaxiSorp plates (Thermo Scientific) were coated with 1 µg/ml of Spike RBD for 48 hr at 4°C. Plates were washed three times with wash buffer (PBS + 0.05% Tween 20). Blocking was performed with blocking solution (PBS + 0.05% Tween 20 + 2% bovine serum albumin), for 4 hr at room temperature. 6 µl of sera was added to 144 µl of blocking solution in the first column of the plate, 1:3 serial dilutions were performed until row 12 for each sample, and plates were incubated for 60 min at room temperature. Plates were washed three times with wash buffer followed by addition of secondary antibody conjugated to horseradish peroxidase, goat anti-human IgG (H + L) (Jackson ImmunoResearch) diluted in blocking solution (1:1000) and 100 µl/well was added and incubated for 60 min at room temperature. After washing plates three times with wash buffer, 100 µl/well of Sure Blue substrate (SeraCare) was added for 1 min. Reaction was stopped using 100 µl/well of KPL TMB Stop Solution (SeraCare). Absorbance was measured at 450 nm using a Spectramax Plus 384 (Molecular Devices). SARS-CoV-2 RBD and N proteins used for ELISA were produced at the Northwestern Recombinant Protein Production Core by Dr. Sergii Pshenychnyi using plasmids that were produced under HHSN272201400008C and obtained from BEI Resources, NIAID, NIH: Vector pCAGGS containing the SARS-related coronavirus 2, Wuhan-Hu-1 spike glycoprotein gene (soluble, stabilized), NR-52394 and receptor binding domain (RBD), NR-52309, nucleocapsid gene NR-53507.

Cell stimulation and IFN-γ/IL-2 ELISPOT

Multiscreen-IP plates (Millipore-Sigma) were coated overnight at 4°C with 2µg/mL anti-IFN-γ (clone 1-D1K, Mabtech) or 5µg/mL anti-IL-2 (clone MT2A91/2C95, Mabtech), washed with sterile PBS, and blocked with complete RPMI-10% FBS. PBMC isolated from CN, CC, and HC subjects were used either freshly isolated or after thawing and resting overnight in media containing 10ng/µL recombinant human IL-15 (Peprotech) at 37°C, 5% CO_2. Cells were then plated at a concentration of $2.5x10^5$ cells/well in 100µL of media and stimulated with the indicated antigen mixtures from SARS-CoV-2 at a concentration of 2µg/mL in complete RPMI medium containing 5% human AB serum (Sigma-Aldrich) and 5ng/mL IL-15. Plates were incubated at 37°C, 5% CO_2 for 20 h and washed 5x with dH_2O and PBS-0.05% Tween-20 (PBS-T). 2µg/mL biotinylated IFN-γ (clone 7-B6-1, Mabtech) or 5µg/mL IL-2 (clone MT8G10, Mabtech) diluted in PBS-10% FBS (PBS-F) was added to the respective wells and plates were incubated for 1.5h at RT. Plates were subsequently incubated for 40 minutes at RT in streptavidin-alkaline phosphatase in PBS-F (Jackson ImmunoResearch) was added after washing plates 5x in PBS-T. ELISPOT plates were developed using an Alkaline Phosphatase Conjugate Substrate Kit according to manufacturer's instructions (Bio-Rad Laboratories, Carlsbad, CA). IFN-γ or IL-2-producing cells were quantified using an ImmunoSpot reader (Cellular Technologies, Ltd., Shaker Heights, OH).

Antibodies and Flow Cytometry

Fresh or frozen PBMCs isolated from the indicated patient groups were stimulated with antigen mixtures as above for 20-22h at 37°C, 5% CO_2. For intracellular staining and cytokine detection, the Brefeldin-A Golgi plug (Biolegend) was added at a 1:1000 concentration 2 hours after antigenic stimulation commenced. Cells were washed with PBS-1% BSA after incubation and incubated with the indicated antibodies for surface phenotyping by AIM assay or for intracellular

cytokine staining (ICS). Cells from each subject were left unstimulated in medium containing 5ng/mL IL-15 ("background") or stimulated in the presence of the indicated antigens. Fixation and permeabilization was performed using Cytofix/Cytoperm (BD Biosciences). Surface staining was done in the dark at 4°C for 30 minutes, while ICS was done in the dark at RT for 45 minutes. Flow cytometry was conducted on 2-5x10^5 cells per condition. Data was acquired on a BD FACSymphony Spectral analyzer and analyzed using FlowJo v10 (BD Biosciences) and SPICE-Pestle (Roederer et al., 2011).

Quantification and Statistical Analysis

Statistical tests to determine significance are described in figure legends and conducted largely in Prism (GraphPad). For pie graphs or heatmaps generated using SPICE analysis, statistics were determined by Permutation test following unstimulated background subtraction, with additional thresholding of 0.03% to account for noise, using SPICE-Pestle. *P*-values lower than 0.05 were considered statistically significant. Quantile stratification was performed within group for CN cohort. Clinical data were collected and managed using REDCap electronic data capture tools hosted at Northwestern University Feinberg School of Medicine (Harris et al., 2009). REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

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Author Contributions

Conceptualization L.V. P.P.M. and I.K; Investigation L.V., B.H., Z.O., P.H.L, and N.P.; Formal Analysis L.V., B.H., E.M.L., P.P.M. and N.P.; Resources L.V., P.P.M., I.K., Data Curation L.V.; Writing L.V. with feedback from all authors; Supervision P.P.M and I.K.; Project Administration L.V.; Funding Acquisition L.V., P.P.M, and I.K.

Declaration of Interests

The authors declare no competing interests.

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802

Figure Legends

Figure 1: Study design and clinical data

A.) Flow chart describing patient populations and experimental assays for each sample. B.) Table showing patient demographics and neurologic symptoms. C.) PROMIS-57 patient reported survey T scores for CN (n=36) and CC (n=13) groups. D.) NIH Toolbox cognitive T scores for CN (n=43). Horizontal black line represents the U.S. national average T score of 50; p values relative to US national average by one sample Wilcoxon signed rank test. *p<0.05, ***p<0.005, ****p<0.0001 by two-tailed Student's t test.

Figure 2: Neuro-PASC long-haulers display altered reactivity to SARS-CoV-2 N and M peptides compared to healthy convalescents.

A-B.) CN (PCR+ or seropositive neuro-PASC) and CC (PCR+ or seropositive healthy COVID convalescent) groups display similar IFN-γ and IL-2 responses to peptides from SARS-CoV-2 Spike protein by ELISPOT. C-D.) CN samples show significantly enhanced IFN-γ responses to the N3 peptide pool (C) and to the M1 and M3 peptide pools (D) compared with CC or healthy controls. E-F.) N- and M- specific IL-2 production did not significantly differ between patient subgroups. G.) Spike RBD IgG endpoint titer quantification for CN, CC, and HC groups. LOD is limit of detection. Data representative of 10 experiments with all conditions plated in duplicate. *p<0.05, **p<0.01, ***p<0.005, ****p<0.0001 by two-way ANOVA with Tukey's posttest.

Figure 3: N-specific $CD4^+$ Tfh cells are more activated and S-specific $CD4^+$ T cells display enhanced polyfunctional granzyme production in neuro-PASC long-haulers.

A.) FACS plots show that Ag-specific CD4 Tfh from CN patients are more highly activated in response to N peptide pool stimulation compared with CC or HC, but less activated by M peptides. B). Quantification of AIM^+ Tfh cell activation. C-D.) N- and M-specific AIM^+ Tfh cell

826 percentages plotted by time sample obtained post-infection for CN and CC. No correlation was
827 found between these parameters. E.) Anti-SARS-CoV-2 nucleocapsid IgG endpoint titers for
828 CN, CC, and HC subjects shown in 3D. CN patients display significantly elevated anti-N IgG
829 titers compared with CC subjects. F.) Pie graphs show $CD4^+$ T cells from CC patients have
830 enhanced production of granzymes A, B, & M compared with CN or HC subjects in response to
831 SARS-CoV-2 Spike protein. G.) Heatmap quantifying polyfunctionality in different categories of
832 cytokine production between groups. CC subjects produced 2-fold more granzymes A/B/M than
833 CN patients. Data combined from 6 independent experiments with CN n=35, CC n=11, and HC
834 n=9. *$p<0.05$ using one-way ANOVA with Bonferroni's posttest (B) or a Permutation test (E).
835 All pie graphs are showing data after subtracting background (unstimulated condition).

836 **Figure 4: Neuro-PASC long-haulers have decreased $CD8^+$ memory T cell activation and**
837 **function compared with healthy convalescents.**

838 A.) Selected FACS plots showing CD8 TEM cells are less activated in CN vs. CC groups after S
839 and N peptide pool stimulation. B.) Quantification of AIM+ CD8 TEM cells after SARS-CoV-2
840 peptide stimulation. C.) $CD8^+$ TEMRA cells accumulate significantly in PBMC from CN
841 subjects compared with CC or HC. D.) $CD8^+$ TEMRA cells from CN patients are less activated
842 by S3/4 and N pools compared with those from CC subjects. E.) Quantification of AIM^+ $CD8^+$
843 TEMRA cells from D. F.) Pie charts demonstrating that S3/4-specific $CD8^+$ TEM are polarized
844 to produce more TNF-α in CC group while those from CN patients produce significantly more
845 IL-6. G.) $CD8^+$ TEMRA from CC patients are polarized towards category 2 cytokine production
846 in response to S3/4 stimulation compared with CN subjects. Black lines demonstrate boundaries
847 of category 2 cytokine production in each group. Data combined from 5 independent
848 experiments with n=35 CN, n=11 CC. *$p<0.05$, **$p<0.01$ using two-tailed Student's t test with

Welch's correction (B, C, E) or permutation test (F, G). All pie graphs are showing data after subtracting background (unstimulated condition).

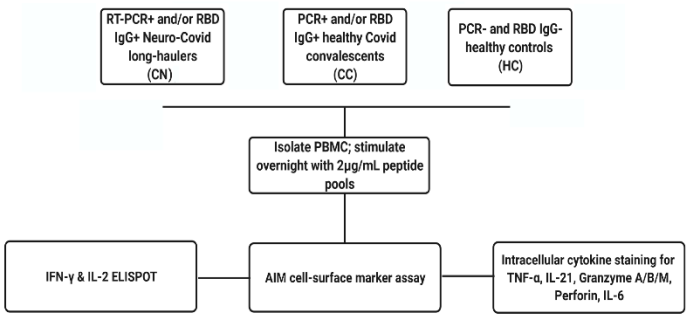
Figure 5: Clinical measures of cognitive dysfunction and depression correlate with altered CD8$^+$ memory T cell function in neuro-PASC long-haulers.

A.) Higher T cell responses to N pool stimulation are positively correlated with high pain scores in CN patients. B.) A positive trend exists between elevated N-specific IFN-γ production and higher depression scores in CN patients. C.) CN patients scoring high on pain interference have Spike-specific CD8$^+$ T cells producing more IL-6 than those scoring low. D.) Heatmap showing CN with low pain scores have Spike-specific CD8$^+$ T cells producing significantly more granzymes A or B than those reporting high pain levels. E.) Pie graph to demonstrate that CN patients in Q4 for depression have significantly enhanced production of granzymes A/B/M compared with patients with low depression scores. F.) Heatmap demonstrating that CN patients with high depression scores had Spike-specific CD8$^+$ TEM producing 3-fold higher levels of granzymes A, B, and M or perforin than those scoring low on depression. G.) Spike-specific CD8$^+$ TCM from CN patients with high scores for executive function are more polarized to produce granzymes A/B/M compared with CN patients from Q1. H.) Heatmap showing Spike-specific CD8$^+$ TCM in Q1 CN patients are more polyfunctional and produce 8-10x more granzymes A/B/M and perforin than those in Q4. Data representative of 5 independent experiments with n=8-9/quartile. All pie graphs showing Ag-specific cytokine production are background subtracted (unstimulated conditions). *p<0.05, **p<0.01, ****p<0.001 using Permutation tests.

Figure 6: Spike-specific T cell responses wane within 4 months after vaccination in CC and HC subjects while remaining high in CN patients over time.

A.) Vaccine study visit timeline. V0 was obtained before the first dose of either Pfizer or Moderna mRNA vaccines. V1 and V2 were conducted 3 weeks after the first and second doses, respectively. B.) Vaccine study subject demographics. C.) Longitudinal anti-Spike RBD IgG responses from V0-V4 across groups. Antibody titers are highest in CN patients and wane most quickly in HC subjects. D.) IFN-γ production from Spike-specific T cells do not significantly increase in CC and HC groups while remaining high in CN up to V4 post-vaccination. Total S-specific IFN-γ SFU calculated by averaging responses from each sub-pool for each participant (data in Fig. S9). Data combined from 10 individual experiments with all ELISA conditions done in triplicate and all ELISPOT conditions plated in duplicate. *$p<0.05$, **$p<0.01$, ***$p<0.005$, ****$p<0.0001$ by two-way ANOVA with Tukey's posttest or by two-tailed Student's t test.

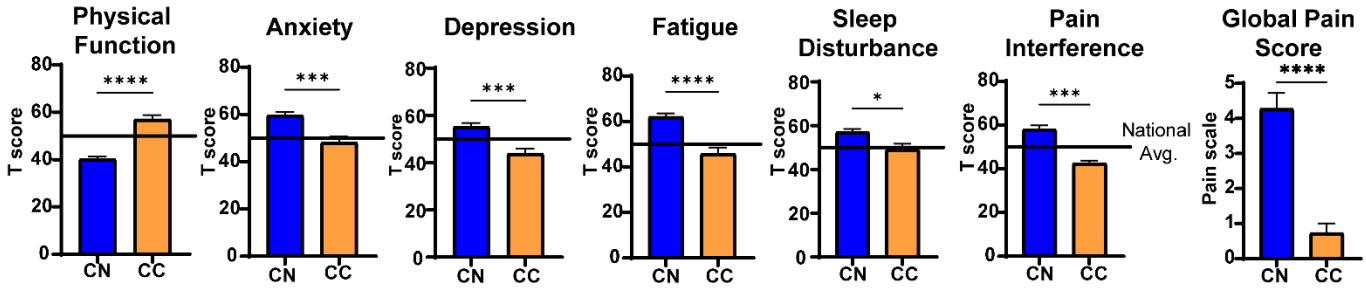
A.



B.

Patient Demographics	CN	CC	HC
Participants	56	24	31
Sex	38F, 18M	16F, 8M	19F, 12M
Race/Ethnicity	39 White 9 Hispanic 4 Asian 4 Black	15 White 6 Hispanic 3 Asian	11 White 5 Hispanic 13 Asian 2 Black
Hospitalization (%)	8 (14.3)	1 (4.2)	0 (0)
Days post-symptom onset (range)	193.4 (20-407)	155.0 (44-458)	NA
Neurologic symptoms (%)	56 (100)	0 (0)	0 (0)
Brain Fog	49 (87.5		
Fatigue	46 (82.1)		
Headache	41 (73.2)		
Dizziness	33 (58.9)		
Anosmia	35 (62.5)		
Dysgeusia	33 (58.9)		
Myalgias	34 (60.7)		

C.



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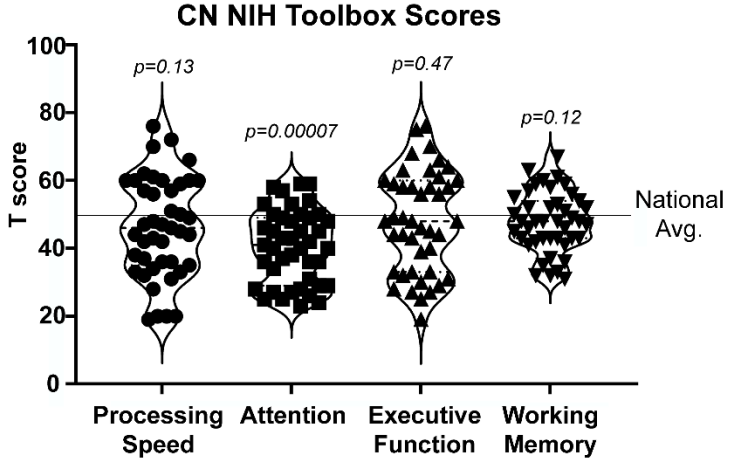


Figure 1

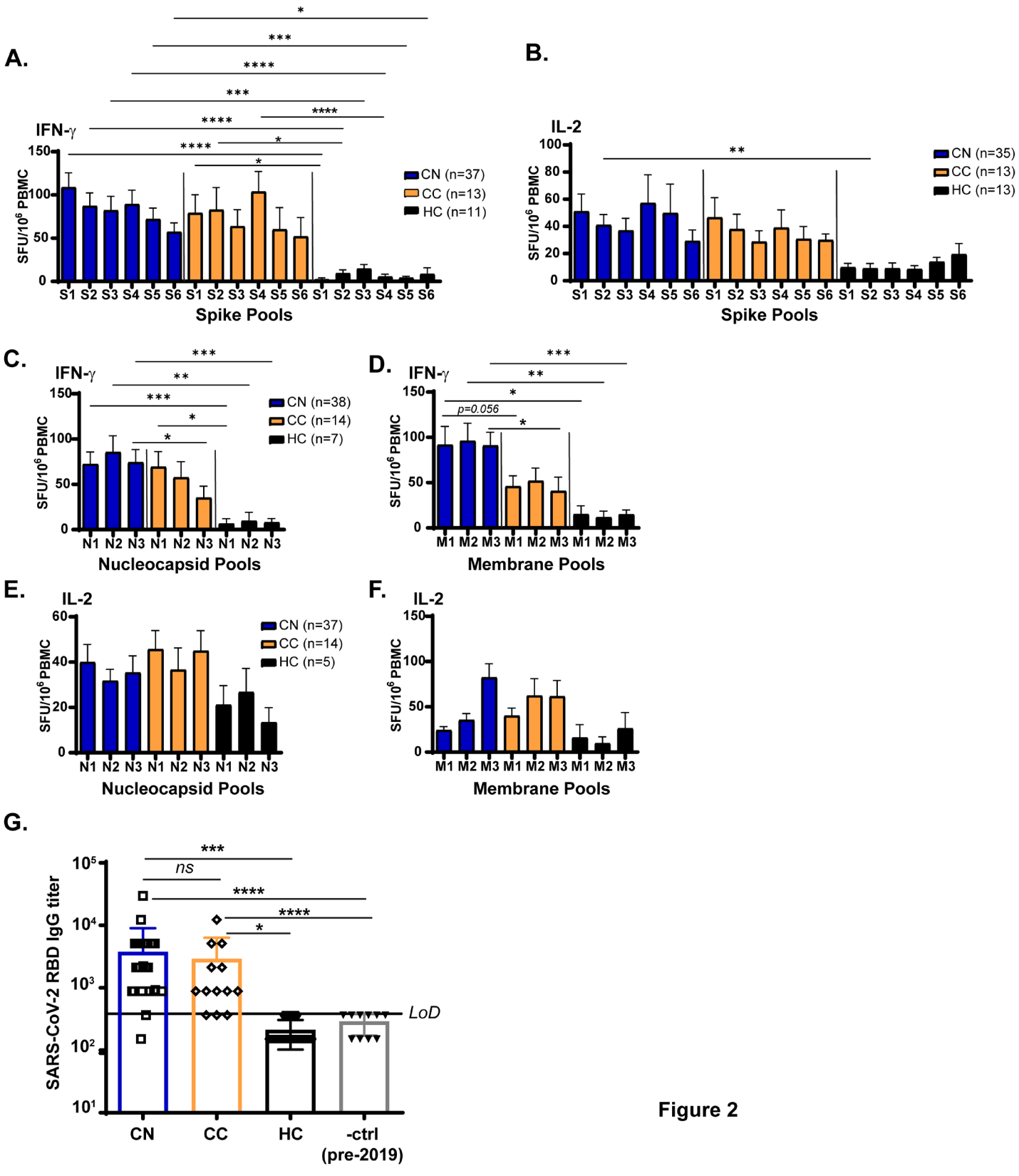
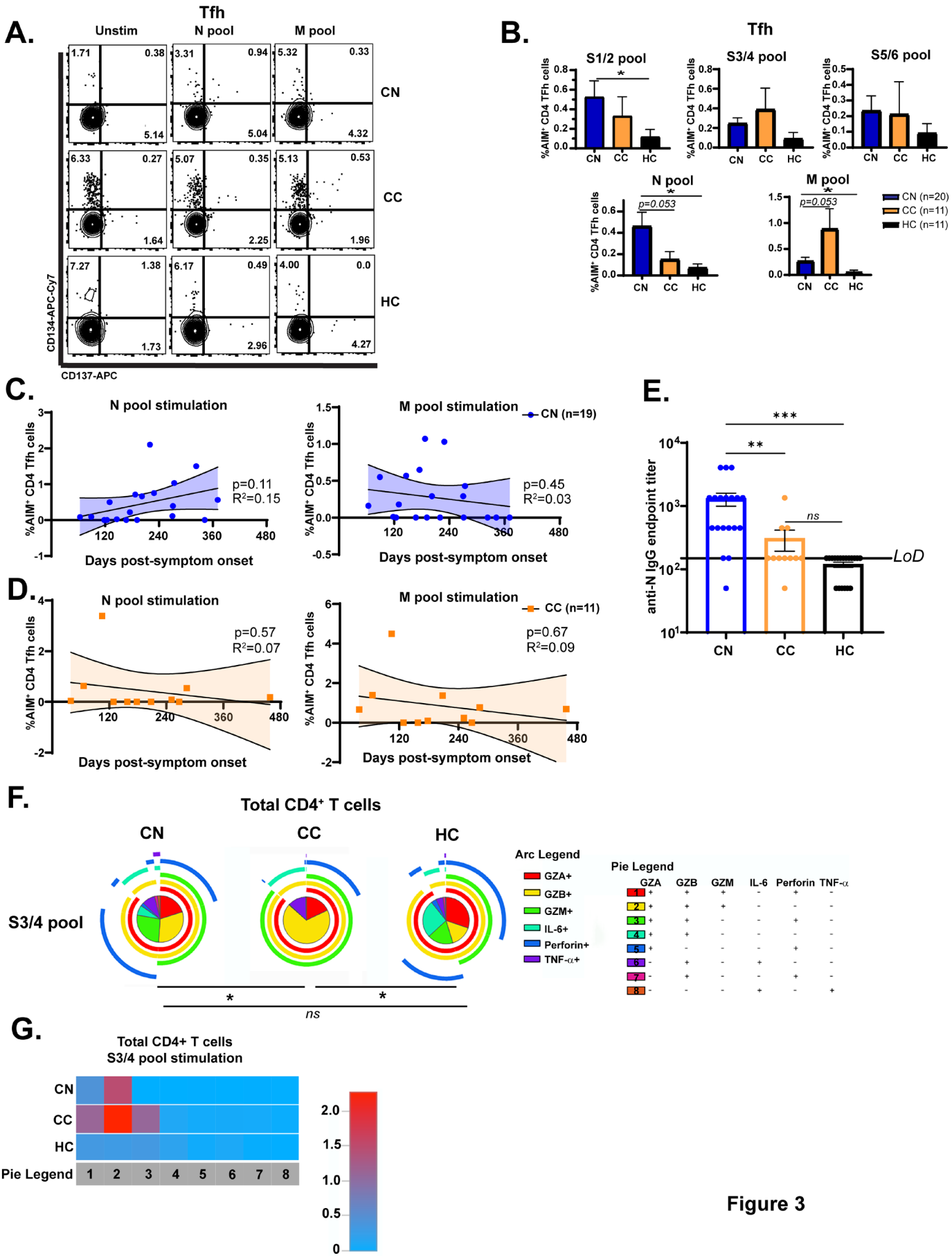


Figure 2



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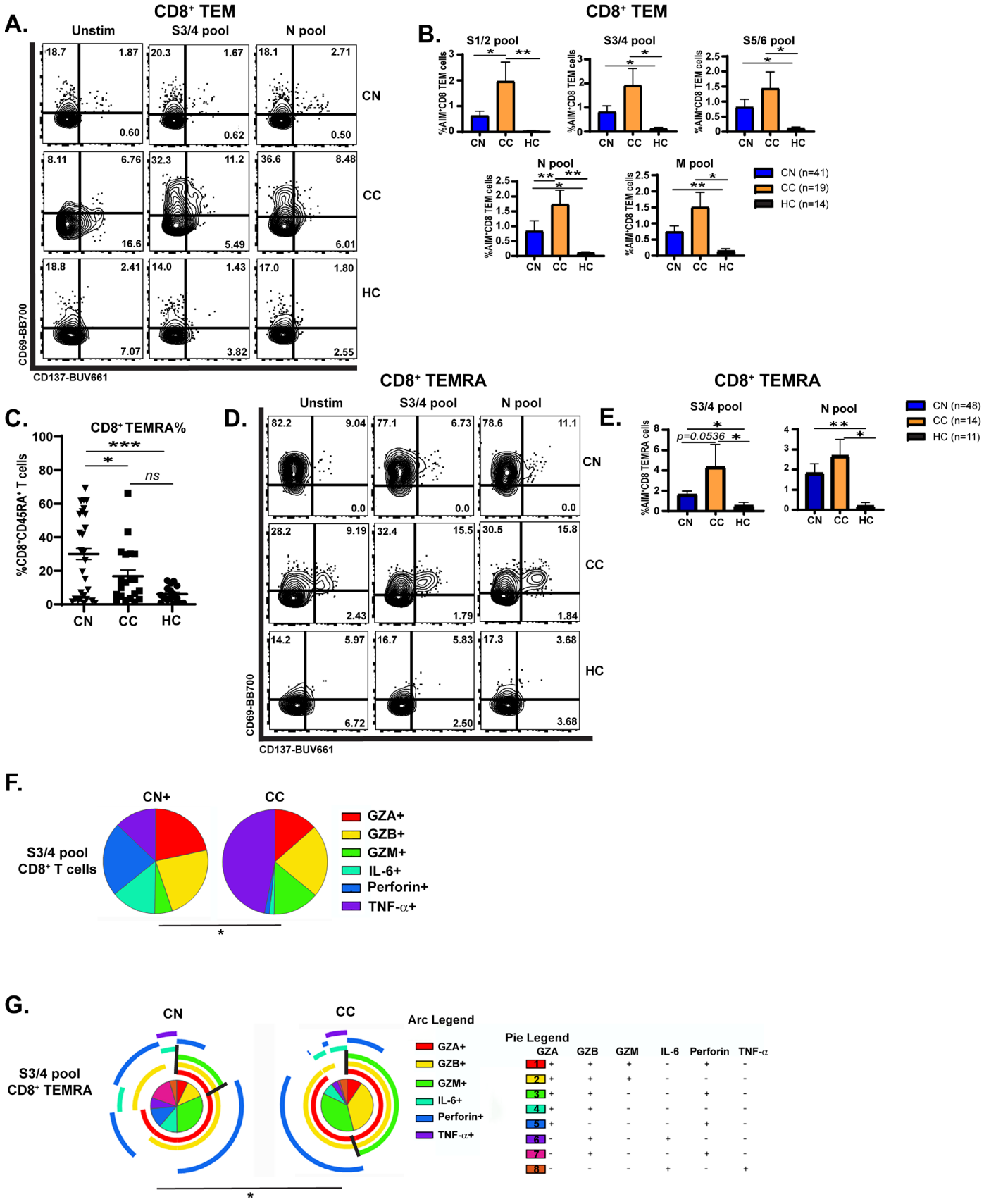


Figure 4

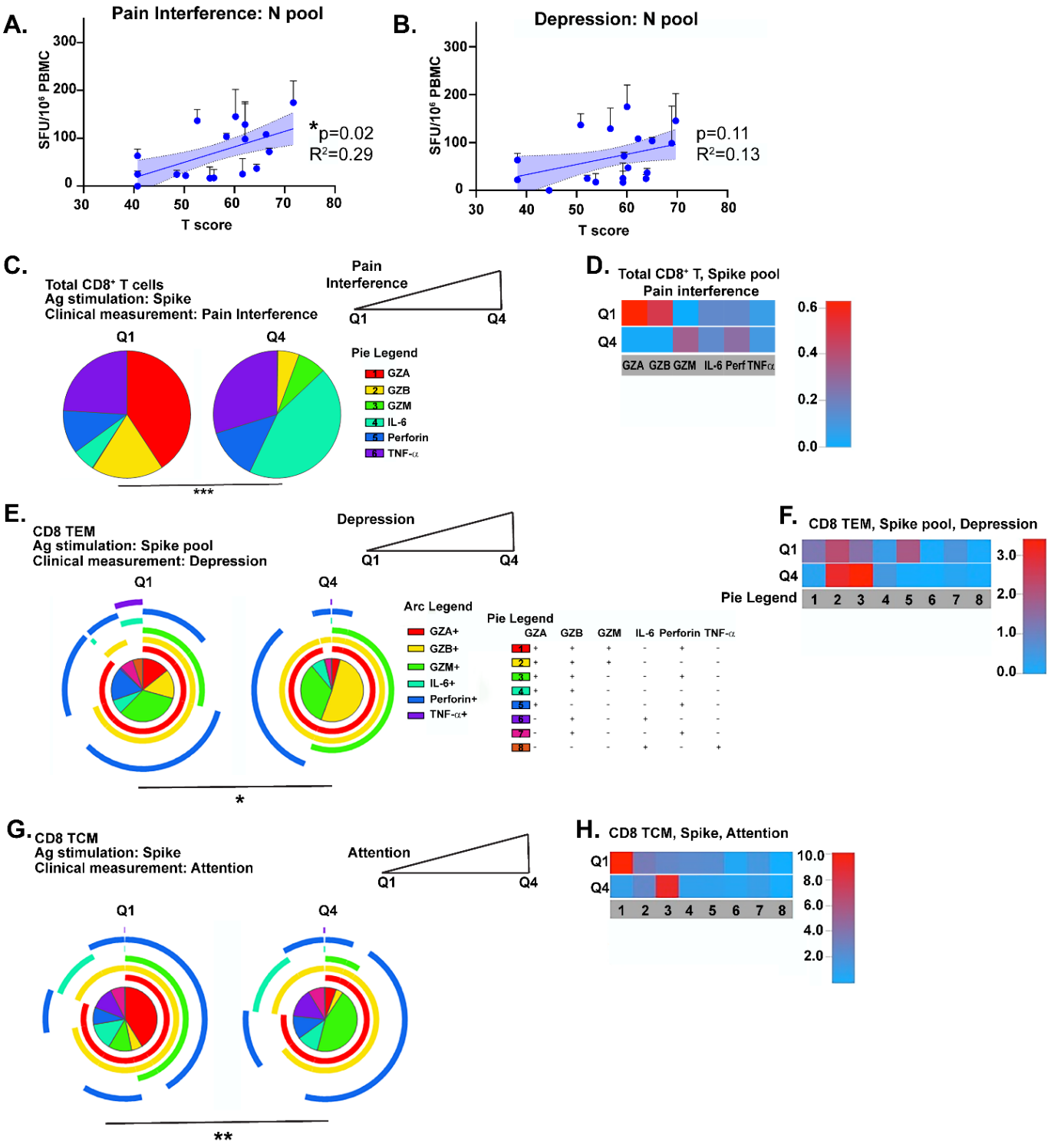
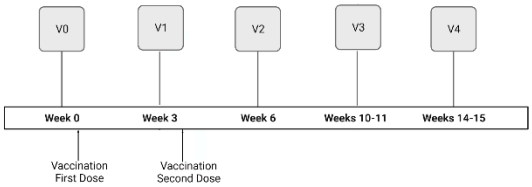


Figure 5

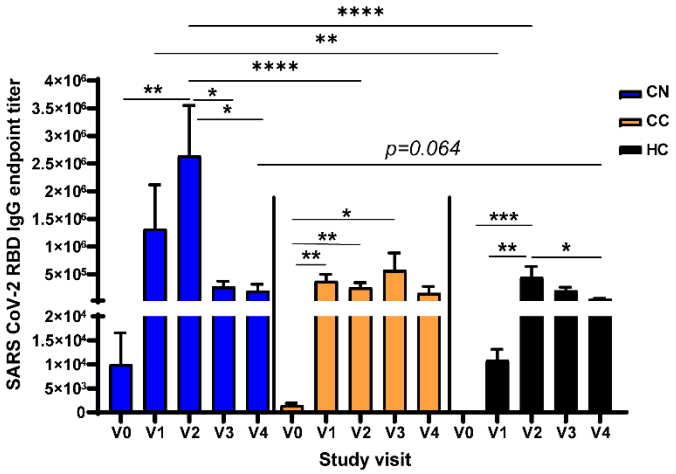
A.



B.

Vaccine Study Demographics	CN	CC	HC
Participants	19	12	20
Mean Age (range)	50.8 (33-70)	29.2 (22-63)	29.9 (22-59)
Sex	13F, 6M	7F, 5M	13F, 7M
Race/Ethnicity	12 White 5 Hispanic 1 Asian 1 Black	8 White 1 Hispanic 3 Asian	8 White 4 Hispanic 7 Asian 1 Black
Vaccine Brand	13 Pfizer 6 Moderna	10 Pfizer 2 Moderna	16 Pfizer 4 Moderna

C.



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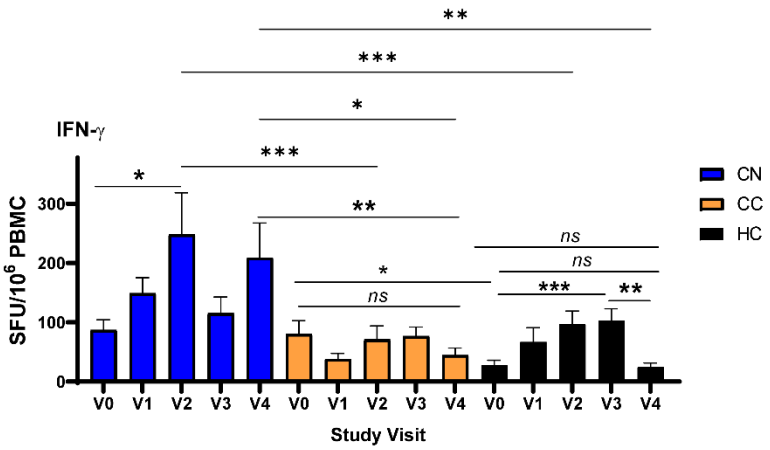

Figure 6

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