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Abstract

Vaccine failure in patients with secondary immunodeficiencies (SIs) is associated with severe infections and mortality. To define a mechanistic basis for this, we applied an integrated multi-omic and functional approach to dissect vaccine responses pre- and post- booster (third dose) COVID-19 mRNA/LNP vaccination from 217 individuals comprising ten clinically distinct SI groups with variable antibody and T cell response rates after vaccination and healthy controls. The mechanisms of antibody impairment differed across SI groups and included reduced B cell frequency, antigen-specific B cell-intrinsic defects, and aberrant functionality of circulating antigen-specific T_{FH} cells. In contrast, defects in effector differentiation, metabolic signalling and functionality of antigen-specific CD8⁺ and CD4⁺ T cells were observed in all individuals with impaired T cell immunity. Together, these data define distinct mechanisms of antibody failure and a unified mechanism of T cell dysfunction that could be targeted to enhance vaccine efficacy in immune vulnerable patients.

Introduction

In healthy populations, COVID-19 mRNA/lipid nanoparticle (mRNA/LNP) vaccines elicit high magnitude SARS-CoV-2 antibody and T cell responses and protect against severe COVID-19^{1, 2}. The magnitude of antigen-specific antibody and IFN γ -producing T cells induced by mRNA/LNP vaccines vary substantially across healthy human populations depending on genetic, epigenetic and environmental factors¹. For several vaccine modalities immune signatures have been identified which predict robust vaccine-induced immune responses, including the absence of pre-vaccine “inflammation” and the frequency of plasma cells 7-days after vaccination^{3, 4, 5, 6, 7}. While informative, these signatures are derived in healthy populations and so capture a spectrum of vaccine responses that range from ‘moderate’ to ‘high’ and are unable to resolve features related to complete or partial vaccine failure, as observed in immune-vulnerable patient populations. Furthermore, there remains a paucity of studies that evaluate immune signatures predictive of vaccine antigen-specific T cell responses, despite the critical role that these cells play in protection from severe disease^{8, 9, 10, 11, 12}.

Individuals with a range of secondary immunodeficiencies (SIs) are unable to generate high titre antibody and T cell responses after multiple doses of mRNA/LNP or adenoviral vectored COVID-19 vaccines, whereas immunocompetent individuals make effective immune responses to the same vaccines^{1, 11, 13}. SIs represent a heterogenous group of conditions caused by chronic diseases and/or immunosuppressive therapeutics, both drugs^{14, 15} and disease¹⁵ have been associated with attenuated immune responsiveness to vaccines.

Common immunological correlates relating to vaccine failure across multiple SIs remain unknown and the perturbed biological pathways that contribute to immune dysfunction and vaccine failure in SIs are currently unexplored^{16, 17}. Identification of specific biological pathways that contribute to vaccine failure across multiple SIs may allow the development of novel vaccine strategies optimised for SI patient populations. This is particularly important in the context of mRNA/LNP vaccination, a vaccine platform which is increasingly applied to clinical settings such as infectious disease and cancer^{18, 19, 20}, and concomitantly to an increasing population of patients with co-morbidities and SIs globally.

To assess the impact of SIs on vaccine outcomes and identify signatures associated with vaccine immune non-responsiveness, we performed a detailed characterisation of pre- and post-third COVID-19 mRNA/LNP vaccine immune responses in 217 individuals from ten clinically distinct immunosuppressive backgrounds with low anti-SARS-CoV-2 spike binding antibody titres (Ig-RBD) after prior COVID-19 vaccination¹⁴. Using systems and functional immune experimental approaches, we provide a detailed assessment of the shared and unique biological pathways of antibody and T cell vaccine non-responsiveness and identify possible targets to optimise mRNA/LNP vaccine strategies for at-risk populations.

Results

Study design and patient demographics

A subset of 217 individuals from 10 clinically distinct SIs were selected from the OCTAVE DUO study for detailed immune characterisation. A minimum of 14 individuals per clinical group were selected to capture the clinical variability within each cohort. This included individuals with cirrhosis (Cirr); ulcerative colitis and Crohn's disease (collectively inflammatory bowel disease [IBD]); ANCA-associated vasculitis (AAV); rheumatoid arthritis (RA); liver and renal transplant (LT and RT); individuals on Ig replacement therapy; autologous haematopoietic stem cell-transplant (HSCT) and individuals with aggressive or indolent B cell non-Hodgkins lymphoma (NHL) (**Fig. 1A**). All participants were SARS-CoV-2 infection naive up until third vaccination (**Methods**). Blood was sampled immediately prior to and 21-days after a third dose of COVID-19 mRNA/LNP vaccine (mRNA-1273 or BNT162b2), and comparisons were made with an additional cohort of healthy control (HC) volunteers (n=14). As expected, age, sex, comorbidity prevalence, BMI, performance status (WHO) and immunosuppressive therapies varied between SI groups, consistent with differences in their underlying clinical phenotypes (**Fig. 1B&C, Supplementary Table 1**).

Variable effect of SI and immunosuppressive therapies on antibody and T cell responses to a booster mRNA/LNP COVID-19 vaccine

Re-analysis of anti-SARS-CoV-2 receptor binding domain (RBD) binding antibody titres (Ig-RBD) and IFN γ ⁺ S-specific T cell responses (IFN γ -S) in the 217-person subset of OCTAVE DUO demonstrated large intra- and inter- SI group variability at V3+21d and no significant increase between pre-V3 and V3+21d Ig-RBD or IFN γ -S in several SI groups (**Extended Data 1A&B**). Stratification of the cohort based on undetectable ('no': <0.8AU/mL), functionally deficient ('low': <1000AU/mL) (**Methods**) or high (>1000AU/mL) V3+21d Ig-RBD titres demonstrated that over 40% of individuals in AAV, RA, LT, RT, PID, HSCT and NHL groups had functionally deficient (low) or undetectable (no) Ig-RBD at V3+21d (**Fig. 1D**). Stratification based on detection of IFN γ -S T cell responses indicated that some SI groups (IBD, AAV, RA and PID) had only one or no individuals with undetectable V3+21d IFN γ -S, whereas 20-50% of individuals in Cirr, LT, RT, HSCT and NHL groups had undetectable IFN γ -S at V3+21d (**Fig. 1E**).

Certain immunotherapies, such as aCD20 and MMF, were particularly associated with reduced Ig-RBD regardless of SI group, whereas SI group was the predominant factor associated with reduced IFN γ -S (**Extended Data Fig. 1C&D**). Notably, there was no correlation between V3+21d RBD-Ig and V3+21d IFN γ -S, highlighting the differential impact of SIs on vaccine-induced antibody, compared with T cell, responses (**Extended Data Fig. 1E**). The disconnect between V3+21d RBD-Ig and IFN γ -S was most pronounced in RA, AAV and cirrhosis groups: AAV and RA had low V3+21d RBD-Ig and high IFN γ -S whereas the opposite was true for cirrhosis patients (**Fig. 1D, Extended Data Fig. 1A-E**). In contrast, transplant recipients (LT, RT and HSCT) on any immunosuppressive therapy had reduced V3+21d Ig-RBD and IFN γ -S compared to HC or IBD patients (**Extended Data Fig. 1A-E**). This indicates a complex interaction between SIs and immunosuppressive therapy that has variable impact on Ig-RBD and IFN γ -S responses to vaccine.

Baseline blood transcriptomic signatures across multiple disease groups are associated with vaccine immunogenicity

To identify immune pathways that contribute to vaccine responsiveness we first assessed the baseline (pre-V3) peripheral blood transcriptome in 231 individuals using whole blood RNA-sequencing. Quantification and consensus k-means clustering of blood transcriptomic modules (**Methods**) identified 10 modules of genesets related to immune function, inflammation, metabolism, haematopoiesis and cell cycling (**Extended Data Fig. 2A-D**). There

was significant heterogeneity across the cohort in the expression of all geneset modules except HLA, NK and cell cycling modules (**Extended Data Fig. 2C&D**).

Models of geneset module expression comparing the expression of SI group and immunosuppressive therapy combinations, while accounting for demographic features (age, sex, obesity, diabetes status), demonstrated SI group and immunosuppressive therapy specific effects (**Fig. 1F**). Given the similarity of the whole-blood transcriptome and clinical phenotype of LT and RT groups, these were analysed together (**Extended Data Fig. 2C&E**). All participants on aCD20 therapy, regardless of SI group, had reduced B cell geneset module expression compared to HC (**Fig. 1F**). While all LT and RT participants had increased IFN signalling compared to HC, only those on triple immunosuppression (calcineurin inhibitors (CNI), mycophenolate mofetil (MMF) and corticosteroids) had reduced B/plasma cell and T cell signalling and increased innate and haematopoiesis/motility expression (**Fig. 1F**). Across the cohort, SI group explained more variation in geneset module expression than immunosuppressive therapies or demographics in all modules except the B cell module, where aCD20 therapy was the single most explanatory variable (**Fig. 1G**).

Correlation analysis between baseline transcriptomic module expression and V3+21d Ig-RBD titres demonstrated that pre-V3 B and plasma and T cell module expression were positively correlated with vaccine-induced Ig-RBD whilst innate, IFN and haematopoiesis/motility module expression was negatively correlated (**Fig. 1H**). Baseline expression of the B and plasma cell module was most strongly correlated with V3+21d Ig-RBD ($\rho = 0.5$). IFN γ -S magnitude at V3+21d was positively correlated with pre-V3 T cell and metabolism geneset modules and negatively correlated with IFN and cell cycle related genesets (**Fig. 1I**).

Impaired vaccine-induced antibody responses in transplant recipients are independent of global B cell phenotype

As immunosuppressive therapies varied between SI groups, we next assessed the association of the baseline transcriptome with V3+21d Ig-RBD stratified by SI group. Correlation analysis between baseline B and plasma module expression and V3+21d Ig-RBD demonstrated that only groups using aCD20 therapy showed significant correlation (**Fig. 2A**), with 47-85% of variance in V3+21d RBD-Ig titre explained by baseline B and plasma module expression, compared with 2% in LT/RT groups (**Fig. 2B**). No geneset module in Cirr, IBD, or RT/LT groups explained more than 20% of variance, suggesting more specific defects were related to vaccine failure in these groups (**Extended Data Fig. 3A**).

To further investigate immune pathways associated with vaccine-induced antibody responses across the different groups, individuals from select SI group and HCs were stratified into four groups: recipients of aCD20 (Indolent B NHL and AAV groups), LT/RT recipients, other non-aCD20/LT/RT SI groups, and HCs, and additional immunophenotyping at pre-V3 and V3+21d timepoints was performed (**Fig. 2C**). Spectral flow cytometry (**Extended Data Fig. 4**) was used to determine pre-V3 global and S1-specific B cell frequencies and phenotype. Analysis of the global B cell compartment demonstrated that only SI groups on aCD20 therapy had reduced B cell frequencies or altered B cell composition (**Extended Data Fig. 3B&C**). Correlation of baseline B cell frequencies with V3+21d Ig-RBD confirmed the lack of relationship between B cell frequency with vaccine-induced antibody titres in RT/LT (**Extended Data Fig. 3D**). This was also observed amongst pre-V3 S1-specific B cells, which were reduced in frequency in all SI groups compared to HC but particularly in aCD20 recipients and lacked an association with V3+21d Ig-RBD only in LT/RT recipients (**Extended Data Fig. 3E-G**). LT/RT participants additionally lacked a relationship between Ig-RBD titre and the capacity to neutralise omicron BA1 virus, suggesting altered antibody functionality (**Extended Data Fig. 3H**). Overall, this suggests that whilst LT/RT recipients can generate antigen-specific memory B cells to vaccines, the quantity and quality of antibodies produced by vaccine-induced B cells is reduced in these SI groups.

Altered class-switch and transcriptomic profile of antigen-specific B cells are associated with impaired antibody responsiveness in LT/RT recipients

To assess why LT/RT recipients had poor antibody responses despite a similar global B cell compartment and similar frequency of S1-specific B cells, we performed scRNA- and scBCR-sequencing on flow cytometry sorted S1-specific B cells and non-antigen enriched CD45⁺ cells for comparative analysis, from 77 individuals across SI groups at V3+21d (**Fig. 2D**). Consistent with baseline, LT/RT recipients had similar frequencies of S1-specific B cells compared to non-aCD20 SI groups and this was not correlated with V3+21d Ig-RBD (**Fig. 2E&F**). S1-specific B cells were integrated with B cells from CD45⁺ scRNA-seq data (background) and clustering identified four naive, four memory, one DN1, one atypical B cell (ABC), and one plasma cell population (**Fig. 2G, Extended Data Fig. 3I**). Memory B cell cluster 4 and ABC-like populations were enriched in S1-specific compared to background B cells and made up the largest proportion of S1-specific B cells (**Fig. 2H, Extended Data Fig. 3J**).

Although the relative frequency of naive and memory B cells amongst the background B cell population was comparable between LT/RT, non-aCD20 SI groups and HCs (consistent with the baseline data) (**Fig. 2I** (left), **Extended Data Fig. 3J**), LT/RT and aCD20 recipients had significantly reduced S1-specific memory B cell frequencies (**Fig. 2I**). The memory B cell cluster 4 population was particularly reduced in LT/RT and was similar to the frequency identified in aCD20 recipients and amongst background B cells (**Fig. 2I&J**). The frequency of memory B cell cluster 4 amongst S1-specific B cells was significantly correlated with V3+21d Ig-RBD and omicron BA.1 neutralising antibody titres in both LT/RT and other groups (**Fig. 2K & Extended Data Fig. 3K**). The memory B cell cluster 4 was enriched for metabolism, and cell cycling pathways – including those related to B cell activation including Myc and mTORC²¹ (**Extended Data Fig. 3L**), pathways that were dampened in the LT/RT participants (**Fig. 2L&M**).

The majority of class-switched S1-specific B cells across the cohort expressed *IGHG1* BCRs that were identified in memory B cluster 4 (**Extended Data Fig. 3M**). Comparison of somatic hypermutation (SHM) in S1-specific class-switched memory/DN B cells indicated that only aCD20 recipients had reduced SHM rates (**Fig. 2N**). In contrast, S1-specific B cells in aCD20 and LT/RT groups had reduced rates of class-switching and were not more class-switched than background memory/DN B cells (**Fig. 2O&P**). Combined, these data suggest that LT/RT recipients have a specific defect in B cell differentiation (class switching and memory differentiation) associated with antibody non-responsiveness.

Exacerbated features typical of “immune aging” are associated with reduced vaccine-induced T cells in SI

Unlike Ig-RBD, there was a notable split in the detection of V3+21d IFN γ -S across SI groups and this was not related to specific immunosuppressive therapies. Individuals in Cirr, LT, RT, HSCT, NHL had high-rates of IFN γ -S non-responsiveness (termed metagroup 1) at V3+21d, whereas IBD, AAV, RA and PID SI groups had one or fewer individuals with undetectable IFN γ -S per group (termed metagroup 2) (**Fig. 1F**). This observation prompted two key questions: 1) what immune features differentiate metagroup 1 from metagroup 2 that may inform why certain SI groups are at risk of T cell non-responsiveness? and 2) within metagroup 1, what differentiates individuals with/without detectable T cell responses after vaccination? We therefore stratified the cohort into three groups for further analysis: 1) metagroup 1 with undetectable V3+21d IFN γ -S (termed MG1-); 2) metagroup 1 with detectable V3+21d IFN γ -S (termed MG1+) and 3) metagroup 2 (termed MG2+) (**Fig. 3A**). This stratification additionally

helped to account for age, which varied between SI groups and was significantly higher in MG1 compared to MG2 (**Extended Data Fig. 5A**). Immunosuppressive therapy usage varied between metagroups (**Fig. 3B**).

Comparison of flow cytometry-derived CD4⁺, CD8⁺, MAIT-like (CD161⁺⁺CD127⁺) and $\gamma\delta$ T cell (**Extended Data Fig. 4**) frequencies between MG1⁻, MG1⁺ and MG2⁺ identified significantly elevated frequencies of CD4⁺ T_H1 (non-naive, non-T_{reg}, CXCR3⁺CCR6⁻) and cT_{FH} (non-naive, non-T_{reg}, CXCR5⁺PD1⁺) populations in MG1⁻ or MG1⁺ compared to MG2⁺ response groups (**Extended Data Fig. 5B**). Notably, phenotypically naive (CD45RA⁺CCR7⁺) CD4⁺ and CD8⁺ T cell frequencies were reduced in MG1⁻ compared to MG1⁺ and MG2⁺ and were negatively correlated with age (**Extended Data Fig. 5B&C**). Phenotypically naive T cells include recent thymic emigrants (RTE, CD95⁻CD38⁺⁺)²², mature naive (CD95⁻CD38^{lo}) and stem cell memory (T_{scm}, CD95⁺) (**Extended Data Fig. 5D**). CD4⁺ and CD8⁺ mature naive and RTE were significantly reduced as a proportion of CD4⁺ or CD8⁺ populations in MG1⁻ compared with MG1⁺ or MG2⁺, but T_{scm} were not (**Extended Data Fig. 5D**), however RTE frequencies were not reduced as a proportion of CD95⁻ CD4⁺/CD8⁺ T cells (**Extended Data Fig. 5E**), suggesting vaccine non-responsiveness was not directly linked to reduced current thymic output or stem-like memory capacity but rather a decreased overall naive T cell pool²³.

In assessing the functional capacity of baseline T cells, individuals in MG1⁻ and MG1⁺ additionally had lower frequencies of cytokine producing cells (IFNG, IL2, TNF, IL-17A or IL-4) in EM and EMRA T cell populations in response to CD3 stimulation compared with MG2⁺ (**Extended Data Fig. 5F**). Taken together these data suggest that the pool of potential antigen-responsive naive T cells was reduced pre-vaccination in individuals with impaired IFN γ -S responses to vaccine. Additionally, these individuals had T cell populations with reduced overall capacity to respond to stimulus. These features are largely consistent with the phenotype of T cells observed in advanced human ageing^{22, 24}.

Distinct effector CD8⁺ T cell populations and clonal expansion induced by vaccination across response groups

To further characterise T cell dysfunction, we performed scRNA-seq on activation induced marker (AIM-seq²⁵) expressing T cells after SARS-CoV-2 spike peptide stimulation (S-responsive T cells) at V3+21d (**Fig. 3C & Extended Data Fig. 6A**) (assessing the same 77 donors with antigen-specific B cell and background CD45⁺ scRNA-seq analyses). We supplemented the dataset with additional S-responsive AIM⁺ T cells from healthy mRNA/LNP

vaccinees²⁵. A total of 134,378 AIM⁺ T cells passed QC, comprising CD4⁺ (84%), CD8⁺ and unconventional T cells (MAIT, V δ 1⁺, V δ 2⁺ and V δ 3⁺ $\gamma\delta$ T) (16%) (**Extended Data Fig. 6B**). V3+21d AIM⁺ T cell frequencies positively correlated ($\rho = 0.48$) with V3+21d IFN γ -S, but there was no significant difference in AIM⁺ T cell frequency between MG1⁻, MG1⁺ or MG2⁺ (**Extended Data Fig. 6C&D**).

Clustering of background and S-responsive conventional CD8⁺ T cells identified 12 distinct clusters, including four with <5% S-responsive cells, four with >95% S-responsive cells and four with mixed S-responsive/background populations (**Fig. 3D-F**). Cluster 4 and 10 contained cytokine producing effector cells, clusters 6, 7, 8 and 9 contained cytotoxic, non-cytokine producing cells, cluster 12 was highly activated but not conventionally functional, cluster 2 had a central memory/stem cell like phenotype and cluster 1, 3, 5 and 11 were phenotypically naive (**Fig. 3E**).

Based on the reduced naive CD8⁺ T cell frequencies of individuals in MG1⁻ (**Extended Data Fig. 5B&C**), we hypothesised that vaccine-responsive clones would be less diverse and/or expanded in individuals in this group. Consistent with this, individuals in SI groups enriched for IFN γ non-responsiveness (MG1⁻ or MG1⁺) had reduced TCR diversity compared to MG2⁺ (**Extended Data Fig. 6E**). SARS-CoV-2 spike-specific public clones and antigen-enriched metaclasses were mostly identified in clusters 4 and 10 (**Extended Data Fig. 6F&G**) and both were more frequent in S-responsive CD8⁺ T cells from individuals in MG2⁺ (**Fig. 3G&H**). The number of antigen-enriched metaclasses positively correlated with V3+21d IFN γ -S responses (**Extended Data Fig. 6H**). One highly enriched metaclass contained public clones specific for a SARS-CoV-2 spike epitope (amino acids 265–277; YLQPPRTFL) (**Extended Data Fig. 6I&J**).

We next compared differences in cellular frequency/functionality that may explain this impairment of clonal expansion. Neighbourhood abundance analysis demonstrated that CD8⁺ clusters 4 and 10 were significantly reduced in frequency in the MG1⁻ and MG1⁺ compared to MG2⁺ (**Fig. 3H**). Notably, clusters 4 and 10 were almost entirely depleted in MG1⁻, whereas only cluster 10 was depleted in MG1⁺ (**Fig. 3I**), indicating that cluster 4 is key a subset of IFN γ producing T cells after vaccination. Comparison of functional/phenotypic genesets demonstrated enrichment of E2F1 cell cycling, oxidative phosphorylation and Myc signalling pathways in cluster 10, whereas cluster 4 was enriched for hypoxia and polyfunctional cytokine production pathways (**Fig. 3K**). Cluster 10 was also enriched for surface protein expression of

CD27 and CD62L compared to cluster 4 (**Extended Data Fig. 6K**). These functional and phenotypic markers suggested that cluster 10 was of a central memory CD8⁺ T cell phenotype, whereas cluster 4 represented a terminally differentiated memory CD8⁺ T cell cluster²⁶. As identified in vaccine-induced antigen-specific B cells (**Fig. 2L**), overall Myc signalling was significantly reduced in S-responsive CD8⁺ effector cells from individuals in MG1 compared to MG2 and was lowest in individuals with undetectable V3+21d IFN γ -S (MG1-) (**Fig. 3L**).

CNIs are known to block Myc signalling²⁷, and as expected, Myc signalling was particularly reduced in LT/RT recipients on CNIs (**Fig. 3M**). Interestingly however, the expression of Myc pathway genes and other pathways that were upregulated in cluster 10 in S-responsive CD8⁺ T cells was significantly correlated with V3+21d IFN γ -S in both individuals on CNI and those not on CNIs (**Fig. 3N**). This indicates that recruitment of a central memory CD8⁺ T cell response capable of Myc signalling is critical to generate a functional response to vaccination, and that this is impaired in individuals without IFN γ -S responses to vaccination independent of specific clinical intervention.

Reduced activation and differentiation of IFN γ producing CD4⁺ T cells is associated with altered T cell signalling and metabolism

We next investigated the S-responsive CD4⁺ T cell compartment. 158,395 S-responsive and background CD4⁺ T cells were analysed, revealing nine CD4⁺ T cell populations that were annotated using canonical markers of T helper and memory cell subsets (**Fig. 4A-C**). The recovered populations were consistent with S-responsive CD4⁺ T cells identified after COVID-19 vaccination of healthy individuals²⁵. Background CD4⁺ T cells were detected within naive, central memory or regulatory clusters and 100% of cells in the cT_{FH} and T_H1/T_H17 populations were S-responsive (**Fig. 4B&C, Extended Data Fig. 7A**).

Associating the S-responsive CD4⁺ T cell phenotype with the functional (IFN γ -S) response demonstrated that T_{em} and T_H1/T_H17 populations were positively correlated with V3+21d IFN γ -S, whilst naive and T_{cm} were inversely correlated (**Fig. 4D**). Analysis of clonal overlap between clusters (**Fig. 4E**) and phenotypic analysis of expanded clones demonstrated that the majority (88% of expanded clones containing cells from greater than three clusters) of clonal sharing occurred across the differentiation trajectory including naive, T_{cm}, T_{em}, cytotoxic and T_H1/T_H17 CD4⁺ T cells (**Fig. 4F, Extended Data Fig. 7B**). To view the continuum of functionality and differentiation across this trajectory, we performed pseudotime analysis (**Fig. 4G**). Several genesets changed in expression linearly (e.g. mTor signalling, cytokine signalling and some

metabolic pathways) or non-linearly (e.g. CD40LG and oxidative phosphorylation signalling) with respect to increasing pseudotime (**Extended Data Fig. 7C**).

Comparison of T cell response groups across pseudotime highlighted clear differences in both CD4⁺ T cell composition and functionality (**Fig. 4H-J**). S-responsive cells from MG1⁻ were highly skewed toward an undifferentiated state (**Fig. 4H**) and, compared to MG1⁺ or MG2⁺, had reduced glycolytic signalling across the entire pseudotime trajectory and functional impairment within activated, differentiated S-responsive CD4⁺ T cells (**Fig. 4J**). Activated, differentiated CD4⁺ T cells from individuals in MG1⁺ appeared intermediate to cells from MG1⁻ and MG2⁺ (**Fig. 4J**).

The TCR repertoire of S-responsive CD4⁺ T cells from individuals in MG1⁻ was of significantly lower average diversity compared to that from individuals in MG1⁺ and/or MG2⁺ groups, and this was also observed within T_{em} and T_{H1}/T_{H17} populations alone (**Extended Data Fig. 7D**). Across the transcriptionally derived pseudotime (**Fig. 4G**), expanded clones from MG1⁻ had reduced mean pseudotime values (**Fig. 4K**) and spanned a lower average pseudotime distance, compared to expanded clones from MG1⁺ or MG2⁺ groups (**Fig. 4L**). This indicates that expanded clones from individuals with impaired IFN γ T cell responses are less differentiated and in a lower state of flux compared to clones with measurable IFN γ responses. In sum, CD4⁺ T cells from patients with impaired IFN γ -S responses had impaired capacity for effector differentiation and altered clonal dynamics.

Skewed functionality of vaccine-induced circulating T_{FH} cells is associated with impaired antibody production in response to vaccination

cT_{FH} cells are associated with vaccine-induced antibody responses and are impaired in certain SIs^{28, 29}. To investigate the role of cT_{FH} in the vaccine-induced antibody response in SIs, we further subclustered the CD4_{Tfh} population. This identified six distinct cT_{FH} subclusters (**Fig. 5A**). Tfh_1 and Tfh_2 appeared phenotypically similar based on expression of canonical cT_{FH} defining genes (**Extended Data Fig. 8A**). Tfh_4 had high expression of *IFNG* and expression of *MKI67*, consistent with a proliferating type 1 T_{FH} cell. The Tfh_3 cluster had overlap with T_{H1}/T_{H17} and T_{H2} populations (**Extended Data Fig. 8A-C**), consistent with a naive-like phenotype. Within cT_{FH} cells, there was substantial clonal sharing between cT_{FH} subclusters 1-4 and the T_{cm} cluster, indicating that these clusters separate from other S-responsive clones early in the differentiation pathway and do not represent distinct terminal differentiation states (**Extended Data Fig. 8D**). Tfh_5 represented a T_{FR} population (**Extended Data Fig. 8A-C**).

To determine which cT_{FH} populations may play a role in interacting with GC B cells to induce an optimal vaccine-induced antibody response, we assessed the predicted interactions between S-responsive cT_{FH} subclusters and the S1-specific B_Mem4 population (**Fig. 2**). Several expected T_{FH}-B cell interactions, such as CD40L-CD40, were predicted between B_Mem4 and all T_{FH} subpopulations (**Extended Data Fig. 8E**), however some interactions were identified only in a subset of the cT_{fh} subpopulations (**Fig. 5B**). There were notable differences between subsets in predicted cytokine/chemokine interactions, whereby only T_{FH} clusters 1, 2, 4 and 6 had predicted IFNG-IFNGR1/2 interactions and 1, 4 and 6 had CXCL13-CXCR3/5 interactions (**Fig. 5B**). Notably, clusters 2 and 4 were uniquely predicted to have IL-21-IL21R and IL2-IL2R interactions.

Given the clonal overlap and high numbers of predicted T_{FH}-B cell interactions, we further annotated cells in clusters 1, 2 and 4 by geneset analysis. Tfh_1 was highly enriched for expression of the T-CellAnnoTator (TCAT) derived Tph and Tfh-2 genesets (**Fig. 5C**), which are upregulated in cancer and inflammation and associated with high expression of *CXCL13*³⁰, also observed in our study (**Fig. 5D**). *CXCR4* and *TOX2*, both related to T_{FH} functionality, were also highly upregulated in Tfh_1 (**Fig. 5D**). There were clear differences in metabolic signalling across the subsets, Tfh_1 was enriched for genesets related to lipid metabolism and cholesterol homeostasis (H_BILE_ACID_METABOLISM and H_HEME_METABOLISM), whereas Tfh_2 and Tfh_4 were enriched for genesets associated with nicotinate/nicotinamide metabolism, pyrimidine metabolism and oxidative phosphorylation (**Fig. 5C**). This suggests that Tfh_1 were more metabolically quiescent compared to Tfh_2 and Tfh_4. Consistent with this, Tfh_2 highly expressed Myc related signalling pathways and *IL2* (**Fig. 5C&D**), associated with early T cell activation; and Tfh_4 expressed cell cycling genesets suggesting ongoing proliferation.

While the overall frequency of S-responsive cT_{FH}'s was not reduced in transplant recipients compared to other SI groups or HCs (**Extended Data Fig. 8F**), cT_{FH} cluster 1 was significantly enriched in frequency in LT/RT (**Fig. 5E**). Strikingly, the composition of S-responsive cT_{FH} composition was strongly associated with V3+21d Ig-RBD in LT/RT but not the rest of the cohort: Tfh_1 frequency was negatively associated, and Tfh_2 and Tfh_4 frequency were each positively associated with V3+21d Ig-RBD (**Fig. 5F**). This suggests that the ability to induce functional cT_{FH} is strongly associated with antibody responsiveness in LT/RT recipients.

T_{FH} cells can also help to promote CD8⁺ T cell responses through *IL2* and *IL21* production, and type 1 cT_{FH} can produce IFN γ ³¹, we therefore investigated the association of T_{FH} subsets with vaccine-induced IFN γ -S. Analysis of interactions between cT_{FH} subsets and S-responsive effector CD8⁺ subsets demonstrated that only the metabolically active cT_{FH} subsets Tfh_1 and Tfh_4 were predicted to interact with CD8⁺ T cells through IL-21-IL21R and IL2-IL2R (**Fig. 5G**). Tfh_4 was significantly reduced in frequency amongst S-responsive cT_{FH} in individuals in MG1 compared to MG2, and its frequency was positively associated with V3+21d IFN γ -S (**Fig. 5H&I**). Activated cT_{FH} are therefore indicative of both antibody and IFN γ T cell responses to vaccination.

Discussion

Although it is widely recognised that patients with secondary immunodeficiencies have an attenuated T and B cell response to COVID-19 vaccines, mechanistic insight into the reasons for this remain poorly understood. Here we undertook a multi-omic and functional immune analysis to identify key characteristics, pre- and post-vaccination, that relate to vaccine responsiveness in ten clinically distinct SIs. Comparison across these disease groups allowed us to demonstrate both common and unique mechanisms of immune impairment of B and T cell responses leading to vaccine failure.

For antibody responses, there was not a unified mechanism that explained vaccine responsiveness. Unlike aCD20 recipients, where B cell frequency limited antibody responses³² – specific deficits in the generation of class-switched, memory antigen-specific B cells were observed in RT/LT recipients and associated with reduced antibody response. Systemic inflammation pre-vaccination, the generation of dysfunctional vaccine-specific cT_{FH} and cell-intrinsic perturbations in critical regulatory circuits (e.g. Myc) and metabolic pathways likely drive this phenotype.

cT_{FH} from LT/RT recipients with poor vaccine-induced antibody responses had high expression of *CXCL13* and *CXCR4*. CXCL13⁺ T_{FH} have been observed in IL-2 depleted tumour microenvironments, suggesting that this population may emerge secondary to IL-2 signalling blockade by calcineurin inhibitors³³. T_{FH} normally localise to the GC light zone, while CXCR4 directs blasting GC B cells into the GC dark zone³⁴. Therefore, expression of CXCR4 on T_{FH} would aberrantly position them within the GC dark zone and thereby disrupt GC organisation and impair antibody responses, as has been reported for CXCR4⁺ T_{FH} in aged mice³⁵. One prior study detected reduced T_{FH} frequencies in lymph nodes of RT patients but

was unable to resolve their phenotype due to low cell frequencies³⁶. Our data suggest that both numeric and functional defects in T_{FH} cell contribute to impaired antibody responses.

In evaluating mechanisms linked to failure to generate a robust T cell response, we identified underpinning mechanisms that were shared across multiple SIs (cirrhosis, SOT, and NHL), suggesting convergence on a common axis of T cell dysfunction independent of specific immunosuppressive therapies. Across both CD4⁺ and CD8⁺ T cell compartments, individuals with impaired vaccine-induced IFN γ T cell responses had reduced naive T cell pool size, TCR repertoire complexity and effector function. This was consistent with a limiting precursor model, where contraction of the underlying naïve T cell pool reduces the number of available clones capable of expansion and differentiation following vaccination. This phenotype shares features of age-associated impairment of vaccine responses^{22, 24, 37}.

Common across impaired B and T cell responses to vaccination were perturbed Myc signalling and metabolic alterations. Myc is blocked by CNIs, however we observed an association between Myc signalling and antibody/T cell responses regardless of prescription of CNIs, suggesting Myc antagonism may occur by other means. Evidence that Myc signalling decreases with increasing age supports this hypothesis^{38, 39, 40}. For B cells, as Myc signalling is required for cell activation and GC formation³⁹ reduced signalling may impair the proliferation required for positive selection and generation of class-switch recombination of the IgG⁺ memory B population associated with antibody responses. For T cells, including T_{FH} cells^{41, 42}, Myc signalling is also required for differentiation and functionality.

Altered cellular metabolism was also associated with impaired antibody and T cell responses. In LT/RT, reduced OxPhos in B and CD8⁺ T cells was associated with impaired antibody and IFN γ T cell responses respectively. In CD4⁺ T cells, reduced glycolysis was associated with impaired IFN γ T cell responses, and a shift toward fatty acid metabolism was observed. Given the importance of OxPhos and glycolysis in supporting T and B cell activation and memory^{43, 44}, impairment of these pathways is a clear driver of reduced vaccine-induced responses across SIs.

Temporary interruption/dose reduction of immunosuppressive agents, where possible, may alleviate the blockade of these key pathways and improve vaccine immunogenicity⁴⁵. The mechanism of impaired T cell responses were unified across SI groups, even those not on immunosuppressive therapies. Furthermore, we noted striking similarities between the

mechanisms identified in SIs and those previously observed in advanced age. Therefore, efforts to modify vaccine formulations through use of molecular adjuvants (e.g. co-administered mRNA/LNPs encoding anti-CD40 monoclonal antibodies or IL-21 protein) or other immune boosting approaches that overcome the deficits described here may be broadly effective at improving vaccine responsiveness. Reverse vaccinology efforts can leverage these insights to improve vaccines developed for at-risk populations.

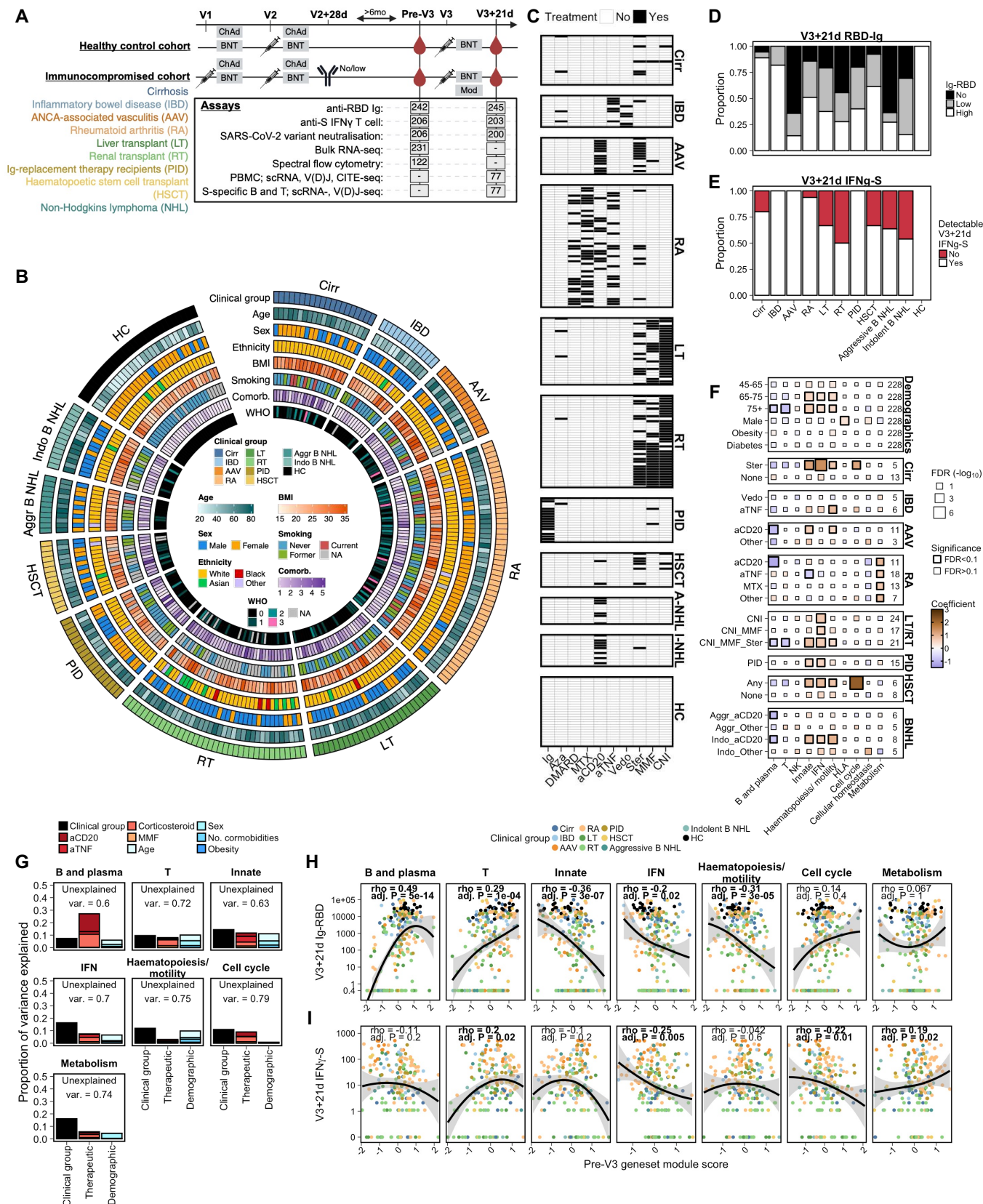


Figure 1) Immune dysfunction and impairment of vaccine-induced antibody and T cell response in individuals with secondary immunodeficiencies

509 **A)** Study overview, sampling timepoints and immune assays. V1-V3 = Vaccine doses 1-3,
510 no/low = no/low V2+28d anti-SARS-CoV-2 spike binding antibody titres (Ig-RBD), numbers in
511 boxes = number of individuals. **B)** Study demographics, WHO = World Health Organisation
512 Performance Score. **C)** Immunosuppressive therapies used across the study. CNI =
513 calcineurin inhibitor, MMF = mycophenolate mofetil, Ster = corticosteroid, Vedo =
514 vedolizumab, aTNF = anti-TNF, aCD20 = anti-CD20, MTX = methotrexate, DMARD = disease
515 modifying anti-rheumatic drug, Aza = azathioprine, Ig = immunoglobulin replacement. **D)**
516 Cohort stratification by no (<0.8AU/mL), low (0.8-1000AU/mL) and high (>1000AU/mL)
517 V3+21d Ig-RBD. **E)** Cohort stratification by V3+21d IFN γ -S response (>1 SFC/10⁶ cells). **F)**
518 Linear models of mean expression of consensus clusters, each group is compared to healthy
519 controls. **G)** Proportion of mean module expression variance explained by clinical group,
520 immunosuppressive therapeutic or demographic variables in linear models for each geneset
521 module. **H)** Correlation of pre-V3 mean consensus cluster expression (geneset module score)
522 with V3+21d anti-SARS-CoV-2 receptor binding domain binding antibody titres (Ig-RBD) (**I**) or
523 V3+21d anti-SARS-CoV-2 spike IFN γ T cell magnitude (**J**).

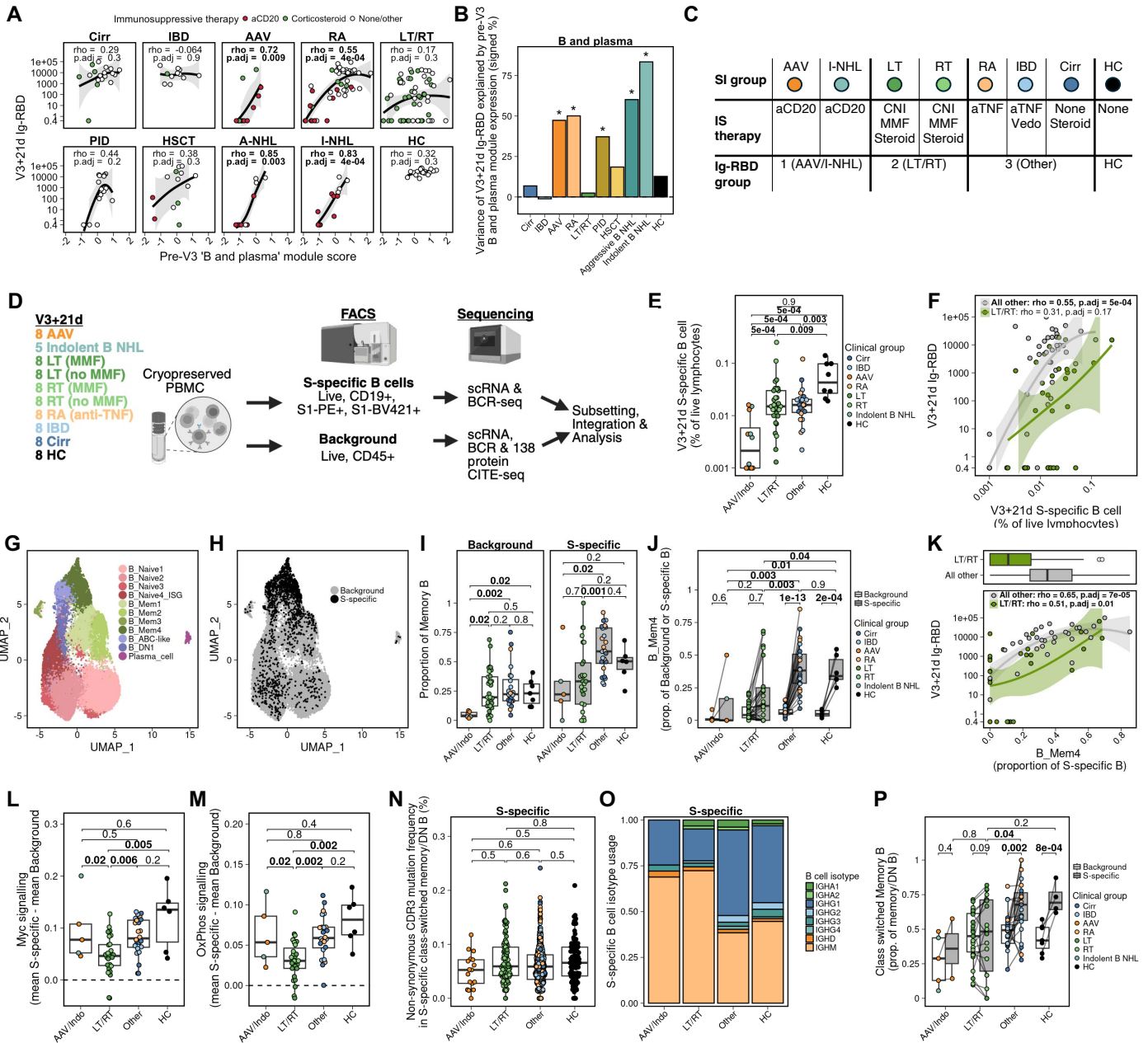


Figure 2) Cytometric and transcriptomic analysis of SARS-CoV-2 spike specific B cells across SI groups

A) Correlation of pre-V3 B and plasma module expression with V3+21d Ig-RBD stratified by clinical group. **B)** Proportion of V3+21d Ig-RBD explained by pre-V3 B and plasma module expression stratified by clinical group. Values are adjusted based on the direction of the model coefficient. * denotes a coefficient that is significantly non-zero. **C)** Schematic of B cell analysis and cohort grouping. **D)** Schematic of experiment for panels E-P. ADT = antibody derived tag. **E&F)** V3+21d S1-specific B cell frequency across B cell comparison groups (E) or correlated with V3+21d Ig-RBD (F). **G&H)** UMAP depiction of integrated V3+21d antigen-specific and

534 background B cells coloured by Leiden clusters (**G**) or experimental condition (**H**). **I**) Proportion
535 of cells in each experimental condition that are within a memory B cluster. **J**) Proportion of
536 B_Mem4 cluster within background or S1-specific B cells. **K**) Correlation of B_Mem4 cluster
537 proportion within S1-specific B cells with V3+21d Ig-RBD. **L&M**) Difference in mean per donor
538 expression of HM_MYC_TARGETS_V1 (**L**) or HM_OXIDATIVE_PHOSPHORYLATION (**M**)
539 genesets in S1-specific compared to background B cells. **N**) Mean per person non-
540 synonymous CDR3b mutation frequency in S-specific switched memory B cells. **O**) B cell
541 receptor isotype usage amongst S-specific B cells across different groups. **P**) Proportion of
542 class-switched (*IGHG1-4*, *IGHA1-2*) memory B cells within memory B or DN B cells.
543

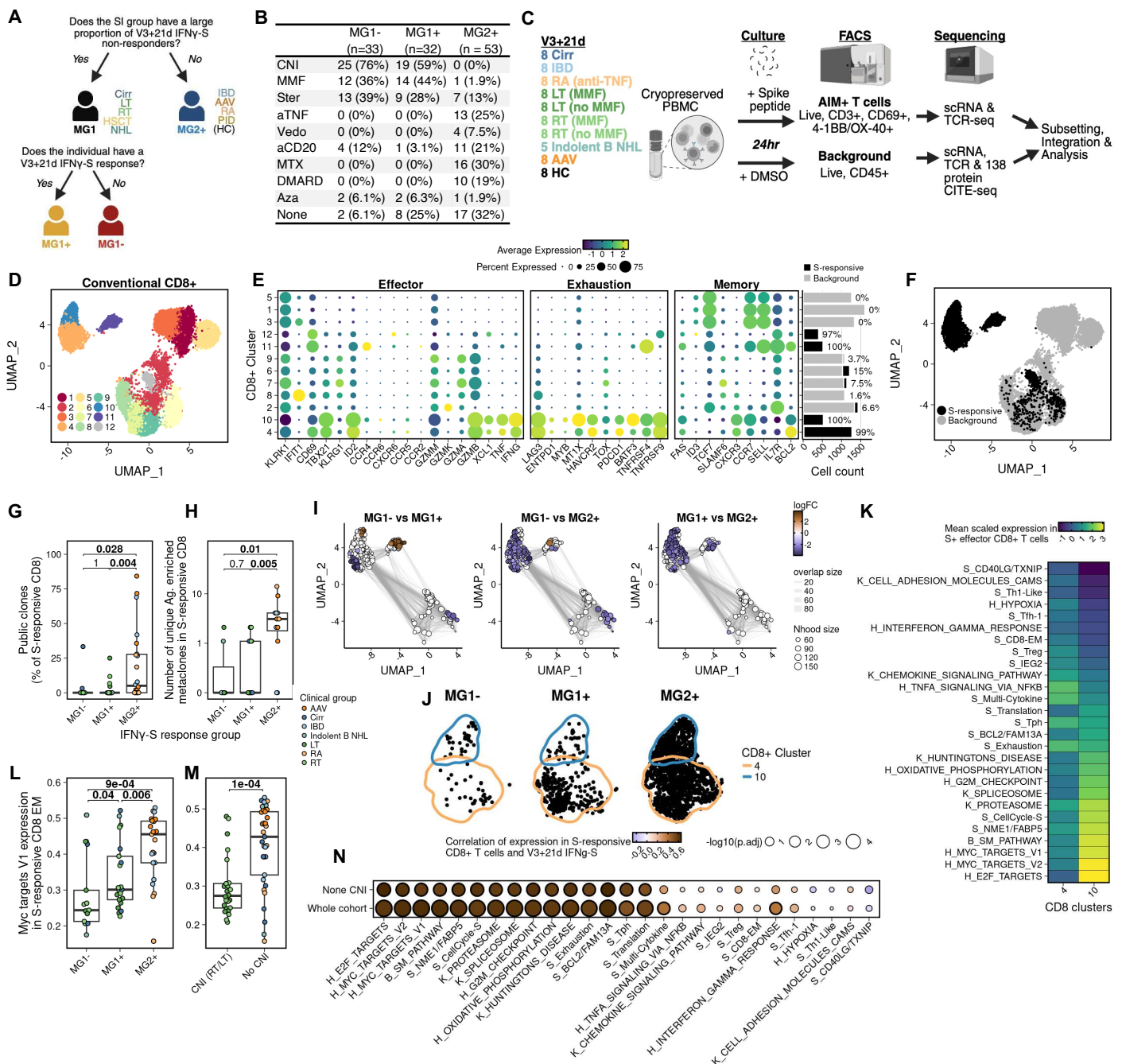


Figure 3) Transcriptomic and T cell receptor repertoire analysis of SARS-CoV-2 spike responsive CD8+ T cells

A) Schematic of cohort grouping. **B)** Immunosuppressive therapy usage in each cohort group. **C)** Schematic of experimental procedure used in Figure 3-5. **D)** UMAP of background (live CD45+) and S-responsive (activation induced marker positive) CD8+ T cell single-cell RNA-sequencing data coloured by Leiden clusters. **E)** Expression of selected mRNA markers and total cell count per cluster and cluster proportion by experimental condition. **F)** As in D but coloured by experimental condition. **G)** Per person proportion of public clones within S-

responsive CD8⁺ T cells. **H)** Number of unique metaclones per person amongst S-responsive CD8⁺ T cells. **I)** MiloR analysis of cell neighbourhood abundance within S-responsive CD8⁺ T cells across IFN γ -S response groups. **J)** Cell abundance of clusters 4 and 10 stratified by IFN γ response groups. **K)** Mean scaled expression of genesets within S-responsive CD8⁺ effector T cells. Only the top differentially expressed genesets between clusters 4 and 10 are shown. **L&M)** Expression of HM_MYC_TARGETS_V1 geneset within pseudobulk total S-responsive CD8⁺ effector T cells, stratified by IFN γ -S response groups (**L**) or by usage of calcineurin inhibitors (CNI) (**M**). **N)** Correlation of the expression of genesets within pseudobulk total S-responsive CD8⁺ effector T cells and V3+21d IFN γ -S in the whole cohort and with patients receiving CNI's removed.

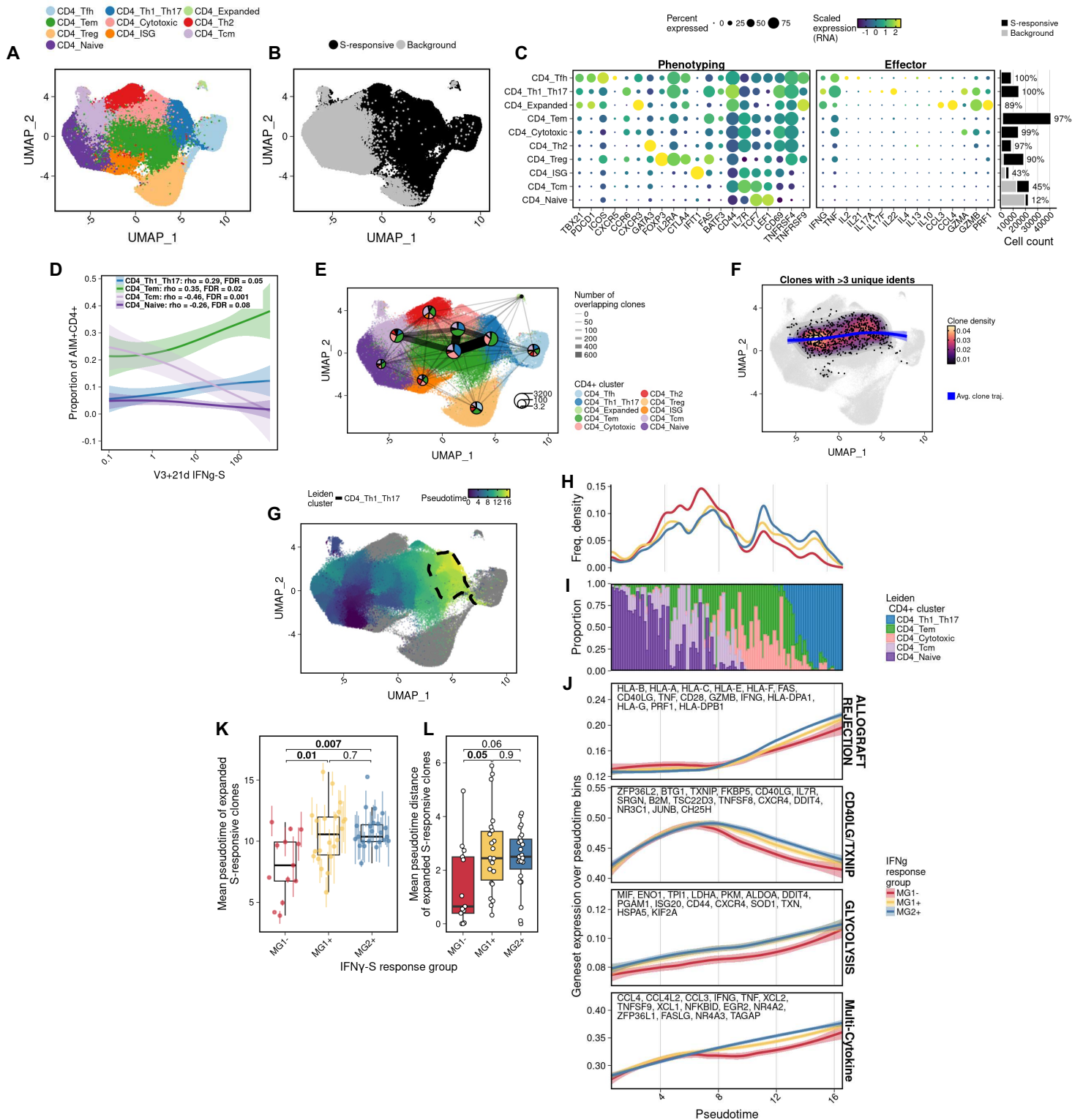
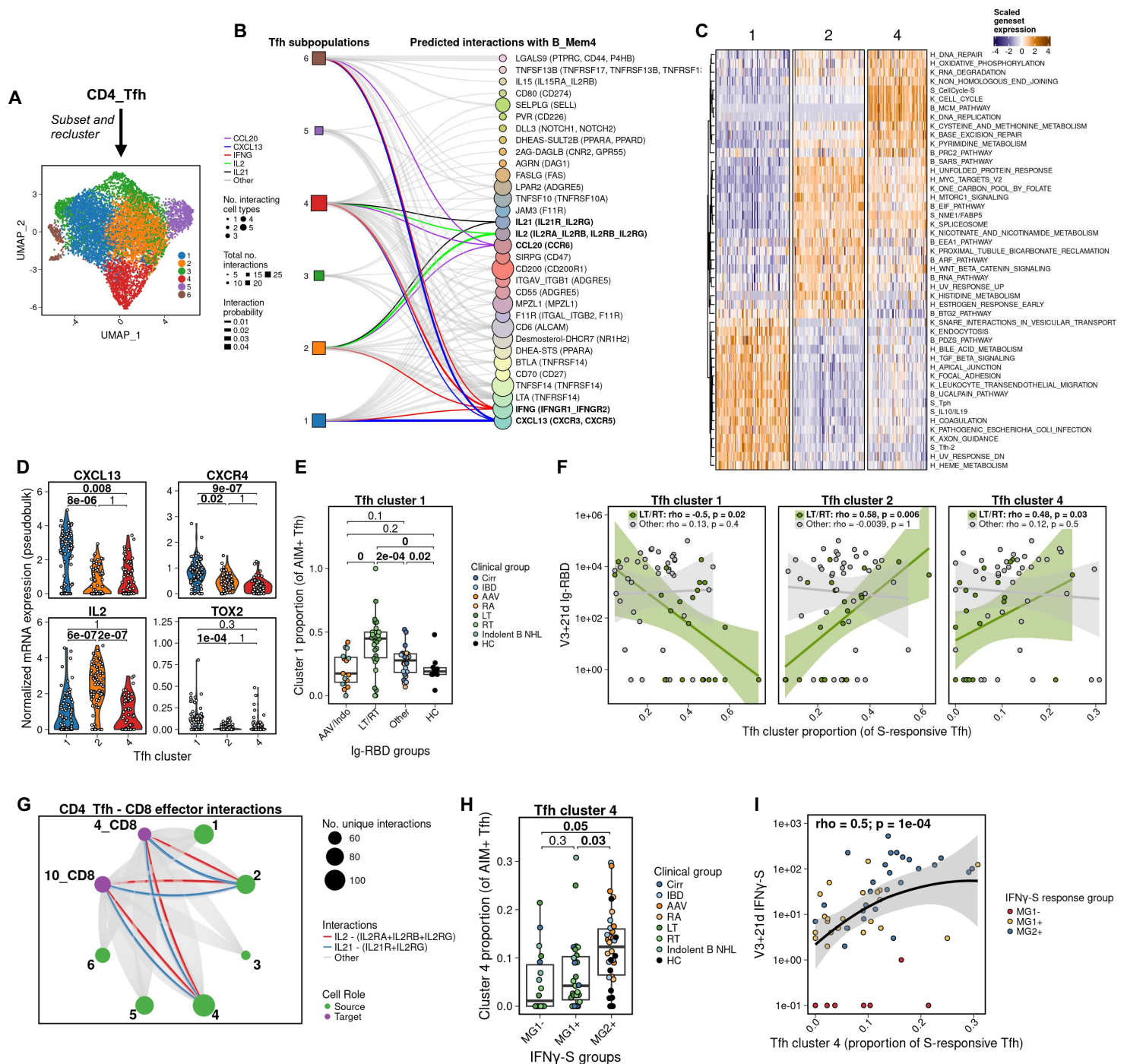


Figure 4) Transcriptomic and T cell receptor repertoire analysis of SARS-CoV-2 spike responsive CD4+ T cells

A&B) UMAP of background (live CD45+) and S-responsive (activation induced marker positive) CD4+ T cell single-cell RNA-sequencing data coloured by Leiden clusters (A) or experimental condition (B). **C)** Scaled expression of selected mRNA markers used for

570 annotation and total cell count and experimental condition proportion (right). **D)** Correlation of
571 S-responsive cell type proportions with V3+21d anti-SARS-CoV-2 IFN γ T cell magnitudes
572 (IFN γ -S). **E)** Clonal sharing between clusters. The pie chart overlaying each Leiden cluster
573 represents the number of expanded clones per cluster and are coloured according to the
574 proportion of expanded clones shared with other clusters. Network weight (transparency and
575 line width) depict the number of overlapping clones per cluster pair. **F)** Density of cells within
576 clones with >3 unique constituent clusters. Line represents loess curve based on UMAP
577 coordinates of selected cells. **G)** Monocle3 pseudotime trajectory of naive, T_{cm}, T_{em}, cytotoxic
578 and Th1_Th17 cells (**Methods**). **H-J)** Cell frequency density (**H**), cluster frequency (**I**) and
579 expression of selected genesets (**J**) over pseudotime. **K)** Point represents the mean
580 pseudotime of all expanded S-responsive clones per individual. Bounds represent the mean
581 of the maximum and minimum pseudotime of each expanded clone in an individual. **L)** Per
582 person mean of pseudotime distance within a clone, as per bounds in panel K.



583

584 **Figure 5) Sub-analysis of a CXCR4⁺ CXCL13⁺ cT_{fh} subset associated with vaccine-**
 585 **induced antibody responses**

586 **A)** UMAP of T_{fh} cells coloured by T_{fh} subclusters. **B)** Predicted interactions between T_{fh}
 587 subclusters and S-specific B_Mem4 cells using CellChat. **C)** Geneset expression in
 588 pseudobulk mRNA per individual x T_{fh} cluster, only the top differentially expressed genesets
 589 are displayed. **D)** Mean normalized expression of selected gene expression programmes or
 590 genes in individuals and clusters. **E)** Proportion of T_{fh} cluster 1 amongst all S-responsive T_{fh}.

591 P values are from Bayesian modelling of cell type composition, controlling for demographic
592 covariates. **F)** Correlation of T_{FH} subpopulations with V3+21d Ig-RBD **G)** Predicted IL2-IL2RA
593 and IL21-IL21R interactions between T_{FH} subclusters and S-specific CD8 effector populations
594 using CellChat. **H)** Proportion of Tfh cluster 4 amongst S-responsive T_{FH}. P values are from
595 Bayesian modelling of cell type composition, controlling for demographic covariates. **I)**
596 Correlation of T_{FH} subpopulation 4 with V3+21d IFN γ -S.
597
598

Online Methods

Data collection:

Study design and sample collection

All individuals with SIs were recruited in the OCTAVE DUO study, an open label multicentre, randomised controlled trial conducted in the UK¹⁴. The study received ethical approval from the London and Fulham Research Ethics Committee (REC 302634). The full study protocol, inclusion and exclusion criteria are available at¹⁴. Briefly, individuals >18 years of age who were immunocompromised and had no or low anti-SARS-CoV-2 spike binding antibody titres 28 days after two doses of COVID-19 vaccine were randomised to receive one of BNT162b2 or mRNA-1273 as a third vaccine dose. Participants were sampled immediately before and 21 days after third vaccine. Samples from a minimum of 14 individuals per select SI groups were selected randomly, with only individuals that were SARS-CoV-2 infection naive (by self-reporting and anti-nucleocapsid antibody titres) at time of third vaccination included for further analysis in this study. Healthy control individuals were recruited in Oxford through the PITCH cohort¹. PITCH is a sub-study of the SIREN study (trial ID: 252 ISRCTN11041050), which was approved by the Berkshire REC, Health Research 250 Authority (IRAS ID: 284460, REC ref.: 20/SC/0230). In Oxford, PITCH participants were recruited under the GI Biobank Study 16/YH/0247, approved by the research ethics committee (REC) at Yorkshire and the Humber - Sheffield Research Ethics Committee on 29 July 2016, which has been amended for this purpose on 8 June 2020. Both PITCH and OCTAVE DUO studies were performed compliance with all relevant ethical regulations for work with human participants and according to the principles of the Declaration of Helsinki (2008) and the Good Clinical Practice guidelines. Written informed consent was obtained from all participants enrolled in the study. PID and HSCT groups were not included in scRNA-seq or flow cytometry datasets due to lack of sample availability.

Serological and IFN γ T cell assays

Serological and IFN γ T cell assays were performed as in¹⁴. Briefly, the Elecsys® Anti-SARS-CoV-2 total Ig (Roche) was used to assess total serum Ig binding to the receptor binding domain (RBD) of SARS-CoV-2 spike and SARS-CoV-2 nucleocapsid proteins. Assays were performed as per manufacturer instructions. The manufacturer defined negative cutoff of 0.8AU/mL was used.

Live neutralisation of SARS-CoV-2 wild-type and Omicron BA.1 strains was performed as previously described⁴⁶. Samples that fell below or above the limit of quantification (LoQ) were

assigned a representative IC₅₀: undetectable = IC₅₀ of 5; detectable but below LoQ = IC₅₀ of 10; above LoQ = IC₅₀ of 4000.

IFN γ T cell responses were measured using the T-Spot assay by Oxford Immunotec. Responses were measured to separate peptide pools covering S1 and S2 domains of SARS-CoV-2 spike and outputs combined additively. Outputs are presented as negative-subtracted spot forming units (SFU) per 1 million PBMCs.

Bulk RNA-sequencing of whole-blood

Whole blood from donors was collected in Tempus™ tubes (ThermoFisher). Samples were thawed at RT in batches and RNA was extracted using Tempus™ Spin RNA Isolation Kit (ThermoFisher) as per manufacturer guidelines. All work surfaces were pre-treated with RNase AWAY (ThermoFisher) and DNAZap (ThermoFisher). Processed RNA was quantified and QC performed using 4200 TapeStation (Agilent) electrophoresis. RNA libraries were prepared and sequenced across two batches using ribosomal and haemoglobin gene depletion followed by poly-A enrichment library preparation protocols. Paired-end 150bp sequencing of prepared libraries was performed using NovaSeq6000 or NovaSeq X Plus sequencers. Samples were sequenced to a minimum of 20million reads per sample.

Spectral flow cytometry – immunophenotyping and intracellular cytokine staining

For immunophenotyping, 2x10⁶ cryopreserved PBMC were thawed and stained at RT in the dark with fluorophore-conjugated antibodies (antibodies and staining procedure presented in **Supp. D1E**), washed 2x in FACS buffer (PBS + 0.05% bovine serum albumin (BSA) + 1 mM EDTA, Sigma-Aldrich) and stored at 4°C prior to acquisition the same day.

For ICS, 96-well flat-bottom plates were prepared by pre-coating one well per donor with 100 μ L of 1 μ g/mL anti-CD3 monoclonal antibody (CD3-2; MabTech) and incubating for 1-2 hours at 37°C. After incubation, wells were washed 3x with PBS and the following stimulation conditions were prepared in 50 μ L R10 (RPMI 1640 (Sigma-Aldrich), 10% FCS, 1% Penicillin-Streptomycin (ThermoFisher)) and added to the plate: 1 μ g/mL DMSO (unstimulated control), 2 μ g/mL 18mer peptides with 11amino acid overlap covering entire ancestral SARS-CoV-2 spike, R10 only (added to anti-CD3 coated wells). 50 μ L of co-stimulation mix (**Supp. D1F**) and 100 μ L of 1x10⁷ PBMC per well was added to all wells and incubated for 2 hours at 37°C. 50 μ L of 1:1000 Brefeldin A and 1:1000 Monensin (BioLegend) in R10 were added to each well, pipette mixed, and samples were incubated for a further 16-18 hours at 37°C. After incubation,

the samples were washed 2x in FACS buffer and stained for surface markers (**Supp. D1F**) for 30 minutes at 4°C in the dark. Cells were then fixed and permeabilised for 30 minutes at 4°C using Cytofix/Cytoperm (BD Biosciences), washed 2x with Perm/Wash buffer (BD Biosciences) and stained for intracellular markers (**Supp. D1F**) for 30 minutes at 4°C in the dark. After staining, samples were washed 2x in Perm/Wash buffer, resuspended in FACS buffer and stored at 4°C in the dark until acquisition the same day.

Single stain reference controls were produced using PBMC or CompBeads (BD Biosciences) and were treated identically to sample conditions. Single stain reference control antibodies were matched with each experiment for batch, fluorophore and specificity. For B cell baits, monoclonal antibodies with the same fluorophore conjugate (BV421 or PE) targeting a marker on the target cell type (B cells) were used. Reference controls were stored and reused across multiple runs of the same flow cytometry panel, up until 3 months after their generation, or a new batch of staining antibody was used in the experiment, whichever occurred first. Samples were acquired on a five-laser Cytex Aurora using SpectroFlo 3.0.3.

AIM assay and FACS sorting for antigen-specific B and T cells and CD45+ cells.

1x vial of PBMC was thawed per participant and plated in U-bottom 96-well plates with 1×10^6 PBMC in R10 per well and cultured for 24-hours at 37°C. Cells were split to one negative control well (equimolar amount of DMSO) and the remaining PBMC split across multiple wells with overlapping peptide pools covering SARS-CoV-2 spike protein at a final concentration of 1ug/mL. 1×10^6 cells per experiment were incubated for 24 hours with 1:500 diluted leukocyte activation cocktail as a positive control for sorting (BioLegend). Plates were washed with FACS buffer and cells incubated with fluorophore-conjugated antibodies and fluorescently tagged streptavidin-conjugated S1 B cell tetramers (**Supp. D1G**) and one unique DNA-oligomer tagged hashing antibody (Biolegend Total-SeqC anti-human Hashtag antibody) per donor per experiment batch. Staining was performed in a final volume of 50µL for 30mins at 4°C in the dark. After staining, cells were washed three times with FACS buffer, resuspended in 100µl of FACS buffer, and transferred to a 1.5mL RNase-free Microfuge tube. SYTOX Green (ThermoFisher) was pre-diluted 1:60 in PBS + 0.04% BSA, then diluted 1:100 in each sample.

All fluorescence-activated cell sorting (FACS) was performed on a BD FACSAria III (BD Biosciences) using an 85-micron nozzle. Sorted cells were collected into sorting buffer (RPMI-1640 + 1% NEAA + 1% Na Pyruvate + 2.5% HEPES + 10% FBS, Sigma-Aldrich) prior to additional processing. An equal number per donor of live, CD45+ cells from each negative

control well was sorted into sorting buffer and pooled. Pooled live CD45⁺ cells were washed in FACS buffer and incubated at 4°C in the dark for 10 minutes with TruStain FcX (Biolegend) and an additional 30 minutes with TotalSeq-C Universal Cocktail (Biolegend). Cells were washed twice with FACS buffer, counted, and diluted to a final concentration of 1000 cells/μl in resuspension buffer (PBS + 0.04% BSA). All live CD3⁺CD69⁺ OX40⁺/4-1BB⁺ and all live CD19⁺S1-PE⁺/S1-BV421⁺ cells from spike peptide wells were sorted into resuspension buffer in a single conical and resuspended in 304μl resuspension buffer.

10X CITE, V(D)J and RNA-sequencing preparations

FACS sorted CD45⁺ cells and SARS-CoV-2 spike specific B and T cells were loaded separately across 8 channels (4 channels CD45⁺, 4 channels spike-specific B/T) of a 10X Genomics Chromium Next GEM Single Cell 5' high throughput (HT) chip and processed using Chromium X (10x Genomics) as per manufacturer guidelines. Approximately 40,000 cells per channel were loaded. Gene expression, cell surface protein (ADT) and V(D)J sequencing libraries were prepared as per manufacturer guidelines. The following library construction kits from 10x Genomics were used: Library Construction Kit (1000190), Chromium 5' Feature Barcode Kit (1000541), Chromium Single Cell TCR and BCR Amplification Kits (1000252, 1000253) and Dual Index Kits TT and TN Set A (1000215, 1000250). The prepared libraries were sequenced using a NovaSeq6000 PE150 (Illumina) sequencer.

Data analysis

Miscellaneous

Continuous data are presented as median, interquartile range and 90% bounds. Unless otherwise stated all differential expression and linear modelling is performed using age, sex and experimental batch as covariates.

Whole blood bulk RNA-sequencing

Data processing and quality control

RNA-sequencing reads were quality controlled and trimmed using the fastp package (v0.23.4)⁴⁷ with default settings. Reads were then quantified using Salmon (v1.8.0)⁴⁸ mapped to a GRCh38.86 reference genome with the seqBias option enabled. Samples from different experimental batches were integrated using the ComBat_seq function from the sva package (v3.5.0)⁴⁹. Adjusted counts were then normalised using median of ratios method (DESeq2, v1.40.2)⁵⁰ and log₂ transformation of counts per million (CPM) with a prior of 1 for downstream analysis.

739

740 *Geneset variation analysis and consensus clustering*

741 Single sample geneset enrichment analysis (ssGSEA) was implemented using the GSVA
742 (v1.48.3) package to quantify expression of genesets on a per sample basis ⁵¹. Genesets used
743 for this method include blood transcriptomic modules ⁵² or genesets from Human MSigDB
744 Hallmark databases. Consensus k-means clustering (ConsensusClusterPlus package) was
745 performed using the ssGSEA values for each geneset as input. Briefly, samples were
746 randomly subsampled to include 80% of the dataset and clustered into k_2 to k_{20} clusters with
747 1000 repeats for each value of k. A consensus matrix, defined as the proportion of clustering
748 runs in which two items are grouped together was calculated for each value of k and split
749 based on hierarchical clustering of the Euclidean distance of the consensus matrix. A K value
750 of 11 was selected based on the cumulative distribution function area under the curve value
751 and cluster stability. Clusters were annotated based on the identities of their constitutive
752 genesets and the mean geneset value for each cluster (geneset module) was calculated.
753 Generalised linear models or correlation analysis was performed using the mean geneset
754 module value.

755

756 Flow cytometry analysis

757 Data from conventional flow cytometry were analysed in FlowJo 11 (FlowJo, LLC). Data from
758 Aurora Cytex was unmixed using SpectroFlo 3.2.1 (Cytex) with auto-fluorescence subtraction.
759 Manual gating of unmixed spectral flow cytometry data was performed using FlowJo 11.

760

761 Single cell RNA-sequencing analysis

762 *Read mapping, quality control and demultiplexing*

763 FASTQ files were generated from BCL files using Illumina bcl2fastq. FASTQ files for all
764 modalities were mapped to the GRCh38-2020-A reference genome and a custom ADT marker
765 list using the Cell Ranger 7.0.0 multi pipeline for count (GEX), ADT and VDJ data, on a per
766 channel basis. The filtered_contig_annotations.csv file was filtered to retain only high-
767 confidence, full-length, productive contigs corresponding to TCR α or TCR β chains.

768

769 Genetic demultiplexing was performed using the outputs from CellRanger in souporecell
770 (v2.5.0)⁵³. The BAM files from each channel were run separately, and variants were called
771 from common GRCh38 single nucleotide polymorphisms (SNPs) sourced from 1000
772 Genomes. Hashtag oligonucleotides (HTO) were separated from ADT data and consensus
773 demultiplexing was performed using multiseq, dropletutils and bff_raw methods implemented

in the GenerateCellHashingCalls in the cellhashR package. Where 2/3 methods agreed on the call, that call was used. The HTO calls were aligned with the clusters derived from souporecell, with >90% agreement between the methods. Where HTO calls were discordant (no consensus), negative or doublets, cell IDs were called based on the aligned souporecell cluster. Paired $\alpha\beta$ TCR chains were aligned to single cells using scRepertoire and T cells with more than two TCR α or TCR β chains were removed as doublets.

Quality control was performed individually on each channel using scAutoQC⁵⁴ with default settings and number of read counts, number of unique genes, percent mitochondrial genes and percent ribosomal genes as quality control metrics. This method calculates sample-specific QC thresholds used to call quality control failures. Cells within each sample undergo dimensional reduction and clustering based on quality control metrics and clusters with over 50% poor quality cells are filtered out.

Integration, multimodal clustering and cell annotation of CD45⁺ cells

GEX and ADT data from each CD45⁺ channel was loaded into a single mudata object. GEX counts were normalized and log transformed per ScanPy defaults and the top 4000 most highly variable genes selected using the Seurat (v3) method. TotalVI was used to learn a joint probabilistic representation of GEX and ADT data to integrate experimental batches and form a joint dimensional reduction⁵⁵. Denoised ADT data (with unwanted protein background subtracted) was extracted for downstream analysis. Uniform manifold approximation and projection (UMAP) and Leiden clustering of the totalVI joint latent embedding was performed. CellTypist⁵⁶ was used to annotate CD45 cells using the Immune_All_Low model on GEX only and some annotation labels were edited for consistency.

Integration, clustering and cell annotation of SARS-CoV-2 spike-specific B and T cells

GEX data from each S-specific B and T channel was normalized and scaled using Seurat (v5.3.0). The top 4000 most highly variable genes were selected using the variable stabilizing transformation method and the top 50 principal components calculated. V(D)J genes were removed from variable features for the purposes of integration, UMAP reduction and clustering using quietVDJgenes (scRepertoire⁵⁷). Harmony integration with a theta value of two was performed to integrate experimental batches and Leiden clustering was performed on the top 30 harmony embeddings. For B cells, Leiden clusters containing spike-specific B cells were subset and integrated with CellTypist labelled B cells from the CD45⁺ dataset using Harmony. For CD8⁺ T cells, Leiden clusters containing CD8⁺, $\gamma\delta$ T and MAIT populations, based on GEX,

were subset. Due to the transcriptional overlap of these T cell subpopulations, additional sub-clustering and manual annotation was performed to identify canonical conventional CD8⁺ T cells, which were integrated with canonical conventional CD8⁺ T cells from the CD45⁺ dataset using Harmony. Canonical naive CD4⁺ and CD8⁺ CD3⁺ S-specific populations also had a high degree of transcriptional similarity, so sub-clustering of these populations was performed to isolate naive CD8⁺ T cells which were integrated with the CD8⁺ object. All other CD3⁺ (non-CD8⁺, MAIT, or $\gamma\delta$ T) cells from the S-specific dataset were integrated with the CD4⁺ cells from CD45⁺ dataset. S-specific CD4⁺ or CD8⁺ T cell clusters from 6 HC individuals vaccinated with two doses of mRNA/LNP vaccine in a long-dosing interval were bioinformatically isolated from ³⁶ and integrated with the CD4⁺/CD8⁺ datasets separately. Leiden clustering was performed on the Harmony integrated CD4⁺ or CD8⁺ datasets separately and manual annotation performed. After clustering, the three individuals in the indolent B NHL group that were misclassified as mRNA vaccinees were removed from subsequent analysis.

Differential abundance analysis

Differential abundance analysis was performed using `sccomp` (v2.1.20)⁵⁸, where models controlling for age, sex and batch were fit and contrasts between comparison groups for each cell type (Leiden cluster/annotation group) were made. Outliers were removed from each cell type using 2000 sampling iterations (as per `sccomp_remove_outliers`).

Differential expression and geneset enrichment analysis

Differential expression analysis was performed on single-cells using MAST, or on pseudobulk GEX values using EdgeR. MAST differential expression analysis was implemented using the Seurat R package, and comparisons were made between comparisons on a per cluster basis, including subject age, sex and batch as latent variables. Where sufficient cells per donor/cell type were available for comparisons, GEX values for each gene x donor x cell type were aggregated additively to calculate pseudobulk GEX values. Genes with fewer than 10 reads in any sample or fewer than 15 reads across all samples were filtered out. Samples with fewer than 50,000 reads across all genes in the pseudobulk sample were also filtered out. Trimmed mean of M-values (TMM) normalisation was performed on each sample, and quasi-likelihood F-tests were implemented between comparison groups for each cell type, accounting for subject age and sex as covariates. Geneset enrichment analysis was performed using the `fgsea` method implemented in the `ClusterProfiler` (v4.18.4)⁵⁹ package, by ranking the differential gene expression lists by descending average log₂ fold change. Genesets with an enrichment with FDR <0.05 were considered significant. For single-cell geneset enrichment

analysis, including of protein-derived genesets, genesets were quantified using UCell (v2.14.0)⁶⁰.

Pseudotime analysis of S-specific CD4⁺ T cells

Pseudotime analysis was performed on CD4_Naive, CD4_Tcm, CD4_Tem, CD4_Cytotoxic and CD4_Th1_Th17 Leiden clusters using Monocle3⁶¹. UMAP was recalculated using the Harmony embeddings of the CD4 subsets, and this UMAP was used to learn a trajectory through UMAP space. A pseudotime root that fell within the Naive CD4⁺ T cell cluster was selected given its biological relevance as the least differentiated T cell state. The pseudotime rank was then overlaid over the original UMAP projection for visualisation.

Cell interaction prediction

The CellChat (v2.1.2)⁶² package was used to predict interactions between T_{FH} subpopulations and B_mem4 cells. The CellChatDB.human interactions database was used. Over expressed genes and interactions were identified and then communication probability calculated using all cells and the truncated mean average gene expression method with a trim value of 0.15.

Single-cell BCR sequencing processing

BCR analysis was performed using the Immcantation BCR analysis pipeline. Filtered_contig.fasta and filtered_contig_annotations outputs generated by CellRanger vdj were used to assign V(D)J genes using IgBLAST⁶³ and formatted to be Adaptive Immune Receptor Repertoire (AIRR) compatible for downstream analysis. AIRR formatted BCR calls were read into R and filtered to remove non-productive sequences. For further BCR analysis, cells with no or with multiple heavy gene calls were removed. Paired heavy and light chain BCR calls were integrated with gene expression and ADT data. B cell clones were identified for each individual separately by spectral clustering of BCR heavy and light chains from the SCOPer (v1.3.0) package^{64, 65}. Germline sequences for the heavy chain of each clone were reconstructed using reference germline V and J sequences from the international ImMunoGeneTics information (IMGT) database. By comparing the observed VDJ sequence with the inferred germline VDJ sequence, the somatic hypermutation (SHM) rate was calculated using SCOPer. Synonymous and non-synonymous mutations were assessed separately, non-synonymous mutations are reported.

Single-cell TCR sequencing processing

Paired $\alpha\beta$ TCR chains were compiled for each cell using the scRepertoire package (v2.0.4), which loads filtered_contig_annotation files (output from 10x CellRanger multi) and combines them with the Seurat object for downstream analysis. Briefly, createHTOContigList was used with groups based on the called hashtag IDs and combineTCR with default settings. Clones were called based on paired CDR3 amino acid chain calling within each individual at each time point. Visualization of overlapping TCR clones was performed using the circlize package (v0.4.15)⁶⁶. Visualization of selected TCR amino acid sequence motifs was performed using the ggseqlogo package (v0.1).

TCR diversity and public clonotype analysis

For TCR diversity analysis, samples from each individual, cluster or individual x cluster combination were randomly downsampled to the smallest overall sample size (minimum n of 6). Random downsampling was performed 1000 times and the mean of the 1000 repeats was used as the per sample diversity. The D50 metric was calculated as the number of unique clones that made up 50% of the sample, scaled to the downsampled sample size. For public clonotype analysis, β TCR chains of reportedly SARS-CoV-2 S-specific T cells were compiled from Immune Epitope Database (IEDB)⁶⁷, VDJdb⁶⁸ and Immcode⁶⁹ databases. All β TCR chains reportedly specific to CMV, EBV and influenza were additionally compiled and randomly downsampled to be equivalent in number to S-specific β TCR chains. Chains with exact CDR3 matches in the CD8⁺ T cell dataset were labelled as public clonotypes.

CoNGA Analysis for TCRs

To identify metaclonotypes that were antigen-enriched we performed analysis using the CoNGA package (v0.1.2)⁷⁰ in Python (v3.9). Filtered, normalized and integrated CD4⁺ and CD8⁺ Seurat objects were separately exported from R and identical clones were reduced to a single clone using default settings. Within the CoNGA package, TCRdist was used to quantify physiochemical scores of the TCRs of each clonotype and find the distance in TCR space between two independent TCR clonotypes. This metric was then used in kernel PCA to reduce the cell x cell TCRdist matrix. The 50 top TCRdist kernel PCs were retained for downstream clustering and dimensionality reduction. To identify TCR meta-clonotypes that were likely to be antigen enriched, we performed TCR clumping analysis using conga.tcr_clumping.assess_tcr_clumping with default settings. This analysis simulates a background VDJ repertoire representative of a simple model of VDJ recombination and tests whether individual TCR neighborhoods are overrepresented in the observed data compared with this null model of VDJ recombination. Meta-clonotypes with >10 participating clonotypes

were selected for further analysis, and clustalOmega within the msa (v1.32.0)⁷¹ package was used to derive consensus sequences for $\alpha\beta$ CDR3 regions separately.

Data and code availability

No custom software was generated for this manuscript. The software used for all analyses is listed in the relevant sections of Methods, and sufficient detail is given to reproduce analyses. Code can be provided upon reasonable request. Derived and processed data for all the datasets generated during this study and reported in this paper are available at the European Genome-Phenome Archive (EGA). Access to data deposited at EGA is controlled by the OCTAVE DUO data access committee.

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Author contributions

Conceptualization: S.M.M, N.M.P, E.B; Data Curation: S.M.M, L.B, A.K; Formal Analysis: S.M.M; Funding Acquisition: C.S.G, I.B.M, P.Kearns, E.B, S.M.M, T.I.d.S, N.M.P, P.Klenerman, S.Dunachie; Investigation: S.M.M, J.R, G.M, V.N.R, S.Dimitriadis, K.D, A.H; Methodology: S.M.M, E.B, A.K, L.B, N.M.P; Resources: C.S.G, E.B, S.S, I.B.M, M.W, M.P, D.C.T, T.I.d.S, A.M.S, A.R, L.B, A.K, P.Kearns, B.K, P.Klenerman, S.Dunachie; Software: S.M.M; Supervision: E.B, N.M.P, P.Klenerman, S.Dunachie; Validation: A.K, L.B; Visualization: S.M.M; Writing – original draft: S.M.M, E.B, N.M.P; Writing – review & editing: all authors.

Declaration of interests

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