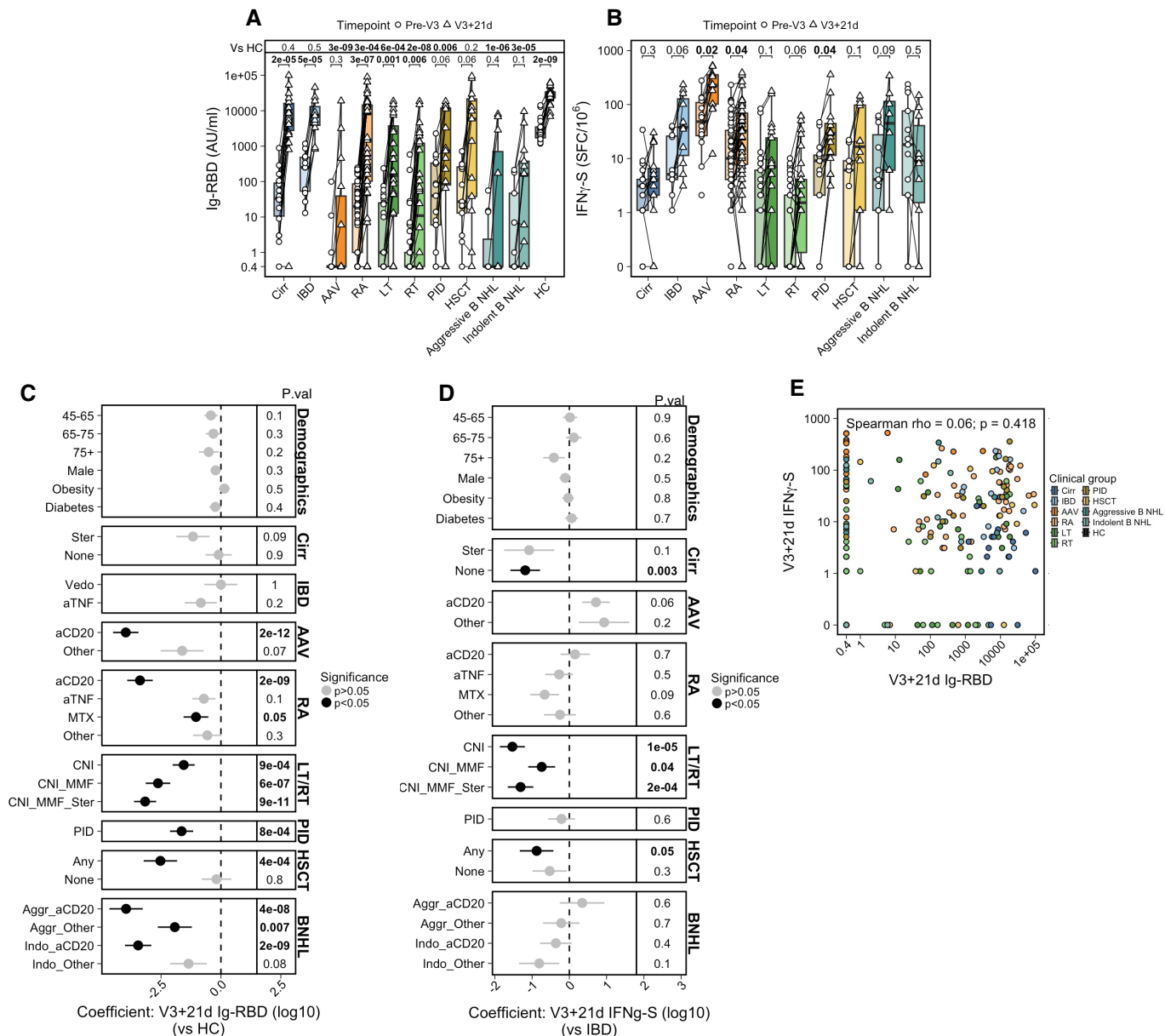
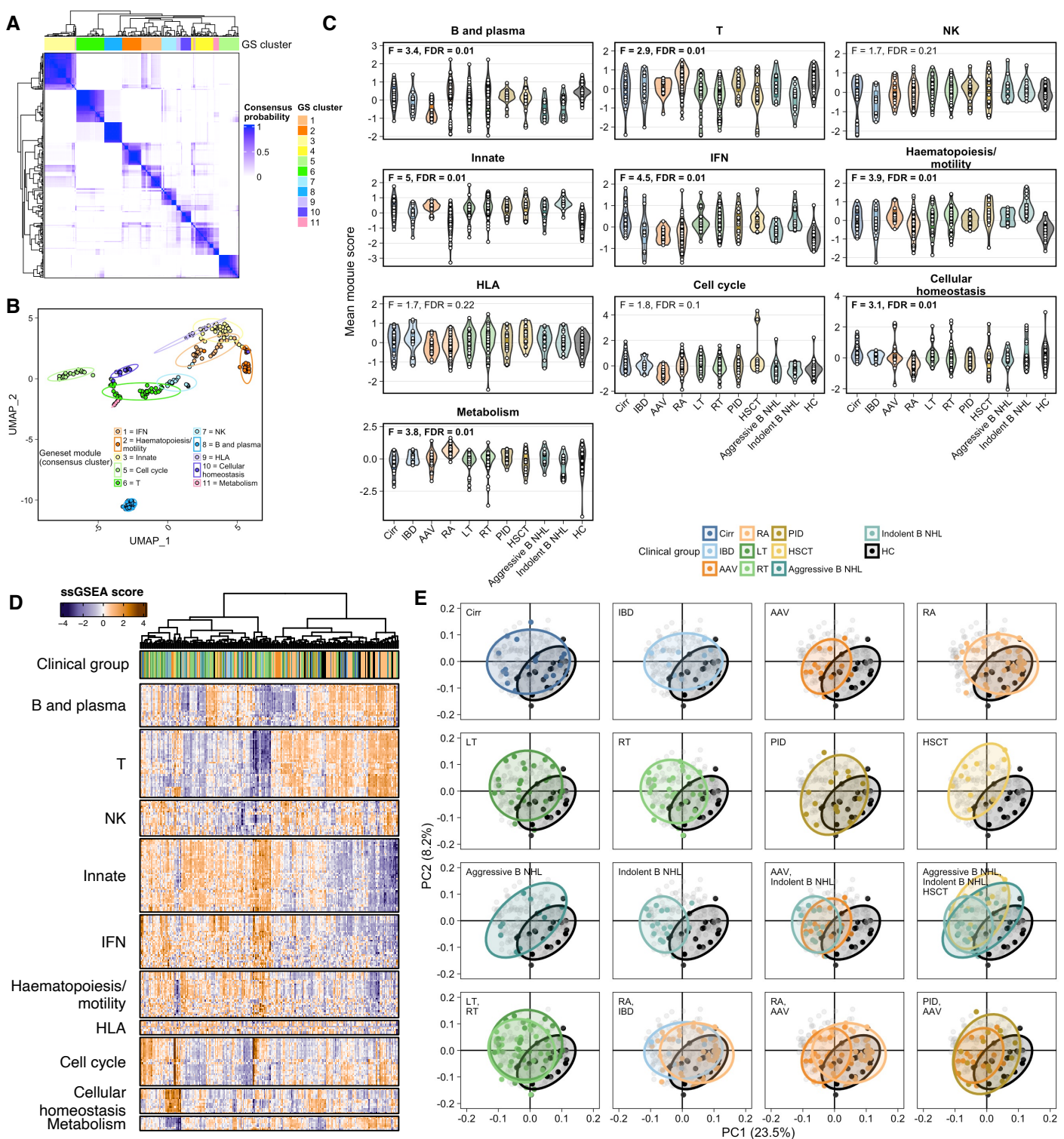




Extended Data Fig. 1) Impact of secondary immunodeficiencies on antibody and T cell responses to a third COVID-19 vaccine.

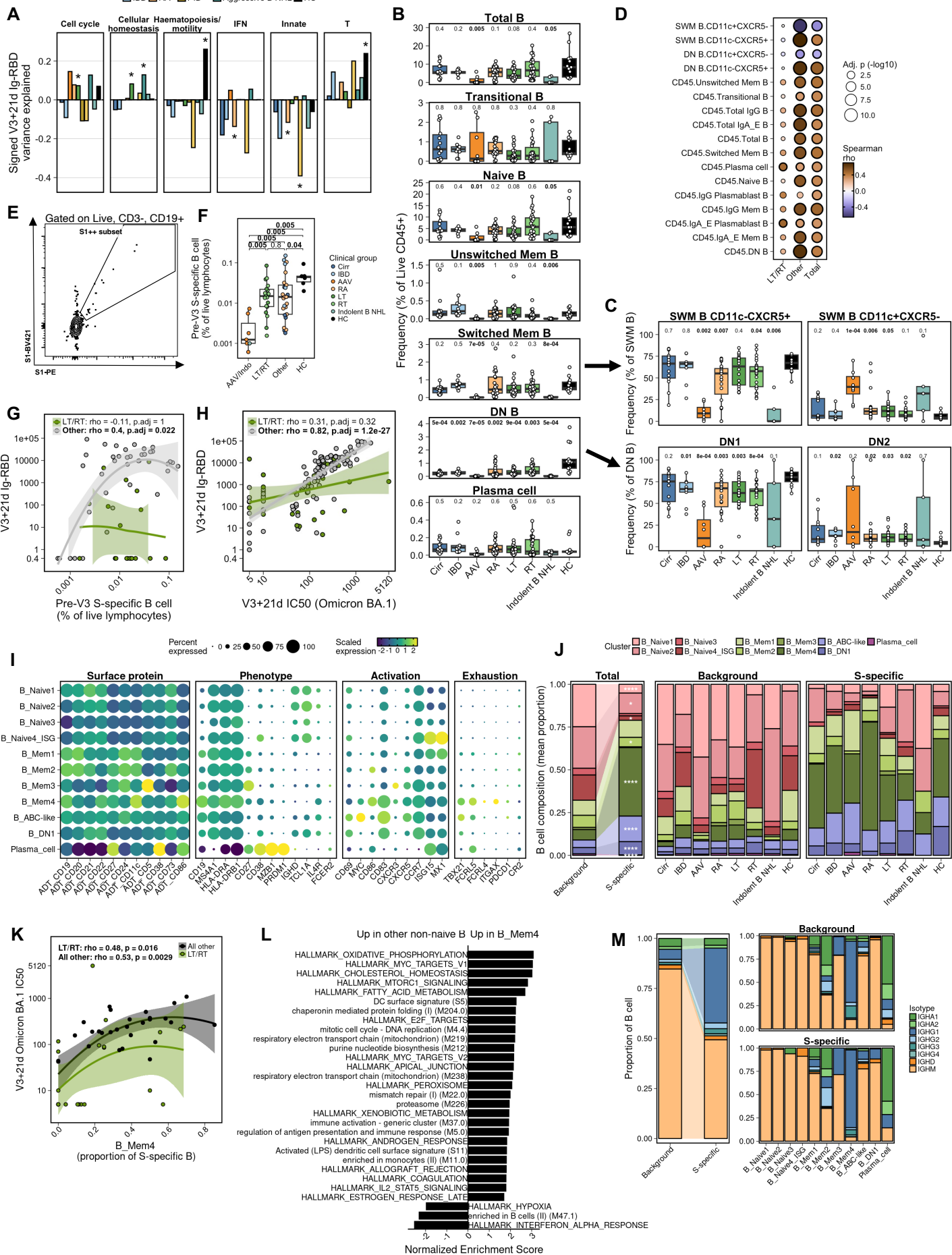
A) Ig-RBD immediately before (pre-V3) and 21-days (V3+21d) after a third vaccine dose. Statistics above horizontal line denote Mann-Whitney U tests compared to HCs, below horizontal line are paired Wilcoxon rank-sum tests. **B)** T cell magnitude to SARS-CoV-2 spike antigen (in IFN γ ELISpot assays (IFN γ -S), showing spot forming cells (SFC), at pre-V3 and V3+21d timepoints. Paired Wilcoxon rank-sum tests. **C)** Linear models of V3+21d anti-SARS-CoV-2 spike binding antibody titres (Ig-RBD). Demographic variables were used as covariates for all clinical/immunosuppressive therapeutic comparisons to healthy controls. **D)** As in C but of V3+21d IFN γ -S, compared to IBD patients. **E)** Correlation of V3+21d Ig-RBD and IFN γ -S



Extended Data Fig. 2) Bulk RNA-sequencing of whole blood delineates secondary immunodeficiencies

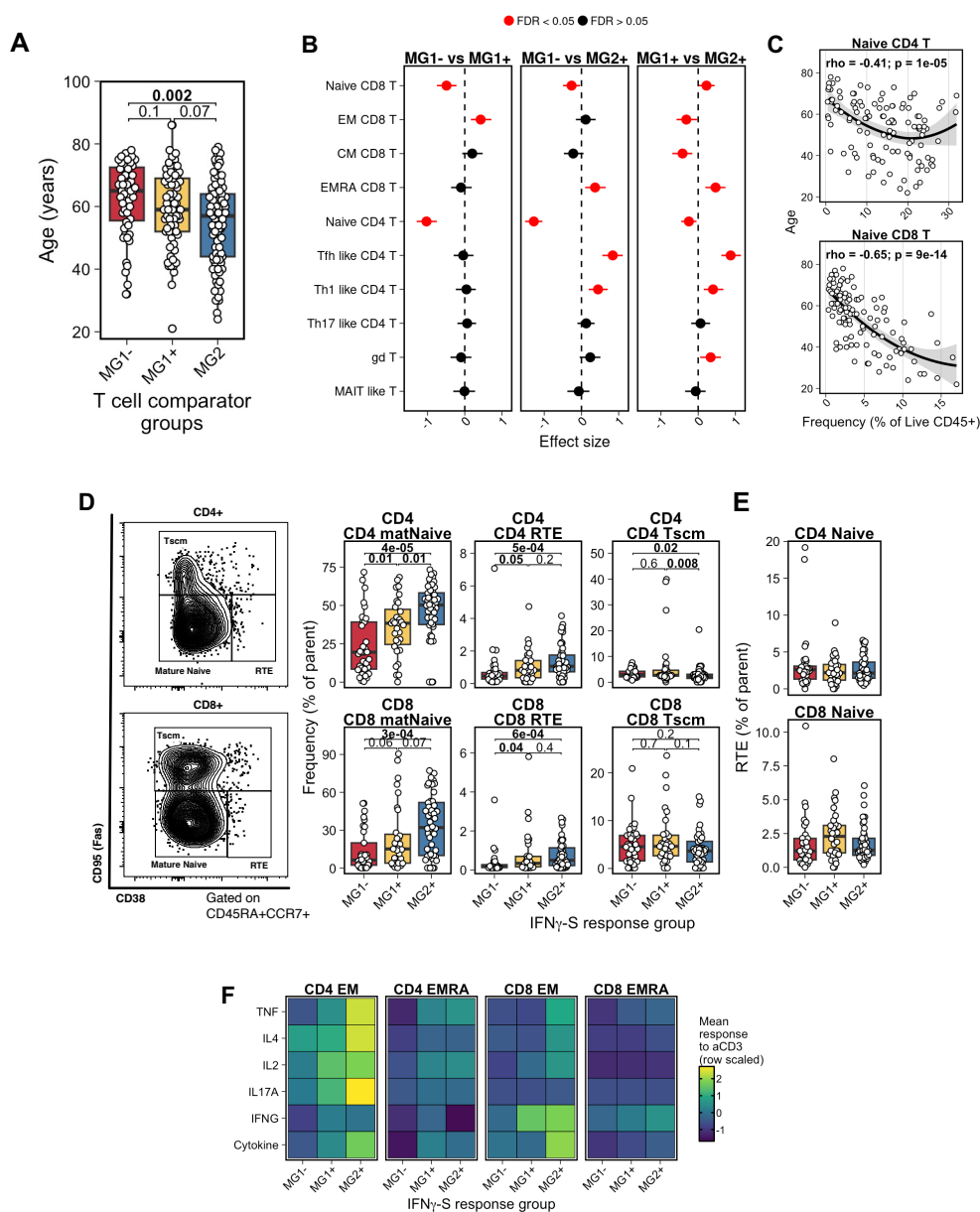
A) Consensus cluster matrix of cluster iterations of single sample geneset enrichment analysis (ssgsea) quantification of blood transcriptomic module genesets (BTM). **B**) UMAP of genesets based on ssgsea expression in the entire cohort. Ellipses are the annotated consensus clusters (geneset modules) identified in A. **C**) Mean per person ssgsea expression of genesets within each geneset module. Stats represent F test and false discovery rate (FDR) comparing across clinical groups. **D**) ssgsea expression of each geneset across the cohort. Genesets are clustered into geneset modules. **E**) PCA analysis based on ssgsea expression of all BTM genesets.

Clinical group: CIRR, IBD, AAV, RA, PID, Aggressive B NHL, Indolent B NHL



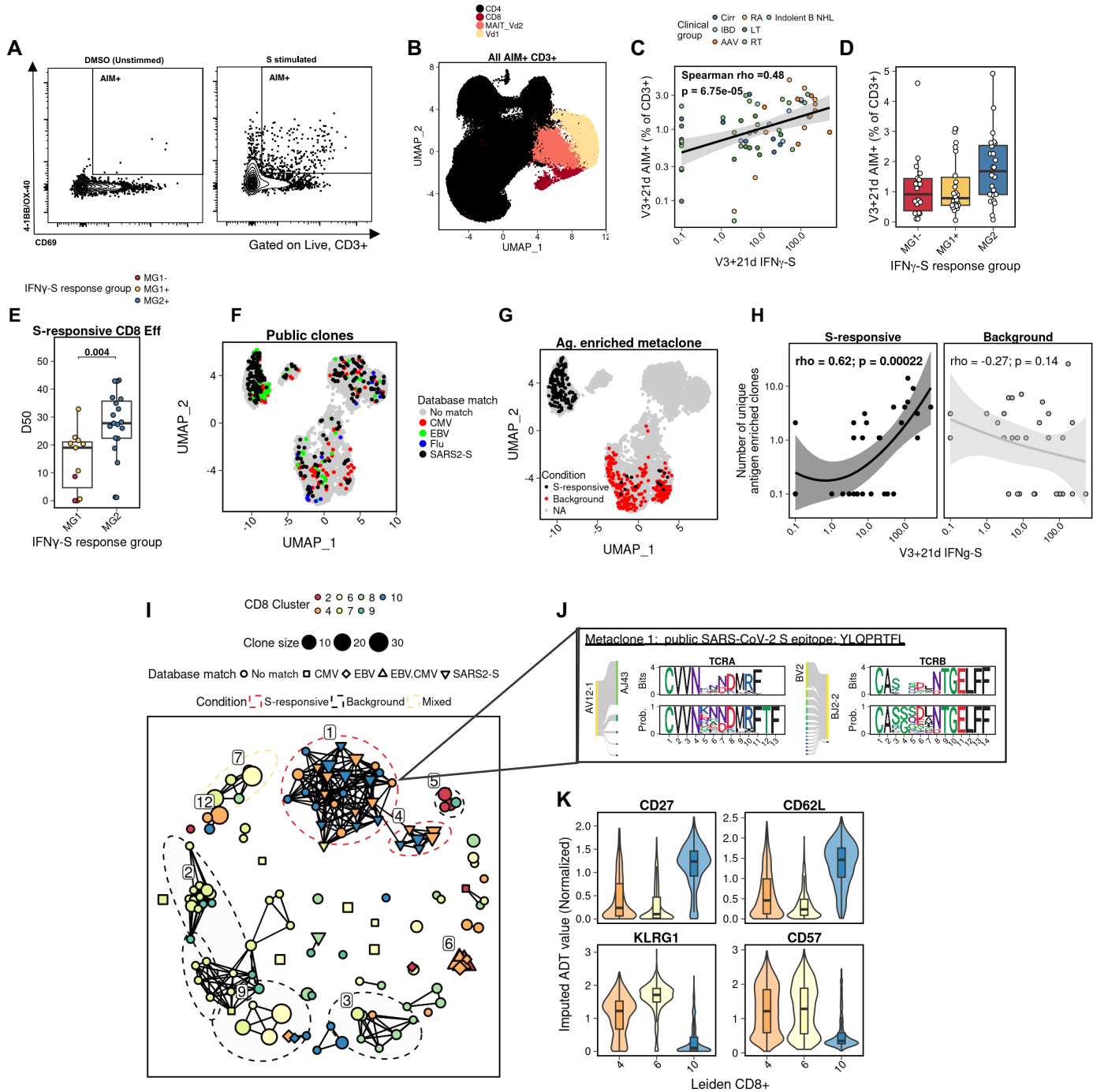
Extended Data Fig. 3) B cell dysfunction across SI groups.

A) Proportion of V3+21d Ig-RBD variance explained by module expression, signed based on the direction of the term coefficient. Generated from linear models of V3+21d Ig-RBD fit separately each clinical group. * denotes a significantly non-zero coefficient. **B&C)** Flow cytometry derived pre-V3 B cell subset frequencies. Number represents false discovery rate (FDR) of Mann-Whitney U test comparing cell frequencies in clinical groups compared to healthy controls. **D)** Correlation of pre-V3 B cell frequencies with V3+21d Ig-RBD, stratified by groups. Thick outlines represent FDR < 0.05. Frequency parent is denoted before the point. **E)** S-specific B cell flow cytometry gating. **F&G)** Pre-V3 S1-specific B cell frequency across B cell comparison groups (**F**) or correlated with V3+21d Ig-RBD (**G**). **H)** Correlation of V3+21d Ig-RBD with V3+21d wildtype (ancestral) or Omicron BA.1 neutralisation 50% inhibitory concentration (IC50). **I)** Mean expression of imputed ADT or mRNA across B cell Leiden clusters. **J)** Leiden cluster composition of cells from each experimental condition. Across all samples (Left) or split into clinical groups and experimental condition (right) * denotes $p < 0.05$ for proportion comparison between conditions. **K)** Correlation of the proportion of the B_Mem4 cluster within S-specific B cells with V3+21d Omicron BA.1 50% inhibitory concentration (IC50). **L)** Geneset enrichment analysis of differentially expressed genes between B_Mem4 and other memory or DN B cell clusters. **M)** B cell receptor (BCR) isotype usage across experiment condition (left) and Leiden clusters (right).



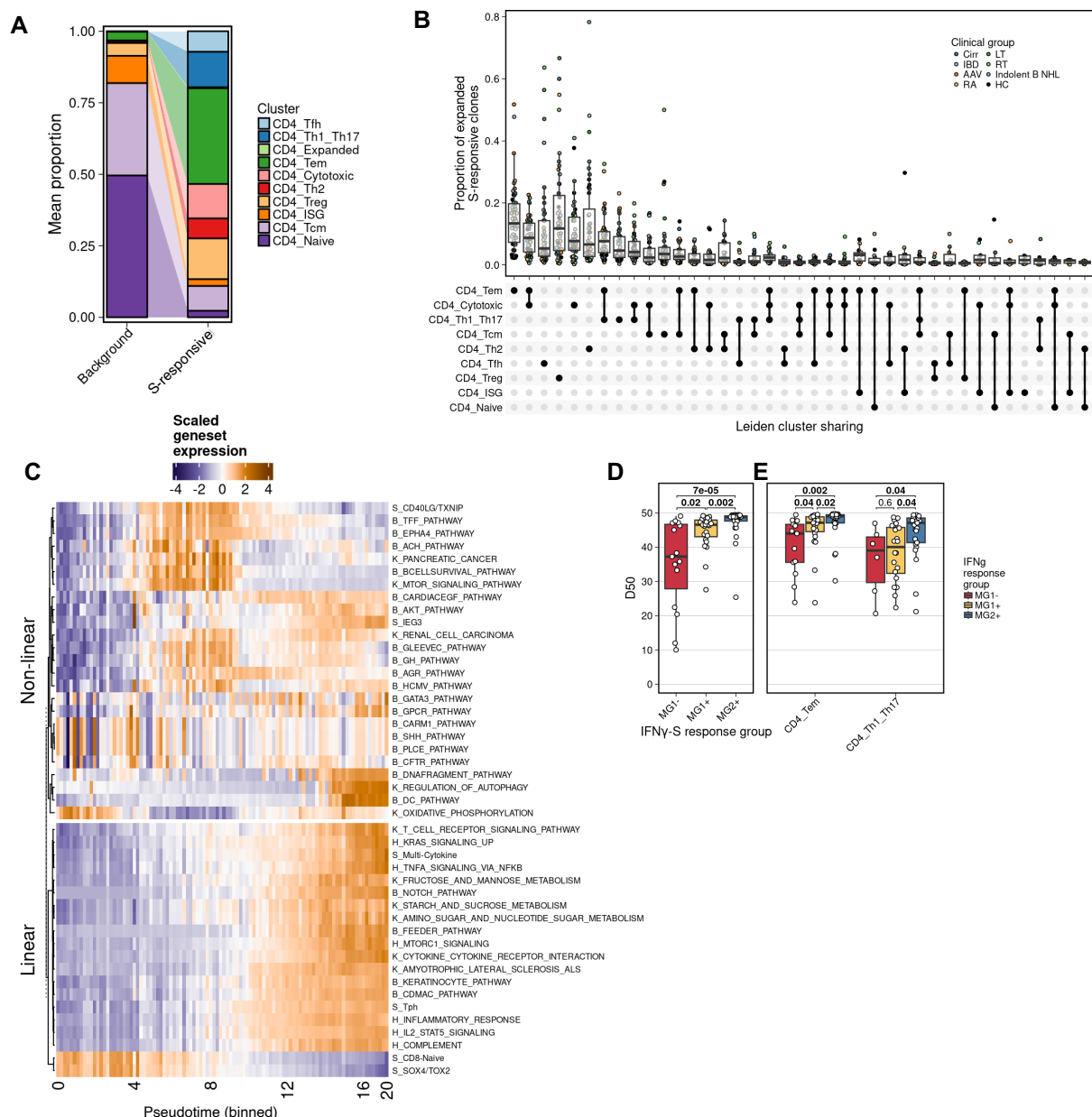
Extended Data Fig. 5) Global phenotypic alterations and impaired T cell responsiveness to stimulation in individuals with secondary immunodeficiencies with poor IFN γ T cell responses to COVID-19 vaccines.

A) Age distribution in IFN γ -S response groups. Adjusted P value is from Wilcoxon-test of age, Benjamini-Hochberg adjusted. **B)** Bayesian model effect sizes of pre-V3 flow-cytometry derived cell type composition (% of live CD45), with sex, batch and age as covariates. **C)** Correlation of naive (CD45RA+CCR7+) T cell frequencies with age. **D)** Gating scheme and frequencies of mature naive, Tscm and recent thymic emigrants (RTE) out of total CD4+ or CD8+ T cells. **E)** Frequencies of RTE out of naive populations. **F)** Row scaled mean cytokine+ frequency after aCD3 + aCD28 + aCD49d stimulation in T cell responsiveness groups. Cytokine frequency is background subtracted



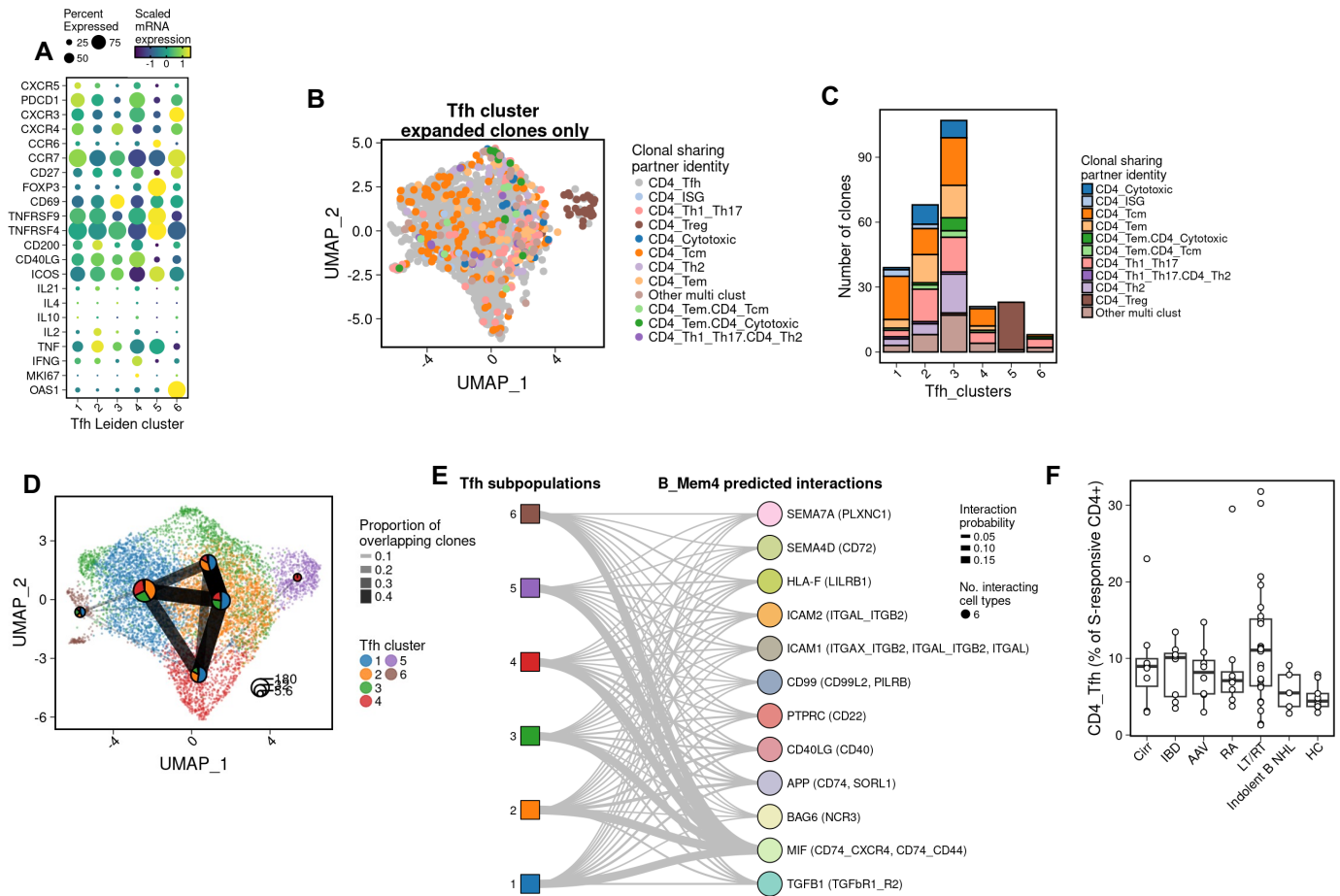
Extended Data Fig. 6) Diverse SARS-CoV-2 spike specific CD8+ T cells are induced by COVID-19 vaccine and altered in individuals with secondary immunodeficiencies

A Flow cytometry gating scheme for activation induced marker (AIM) positive T cells. **B** UMAP of single-cell RNA-sequencing data from AIM+ S-responsive T cells at V3+21d. **C** Correlation of flow cytometry derived V3+21d AIM+ T cell frequency with V3+21d IFN γ -S. **D** S-responsive AIM+ T cell frequency by T cell response group. **E** Per person D50 value based on bootstrapped subsampling of total S-responsive CD8 effector cells (CD8 clusters 4, 6 and 10). **F** Clones with a CDR3b amino acid sequencing matching that of a public database of TCRs for the given specificity overlaid onto CD8+ T cell UMAP. **G** Cells within antigen-enriched metaclonotypes coloured by the experimental condition from which the cells are derived. **H** Correlation between the number of antigen-enriched metaclonotypes in background and S-responsive CD8+ T cells with V3+21d anti-SARS-CoV-2 spike IFN γ T cell magnitude. **I** Antigen-enriched CD8+ T cell metaclonotypes. Each dot represents a unique clone, with size representing number of cells, fill colour representing Leiden cluster and point shape representing a match to a public clone with the given specificity. Network weight is TCRdist score, dashed ellipses and numbers represent metaclonotypes, and the ellipse colour represents the experimental condition the cells were identified in. **J** α TTCR gene usage and CDR3 regions for clones in metaclonotypes 1. **K** Imputed surface protein expression of selected CD8+ T cell markers in S-responsive CD8+ T cells.



Extended Data Fig. 7) Vaccine-induced SARS-CoV-2 spike-specific CD4+ T cells have a diverse phenotype which is varied over secondary immunodeficiencies

A) Leiden cluster composition of background and S-responsive CD4+ T cells. **B)** Per person proportion of expanded S-responsive clones belonging to the given Leiden cluster combination. Cluster combinations with <4 unique constituent clusters are shown. **C)** Binned expression of genesets within pseudobulked bins across pseudotime (100 bins). Selected genesets shown based on linear or non-linear correlations with pseudotime. For linear correlations, only the top 20 most significantly correlated genesets are shown. **D&E)** Per person D50 value based on bootstrapped subsampling of total S-responsive cells (subsample n = 30) (**D**) or S-responsive CD4+ clusters (subsample n = 6) (**E**).



Extended Data Fig. 8) Vaccine-induced CXCR4⁺ cT_{FH}'s are associated with antibody non-responsiveness to vaccination.

A) Expression of select mRNA in cT_{FH} subclusters. **B)** Clonal sharing of expanded cT_{FH} clones with CD4⁺ T cell clusters. **C)** Number of clones within each CD4_Tfh subcluster that are shared with the given S-responsive CD4⁺ T cell cluster. **D)** Clonal sharing of expanded cT_{FH} clones between cT_{FH} subpopulations. **E)** Predicted interactions (**Methods**) between cT_{FH} subclusters and S-specific B_Mem4 cells, shown are interactions that appear in all cT_{FH} subpopulations. **F)** Frequency of the CD4_Tfh cluster amongst all S-responsive CD4⁺ T cells.

	Cirr N = 20 [†]	IBD N = 13 [†]	AAV N = 15 [†]	RA N = 49 [†]	LT N = 27 [†]	RT N = 37 [†]	PID N = 18 [†]	HSCT N = = 14 [†]	Aggr. B NHL N = 11 [†]	Indolent B NHL N = 13 [†]	HC N = 28 [†]	Overall N = 245 [†]
Sex												
Female	12 (60%)	2 (15%)	7 (47%)	42 (86%)	7 (26%)	9 (24%)	11 (61%)	6 (43%)	6 (55%)	3 (23%)	23 (82%)	128 (52%)
Male	8 (40%)	11 (85%)	8 (53%)	7 (14%)	20 (74%)	28 (76%)	7 (39%)	8 (57%)	5 (45%)	10 (77%)	5 (18%)	117 (48%)
Age												
	62 (51 – 69)	43 (33 – 51)	56 (39 – 71)	58 (50 – 64)	63 (54 – 68)	59 (50 – 67)	62 (43 – 68)	55 (50 – 70)	74 (60 – 77)	73 (53 – 76)	34 (26 – 46)	58 (44 – 67)
Ethnicity												
Asian	0 (0%)	1 (7.7%)	0 (0%)	0 (0%)	1 (3.7%)	8 (22%)	0 (0%)	1 (7.1%)	0 (0%)	1 (7.7%)	2 (7.1%)	14 (5.8%)
Black	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.7%)	3 (8.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (1.7%)
Other	0 (0%)	1 (7.7%)	0 (0%)	2 (4.3%)	0 (0%)	2 (5.4%)	1 (5.6%)	1 (7.1%)	1 (9.1%)	0 (0%)	0 (0%)	8 (3.3%)
White	20 (100%)	11 (85%)	13 (100%)	45 (96%)	25 (93%)	24 (65%)	17 (94%)	12 (86%)	10 (91%)	12 (92%)	26 (93%)	215 (89%)
Unknown	0	0	2	2	0	0	0	0	0	0	0	4
BMI												
	30.2 (26.4 – 32.5)	24.4 (21.0 – 28.4)	32.3 (26.7 – 37.6)	25.5 (24.1 – 32.5)	26.5 (23.3 – 32.2)	26.4 (25.0 – 30.0)	24.5 (23.8 – 28.4)	26.3 (24.3 – 27.4)	27.0 (24.1 – 30.1)	28.0 (26.9 – 29.4)	24.4 (21.0 – 26.9)	26.5 (24.2 – 30.4)
Unknown	0	0	0	1	0	7	5	2	0	0	1	16
WHO Performance status												
0	12 (60%)	11 (92%)	6 (40%)	14 (29%)	18 (67%)	20 (67%)	14 (82%)	6 (50%)	9 (82%)	10 (77%)	28 (100%)	148 (63%)
1	2 (10%)	1 (8.3%)	7 (47%)	19 (39%)	8 (30%)	9 (30%)	2 (12%)	5 (42%)	2 (18%)	1 (7.7%)	0 (0%)	56 (24%)
2	5 (25%)	0 (0%)	1 (6.7%)	12 (24%)	1 (3.7%)	1 (3.3%)	1 (5.9%)	1 (8.3%)	0 (0%)	2 (15%)	0 (0%)	24 (10%)
3	1 (5.0%)	0 (0%)	1 (6.7%)	4 (8.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (2.6%)
Unknown	0	1	0	0	0	7	1	2	0	0	0	11
Smoking status												
Currently	4 (20%)	1 (7.7%)	2 (15%)	8 (16%)	1 (3.7%)	0 (0%)	0 (0%)	0 (0%)	1 (11%)	0 (0%)	0 (0%)	17 (8.2%)
Previously	5 (25%)	3 (23%)	4 (31%)	16 (33%)	10 (37%)	3 (21%)	6 (33%)	3 (43%)	3 (33%)	5 (56%)	3 (11%)	61 (29%)
Never	11 (55%)	9 (69%)	7 (54%)	25 (51%)	16 (59%)	11 (79%)	12 (67%)	4 (57%)	5 (56%)	4 (44%)	25 (89%)	129 (62%)
Unknown	0	0	2	0	0	23	0	7	2	4	0	38
Comorbidities												
CVD	2 (10%)	0 (0%)	3 (20%)	4 (8.3%)	2 (7.4%)	14 (38%)	1 (6.3%)	0 (0%)	4 (36%)	3 (23%)	0 (0%)	33 (14%)
Stroke	0 (0%)	0 (0%)	1 (7.1%)	3 (6.3%)	1 (3.7%)	2 (5.4%)	0 (0%)	0 (0%)	3 (27%)	0 (0%)	0 (0%)	10 (4.1%)
Diabetes	11 (55%)	0 (0%)	3 (20%)	6 (13%)	11 (41%)	16 (43%)	1 (6.3%)	3 (21%)	2 (18%)	0 (0%)	0 (0%)	53 (22%)
Type 1	0 (0%)	0 (0%)	0 (0%)	2 (33%)	0 (0%)	2 (13%)	0 (0%)	2 (67%)	0 (0%)	0 (0%)	0 (0%)	6 (11%)
Type 2	11 (100%)	0 (0%)	2 (100%)	4 (67%)	10 (91%)	11 (69%)	1 (100%)	1 (33%)	2 (100%)	0 (0%)	0 (0%)	42 (76%)
Asthma	3 (15%)	1 (7.7%)	1 (6.7%)	6 (13%)	1 (3.7%)	1 (2.7%)	5 (31%)	3 (21%)	0 (0%)	0 (0%)	2 (7.7%)	23 (9.6%)
COPD	0 (0%)	0 (0%)	0 (0%)	1 (2.1%)	1 (3.7%)	1 (2.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.6%)	4 (1.7%)
Hypertension	11 (55%)	0 (0%)	6 (40%)	10 (21%)	8 (30%)	34 (92%)	4 (25%)	2 (14%)	2 (18%)	3 (23%)	1 (3.6%)	81 (33%)
Unknown	0	0	0	1	0	0	2	0	0	0	0	3
Number of comorbidities												
0	3 (15%)	10 (77%)	3 (20%)	16 (33%)	5 (19%)	1 (2.7%)	6 (33%)	2 (14%)	4 (36%)	4 (31%)	22 (79%)	76 (31%)
1	5 (25%)	3 (23%)	4 (27%)	20 (41%)	9 (33%)	13 (35%)	5 (28%)	4 (29%)	2 (18%)	4 (31%)	6 (21%)	75 (31%)
2	5 (25%)	0 (0%)	6 (40%)	8 (16%)	7 (26%)	7 (19%)	1 (5.6%)	4 (29%)	3 (27%)	5 (38%)	0 (0%)	46 (19%)
3	3 (15%)	0 (0%)	2 (13%)	3 (6.1%)	3 (11%)	13 (35%)	3 (17%)	3 (21%)	2 (18%)	0 (0%)	0 (0%)	32 (13%)
4	3 (15%)	0 (0%)	0 (0%)	2 (4.1%)	3 (11%)	2 (5.4%)	1 (5.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	11 (4.5%)
5	1 (5.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2.7%)	2 (11%)	1 (7.1%)	0 (0%)	0 (0%)	0 (0%)	5 (2.0%)
Prior vaccine type												
AZD1222	18 (90%)	13 (100%)	15 (100%)	45 (92%)	24 (89%)	21 (57%)	12 (67%)	7 (50%)	4 (36%)	6 (46%)	4 (14%)	169 (69%)
BNT162b2	2 (10%)	0 (0%)	0 (0%)	4 (8.2%)	3 (11%)	16 (43%)	6 (33%)	7 (50%)	7 (64%)	7 (54%)	23 (82%)	75 (31%)
mRNA-1273	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.6%)	1 (0.4%)
V2+28d antibody response status												
High	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	28 (100%)	28 (11%)
Low	19 (95%)	13 (100%)	4 (27%)	33 (67%)	13 (48%)	4 (11%)	5 (28%)	9 (64%)	2 (18%)	2 (15%)	0 (0%)	104 (42%)
None	1 (5.0%)	0 (0%)	11 (73%)	16 (33%)	14 (52%)	33 (89%)	13 (72%)	5 (36%)	9 (82%)	11 (85%)	0 (0%)	113 (46%)
V3 vaccine type												
BNT162b2	10 (50%)	6 (46%)	8 (53%)	26 (53%)	13 (48%)	20 (54%)	11 (61%)	7 (50%)	6 (55%)	6 (46%)	25 (89%)	138 (56%)
mRNA-1273	10 (50%)	7 (54%)	7 (47%)	23 (47%)	14 (52%)	17 (46%)	7 (39%)	7 (50%)	5 (45%)	7 (54%)	3 (11%)	107 (44%)
Immunosuppressive therapy												
MMF	1 (5.0%)	0 (0%)	1 (6.7%)	0 (0%)	13 (48%)	25 (68%)	2 (11%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	42 (17%)
CNI	2 (10%)	0 (0%)	0 (0%)	0 (0%)	25 (93%)	34 (92%)	0 (0%)	3 (21%)	0 (0%)	0 (0%)	0 (0%)	64 (26%)
Anti-CD20	0 (0%)	0 (0%)	12 (80%)	11 (22%)	0 (0%)	0 (0%)	0 (0%)	2 (14%)	6 (55%)	8 (62%)	0 (0%)	39 (16%)
Anti-TNF	0 (0%)	6 (46%)	0 (0%)	18 (37%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	24 (9.8%)
Methotrexate	0 (0%)	0 (0%)	0 (0%)	29 (59%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	29 (12%)
DMARD	0 (0%)	0 (0%)	0 (0%)	18 (37%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	18 (7.3%)
Corticosteroid	5 (25%)	1 (7.7%)	10 (67%)	6 (12%)	10 (37%)	21 (57%)	0 (0%)	6 (43%)	0 (0%)	1 (7.7%)	0 (0%)	60 (24%)
Azathioprine	2 (10%)	2 (15%)	0 (0%)	0 (0%)	3 (11%)	1 (2.7%)	1 (5.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (3.7%)
Vedolizumab	0 (0%)	5 (38%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (2.0%)
Ig therapy	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	15 (83%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	15 (6.1%)
Number of immunosuppressive therapeutics												
1	1 (5.0%)	9 (69%)	7 (47%)	20 (41%)	6 (22%)	8 (22%)	13 (72%)	2 (14%)	6 (55%)	7 (54%)	0 (0%)	79 (32%)
2	3 (15%)	3 (23%)	8 (53%)	15 (31%)	18 (67%)	14 (38%)	3 (17%)	4 (29%)	0 (0%)	0 (0%)	0 (0%)	68 (28%)
3	1 (5.0%)	0 (0%)	0 (0%)	7 (14%)	3 (11%)	15 (41%)	1 (5.6%)	1 (7.1%)	0 (0%)	1 (7.7%)	0 (0%)	29 (12%)
4	0 (0%)	0 (0%)	0 (0%)	3 (6.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (1.2%)

[†]n (%); Median (Q1, Q3)

Extended Data Table 1) Description of cohort.