## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1. $Nuc_{322-331}$ of SARS-CoV-2 is conserved in the SARS-CoV-2 variants-of-concern.** $Nuc_{322-331}$ resides near the C-terminus of the nucleocapsid protein of SARS-CoV-2, and its sequence, MEVTPSGTWL, is 100% conserved in the B.1.1.7, B.1.351, P.1, and B429/CAL20C first detected in the United Kingdom, South Africa, Brazil, and California, respectively. It is also 100% conserved in SARS-CoV-1, but no orthologs are present in the common cold coronavirus strains 229E, NL63, OC43, and HKU1. Shown are the location of the nucleocapsid gene within the SARS-CoV-2 genome, the location of the sequence encoding the peptide within the nucleocapsid gene, and both the nucleotide and amino acid sequences of corresponding to $Nuc_{322-331}$.

**Figure S2. Expression levels of CyTOF antigens in $Nuc_{322-331}$-specific CD8+ T cells from PID4103.** The $Nuc_{322-331}$-specific cells were identified either as unstimulated tetramer+ cells (*red*) or as cells producing IFNγ after peptide stimulation (*green*). For each antigen, the histograms represent merges of the five timepoints analyzed in this study.

**Figure S3. Expression levels of CyTOF antigens in $Nuc_{322-331}$-specific CD8+ T cells from PID4103 as a function of time.**

Line graphs depict the antigens' median staining intensities, as measured by CyTOF, among the tetramer+ cells in the unstimulated sample (*red*), and the IFNγ+ cells in the $Nuc_{322-331}$-stimulated samples (*green*), at the five timepoints analyzed in this study.

**Figure S4. Approximately half of tetramer+ cells in $Nuc_{322-331}$-stimulated samples do not secrete IFNγ or TNFα.**

PBMCs from PID4103 were stimulated with $Nuc_{322-331}$, stained with HLA-B*40:01/$Nuc_{322-331}$ tetramers, and analyzed by CyTOF. A total of 54.1% of tetramer+ cells expressed neither IFNγ nor TNFα, suggesting that approximately half of tetramer+ cells are not identified using the cytokine secretion assay.

**Figure S5. Longitudinal assessment of the CD8+ and CD4+ T cells of PID4103 directed against the nucleocapsid and spike proteins.**

CD8+ (A) and CD4+ (B) T cells specifically responding to stimulation with overlapping peptides spanning the entire nucleocapsid or spike proteins were identified by gating on the IFNγ+ cells. The cells were phenotyped by CyTOF at baseline, or following 4 hours of co-stimulation with αCD49d/CD28 and either the nucleocapsid or spike peptides. Stimulations were conducted in the presence of brefeldin A to enable detection of intracellular cytokines. Numbers correspond

to the percentage of cells in each sample. Results are gated on live, singlet CD3+CD8+ cells (A) or live, singlet CD3+CD4+ cells (B). Timepoints correspond to days post symptom onset.

## SUPPLEMENTARY TABLES

### Table S1. Tetramers screened by FACS

| Tetramer ID | Epitope | Protein location | MHC allele |
|---|---|---|---|
| $Nuc_{322-331}$ (N1) | MEVTPSGTWL | Nucleocapsid 322-331 | HLA-B*40:01 |
| N2 | LLLDRLNQL | Nucleocapsid 222-230 | HLA-A*02:01 |
| S1 | ALNTLVKQL | Spike 958-996 | HLA-A*02:01 |
| S2 | VLNDILSRL | Spike 976-984 | HLA-A*02:01 |
| S3 | LITGRLQSL | Spike 996-1004 | HLA-A*02:01 |
| S4 | RLNEVAKNL | Spike 1185-1193 | HLA-A*02:01 |
| S5 | NLNESLIDL | Spike 1192-1200 | HLA-A*02:01 |
| S6 | FIAGLIAIV | Spike 1220-1228 | HLA-A*02:01 |
| M1 | HLRIAGHHL | Membrane Protein 148-156 | HLA-B*08:01 |

### Table S2. Participant Characteristics

| Patient ID | Gender | Age (years) | Date of symptom onset | Date of First PCR+ test | Date of Blood Draw(s) | Time Between Symptom Onset and analysis | Time Between first PCR+ test and analysis |
|---|---|---|---|---|---|---|---|
| PID4103 | Female | 42 | 3/13/20 | 04/09/20 | 04/29/20 (PCR+) | 46 days | 20 days |
| | | | | | 05/06/20 (PCR-) | 53 days | 27 days |
| | | | | | 05/20/20 (PCR-) | 67 days | 41 days |
| | | | | | 07/08/20 (PCR-) | 117 days | 90 days |
| | | | | | 10/07/20 (PCR-) | 207 days | 181 days |

**Table S3. List of CyTOF staining antibodies**

| Antibody | Metal label | Clone | Vendor |
|---|---|---|---|
| CD49d (α4) | 141Pr | 9F10 | Fluidigm |
| CD19 | 142Nd | HIB19 | Fluidigm |
| CCR5 | 144Nd | NP6G4 | Fluidigm |
| CD8 | 146Nd | RPAT8 | Fluidigm |
| CD7 | 147Sm | CD76B7 | Fluidigm |
| ICOS | 148Nd | C398.4A | Fluidigm |
| CCR4 | 149Sm | L291H4 | Fluidigm |
| CD62L | 153Eu | DREG56 | Fluidigm |
| TIGIT | 154Sm | MBSA43 | Fluidigm |
| CCR6 | 155Gd | G034E3 | In-house |
| CD29 (β1) | 156Gd | TS2/16 | Fluidigm |
| OX40 | 158Gd | ACT35 | Fluidigm |
| CCR7 | 159Tb | G043H7 | Fluidigm |
| CD28 | 160Gd | CD28.2 | Fluidigm |
| CD45RO | 161Dy | UCHL1 | In-house |
| CD69 | 162Dy | FN50 | Fluidigm |
| Tetramer | 163Dy | N/A | In-house |
| PD1 | 164Dy | EH12.1 | In-house |
| CD127 | 165Ho | A019D5 | Fluidigm |
| CXCR5 | 166Er | RF8B2 | In-house |
| CD27 | 167Er | L128 | Fluidigm |
| CD45RA | 169Tm | HI100 | Fluidigm |
| CD3 | 170Er | UCHT1 | Fluidigm |
| CD57 | 171Yb | HCD57 | In-house |
| CD38 | 172Yb | HIT2 | Fluidigm |
| Tetramer | 173Yb | N/A | In-house |
| CD4 | 174Yb | SK3 | Fluidigm |
| CD25 | 176Yb | M-A251 | In-house |
| HLADR | 112Cd | Tu36 | Invitrogen |
| NFAT1# | 143Nd | D43B1 | Fluidigm |
| BIRC5# | 145Nd | 91630 | In-house |

| | | | |
|---|---|---|---|
| TNFα[#] | 150Nd | MAb11 | In-house |
| CD107a[#] | 151Eu | H4A3 | Fluidigm |
| Granzyme B[#] | 152Sm | O94E6/GZMB | In-house |
| CTLA4[#] | 157Gd | 14D3 | In-house |
| IFNγ[#] | 168Er | B27 | Fluidigm |
| Perforin[#] | 175Lu | B-D48 | Fluidigm |
| IL-6[#] | 209Bi | MQ2-13A5 | In-house |

[#]*Intracellular antibodies*

Figure S1


SARS-COV-2
whole genome
5000
10,000
15,000
20,000
25,000
ORF1ab
S
ORF3a
E
M
ORF7a
ORF8
N
ORF10
SARS-COV-2
Nucleocapsid
28,500
28,750
29,000
29,250
29,500
GU280_gp10: ORF9, nucleocapsid phosphoprotein
SARS-COV-2
Nucleocapsid
peptide
atggaagtcacaccttcgggaacgtggttg
taccttcagtgtggaagcccttgcaccaac
325
330
M E V T P S G T W L
SARS-CoV-2 original
SARS-CoV-2 B.1.1.7 (UK)
SARS-CoV-2 B.1.351 (South Africa)
SARS-CoV-2 P.1 (Brazil)
SARS-CoV-2 B429/CAL20C (California)
SARS-CoV-1
No ortholog: 229E, NL63, OC43, HKU1
NUC22331

Figure S2

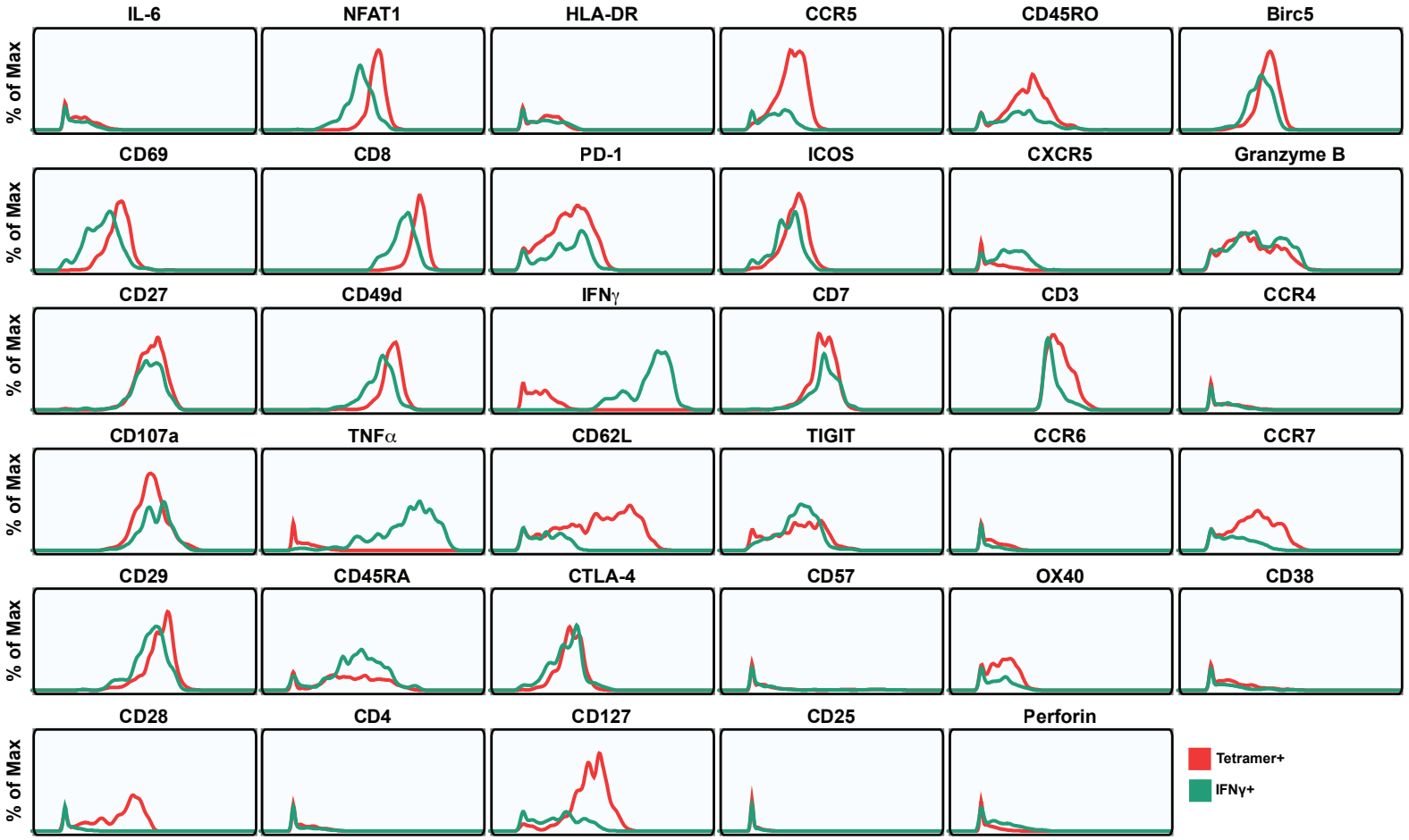

IL-6
NFAT1
HLA-DR
CCR5
CD45RO
Birc5
CD69
CD8
PD-1
ICOS
CXCR5
Granzyme B
CD27
CD49d
IFNγ
CD7
CD3
CCR4
CD107a
TNFα
CD62L
TIGIT
CCR6
CCR7
CD29
CD45RA
CTLA-4
CD57
OX40
CD38
CD28
CD4
CD127
CD25
Perforin
% of Max
Tetramer+
IFNγ+

Figure S3

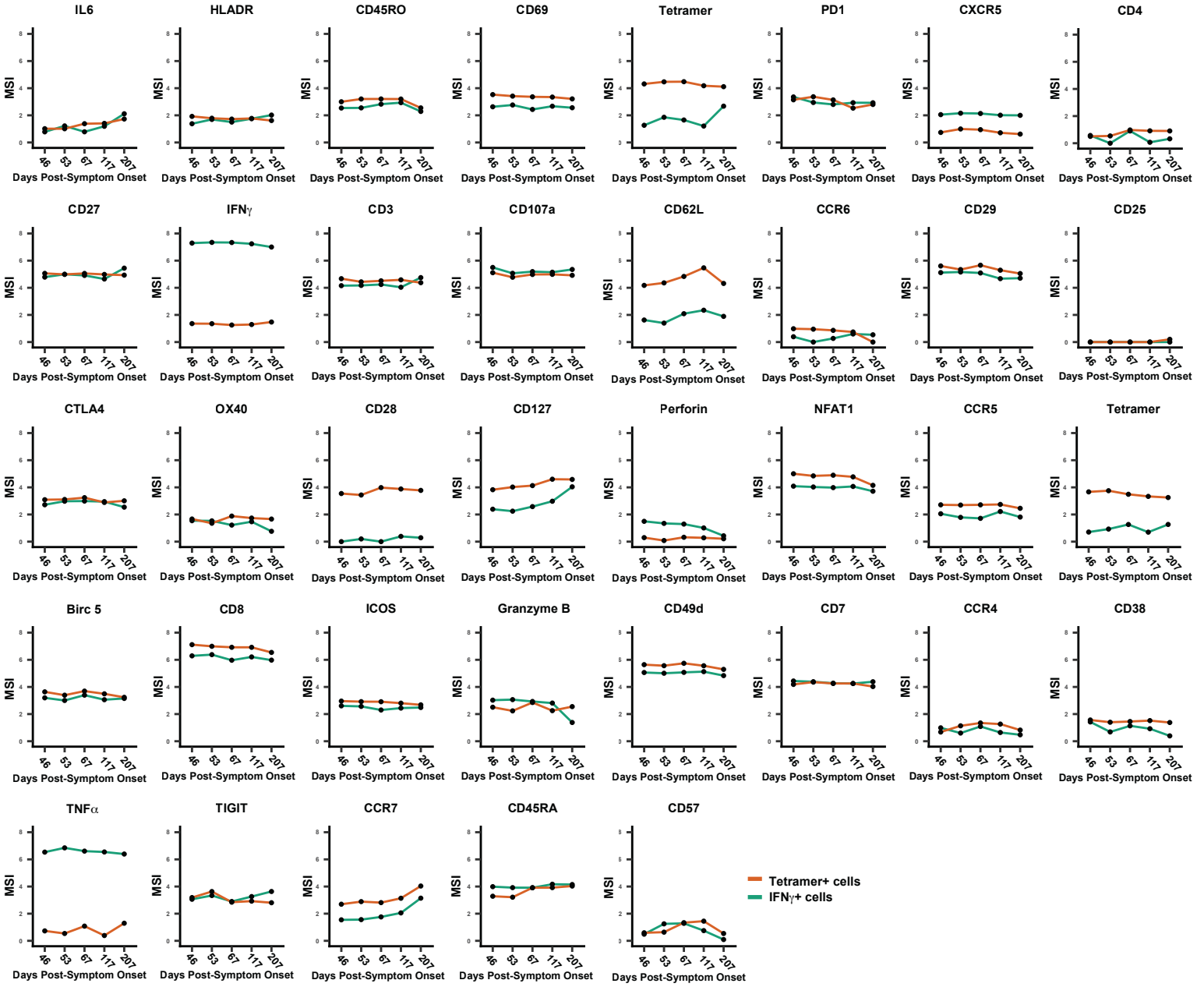

IL6
HLADR
CD45RO
CD69
Tetramer
PD1
CXCR5
CD4
CD27
IFNγ
CD3
CD107a
CD62L
CCR6
CD29
CD25
CTLA4
OX40
CD28
CD127
Perforin
NFAT1
CCR5
Tetramer
Birc 5
CD8
ICOS
Granzyme B
CD49d
CD7
CCR4
CD38
TNFα
TIGIT
CCR7
CD45RA
CD57
MSI
Days Post-Symptom Onset
46
53
67
117
207
Tetramer+ cells
IFNγ+ cells

Figure S4

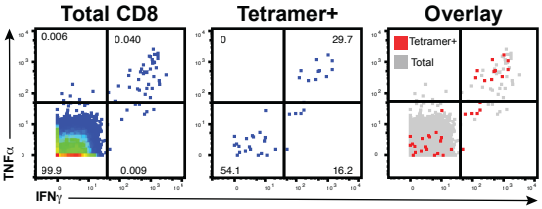
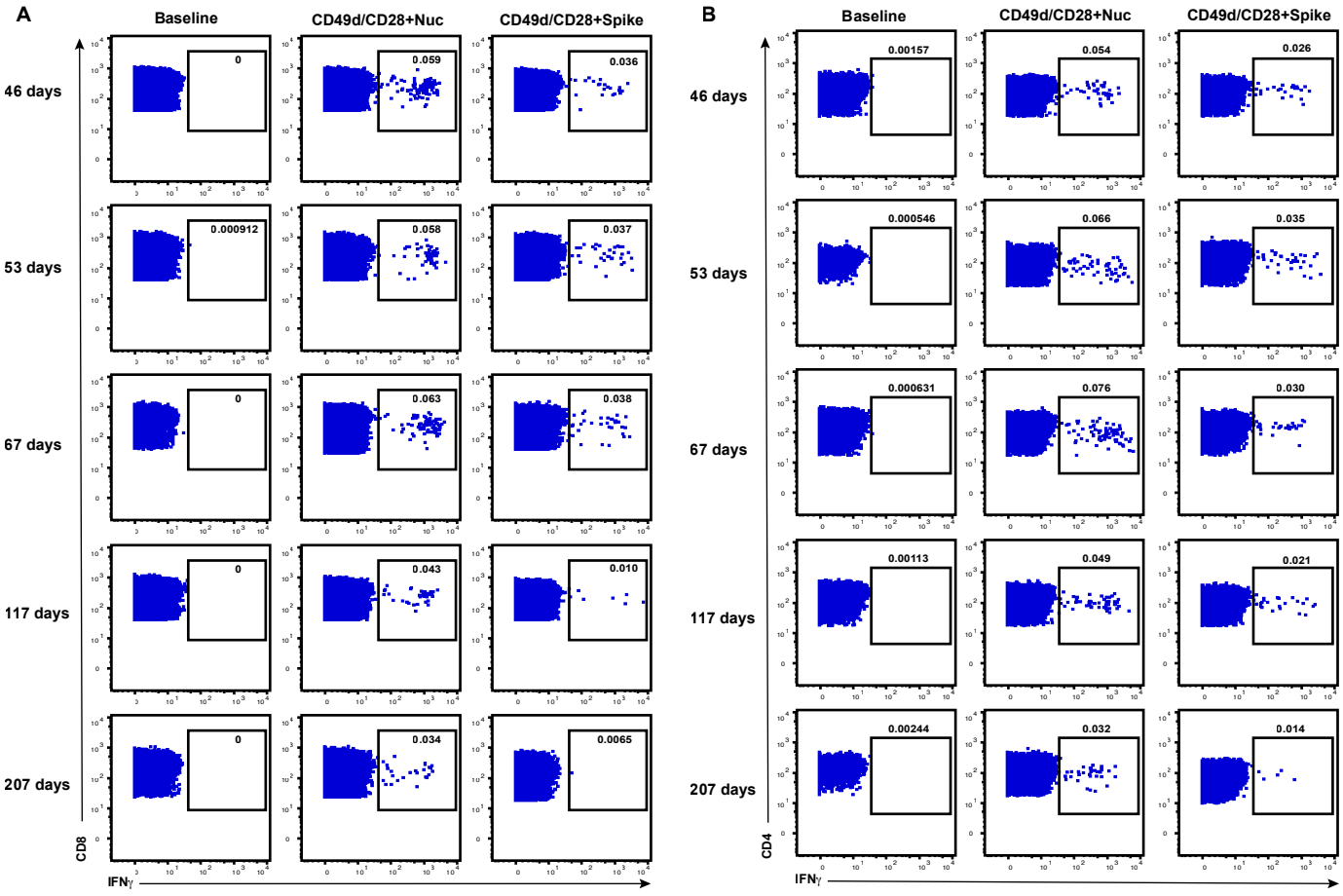

Total CD8
Tetramer+
Overlay
0.006
0.040
99.9
0.009
0
29.7
54.1
16.2
Tetramer+
Total
TNFα
IFNγ

Figure S5


A
Baseline
CD49d/CD28+Nuc
CD49d/CD28+Spike
46 days
53 days
67 days
117 days
207 days
0
0.059
0.036
0.000912
0.058
0.037
0
0.063
0.038
0
0.043
0.010
0
0.034
0.0065
CD8
IFNγ
B
Baseline
CD49d/CD28+Nuc
CD49d/CD28+Spike
46 days
53 days
67 days
117 days
207 days
0.00157
0.054
0.026
0.000546
0.066
0.035
0.000631
0.076
0.030
0.00113
0.049
0.021
0.00244
0.032
0.014
CD4
IFNγ