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Supplemental information

SARS-CoV-2 breakthrough infection induces rapid memory and *de novo* T cell responses

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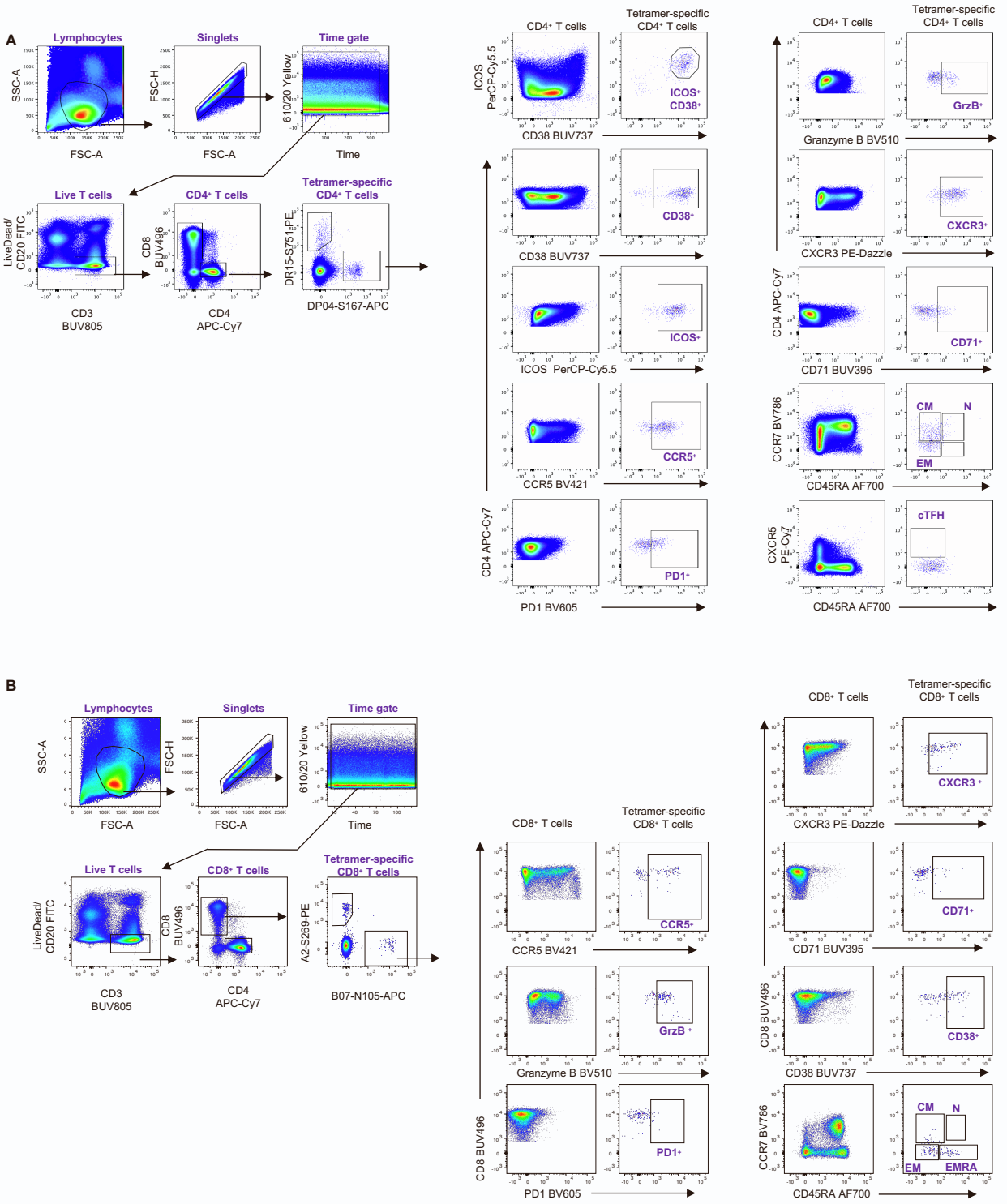


Figure S1. Gating strategy for the identification of pMHC-specific CD4⁺ and CD8⁺ T cells and their phenotypic characterisation, related to Figures 1-4. (A) Lymphocytes were identified by FSC-A vs SSC-A gating, followed by doublet exclusion (FSC- A vs FSC-H), a time gate and then gating on live T cells ($CD3^+CD19^-$) and subsequently $CD4^+CD8^-$ cells. HLA-DR*15-S_{751} and HLA-DP*04-S_{167}-specific cells were identified within $CD4^+$ T cells, and phenotyped as indicated, with the total $CD4^+$ T cell population serving as a reference for gating of phenotypic markers. **(B)** $CD8^+$ T cell were identified as $CD4^-CD8^+$ within live T cells gated as in (A). HLA-A*02-S_{269}, HLA-A*03-S_{378} and HLA-A*24-S_{1208} and HLA-B*07-N_{105}-specific $CD8^+$ T cells were identified within $CD8^+$ T cells and phenotyped as indicated, with the total $CD8^+$ T cell population serving as a reference for gating of phenotypic markers.

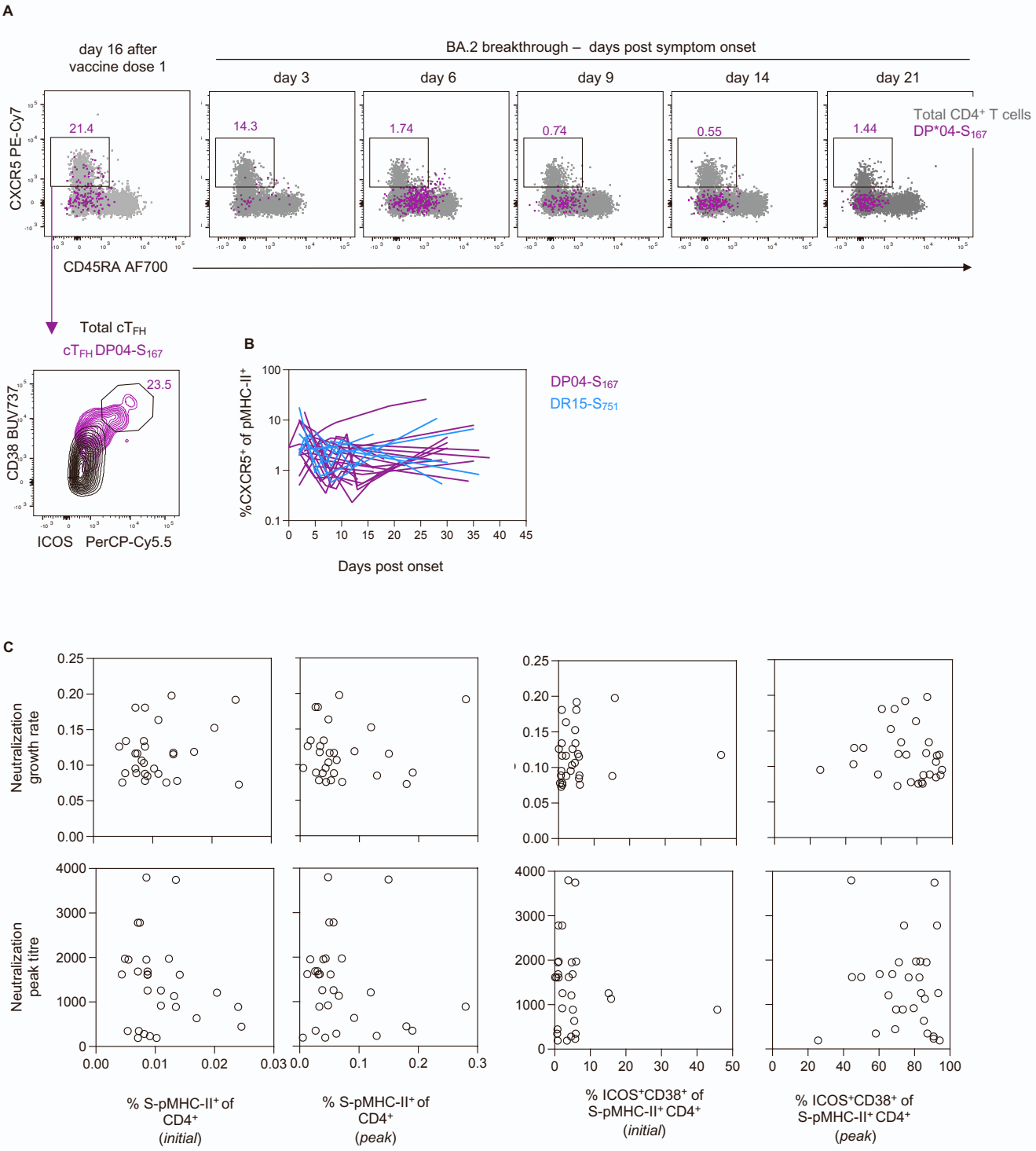


Figure S2. Limited cTFH phenotype following BTI, related to Figure 2. (A) Representative flow cytometry plots of the cTFH phenotype ($CXCR5^+CD45RA^-$) for HLA-DP*04-S_{167}-specific $CD4^+$ T cells. A post-vaccination sample was included in acquisition and analysis, serving as a reference for the cTFH activation and cTFH activation (CD38/ICOS expression). **(B)** Kinetics of $CXCR5^+$ cells for both pMHC-II populations, n=19 for DP*04-S_{167} and n=9 for DR*15-S_{751}. **(C)** Correlations between the initial or peak frequency of S-pMHC-II^+ $CD4^+$ T cells and initial or peak frequency of $ICOS^+CD38^+$ S-pMHC-II^+ $CD4^+$ T cells with the growth rate and peak value of neutralising antibody titres. Spearman correlation coefficient and p-values along with a linear regression line are shown for statistically significant comparisons ($p<0.05$), n=29 datapoints, pooled for both S-pMHC-II^+ $CD4^+$ T cell populations.

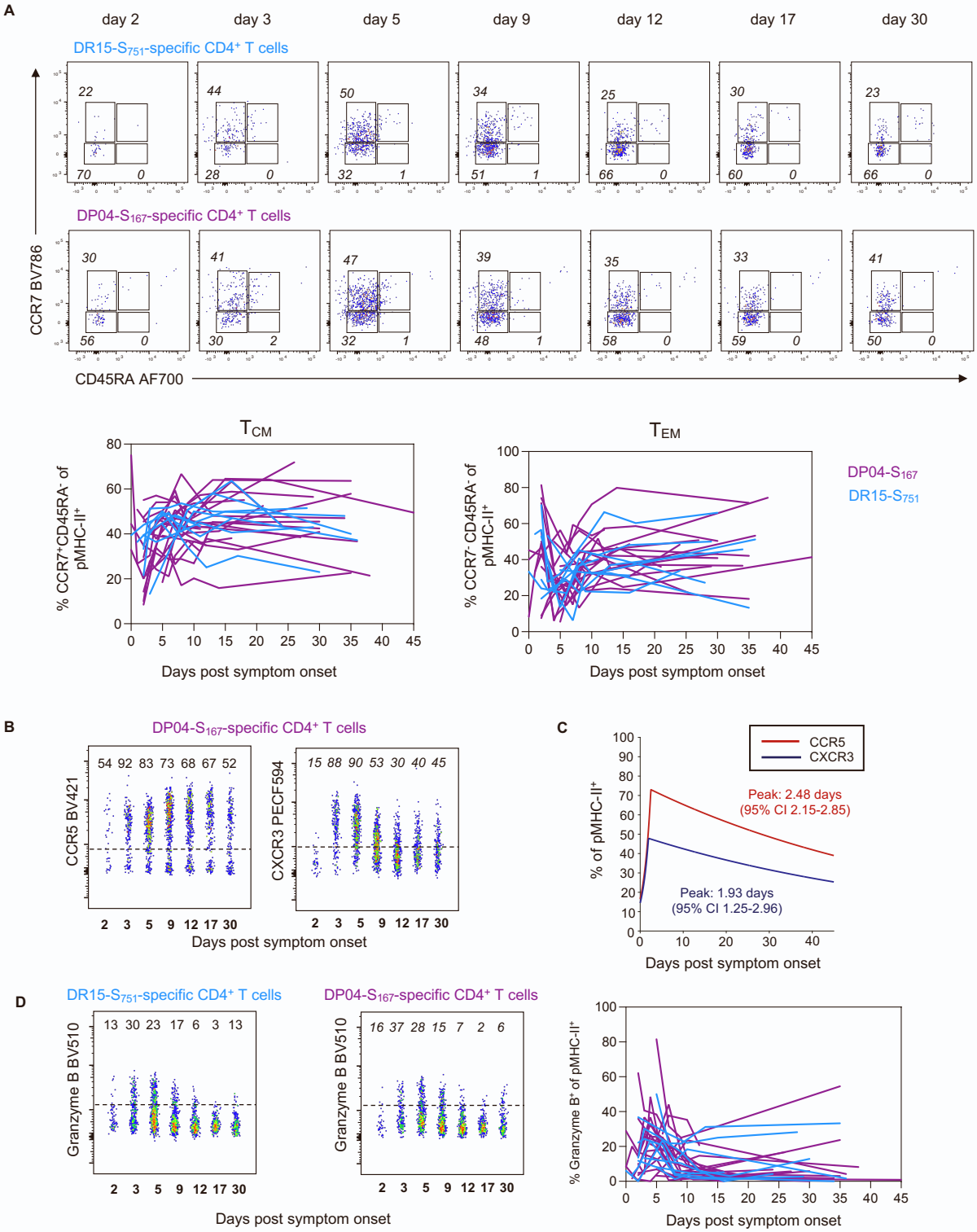
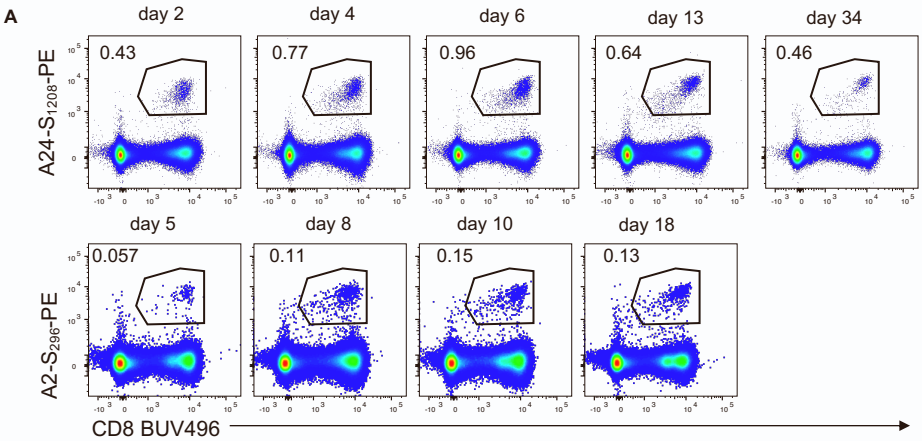
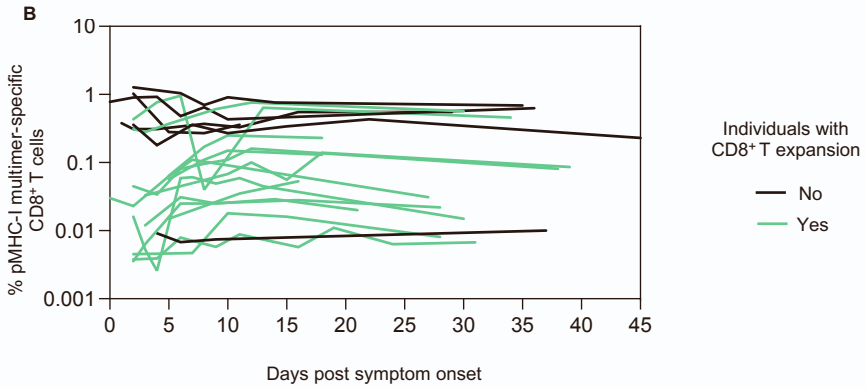
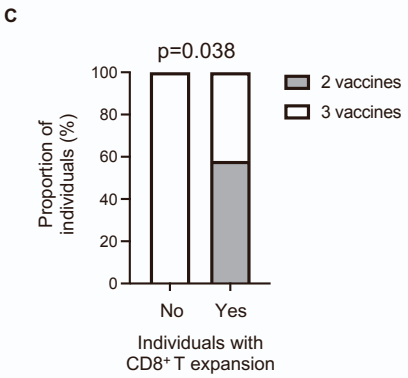
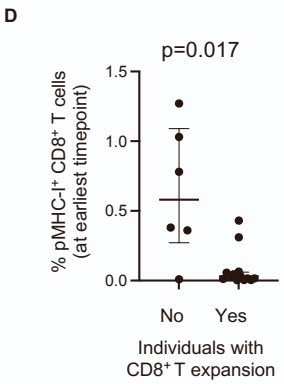

A
day 2
day 3
day 5
day 9
day 12
day 17
day 30
DR15-S751-specific CD4+ T cells
22 70 0
44 28 0
50 32 1
34 51 1
25 66 0
30 60 0
23 66 0
DP04-S167-specific CD4+ T cells
30 56 0
41 30 2
47 32 1
39 48 1
35 58 0
33 59 0
41 50 0
CCR7 BV786
CD45RA AF700
TCM
% CCR7+CD45RA- of pMHC-II+
Days post symptom onset
TEM
% CCR7- CD45RA- of pMHC-II+
Days post symptom onset
DP04-S167
DR15-S751
B
DP04-S167-specific CD4+ T cells
54 92 83 73 68 67 52
15 88 90 53 30 40 45
CCR5 BV421
CXCR3 PECF594
2 3 5 9 12 17 30
Days post symptom onset
C
CCR5
CXCR3
Peak: 2.48 days
(95% CI 2.15-2.85)
Peak: 1.93 days
(95% CI 1.25-2.96)
% of pMHC-II+
Days post symptom onset
D
DR15-S751-specific CD4+ T cells
DP04-S167-specific CD4+ T cells
13 30 23 17 6 3 13
16 37 28 15 7 2 6
Granzyme B BV510
2 3 5 9 12 17 30
Days post symptom onset
% Granzyme B+ of pMHC-II+
Days post symptom onset

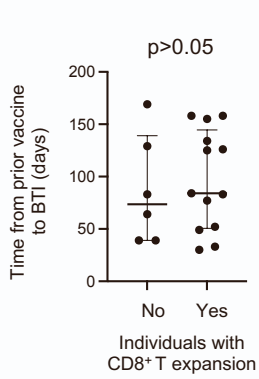
Figure S3. Phenotype of S-specific $CD4^+$ T cells, related to Figure 2. **(A)** Representative flow cytometry plots of HLA-DR*15-S_{751} and HLA-DP*04-S_{167}-specific $CD4^+$ T cells from a single participant showing co-expression of CCR7 and CD45RA. Kinetics of T_{CM} and T_{EM} for both pMHC-II populations. **(B)** Flow cytometry plots of HLA-DP*04-S_{167}-specific $CD4^+$ T cells from a single participant for CCR5 and CXCR5 expression, representative of the data shown in Fig 2. **(C)** Estimated kinetics of CCR5 and CXCR3 expression. The lines indicate the mean estimate for measurement from the piecewise linear regression model, using pooled data from both pMHC-II populations as no significant differences were found between the two. **(D)** Flow cytometry plots of HLA-DR*15-S_{751} and HLA-DP*04-S_{167}-specific $CD4^+$ T cells from a single participant showing expression of Granzyme B, with kinetics from all participants shown both pMHC-II populations. Throughout the figure, coloured lines represent individual donors for each pMHC-specific population, n=19 for DP*04-S_{167} and n=9 for DR*15-S_{751}.


A
day 2
day 4
day 6
day 13
day 34
0.43
0.77
0.96
0.64
0.46
A24-S1208-PE
day 5
day 8
day 10
day 18
0.057
0.11
0.15
0.13
A2-S296-PE
CD8 BUV496


B
% pMHC-I multimer-specific CD8+ T cells
Days post symptom onset
Individuals with CD8+ T expansion
No
Yes


C
p=0.038
Proportion of individuals (%)
2 vaccines
3 vaccines
No
Yes
Individuals with CD8+ T expansion


D
p=0.017
% pMHC-I+ CD8+ T cells (at earliest timepoint)
No
Yes
Individuals with CD8+ T expansion


E
p>0.05
Time from prior vaccine to BTI (days)
No
Yes
Individuals with CD8+ T expansion

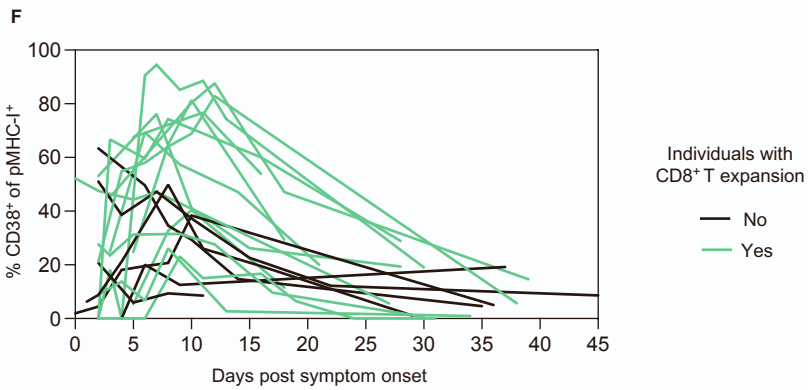

F
% CD38+ of pMHC-I+
Days post symptom onset
Individuals with CD8+ T expansion
No
Yes

Figure S4. $CD8^+$ T cell responses following BTI, related to Figure 3. **(A)** Representative flow cytometry plots of HLA-A*02-S_{269} and HLA-A*24-S_{1208} -specific $CD8^+$ T cell kinetics. **(B)** pMHC-I^+ $CD8^+$ T cell kinetics colour coded for individuals with an observable expansion and those without. **(C)** Vaccination history of individuals with an observable expansion and those without. **(D)** pMHC-I^+ $CD8^+$ T cell frequency at earliest available timepoint for individuals with an observable expansion and those without. **(E)** Time from last vaccination to BTI for individuals with an observable and those without. **(F)** $CD38^+$ phenotype for pMHC-I^+ $CD8^+$ T cell kinetics colour coded for individuals with an observable expansion and those without.

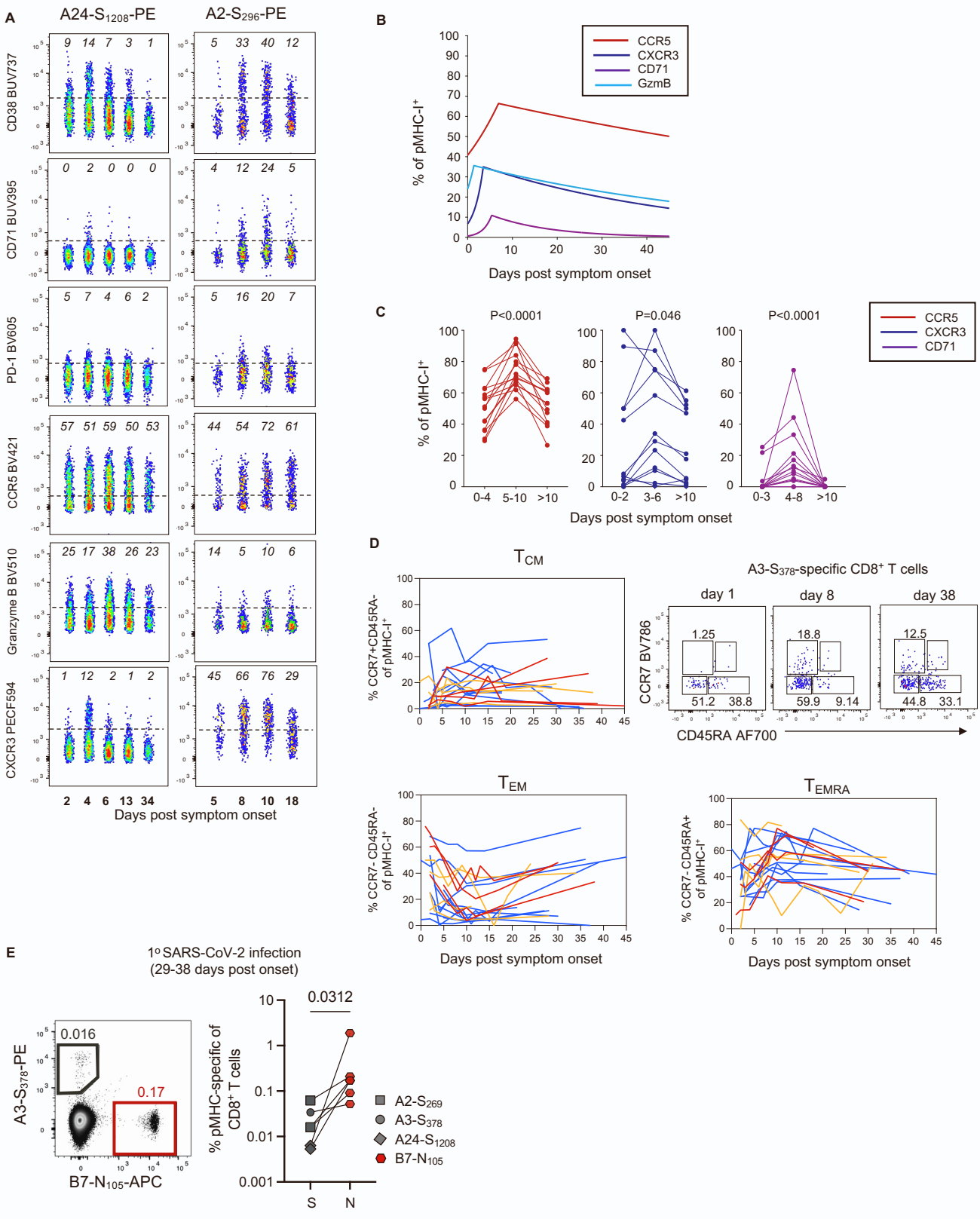

A
A24-S1208-PE
A2-S296-PE
CD38 BUV737
CD71 BUV395
PD-1 BV605
CCR5 BV421
Granzyme B BV510
CXCR3 PECF594
9 14 7 3 1
5 33 40 12
0 2 0 0 0
4 12 24 5
5 7 4 6 2
5 16 20 7
57 51 59 50 53
44 54 72 61
25 17 38 26 23
14 5 10 6
1 12 2 1 2
45 66 76 29
2 4 6 13 34
5 8 10 18
Days post symptom onset
B
CCR5
CXCR3
CD71
GzmB
% of pMHC-I+
Days post symptom onset
C
P<0.0001
P=0.046
P<0.0001
CCR5
CXCR3
CD71
% of pMHC-I+
0-4 5-10 >10
0-2 3-6 >10
0-3 4-8 >10
Days post symptom onset
D
TCM
% CCR7+CD45RA- of pMHC-I+
A3-S378-specific CD8+ T cells
day 1
day 8
day 38
1.25
51.2 38.8
18.8
59.9 9.14
12.5
44.8 33.1
CCR7 BV786
CD45RA AF700
TEM
% CCR7- CD45RA- of pMHC-I+
Days post symptom onset
TEMRA
% CCR7- CD45RA+ of pMHC-I+
Days post symptom onset
E
1º SARS-CoV-2 infection
(29-38 days post onset)
0.016
0.17
A3-S378-PE
B7-N105-APC
% pMHC-specific of CD8+ T cells
0.0312
A2-S269
A3-S378
A24-S1208
B7-N105
S
N

Figure S5. Phenotype of S-specific CD8$^+$ T cells, related to Figures 3 and 4. (A) Flow cytometry plots of HLA-A*02-S_{269} and HLA-A*24-S_{1208}-specific CD8$^+$ T cells from a single participant for different phenotypic markers, representative of the data shown in Fig 3. **(B)** Estimated kinetics of CCR5, CXCR3, CD71 and GzmB expression. The lines indicate the mean estimate for measurement from the piecewise linear regression model, using pooled data from both pMHC-I populations as no significant differences were found between the two. **(C)** Changes in CCR5, CXCR3 and CD71 expression during the course of BTI, binned according to the kinetics estimated by linear regression (n=12-15). Statistics assessed by Friedman test. Data are shown for individuals with samples available at all timepoints indicated.**(D)** Representative flow cytometry plots of HLA-A*03-S_{378} -specific CD8$^+$ T cells from a single participant showing co-expression of CCR7 and CD45RA. Kinetics of T_{CM} T_{EM} and T_{EMRA} for all 3 pMHC-I populations. Throughout the figure, coloured lines represent individual donors for each pMHC-I-specific population, n=11 for A*02-S_{269}, n=4 A*03-S_{378} and n=4 for A*24-S_{1208}. **(E)** Frequency of spike-specific and nucleoprotein-specific CD8$^+$ T cells in convalescent samples from primary SARS-CoV-2 infection. For (C), n=6 donors with paired analysis of spike-specific CD8$^+$ T cells (either A*02, A*03 and A*24) and B*07-N_{105}.

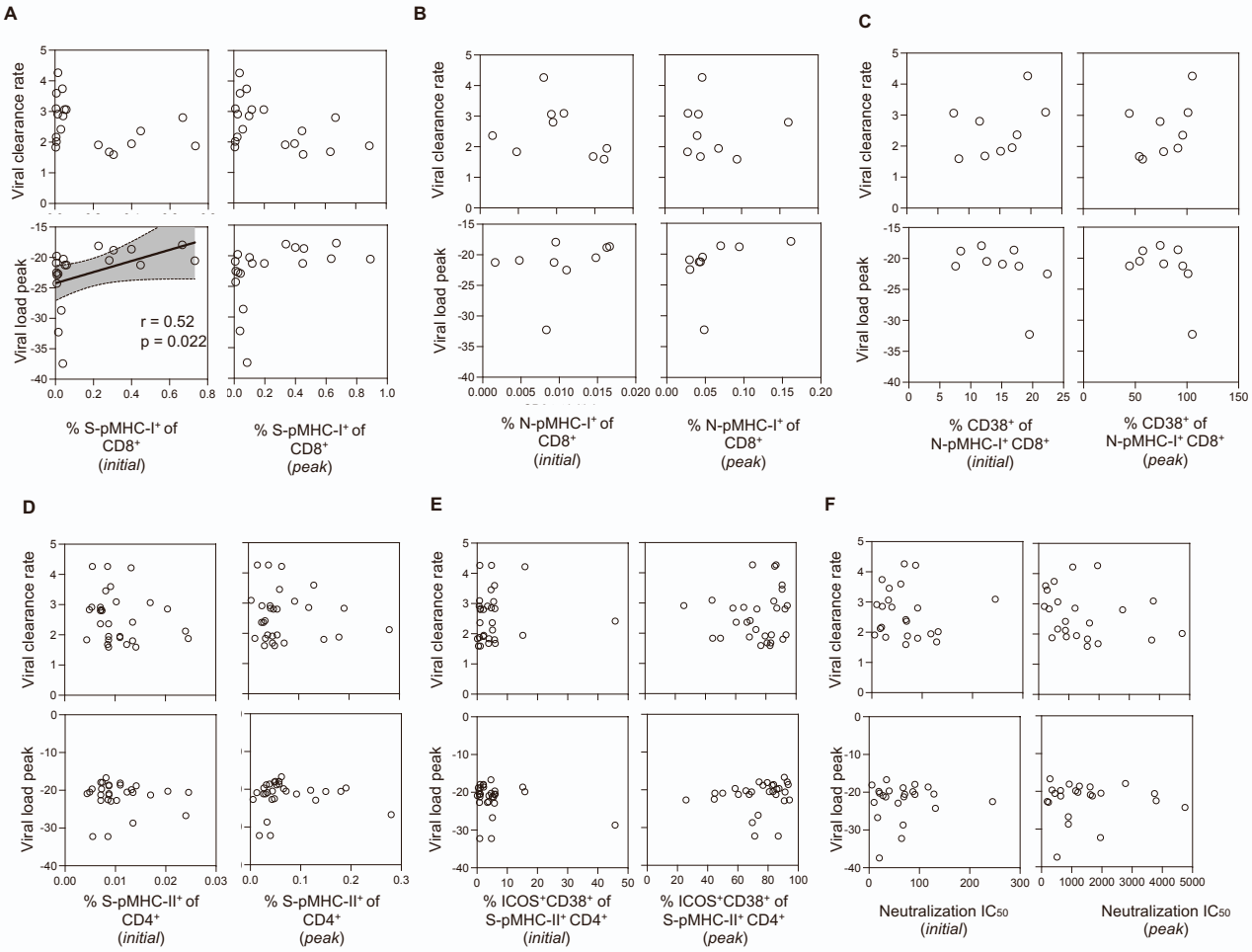


Figure S6. Correlations between immune recall and viral clearance, related to Figure 5. Correlations between the initial or peak frequency of **(A)** S-pMHC-I$^+$ CD8$^+$ T cells, **(B)** N-pMHC-I$^+$ CD8$^+$ T cells or **(C)** CD38$^+$ N-pMHC-I$^+$ CD8$^+$ T cells, **(D)** S-pMHC-II$^+$ CD4$^+$ T cells, **(E)** ICOS$^+$CD38$^+$S-pMHC-II$^+$ CD4$^+$ T cells, **(F)** neutralisation IC_{50} titre against infecting or antigenically similar live virus and viral clearance rate or peak Ct value (amongst available timepoints). Throughout the figure Spearman correlation coefficient and p-values along with a linear regression line are shown for statistically significant comparisons ($p<0.05$), n=19 for (A), n=9 for (B-C), n=29 datapoints, pooled for both S-pMHC-II$^+$ CD4$^+$ T cell populations for (D-E), n=23 for (F).

Supplementary Table 1. Cohort demographics, related to Figure 1

Subject	Gender	Age	# Prior Vaccine doses	Last vaccine to symptom onset (days)	Class I Alleles (relevant to this study)	Class II Alleles (relevant to this study)	VOC
CP105	M	29	2	N.D.	A*02:01	--	Delta
CP106	F	30	2	139	--	DP*04:01	Delta
CP107	F	31	2	77-106	A*02:01	DP*04:01	Delta
CP108	M	29	2	132	--	DP*04:01	Delta
COR034	M	31	2	126	A*02:01, B*07:02	DR*15:01, DP*04:01	Delta
COR136	F	50	2	158	A*02:01, A*03:01	DP*04:01	Delta
COR015	F	27	3	39	A*02:01	--	BA.1
COR032	F	23	3	33	A*24:02, B*07:02	DR*15:01, DP*04:01	BA.1
CP110	M	24	2	84	A*02:01	DP*04:01	BA.1
CP111	M	34	2	83	A*24:02	--	BA.1
CP112	F	35	2	90	A*02:01^	DP*04:01	BA.1
COR198	F	60	3	39	A*02:01	DP*04:02	BA.1
CP69*	F	36	2	111	A*02:01	DR*15:01, DP*04:01	BA.1
CP40*	M	63	3	49	--	DR*15:01, DP*04:01	BA.2
COR291	M	50	3	134	A*02:01	DR*15:01	BA.2
COR043	F	55	3	64	A*02:01	DP*04:01	BA.2
CP117	M	40	3	52	A*03:01, B*07:02	DR*15:01, DP*04:01	BA.2
COR281	F	43	3	129	A*03:01, B*07:02	DR*15:01, DP*04:01	BA.2
CP118	M	66	3	100	--	DP*04:01	BA.2
COR274	F	57	3	155	A*03:01	DP*04:01, 04:02	BA.2
COR275	M	62	3	83	A*02:01, B*07:02	DR*15:01, DP*04:01	BA.2
COR215	F	55	3	125	A*24:02, B*07:02	DR*15:01, DP*04:01	BA.2
COR039	F	58	3	169	A*24:02, B*07:02	DR*15:01, DP*04:01	BA.2

*Previously infected during Hu-1 wave. ^Data not shown, due to lack of A*02:01 pentamer binding in any longitudinal samples from participant; N.D., not determined.

Supplementary Table 2. Piecewise linear regression parameters of viral kinetics and neutralising antibodies (with 95% CI), related to Figure 1. Values in bold indicate a significant difference between VOCs.

		Initial value	**Delay (days PSO)**	**Growth Rate (per day)**	**Peak Time (days PSO)**	**Decay Rate (per day)**
Viral RNA (ΔCt)	Delta	36.5 (44.71 – 28.29)	N/A	5.47 (2.83 – 10.57)	3.22 (2.53 – 4.1)	**3.97** (3.03 – 5.22)
	BA.1	31.79 (49.98 – 13.6)	N/A	4.41 (0.82 – 23.77)	2.43 (1.24 – 4.78)*	**2.13** (1.03 – 4.43)
	BA.2	24.2 (49.5 – 1.1)	N/A	6.73 (0.32 – 139.23)	1.12 (0.47 – 2.63)	**2.04** (1.12 – 3.73)
	Nab (IC_{50})	51.29 (30.52 – 86.18)	4.3 (2.2 – 8.42)	0.12 (0.084 – 0.18)	14.58 (12.23 – 17.39)	N/A

Supplementary Table 3. Estimates of spike-specific $CD4^+$ T cell expansion, activation and phenotypic parameters (with 95% CI), related to Figures 2 and 5. Pooled estimates from both epitopes are shown if the epitope-specific estimates were not significantly different for any parameter. Separate epitope-specific estimates are shown if at least one of the parameters of that makers were significantly different between epitopes. Values in bold indicate a significant difference between epitopes for the indicated marker.

	Initial (%)	**Delay (days PSO)**	**Growth Rate (per day)**	**Peak Time (days PSO)**	**Decay Rate (per day)**
$\%pMCH^+$ of $CD4^+$	0.0093 (0.0071 – 0.012)	2.51 (1.86 – 3.4)	0.24 (0.16 – 0.35)	5.42 (4.81 – 6.1)	0.014 (0.01 – 0.019)
$\%CD38^+$ $ICOS^+$ of $pMCH^+$	2.44 (1.1 – 5.42)	1.12 (0.7 – 1.8)	0.62 (0.5 – 0.76)	3.60 (3.22 – 4.01)	0.057 (0.051 – 0.063)

	Initial (%)	**Growth Rate (per day)**	**Peak Time (days PSO)**	**Decay Rate (per day)**
$\%CCR5^+$ *(pooled epitopes)*	16.59 (12 – 22.96)	0.26 (0.19 0 0.35)	2.48 (2.15 – 2.85)	0.0064 (0.0043 – 0.0095)
$\%CXCR3^+$ *(pooled epitopes)*	14.4 (7.51 – 27.8)	0.27 (0.13 – 0.54)	1.93 (1.25 – 2.96)	0.0064 (0.0032 – 0.013)
$\%GzmB^+$ *(pooled epitopes)*	7.34 (4.27 – 12.62)	0.13 (0.04 – 0.4)	1.97 (0.88 – 4.38)	0.019 (0.013 – 0.029)
$\%PD\text{-}1^+$ *(DP04)*	22.38 (10.49 – 47.78)	0.11 (0.05 – 0.26)	4.05 (3.29 – 5)	**0.0078** (0.004 – 0.015)
$\%PD\text{-}1^+$ *(DR15)*	34.04 (6.7 – 172.82)	0.12 (0.019 – 0.78)	2.71 (1.46 – 5.03)	**0.00047** (0.00023 – 0.0093)
$\%CD71^+$ *(DP04)*	1.05 (0.39 – 2.86)	0.38 (0.23 – 0.62)	3.35 (2.73 – 4.11)	**0.077** (0.056 – 0.11)
$\%CD71^+$ *(DR15)*	1.32 (0.08 – 21.71)	0.45 (0.00011 – 1.821)	1.64 (0.001 – 2.588)	**0.038** (0.014 – 0.1)

Supplementary Table 4. Estimates of S-specific $CD8^+$ T cell expansion and activation (with 95% CI), related to Figures 3 and 5. Pooled estimates from all three epitopes are shown.

	Initial (%)	**Delay (days PSO)**	**Growth Rate (per day)**	**Peak Time (days PSO)**	**Decay Rate (per day)**
$\%pMCH^+$ of $CD8^+$	0.055 (0.024 – 0.12)	4.39 (3.4 – 5.68)	0.15 (0.07 – 0.3)	5.81 (4.87 – 6.94)	0.00068 (4.7e-7 – 0.98)
$\%CD38^+$ of $pMCH^+$	15.85 (10.82 – 23.21)	0.24 (0.019 – 3.06)	0.082 (0.055 – 0.12)	7.1 (5.86 – 8.6)	0.036 (0.028 – 0.046)

	Initial (%)	**Growth Rate (per day)**	**Peak Time (days PSO)**	**Decay Rate (per day)**
$\%CCR5^+$	40.74 (34.41 – 48.23)	0.031 (0.018 – 0.052)	6.89 (5.17 – 9.17)	0.0032 (0.0015 – 0.0068)
$\%CXCR3^+$	6.68 (2.93 – 15.26)	0.2 (0.12 – 0.34)	3.49 (2.56 – 4.75)	0.0093 (0.0049 – 0.017)
$\%GzmB^+$	23.99 (13.77 – 41.79)	0.12 (0.007 – 2.14)	1.4 (0.15 – 12.58)	0.0069 (0.0039 – 0.012)
$\%CD71^+$	0.64 (0.2 – 2)	0.23 (0.14 – 0.38)	5.36 (4.25 – 6.77)	0.033 (0.022 – 0.049)
$\%PD\text{-}1^+$	7.52 (4.53 – 12.46)	0.096 (0.04 – 0.23)	3.32 (2.14 – 5.16)	0.0079 (0.0047 – 0.013)

Supplementary Table 5. Estimates of S- and N-specific $CD8^+$ T cell expansion and activation (with 95% CI), related to Figures 4 and 5. Values in bold indicate a significant difference between epitopes for the indicated marker.

	Initial (%)	**Delay (days PSO)**	**Growth Rate (per day)**	**Peak Time (days PSO)**	**Decay Rate (per day)**
%$pMCH^+$ of $CD8^+$ **(S)**	**0.098** (0.012 – 0.82)	6.29 (1.49 – 26.46)	0.087 (0.013 – 0.556)	7.69 (3.56 – 16.57)	0 (NA)
%$pMCH^+$ of $CD8^+$ **(N)**	**0.012** (0.0048 – 0.03)	3.06 (1.62 -5.81)	0.12 (0.071 – 0.2)	8.17 (6.32 – 10.56)	0 (NA)
%$CD38^+$ **(S)**	10.16 (1.66 – 62.36)	0.95 (0.001 – 86.85)	0.073 (0.01 – 0.52)	8.29 (4.45 – 15.43)	**0.047** (0.014 – 0.16)
%$CD38^+$ **(N)**	17.78 (9.58 – 33)	3.63 (2.28 – 5.79)	0.19 (0.077 – 0.45)	6.89 (5.26 – 9.02)	**0.019** (0.011 – 0.034)

Supplementary Table 6. Estimates of S- and N-specific $CD8^+$ T cell phenotypic parameters (with 95% CI) for individuals presented in Figure 4. Estimates for N-specific $CD8^+$ T cells only are shown if the S -and N-specific estimates were not significantly different for any parameter (estimates for S only are shown in Supplementary Table 6. Separate epitope-specific estimates are shown if at least one of the parameters of that makers were significantly different between epitopes. Values in bold indicate a significant difference between epitopes for the indicated marker. Growth and decay parameters for PD-1 expression kinetics could not be determined.

	Initial (%)	**Growth Rate (per day)**	**Peak Time (days PSO)**	**Decay Rate (per day)**
%GzmB$^+$ **(S)**	**22.75** (4.81 – 135.88)	0.100 (0.0002 – 5.43)	1.22 (0.01 – 141.04)	0.0081 (0.0015 – 0.042)
%GzmB$^+$ **(N)**	**6.83** (3.4 – 13.76)	0.08 (0.045 – 0.17)	5.26 (3.14 – 8.81)	0.021 (0.012 – 0.038)
%CCR5$^+$ **(S)**	**40.93** (14.96 – 111.96)	**0.017** (0.0043 – 0.071)	7.52 (2.69 – 21.05)	0.0002 (1e-13 – 3.6)
%CCR5$^+$ **(N)**	**2.15** (1.32 – 3.48)	**0.29** (0.2 – 0.41)	4.95 (3.75 – 6.52)	0.01 (0.0066 – 0.016)
%CD71$^+$ **(N)**	0.74 (0.11 - 5.05)	0.36 (0.16 - 0.85)	4.13 (3.09 - 5.54)	0.045 (0.027 - 0.073)
%CXCR3$^+$ **(N)**	12.89 (6.79 - 24.45)	0.13 (0.062 - 0.28)	4.75 (3.08 - 7.33)	0.0038 (0.0006 - 0.025)