Figure 1A

	1	2	3	4	5	
PE	Peptide 4	Peptide 4	Peptide 4	Peptide 4	Peptide 4	**UV-exchange**
APC-Cy7	Naïve	Digested Un-exchanged	Peptide 5	Dengue 1	Dengue 2	

	6	7	8	9	10	
PE	Peptide 4	Peptide 4	Peptide 4	Peptide 4	Peptide 4	**Enzyme exchange TRIS buffer PH 8 RT 16 hours**
APC-Cy7	Naïve	Digested Un-exchanged	Peptide 5	Dengue 1	Dengue 2	

	11	12	13	14	15	
PE	Peptide 4	Peptide 4	Peptide 4	Peptide 4	Peptide 4	**Enzyme exchange Citrate buffer PH 6.2 RT for 16 hours**
APC-Cy7	Naïve	Digested Un-exchanged	Peptide 5	Dengue 1	Dengue 2	

Positive control:

Peptide 4: AVFDRKSDAK (SEQ ID NO: 5)

Peptide 5: IVTDFSVIK (SEQ ID NO: 39)

Negative controls:

Dengue 1: GTSGSPIVNR (SEQ ID NO: 44)

Dengue 2: ATYGWNLVK (SEQ ID NO: 45)

Figure 1B

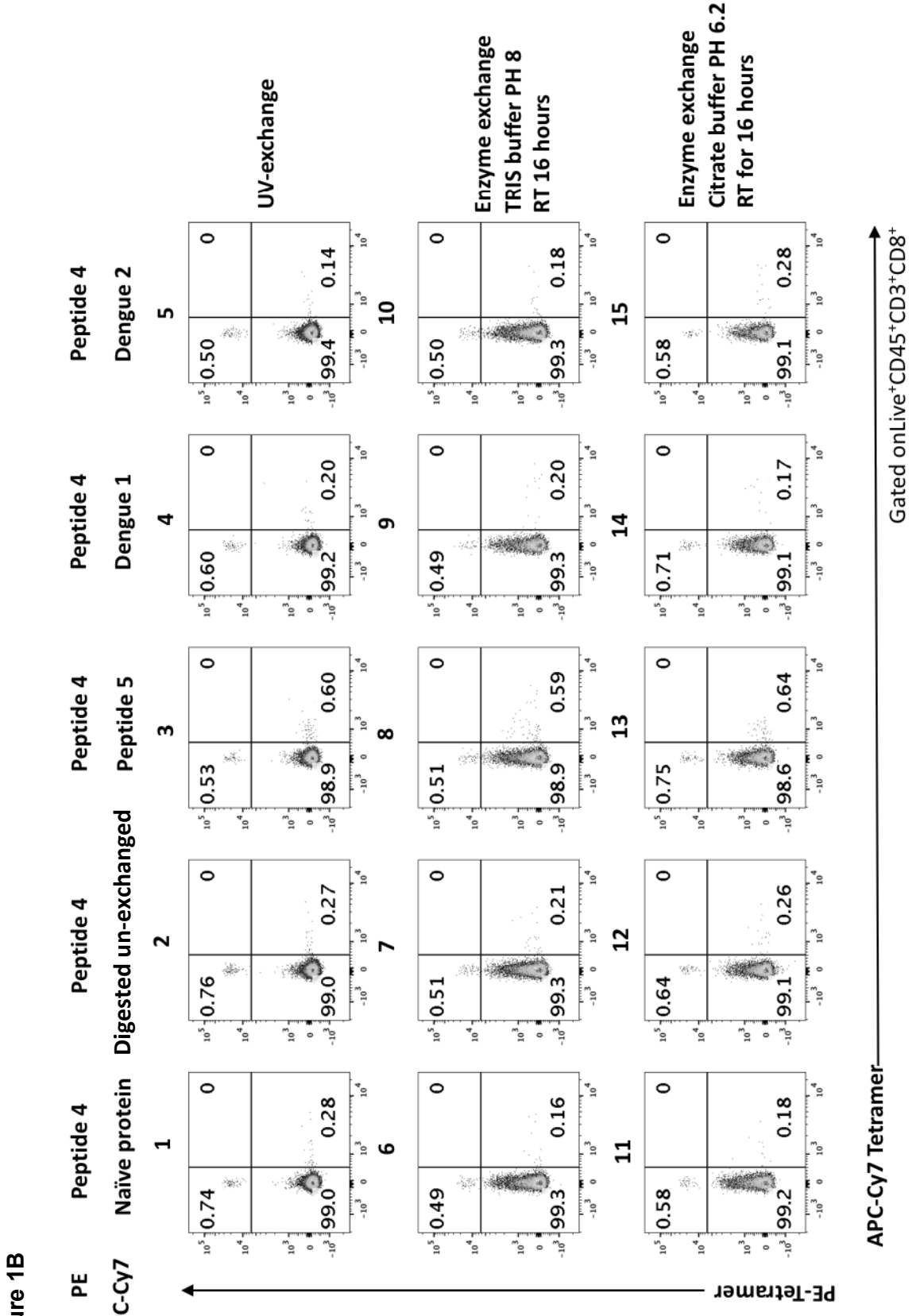

PE
APC-Cy7
Peptide 4
Naïve protein
Peptide 4
Digested un-exchanged
Peptide 4
Peptide 5
Peptide 4
Dengue 1
Peptide 4
Dengue 2
UV-exchange
Enzyme exchange
TRIS buffer PH 8
RT 16 hours
Enzyme exchange
Citrate buffer PH 6.2
RT for 16 hours
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
0.74
0
99.0
0.28
0.76
0
99.0
0.27
0.53
0
98.9
0.60
0.60
0
99.2
0.20
0.50
0
99.4
0.14
0.49
0
99.3
0.16
0.51
0
99.3
0.21
0.51
0
98.9
0.59
0.49
0
99.3
0.20
0.50
0
99.3
0.18
0.58
0
99.2
0.18
0.64
0
99.1
0.26
0.75
0
98.6
0.64
0.71
0
99.1
0.17
0.58
0
99.1
0.28
PE-Tetramer
APC-Cy7 Tetramer
Gated onLive+CD45+CD3+CD8+

Figure 2

A

No.	HLA-A*11:01 Control peptides Sequence	SAV 1	SAV 2	SAV 3	
1	SIIPSGPLK	152	166	175	SEQ ID NO: 46
2	RMVLASTTAK	166	174	175	SEQ ID NO: 47
3	KSMREEYRK	152	174	175	SEQ ID NO: 48
4	AVFDRKSDAK (influenza 1)	164	166	174	SEQ ID NO: 5
5	IVTDFSVIK (EBV 1)	152	154	166	SEQ ID NO: 39
6	NTLEQTVKK (EBV 2)	152	164	166	SEQ ID NO: 40
7	ATIGTAMYK	154	164	166	SEQ ID NO: 49
8	SSCSSCPLSK	152	166	174	SEQ ID NO: 50
9	YVNVNMGLK	154	164	175	SEQ ID NO: 51
10	NAIVE	154	166	174	
11	Digested un-exchanged	152	164	174	

B **EBV peptide 1 (IVTDFSVIK): 166-152-154**

(SEQ ID NO: 39)

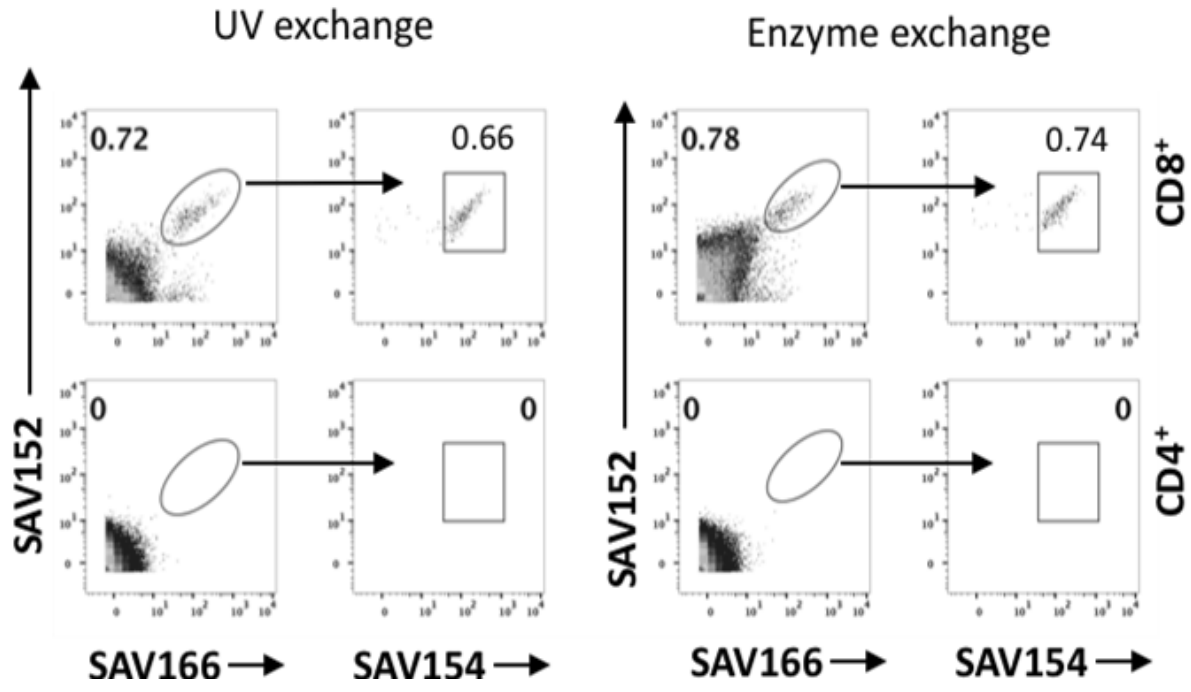
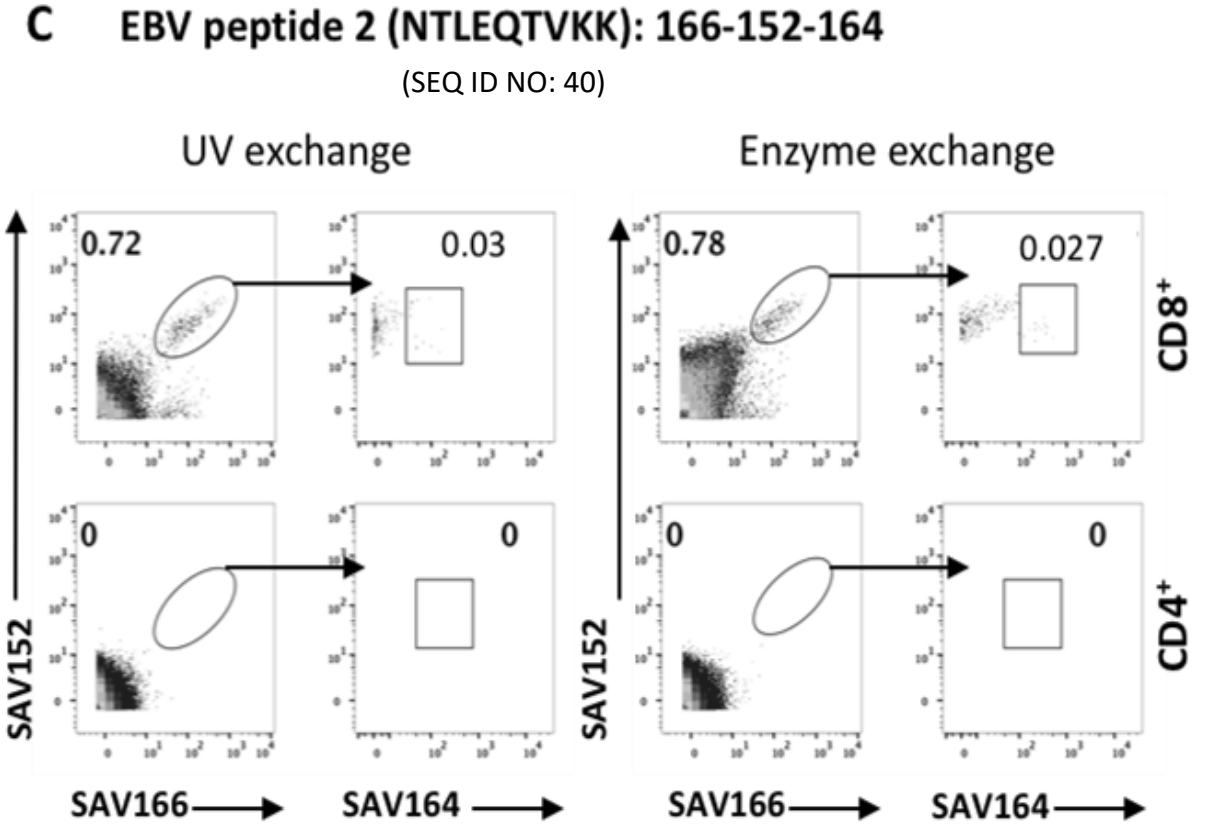


Figure 2 (continued)



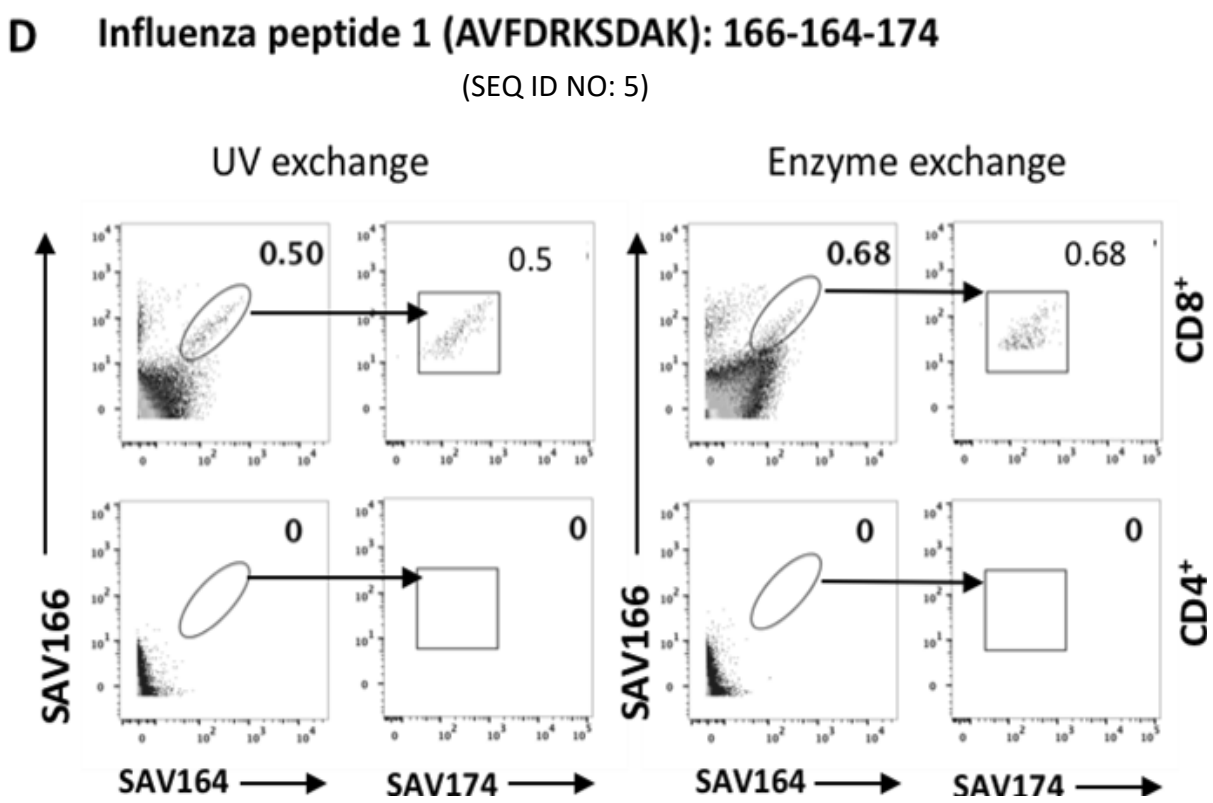


Figure 3A

PE	Peptide 1	Peptide 1	Peptide 1	Peptide 1	Peptide 1	UV-exchange (1.2mg/ml)
APC	Naive	Digested Unexchanged	Peptide 2	Peptide 3	Peptide 15	
PE	Peptide 1	Peptide 1	Peptide 1	Peptide 1	Peptide 1	UV-exchange (5mg/ml)
APC	Naive	Digested Unexchanged	Peptide 2	Peptide 3	Peptide 15	
PE	Peptide 1	Peptide 1	Peptide 1	Peptide 1	Peptide 1	Enzyme exchange TRIS buffer PH 8 RT 16 hours
APC	Naive	Digested Unexchanged	Peptide 2	Peptide 3	Peptide 15	
PE	Peptide 1		Peptide 1	Peptide 1	Peptide 1	Enzyme exchange TRIS Buffer PH 8 37°C 16 hours
APC	Naive		Peptide 2	Peptide 3	Peptide 15	
PE	Peptide 1		Peptide 1	Peptide 1	Peptide 1	Enzyme exchange Citrate buffer PH 6.2 RT for 16 hours
APC	Naive		Peptide 2	Peptide 3	Peptide 15	
PE	Peptide 1		Peptide 1	Peptide 1	Peptide 1	Enzyme exchange Citrate buffer PH 6.2 37°C for 16 hours
APC	Naive		Peptide 2	Peptide 3	Peptide 15	

Positive control:

Peptide 1: NLVPMVATV (SEQ ID NO: 41)

Negative controls:

Peptide 2: VLEETSVML (SEQ ID NO: 52)

Peptide 3: GTSGSPIVNR (SEQ ID NO: 44)

Peptide 15: FLDKGTYTL (SEQ ID NO: 53)

Figure 3B

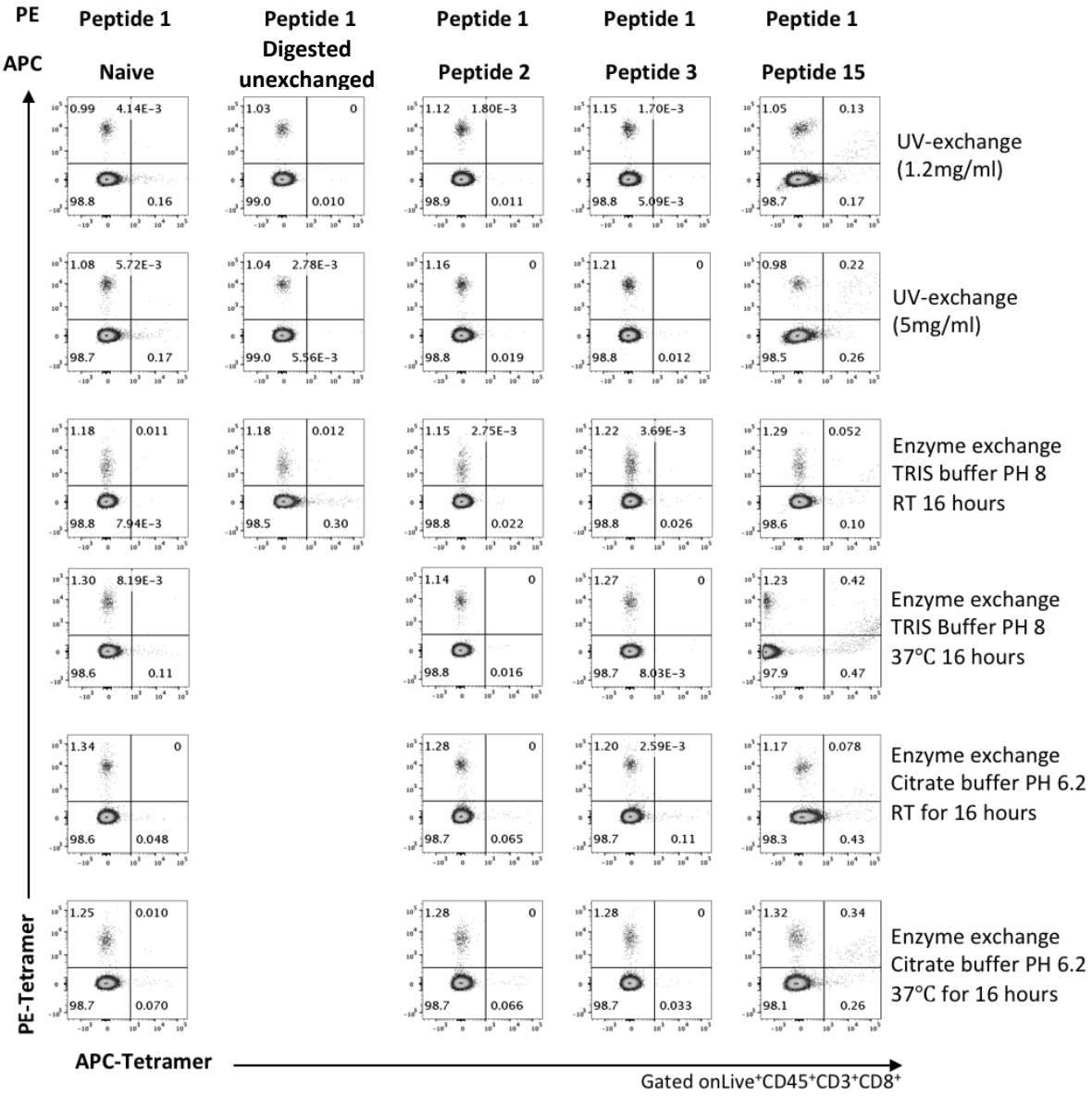

PE
APC
Peptide 1
Naive
Peptide 1
Digested
unexchanged
Peptide 1
Peptide 2
Peptide 1
Peptide 3
Peptide 1
Peptide 15
0.99 4.14E-3
98.8 0.16
1.03 0
99.0 0.010
1.12 1.80E-3
98.9 0.011
1.15 1.70E-3
98.8 5.09E-3
1.05 0.13
98.7 0.17
UV-exchange
(1.2mg/ml)
1.08 5.72E-3
98.7 0.17
1.04 2.78E-3
99.0 5.56E-3
1.16 0
98.8 0.019
1.21 0
98.8 0.012
0.98 0.22
98.5 0.26
UV-exchange
(5mg/ml)
1.18 0.011
98.8 7.94E-3
1.18 0.012
98.5 0.30
1.15 2.75E-3
98.8 0.022
1.22 3.69E-3
98.8 0.026
1.29 0.052
98.6 0.10
Enzyme exchange
TRIS buffer PH 8
RT 16 hours
1.30 8.19E-3
98.6 0.11
1.14 0
98.8 0.016
1.27 0
98.7 8.03E-3
1.23 0.42
97.9 0.47
Enzyme exchange
TRIS Buffer PH 8
37°C 16 hours
1.34 0
98.6 0.048
1.28 0
98.7 0.065
1.20 2.59E-3
98.7 0.11
1.17 0.078
98.3 0.43
Enzyme exchange
Citrate buffer PH 6.2
RT for 16 hours
1.25 0.010
98.7 0.070
1.28 0
98.7 0.066
1.28 0
98.7 0.033
1.32 0.34
98.1 0.26
Enzyme exchange
Citrate buffer PH 6.2
37°C for 16 hours
PE-Tetramer
APC-Tetramer
Gated onLive+CD45+CD3+CD8+

Figure 4A

PE	Peptide 1	Peptide 1	Peptide 1	Peptide 1	**UV-exchange**
APC	Naive	Digested unexchanged	Peptide 2	Peptide 11	
PE	Peptide 1	Peptide 1	Peptide 1	Peptide 1	**Enzyme exchange Cacodylate buffer PH 6.2 RT for 16 hours**
APC	Naive	Digested unexchanged	Peptide 2	Peptide 11	
PE	Peptide 1	Peptide 1	Peptide 1	Peptide 1	**Enzyme exchange TRIS buffer PH 8 RT 16 hours**
APC	Naive	Digested unexchanged	Peptide 2	Peptide 11	

Positive control:
Peptide 1: NLVPMVATV (SEQ ID NO: 41)

Negative controls:
Peptide 2: VLEETSVML (SEQ ID NO: 52)
Peptide 11: FLYALALLL (SEQ ID NO: 54)

Figure 4B

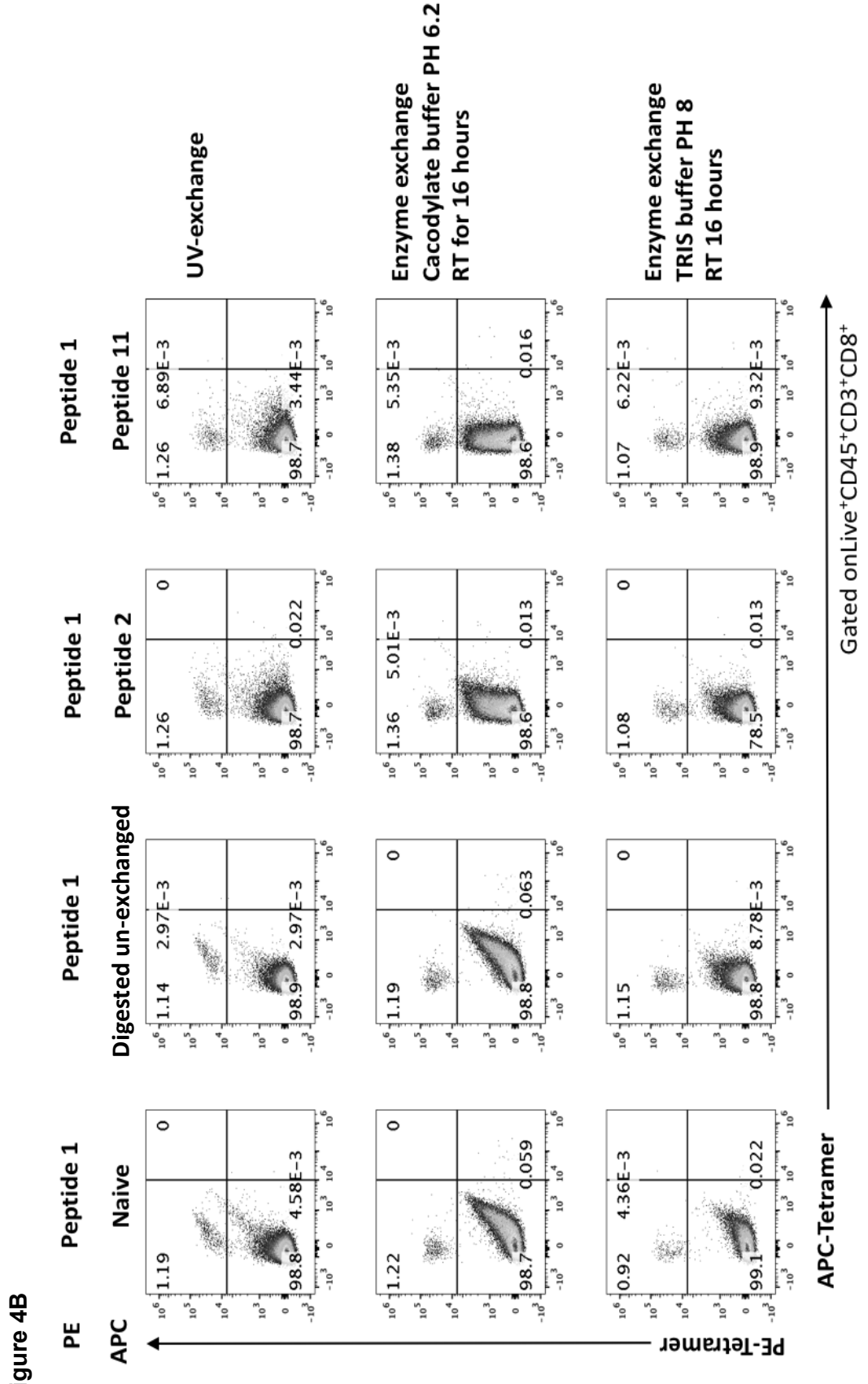
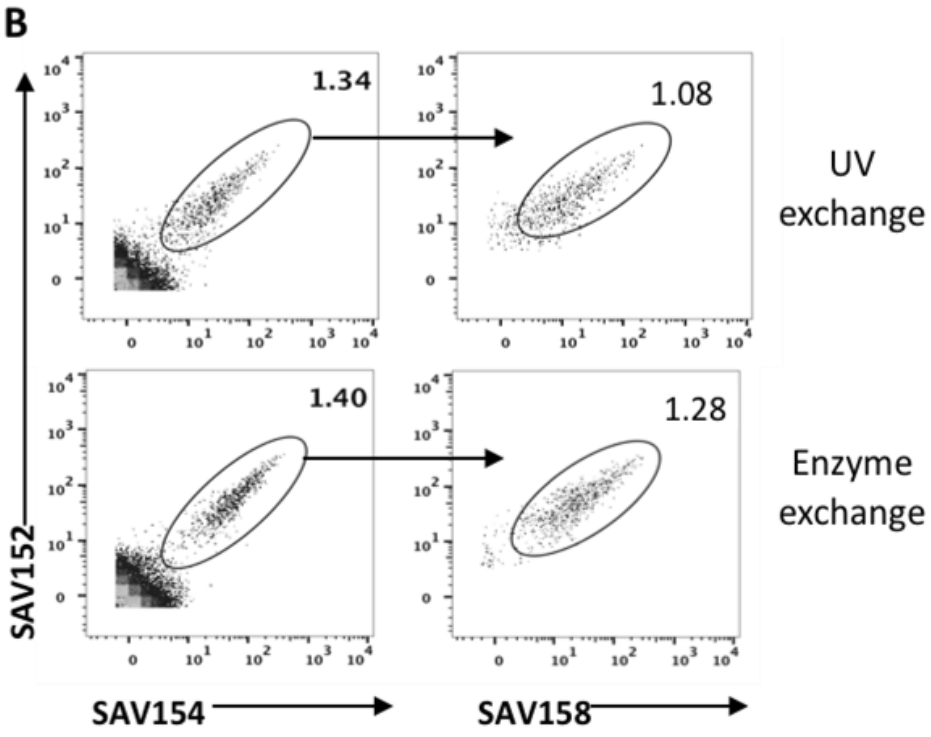

PE
Peptide 1
Peptide 1
Peptide 1
Peptide 1
APC
Naive
Digested un-exchanged
Peptide 2
Peptide 11
UV-exchange
Enzyme exchange
Cacodylate buffer PH 6.2
RT for 16 hours
Enzyme exchange
TRIS buffer PH 8
RT 16 hours
PE-Tetramer
APC-Tetramer
Gated onLive+CD45+CD3+CD8+

Figure 5

A

No.	Peptide	SAV Codes	
1	NLVPMVATV (CMV)	152, 154, 158	SEQ ID NO: 41
2	VLEETSVML	164, 166, 171	SEQ ID NO: 52
3	FLDKGTYTL	166,171, 175	SEQ ID NO: 53
4	FLYALALLL	164, 166, 175	SEQ ID NO: 54
5	Digested-Unexchanged	164, 173, 175	
6	Naïve Protein	171, 173, 175	

B



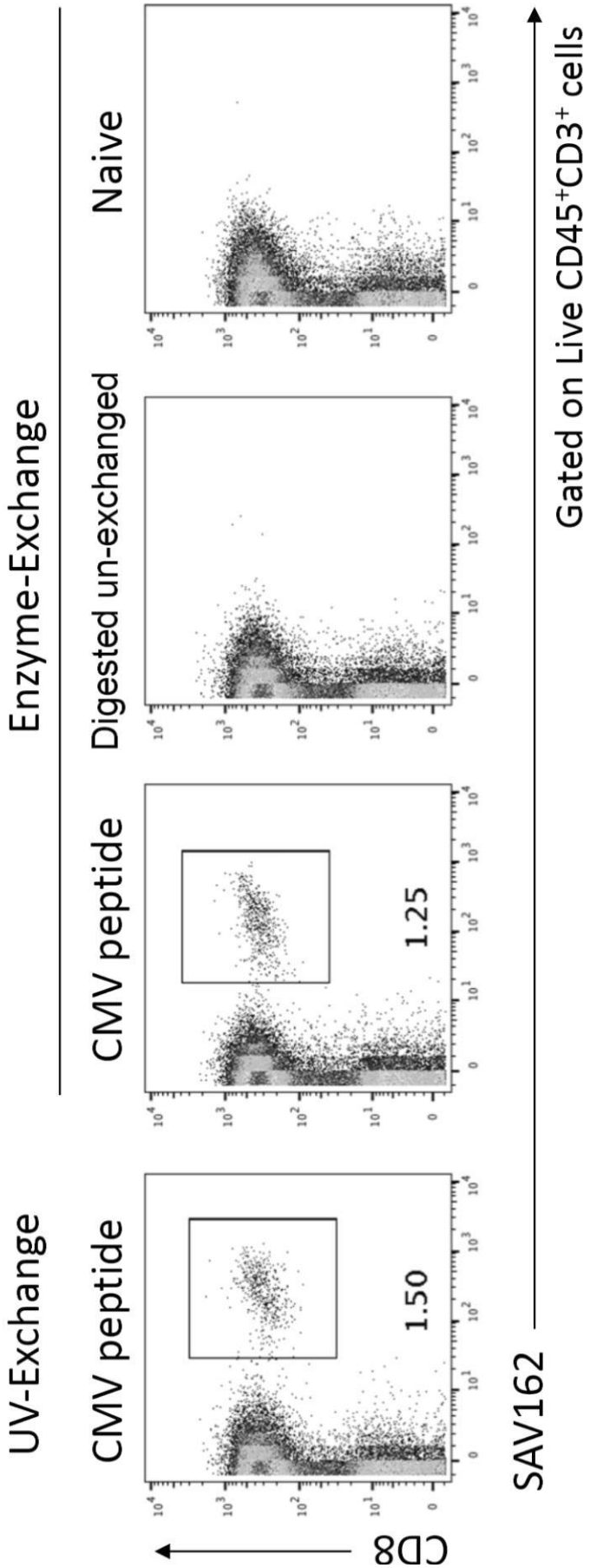

UV-Exchange
Enzyme-Exchange
CMV peptide
CMV peptide
Digested un-exchanged
Naive
1.50
1.25
CD8
SAV162
Gated on Live CD45+CD3+ cells

Figure 6