Figure 1

A

TCR	TRAV	TRAJ	CDR3α	TRBV	TRBJ	CDR3β
6218	21	53	ILSGGSNYKLT SEQ ID NO: 199	29	2-7	ASSFGREQY SEQ ID NO: 201
6218αC	21	53	ILSGGCNYKLT SEQ ID NO: 200	29	2-7	ASSFGREQY SEQ ID NO: 201
6218βC	21	53	ILSGGSNYKLT SEQ ID NO: 199	29	2-7	ASSFCREQY SEQ ID NO: 202

B

Peptide	Sequence
PA	SSLENFRAYV SEQ ID NO: 203
PA4C	SSLCNFRAYV SEQ ID NO: 204
PA7K	SSLENFKAYV SEQ ID NO: 205
PA7A	SSLENFAAYV SEQ ID NO: 206
PA7L	SSLENFLAYV SEQ ID NO: 207
PA4C7K	SSLCNFKAYV SEQ ID NO: 208
PA4C7A	SSLCNFAAYV SEQ ID NO: 209
PA4C7L	SSLCNFLAYV SEQ ID NO: 210

Figure 2

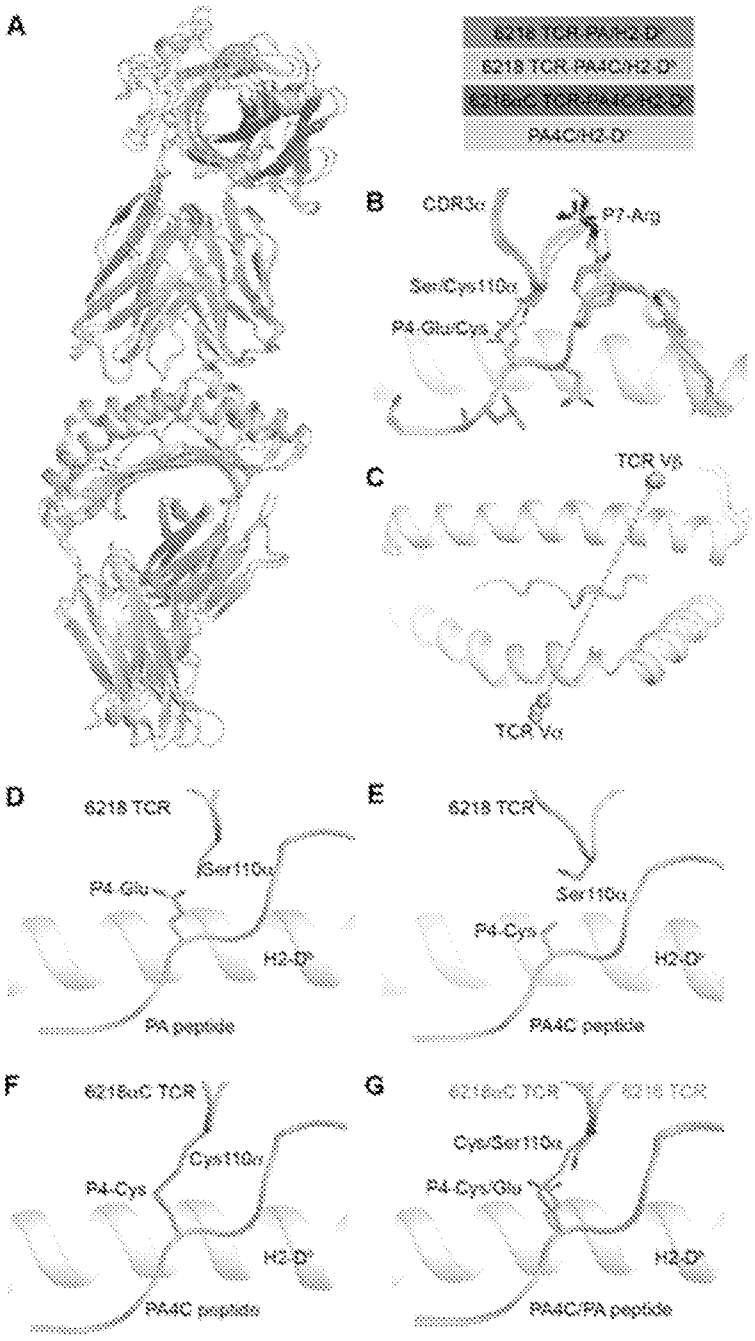

A
B
CDR3α
P7-Arg
Ser/Cys110α
P4-Glu/Cys
C
TCR Vβ
TCR Vα
D
6218 TCR
Ser110α
P4-Glu
H2-Dᵈ
PA peptide
E
6218 TCR
Ser110α
P4-Cys
H2-Dᵈ
PA4C peptide
F
6218αC TCR
Cys110α
P4-Cys
H2-Dᵈ
PA4C peptide
G
6218αC TCR
6218 TCR
Cys/Ser110α
P4-Cys/Glu
H2-Dᵈ
PA4C/PA peptide

Figure 3

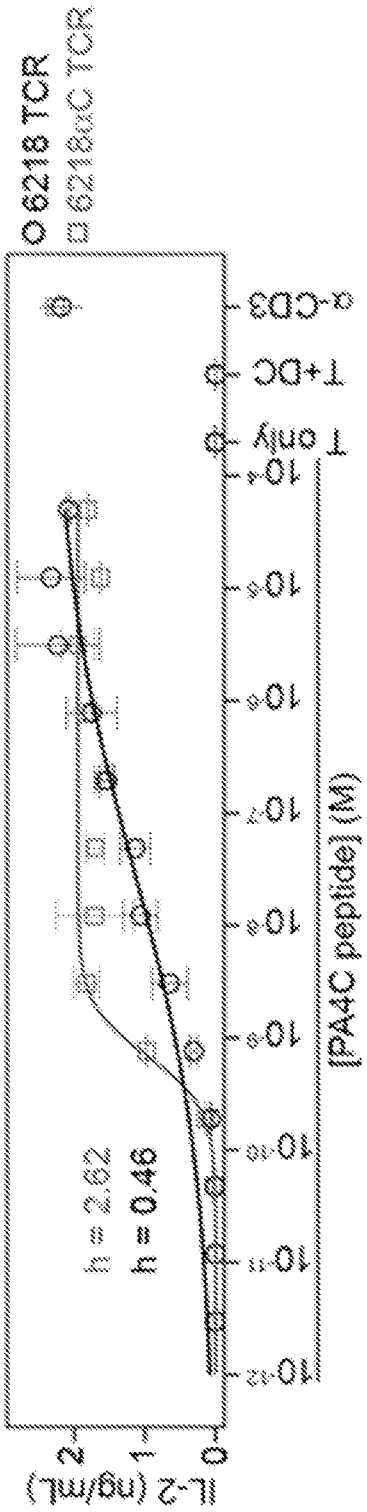

6218 TCR
6218αC TCR
α-CD3
T+DC
T only
10⁻⁴
10⁻⁵
10⁻⁶
10⁻⁷
10⁻⁸
10⁻⁹
10⁻¹⁰
10⁻¹¹
10⁻¹²
[PA4C peptide] (M)
h = 2.62
h = 0.46
2
1
0
IL-2 (ng/mL)

Figure 4

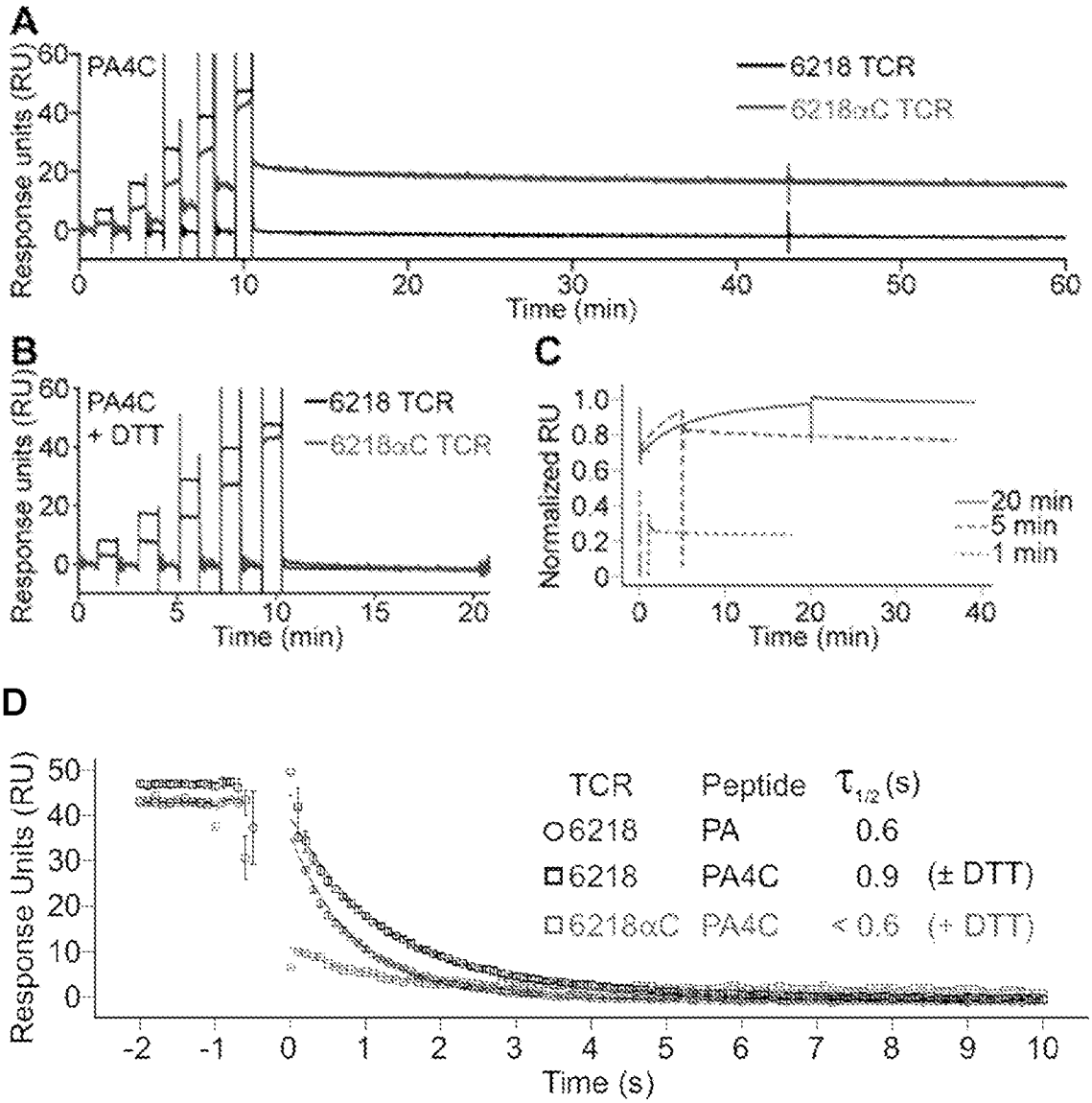

A
PA4C
6218 TCR
6218αC TCR
Response units (RU)
Time (min)
B
PA4C
+ DTT
6218 TCR
6218αC TCR
Response units (RU)
Time (min)
C
Normalized RU
20 min
5 min
1 min
Time (min)
D
Response Units (RU)
TCR Peptide τ1/2 (s)
6218 PA 0.6
6218 PA4C 0.9 (± DTT)
6218αC PA4C < 0.6 (+ DTT)
Time (s)

Figure 5

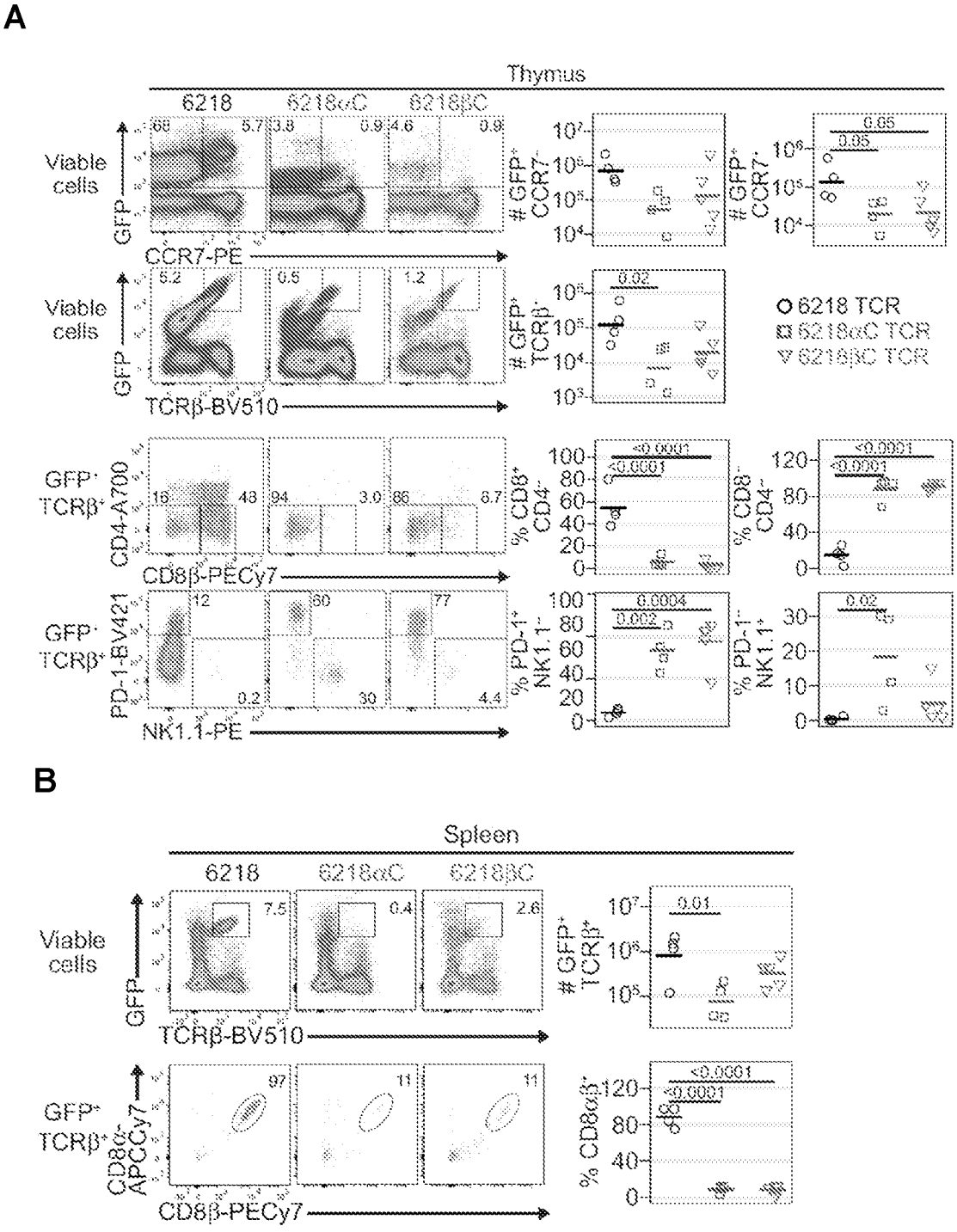

A
Thymus
6218
6218αC
6218βC
Viable cells
GFP
CCR7-PE
TCRβ-BV510
GFP+ CCR7-
GFP+ TCRβ-
6218 TCR
6218αC TCR
6218βC TCR
GFP+ TCRβ+
CD4-A700
CD8β-PECy7
% CD8+ CD4-
PD-1-BV421
NK1.1-PE
% PD-1+ NK1.1+
B
Spleen
CD8α-APCCy7
% CD8αβ+

Figure 5 continued

C

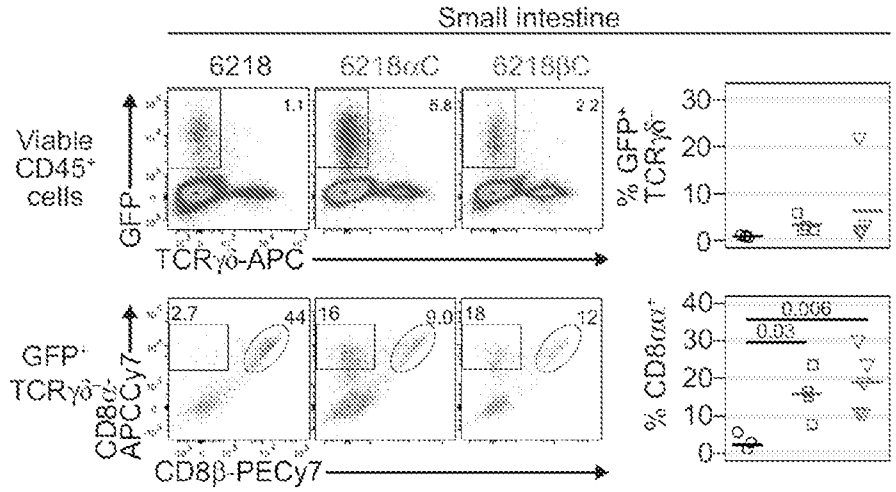


Figure 6

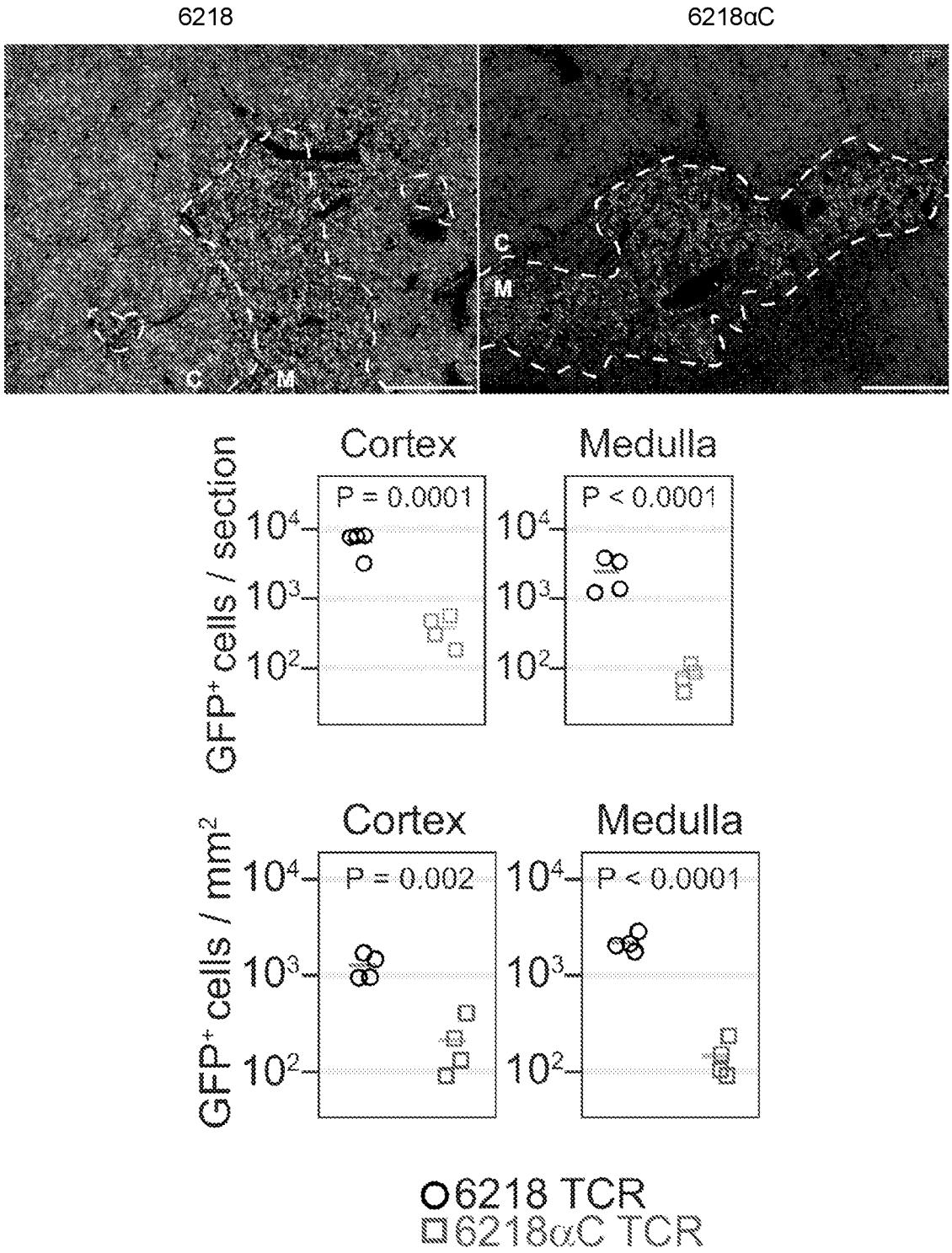

6218
6218αC
C
M
C
M
Cortex
Medulla
P = 0.0001
P < 0.0001
GFP+ cells / section
10^4
10^3
10^2
Cortex
Medulla
P = 0.002
P < 0.0001
GFP+ cells / mm2
10^4
10^3
10^2
6218 TCR
6218αC TCR

Figure 7

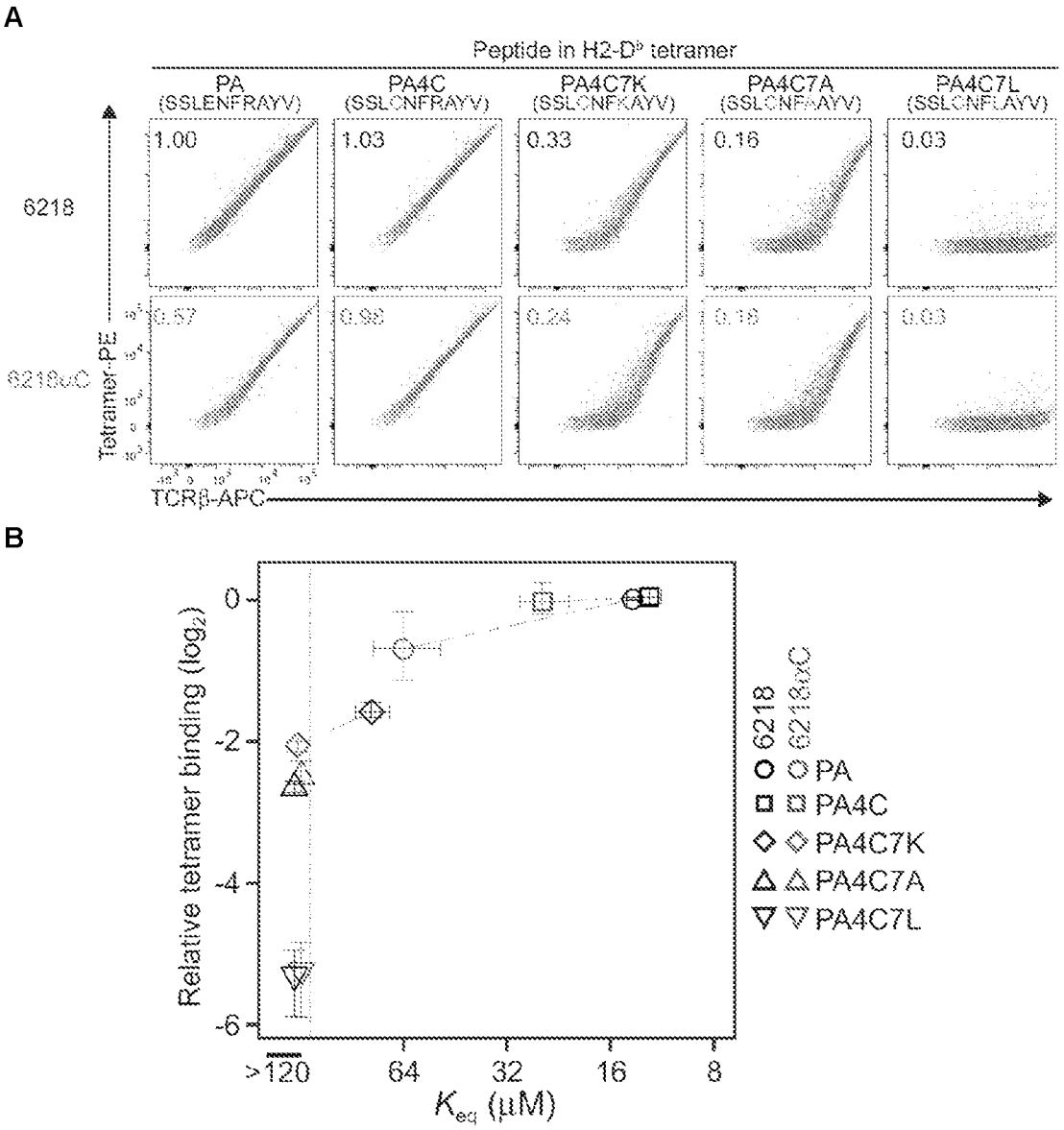
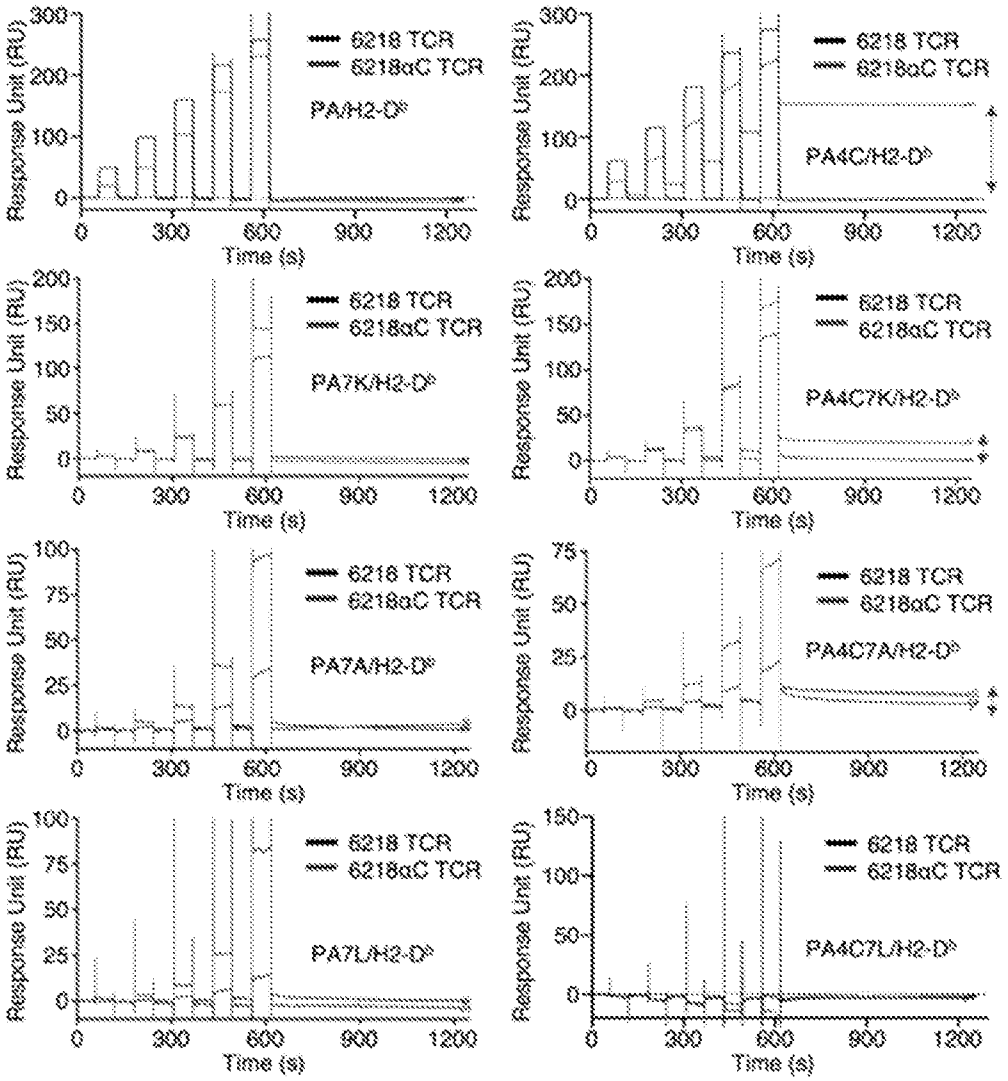

A
Peptide in H2-D^b tetramer
PA (SSLENFRAYV)
PA4C (SSLCNFRAYV)
PA4C7K (SSLCNFKAYV)
PA4C7A (SSLCNFAAYV)
PA4C7L (SSLCNFLAYV)
6218
6218αC
1.00
1.03
0.33
0.16
0.03
0.57
0.90
0.24
0.16
0.03
Tetramer-PE
TCRβ-APC
B
Relative tetramer binding (log$_2$)
0
-2
-4
-6
>120
64
32
16
8
K_{eq} (μM)
6218
6218αC
PA
PA4C
PA4C7K
PA4C7A
PA4C7L

Figure 8



6218 TCR	
Peptide	K_D (μM)
PA	13.8 ± 0.5
PA7K	98.0 ± 1.0
PA7A	>120
PA7L	>240
PA4C	9.1 ± 0.5
PA4C7K	>120
PA4C7A	>120
PA4C7L	No binding

6218αC TCR	
Peptide	K_D (μM)
PA	64.1 ± 14.0
PA7K	>120
PA7A	>120
PA7L	>240
PA4C	25.2 ± 4.1
PA4C7K	80.0 ± 16.0
PA4C7A	>120
PA4C7L	No binding

Figure 9

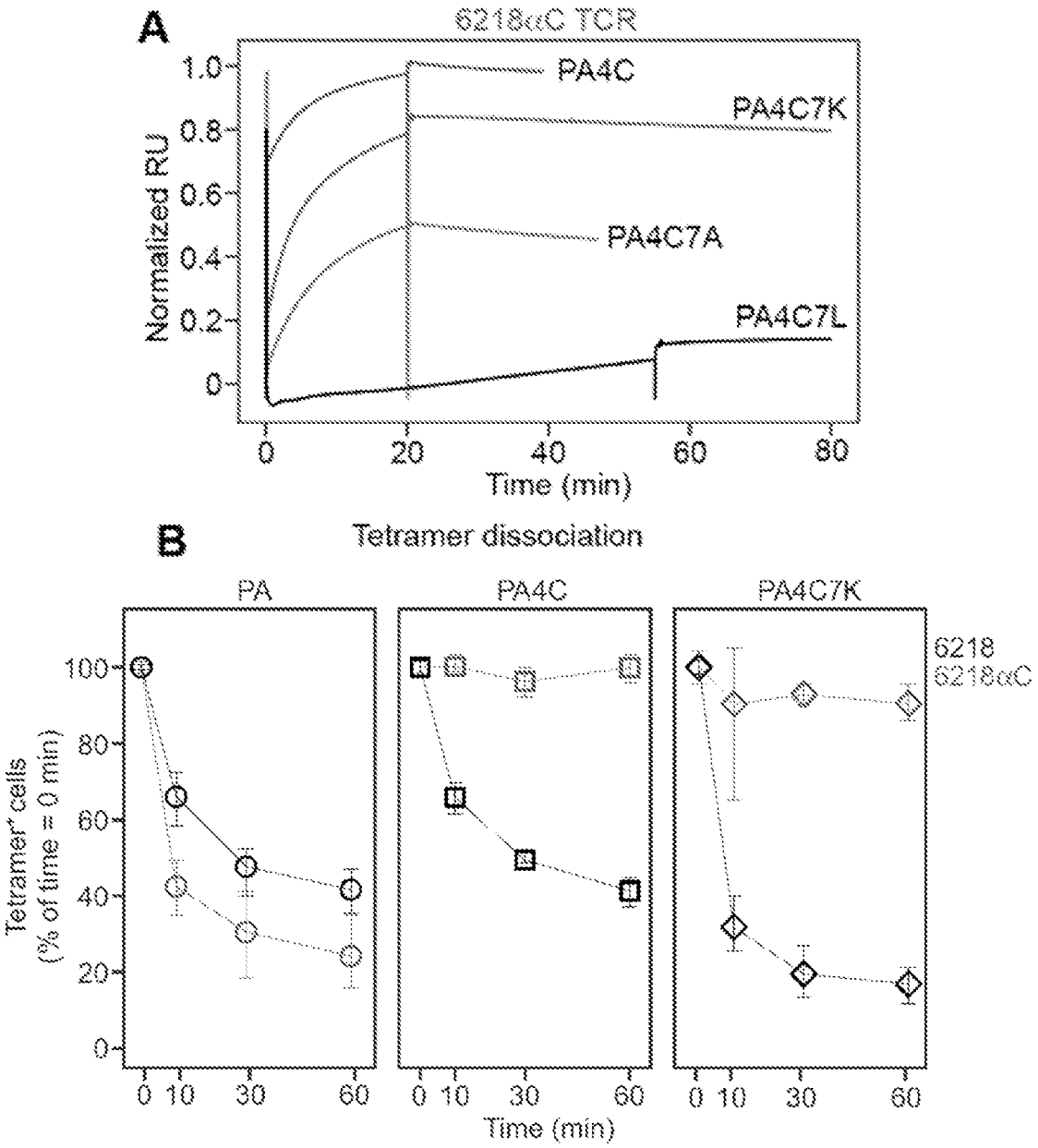

A
6218αC TCR
PA4C
PA4C7K
PA4C7A
PA4C7L
Normalized RU
1.0
0.8
0.6
0.4
0.2
0
0
20
40
60
80
Time (min)
B
Tetramer dissociation
PA
PA4C
PA4C7K
6218
6218αC
Tetramer+ cells
(% of time = 0 min)
100
80
60
40
20
0
0 10 30 60
Time (min)

Figure 10

A

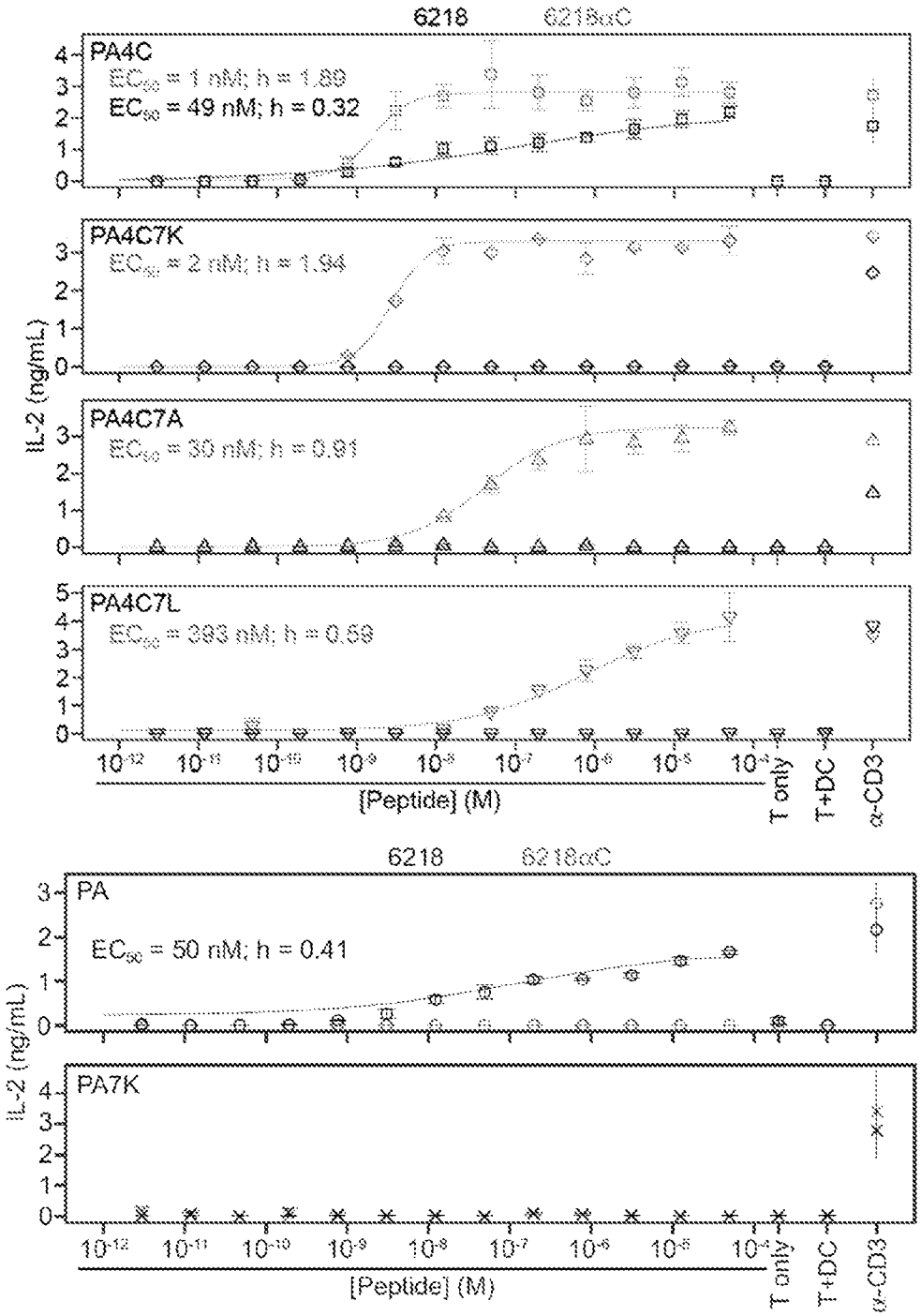

6218
6218αC
PA4C
EC50 = 1 nM; h = 1.89
EC50 = 49 nM; h = 0.32
PA4C7K
EC50 = 2 nM; h = 1.94
PA4C7A
EC50 = 30 nM; h = 0.91
PA4C7L
EC50 = 383 nM; h = 0.59
IL-2 (ng/mL)
[Peptide] (M)
T only
T+DC
α-CD3
PA
EC50 = 50 nM; h = 0.41
PA7K

Figure 10 continued

B

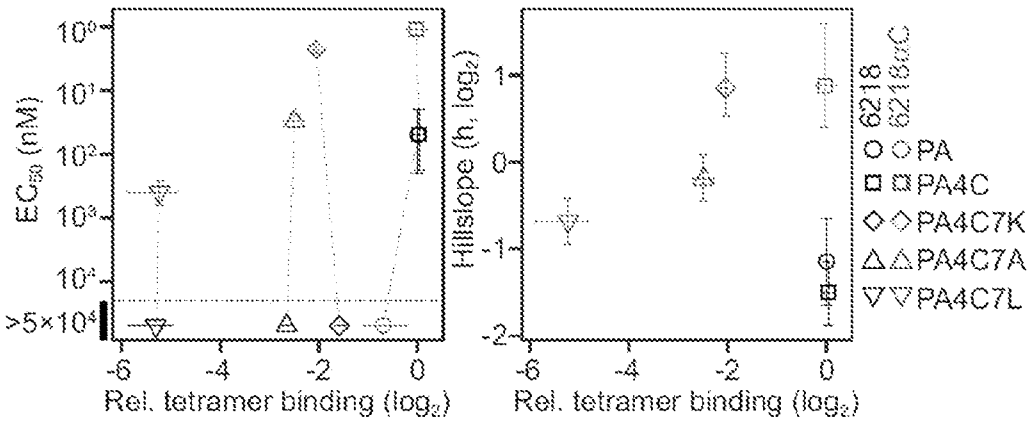


Figure 11

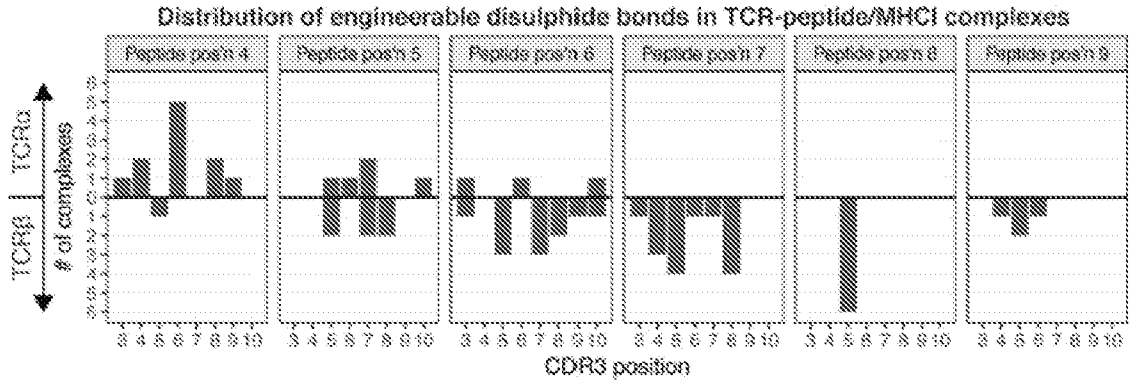

Distribution of engineerable disulphide bonds in TCR-peptide/MHCI complexes
Peptide pos'n 4
Peptide pos'n 5
Peptide pos'n 6
Peptide pos'n 7
Peptide pos'n 8
Peptide pos'n 9
TCRα
TCRβ
# of complexes
CDR3 position

Figure 12

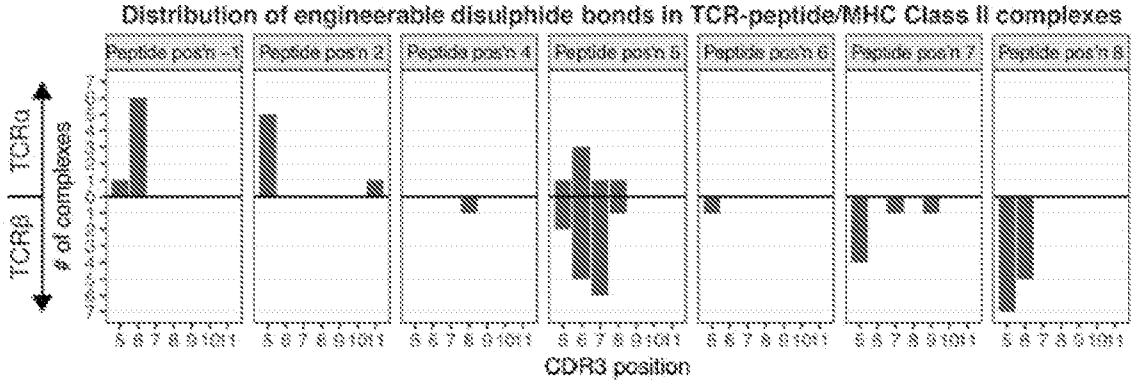

Distribution of engineerable disulphide bonds in TCR-peptide/MHC Class II complexes
Peptide pos'n -1
Peptide pos'n 2
Peptide pos'n 4
Peptide pos'n 5
Peptide pos'n 6
Peptide pos'n 7
Peptide pos'n 8
TCRα
TCRβ
# of complexes
5 6 7 8 9 10 11
CDR3 position

Figure 13

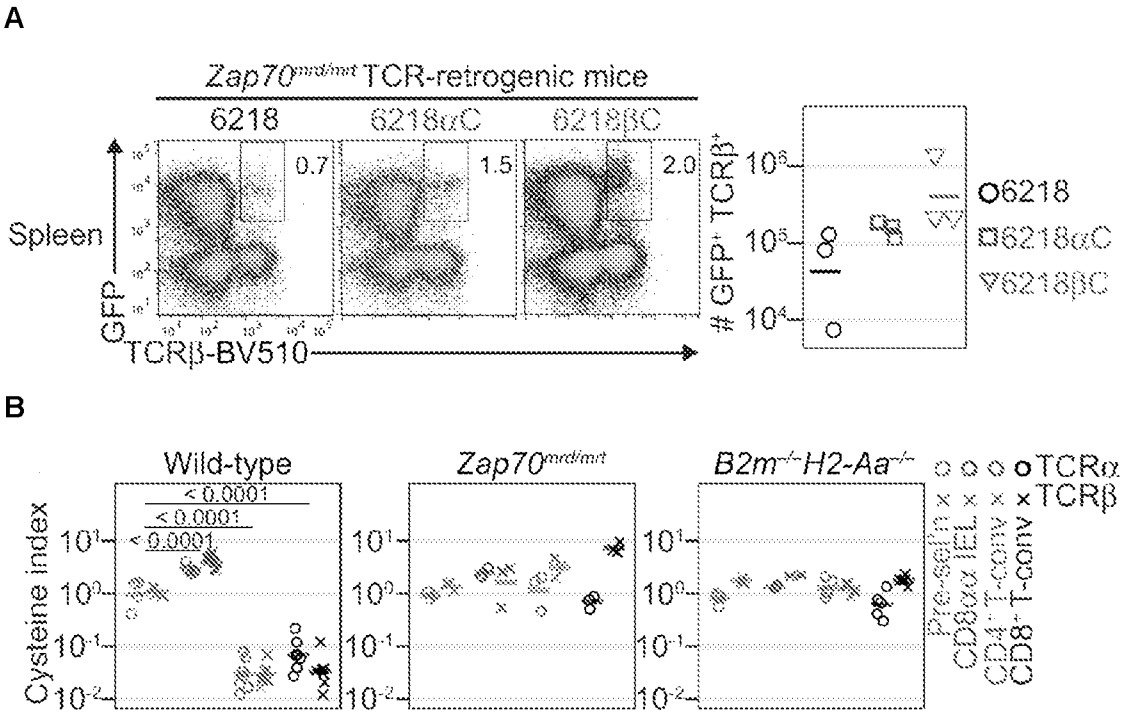

A
Zap70mrd/mrt TCR-retrogenic mice
6218
6218αC
6218βC
Spleen
GFP
TCRβ-BV510
0.7
1.5
2.0
GFP+ TCRβ+
6218
6218αC
6218βC
B
Wild-type
Zap70mrd/mrt
B2m−/−H2-Aa−/−
Cysteine index
< 0.0001
< 0.0001
< 0.0001
TCRα
TCRβ
Pre-selection
CD8αα IEL
CD4+ T-conv
CD8+ T-conv

Figure 14

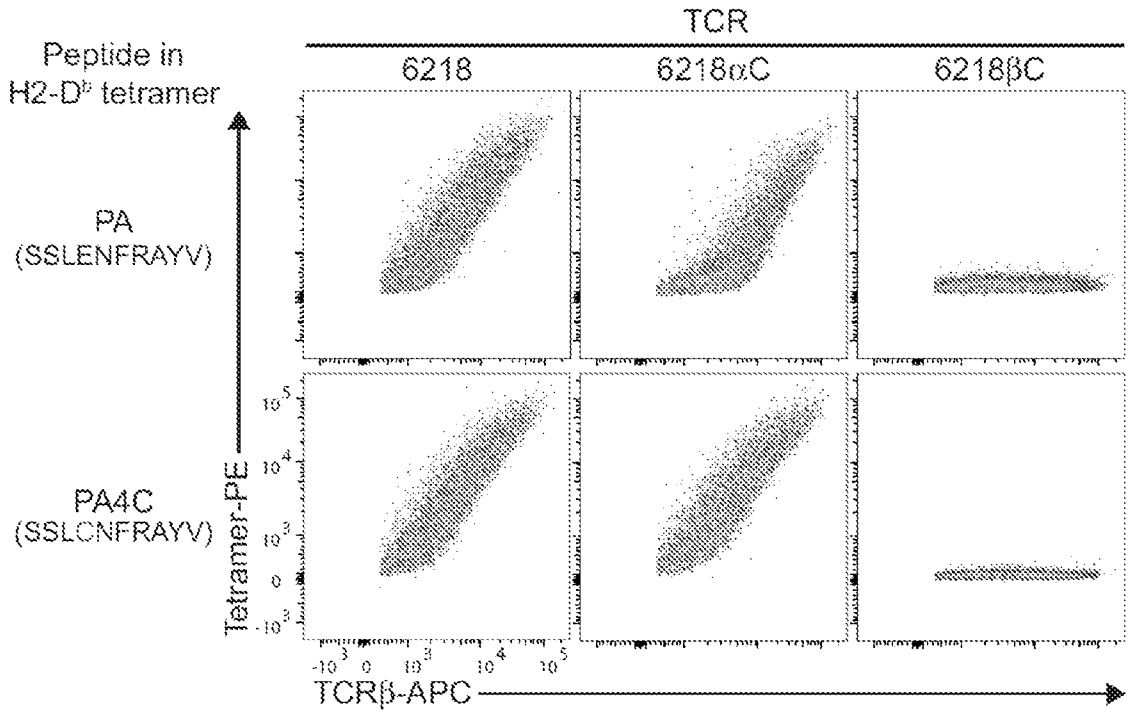

TCR
6218
6218αC
6218βC
Peptide in
H2-Db tetramer
PA
(SSLENFRAYV)
PA4C
(SSLCNFRAYV)
Tetramer-PE
TCRβ-APC

Figure 15

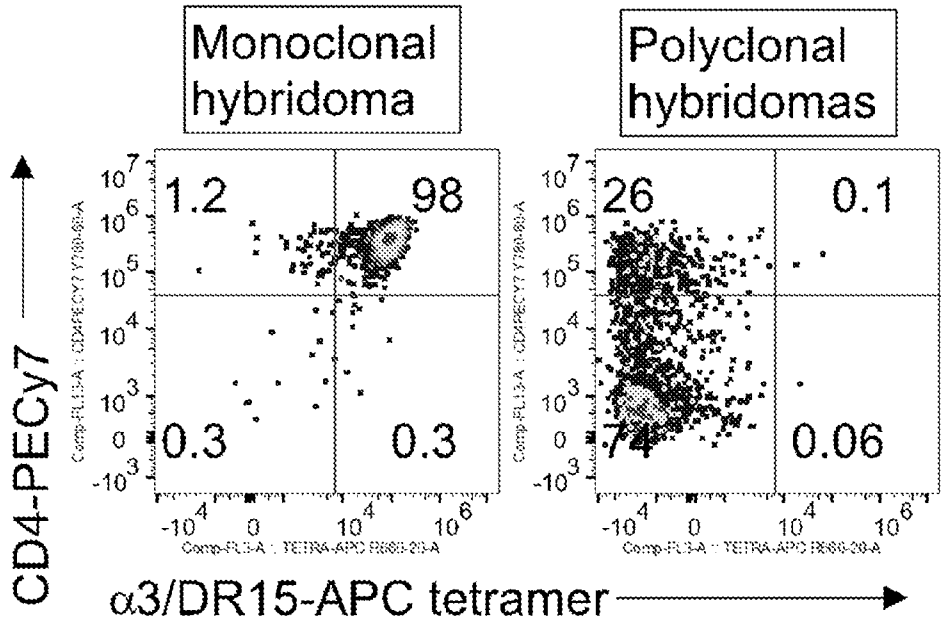

Monoclonal hybridoma
Polyclonal hybridomas
1.2
98
0.3
0.3
26
0.1
74
0.06
CD4-PECy7
α3/DR15-APC tetramer

Figure 16

A

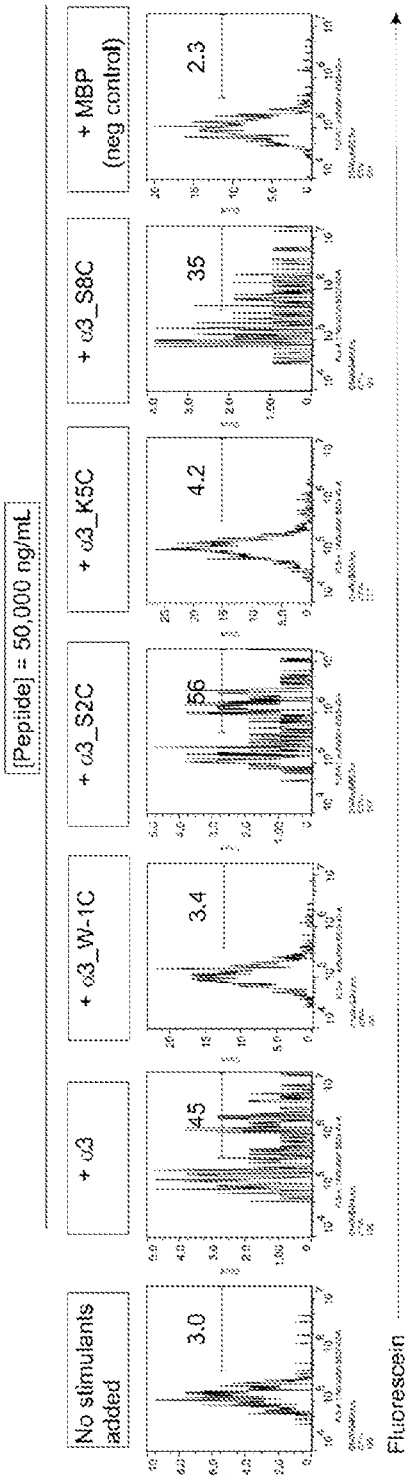

[Peptide] = 50,000 ng/mL
No stimulants added
+ ω3
+ ω3_W-1C
+ ω3_S2C
+ ω3_K5C
+ ω3_S8C
+ MBP (neg control)
3.0
45
3.4
56
4.2
35
2.3
Fluorescein

Figure 16 continued

B

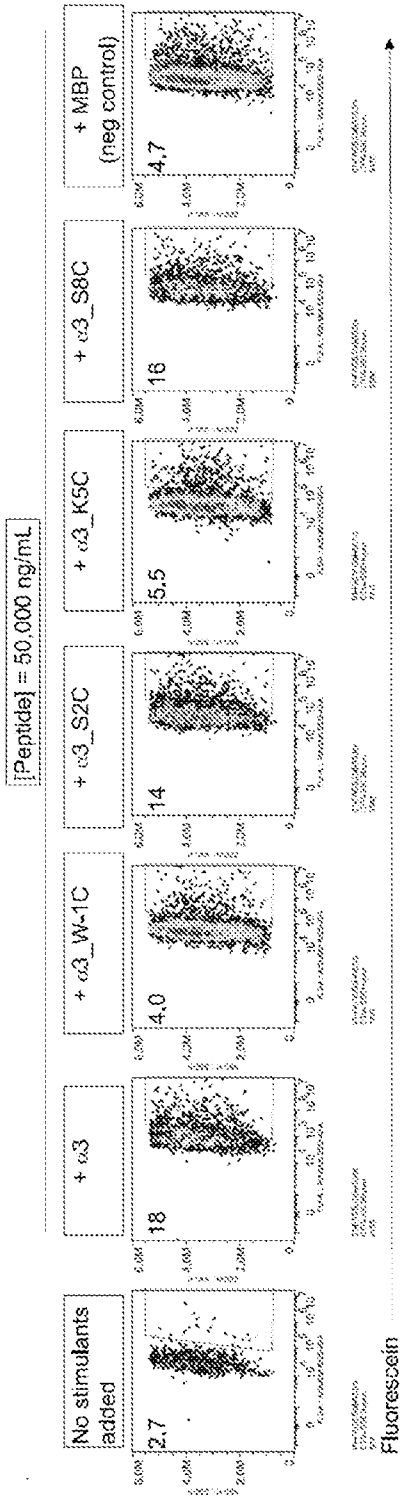


Figure 17

TCR	TRAV	TRAJ	CDR3α	TRBV	TRBJ	CDR3β
LS1	12N-3	22	ALS***SGSWQ***LI (SEQ ID NO: 224)	13-2	1-4	ASGE***GQGRG***ERLF (SEQ ID NO: 225)