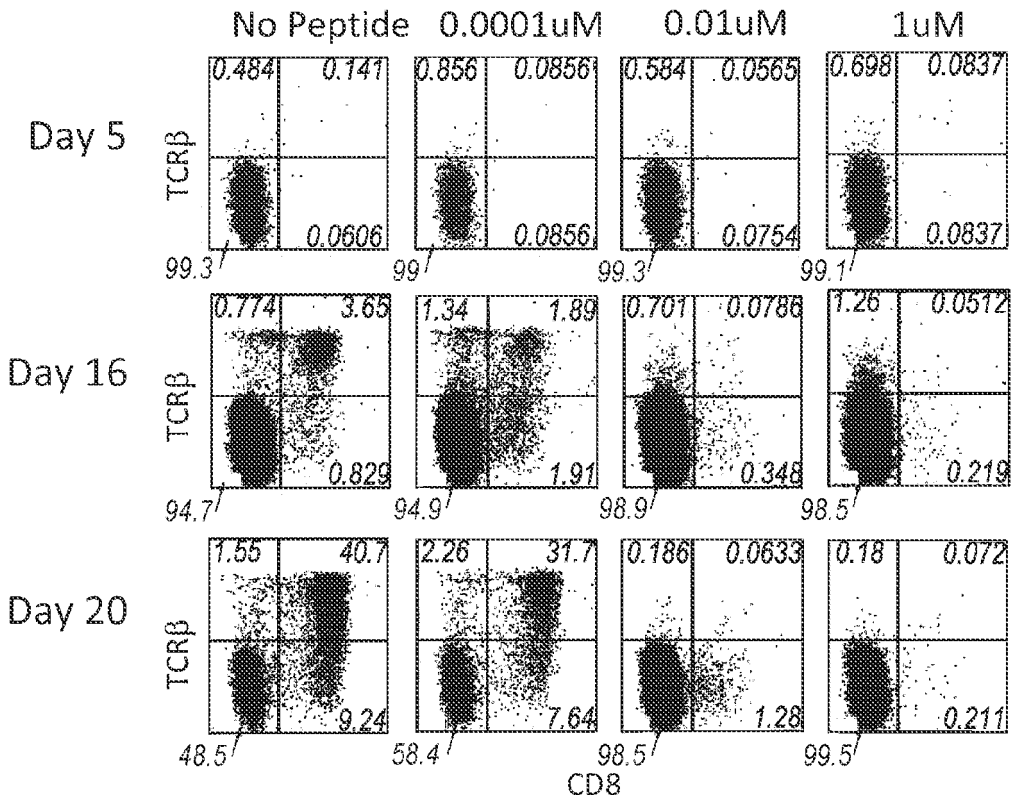

No Peptide
0.0001uM
0.01uM
1uM
Day 5
Day 16
Day 20
TCRβ
0.484
0.141
0.0606
99.3
0.856
0.0856
0.0856
99
0.584
0.0565
0.0754
99.3
0.698
0.0837
0.0837
99.1
0.774
3.65
0.829
94.7
1.34
1.89
1.91
94.9
0.701
0.0786
0.348
98.9
1.26
0.0512
0.219
98.5
1.55
40.7
9.24
48.5
2.26
31.7
7.64
58.4
0.186
0.0633
1.28
98.5
0.18
0.072
0.211
99.5
CD8

Fig. 1A

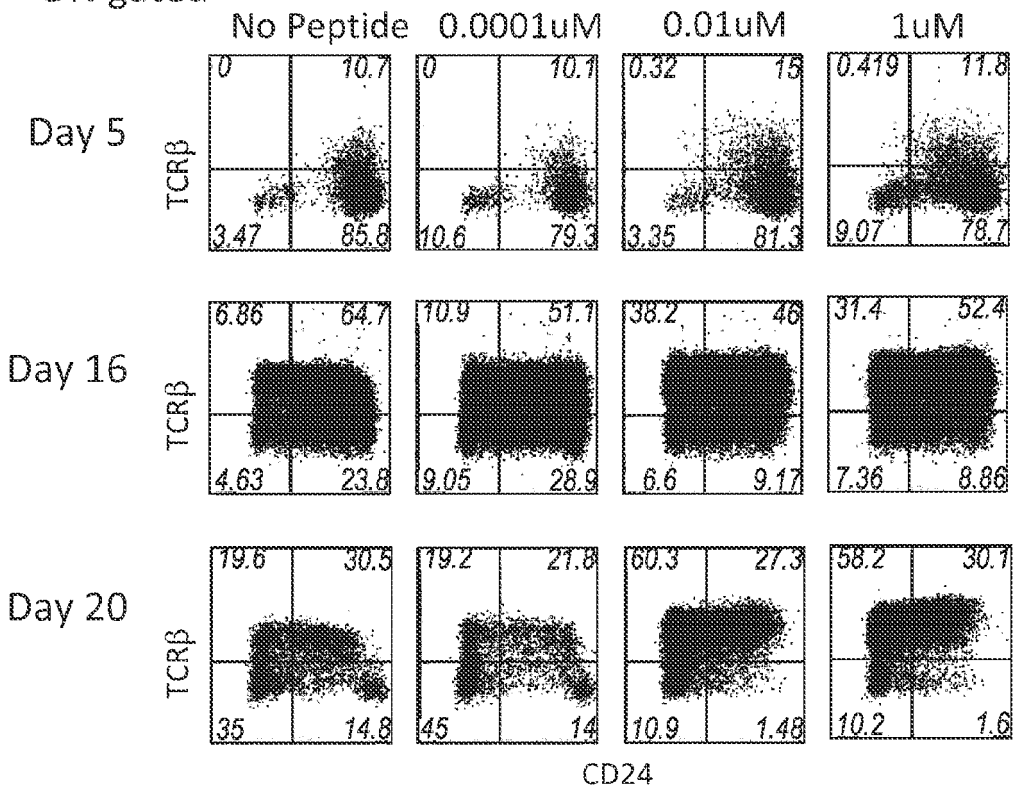

DN gated
No Peptide
0.0001uM
0.01uM
1uM
Day 5
Day 16
Day 20
TCRβ
0
10.7
3.47
85.8
0
10.1
10.6
79.3
0.32
15
3.35
81.3
0.419
11.8
9.07
78.7
6.86
64.7
4.63
23.8
10.9
51.1
9.05
28.9
38.2
46
6.6
9.17
31.4
52.4
7.36
8.86
19.6
30.5
35
14.8
19.2
21.8
45
14
60.3
27.3
10.9
1.48
58.2
30.7
10.2
1.6
CD24

Fig. 1B

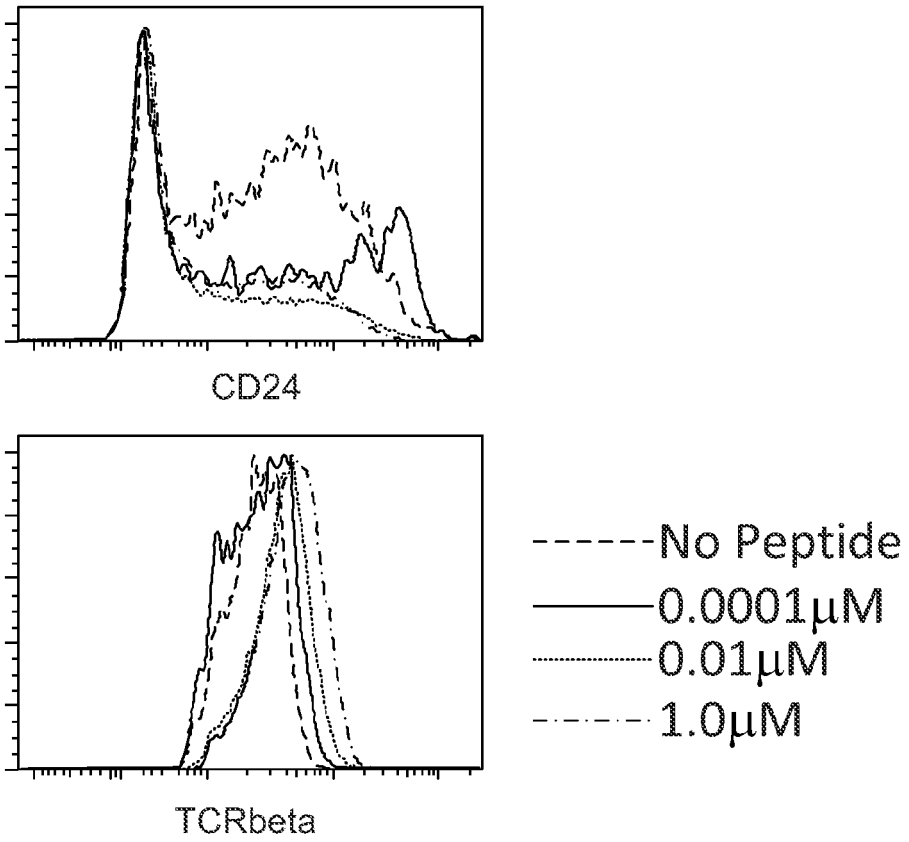

CD24
TCRbeta
No Peptide
0.0001μM
0.01μM
1.0μM

Fig. 1C

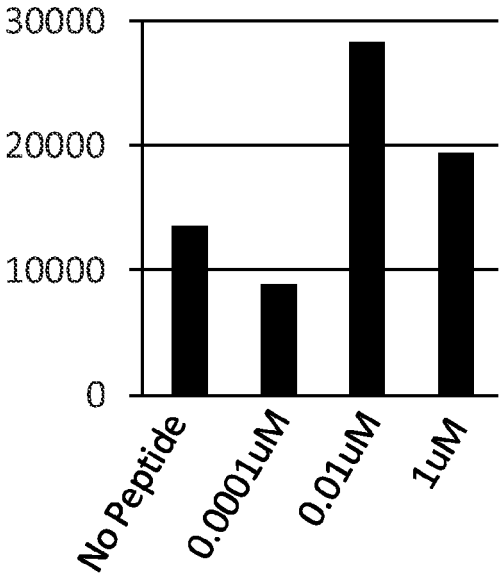

30000
20000
10000
0
No Peptide
0.0001uM
0.01uM
1uM

Fig. 1D

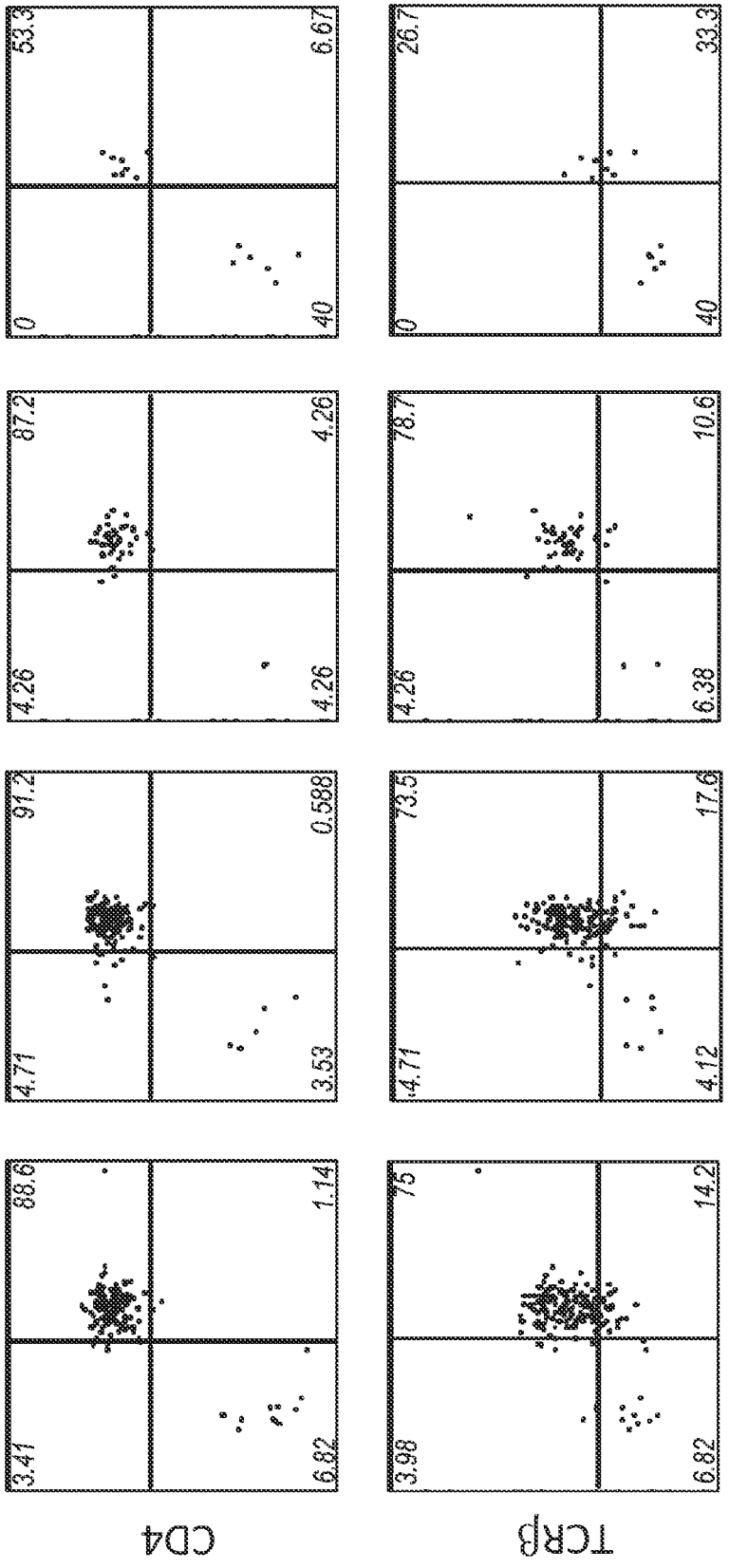

53.3
6.67
0
40
26.7
33.3
0
40
87.2
4.26
4.26
4.26
78.7
10.6
4.26
6.38
91.2
0.588
4.71
3.53
73.5
17.6
4.71
4.12
88.6
1.14
3.41
6.82
75
14.2
3.98
6.82
CD4
TCRβ
CD8

Fig. 2

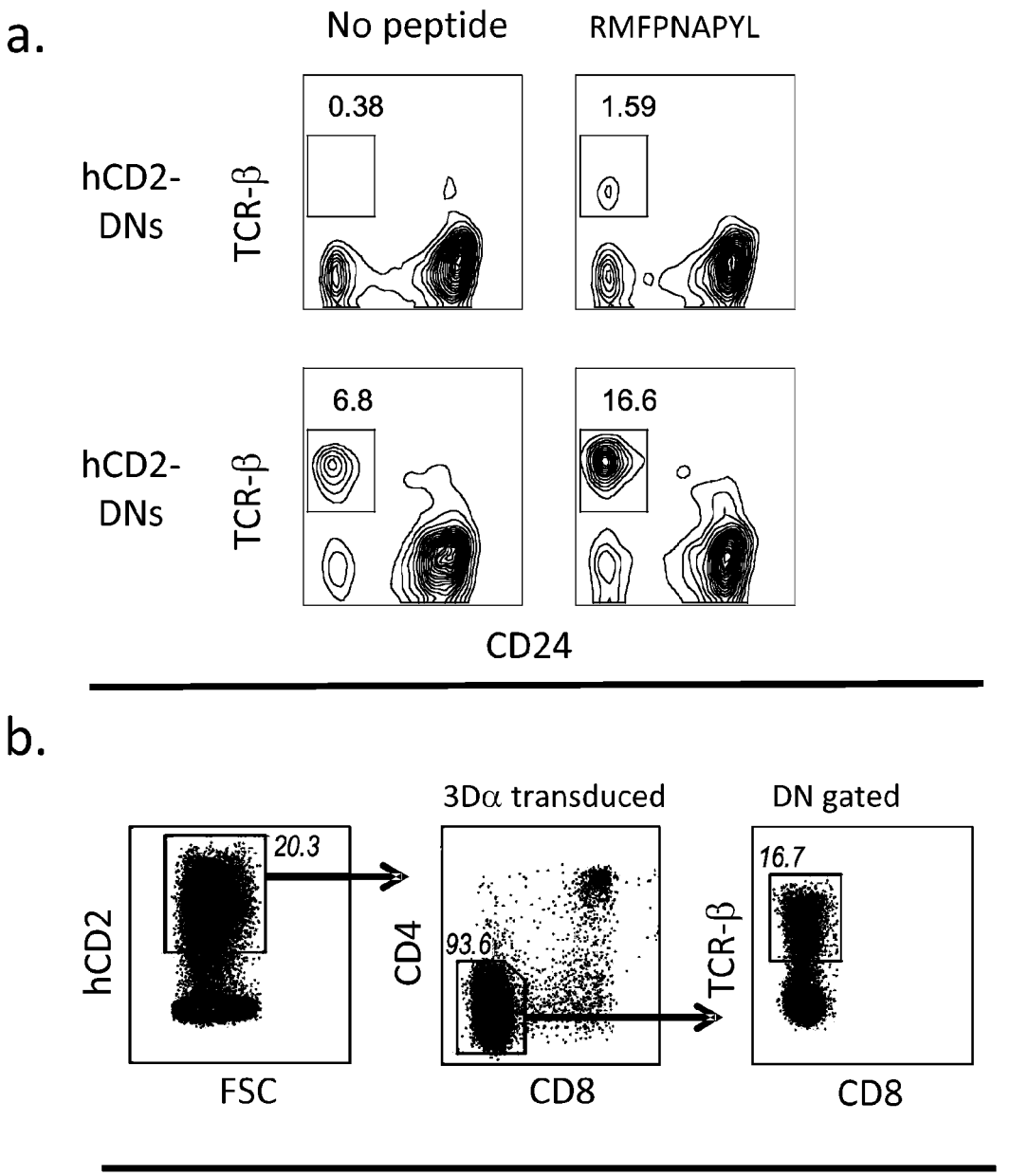

a.
No peptide
RMFPNAPYL
0.38
1.59
hCD2-
DNs
TCR-β
6.8
16.6
hCD2-
DNs
TCR-β
CD24
b.
3Dα transduced
DN gated
20.3
hCD2
FSC
CD4
93.6
CD8
16.7
TCR-β
CD8
c.
• Clone Vβ10 TCRβ chains by PCR
• Directional cloning into pENTR
• Transfer to retroviral vector MigR1 (IRES-GFP)
• Transduce and screen 58-/-cells

Fig. 3

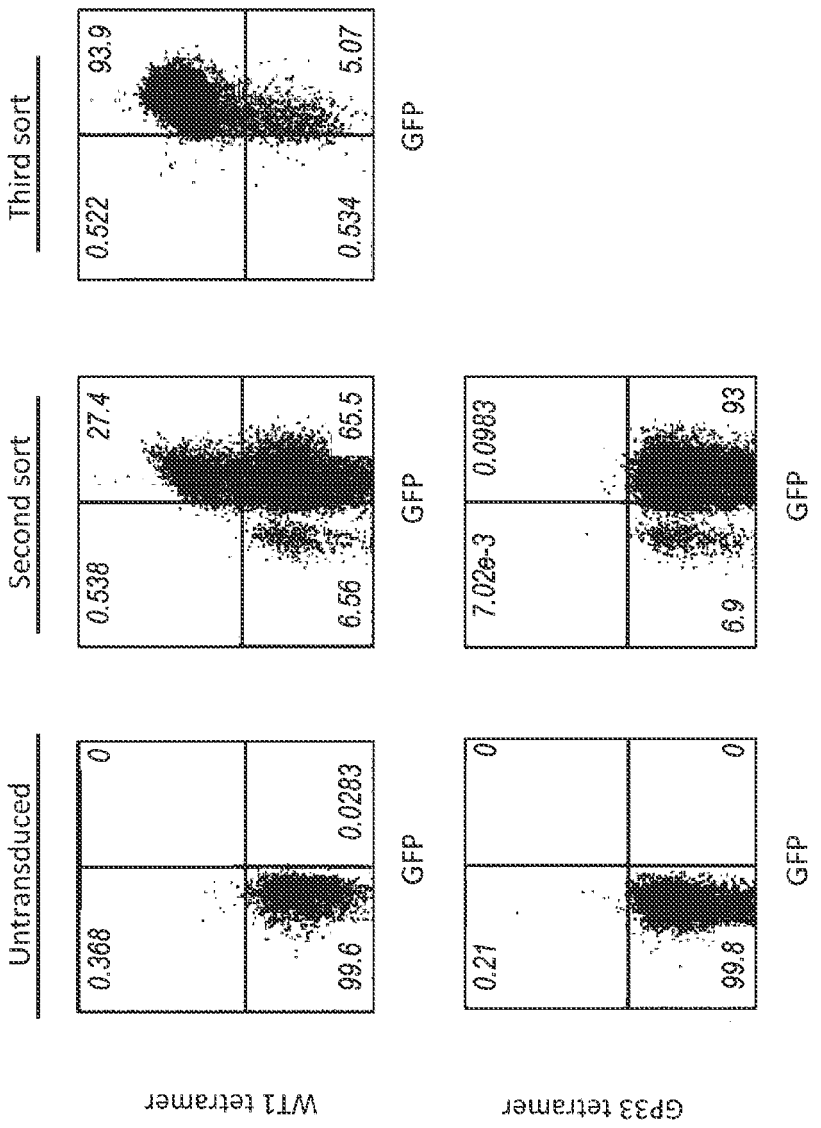

Untransduced
Second sort
Third sort
0.368
0
0.0283
99.6
0.538
27.4
65.5
6.56
0.522
93.9
5.07
0.534
0.21
0
0
99.8
7.02e-3
0.0983
93
6.9
GFP
GFP
GFP
GFP
GFP
GFP
WT1 tetramer
GP33 tetramer

Fig. 4A

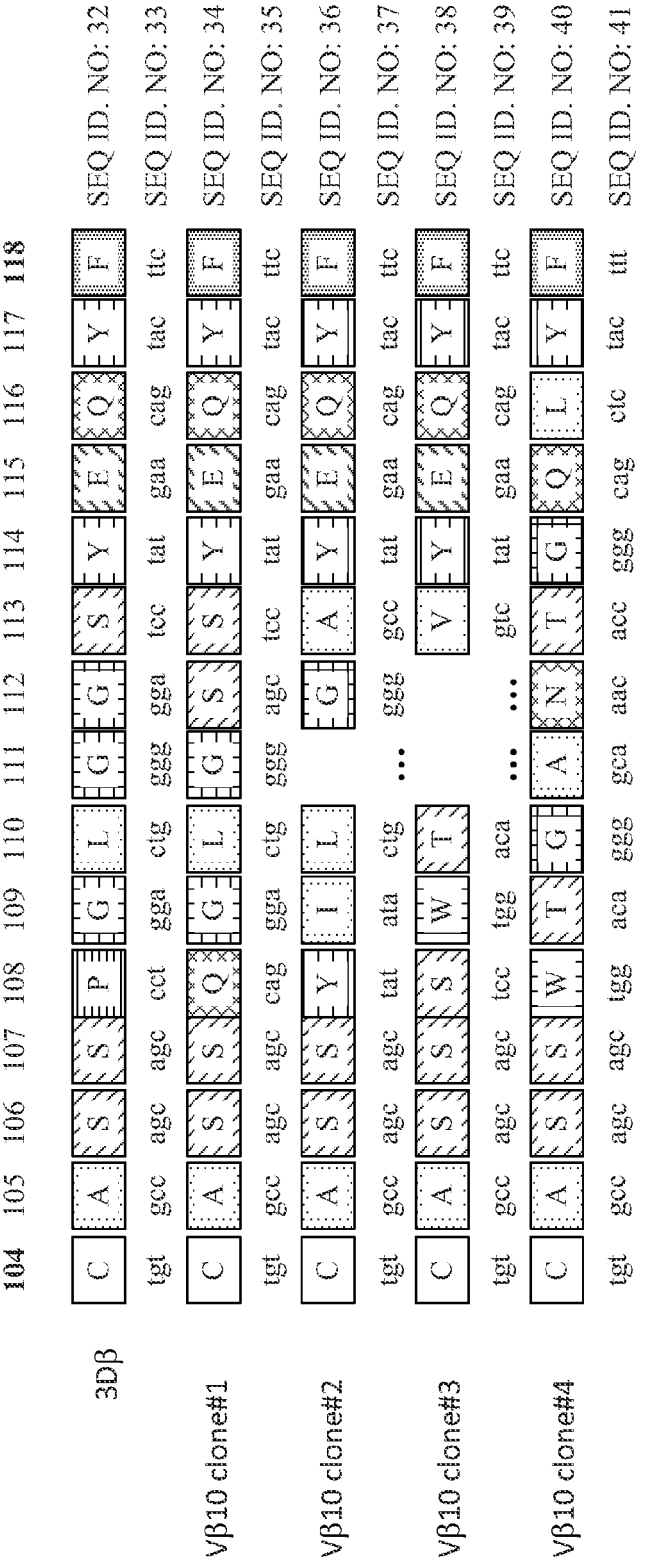

104 105 106 107 108 109 110 111 112 113 114 115 116 117 118
3Dβ
C A S S S P G L G G S Y E Q Y F
tgt gcc agc agc cct gga ctg ggg gga tcc tat gaa cag tac ttc
SEQ ID. NO: 32
Vβ10 clone#1
C A S S S Q G L G S S Y E Q Y F
tgt gcc agc agc cag gga ctg ggg agc tcc tat gaa cag tac ttc
SEQ ID. NO: 33
Vβ10 clone#2
C A S S S Y I L G A Y E Q Y F
tgt gcc agc agc tat ata ctg ... ggg gcc tat gaa cag tac ttc
SEQ ID. NO: 34
Vβ10 clone#3
C A S S S W T G V Y E Q Y F
tgt gcc agc agc tcc tgg aca ... ... gtc tat gaa cag tac ttc
SEQ ID. NO: 35
Vβ10 clone#4
C A S S S W T G A N T G Q L Y F
tgt gcc agc agc tgg aca ggg gca aac acc ggg cag ctc tac ttt
SEQ ID. NO: 36
SEQ ID. NO: 37
SEQ ID. NO: 38
SEQ ID. NO: 39
SEQ ID. NO: 40
SEQ ID. NO: 41

Fig. 4B

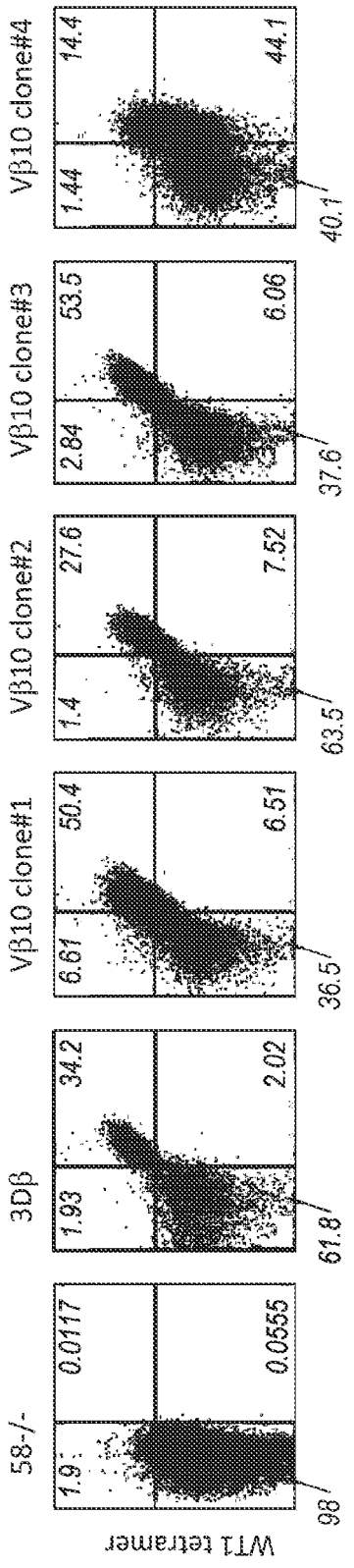

58-/-
3Dβ
Vβ10 clone#1
Vβ10 clone#2
Vβ10 clone#3
Vβ10 clone#4
WT1 tetramer
TCR β
1.9
0.0117
0.0555
98
1.93
34.2
2.02
61.8
6.61
50.4
6.51
36.5
1.4
27.6
7.52
63.5
2.84
53.5
6.06
37.6
1.44
14.4
44.1
40.1

Fig. 4C

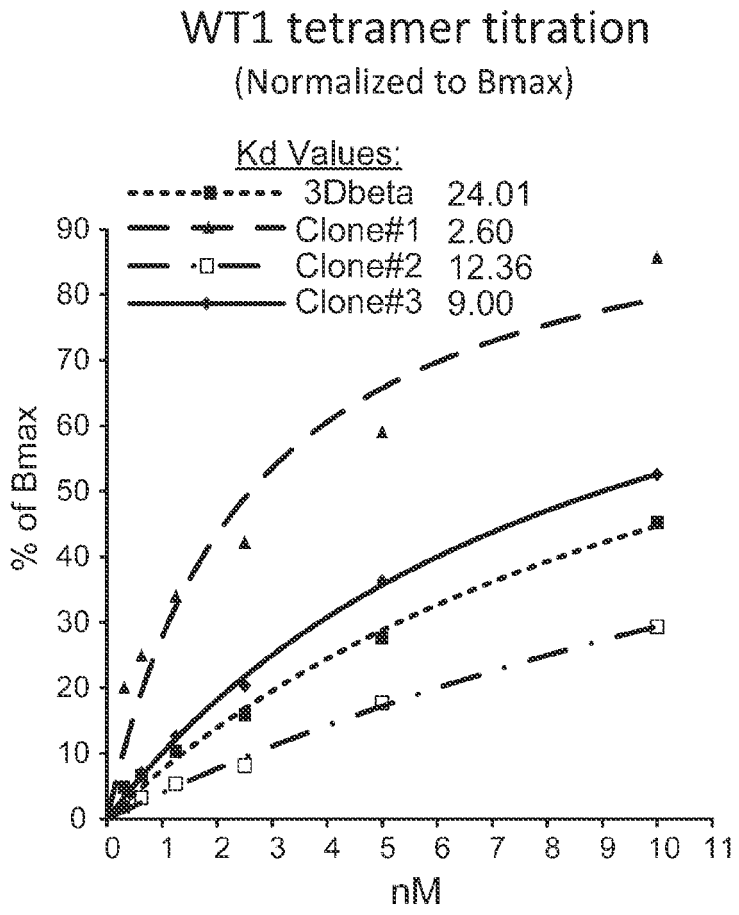

WT1 tetramer titration
(Normalized to Bmax)
Kd Values:
3Dbeta 24.01
Clone#1 2.60
Clone#2 12.36
Clone#3 9.00
% of Bmax
0
10
20
30
40
50
60
70
80
90
0 1 2 3 4 5 6 7 8 9 10 11
nM

Fig. 5A

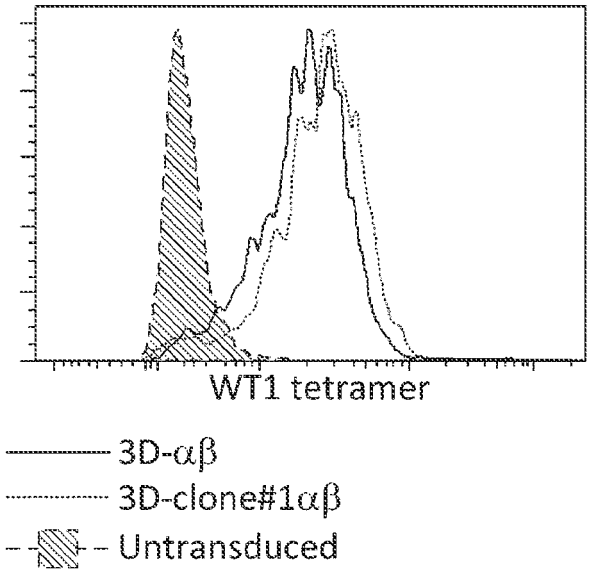

WT1 tetramer
3D-αβ
3D-clone#1αβ
Untransduced

Fig. 5B

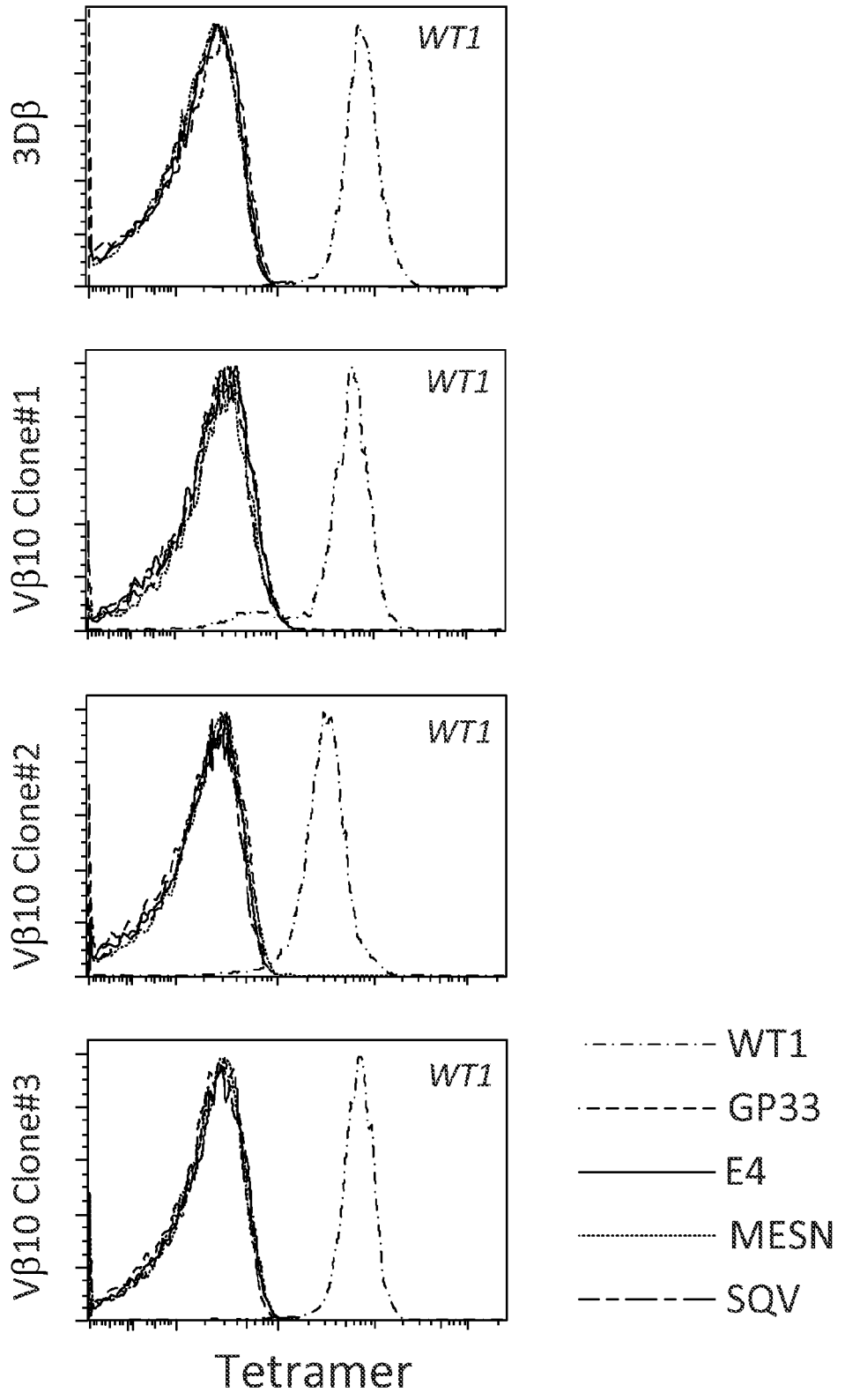

3Dβ
Vβ10 Clone#1
Vβ10 Clone#2
Vβ10 Clone#3
WT1
WT1
WT1
WT1
WT1
GP33
E4
MESN
SQV
Tetramer

Fig. 5C

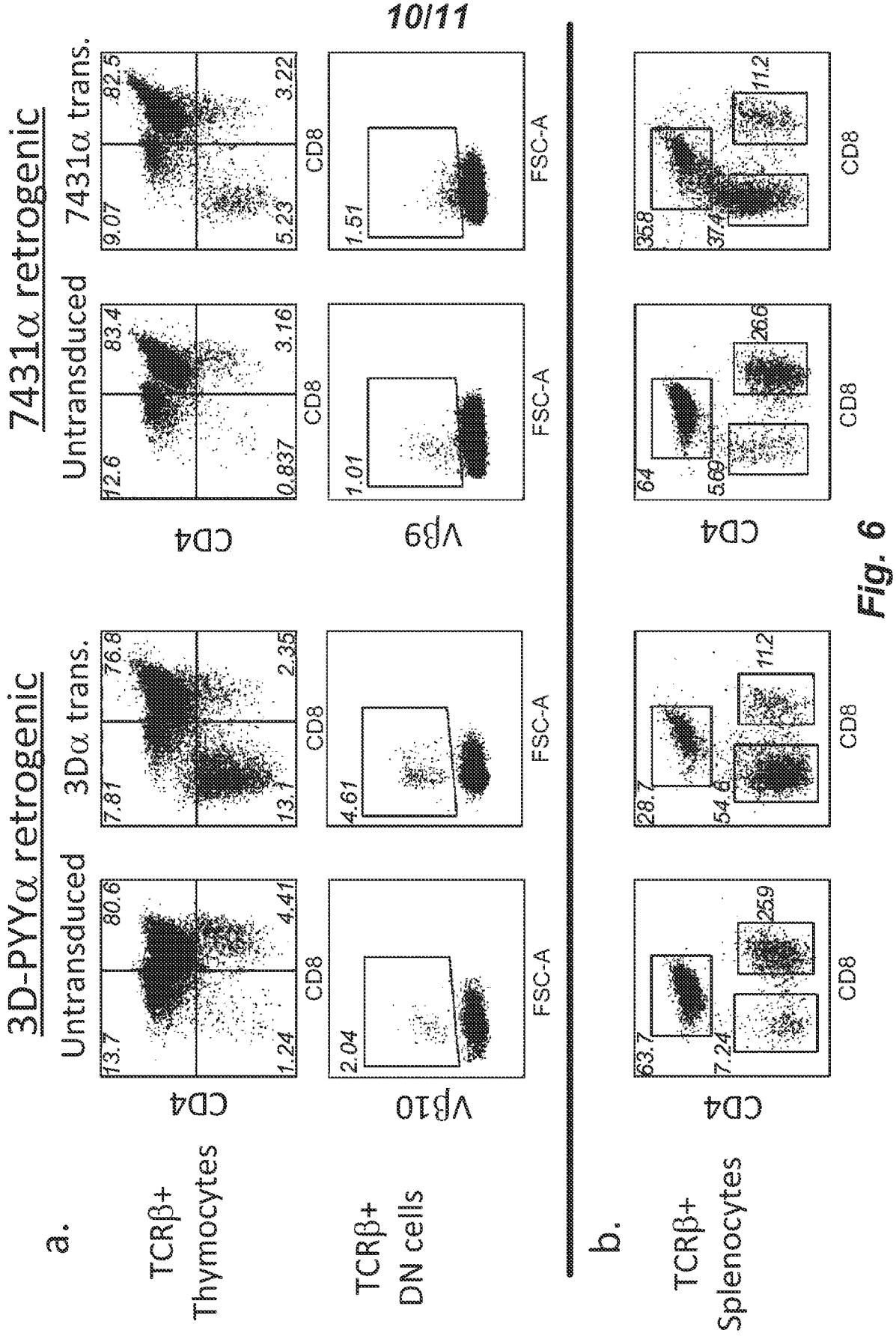

a.
TCRβ+
Thymocytes
3D-PYYα retrogenic
Untransduced
3Dα trans.
CD4
CD8
13.7
80.6
1.24
4.41
7.81
76.8
13.1
2.35
Vβ10
FSC-A
2.04
4.61
TCRβ+
DN cells
7431α retrogenic
Untransduced
7431α trans.
12.6
83.4
0.837
3.16
9.07
82.5
5.23
3.22
Vβ9
1.01
1.51
b.
TCRβ+
Splenocytes
63.7
7.24
25.9
28.7
54.6
11.2
64
5.69
26.6
35.8
37.4
11.2

Fig. 6

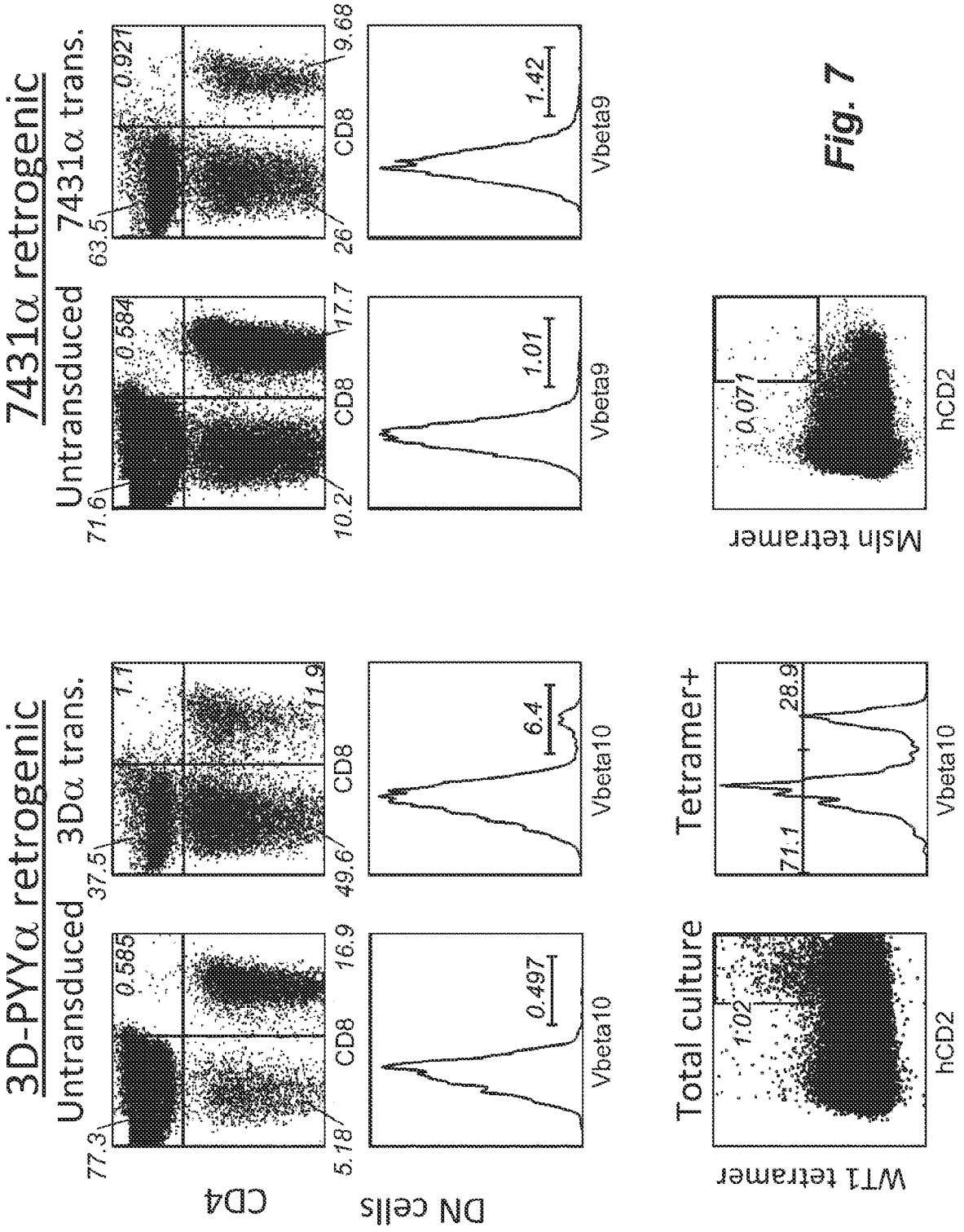

3D-PYYα retrogenic
Untransduced
3Dα trans.
7431α retrogenic
Untransduced
7431α trans.
CD4
CD8
DN cells
Vbeta10
Vbeta9
Total culture
Tetramer+
WT1 tetramer
Msln tetramer
hCD2
77.3
0.585
16.9
5.18
37.5
1.1
11.9
49.6
0.497
6.4
71.6
0.584
17.7
10.2
1.01
63.5
0.921
9.68
26
1.42
1.02
71.1
28.9
0.07

Fig. 7