Figure 1

Alignment of human Vγ4, human Vγ2 and mouse Vγ7 V regions

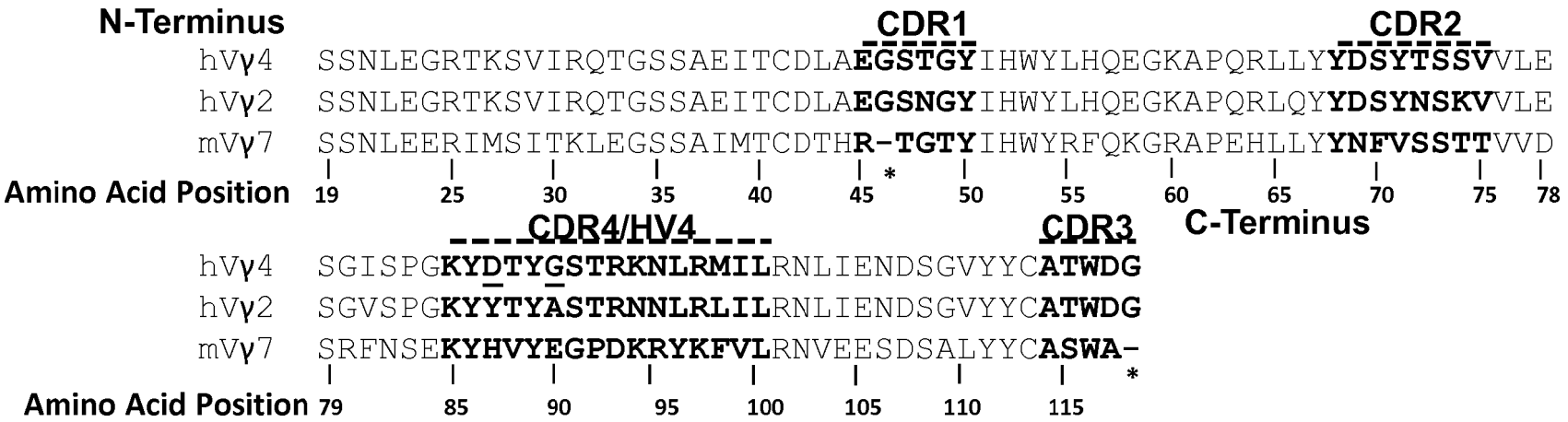


Figure 2

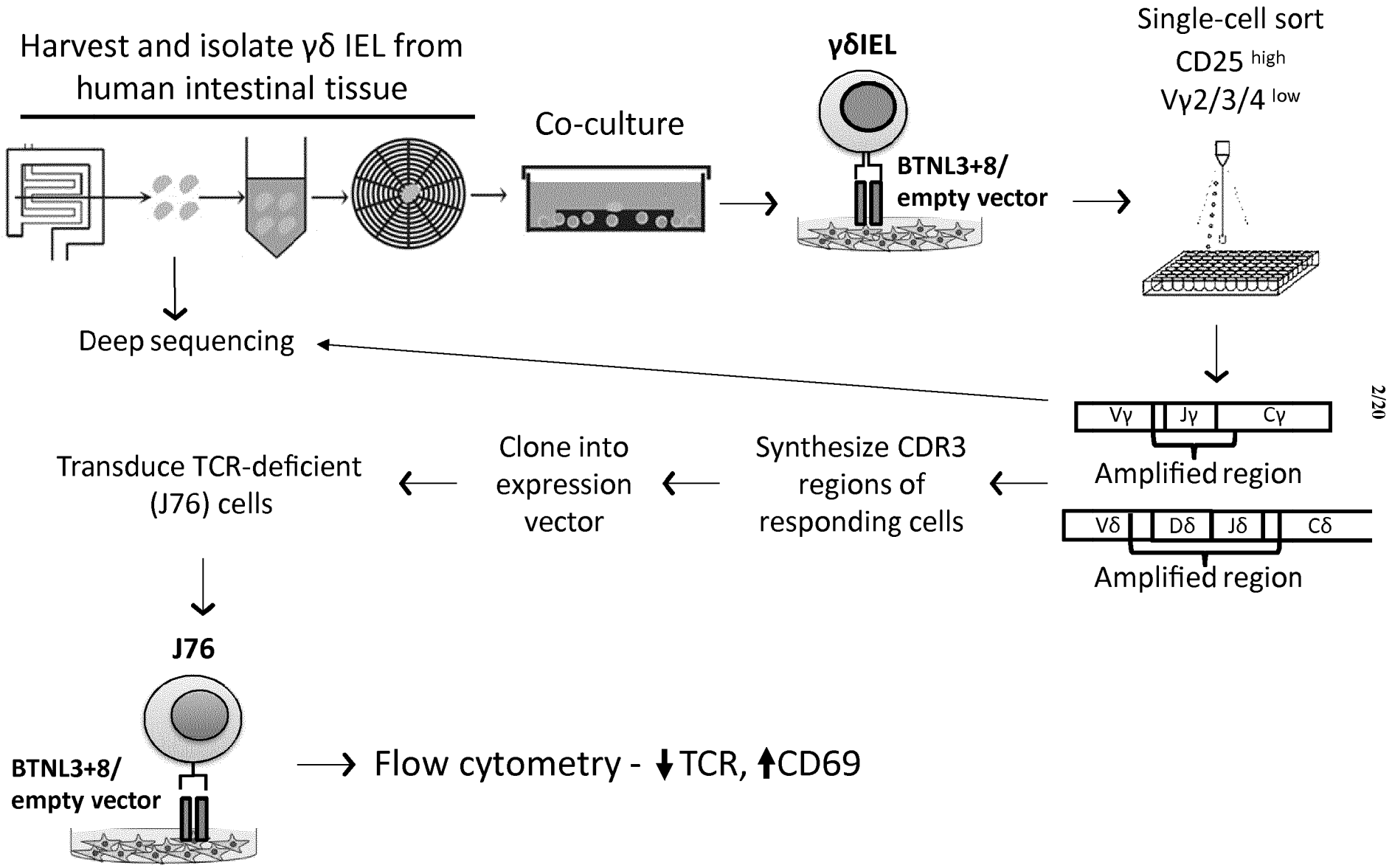

Harvest and isolate γδ IEL from human intestinal tissue
Co-culture
γδIEL
BTNL3+8/ empty vector
Single-cell sort
CD25 high
Vγ2/3/4 low
Deep sequencing
Vγ
Jγ
Cγ
Amplified region
Vδ
Dδ
Jδ
Cδ
Amplified region
Synthesize CDR3 regions of responding cells
Clone into expression vector
Transduce TCR-deficient (J76) cells
J76
BTNL3+8/ empty vector
Flow cytometry - ↓TCR, ↑CD69

Figure 3A

Lentiviral vector backbone used for expression (custom-designed)



Figure 3B

Example of TCR transduction in the Jurkat-derived TCR$\alpha\beta^{-/-}$ cell line J76 (72h post-transduction)

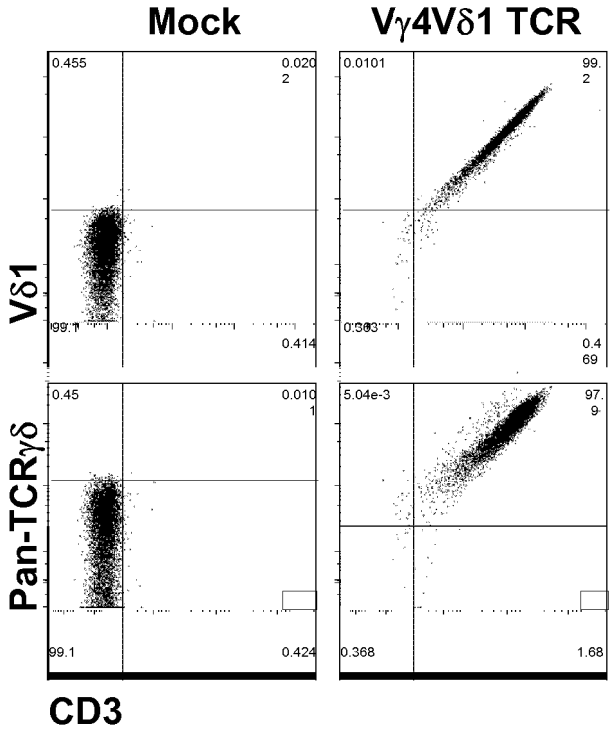
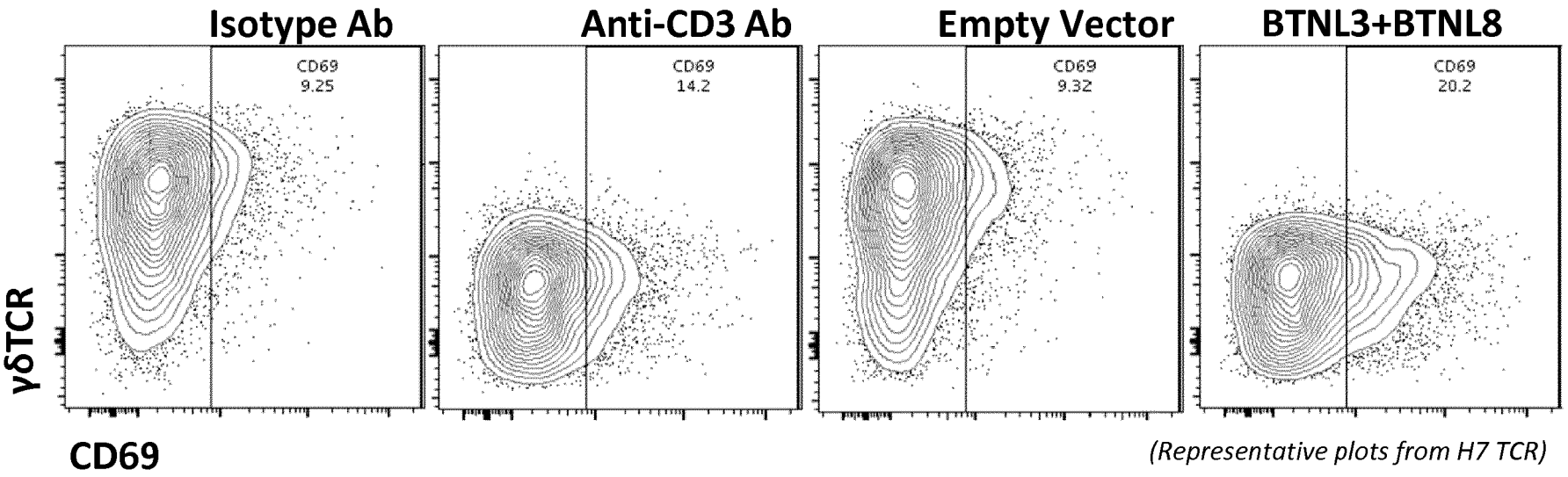


Figure 4A

Response of transduced J76 cells to BTNL3+BTNL8-expressing HEK-293T cells



(Representative plots from H7 TCR)

Figure 4B

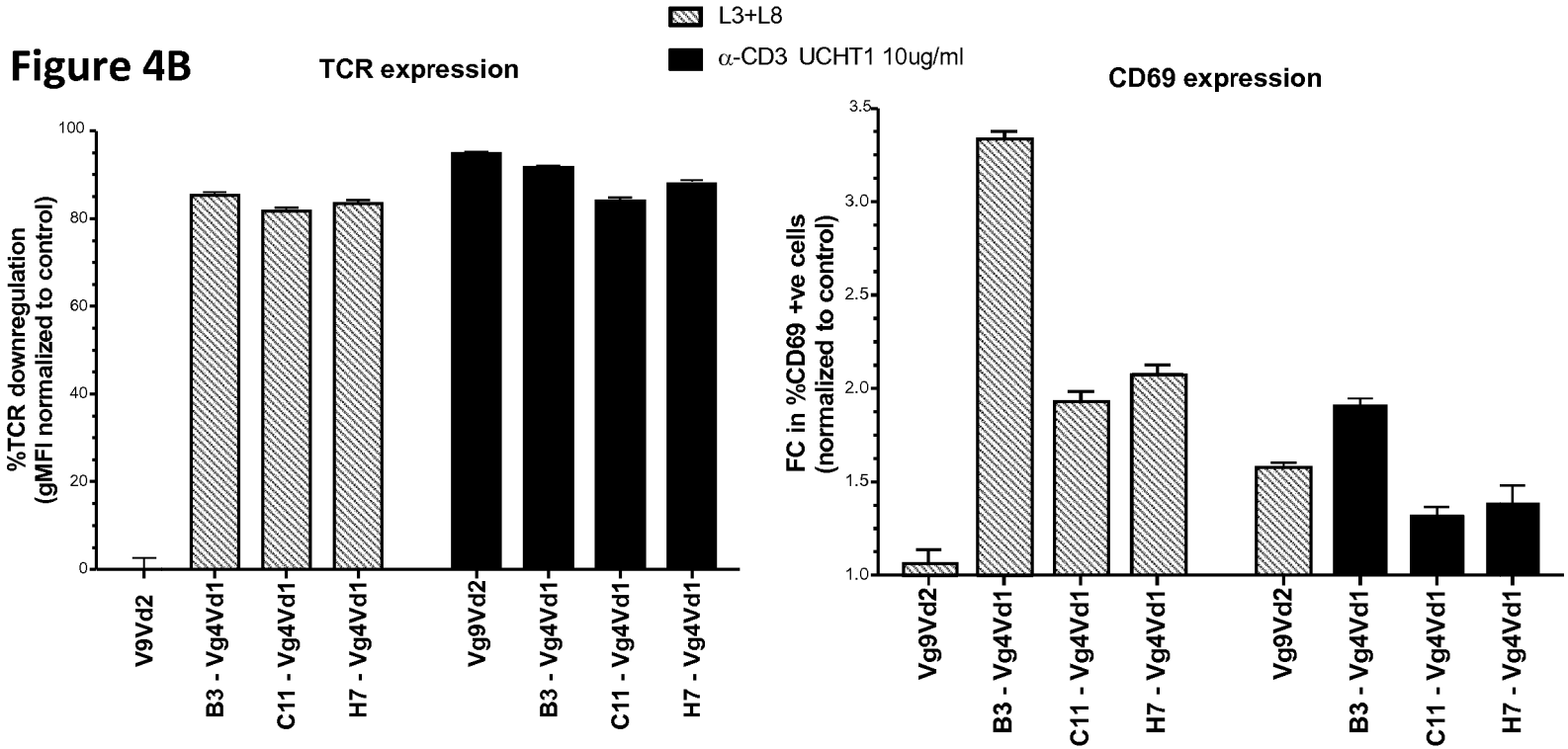


Figure 5

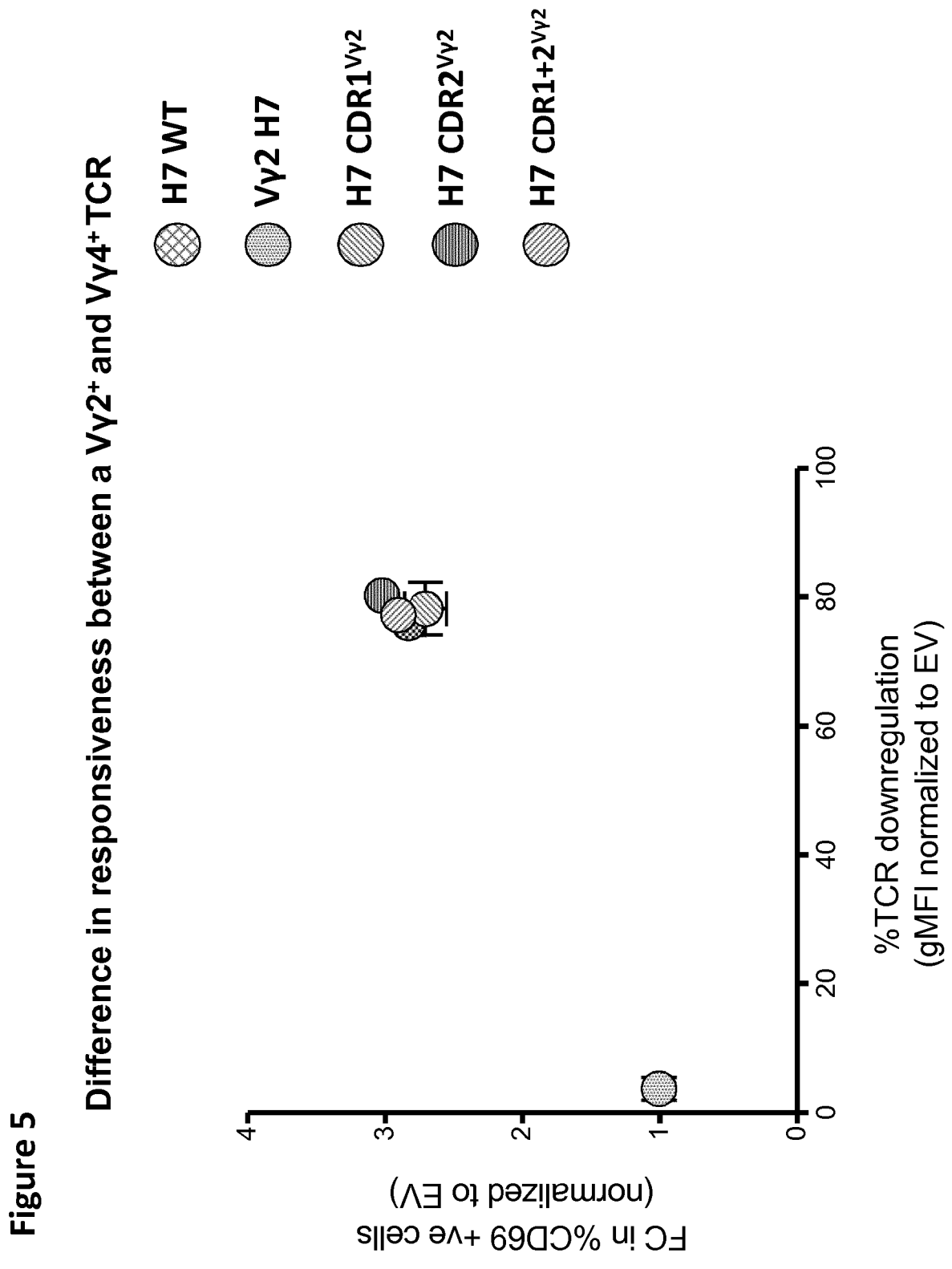
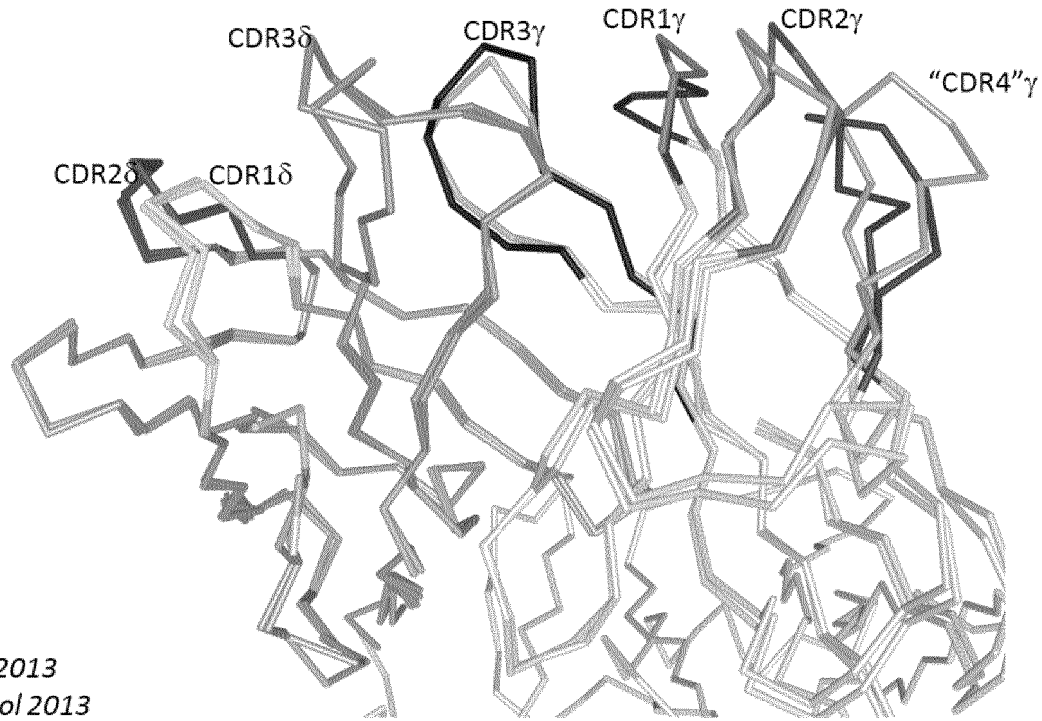

Difference in responsiveness between a Vγ2+ and Vγ4+ TCR
H7 WT
Vγ2 H7
H7 CDR1Vγ2
H7 CDR2Vγ2
H7 CDR1+2Vγ2
FC in %CD69 +ve cells
(normalized to EV)
0
1
2
3
4
0
20
40
60
80
100
%TCR downregulation
(gMFI normalized to EV)

Figure 6A

```
TRGV2   SSNLEGRTKSVIRQTGSSAEITCDLAEGSNGYIHWYLHQEGKAPQRLQYYDSYNSKVVLESGVSPGKYYTYASTRNNLRLILRNLIENDSGVYYCATWDG
TRGV4   SSNLEGRTKSVIRQTGSSAEITCDLAEGSTGYIHWYLHQEGKAPQRLLYYDSYTSSVVLESGISPGKYDTYGSTRKNLRMILRNLIENDSGVYYCATWDG
                                  CDR1                CDR2              "CDR4"                CDR3
```

Figure 6B



Structures from:
Luoma, Immunity 2013
Ulrich, Nat Immunol 2013

Figure 7

The "CDR4" (AKA HV4, HVR4, FR3) loop sequence of the Vg4 TCR is critical for responsiveness to BTNL3+L8

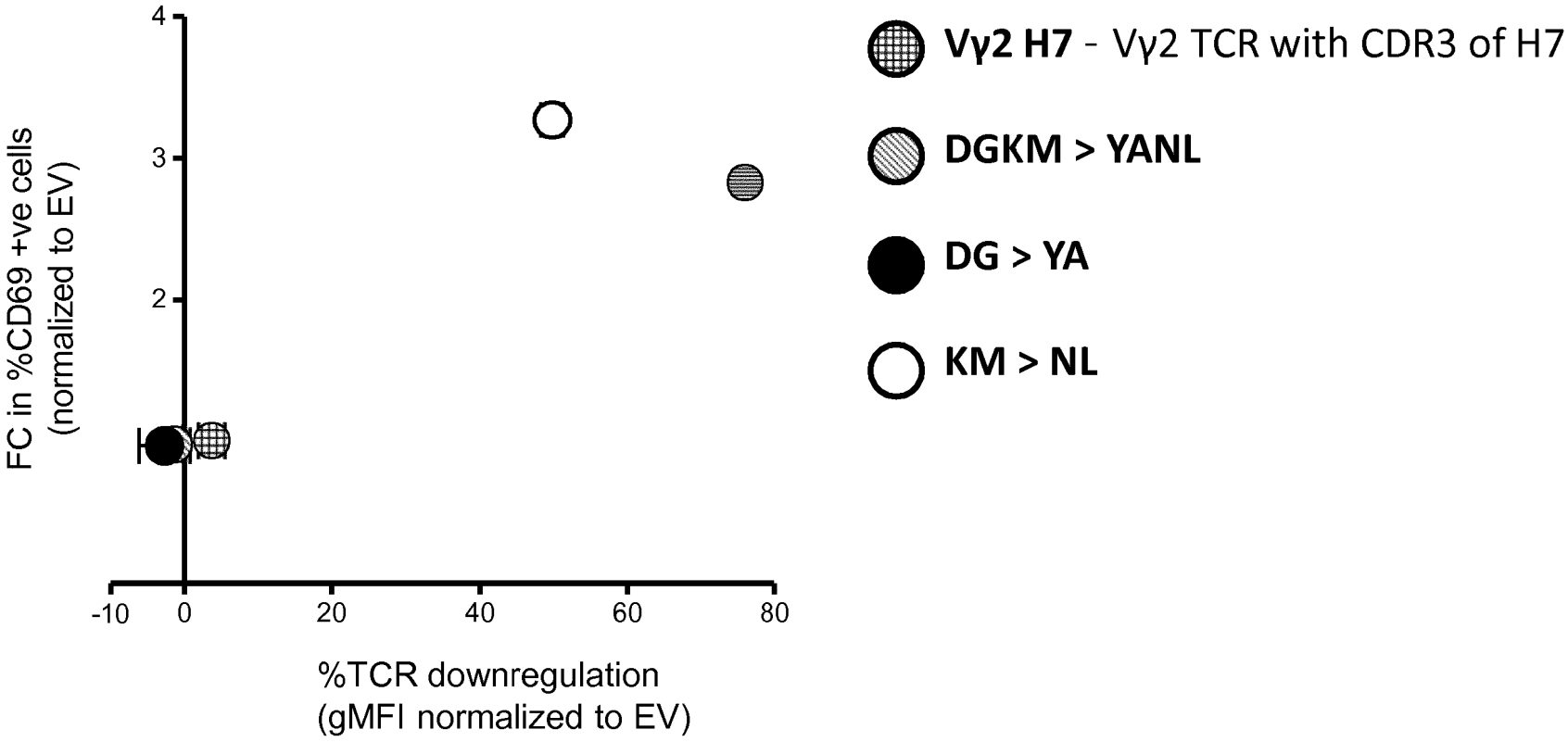




Figure 8

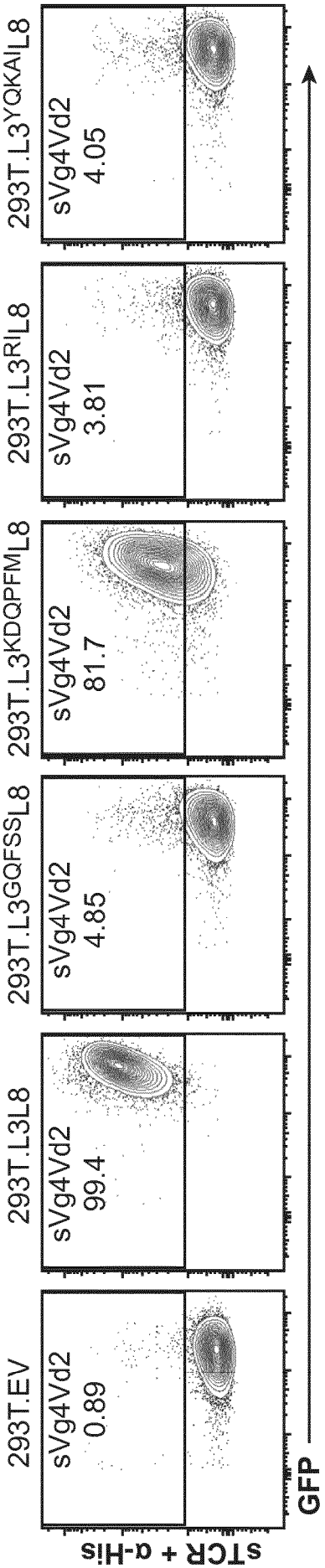

293T.EV
sVg4Vd2
0.89
293T.L3L8
sVg4Vd2
99.4
293T.L3GQFSSL8
sVg4Vd2
4.85
293T.L3KDQPFML8
sVg4Vd2
81.7
293T.L3RIL8
sVg4Vd2
3.81
293T.L3YQKAIL8
sVg4Vd2
4.05
sTCR + α-His
GFP

FIG. 9A

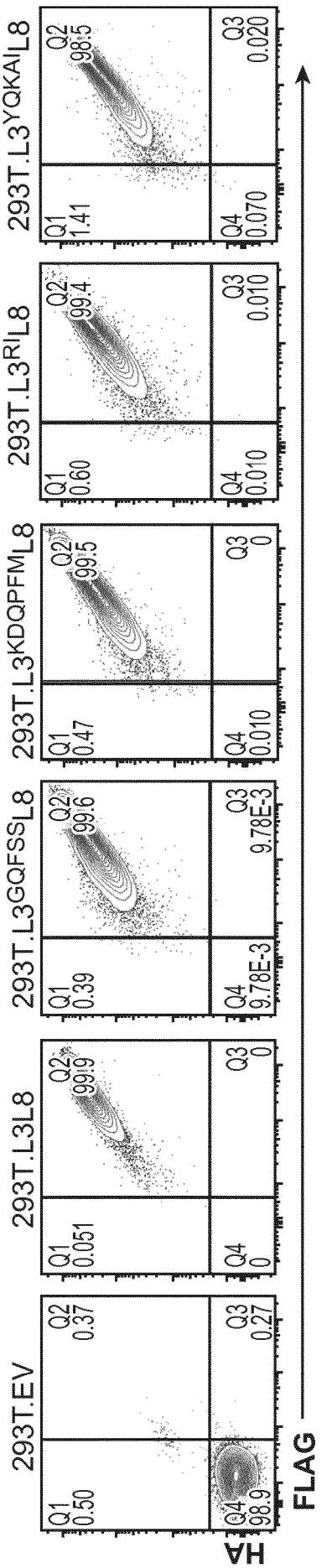

293T.EV
Q1 0.50
Q2 0.37
Q4 98.9
Q3 0.27
293T.L3L8
Q1 0.051
Q2 99.9
Q4 0
Q3 0
293T.L3GQFSSL8
Q1 0.39
Q2 99.6
Q4 9.78E-3
Q3 9.78E-3
293T.L3KDQPFML8
Q1 0.47
Q2 99.5
Q4 0.010
Q3 0
293T.L3RIL8
Q1 0.60
Q2 99.4
Q4 0.010
Q3 0.010
293T.L3YQKAIL8
Q1 1.41
Q2 98.5
Q4 0.070
Q3 0.020
HA
FLAG

FIG. 9B

Figure 10

Cells stained with indicated soluble TCR constructs (His-tagged) + αHis-APC

Numbers above plots indicate dilutions of the TCR + αHis-APC

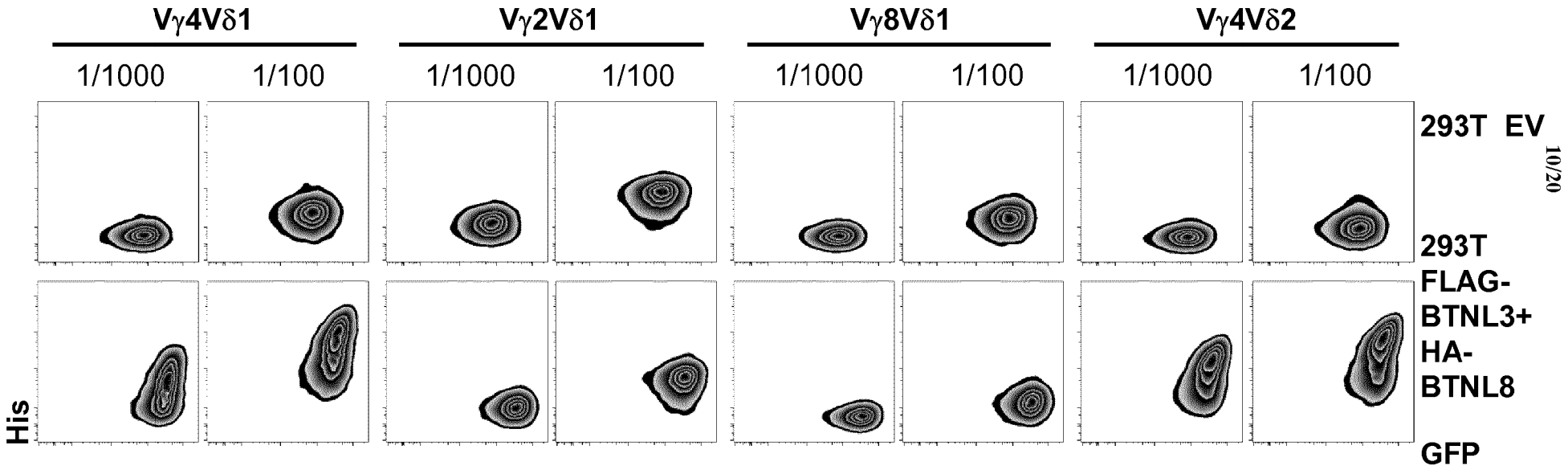


Figure 11A

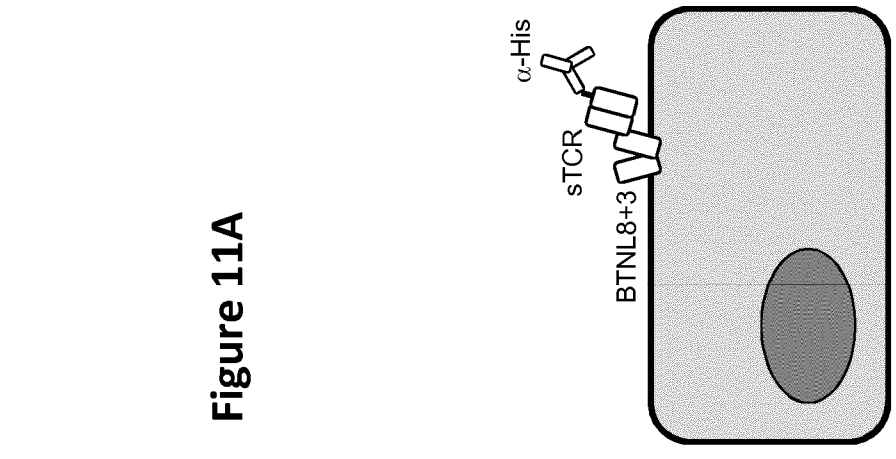
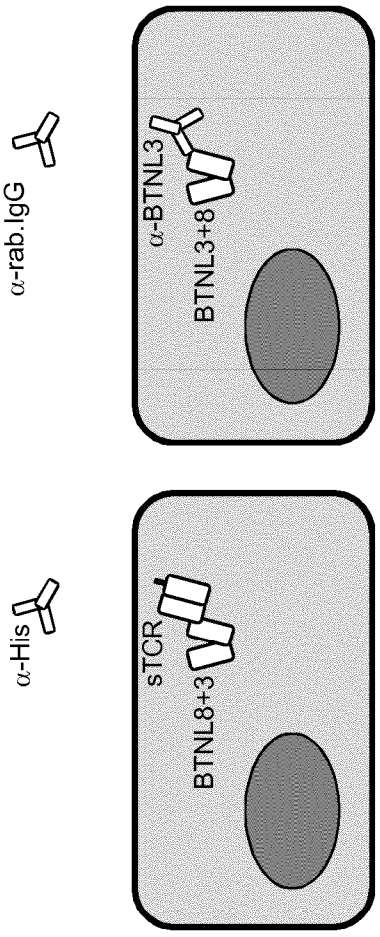


Figure 11B



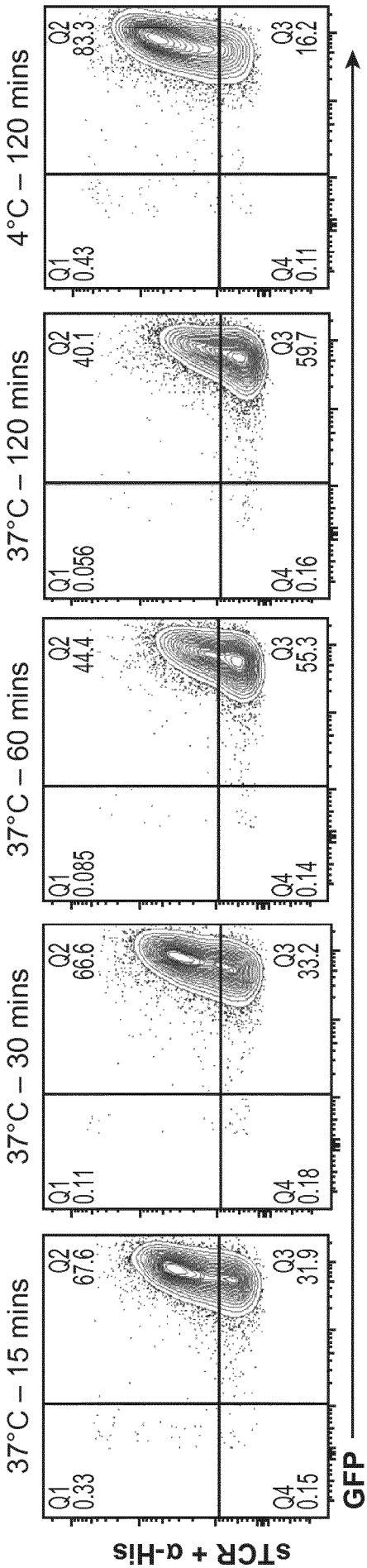
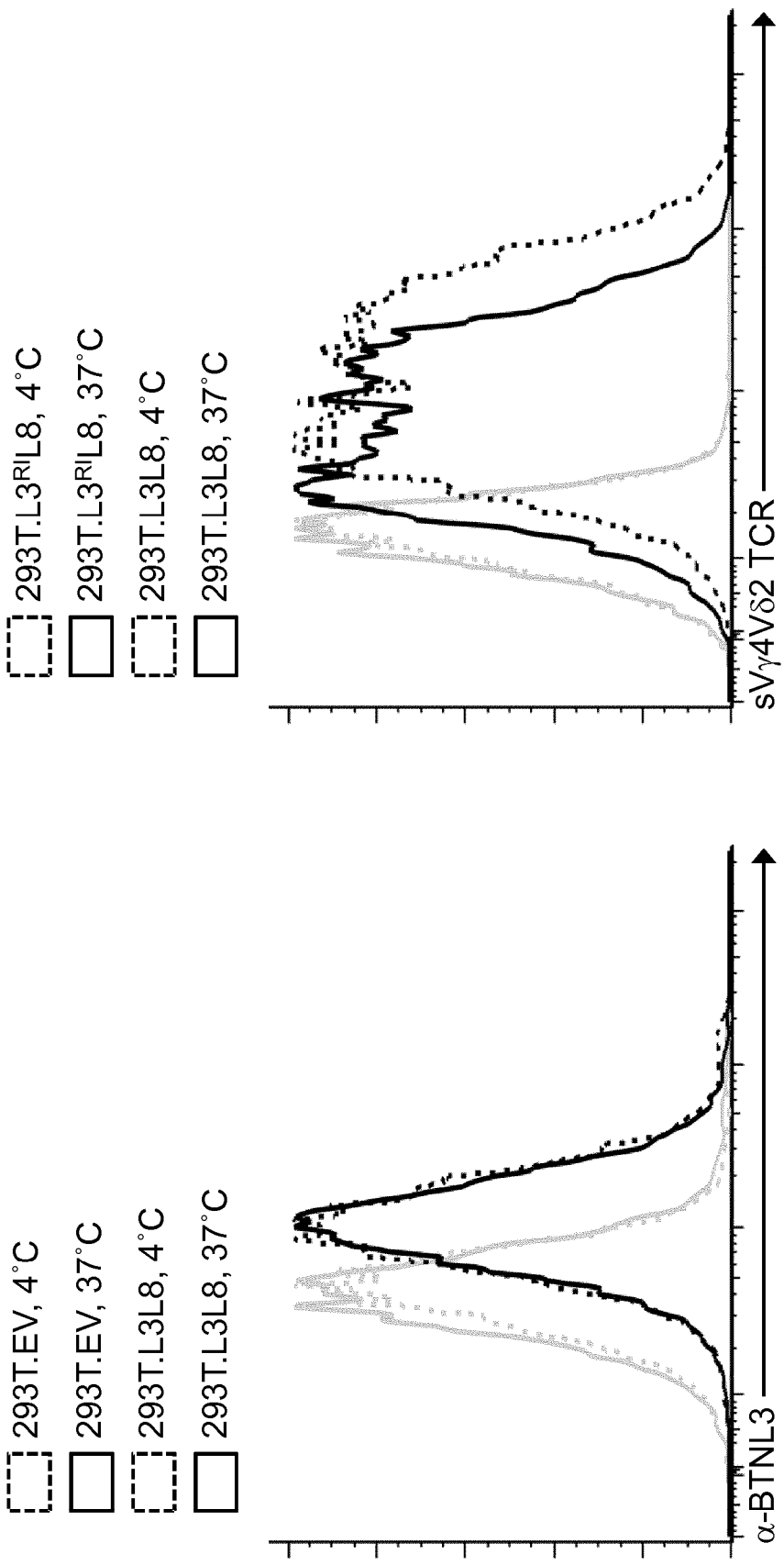

37°C – 15 mins
37°C – 30 mins
37°C – 60 mins
37°C – 120 mins
4°C – 120 mins
Q1 0.33
Q2 67.6
Q4 0.15
Q3 31.9
Q1 0.11
Q2 66.6
Q4 0.18
Q3 33.2
Q1 0.085
Q2 44.4
Q4 0.14
Q3 55.3
Q1 0.056
Q2 40.1
Q4 0.16
Q3 59.7
Q1 0.43
Q2 83.3
Q4 0.11
Q3 16.2
sTCR + α-His
GFP

FIG. 12

Figure 13A


293T.EV, 4˚C
293T.EV, 37˚C
293T.L3L8, 4˚C
293T.L3L8, 37˚C
293T.L3RIL8, 4˚C
293T.L3RIL8, 37˚C
293T.L3L8, 4˚C
293T.L3L8, 37˚C
α-BTNL3
sVγ4Vδ2 TCR

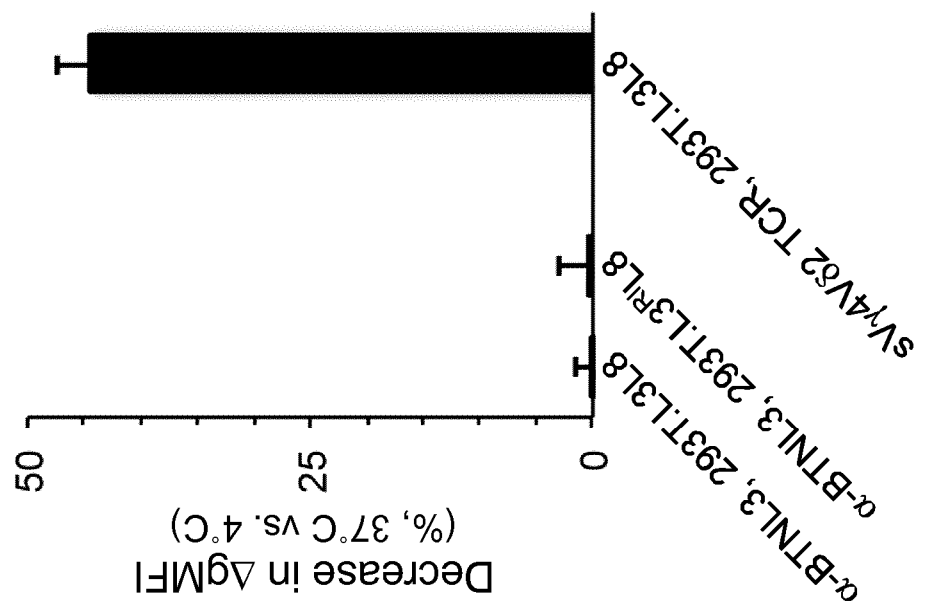
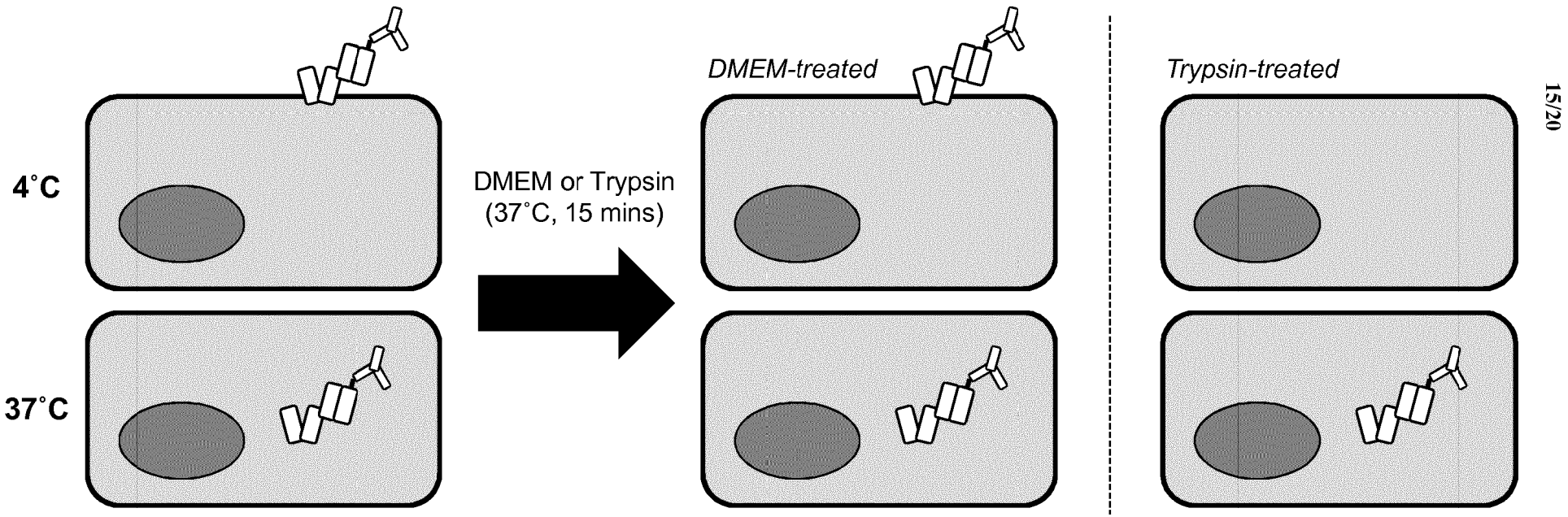

Decrease in ΔgMFI
(%, 37°C vs. 4°C)
50
25
0
α-BTNL3, 293T.L3L8
α-BTNL3, 293T.L3RL8
sVγ4Vδ2 TCR, 293T.L3L8

Figure 13B

Figure 14


4˚C
37˚C
DMEM or Trypsin
(37˚C, 15 mins)
DMEM-treated
Trypsin-treated

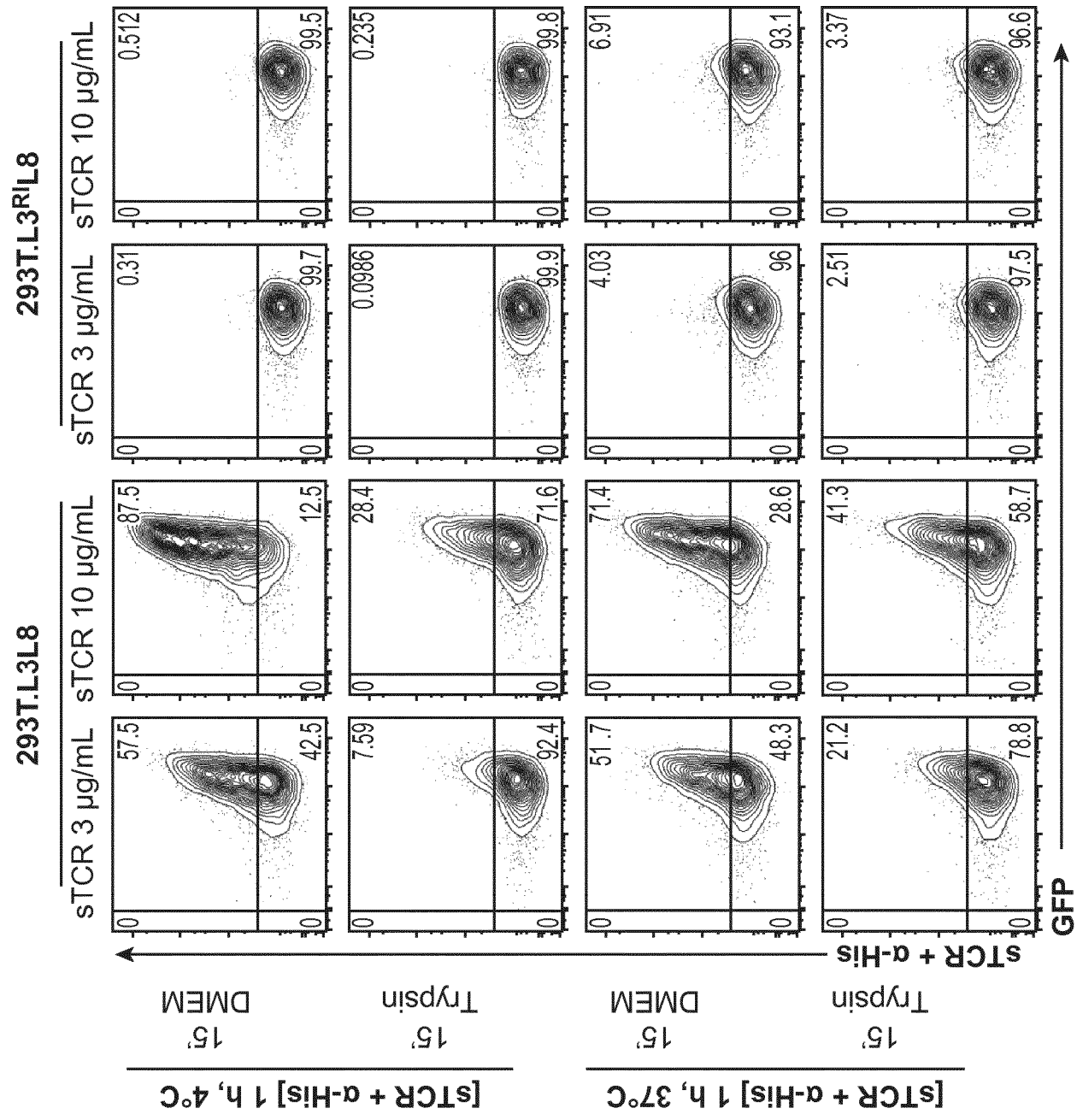

293T.L3L8
293T.L3RIL8
sTCR 3 µg/mL
sTCR 10 µg/mL
sTCR 3 µg/mL
sTCR 10 µg/mL
57.5
42.5
87.5
12.5
0.31
99.7
0.512
99.5
7.59
92.4
28.4
71.6
0.0986
99.9
0.235
99.8
51.7
48.3
71.4
28.6
4.03
96
6.91
93.1
21.2
78.8
41.3
58.7
2.51
97.5
3.37
96.6
[sTCR + α-His] 1 h, 37°C
[sTCR + α-His] 1 h, 4°C
15'
Trypsin
15'
DMEM
15'
Trypsin
15'
DMEM
sTCR + α-His
GFP

FIG. 15A

Figure 15B

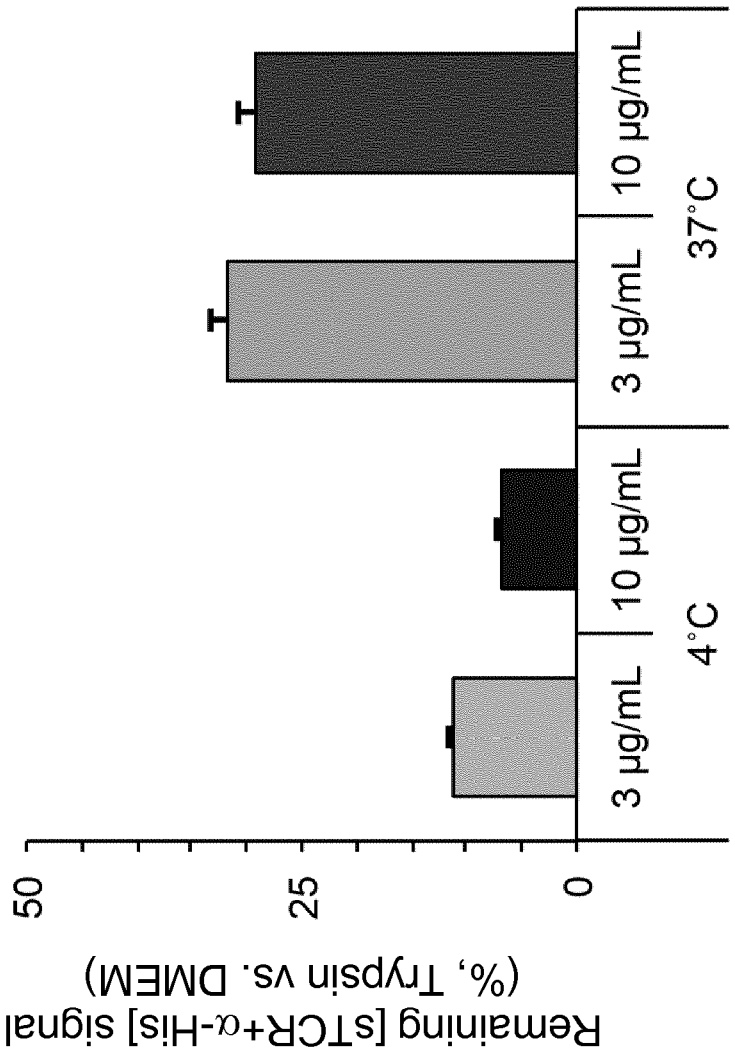

Remaining [sTCR+α-His] signal
(%, Trypsin vs. DMEM)
50
25
0
3 µg/mL
10 µg/mL
3 µg/mL
10 µg/mL
4°C
37°C

Figure 16A

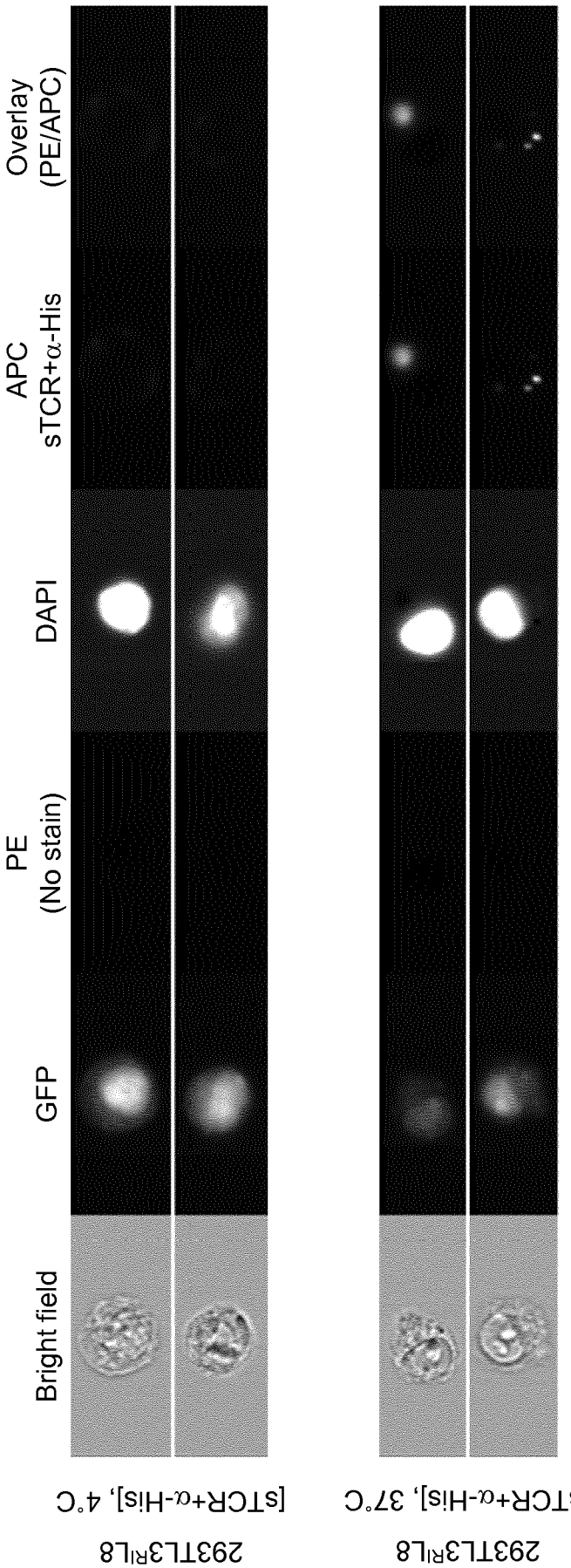
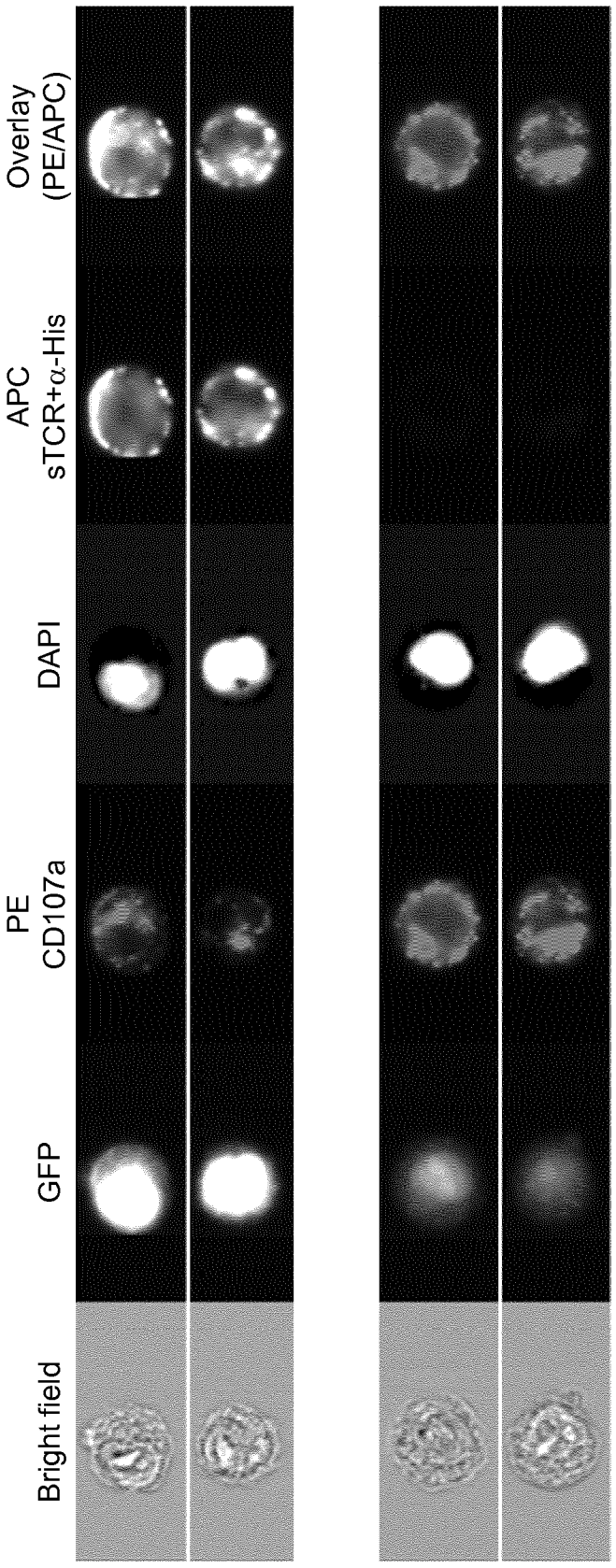

Bright field
GFP
PE
(No stain)
DAPI
APC
sTCR+α-His
Overlay
(PE/APC)
293TL3RIL8
[sTCR+α-His], 37°C
293TL3RIL8
[sTCR+α-His], 4°C

Figure 16B


Bright field
GFP
PE
CD107a
DAPI
APC
sTCR+α-His
Overlay
(PE/APC)
293TL3L8
[sTCR+α-His], 4°C
DMEM
293TL3L8
[sTCR+α-His], 4°C
TRYPSIN

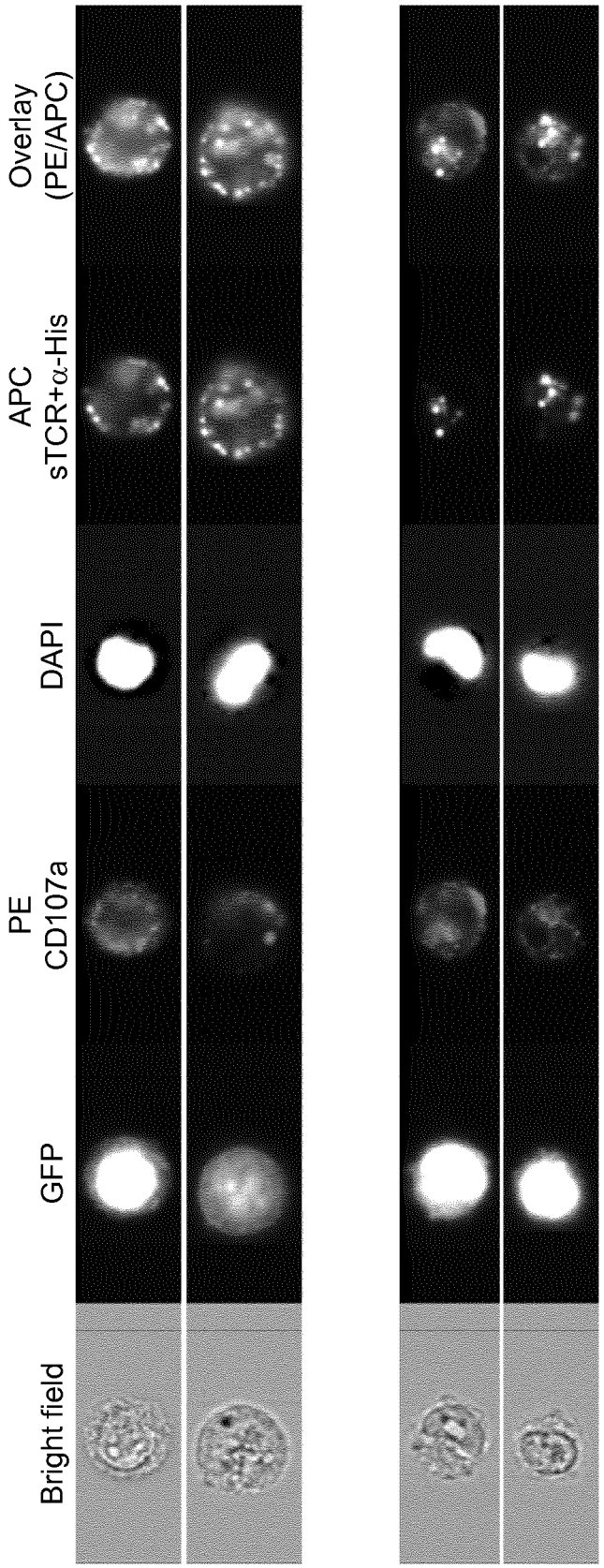

Bright field
GFP
PE
CD107a
DAPI
APC
sTCR+α-His
Overlay
(PE/APC)
293TL3L8
[sTCR+α-His], 37°C
TRYPSIN
293TL3L8
[sTCR+α-His], 37°C
DMEM

Figure 16C