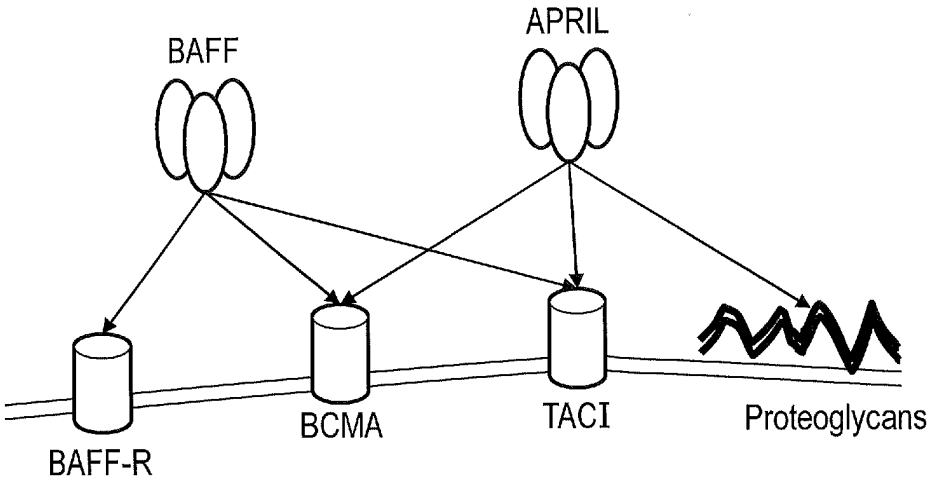

BAFF
APRIL
BAFF-R
BCMA
TACI
Proteoglycans

FIG. 1

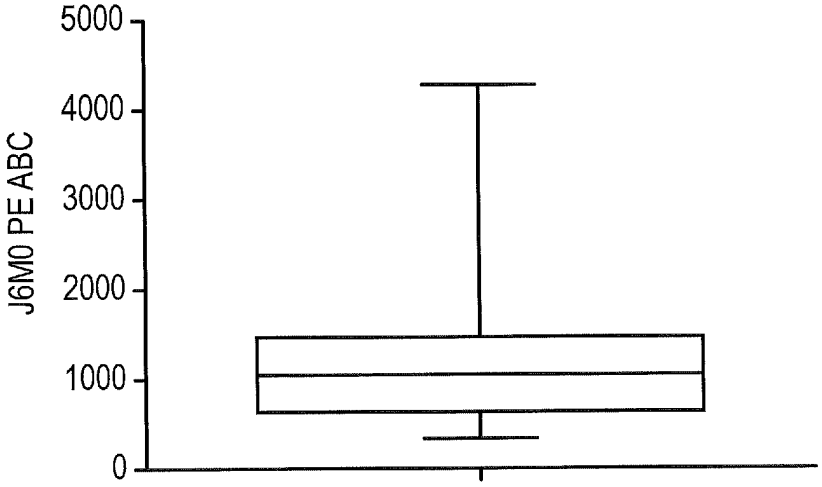

5000
4000
3000
2000
1000
0
J6M0 PE ABC

FIG. 2

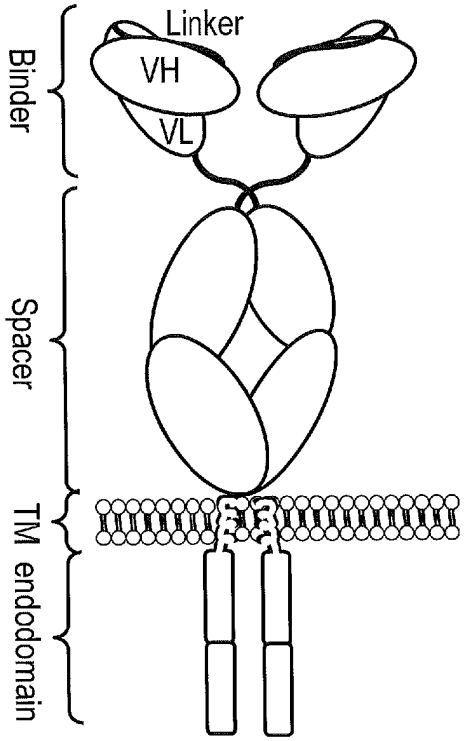

Linker
VH
VL
Binder
Spacer
TM
endodomain

FIG. 3

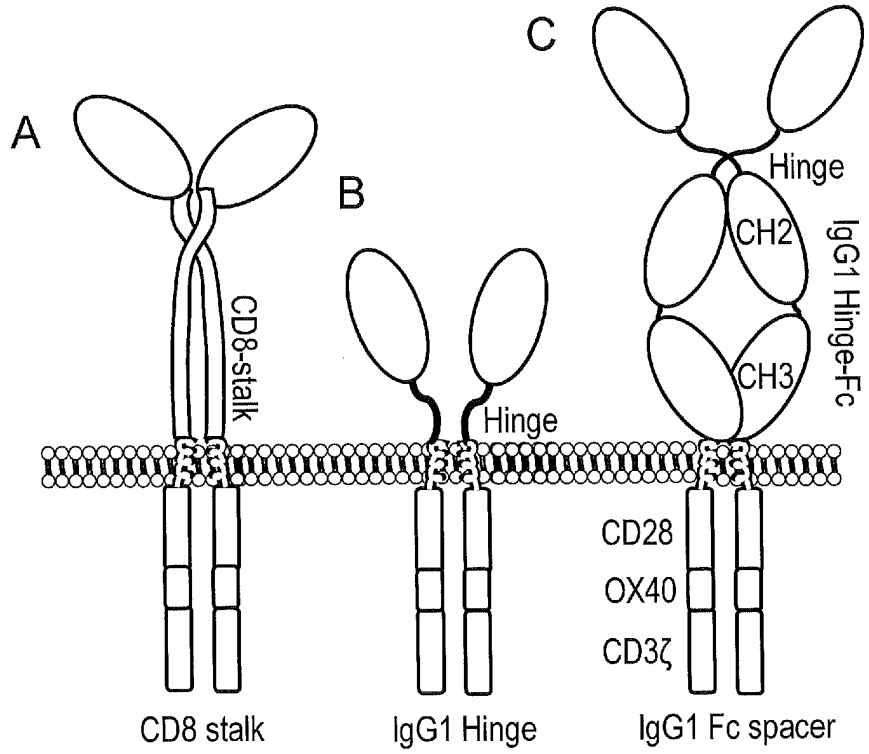

A
B
C
CD8-stalk
Hinge
Hinge
CH2
CH3
IgG1 Hinge-Fc
CD28
OX40
CD3ζ
CD8 stalk
IgG1 Hinge
IgG1 Fc spacer

FIG. 4

A

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQ
VLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPH
GTFLGFVKL*SGGGSDP*TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVLVVVGGVL
ACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGG
SFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

B

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQ
VLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPH
GTFLGFVKL*SGGGSDP*AEPKSPDKTHTCPPCPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHS
DYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSR
SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

C

METDTLLLWVLLLWVPGSTGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQ
VLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVIIPRARAKLNLSPH
GTFLGFVKL*SGGGSDP*AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM
NMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSAD
APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR
GKGHDGLYQGLSTATKDTYDALHMQALPPR

Signal Peptide	Efficient signal peptide
dAPRIL	Truncated APRIL
Spacer	Either hinge-CH2CH3 of human IgG1, human CD8α stalk and human IgG1 hinge
TM and endodomain	Compound endodomain comprising of the CD28TM domain, CD28 endodomain and OX40 and CD3-Zeta endodomains

FIG. 5

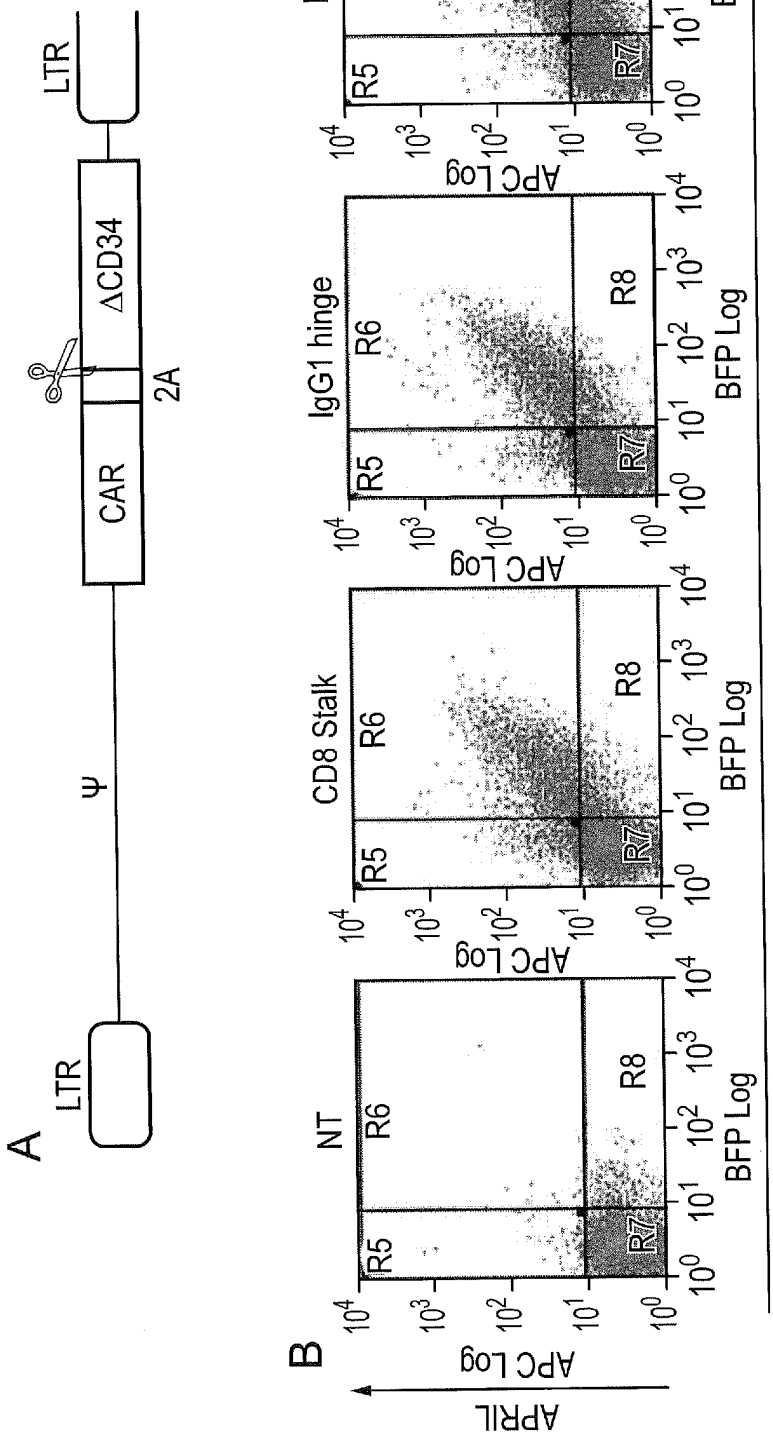

A
LTR
Ψ
CAR
2A
ΔCD34
LTR
B
APRIL
APC Log
BFP Log
dCD34
NT
CD8 Stalk
IgG1 hinge
FcPvaa
R5
R6
R7
R8

FIG. 6

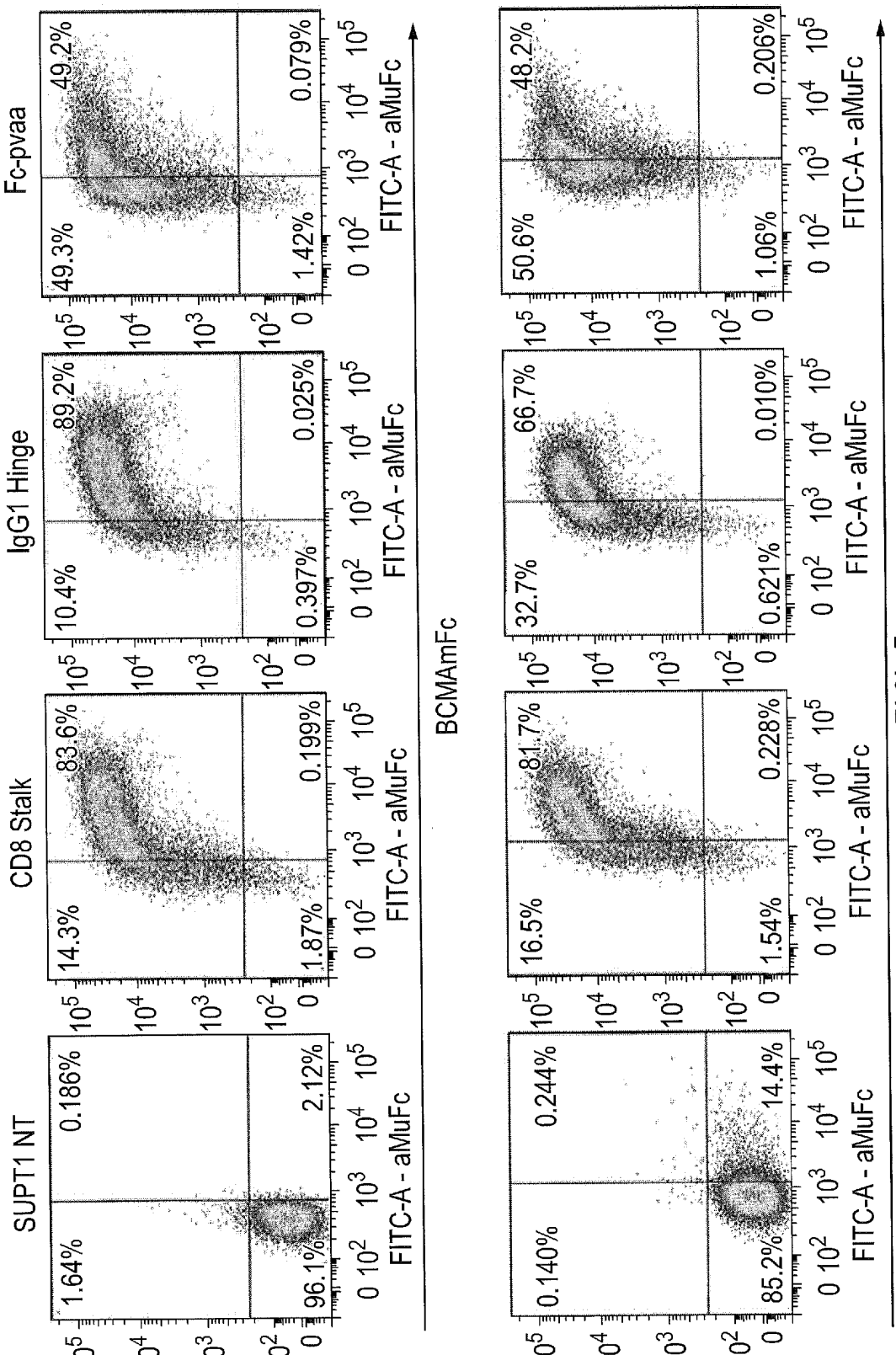


FIG. 6 (continued)

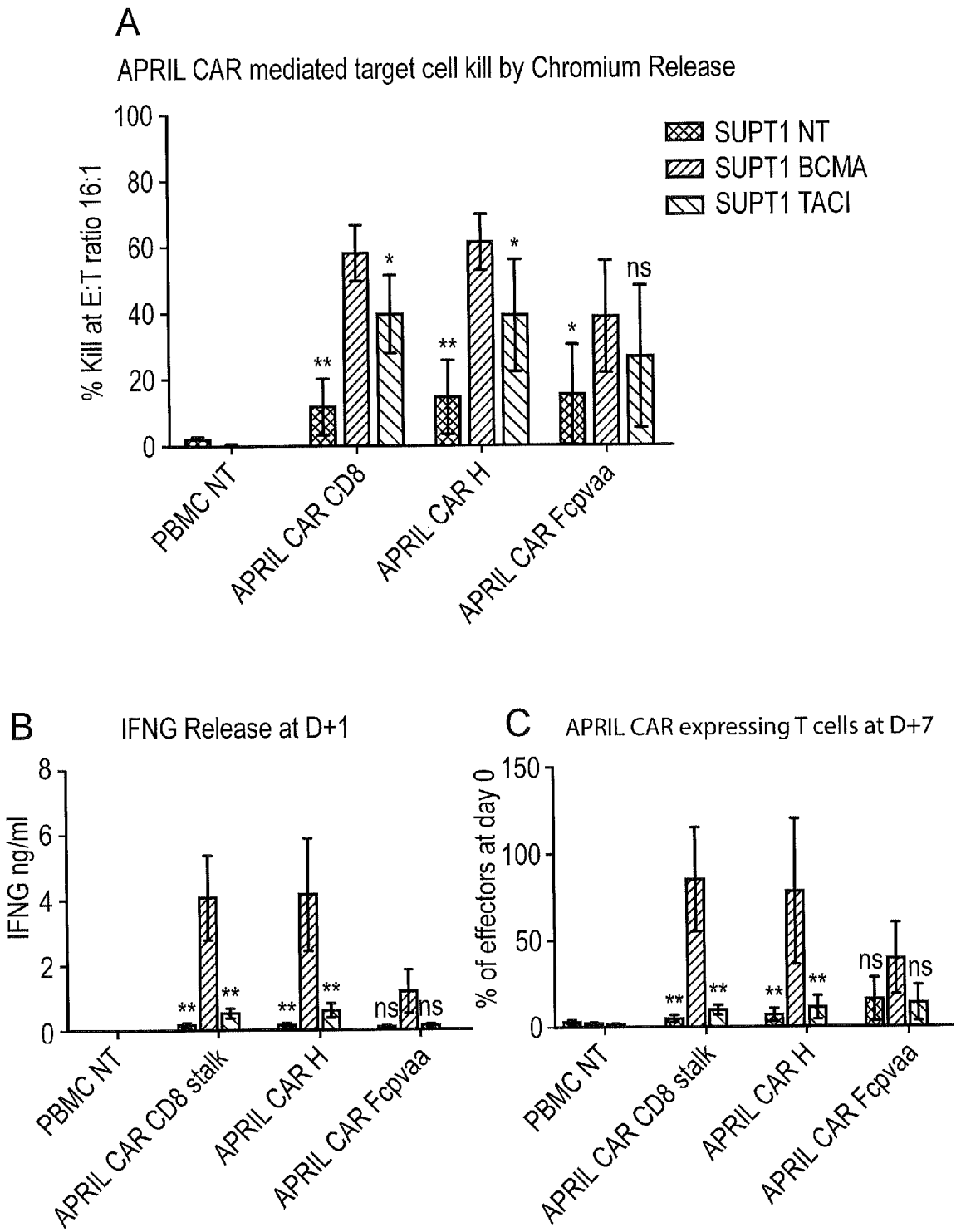

A
APRIL CAR mediated target cell kill by Chromium Release
% Kill at E:T ratio 16:1
100
80
60
40
20
0
SUPT1 NT
SUPT1 BCMA
SUPT1 TACI
**
*
**
*
*
ns
PBMC NT
APRIL CAR CD8
APRIL CAR H
APRIL CAR Fcpvaa
B
IFNG Release at D+1
IFNG ng/ml
8
6
4
2
0
**
**
**
**
ns
ns
PBMC NT
APRIL CAR CD8 stalk
APRIL CAR H
APRIL CAR Fcpvaa
C
APRIL CAR expressing T cells at D+7
% of effectors at day 0
150
100
50
0
**
**
**
**
ns
ns
PBMC NT
APRIL CAR CD8 stalk
APRIL CAR H
APRIL CAR Fcpvaa

FIG. 7

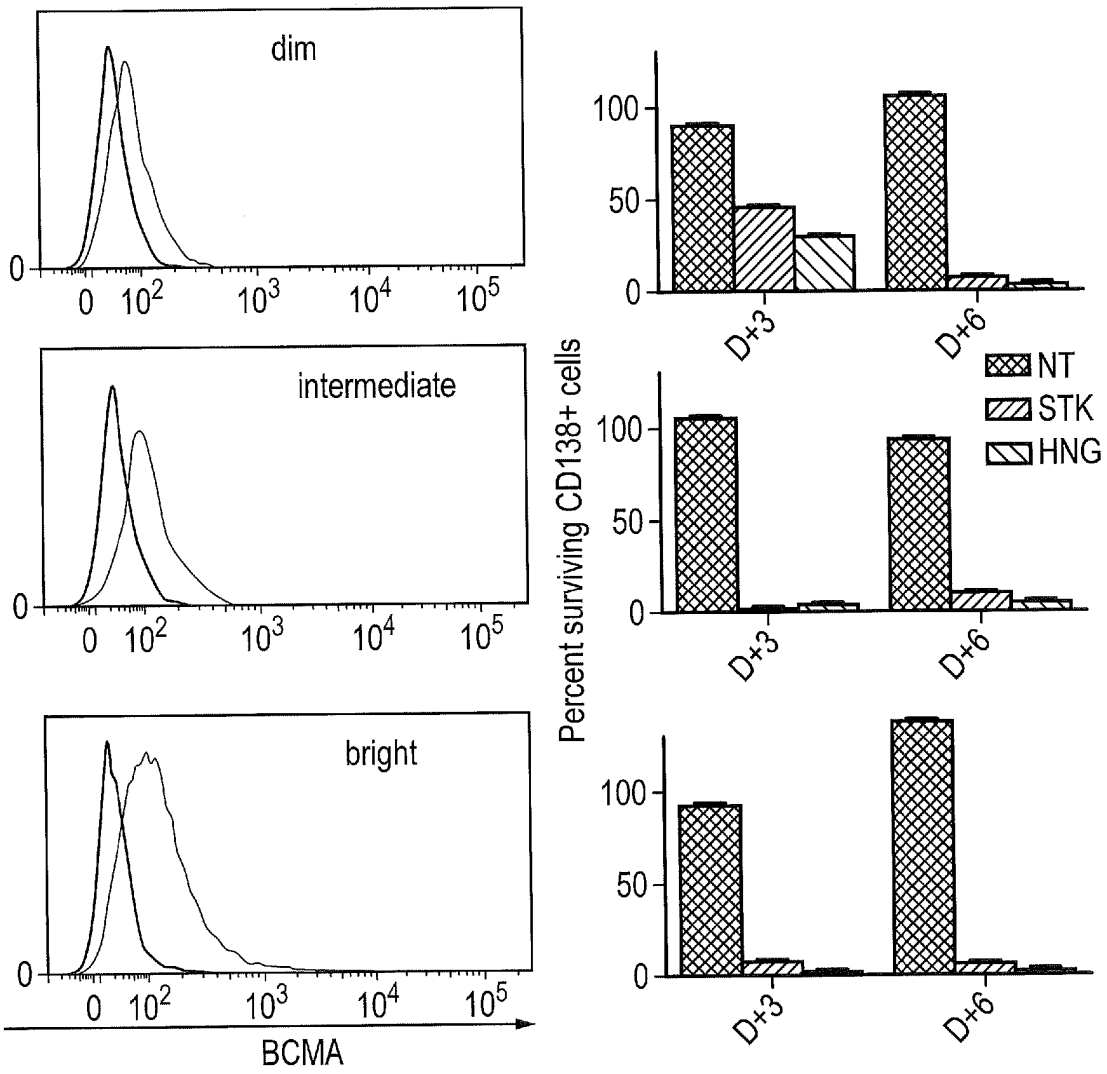

dim
intermediate
bright
0
0 10^2 10^3 10^4 10^5
BCMA
Percent surviving CD138+ cells
100
50
0
D+3
D+6
NT
STK
HNG

FIG. 8

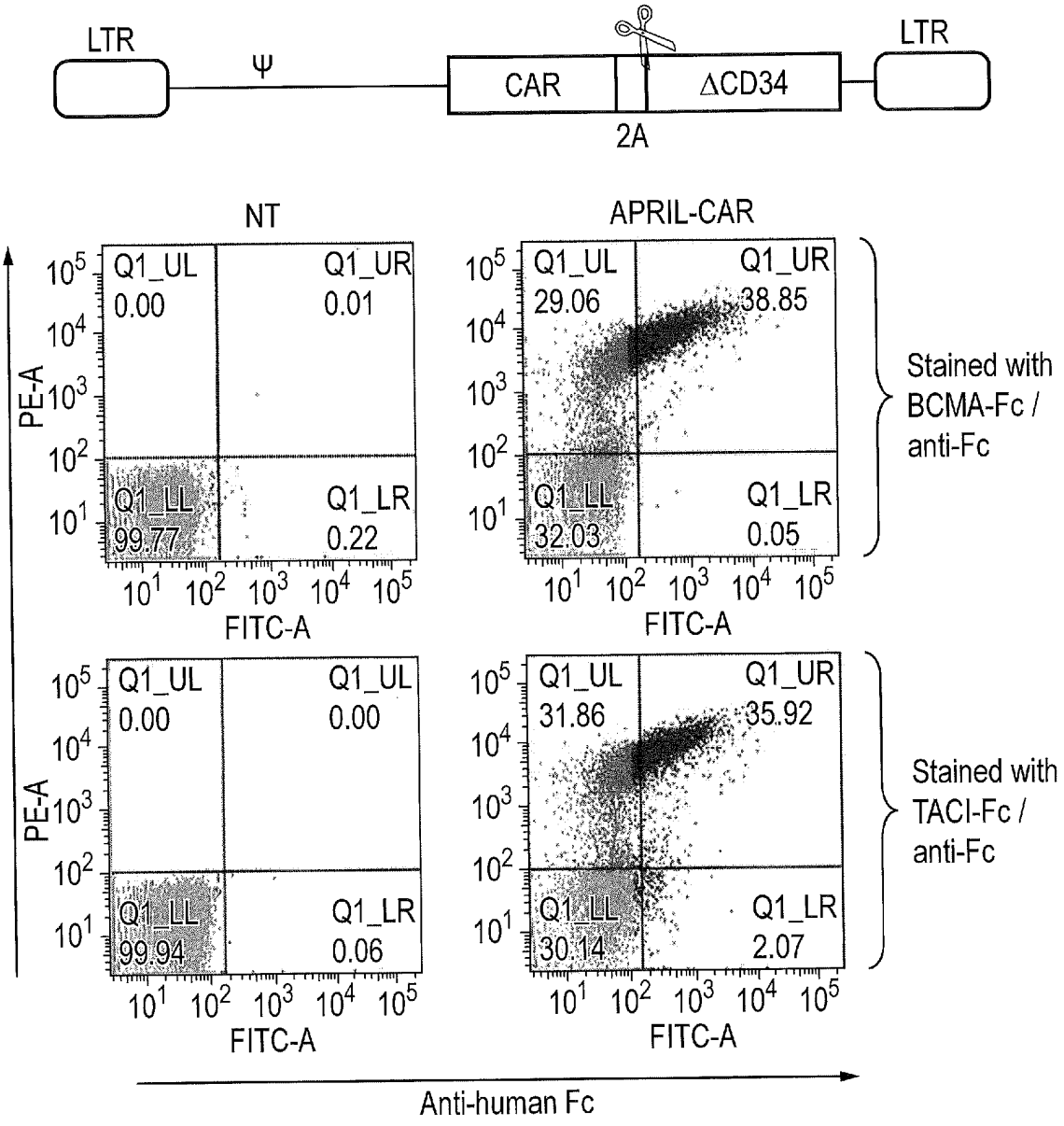

LTR
Ψ
CAR
ΔCD34
2A
LTR
NT
APRIL-CAR
Q1_UL
0.00
Q1_UR
0.01
Q1_LL
99.77
Q1_LR
0.22
Q1_UL
29.06
Q1_UR
38.85
Q1_LL
32.03
Q1_LR
0.05
Stained with BCMA-Fc / anti-Fc
Q1_UL
0.00
Q1_UL
0.00
Q1_LL
99.94
Q1_LR
0.06
Q1_UL
31.86
Q1_UR
35.92
Q1_LL
30.14
Q1_LR
2.07
Stained with TACI-Fc / anti-Fc
PE-A
FITC-A
Anti-human Fc

FIG. 9

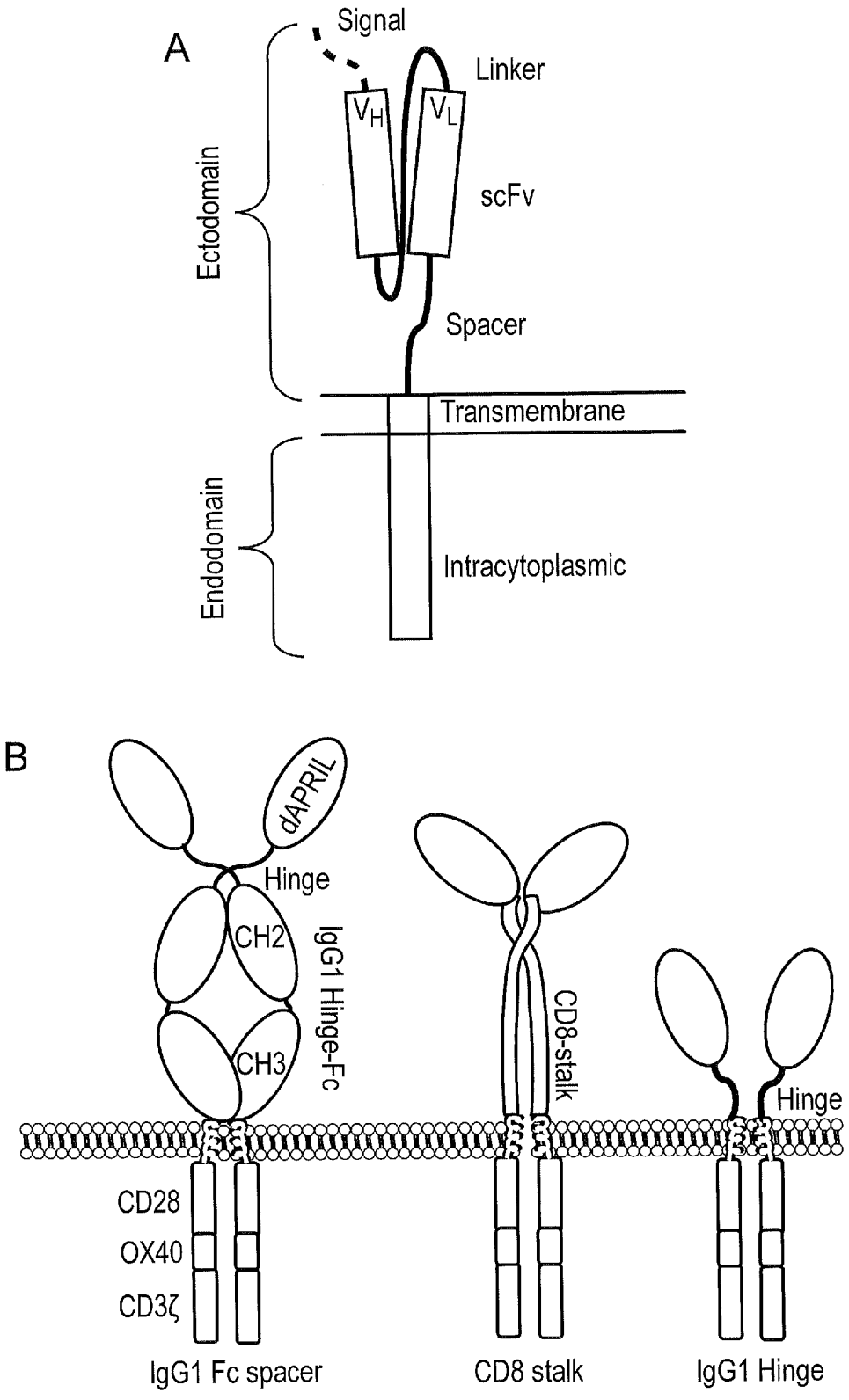

A
Signal
Linker
V_H
V_L
scFv
Ectodomain
Spacer
Transmembrane
Endodomain
Intracytoplasmic
B
dAPRIL
Hinge
CH2
CH3
IgG1 Hinge-Fc
CD8-stalk
Hinge
CD28
OX40
CD3ζ
IgG1 Fc spacer
CD8 stalk
IgG1 Hinge

FIG. 10

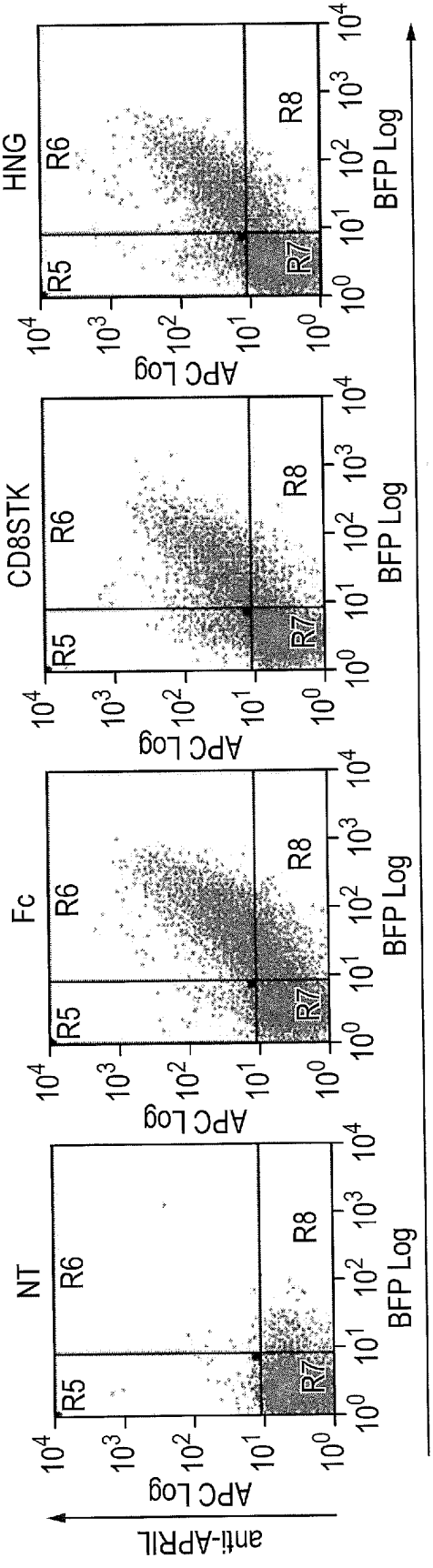


FIG. 11

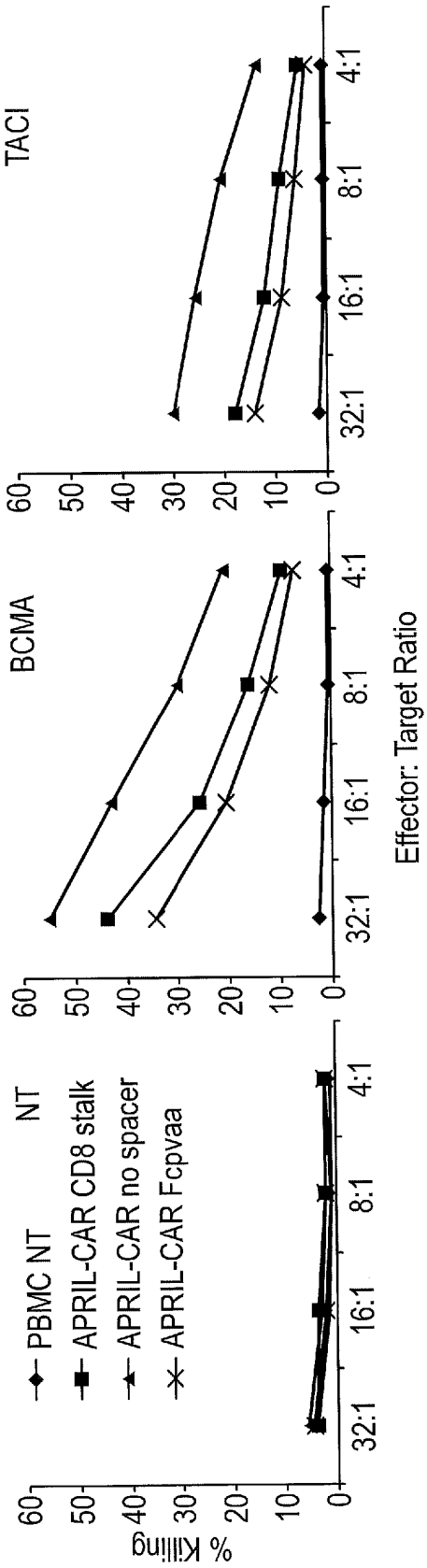

% Killing
PBMC NT
APRIL-CAR CD8 stalk
APRIL-CAR no spacer
APRIL-CAR Fcpvaa
NT
BCMA
TACI
Effector: Target Ratio
32:1
16:1
8:1
4:1
0
10
20
30
40
50
60

FIG. 12

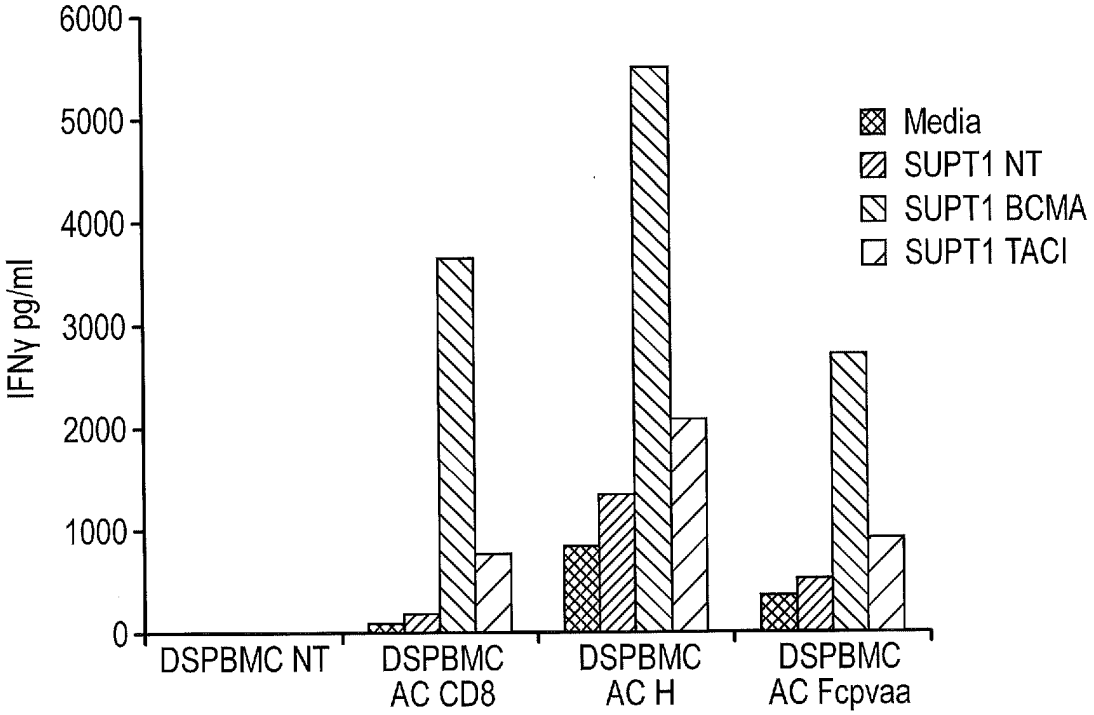

6000
5000
4000
3000
2000
1000
0
IFNγ pg/ml
Media
SUPT1 NT
SUPT1 BCMA
SUPT1 TACI
DSPBMC NT
DSPBMC AC CD8
DSPBMC AC H
DSPBMC AC Fcpvaa

FIG. 13

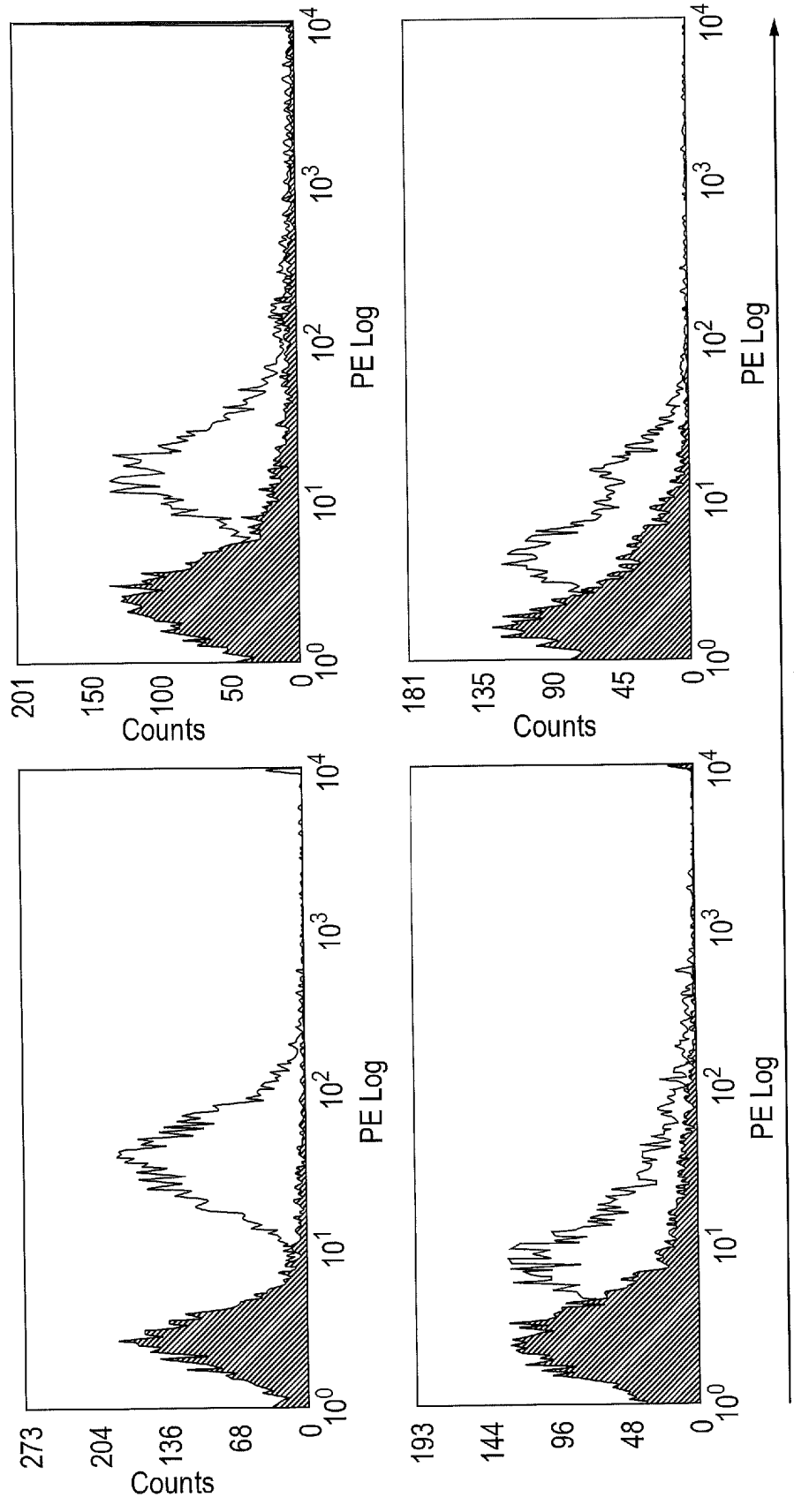

201
150
100
50
0
Counts
10^0
10^1
10^2
10^3
10^4
PE Log
181
135
90
45
0
Counts
10^0
10^1
10^2
10^3
10^4
PE Log
273
204
136
68
0
Counts
10^0
10^1
10^2
10^3
10^4
PE Log
193
144
96
48
0
10^0
10^1
10^2
10^3
10^4
PE Log
BCMA (PE)

FIG. 14

A

MGTSLLCWMALCLLGADHADGKPTPNPPLEGLDSTSGGGGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQ
AQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQG
DILSVIIPRARAKLNLSPHGTFLGFVKLSGGGSDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMI
ARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVA
FIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQ
ADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ
KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

B

MGTSLLCWMALCLLGADHADGKPTPNPPLEGLDSTSGGGGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQ
AQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQG
DILSVIIPRARAKLNLSPHGTFLGFVKLSGGGSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR
GLDFACDIFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAA
YRSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ
ALPPR

C

MGTSLLCWMALCLLGADHADGKPTPNPPLEGLDSTSGGGGSVLHLVPINATSKDDSDVTEVMWQPALRRGRGLQ
AQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQVVSREGQGRQETLFRCIRSMPSHPDRAYNSCYSAGVFHLHQG
DILSVIIPRARAKLNLSPHGTFLGFVKLSGGGSDPAEPKSPDKTHTCPPCPKDPKFWVLVVVGGVLACYSLLV
TVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQ
EEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN
ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Signal Peptide	Efficient signal peptide
tag and linker	Epiptope tag and linker (will be removed for production version, here for Western blotting etc).
dAPRIL	Truncated APRIL
spacer	Either hinge-CH2CH3 of human IgG1, human CD8α stalk and human IgG1 hinge
TM and endodomain	Compound endodomain comprising of the CD28TM domain, CD28 endodomain and OX40 and CD3-Zeta endodomains

FIG. 15

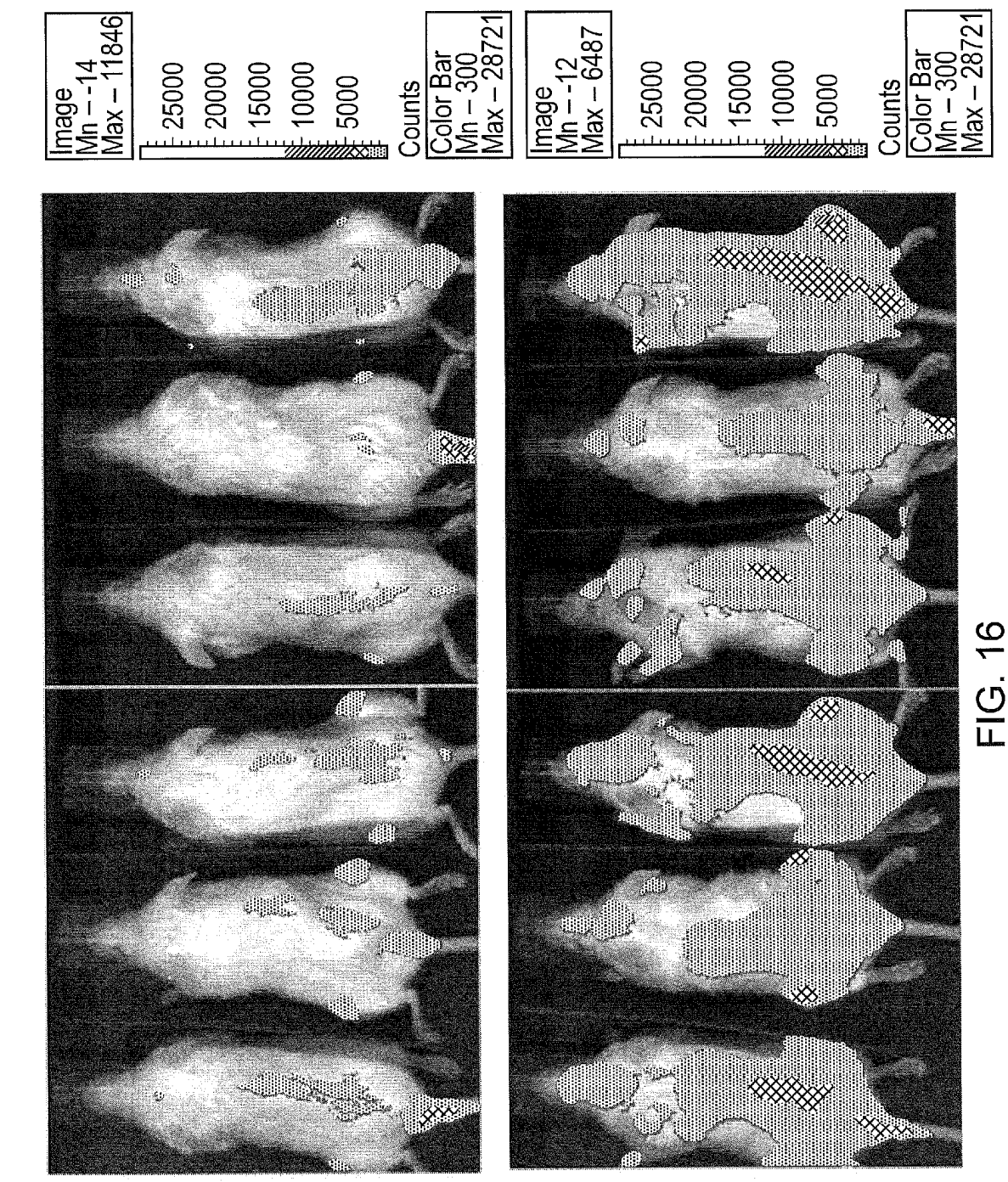

Image
Mn – -14
Max – 11846
25000
20000
15000
10000
5000
Counts
Color Bar
Mn – 300
Max – 28721
Image
Mn – -12
Max – 6487
25000
20000
15000
10000
5000
Counts
Color Bar
Mn – 300
Max – 28721
D+8
D+13
T-cells IV

FIG. 16

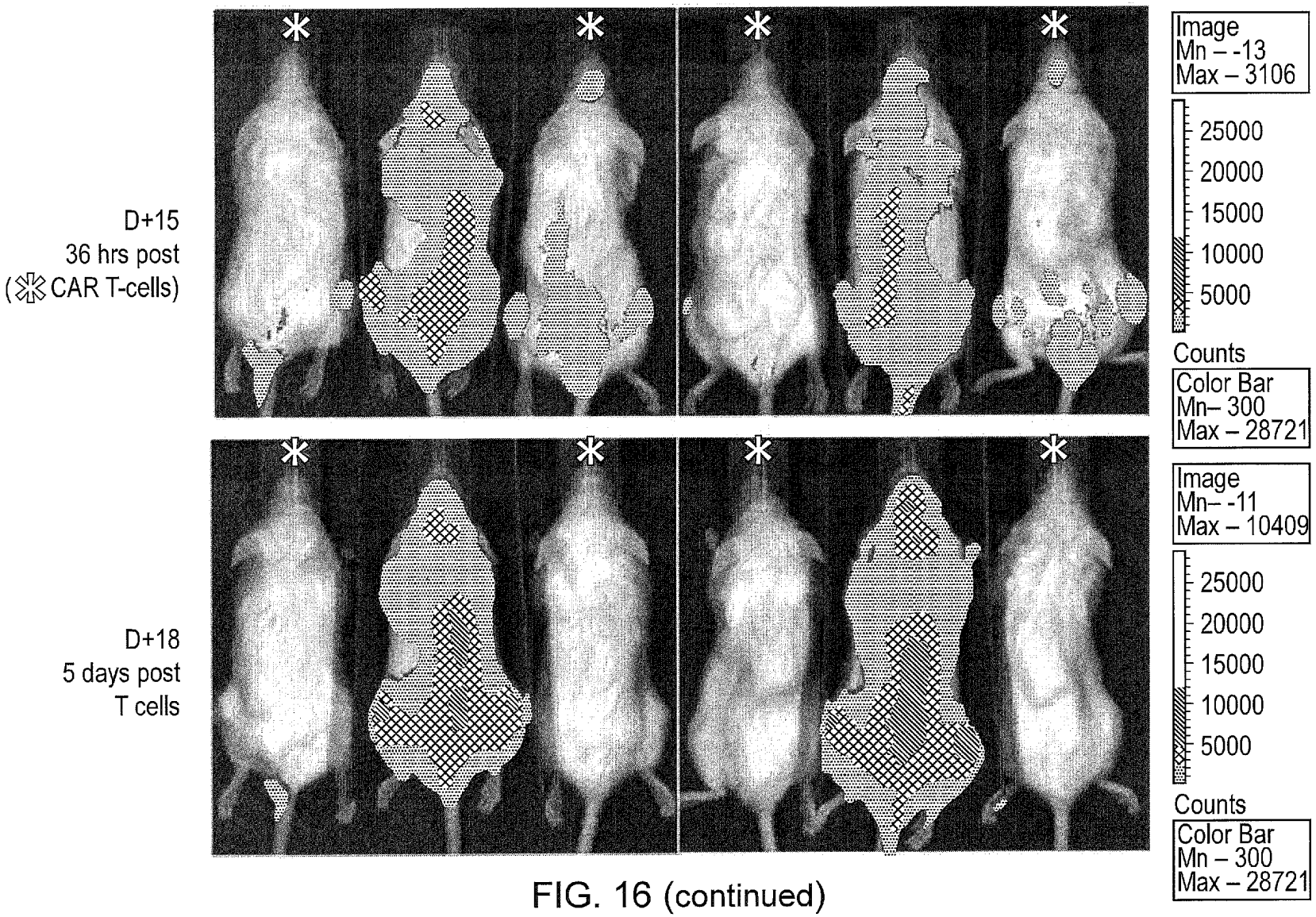


FIG. 16 (continued)