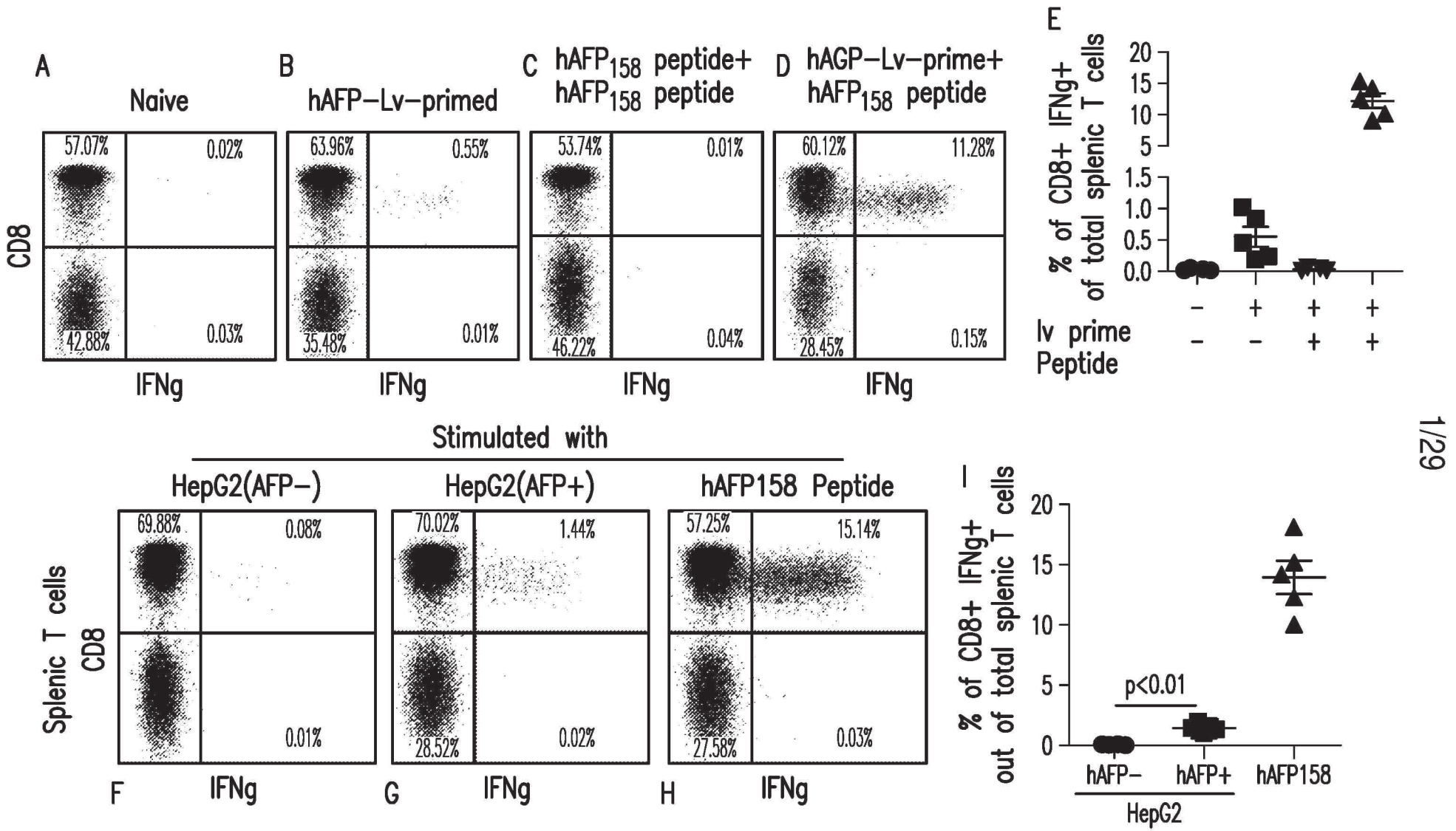

A
Naive
B
hAFP−Lv−primed
C hAFP158 peptide+ hAFP158 peptide
D hAGP−Lv−prime+ hAFP158 peptide
CD8
57.07%
0.02%
42.88%
0.03%
63.96%
0.55%
35.48%
0.01%
53.74%
0.01%
46.22%
0.04%
60.12%
11.28%
28.45%
0.15%
IFNg
E
% of CD8+ IFNg+ of total splenic T cells
20
15
10
5
1.5
1.0
0.5
0.0
lv prime
Peptide
Stimulated with
HepG2(AFP−)
HepG2(AFP+)
hAFP158 Peptide
Splenic T cells
CD8
69.88%
0.08%
0.01%
70.02%
1.44%
28.52%
0.02%
57.25%
15.14%
27.58%
0.03%
F
G
H
I
% of CD8+ IFNg+ out of total splenic T cells
p<0.01
hAFP−
hAFP+
hAFP158
HepG2

FIG. 1A-1I

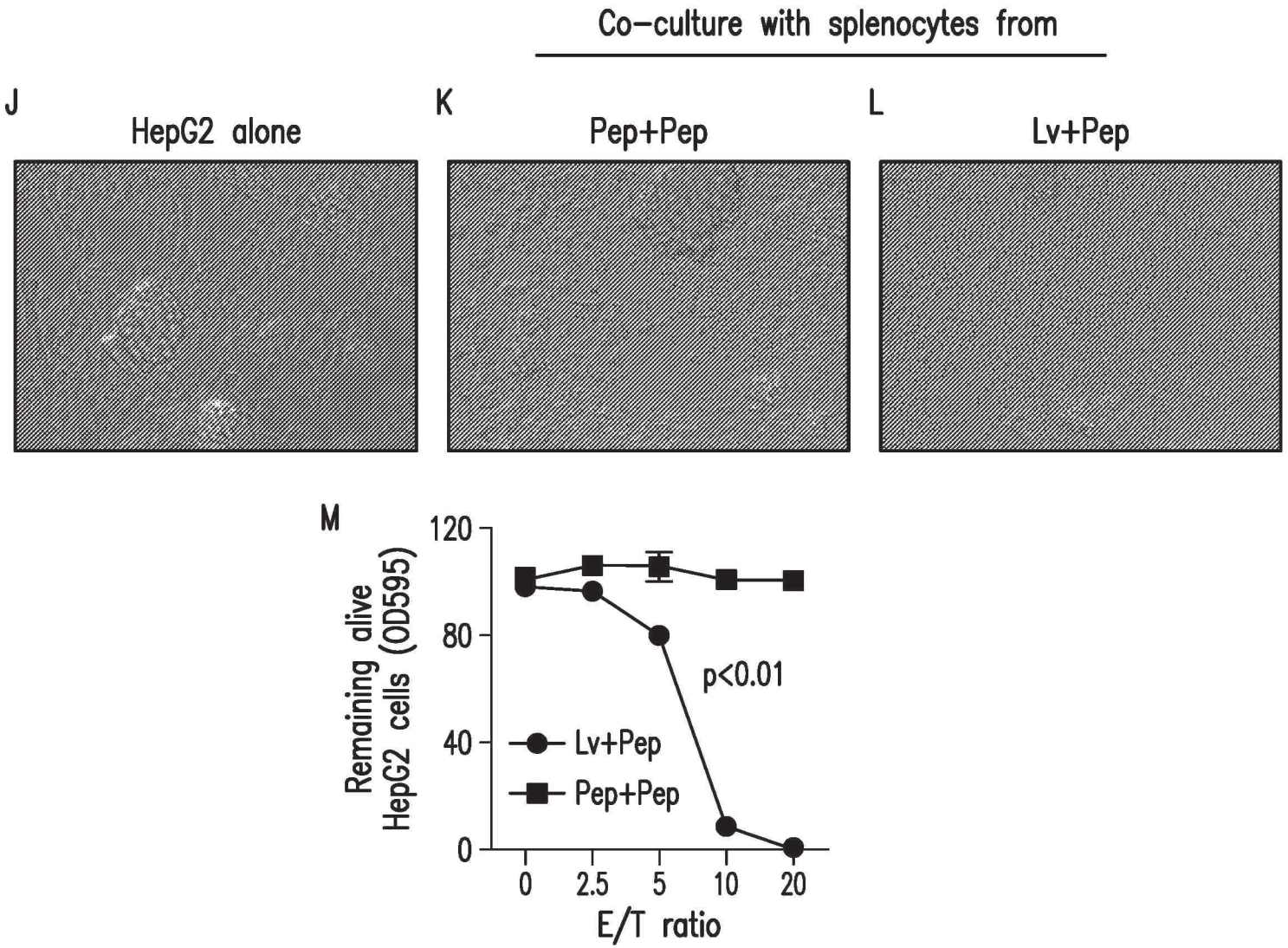

Co-culture with splenocytes from
J
HepG2 alone
K
Pep+Pep
L
Lv+Pep
M
Remaining alive HepG2 cells (OD595)
120
80
40
0
p<0.01
Lv+Pep
Pep+Pep
0
2.5
5
10
20
E/T ratio

FIG. 1J-1M

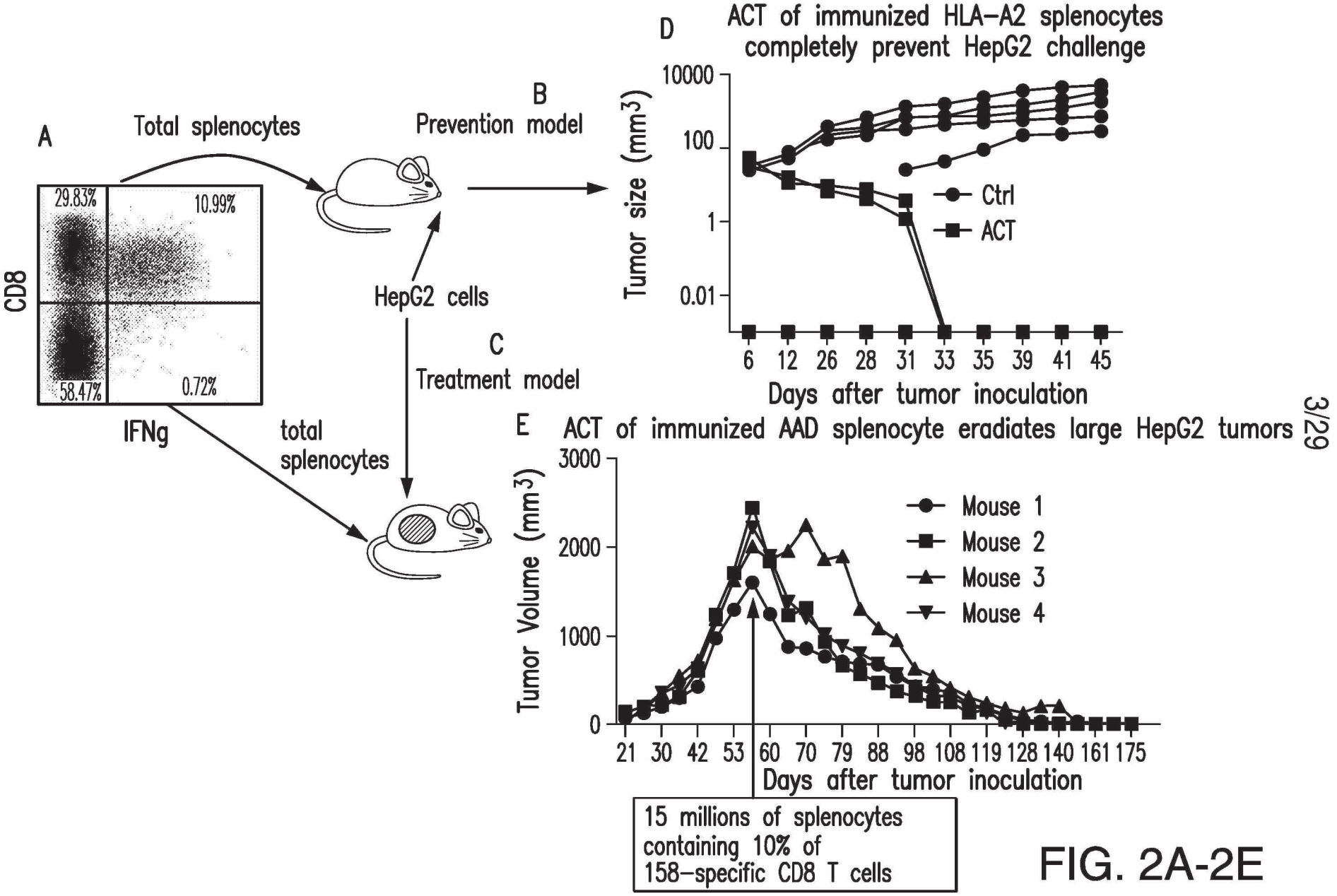
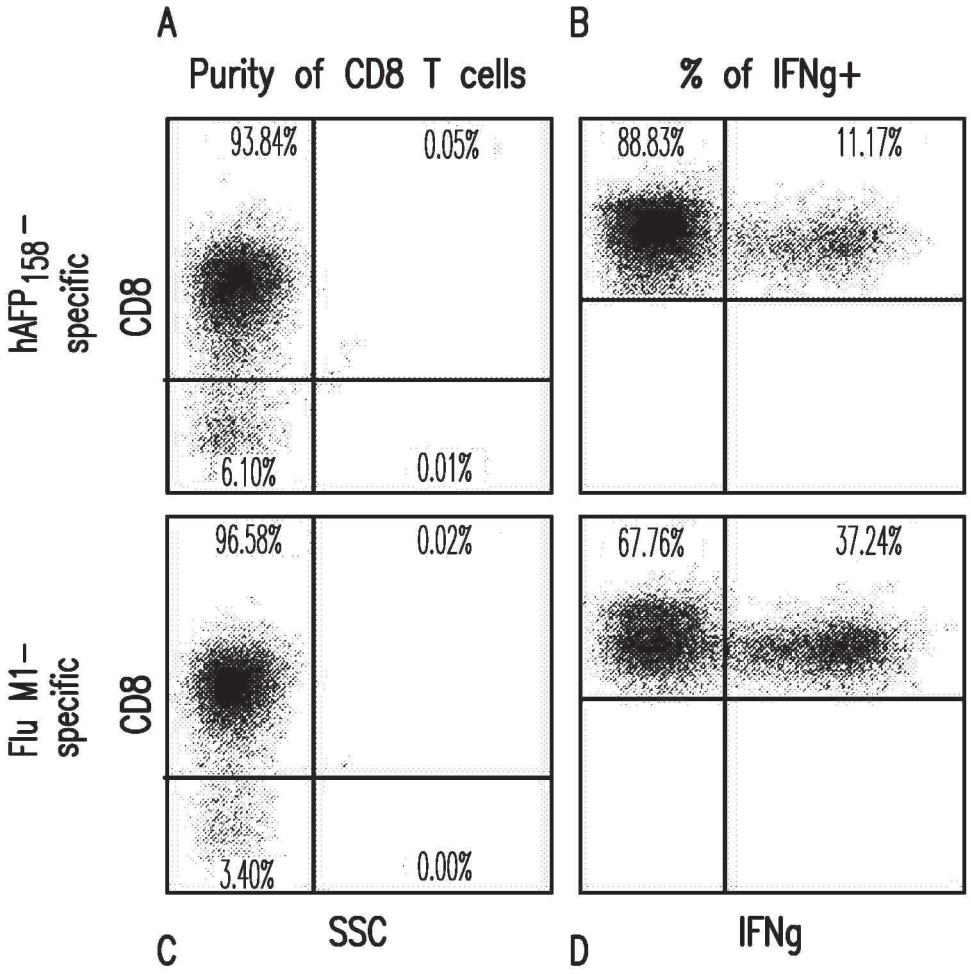

A
Total splenocytes
29.83%
10.99%
58.47%
0.72%
CD8
IFNg
B
Prevention model
HepG2 cells
C
Treatment model
total
splenocytes
D
ACT of immunized HLA-A2 splenocytes
completely prevent HepG2 challenge
Tumor size (mm3)
10000
100
1
0.01
Ctrl
ACT
6 12 26 28 31 33 35 39 41 45
Days after tumor inoculation
E
ACT of immunized AAD splenocyte eradiates large HepG2 tumors
Tumor Volume (mm3)
3000
2000
1000
0
Mouse 1
Mouse 2
Mouse 3
Mouse 4
21 30 42 53 60 70 79 88 98 108 119 128 140 161 175
Days after tumor inoculation
15 millions of splenocytes
containing 10% of
158-specific CD8 T cells

FIG. 2A-2E


A
Purity of CD8 T cells
B
% of IFNg+
93.84%
0.05%
6.10%
0.01%
88.83%
11.17%
96.58%
0.02%
3.40%
0.00%
67.76%
37.24%
hAFP158–specific CD8
Flu M1–specific CD8
C
SSC
D
IFNg

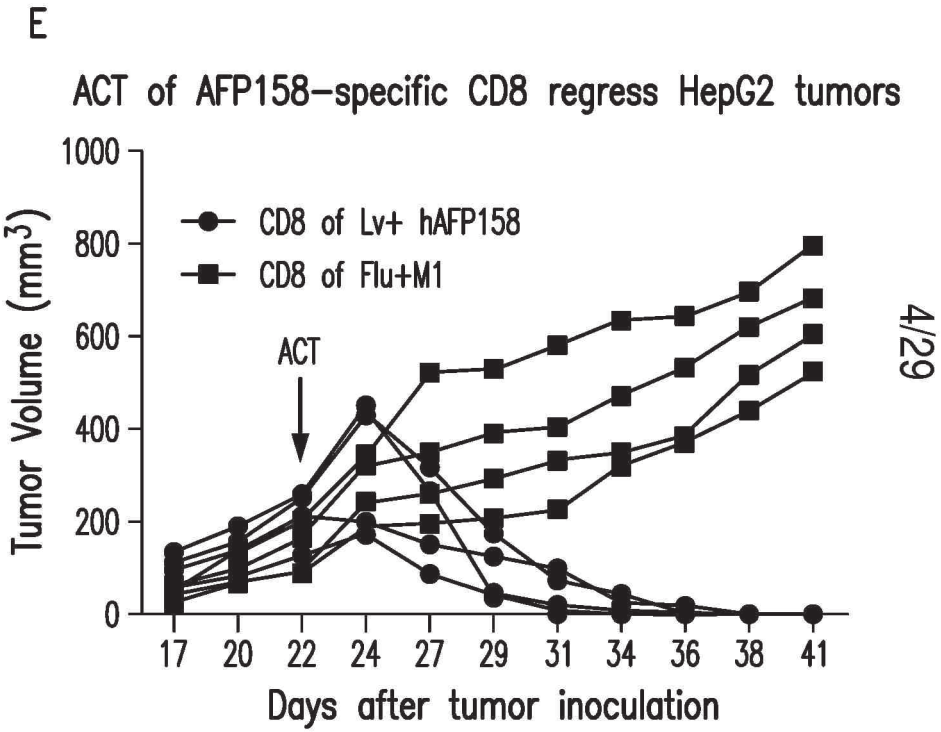

E
ACT of AFP158–specific CD8 regress HepG2 tumors
Tumor Volume (mm3)
1000
800
600
400
200
0
CD8 of Lv+ hAFP158
CD8 of Flu+M1
ACT
17
20
22
24
27
29
31
34
36
38
41
Days after tumor inoculation

FIG. 3A-3E

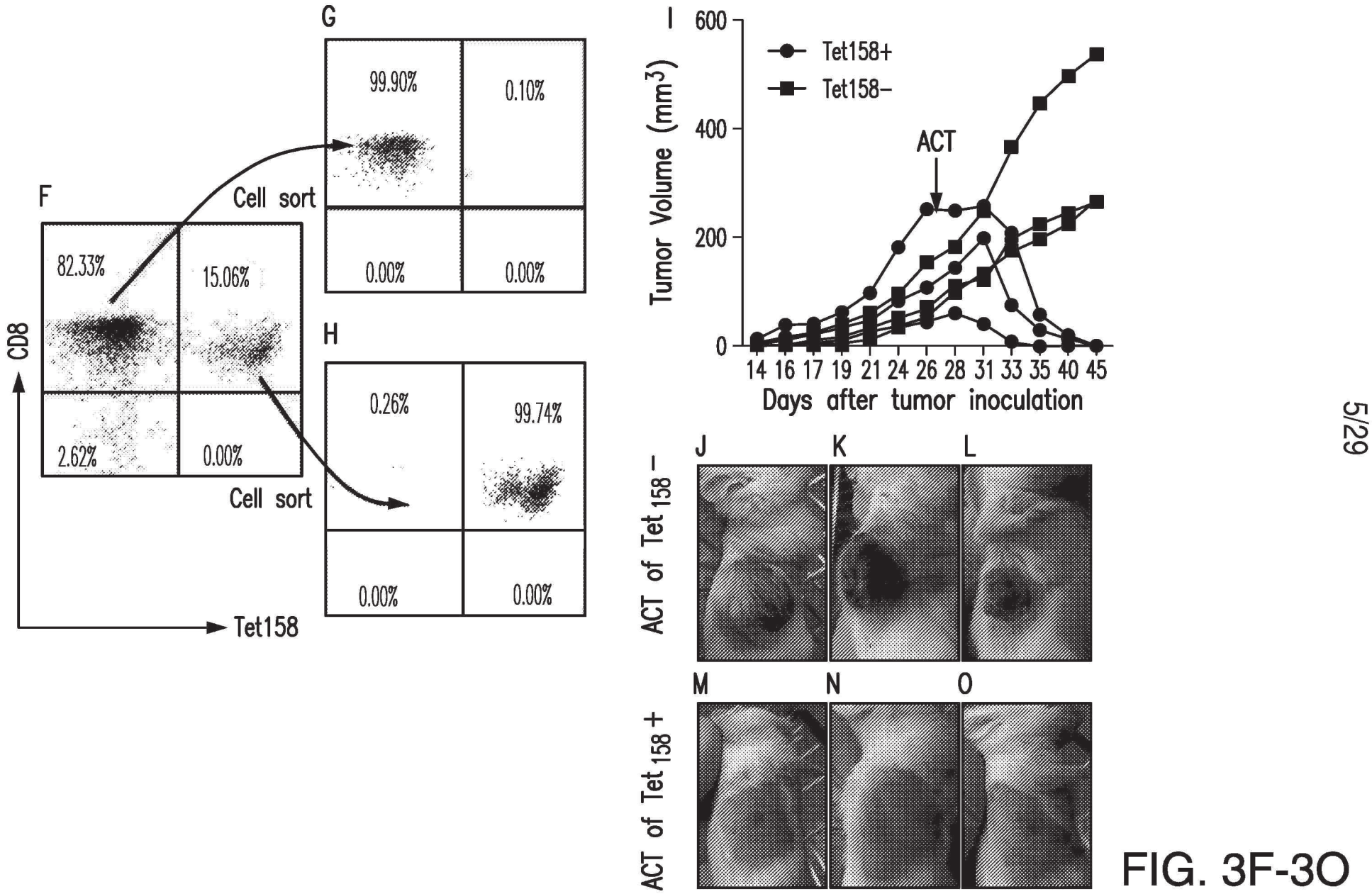

F
G
H
I
J
K
L
M
N
O
CD8
Tet158
82.33%
15.06%
2.62%
0.00%
Cell sort
99.90%
0.10%
0.00%
0.00%
Cell sort
0.26%
99.74%
0.00%
0.00%
Tumor Volume (mm3)
600
400
200
0
Tet158+
Tet158−
ACT
14 16 17 19 21 24 26 28 31 33 35 40 45
Days after tumor inoculation
ACT of Tet158 −
ACT of Tet158 +

FIG. 3F-3O

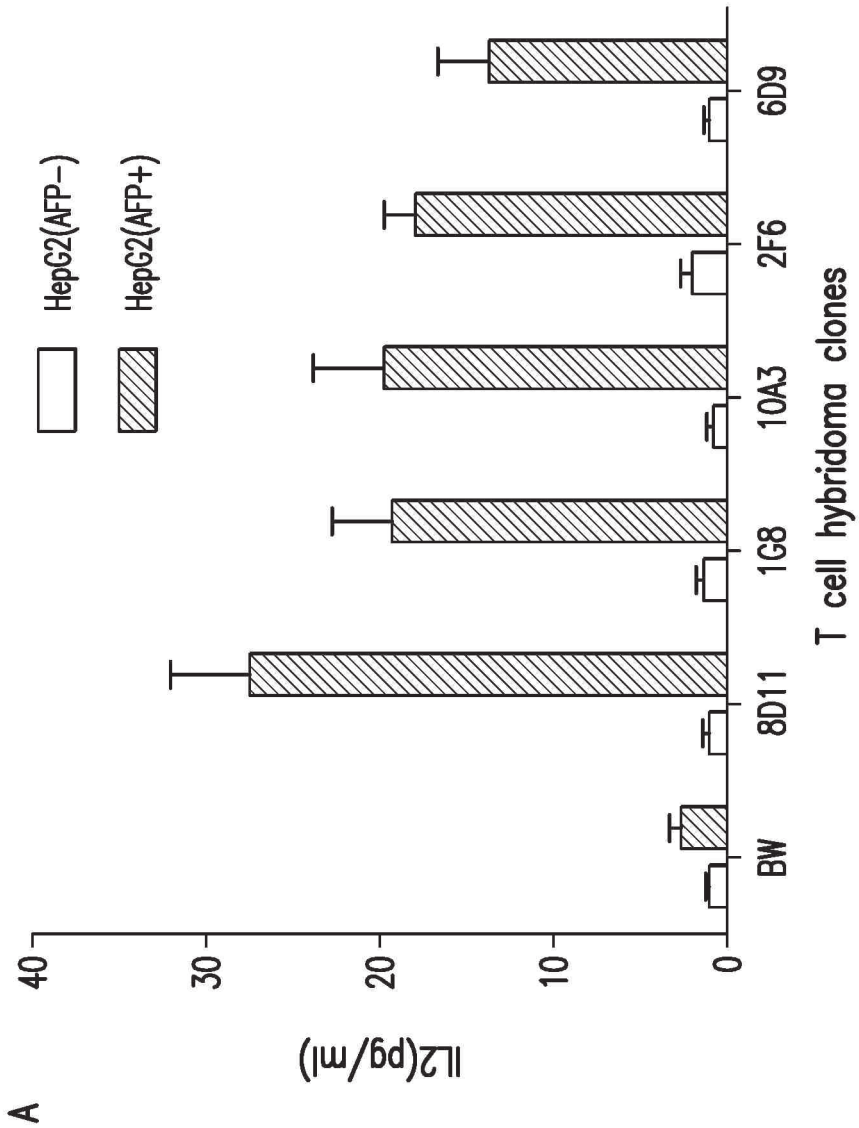

A
IL2(pg/ml)
0
10
20
30
40
HepG2(AFP−)
HepG2(AFP+)
BW
8D11
1G8
10A3
2F6
6D9
T cell hybridoma clones

FIG. 4A

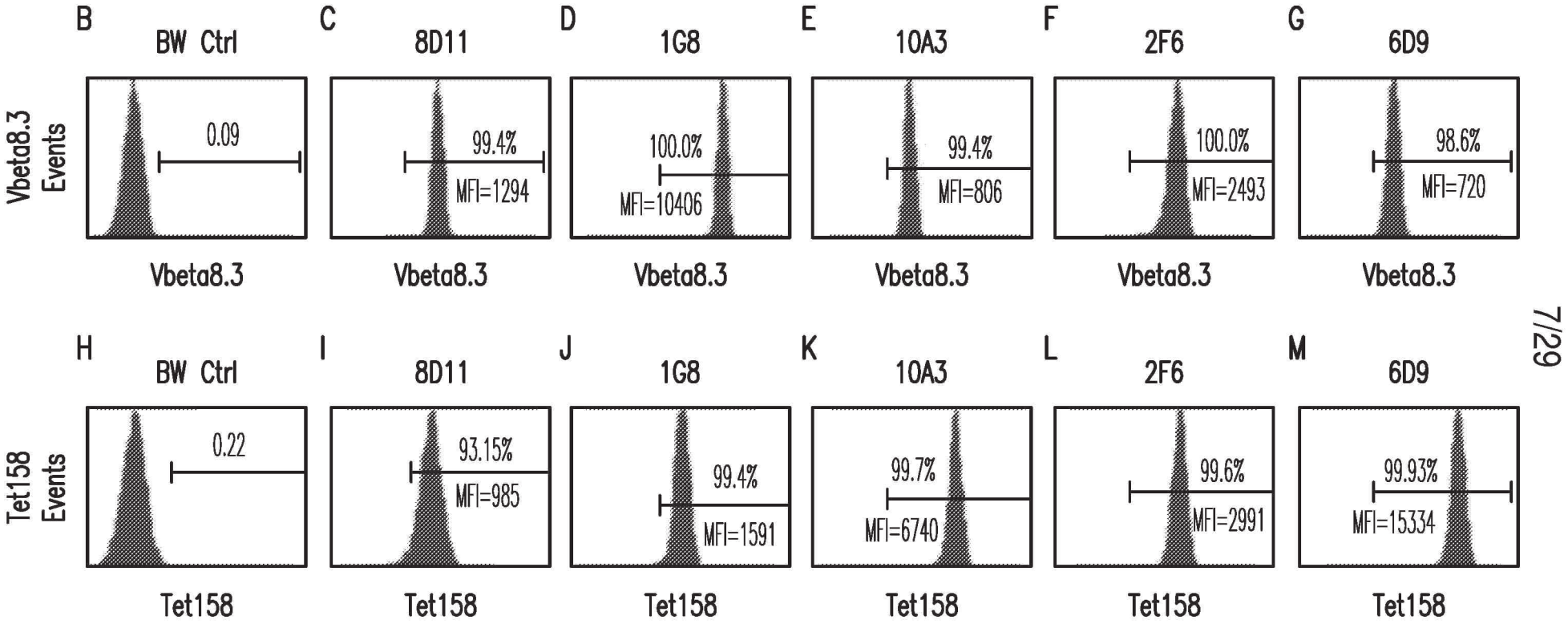

B
BW Ctrl
C
8D11
D
1G8
E
10A3
F
2F6
G
6D9
Vbeta8.3 Events
0.09
99.4%
MFI=1294
100.0%
MFI=10406
99.4%
MFI=806
100.0%
MFI=2493
98.6%
MFI=720
Vbeta8.3
H
BW Ctrl
I
8D11
J
1G8
K
10A3
L
2F6
M
6D9
Tet158 Events
0.22
93.15%
MFI=985
99.4%
MFI=1591
99.7%
MFI=6740
99.6%
MFI=2991
99.93%
MFI=15334
Tet158

FIG. 4B-4M

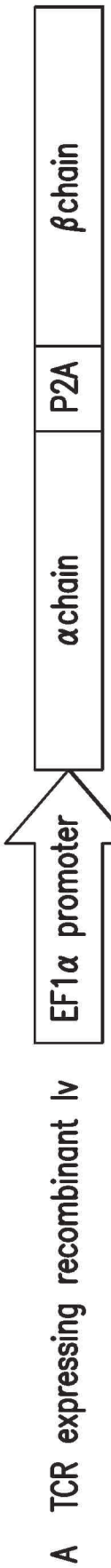

A TCR expressing recombinant lv
EF1α promoter
αchain
P2A
βchain

FIG. 5A

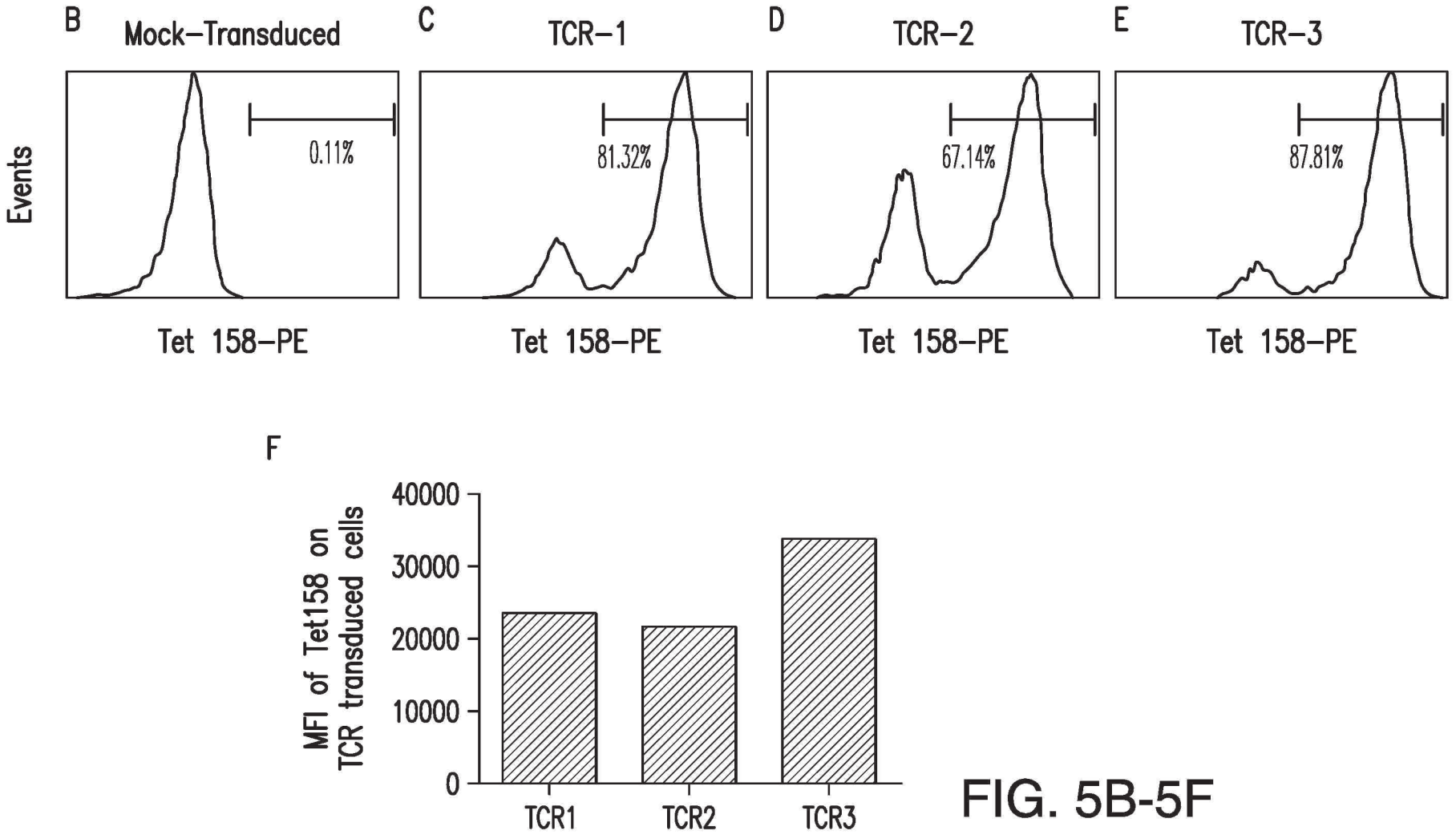

B
Mock–Transduced
C
TCR–1
D
TCR–2
E
TCR–3
Events
0.11%
81.32%
67.14%
87.81%
Tet 158–PE
Tet 158–PE
Tet 158–PE
Tet 158–PE
F
MFI of Tet158 on TCR transduced cells
40000
30000
20000
10000
0
TCR1
TCR2
TCR3

FIG. 5B-5F

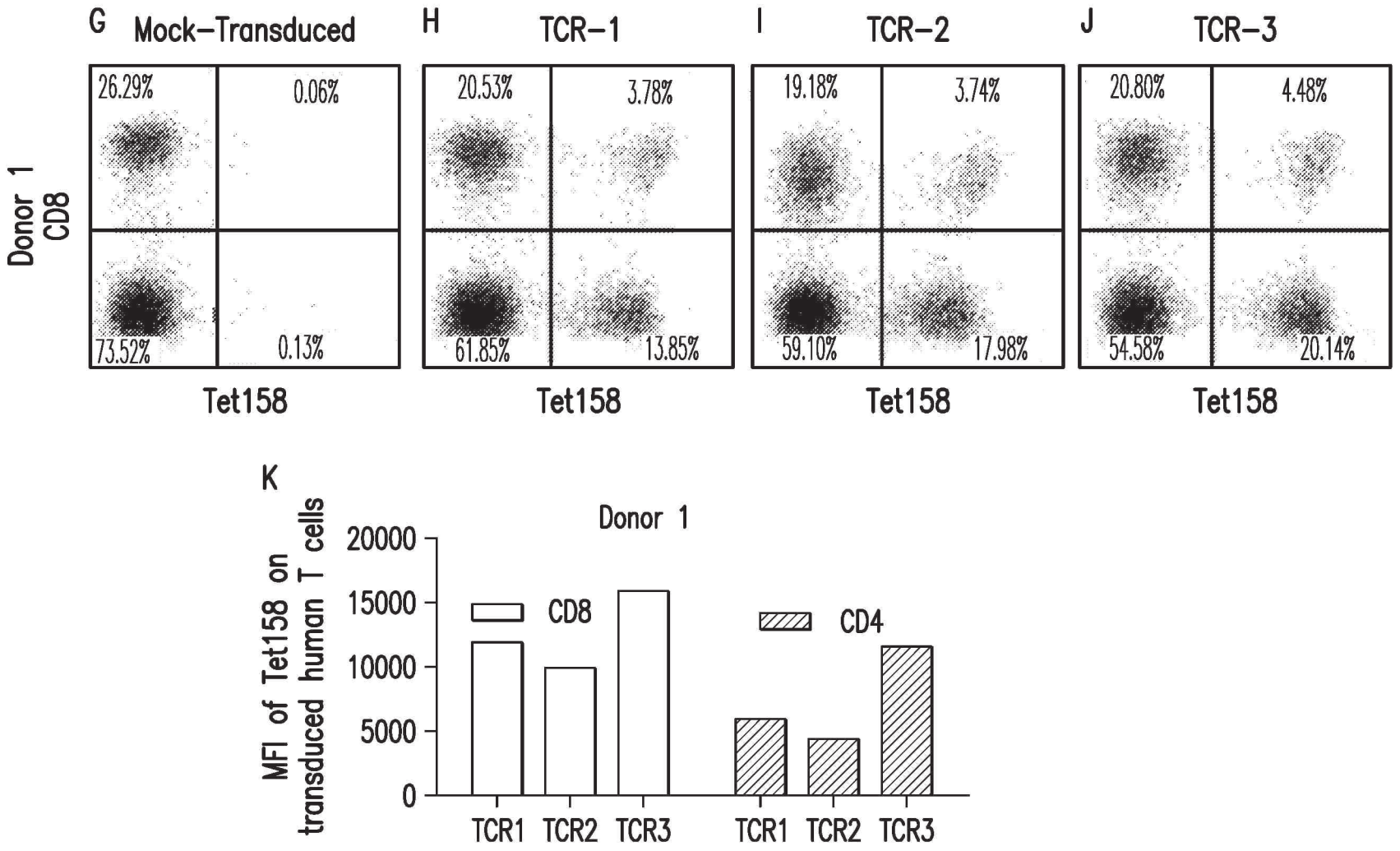

G
Mock-Transduced
H
TCR-1
I
TCR-2
J
TCR-3
Donor 1
CD8
26.29%
0.06%
73.52%
0.13%
20.53%
3.78%
61.85%
13.85%
19.18%
3.74%
59.10%
17.98%
20.80%
4.48%
54.58%
20.14%
Tet158
Tet158
Tet158
Tet158
K
Donor 1
MFI of Tet158 on transduced human T cells
20000
15000
10000
5000
0
CD8
CD4
TCR1
TCR2
TCR3
TCR1
TCR2
TCR3

FIG. 5G-5K

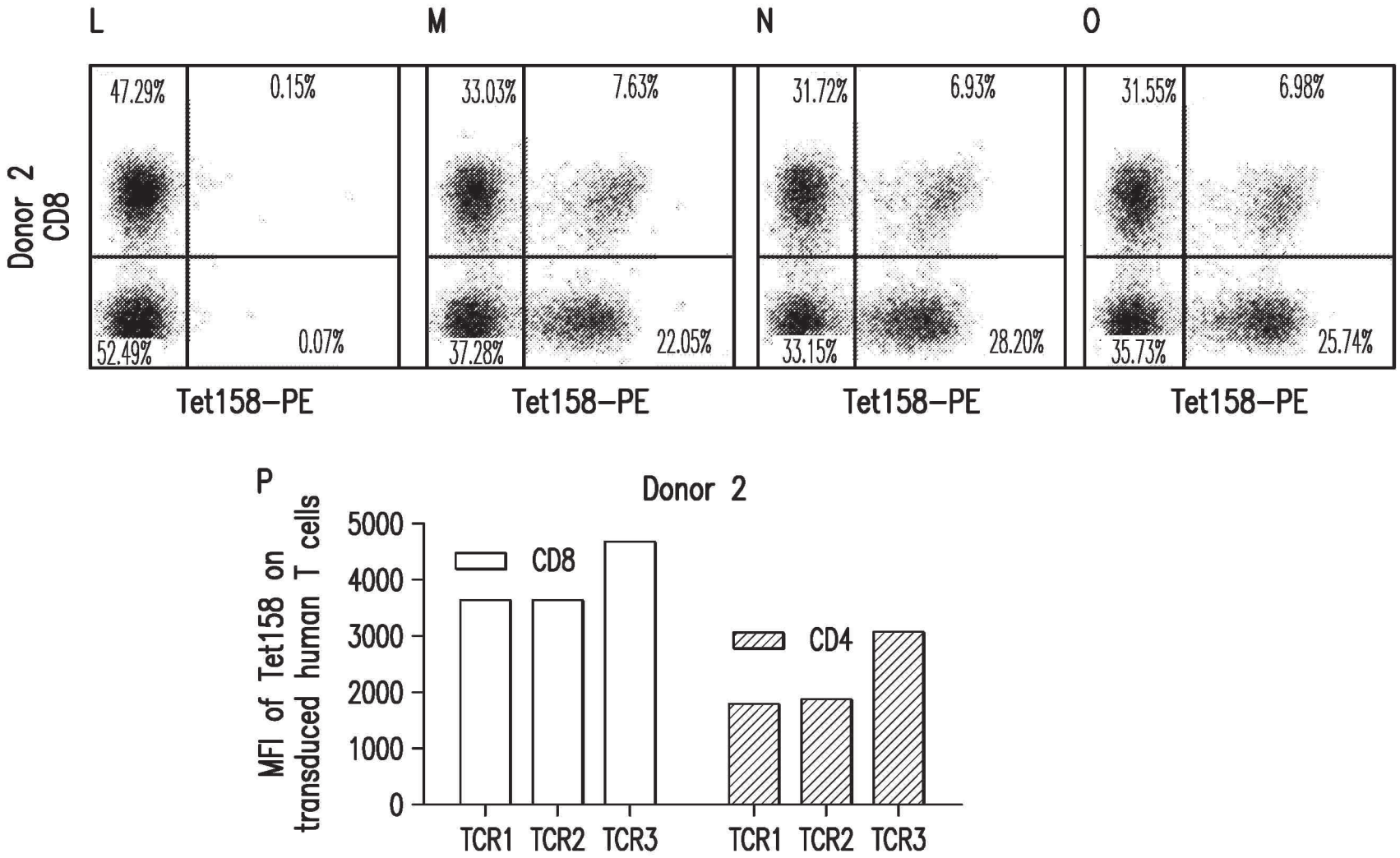
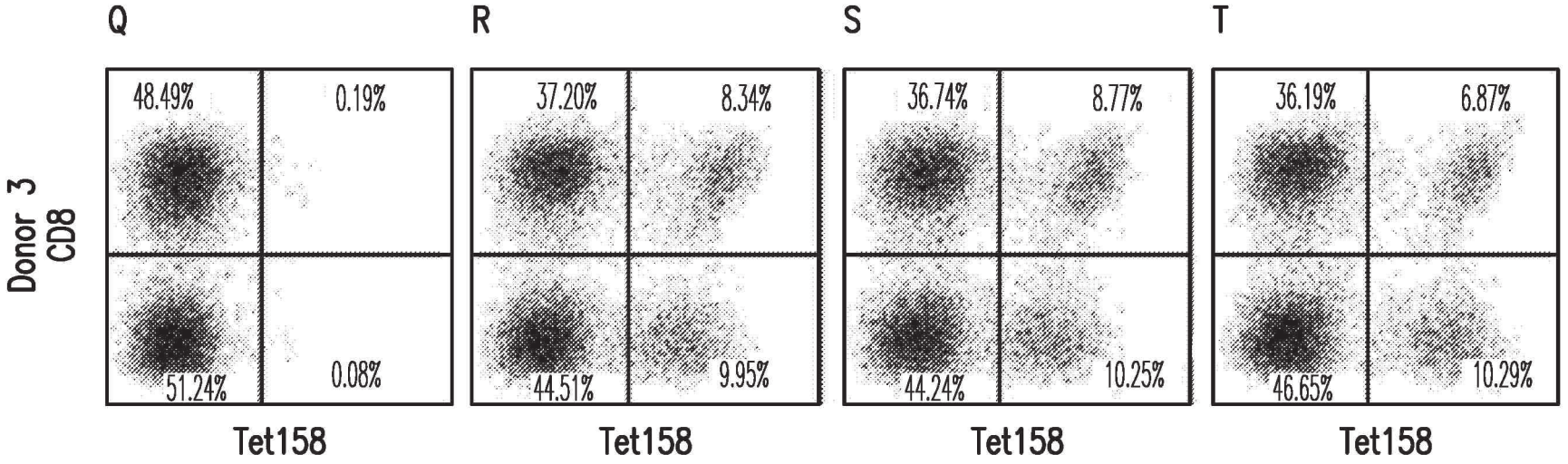

L
M
N
O
Donor 2
CD8
47.29%
0.15%
52.49%
0.07%
33.03%
7.63%
37.28%
22.05%
31.72%
6.93%
33.15%
28.20%
31.55%
6.98%
35.73%
25.74%
Tet158-PE
Tet158-PE
Tet158-PE
Tet158-PE
P
Donor 2
MFI of Tet158 on transduced human T cells
5000
4000
3000
2000
1000
0
CD8
CD4
TCR1
TCR2
TCR3
TCR1
TCR2
TCR3

FIG. 5L-5P


Q
R
S
T
Donor 3
CD8
48.49%
0.19%
51.24%
0.08%
37.20%
8.34%
44.51%
9.95%
36.74%
8.77%
44.24%
10.25%
36.19%
6.87%
46.65%
10.29%
Tet158
Tet158
Tet158
Tet158

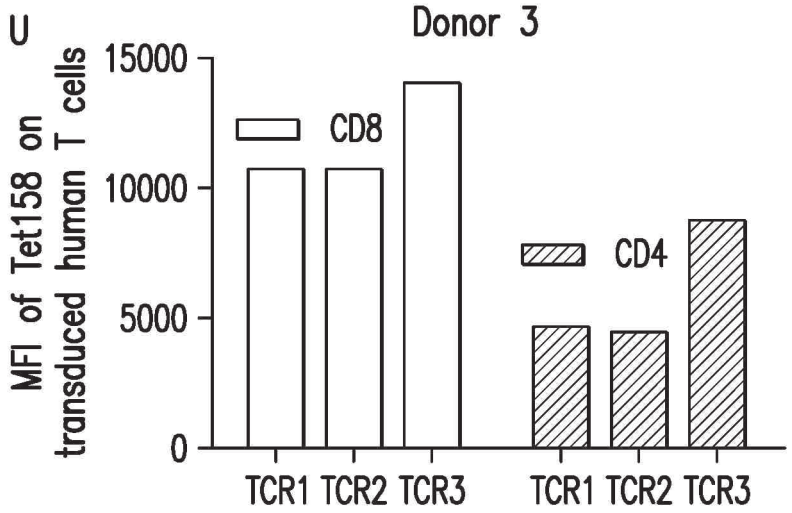

U
Donor 3
MFI of Tet158 on transduced human T cells
15000
10000
5000
0
CD8
CD4
TCR1 TCR2 TCR3
TCR1 TCR2 TCR3

FIG. 5Q-5U

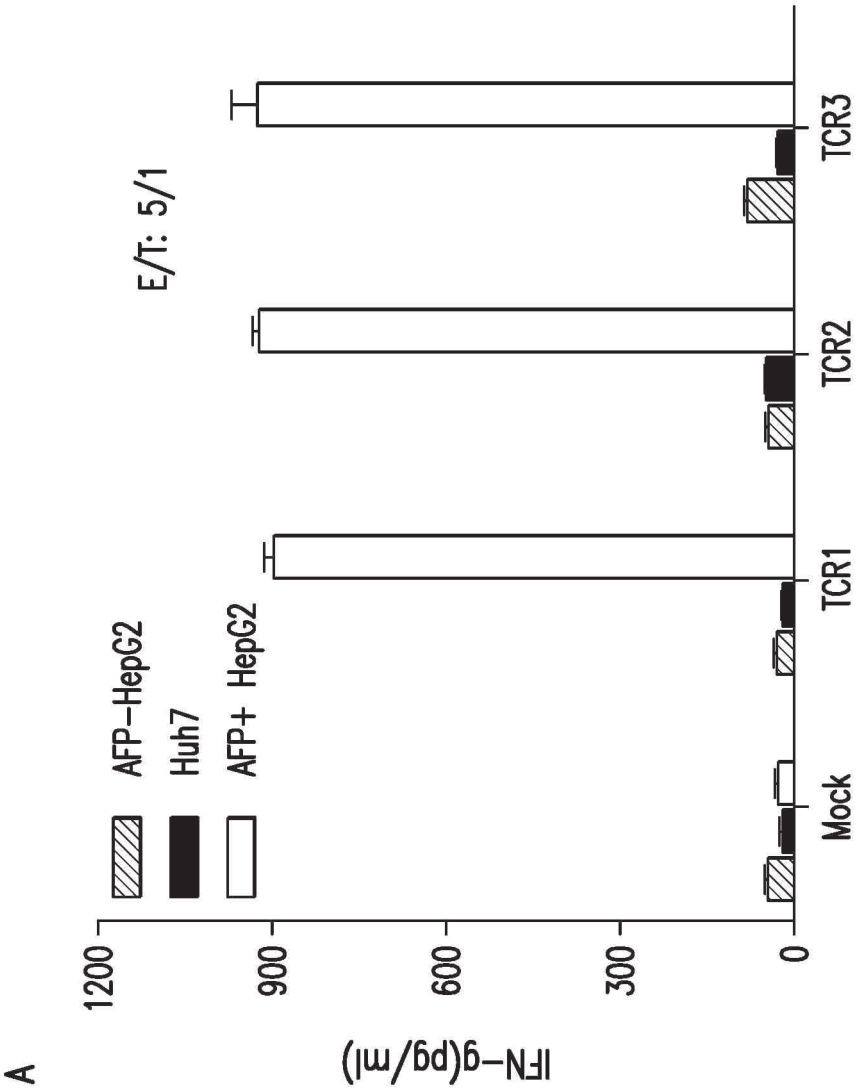

A
IFN-g(pg/ml)
1200
900
600
300
0
E/T: 5/1
AFP-HepG2
Huh7
AFP+ HepG2
Mock
TCR1
TCR2
TCR3
HLA-A2+ primary human T cells
transduced with TCR genes

FIG. 6A

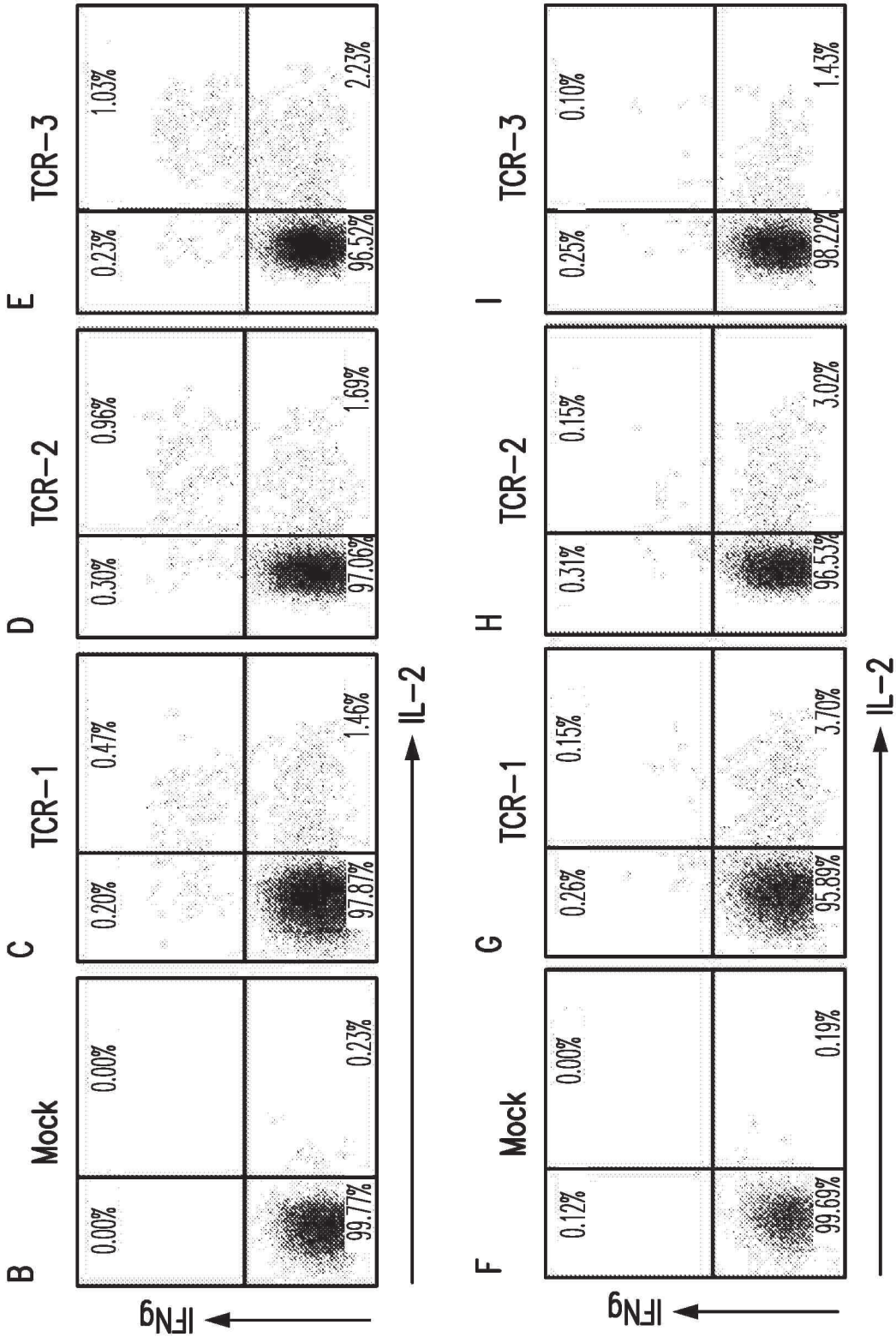

B
Mock
C
TCR-1
D
TCR-2
E
TCR-3
F
Mock
G
TCR-1
H
TCR-2
I
TCR-3
IFNg
IL-2
0.00%
0.00%
99.77%
0.23%
0.20%
0.47%
97.87%
1.46%
0.30%
0.96%
97.06%
1.69%
0.23%
1.03%
96.52%
2.23%
0.12%
0.00%
99.69%
0.19%
0.26%
0.15%
95.89%
3.70%
0.31%
0.15%
96.53%
3.02%
0.25%
0.10%
98.22%
1.43%

FIG. 6B-6I

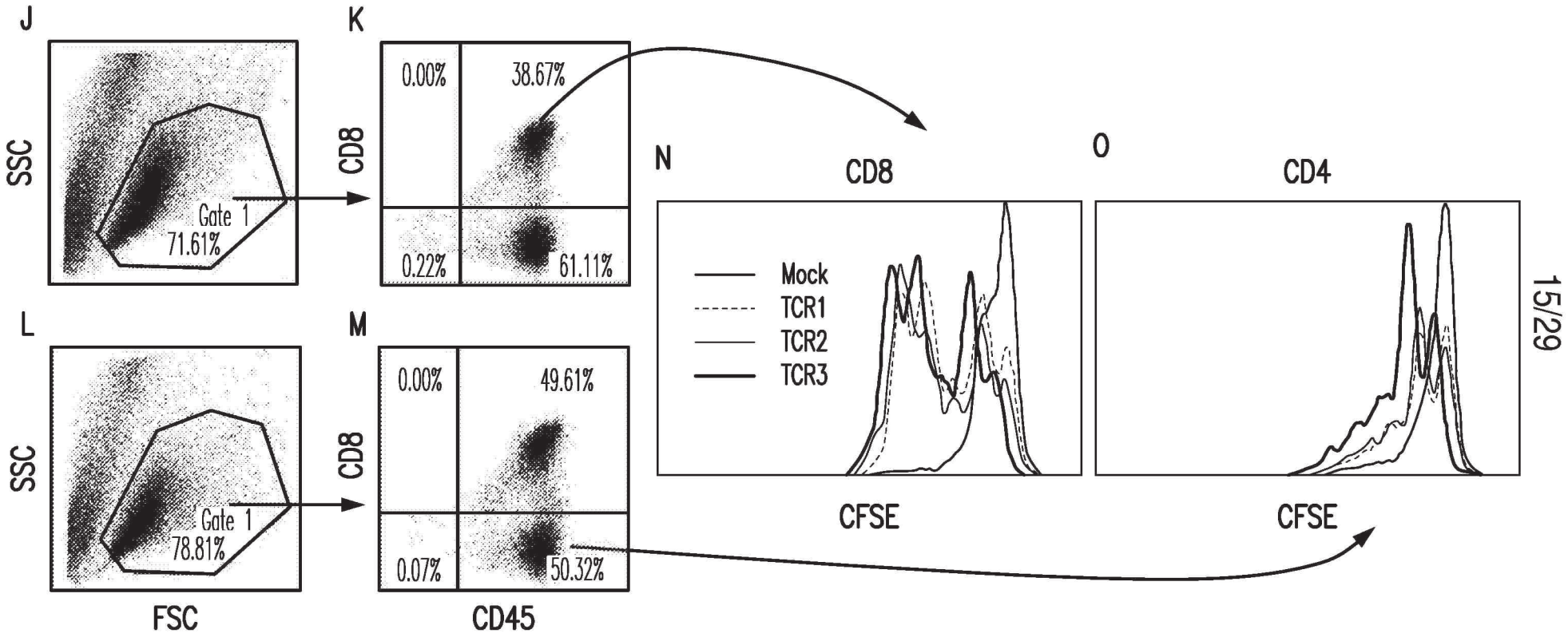

J
SSC
Gate 1
71.61%
K
CD8
0.00%
38.67%
0.22%
61.11%
L
SSC
Gate 1
78.81%
FSC
M
CD8
0.00%
49.61%
0.07%
50.32%
CD45
N
CD8
Mock
TCR1
TCR2
TCR3
CFSE
O
CD4
CFSE

FIG. 6J-6O

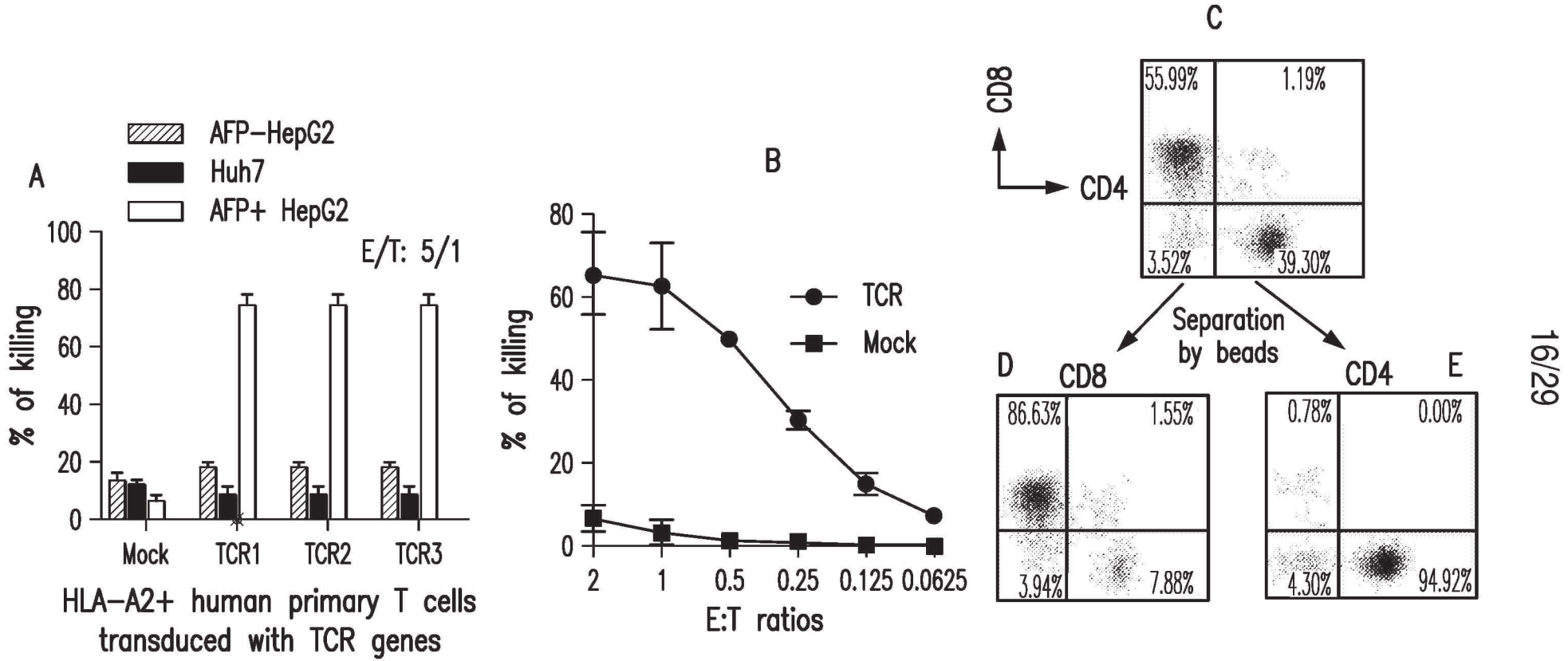

A
AFP−HepG2
Huh7
AFP+ HepG2
E/T: 5/1
% of killing
100
80
60
40
20
0
Mock
TCR1
TCR2
TCR3
HLA−A2+ human primary T cells
transduced with TCR genes
B
% of killing
80
60
40
20
0
TCR
Mock
2
1
0.5
0.25
0.125
0.0625
E:T ratios
C
CD8
CD4
55.99%
1.19%
3.52%
39.30%
Separation
by beads
D
CD8
86.63%
1.55%
3.94%
7.88%
CD4
E
0.78%
0.00%
4.30%
94.92%

FIG. 7A-7E

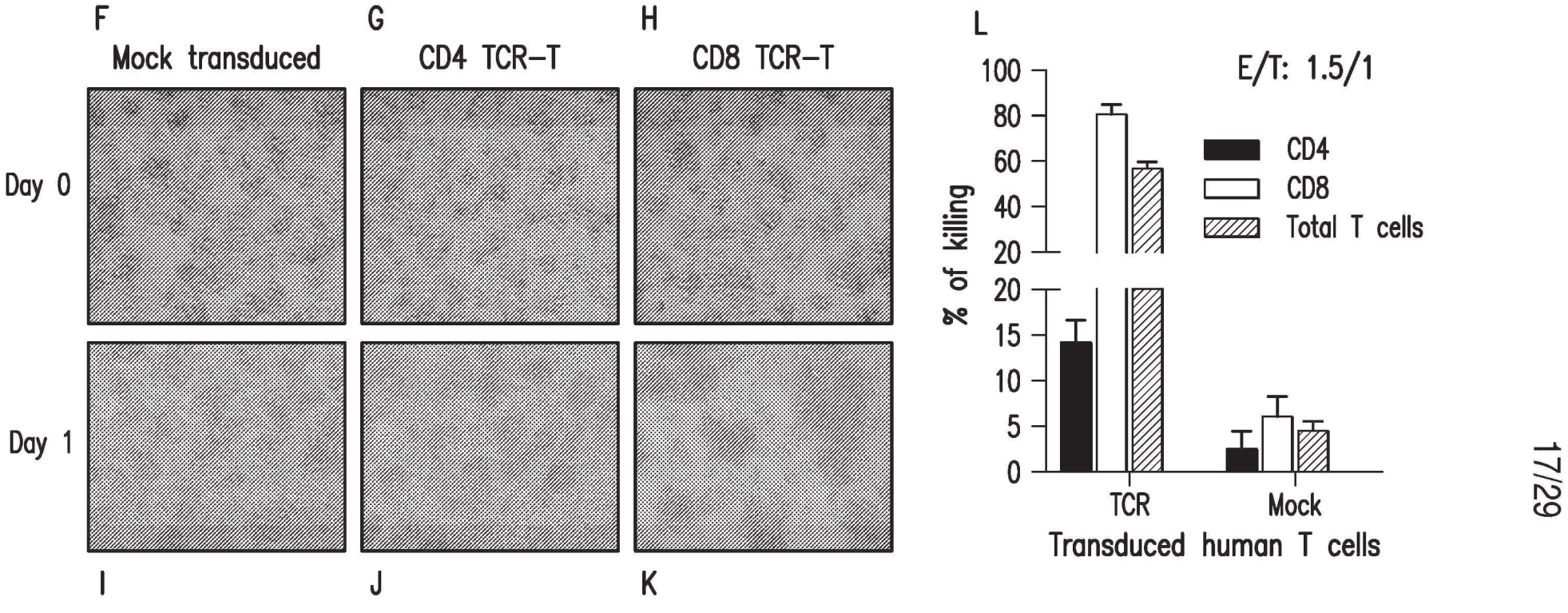

F
Mock transduced
G
CD4 TCR-T
H
CD8 TCR-T
Day 0
Day 1
I
J
K
L
E/T: 1.5/1
% of killing
100
80
60
40
20
20
15
10
5
0
CD4
CD8
Total T cells
TCR
Mock
Transduced human T cells
M
Mock transduced
N
CD4 TCR-T
O
CD8 TCR-T

FIG. 7F-7O

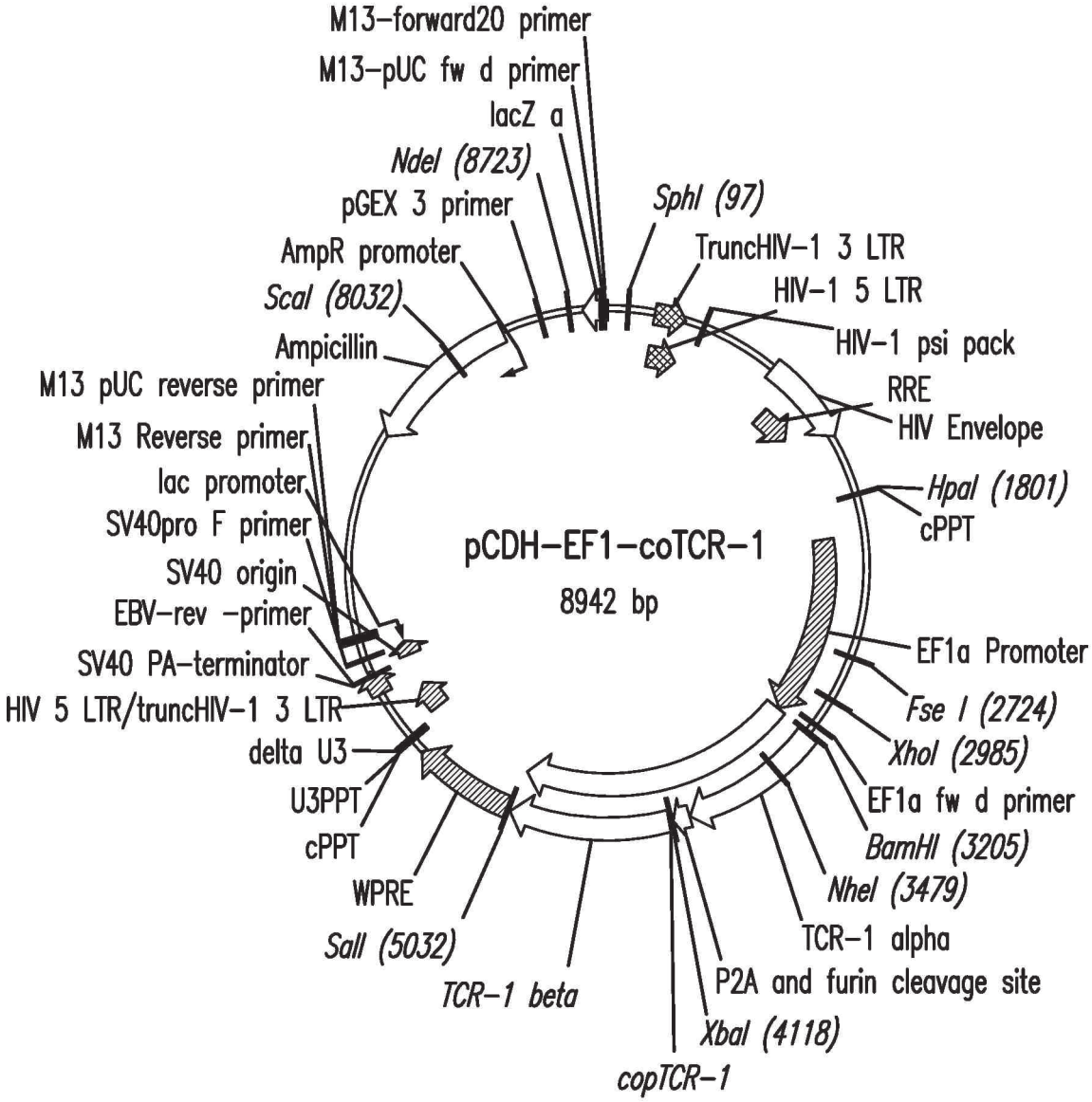

M13–forward20 primer
M13–pUC fw d primer
lacZ a
NdeI (8723)
pGEX 3 primer
AmpR promoter
ScaI (8032)
Ampicillin
M13 pUC reverse primer
M13 Reverse primer
lac promoter
SV40pro F primer
SV40 origin
EBV–rev –primer
SV40 PA–terminator
HIV 5 LTR/truncHIV–1 3 LTR
delta U3
U3PPT
cPPT
WPRE
SalI (5032)
TCR–1 beta
copTCR–1
XbaI (4118)
P2A and furin cleavage site
TCR–1 alpha
NheI (3479)
BamHI (3205)
EF1a fw d primer
XhoI (2985)
Fse I (2724)
EF1a Promoter
cPPT
HpaI (1801)
HIV Envelope
RRE
HIV–1 psi pack
HIV–1 5 LTR
TruncHIV–1 3 LTR
SphI (97)
pCDH–EF1–coTCR–1
8942 bp

FIG. 8

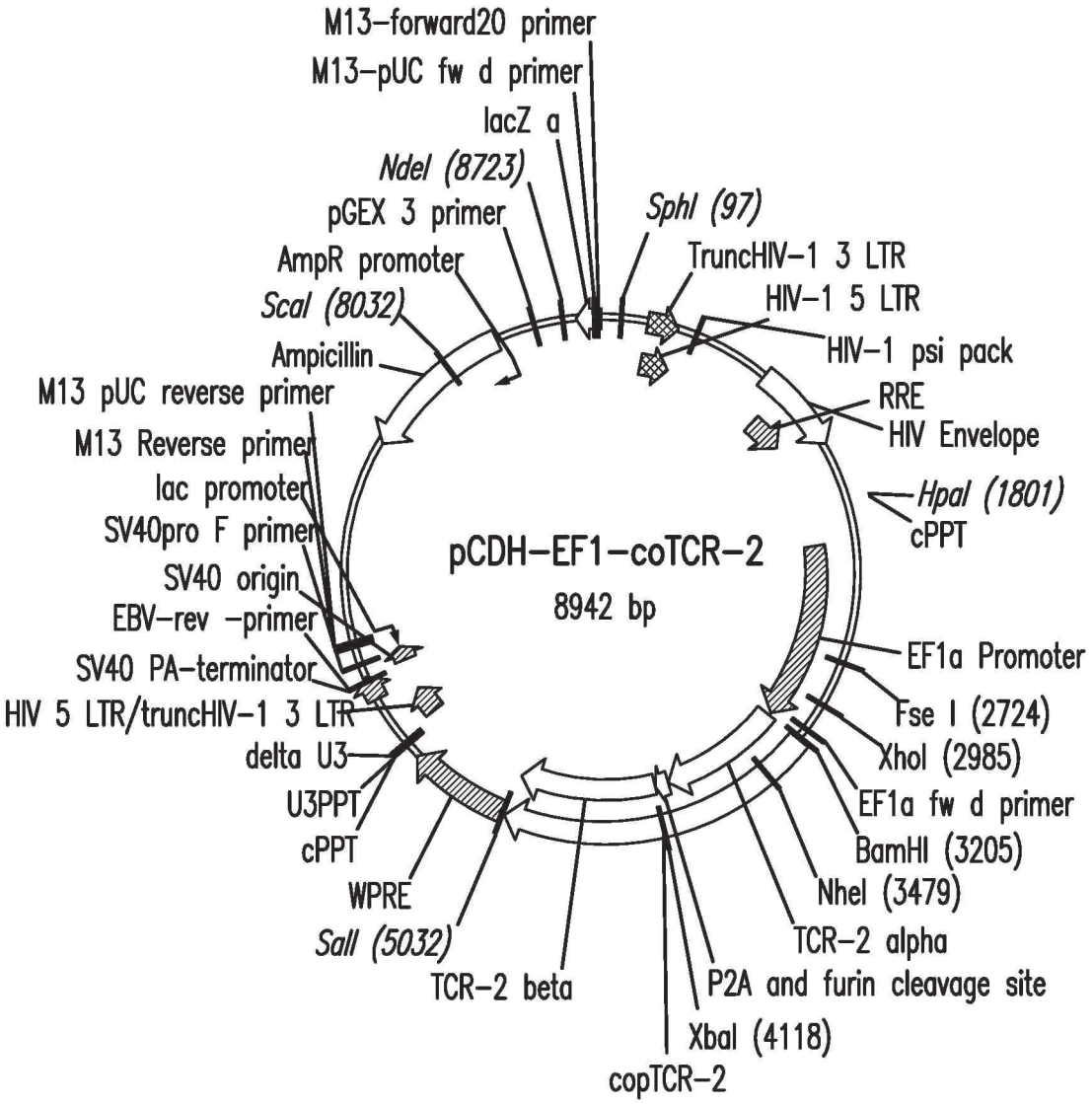

M13–forward20 primer
M13–pUC fw d primer
lacZ a
NdeI (8723)
pGEX 3 primer
SphI (97)
AmpR promoter
TruncHIV–1 3 LTR
ScaI (8032)
HIV–1 5 LTR
Ampicillin
HIV–1 psi pack
M13 pUC reverse primer
RRE
M13 Reverse primer
HIV Envelope
lac promoter
HpaI (1801)
SV40pro F primer
cPPT
pCDH–EF1–coTCR–2
SV40 origin
8942 bp
EBV–rev –primer
EF1a Promoter
SV40 PA–terminator
Fse I (2724)
HIV 5 LTR/truncHIV–1 3 LTR
XhoI (2985)
delta U3
EF1a fw d primer
U3PPT
BamHI (3205)
cPPT
NheI (3479)
WPRE
TCR–2 alpha
SalI (5032)
P2A and furin cleavage site
TCR–2 beta
XbaI (4118)
copTCR–2

FIG. 9

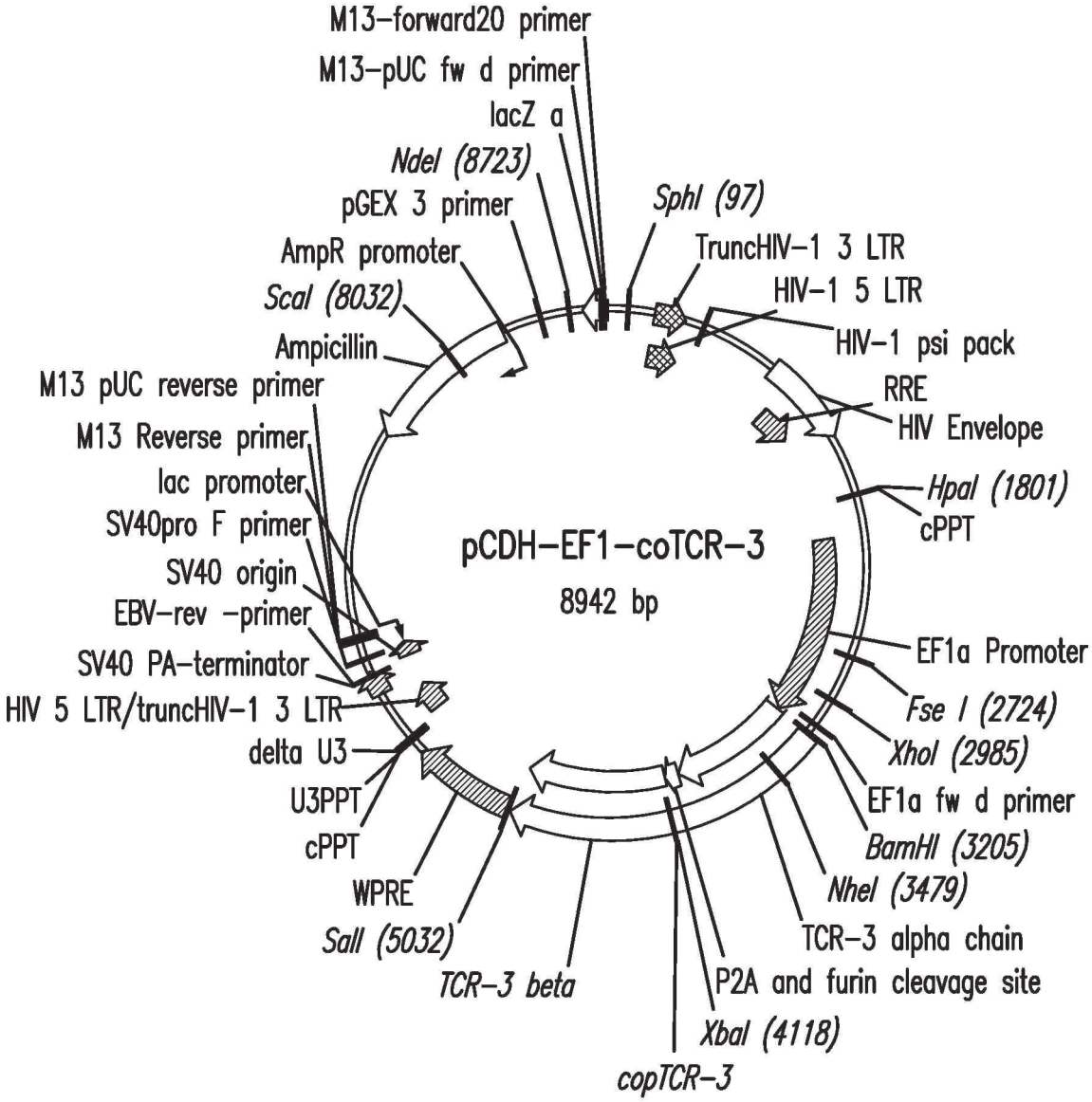

M13–forward20 primer
M13–pUC fw d primer
lacZ a
NdeI (8723)
pGEX 3 primer
AmpR promoter
ScaI (8032)
Ampicillin
M13 pUC reverse primer
M13 Reverse primer
lac promoter
SV40pro F primer
SV40 origin
EBV–rev –primer
SV40 PA–terminator
HIV 5 LTR/truncHIV–1 3 LTR
delta U3
U3PPT
cPPT
WPRE
SalI (5032)
TCR–3 beta
copTCR–3
XbaI (4118)
P2A and furin cleavage site
TCR–3 alpha chain
NheI (3479)
BamHI (3205)
EF1a fw d primer
XhoI (2985)
Fse I (2724)
EF1a Promoter
HpaI (1801)
cPPT
RRE
HIV Envelope
HIV–1 psi pack
HIV–1 5 LTR
TruncHIV–1 3 LTR
SphI (97)
pCDH–EF1–coTCR–3
8942 bp

FIG. 10

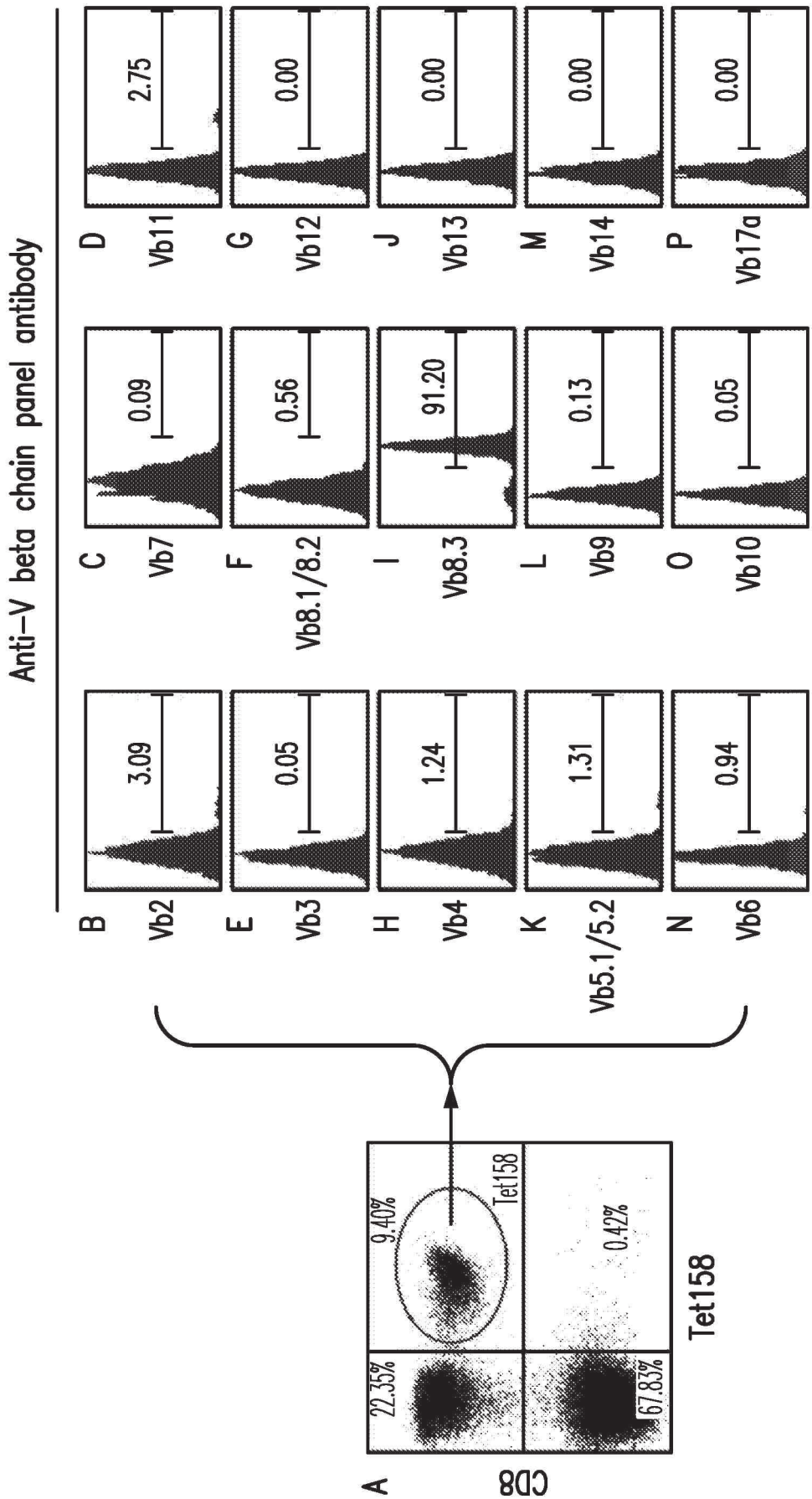

Anti-V beta chain panel antibody
A
CD8
Tet158
22.35%
9.40%
Tet158
0.42%
67.83%
B
Vb2
3.09
C
Vb7
0.09
D
Vb11
2.75
E
Vb3
0.05
F
Vb8.1/8.2
0.56
G
Vb12
0.00
H
Vb4
1.24
I
Vb8.3
91.20
J
Vb13
0.00
K
Vb5.1/5.2
1.31
L
Vb9
0.13
M
Vb14
0.00
N
Vb6
0.94
O
Vb10
0.05
P
Vb17a
0.00

FIG. 11A-11P

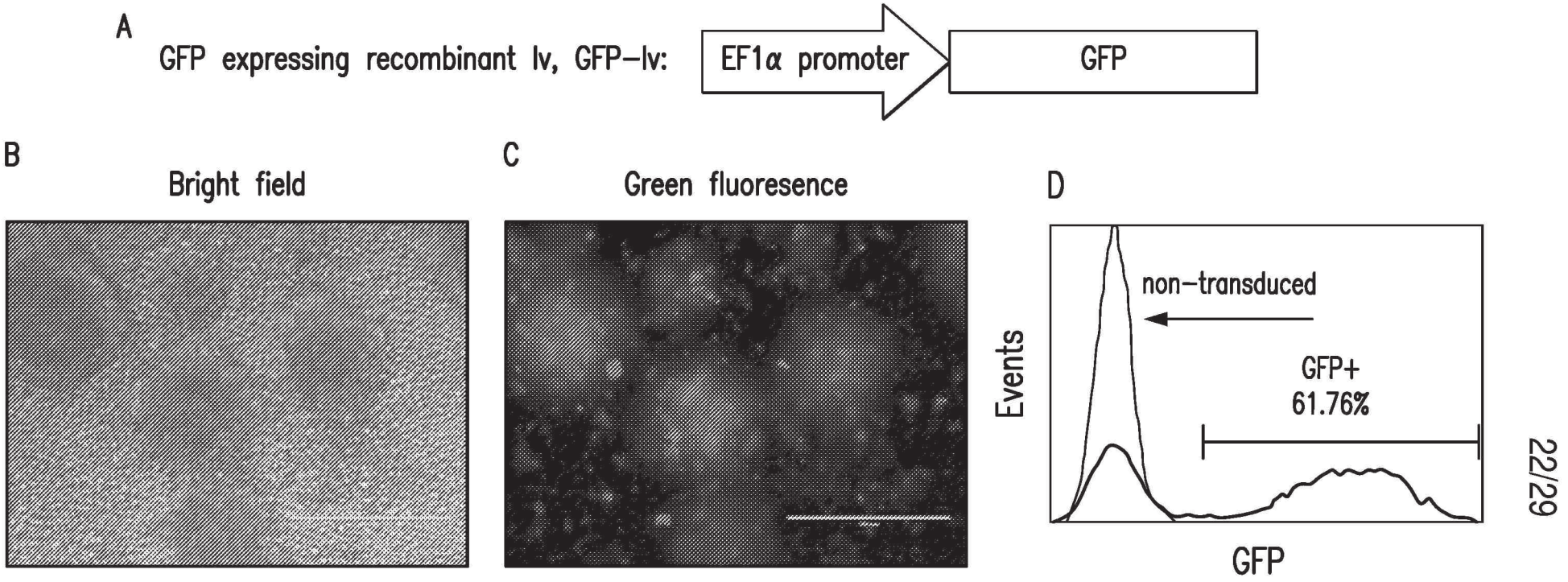


FIG. 12A-12D

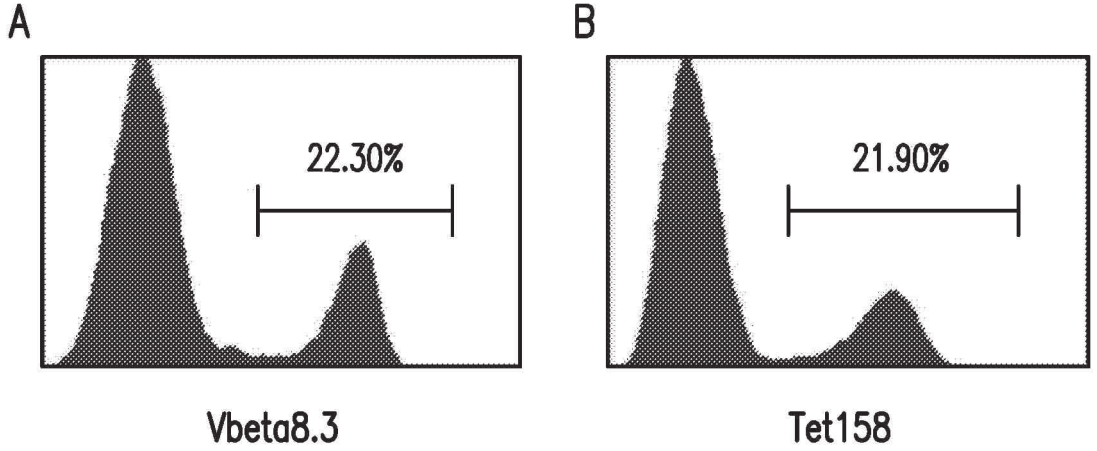


FIG. 13A-13B

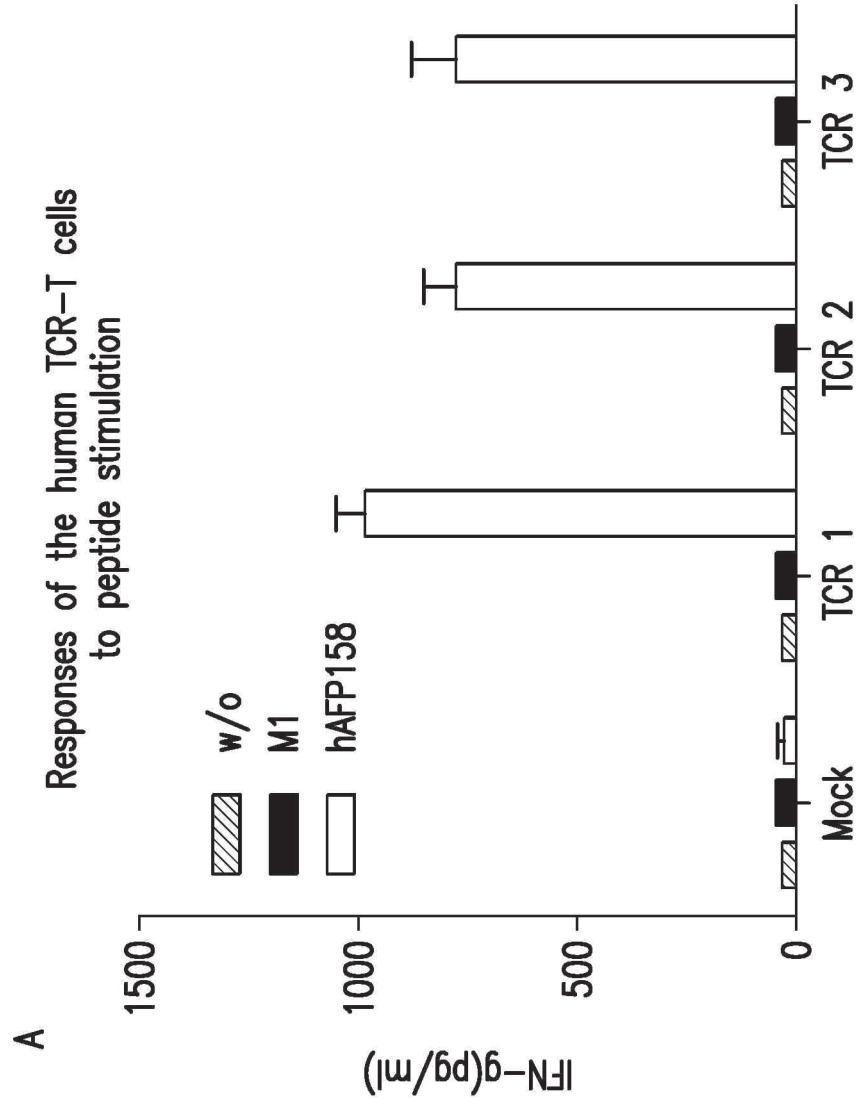

A
Responses of the human TCR-T cells
to peptide stimulation
IFN-g(pg/ml)
1500
1000
500
0
w/o
M1
hAFP158
Mock
TCR 1
TCR 2
TCR 3
HLA-A2+ human primary T cells
transduced with TCR genes

FIG. 14A

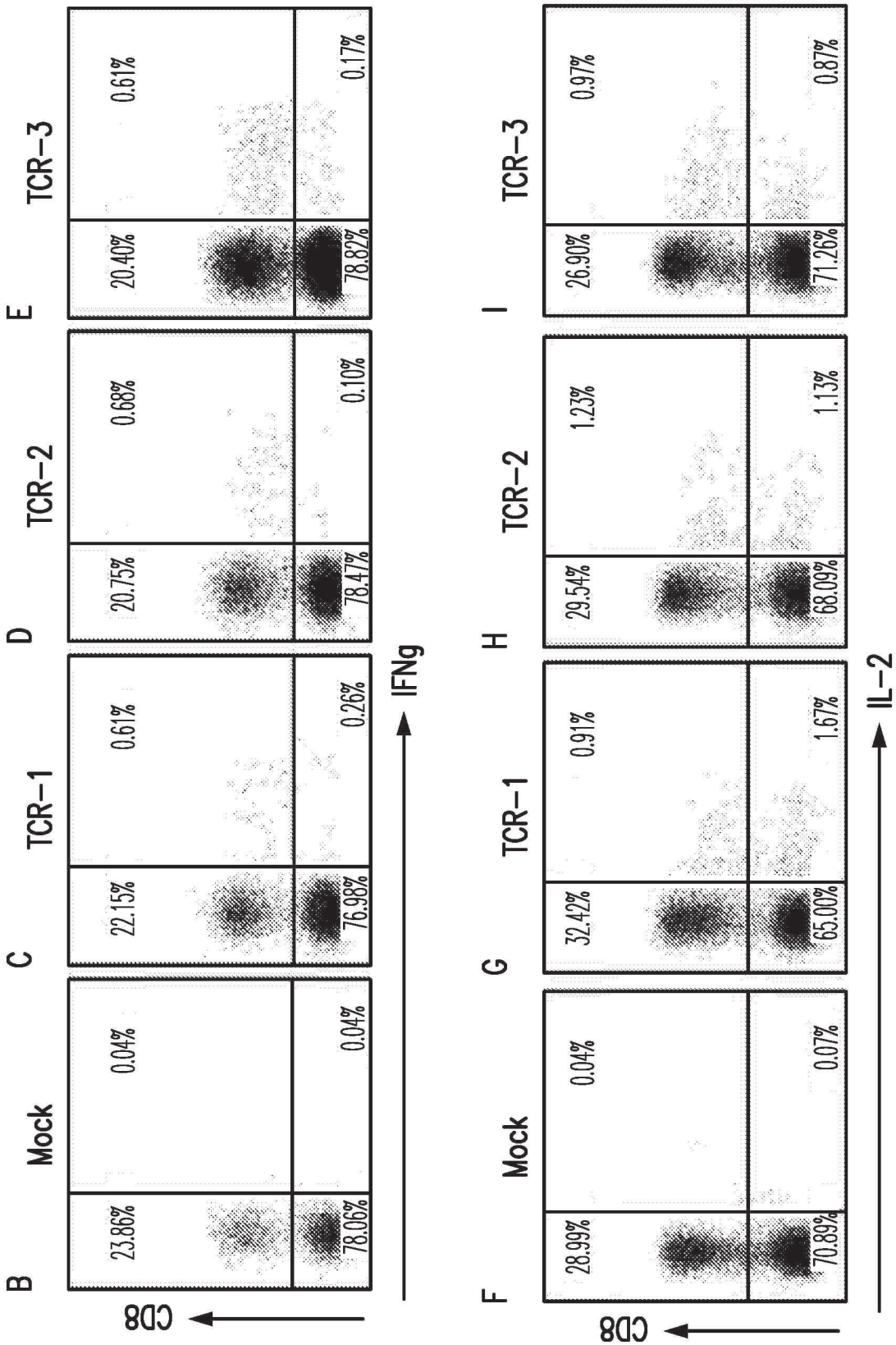

B
Mock
C
TCR−1
D
TCR−2
E
TCR−3
23.86%
0.04%
22.15%
0.61%
20.75%
0.68%
20.40%
0.61%
78.06%
0.04%
76.98%
0.26%
78.47%
0.10%
78.82%
0.17%
CD8
IFNg
F
Mock
G
TCR−1
H
TCR−2
I
TCR−3
28.99%
0.04%
32.42%
0.91%
29.54%
1.23%
26.90%
0.97%
70.89%
0.07%
65.00%
1.67%
68.09%
1.13%
71.26%
0.87%
CD8
IL−2

FIG. 14B-14I

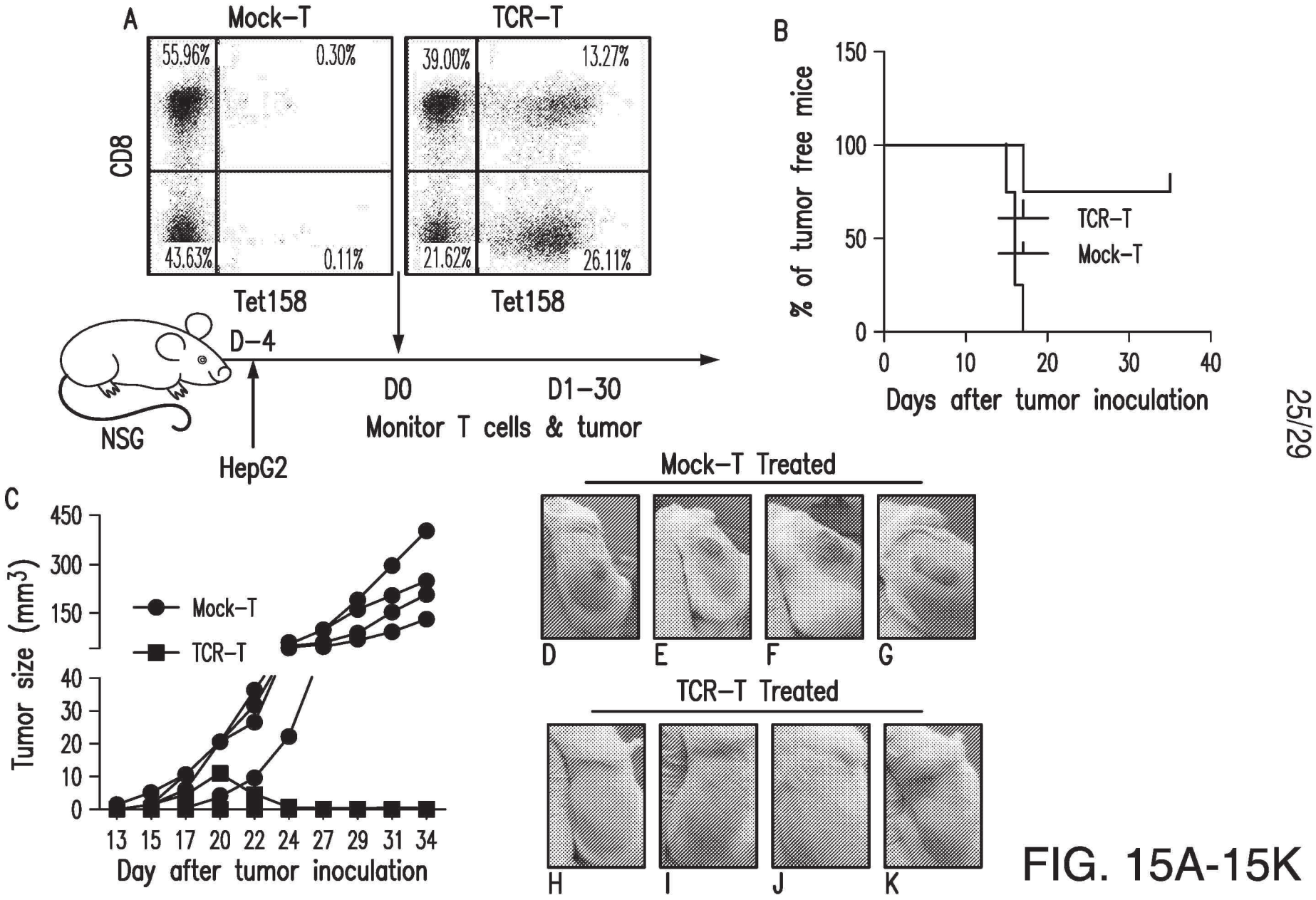

A
Mock-T
TCR-T
55.96%
0.30%
43.63%
0.11%
39.00%
13.27%
21.62%
26.11%
CD8
Tet158
Tet158
D-4
D0
D1-30
Monitor T cells & tumor
NSG
HepG2
B
% of tumor free mice
150
100
50
0
0
10
20
30
40
Days after tumor inoculation
TCR-T
Mock-T
C
Tumor size (mm3)
450
300
150
40
30
20
10
0
Mock-T
TCR-T
13 15 17 20 22 24 27 29 31 34
Day after tumor inoculation
Mock-T Treated
D
E
F
G
TCR-T Treated
H
I
J
K

FIG. 15A-15K

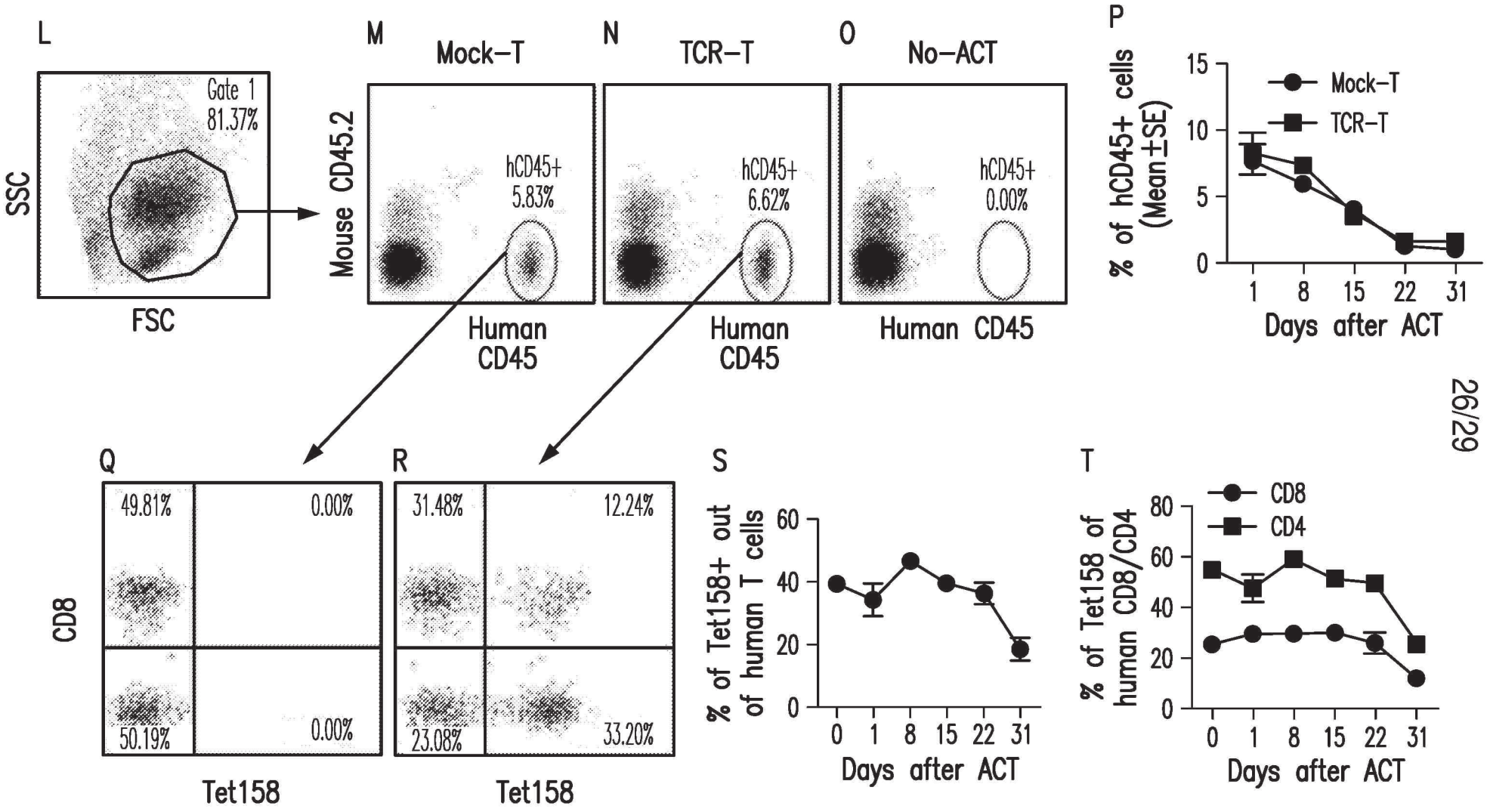

L
Gate 1
81.37%
SSC
FSC
M
Mock–T
N
TCR–T
O
No–ACT
Mouse CD45.2
hCD45+
5.83%
hCD45+
6.62%
hCD45+
0.00%
Human
CD45
Human
CD45
Human CD45
P
% of hCD45+ cells
(Mean±SE)
Mock–T
TCR–T
Days after ACT
Q
R
CD8
49.81%
0.00%
50.19%
0.00%
31.48%
12.24%
23.08%
33.20%
Tet158
Tet158
S
% of Tet158+ out
of human T cells
Days after ACT
T
CD8
CD4
% of Tet158 of
human CD8/CD4
Days after ACT

FIG. 15L-15T

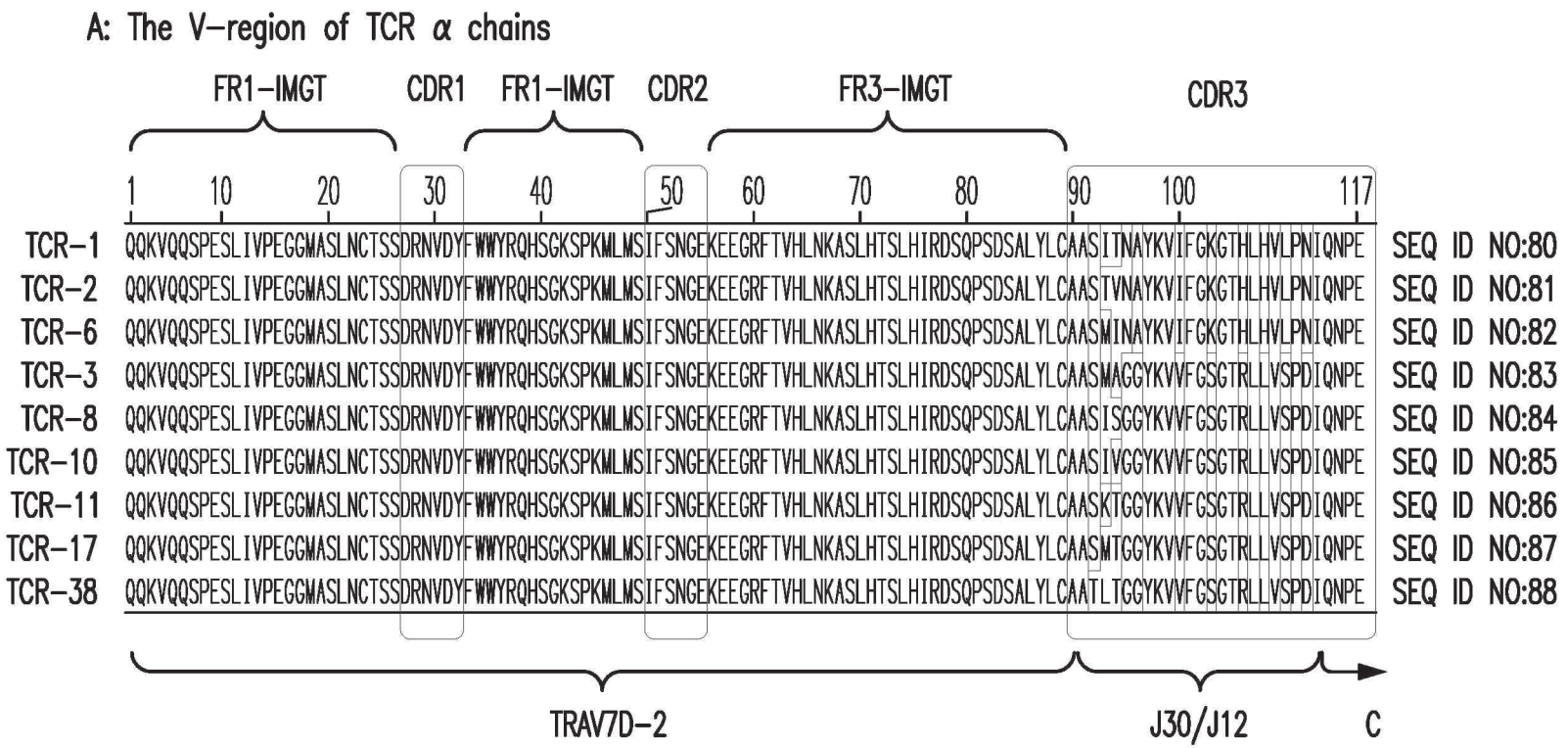

A: The V-region of TCR α chains
FR1-IMGT
CDR1
FR1-IMGT
CDR2
FR3-IMGT
CDR3
1 10 20 30 40 50 60 70 80 90 100 117
TCR-1 QQKVQQSPESLIVPEGGMASLNCTSSDRNVDYFWWYRQHSGKSPKMLMSIFSNGEKEEGRFTVHLNKASLHTSLHIRDSQPSDSALYLCAASITNAYKVIFGKGTHLHVLPNIQNPE SEQ ID NO:80
TCR-2 QQKVQQSPESLIVPEGGMASLNCTSSDRNVDYFWWYRQHSGKSPKMLMSIFSNGEKEEGRFTVHLNKASLHTSLHIRDSQPSDSALYLCAASTVNAYKVIFGKGTHLHVLPNIQNPE SEQ ID NO:81
TCR-6 QQKVQQSPESLIVPEGGMASLNCTSSDRNVDYFWWYRQHSGKSPKMLMSIFSNGEKEEGRFTVHLNKASLHTSLHIRDSQPSDSALYLCAASMINAYKVIFGKGTHLHVLPNIQNPE SEQ ID NO:82
TCR-3 QQKVQQSPESLIVPEGGMASLNCTSSDRNVDYFWWYRQHSGKSPKMLMSIFSNGEKEEGRFTVHLNKASLHTSLHIRDSQPSDSALYLCAASMAGGYKVVFGSGTRLLVSPDIQNPE SEQ ID NO:83
TCR-8 QQKVQQSPESLIVPEGGMASLNCTSSDRNVDYFWWYRQHSGKSPKMLMSIFSNGEKEEGRFTVHLNKASLHTSLHIRDSQPSDSALYLCAASISGGYKVVFGSGTRLLVSPDIQNPE SEQ ID NO:84
TCR-10 QQKVQQSPESLIVPEGGMASLNCTSSDRNVDYFWWYRQHSGKSPKMLMSIFSNGEKEEGRFTVHLNKASLHTSLHIRDSQPSDSALYLCAASIVGGYKVVFGSGTRLLVSPDIQNPE SEQ ID NO:85
TCR-11 QQKVQQSPESLIVPEGGMASLNCTSSDRNVDYFWWYRQHSGKSPKMLMSIFSNGEKEEGRFTVHLNKASLHTSLHIRDSQPSDSALYLCAASKTGGYKVVFGSGTRLLVSPDIQNPE SEQ ID NO:86
TCR-17 QQKVQQSPESLIVPEGGMASLNCTSSDRNVDYFWWYRQHSGKSPKMLMSIFSNGEKEEGRFTVHLNKASLHTSLHIRDSQPSDSALYLCAASMTGGYKVVFGSGTRLLVSPDIQNPE SEQ ID NO:87
TCR-38 QQKVQQSPESLIVPEGGMASLNCTSSDRNVDYFWWYRQHSGKSPKMLMSIFSNGEKEEGRFTVHLNKASLHTSLHIRDSQPSDSALYLCAATLTGGYKVVFGSGTRLLVSPDIQNPE SEQ ID NO:88
TRAV7D-2
J30/J12
C

FIG. 16

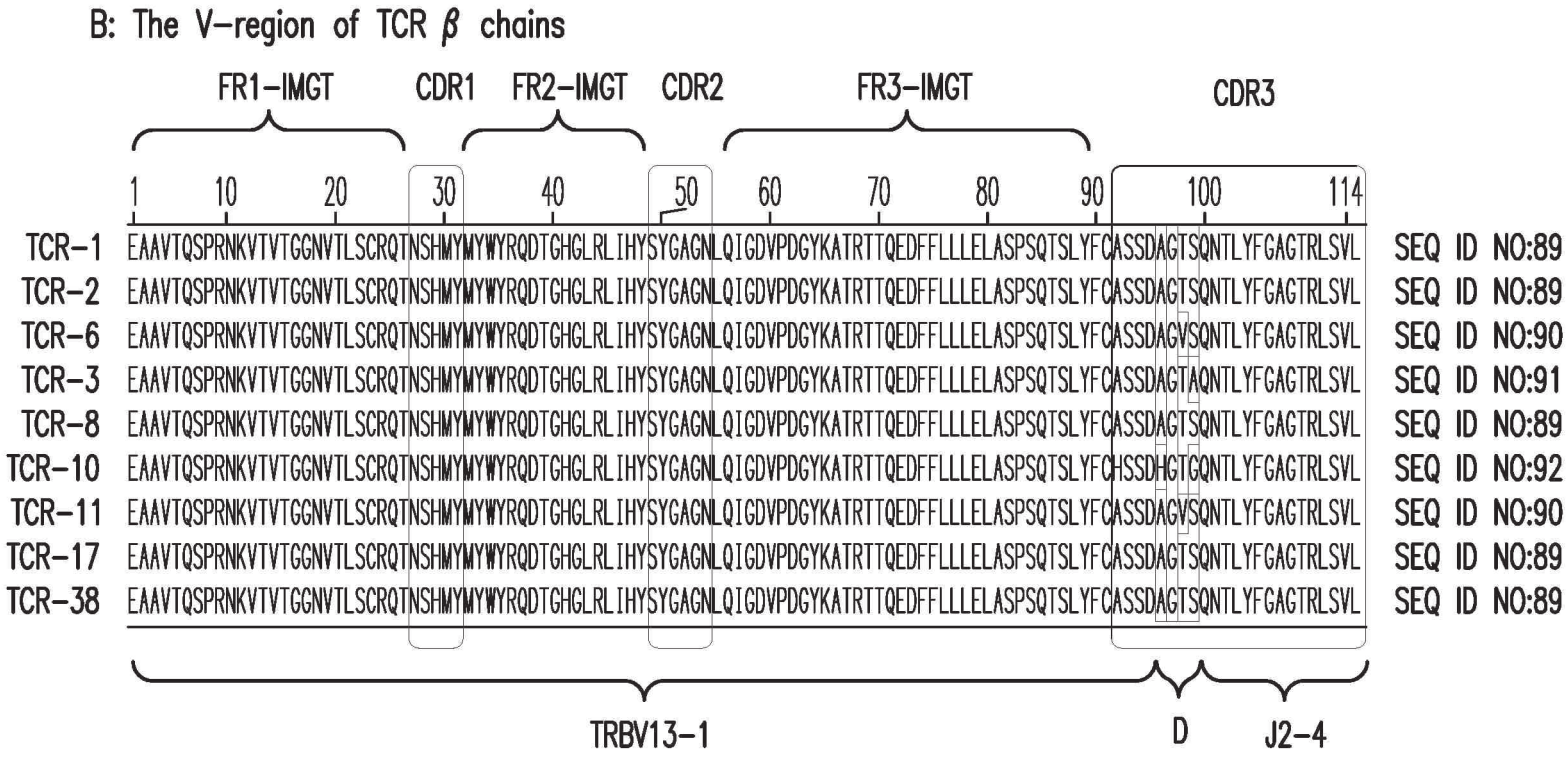


FIG. 16 continued

CDR3 OF TCR α chains

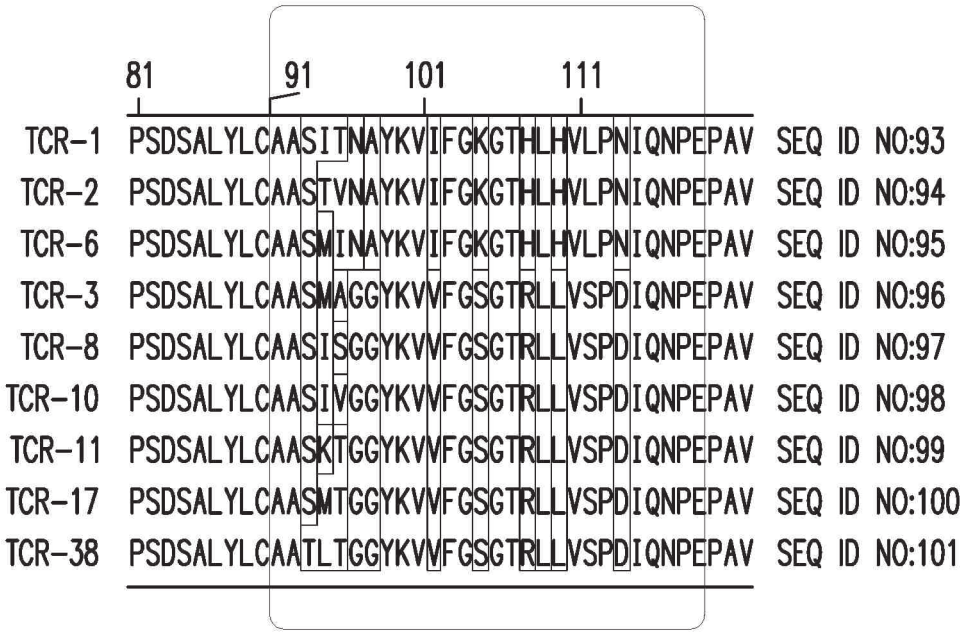


FIG. 17A

CDR3 OF TCR β chains

FIG. 17B