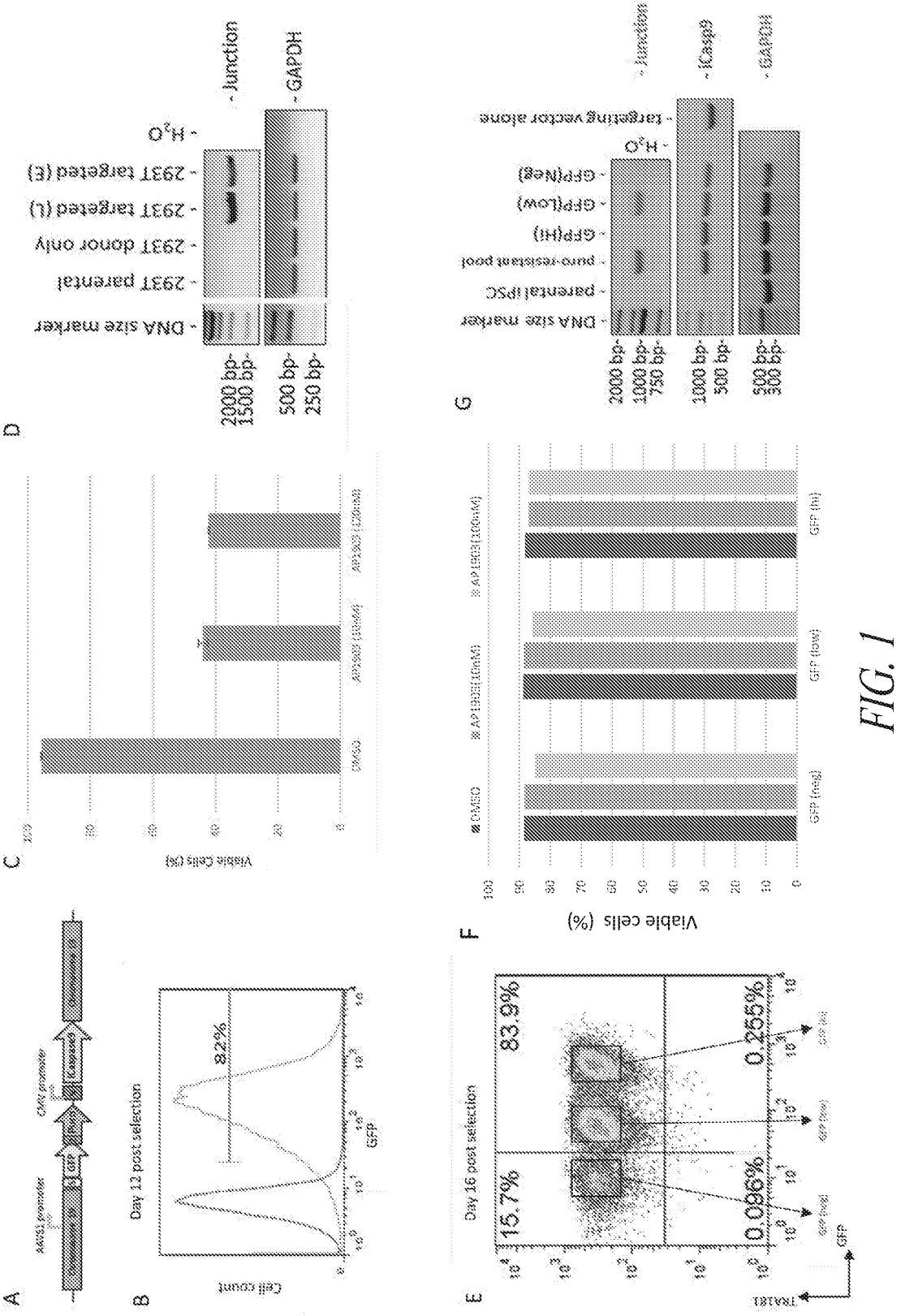

A
AAVS1 promoter
GFP
Puro
CMV promoter
iCaspase9
Truncated LNGFR
B
Day 13 post selection
Cell count
8.2%
GFP
C
Viable Cells (%)
DMSO
AP1903 (10nM)
AP1903 (100nM)
D
- DNA size marker
- 293T parental
- 293T donor only
- 293T targeted (L)
- 293T targeted (E)
- H2O
2000 bp-
1500 bp-
500 bp-
250 bp-
- Junction
- GAPDH
E
Day 16 post selection
83.9%
15.7%
0.255%
0.086%
TRA1B1
GFP
F
Viable cells (%)
DMSO
AP1903(10nM)
AP1903(100nM)
GFP (neg)
GFP (low)
GFP (hi)
G
- DNA size marker
- parental iPSC
- puro-resistant pool
- GFP(Hi)
- GFP(Low)
- GFP(Neg)
- H2O
- targeting vector alone
2000 bp-
1000 bp-
750 bp-
1000 bp-
500 bp-
500 bp-
300 bp-
- Junction
- iCasp9
- GAPDH

FIG. 1

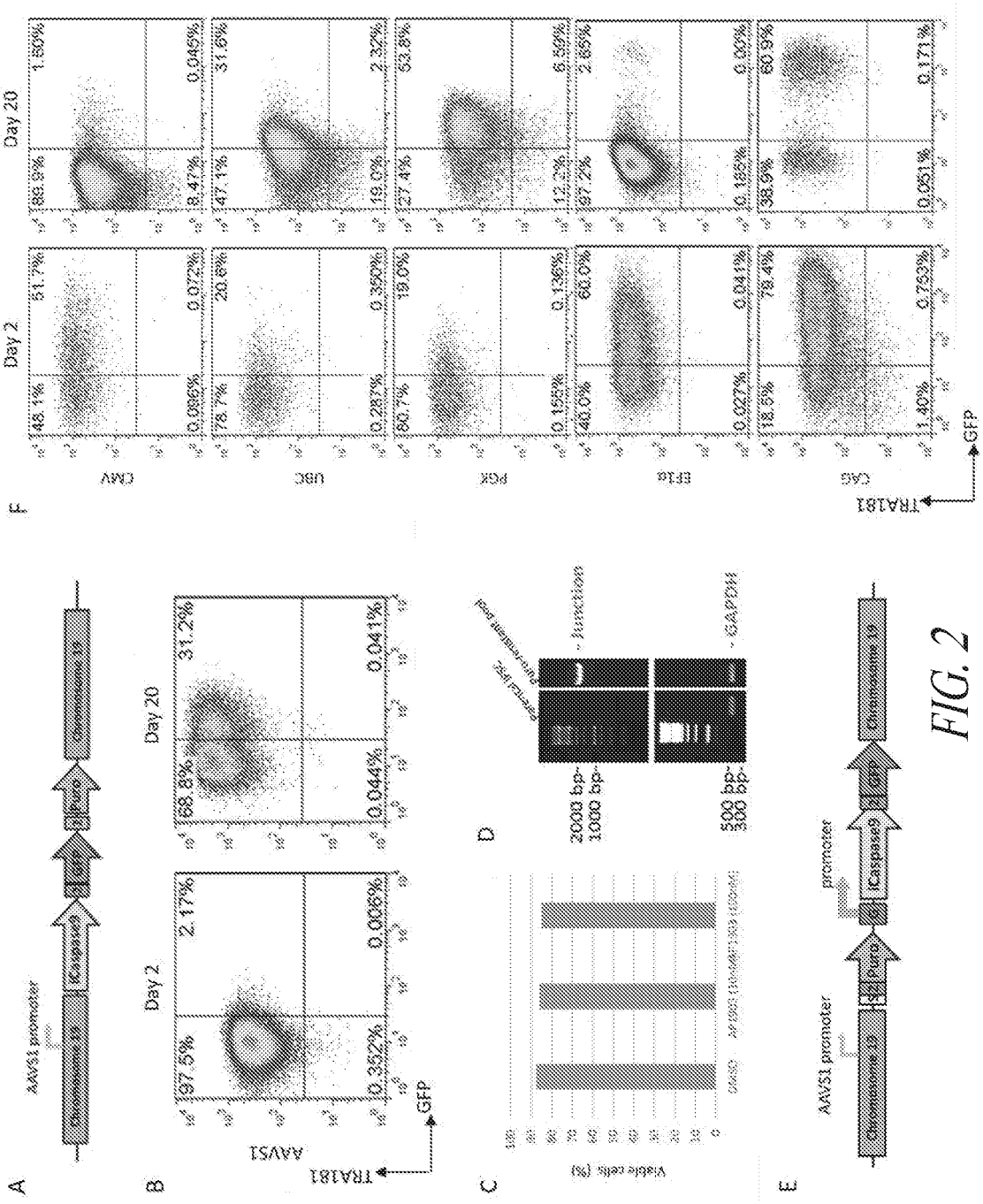

A
AAVS1 promoter
Caspase 9
GFP
Puro
Chromosome 19
B
Day 2
Day 20
97.5%
2.17%
0.352%
0.006%
68.8%
31.2%
0.044%
0.041%
AAVS1
TRA1B1
GFP
C
Viable cells (%)
D
Fold activation over non-cleaved
2000 bp-
1000 bp-
500 bp-
300 bp-
- Junction
- GAPDH
E
AAVS1 promoter
Caspase 9
GFP
Puro
Chromosome 19
promoter
F
Day 2
Day 20
CMV
UBC
PGK
EF1a
CAG
48.1%
51.7%
0.086%
0.072%
78.7%
20.6%
0.281%
0.352%
80.7%
19.0%
0.155%
0.136%
40.0%
60.0%
0.027%
0.041%
18.6%
78.4%
1.40%
0.763%
86.9%
8.47%
1.85%
0.045%
47.1%
19.0%
31.6%
2.32%
27.4%
12.2%
63.8%
6.56%
97.2%
0.185%
2.85%
0.05%
38.9%
0.051%
60.9%
0.171%
TRA1B1
GFP

FIG. 2

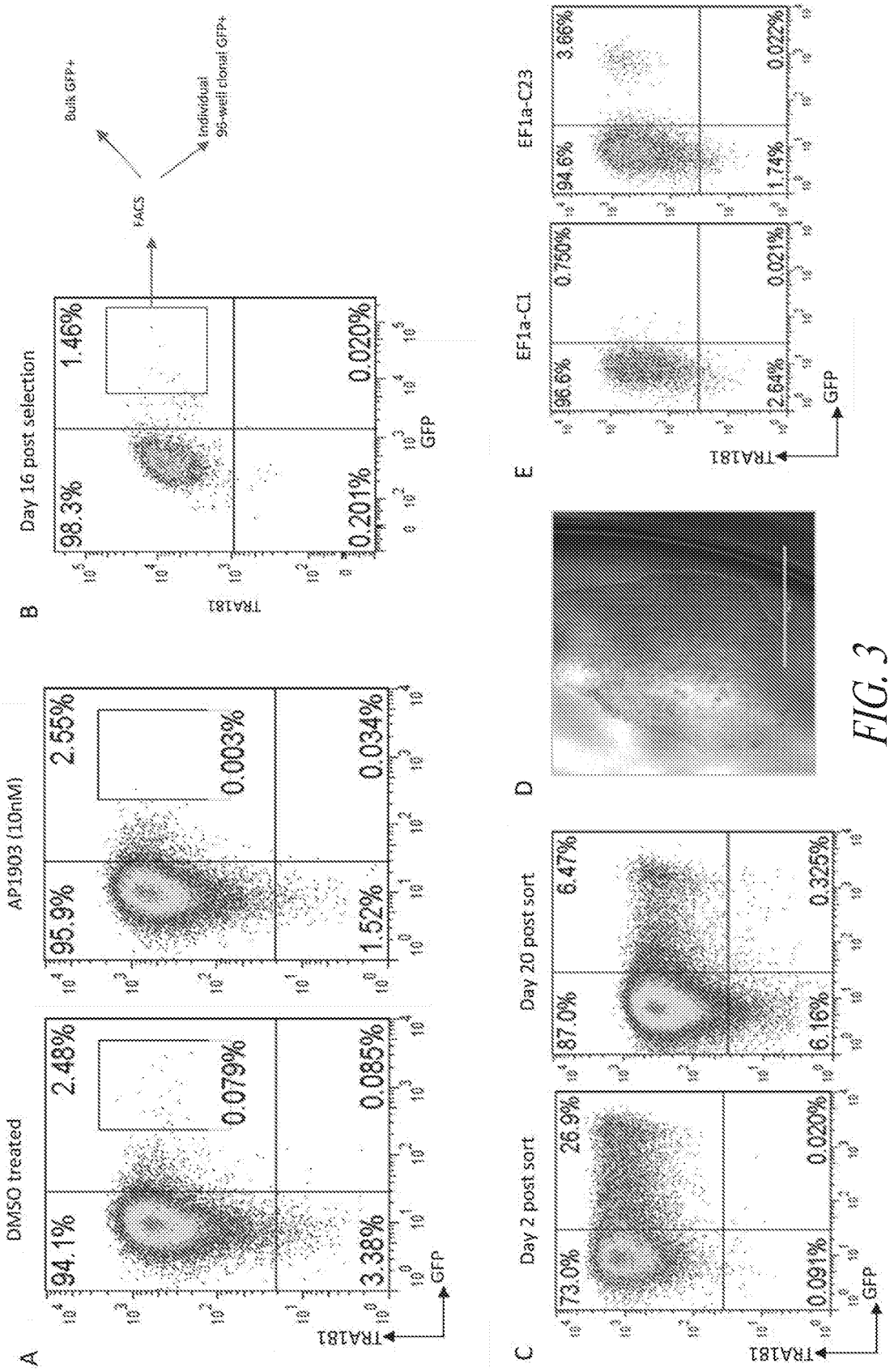

A
DMSO treated
AP1903 (10nM)
94.1%
2.48%
0.079%
3.38%
0.085%
95.9%
2.55%
0.003%
1.52%
0.034%
TRA1B1
GFP
B
Day 16 post selection
98.3%
1.46%
0.201%
0.020%
FACS
Bulk GFP+
Individual 96-well clonal GFP+
C
Day 2 post sort
73.0%
26.9%
0.091%
0.020%
Day 20 post sort
87.0%
6.47%
6.16%
0.325%
D
E
EF1a-C1
96.6%
0.750%
2.64%
0.021%
EF1a-C23
94.6%
3.66%
1.74%
0.022%

FIG. 3

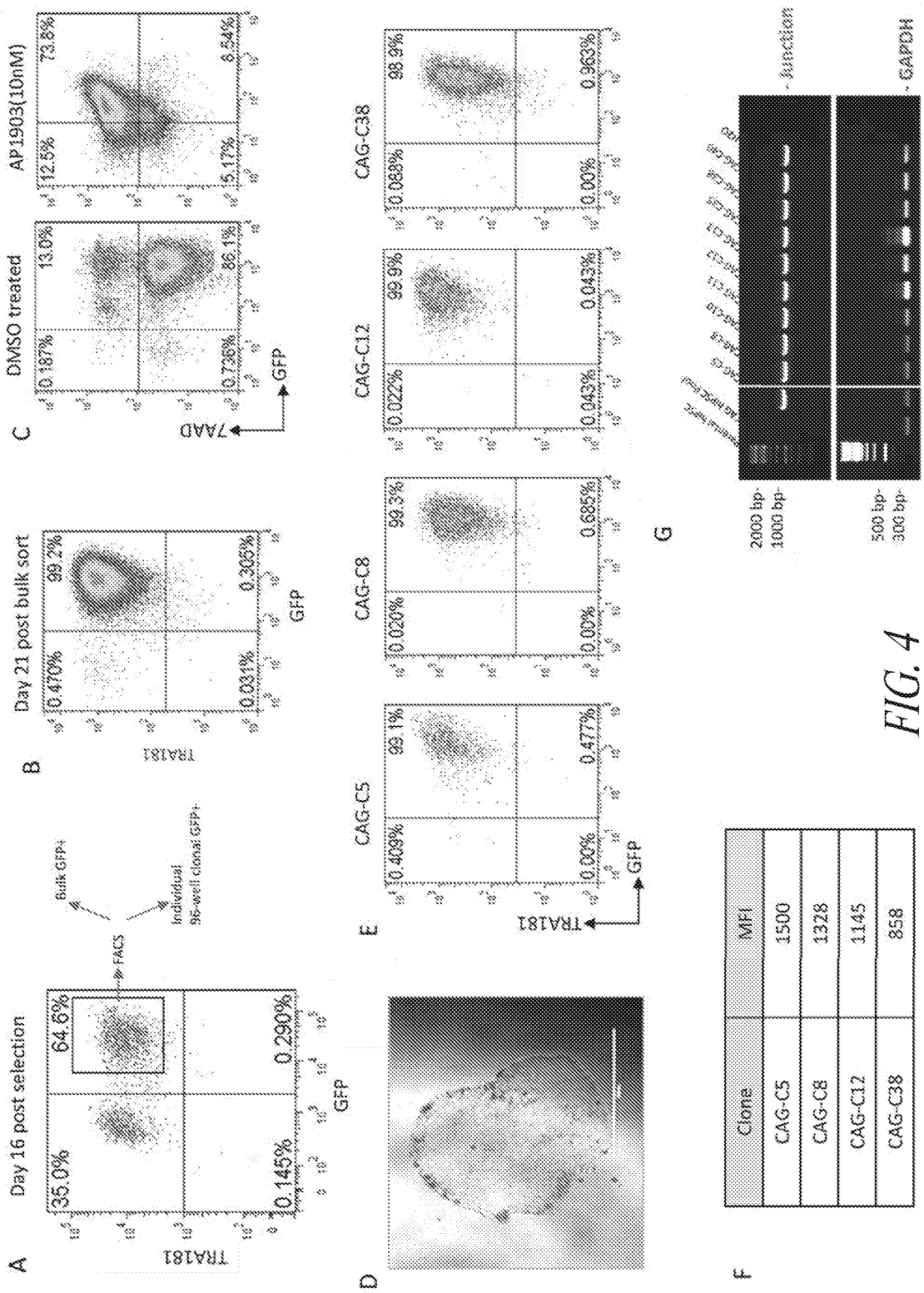

A
Day 16 post selection
TRA181
GFP
35.0%
64.6%
0.145%
0.290%
FACS
Bulk GFP+
Individual 96-well clonal GFP+
B
Day 21 post bulk sort
TRA181
GFP
0.470%
99.2%
0.031%
0.306%
C
DMSO treated
AP1903(10nM)
7AAD
GFP
10.187%
13.0%
0.736%
86.1%
12.5%
73.8%
5.17%
8.54%
D
E
CAG-C5
CAG-C8
CAG-C12
CAG-C38
TRA181
GFP
0.409%
99.1%
0.00%
0.477%
0.029%
99.3%
0.00%
0.685%
0.022%
99.9%
0.043%
0.043%
0.088%
98.9%
0.00%
0.363%
F
Clone
MFI
CAG-C5
1500
CAG-C8
1328
CAG-C12
1145
CAG-C38
858
G
Junction
GAPDH
2000 bp
1000 bp
500 bp
300 bp

FIG. 4

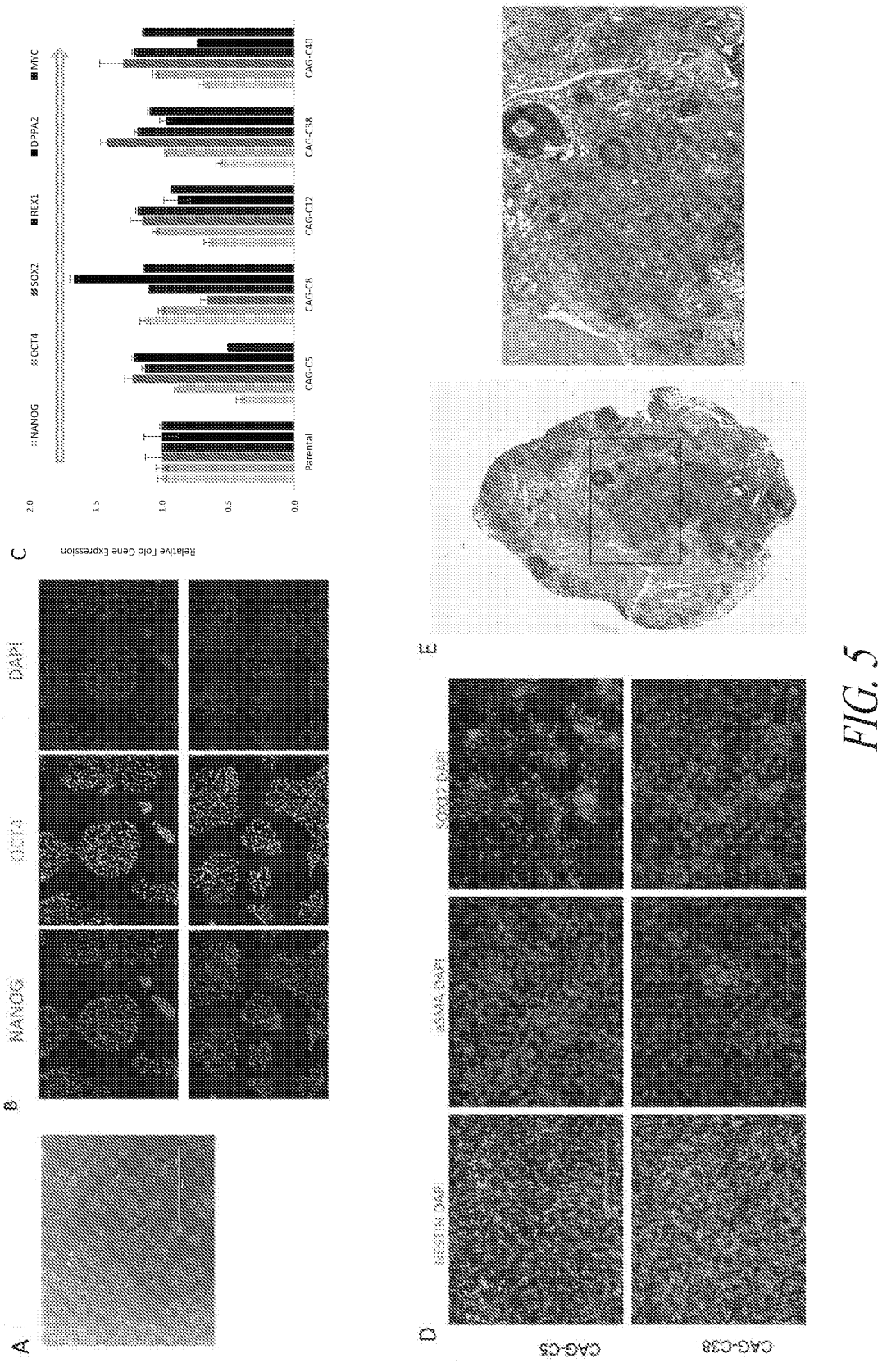

A
B
NANOG
OCT4
DAPI
C
Relative Fold Gene Expression
NANOG
OCT4
SOX2
REX1
DPPA2
MYC
Parental
CAG-C5
CAG-C8
CAG-C12
CAG-C38
CAG-C40
D
CAG-C5
CAG-C38
E

FIG. 5

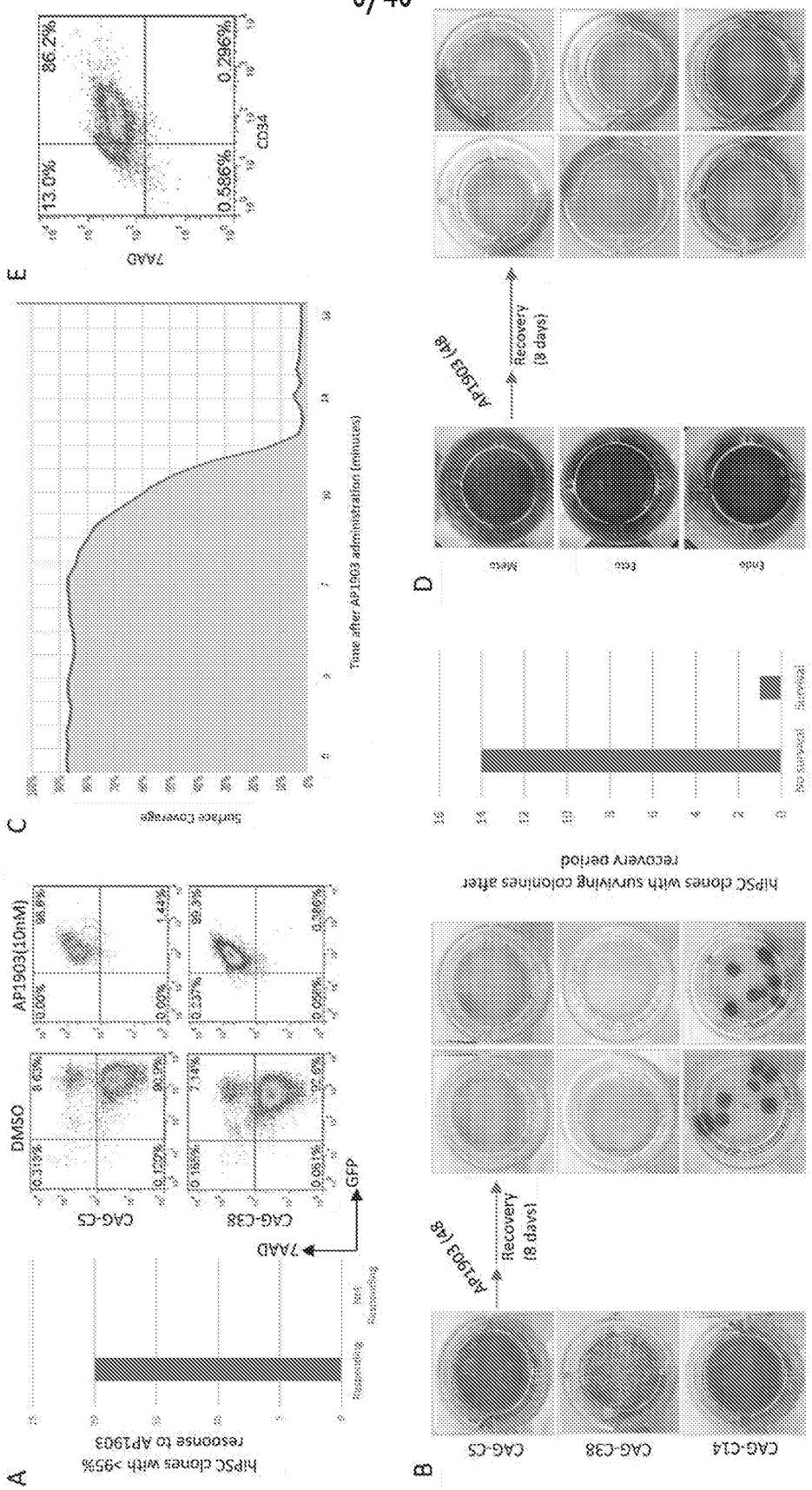

A
hiPSC clones with >95%
response to AP1903
Recovery
Survival
DMSO
AP1903(10nM)
CAG-C5
CAG-C38
7AAD
GFP
B
CAG-C5
CAG-C38
CAG-C14
AP1903 (48
Recovery
(8 days)
C
Surface Coverage
Time after AP1903 administration (minutes)
D
hiPSC clones with surviving colonies after
recovery period
Survival
Meso
Ecto
Endo
AP1903 (48
Recovery
(8 days)
E
7AAD
CD34
86.2%
13.0%
0.586%
0.296%

FIG. 6

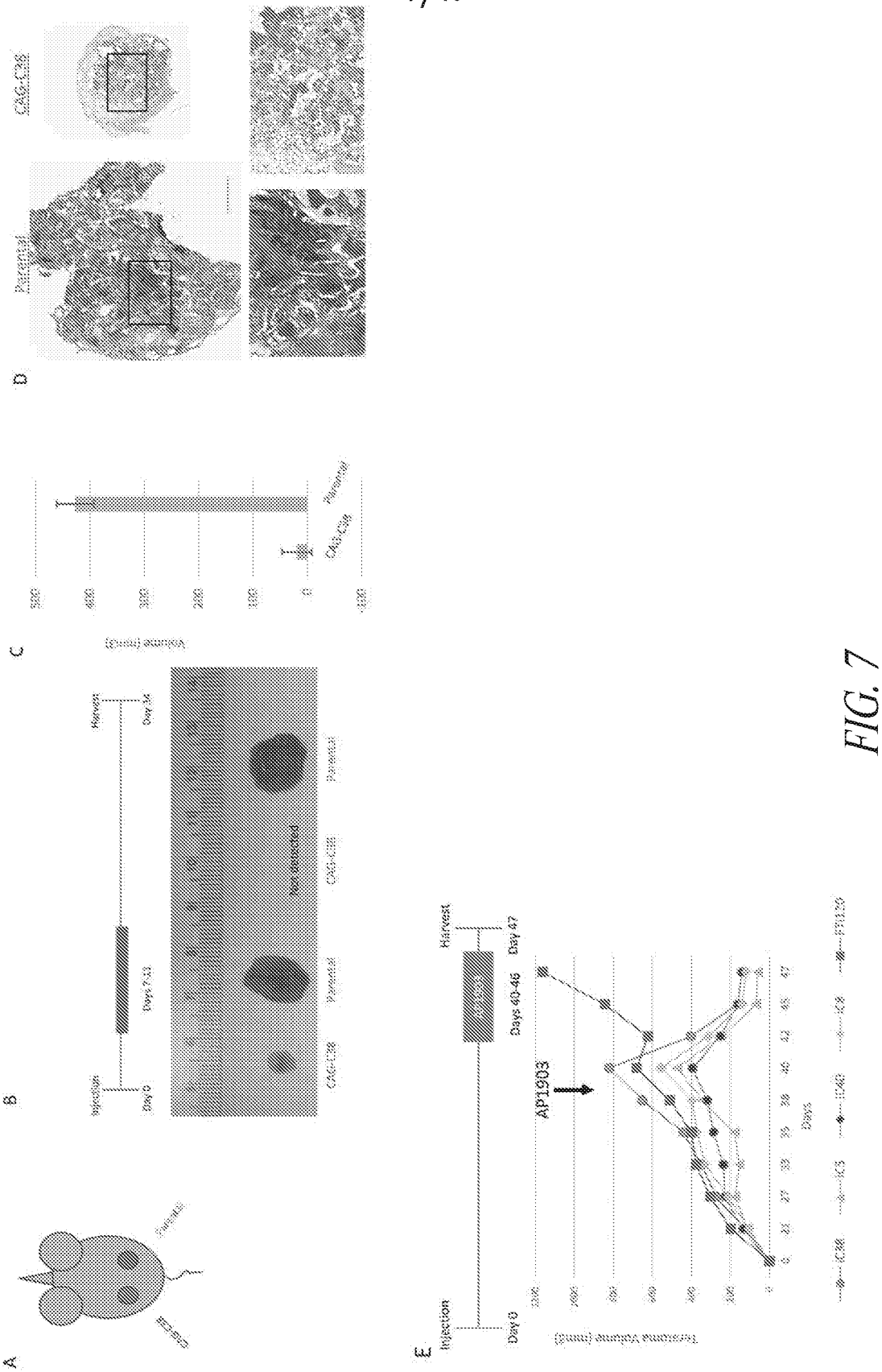

A
Control
AP1903
B
Injection
Day 0
Days 7-13
Harvest
Day 14
Not injected
CAG-C38
Parental
C
Tumor Volume (mm3)
500
400
300
200
100
0
-100
CAG-C38
Parental
D
CAG-C38
Parental
E
Injection
Day 0
Days 40-46
Harvest
Day 47
AP1903
Tumor Volume (mm3)
Days
C38
IC3
IL40
IC8
FP1129

FIG. 7

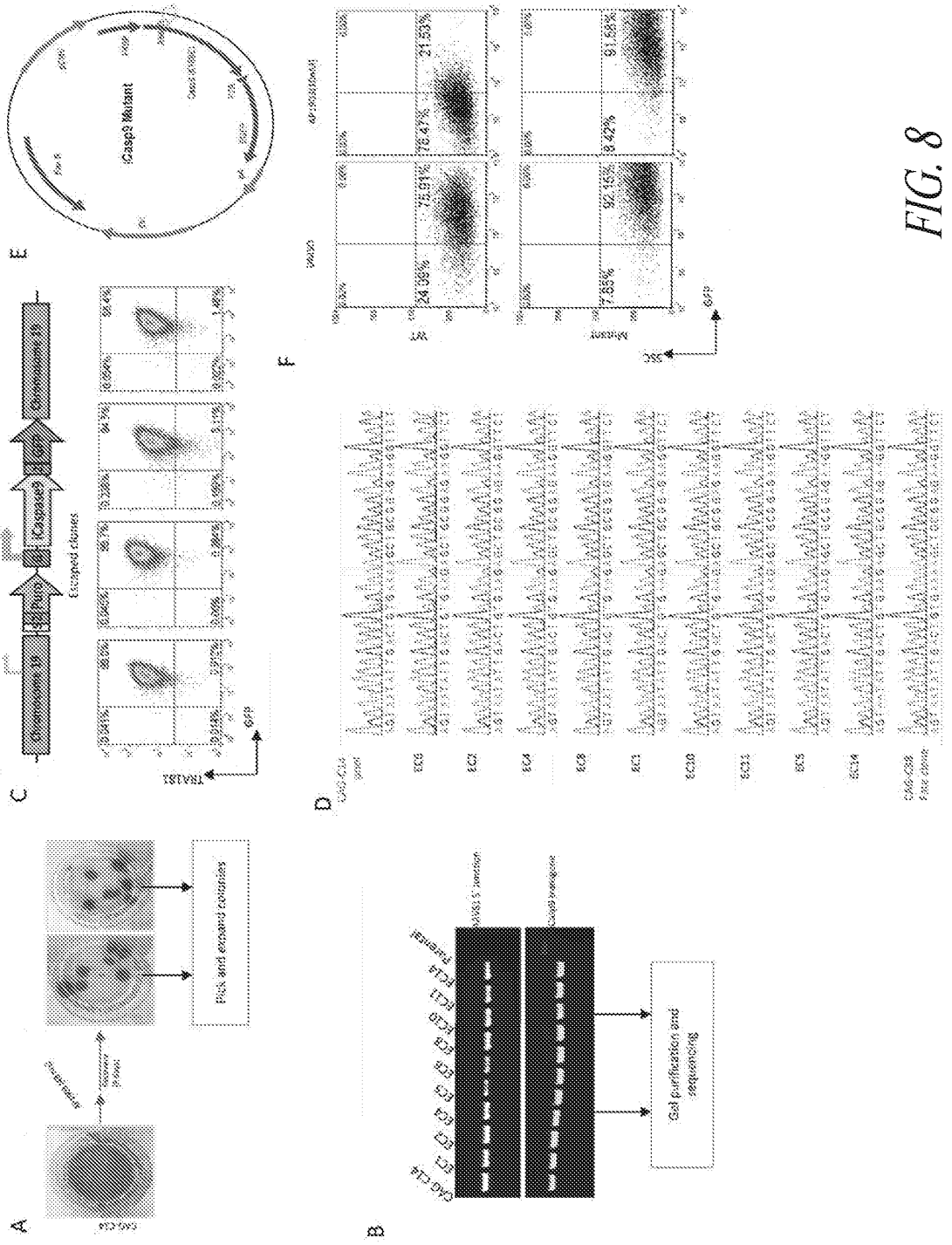


FIG. 8

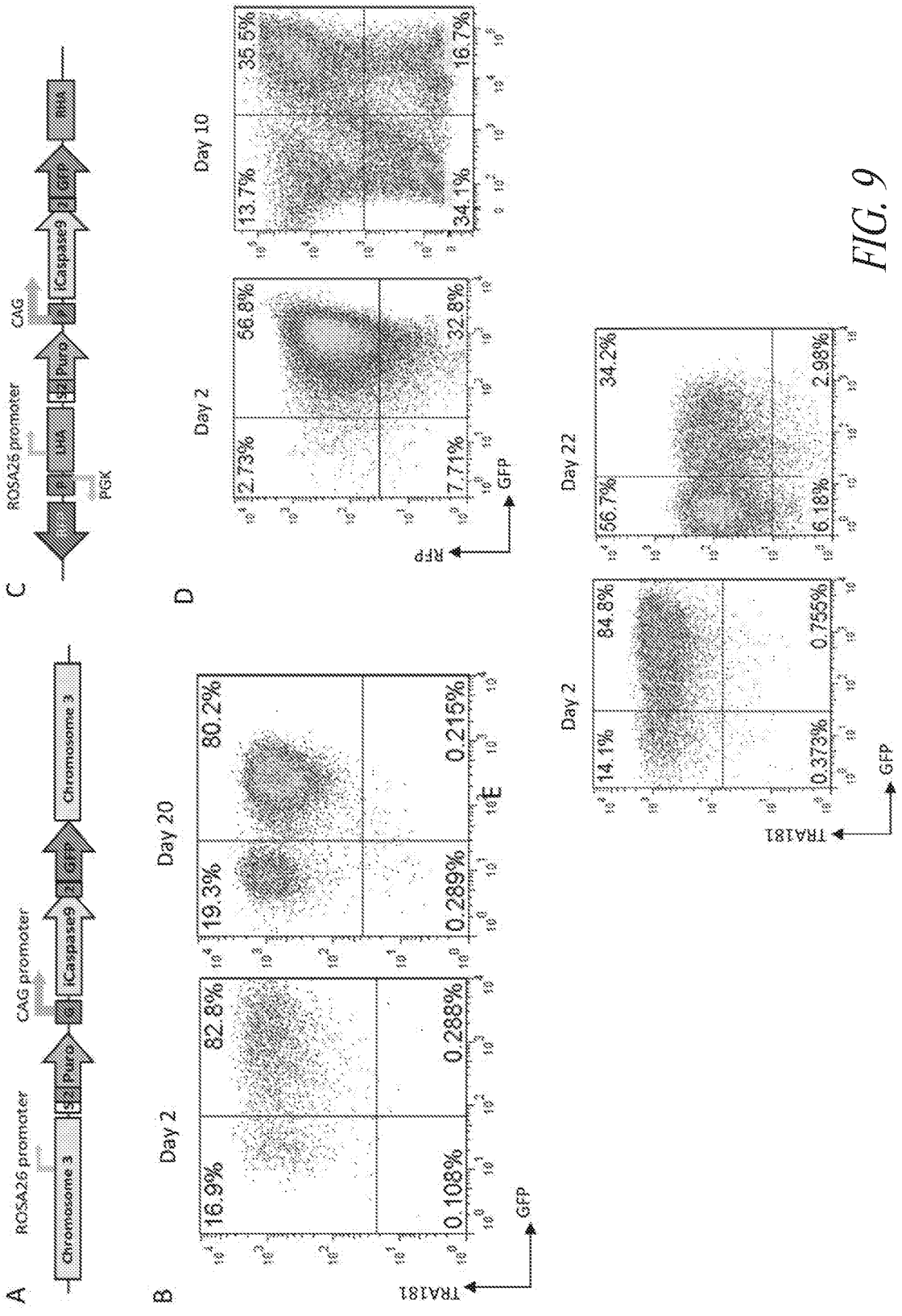

A
ROSA26 promoter
Chromosome 3
SA
Puro
CAG promoter
iCaspase9
2A
GFP
Chromosome 3
C
ROSA26 promoter
CAG
Chromosome 3
SA
IRES
Puro
2A
iCaspase9
2A
GFP
bGH
B
Day 2
16.9%
82.8%
0.108%
0.288%
Day 20
19.3%
80.2%
0.289%
0.215%
TRA181
GFP
D
Day 2
2.73%
66.8%
7.71%
32.8%
Day 10
13.7%
35.5%
34.1%
16.7%
RFP
GFP
E
Day 2
14.1%
84.8%
0.373%
0.755%
Day 22
16.7%
34.2%
16.18%
2.98%
TRA181
GFP

FIG. 9

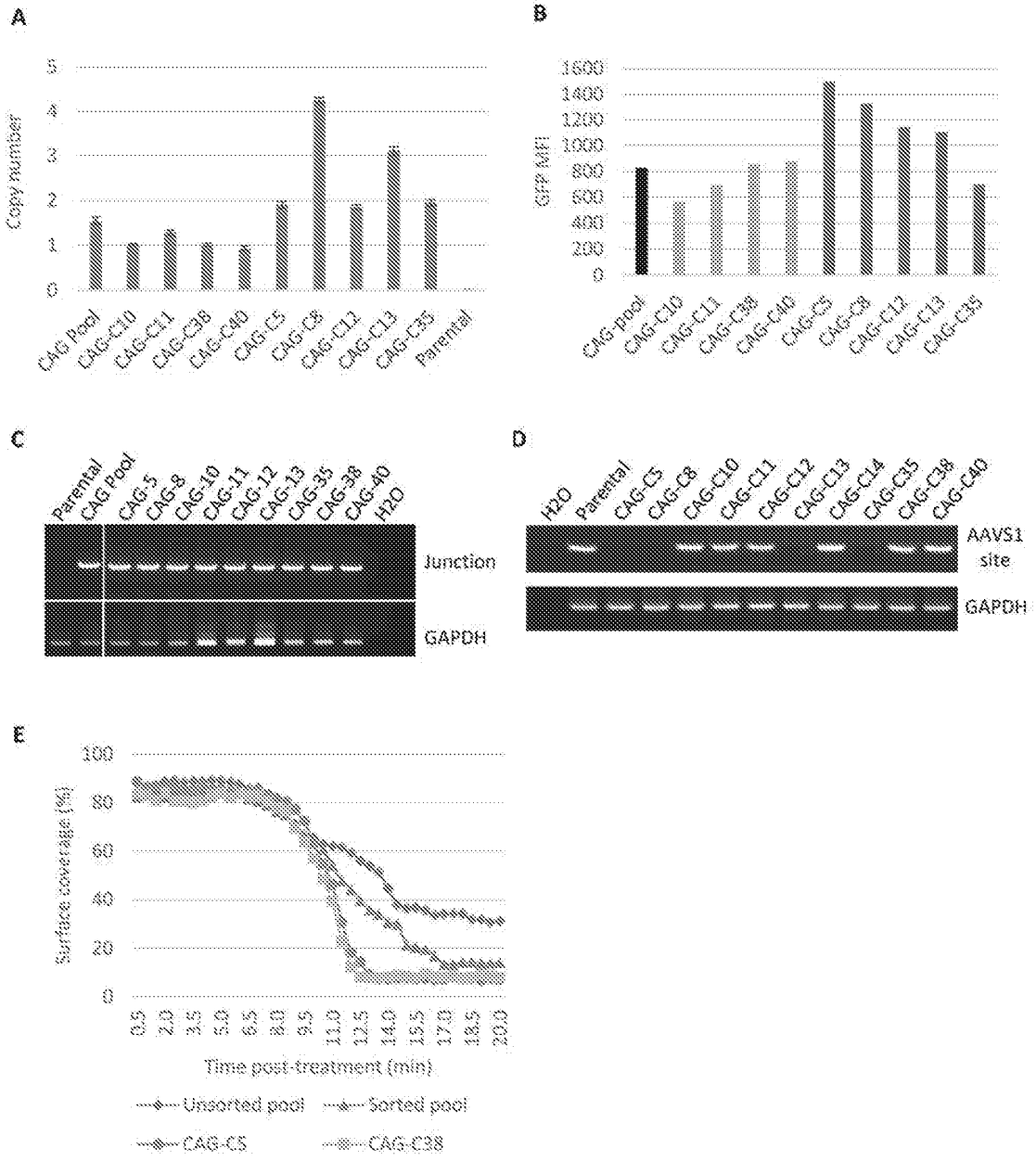

A
Copy number
0
1
2
3
4
5
CAG Pool
CAG-C10
CAG-C11
CAG-C38
CAG-C40
CAG-C5
CAG-C8
CAG-C12
CAG-C13
CAG-C35
Parental
B
GFP MFI
0
200
400
600
800
1000
1200
1400
1600
CAG-pool
CAG-C10
CAG-C11
CAG-C38
CAG-C40
CAG-C5
CAG-C8
CAG-C12
CAG-C13
CAG-C35
C
Parental
CAG Pool
CAG-5
CAG-8
CAG-10
CAG-11
CAG-12
CAG-13
CAG-35
CAG-38
CAG-40
H2O
Junction
GAPDH
D
H2O
Parental
CAG-C5
CAG-C8
CAG-C10
CAG-C11
CAG-C12
CAG-C13
CAG-C14
CAG-C35
CAG-C38
CAG-C40
AAVS1 site
GAPDH
E
Surface coverage (%)
0
20
40
60
80
100
0.5
2.0
3.5
5.0
6.5
8.0
9.5
11.0
12.5
14.0
15.5
17.0
18.5
20.0
Time post-treatment (min)
Unsorted pool
Sorted pool
CAG-C5
CAG-C38

FIG. 10

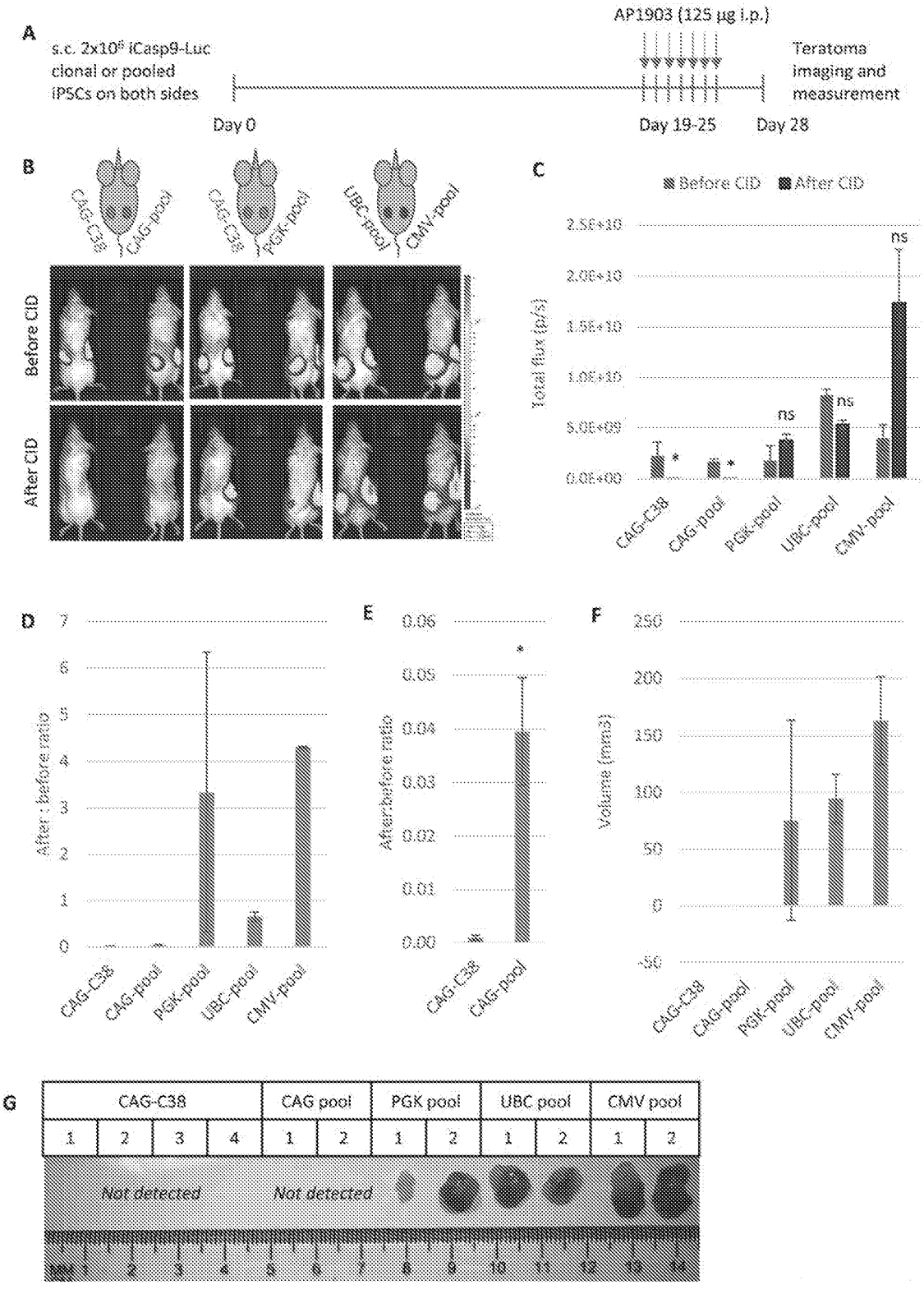

A
s.c. 2x10⁶ iCasp9-Luc clonal or pooled iPSCs on both sides
AP1903 (125 µg i.p.)
Teratoma imaging and measurement
Day 0
Day 19-25
Day 28
B
CAG-C38
CAG-pool
PGK-pool
UBC-pool
CMV-pool
Before CID
After CID
C
Before CID
After CID
Total flux (p/s)
2.5E+10
2.0E+10
1.5E+10
1.0E+10
5.0E+09
0.0E+00
ns
*
D
After : before ratio
E
After:before ratio
F
Volume (mm3)
G
CAG-C38
CAG pool
PGK pool
UBC pool
CMV pool
Not detected
Not detected

FIG. 11

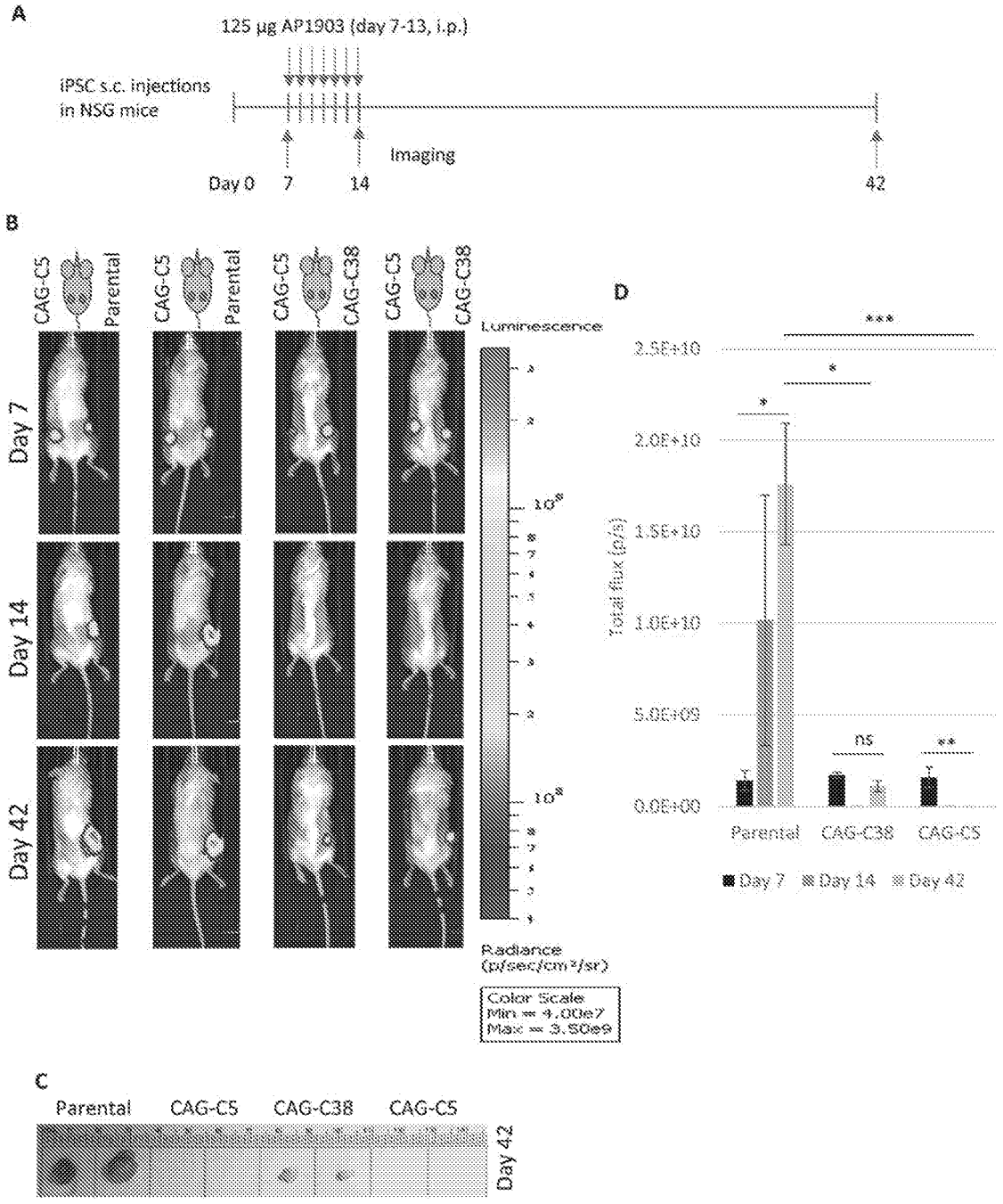

A
125 µg AP1903 (day 7-13, i.p.)
iPSC s.c. injections in NSG mice
Imaging
Day 0
7
14
42
B
CAG-C5
Parental
CAG-C5
Parental
CAG-C5
CAG-C38
CAG-C5
CAG-C38
Day 7
Day 14
Day 42
Luminescence
Radiance (p/sec/cm²/sr)
Color Scale
Min = 4.00e7
Max = 3.50e9
D
***
*
*
2.5E+10
2.0E+10
1.5E+10
1.0E+10
5.0E+09
0.0E+00
Total flux (p/s)
ns
**
Parental
CAG-C38
CAG-C5
Day 7
Day 14
Day 42
C
Parental
CAG-C5
CAG-C38
CAG-C5
Day 42

FIG. 12

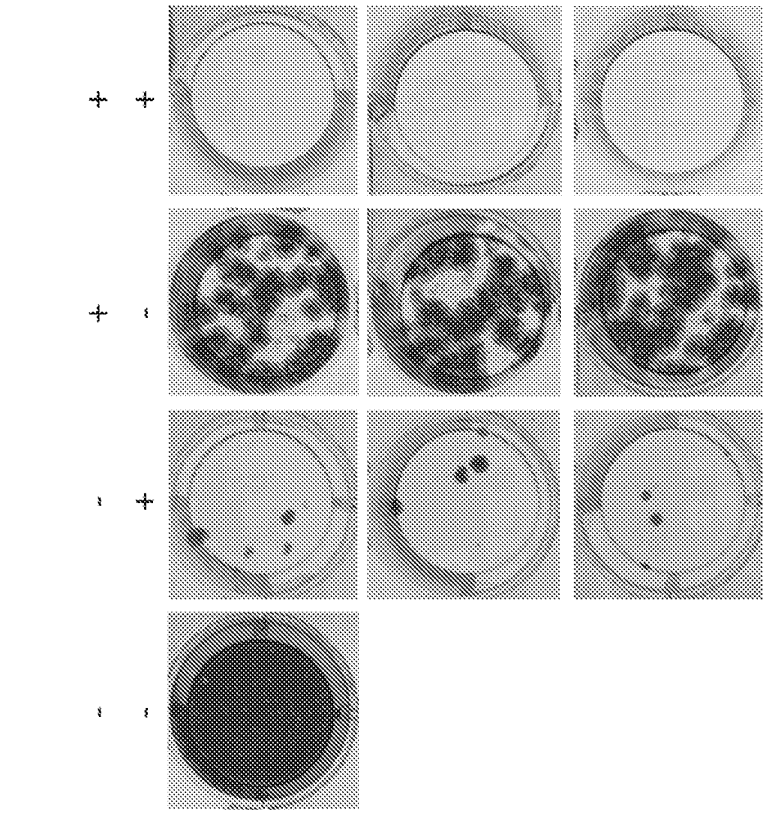

A
TK-iPS
Wt iPS
TK
TK-Bsd junction
H11-transgene junction
GAPDH
B
GCV:
AP1903:
- -
- +
+ -
+ +
(i)
(ii)
(iii)
(iv)

FIG. 13

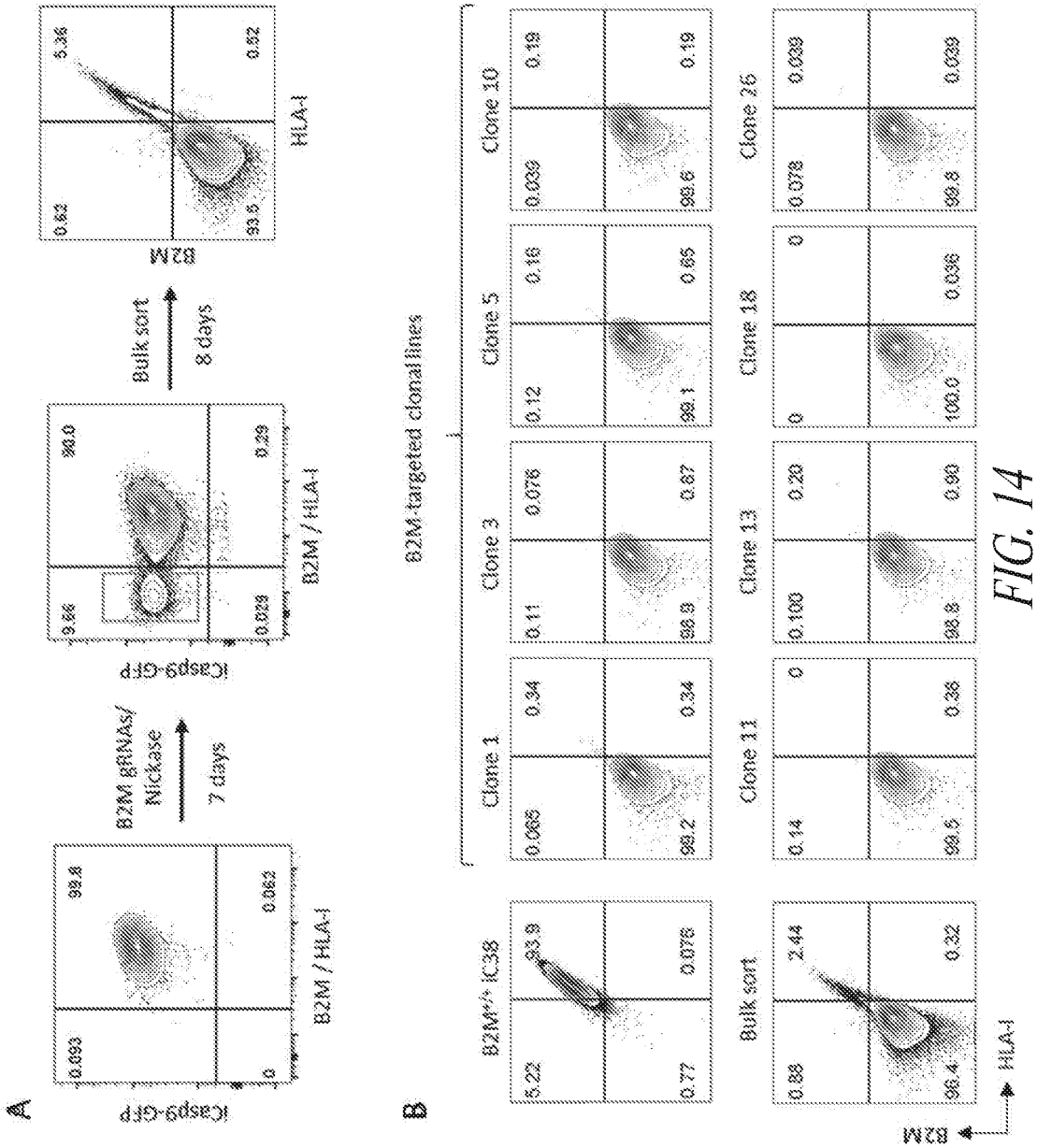

A
iCasp9-GFP
B2M / HLA-I
B2M gRNAs/
Nickase
7 days
Bulk sort
8 days
B2M
HLA-I
B
B2M targeted clonal lines
B2M+/+ IC38
Clone 1
Clone 3
Clone 5
Clone 10
Clone 11
Clone 13
Clone 18
Clone 26
Bulk sort
B2M
HLA-I

FIG. 14

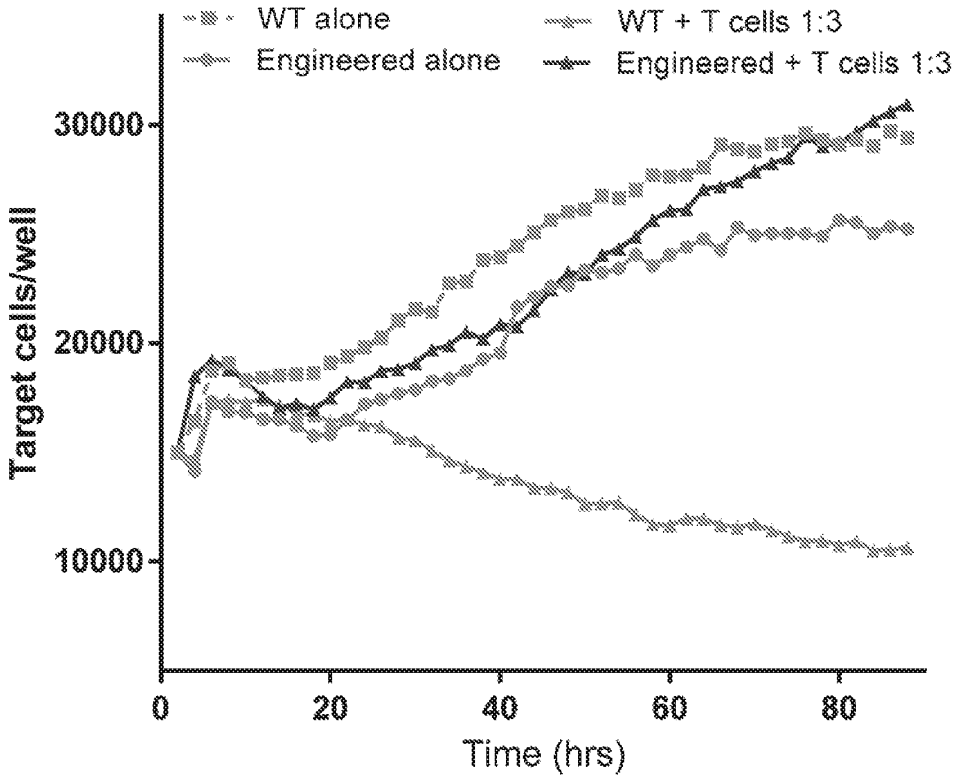

WT alone
Engineered alone
WT + T cells 1:3
Engineered + T cells 1:3
Target cells/well
30000
20000
10000
0
20
40
60
80
Time (hrs)

FIG. 15A

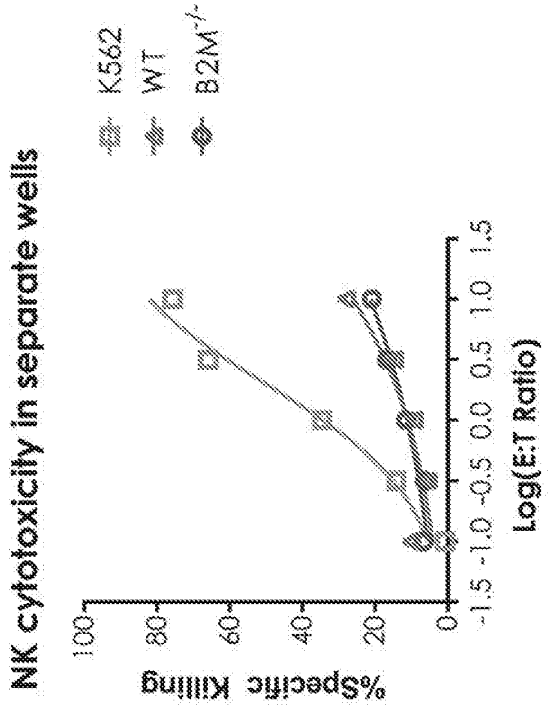


FIG. 15C

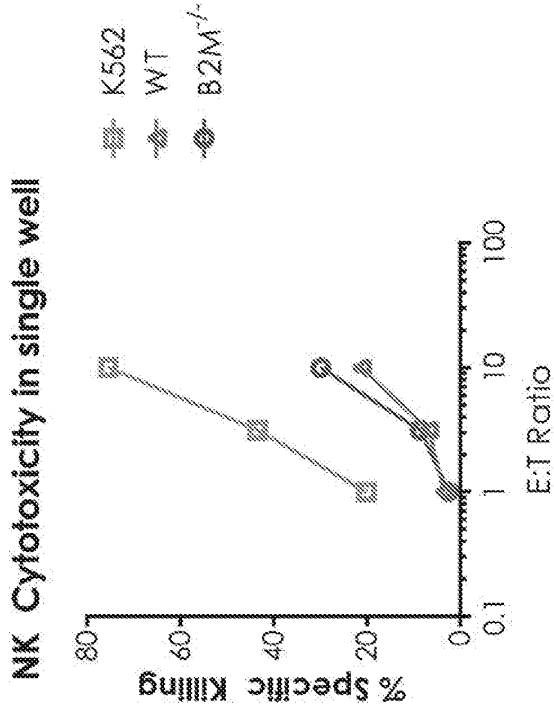


FIG. 15B

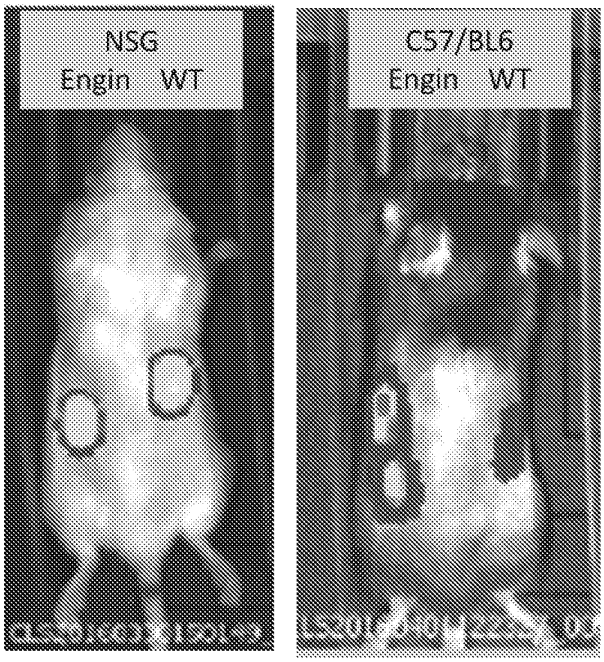


FIG. 15D

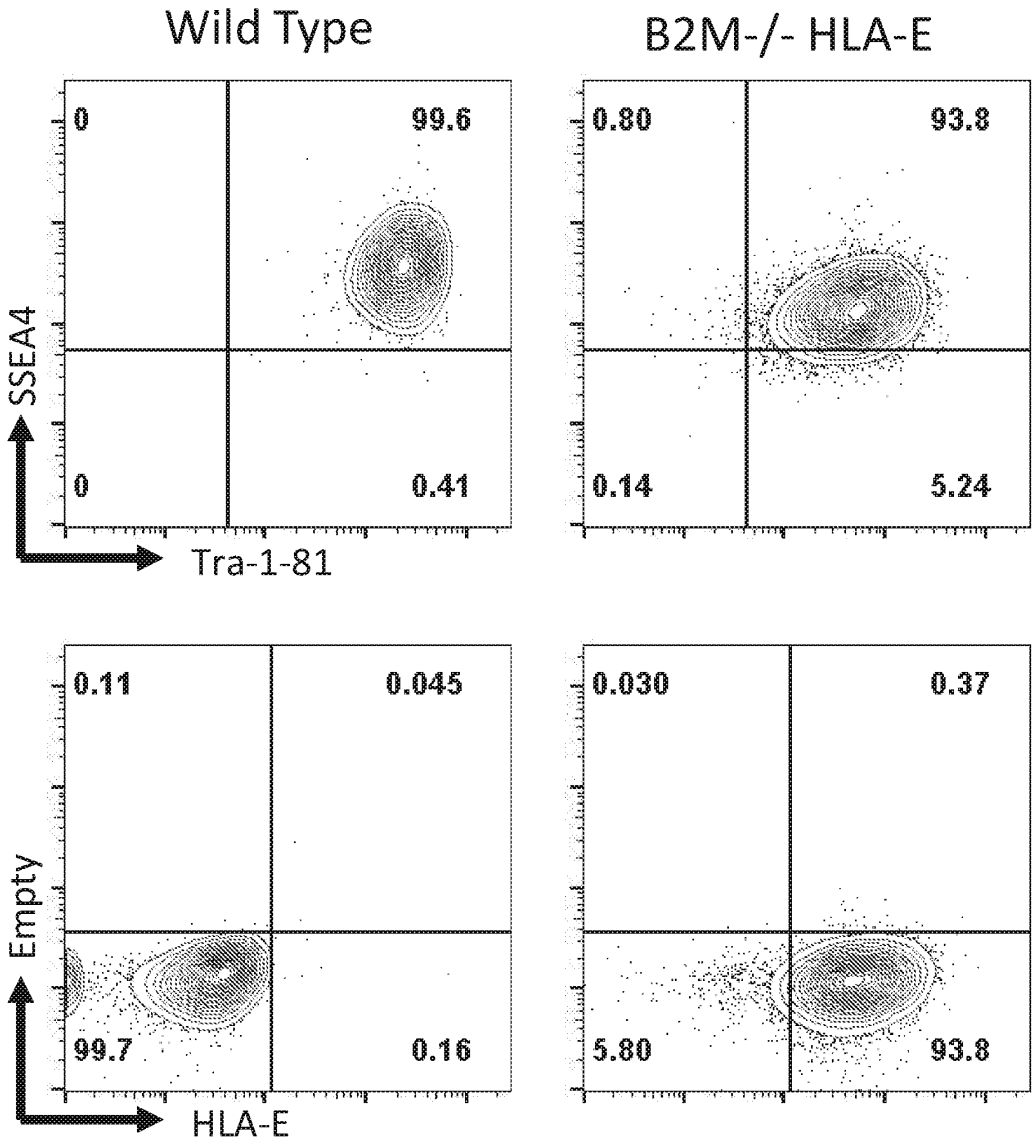

Wild Type
B2M-/- HLA-E
SSEA4
Tra-1-81
0
99.6
0
0.41
0.80
93.8
0.14
5.24
Empty
HLA-E
0.11
0.045
99.7
0.16
0.030
0.37
5.80
93.8

FIG. 16A

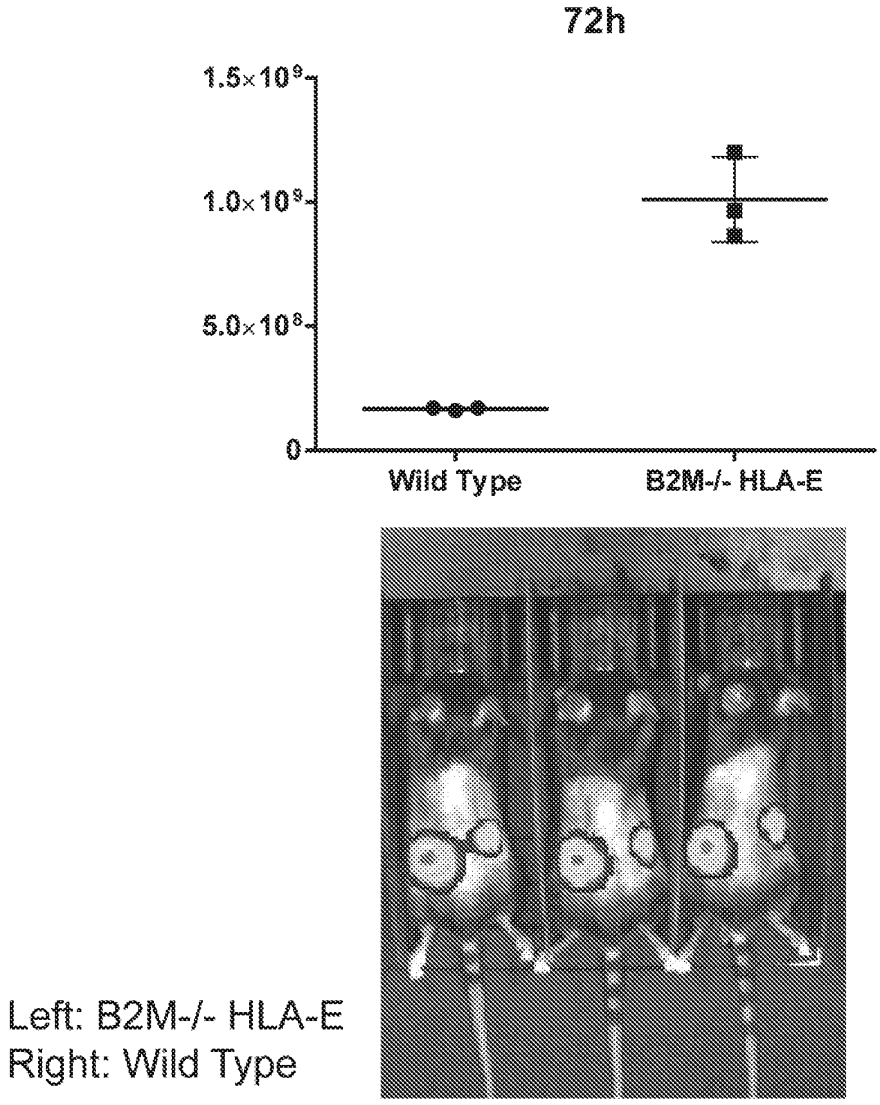

72h
1.5×10⁹
1.0×10⁹
5.0×10⁸
0
Wild Type
B2M-/- HLA-E
Left: B2M-/- HLA-E
Right: Wild Type

FIG. 16B

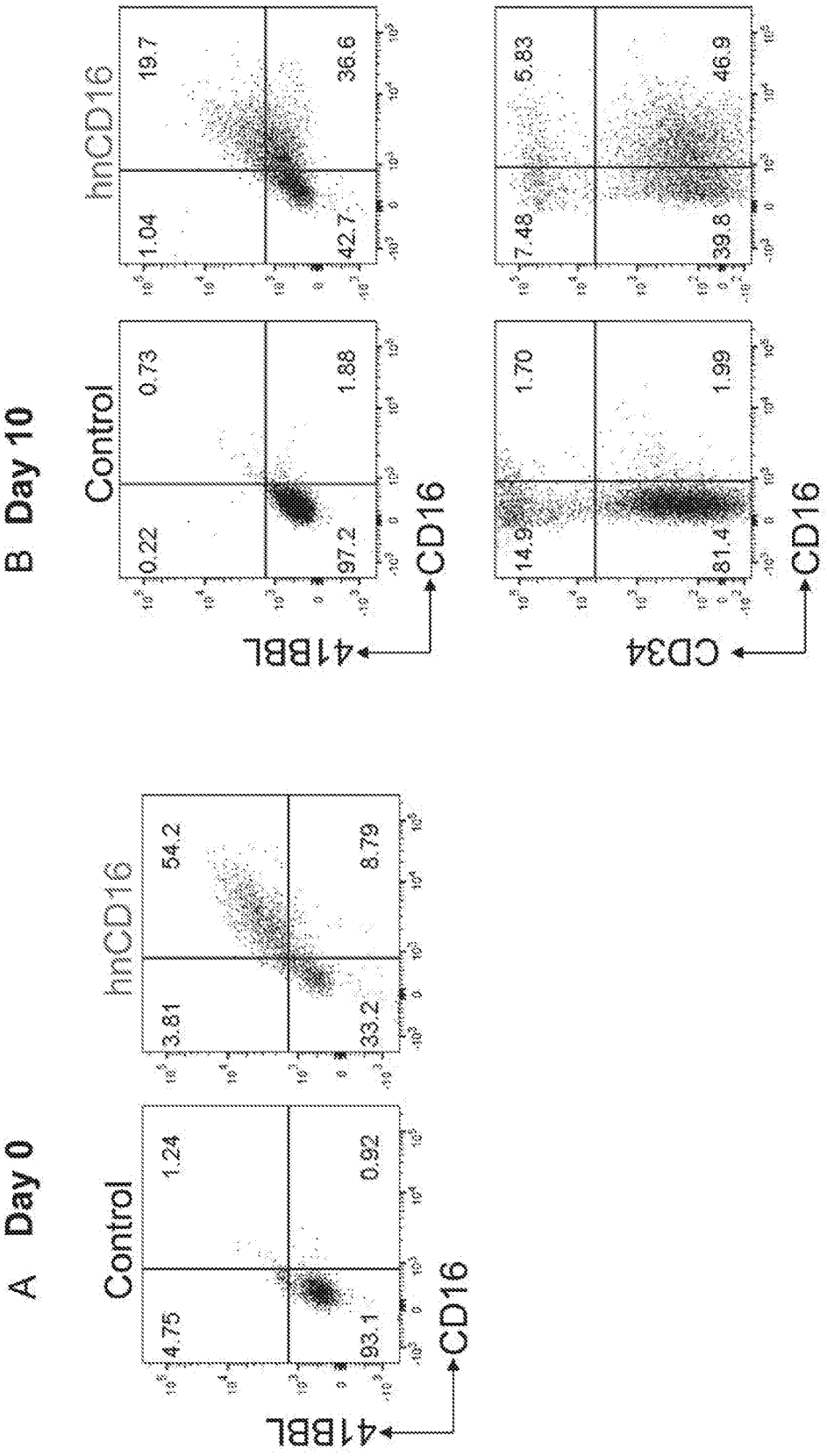

A Day 0
Control
4.75
1.24
93.1
0.92
4-1BBL
CD16
hnCD16
3.81
54.2
33.2
8.79
B Day 10
Control
0.22
0.73
97.2
1.88
4-1BBL
CD16
hnCD16
1.04
19.7
42.7
36.6
CD34
CD16
14.9
1.70
81.4
1.99
7.48
5.83
39.8
46.9

FIG. 17

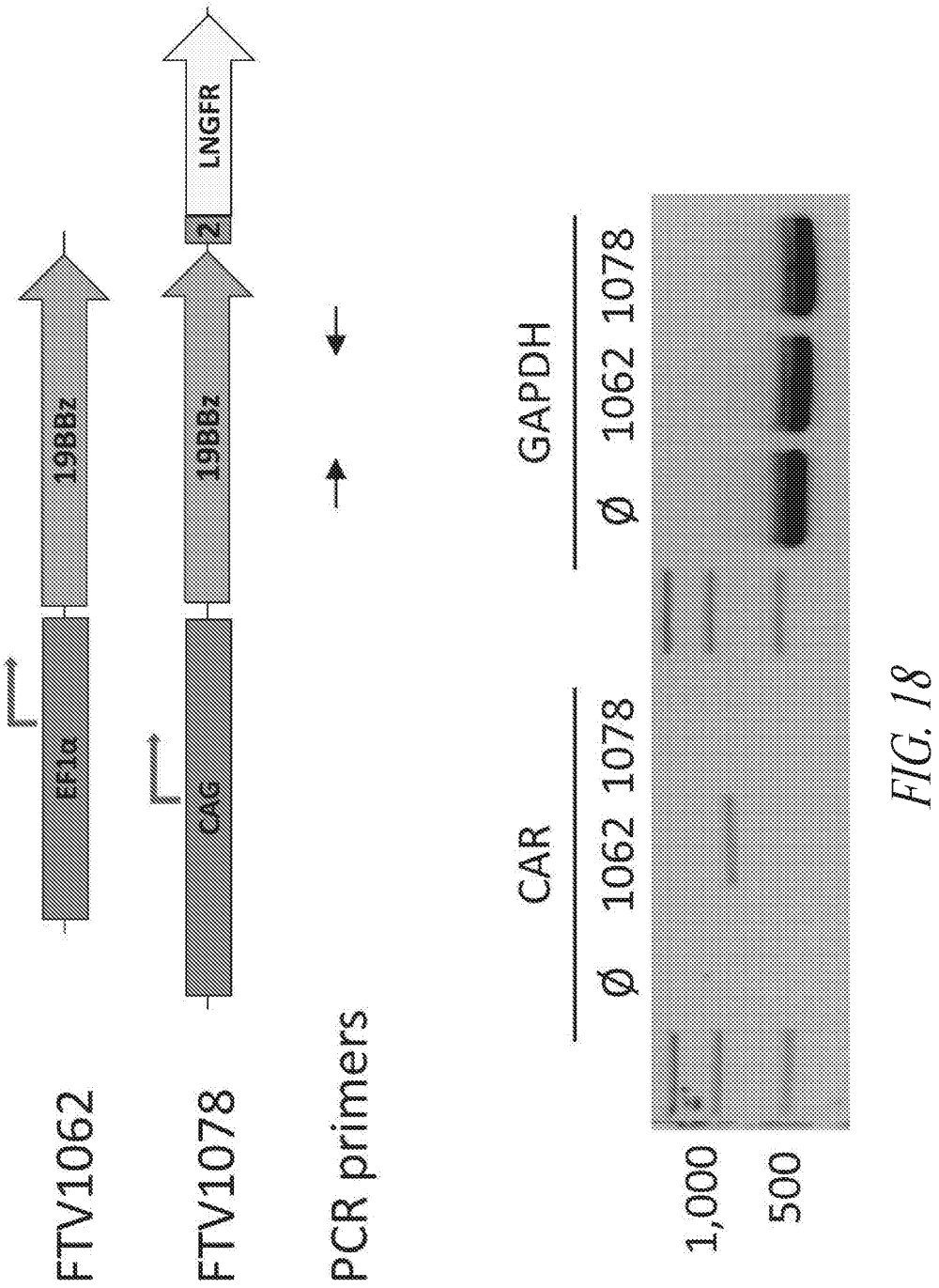

FTV1062
EF1α
19BBz
FTV1078
CAG
19BBz
2
LNGFR
PCR primers
CAR
Ø 1062 1078
GAPDH
Ø 1062 1078
1,000
500

FIG. 18

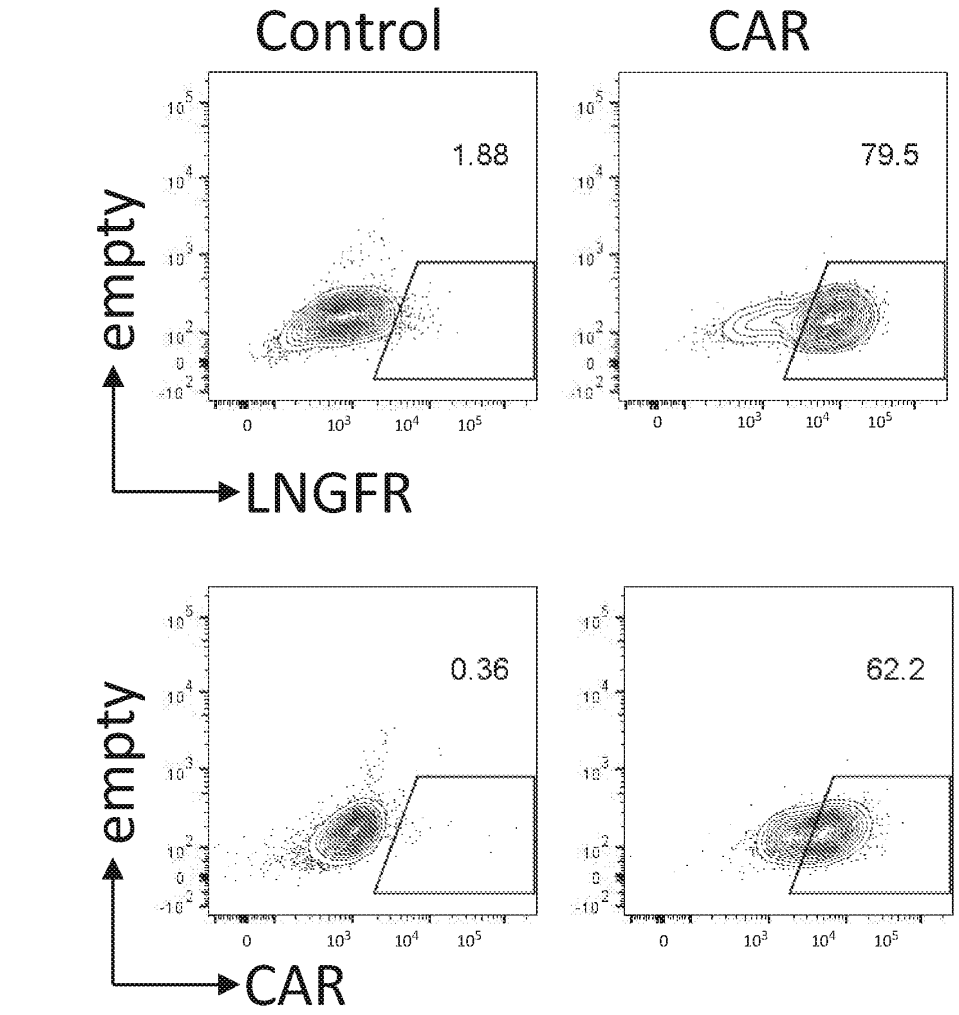


FIG. 19A

Day 10

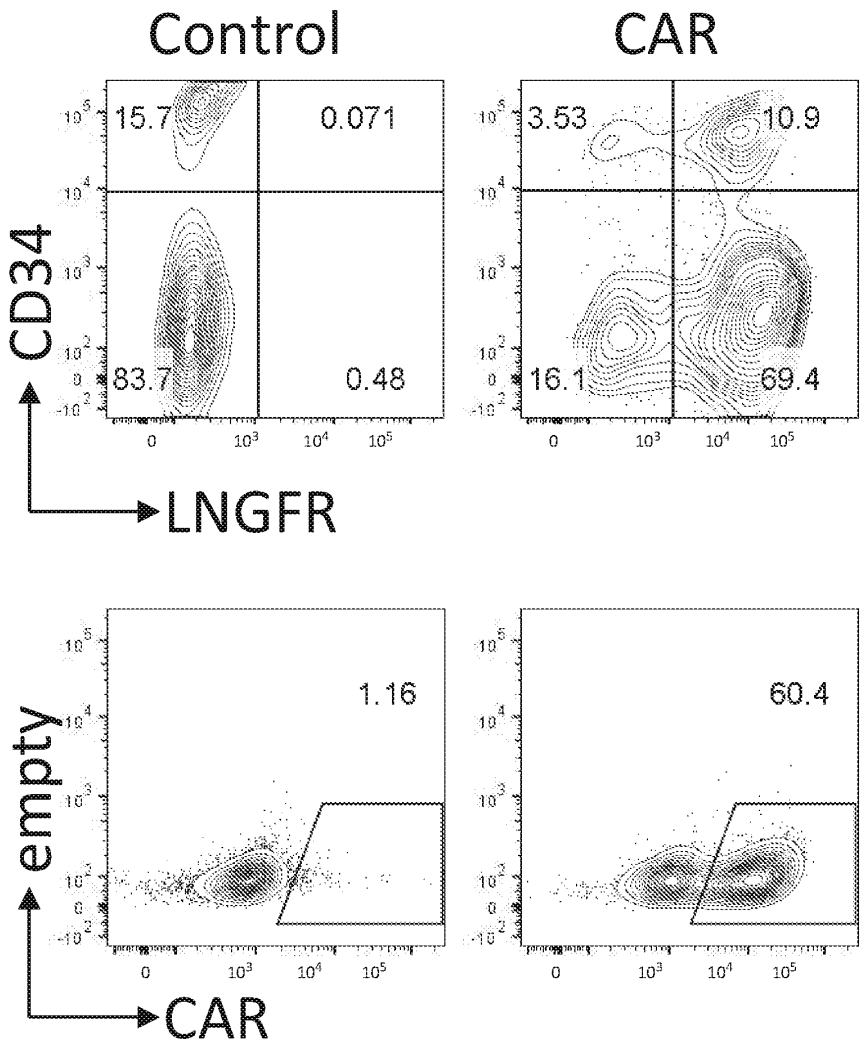


FIG. 19B

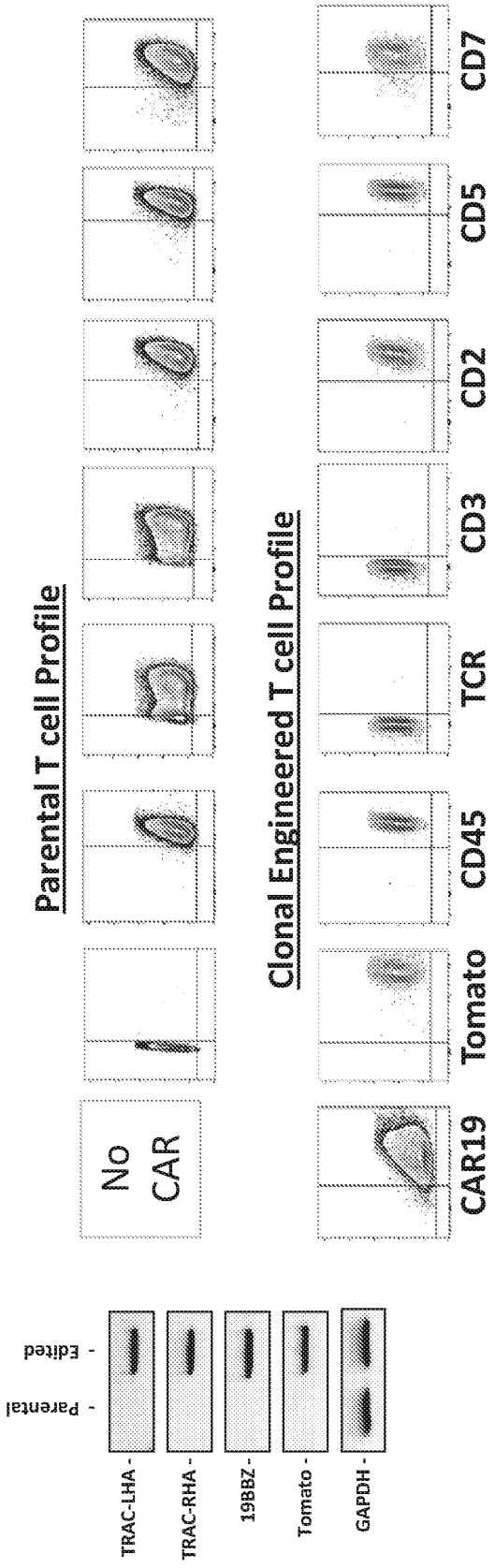

Parental T cell Profile
Clonal Engineered T cell Profile
No CAR
CAR19
Tomato
CD45
TCR
CD3
CD2
CD5
CD7
TRAC-LHA
TRAC-RHA
19BBZ
Tomato
GAPDH
Parental
Edited

FIG. 20

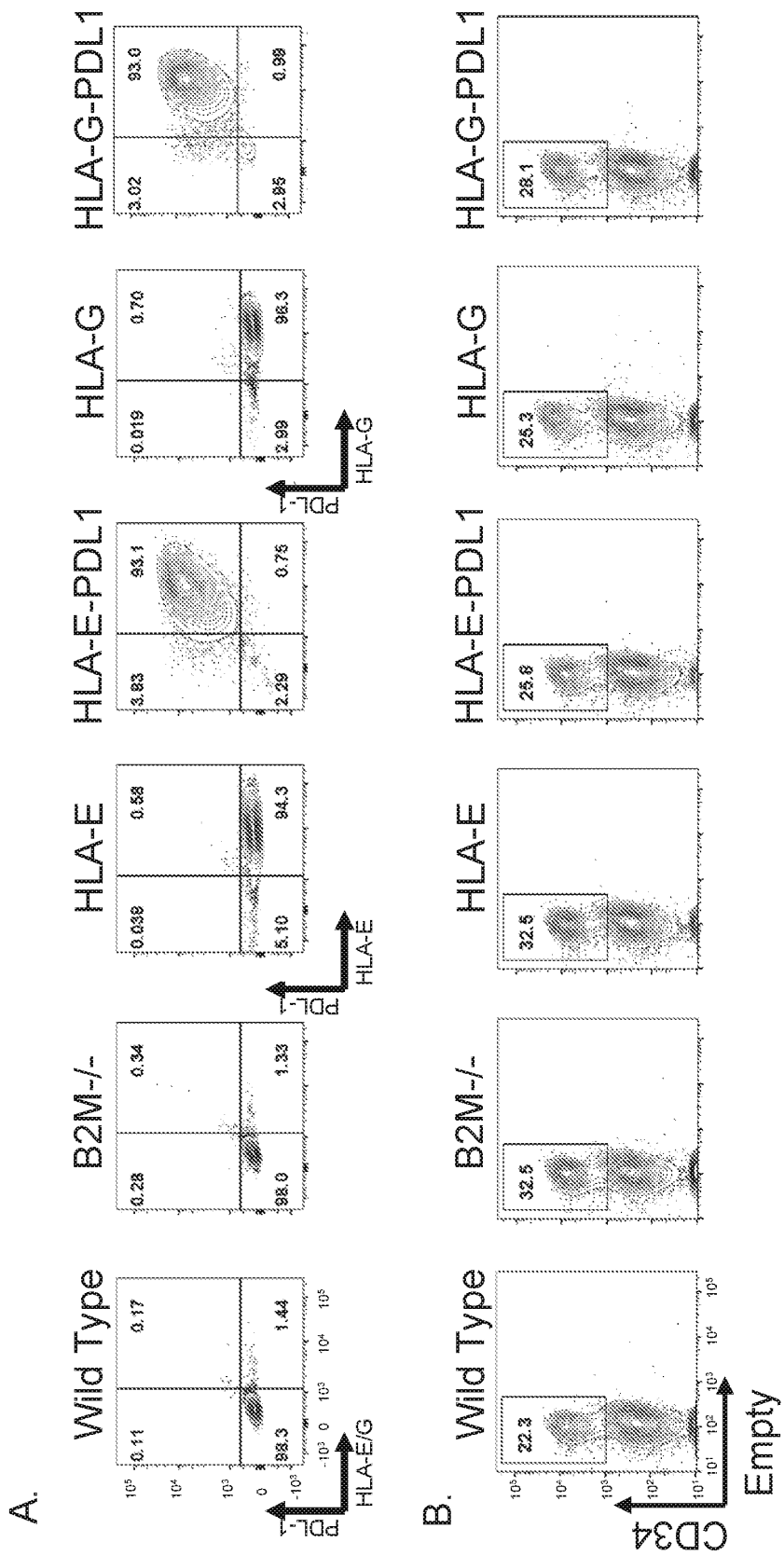

A.
Wild Type
B2M-/-
HLA-E
HLA-E-PDL1
HLA-G
HLA-G-PDL1
0.11
0.17
98.3
1.44
0.28
0.34
98.0
1.33
0.039
0.58
5.10
94.3
3.83
93.1
2.29
0.76
0.019
0.70
2.99
96.3
3.02
93.0
2.85
0.99
PDL-1
HLA-E/G
HLA-E
HLA-G
B.
Wild Type
B2M-/-
HLA-E
HLA-E-PDL1
HLA-G
HLA-G-PDL1
22.3
32.5
32.5
25.8
25.2
28.1
CD34
Empty

FIG. 21

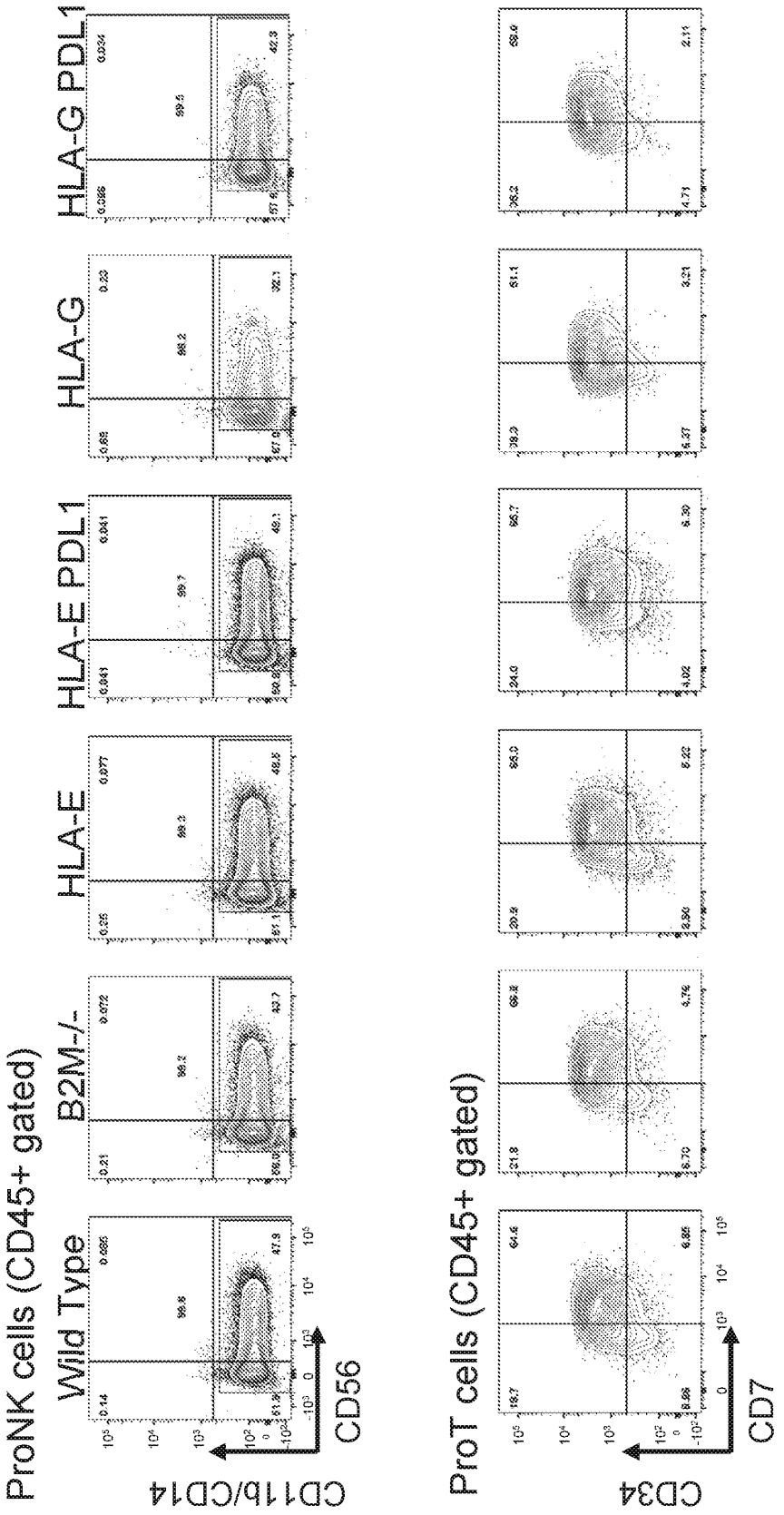

ProNK cells (CD45+ gated)
Wild Type
B2M-/-
HLA-E
HLA-E PDL1
HLA-G
HLA-G PDL1
CD11b/CD14
CD56
ProT cells (CD45+ gated)
CD34
CD7

FIG. 22

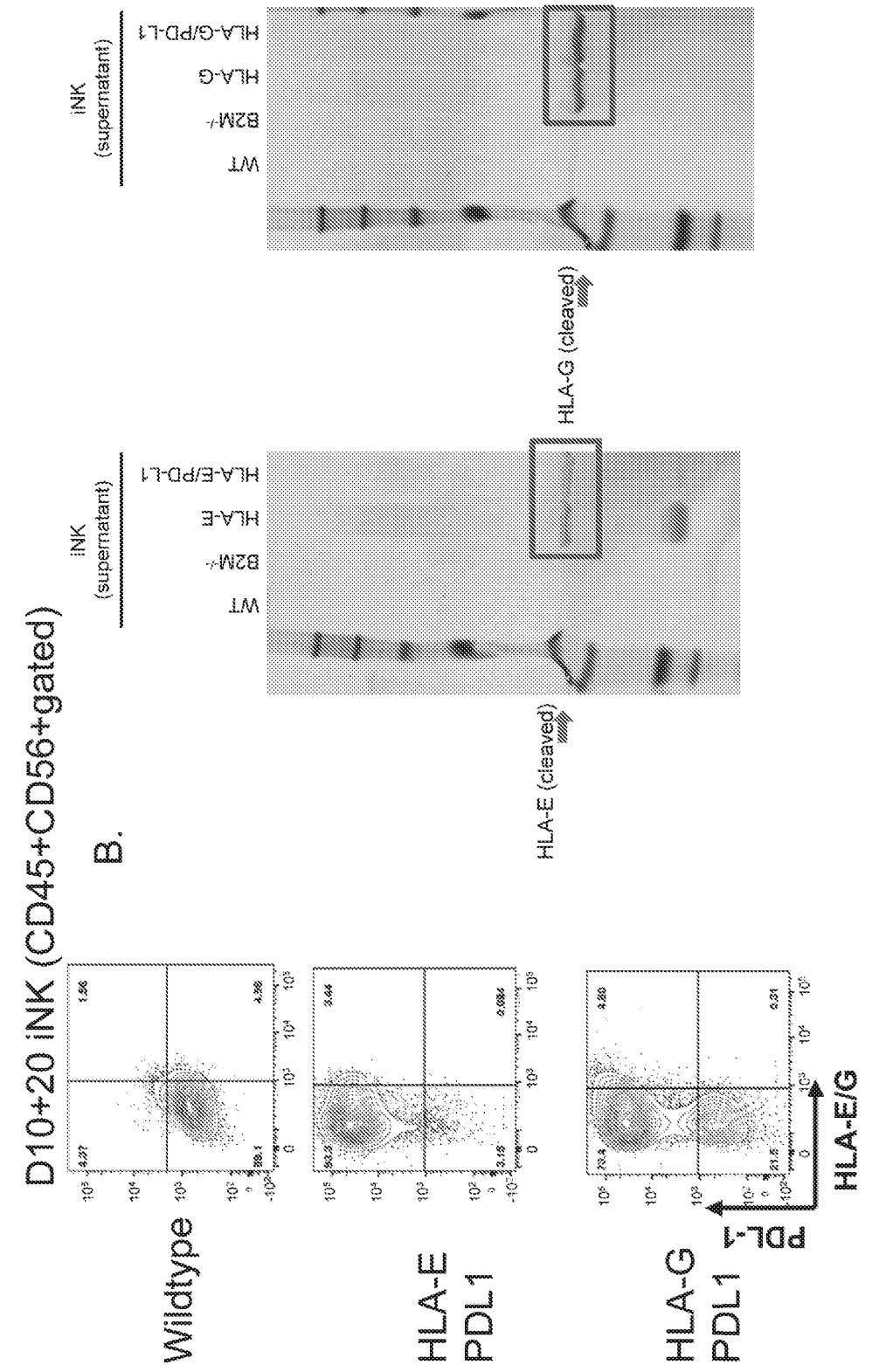
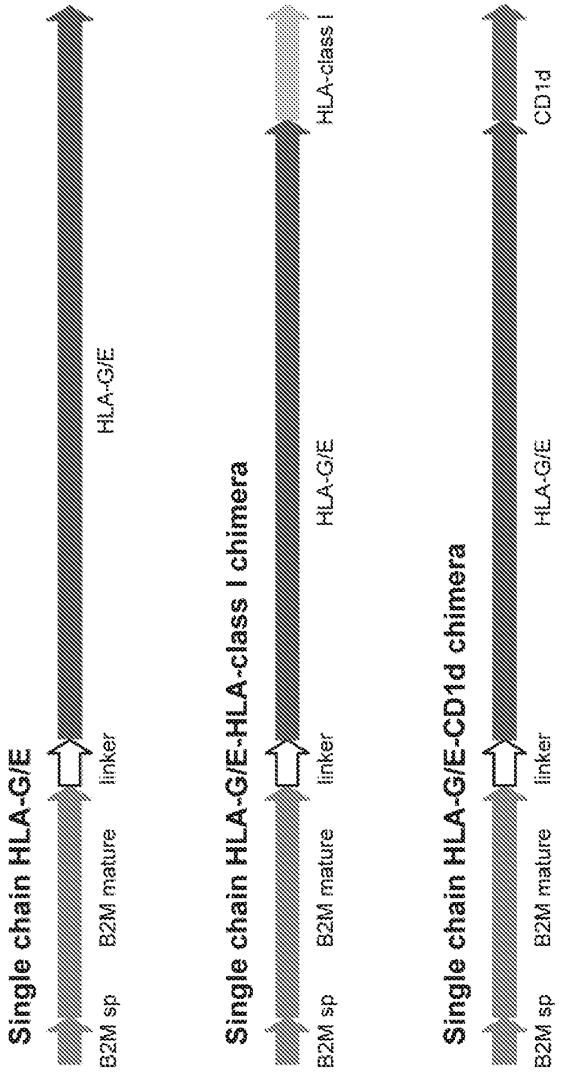

A.
D10+20 iNK (CD45+CD56+gated)
Wildtype
HLA-E PDL1
HLA-G PDL1
PDL-1
HLA-E/G
B.
iNK (supernatant)
WT
B2M-/-
HLA-E
HLA-E/PD-L1
HLA-E (cleaved)
HLA-G
HLA-G/PD-L1
HLA-G (cleaved)

FIG. 23


A.
Single chain HLA-G/E
B2M sp
B2M mature
linker
HLA-G/E
Single chain HLA-G/E-HLA-class I chimera
B2M sp
B2M mature
linker
HLA-G/E
HLA-class I
Single chain HLA-G/E-CD1d chimera
B2M sp
B2M mature
linker
HLA-G/E
CD1d

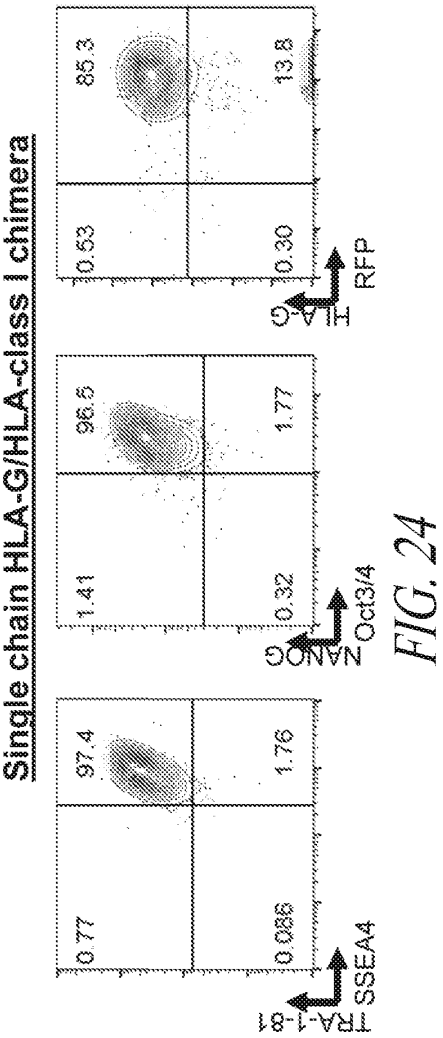

B.
Single chain HLA-G/HLA-class I chimera
0.77
97.4
0.086
1.76
1.41
96.6
0.32
1.77
0.53
85.3
0.30
13.8
TRA-1-81
SSEA4
NANOG
Oct3/4
HLA-G
RFP

FIG. 24

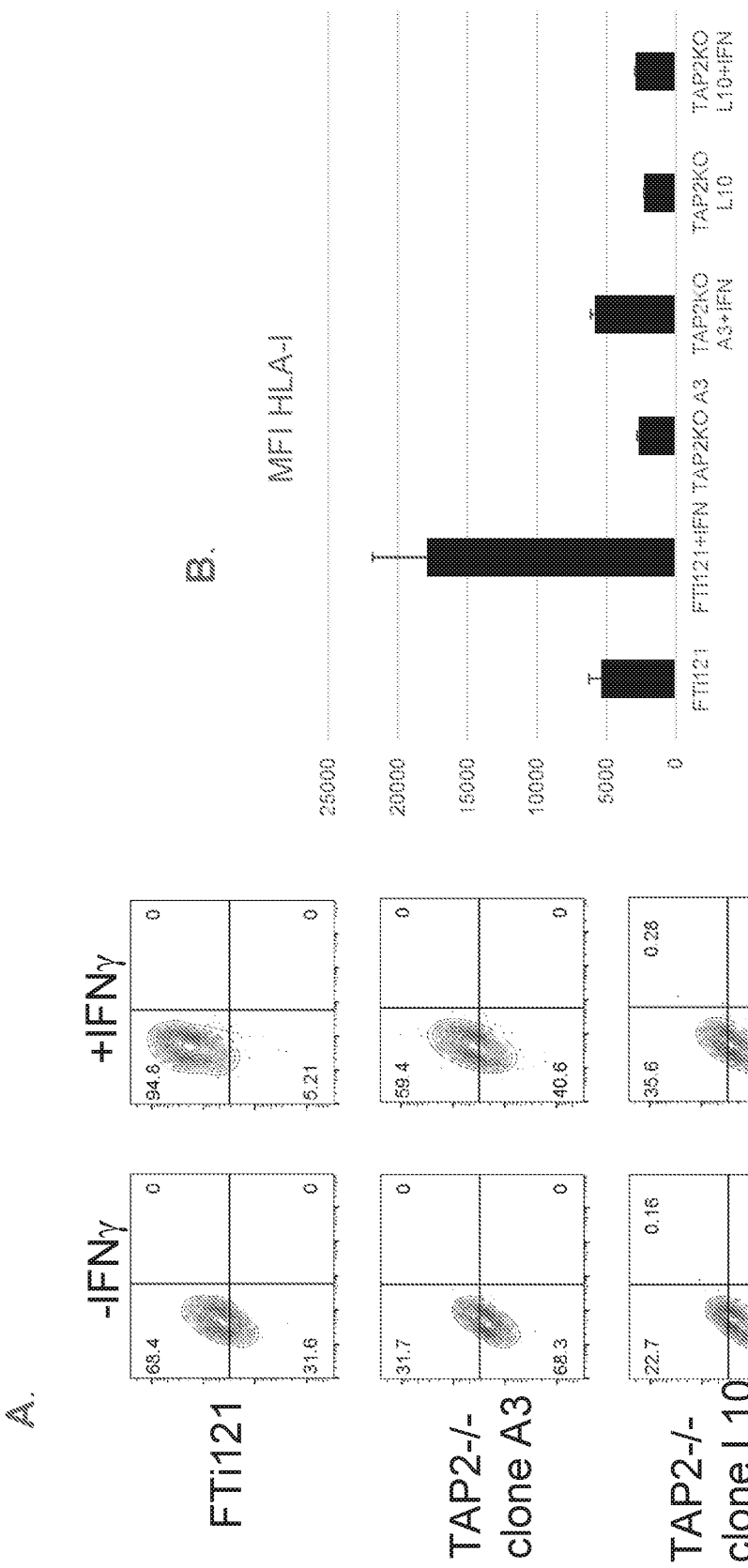

A.
-IFNγ
+IFNγ
FTi121
TAP2-/- clone A3
TAP2-/- clone L10
68.4
31.6
0
0
94.8
5.21
31.7
68.3
59.4
40.6
22.7
77.2
0.16
35.6
64.1
0.28
HLA-I
GFP
(gated on B2Mnull, not shown)
B.
MFI HLA-I
25000
20000
15000
10000
5000
0
FTi121
FTi121+IFN
TAP2KO A3
TAP2KO A3+IFN
TAP2KO L10
TAP2KO L10+IFN

FIG. 25

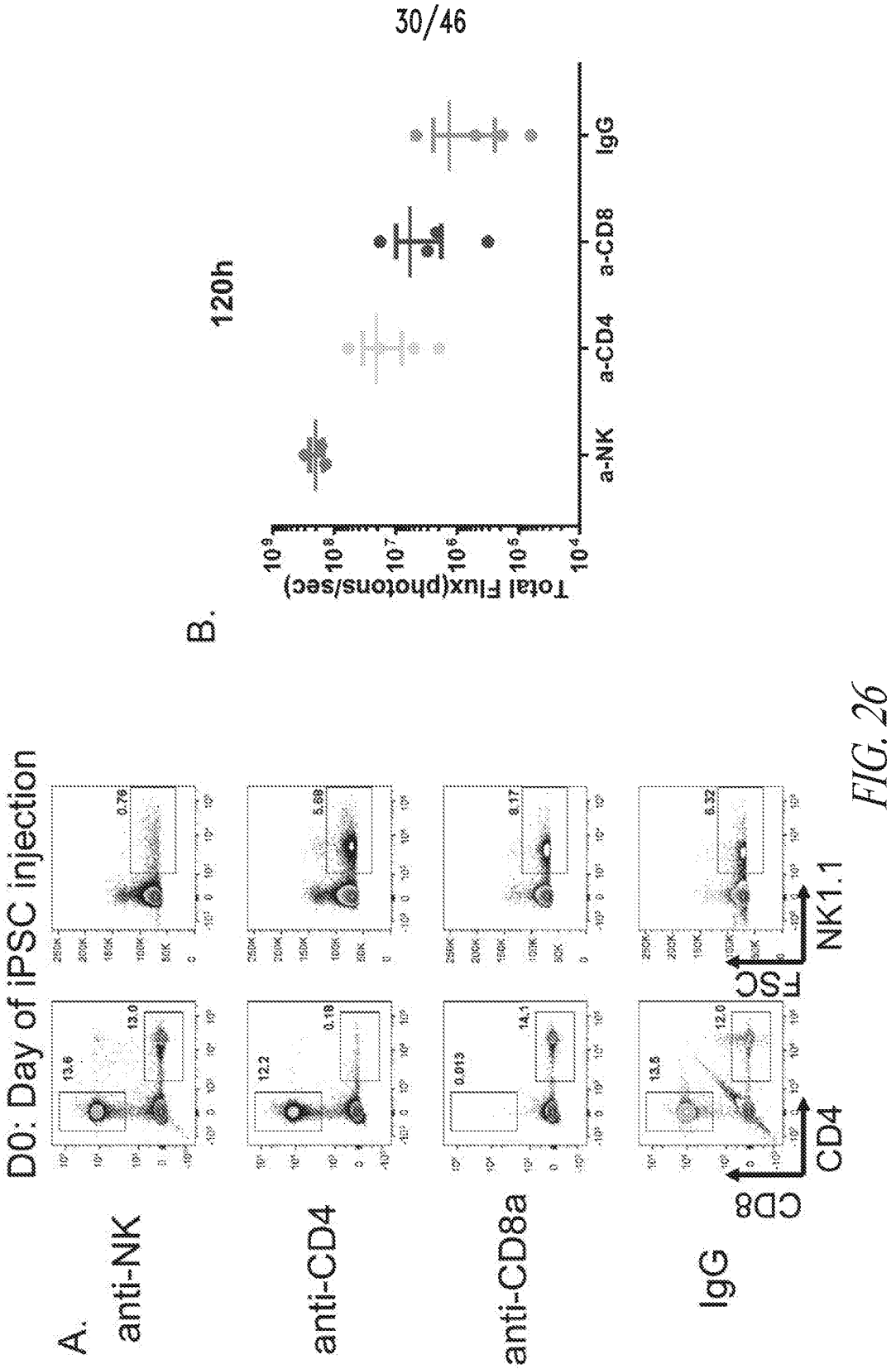

A.
D0: Day of iPSC injection
anti-NK
anti-CD4
anti-CD8a
IgG
CD8
CD4
FSC
NK1.1
13.6
13.0
0.76
12.2
0.18
5.88
0.013
14.1
8.17
13.8
12.0
8.32
B.
120h
Total Flux(photons/sec)
a-NK
a-CD4
a-CD8
IgG

FIG. 26

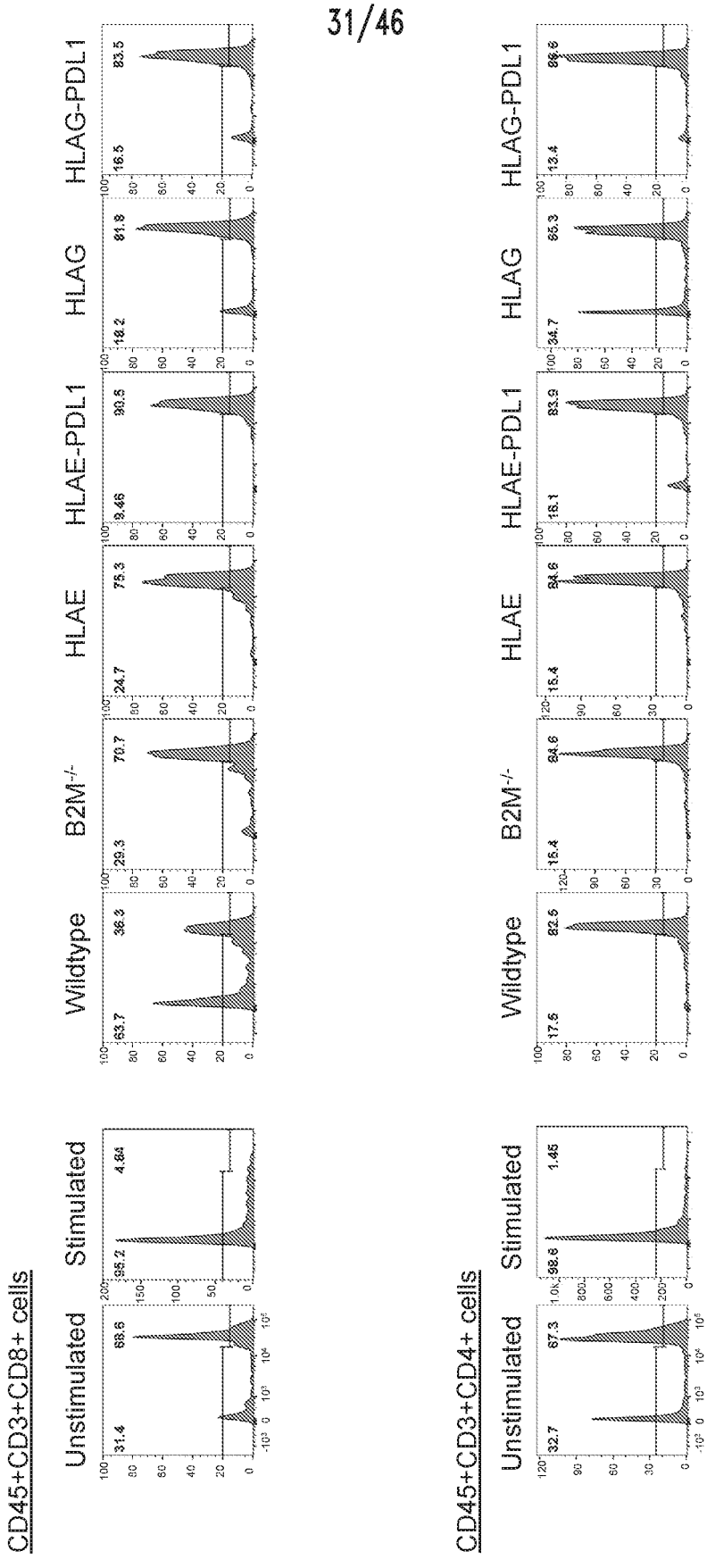

CD45+CD3+CD8+ cells
Unstimulated
Stimulated
Wildtype
B2M-/-
HLAE
HLAE-PDL1
HLAG
HLAG-PDL1
31.4
68.6
95.2
4.84
63.7
36.3
29.3
70.7
24.7
75.3
8.46
90.5
18.2
81.8
16.5
83.5
CD45+CD3+CD4+ cells
32.7
67.3
98.6
1.45
17.6
82.5
15.4
84.6
15.4
84.6
18.1
83.9
34.7
65.3
13.4
86.6
CellTraceV

FIG. 27A

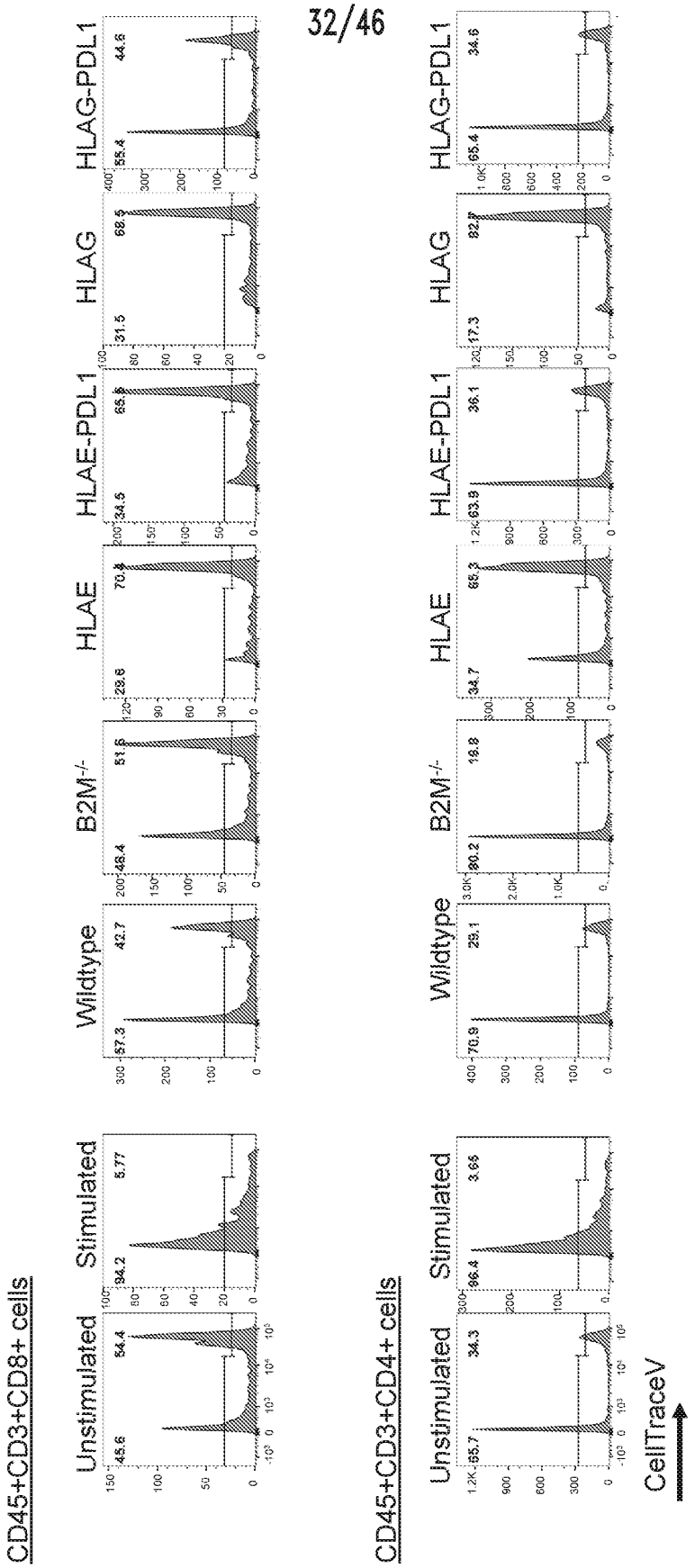

CD45+CD3+CD8+ cells
CD45+CD3+CD4+ cells
Unstimulated
Stimulated
Wildtype
B2M-/-
HLAE
HLAE-PDL1
HLAG
HLAG-PDL1
CellTraceV

FIG. 27B

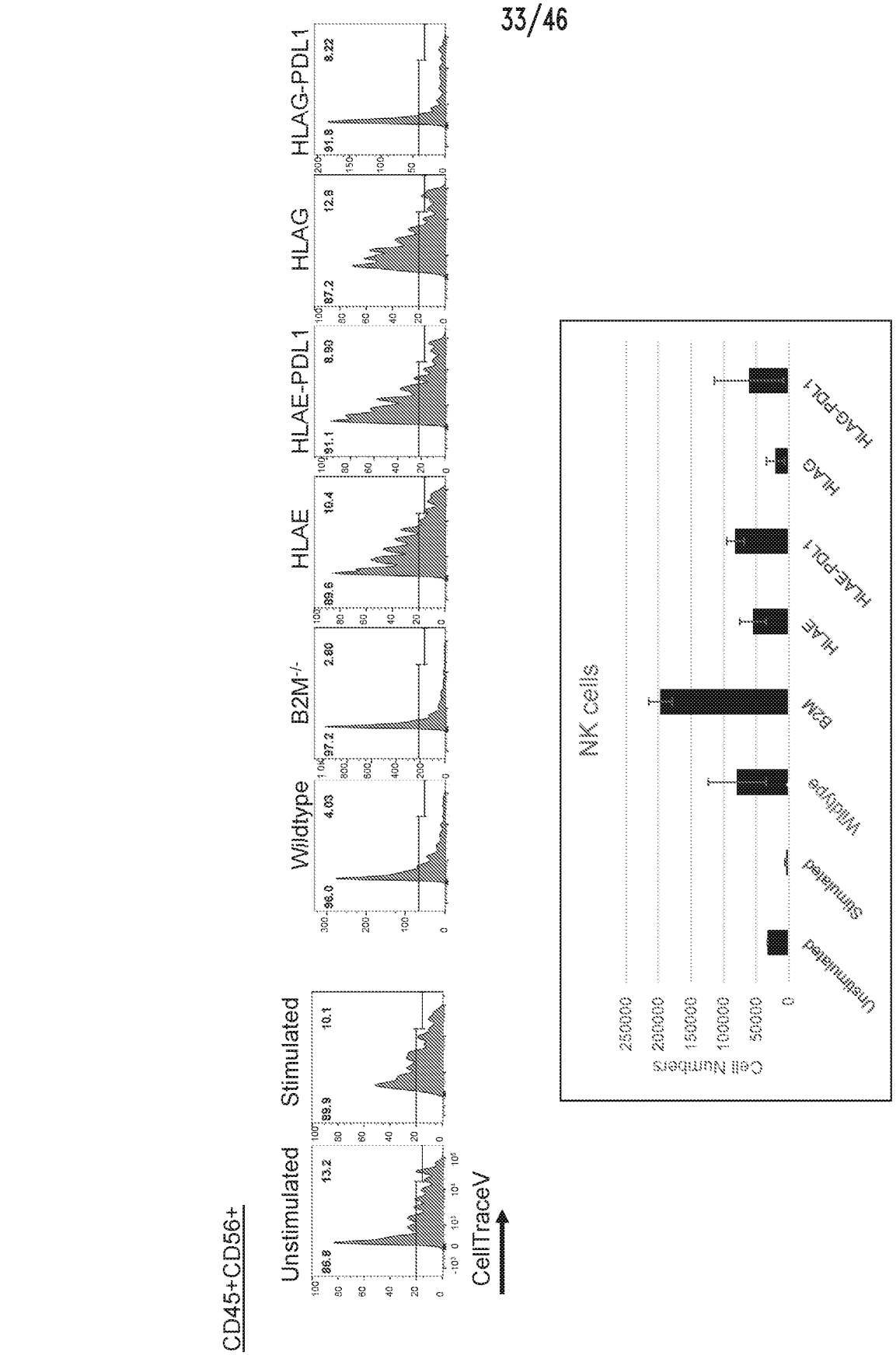

CD45+CD56+
Unstimulated
Stimulated
CellTraceV
Wildtype
B2M-/-
HLAE
HLAE-PDL1
HLAG
HLAG-PDL1
86.8
13.2
99.9
0.1
96.0
4.03
97.2
2.80
99.6
10.4
91.1
8.93
87.2
12.8
91.8
8.22
NK cells
Cell Numbers
250000
200000
150000
100000
50000
0
Unstimulated
Stimulated
Wildtype
B2M
HLAE
HLAE-PDL1
HLAG
HLAG-PDL1

FIG. 27C

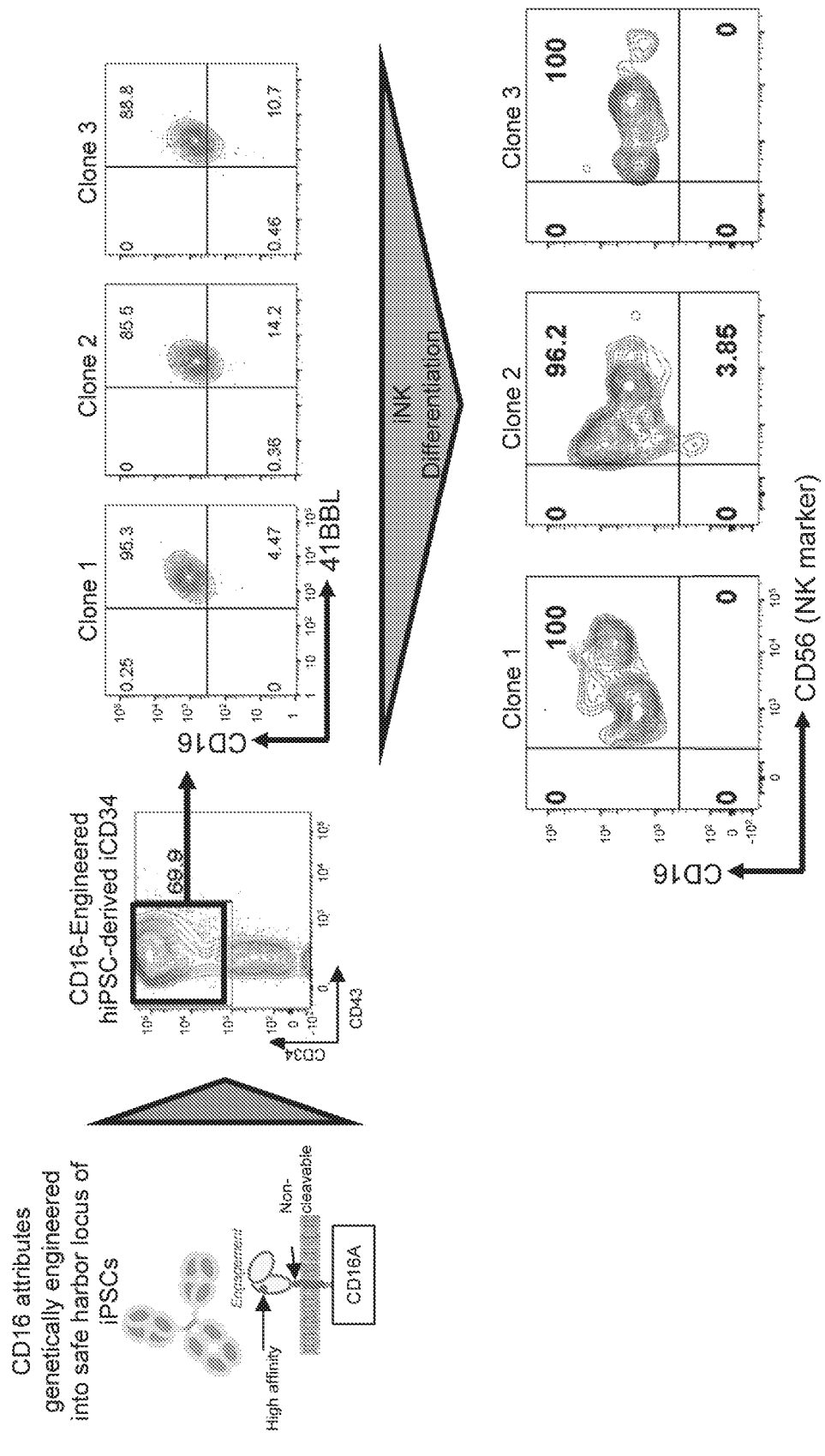

CD16 attributes genetically engineered into safe harbor locus of iPSCs
High affinity
Engagement
Non-cleavable
CD16A
CD16-Engineered hiPSC-derived iCD34
69.9
CD34
CD43
Clone 1
0.25
95.3
0
4.47
Clone 2
0
85.5
0.38
14.2
Clone 3
0
89.8
0.46
10.7
CD16
41BBL
iNK Differentiation
Clone 1
100
0
0
Clone 2
96.2
0
3.85
Clone 3
100
0
0
CD16
CD56 (NK marker)

FIG. 28A

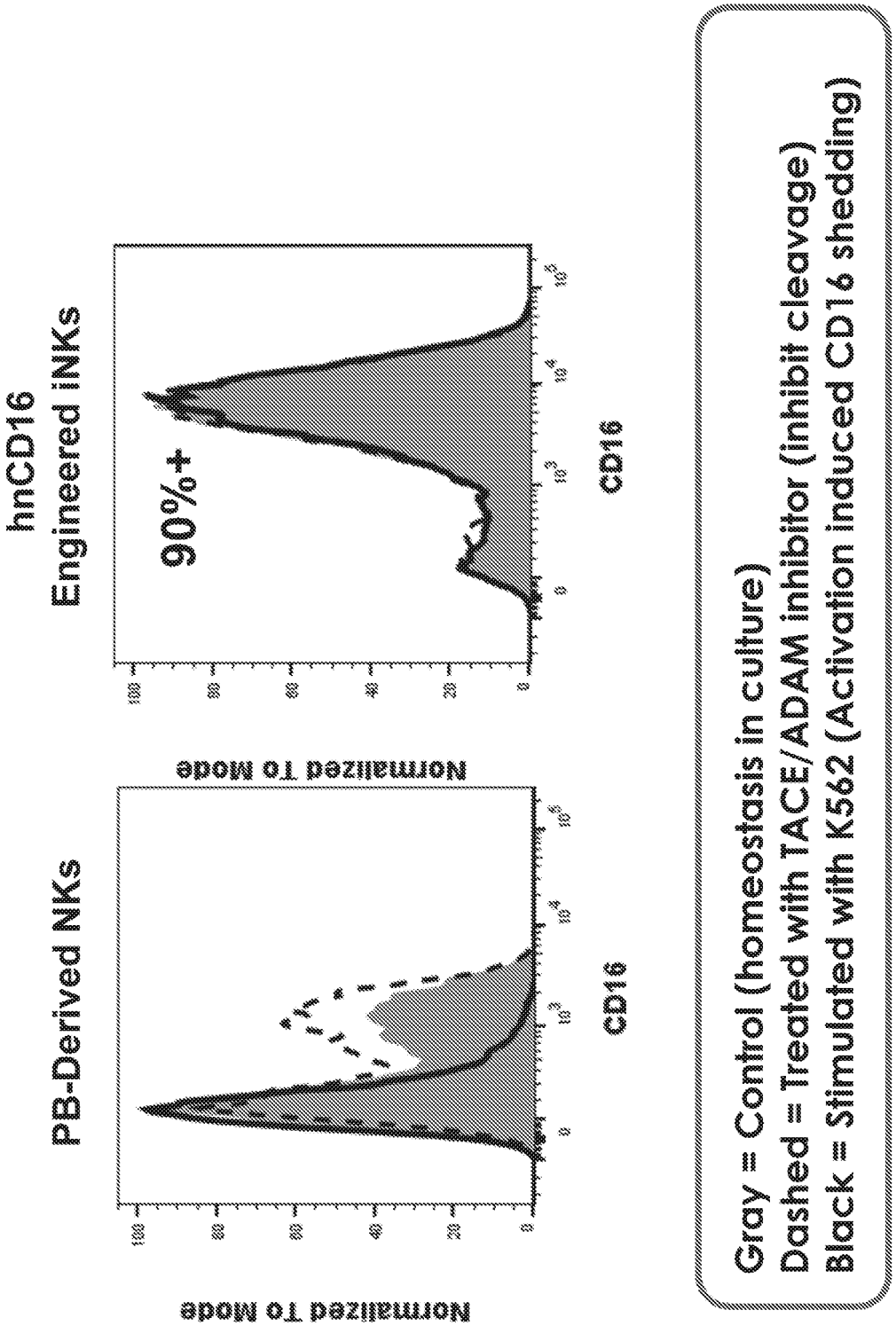

hnCD16
Engineered iNKs
90%+
CD16
Normalized To Mode
PB-Derived NKs
CD16
Normalized To Mode
Gray = Control (homeostasis in culture)
Dashed = Treated with TACE/ADAM inhibitor (inhibit cleavage)
Black = Stimulated with K562 (Activation induced CD16 shedding)

FIG. 28B

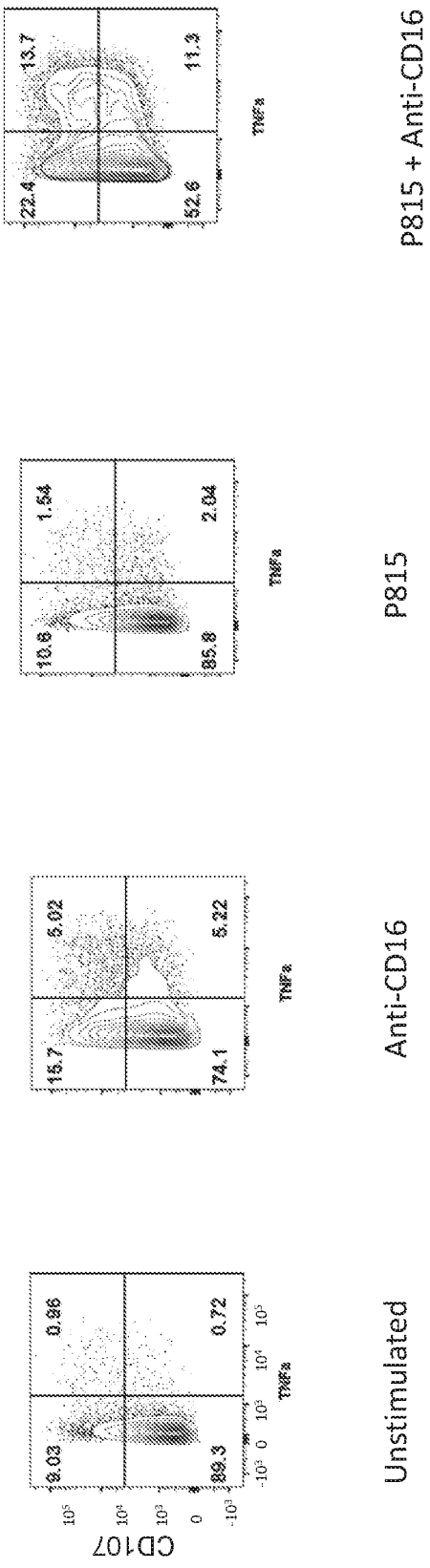

CD107
TNFa
0.03
0.96
89.3
0.72
Unstimulated
15.7
5.02
74.1
5.22
Anti-CD16
10.8
1.54
85.8
2.04
P815
22.4
13.7
52.6
11.3
P815 + Anti-CD16

FIG. 29A

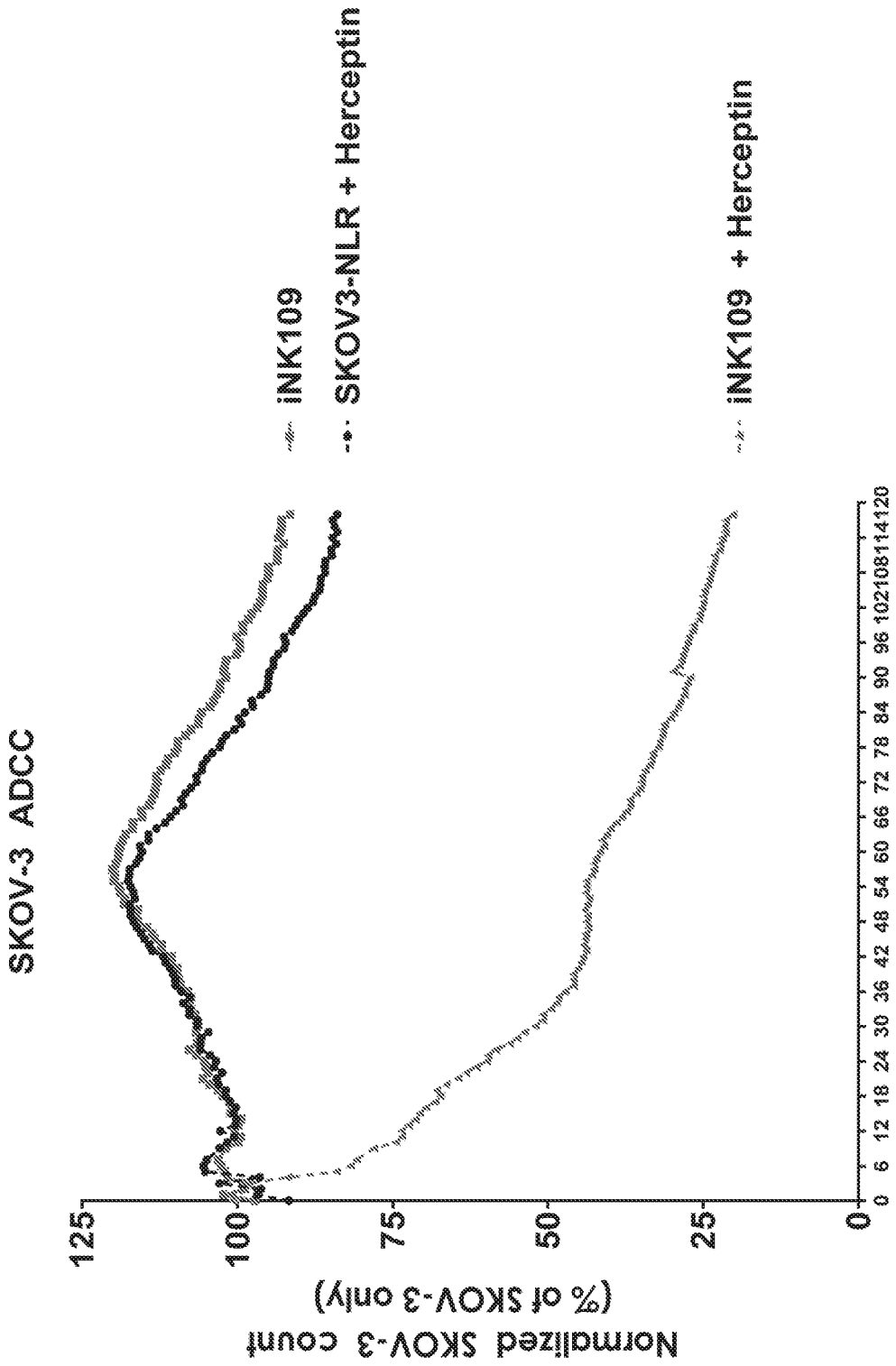

SKOV-3 ADCC
iNK109
SKOV3-NLR + Herceptin
iNK109 + Herceptin
Normalized SKOV-3 count
(% of SKOV-3 only)
125
100
75
50
25
0
0 6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96 102108114120
Time, hrs

FIG. 29B

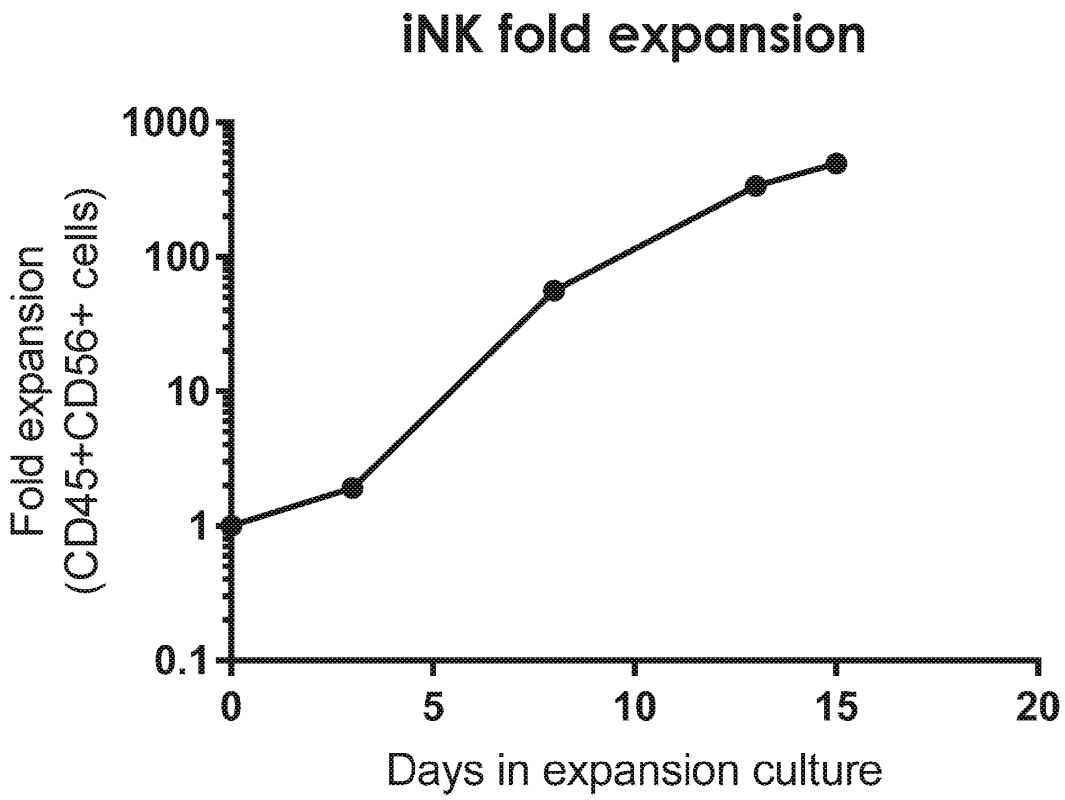

iNK fold expansion
Fold expansion
(CD45+CD56+ cells)
1000
100
10
1
0.1
0
5
10
15
20
Days in expansion culture

FIG. 30

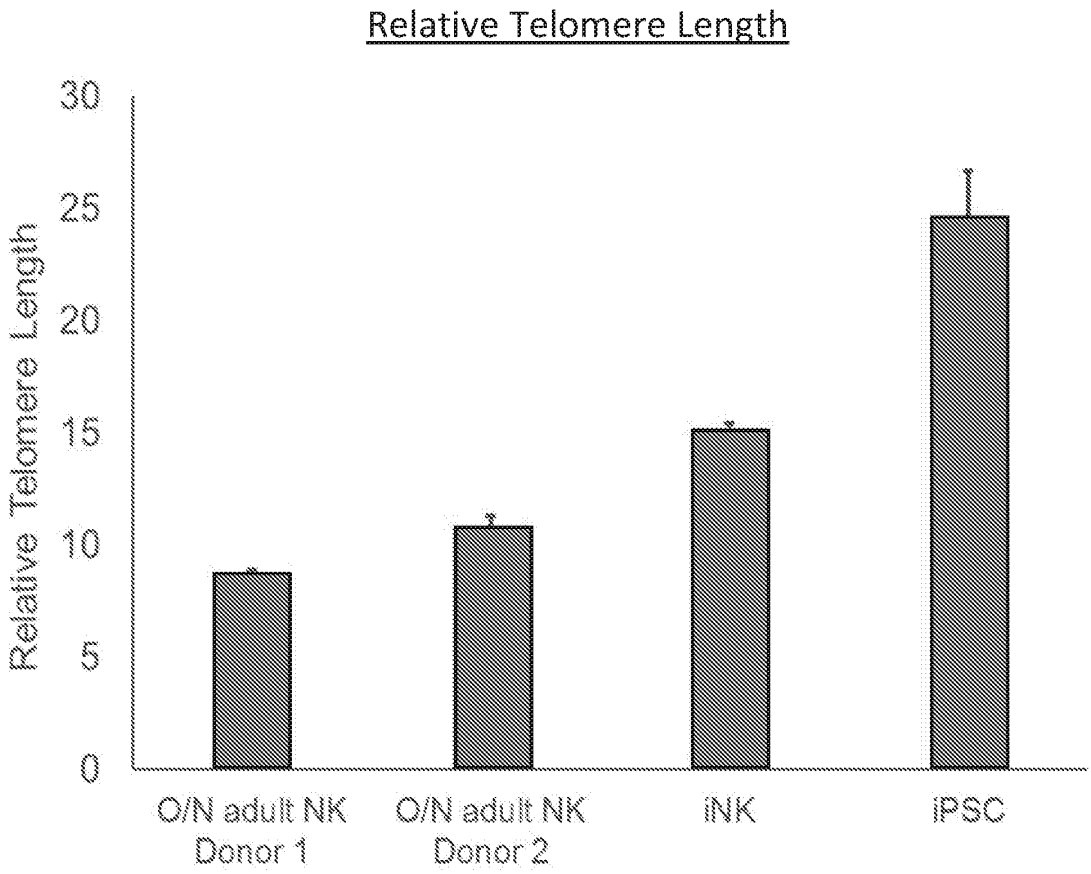

Relative Telomere Length
Relative Telomere Length
30
25
20
15
10
5
0
O/N adult NK Donor 1
O/N adult NK Donor 2
iNK
iPSC

FIG. 31

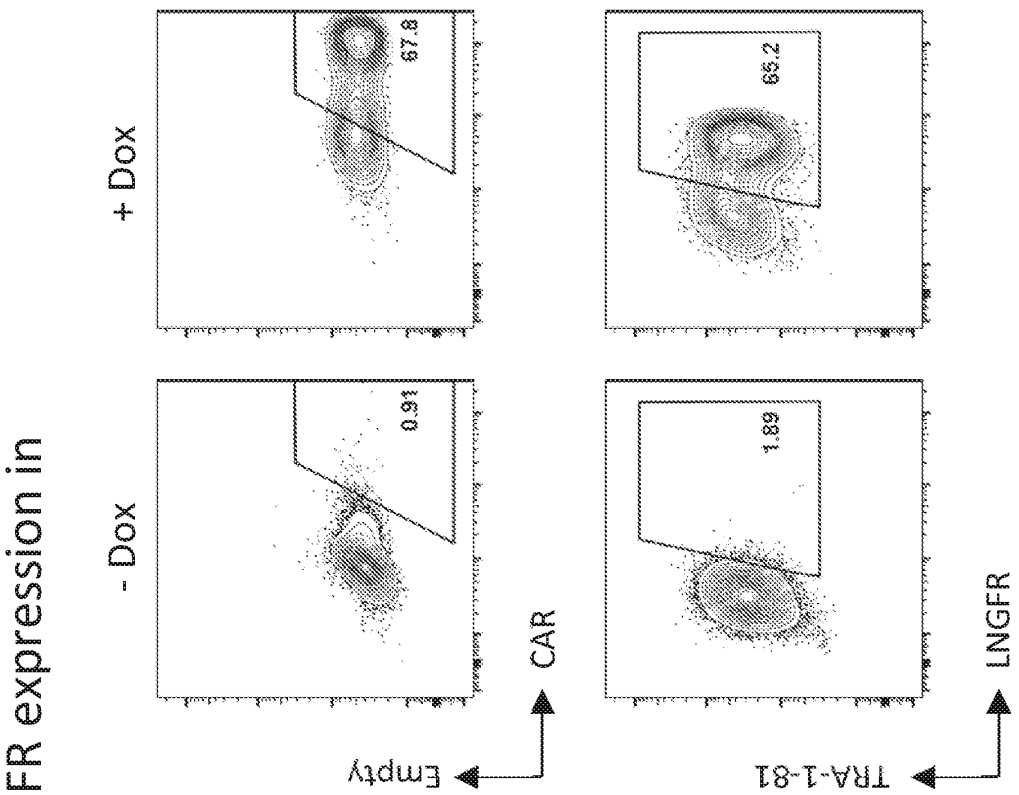

Tet-inducible CAR-2A-LNGFR expression in transduced iPSCs
- Dox
+ Dox
0.91
67.8
1.89
66.2
Empty
CAR
TRA-1-81
LNGFR

FIG. 32A

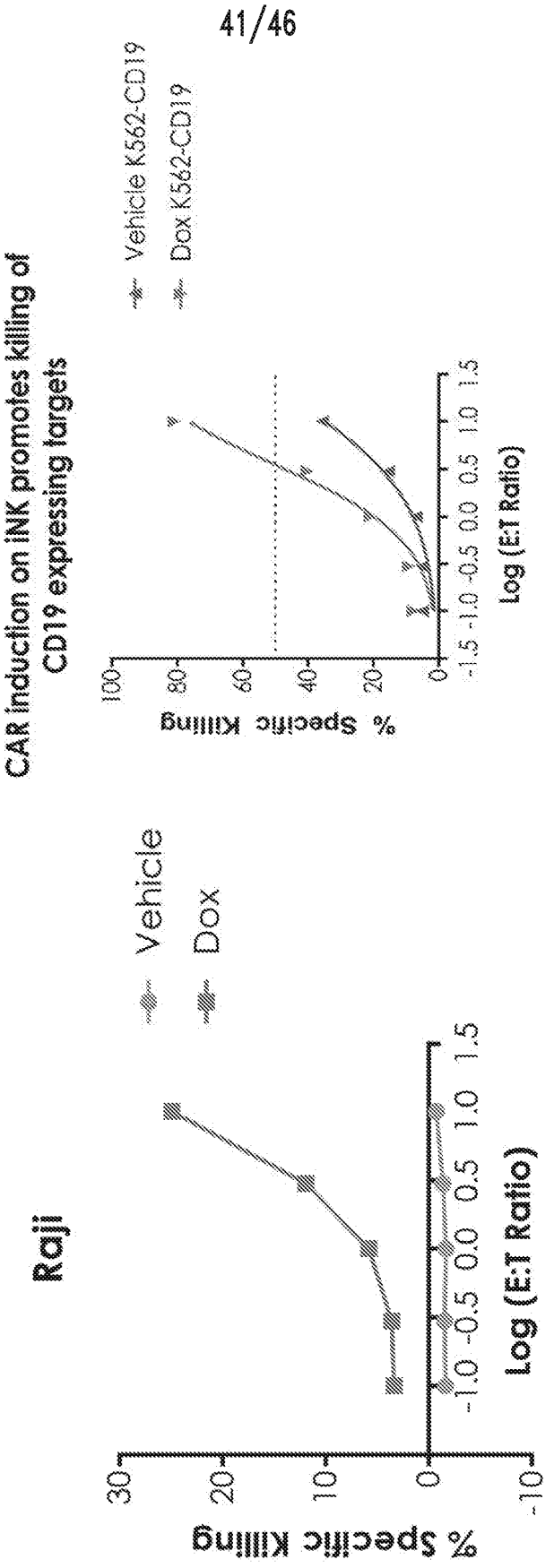

CAR induction on iNK promotes killing of CD19 expressing targets
Vehicle K562-CD19
Dox K562-CD19
% Specific Killing
Log (E:T Ratio)
Raji
Vehicle
Dox
% Specific Killing
Log (E:T Ratio)

FIG. 32B

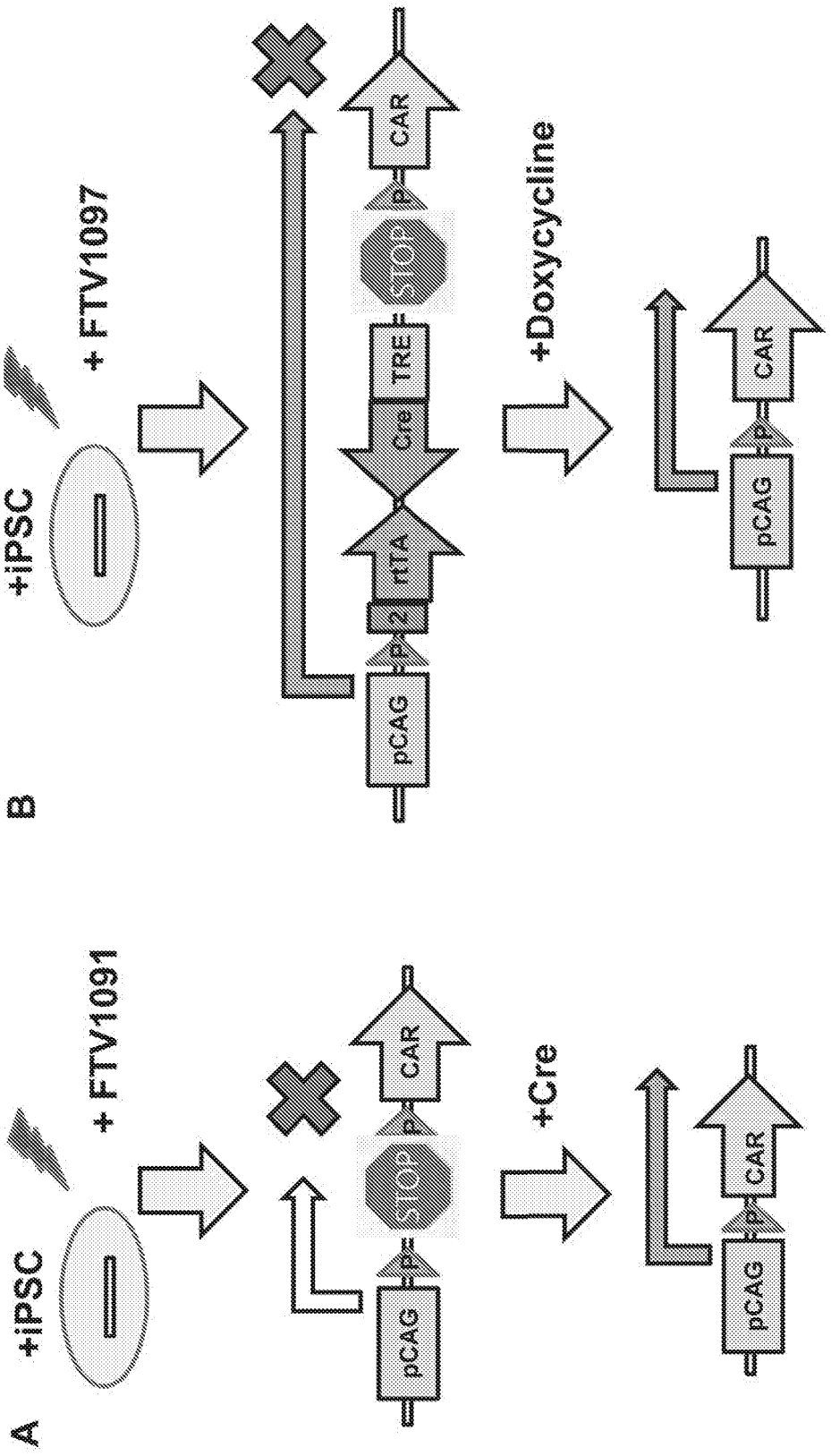

A
+iPSC
+ FTV1091
pCAG
STOP
CAR
+Cre
B
+iPSC
+ FTV1097
pCAG
rtTA
TRE
Cre
STOP
CAR
+Doxycycline

FIG. 33

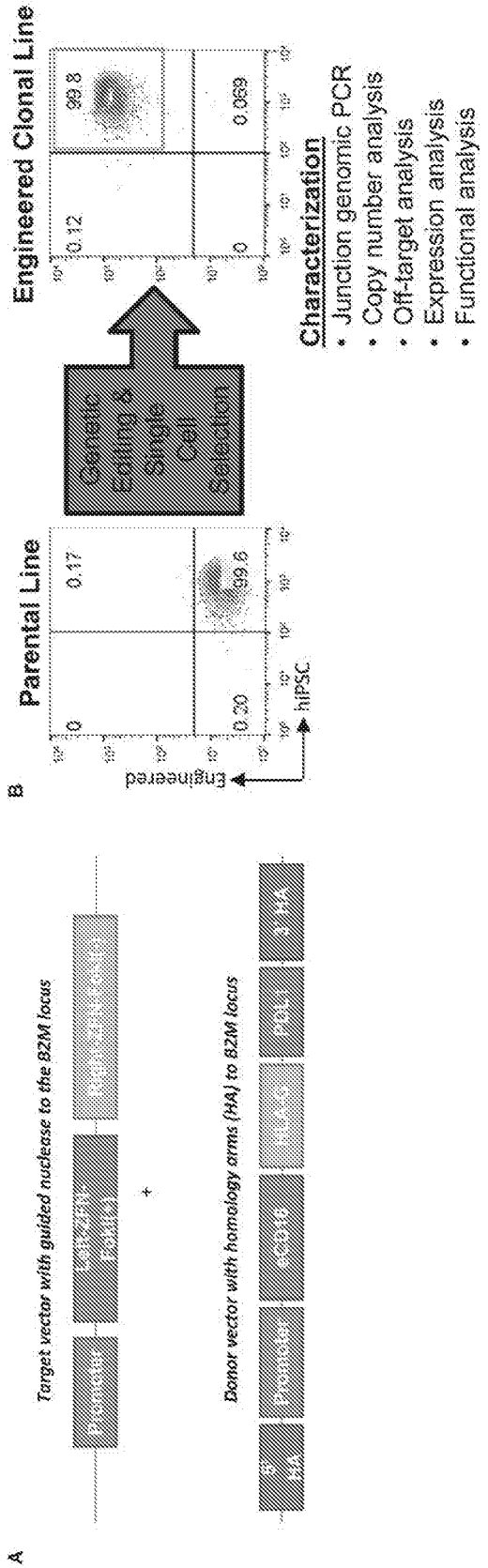

A
Target vector with guided nuclease to the B2M locus
Donor vector with homology arms (HA) to B2M locus
B
Parental Line
Engineered
hiPSC
0
0.17
0.20
99.6
Genetic Editing & Single Cell Selection
Engineered Clonal Line
0.12
99.8
0
0.069
Characterization
• Junction genomic PCR
• Copy number analysis
• Off-target analysis
• Expression analysis
• Functional analysis

FIG. 34

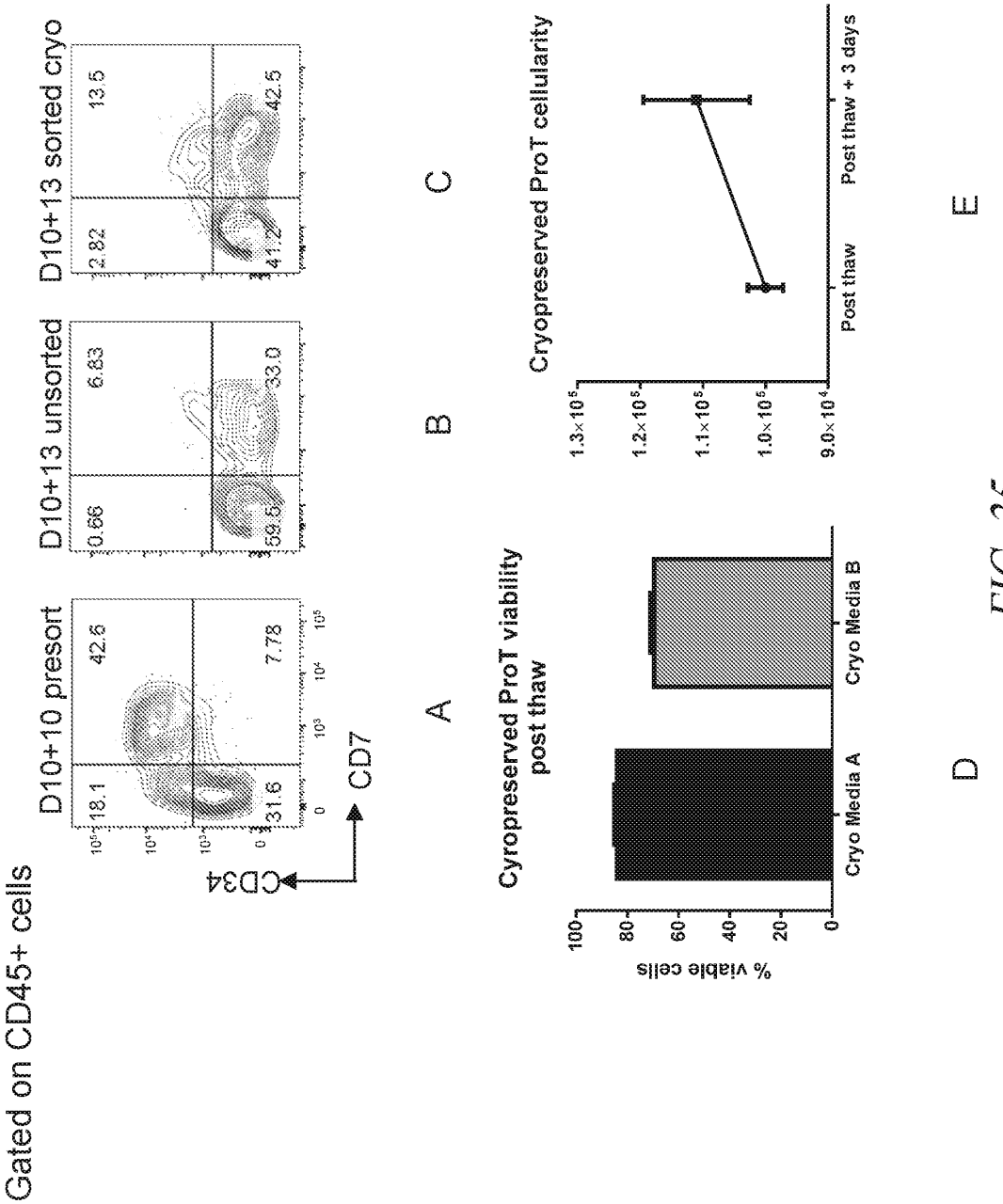

Gated on CD45+ cells
D10+10 presort
D10+13 unsorted
D10+13 sorted cryo
18.1
42.6
31.6
7.78
0.66
6.83
59.6
33.0
2.82
13.5
41.2
42.5
CD34
CD7
A
B
C
Cyropreserved ProT viability post thaw
% viable cells
Cryo Media A
Cryo Media B
Cryopreserved ProT cellularity
Post thaw
Post thaw + 3 days
D
E

FIG. 35

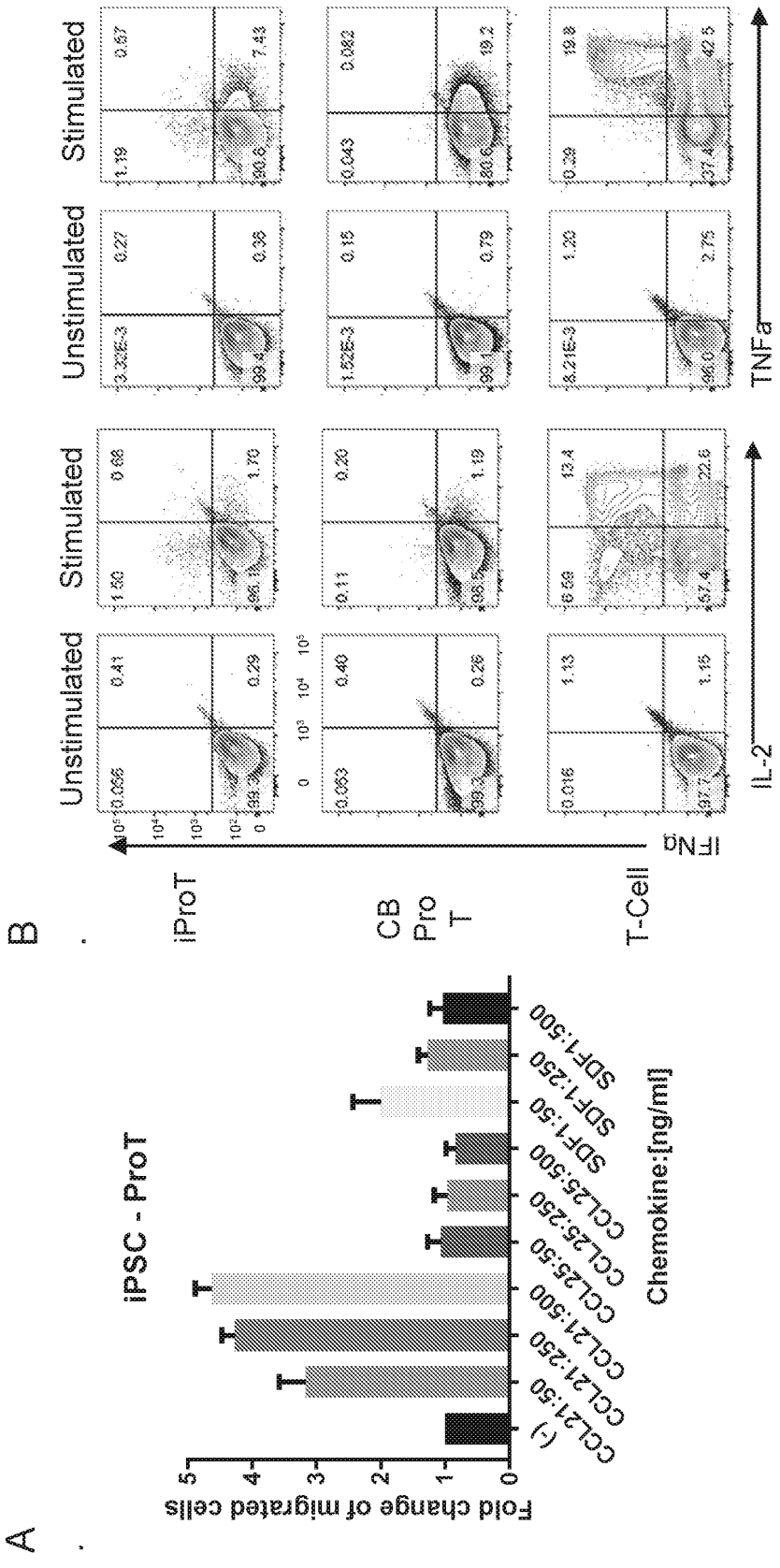

A
iPSC - ProT
Fold change of migrated cells
Chemokine:[ng/ml]
(-)
CCL21:50
CCL21:250
CCL21:500
CCL25:50
CCL25:250
CCL25:500
SDF1:50
SDF1:250
SDF1:500
B
iProT
CB Pro T
T-Cell
Unstimulated
Stimulated
IFNg
IL-2
TNFa

FIG. 36

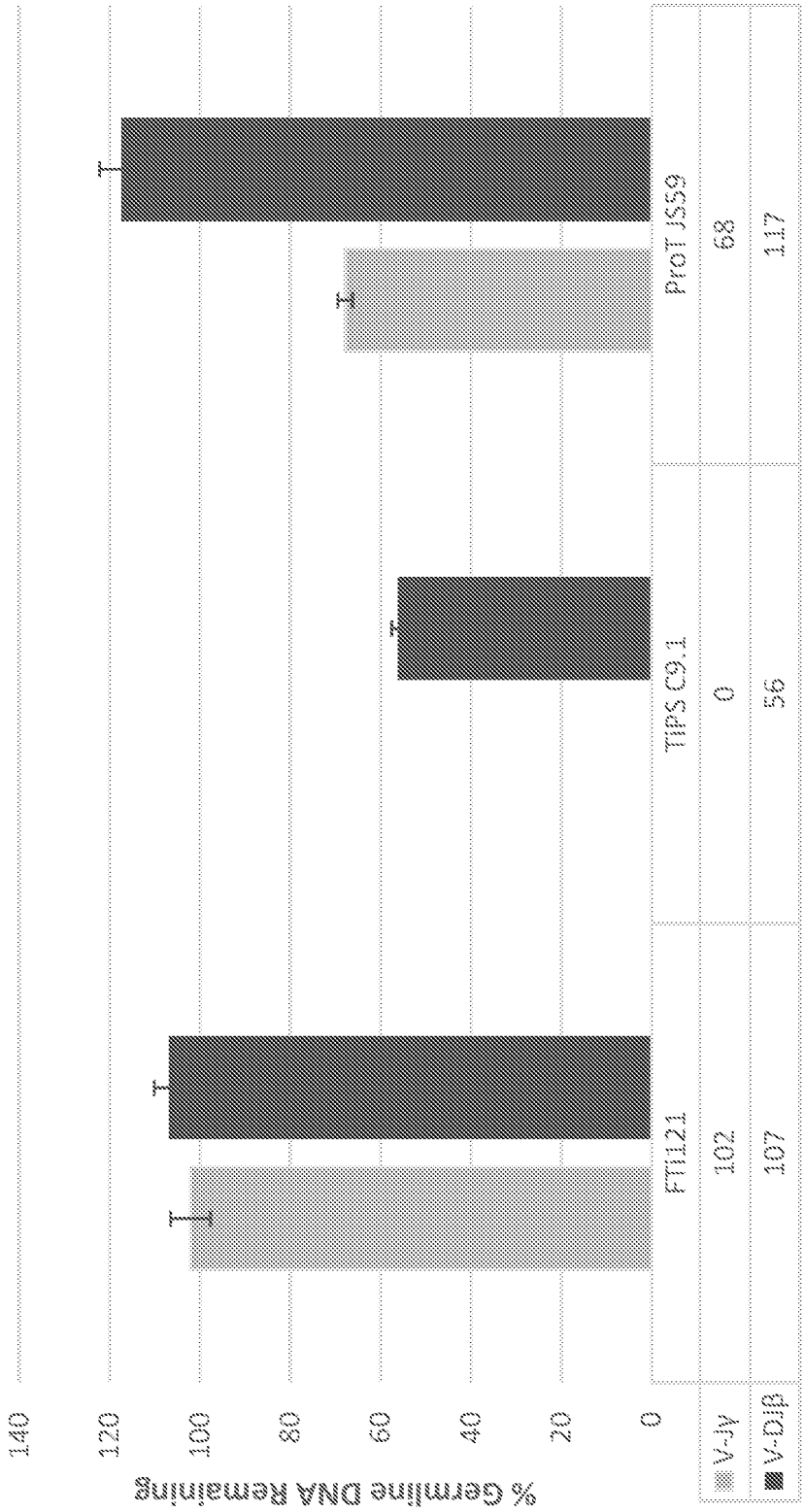

% Germline DNA Remaining
140
120
100
80
60
40
20
0
V-Jγ
V-DJβ
FTL121
102
107
TIPS C9.1
0
56
ProT JS59
68
117
V-Jγ
V-DJβ

FIG. 37