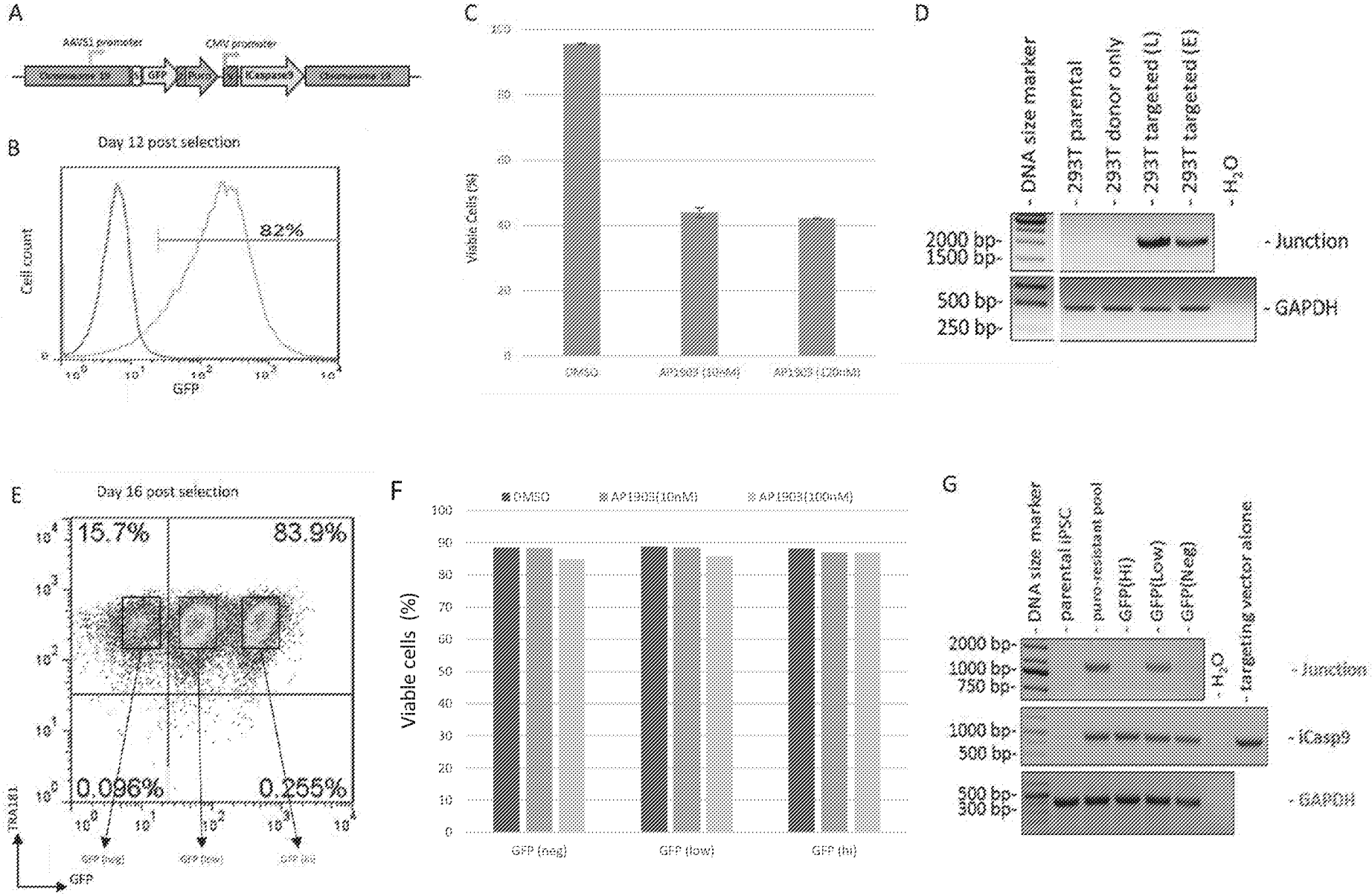

A
AAVS1 promoter
CMV promoter
GFP
Puro
iCaspase9
B
Day 12 post selection
Cell count
8.2%
GFP
C
Viable Cells (%)
DMSO
AP1903 (10nM)
AP1903 (100nM)
D
- DNA size marker
- 293T parental
- 293T donor only
- 293T targeted (L)
- 293T targeted (E)
- H2O
2000 bp-
1500 bp-
- Junction
500 bp-
250 bp-
- GAPDH
E
Day 16 post selection
15.7%
83.9%
0.096%
0.255%
TRA181
GFP
F
DMSO
AP1903(10nM)
AP1903(100nM)
Viable cells (%)
GFP (neg)
GFP (low)
GFP (hi)
G
- DNA size marker
- parental iPSC
- puro-resistant pool
- GFP(Hi)
- GFP(Low)
- GFP(Neg)
- targeting vector alone
- H2O
2000 bp-
1000 bp-
750 bp-
- Junction
1000 bp-
500 bp-
- iCasp9
500 bp-
300 bp-
- GAPDH

FIG. 1

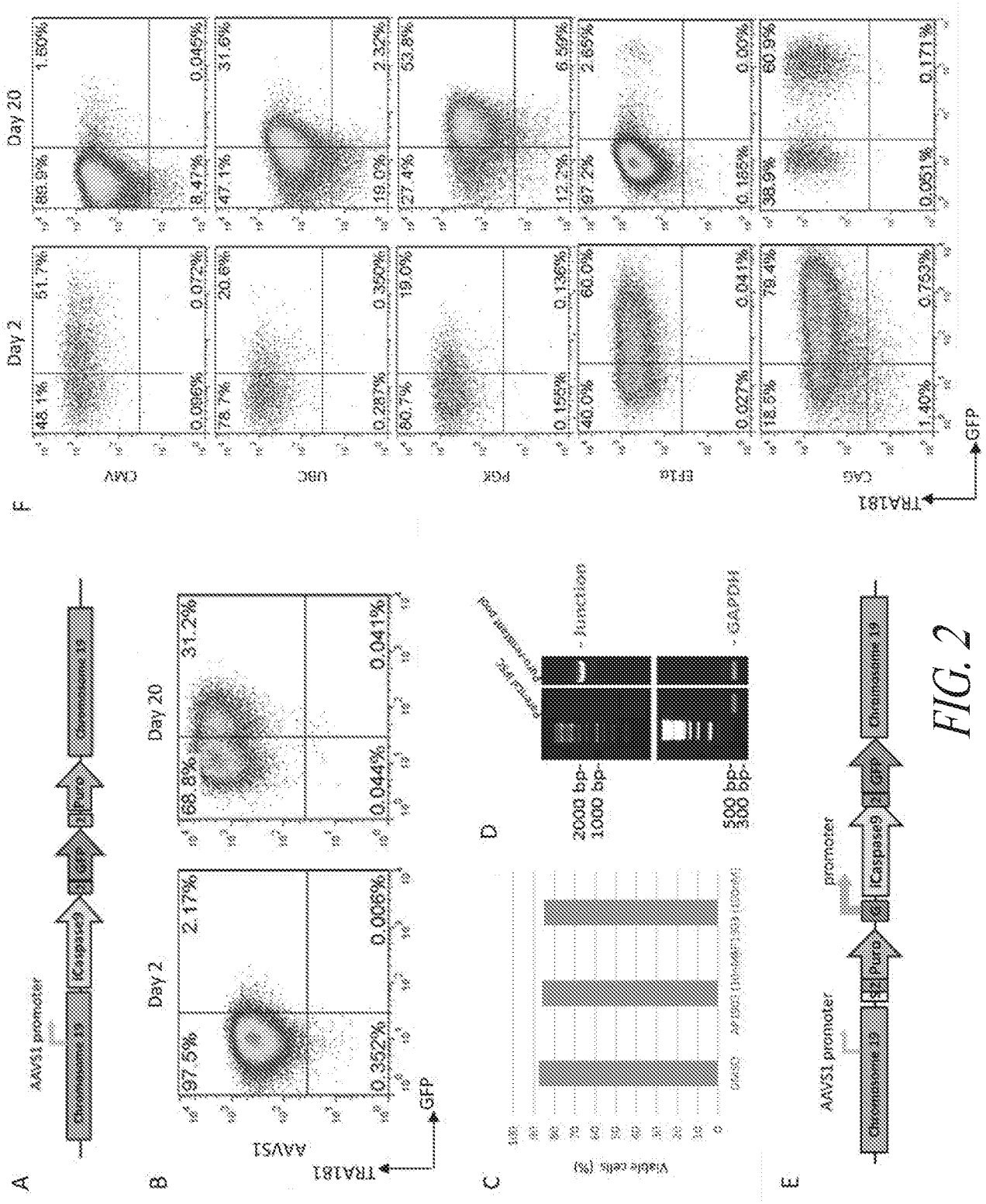

A
AAVS1 promoter
Puro
iCaspase9
GFP
Doxycycline 1g
B
Day 2
Day 20
97.5%
2.17%
0.352%
0.006%
68.8%
31.2%
0.044%
0.041%
AAVS1
TRA1B1
GFP
C
Viable cells (%)
D
2000 bp-
1000 bp-
500 bp-
300 bp-
- Junction
- GAPDH
E
AAVS1 promoter
Puro
iCaspase9
GFP
Doxycycline 1g
F
Day 2
Day 20
CMV
UBC
PGK
EF1α
CAG
48.1%
51.7%
0.096%
0.072%
78.7%
20.8%
0.287%
0.352%
80.7%
19.0%
0.153%
0.136%
40.0%
60.0%
0.027%
0.041%
18.6%
78.4%
1.40%
0.753%
88.9%
1.60%
8.47%
0.045%
47.1%
31.6%
19.0%
2.32%
27.4%
53.8%
12.2%
6.56%
67.2%
2.65%
0.185%
0.007%
38.9%
60.9%
0.051%
0.171%
TRA1B1
GFP

FIG. 2

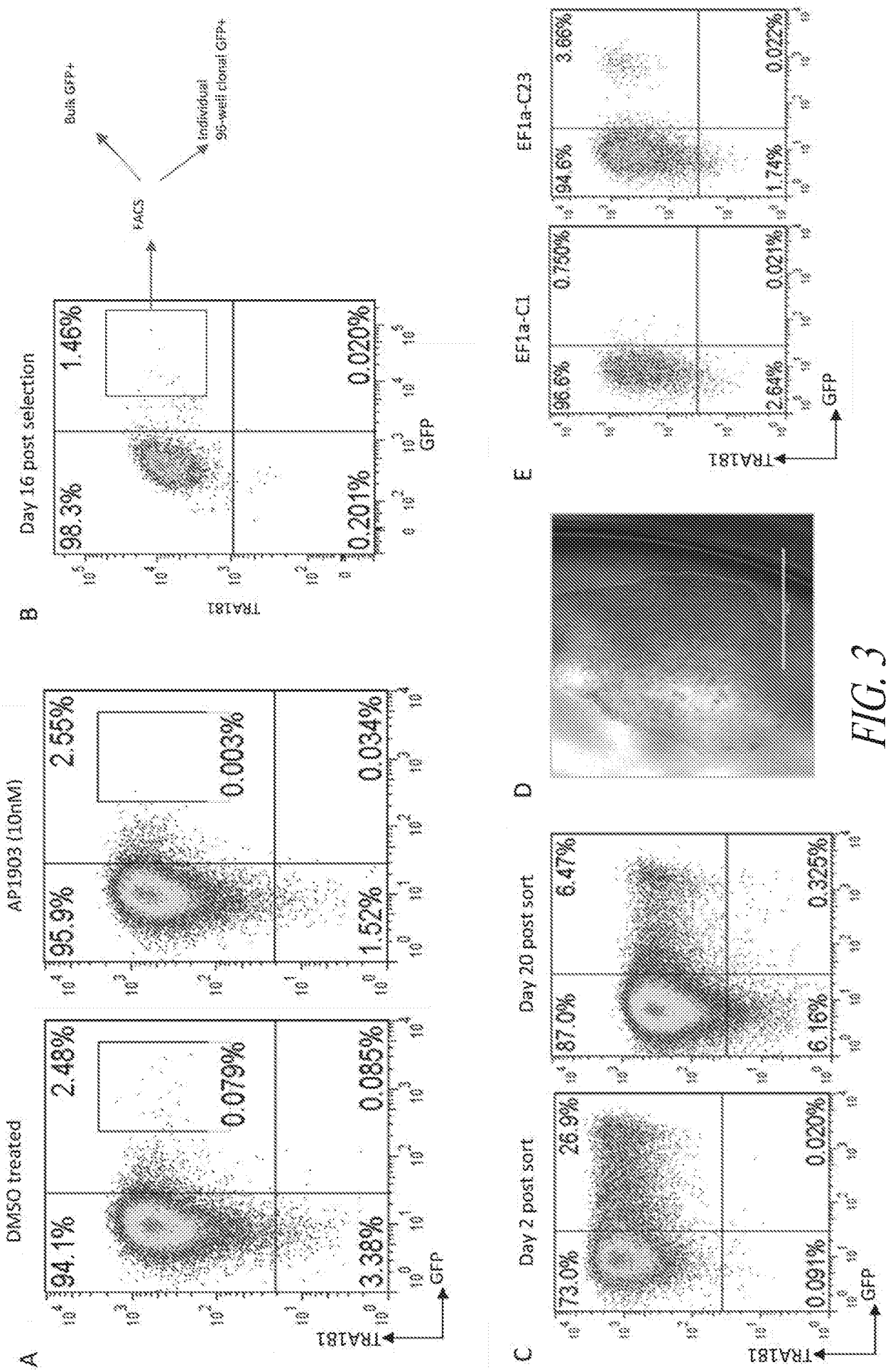

A
DMSO treated
AP1903 (10nM)
94.1%
2.48%
0.079%
3.38%
0.085%
95.9%
2.55%
0.003%
1.52%
0.034%
TRA1B1
GFP
B
Day 16 post selection
98.3%
1.46%
0.201%
0.020%
FACS
Bulk GFP+
individual
96-well clonal GFP+
C
Day 2 post sort
Day 20 post sort
73.0%
26.9%
0.091%
0.020%
87.0%
6.47%
6.16%
0.325%
D
E
EF1a-C1
EF1a-C23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

FIG. 3

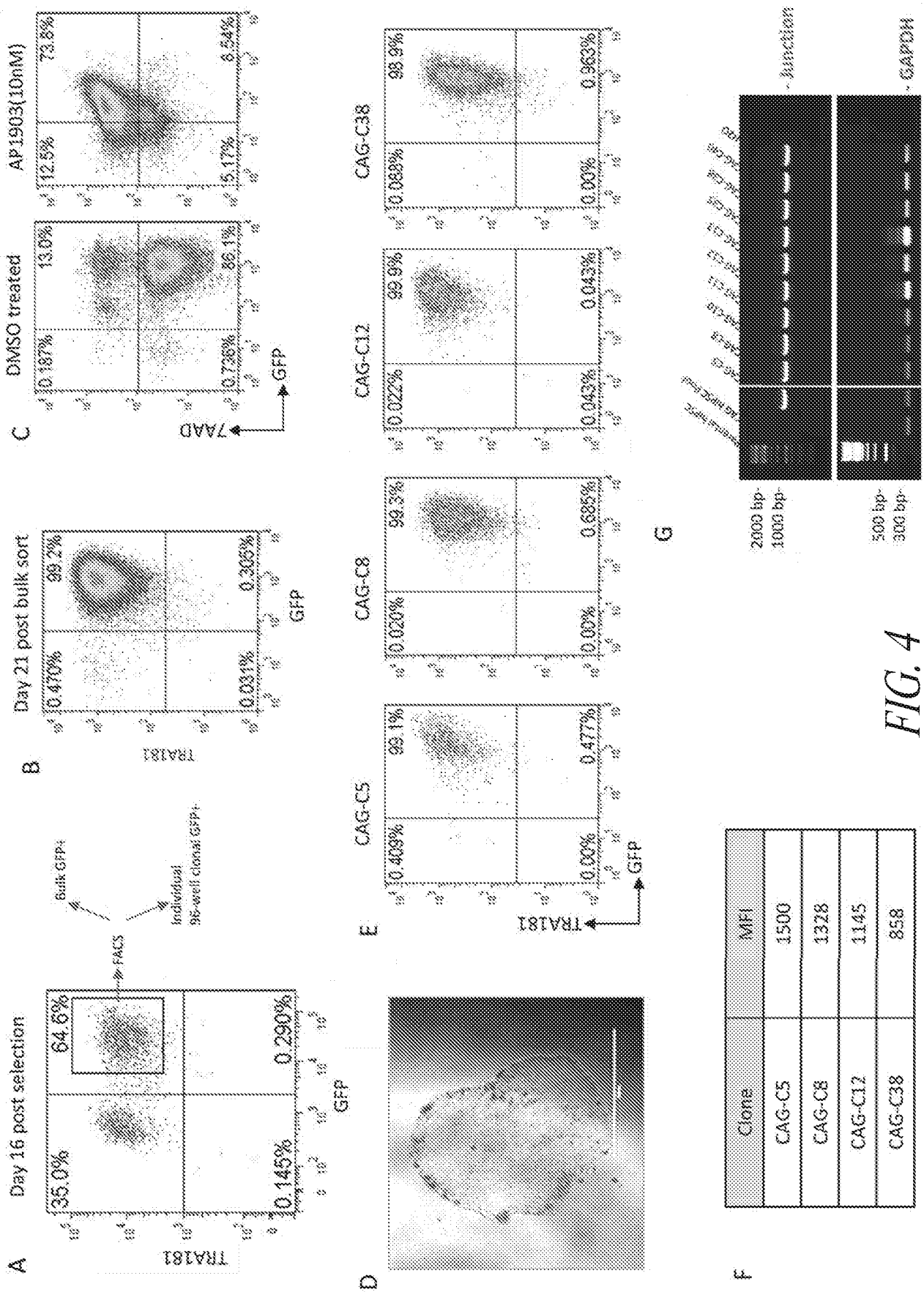

A
Day 16 post selection
TRA181
GFP
35.0%
64.6%
0.145%
0.290%
FACS
Bulk GFP+
Individual
96-well clonal GFP+
B
Day 21 post bulk sort
0.470%
99.2%
0.031%
0.305%
C
DMSO treated
AP1903(10nM)
7AAD
GFP
0.187%
13.0%
0.736%
86.1%
12.5%
73.8%
5.17%
6.54%
D
E
CAG-C5
CAG-C8
CAG-C12
CAG-C38
0.409%
99.1%
0.00%
0.477%
0.020%
99.3%
0.685%
0.022%
99.9%
0.043%
0.088%
98.9%
0.363%
F
Clone
MFI
CAG-C5
1500
CAG-C8
1328
CAG-C12
1145
CAG-C38
858
G
2000 bp
1000 bp
Junction
500 bp
300 bp
GAPDH

FIG. 4

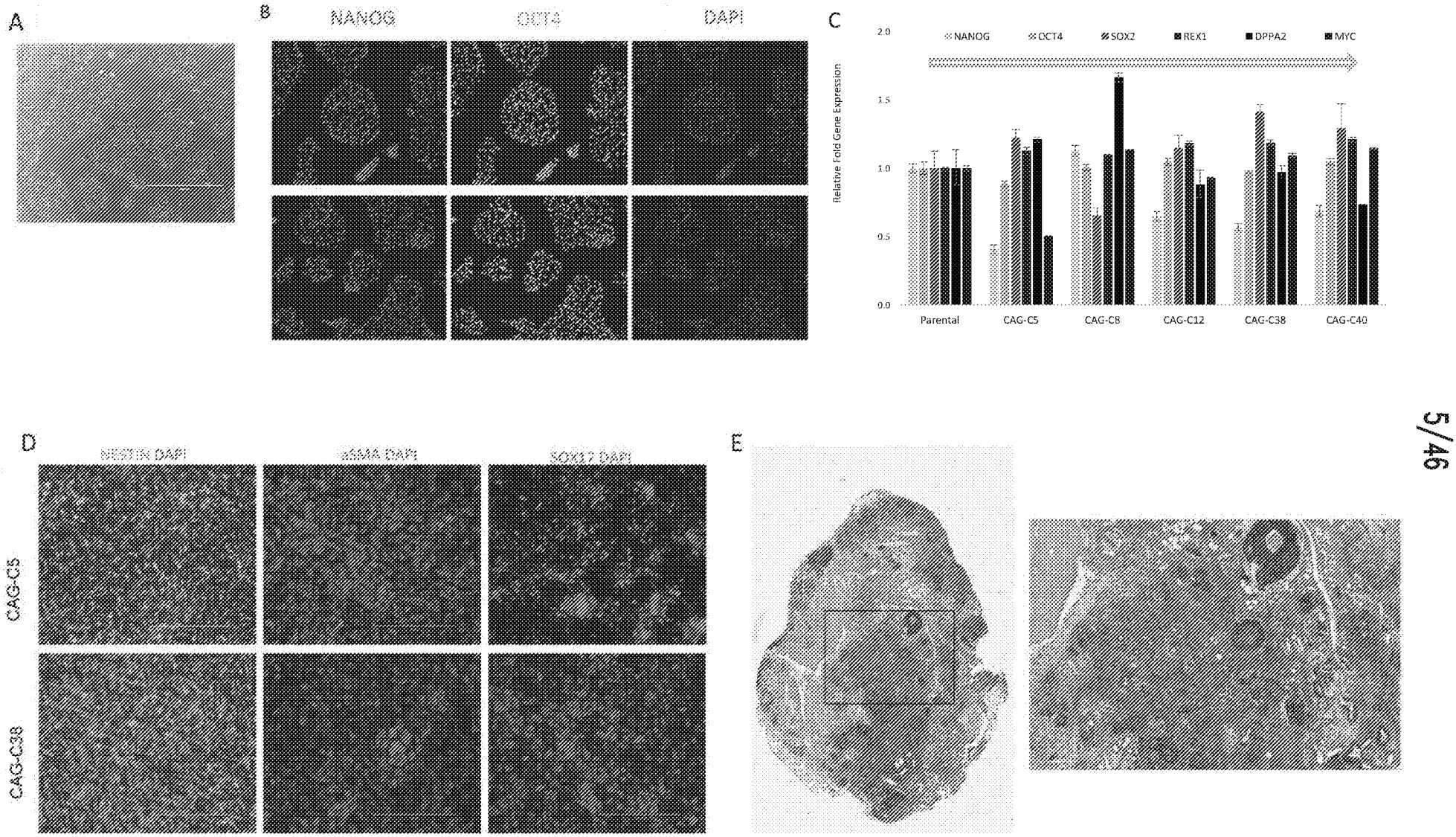

A
B
NANOG
OCT4
DAPI
C
NANOG
OCT4
SOX2
REX1
DPPA2
MYC
Relative Fold Gene Expression
2.0
1.5
1.0
0.5
0.0
Parental
CAG-C5
CAG-C8
CAG-C12
CAG-C38
CAG-C40
D
NESTIN DAPI
αSMA DAPI
SOX17 DAPI
CAG-C5
CAG-C38
E

FIG. 5

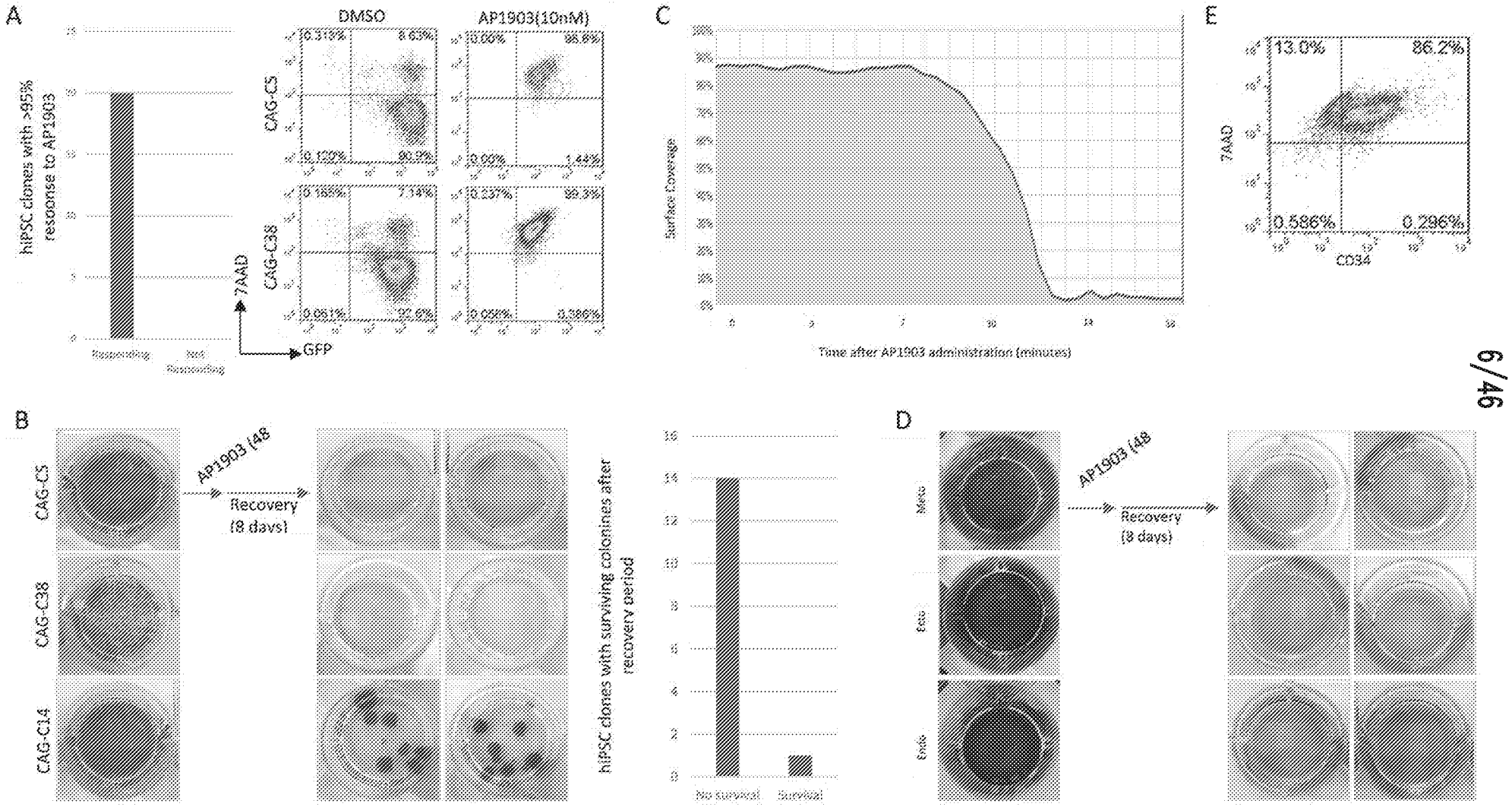

A
hiPSC clones with >95% response to AP1903
Responding
Non Responding
DMSO
AP1903(10nM)
CAG-C5
CAG-C38
7AAD
GFP
0.319%
8.63%
0.120%
90.9%
0.00%
99.8%
0.00%
1.44%
0.185%
7.14%
92.6%
0.237%
99.3%
0.051%
0.086%
0.00%
C
Surface Coverage
Time after AP1903 administration (minutes)
E
13.0%
86.2%
0.586%
0.296%
7AAD
CD34
B
CAG-C5
CAG-C38
CAG-C14
AP1903 (48
Recovery
(8 days)
hiPSC clones with surviving colonines after recovery period
No survival
Survival
D
Mock
Exo1
Exo2
AP1903 (48
Recovery
(8 days)

FIG. 6

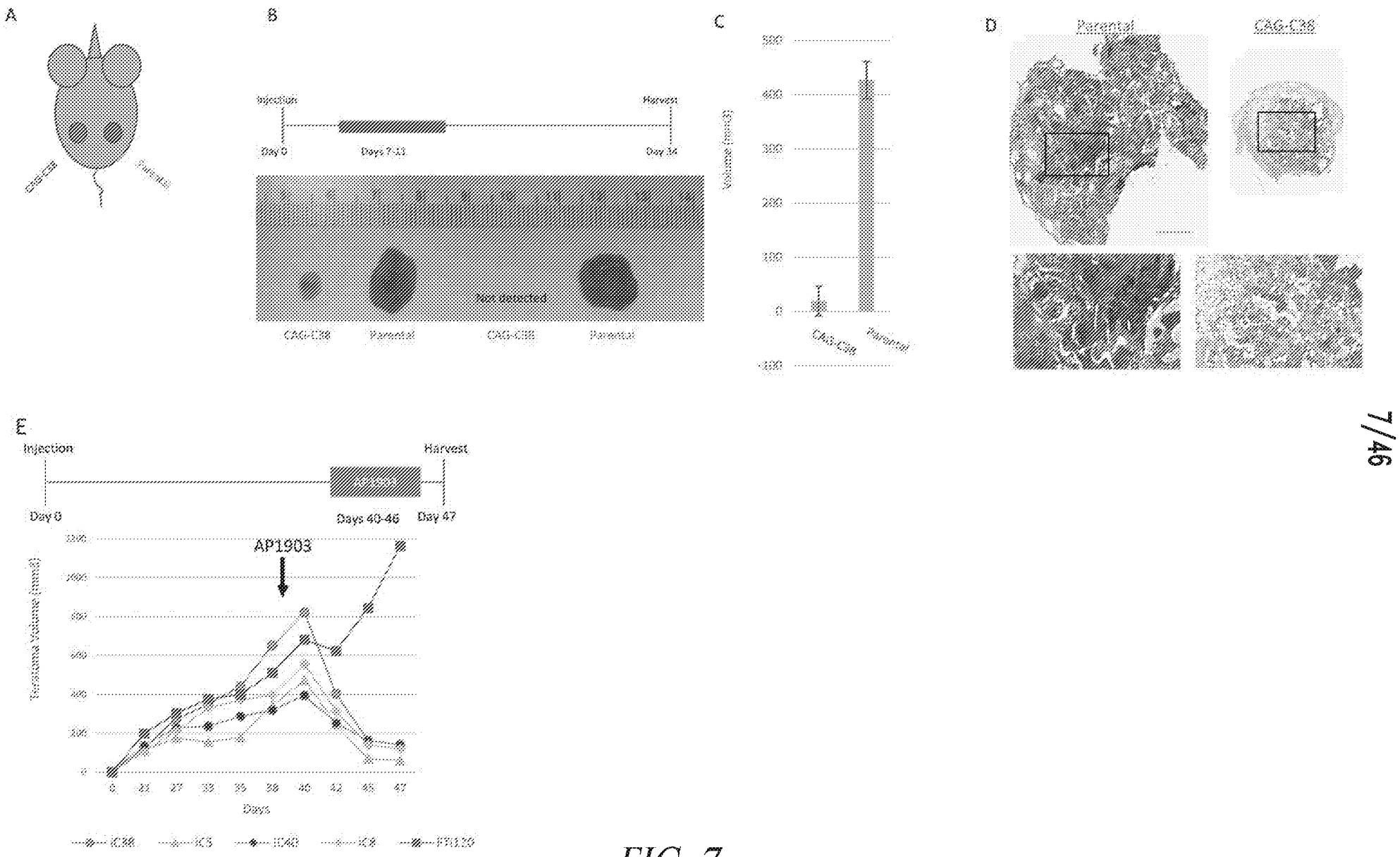

A
CAG-C38
Parental
B
Injection
Harvest
Day 0
Days 7-33
Day 34
Not detected
CAG-C38
Parental
CAG-C38
Parental
C
Volume (mm3)
500
400
300
200
100
0
-100
CAG-C38
Parental
D
Parental
CAG-C36
E
Injection
Harvest
Day 0
Days 40-46
Day 47
AP1903
Tumour Volume (mm3)
0
21
27
33
35
38
40
42
45
47
Days
IC38
IC5
IC40
IC8
FP1120

FIG. 7

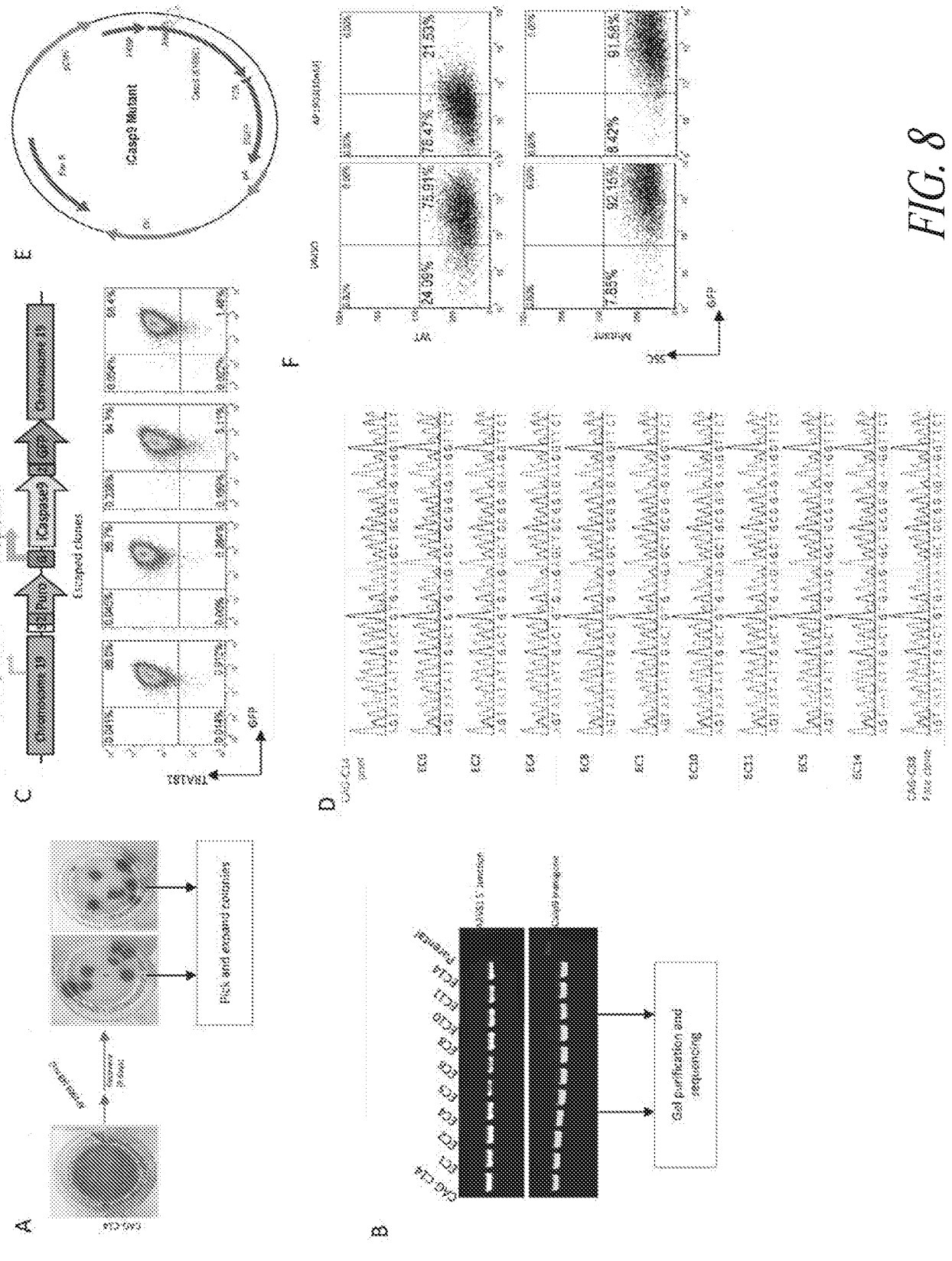

A
Pick and expand colonies
B
Gel purification and sequencing
C
AAVS1 promoter
CAG promoter
Escaped clones
GFP
D
E
iCasp9 Mutant
F
AAVS1
CAG-iCas9
GFP

FIG. 8

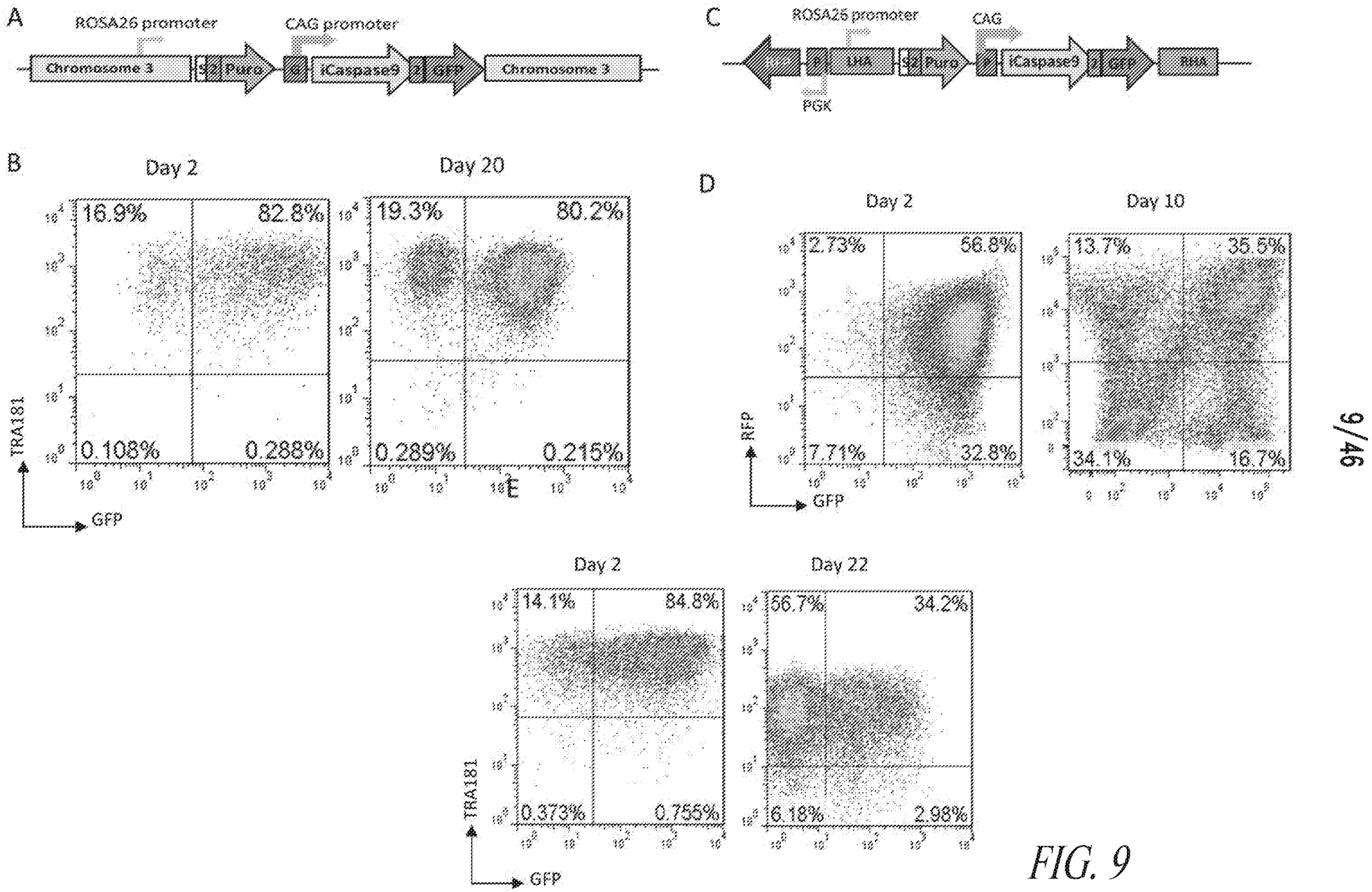

A
ROSA26 promoter
CAG promoter
Chromosome 3
Puro
iCaspase9
GFP
Chromosome 3
C
ROSA26 promoter
CAG
PGK
Puro
iCaspase9
B
Day 2
Day 20
16.9%
82.8%
0.108%
0.288%
19.3%
80.2%
0.289%
0.215%
TRA181
GFP
D
Day 2
Day 10
2.73%
56.8%
7.71%
32.8%
13.7%
35.5%
34.1%
16.7%
RFP
GFP
E
Day 2
Day 22
14.1%
84.8%
0.373%
0.755%
56.7%
34.2%
6.18%
2.98%
TRA181
GFP

FIG. 9

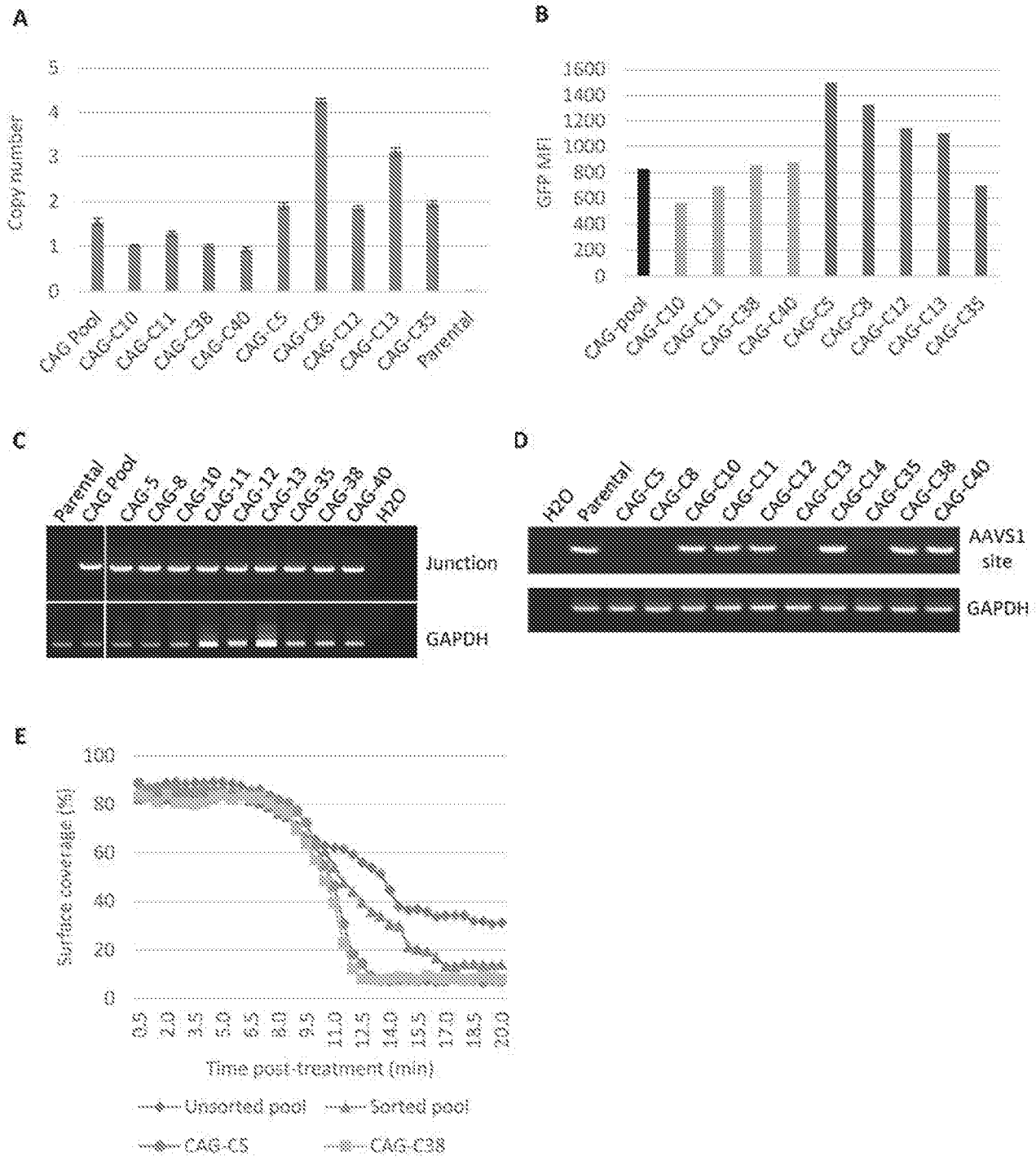

A
Copy number
0
1
2
3
4
5
CAG Pool
CAG-C10
CAG-C11
CAG-C38
CAG-C40
CAG-C5
CAG-C8
CAG-C12
CAG-C13
CAG-C35
Parental
B
GFP MFI
0
200
400
600
800
1000
1200
1400
1600
CAG-pool
CAG-C10
CAG-C11
CAG-C38
CAG-C40
CAG-C5
CAG-C8
CAG-C12
CAG-C13
CAG-C35
C
Parental
CAG Pool
CAG-5
CAG-8
CAG-10
CAG-11
CAG-12
CAG-13
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

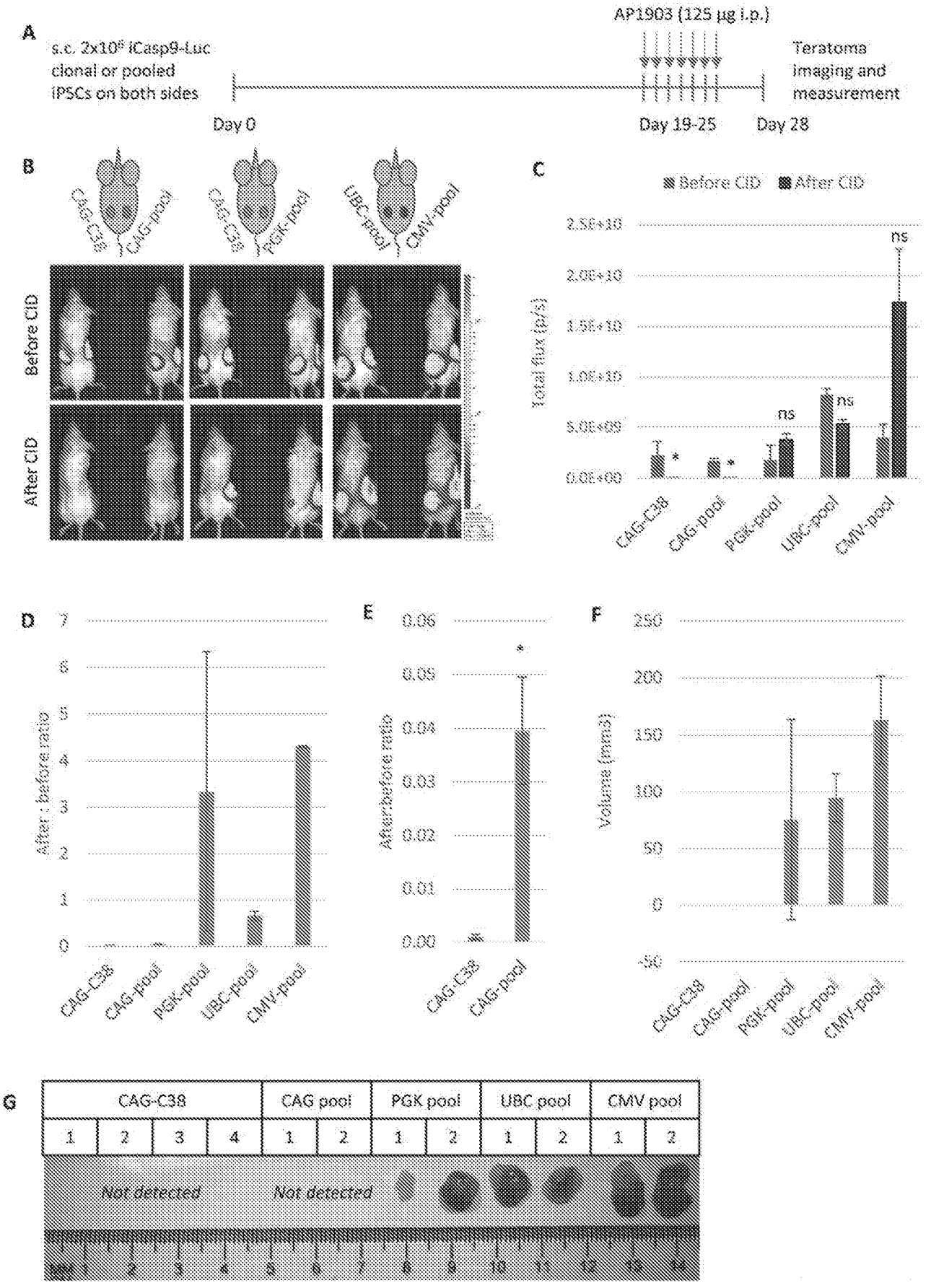

A
s.c. 2x10⁶ iCasp9-Luc clonal or pooled iPSCs on both sides
AP1903 (125 µg i.p.)
Teratoma imaging and measurement
Day 0
Day 19-25
Day 28
B
CAG-C38
CAG-pool
PGK-pool
UBC-pool
CMV-pool
Before CID
After CID
C
Before CID
After CID
Total flux (p/s)
2.5E+10
2.0E+10
1.5E+10
1.0E+10
5.0E+09
0.0E+00
ns
*
D
After : before ratio
E
After:before ratio
F
Volume (mm3)
250
200
150
100
50
0
-50
G
CAG-C38
CAG pool
PGK pool
UBC pool
CMV pool
Not detected
Not detected

FIG. 11

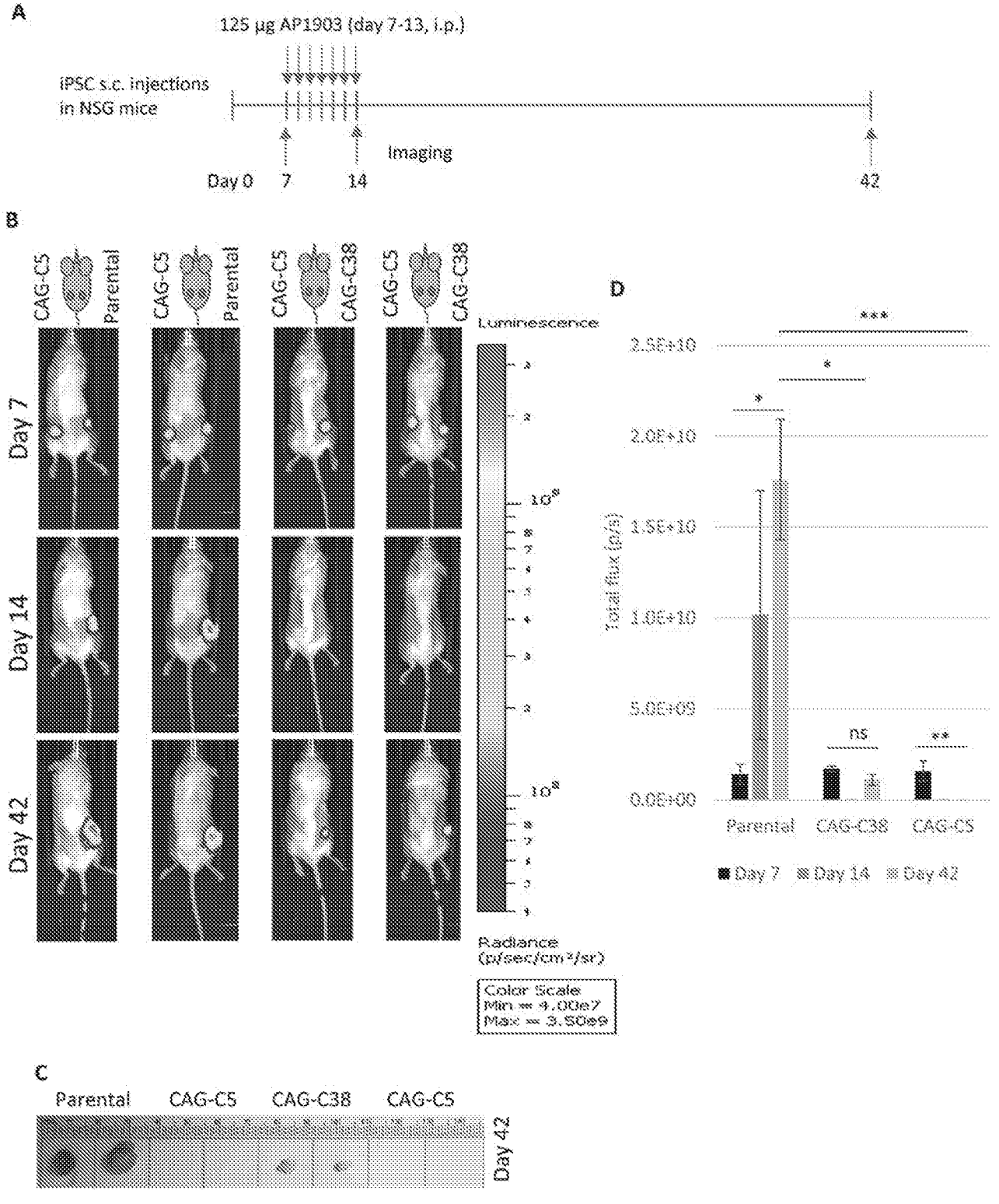

A
125 µg AP1903 (day 7-13, i.p.)
iPSC s.c. injections in NSG mice
Imaging
Day 0
7
14
42
B
CAG-C5
Parental
CAG-C38
Day 7
Day 14
Day 42
Luminescence
Radiance (p/sec/cm²/sr)
Color Scale
Min = 4.00e7
Max = 3.50e9
C
Parental
CAG-C5
CAG-C38
CAG-C5
Day 42
D
Total flux (p/s)
2.5E+10
2.0E+10
1.5E+10
1.0E+10
5.0E+09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

FIG. 12

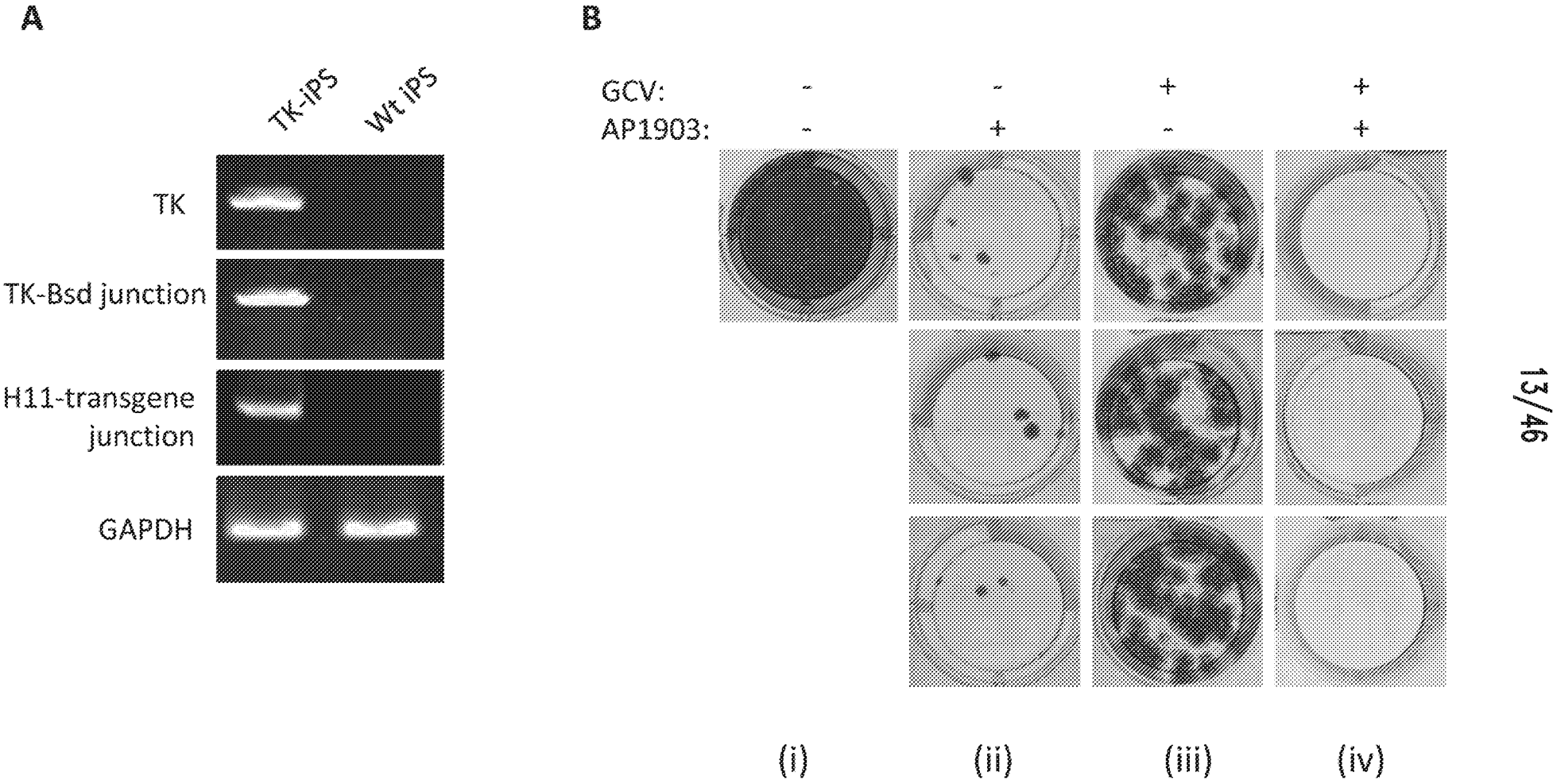

A
TK-iPS
Wt iPS
TK
TK-Bsd junction
H11-transgene junction
GAPDH
B
GCV:
AP1903:
(i)
(ii)
(iii)
(iv)

FIG. 13

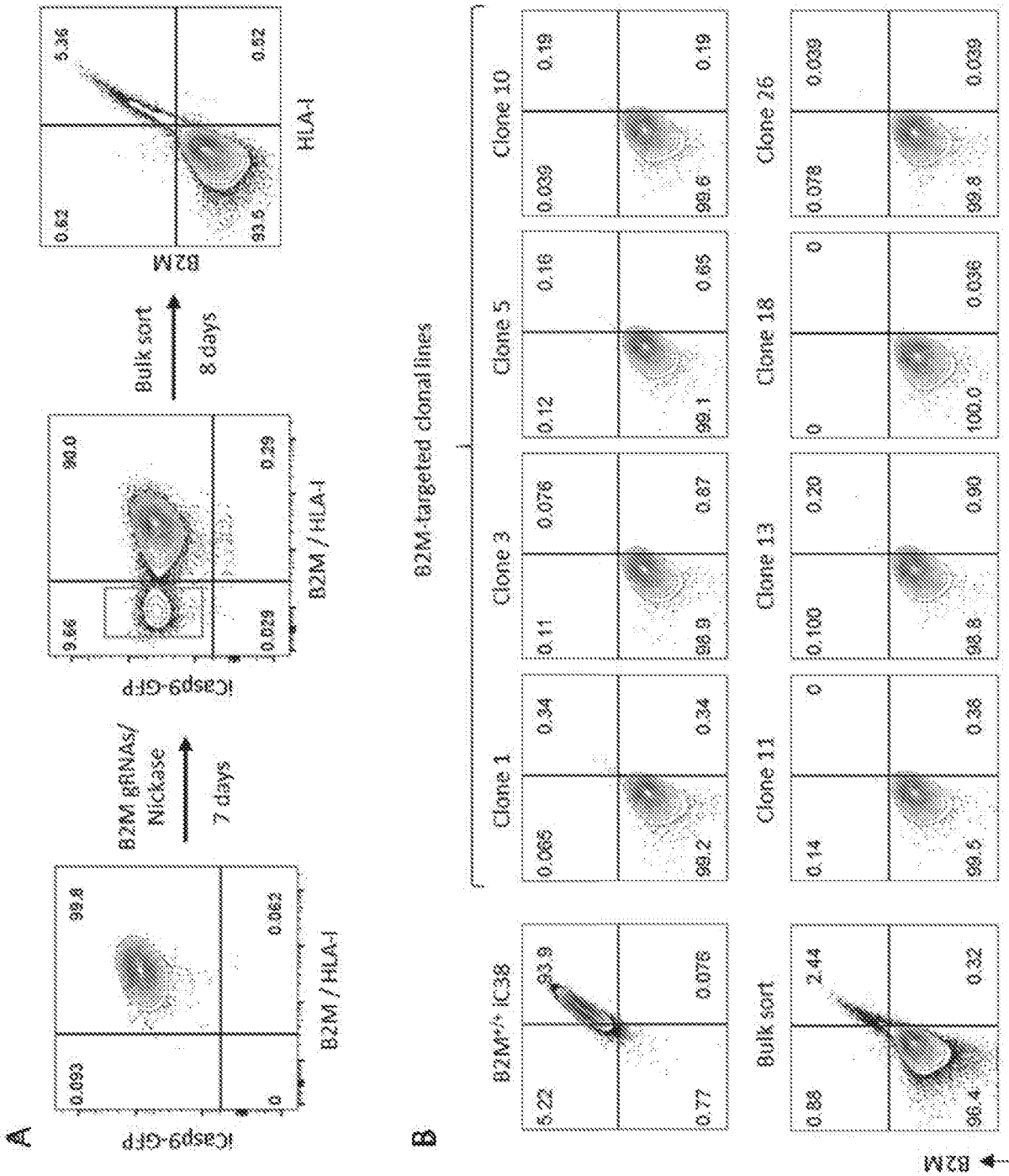

A
B2M gRNAs/
Nickase
7 days
Bulk sort
8 days
iCasp9-GFP
B2M / HLA-I
0.093
99.8
0
0.063
9.66
90.0
0.029
0.29
0.63
5.36
93.6
0.52
B2M
HLA-I
B
B2M-targeted clonal lines
Clone 1
Clone 3
Clone 5
Clone 10
Clone 11
Clone 13
Clone 18
Clone 26
B2M-/- IC38
Bulk sort
5.22
93.9
0.77
0.076
0.065
0.34
99.2
0.34
0.11
0.076
98.9
0.87
0.12
0.16
99.1
0.65
0.039
0.19
99.6
0.19
0.14
0
99.5
0.36
0.100
0.20
98.8
0.90
0
0
100.0
0.036
0.078
0.029
99.8
0.039
0.88
2.44
96.4
0.32
B2M
HLA-I

FIG. 14

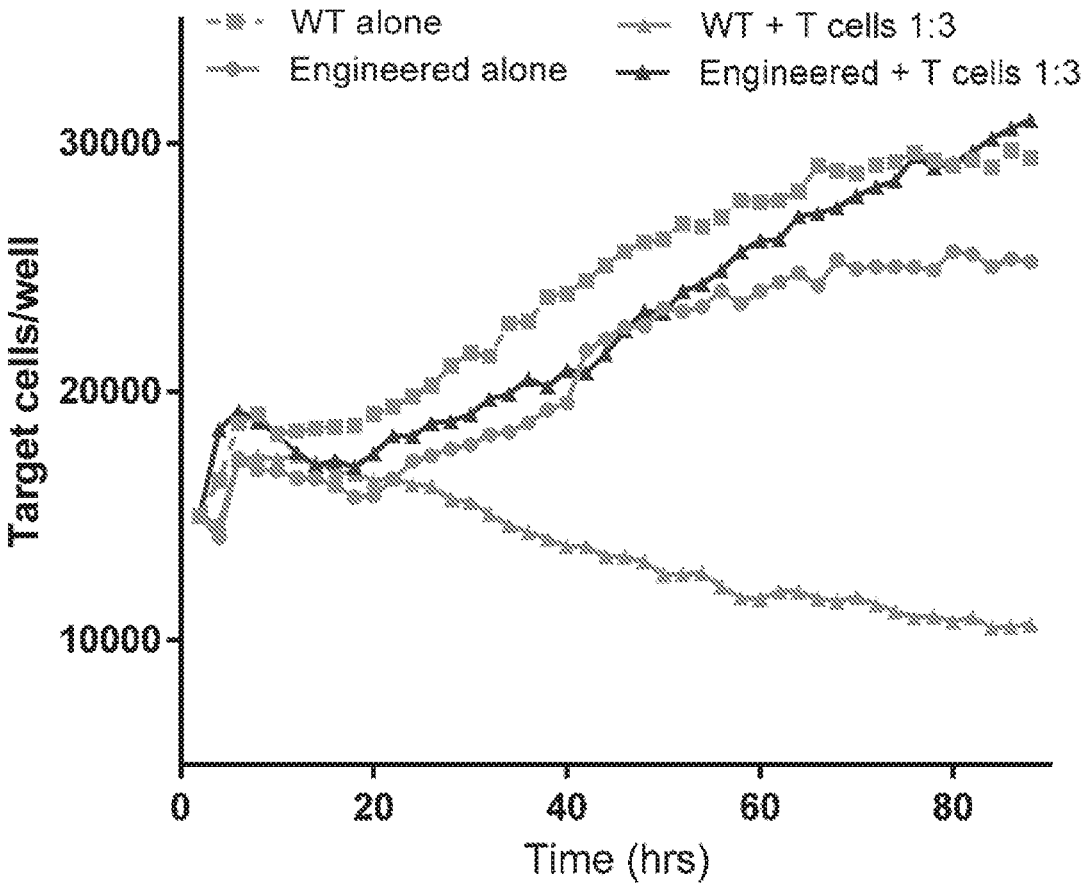

WT alone
Engineered alone
WT + T cells 1:3
Engineered + T cells 1:3
Target cells/well
30000
20000
10000
0
20
40
60
80
Time (hrs)

FIG. 15A

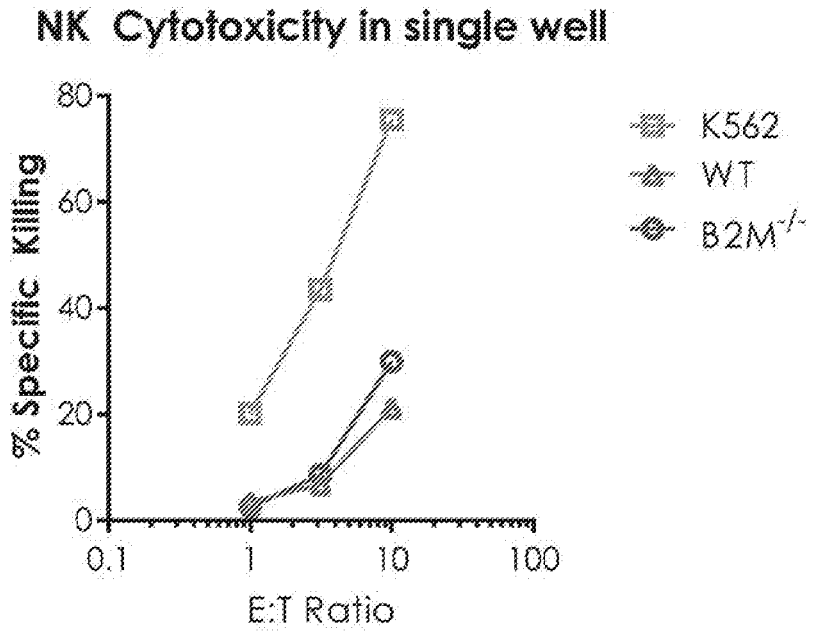


FIG. 15B

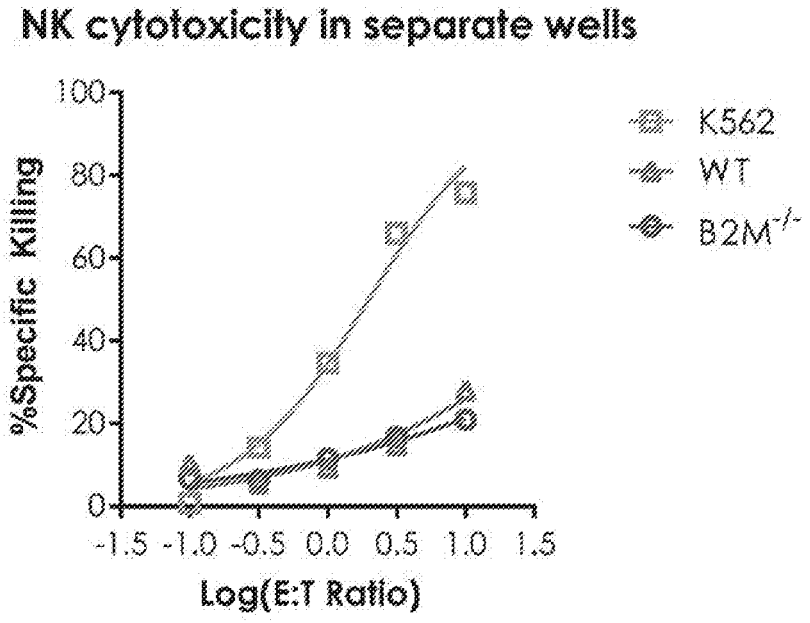


FIG. 15C

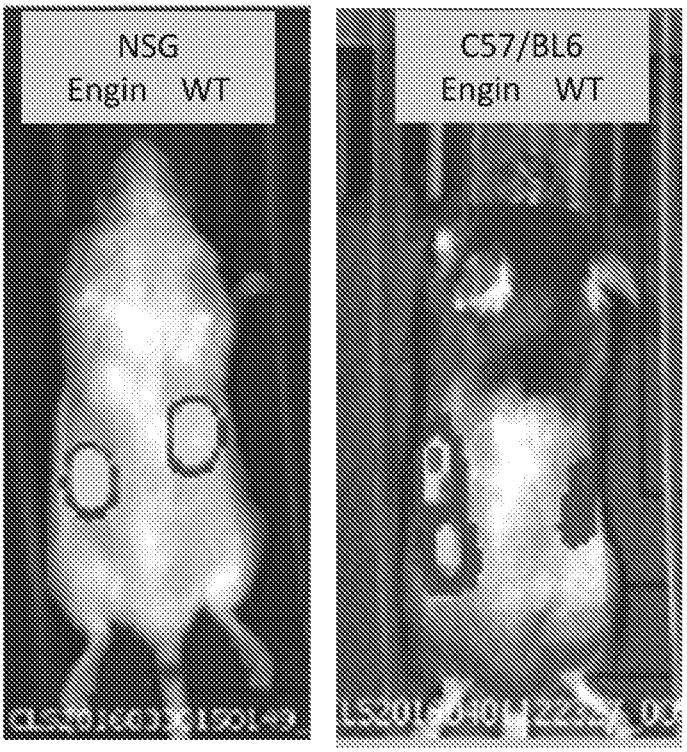


FIG. 15D

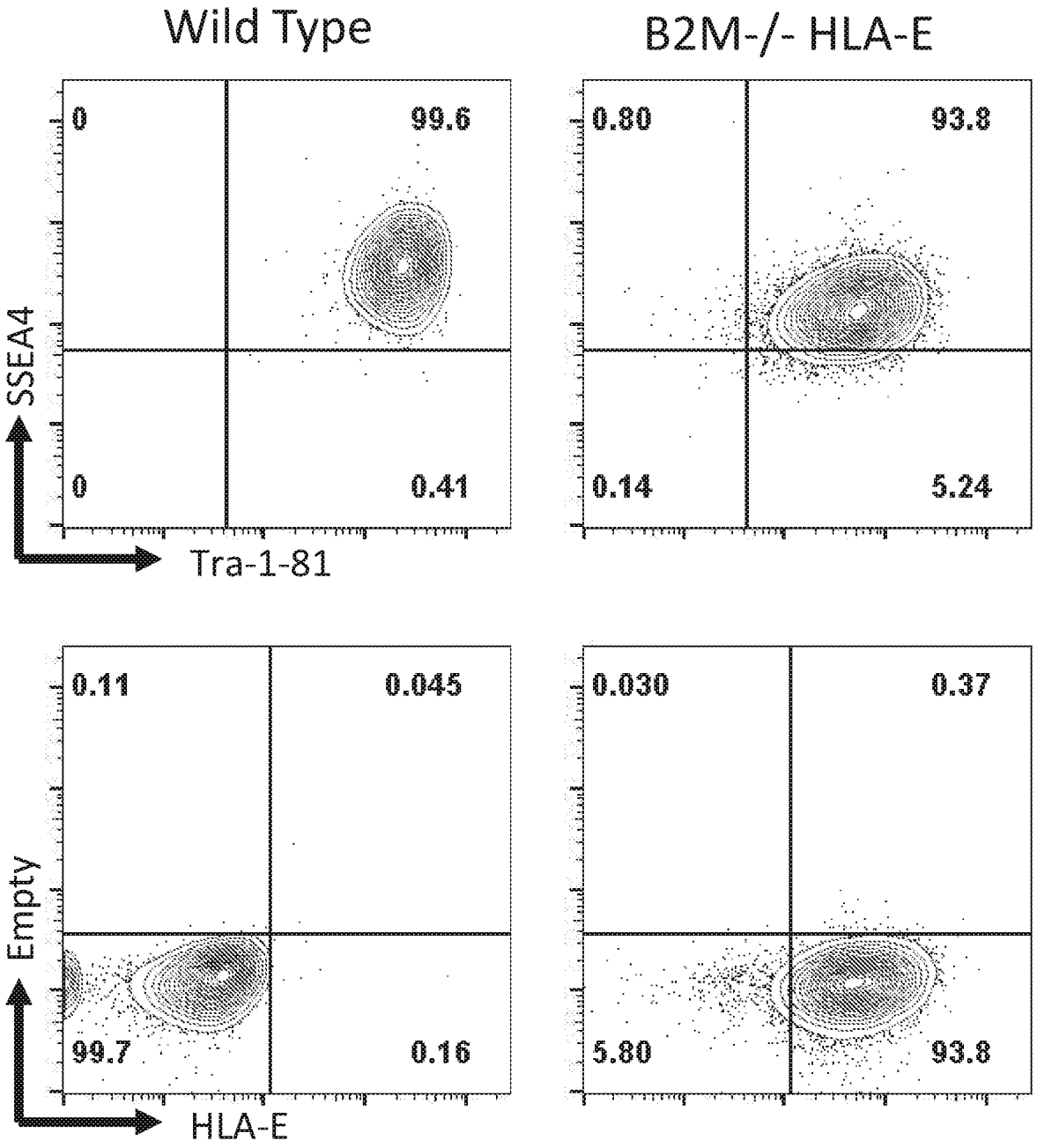
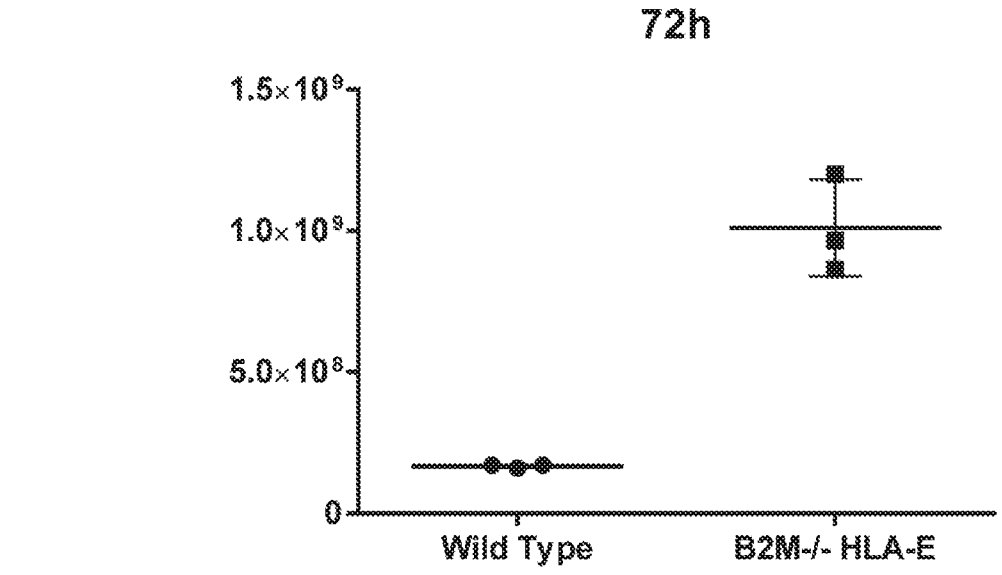
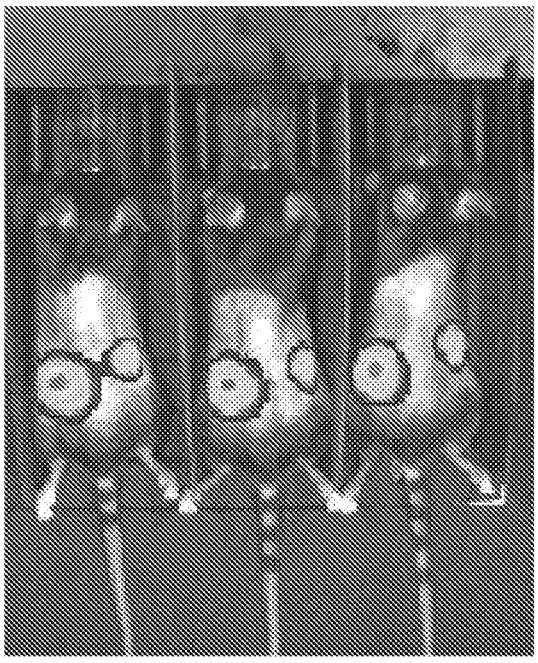

Wild Type
B2M-/- HLA-E
SSEA4
Tra-1-81
0
99.6
0
0.41
0.80
93.8
0.14
5.24
Empty
HLA-E
0.11
0.045
99.7
0.16
0.030
0.37
5.80
93.8

FIG. 16A





Left: B2M-/- HLA-E
Right: Wild Type

FIG. 16B

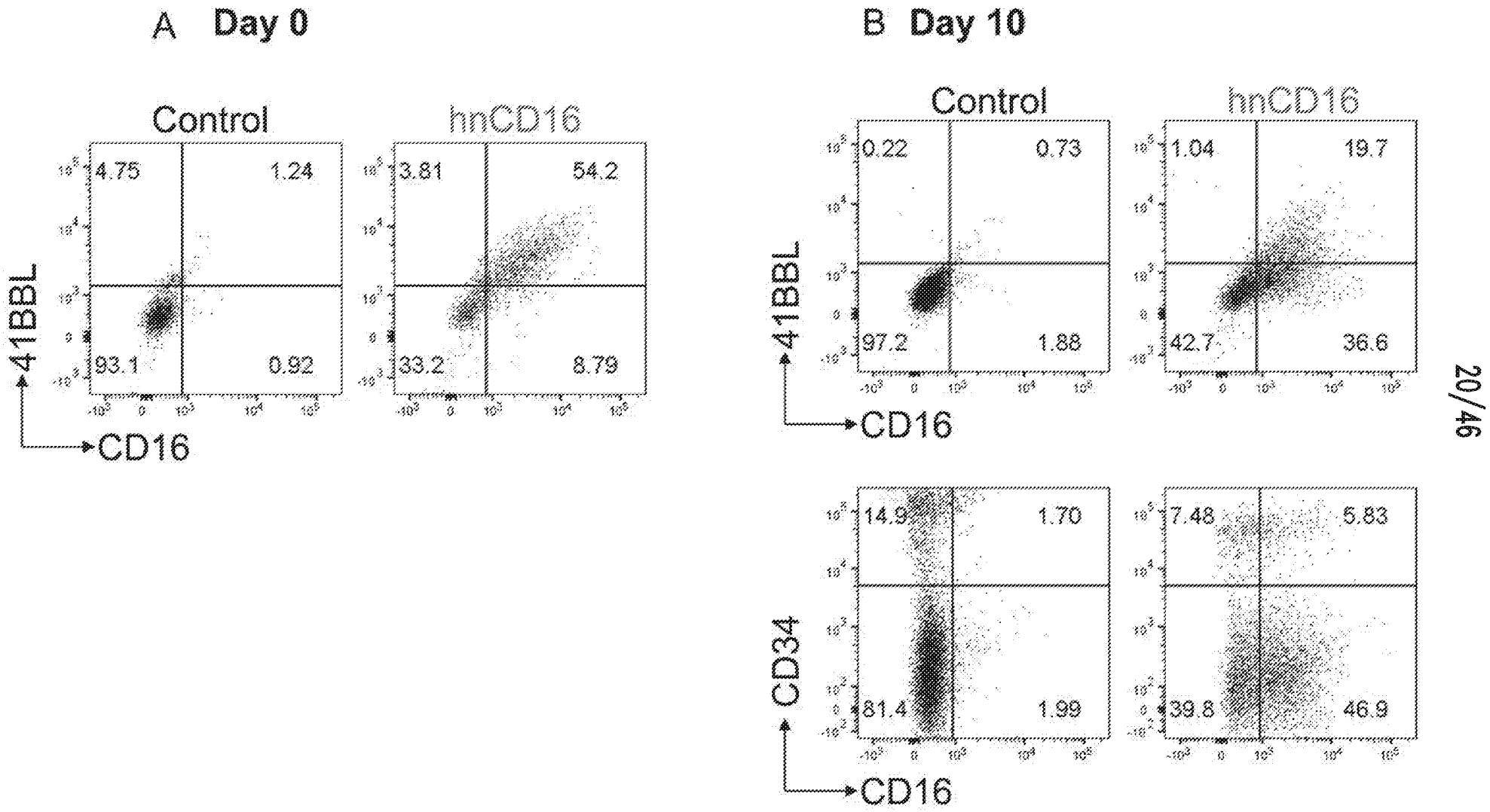

A Day 0
Control
hnCD16
4.75
1.24
93.1
0.92
3.81
54.2
33.2
8.79
41BBL
CD16
B Day 10
Control
hnCD16
0.22
0.73
97.2
1.88
1.04
19.7
42.7
36.6
41BBL
CD16
14.9
1.70
81.4
1.99
7.48
5.83
39.8
46.9
CD34
CD16

FIG. 17

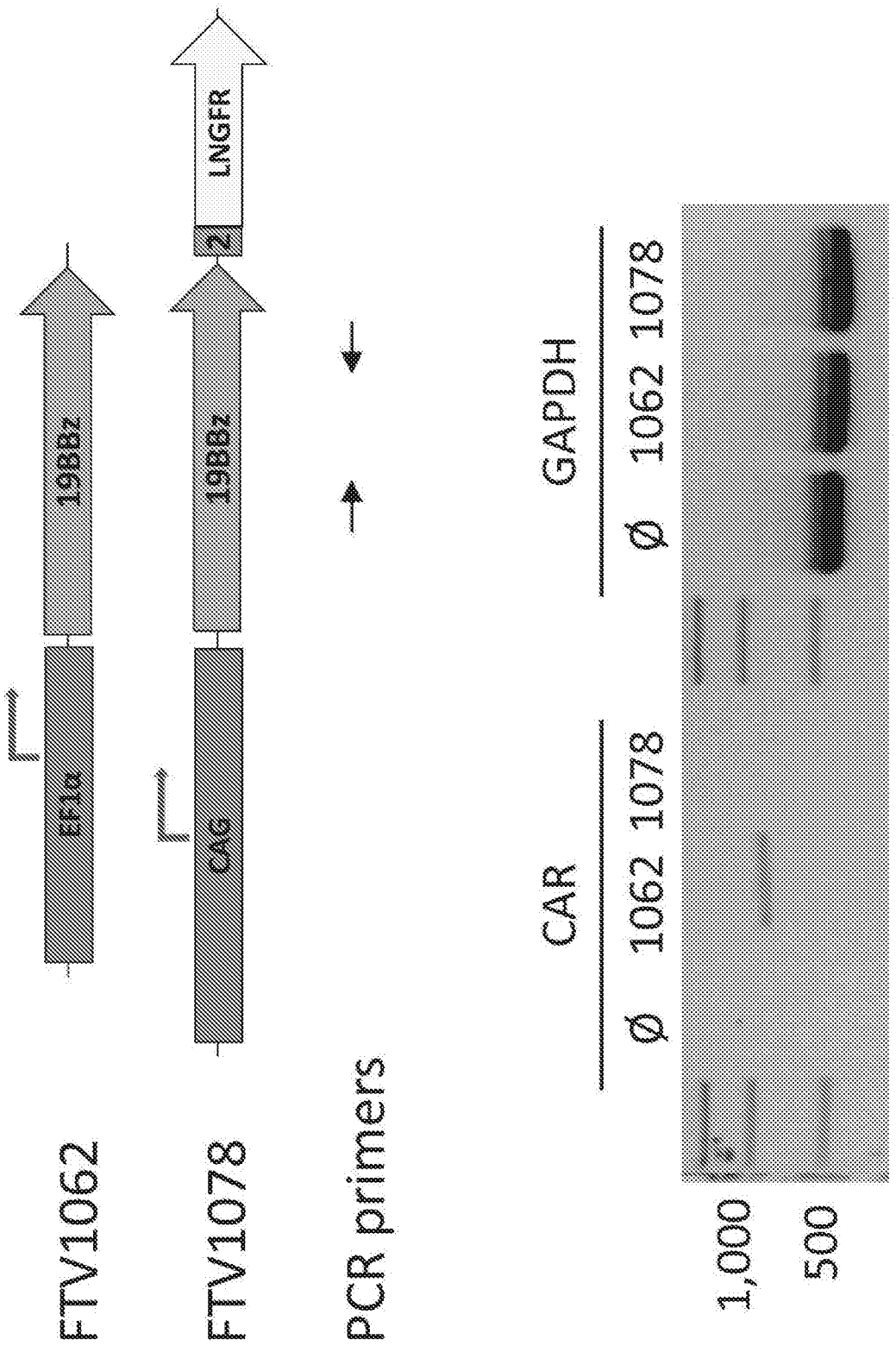

FTV1062
EF1α
19BBz
FTV1078
CAG
19BBz
2A
LNGFR
PCR primers
CAR
Ø
1062
1078
GAPDH
Ø
1062
1078
1,000
500

FIG. 18

Day 0

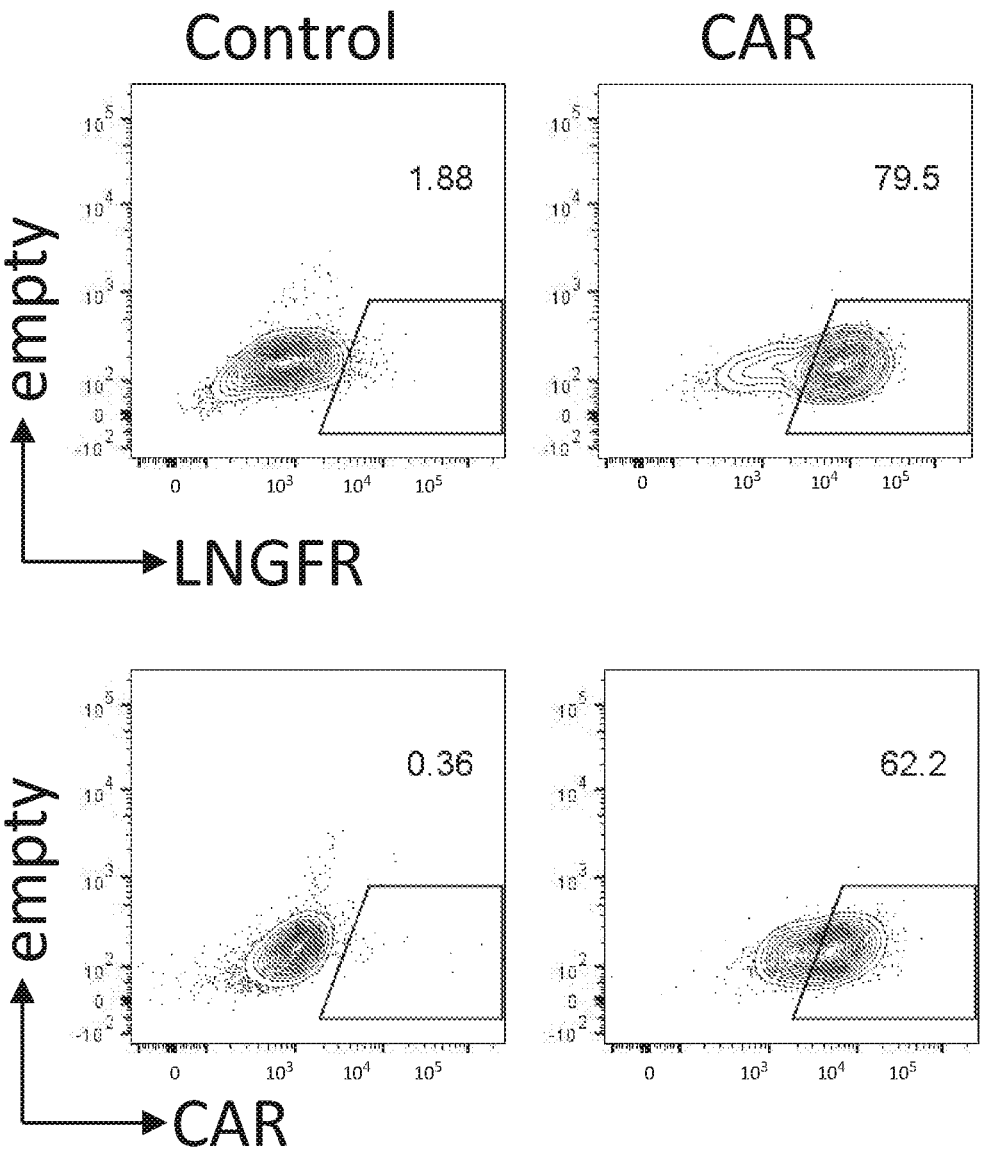


FIG. 19A

Day 10

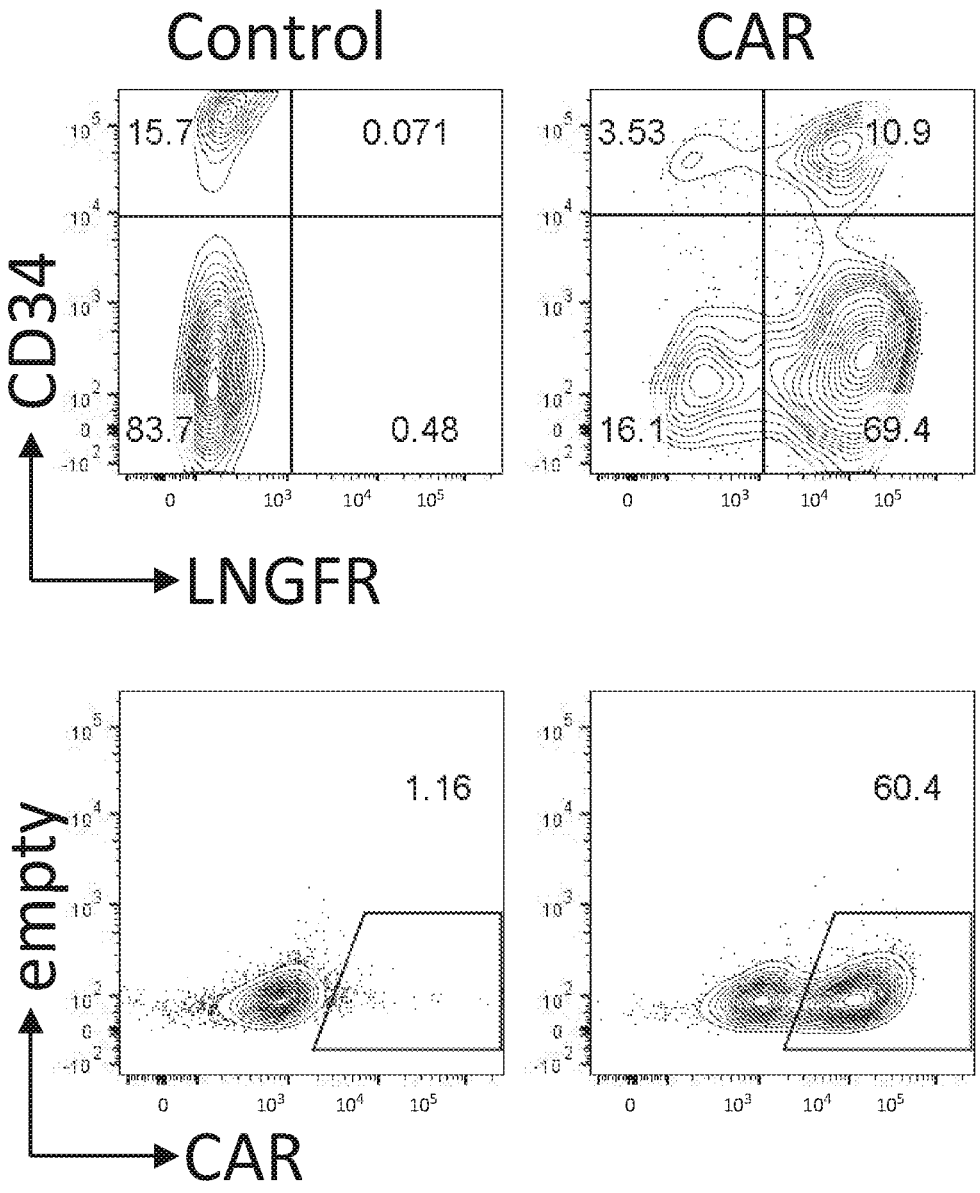


FIG. 19B

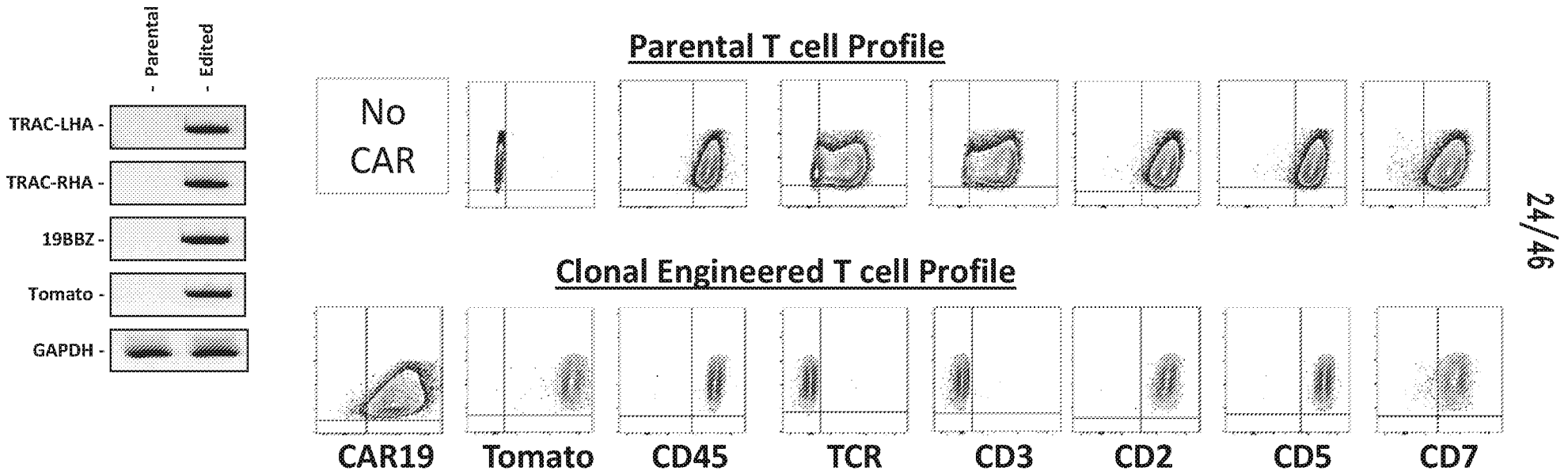

- Parental
- Edited
TRAC-LHA -
TRAC-RHA -
19BBZ -
Tomato -
GAPDH -
Parental T cell Profile
No CAR
Clonal Engineered T cell Profile
CAR19
Tomato
CD45
TCR
CD3
CD2
CD5
CD7

FIG. 20

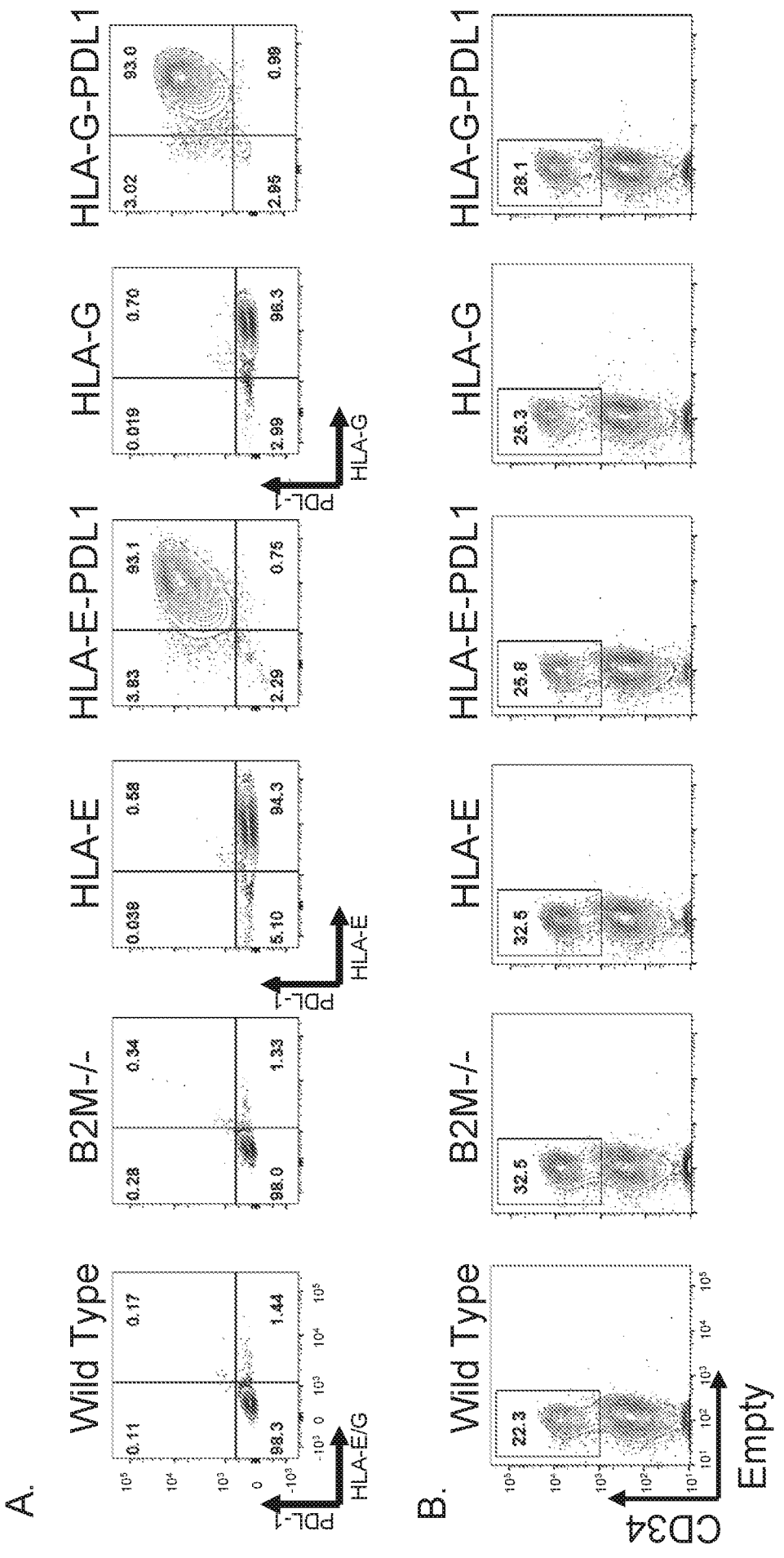

A.
B.
Wild Type
B2M-/-
HLA-E
HLA-E-PDL1
HLA-G
HLA-G-PDL1
PDL-1
HLA-E/G
HLA-E
HLA-G
CD34
Empty
0.11
0.17
98.3
1.44
0.28
0.34
98.0
1.33
0.039
0.58
5.10
94.3
3.83
93.1
2.29
0.75
0.019
0.70
2.99
96.3
3.02
93.0
2.85
0.99
22.3
32.5
25.8
25.3
28.1

FIG. 21

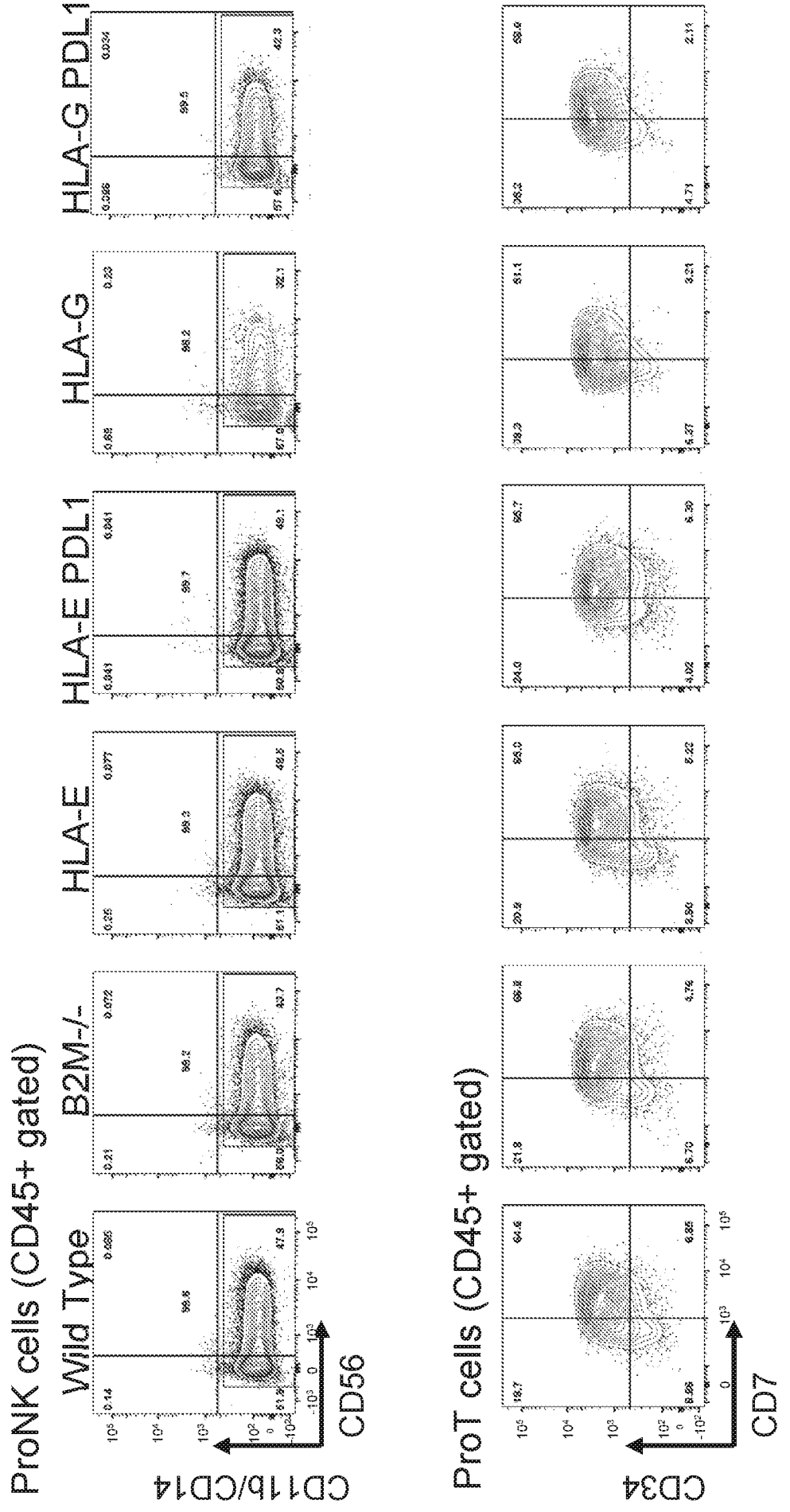

ProNK cells (CD45+ gated)
Wild Type
B2M-/-
HLA-E
HLA-E PDL1
HLA-G
HLA-G PDL1
CD11b/CD14
CD56
ProT cells (CD45+ gated)
CD34
CD7

FIG. 22

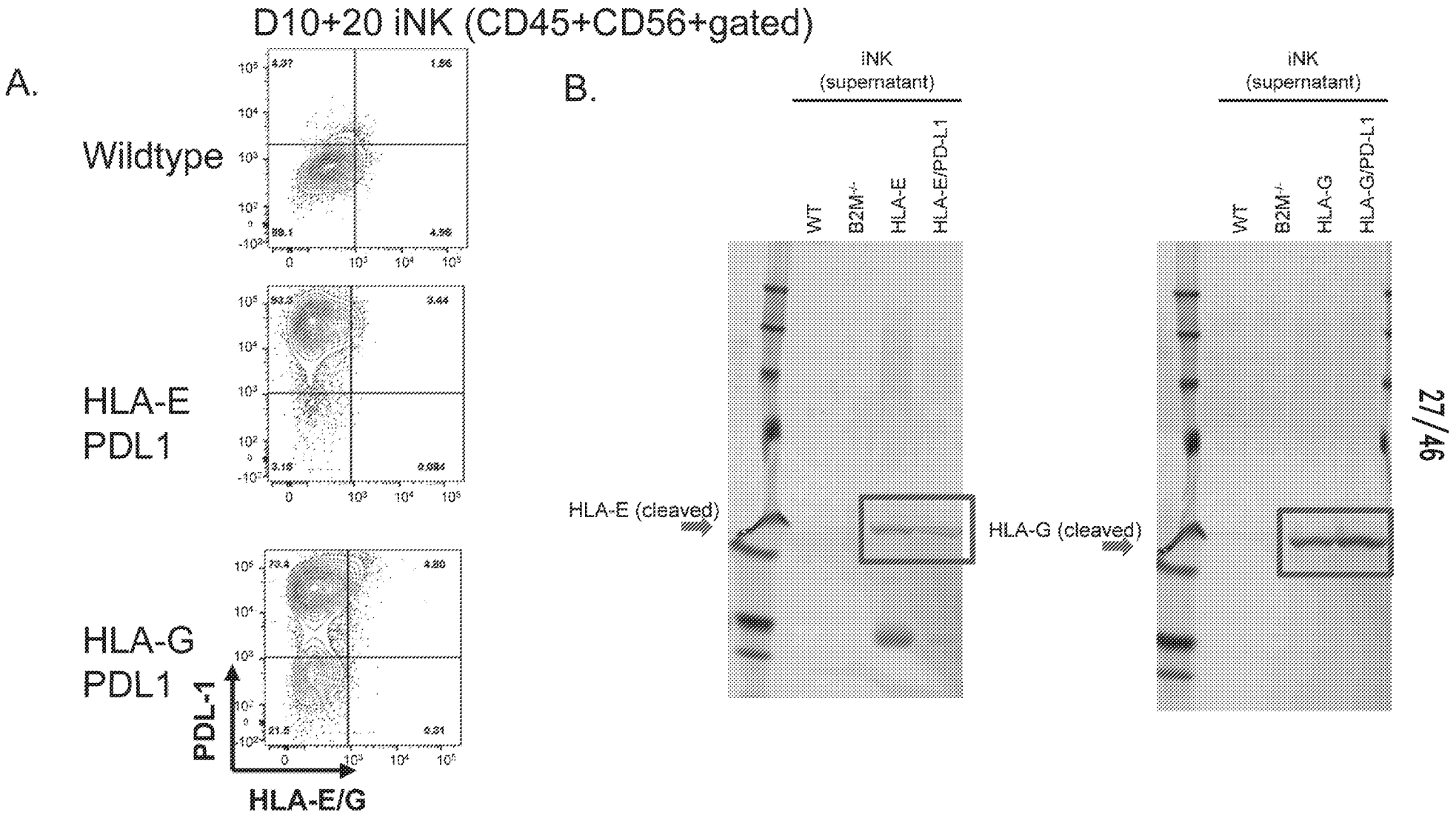
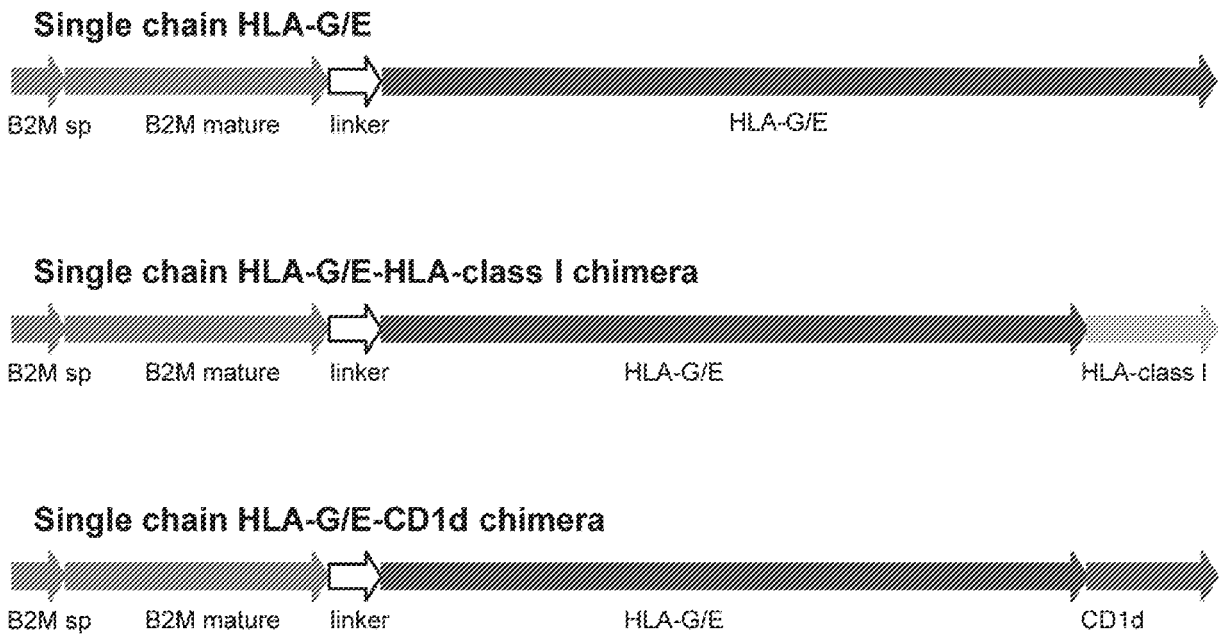

D10+20 iNK (CD45+CD56+gated)
A.
Wildtype
HLA-E
PDL1
HLA-G
PDL1
PDL-1
HLA-E/G
B.
iNK
(supernatant)
WT
B2M-/-
HLA-E
HLA-E/PD-L1
HLA-E (cleaved)
iNK
(supernatant)
WT
B2M-/-
HLA-G
HLA-G/PD-L1
HLA-G (cleaved)

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.

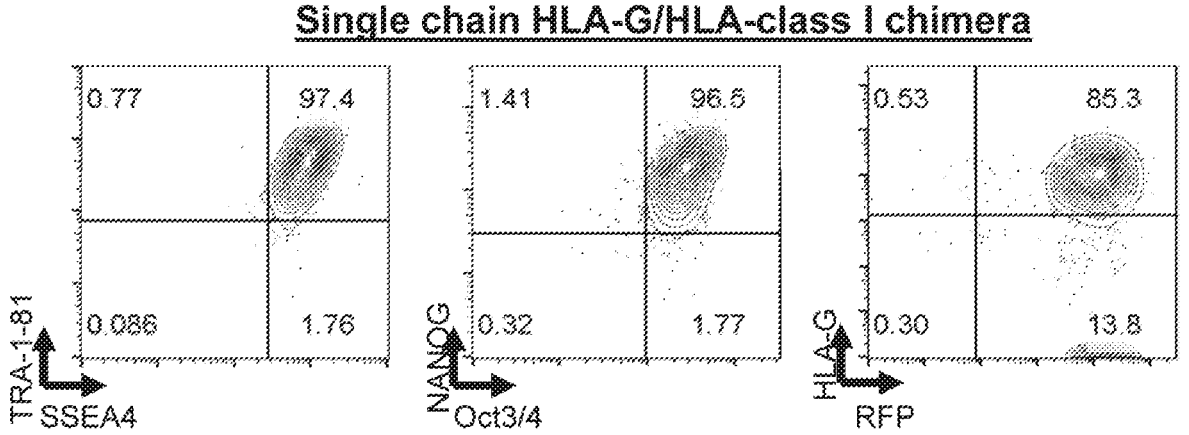

Single chain HLA-G/HLA-class I chimera
0.77
97.4
0.086
1.76
TRA-1-81
SSEA4
1.41
96.6
0.32
1.77
NANOG
Oct3/4
0.53
85.3
0.30
13.8
HLA-G
RFP

FIG. 24

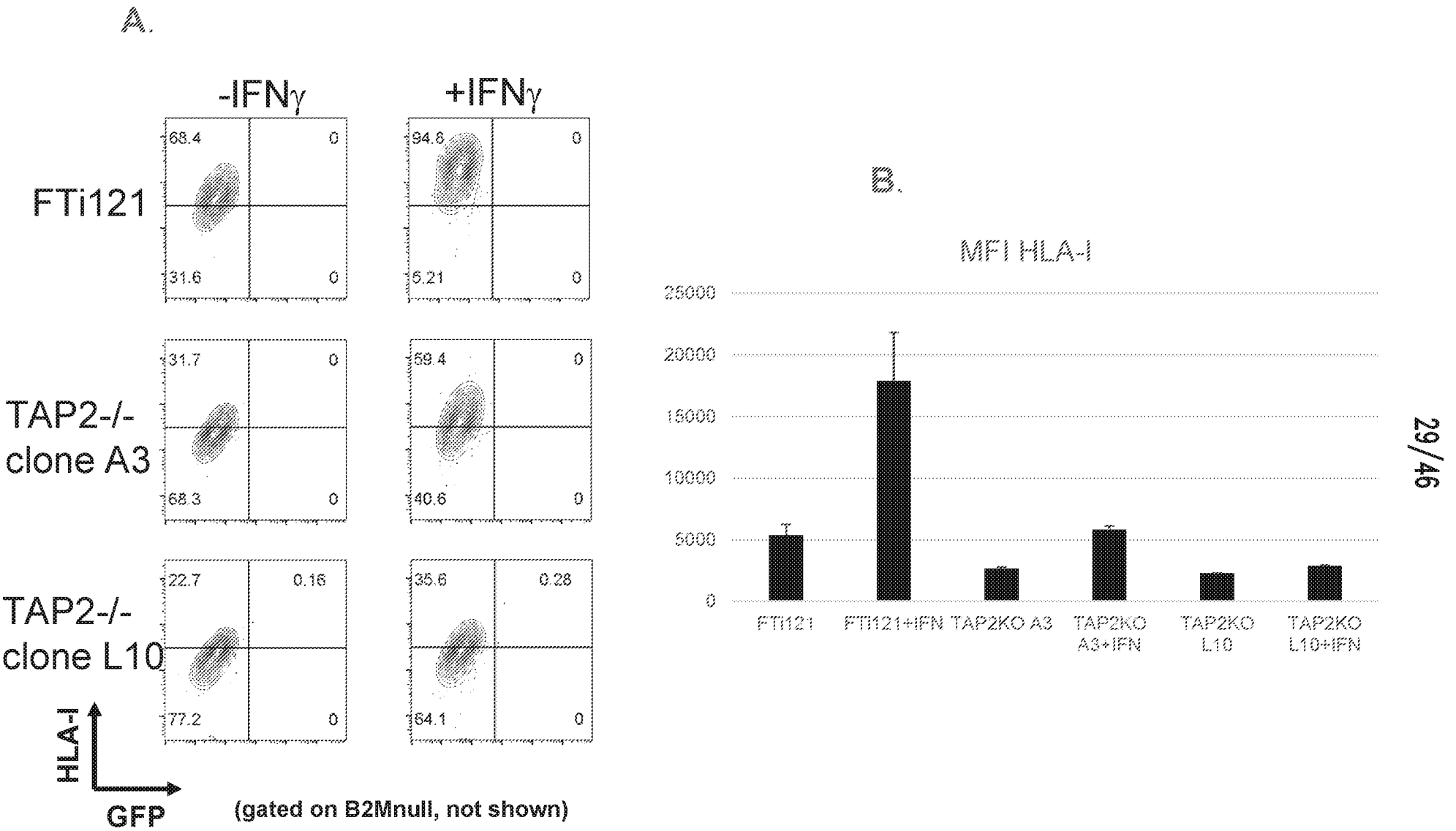

A.
-IFNγ
+IFNγ
FTi121
TAP2-/- clone A3
TAP2-/- clone L10
68.4
0
31.6
0
94.6
0
5.21
0
31.7
0
68.3
0
59.4
0
40.6
0
22.7
0.16
77.2
0
35.6
0.28
64.1
0
HLA-I
GFP
(gated on B2Mnull, not shown)
B.
MFI HLA-I
25000
20000
15000
10000
5000
0
FTi121
FTi121+IFN
TAP2KO A3
TAP2KO A3+IFN
TAP2KO L10
TAP2KO L10+IFN

FIG. 25

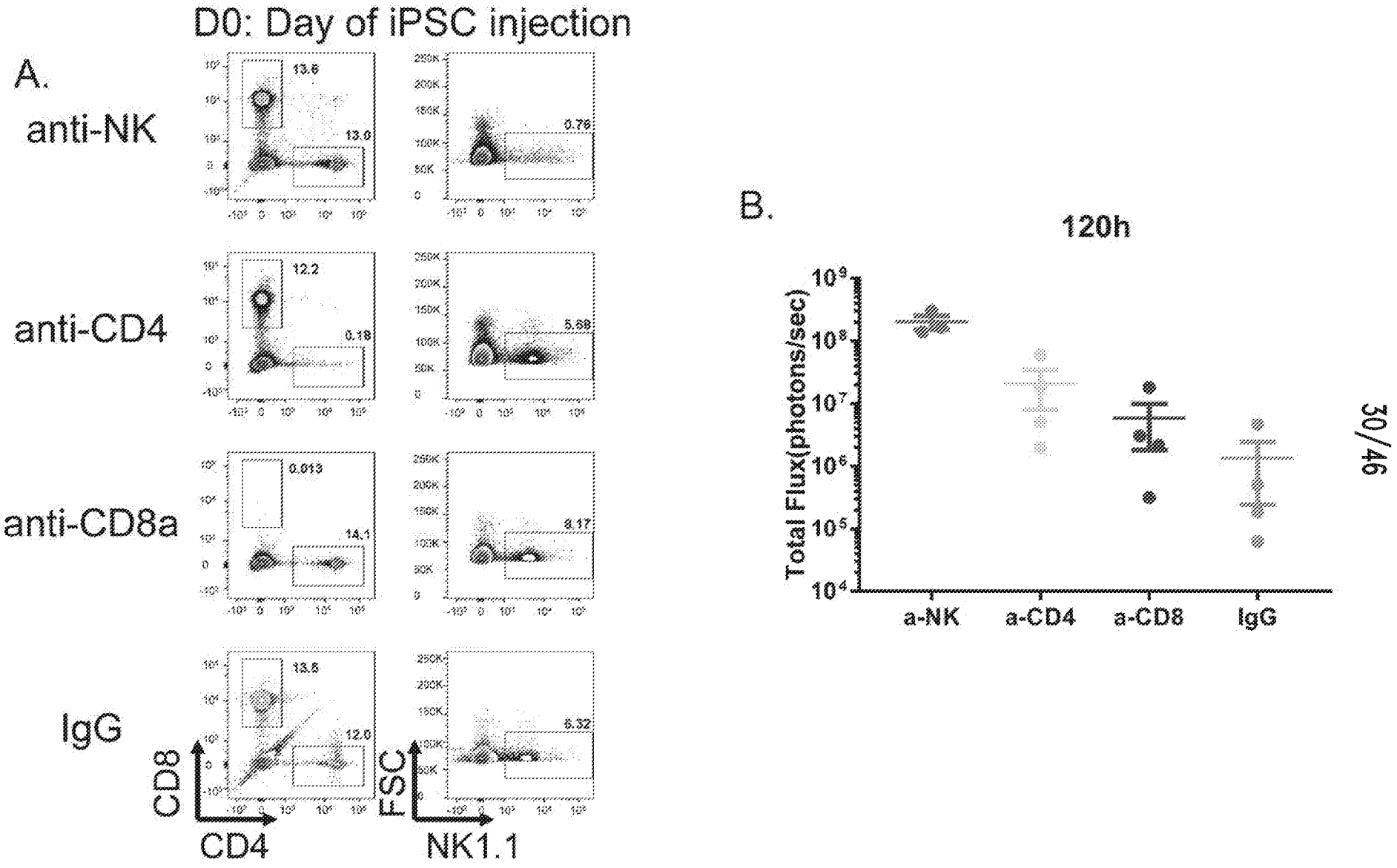

D0: Day of iPSC injection
A.
anti-NK
anti-CD4
anti-CD8a
IgG
13.6
13.0
0.76
12.2
0.18
5.58
0.013
14.1
8.17
13.5
12.0
6.32
CD8
CD4
FSC
NK1.1
B.
120h
Total Flux(photons/sec)
a-NK
a-CD4
a-CD8
IgG
30/46

FIG. 26

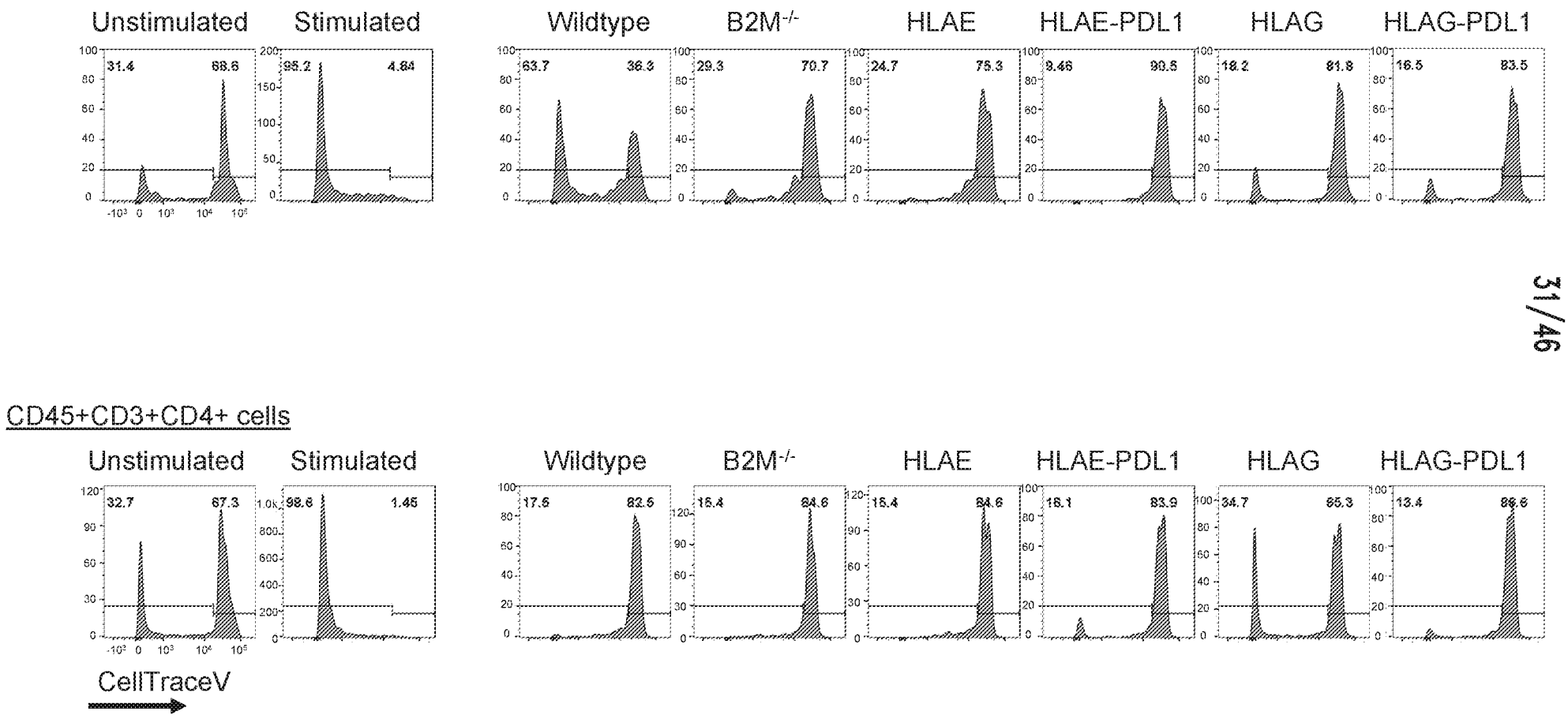

CD45+CD3+CD8+ cells
Unstimulated
Stimulated
Wildtype
B2M-/-
HLAE
HLAE-PDL1
HLAG
HLAG-PDL1
31.4
68.6
95.2
4.84
63.7
36.3
29.3
70.7
24.7
75.3
9.46
90.5
18.2
81.8
16.5
83.5
CD45+CD3+CD4+ cells
Unstimulated
Stimulated
Wildtype
B2M-/-
HLAE
HLAE-PDL1
HLAG
HLAG-PDL1
32.7
67.3
98.6
1.45
17.6
82.5
15.4
84.6
15.4
84.6
16.1
83.9
34.7
65.3
13.4
86.6
CellTraceV

FIG. 27A

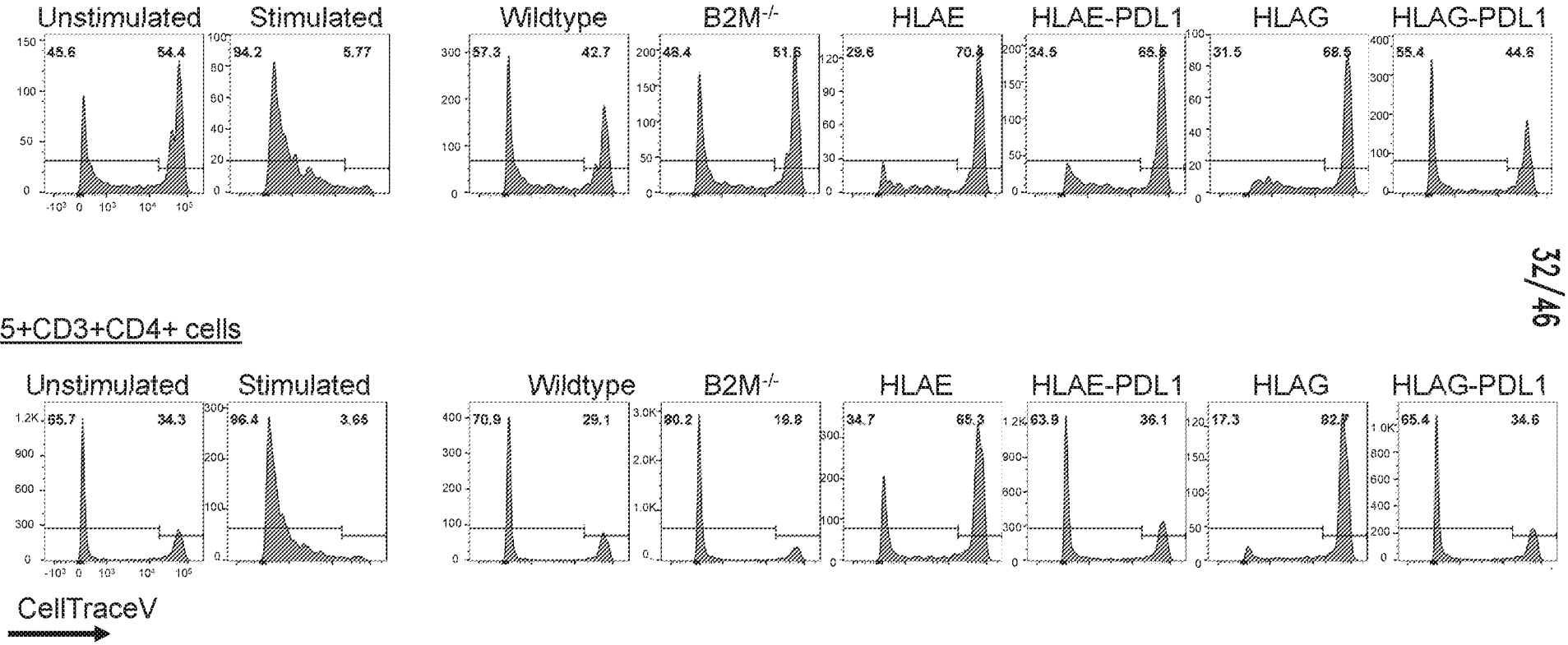
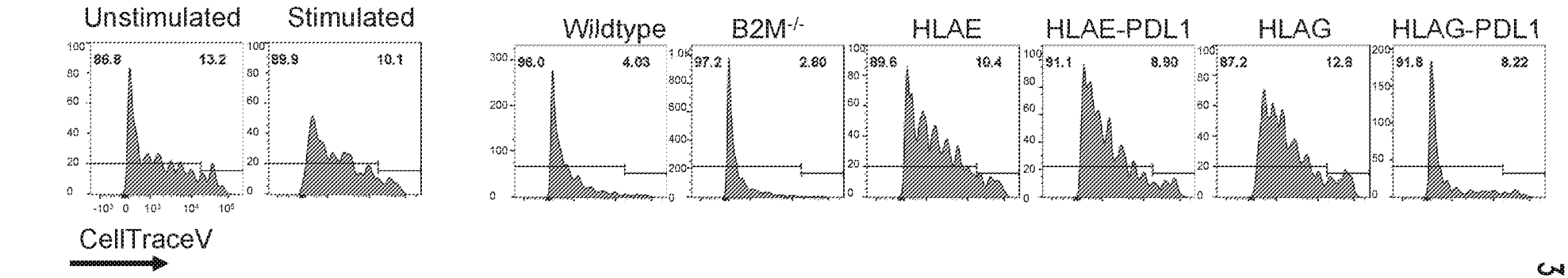

CD45+CD3+CD8+ cells
Unstimulated
Stimulated
Wildtype
B2M-/-
HLAE
HLAE-PDL1
HLAG
HLAG-PDL1
45.6
54.4
94.2
5.77
57.3
42.7
48.4
51.6
29.6
70.4
34.5
65.5
31.5
68.5
55.4
44.6
CD45+CD3+CD4+ cells
Unstimulated
Stimulated
Wildtype
B2M-/-
HLAE
HLAE-PDL1
HLAG
HLAG-PDL1
65.7
34.3
96.4
3.65
70.9
29.1
80.2
19.8
34.7
65.3
63.9
36.1
17.3
82.7
65.4
34.6
CellTraceV

FIG. 27B


CD45+CD56+
Unstimulated
Stimulated
Wildtype
B2M-/-
HLAE
HLAE-PDL1
HLAG
HLAG-PDL1
86.8
13.2
89.9
10.1
96.0
4.03
97.2
2.80
89.6
10.4
91.1
8.90
87.2
12.8
91.8
8.22
CellTraceV

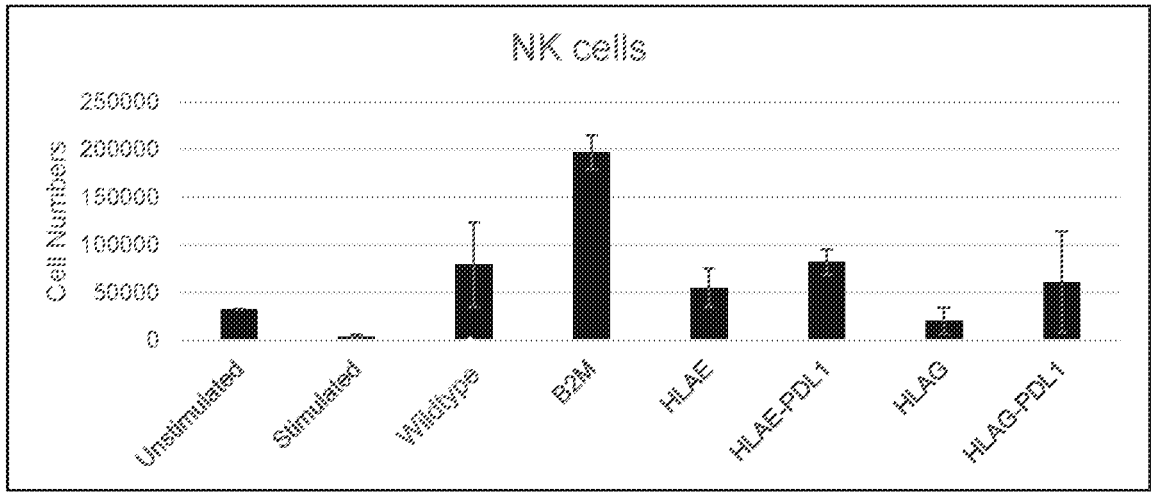

NK cells
Cell Numbers
250000
200000
150000
100000
50000
0
Unstimulated
Stimulated
Wildtype
B2M
HLAE
HLAE-PDL1
HLAG
HLAG-PDL1

FIG. 27C

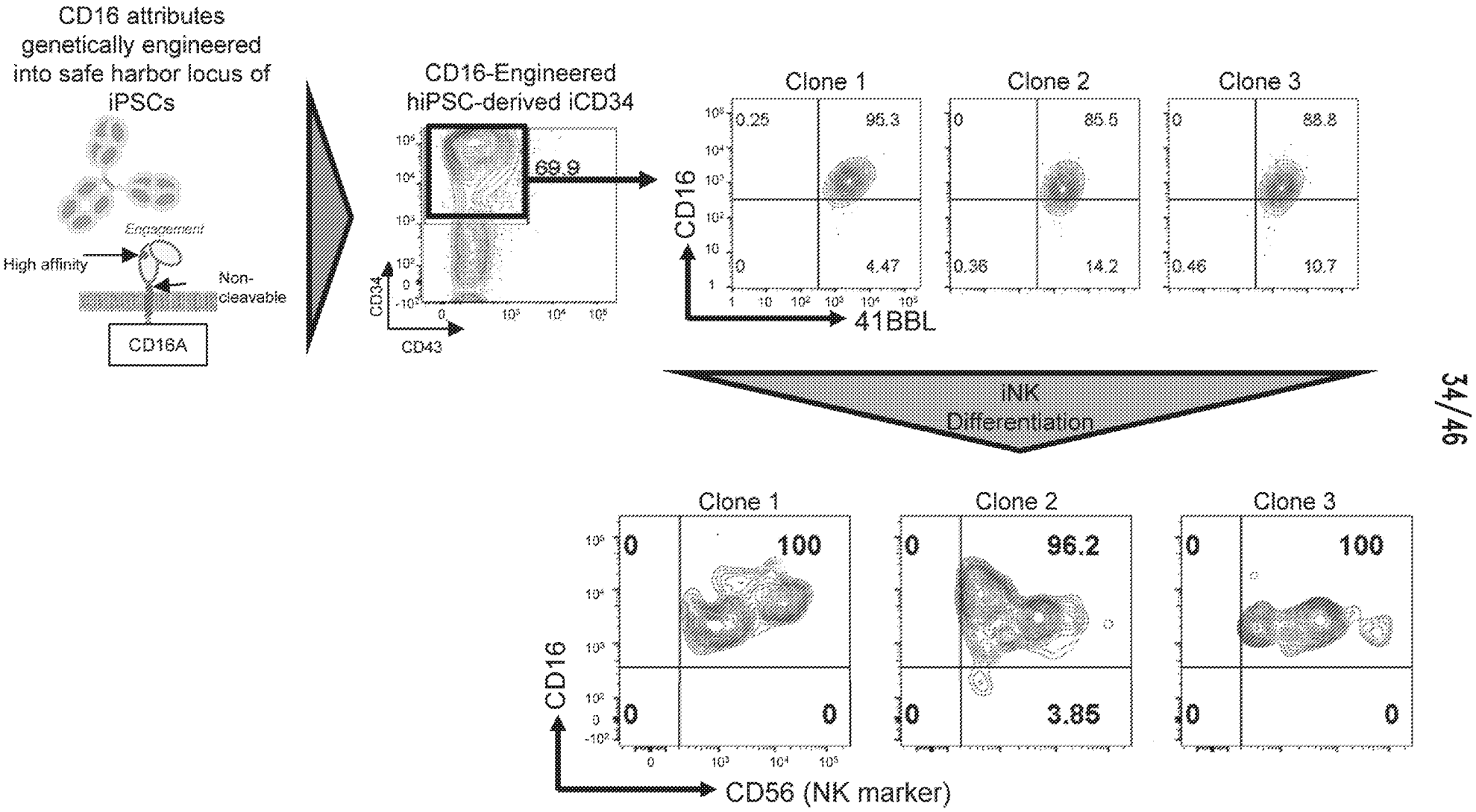
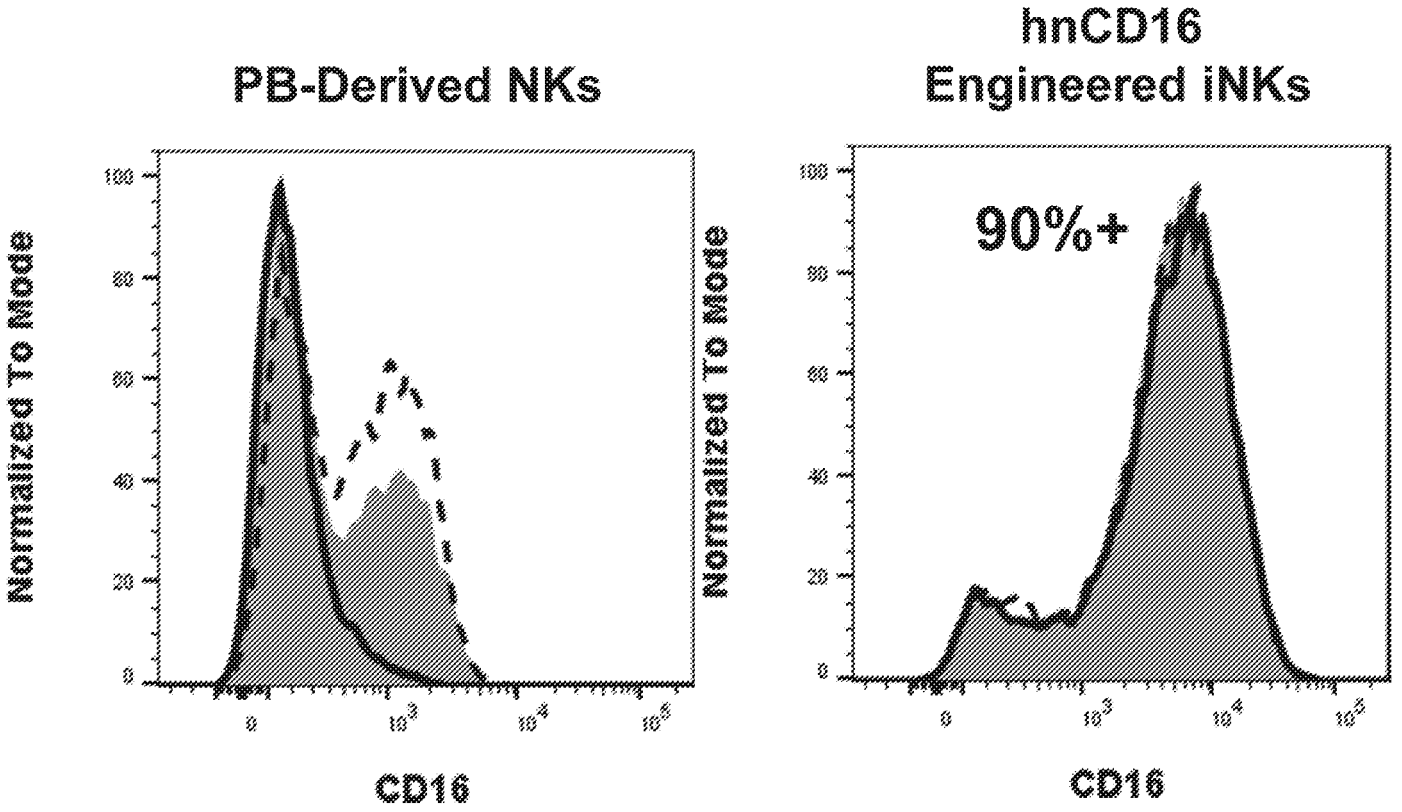
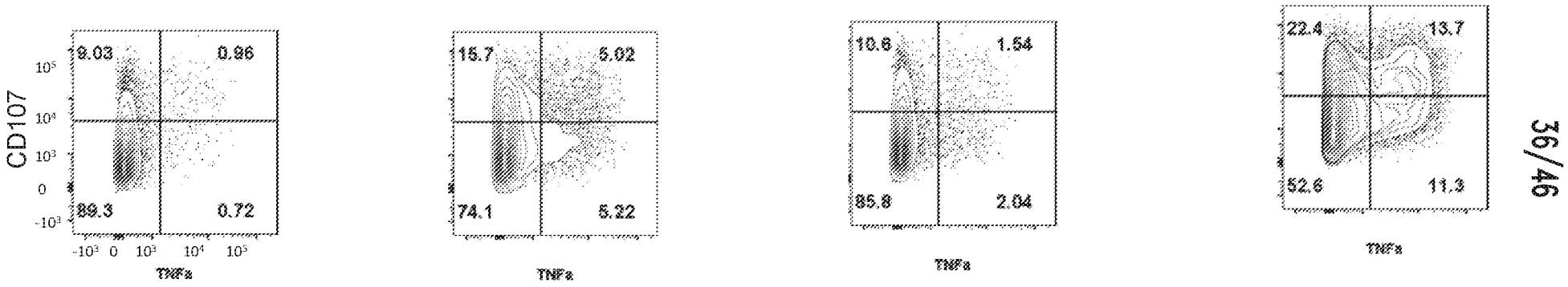

CD16 attributes genetically engineered into safe harbor locus of iPSCs
Engagement
High affinity
Non-cleavable
CD16A
CD16-Engineered hiPSC-derived iCD34
69.9
CD34
CD43
Clone 1
Clone 2
Clone 3
0.25
95.3
4.47
0
85.6
0.36
14.2
88.8
0.46
10.7
CD16
41BBL
iNK Differentiation
100
96.2
3.85
CD56 (NK marker)

FIG. 28A



Gray = Control (homeostasis in culture)
Dashed = Treated with TACE/ADAM inhibitor (inhibit cleavage)
Black = Stimulated with K562 (Activation induced CD16 shedding)

FIG. 28B



Unstimulated Anti-CD16 P815 P815 + Anti-CD16

FIG. 29A

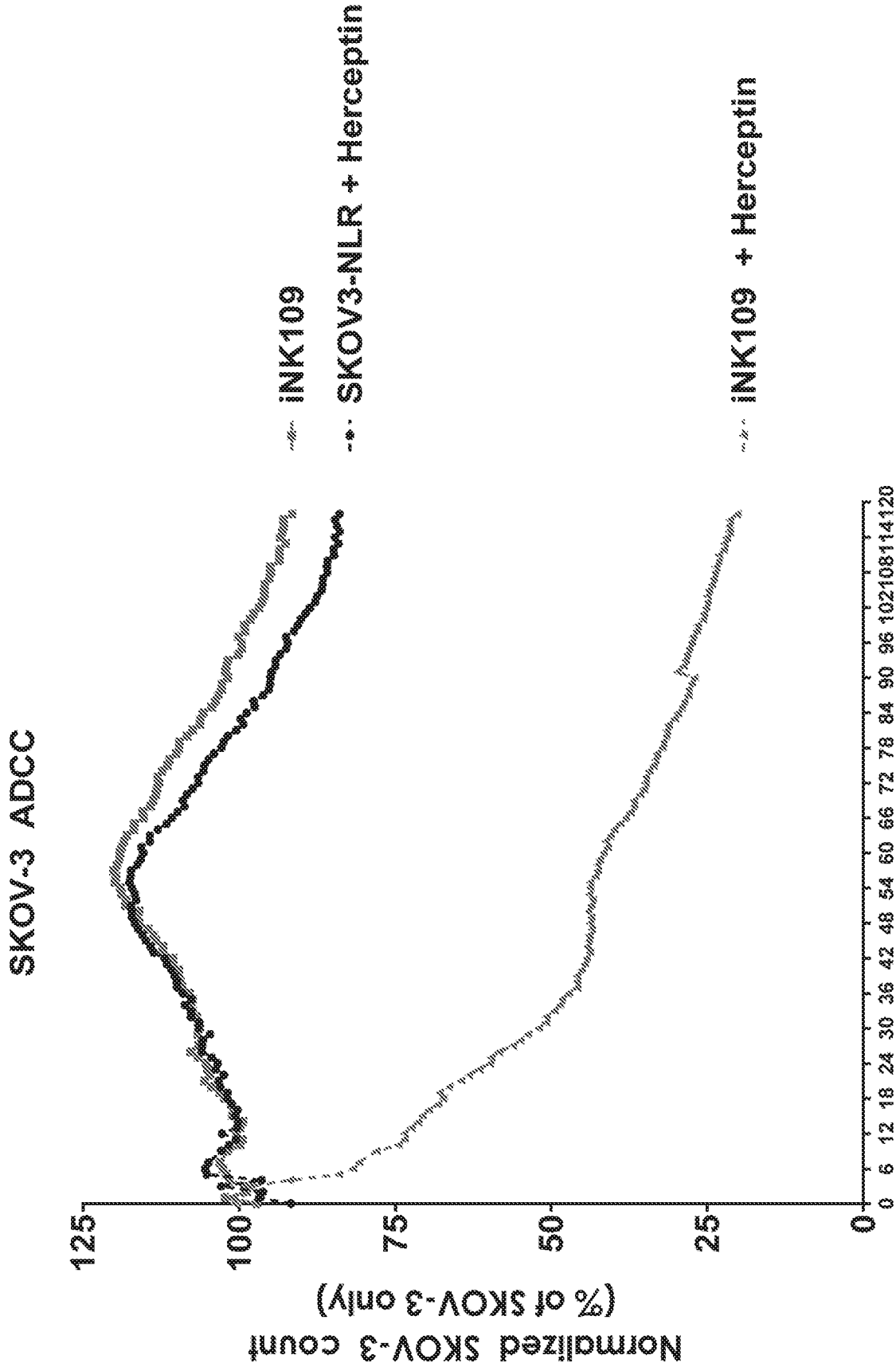

SKOV-3 ADCC
iNK109
SKOV3-NLR + Herceptin
iNK109 + Herceptin
Normalized SKOV-3 count
(% of SKOV-3 only)
125
100
75
50
25
0
0 6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96 102108114120
Time, hrs

FIG. 29B

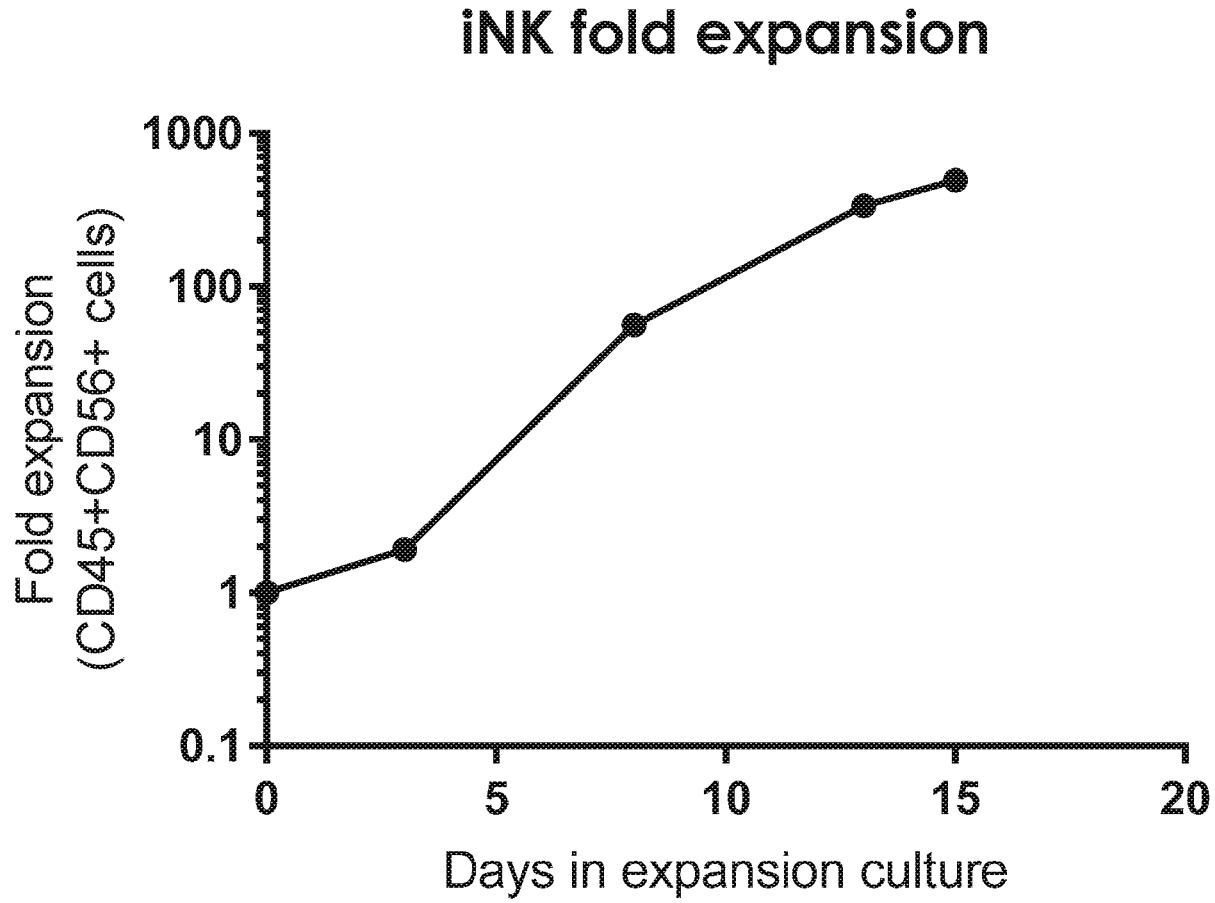

iNK fold expansion
Fold expansion
(CD45+CD56+ cells)
1000
100
10
1
0.1
0
5
10
15
20
Days in expansion culture

FIG. 30

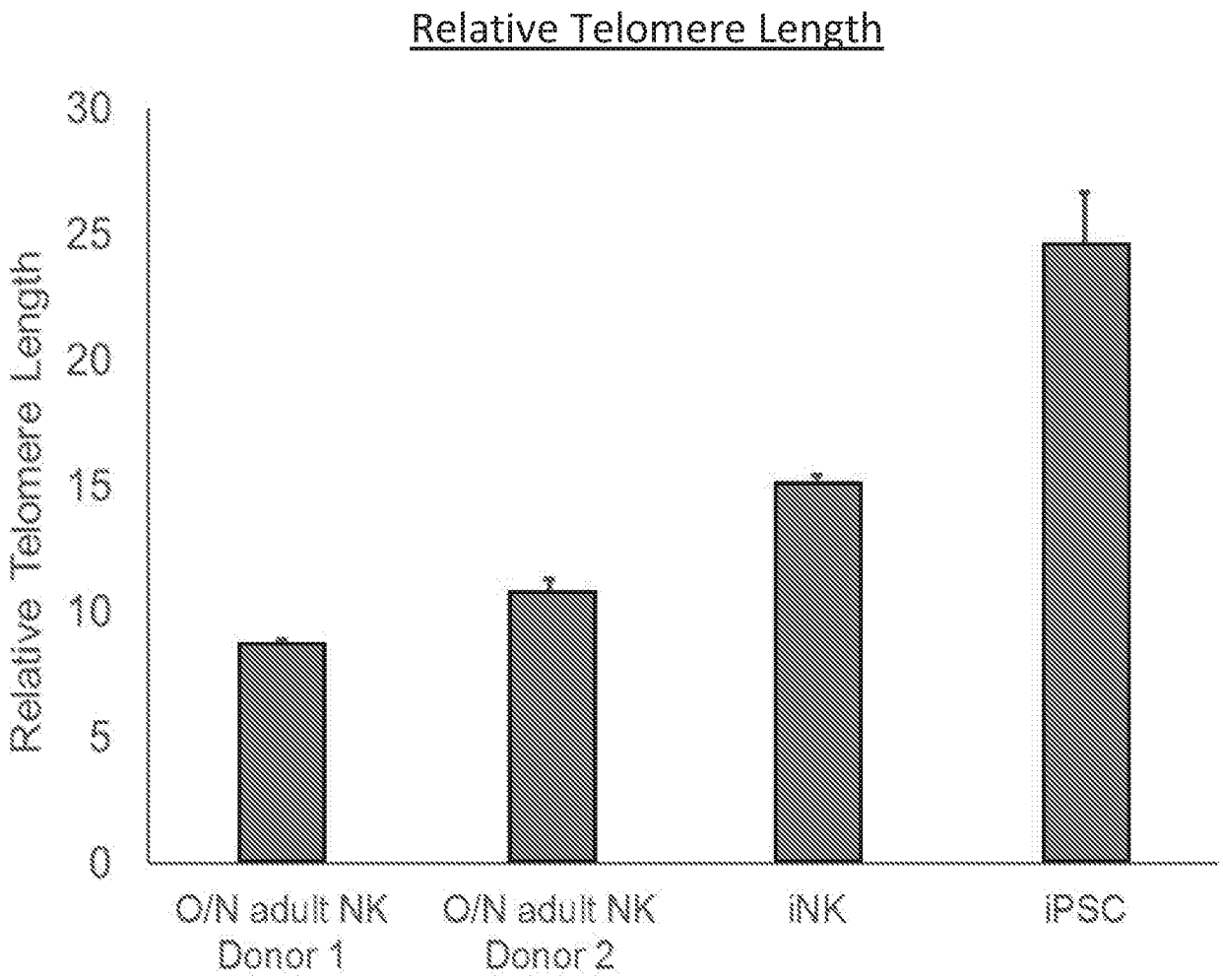

Relative Telomere Length
Relative Telomere Length
30
25
20
15
10
5
0
O/N adult NK Donor 1
O/N adult NK Donor 2
iNK
iPSC

FIG. 31

Tet-inducible CAR-2A-LNGFR expression in transduced iPSCs

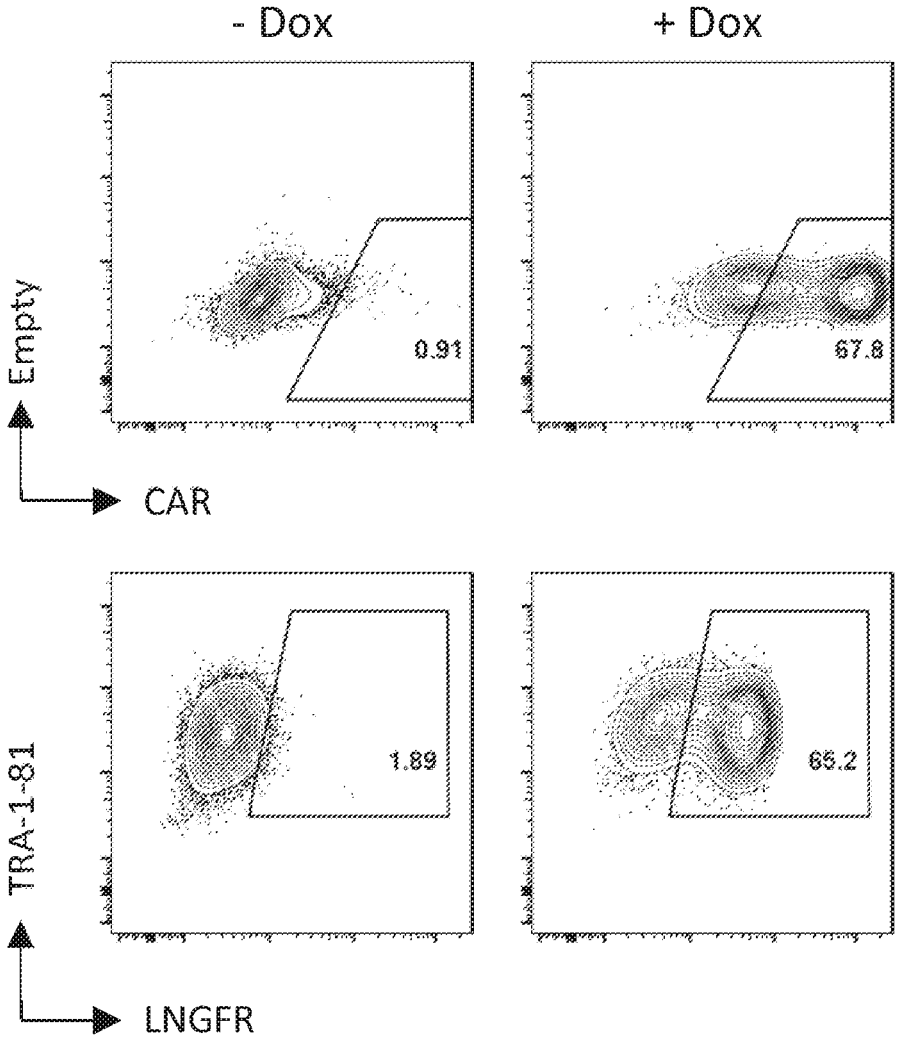


FIG. 32A

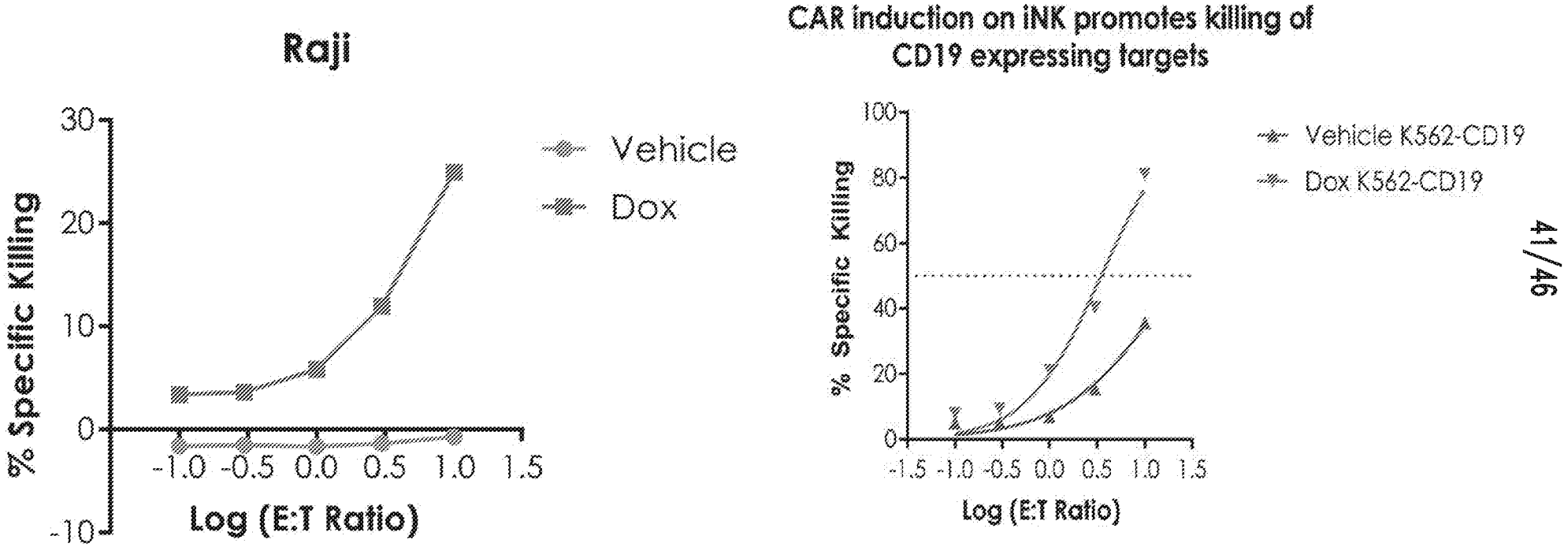

Raji
30
20
10
0
-10
% Specific Killing
-1.0 -0.5 0.0 0.5 1.0 1.5
Log (E:T Ratio)
Vehicle
Dox
CAR induction on iNK promotes killing of
CD19 expressing targets
100
80
60
40
20
0
% Specific Killing
-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5
Log (E:T Ratio)
Vehicle K562-CD19
Dox K562-CD19

FIG. 32B

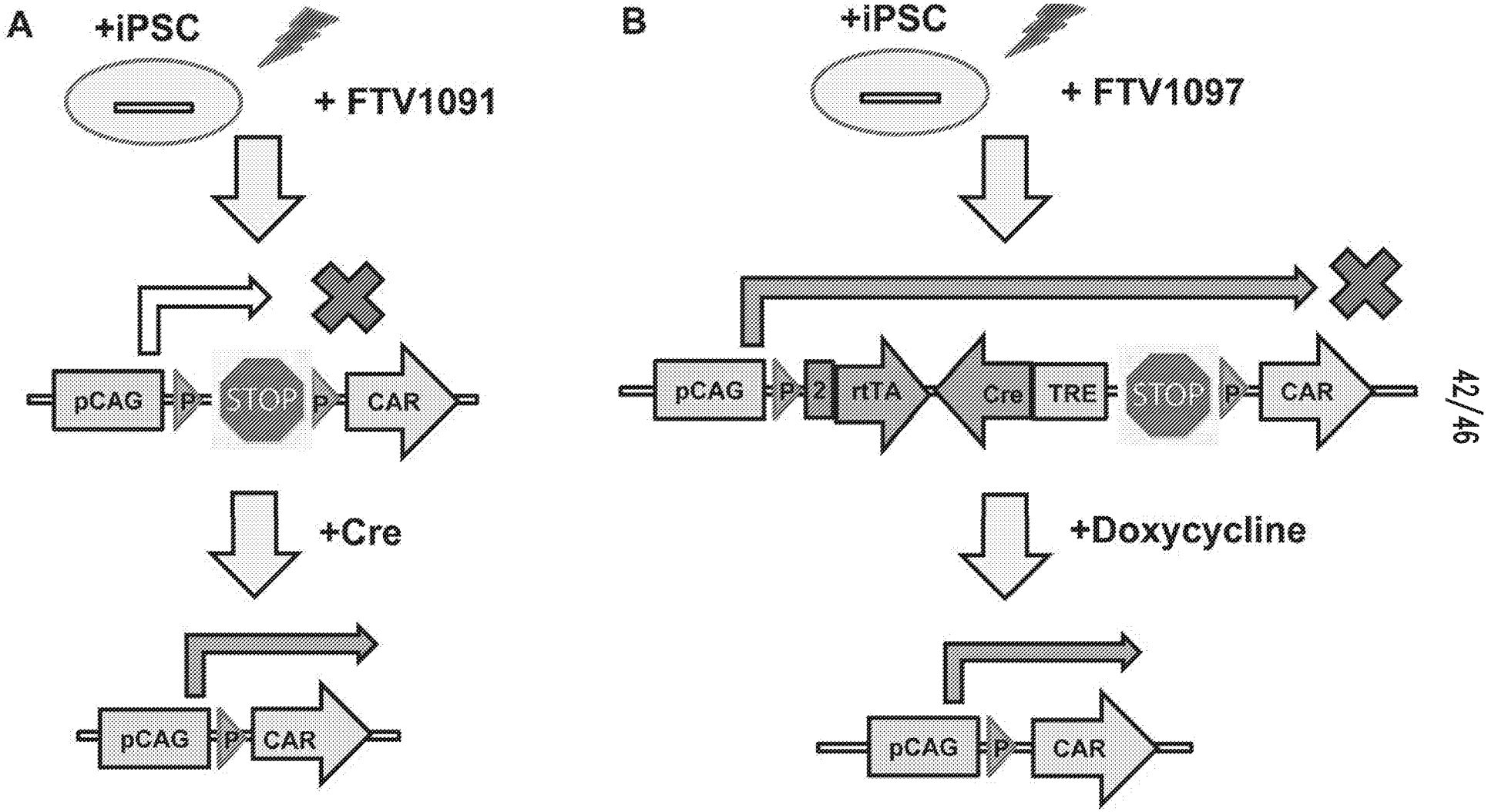

A
+iPSC
+ FTV1091
pCAG
P
STOP
P
CAR
+Cre
pCAG
P
CAR
B
+iPSC
+ FTV1097
pCAG
P
2
rtTA
Cre
TRE
STOP
P
CAR
+Doxycycline
pCAG
P
CAR

FIG. 33

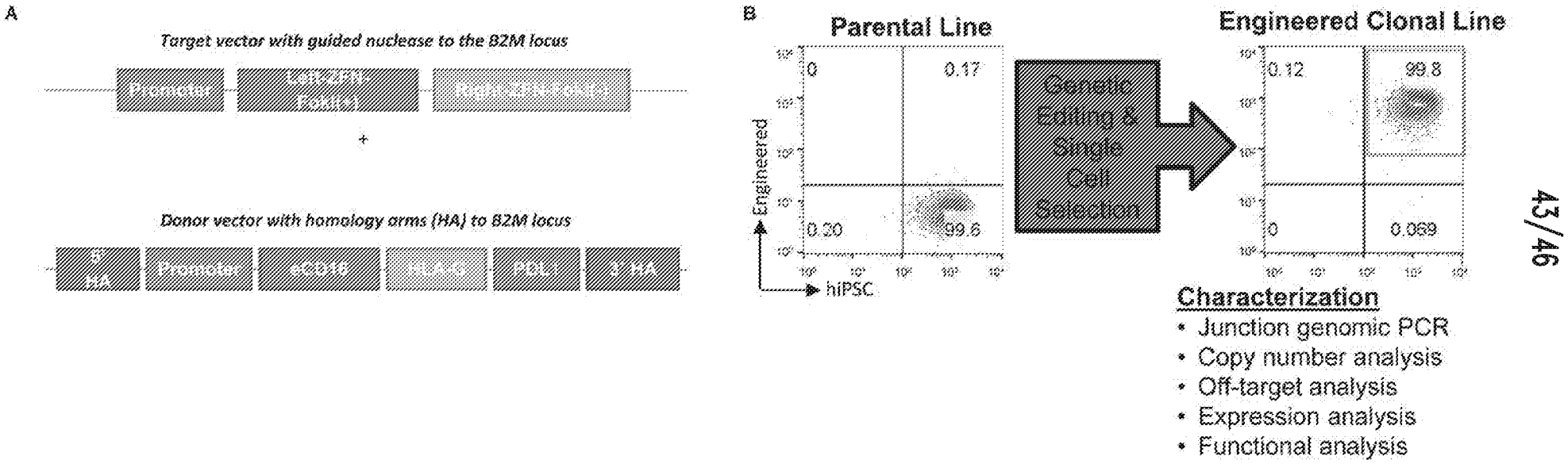

A
Target vector with guided nuclease to the B2M locus
Promoter
Left 2A-FP-Puro(R)
Right 2A-Neo(R)
+
Donor vector with homology arms (HA) to B2M locus
5' HA
Promoter
sCD19
PD-L1
3' HA
B
Parental Line
Engineered
hiPSC
0
0.17
0.30
99.6
Genetic Editing & Single Cell Selection
Engineered Clonal Line
0.12
99.8
0
0.069
Characterization
• Junction genomic PCR
• Copy number analysis
• Off-target analysis
• Expression analysis
• Functional analysis

FIG. 34

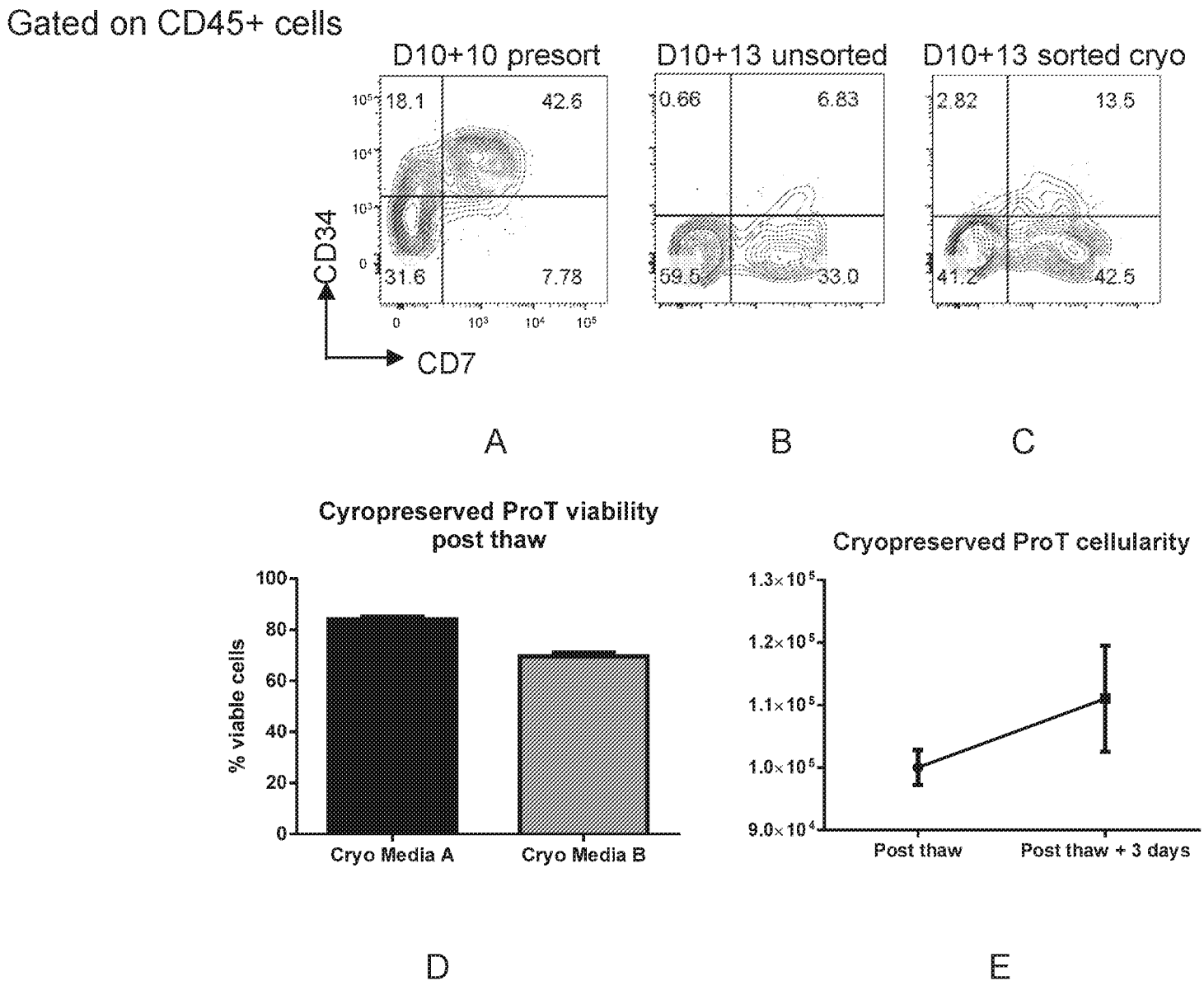

Gated on CD45+ cells
D10+10 presort
D10+13 unsorted
D10+13 sorted cryo
18.1
42.6
31.6
7.78
0.66
6.83
59.5
33.0
2.82
13.5
41.2
42.5
CD34
CD7
A
B
C
Cyropreserved ProT viability post thaw
% viable cells
Cryo Media A
Cryo Media B
Cryopreserved ProT cellularity
Post thaw
Post thaw + 3 days
D
E

FIG. 35

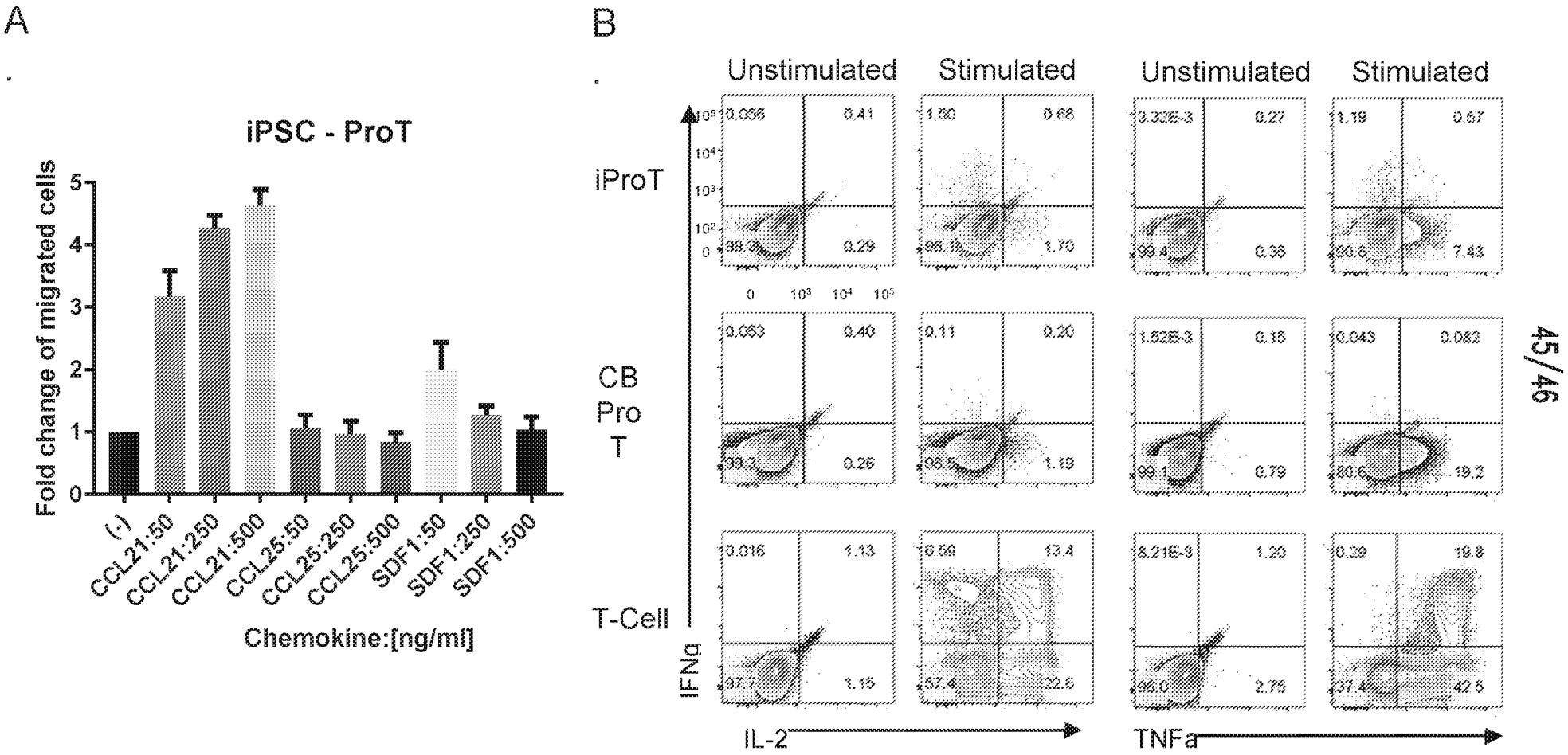

A
iPSC - ProT
Fold change of migrated cells
0
1
2
3
4
5
(-)
CCL21:50
CCL21:250
CCL21:500
CCL25:50
CCL25:250
CCL25:500
SDF1:50
SDF1:250
SDF1:500
Chemokine:[ng/ml]
B
Unstimulated
Stimulated
Unstimulated
Stimulated
iProT
CB Pro T
T-Cell
IFNg
IL-2
TNFa
0.056
0.41
1.50
0.68
3.32E-3
0.27
1.19
0.67
99.3
0.29
96.1
1.70
99.4
0.38
90.6
7.43
0.053
0.40
0.11
0.20
1.52E-3
0.15
0.043
0.082
99.3
0.26
98.5
1.19
99.1
0.79
80.6
19.2
0.016
1.13
6.59
13.4
8.21E-3
1.20
0.29
19.8
97.7
1.15
57.4
22.6
96.0
2.75
37.4
42.5

FIG. 36

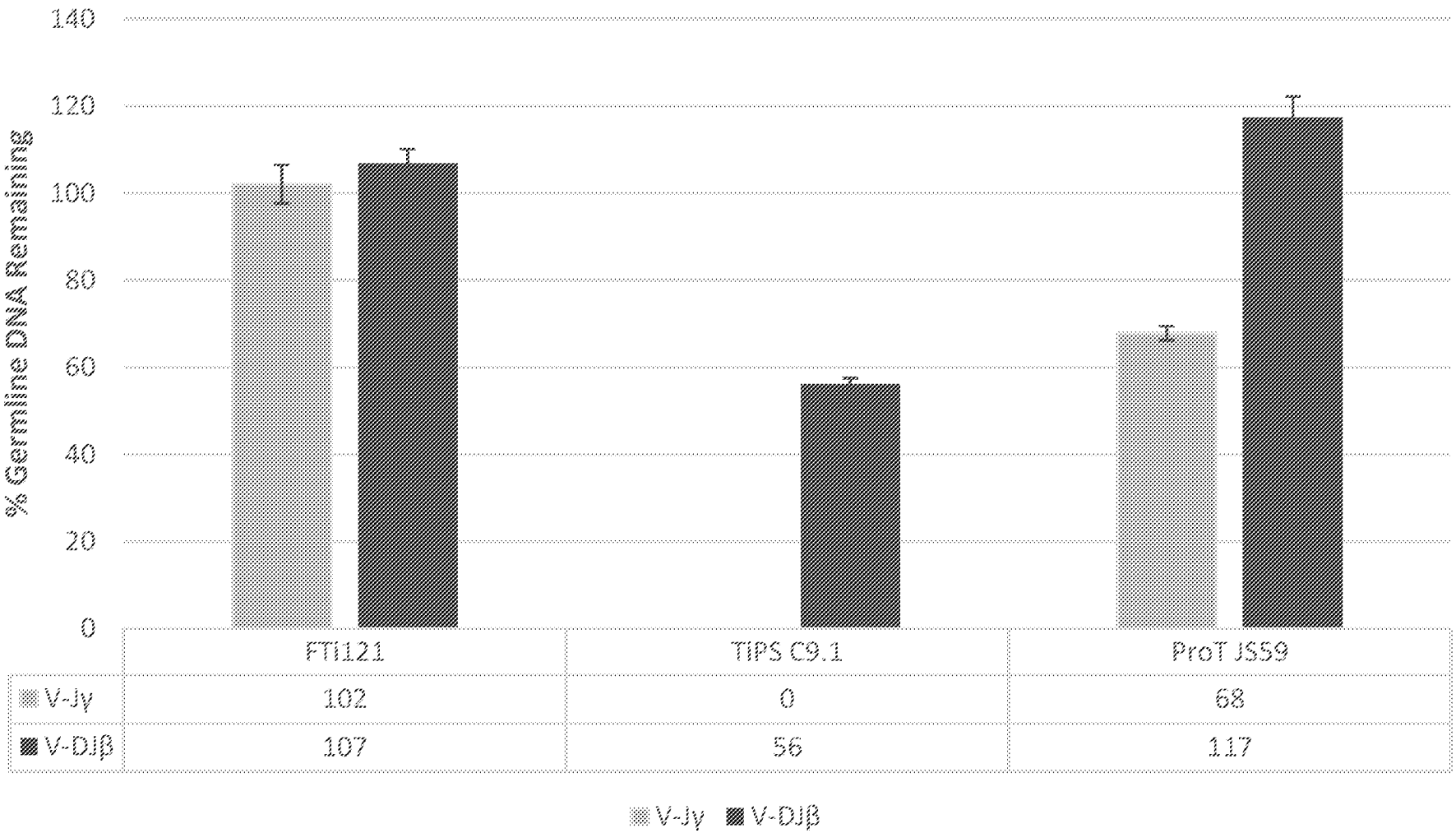

% Germline DNA Remaining
140
120
100
80
60
40
20
0
FTI121
TIPS C9.1
ProT JS59
V-Jγ
V-DJβ
102
0
68
107
56
117
V-Jγ
V-DJβ

FIG. 37