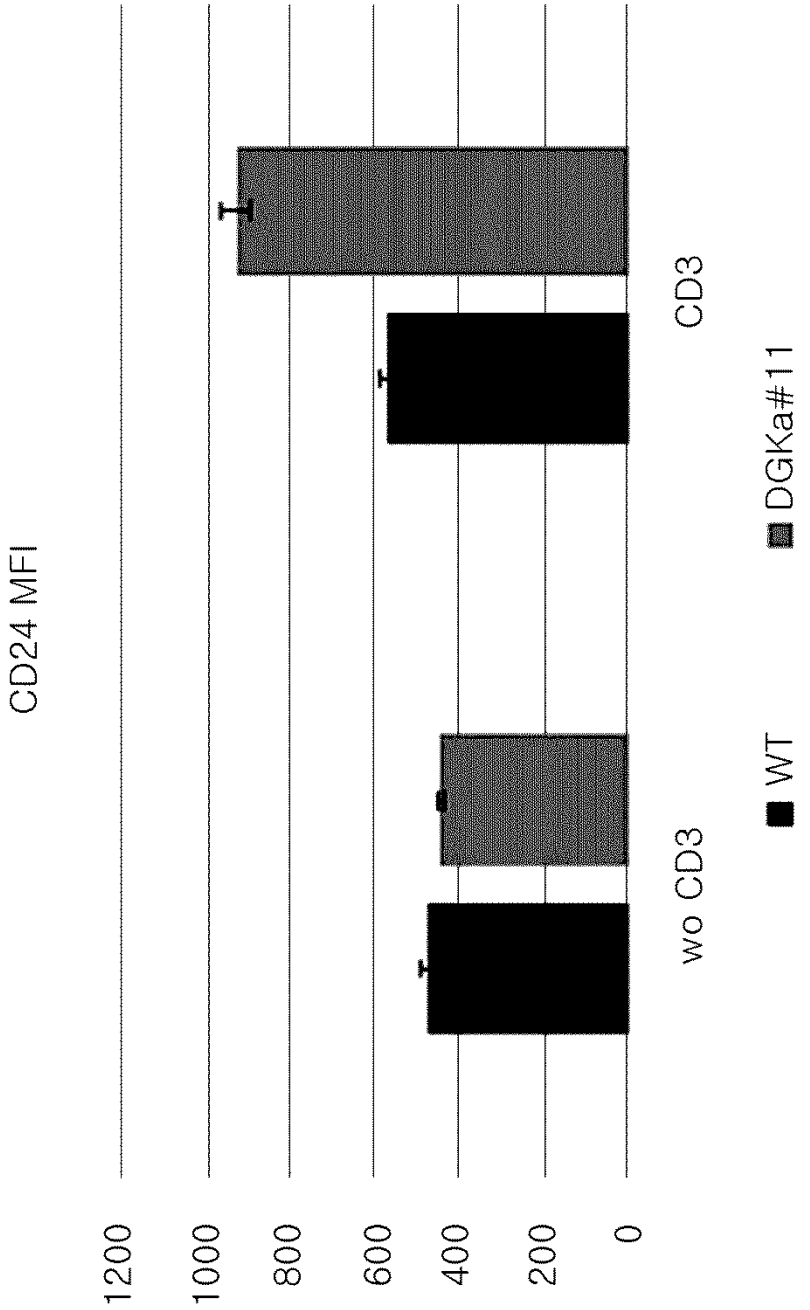
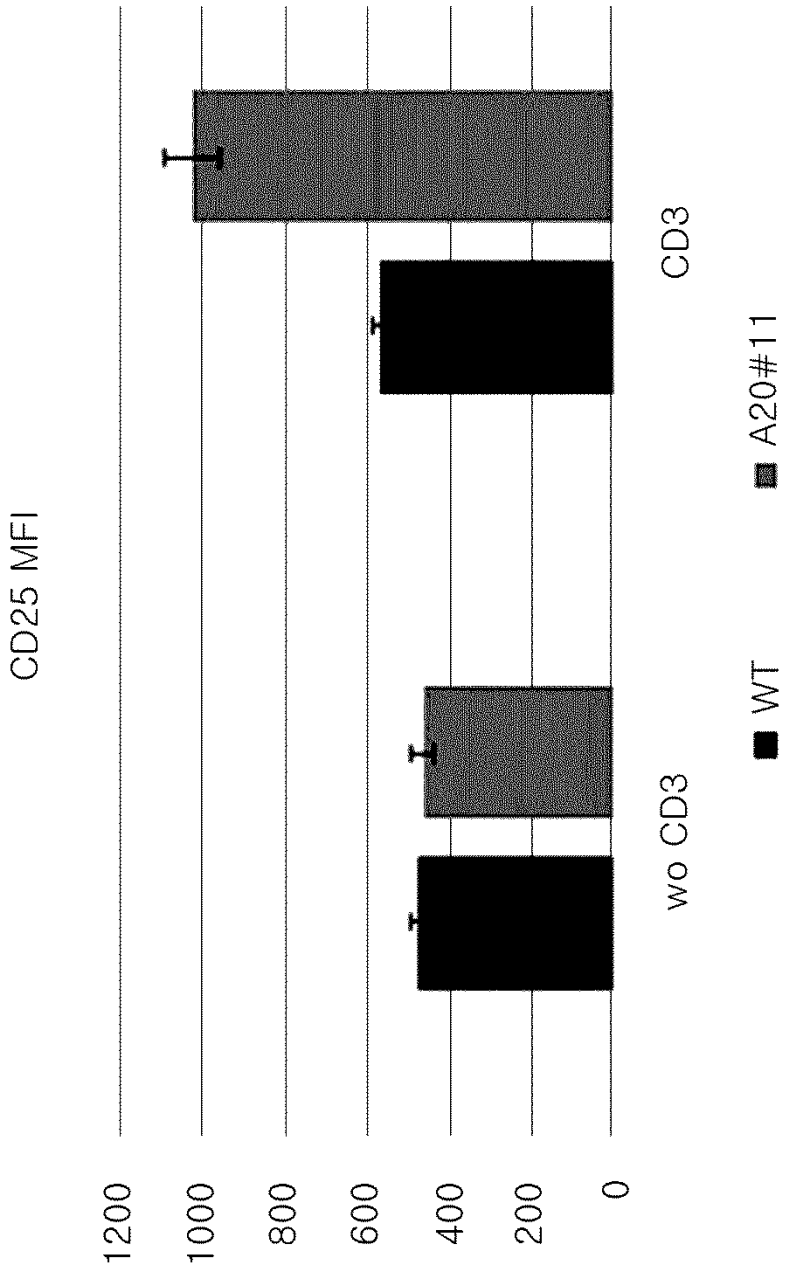
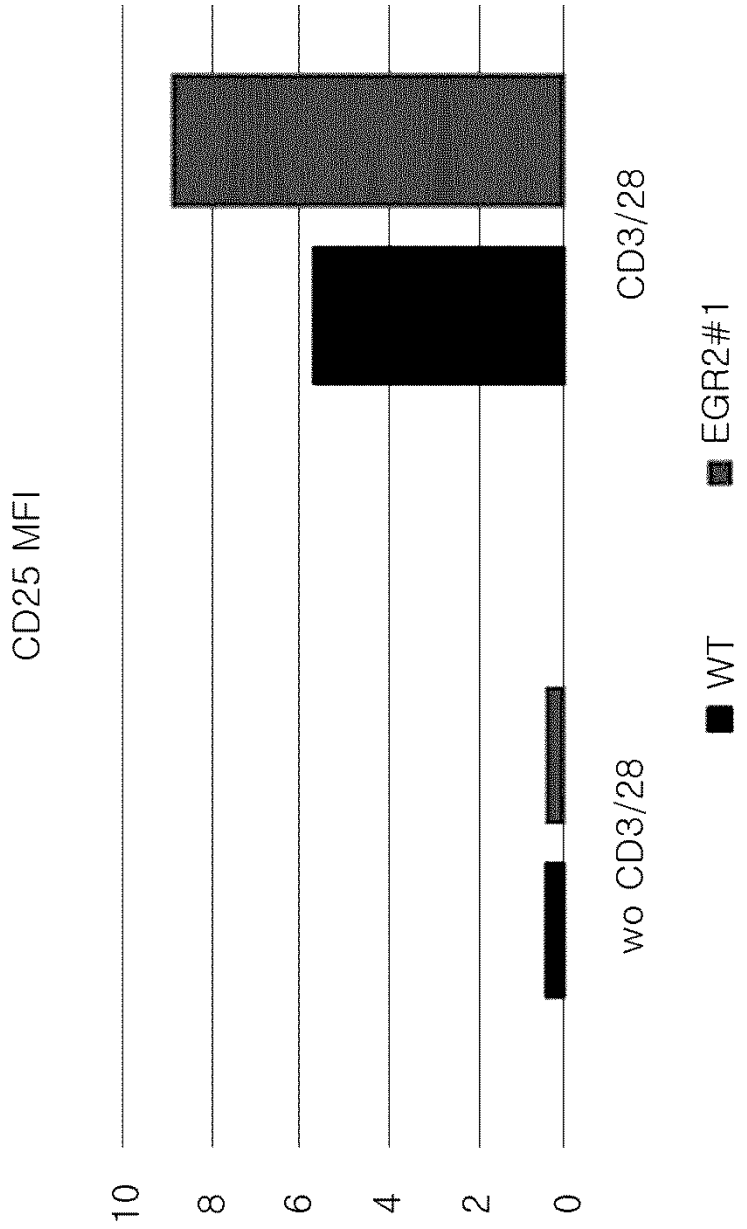
【FIG. 1】



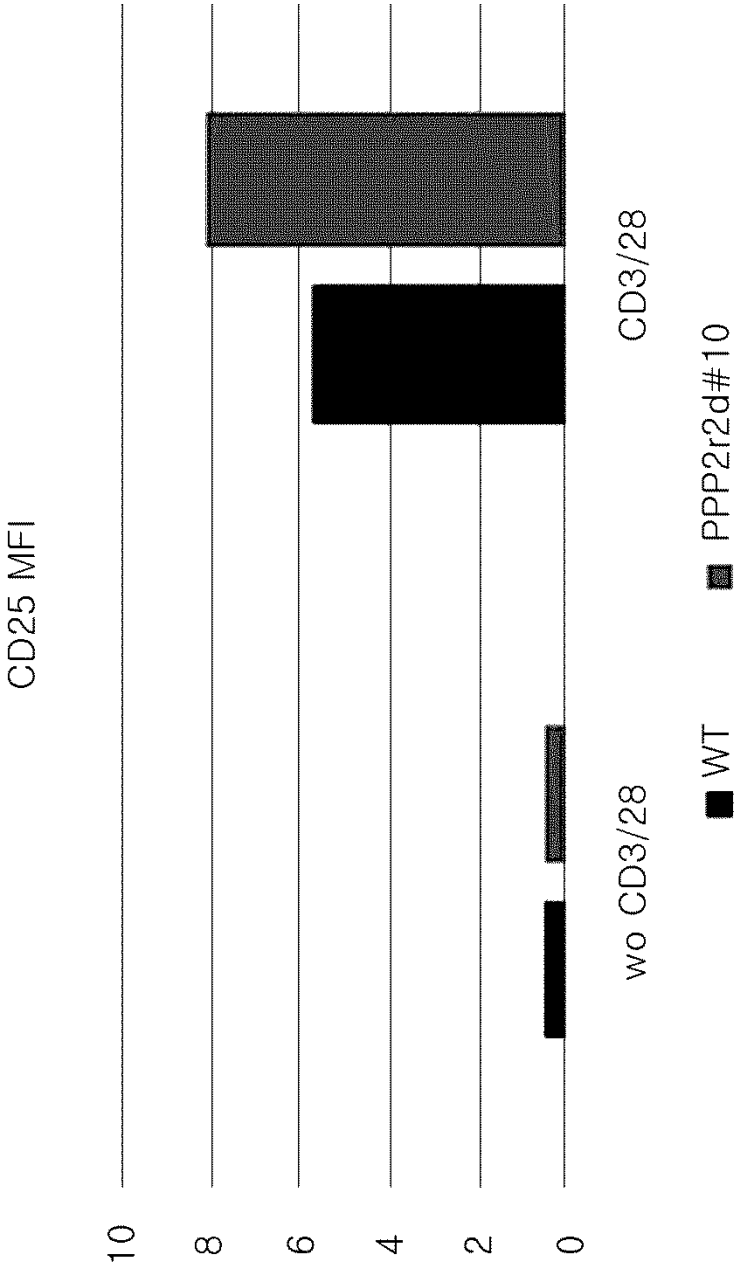
【FIG. 2】



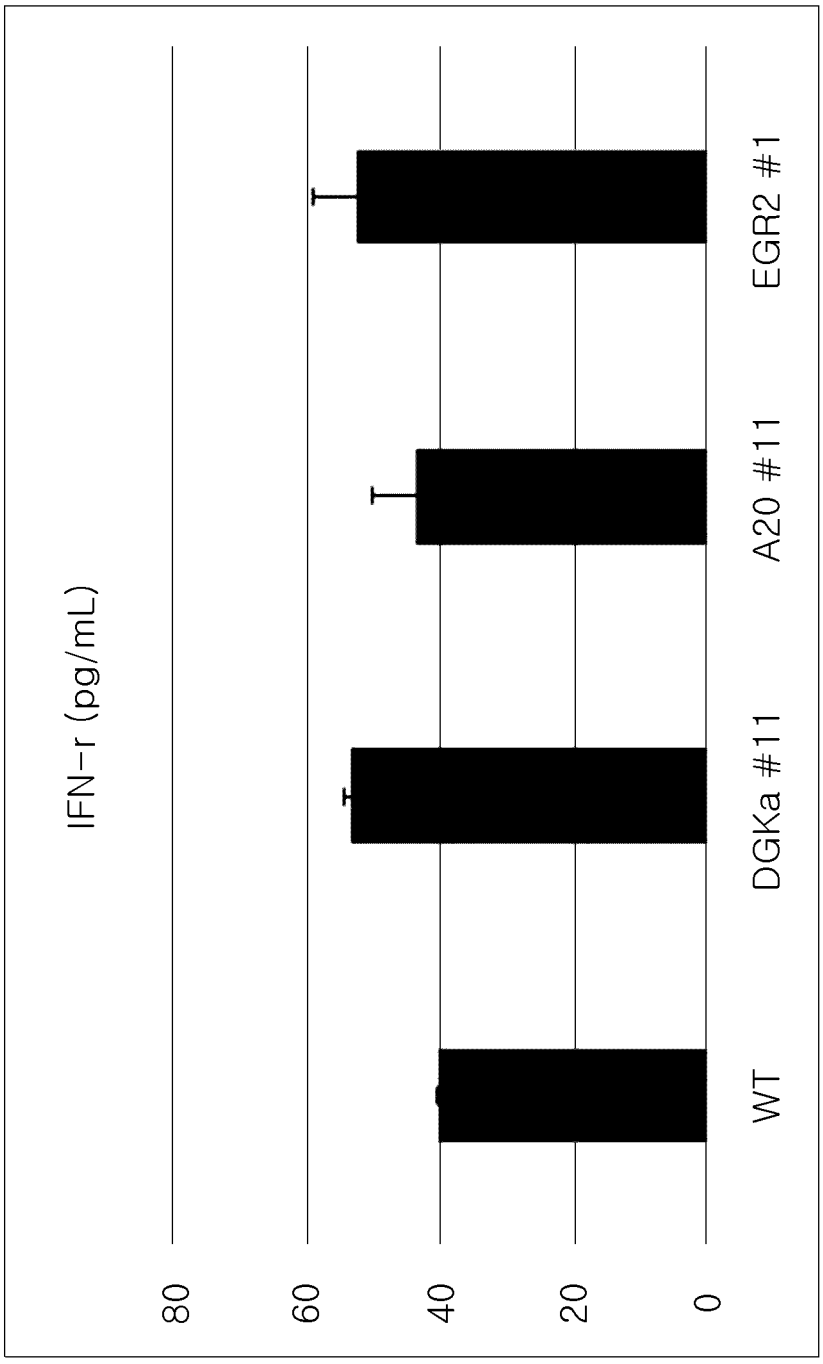
【FIG. 3】



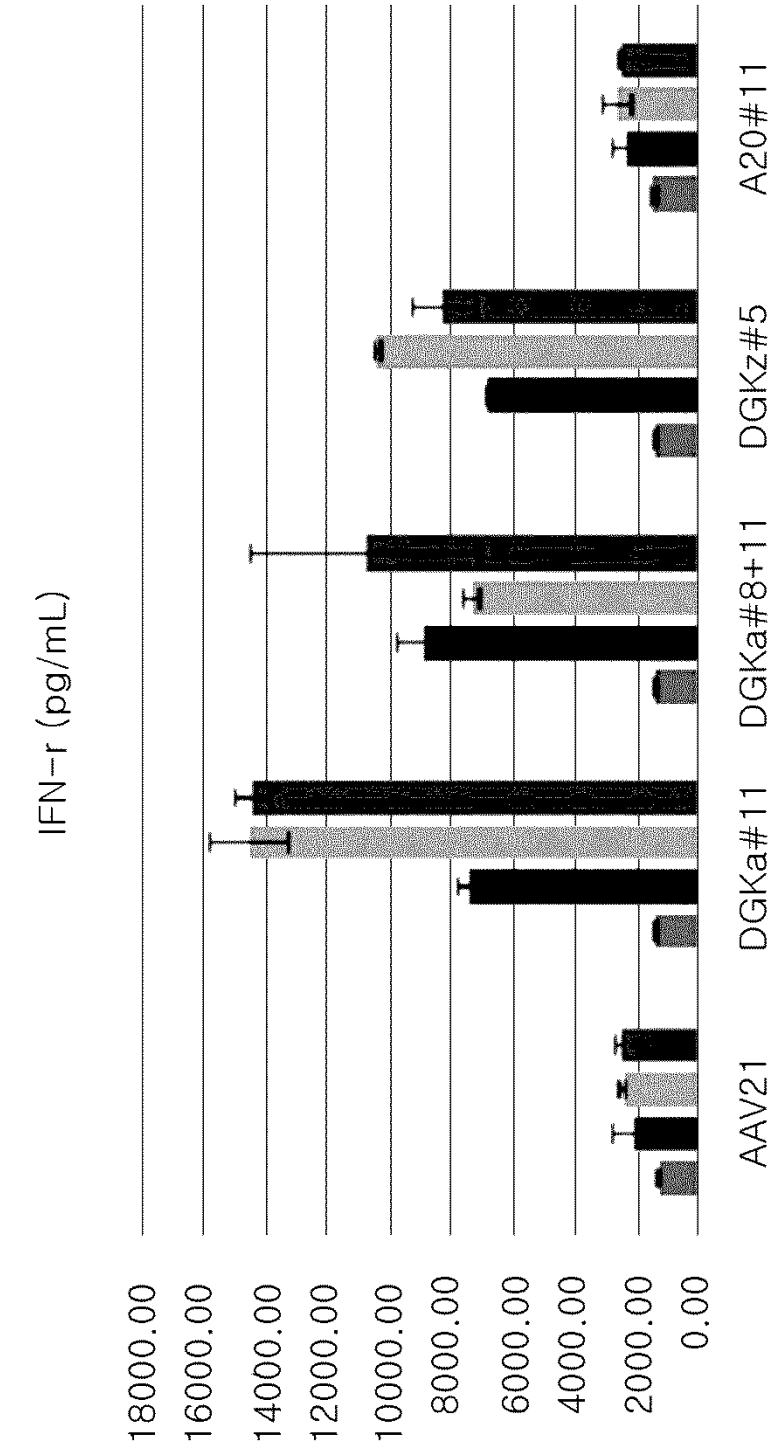
【FIG. 4】



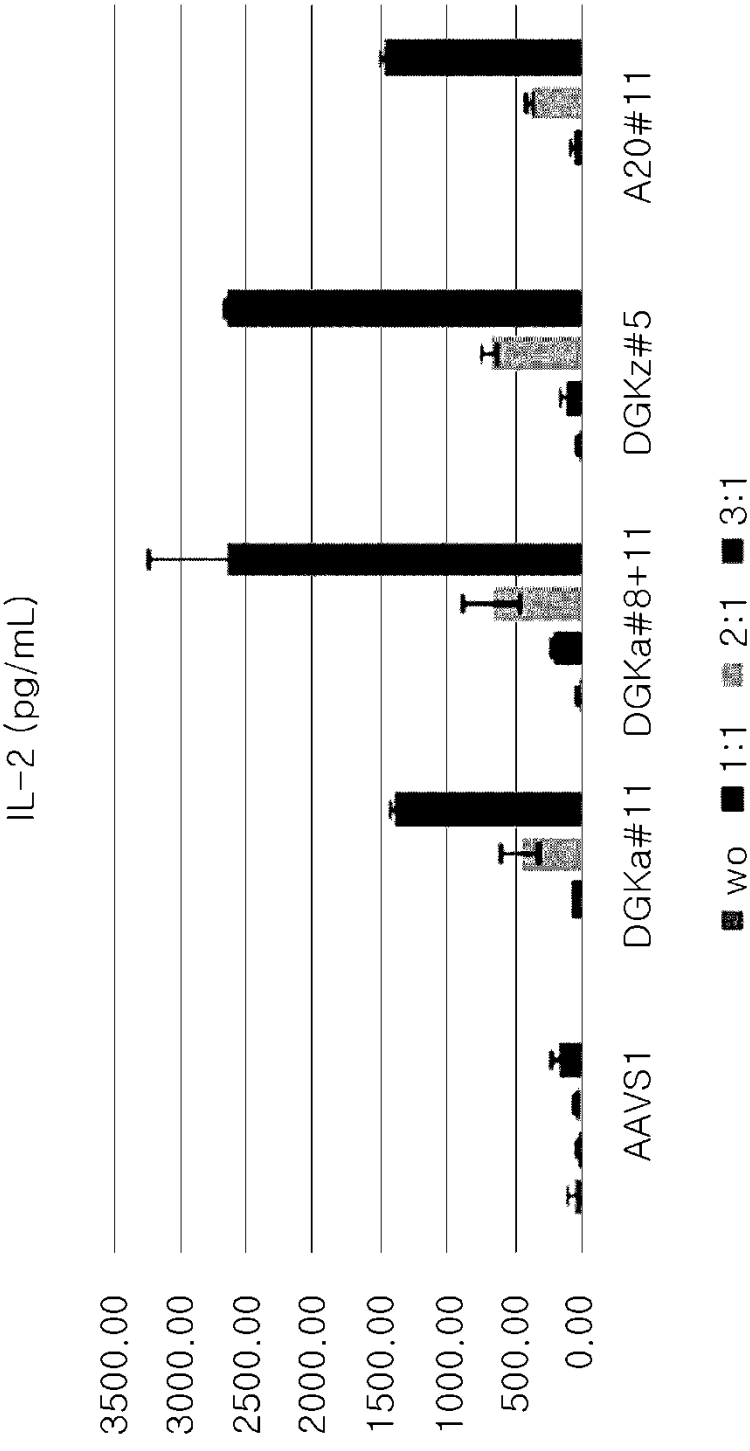
【FIG. 5】



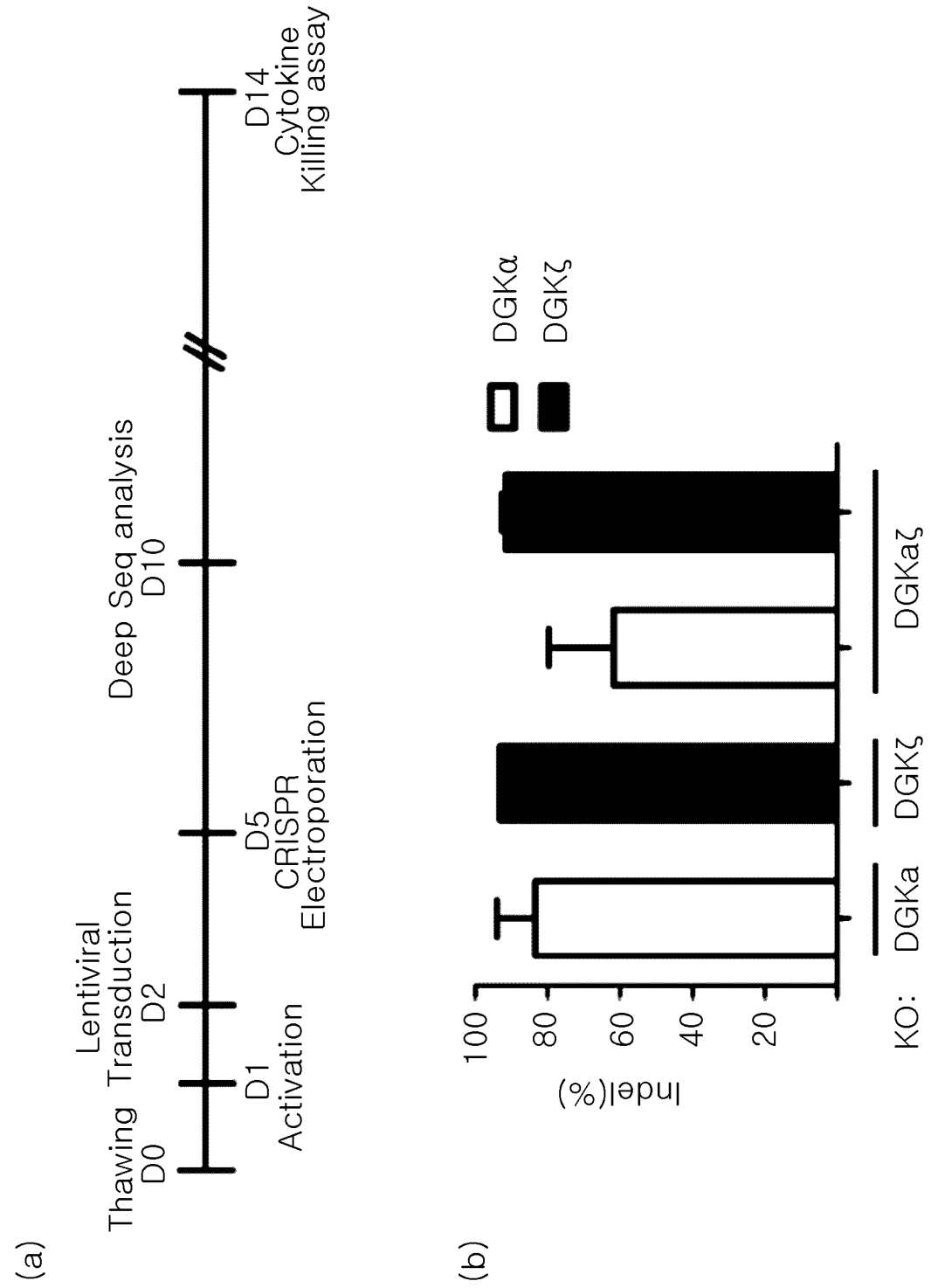
【FIG. 6】


IFN-r (pg/mL)
18000.00
16000.00
14000.00
12000.00
10000.00
8000.00
6000.00
4000.00
2000.00
0.00
AAV21
DGKa#11
DGKa#8+11
DGKz#5
A20#11
wo
1:1
2:1
3:1

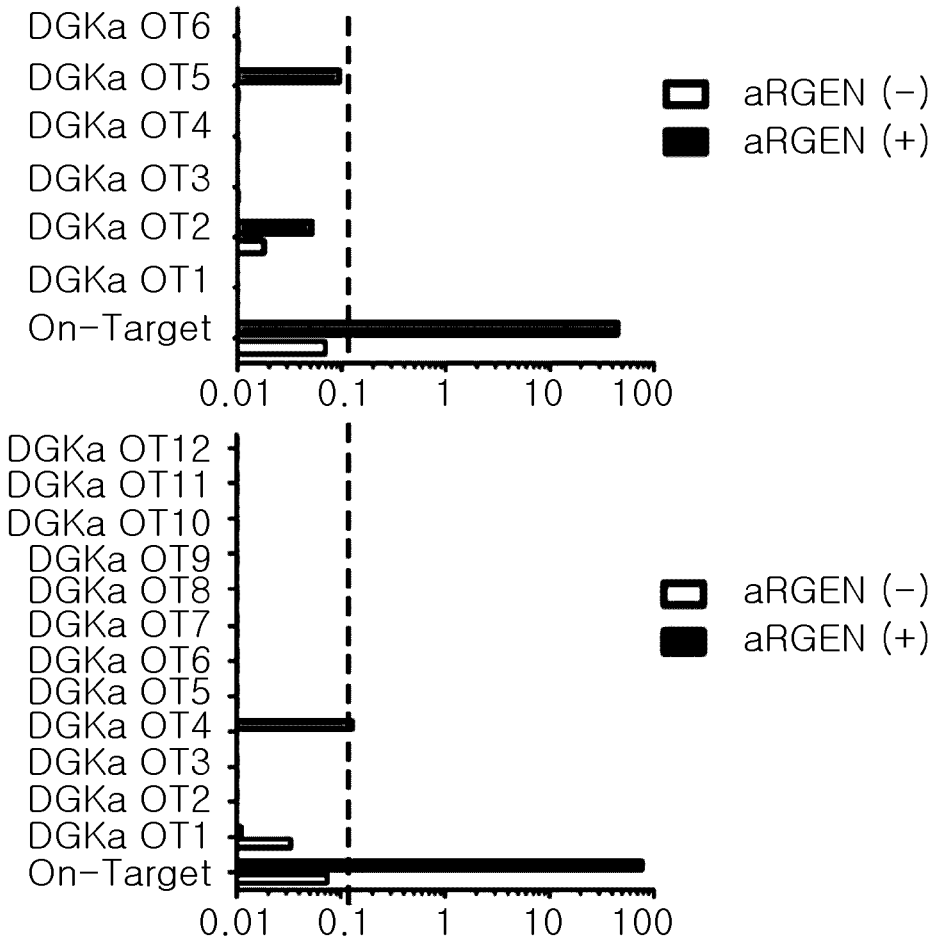
【FIG. 7】


IL-2 (pg/mL)
3500.00
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
AAVS1
DGKa#11
DGKa#8+11
DGKz#5
A20#11
wo
1:1
2:1
3:1

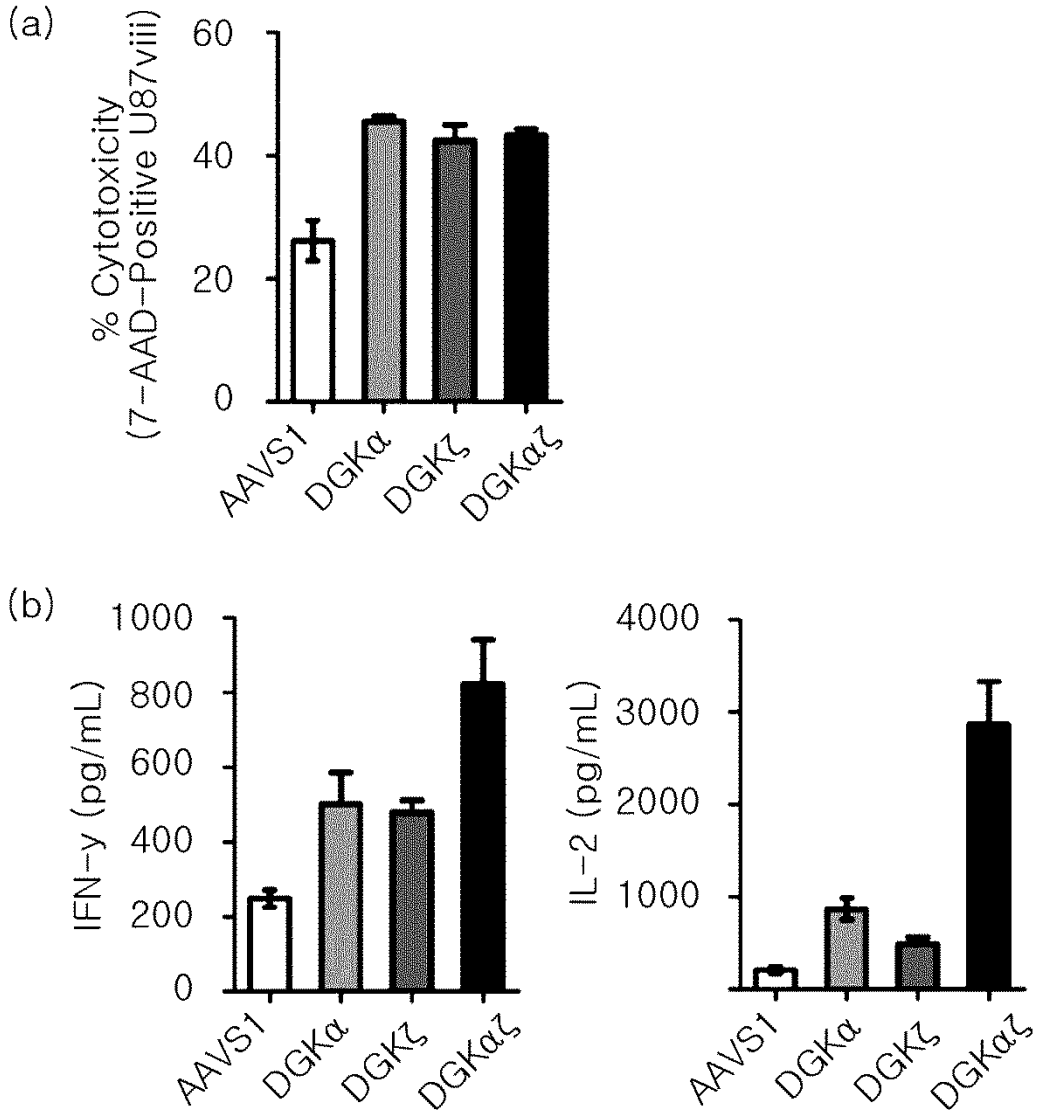
【FIG. 8a】


(a)
Thawing D0
Lentiviral Transduction D2
D1 Activation
D5 CRISPR Electroporation
Deep Seq analysis D10
D14 Cytokine Killing assay
(b)
DGKα
DGKζ
Indel(%)
100
80
60
40
20
KO: DGKa DGKζ DGKaζ

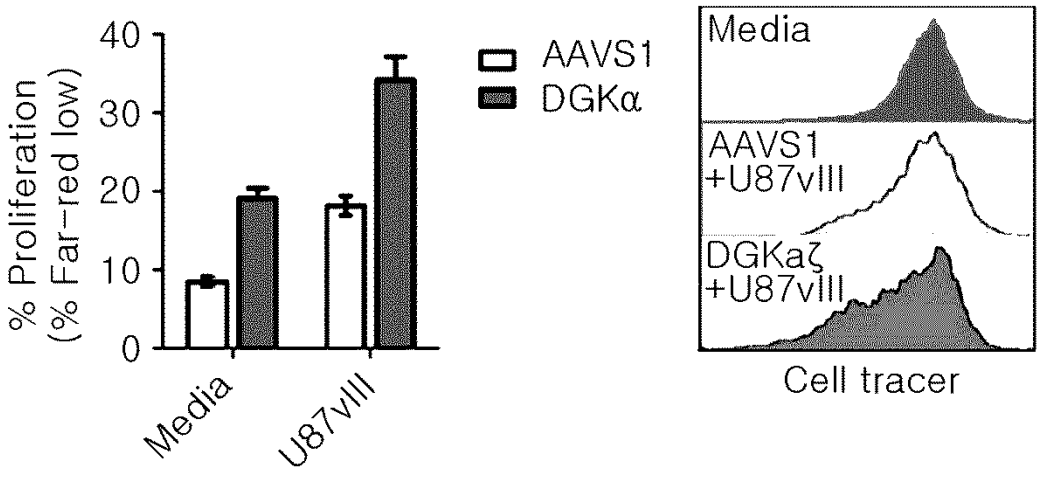
【FIG. 8b】


DGKa OT6
DGKa OT5
DGKa OT4
DGKa OT3
DGKa OT2
DGKa OT1
On-Target
0.01
0.1
1
10
100
aRGEN (-)
aRGEN (+)
DGKa OT12
DGKa OT11
DGKa OT10
DGKa OT9
DGKa OT8
DGKa OT7
DGKa OT6
DGKa OT5
DGKa OT4
DGKa OT3
DGKa OT2
DGKa OT1
On-Target
0.01
0.1
1
10
100
aRGEN (-)
aRGEN (+)

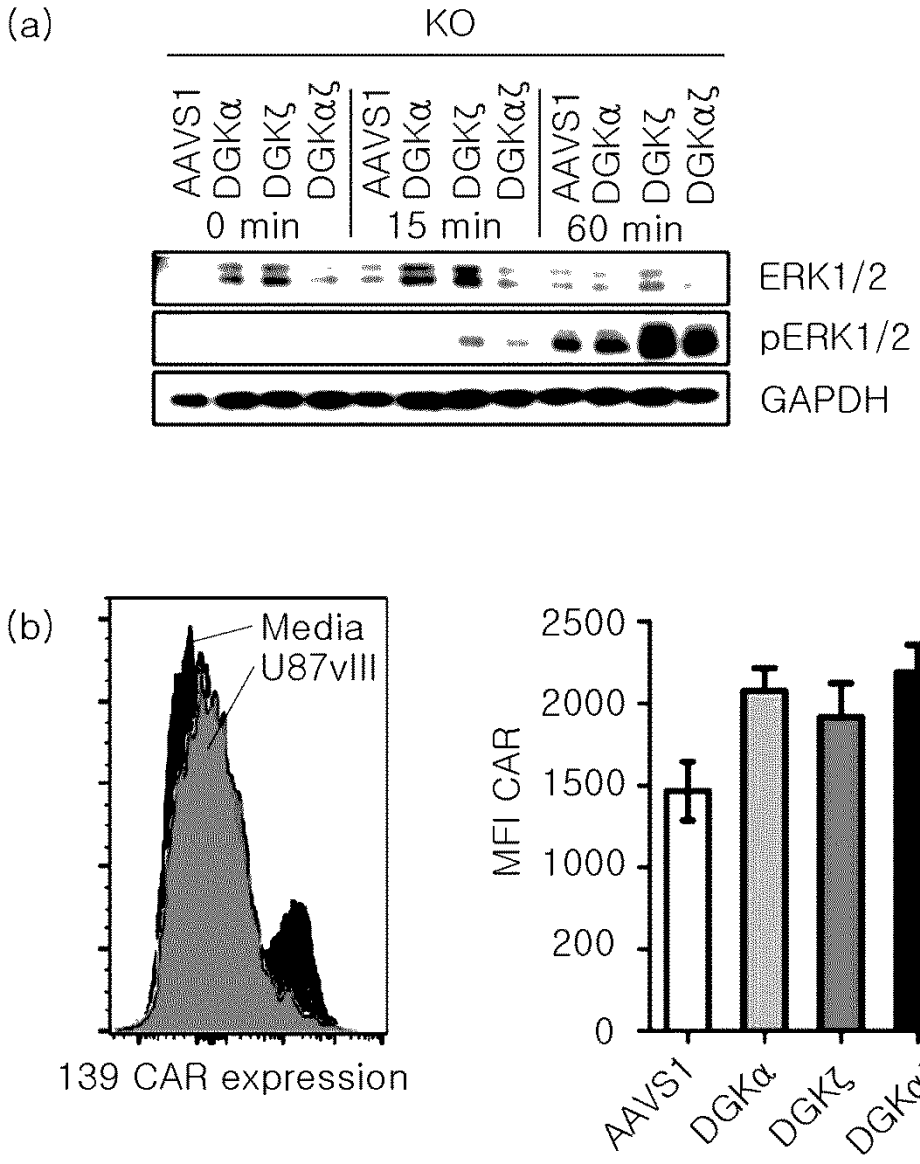
【FIG. 9a】


(a)
% Cytotoxicity
(7-AAD-Positive U87viii)
60
40
20
0
AAVS1
DGKα
DGKζ
DGKαζ
(b)
IFN-γ (pg/mL)
1000
800
600
400
200
0
AAVS1
DGKα
DGKζ
DGKαζ
IL-2 (pg/mL)
4000
3000
2000
1000
AAVS1
DGKα
DGKζ
DGKαζ

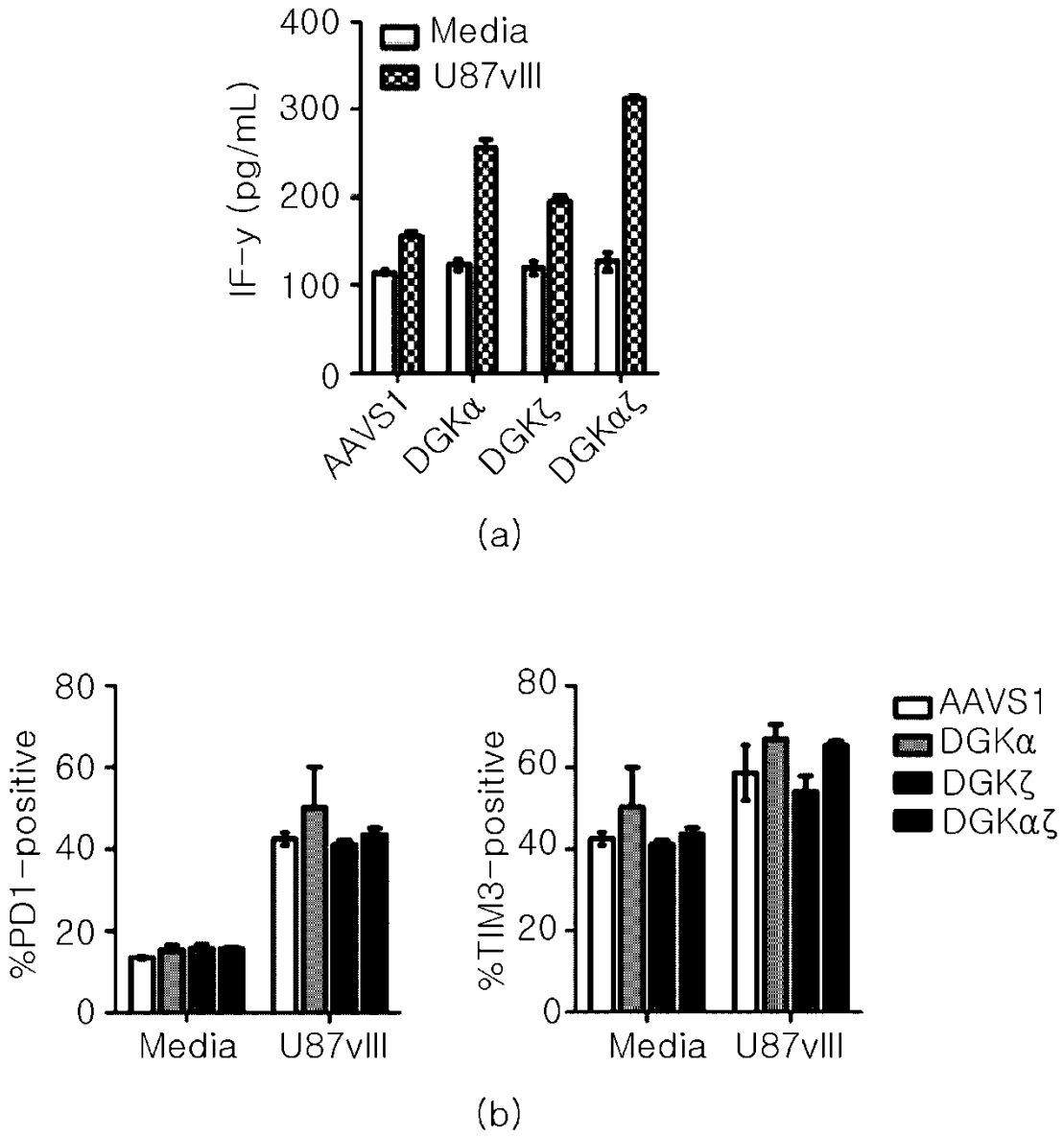
【FIG. 9b】


% Proliferation
(% Far-red low)
40
30
20
10
0
Media
U87vIII
AAVS1
DGKα
Media
AAVS1
+U87vIII
DGKaζ
+U87vIII
Cell tracer

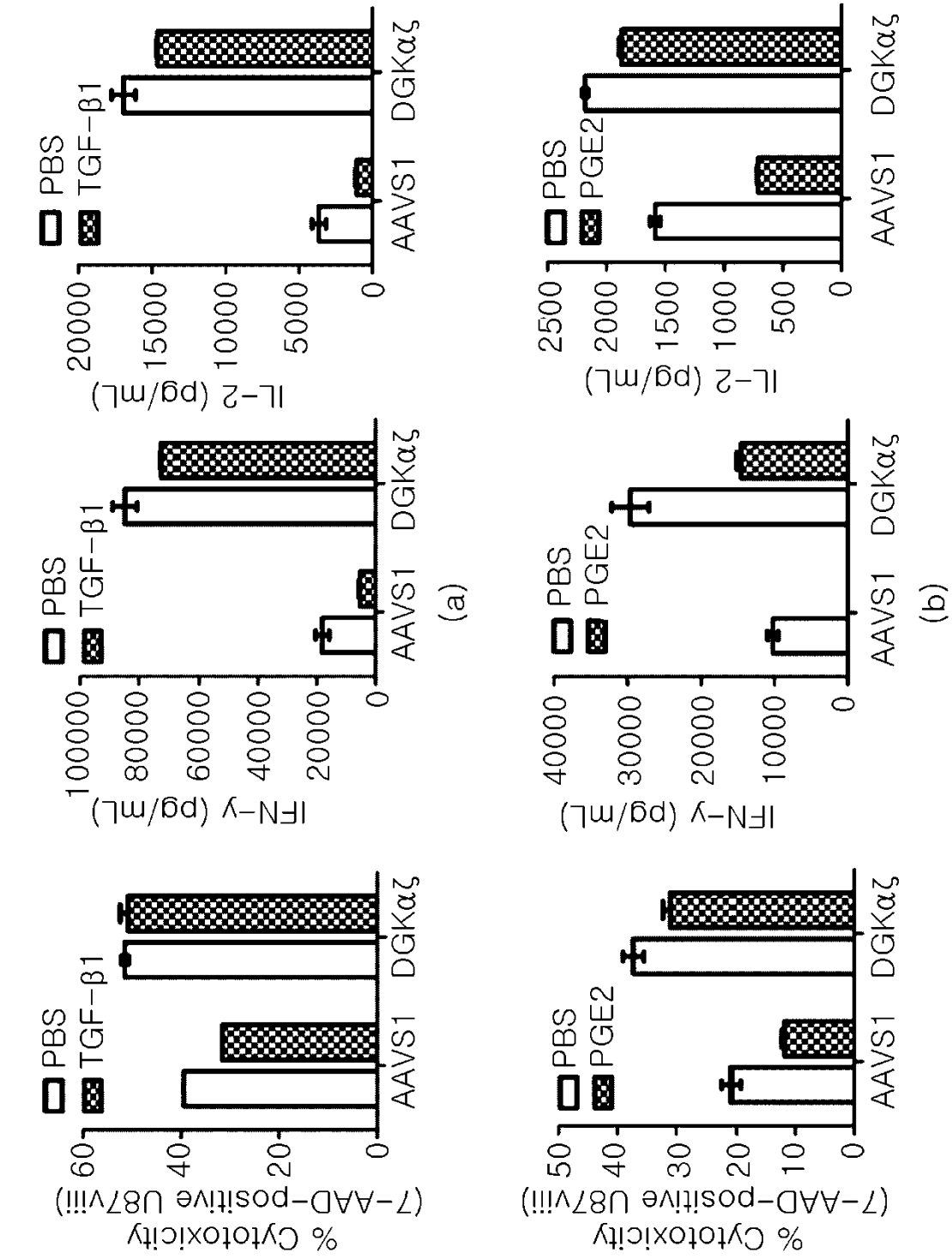
【FIG. 10】


(a)
KO
AAVS1
DGKα
DGKζ
DGKαζ
0 min
15 min
60 min
ERK1/2
pERK1/2
GAPDH
(b)
Media
U87vIII
139 CAR expression
MFI CAR
2500
2000
1500
1000
200
0
AAVS1
DGKα
DGKζ
DGKαζ

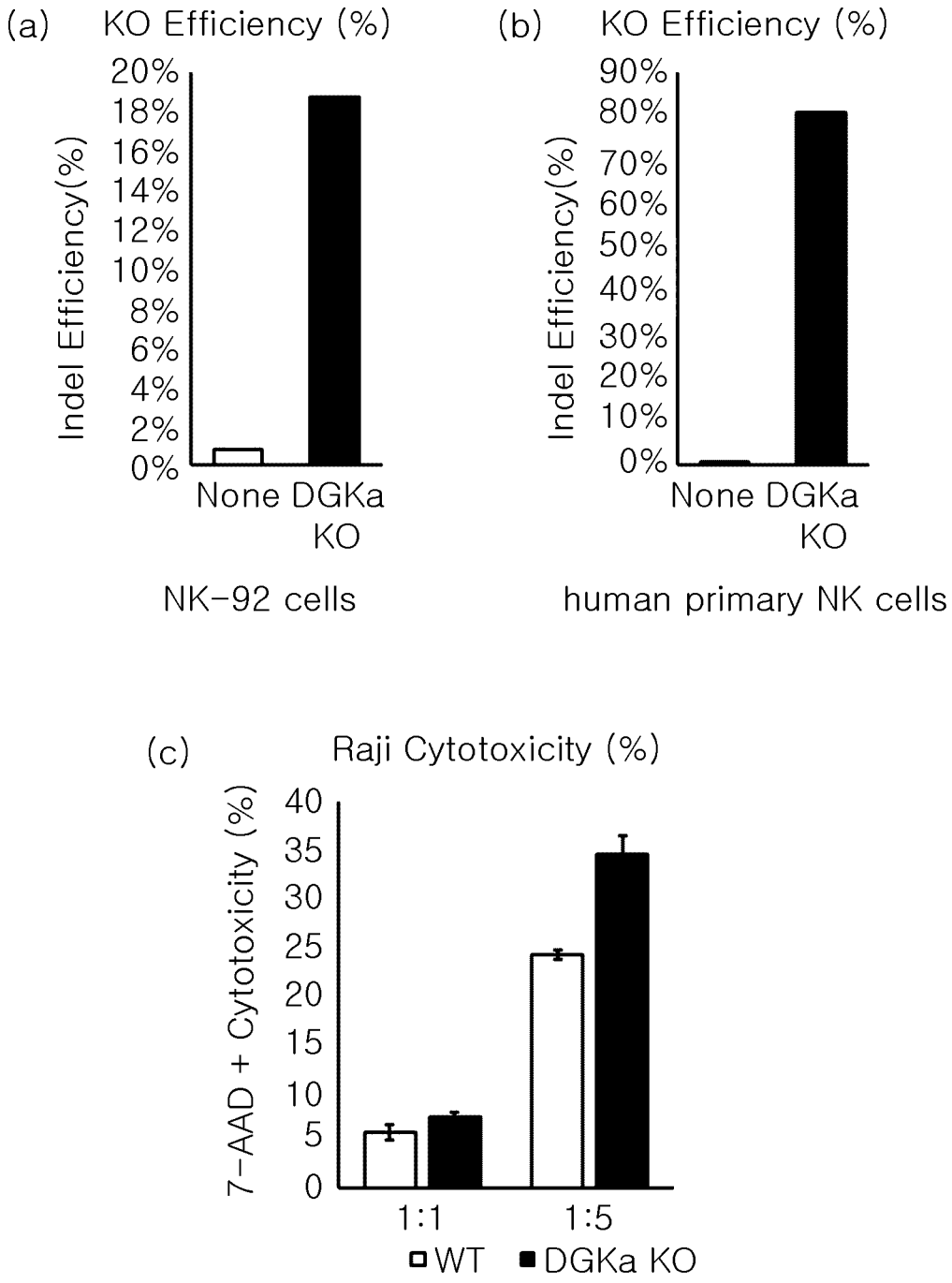
【FIG. 11】


400
300
200
100
0
IF-γ (pg/mL)
Media
U87vIII
AAVS1
DGKα
DGKζ
DGKαζ
(a)
80
60
40
20
0
%PD1-positive
Media
U87vIII
%TIM3-positive
AAVS1
DGKα
DGKζ
DGKαζ
(b)

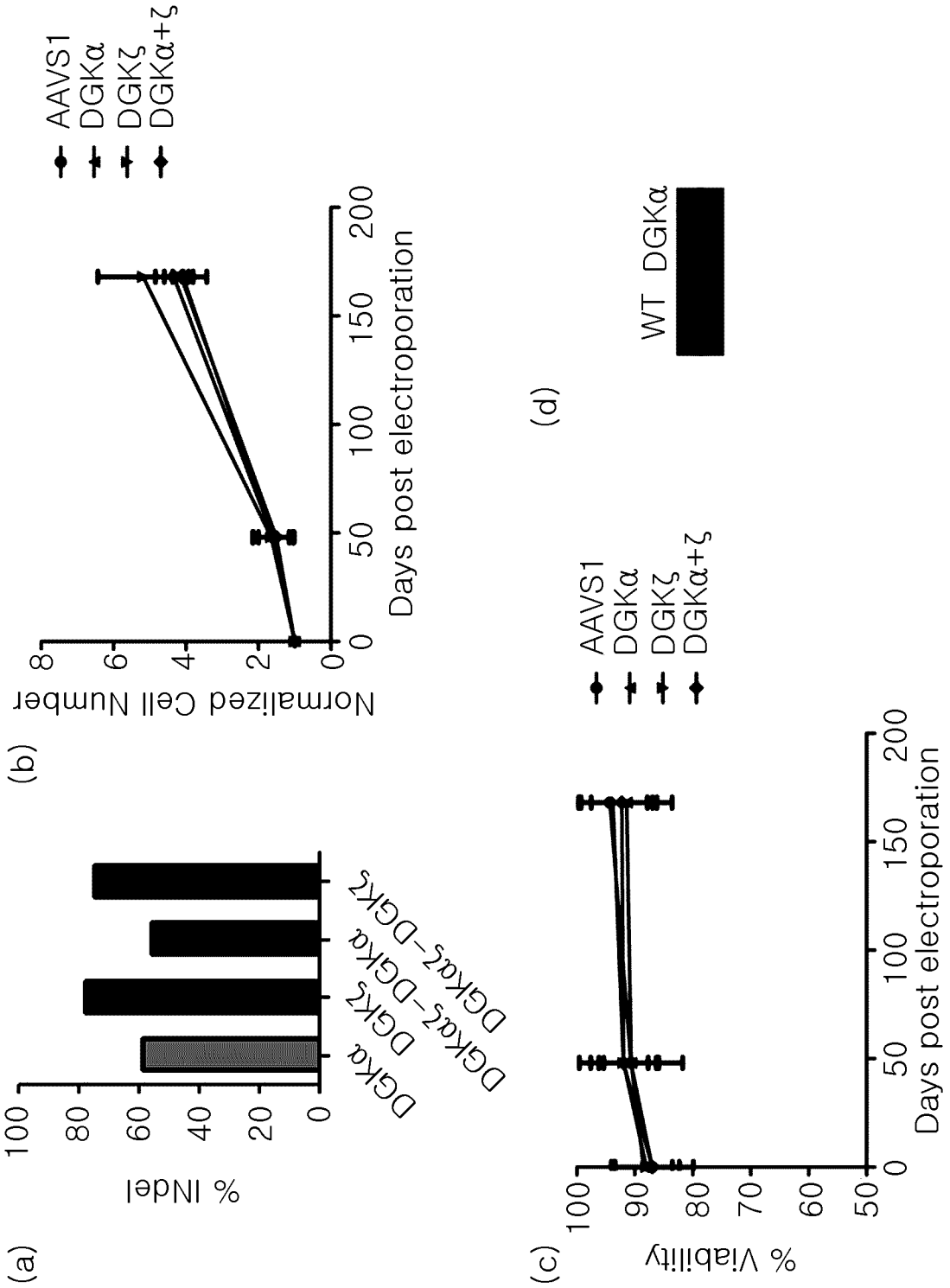
【FIG. 12】


% Cytotoxicity (7-AAD-positive U87vIII)
PBS
TGF-β1
PGE2
AAVS1
DGKαζ
IFN-γ (pg/mL)
IL-2 (pg/mL)
(a)
(b)

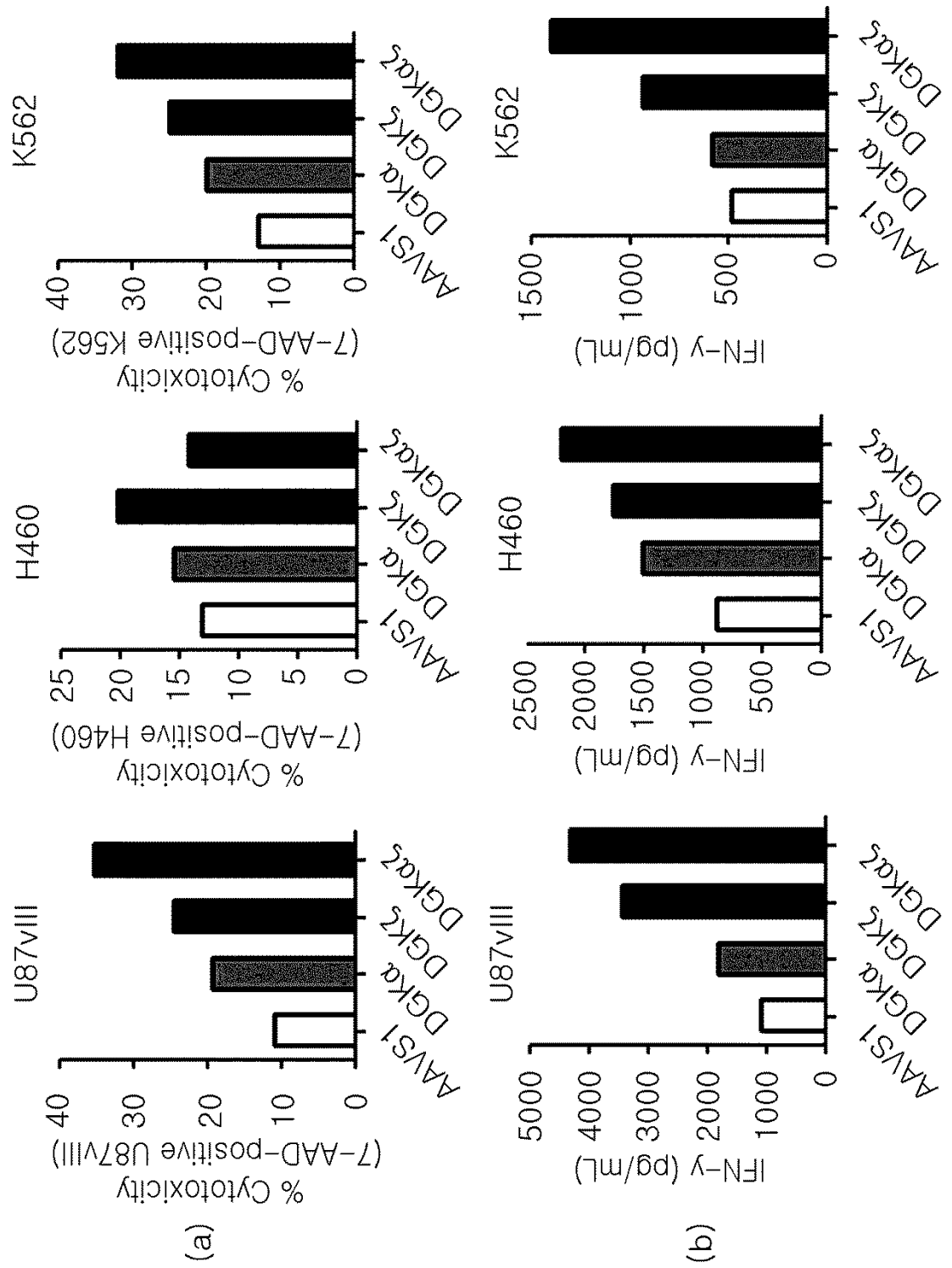
【FIG. 13】


(a)
KO Efficiency (%)
Indel Efficiency(%)
20%
18%
16%
14%
12%
10%
8%
6%
4%
2%
0%
None
DGKa KO
NK-92 cells
(b)
KO Efficiency (%)
Indel Efficiency(%)
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
None
DGKa KO
human primary NK cells
(c)
Raji Cytotoxicity (%)
7-AAD + Cytotoxicity (%)
40
35
30
25
20
15
10
5
0
1:1
1:5
WT
DGKa KO

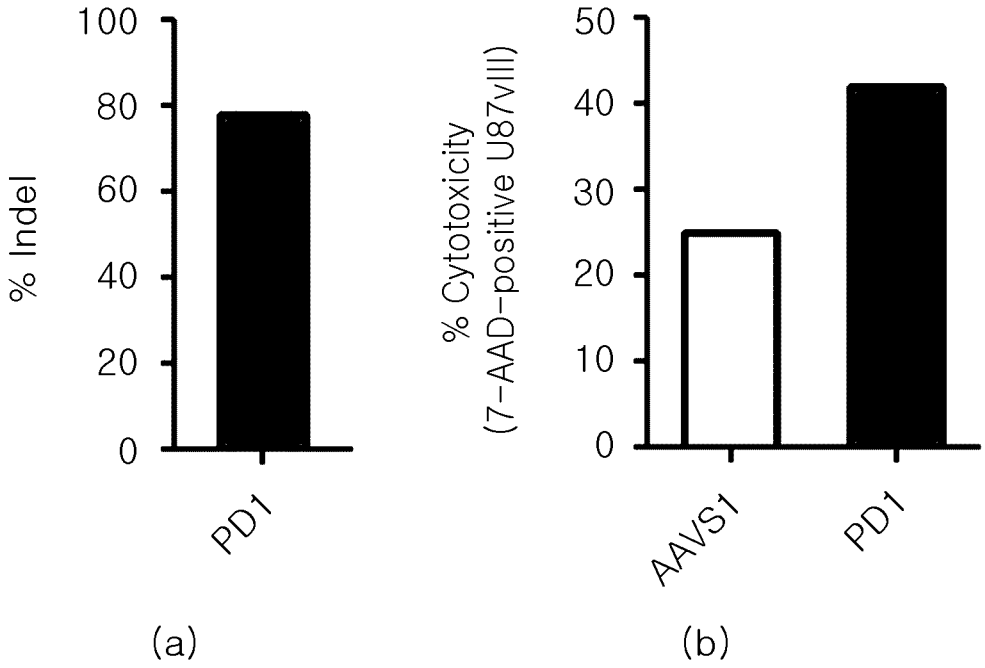
【FIG. 14】


(a)
% INdel
100
80
60
40
20
0
DGKα
DGKζ
DGKαζ-DGKα
DGKαζ-DGKζ
(b)
Normalized Cell Number
8
6
4
2
0
0
50
100
150
200
Days post electroporation
AAVS1
DGKα
DGKζ
DGKα+ζ
(c)
% Viability
100
90
80
70
60
50
0
50
100
150
200
Days post electroporation
AAVS1
DGKα
DGKζ
DGKα+ζ
(d)
WT DGKα

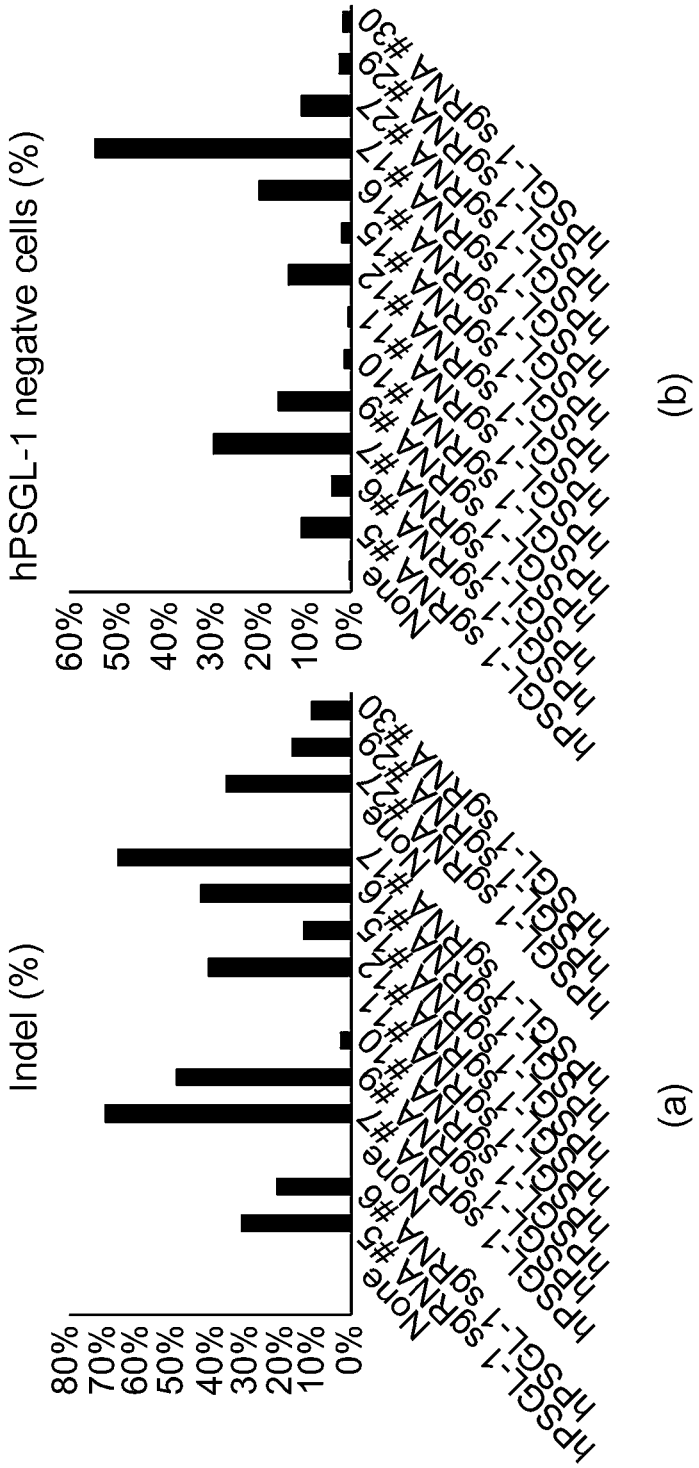
【FIG. 15】


(a)
U87vIII
H460
K562
% Cytotoxicity
(7-AAD-positive U87vIII)
(7-AAD-positive H460)
(7-AAD-positive K562)
0
10
20
30
40
5
15
25
AAVS1
DGKα
DGKζ
DGKαζ
(b)
IFN-γ (pg/mL)
0
1000
2000
3000
4000
5000
500
1500
2500

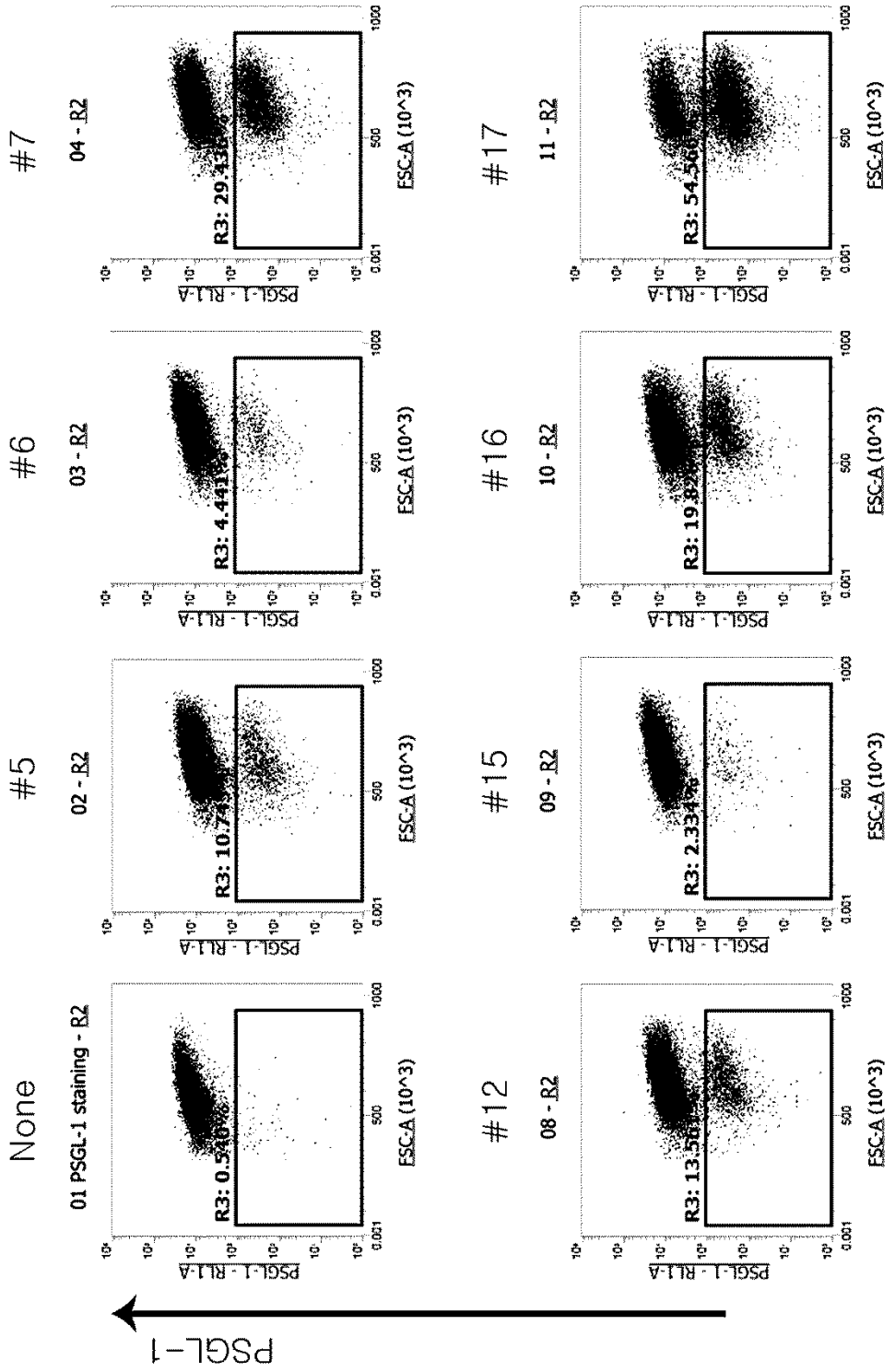
【FIG. 16】


100
80
60
40
20
0
% Indel
PD1
(a)
50
40
30
20
10
0
% Cytotoxicity
(7-AAD-positive U87vIII)
AAVS1
PD1
(b)

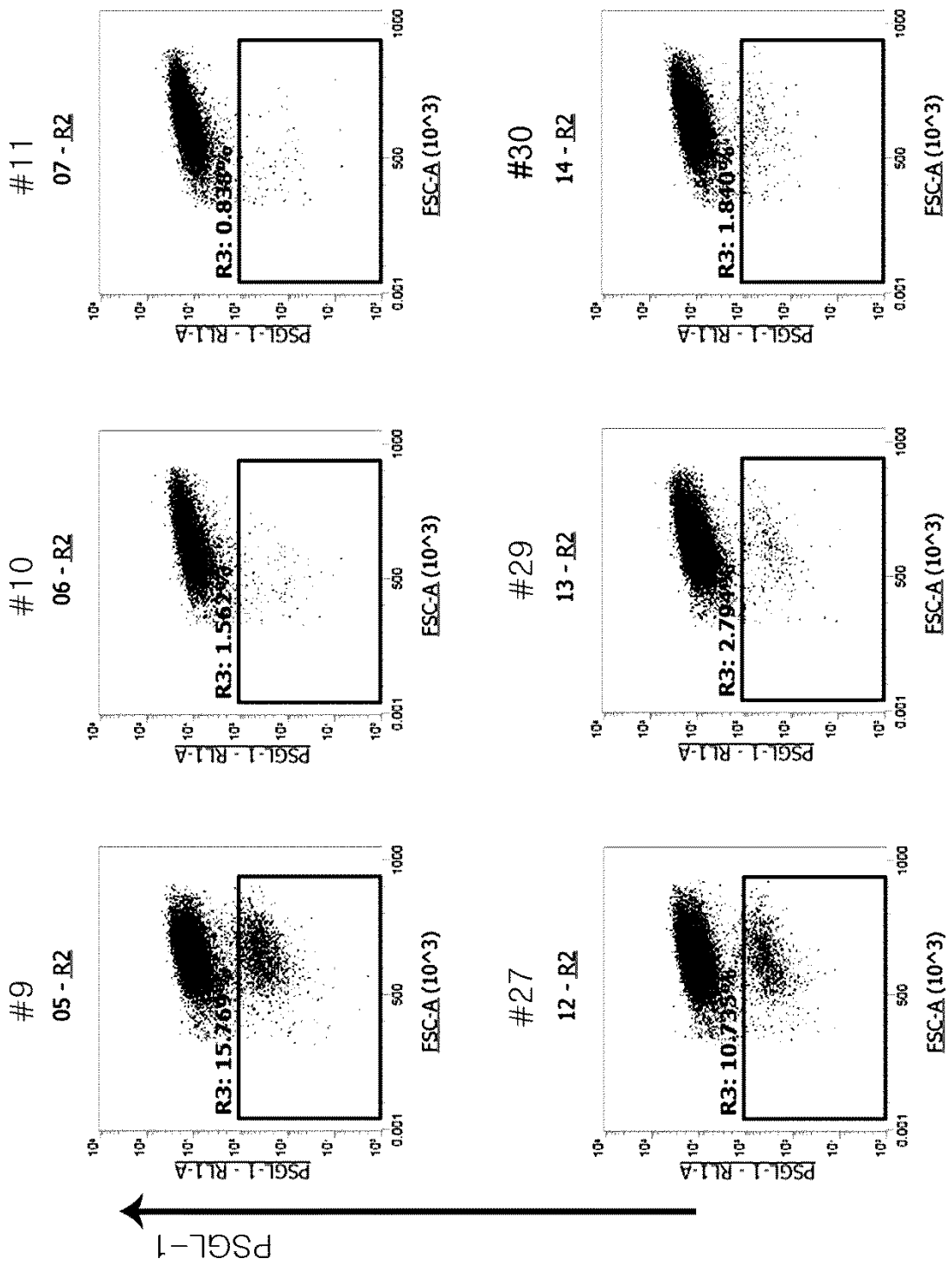
【FIG. 17a】


hPSGL-1 negative cells (%)
60%
50%
40%
30%
20%
10%
0%
None
sgRNA #5
sgRNA #6
sgRNA #7
sgRNA #9
sgRNA #10
sgRNA #1
sgRNA #2
sgRNA #3
sgRNA #6
sgRNA #7
sgRNA #27
sgRNA #29
sgRNA #30
hPSGL-1
(b)
Indel (%)
80%
70%
60%
50%
40%
30%
20%
10%
0%
None
sgRNA #5
sgRNA #6
sgRNA #7
sgRNA #9
sgRNA #10
sgRNA #1
sgRNA #2
sgRNA #3
sgRNA #6
sgRNA #7
sgRNA #27
sgRNA #29
sgRNA #30
hPSGL-1
(a)

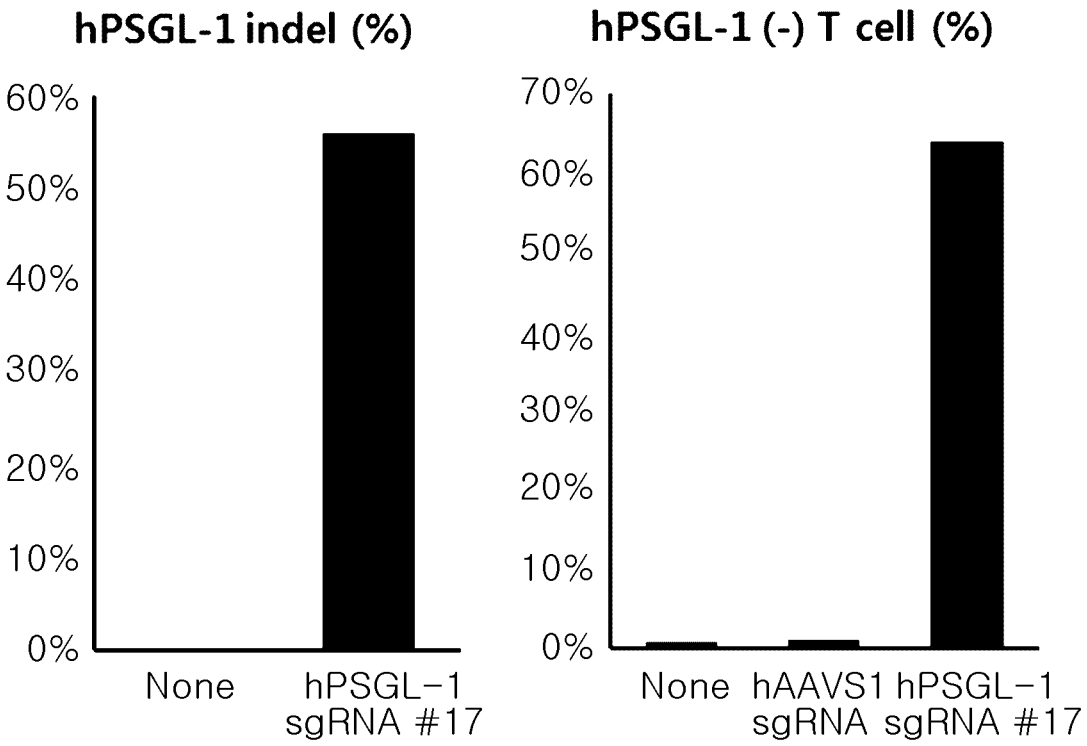
【FIG. 17b】

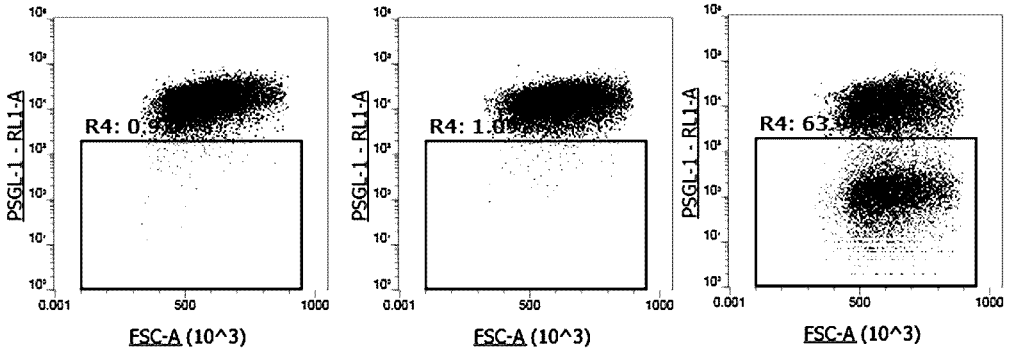

None
#5
#12
#15
#6
#16
#7
#17
PSGL-1
01 PSGL-1 staining - R2
02 - R2
08 - R2
09 - R2
03 - R2
10 - R2
04 - R2
11 - R2
R3: 0.540
R3: 10.7
R3: 13.5
R3: 2.33
R3: 4.441
R3: 19.8
R3: 29.43
R3: 54.56
PSGL-1 - R1-A
FSC-A (10^3)

【FIG. 17c】


#9
05 - R2
R3: 15.76%
#10
06 - R2
R3: 1.56%
#11
07 - R2
R3: 0.83%
#27
12 - R2
R3: 10.73%
#29
13 - R2
R3: 2.79%
#30
14 - R2
R3: 1.84%
PSGL-1 - R1-A
ESC-A (10^3)
PSGL-1

【FIG. 18】


hPSGL-1 indel (%)
60%
50%
40%
30%
20%
10%
0%
None
hPSGL-1 sgRNA #17
(a)
hPSGL-1 (-) T cell (%)
70%
60%
50%
40%
30%
20%
10%
0%
None
hAAVS1 sgRNA
hPSGL-1 sgRNA #17
(b)


PSGL-1 - RL1-A
FSC-A (10^3)
0.001
500
1000
(c)