FIG. 1A

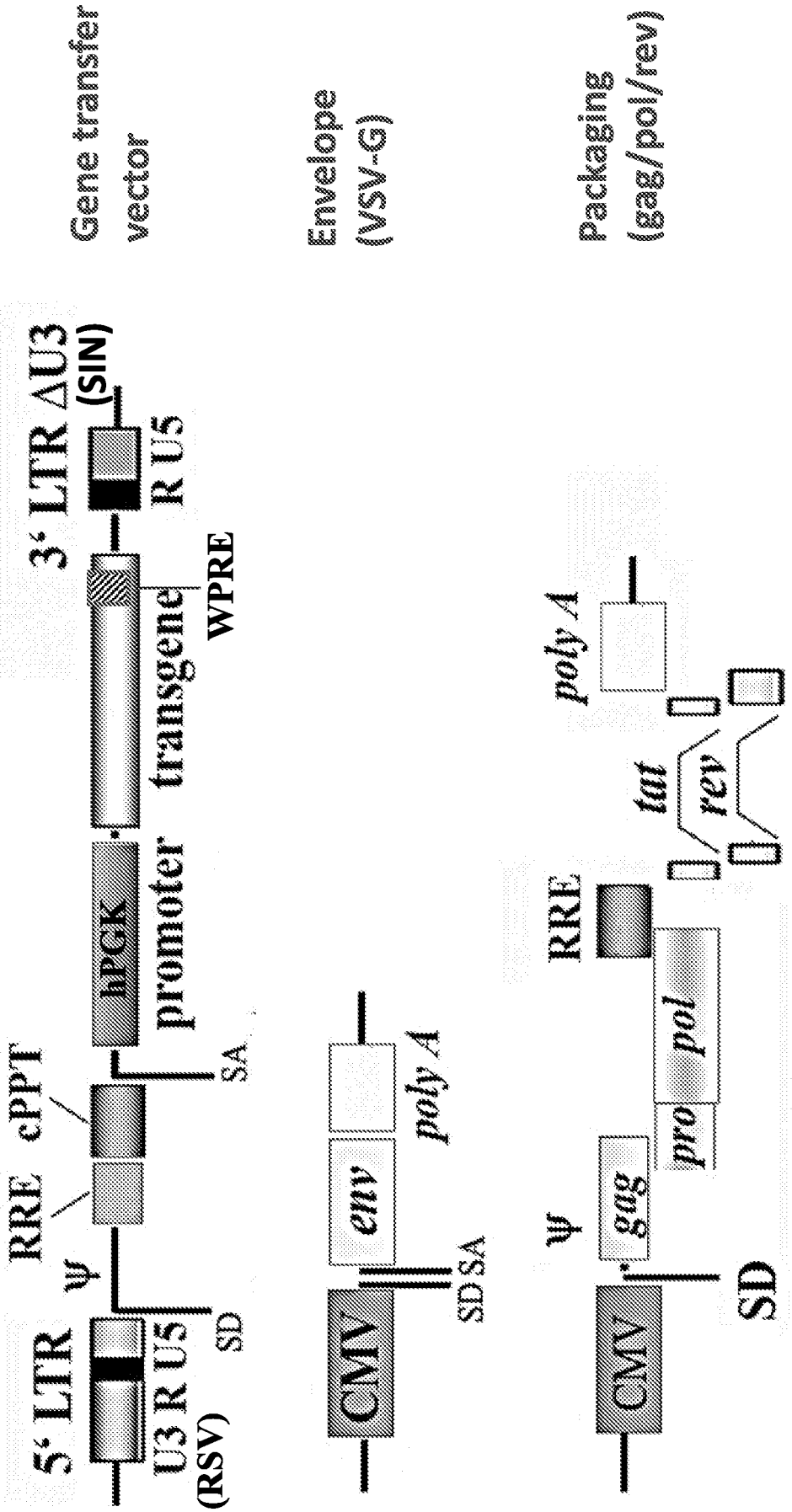

Gene transfer vector
Envelope (VSV-G)
Packaging (gag/pol/rev)
5' LTR
U3 R U5 (RSV)
Ψ
RRE
cPPT
SD
SA
hPGK
promoter
transgene
WPRE
3' LTR ΔU3 (SIN)
R U5
CMV
env
poly A
SD SA
CMV
Ψ
gag
RRE
pro
pol
tat
rev
poly A
SD

FIG. 1B

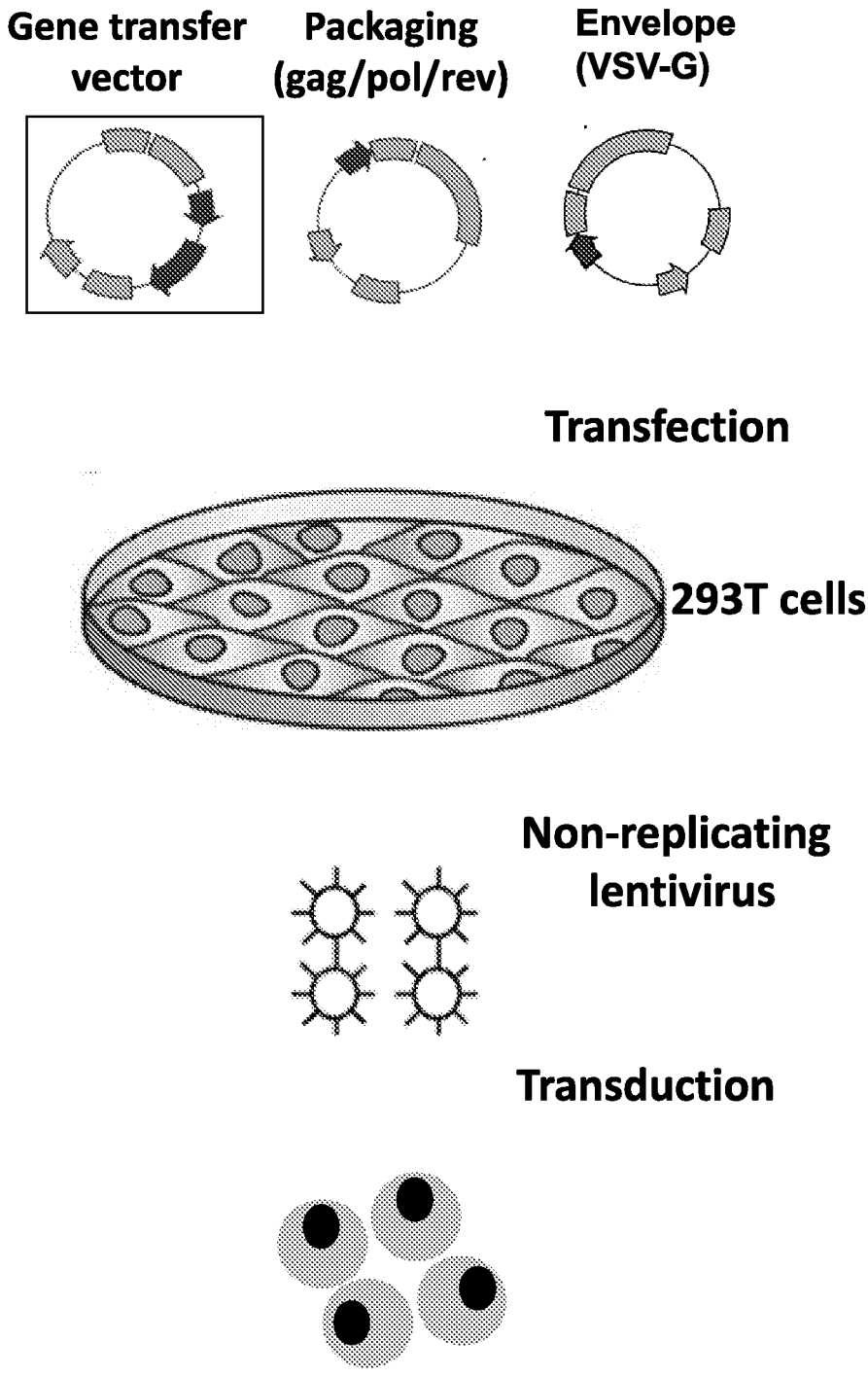

Gene transfer vector
Packaging (gag/pol/rev)
Envelope (VSV-G)
Transfection
293T cells
Non-replicating lentivirus
Transduction

FIG. 2

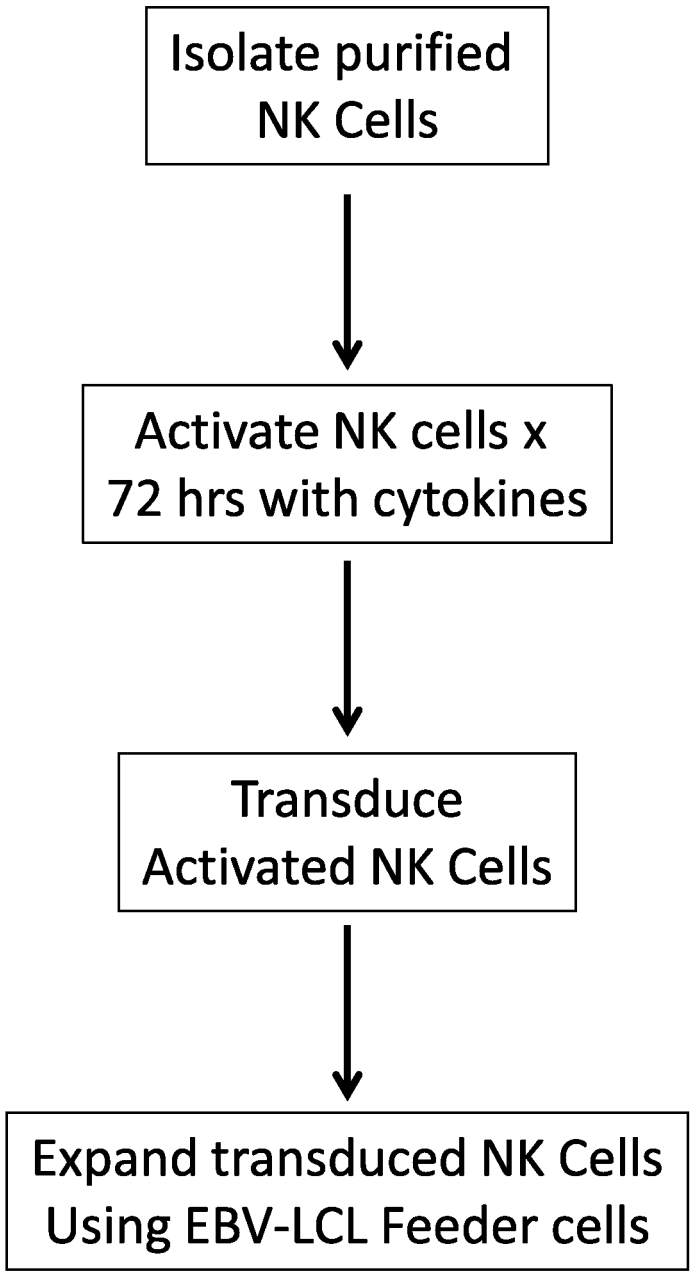

Isolate purified NK Cells
Activate NK cells x 72 hrs with cytokines
Transduce Activated NK Cells
Expand transduced NK Cells Using EBV-LCL Feeder cells

FIG. 3

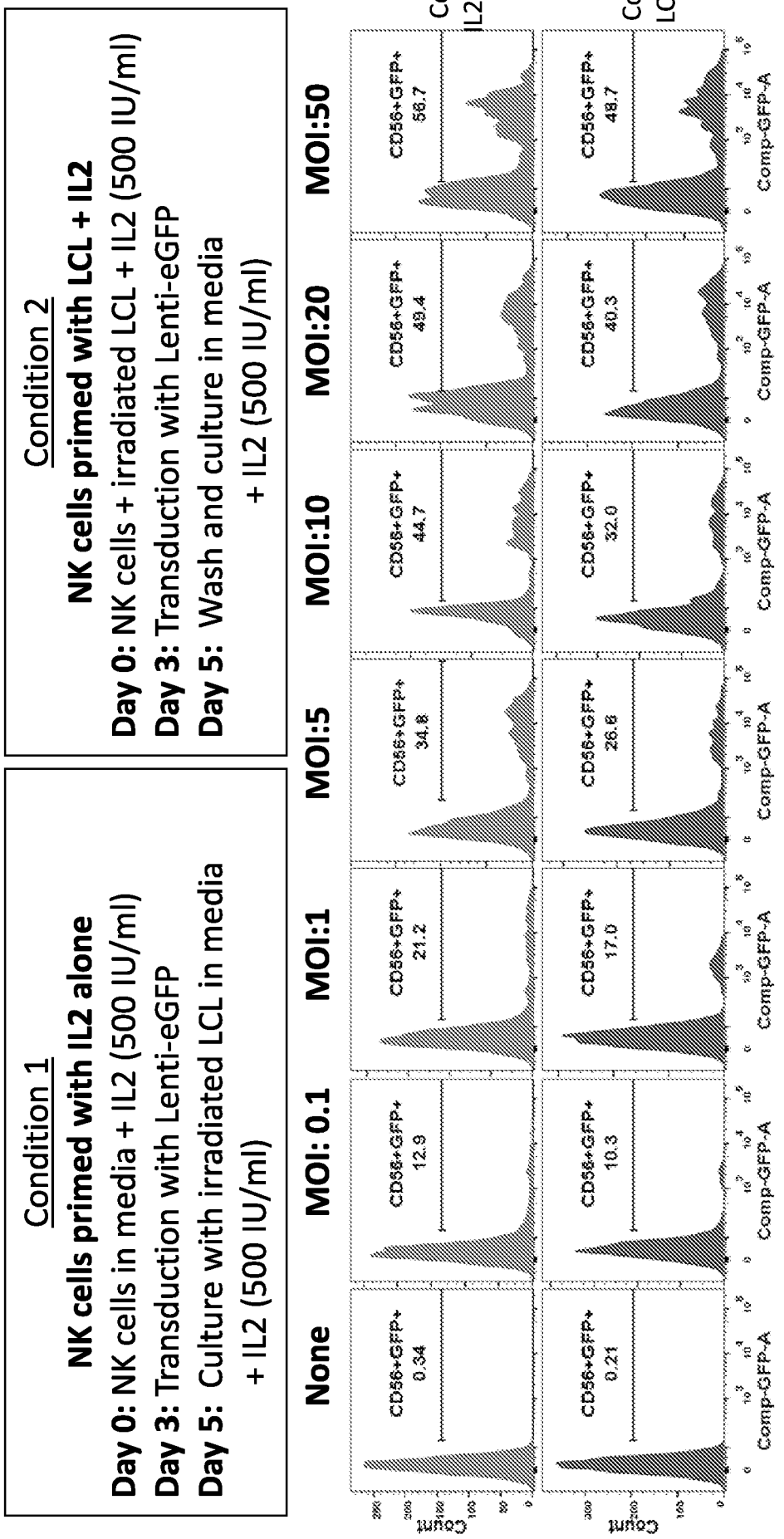

Condition 1
NK cells primed with IL2 alone
Day 0: NK cells in media + IL2 (500 IU/ml)
Day 3: Transduction with Lenti-eGFP
Day 5: Culture with irradiated LCL in media + IL2 (500 IU/ml)
Condition 2
NK cells primed with LCL + IL2
Day 0: NK cells + irradiated LCL + IL2 (500 IU/ml)
Day 3: Transduction with Lenti-eGFP
Day 5: Wash and culture in media + IL2 (500 IU/ml)
None
MOI: 0.1
MOI:1
MOI:5
MOI:10
MOI:20
MOI:50
Cond. 1 IL2 primed
Cond. 2 LCL + IL2
CD56+GFP+ 0.34
CD56+GFP+ 12.9
CD56+GFP+ 21.2
CD56+GFP+ 34.8
CD56+GFP+ 44.7
CD56+GFP+ 49.4
CD56+GFP+ 58.7
CD56+GFP+ 0.21
CD56+GFP+ 10.3
CD56+GFP+ 17.0
CD56+GFP+ 26.6
CD56+GFP+ 32.0
CD56+GFP+ 40.3
CD56+GFP+ 48.7
Count
Comp-GFP-A

FIG. 4

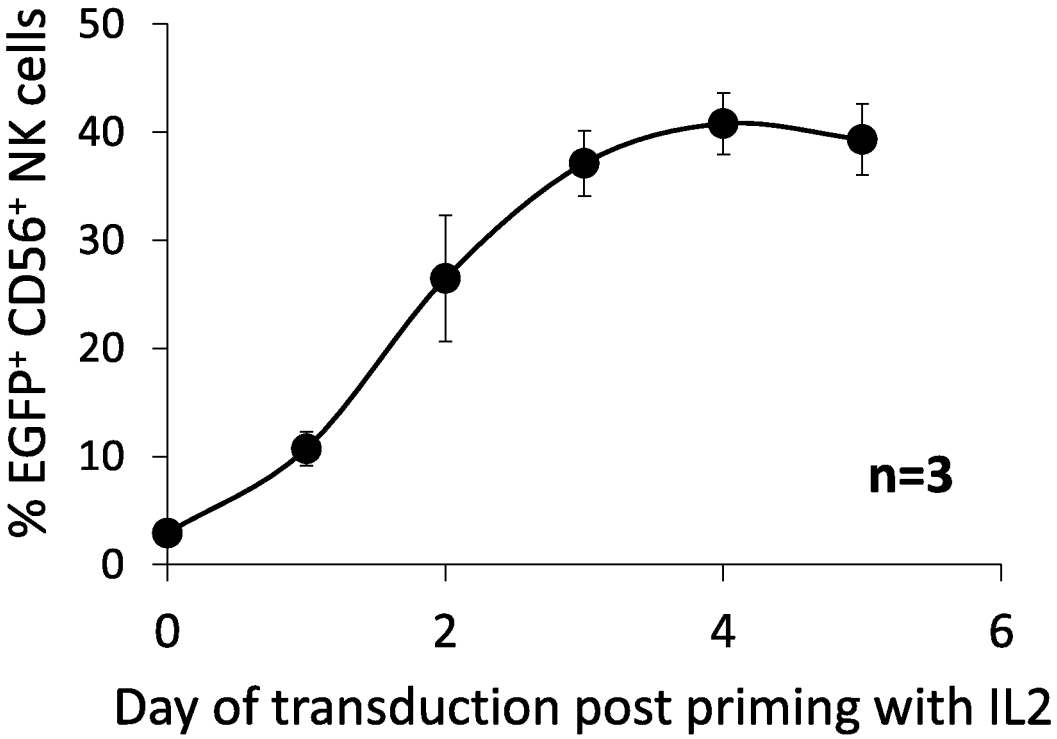

% EGFP+ CD56+ NK cells
50
40
30
20
10
0
n=3
0
2
4
6
Day of transduction post priming with IL2

FIG. 5A

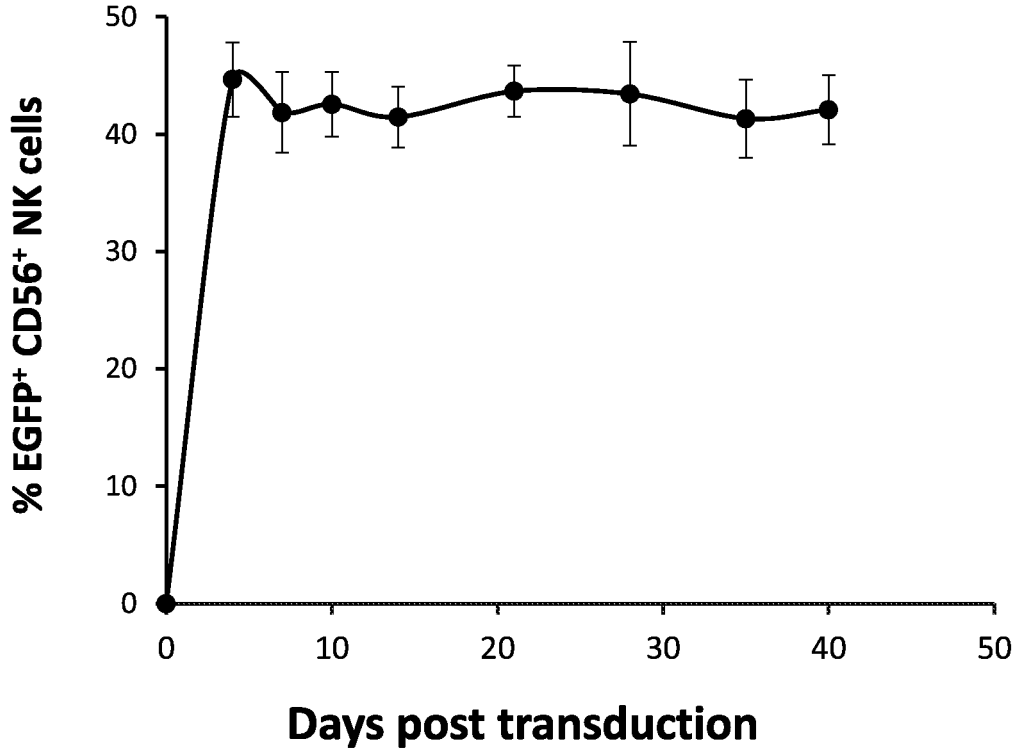

% EGFP+ CD56+ NK cells
50
40
30
20
10
0
0
10
20
30
40
50
Days post transduction

FIG. 5B

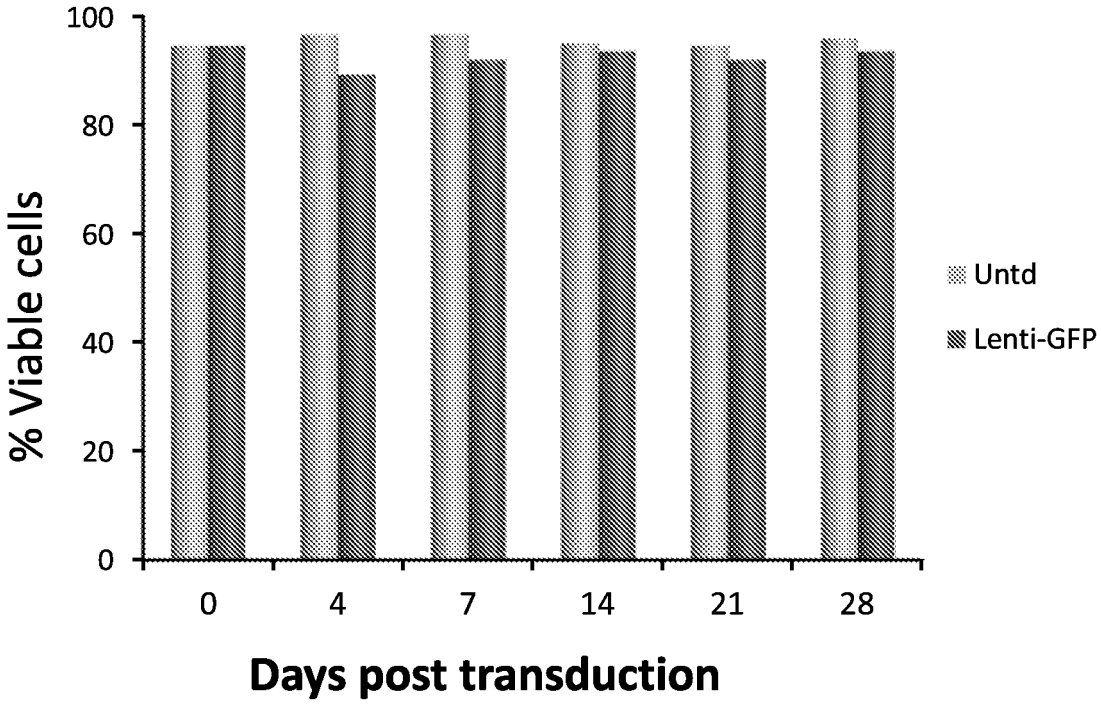

100
80
60
40
20
0
% Viable cells
0
4
7
14
21
28
Days post transduction
Untd
Lenti-GFP

FIG. 6

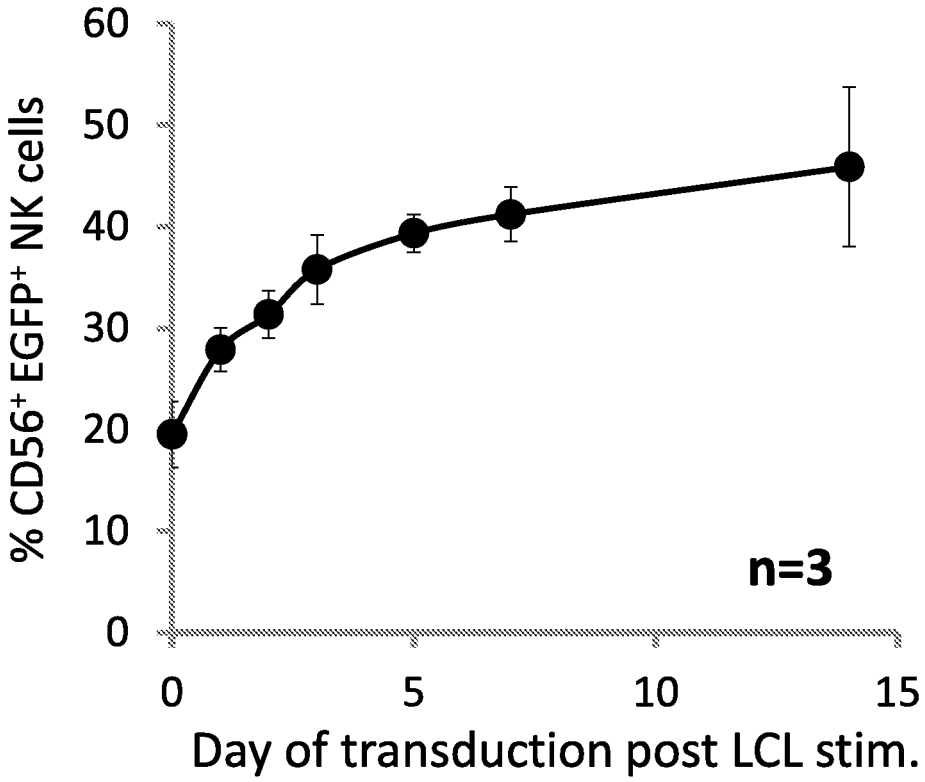

60
50
40
30
20
10
0
% CD56+ EGFP+ NK cells
n=3
0
5
10
15
Day of transduction post LCL stim.

FIG. 7

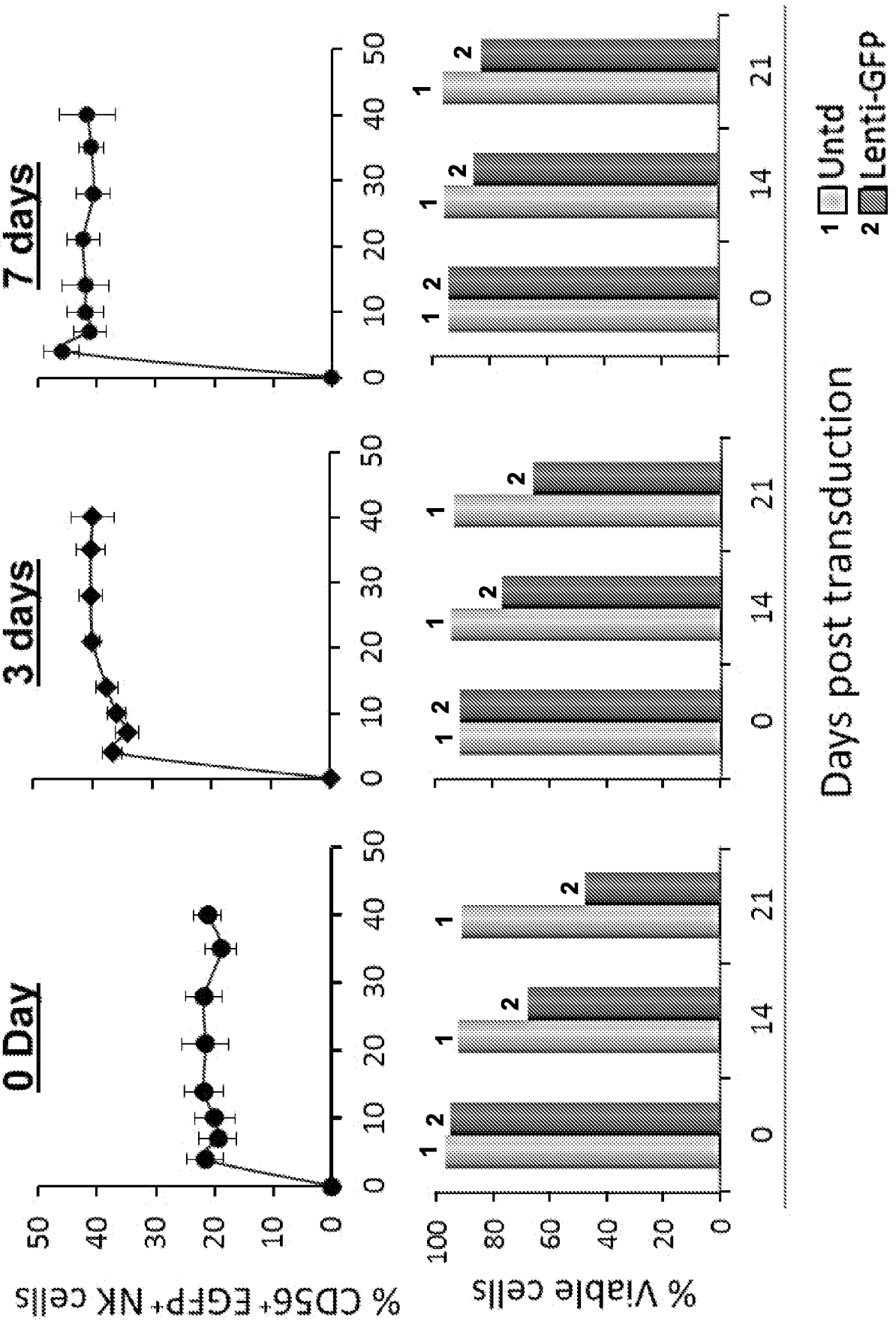

0 Day
3 days
7 days
% CD56+ EGFP+ NK cells
% Viable cells
Days post transduction
1 Untd
2 Lenti-GFP

FIG. 8A

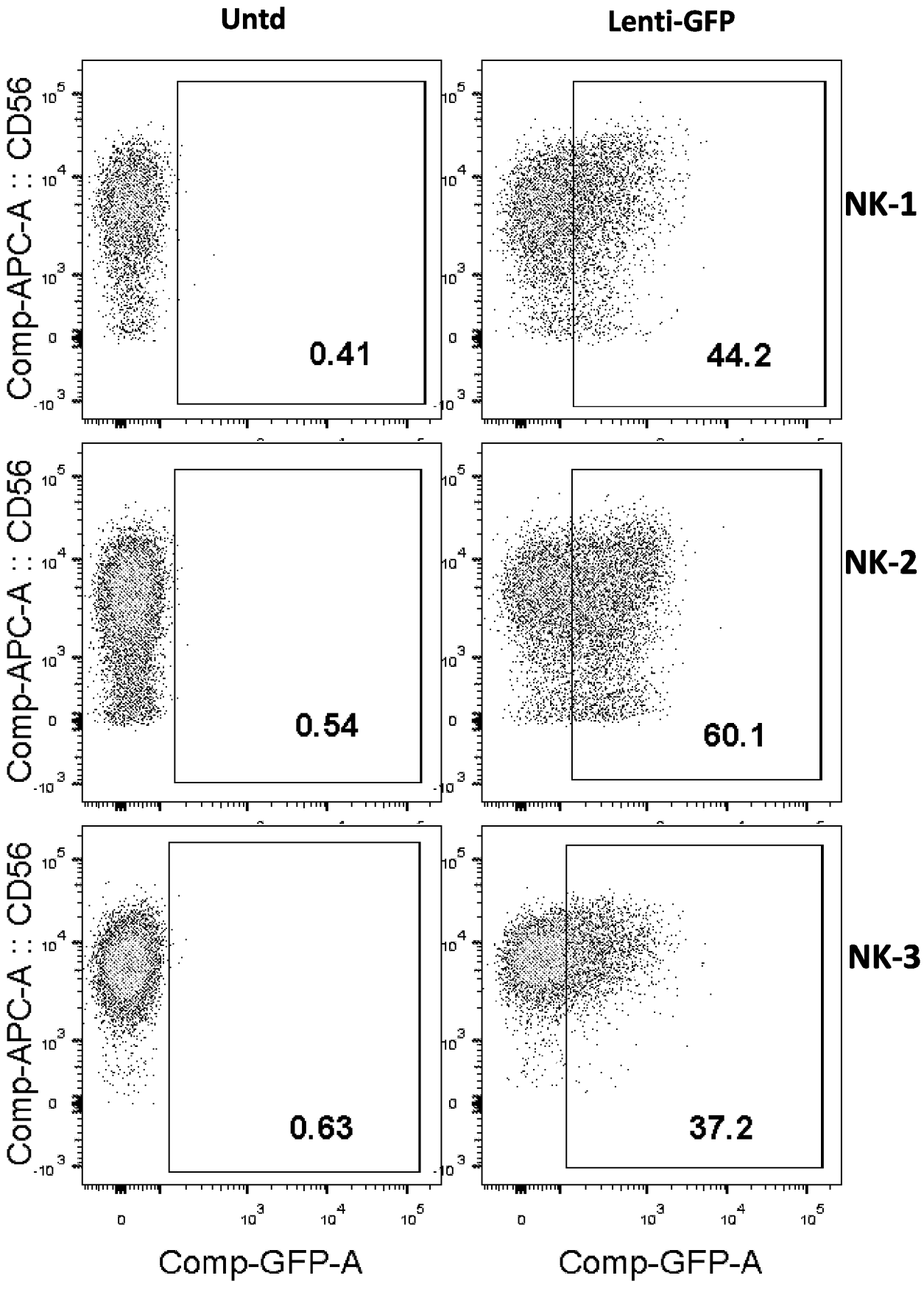

Untd
Lenti-GFP
Comp-APC-A :: CD56
Comp-GFP-A
0.41
44.2
0.54
60.1
0.63
37.2
NK-1
NK-2
NK-3

FIG. 8B

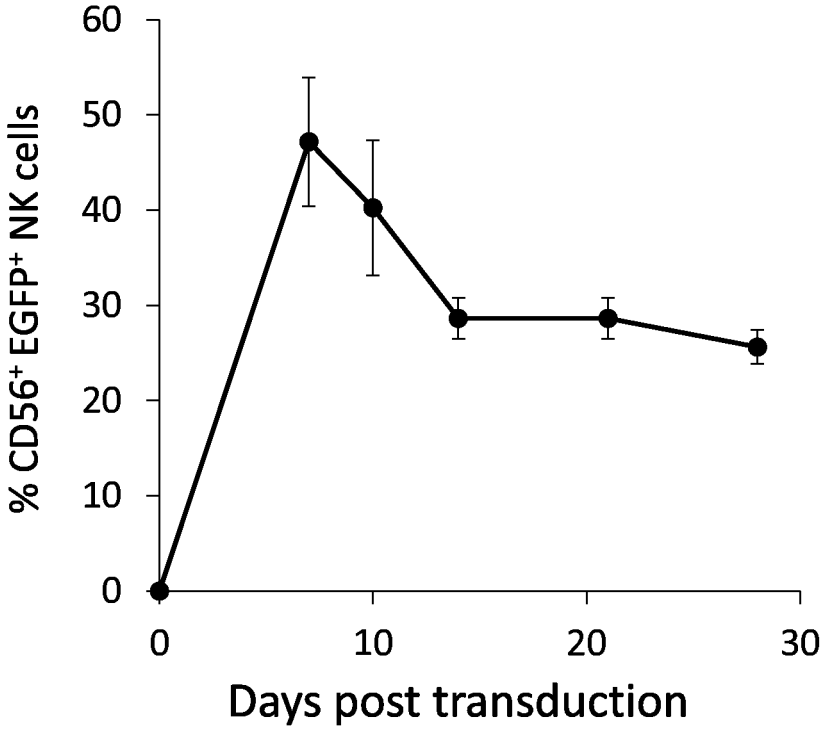

60
50
40
30
20
10
0
% CD56+ EGFP+ NK cells
0
10
20
30
Days post transduction

FIG. 9

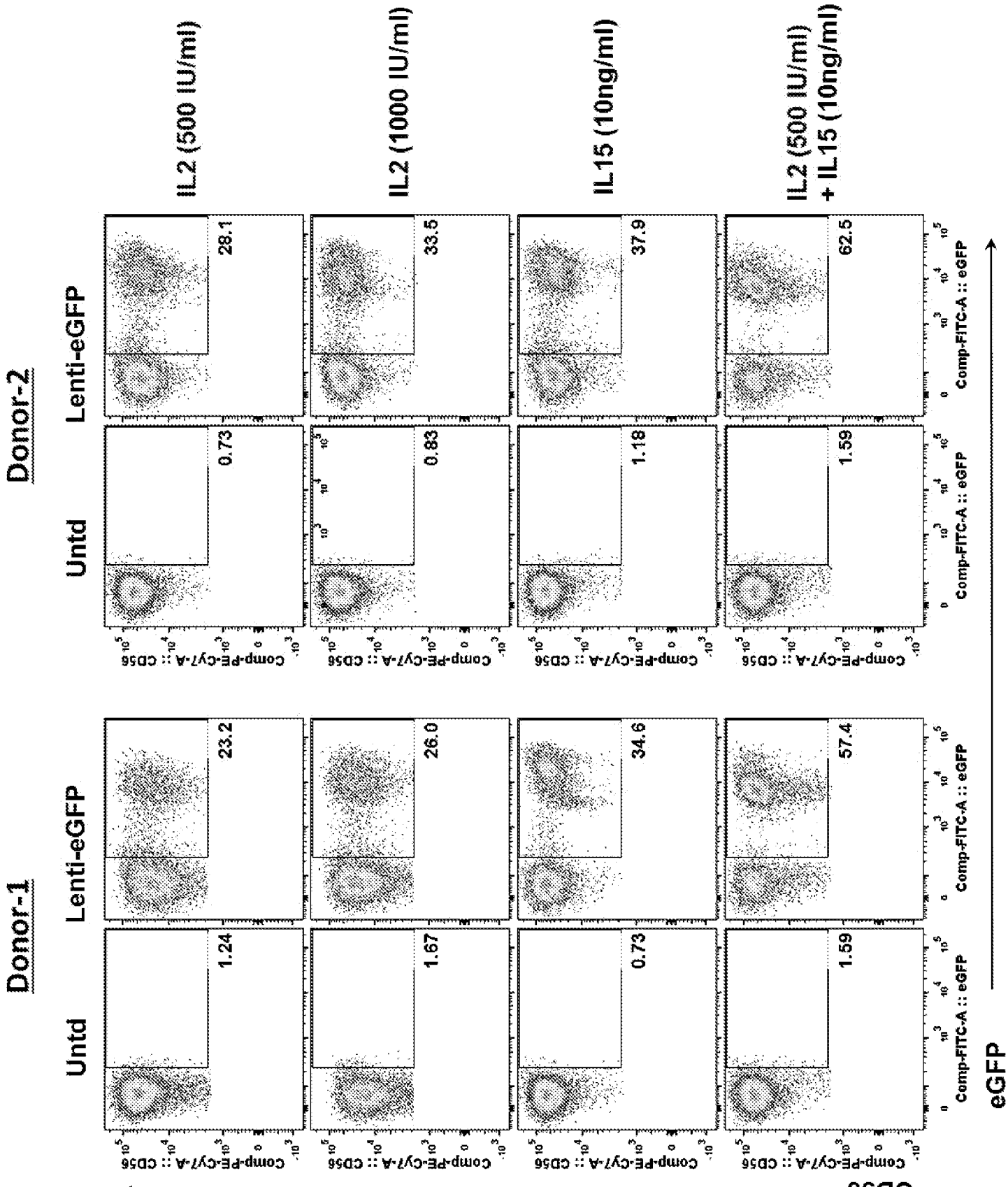

Donor-1
Donor-2
Untd
Lenti-eGFP
IL2 (500 IU/ml)
IL2 (1000 IU/ml)
IL15 (10ng/ml)
IL2 (500 IU/ml) + IL15 (10ng/ml)
1.24
23.2
0.73
28.1
1.67
26.0
0.83
33.5
0.73
34.6
1.18
37.9
1.59
57.4
1.59
62.5
CD56
eGFP
Comp-PE-Cy7-A :: CD56
Comp-FITC-A :: eGFP

FIG. 10

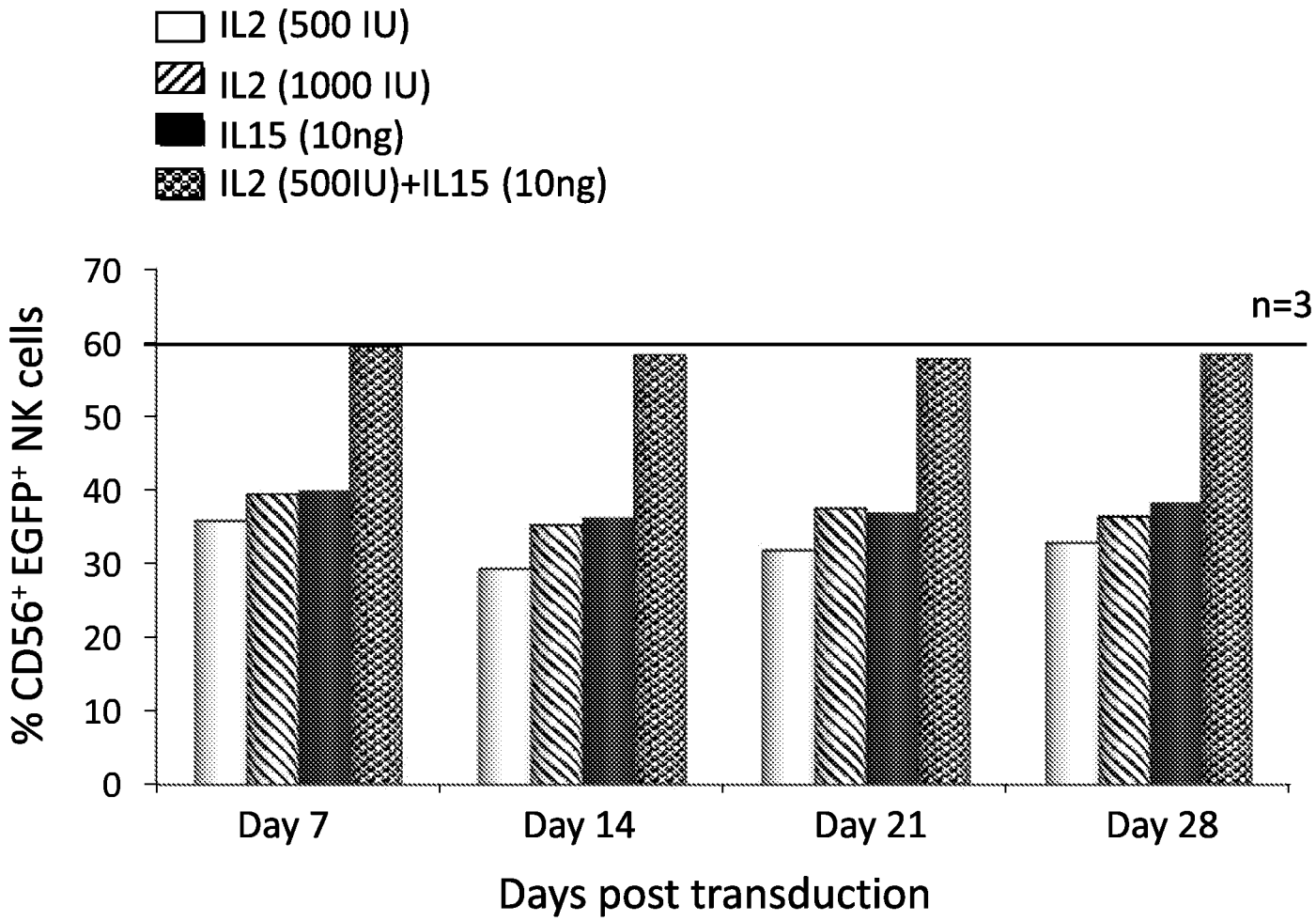

IL2 (500 IU)
IL2 (1000 IU)
IL15 (10ng)
IL2 (500IU)+IL15 (10ng)
% CD56+ EGFP+ NK cells
70
60
50
40
30
20
10
0
n=3
Day 7
Day 14
Day 21
Day 28
Days post transduction

FIG. 11

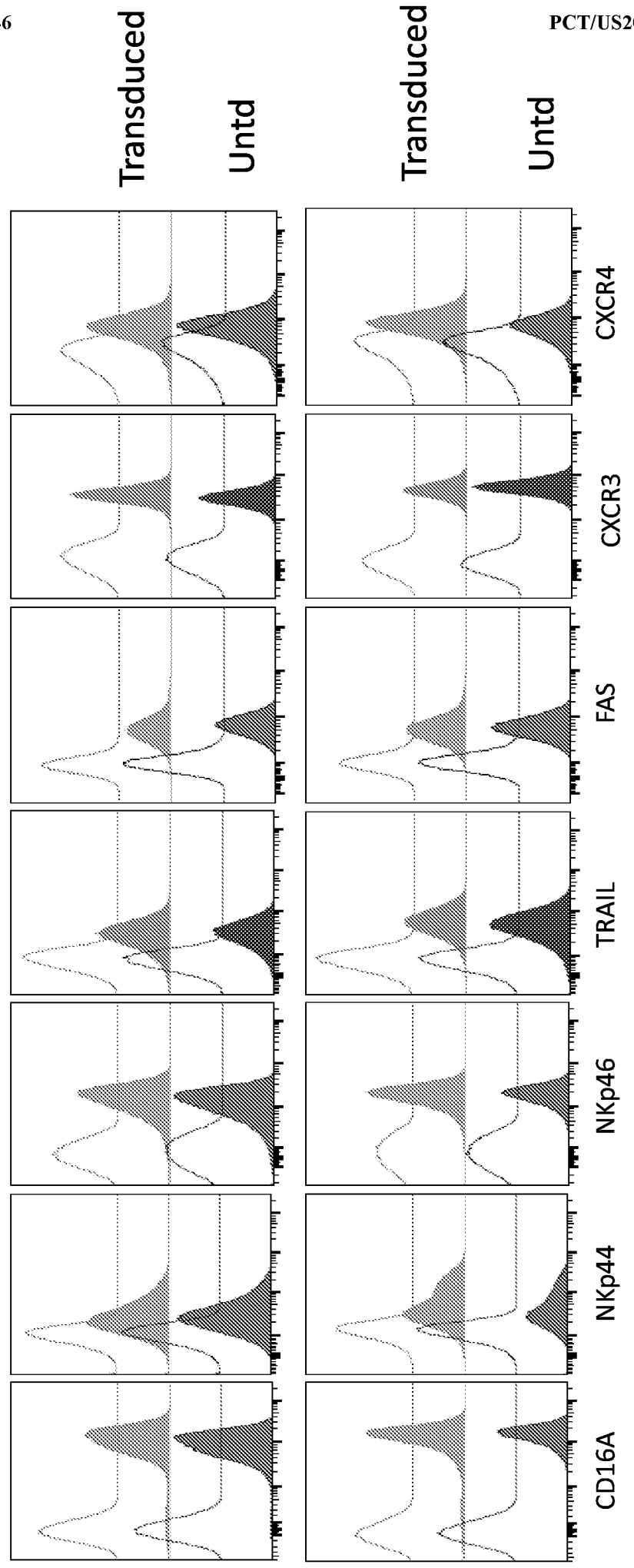

Transduced
Untd
Transduced
Untd
CD16A
NKp44
NKp46
TRAIL
FAS
CXCR3
CXCR4

FIG. 12

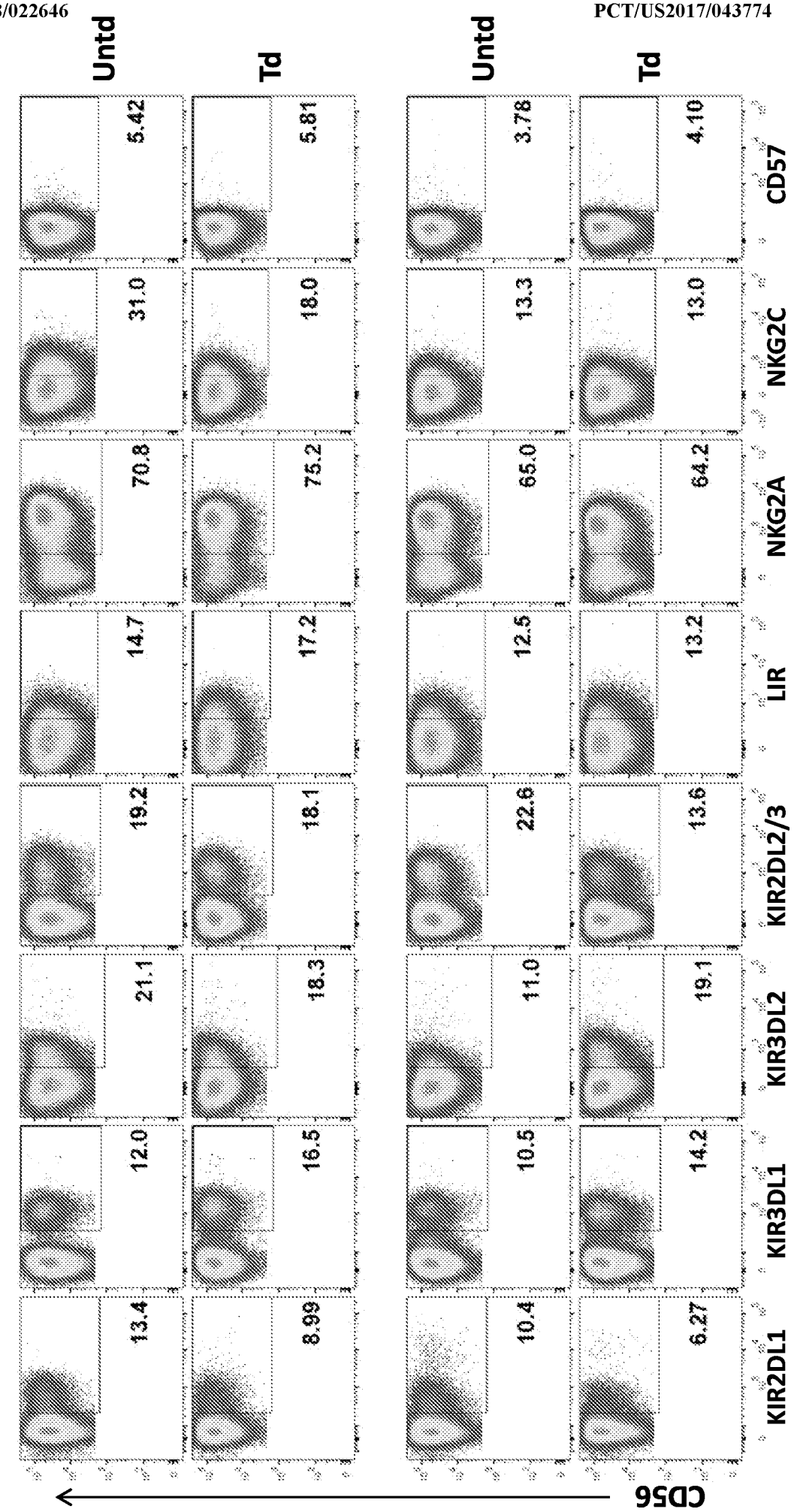

Untd
Td
KIR2DL1
KIR3DL1
KIR3DL2
KIR2DL2/3
LIR
NKG2A
NKG2C
CD57
CD56
13.4
12.0
21.1
19.2
14.7
70.8
31.0
5.42
10.4
10.5
11.0
22.6
12.5
65.0
13.3
3.78
8.99
16.5
18.3
18.1
17.2
75.2
18.0
5.81
6.27
14.2
19.1
13.6
13.2
64.2
13.0
4.10

FIG. 13

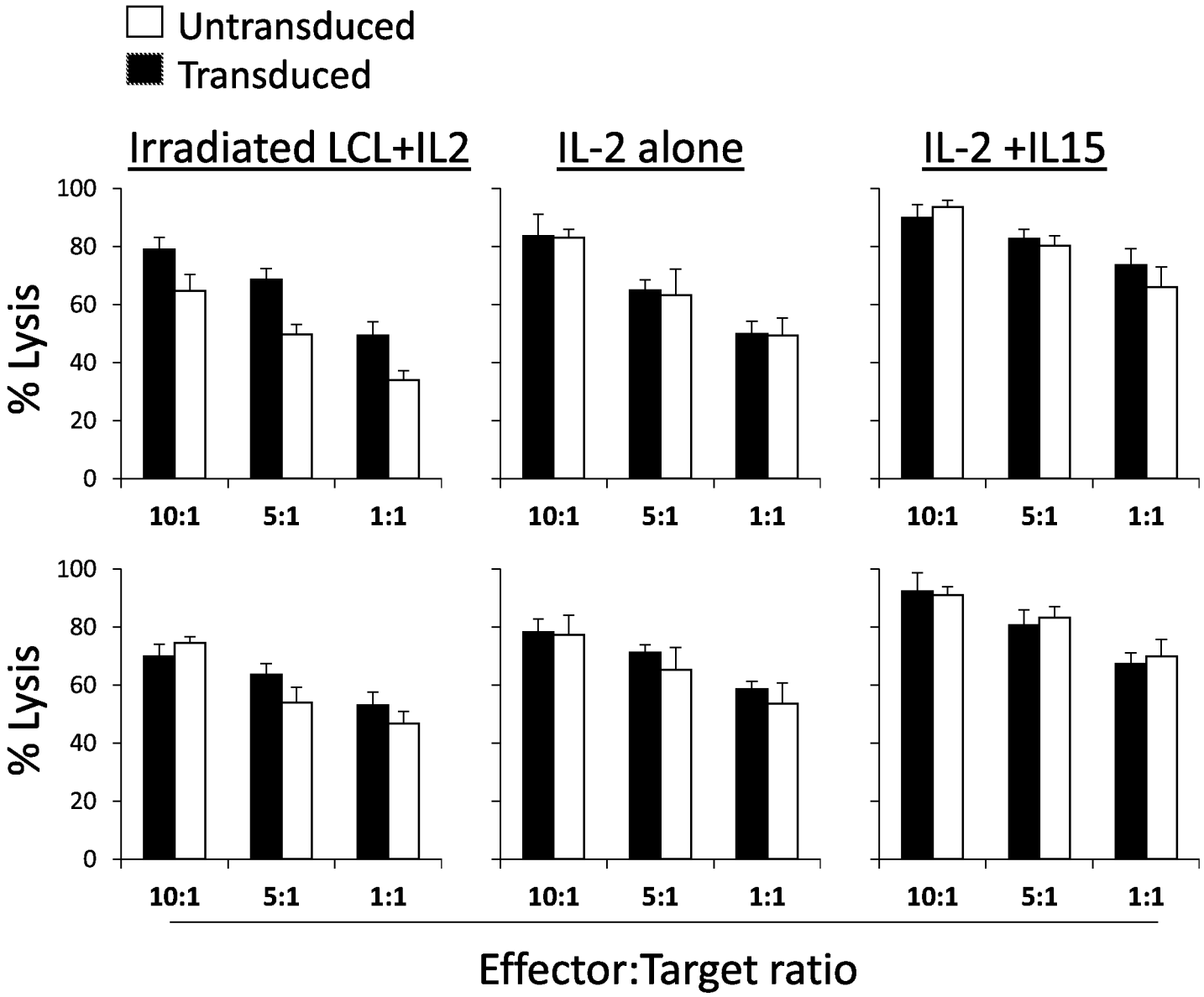

Untransduced
Transduced
Irradiated LCL+IL2
IL-2 alone
IL-2 +IL15
% Lysis
100
80
60
40
20
0
10:1
5:1
1:1
Effector:Target ratio

FIG. 14

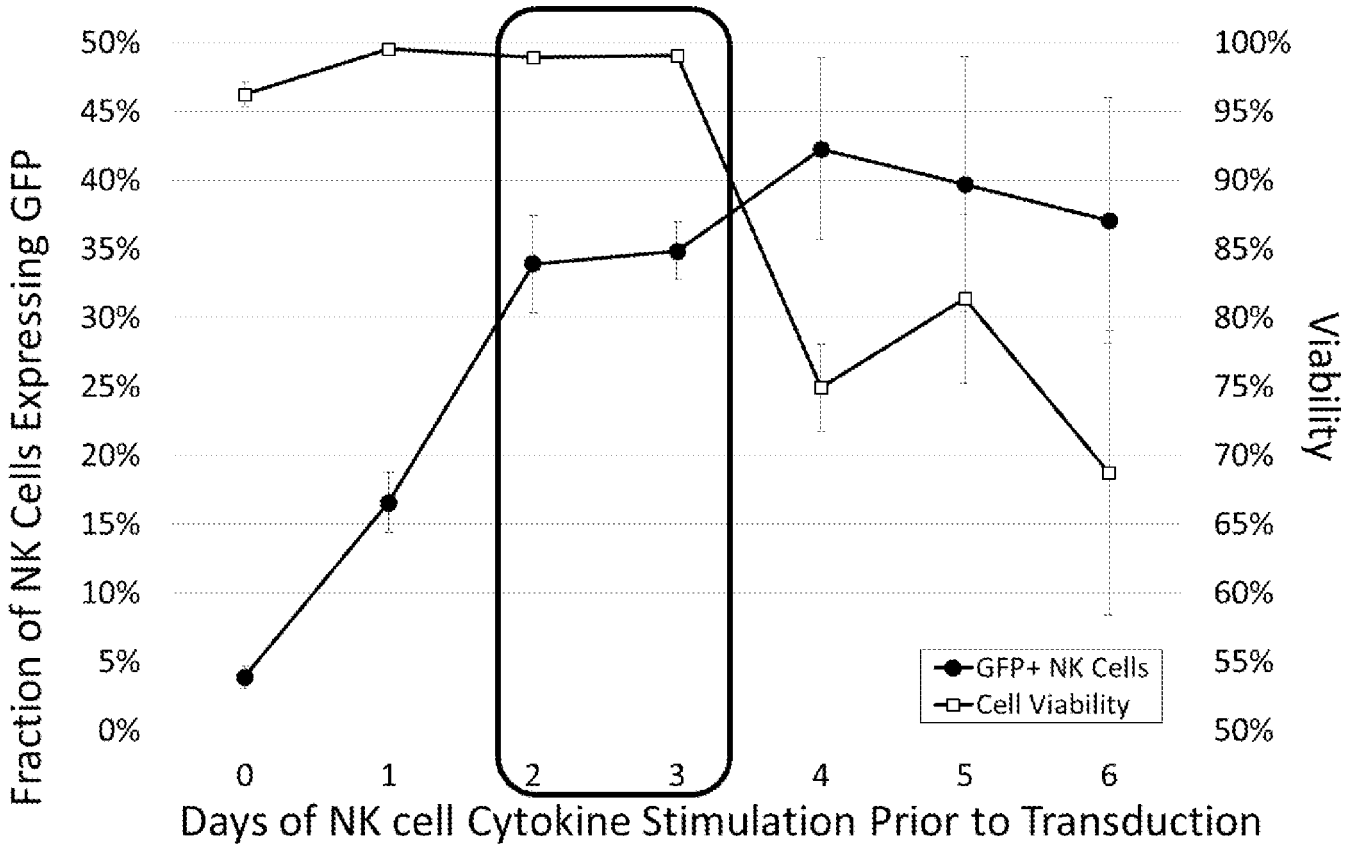

Fraction of NK Cells Expressing GFP
Viability
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
100%
95%
90%
85%
80%
75%
70%
65%
60%
55%
50%
0
1
2
3
4
5
6
GFP+ NK Cells
Cell Viability
Days of NK cell Cytokine Stimulation Prior to Transduction

FIG. 15

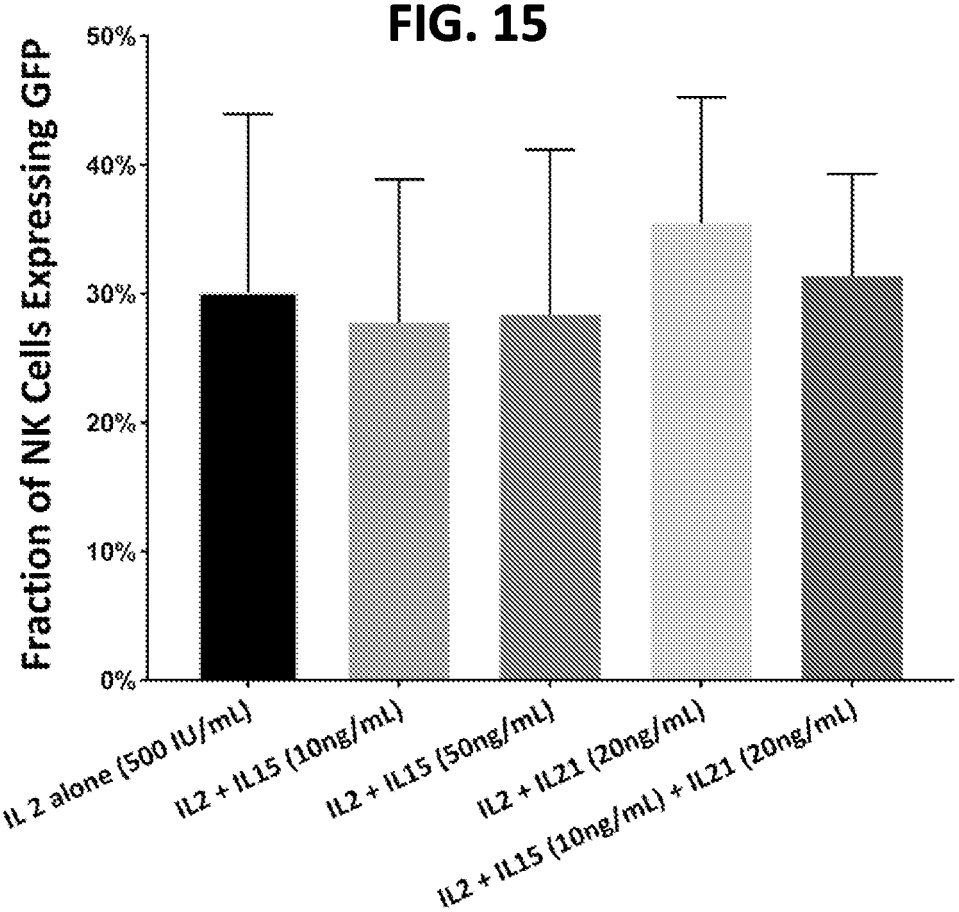

Fraction of NK Cells Expressing GFP
50%
40%
30%
20%
10%
0%
IL 2 alone (500 IU/mL)
IL2 + IL15 (10ng/mL)
IL2 + IL15 (50ng/mL)
IL2 + IL21 (20ng/mL)
IL2 + IL15 (10ng/mL) + IL21 (20ng/mL)

FIG. 16A

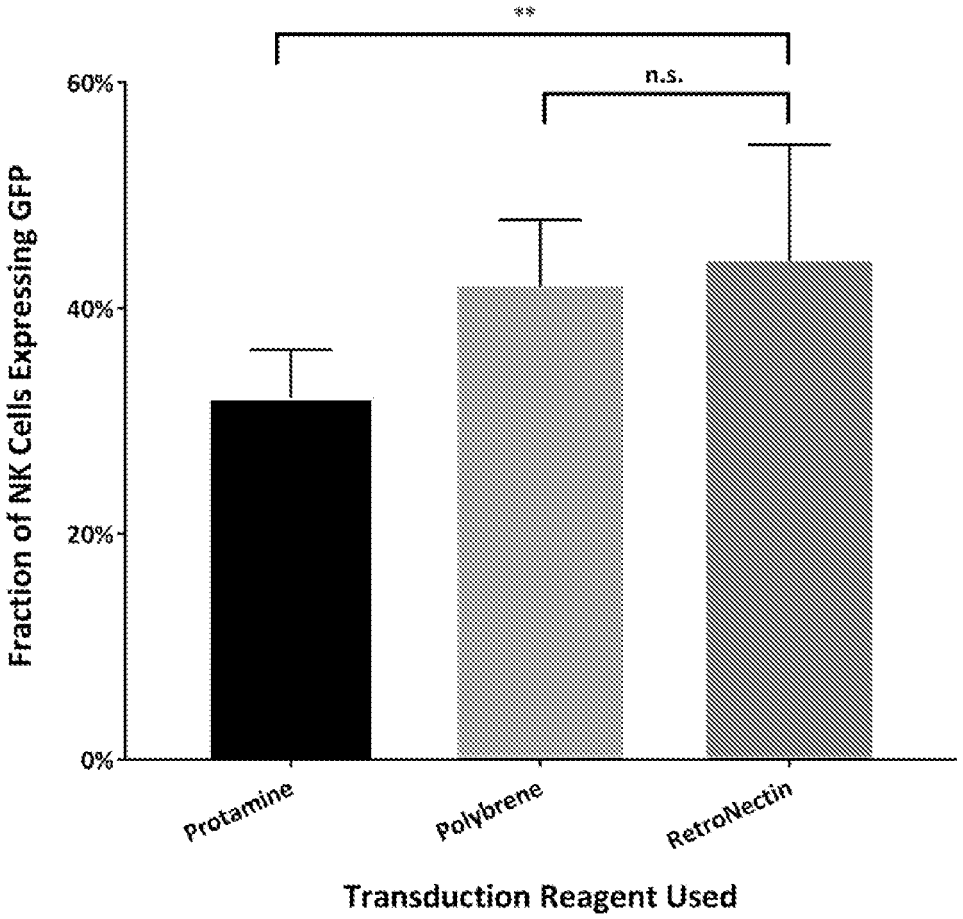
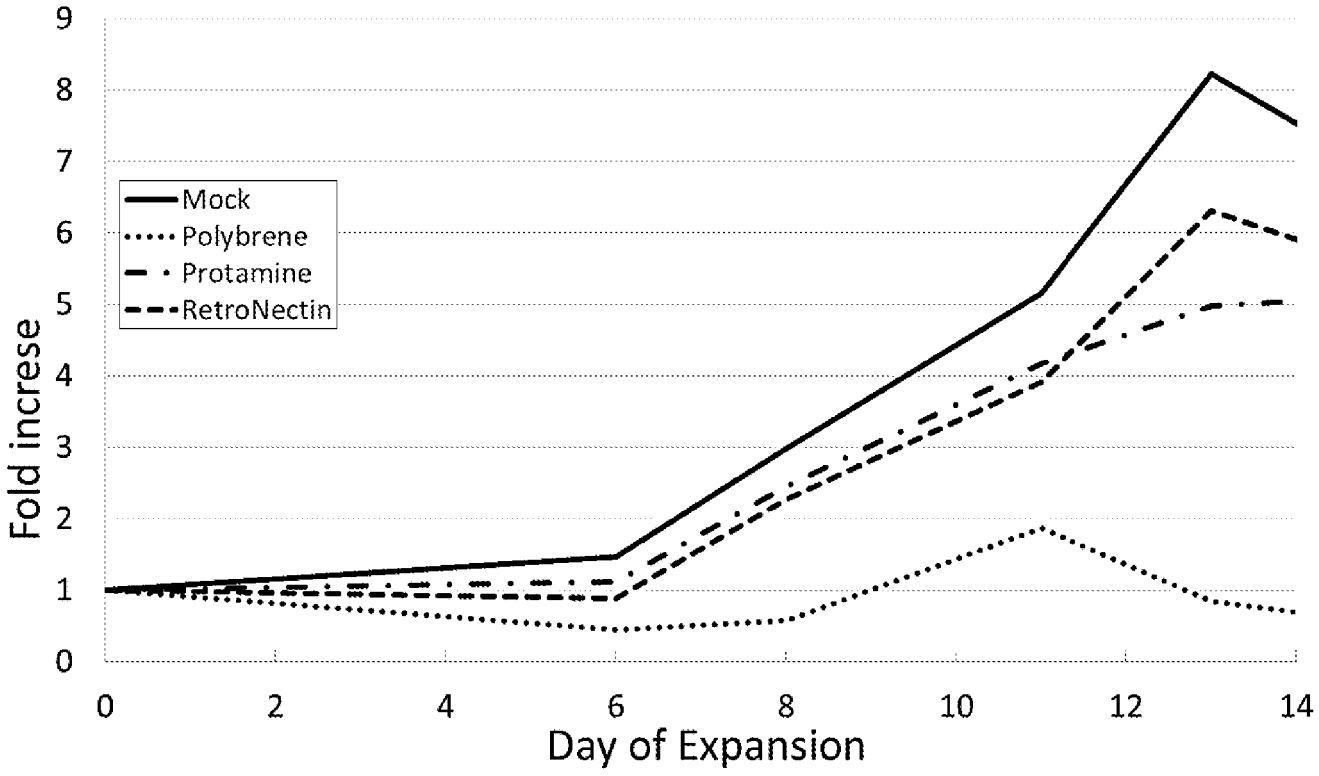


FIG. 16B



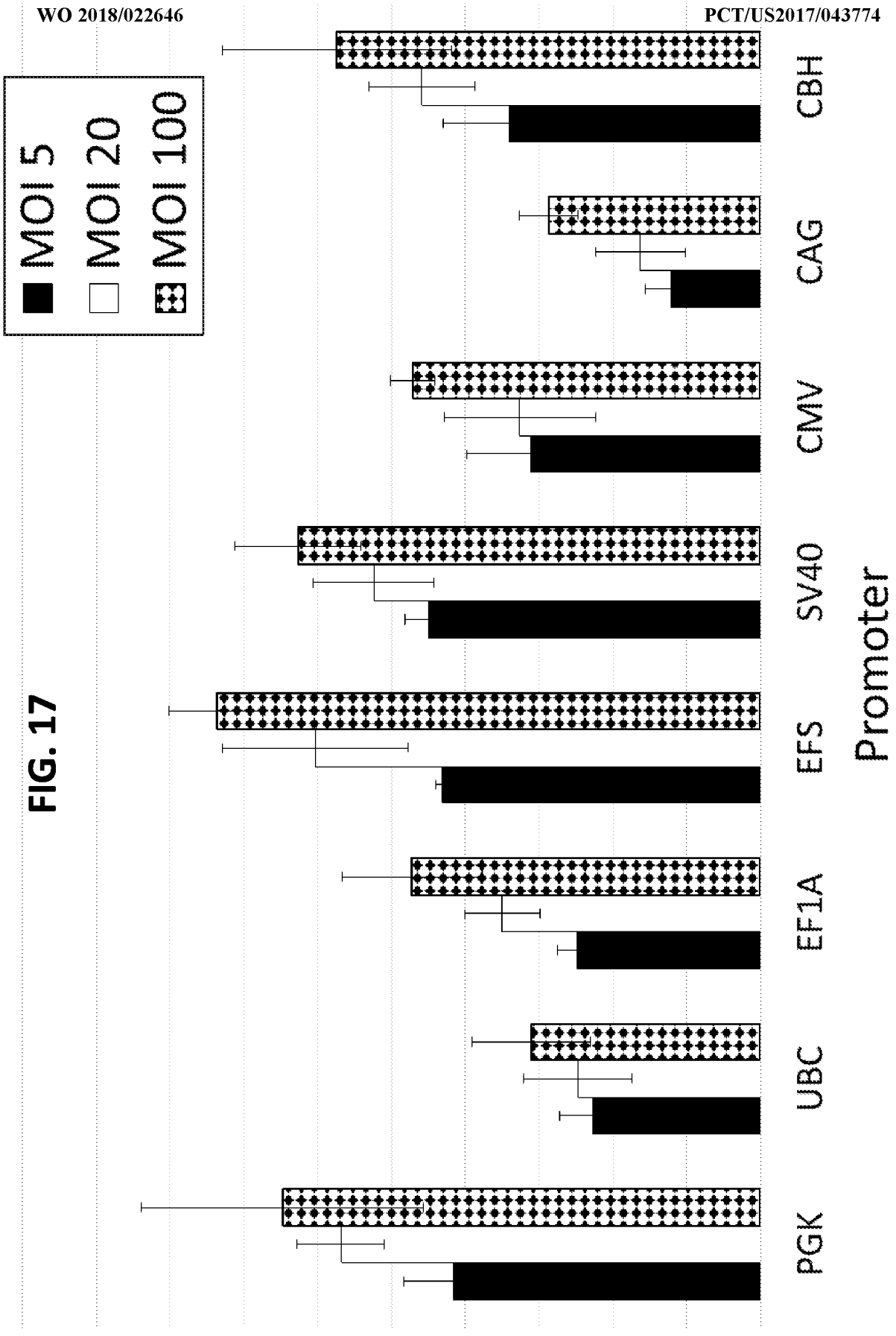

MOI 5
MOI 20
MOI 100
Fraction of NK Cells Expressing GFP
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
PGK
UBC
EF1A
EFS
SV40
CMV
CAG
CBH
Promoter

FIG. 17

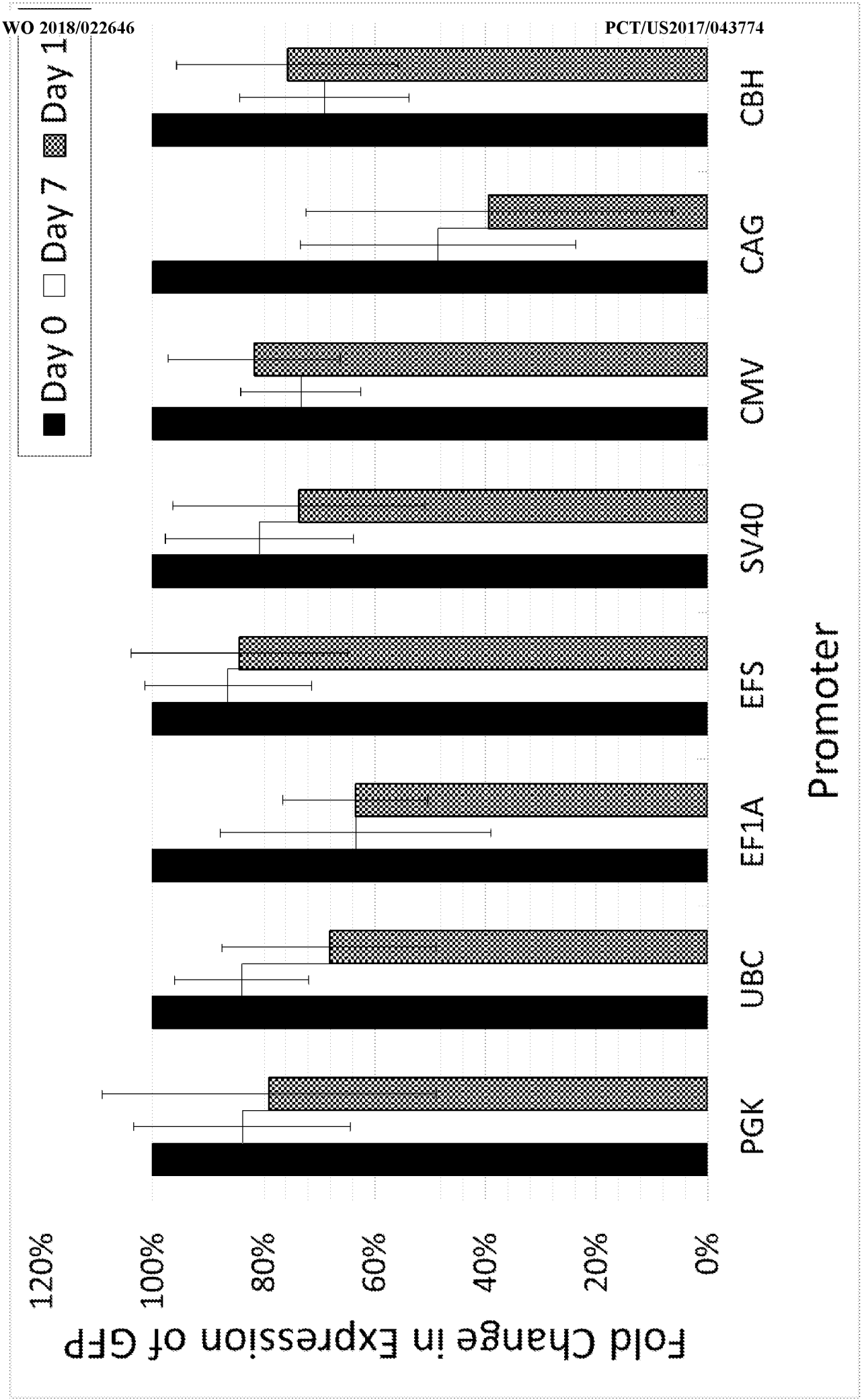

Day 0
Day 7
Day 1
Fold Change in Expression of GFP
120%
100%
80%
60%
40%
20%
0%
PGK
UBC
EF1A
EFS
SV40
CMV
CAG
CBH
Promoter

FIG. 18

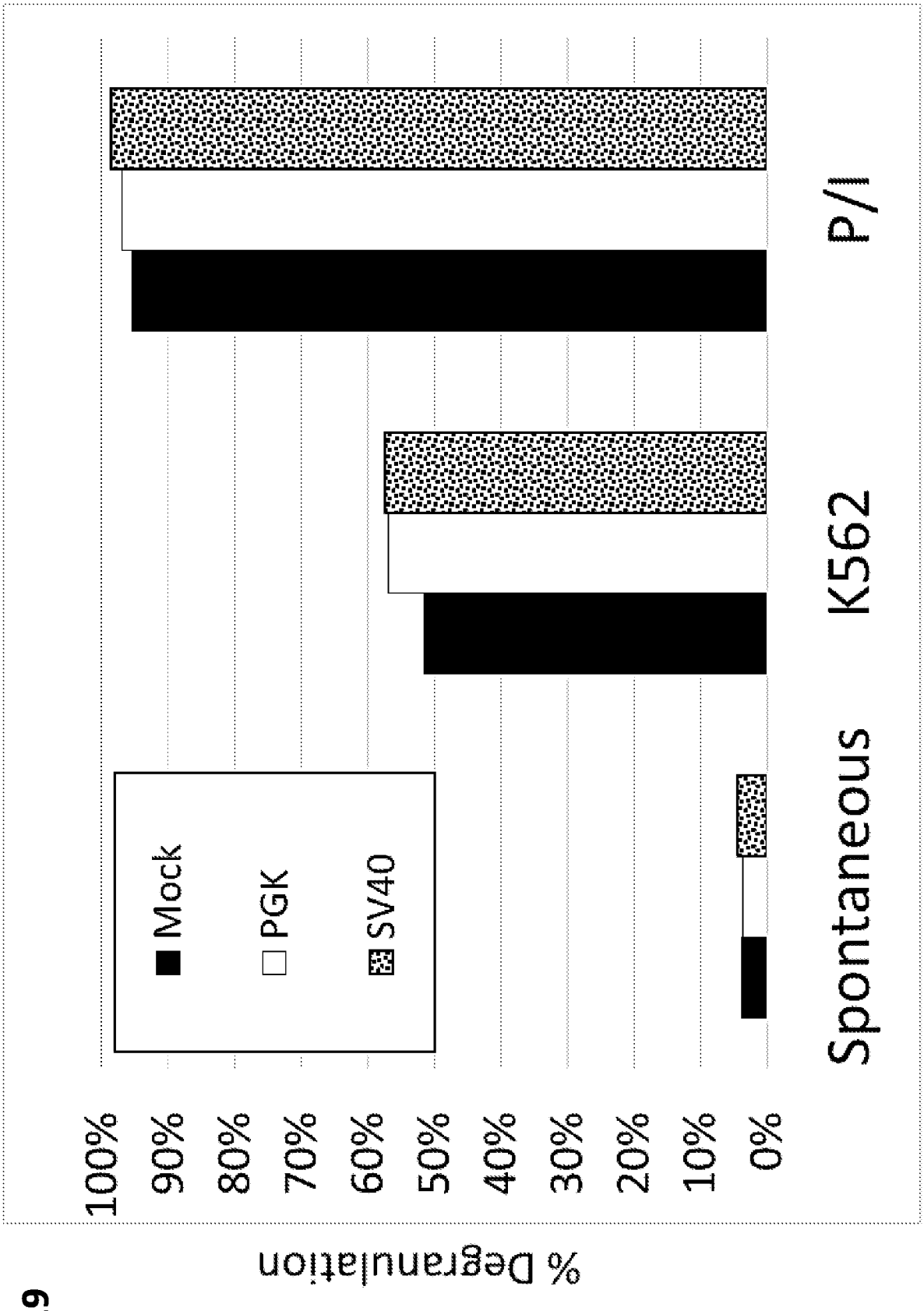

% Degranulation
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Mock
PGK
SV40
Spontaneous
K562
P/I

FIG. 19

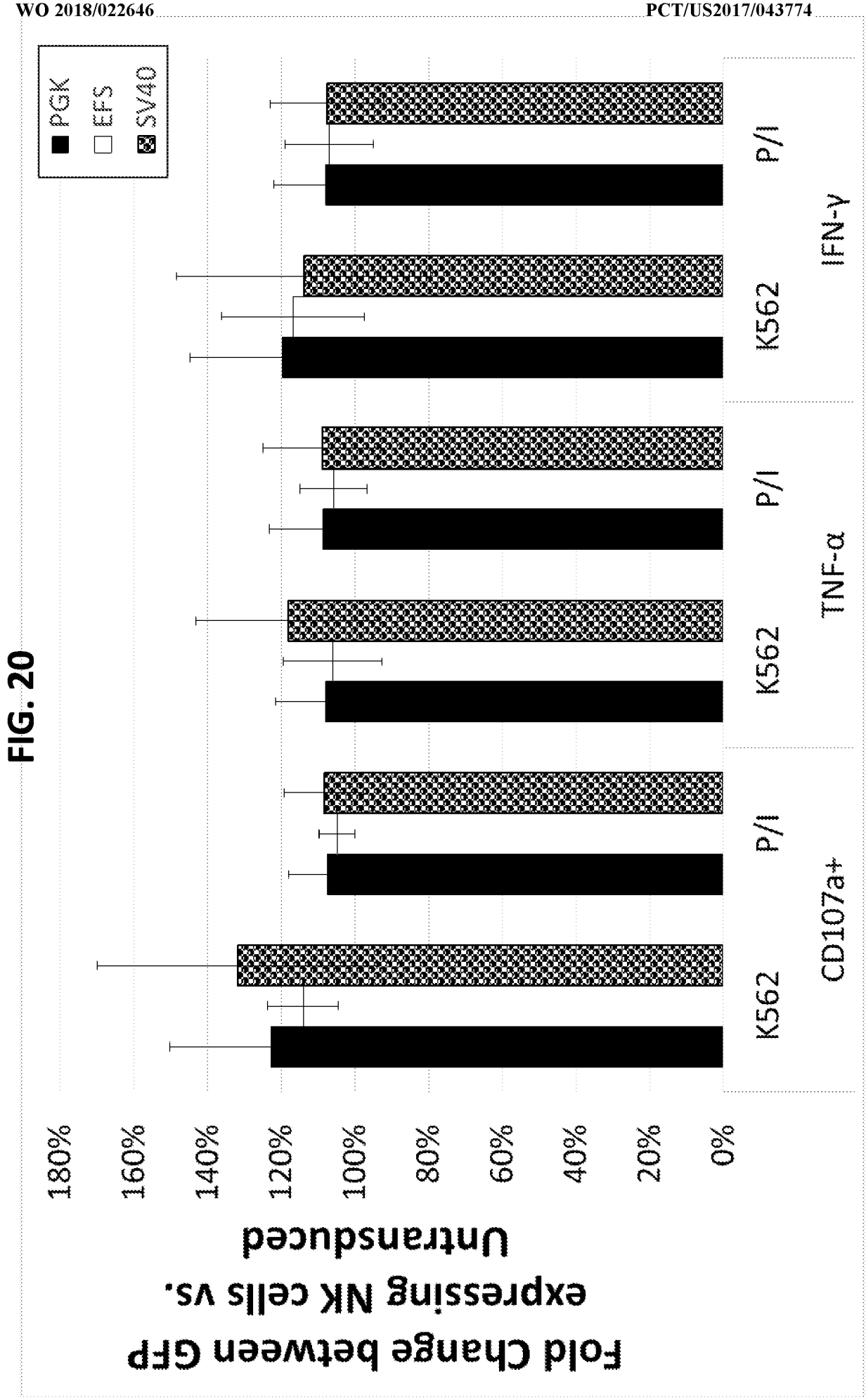

Fold Change between GFP expressing NK cells vs. Untransduced
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
PGK
EFS
SV40
CD107a+
TNF-α
IFN-γ
K562
P/I

FIG. 20

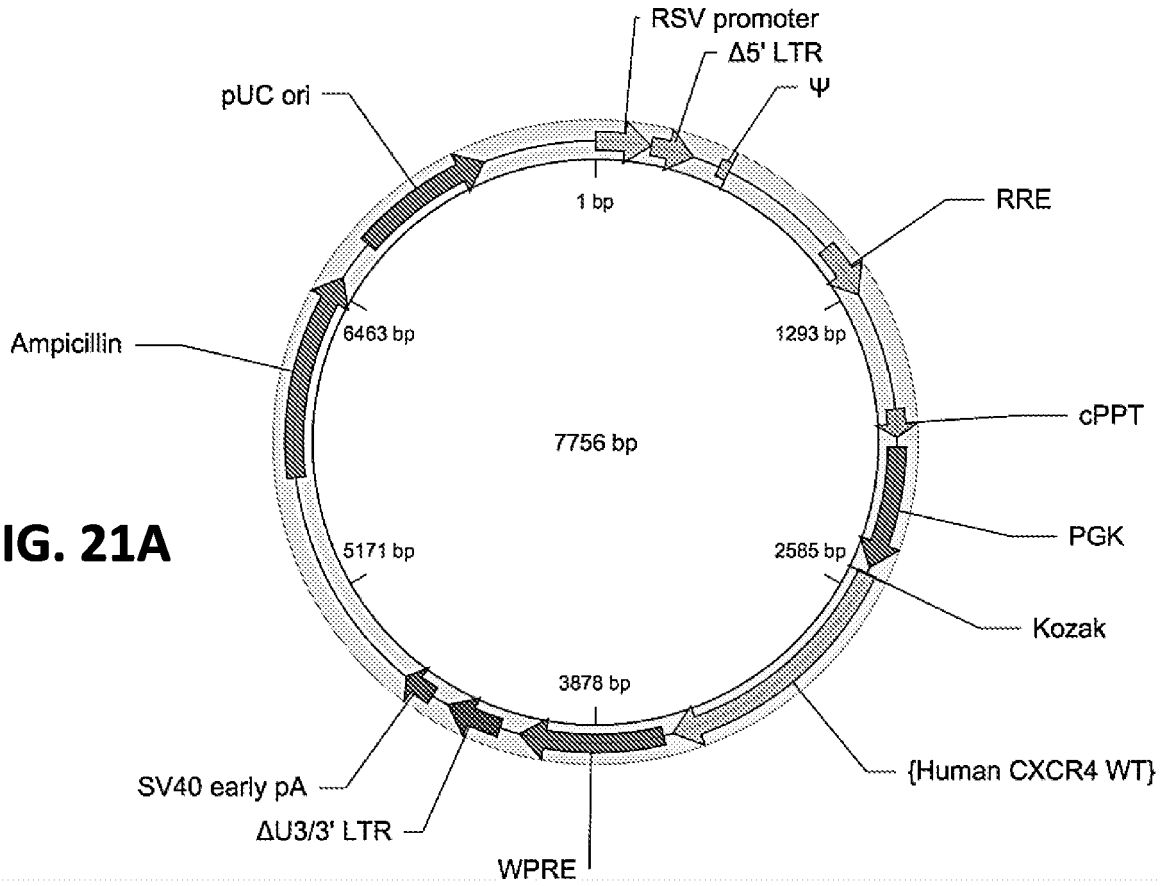


FIG. 21A

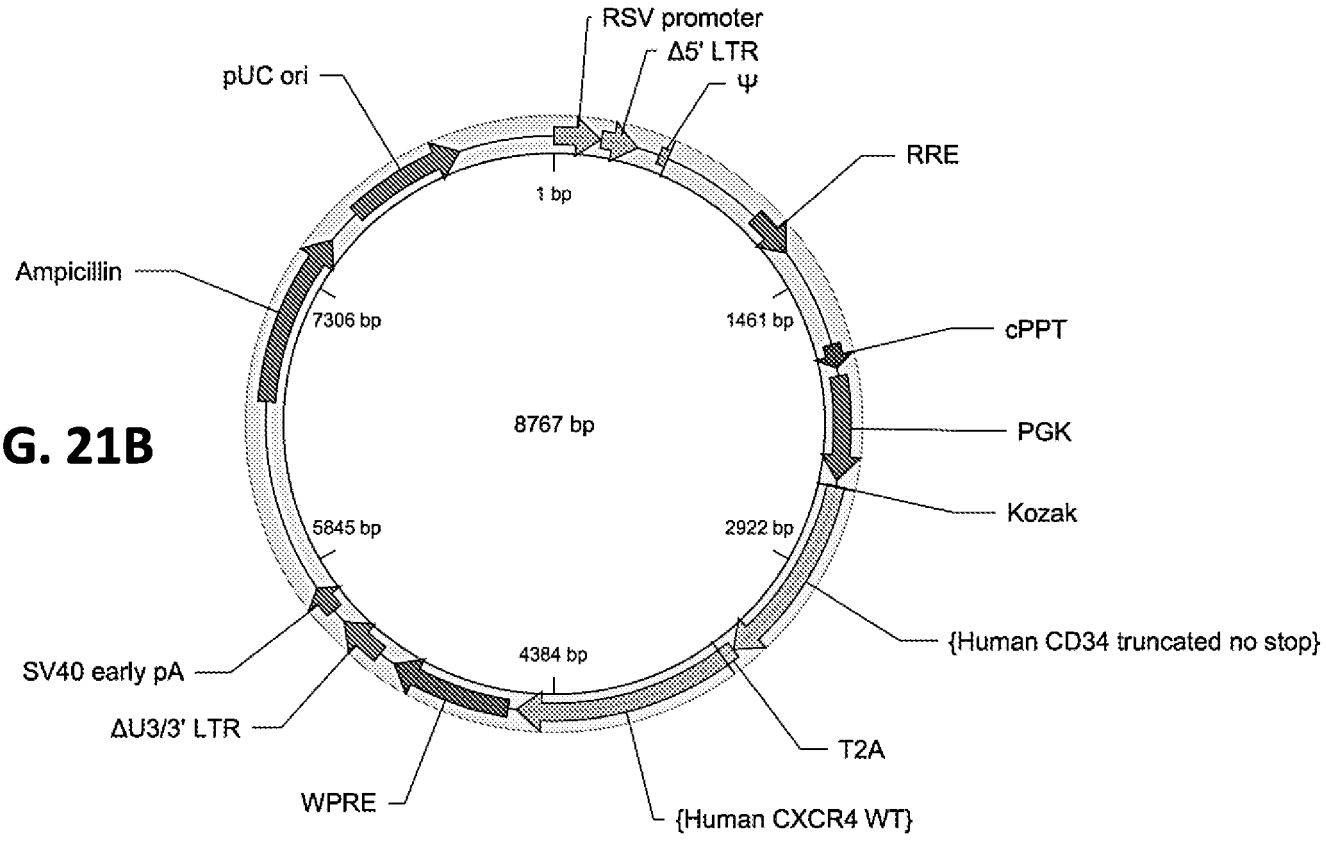


FIG. 21B

FIG. 22A

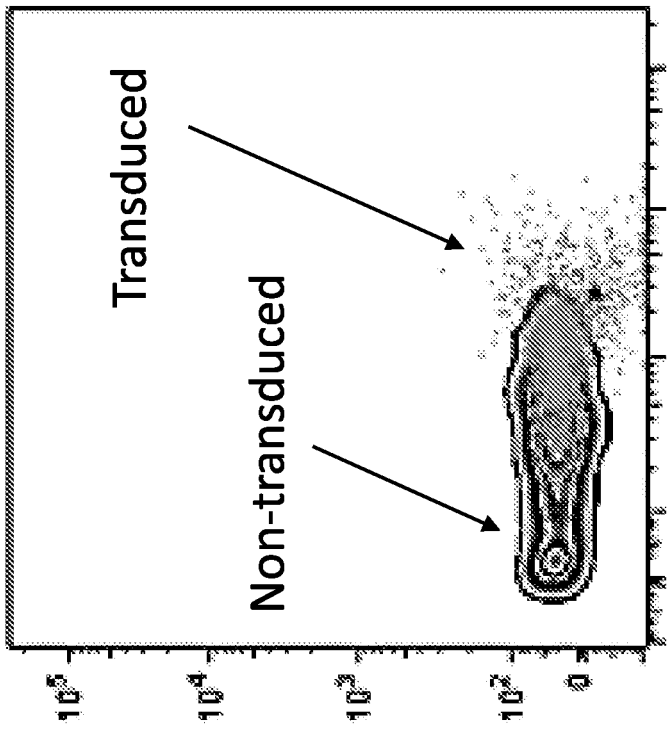


FIG. 22B

