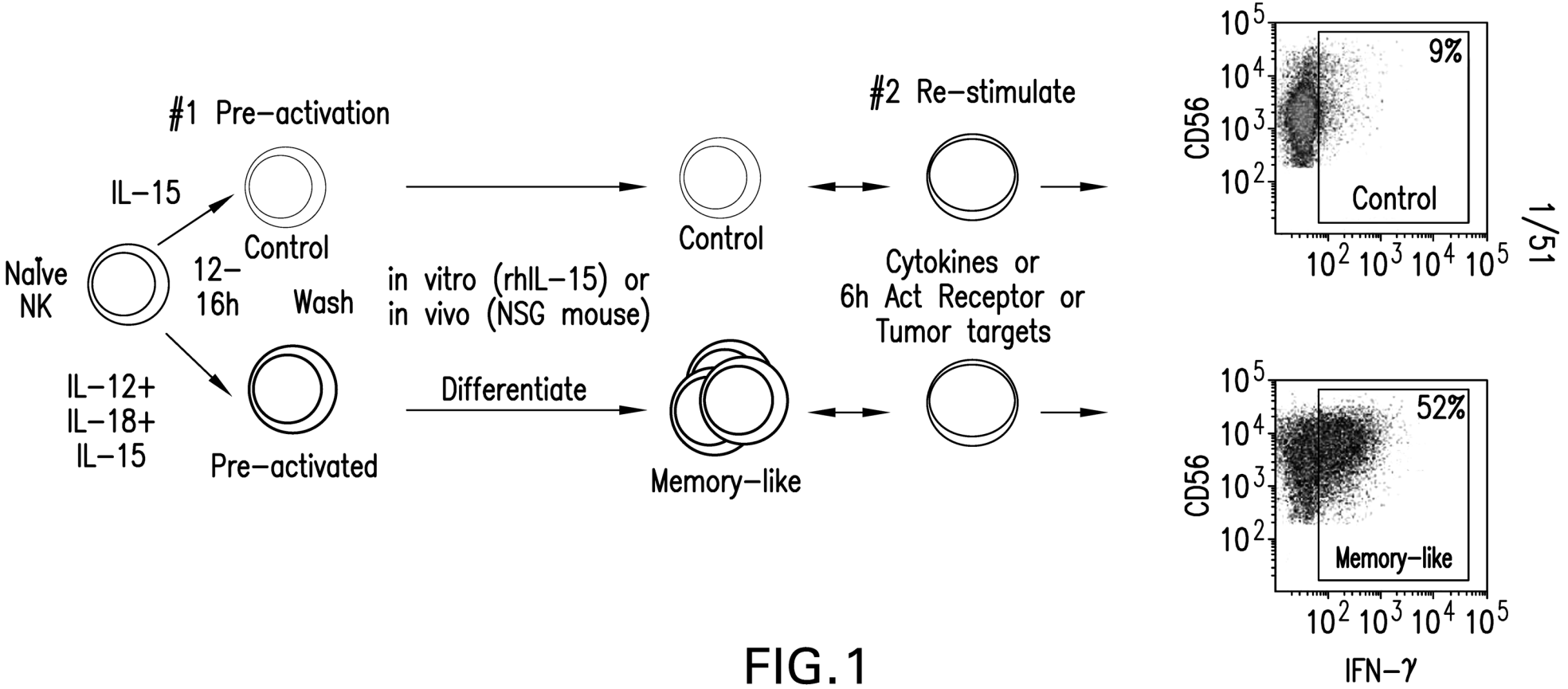

#1 Pre-activation
IL-15
Control
Naïve NK
12-16h
Wash
IL-12+ IL-18+ IL-15
Pre-activated
in vitro (rhIL-15) or in vivo (NSG mouse)
Differentiate
Control
Memory-like
#2 Re-stimulate
Cytokines or 6h Act Receptor or Tumor targets
CD56
IFN-γ
9%
Control
52%
Memory-like
1/51

FIG. 1

cNK: Conventional NK cells
CIML NK: Cytokine-induced memory-like NK cells

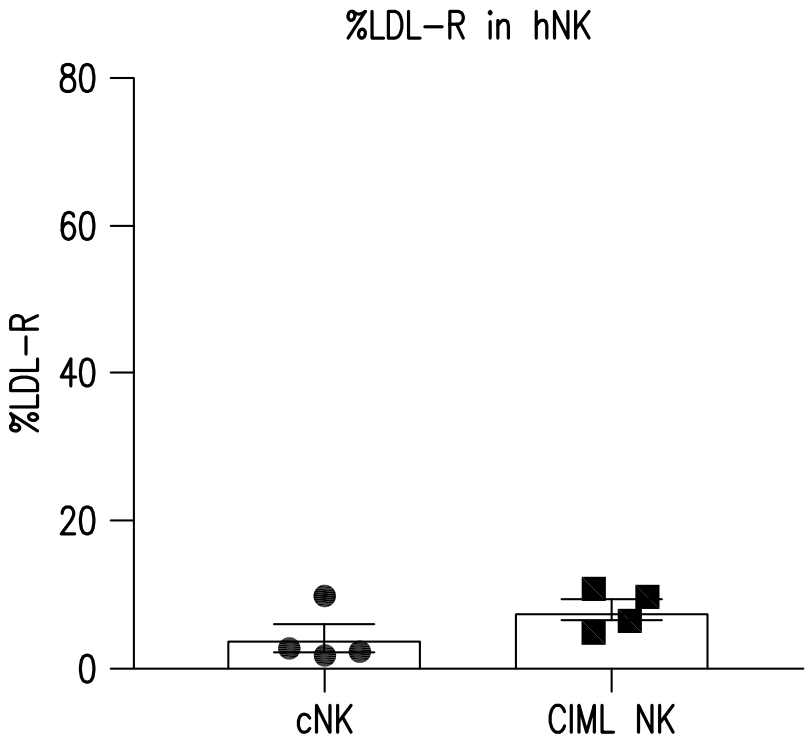


FIG.2A

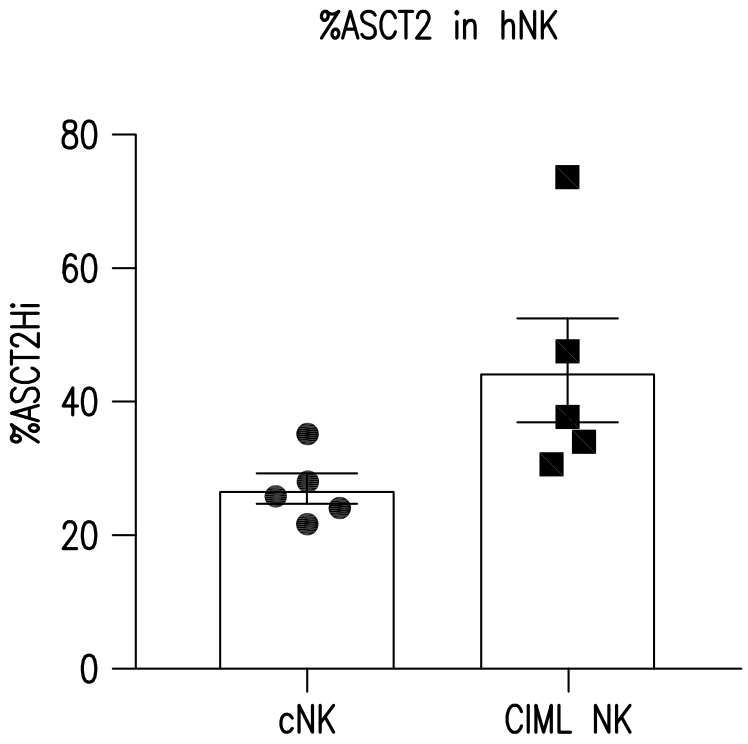


FIG.2B

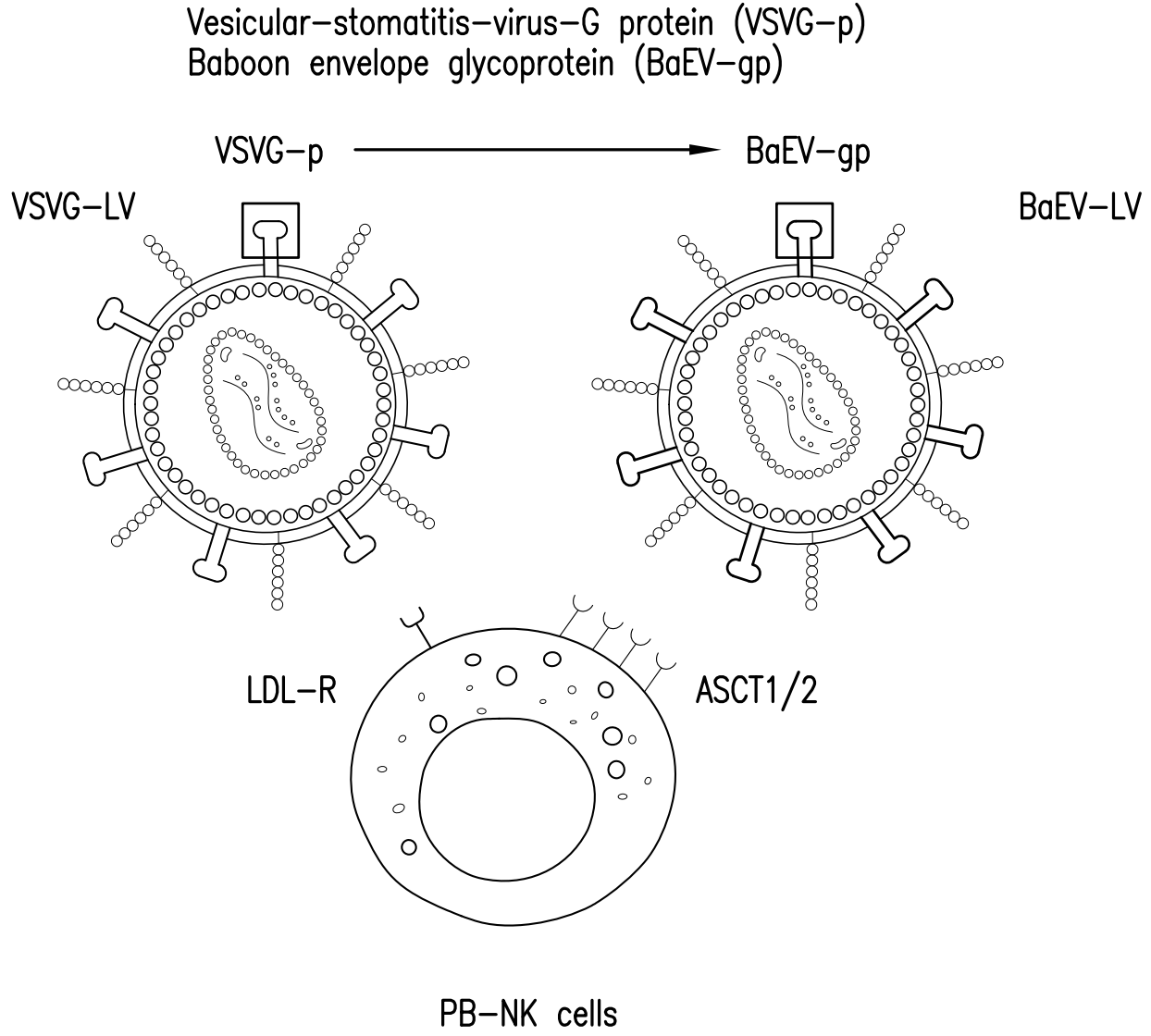

Vesicular-stomatitis-virus-G protein (VSVG-p)
Baboon envelope glycoprotein (BaEV-gp)
VSVG-p
BaEV-gp
VSVG-LV
BaEV-LV
LDL-R
ASCT1/2
PB-NK cells

FIG.2C

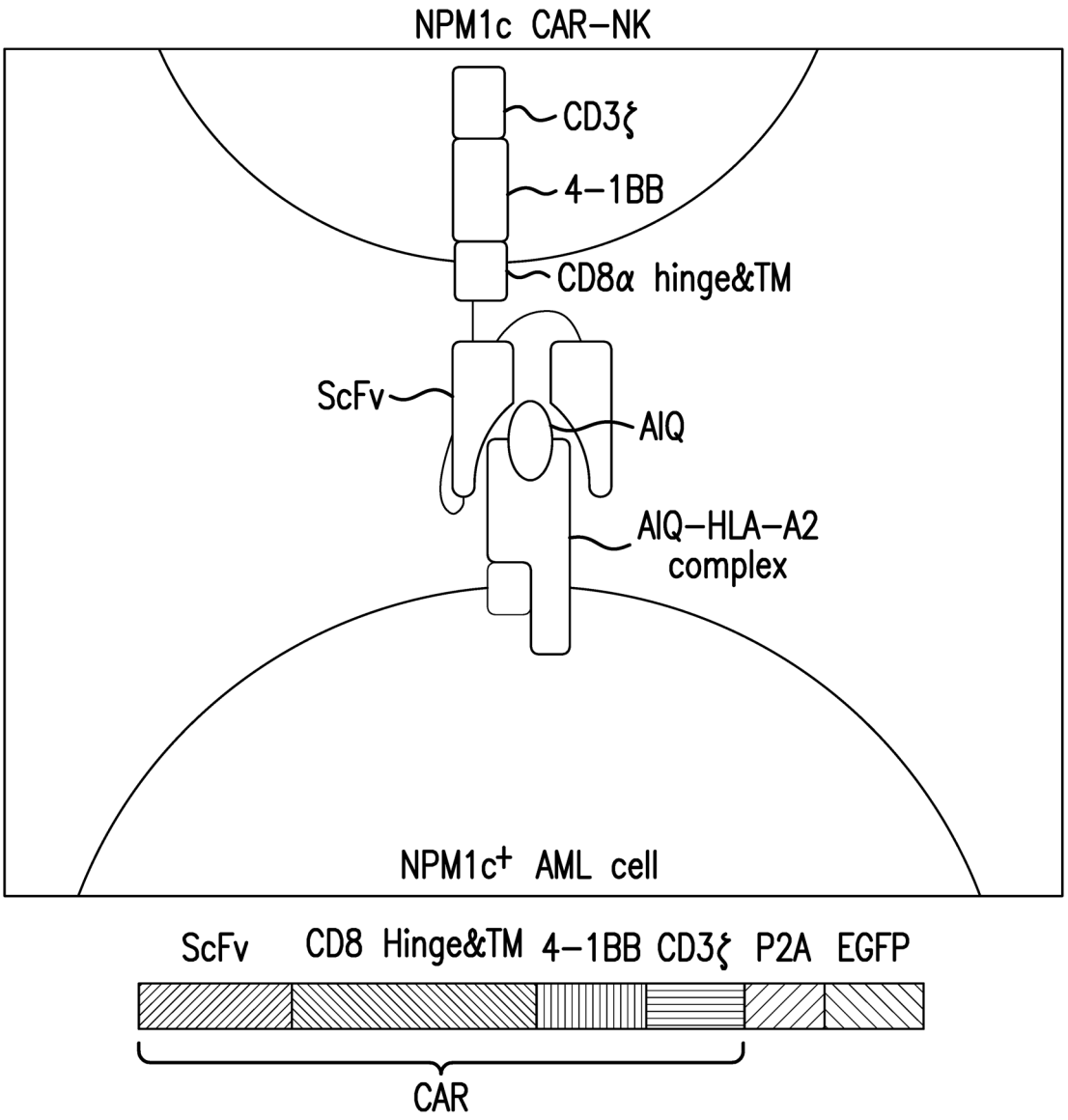

NPM1c CAR-NK
CD3ζ
4-1BB
CD8α hinge&TM
ScFv
AIQ
AIQ-HLA-A2
complex
NPM1c+ AML cell
ScFv
CD8 Hinge&TM
4-1BB
CD3ζ
P2A
EGFP
CAR

FIG.3A

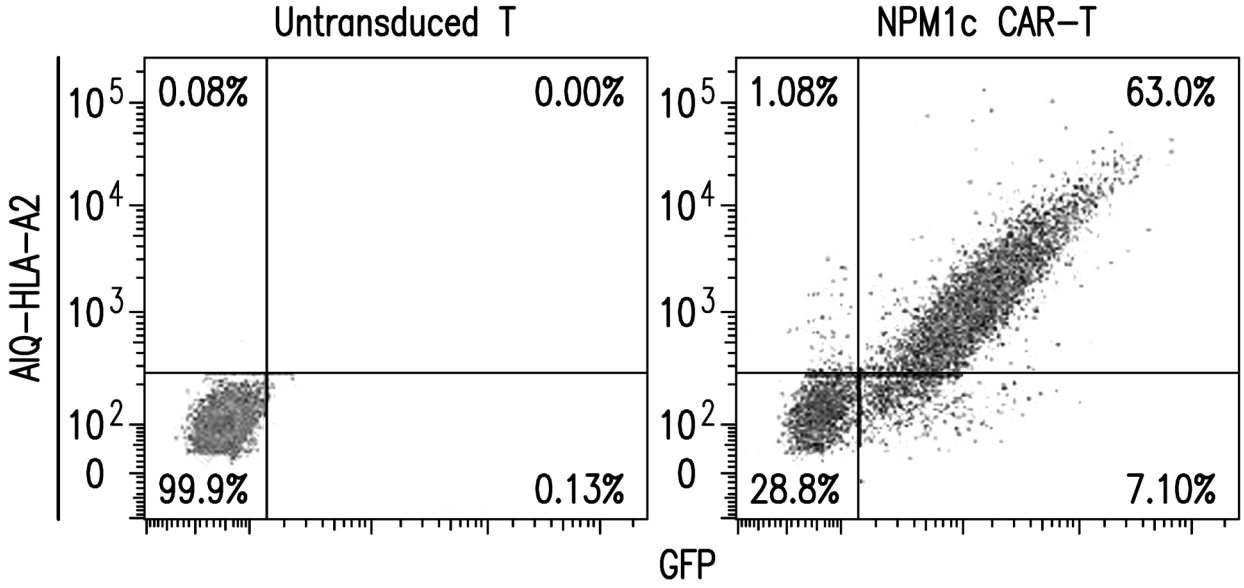

Untransduced T
NPM1c CAR-T
AIQ-HLA-A2
GFP
0.08%
0.00%
99.9%
0.13%
1.08%
63.0%
28.8%
7.10%

FIG.3B

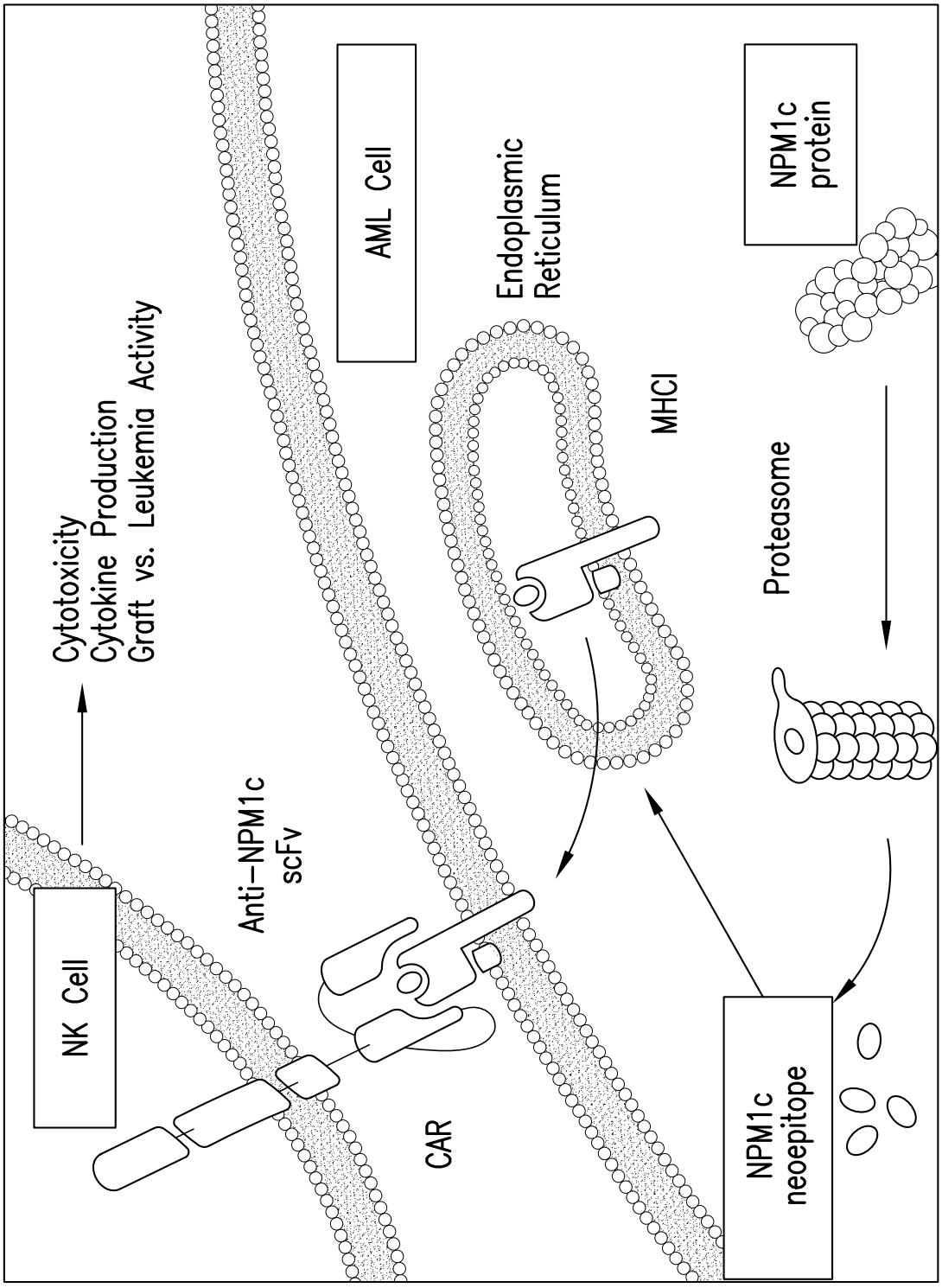


FIG.4

CAR targeting a neoepitope derived from NPM1c protein

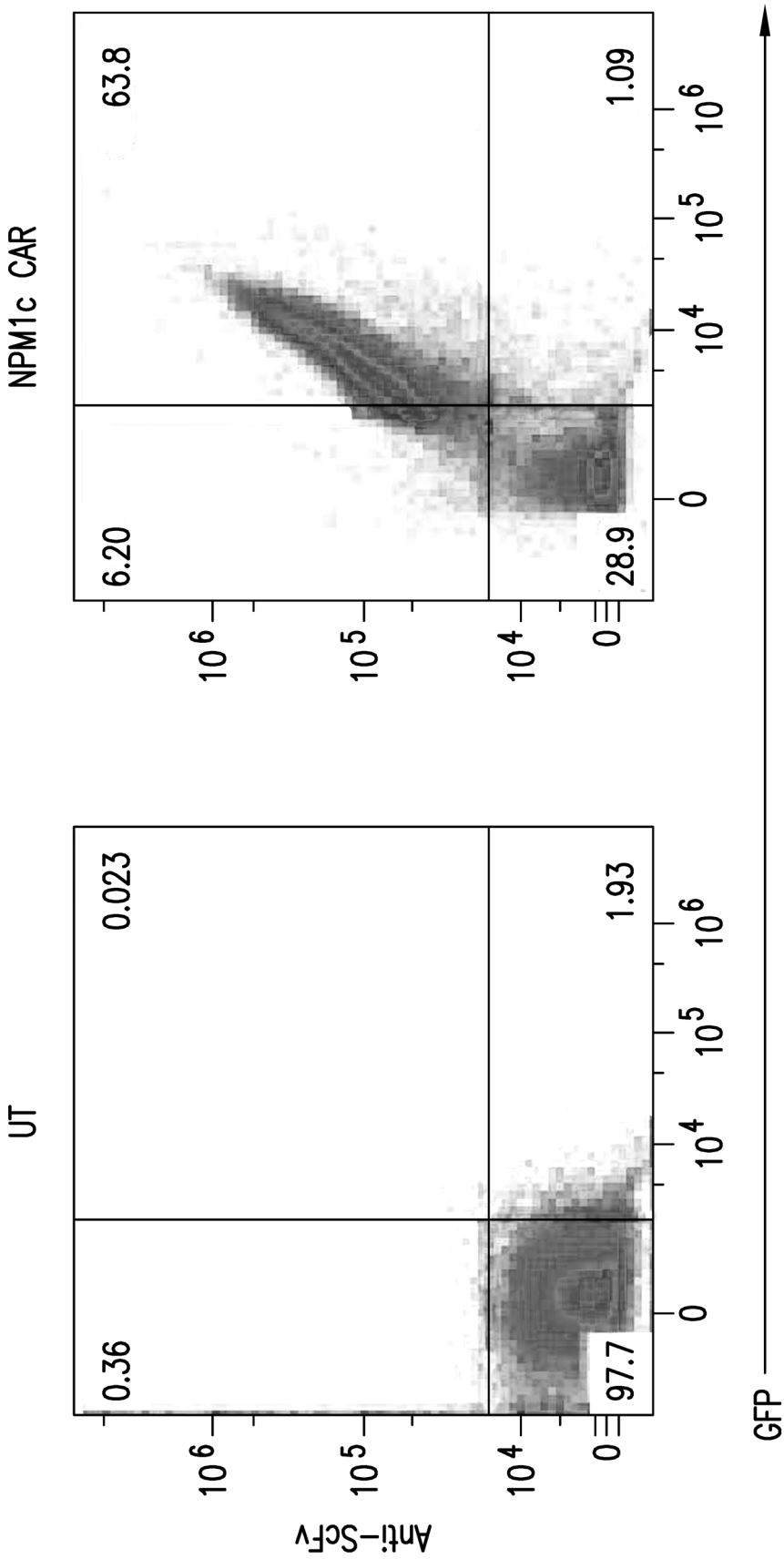

UT
NPM1c CAR
Anti-ScFv
GFP
0.36
0.023
97.7
1.93
6.20
63.8
28.9
1.09
0
10^4
10^5
10^6

FIG.5

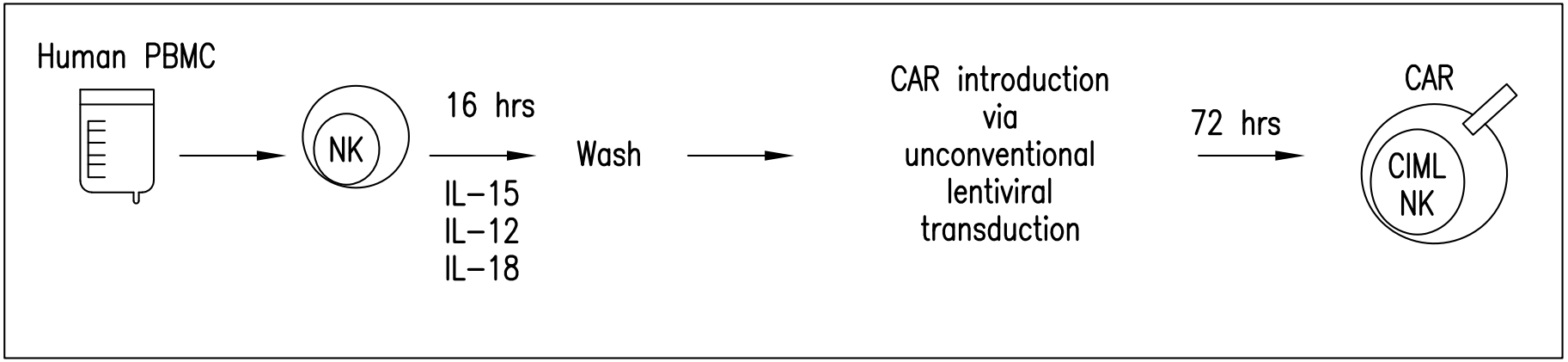

Human PBMC
NK
16 hrs
IL-15
IL-12
IL-18
Wash
CAR introduction
via
unconventional
lentiviral
transduction
72 hrs
CAR
CIML
NK

FIG.6A

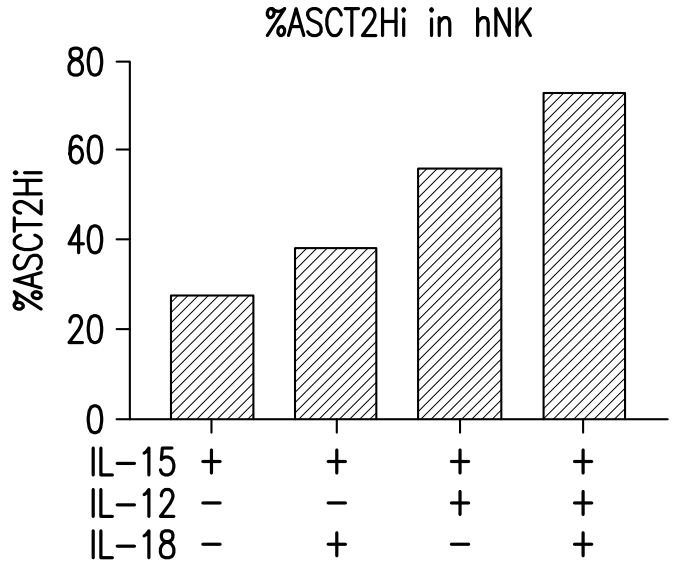

%ASCT2Hi in hNK
%ASCT2Hi
80
60
40
20
0
IL-15 + + + +
IL-12 - - + +
IL-18 - + - +

FIG.6B

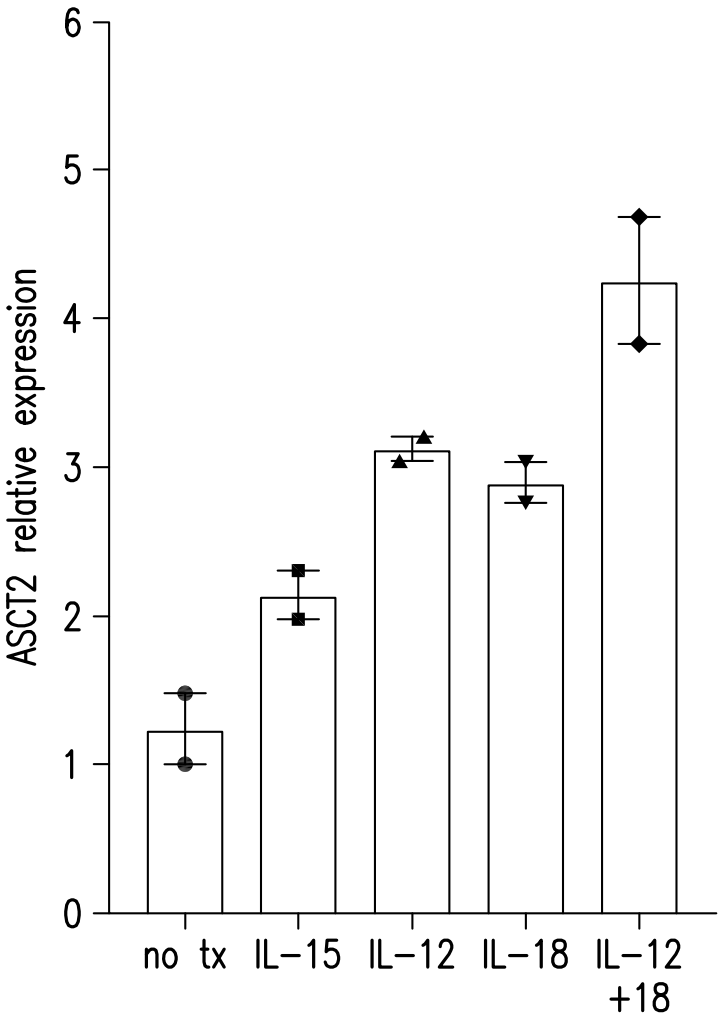


FIG.6C

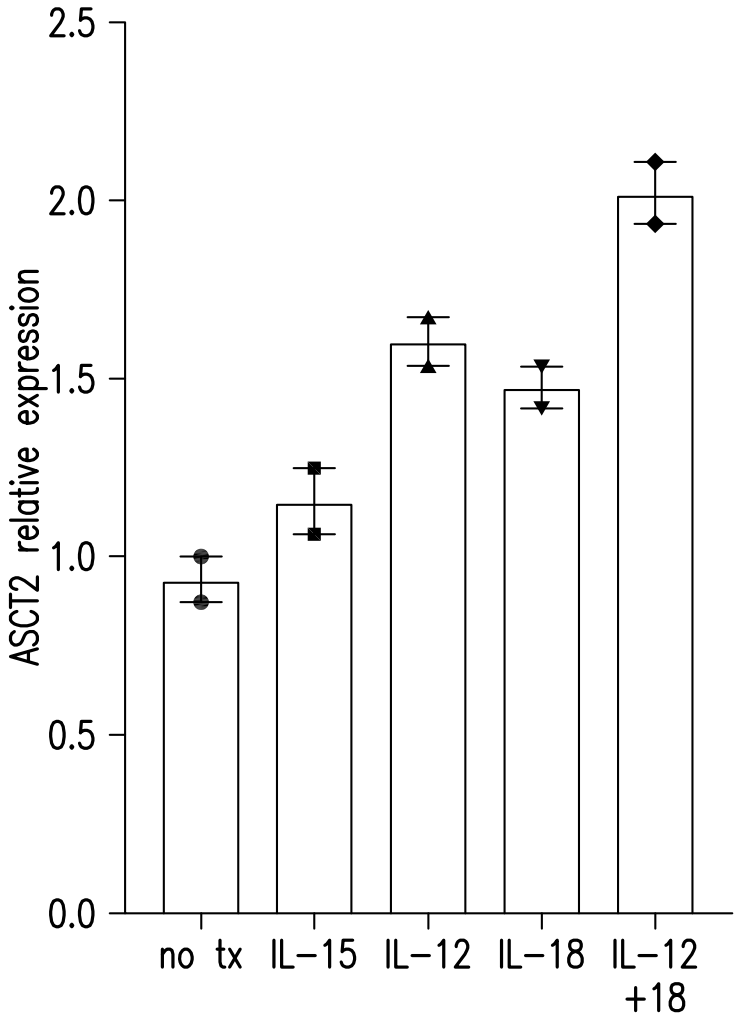


FIG.6D

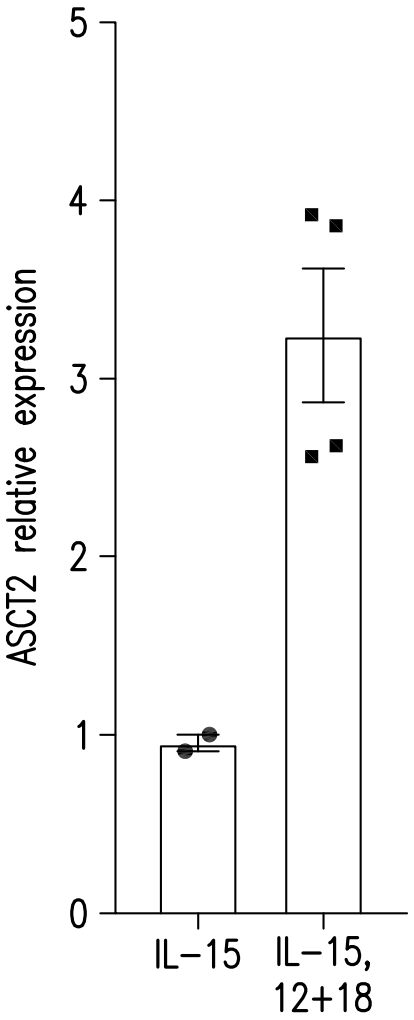


FIG.6E

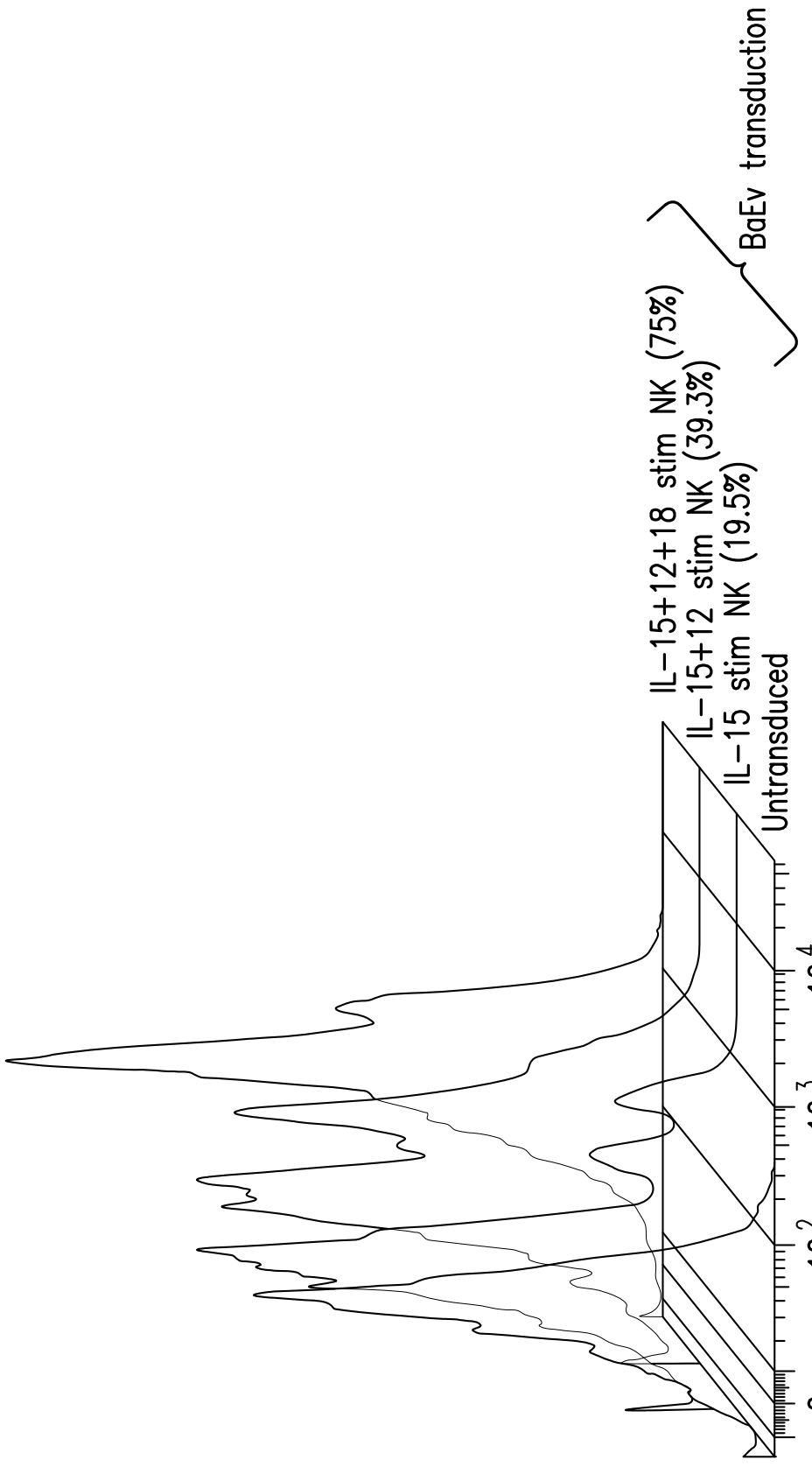
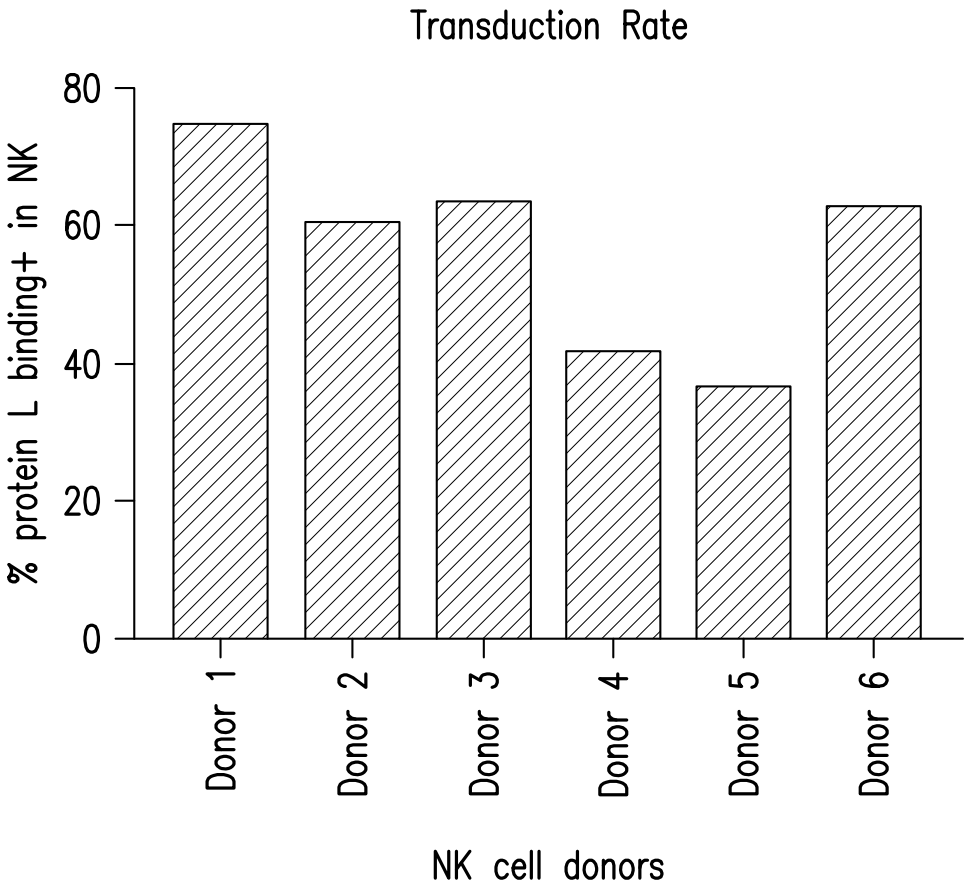

BaEv transduction
IL−15+12+18 stim NK (75%)
IL−15+12 stim NK (39.3%)
IL−15 stim NK (19.5%)
Untransduced
0
10^2
10^3
10^4
GFP (transduction reporter)

FIG.6F


Transduction Rate
% protein L binding+ in NK
80
60
40
20
0
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
Donor 6
NK cell donors

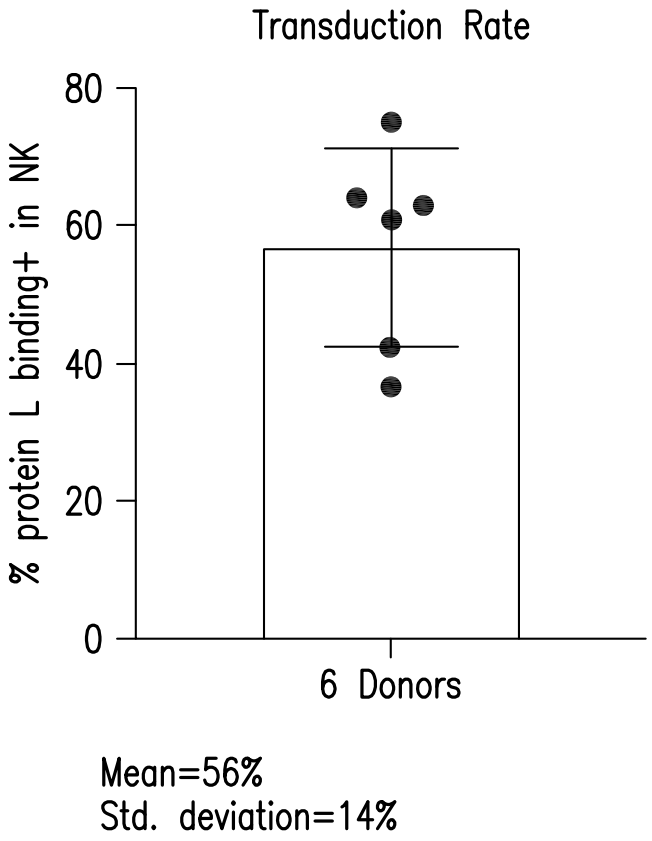

Transduction Rate
% protein L binding+ in NK
80
60
40
20
0
6 Donors
Mean=56%
Std. deviation=14%

FIG.7A

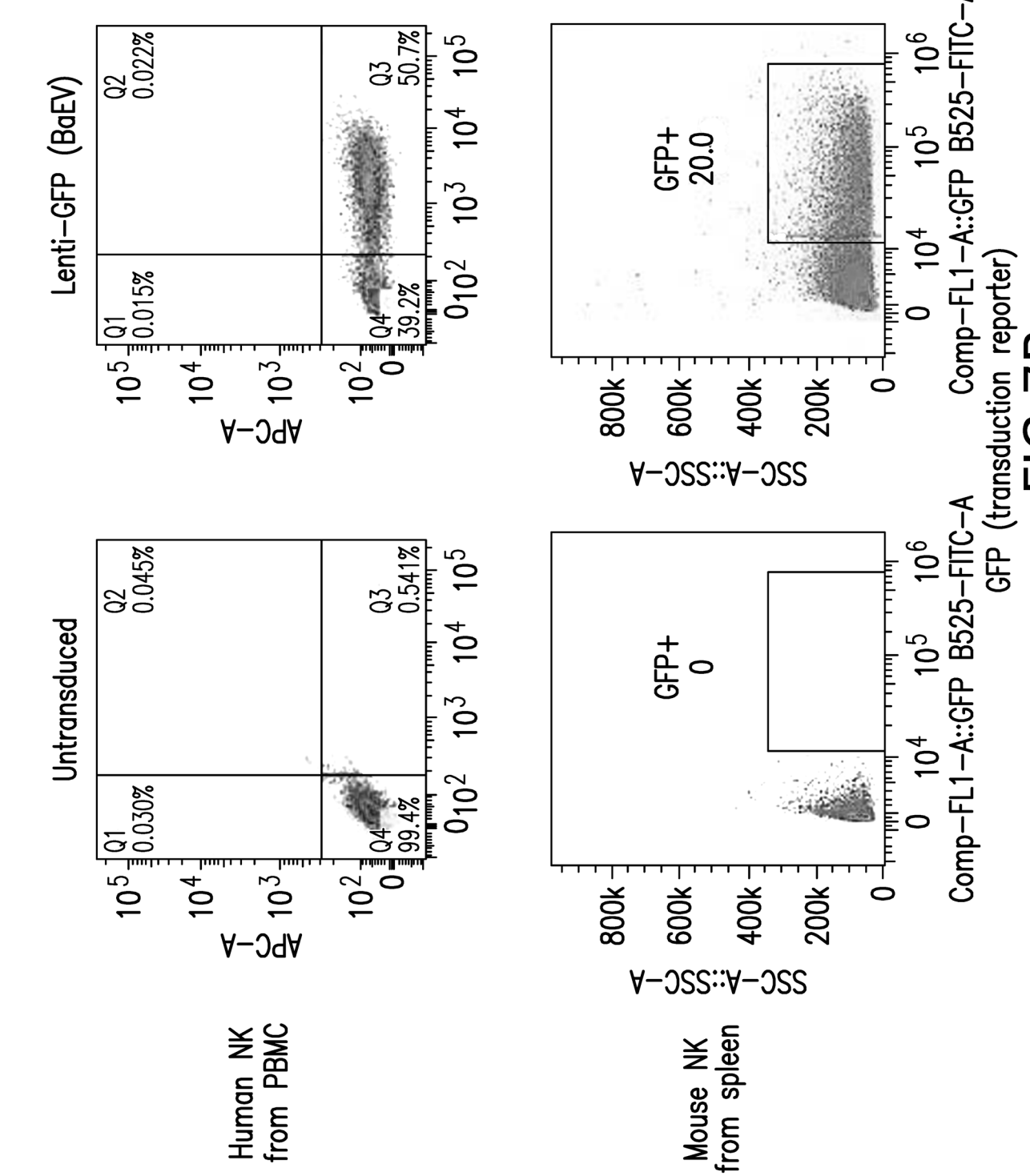

Untransduced
Lenti-GFP (BaEV)
Human NK from PBMC
Mouse NK from spleen
APC-A
Q1 0.030%
Q2 0.045%
Q4 99.4%
Q3 0.541%
Q1 0.015%
Q2 0.022%
Q4 39.2%
Q3 50.7%
SSC-A::SSC-A
GFP+ 0
GFP+ 20.0
Comp-FL1-A::GFP B525-FITC-A
GFP (transduction reporter)

FIG.7B

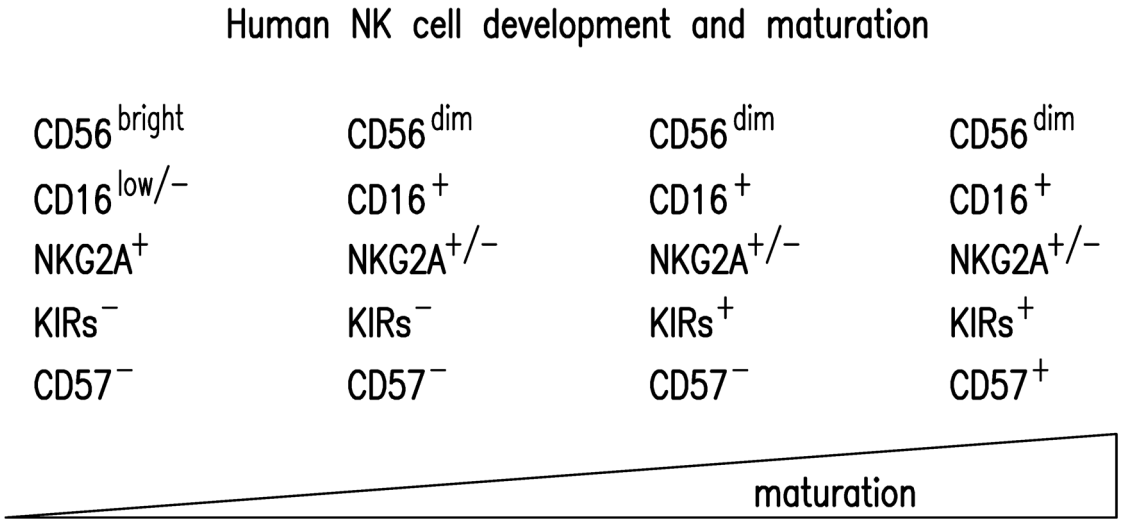


FIG.7C

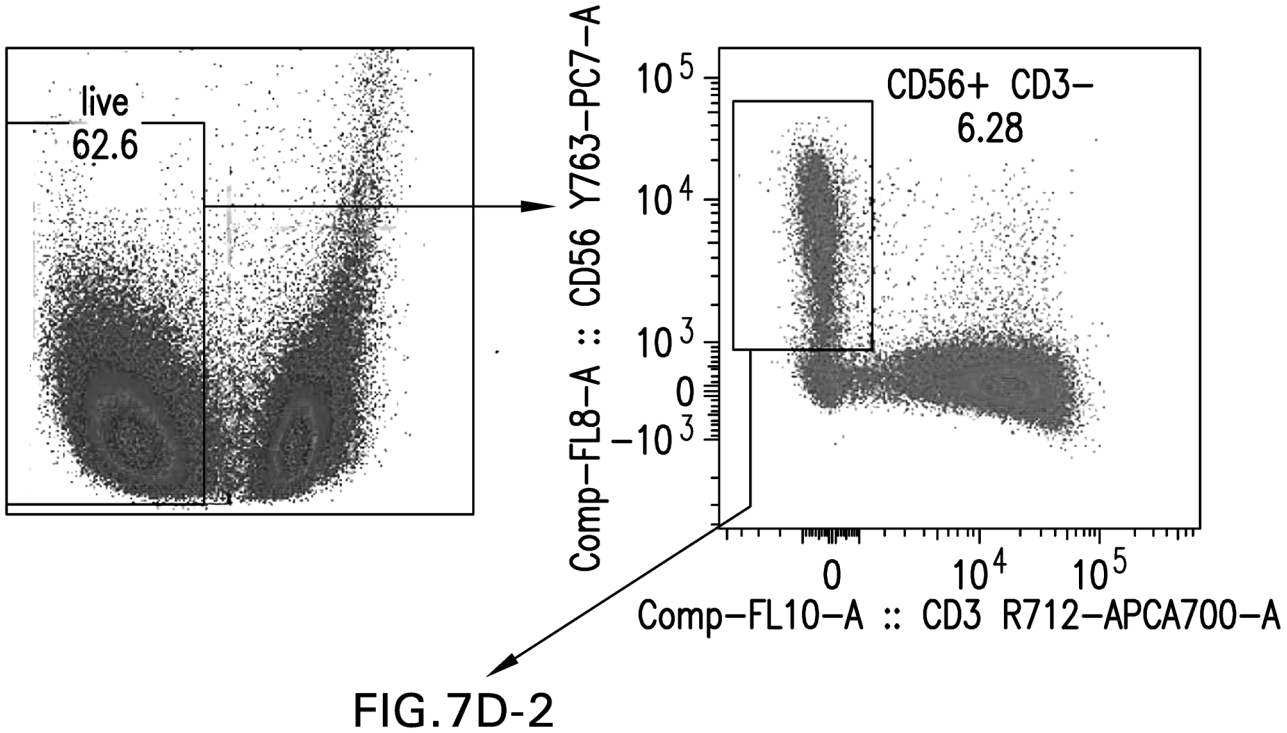


FIG.7D-2

FIG.7D-1

FIG.7D-1

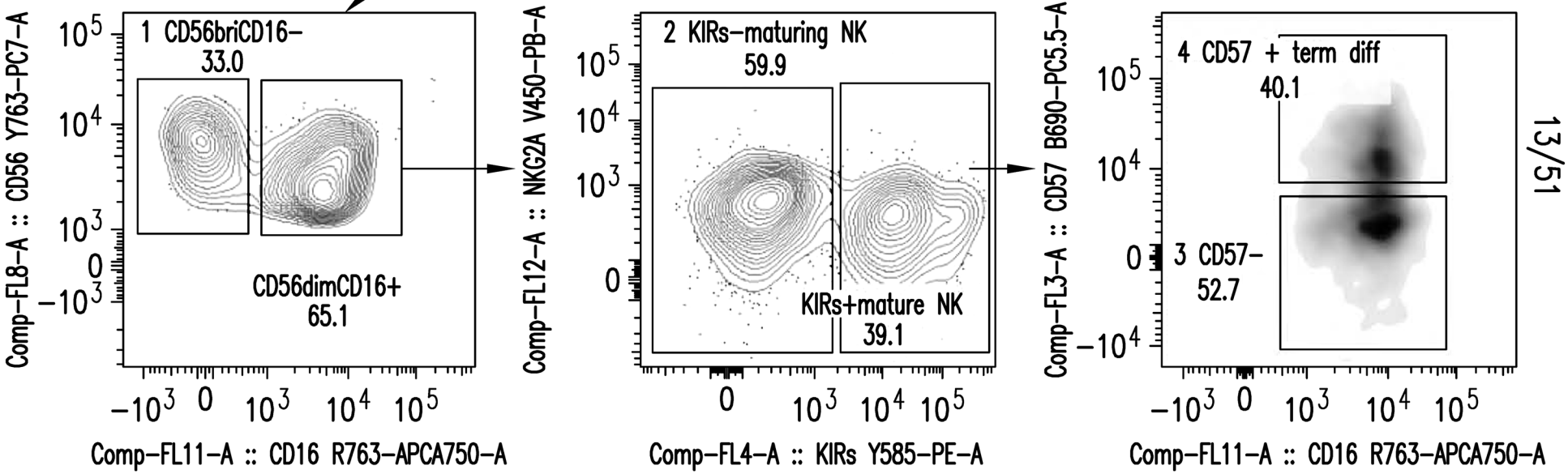


FIG.7D-2

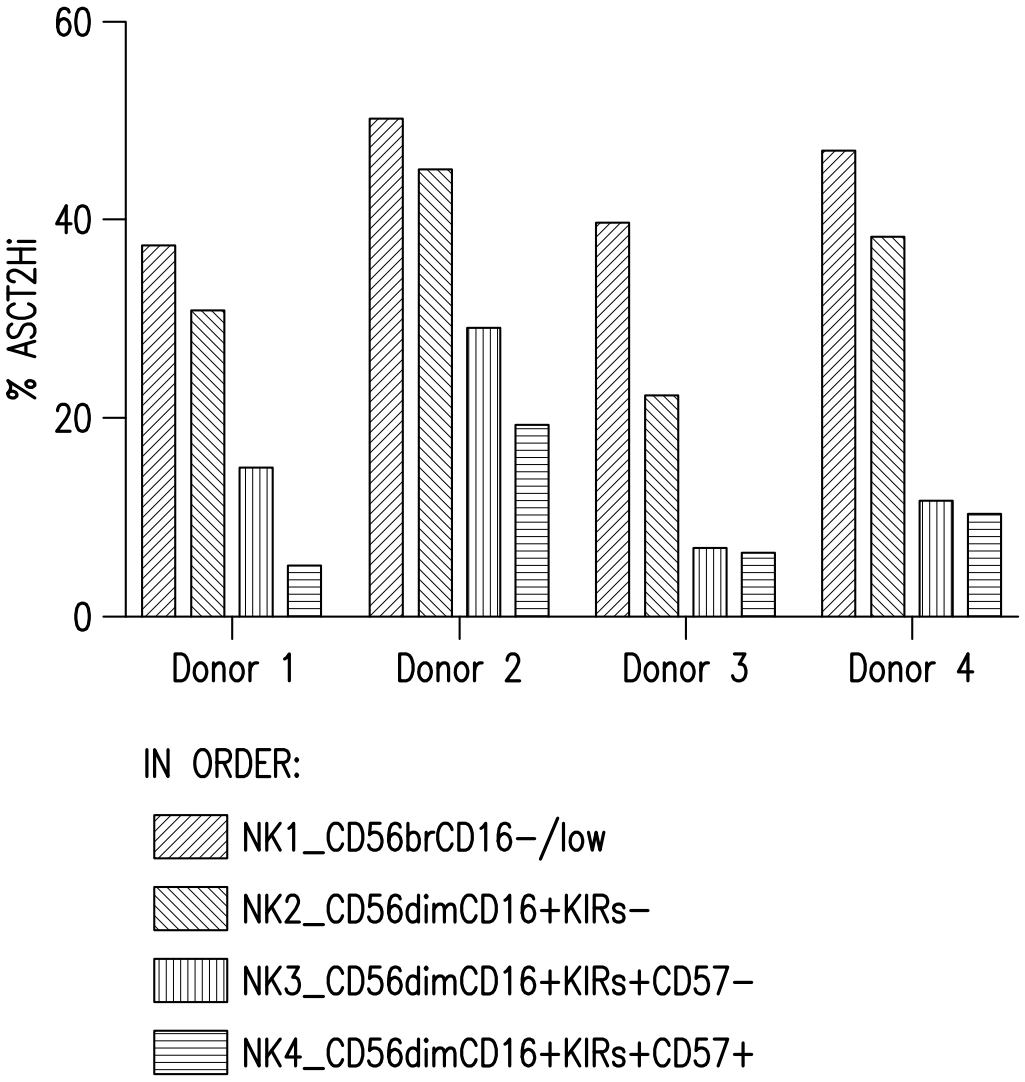

60
40
20
0
% ASCT2Hi
Donor 1
Donor 2
Donor 3
Donor 4
IN ORDER:
NK1_CD56brCD16−/low
NK2_CD56dimCD16+KIRs−
NK3_CD56dimCD16+KIRs+CD57−
NK4_CD56dimCD16+KIRs+CD57+

FIG.7E

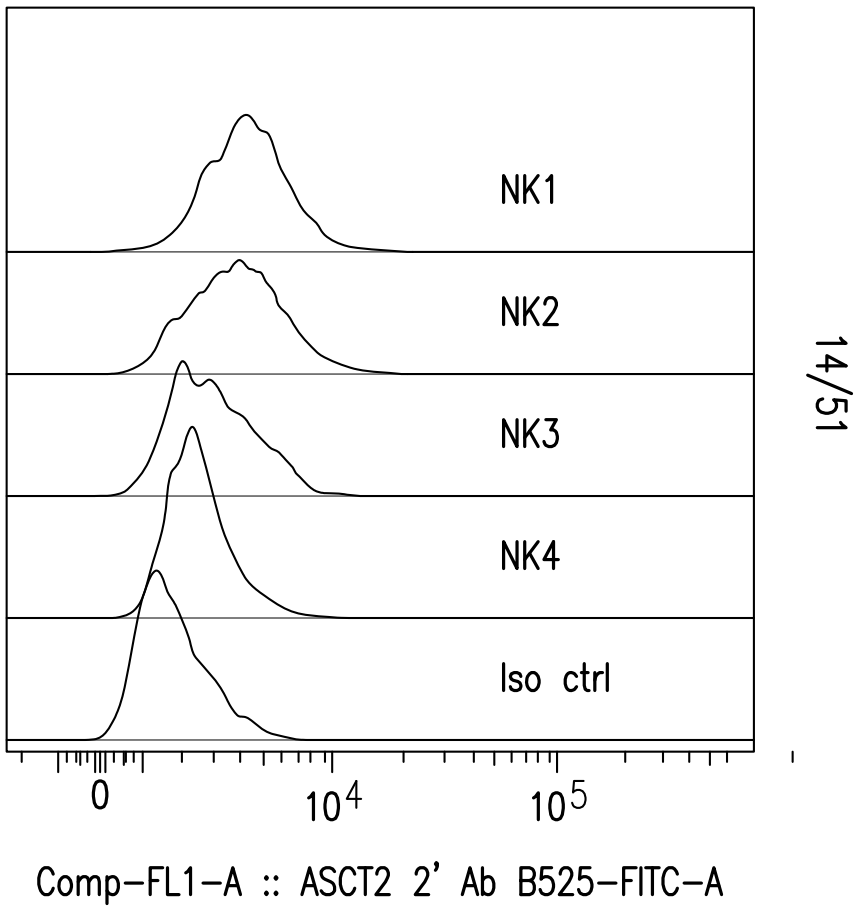

NK1
NK2
NK3
NK4
Iso ctrl
0
10^4
10^5
Comp−FL1−A :: ASCT2 2' Ab B525−FITC−A

FIG.7F

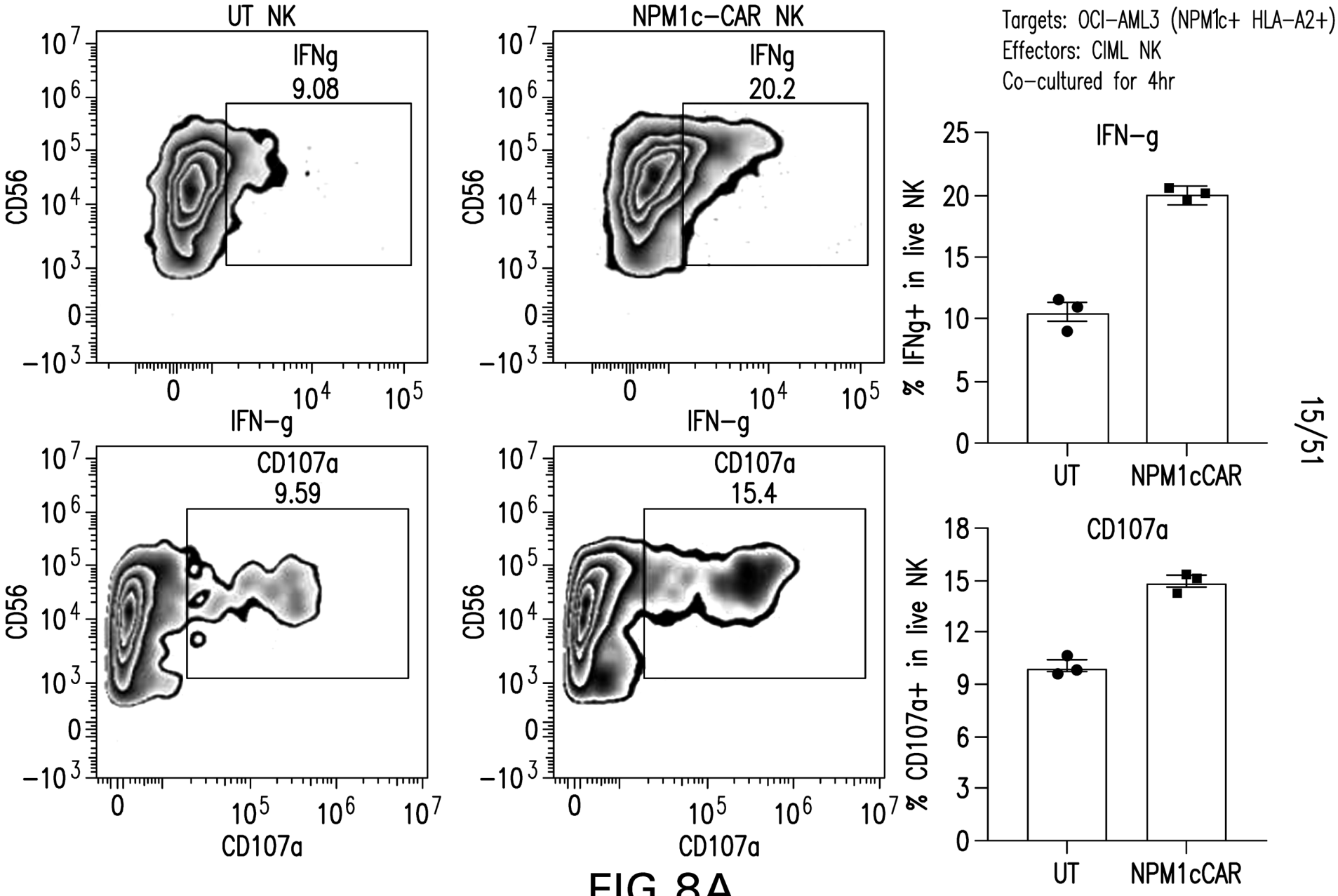

UT NK
IFNg
9.08
CD56
IFN-g
CD107a
9.59
CD107a
NPM1c-CAR NK
IFNg
20.2
CD56
IFN-g
CD107a
15.4
CD107a
Targets: OCI-AML3 (NPM1c+ HLA-A2+)
Effectors: CIML NK
Co-cultured for 4hr
IFN-g
% IFNg+ in live NK
UT
NPM1cCAR
CD107a
% CD107a+ in live NK
UT
NPM1cCAR

FIG.8A

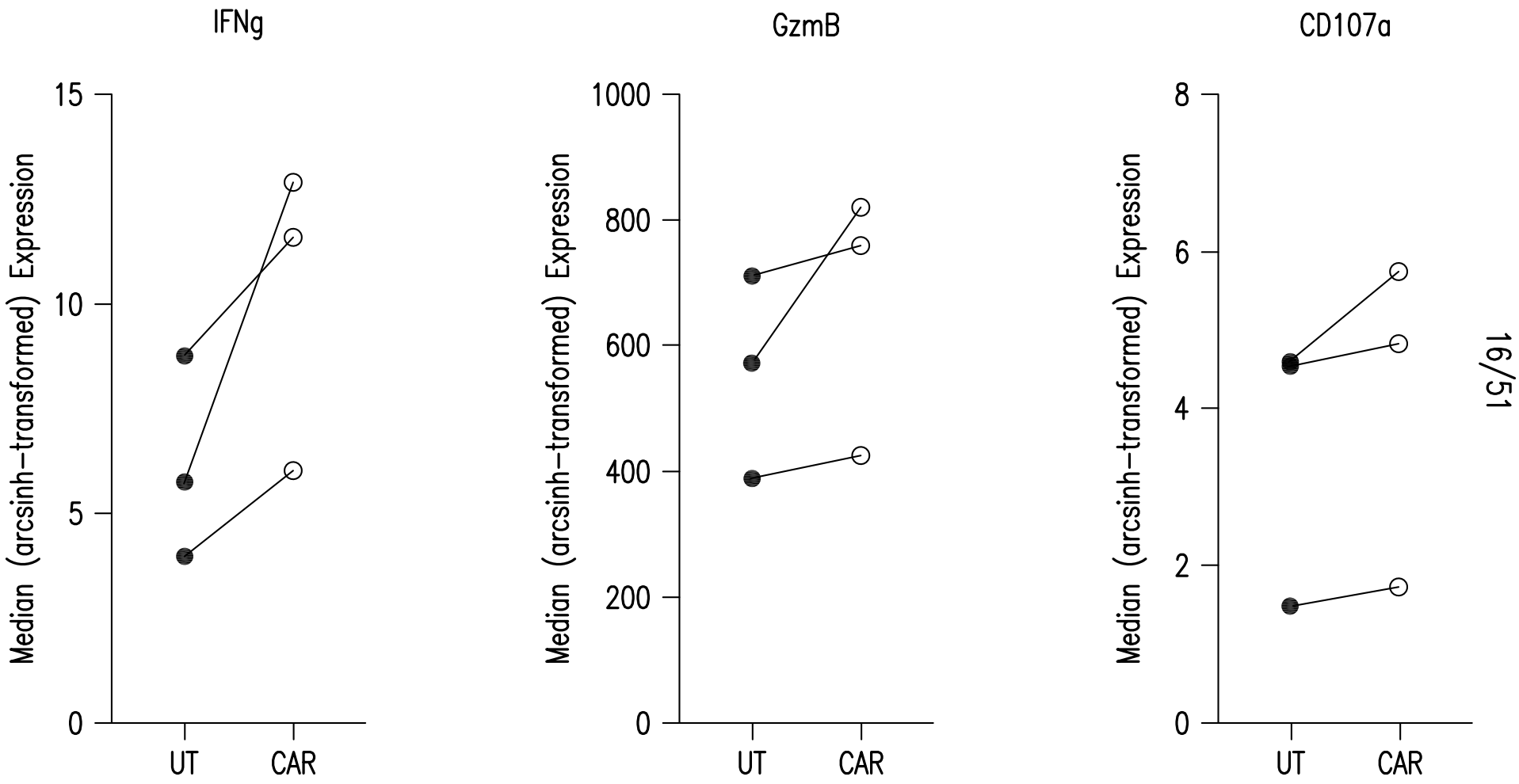

IFNg
GzmB
CD107a
Median (arcsinh−transformed) Expression
15
10
5
0
1000
800
600
400
200
8
6
4
2
UT
CAR

FIG.8B

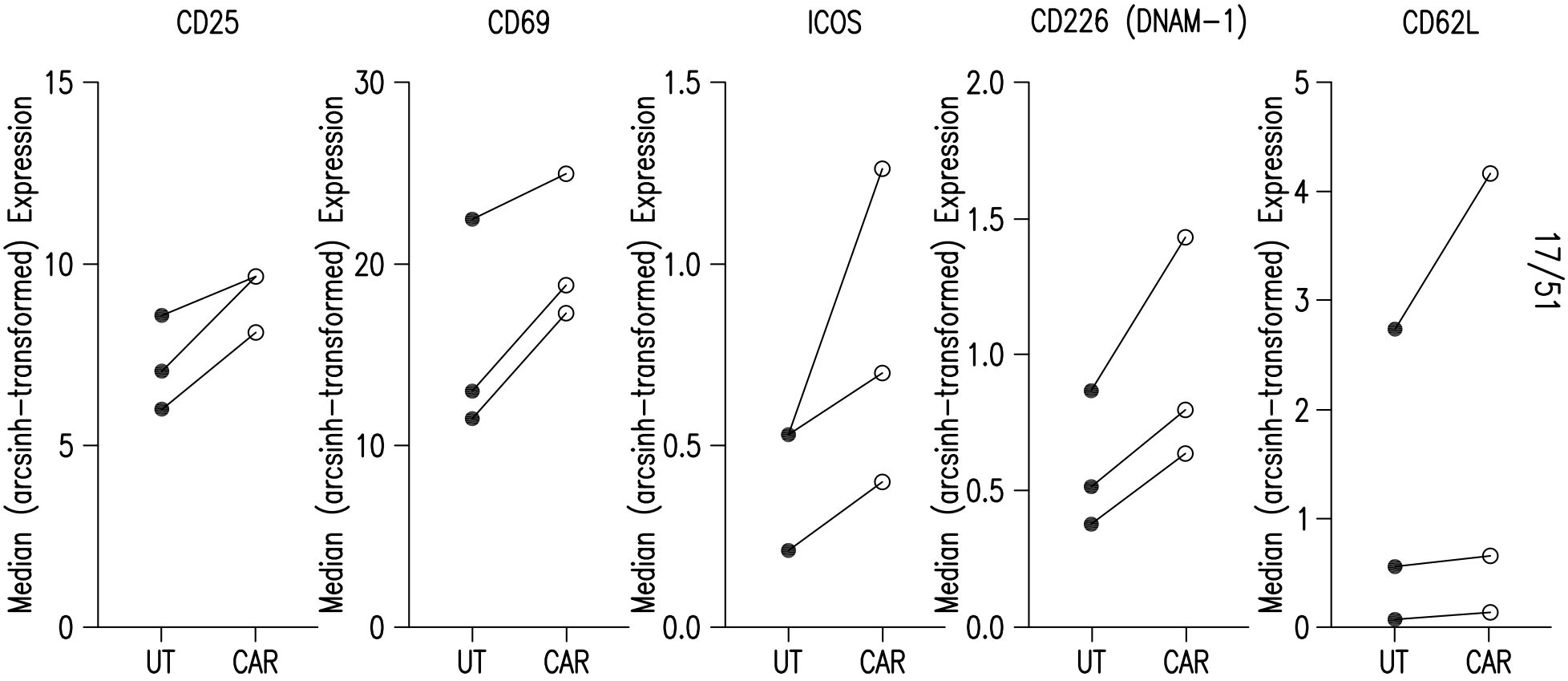

CD25
CD69
ICOS
CD226 (DNAM-1)
CD62L
Median (arcsinh-transformed) Expression
15
10
5
0
30
20
10
0
1.5
1.0
0.5
0.0
2.0
1.5
1.0
0.5
0.0
5
4
3
2
1
0
UT
CAR

FIG.8C

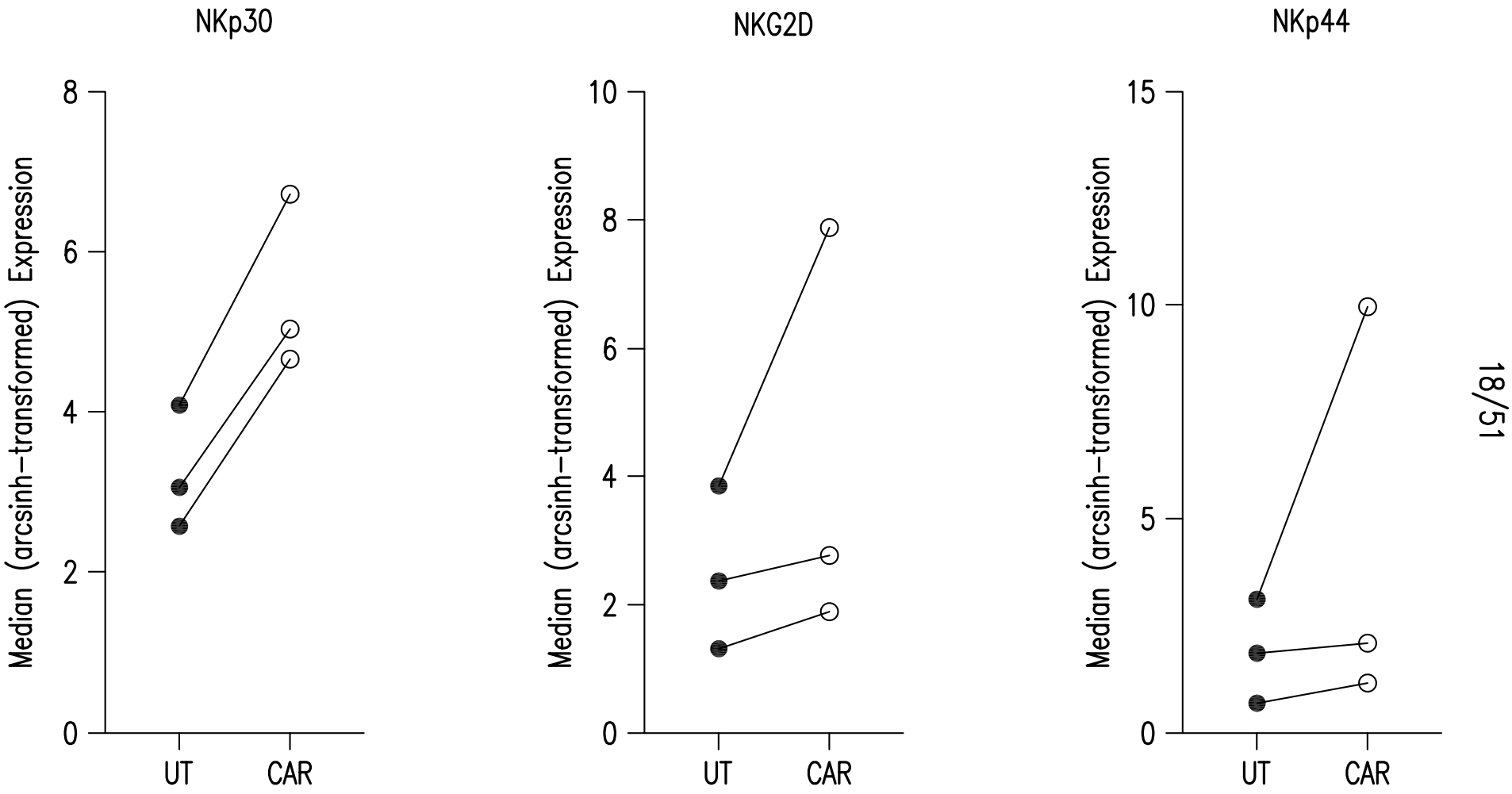

NKp30
Median (arcsinh−transformed) Expression
0
2
4
6
8
UT
CAR
NKG2D
Median (arcsinh−transformed) Expression
0
2
4
6
8
10
UT
CAR
NKp44
Median (arcsinh−transformed) Expression
0
5
10
15
UT
CAR

FIG.8D

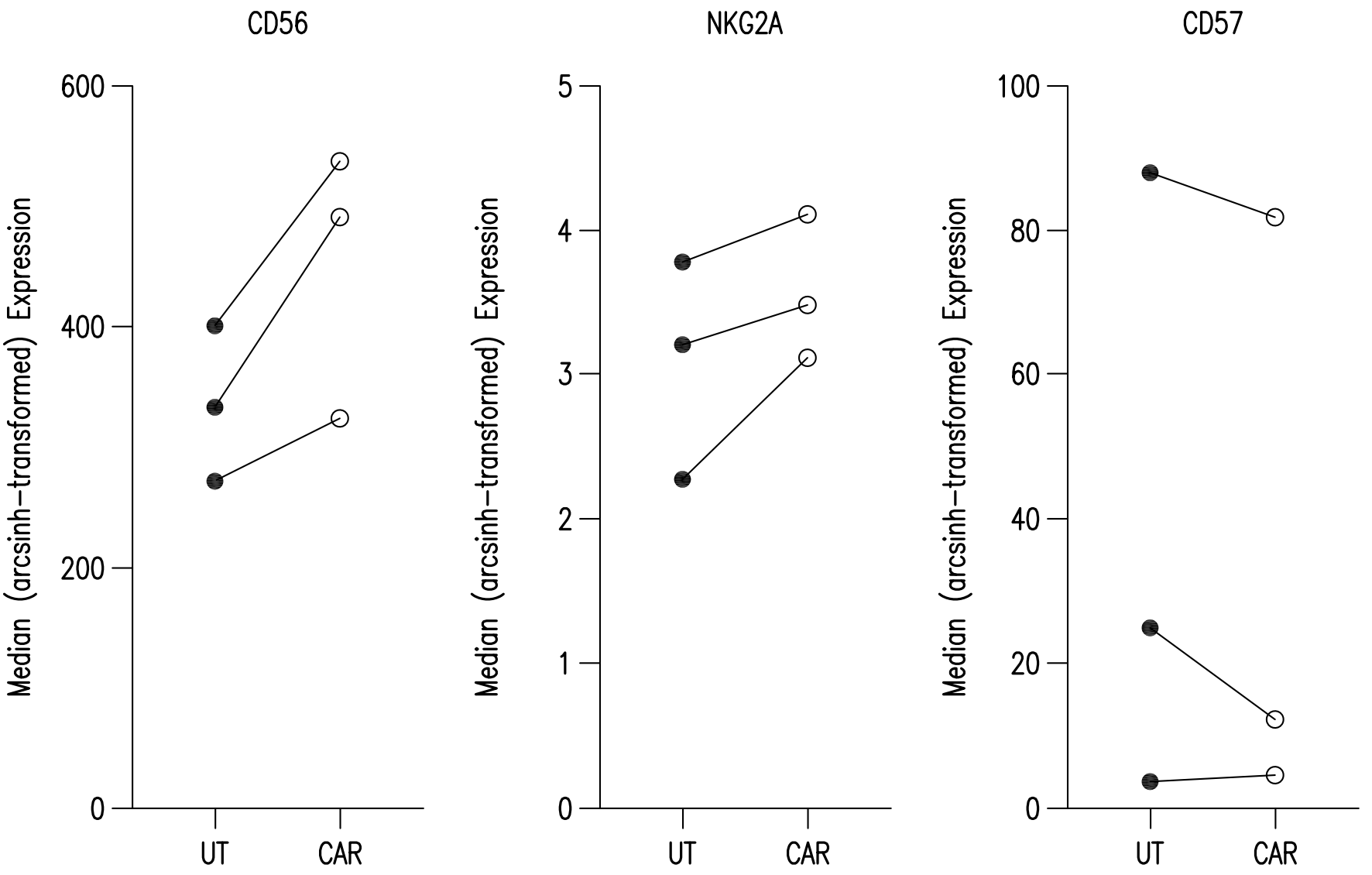

CD56
NKG2A
CD57
Median (arcsinh-transformed) Expression
600
400
200
0
5
4
3
2
1
100
80
60
40
20
UT
CAR

FIG.8E

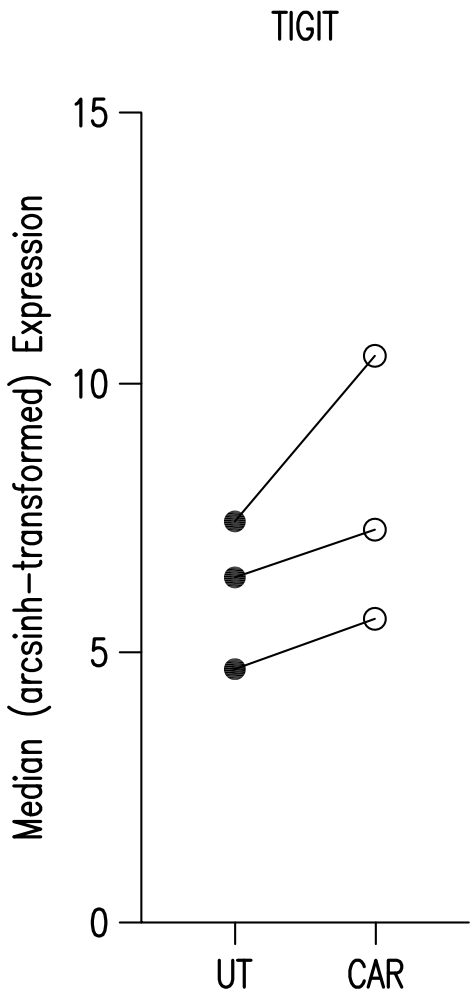

TIGIT
Median (arcsinh−transformed) Expression
15
10
5
0
UT
CAR

FIG.8F

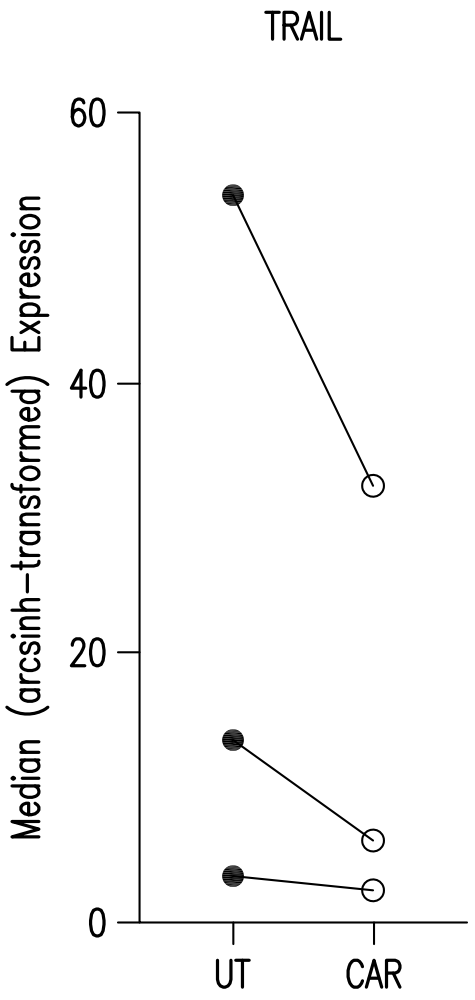

TRAIL
Median (arcsinh−transformed) Expression
60
40
20
0
UT
CAR

FIG.8G

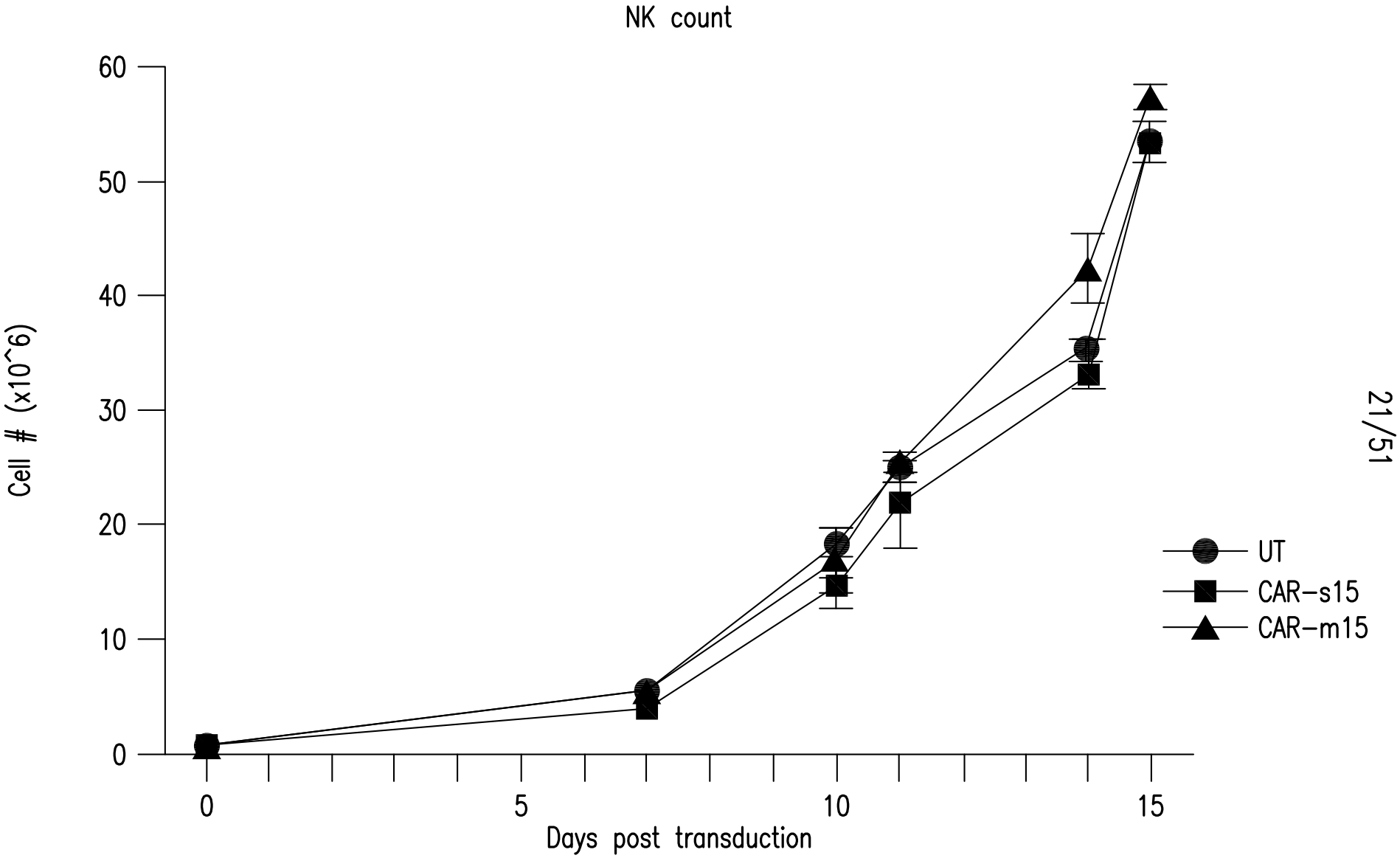

NK count
Cell # (x10^6)
60
50
40
30
20
10
0
0
5
10
15
Days post transduction
UT
CAR-s15
CAR-m15

FIG.9A

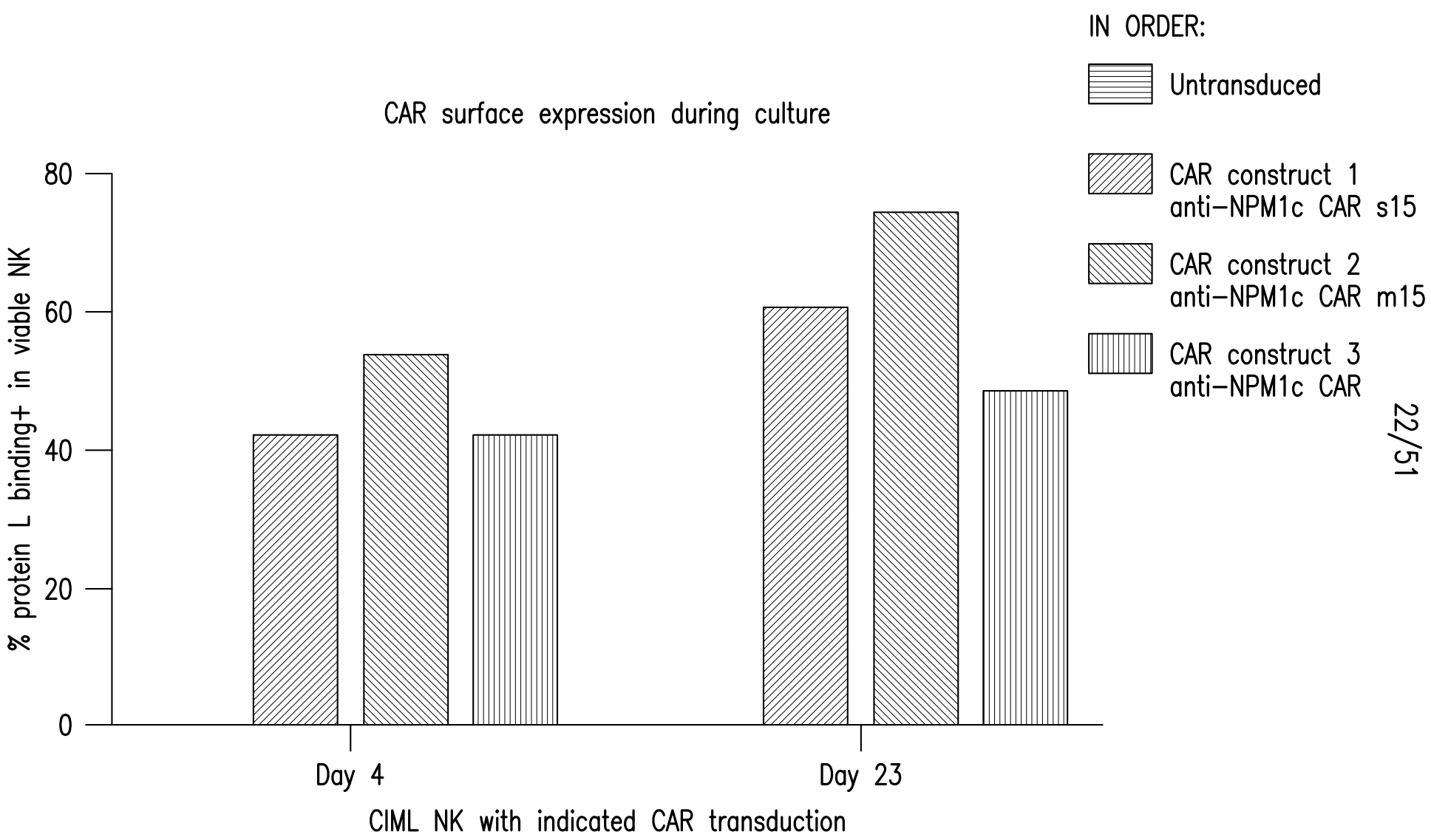

CAR surface expression during culture
% protein L binding+ in viable NK
80
60
40
20
0
Day 4
Day 23
CIML NK with indicated CAR transduction
IN ORDER:
Untransduced
CAR construct 1
anti-NPM1c CAR s15
CAR construct 2
anti-NPM1c CAR m15
CAR construct 3
anti-NPM1c CAR

FIG.9B

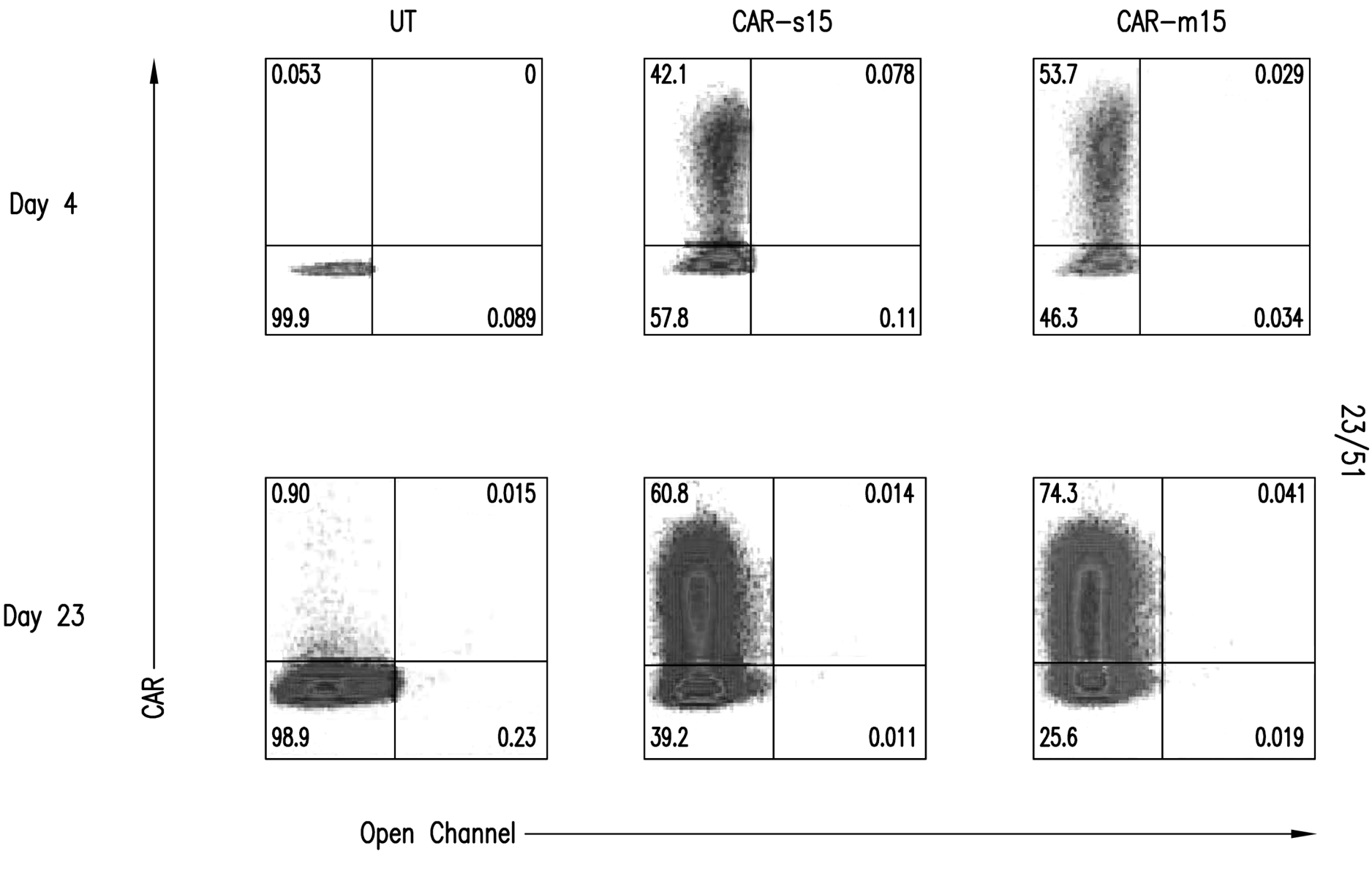

UT
CAR-s15
CAR-m15
Day 4
Day 23
CAR
0.053
0
99.9
0.089
42.1
0.078
57.8
0.11
53.7
0.029
46.3
0.034
0.90
0.015
98.9
0.23
60.8
0.014
39.2
0.011
74.3
0.041
25.6
0.019
Open Channel

FIG.9C

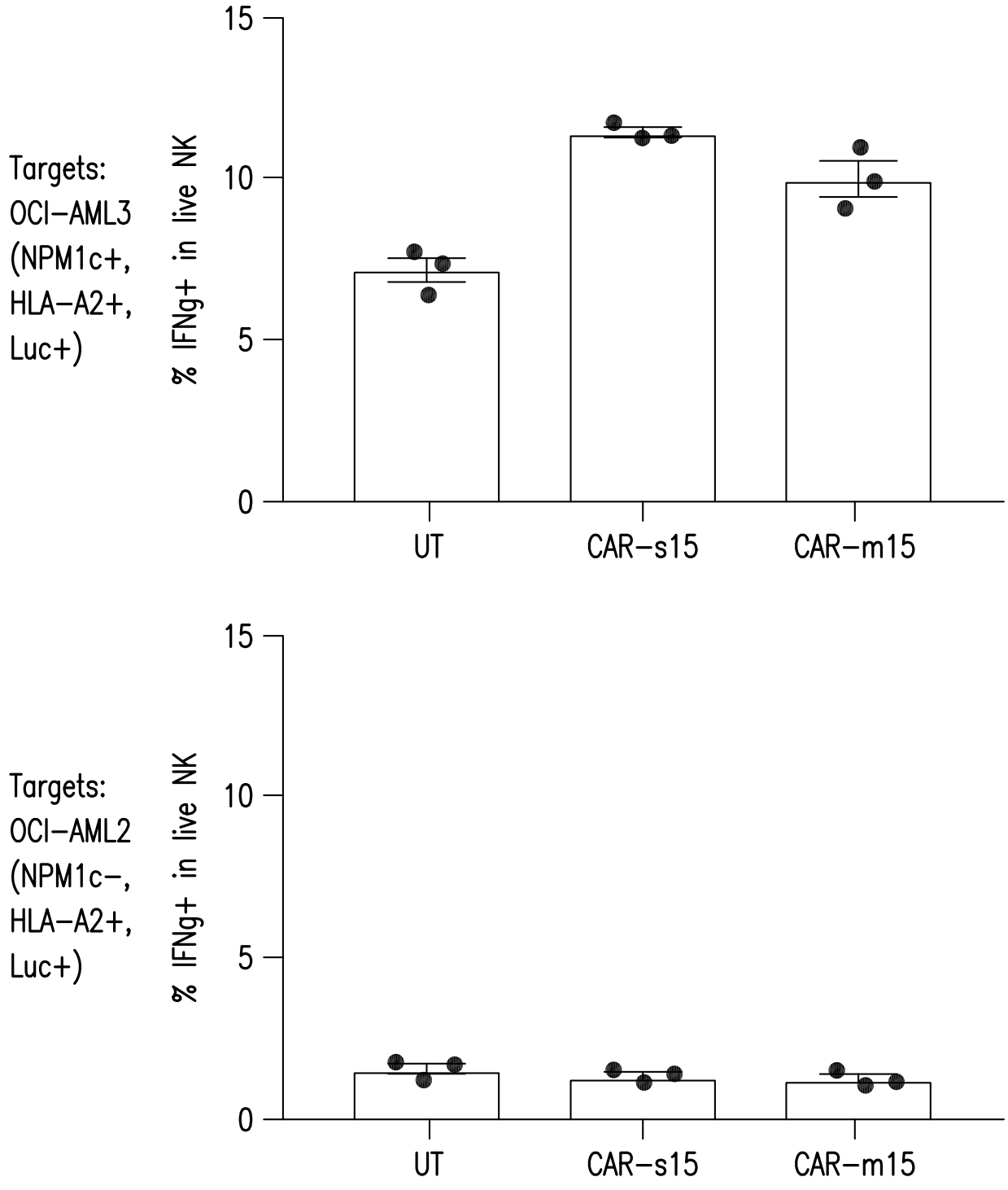

Targets:
OCI-AML3
(NPM1c+,
HLA-A2+,
Luc+)
% IFNg+ in live NK
15
10
5
0
UT
CAR-s15
CAR-m15
Targets:
OCI-AML2
(NPM1c-,
HLA-A2+,
Luc+)
% IFNg+ in live NK
15
10
5
0
UT
CAR-s15
CAR-m15

FIG.10A

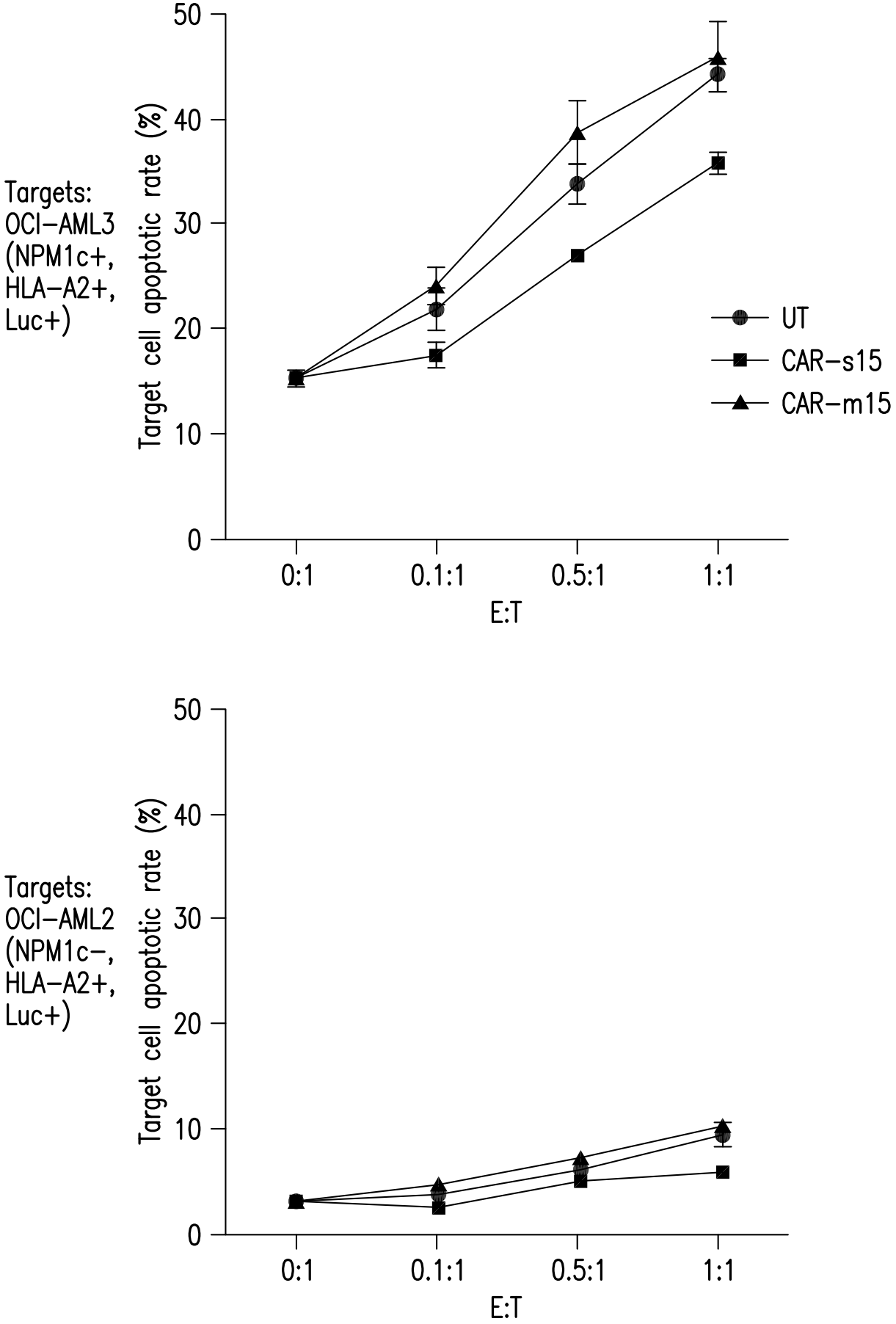

Targets:
OCI-AML3
(NPM1c+,
HLA-A2+,
Luc+)
Target cell apoptotic rate (%)
50
40
30
20
10
0
0:1
0.1:1
0.5:1
1:1
E:T
UT
CAR-s15
CAR-m15
Targets:
OCI-AML2
(NPM1c-,
HLA-A2+,
Luc+)
Target cell apoptotic rate (%)
50
40
30
20
10
0
0:1
0.1:1
0.5:1
1:1
E:T

FIG.10B

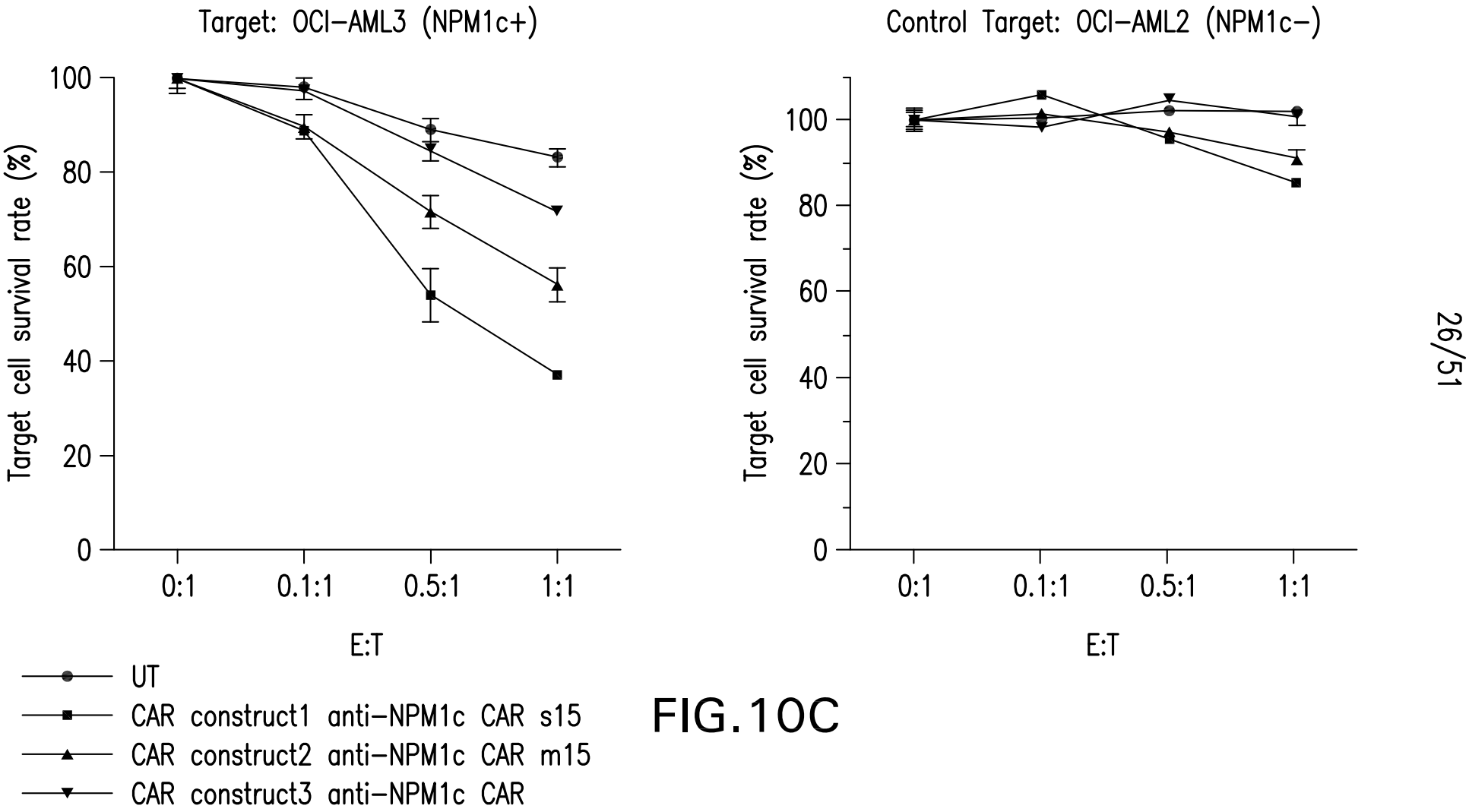

Target: OCI-AML3 (NPM1c+)
Target cell survival rate (%)
100
80
60
40
20
0
0:1
0.1:1
0.5:1
1:1
E:T
Control Target: OCI-AML2 (NPM1c-)
Target cell survival rate (%)
100
80
60
40
20
0
0:1
0.1:1
0.5:1
1:1
E:T
UT
CAR construct1 anti-NPM1c CAR s15
CAR construct2 anti-NPM1c CAR m15
CAR construct3 anti-NPM1c CAR

FIG.10C

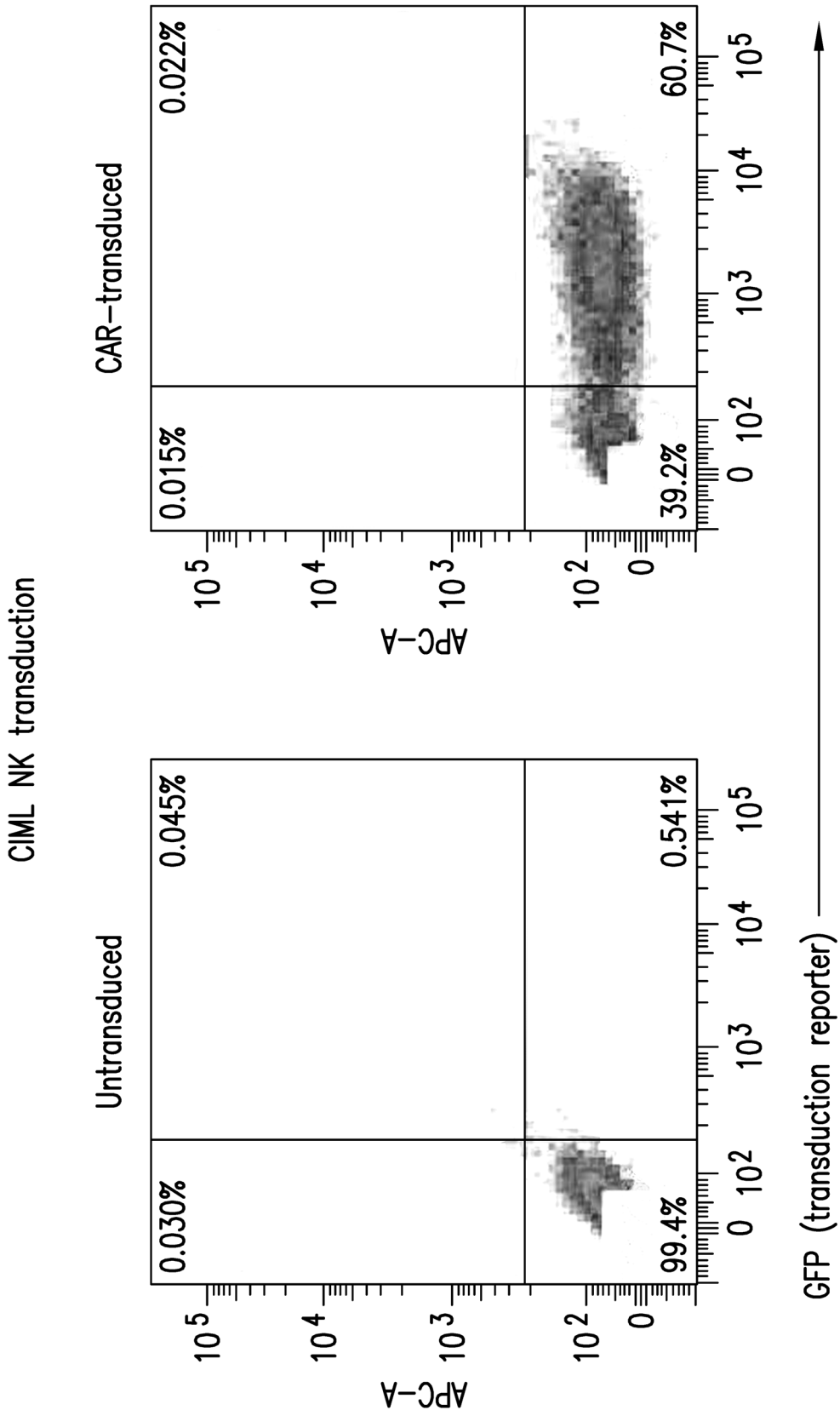

CIML NK transduction
Untransduced
CAR-transduced
0.030%
0.045%
99.4%
0.541%
0.015%
0.022%
39.2%
60.7%
APC-A
GFP (transduction reporter)
0
10^2
10^3
10^4
10^5

FIG.10D

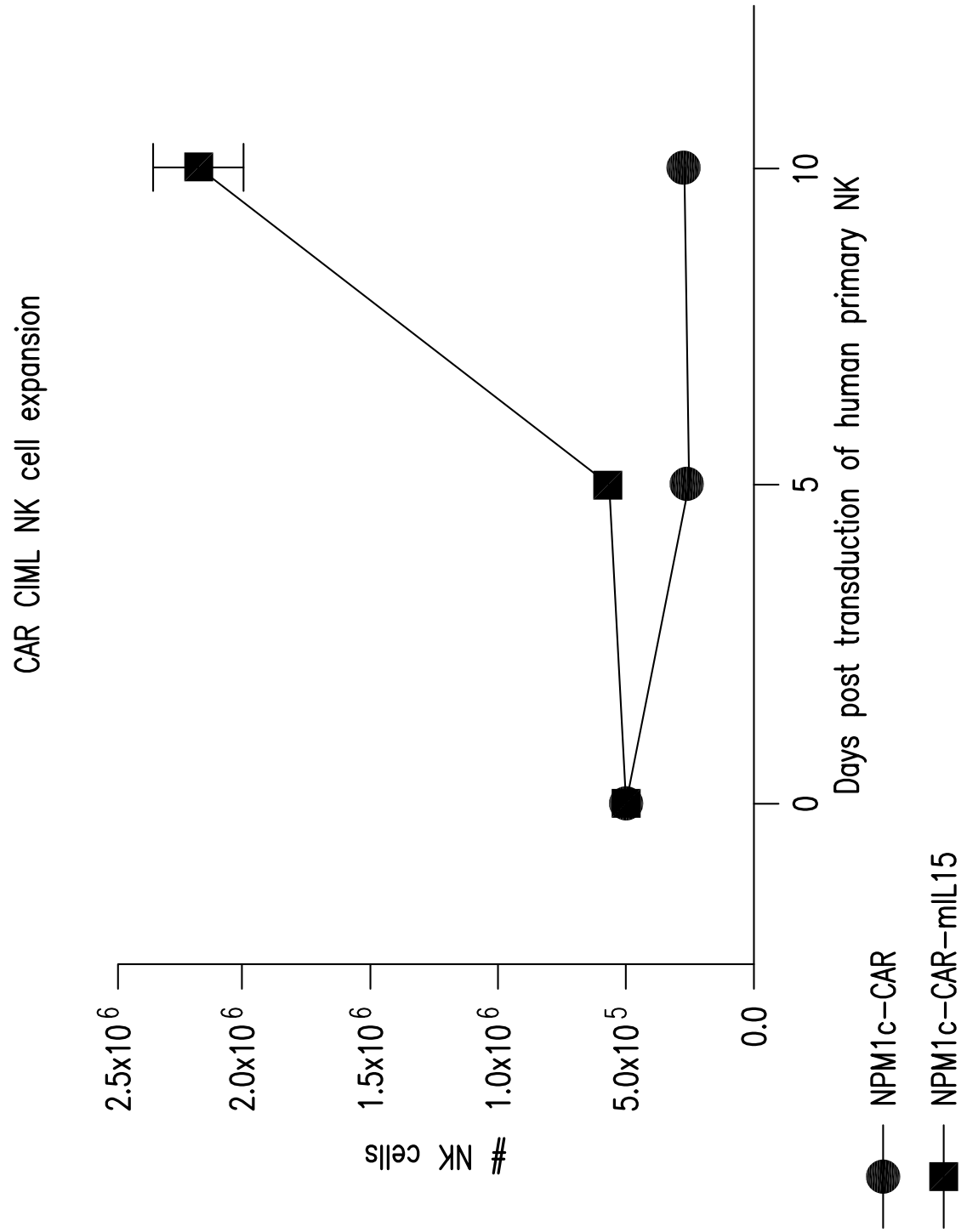

CAR CIML NK cell expansion
NK cells
2.5x10^6
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
0
5
10
Days post transduction of human primary NK
NPM1c-CAR
NPM1c-CAR-mIL15

FIG.10E

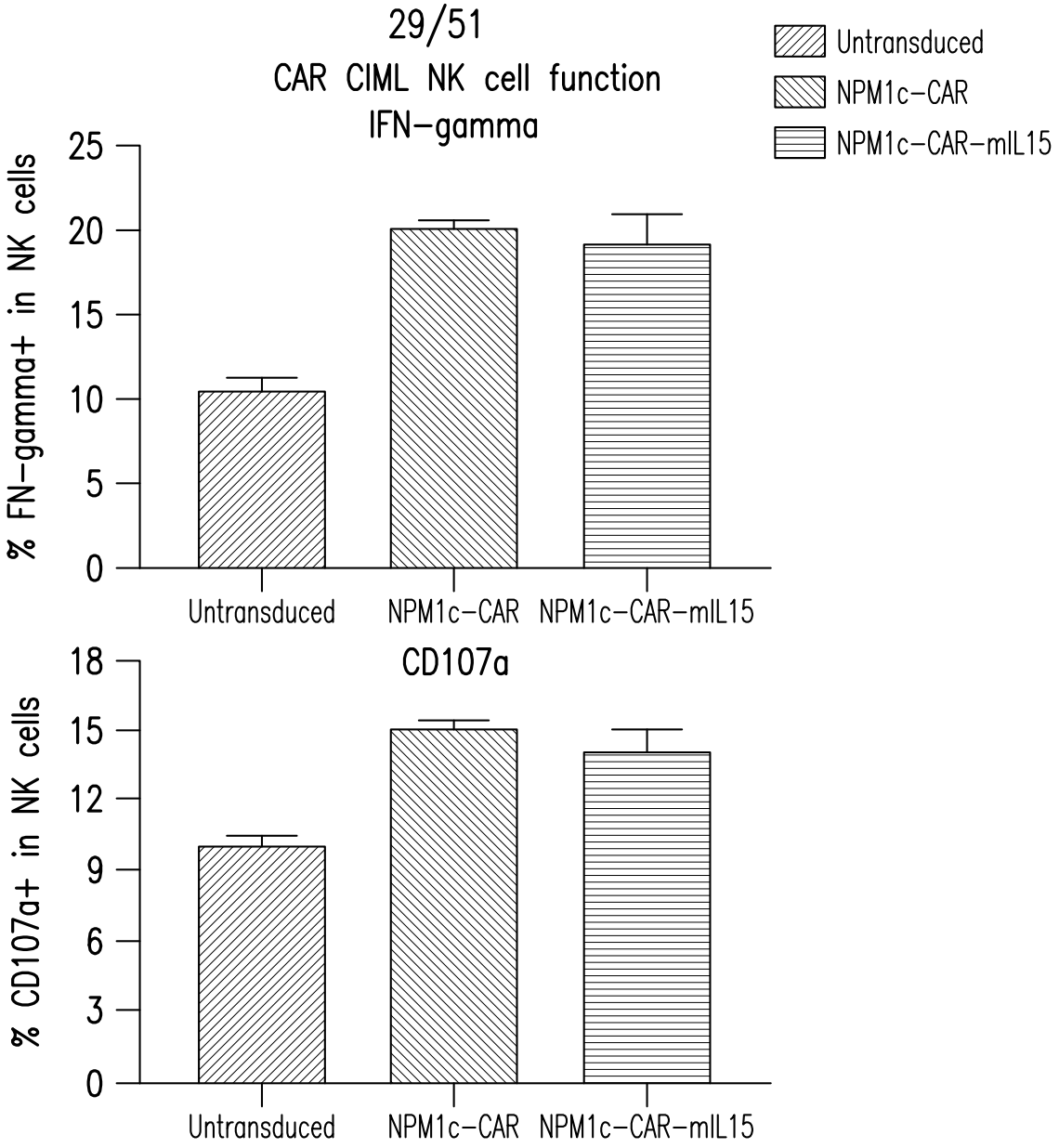


FIG.10F

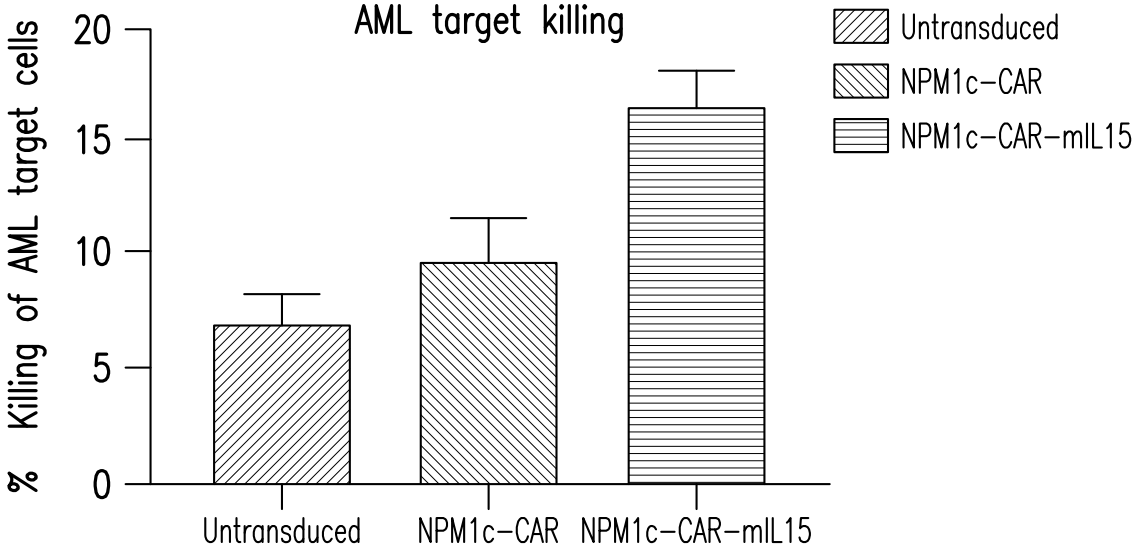


FIG.10G

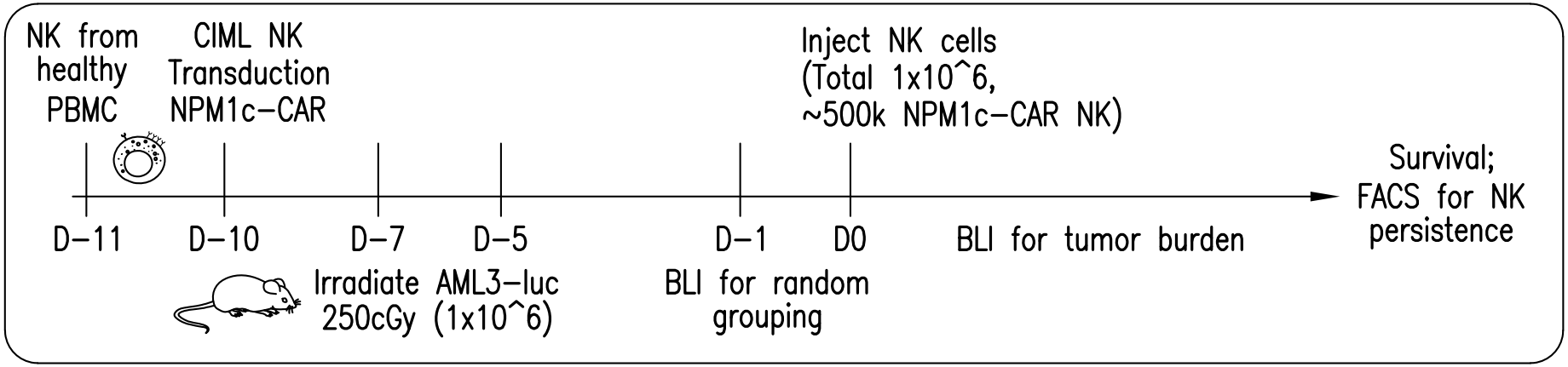

NK from healthy PBMC
CIML NK Transduction NPM1c-CAR
Inject NK cells (Total 1x10^6, ~500k NPM1c-CAR NK)
Survival; FACS for NK persistence
D-11
D-10
D-7
D-5
D-1
D0
BLI for tumor burden
Irradiate 250cGy
AML3-luc (1x10^6)
BLI for random grouping

FIG.11A

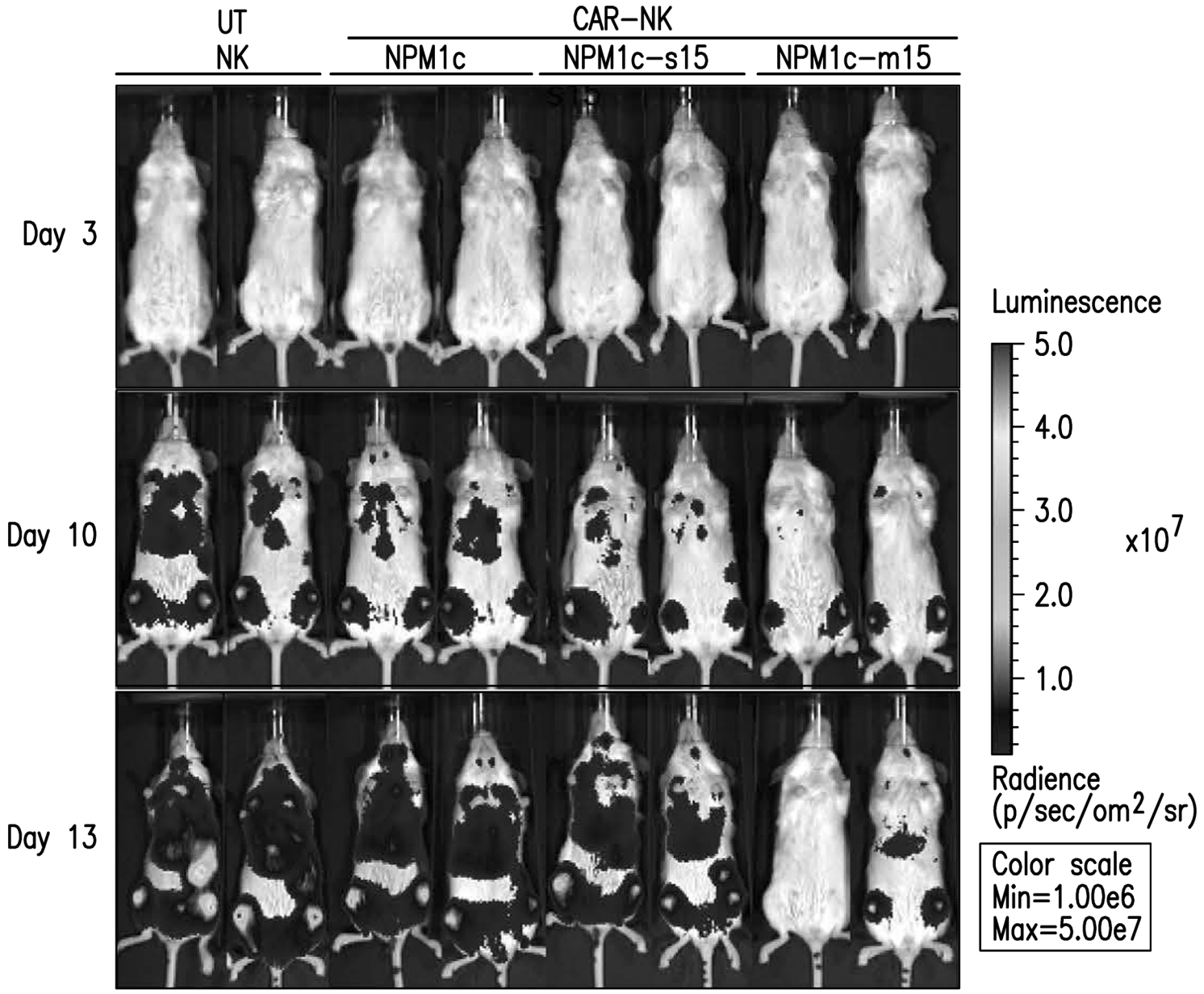

UT
NK
CAR-NK
NPM1c
NPM1c-s15
NPM1c-m15
Day 3
Day 10
Day 13
Luminescence
5.0
4.0
3.0
2.0
1.0
x10^7
Radience
(p/sec/om^2/sr)
Color scale
Min=1.00e6
Max=5.00e7

FIG.11B

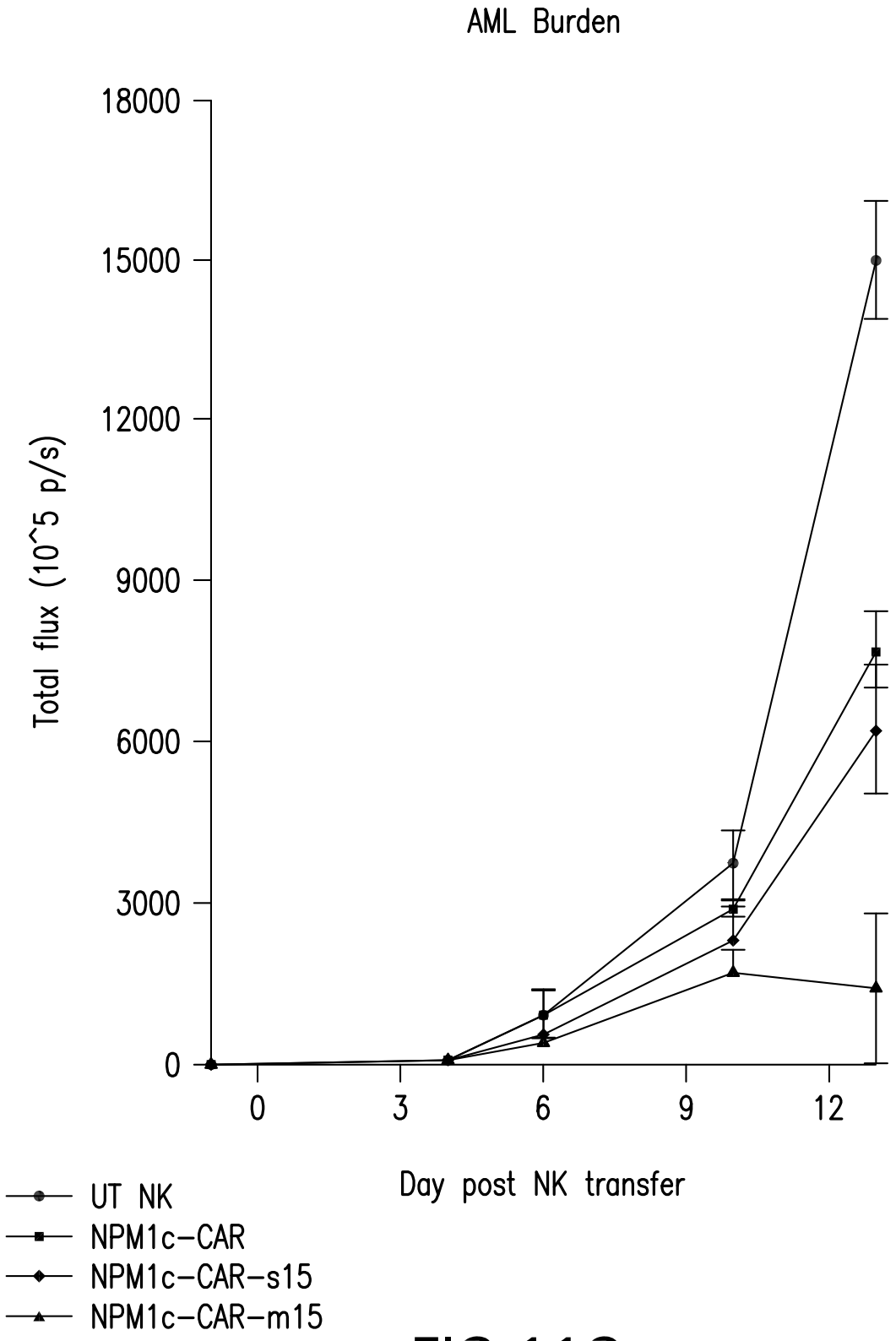

AML Burden
18000
15000
12000
9000
6000
3000
0
Total flux (10^5 p/s)
0
3
6
9
12
Day post NK transfer
UT NK
NPM1c-CAR
NPM1c-CAR-s15
NPM1c-CAR-m15

FIG.11C

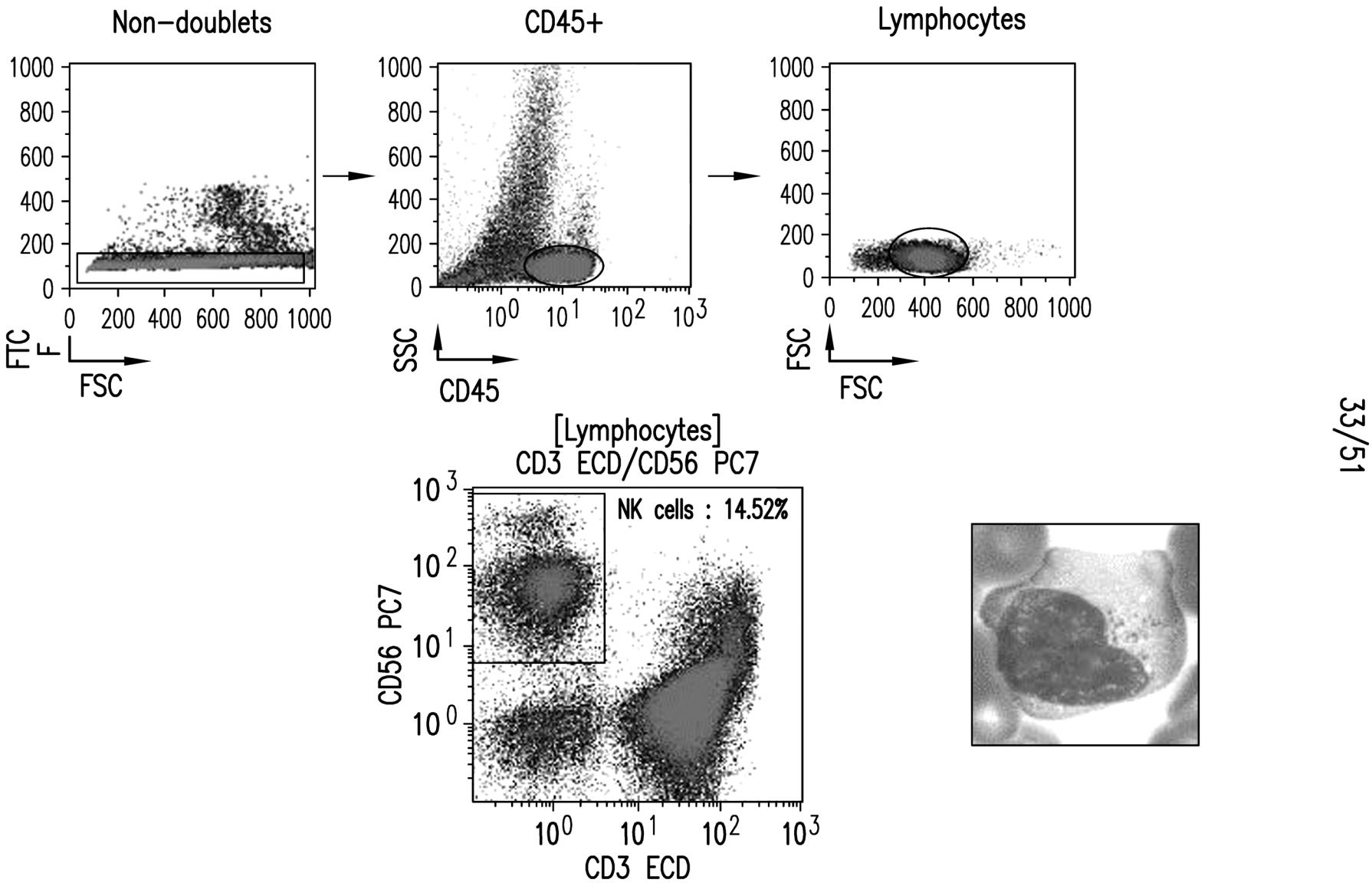

Non−doublets
CD45+
Lymphocytes
1000
800
600
400
200
0
0 200 400 600 800 1000
FTC
F
FSC
SSC
CD45
FSC
[Lymphocytes]
CD3 ECD/CD56 PC7
NK cells : 14.52%
CD56 PC7
CD3 ECD

FIG.12

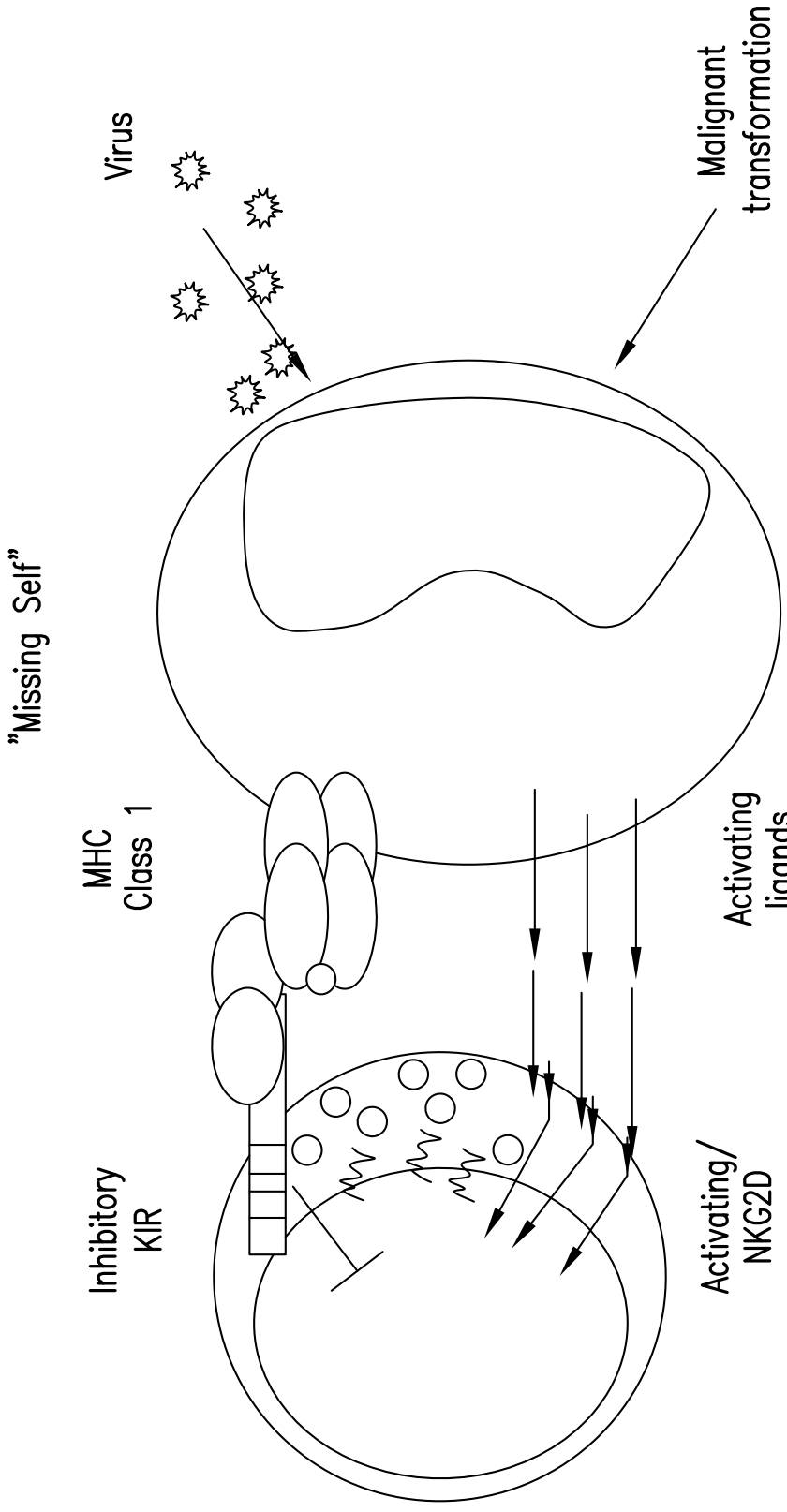
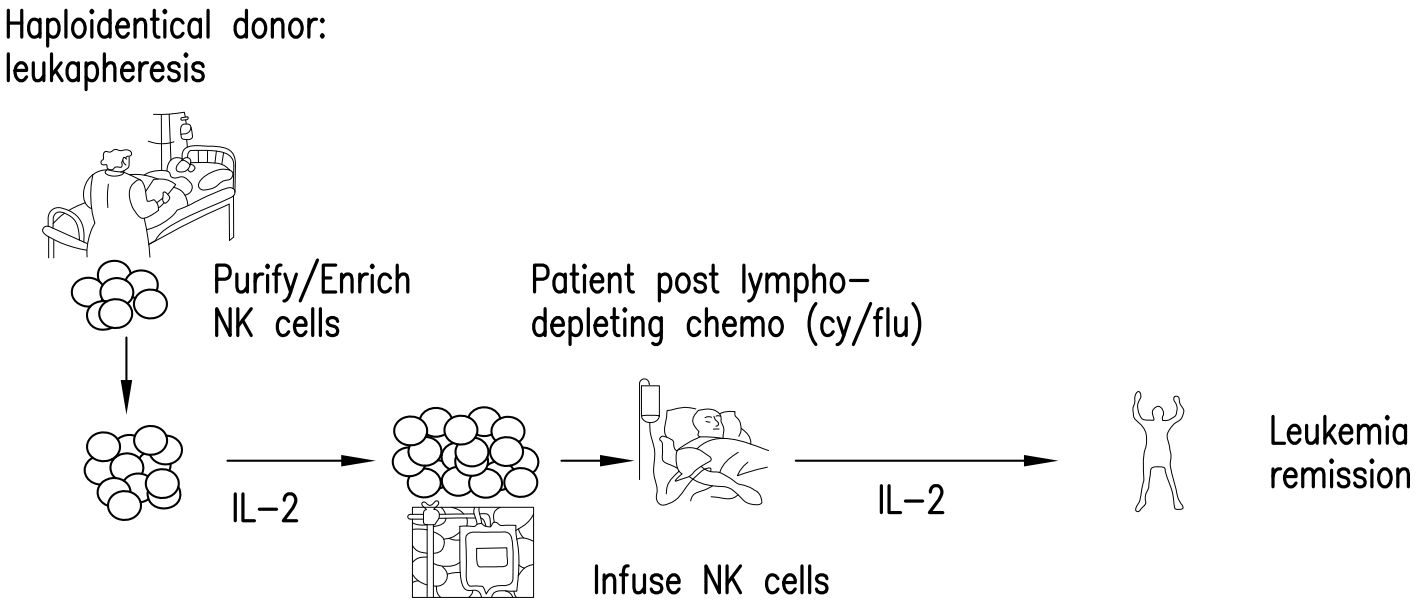

"Missing Self"
Virus
Malignant transformation
MHC Class 1
Activating ligands MIC-A/MIC-B
Inhibitory KIR
Activating/ NKG2D

FIG.13



Conventional NK cells have a life span of around 12–14 days
Limited expansion and persistence *in vivo*
Modest success mostly restricted to AML and MDS patients (~5–25%)
Primary NK cells are resistant to genetic manipulation

FIG. 14

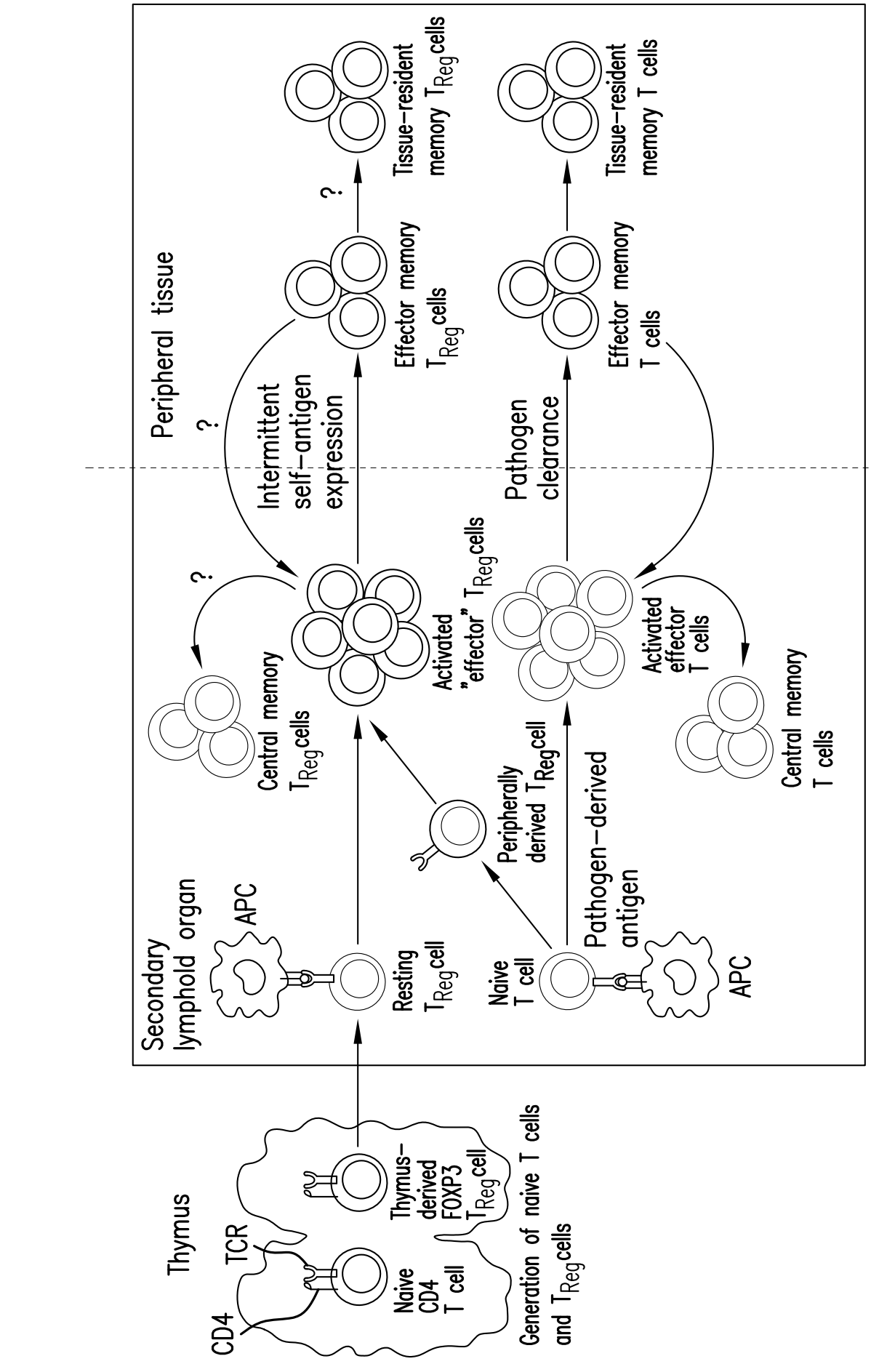

Thymus
CD4
TCR
Naive CD4 T cell
Thymus-derived FOXP3 TReg cell
Generation of naive T cells and TReg cells
Secondary lymphoid organ
APC
Resting TReg cell
Central memory TReg cells
Peripherally derived TReg cell
Activated "effector" TReg cells
Intermittent self-antigen expression
Effector memory TReg cells
Tissue-resident memory TReg cells
Naive T cell
Pathogen-derived antigen
APC
Pathogen clearance
Effector memory T cells
Tissue-resident memory T cells
Activated effector T cells
Central memory T cells
Peripheral tissue
?
?
?
?

FIG.15

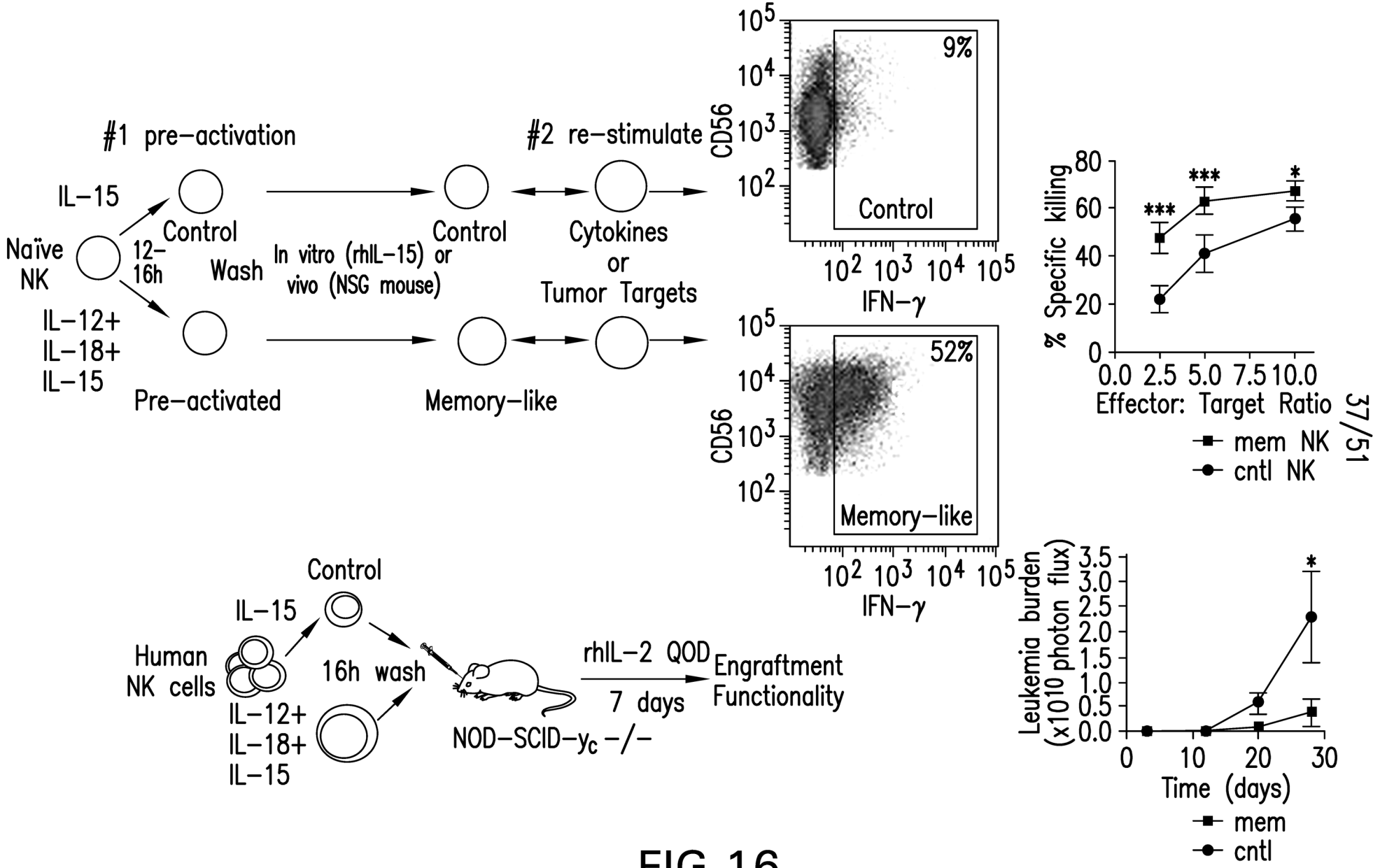
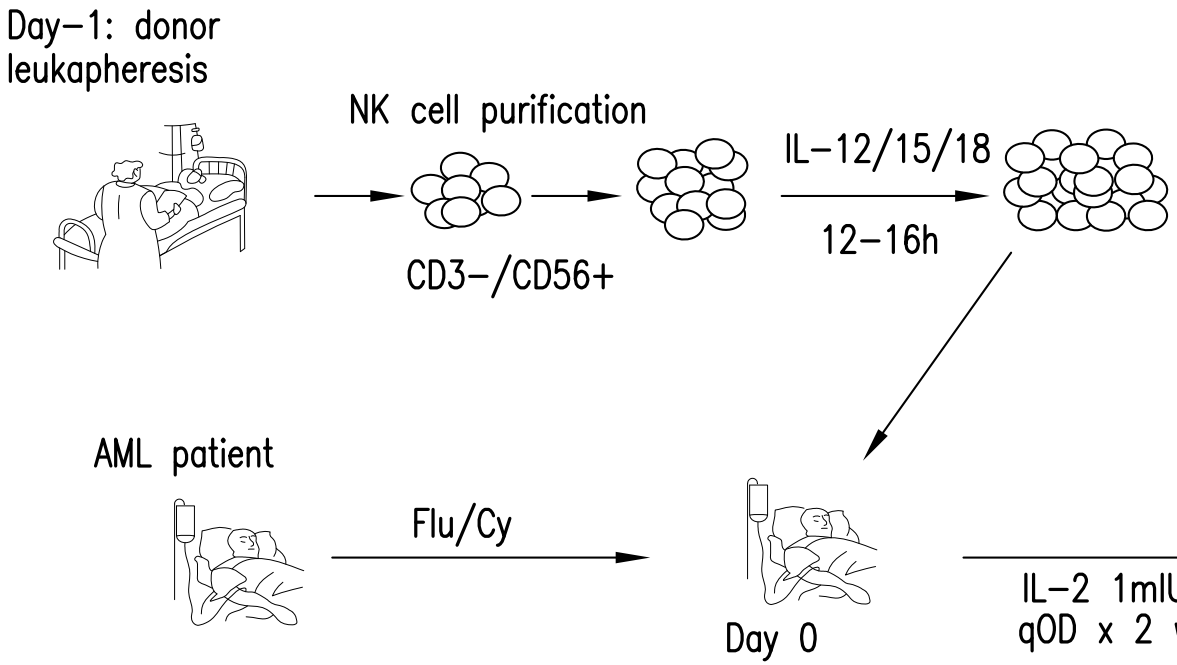

#1 pre-activation
#2 re-stimulate
IL-15
Control
Naïve NK
12-16h
Wash
In vitro (rhIL-15) or vivo (NSG mouse)
Control
Cytokines or Tumor Targets
IL-12+ IL-18+ IL-15
Pre-activated
Memory-like
CD56
IFN-γ
9%
Control
52%
Memory-like
% Specific killing
Effector: Target Ratio
mem NK
cntl NK
37/51
Control
IL-15
Human NK cells
16h wash
IL-12+ IL-18+ IL-15
NOD-SCID-γc -/-
rhIL-2 QOD
7 days
Engraftment Functionality
Leukemia burden (x1010 photon flux)
Time (days)
mem
cntl

FIG.16



Cell dose levels:

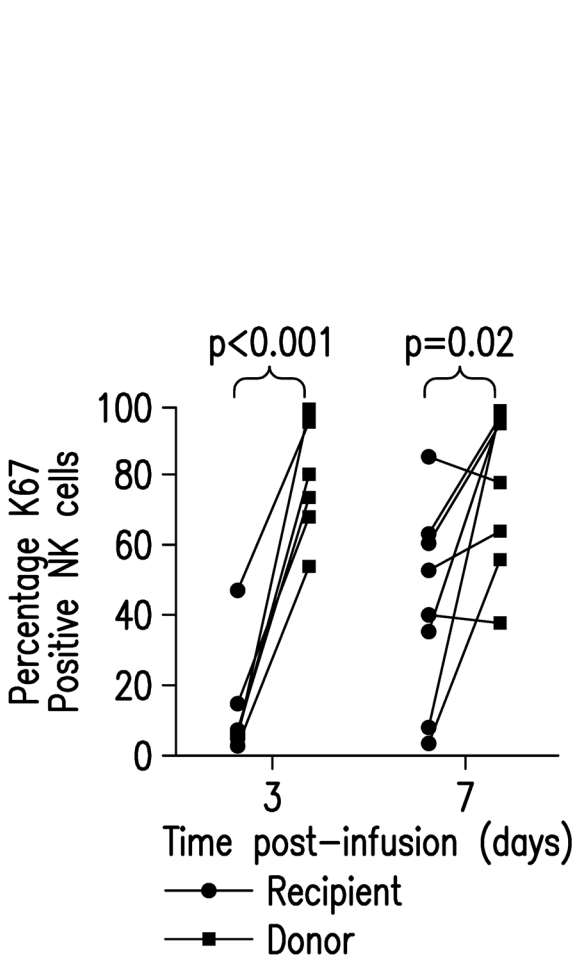
1. 0.5 x 10^6/kg
2. 1.0 x 10^6/kg
3. Max capped at $10x10^6$/kg

Endpoints:

Primary:
Safety/MTD

Secondary:
Leukemia Response
In vivo biology

FIG.17



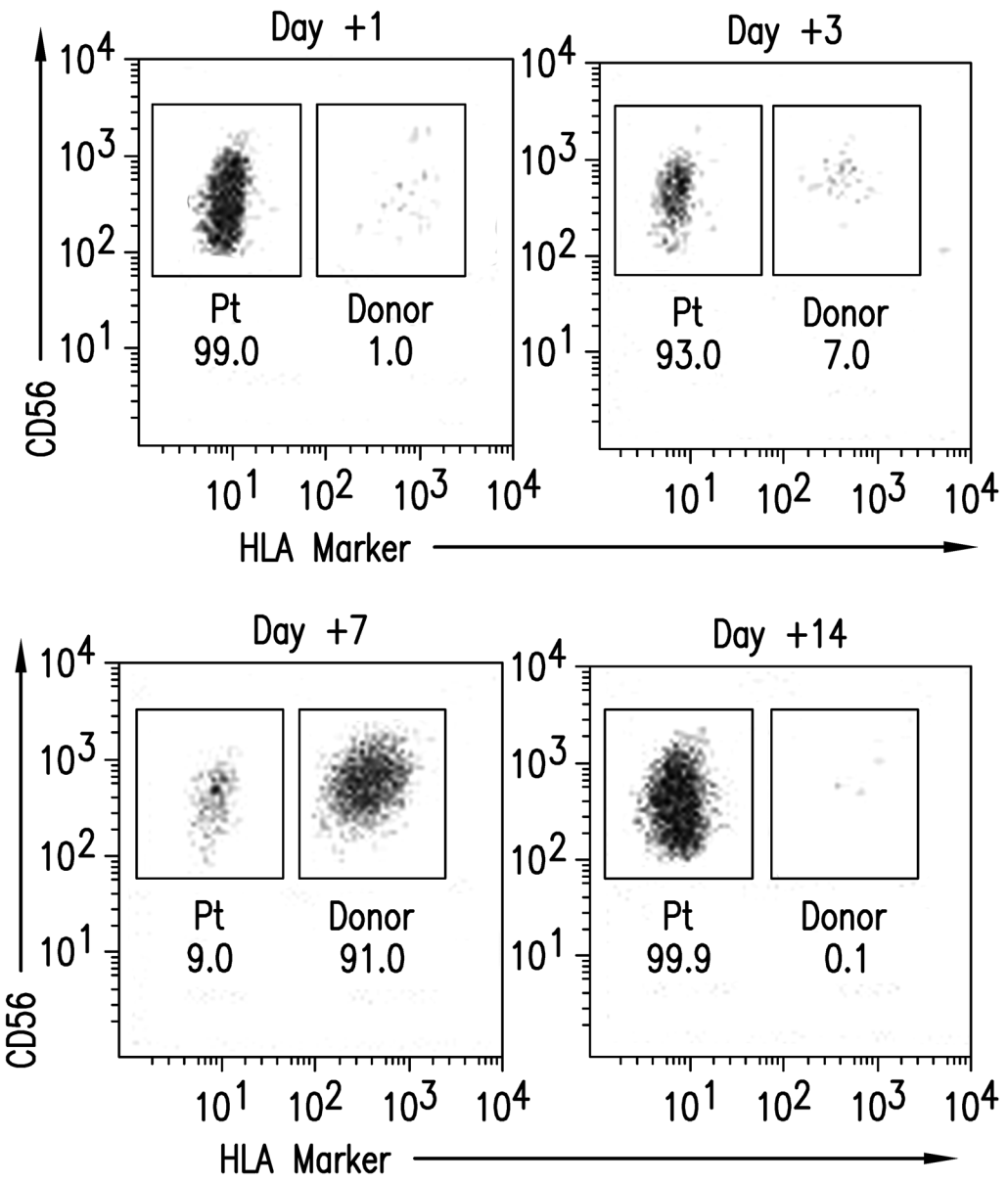


FIG.18
CONTINUED

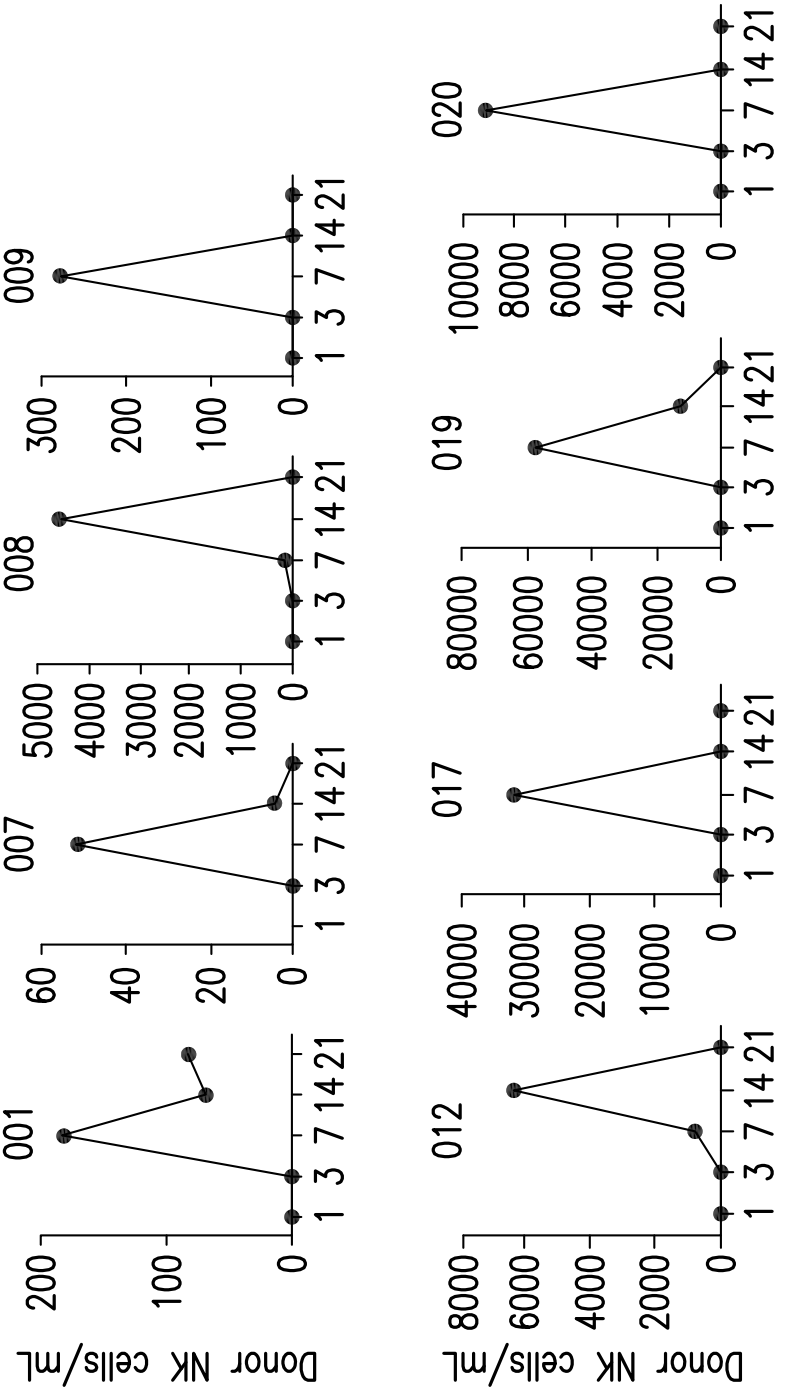


FIG.18
CONTINUED

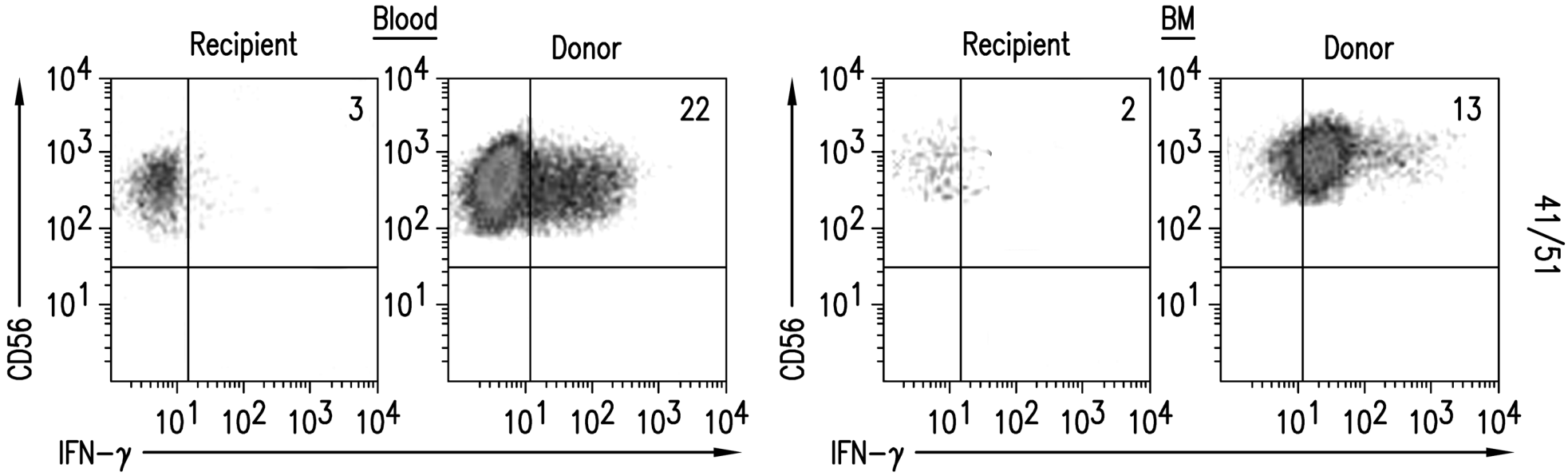


FIG.18
CONTINUED

UPN	Dose Level	Gender	Age (years)	WHO Dx	BM Blast (%)	KIR Ligand Mismatch	IWG Response	DLT	GVHD
001	1	M	73	M2	16	Yes	TF–PD	No	No
006	1	M	70	M0	28	Yes	TF–PD	No	No
007	1	M	77	M0	47	Yes	CR	No	No
008	2	M	76	t–AML	17	Yes	TF–PD	No	No
009	2	F	73	M1	80	No	MLFS	No	No
012	2	F	71	M5	15	Yes	CR	No	No
017	3	M	64	t–AML	69	Yes	TF–PD	No	No
019	3	F	71	M5	15	Yes	CR	No	No
020	3	M	60	MDS–AML	13	Yes	CRp	No	No
023	3	M	83	M1	86	No	CRp	No	No
025	3	M	72	t–AML	1	No	CR	No	No
027	3	M	76	M1	In CR	No	CRp	No	No
028	3	F	69	HMA failed MDS	6	No	Marrow CR	No	No

>50% CR Rate

2 Long term survivors

1 Died in CR

FIG.19

Relapse remains most common cause of death.

Donor lymphocyte infusion (DLI) with suboptimal outcomes (15–20% response)

Potential for more GVHD due to HLA mismatch

Unmet need

Phase 1 a/b study in relapsed patients

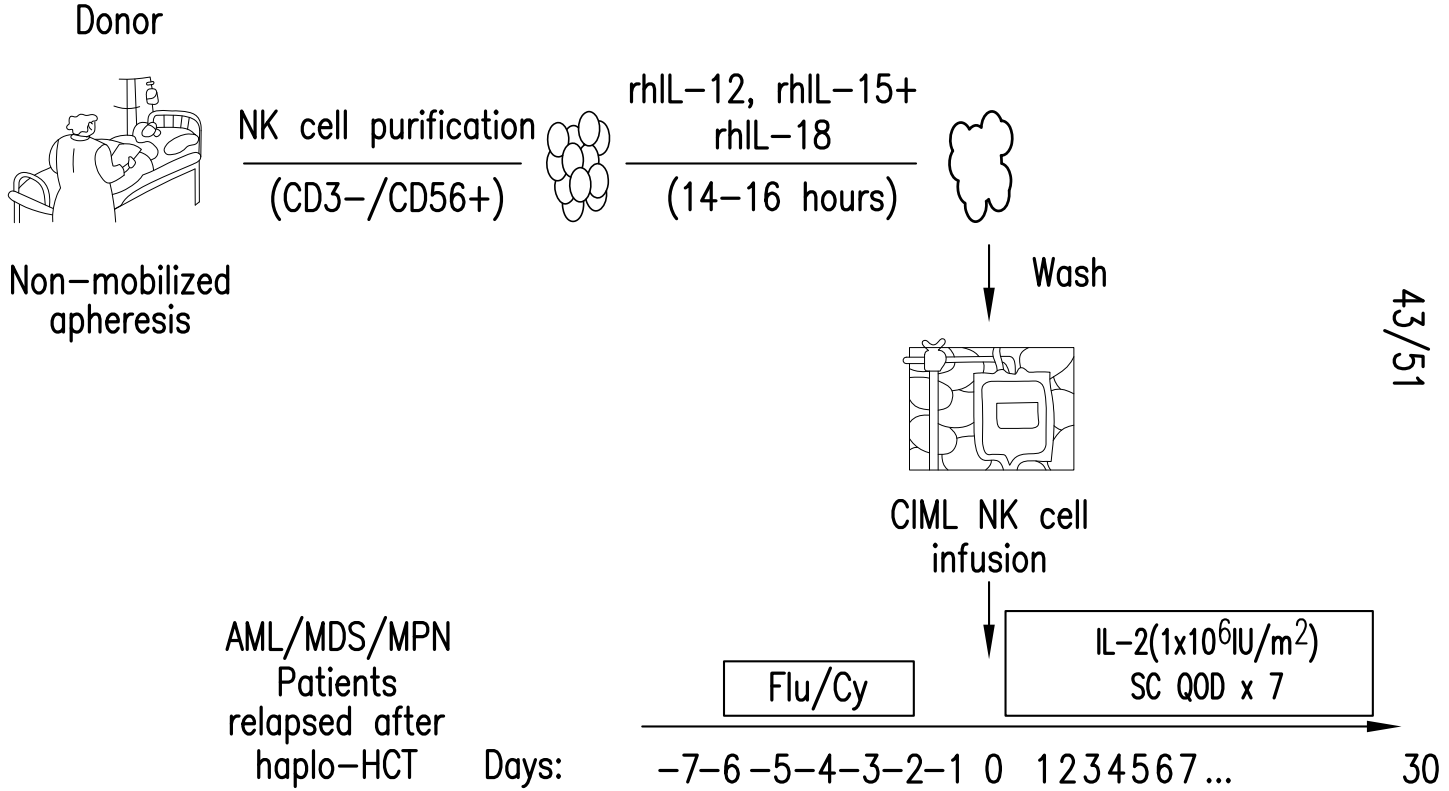


FIG.20

First 4 patients cleared disease, 2nd patient *(TP53 mutated)* MRD– by flow & RHP

Massive expansion & prolonged persistence

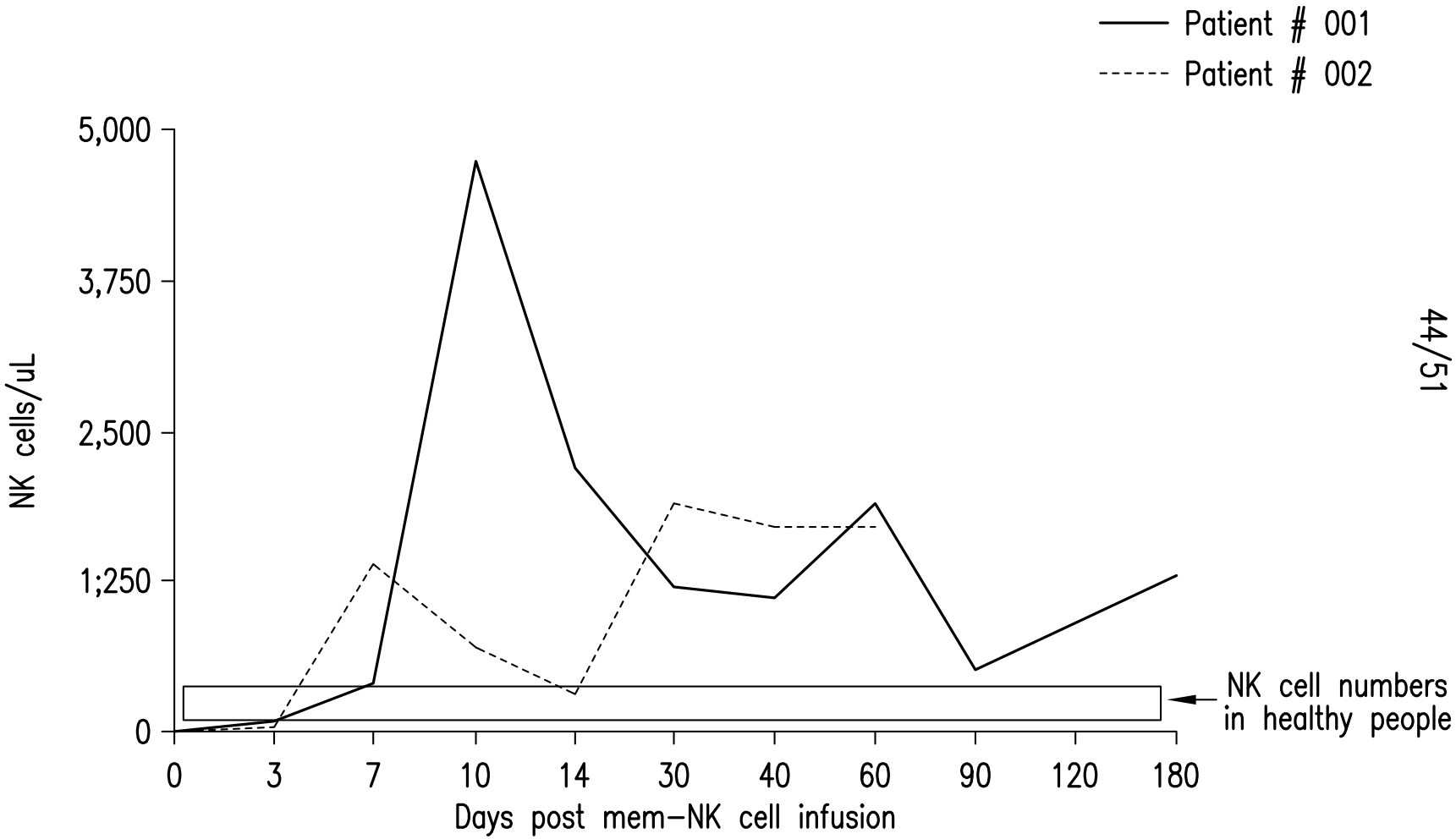


FIG.21

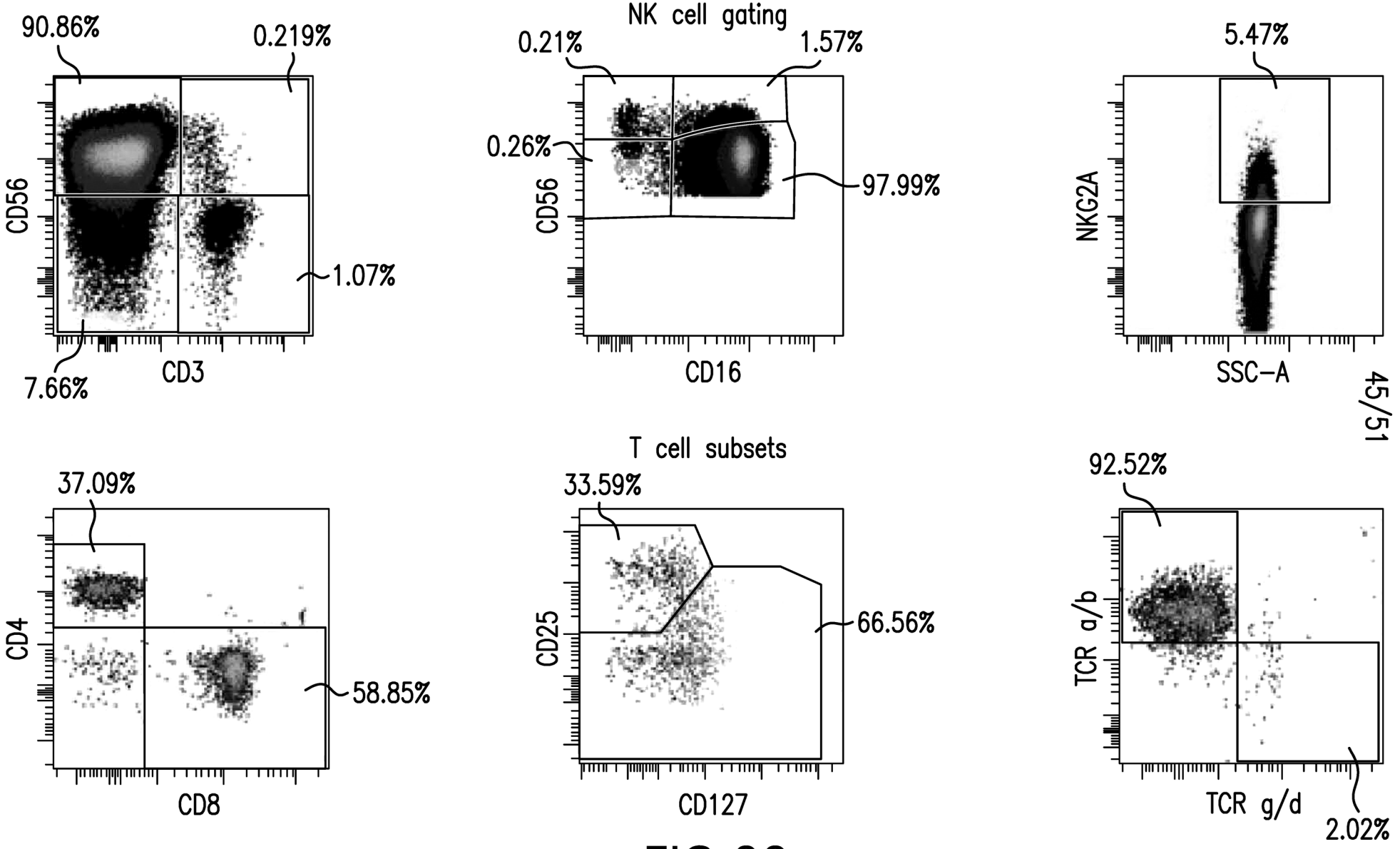
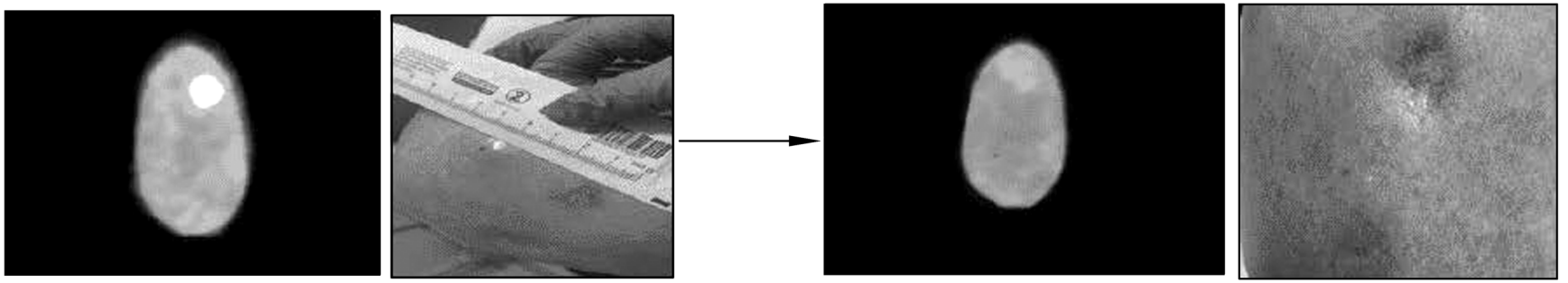

90.86%
0.219%
CD56
1.07%
7.66%
CD3
NK cell gating
0.21%
1.57%
0.26%
97.99%
CD56
CD16
5.47%
NKG2A
SSC-A
45/51
37.09%
CD4
58.85%
CD8
T cell subsets
33.59%
CD25
66.56%
CD127
92.52%
TCR a/b
TCR g/d
2.02%

FIG.22

66M, diagnosed in 5/2019, achieves CR with tagraxofusp (SL-401)
Underwent RIC Haplo HCT on 9/2019 but relapsed 5 months after Haplo HCT

Underwent memory-like NK cell immunotherapy in 8/2020 In CR!



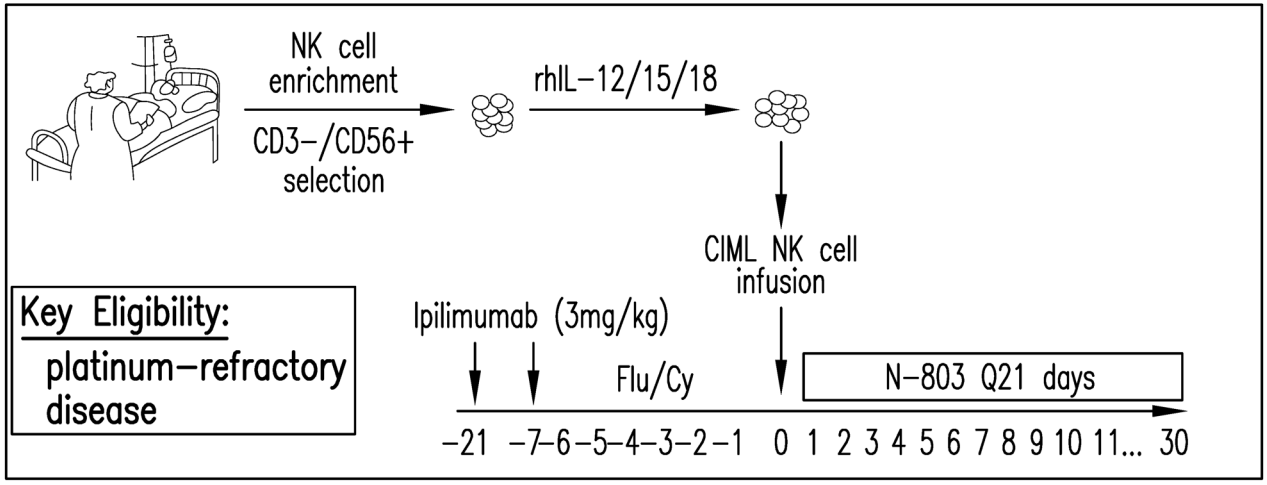
Pre-NK cell infusion Day 30 post NK cell infusion

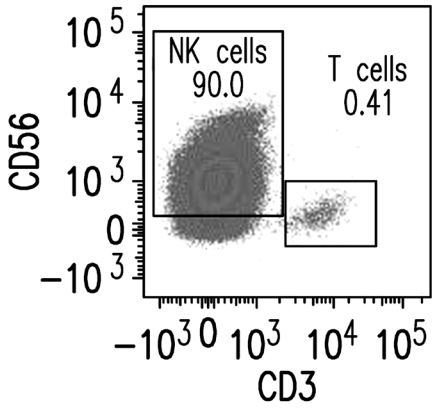
Mature NK cells constituted 75% of the 1350/uL peripheral blood lymphocytes at day +28 after infusion

In vitro BPDCN targeting with ML-NK cells

FIG.23

Anti–CTLA–4 antibody leads to Treg depletion to facilitate NK cell activity
NK cell infiltration associated with favorable outcomes in H&N ca patients
N–803 is an IL–15 super–agonist which enhances NK cell activation in vivo





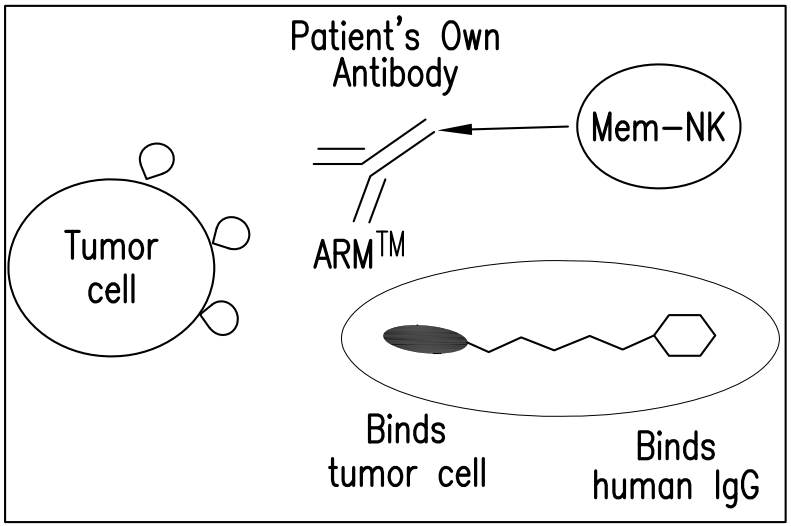
First patient treated on 9/15 (platinum/cetux/pembro failure)

>2500 mature NK cells/uL on day 21

IL–15 injection site rash



FIG.24


Patient's Own
Antibody
Mem–NK
Tumor
cell
ARM™
Binds
tumor cell
Binds
human IgG

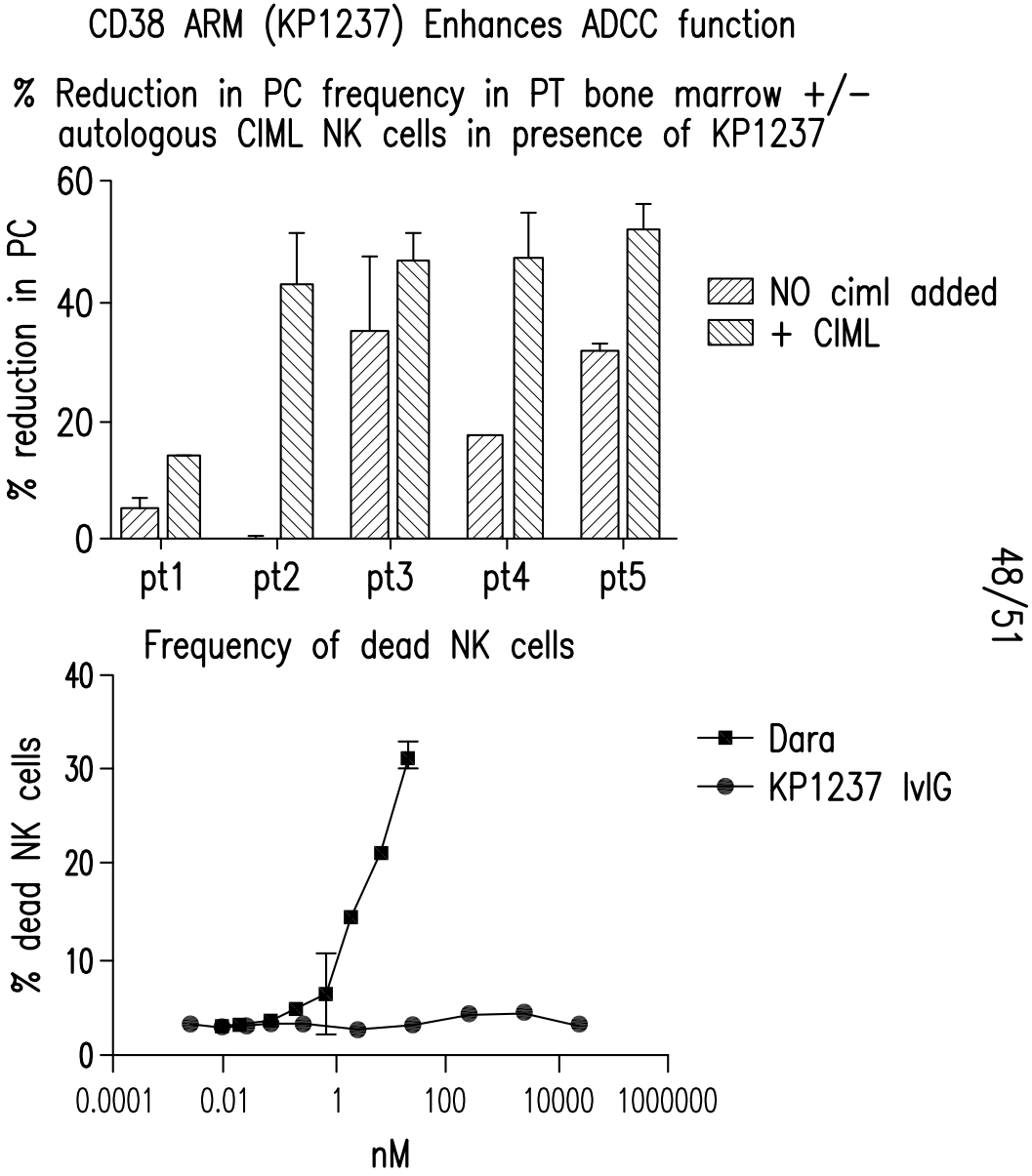

CD38 ARM (KP1237) Enhances ADCC function
% Reduction in PC frequency in PT bone marrow +/−
autologous CIML NK cells in presence of KP1237
% reduction in PC
60
40
20
0
pt1
pt2
pt3
pt4
pt5
NO ciml added
+ CIML
Frequency of dead NK cells
% dead NK cells
40
30
20
10
0
0.0001
0.01
1
100
10000
1000000
nM
Dara
KP1237 IvIG

FIG.25

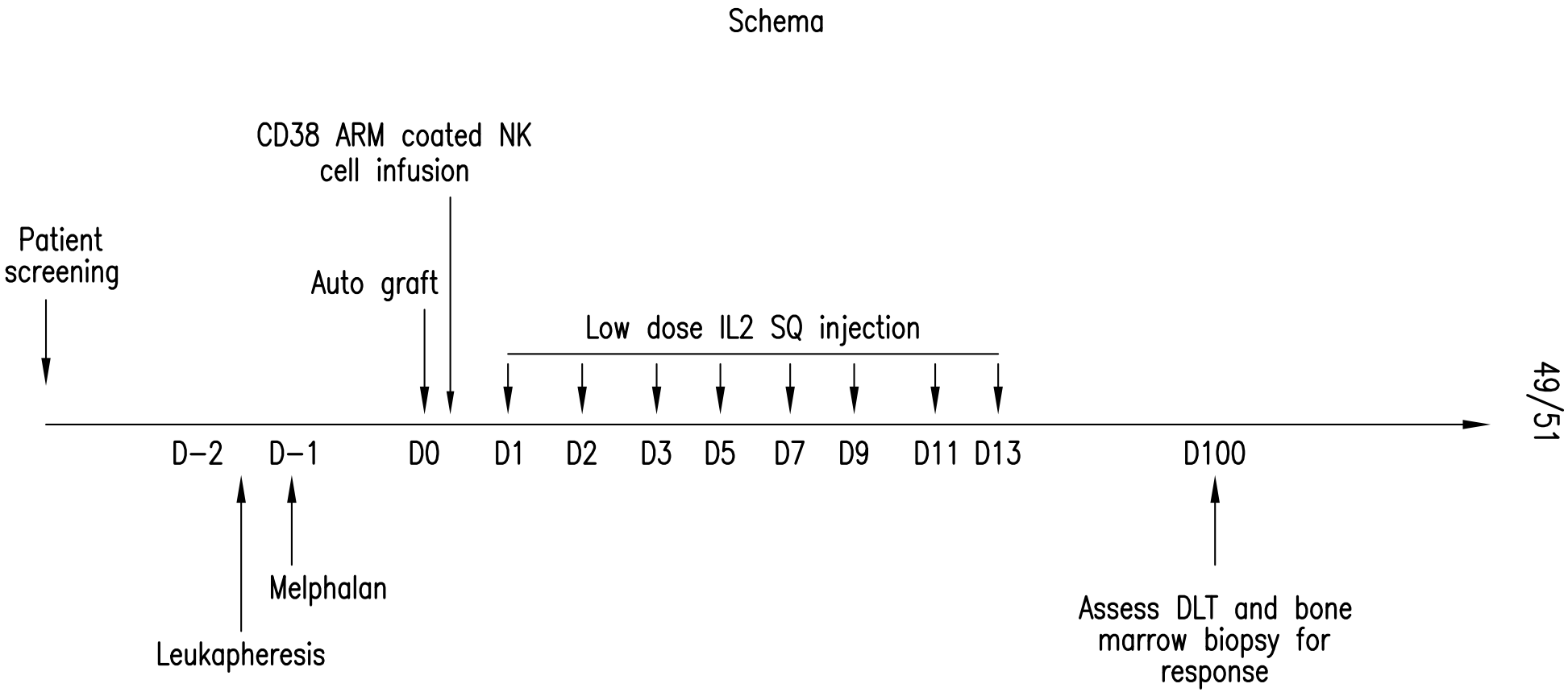

Schema
CD38 ARM coated NK cell infusion
Patient screening
Auto graft
Low dose IL2 SQ injection
D−2
D−1
D0
D1
D2
D3
D5
D7
D9
D11
D13
D100
Melphalan
Leukapheresis
Assess DLT and bone marrow biopsy for response

FIG.26

NK cell CARs are safe (no CRS/no neurotoxicity), response rate similar to CAR T cells

Limited efficacy of CAR T cells in AML/MDS

Dual targeting of myeloid blasts by Mem-NK cells armed with CARs *(antigen expression triggers CAR vs HLA loss triggers direct NK cell cytotoxicity)*

Primary Peripheral blood NK cells are resistant to transduction

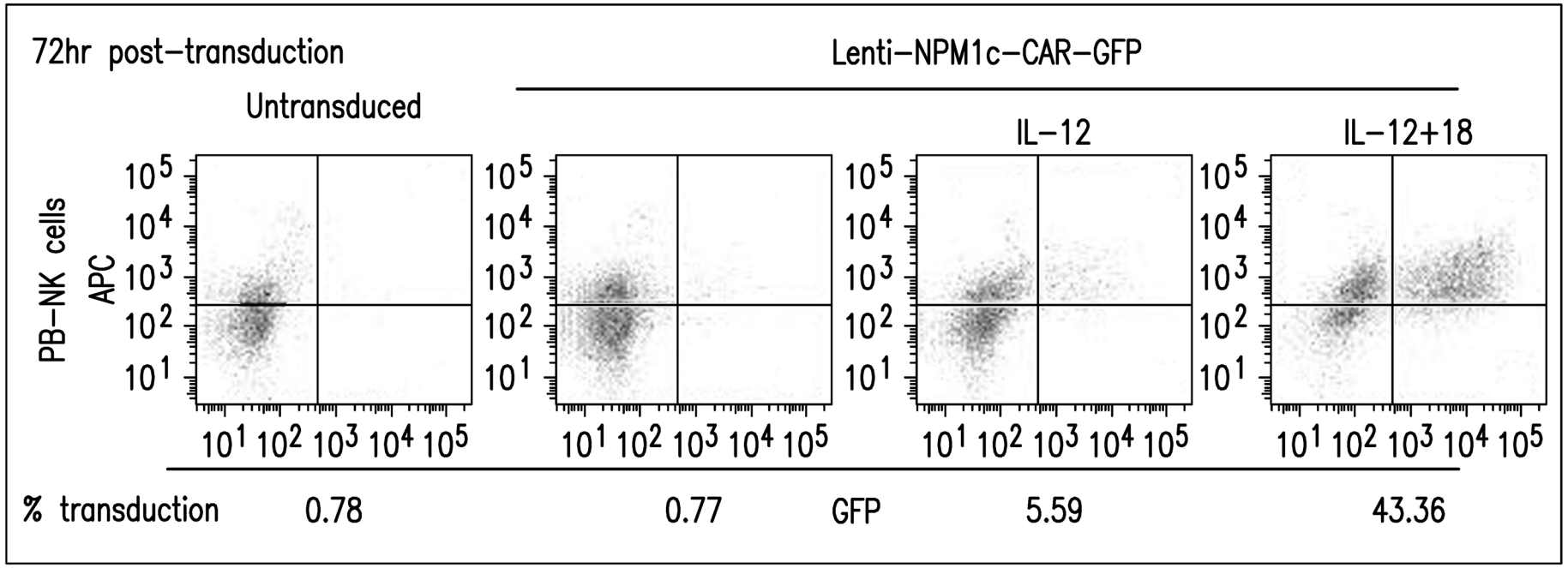


FIG.27

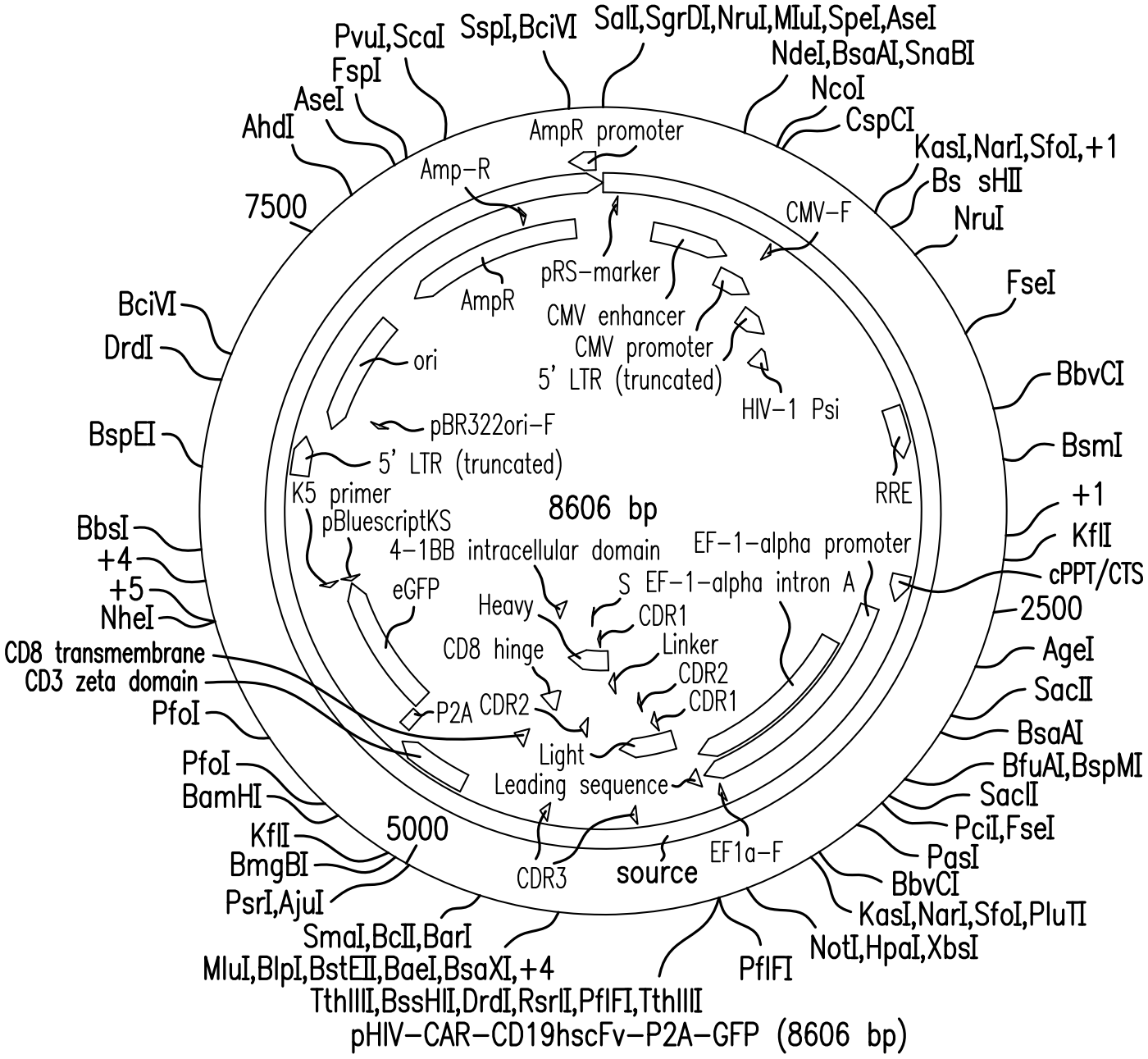

PvuI,ScaI
SspI,BciVI
SalI,SgrDI,NruI,MluI,SpeI,AseI
FspI
NdeI,BsaAI,SnaBI
AseI
NcoI
AhdI
CspCI
AmpR promoter
KasI,NarI,SfoI,+1
Amp-R
Bs sHII
7500
CMV-F
NruI
pRS-marker
AmpR
FseI
BciVI
CMV enhancer
DrdI
CMV promoter
ori
5' LTR (truncated)
BbvCI
HIV-1 Psi
pBR322ori-F
BspEI
BsmI
5' LTR (truncated)
RRE
K5 primer
+1
8606 bp
pBluescriptKS
BbsI
4-1BB intracellular domain
EF-1-alpha promoter
KflI
+4
eGFP
S
EF-1-alpha intron A
cPPT/CTS
+5
Heavy
CDR1
2500
NheI
CD8 hinge
Linker
AgeI
CD8 transmembrane
CDR2
CD3 zeta domain
SacII
P2A
CDR2
CDR1
PfoI
BsaAI
Light
BfuAI,BspMI
PfoI
Leading sequence
SacII
BamHI
PciI,FseI
5000
KflI
EF1a-F
PasI
BmgBI
CDR3
source
BbvCI
PsrI,AjuI
KasI,NarI,SfoI,PluTI
SmaI,BclI,BarI
NotI,HpaI,XbsI
MluI,BlpI,BstEII,BaeI,BsaXI,+4
PflFI
TthIIII,BssHII,DrdI,RsrII,PflFI,TthIIII
pHIV-CAR-CD19hscFv-P2A-GFP (8606 bp)

FIG.28