FIGURES

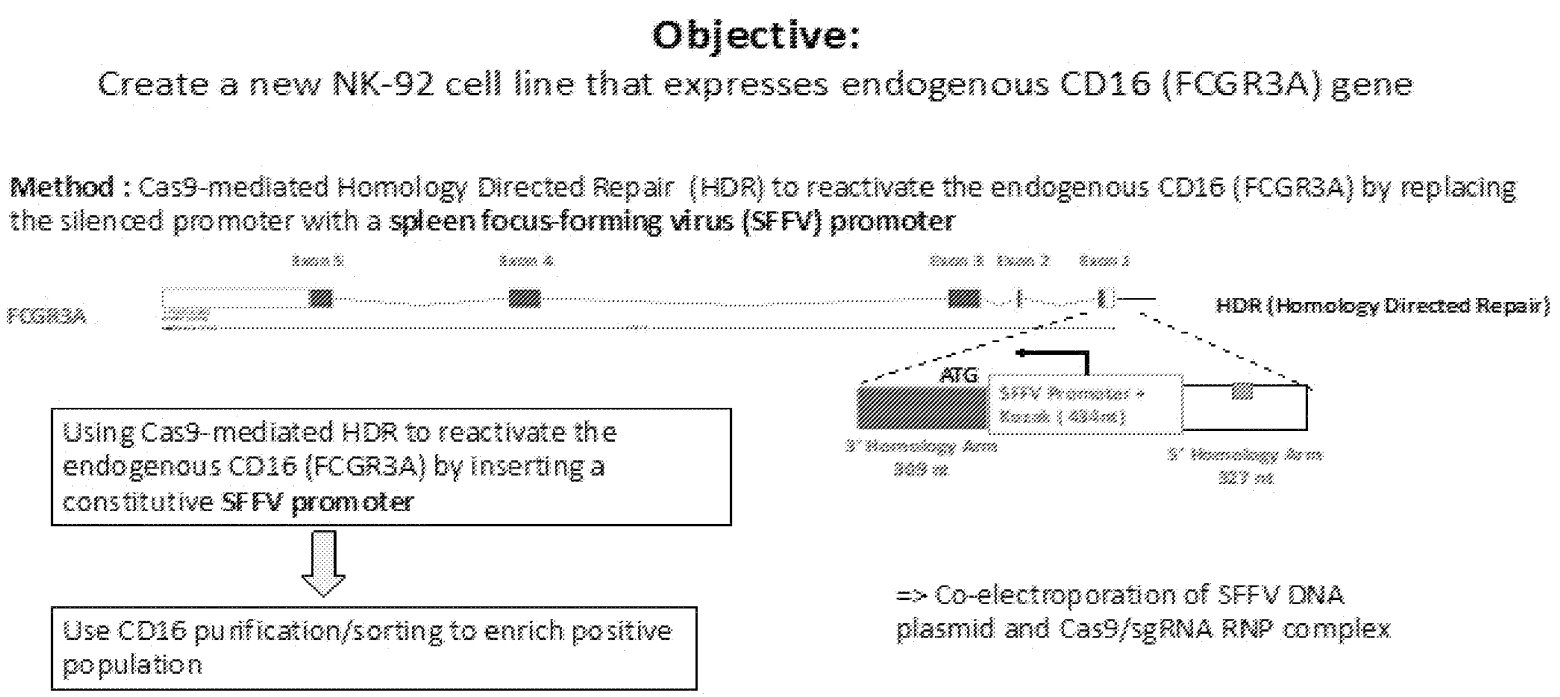


FIG.1

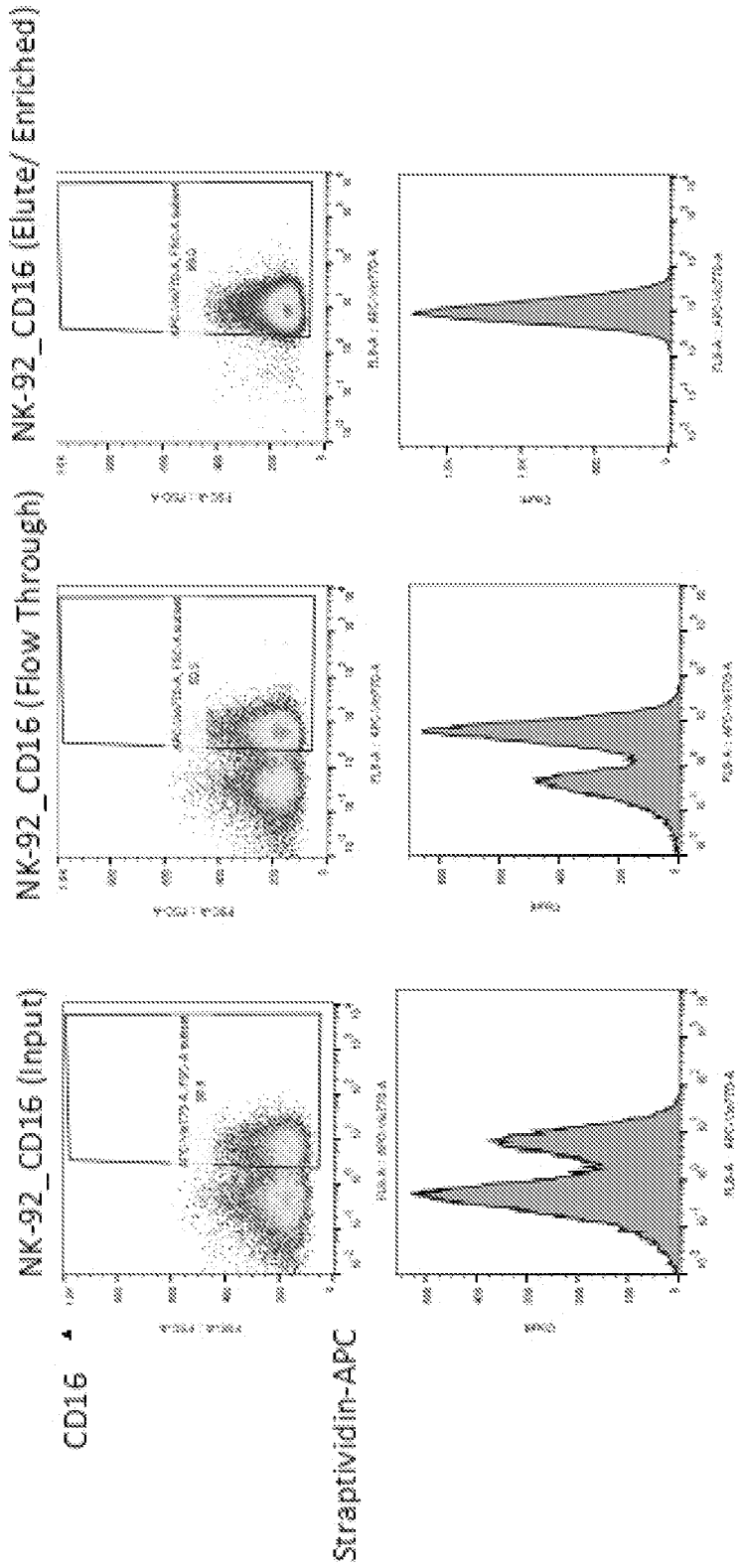

NK-92_CD16 (Input)
NK-92_CD16 (Flow Through)
NK-92_CD16 (Elute/ Enriched)
CD16
Streptividin-APC

FIG.2

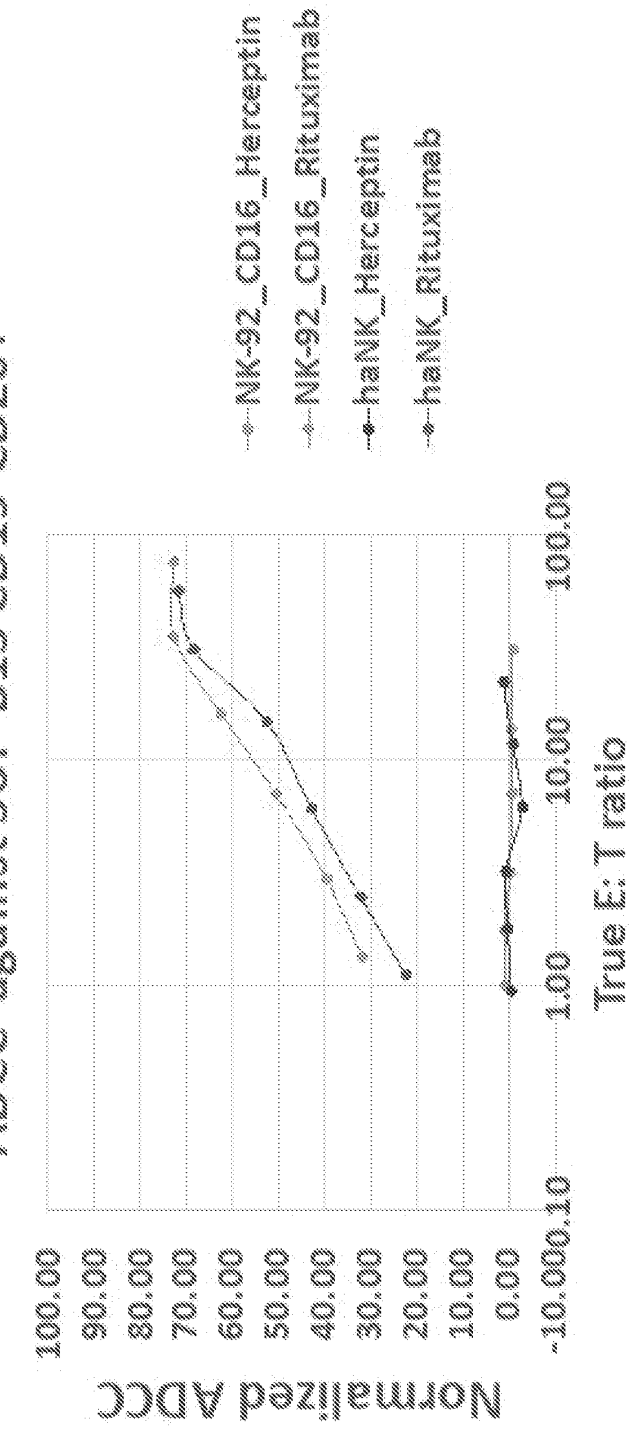

ADCC against SUP-B15 CD19-CD20+
NK-92_CD16_Herceptin
NK-92_CD16_Rituximab
haNK_Herceptin
haNK_Rituximab
Normalized ADCC
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
-10.00
0.10
1.00
10.00
100.00
True E: T ratio

FIG.3

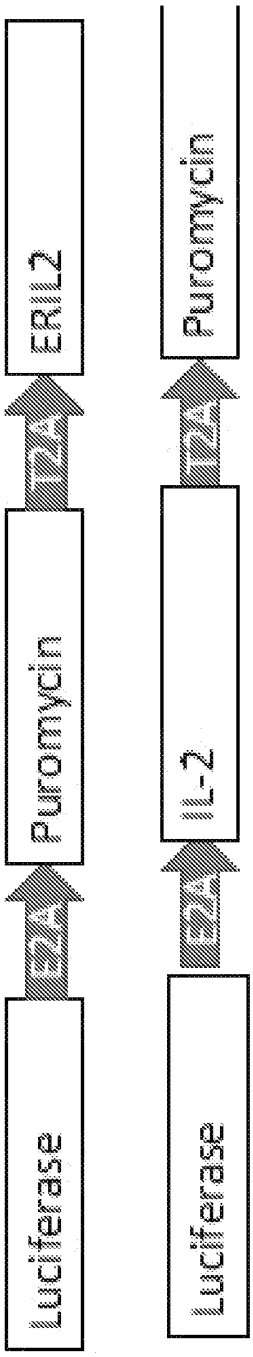

Luciferase
E2A
Puromycin
T2A
ERIL2
Luciferase
E2A
IL-2
T2A
Puromycin

FIG.4

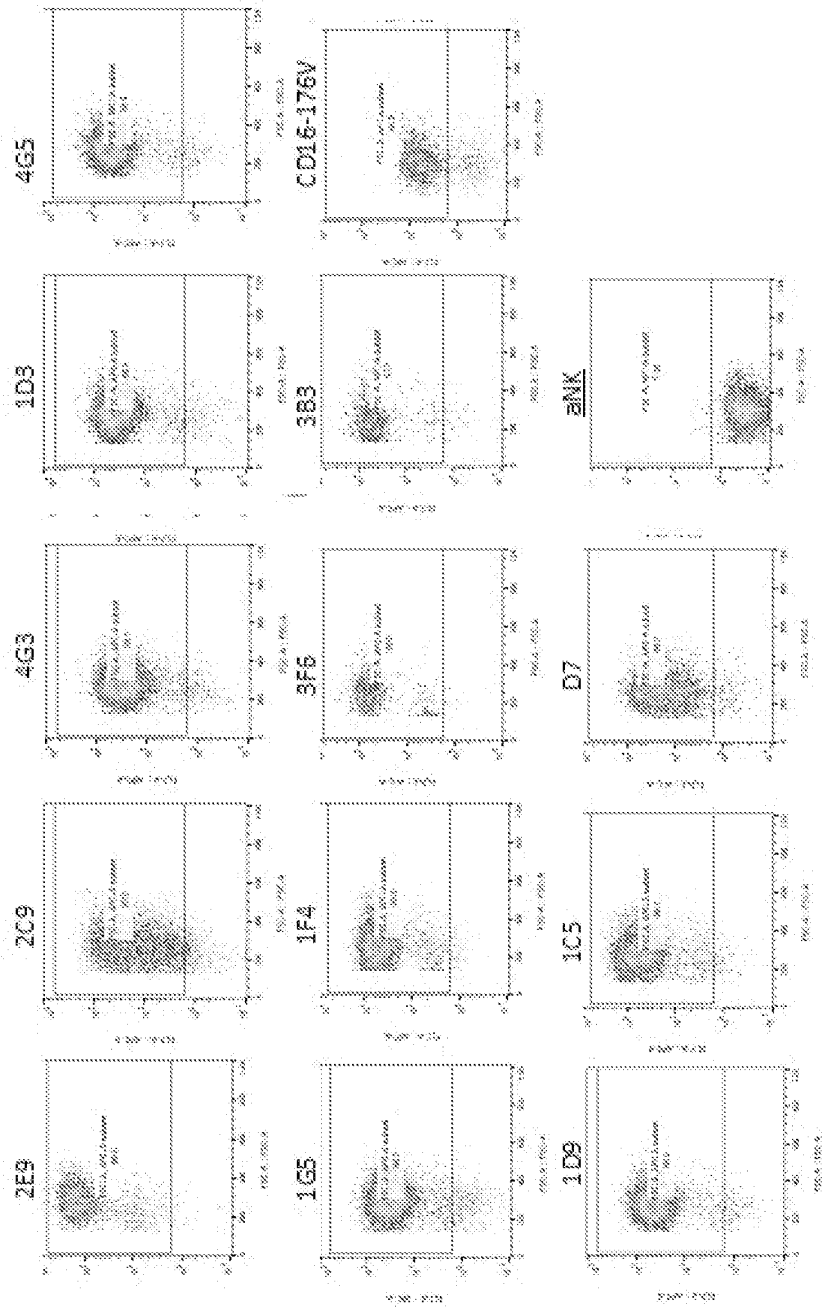


FIG.5

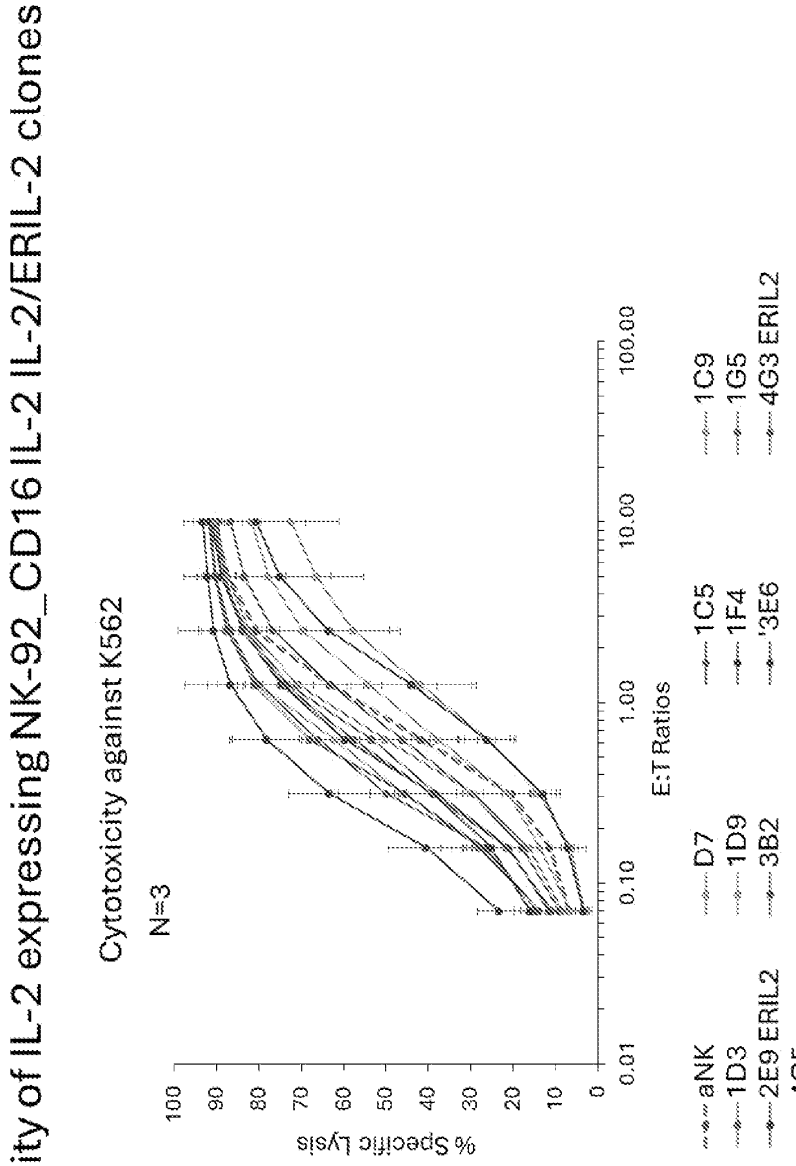

Cytotoxicity of IL-2 expressing NK-92_CD16 IL-2 IL-2/ERIL-2 clones
Cytotoxicity against K562
N=3
% Specific Lysis
100
90
80
70
60
50
40
30
20
10
0
0.01
0.10
1.00
10.00
100.00
E:T Ratios
aNK
1D3
2E9 ERIL2
4G5
D7
1D9
3B2
1C5
1F4
'3E6
1C9
1G5
4G3 ERIL2

FIG.6

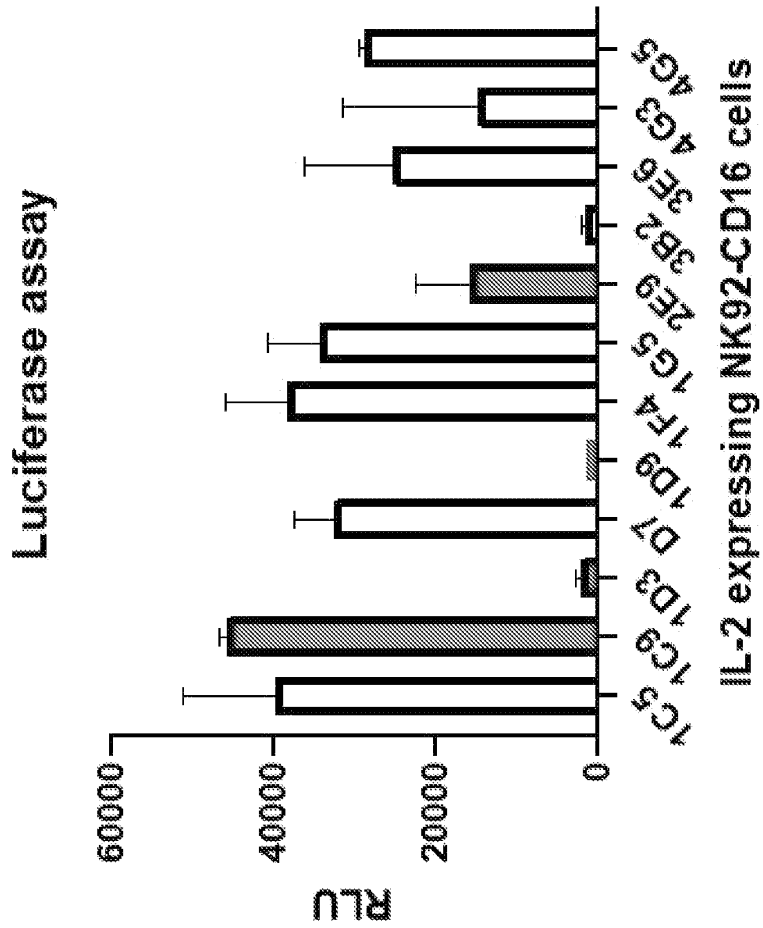

Luciferase assay
RLU
60000
40000
20000
0
1C5
1C9
1D3
1D7
1D9
1F4
1G5
2E9
3B2
3E6
4G3
4G5
IL-2 expressing NK92-CD16 cells

FIG.7

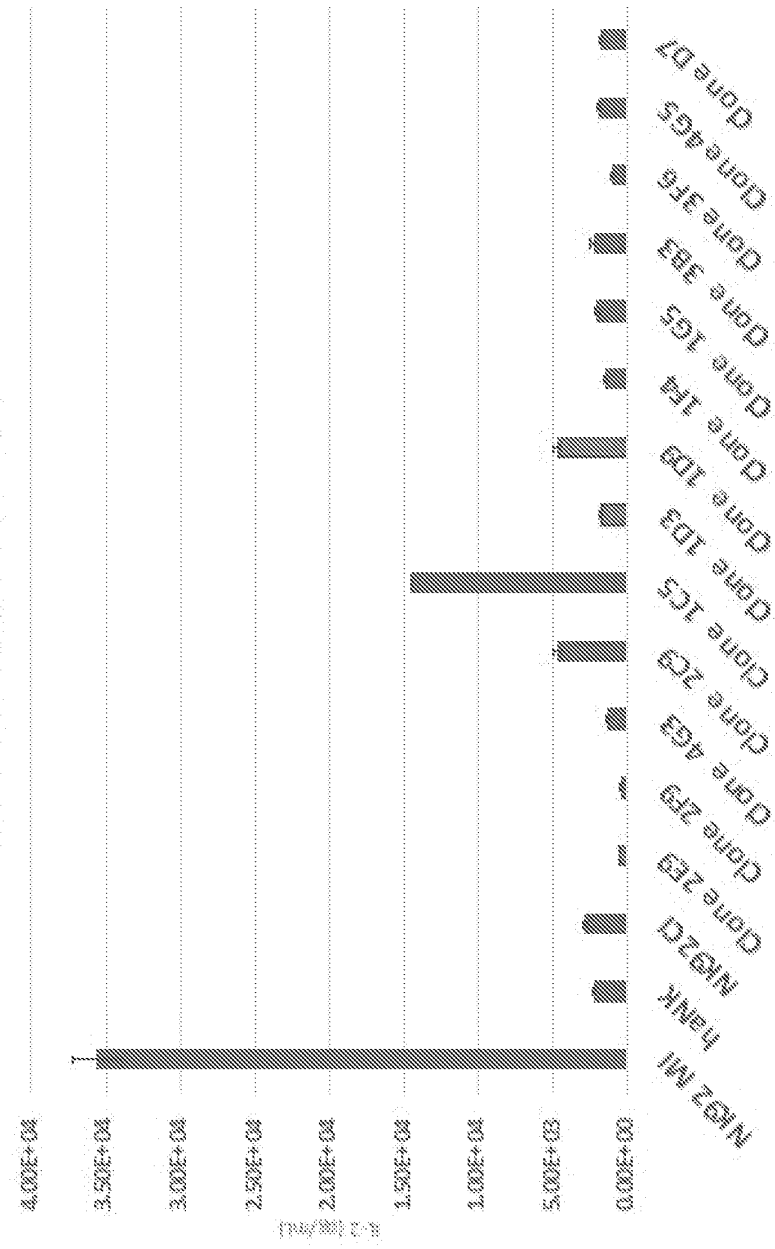

IL-2 in Cell supernatant post 24 Hours
(ng/ml) IL-2
4.00E+04
3.50E+04
3.00E+04
2.50E+04
2.00E+04
1.50E+04
1.00E+04
5.00E+03
0.00E+00
1
NB2 M1
hANK
NB2C
Clone 2E3
Clone 2F3
Clone 4G3
Clone 2C3
Clone 1C5
Clone 1D3
Clone 1D8
Clone 1F4
Clone 1G5
Clone 3B3
Clone 3F6
Clone 4G5
Clone D7

FIG.8

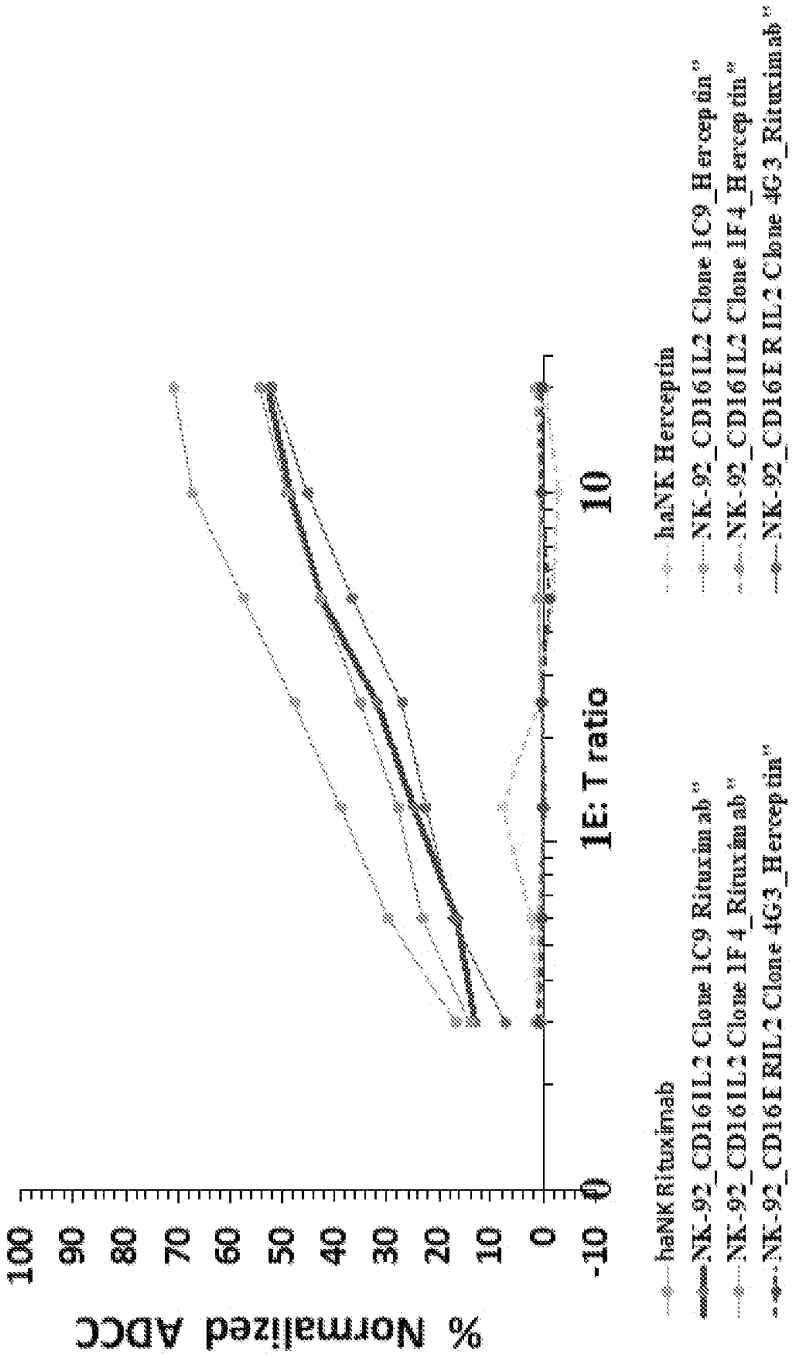

% Normalized ADCC
100
90
80
70
60
50
40
30
20
10
0
-10
0
10
1E: T ratio
haNK Rituximab
NK-92_CD16IL2 Clone 1C9 Rituximab"
NK-92_CD16IL2 Clone 1F4_Rituximab"
NK-92_CD16I RIL2 Clone 4G3_Herceptin"
haNK Herceptin
NK-92_CD16IL2 Clone 1C9_Herceptin"
NK-92_CD16IL2 Clone 1F4_Herceptin"
NK-92_CD16I RIL2 Clone 4G3_Rituximab"

FIG.9

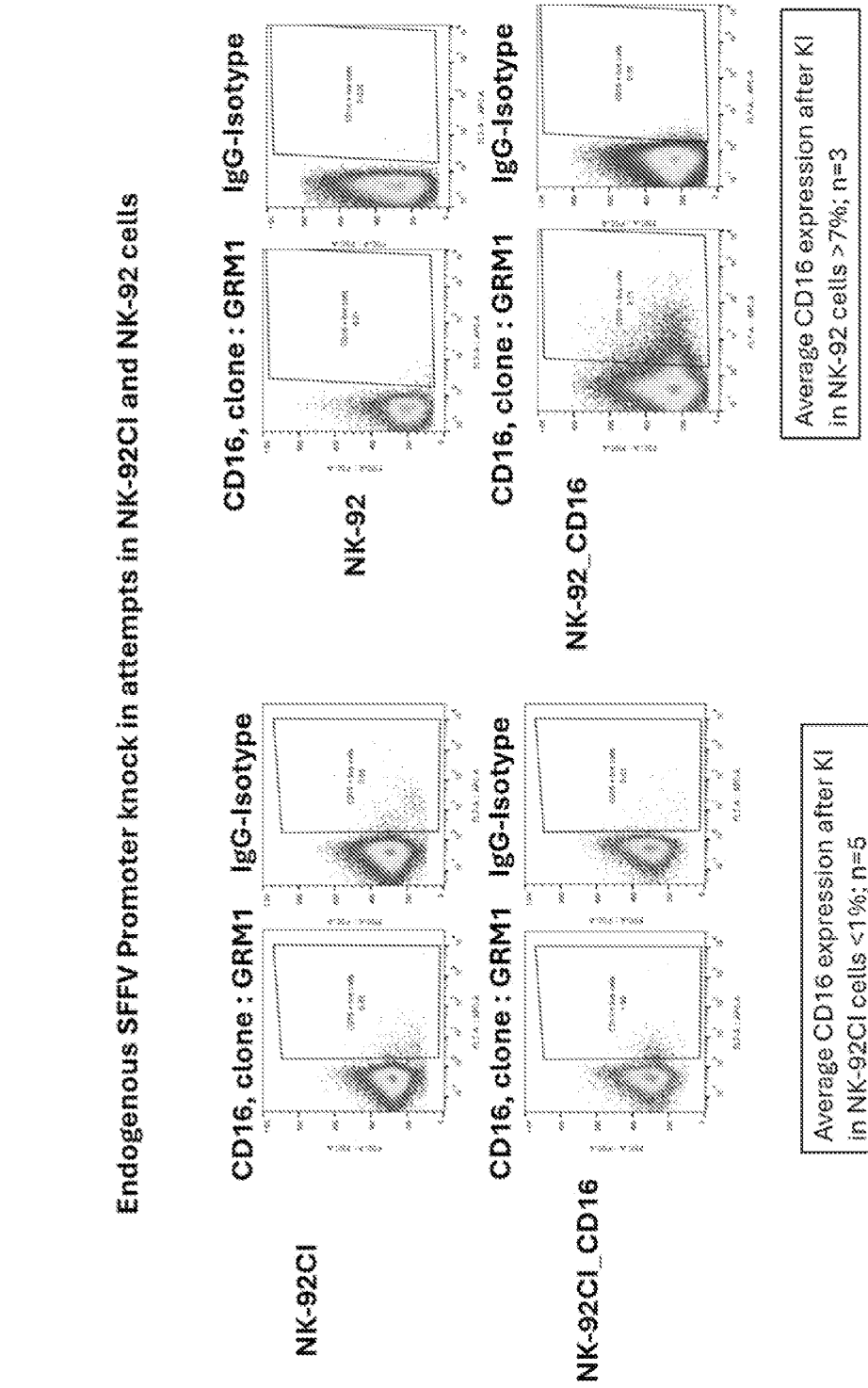

Endogenous SFFV Promoter knock in attempts in NK-92Cl and NK-92 cells
CD16, clone : GRM1
IgG-Isotype
NK-92
NK-92_CD16
NK-92Cl
NK-92Cl_CD16
Average CD16 expression after KI in NK-92 cells >7%; n=3
Average CD16 expression after KI in NK-92Cl cells <1%; n=5

FIG.10

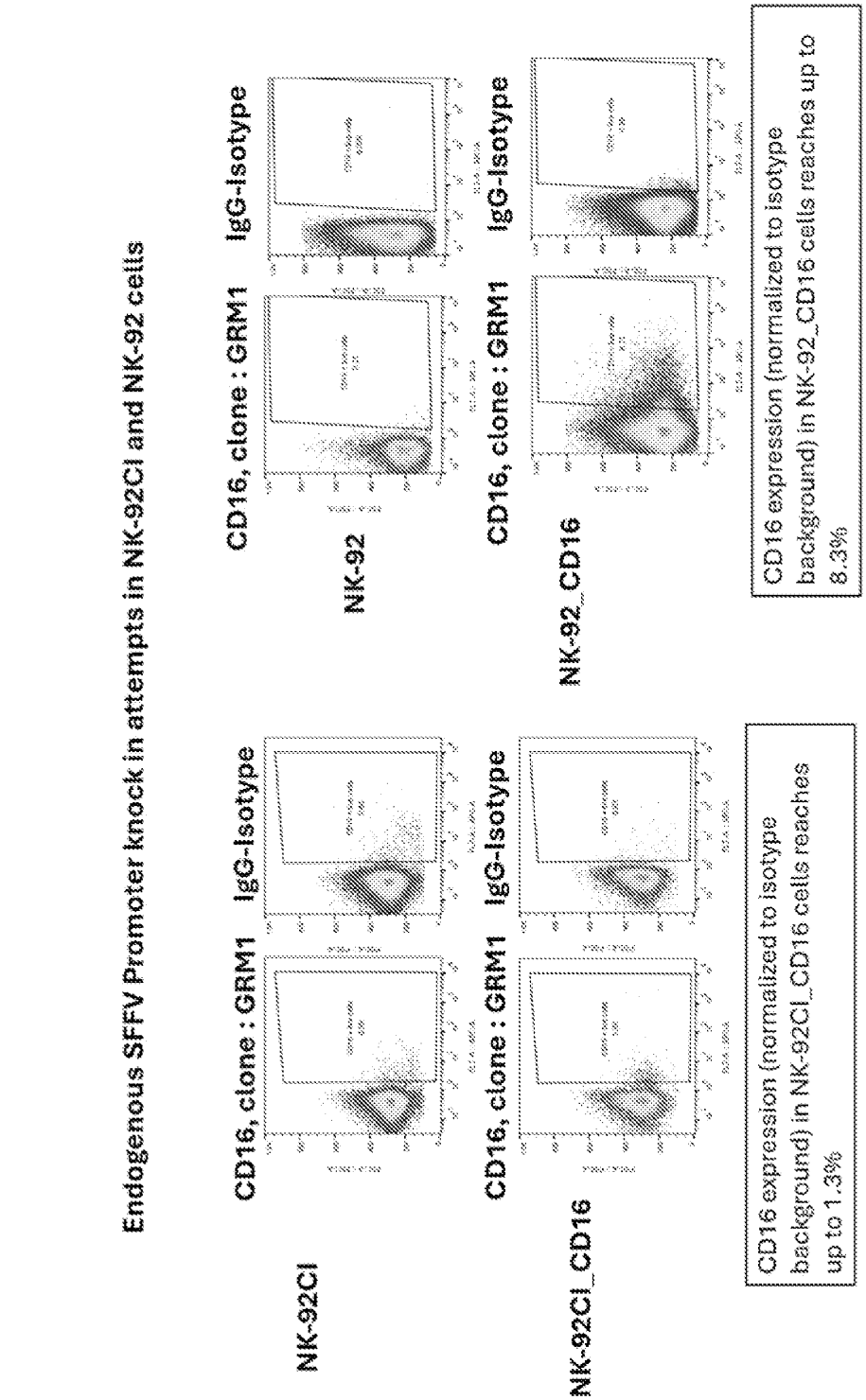

Endogenous SFFV Promoter knock in attempts in NK-92CI and NK-92 cells
NK-92CI
CD16, clone : GRM1
IgG-Isotype
NK-92CI_CD16
CD16, clone : GRM1
IgG-Isotype
CD16 expression (normalized to isotype background) in NK-92CI_CD16 cells reaches up to 1.3%
NK-92
CD16, clone : GRM1
IgG-Isotype
NK-92_CD16
CD16, clone : GRM1
IgG-Isotype
CD16 expression (normalized to isotype background) in NK-92_CD16 cells reaches up to 8.3%

FIG.11

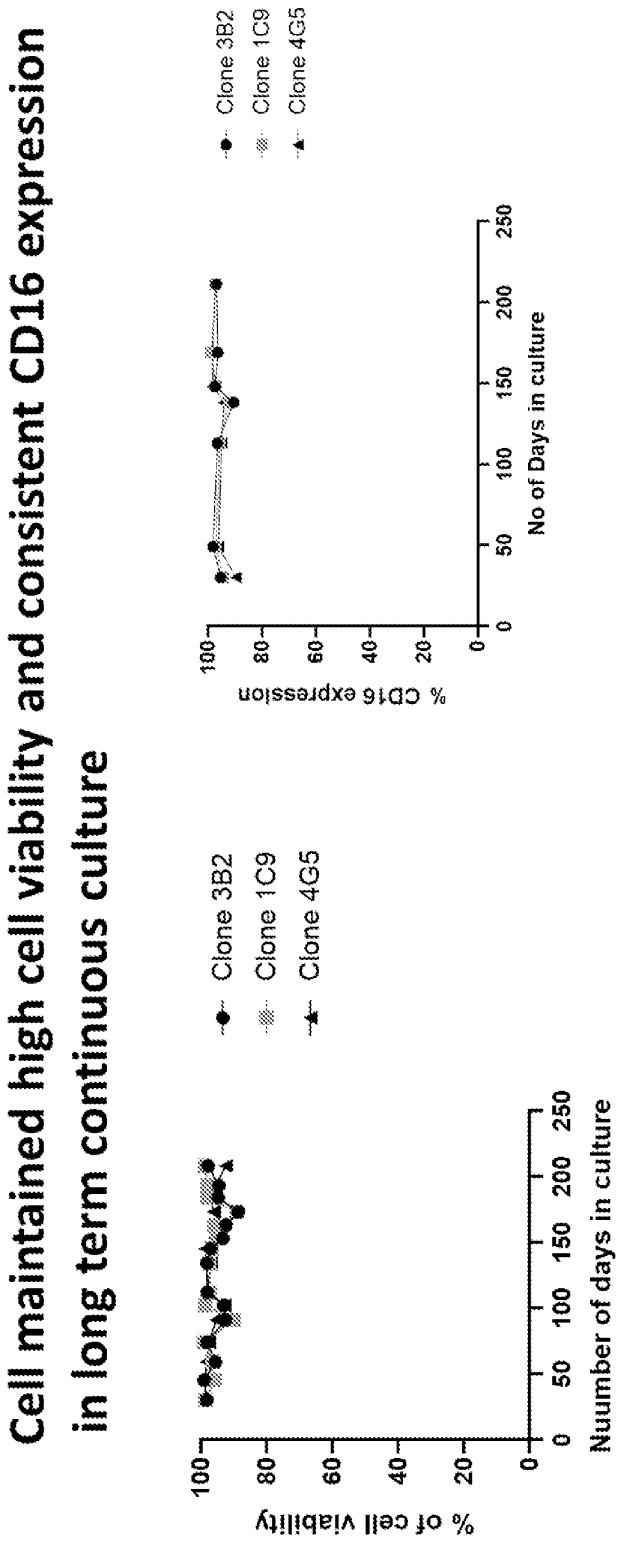

Cell maintained high cell viability and consistent CD16 expression in long term continuous culture
% of cell viability
Nuumber of days in culture
Clone 3B2
Clone 1C9
Clone 4G5
% CD16 expression
No of Days in culture
0
20
40
60
80
100
0
50
100
150
200
250

FIG.12

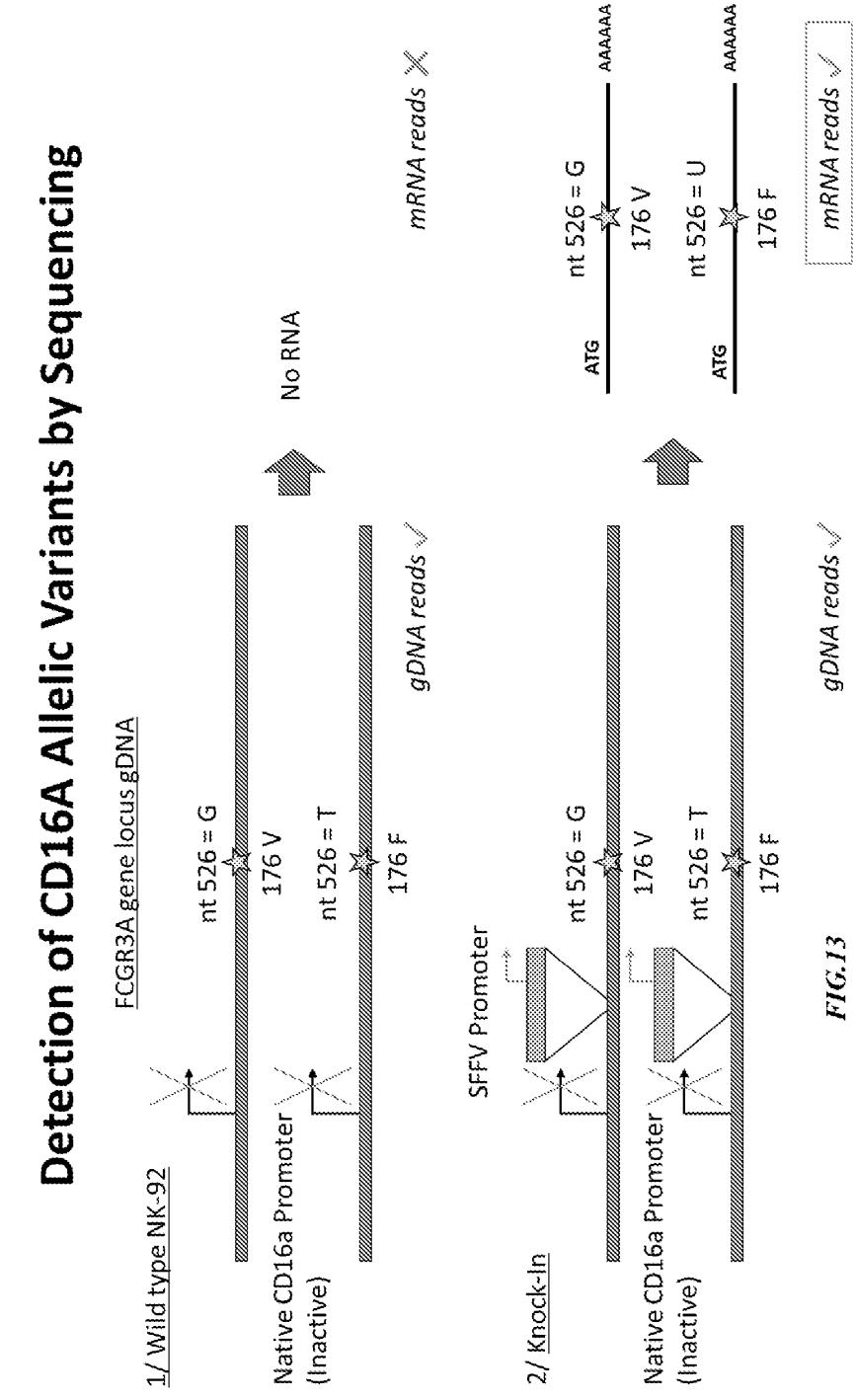

Detection of CD16A Allelic Variants by Sequencing
1/ Wild type NK-92
FCGR3A gene locus gDNA
nt 526 = G
176 V
nt 526 = T
176 F
Native CD16a Promoter
(Inactive)
No RNA
mRNA reads ✗
gDNA reads ✓
2/ Knock-In
SFFV Promoter
nt 526 = G
176 V
nt 526 = T
176 F
Native CD16a Promoter
(Inactive)
ATG
AAAAAA
nt 526 = G
176 V
nt 526 = U
176 F
mRNA reads ✓
gDNA reads ✓

FIG.13

Determination of CD16A allelic variations in NK-92 cells (gDNA seq)

Sample	Analyte	Date	Reference Allele Support	Alternate Allele Support	Alt. Allele Frequency (95% CI)
NK-92	WGS DNA	1-Jun-15	48	34	**41.5** (30.7, 52.9)
NK-92.C (= ATCC)	WGS DNA	11-Sep-15	25	24	**49.0** (34.4, 63.7)
NK-92 WT (= aNK)	WGS DNA	11-Sep-15	23	18	**43.9** (28.5, 60.3)
NK-92 FcR-IL2ER/haNK	WGS DNA	11-Sep-15	25	15	37.5 (22.7, 54.2)
Normal PBMCs DNA	WGS DNA	20-Aug-16	5	29	**85.3** (68.9, 95.0)

Reference Allele = CD16 158V

Alternate Allele = CD16 158F

NK-92 = ~50% F vs. V

FIG.14

Determination of CD16A allelic variations in NK-92 cells (bulk RNAseq)

Reference sequence = CD16 158F			1C9			3B2			4G5			NK-92.CD16 176V		
Nucleotide Position	Reference Allele	Variant Allele	Variant Count	Coverage	Variant Frequency	Variant Count	Coverage	Variant Frequency	Variant Count	Coverage	Variant Frequency	Variant Count	Coverage	Variant Frequency
197	T	A	107	108	99.07%	211	212	99.53%	135	137	98.54%	n.d	n.d	n.d
526	**T**	**G**	**58**	**59**	98.31%	**150**	**152**	98.68%	**78**	**78**	100%	**417**	**421**	99.05%

Nucleotide position 526 = 158F -> V mutation

n.d. = Not Determined

All clones appear to only express the CD16 158V variant

FIG.15

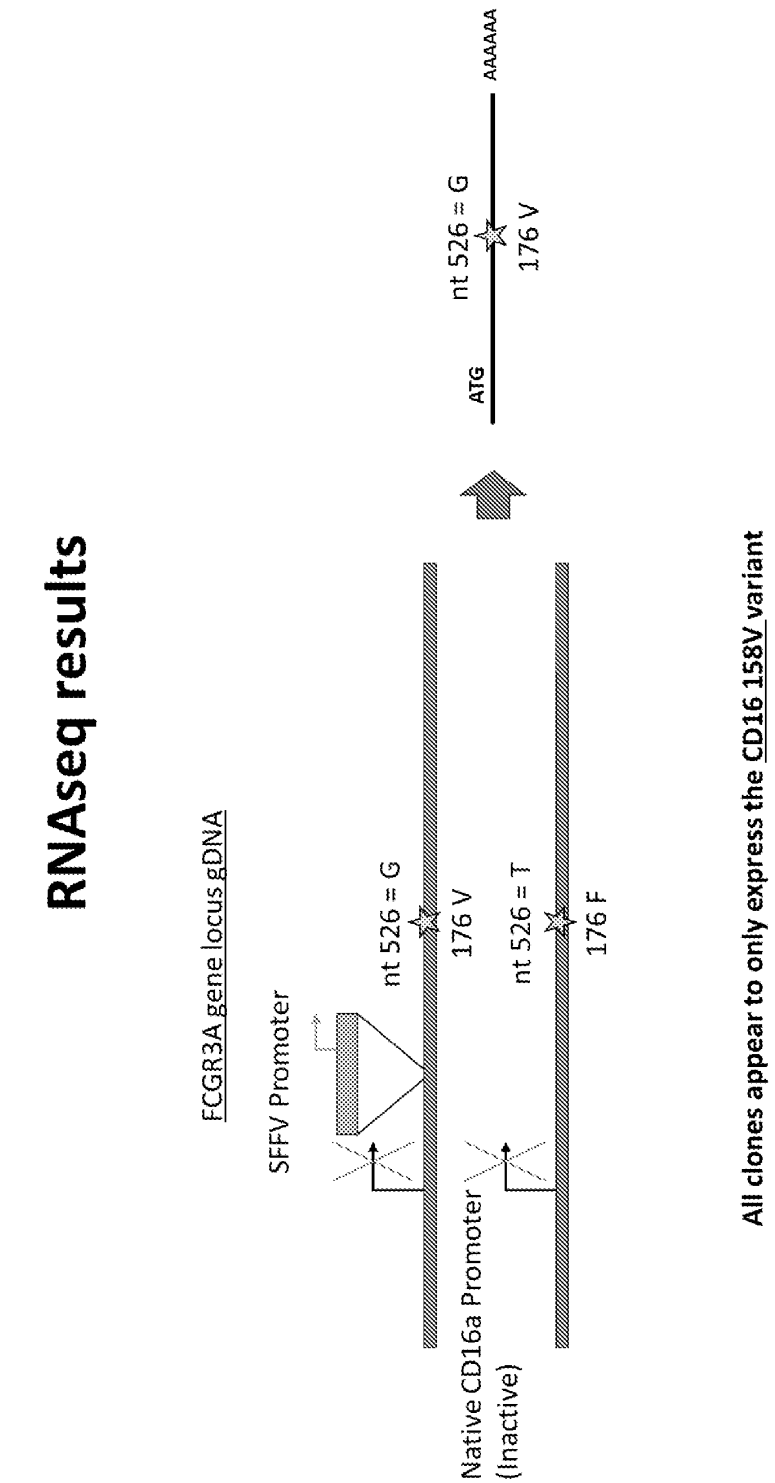

RNAseq results
FCGR3A gene locus gDNA
SFFV Promoter
nt 526 = G
176 V
nt 526 = T
176 F
Native CD16a Promoter
(Inactive)
ATG
nt 526 = G
176 V
AAAAA
All clones appear to only express the CD16 158V variant

FIG.16

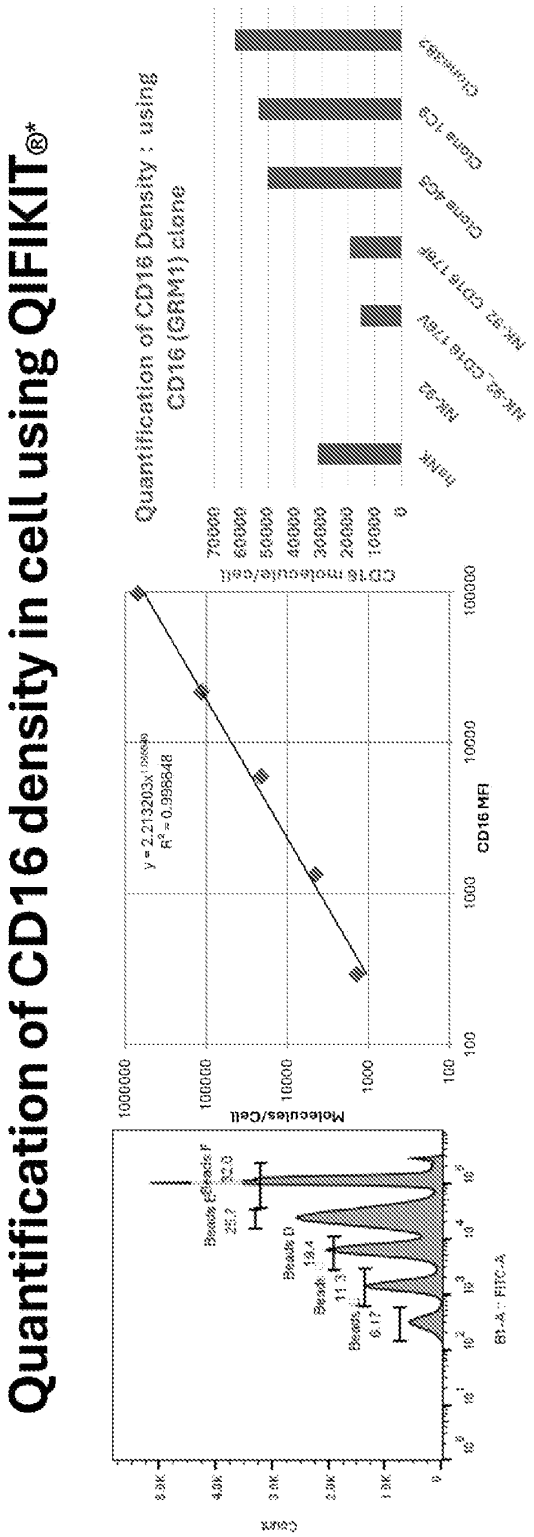

Quantification of CD16 density in cell using QIFIKIT®*
Quantification of CD16 Density : using CD16 (GRM1) clone
CD16 molecule/cell
70000
60000
50000
40000
30000
20000
10000
0
NK-92
NK-92_CD16 176V
NK-92_CD16 176F
Clone 2G3
Clone 1D3
Clone 3G8
Molecules/Cell
1000000
100000
10000
1000
100
y = 2.213203x
R² = 0.998648
CD16 MFI
100
1000
10000
100000
Beads F
Beads E
Beads D
Beads C
Beads B
25.2
82.3
18.4
11.3
6.17
B1-A :: FITC-A

FIG.17

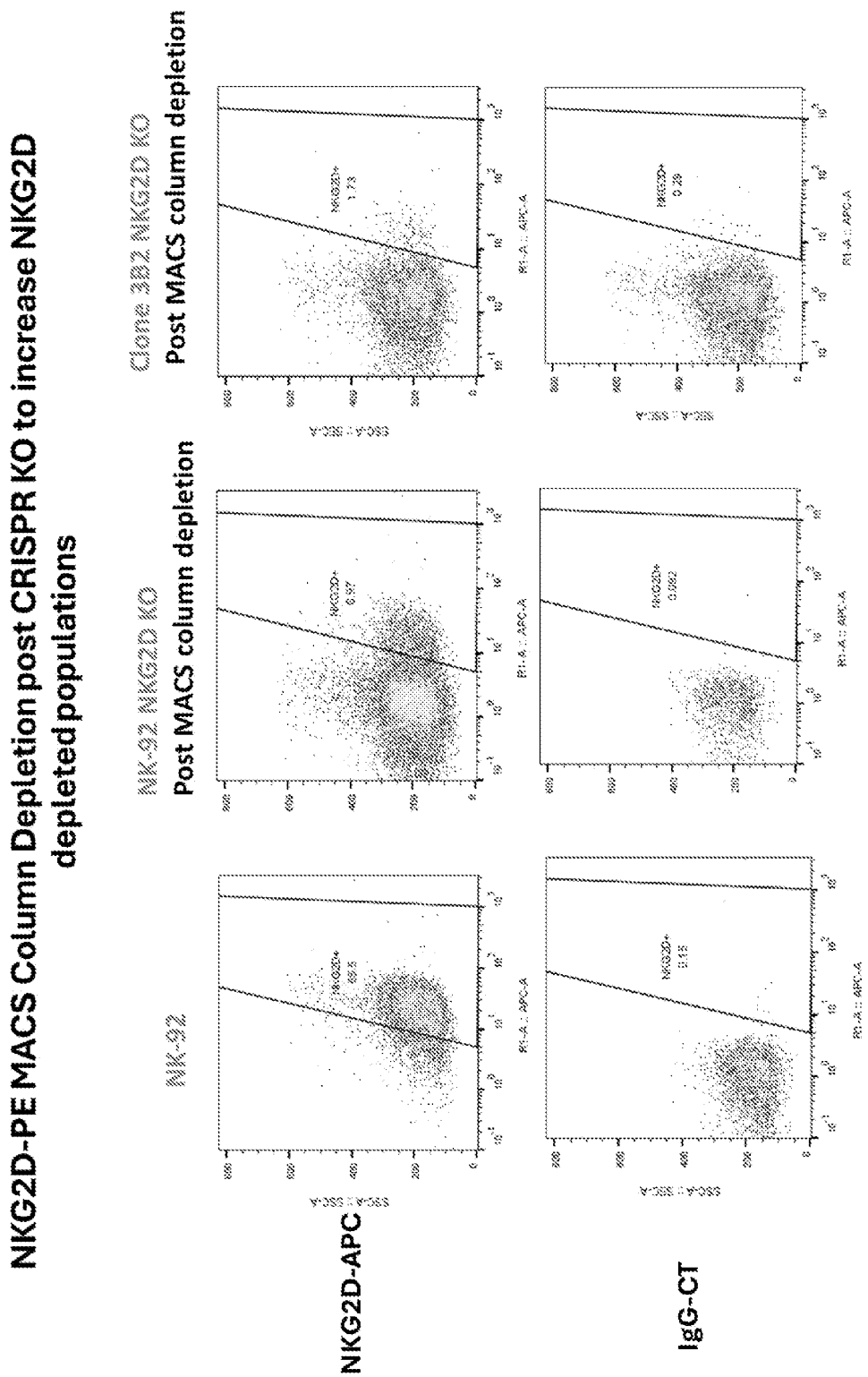

NKG2D-PE MACS Column Depletion post CRISPR KO to increase NKG2D depleted populations
NK-92
NK-92 NKG2D KO
Post MACS column depletion
Clone 3B2 NKG2D KO
Post MACS column depletion
NKG2D-APC
IgG-CT

FIG.18

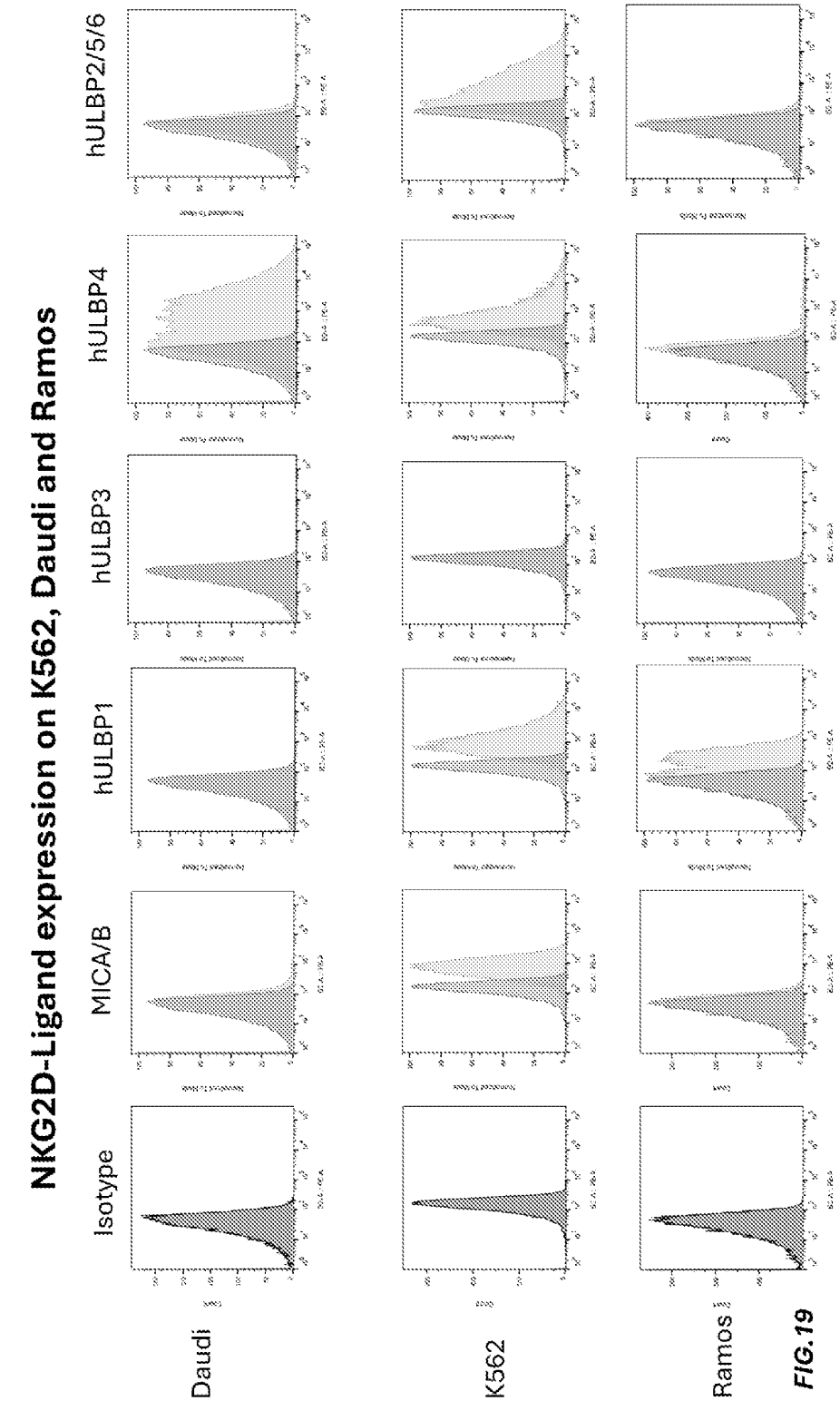

NKG2D-Ligand expression on K562, Daudi and Ramos
Isotype
MICA/B
hULBP1
hULBP3
hULBP4
hULBP2/5/6
Daudi
K562
Ramos

FIG.19

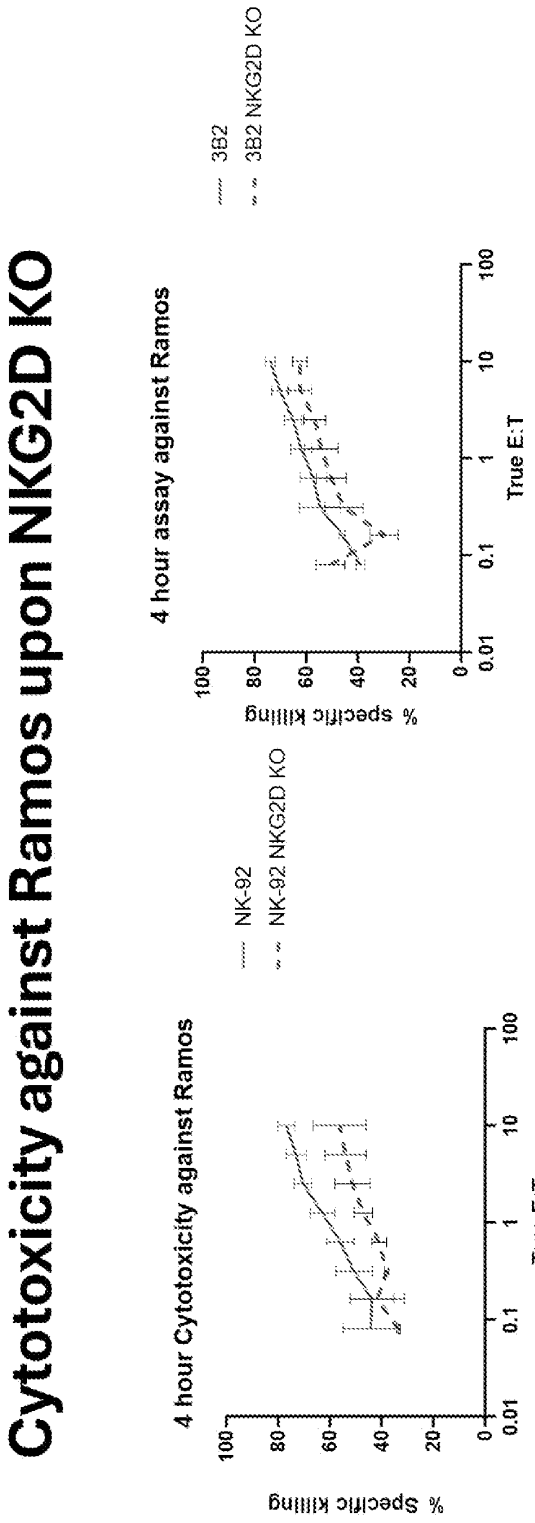

Cytotoxicity against Ramos upon NKG2D KO
4 hour Cytotoxicity against Ramos
% Specific killing
True E:T
NK-92
NK-92 NKG2D KO
4 hour assay against Ramos
% specific killing
True E:T
3B2
3B2 NKG2D KO

FIG.20

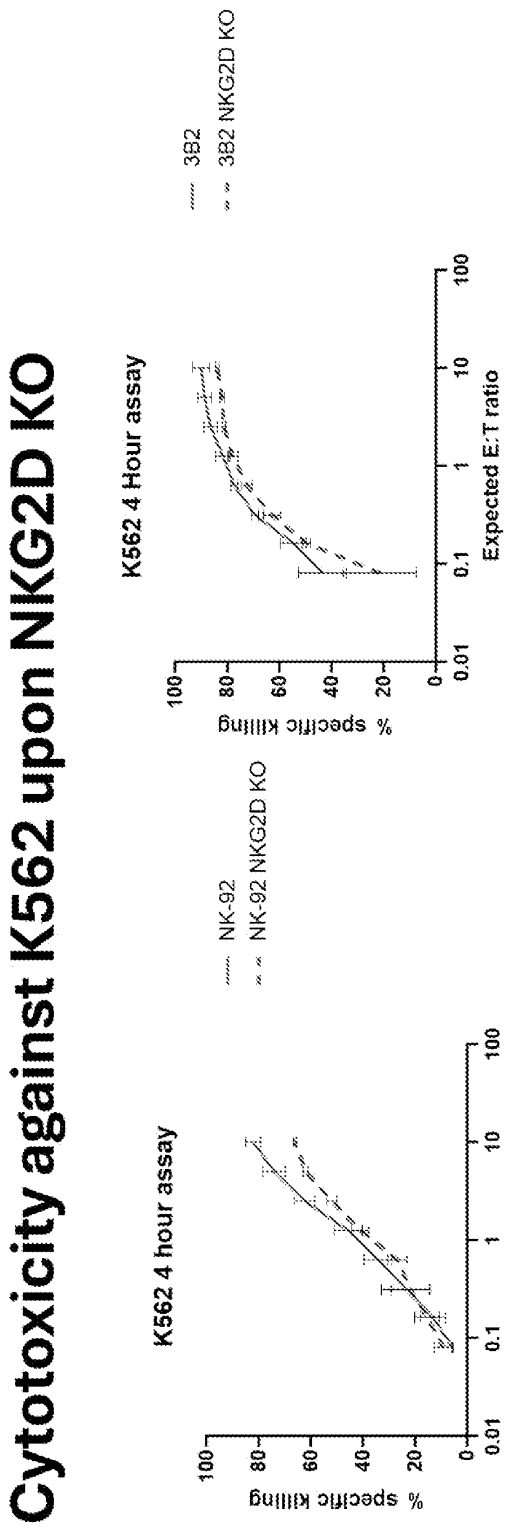

Cytotoxicity against K562 upon NKG2D KO
K562 4 hour assay
% specific killing
Expected E:T ratio
NK-92
NK-92 NKG2D KO
N=3
K562 4 Hour assay
% specific killing
Expected E:T ratio
3B2
3B2 NKG2D KO
N=3

FIG.21

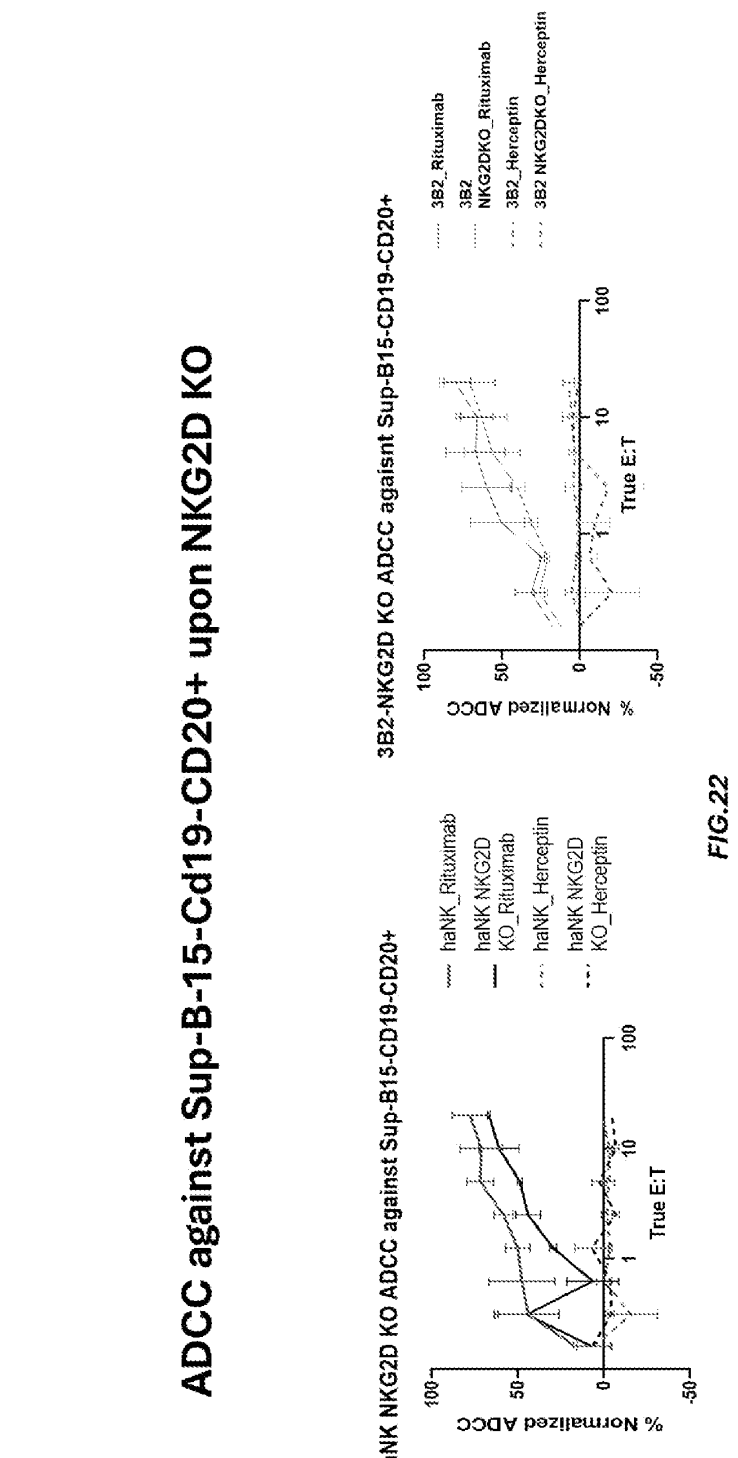
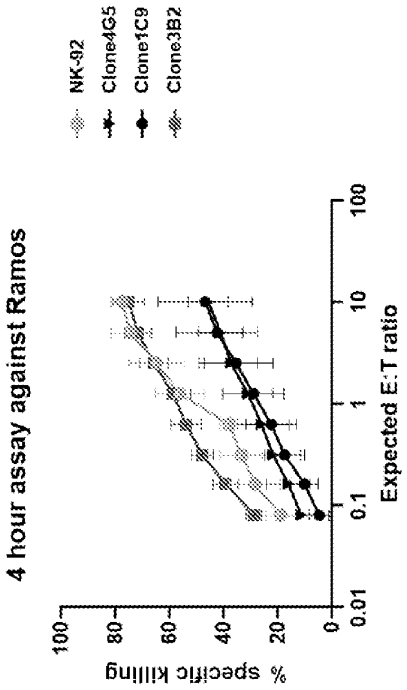

ADCC against Sup-B-15-Cd19-CD20+ upon NKG2D KO
haNK NKG2D KO ADCC against Sup-B15-CD19-CD20+
% Normalized ADCC
True E:T
haNK_Rituximab
haNK NKG2D KO_Rituximab
haNK_Herceptin
haNK NKG2D KO_Herceptin
3B2-NKG2D KO ADCC agaisnt Sup-B15-CD19-CD20+
% Normalized ADCC
True E:T
3B2_Rituximab
3B2 NKG2DKO_Rituximab
3B2_Herceptin
3B2 NKG2DKO_Herceptin

FIG.22

Cytotoxicity Characterization against K562, Ramos



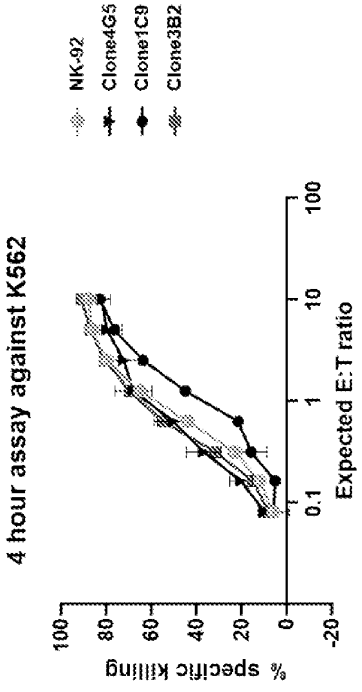


FIG.23