FIGURES

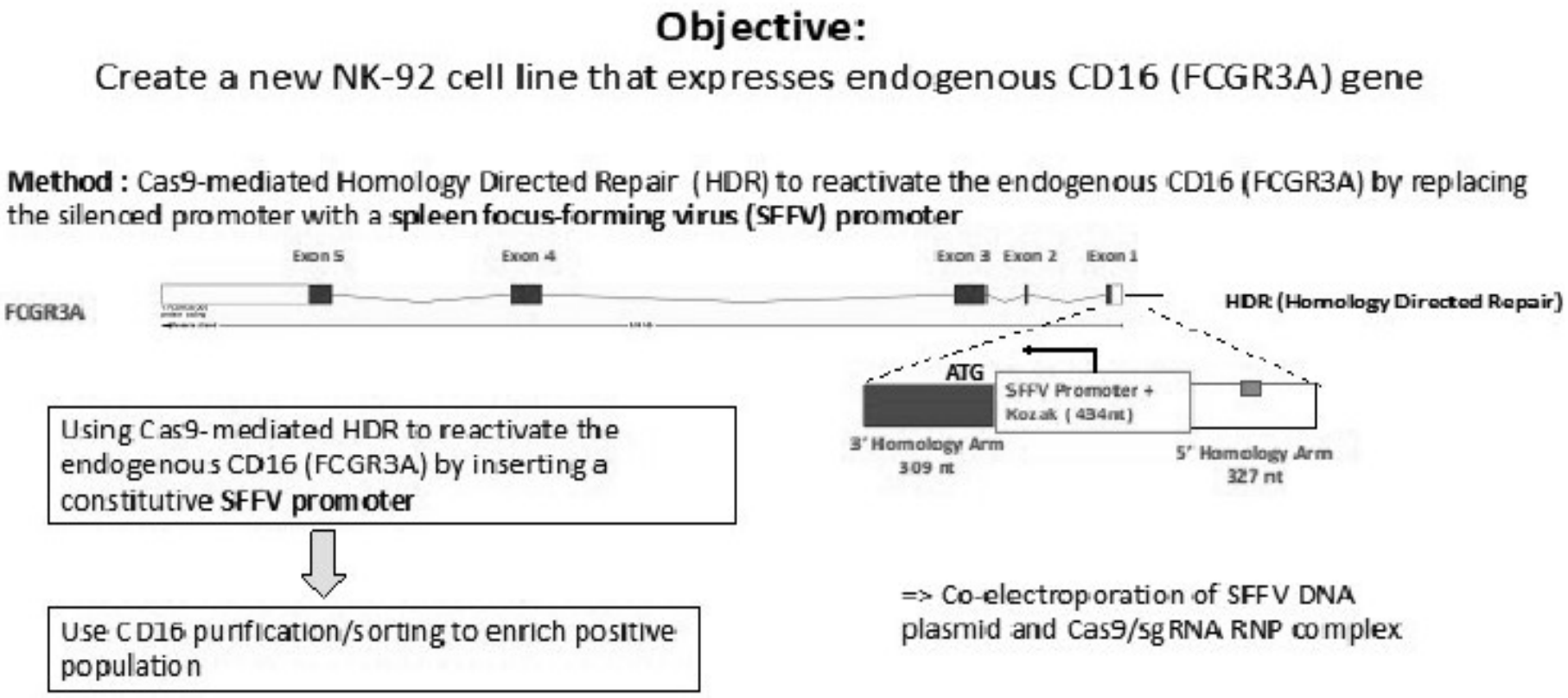


FIG.1

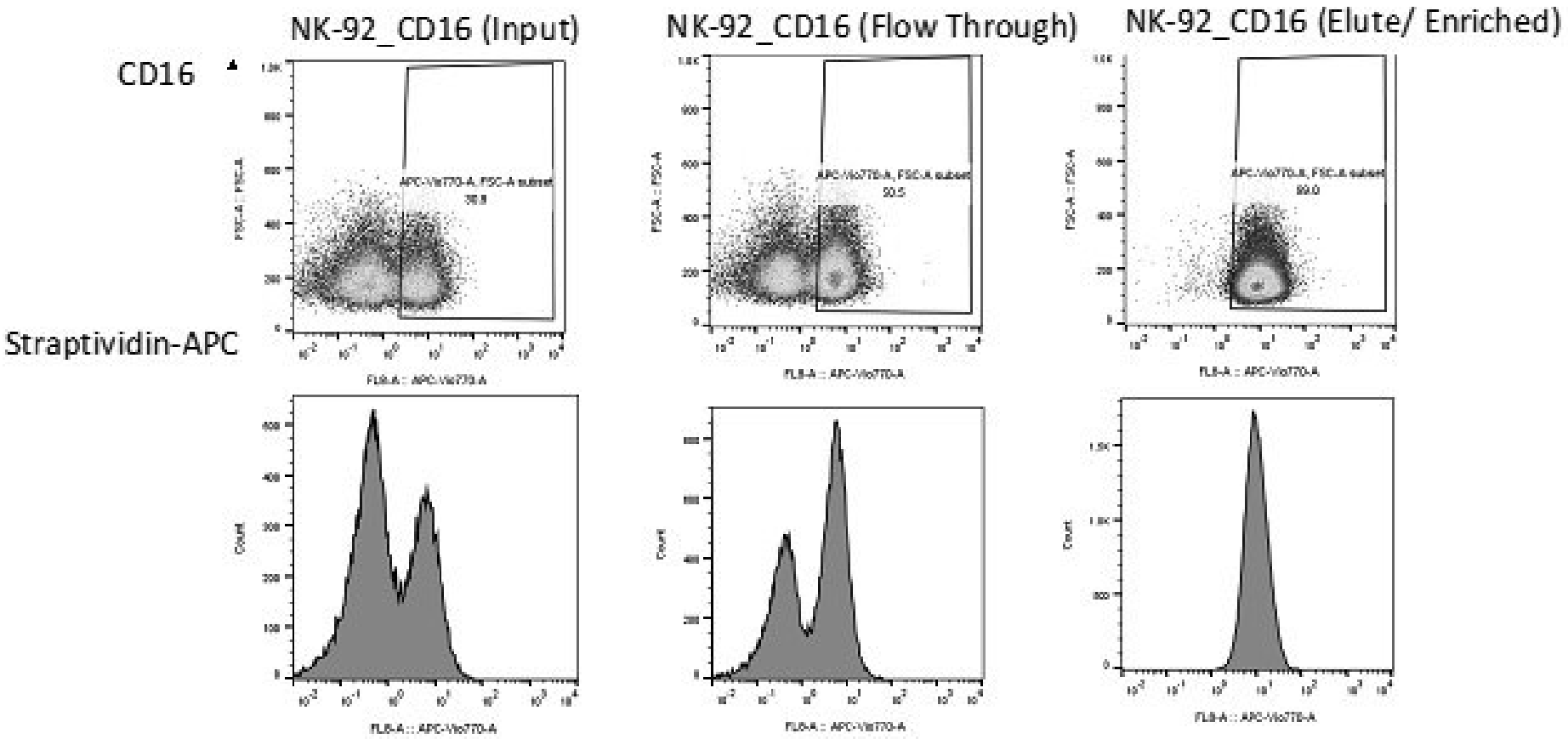

NK-92_CD16 (Input)
NK-92_CD16 (Flow Through)
NK-92_CD16 (Elute/ Enriched)
CD16
Straptividin-APC
Count
FL8-A :: APC-Vio770-A

FIG.2

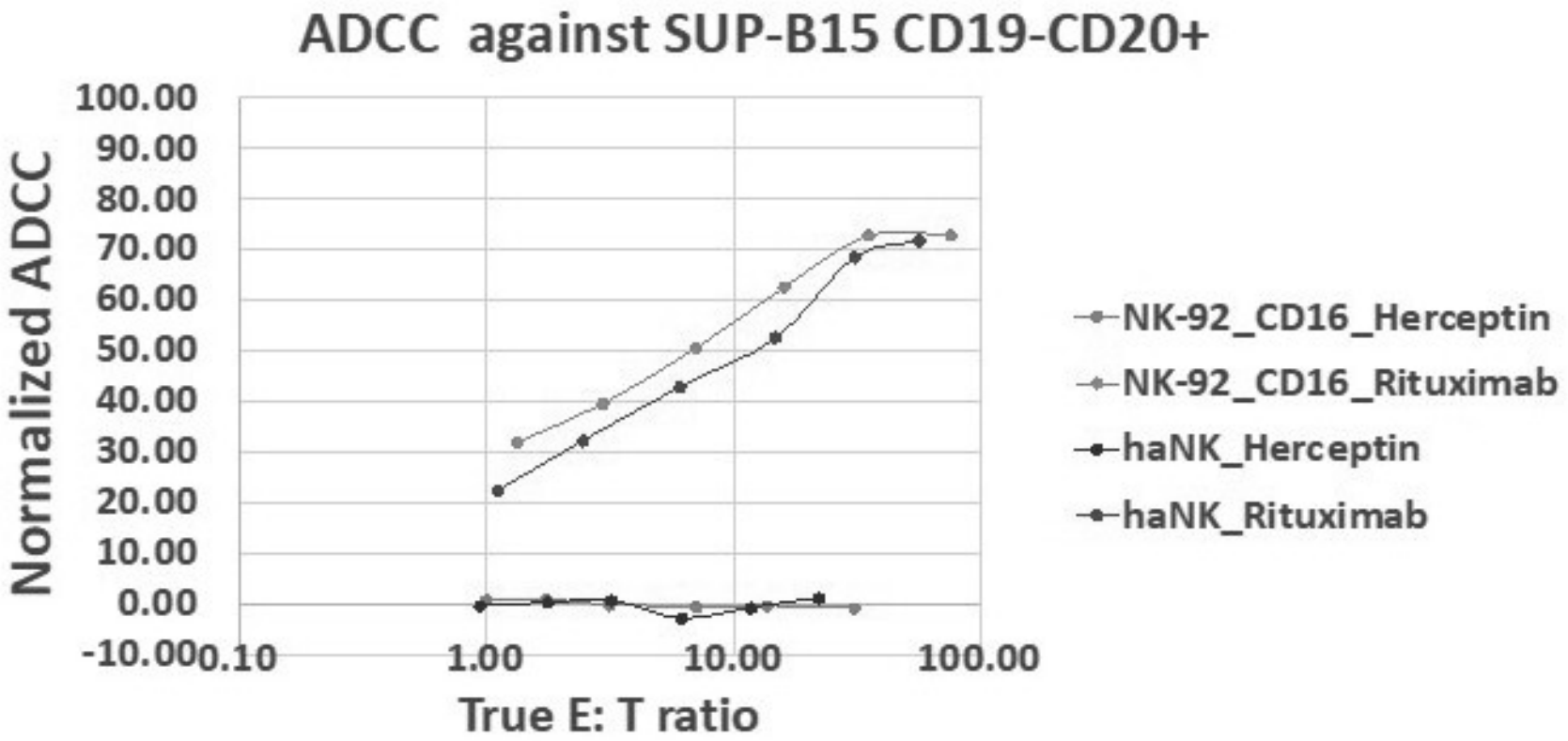

ADCC against SUP-B15 CD19-CD20+
Normalized ADCC
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
-10.00
0.10
1.00
10.00
100.00
True E: T ratio
NK-92_CD16_Herceptin
NK-92_CD16_Rituximab
haNK_Herceptin
haNK_Rituximab

FIG.3

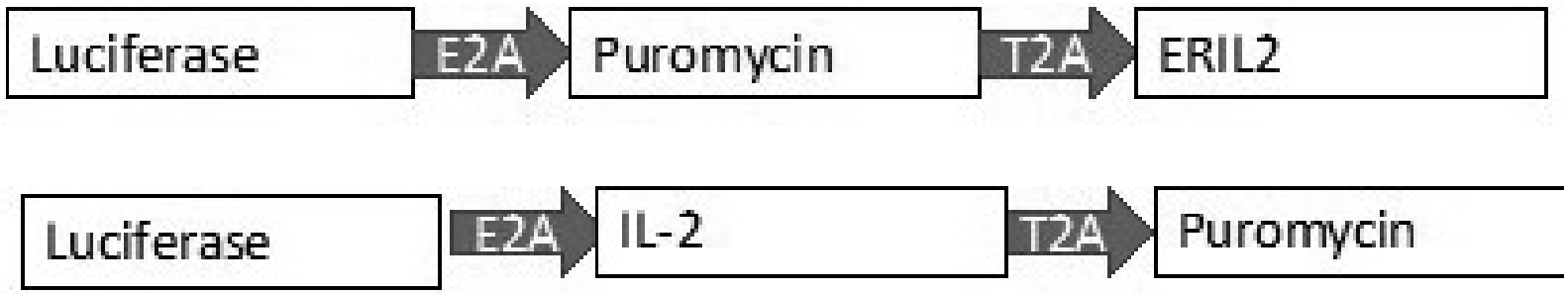

Luciferase
E2A
Puromycin
T2A
ERIL2
Luciferase
E2A
IL-2
T2A
Puromycin

FIG.4

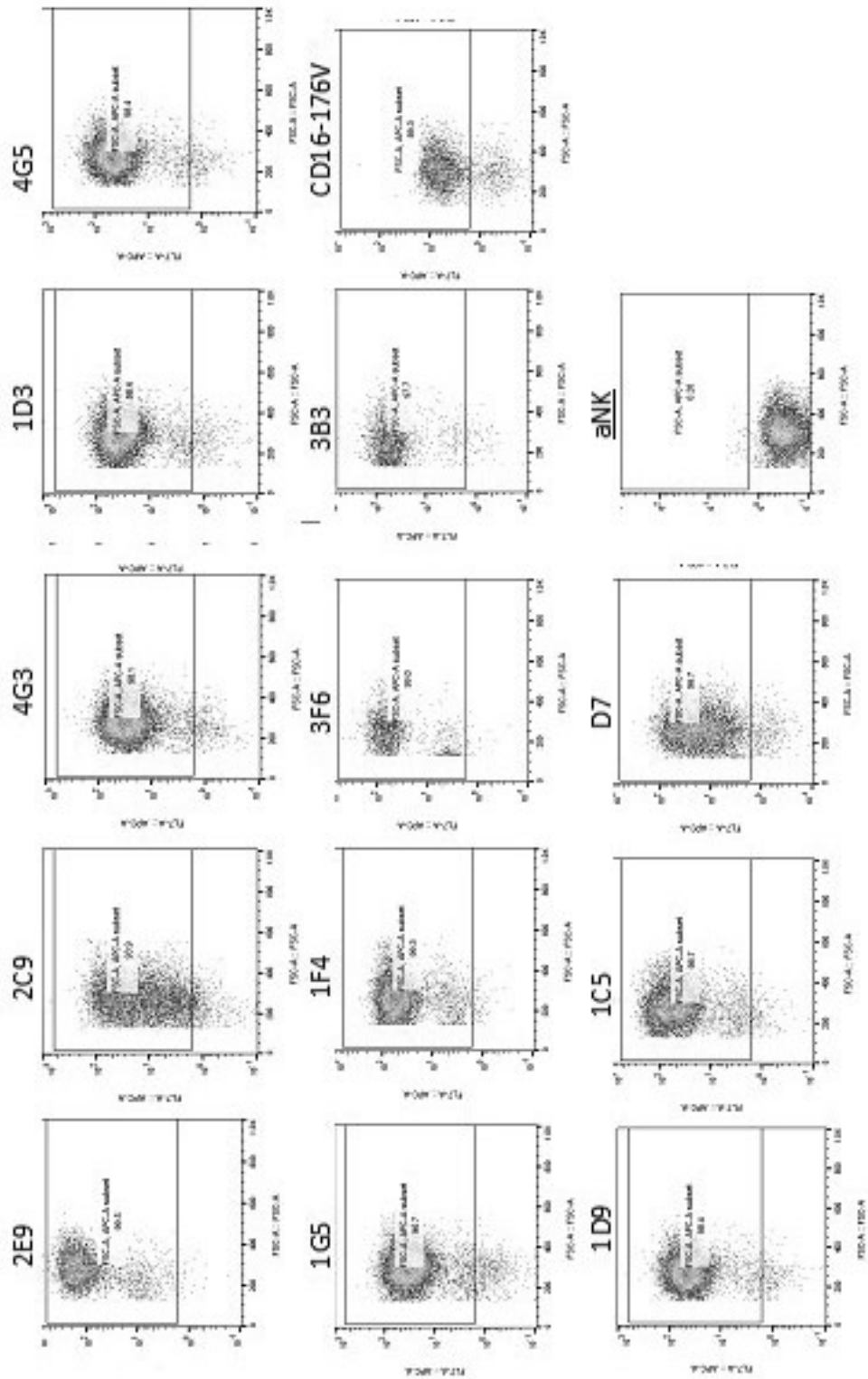


FIG.5

Cytotoxicity of IL-2 expressing NK-92_CD16 IL-2 IL-2/ERIL-2 clones

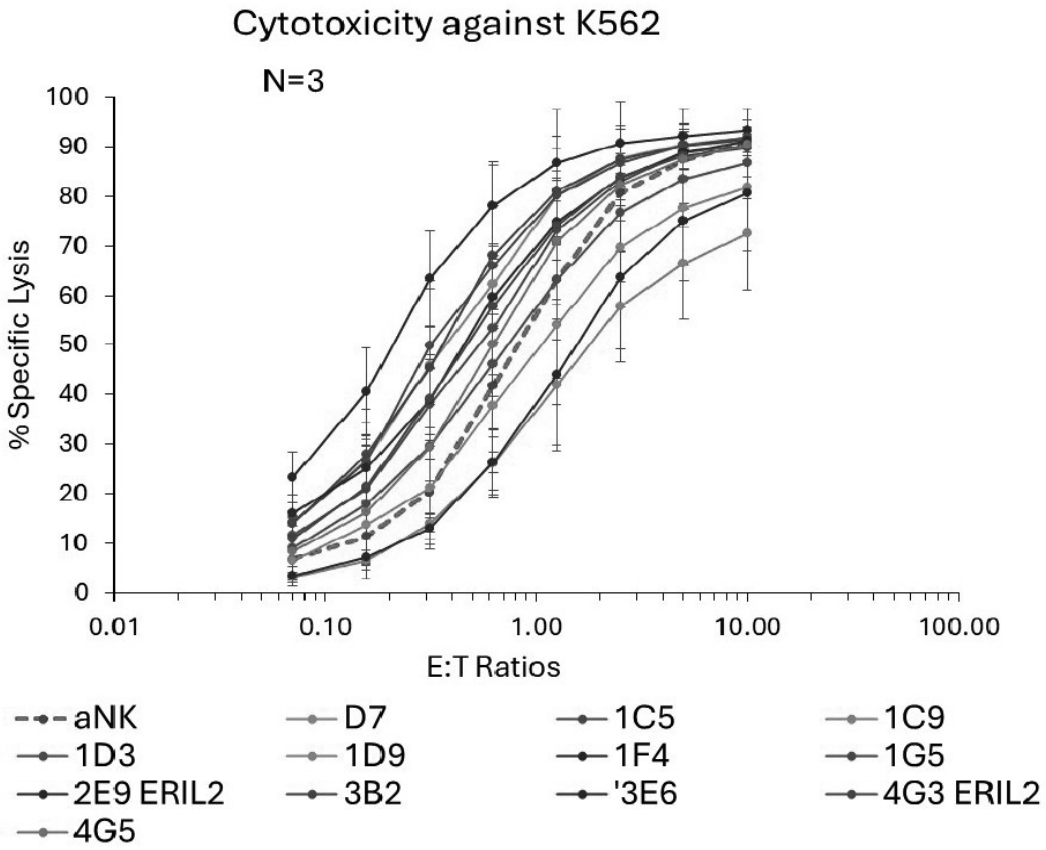


FIG.6

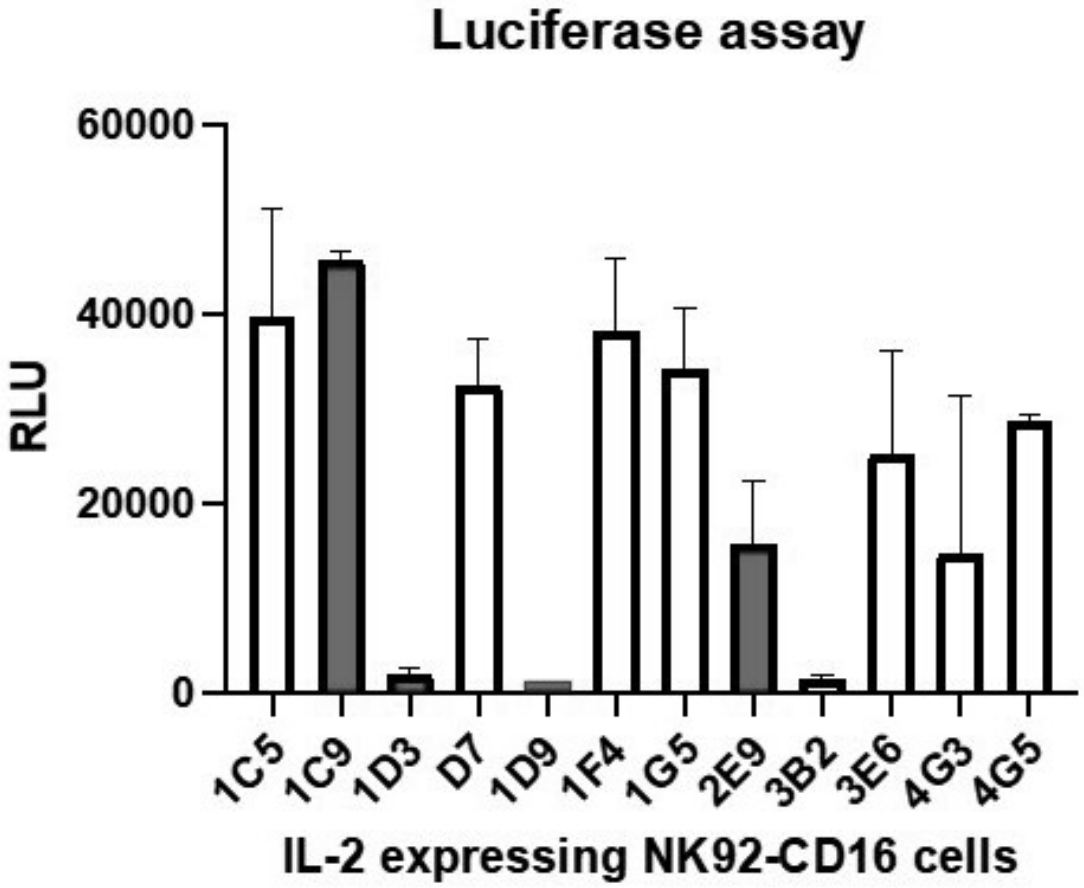

Luciferase assay
RLU
60000
40000
20000
0
1C5
1C9
1D3
D7
1D9
1F4
1G5
2E9
3B2
3E6
4G3
4G5
IL-2 expressing NK92-CD16 cells

FIG. 7

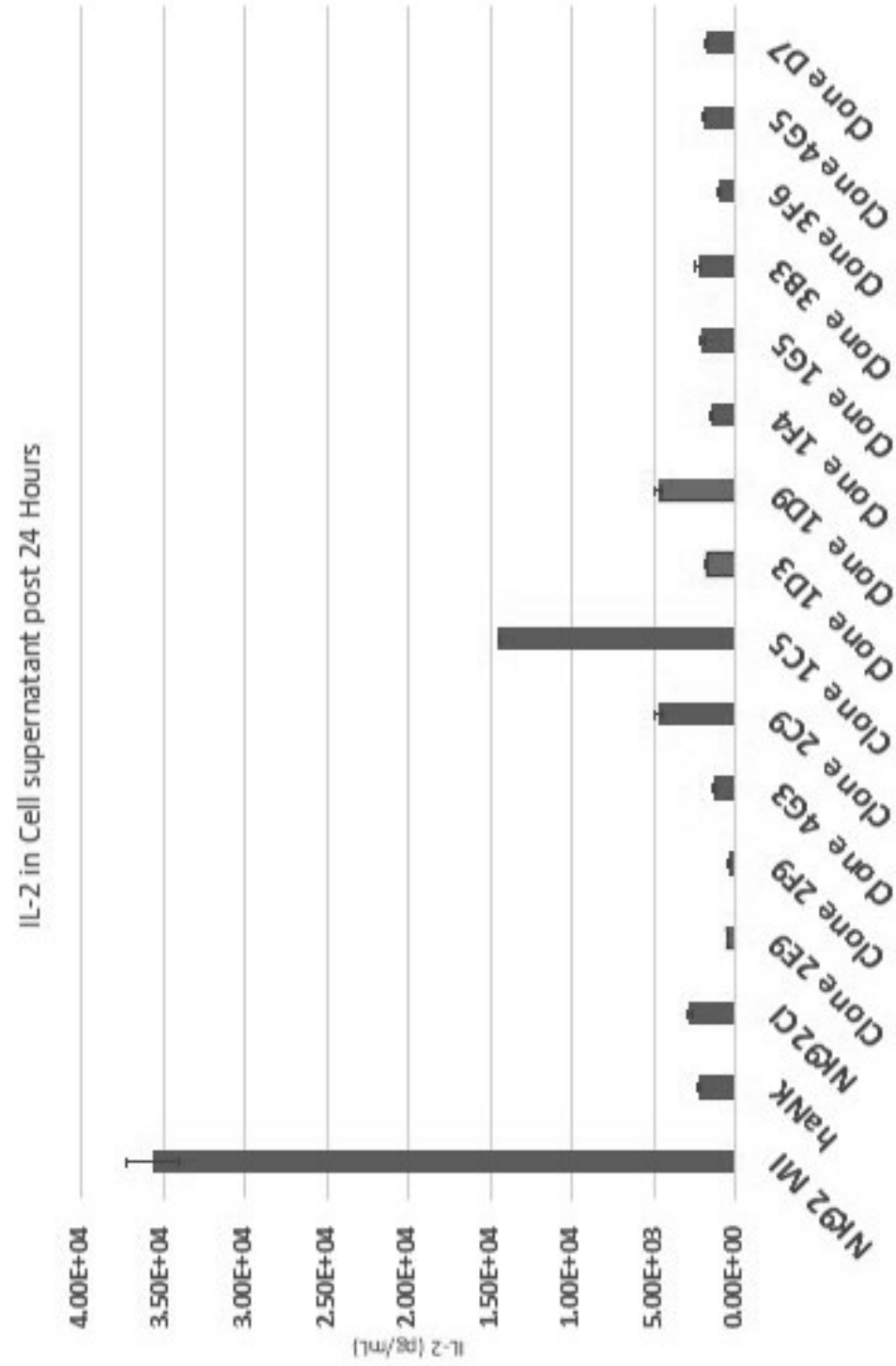

IL-2 in Cell supernatant post 24 Hours
IL-2 (pg/mL)
4.00E+04
3.50E+04
3.00E+04
2.50E+04
2.00E+04
1.50E+04
1.00E+04
5.00E+03
0.00E+00
NK92 MI
haNk
NK92CI
Clone 2E9
Clone 2F9
Clone 4G3
Clone 2C9
Clone 1C5
Clone 1D3
Clone 1D9
Clone 1F4
Clone 1G5
Clone 3B3
Clone 3F6
Clone 4G5
Clone D7

FIG.8

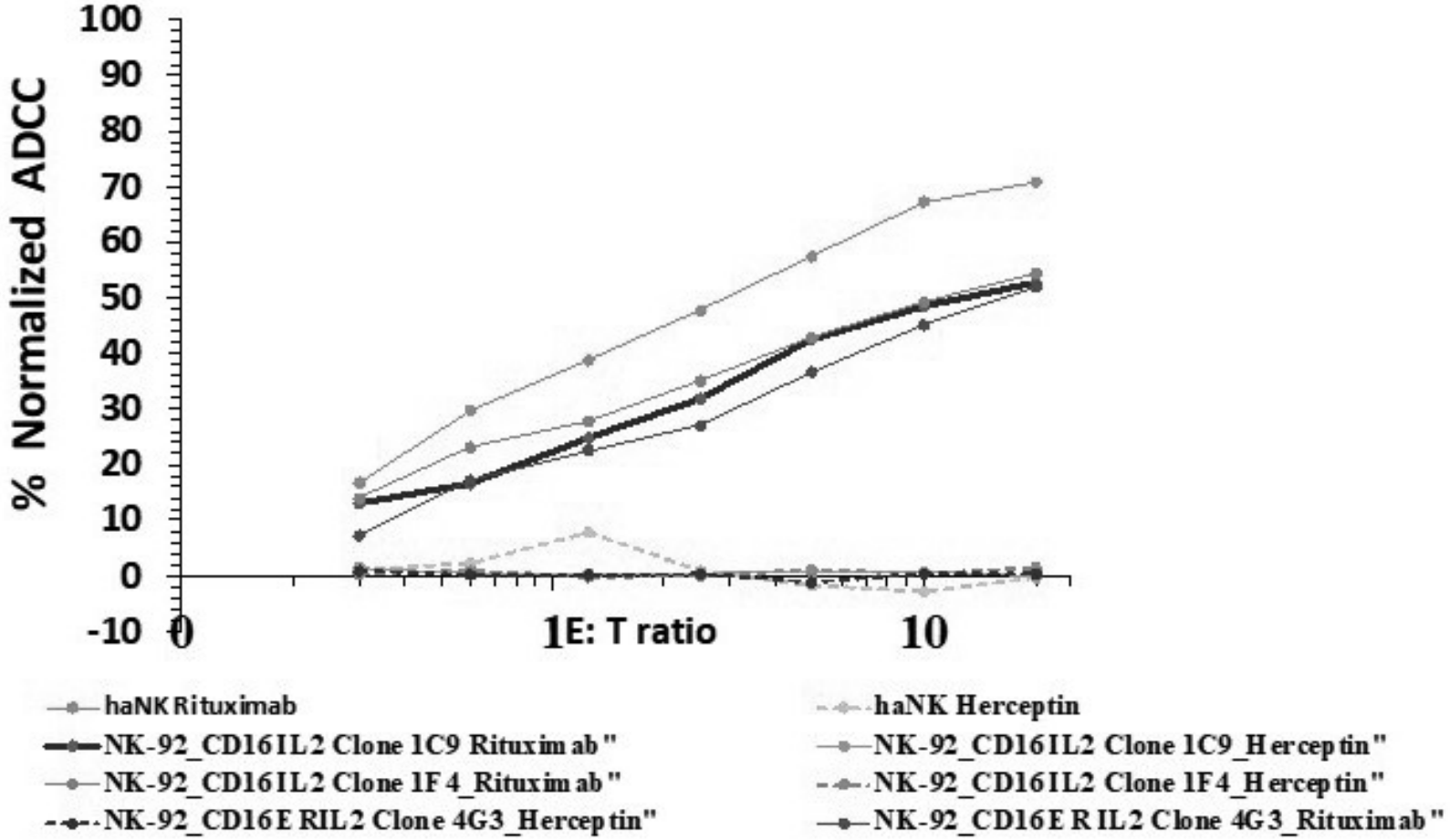
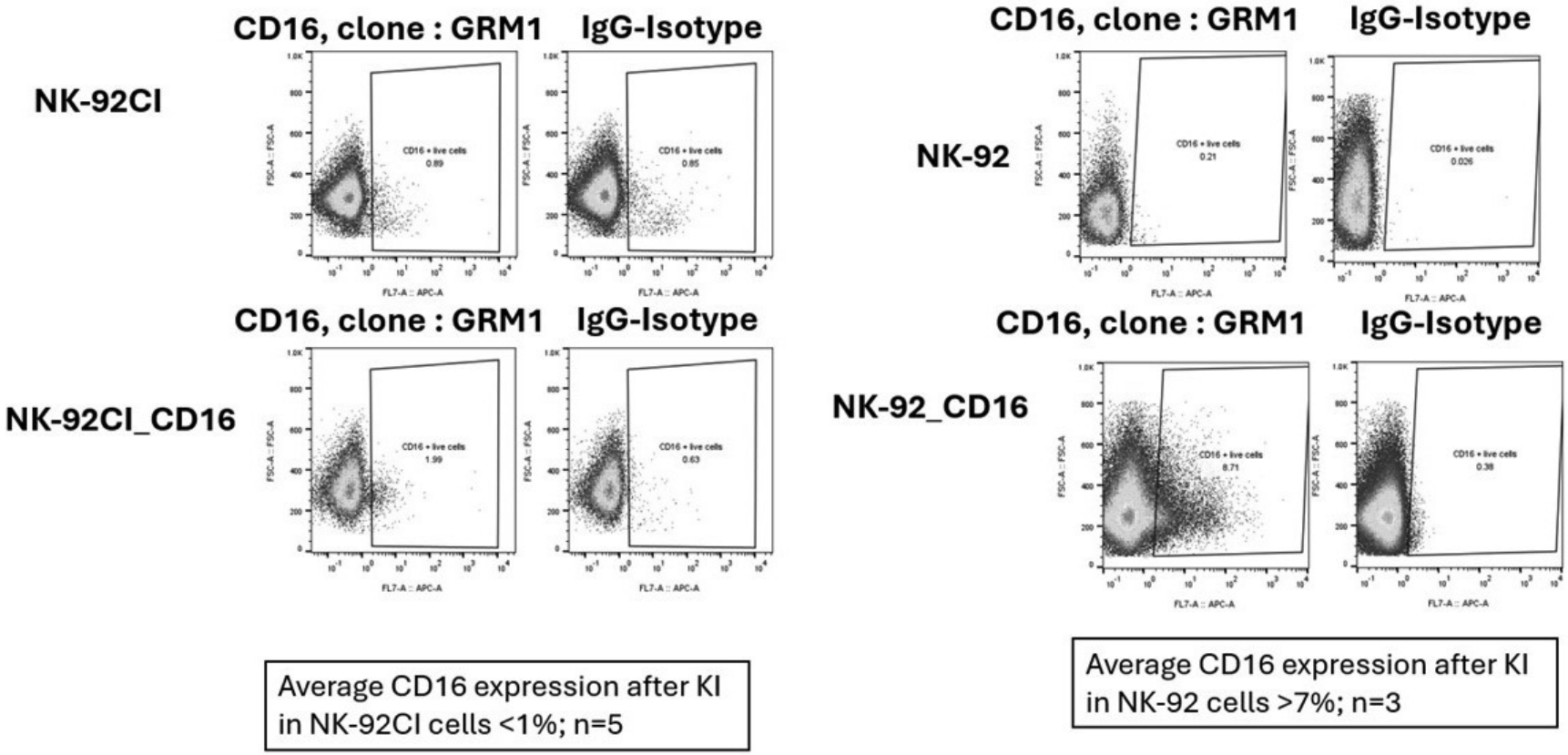

% Normalized ADCC
100
90
80
70
60
50
40
30
20
10
0
-10
0
1E: T ratio
10
haNK Rituximab
haNK Herceptin
NK-92_CD16IL2 Clone 1C9 Rituximab"
NK-92_CD16IL2 Clone 1C9_Herceptin"
NK-92_CD16IL2 Clone 1F4_Rituximab"
NK-92_CD16IL2 Clone 1F4_Herceptin"
NK-92_CD16ERIL2 Clone 4G3_Herceptin"
NK-92_CD16ERIL2 Clone 4G3_Rituximab"

FIG.9

Endogenous SFFV Promoter knock in attempts in NK-92CI and NK-92 cells

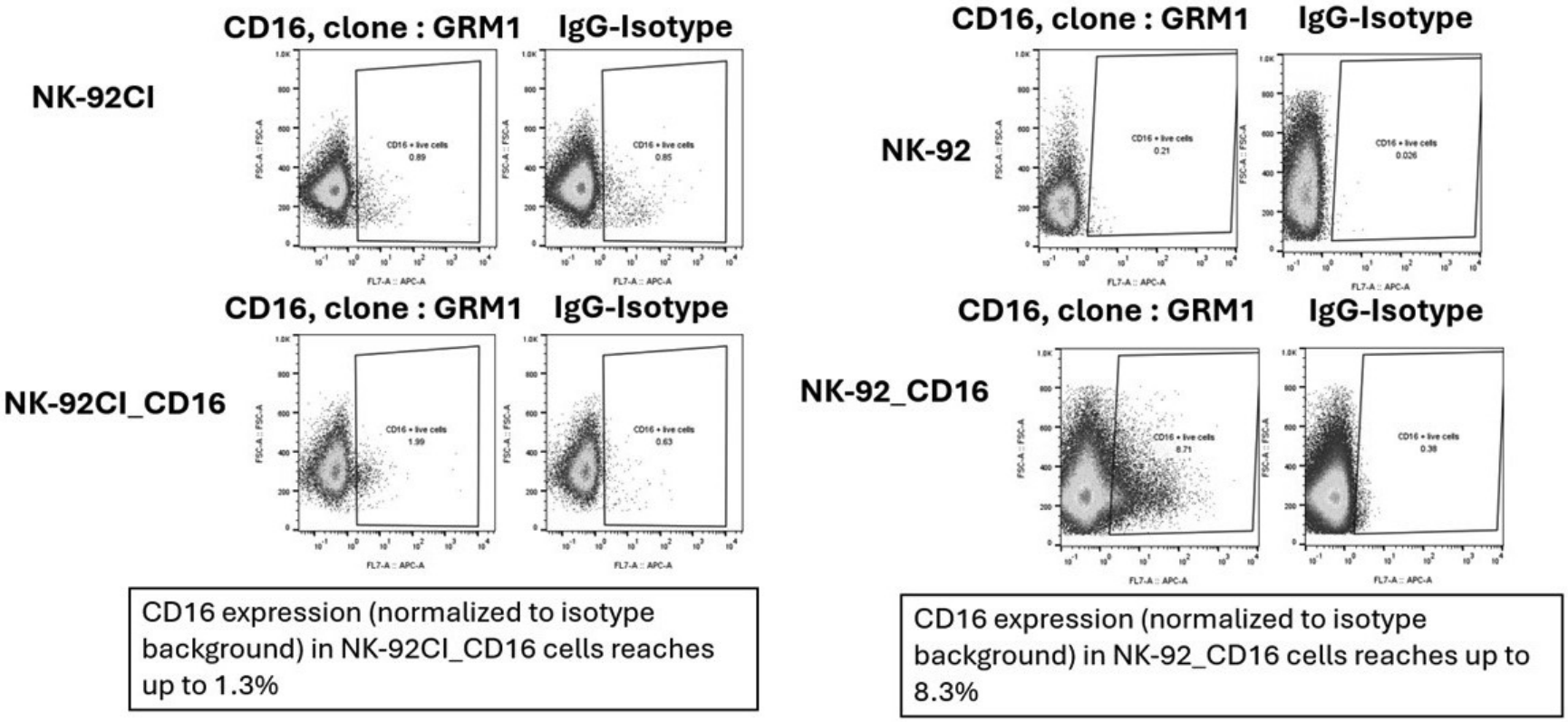


Average CD16 expression after KI in NK-92CI cells <1%; n=5

Average CD16 expression after KI in NK-92 cells >7%; n=3

FIG.10

Endogenous SFFV Promoter knock in attempts in NK-92CI and NK-92 cells

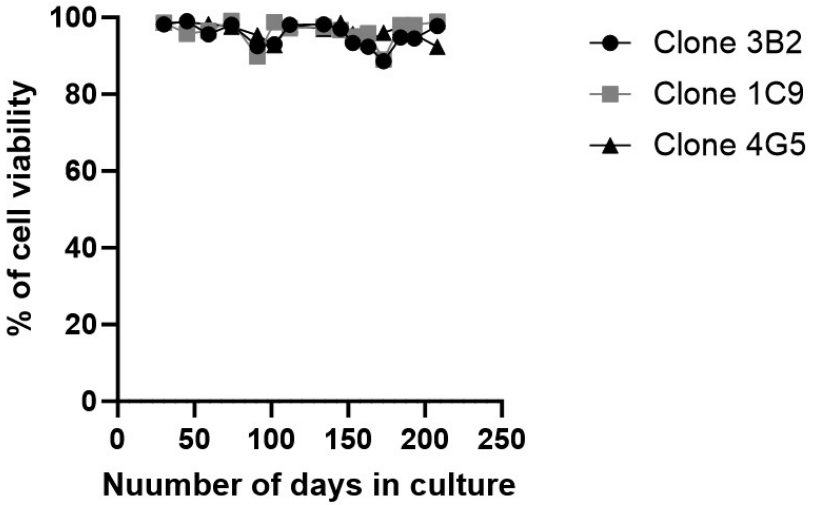


CD16 expression (normalized to isotype background) in NK-92CI_CD16 cells reaches up to 1.3%

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



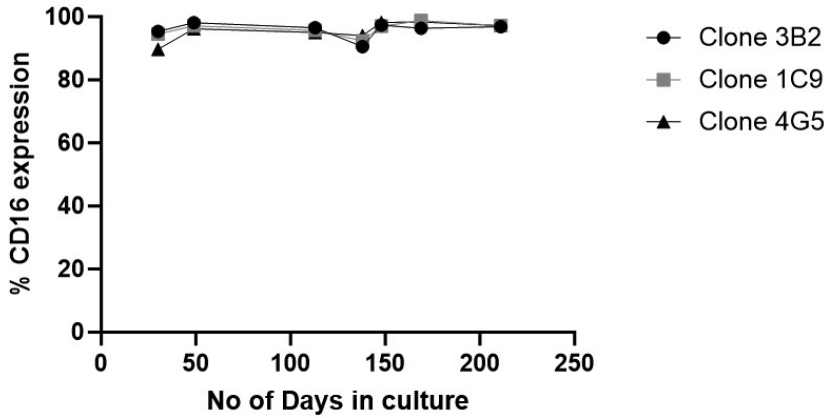


FIG.12

Detection of CD16A Allelic Variants by Sequencing

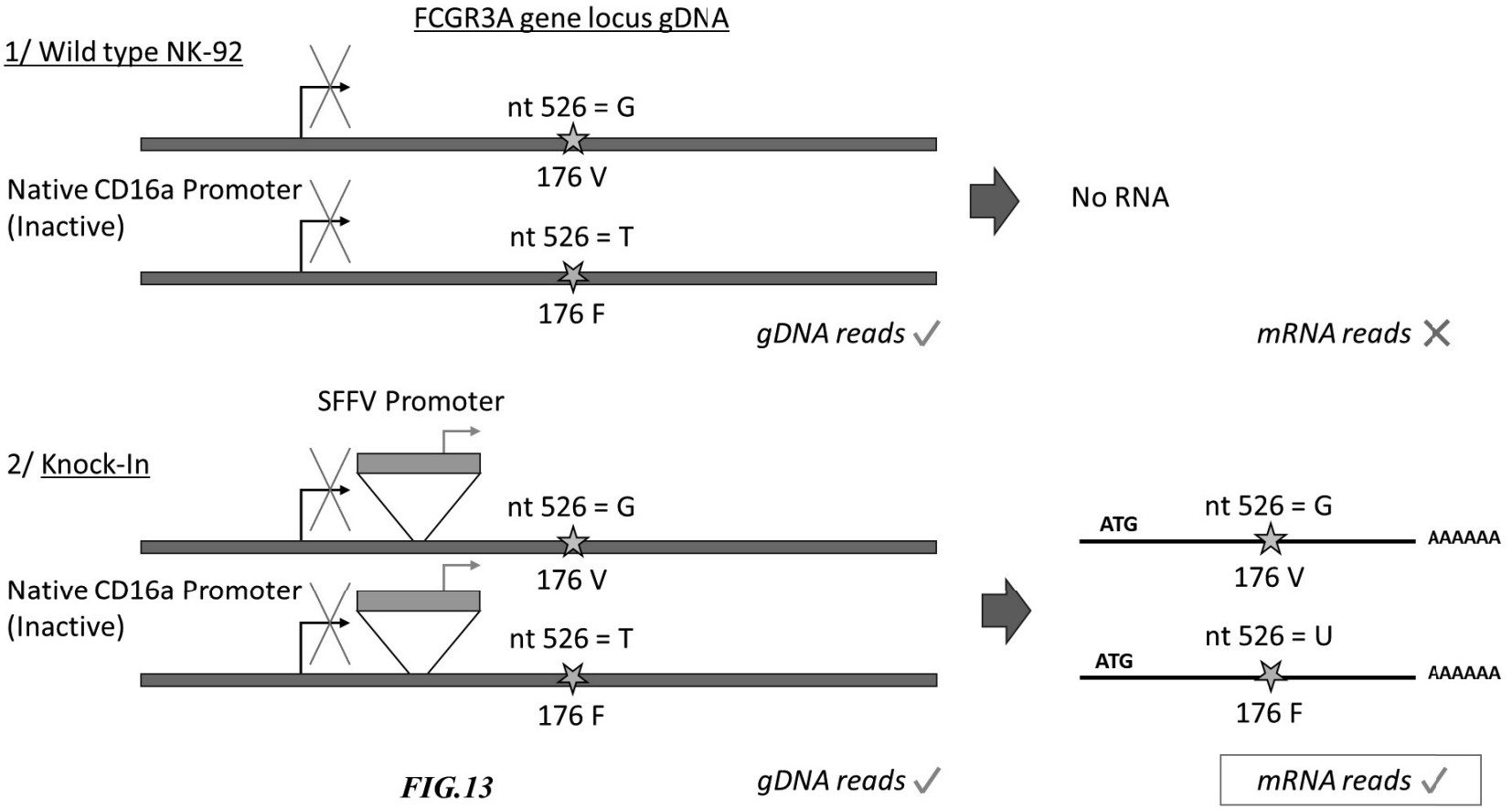


FIG.13

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%**

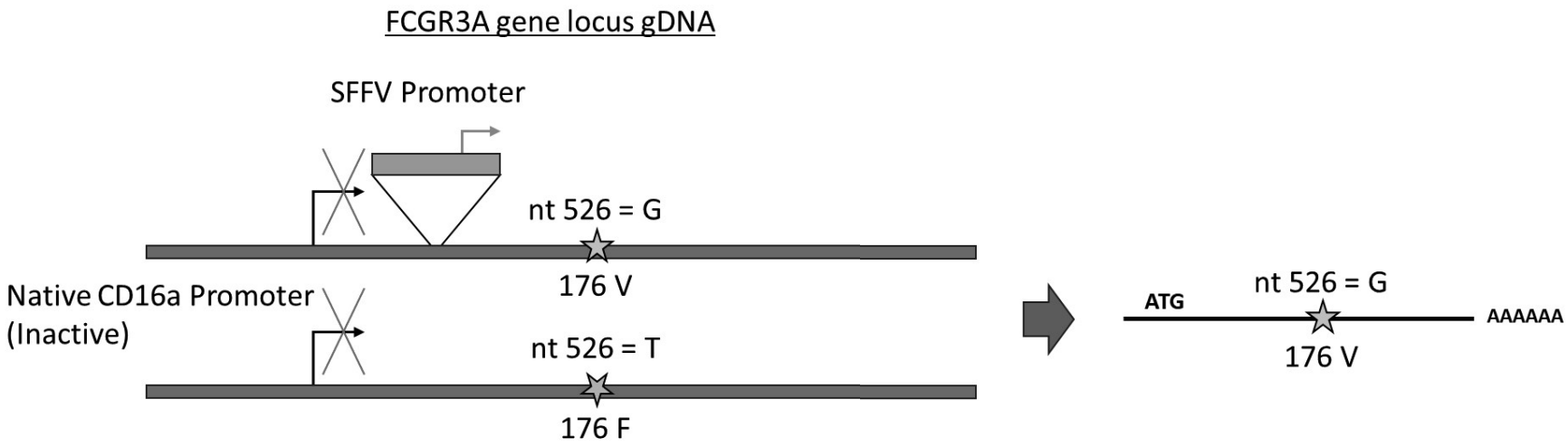
Nucleotide position 526 = 158F -> V mutation

n.d. = Not Determined

All clones appear to only express the CD16 158V variant

FIG.15

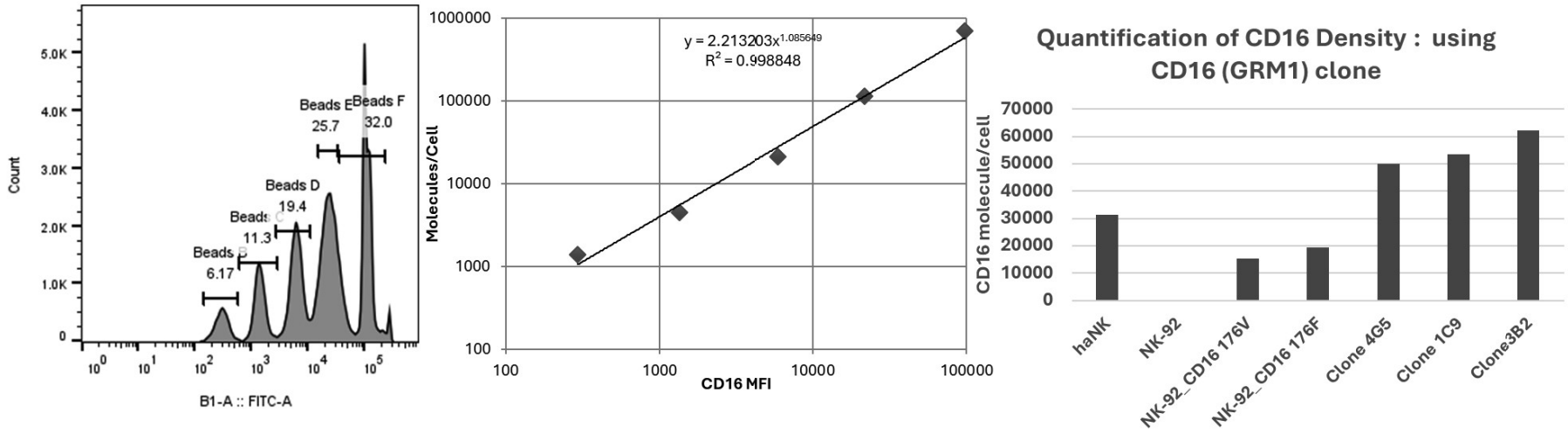
RNAseq results



All clones appear to only express the CD16 158V variant

FIG.16

Quantification of CD16 density in cell using QIFIKIT®*



PJ2024-04-09Cakibration beadsH1.0001.fcs
singlets
129099

FIG.17

NKG2D-PE MACS Column Depletion post CRISPR KO to increase NKG2D depleted populations

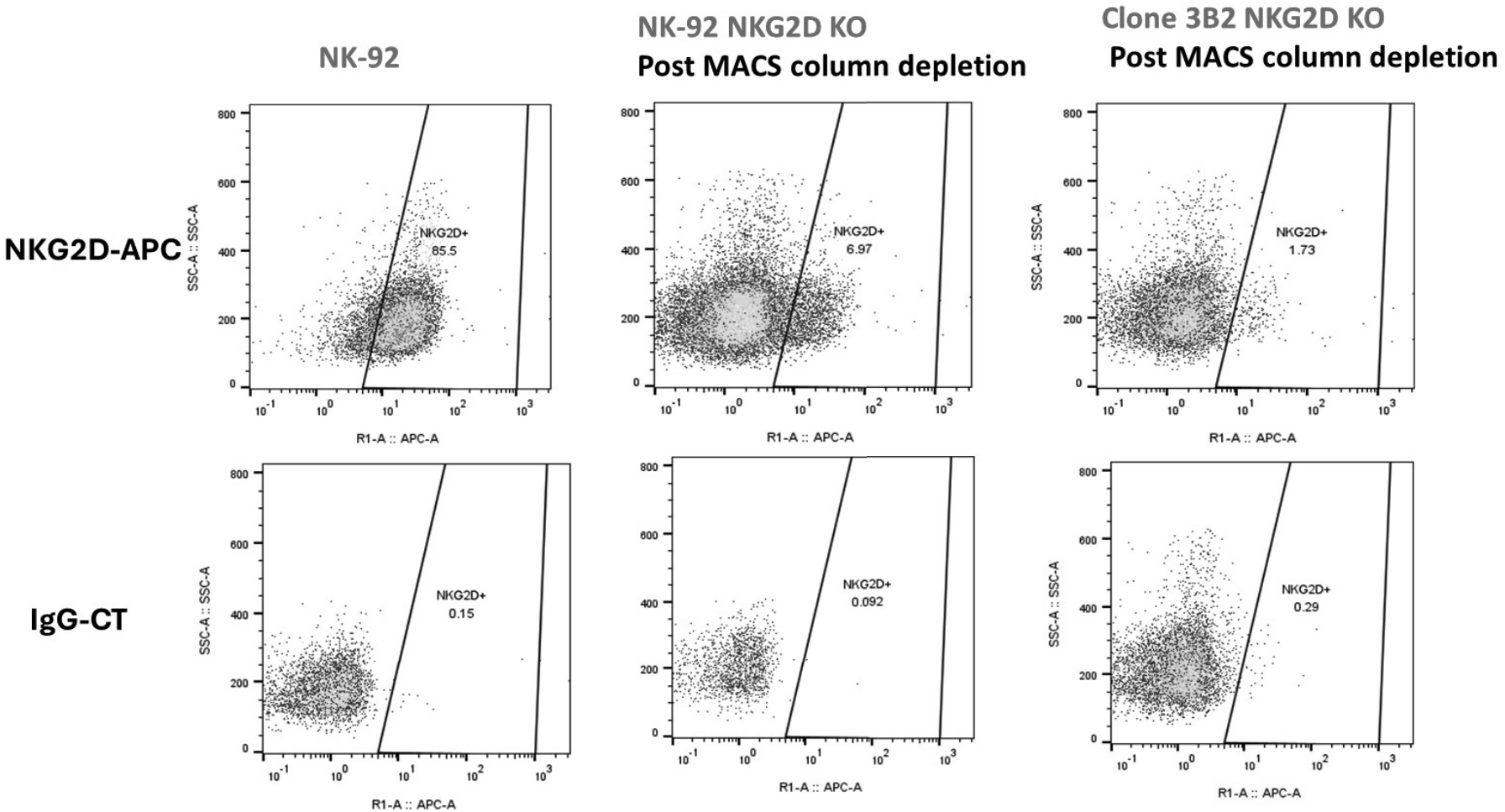


FIG.18

NKG2D-Ligand expression on K562, Daudi and Ramos

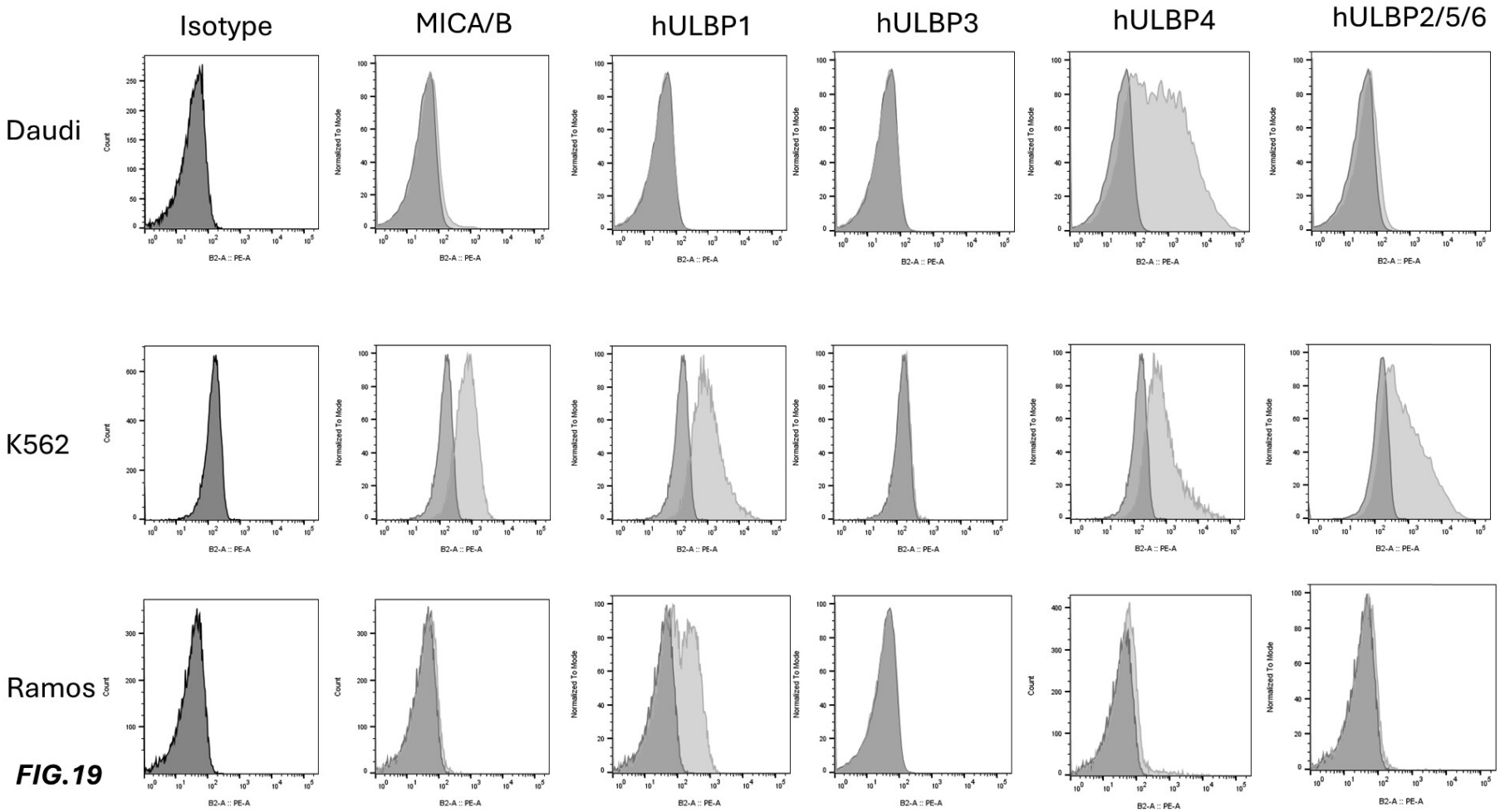


FIG.19

Cytotoxicity against Ramos upon NKG2D KO

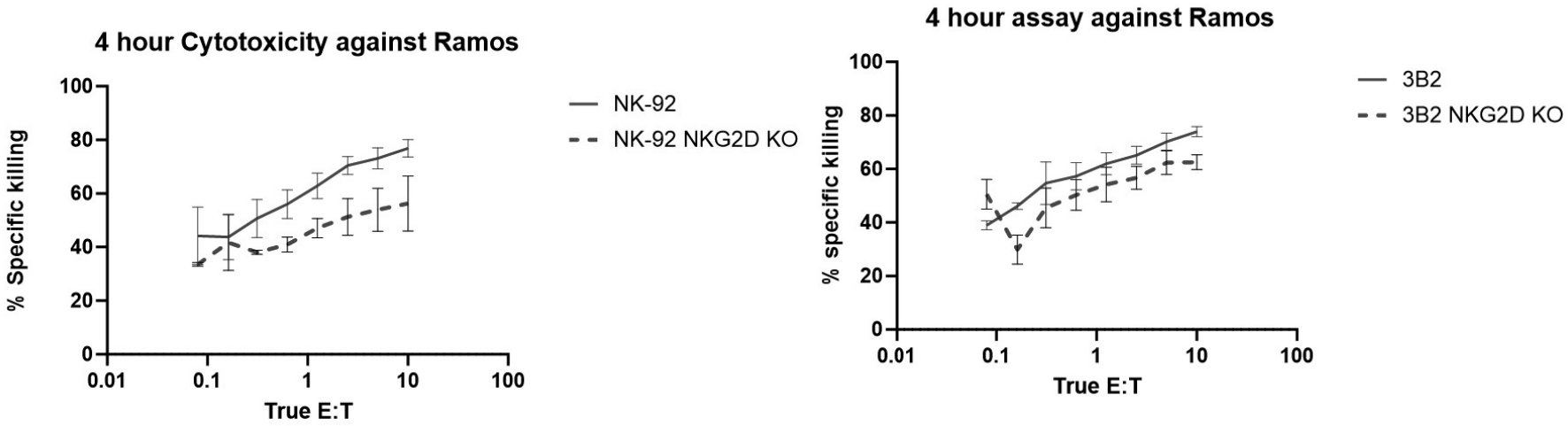
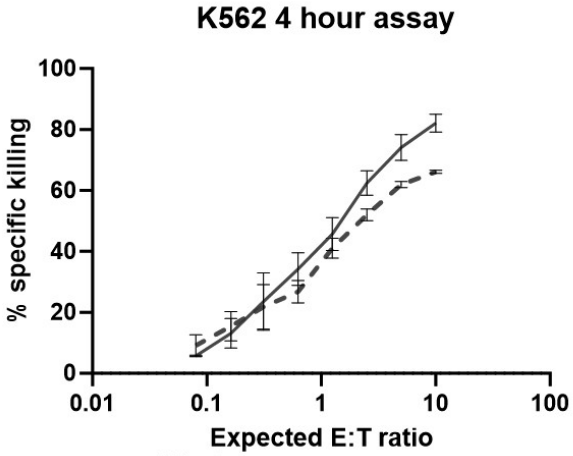


FIG.20

Cytotoxicity against K562 upon NKG2D KO



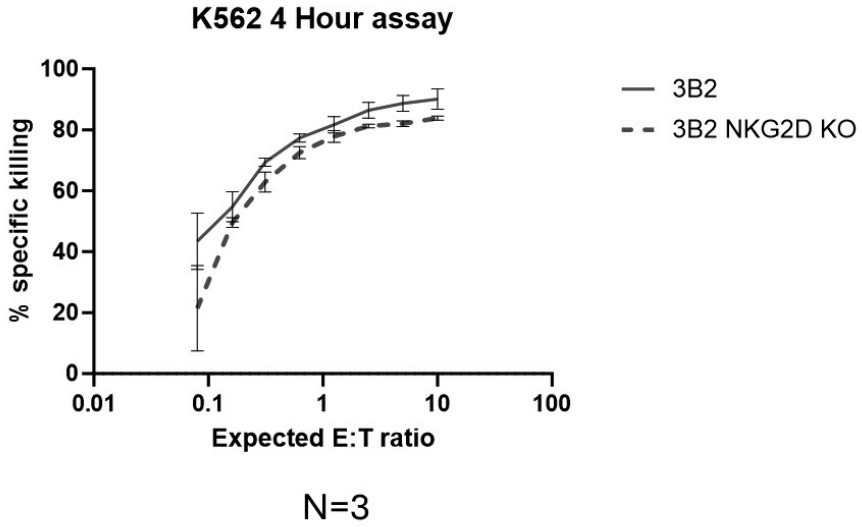


FIG.21

ADCC against Sup-B-15-Cd19-CD20+ upon NKG2D KO

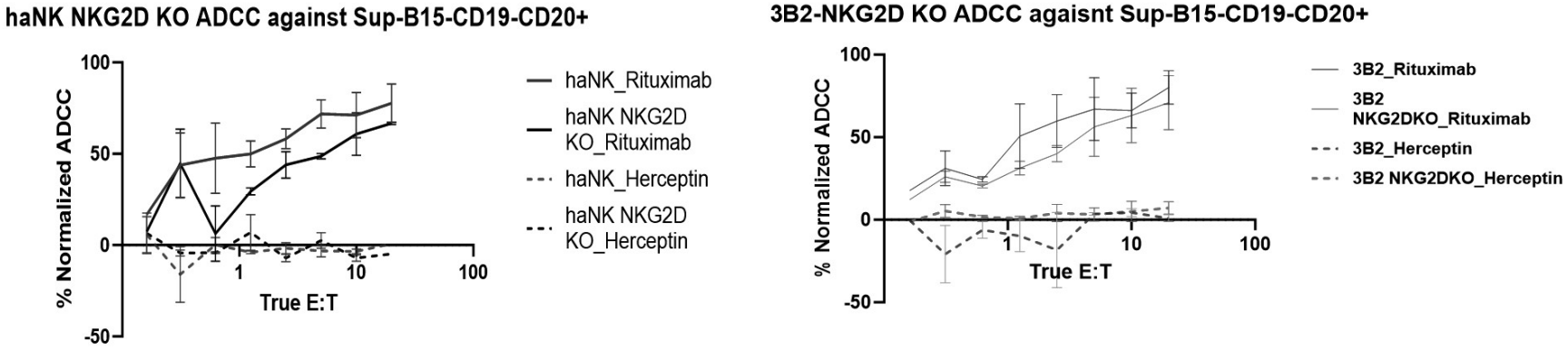


FIG.22

Cytotoxicity Characterization against K562, Ramos

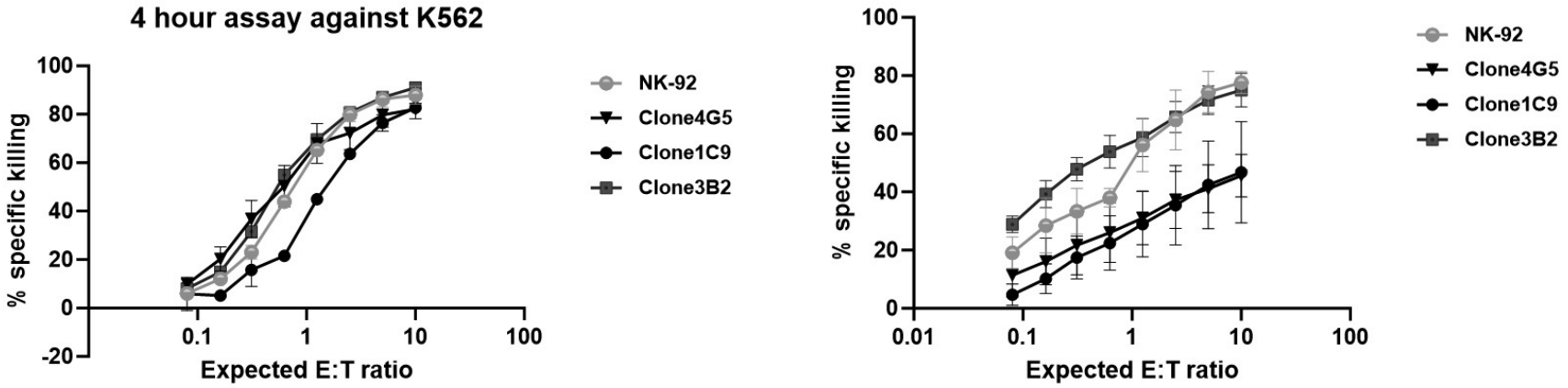


FIG.23