Lineage Antigens
Type 0
Antigen is lineage specific
But
a) Antigen is required for survival
b) OR cell-type carrying antigen is required for survival
c) Example: Lung, Glial cell or CD31 (endothelial cell)
These make poor targets for CAR-T therapy
Type 1
Antigen is lineage specific
But
a) Antigen is not required for survival
b) Cell-type carrying antigen is not required for survival
Example: CD307 expressed in Myeloma cells and Plasma Cells
These make good targets for CAR-T therapy
Type 2
Antigen is lineage specific
But
a) Antigen is not required for survival
b) Cell-type carrying antigen is required for survival
c) But ANTIGEN CAN BE CRISPED in NORMAL CELL OR STEM CELL
Example: CD33 expressed in AML and Myeloid cells and stem cells
These make good targets for CAR-T therapy if they can be combined with CRISPR-mediated deletion of antigen in normal cells
Type 3
Antigen is lineage specific (or tumor specific)
But
a) Antigen is not required for survival
b) Cell-type carrying antigen is not required for survival
Example: LMP2 form EBV expressed in EBV-derived tumors
These make good targets for CAR-T therapy

FIG. 1

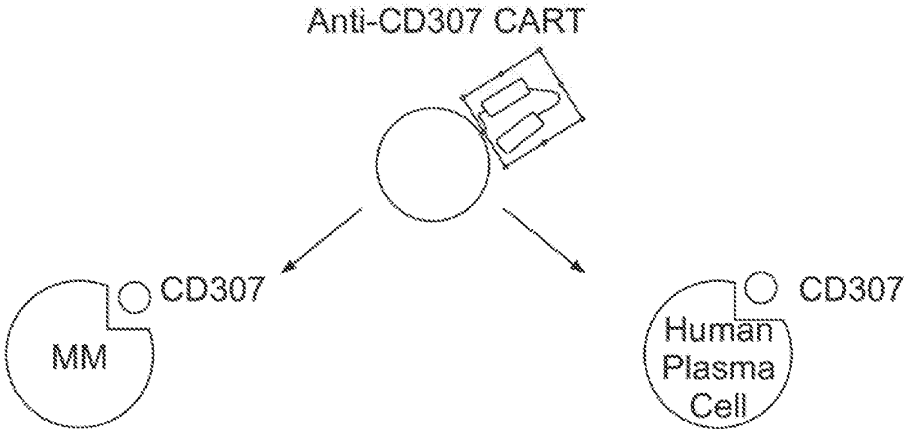

Anti-CD307 CART
CD307
MM
CD307
Human
Plasma
Cell

FIG. 2

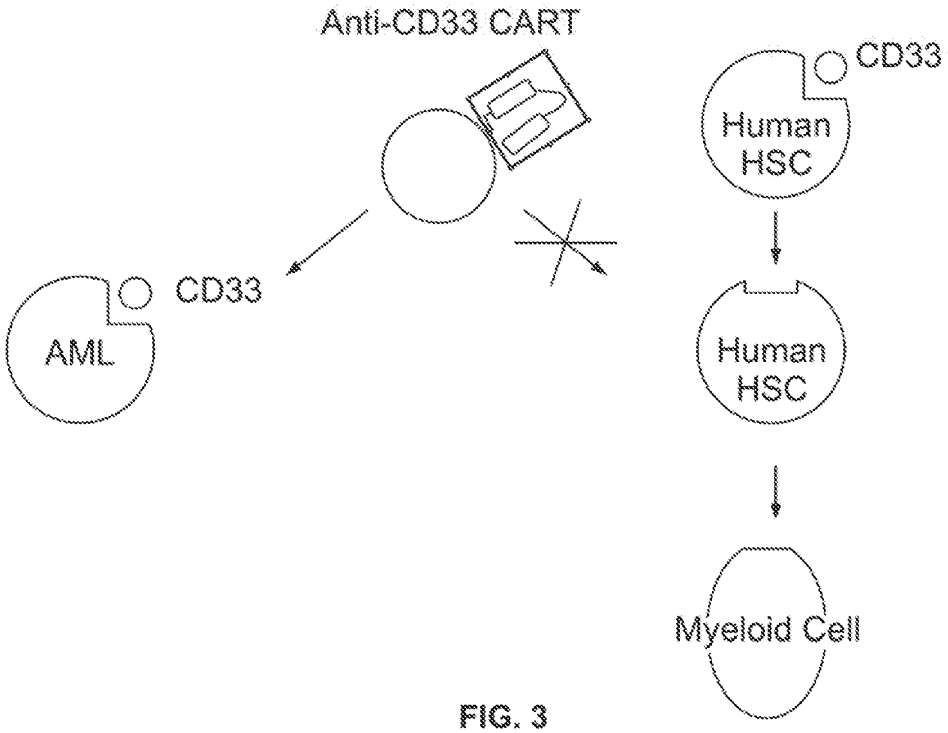

Anti-CD33 CART
CD33
AML
CD33
Human
HSC
Human
HSC
Myeloid Cell

FIG. 3

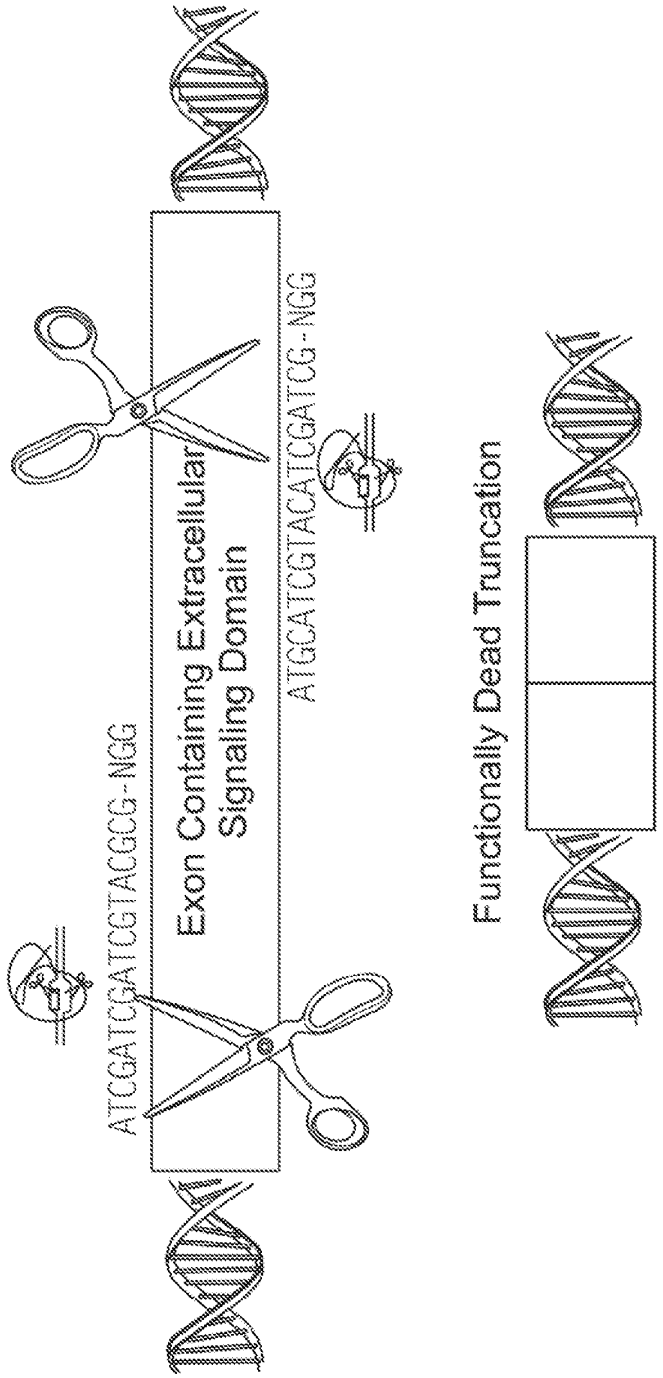

ATCGATCGATCGTACGGCG-NGG
Exon Containing Extracellular Signaling Domain
ATGCATCGTACATCGATCG-NGG
Functionally Dead Truncation

FIG. 4

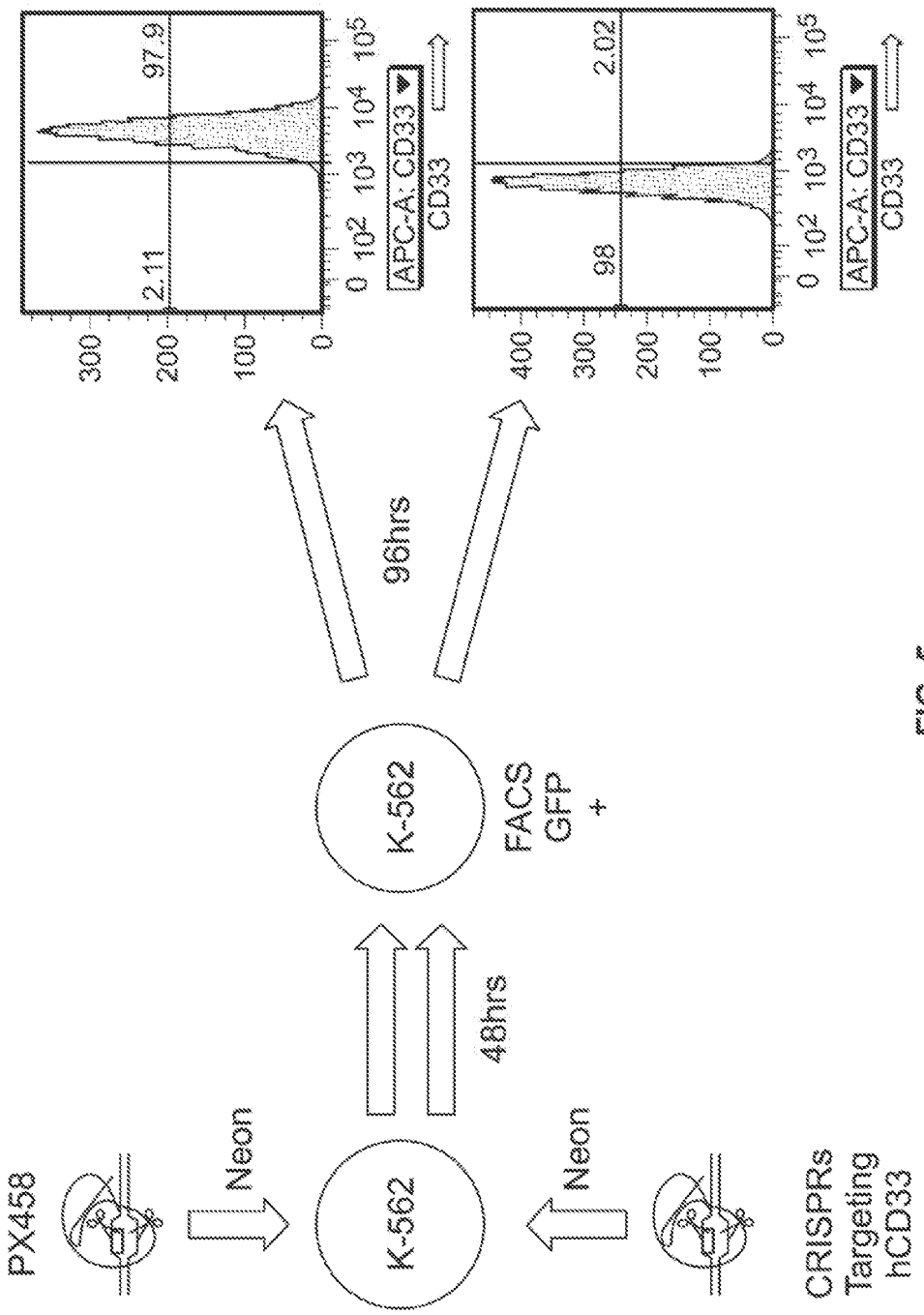

PX458
Neon
K-562
48hrs
Neon
CRISPRs
Targeting
hCD33
K-562
FACS
GFP
+
96hrs
97.9
2.11
APC-A: CD33
CD33
2.02
98
APC-A: CD33
CD33

FIG. 5

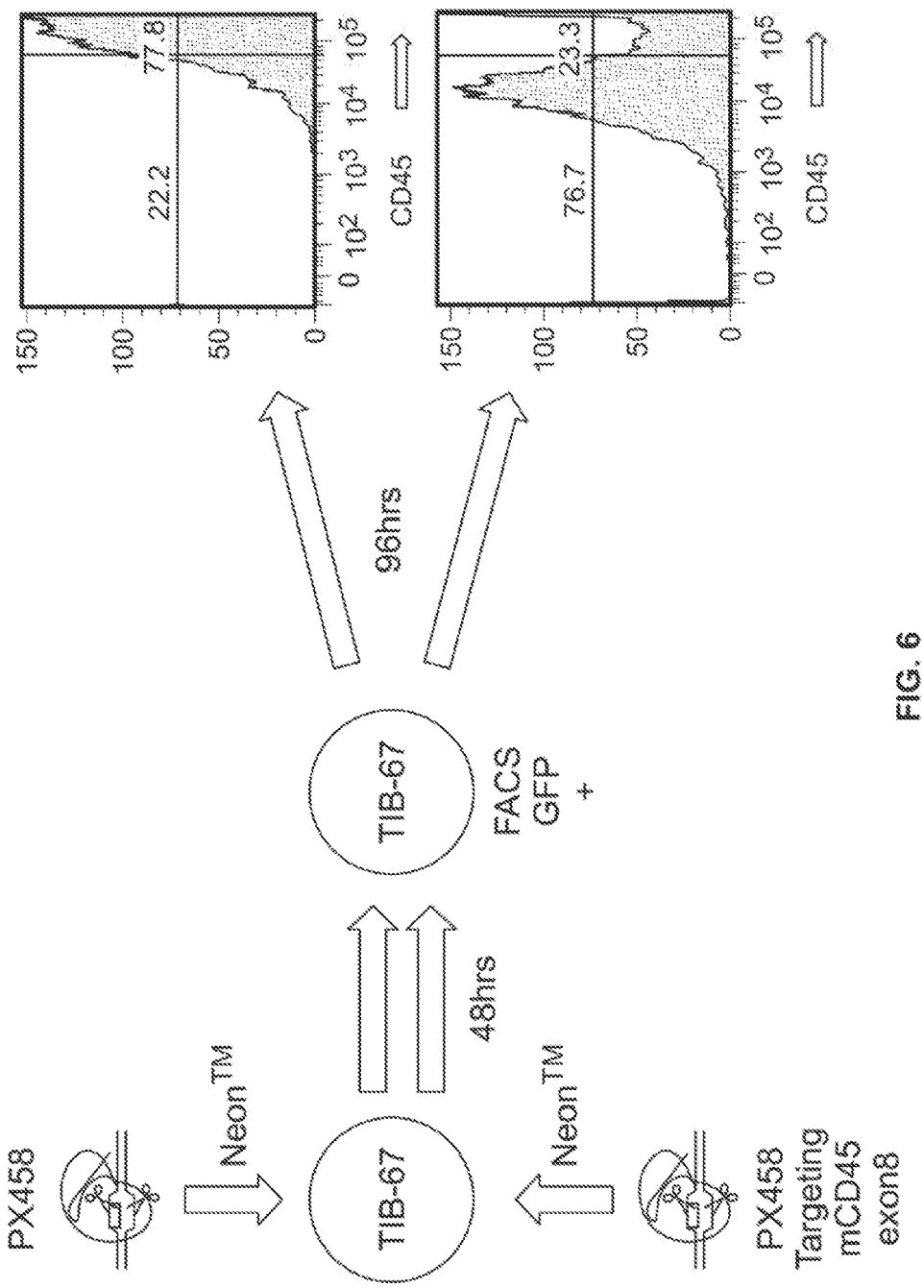

PX458
Neon™
TIB-67
48hrs
TIB-67
FACS
GFP +
96hrs
PX458
Targeting
mCD45
exon8
Neon™
77.8
22.2
CD45
23.3
76.7
CD45

FIG. 6

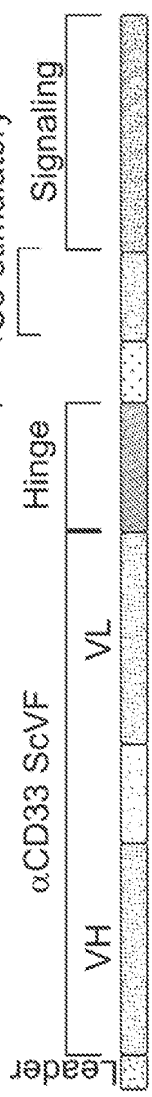


FIG. 7A

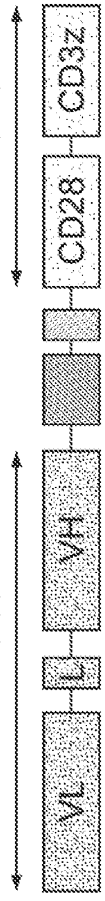


FIG. 7B

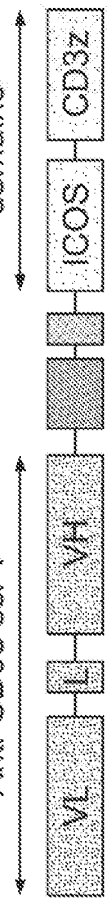


FIG. 7C

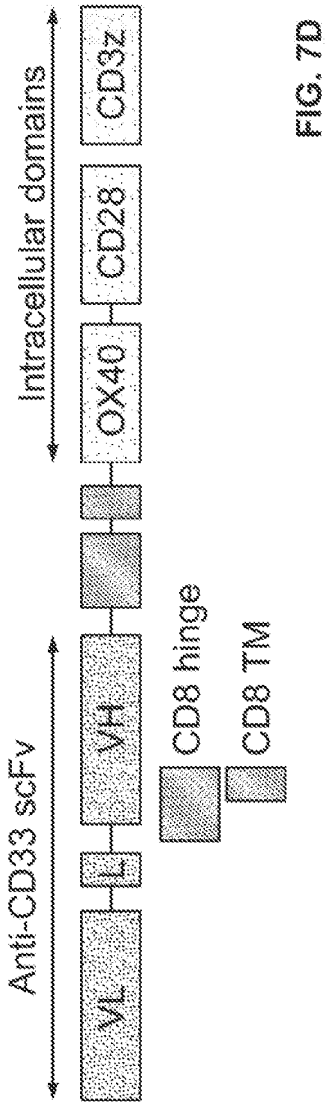

Anti-CD33 scFv
Intracellular domains
VL
L
VH
CD8 hinge
CD8 TM
OX40
CD28
CD3z

FIG. 7D

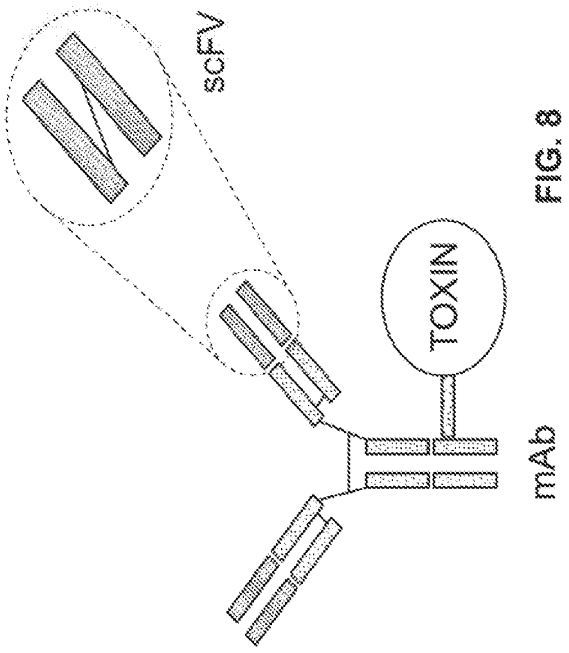
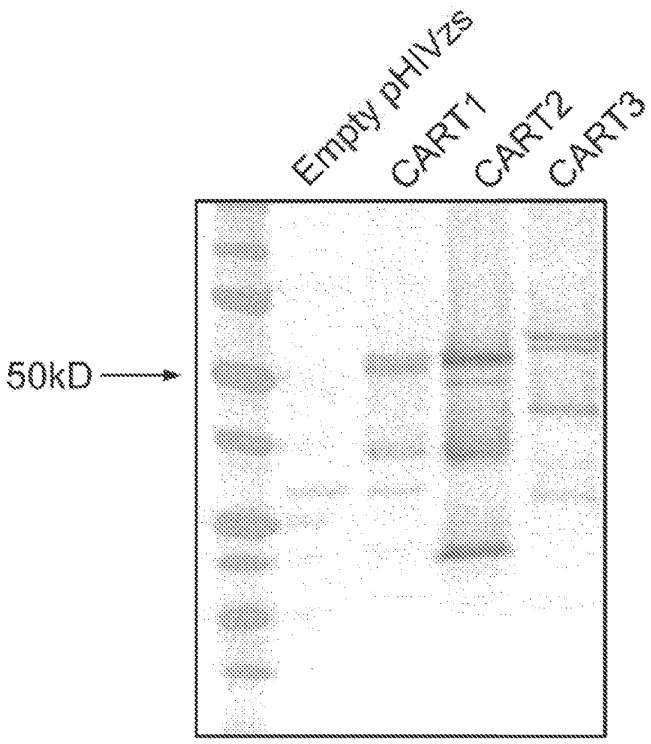

scFV
TOXIN
mAb

FIG. 8



Stain with anti-CD3TCRz, ab
Epitope: C-term CD3z

Construct	MW kDa
CART1	55.3AAAA
CART2	56.3
CART3	61

FIG. 9A

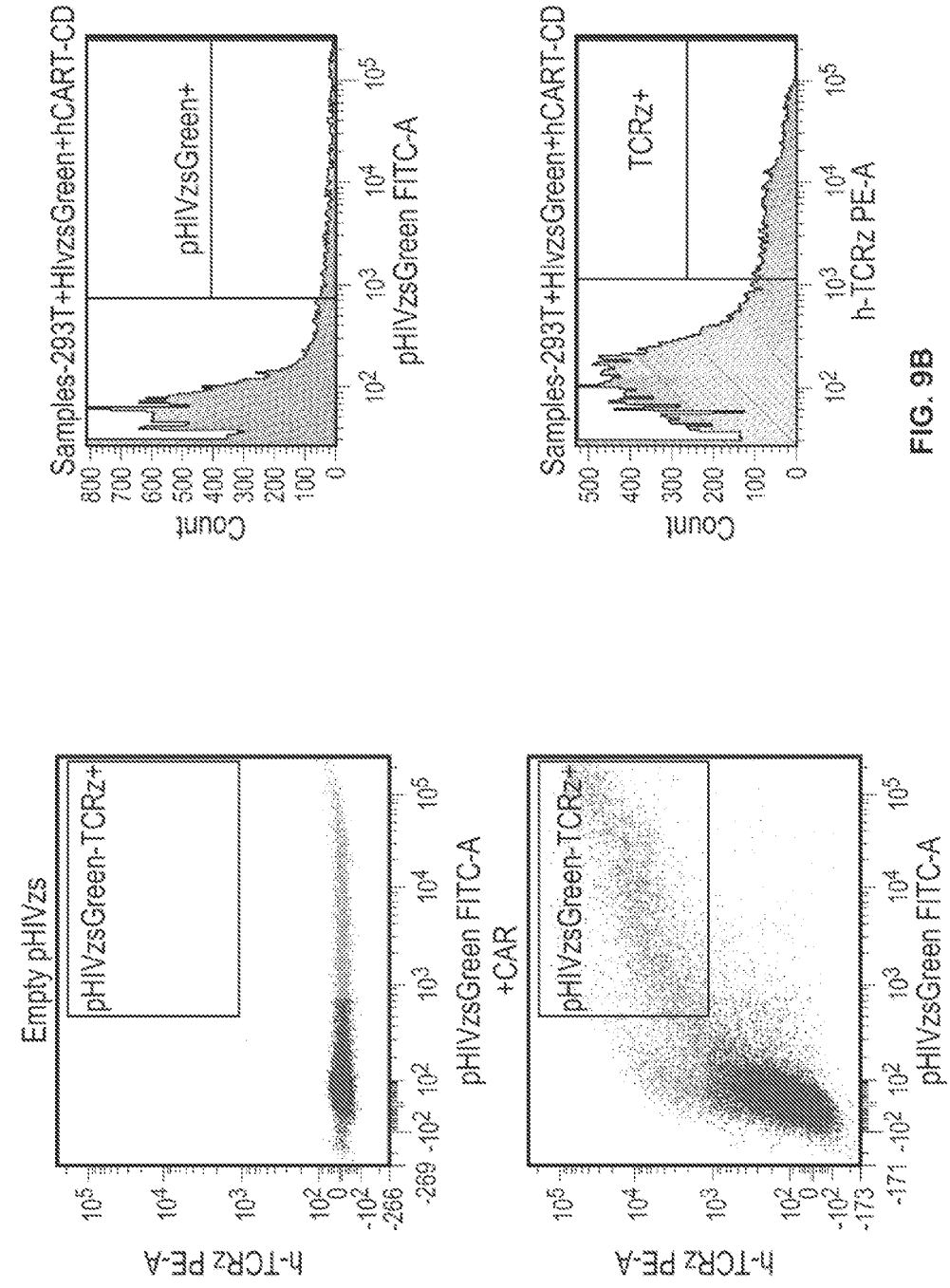

Samples-293T+HlvzsGreen+hCART-CD
Count
pHlVzsGreen+
pHlVzsGreen FITC-A
Samples-293T+HlvzsGreen+hCART-CD
Count
TCRz+
h-TCRz PE-A
Empty pHlVzs
pHlVzsGreen-TCRz+
h-TCRz PE-A
pHlVzsGreen FITC-A
+CAR
pHlVzsGreen-TCRz+
h-TCRz PE-A
pHlVzsGreen FITC-A

FIG. 9B

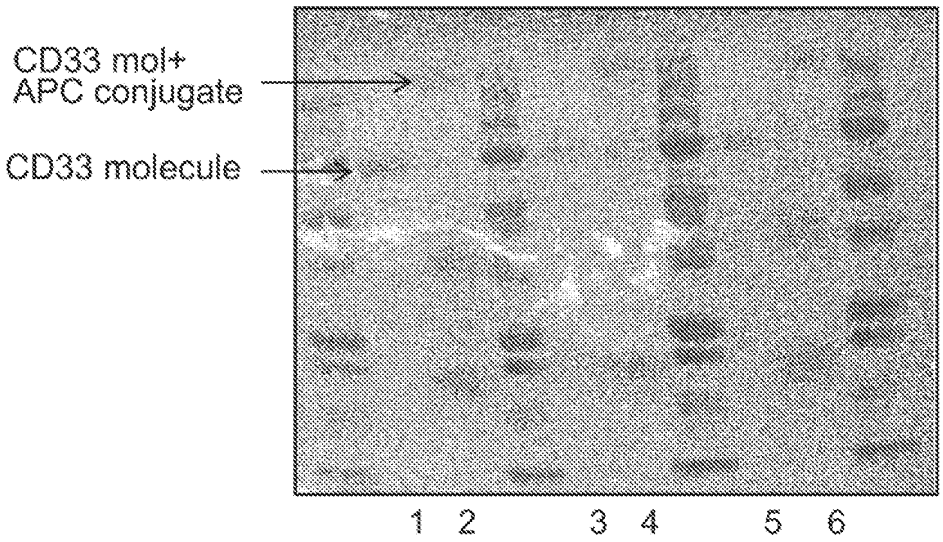


FIG. 10A

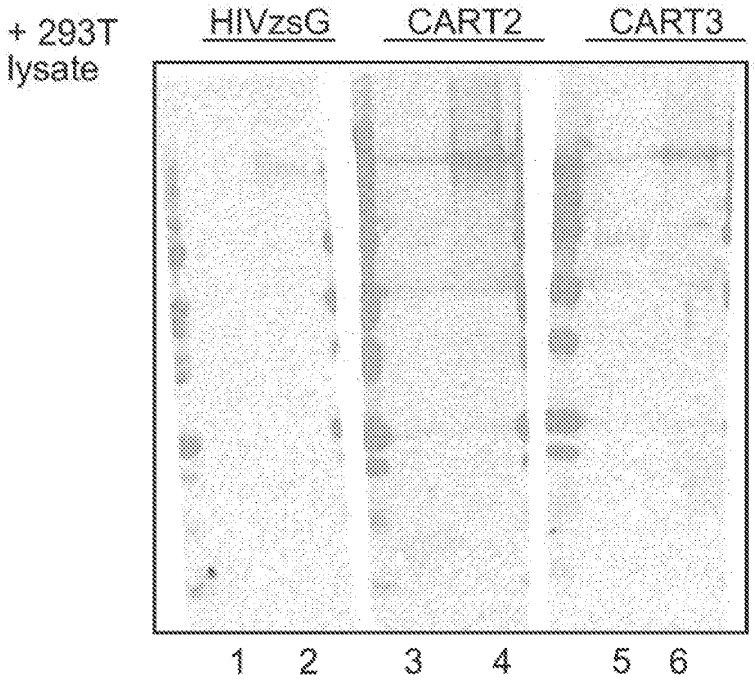


FIG. 10B

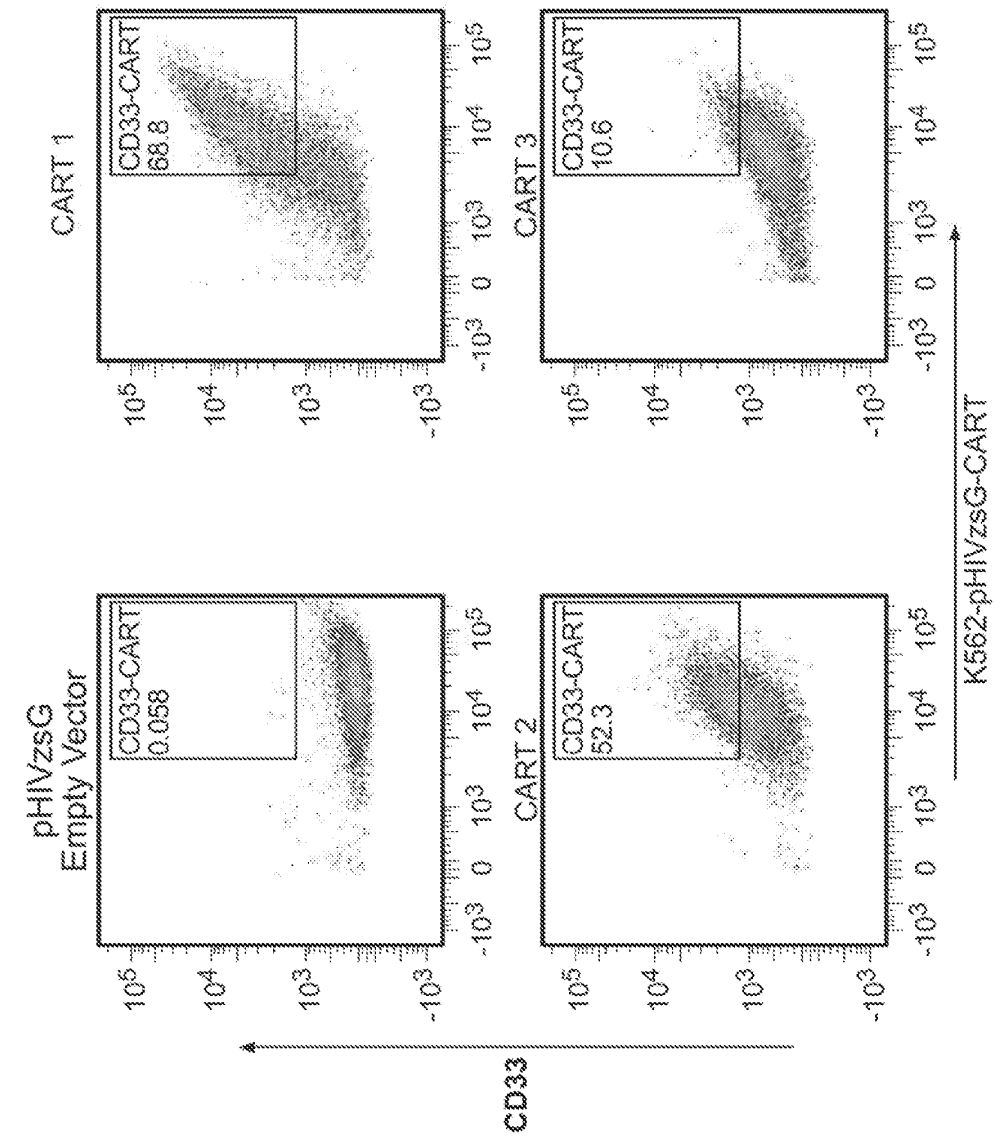

pHIVzsG
Empty Vector
CART 1
CART 2
CART 3
CD33-CART
0.058
CD33-CART
68.8
CD33-CART
52.3
CD33-CART
10.6
CD33
K562-pHIVzsG-CART

FIG. 10C

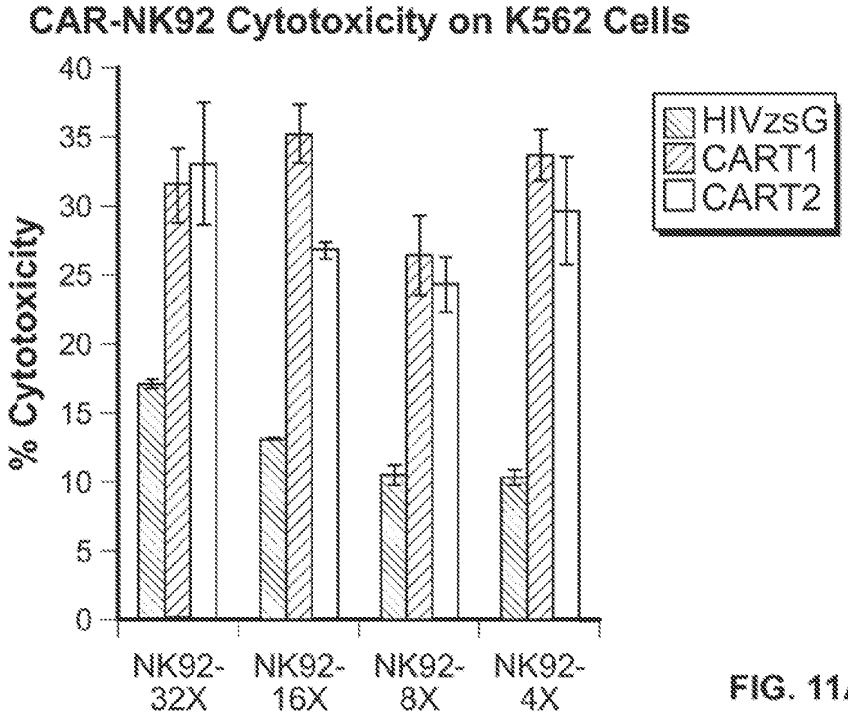


FIG. 11A

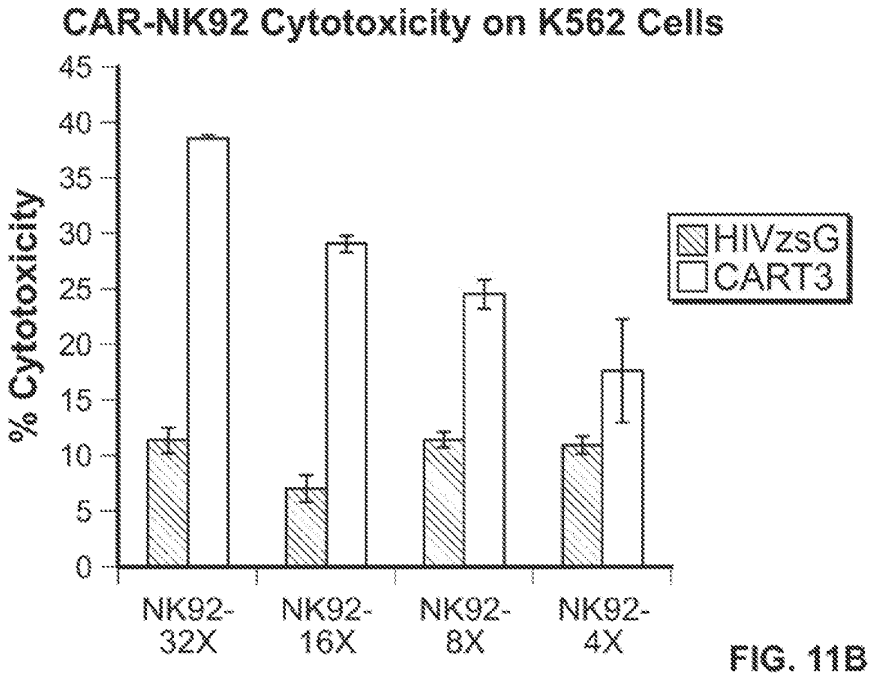


FIG. 11B

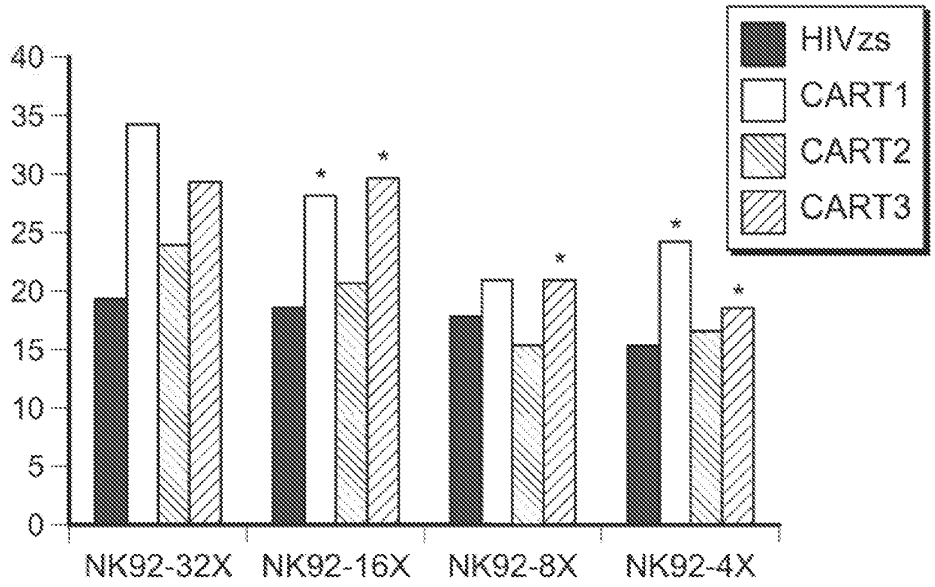


FIG. 12A

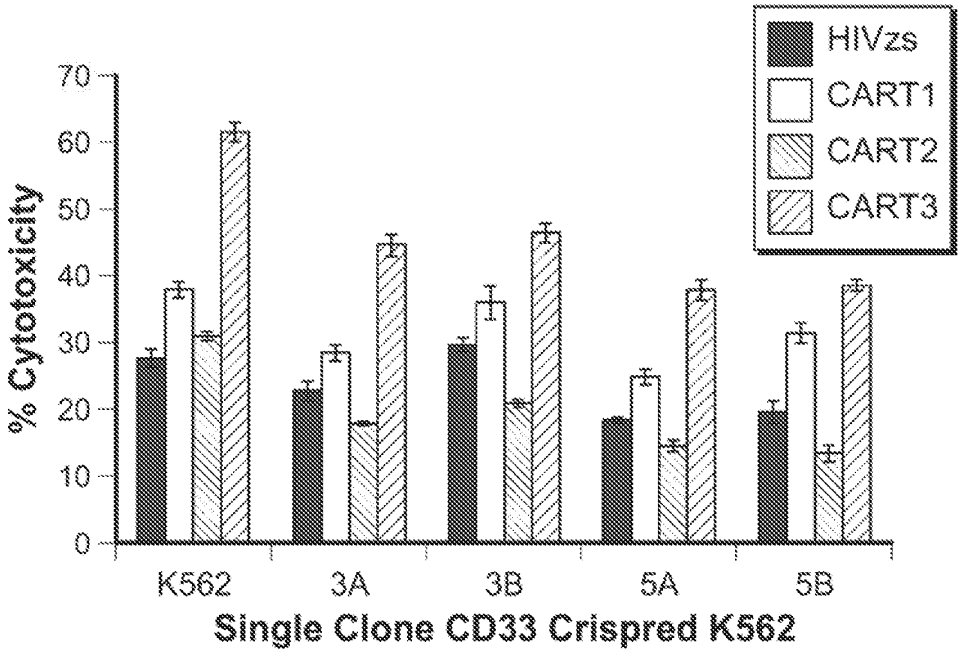


FIG. 12B

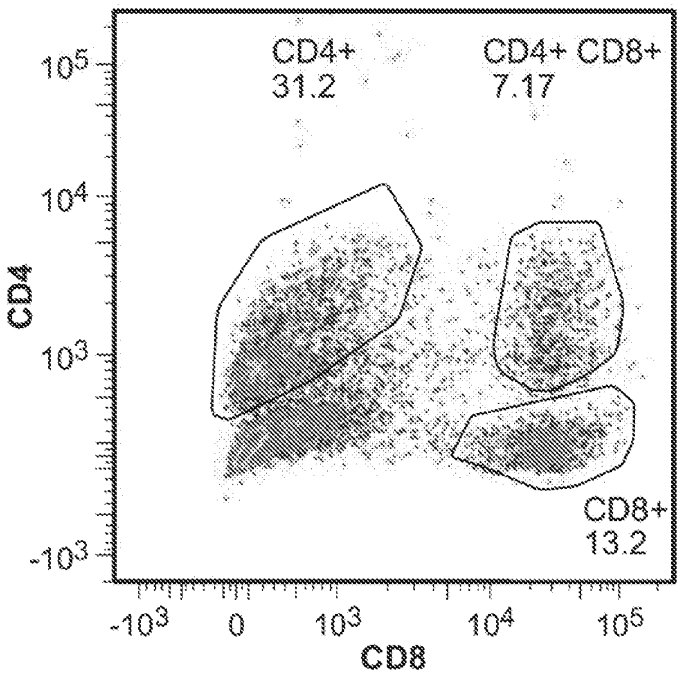


FIG. 13A

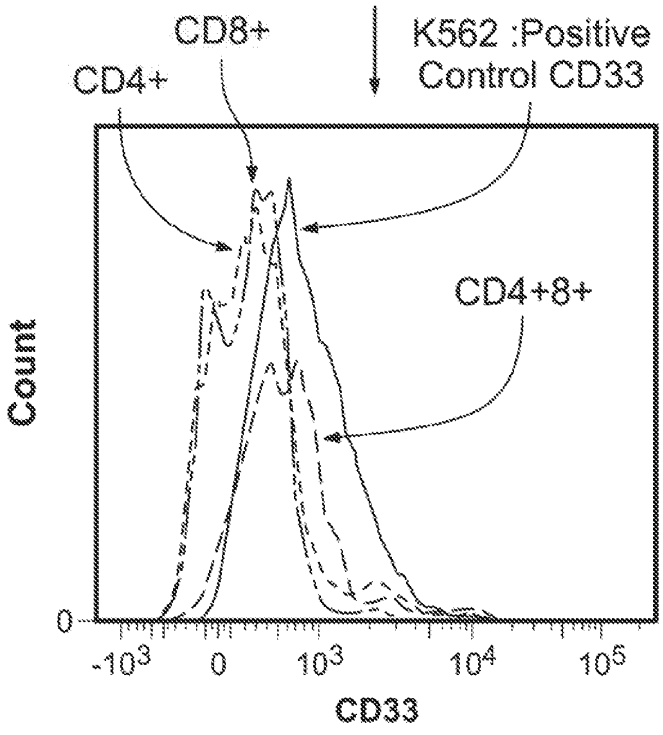


FIG. 13B

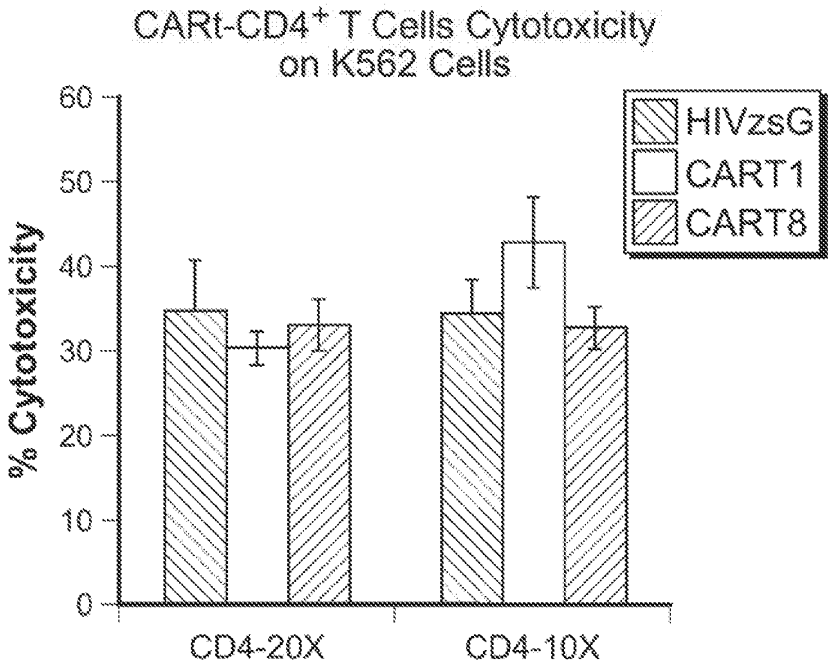


FIG. 14A

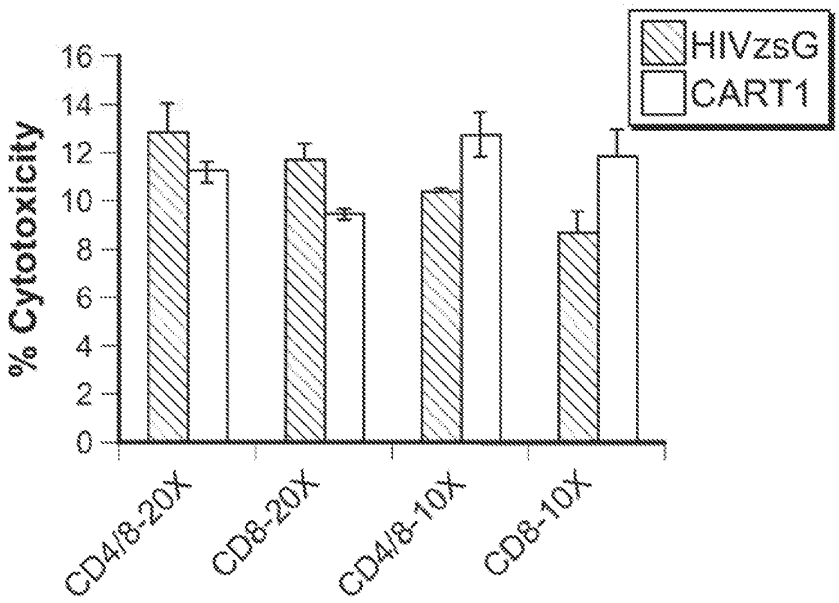


FIG. 14B

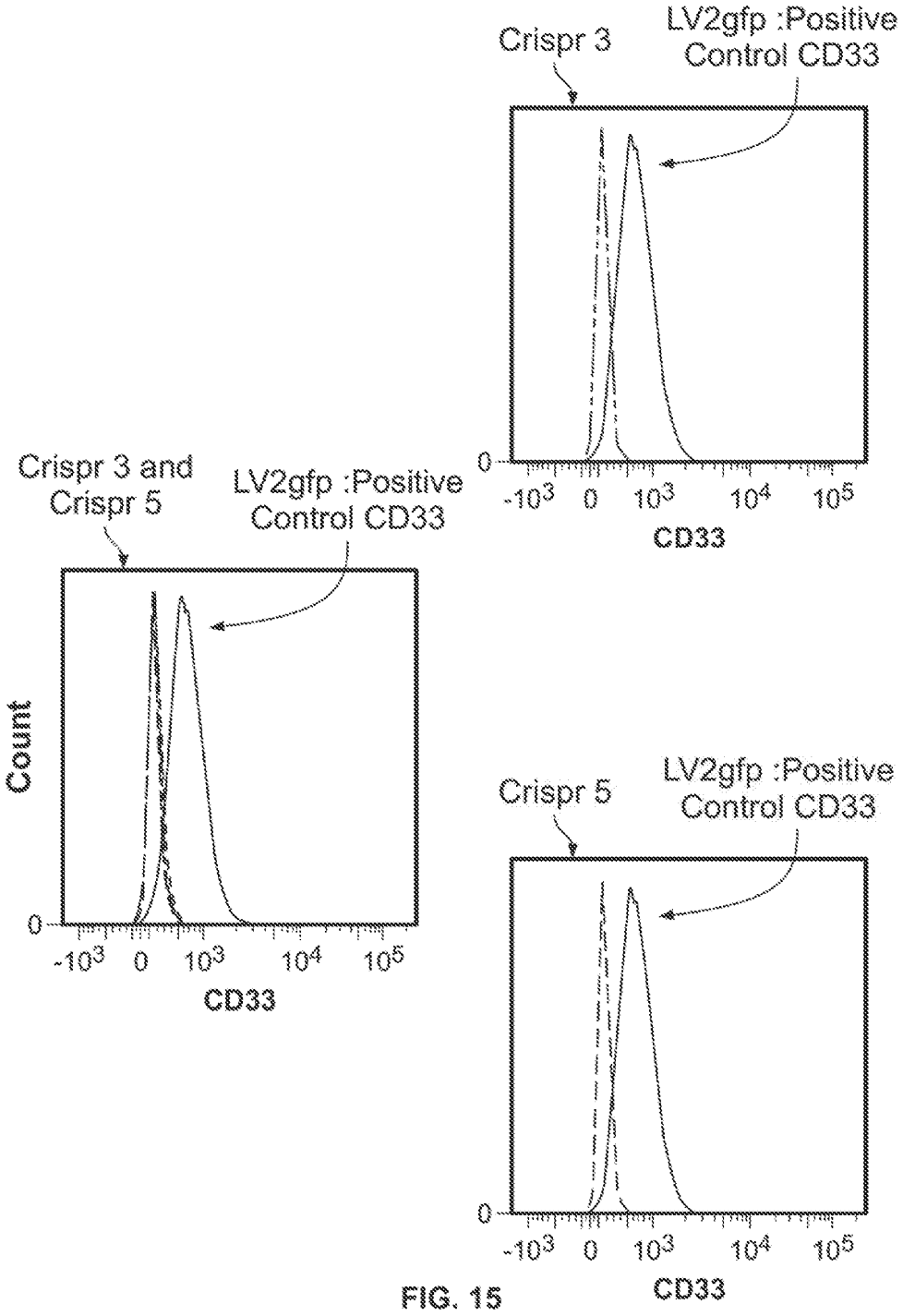

Crispr 3
LV2gfp :Positive Control CD33
0
-10^3 0 10^3 10^4 10^5
CD33
Crispr 3 and Crispr 5
LV2gfp :Positive Control CD33
Count
0
-10^3 0 10^3 10^4 10^5
CD33
Crispr 5
LV2gfp :Positive Control CD33
0
-10^3 0 10^3 10^4 10^5
CD33

FIG. 15

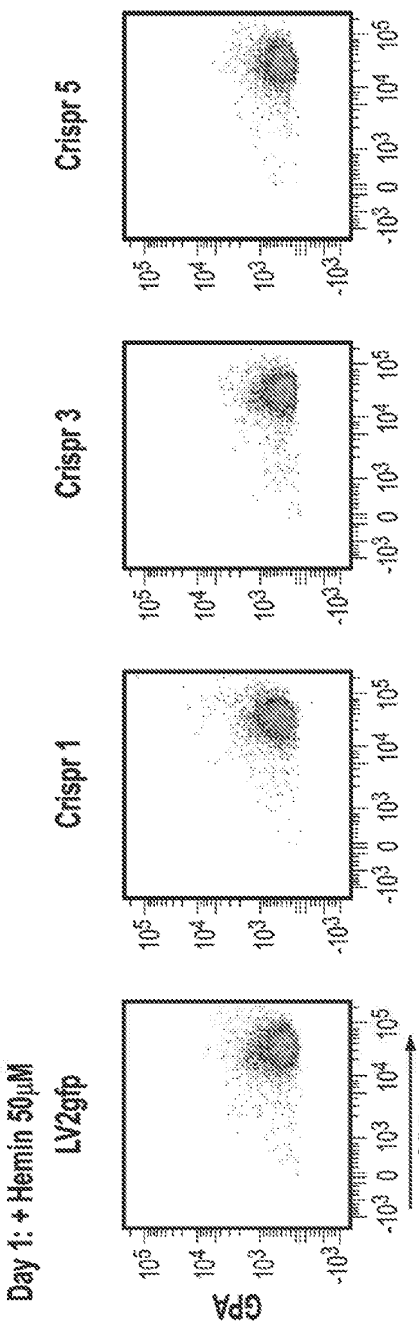


FIG. 16A

FIG. 16B

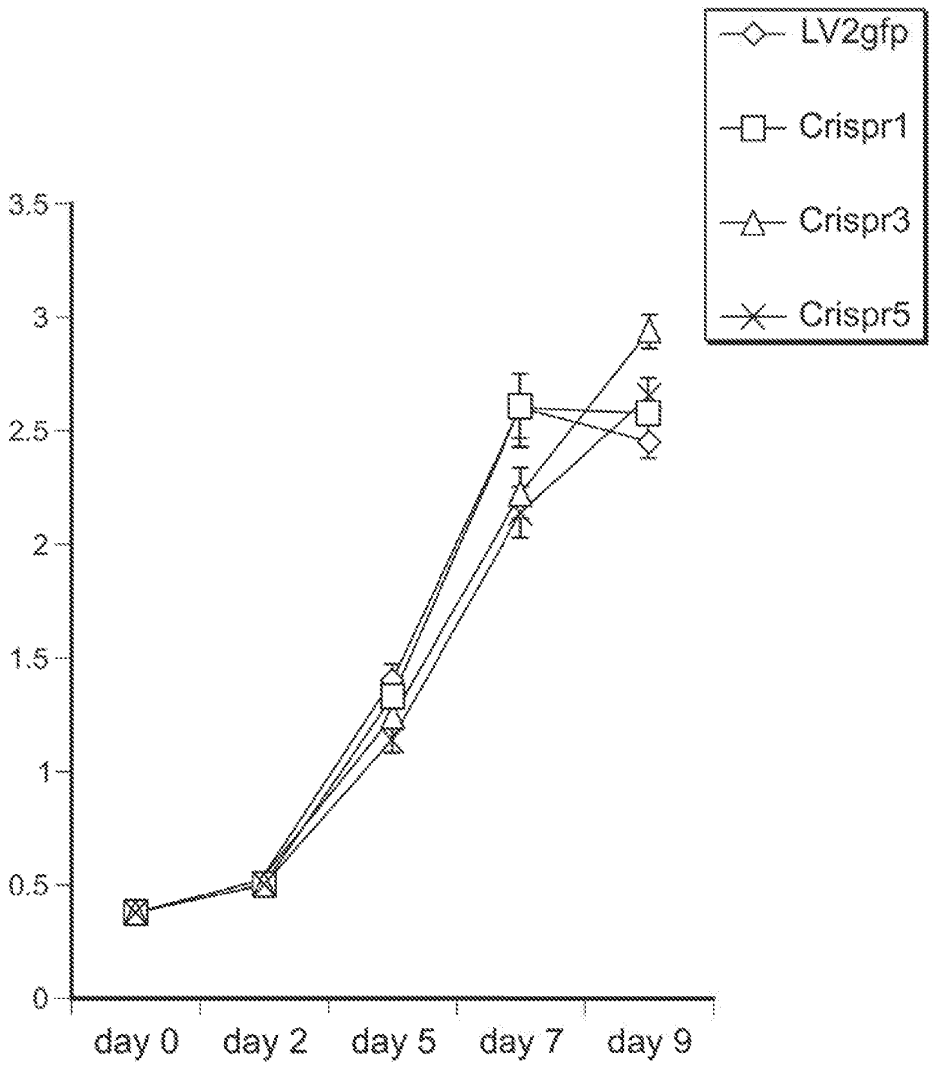

LV2gfp
Crispr1
Crispr3
Crispr5
3.5
3
2.5
2
1.5
1
0.5
0
day 0
day 2
day 5
day 7
day 9

FIG. 16C

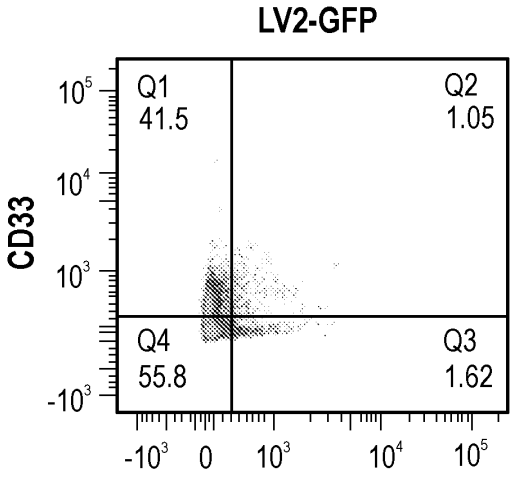


FIG. 17A

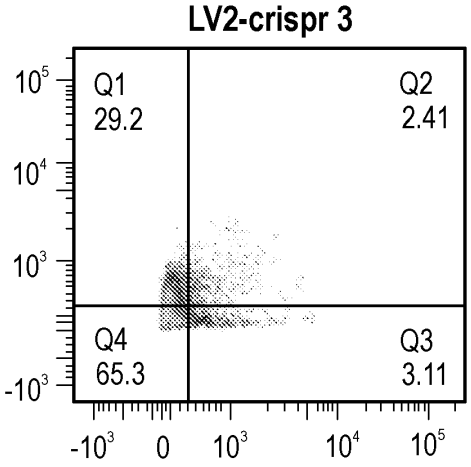


FIG. 17B

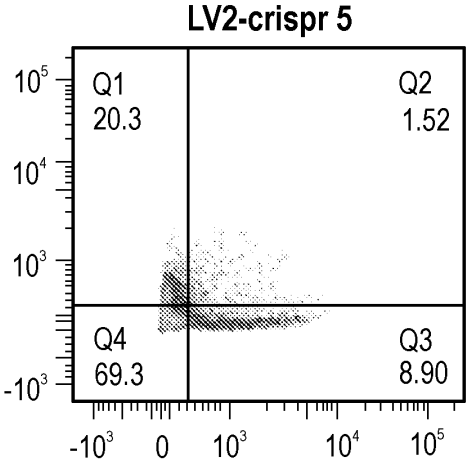


FIG. 17C

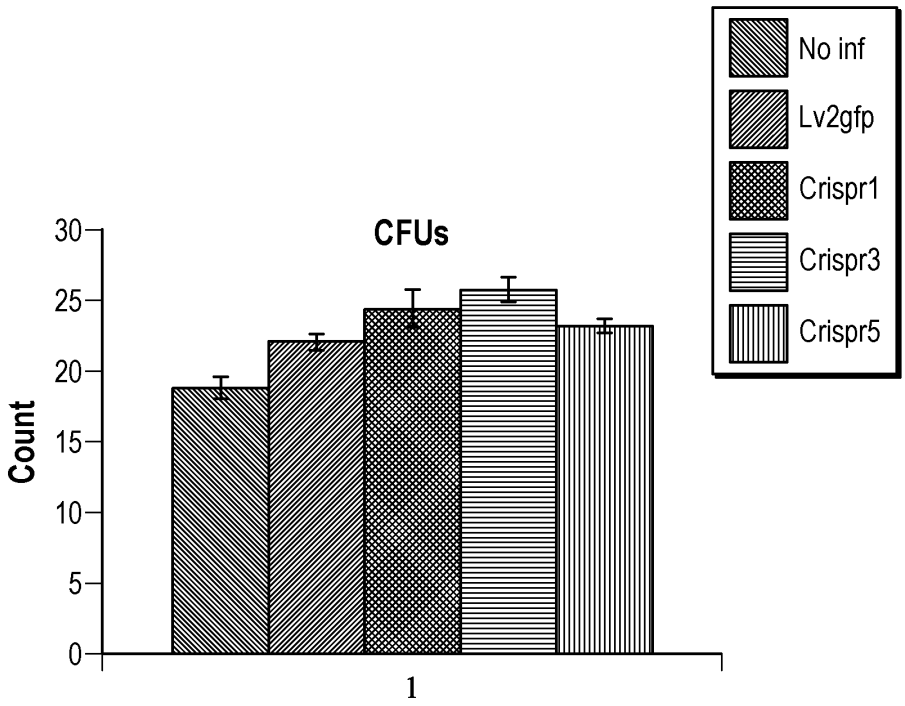

CFUs
Count
30
25
20
15
10
5
0
1
No inf
Lv2gfp
Crispr1
Crispr3
Crispr5

FIG. 18

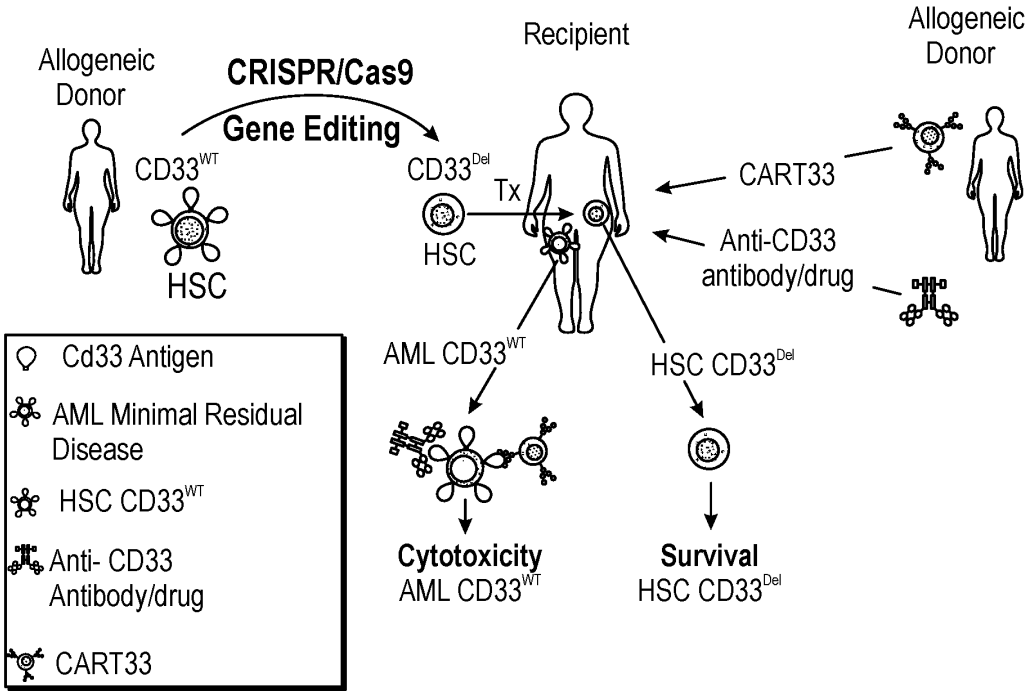


FIG. 19A

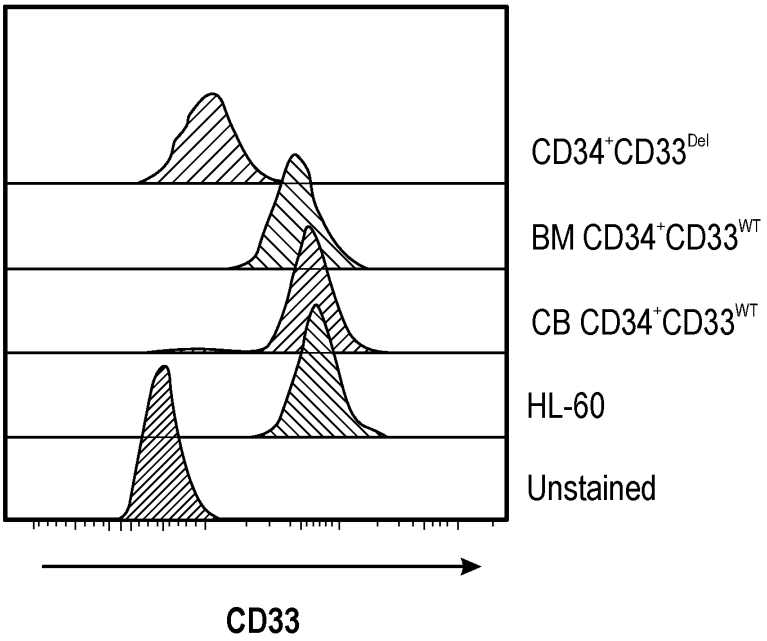


FIG. 19B

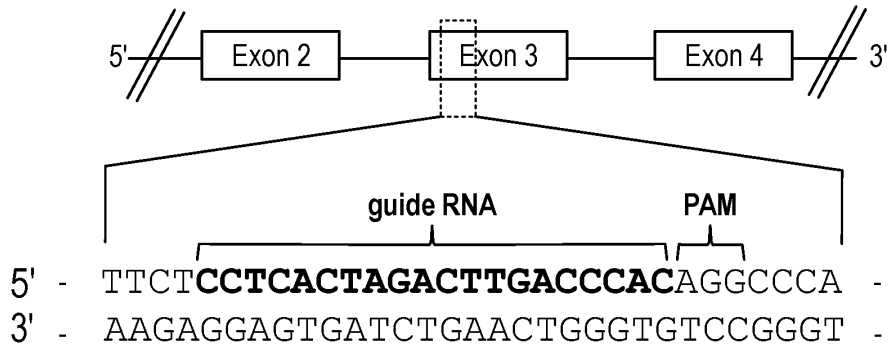


FIG. 19C

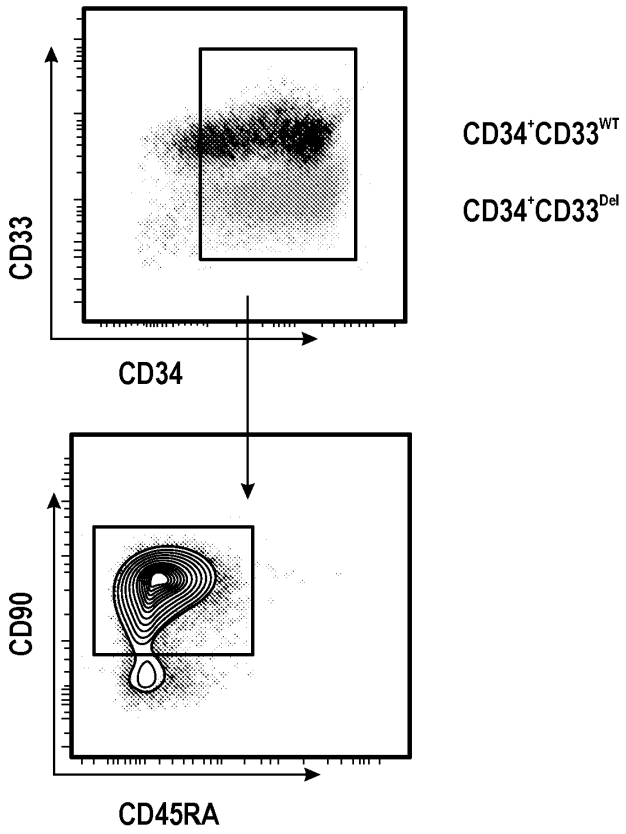


FIG. 19D

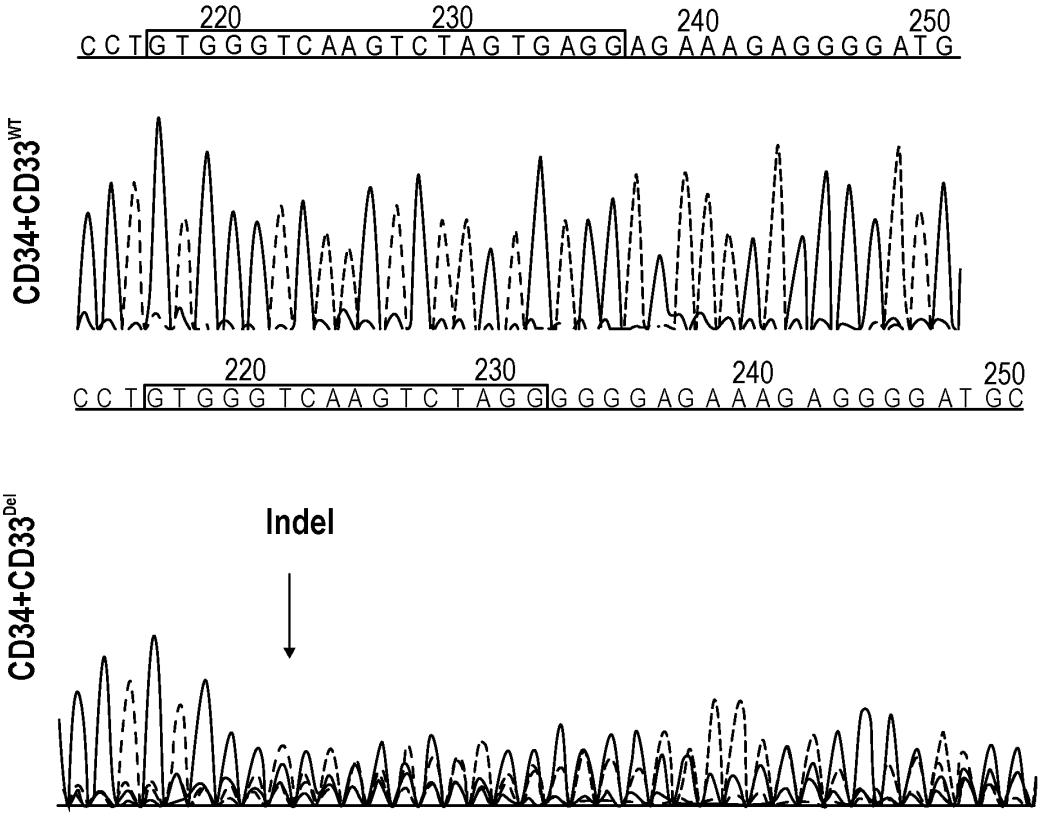


FIG. 19E

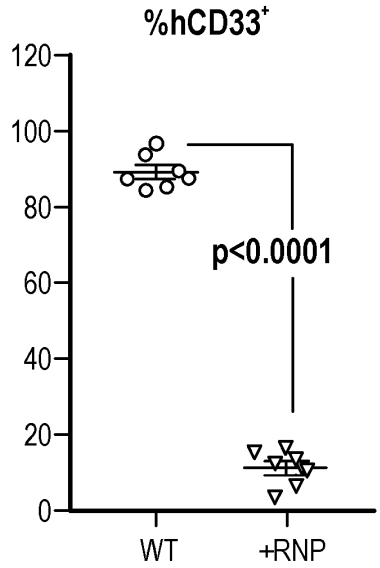


FIG. 19F

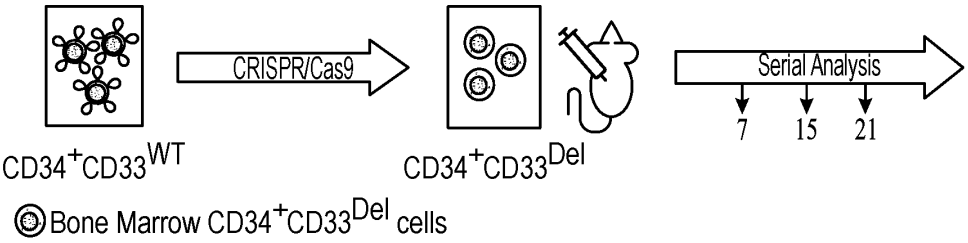


FIG. 20A

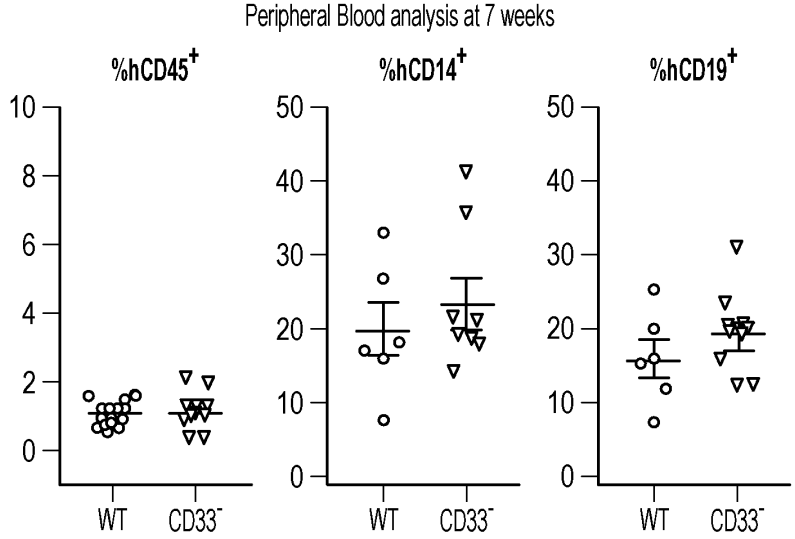


FIG. 20B

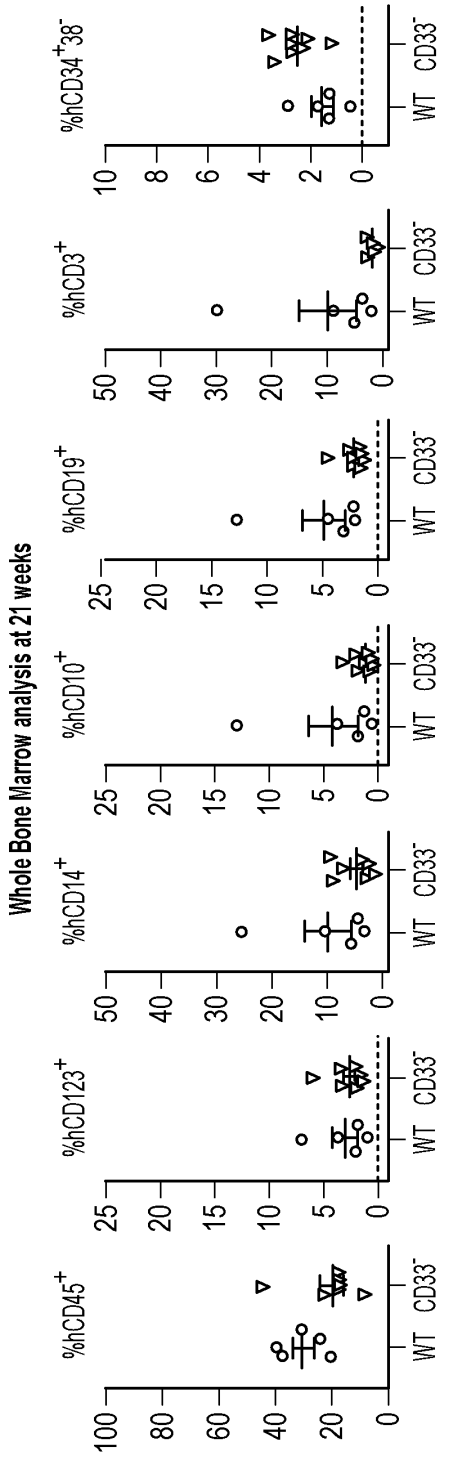

Whole Bone Marrow analysis at 21 weeks
%hCD45+
%hCD123+
%hCD14+
%hCD10+
%hCD19+
%hCD3+
%hCD34+38-
WT
CD33-

FIG. 20C

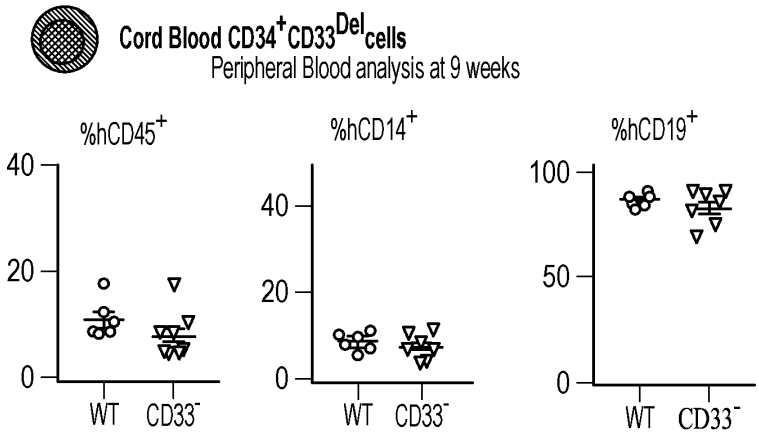

Cord Blood CD34+CD33Delcells
Peripheral Blood analysis at 9 weeks
%hCD45+
40
20
0
WT
CD33-
%hCD14+
40
20
0
WT
CD33-
%hCD19+
100
50
0
WT
CD33-

FIG. 20D

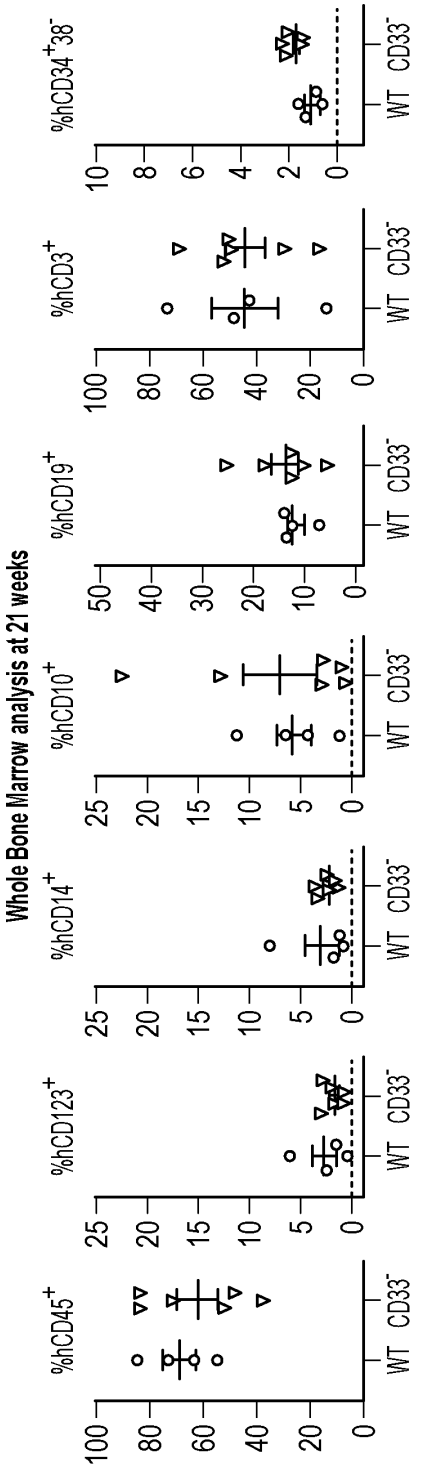

Whole Bone Marrow analysis at 21 weeks
%hCD45+
%hCD123+
%hCD14+
%hCD10+
%hCD19+
%hCD3+
%hCD34+38-
WT
CD33-

FIG. 20E

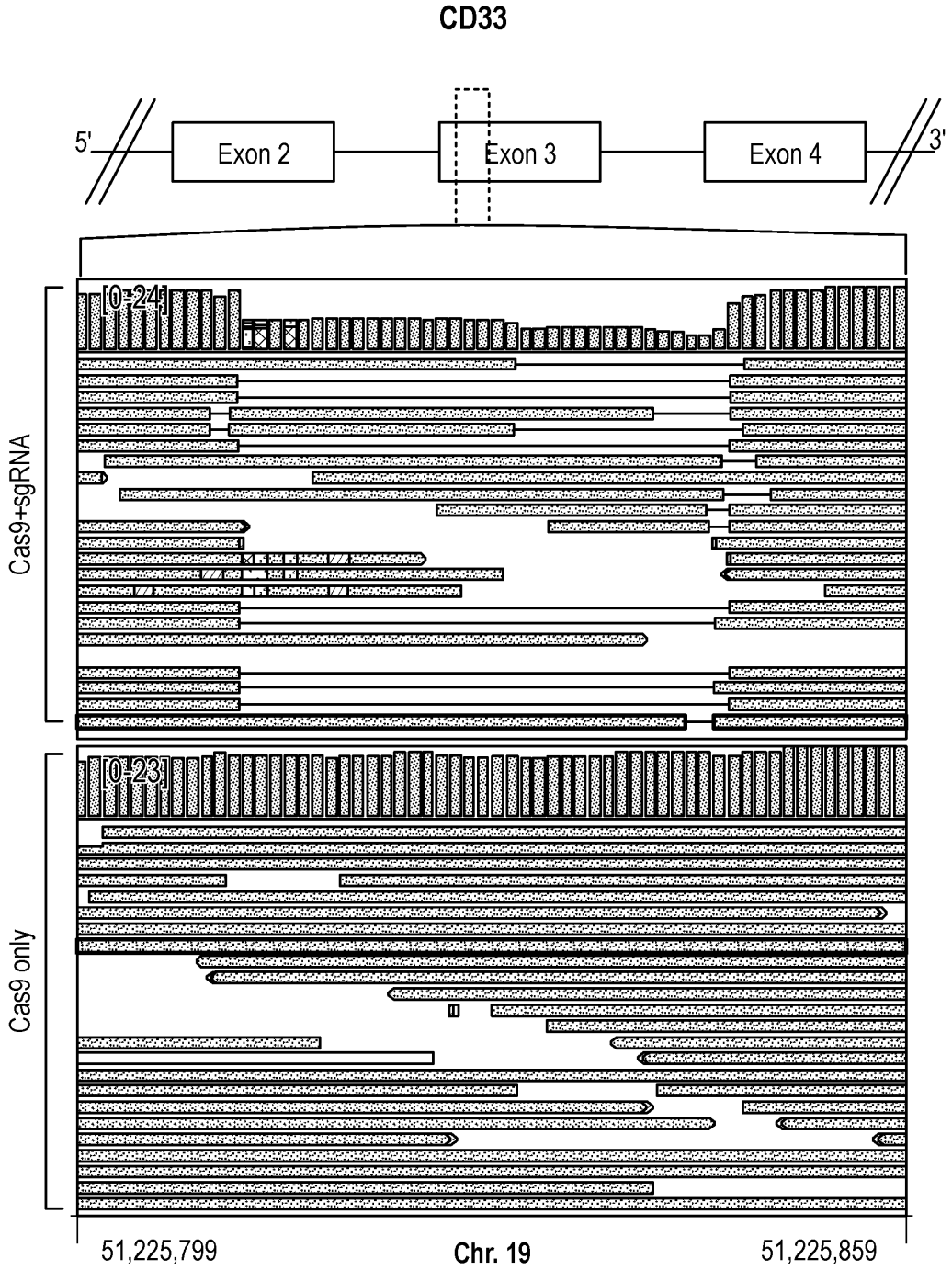

CD33
5'
Exon 2
Exon 3
Exon 4
3'
[0-24]
Cas9+sgRNA
[0-23]
Cas9 only
51,225,799
Chr. 19
51,225,859

FIG. 21A

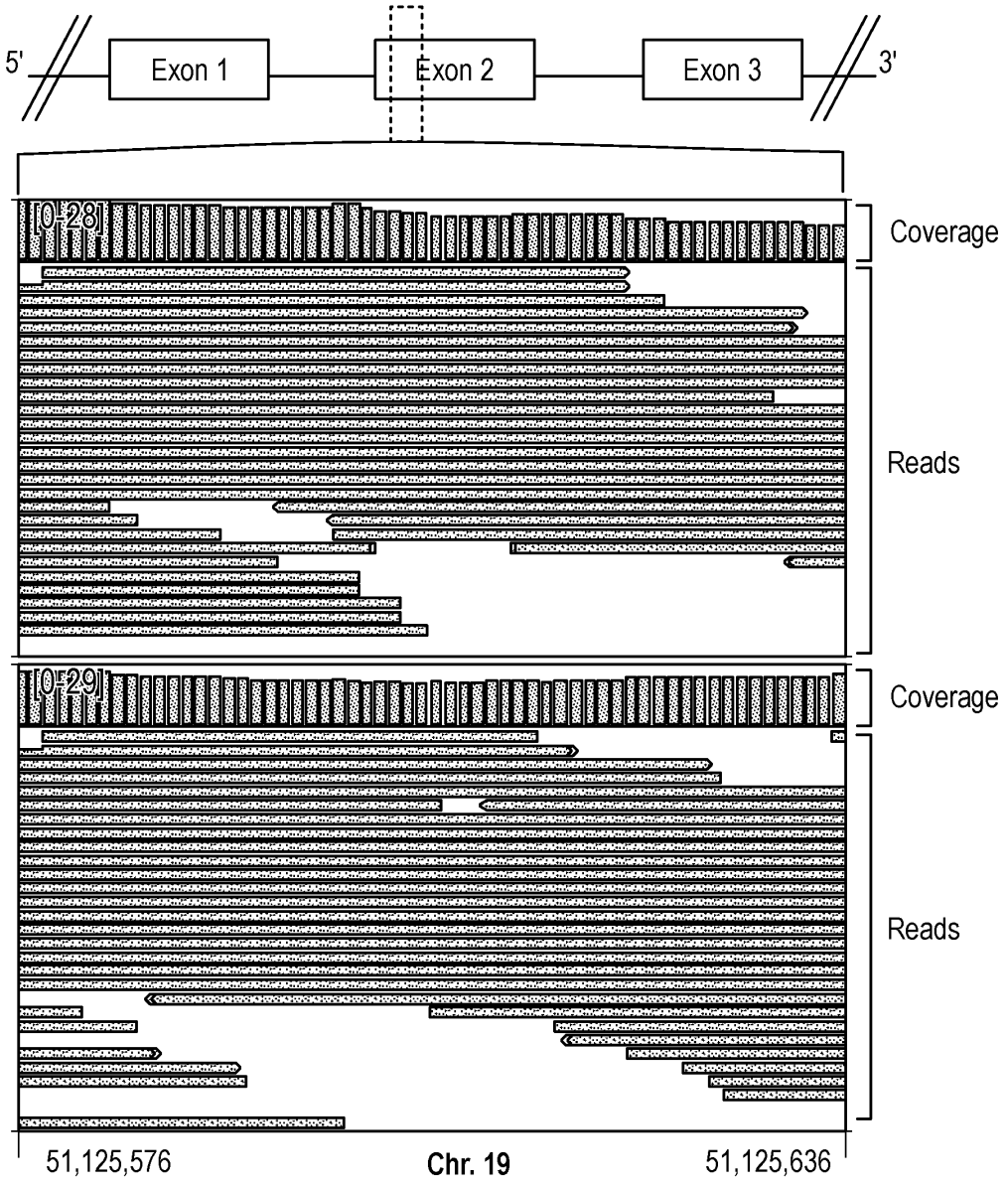

SIGLEC9
5'
Exon 1
Exon 2
Exon 3
3'
[0-28]
Coverage
Reads
[0-29]
Coverage
Reads
51,125,576
Chr. 19
51,125,636

FIG. 21B

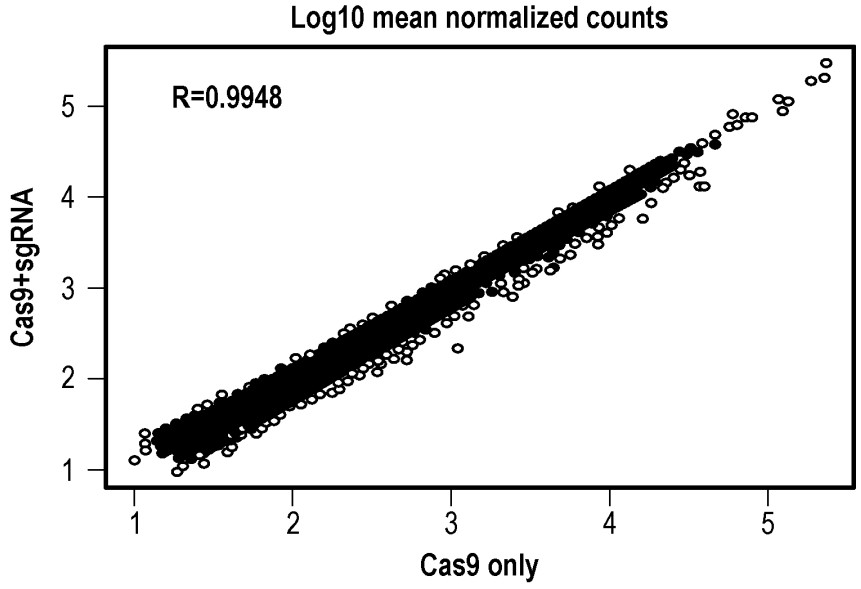


FIG. 21C

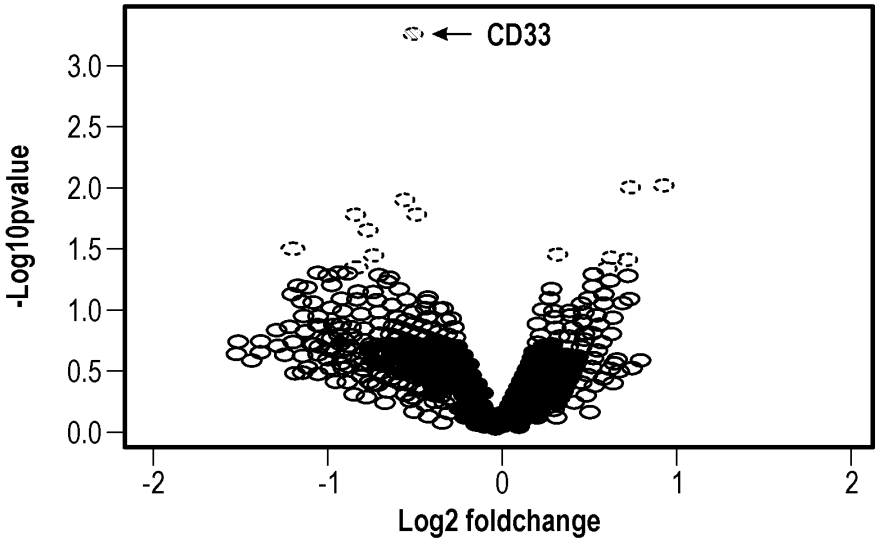


FIG. 21D

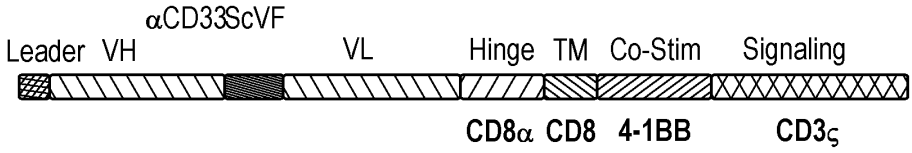


FIG. 22A

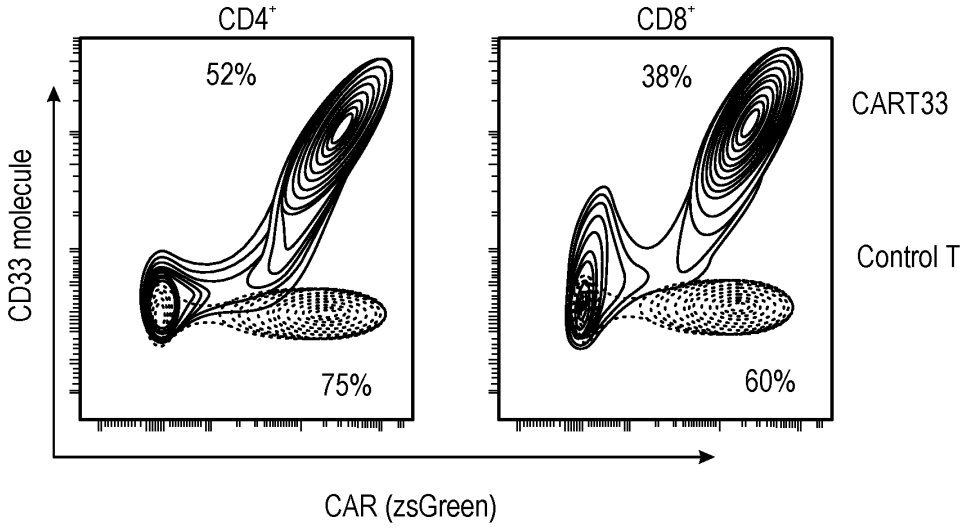


FIG. 22B

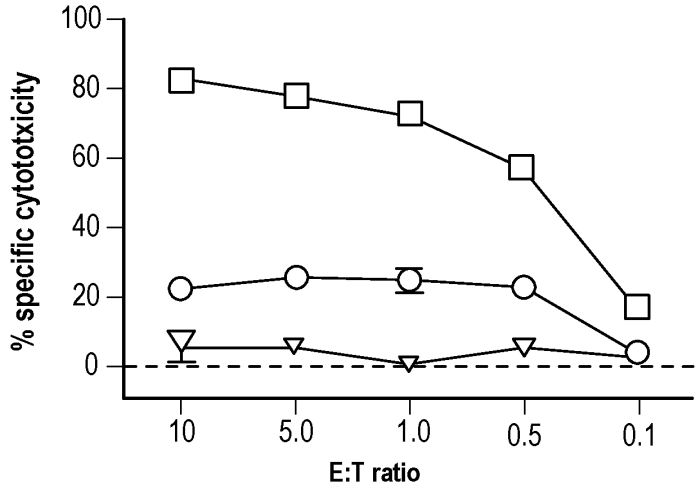


FIG. 22C

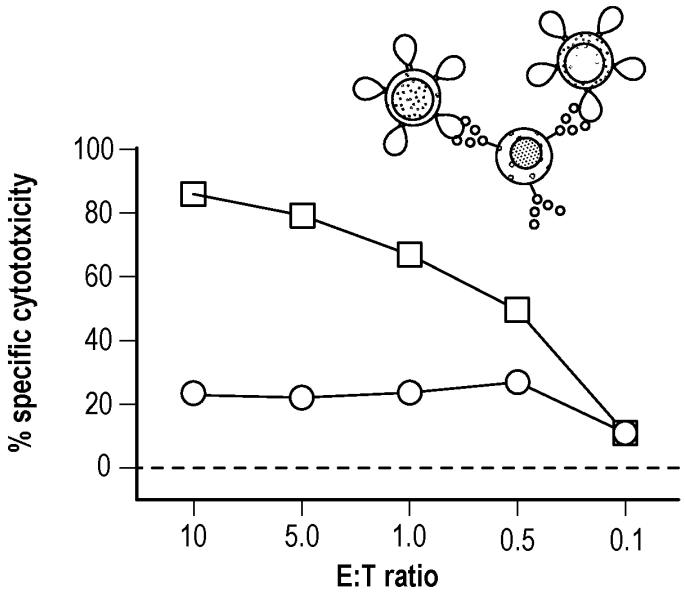


FIG. 22D

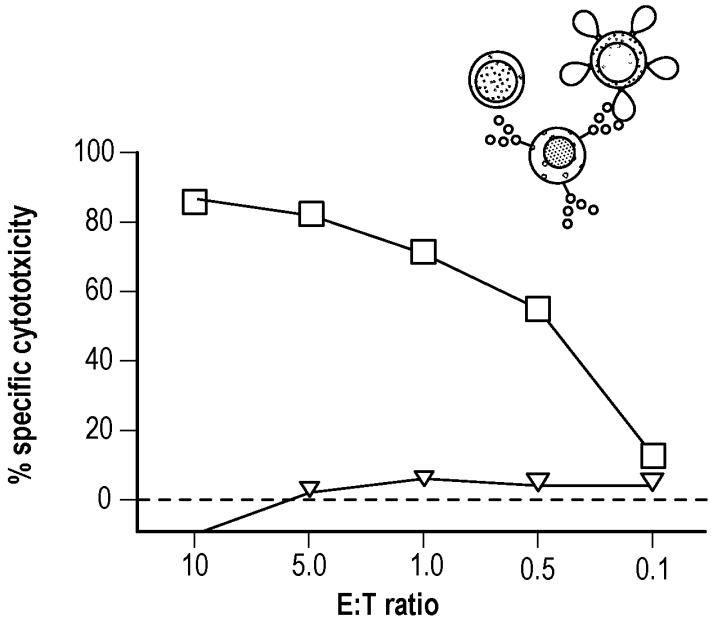


FIG. 22E

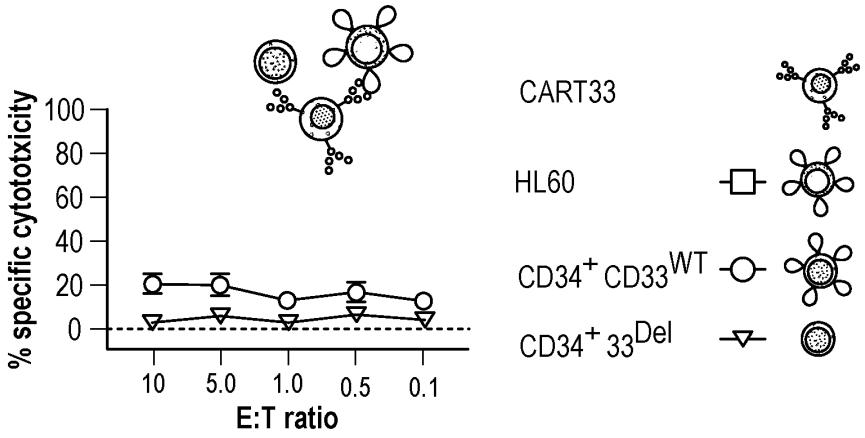


FIG. 22F

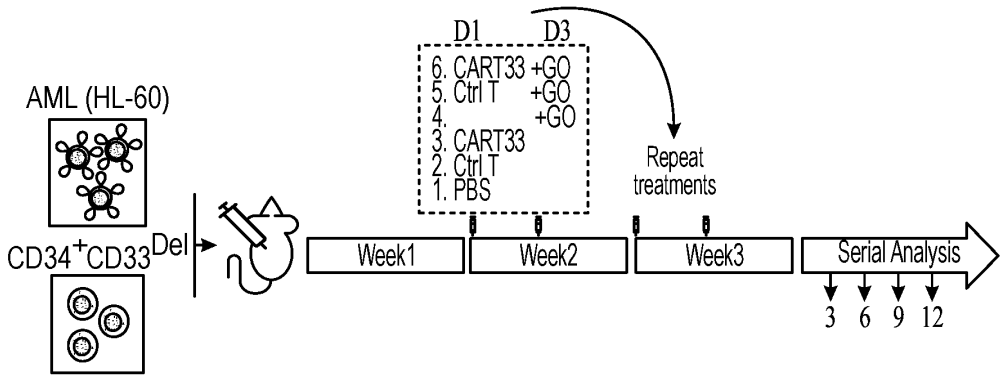
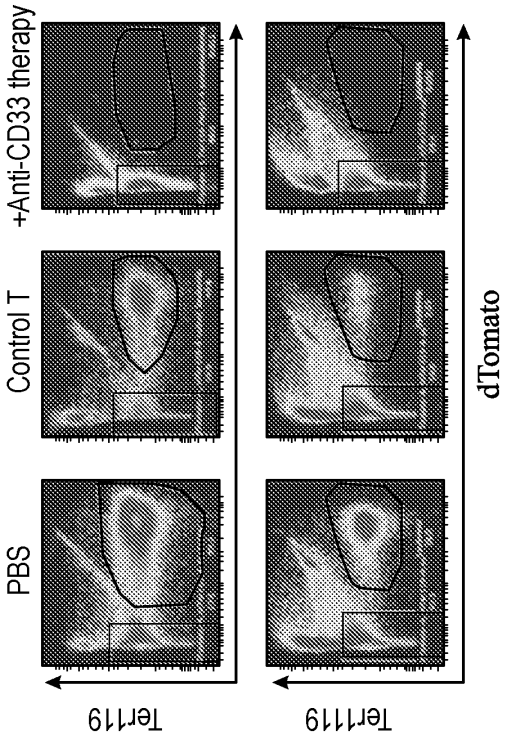


FIG. 23A


PBS
Control T
+Anti-CD33 therapy
Ter119
Ter119
dTomato

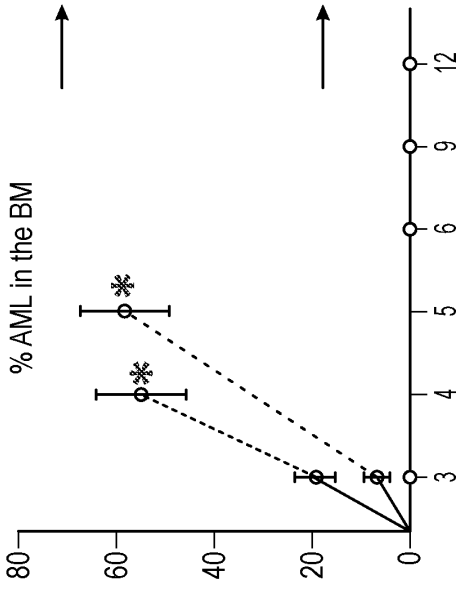

% AML in the BM
80
60
40
20
0
3
4
5
6
9
12

FIG. 23B

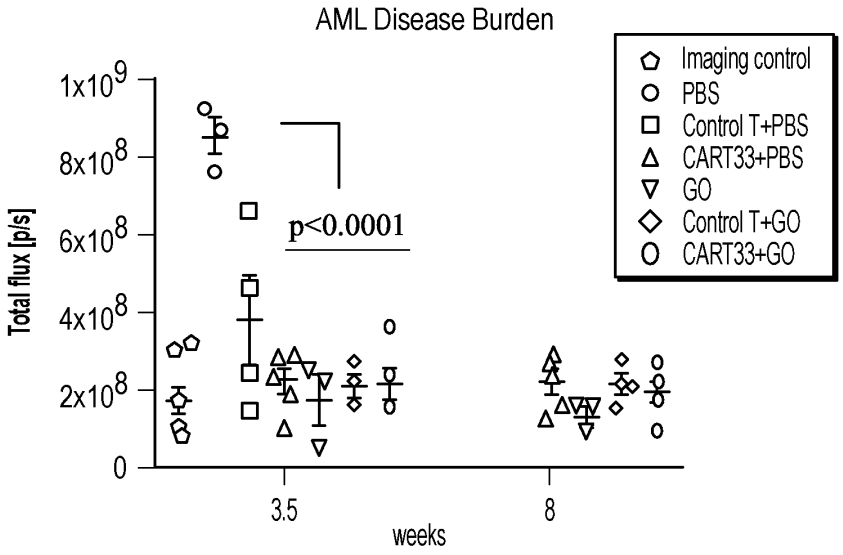

AML Disease Burden
Total flux [p/s]
1x10^9
8x10^8
6x10^8
4x10^8
2x10^8
0
p<0.0001
3.5
8
weeks
Imaging control
PBS
Control T+PBS
CART33+PBS
GO
Control T+GO
CART33+GO

FIG. 23C

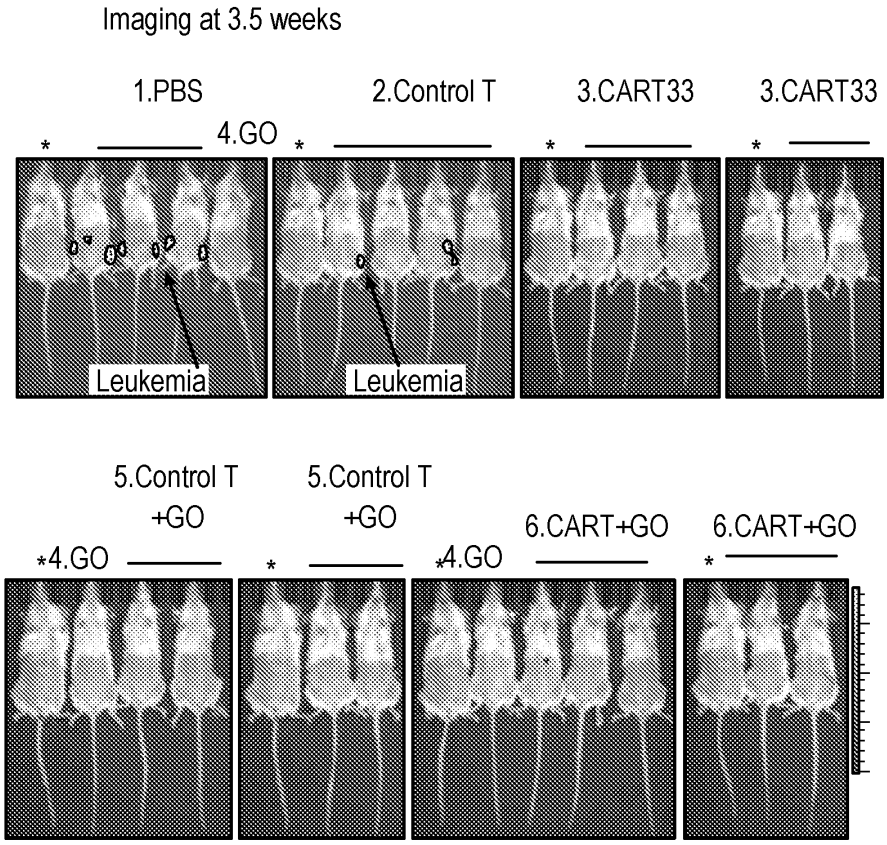


FIG. 23D

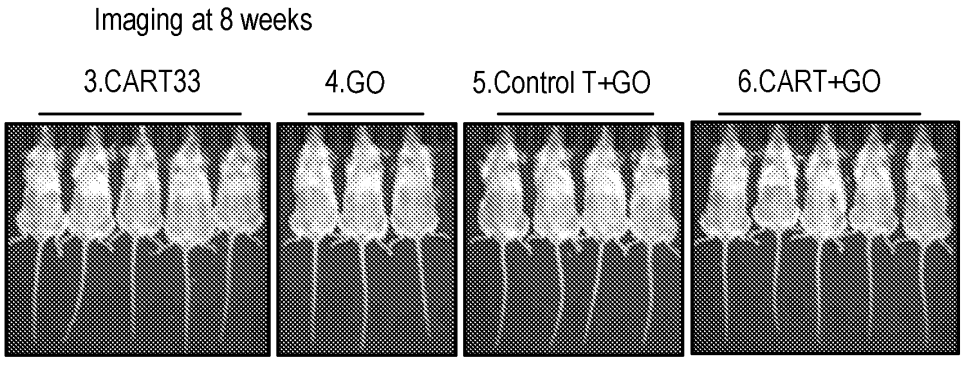


FIG. 23E

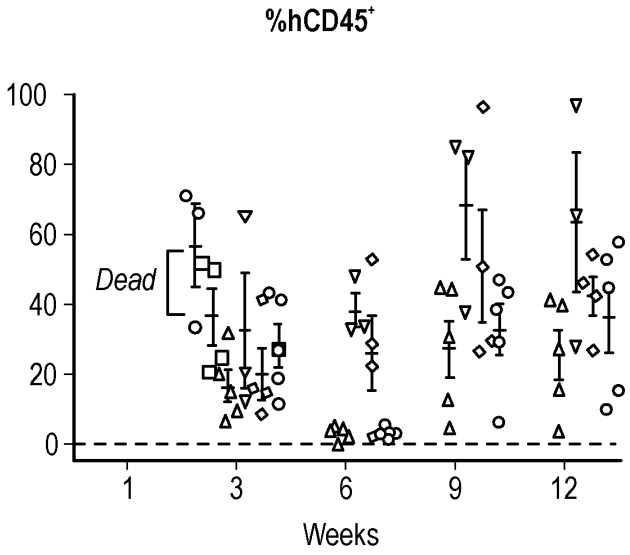


FIG. 23F

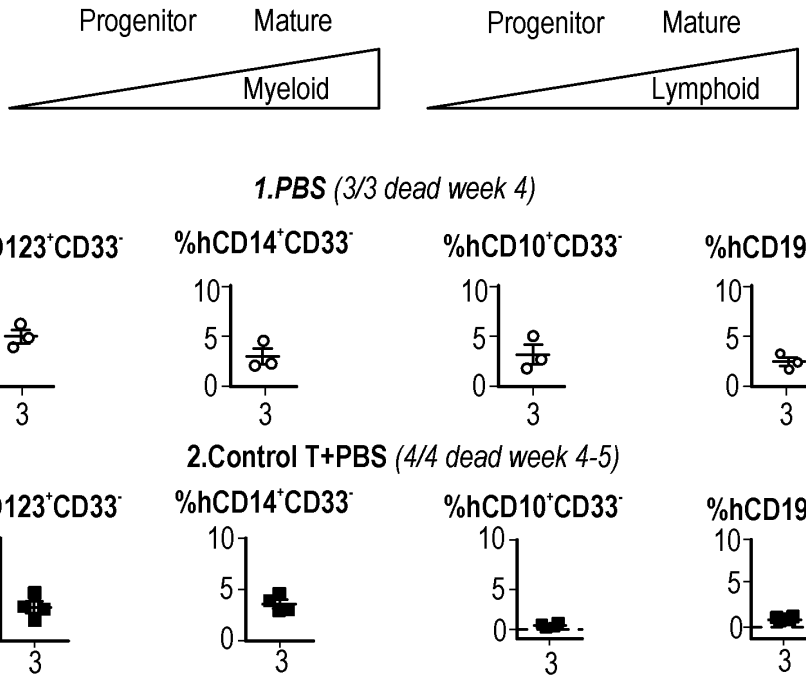


FIG. 24A

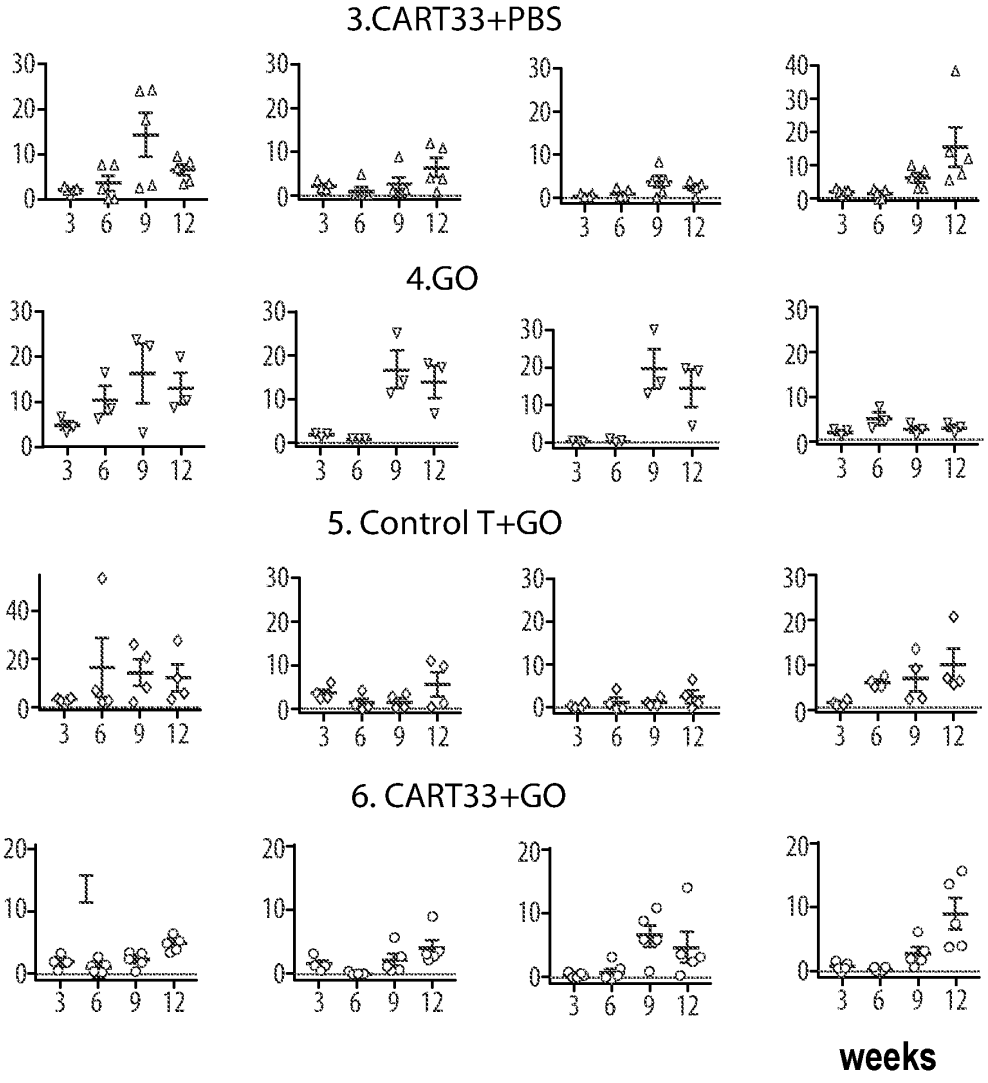


FIG. 24A (continued)

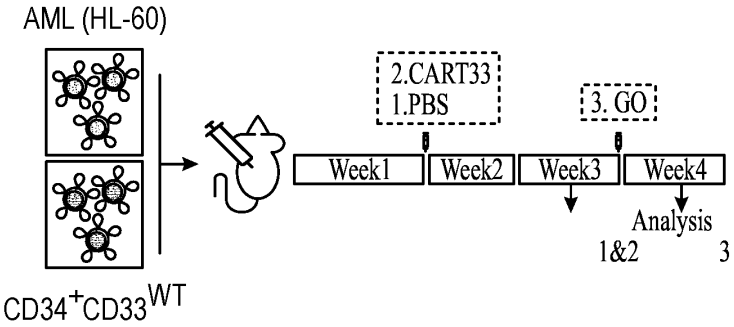


FIG. 24B

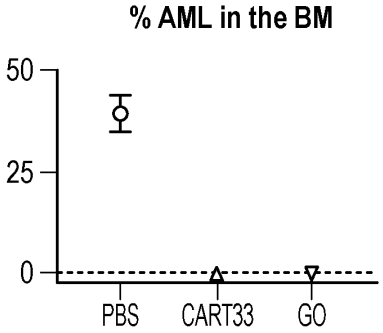


FIG. 24C

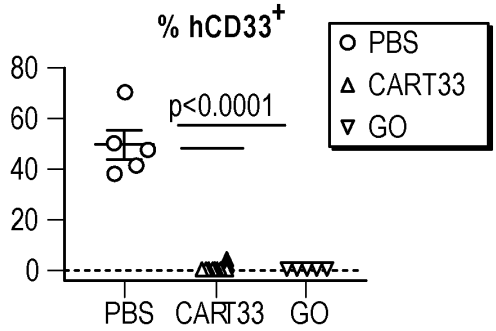


FIG. 24D

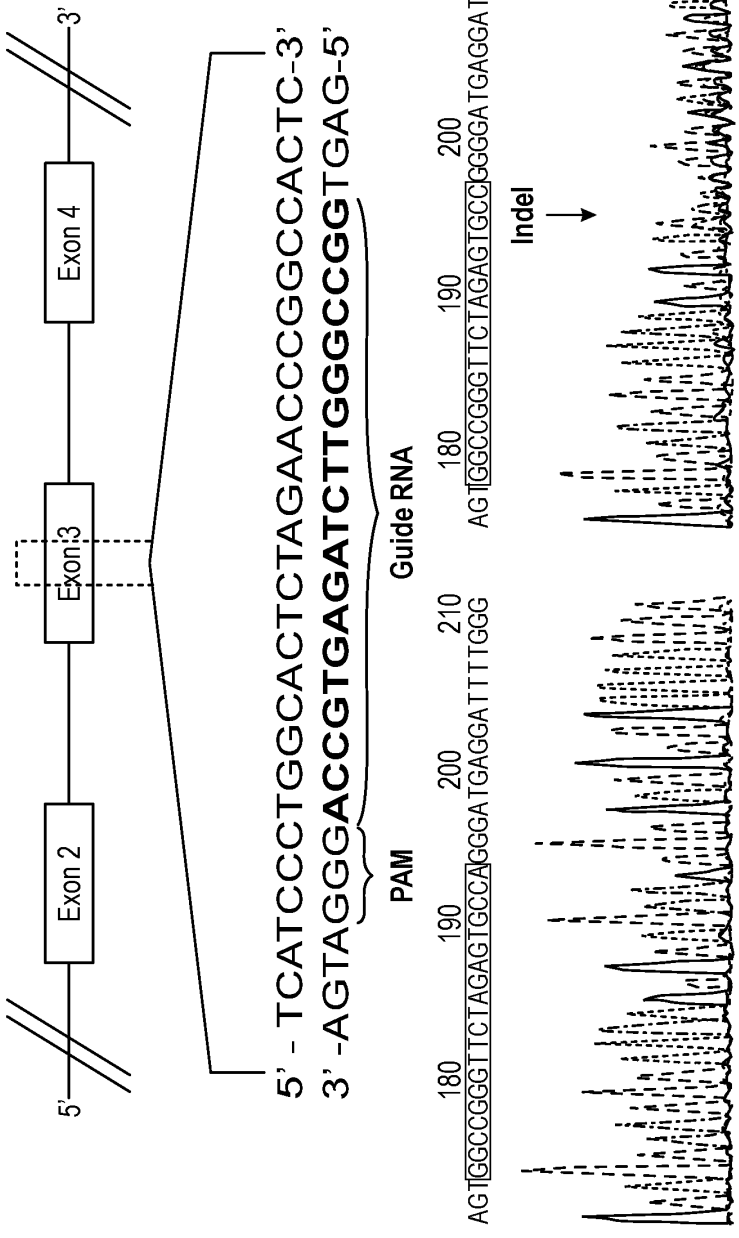

5'
Exon 2
Exon 3
Exon 4
3'
5' - TCATCCCTGGCACTCTAGAACCCGGCCACTC-3'
3' -AGTAGGGACCGTGAGATCTTGGGCCGGTGAG-5'
PAM
Guide RNA
180 190 200 210
AGTGGCCGGGTTCTAGAGTGCCAGGGATGAGGATTTTGGG
Indel
CD34+CD33WT
CD34+CD33Del

FIG. 25A

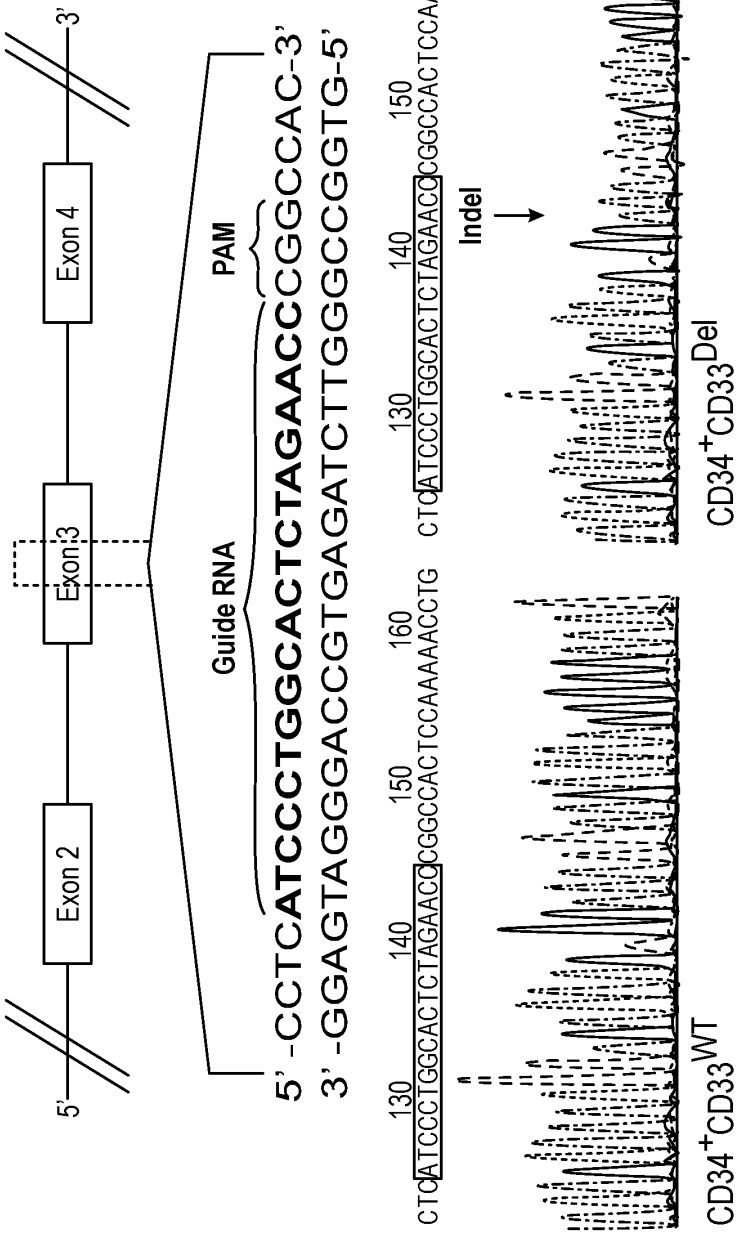

5'
3'
Exon 2
Exon 3
Exon 4
Guide RNA
PAM
5' -CCTCATCCCTGGCACTCTAGAACCCGGCCAC-3'
3' -GGAGTAGGGACCGTGAGATCTTGGGCCGGTG-5'
130 140 150 160
CTCATCCCTGGCACTCTAGAACCGGCCACTCCAAAAACCTG
Indel
CD34+CD33WT
CD34+CD33Del

FIG. 25B

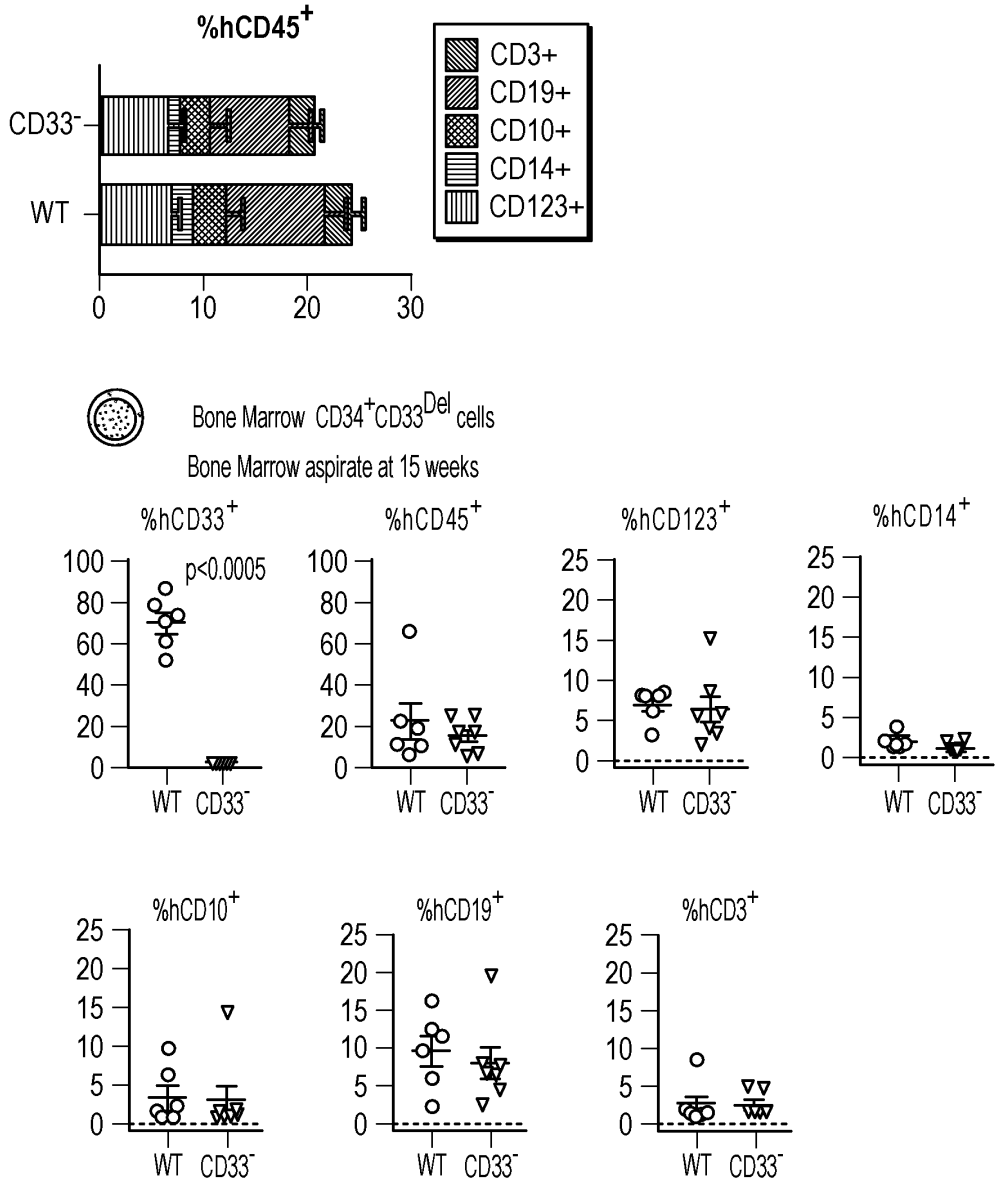

%hCD45+
CD33-
WT
0
10
20
30
CD3+
CD19+
CD10+
CD14+
CD123+
Bone Marrow CD34+CD33Del cells
Bone Marrow aspirate at 15 weeks
%hCD33+
p<0.0005
%hCD45+
%hCD123+
%hCD14+
%hCD10+
%hCD19+
%hCD3+
WT
CD33-

FIG. 26A

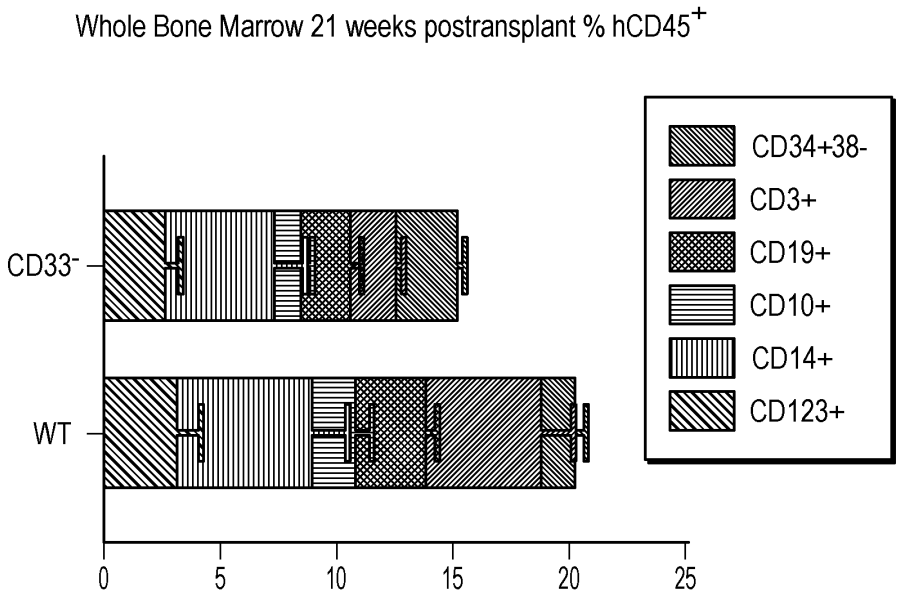
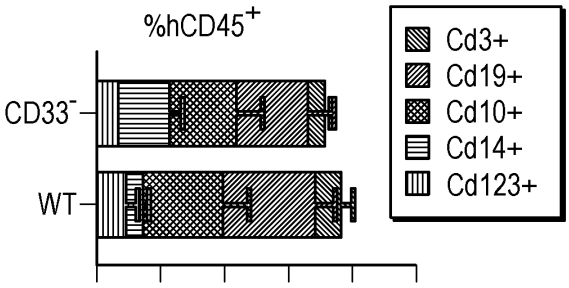

Whole Bone Marrow 21 weeks postransplant % hCD45+
CD33-
WT
0
5
10
15
20
25
CD34+38-
CD3+
CD19+
CD10+
CD14+
CD123+

FIG. 26B


%hCD45+
CD33-
WT
Cd3+
Cd19+
Cd10+
Cd14+
Cd123+

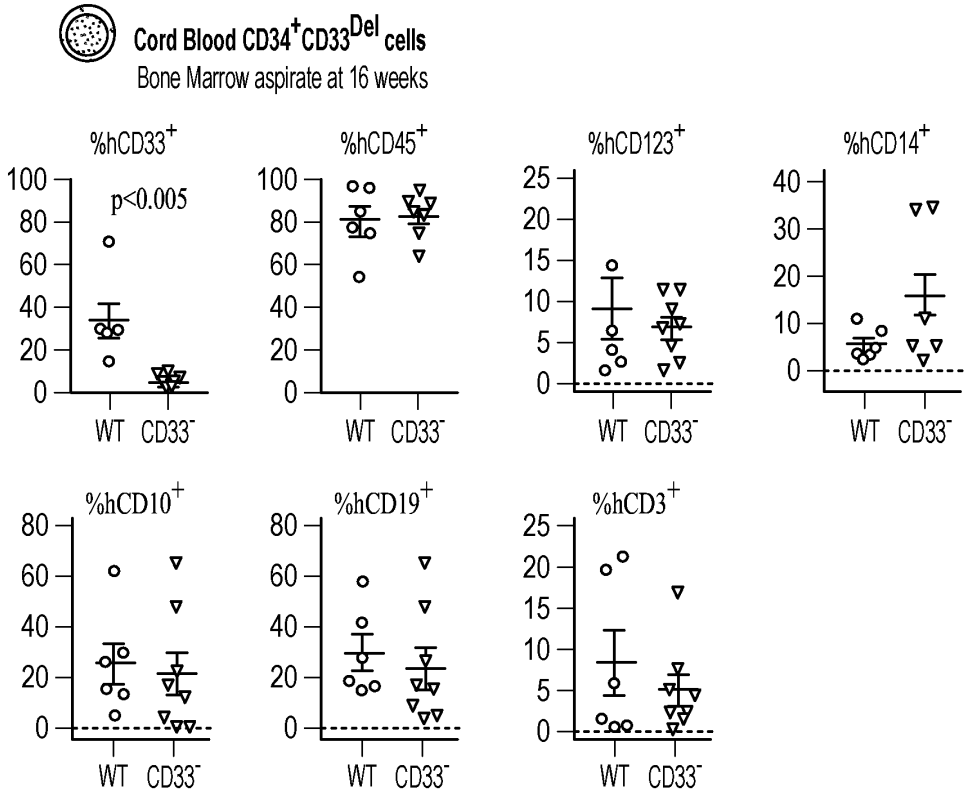

Cord Blood CD34+CD33Del cells
Bone Marrow aspirate at 16 weeks
%hCD33+
p<0.005
%hCD45+
%hCD123+
%hCD14+
%hCD10+
%hCD19+
%hCD3+
WT
CD33-

FIG. 26C

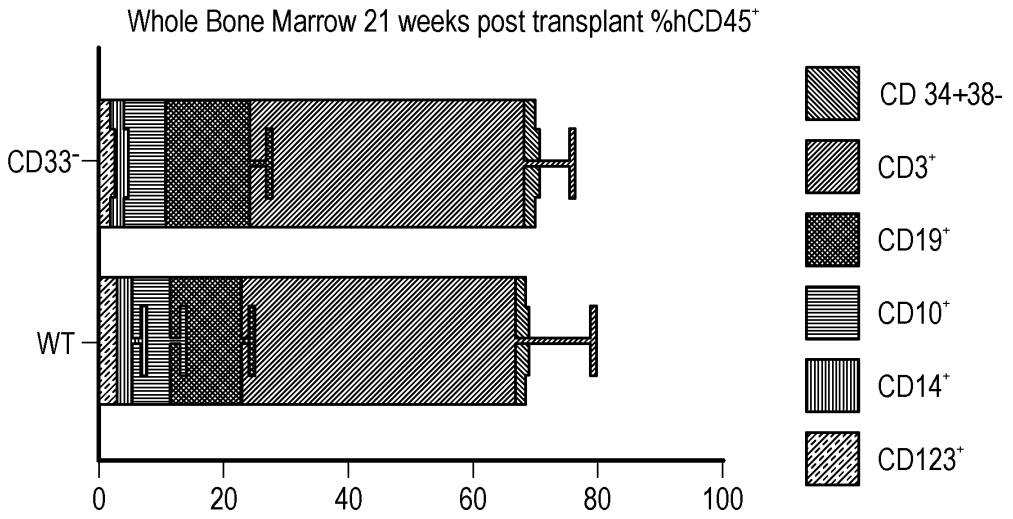

Whole Bone Marrow 21 weeks post transplant %hCD45+
CD33-
WT
0
20
40
60
80
100
CD 34+38-
CD3+
CD19+
CD10+
CD14+
CD123+

FIG. 26D

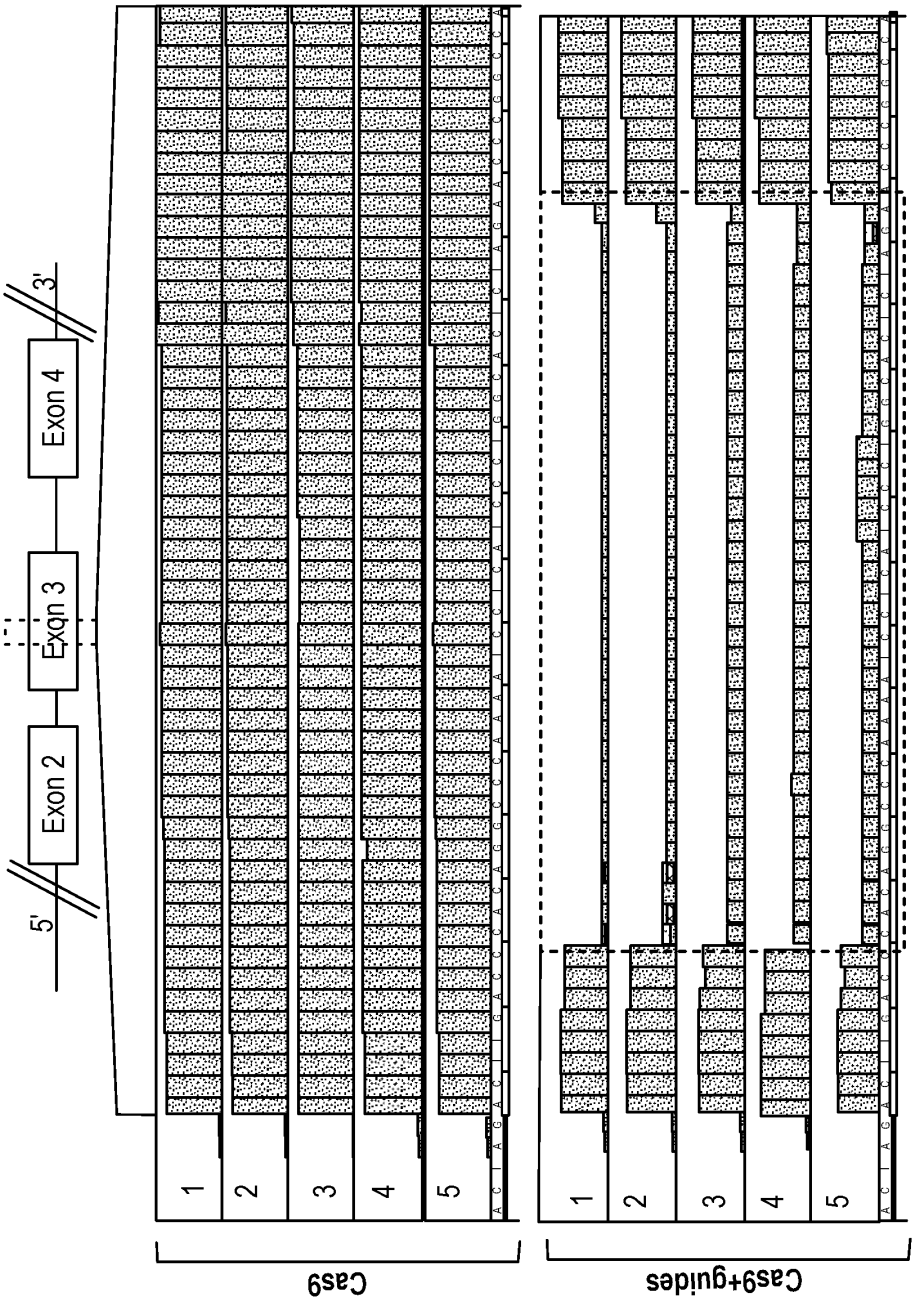


FIG. 27

	Cas9			Cas9+sgRNA		
Total PF Reads	629,724,054			659,744,702		
Percent Q30 Bases	93.46%			93.43%		
	SNVs	Insertions	Deletions	SNVs	Insertions	Deletions
Small Variants Summary						
Total Passing	3,743,126	401,796	418,883	3,744,331	401,386	418,187
Percent Found in dbSNP	94.24%	85.75%	86.70%	94.25%	85.82%	86.89%
Het/Hom Ratio	1.613	2.008	2.358	1.614	2	2.343
Ts/TV Ratio	2.063	-	-	2.063	-	-
Variants by Consequence						
Frameshifts	-	58	67	-	57	67
Non-Synonymous	10,248	144	192	10,300	157	191
Synonymous	11,638	-	-	11,625	-	-
Stop Gained	77	6	0	77	6	0
Stop Lost	19	0	1	19	0	1

FIG. 28

OFF TARGET SITE	MISMATCHES	CHROMOSOME	CUT SITE	PAM	GENE	Indels
ATCCCTGGCACTC**AG**GA**G**CC	3	chr9	129,640,094	AGG	ASB6	None
AA**A**CCCTGGC**T**CTCTA**A**AACC	3	chr12	112,995,250	AGG	RP1-71H24_1	None
ATCCCTGGCAC**C**CT**G**G**C**ACC	3	chr14	99,627,517	TGG		None
ATCCCTGGCACTC**C**AG**G**GCC	3	chr17	9,253,589	TGG	STX8	None
A**G**CCCTGGCA**G**TCT**G**GAACC	3	chr8	52,941,254	TGG		None
ATCCCA**G**GC**C**CTCTA**T**AACC	3	chr1	236,691,946	TGG	ACTN2	None
ATCC**A**TGGT**A**CTCT**G**GAACC	3	chr22	35,609,583	AGG	MB	None
AA**A**CCCA**G**GCACTCTA**G**CACC	3	chr2	203,480,837	AGG	RAPH1	None
A**C**CCCT**A**GCACTCTAGA**G**CC	3	chr1	26,560,691	AGG	RPS6KA1	None
A**C**CCCTGGCA**G**TCTAGA**G**CC	3	chr4	25,435,756	AGG		None
ATCCCTG**A**CACTCT**G**G**G**ACC	3	chr12	3,595,172	TGG		None
A**G**CCCT**C**GCACTCT**G**GAACC	3	chr14	74,598,895	AGG	LTBP2	None
ATC**T**CTGGCA**G**ACTAGAACC	3	chr5	94,207,405	AGG	KIAA0825	None
A**C**CCCTG**A**CACTCTAGAA**AG**	4	chr16	57,399,318	AGG		None
ATCCCTGGCA**C**ACT**G**G**C**CC	4	chr2	106,853,582	AGG	ST6GAL2	None
ATC**A**CTTG**T**CT**C**C**C**TAGAACC	4	chr9	125,259,035	TGG		None
ATC**C**CTG**A**C**C**CTC**C**AGAACA	4	chr9	131,545,888	TGG	RP11-40A7.2	None
A**C**CCCTGGCT**C**C**C**TAGA**G**CC	4	chr10	125,810,087	TGG	UROS	None
CTGCCTGGC**T**CTCTAG**C**ACC	4	chr11	205,054	TGG	BET1L	None
GTCTCTTGGCACTCTAG**GC**CC	4	chr11	2,689,716	AGG	KCNQ1	None

FIG. 29

OFF TARGET SITE	MISMATCHES	CHROMOSOME	CUT SITE	PAM	GENE	Indels
CCTCAC**A**AGAT**TA**GACCCAC	3	chr12	86,341,556	TGG	MGAT4C	None
TCTCACT**GC**CTTGACCCAC	3	chr9	134,687,890	TGG	COL5A1	None
CCTCACT**G**GACTTGAC**T**CA**G**	3	chr1	209,621,235	GGG	LAMB3	None
CCTCACT**G**GAC**AA**GACCCAC	3	chr12	19,574,394	TGG	AEBP2	None
CCTCACTAGACT**A**GAT**C**CA**T**	3	chr9	973,903	TGG		None
CCTC**G**CTAG**C**CTT**T**ACCCAC	3	chr7	157,876,963	AGG	PTPRN2	None
CCTCAC**A**AGAC**AA**GACCCAC	3	chr8	62,858,829	TGG	NKAIN3	None
C**T**TCAC**C**AG**C**CTTGACCCAC	3	chr19	51,125,605	AGG	SIGLEC9	None
CCTCAC**C**AG**C**C**C**TGACCCAC	3	chr19	51,143,004	AGG	SIGLEC7	None
C**T**TCAC**C**AG**C**CTTGACCCAC	3	chr19	51,144,414	AGG	SIGLEC7	None
CCTCAC**C**AG**C**C**C**TGACCCAC	3	chr19	51,185,448	AGG	SIGLEC20P	None
CCTCAC**C**AG**C**C**C**TGACCCAC	3	chr19	51,211,800	AGG	SIGLEC22P	None
CCTCAC**C**AG**C**C**C**TGACCCAC	3	chr19	51,501,106	AGG	SIGLEC12	None
CCTCAC**C**AG**C**C**C**TGACCCAC	3	chr19	51,509,057	AGG	SIGLEC27P	None
CCTCAC**C**AG**C**C**C**TGACCCAC	3	chr19	51,530,952	AGG	SIGLEC6	None
TCACACT**GC**CTTGACCCAC	4	chr9	130,997,236	TGG		None
CCT**T**ACTAG**C**CT**C**CACCCAC	4	chr5	127,098,351	GGG		None
CCTCAC**AA**GAT**AA**GACCCAC	4	chr2	223,246,492	TGG		None
CCT**A**ACTAGAA**A**TTG**C**C**A**CAC	4	chr2	228,144,164	AGG	SPHKAP	None
CCTC**TA**T**A**TACTTG**T**CCCAC	4	chr1	46,408,719	AGG	FAAH	None

FIG. 30

Gene	Log2 Fold Change	Pvalue	Padj	Description
CD33	-0.837	0.000455048	0.999	Myeloid call surface antigen CD33
CDON	1.033	0.009579405	0.999	Call adhesion molecule-related
AC027279.1	0.824	0.01015653	0.999	Non-coding RNA
LDLR	-0.563	0.012597131	0.999	Low-density lipoprotein receptor
PRAME	-0.87	0.016800466	0.999	Melanoma antigen preferentially expressed in tumors
INSIG1	-0.494	0.017206864	0.999	Insulin-induced gene 1 protein
HMGB1P31	-0.797	0.022454929	0.999	Non-coding RNA
AP005019.1	-1.261	0.032232654	0.999	Non-coding RNA
PCLAF	0.382	0.035486149	0.999	PCNA-associated factor:PCLAF
CD22	-0.76	0.03640001	0.999	B-cell receptor CD22
TPTE2	0.712	0.037739962	0.999	Phosphatidylinositor 3,4,5-trisphosphate 3-phosphatase TPTE2
ZNF469	0.811	0.039222723	0.999	Zinc finger protein 469
SVOPL	-0.867	0.04599215	0.999	Putative transporter SVOPL
FFAR4	0.678	0.046205968	0.999	Free fatty acid receptor 4

FIG. 31

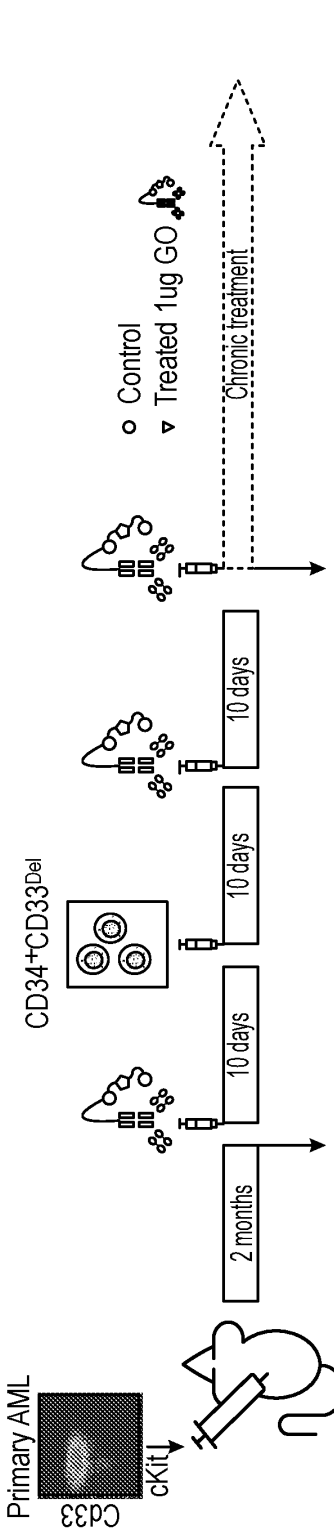


FIG. 32A

FIG. 32B

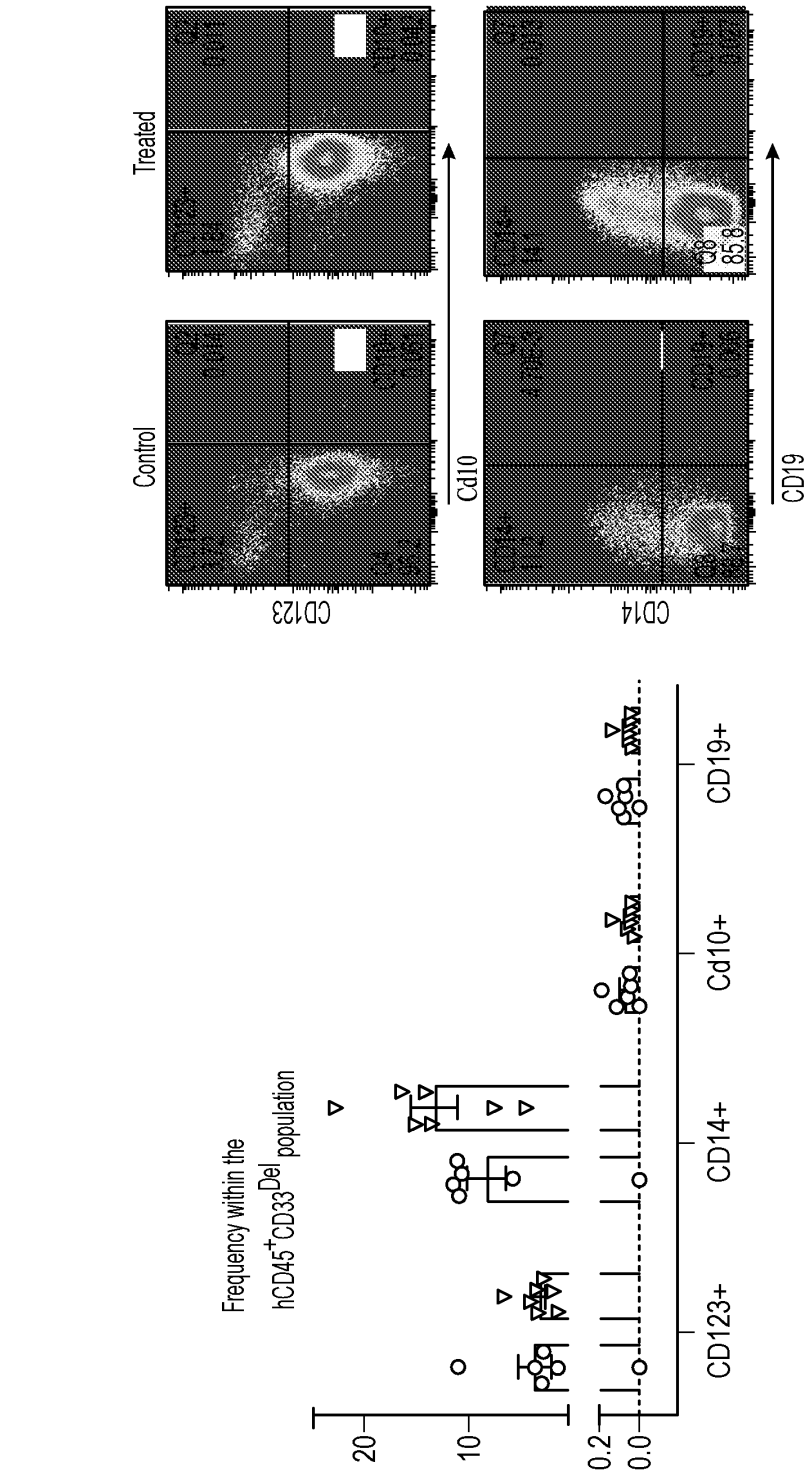


FIG. 32B (Continued)

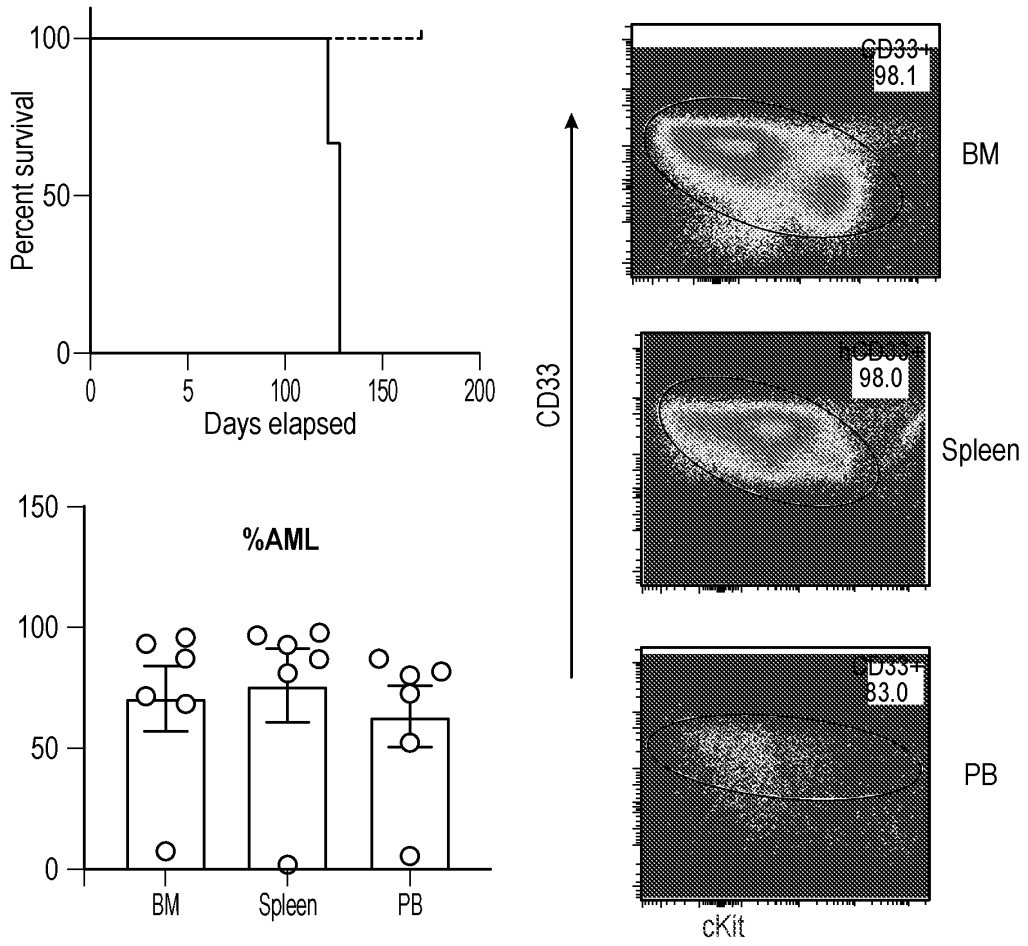

100
50
0
Percent survival
0
5
100
150
200
Days elapsed
150
100
50
0
%AML
BM
Spleen
PB
CD33
CD33+
98.1
BM
98.0
Spleen
83.0
PB
cKit

FIG. 32C

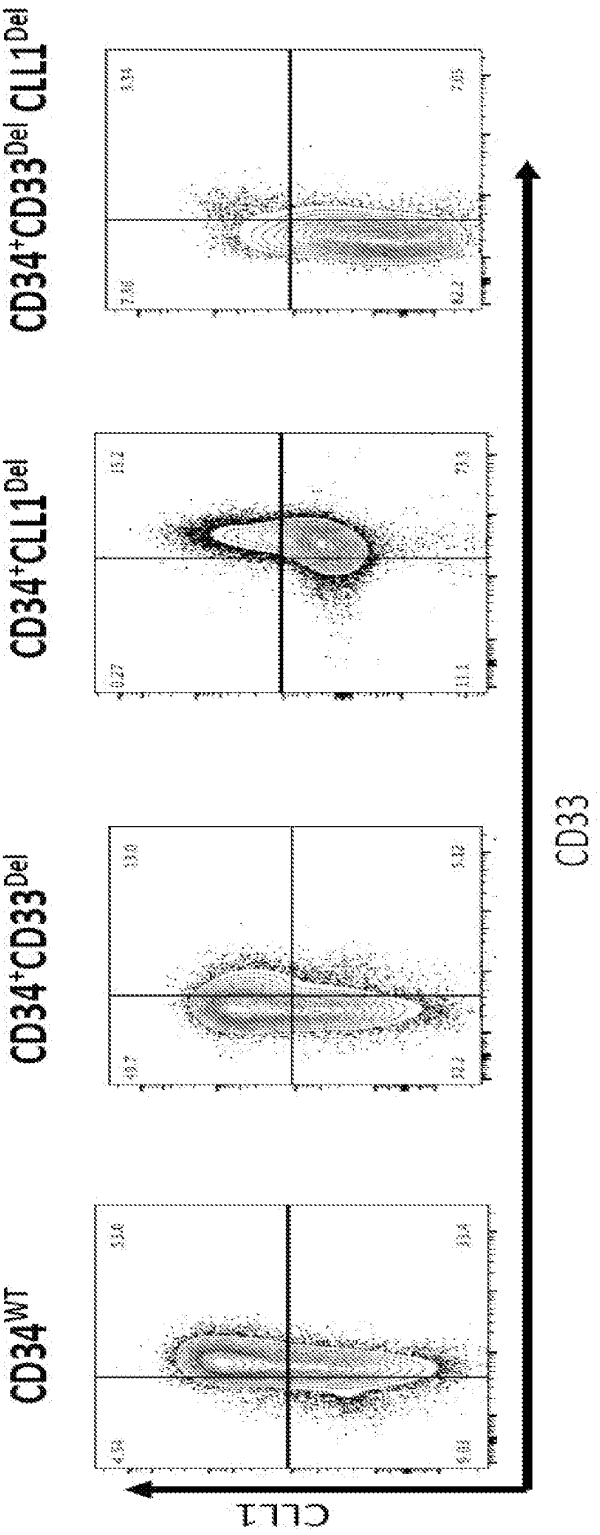

CD34WT
CD34+CD33Del
CD34+CLL1Del
CD34+CD33Del CLL1Del
CLL1
CD33

FIG. 33A

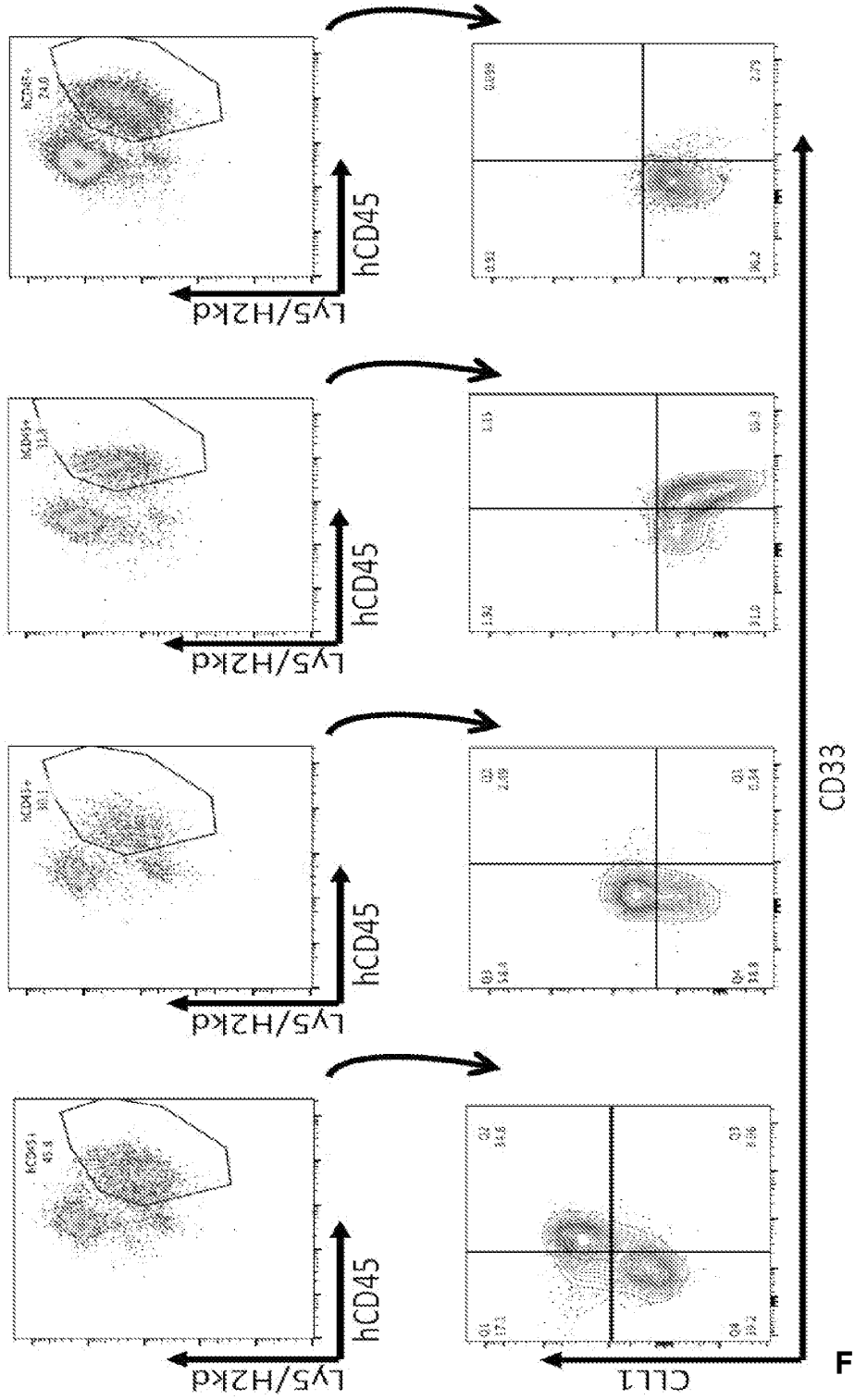


FIG. 33B

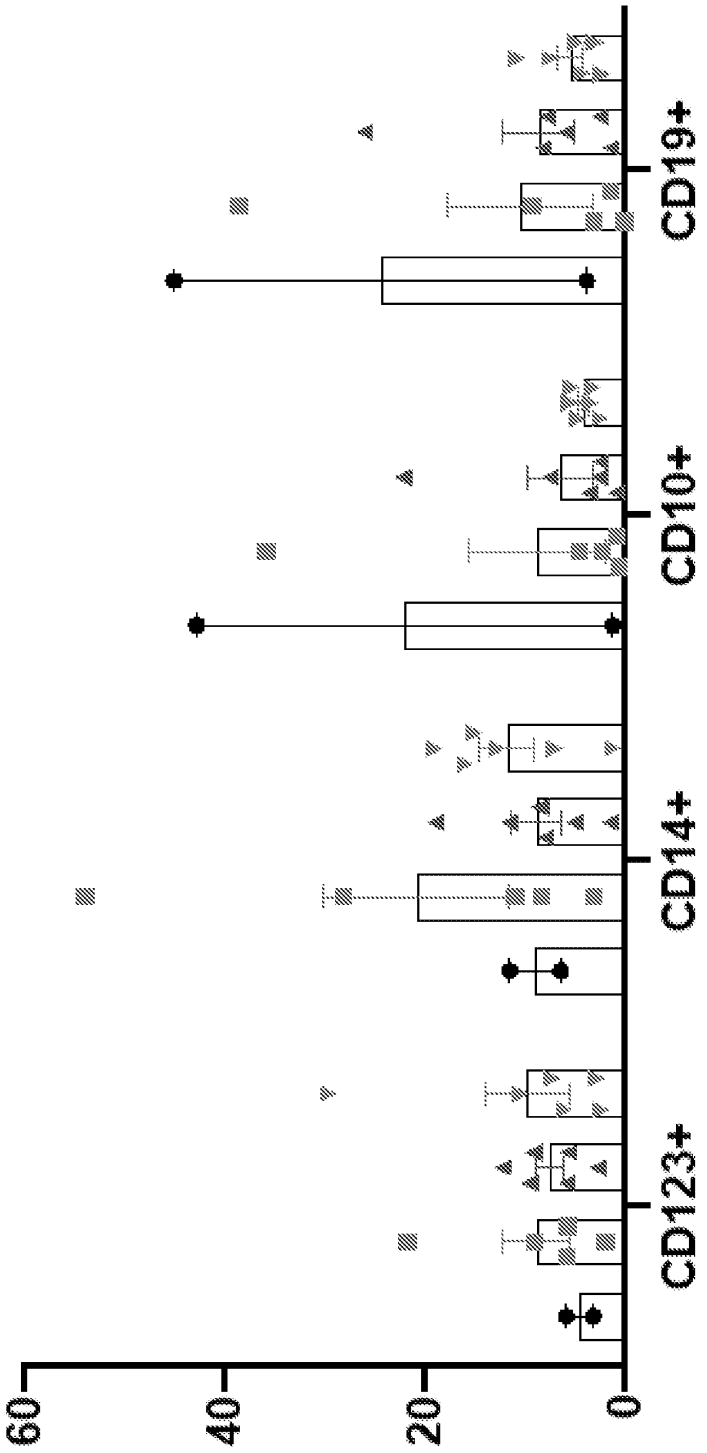


FIG. 33C

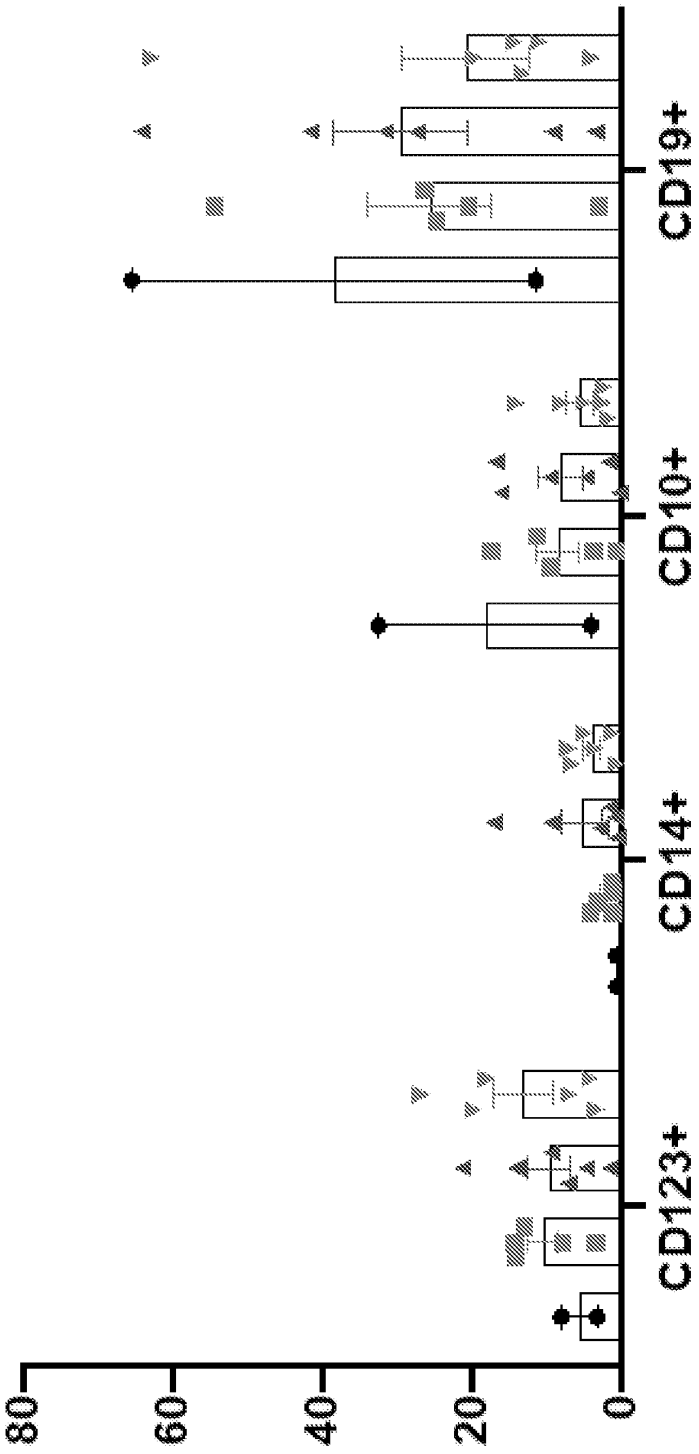


FIG. 33D