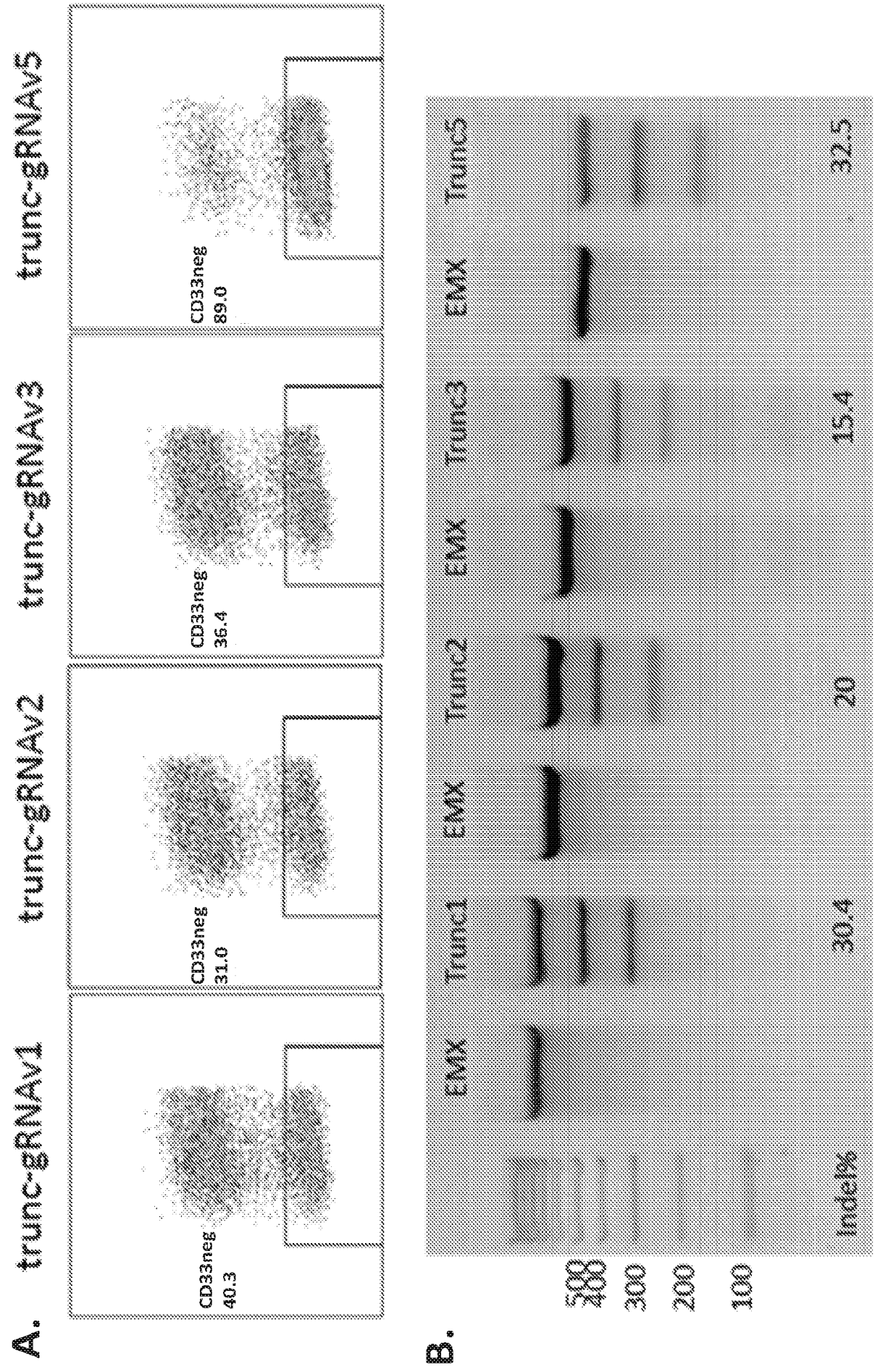
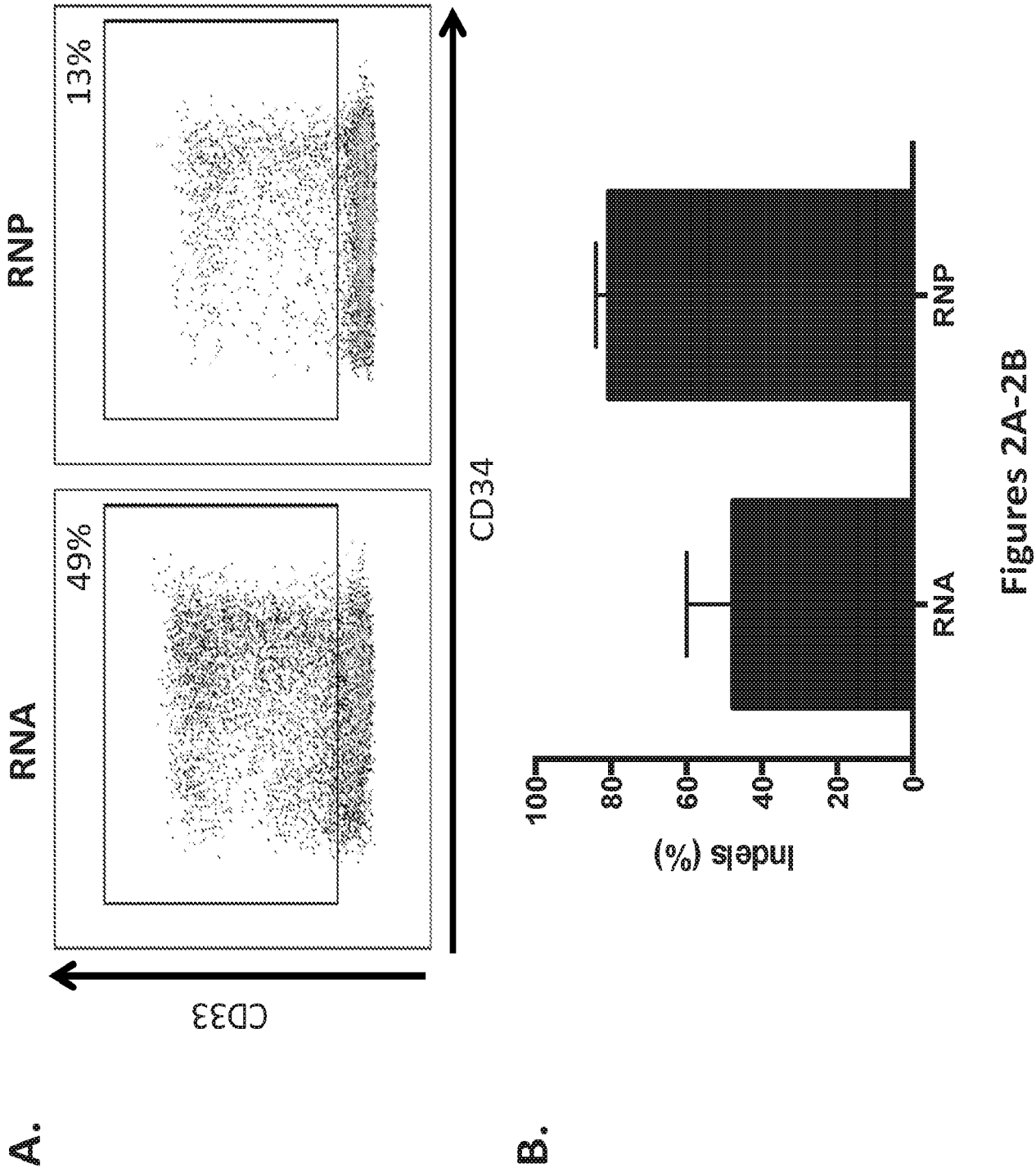
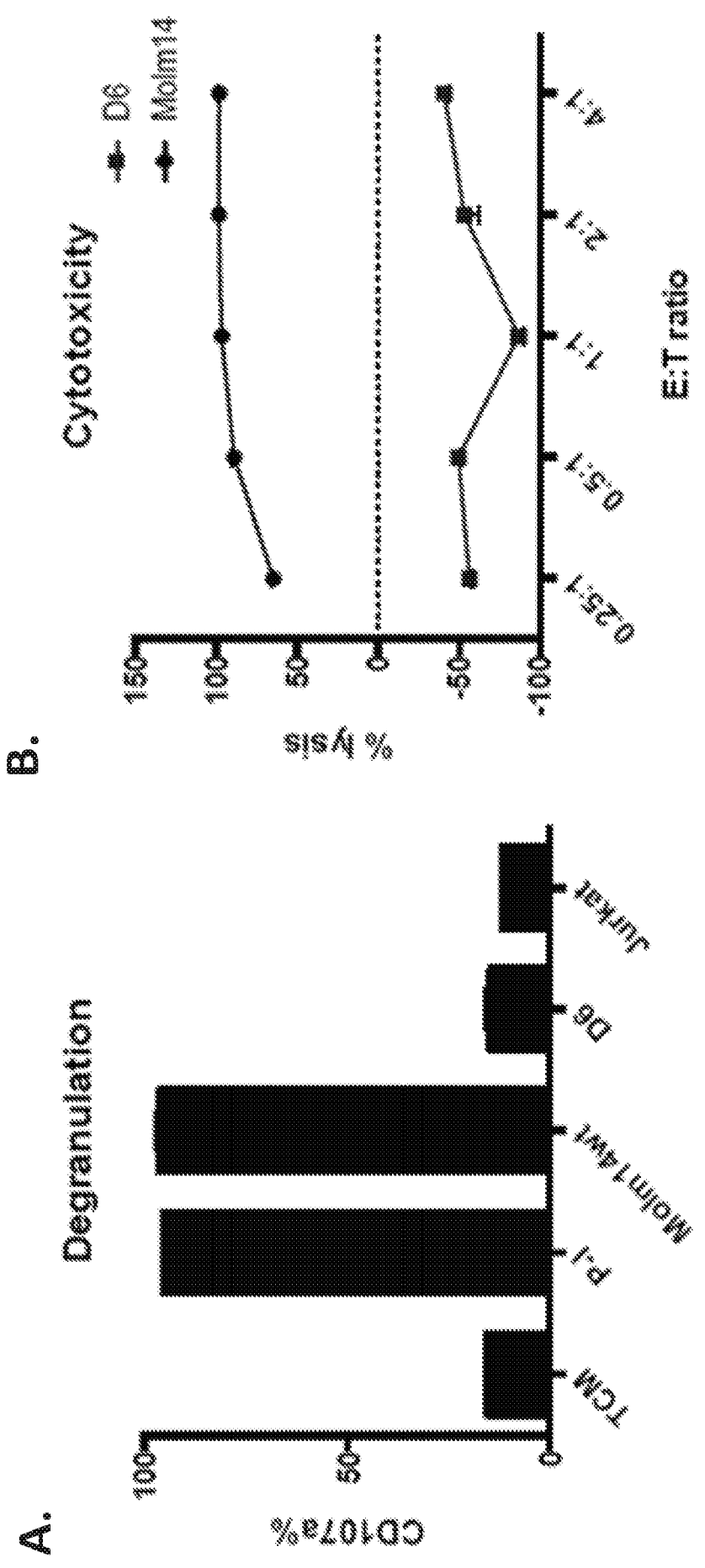


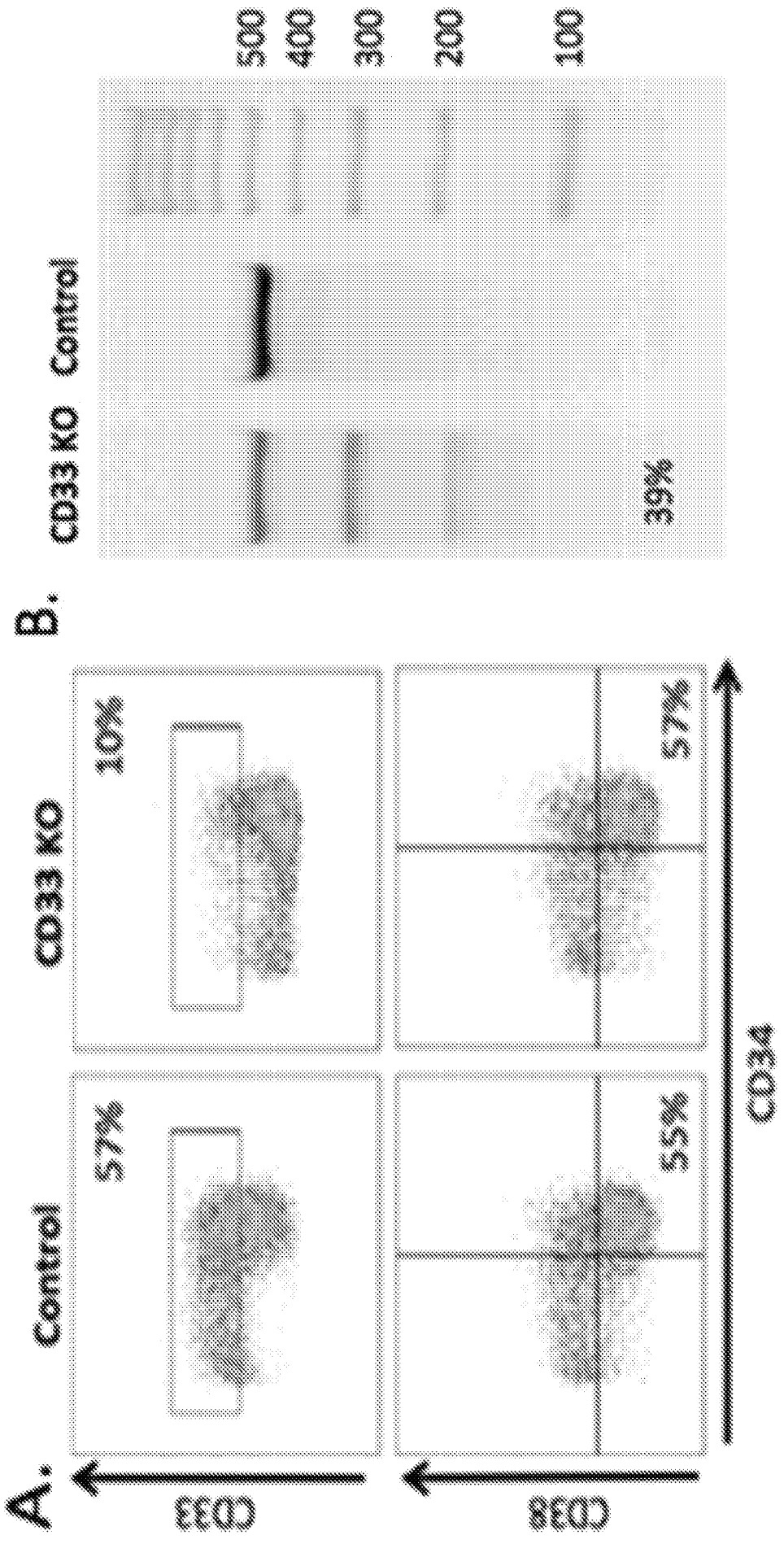
Figures 1A-1B



Figures 2A-2B



Figures 3A-3B



Figures 4A-4B

Figure 4C

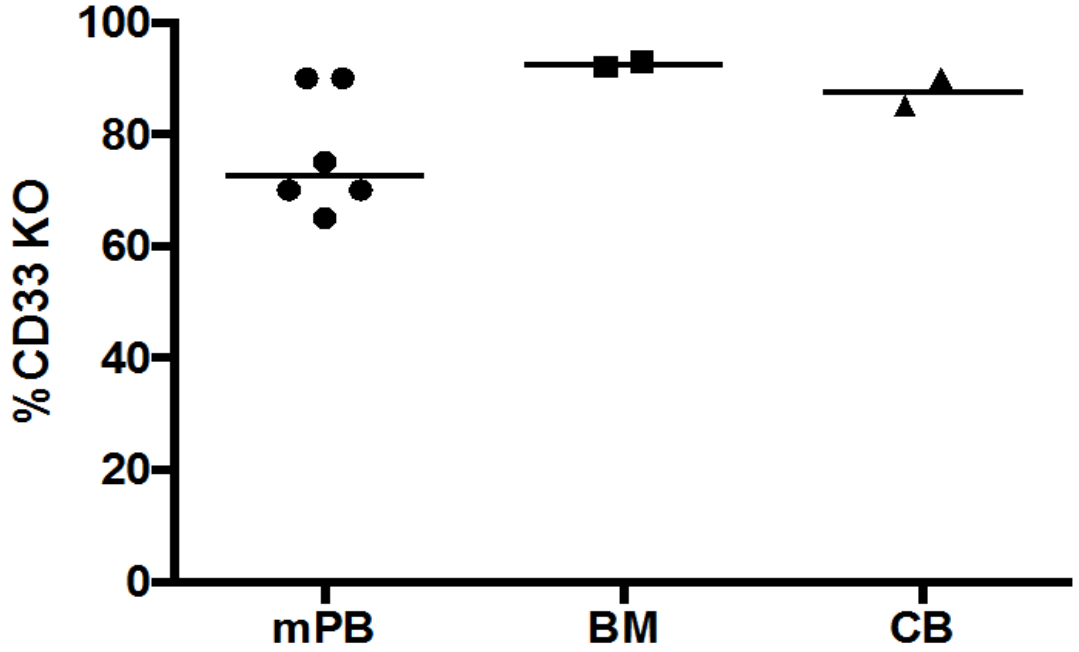
(SEQ ID NO: 3)

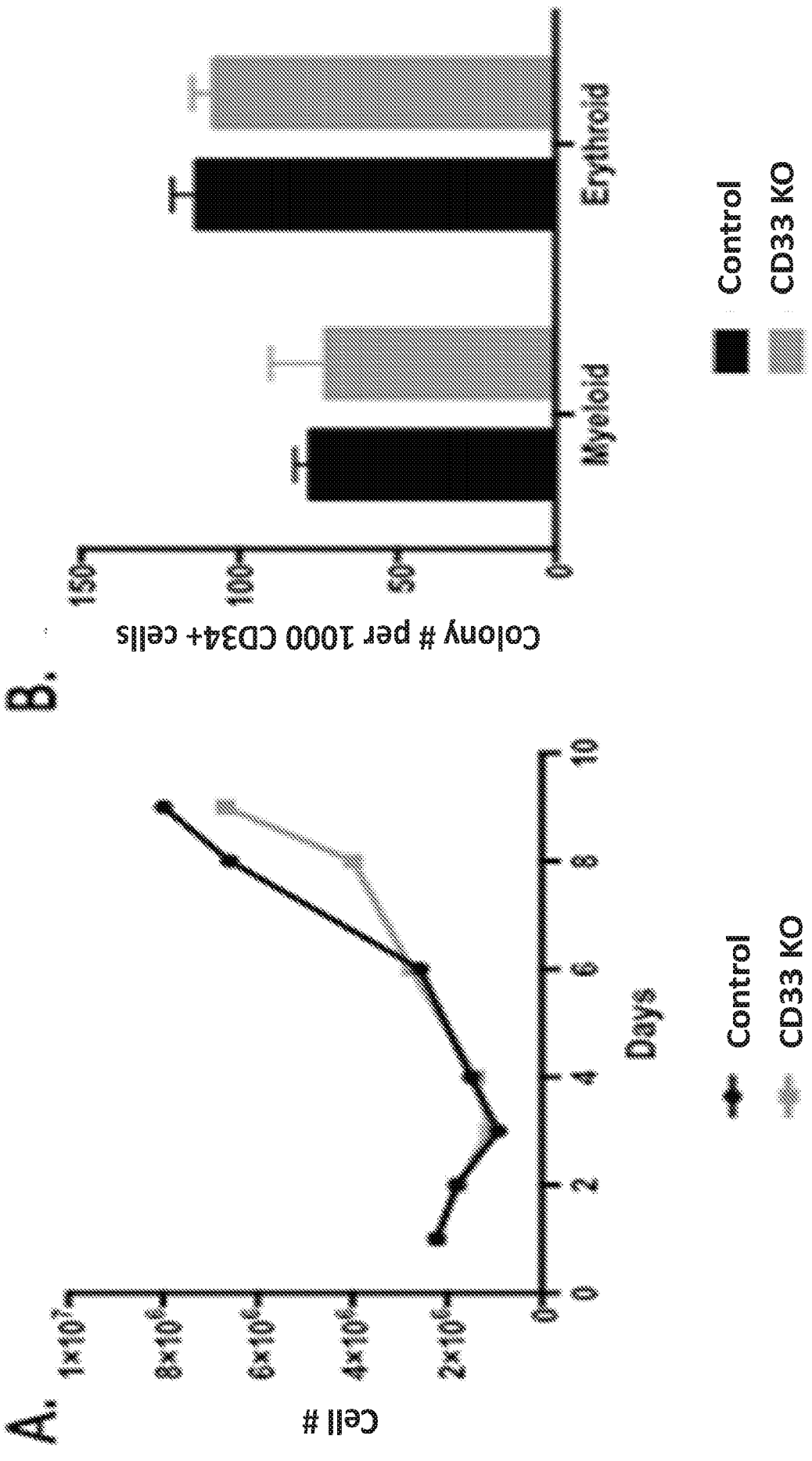
CD33-gRNA ▼ PAM

5’-GCAGGAGTCAGTGACGGTACAGGAGGGTTTGTGCGTCC-3’ (SEQ ID NO: 4)

wt	GCAGGAGTCAGTGACGGTACA GGAGGGTTTGTGCGTCC	(SEQ ID NO: 4)
+1	GCAGGAGTCAGTGACGGTACAAGGAGGGTTTGTGCGTCC	(SEQ ID NO: 5)
D2	GCAGGAGTCAGTGACGGTA - - GGAGGGTTTGTGCGTCC	(SEQ ID NO: 6)
D14	GCAGGAGTCAGTGACGGT - - - - - - - - - - - GCGTCC	(SEQ ID NO: 7)
D15	GCAGGAGTCAGTGACG - - - - - - - - - - - TGCGTCC	(SEQ ID NO: 8)
D18	GCAGGAG - - - - - - - - - - - - - - - - - GTTTGTGCGTCC	(SEQ ID NO: 9)
D20	GCAGGAGTCAGTG - - - - - - - - - - - - - - - CGTCC	(SEQ ID NO: 10)

Figure 4D





Figures 5A-5B

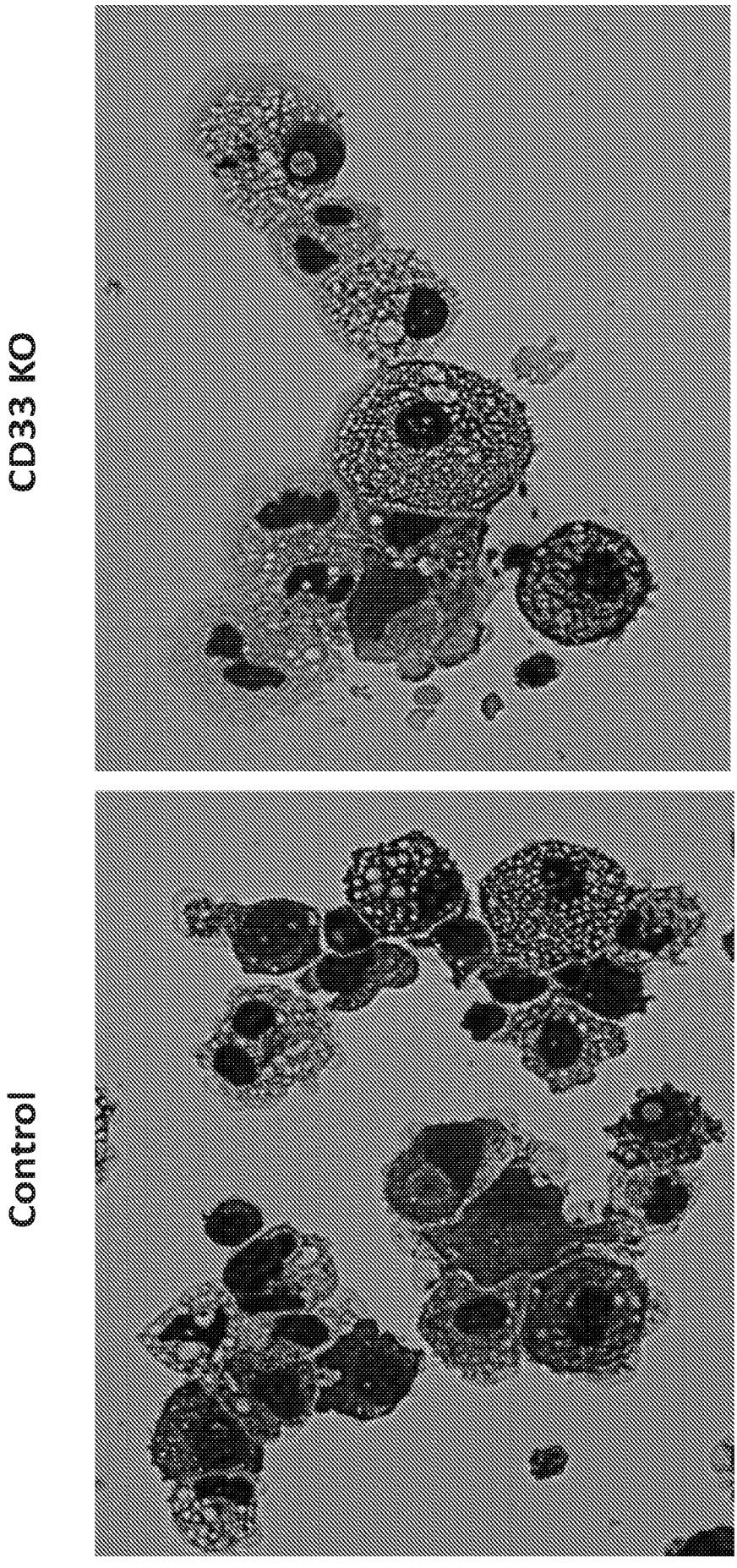


Figure 5C

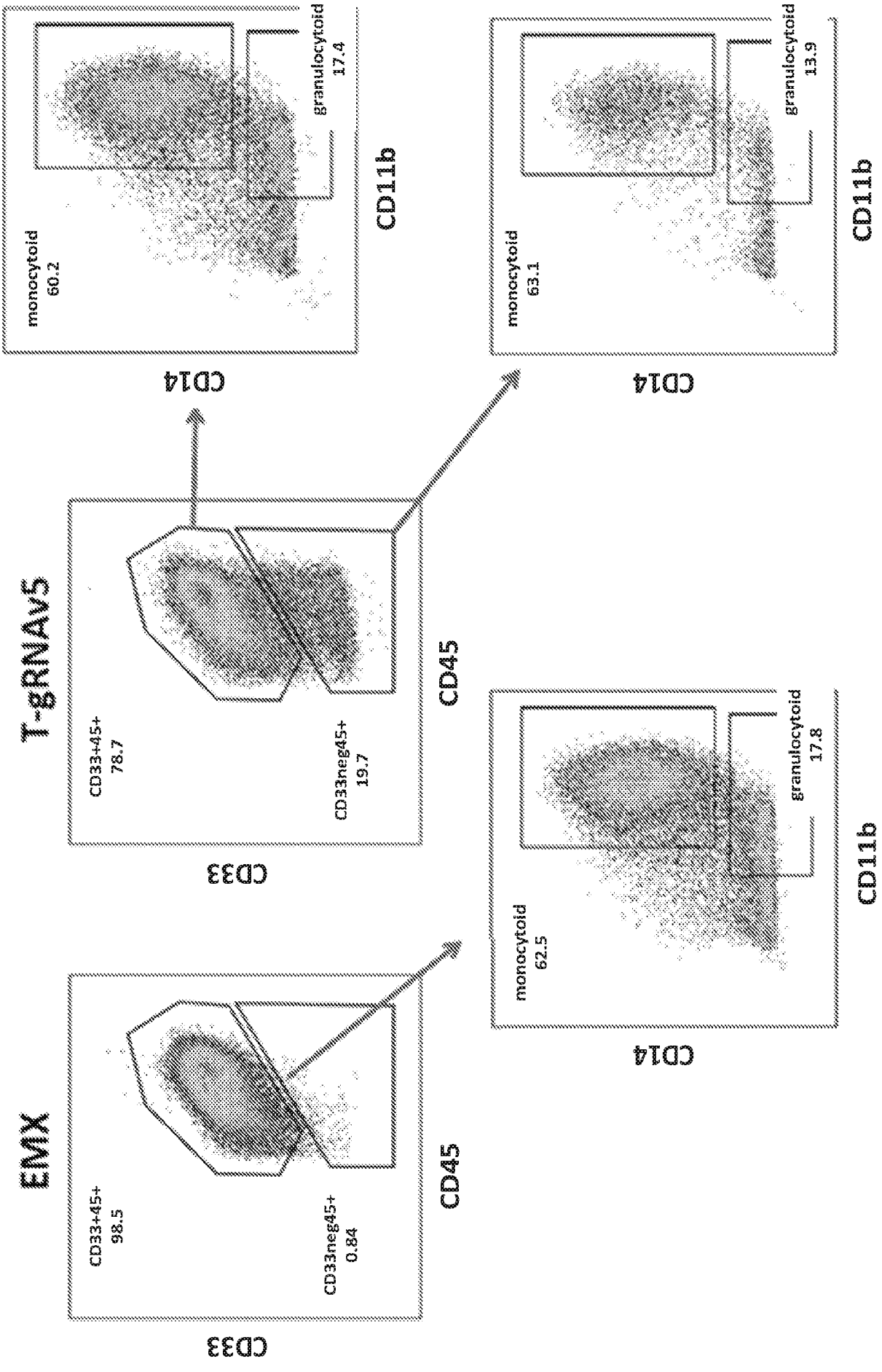

T-gRNAv5
CD33
CD45
CD33+45+
78.7
CD33neg45+
19.7
CD14
CD11b
monocytoid
60.2
granulocytoid
17.4
CD14
CD11b
monocytoid
63.1
granulocytoid
13.9
EMX
CD33
CD45
CD33+45+
98.5
CD33neg45+
0.84
CD14
CD11b
monocytoid
62.5
granulocytoid
17.8

Figure 6

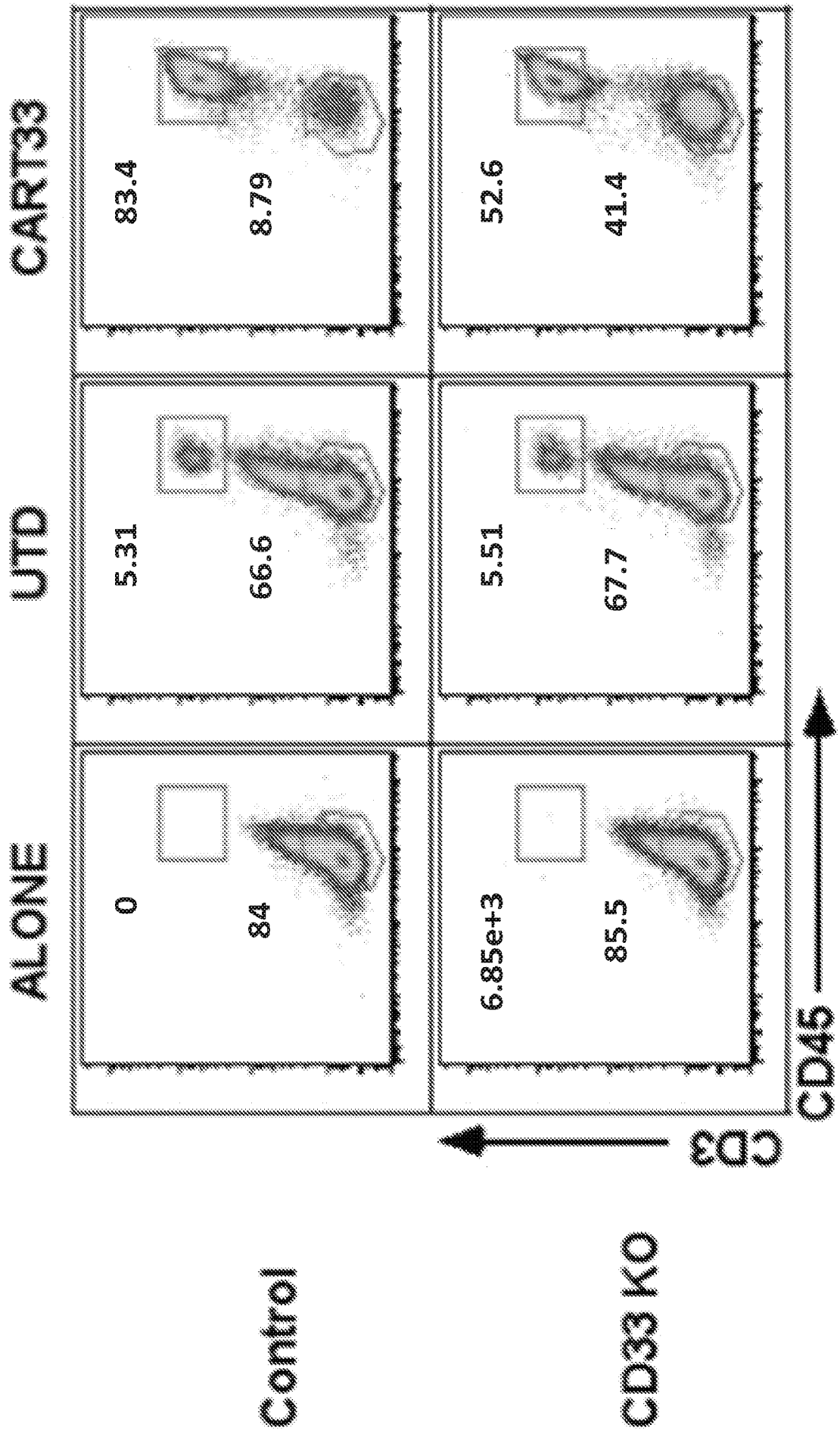

ALONE
UTD
CART33
0
84
5.31
66.6
83.4
8.79
6.85e+3
85.5
5.51
67.7
52.6
41.4
CD3
CD45
Control
CD33 KO

Figure 7A

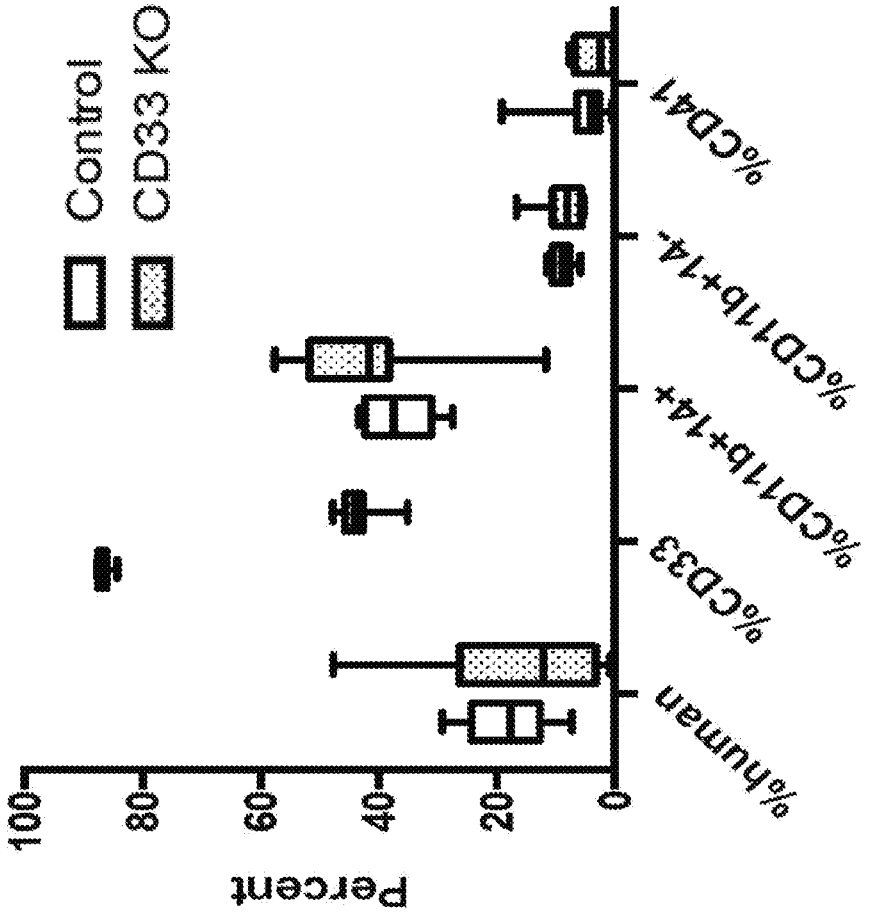


Figure 8

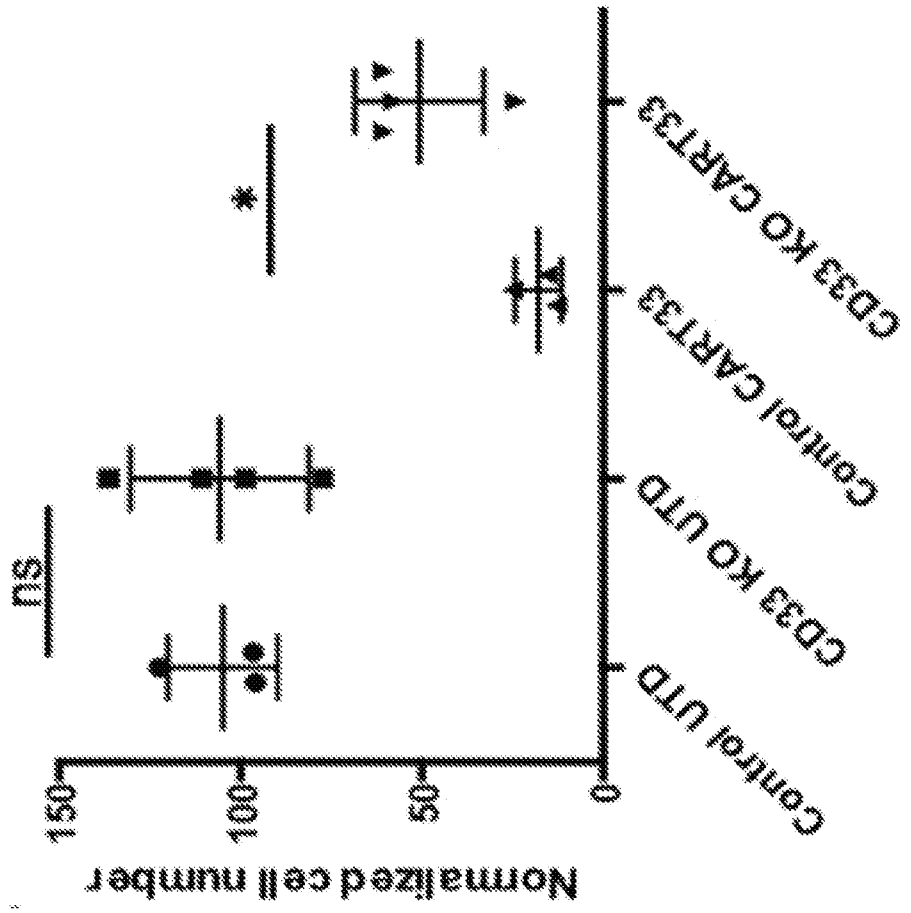


Figure 7B

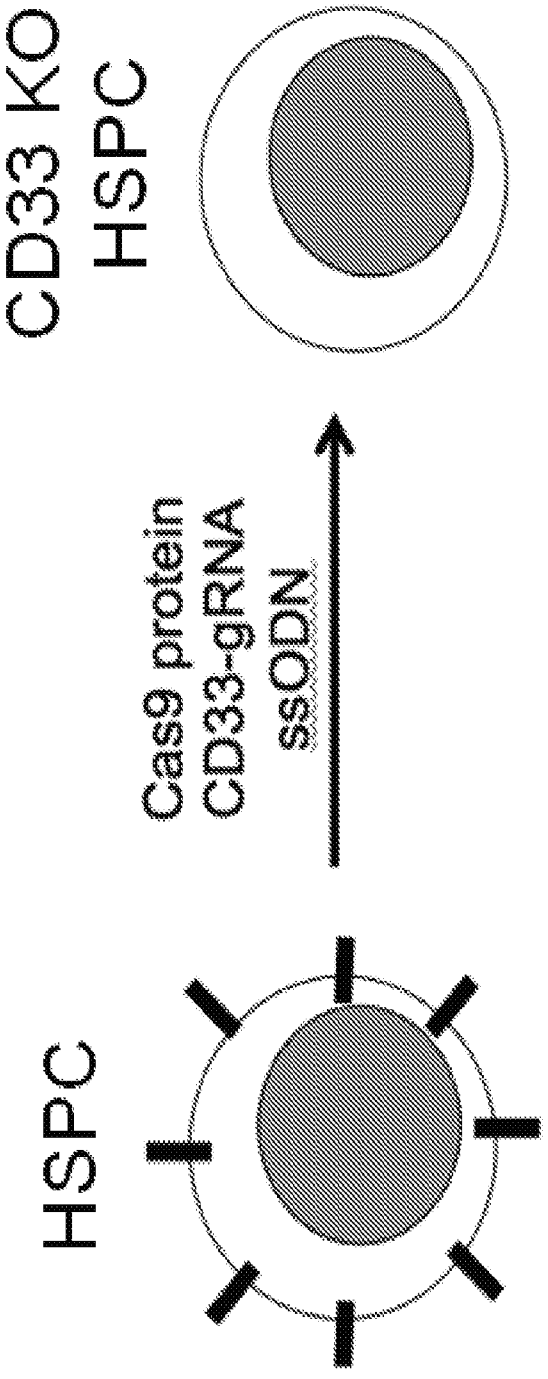


Figure 9A

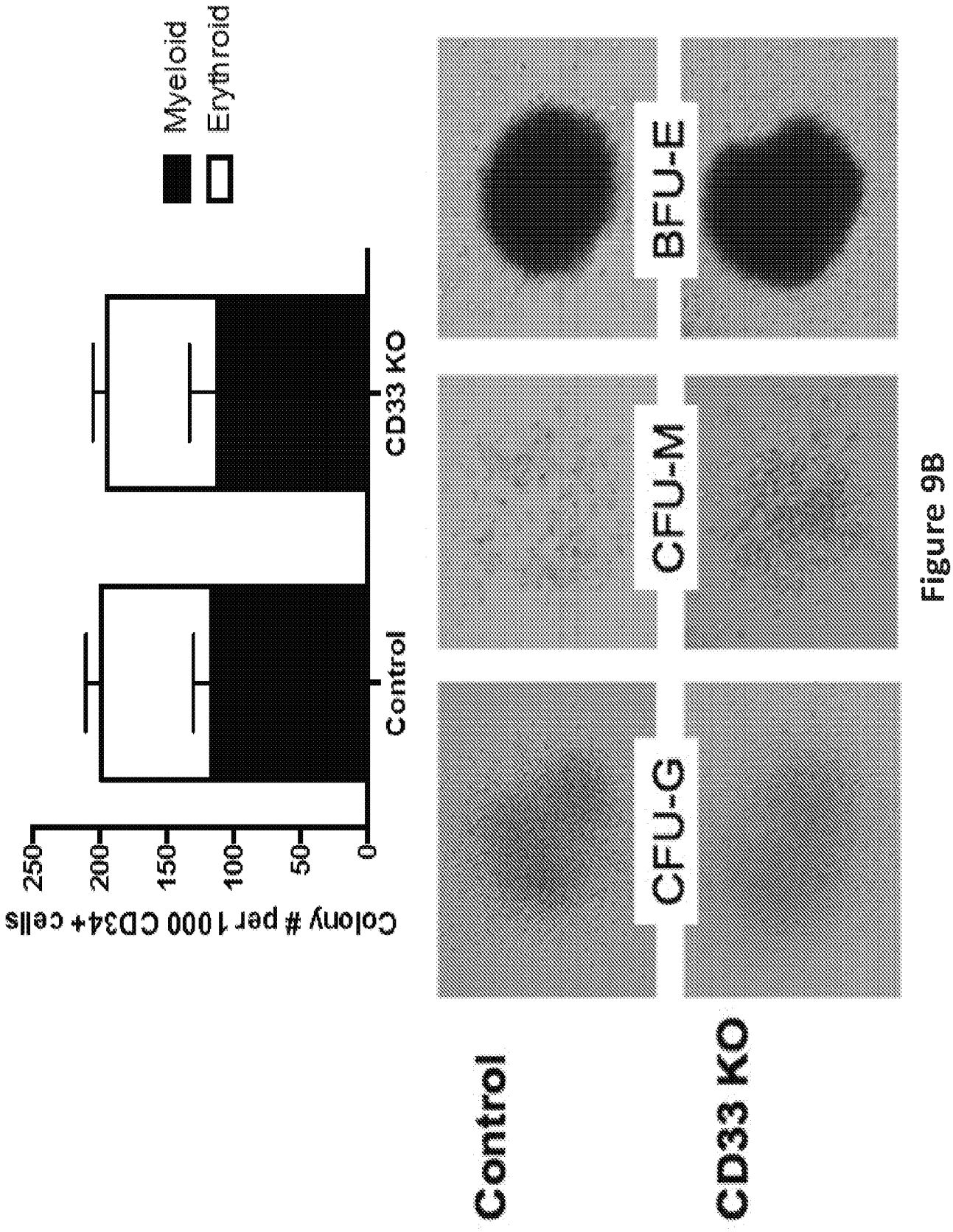

Myeloid
Erythroid
Colony # per 1000 CD34+ cells
250
200
150
100
50
0
Control
CD33 KO
Control
CD33 KO
CFU-G
CFU-M
BFU-E

Figure 9B

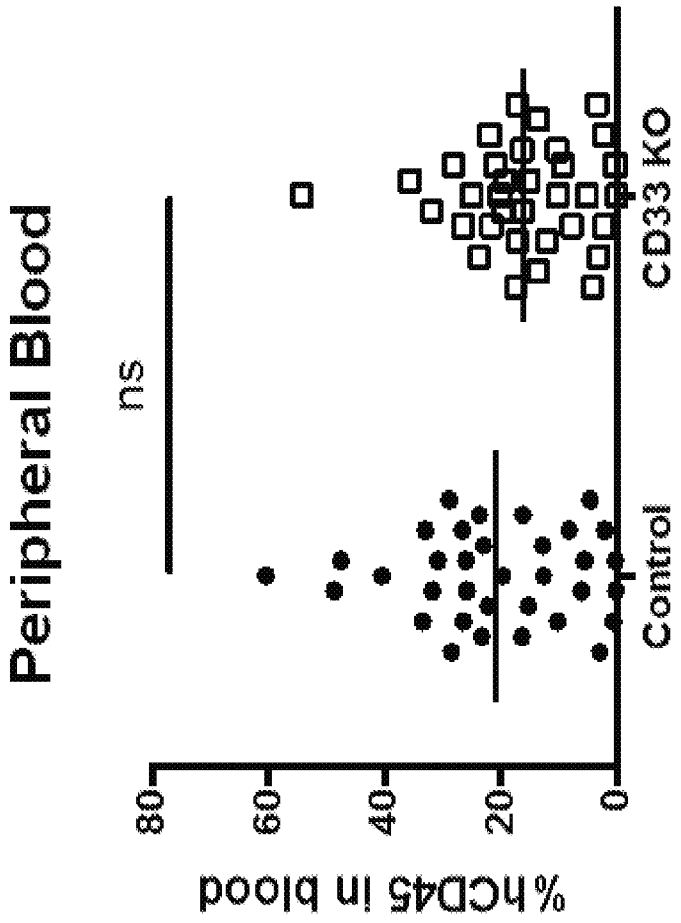

Peripheral Blood
ns
% hCD45 in blood
80
60
40
20
0
Control
CD33 KO

Figure 9C

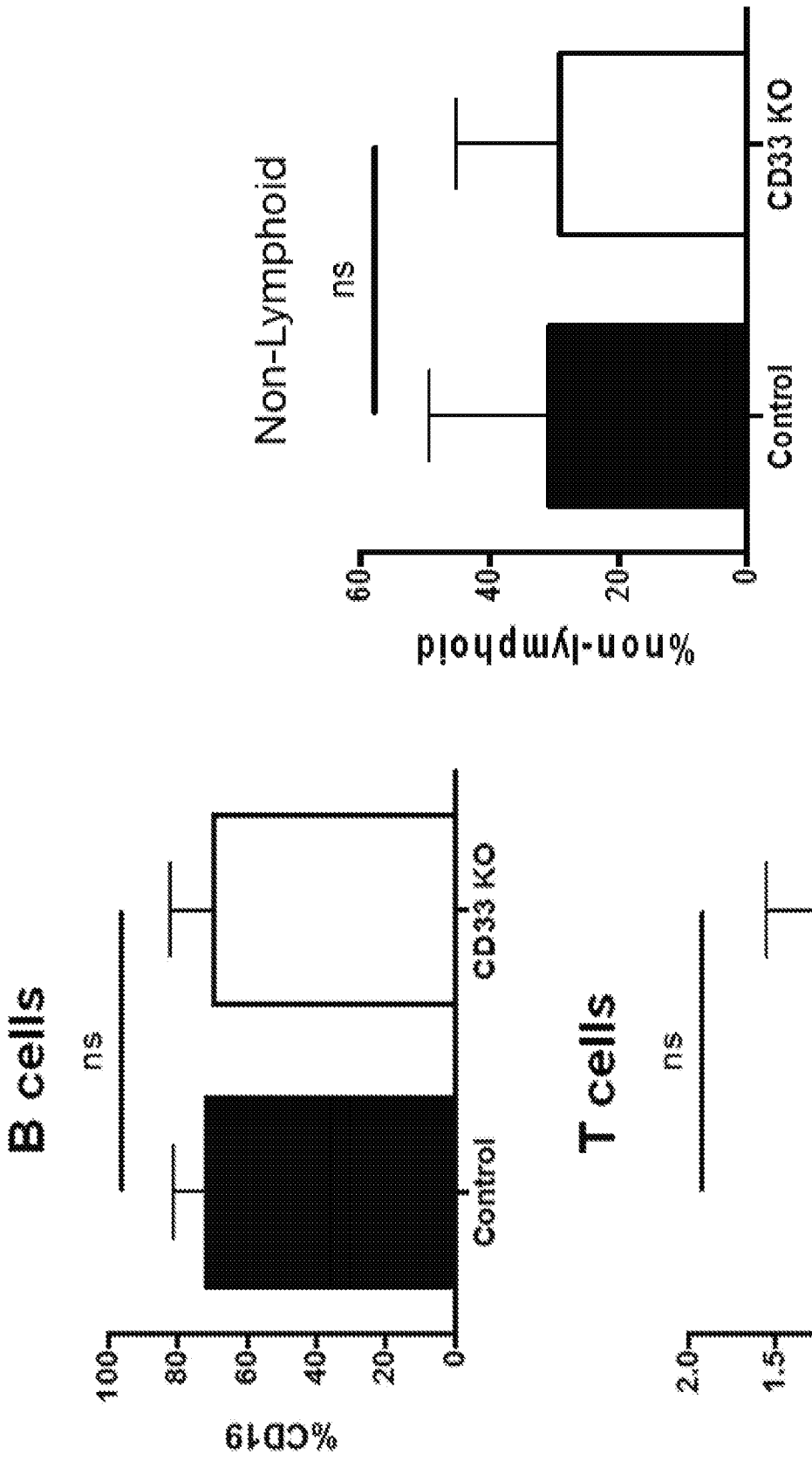

B cells
ns
CD33 KO
Control
%CD19
0
20
40
60
80
100
T cells
ns
CD33 KO
Control
%CD3
0.0
0.5
1.0
1.5
2.0
Non-Lymphoid
ns
CD33 KO
Control
%non-lymphoid
0
20
40
60

Figure 9D

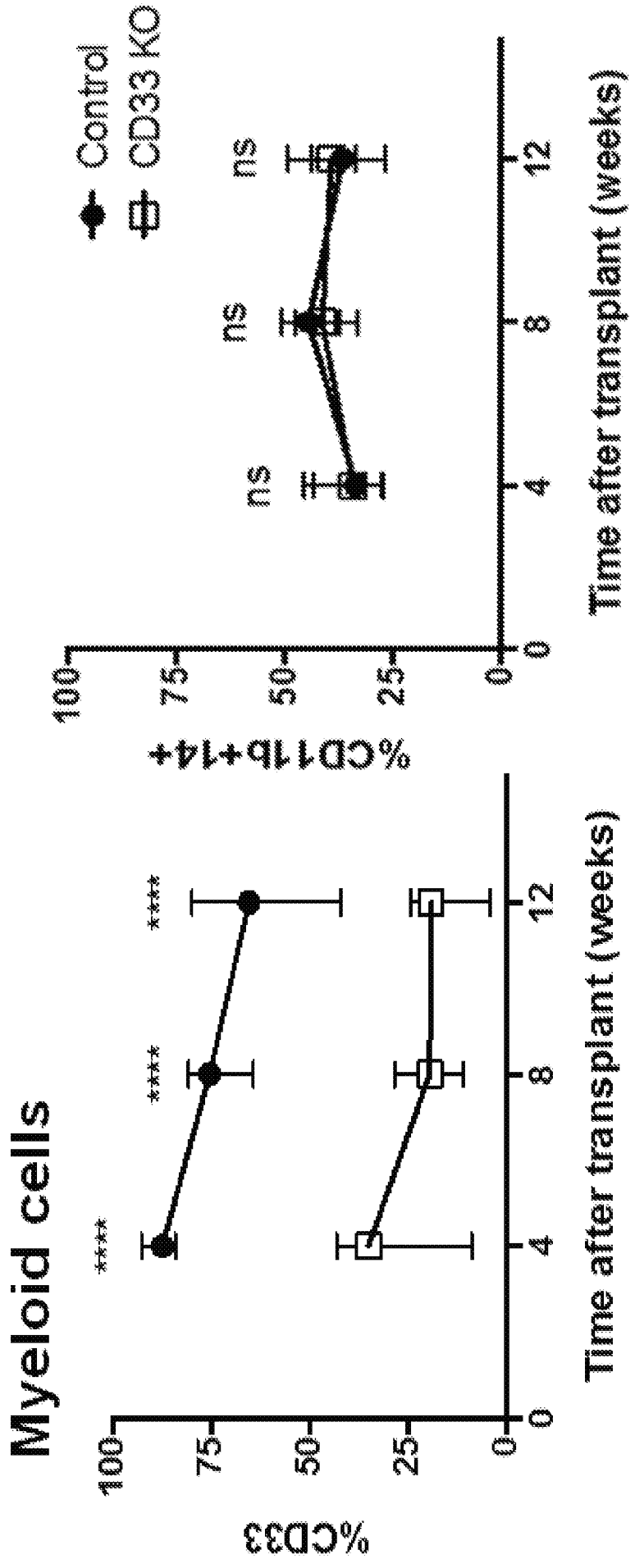

Myeloid cells
%CD33
100
75
50
25
0
0
4
8
12
Time after transplant (weeks)
****
****
****
%CD11b+14+
100
75
50
25
0
0
4
8
12
Time after transplant (weeks)
ns
ns
ns
Control
CD33 KO

Figure 9E

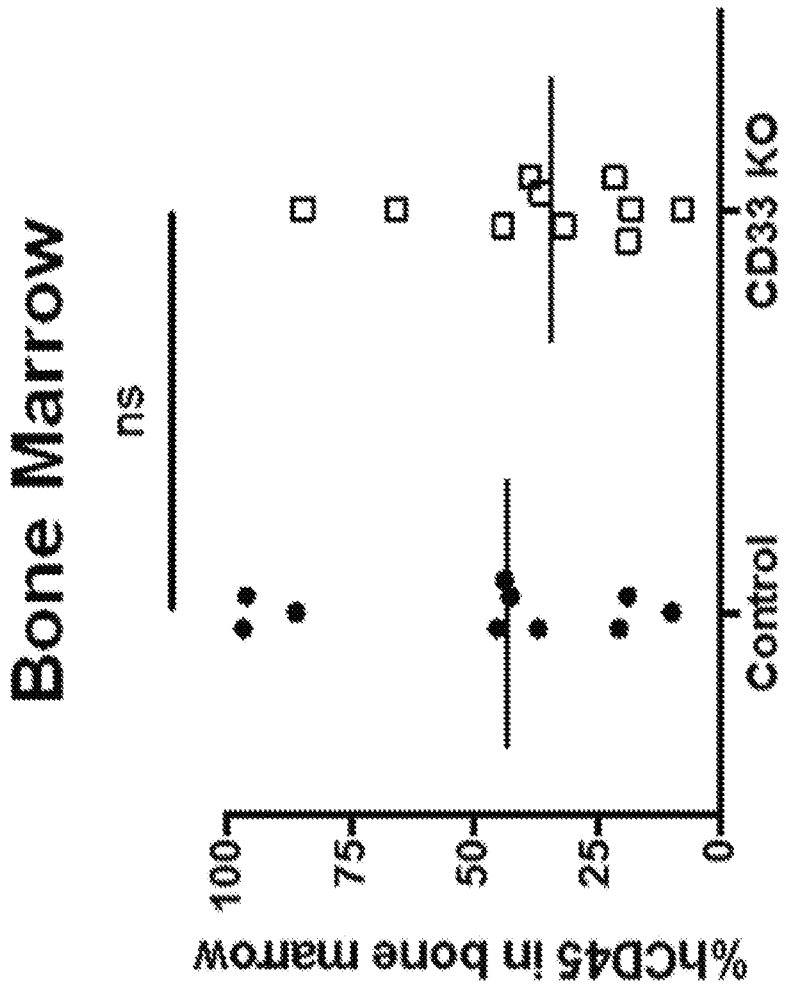
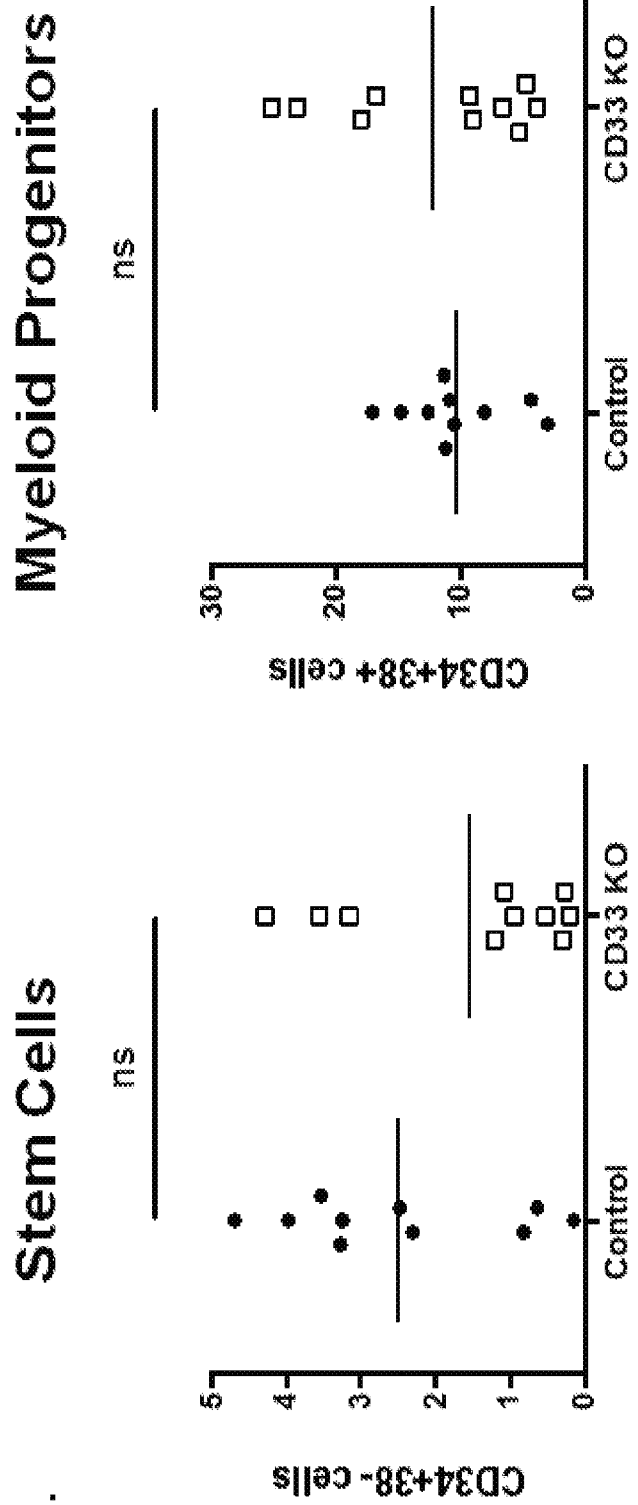

Bone Marrow
ns
Control
cd33 KO
0
25
50
75
100
%hCD45 in bone marrow

Figure 9F

G.



H.

	1° hCD45%	CD33 KO	2° hCD45%	CD33 KO
EMX	49.1	0	1.45	0
EMX	20.1	0	0.04	0
CD33	35.1	61%	8.6	66%
CD33	30.3	72%	0.10	84%

Figures 9G-9H

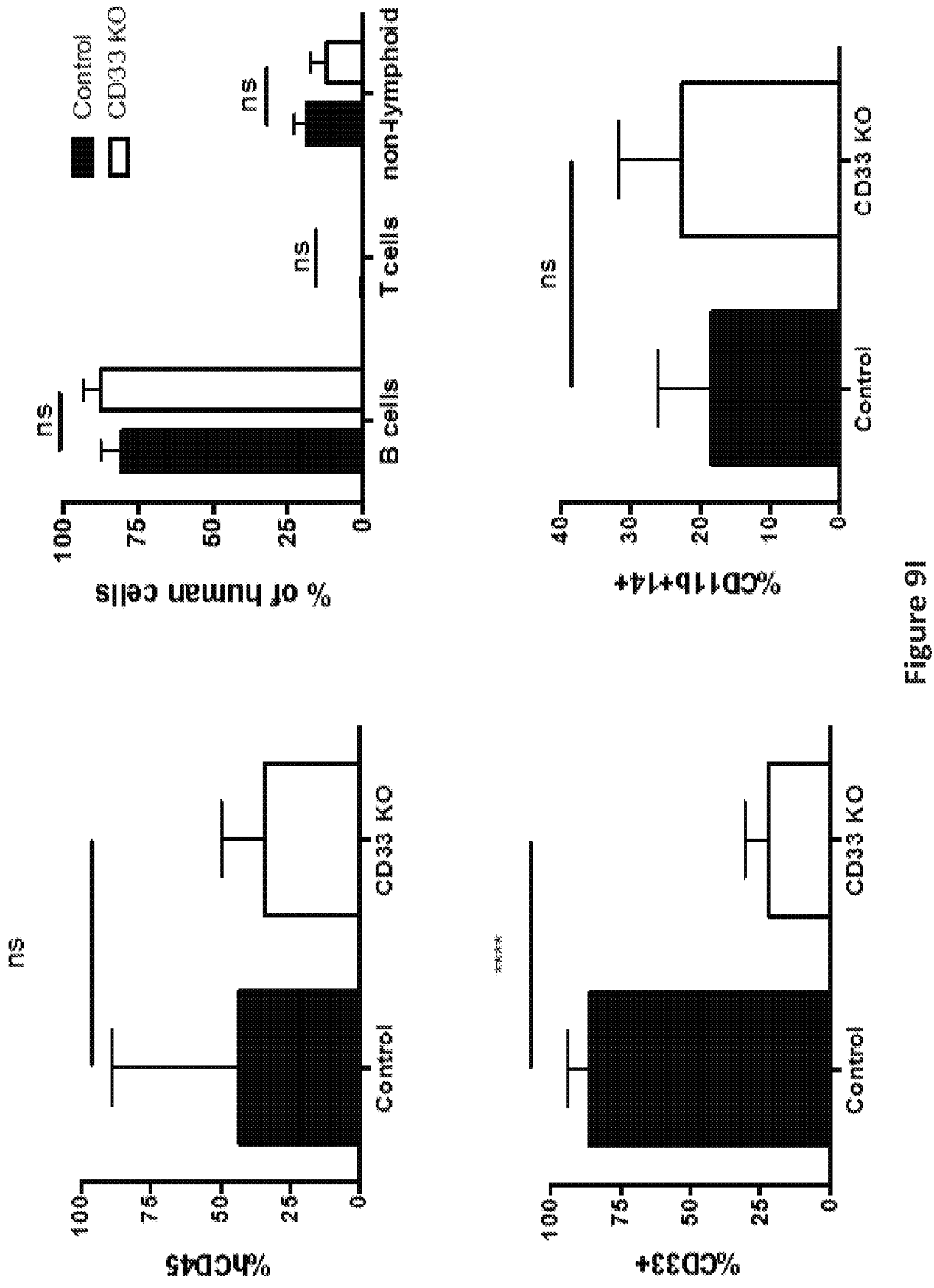

Control
CD33 KO
% of human cells
100
75
50
25
0
ns
ns
ns
B cells
T cells
non-lymphoid
%CD11b+14+
40
30
20
10
0
ns
Control
CD33 KO
%hCD45
100
75
50
25
0
ns
Control
CD33 KO
%CD33+
100
75
50
25
0
****
Control
CD33 KO

Figure 9I

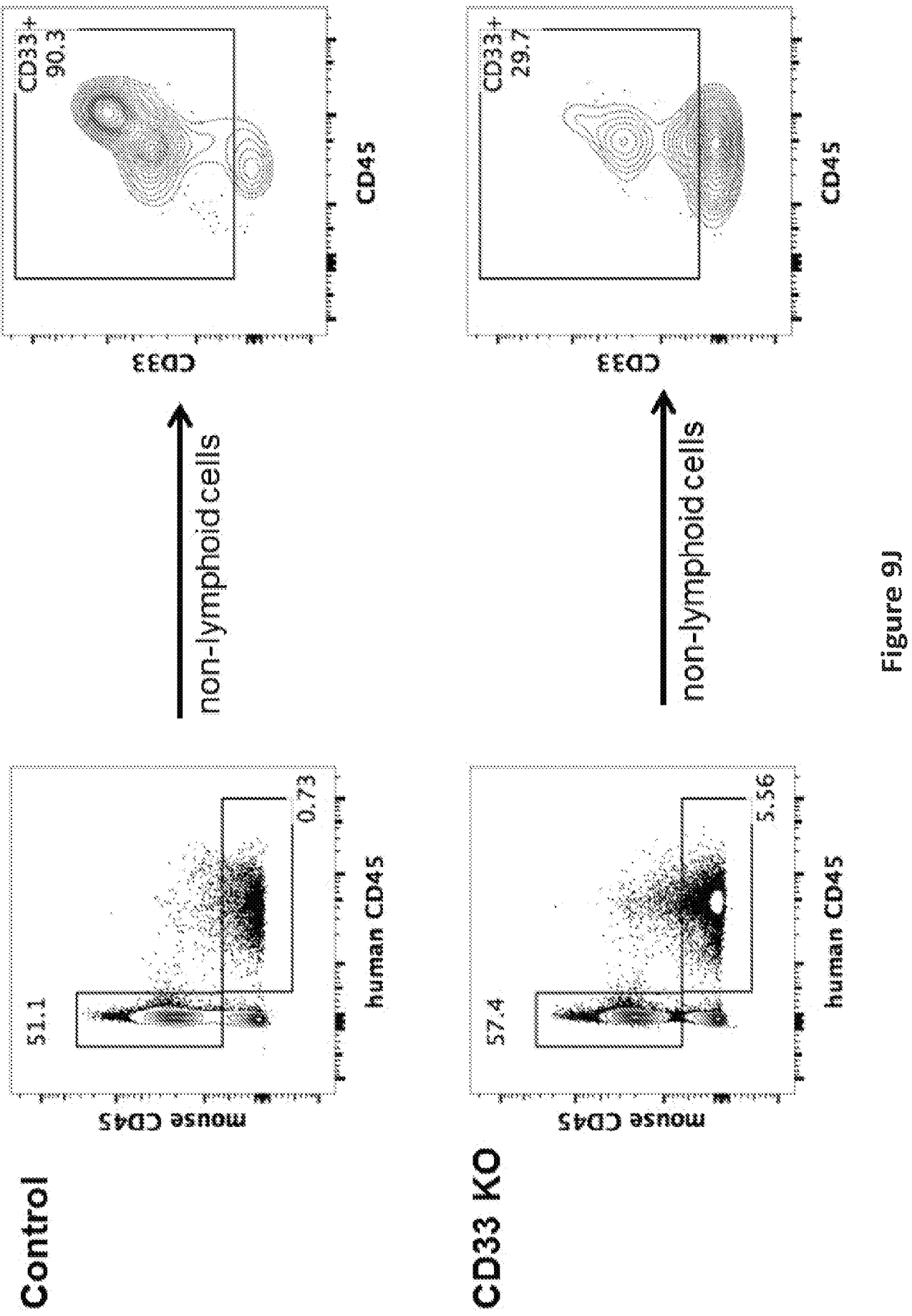
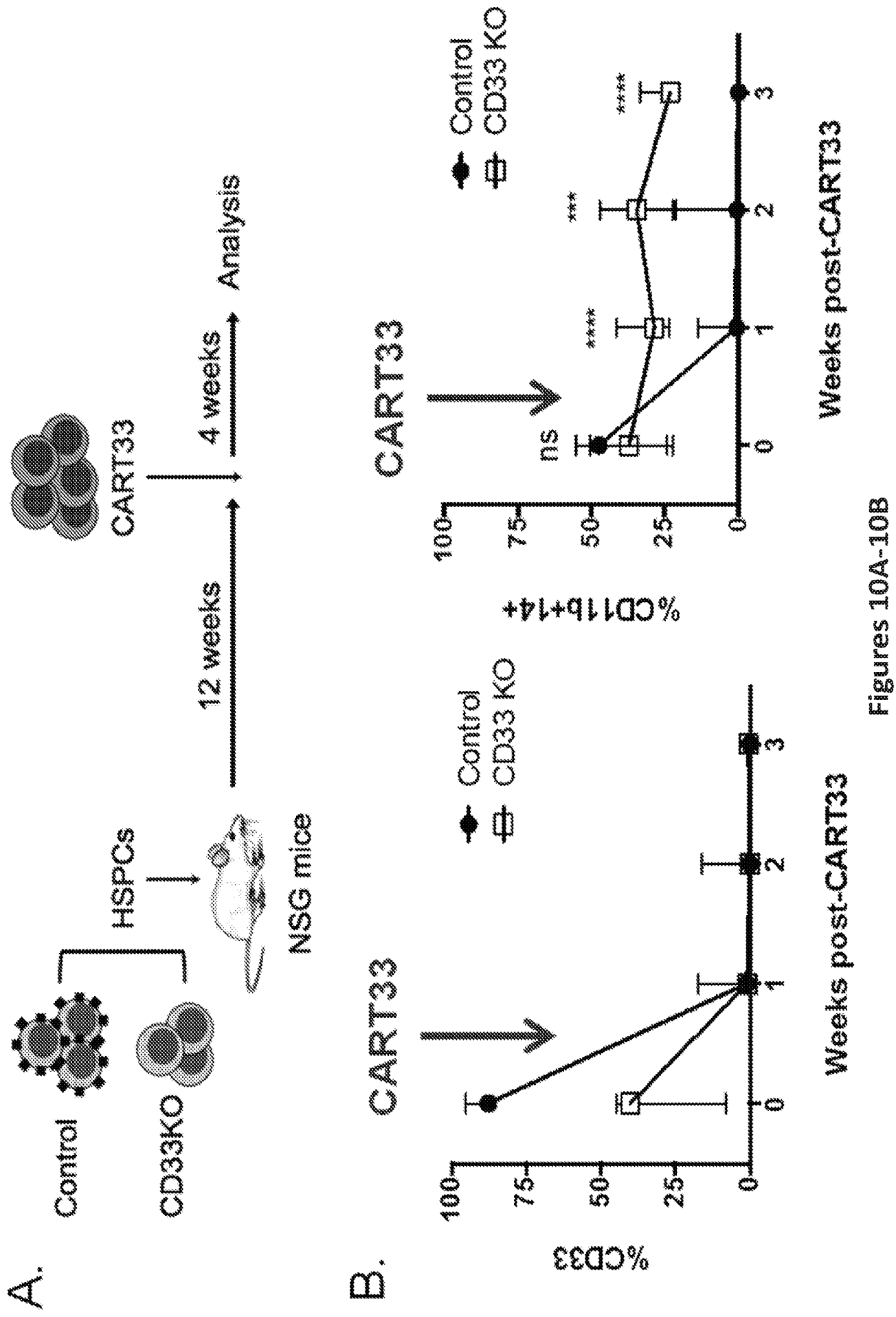

Control
CD33 KO
mouse CD45
human CD45
51.1
0.73
57.4
5.56
non-lymphoid cells
CD33
CD45
CD33+
90.3
CD33+
29.7

Figure 9J



Figures 10A-10B

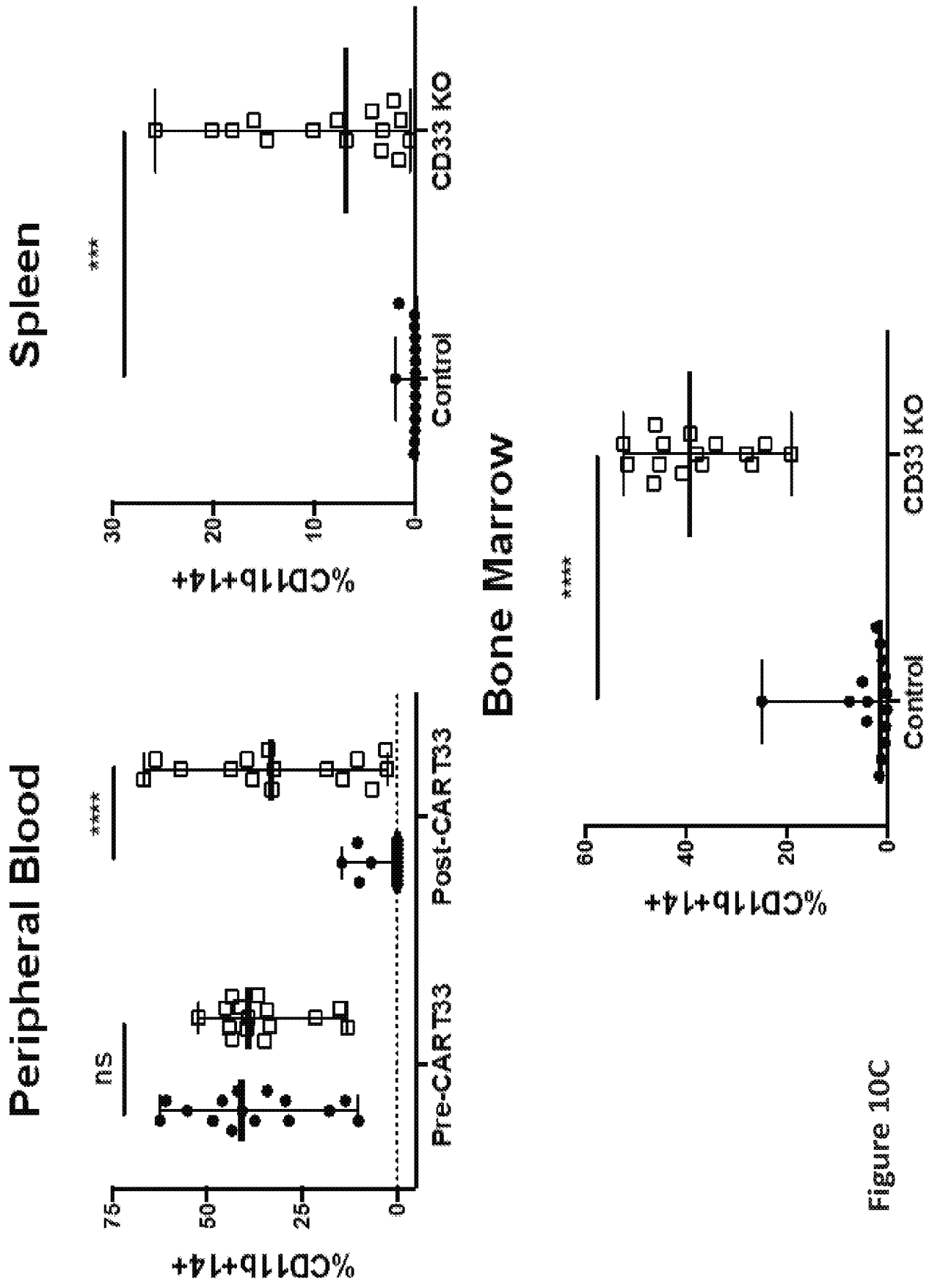

Peripheral Blood
ns
****
%CD11b+14+
75
50
25
0
Pre-CAR T33
Post-CAR T33
Spleen
***
%CD11b+14+
30
20
10
0
Control
CD33 KO
Bone Marrow
****
%CD11b+14+
60
40
20
0
Control
CD33 KO

Figure 10C

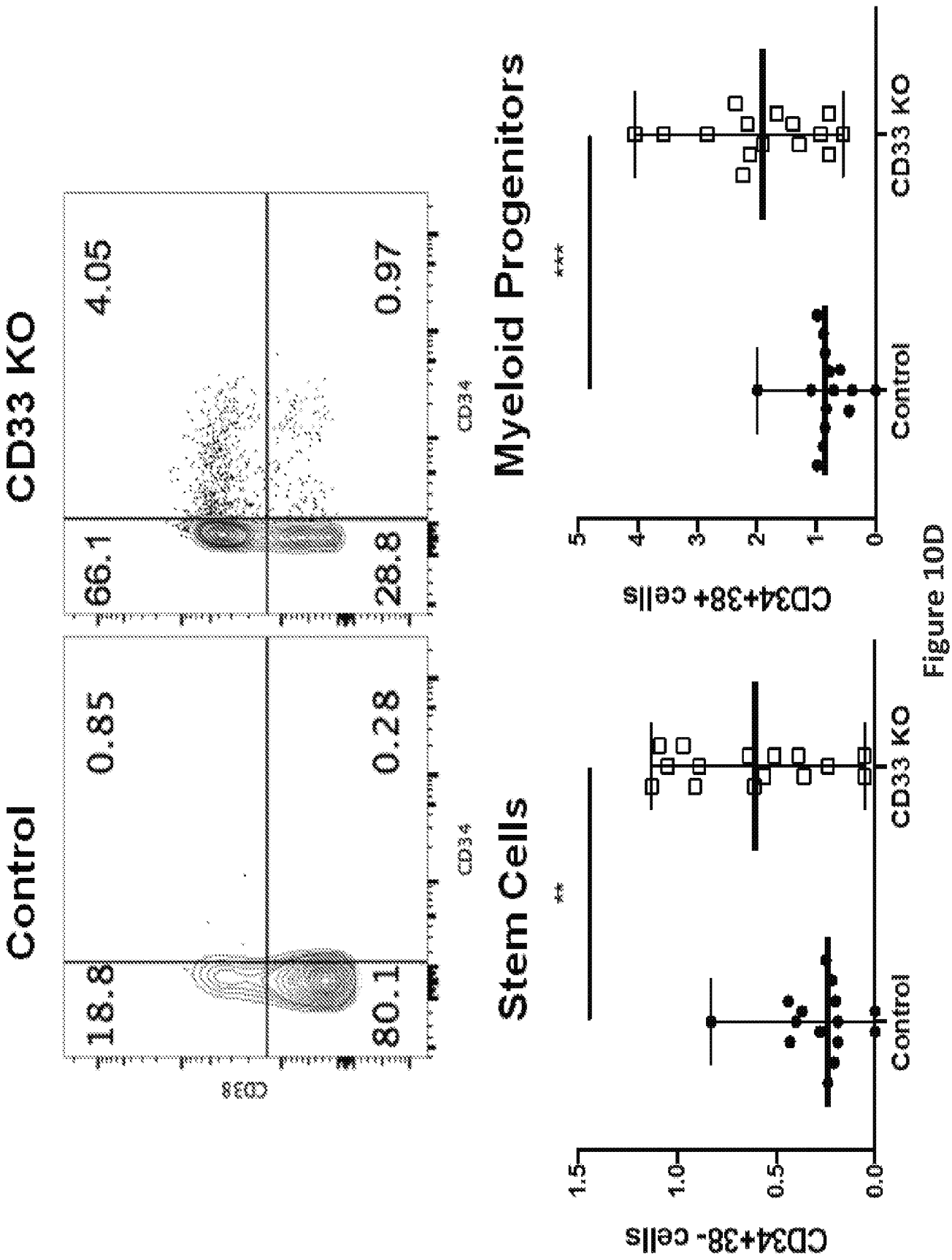
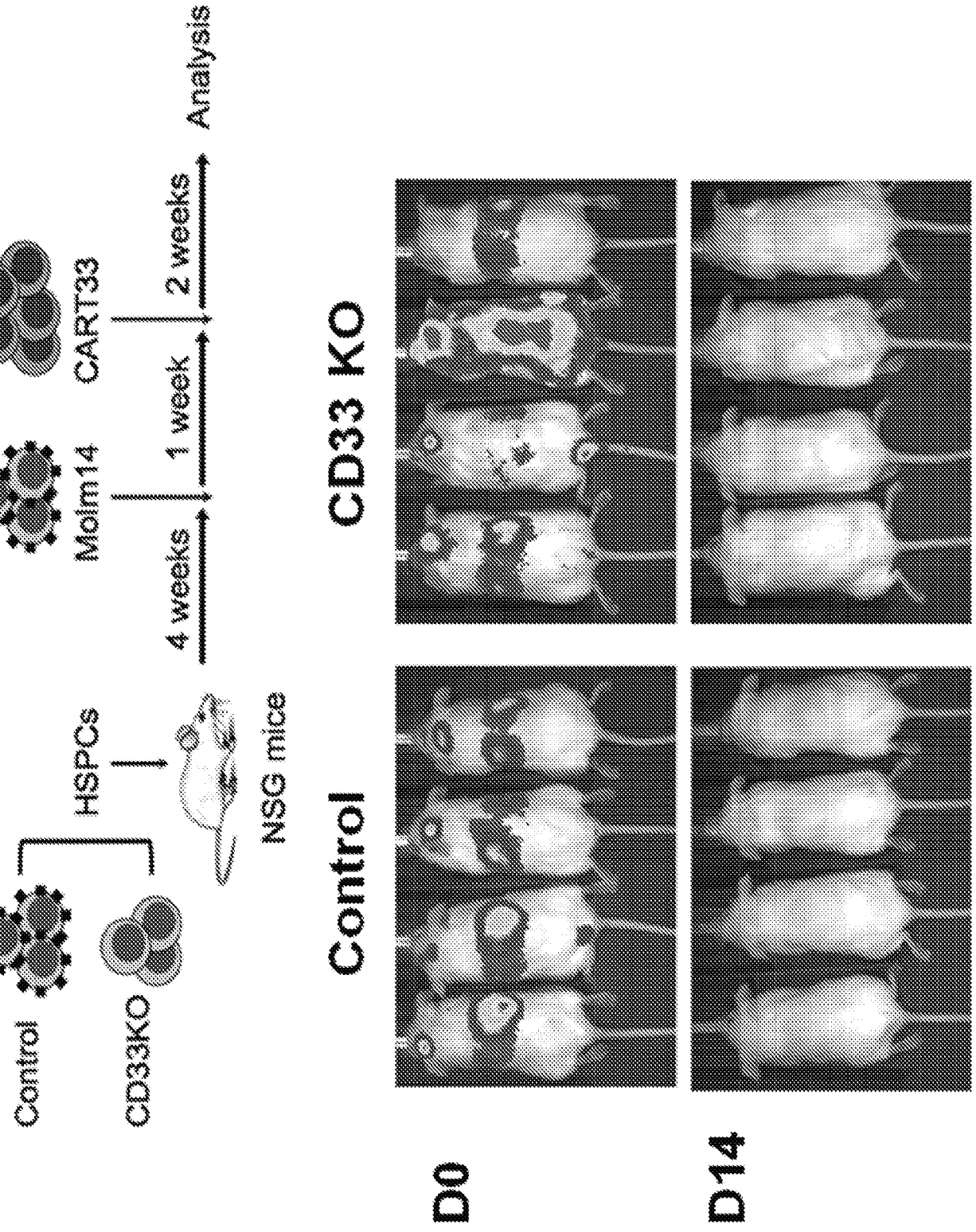
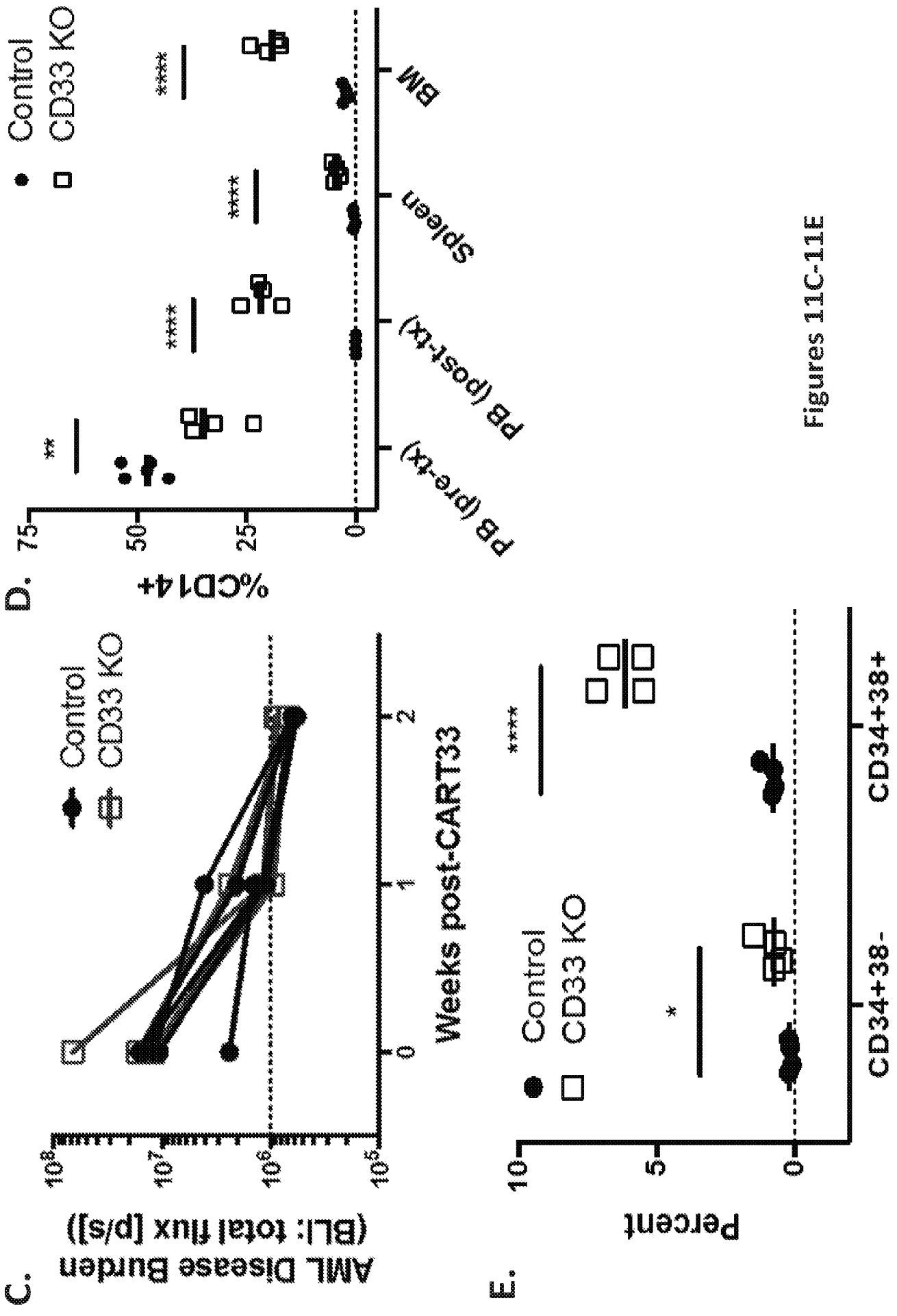

CD33 KO
Control
4.05
0.97
66.1
28.8
0.85
0.28
18.8
80.1
CD34
CD38
Myeloid Progenitors
Stem Cells
***
**
CD34+38+ cells
CD34+38- cells
Control
CD33 KO
0
1
2
3
4
5
0.0
0.5
1.0
1.5

Figure 10D



Figures 11A-11B



Figures 11C-11E

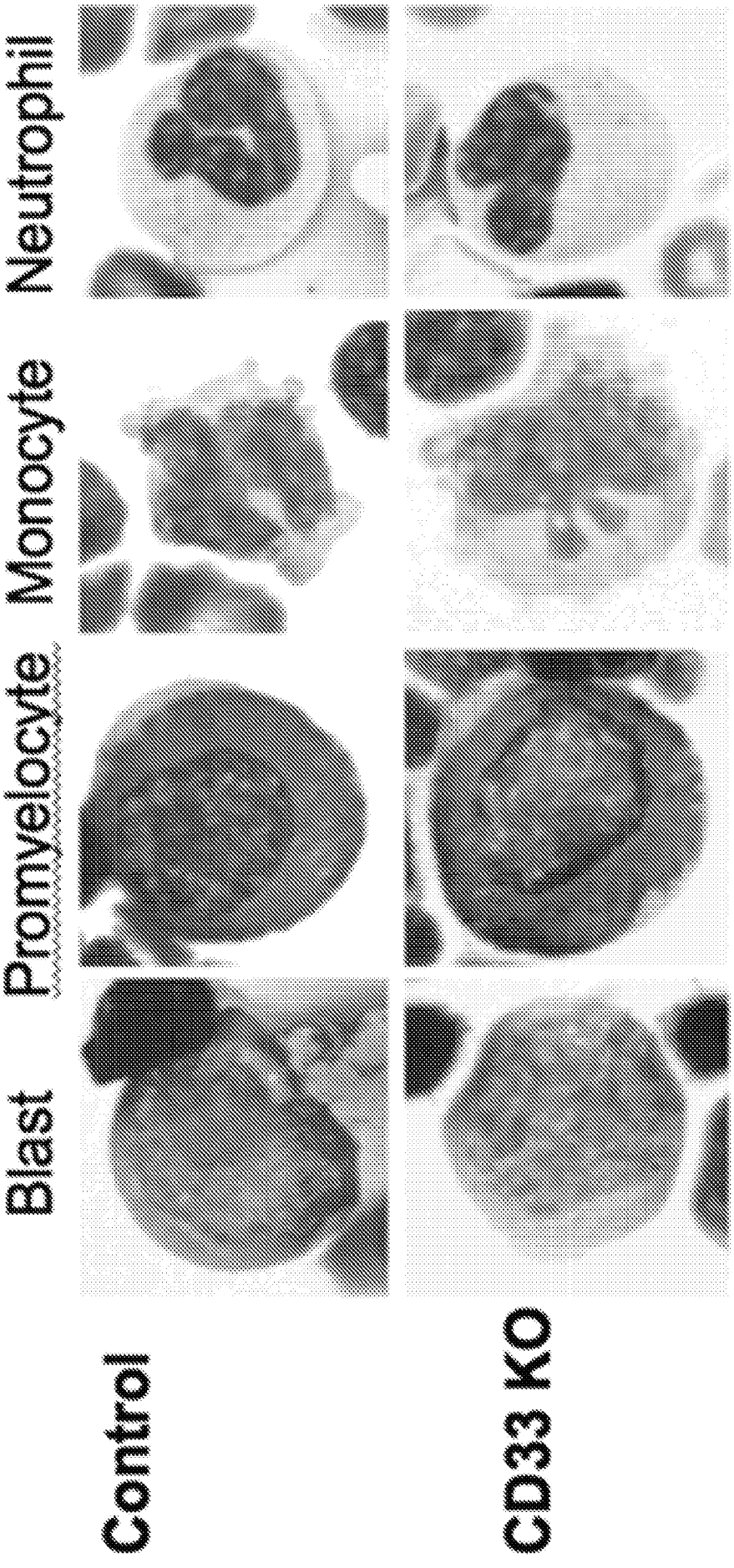


Figure 12A

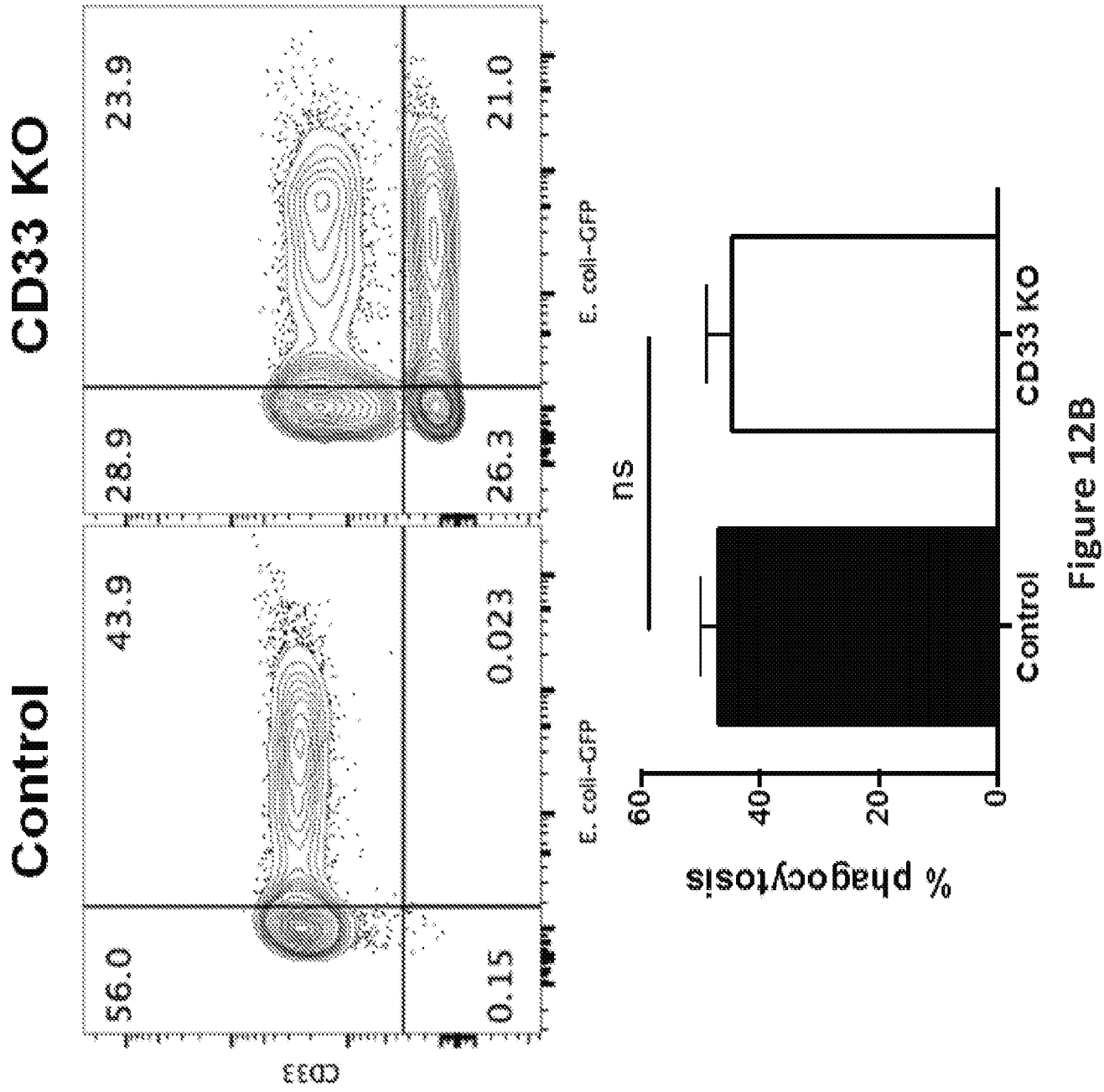

Control
CD33 KO
56.0
43.9
0.15
0.023
23.9
28.9
21.0
26.3
CD33
E. coli-GFP
E. coli-GFP
ns
% phagocytosis
60
40
20
0
Control
CD33 KO

Figure 12B

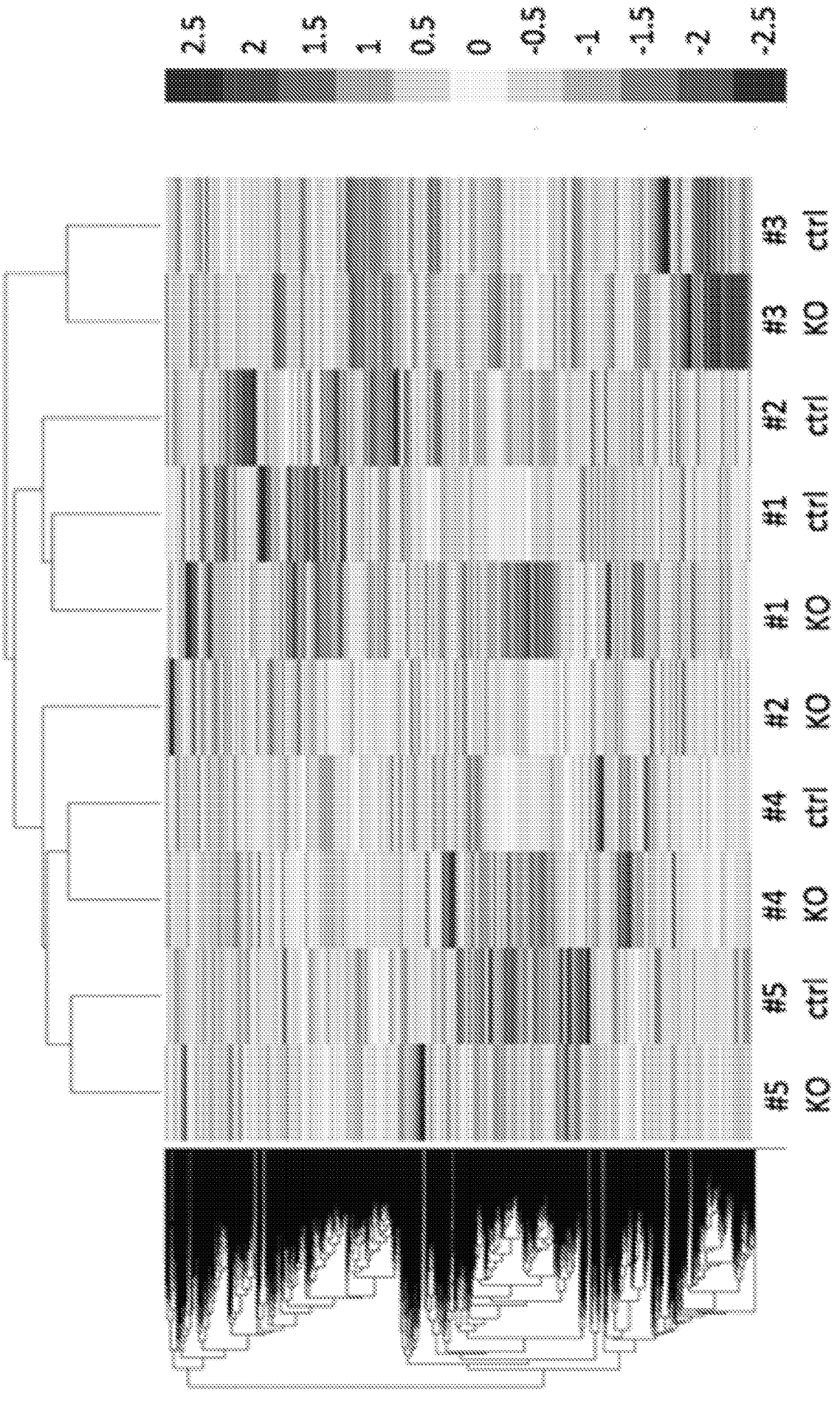

2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
#5 KO
#5 ctrl
#4 KO
#4 ctrl
#2 KO
#1 KO
#1 ctrl
#2 ctrl
#3 KO
#3 ctrl

Figure 12C

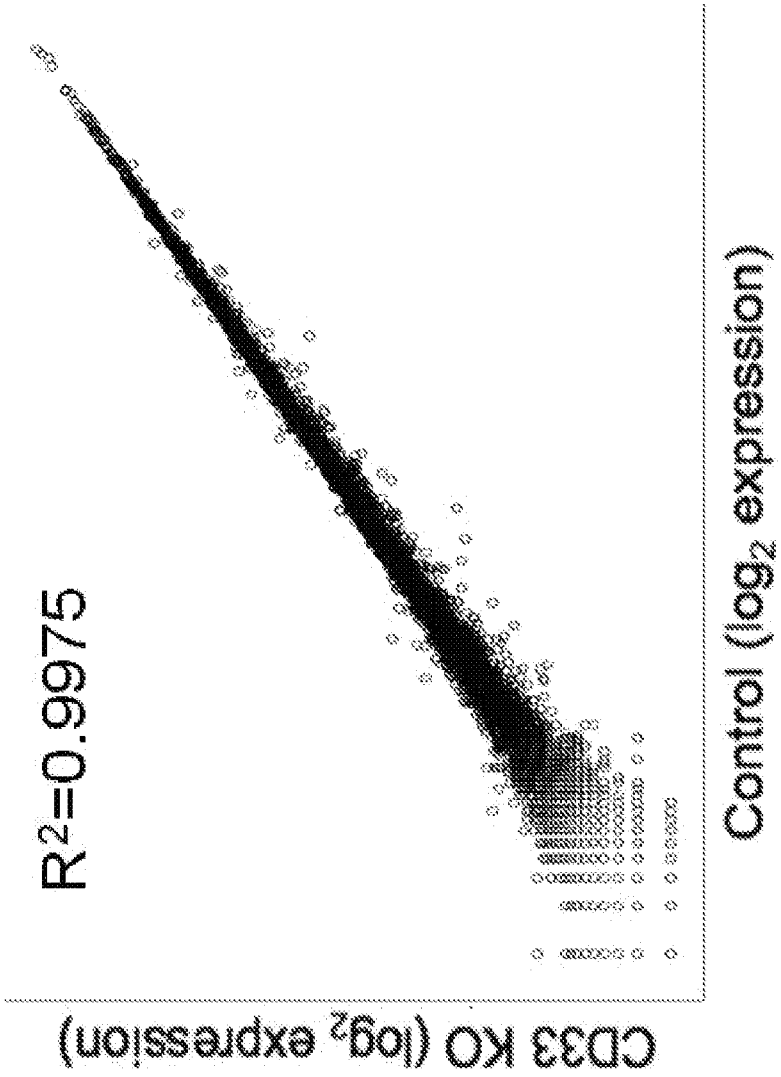


Figure 12D

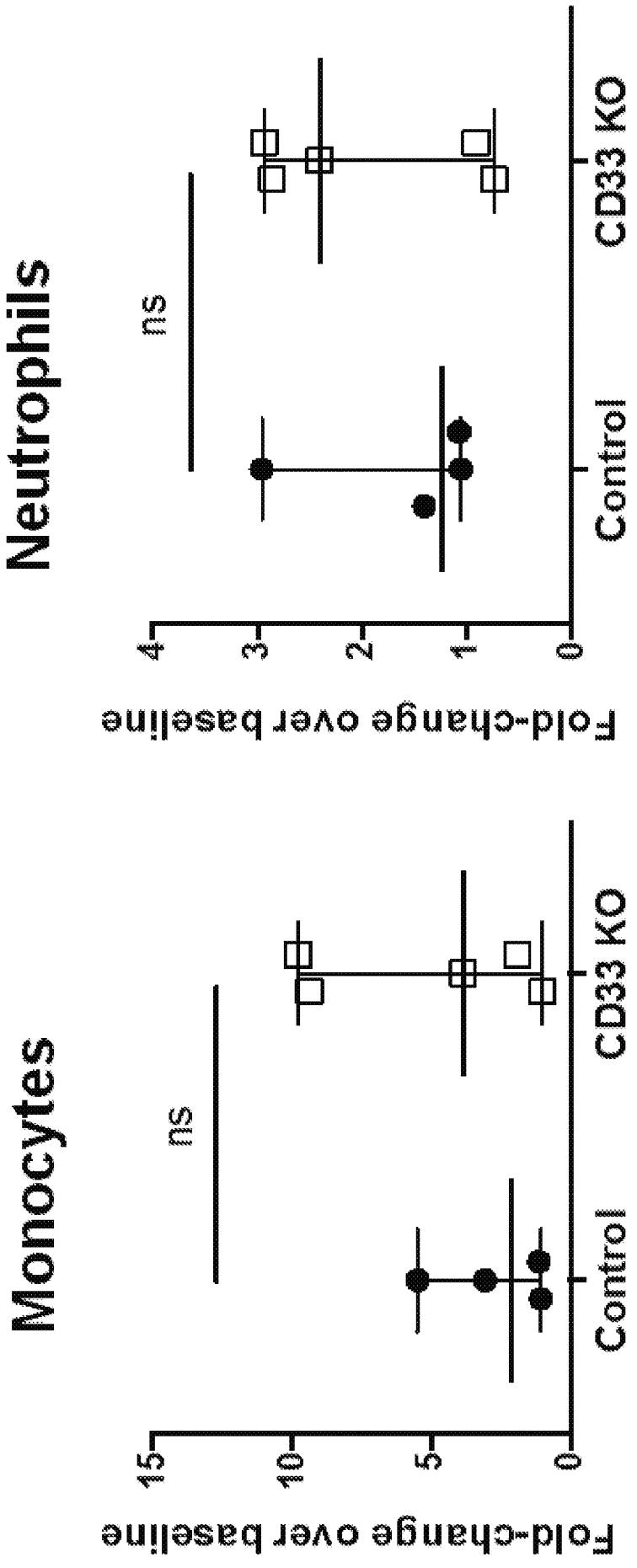

Neutrophils
ns
Control
CD33 KO
Fold-change over baseline
0
1
2
3
4
Monocytes
ns
Control
CD33 KO
Fold-change over baseline
0
5
10
15

Figure 12E

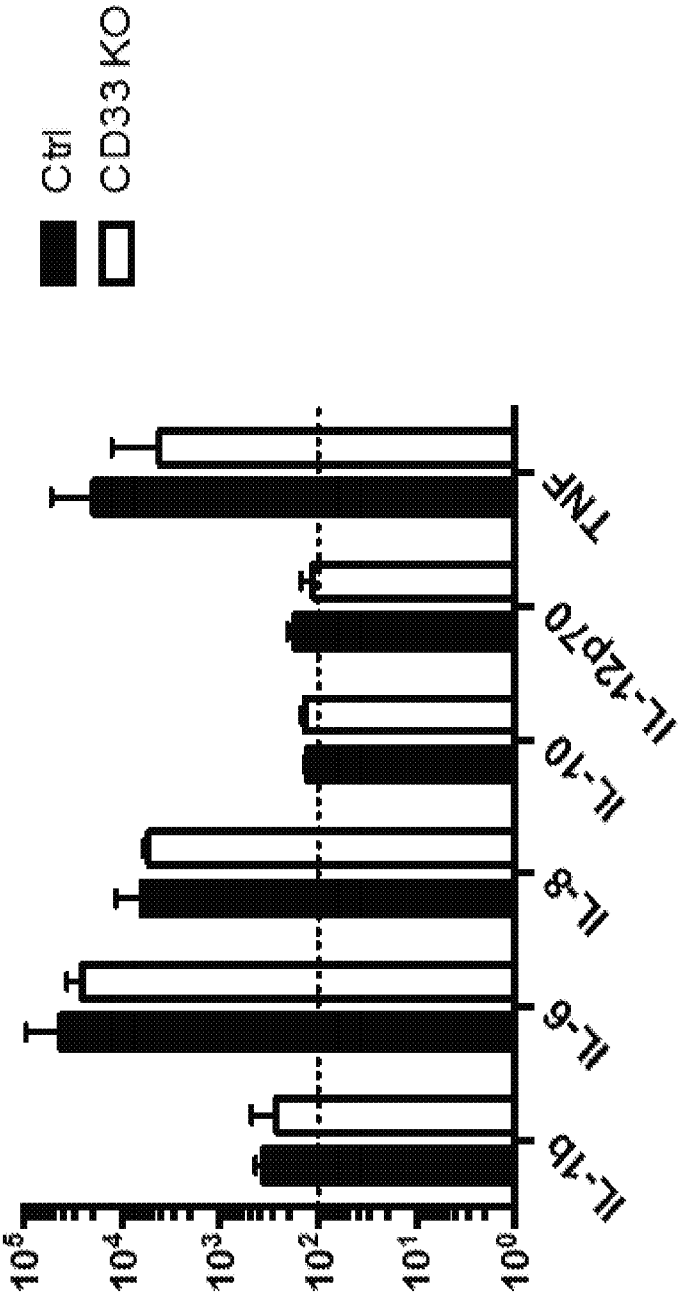

Ctrl
CD33 KO
TNF
IL-12P70
IL-10
IL-8
IL-6
IL-1b
10^5
10^4
10^3
10^2
10^1
10^0

Figure 12F

MIT (http://crispr.mit.edu/)

CCTop (http://crispr.cos.uni-heidelberg.de/)

	Location	#MM	MIT score	CCTop rank	Position
CD33	chr19	0	100	1	Exon
SIGLEC22P	chr19	0	100	2	Exon
Chr18	chr18	3	1.56		Intergenic
ARHGEF10					
L	chr1	3	1.52	3	Intron
SIGLEC6	chr19	2	1.38	7	Exon
SIGLEC17P	chr19	2	1.31	5	Exon
GHDC	chr17	4	0.77		Exon
SIGLEC5	chr19	3	0.51	8	Exon
SIGLEC12	chr19	3	0.51	10	Exon
SIGLEC14	chr19	3	0.51	9	Exon
VMO1	chr17	4	0.38		Exon
Chr2	chr2	3	0.29	6	Intergenic
Chr1	chr1	3	0	4	Intergenic

Figure 13A

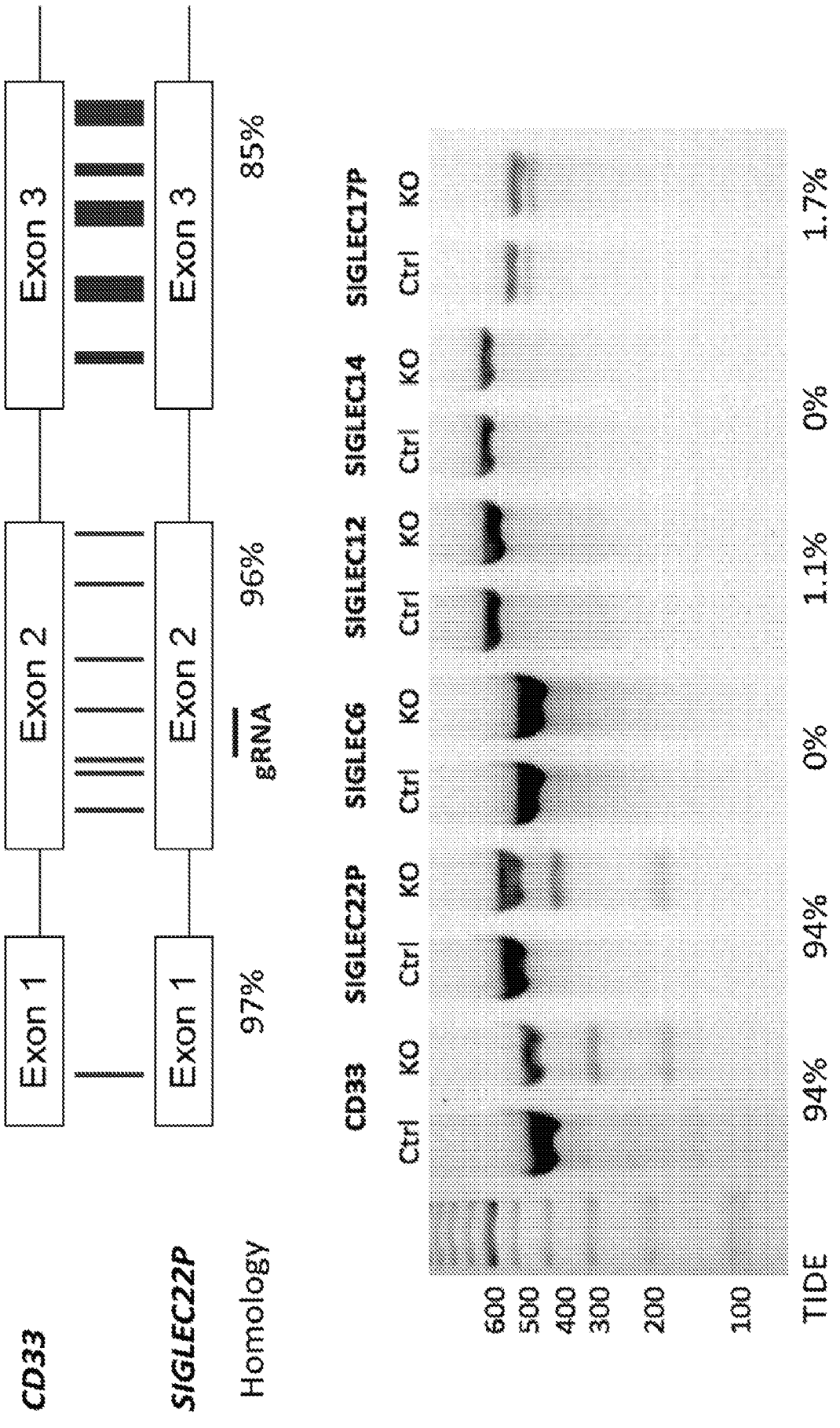

CD33
Exon 1
Exon 2
Exon 3
SIGLEC22P
Exon 1
Exon 2
Exon 3
Homology
97%
96%
85%
gRNA
CD33 KO
SIGLEC22P KO
SIGLEC6 KO
SIGLEC12 KO
SIGLEC14 KO
SIGLEC17P KO
Ctrl
Ctrl
Ctrl
Ctrl
Ctrl
Ctrl
600
500
400
300
200
100
TIDE
94%
94%
0%
1.1%
0%
1.7%

Figure 13B

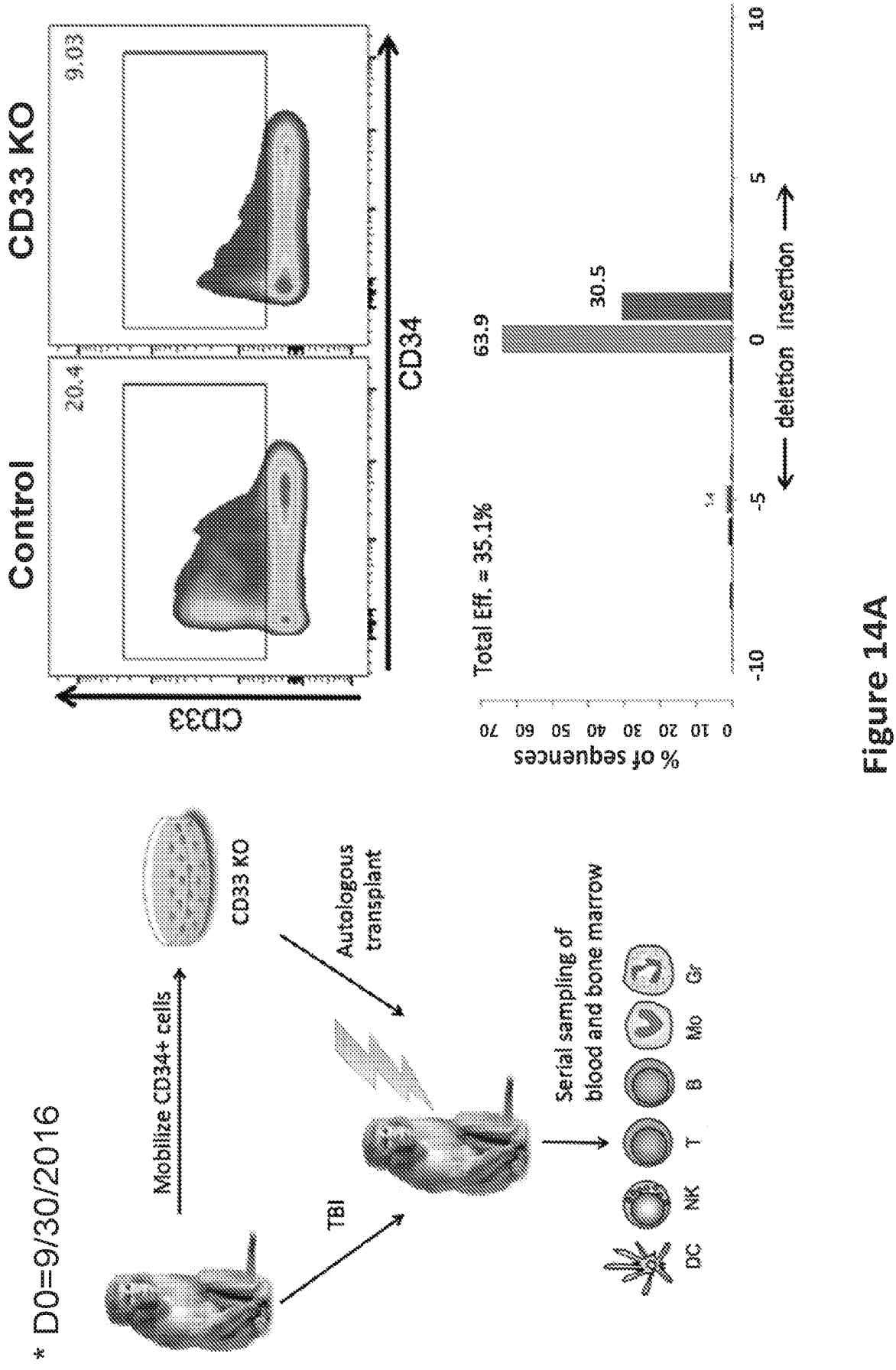

* D0=9/30/2016
Mobilize CD34+ cells
CD33 KO
TBI
Autologous transplant
Serial sampling of blood and bone marrow
DC
NK
T
B
Mo
Gr
Control
CD33 KO
20.4
9.03
CD33
CD34
Total Eff. = 35.1%
63.9
30.5
% of sequences
deletion
insertion

Figure 14A

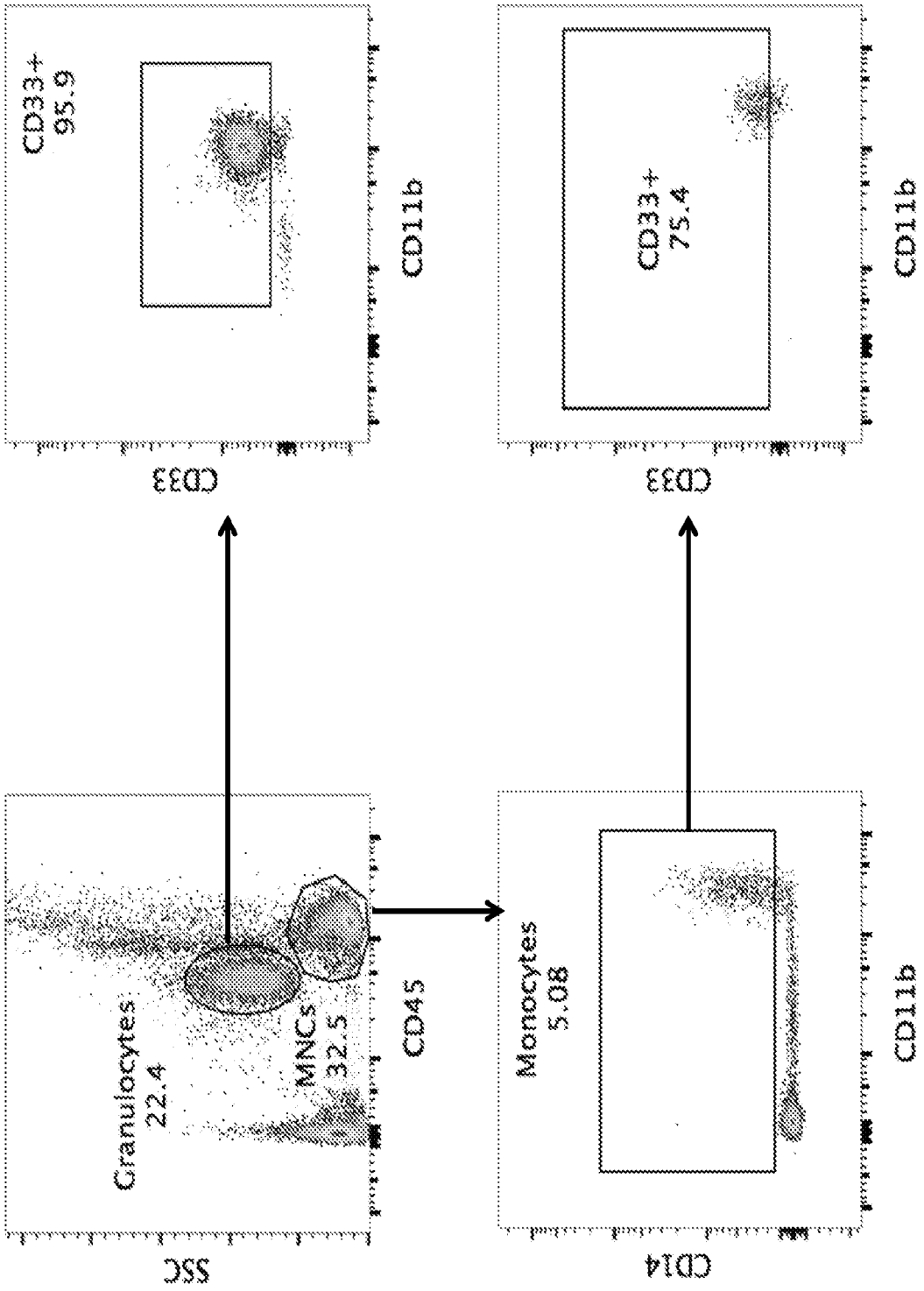

SSC
CD45
Granulocytes
22.4
MNCs
32.5
CD14
CD11b
Monocytes
5.08
CD33
CD11b
CD33+
75.4
CD33
CD11b
CD33+
95.9

Figure 14B