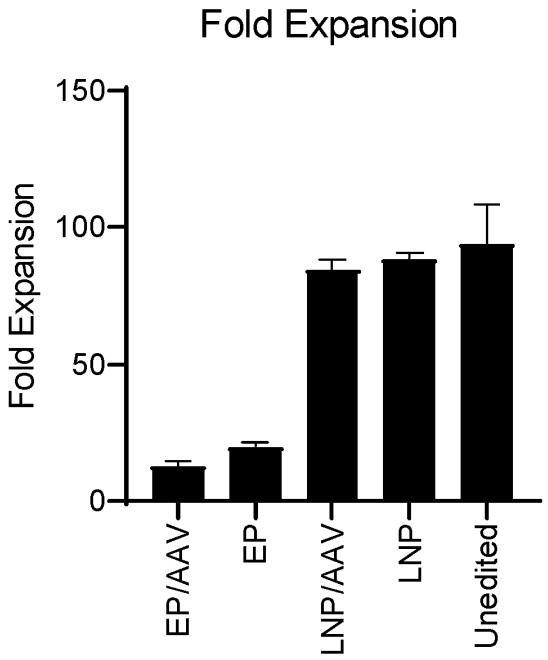

Fold Expansion
Fold Expansion
150
100
50
0
EP/AAV
EP
LNP/AAV
LNP
Unedited

FIG. 1

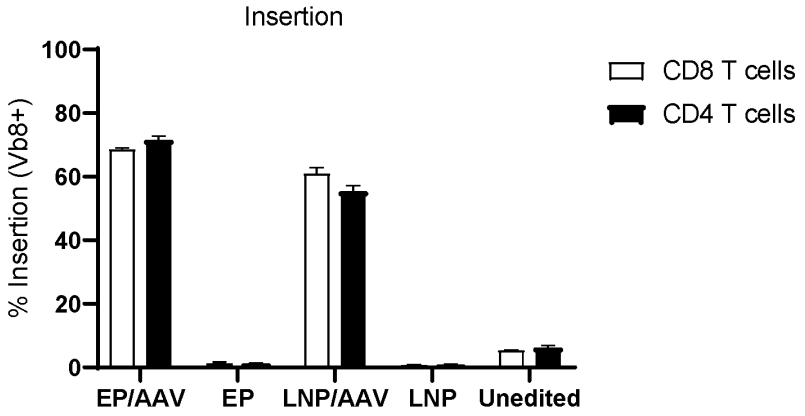

Insertion
% Insertion (Vb8+)
100
80
60
40
20
0
CD8 T cells
CD4 T cells
EP/AAV
EP
LNP/AAV
LNP
Unedited

FIG. 2

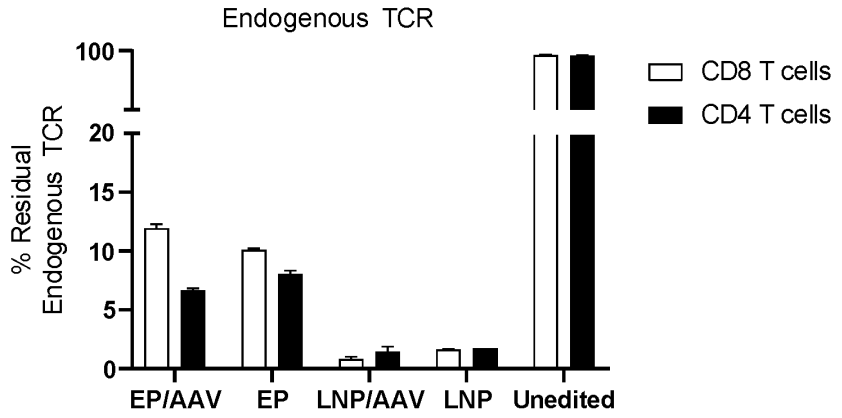

Endogenous TCR
% Residual Endogenous TCR
100
20
15
10
5
0
CD8 T cells
CD4 T cells
EP/AAV
EP
LNP/AAV
LNP
Unedited

FIG. 3

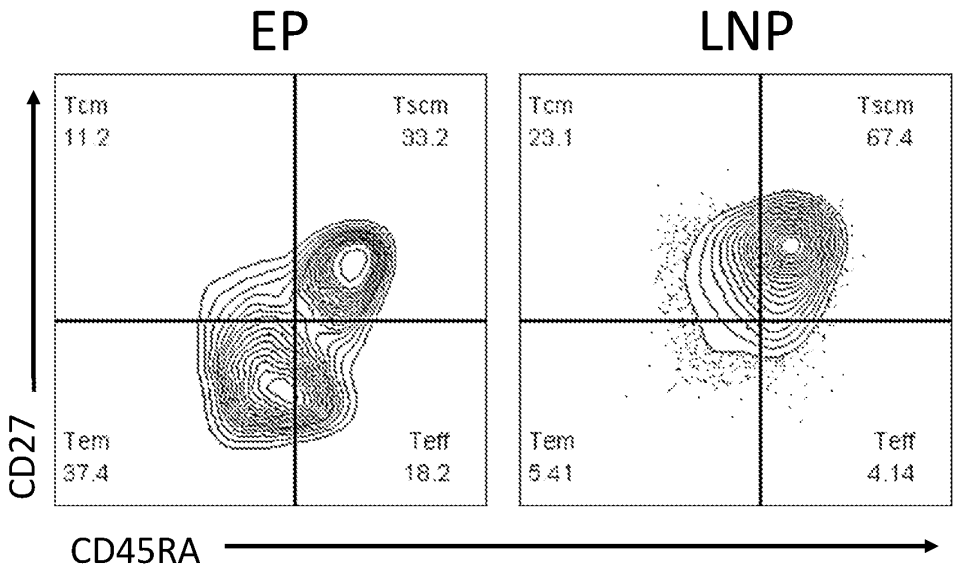

EP
LNP
Tcm
11.2
Tscm
33.2
Tem
37.4
Teff
18.2
Tcm
23.1
Tscm
67.4
Tem
5.41
Teff
4.14
CD27
CD45RA

FIG. 4

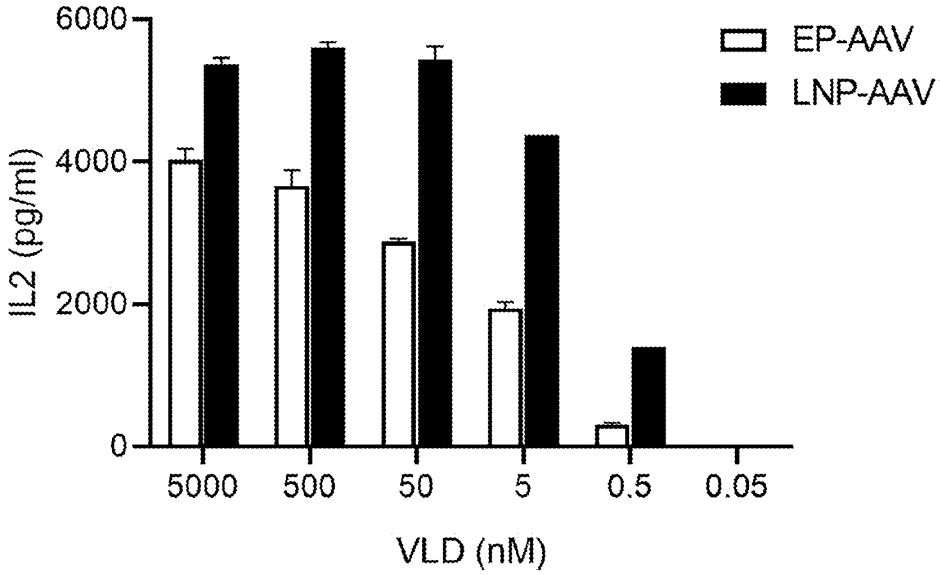

6000
4000
2000
0
IL2 (pg/ml)
EP-AAV
LNP-AAV
5000
500
50
5
0.5
0.05
VLD (nM)

FIG. 5

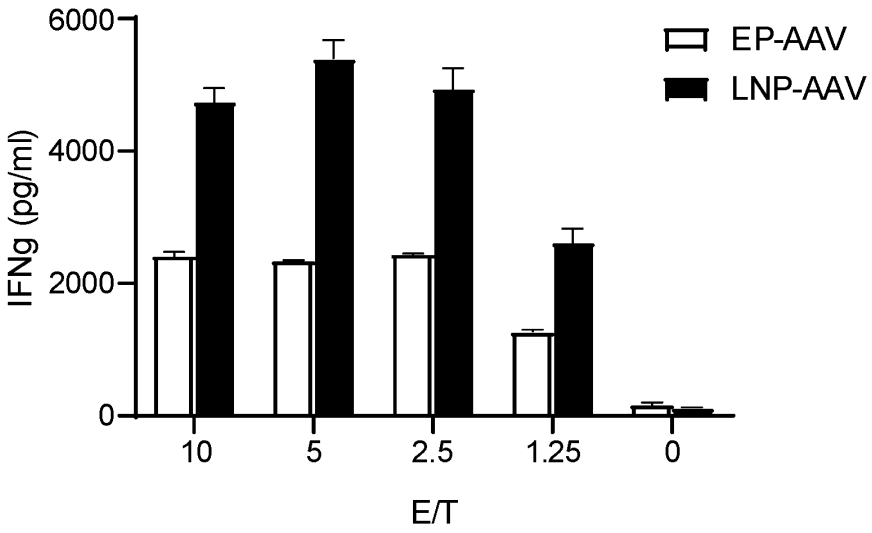

6000
4000
2000
0
IFNg (pg/ml)
EP-AAV
LNP-AAV
10
5
2.5
1.25
0
E/T

FIG. 6

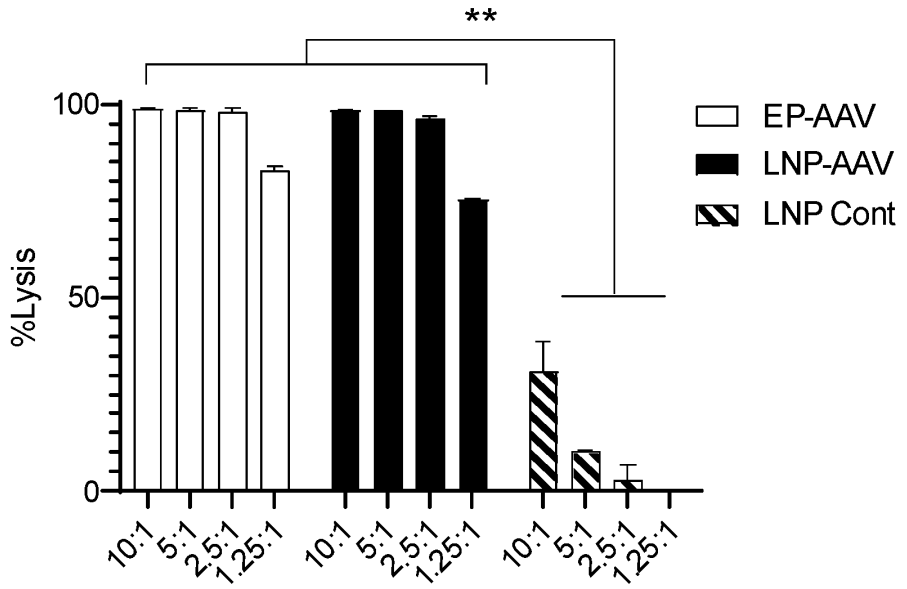

**
100
50
0
%Lysis
EP-AAV
LNP-AAV
LNP Cont
10:1
5:1
2.5:1
1.25:1
10:1
5:1
2.5:1
1.25:1
10:1
5:1
2.5:1
1.25:1

FIG. 7

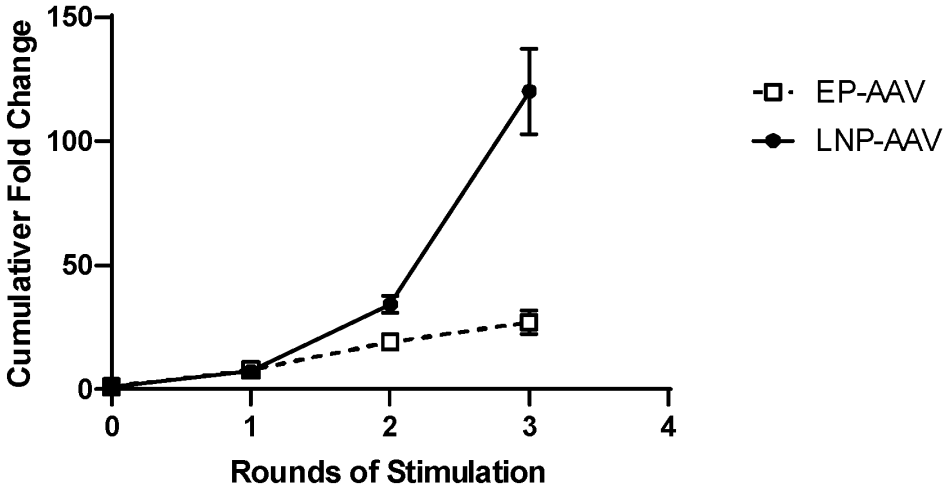

150
100
50
0
Cumulativer Fold Change
0
1
2
3
4
Rounds of Stimulation
EP-AAV
LNP-AAV

FIG. 8

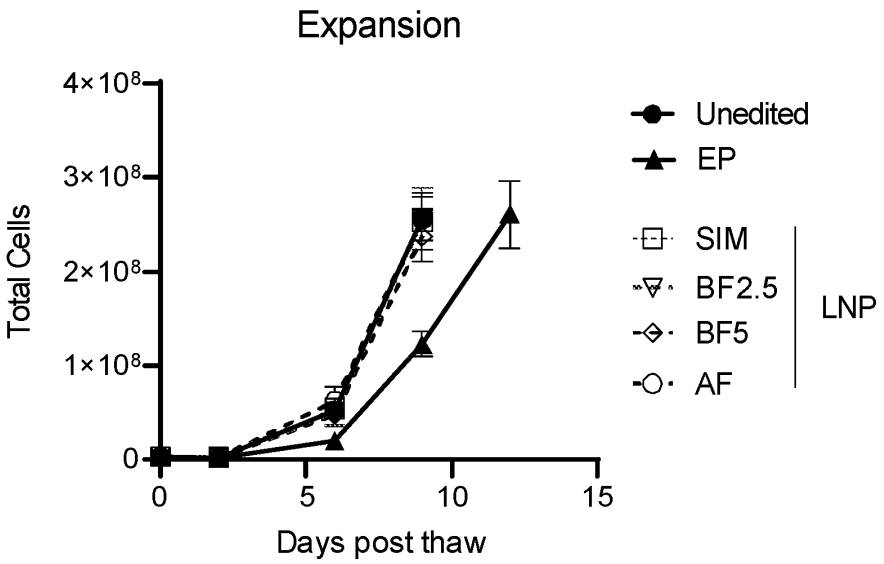

Expansion
Total Cells
4×10^8
3×10^8
2×10^8
1×10^8
0
0
5
10
15
Days post thaw
Unedited
EP
SIM
BF2.5
BF5
AF
LNP

FIG. 9

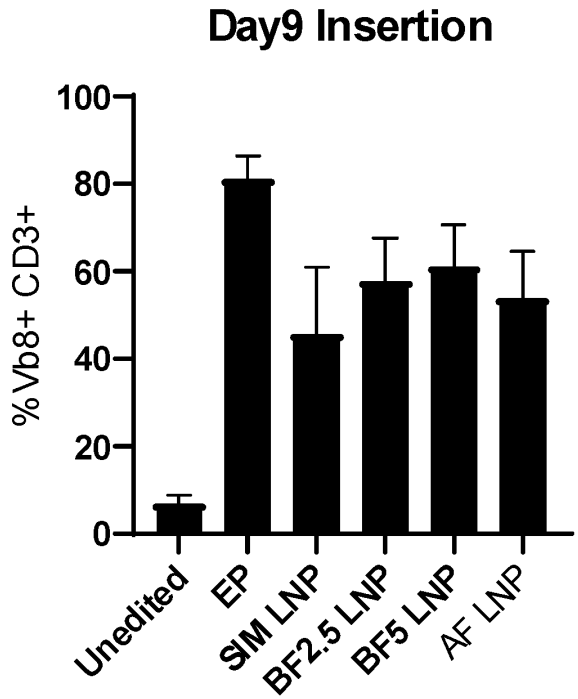

Day9 Insertion
% Vb8+ CD3+
100
80
60
40
20
0
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP

FIG. 10

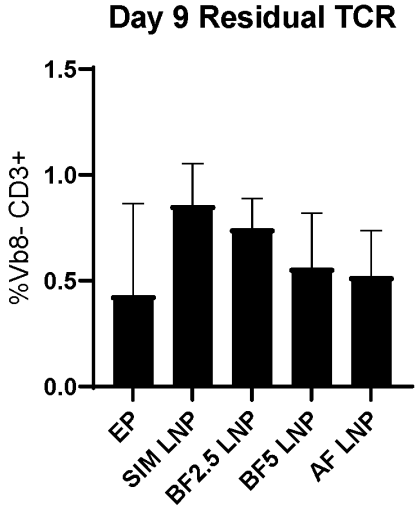

Day 9 Residual TCR
%Vb8- CD3+
1.5
1.0
0.5
0.0
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP

FIG. 11

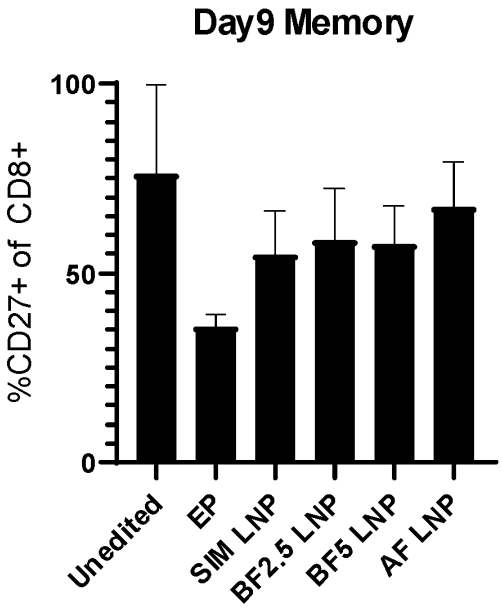

Day9 Memory
%CD27+ of CD8+
100
50
0
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP

FIG. 12

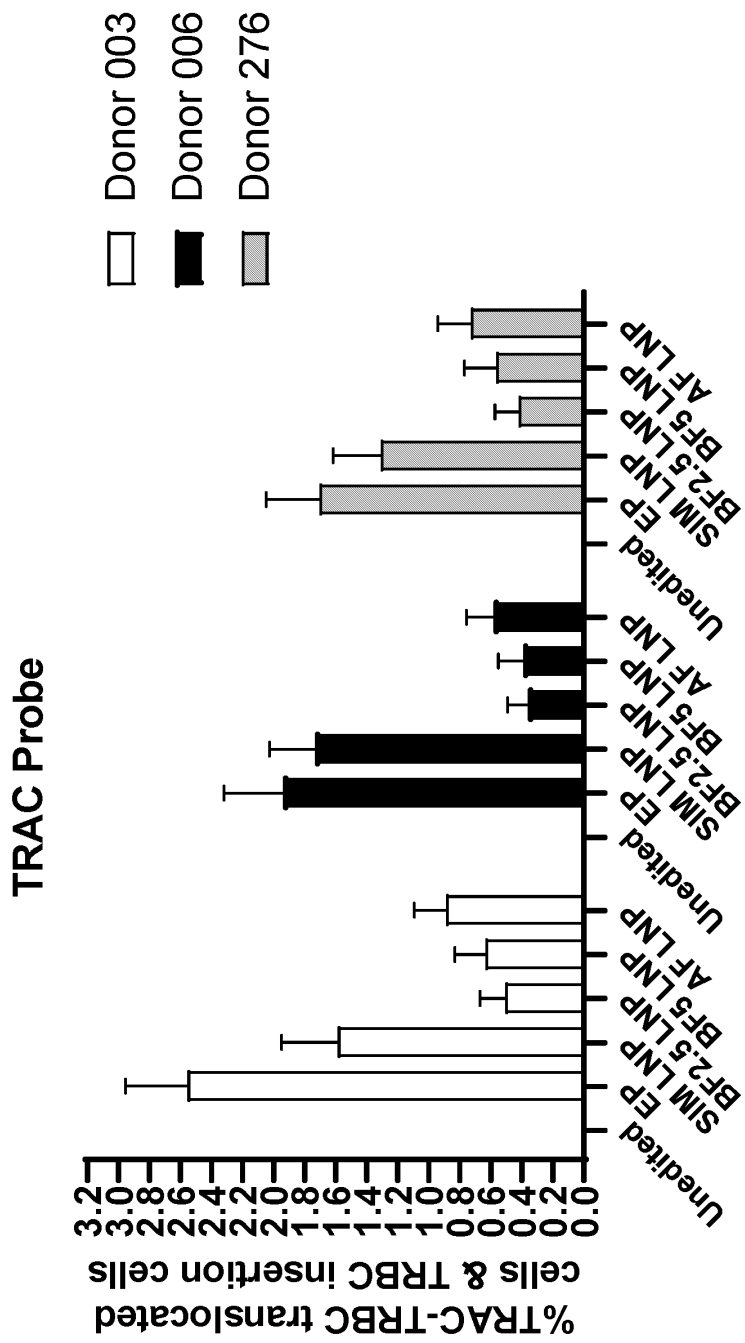

Donor 003
Donor 006
Donor 276
TRAC Probe
%TRAC-TRBC translocated cells & TRBC insertion cells
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP

FIG. 13A

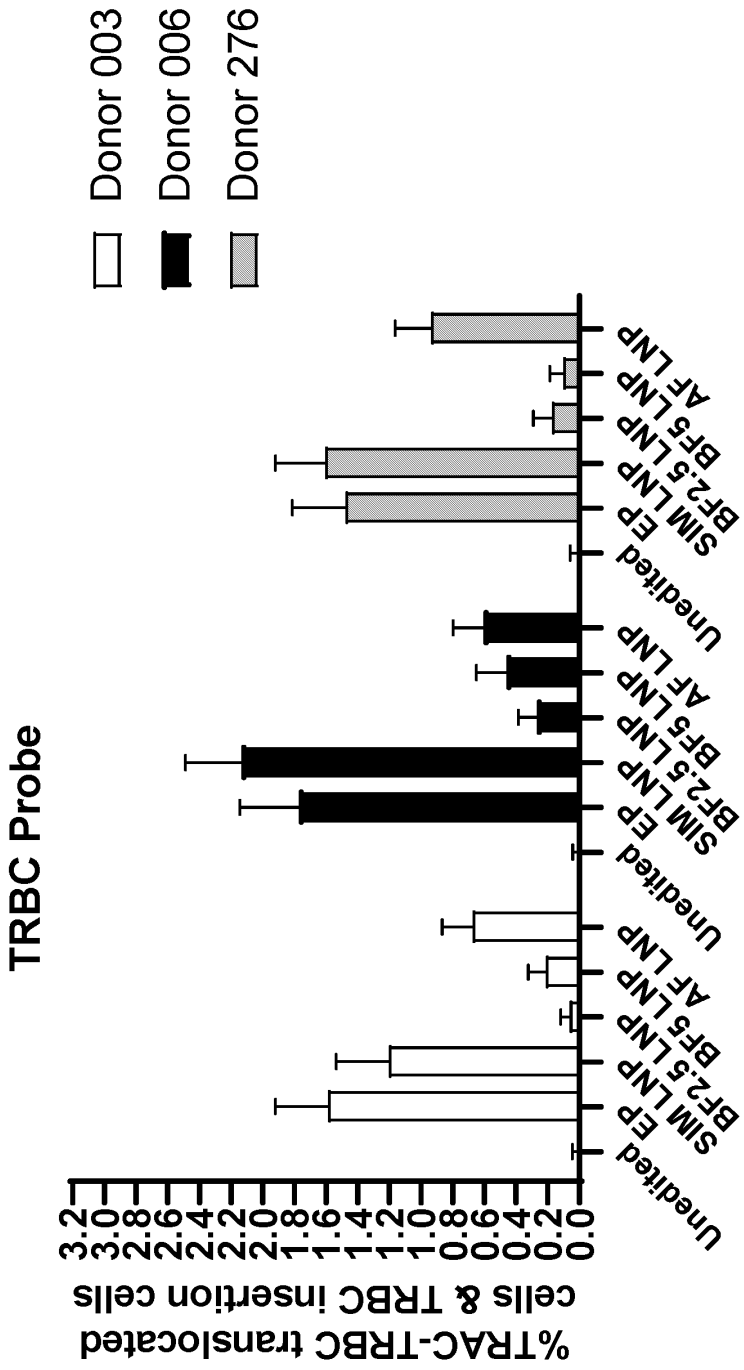

Donor 003
Donor 006
Donor 276
TRBC Probe
%TRAC-TRBC translocated cells & TRBC insertion cells
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP

FIG. 13B

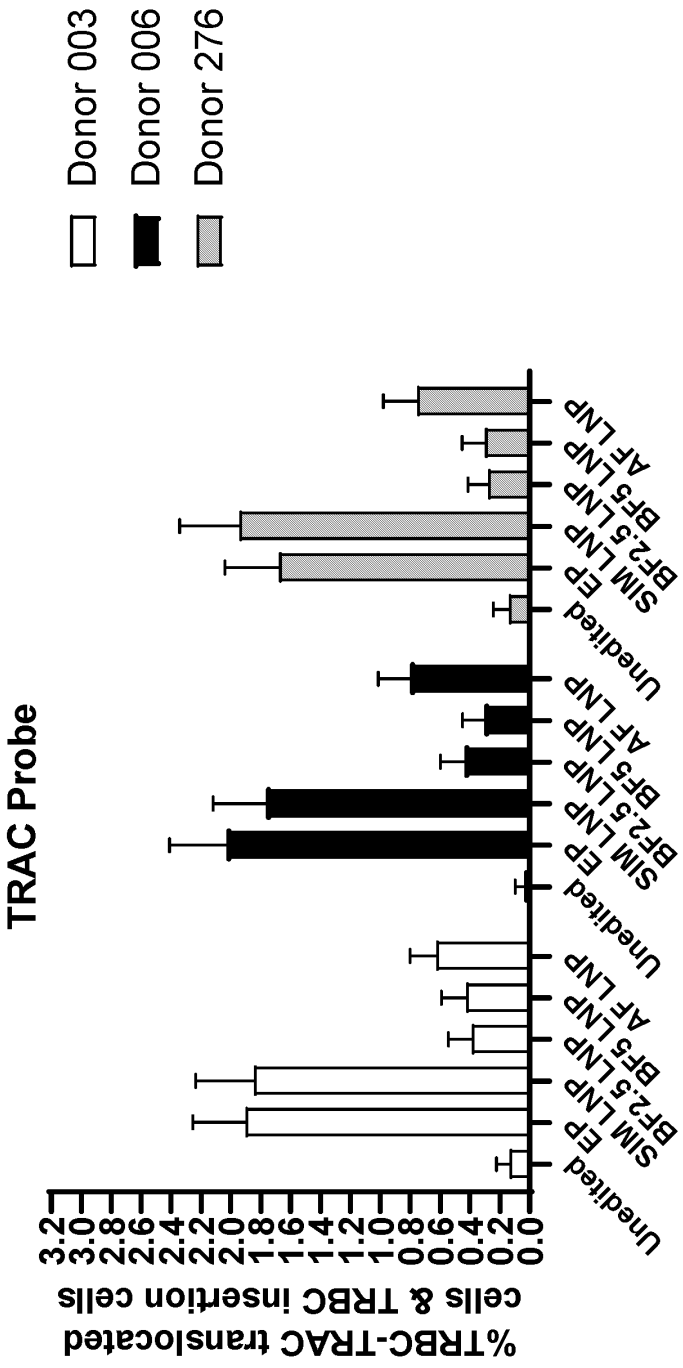

Donor 003
Donor 006
Donor 276
TRAC Probe
%TRBC-TRAC translocated cells & TRBC insertion cells
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP

FIG. 14A

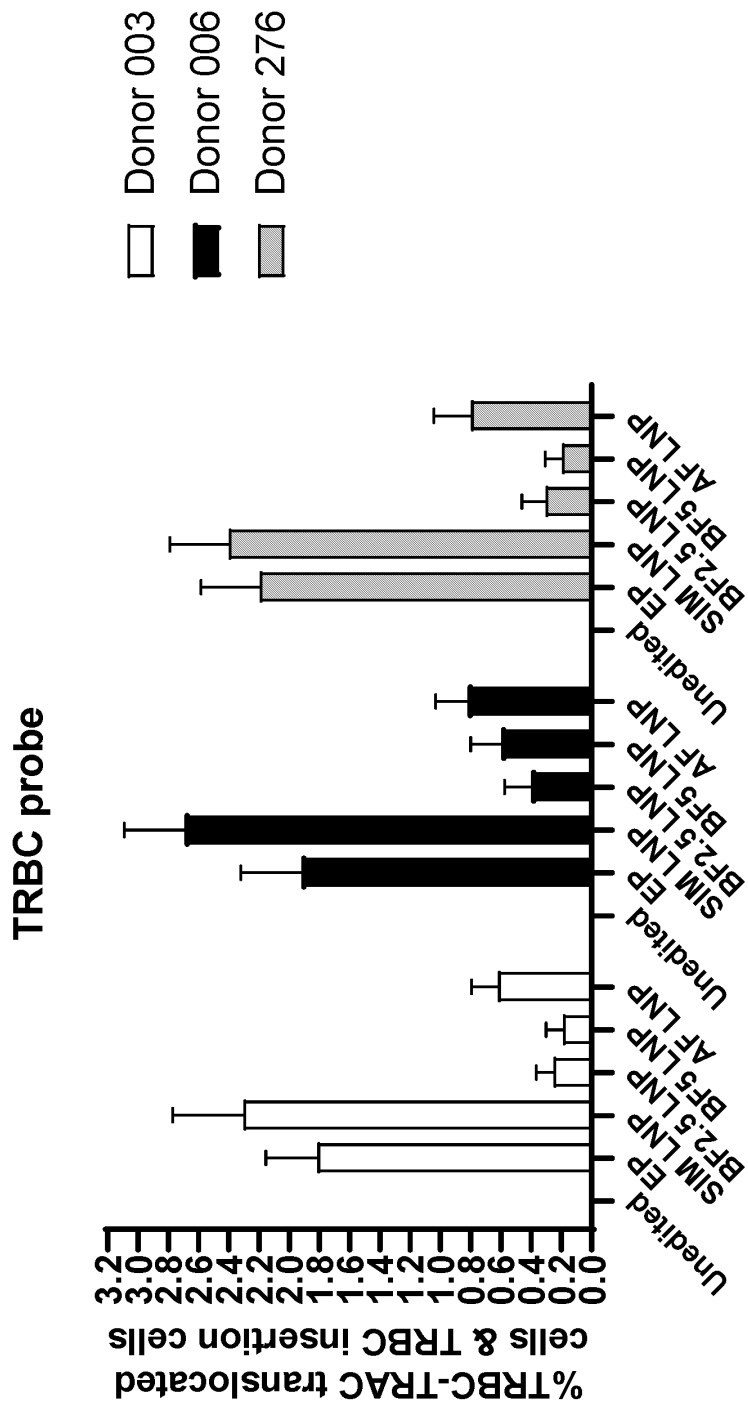

Donor 003
Donor 006
Donor 276
TRBC probe
%TRBC-TRAC translocated cells & TRBC insertion cells
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP
Unedited
EP
SIM LNP
BF2.5 LNP
BF5 LNP
AF LNP

FIG. 14B

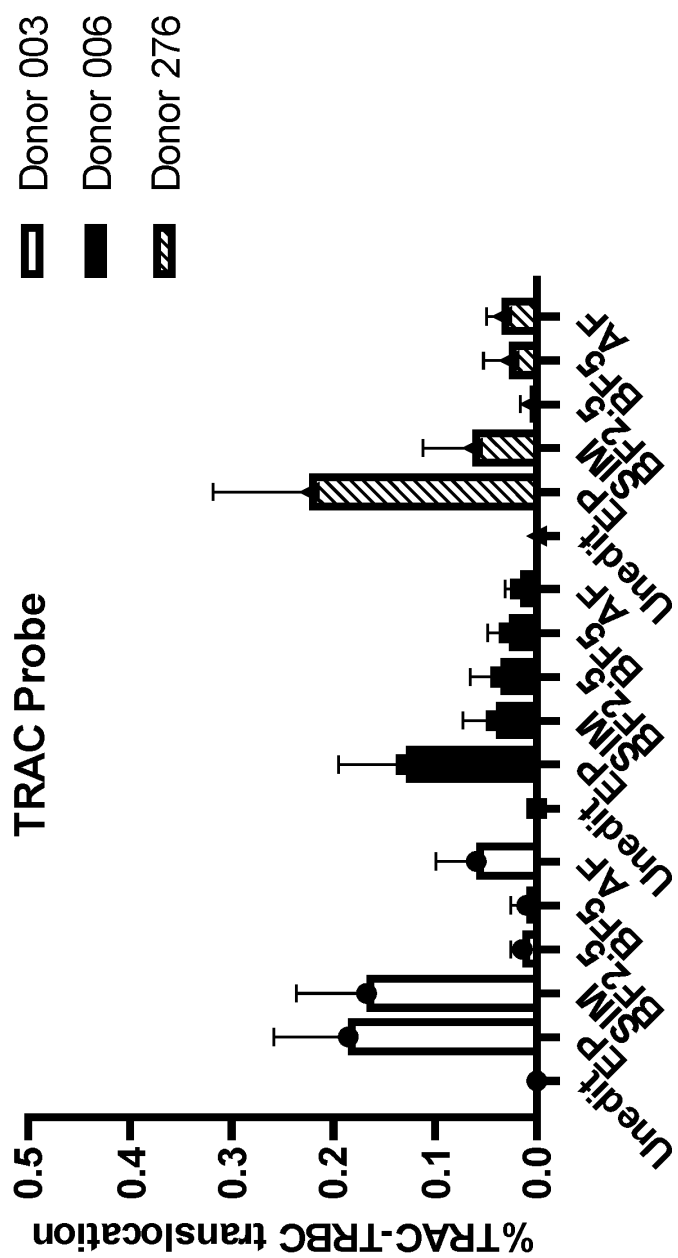

Donor 003
Donor 006
Donor 276
TRAC Probe
%TRAC-TRBC translocation
0.5
0.4
0.3
0.2
0.1
0.0
Unedited
5'ssEBT2-WIS5'ssEBT2-BF
Unedited
5'ssEBT2-WIS5'ssEBT2-AF
Unedited
5'ssEBT2-WIS5'ssEBT2-BF
Unedited
5'ssEBT2-WIS5'ssEBT2-AF

FIG. 14C

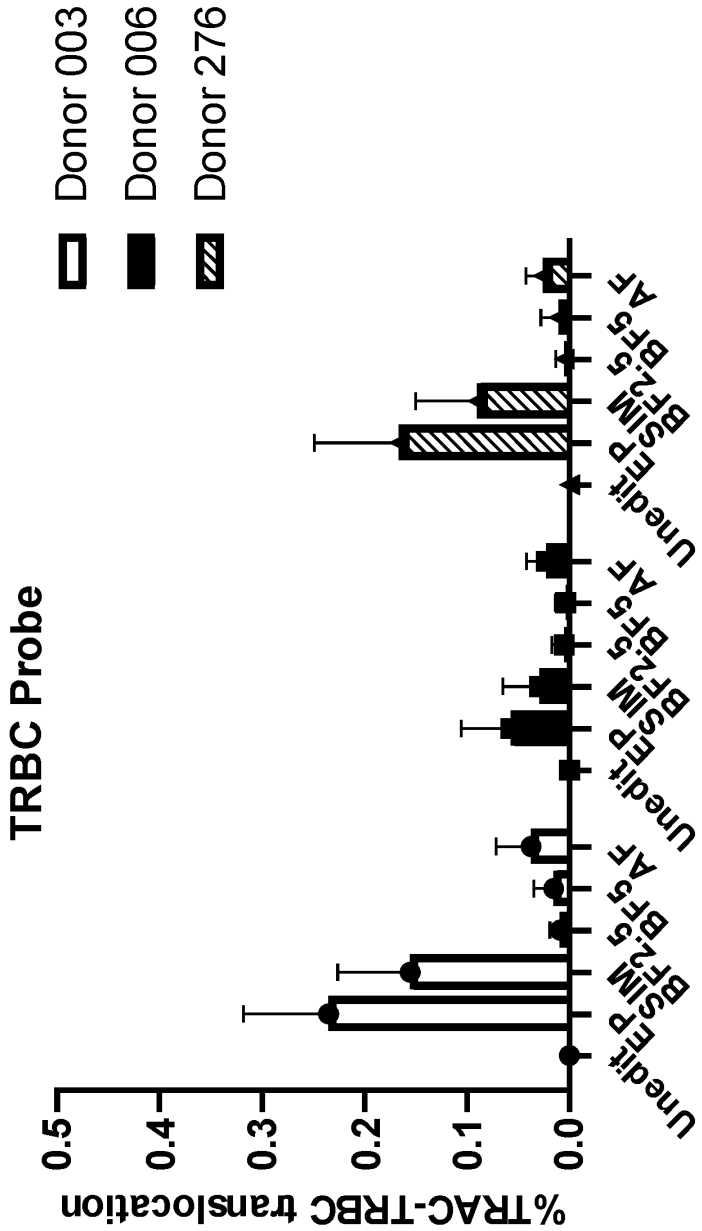

Donor 003
Donor 006
Donor 276
TRBC Probe
%TRAC-TRBC translocation
0.5
0.4
0.3
0.2
0.1
0.0
Unedit
BsTEV1 2.5 μM
BsTEV1 2.5 μM + BsTEV2 2.5 μM
Unedit
BsTEV1 2.5 μM
BsTEV1 2.5 μM + BsTEV2 2.5 μM
Unedit
BsTEV1 2.5 μM
BsTEV1 2.5 μM + BsTEV2 2.5 μM

FIG. 14D

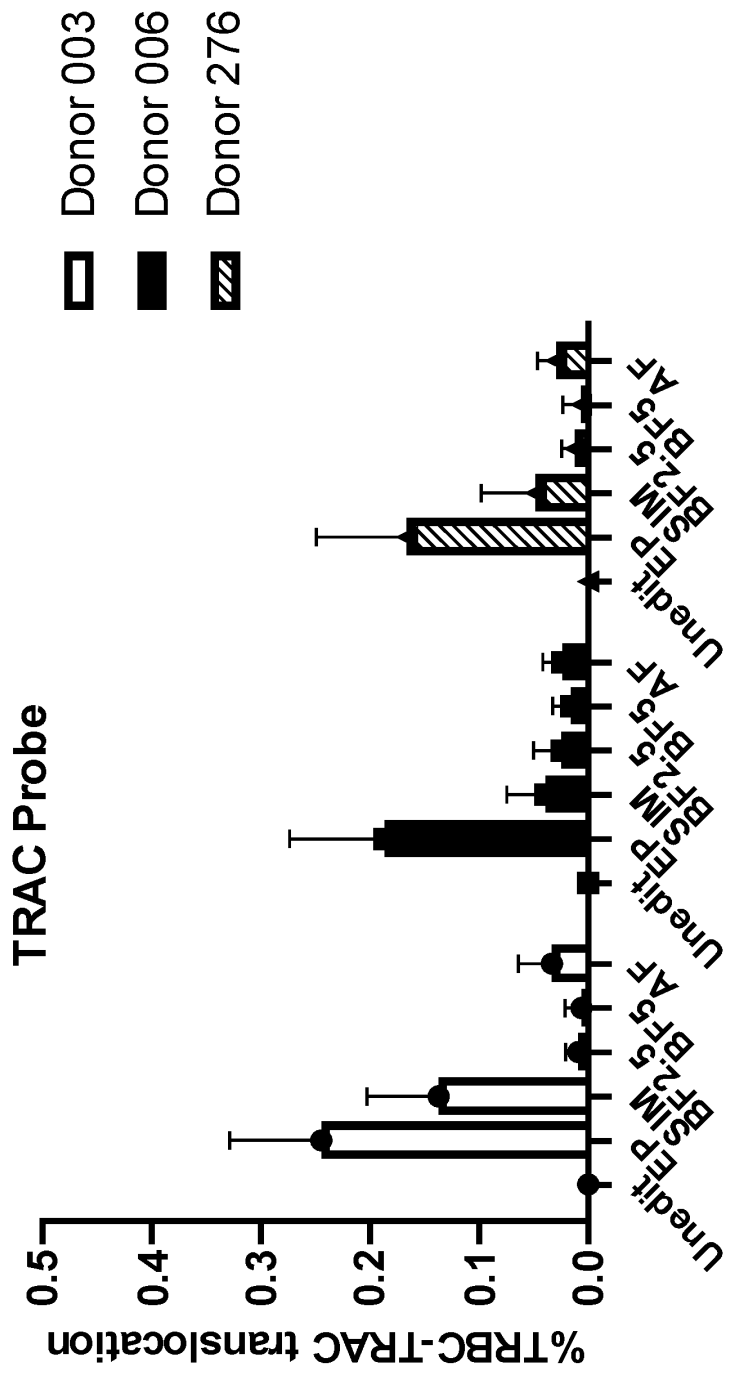

Donor 003
Donor 006
Donor 276
TRAC Probe
%TRBC-TRAC translocation
0.0
0.1
0.2
0.3
0.4
0.5
Unedited
SIM 5'
BFP 2'
BFP 5' AF
Unedited
SIM 5'
BFP 2'
BFP 5' AF
Unedited
SIM 5'
BFP 2'
BFP 5' AF

FIG. 14E

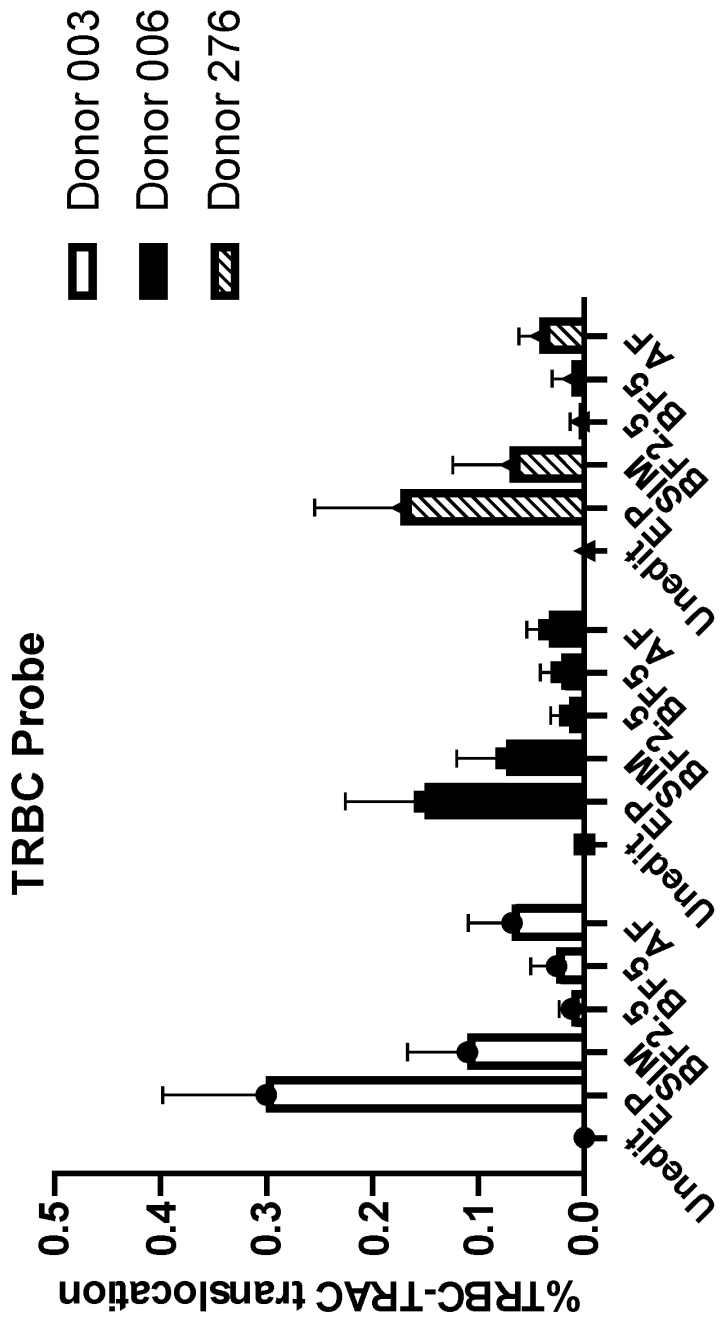

Donor 003
Donor 006
Donor 276
TRBC Probe
%TRBC-TRAC translocation
0.5
0.4
0.3
0.2
0.1
0.0
Unedit
BFP5 2WIS5 BFP5 AF
Unedit
BFP5 2WIS5 BFP5 AF
Unedit
BFP5 2WIS5 BFP5 AF

FIG. 14F

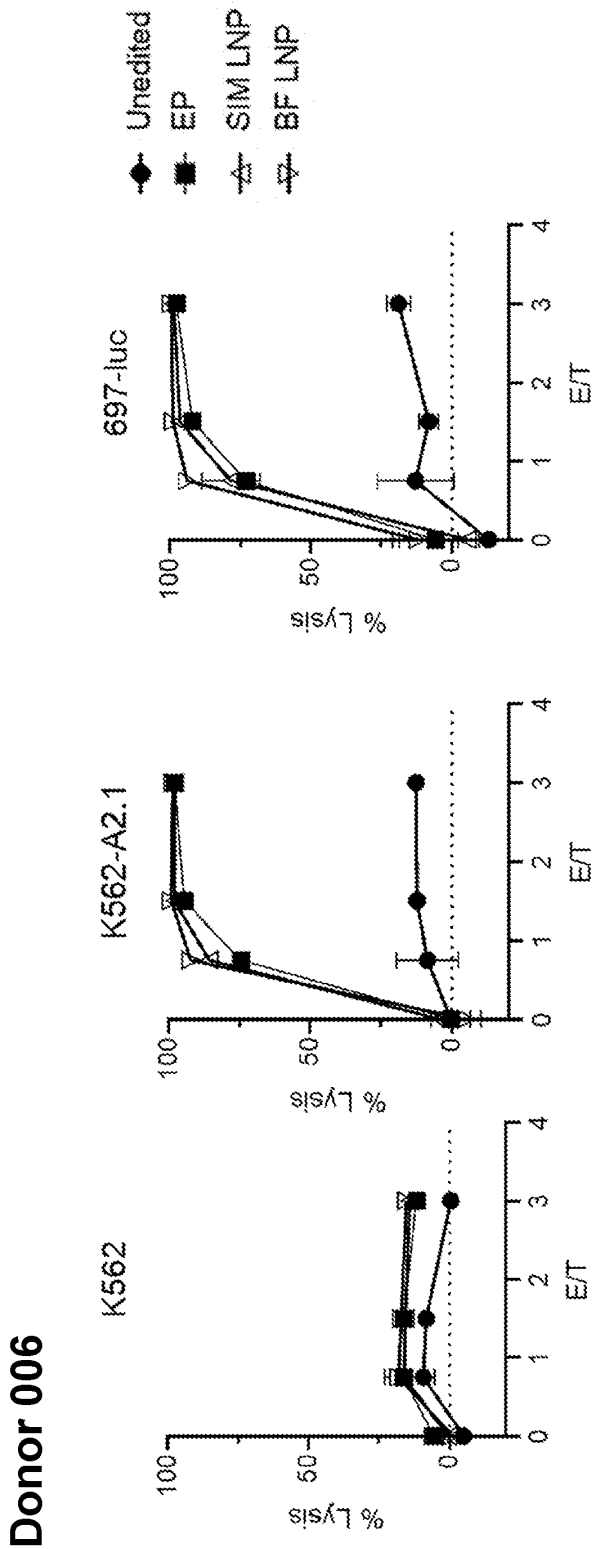


FIG. 15A

FIG. 15B

FIG. 15C

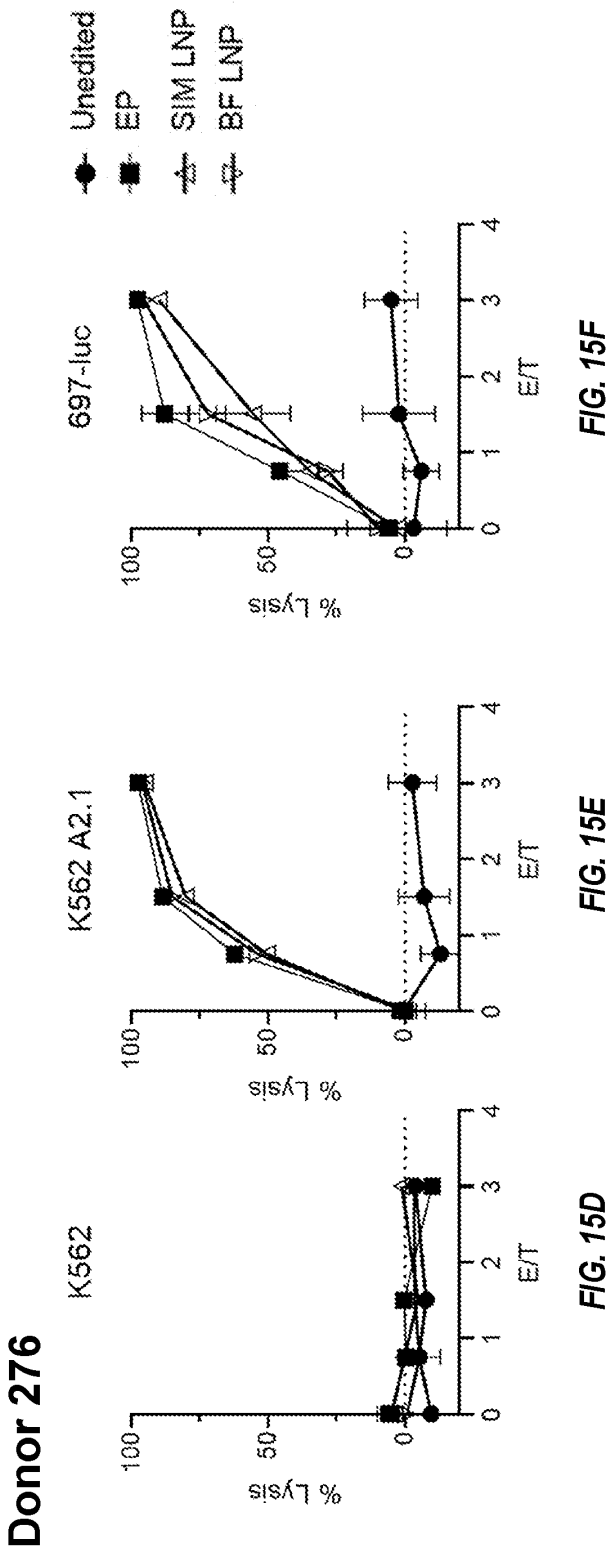


FIG. 15D

FIG. 15E

FIG. 15F

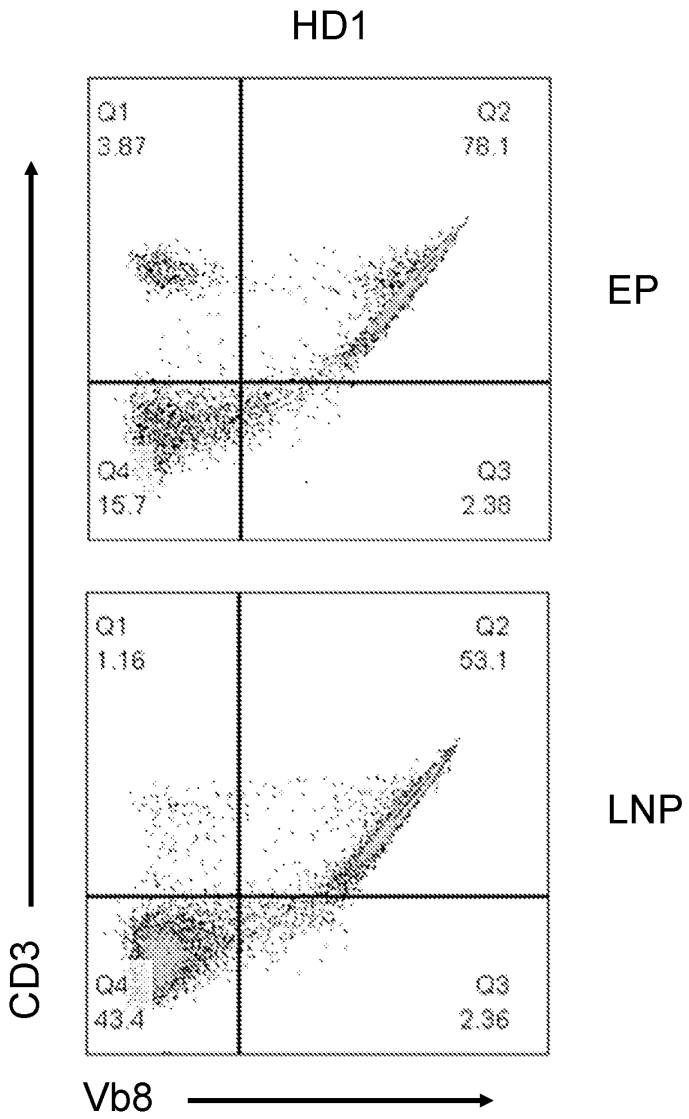

HD1
Q1
3.87
Q2
78.1
Q4
15.7
Q3
2.38
EP
Q1
1.16
Q2
53.1
Q4
43.4
Q3
2.36
LNP
CD3
Vb8

FIG. 16

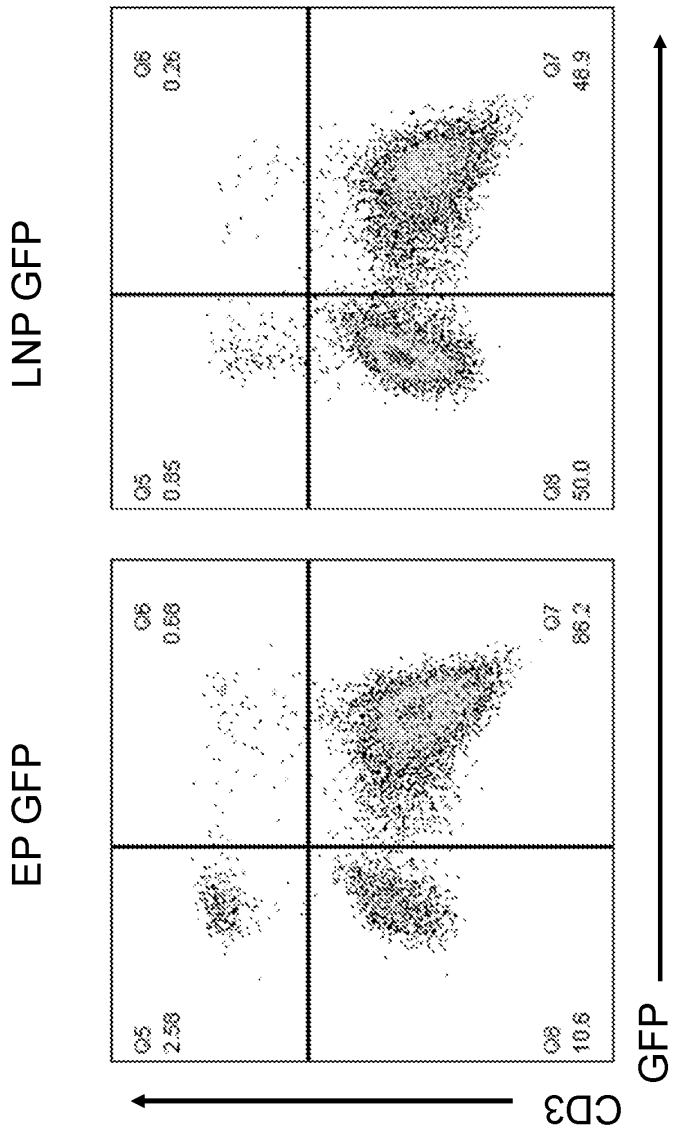

LNP GFP
EP GFP
Q6
0.26
Q7
46.9
Q5
0.55
Q8
50.0
Q6
0.68
Q7
86.2
Q5
2.58
Q8
10.6
CD3
GFP

FIG. 17

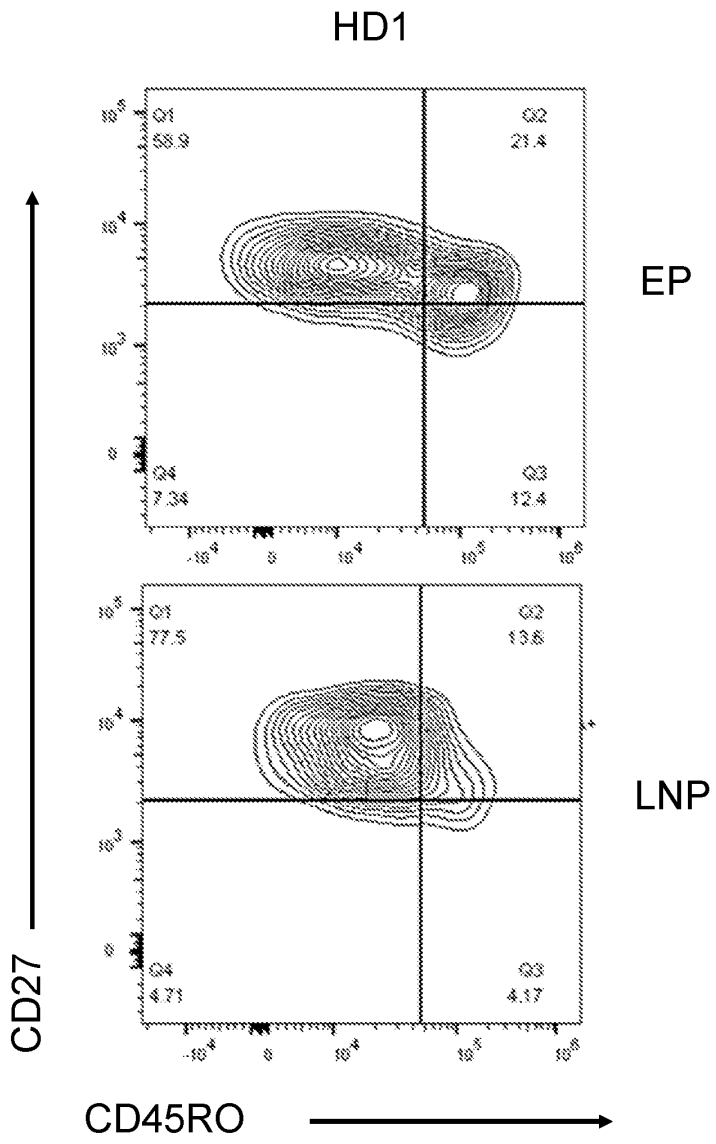

HD1
Q1
65.9
Q2
21.4
Q4
7.34
Q3
12.4
EP
Q1
77.5
Q2
13.6
Q4
4.71
Q3
4.17
LNP
CD27
CD45RO

FIG. 18

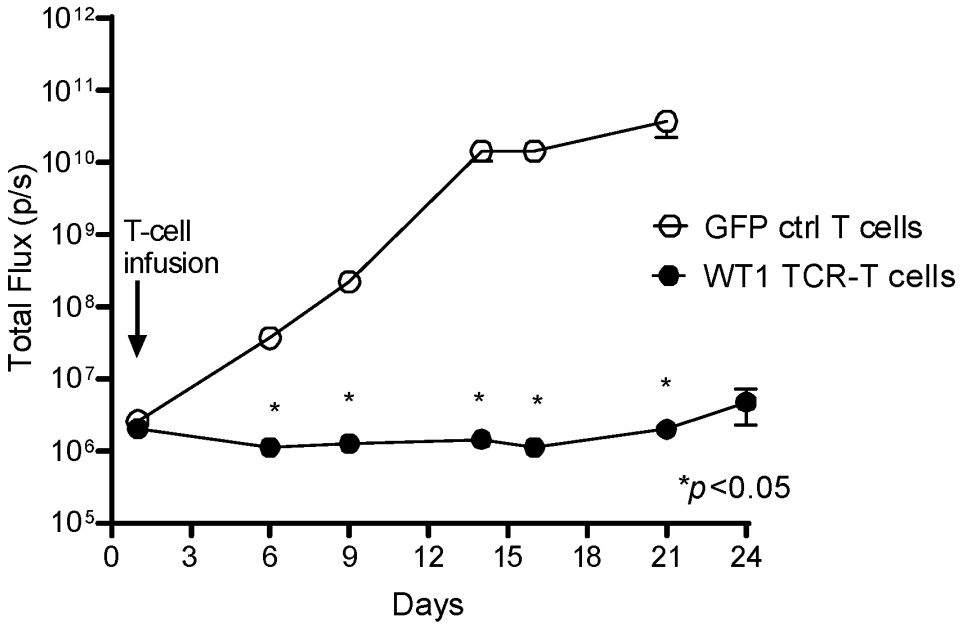

Total Flux (p/s)
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
T-cell infusion
GFP ctrl T cells
WT1 TCR-T cells
*
*p<0.05
0 3 6 9 12 15 18 21 24
Days

FIG. 19

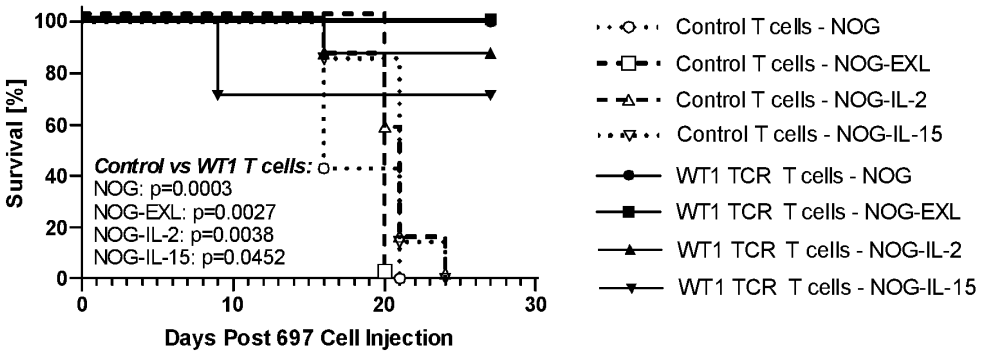

Survival [%]
100
80
60
40
20
0
Control vs WT1 T cells:
NOG: p=0.0003
NOG-EXL: p=0.0027
NOG-IL-2: p=0.0038
NOG-IL-15: p=0.0452
0 10 20 30
Days Post 697 Cell Injection
Control T cells - NOG
Control T cells - NOG-EXL
Control T cells - NOG-IL-2
Control T cells - NOG-IL-15
WT1 TCR T cells - NOG
WT1 TCR T cells - NOG-EXL
WT1 TCR T cells - NOG-IL-2
WT1 TCR T cells - NOG-IL-15

FIG. 20

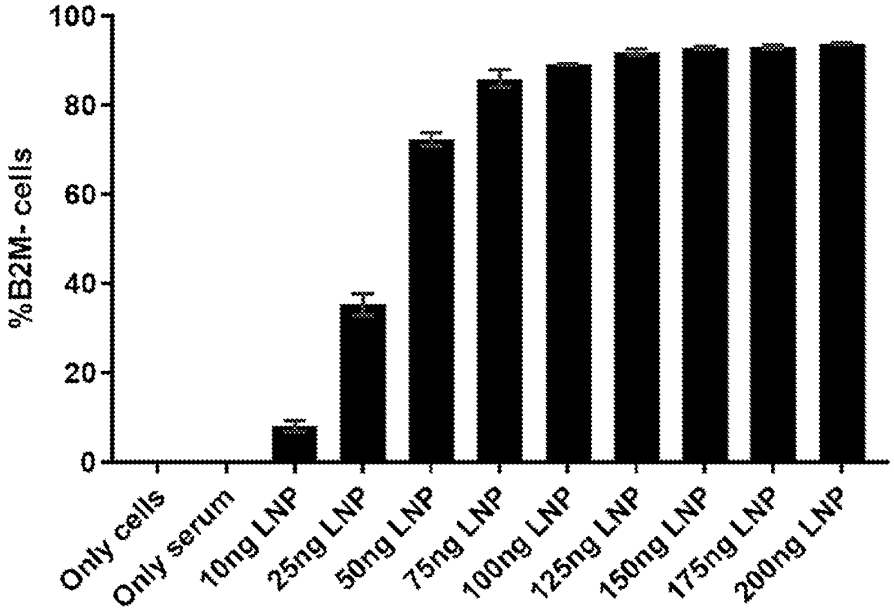


FIG. 21A

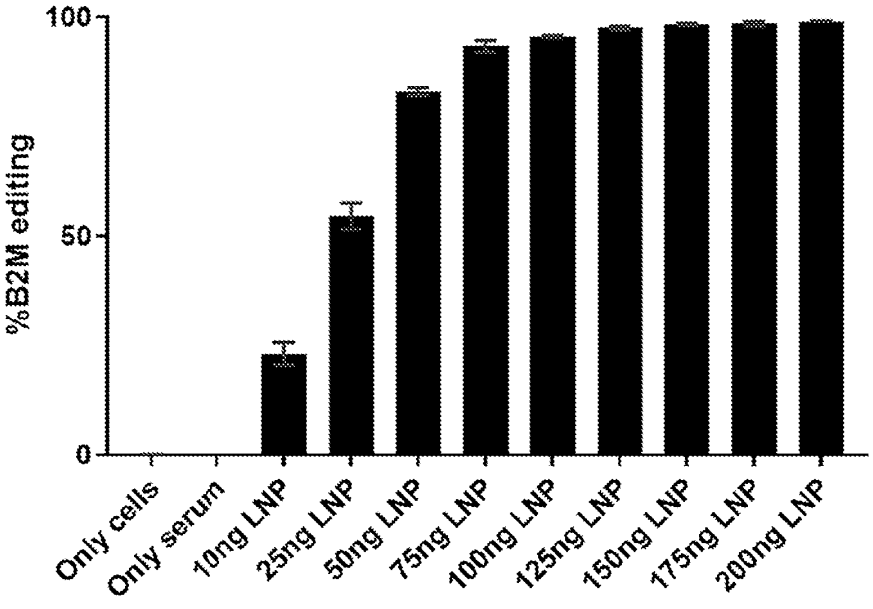


FIG. 21B

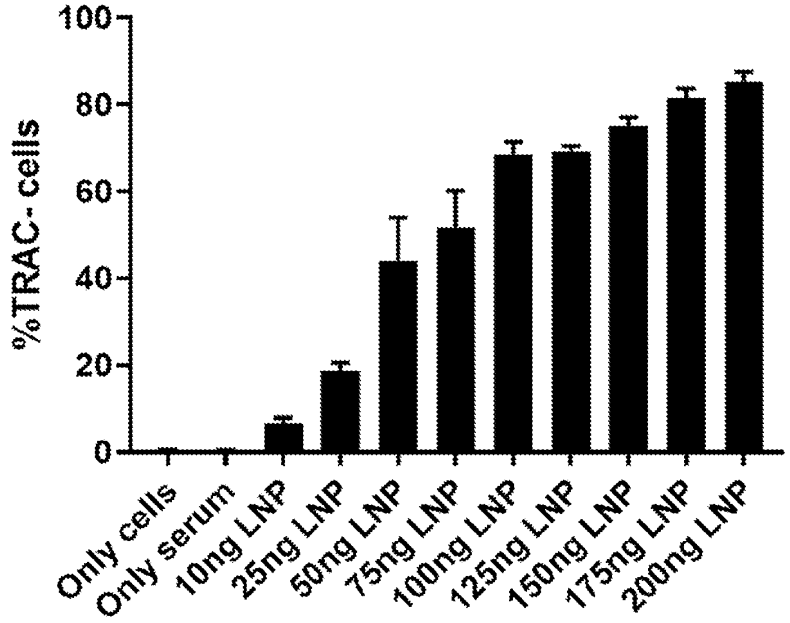


FIG. 22A

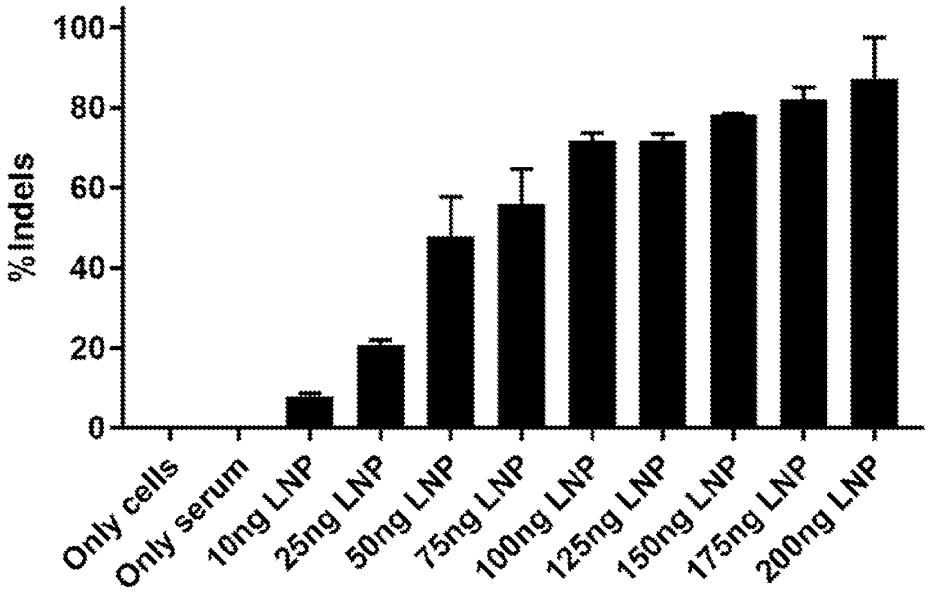


FIG. 22B

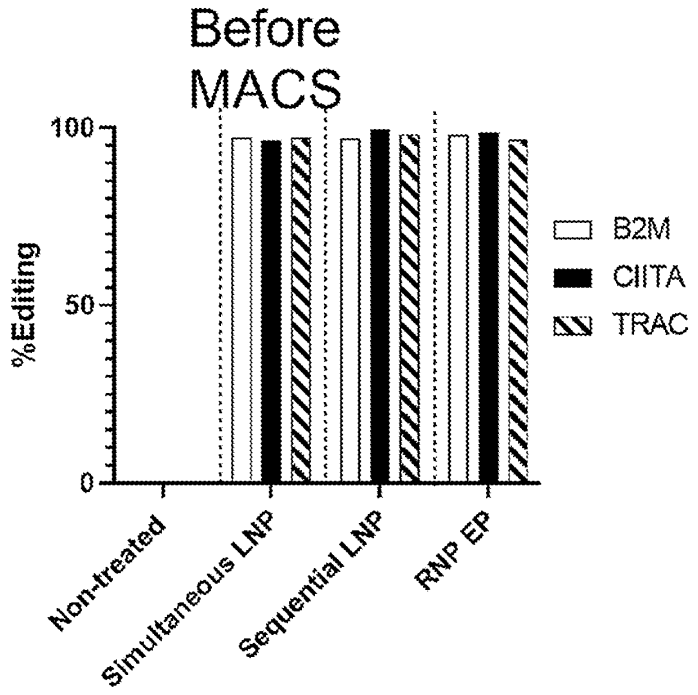


FIG. 23A

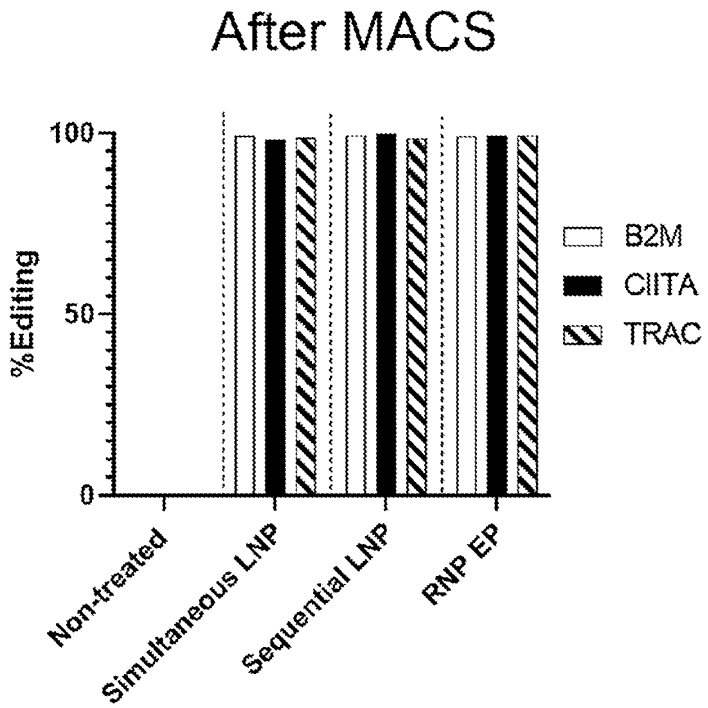


FIG. 23B

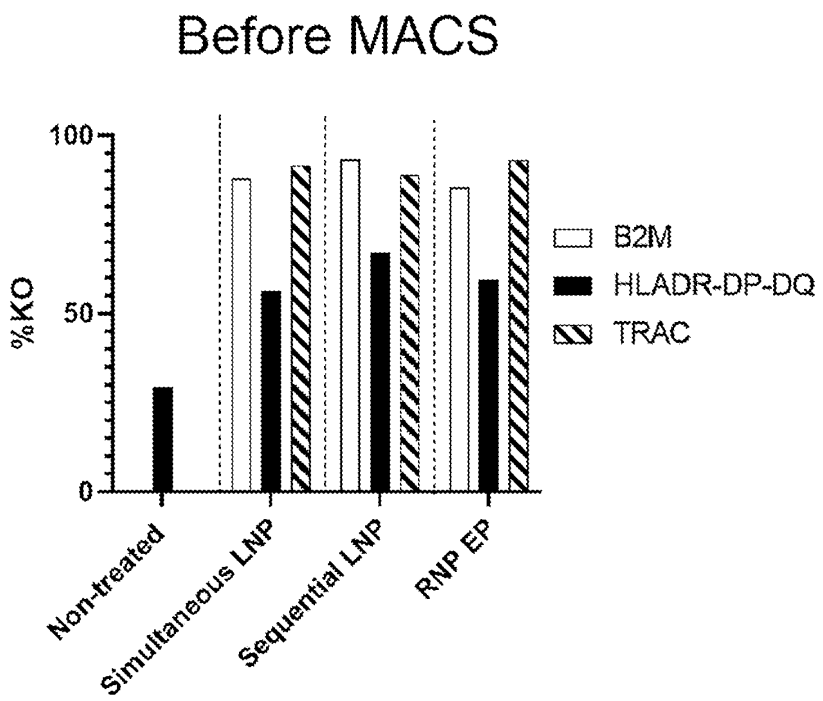


FIG. 24A

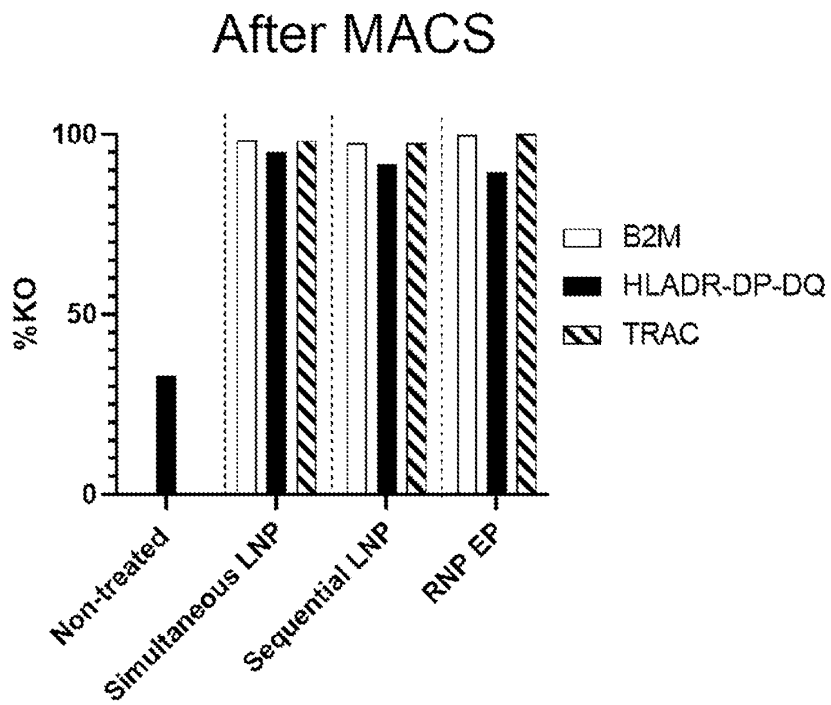


FIG. 24B

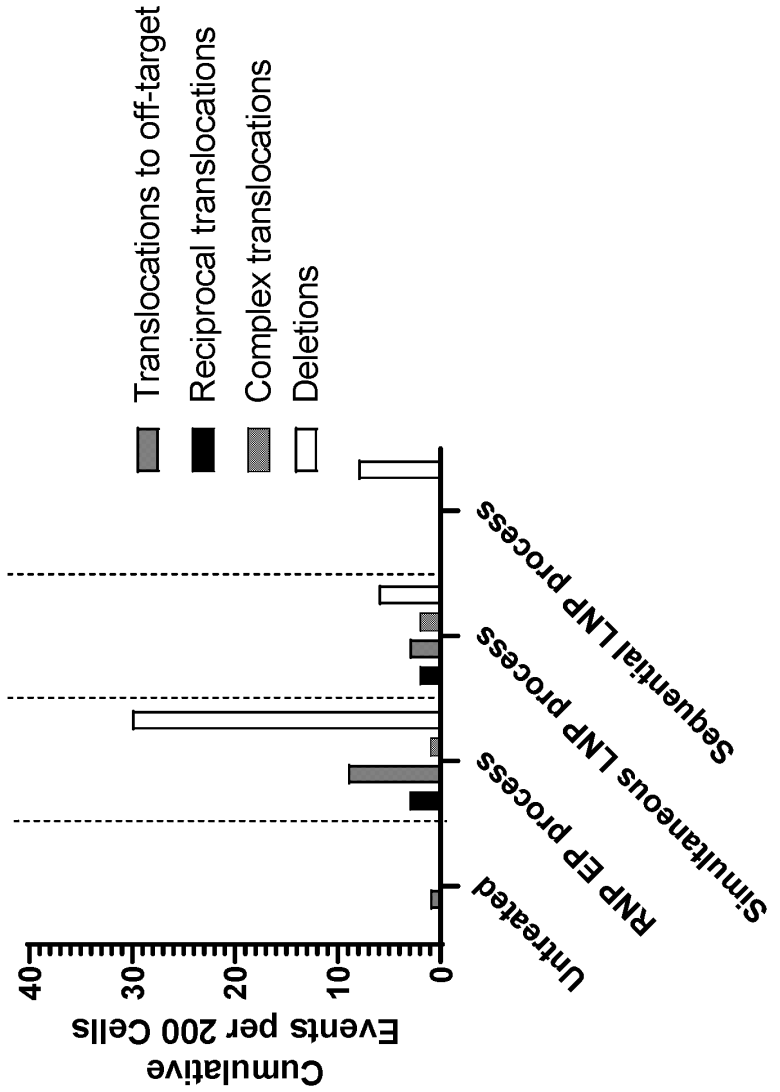

Translocations to off-target
Reciprocal translocations
Complex translocations
Deletions
Cumulative Events per 200 Cells
0
10
20
30
40
Untreated
RNP EP process
Simultaneous LNP process
Sequential LNP process

FIG. 25

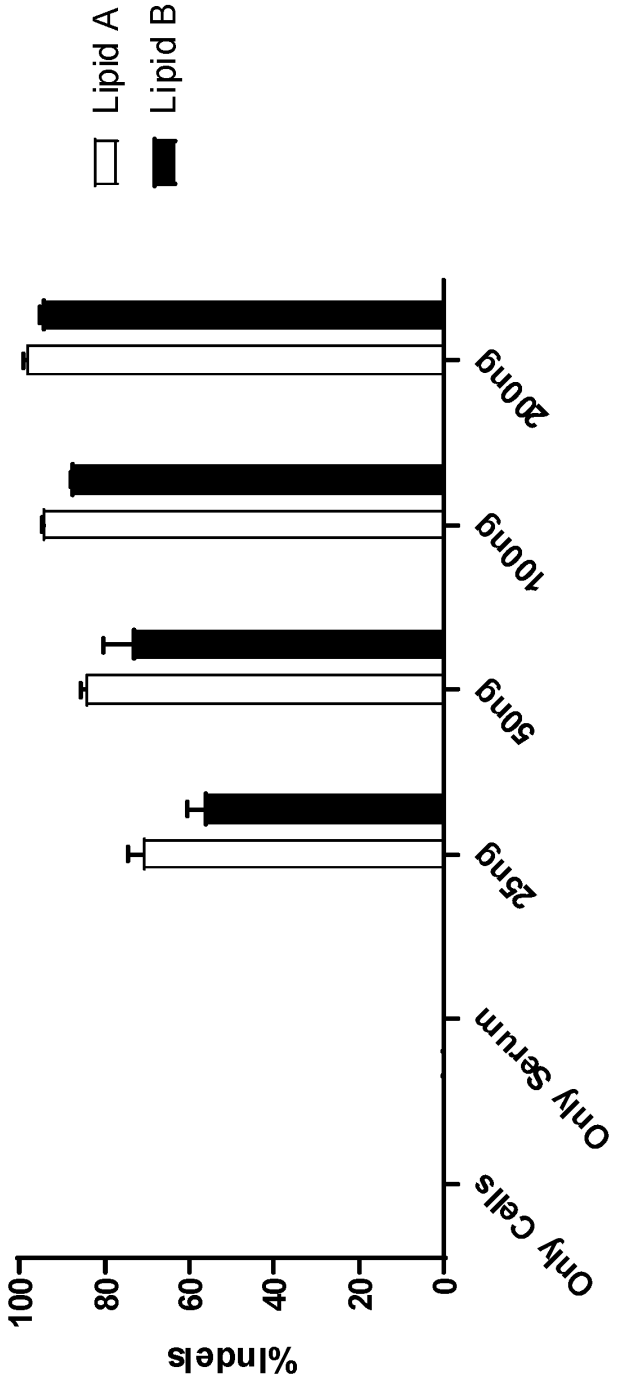

Lipid A
Lipid B
%Indels
100
80
60
40
20
0
Only Cells
Only Serum
25ng
50ng
100ng
200ng

FIG. 26

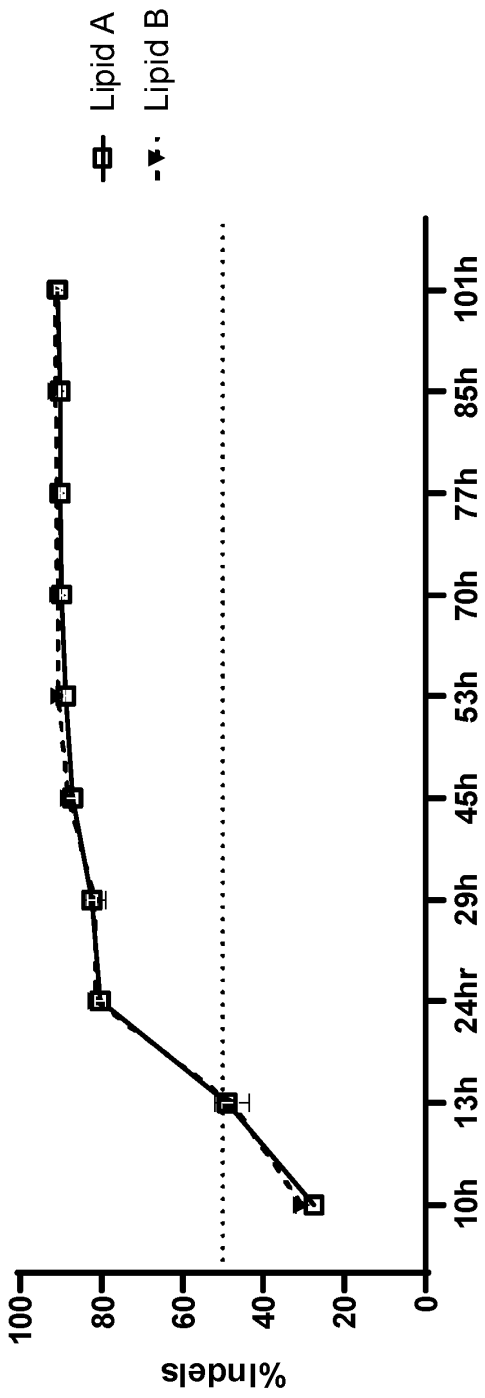

Lipid A
Lipid B
%Indels
100
80
60
40
20
0
10h
13h
24hr
29h
45h
53h
70h
77h
85h
101h
Hours

FIG. 27

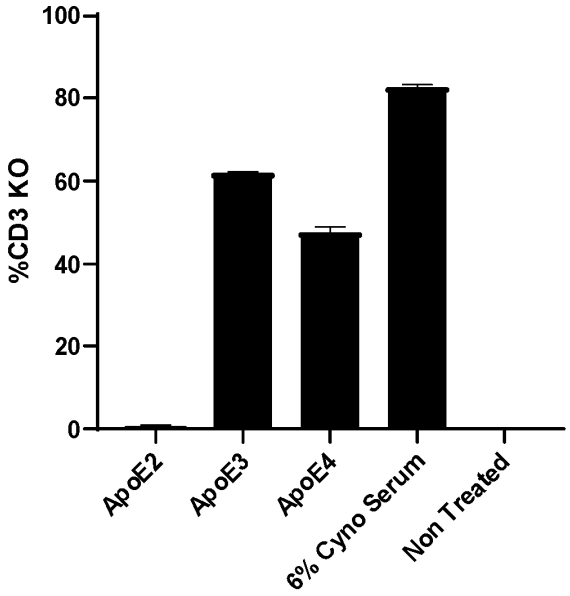

100
80
60
40
20
0
%CD3 KO
ApoE2
ApoE3
ApoE4
6% Cyno Serum
Non Treated

FIG. 28

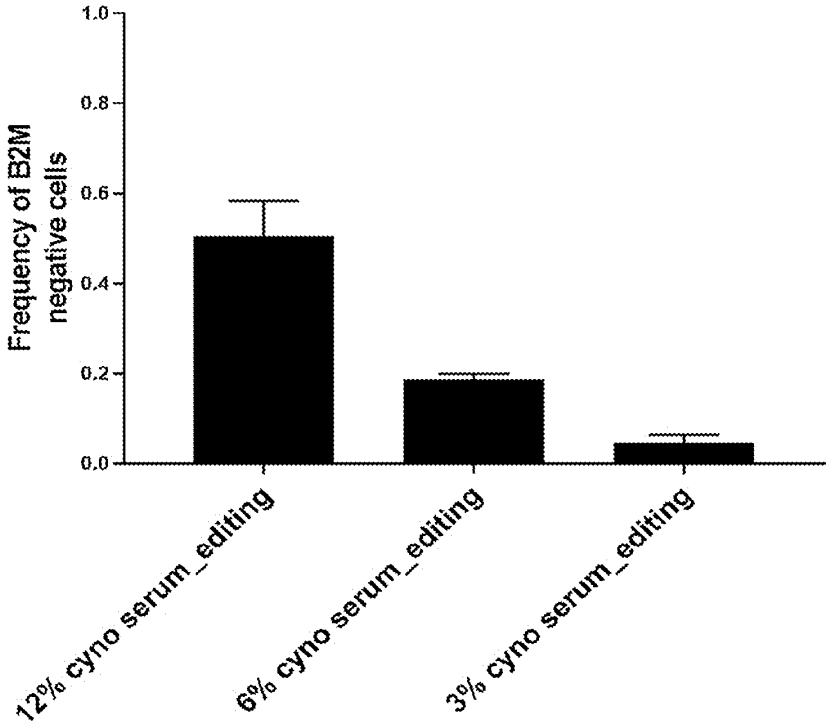

1.0
0.8
0.6
0.4
0.2
0.0
Frequency of B2M negative cells
12% cyno serum_editing
6% cyno serum_editing
3% cyno serum_editing

FIG. 29

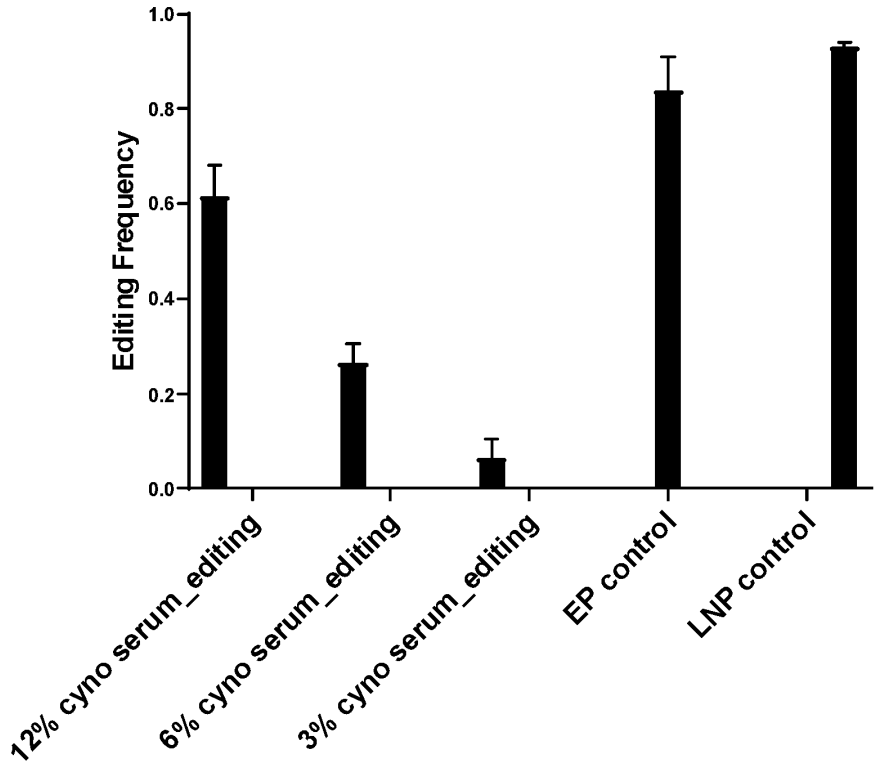

Editing Frequency
1.0
0.8
0.6
0.4
0.2
0.0
12% cyno serum_editing
6% cyno serum_editing
3% cyno serum_editing
EP control
LNP control

FIG. 30

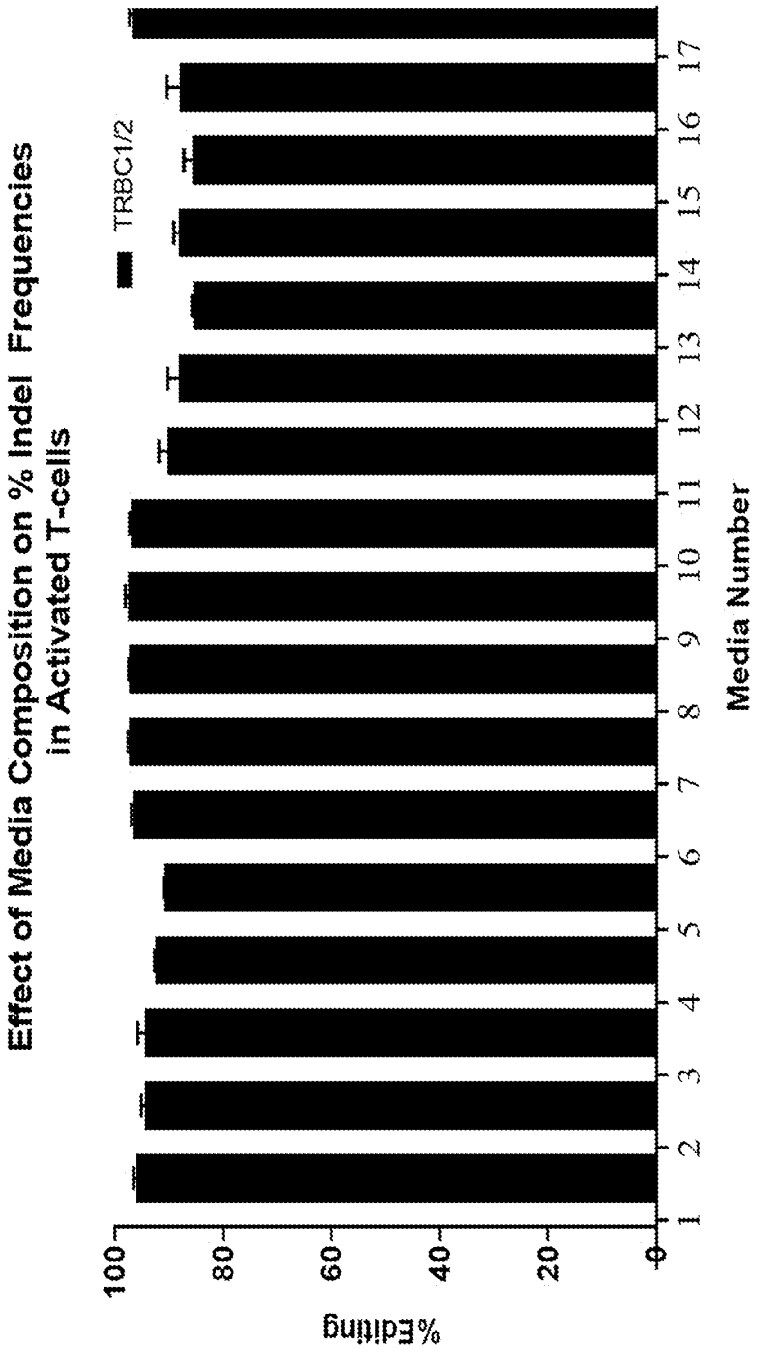

Effect of Media Composition on % Indel Frequencies in Activated T-cells
TRBC1/2
%Editing
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
Media Number

FIG. 31

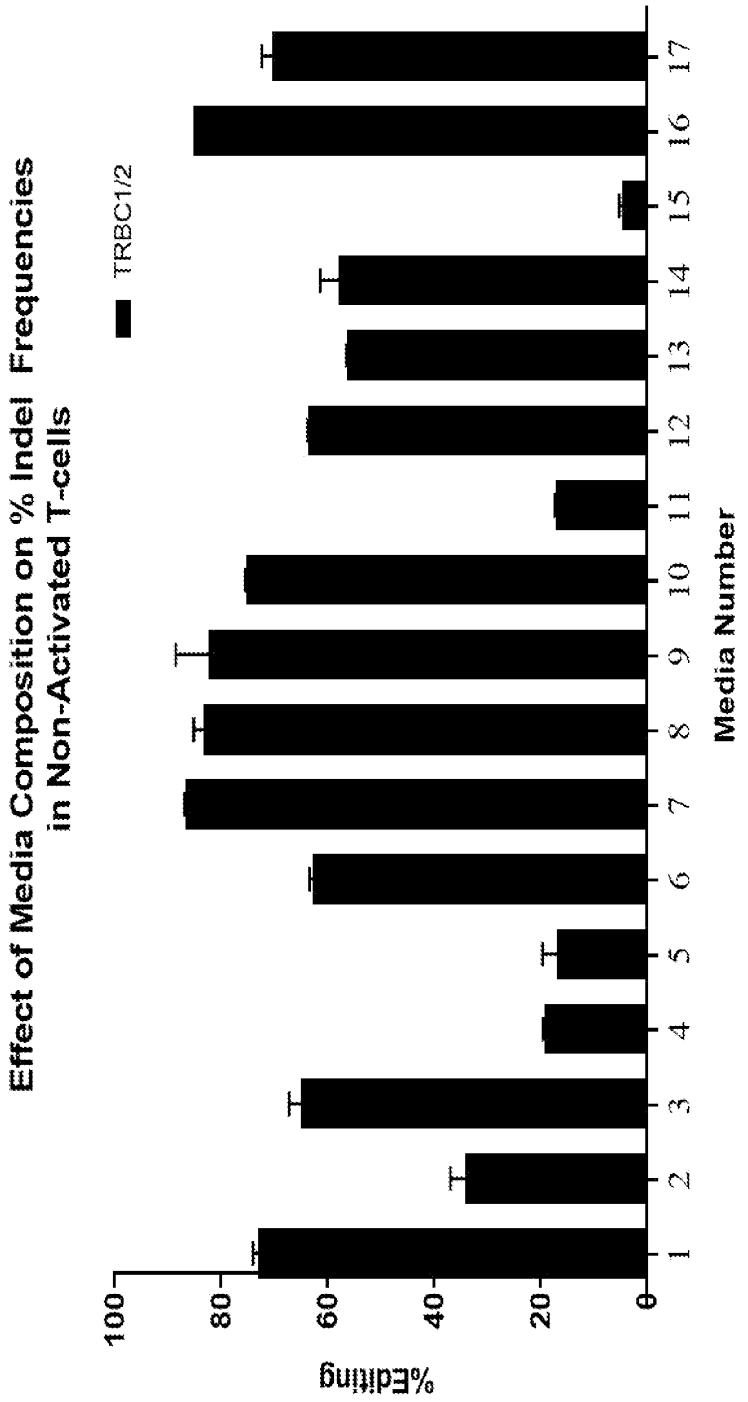

Effect of Media Composition on % Indel Frequencies in Non-Activated T-cells
TRBC1/2
%Editing
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
Media Number

FIG. 32

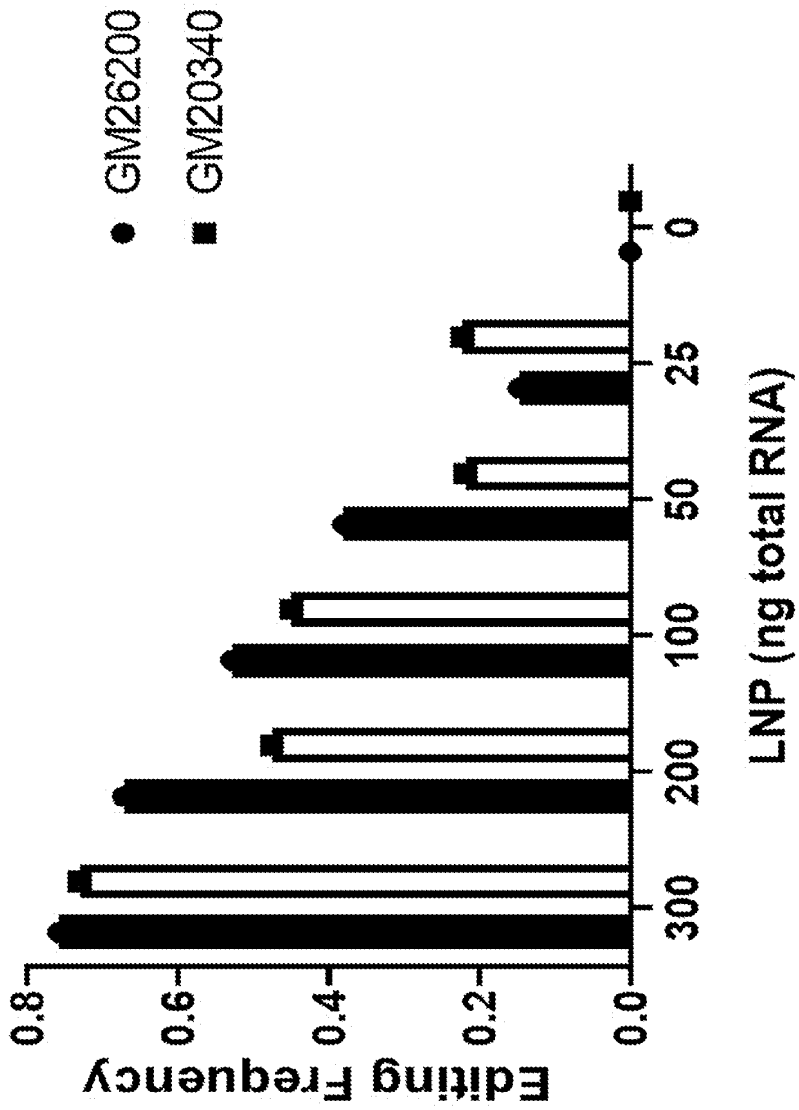

GM26200
GM20340
Editing Frequency
0.0
0.2
0.4
0.6
0.8
LNP (ng total RNA)
0
25
50
100
200
300

FIG. 33

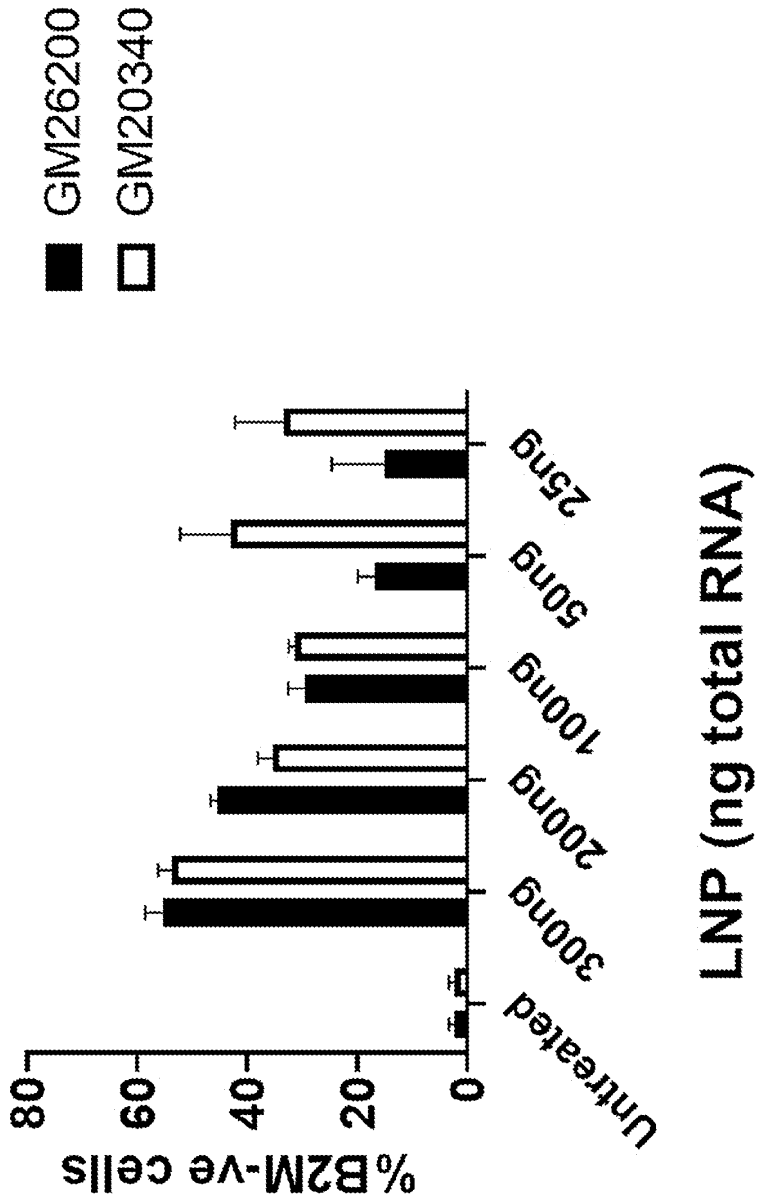

GM26200
GM20340
% B2M-ve cells
0
20
40
60
80
Untreated
30ng
200ng
100ng
50ng
25ng
LNP (ng total RNA)

FIG. 34

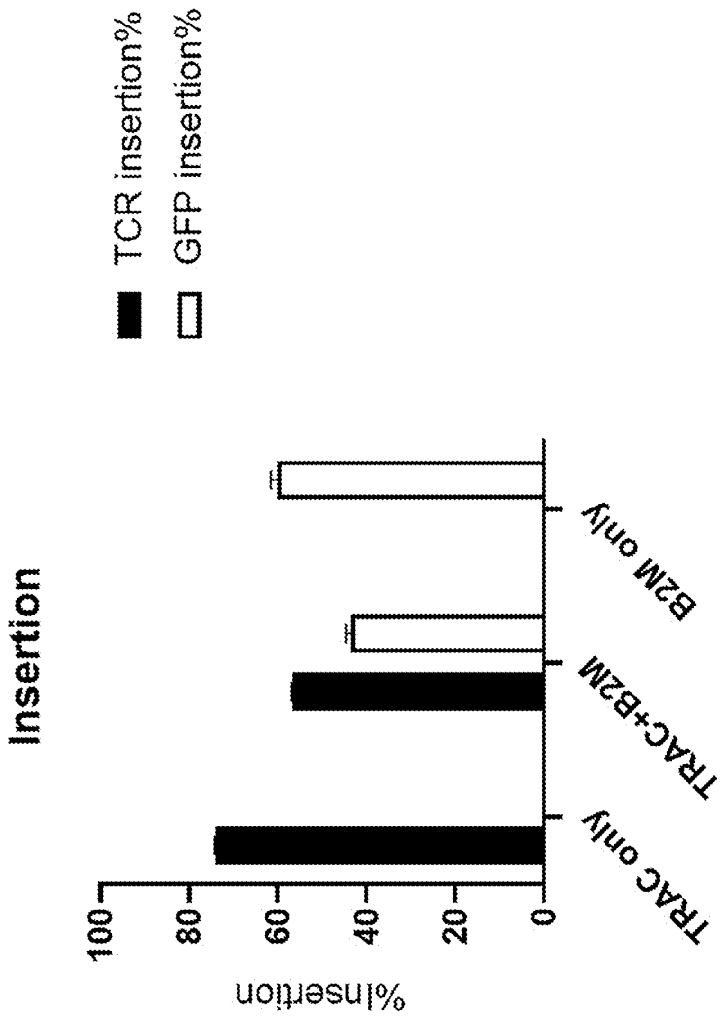

TCR insertion%
GFP insertion%
Insertion
%Insertion
100
80
60
40
20
0
TRAC only
TRAC+B2M
B2M only

FIG. 35

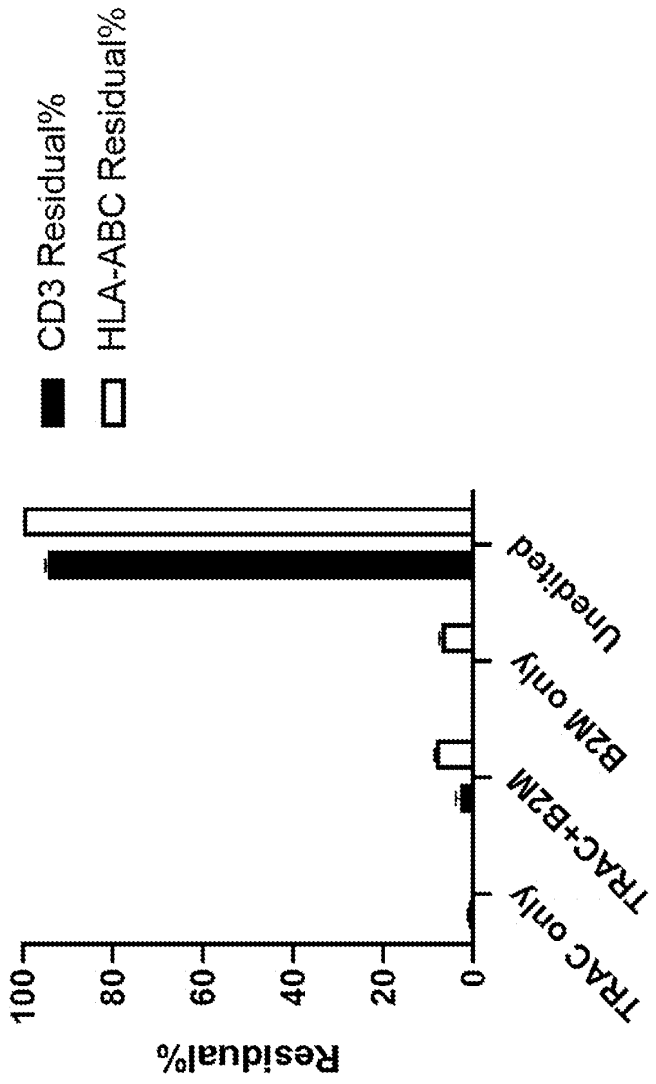

CD3 Residual%
HLA-ABC Residual%
Residual%
100
80
60
40
20
0
Unedited
B2M only
TRAC+B2M
TRAC only

FIG. 36

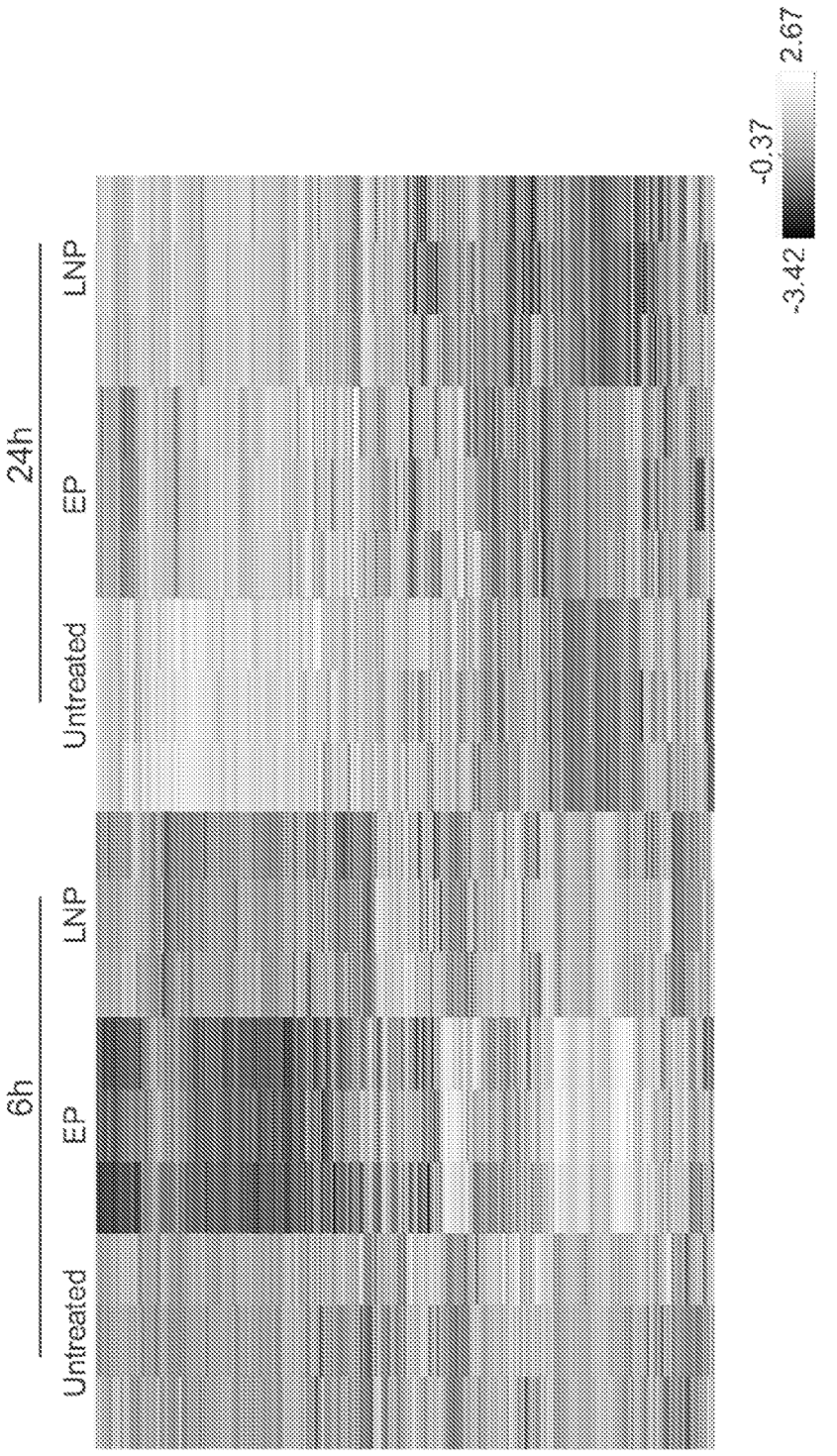


FIG. 37

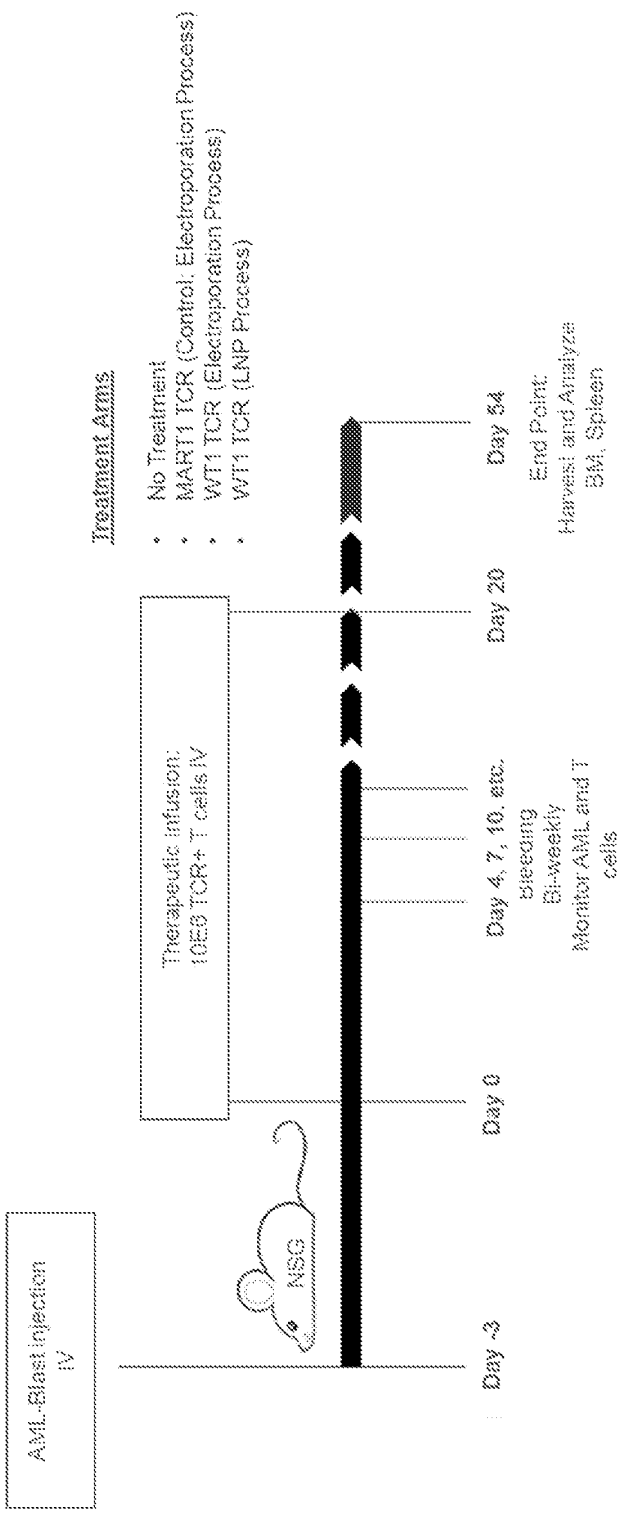

Treatment Arms
No Treatment
MART1 TCR (Control, Electroporation Process)
WT1 TCR (Electroporation Process)
WT1 TCR (LNP Process)
AML Blast Injection IV
NSG
Therapeutic infusion: 10E6 TCR+ T cells IV
Day -3
Day 0
Day 4, 7, 10, etc.
bleeding
Bi-weekly
Monitor AML and T cells
Day 20
Day 84
End Point:
Harvest and Analyze
BM, Spleen

FIG. 38A

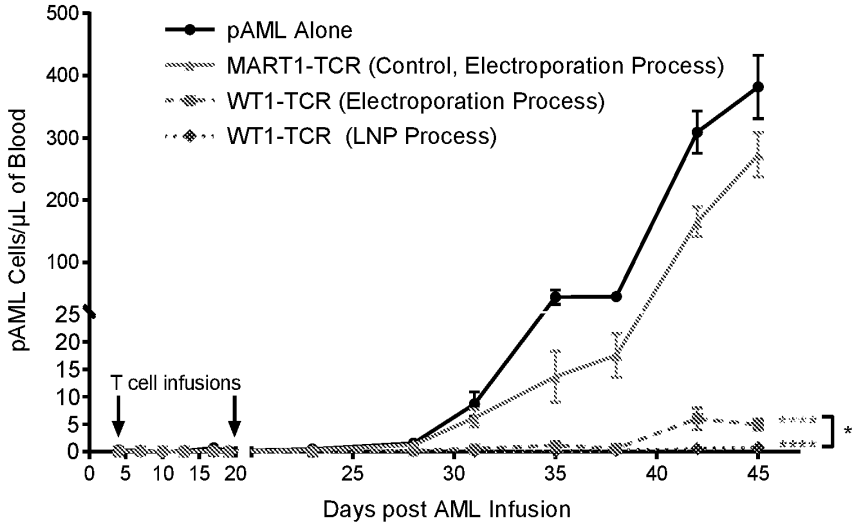

pAML Alone
MART1-TCR (Control, Electroporation Process)
WT1-TCR (Electroporation Process)
WT1-TCR (LNP Process)
pAML Cells/µL of Blood
500
400
300
200
100
25
20
15
10
5
0
T cell infusions
0 5 10 15 20 25 30 35 40 45
Days post AML Infusion
****
***
*

FIG. 38B

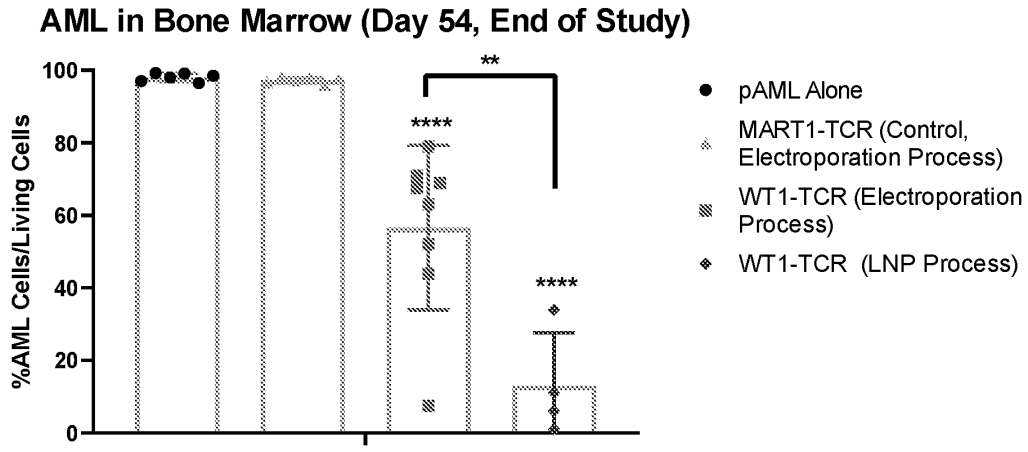


FIG. 38C

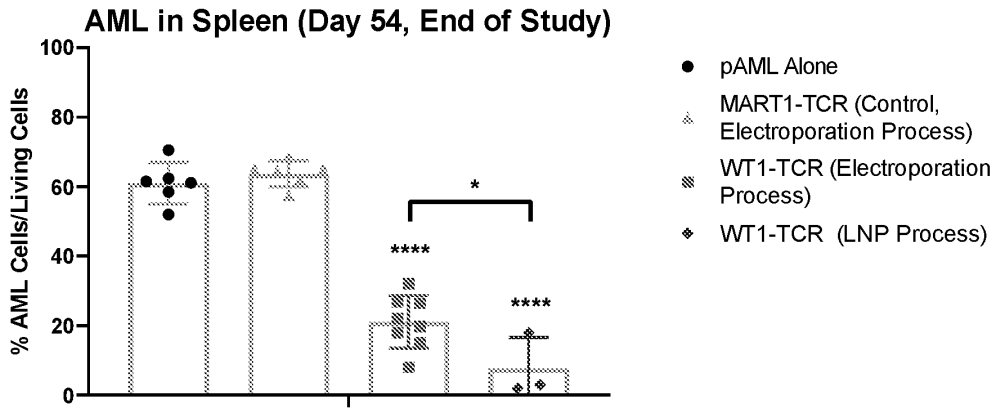


FIG. 38D

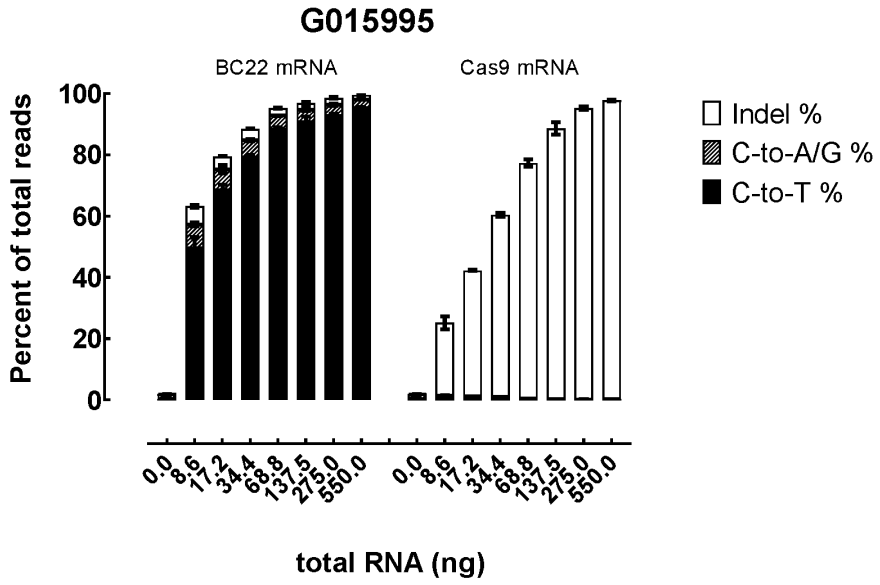


FIG. 39A

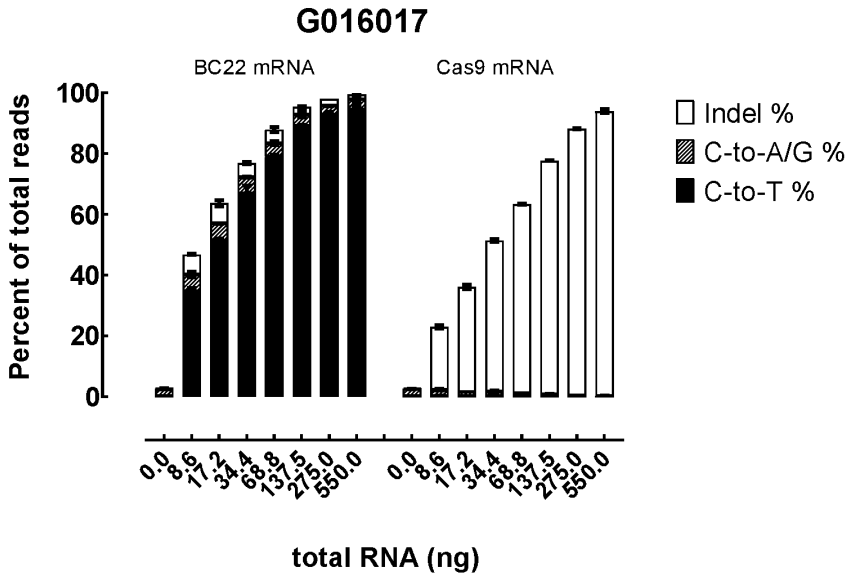


FIG. 39B

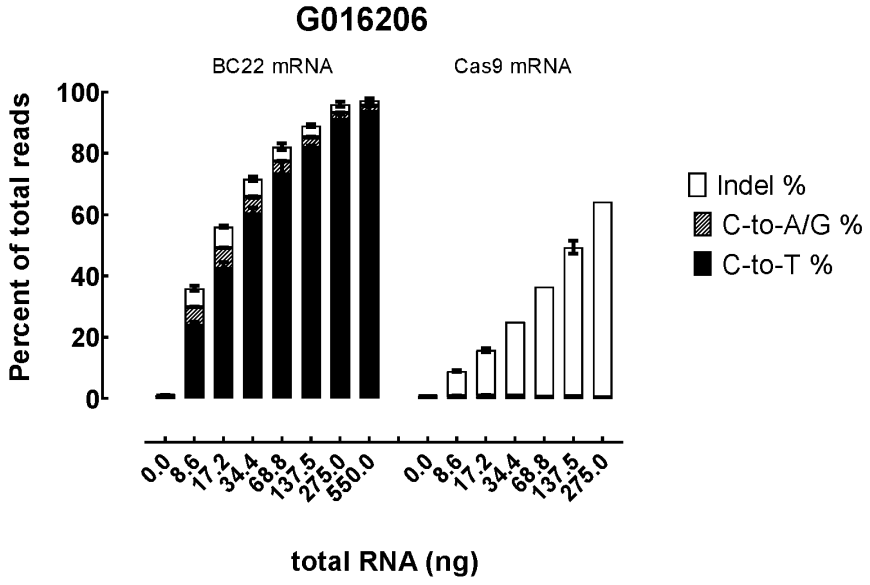


FIG. 39C

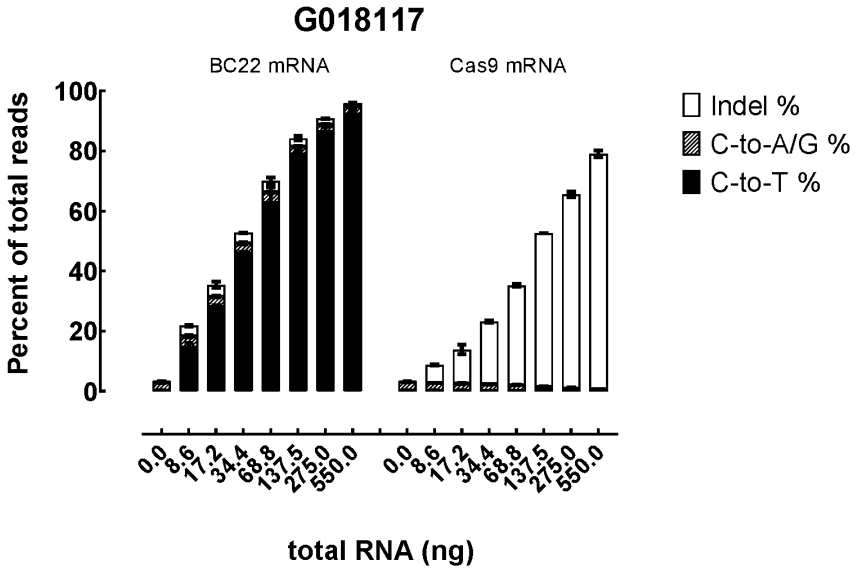


FIG. 39D

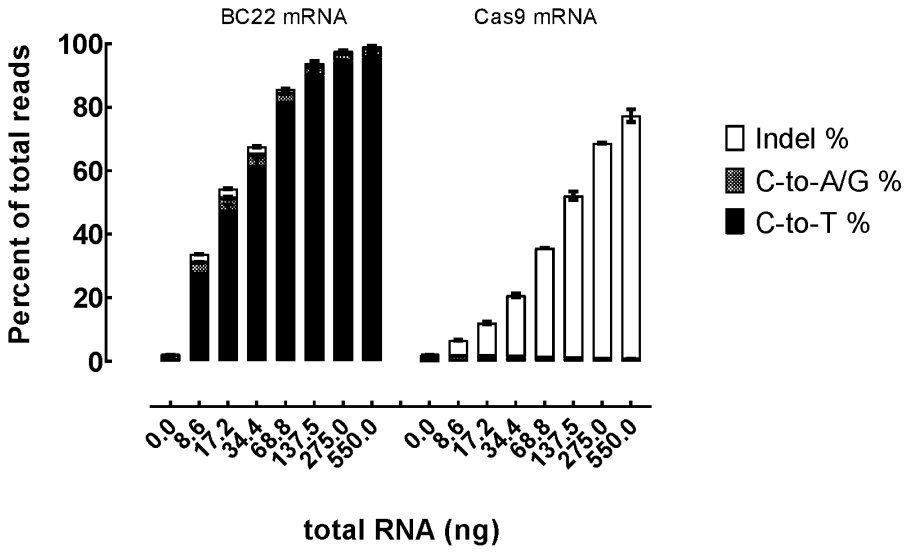


FIG. 40A

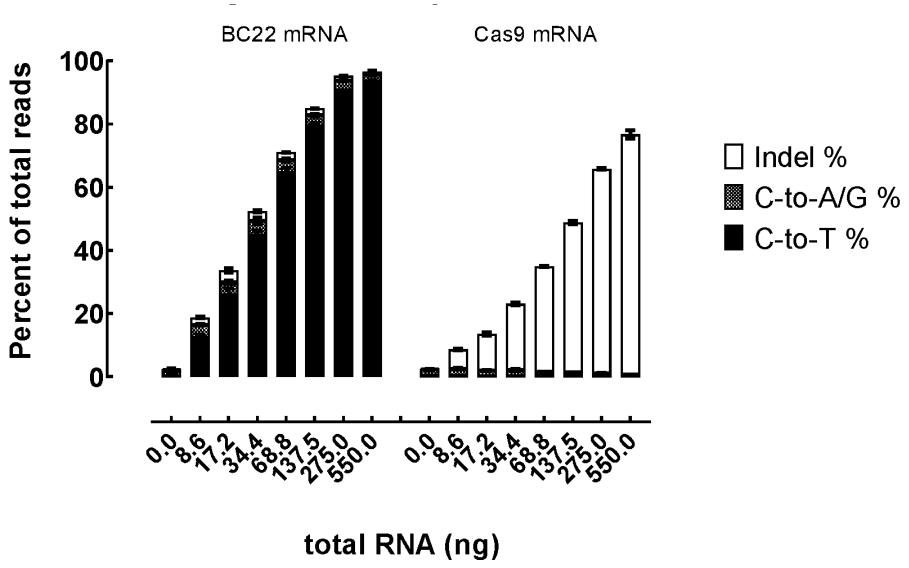


FIG. 40B

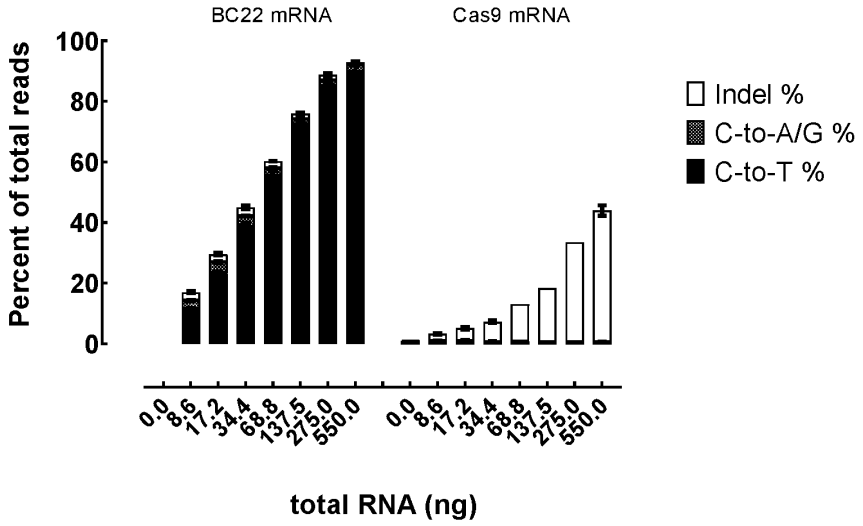


FIG. 40C

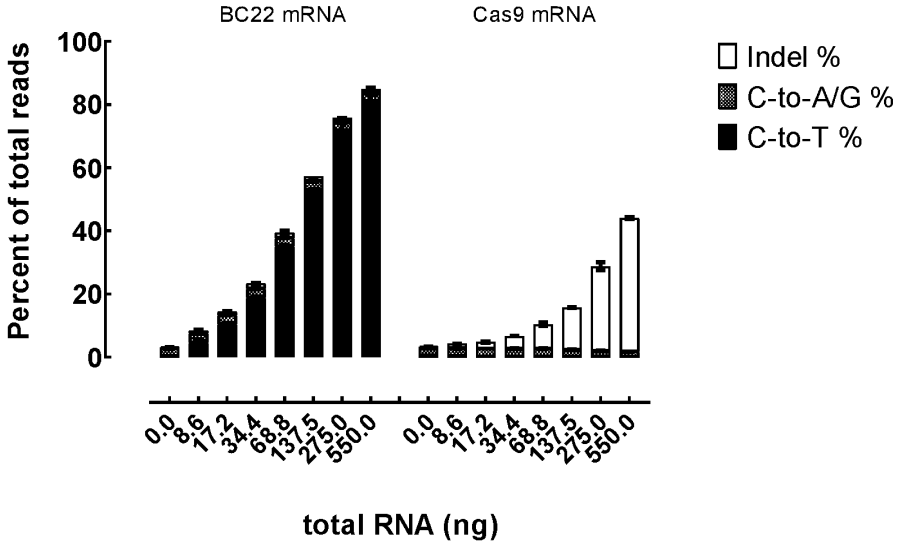


FIG. 40D

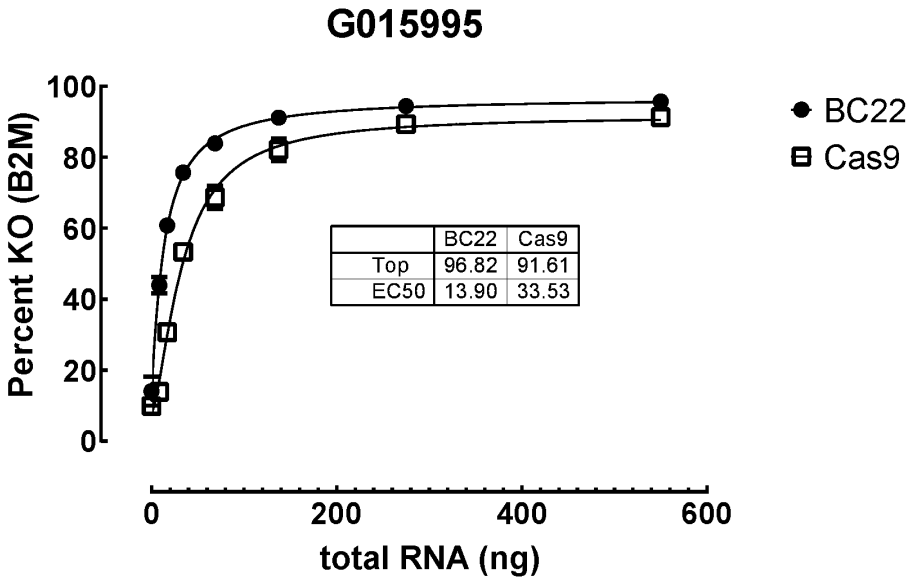


FIG. 41A

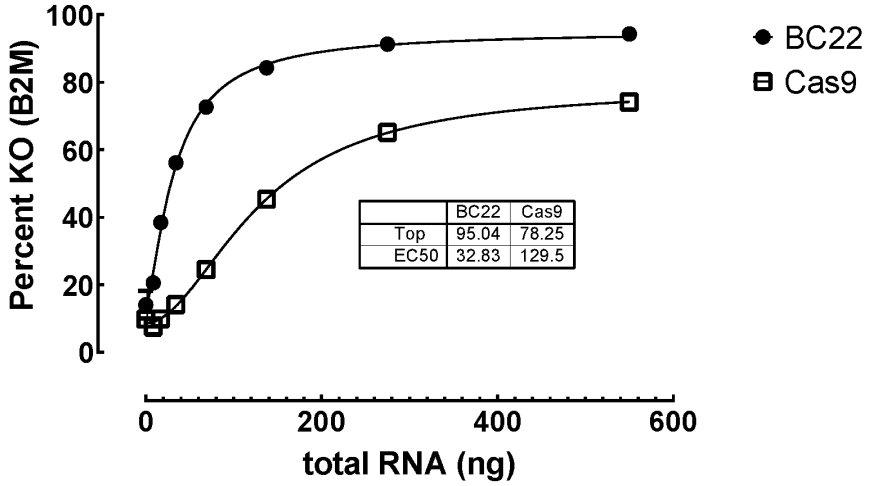


FIG. 41B

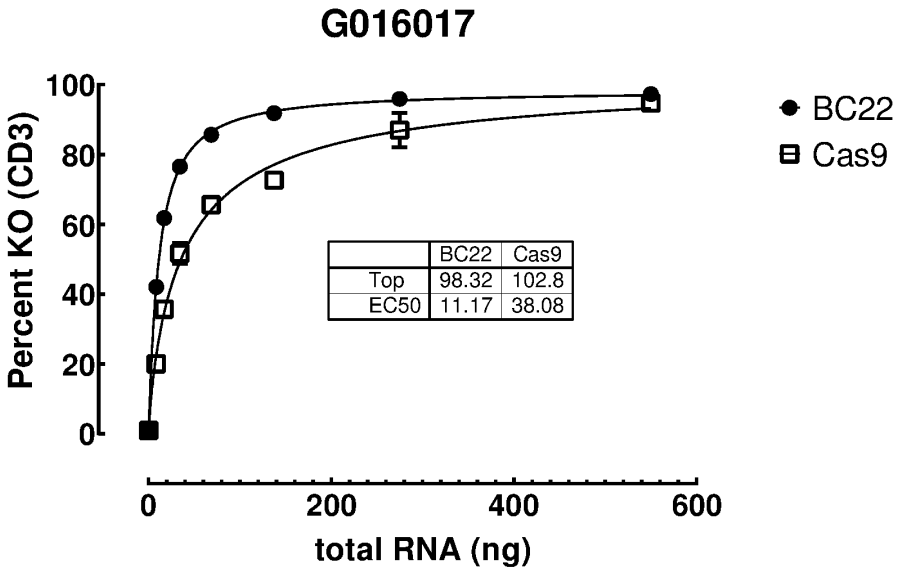


FIG. 41C

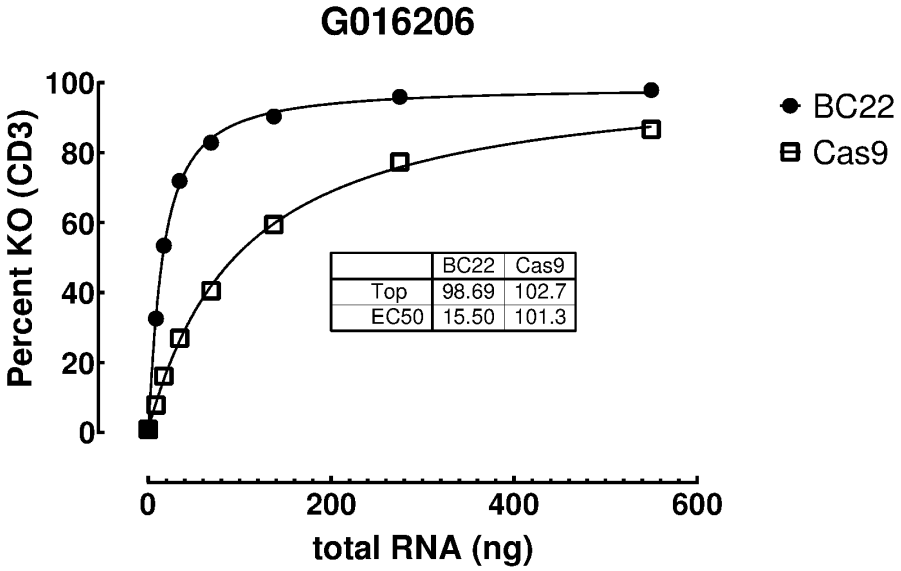


FIG. 41D

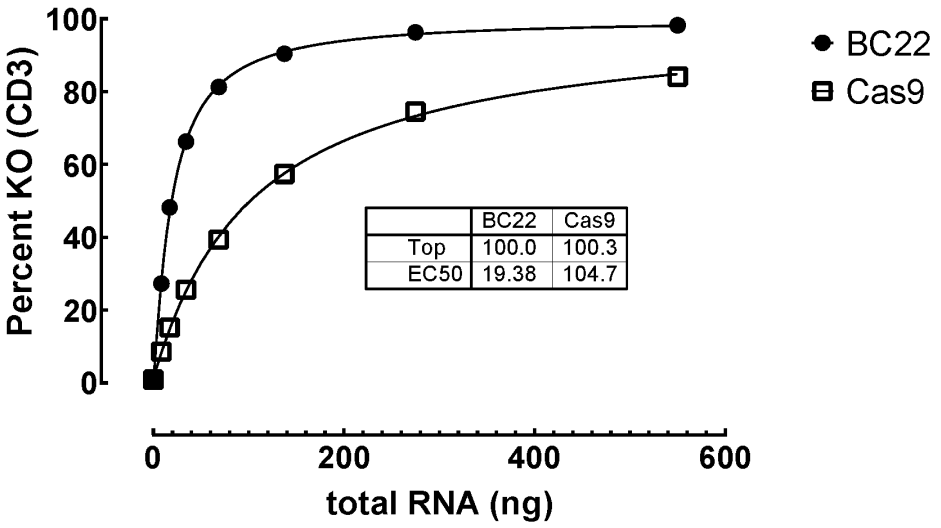


FIG. 41E

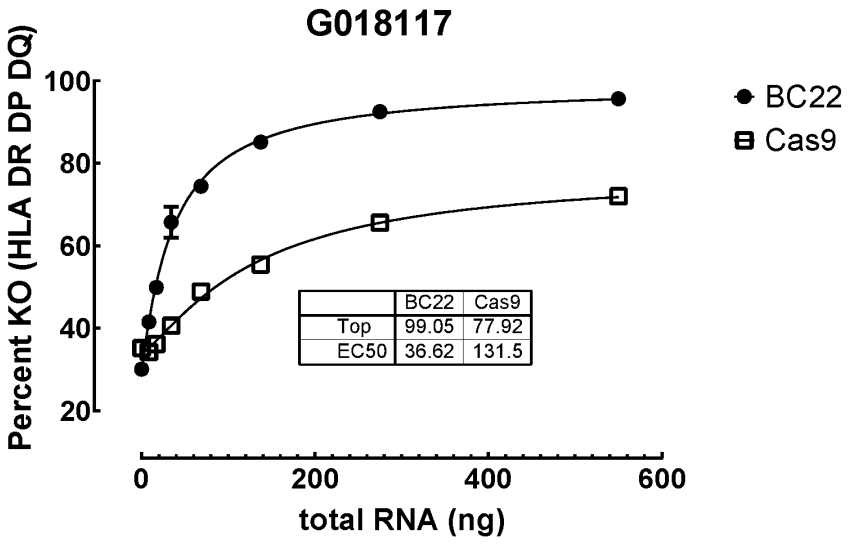


FIG. 41F

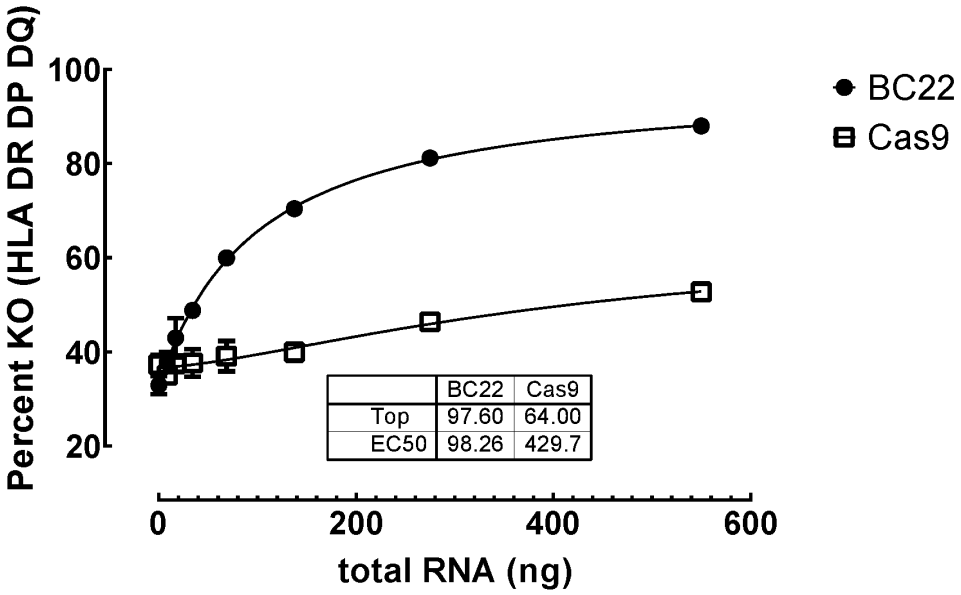


FIG. 41G

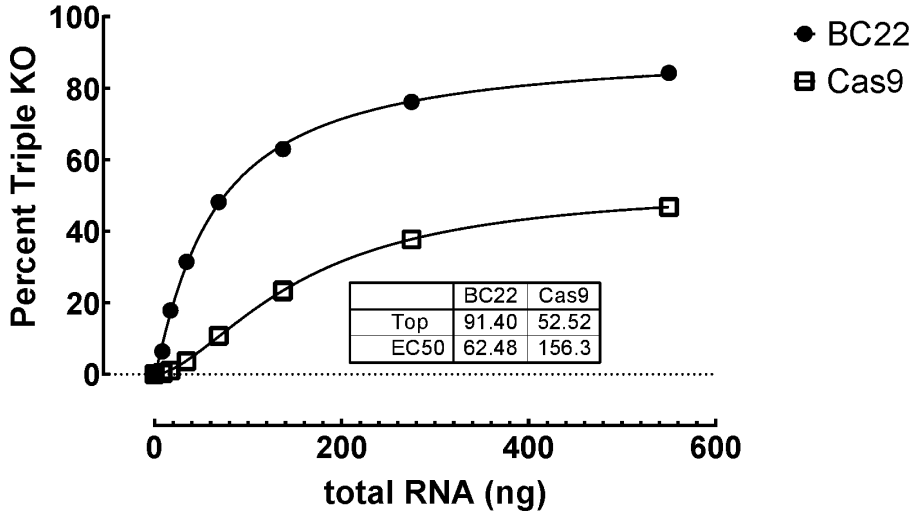


FIG. 41H

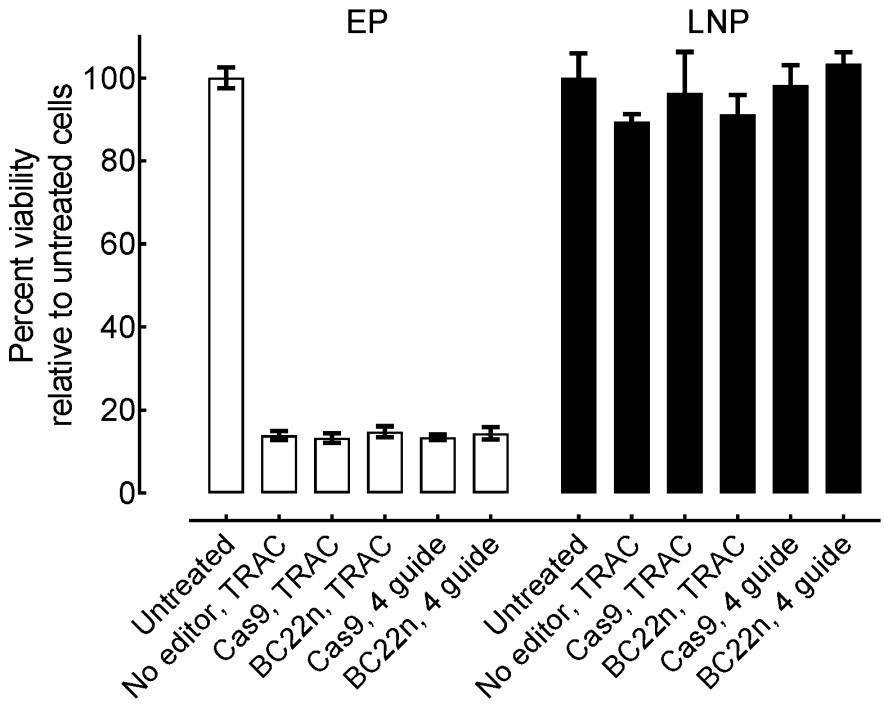

EP
LNP
Percent viability
relative to untreated cells
100
80
60
40
20
0
Untreated
No editor, TRAC
Cas9, TRAC
BC22n, TRAC
Cas9, 4 guide
BC22n, 4 guide
Untreated
No editor, TRAC
Cas9, TRAC
BC22n, TRAC
Cas9, 4 guide
BC22n, 4 guide

FIG. 42

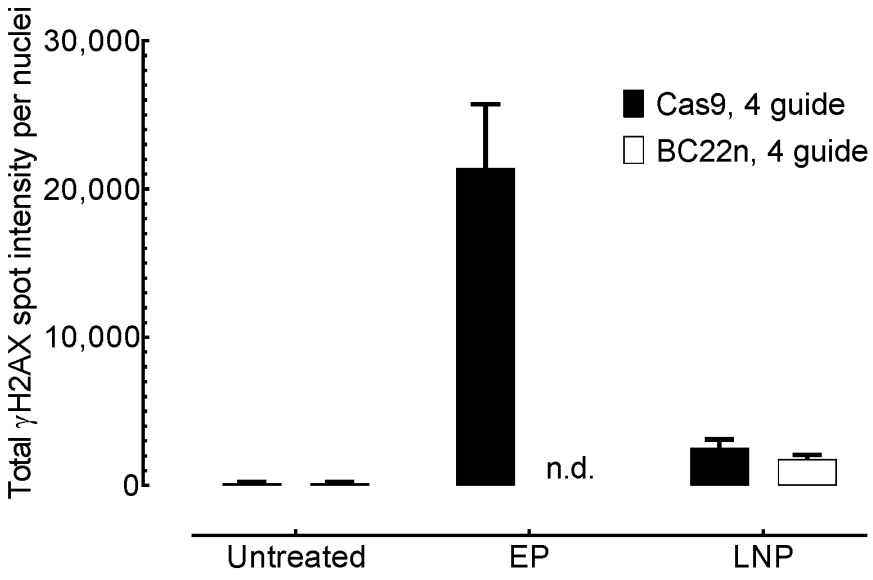

Total γH2AX spot intensity per nuclei
30,000
20,000
10,000
0
Cas9, 4 guide
BC22n, 4 guide
n.d.
Untreated
EP
LNP

FIG. 43

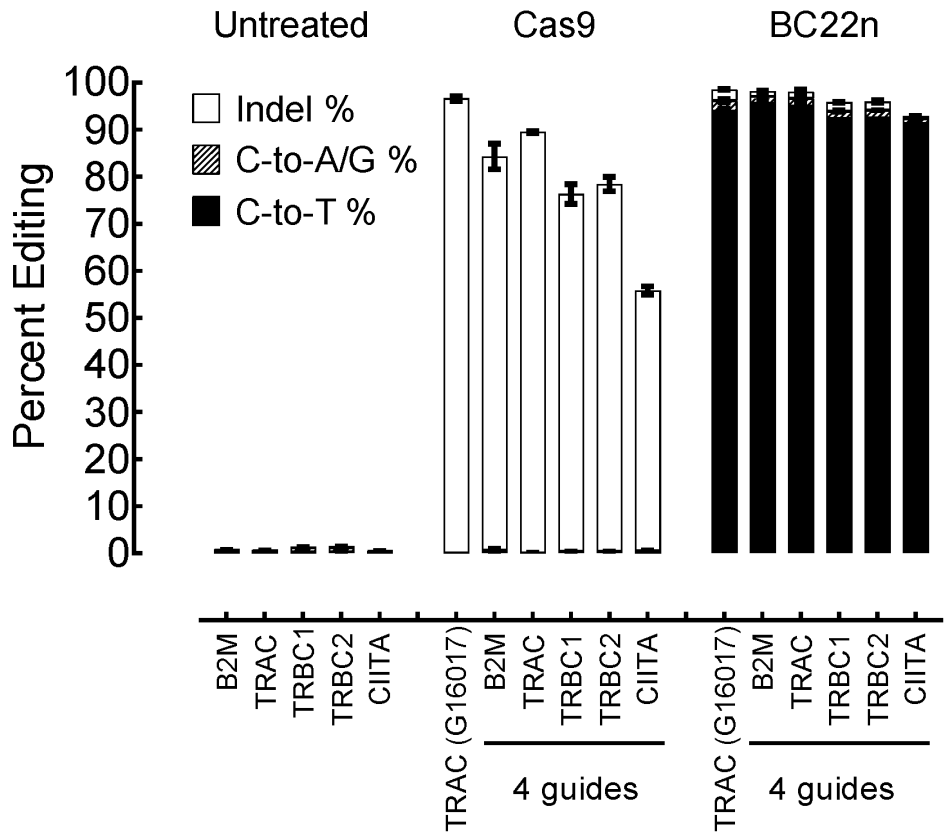

Untreated
Cas9
BC22n
Percent Editing
100
90
80
70
60
50
40
30
20
10
0
Indel %
C-to-A/G %
C-to-T %
B2M
TRAC
TRBC1
TRBC2
CIITA
TRAC (G16017)
B2M
TRAC
TRBC1
TRBC2
CIITA
4 guides
TRAC (G16017)
B2M
TRAC
TRBC1
TRBC2
CIITA
4 guides

FIG. 44

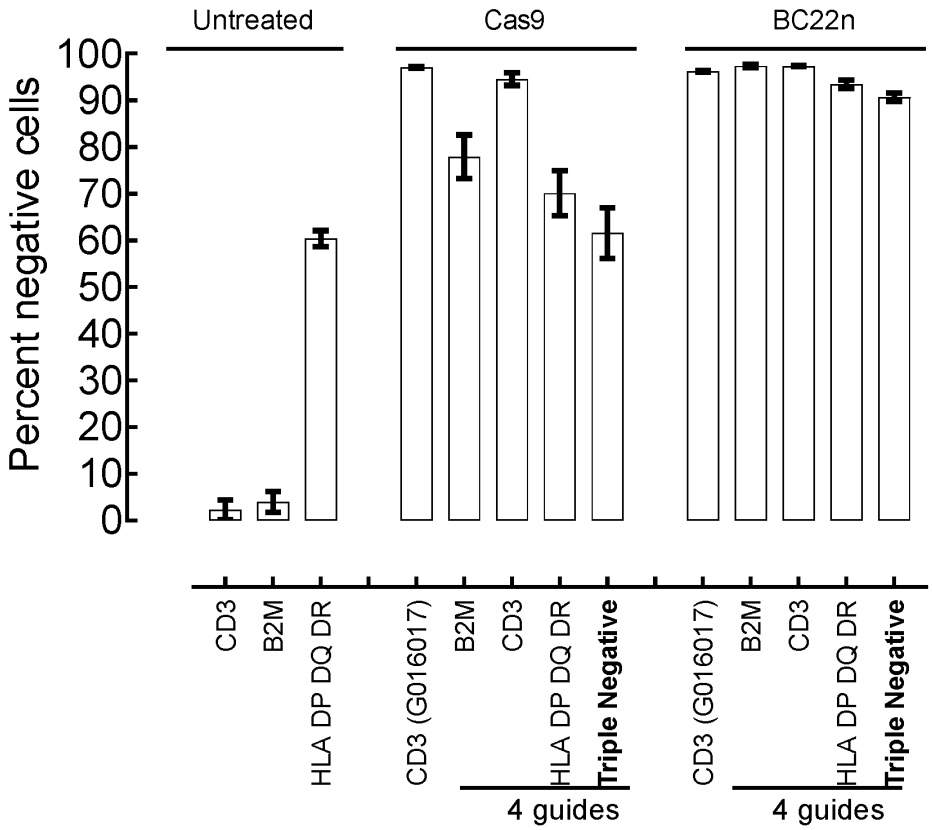

Untreated
Cas9
BC22n
Percent negative cells
100
90
80
70
60
50
40
30
20
10
0
CD3
B2M
HLA DP DQ DR
CD3 (G016017)
B2M
CD3
HLA DP DQ DR
Triple Negative
4 guides
CD3 (G016017)
B2M
CD3
HLA DP DQ DR
Triple Negative
4 guides

FIG. 45

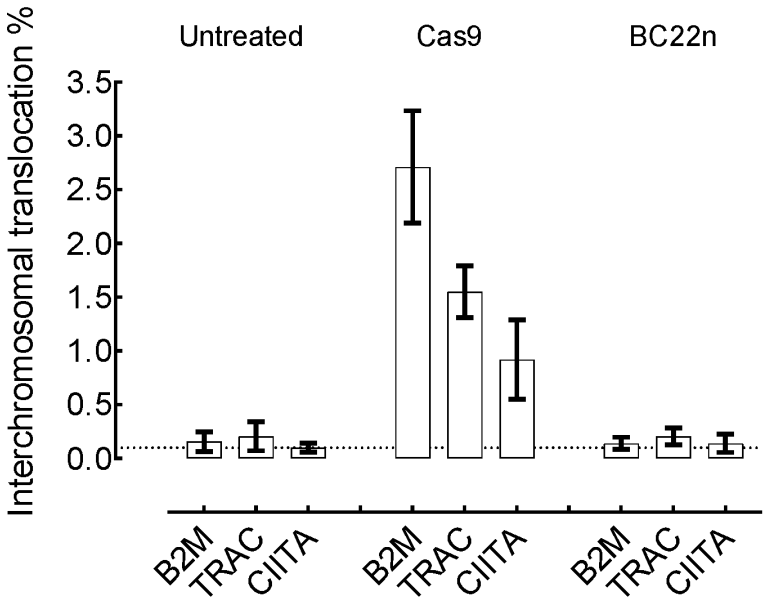

Untreated
Cas9
BC22n
Interchromosomal translocation %
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
B2M
TRAC
CIITA
B2M
TRAC
CIITA
B2M
TRAC
CIITA

FIG. 46

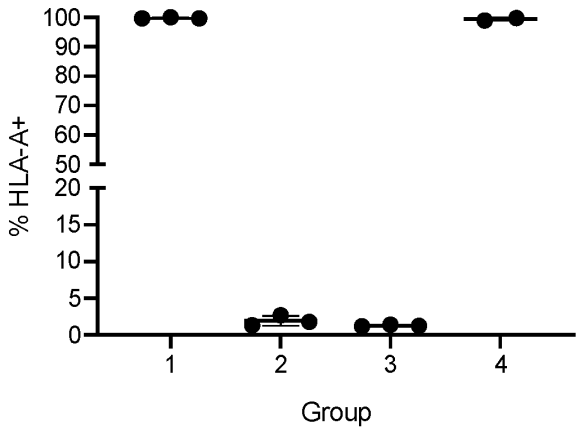


FIG. 47A

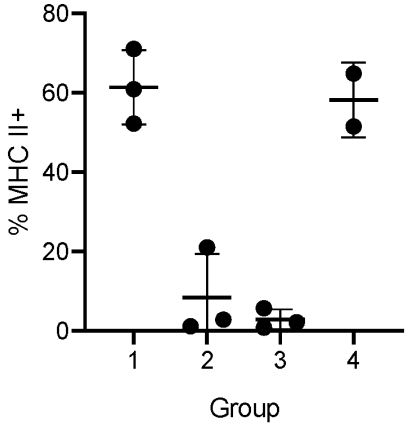


FIG. 47B

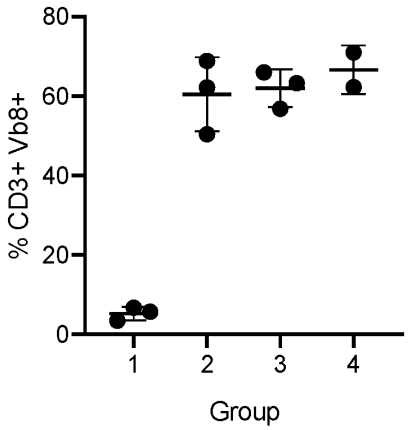


FIG. 47C

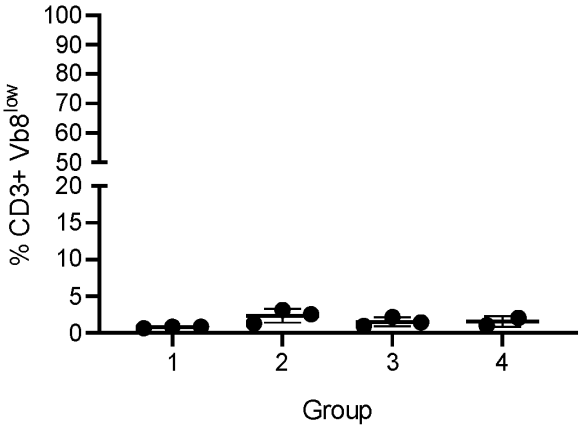


FIG. 47D

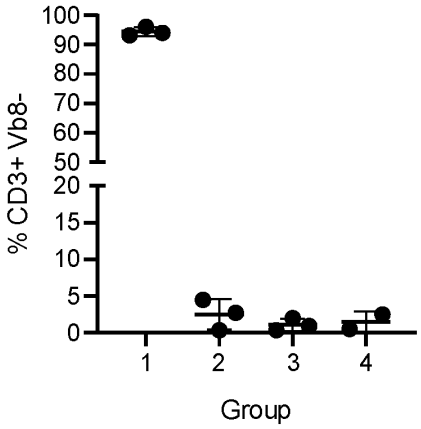


FIG. 47E

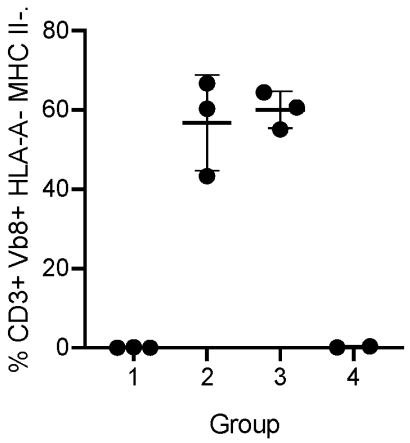


FIG. 47F

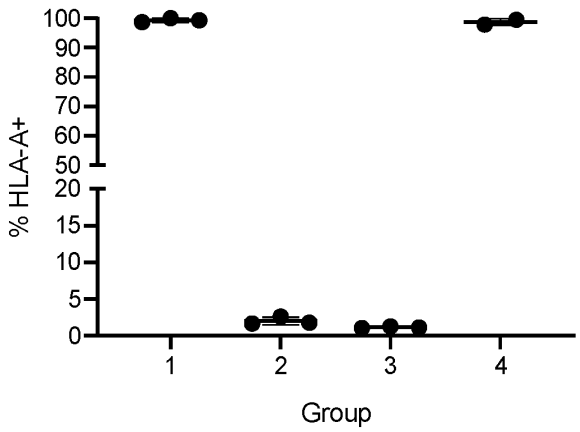


FIG. 48A

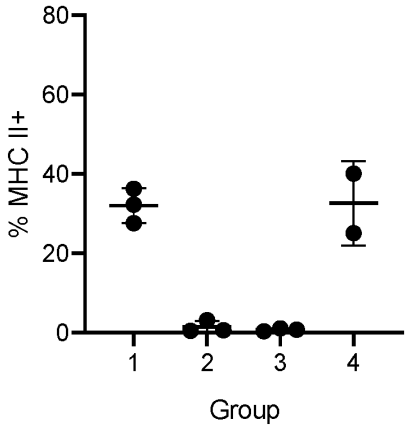


FIG. 48B

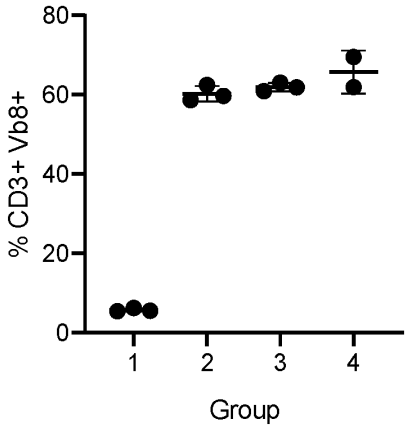


FIG. 48C

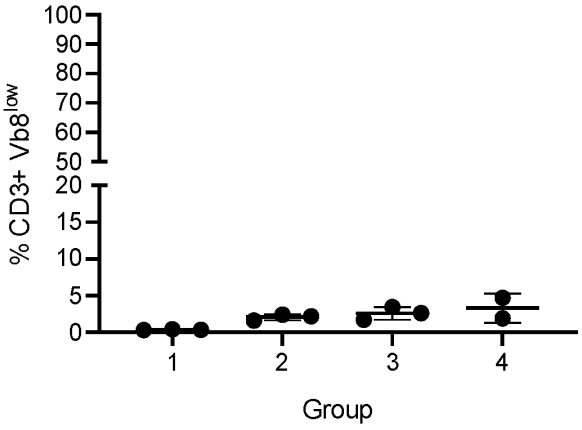


FIG. 48D

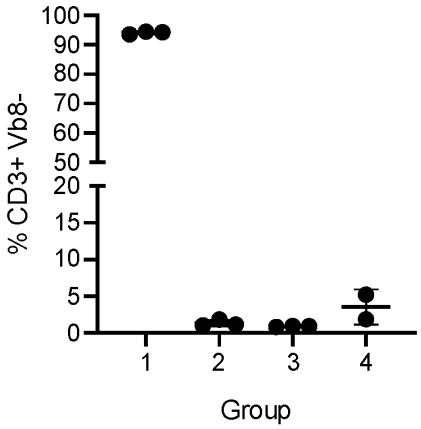


FIG. 48E

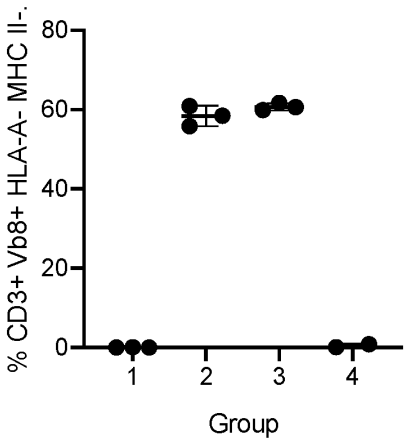


FIG. 48F

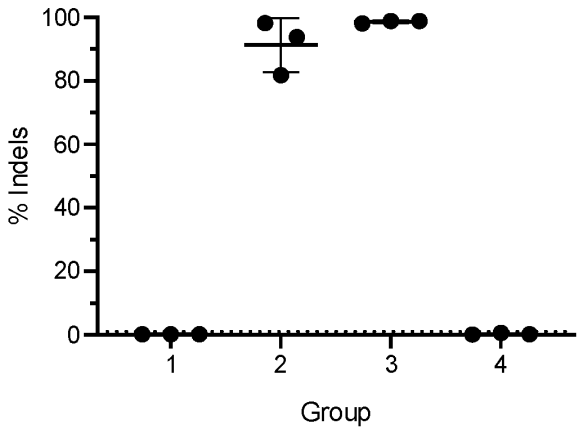


FIG. 49A

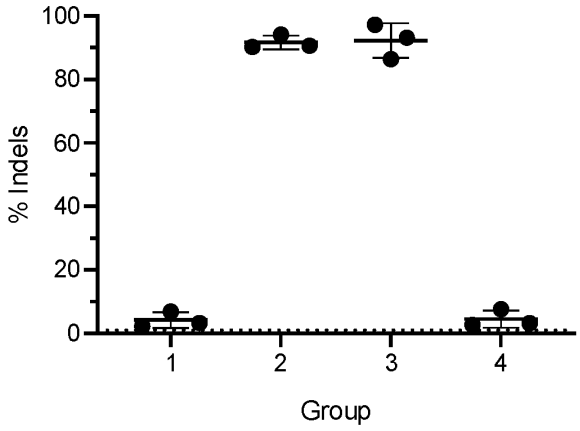


FIG. 49B

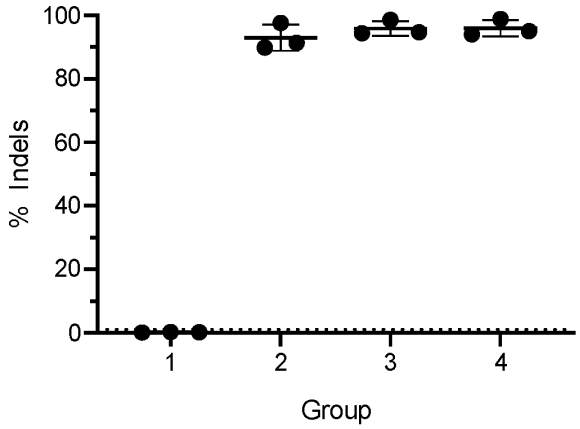


FIG. 49C

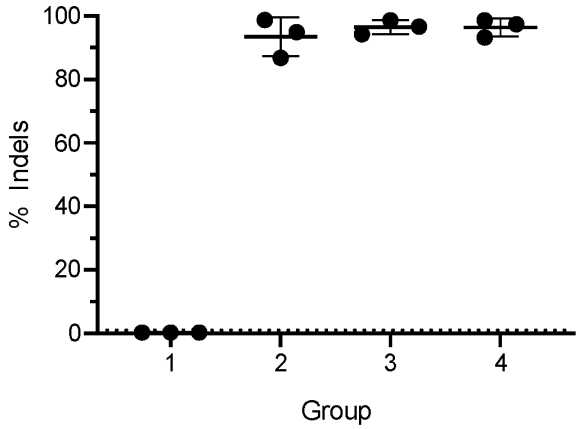


FIG. 49D

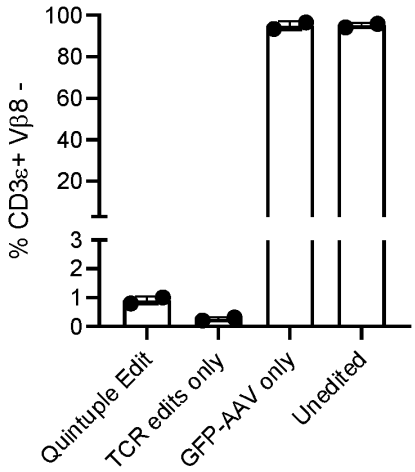


FIG. 50A

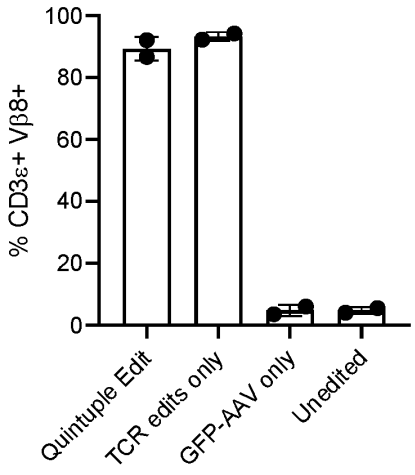


FIG. 50B

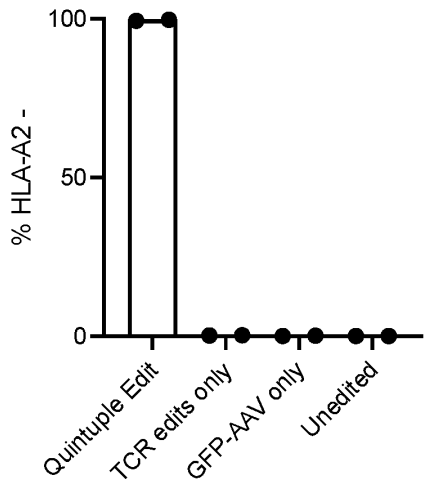


FIG. 50C

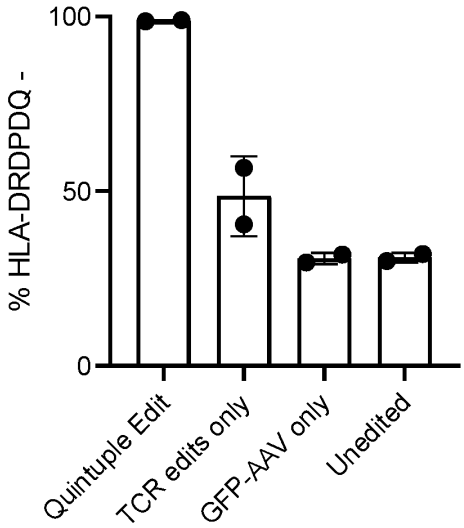


FIG. 50D

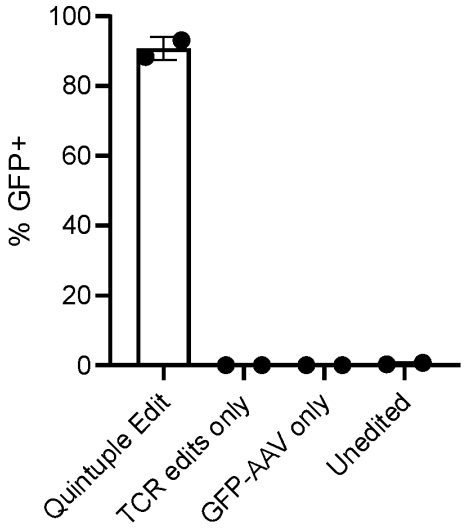


FIG. 50E

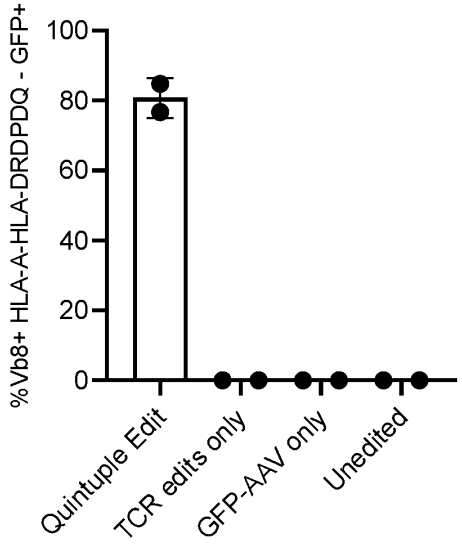


FIG. 50F

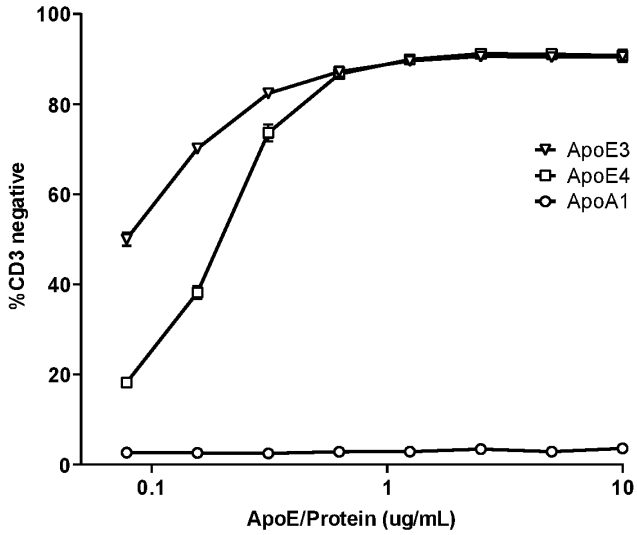


FIG. 51A

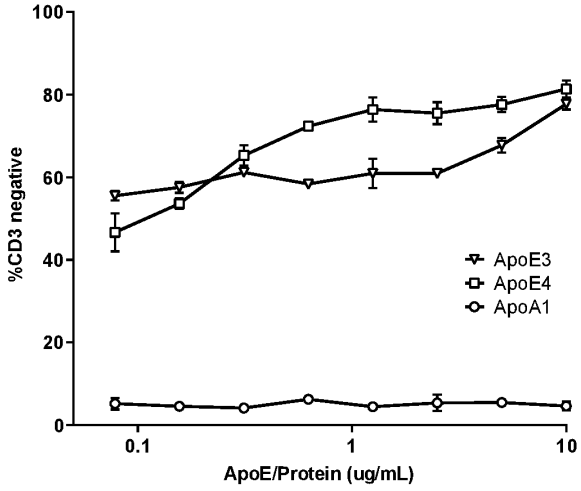


FIG. 51B

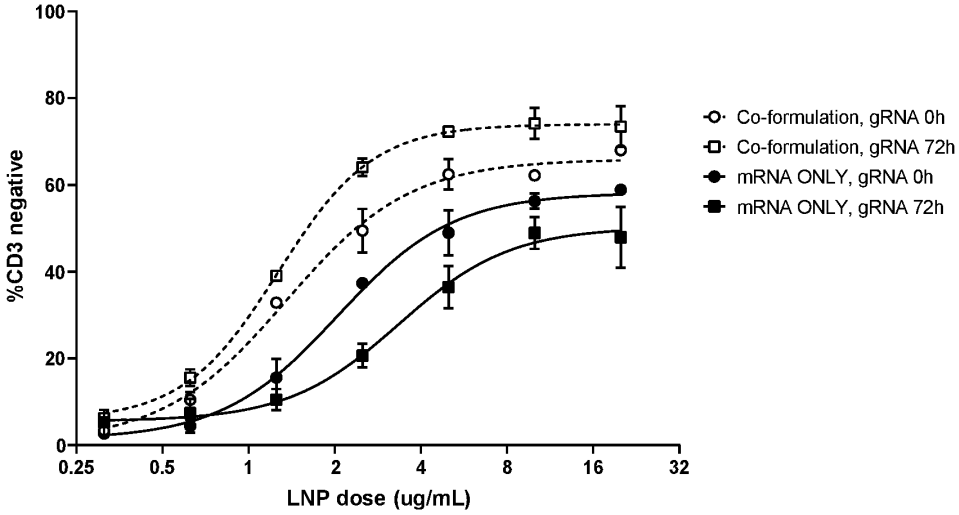


FIG. 52A

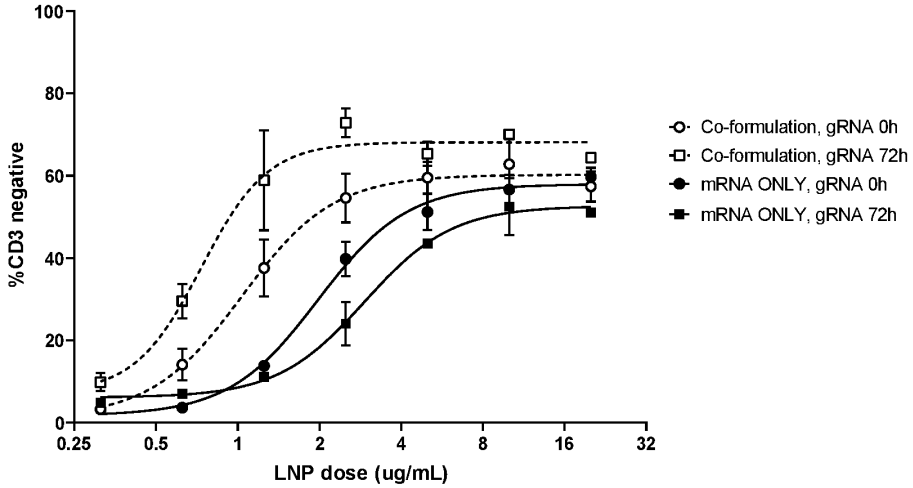


FIG. 52B

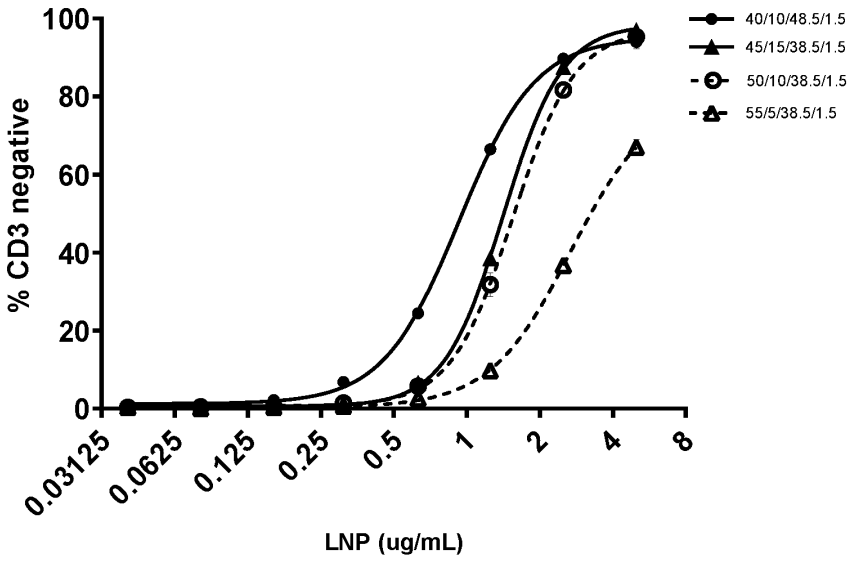


FIG. 53A

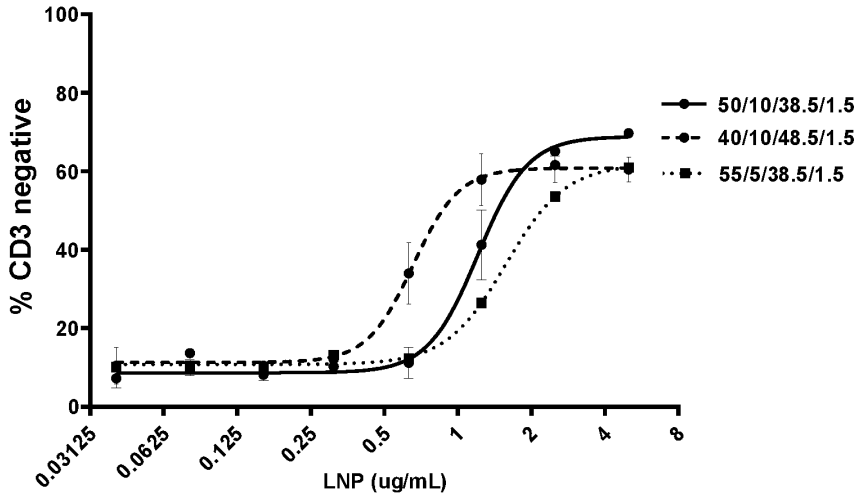


FIG. 53B

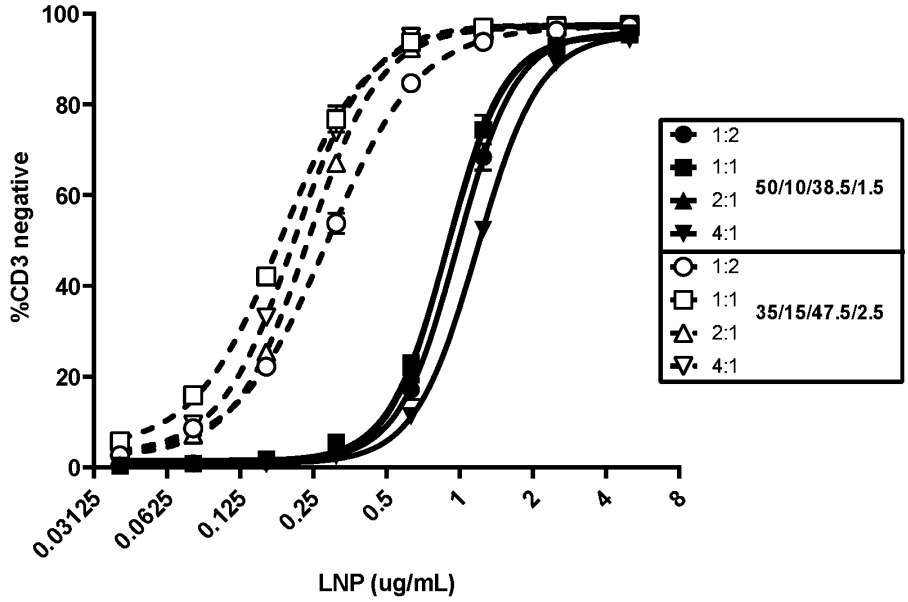

100
80
60
40
20
0
%CD3 negative
0.03125
0.0625
0.125
0.25
0.5
1
2
4
8
LNP (ug/mL)
1:2
1:1
2:1
4:1
50/10/38.5/1.5
1:2
1:1
2:1
4:1
35/15/47.5/2.5

FIG. 54

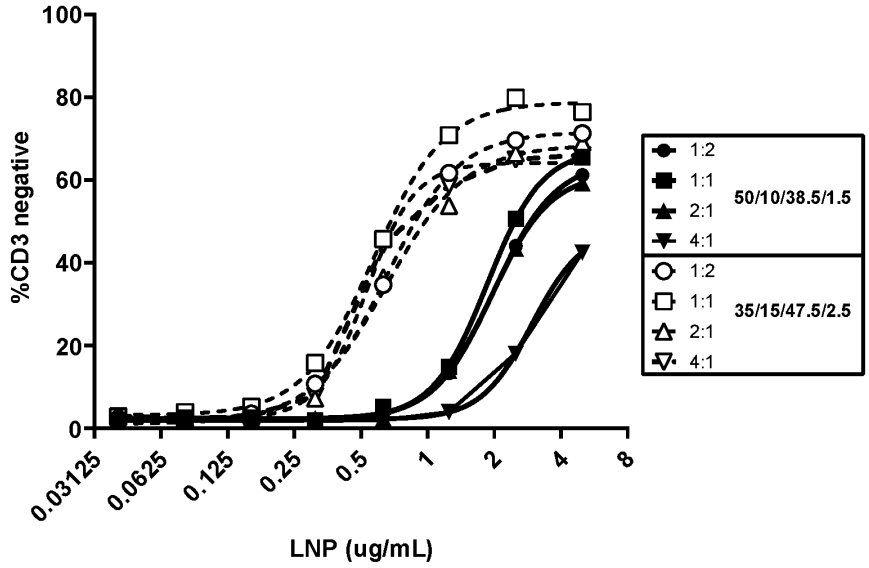


FIG. 55A

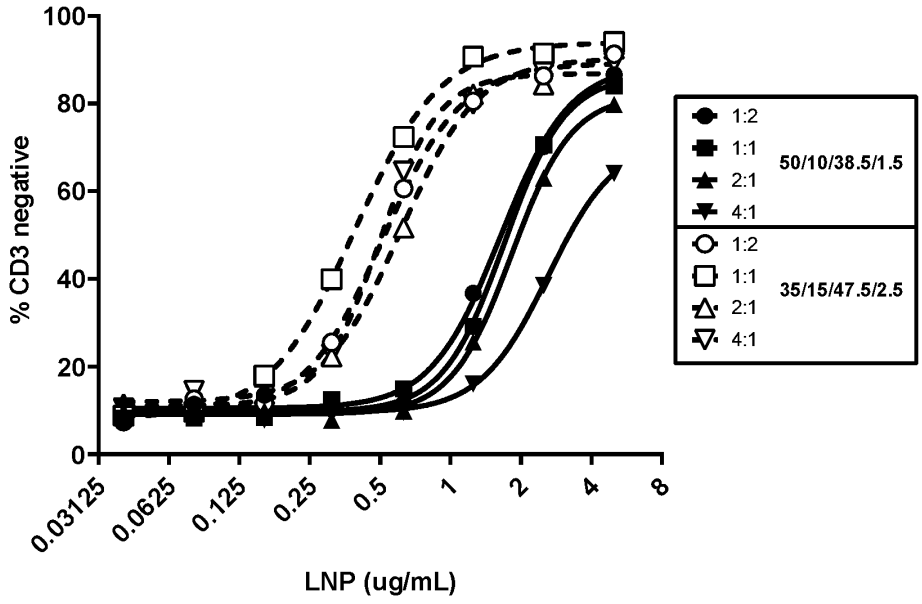


FIG. 55B

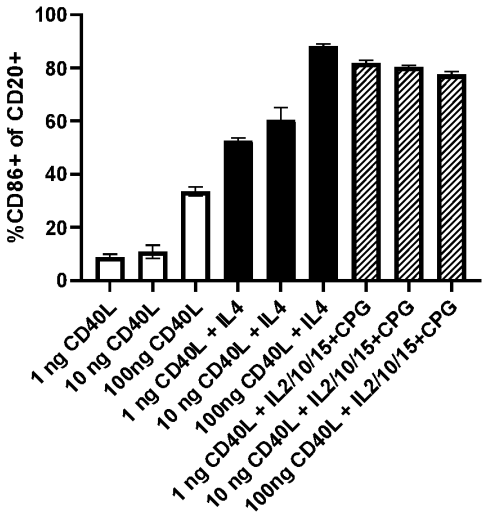


FIG. 56A

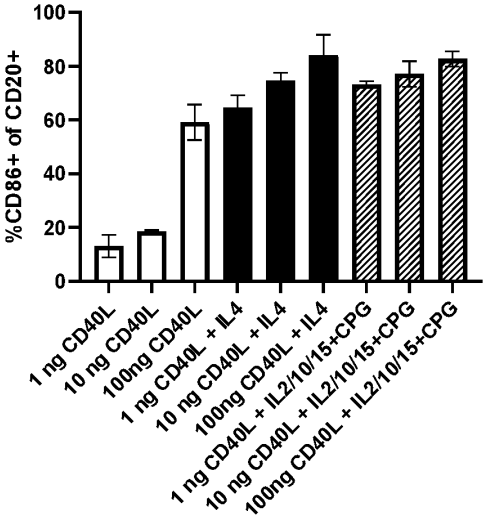


FIG. 56B

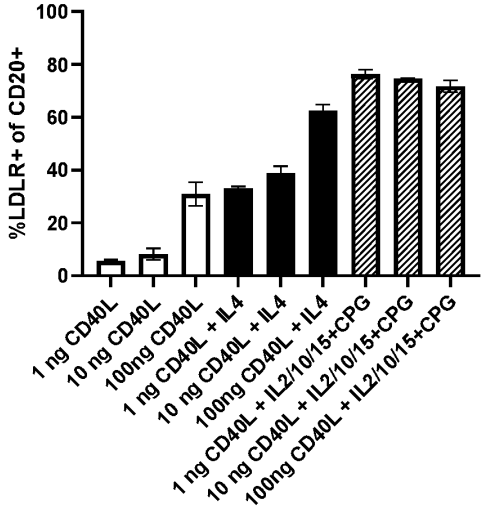


FIG. 56C

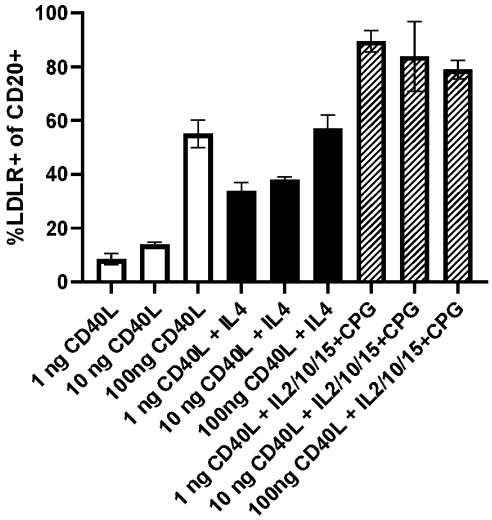


FIG. 56D

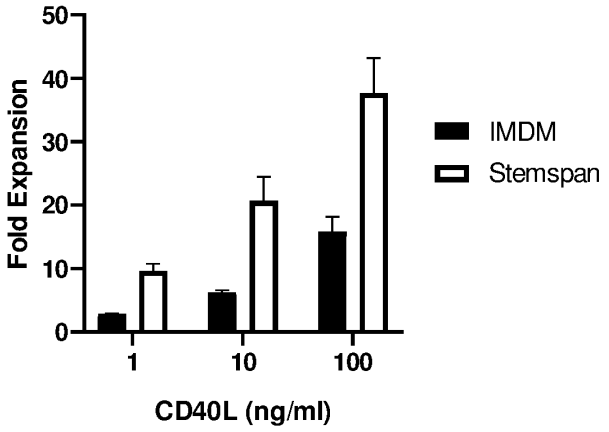


Fig 57A

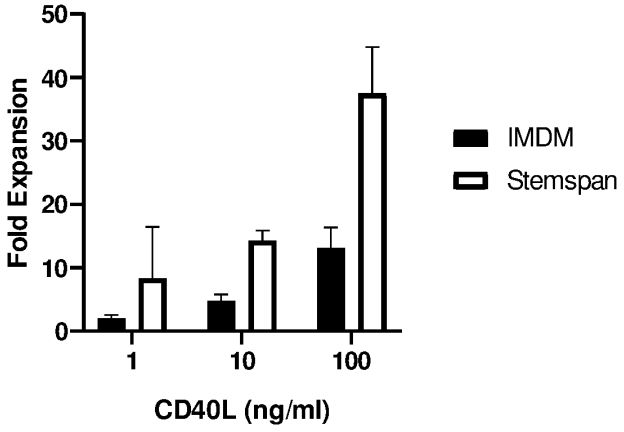


FIG. 57B

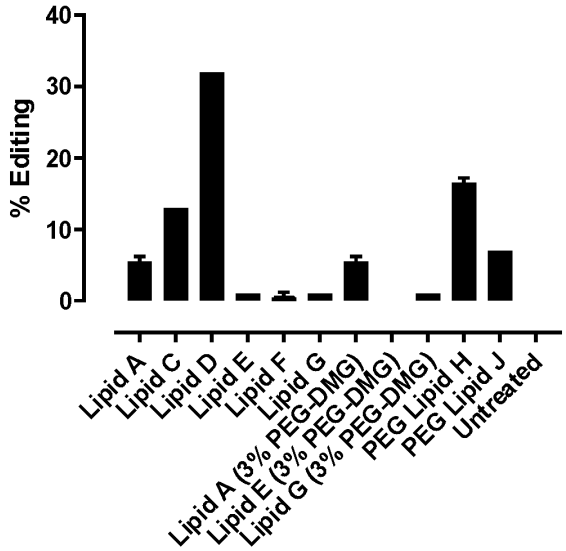


FIG. 58A

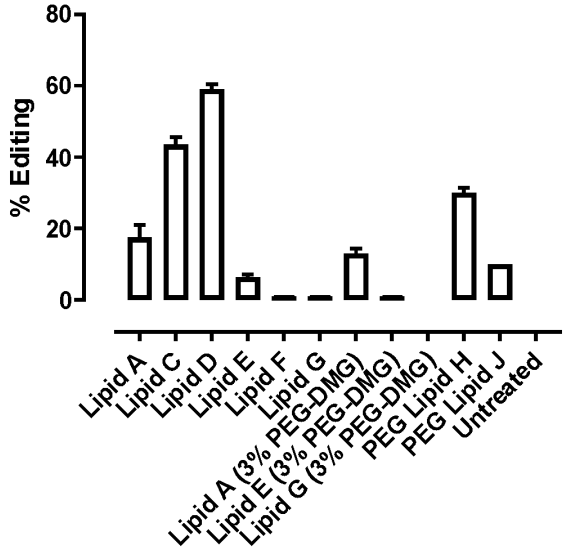


FIG. 58B

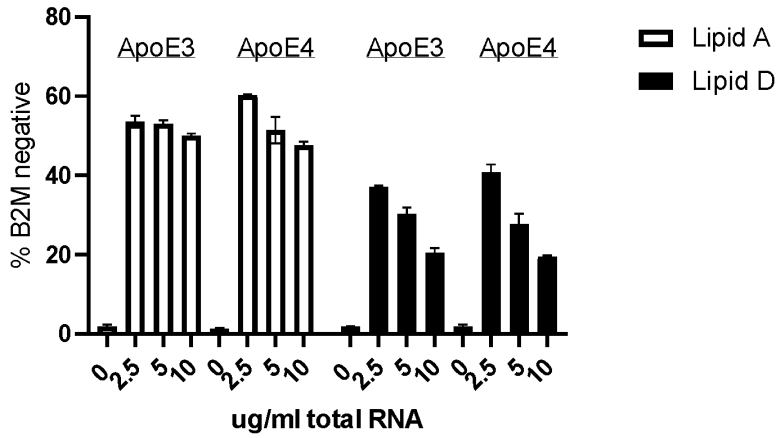

80
60
40
20
0
% B2M negative
ApoE3
ApoE4
ApoE3
ApoE4
Lipid A
Lipid D
0
2.5
5
10
0
2.5
5
10
0
2.5
5
10
0
2.5
5
10
ug/ml total RNA

FIG. 59

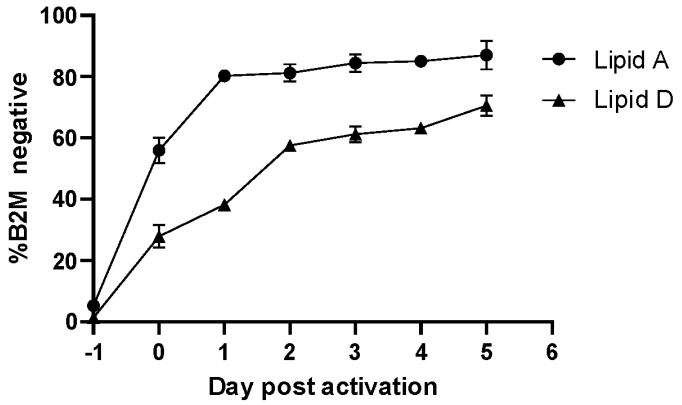


FIG. 60A

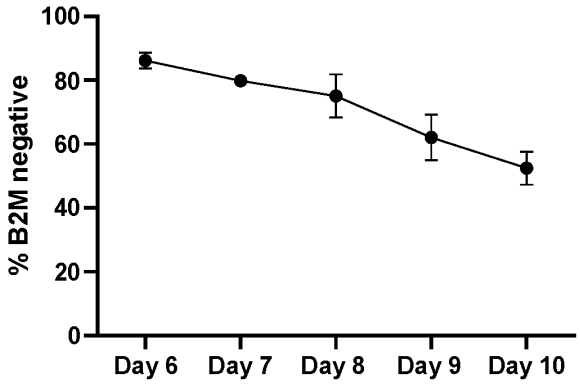


FIG. 60B

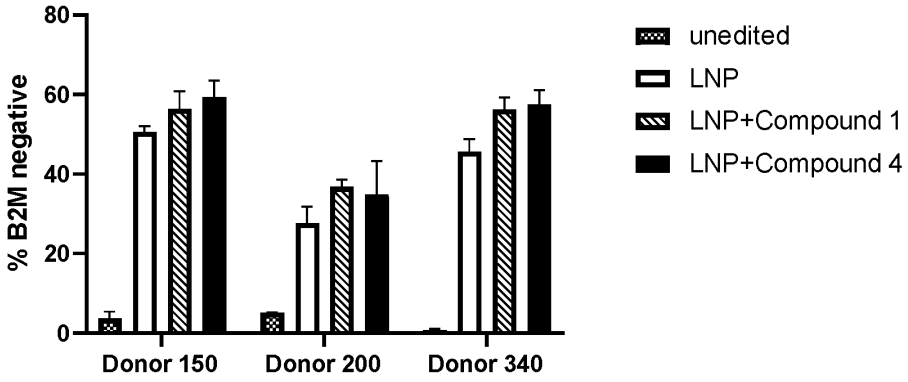

80
60
40
20
0
% B2M negative
Donor 150
Donor 200
Donor 340
unedited
LNP
LNP+Compound 1
LNP+Compound 4

FIG. 61

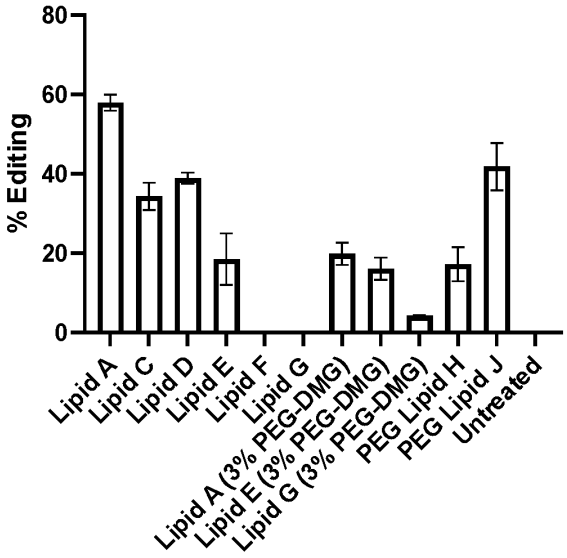

% Editing
80
60
40
20
0
Lipid A
Lipid C
Lipid D
Lipid E
Lipid F
Lipid G
Lipid A (3% PEG-DMG)
Lipid E (3% PEG-DMG)
Lipid G (3% PEG-DMG)
PEG Lipid H
PEG Lipid J
Untreated

FIG. 62

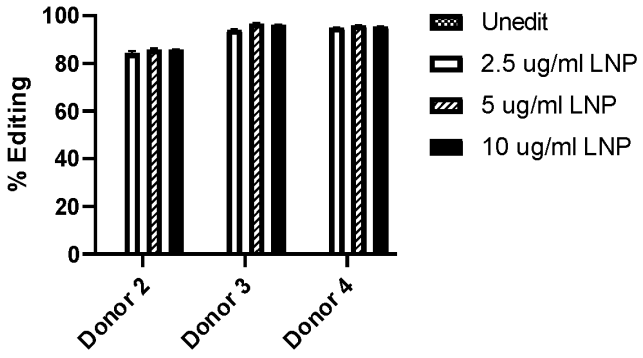

100
80
60
40
20
0
% Editing
Donor 2
Donor 3
Donor 4
Unedit
2.5 ug/ml LNP
5 ug/ml LNP
10 ug/ml LNP

FIG. 63

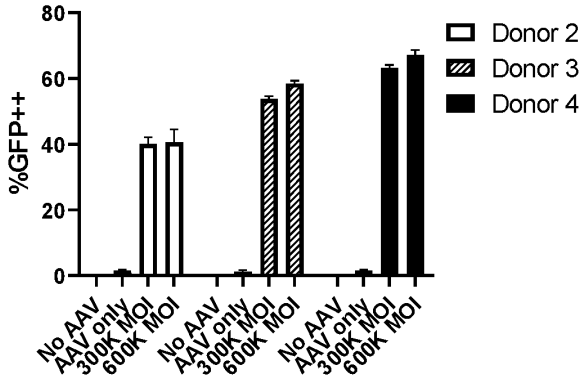

80
60
40
20
0
%GFP++
Donor 2
Donor 3
Donor 4
No AAV
AAV only
300K MOI
600K MOI
No AAV
AAV only
300K MOI
600K MOI
No AAV
AAV only
300K MOI
600K MOI

FIG. 64

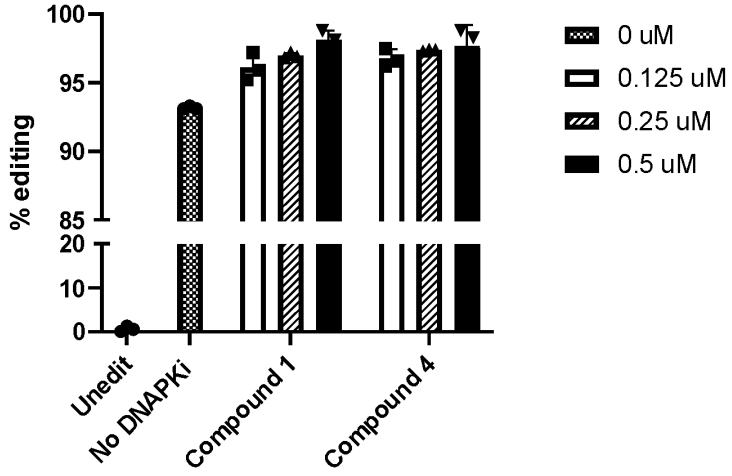


FIG. 65A

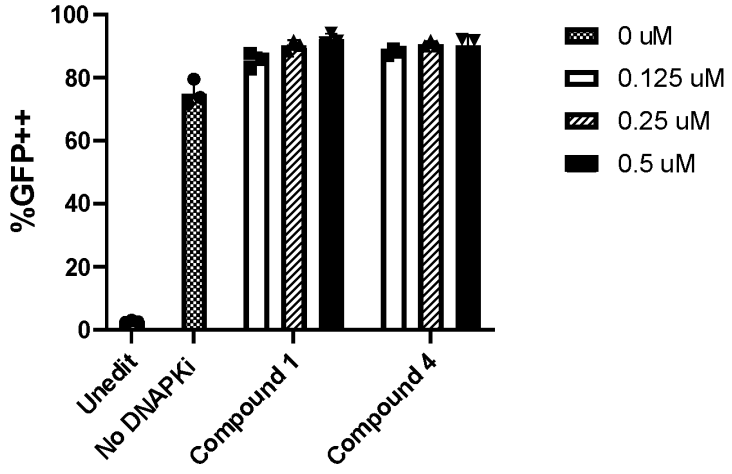


FIG. 65B

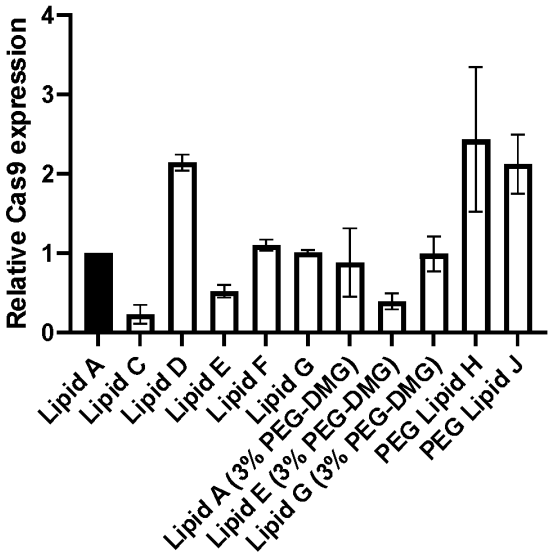

Relative Cas9 expression
4
3
2
1
0
Lipid A
Lipid C
Lipid D
Lipid E
Lipid F
Lipid G
Lipid A (3% PEG-DMG)
Lipid E (3% PEG-DMG)
Lipid G (3% PEG-DMG)
PEG Lipid H
PEG Lipid J

FIG. 66

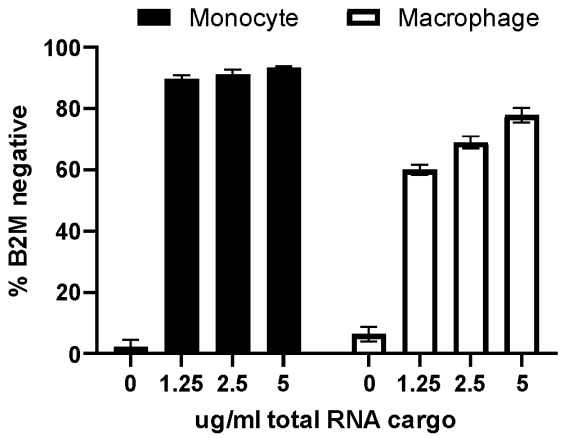

Monocyte
Macrophage
% B2M negative
100
80
60
40
20
0
0
1.25
2.5
5
0
1.25
2.5
5
ug/ml total RNA cargo

FIG. 67

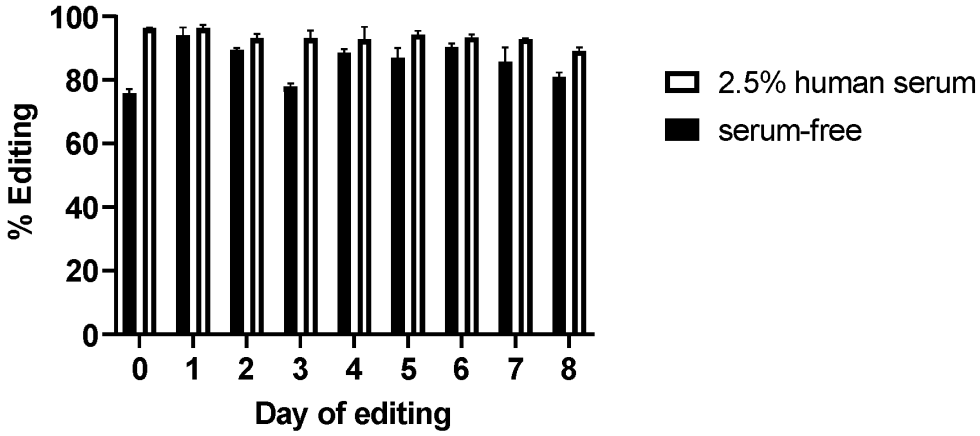

100
80
60
40
20
0
% Editing
0 1 2 3 4 5 6 7 8
Day of editing
2.5% human serum
serum-free

FIG. 68

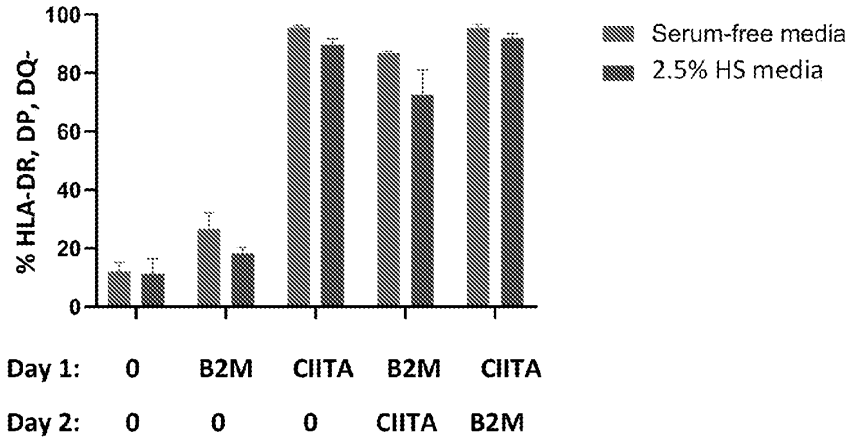


FIG. 69A

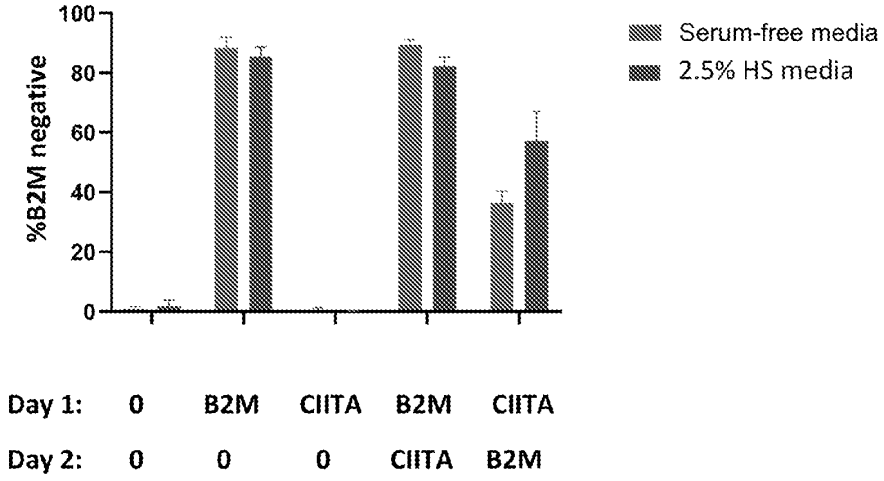


FIG. 69B

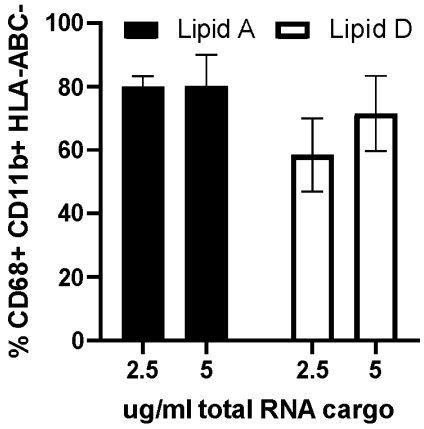

% CD68+ CD11b+ HLA-ABC-
100
80
60
40
20
0
Lipid A
Lipid D
2.5
5
2.5
5
ug/ml total RNA cargo

FIG. 70