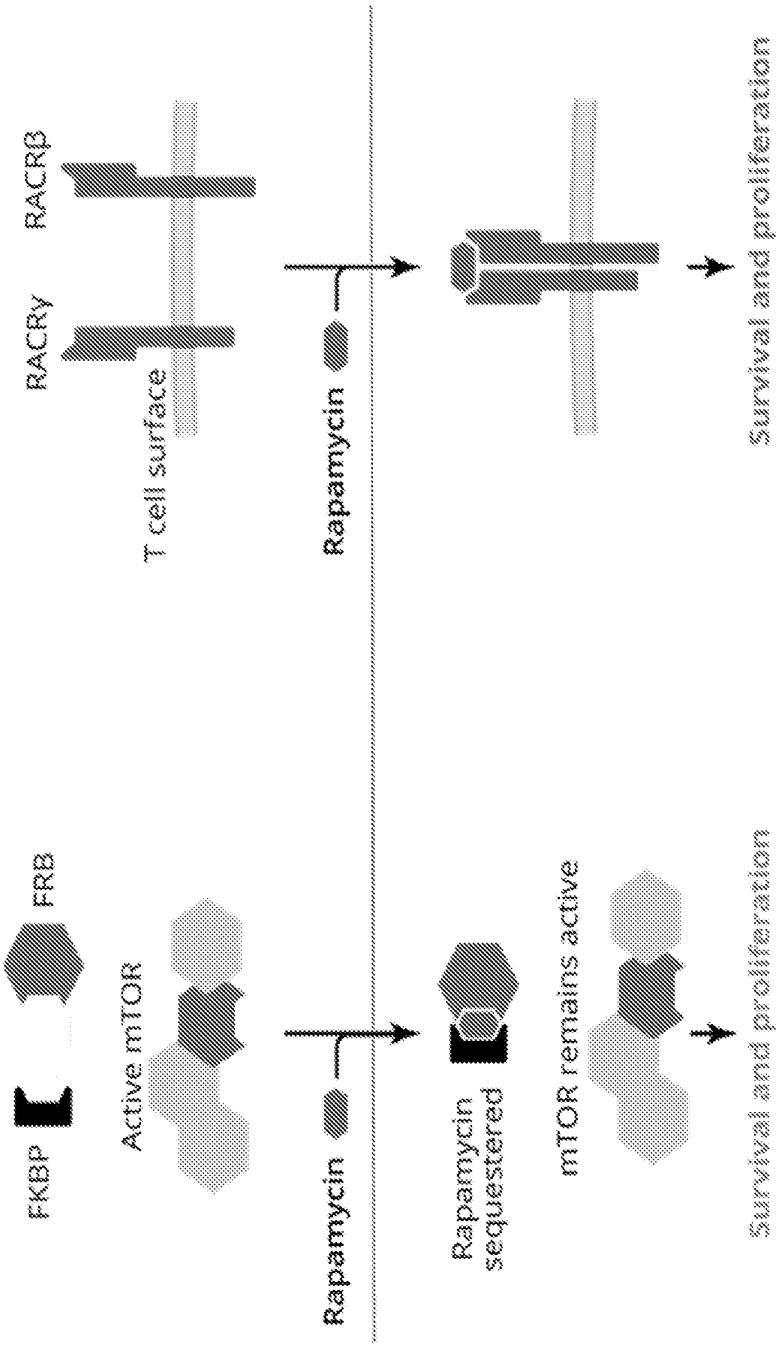

FKBP
FRB
Active mTOR
Rapamycin
Rapamycin sequestered
mTOR remains active
Survival and proliferation
T cell surface
RACRγ
RACRβ
Rapamycin
Survival and proliferation

FIG. 1

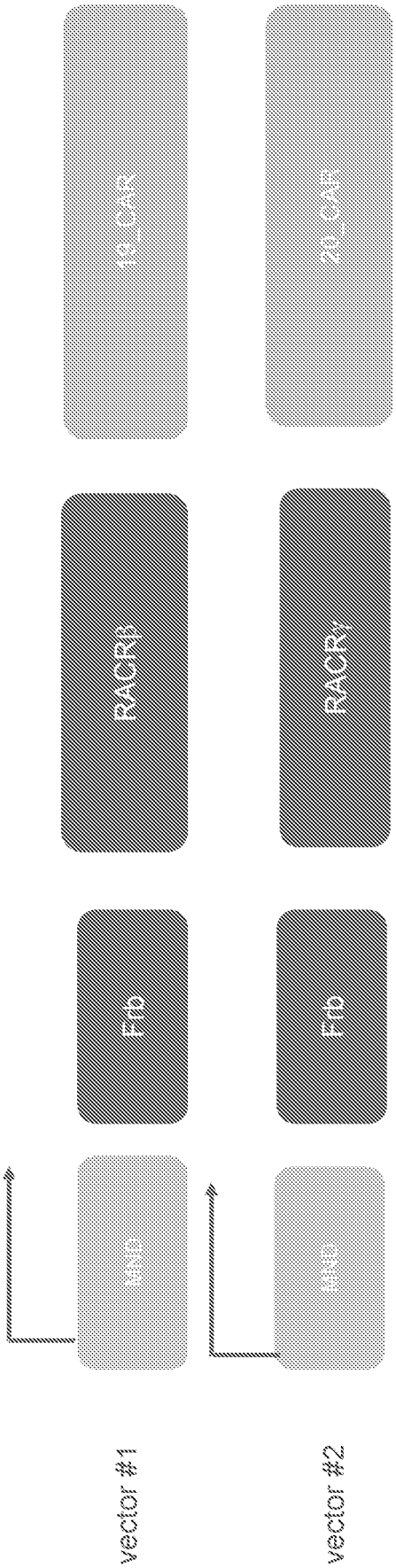


FIG. 2

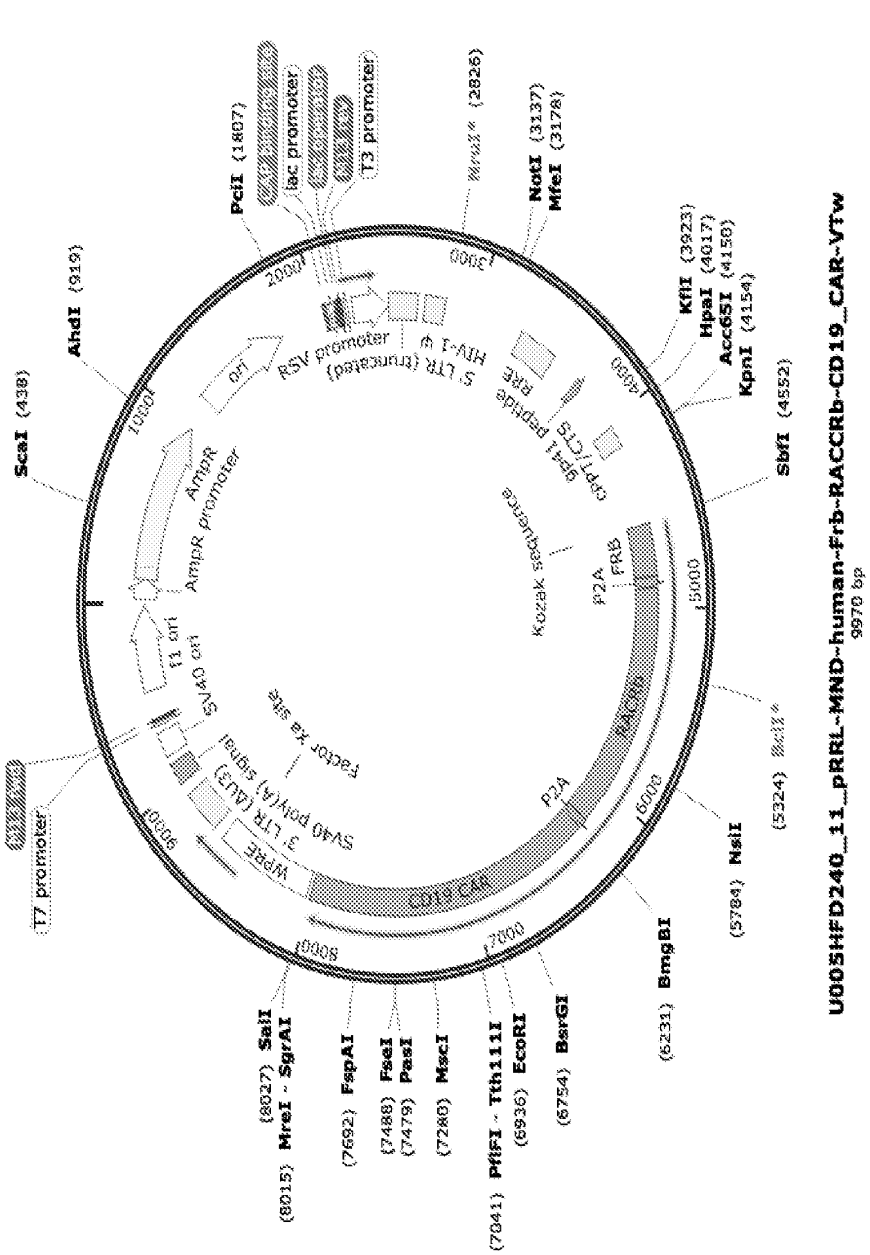

U005HFD240_11_pRRL-MND-human-Frb-RACCRb-CD19_CAR-VTw
9870 bp
ScaI (438)
AhdI (919)
PciI (1867)
lac promoter
T3 promoter
MreI (2826)
NotI (3137)
MfeI (3178)
KflI (3923)
HpaI (4017)
Acc65I (4158)
KpnI (4154)
SbfI (4552)
MreI (5324)
NsiI (5784)
BmgBI (6231)
BsrGI (6754)
EcoRI (6936)
PflFI - Tth111I (7041)
MscI (7280)
PasI (7479)
FseI (7488)
FspAI (7692)
MreI - SgrAI (8015)
SalI (8027)
T7 promoter
AmpR promoter
f1 ori
SV40 ori
Factor Xa site
SV40 poly(A) signal
3' LTR (ΔU3)
WPRE
CD19 CAR
P2A
FRB
Kozak sequence
gpt-FCYS
gp41 peptide
RRE
HIV-1 Ψ
5' LTR (truncated)
RSV promoter
ori

FIG. 3

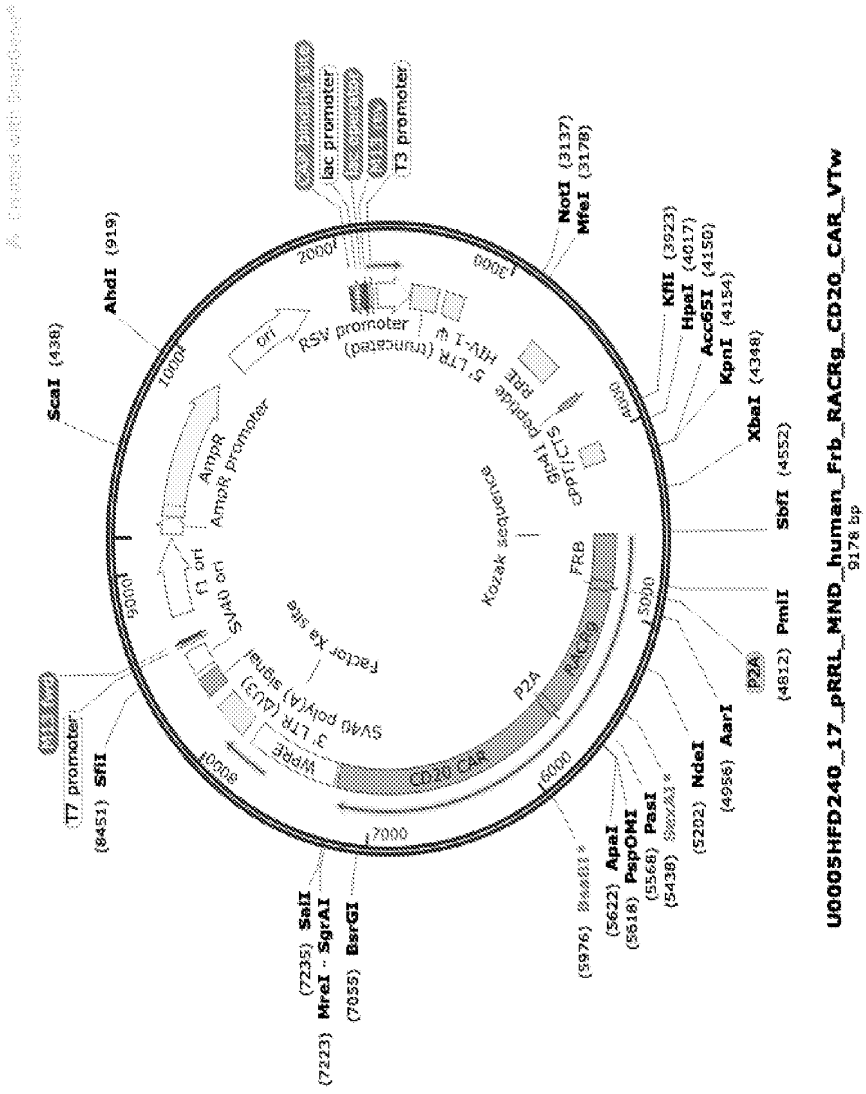

U0005HFD240_17_pRRL_MND_human_Frb_RACRg_CD20_CAR_VTw
9178 bp
lac promoter
T3 promoter
NotI (3137)
MfeI (3178)
KflI (3923)
HpaI (4017)
Acc65I (4150)
KpnI (4154)
XbaI (4348)
SbfI (4552)
PmlI (4812)
P2A
AarI (4956)
NdeI (5202)
PasI (5438)
PspOMI (5566)
ApaI (5622)
BsrGI (7055)
SgrAI (7223)
SalI (7235)
SfiI (8451)
T7 promoter
ScaI (438)
AbdI (919)
ori
RSV promoter (truncated)
5' LTR HIV-1
RRE
Gag/Pol
Kozak sequence
FRB
P2A
CD20 CAR
WPRE
3' LTR (ΔU3)
SV40 poly(A) signal
factor Xa site
SV40 ori
f1 ori
AmpR promoter
AmpR

FIG. 4

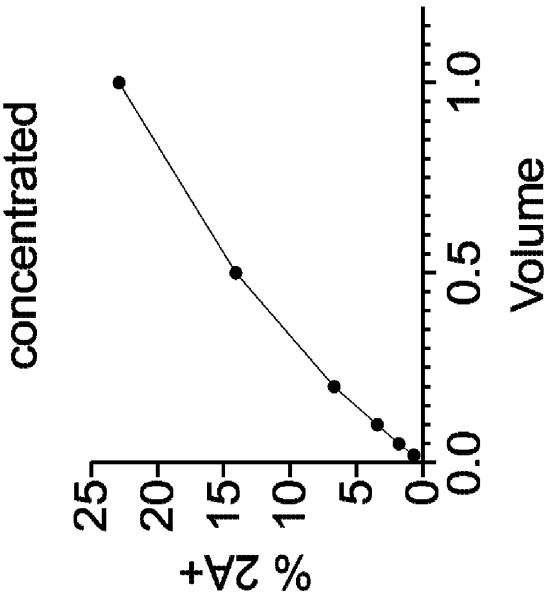

concentrated
% 2A+
25
20
15
10
5
0
0.0
0.5
1.0
Volume

FIG. 5B

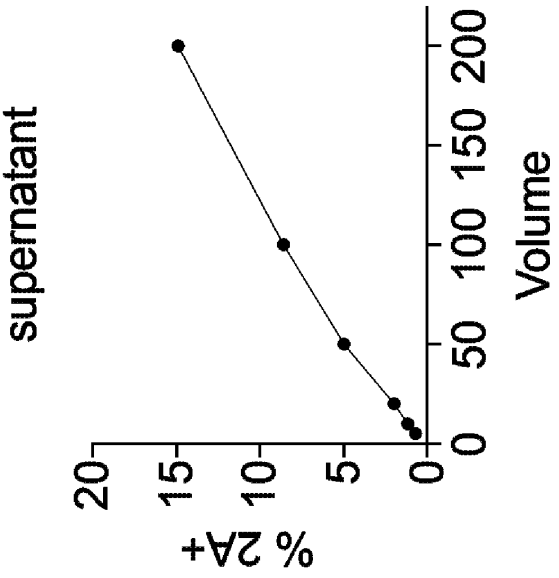

supernatant
% 2A+
20
15
10
5
0
0
50
100
150
200
Volume

FIG. 5A

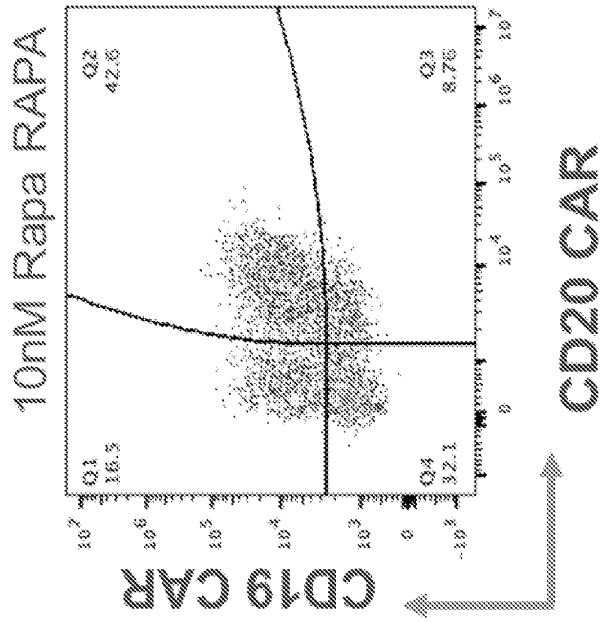

10nM Rapa RAPA
Q2
42.6
Q3
8.76
Q1
16.5
Q4
32.1
CD19 CAR
CD20 CAR

FIG. 6B

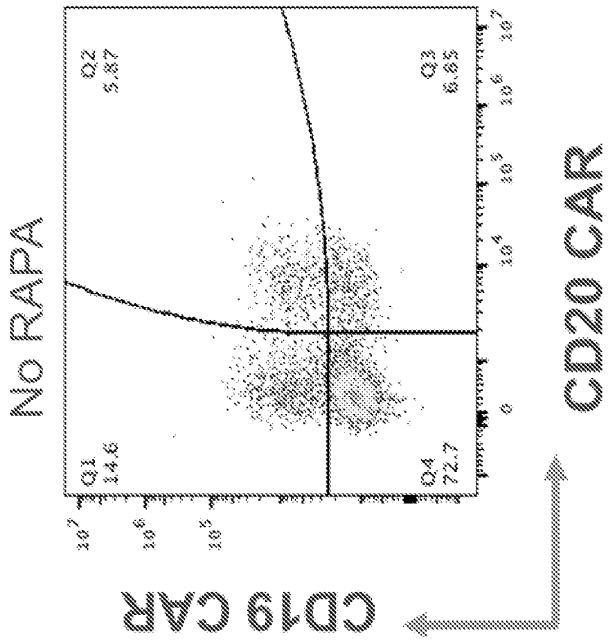

No RAPA
Q2
5.87
Q3
6.65
Q1
14.6
Q4
72.7
CD19 CAR
CD20 CAR

FIG. 6A

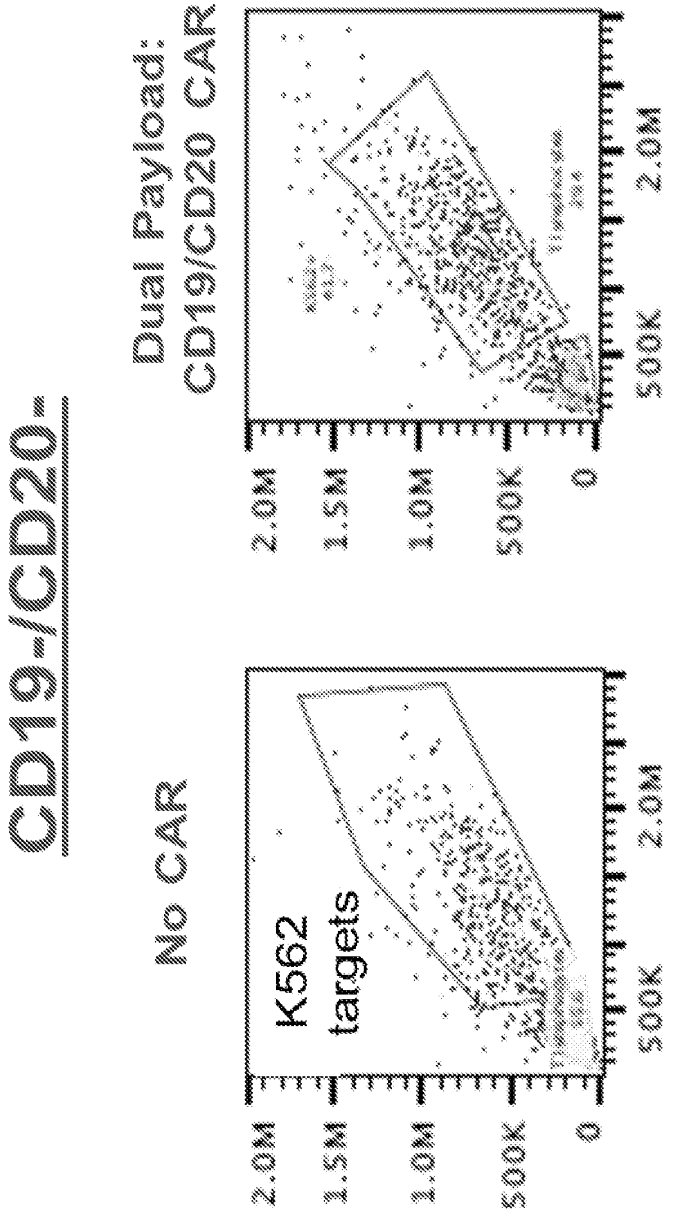

CD19-/CD20-
No CAR
K562
targets
Dual Payload:
CD19/CD20 CAR
2.0M
1.5M
1.0M
500K
0
500K
2.0M

FIG. 7A

FIG. 7B

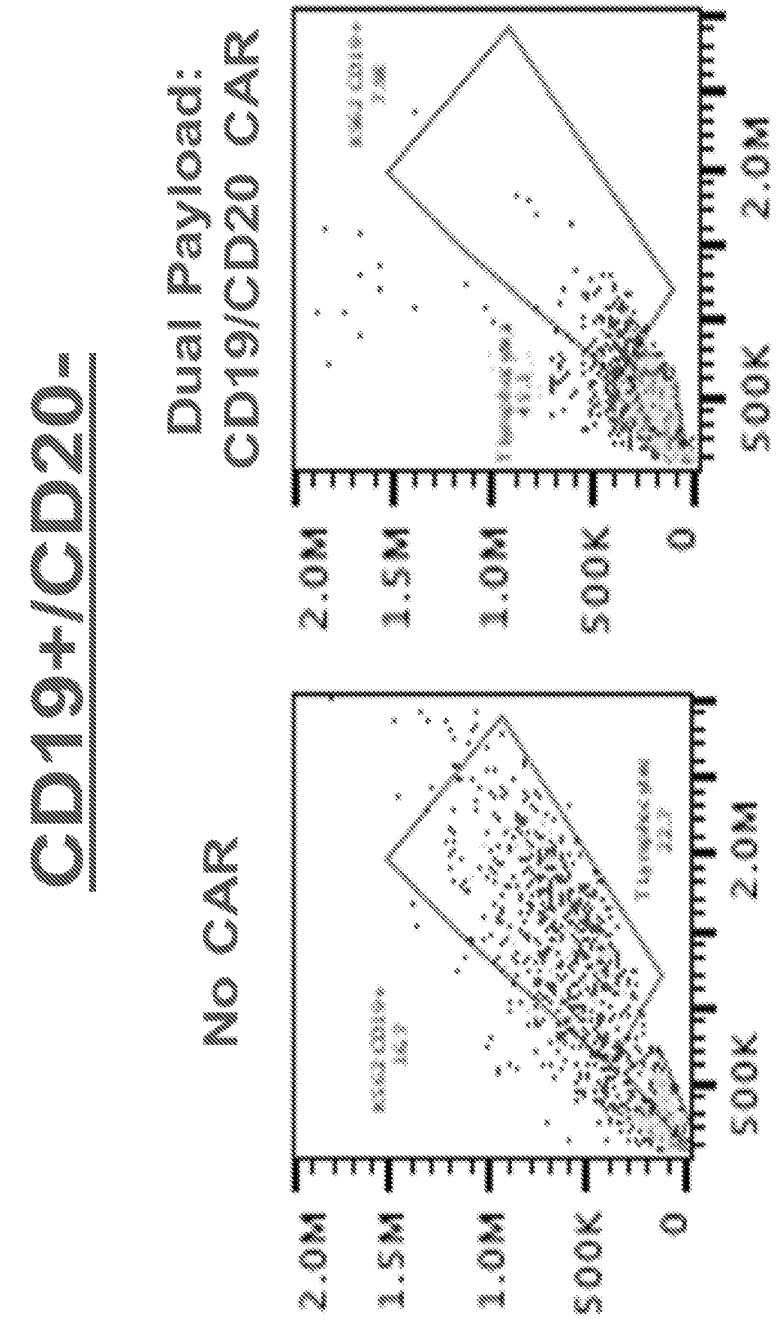

CD19+/CD20-
No CAR
Dual Payload:
CD19/CD20 CAR
2.0M
1.5M
1.0M
500K
0
500K
2.0M

FIG. 7C

FIG. 7D

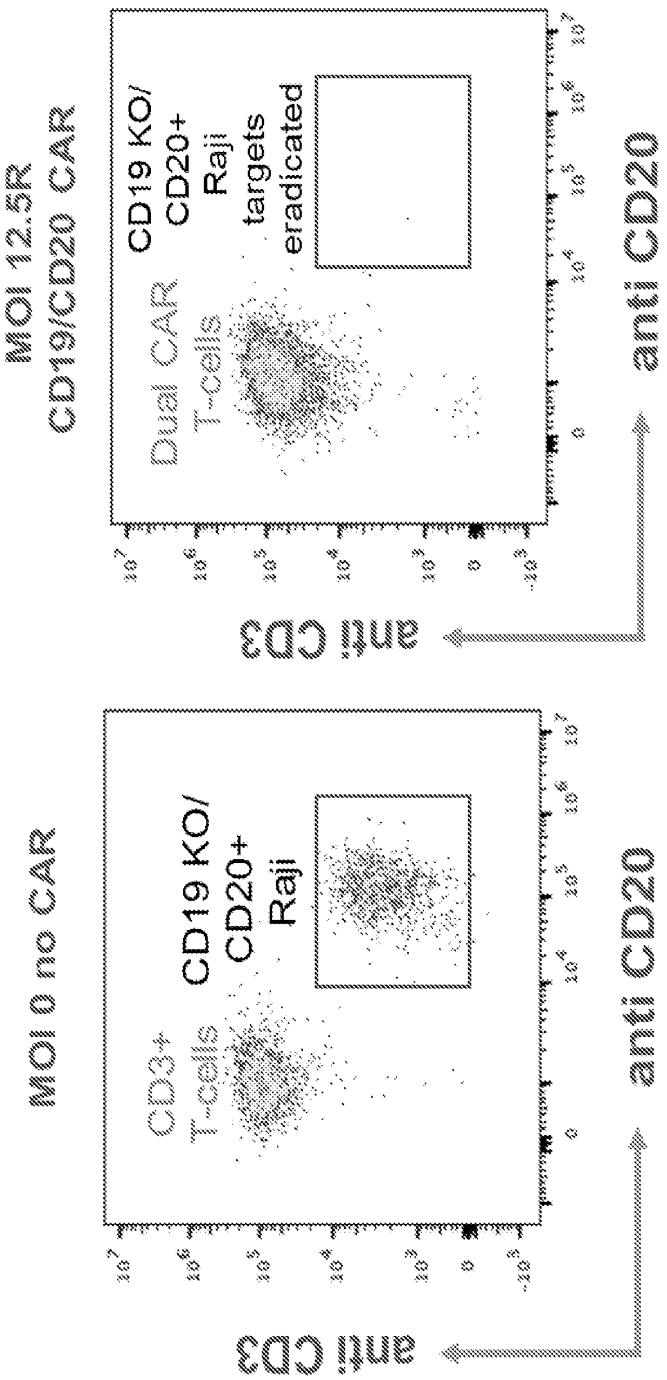

MOI 12.5R
CD19/CD20 CAR
Dual CAR
T-cells
CD19 KO/
CD20+
Raji
targets
eradicated
anti CD3
anti CD20
MOI 0 no CAR
CD3+
T-cells
CD19 KO/
CD20+
Raji
anti CD3
anti CD20

FIG. 8B

FIG. 8A

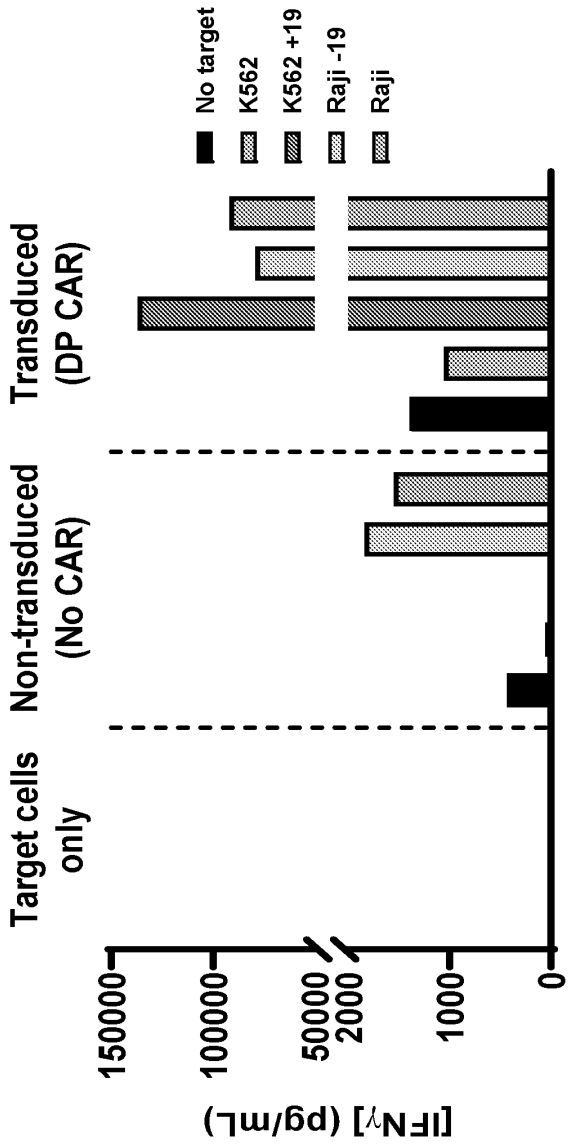

No target
K562
K562 +19
Raji -19
Raji
Transduced (DP CAR)
Non-transduced (No CAR)
Target cells only
150000
100000
50000
2000
1000
0
[IFNγ] (pg/mL)

FIG. 9

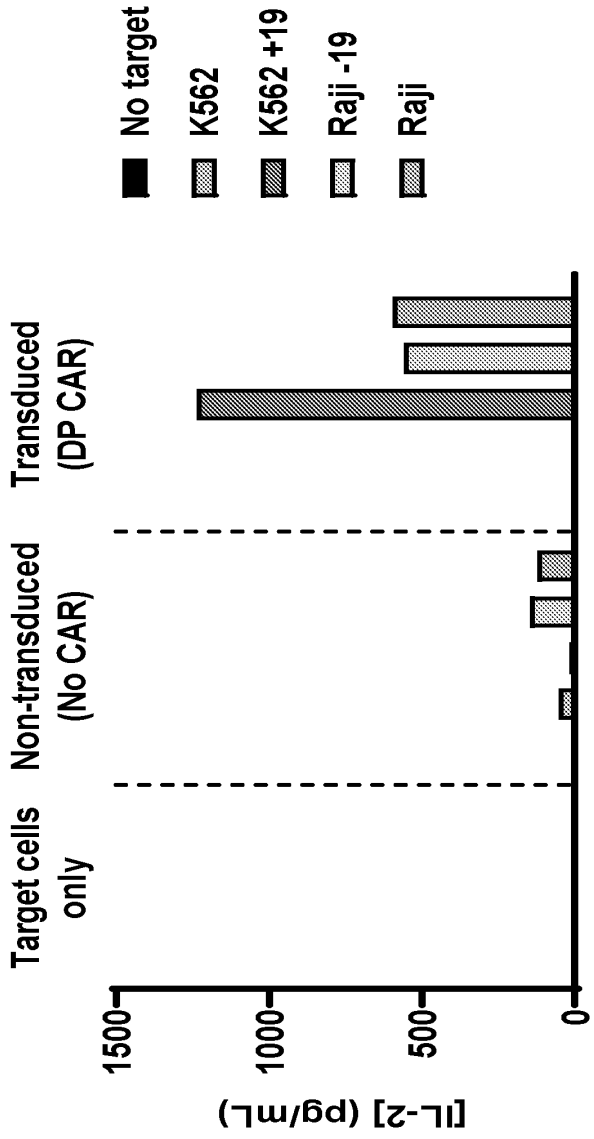

No target
K562
K562 +19
Raji -19
Raji
Transduced (DP CAR)
Non-transduced (No CAR)
Target cells only
1500
1000
500
0
[IL-2] (pg/mL)

FIG. 10

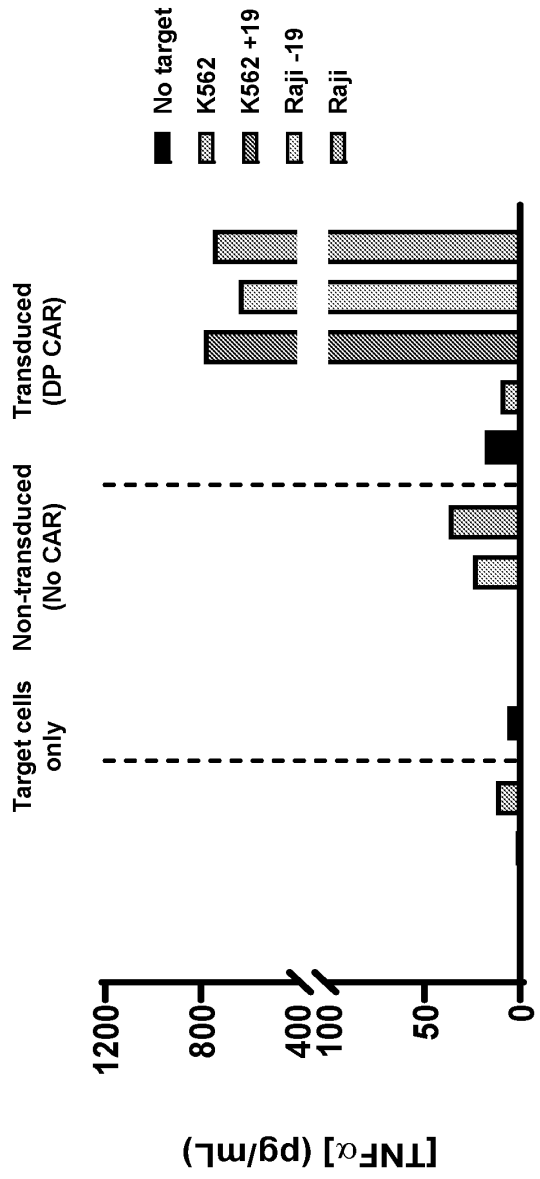

No target
K562
K562 +19
Raji -19
Raji
Transduced (DP CAR)
Non-transduced (No CAR)
Target cells only
1200
800
400
100
50
0
[TNFα] (pg/mL)

FIG. 11

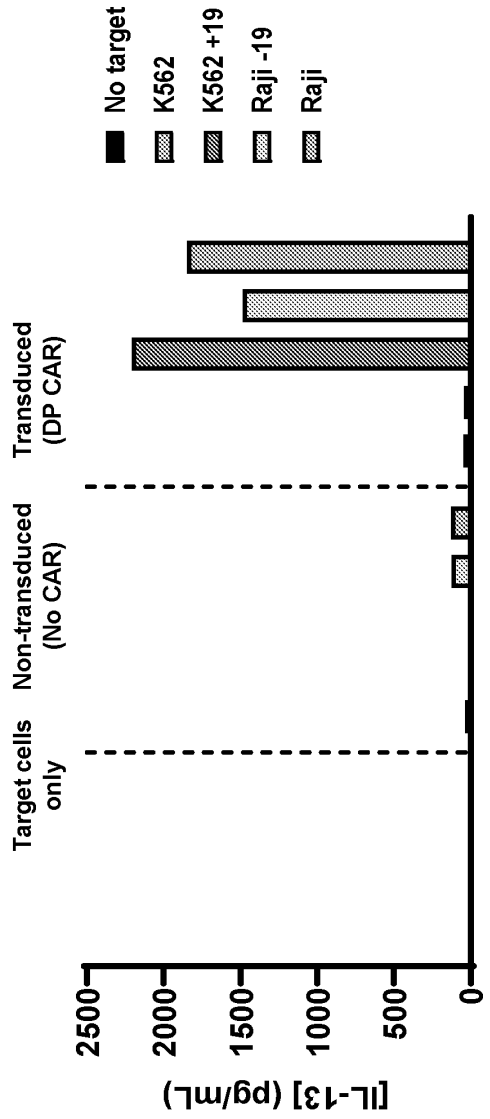

No target
K562
K562 +19
Raji -19
Raji
Transduced (DP CAR)
Non-transduced (No CAR)
Target cells only
2500
2000
1500
1000
500
0
[IL-13] (pg/mL)

FIG. 12

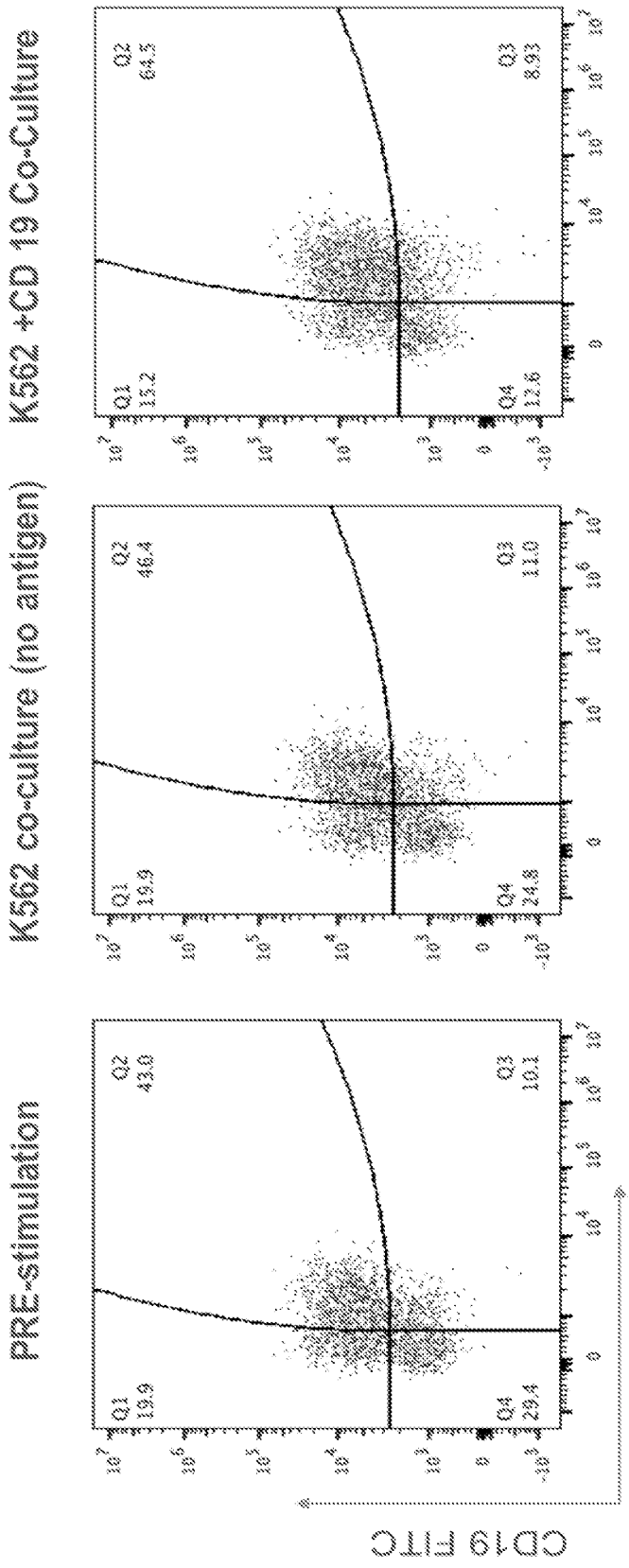


FIG. 13A

FIG. 13B

FIG. 13C

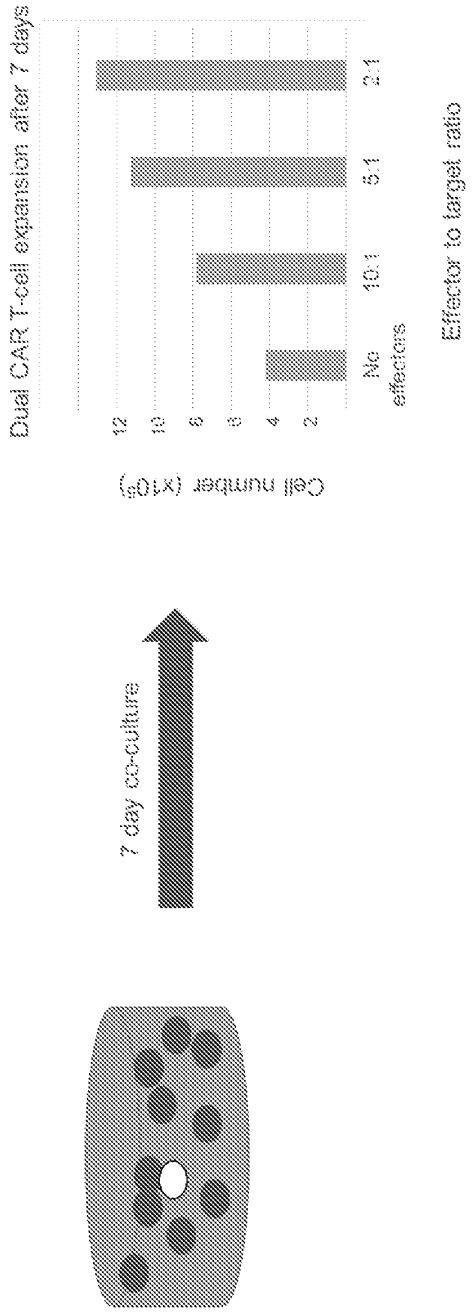

Dual CAR T-cell expansion after 7 days
Cell number (x10⁵)
12
10
8
6
4
2
No effectors
10:1
5:1
2:1
Effector to target ratio
7 day co-culture

FIG. 14