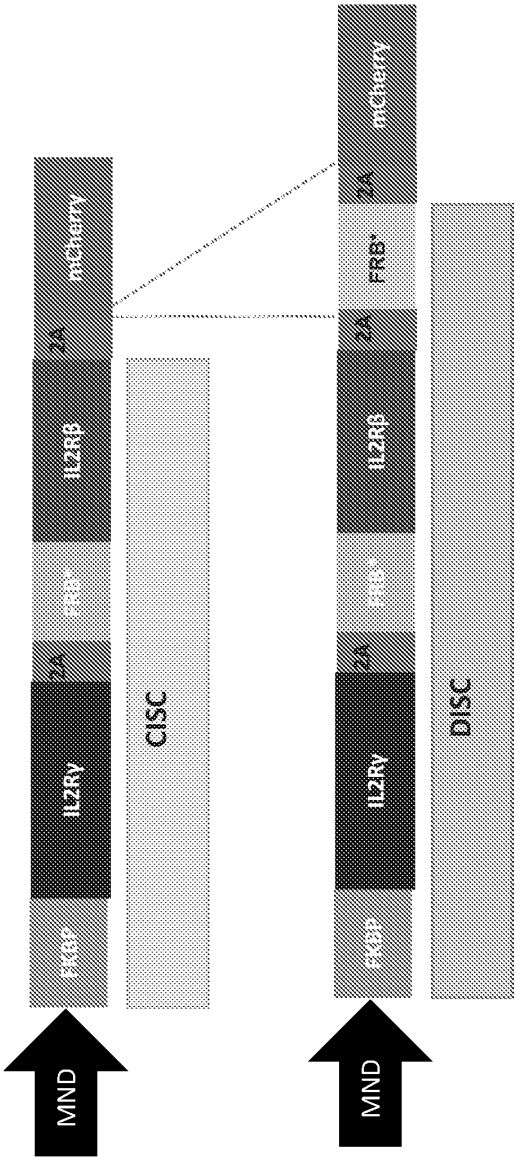

MND
FKBP
IL2Rγ
2A
FRB*
IL2Rβ
2A
mCherry
CISC
MND
FKBP
IL2Rγ
2A
FRB*
IL2Rβ
2A
FRB*
2A
mCherry
DISC

FIG. 1

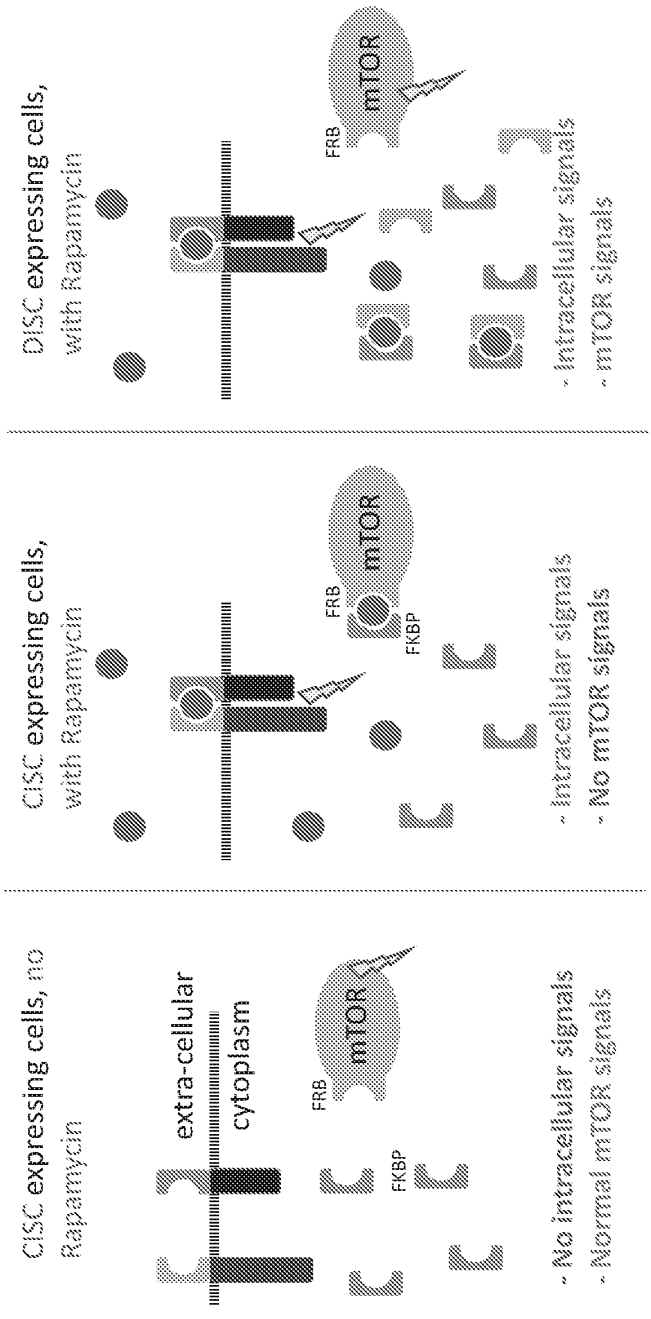

CISC expressing cells, no Rapamycin
extra-cellular
cytoplasm
FRB
mTOR
FKBP
- No intracellular signals
- Normal mTOR signals
CISC expressing cells, with Rapamycin
FRB
mTOR
FKBP
- Intracellular signals
- No mTOR signals
DISC expressing cells, with Rapamycin
FRB
mTOR
- Intracellular signals
- mTOR signals

FIG. 2

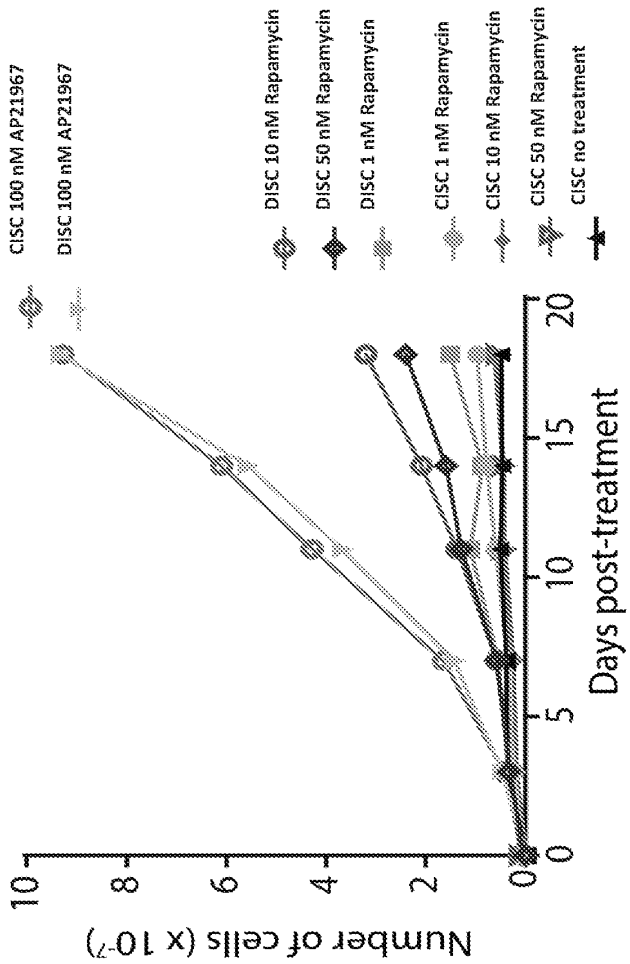

CISC 100 nM AP21967
DISC 100 nM AP21967
DISC 10 nM Rapamycin
DISC 50 nM Rapamycin
DISC 1 nM Rapamycin
CISC 1 nM Rapamycin
CISC 10 nM Rapamycin
CISC 50 nM Rapamycin
CISC no treatment
Number of cells (x 10^7)
0
2
4
6
8
10
0
5
10
15
20
Days post-treatment

FIG. 3

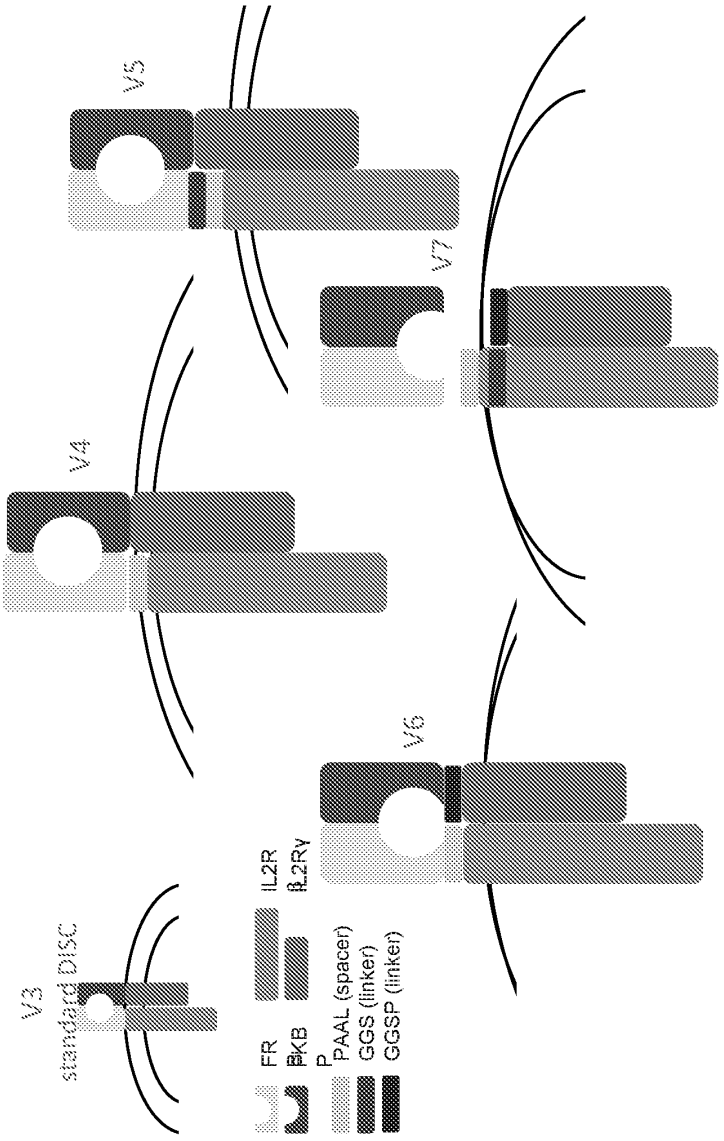

V5
V7
V4
V6
V3
standard Disc
IL2R
IL2Rγ
FR
BKB
P
PAAL (spacer)
GGS (linker)
GGSP (linker)

FIG. 4

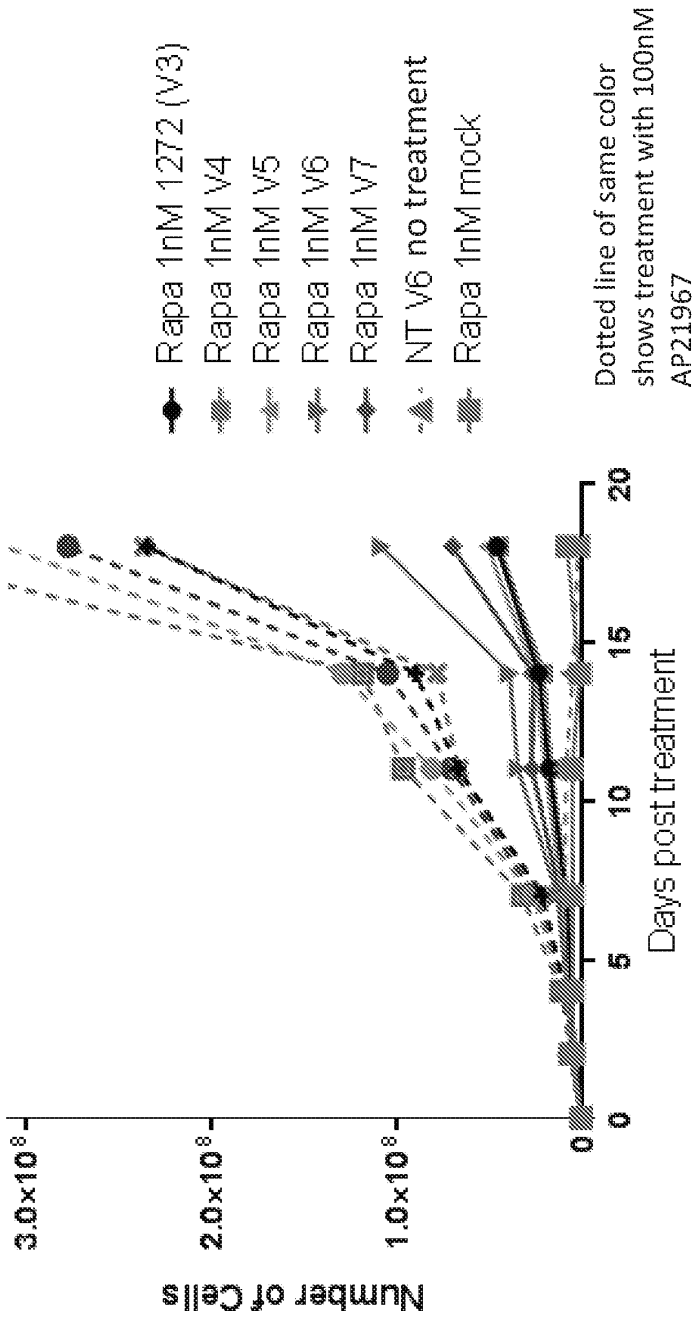

Rapa 1nM 1272 (V3)
Rapa 1nM V4
Rapa 1nM V5
Rapa 1nM V6
Rapa 1nM V7
NT V6 no treatment
Rapa 1nM mock
Dotted line of same color shows treatment with 100nM AP21967
Number of Cells
3.0x10^8
2.0x10^8
1.0x10^8
0
0
5
10
15
20
Days post treatment

FIG. 5

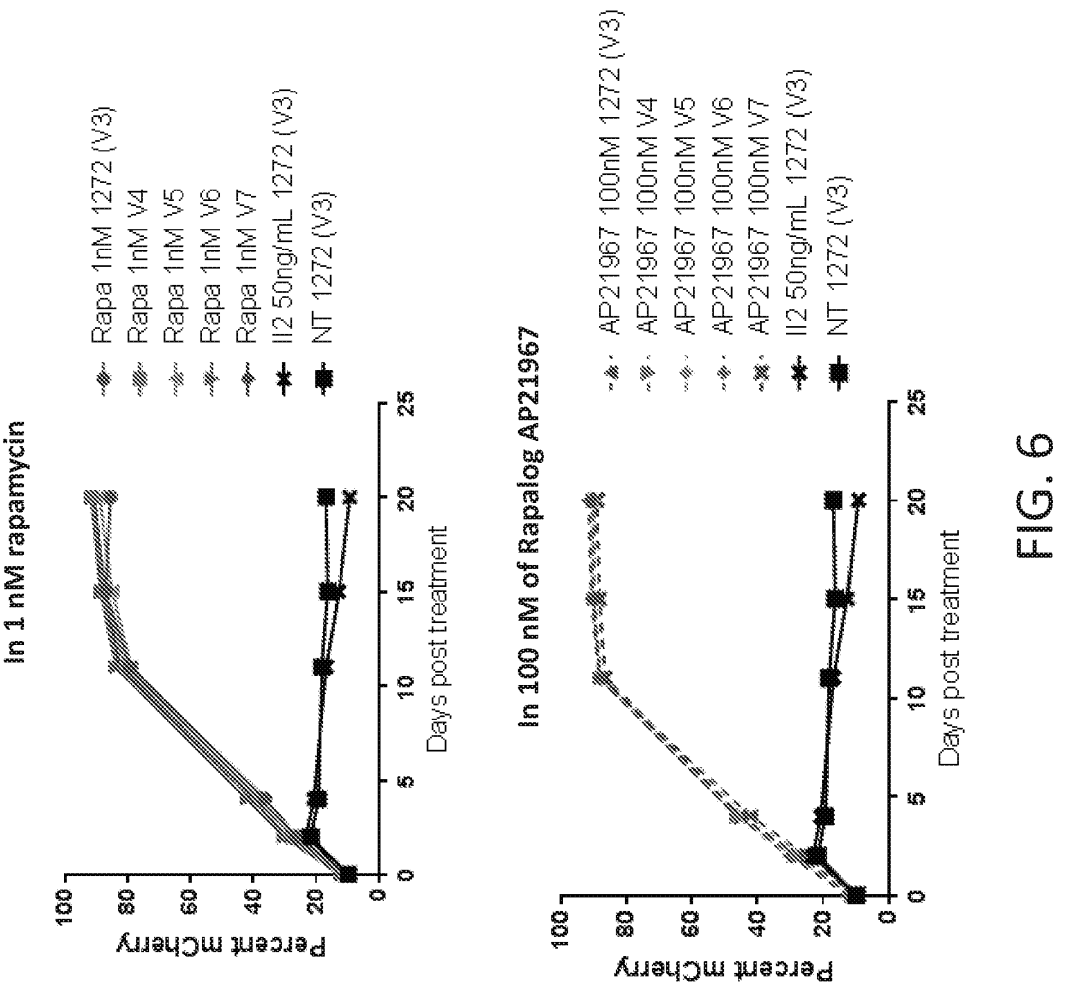

In 1 nM rapamycin
Rapa 1nM 1272 (V3)
Rapa 1nM V4
Rapa 1nM V5
Rapa 1nM V6
Rapa 1nM V7
Il2 50ng/mL 1272 (V3)
NT 1272 (V3)
Percent mCherry
Days post treatment
In 100 nM of Rapalog AP21967
AP21967 100nM 1272 (V3)
AP21967 100nM V4
AP21967 100nM V5
AP21967 100nM V6
AP21967 100nM V7
Il2 50ng/mL 1272 (V3)
NT 1272 (V3)
Percent mCherry
Days post treatment

FIG. 6

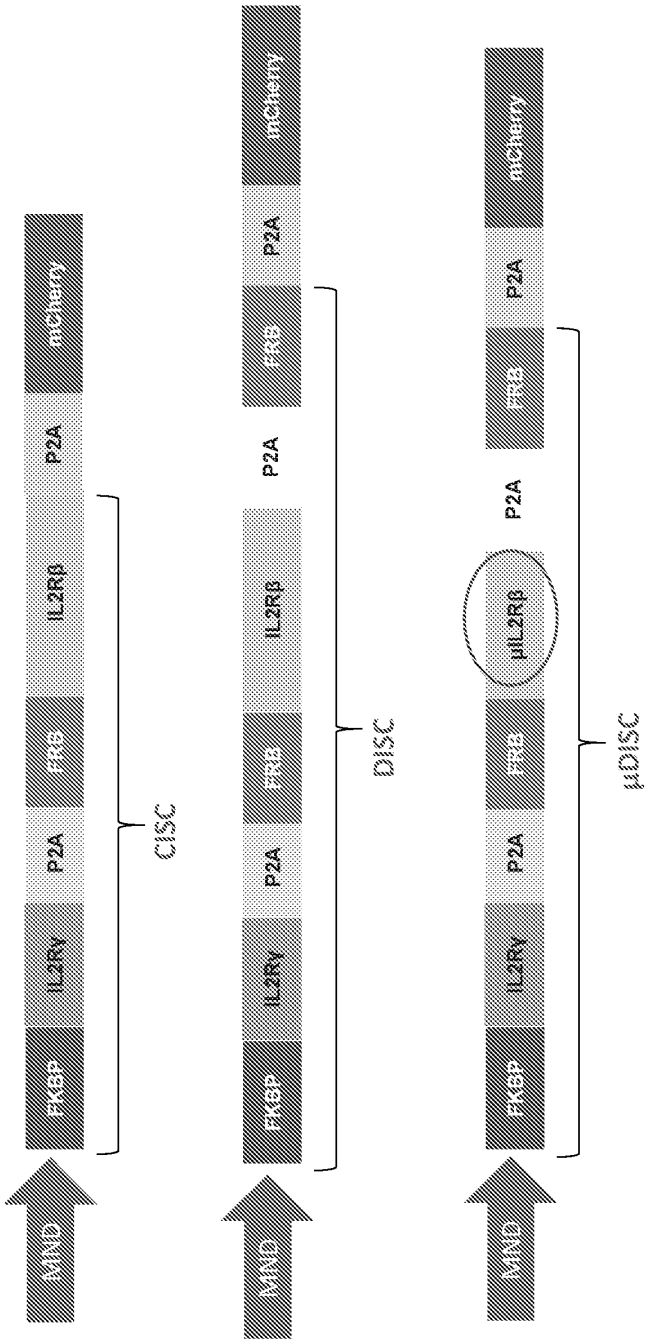

MND
FKBP
IL2RγC
P2A
FRB
IL2Rβ
P2A
mCherry
CISC
MND
FKBP
IL2RγC
P2A
FRB
IL2Rβ
P2A
FRB
P2A
mCherry
DISC
MND
FKBP
IL2RγC
P2A
FRB
μIL2Rβ
P2A
FRB
P2A
mCherry
μDISC

FIG. 7

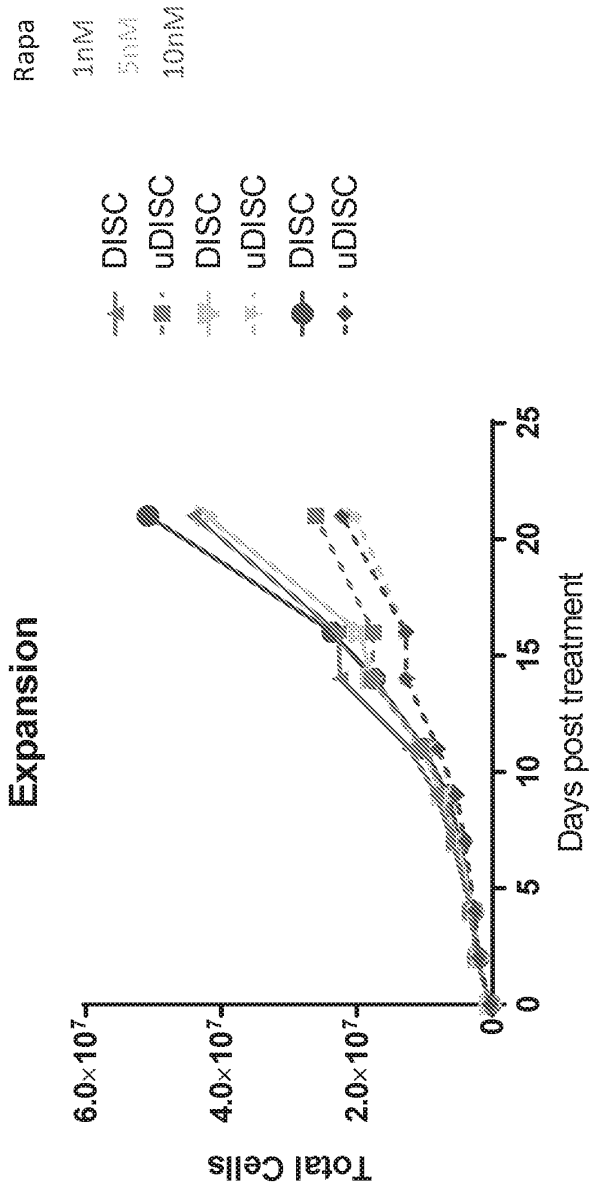

Rapa
1nM
5nM
10nM
DISC
uDISC
DISC
uDISC
DISC
uDISC
Expansion
Total Cells
6.0×10^7
4.0×10^7
2.0×10^7
0
0
5
10
15
20
25
Days post treatment

FIG. 8

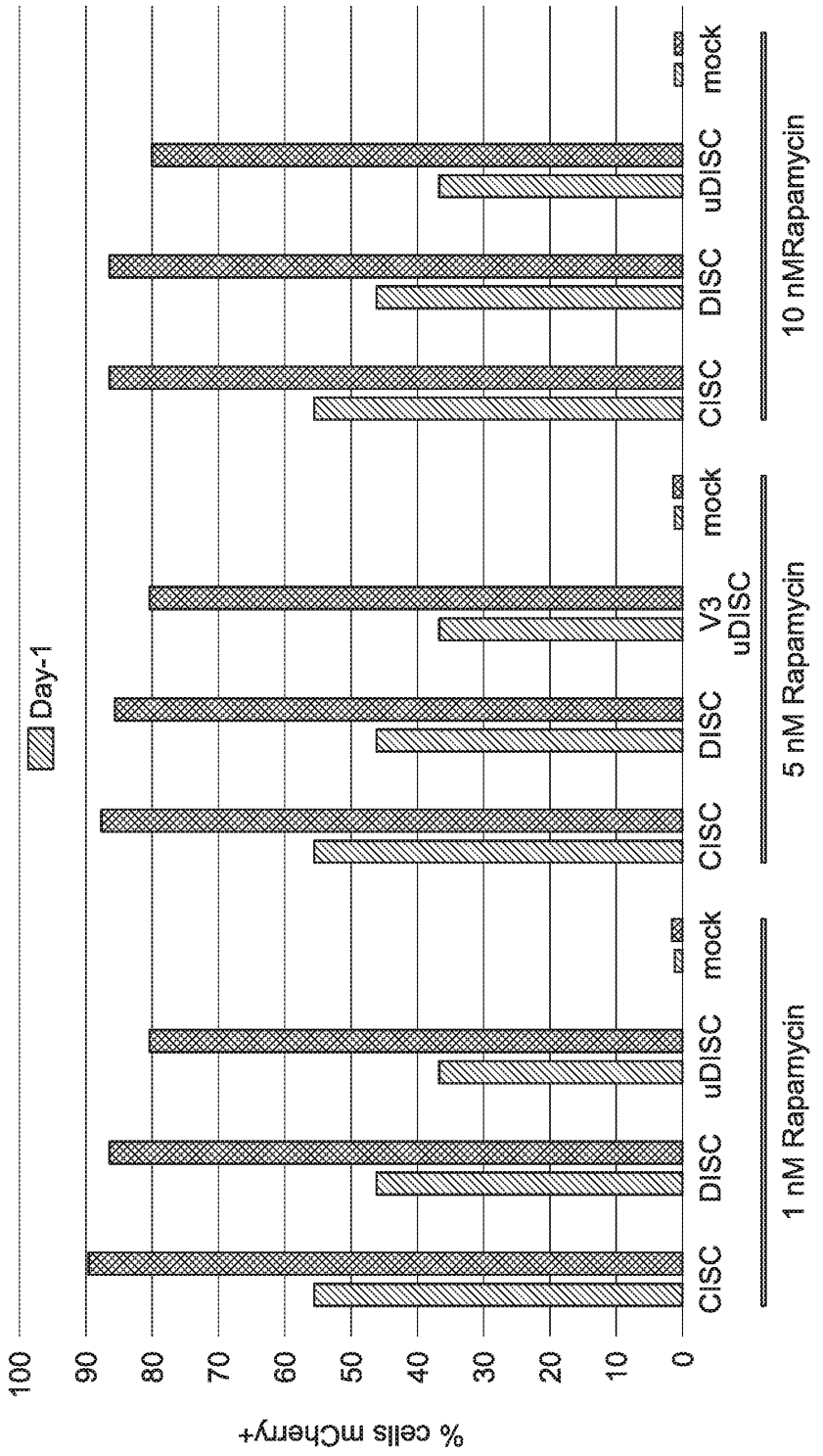

% cells mCherry+
100
90
80
70
60
50
40
30
20
10
0
Day-1
CISC
DISC
uDISC
mock
CISC
DISC
V3 uDISC
mock
CISC
DISC
uDISC
mock
1 nM Rapamycin
5 nM Rapamycin
10 nMRapamycin

FIG. 9

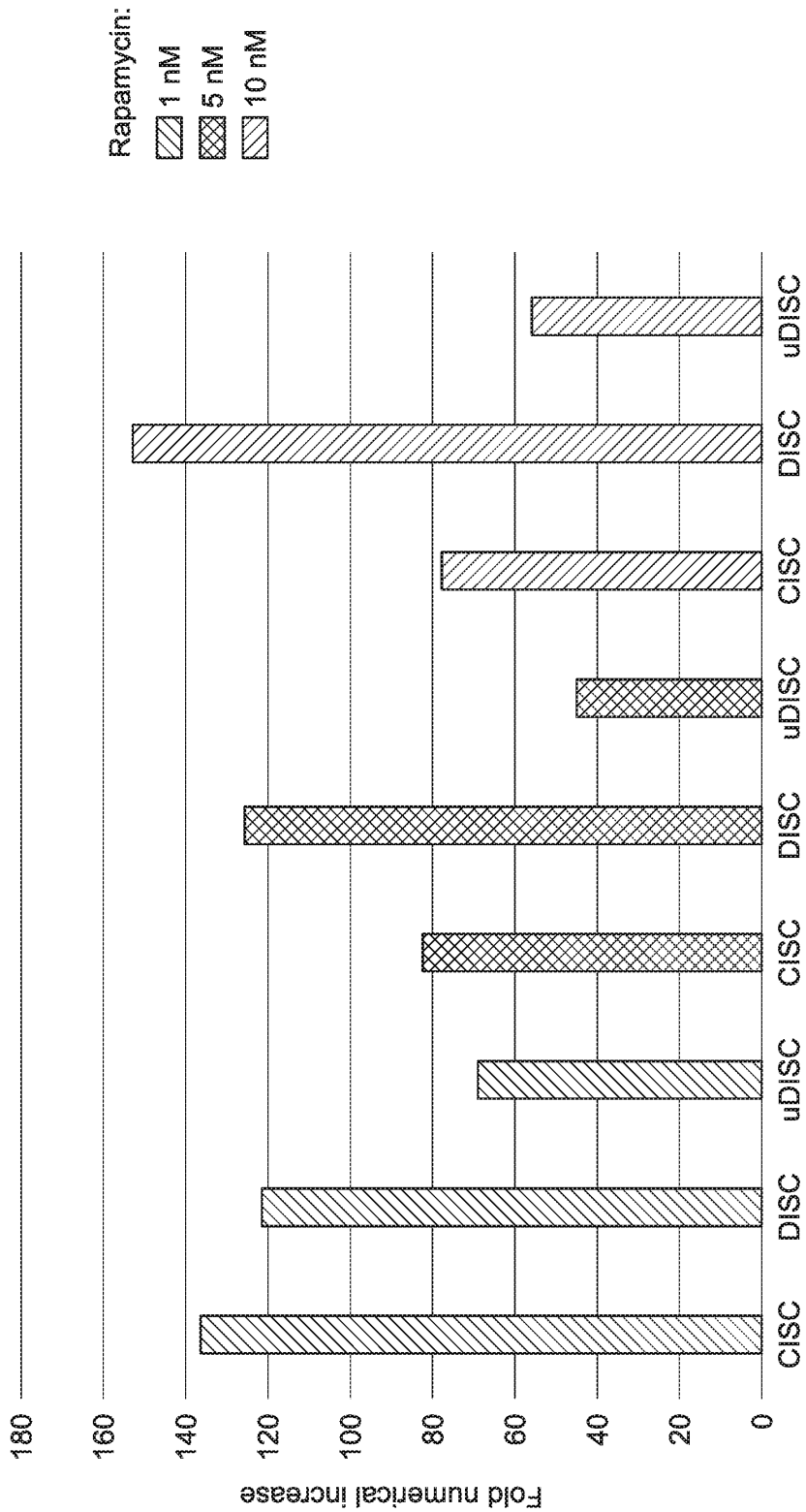

Rapamycin:
1 nM
5 nM
10 nM
Fold numerical increase
0
20
40
60
80
100
120
140
160
180
CISC
DISC
uDISC
CISC
DISC
uDISC
CISC
DISC
uDISC

FIG. 10

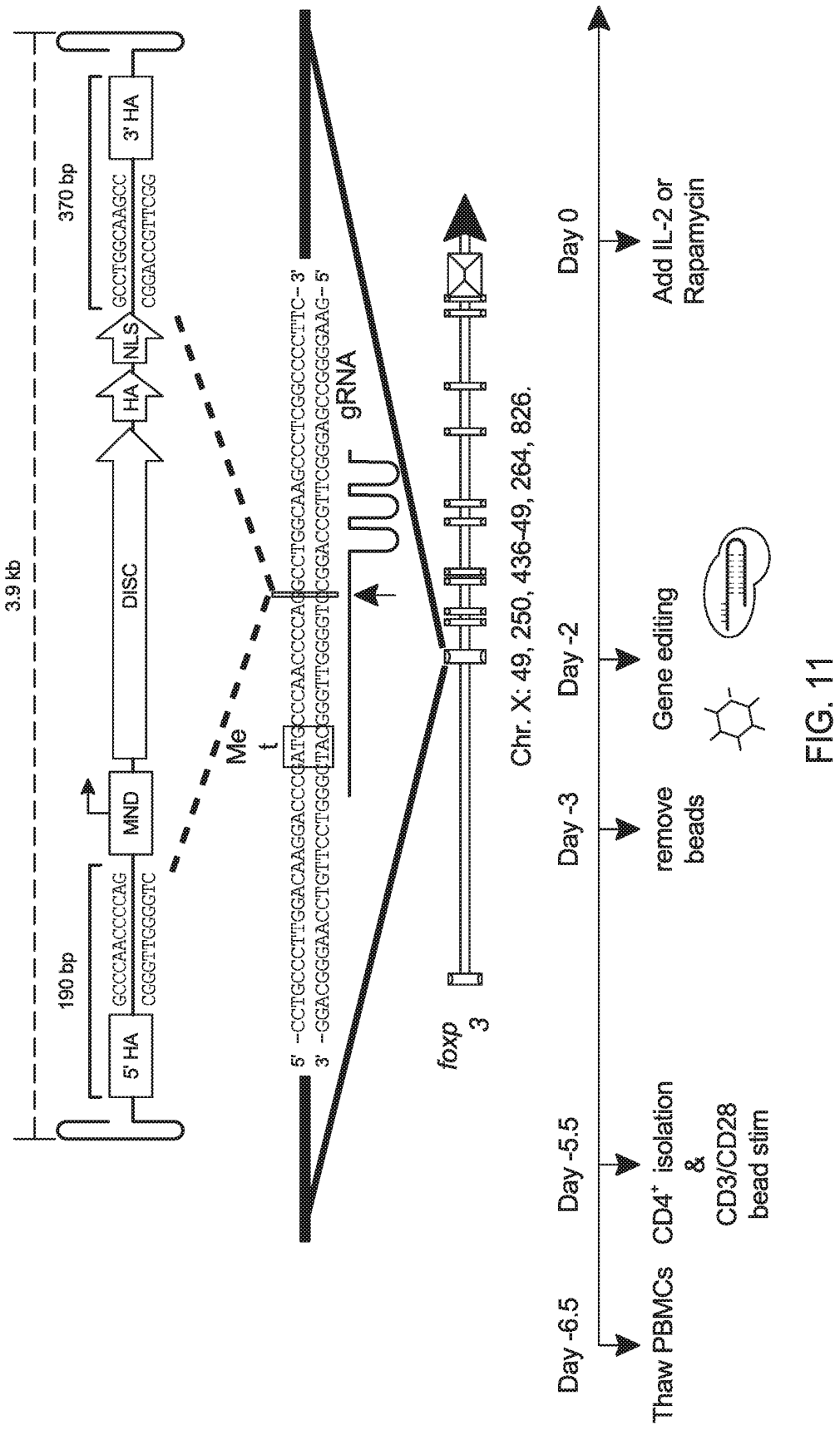

3.9 kb
190 bp
5' HA
GCCCAACCCCAG
CGGGTTGGGGTC
MND
DISC
HA
NLS
370 bp
GCCTGGCAAGCC
CGGACCGTTCGG
3' HA
Me
t
5' -CCTGCCCTTGGACAAGGACCCCATGCCCAACCCCAGGCCTGGCAAGCCCTCGGCCCCTTC- 3'
3' -GGACGGGAACCTGTTCCTGGGGTACGGGTTGGGGTCCGGACCGTTCGGGAGCCGGGGAAG- 5'
gRNA
foxp
3
Chr. X: 49, 250, 436-49, 264, 826.
Day -6.5
Day -5.5
Day -3
Day -2
Day 0
Thaw PBMCs
CD4+ isolation
&
CD3/CD28
bead stim
remove
beads
Gene editing
Add IL-2 or
Rapamycin

FIG. 11

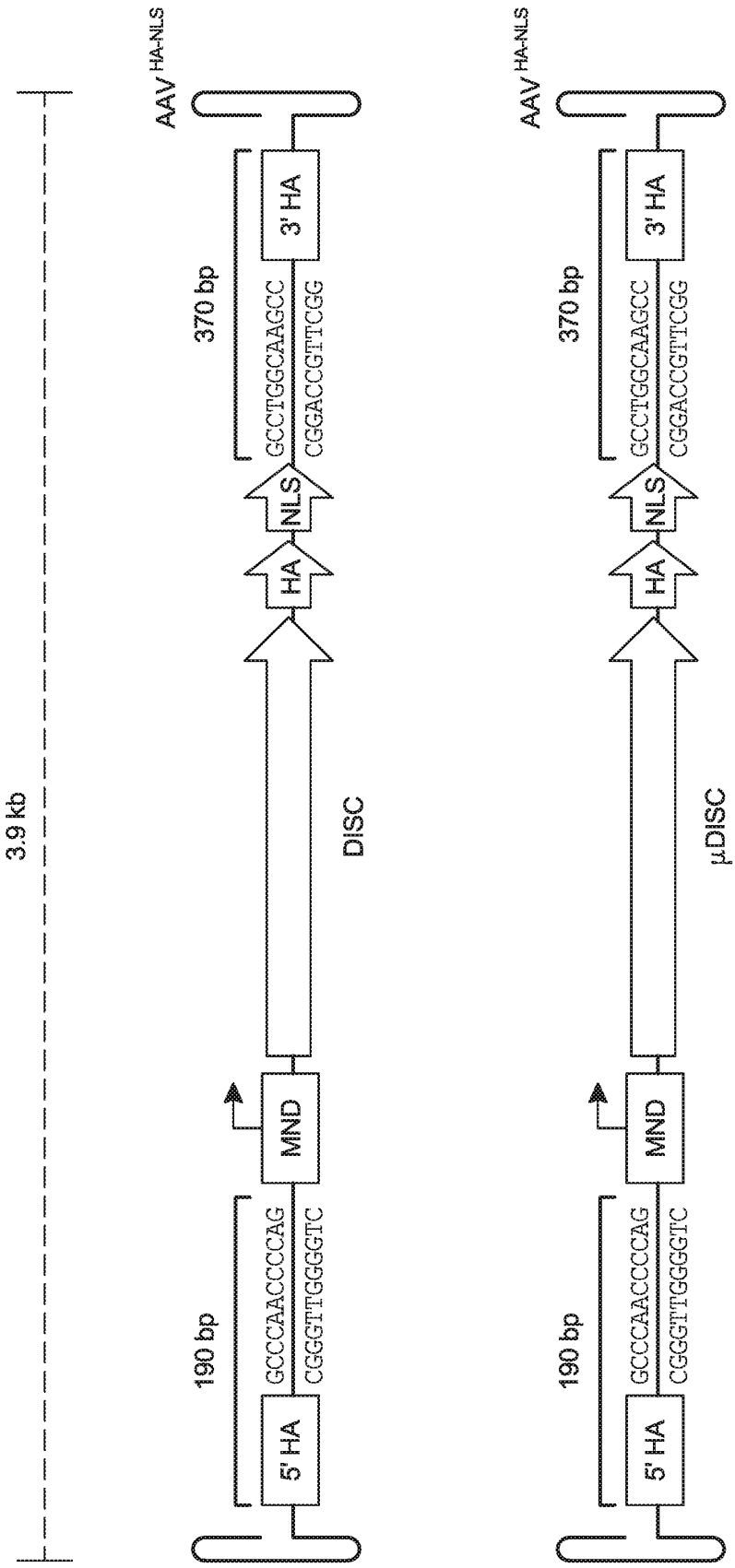
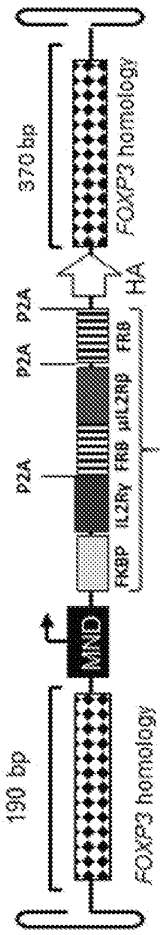

3.9 kb
AAV
HA-NLS
370 bp
GCCTGGCAAGCC
CGGACCGTTCGG
3' HA
NLS
HA
MND
DISC
190 bp
GCCCAACCCCAG
CGGGTTGGGGTC
5' HA
AAV
HA-NLS
370 bp
GCCTGGCAAGCC
CGGACCGTTCGG
3' HA
NLS
HA
MND
µDISC
190 bp
GCCCAACCCCAG
CGGGTTGGGGTC
5' HA

FIG. 12



μDISC

FIG. 13A

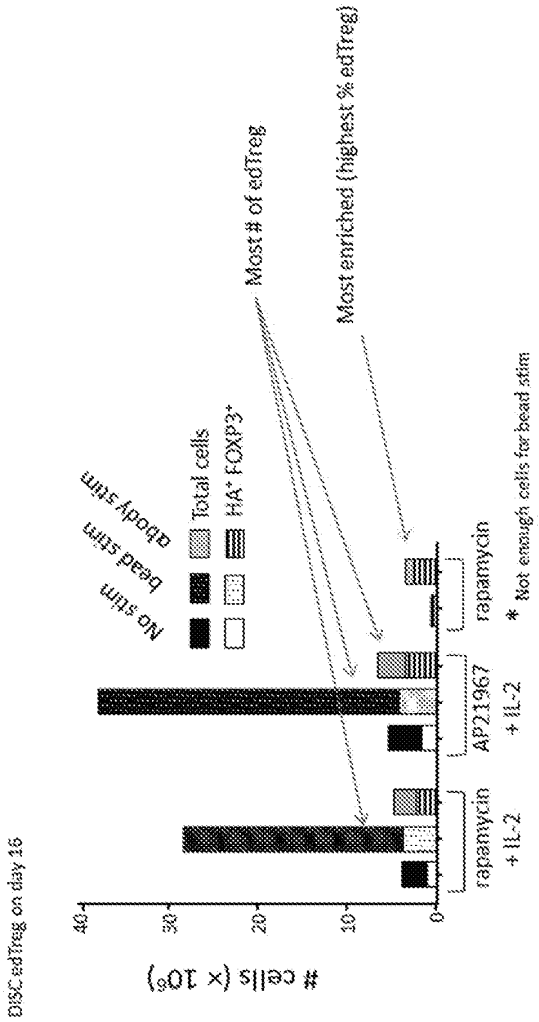


FIG. 13B

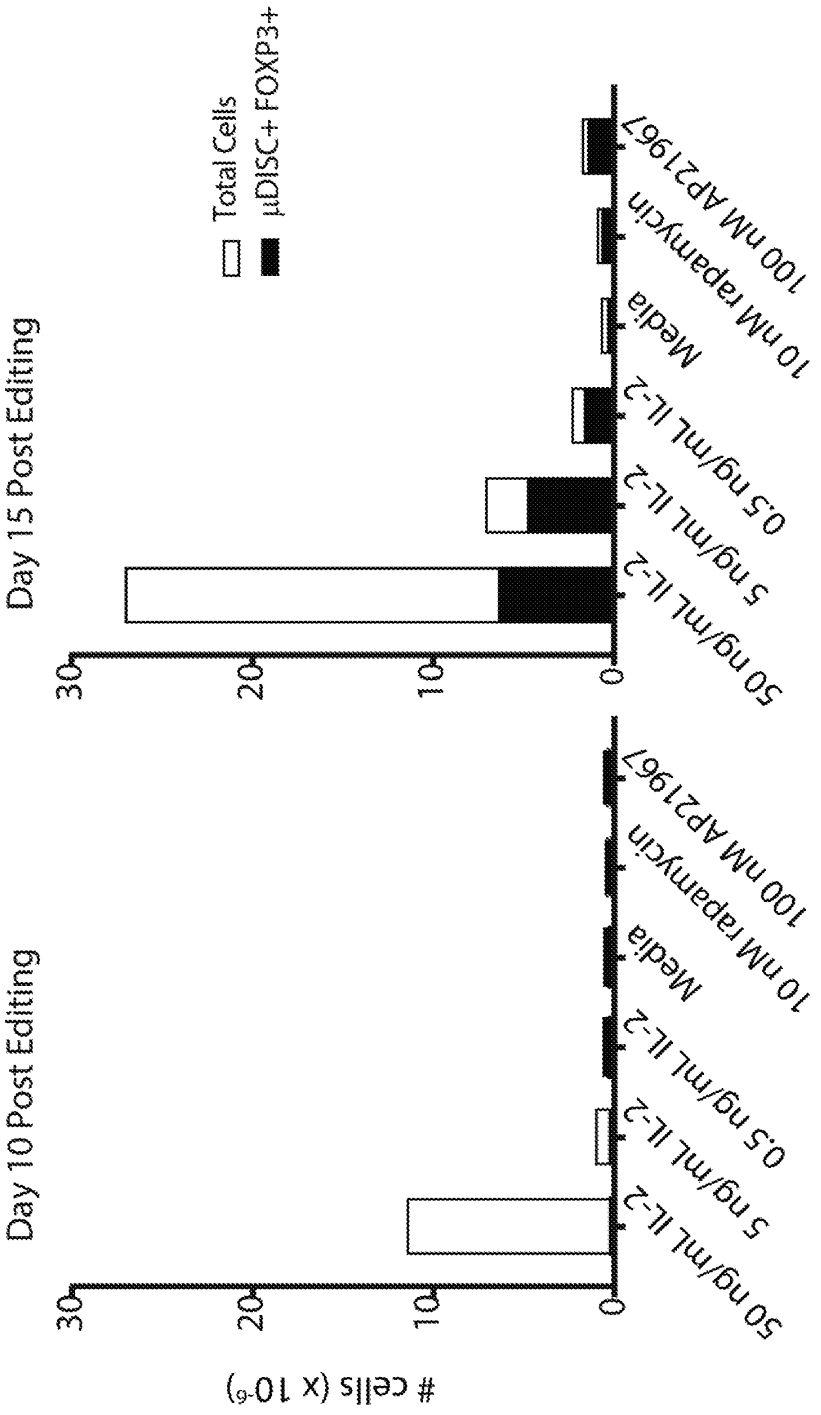


FIG. 14B

FIG. 14A

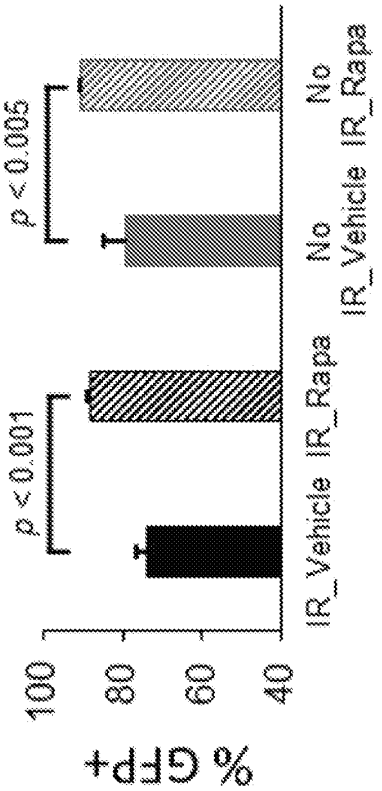


FIG. 15A

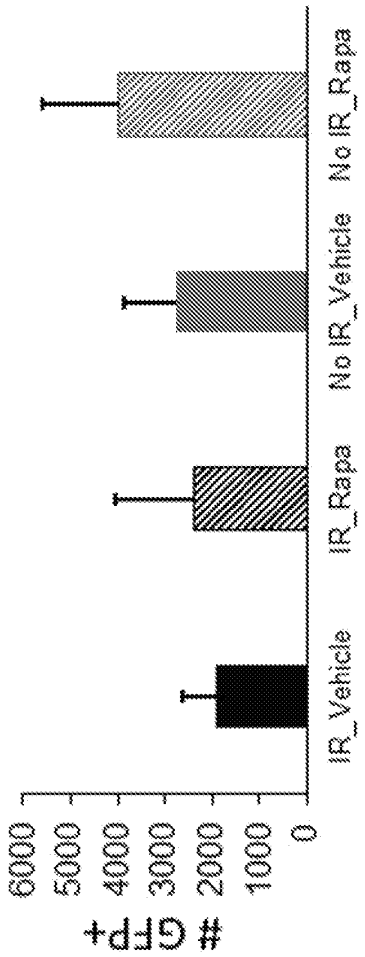


FIG. 15B

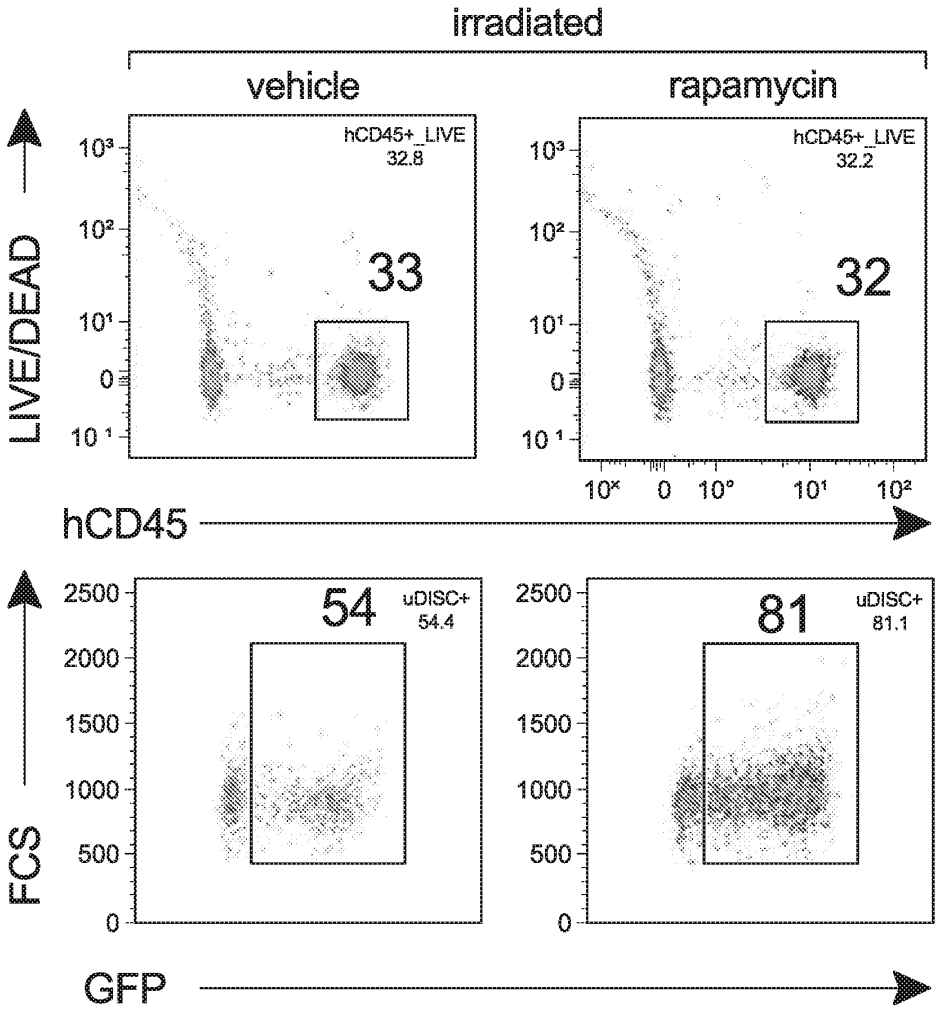

irradiated
vehicle
rapamycin
hCD45+_LIVE
32.8
33
hCD45+_LIVE
32.2
32
LIVE/DEAD
hCD45
54
uDISC+
54.4
81
uDISC+
81.1
FCS
GFP

FIG. 16A

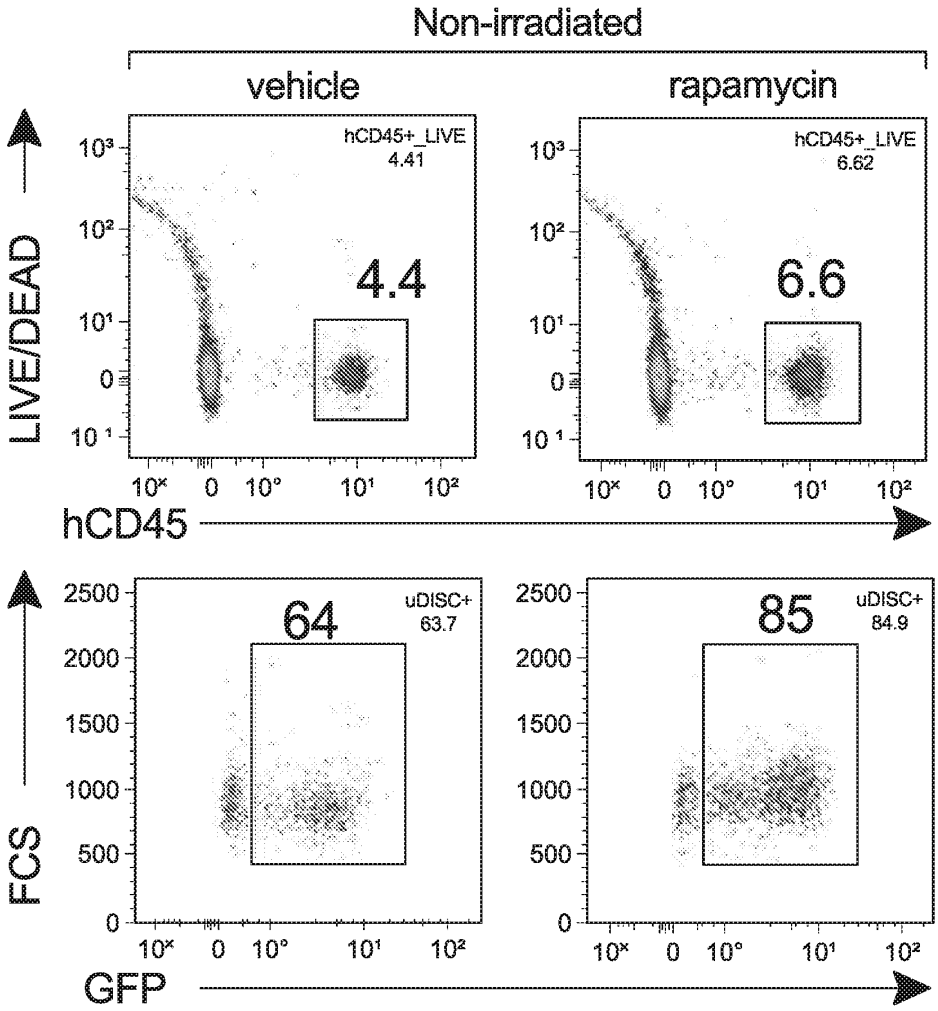

Non-irradiated
vehicle
rapamycin
hCD45+_LIVE
4.41
4.4
hCD45+_LIVE
6.62
6.6
LIVE/DEAD
hCD45
64
uDISC+
63.7
85
uDISC+
84.9
FCS
GFP

FIG. 16A (Con'd)

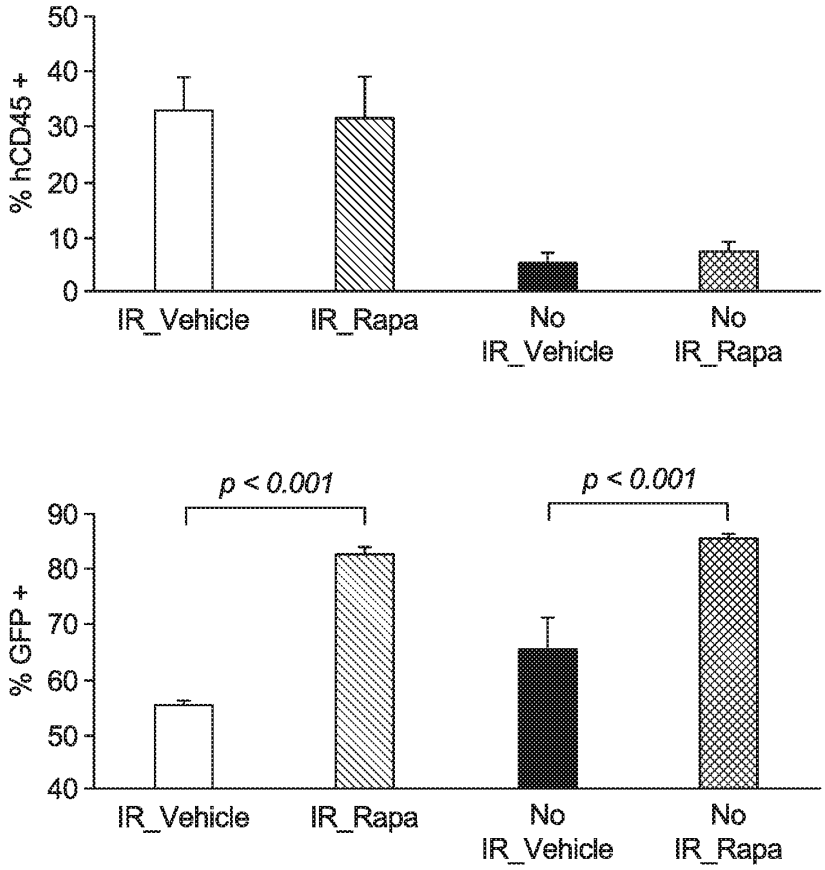

% hCD45 +
50
40
30
20
10
0
IR_Vehicle
IR_Rapa
No IR_Vehicle
No IR_Rapa
p < 0.001
p < 0.001
% GFP +
90
80
70
60
50
40
IR_Vehicle
IR_Rapa
No IR_Vehicle
No IR_Rapa

FIG. 16A (Con'd)

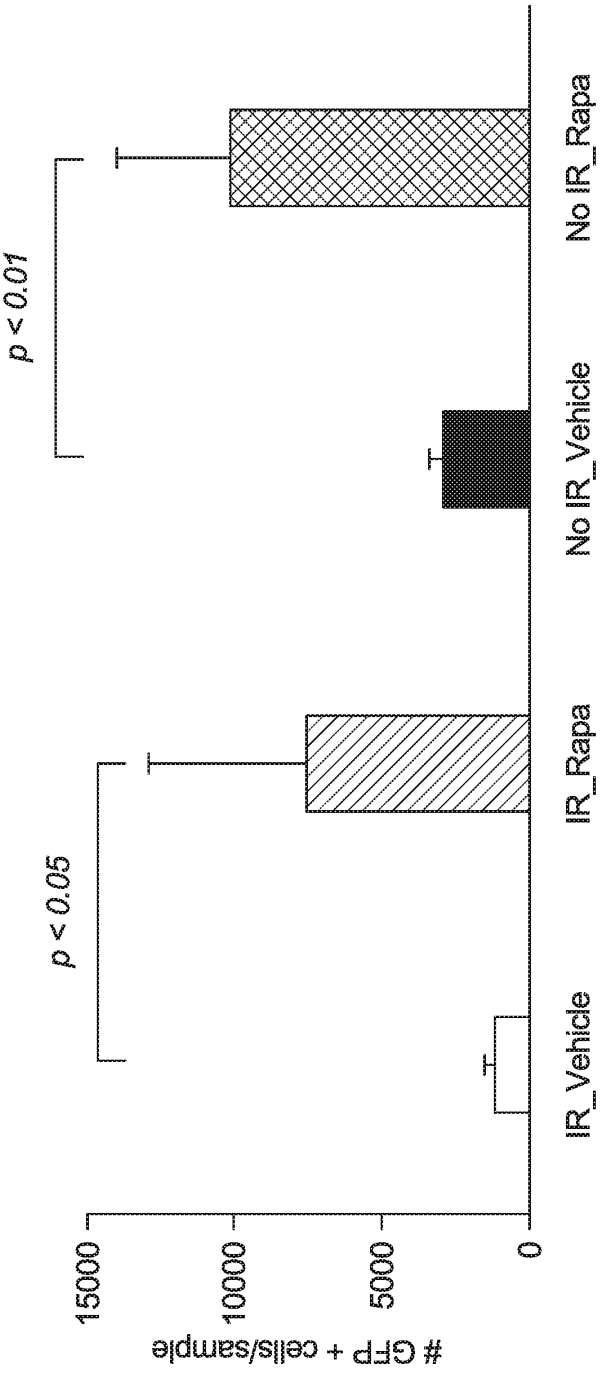

p < 0.01
p < 0.05
IR_Vehicle
IR_Rapa
No IR_Vehicle
No IR_Rapa
15000
10000
5000
0
GFP + cells/sample

FIG. 16B

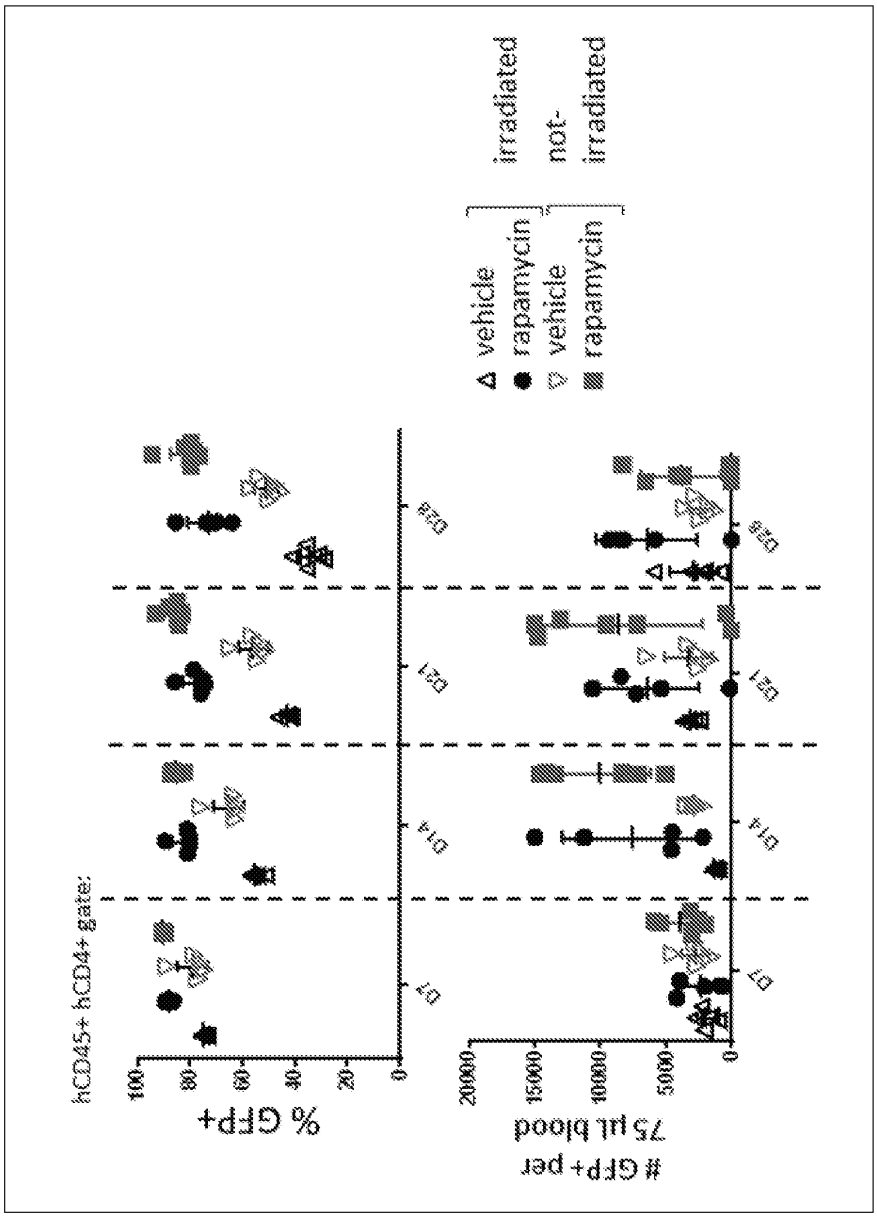

irradiated
not-irradiated
vehicle
rapamycin
vehicle
rapamycin
hCD45+ hCD4+ gate:
% GFP+
# GFP+ per 75 µL blood
D1
D14
D28
D56
D84

FIG. 17

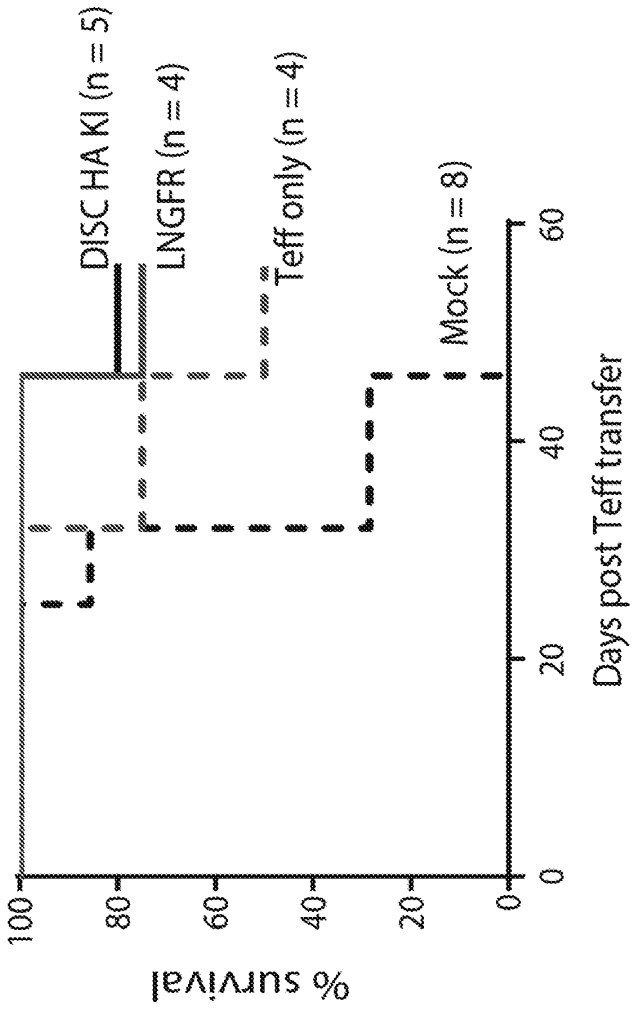

DISC HA KI (n = 5)
LNGFR (n = 4)
Teff only (n = 4)
Mock (n = 8)
% survival
100
80
60
40
20
0
0
20
40
60
Days post Teff transfer

FIG. 18