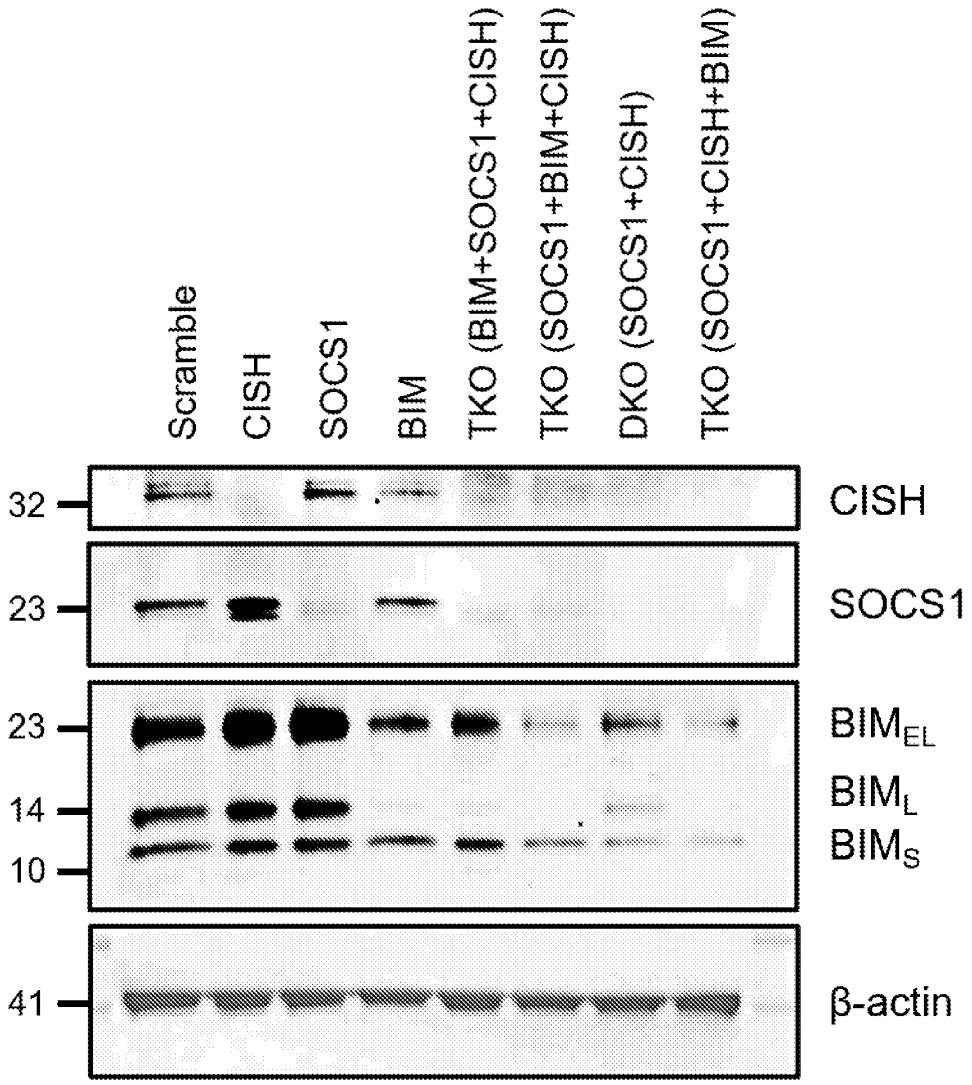

Scramble
CISH
SOCS1
BIM
TKO (BIM+SOCS1+CISH)
TKO (SOCS1+BIM+CISH)
DKO (SOCS1+CISH)
TKO (SOCS1+CISH+BIM)
32
CISH
23
SOCS1
23
14
10
BIM$_{EL}$
BIM$_{L}$
BIM$_{S}$
41
β-actin

FIG. 1

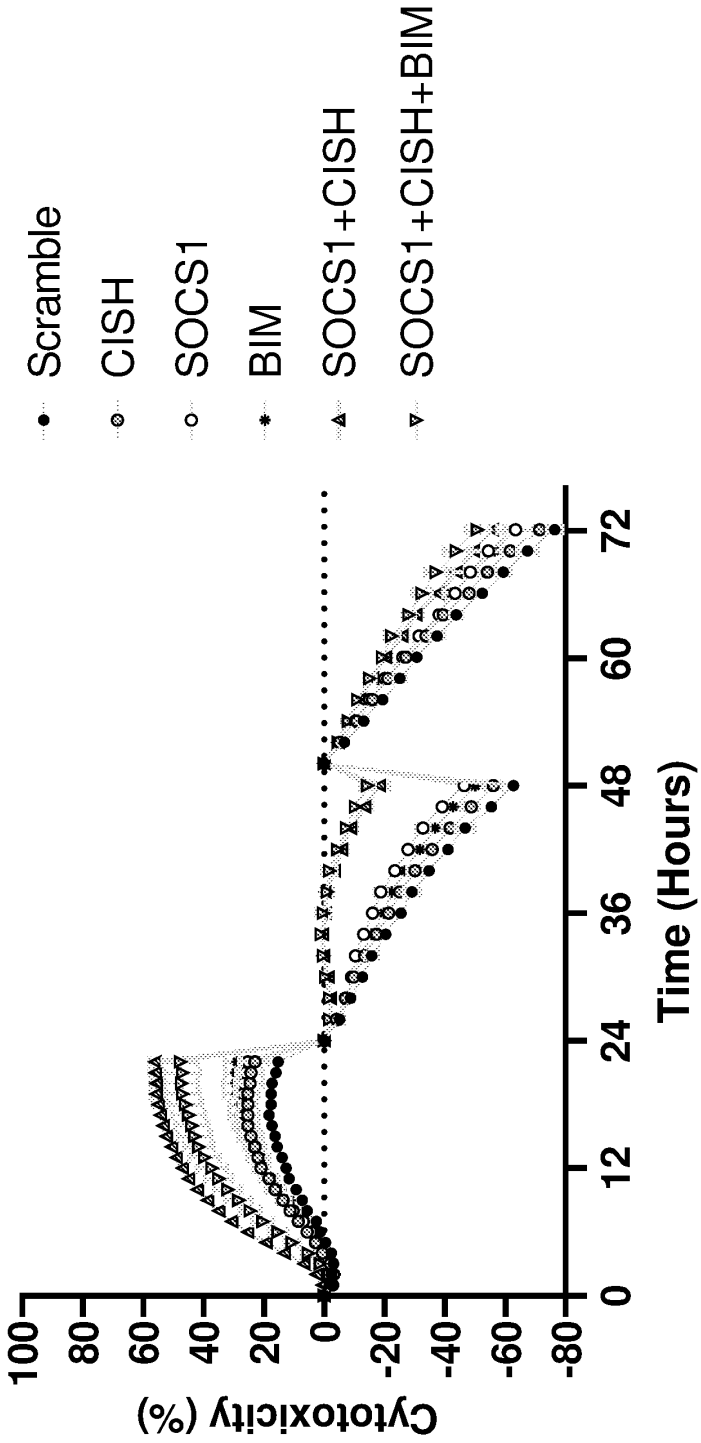

Scramble
CISH
SOCS1
BIM
SOCS1+CISH
SOCS1+CISH+BIM
Zol(-) E:T=2:1
Cytotoxicity (%)
100
80
60
40
20
0
-20
-40
-60
-80
0
12
24
36
48
60
72
Time (Hours)

FIG. 2A

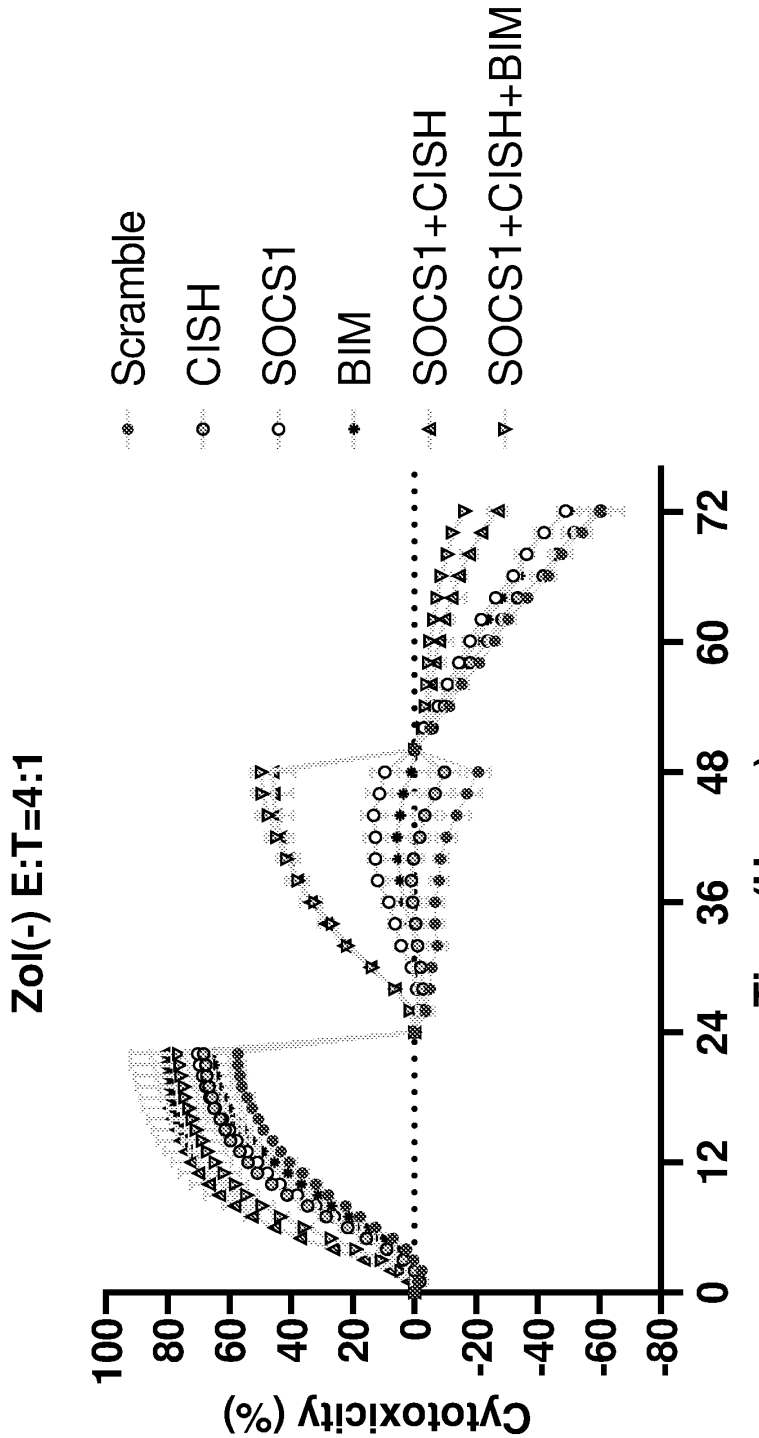

Scramble
CISH
SOCS1
BIM
SOCS1+CISH
SOCS1+CISH+BIM
Zol(-) E:T=4:1
Cytotoxicity (%)
100
80
60
40
20
0
-20
-40
-60
-80
0
12
24
36
48
60
72
Time (Hours)

FIG. 2B

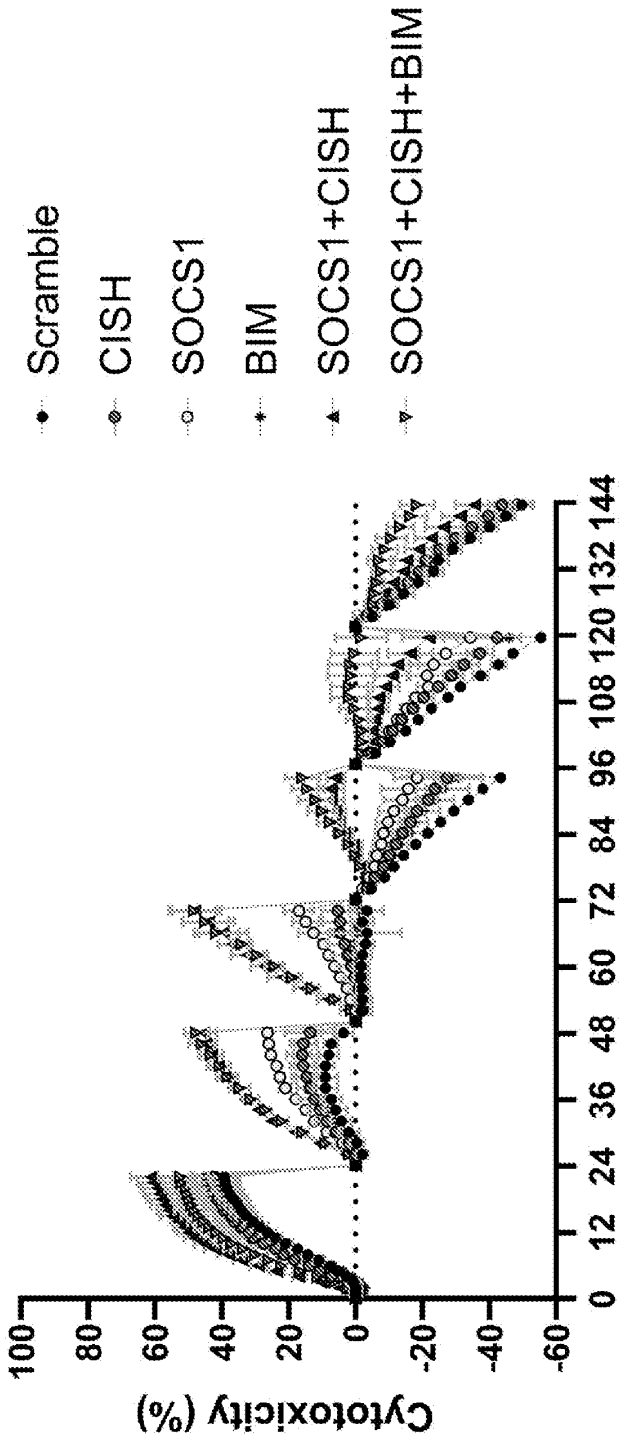

Scramble
CISH
SOCS1
BIM
SOCS1+CISH
SOCS1+CISH+BIM
Zol(+) E:T=2:1
Cytotoxicity (%)
100
80
60
40
20
0
-20
-40
-60
0
12
24
36
48
60
72
84
96
108
120
132
144
Time (Hours)

FIG. 2C

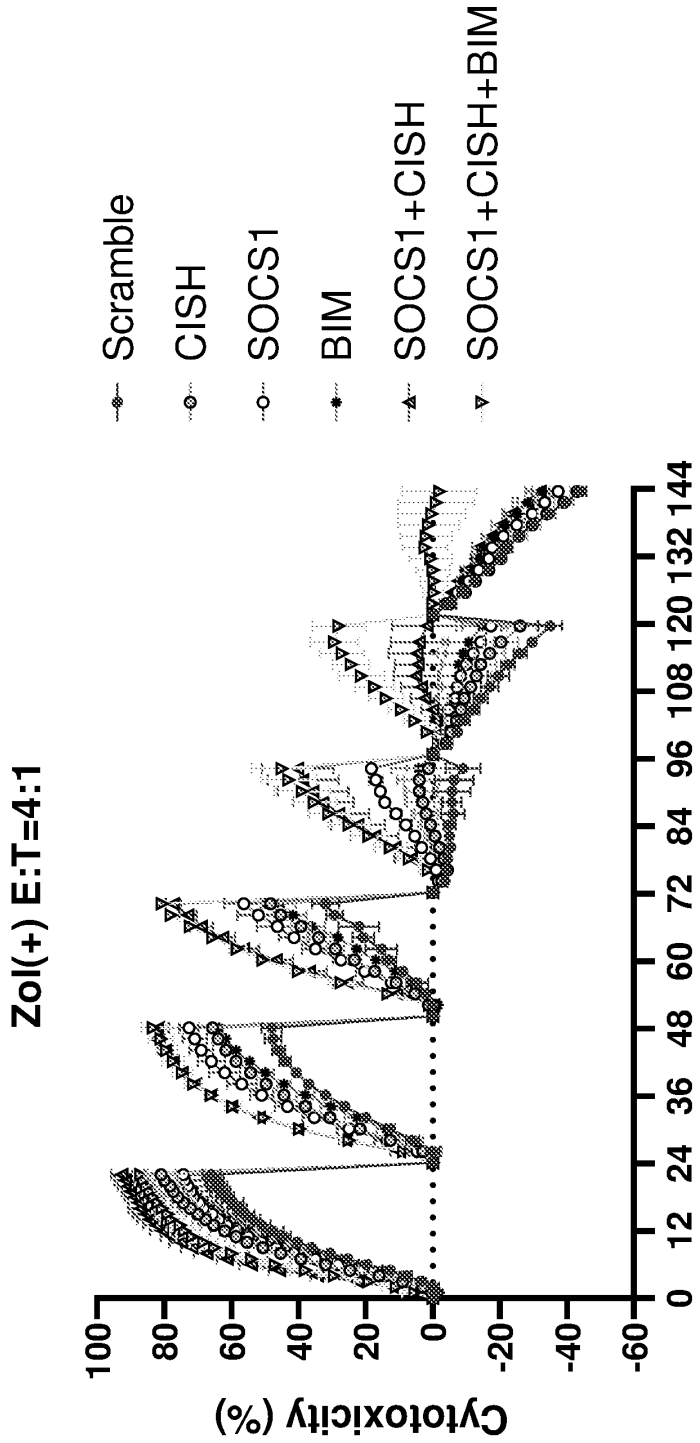

Scramble
CISH
SOCS1
BIM
SOCS1+CISH
SOCS1+CISH+BIM
Zol(+) E:T=4:1
Cytotoxicity (%)
100
80
60
40
20
0
-20
-40
-60
0
12
24
36
48
60
72
84
96
108
120
132
144
Time (Hours)

FIG. 2D

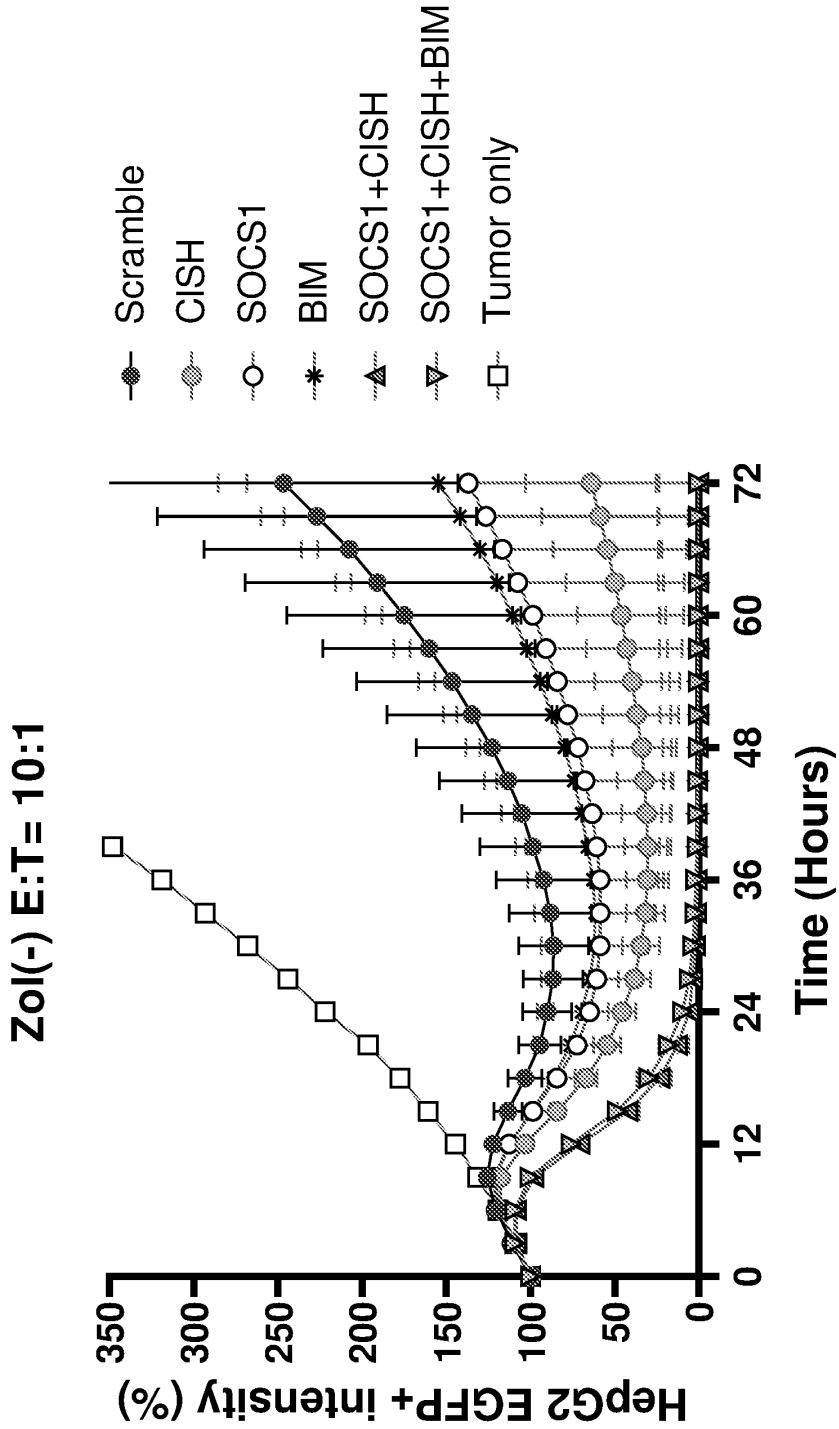

Zol(-) E:T= 10:1
HepG2 EGFP+ intensity (%)
350
300
250
200
150
100
50
0
0
12
24
36
48
60
72
Time (Hours)
Scramble
CISH
SOCS1
BIM
SOCS1+CISH
SOCS1+CISH+BIM
Tumor only

FIG. 2E

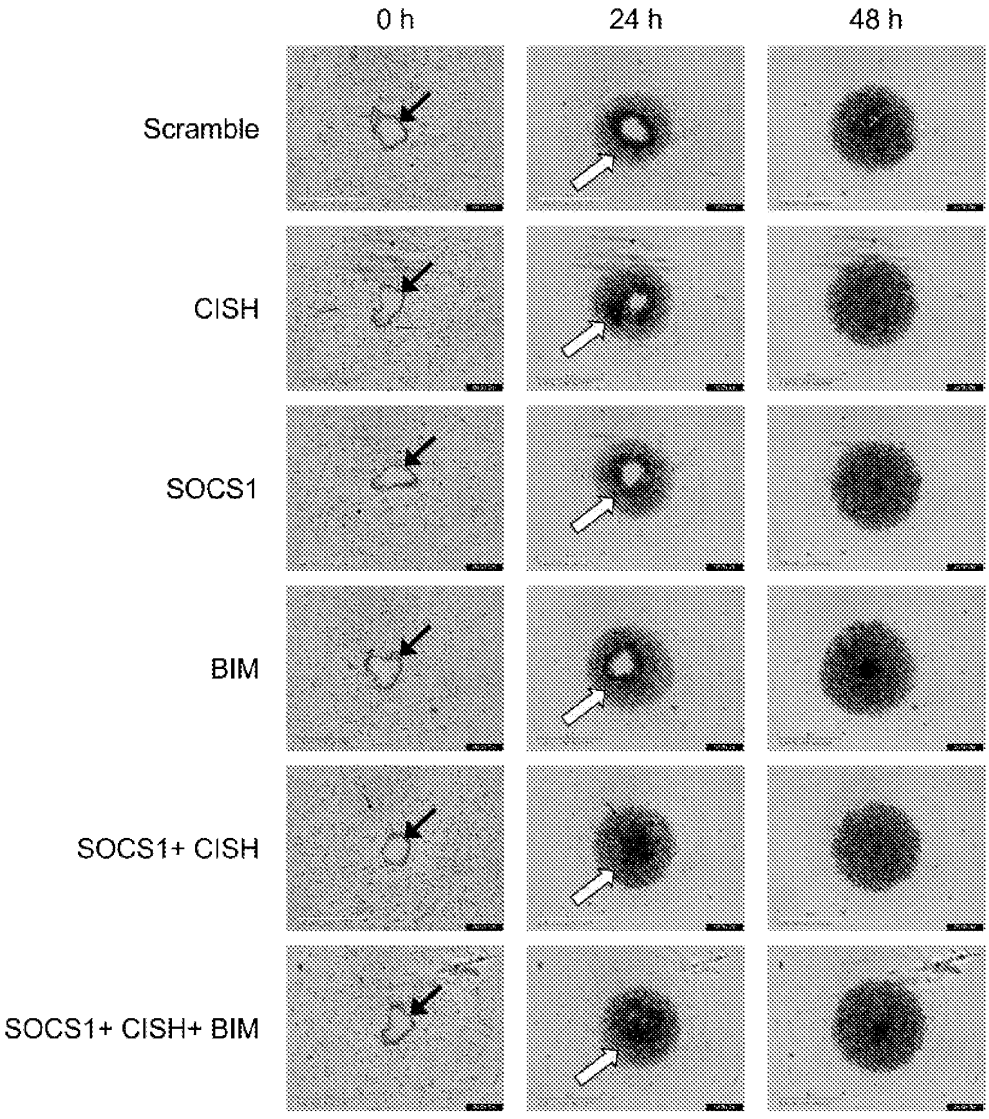


FIG. 2F

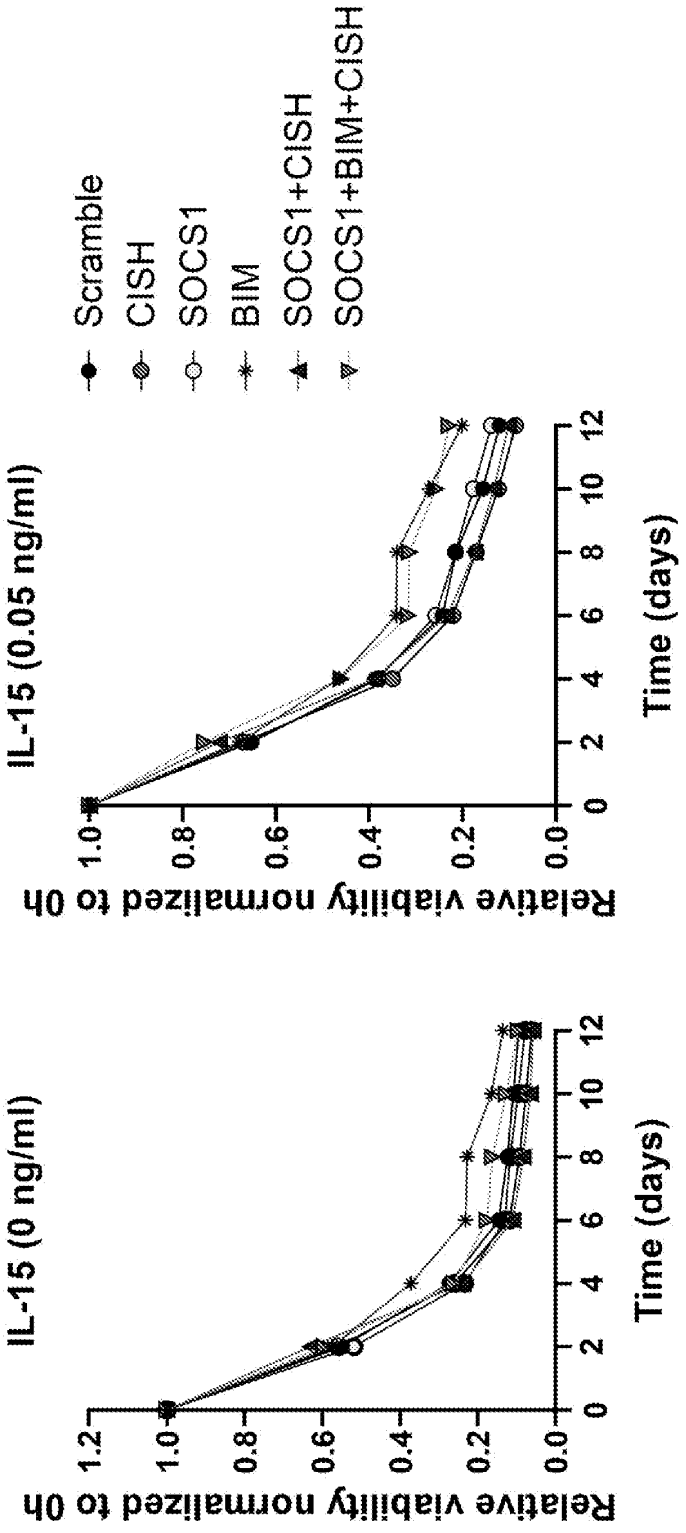

Scramble
CISH
SOCS1
BIM
SOCS1+CISH
SOCS1+BIM+CISH
IL-15 (0.05 ng/ml)
Relative viability normalized to 0h
Time (days)
IL-15 (0 ng/ml)
Relative viability normalized to 0h
Time (days)

FIG. 3

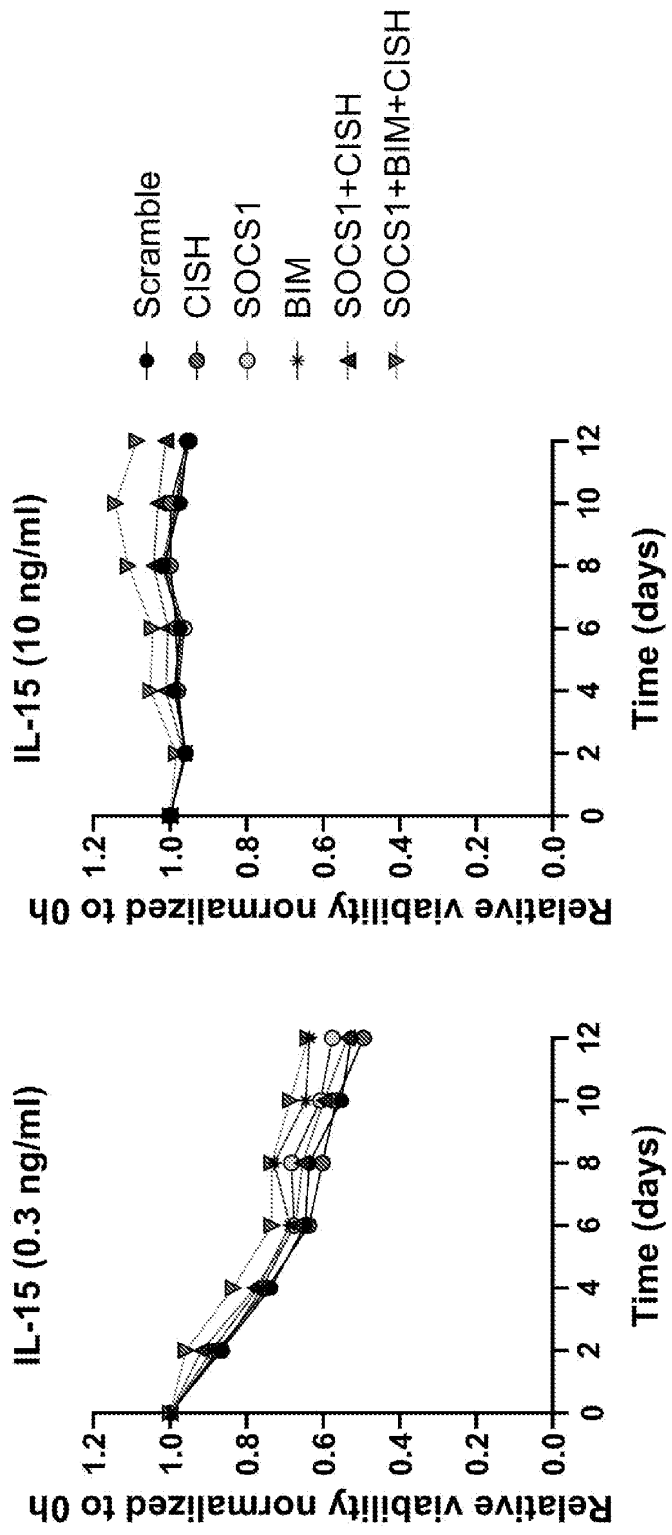

IL-15 (0.3 ng/ml)
IL-15 (10 ng/ml)
Relative viability normalized to 0h
Time (days)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
2
4
6
8
10
12
Scramble
CISH
SOCS1
BIM
SOCS1+CISH
SOCS1+BIM+CISH

FIG. 3 (cont.)

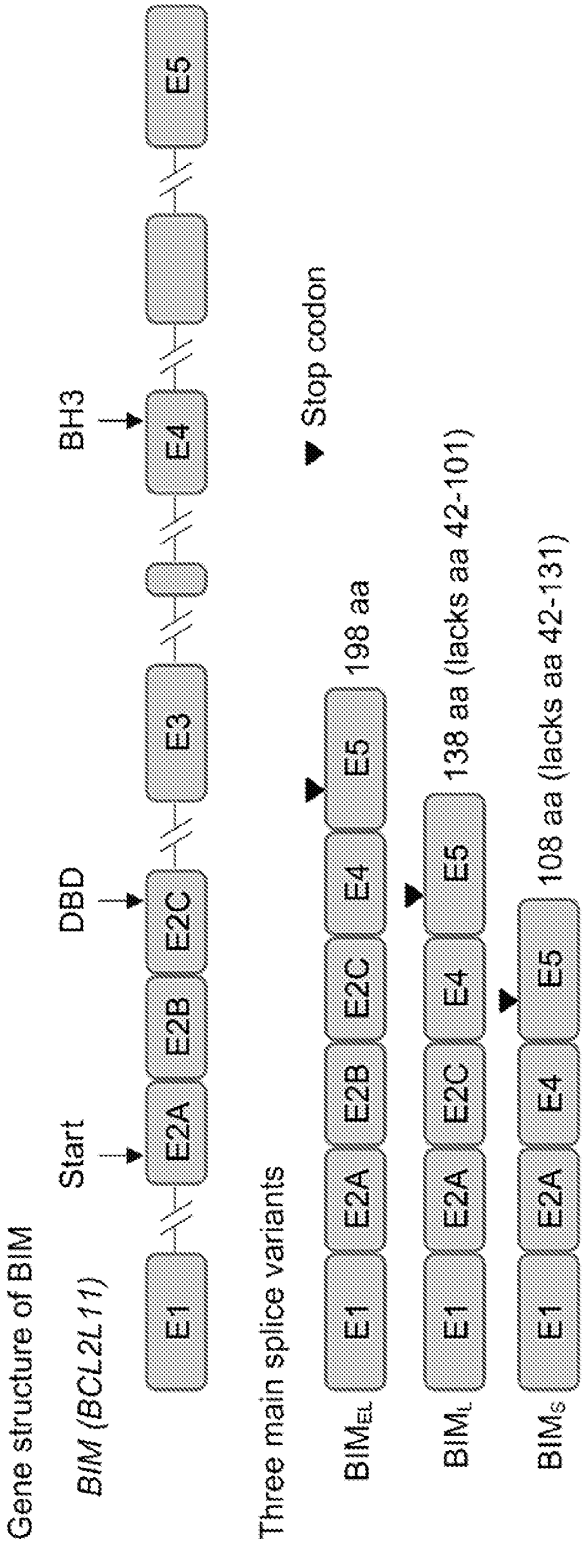

Gene structure of BIM
BIM (BCL2L11)
Start
DBD
BH3
E1
E2A
E2B
E2C
E3
E4
E5
Three main splice variants
Stop codon
BIMEL
198 aa
BIML
138 aa (lacks aa 42-101)
BIMS
108 aa (lacks aa 42-131)

FIG. 4A

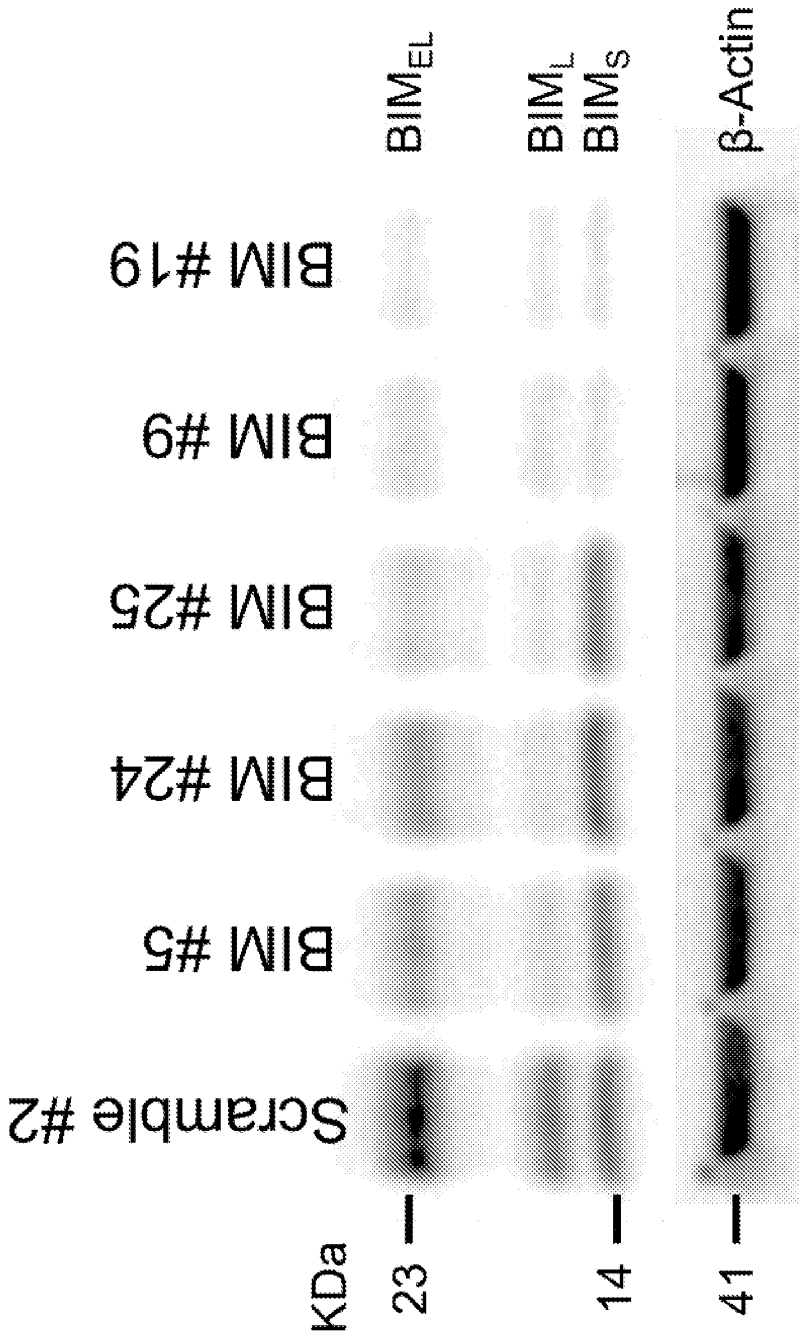

KDa
23
14
41
BIM$_{EL}$
BIM$_L$
BIM$_S$
β-Actin
Scramble #2
BIM #5
BIM #24
BIM #25
BIM #9
BIM #19

FIG. 4B

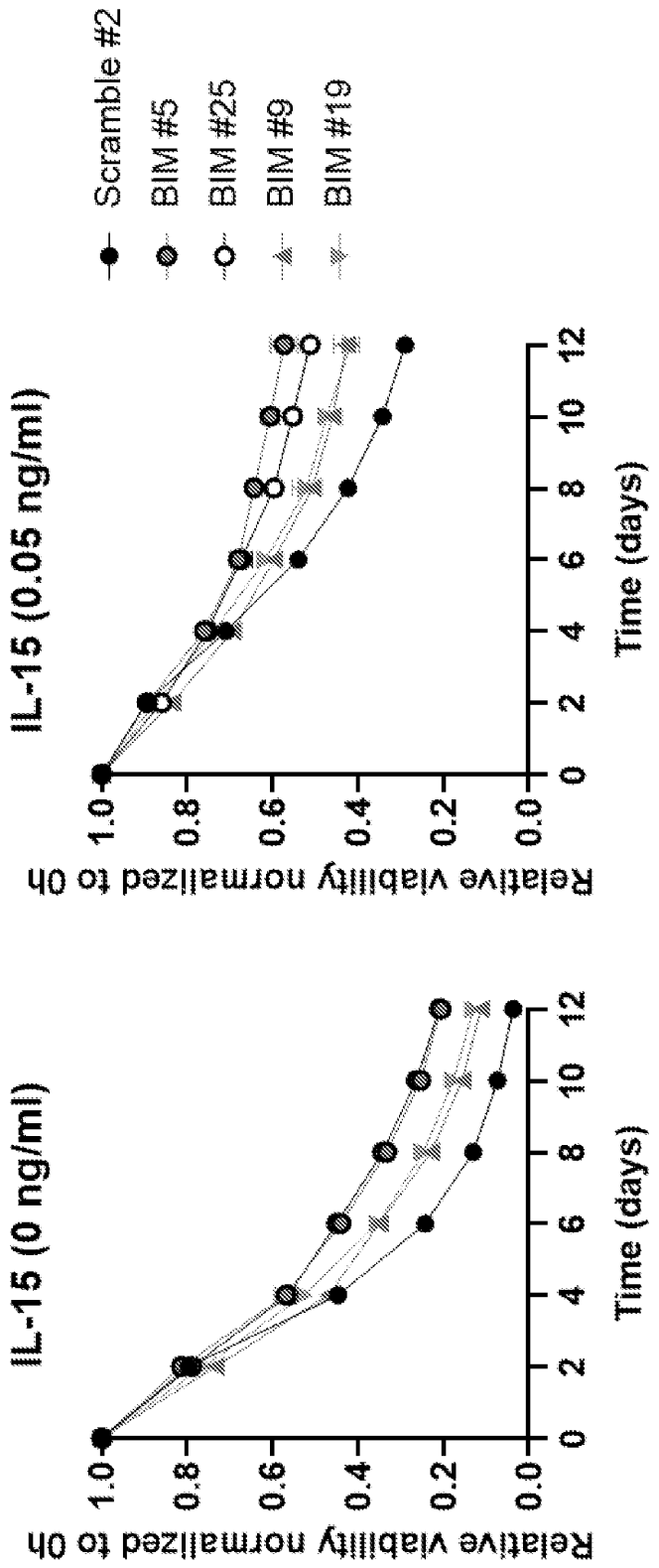

Scramble #2
BIM #5
BIM #25
BIM #9
BIM #19
IL-15 (0.05 ng/ml)
Relative viability normalized to 0h
Time (days)
IL-15 (0 ng/ml)
Relative viability normalized to 0h
Time (days)

FIG. 4C

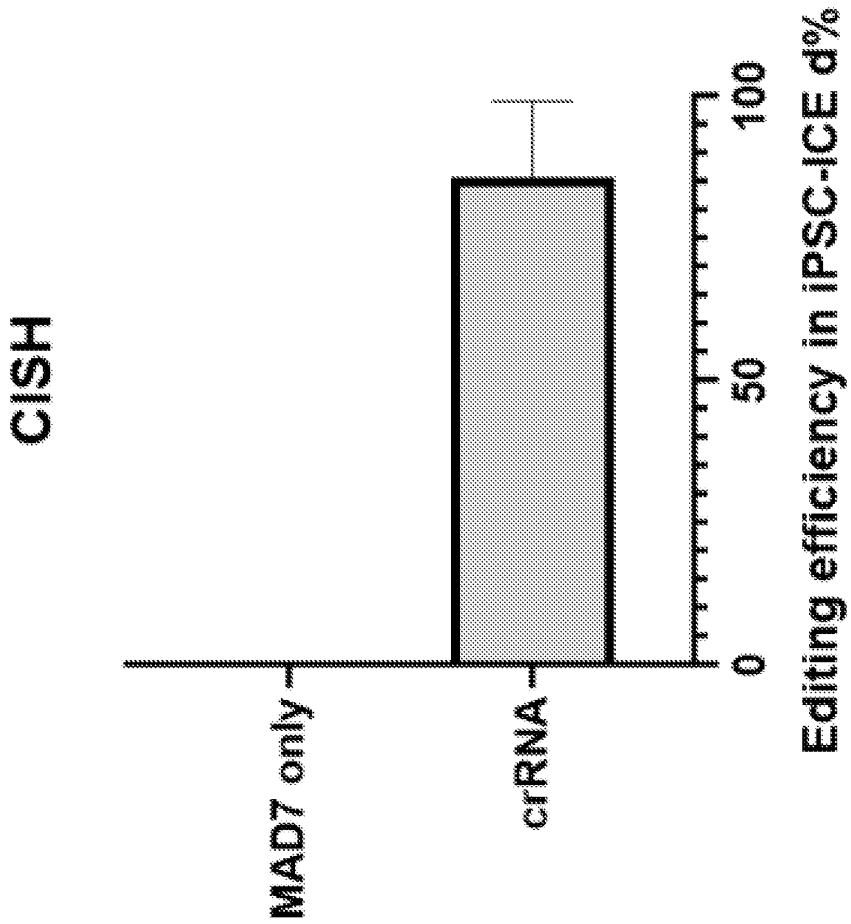

CISH
Editing efficiency in iPSC-ICE d%
0
50
100
MAD7 only
crRNA

FIG. 5A

FIG. 5B

Sequential KOs (2 consecutive electroporations for each gene)	# of clones obtained	Successfully sequenced	Indels detected (ICEd >10%)	Heterozygous KO	Homozygous KO (ICEd >90%)	Pooled sample Indel determination ICEd
CISH	198/288 (68.75%)	144	137 (95.14%)	46 (31.94%)	91 (63.19%)	85%

FIG. 5C

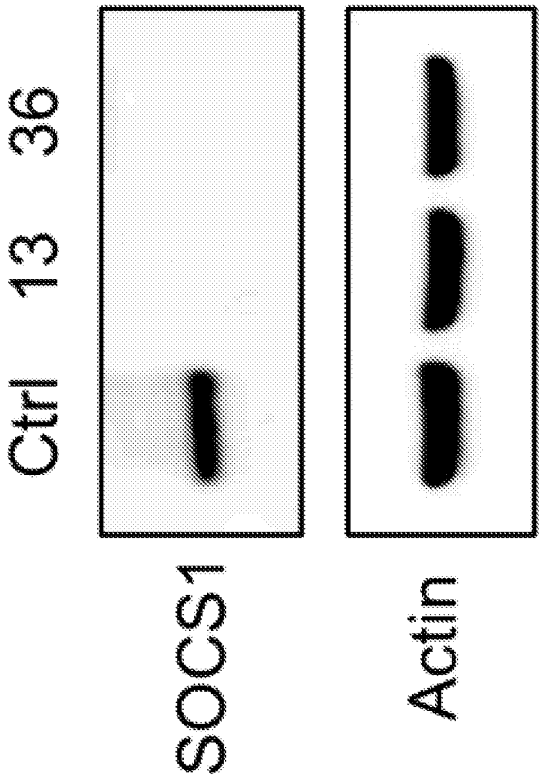

Ctrl
13
36
SOCS1
Actin

FIG. 6B

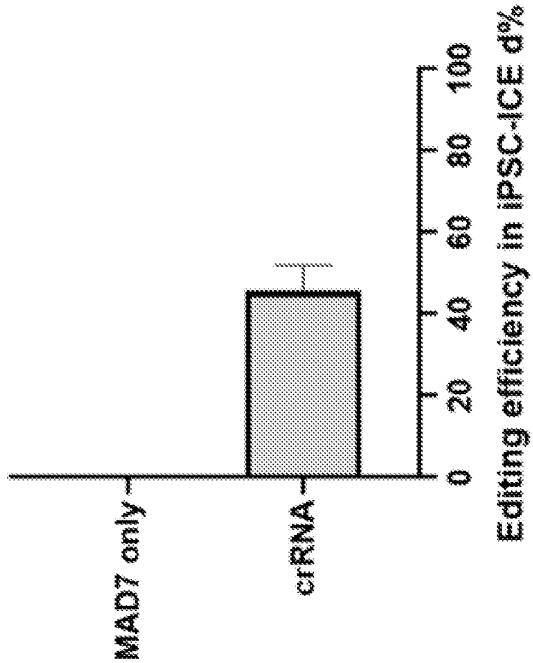

MAD7 only
crRNA
0
20
40
60
80
100
Editing efficiency in iPSC-ICE d%

FIG. 6A

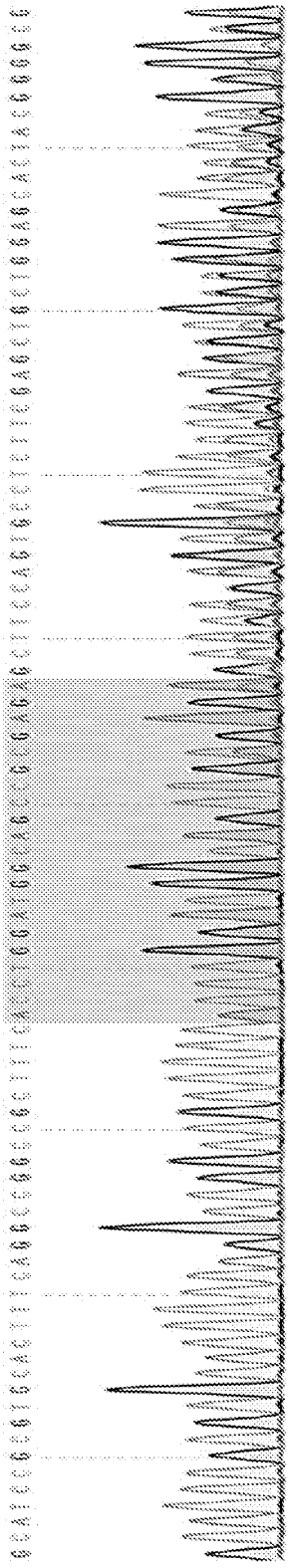


FIG. 6C

Sequential KOs (2 consecutive electroporations for each gene)	# of clones obtained	Successfully sequenced	Indels detected (ICEd >10%)	Heterozygous KO	Homozygous KO (ICEd >90%)	Pooled sample Indel determination ICEd
SOCS1	129/480 (26.88%)	119	78 (65.55%)	65 (54.62%)	13 (10.92%)	45%

FIG. 6D

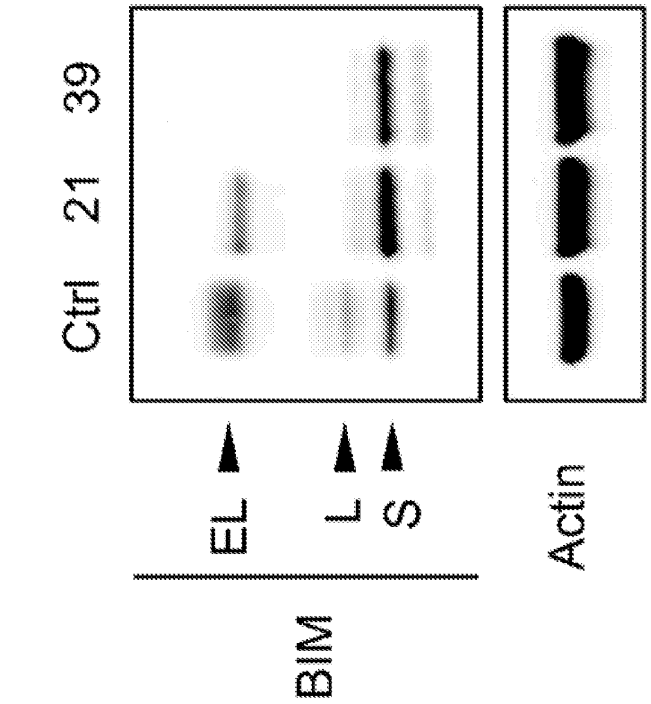

Ctrl
21
39
EL
L
S
BIM
Actin

FIG. 7B

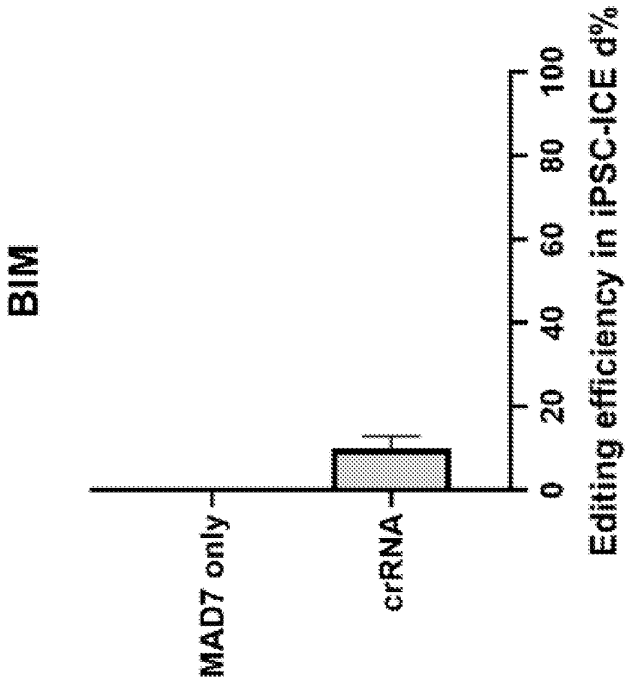

BIM
MAD7 only
crRNA
0
20
40
60
80
100
Editing efficiency in iPSC-ICE d%

FIG. 7A

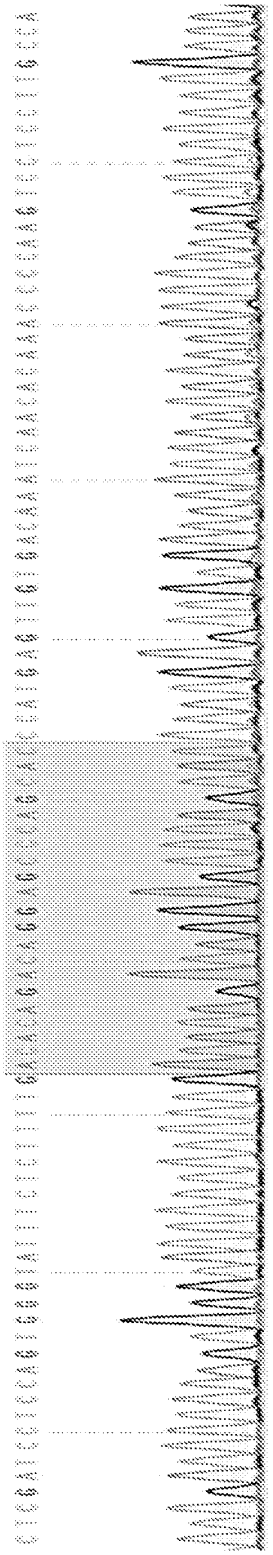


FIG. 7C

Sequential KOs (2 consecutive electroporations for each gene)	# of clones obtained	Successfully sequenced	Indels detected (ICEd >10%)	Heterozygous KO	Homozygous KO (ICEd >90%)	Pooled sample Indel determination ICEd
BIM	168/480 (35%)	159	62 (38.99%)	57 (35.85%)	5 (3.14%)	10%

FIG. 7D

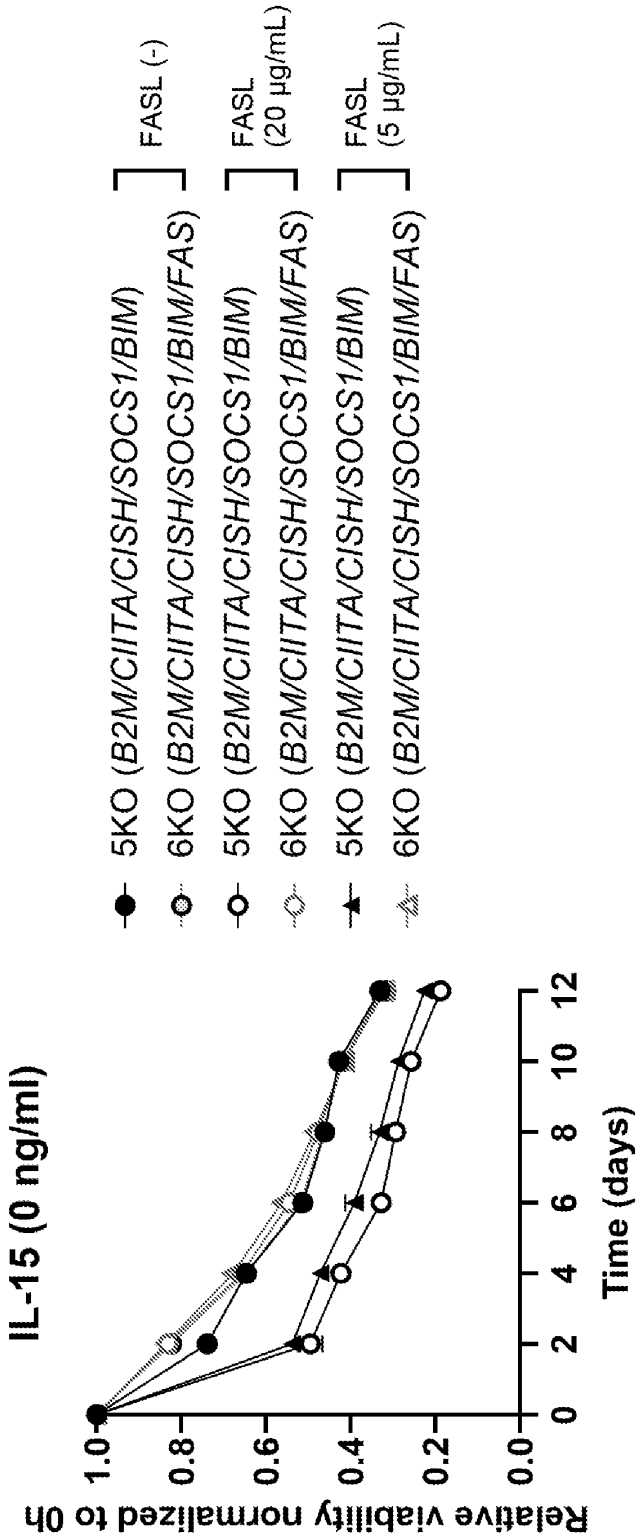

IL-15 (0 ng/ml)
Relative viability normalized to 0h
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
Time (days)
5KO (B2M/CIITA/CISH/SOCS1/BIM)
6KO (B2M/CIITA/CISH/SOCS1/BIM/FAS)
5KO (B2M/CIITA/CISH/SOCS1/BIM)
6KO (B2M/CIITA/CISH/SOCS1/BIM/FAS)
5KO (B2M/CIITA/CISH/SOCS1/BIM)
6KO (B2M/CIITA/CISH/SOCS1/BIM/FAS)
FASL (-)
FASL (20 µg/mL)
FASL (5 µg/mL)

FIG. 8

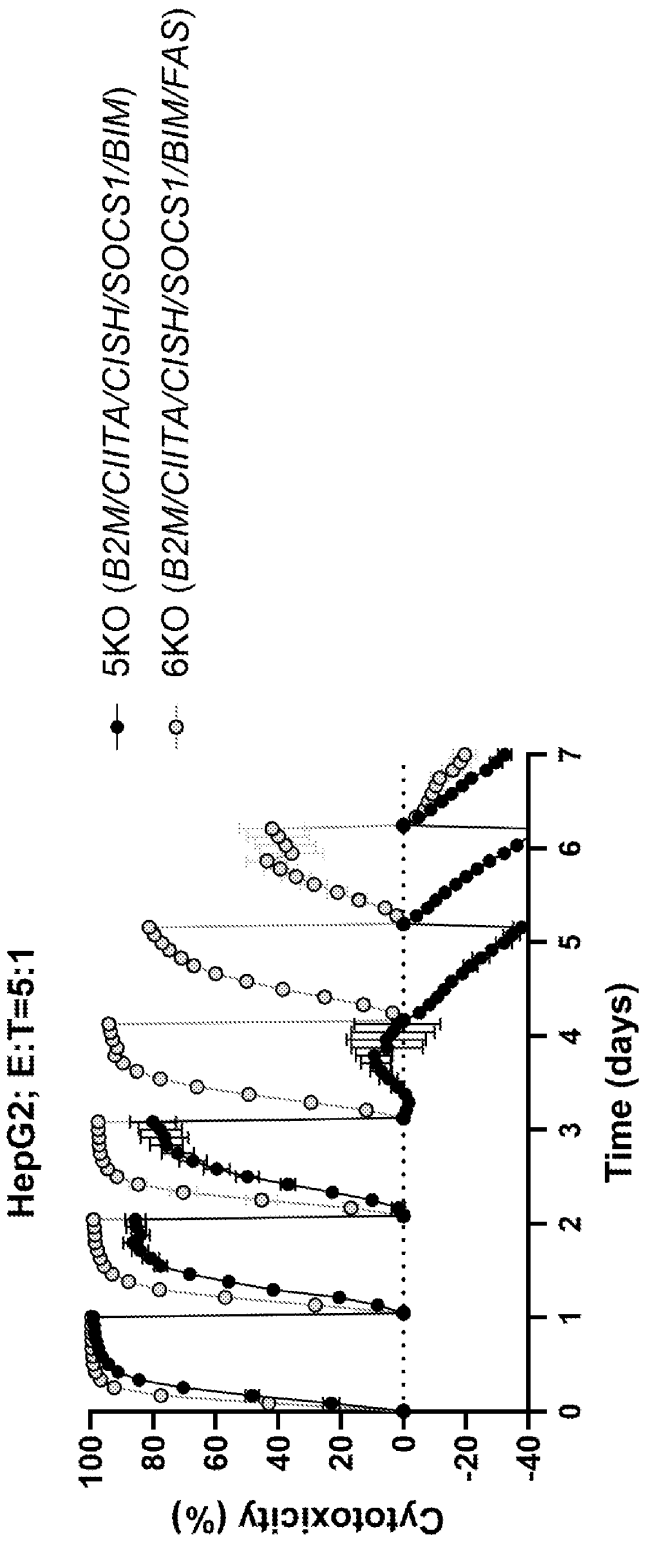

HepG2; E:T=5:1
5KO (B2M/CIITA/CISH/SOCS1/BIM)
6KO (B2M/CIITA/CISH/SOCS1/BIM/FAS)
Cytotoxicity (%)
100
80
60
40
20
0
-20
-40
0
1
2
3
4
5
6
7
Time (days)

FIG. 9A

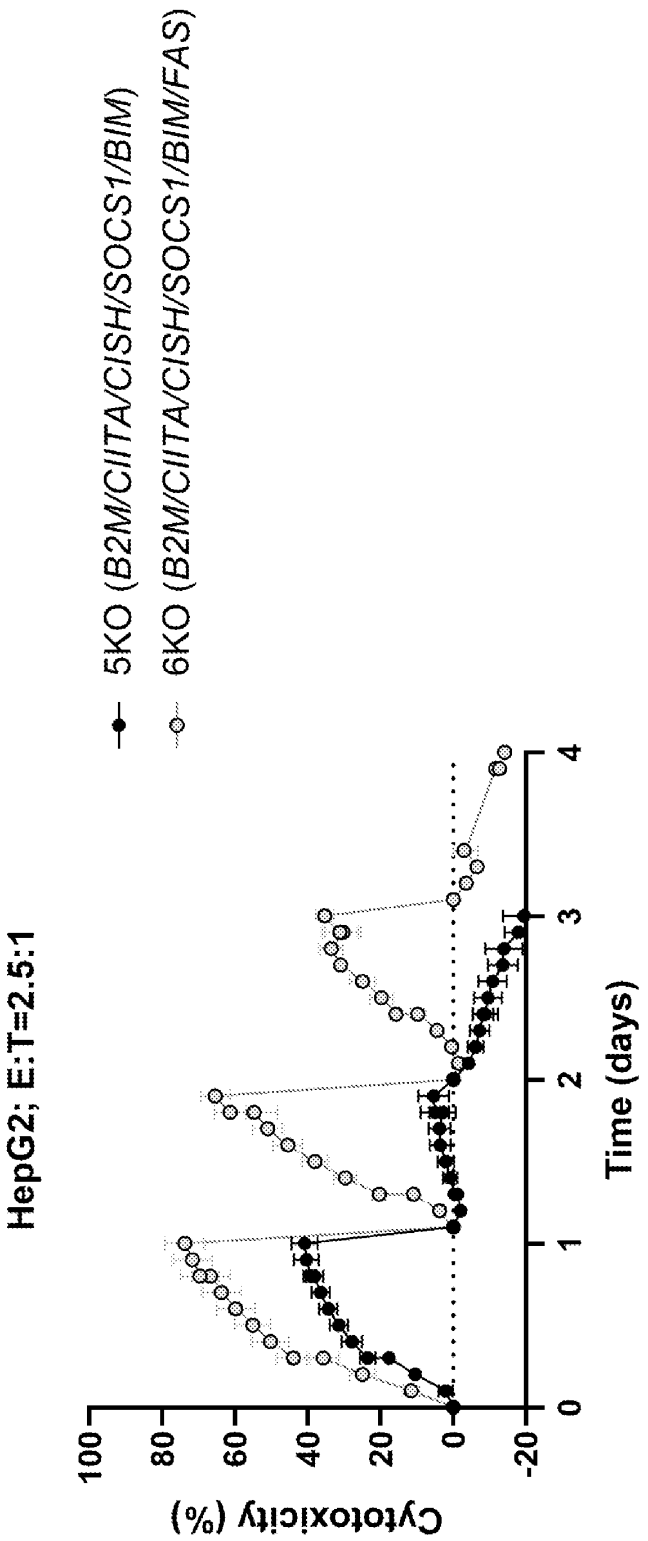

HepG2; E:T=2.5:1
5KO (B2M/CIITA/CISH/SOCS1/BIM)
6KO (B2M/CIITA/CISH/SOCS1/BIM/FAS)
Cytotoxicity (%)
100
80
60
40
20
0
-20
0
1
2
3
4
Time (days)

FIG. 9B

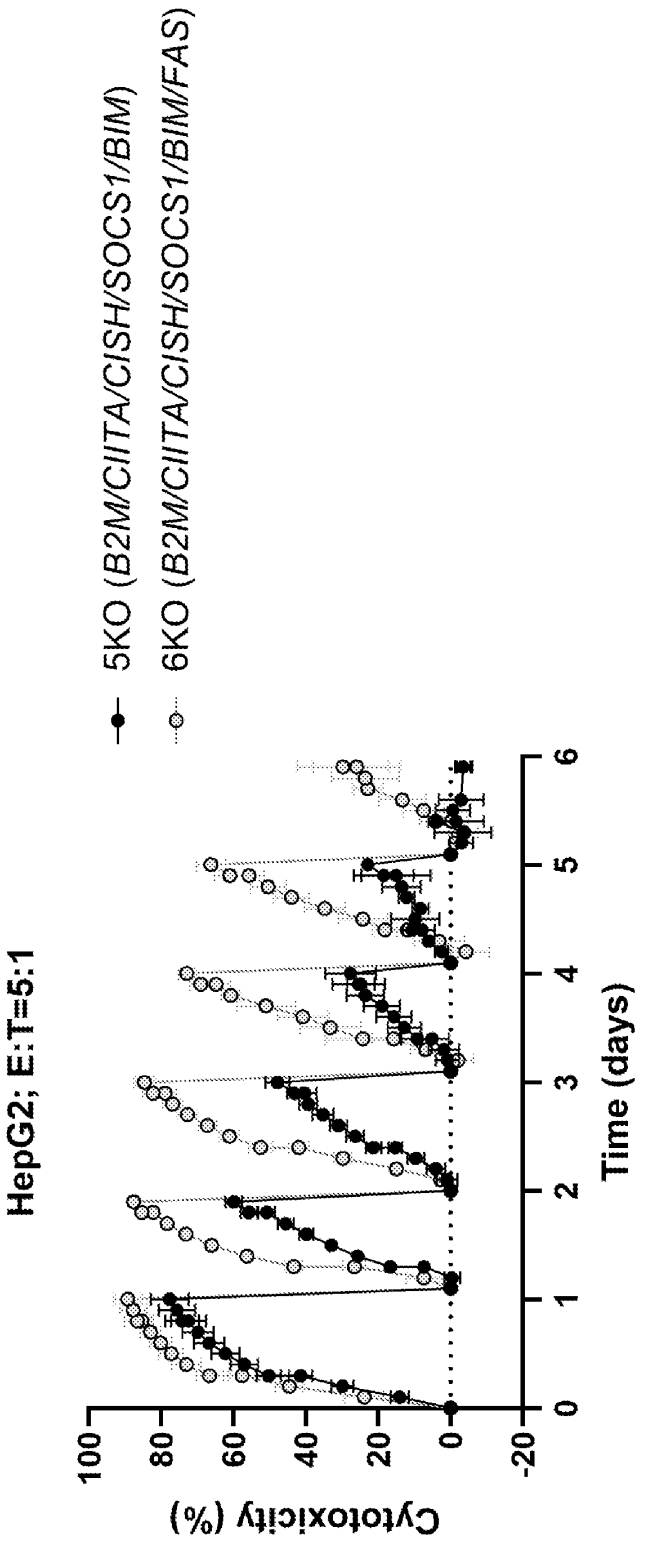

5KO (B2M/CIITA/CISH/SOCS1/BIM)
6KO (B2M/CIITA/CISH/SOCS1/BIM/FAS)
HepG2; E:T=5:1
Cytotoxicity (%)
100
80
60
40
20
0
-20
0
1
2
3
4
5
6
Time (days)

FIG. 9C

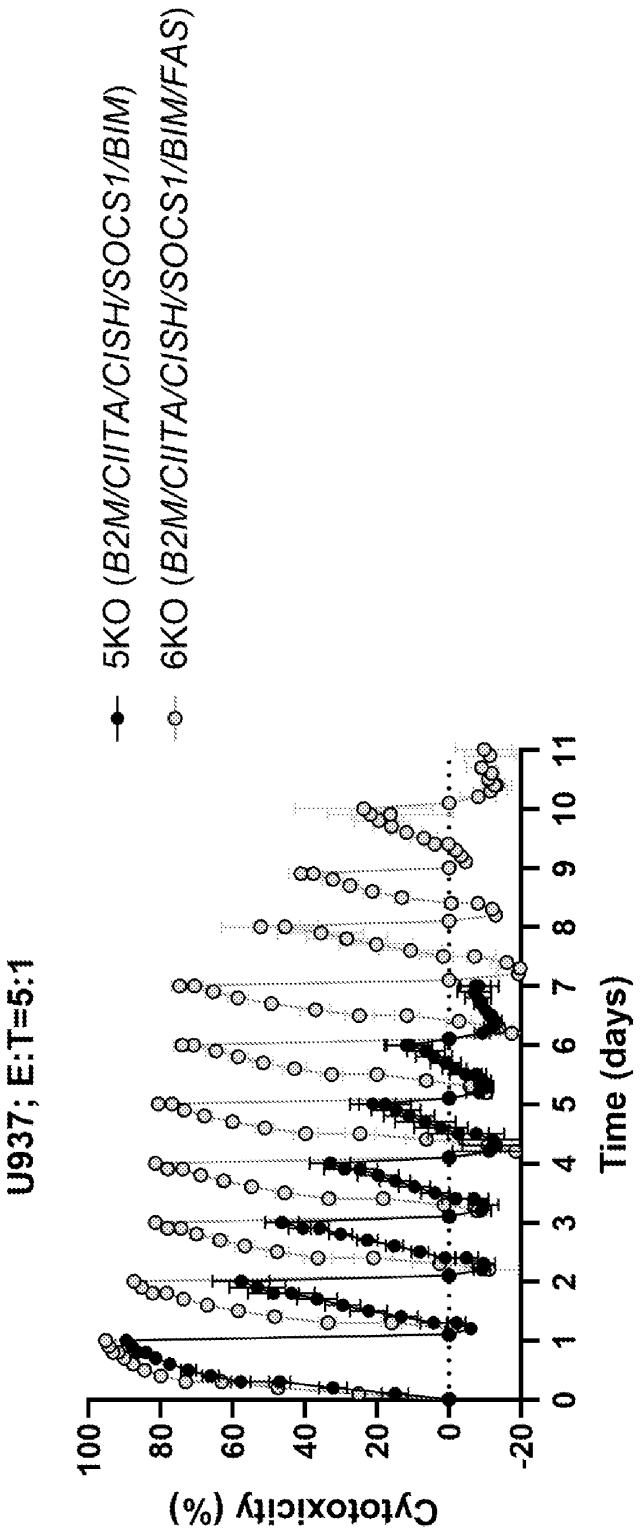

5KO (B2M/CIITA/CISH/SOCS1/BIM)
6KO (B2M/CIITA/CISH/SOCS1/BIM/FAS)
U937; E:T=5:1
Cytotoxicity (%)
100
80
60
40
20
0
-20
0
1
2
3
4
5
6
7
8
9
10
11
Time (days)

FIG. 9D

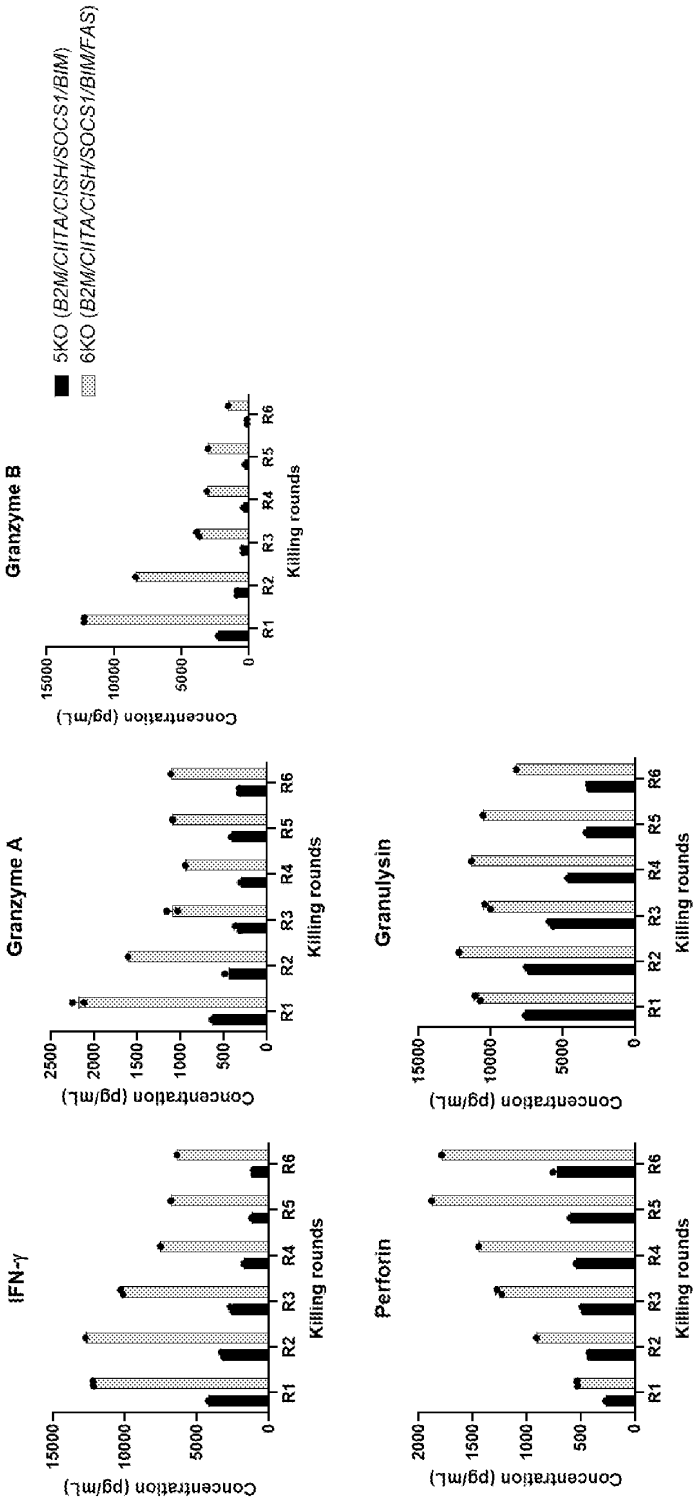

5KO (B2M/CIITA/CISH/SOCS1/BIM)
6KO (B2M/CIITA/CISH/SOCS1/BIM/FAS)
Granzyme B
Granzyme A
Granulysin
IFN-γ
Perforin
Concentration (pg/mL)
Killing rounds
R1
R2
R3
R4
R5
R6

FIG. 10

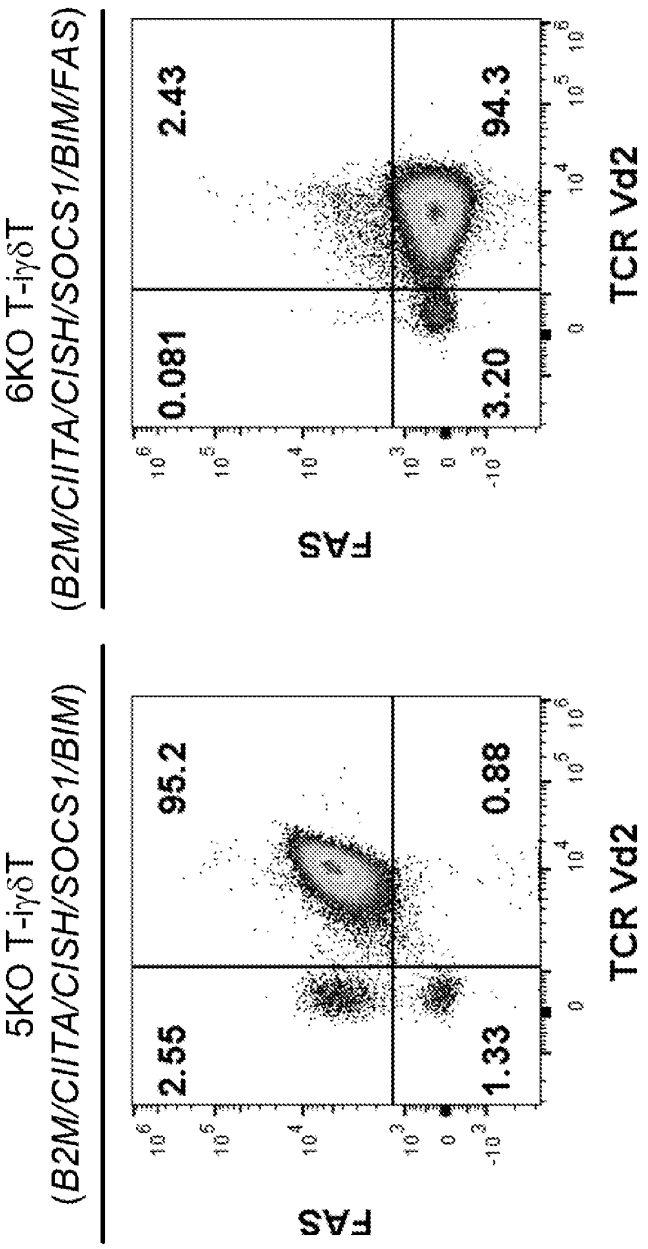

5KO T-iγδT
(B2M/CIITA/CISH/SOCS1/BIM)
2.55
95.2
1.33
0.88
FAS
TCR Vd2
6KO T-iγδT
(B2M/CIITA/CISH/SOCS1/BIM/FAS)
0.081
2.43
3.20
94.3
FAS
TCR Vd2

FIG. 11A

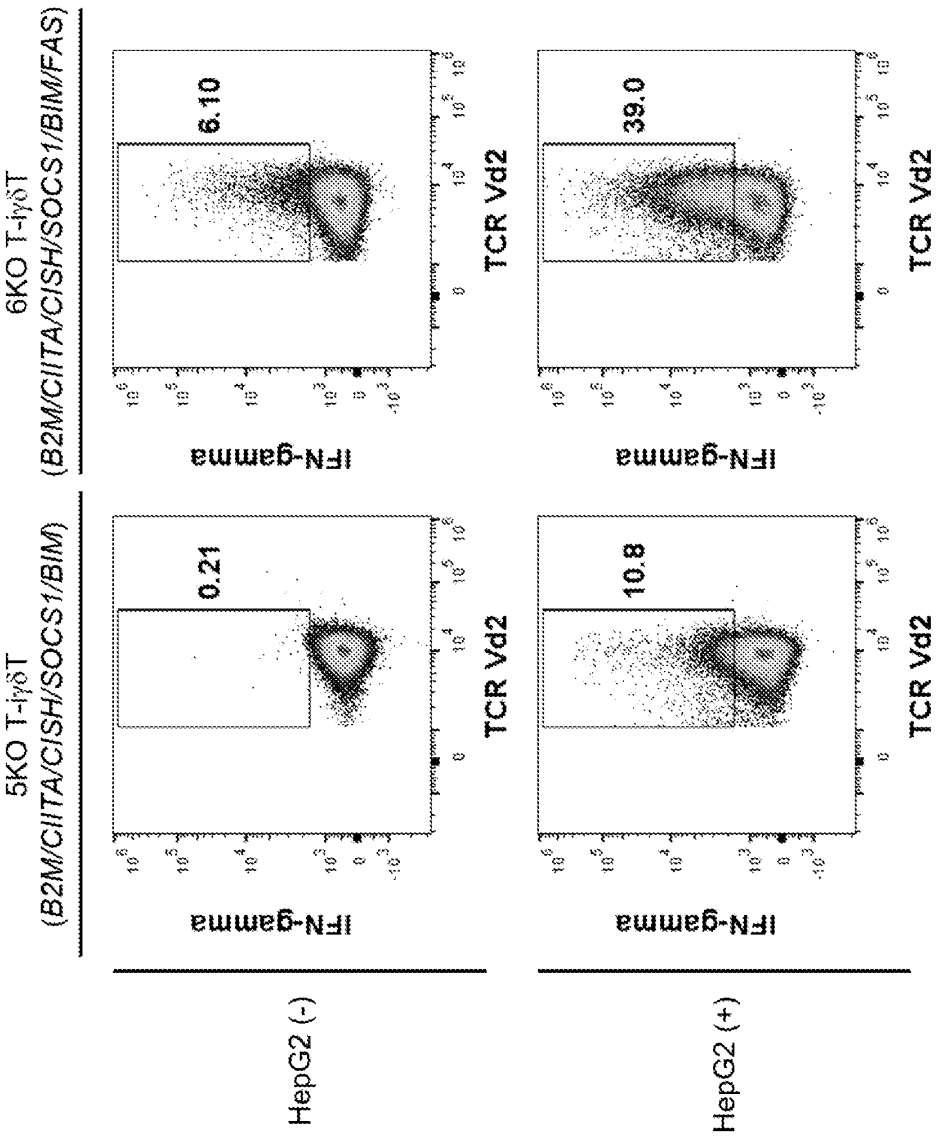

5KO T-iγδT
(B2M/CIITA/CISH/SOCS1/BIM)
6KO T-iγδT
(B2M/CIITA/CISH/SOCS1/BIM/FAS)
HepG2 (-)
HepG2 (+)
IFN-gamma
TCR Vd2
0.21
6.10
10.8
39.0

FIG. 11B

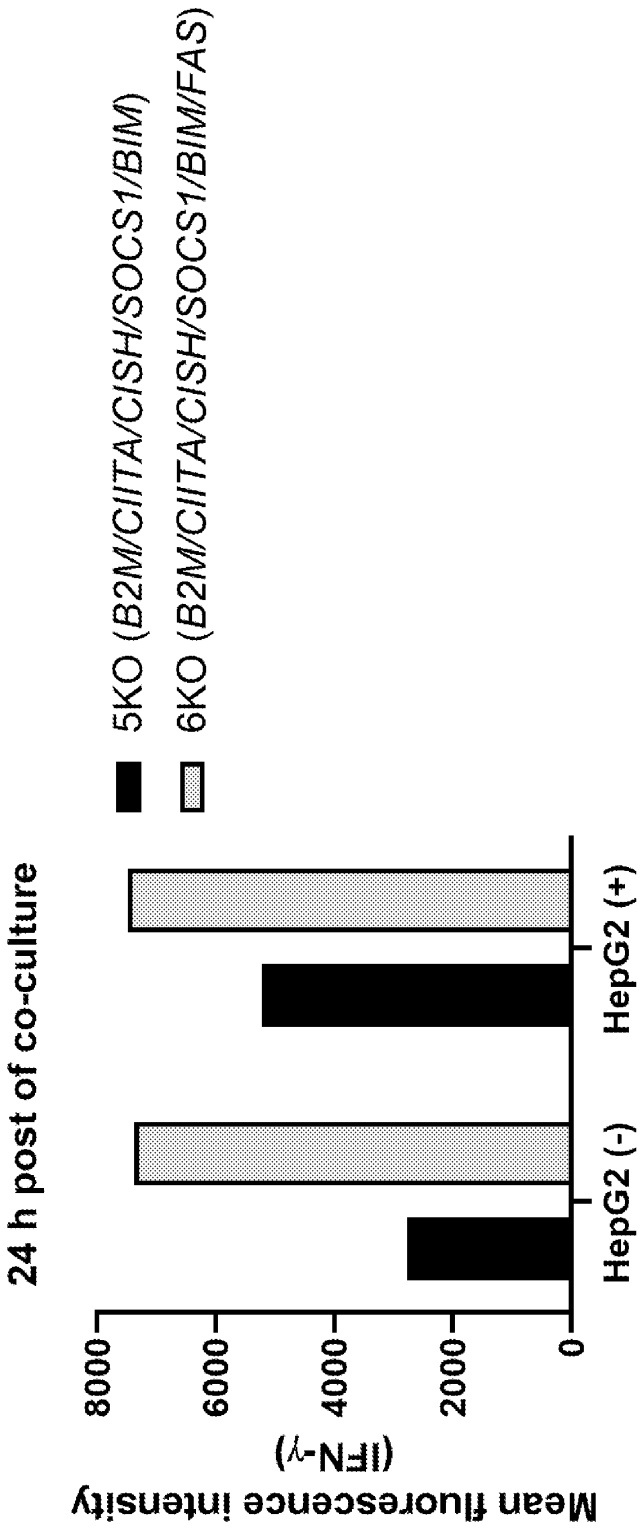

24 h post of co-culture
5KO (B2M/CIITA/CISH/SOCS1/BIM)
6KO (B2M/CIITA/CISH/SOCS1/BIM/FAS)
Mean fluorescence intensity
(IFN-γ)
8000
6000
4000
2000
0
HepG2 (-)
HepG2 (+)

FIG. 11C

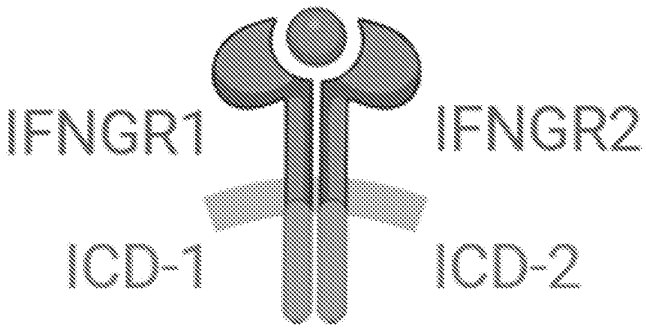


FIG. 12

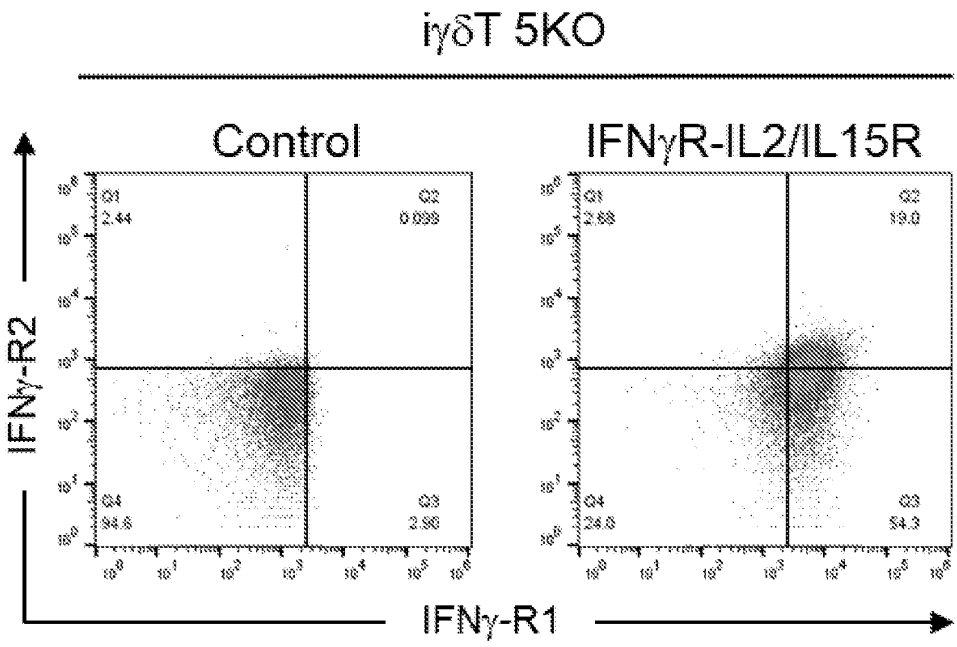

iγδT 5KO
Control
IFNγR-IL2/IL15R
IFNγ-R2
IFNγ-R1
Q1
2.44
Q2
0.029
Q4
94.6
Q3
2.90
Q1
2.68
Q2
19.0
Q4
24.0
Q3
54.3

FIG. 13A

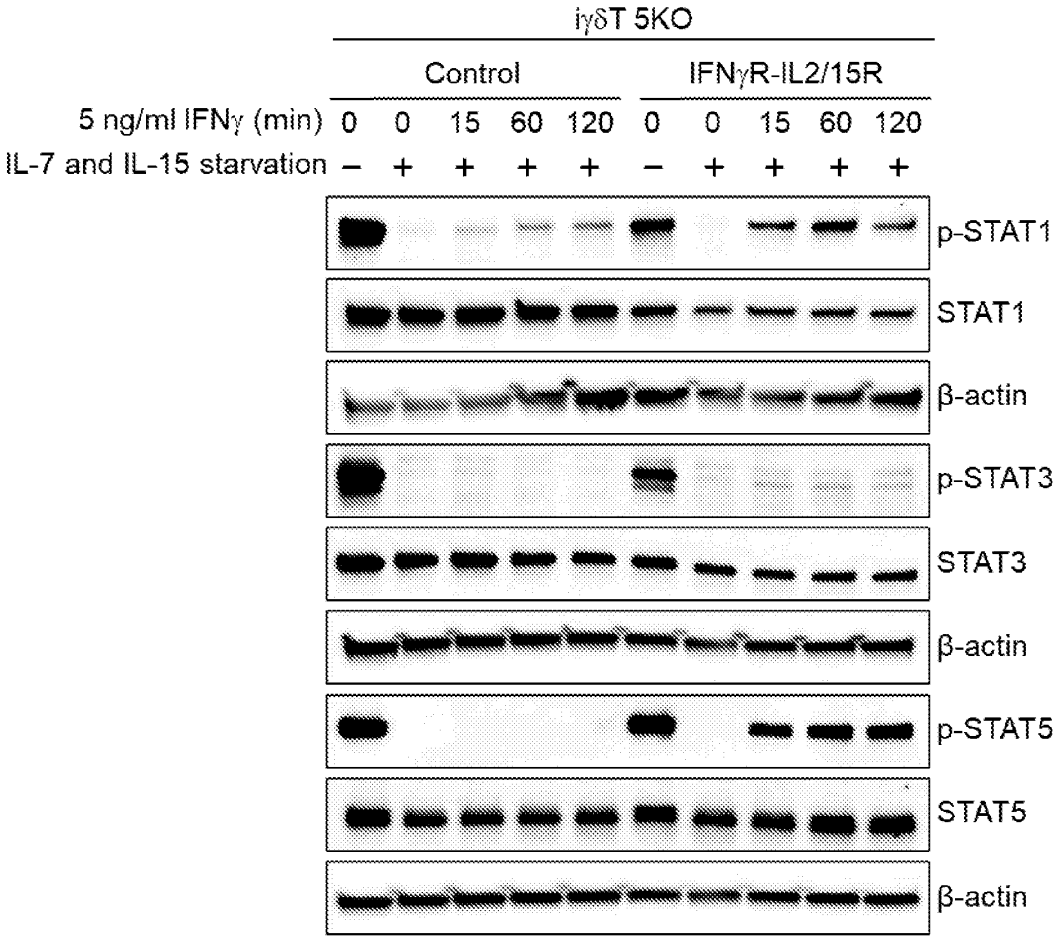

iγδT 5KO
Control
IFNγR-IL2/15R
5 ng/ml IFNγ (min) 0 0 15 60 120 0 0 15 60 120
IL-7 and IL-15 starvation − + + + + − + + + +
p-STAT1
STAT1
β-actin
p-STAT3
STAT3
β-actin
p-STAT5
STAT5
β-actin

FIG. 13B

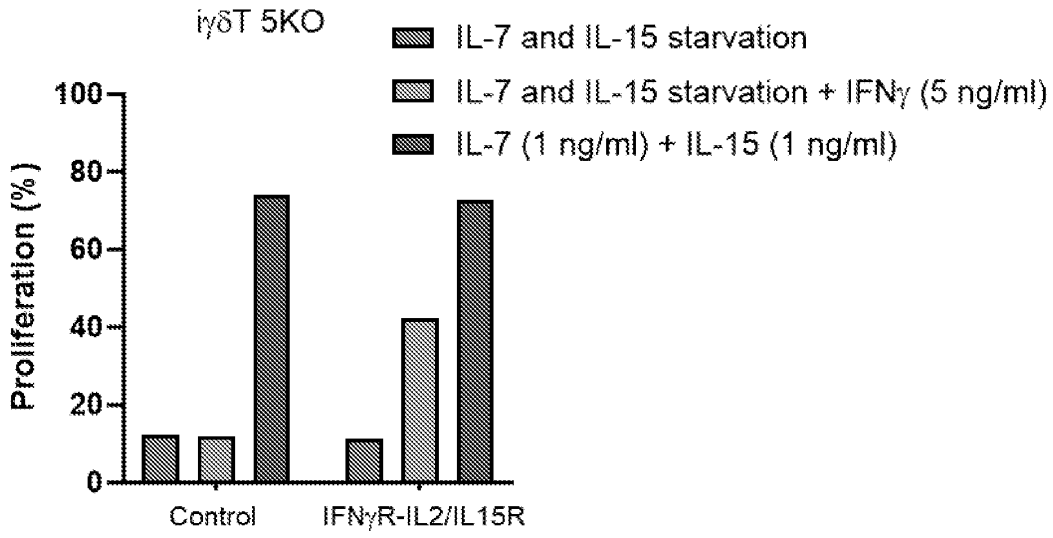

iγδT 5KO
IL-7 and IL-15 starvation
IL-7 and IL-15 starvation + IFNγ (5 ng/ml)
IL-7 (1 ng/ml) + IL-15 (1 ng/ml)
Proliferation (%)
100
80
60
40
20
0
Control
IFNγR-IL2/IL15R

FIG. 14

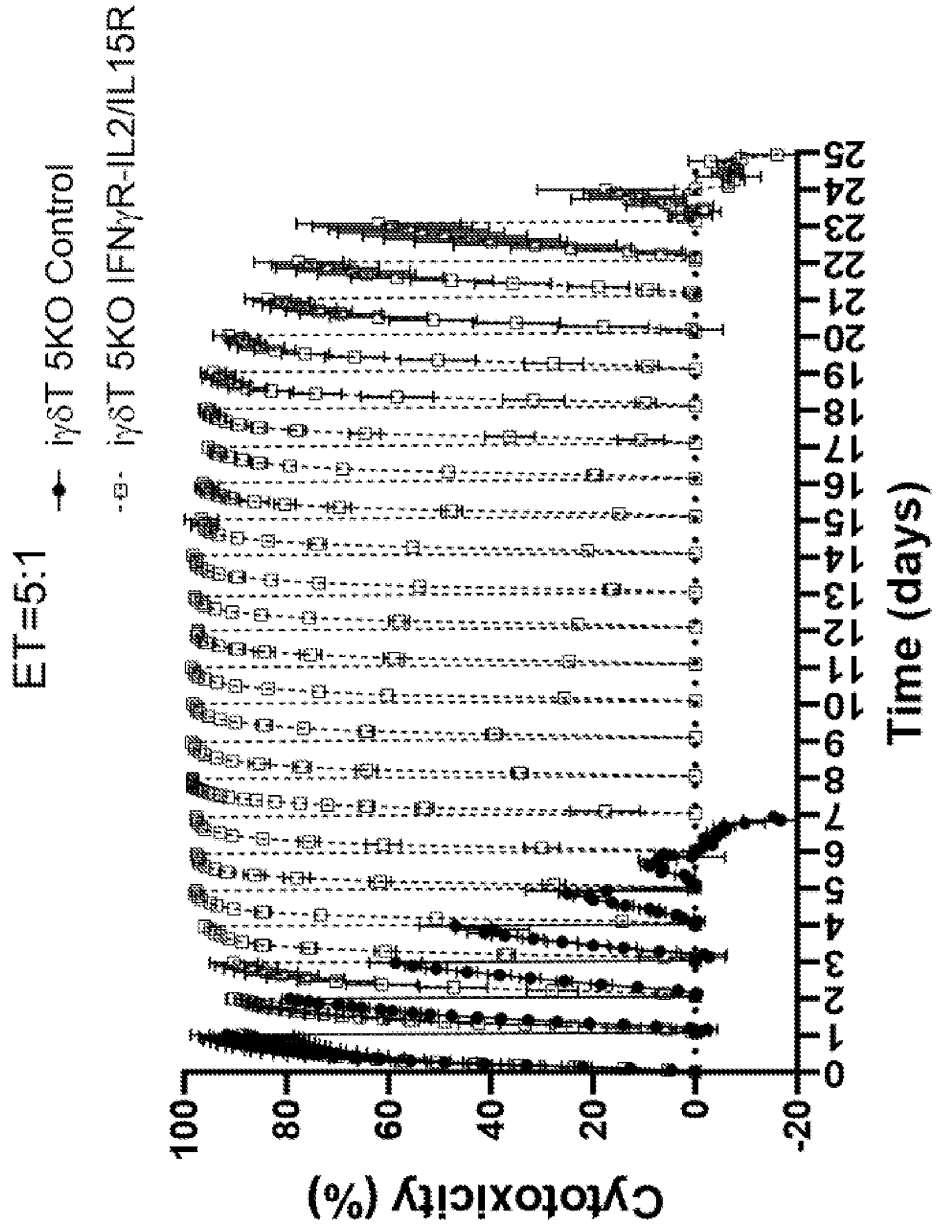

ET=5:1
iγδT 5KO Control
iγδT 5KO IFNγR-IL2/IL15R
Cytotoxicity (%)
100
80
60
40
20
0
-20
Time (days)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

FIG. 15

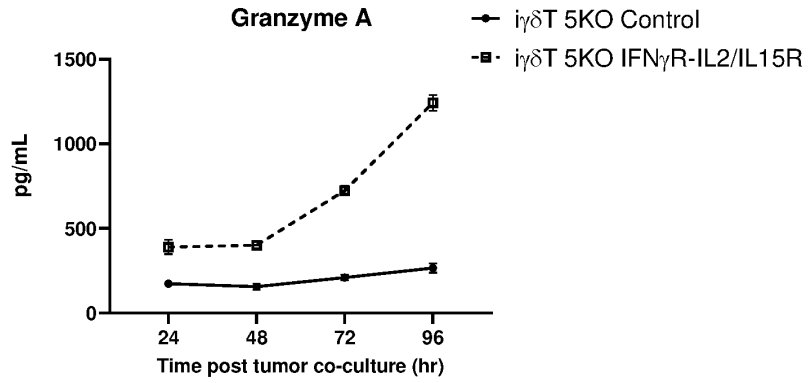


FIG. 16A

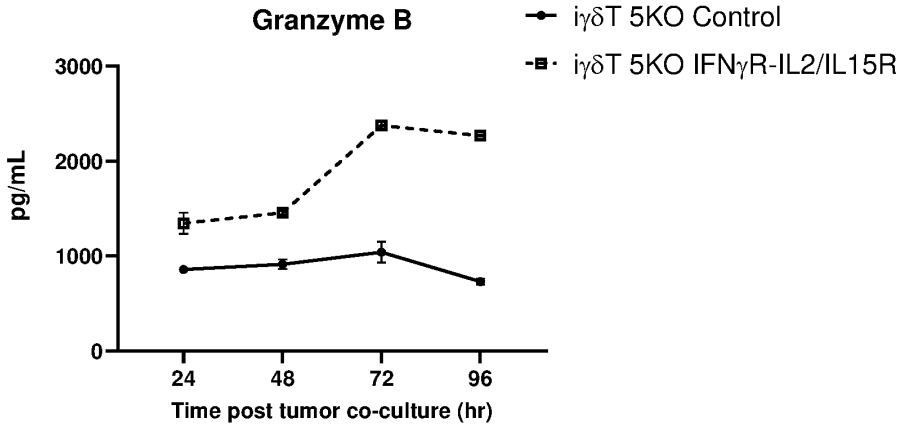


FIG. 16B

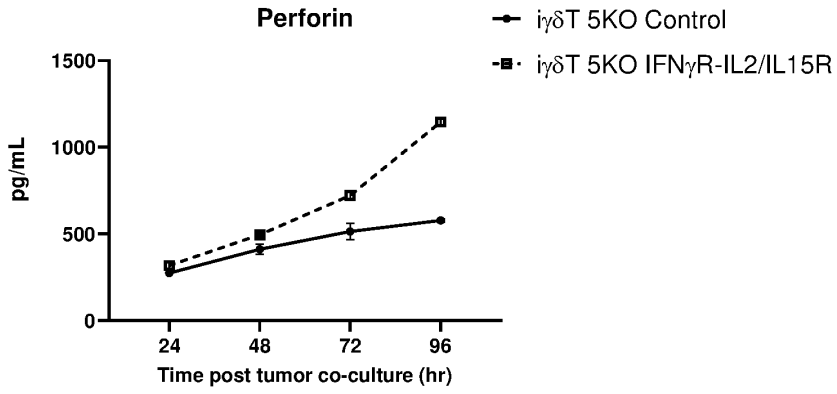


FIG. 16C

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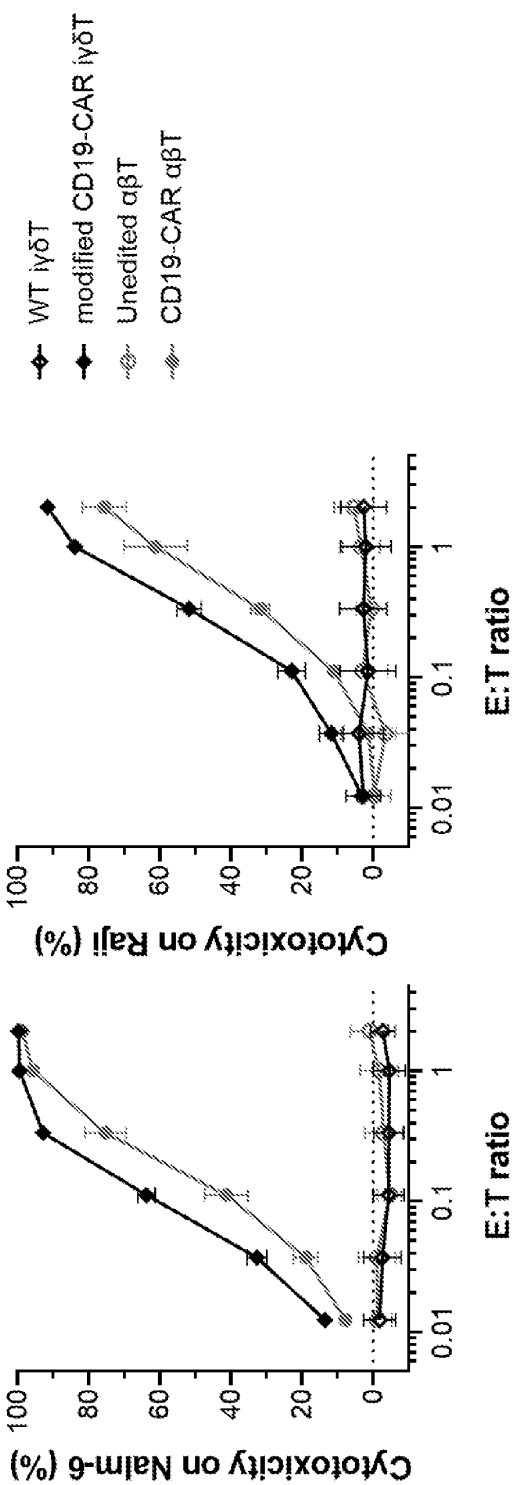

WT iγδT
modified CD19-CAR iγδT
Unedited αβT
CD19-CAR αβT
Cytotoxicity on Raji (%)
Cytotoxicity on Nalm-6 (%)
E:T ratio
E:T ratio
0
20
40
60
80
100
0.01
0.1
1

FIG. 17A

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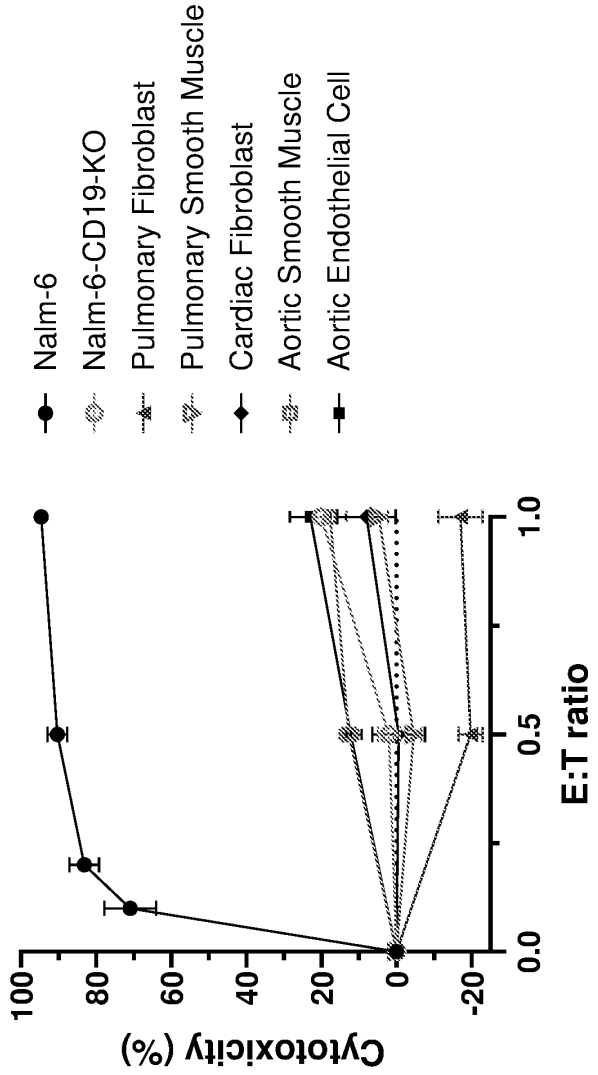

Nalm-6
Nalm-6-CD19-KO
Pulmonary Fibroblast
Pulmonary Smooth Muscle
Cardiac Fibroblast
Aortic Smooth Muscle
Aortic Endothelial Cell
Cytotoxicity (%)
100
80
60
40
20
0
-20
0.0
0.5
1.0
E:T ratio

FIG. 17B

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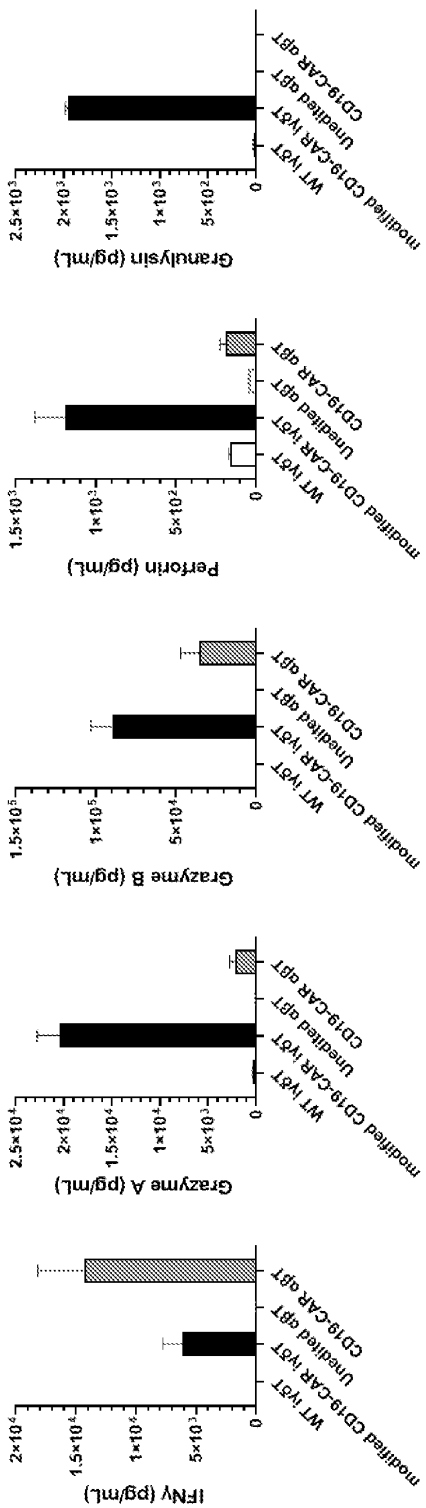

IFNγ (pg/mL)
Grazyme A (pg/mL)
Grazyme B (pg/mL)
Perforin (pg/mL)
Granulysin (pg/mL)
WT iγδT
Unedited αβT
CD19-CAR iγδT
modified CD19-CAR αβT

FIG. 18A

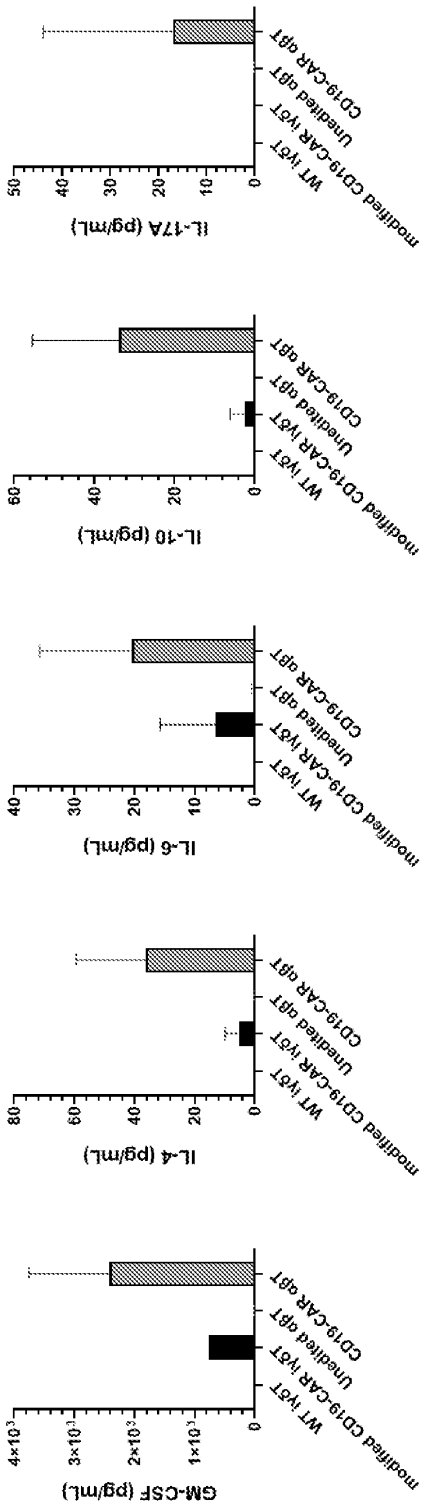

GM-CSF (pg/mL)
IL-4 (pg/mL)
IL-6 (pg/mL)
IL-10 (pg/mL)
IL-17A (pg/mL)
WT iγδT
Unedited αβT
CD19-CAR αβT
modified CD19-CAR iγδT

FIG. 18B

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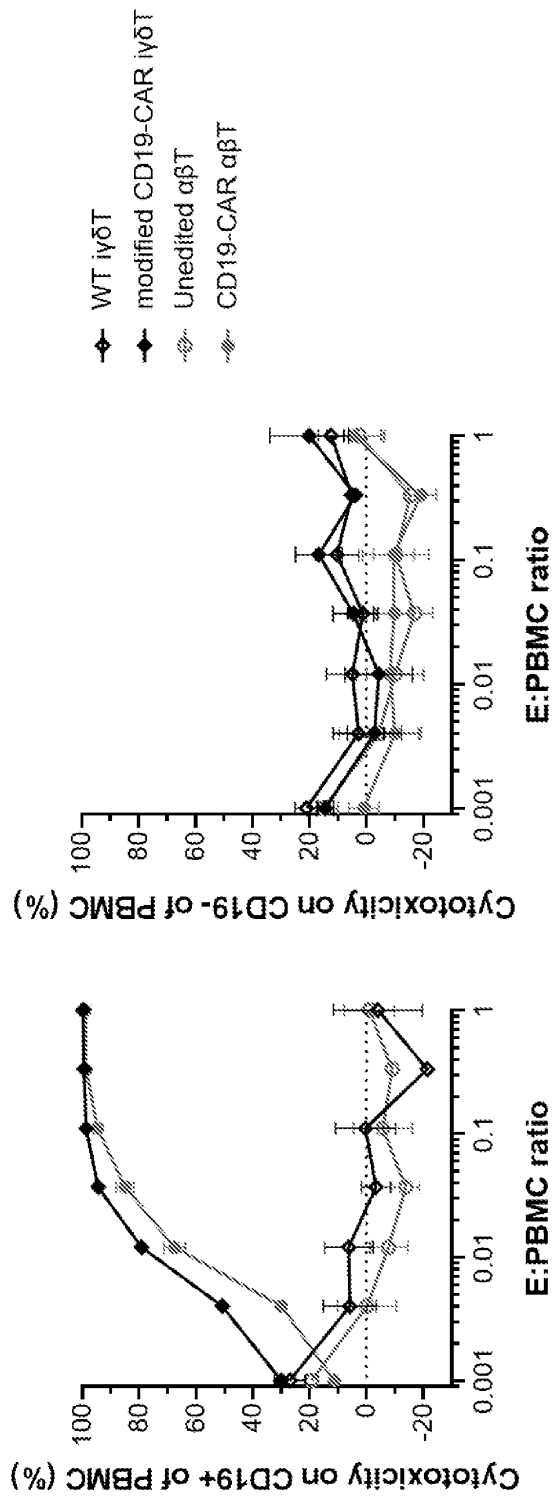

WT iγδT
modified CD19-CAR iγδT
Unedited αβT
CD19-CAR αβT
Cytotoxicity on CD19- of PBMC (%)
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
E:PBMC ratio
Cytotoxicity on CD19+ of PBMC (%)
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
E:PBMC ratio

FIG. 19A

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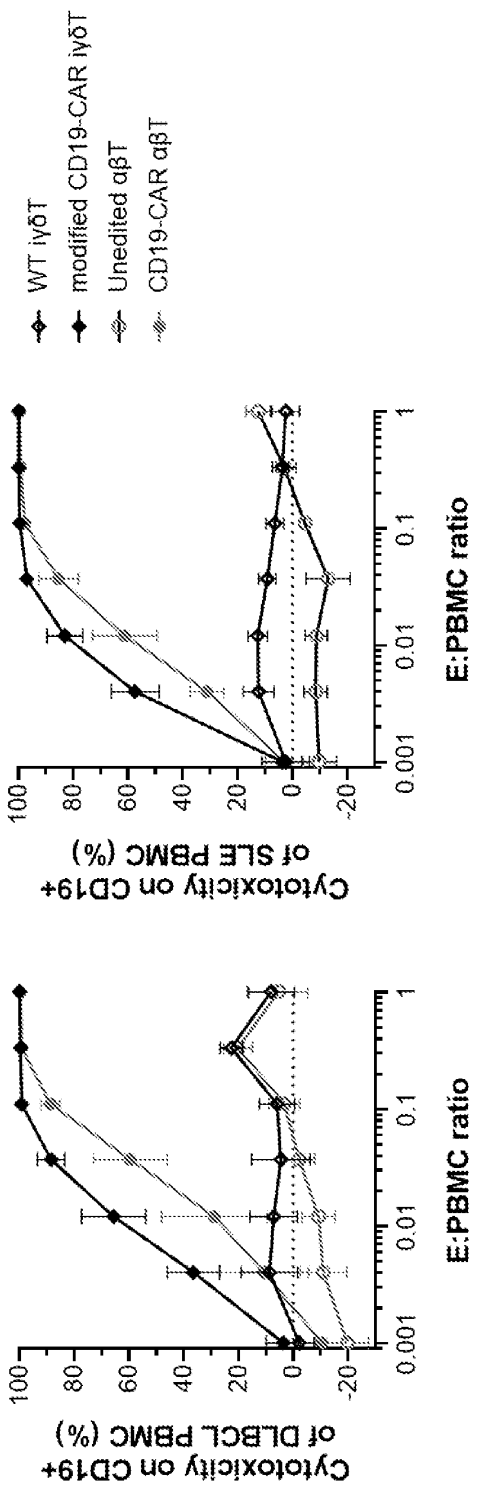

WT iγδT
modified CD19-CAR iγδT
Unedited αβT
CD19-CAR αβT
Cytotoxicity on CD19+ of SLE PBMC (%)
Cytotoxicity on CD19+ of DLBCL PBMC (%)
E:PBMC ratio
E:PBMC ratio
100
80
60
40
20
0
-20
0.001
0.01
0.1
1

FIG. 19B

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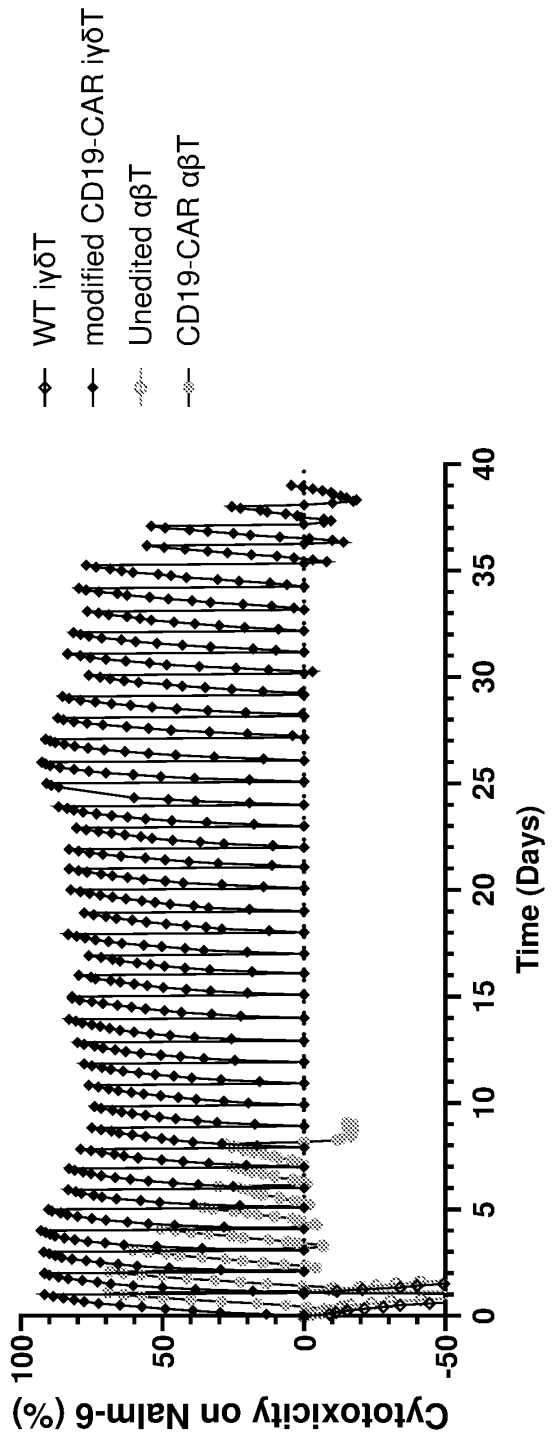

WT iγδT
modified CD19-CAR iγδT
Unedited αβT
CD19-CAR αβT
Cytotoxicity on Nalm-6 (%)
100
50
0
-50
0
5
10
15
20
25
30
35
40
Time (Days)

FIG. 20A

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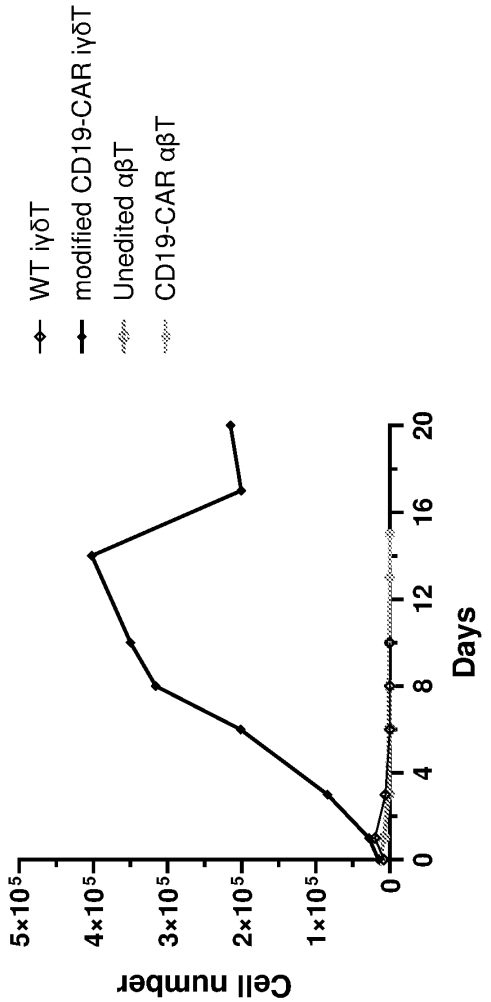

WT iγδT
modified CD19-CAR iγδT
Unedited αβT
CD19-CAR αβT
Cell number
5×10^5
4×10^5
3×10^5
2×10^5
1×10^5
0
0
4
8
12
16
20
Days

FIG. 20B

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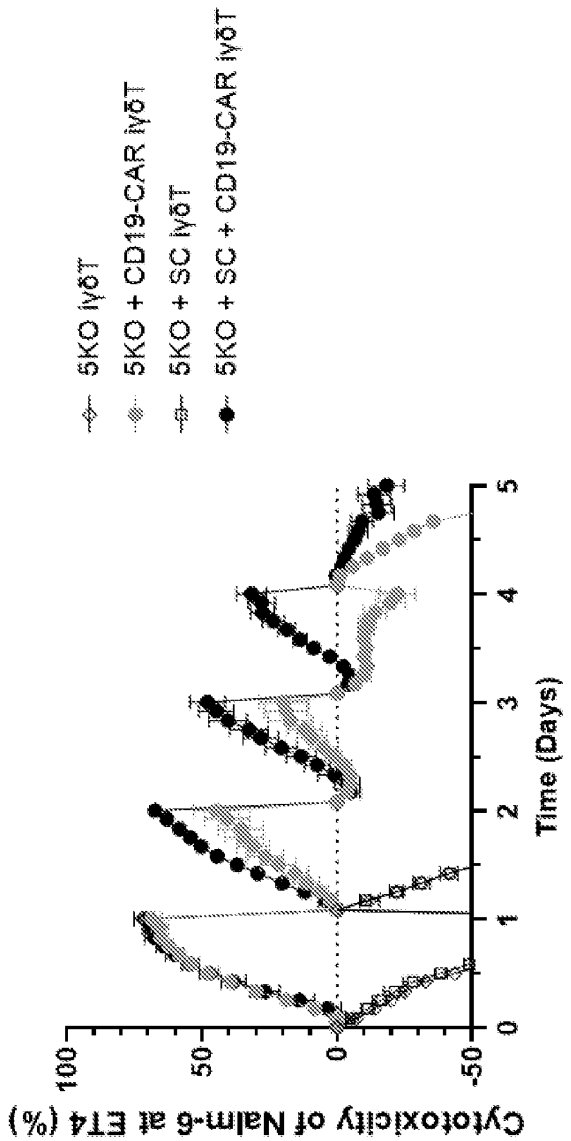

5KO iγδT
5KO + CD19-CAR iγδT
5KO + SC iγδT
5KO + SC + CD19-CAR iγδT
Cytotoxicity of Nalm-6 at ET4 (%)
100
50
0
-50
0
1
2
3
4
5
Time (Days)

FIG. 21A

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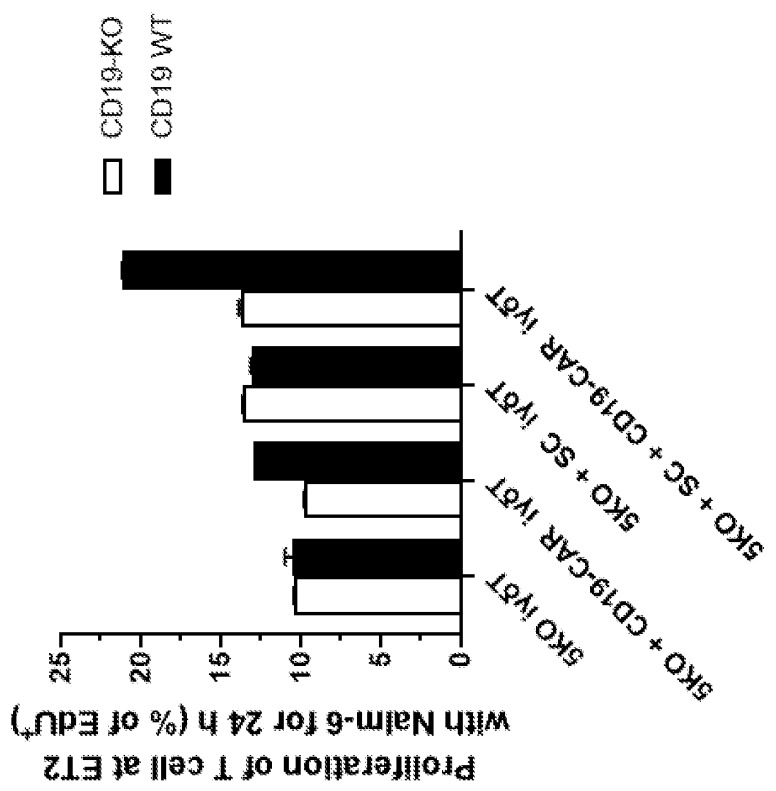

CD19-KO
CD19 WT
Proliferation of T cell at ET2
with Nalm-6 for 24 h (% of EdU+)
0
5
10
15
20
25
5KO IVGT
5KO + CD19-CAR IVGT
5KO + SC IVGT
5KO + SC + CD19-CAR IVGT

FIG. 21B