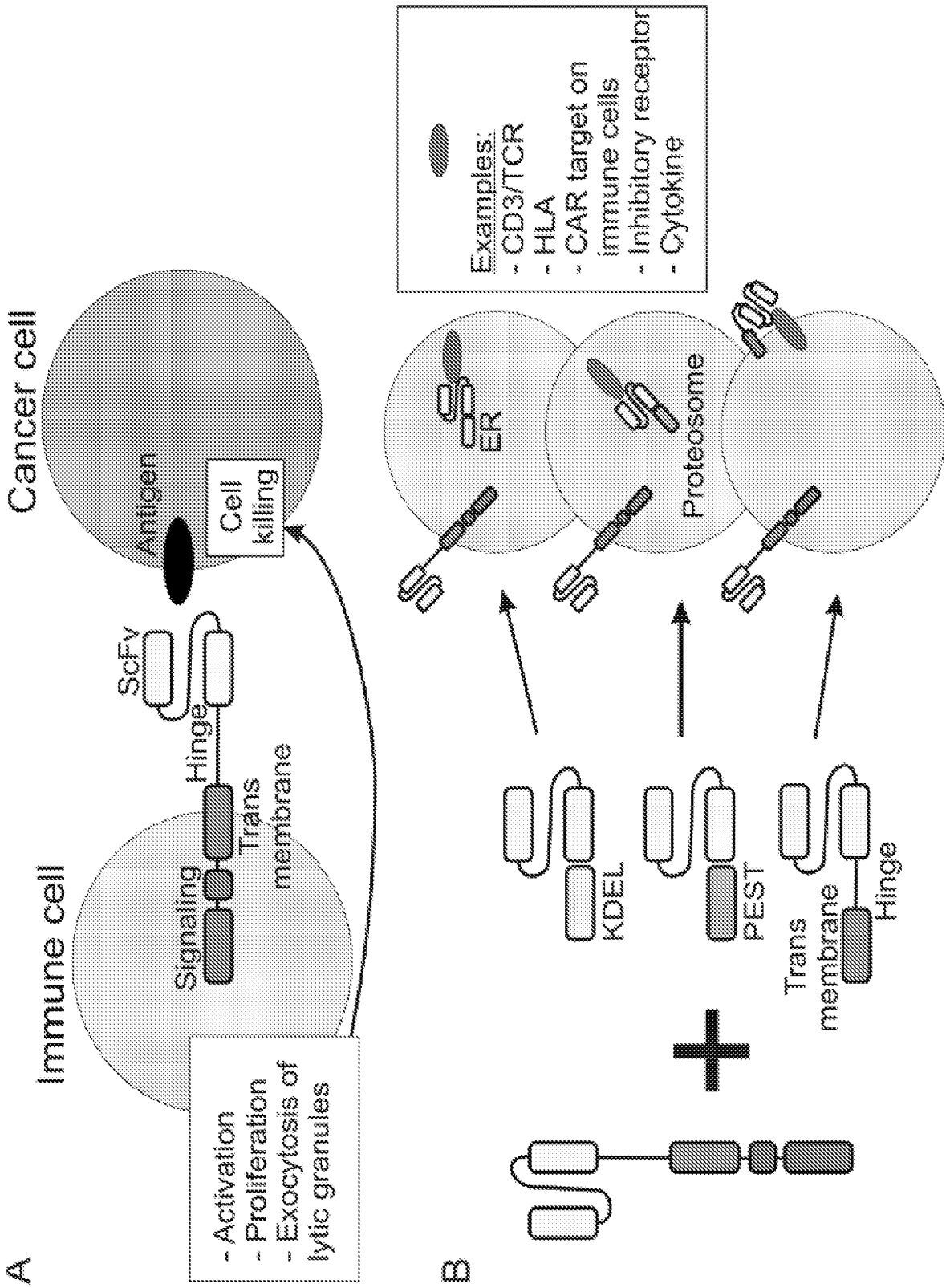

A
Immune cell
Signaling
Trans membrane
Hinge
ScFv
Cancer cell
Antigen
Cell killing
- Activation
- Proliferation
- Exocytosis of lytic granules
B
KDEL
PEST
Trans membrane
Hinge
ER
Proteosome
Examples:
- CD3/TCR
- HLA
- CAR target on immune cells
- Inhibitory receptor
- Cytokine

FIG. 1

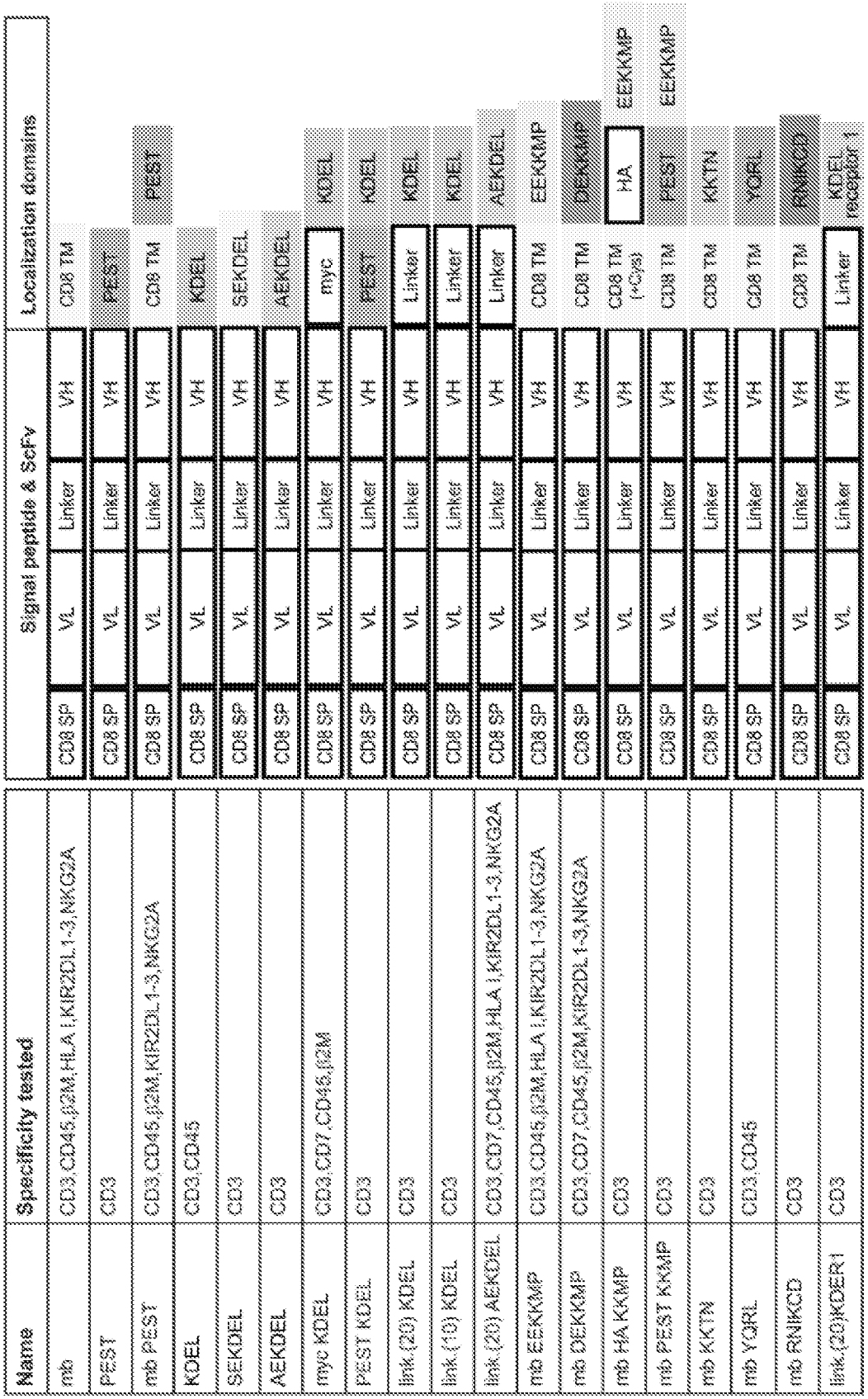
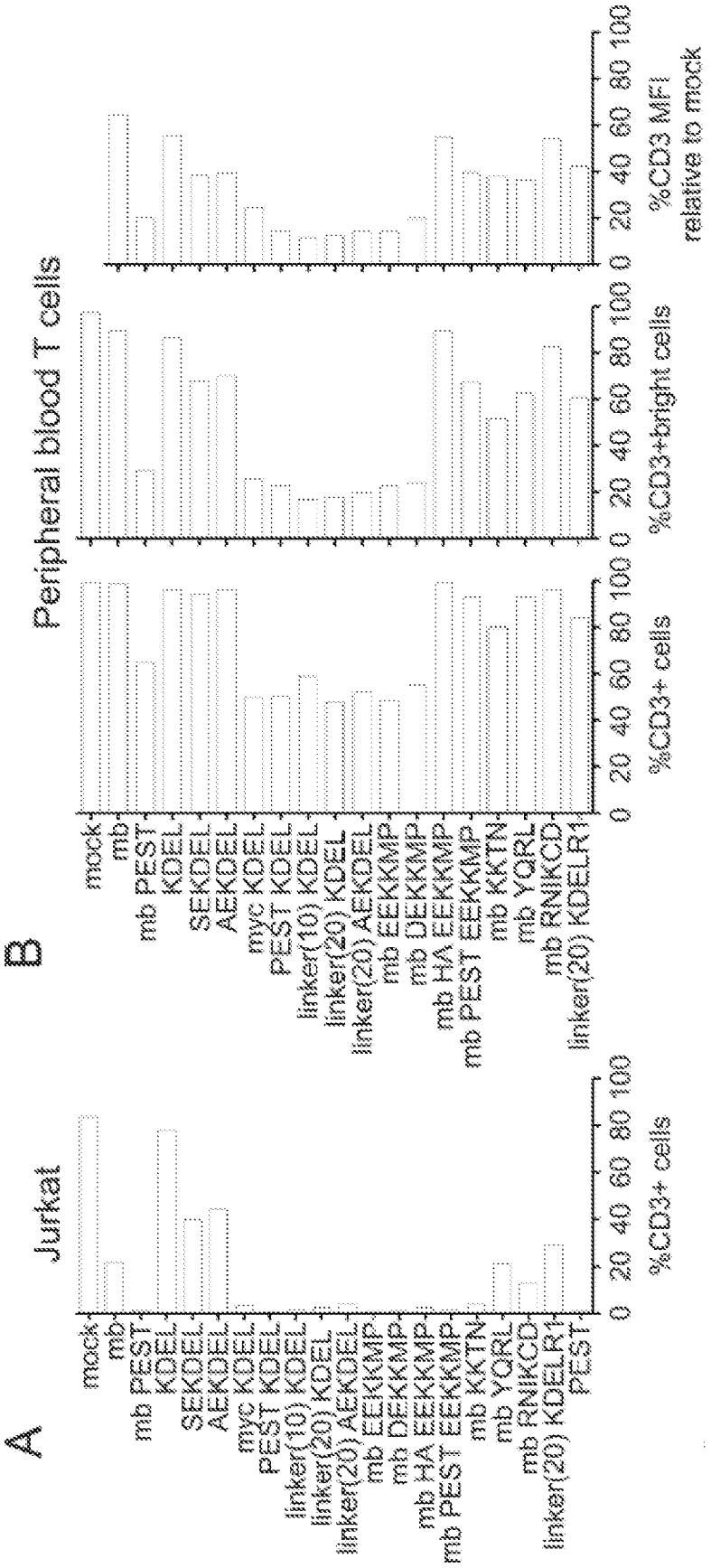

Name
Specificity tested
Signal peptide & ScFv
Localization domains
mb
CD3,CD45,β2M,HLA I,KIR2DL1-3,NKG2A
CD8 SP
VL
Linker
VH
CD8 TM
PEST
CD3
CD8 SP
VL
Linker
VH
PEST
mb PEST
CD3,CD45,β2M,KIR2DL1-3,NKG2A
CD8 SP
VL
Linker
VH
CD8 TM
PEST
KDEL
CD3,CD45
CD8 SP
VL
Linker
VH
KDEL
SEKDEL
CD3
CD8 SP
VL
Linker
VH
SEKDEL
AEKDEL
CD3
CD8 SP
VL
Linker
VH
AEKDEL
myc KDEL
CD3,CD7,CD45,β2M
CD8 SP
VL
Linker
VH
myc
KDEL
PEST KDEL
CD3
CD8 SP
VL
Linker
VH
PEST
KDEL
link.(20) KDEL
CD3
CD8 SP
VL
Linker
VH
Linker
KDEL
link.(10) KDEL
CD3
CD8 SP
VL
Linker
VH
Linker
KDEL
link.(20) AEKDEL
CD3,CD7,CD45,β2M,HLA I,KIR2DL1-3,NKG2A
CD8 SP
VL
Linker
VH
Linker
AEKDEL
mb EEKKMP
CD3,CD45,β2M,HLA I,KIR2DL1-3,NKG2A
CD8 SP
VL
Linker
VH
CD8 TM
EEKKMP
mb DEKKMP
CD3,CD7,CD45,β2M,KIR2DL1-3,NKG2A
CD8 SP
VL
Linker
VH
CD8 TM
DEKKMP
mb HA KKMP
CD3
CD8 SP
VL
Linker
VH
CD8 TM (+Cys)
HA
EEKKMP
mb PEST KKMP
CD3
CD8 SP
VL
Linker
VH
CD8 TM
PEST
EEKKMP
mb KKTN
CD3
CD8 SP
VL
Linker
VH
CD8 TM
KKTN
mb YQRL
CD3,CD45
CD8 SP
VL
Linker
VH
CD8 TM
YQRL
mb RNIKCD
CD3
CD8 SP
VL
Linker
VH
CD8 TM
RNIKCD
link.(20)KDER1
CD3
CD8 SP
VL
Linker
VH
Linker
KDEL receptor 1

FIG. 2


A
Jurkat
mock
mb
mb PEST
KDEL
SEKDEL
AEKDEL
myc KDEL
PEST KDEL
linker(10) KDEL
linker(20) KDEL
linker(20) AEKDEL
mb EEKKMP
mb DEKKMP
mb HA EEKKMP
mb PEST EEKKMP
mb KKTN
mb YQRL
mb RNIKCD
linker(20) KDELR1
PEST
0 20 40 60 80 100
%CD3+ cells
B
Peripheral blood T cells
mock
mb
mb PEST
KDEL
SEKDEL
AEKDEL
myc KDEL
PEST KDEL
linker(10) KDEL
linker(20) KDEL
linker(20) AEKDEL
mb EEKKMP
mb DEKKMP
mb HA EEKKMP
mb PEST EEKKMP
mb KKTN
mb YQRL
mb RNIKCD
linker(20) KDELR1
0 20 40 60 80 100
%CD3+ cells
0 20 40 60 80 100
%CD3+bright cells
0 20 40 60 80 100
%CD3 MFI
relative to mock

FIGS. 3A-3B

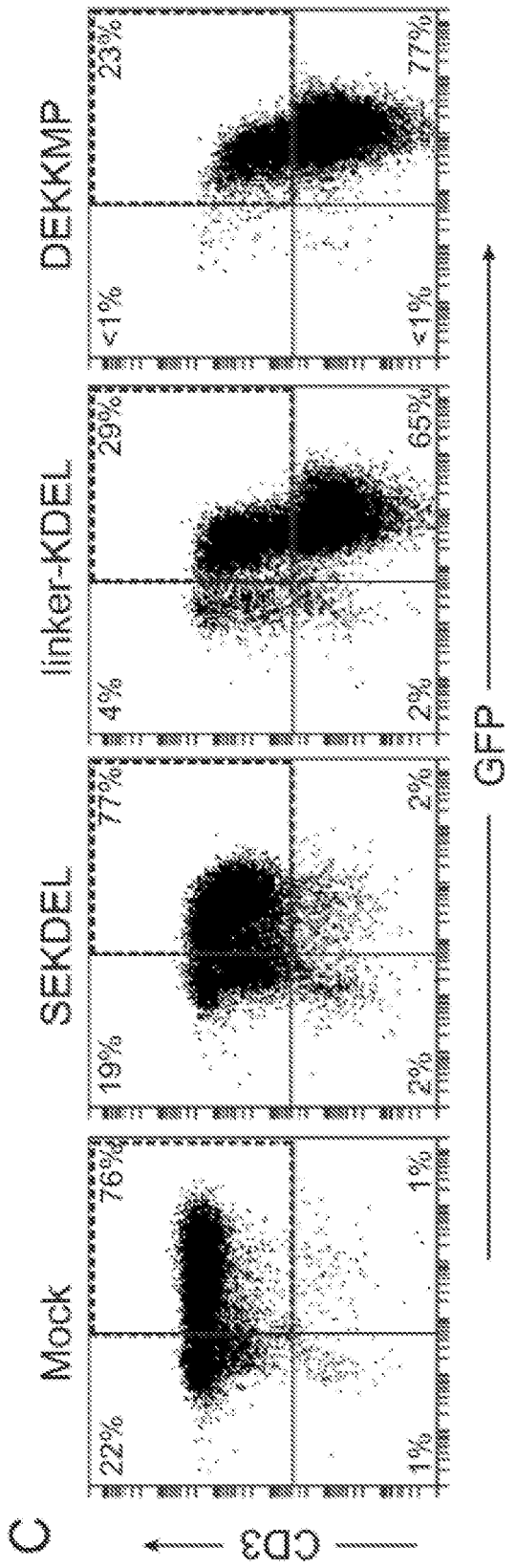

C
CD3
GFP
Mock
SEKDEL
linker-KDEL
DEKKMP
22%
1%
76%
1%
19%
2%
77%
2%
4%
2%
29%
65%
<1%
<1%
23%
77%

FIG. 3C

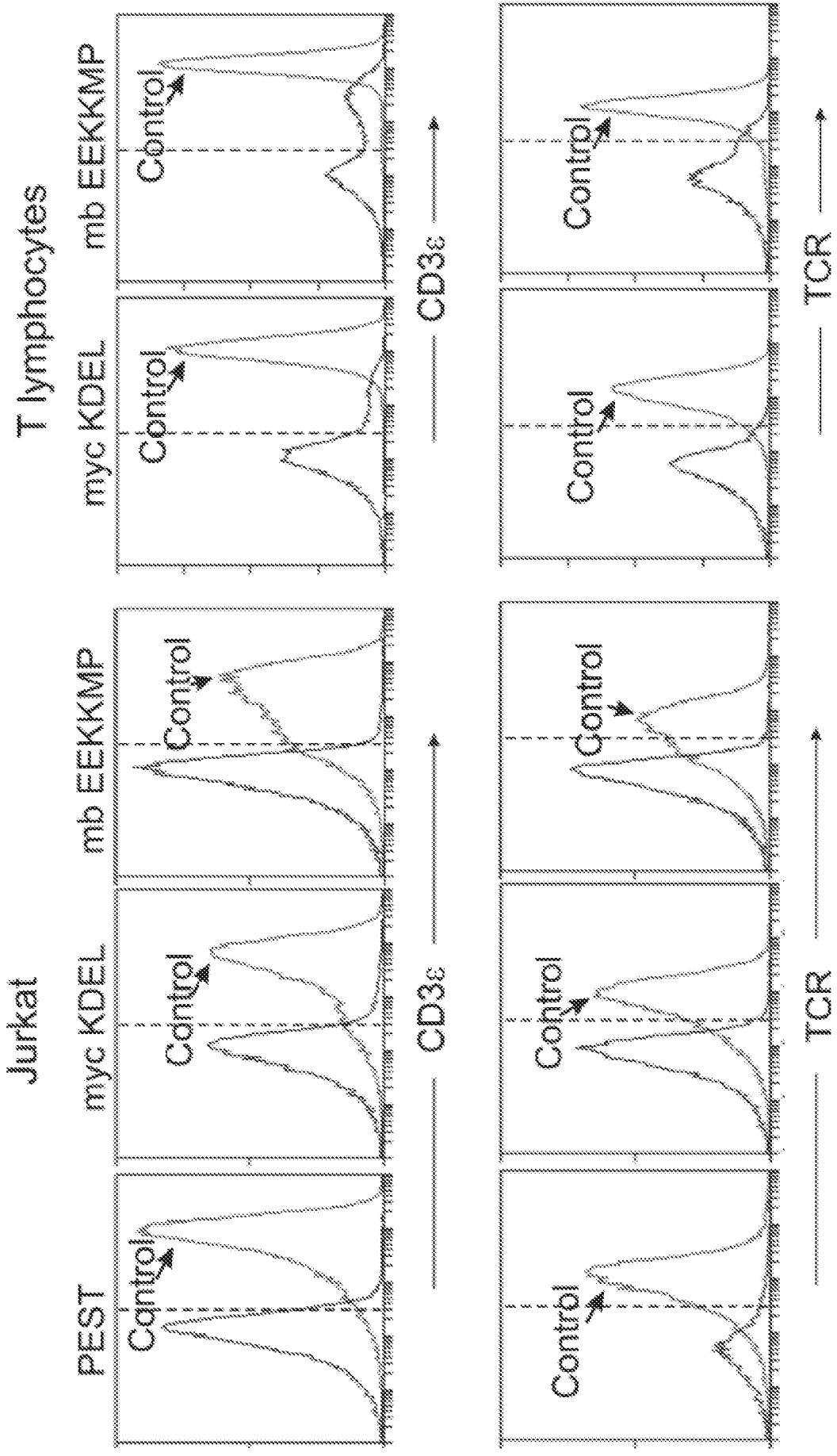

Jurkat
T lymphocytes
PEST
myc KDEL
mb EEKKMP
Control
CD3ε
TCR

FIG. 4

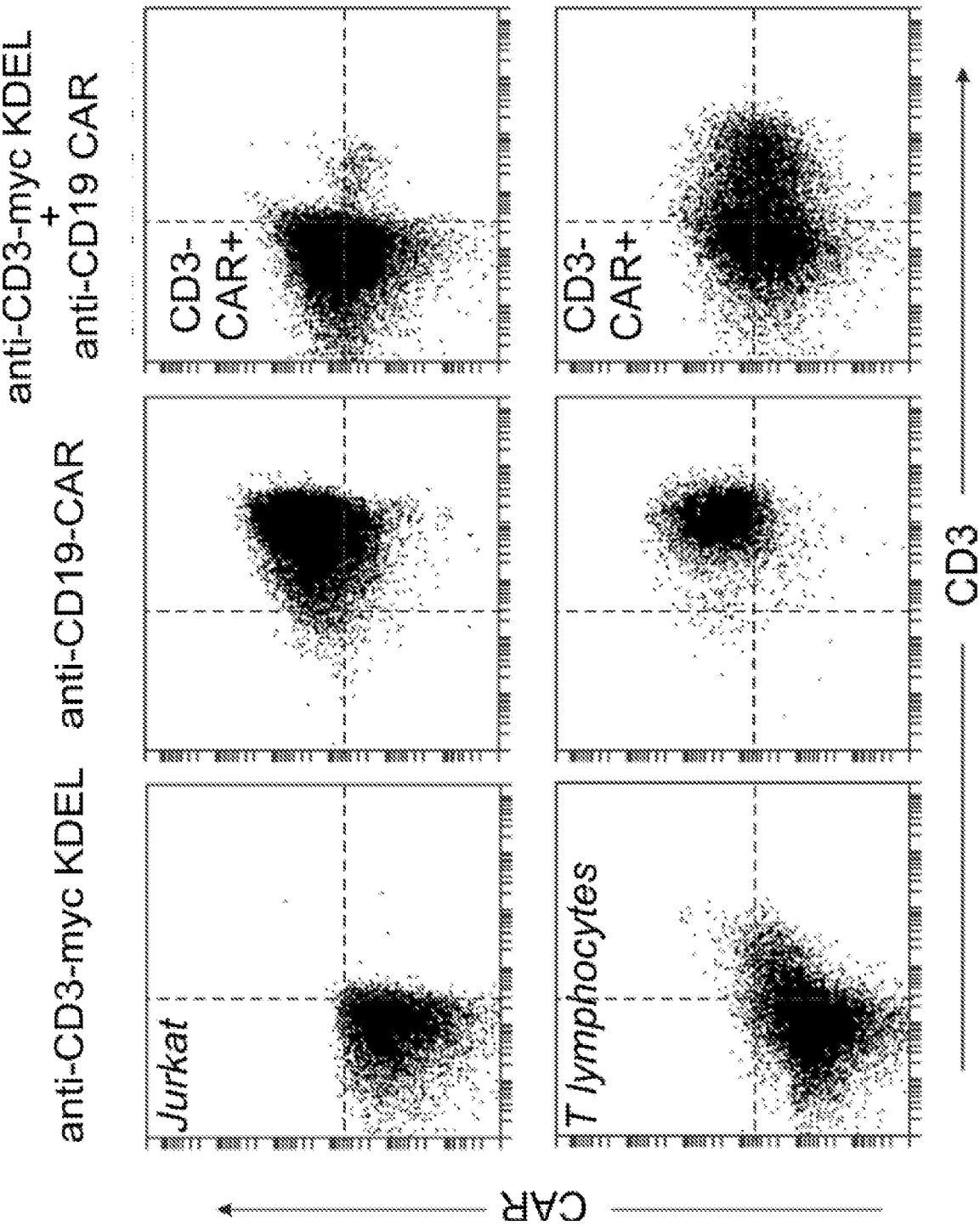

anti-CD3-myc KDEL
anti-CD19-CAR
anti-CD3-myc KDEL
+
anti-CD19 CAR
Jurkat
T lymphocytes
CD3-
CAR+
CD3-
CAR+
CAR
CD3

FIG. 5

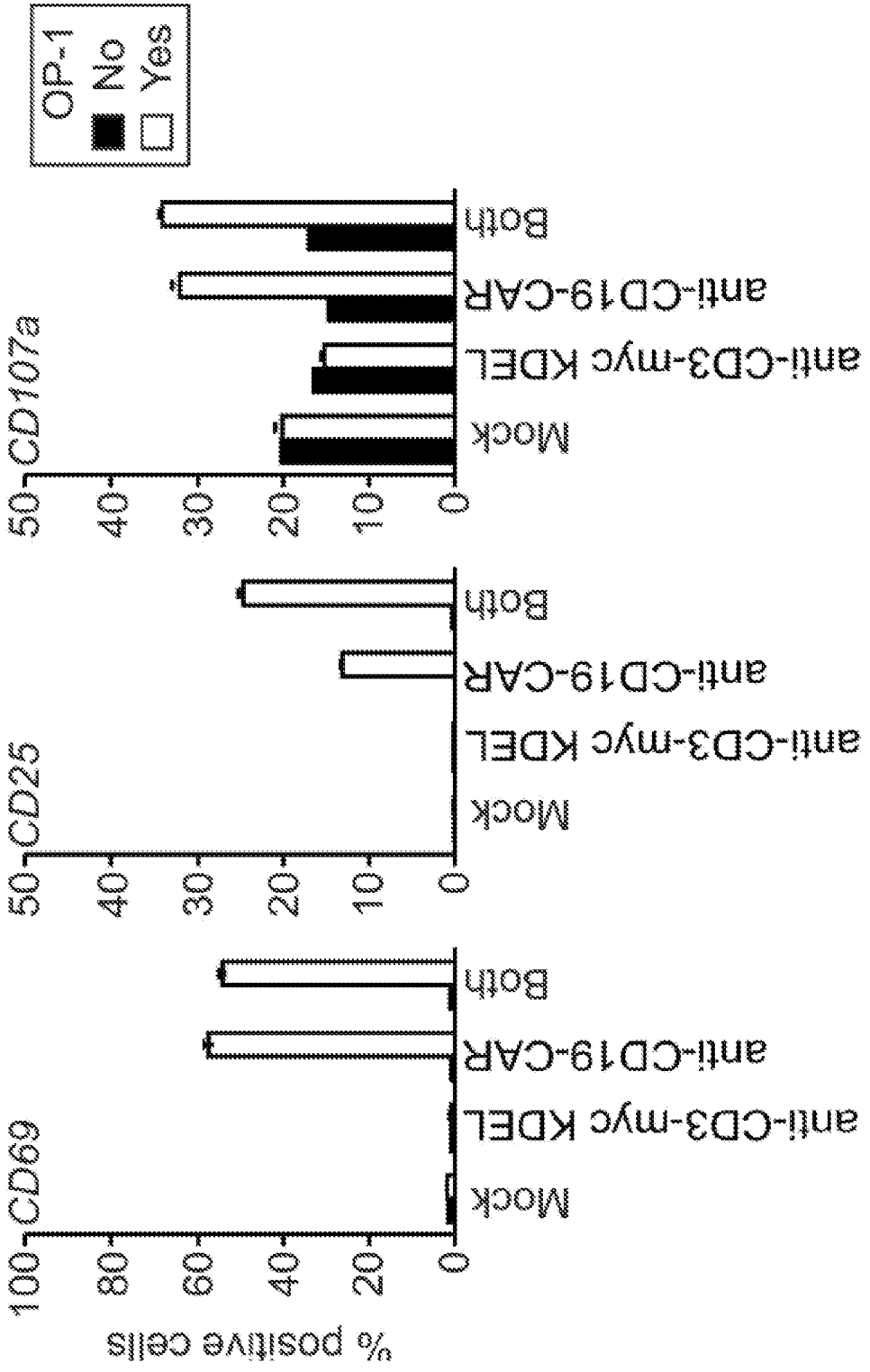

OP-1
No
Yes
% positive cells
CD69
CD25
CD107a
100
80
60
40
20
0
50
40
30
20
10
0
Mock
anti-CD3-myc KDEL
anti-CD19-CAR
Both

FIG. 6

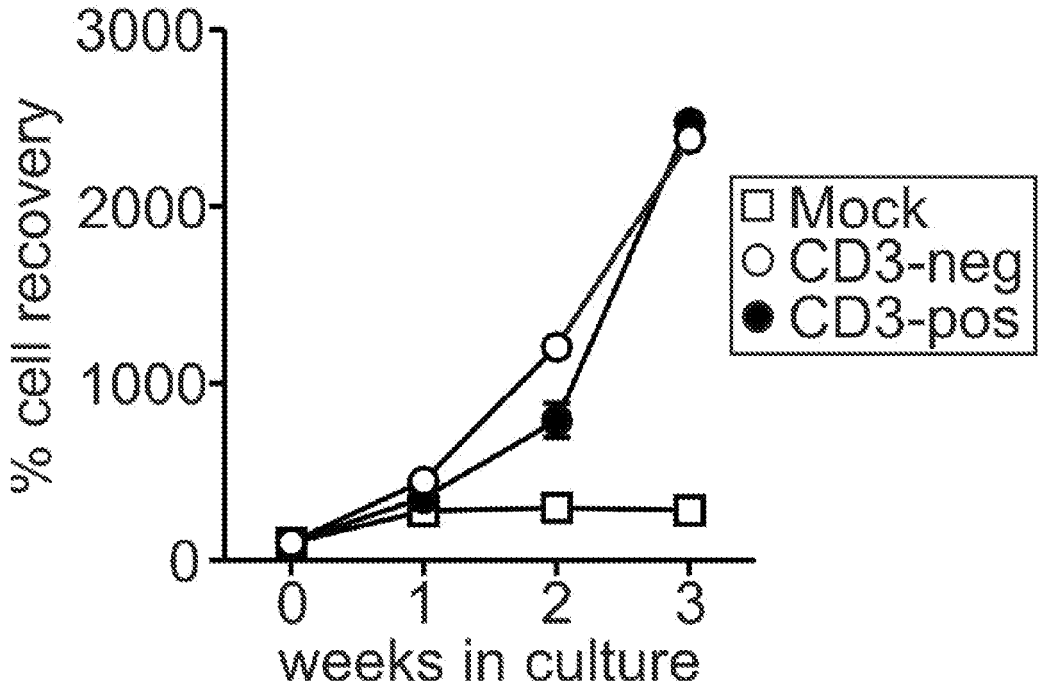

3000
2000
1000
0
% cell recovery
0
1
2
3
weeks in culture
Mock
CD3-neg
CD3-pos

FIG. 7

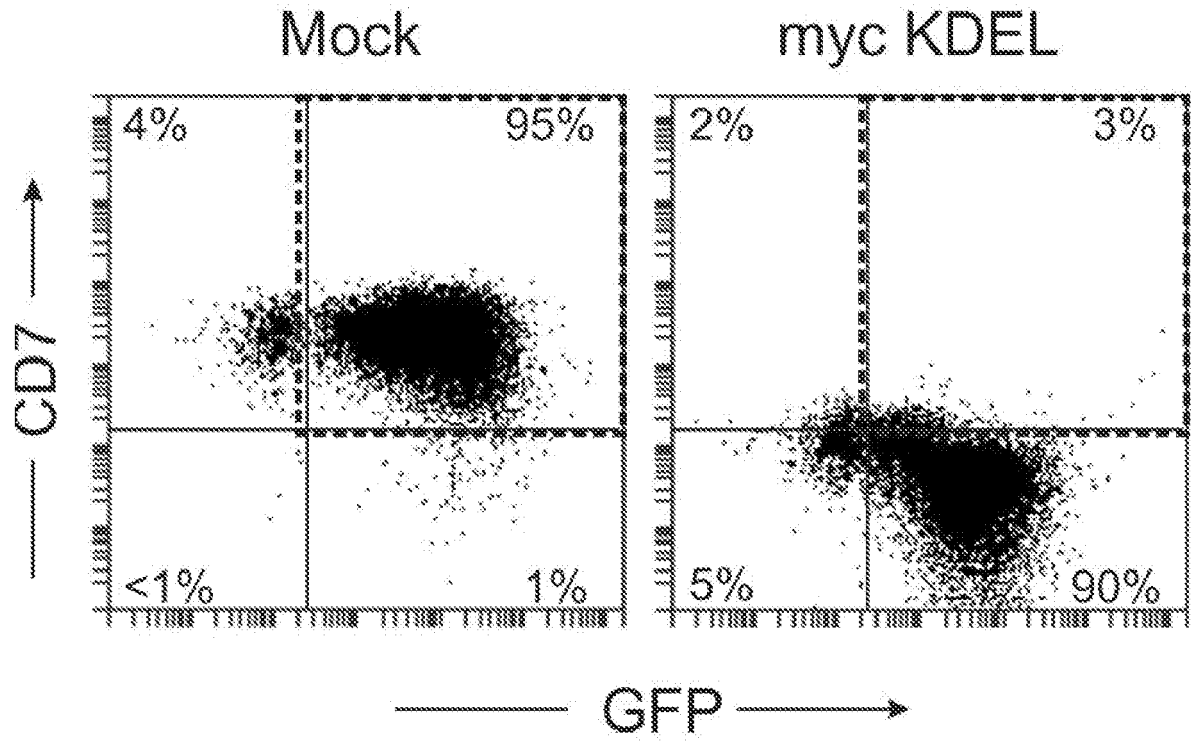

Mock
myc KDEL
4%
95%
<1%
1%
2%
3%
5%
90%
CD7
GFP

FIG. 8

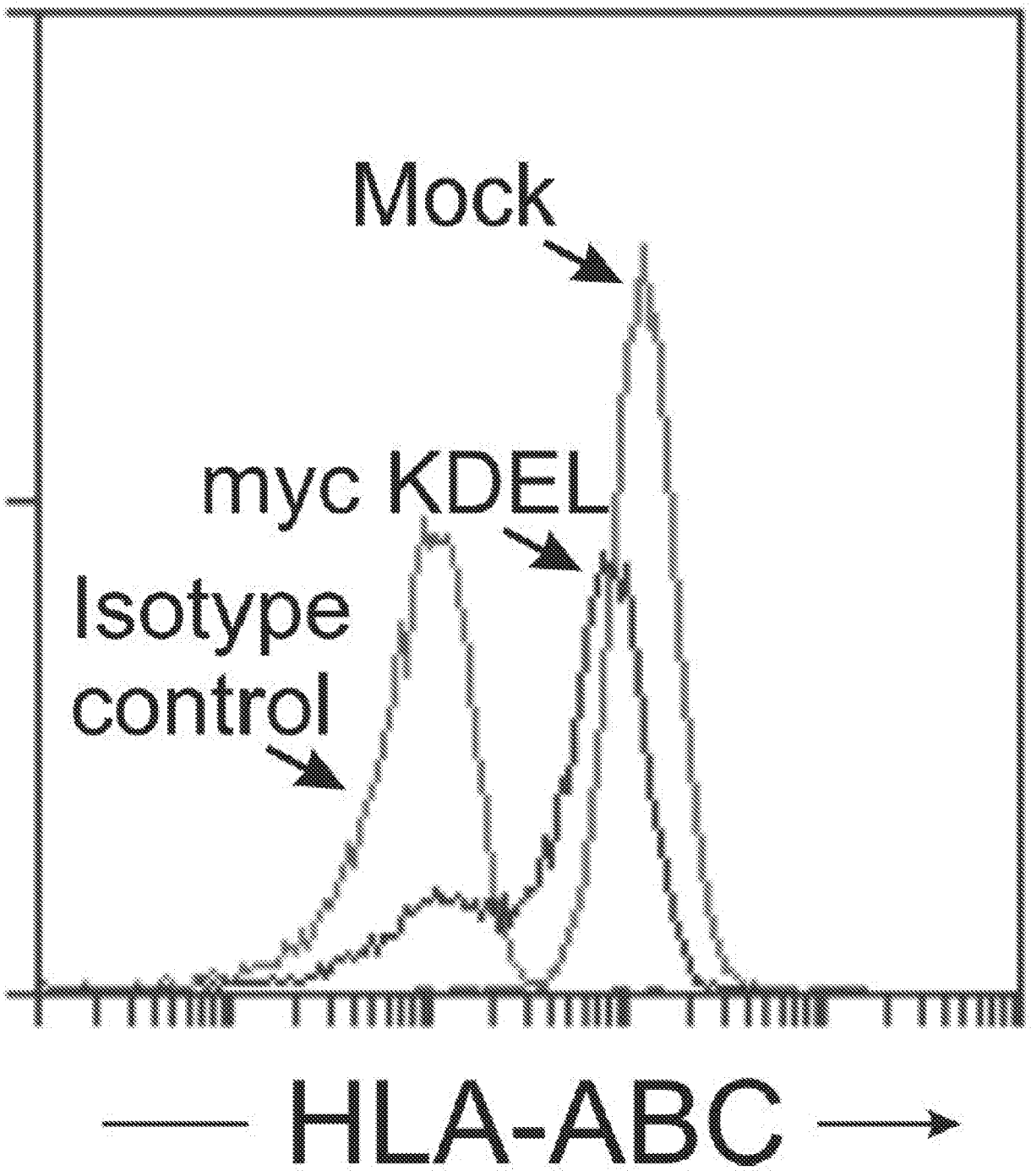


FIG. 9

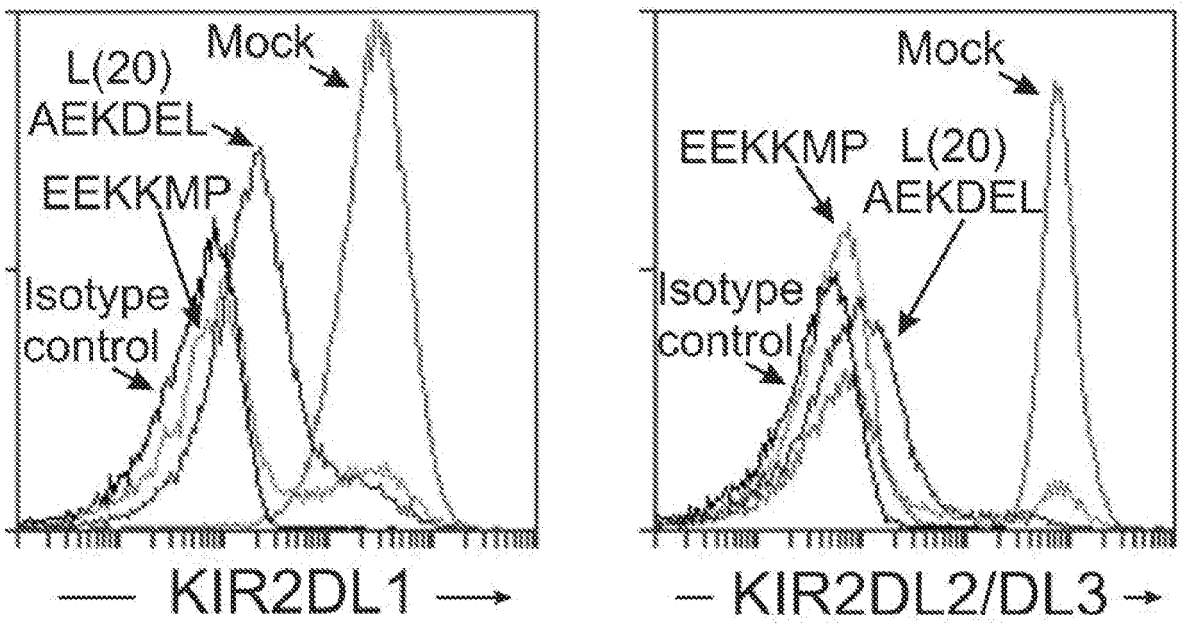

Mock
L(20)
AEKDEL
EEKKMP
Isotype
control
KIR2DL1
Mock
EEKKMP
L(20)
AEKDEL
Isotype
control
KIR2DL2/DL3

FIG. 10

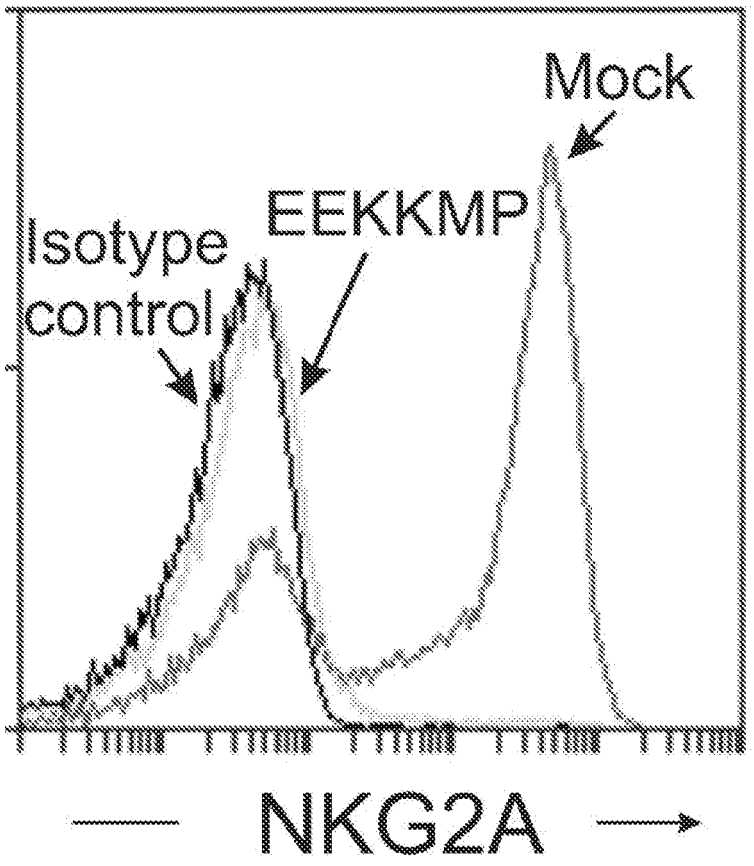

Mock
EEKKMP
Isotype
control
NKG2A

FIG. 11