FIG. 1A

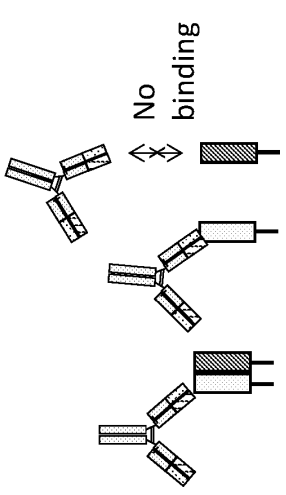


FIG. 1B

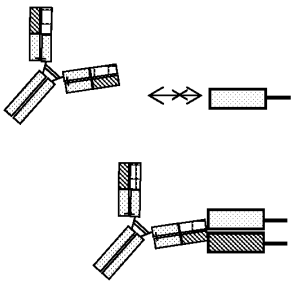


FIG. 1C

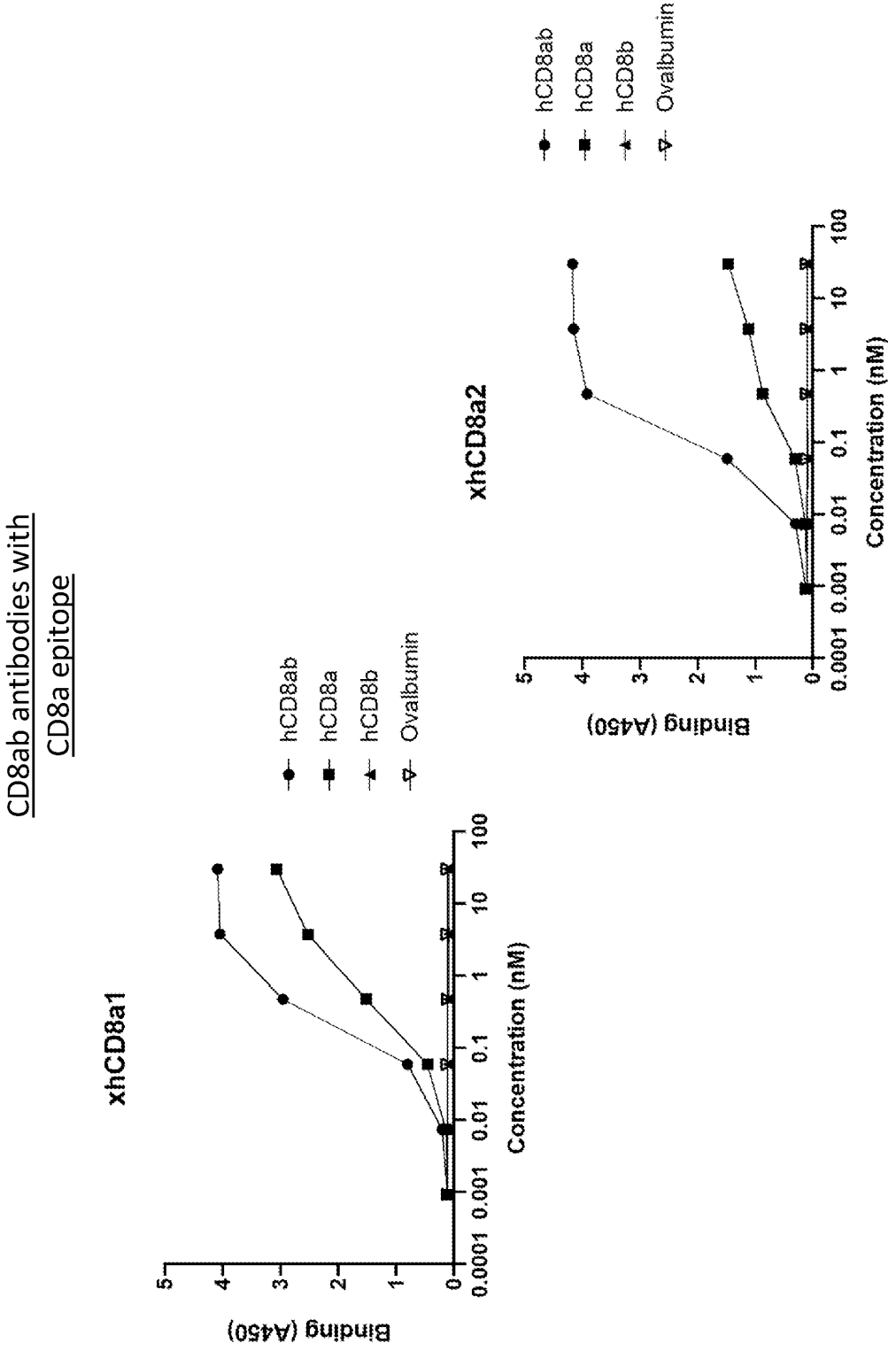

CD8ab antibodies with
CD8a epitope
xhCD8a1
xhCD8a2
hCD8ab
hCD8a
hCD8b
Ovalbumin
Binding (A450)
Concentration (nM)
0
1
2
3
4
5
0.0001
0.001
0.01
0.1
1
10
100

FIG. 2A

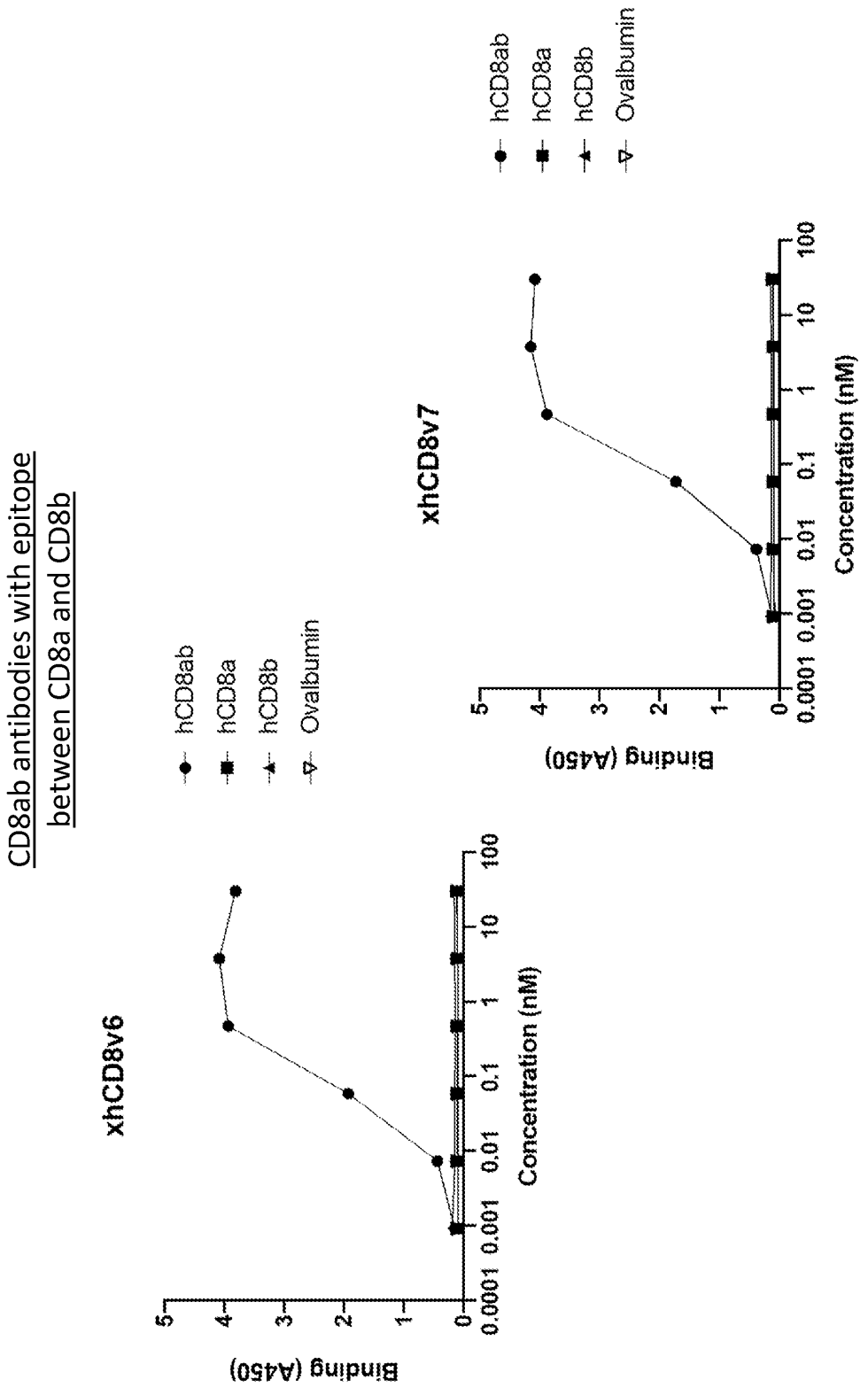

CD8ab antibodies with epitope
between CD8a and CD8b
xhCD8v6
xhCD8v7
hCD8ab
hCD8a
hCD8b
Ovalbumin
Binding (A450)
Concentration (nM)
0
1
2
3
4
5
0.0001
0.001
0.01
0.1
1
10
100

FIG. 2B

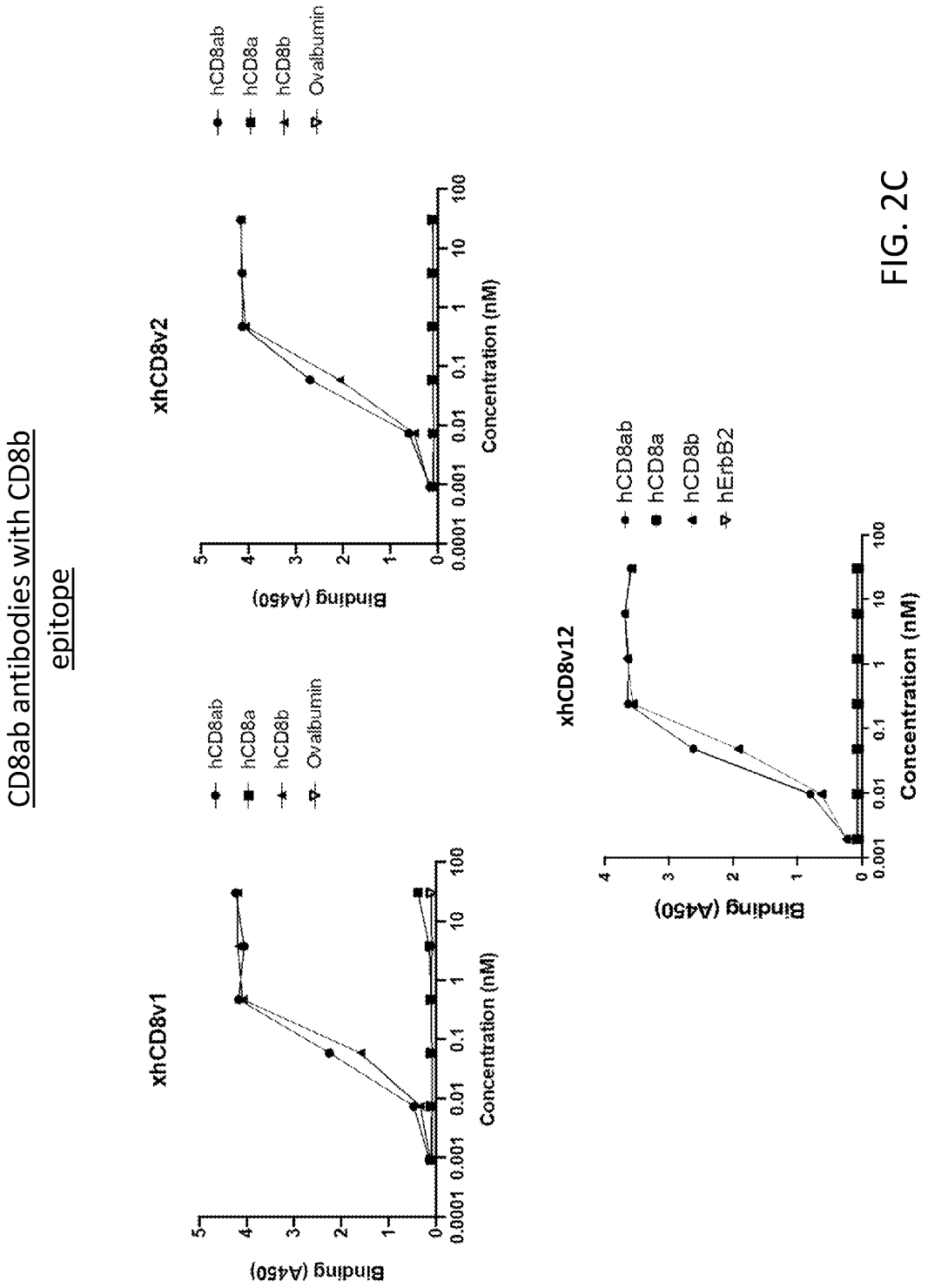

CD8ab antibodies with CD8b epitope
xhCD8v1
xhCD8v2
xhCD8v12
hCD8ab
hCD8a
hCD8b
Ovalbumin
hErbB2
Binding (A450)
Concentration (nM)
0.0001
0.001
0.01
0.1
1
10
100

FIG. 2C

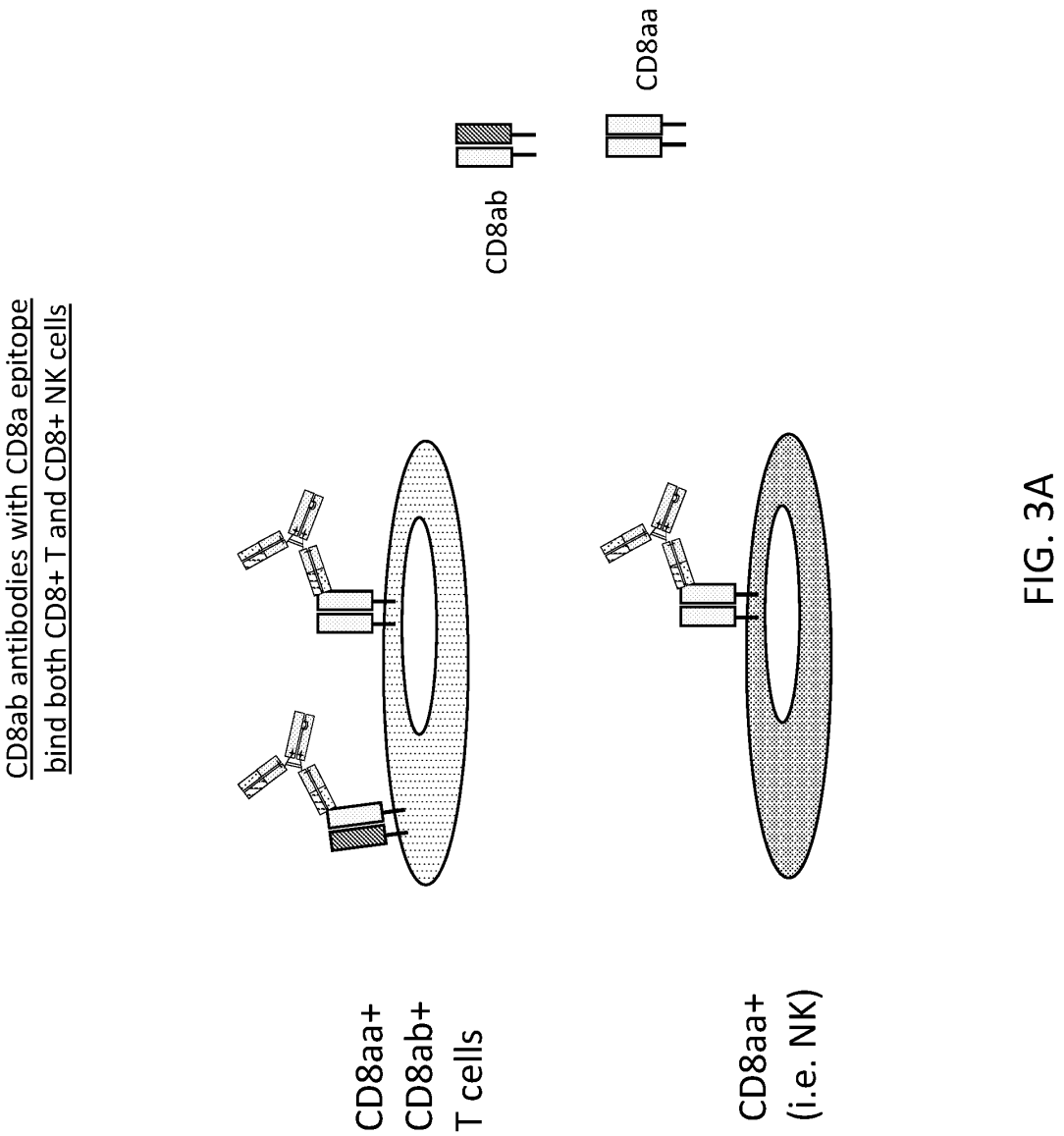

CD8ab antibodies with CD8a epitope
bind both CD8+ T and CD8+ NK cells
CD8ab
CD8aa
CD8aa+
CD8ab+
T cells
CD8aa+
(i.e. NK)

FIG. 3A

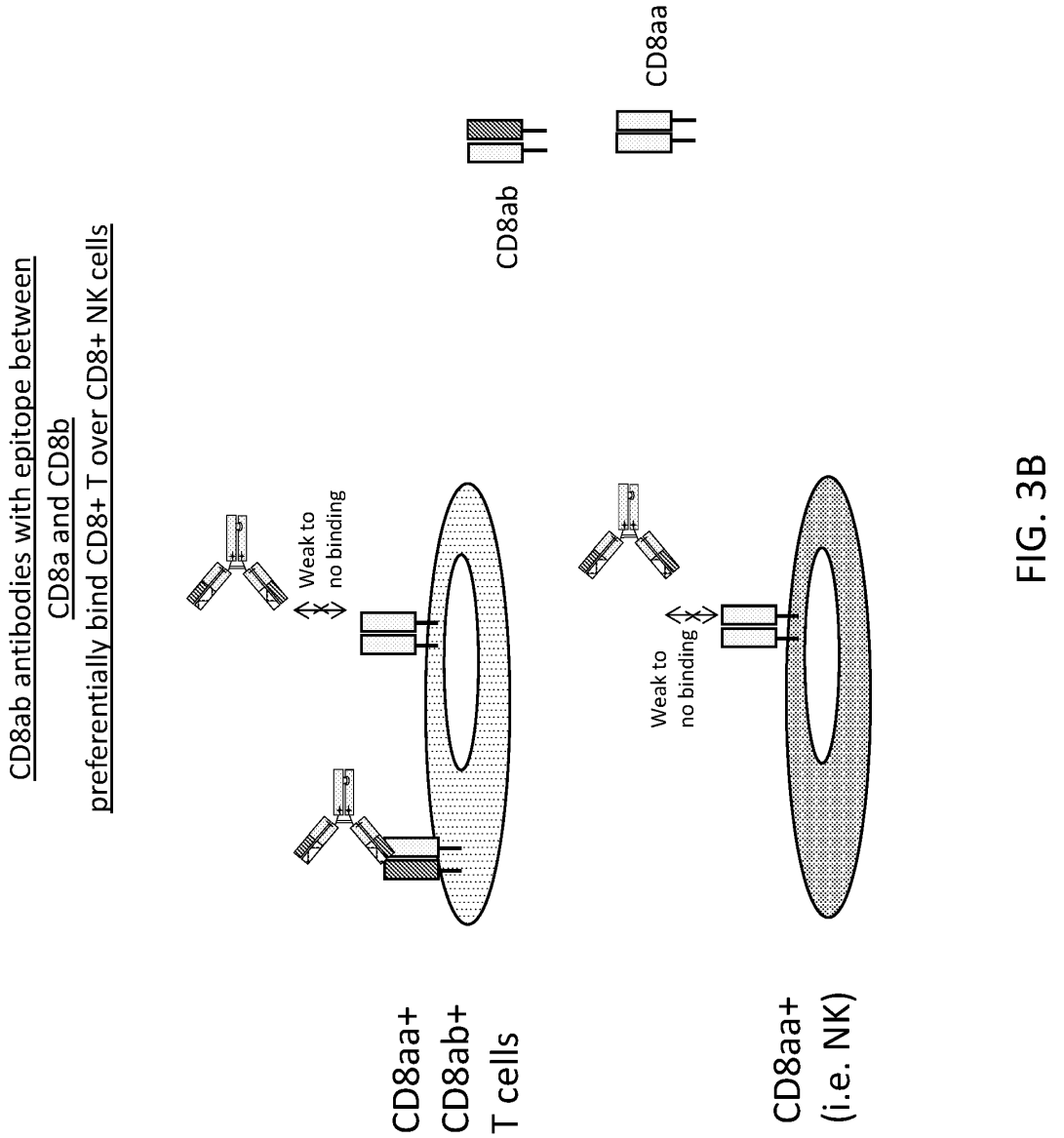

CD8ab antibodies with epitope between CD8a and CD8b
preferentially bind CD8+ T over CD8+ NK cells
CD8ab
CD8aa
Weak to no binding
Weak to no binding
CD8aa+ CD8ab+ T cells
CD8aa+ (i.e. NK)

FIG. 3B

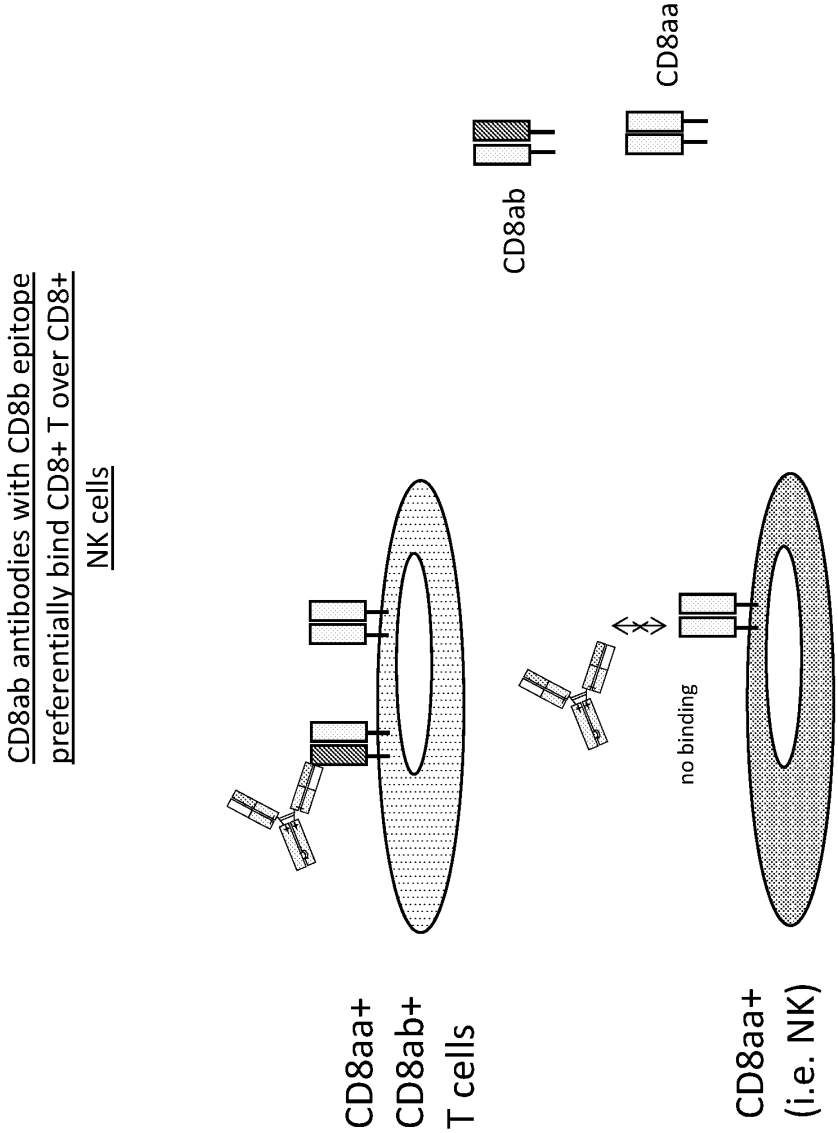

CD8ab antibodies with CD8b epitope preferentially bind CD8+ T over CD8+ NK cells
CD8ab
CD8aa
CD8aa+ CD8ab+ T cells
no binding
CD8aa+ (i.e. NK)

FIG. 3C

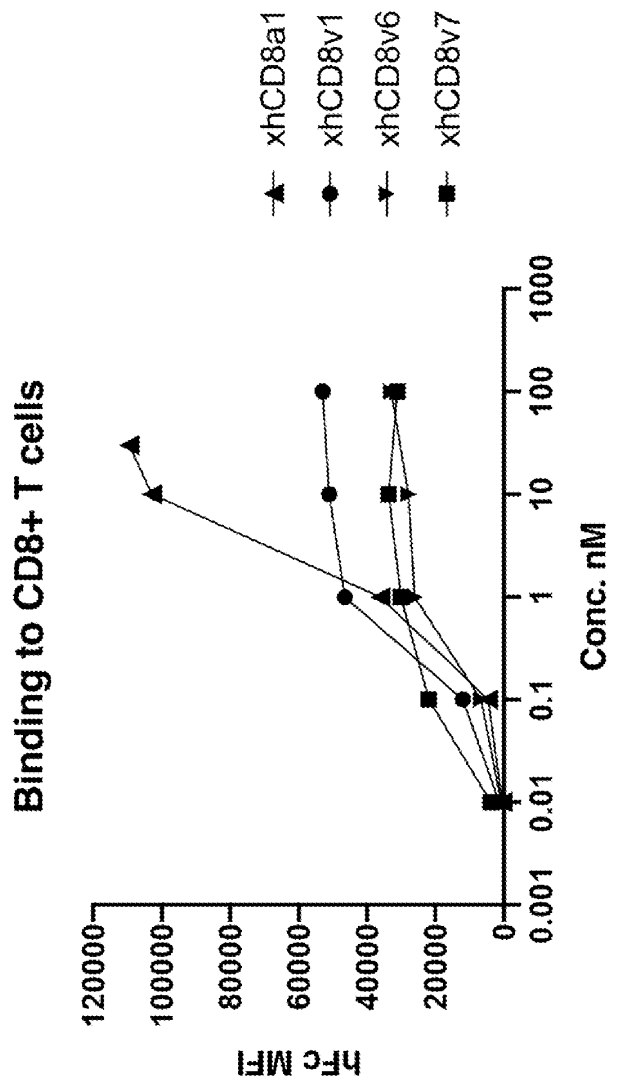

Binding to CD8+ T cells
hFc MFI
120000
100000
80000
60000
40000
20000
0
0.001
0.01
0.1
1
10
100
1000
Conc. nM
xhCD8a1
xhCD8v1
xhCD8v6
xhCD8v7

FIG. 4

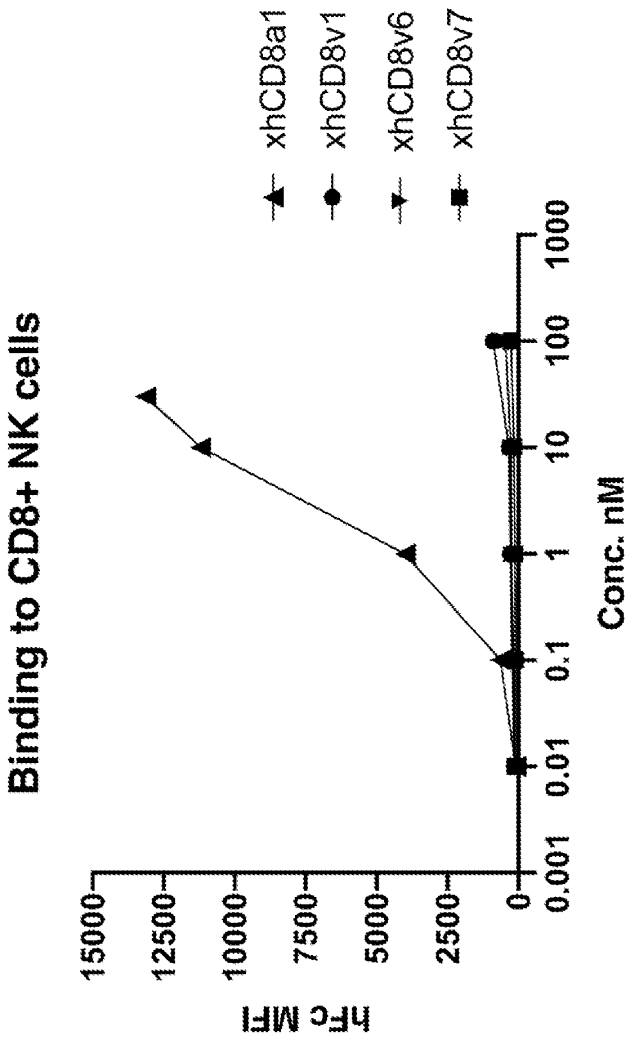

Binding to CD8+ NK cells
hFc MFI
15000
12500
10000
7500
5000
2500
0
0.001
0.01
0.1
1
10
100
1000
Conc. nM
xhCD8a1
xhCD8v1
xhCD8v6
xhCD8v7

FIG. 5

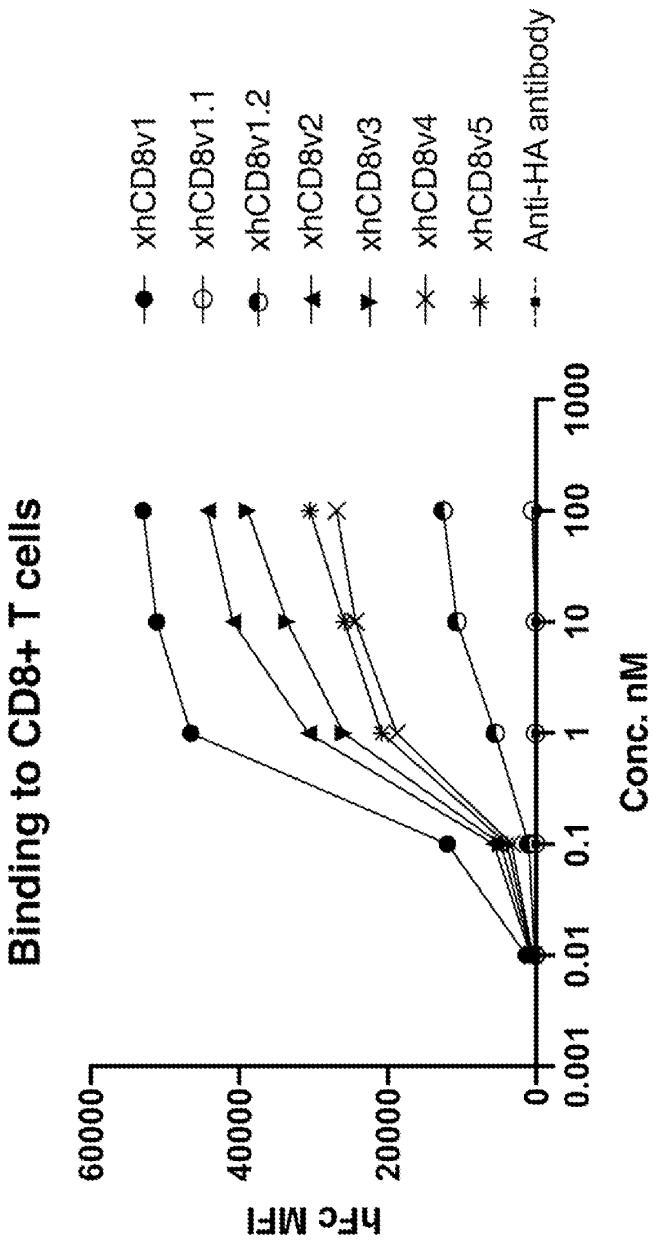

Binding to CD8+ T cells
hFc MFI
60000
40000
20000
0
0.001
0.01
0.1
1
10
100
1000
Conc. nM
xhCD8v1
xhCD8v1.1
xhCD8v1.2
xhCD8v2
xhCD8v3
xhCD8v4
xhCD8v5
Anti-HA antibody

FIG. 6

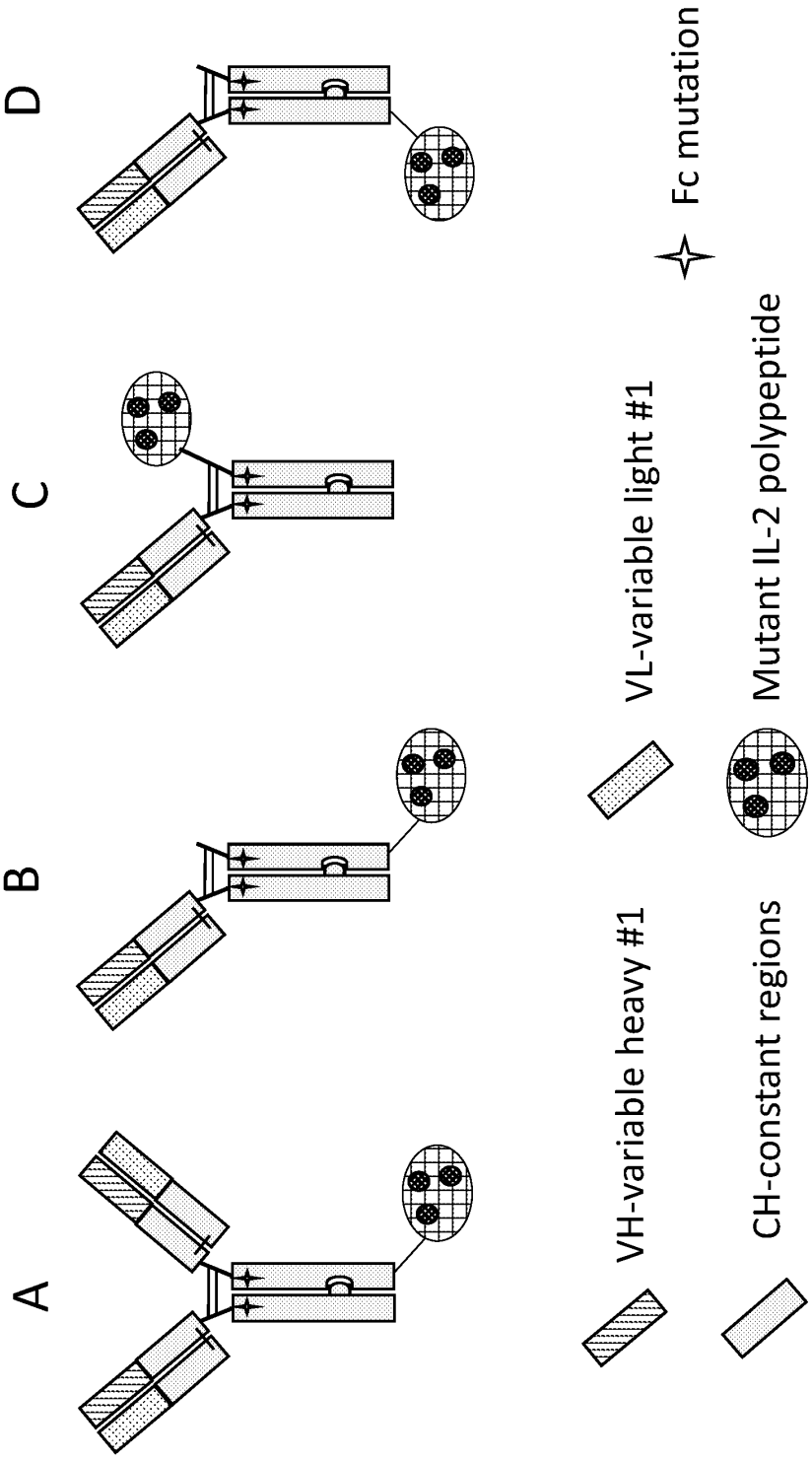

A
B
C
D
VH-variable heavy #1
VL-variable light #1
CH-constant regions
Mutant IL-2 polypeptide
Fc mutation

FIG. 7

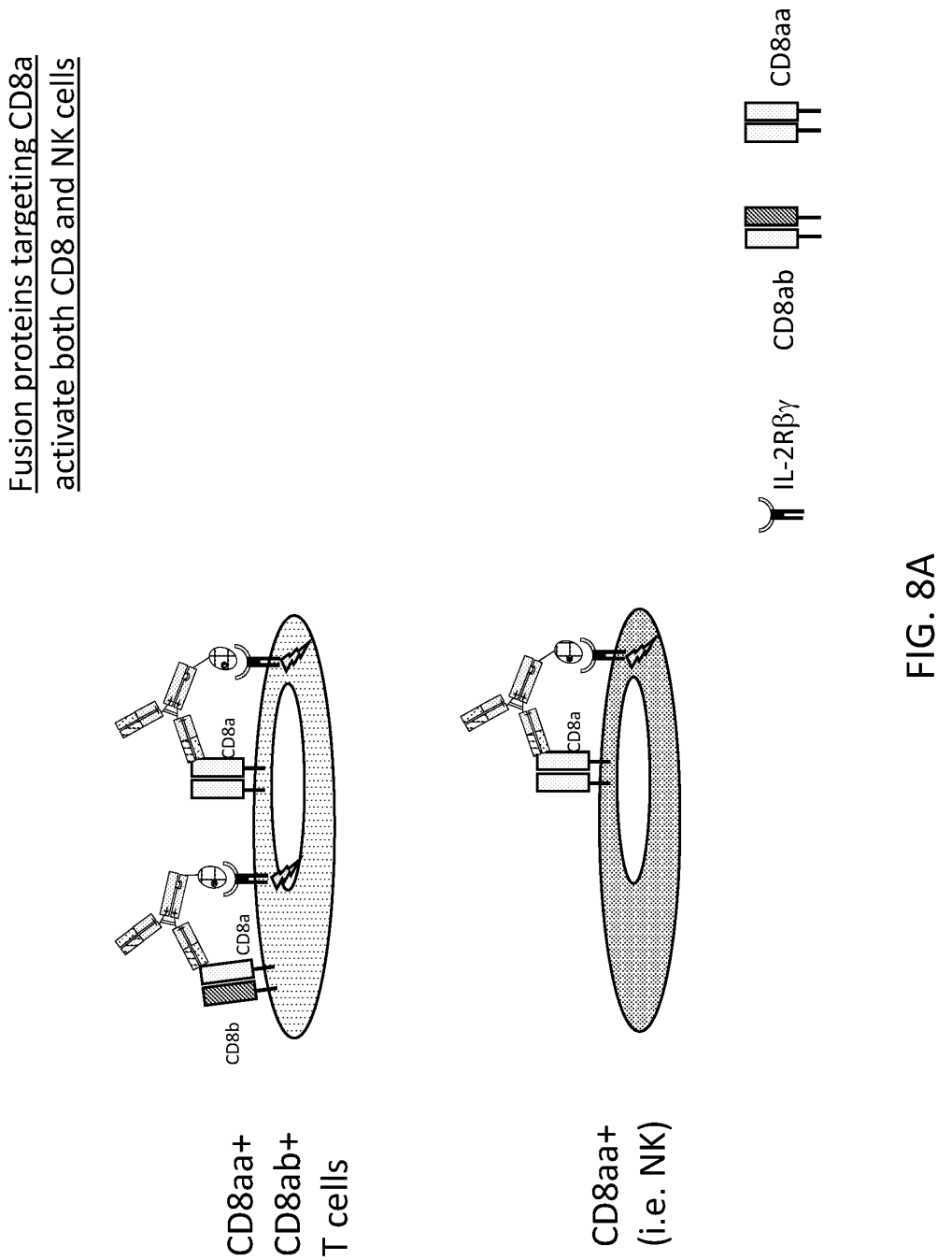

Fusion proteins targeting CD8a
activate both CD8 and NK cells
IL-2Rβγ
CD8ab
CD8aa
CD8a
CD8a
CD8b
CD8aa+
CD8ab+
T cells
CD8a
CD8aa+
(i.e. NK)

FIG. 8A

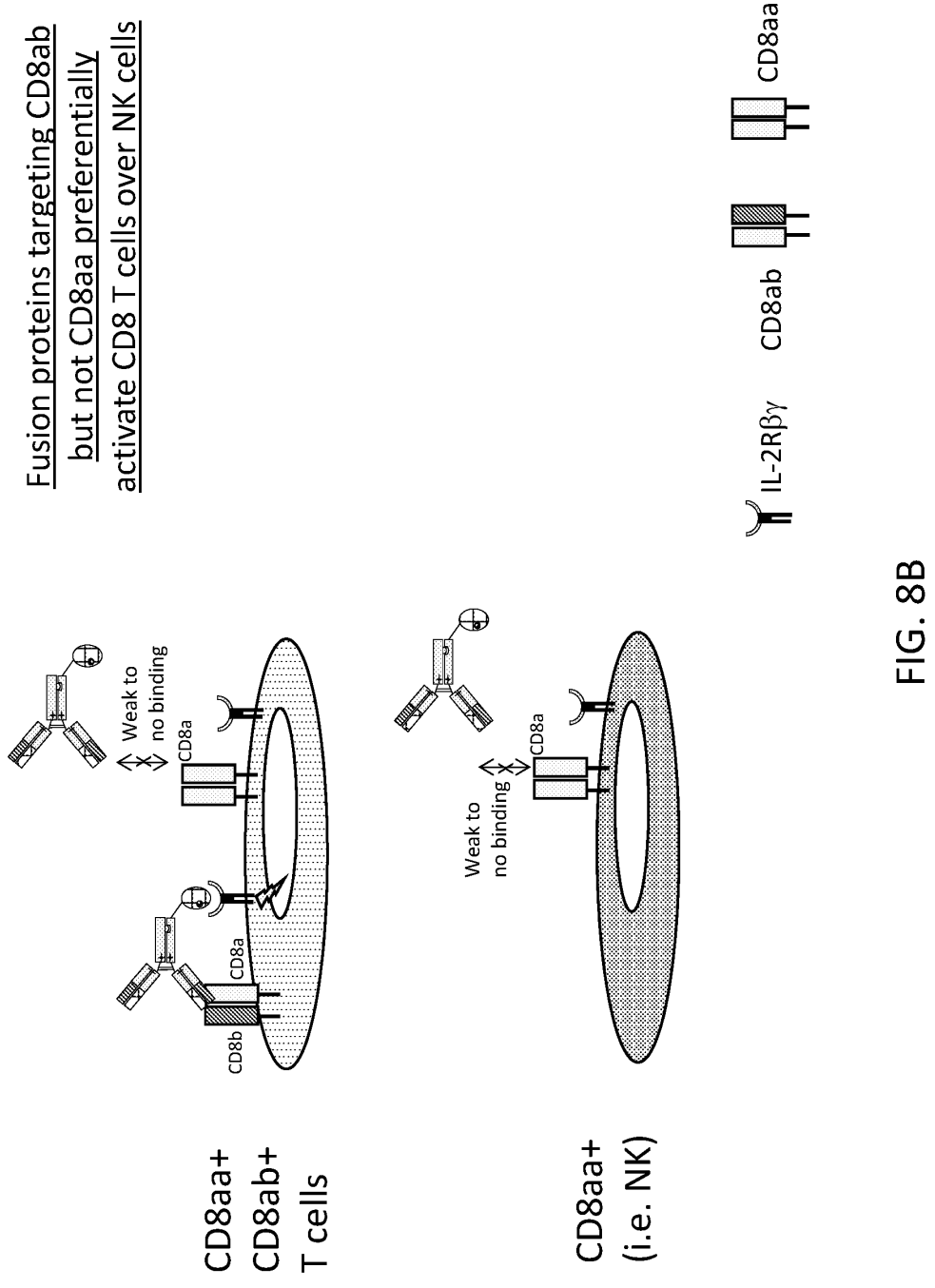

Fusion proteins targeting CD8ab
but not CD8aa preferentially
activate CD8 T cells over NK cells
Weak to
no binding
CD8a
CD8a
CD8b
CD8aa+
CD8ab+
T cells
Weak to
no binding
CD8a
CD8aa+
(i.e. NK)
IL-2Rβγ
CD8ab
CD8aa

FIG. 8B

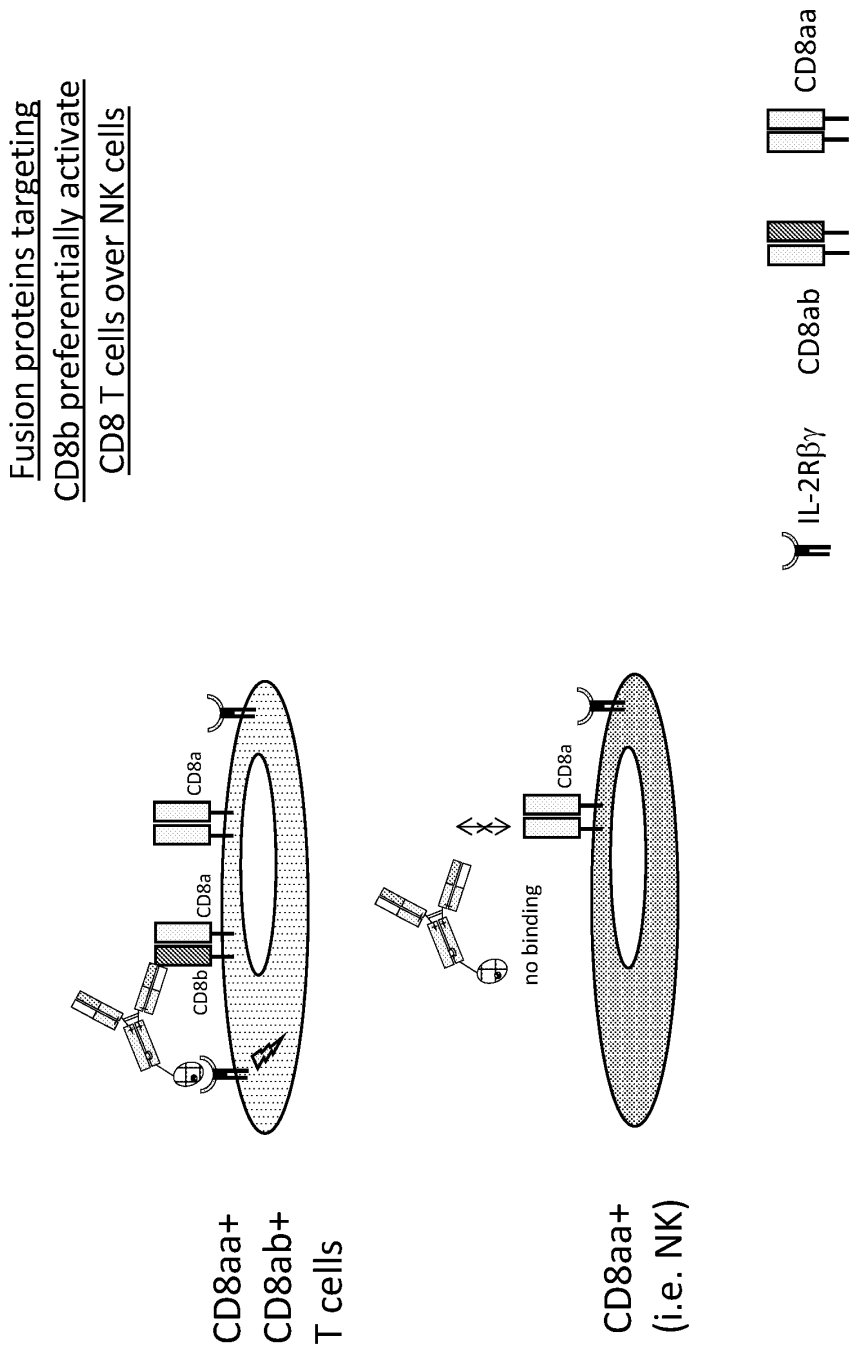

Fusion proteins targeting
CD8b preferentially activate
CD8 T cells over NK cells
CD8a
CD8a
CD8b
CD8aa+
CD8ab+
T cells
no binding
CD8a
CD8aa+
(i.e. NK)
IL-2Rβγ
CD8ab
CD8aa

FIG. 8C

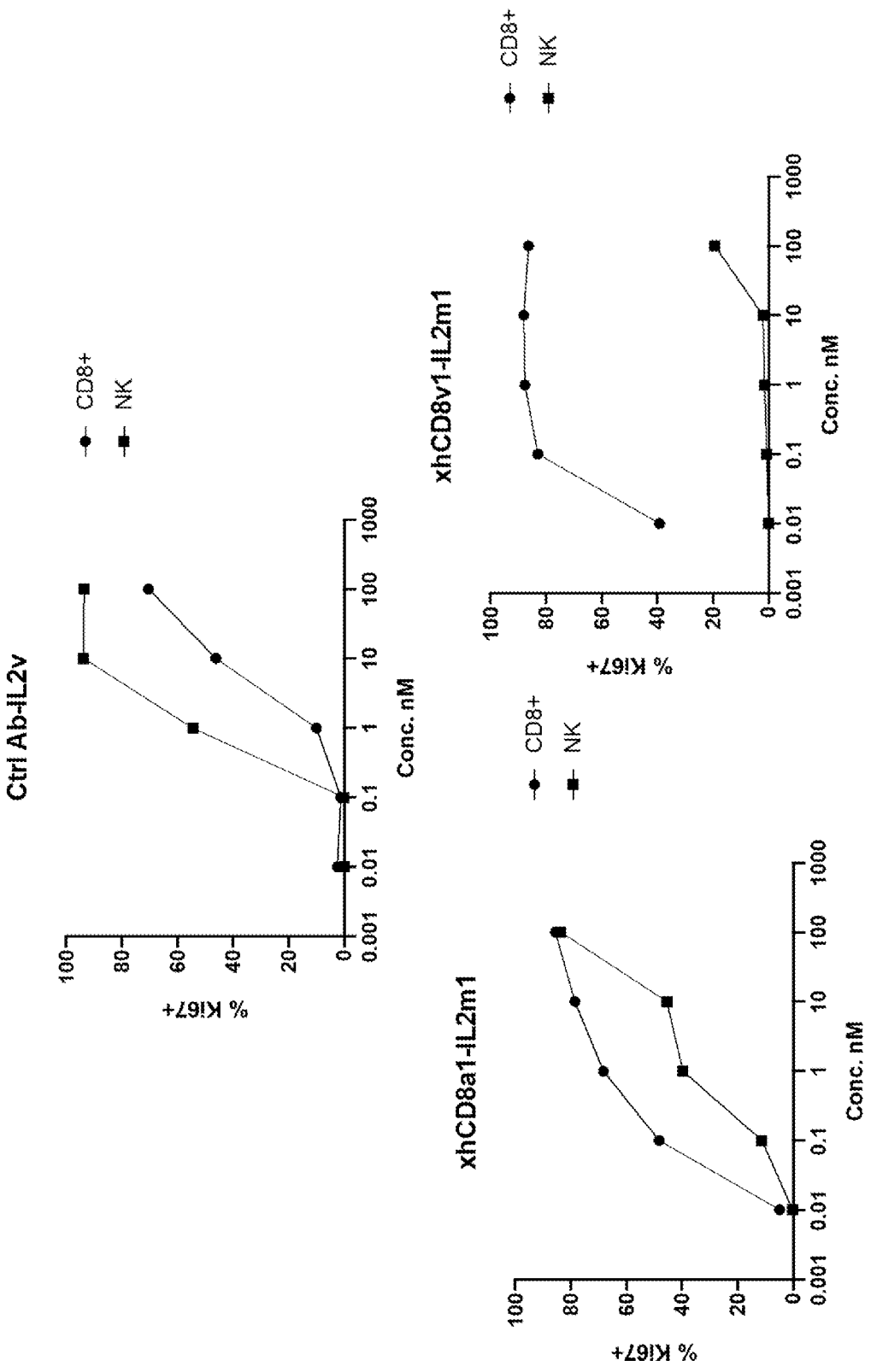

Ctrl Ab-IL2v
xhCD8v1-IL2m1
xhCD8a1-IL2m1
CD8+
NK
% KI67+
Conc. nM
0
20
40
60
80
100
0.001
0.01
0.1
1
10
100
1000

FIG. 9

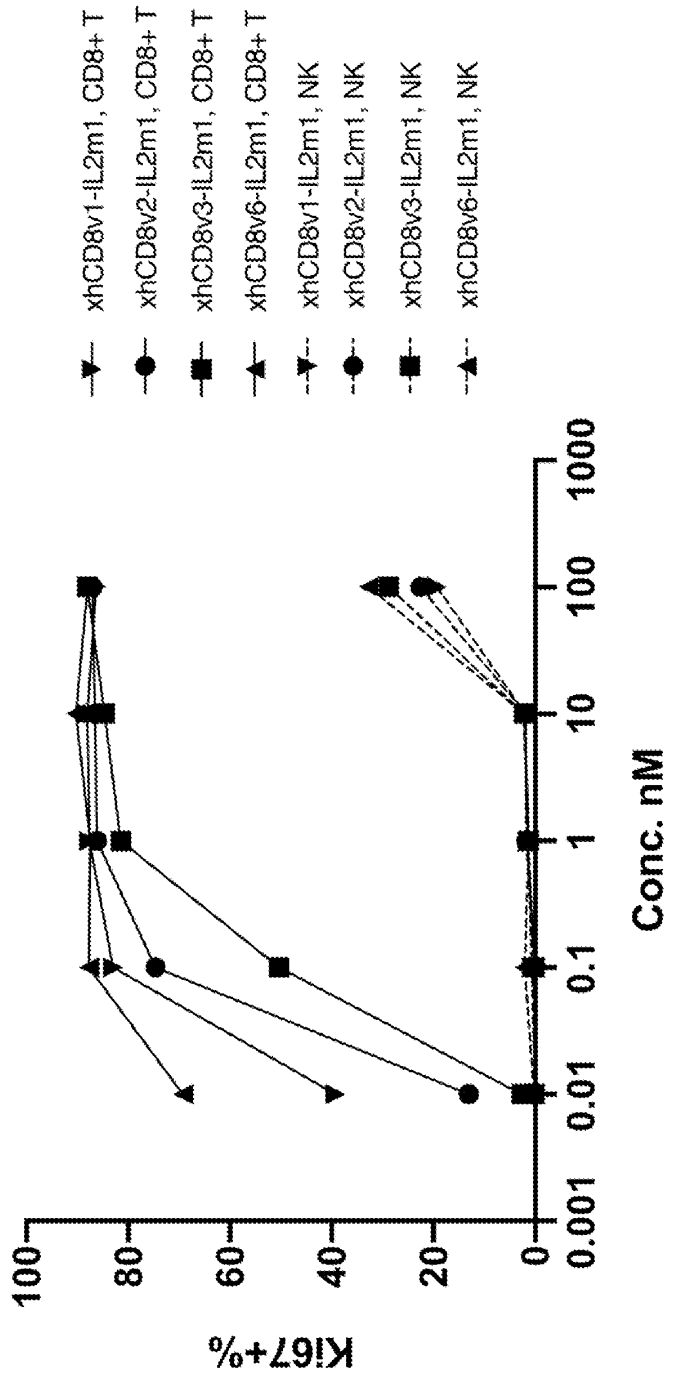

xhCD8v1-IL2m1, CD8+ T
xhCD8v2-IL2m1, CD8+ T
xhCD8v3-IL2m1, CD8+ T
xhCD8v6-IL2m1, CD8+ T
xhCD8v1-IL2m1, NK
xhCD8v2-IL2m1, NK
xhCD8v3-IL2m1, NK
xhCD8v6-IL2m1, NK
KI67+%
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
Conc. nM

FIG. 10A

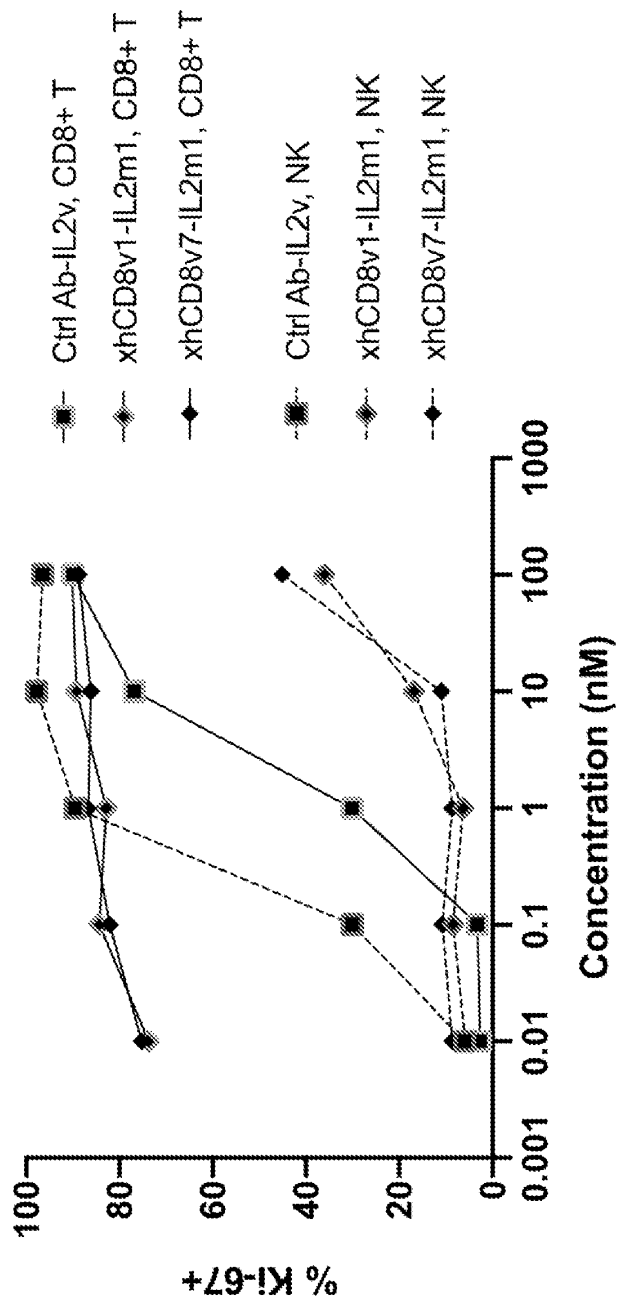

Ctrl Ab-IL2v, CD8+ T
xhCD8v1-IL2m1, CD8+ T
xhCD8v7-IL2m1, CD8+ T
Ctrl Ab-IL2v, NK
xhCD8v1-IL2m1, NK
xhCD8v7-IL2m1, NK
% Ki-67+
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 10B

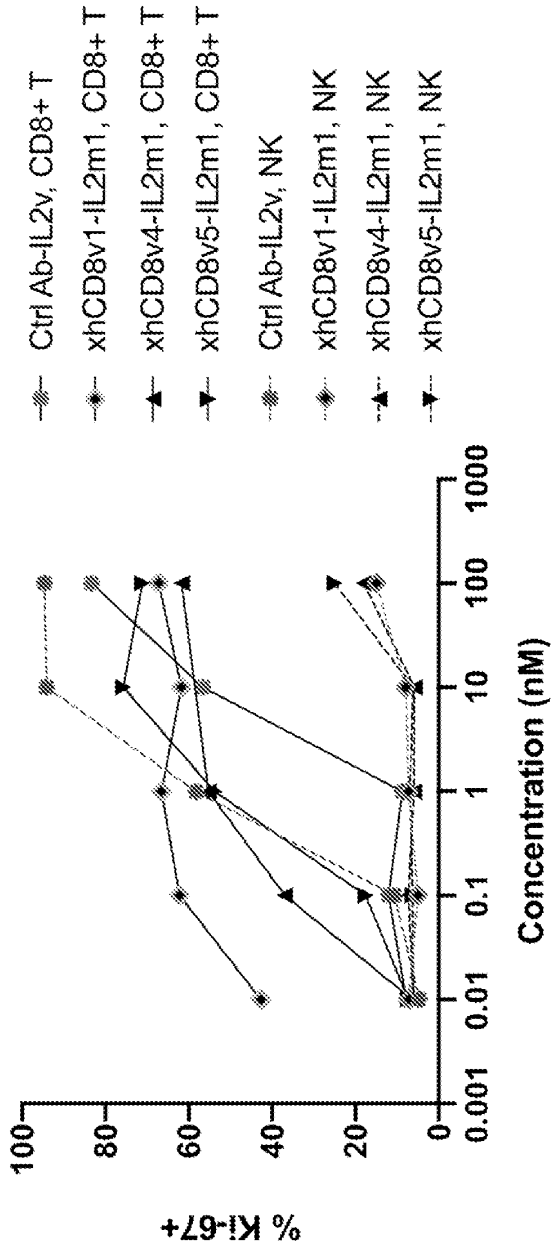

Ctrl Ab-IL2v, CD8+ T
xhCD8v1-IL2m1, CD8+ T
xhCD8v4-IL2m1, CD8+ T
xhCD8v5-IL2m1, CD8+ T
Ctrl Ab-IL2v, NK
xhCD8v1-IL2m1, NK
xhCD8v4-IL2m1, NK
xhCD8v5-IL2m1, NK
% Ki-67+
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 10C

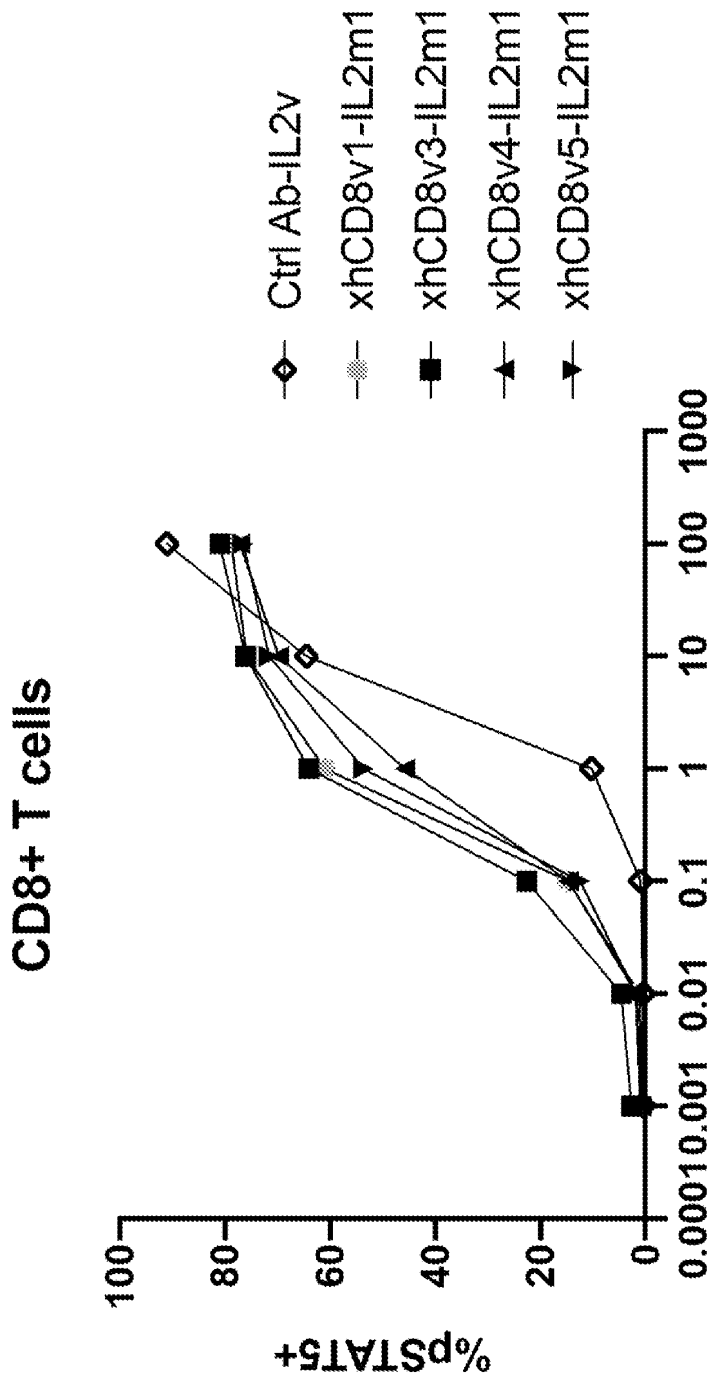

CD8+ T cells
%pSTAT5+
100
80
60
40
20
0
0.00010.001 0.01 0.1 1 10 100 1000
Concentration (nM)
Ctrl Ab-IL2v
xhCD8v1-IL2m1
xhCD8v3-IL2m1
xhCD8v4-IL2m1
xhCD8v5-IL2m1

FIG. 11A

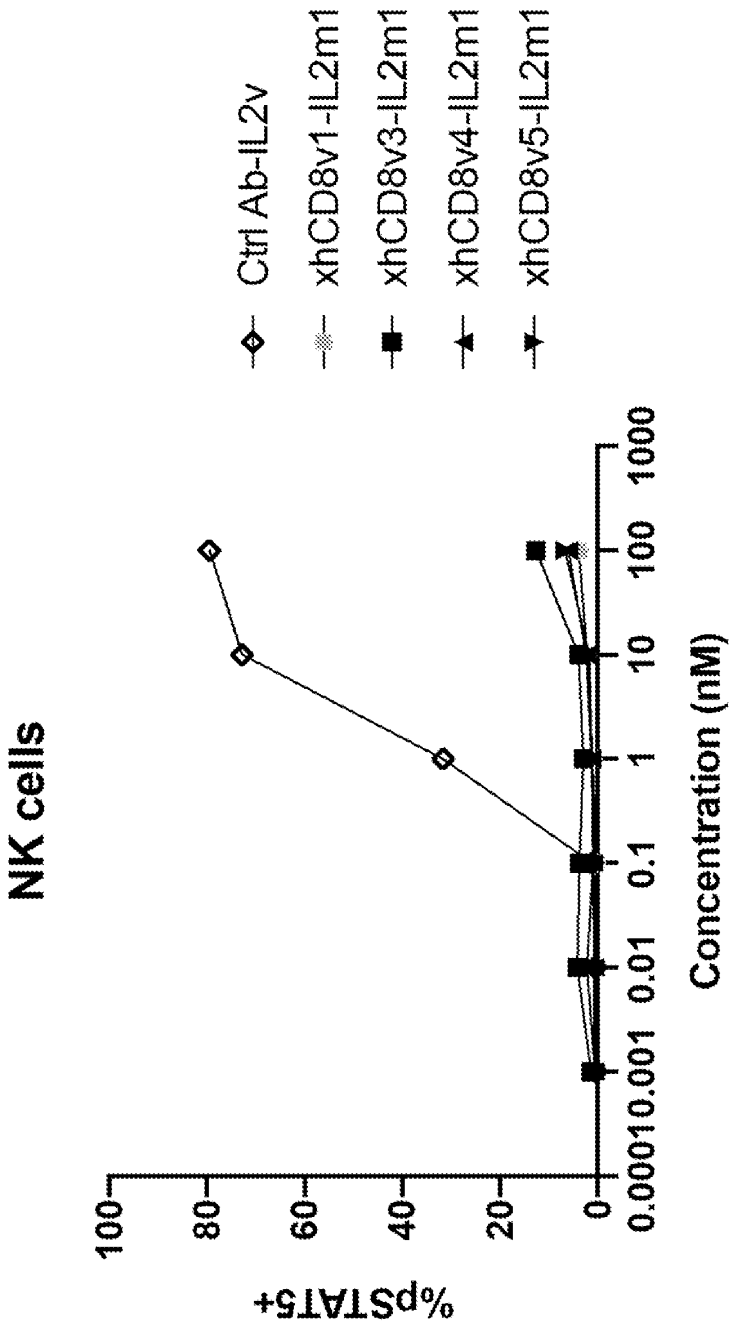

NK cells
%pSTAT5+
100
80
60
40
20
0
0.00010.001 0.01 0.1 1 10 100 1000
Concentration (nM)
Ctrl Ab-IL2v
xhCD8v1-IL2m1
xhCD8v3-IL2m1
xhCD8v4-IL2m1
xhCD8v5-IL2m1

FIG. 11B

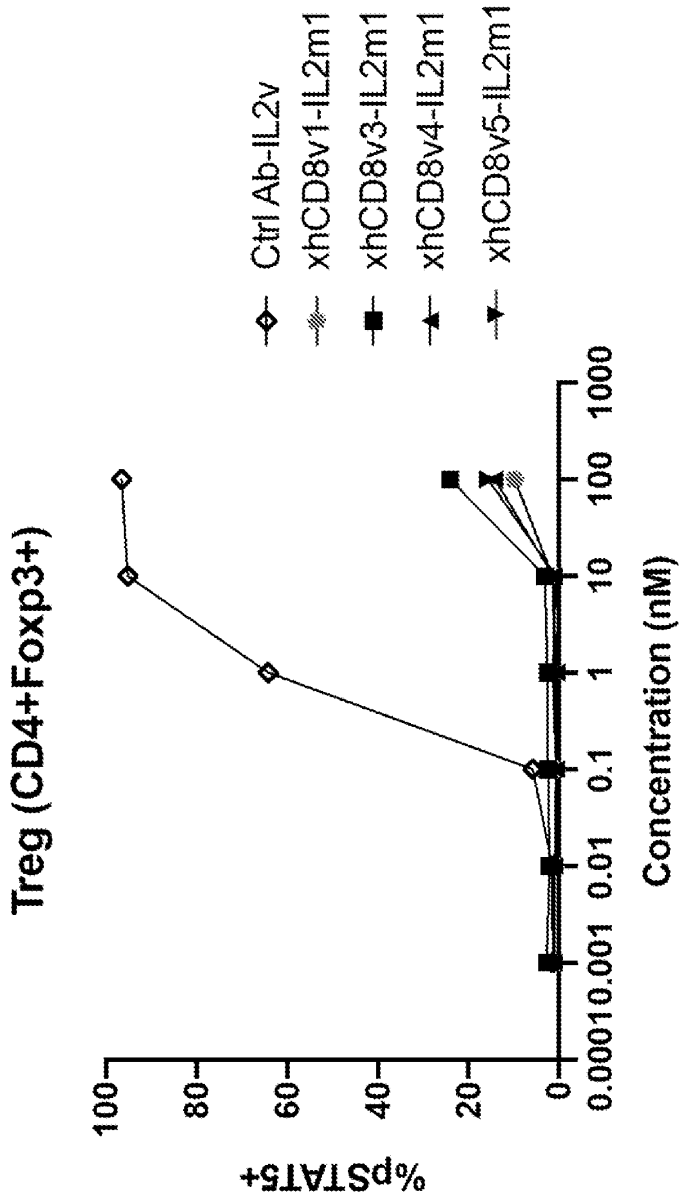

Treg (CD4+Foxp3+)
%pSTAT5+
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)
Ctrl Ab-IL2v
xhCD8v1-IL2m1
xhCD8v3-IL2m1
xhCD8v4-IL2m1
xhCD8v5-IL2m1

FIG. 11C

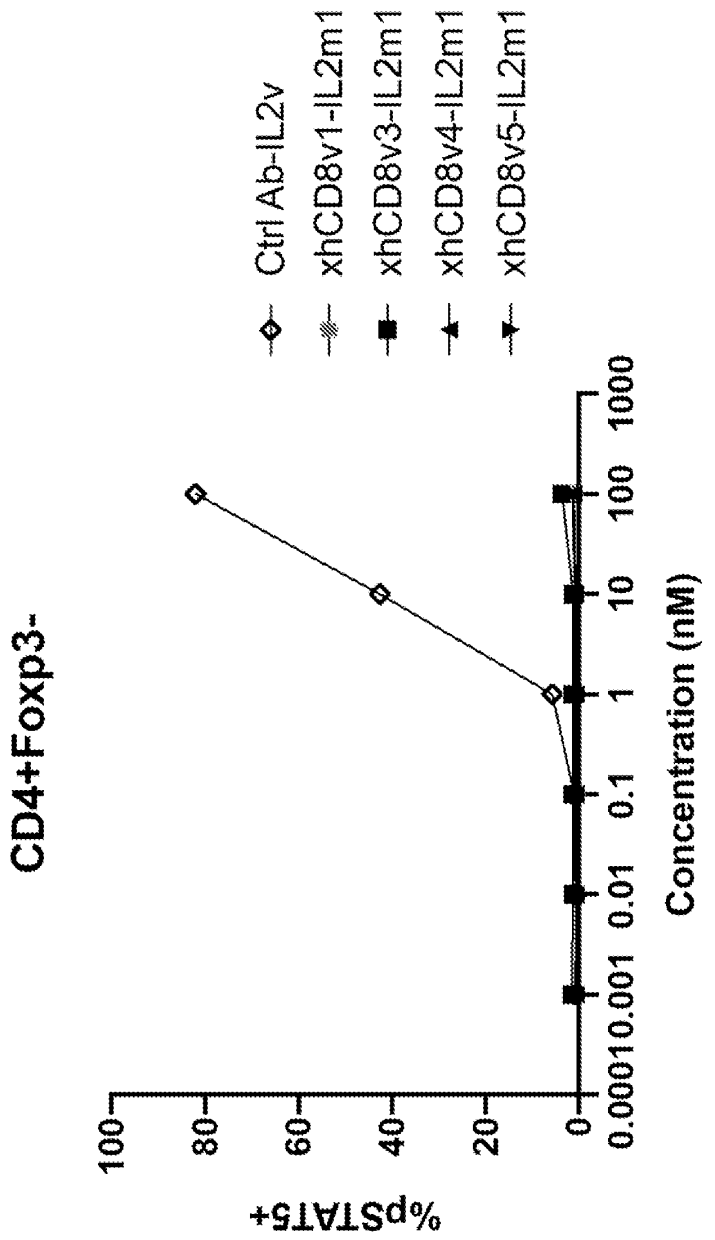

CD4+Foxp3-
%pSTAT5+
100
80
60
40
20
0
0.00010.001 0.01 0.1 1 10 100 1000
Concentration (nM)
Ctrl Ab-IL2v
xhCD8v1-IL2m1
xhCD8v3-IL2m1
xhCD8v4-IL2m1
xhCD8v5-IL2m1

FIG. 11D

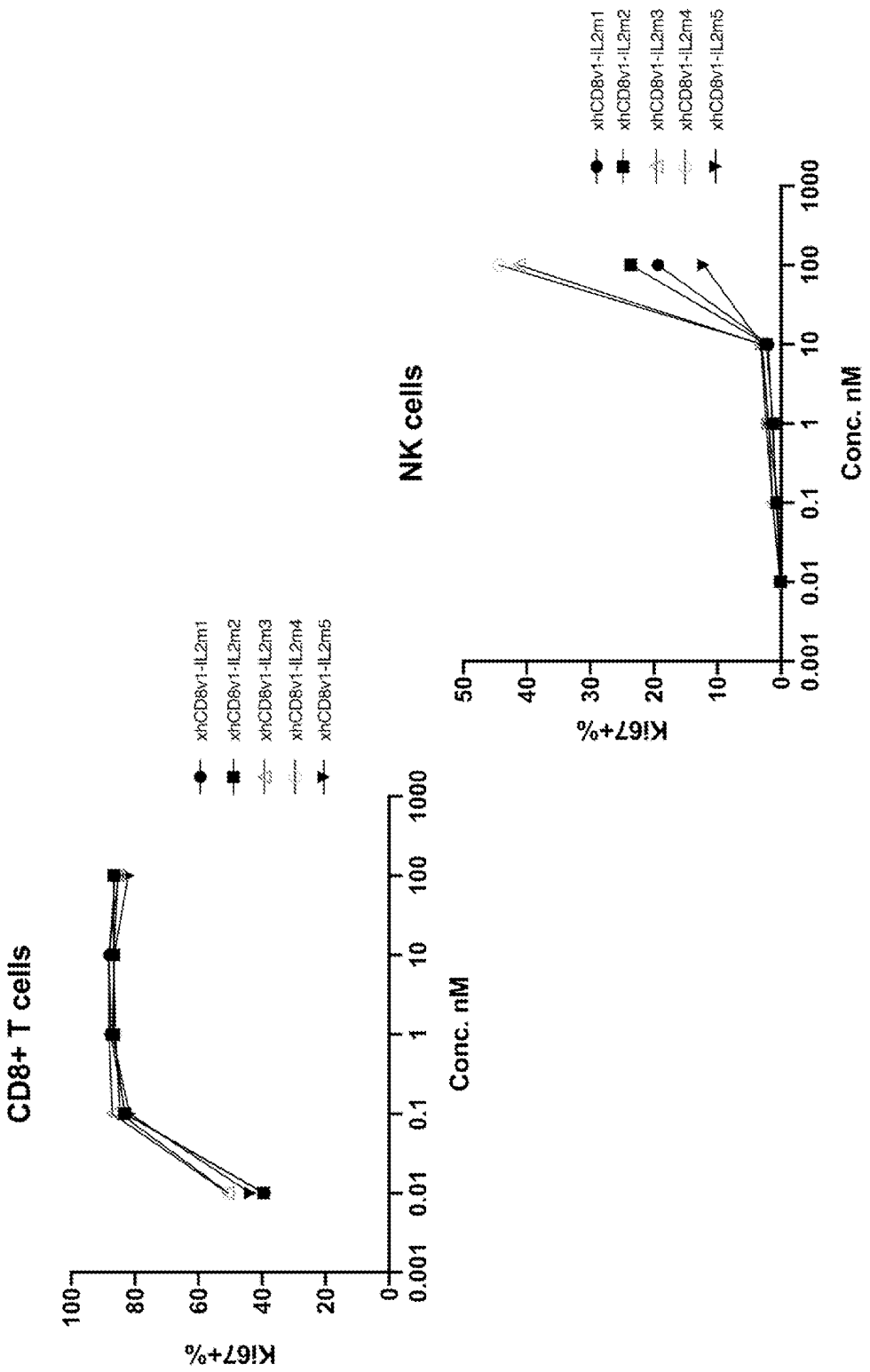

CD8+ T cells
NK cells
KI67+%
Conc. nM
0.001
0.01
0.1
1
10
100
1000
0
20
40
60
80
100
10
30
50
xhCD8v1-IL2m1
xhCD8v1-IL2m2
xhCD8v1-IL2m3
xhCD8v1-IL2m4
xhCD8v1-IL2m5

FIG. 12

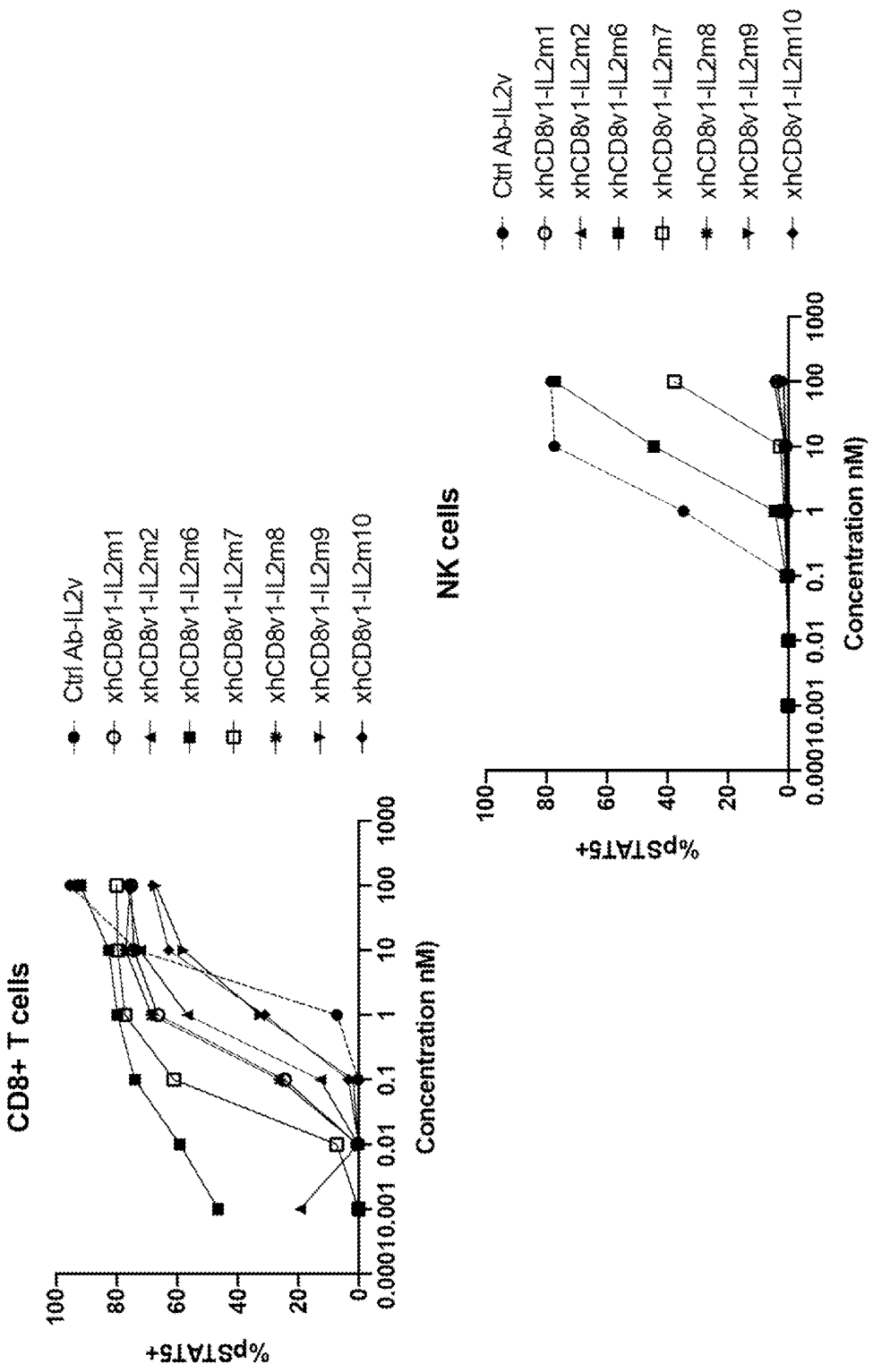

CD8+ T cells
%pSTAT5+
Concentration nM)
Ctrl Ab-IL2v
xhCD8v1-IL2m1
xhCD8v1-IL2m2
xhCD8v1-IL2m6
xhCD8v1-IL2m7
xhCD8v1-IL2m8
xhCD8v1-IL2m9
xhCD8v1-IL2m10
NK cells
%pSTAT5+
Concentration nM)
Ctrl Ab-IL2v
xhCD8v1-IL2m1
xhCD8v1-IL2m2
xhCD8v1-IL2m6
xhCD8v1-IL2m7
xhCD8v1-IL2m8
xhCD8v1-IL2m9
xhCD8v1-IL2m10

FIG. 13A

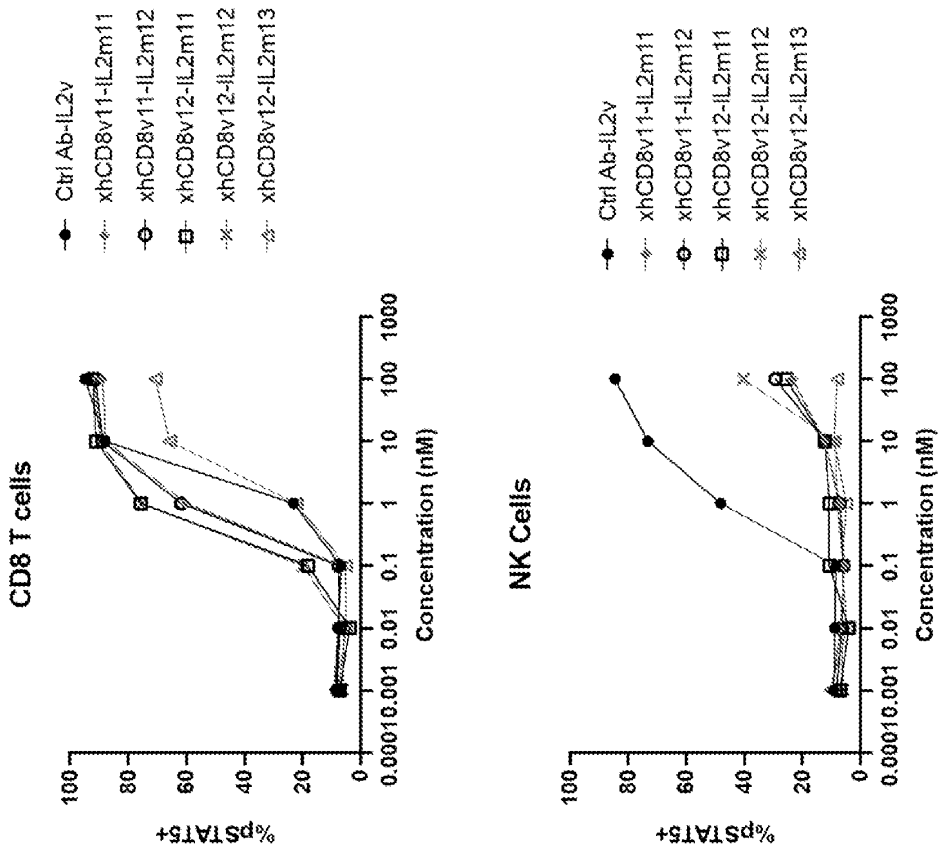

CD8 T cells
NK Cells
Ctrl Ab-IL2v
xhCD8v11-IL2m11
xhCD8v11-IL2m12
xhCD8v12-IL2m11
xhCD8v12-IL2m12
xhCD8v12-IL2m13
%pSTAT5+
Concentration (nM)
0
20
40
60
80
100
0.0001
0.001
0.01
0.1
1
10
100
1000

FIG. 13B

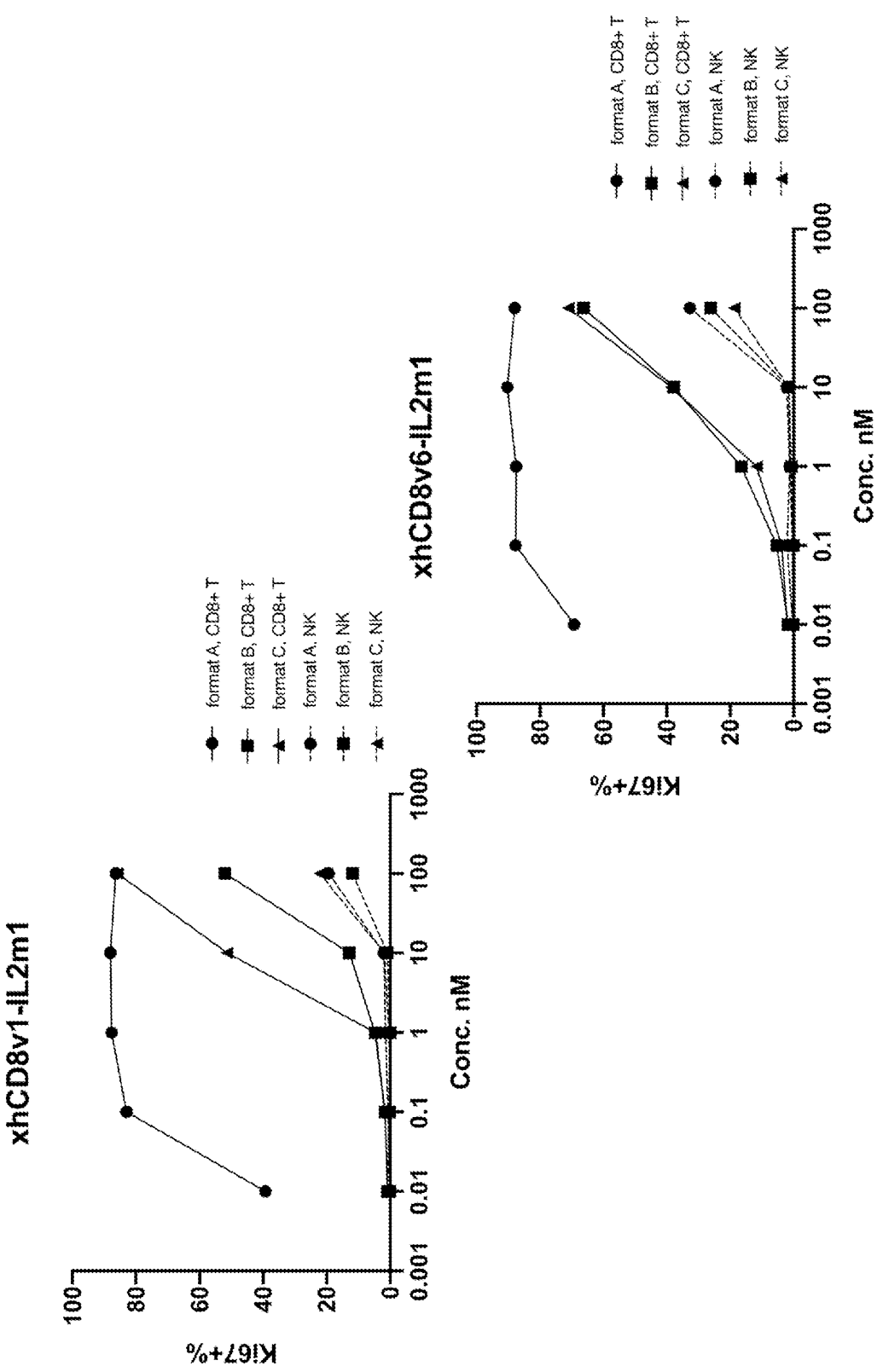

xhCD8v1-IL2m1
xhCD8v6-IL2m1
KI67+%
Conc. nM
0
20
40
60
80
100
0.001
0.01
0.1
1
10
100
1000
format A, CD8+ T
format B, CD8+ T
format C, CD8+ T
format A, NK
format B, NK
format C, NK

FIG. 14A

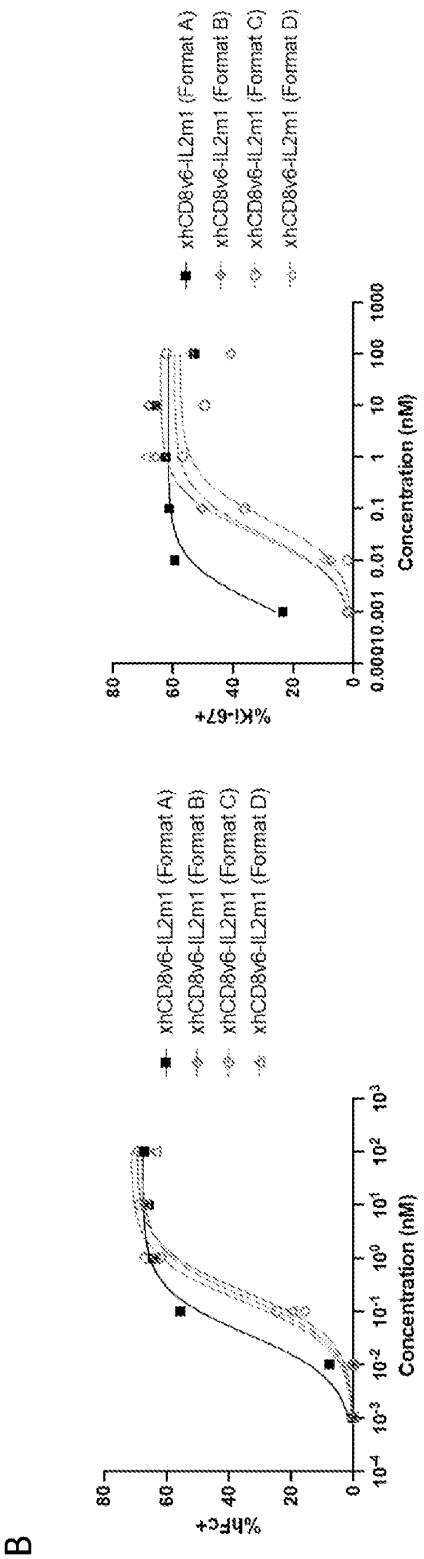

B
%hFc+
%Ki-67+
Concentration (nM)
xhCD8v6-IL2m1 (Format A)
xhCD8v6-IL2m1 (Format B)
xhCD8v6-IL2m1 (Format C)
xhCD8v6-IL2m1 (Format D)

FIG. 14B

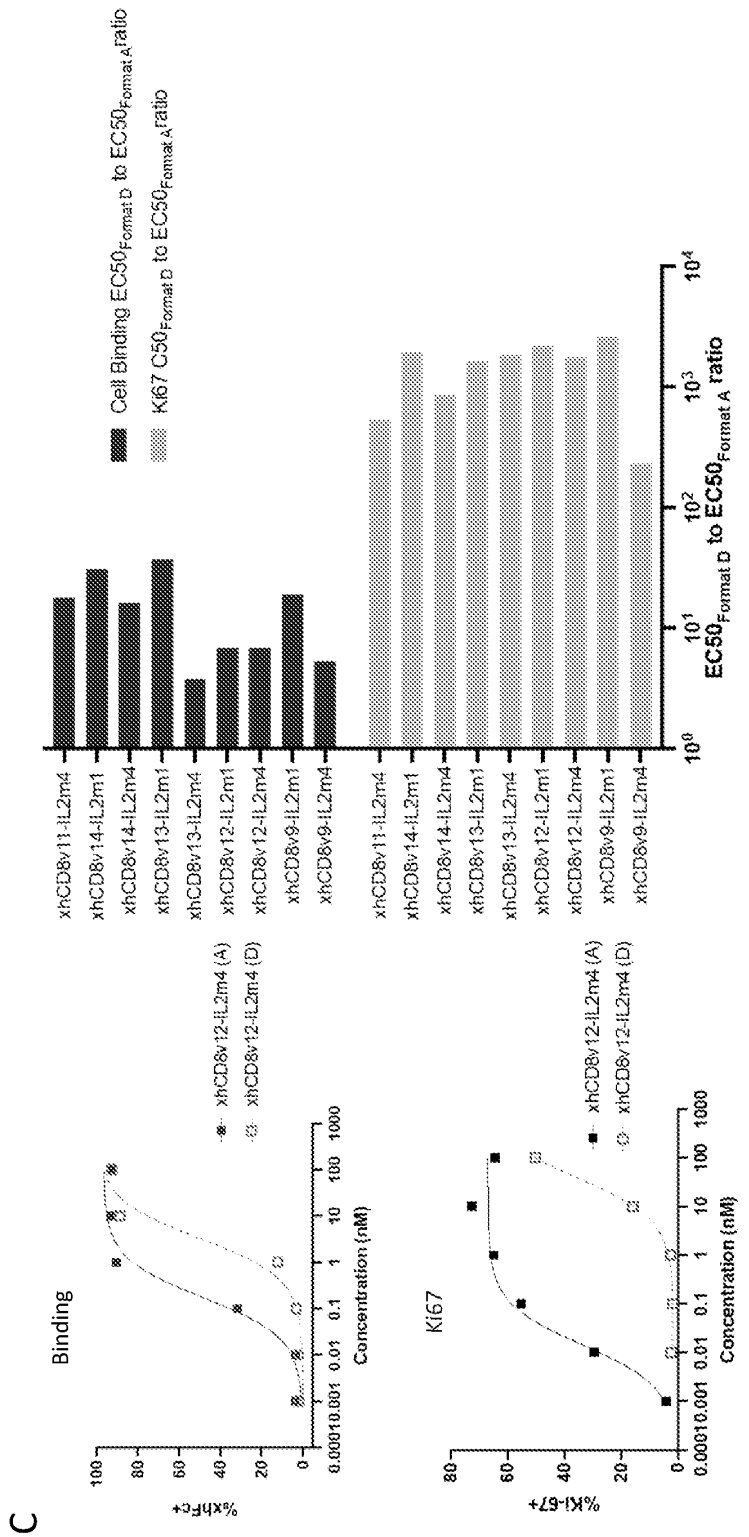

C
Binding
%xhFc+
Concentration (nM)
xhCD8v12-IL2m4 (A)
xhCD8v12-IL2m4 (D)
Ki67
%Ki-67+
Concentration (nM)
xhCD8v12-IL2m4 (A)
xhCD8v12-IL2m4 (D)
Cell Binding EC50Format D to EC50Format A ratio
Ki67 C50Format D to EC50Format A ratio
xhCD8v11-IL2m4
xhCD8v14-IL2m1
xhCD8v14-IL2m4
xhCD8v13-IL2m1
xhCD8v13-IL2m4
xhCD8v12-IL2m1
xhCD8v12-IL2m4
xhCD8v9-IL2m1
xhCD8v9-IL2m4
EC50Format D to EC50Format A ratio

FIG. 14C

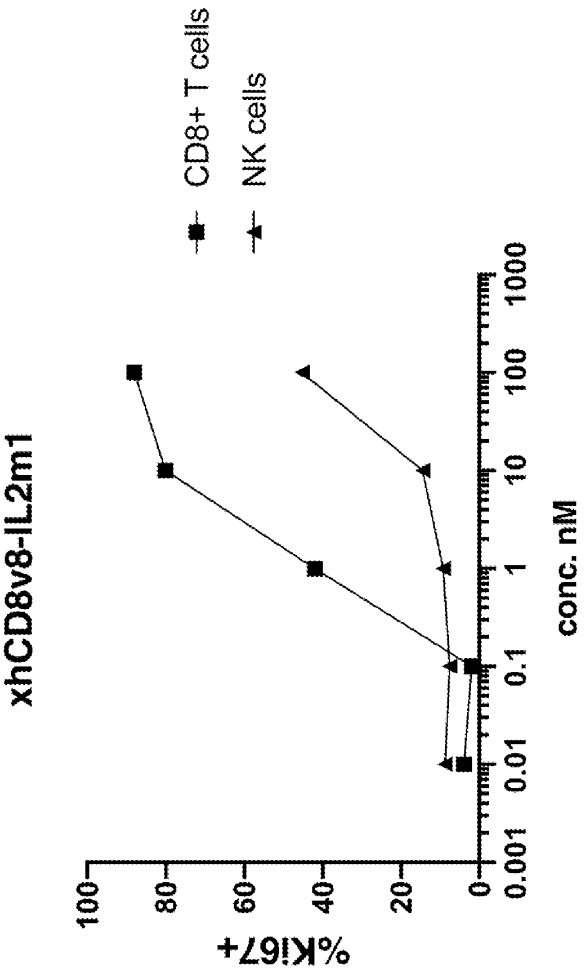
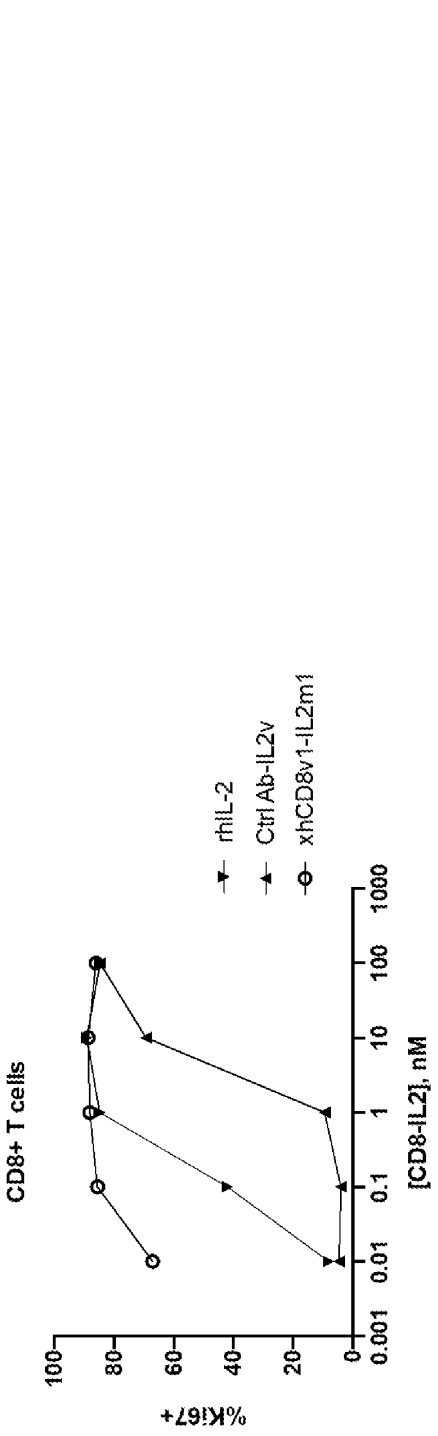

xhCD8v8-IL2m1
CD8+ T cells
NK cells
%Ki67+
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
conc. nM

FIG. 15


CD8+ T cells
rhIL-2
Ctrl Ab-IL2v
xhCD8v1-IL2m1
%Ki67+
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
[CD8-IL2], nM

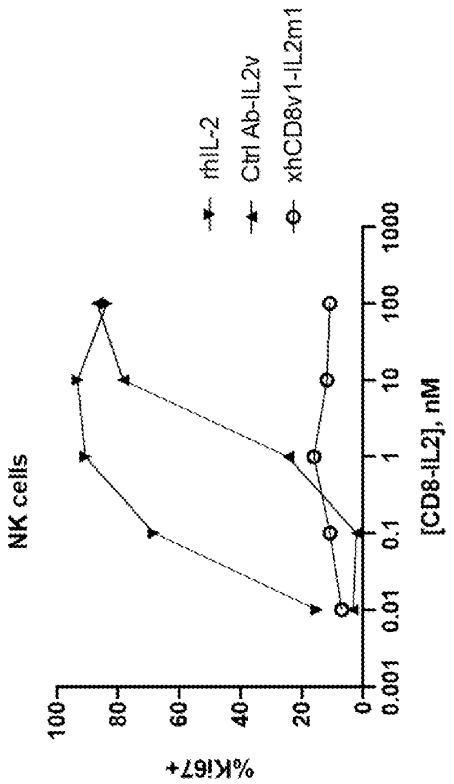

NK cells
rhIL-2
Ctrl Ab-IL2v
xhCD8v1-IL2m1
%Ki67+
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
1000
[CD8-IL2], nM

FIG. 16A

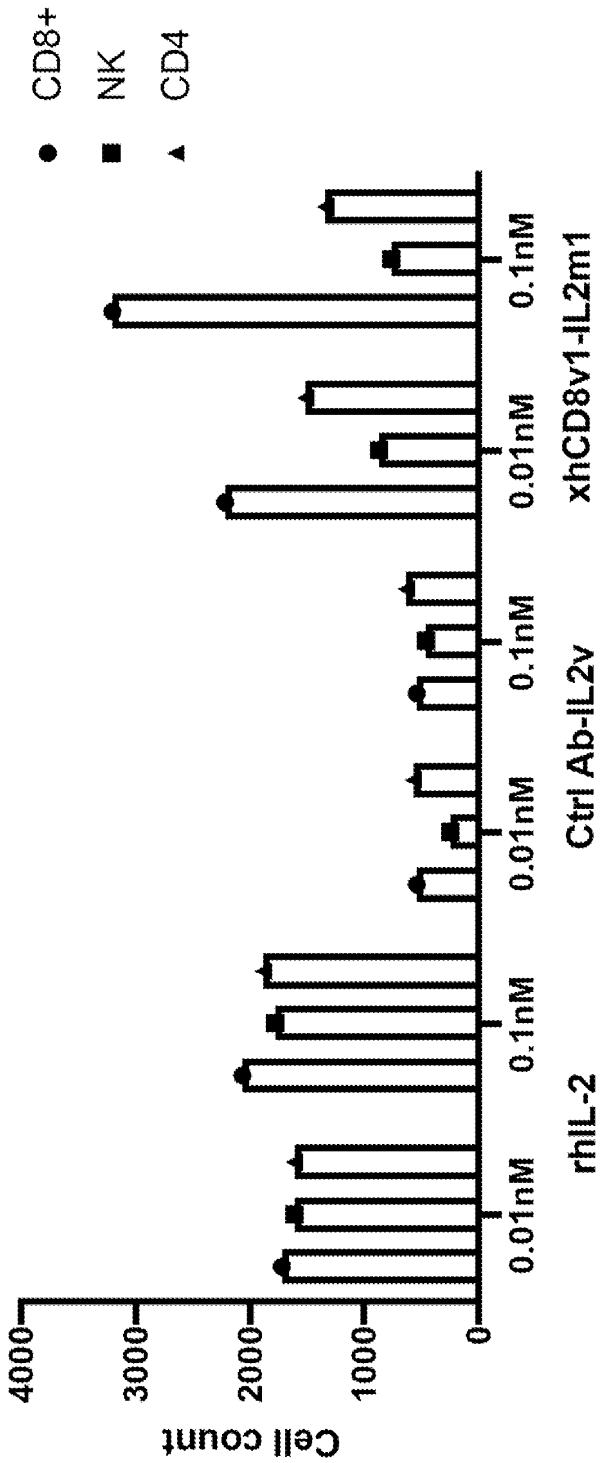

Tumor infiltrating immune cells
CD8+
NK
CD4
Cell count
4000
3000
2000
1000
0
0.01nM
0.1nM
0.01nM
0.1nM
0.01nM
0.1nM
rhIL-2
Ctrl Ab-IL2v
xhCD8v1-IL2m1

FIG. 16B

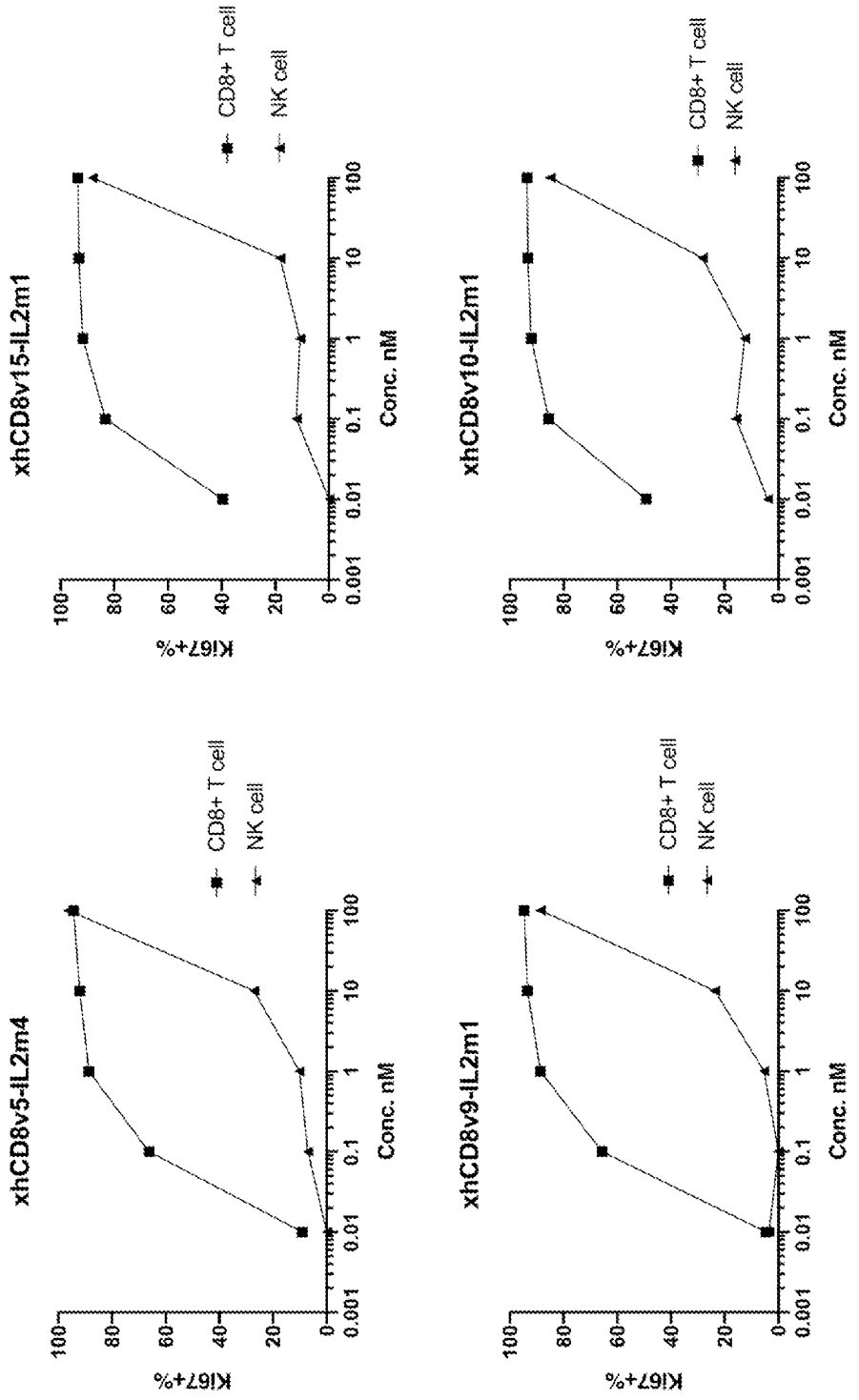

xhCD8v15-IL2m1
xhCD8v10-IL2m1
xhCD8v5-IL2m4
xhCD8v9-IL2m1
CD8+ T cell
NK cell
KI67+%
Conc. nM
0
20
40
60
80
100
0.001
0.01
0.1
1
10
100

FIG. 17A

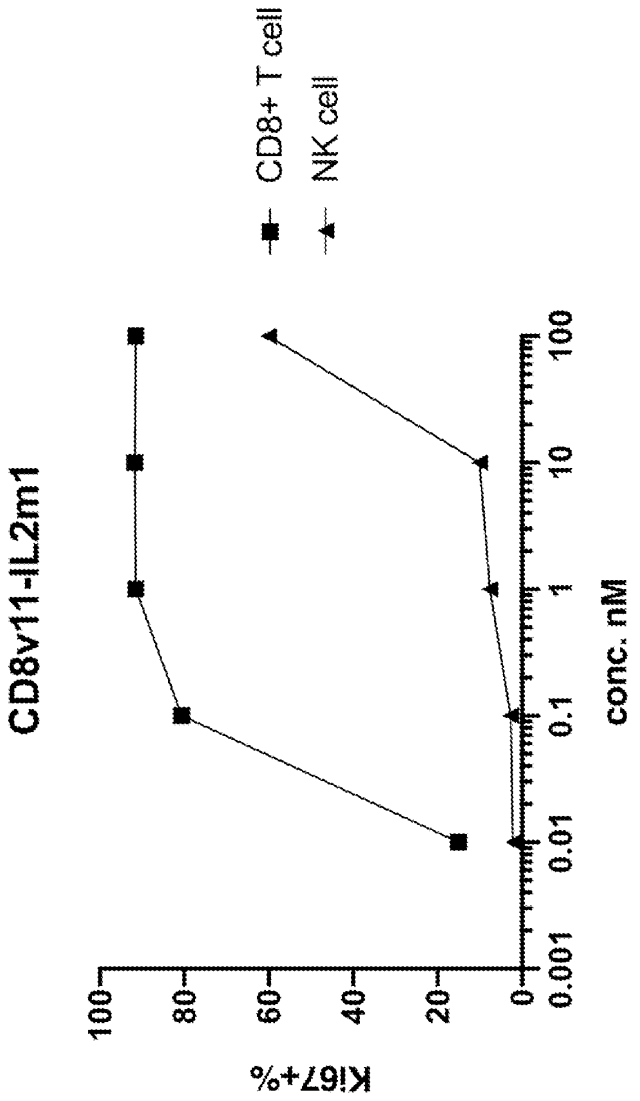

CD8v11-IL2m1
CD8+ T cell
NK cell
Ki67+%
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
conc. nM

FIG. 17B

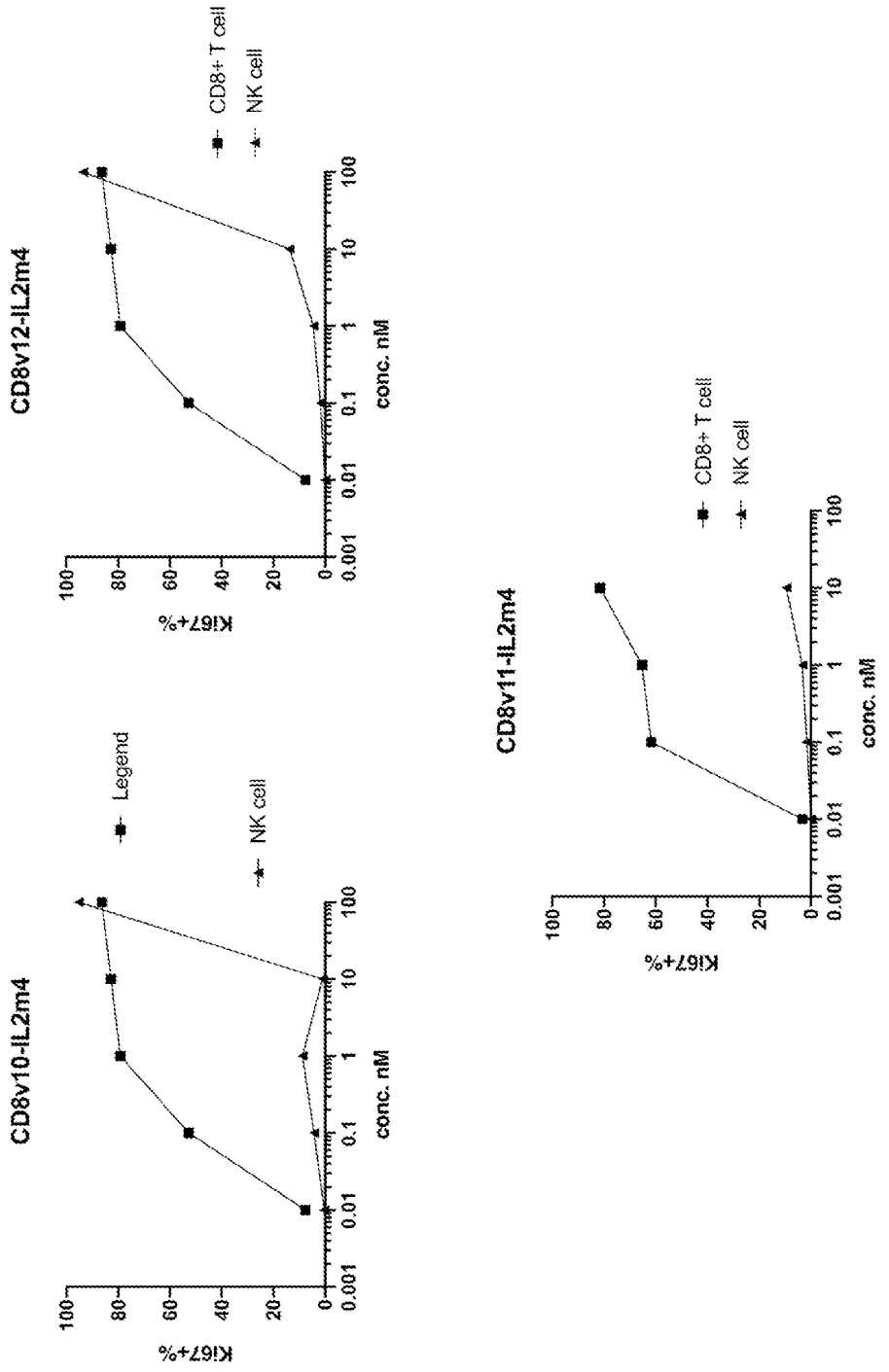

CD8v12-IL2m4
CD8v10-IL2m4
CD8v11-IL2m4
Legend
CD8+ T cell
NK cell
Ki67+%
conc. nM
0
20
40
60
80
100
0.001
0.01
0.1
1
10
100

FIG. 17C

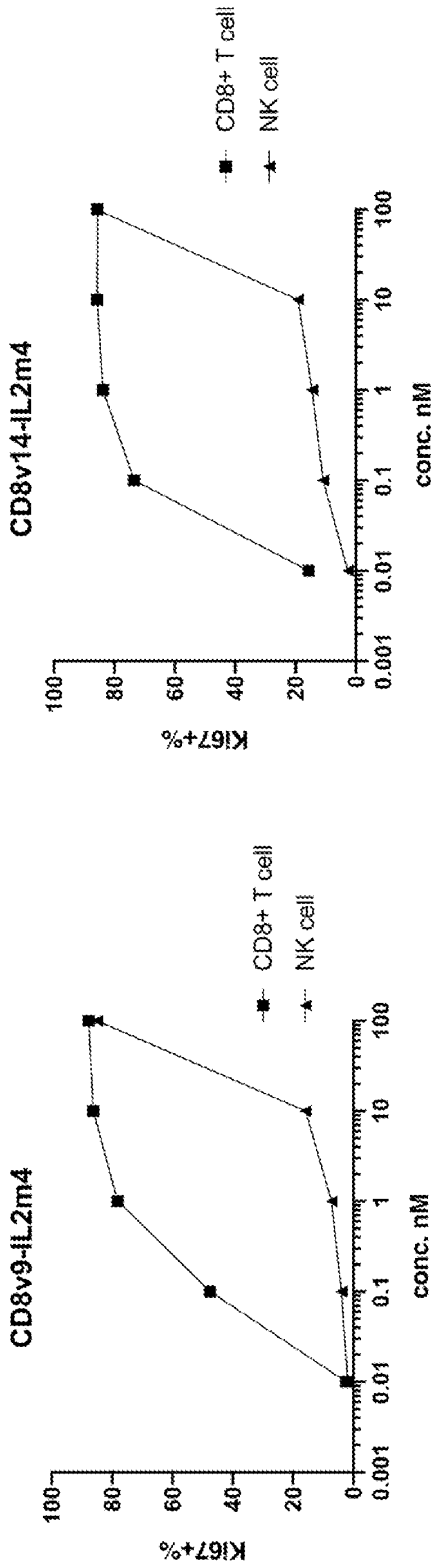

CD8v14-IL2m4
CD8+ T cell
NK cell
Ki67+%
conc. nM
0
20
40
60
80
100
0.001
0.01
0.1
1
10
100
CD8v9-IL2m4
CD8+ T cell
NK cell
Ki67+%
conc. nM

FIG. 17D

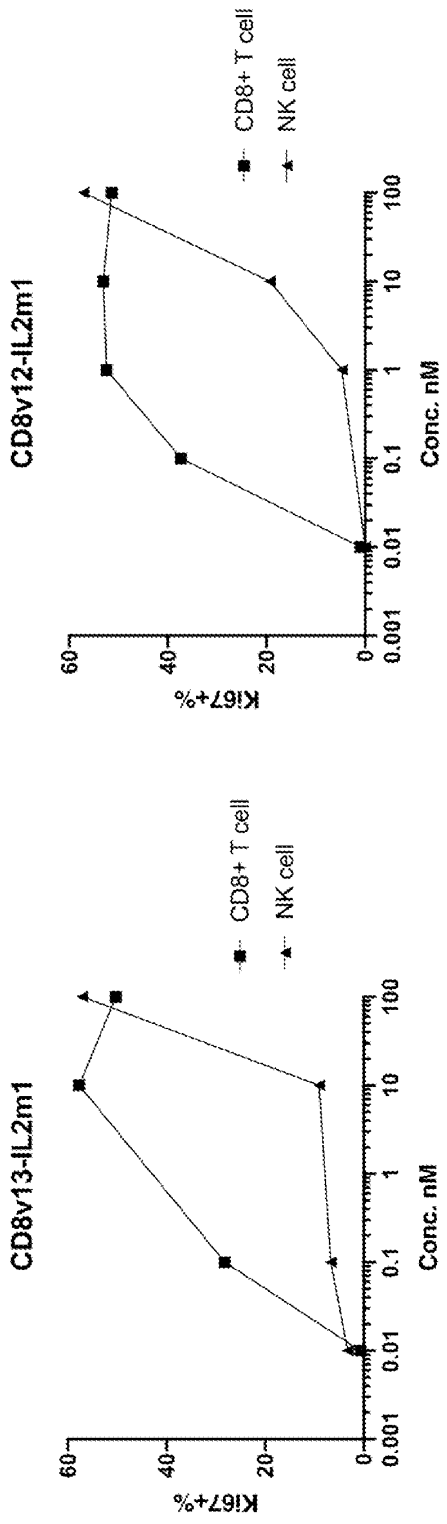

CD8v12-IL2m1
CD8+ T cell
NK cell
Ki67+%
Conc. nM
0
20
40
60
0.001
0.01
0.1
1
10
100
CD8v13-IL2m1
CD8+ T cell
NK cell
Ki67+%
Conc. nM

FIG. 17E

	# of amino acid liability	% similar to human germline				
		VH	# of a.a. mismatch	VK	# of a.a. mismatch	Total # of a.a. mismatch
xhCD8v2	2	91%	9	92%	7	16
xhCD8v3	1	91%	9	92%	7	16
xhCD8v4	2	93%	7	92%	7	14
xhCD8v5	3	91%	9	92%	7	16
xhCD8v6	5	86%	14	98%	2	16
xhCD8v9	0	97%	3	92%	7	10
xhCD8v10	2	92%	8	98%	2	10
xhCD8v11	2	92%	8	98%	2	10
xhCD8v12	0	97%	3	94%	5	8
xhCD8v13	0	97%	3	92%	7	10
xhCD8v14	2	93%	7	98%	2	9
xhCD8v15	2	93%	7	98%	2	9

FIG. 18A

```
xhCD8v1 VH          QVHLQQSGPELVKPGASVKMSCKTSGYTFTKYTMHWVKQGHEESLEWIGHFNPNNDETKY
VH1-69 germline     QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANY
xhCD8v1.1 VH        QVQLVQSGAEVKKPGSSVKVSCKASGYTFTKYAISWVRQAPGQGLEWMGHFNPNNDETKY
                    **:* ***.*: ***:***:***:** **:.*:: **:*.  :.***:* : *  . ::*

xhCD8v1 VH          NQKFTGKATLTVDKSSTTAYMELRSLTSDDSALYYCARDGLGLRLFADWGQGTLITVSA
VH1-69 germline     AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAR
xhCD8v1.1 VH        NQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDGLGLRLFADWGQGTLVTVSS
                    *** *:.*:*.*:*::****** ** *:*:*:*****

xhCD8v1 VK          DIQMTQSPASLSASVGETVTITCGASENIYGALNWYQRKQGKSPQLLIFGATNLADGVSS
VK1-39 germline     DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS
xhCD8v1.1 VK        DIQMTQSPSSLSASVGDRVTITCRASENIYGALNWYQQKPGKAPKLLIYGATNLADGVPS
                    ********:*******: ***** **:.* . *****:* **:*:***:.*:.* .**.*

xhCD8v1 VK          RFSGSGSDRQYSLKISSLHPDDVATYYCQNILDTPWTFGGGTKLEIK
VK1-39 germline     RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP
xhCD8v1.1 VK        RFSGSGSGTDFTLTISSLQPEDFATYYCQNILDTPWTFGGGTKLEIK
                    *******. :::*.****:*:*.******:  .**
```

FIG. 18B

```
xhCD8v1.1  QVQLVQSGAEVKKPGSSVKVSCKASGYTFTKYAISWVRQAPGQGLEWMGHFNPNNDETKY
xhCD8v2    QVQLVQSGAEVKKPGSSVKVSCKASGYRFHNFAISWVRQAPGQGLEWMGGIIPGHAKANY
xhCD8v3    QVQLVQSGAEVKKPGSSVKVSCKASGSRFYKFAISWVRQAPGQGLEWMGGIIPGHAKANY
xhCD8v4    QVQLVQSGAEVKKPGSSVKVSCKASGYTFTKYAISWVRQAPGQGLEWMGGIIPGHAKANY
xhCD8v5    QVQLVQSGAEVKKPGSSVKVSCKASGSGFRGHAISWVRQAPGQGLEWMGGIIPGHAKANY
xhCD8v9    QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPGAATANY
xhCD8v12   QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPGYATANY
xhCD8v13   QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPGYATANY
           ************************************ : *.    ::*

xhCD8v1.1  NQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDGLGLRLFADWGQGTLVTVSS
xhCD8v2    AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDGLGIRLFADWGQGTLVTVSS
xhCD8v3    AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDGLGIRLFADWGQGTLVTVSS
xhCD8v4    AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDGLGIRLFADWGQGTLVTVSS
xhCD8v5    AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDGLGIRLFADWGQGTLVTVSS
xhCD8v9    AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDAAGIRLFADWGQGTLVTVSS
xhCD8v12   AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDAAGIRLFADWGQGTLVTVSS
xhCD8v13   AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDAAGIRLFADWGQGTLVTVSS
           ************************************. *:*********************
```

FIG. 18C

```
xhCD8v1.1   DIQMTQSPSSLSASVGDRVTITCRASENIYGALNWYQQKPGKAPKLLIYGATNLADGVPS
xhCD8v2     DIQMTQSPSSLSASVGDRVTITCRASQEIYGALNWYQQKPGKAPKLLIYGATNLQSGVPS
xhCD8v3     DIQMTQSPSSLSASVGDRVTITCRASQEIYGALNWYQQKPGKAPKLLIYGATNLQSGVPS
xhCD8v4     DIQMTQSPSSLSASVGDRVTITCRASQKIYGALNWYQQKPGKAPKLLIYGATNLQSGVPS
xhCD8v5     DIQMTQSPSSLSASVGDRVTITCRASQKIYGALNWYQQKPGKAPKLLIYGATNLQSGVPS
xhCD8v9     DIQMTQSPSSLSASVGDRVTITCRASQEIYGALNWYQQKPGKAPKLLIYGATNLQSGVPS
xhCD8v12    DIQMTQSPSSLSASVGDRVTITCRASQSIYGALNWYQQKPGKAPKLLIYGASNLQSGVPS
xhCD8v13    DIQMTQSPSSLSASVGDRVTITCRASQEIYGALNWYQQKPGKAPKLLIYGATNLQSGVPS
            ************************:.**********************:**  .****

xhCD8v1.1   RFSGSGSGTDFTLTISSLQPEDFATYYCQNILDTPWTFGGGTKLEIK
xhCD8v2     RFSGSGSGTDFTLTISSLQPEDFATYYCQDIYDAPWTFGGGTKVEIK
xhCD8v3     RFSGSGSGTDFTLTISSLQPEDFATYYCQDIYDAPWTFGGGTKVEIK
xhCD8v4     RFSGSGSGTDFTLTISSLQPEDFATYYCQNTYDTPWTFGGGTKVEIK
xhCD8v5     RFSGSGSGTDFTLTISSLQPEDFATYYCQNTYDTPWTFGGGTKVEIK
xhCD8v9     RFSGSGSGTDFTLTISSLQPEDFATYYCQSTYDAPWTFGGGTKVEIK
xhCD8v12    RFSGSGSGTDFTLTISSLQPEDFATYYCQSTYTAPWTFGGGTKVEIK
xhCD8v13    RFSGSGSGTDFTLTISSLQPEDFATYYCQSTYDAPWTFGGGTKVEIK
            **********************.    :**********:***
```

FIG. 18D

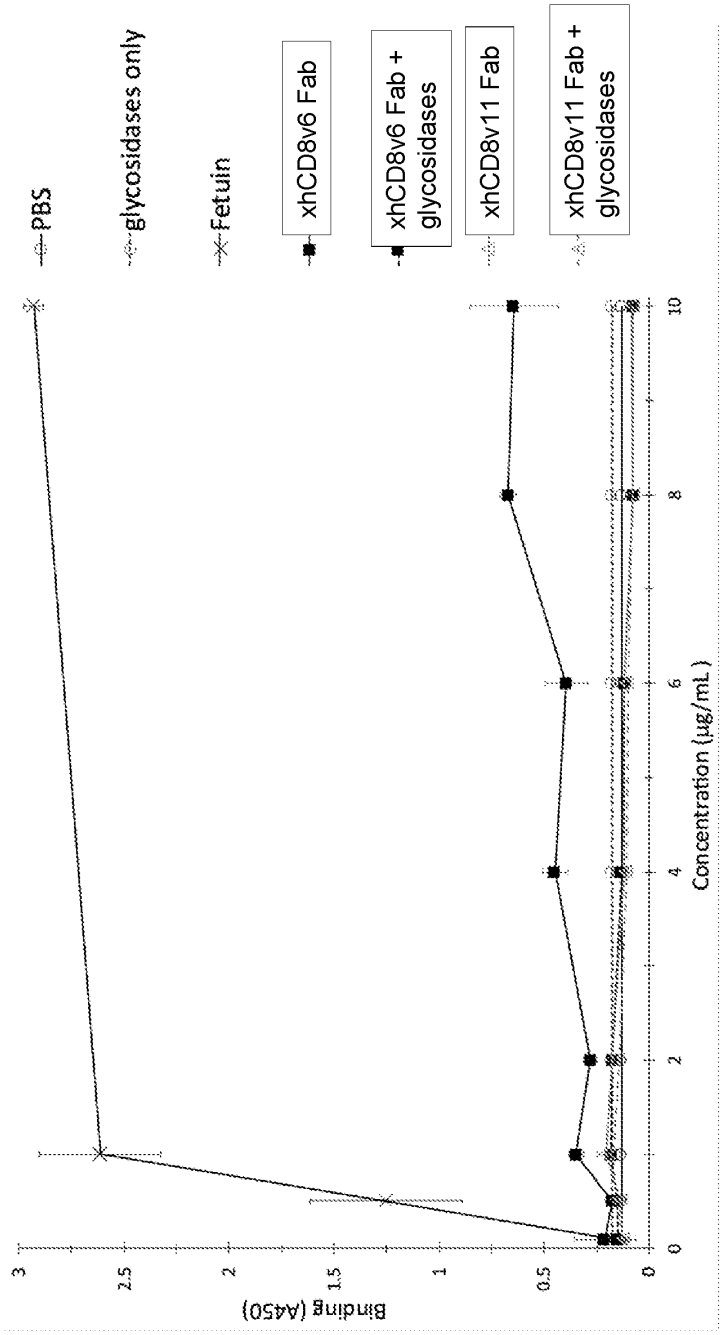

PBS
glycosidases only
Fetuin
xhCD8v6 Fab
xhCD8v6 Fab + glycosidases
xhCD8v11 Fab
xhCD8v11 Fab + glycosidases
Binding (A450)
Concentration (µg/mL)
0
0.5
1
1.5
2
2.5
3
0
2
4
6
8
10

FIG. 19