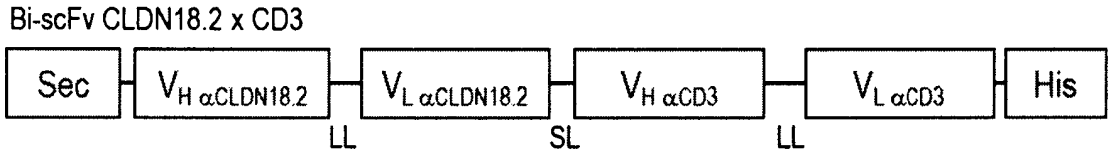
Figure 1

A


Bi-scFv CLDN18.2 x CD3
Sec
$V_{H\ \alpha CLDN18.2}$
$V_{L\ \alpha CLDN18.2}$
$V_{H\ \alpha CD3}$
$V_{L\ \alpha CD3}$
His
LL
SL
LL

B

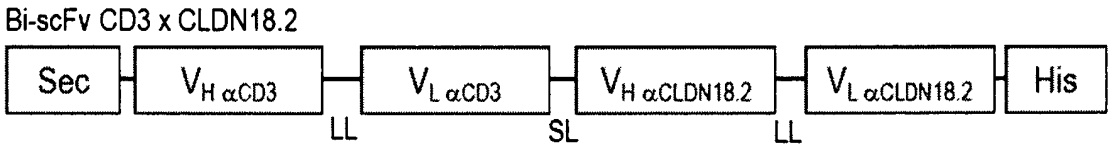

Bi-scFv CD3 x CLDN18.2
Sec
$V_{H\ \alpha CD3}$
$V_{L\ \alpha CD3}$
$V_{H\ \alpha CLDN18.2}$
$V_{L\ \alpha CLDN18.2}$
His
LL
SL
LL

Figure 2

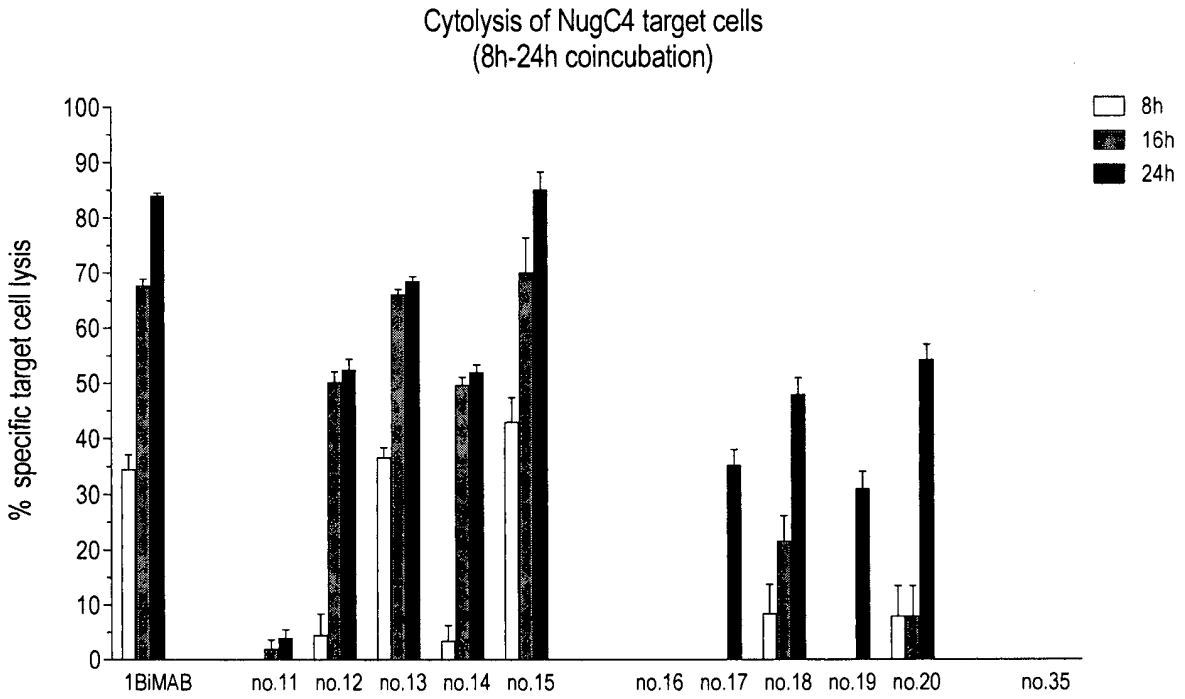
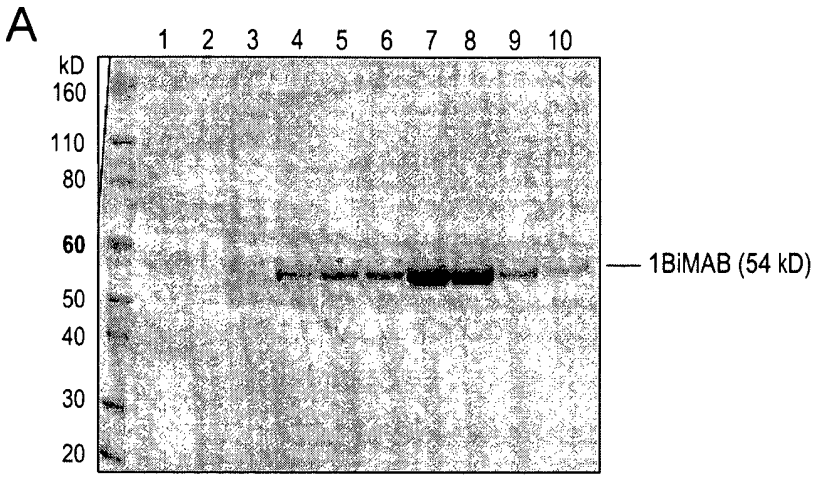

Cytolysis of NugC4 target cells
(8h-24h coincubation)
% specific target cell lysis
100
90
80
70
60
50
40
30
20
10
0
8h
16h
24h
1BiMAB
no.11
no.12
no.13
no.14
no.15
no.16
no.17
no.18
no.19
no.20
no.35

Figure 3


A
1 2 3 4 5 6 7 8 9 10
kD
160
110
80
60
50
40
30
20
1BiMAB (54 kD)

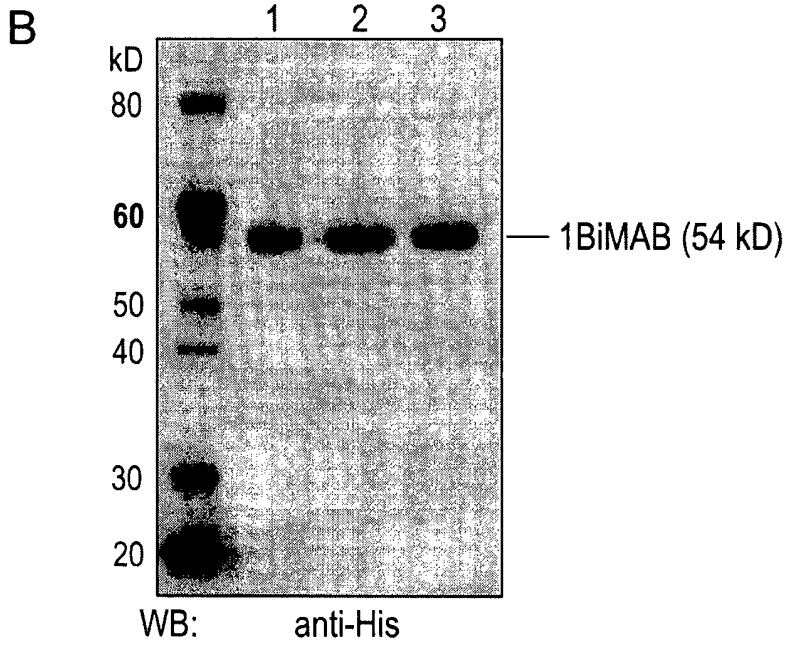
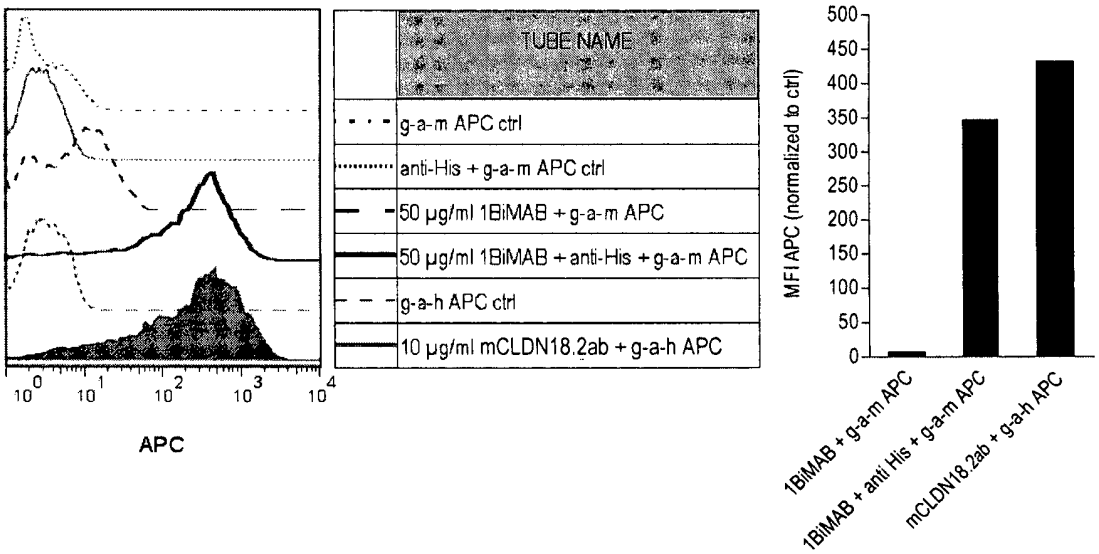

B
1 2 3
kD
80
60
50
40
30
20
1BiMAB (54 kD)
WB: anti-His

Figure 4

A


NugC4 cells
TUBE NAME
g-a-m APC ctrl
anti-His + g-a-m APC ctrl
50 µg/ml 1BiMAB + g-a-m APC
50 µg/ml 1BiMAB + anti-His + g-a-m APC
g-a-h APC ctrl
10 µg/ml mCLDN18.2ab + g-a-h APC
APC
MFI APC (normalized to ctrl)
1BiMAB + g-a-m APC
1BiMAB + anti His + g-a-m APC
mCLDN18.2ab + g-a-h APC

B

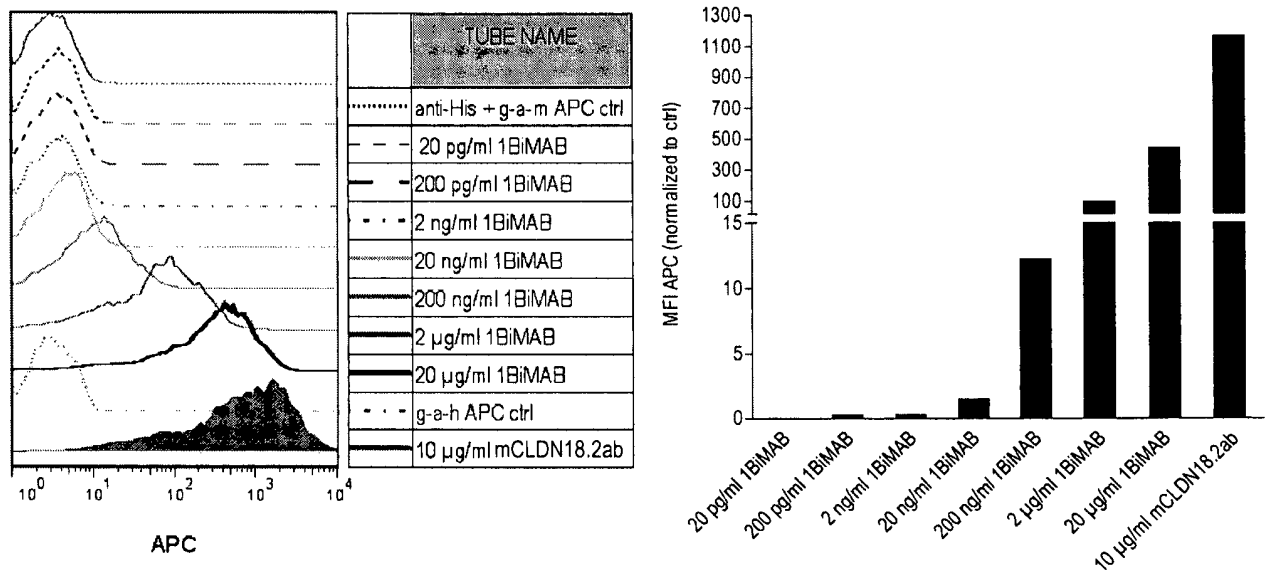
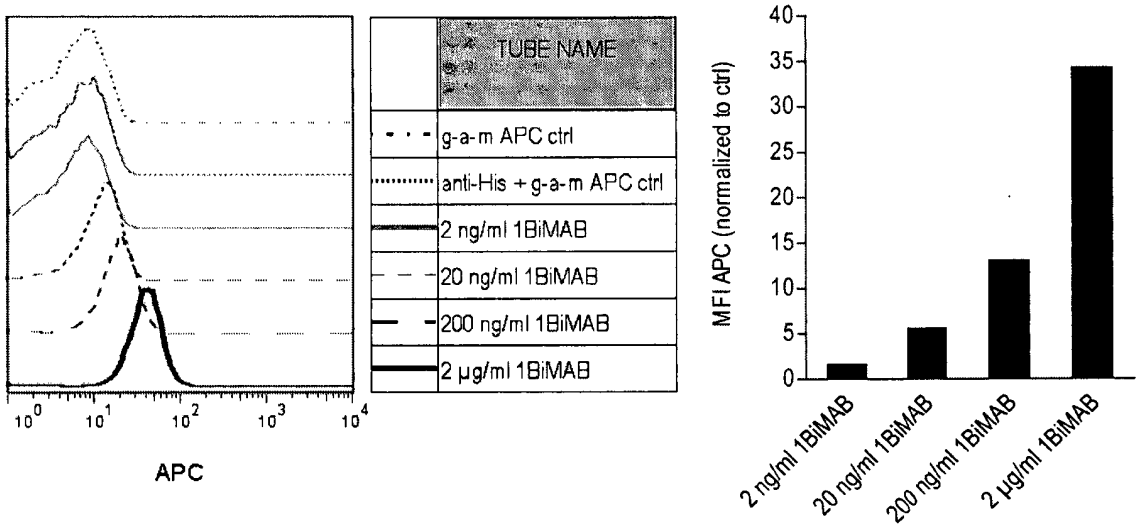

NugC4 cells
TUBE NAME
anti-His + g-a-m APC ctrl
20 pg/ml 1BiMAB
200 pg/ml 1BiMAB
2 ng/ml 1BiMAB
20 ng/ml 1BiMAB
200 ng/ml 1BiMAB
2 µg/ml 1BiMAB
20 µg/ml 1BiMAB
g-a-h APC ctrl
10 µg/ml mCLDN18.2ab
APC
MFI APC (normalized to ctrl)
20 pg/ml 1BiMAB
200 pg/ml 1BiMAB
2 ng/ml 1BiMAB
20 ng/ml 1BiMAB
200 ng/ml 1BiMAB
2 µg/ml 1BiMAB
20 µg/ml 1BiMAB
10 µg/ml mCLDN18.2ab

Figure 4

C

Human TL



D

CLDN18.2 negative cell line (PA-1)

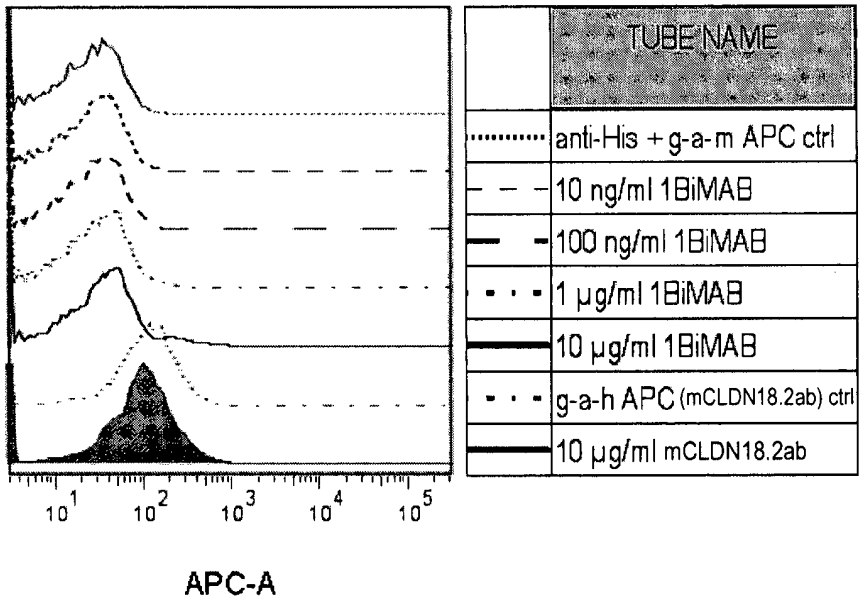


Figure 5

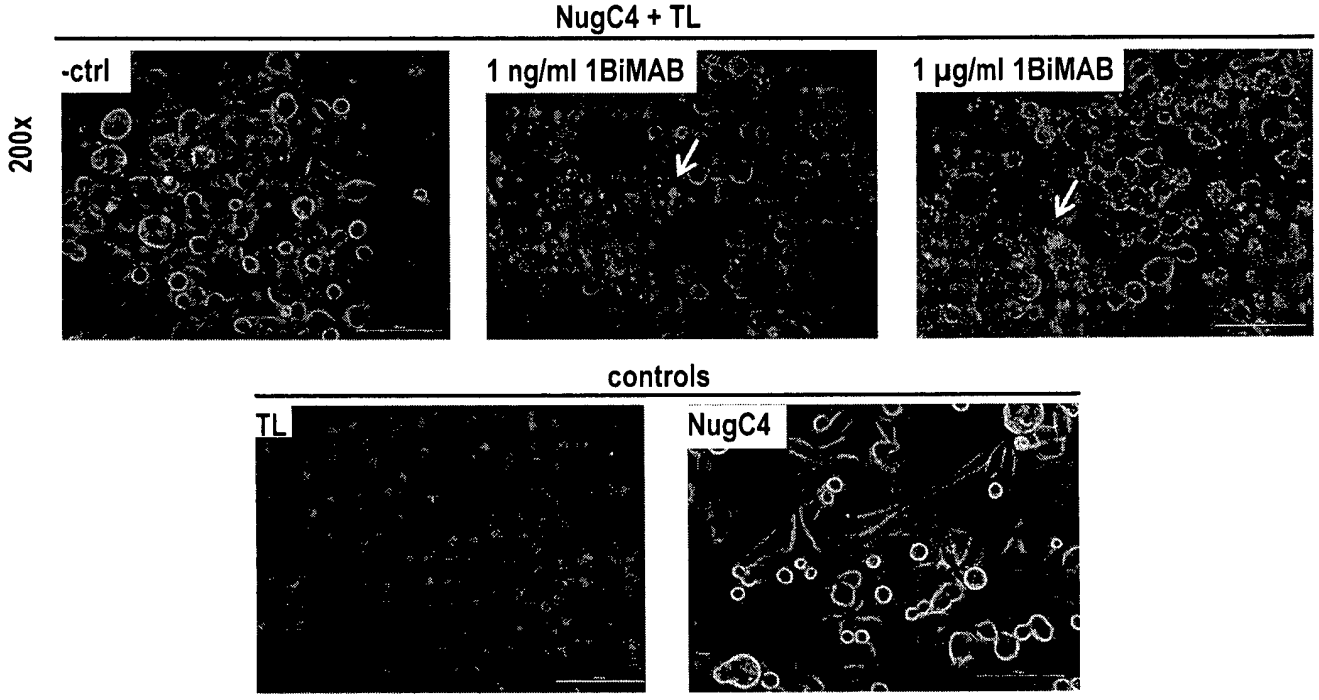

NugC4 + TL
-ctrl
1 ng/ml 1BiMAB
1 µg/ml 1BiMAB
200x
controls
TL
NugC4

Figure 6

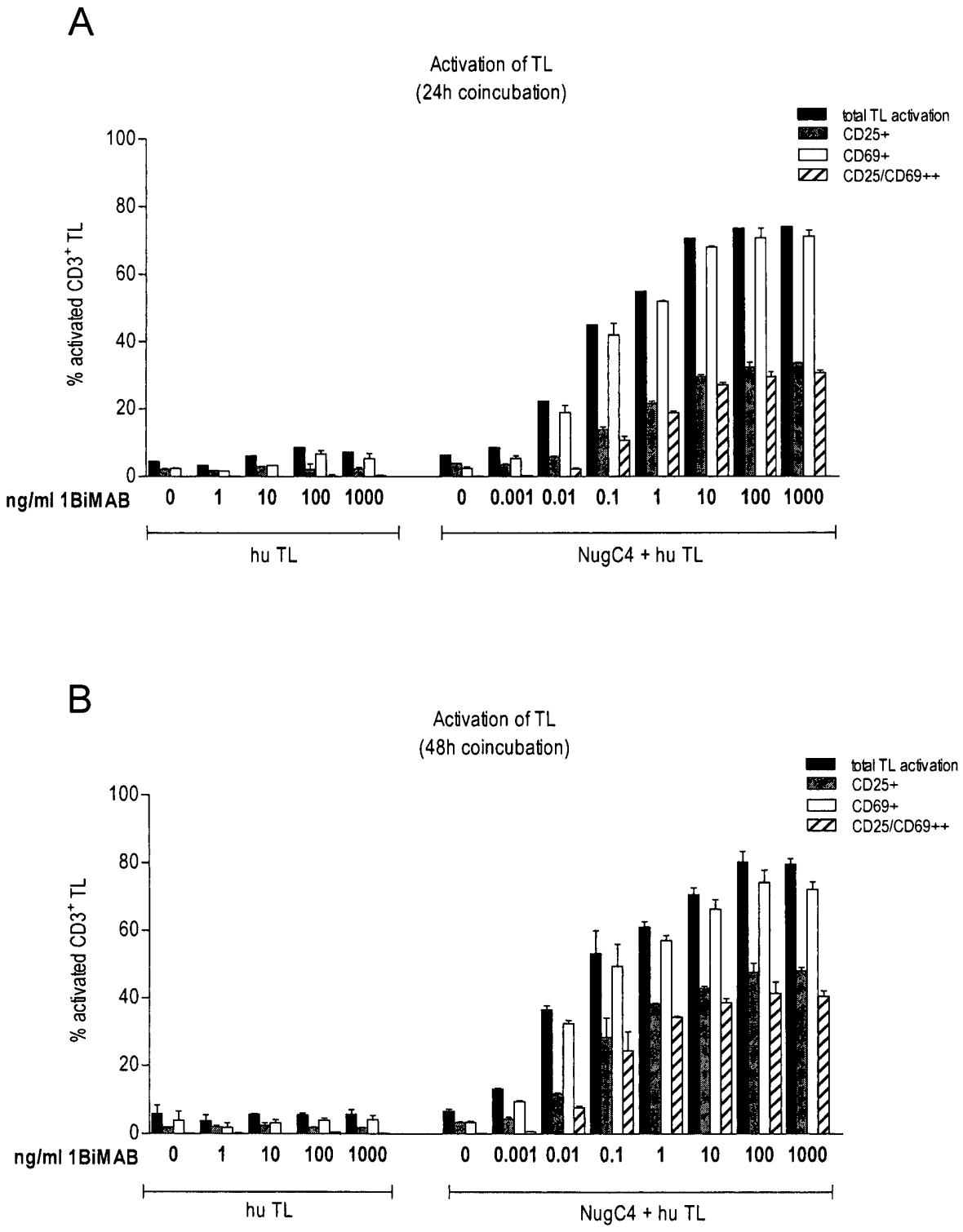
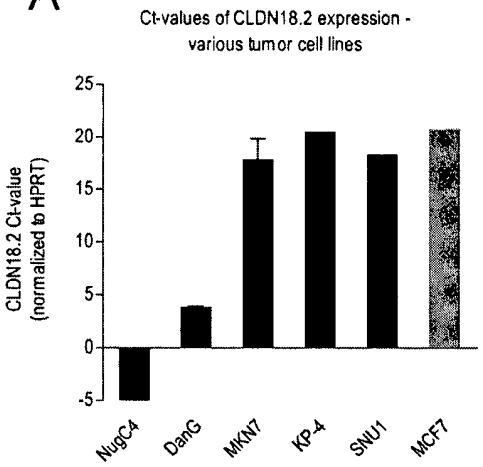

A
Activation of TL
(24h coincubation)
total TL activation
CD25+
CD69+
CD25/CD69++
% activated CD3+ TL
100
80
60
40
20
0
ng/ml 1BiMAB
0 1 10 100 1000
0 0.001 0.01 0.1 1 10 100 1000
hu TL
NugC4 + hu TL
B
Activation of TL
(48h coincubation)
total TL activation
CD25+
CD69+
CD25/CD69++
% activated CD3+ TL
100
80
60
40
20
0
ng/ml 1BiMAB
0 1 10 100 1000
0 0.001 0.01 0.1 1 10 100 1000
hu TL
NugC4 + hu TL

Figure 7

A



Cell line	Cancer type
NugC4	Gastric lymph node cancer
DanG	Pancreatic cancer
MKN7	Gastric cancer
KP-4	Pancreatic cancer
SNU1	Gastric cancer
MCF7	Breast cancer

B

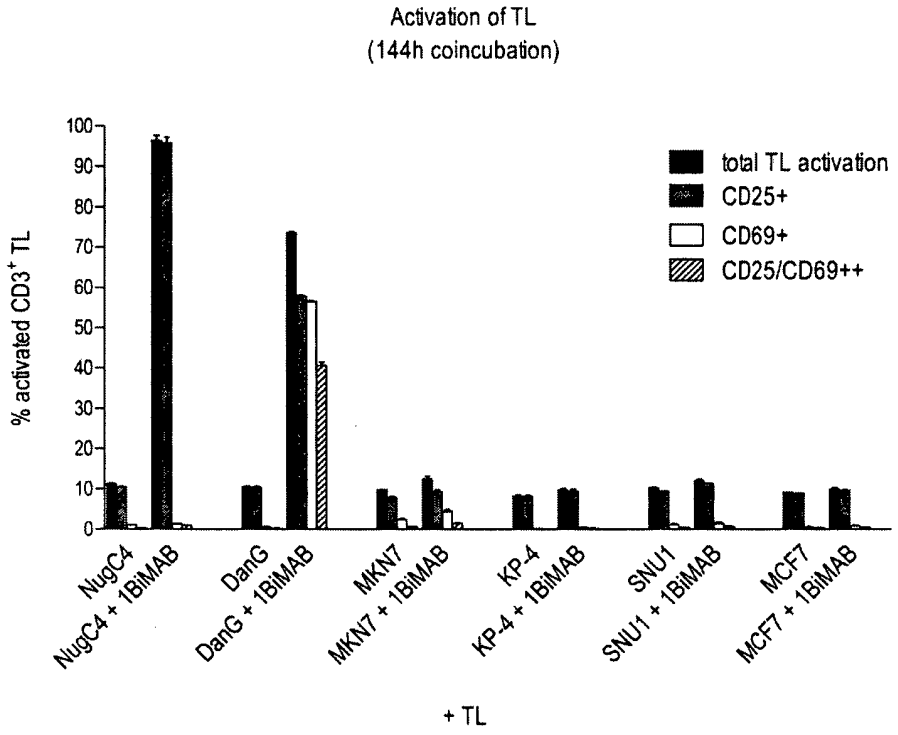
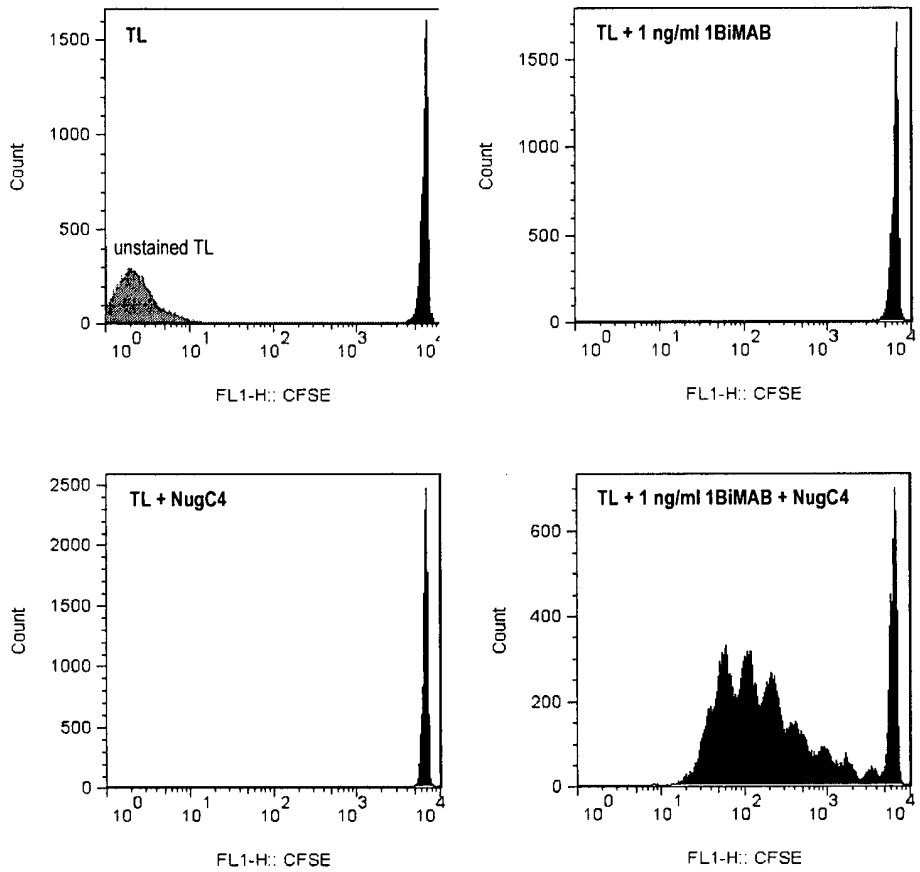


Figure 8

A


TL
unstained TL
TL + 1 ng/ml 1BiMAB
TL + NugC4
TL + 1 ng/ml 1BiMAB + NugC4
Count
FL1-H:: CFSE

B

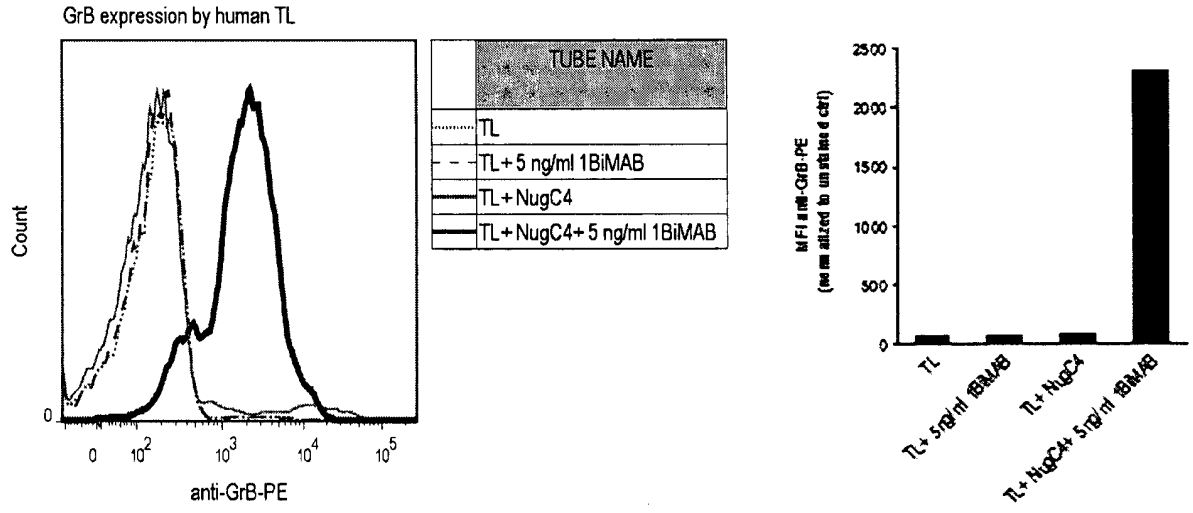

GrB expression by human TL
Count
anti-GrB-PE
TUBE NAME
TL
TL+5 ng/ml 1BiMAB
TL+NugC4
TL+NugC4+5 ng/ml 1BiMAB

Figure 9

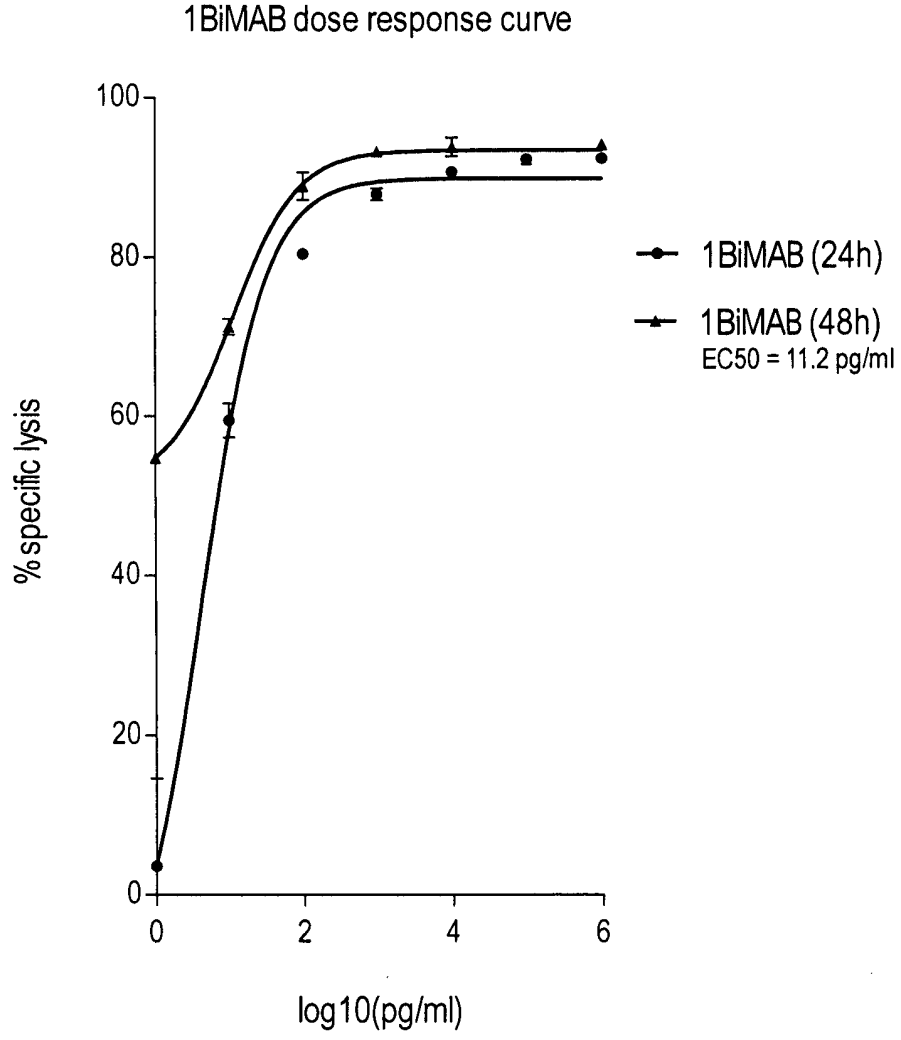
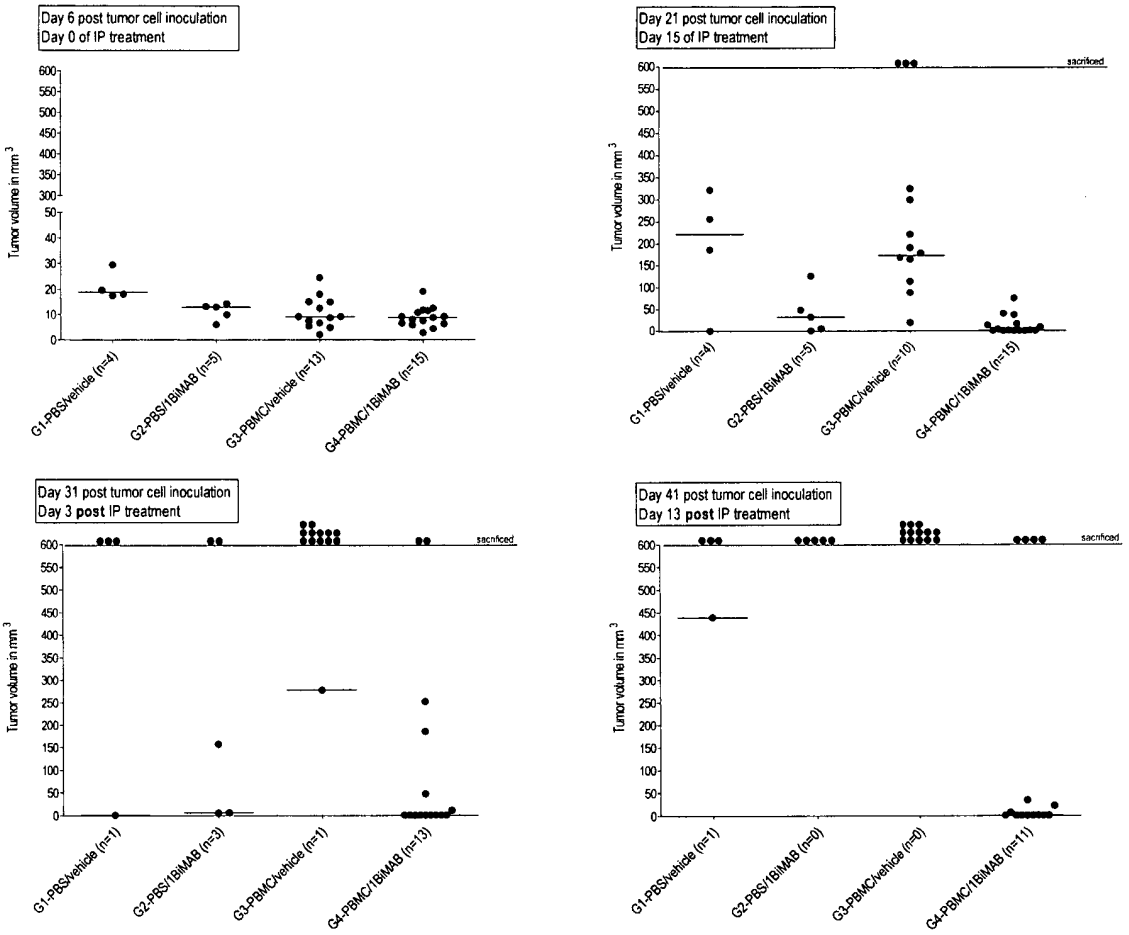

1BiMAB dose response curve
100
80
60
40
20
0
% specific lysis
0
2
4
6
log10(pg/ml)
1BiMAB (24h)
1BiMAB (48h)
EC50 = 11.2 pg/ml

Figure 10

A


Day 6 post tumor cell inoculation
Day 0 of IP treatment
Day 21 post tumor cell inoculation
Day 15 of IP treatment
Day 31 post tumor cell inoculation
Day 3 post IP treatment
Day 41 post tumor cell inoculation
Day 13 post IP treatment
Tumor volume in mm 3
sacrificed
G1-PBS/vehicle (n=4)
G2-PBS/1BiMAB (n=5)
G3-PBMC/vehicle (n=13)
G4-PBMC/1BiMAB (n=15)
G3-PBMC/vehicle (n=10)
G1-PBS/vehicle (n=1)
G2-PBS/1BiMAB (n=3)
G3-PBMC/vehicle (n=1)
G4-PBMC/1BiMAB (n=13)
G2-PBS/1BiMAB (n=0)
G3-PBMC/vehicle (n=0)
G4-PBMC/1BiMAB (n=11)

B

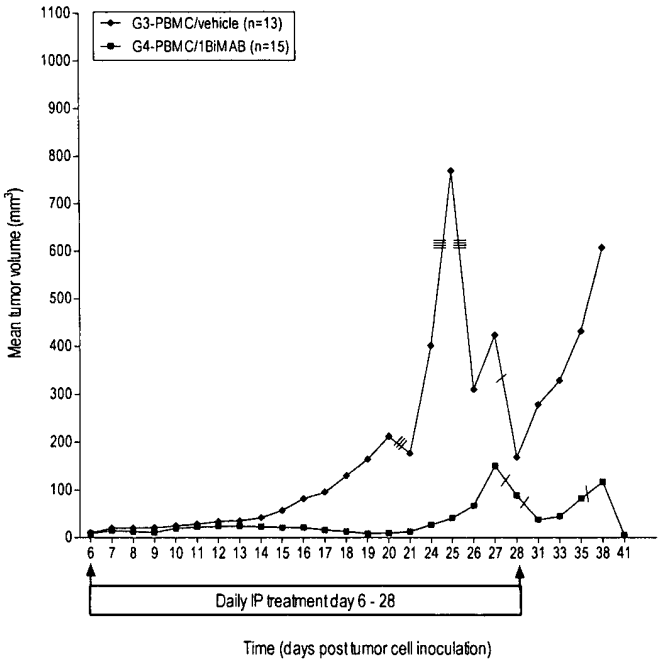
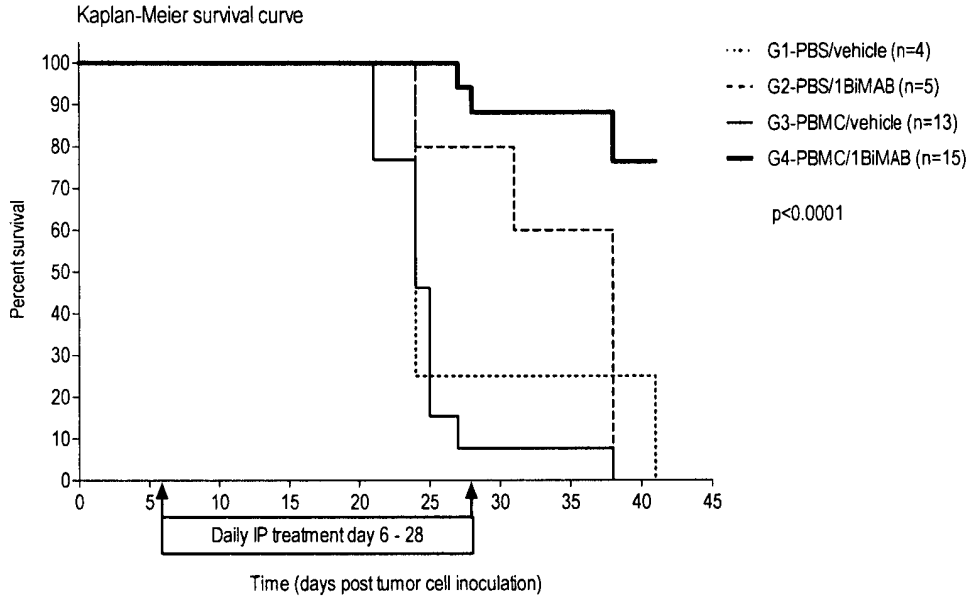

G3-PBMC/vehicle (n=13)
G4-PBMC/1BiMAB (n=15)
Mean tumor volume (mm3)
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 24 25 26 27 28 31 33 35 38 41
Daily IP treatment day 6 - 28
Time (days post tumor cell inoculation)

Figure 10

C


Kaplan-Meier survival curve
Percent survival
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
Daily IP treatment day 6 - 28
Time (days post tumor cell inoculation)
G1-PBS/vehicle (n=4)
G2-PBS/1BiMAB (n=5)
G3-PBMC/vehicle (n=13)
G4-PBMC/1BiMAB (n=15)
p<0.0001

D

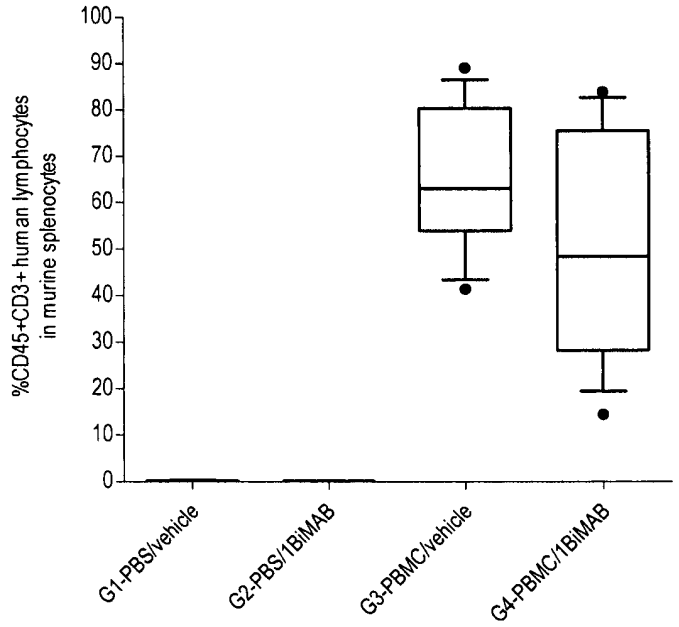
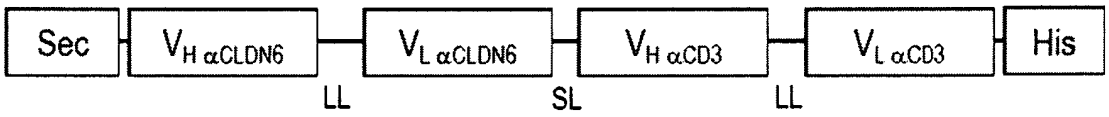

%CD45+CD3+ human lymphocytes in murine splenocytes
100
90
80
70
60
50
40
30
20
10
0
G1-PBS/vehicle
G2-PBS/1BiMAB
G3-PBMC/vehicle
G4-PBMC/1BiMAB

Figure 11

A

Bi-scFv CLDN6 x CD3 (6PHU5)



B

Bi-scFv CD3 x CLDN6 (6PHU3)

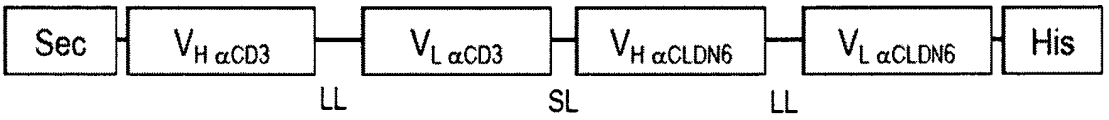


Figure 12

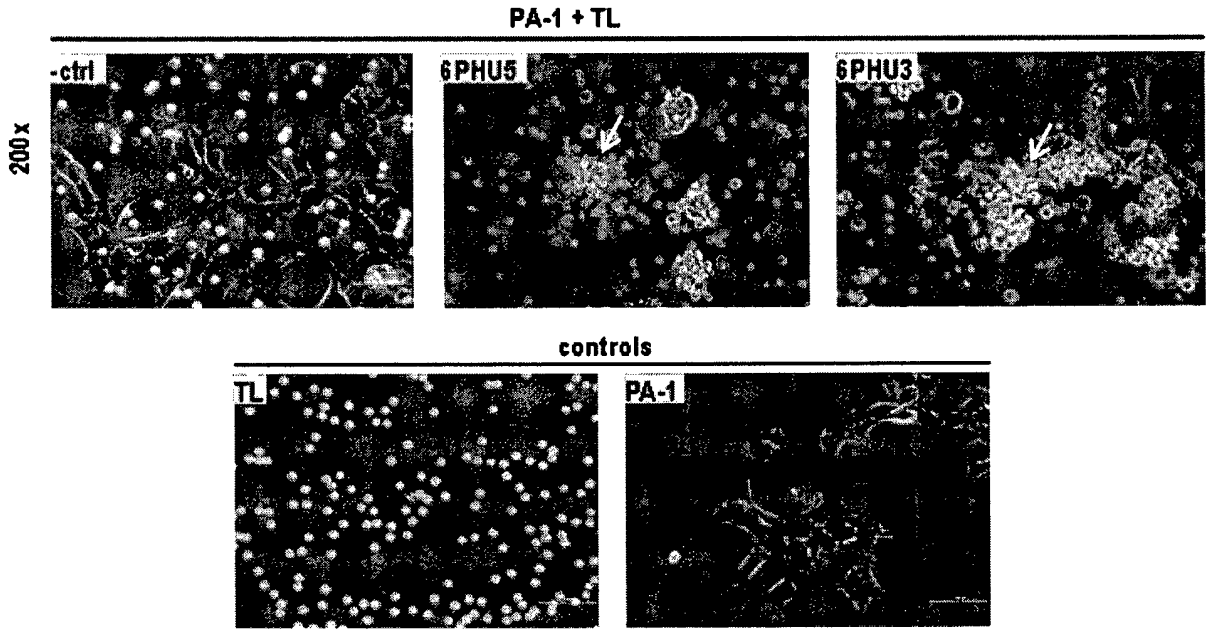


Figure 13

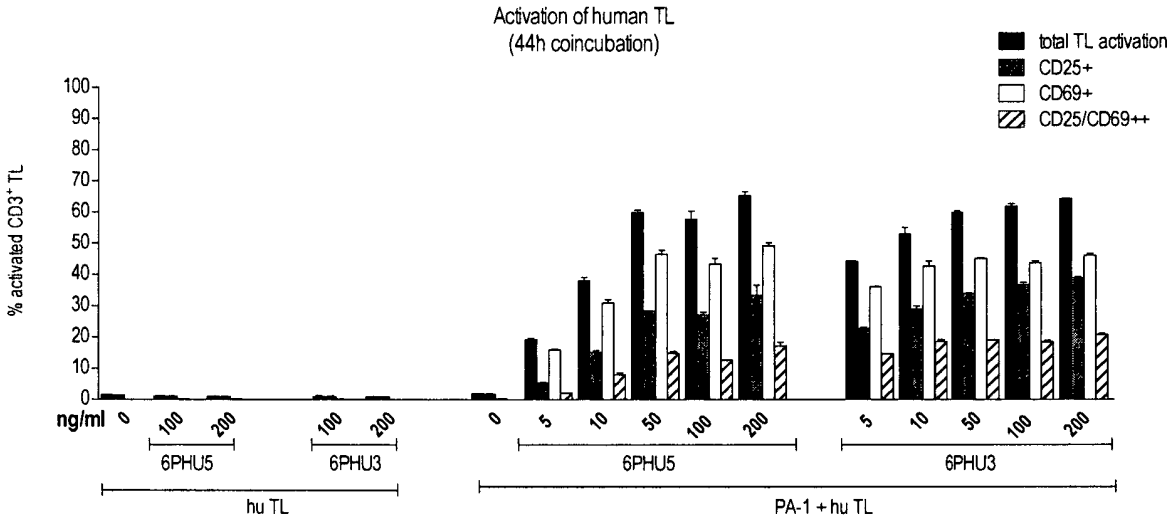
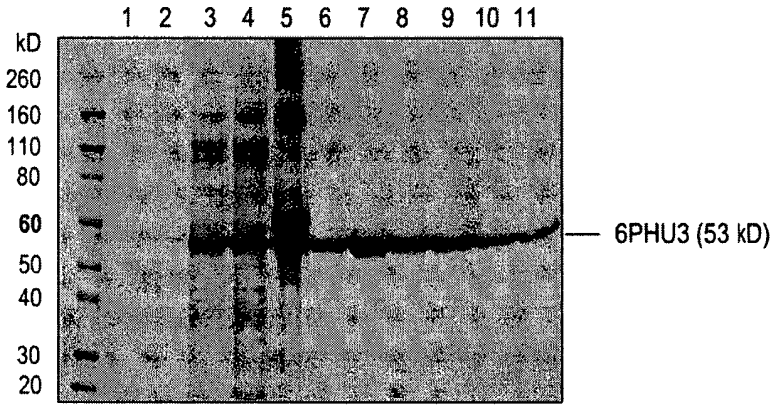

Activation of human TL
(44h coincubation)
total TL activation
CD25+
CD69+
CD25/CD69++
% activated CD3+ TL
100
90
80
70
60
50
40
30
20
10
0
ng/ml
0
100
200
100
200
0
5
10
50
100
200
5
10
50
100
200
6PHU5
6PHU3
6PHU5
6PHU3
hu TL
PA-1 + hu TL

Figure 14

A


1 2 3 4 5 6 7 8 9 10 11
kD
260
160
110
80
60
50
40
30
20
6PHU3 (53 kD)

B

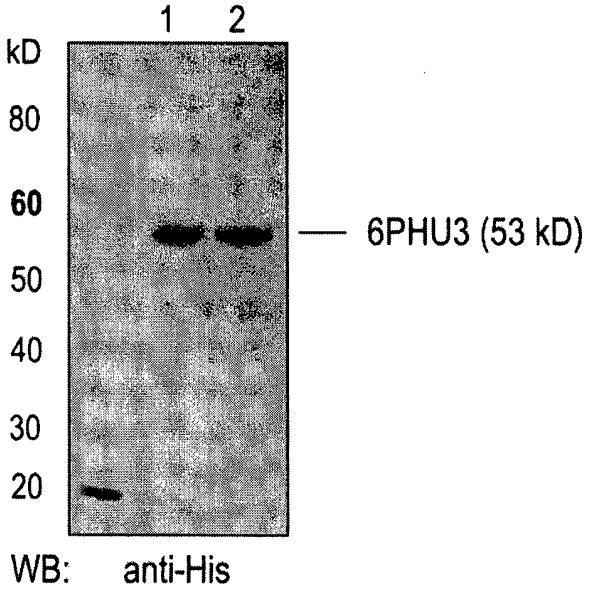

1 2
kD
80
60
50
40
30
20
6PHU3 (53 kD)
WB: anti-His

Figure 15

A

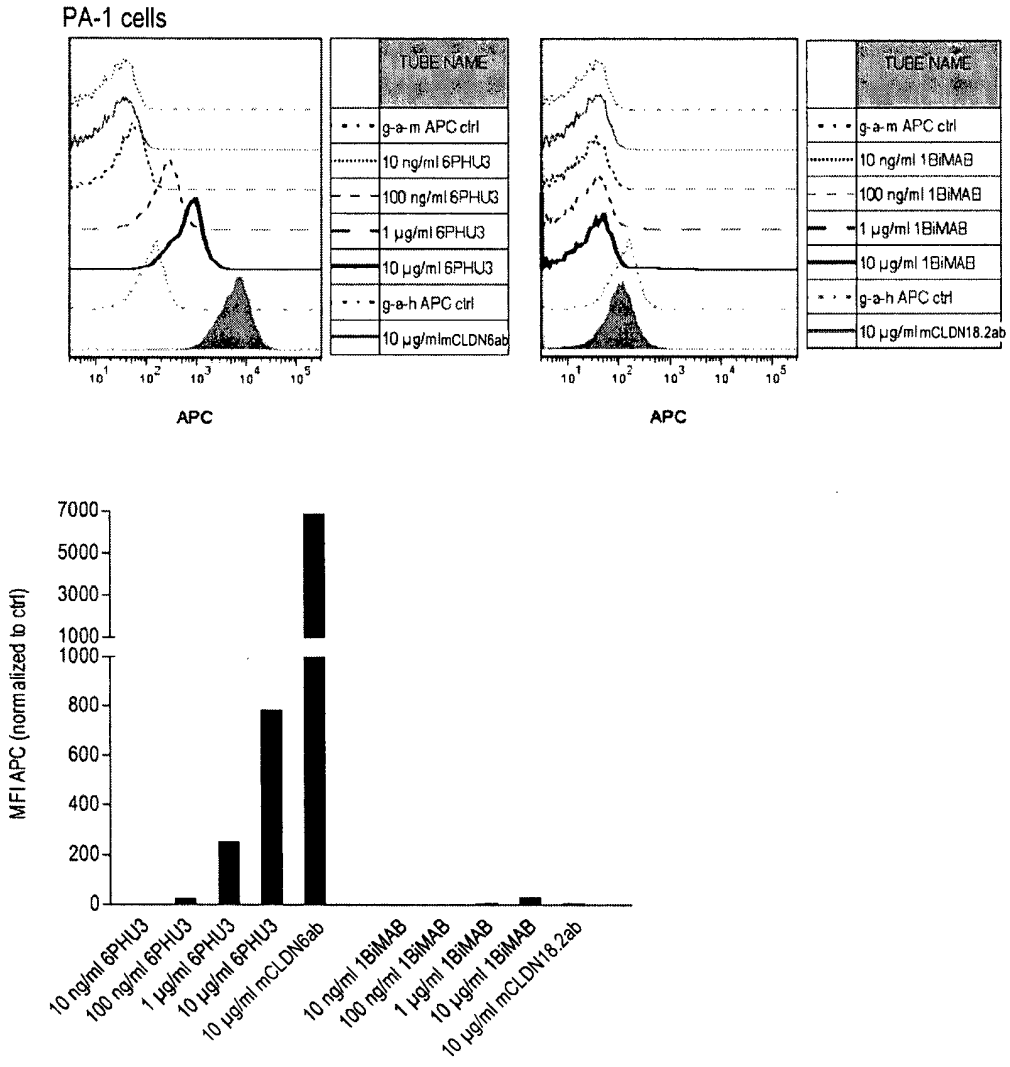
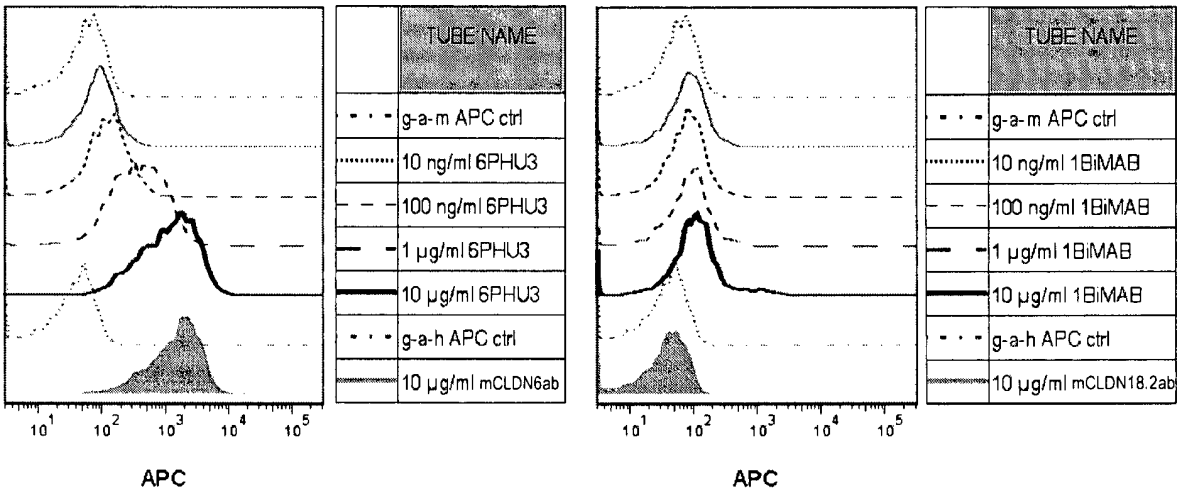

PA-1 cells
TUBE NAME
g-a-m APC ctrl
10 ng/ml 6PHU3
100 ng/ml 6PHU3
1 µg/ml 6PHU3
10 µg/ml 6PHU3
g-a-h APC ctrl
10 µg/ml mCLDN6ab
APC
TUBE NAME
g-a-m APC ctrl
10 ng/ml 1BiMAB
100 ng/ml 1BiMAB
1 µg/ml 1BiMAB
10 µg/ml 1BiMAB
g-a-h APC ctrl
10 µg/ml mCLDN18.2ab
APC
MFI APC (normalized to ctrl)
7000
5000
3000
1000
1000
800
600
400
200
0
10 ng/ml 6PHU3
100 ng/ml 6PHU3
1 µg/ml 6PHU3
10 µg/ml 6PHU3
10 µg/ml mCLDN6ab
10 ng/ml 1BiMAB
100 ng/ml 1BiMAB
1 µg/ml 1BiMAB
10 µg/ml 1BiMAB
10 µg/ml mCLDN18.2ab

Figure 15

A

OV-90 cells



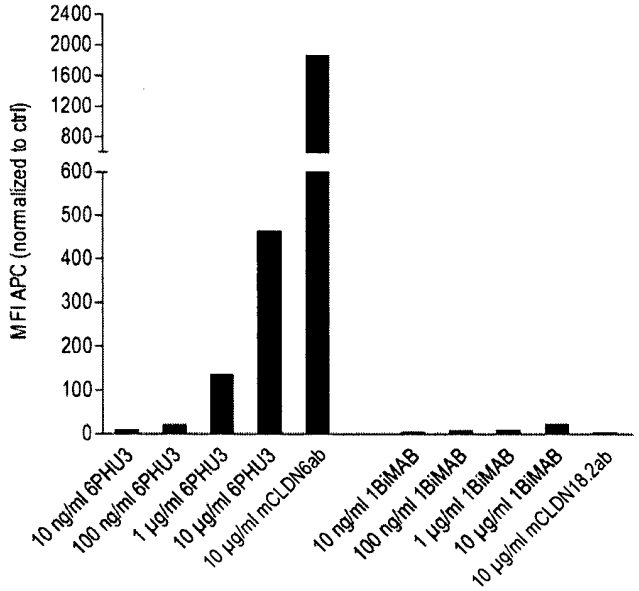


Figure 15

B

Human TL

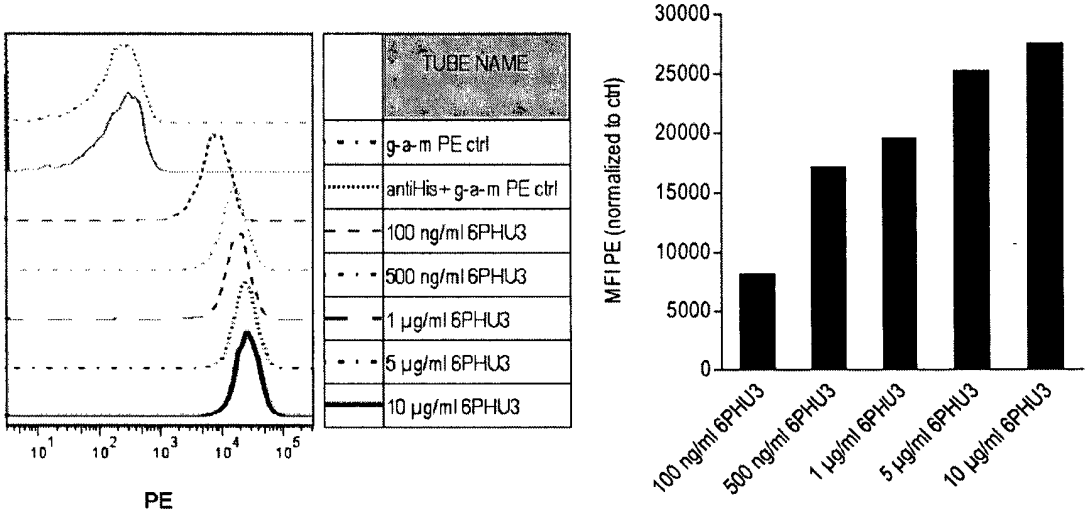
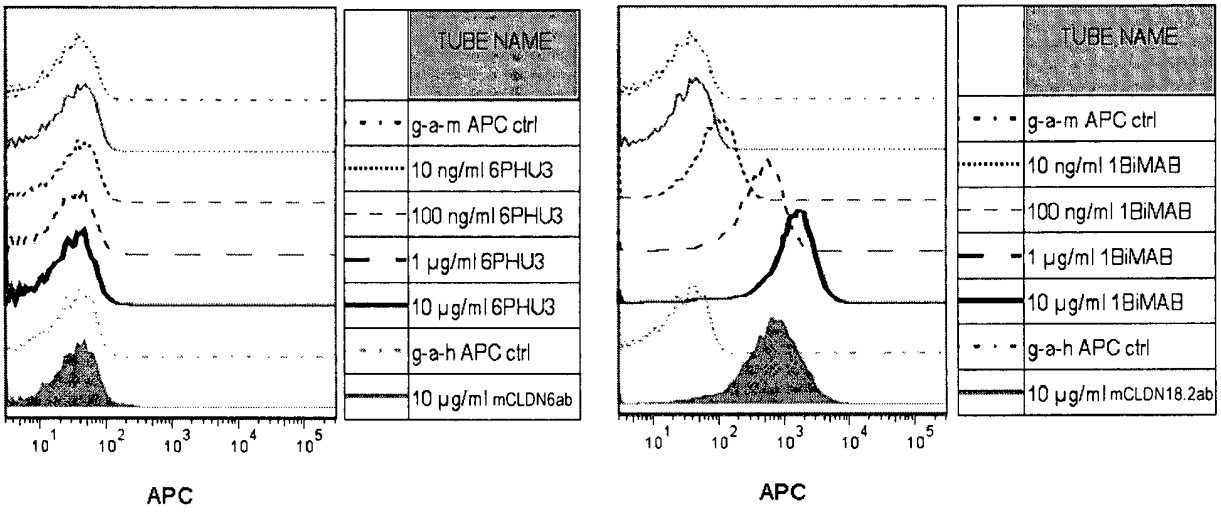


Figure 15

C

CLDN6 negative cell line (NugC4)



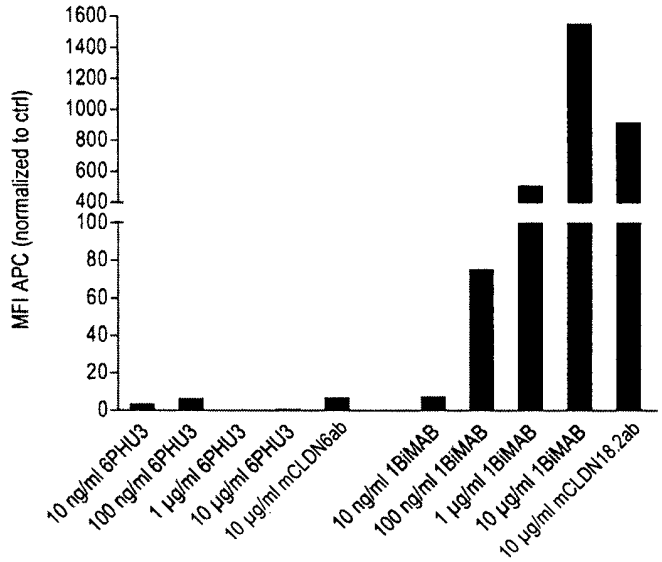
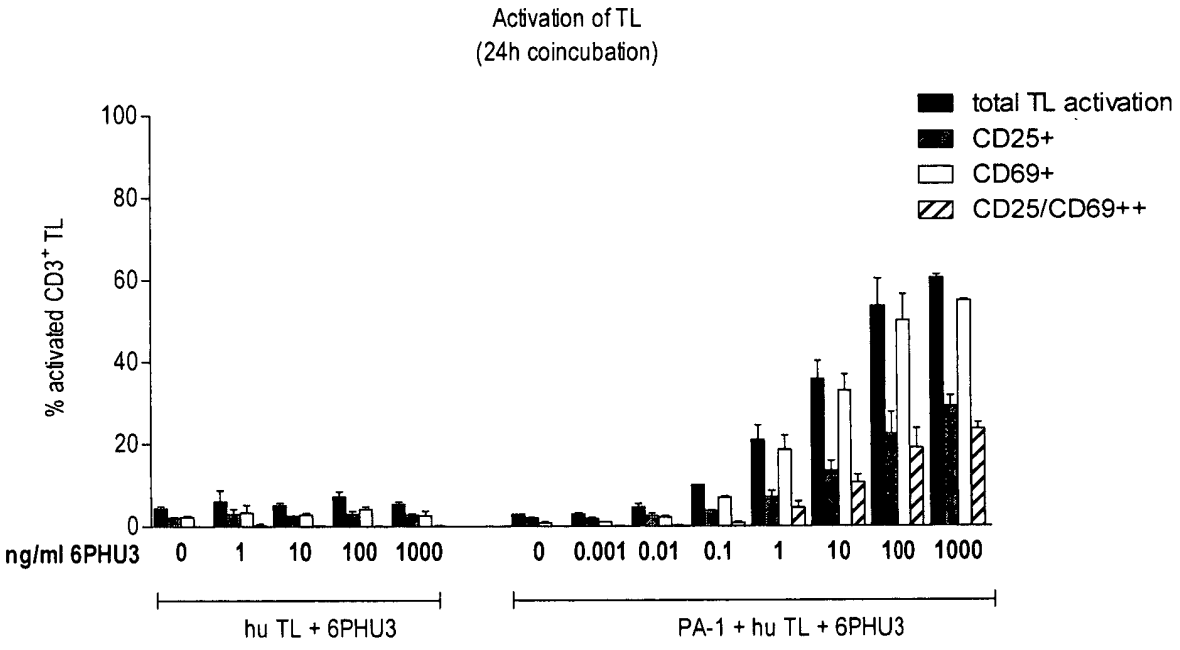


Figure 16

A


Activation of TL
(24h coincubation)
total TL activation
CD25+
CD69+
CD25/CD69++
% activated CD3+ TL
100
80
60
40
20
0
ng/ml 6PHU3
0 1 10 100 1000
0 0.001 0.01 0.1 1 10 100 1000
hu TL + 6PHU3
PA-1 + hu TL + 6PHU3

B

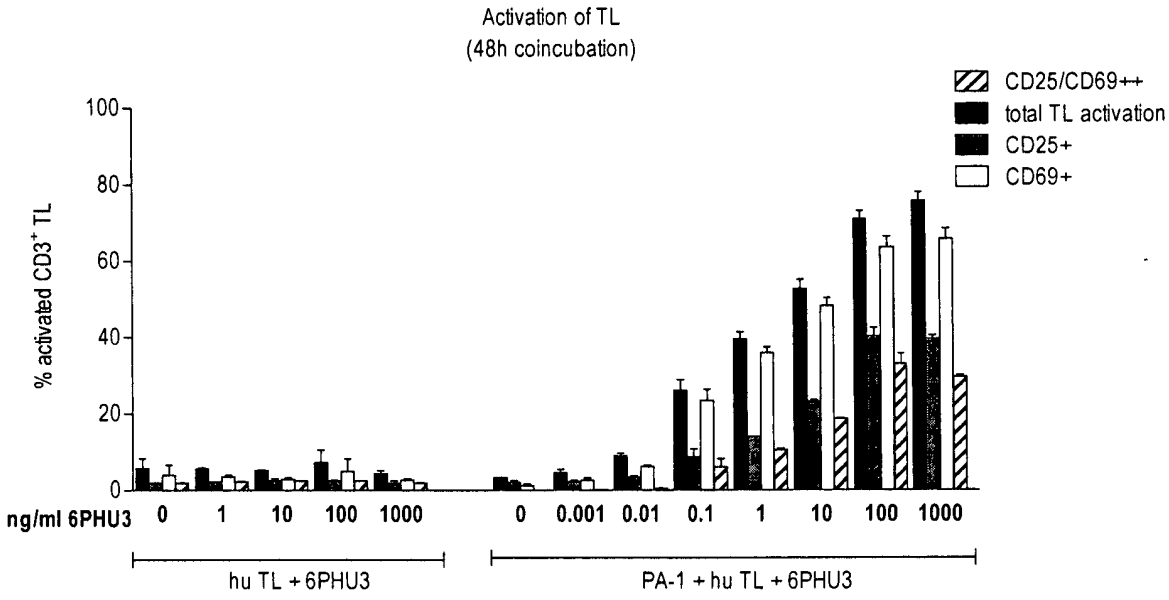

Activation of TL
(48h coincubation)
CD25/CD69++
total TL activation
CD25+
CD69+
% activated CD3+ TL
100
80
60
40
20
0
ng/ml 6PHU3
0 1 10 100 1000
0 0.001 0.01 0.1 1 10 100 1000
hu TL + 6PHU3
PA-1 + hu TL + 6PHU3

Figure 17

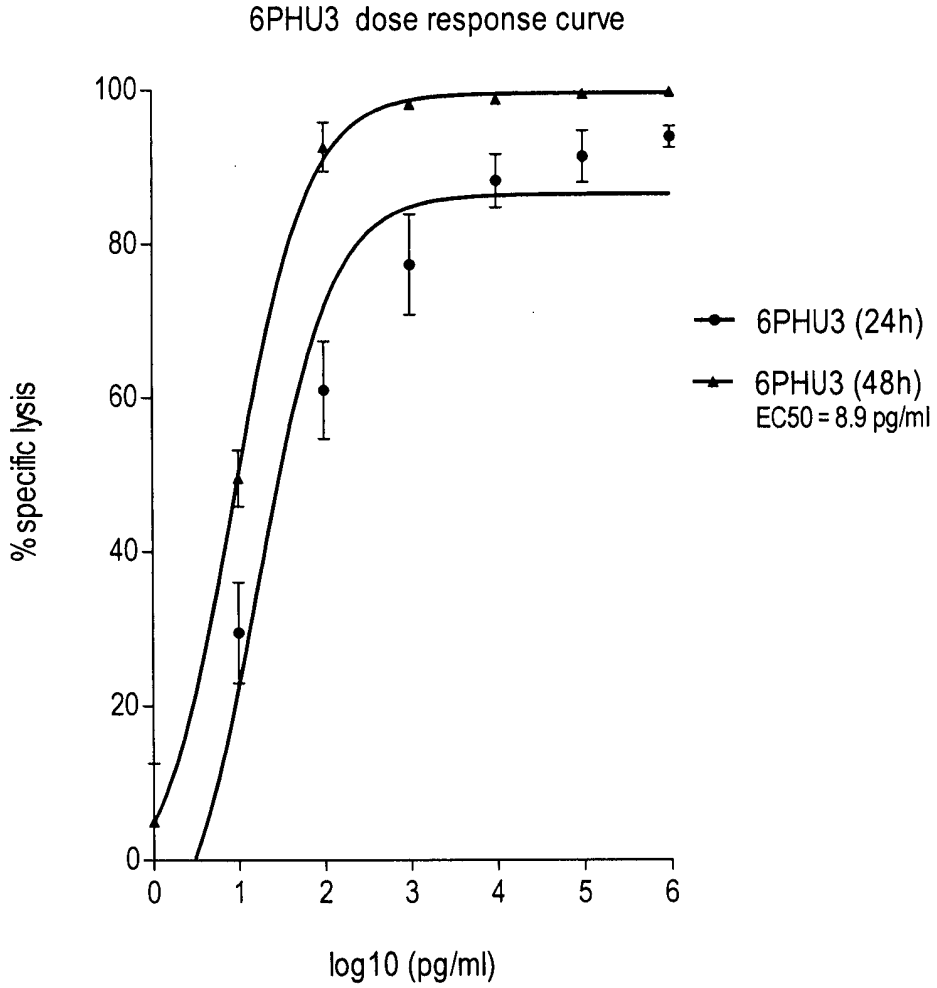

6PHU3 dose response curve
% specific lysis
100
80
60
40
20
0
0
1
2
3
4
5
6
log10 (pg/ml)
6PHU3 (24h)
6PHU3 (48h)
EC50 = 8.9 pg/ml

Figure 18

A

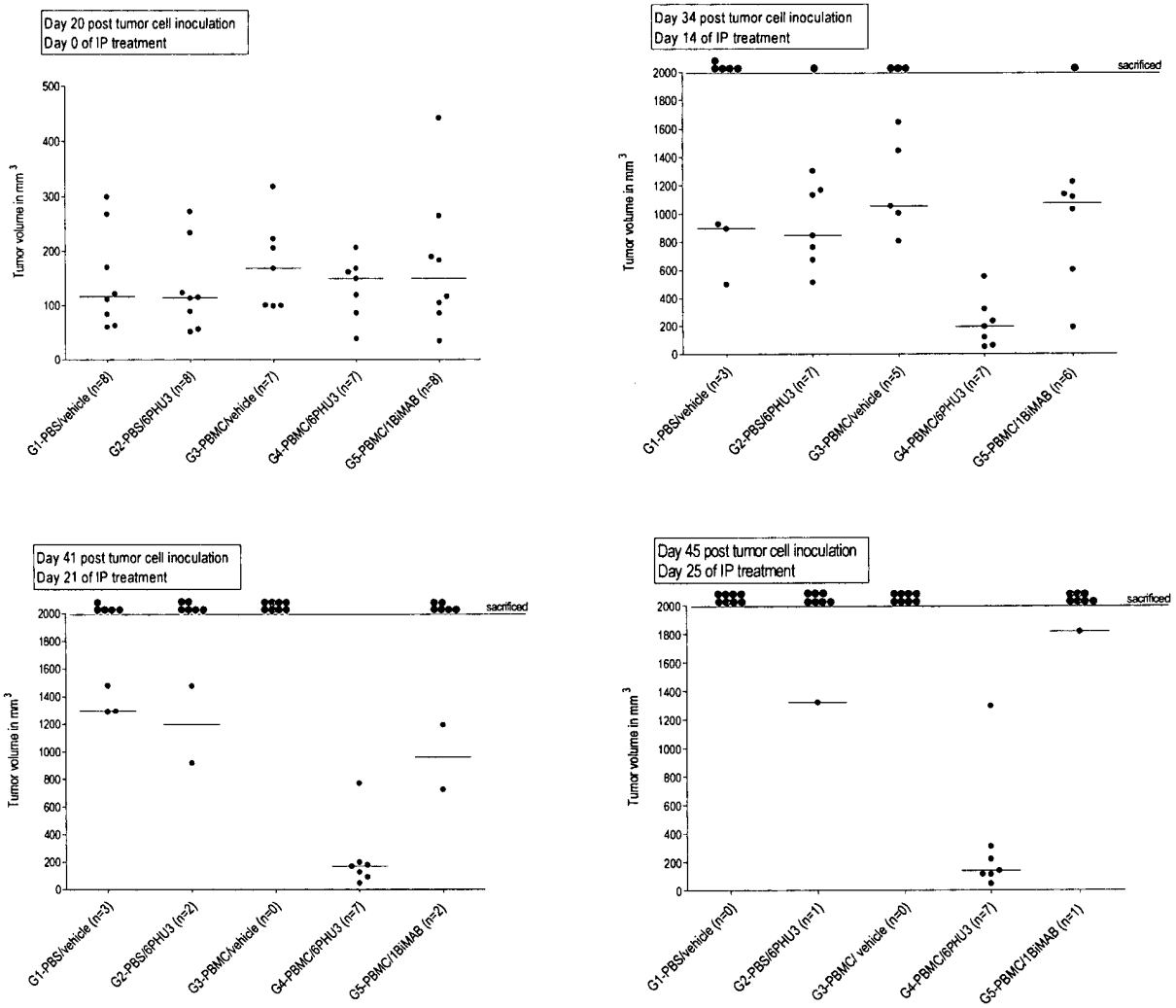
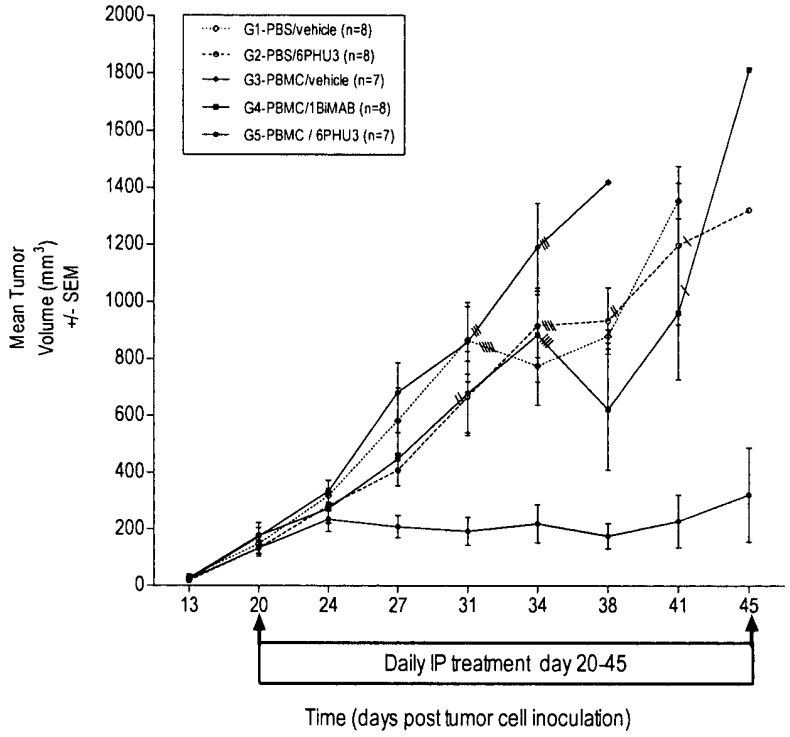

Day 20 post tumor cell inoculation
Day 0 of IP treatment
Tumor volume in mm 3
G1-PBS/vehicle (n=8)
G2-PBS/6PHU3 (n=8)
G3-PBMC/vehicle (n=7)
G4-PBMC/6PHU3 (n=7)
G5-PBMC/1BiMAB (n=8)
Day 34 post tumor cell inoculation
Day 14 of IP treatment
sacrificed
G1-PBS/vehicle (n=3)
G2-PBS/6PHU3 (n=7)
G3-PBMC/vehicle (n=5)
G4-PBMC/6PHU3 (n=7)
G5-PBMC/1BiMAB (n=6)
Day 41 post tumor cell inoculation
Day 21 of IP treatment
G1-PBS/vehicle (n=3)
G2-PBS/6PHU3 (n=2)
G3-PBMC/vehicle (n=0)
G4-PBMC/6PHU3 (n=7)
G5-PBMC/1BiMAB (n=2)
Day 45 post tumor cell inoculation
Day 25 of IP treatment
G1-PBS/vehicle (n=0)
G2-PBS/6PHU3 (n=1)
G3-PBMC/ vehicle (n=0)
G4-PBMC/6PHU3 (n=7)
G5-PBMC/1BiMAB (n=1)

Figure 18

B


G1-PBS/vehicle (n=8)
G2-PBS/6PHU3 (n=8)
G3-PBMC/vehicle (n=7)
G4-PBMC/1BiMAB (n=8)
G5-PBMC / 6PHU3 (n=7)
Mean Tumor Volume (mm3) +/- SEM
2000
1800
1600
1400
1200
1000
800
600
400
200
0
13
20
24
27
31
34
38
41
45
Daily IP treatment day 20-45
Time (days post tumor cell inoculation)

C

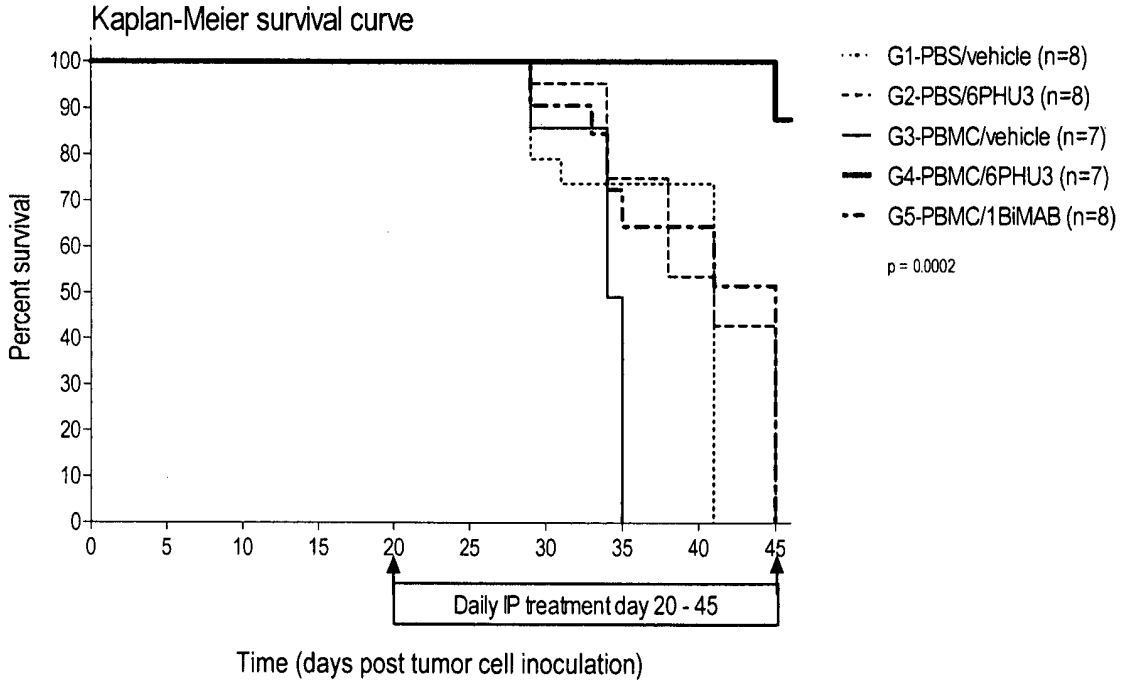

Kaplan-Meier survival curve
Percent survival
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
Daily IP treatment day 20 - 45
Time (days post tumor cell inoculation)
G1-PBS/vehicle (n=8)
G2-PBS/6PHU3 (n=8)
G3-PBMC/vehicle (n=7)
G4-PBMC/6PHU3 (n=7)
G5-PBMC/1BiMAB (n=8)
p = 0.0002

Figure 18

D

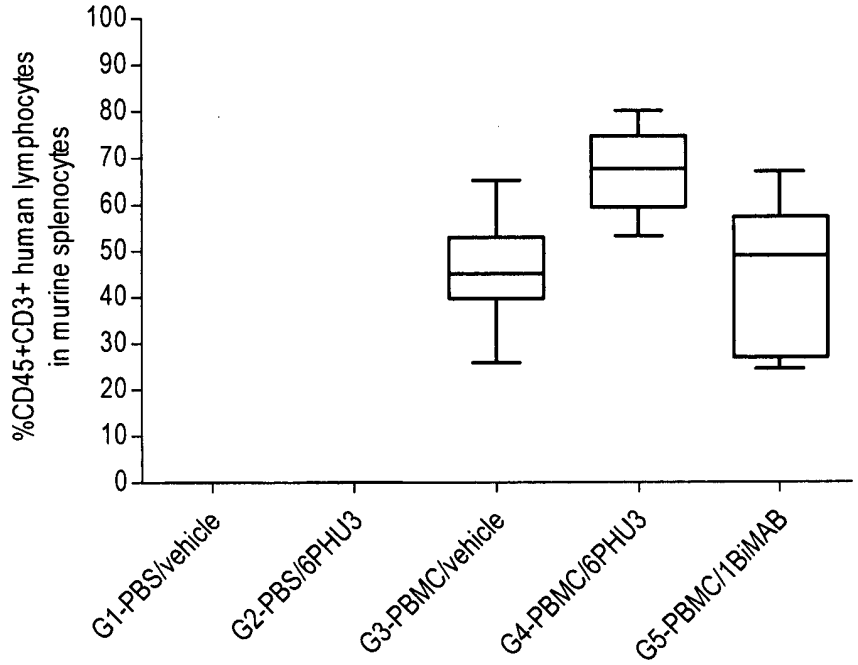
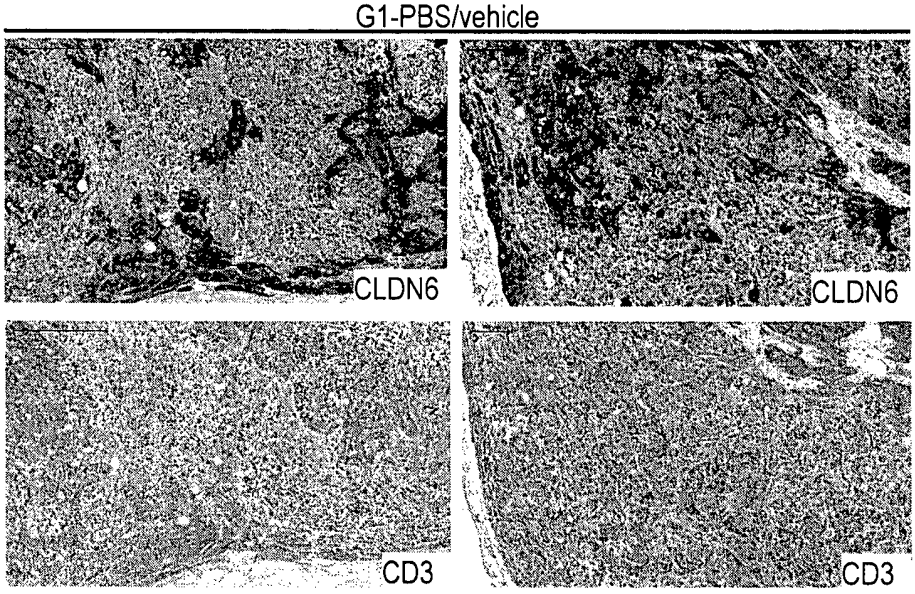

%CD45+CD3+ human lymphocytes in murine splenocytes
100
90
80
70
60
50
40
30
20
10
0
G1-PBS/vehicle
G2-PBS/6PHU3
G3-PBMC/vehicle
G4-PBMC/6PHU3
G5-PBMC/1BiMAB

Figure 19

A


G1-PBS/vehicle
CLDN6
CLDN6
CD3
CD3

B

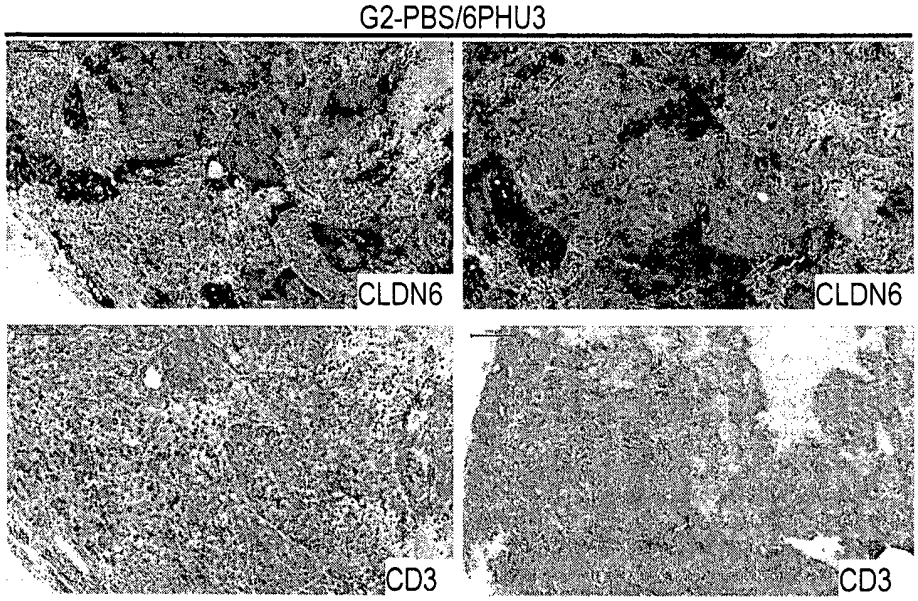
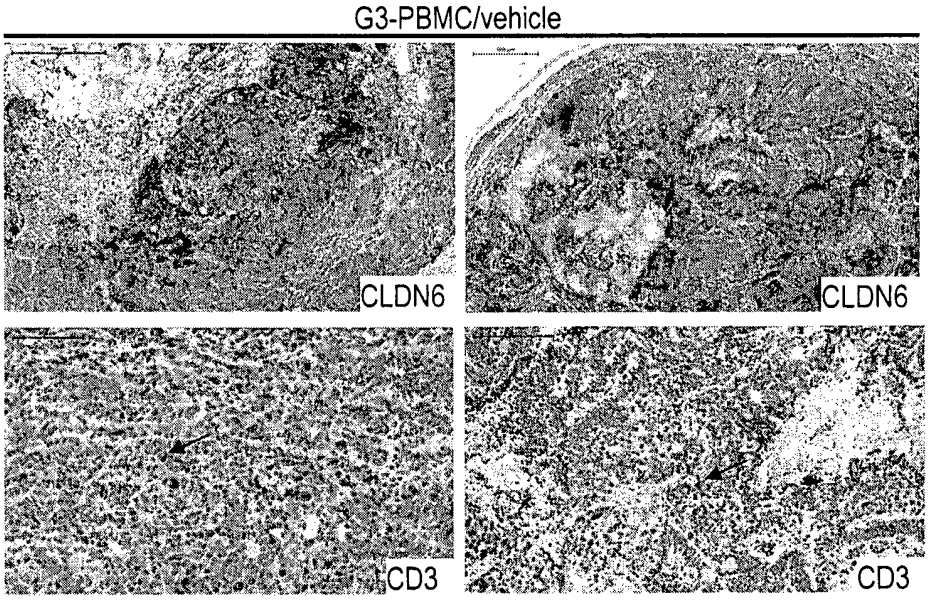

G2-PBS/6PHU3
CLDN6
CLDN6
CD3
CD3

Figure 19

C


G3-PBMC/vehicle
CLDN6
CLDN6
CD3
CD3

D

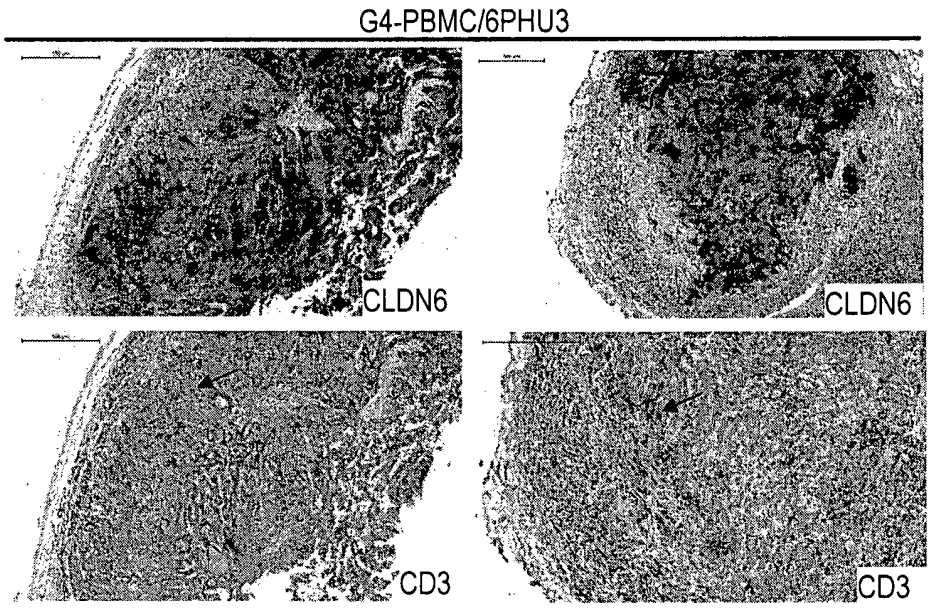

G4-PBMC/6PHU3
CLDN6
CLDN6
CD3
CD3

Figure 19

E

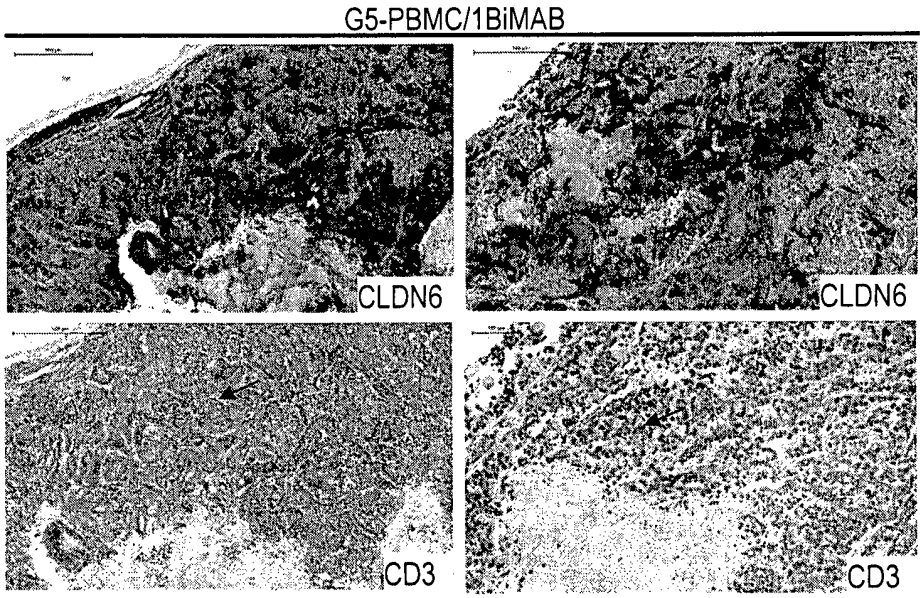
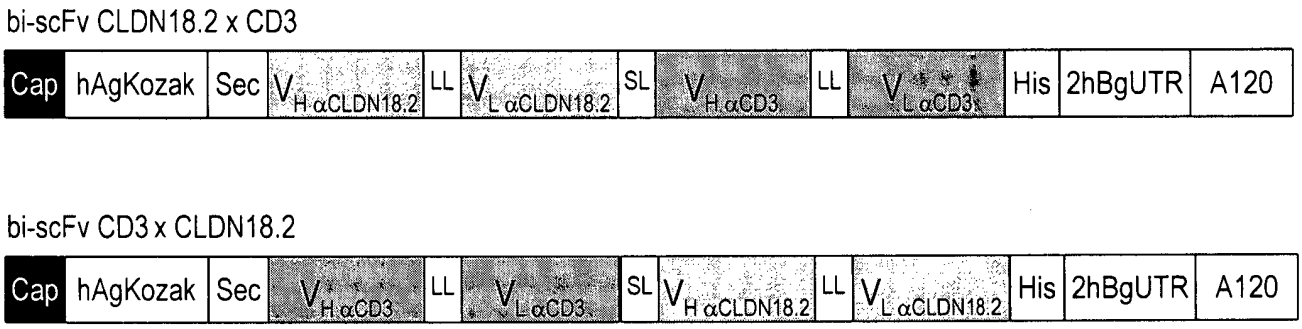


Figure 20

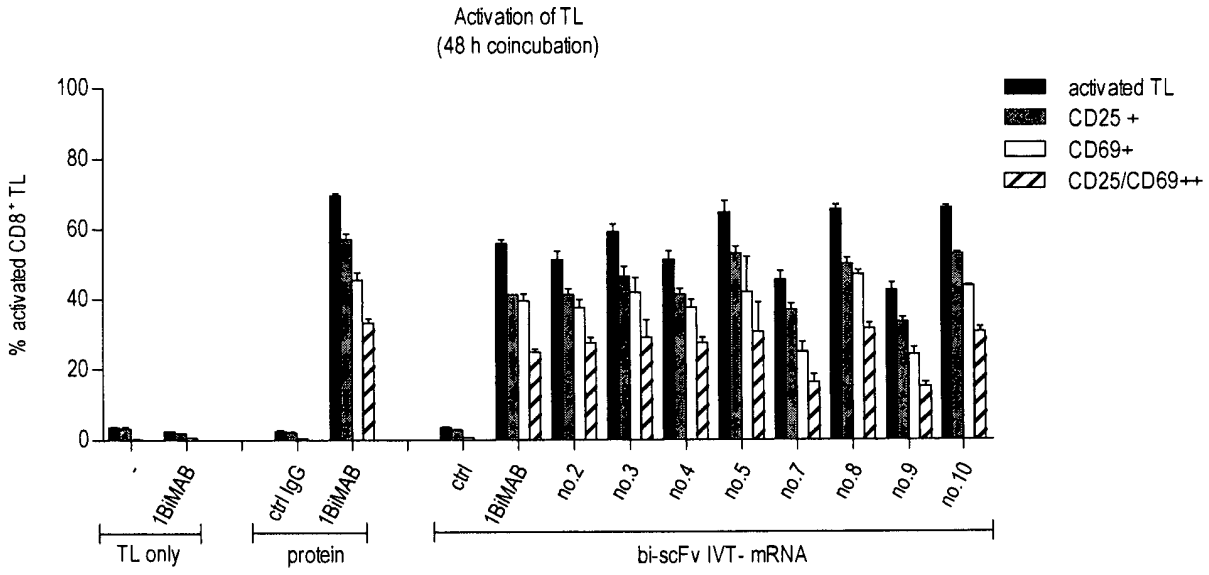
A


bi-scFv CLDN18.2 x CD3
Cap
hAgKozak
Sec
V H αCLDN18.2
LL
V L αCLDN18.2
SL
V H αCD3
LL
V L αCD3
His
2hBgUTR
A120
bi-scFv CD3 x CLDN18.2
Cap
hAgKozak
Sec
V H αCD3
LL
V L αCD3
SL
V H αCLDN18.2
LL
V L αCLDN18.2
His
2hBgUTR
A120

B


Cap
5'UTR
nsP1-4
sgP
Sec
V H αCLDN18.2
LL
V L αCLDN18.2
SL
V H αCD3
LL
V L αCD3
His
3'UTR
A120

Figure 21


Activation of TL
(48 h coincubation)
% activated CD8+ TL
100
80
60
40
20
0
activated TL
CD25 +
CD69+
CD25/CD69++
-
1BiMAB
ctrl IgG
1BiMAB
ctrl
1BiMAB
no.2
no.3
no.4
no.5
no.7
no.8
no.9
no.10
TL only
protein
bi-scFv IVT- mRNA

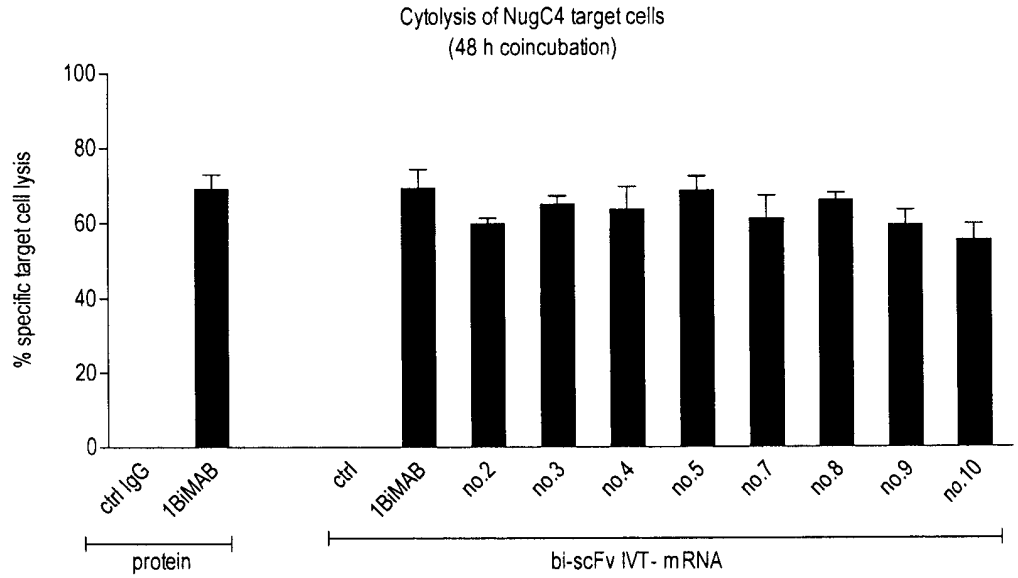

Cytolysis of NugC4 target cells
(48 h coincubation)
% specific target cell lysis
100
80
60
40
20
0
ctrl IgG
1BiMAB
ctrl
1BiMAB
no.2
no.3
no.4
no.5
no.7
no.8
no.9
no.10
protein
bi-scFv IVT- mRNA

Figure 22

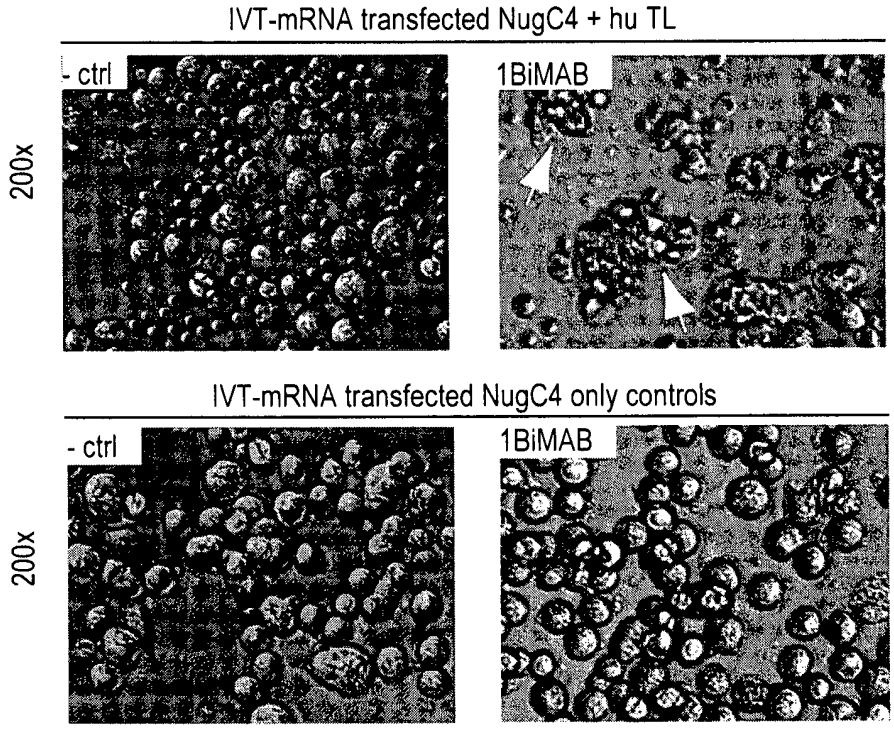
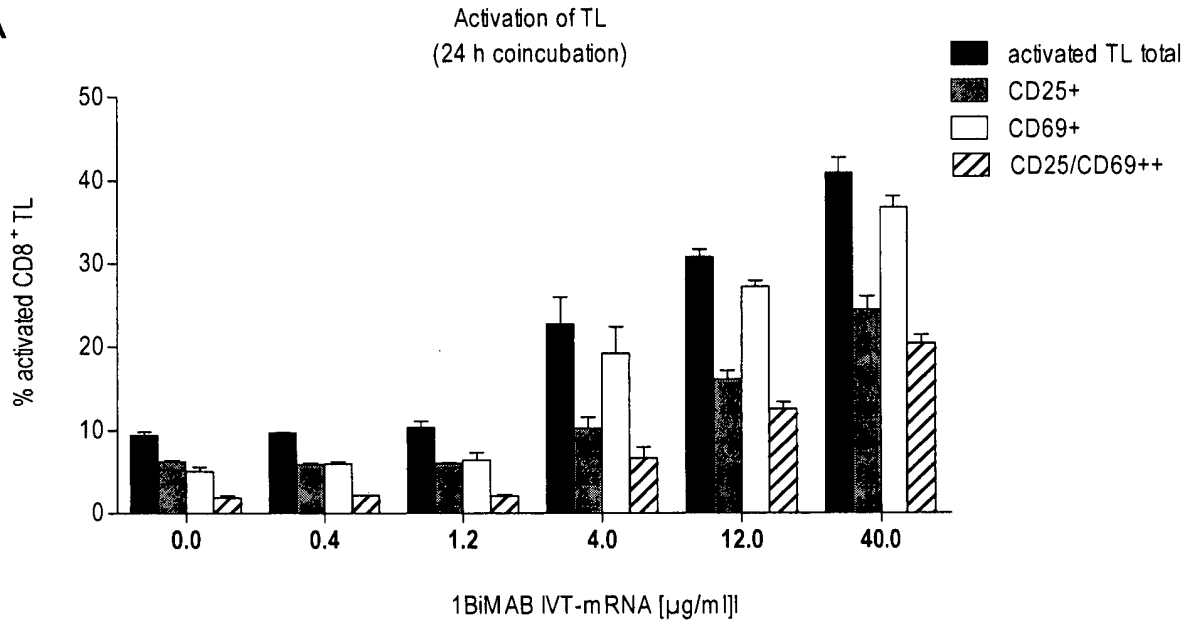

IVT-mRNA transfected NugC4 + hu TL
- ctrl
1BiMAB
200x
IVT-mRNA transfected NugC4 only controls
- ctrl
1BiMAB
200x

Figure 23

A


Activation of TL
(24 h coincubation)
activated TL total
CD25+
CD69+
CD25/CD69++
% activated CD8+ TL
50
40
30
20
10
0
0.0
0.4
1.2
4.0
12.0
40.0
1BiMAB IVT-mRNA [µg/ml]]

B

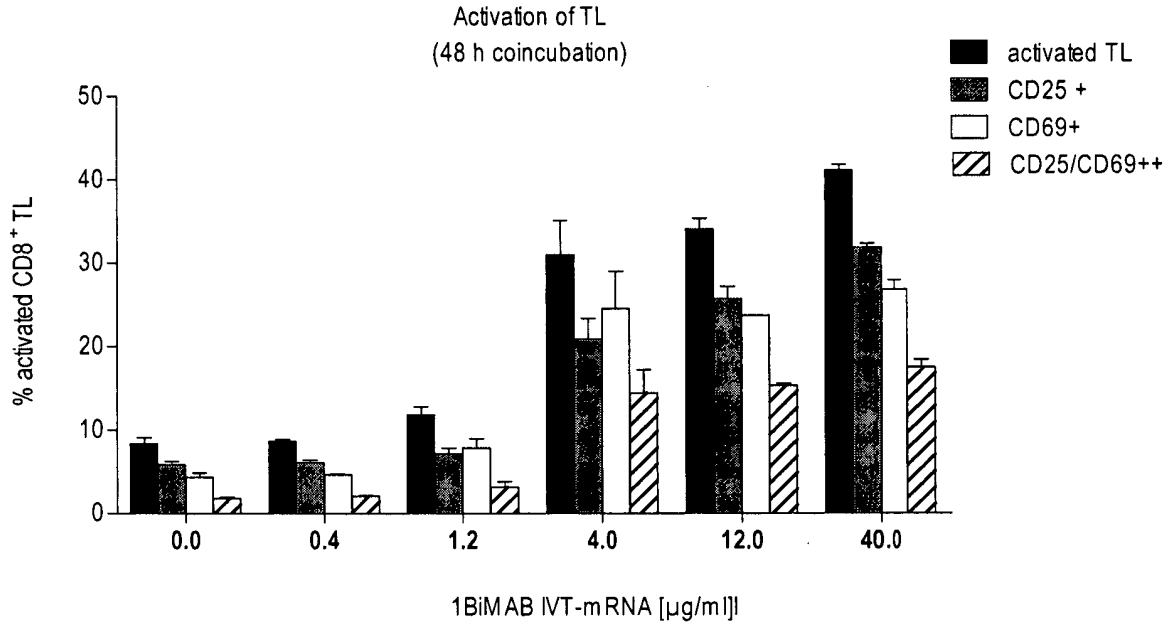

Activation of TL
(48 h coincubation)
activated TL
CD25 +
CD69+
CD25/CD69++
% activated CD8+ TL
50
40
30
20
10
0
0.0
0.4
1.2
4.0
12.0
40.0
1BiMAB IVT-mRNA [µg/ml]]

Figure 24

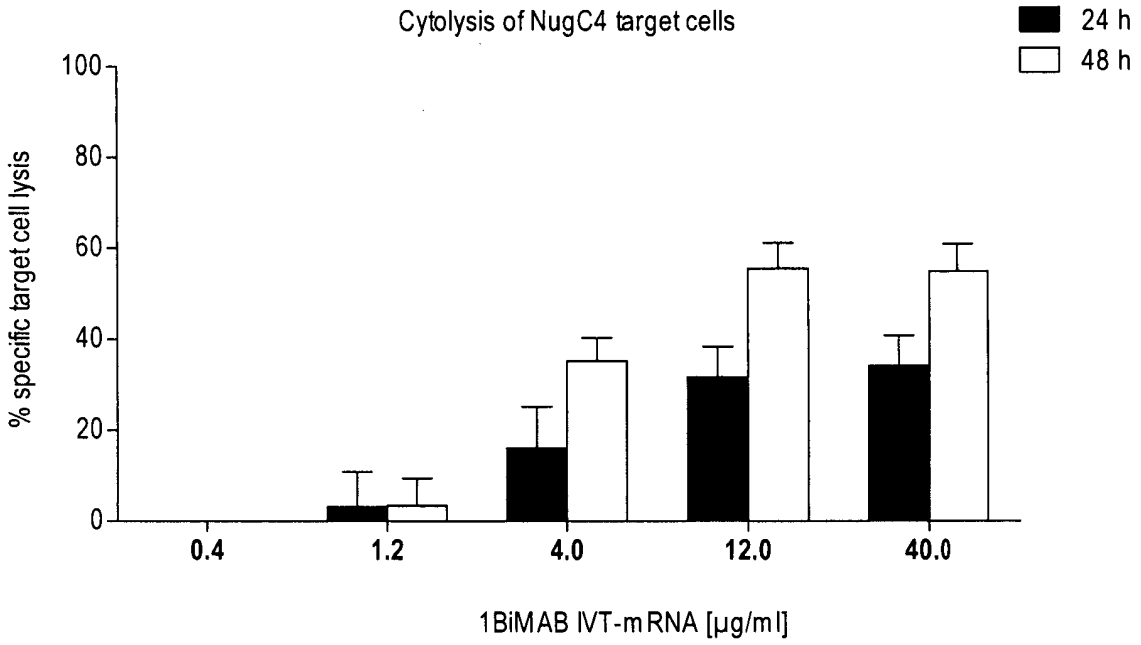

Cytolysis of NugC4 target cells
24 h
48 h
% specific target cell lysis
100
80
60
40
20
0
0.4
1.2
4.0
12.0
40.0
1BiMAB IVT-mRNA [µg/ml]

Figure 25

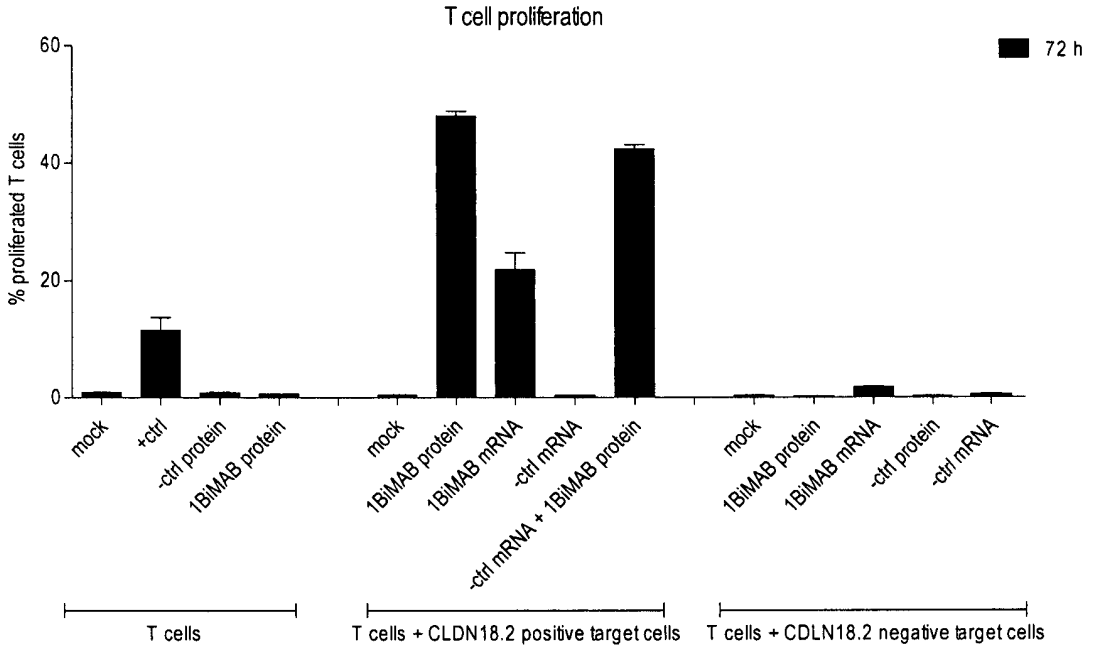
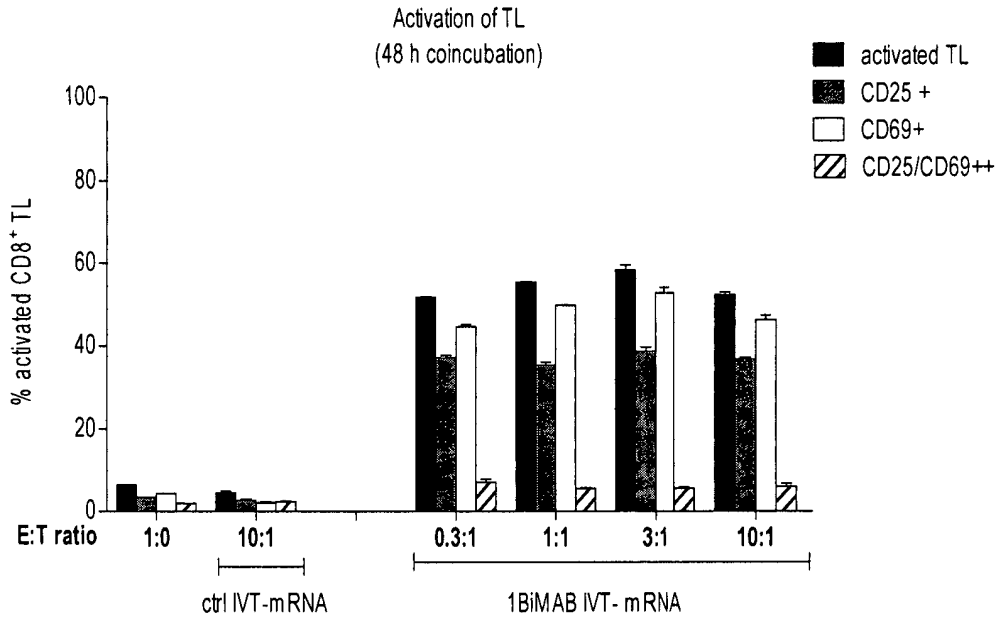

T cell proliferation
72 h
% proliferated T cells
60
40
20
0
mock
+ctrl
-ctrl protein
1BiMAB protein
mock
1BiMAB protein
1BiMAB mRNA
-ctrl mRNA
-ctrl mRNA + 1BiMAB protein
mock
1BiMAB protein
1BiMAB mRNA
-ctrl protein
-ctrl mRNA
T cells
T cells + CLDN18.2 positive target cells
T cells + CDLN18.2 negative target cells

Figure 26

A


Activation of TL
(48 h coincubation)
activated TL
CD25 +
CD69+
CD25/CD69++
% activated CD8+ TL
100
80
60
40
20
0
E:T ratio
1:0
10:1
0.3:1
1:1
3:1
10:1
ctrl IVT-mRNA
1BiMAB IVT- mRNA

B

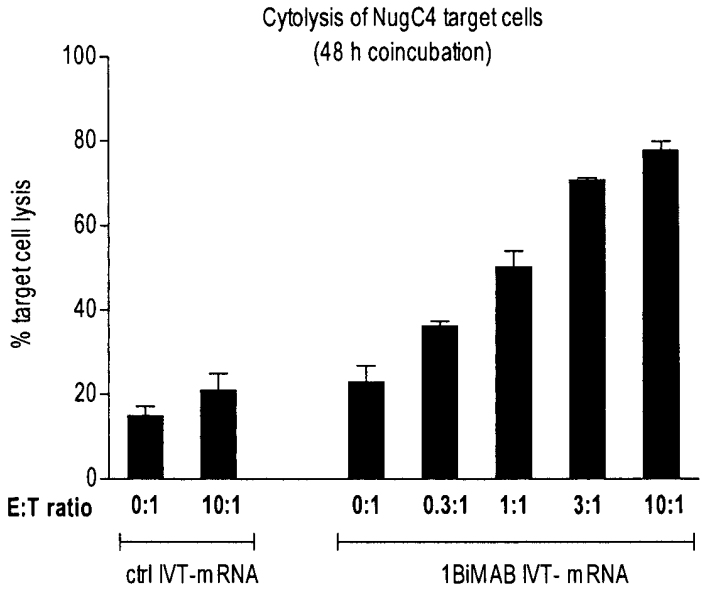
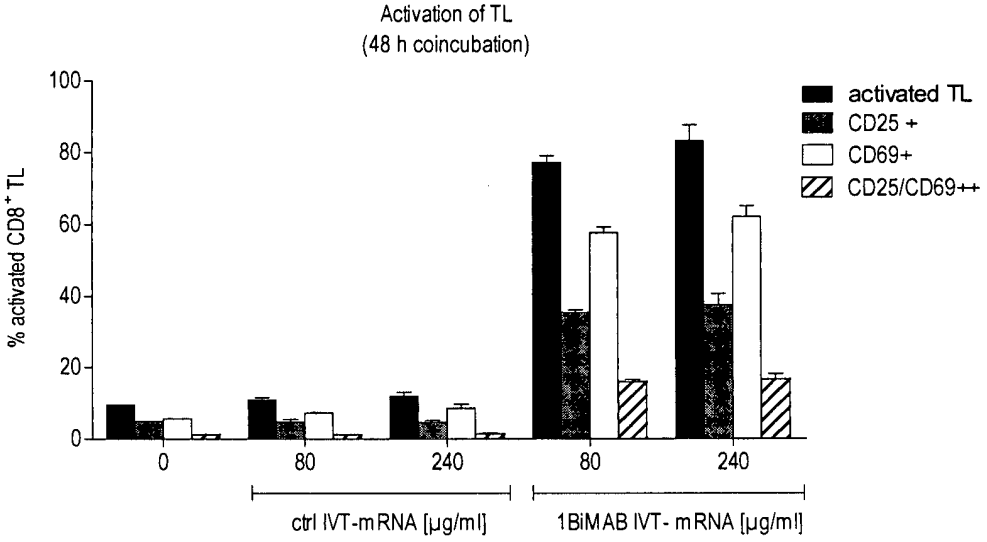

Cytolysis of NugC4 target cells
(48 h coincubation)
% target cell lysis
100
80
60
40
20
0
E:T ratio
0:1
10:1
0:1
0.3:1
1:1
3:1
10:1
ctrl IVT-mRNA
1BiMAB IVT- mRNA

Figure 27

A


Activation of TL
(48 h coincubation)
% activated CD8+ TL
100
80
60
40
20
0
activated TL
CD25 +
CD69+
CD25/CD69++
0
80
240
80
240
ctrl IVT-mRNA [µg/ml]
1BiMAB IVT- mRNA [µg/ml]

B

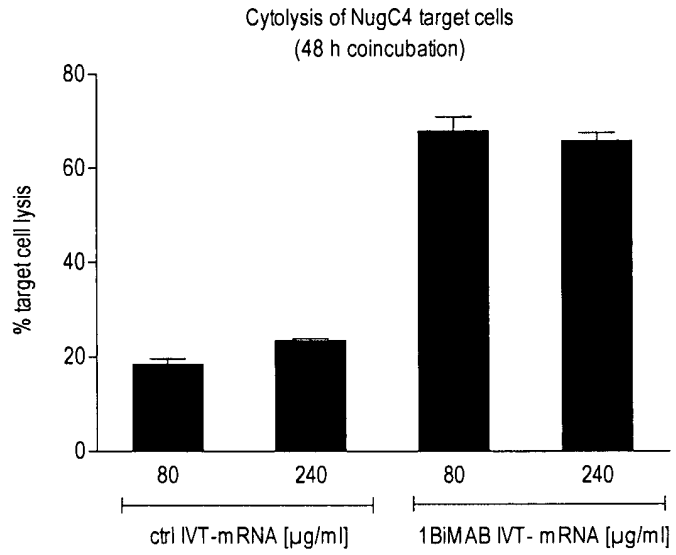

Cytolysis of NugC4 target cells
(48 h coincubation)
% target cell lysis
80
60
40
20
0
80
240
80
240
ctrl IVT-mRNA [µg/ml]
1BiMAB IVT- mRNA [µg/ml]

Figure 28

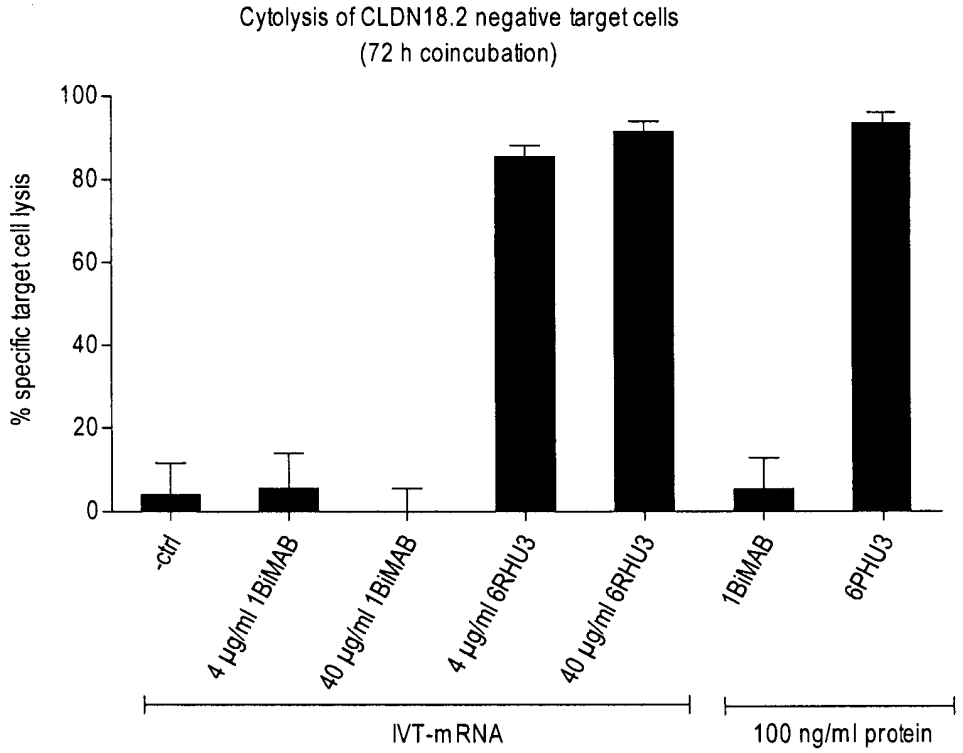
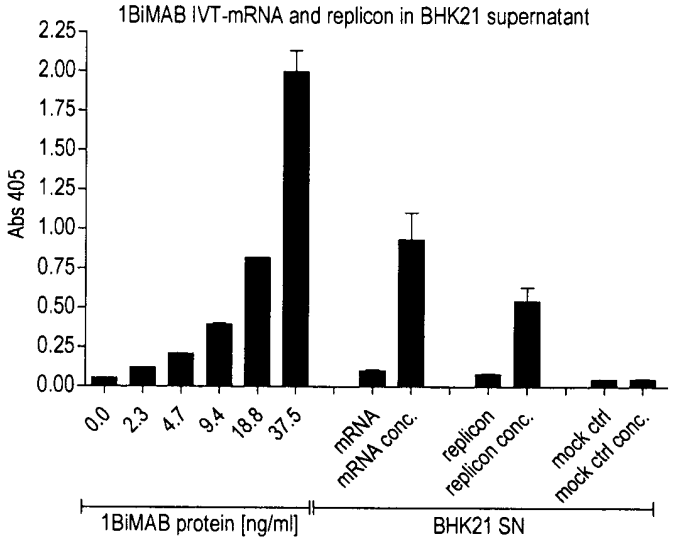
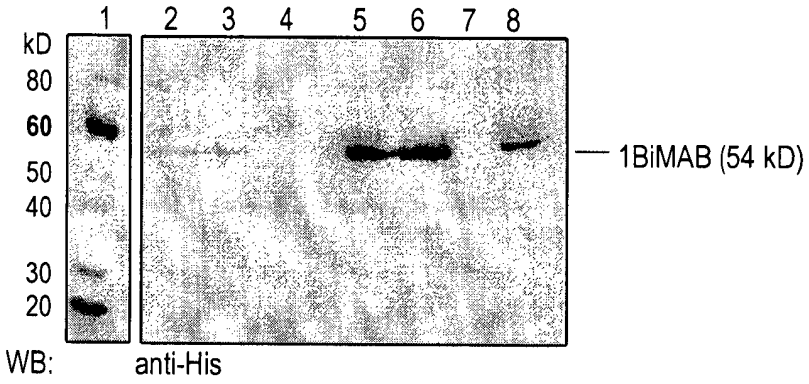

Cytolysis of CLDN18.2 negative target cells
(72 h coincubation)
% specific target cell lysis
100
80
60
40
20
0
-ctrl
4 µg/ml 1BiMAB
40 µg/ml 1BiMAB
4 µg/ml 6RHU3
40 µg/ml 6RHU3
1BiMAB
6PHU3
IVT-mRNA
100 ng/ml protein

Figure 29

A

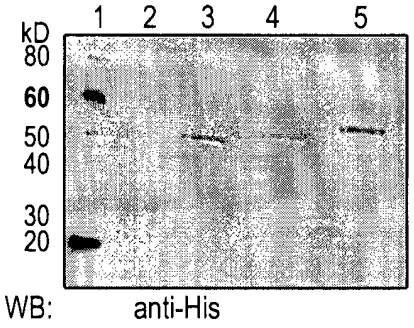


B



lane	sample
1	Protein standard
2	1BiMAB IVT-mRNA in SN
3	1BiMAB IVT-replicon in SN
4	Mock ctrl SN
5	1BiMAB IVT-mRNA in cell lysate
6	1BiMAB IVT-replicon in cell lysate
7	Mock ctrl cell lysate
8	0.1 µg purified 1BiMAB protein

C



lane	sample
1	Protein standard
2	Mock ctrl SN
3	Ctrl BiMAB in SN
4	1BiMAB in SN
5	0.1 µg purified 1BiMAB protein

Figure 30

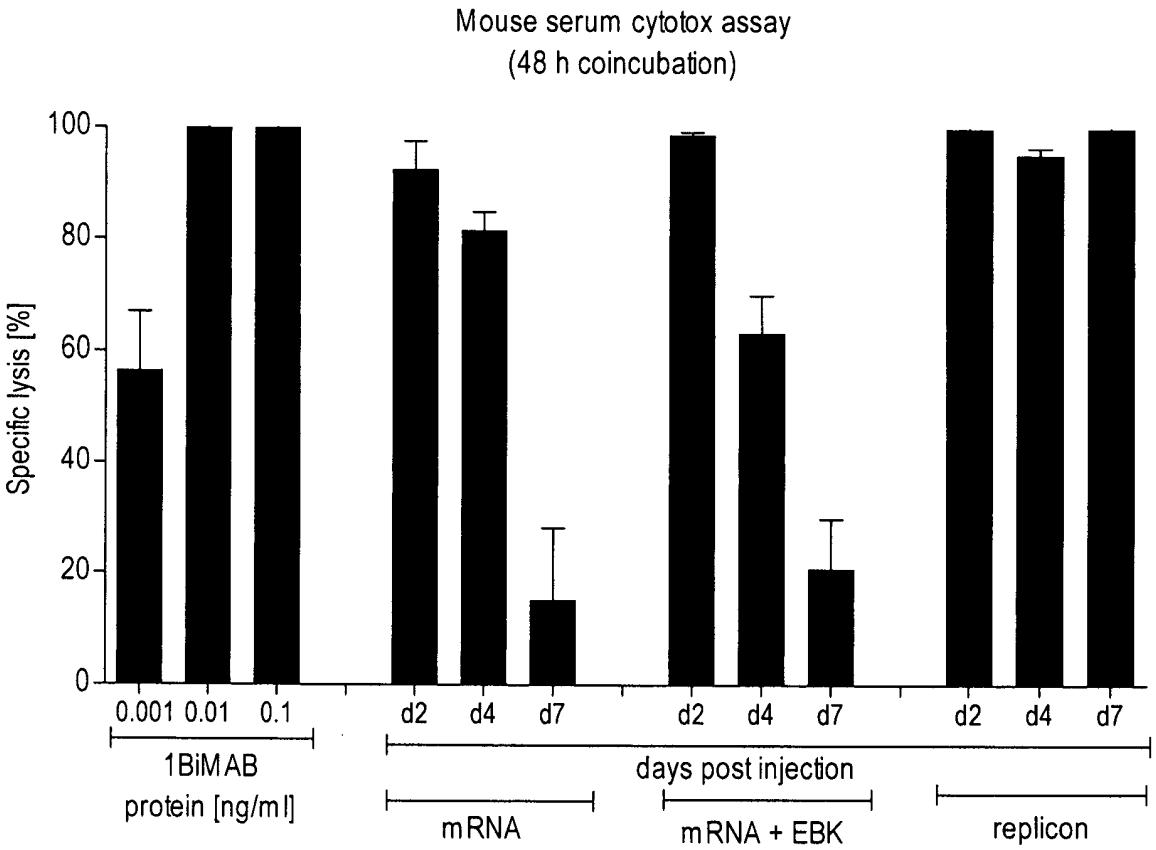
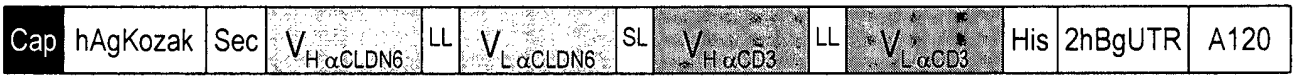

Mouse serum cytotox assay
(48 h coincubation)
Specific lysis [%]
100
80
60
40
20
0
0.001 0.01 0.1
d2 d4 d7
d2 d4 d7
d2 d4 d7
1BiMAB
protein [ng/ml]
days post injection
mRNA
mRNA + EBK
replicon

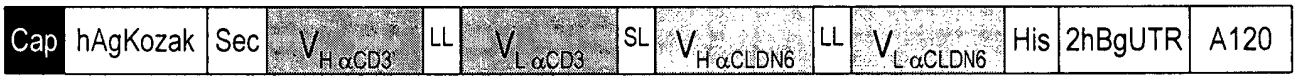
Figure 31

A

bi-scFv CLDN6 x CD3



bi-scFv CD3 x CLDN6



B

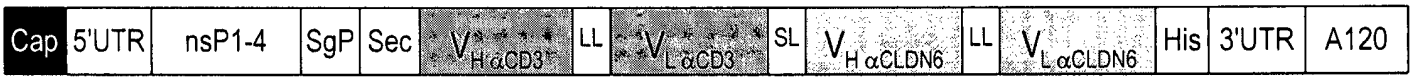


Figure 32

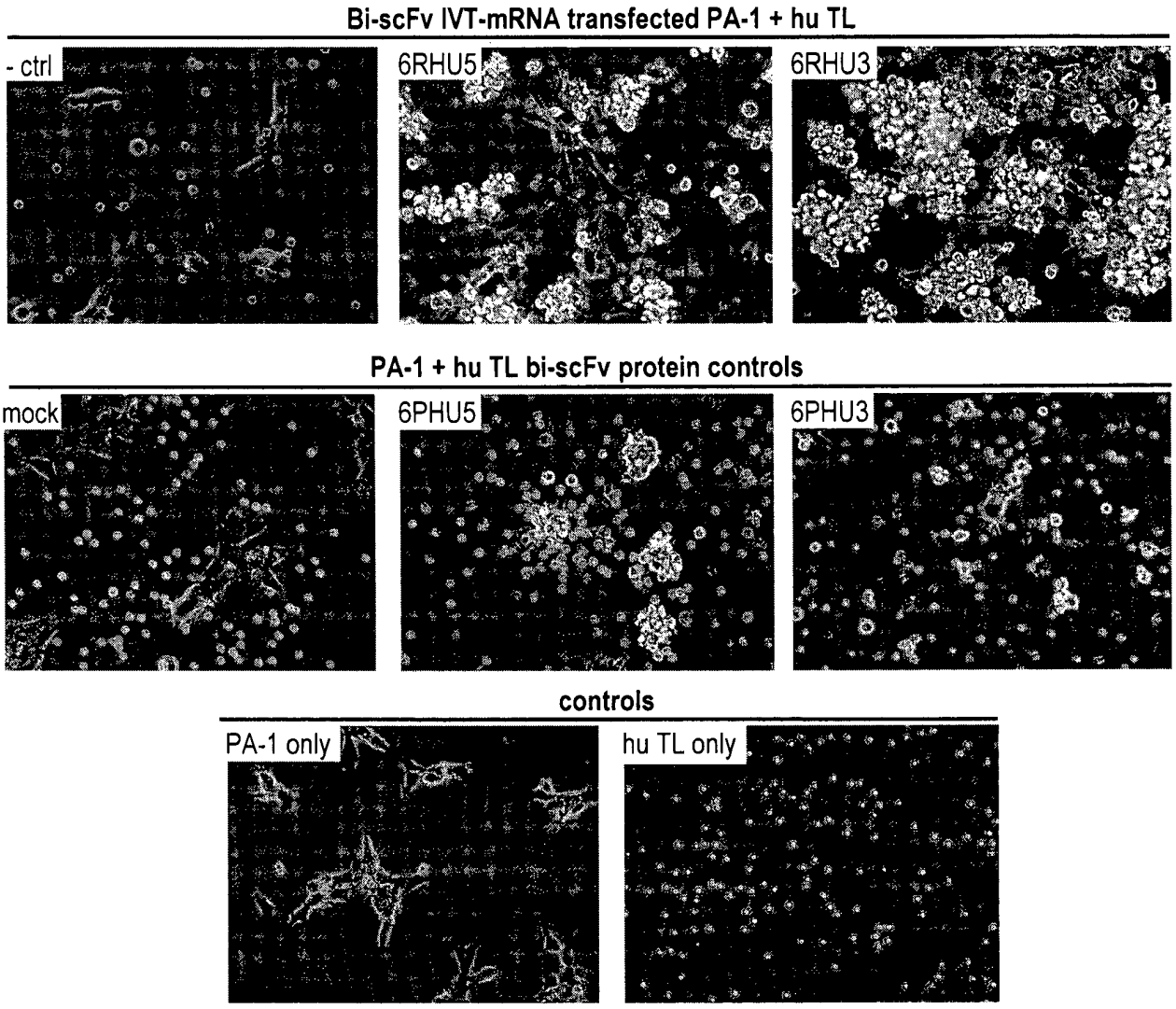

Bi-scFv IVT-mRNA transfected PA-1 + hu TL
- ctrl
6RHU5
6RHU3
PA-1 + hu TL bi-scFv protein controls
mock
6PHU5
6PHU3
controls
PA-1 only
hu TL only

Figure 33

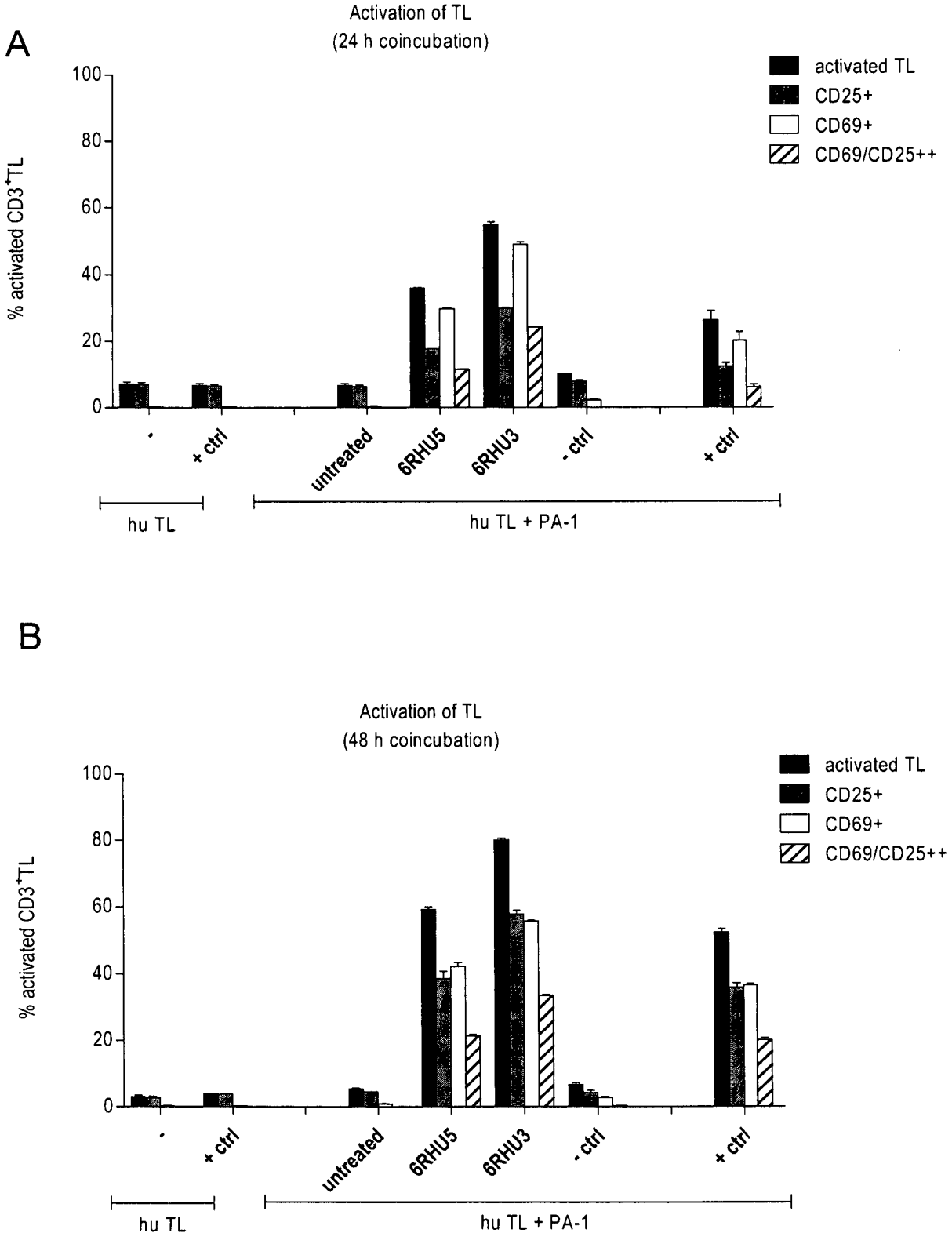

A
Activation of TL
(24 h coincubation)
% activated CD3+TL
100
80
60
40
20
0
activated TL
CD25+
CD69+
CD69/CD25++
-
+ ctrl
untreated
6RHU5
6RHU3
- ctrl
+ ctrl
hu TL
hu TL + PA-1
B
Activation of TL
(48 h coincubation)
% activated CD3+TL
100
80
60
40
20
0
activated TL
CD25+
CD69+
CD69/CD25++
-
+ ctrl
untreated
6RHU5
6RHU3
- ctrl
+ ctrl
hu TL
hu TL + PA-1

Figure 34

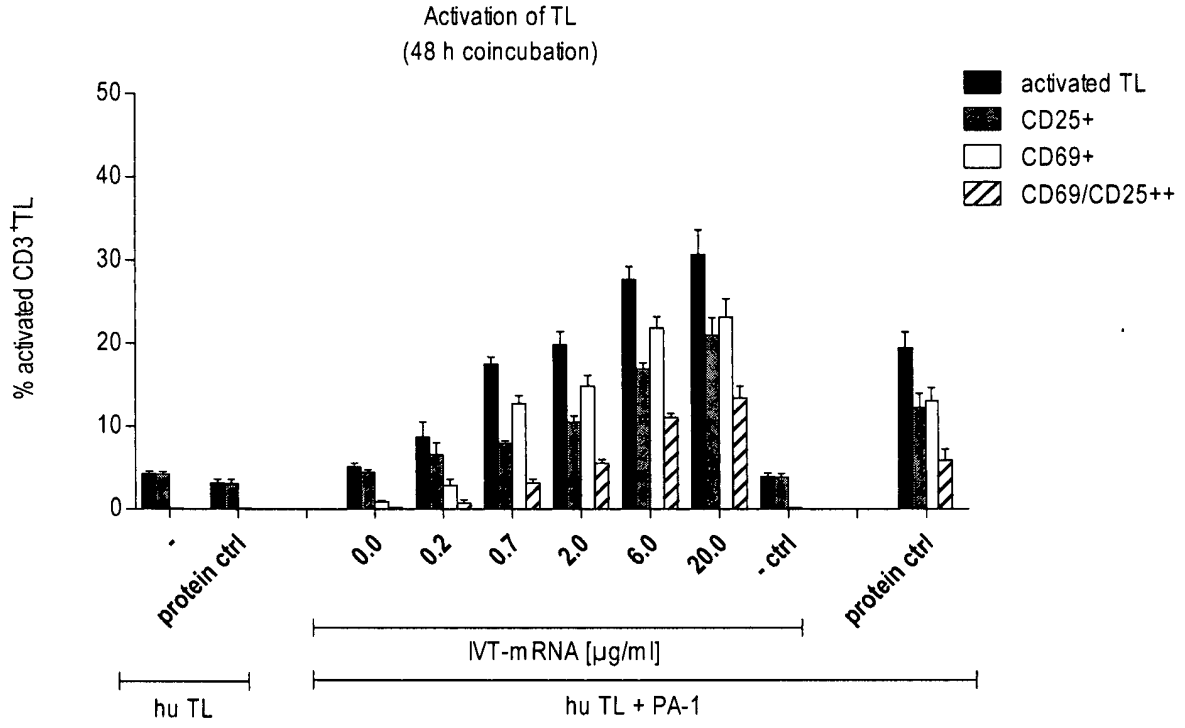

Activation of TL
(48 h coincubation)
activated TL
CD25+
CD69+
CD69/CD25++
% activated CD3+TL
50
40
30
20
10
0
-
protein ctrl
0.0
0.2
0.7
2.0
6.0
20.0
- ctrl
protein ctrl
IVT-mRNA [µg/ml]
hu TL
hu TL + PA-1

Figure 35

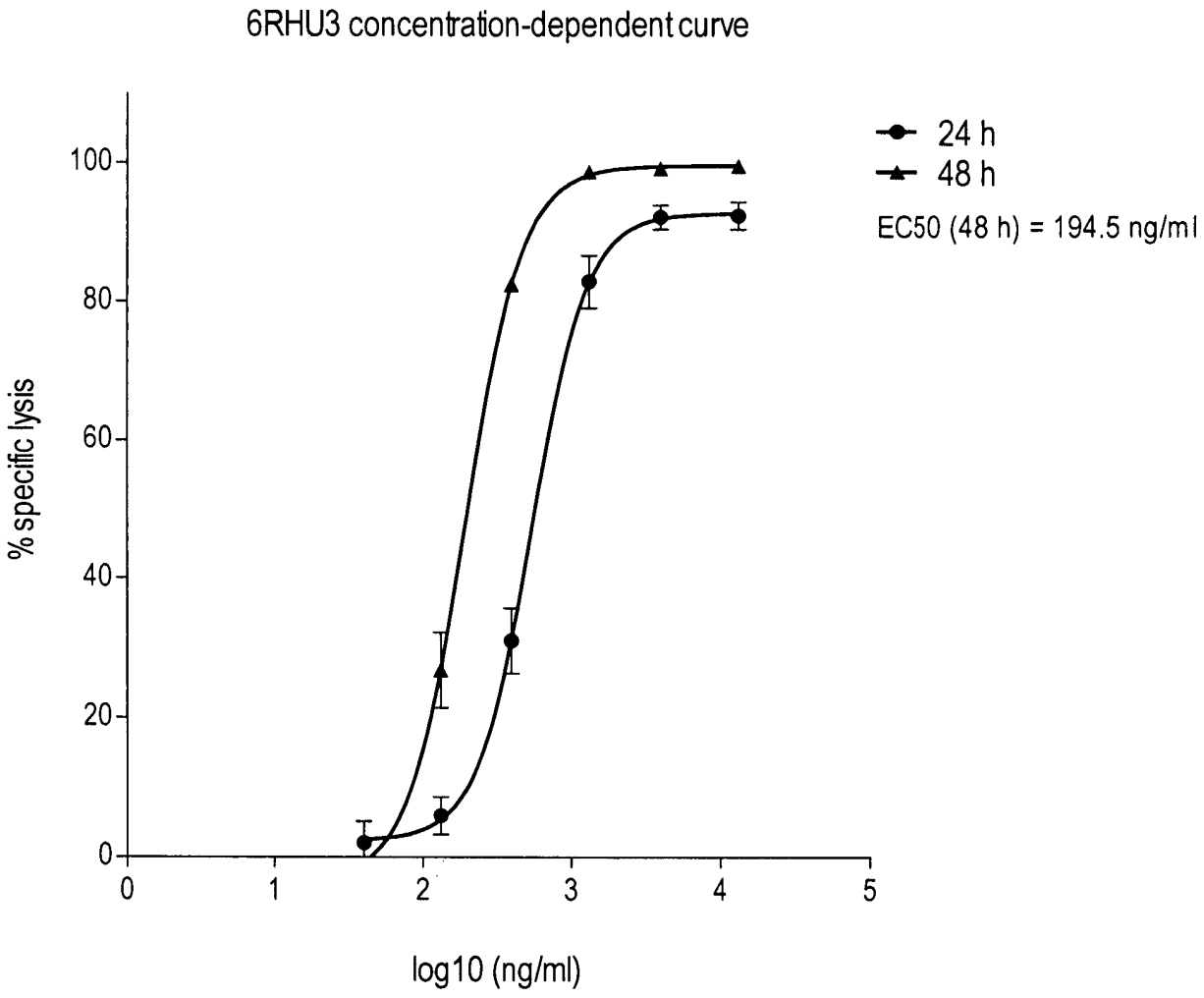

6RHU3 concentration-dependent curve
24 h
48 h
EC50 (48 h) = 194.5 ng/ml
% specific lysis
100
80
60
40
20
0
0
1
2
3
4
5
log10 (ng/ml)

Figure 36

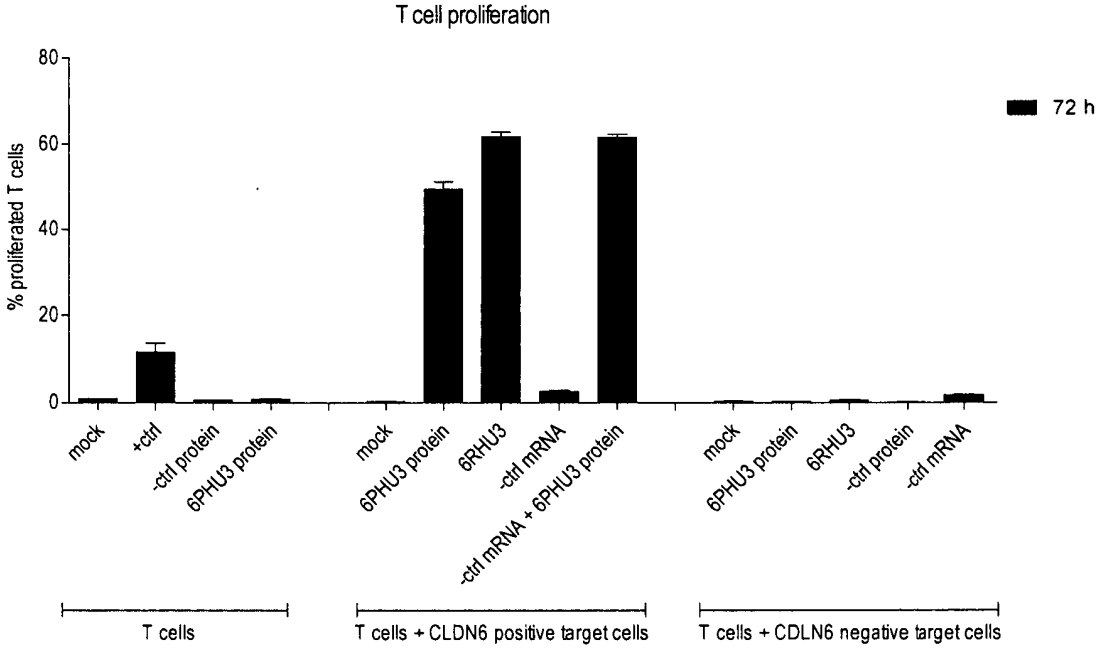
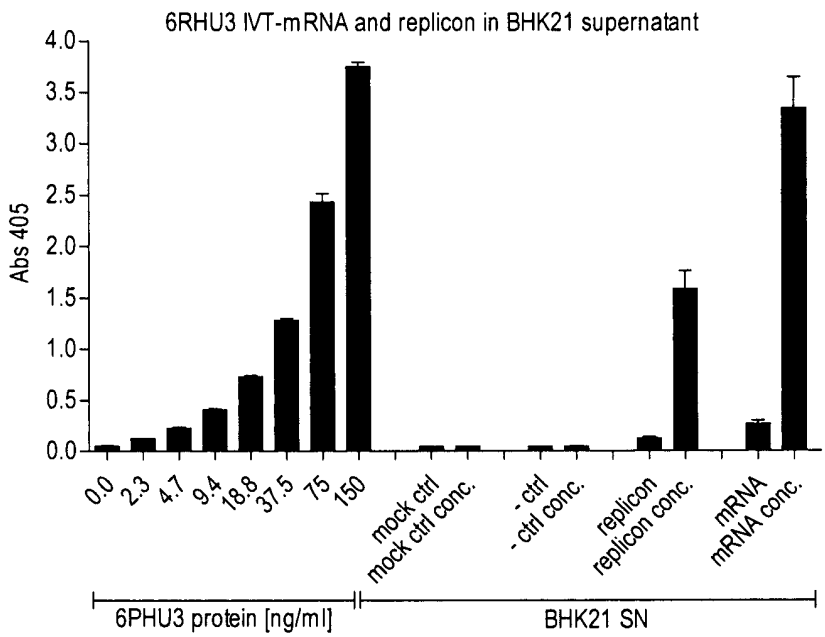
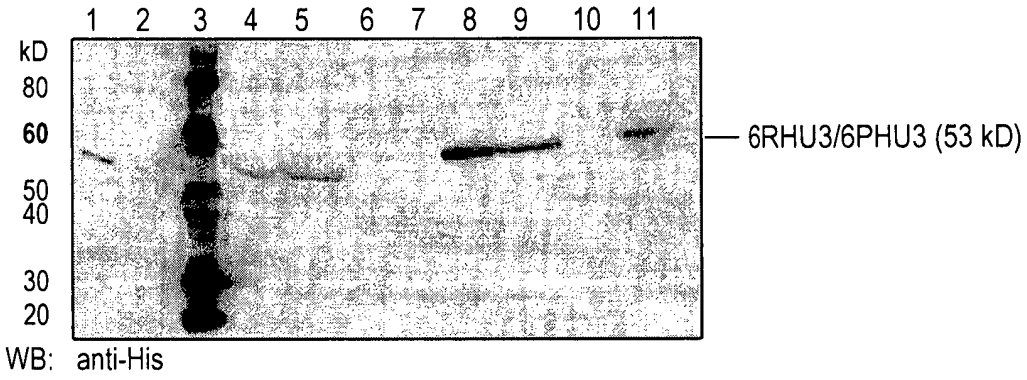

T cell proliferation
% proliferated T cells
80
60
40
20
0
72 h
mock
+ctrl
-ctrl protein
6PHU3 protein
mock
6PHU3 protein
6RHU3
-ctrl mRNA
-ctrl mRNA + 6PHU3 protein
mock
6PHU3 protein
6RHU3
-ctrl protein
-ctrl mRNA
T cells
T cells + CLDN6 positive target cells
T cells + CDLN6 negative target cells

Figure 37

A

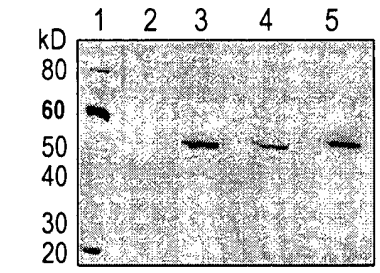


B



lane	sample
1	No.25 in SN
2	No.25 in cell lysate
3	Protein standard
4	6RHU3 IVT-mRNA in SN
5	6RHU3 IVT-replicon in SN
6	Mock ctrl SN
7	-
8	6RHU3 IVT-mRNA in cell lysate
9	6RHU3 IVT-replicon in cell lysate
10	Mock ctrl cell lysate
11	0.1 µg purified 6PHU3 protein

C



WB: anti-His

lane	sample
1	Protein standard
2	Mock ctrl SN
3	Ctrl BiMAB in SN
4	6RHU3 in SN
5	0.1 µg purified 6PHU3 protein

Figure 38

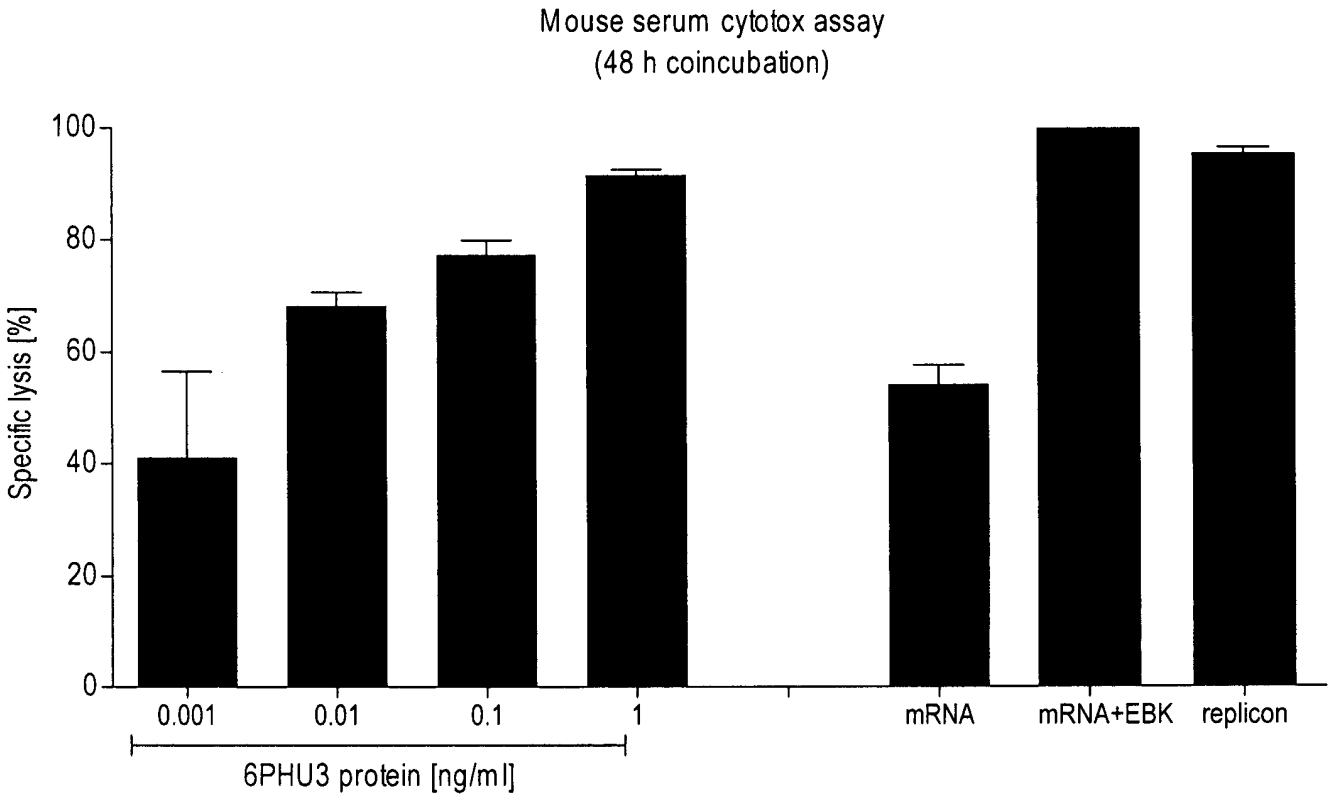

Mouse serum cytotox assay
(48 h coincubation)
Specific lysis [%]
100
80
60
40
20
0
0.001
0.01
0.1
1
6PHU3 protein [ng/ml]
mRNA
mRNA+EBK
replicon

Figure 39 a

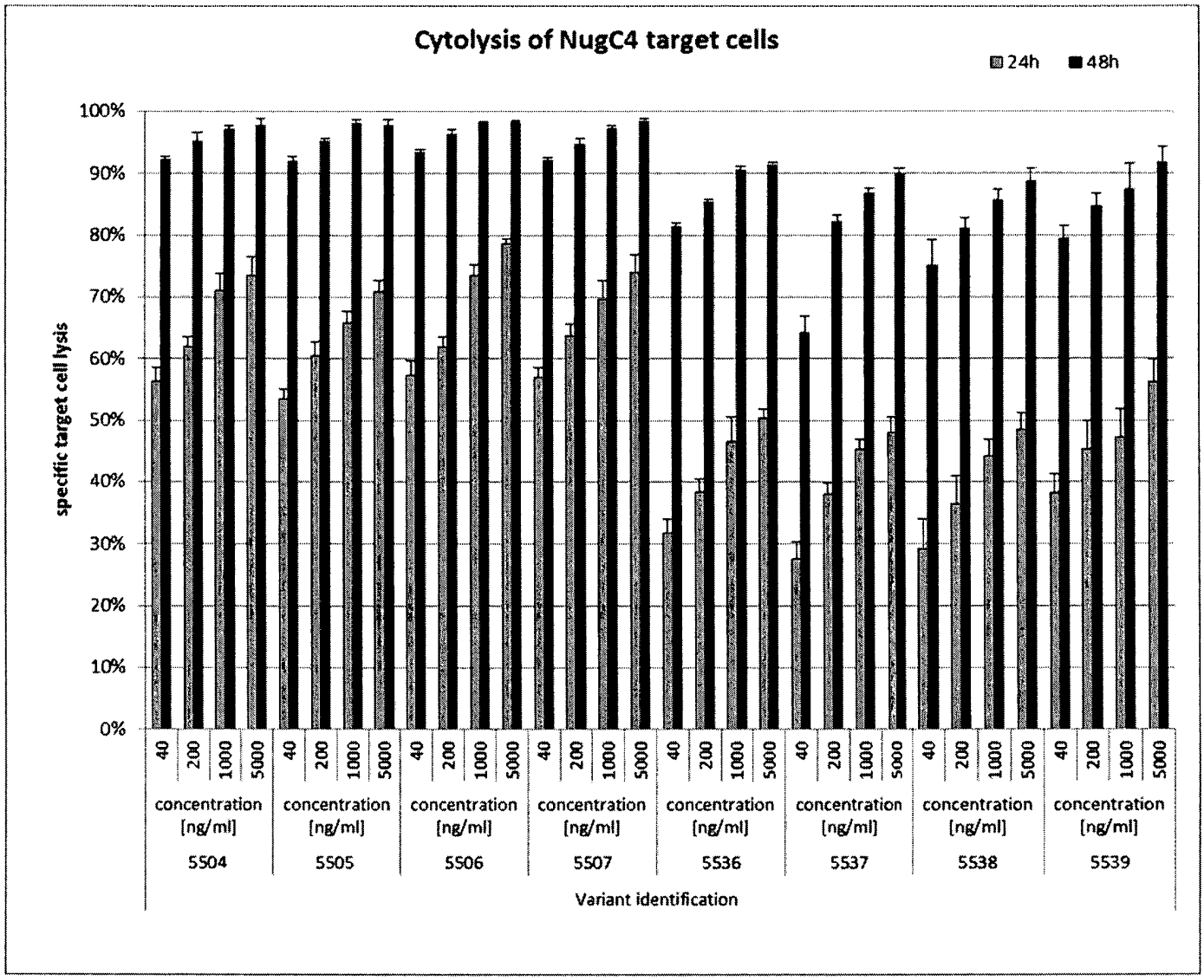

Cytolysis of NugC4 target cells
24h
48h
specific target cell lysis
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
40
200
1000
5000
concentration [ng/ml]
5504
5505
5506
5507
5536
5537
5538
5539
Variant identification

Figure 39 b

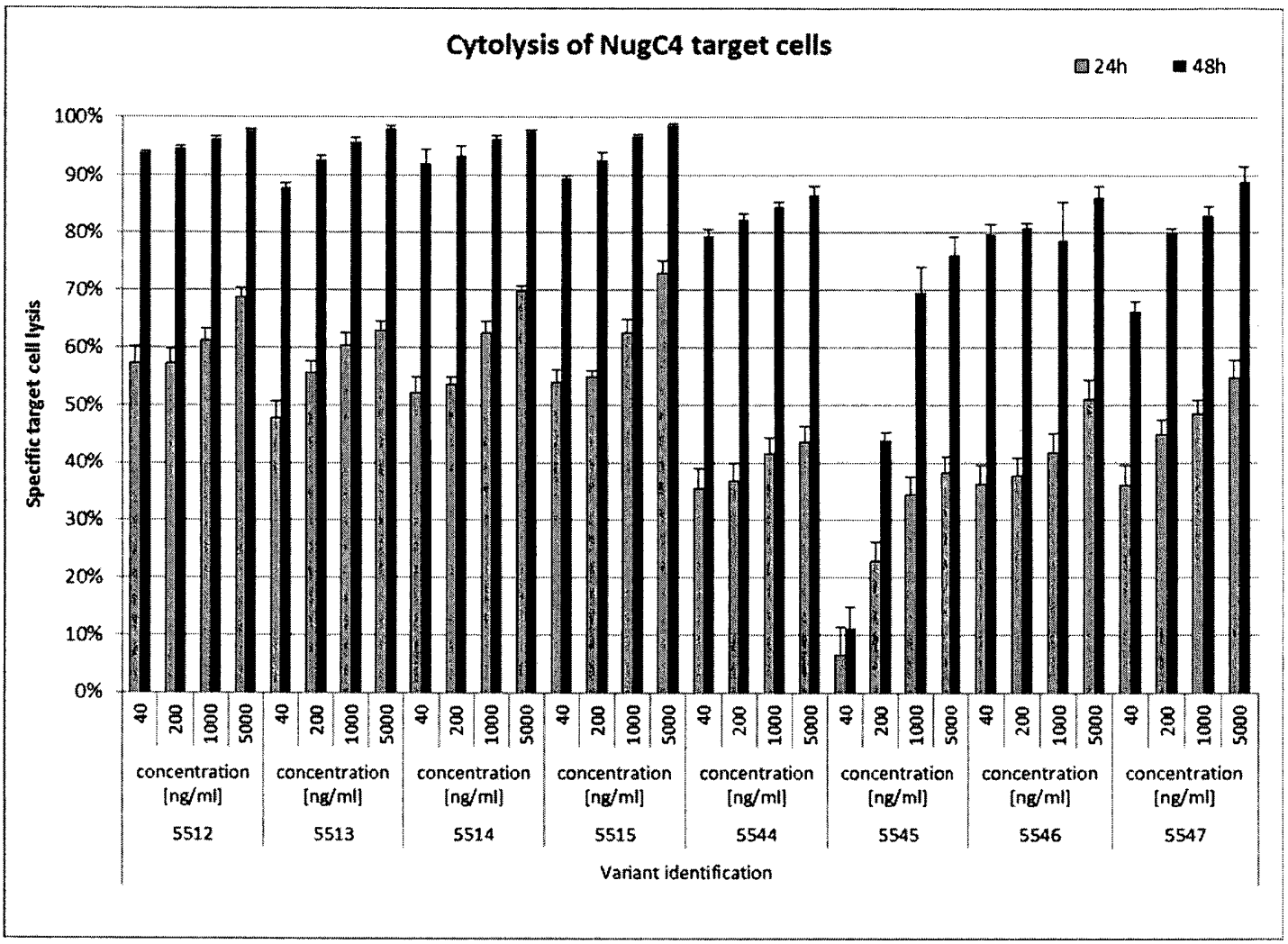

Cytolysis of NugC4 target cells
24h
48h
Specific target cell lysis
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
40
200
1000
5000
concentration [ng/ml]
5512
5513
5514
5515
5544
5545
5546
5547
Variant identification

Figure 39 c

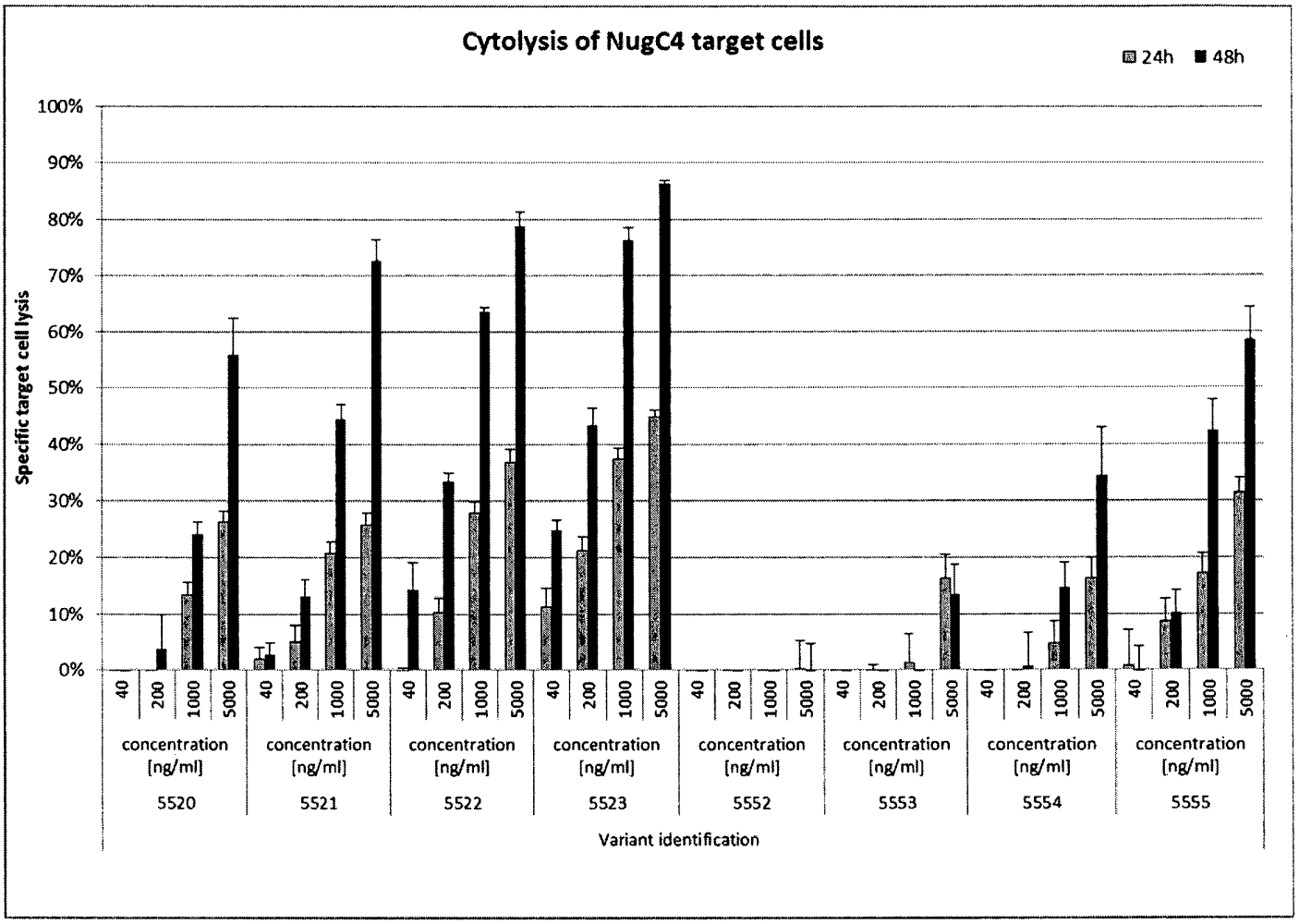

Cytolysis of NugC4 target cells
24h
48h
Specific target cell lysis
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
40
200
1000
5000
concentration [ng/ml]
5520
5521
5522
5523
5552
5553
5554
5555
Variant identification

Figure 39 d

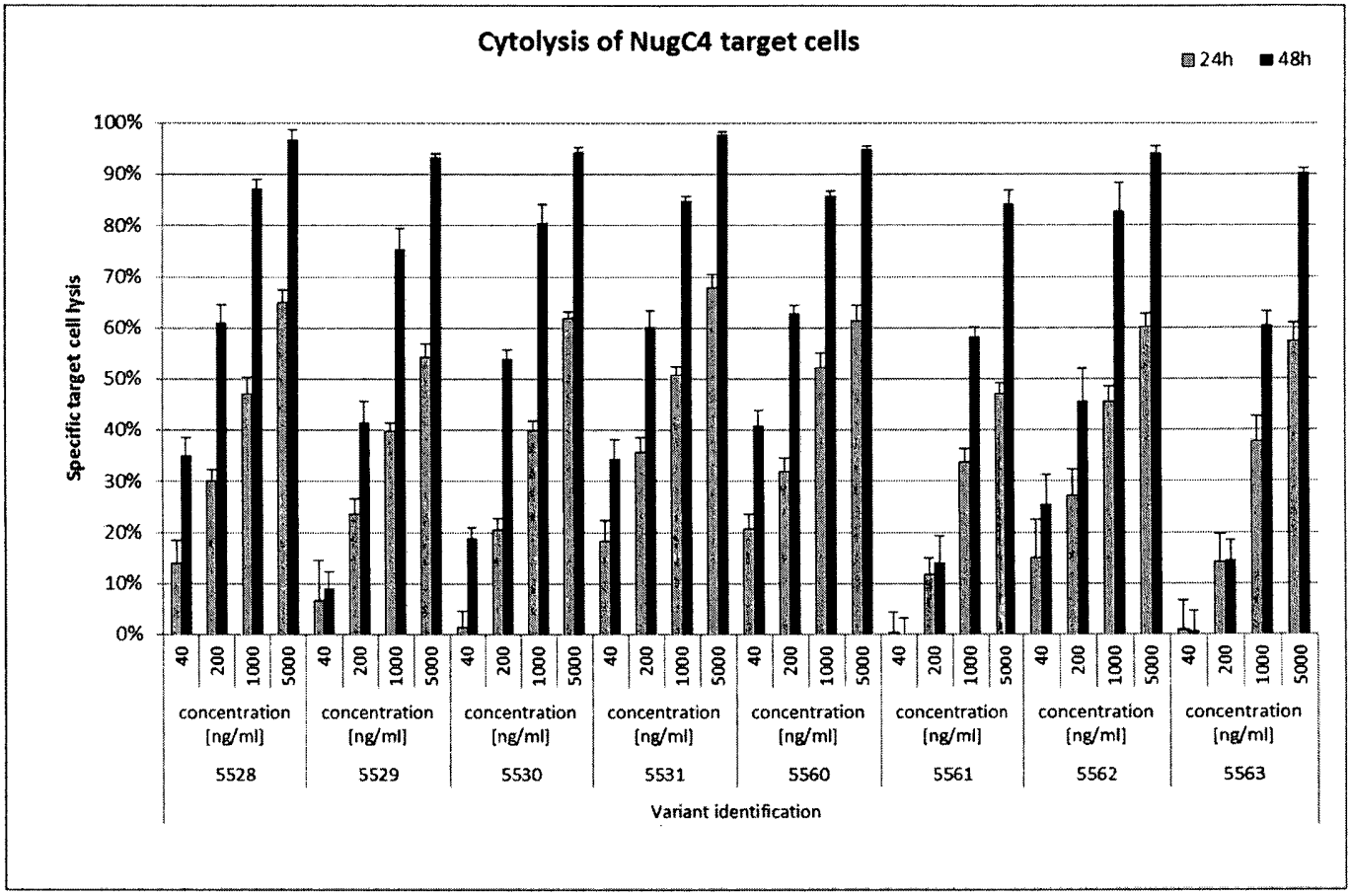
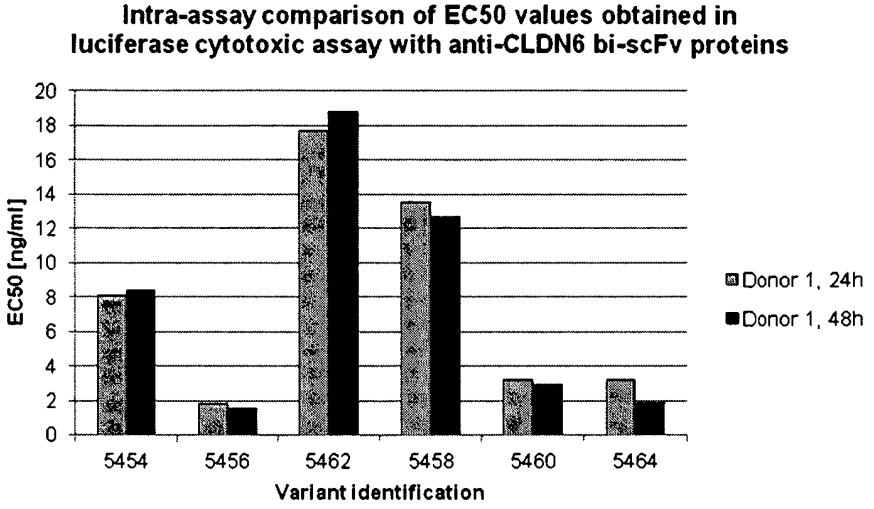
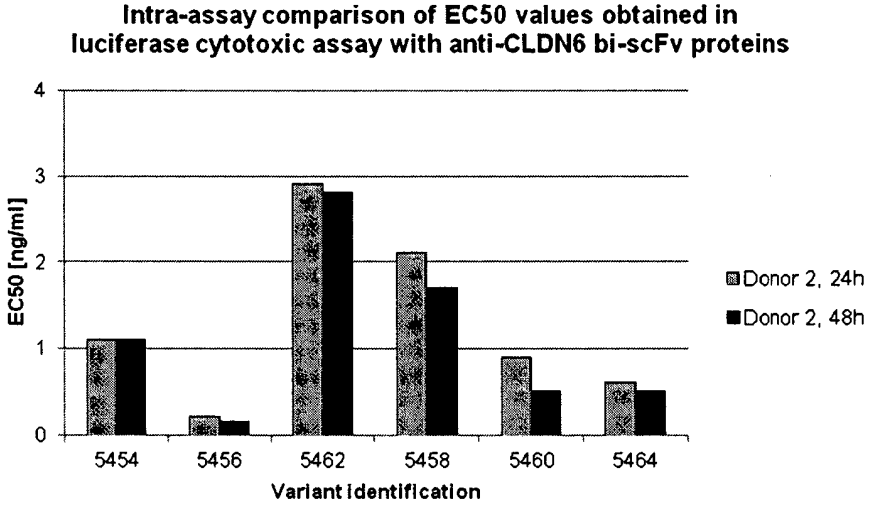

Cytolysis of NugC4 target cells
24h
48h
Specific target cell lysis
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
40
200
1000
5000
concentration [ng/ml]
5528
5529
5530
5531
5560
5561
5562
5563
Variant identification

Figure 40

A



B



C

