Fig. 1

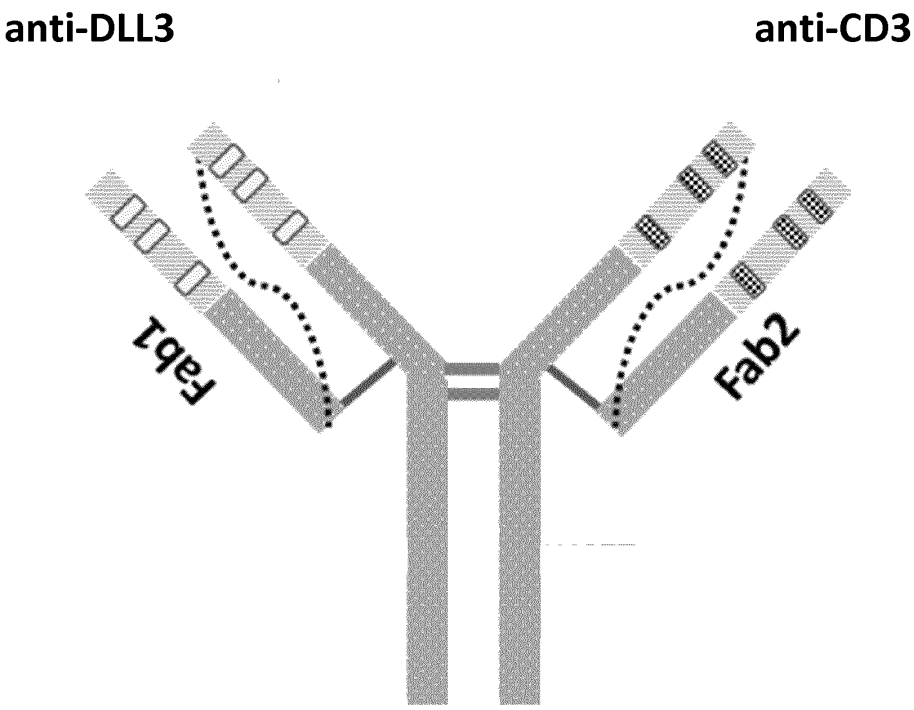

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Fig. 2

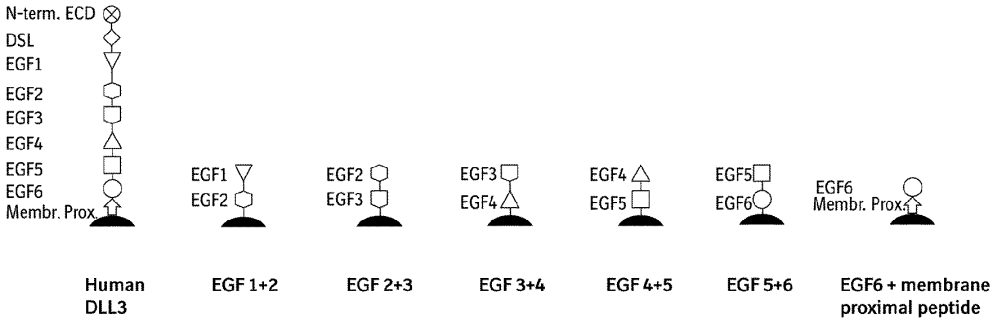
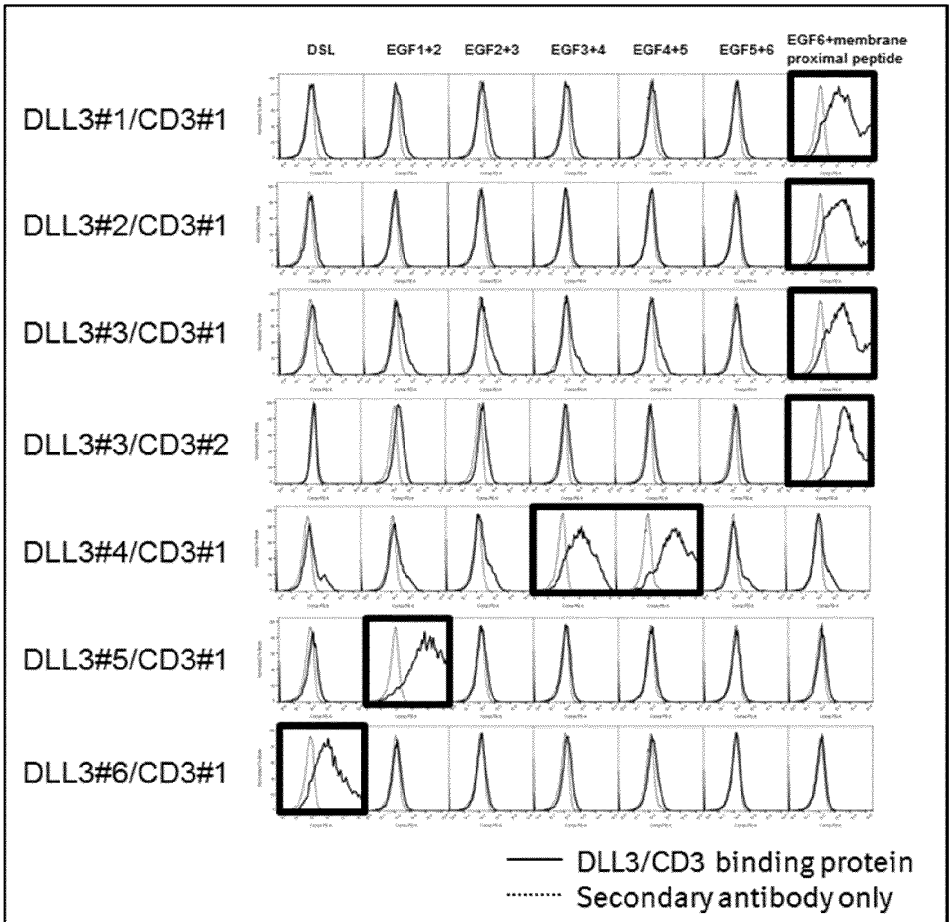
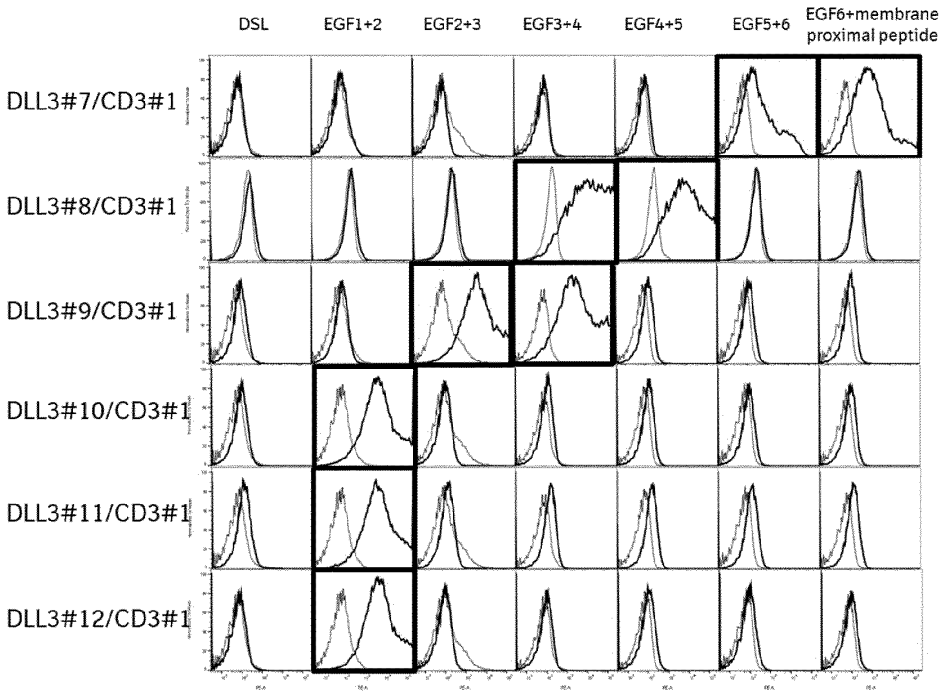

N-term. ECD
DSL
EGF1
EGF2
EGF3
EGF4
EGF5
EGF6
Membr. Prox.
EGF1
EGF2
EGF2
EGF3
EGF3
EGF4
EGF4
EGF5
EGF5
EGF6
EGF6
Membr. Prox.
Human DLL3
EGF 1+2
EGF 2+3
EGF 3+4
EGF 4+5
EGF 5+6
EGF6 + membrane proximal peptide

Fig. 3A



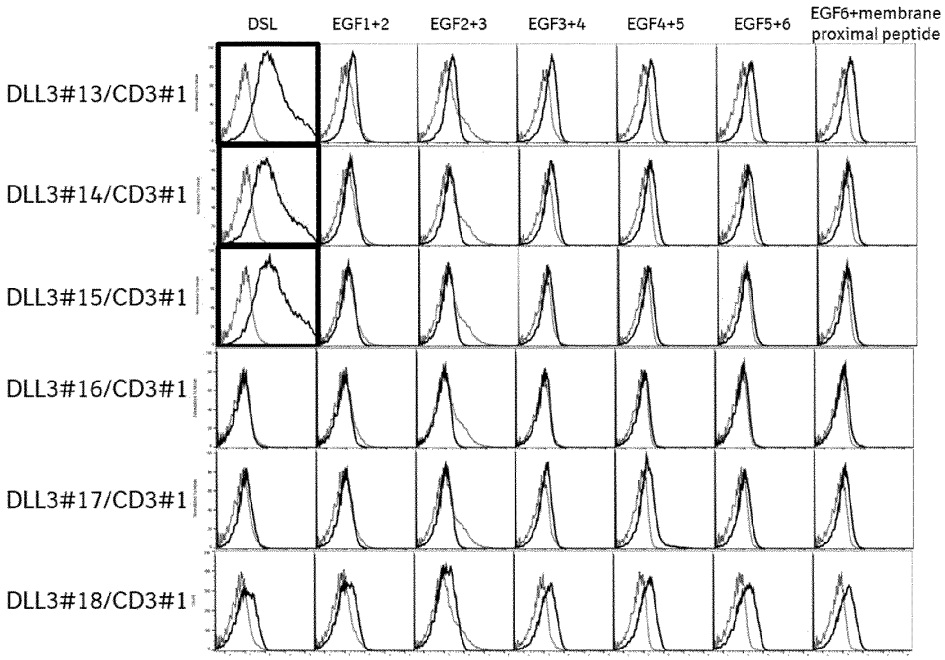
DLL3/CD3 binding proteins	Epitope Domain
DLL3#1/CD3#1	Membrane proximal peptide
DLL3#2/CD3#1	Membrane proximal peptide
DLL3#3/CD3#1	Membrane proximal peptide
DLL3#3/CD3#2	Membrane proximal peptide
DLL3#4/CD3#1	EGF4
DLL3#5/CD3#1	EGF1
DLL3#6/CD3#1	DSL

Fig. 3B



DLL3/CD3 Binding Proteins	Epitope Domain
DLL3#7/CD3#1	EGF6
DLL3#8/CD3#1	EGF4
DLL3#9/CD3#1	EGF3
DLL3#10/CD3#1	EGF1
DLL3#11/CD3#1	EGF1
DLL3#12/CD3#1	EGF1

Fig. 3C



DLL3/CD3 Binding Proteins	Epitope Domain
DLL3#6/CD3#1	DSL
DLL3#13/CD3#1	DSL
DLL3#14/CD3#1	DSL
DLL3#15/CD3#1	DSL
DLL3#16/CD3#1	Non-DSL – non-EGF1-6—non-membrane proximal peptide
DLL3#17/CD3#1	Non-DSL – non-EGF1-6—non-membrane proximal peptide
DLL3#18/CD3#1	Non-DSL – non-EGF1-6—non-membrane proximal peptide

Fig. 4

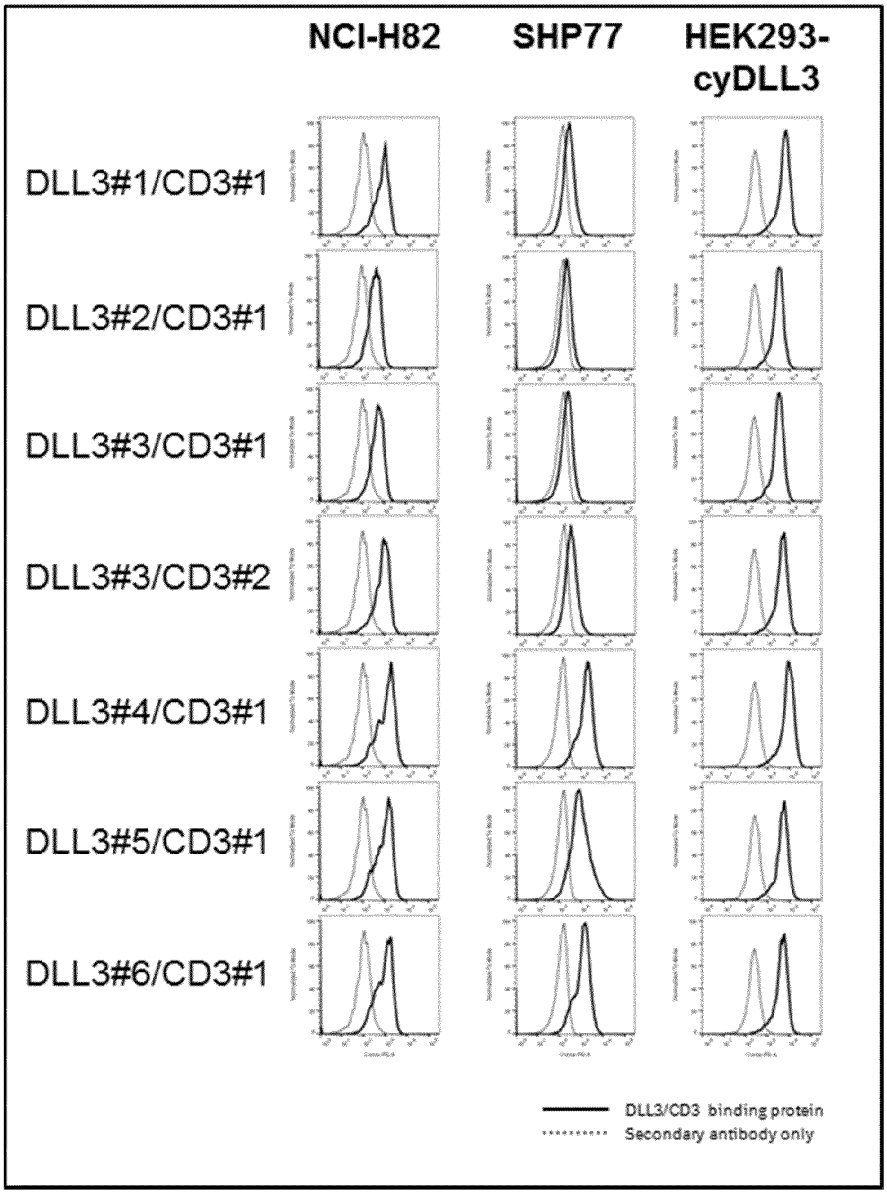

NCI-H82
SHP77
HEK293-cyDLL3
DLL3#1/CD3#1
DLL3#2/CD3#1
DLL3#3/CD3#1
DLL3#3/CD3#2
DLL3#4/CD3#1
DLL3#5/CD3#1
DLL3#6/CD3#1
DLL3/CD3 binding protein
Secondary antibody only

Fig. 5

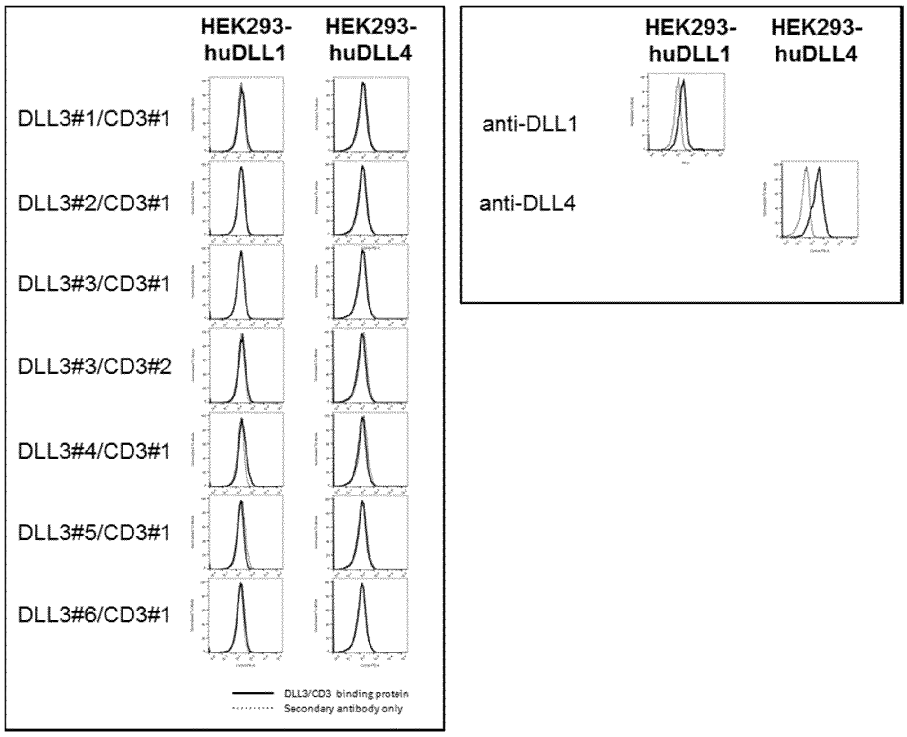

HEK293-huDLL1
HEK293-huDLL4
DLL3#1/CD3#1
DLL3#2/CD3#1
DLL3#3/CD3#1
DLL3#3/CD3#2
DLL3#4/CD3#1
DLL3#5/CD3#1
DLL3#6/CD3#1
DLL3/CD3 binding protein
Secondary antibody only
HEK293-huDLL1
HEK293-huDLL4
anti-DLL1
anti-DLL4

Fig. 6A

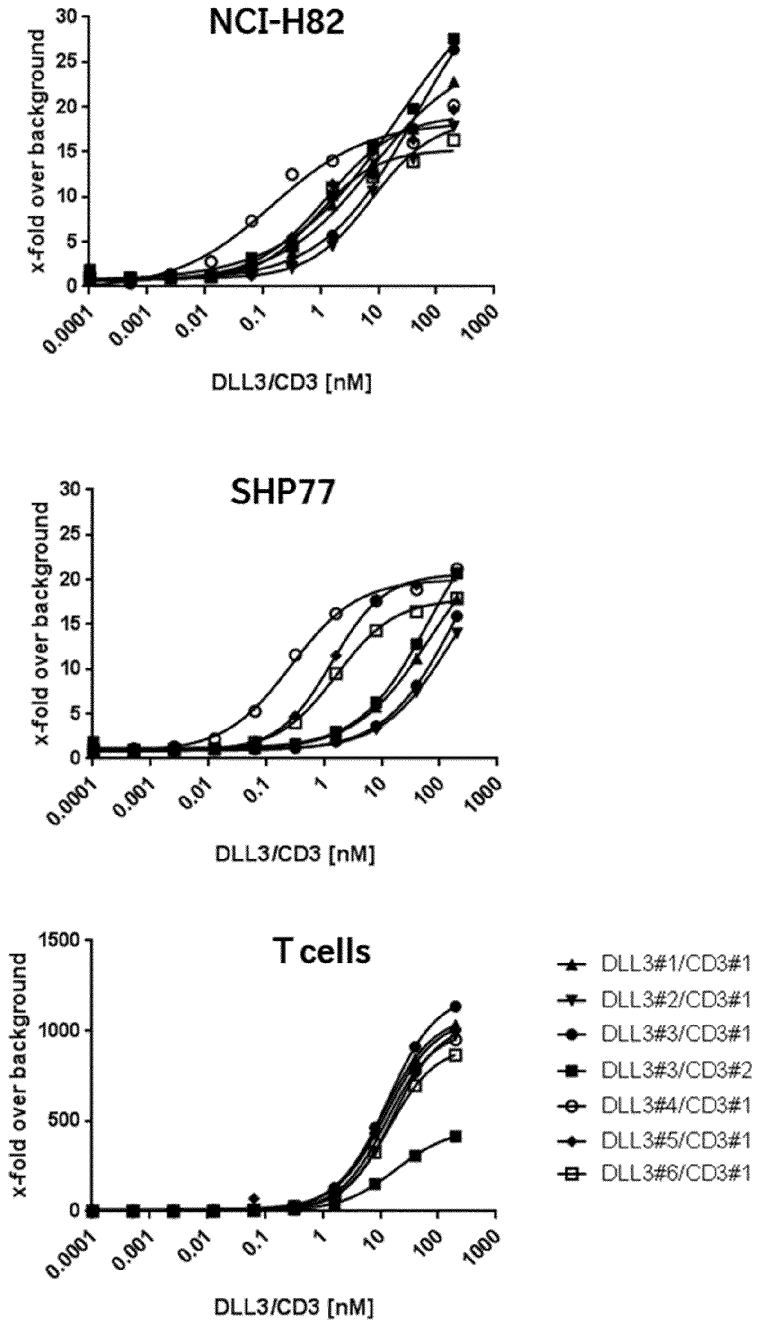

NCI-H82
x-fold over background
DLL3/CD3 [nM]
SHP77
x-fold over background
DLL3/CD3 [nM]
T cells
x-fold over background
DLL3/CD3 [nM]
DLL3#1/CD3#1
DLL3#2/CD3#1
DLL3#3/CD3#1
DLL3#3/CD3#2
DLL3#4/CD3#1
DLL3#5/CD3#1
DLL3#6/CD3#1

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Fig. 6B

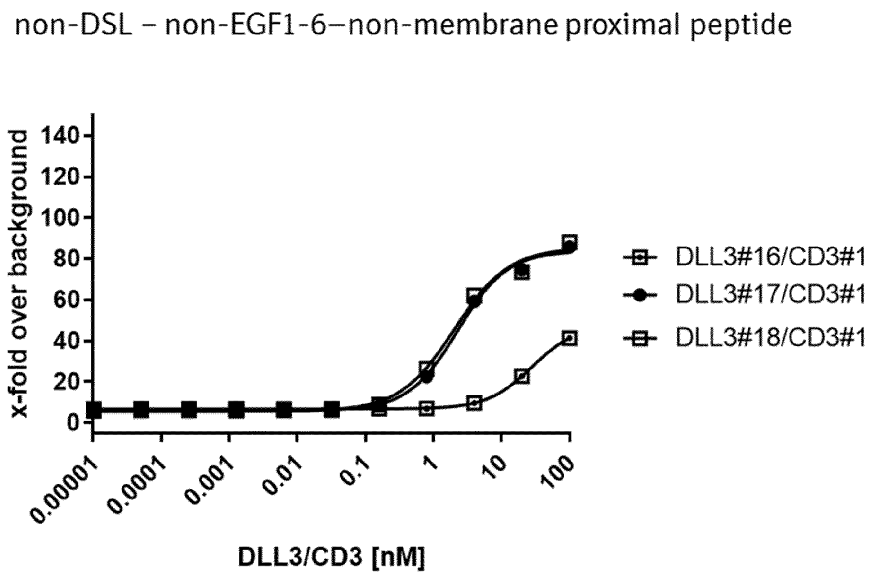
non-DSL – non-EGF1-6–non-membrane proximal peptide
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#16/CD3#1
DLL3#17/CD3#1
DLL3#18/CD3#1

Fig. 6C

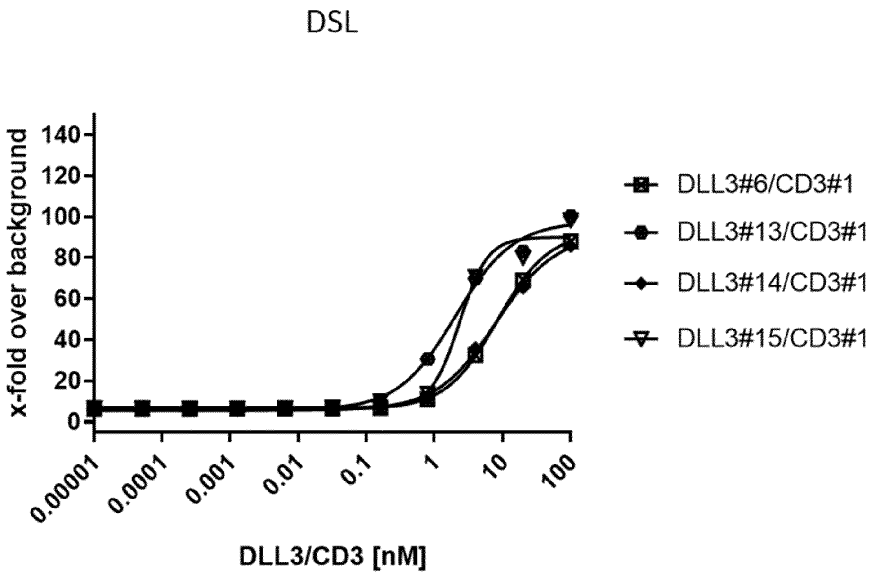
DSL
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#6/CD3#1
DLL3#13/CD3#1
DLL3#14/CD3#1
DLL3#15/CD3#1

Fig. 6D

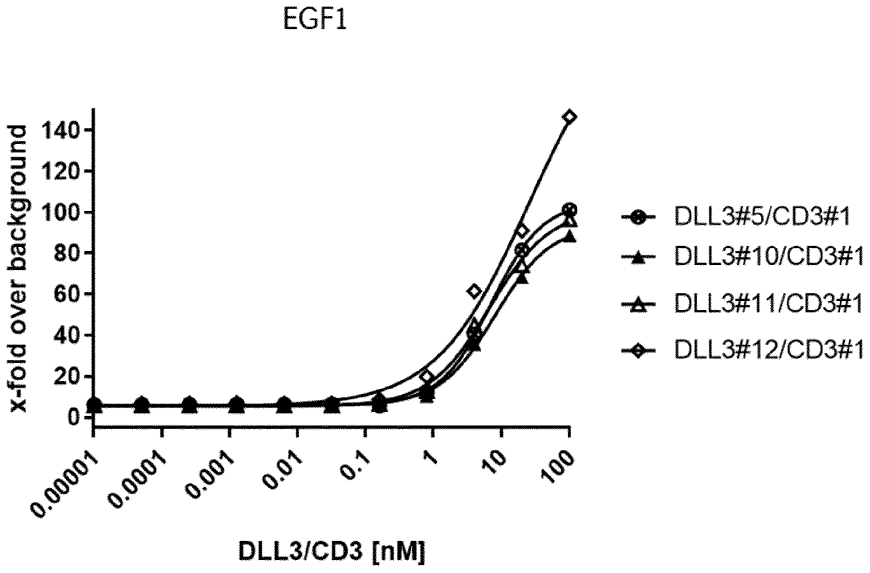
EGF1
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#5/CD3#1
DLL3#10/CD3#1
DLL3#11/CD3#1
DLL3#12/CD3#1

Fig. 6E

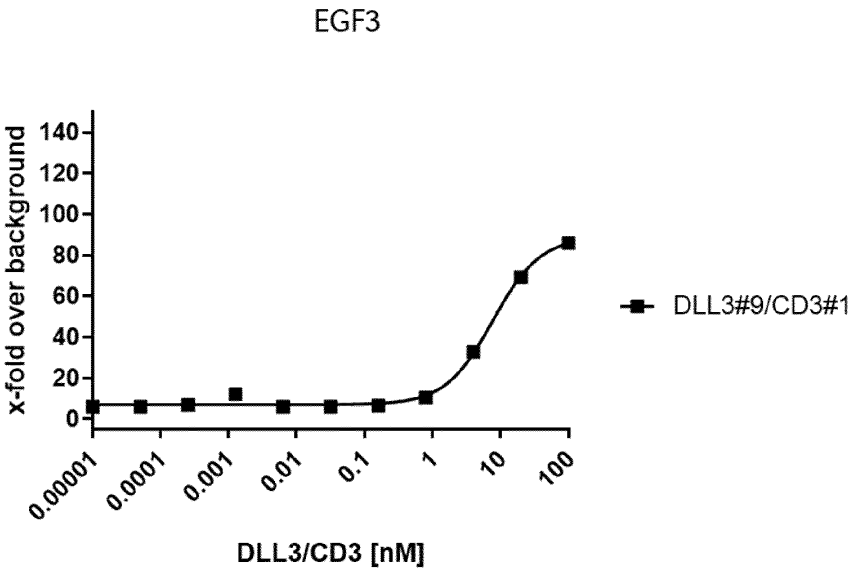
EGF3
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#9/CD3#1

Fig. 6F

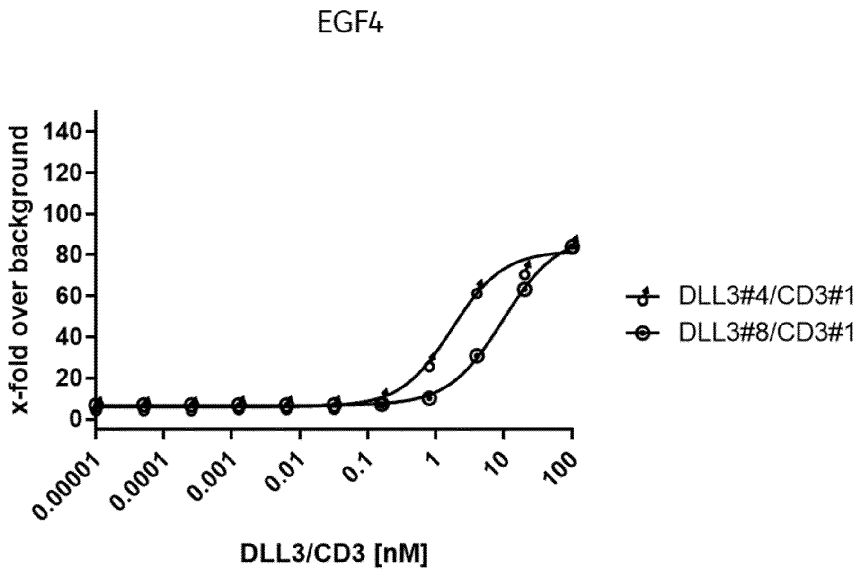
EGF4
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#4/CD3#1
DLL3#8/CD3#1

Fig. 6G

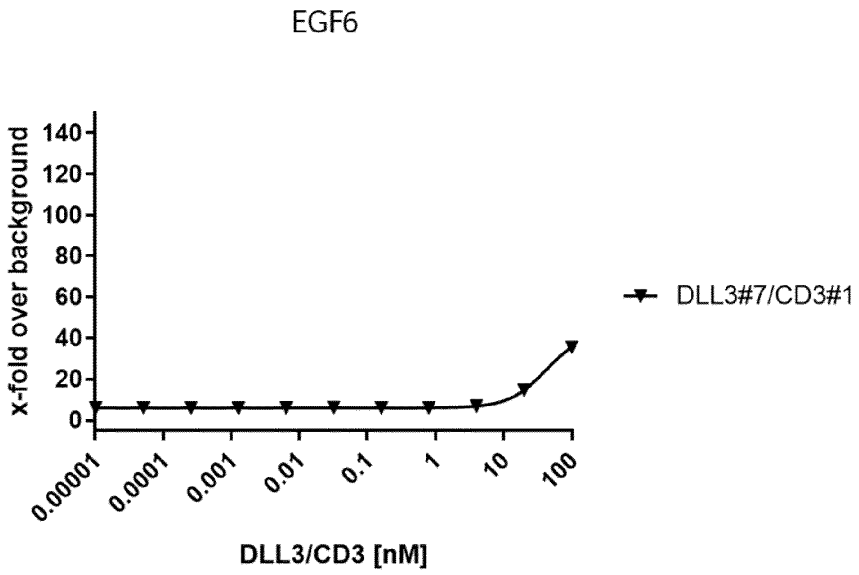
EGF6
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#7/CD3#1

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Fig. 6H

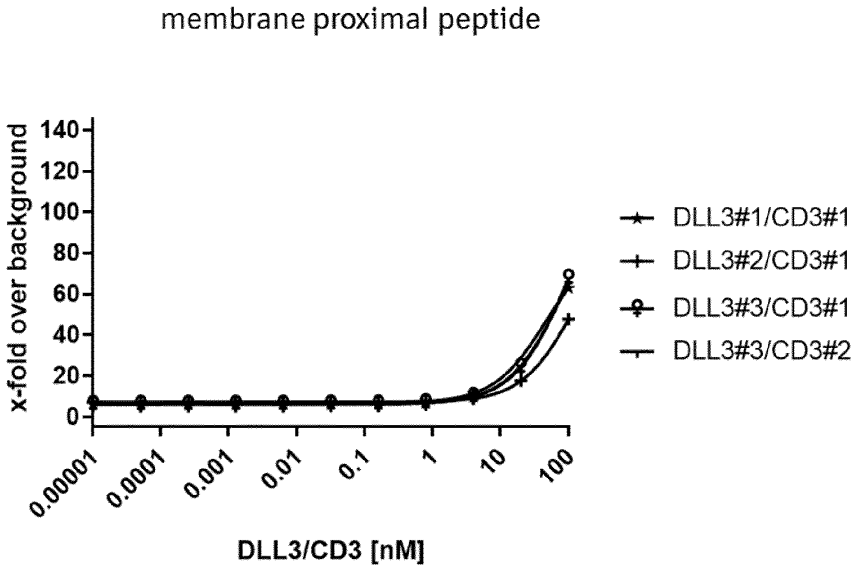
membrane proximal peptide
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#1/CD3#1
DLL3#2/CD3#1
DLL3#3/CD3#1
DLL3#3/CD3#2

Fig. 6I

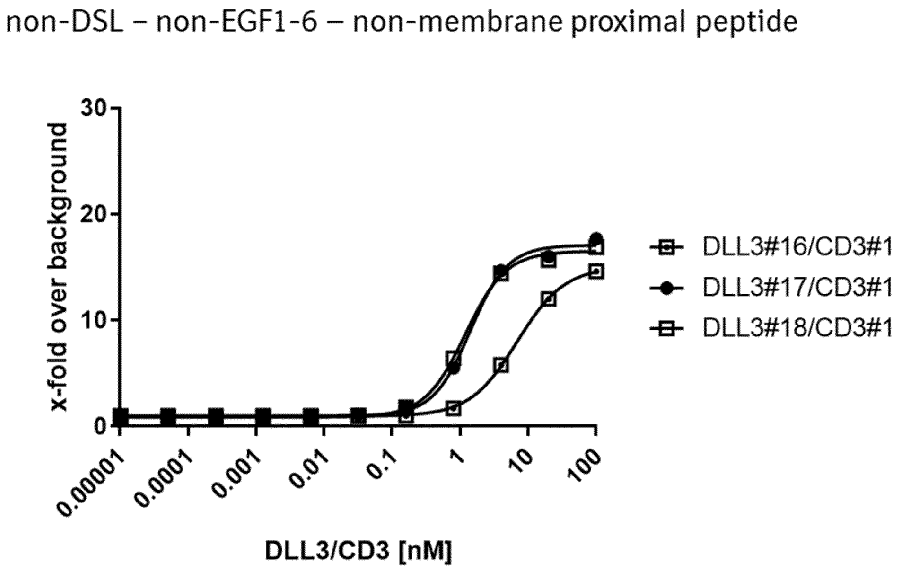
non-DSL – non-EGF1-6 – non-membrane proximal peptide
x-fold over background
30
20
10
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#16/CD3#1
DLL3#17/CD3#1
DLL3#18/CD3#1

Fig. 6J

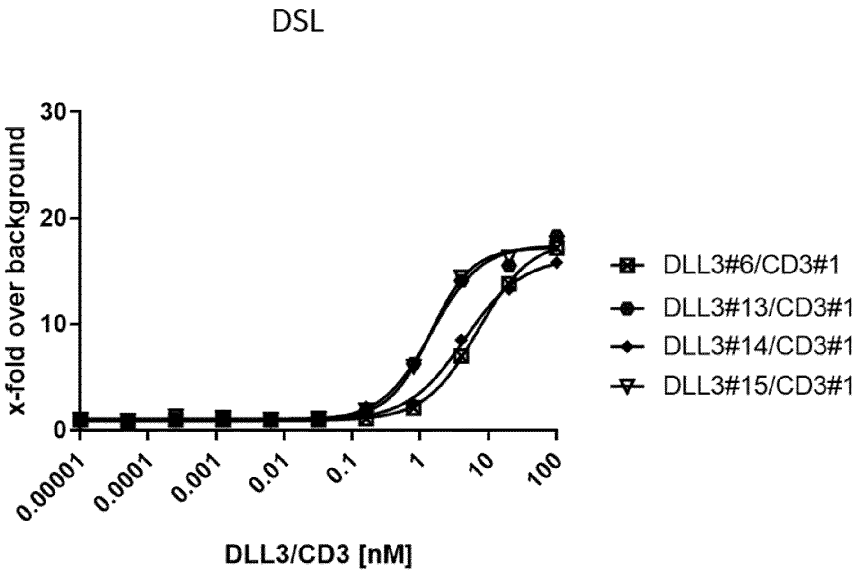
DSL
x-fold over background
30
20
10
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#6/CD3#1
DLL3#13/CD3#1
DLL3#14/CD3#1
DLL3#15/CD3#1

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Fig. 6K

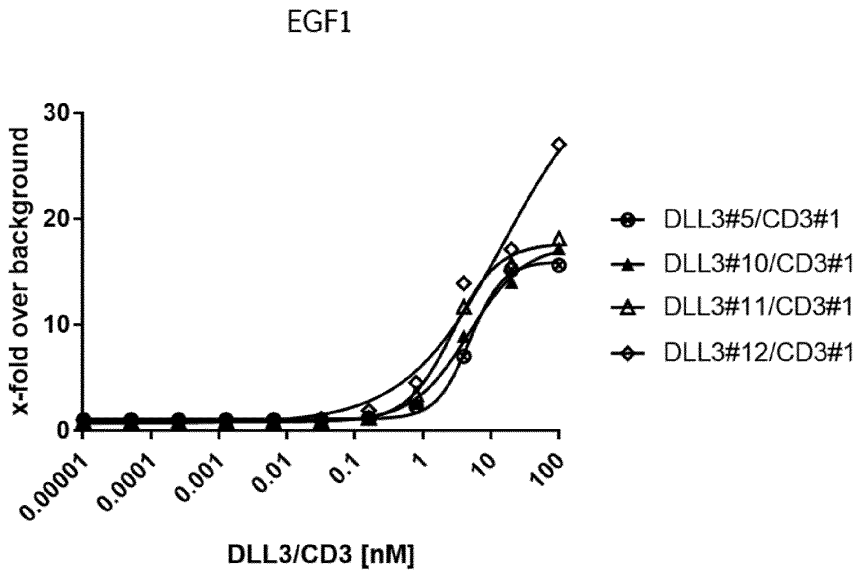
EGF1
x-fold over background
30
20
10
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#5/CD3#1
DLL3#10/CD3#1
DLL3#11/CD3#1
DLL3#12/CD3#1

Fig. 6L

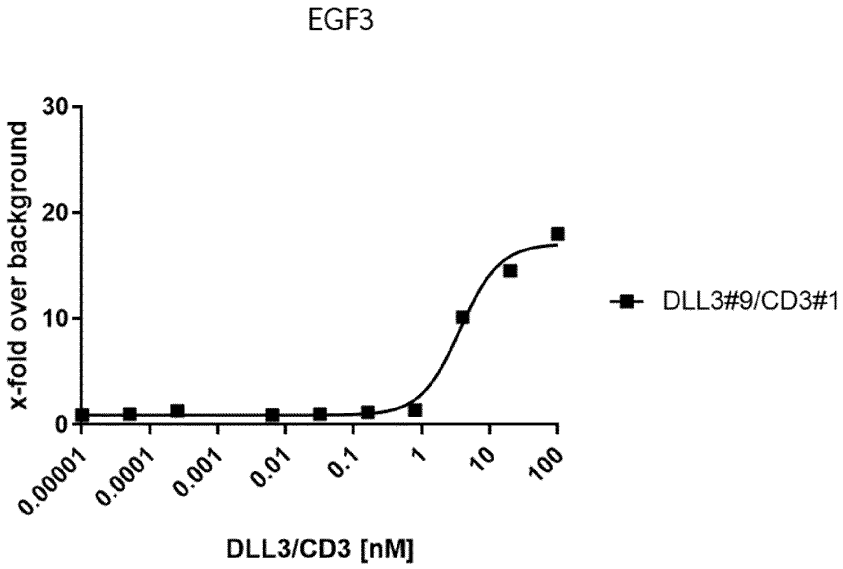
EGF3
x-fold over background
30
20
10
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#9/CD3#1

Fig. 6M

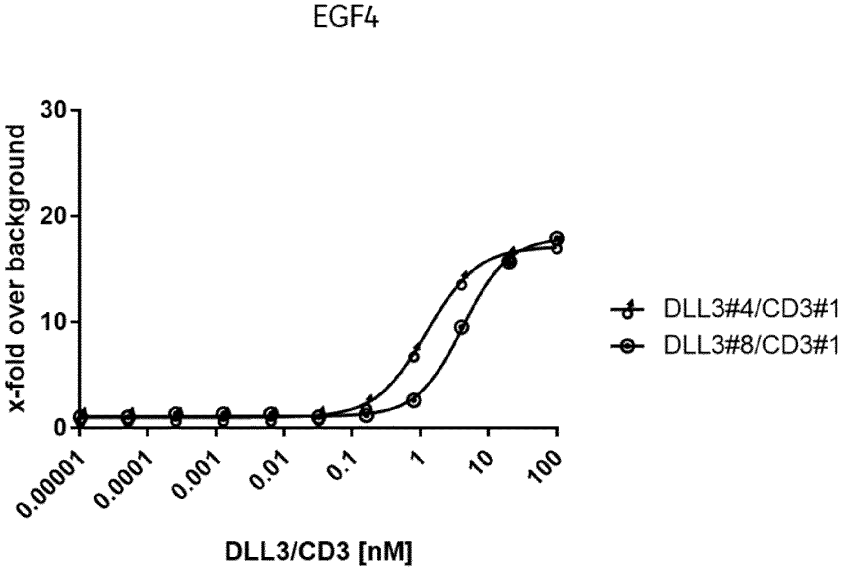
EGF4
x-fold over background
30
20
10
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#4/CD3#1
DLL3#8/CD3#1

Fig. 6N

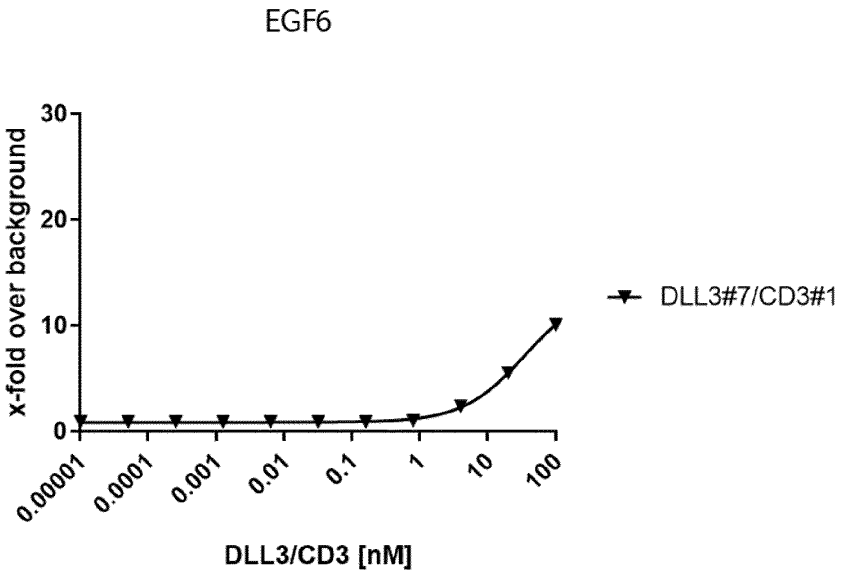
EGF6
x-fold over background
30
20
10
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#7/CD3#1

Fig. 6O

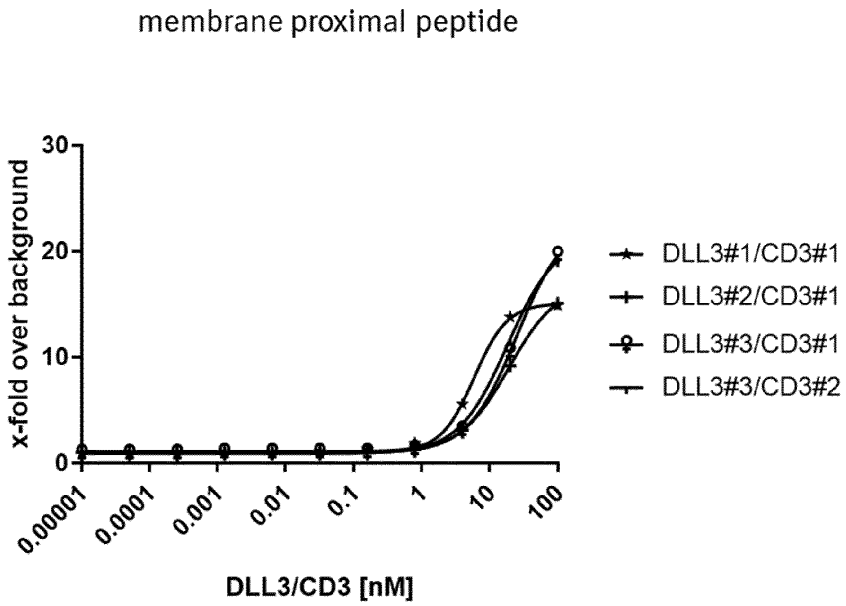
membrane proximal peptide
x-fold over background
30
20
10
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#1/CD3#1
DLL3#2/CD3#1
DLL3#3/CD3#1
DLL3#3/CD3#2

Fig. 6P

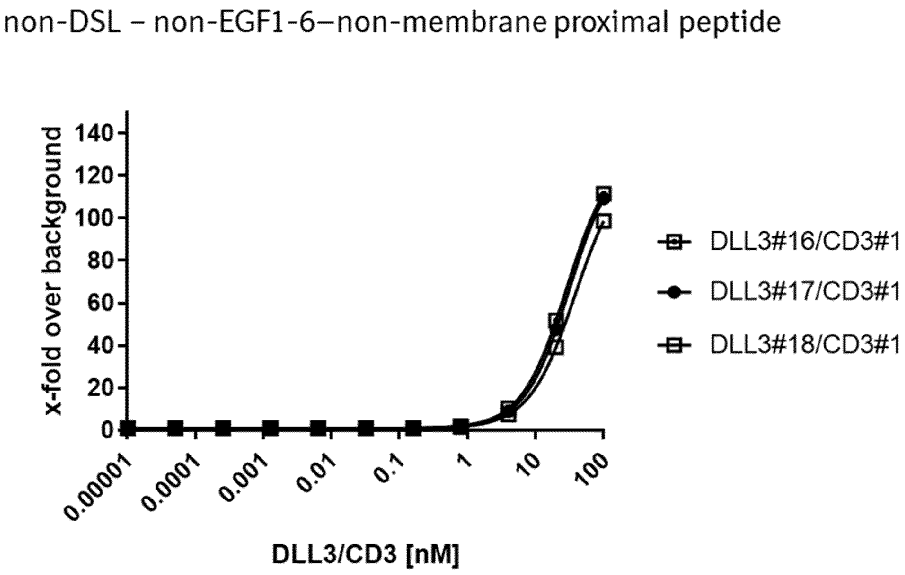
non-DSL – non-EGF1-6–non-membrane proximal peptide
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#16/CD3#1
DLL3#17/CD3#1
DLL3#18/CD3#1

Fig. 6Q

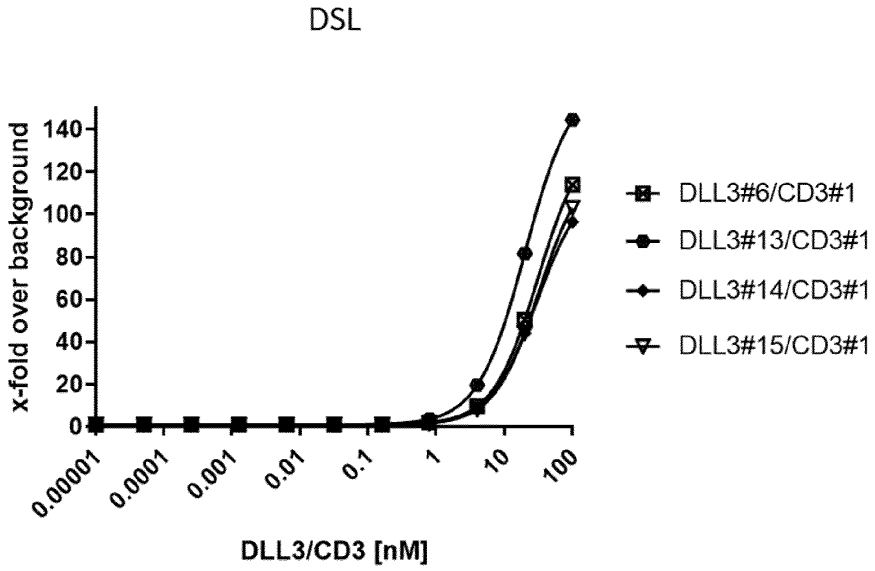
DSL
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#6/CD3#1
DLL3#13/CD3#1
DLL3#14/CD3#1
DLL3#15/CD3#1

Fig. 6R

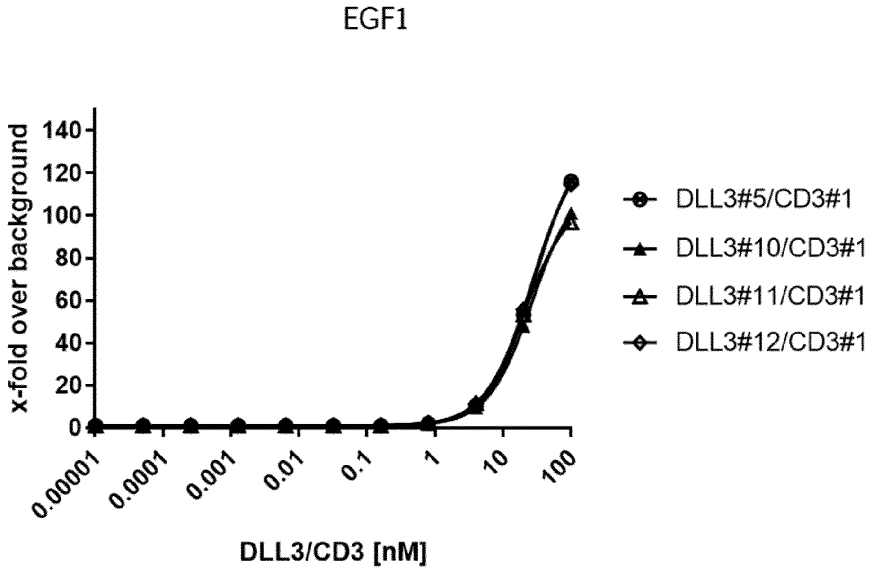
EGF1
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#5/CD3#1
DLL3#10/CD3#1
DLL3#11/CD3#1
DLL3#12/CD3#1

Fig. 6S

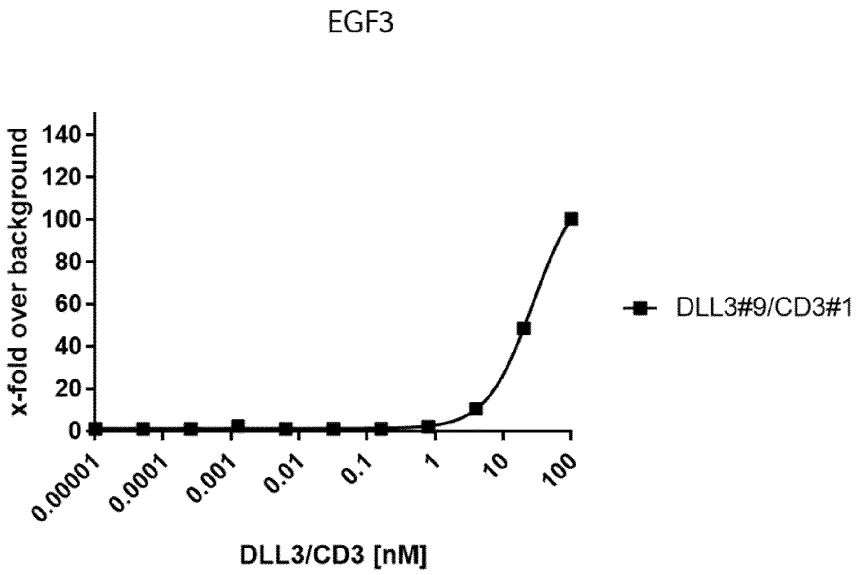
EGF3
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#9/CD3#1

Fig. 6T

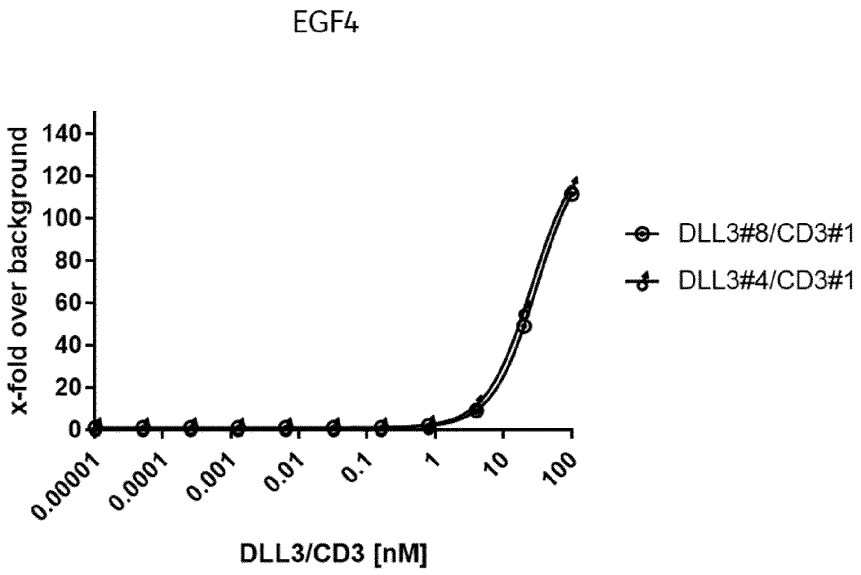
EGF4
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#8/CD3#1
DLL3#4/CD3#1

Fig. 6U

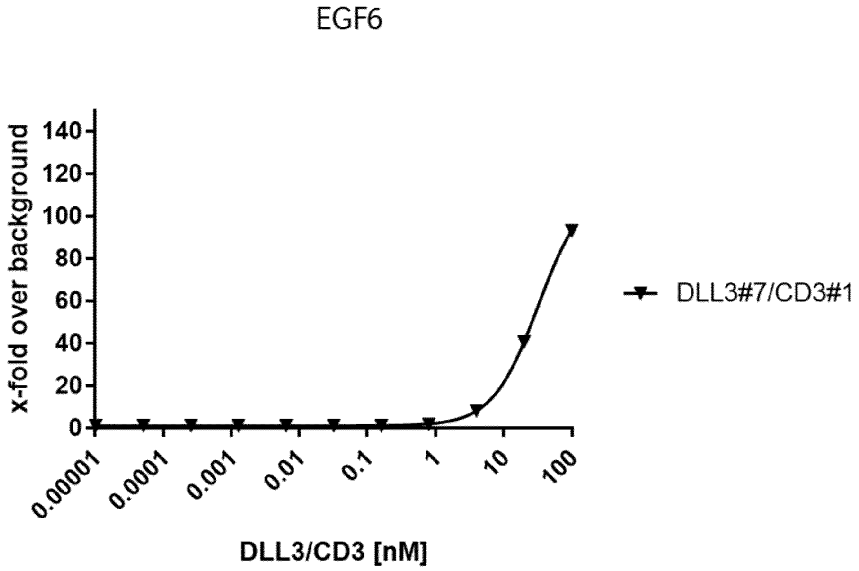
EGF6
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#7/CD3#1

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Fig. 6V

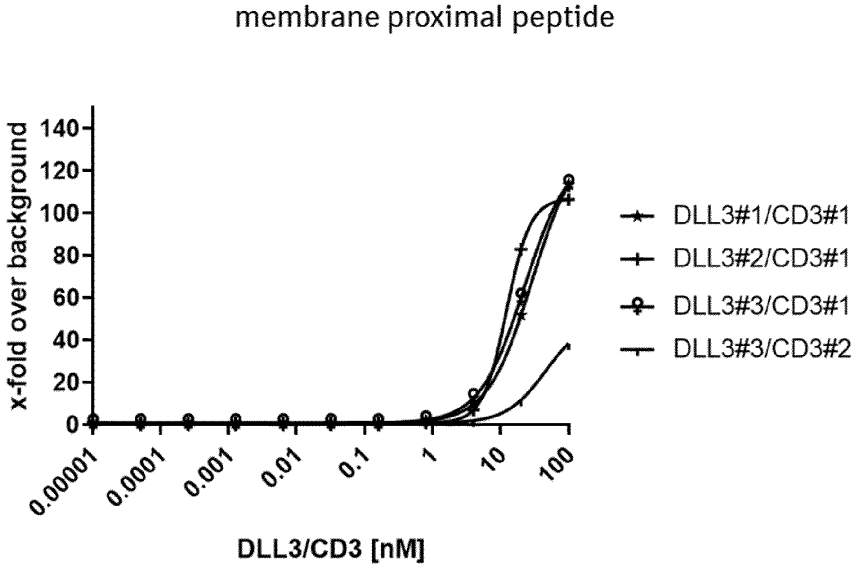
membrane proximal peptide
x-fold over background
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#1/CD3#1
DLL3#2/CD3#1
DLL3#3/CD3#1
DLL3#3/CD3#2

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Fig. 7A

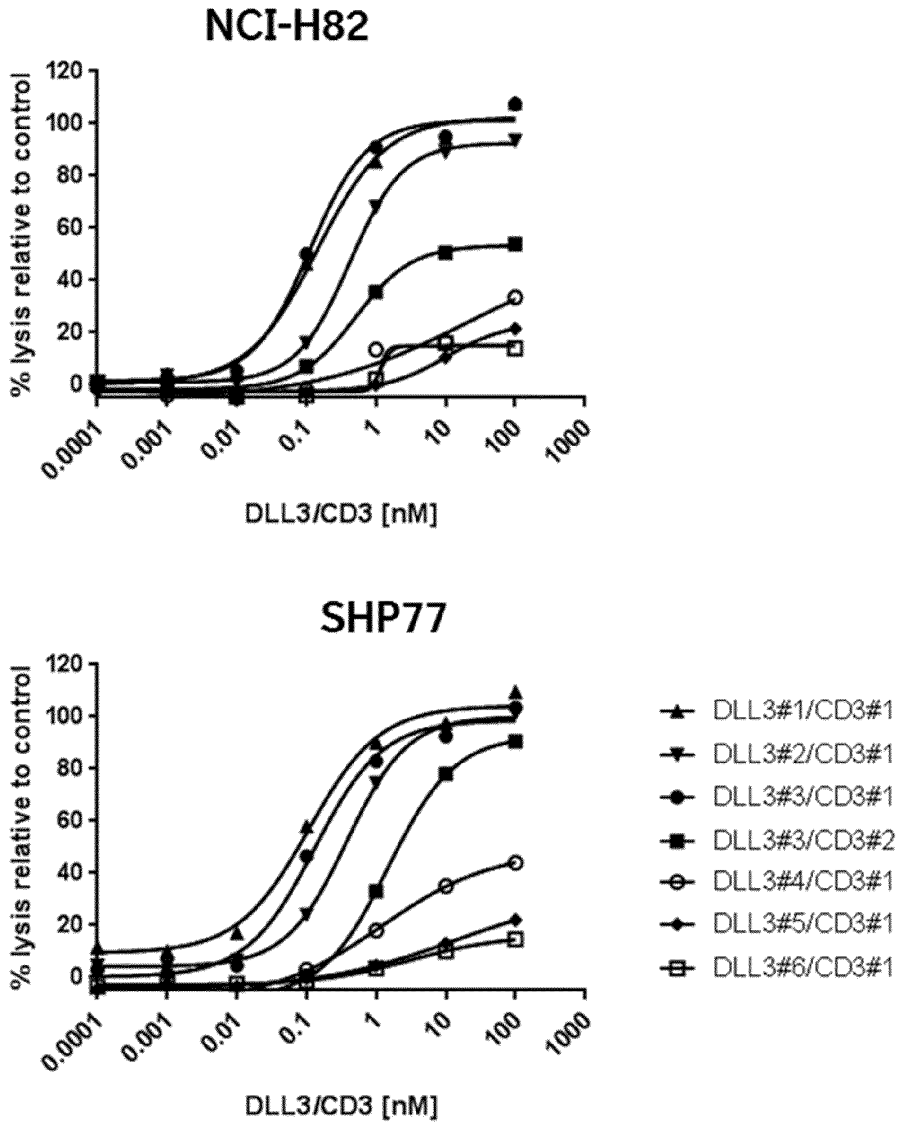
NCI-H82
% lysis relative to control
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
1000
DLL3/CD3 [nM]
SHP77
% lysis relative to control
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
1000
DLL3/CD3 [nM]
DLL3#1/CD3#1
DLL3#2/CD3#1
DLL3#3/CD3#1
DLL3#3/CD3#2
DLL3#4/CD3#1
DLL3#5/CD3#1
DLL3#6/CD3#1

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Fig. 7B

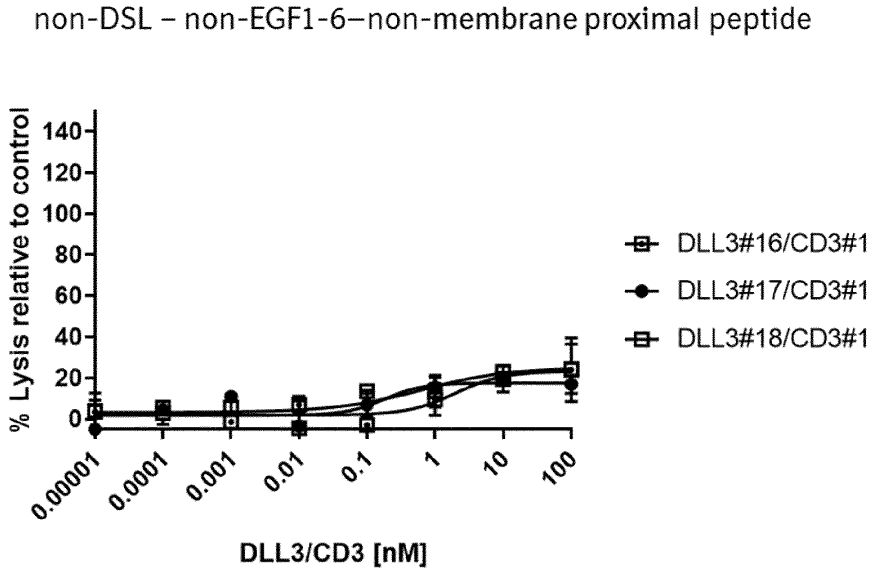

non-DSL – non-EGF1-6–non-membrane proximal peptide
% Lysis relative to control
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#16/CD3#1
DLL3#17/CD3#1
DLL3#18/CD3#1

Fig. 7 C

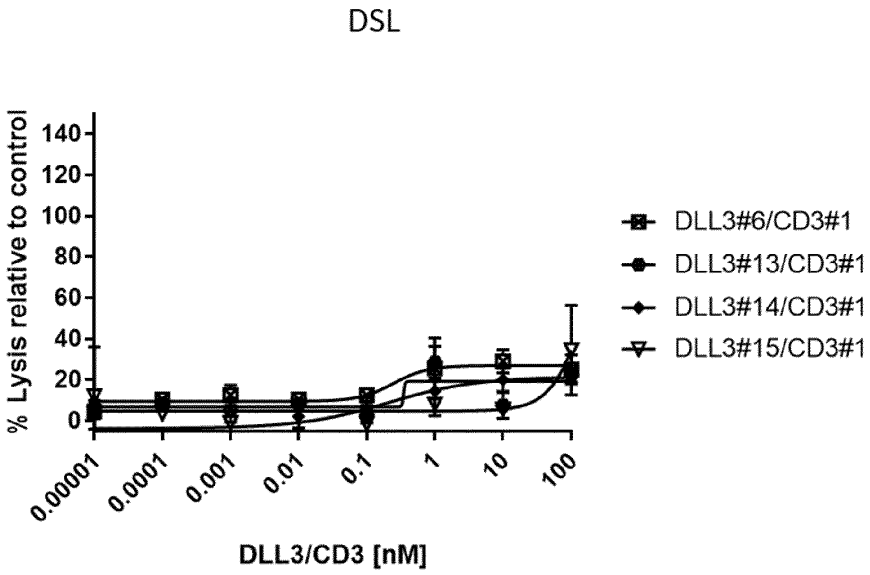

DSL
% Lysis relative to control
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#6/CD3#1
DLL3#13/CD3#1
DLL3#14/CD3#1
DLL3#15/CD3#1

Fig. 7D

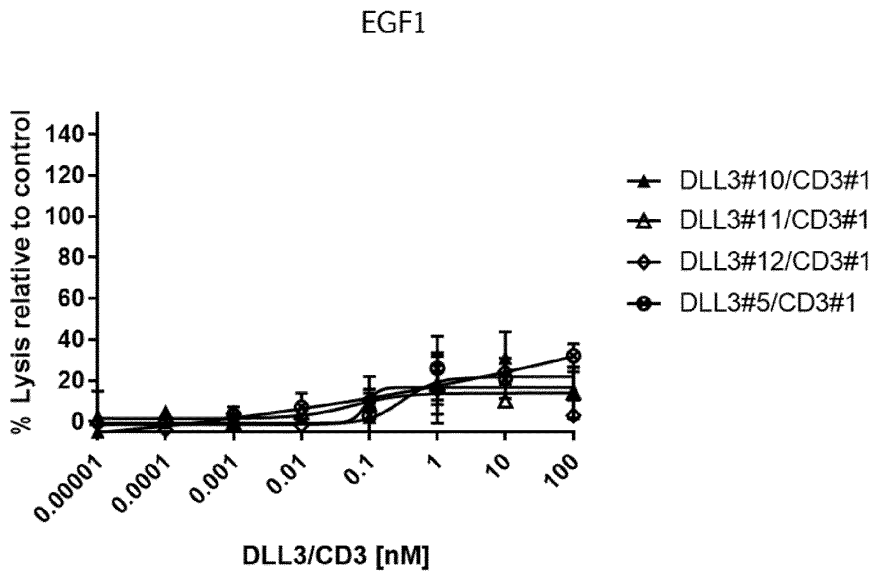

EGF1
% Lysis relative to control
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#10/CD3#1
DLL3#11/CD3#1
DLL3#12/CD3#1
DLL3#5/CD3#1

Fig. 7E

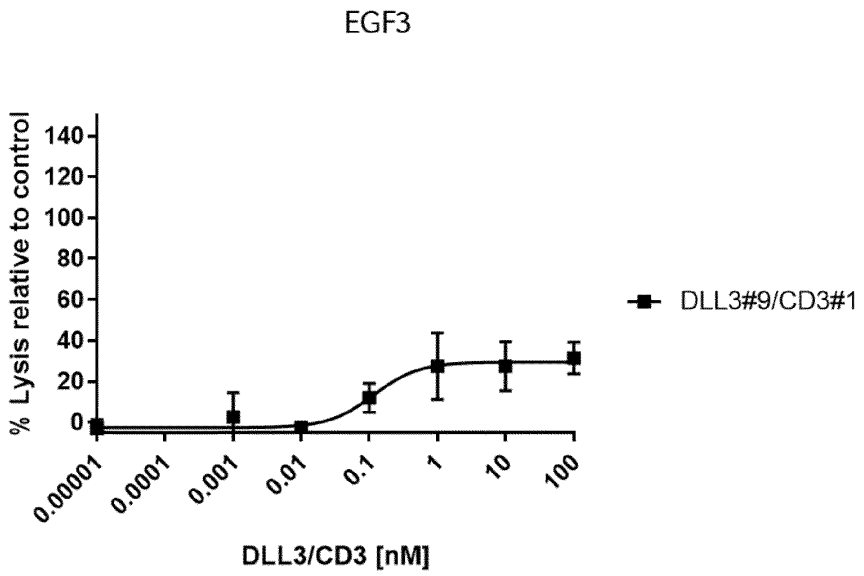

EGF3
% Lysis relative to control
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#9/CD3#1

Fig. 7F

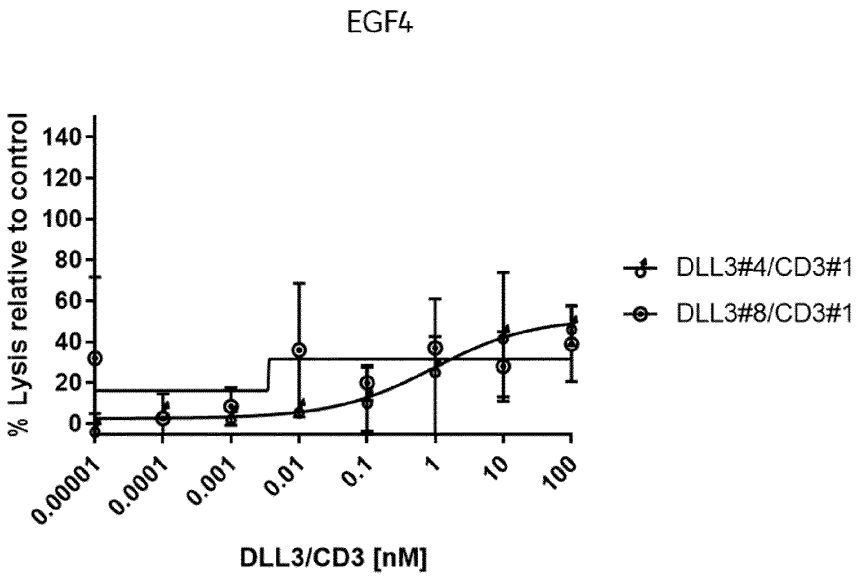

EGF4
% Lysis relative to control
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#4/CD3#1
DLL3#8/CD3#1

Fig. 7G

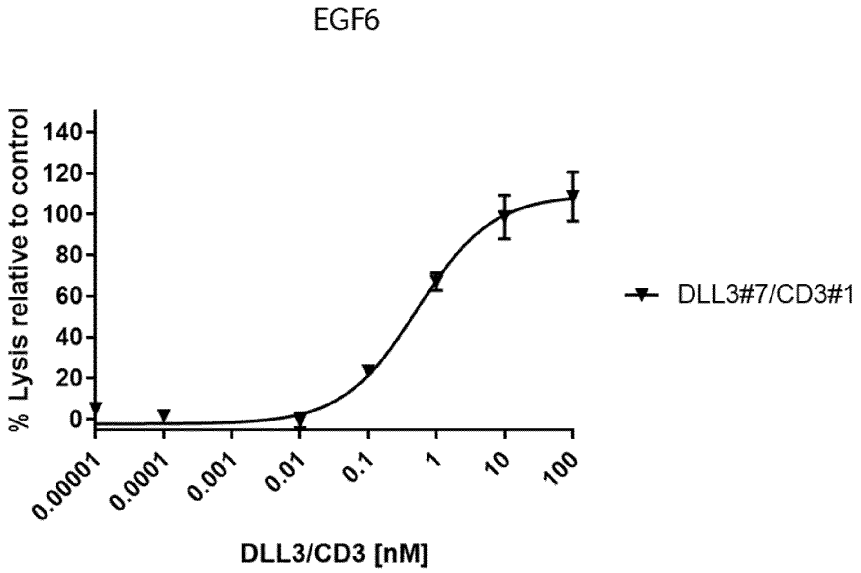

EGF6
% Lysis relative to control
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#7/CD3#1

Fig. 7H

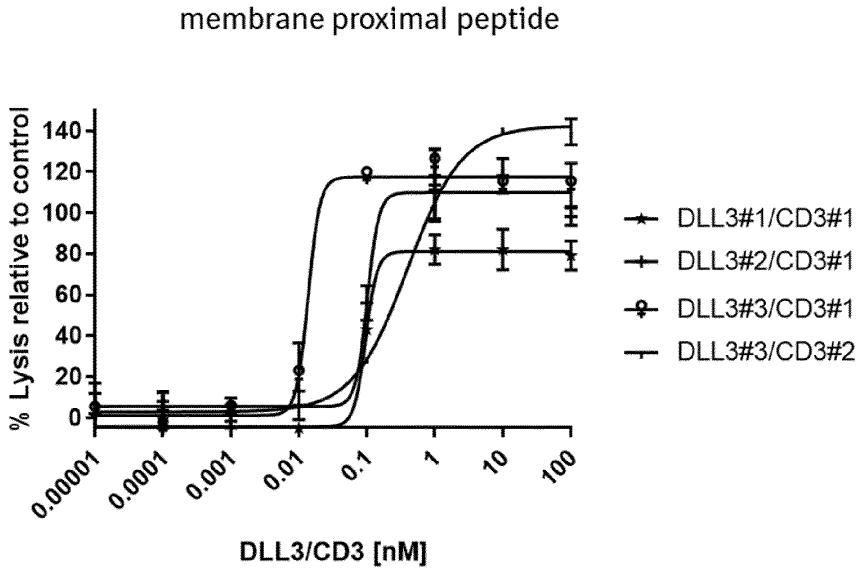

membrane proximal peptide
% Lysis relative to control
140
120
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#1/CD3#1
DLL3#2/CD3#1
DLL3#3/CD3#1
DLL3#3/CD3#2

Fig. 7I

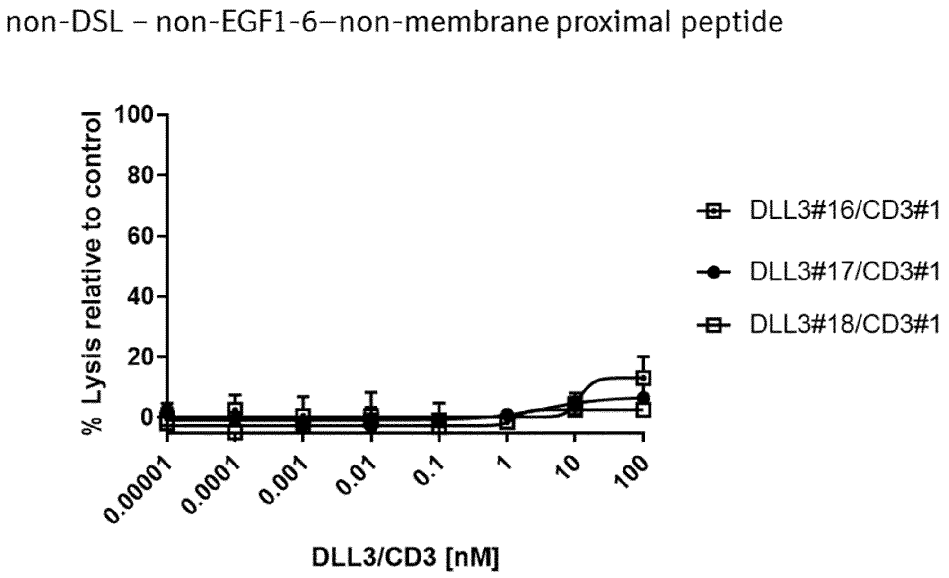

non-DSL – non-EGF1-6–non-membrane proximal peptide
% Lysis relative to control
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#16/CD3#1
DLL3#17/CD3#1
DLL3#18/CD3#1

Fig. 7J

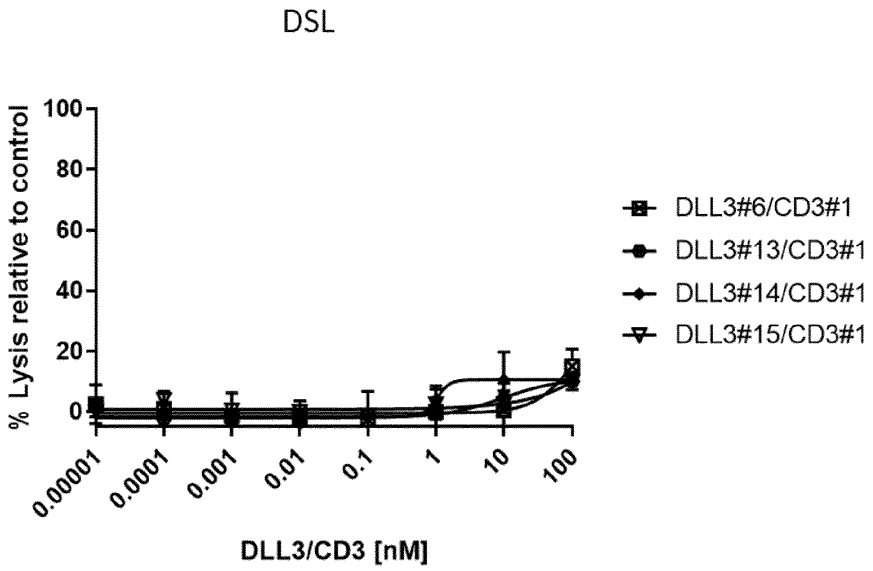

DSL
% Lysis relative to control
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#6/CD3#1
DLL3#13/CD3#1
DLL3#14/CD3#1
DLL3#15/CD3#1

Fig. 7K

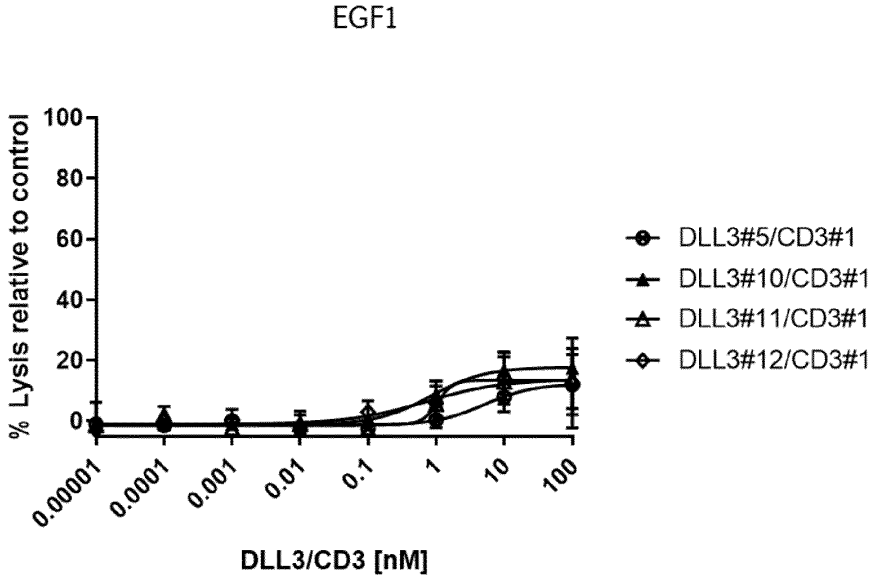

EGF1
% Lysis relative to control
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#5/CD3#1
DLL3#10/CD3#1
DLL3#11/CD3#1
DLL3#12/CD3#1

Fig. 7L

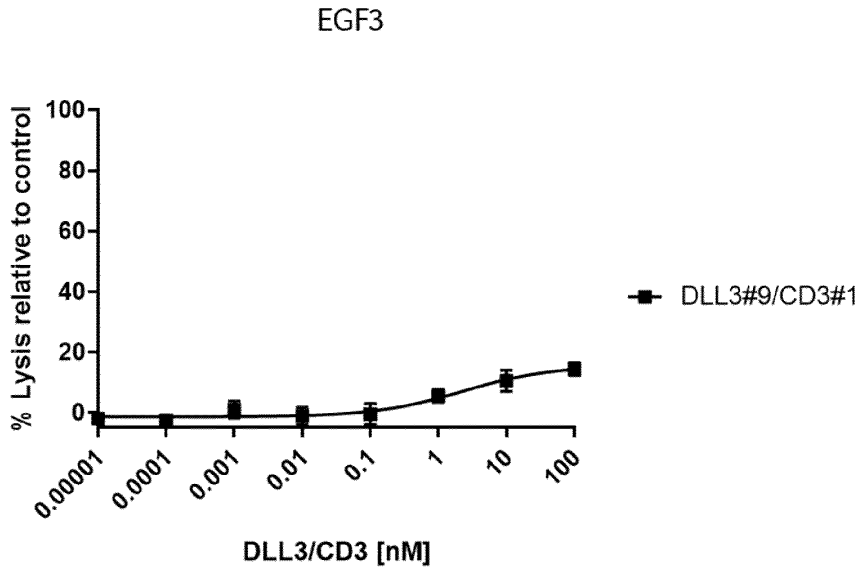

EGF3
% Lysis relative to control
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#9/CD3#1

Fig. 7M

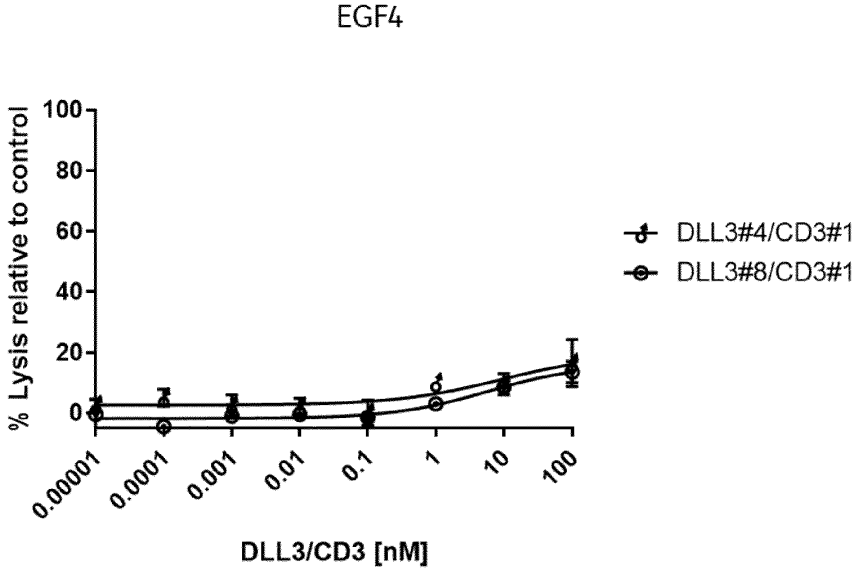

EGF4
% Lysis relative to control
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#4/CD3#1
DLL3#8/CD3#1

Fig. 7N

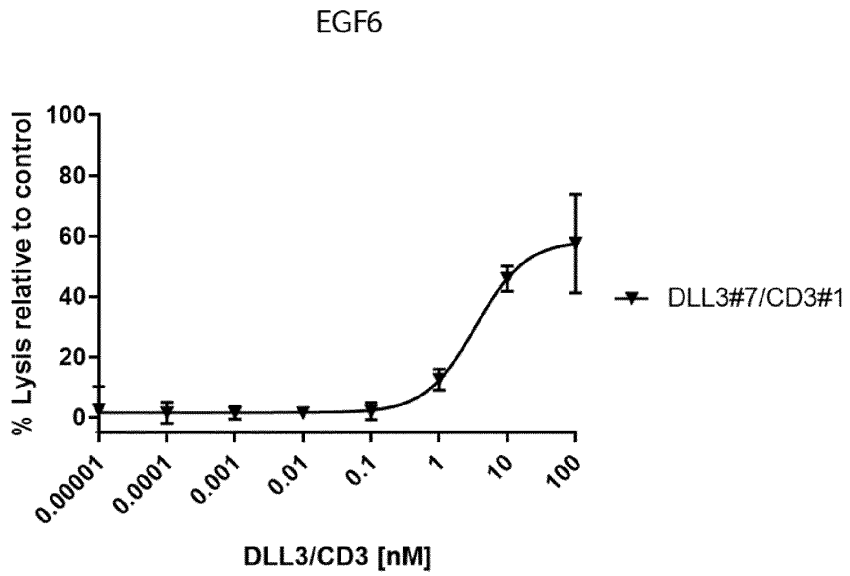

EGF6
% Lysis relative to control
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#7/CD3#1

Fig. 7O

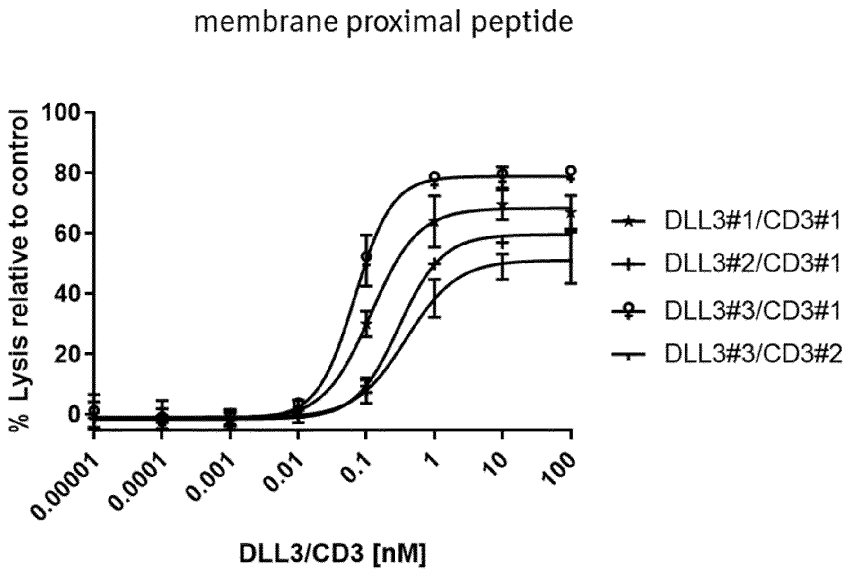
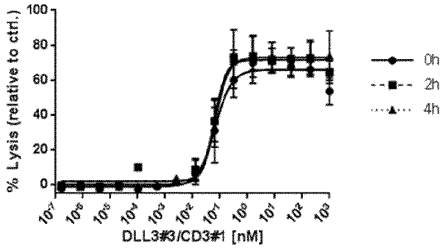

membrane proximal peptide
% Lysis relative to control
100
80
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
DLL3#1/CD3#1
DLL3#2/CD3#1
DLL3#3/CD3#1
DLL3#3/CD3#2

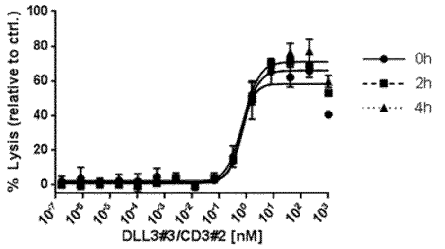
Fig. 8

DLL3#3/CD3#1



Pre-incubation	EC_{90} [nM]
0 h	0.3
2h	0.2
4h	0.2

DLL3#3/CD3#2



Pre-incubation	EC_{90} [nM]
0 h	2
2h	2.9
4h	3.4

Fig. 9

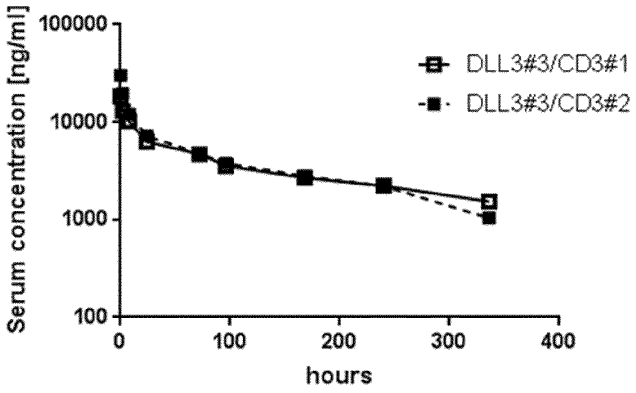

Serum concentration [ng/ml]
100000
10000
1000
100
0
100
200
300
400
hours
DLL3#3/CD3#1
DLL3#3/CD3#2

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Fig. 10

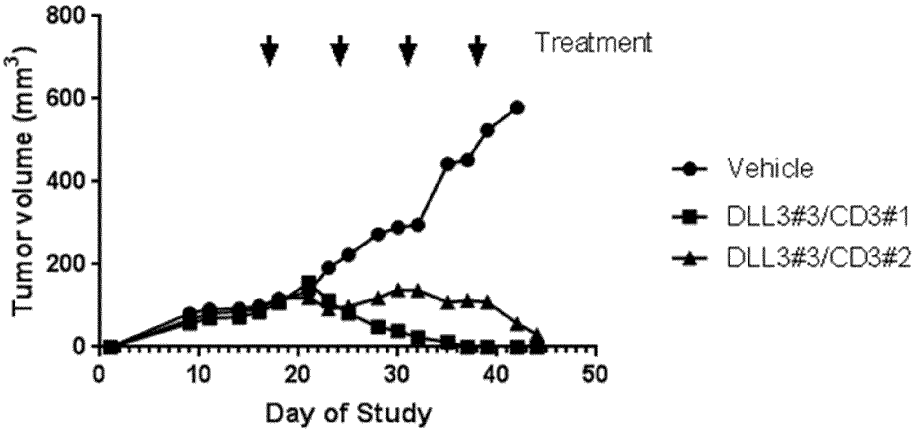
800
600
400
200
0
Tumor volume (mm3)
0
10
20
30
40
50
Day of Study
Treatment
Vehicle
DLL3#3/CD3#1
DLL3#3/CD3#2

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Fig. 11

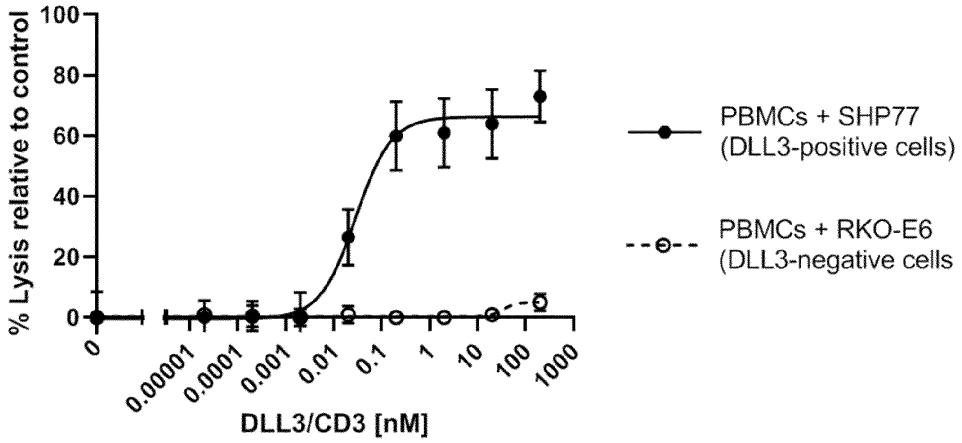
% Lysis relative to control
100
80
60
40
20
0
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
DLL3/CD3 [nM]
PBMCs + SHP77
(DLL3-positive cells)
PBMCs + RKO-E6
(DLL3-negative cells

Fig. 12

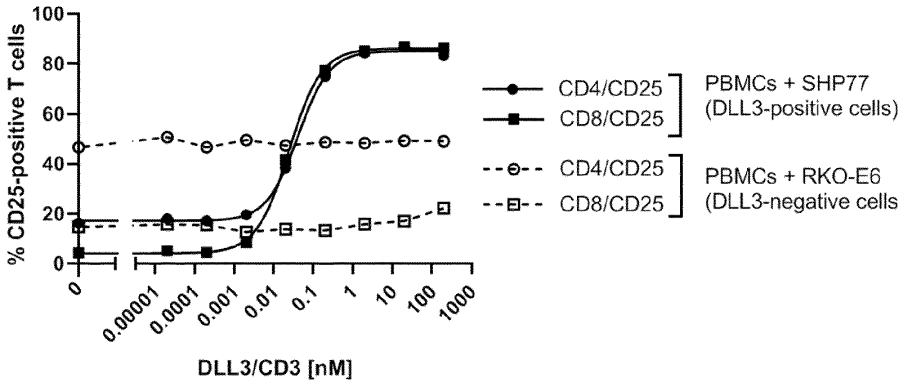
% CD25-positive T cells
100
80
60
40
20
0
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
DLL3/CD3 [nM]
CD4/CD25
CD8/CD25
PBMCs + SHP77
(DLL3-positive cells)
CD4/CD25
CD8/CD25
PBMCs + RKO-E6
(DLL3-negative cells

Fig. 13

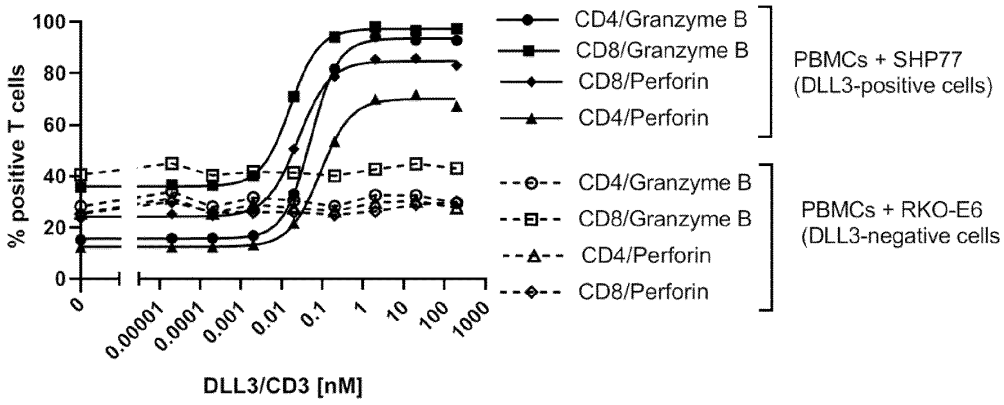
% positive T cells
100
80
60
40
20
0
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
DLL3/CD3 [nM]
CD4/Granzyme B
CD8/Granzyme B
CD8/Perforin
CD4/Perforin
PBMCs + SHP77
(DLL3-positive cells)
CD4/Granzyme B
CD8/Granzyme B
CD4/Perforin
CD8/Perforin
PBMCs + RKO-E6
(DLL3-negative cells

Fig. 14A

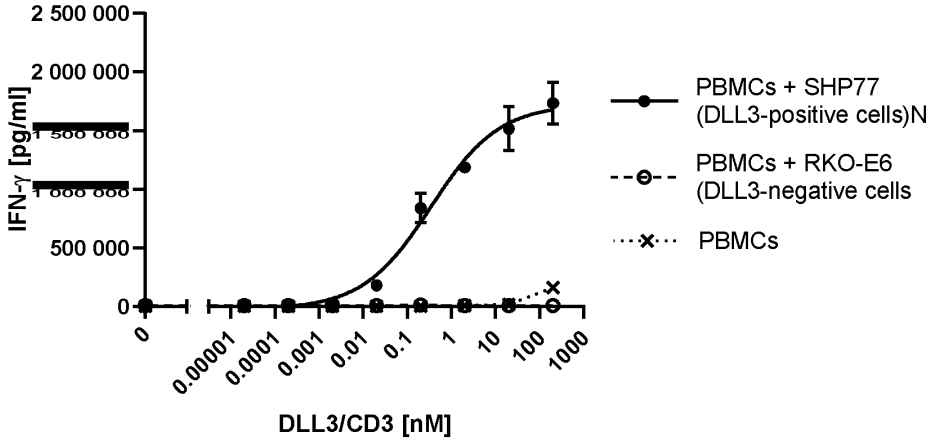
2 500 000
2 000 000
500 000
0
IFN-γ [pg/ml]
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
DLL3/CD3 [nM]
PBMCs + SHP77
(DLL3-positive cells)N
PBMCs + RKO-E6
(DLL3-negative cells
PBMCs

Fig. 14B

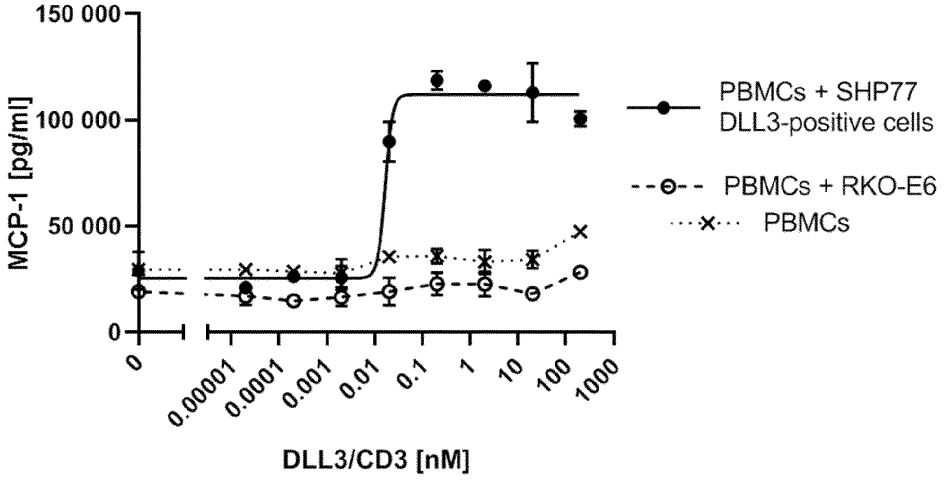
150 000
100 000
50 000
0
MCP-1 [pg/ml]
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
DLL3/CD3 [nM]
PBMCs + SHP77
DLL3-positive cells
PBMCs + RKO-E6
PBMCs

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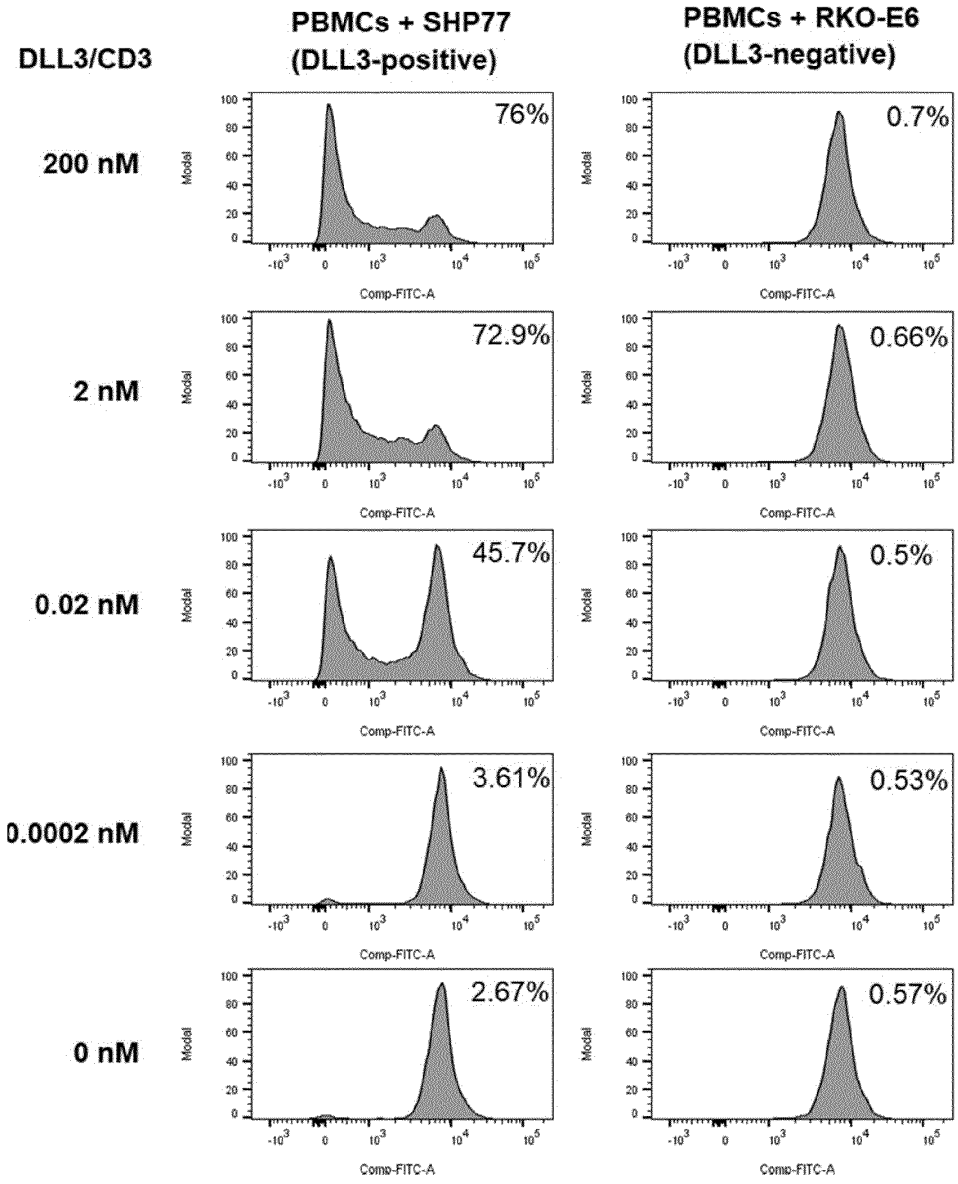
Fig. 15


DLL3/CD3
PBMCs + SHP77
(DLL3-positive)
PBMCs + RKO-E6
(DLL3-negative)
200 nM
2 nM
0.02 nM
0.0002 nM
0 nM
76%
0.7%
72.9%
0.66%
45.7%
0.5%
3.61%
0.53%
2.67%
0.57%
Modal
Comp-FITC-A

54/63

Fig. 16

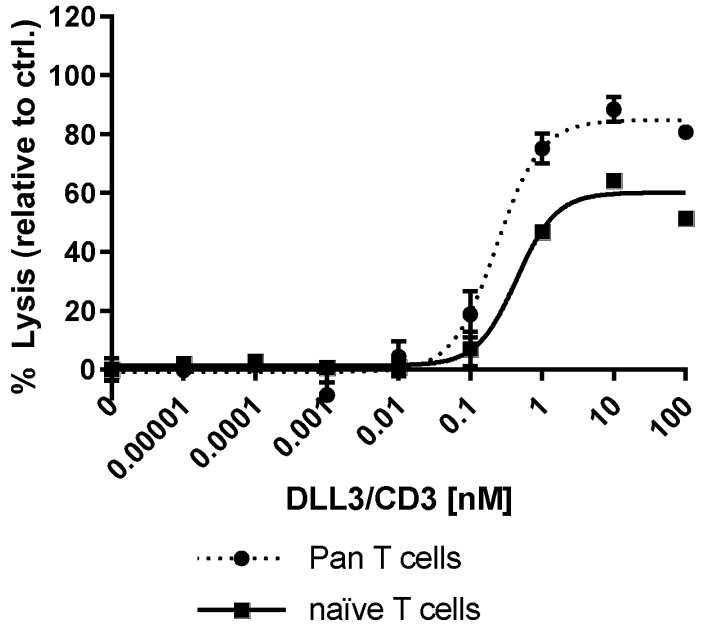

% Lysis (relative to ctrl.)
120
100
80
60
40
20
0
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
Pan T cells
naïve T cells

Fig. 17

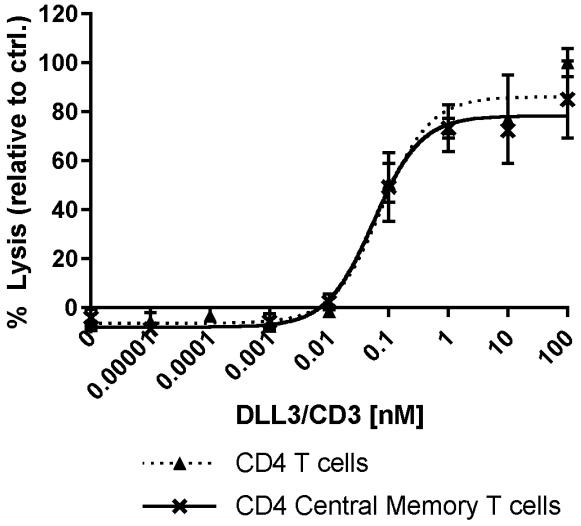

% Lysis (relative to ctrl.)
120
100
80
60
40
20
0
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
CD4 T cells
CD4 Central Memory T cells

Fig. 18

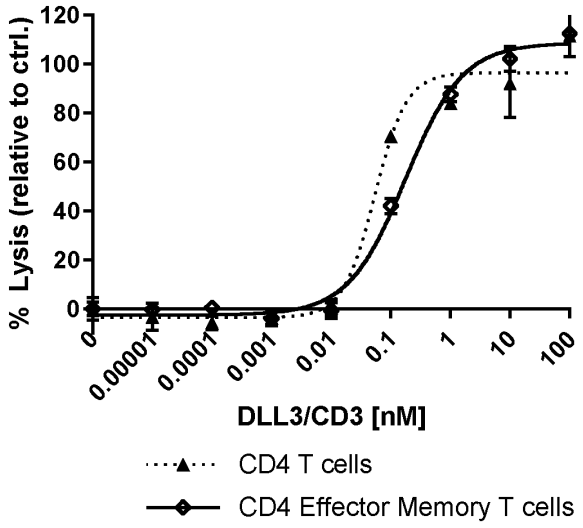

% Lysis (relative to ctrl.)
120
100
80
60
40
20
0
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
CD4 T cells
CD4 Effector Memory T cells

Fig. 19

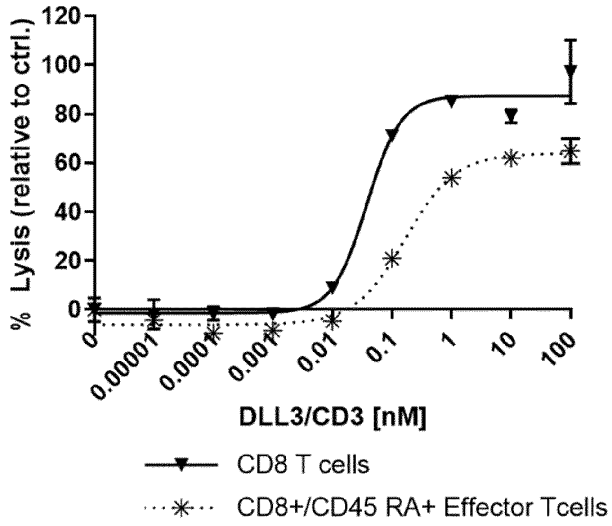

% Lysis (relative to ctrl.)
120
100
80
60
40
20
0
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
CD8 T cells
CD8+/CD45 RA+ Effector Tcells

Fig. 20

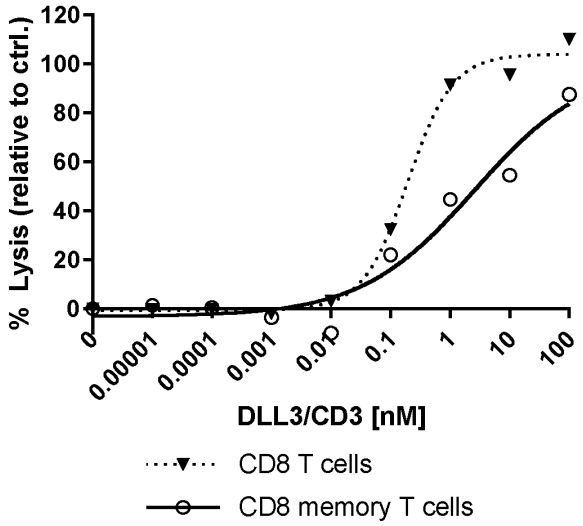

% Lysis (relative to ctrl.)
120
100
80
60
40
20
0
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
DLL3/CD3 [nM]
CD8 T cells
CD8 memory T cells

Fig. 21

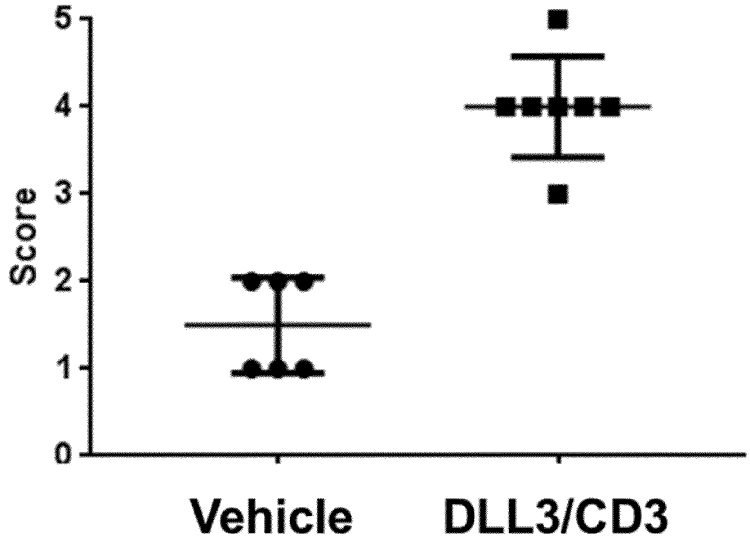


Fig. 22

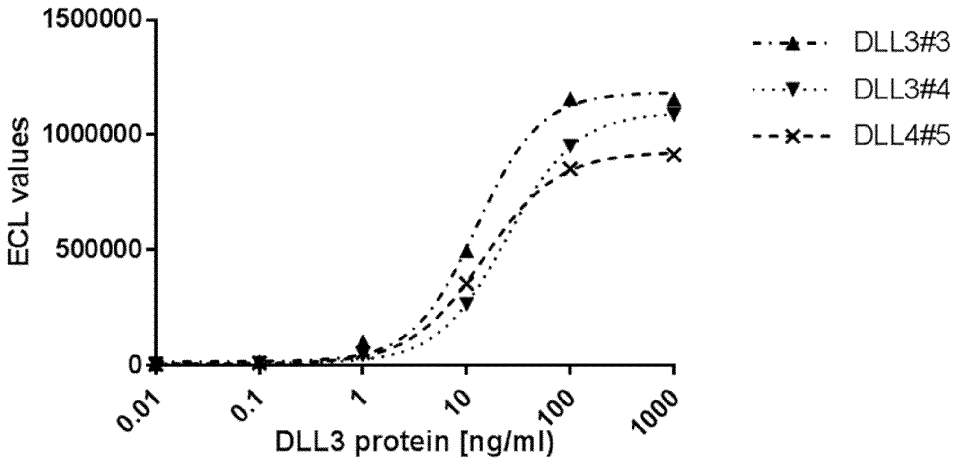

1500000
1000000
500000
0
ECL values
0.01
0.1
1
10
100
1000
DLL3 protein [ng/ml]
DLL3#3
DLL3#4
DLL4#5

Fig. 23

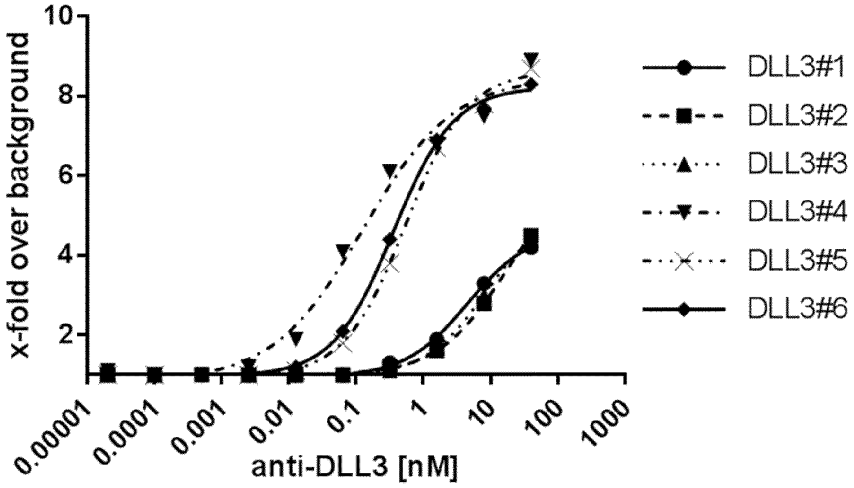

10
8
6
4
2
x-fold over background
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
anti-DLL3 [nM]
DLL3#1
DLL3#2
DLL3#3
DLL3#4
DLL3#5
DLL3#6

Fig. 24

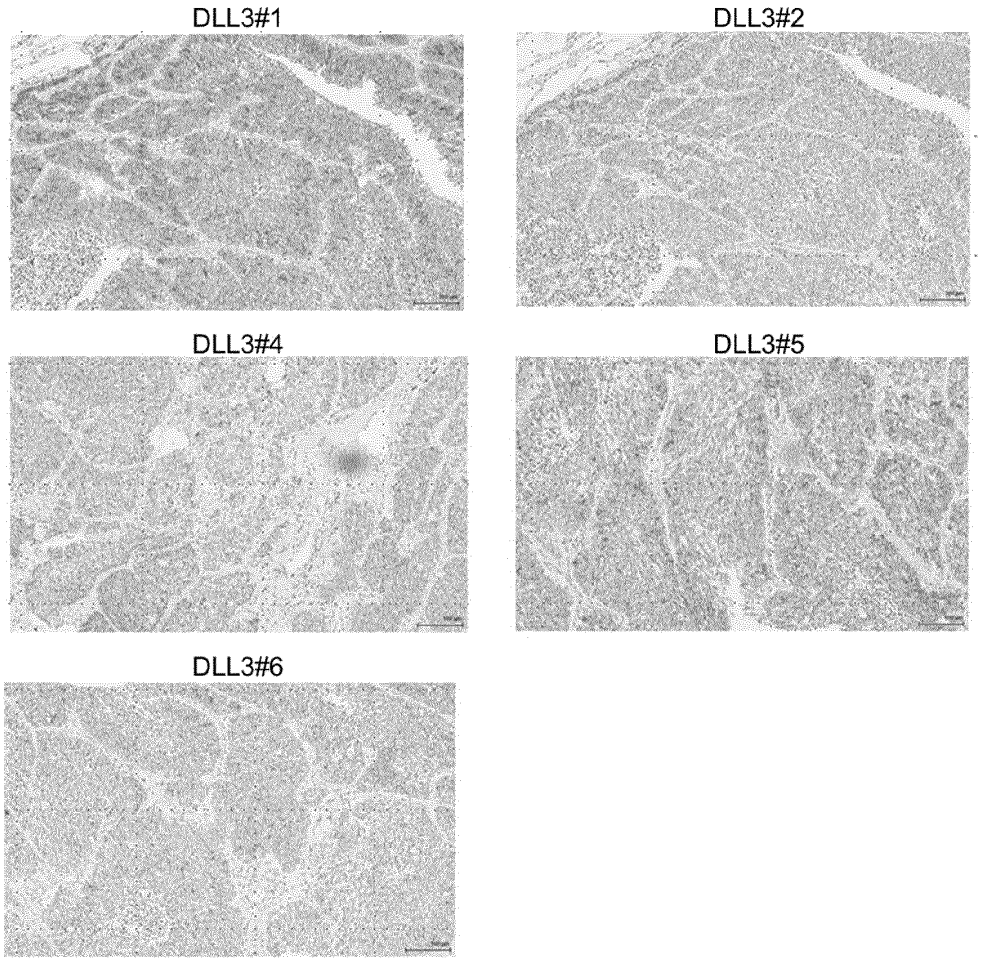
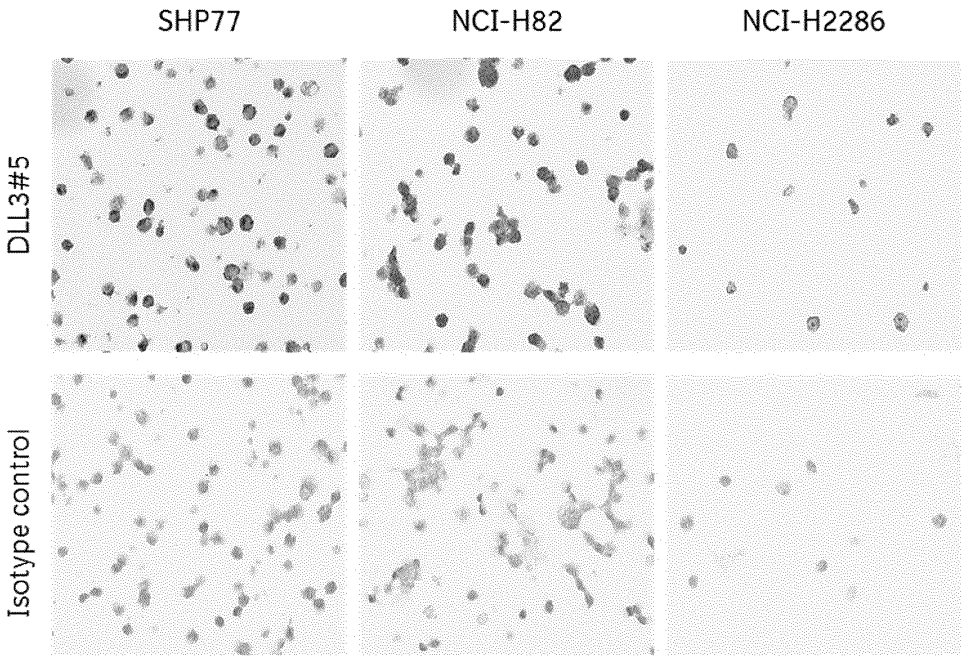



Fig. 25