Figure 1

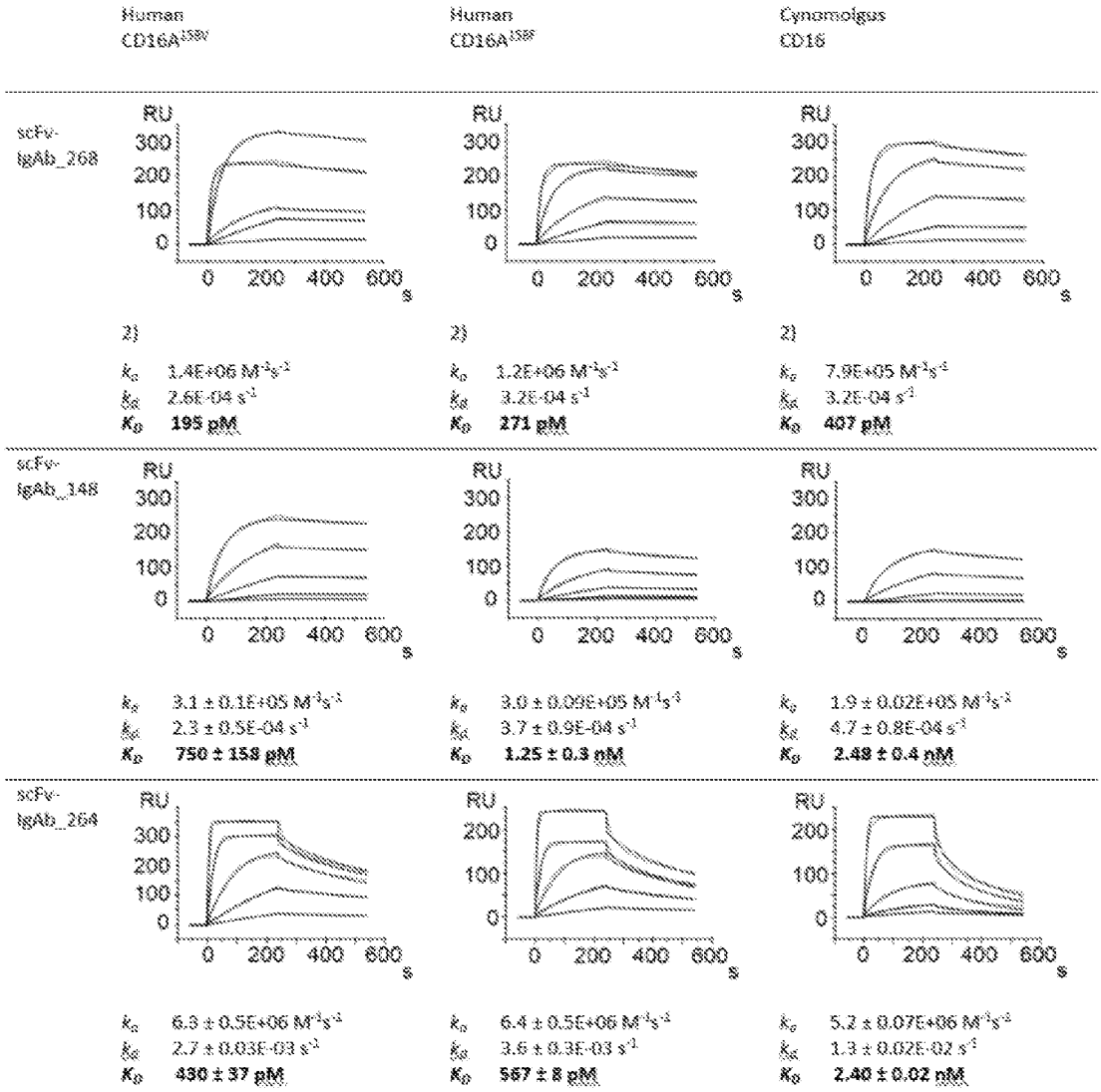

Human CD16A158V
Human CD16A158F
Cynomolgus CD16
scFv-IgAb_268
scFv-IgAb_148
scFv-IgAb_264
RU
s
2)
k_a 1.4E+06 $M^{-1}s^{-1}$
k_d 2.6E-04 s^{-1}
K_D 195 pM
k_a 1.2E+06 $M^{-1}s^{-1}$
k_d 3.2E-04 s^{-1}
K_D 271 pM
k_a 7.9E+05 $M^{-1}s^{-1}$
k_d 3.2E-04 s^{-1}
K_D 407 pM
k_a 3.1 ± 0.1E+05 $M^{-1}s^{-1}$
k_d 2.3 ± 0.5E-04 s^{-1}
K_D 750 ± 158 pM
k_a 3.0 ± 0.09E+05 $M^{-1}s^{-1}$
k_d 3.7 ± 0.9E-04 s^{-1}
K_D 1.25 ± 0.3 nM
k_a 1.9 ± 0.02E+05 $M^{-1}s^{-1}$
k_d 4.7 ± 0.8E-04 s^{-1}
K_D 2.48 ± 0.4 nM
k_a 6.3 ± 0.5E+06 $M^{-1}s^{-1}$
k_d 2.7 ± 0.03E-03 s^{-1}
K_D 430 ± 37 pM
k_a 6.4 ± 0.5E+06 $M^{-1}s^{-1}$
k_d 3.6 ± 0.3E-03 s^{-1}
K_D 567 ± 8 pM
k_a 5.2 ± 0.07E+06 $M^{-1}s^{-1}$
k_d 1.3 ± 0.02E-02 s^{-1}
K_D 2.40 ± 0.02 nM

Figure 2

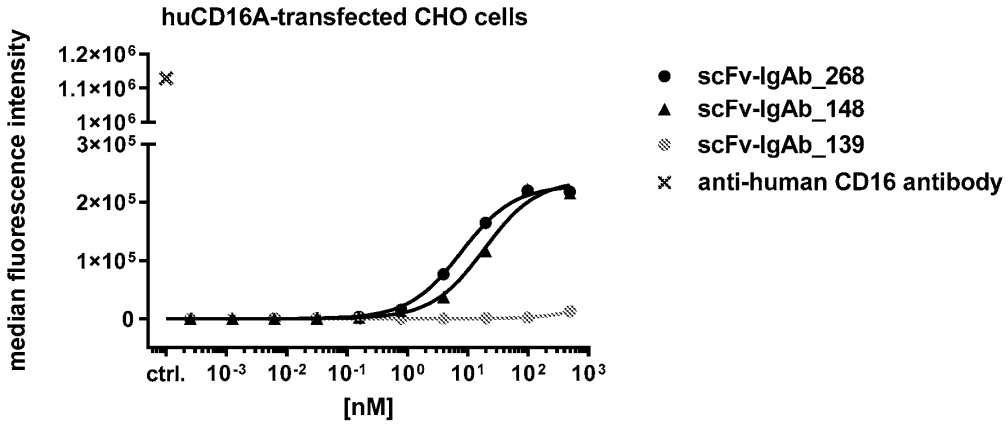

huCD16A-transfected CHO cells
median fluorescence intensity
1.2×10⁶
1.1×10⁶
1×10⁶
3×10⁵
2×10⁵
1×10⁵
0
ctrl.
10⁻³
10⁻²
10⁻¹
10⁰
10¹
10²
10³
[nM]
scFv-IgAb_268
scFv-IgAb_148
scFv-IgAb_139
anti-human CD16 antibody

Figure 3

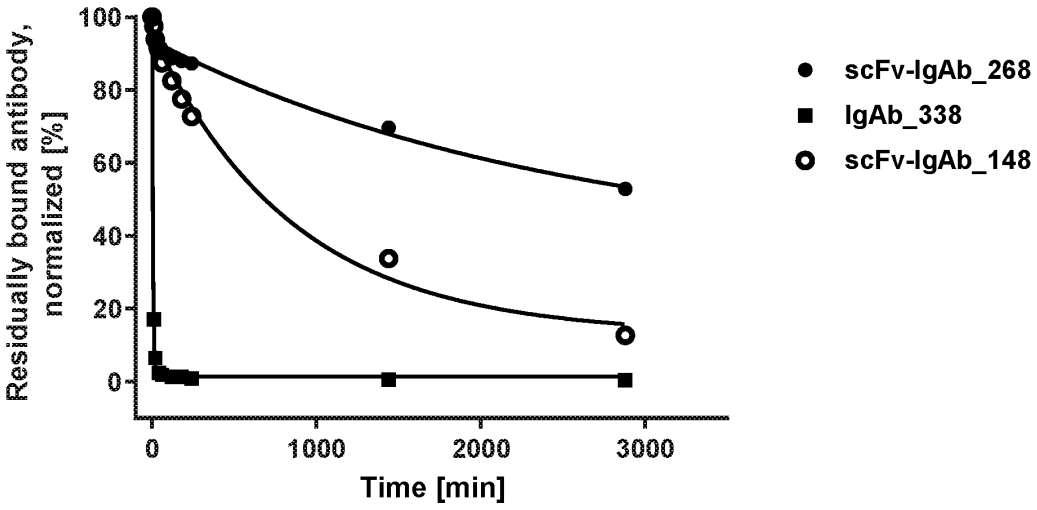

Residually bound antibody, normalized [%]
100
80
60
40
20
0
0
1000
2000
3000
Time [min]
scFv-IgAb_268
IgAb_338
scFv-IgAb_148

Figure 4

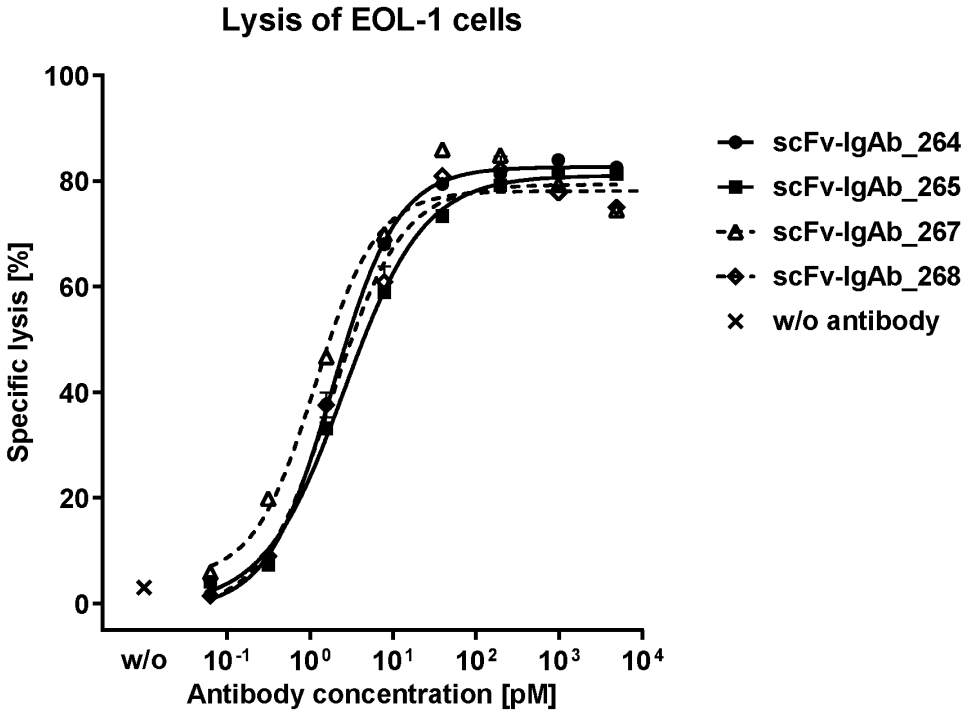

Lysis of EOL-1 cells
Specific lysis [%]
100
80
60
40
20
0
w/o
10-1
100
101
102
103
104
Antibody concentration [pM]
scFv-IgAb_264
scFv-IgAb_265
scFv-IgAb_267
scFv-IgAb_268
w/o antibody

Figure 5A

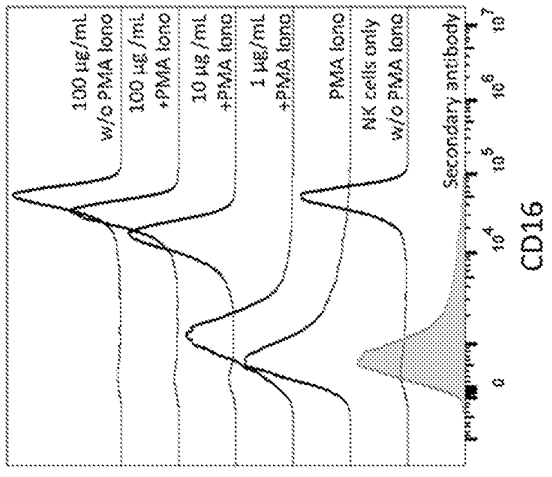


Figure 5B

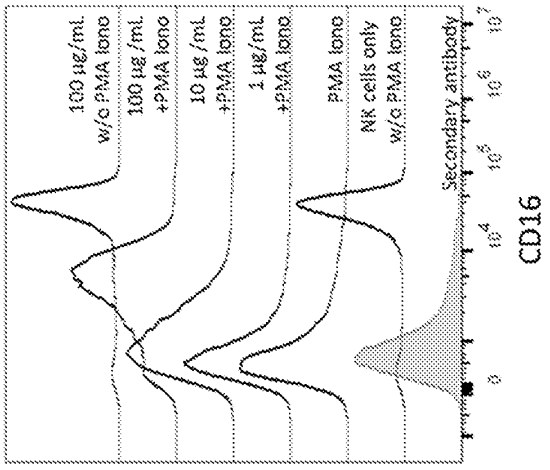


Figure 5C

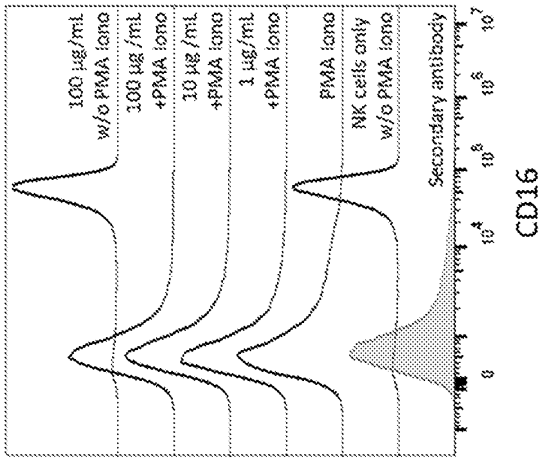


Figure 6C

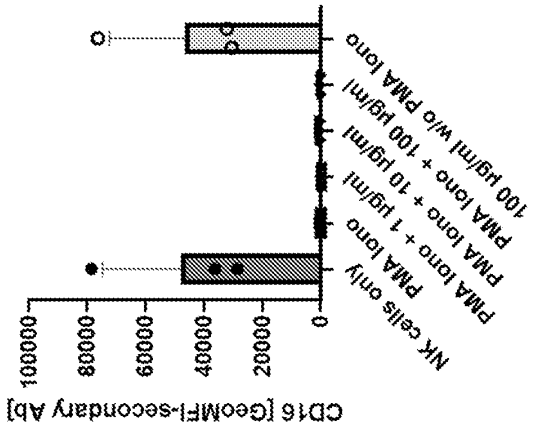


Figure 6B

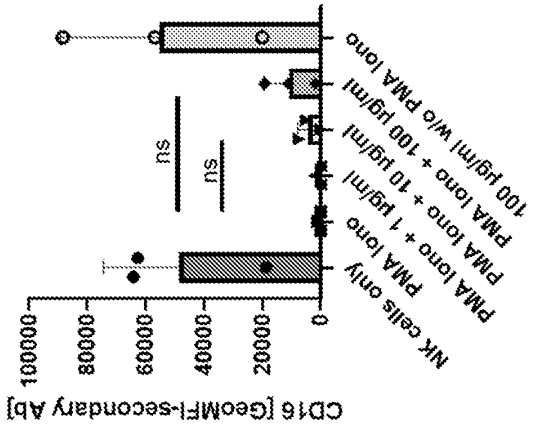


Figure 6A

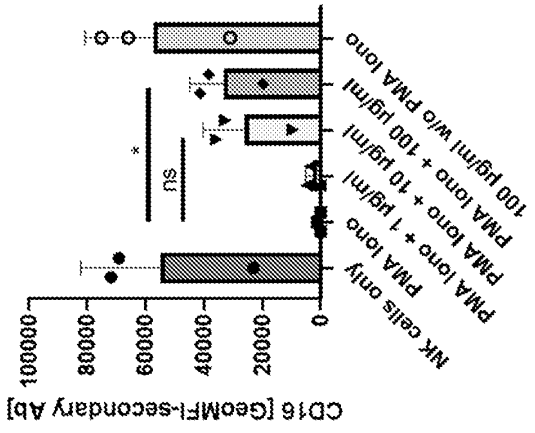


Figure 7

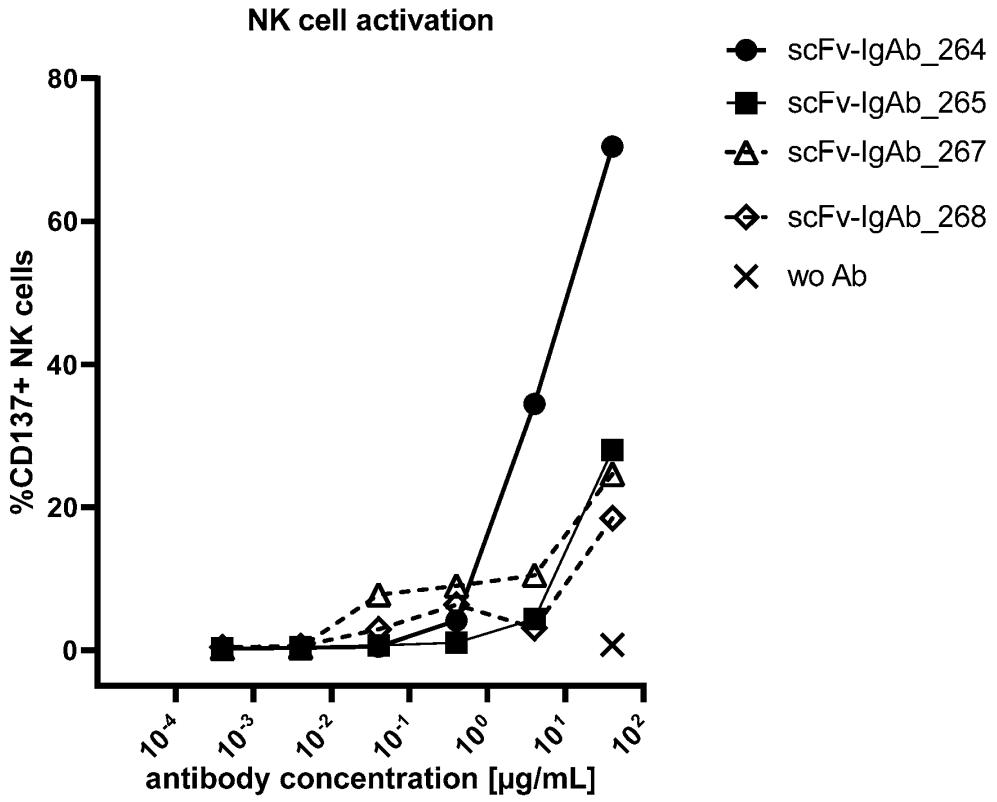

NK cell activation
%CD137+ NK cells
80
60
40
20
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
antibody concentration [µg/mL]
scFv-IgAb_264
scFv-IgAb_265
scFv-IgAb_267
scFv-IgAb_268
wo Ab

Figure 8

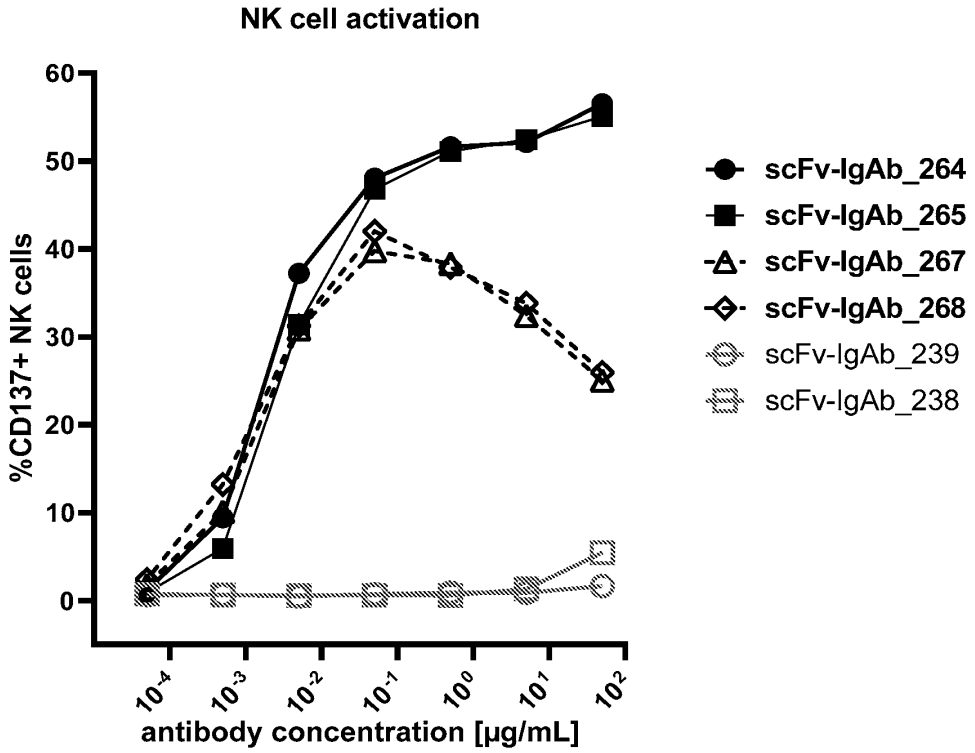

NK cell activation
%CD137+ NK cells
60
50
40
30
20
10
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
antibody concentration [µg/mL]
scFv-IgAb_264
scFv-IgAb_265
scFv-IgAb_267
scFv-IgAb_268
scFv-IgAb_239
scFv-IgAb_238

Figure 9A

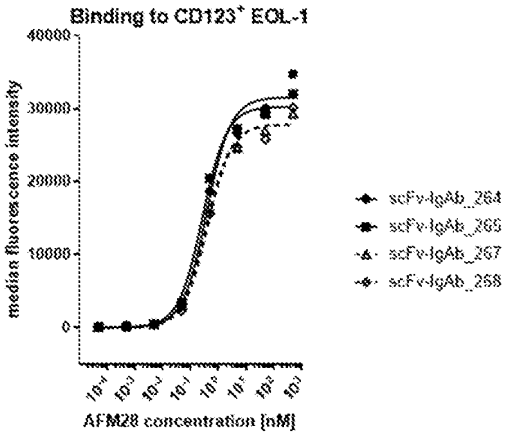


Figure 9B

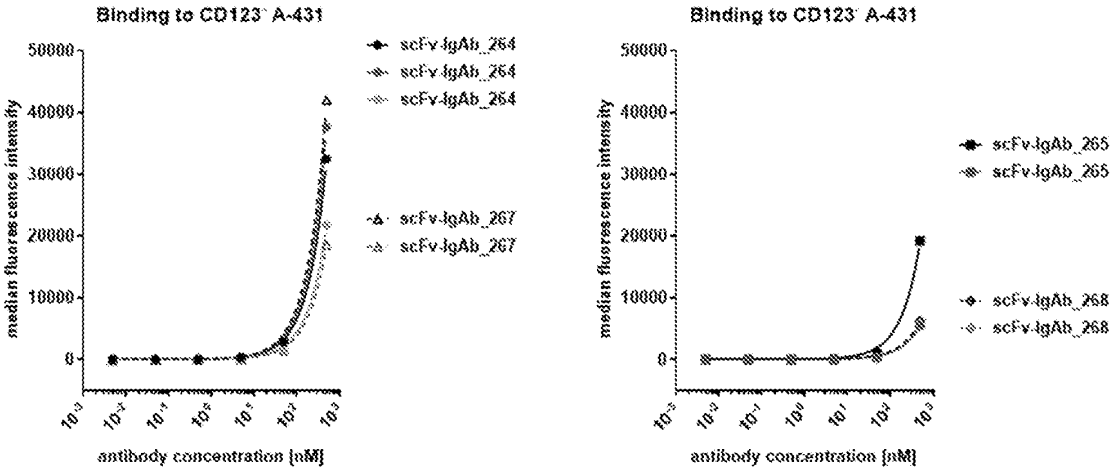
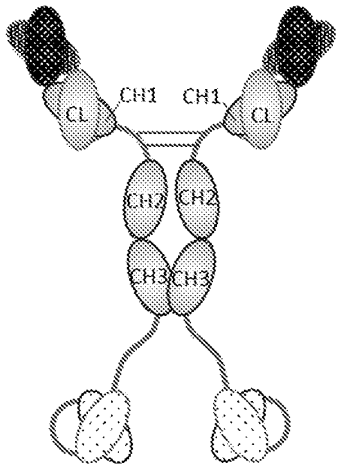


Figure 10



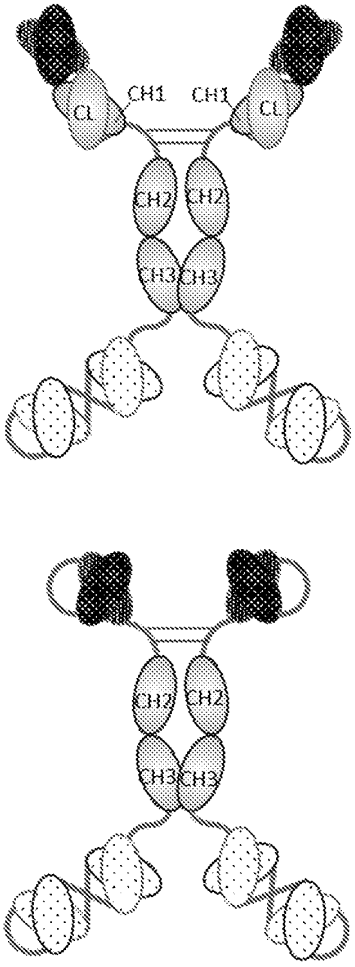
variable heavy chain of first binding domain (A)

variable light chain of first binding domain (A)

variable heavy chain of second binding domain (B)

variable light chain of second binding domain (B)

Figure 11



variable heavy chain of first binding domain (A)

variable light chain of first binding domain (A)

variable heavy chain of second binding domain (B)

variable light chain of second binding domain (B)

Figure 12A

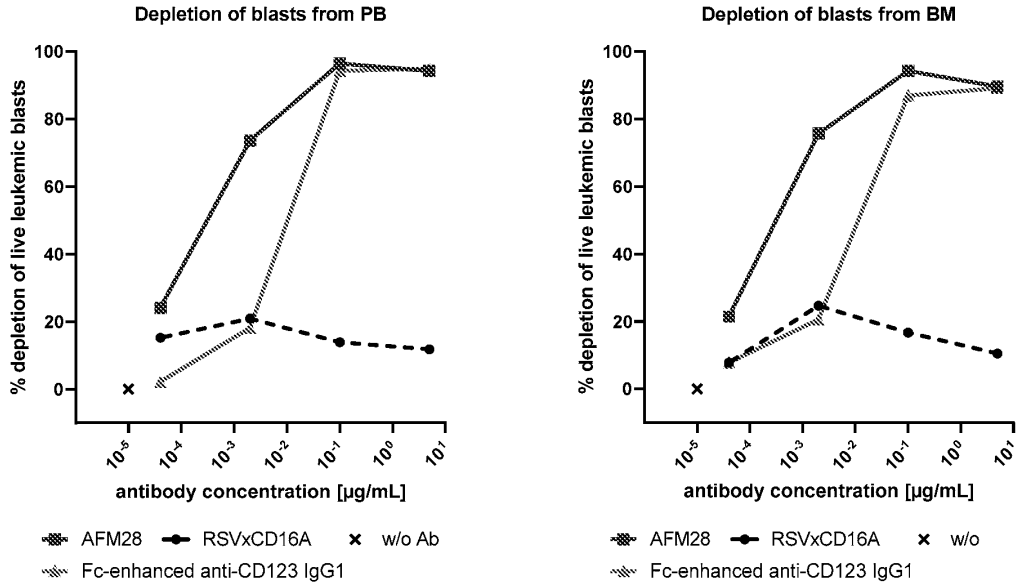


Figure 12B

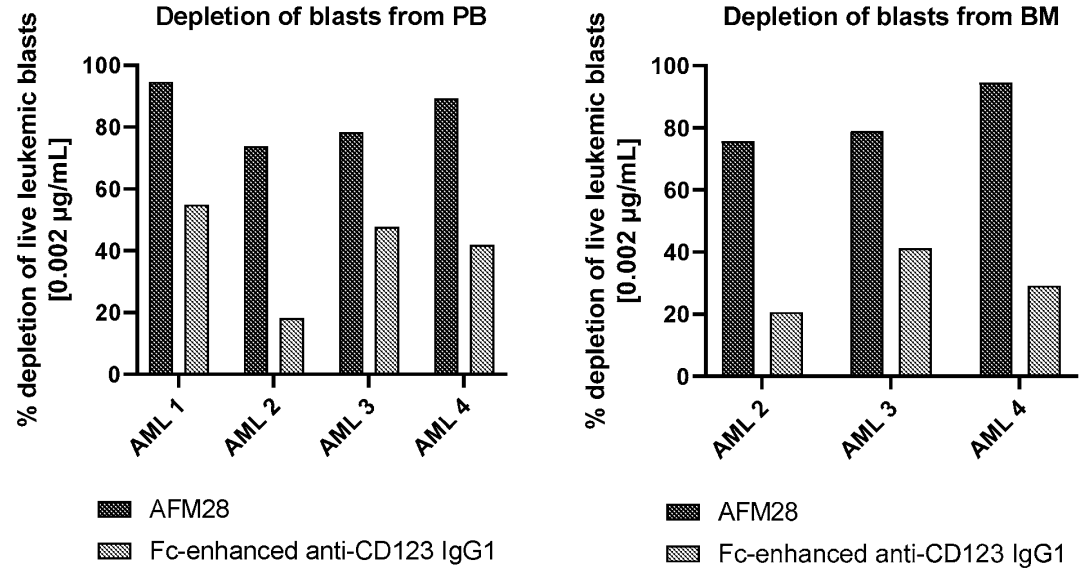


Figure 13A

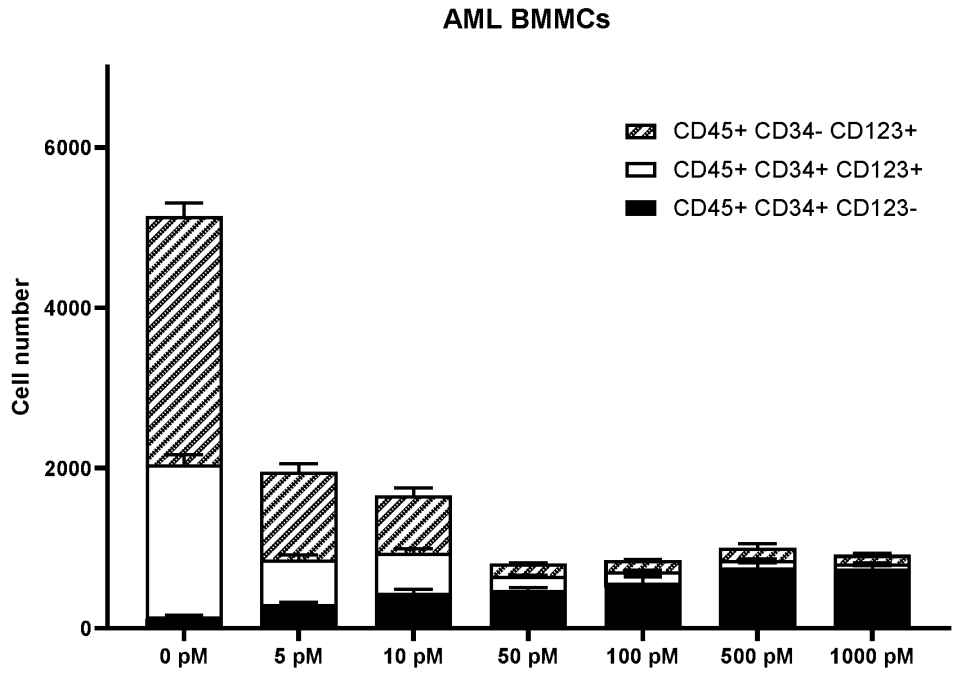


Figure 13B

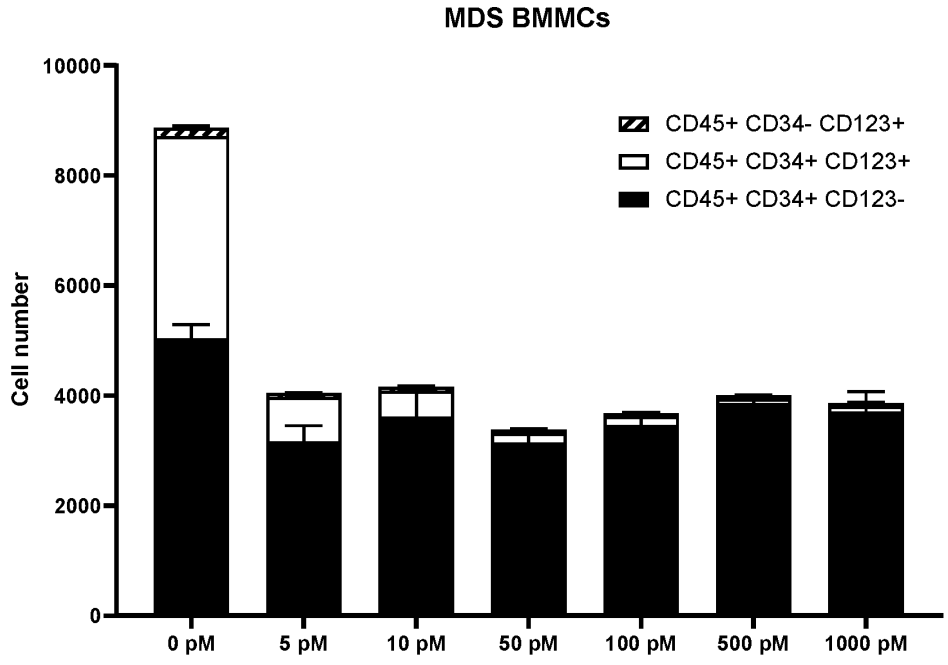
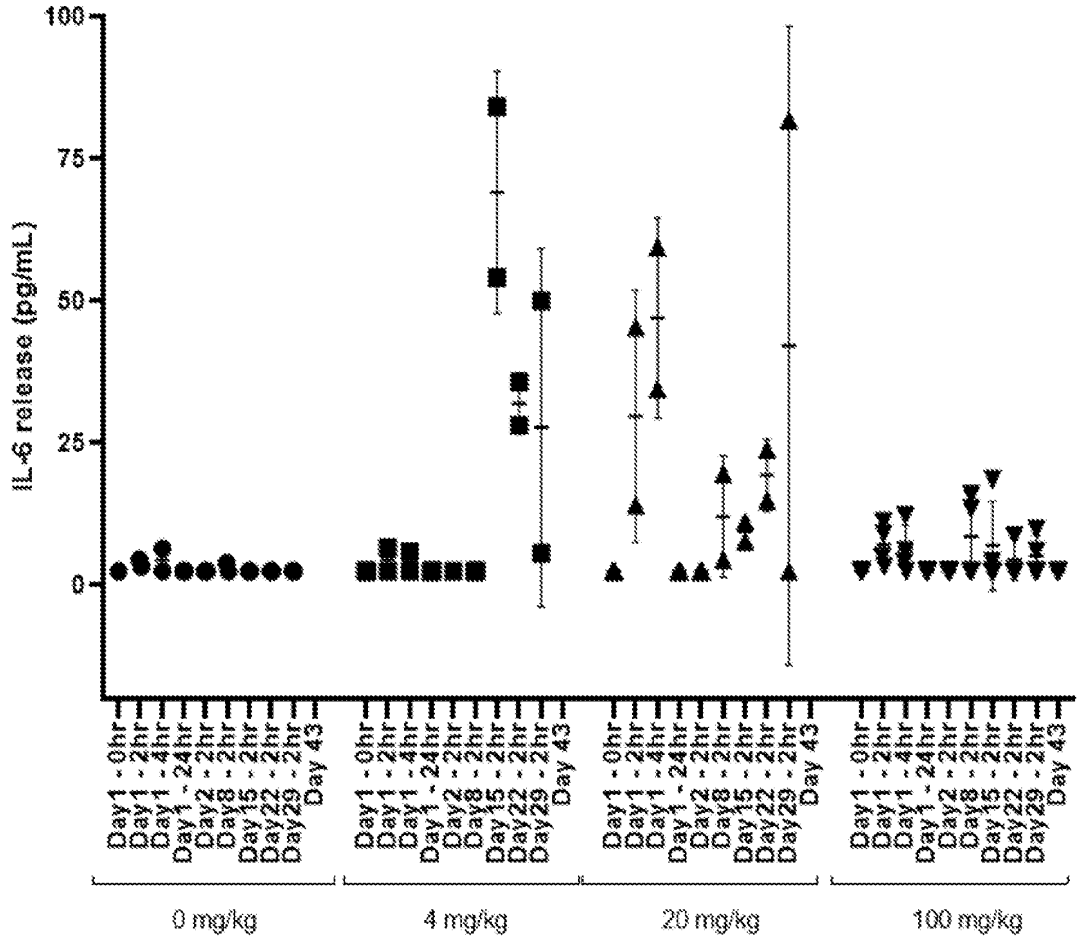


Figure 14


IL-6 release (pg/mL)
100
75
50
25
0
Day1 - 0hr
Day1 - 2hr
Day1 - 4hr
Day1 - 24hr
Day2 - 2hr
Day8 - 2hr
Day15 - 2hr
Day22 - 2hr
Day29 - 2hr
Day 43
0 mg/kg
4 mg/kg
20 mg/kg
100 mg/kg

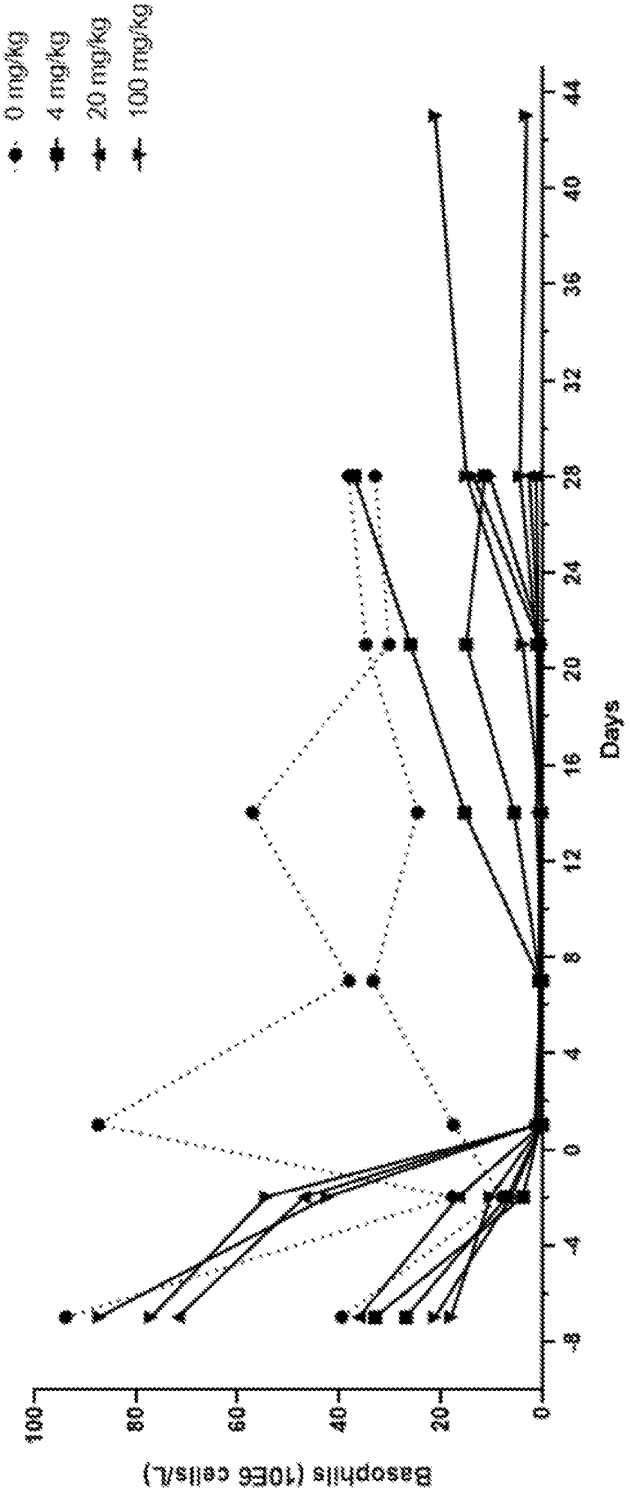

0 mg/kg
4 mg/kg
20 mg/kg
100 mg/kg
Basophils (10E6 cells/L)
0
20
40
60
80
100
-8
-4
0
4
8
12
16
20
24
28
32
36
40
44
Days

Figure 15

Figure 16A

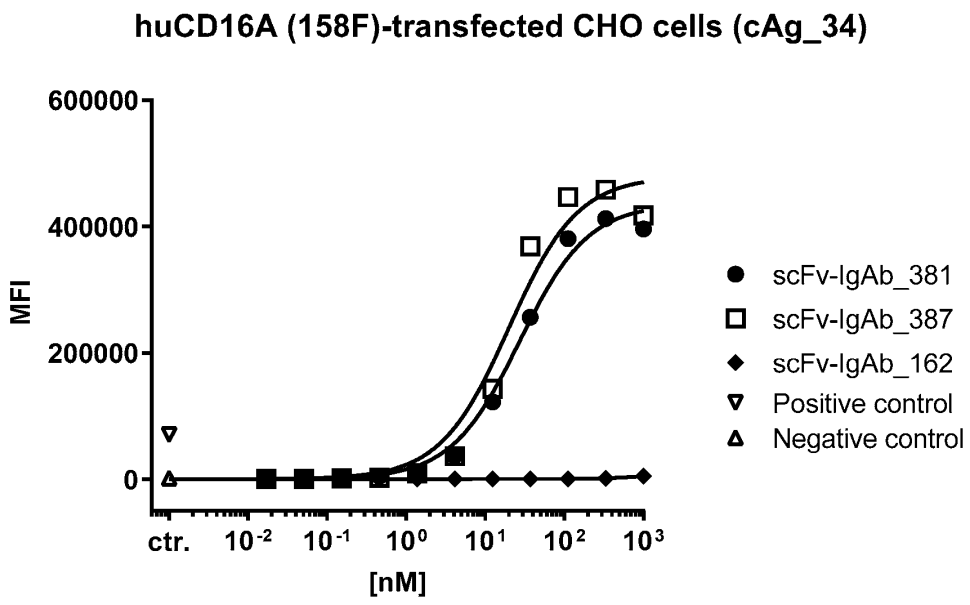


Figure 16B

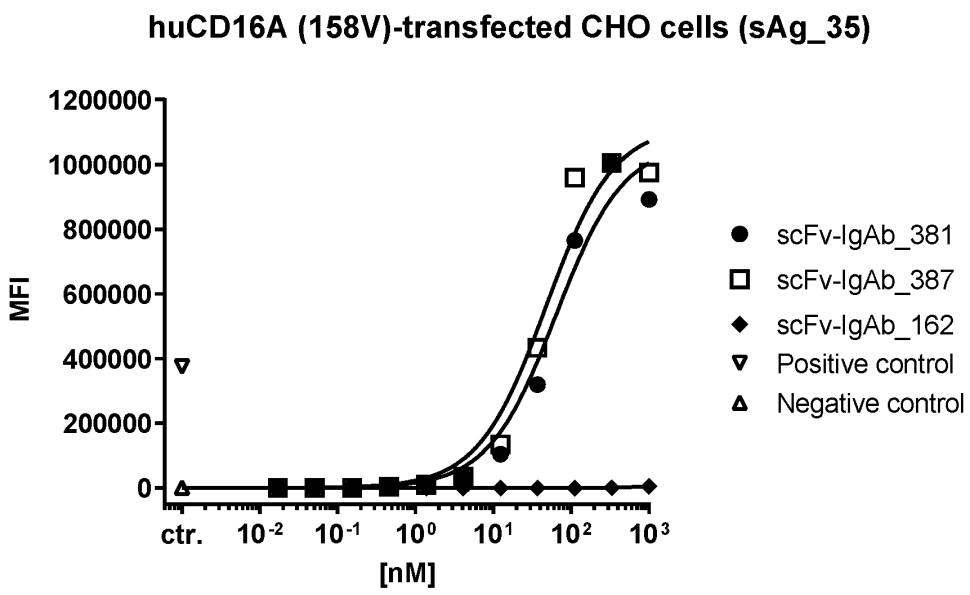


Figure 16C

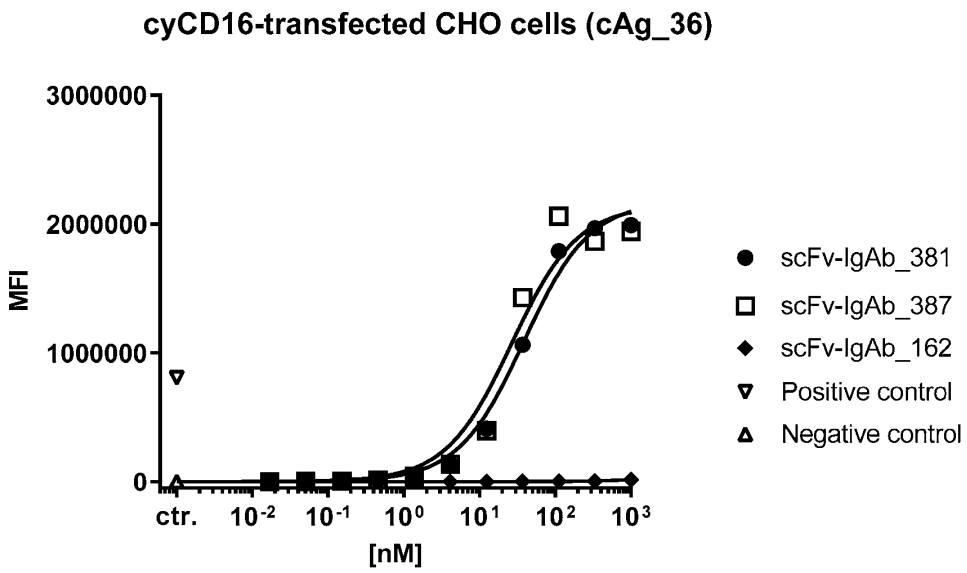

cyCD16-transfected CHO cells (cAg_36)
3000000
2000000
1000000
0
MFI
ctr.
10-2
10-1
100
101
102
103
[nM]
scFv-IgAb_381
scFv-IgAb_387
scFv-IgAb_162
Positive control
Negative control

Figure 17

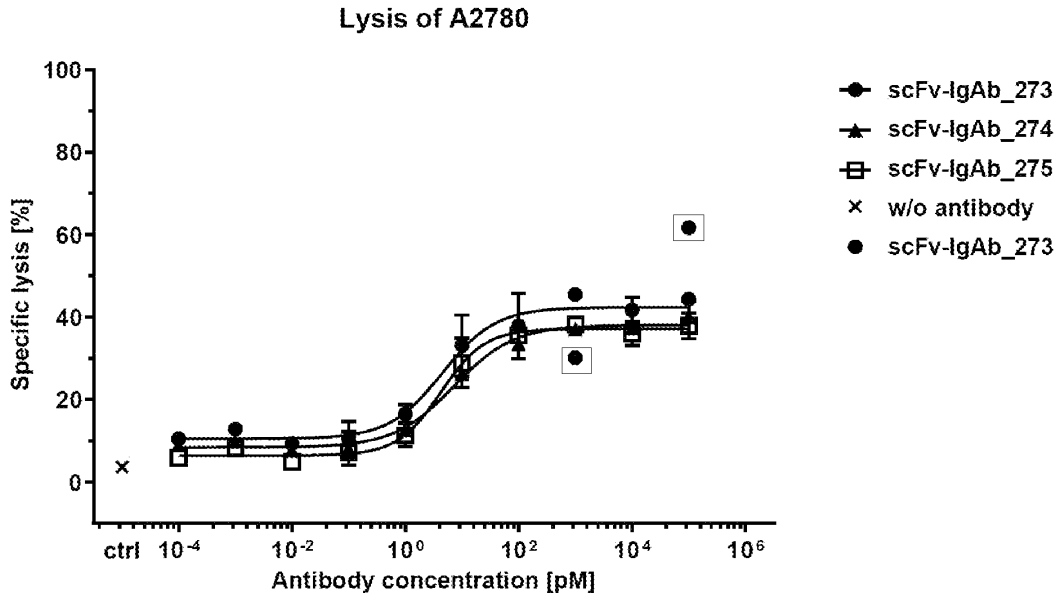

Lysis of A2780
Specific lysis [%]
100
80
60
40
20
0
ctrl
10-4
10-2
100
102
104
106
Antibody concentration [pM]
scFv-IgAb_273
scFv-IgAb_274
scFv-IgAb_275
w/o antibody
scFv-IgAb_273

Figure 18A

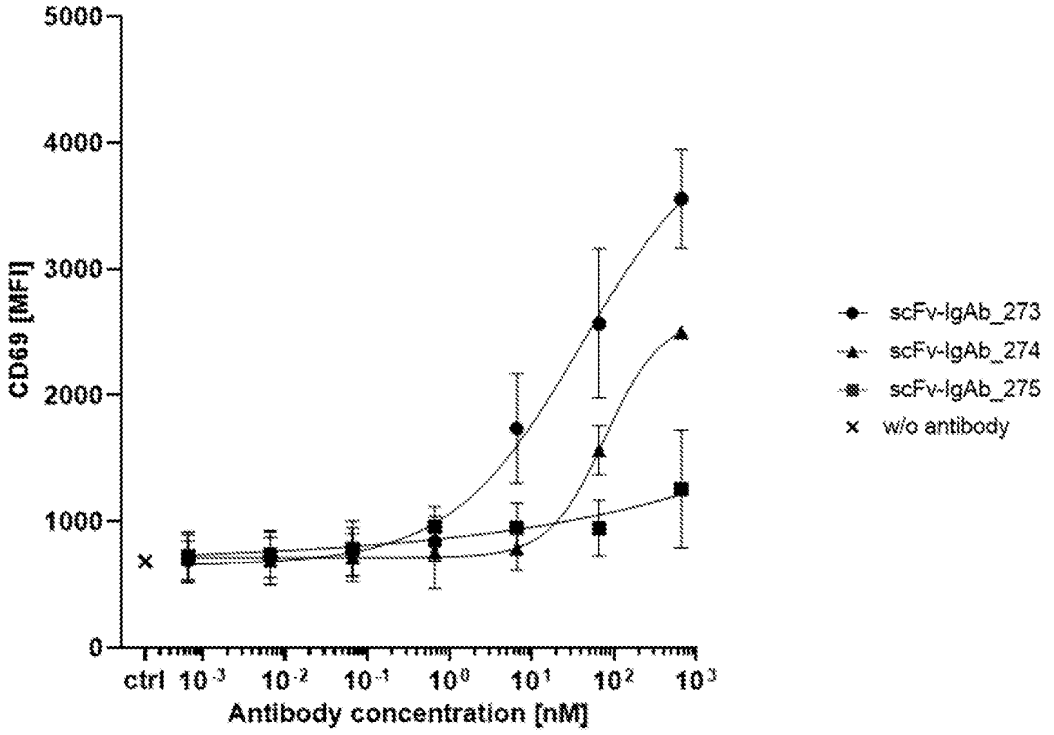


Figure 18B

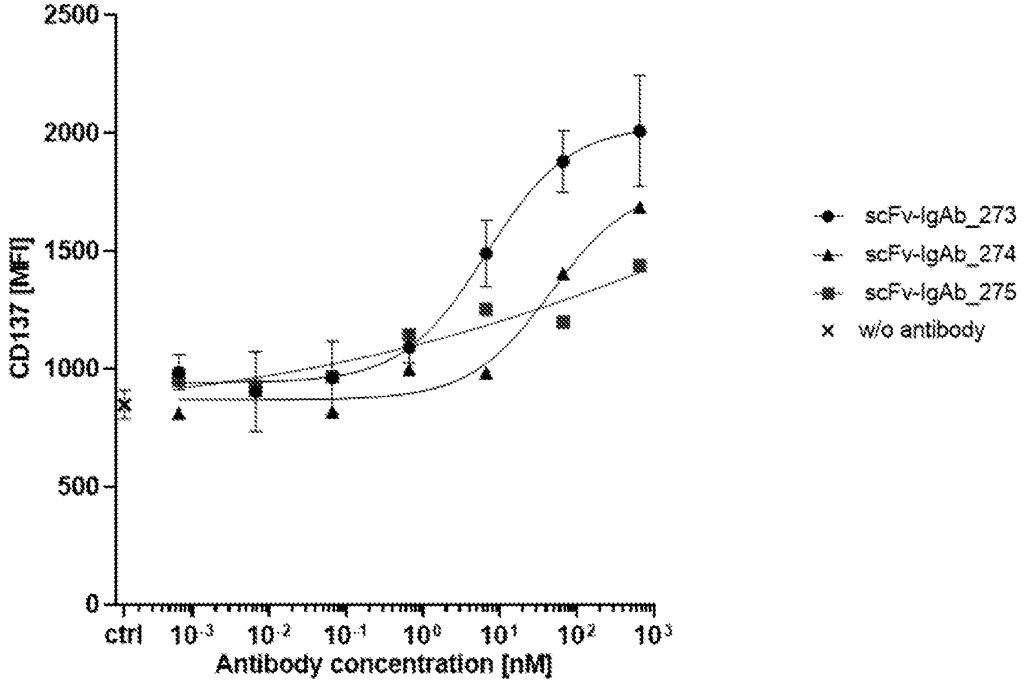


Figure 19A

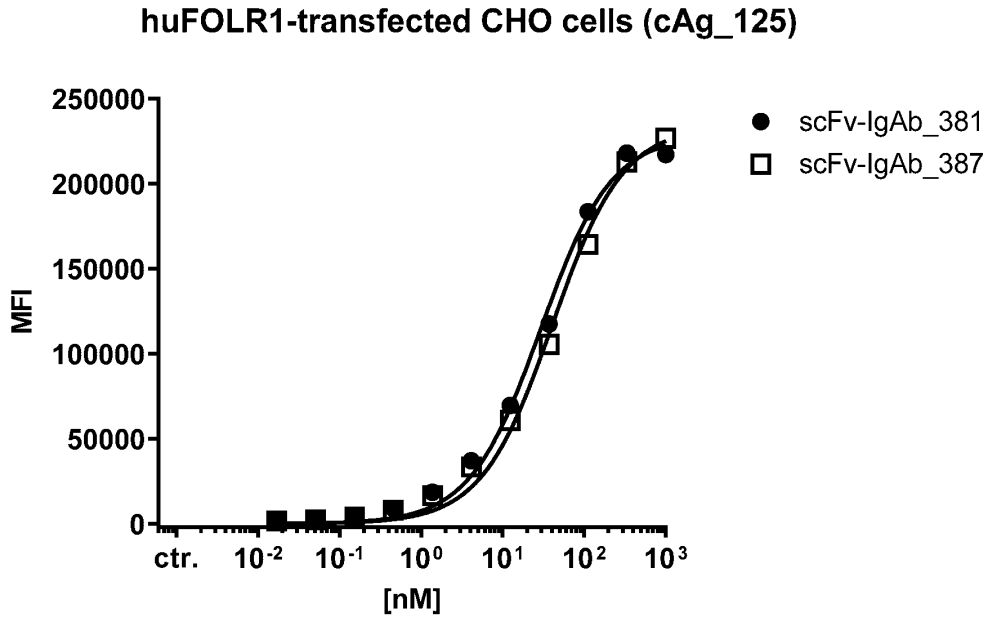


Figure 19B

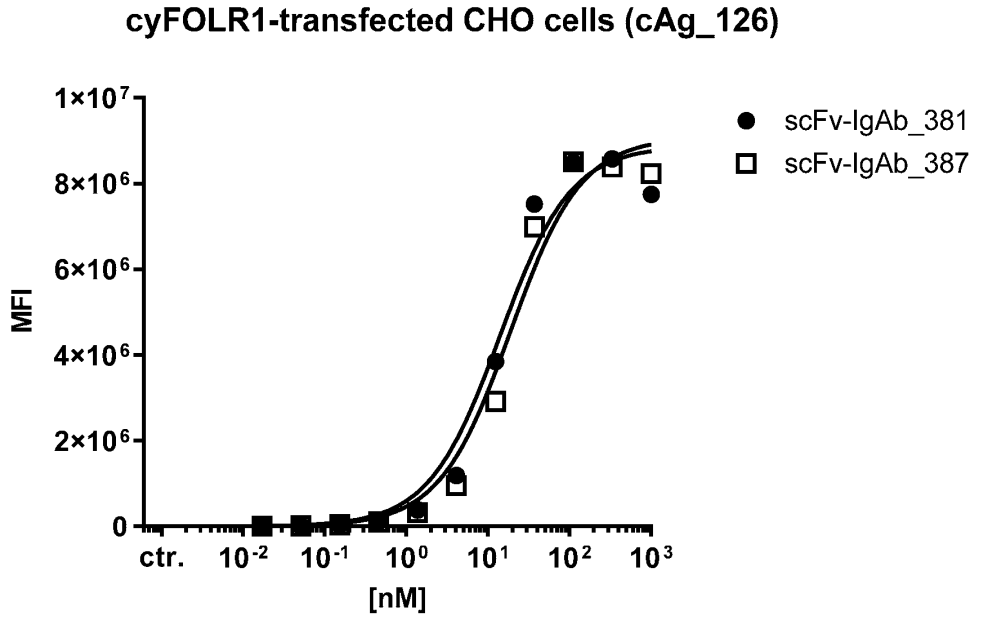


Figure 20

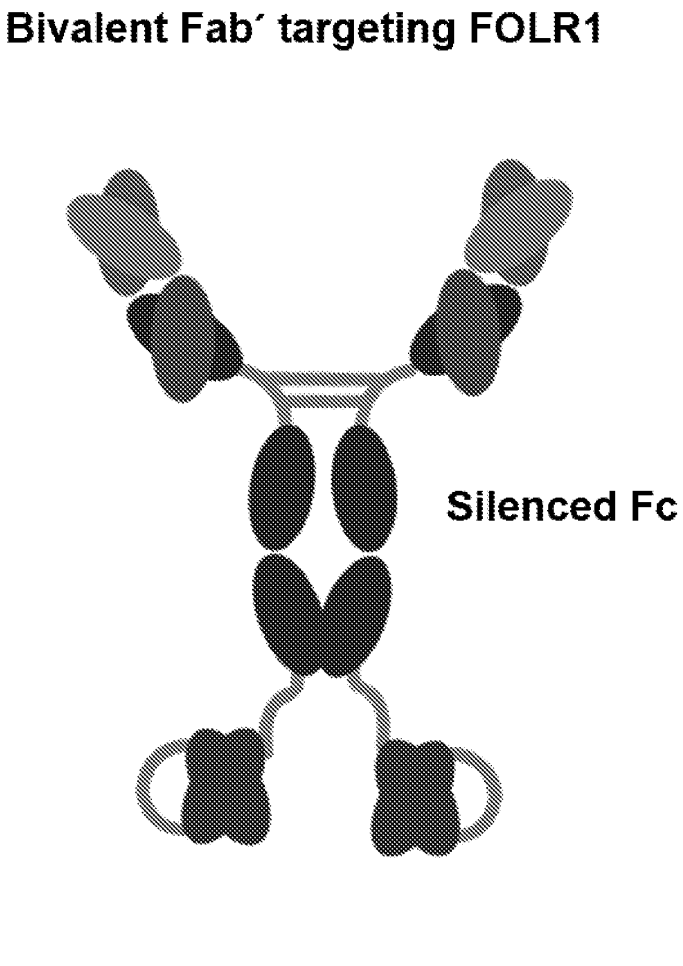

Bivalent Fab´ targeting FOLR1
Silenced Fc
Bivalent scFv targeting CD16A

Figure 21

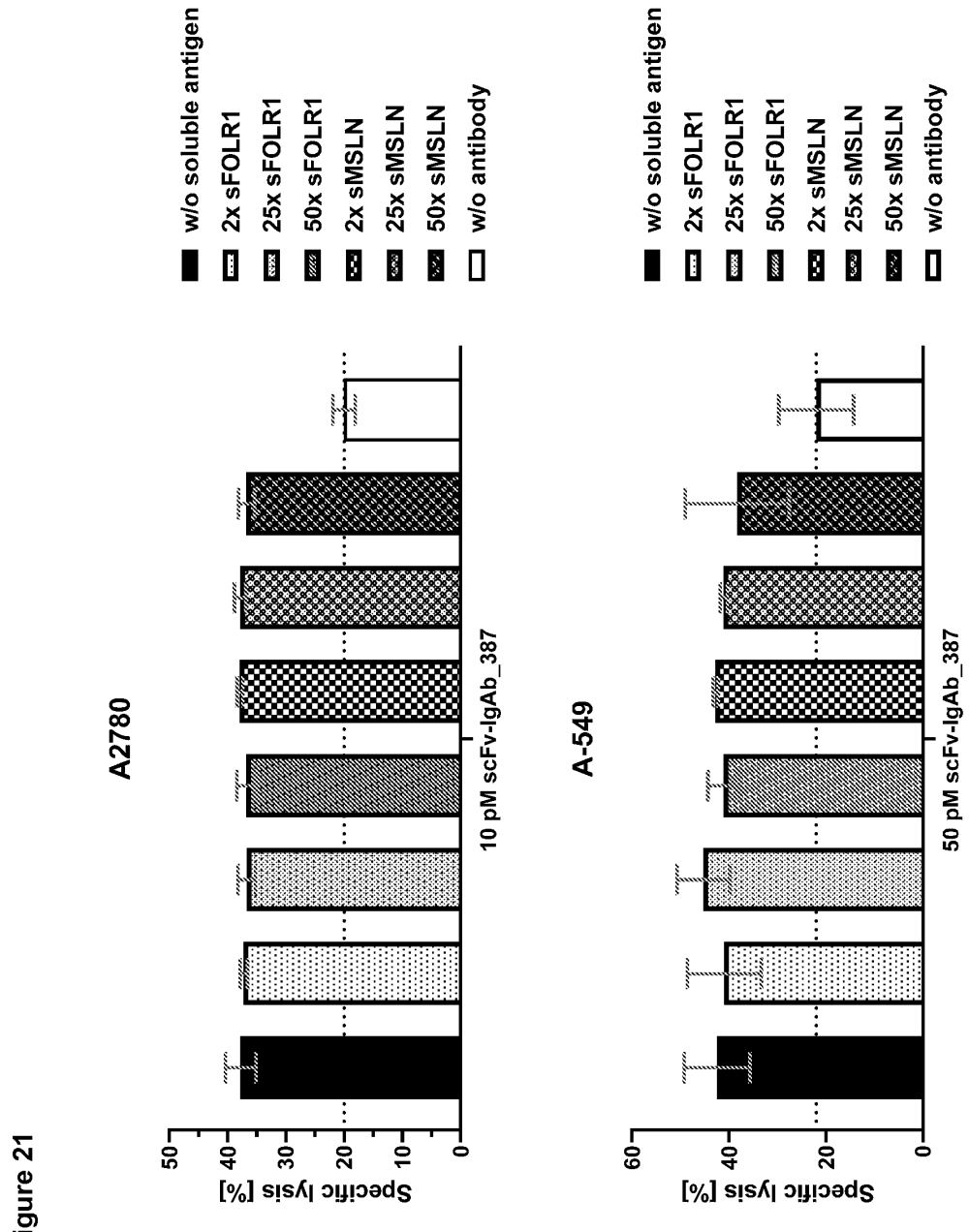

A2780
w/o soluble antigen
2x sFOLR1
25x sFOLR1
50x sFOLR1
2x sMSLN
25x sMSLN
50x sMSLN
w/o antibody
Specific lysis [%]
0
10
20
30
40
50
10 pM scFv-IgAb_387
A-549
w/o soluble antigen
2x sFOLR1
25x sFOLR1
50x sFOLR1
2x sMSLN
25x sMSLN
50x sMSLN
w/o antibody
Specific lysis [%]
0
20
40
60
50 pM scFv-IgAb_387

Figure 22

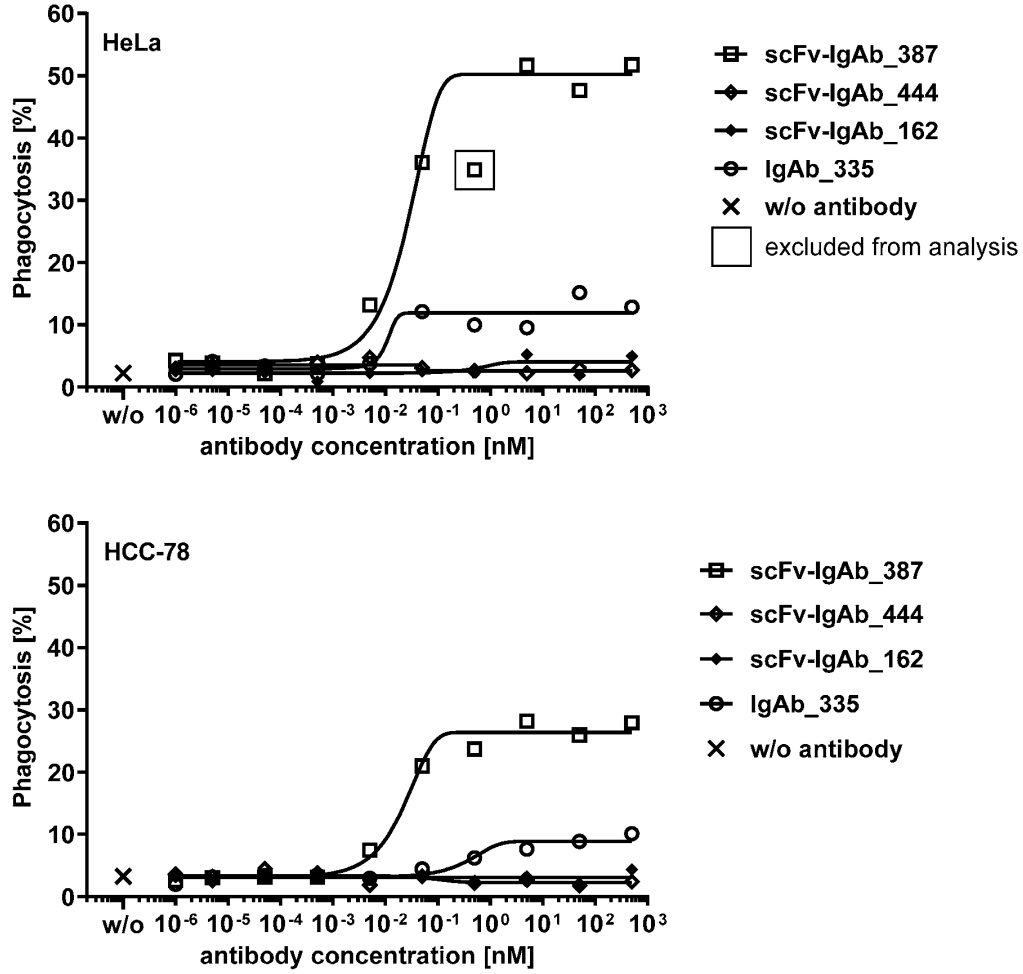
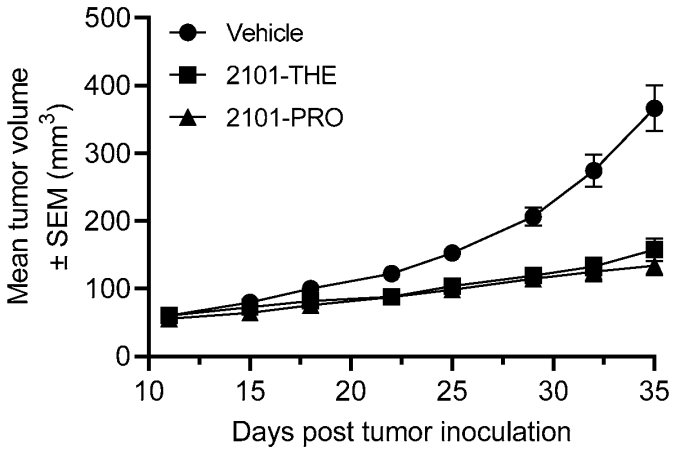

HeLa
Phagocytosis [%]
60
50
40
30
20
10
0
w/o 10-6 10-5 10-4 10-3 10-2 10-1 100 101 102 103
antibody concentration [nM]
scFv-IgAb_387
scFv-IgAb_444
scFv-IgAb_162
IgAb_335
w/o antibody
excluded from analysis
HCC-78
Phagocytosis [%]
60
50
40
30
20
10
0
w/o 10-6 10-5 10-4 10-3 10-2 10-1 100 101 102 103
antibody concentration [nM]
scFv-IgAb_387
scFv-IgAb_444
scFv-IgAb_162
IgAb_335
w/o antibody

Figure 23


Vehicle
2101-THE
2101-PRO
Mean tumor volume ± SEM (mm3)
500
400
300
200
100
0
10
15
20
25
30
35
Days post tumor inoculation

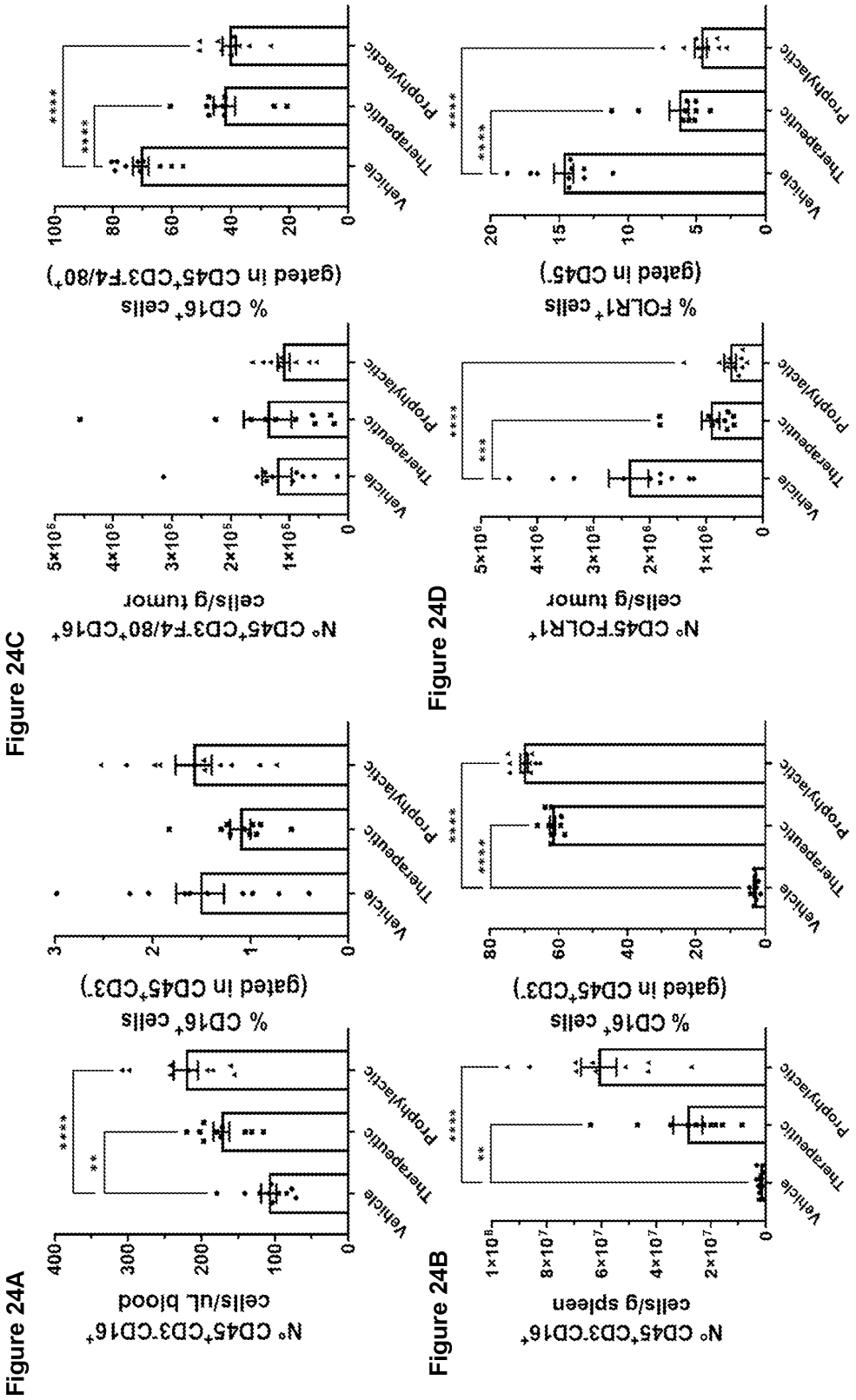


Figure 24A
N° CD45+CD3-CD16+ cells/uL blood
% CD16+ cells (gated in CD45+CD3-)
Figure 24B
N° CD45+CD3-CD16+ cells/g spleen
% CD16+ cells (gated in CD45+CD3-)
Figure 24C
N° CD45+CD3-F4/80+CD16+ cells/g tumor
% CD16+ cells (gated in CD45+CD3-F4/80+)
Figure 24D
N° CD45-FOLR1+ cells/g tumor
% FOLR1+ cells (gated in CD45-)
Vehicle
Therapeutic
Prophylactic

Figure 25

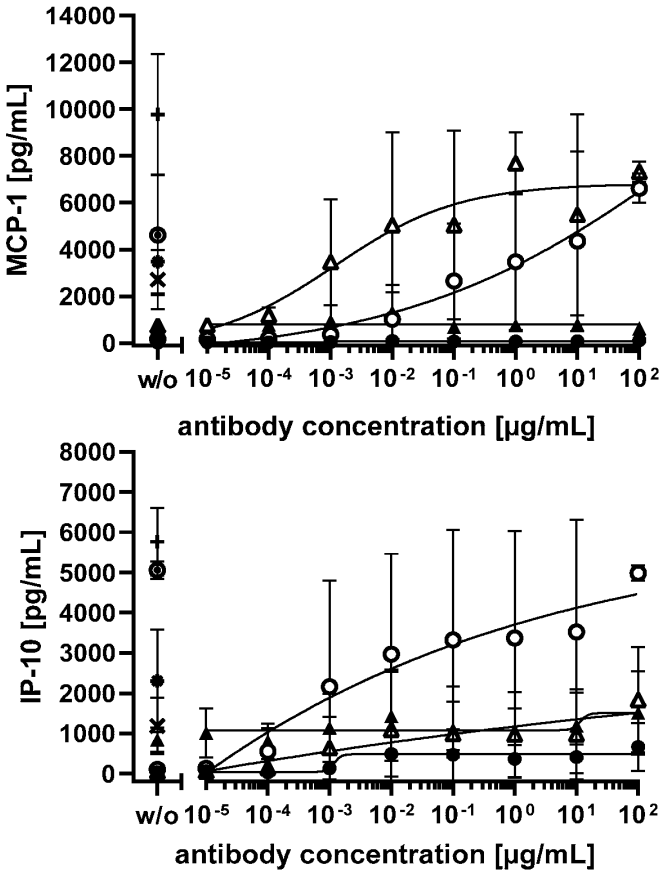

MCP-1 [pg/mL]
14000
12000
10000
8000
6000
4000
2000
0
w/o 10-5 10-4 10-3 10-2 10-1 100 101 102
antibody concentration [µg/mL]
IP-10 [pg/mL]
8000
7000
6000
5000
4000
3000
2000
1000
0
w/o 10-5 10-4 10-3 10-2 10-1 100 101 102
antibody concentration [µg/mL]
PBMCs only
PBMC + OVCAR-3
PBMC + A-549
PBMC + Raji
CD3/CD28 in PBMCs only
CD3/CD28 in PBMC + OVCAR-3
CD3/CD28 in PBMC + A-549
CD3/CD28 in PBMC + Raji

Figure 26

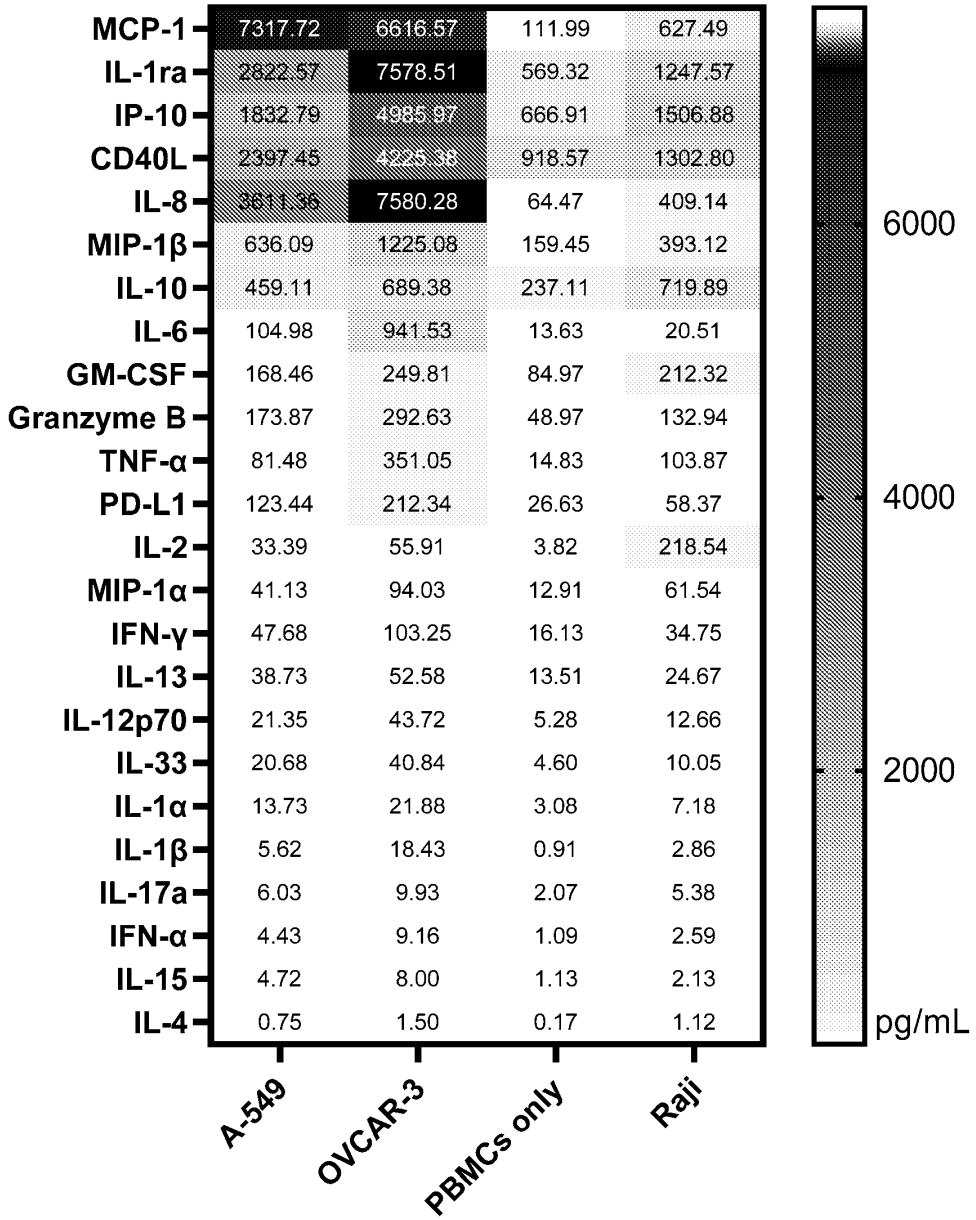

MCP-1 7317.72 6616.57 111.99 627.49
IL-1ra 2822.57 7578.51 569.32 1247.57
IP-10 1832.79 4985.97 666.91 1506.88
CD40L 2397.45 4225.38 918.57 1302.80
IL-8 3611.35 7580.28 64.47 409.14
MIP-1β 636.09 1225.08 159.45 393.12
IL-10 459.11 689.38 237.11 719.89
IL-6 104.98 941.53 13.63 20.51
GM-CSF 168.46 249.81 84.97 212.32
Granzyme B 173.87 292.63 48.97 132.94
TNF-α 81.48 351.05 14.83 103.87
PD-L1 123.44 212.34 26.63 58.37
IL-2 33.39 55.91 3.82 218.54
MIP-1α 41.13 94.03 12.91 61.54
IFN-γ 47.68 103.25 16.13 34.75
IL-13 38.73 52.58 13.51 24.67
IL-12p70 21.35 43.72 5.28 12.66
IL-33 20.68 40.84 4.60 10.05
IL-1α 13.73 21.88 3.08 7.18
IL-1β 5.62 18.43 0.91 2.86
IL-17a 6.03 9.93 2.07 5.38
IFN-α 4.43 9.16 1.09 2.59
IL-15 4.72 8.00 1.13 2.13
IL-4 0.75 1.50 0.17 1.12
A-549
OVCAR-3
PBMCs only
Raji
6000
4000
2000
pg/mL

Figure 27A

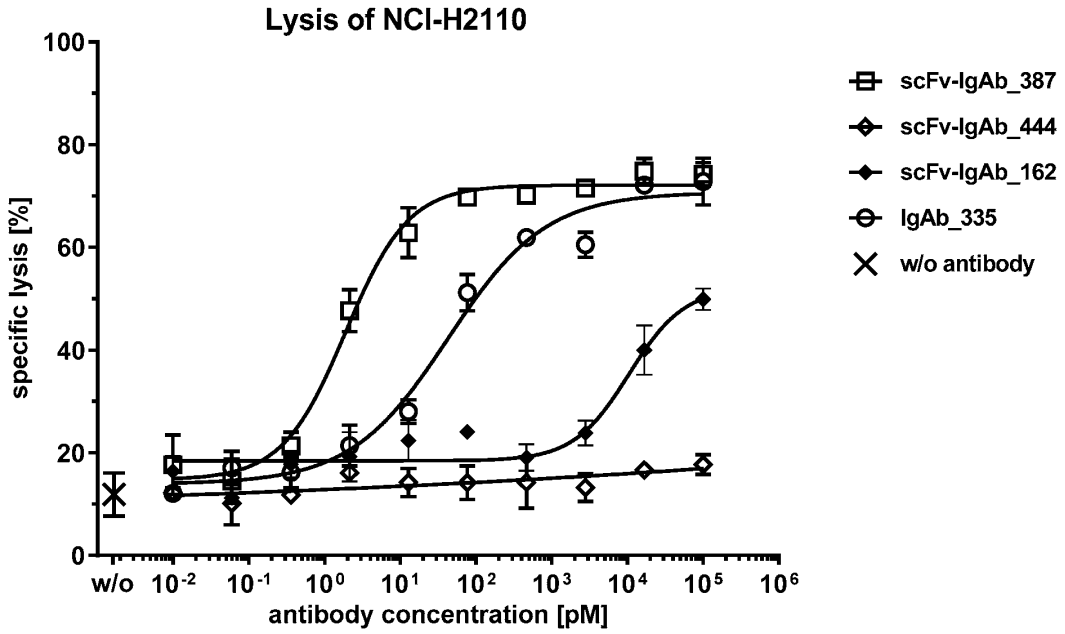


Figure 27B

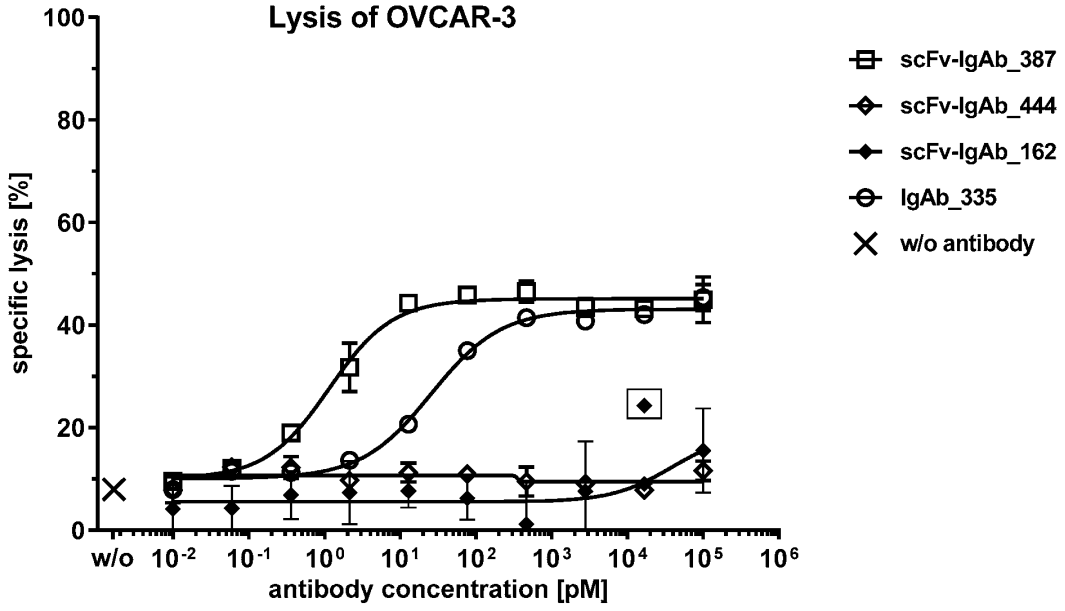


Figure 27C

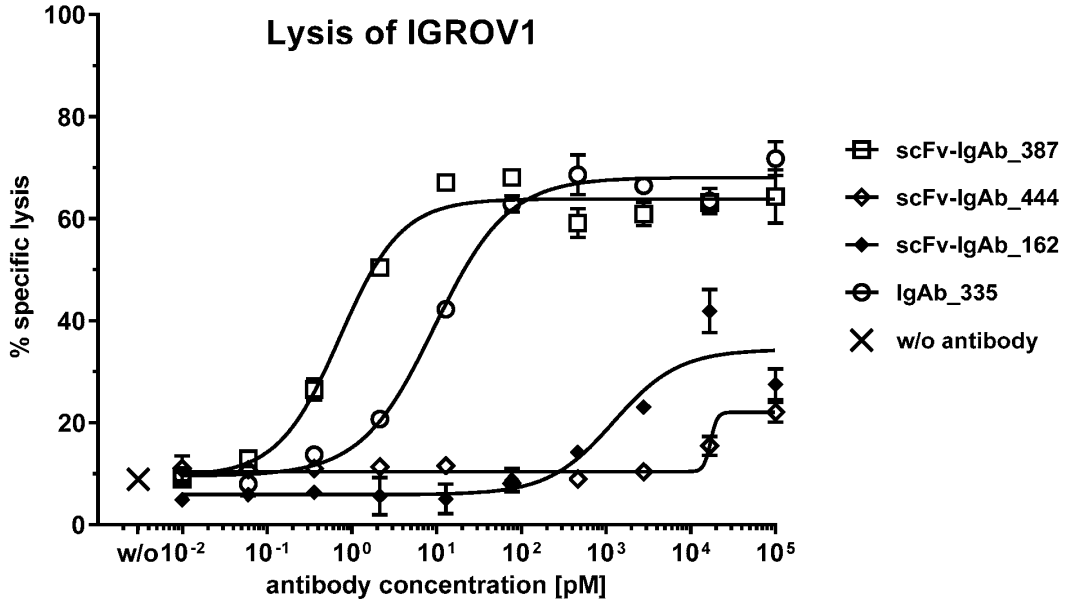


Figure 27D

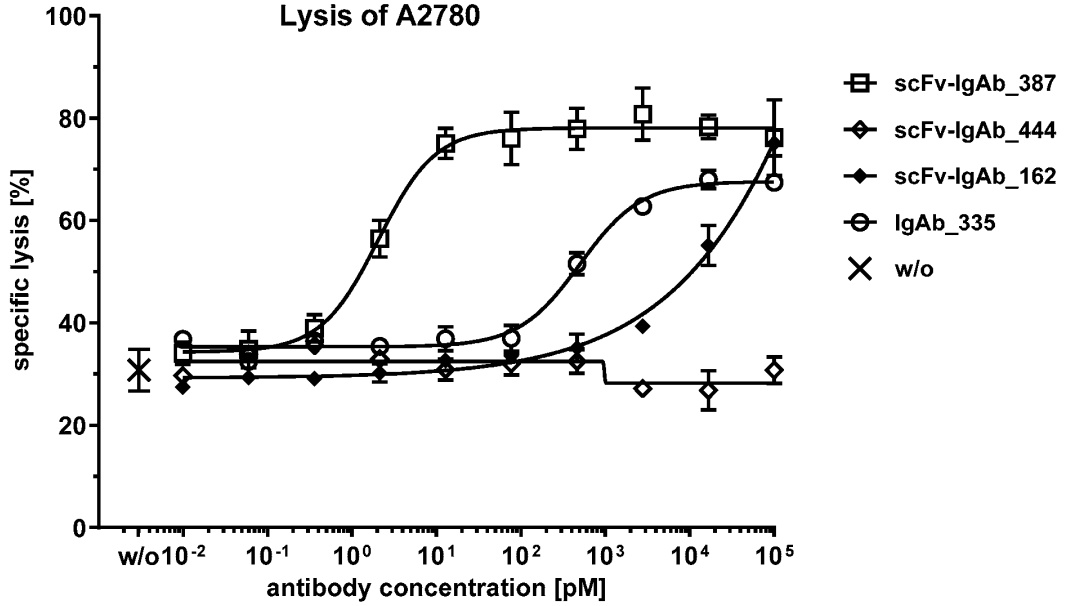


Figure 27E

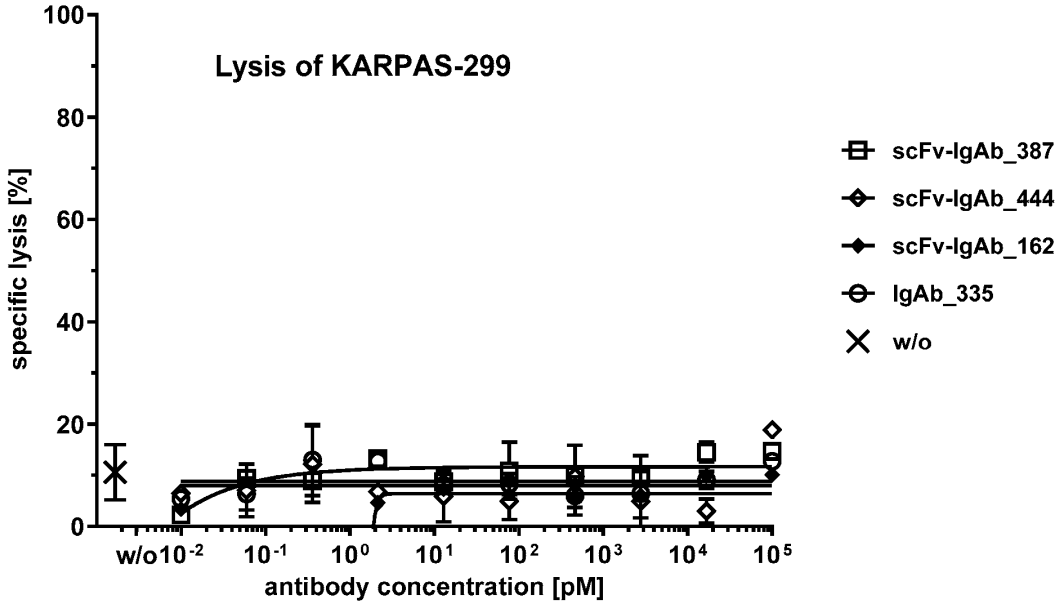


Figure 27F

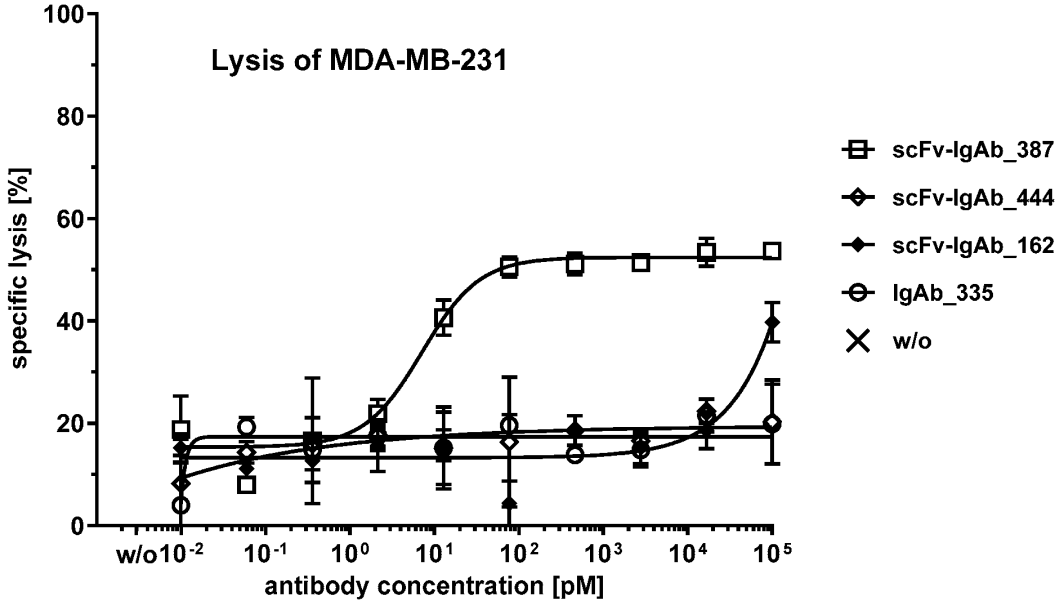


Figure 27G

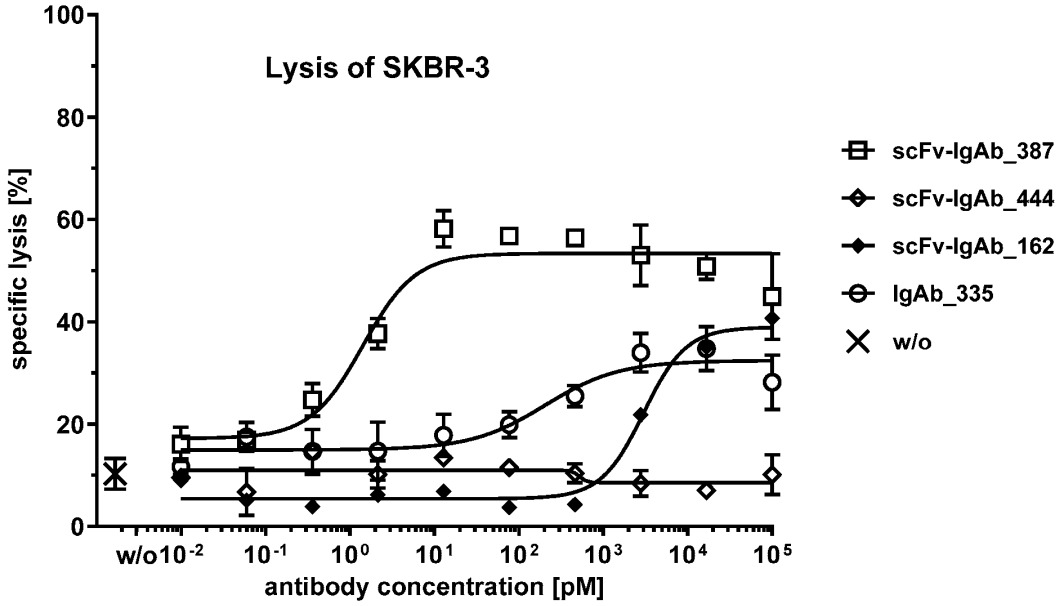

Lysis of SKBR-3
specific lysis [%]
100
80
60
40
20
0
w/o
10-2
10-1
100
101
102
103
104
105
antibody concentration [pM]
scFv-IgAb_387
scFv-IgAb_444
scFv-IgAb_162
IgAb_335
w/o

Figure 28

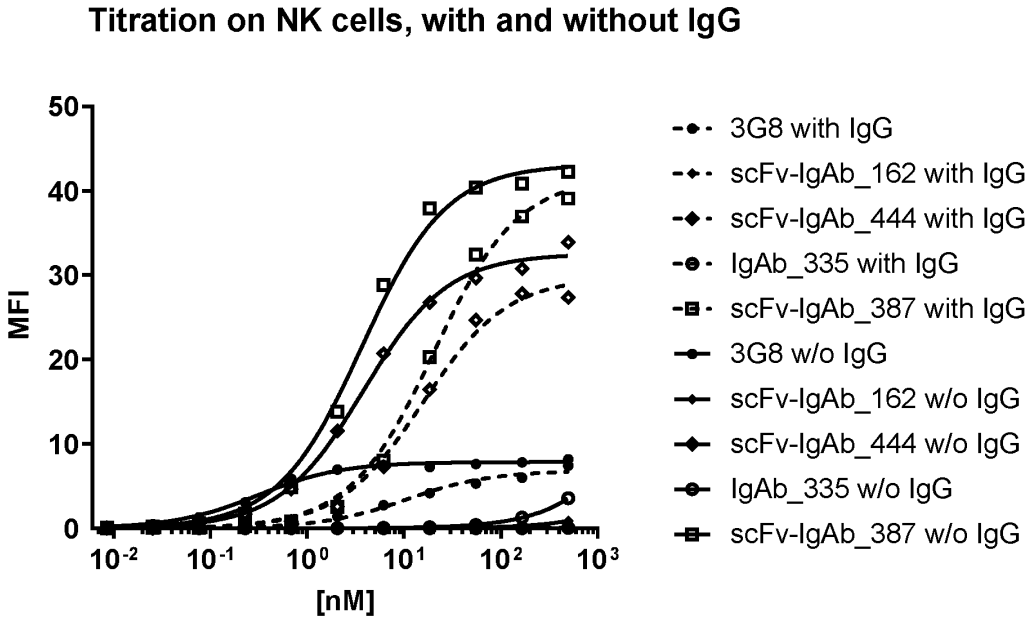

Titration on NK cells, with and without IgG
MFI
50
40
30
20
10
0
10-2
10-1
100
101
102
103
[nM]
3G8 with IgG
scFv-IgAb_162 with IgG
scFv-IgAb_444 with IgG
IgAb_335 with IgG
scFv-IgAb_387 with IgG
3G8 w/o IgG
scFv-IgAb_162 w/o IgG
scFv-IgAb_444 w/o IgG
IgAb_335 w/o IgG
scFv-IgAb_387 w/o IgG

Figure 29A

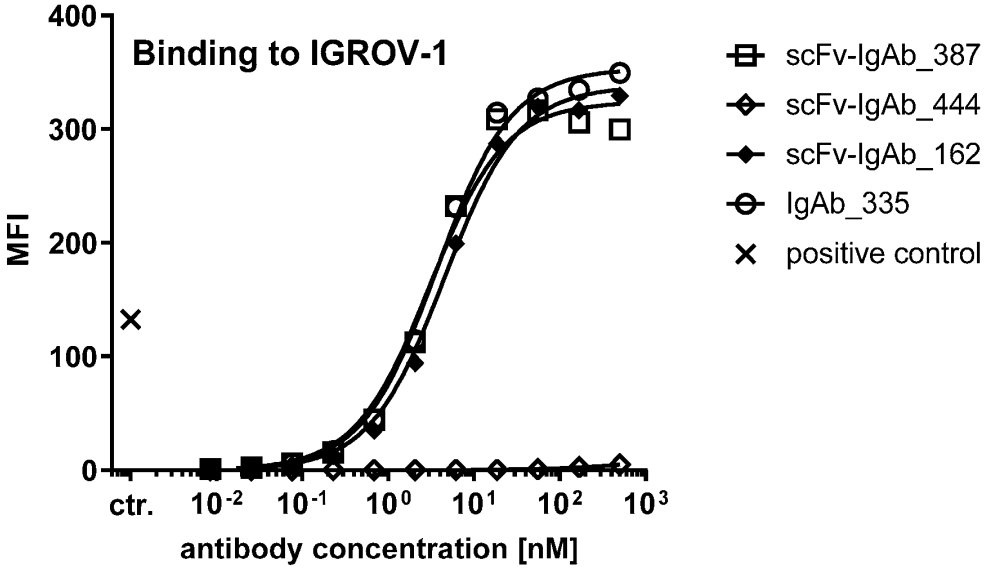


Figure 29B

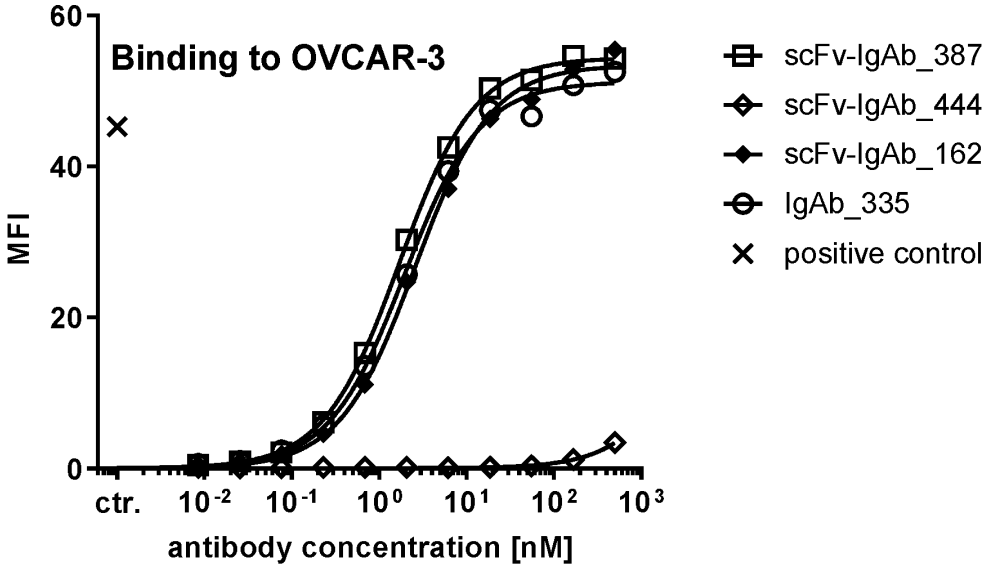


Figure 30A

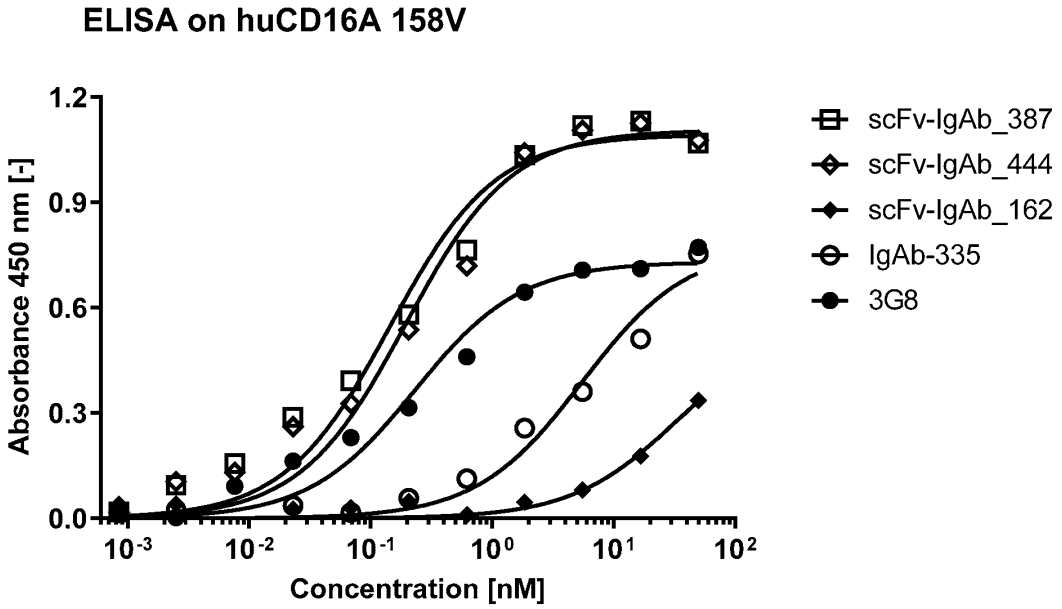


Figure 30B

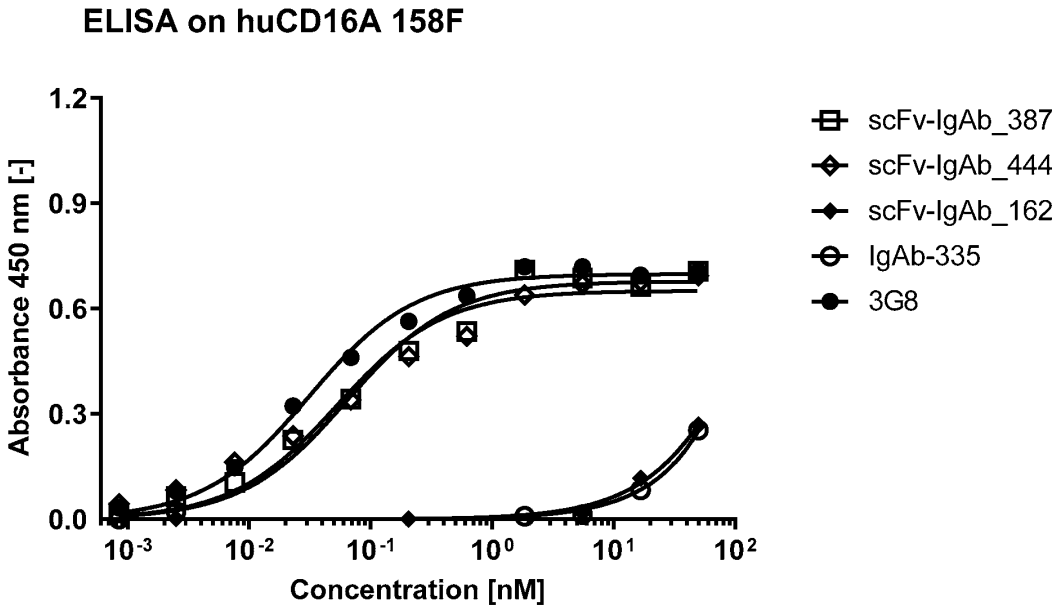
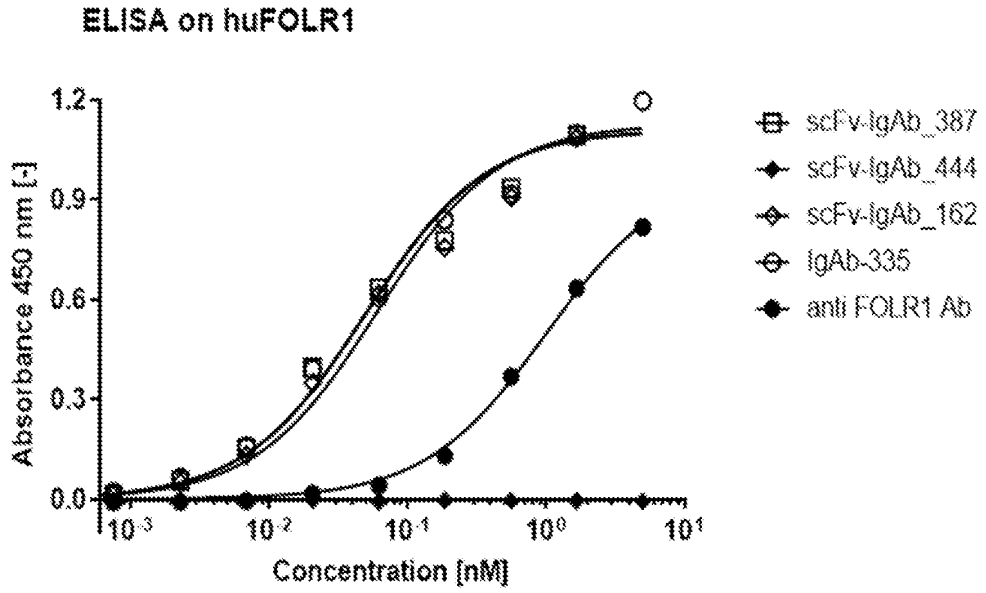


Figure 31


ELISA on huFOLR1
Absorbance 450 nm [-]
1.2
0.9
0.6
0.3
0.0
10 -3
10 -2
10 -1
10 0
10 1
Concentration [nM]
scFv-IgAb_387
scFv-IgAb_444
scFv-IgAb_162
IgAb-335
anti FOLR1 Ab