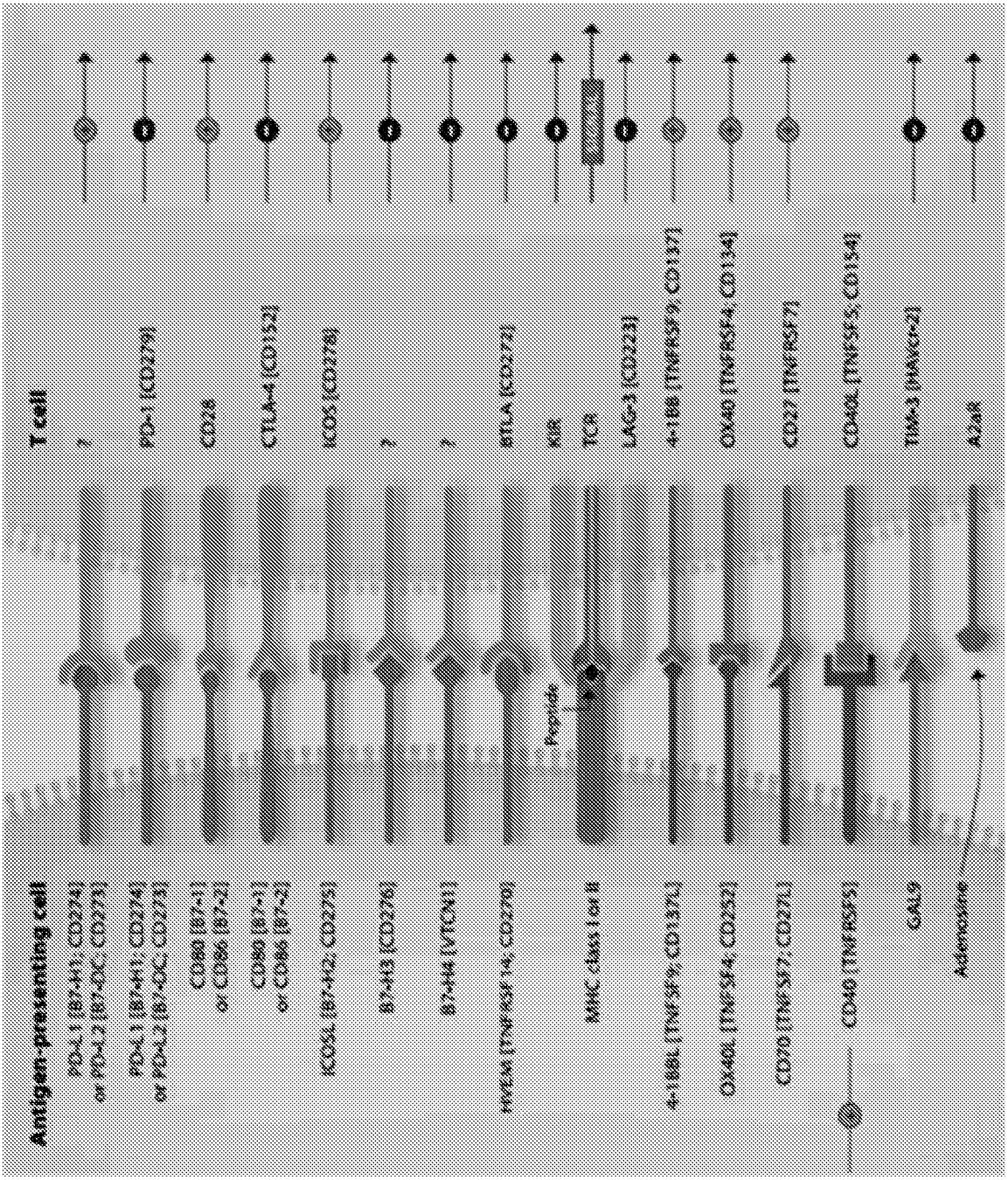
Fig. 1


Antigen-presenting cell
PD-L1 [B7-H1; CD274]
or PD-L2 [B7-DC; CD273]
PD-L1 [B7-H1; CD274]
or PD-L2 [B7-DC; CD273]
CD80 [B7-1]
or CD86 [B7-2]
CD80 [B7-1]
or CD86 [B7-2]
ICOSL [B7-H2; CD275]
B7-H3 [CD276]
B7-H4 [VTCN1]
HVEM [TNFRSF14; CD270]
MHC class I or II
+1BBL [TNFSF9; CD137L]
OX40L [TNFSF4; CD252]
CD70 [TNFSF7; CD27L]
CD40 [TNFRSF5]
GAL9
Adenosine
T cell
?
PD-1 [CD279]
CD28
CTLA-4 [CD152]
ICOS [CD278]
?
?
BTLA [CD272]
KIR
TCR
LAG-3 [CD223]
+1BB [TNFRSF9; CD137]
OX40 [TNFRSF4; CD134]
CD27 [TNFRSF7]
CD40L [TNFSF5; CD154]
TIM-3 [HAVCR2]
A2aR

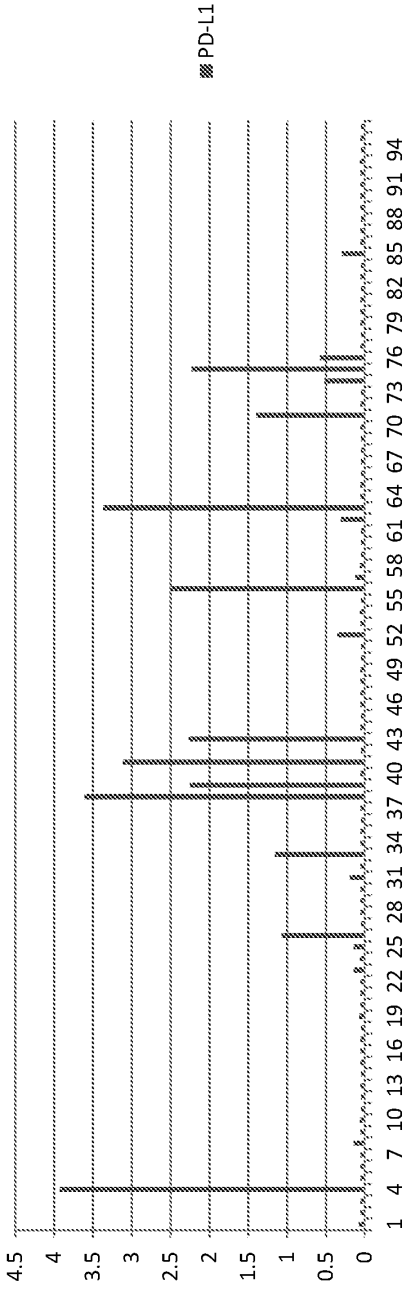

PD-L1
PD-L1
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94

Fig. 2A

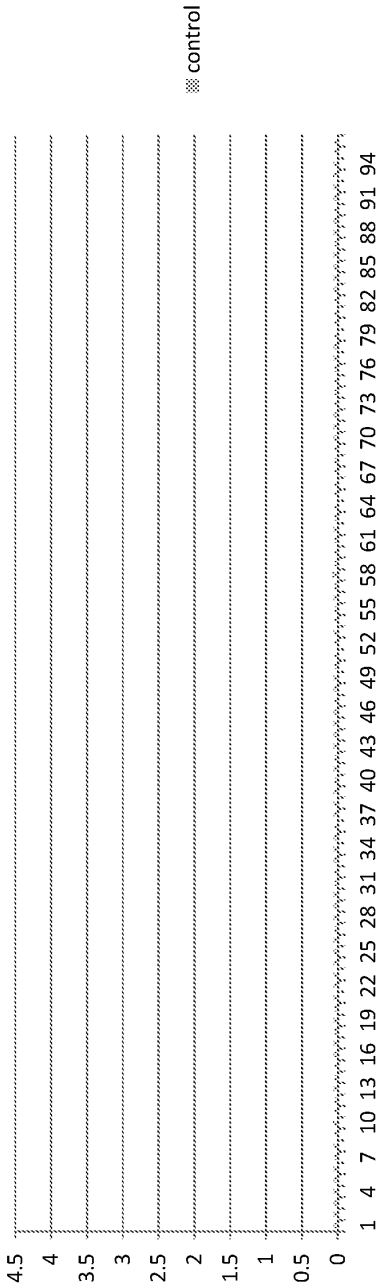

control
control
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94

Fig. 2B

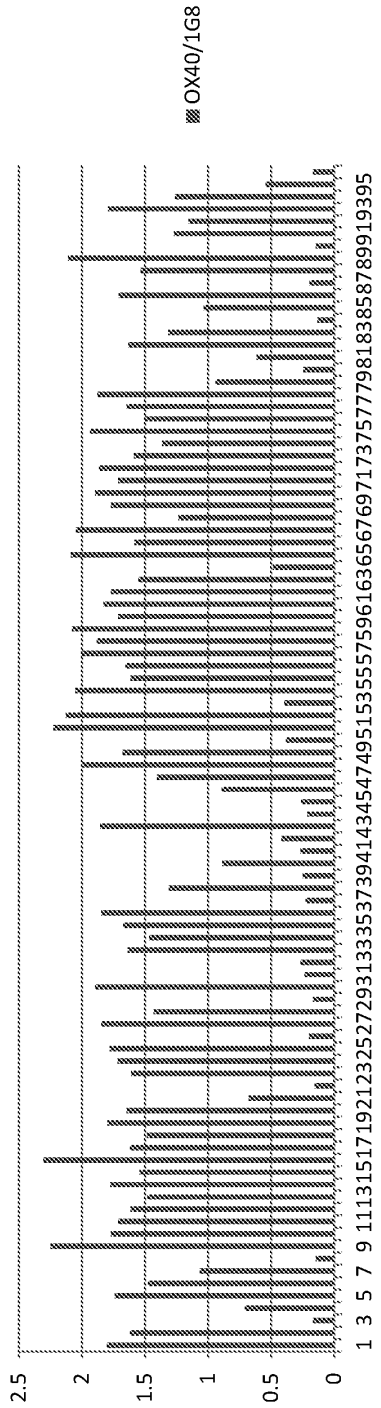

OX40/1G8
2.5
2
1.5
1
0.5
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65 67 69 71 73 75 77 79 81 83 85 87 89 91 93 95

Fig. 3A

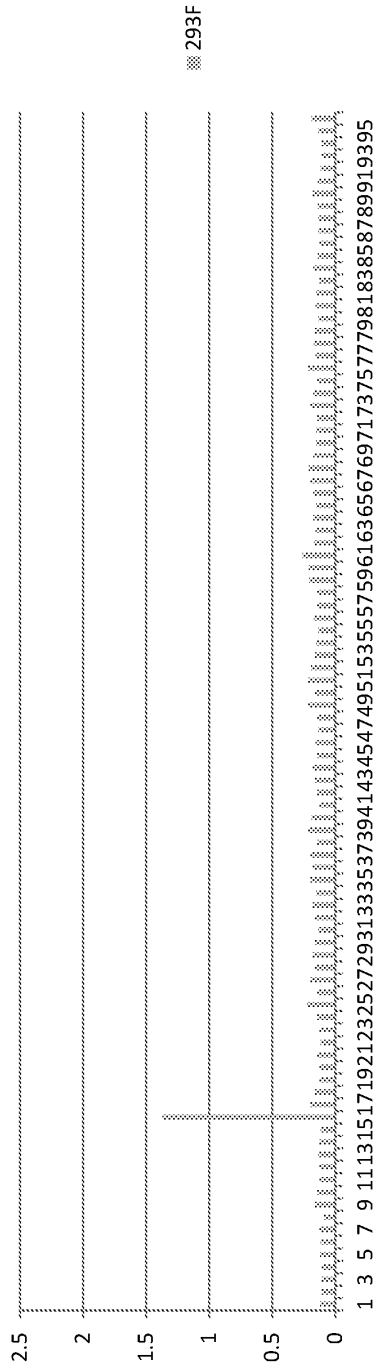

293F
2.5
2
1.5
1
0.5
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61 63 65 67 69 71 73 75 77 79 81 83 85 87 89 91 93 95

Fig. 3B

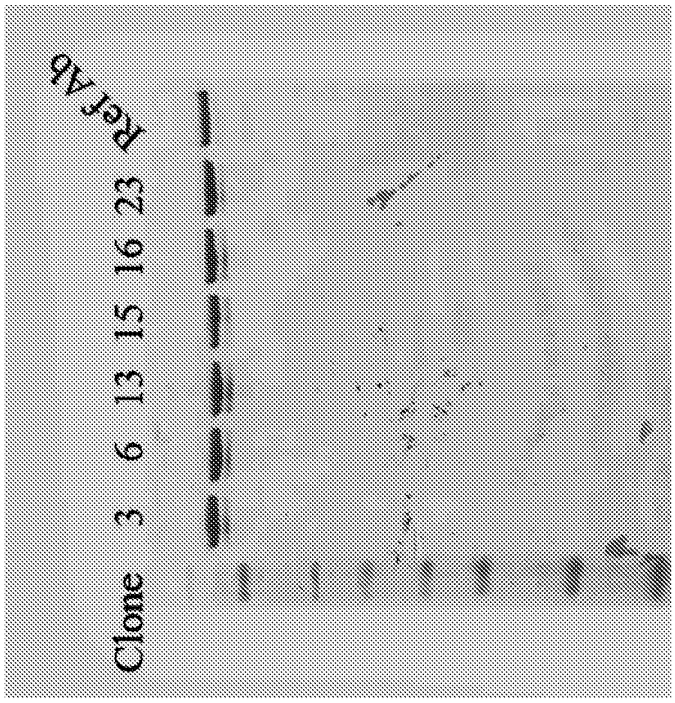


Fig. 4

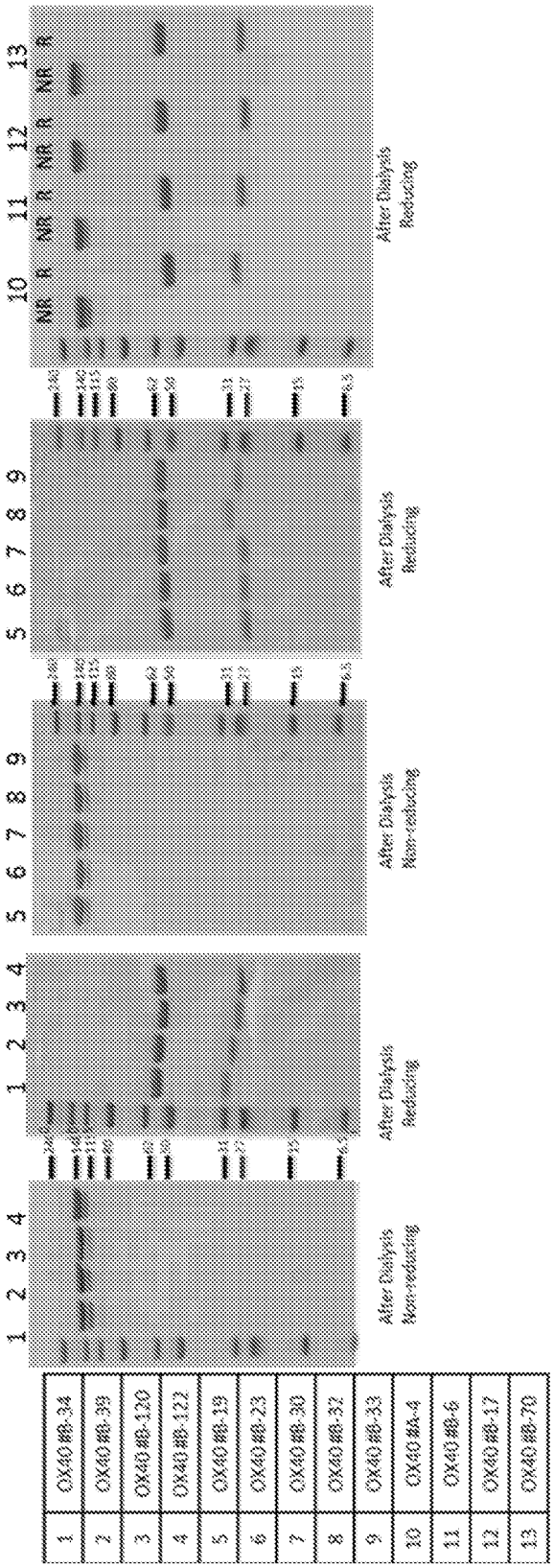

1 2 3 4
After Dialysis Non-reducing
1 2 3 4
After Dialysis Reducing
5 6 7 8 9
After Dialysis Non-reducing
5 6 7 8 9
After Dialysis Reducing
10 11 12 13
NR R NR R NR R NR R
After Dialysis Reducing
1 OX40 #B-34
2 OX40 #B-39
3 OX40 #B-120
4 OX40 #B-122
5 OX40 #B-19
6 OX40 #B-23
7 OX40 #B-30
8 OX40 #B-32
9 OX40 #B-33
10 OX40 #A-4
11 OX40 #B-6
12 OX40 #B-17
13 OX40 #B-70

Fig. 5

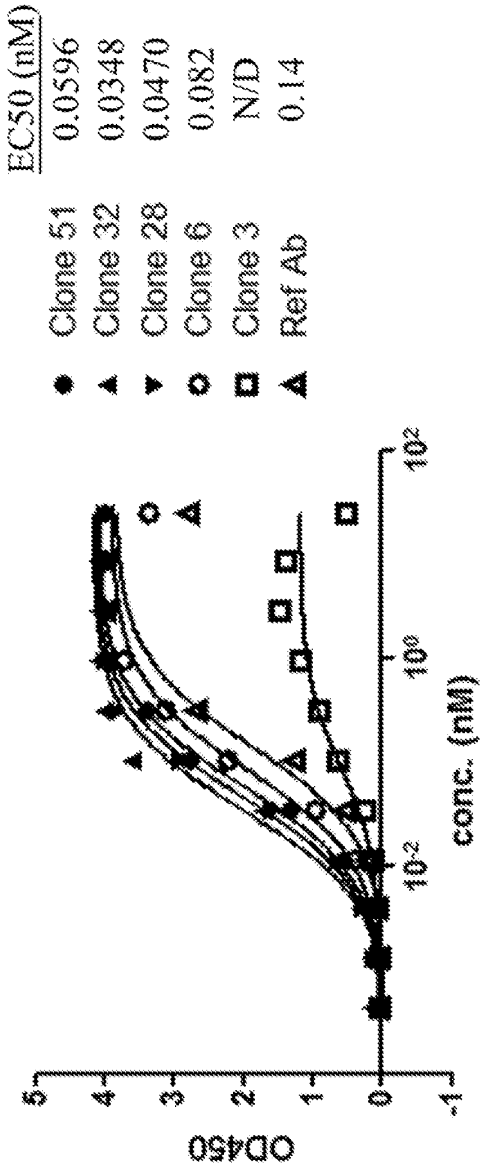

EC50 (nM)
Clone 51 0.0596
Clone 32 0.0348
Clone 28 0.0470
Clone 6 0.082
Clone 3 N/D
Ref Ab 0.14
OD450
conc. (nM)

Fig. 6

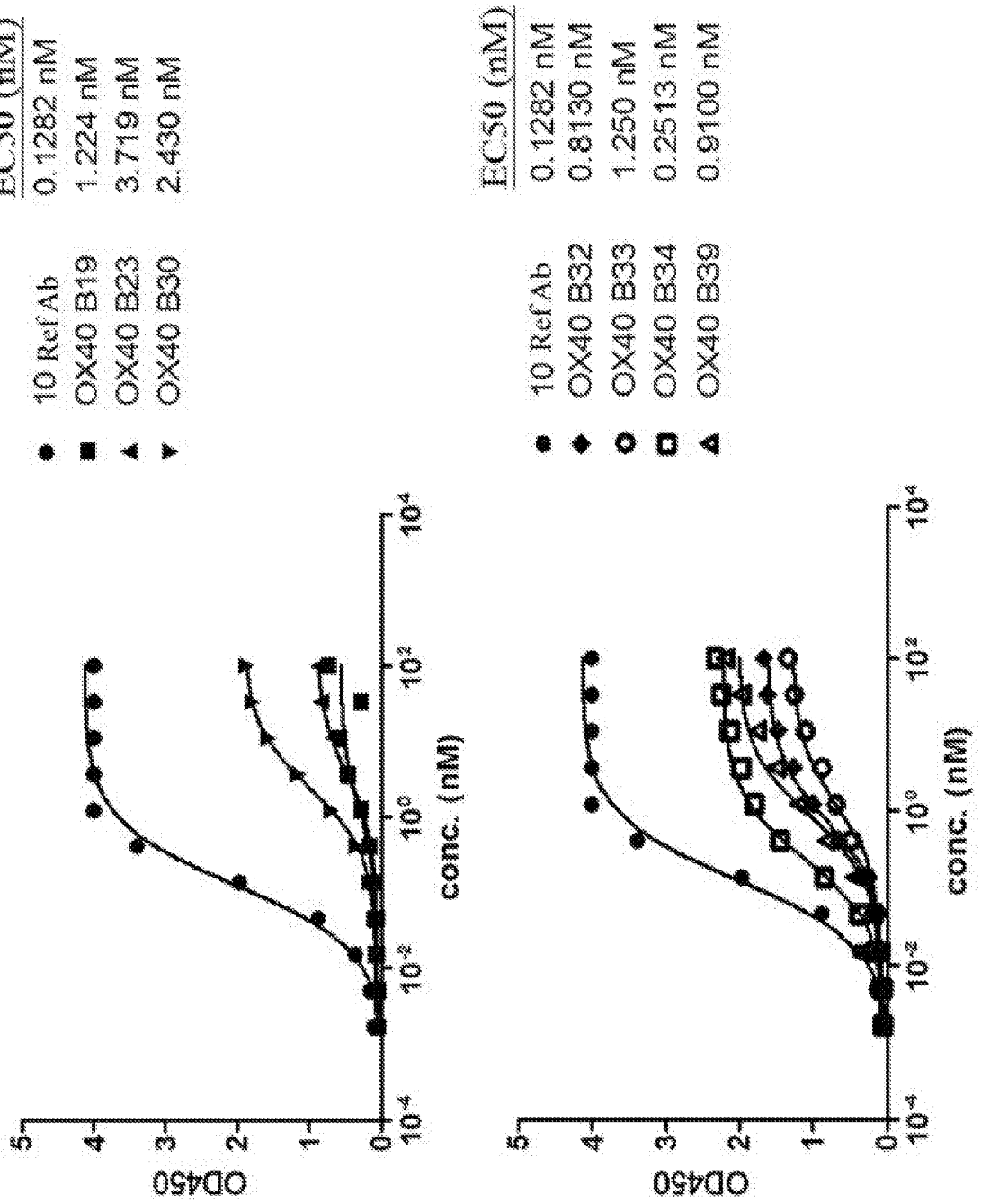

EC50 (nM)
10 Ref Ab 0.1282 nM
OX40 B19 1.224 nM
OX40 B23 3.719 nM
OX40 B30 2.430 nM
OD450
conc. (nM)
EC50 (nM)
10 Ref Ab 0.1282 nM
OX40 B32 0.8130 nM
OX40 B33 1.250 nM
OX40 B34 0.2513 nM
OX40 B39 0.9100 nM
OD450
conc. (nM)

Fig. 7

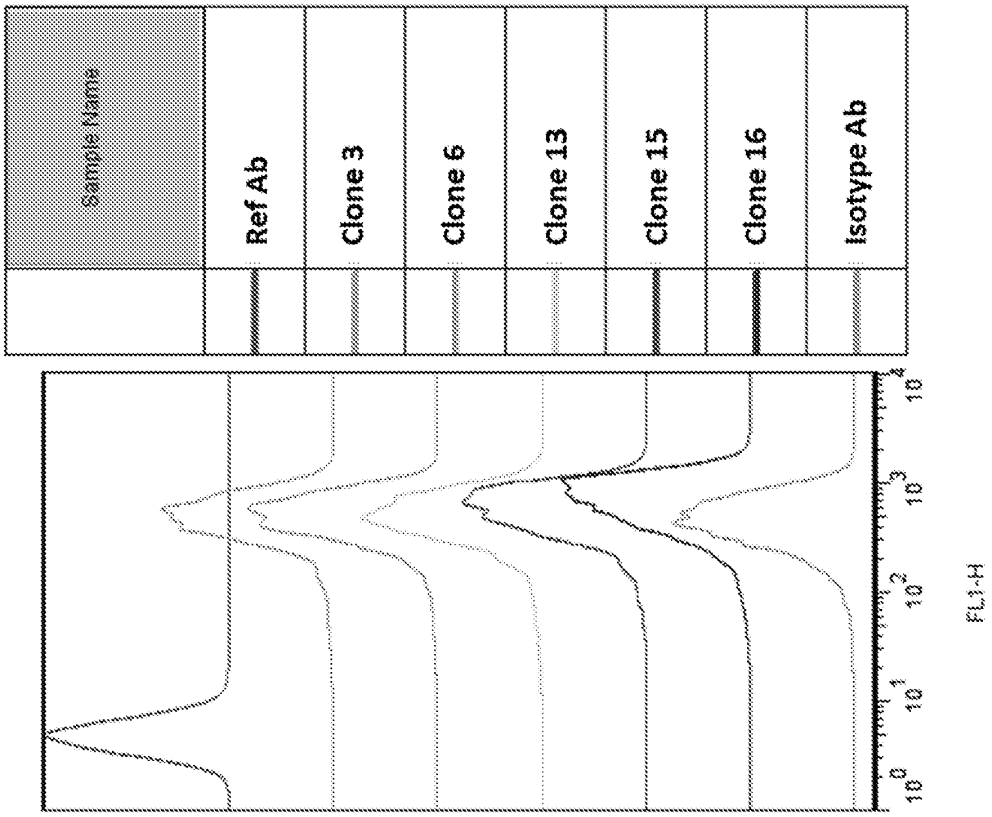

Sample Name
Ref Ab
Clone 3
Clone 6
Clone 13
Clone 15
Clone 16
Isotype Ab
FL1-H
10^0
10^1
10^2
10^3
10^4

Fig. 8

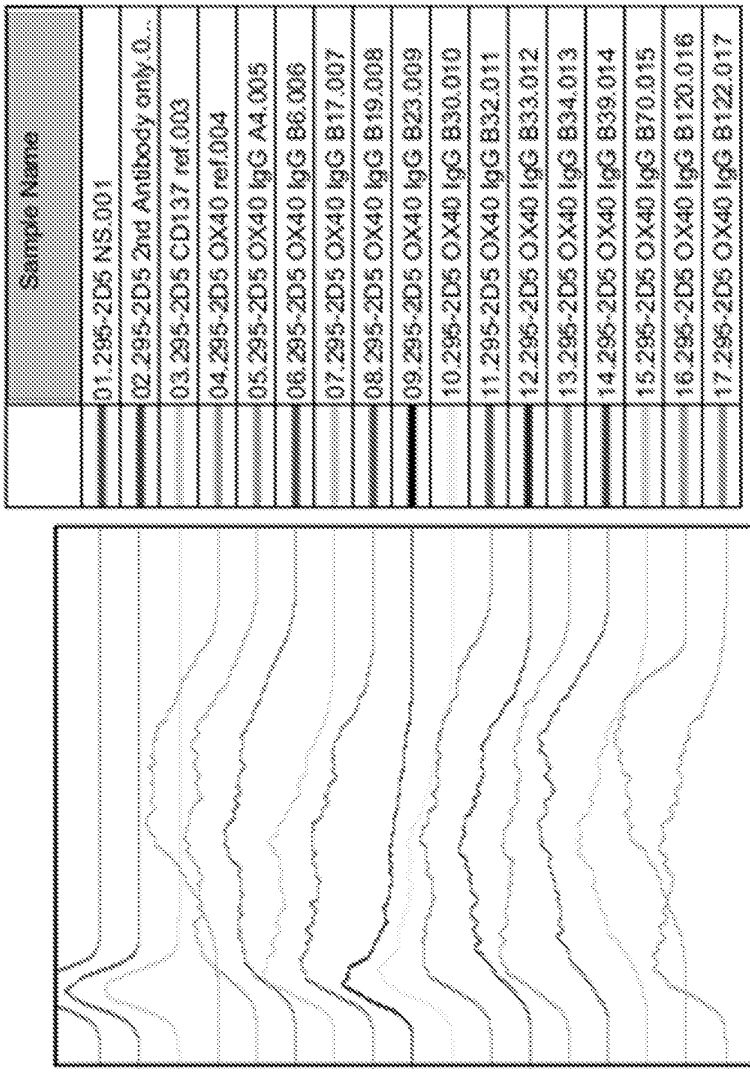

Sample Name
01.295-2D5 NS.001
02.295-2D5 2nd Antibody only.0...
03.295-2D5 CD137 ref.003
04.295-2D5 OX40 ref.004
05.295-2D5 OX40 IgG A4.005
06.295-2D5 OX40 IgG B6.006
07.295-2D5 OX40 IgG B17.007
08.295-2D5 OX40 IgG B19.008
09.295-2D5 OX40 IgG B23.009
10.295-2D5 OX40 IgG B30.010
11.295-2D5 OX40 IgG B32.011
12.295-2D5 OX40 IgG B33.012
13.295-2D5 OX40 IgG B34.013
14.295-2D5 OX40 IgG B39.014
15.295-2D5 OX40 IgG B70.015
16.295-2D5 OX40 IgG B120.016
17.295-2D5 OX40 IgG B122.017
10^0
10^1
10^2
10^3
10^4
FL1-H:: Anti-hIgG-A488

Fig. 9

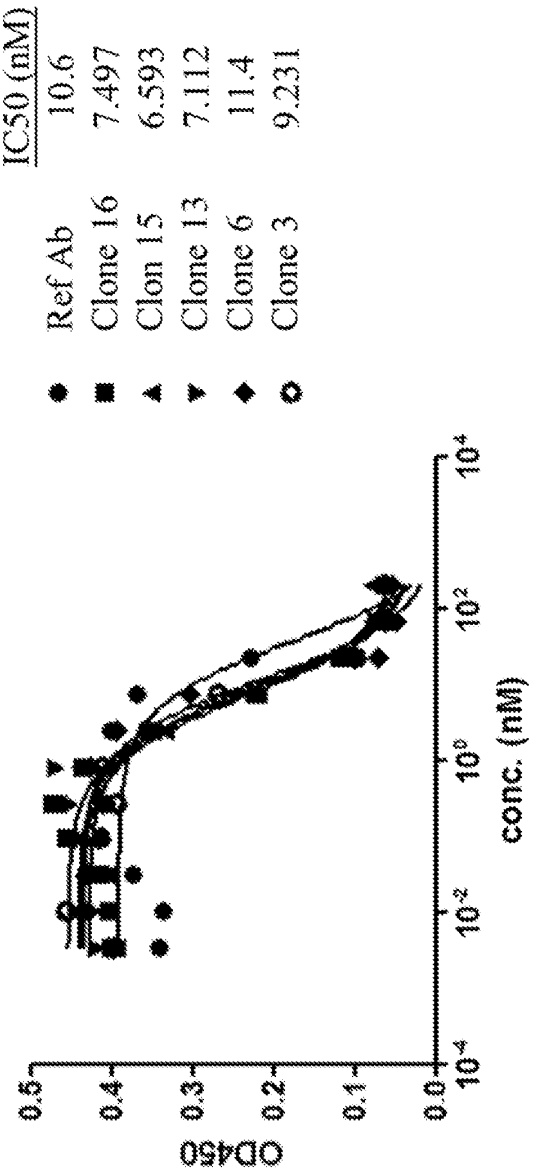

IC50 (nM)
10.6
7.497
6.593
7.112
11.4
9.231
Ref Ab
Clone 16
Clon 15
Clone 13
Clone 6
Clone 3
OD450
0.0
0.1
0.2
0.3
0.4
0.5
conc. (nM)
10^-4
10^-2
10^0
10^2
10^4

Fig. 10

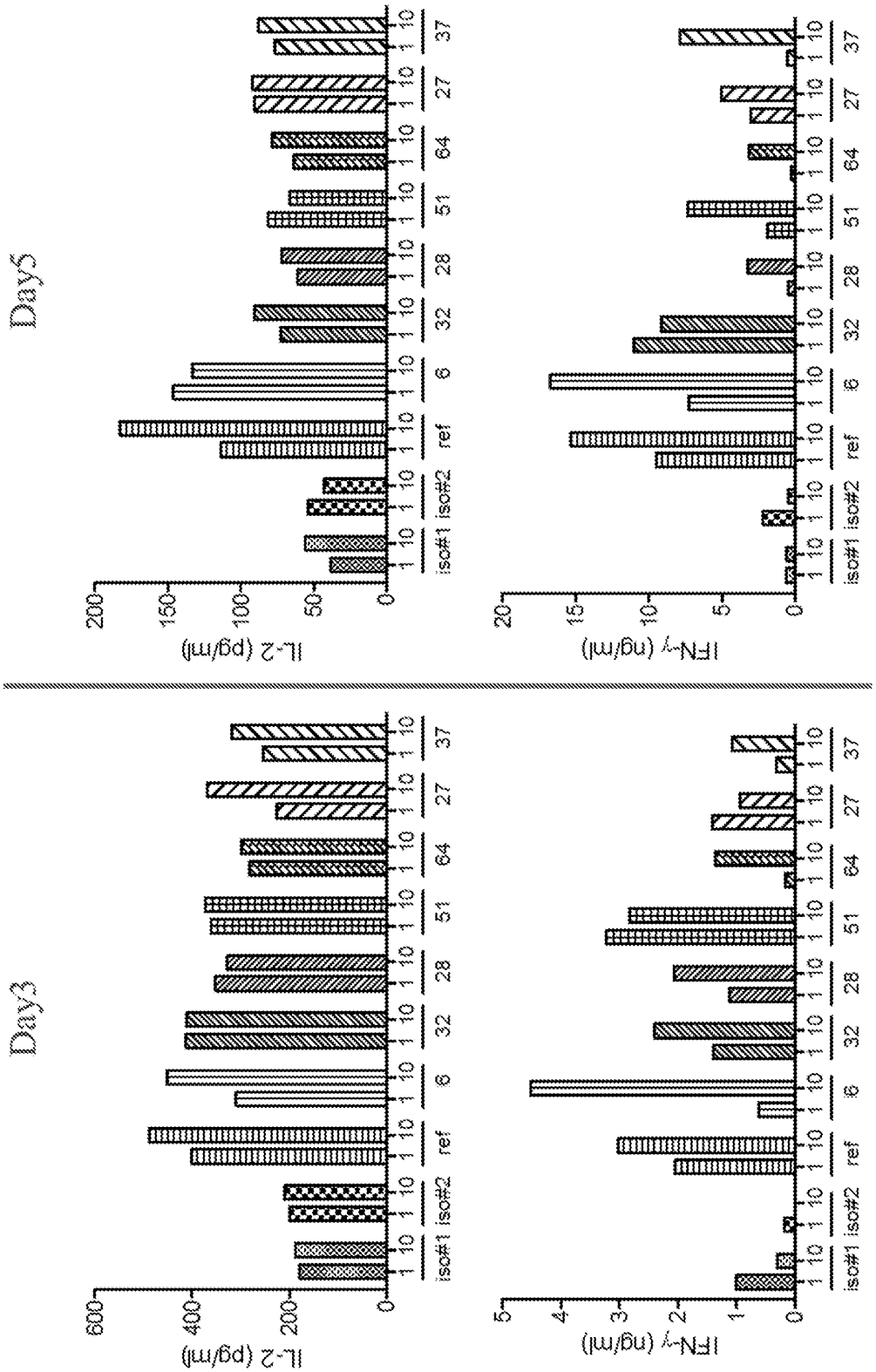


Fig. 11B

Fig. 11A

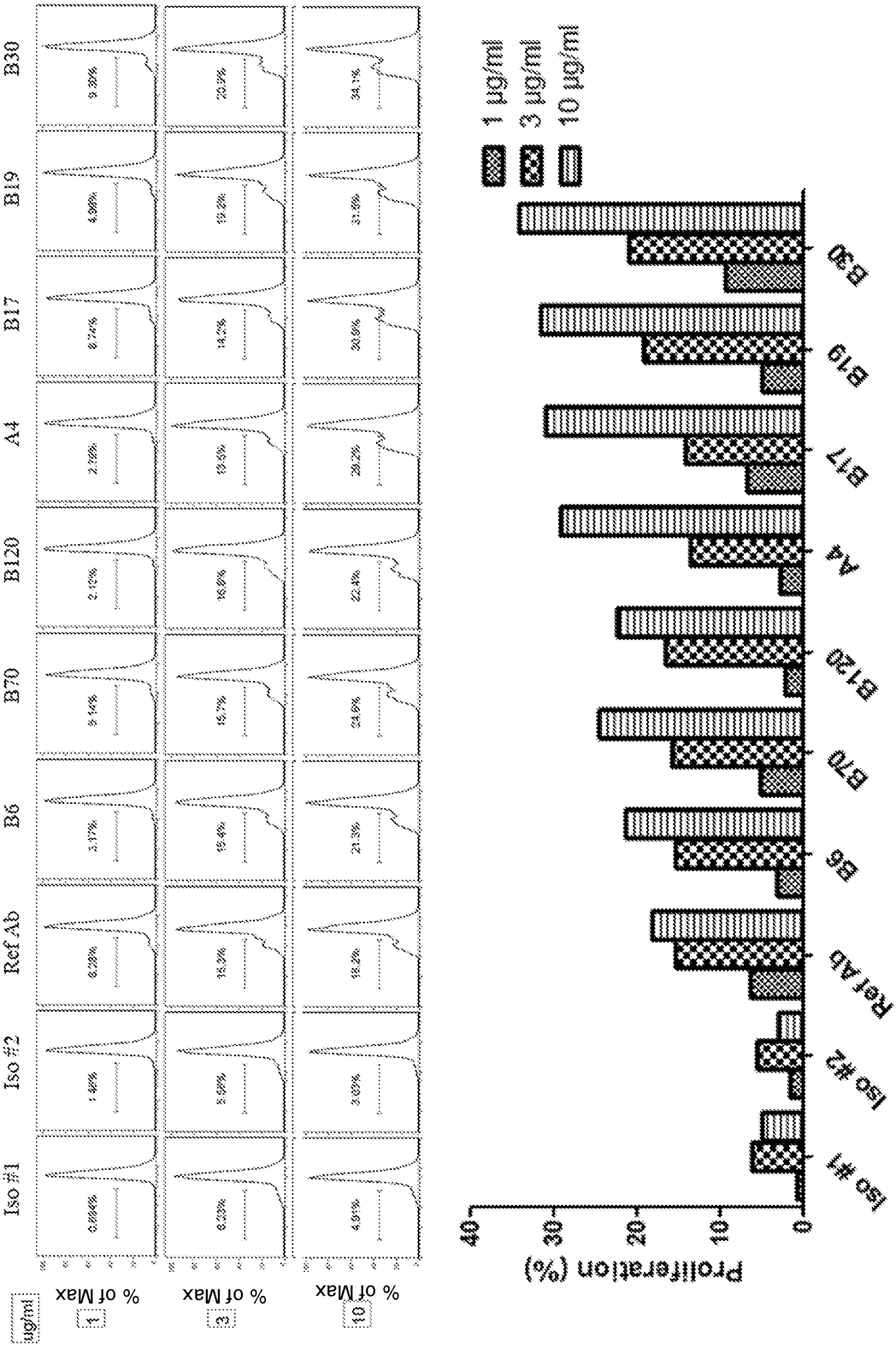

Iso #1
Iso #2
Ref Ab
B6
B70
B120
A4
B17
B19
B30
ug/ml
1
3
10
% of Max
% of Max
% of Max
1 µg/ml
3 µg/ml
10 µg/ml
Proliferation (%)
0
10
20
30
40
Iso #1
Iso #2
Ref Ab
B6
B70
B120
A4
B17
B19
B30

Fig. 12A

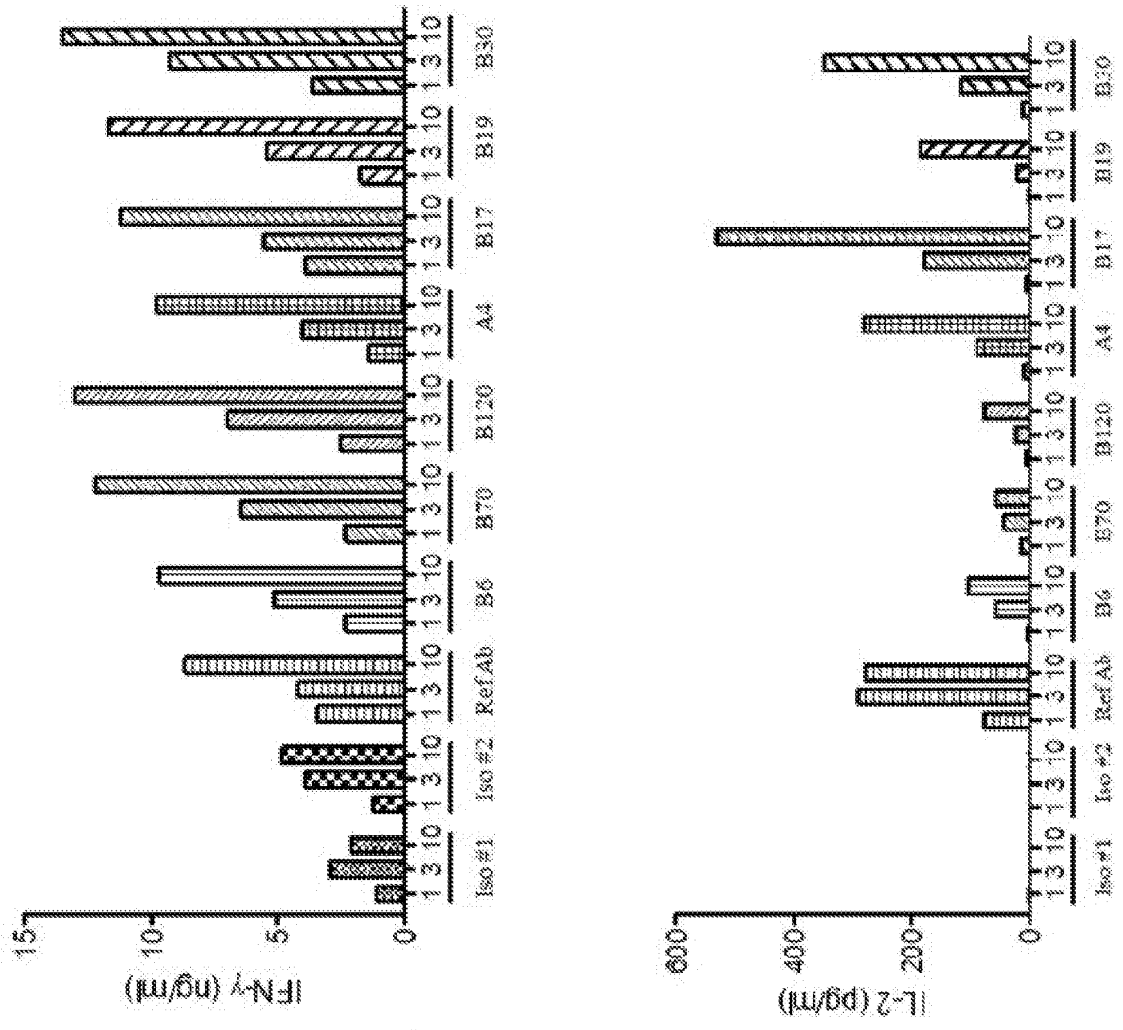

IFN-γ (ng/ml)
0
5
10
15
IL-2 (pg/ml)
0
200
400
600
Iso #1
Iso #2
Ref Ab
B6
B70
B120
A4
B17
B19
B30
1
3
10

Fig. 12B

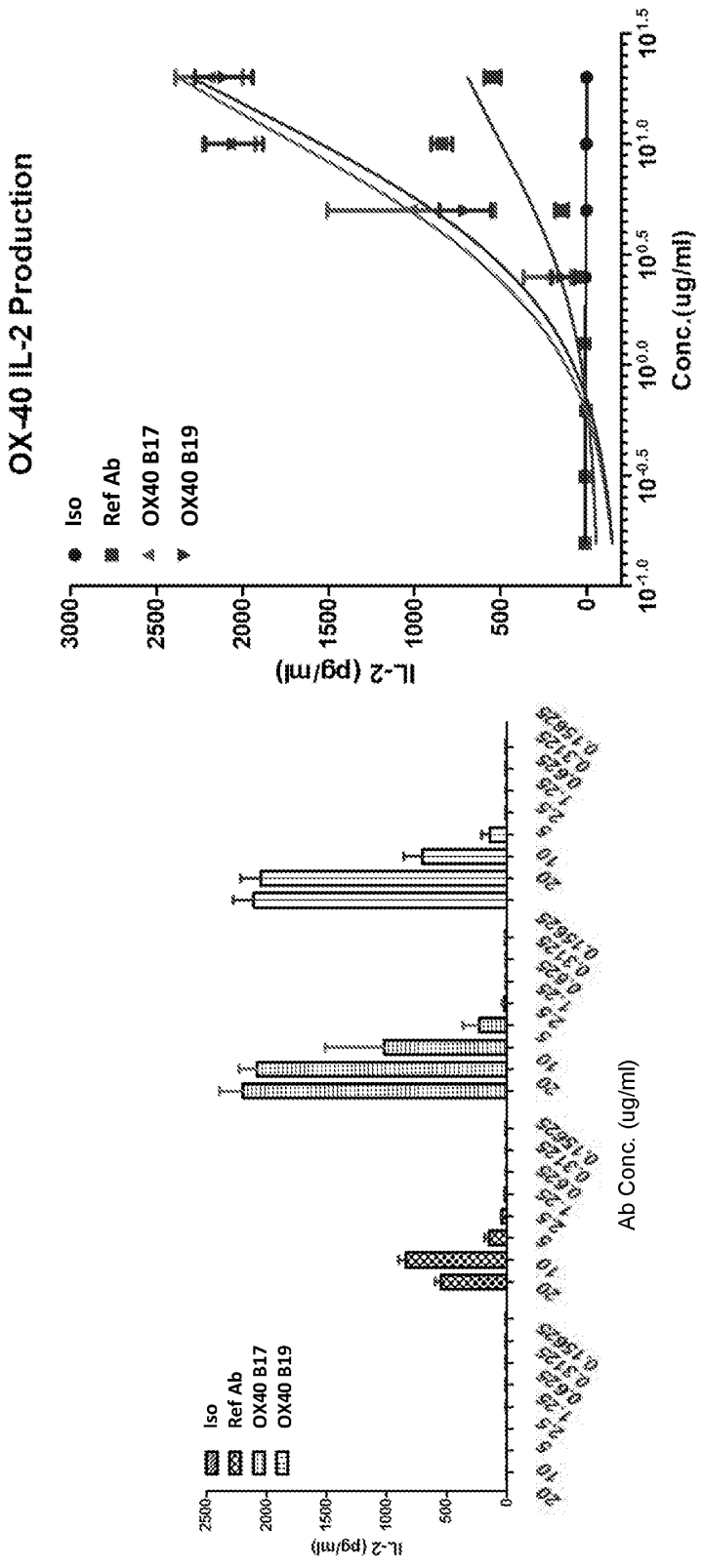

OX-40 IL-2 Production
Iso
Ref Ab
OX40 B17
OX40 B19
IL-2 (pg/ml)
Conc.(ug/ml)
Ab Conc. (ug/ml)

Fig. 13A

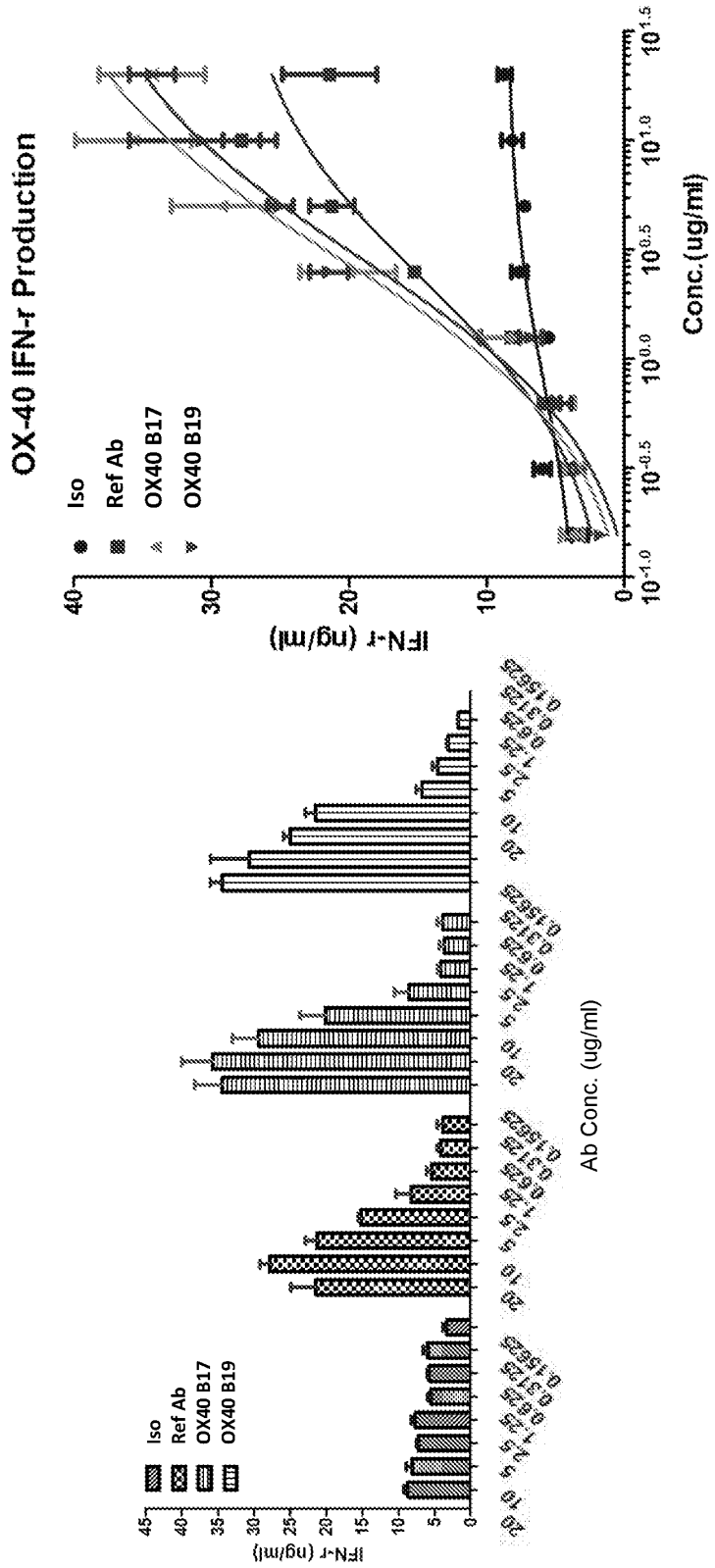

OX-40 IFN-r Production
Iso
Ref Ab
OX40 B17
OX40 B19
IFN-r (ng/ml)
Conc.(ug/ml)
Ab Conc. (ug/ml)

Fig. 13B

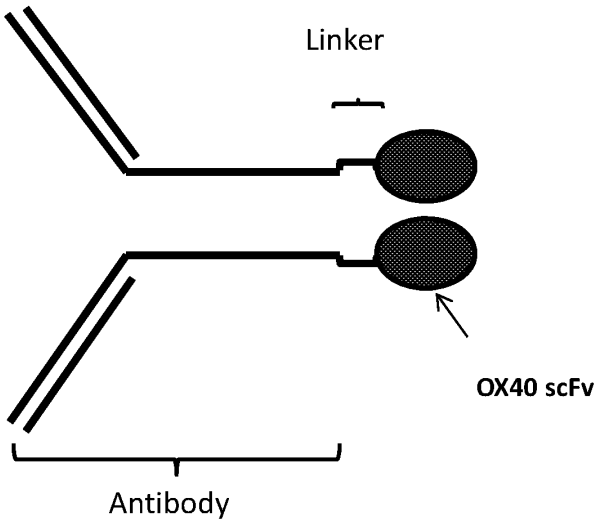


Fig. 14

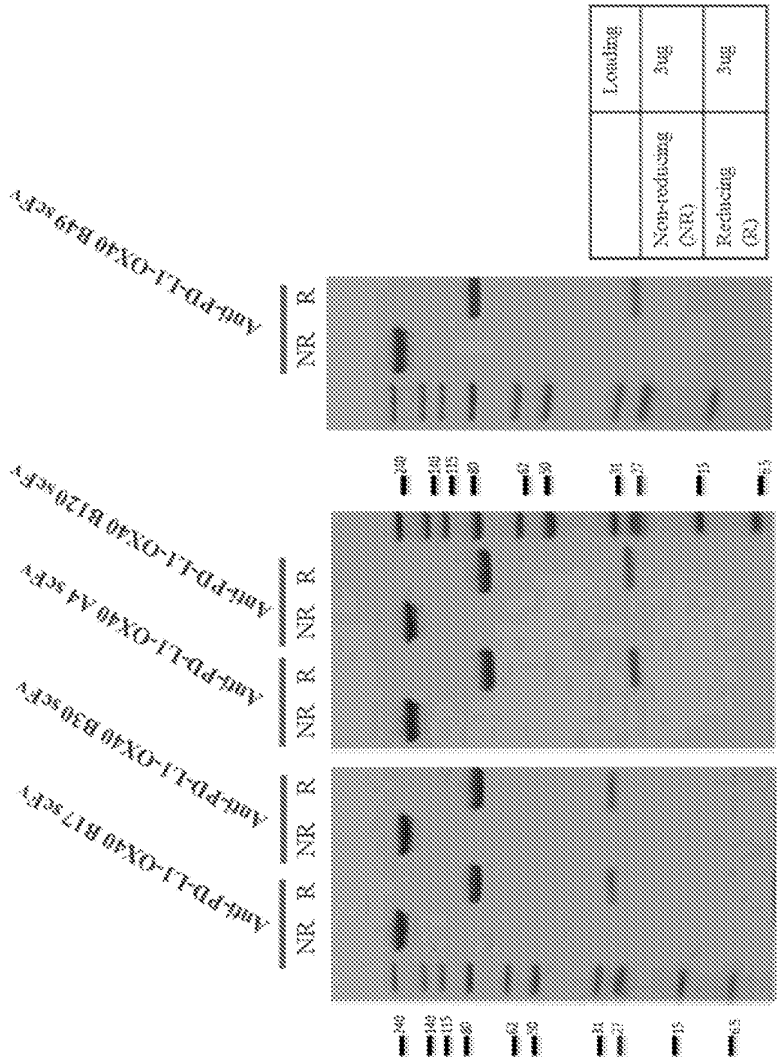

Loading
3ug
3ug
Non-reducing (NR)
Reducing (R)
Anti-PD-L1-OX40 B49 scFv
Anti-PD-L1-OX40 B120 scFv
Anti-PD-L1-OX40 A4 scFv
Anti-PD-L1-OX40 B30 scFv
Anti-PD-L1-OX40 B17 scFv
NR
R
kDa

Fig. 15

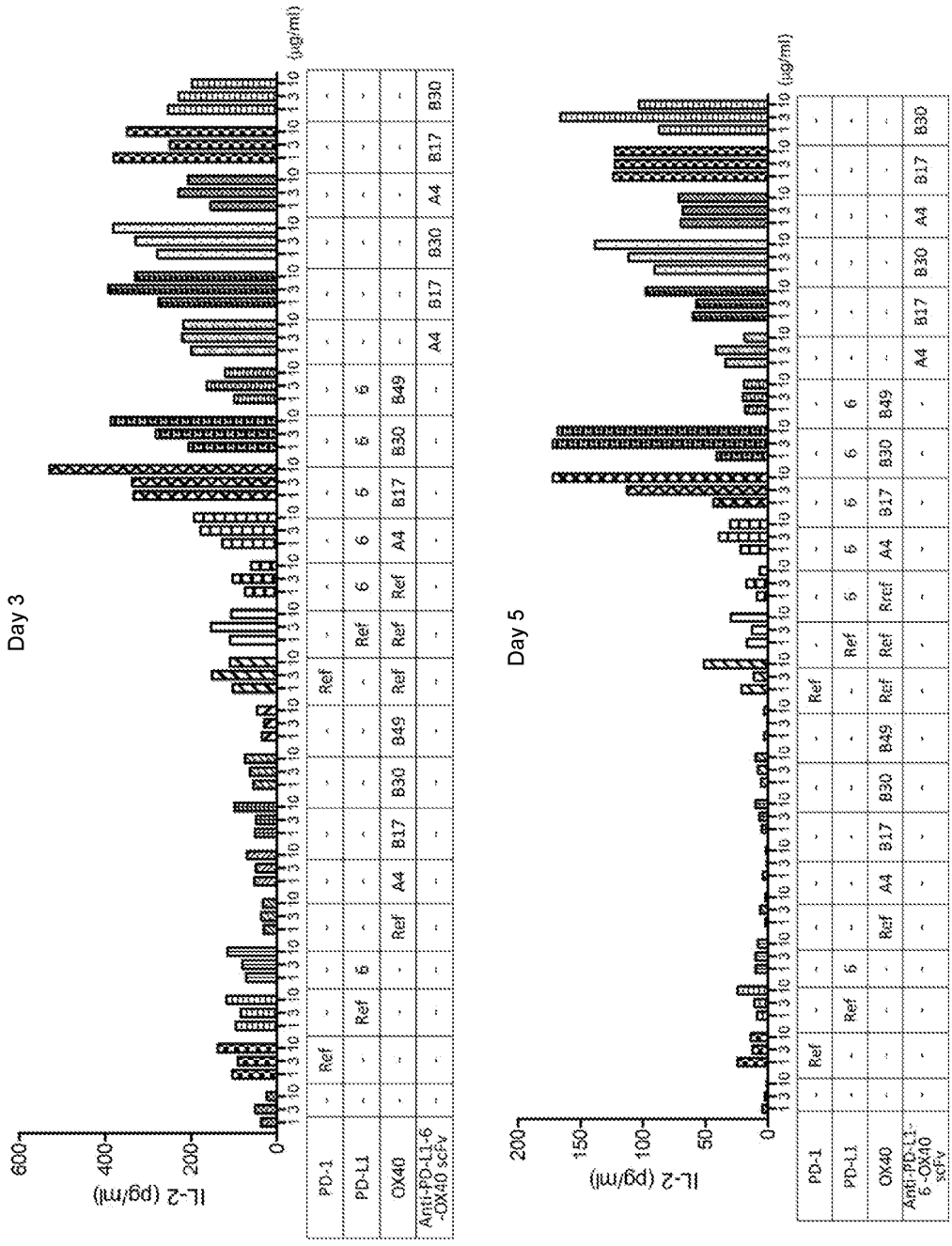

Day 3
IL-2 (pg/ml)
600
400
200
0
(μg/ml)
PD-1
PD-L1
OX40
Anti-PD-L1-6-OX40 scFv
Day 5
IL-2 (pg/ml)
200
150
100
50
0
(μg/ml)
PD-1
PD-L1
OX40
Anti-PD-L1-6-OX40 scFv

Fig. 16A

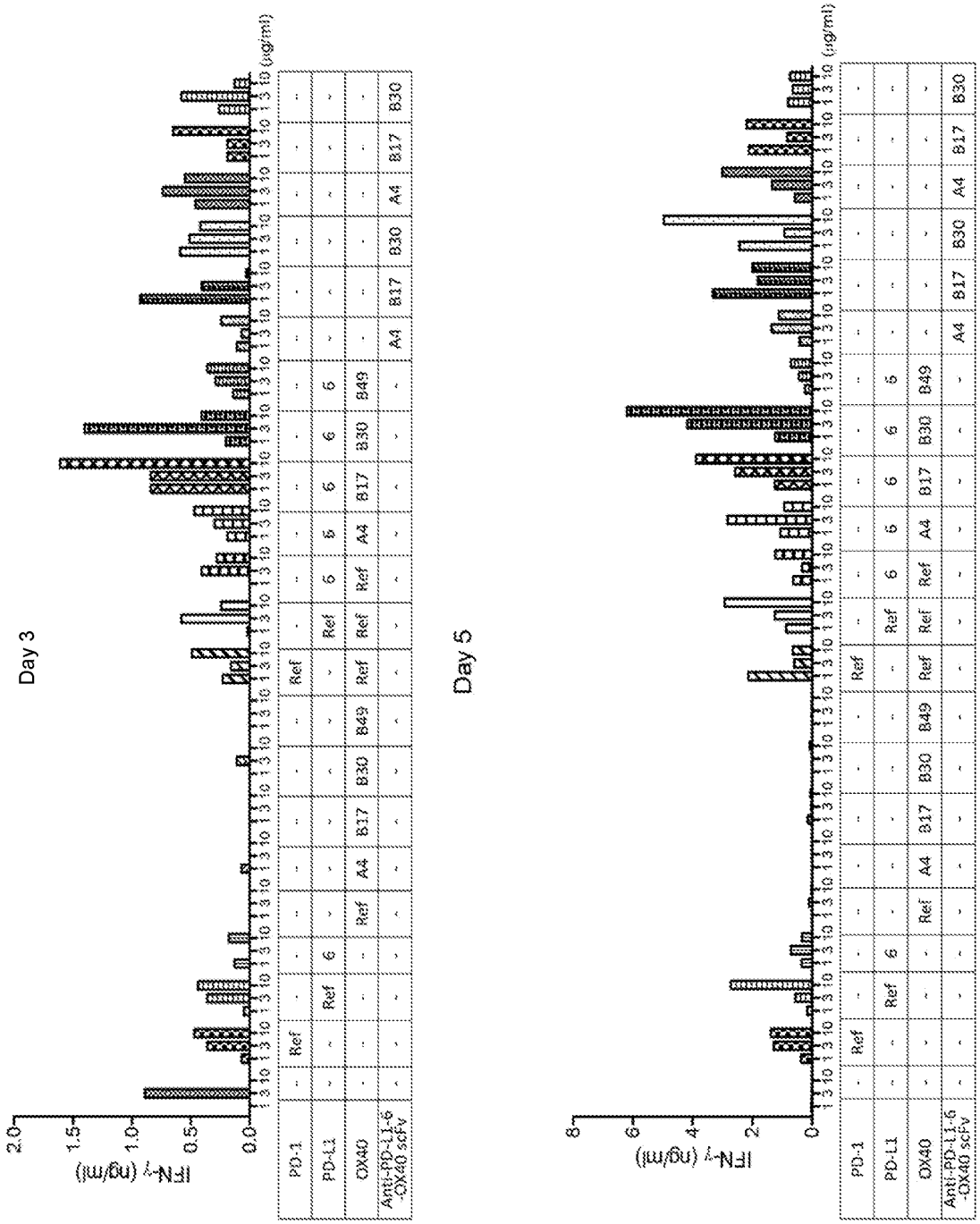

Day 3
Day 5
IFN-γ (ng/ml)
PD-1
PD-L1
OX40
Anti-PD-L1-6-OX40 scFv
Ref
A4
B17
B30
B49
6
(μg/ml)

Fig. 16B

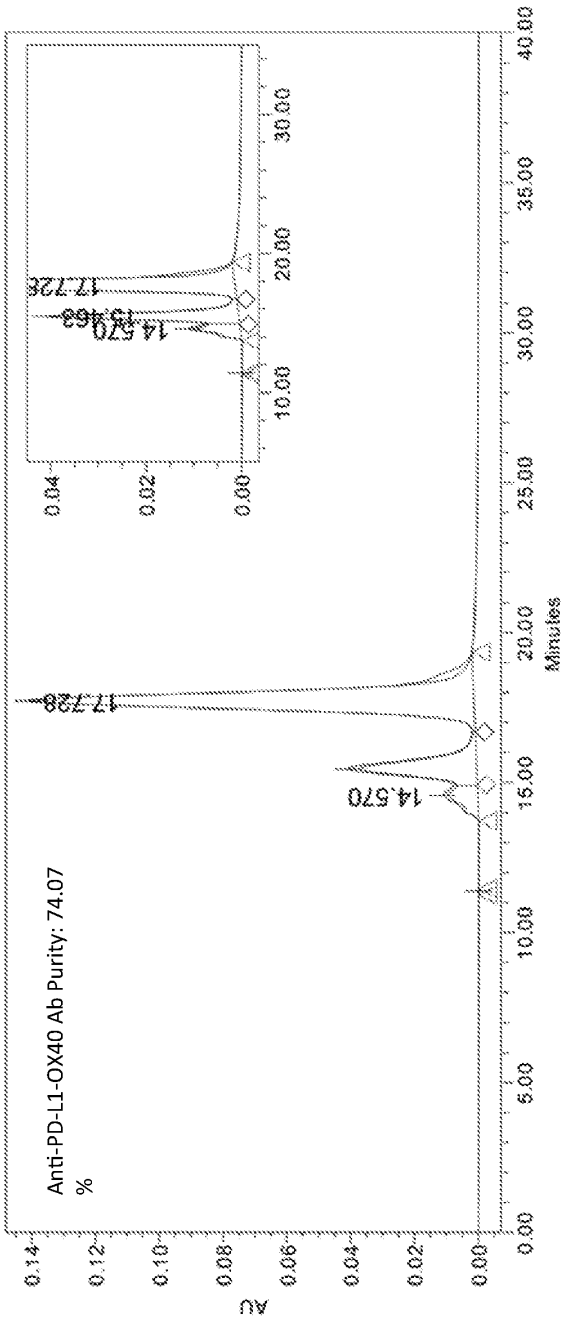

Anti-PD-L1-OX40 Ab Purity: 74.07
%
14.570
17.728
14.570
15.463
17.726
AU
Minutes

Fig. 17A

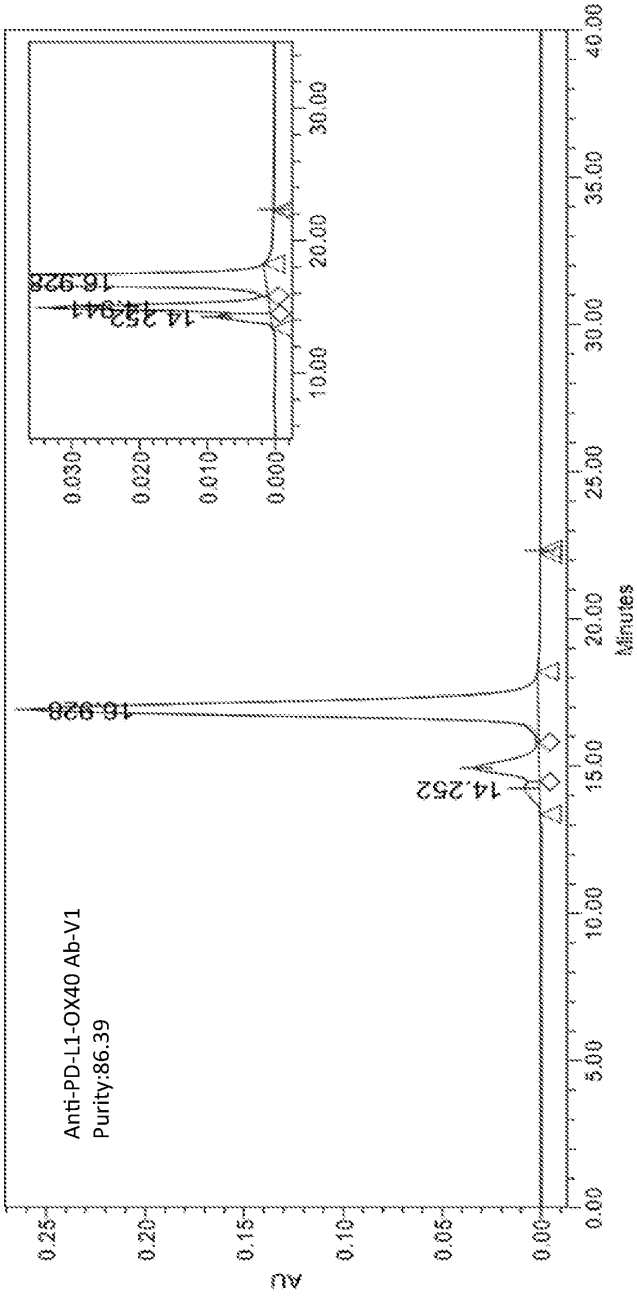


Fig. 17B

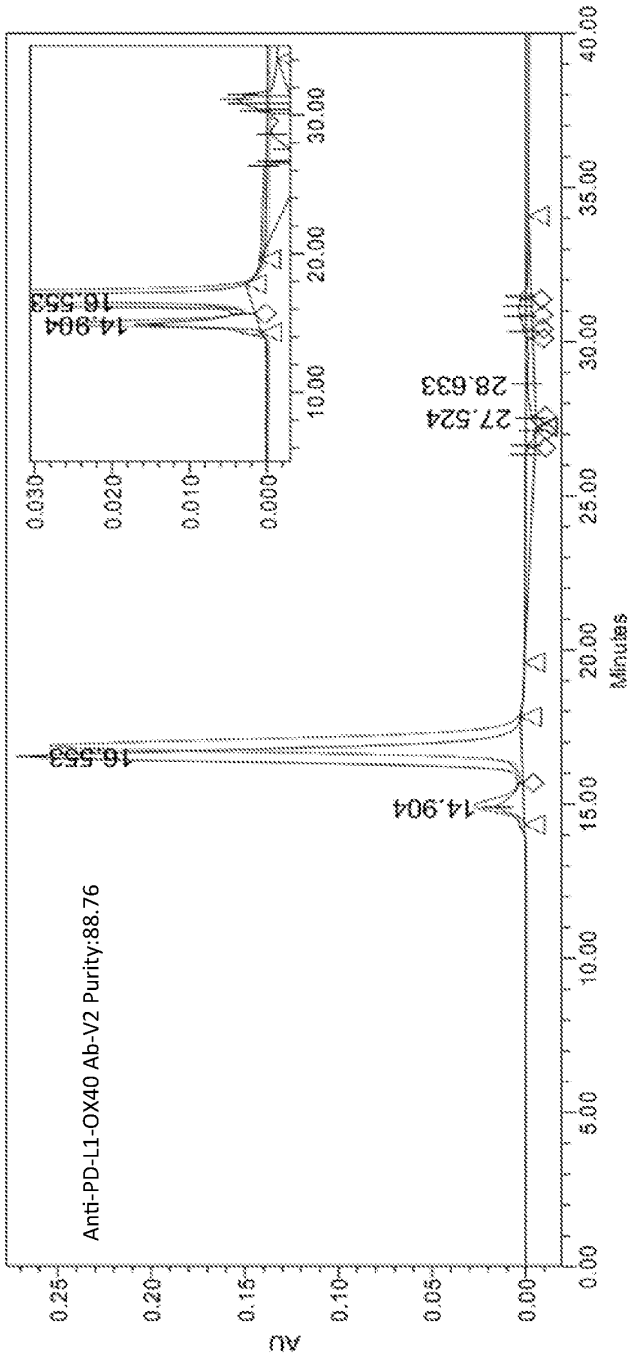

Anti-PD-L1-OX40 Ab-V2 Purity:88.76
AU
Minutes
16.553
14.904
27.524
28.633
0.25
0.20
0.15
0.10
0.05
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
0.030
0.020
0.010
0.000

Fig. 17C

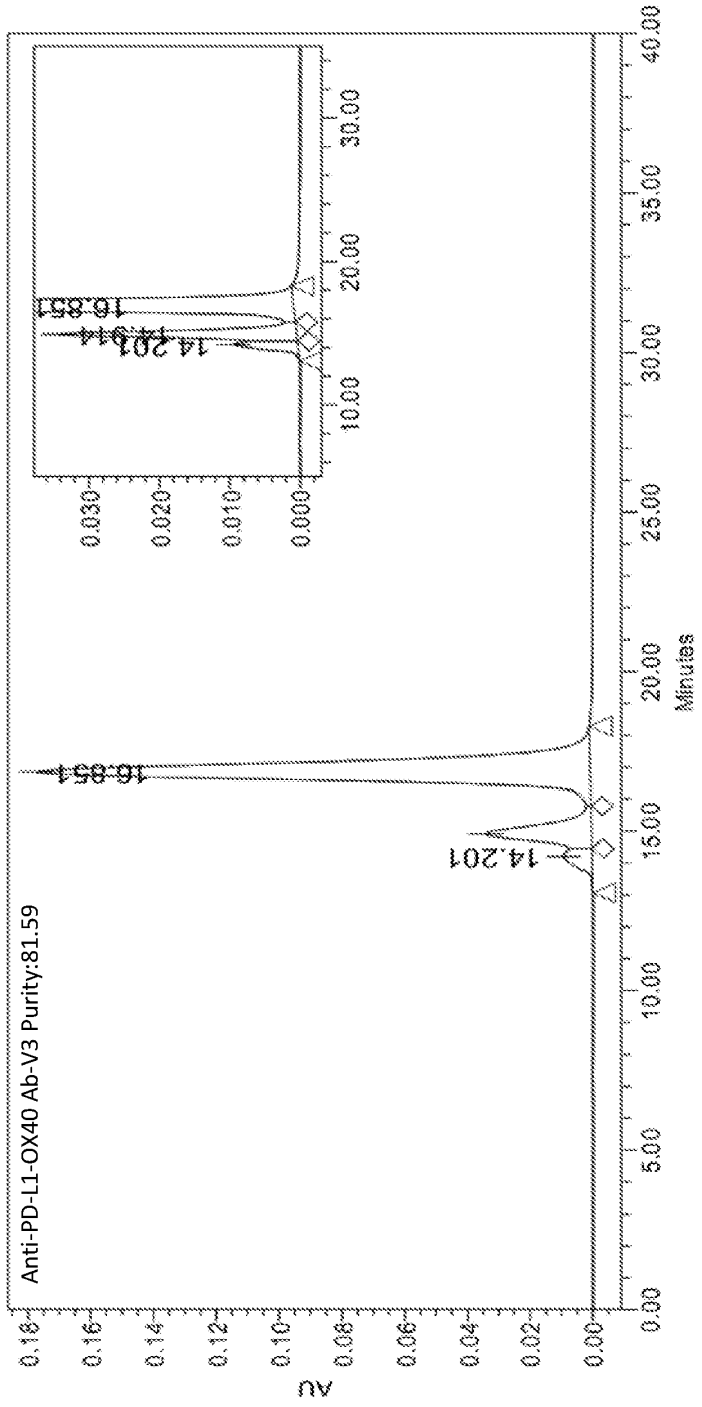


Fig. 17D

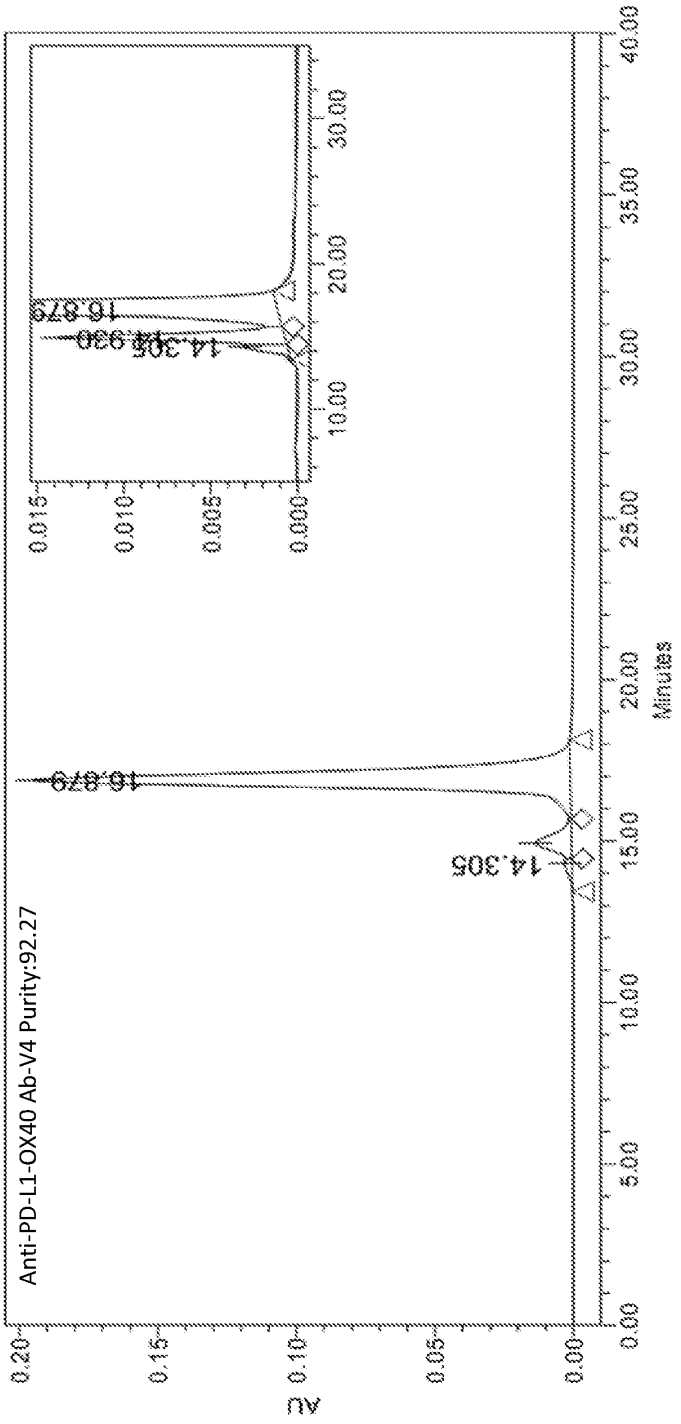

Anti-PD-L1-OX40 Ab-V4 Purity:92.27
16.879
14.305
AU
Minutes
0.20
0.15
0.10
0.05
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
0.015
0.010
0.005
0.000
10.00
20.00
30.00

Fig. 17E

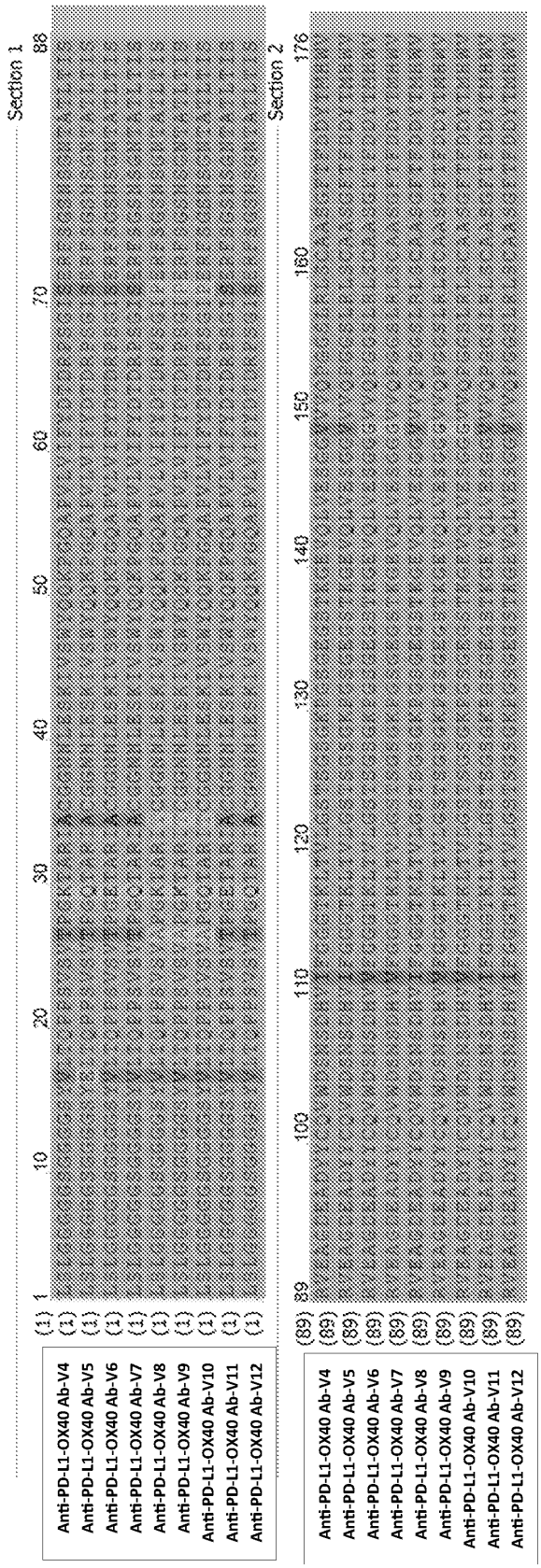

Section 1
Section 2
Anti-PD-L1-OX40 Ab-V4
Anti-PD-L1-OX40 Ab-V5
Anti-PD-L1-OX40 Ab-V6
Anti-PD-L1-OX40 Ab-V7
Anti-PD-L1-OX40 Ab-V8
Anti-PD-L1-OX40 Ab-V9
Anti-PD-L1-OX40 Ab-V10
Anti-PD-L1-OX40 Ab-V11
Anti-PD-L1-OX40 Ab-V12

Fig. 18

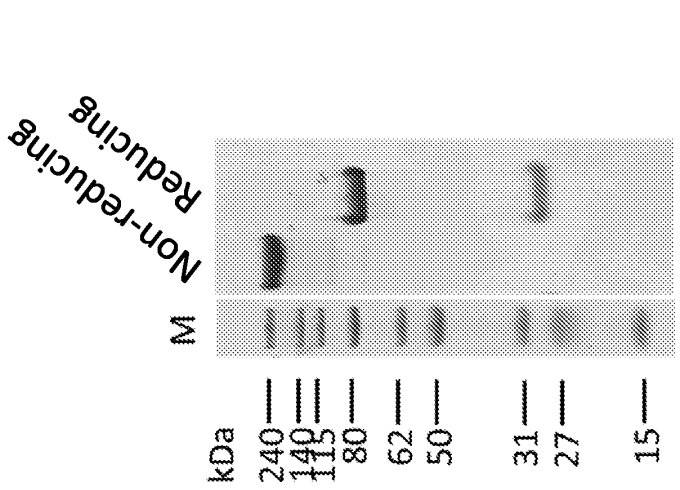

Anti-PD-L1-OX40 Ab-V5
Non-reducing
Reducing
M
kDa
240
140
115
80
62
50
31
27
15

Fig. 19

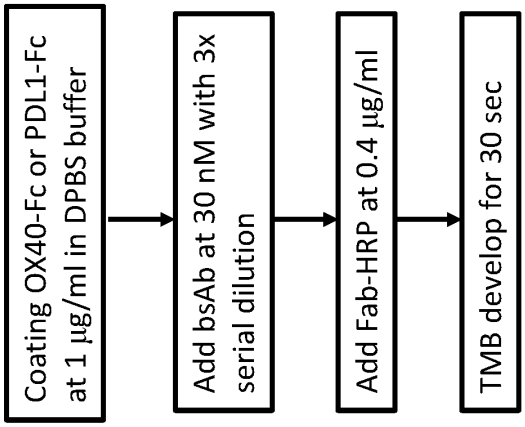

Coating OX40-Fc or PDL1-Fc at 1 µg/ml in DPBS buffer
Add bsAb at 30 nM with 3x serial dilution
Add Fab-HRP at 0.4 µg/ml
TMB develop for 30 sec

Fig. 20

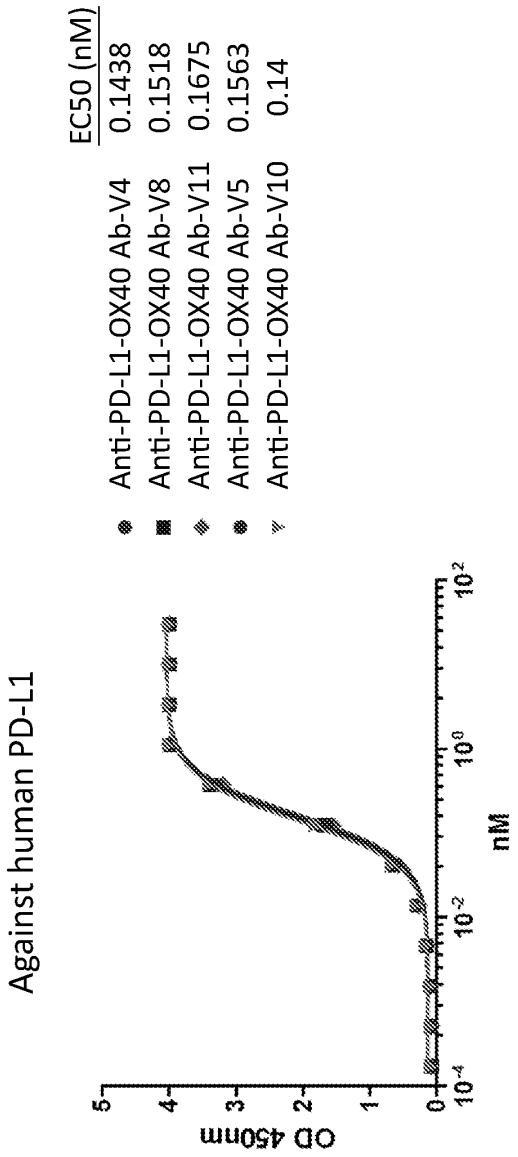

Against human PD-L1
EC50 (nM)
Anti-PD-L1-OX40 Ab-V4 0.1438
Anti-PD-L1-OX40 Ab-V8 0.1518
Anti-PD-L1-OX40 Ab-V11 0.1675
Anti-PD-L1-OX40 Ab-V5 0.1563
Anti-PD-L1-OX40 Ab-V10 0.14
OD 450nm
0
1
2
3
4
5
10-4
10-2
100
102
nM

Fig. 21

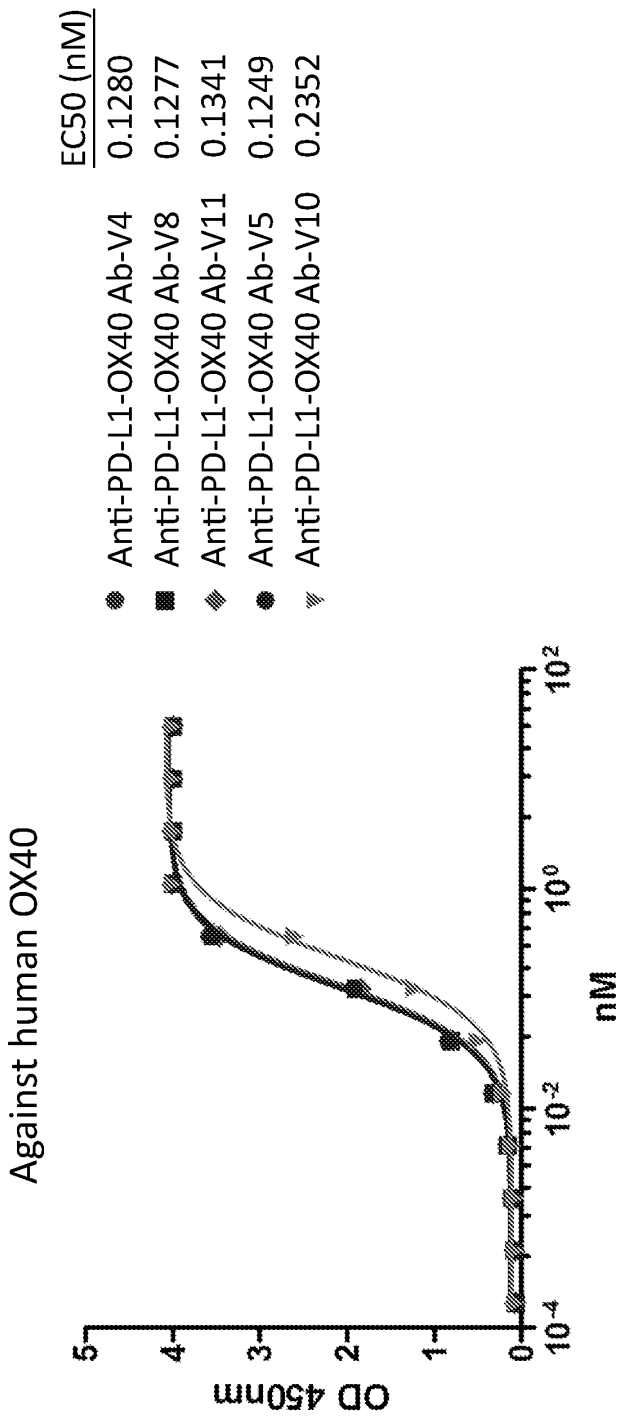

EC50 (nM)
Anti-PD-L1-OX40 Ab-V4 0.1280
Anti-PD-L1-OX40 Ab-V8 0.1277
Anti-PD-L1-OX40 Ab-V11 0.1341
Anti-PD-L1-OX40 Ab-V5 0.1249
Anti-PD-L1-OX40 Ab-V10 0.2352
Against human OX40
OD 450nm
nM

Fig. 22

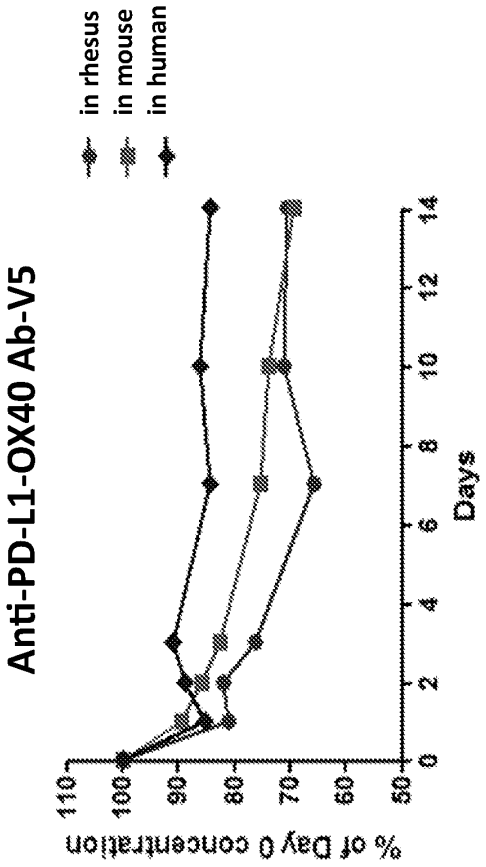

Anti-PD-L1-OX40 Ab-V5
in rhesus
in mouse
in human
% of Day 0 concentration
110
100
90
80
70
60
50
0
2
4
6
8
10
12
14
Days

Fig. 23

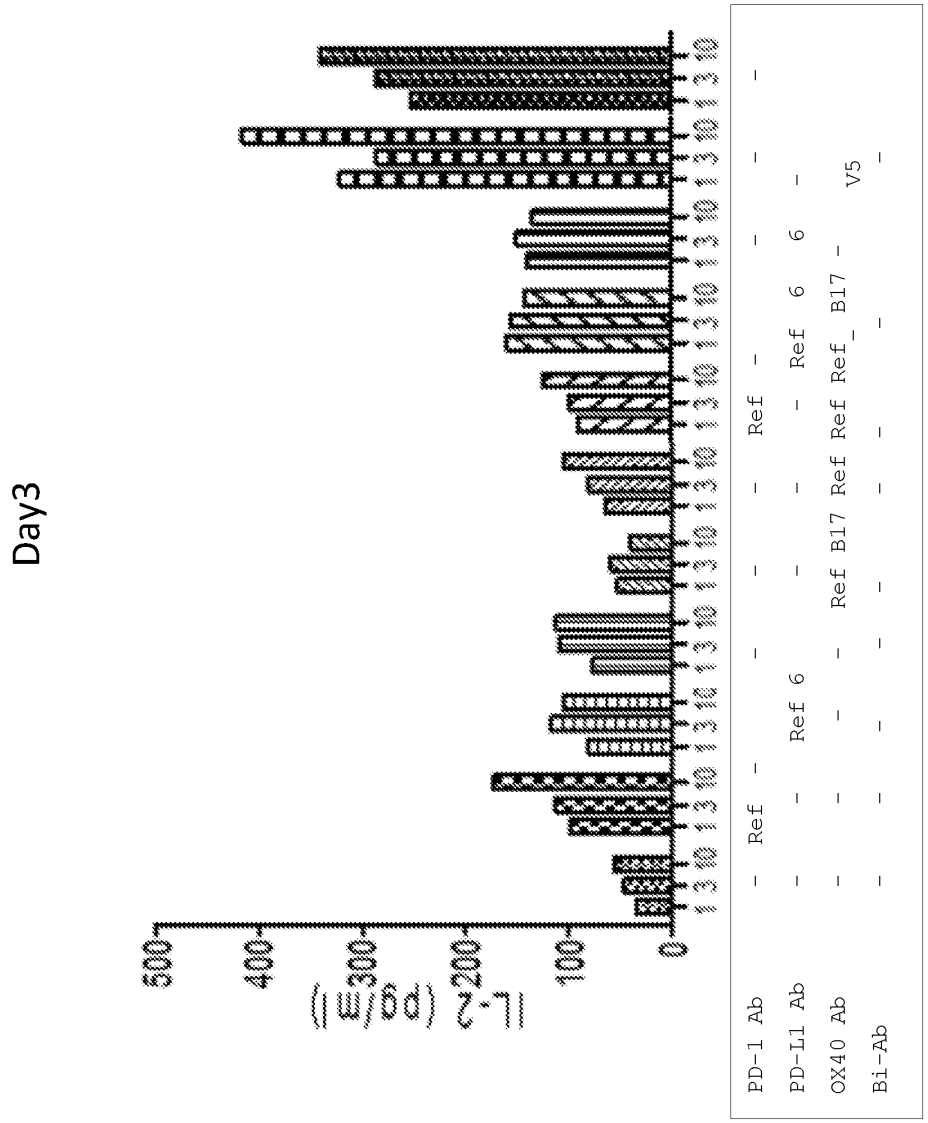

Day3
IL-2 (pg/ml)
0
100
200
300
400
500
PD-1 Ab
PD-L1 Ab
OX40 Ab
Bi-Ab
Ref
Ref 6
Ref B17
Ref Ref
Ref_ B17
V5

Fig. 24A

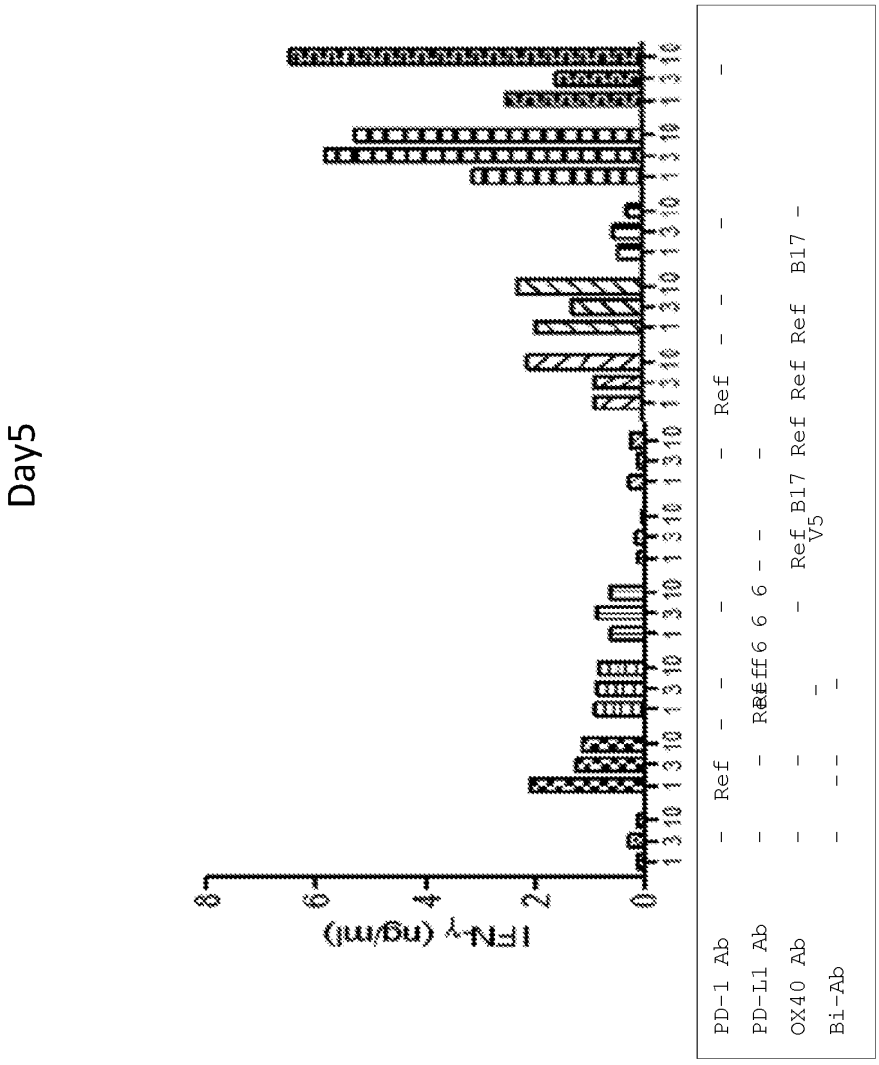

Day5
IFN-γ (ng/ml)
0
2
4
6
8
1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10 1 3 10
PD-1 Ab - Ref - - - - - Ref - - - -
PD-L1 Ab - - Ref Ref 6 6 6 6 - - -
OX40 Ab - - - - Ref B17 Ref Ref Ref B17 -
V5
Bi-Ab - - - -

Fig. 24B

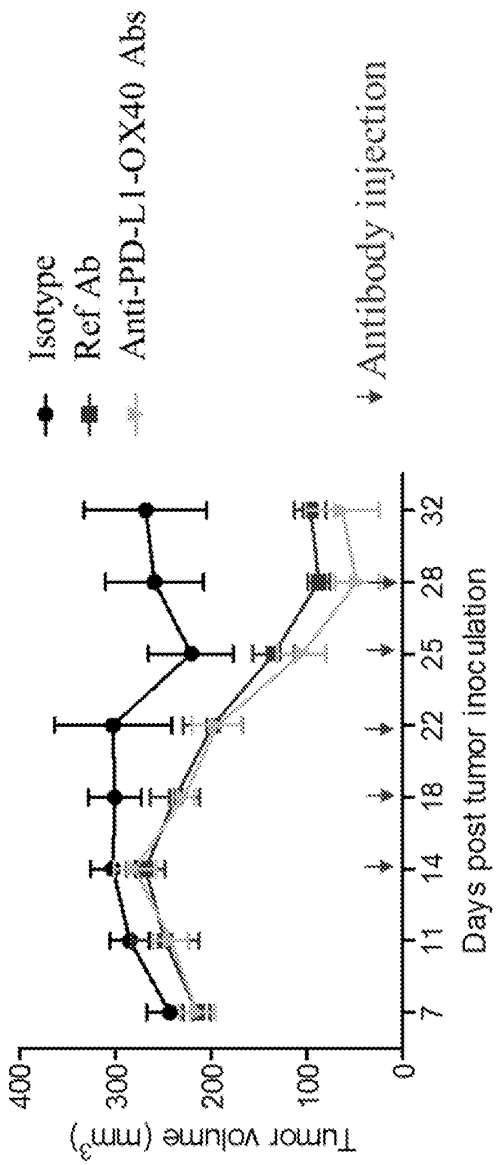

Isotype
Ref Ab
Anti-PD-L1-OX40 Abs
Antibody injection
Tumor volume (mm3)
400
300
200
100
0
7
11
14
18
22
25
28
32
Days post tumor inoculation

Fig. 25