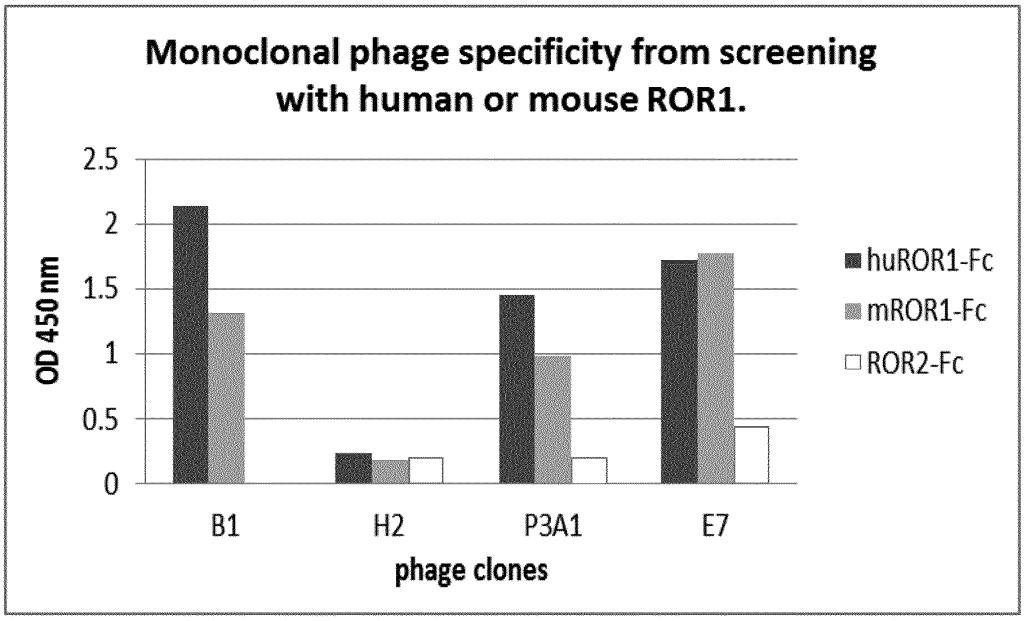


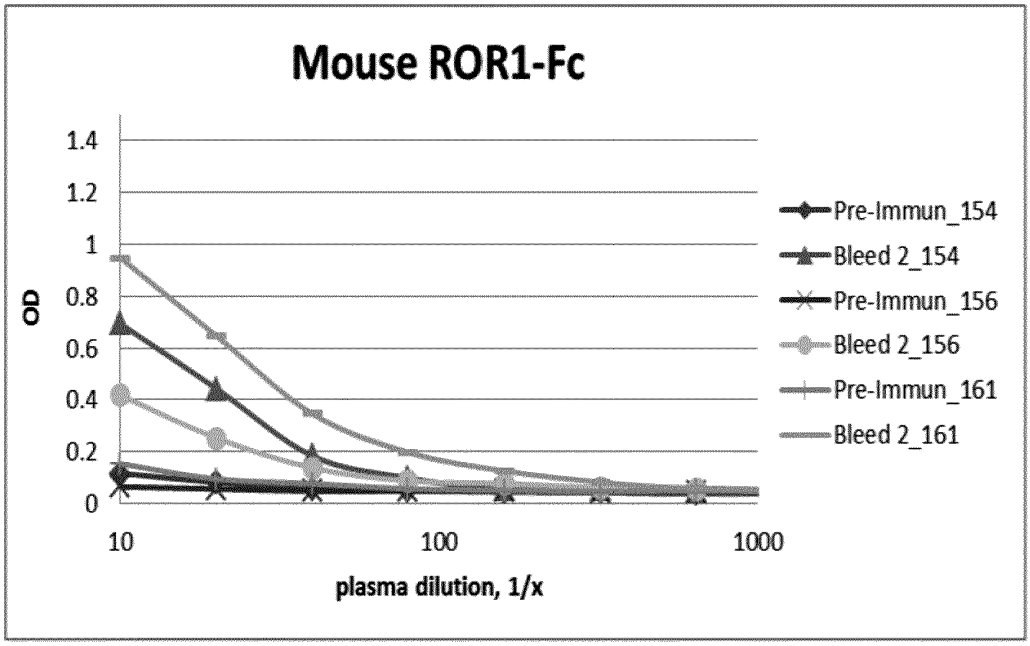
Fig. 1

```
                    10        20        30        40        50        60        70
E7 Protein  - TRVDQTPRTATKETGESLTINCVVTGAKYGLAATYWYRKNPGSSNQERISISGRYVESVNKRTMSFSLRI
CPF7 Protein- TRVDQTPRTATKETGESLTINCVVTGAKYGLFATYWYRKNPGSSNQERISISGRYVESVNKRTMSFSLRI
P3A1 Protein- TRVDQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMSIGGRYVESVNKGAKSFSLRI
B1 Protein  - ASVNQTPRTATKETGESLTINCVVTGANYGLAATYWYRKNPGSSNQERISISGRYVESVNKRTMSFSLRI
                    80        90        100       110
E7 Protein  - KDLTVADSATYYCKAY----PWAMWG----QWYDGAGTVLTVN  105 SEQ ID NO: 102
CPF7 Protein- KDLTVADSATYYCKAVFMPQHWHPAA----HWYDGAGTVLTVN  109 SEQ ID NO: 103
P3A1 Protein- KDLTVADSATYYCKAR---EARHPWLR---QWYDGAGTVLTVN  107 SEQ ID NO: 47
B1 Protein  - KDLTVADSATYYCKAY----PWGAGAPWLVQWYDGAGTVLTVN  109 SEQ ID NO: 44
```

```
                    10        20        30        40        50        60        70
E7 Protein  - TRVDQTPRTATKETGESLTINCVVTGAKYGLAATYWYRKNPGSSNQERISISGRYVESVNKRTMSFSLRI
CPF7 Protein- TRVDQTPRTATKETGESLTINCVVTGAKYGLFATYWYRKNPGSSNQERISISGRYVESVNKRTMSFSLRI
P3A1 Protein- TRVDQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMSIGGRYVESVNKGAKSFSLRI
B1 Protein  - ASVNQTPRTATKETGESLTINCVVTGANYGLAATYWYRKNPGSSNQERISISGRYVESVNKRTMSFSLRI
                    80        90        100       110       120       130
E7 Protein  - KDLTVADSATYYCKAY----PWAMWG----QWYDGAGTVLTVNQASGAHHHHHHGAEFEQKLISEEDL  130
CPF7 Protein- KDLTVADSATYYCKAVFMPQHWHPAA----HWYDGAGTVLTVNQASGAHHHHHHGAEFEQKLISEEDL  134
P3A1 Protein- KDLTVADSATYYCKAR---EARHPWLR---QWYDGAGTVLTVNQASGAHHHHHHGAEFEQKLISEEDL  132
B1 Protein  - KDLTVADSATYYCKAY----PWGAGAPWLVQWYDGAGTVLTVNQASGAHHHHHHGAEFEQKLISEEDL  134
```

SEQ ID NO: 104
SEQ ID NO: 105
SEQ ID NO: 106
SEQ ID NO: 107

Fig. 2


Mouse ROR1-Fc
OD
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
100
1000
plasma dilution, 1/x
Pre-Immun_154
Bleed 2_154
Pre-Immun_156
Bleed 2_156
Pre-Immun_161
Bleed 2_161

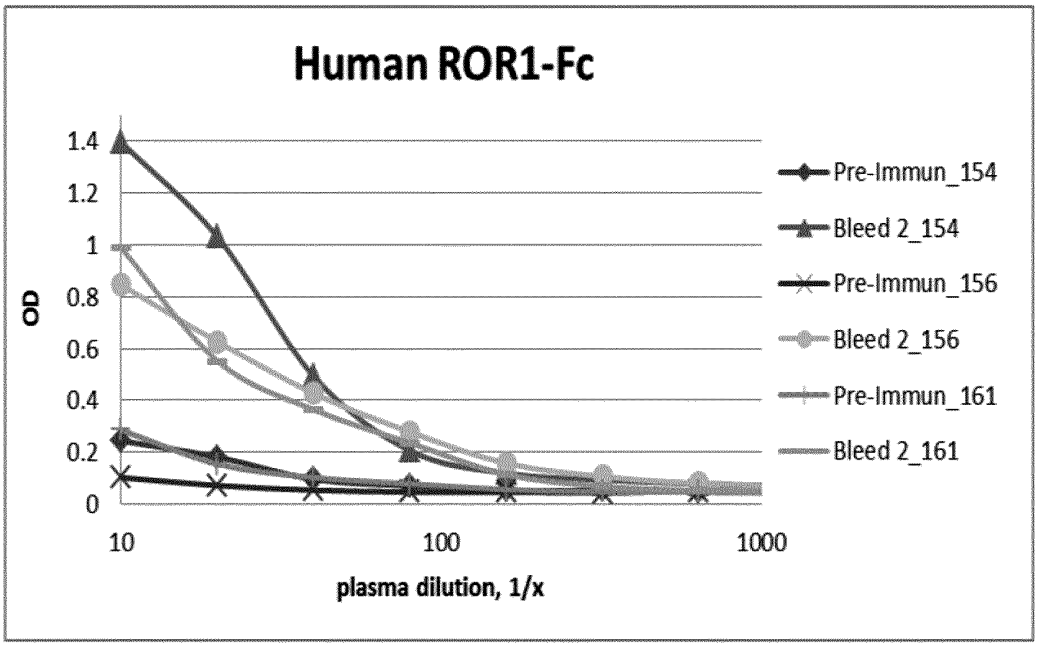

Human ROR1-Fc
OD
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
100
1000
plasma dilution, 1/x
Pre-Immun_154
Bleed 2_154
Pre-Immun_156
Bleed 2_156
Pre-Immun_161
Bleed 2_161

Fig. 3

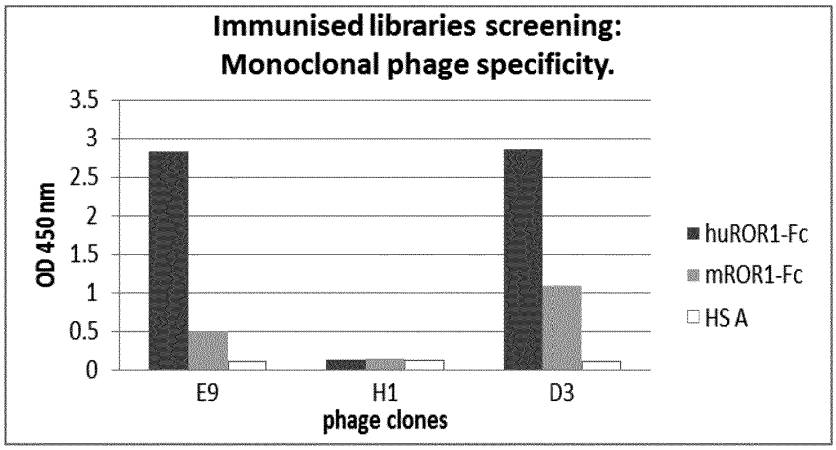


Fig. 4

```
                    10        20        30        40        50        60        70
D3 protein - ASVNQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMSIGGRYVESVNKRAKSFSLRI
E9 protein - AKVDQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMSIGGRYVESVNKRAKSFSLRI
                    80        90       100       110
D3 protein - KDLTVADSATYYCKAQSGMAISTGSGHGYNWYDGAGTVLTVN  112  SEQ ID NO: 39
E9 protein - KDLTVADSATYYCKAQSGMAIDIGSGHGYNWYDGAGTVLTVN  112  SEQ ID NO: 40
```

```
                    10        20        30        40        50        60        70
D3 protein - ASVNQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMSIGGRYVESVNKRAKSFSLRI
E9 protein - AKVDQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMSIGGRYVESVNKRAKSFSLRI
                    80        90       100       110       120       130
D3 protein - KDLTVADSATYYCKAQSGMAISTGSGHGYNWYDGAGTVLTVNQASGAHHHHHHGAEFEQKLISEEDL  137
E9 protein - KDLTVADSATYYCKAQSGMAIDIGSGHGYNWYDGAGTVLTVNQASGAHHHHHHGAEFEQKLISEEDL  137
```

SEQ ID NO: 108
SEQ ID NO: 109

Fig. 5

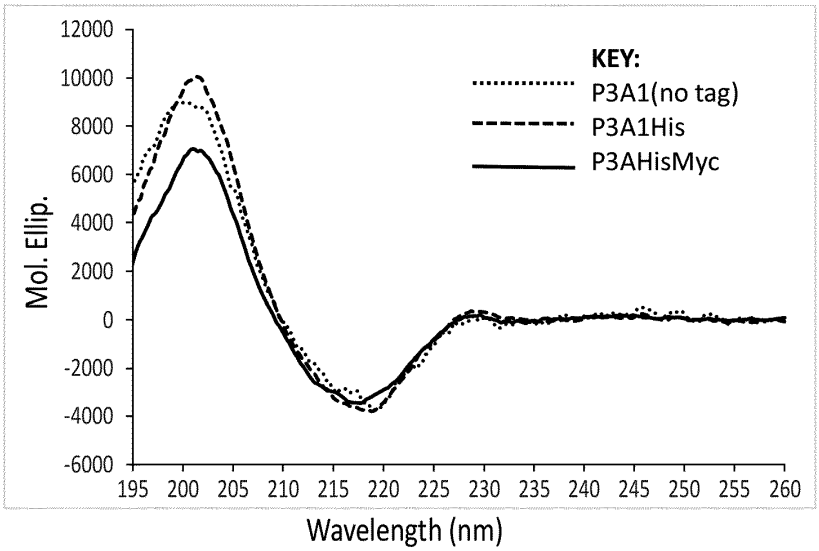


Fig. 6

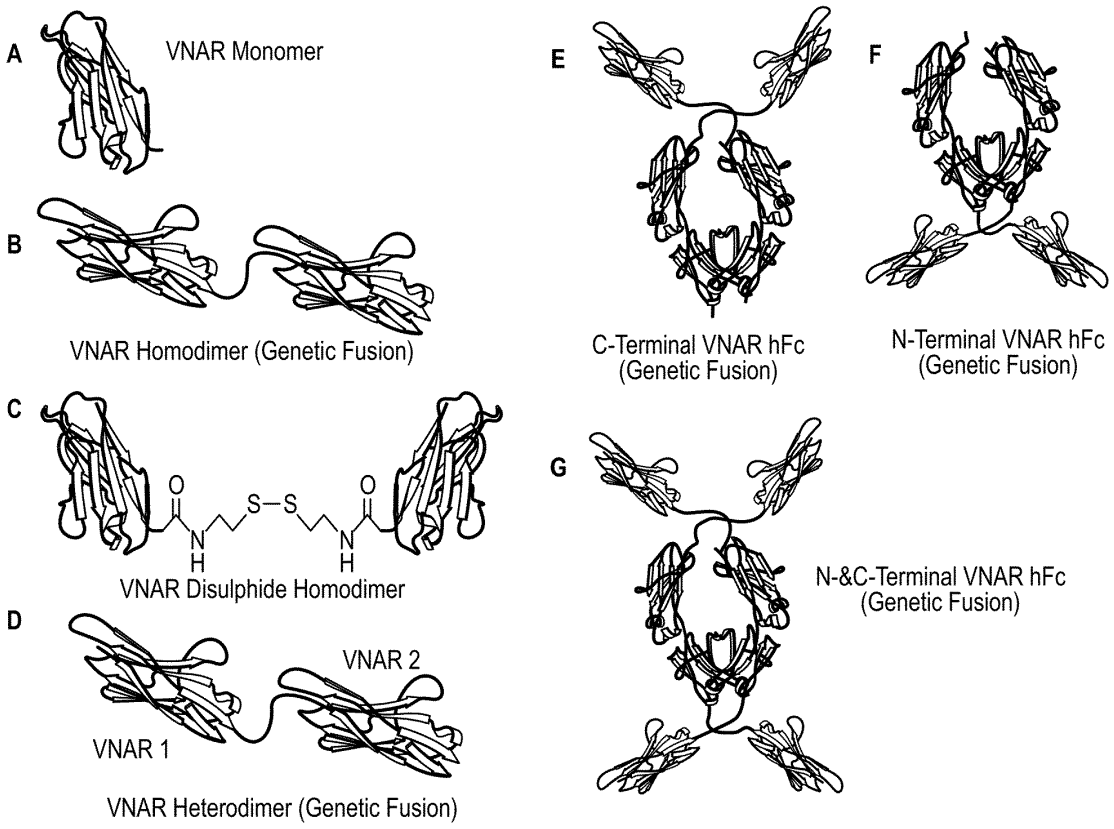

A
VNAR Monomer
B
VNAR Homodimer (Genetic Fusion)
C
O
S—S
O
N
H
N
H
VNAR Disulphide Homodimer
D
VNAR 2
VNAR 1
VNAR Heterodimer (Genetic Fusion)
E
C-Terminal VNAR hFc
(Genetic Fusion)
F
N-Terminal VNAR hFc
(Genetic Fusion)
G
N-&C-Terminal VNAR hFc
(Genetic Fusion)

Fig. 7

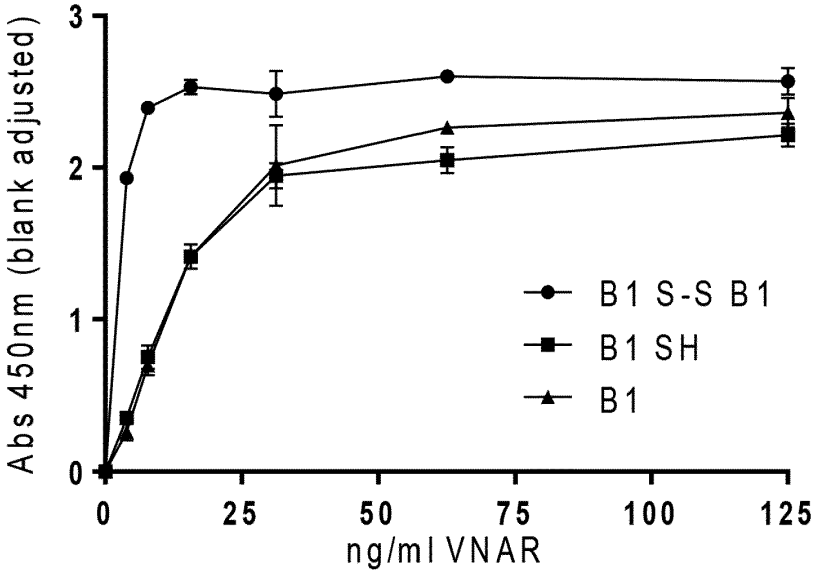

Abs 450nm (blank adjusted)
3
2
1
0
0
25
50
75
100
125
ng/ml VNAR
B1 S-S B1
B1 SH
B1

Fig. 8

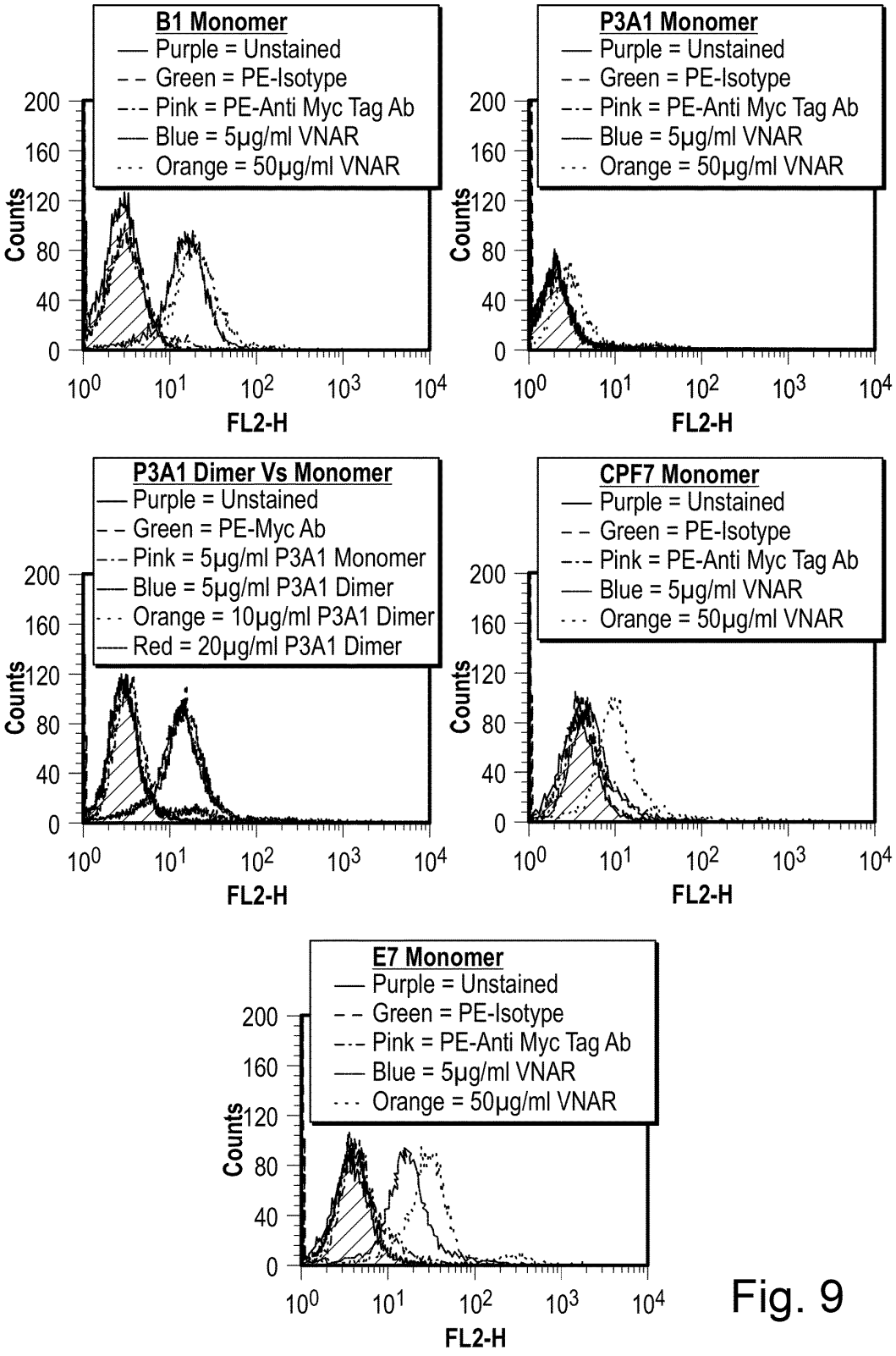

B1 Monomer
Purple = Unstained
Green = PE-Isotype
Pink = PE-Anti Myc Tag Ab
Blue = 5µg/ml VNAR
Orange = 50µg/ml VNAR
P3A1 Monomer
Purple = Unstained
Green = PE-Isotype
Pink = PE-Anti Myc Tag Ab
Blue = 5µg/ml VNAR
Orange = 50µg/ml VNAR
P3A1 Dimer Vs Monomer
Purple = Unstained
Green = PE-Myc Ab
Pink = 5µg/ml P3A1 Monomer
Blue = 5µg/ml P3A1 Dimer
Orange = 10µg/ml P3A1 Dimer
Red = 20µg/ml P3A1 Dimer
CPF7 Monomer
Purple = Unstained
Green = PE-Isotype
Pink = PE-Anti Myc Tag Ab
Blue = 5µg/ml VNAR
Orange = 50µg/ml VNAR
E7 Monomer
Purple = Unstained
Green = PE-Isotype
Pink = PE-Anti Myc Tag Ab
Blue = 5µg/ml VNAR
Orange = 50µg/ml VNAR
Counts
FL2-H
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4

Fig. 9

Linker mouse IgG2a sequence for VNAR fusions

GGGGSGGGGSGGGGSEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISL
SPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQ
HQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQV
TLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKN
WVERNSYSCSVVHEGLHNHHTTKSFSRTPGK (SEQ ID NO: 106)

Linker human IgG1 sequences for VNAR fusions

N-terminal VNAR hFc (linker-hFc sequence)

GGGGSGGGGSGGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 107)

Cysteine engineered variants for bioconjugation

GGGGSGGGGSGGGGSEPKSSDKTHTCPPCPAPELLGGPCVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 108)

GGGGSGGGGSGGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLCLSPGK (SEQ ID NO: 109)

C-terminal hFc VNAR (hFc-linker sequence)

DKTHTCPPCPAPELLGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 110)

Fig. 10

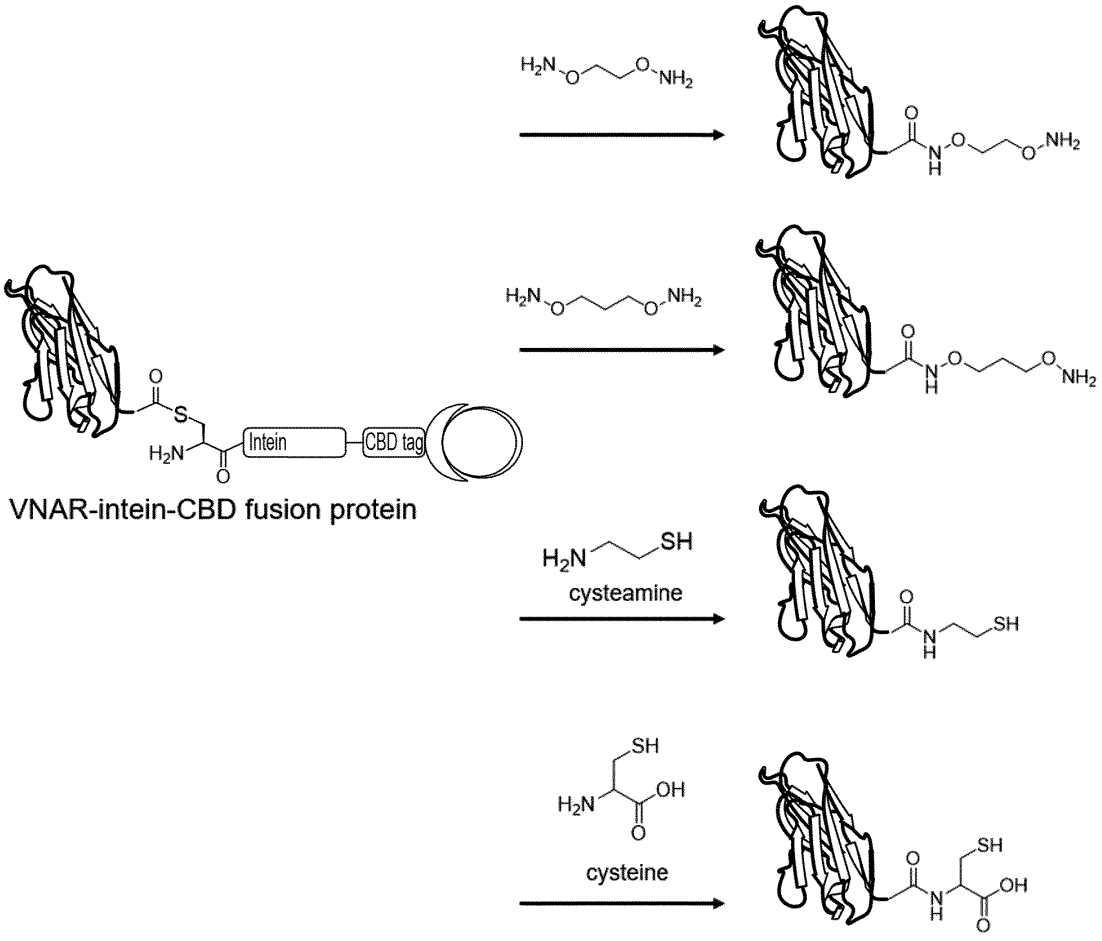

Intein
CBD tag
VNAR-intein-CBD fusion protein
cysteamine
cysteine

Fig. 11

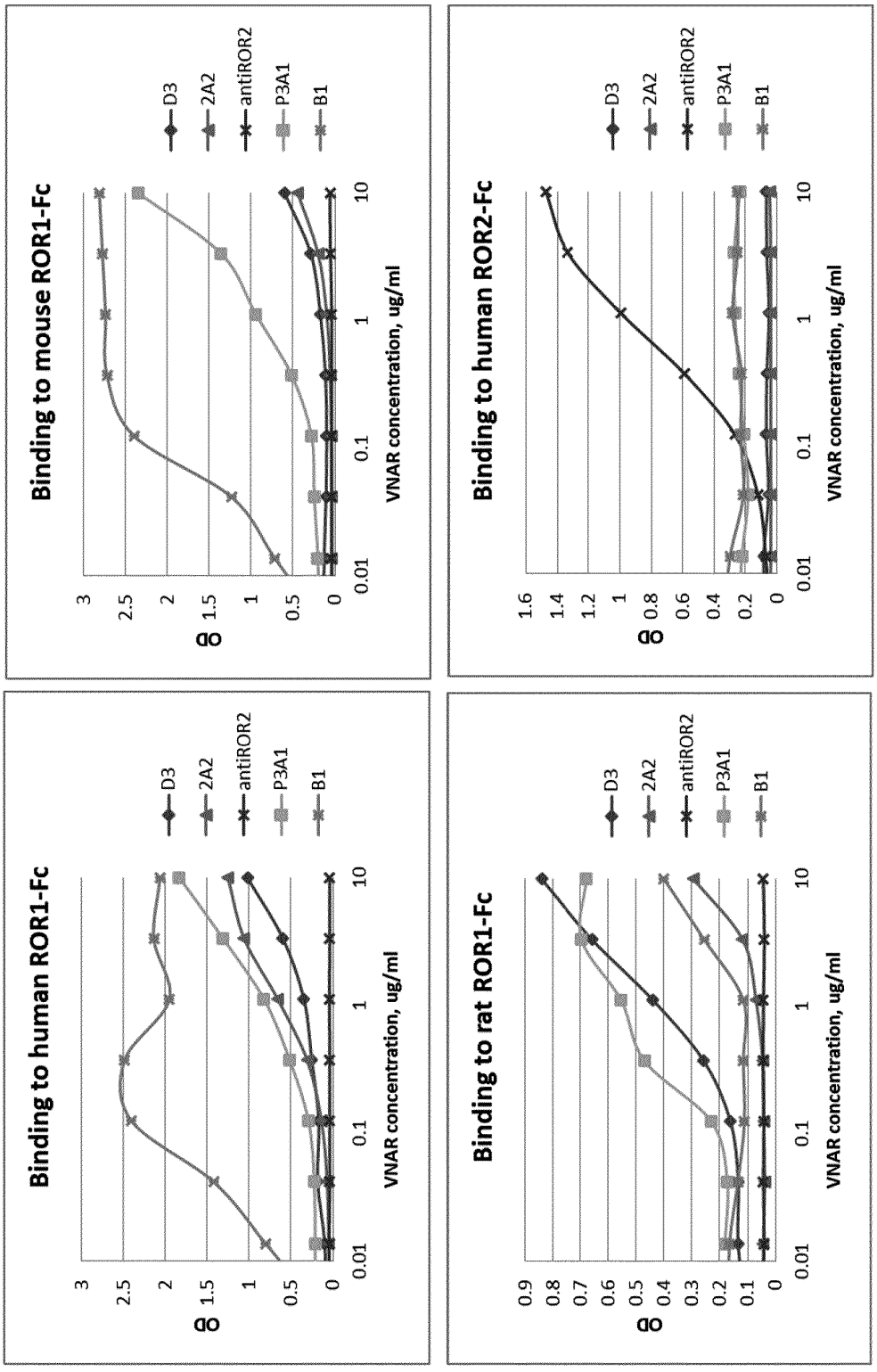

Binding to mouse ROR1-Fc
Binding to human ROR2-Fc
Binding to human ROR1-Fc
Binding to rat ROR1-Fc
D3
2A2
antiROR2
P3A1
B1
OD
VNAR concentration, ug/ml

Fig. 12

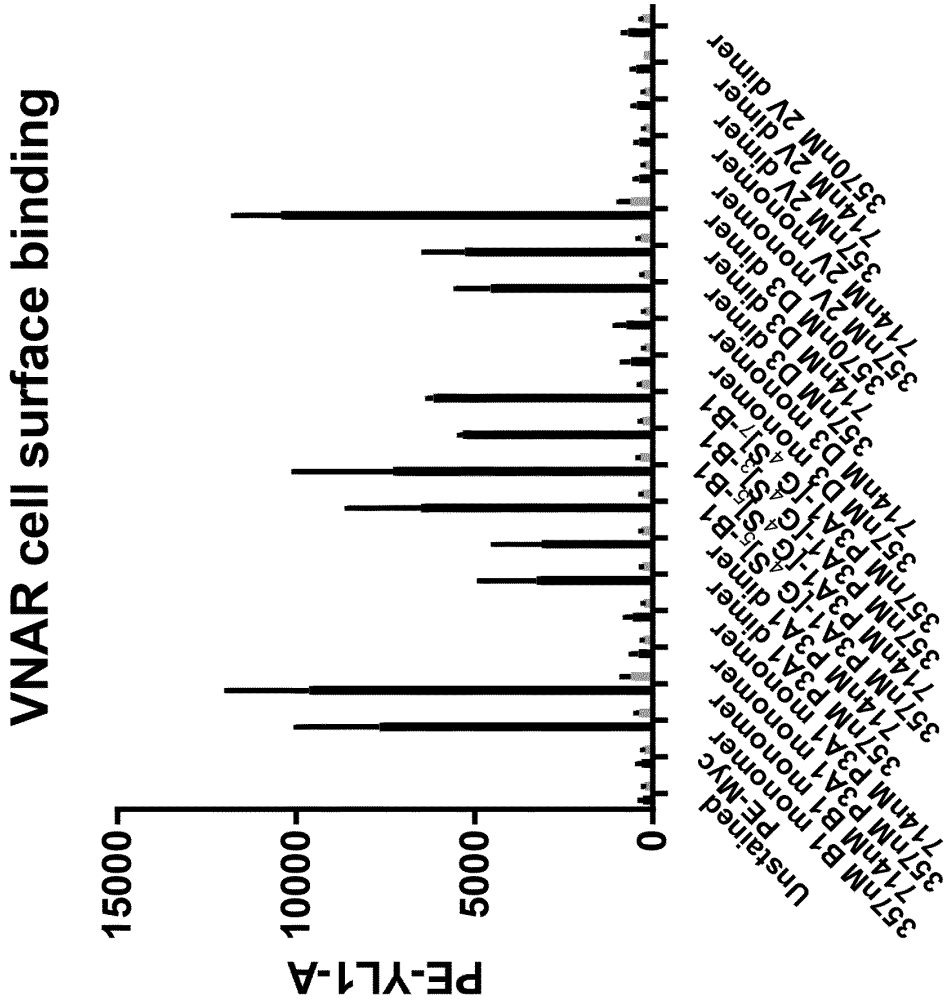
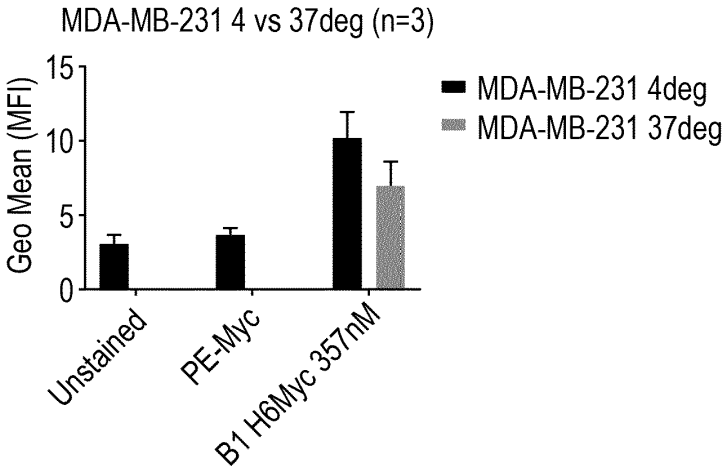
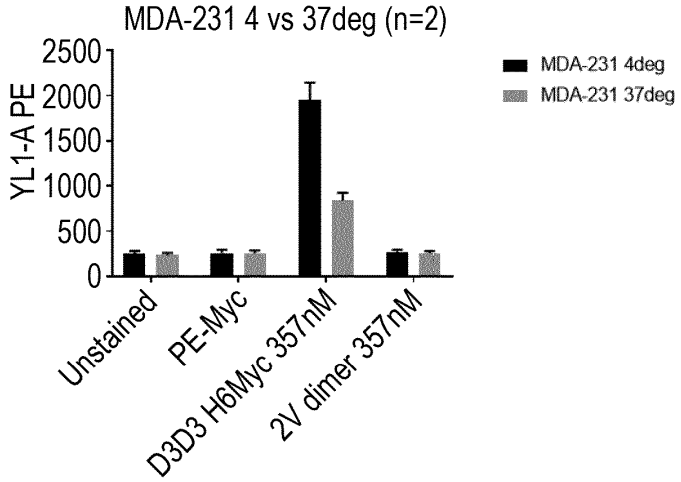

VNAR cell surface binding
A549
A427
PE-YL1-A
15000
10000
5000
0
Unstained
PE-Myc monomer
357nM B1 monomer
714nM B1 monomer
357nM P3A1 monomer
714nM P3A1 monomer
357nM P3A1 dimer
714nM P3A1 dimer
357nM P3A1-IG4S15-B1
714nM P3A1-IG4S15-B1
357nM P3A1-IG4S13-B1
714nM P3A1-IG4S17-B1
357nM D3 monomer
714nM D3 monomer
357nM D3 dimer
714nM D3 dimer
357nM 2V monomer
714nM 2V monomer
357nM 2V dimer
714nM 2V dimer
357nM 2V dimer

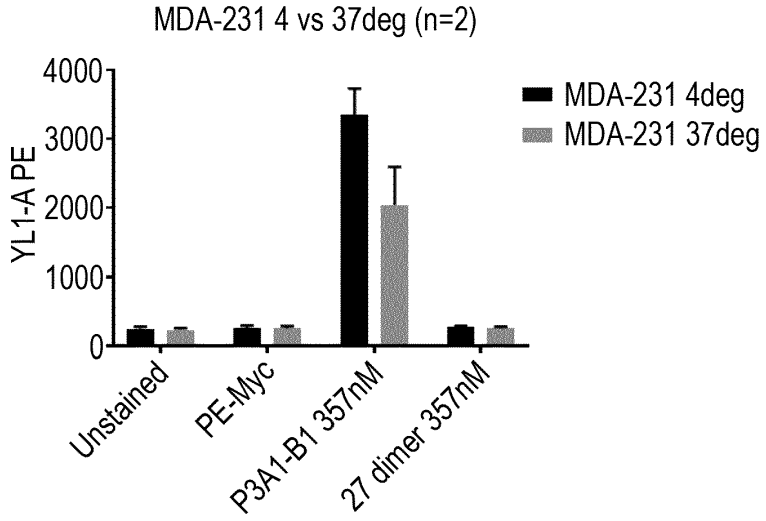
Fig. 13



33% reduction in B1 VNAR cell surface signal at 37°C



57% reduction in D3D3 VNAR cell surface signal at 37°C



39% reduction in P3A1-$(G_4S)_5$-B1 signal at 37°C

Fig. 14

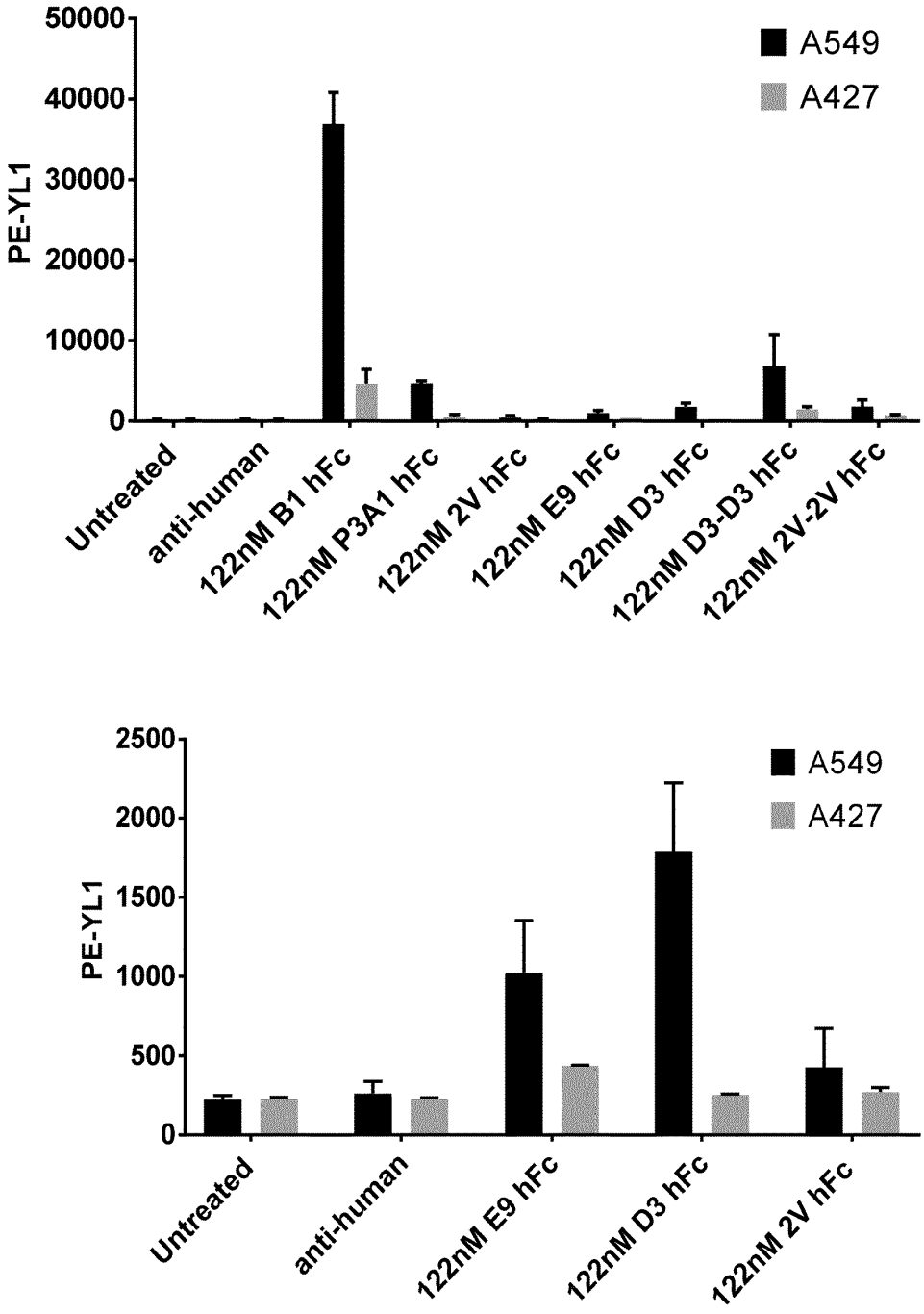
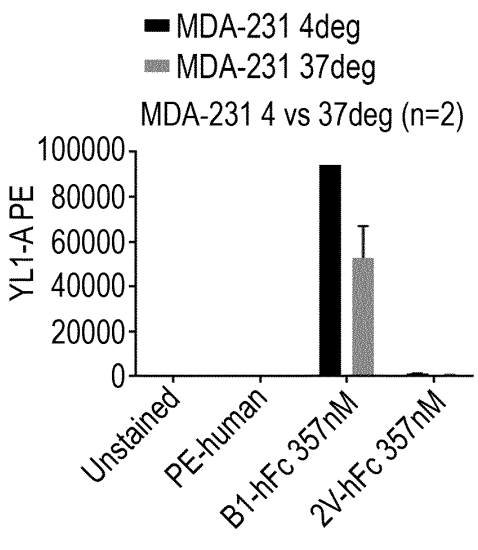
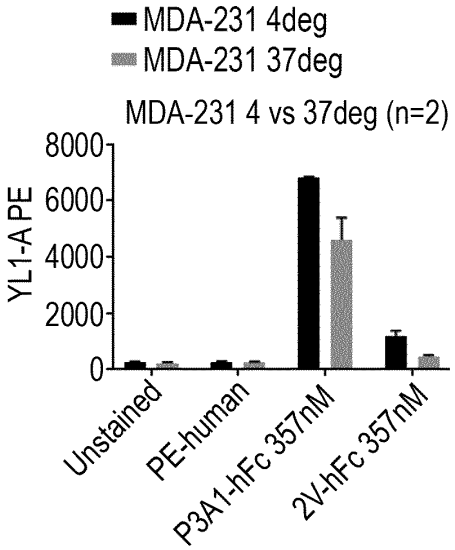

50000
40000
30000
20000
10000
0
PE-YL1
A549
A427
Untreated
anti-human
122nM B1 hFc
122nM P3A1 hFc
122nM 2V hFc
122nM E9 hFc
122nM D3 hFc
122nM D3-D3 hFc
122nM 2V-2V hFc
2500
2000
1500
1000
500
0
PE-YL1
A549
A427
Untreated
anti-human
122nM E9 hFc
122nM D3 hFc
122nM 2V hFc

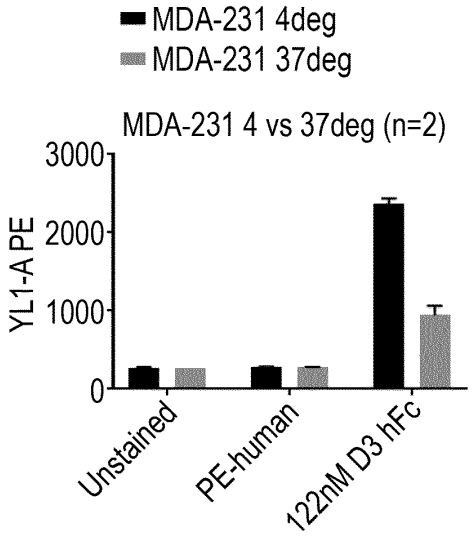
Fig. 15



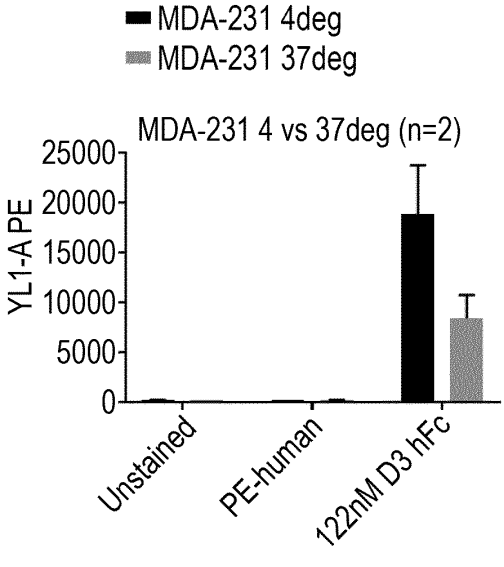
B1 hFc: 44% reduction in signal at 37°C



P3A1 hFc: 33% reduction in signal at 37°C



D3 hFc: 60% reduction in signal at 37°C



D3D3 hFc: 55% reduction in signal at 37°C

Fig. 16

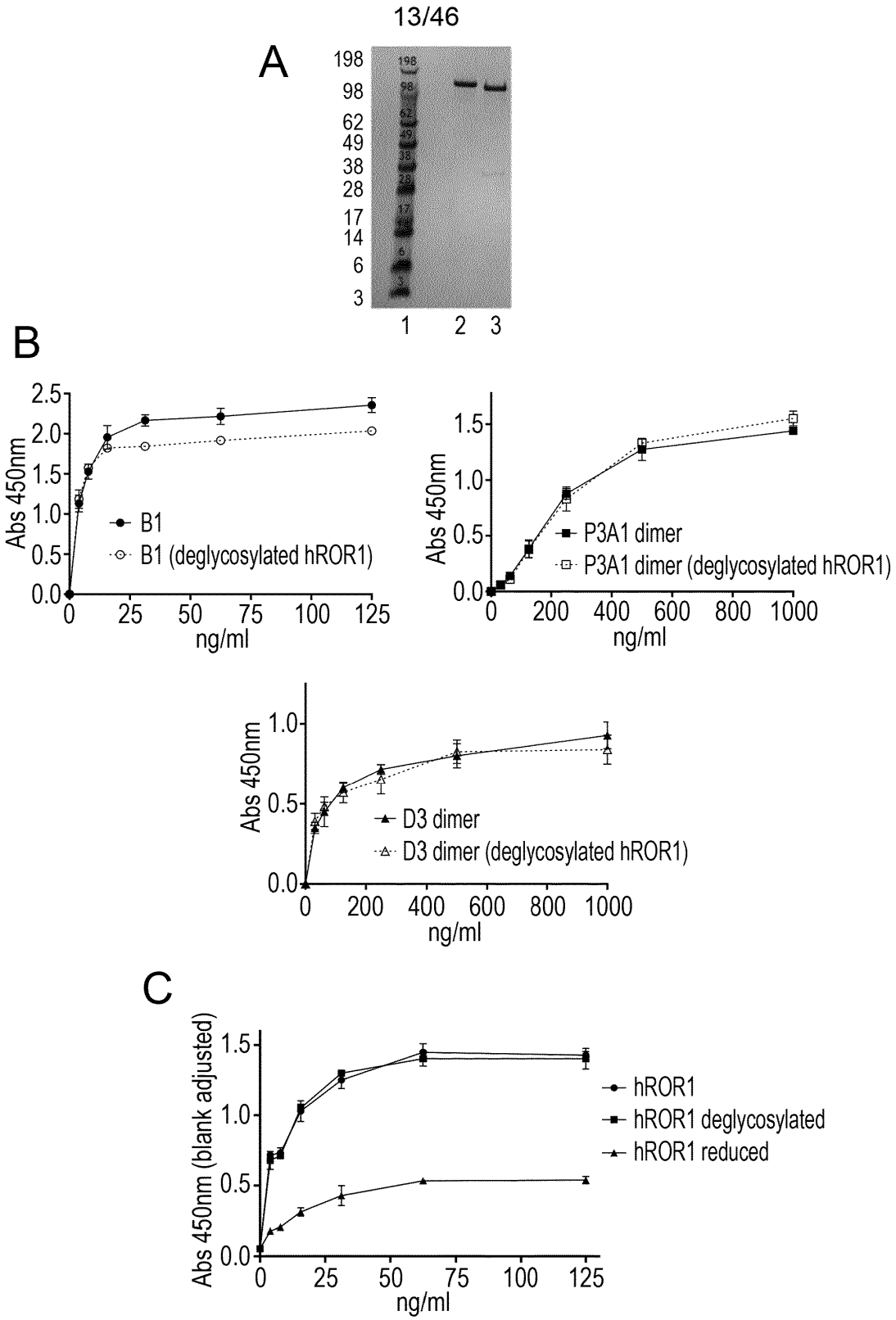

A
198
98
62
49
38
28
17
14
6
3
1 2 3
B
Abs 450nm
B1
B1 (deglycosylated hROR1)
ng/ml
P3A1 dimer
P3A1 dimer (deglycosylated hROR1)
D3 dimer
D3 dimer (deglycosylated hROR1)
C
Abs 450nm (blank adjusted)
hROR1
hROR1 deglycosylated
hROR1 reduced

Fig. 17

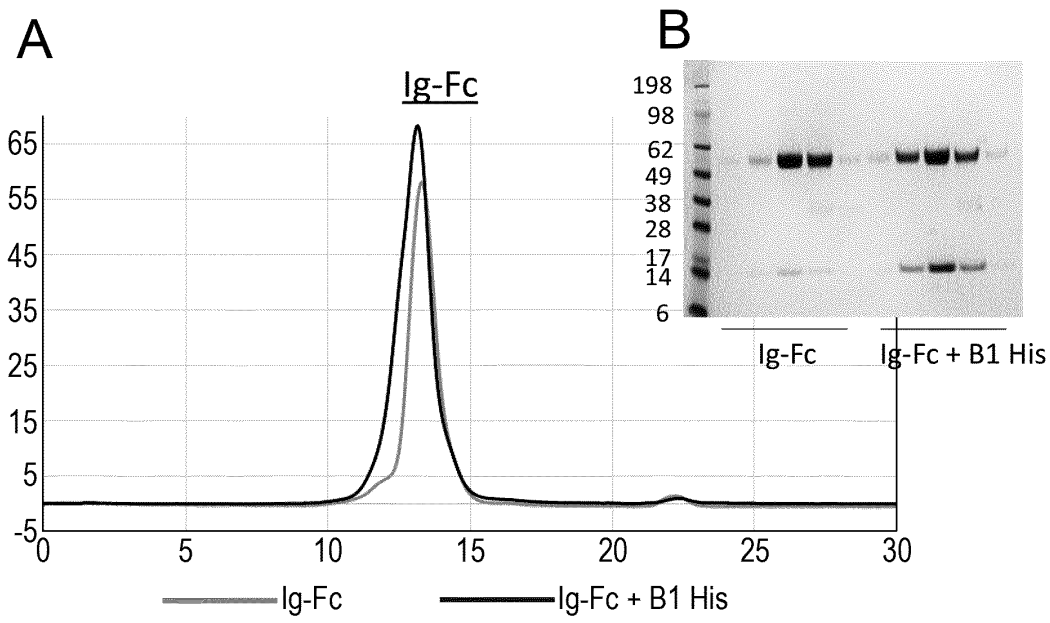
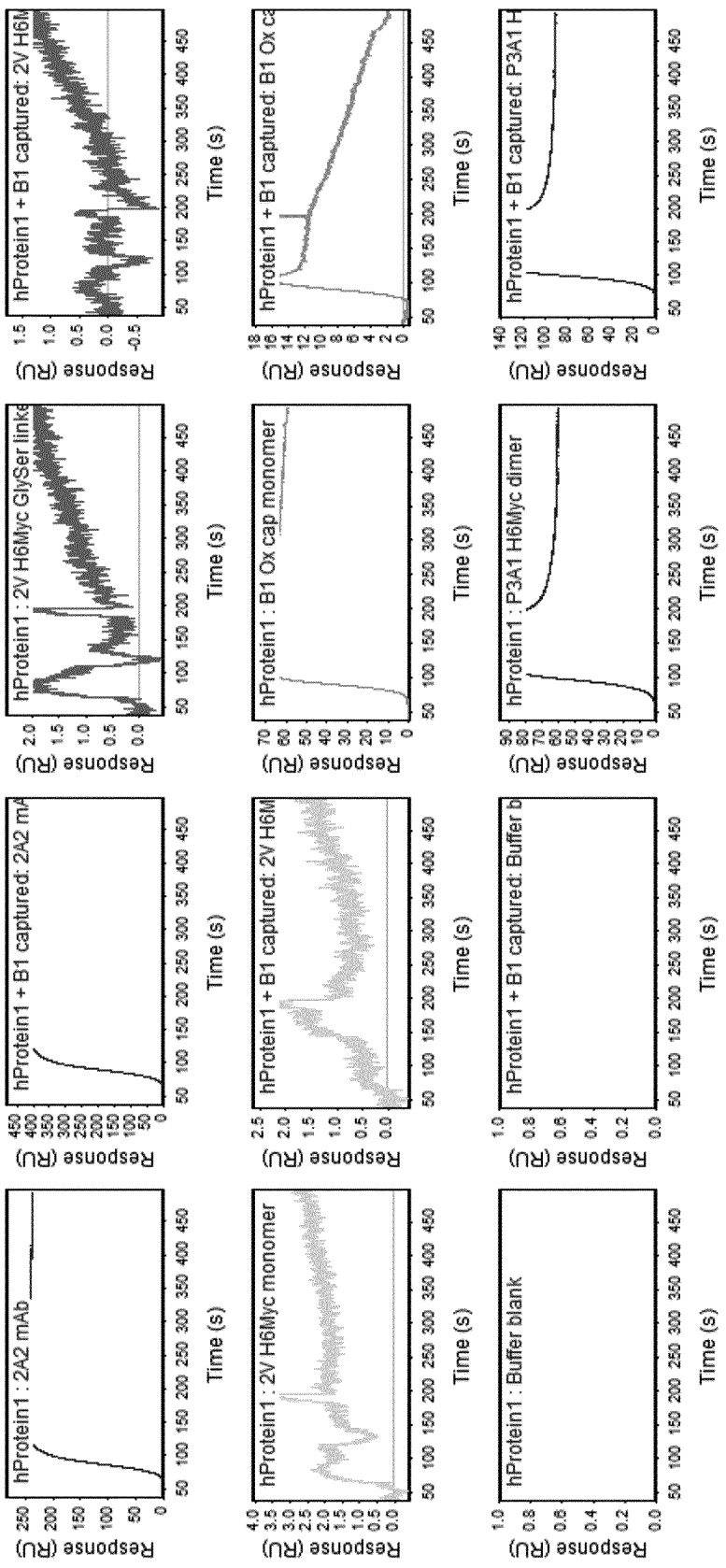

A
Ig-Fc
65
55
45
35
25
15
5
-5
0
5
10
15
20
25
30
Ig-Fc
Ig-Fc + B1 His
B
198
98
62
49
38
28
17
14
6
Ig-Fc
Ig-Fc + B1 His

Fig. 18



B1 captured to FC1/hROR1
FC3 = hROR1

Fig. 19

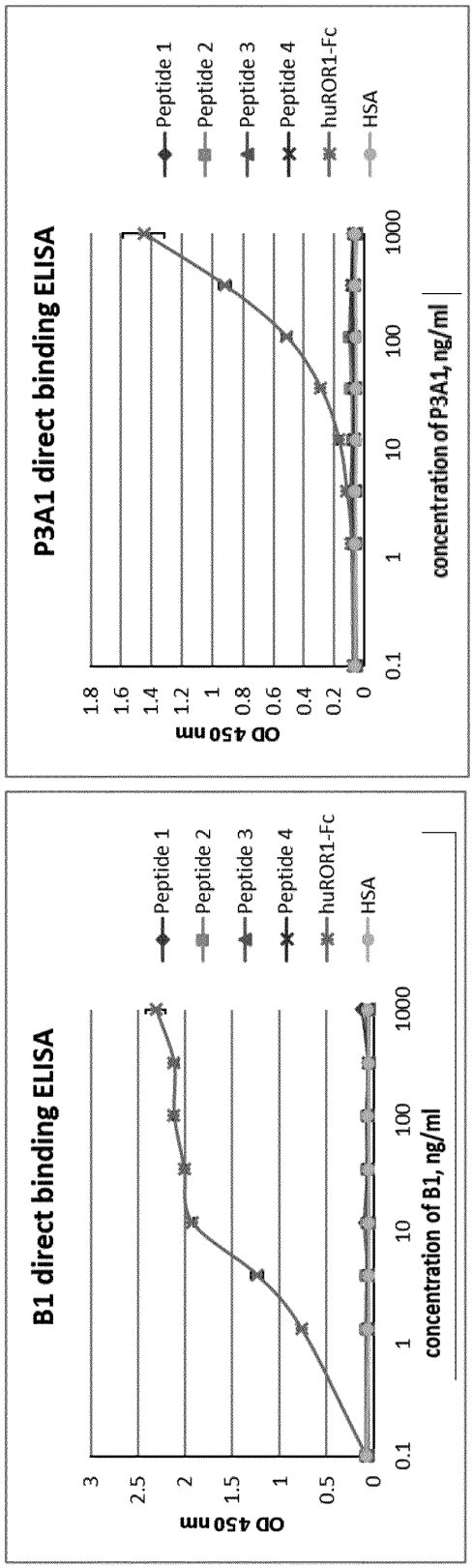

B1 direct binding ELISA
OD 450 nm
concentration of B1, ng/ml
Peptide 1
Peptide 2
Peptide 3
Peptide 4
huROR1-Fc
HSA
P3A1 direct binding ELISA
OD 450 nm
concentration of P3A1, ng/ml
Peptide 1
Peptide 2
Peptide 3
Peptide 4
huROR1-Fc
HSA

Fig. 20

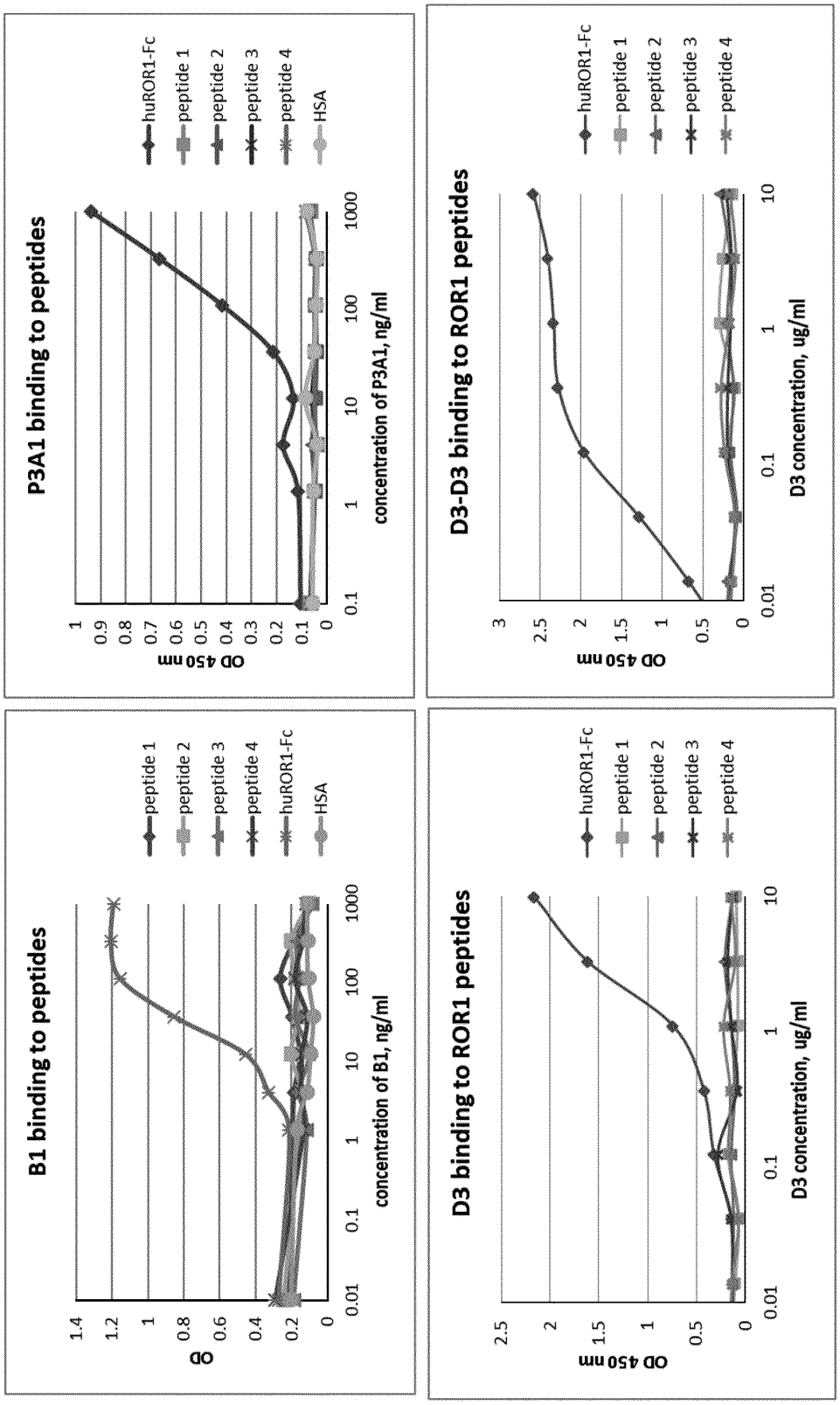

P3A1 binding to peptides
OD 450 nm
concentration of P3A1, ng/ml
huROR1-Fc
peptide 1
peptide 2
peptide 3
peptide 4
HSA
D3-D3 binding to ROR1 peptides
OD 450 nm
D3 concentration, ug/ml
B1 binding to peptides
OD
concentration of B1, ng/ml
D3 binding to ROR1 peptides
OD 450 nm
D3 concentration, ug/ml

Fig. 21

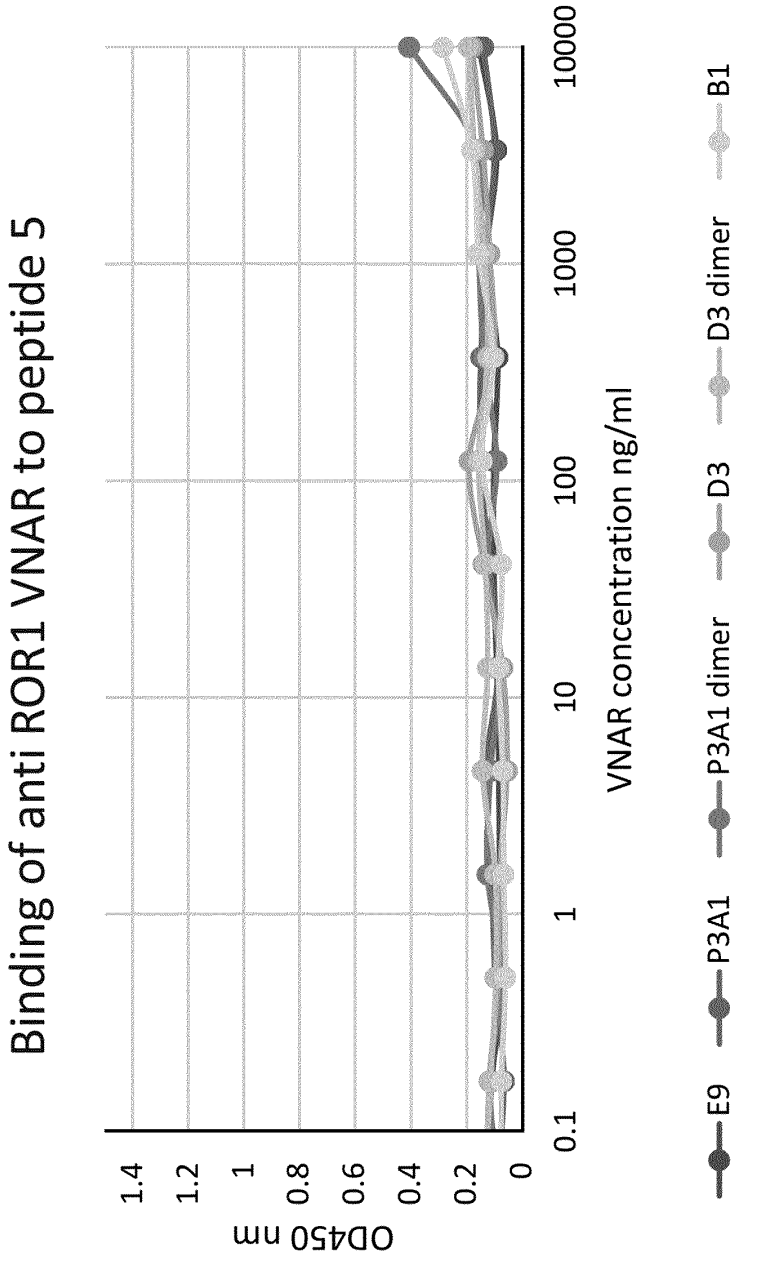


Fig. 21 (continued)

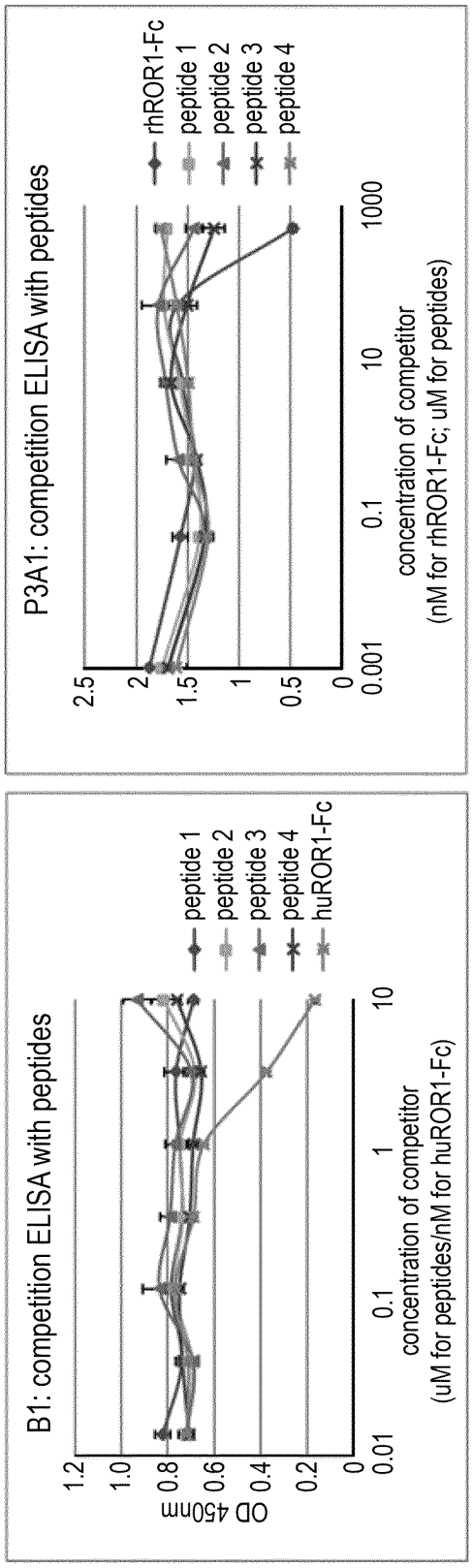

P3A1: competition ELISA with peptides
rhROR1-Fc
peptide 1
peptide 2
peptide 3
peptide 4
2.5
2
1.5
1
0.5
0
0.001
0.1
10
1000
concentration of competitor
(nM for rhROR1-Fc; uM for peptides)
B1: competition ELISA with peptides
peptide 1
peptide 2
peptide 3
peptide 4
huROR1-Fc
OD 450nm
1.2
1.0
0.8
0.6
0.4
0.2
0
0.01
0.1
1
10
concentration of competitor
(uM for peptides/nM for huROR1-Fc)

Fig. 22

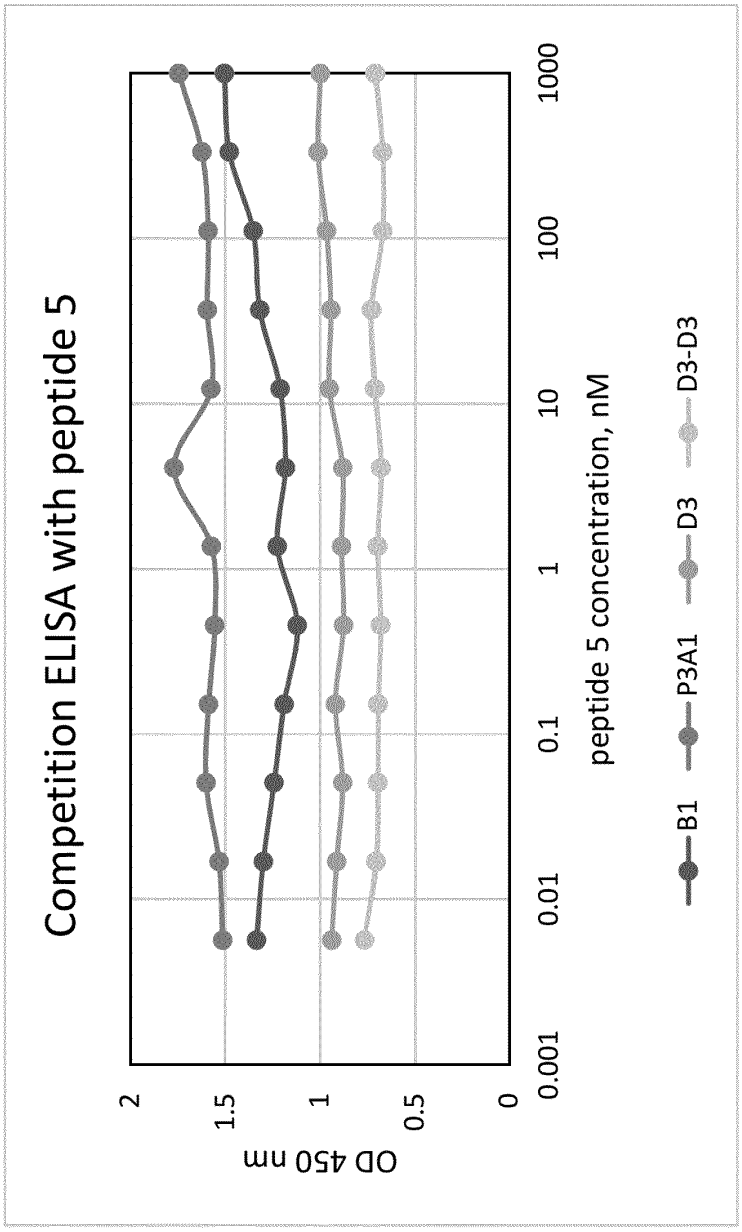


Fig. 22 (continued)

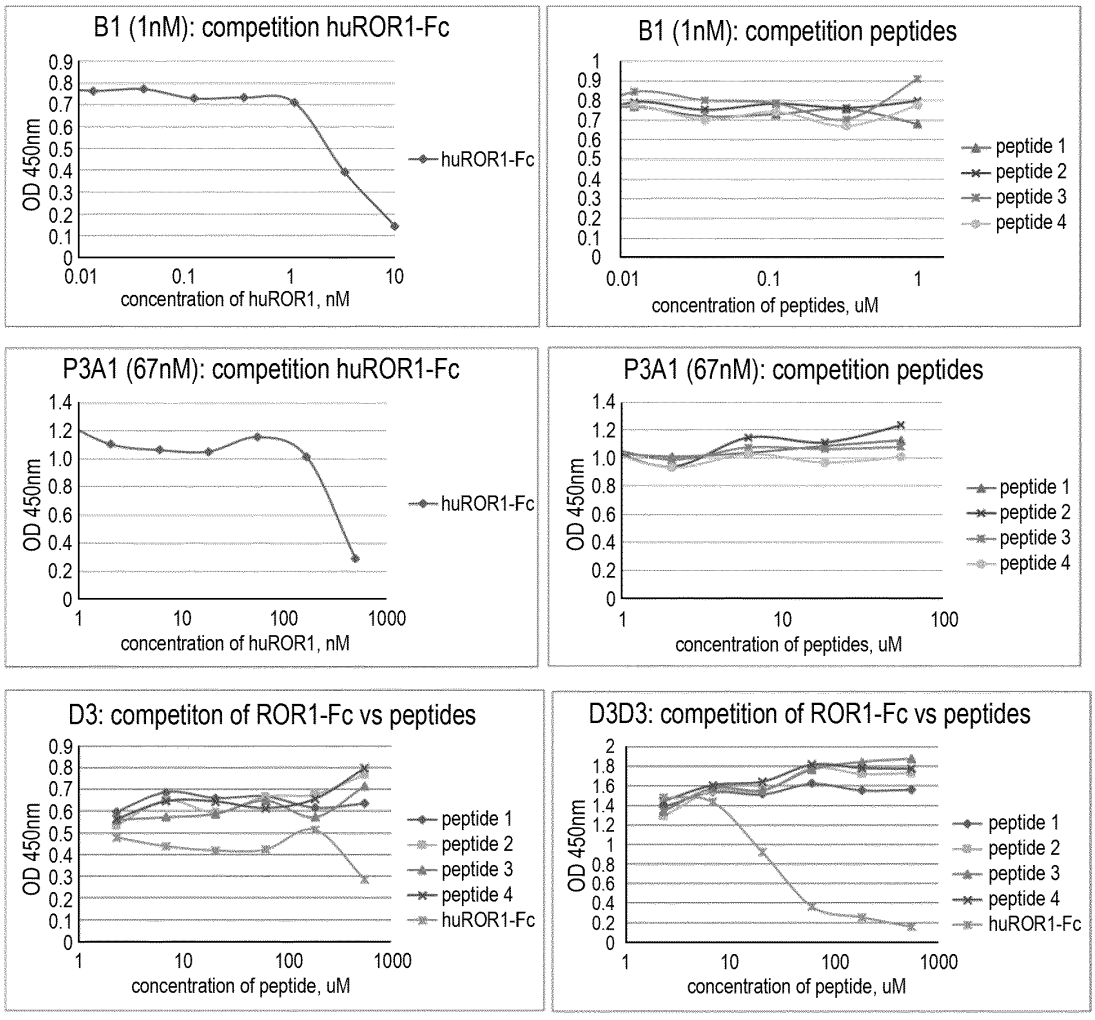

B1 (1nM): competition huROR1-Fc
OD 450nm
concentration of huROR1, nM
huROR1-Fc
B1 (1nM): competition peptides
concentration of peptides, uM
peptide 1
peptide 2
peptide 3
peptide 4
P3A1 (67nM): competition huROR1-Fc
P3A1 (67nM): competition peptides
D3: competiton of ROR1-Fc vs peptides
concentration of peptide, uM
D3D3: competition of ROR1-Fc vs peptides

Fig. 23

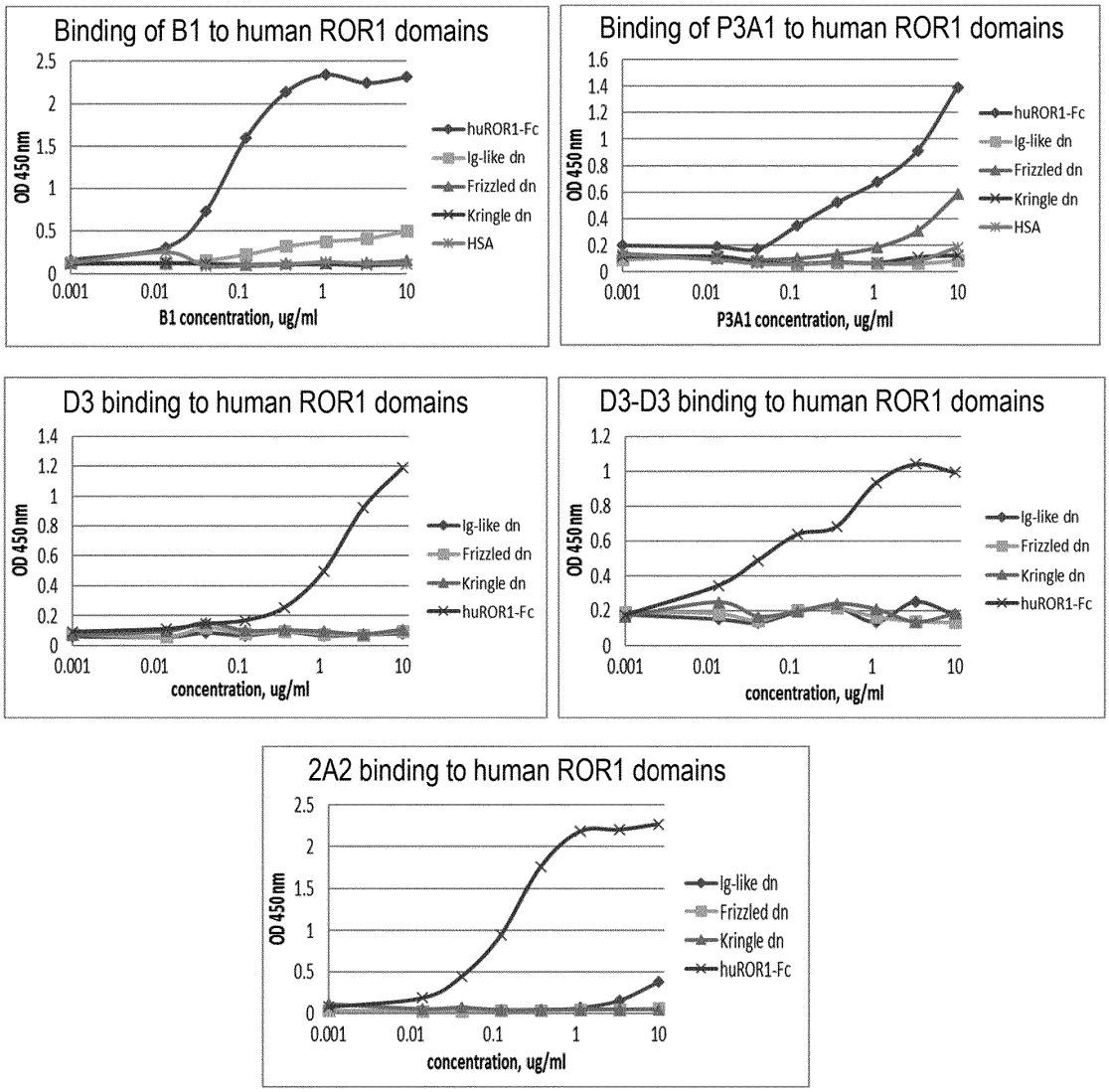

Binding of B1 to human ROR1 domains
OD 450 nm
B1 concentration, ug/ml
huROR1-Fc
Ig-like dn
Frizzled dn
Kringle dn
HSA
Binding of P3A1 to human ROR1 domains
OD 450 nm
P3A1 concentration, ug/ml
huROR1-Fc
Ig-like dn
Frizzled dn
Kringle dn
HSA
D3 binding to human ROR1 domains
OD 450 nm
concentration, ug/ml
Ig-like dn
Frizzled dn
Kringle dn
huROR1-Fc
D3-D3 binding to human ROR1 domains
OD 450 nm
concentration, ug/ml
Ig-like dn
Frizzled dn
Kringle dn
huROR1-Fc
2A2 binding to human ROR1 domains
OD 450 nm
concentration, ug/ml
Ig-like dn
Frizzled dn
Kringle dn
huROR1-Fc

Fig. 24

Fig. 25

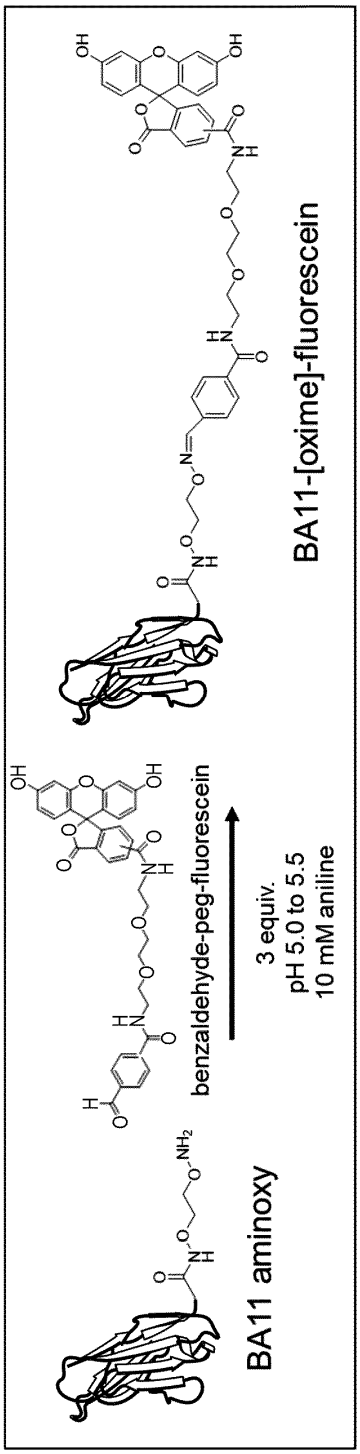


Fig. 26

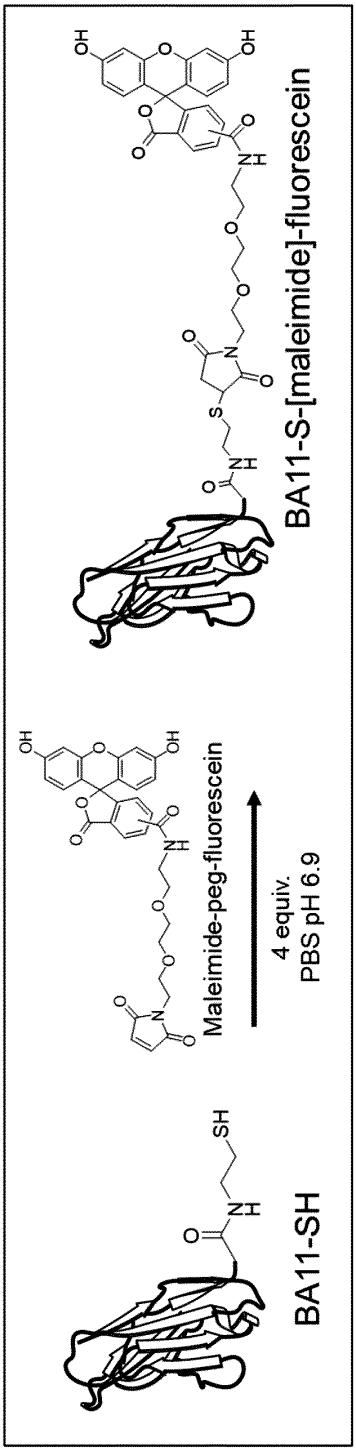
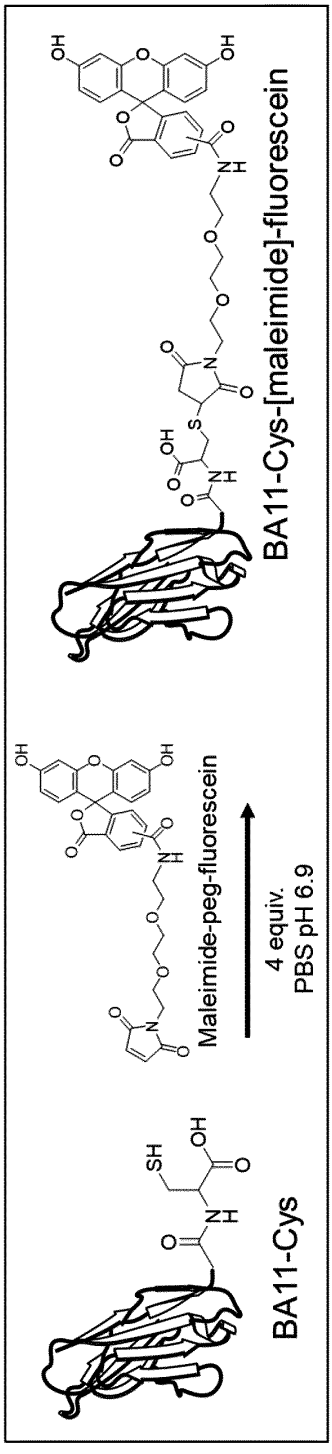


Fig. 27



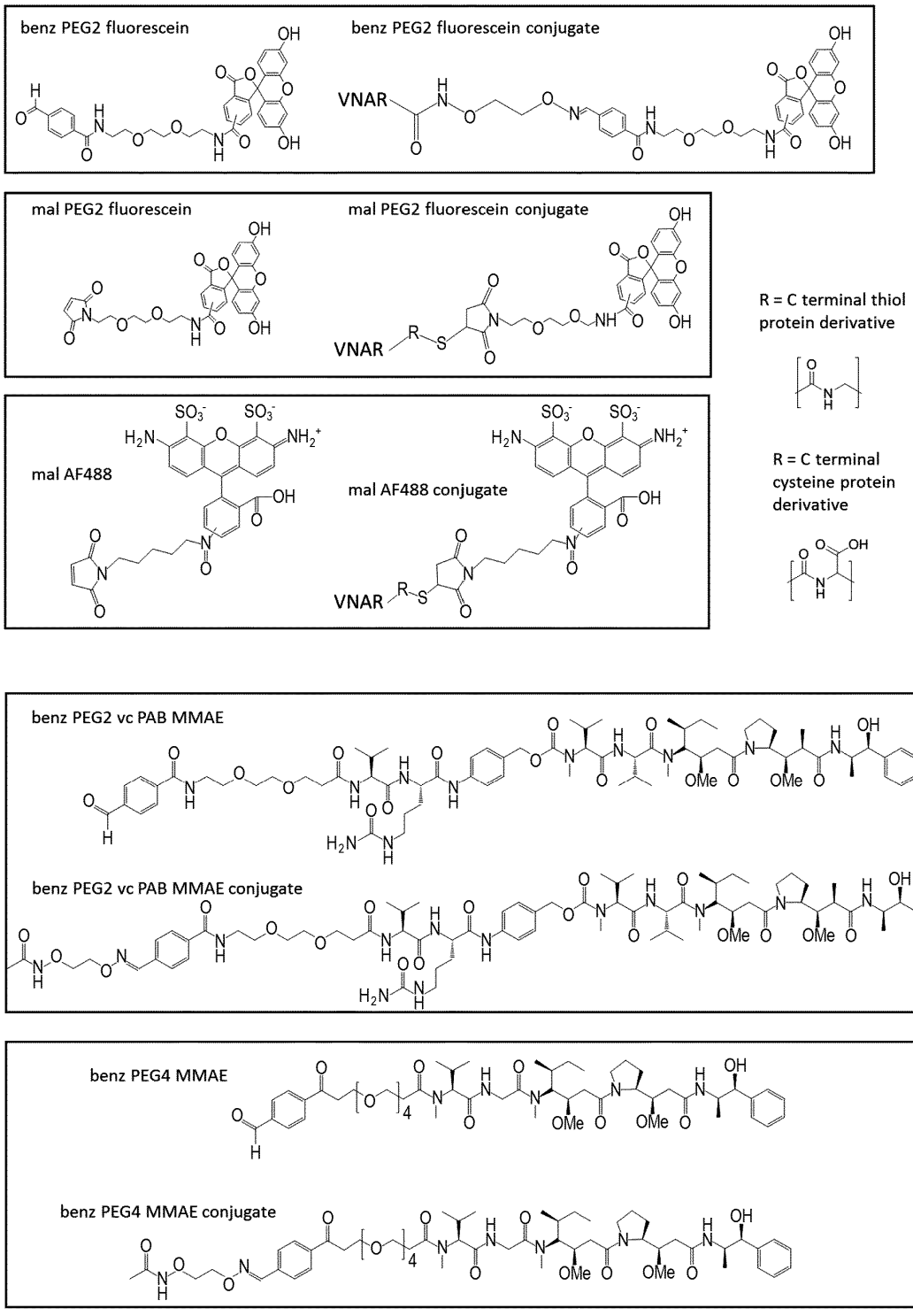

benz PEG2 fluorescein
benz PEG2 fluorescein conjugate
VNAR
mal PEG2 fluorescein
mal PEG2 fluorescein conjugate
VNAR
mal AF488
mal AF488 conjugate
VNAR
R = C terminal thiol protein derivative
R = C terminal cysteine protein derivative
benz PEG2 vc PAB MMAE
benz PEG2 vc PAB MMAE conjugate
benz PEG4 MMAE
benz PEG4 MMAE conjugate

Fig. 28

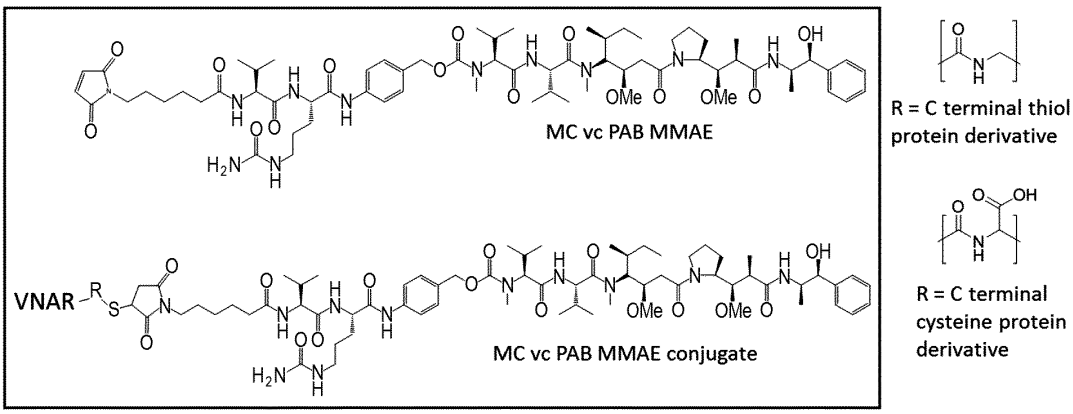


Fig. 28 (continued)

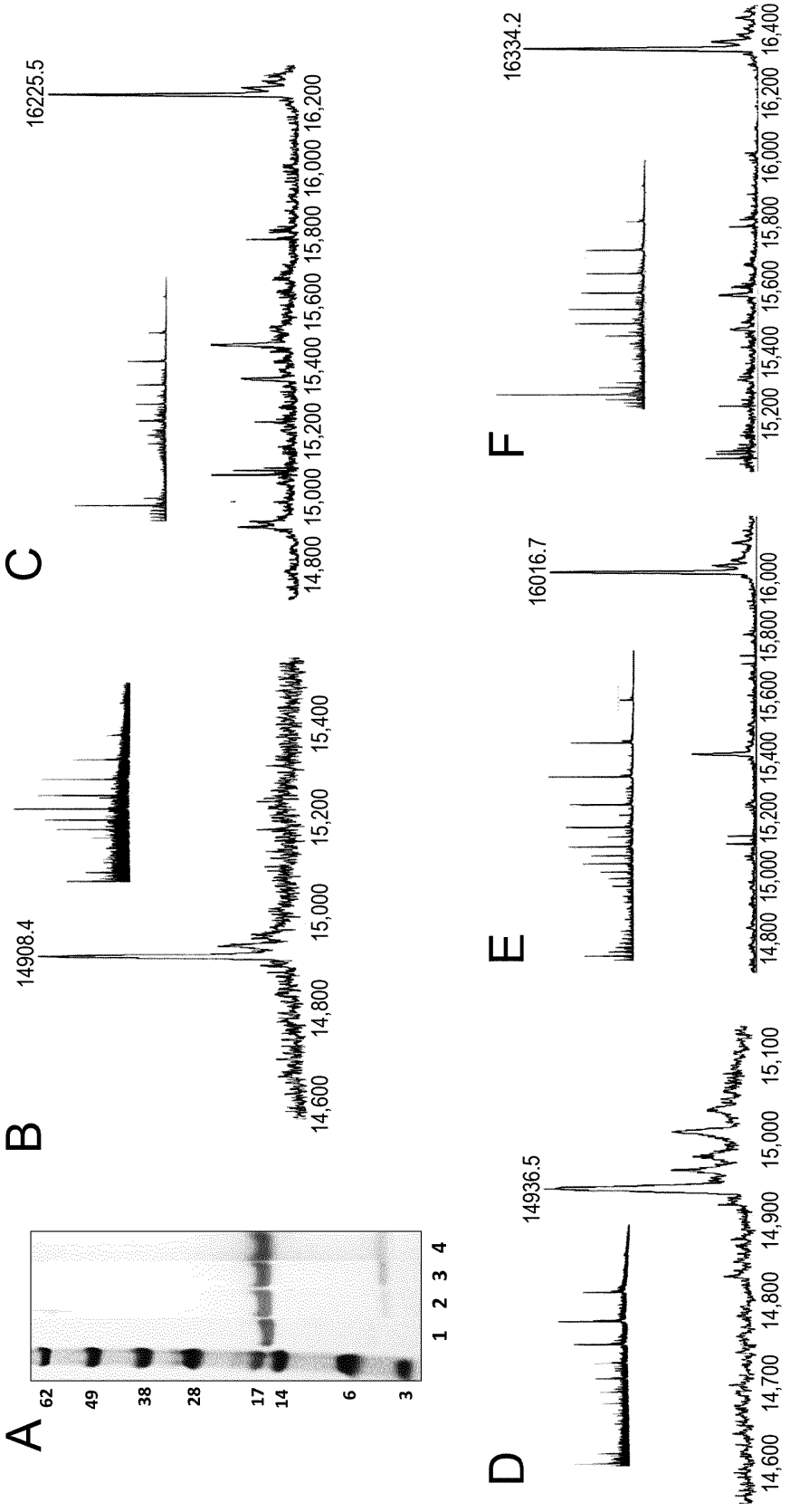

A
62
49
38
28
17
14
6
3
1 2 3 4
B
14908.4
14,600
14,800
15,000
15,200
15,400
C
16225.5
14,800
15,000
15,200
15,400
15,600
15,800
16,000
16,200
D
14936.5
14,600
14,700
14,800
14,900
15,000
15,100
E
16016.7
14,800
15,000
15,200
15,400
15,600
15,800
16,000
F
16334.2
15,200
15,400
15,600
15,800
16,000
16,200
16,400

Fig. 29

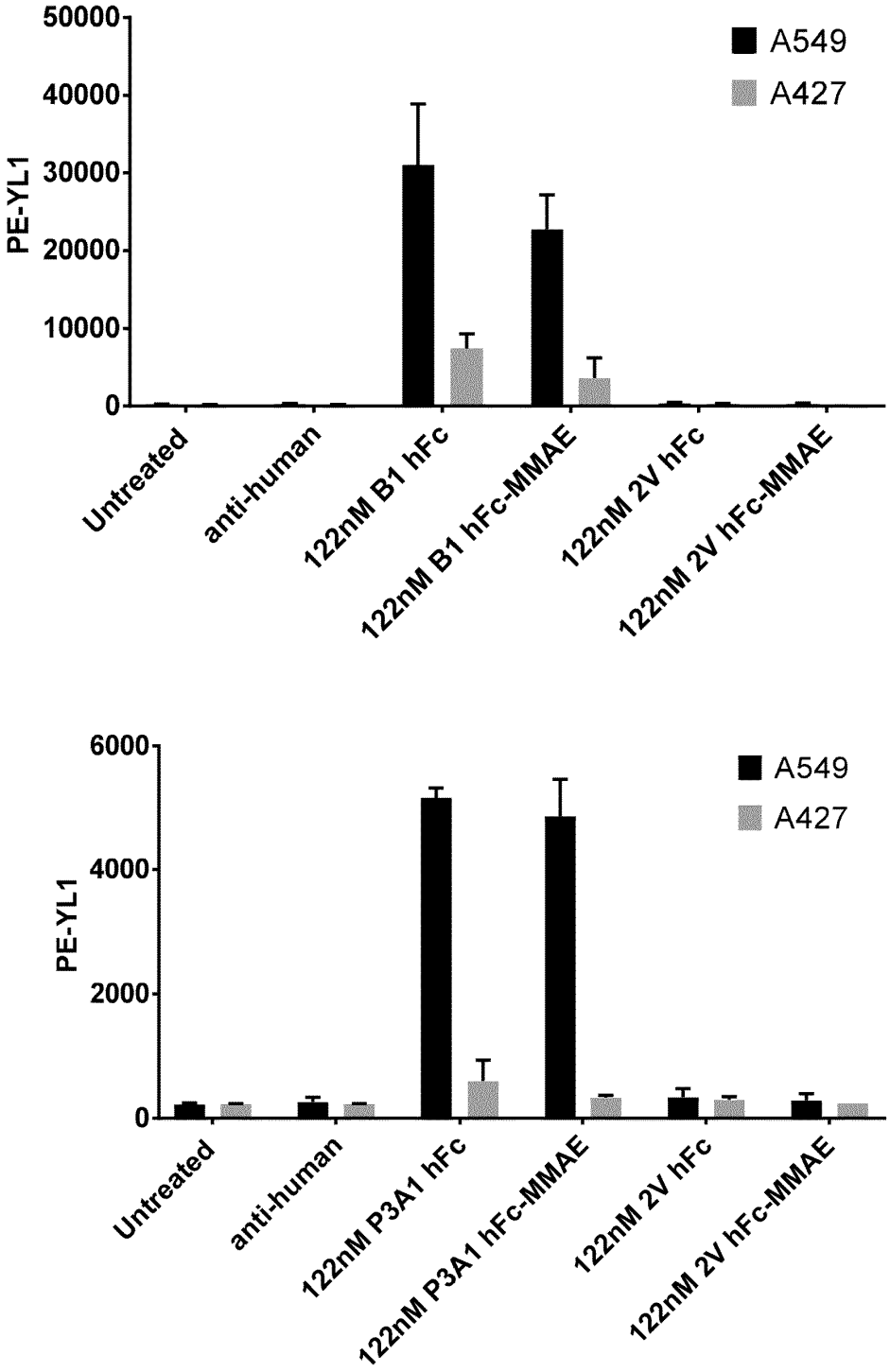

50000
40000
30000
20000
10000
0
PE-YL1
A549
A427
Untreated
anti-human
122nM B1 hFc
122nM B1 hFc-MMAE
122nM 2V hFc
122nM 2V hFc-MMAE
6000
4000
2000
0
PE-YL1
A549
A427
Untreated
anti-human
122nM P3A1 hFc
122nM P3A1 hFc-MMAE
122nM 2V hFc
122nM 2V hFc-MMAE

Fig. 30

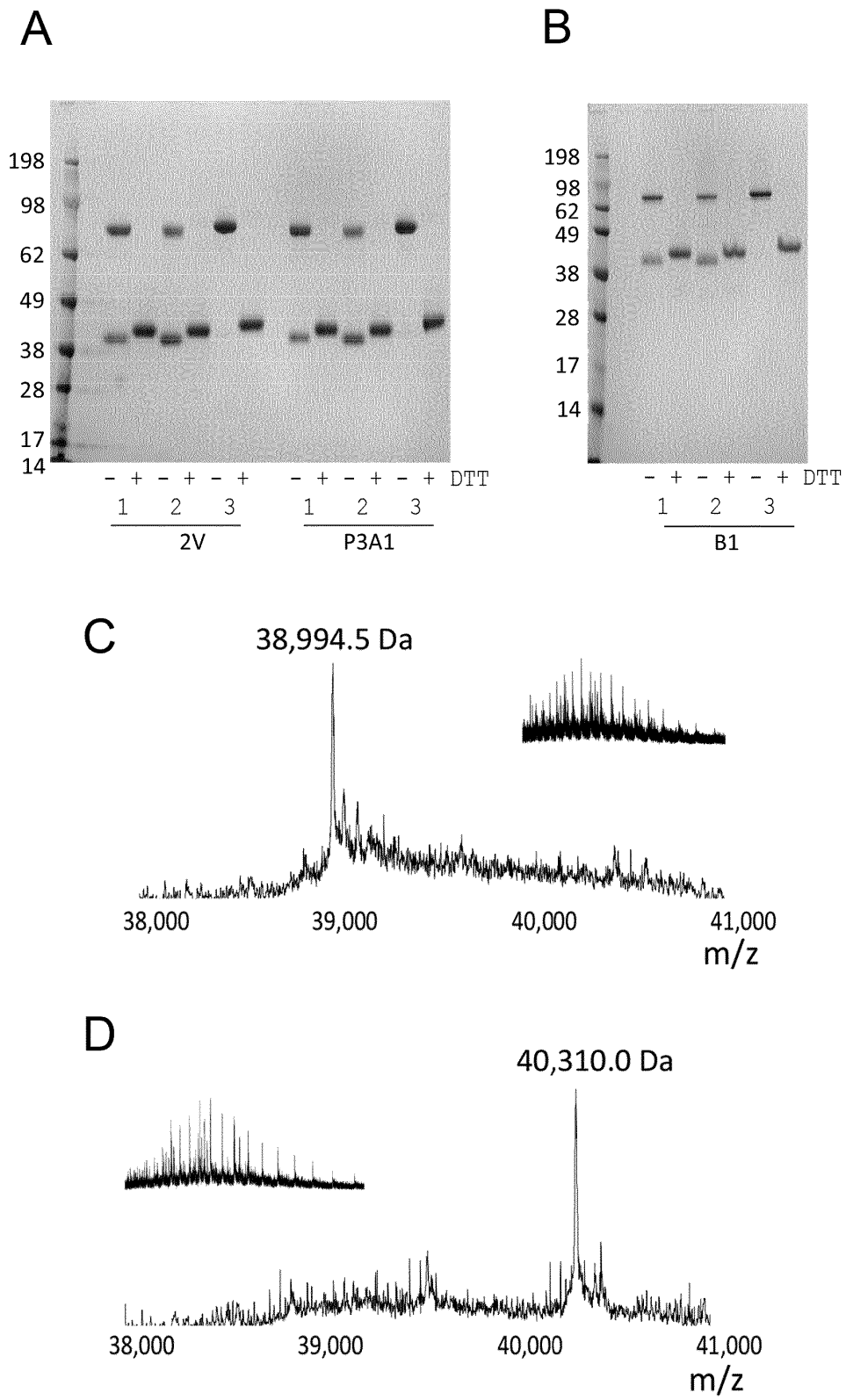

A
198
98
62
49
38
28
17
14
- + - + - + - + - + - + DTT
1 2 3 1 2 3
2V
P3A1
B
198
98
62
49
38
28
17
14
- + - + - + DTT
1 2 3
B1
C
38,994.5 Da
38,000
39,000
40,000
41,000
m/z
D
40,310.0 Da
38,000
39,000
40,000
41,000
m/z

Fig. 31

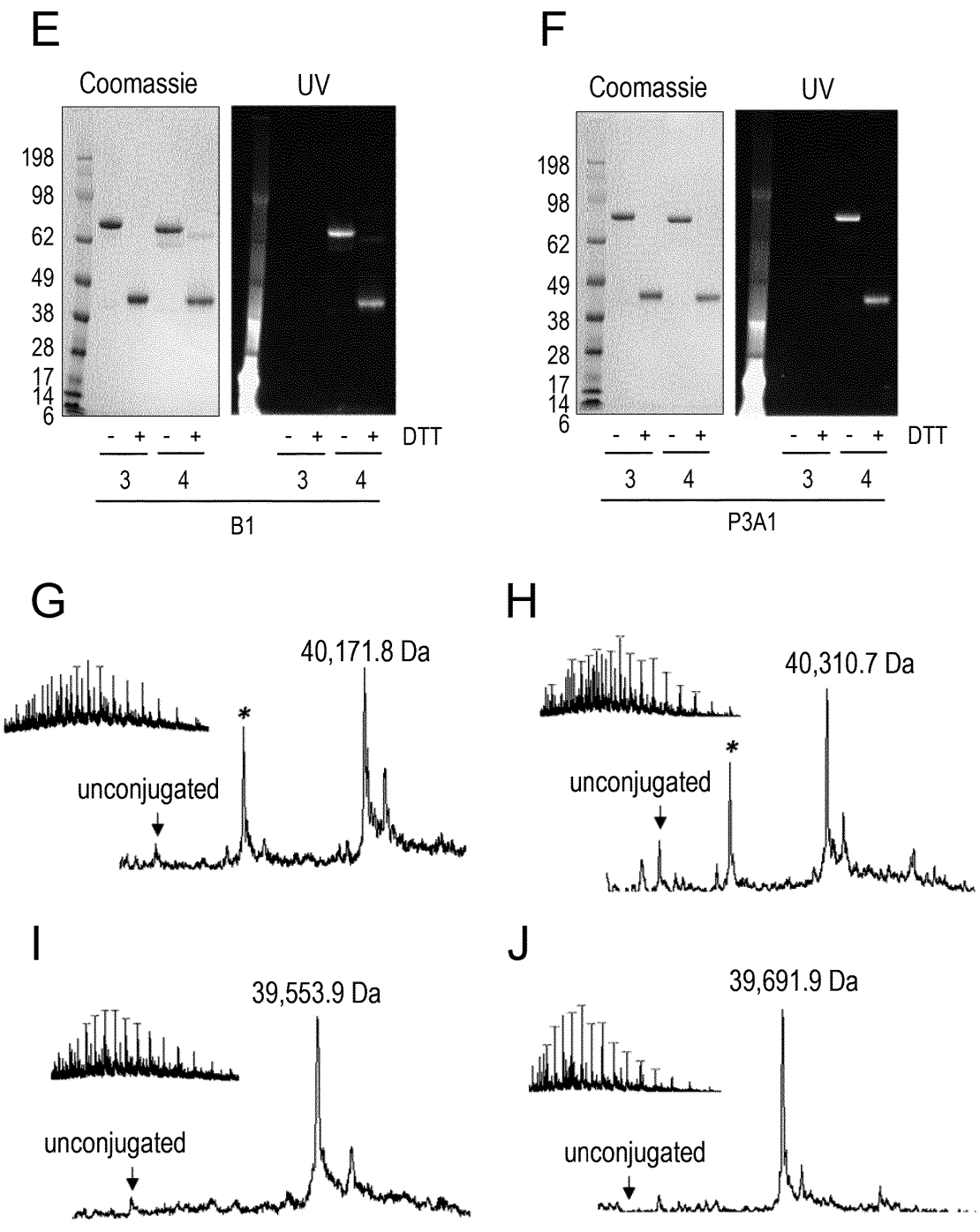
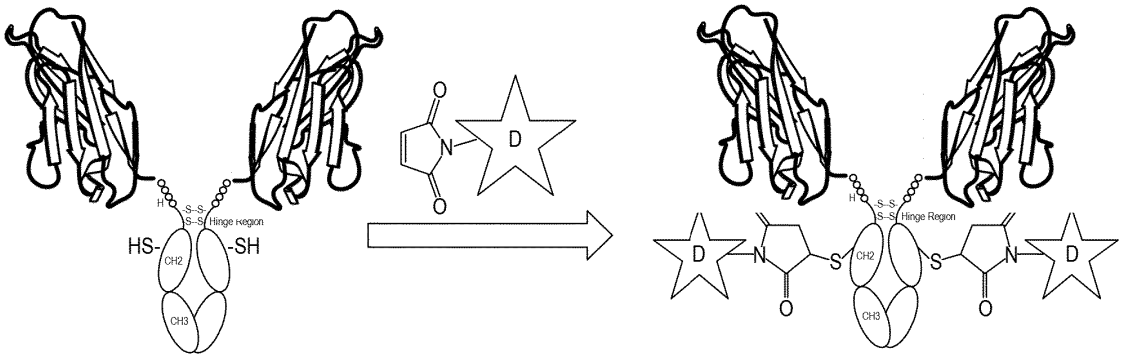


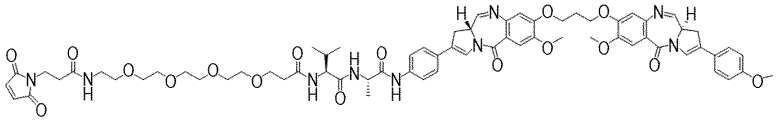
Fig. 31 (continued)



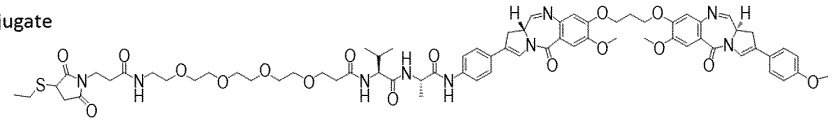
VNAR hFc conjugate

Examples of drug labels (D) and corresponding conjugate:

MA PEG4 va PBD

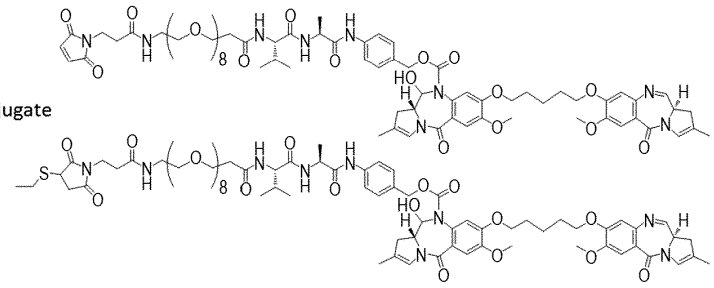


MA PEG4 va PBD conjugate

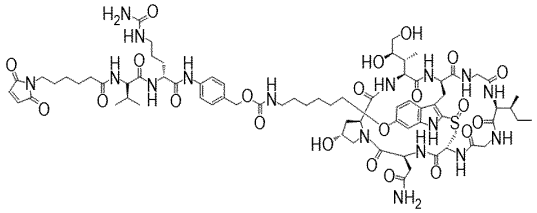


MA PEG8 va PAB SG3199

MA PEG8 va PAB SG3199 conjugate



MC vc PAB NHC_6 α-amanitin



MC vc PAB NHC_6 α-amanitin conjugate

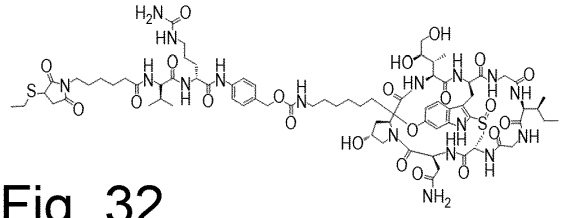
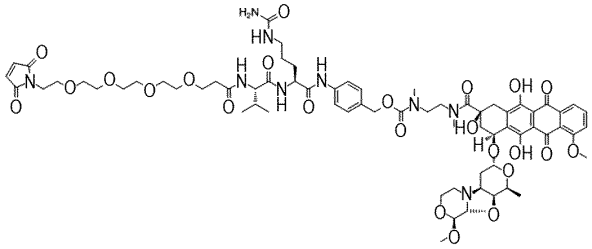


Fig. 32

MA PEG4 vc PAB PNU 159682



MA PEG4 vc PAB PNU 159682 conjugate

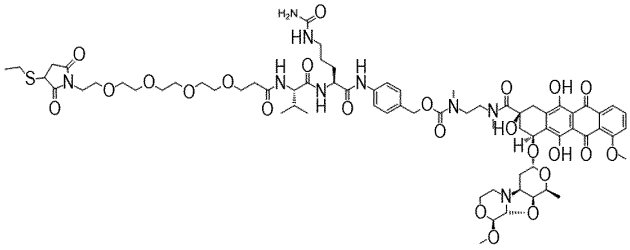
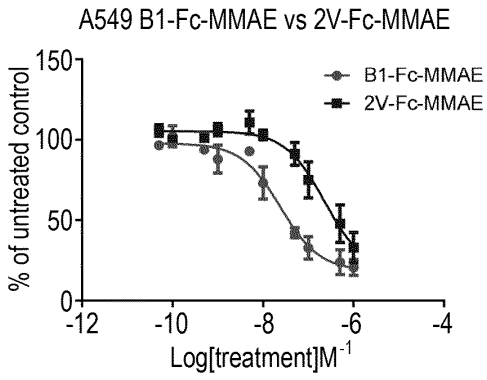
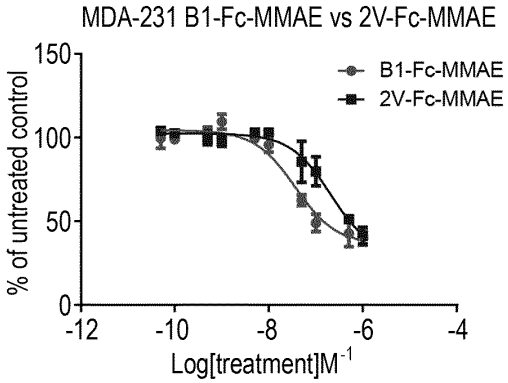


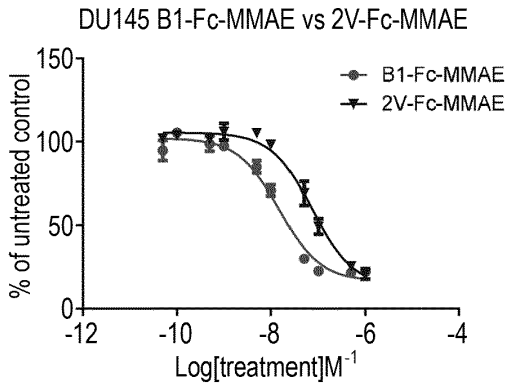
Fig. 32 (continued)



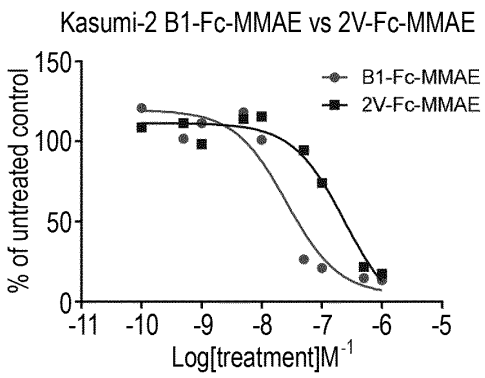
	B1-Fc-MMAE	2V-Fc-MMAE
IC50	2.418e-008	2.277e-007



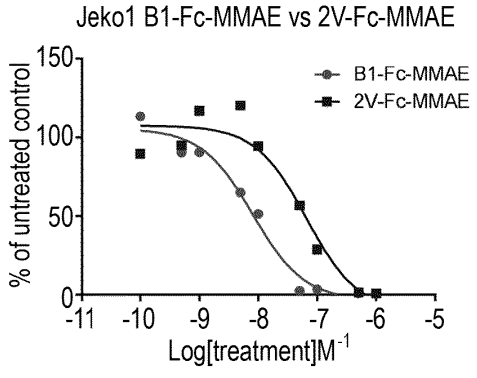
	B1-Fc-MMAE	2V-Fc-MMAE
IC50	3.660e-008	2.119e-007



	B1-Fc-MMAE	2V-Fc-MMAE
IC50	1.501e-008	7.506e-008



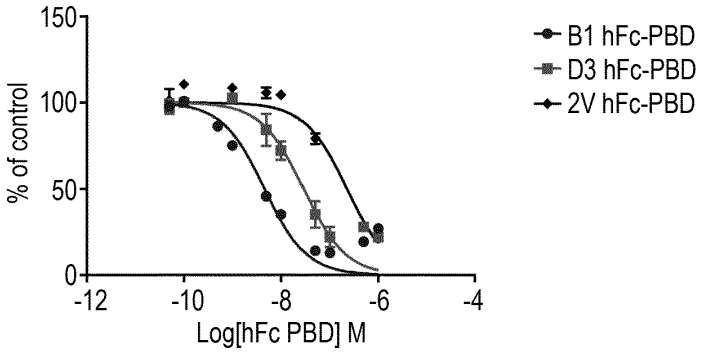
	B1-Fc-MMAE	2V-Fc-MMAE
IC50	2.599e-008	2.397e-007



	B1-Fc-MMAE	2V-Fc-MMAE
IC50	8.100e-009	6.611e-008

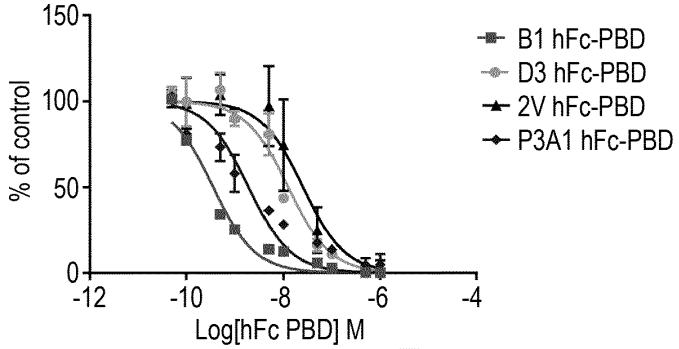
Fig. 33

DU145 cells (96hr cell viability)



	B1 hFc-PBD	D3 hFc-PBD	2V hFc-PBD
IC50	4.612e-009	2.917e-008	2.262e-007

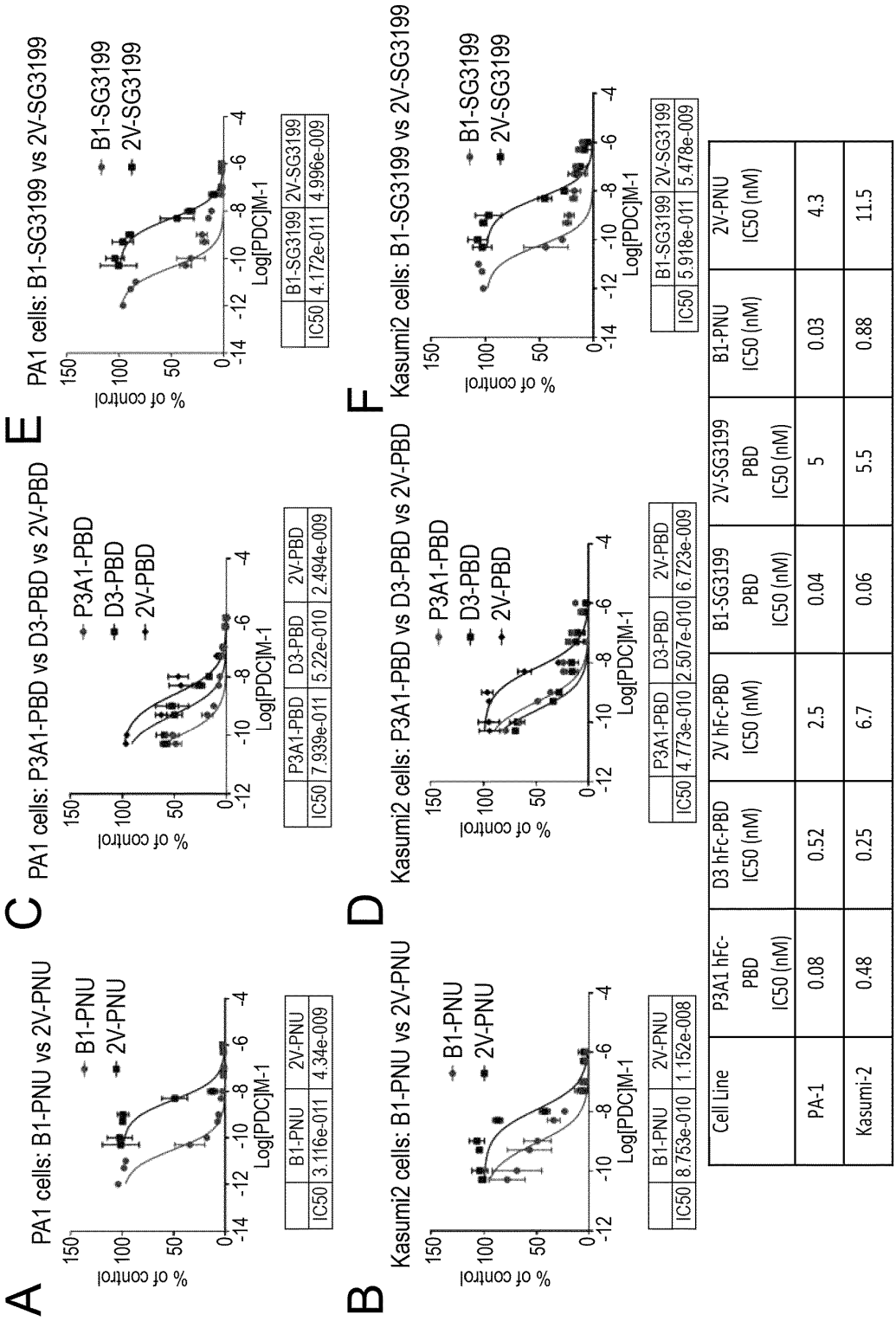
Jeko-1 (96hr cell viability)



	B1 hFc-PBD	P3A1 hFc-PBD	D3 hFc-PBD	2V hFc-PBD
IC50	3.607e-010	1.939e-009	1.256e-008	2.539e-008

Cell Line	B1 hFc-PBD IC50 (nM)	P3A1 hFc-PBD IC50 (nM)	D3 hFc-PBD IC50 (nM)	2V hFc-PBD IC50 (nM)
DU145	4.6	/	29.2	226.2
JeKo-1	0.36	1.9	12.6	25.4

Fig. 34



Cell Line	P3A1 hFc-PBD IC50 (nM)	D3 hFc-PBD IC50 (nM)	2V hFc-PBD IC50 (nM)	B1-SG3199 PBD IC50 (nM)	2V-SG3199 PBD IC50 (nM)	B1-PNU IC50 (nM)	2V-PNU IC50 (nM)
PA-1	0.08	0.52	2.5	0.04	5	0.03	4.3
Kasumi-2	0.48	0.25	6.7	0.06	5.5	0.88	11.5

Fig. 35

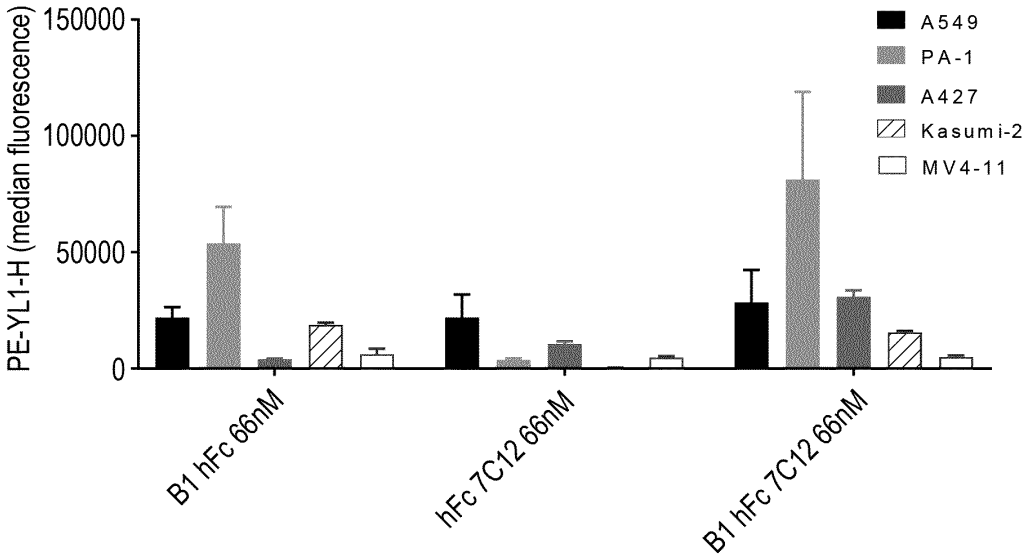


Fig. 36A

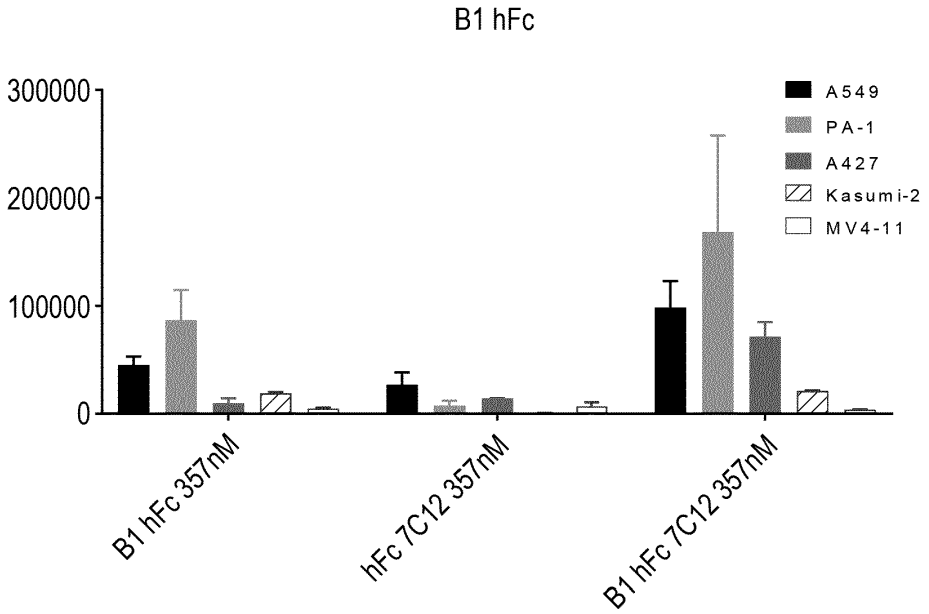


Fig. 36B

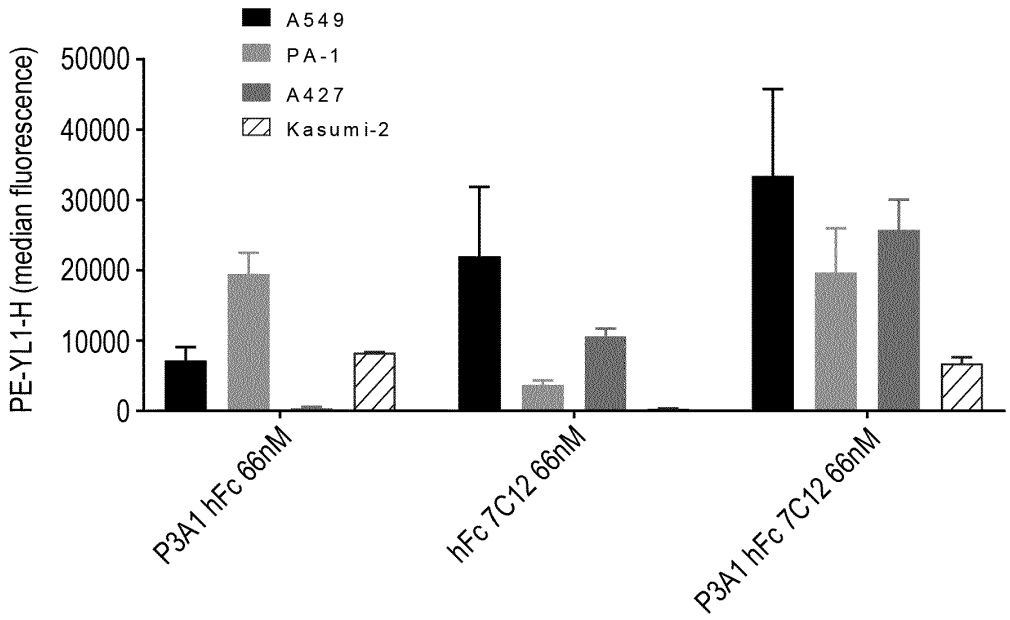


Fig. 36C

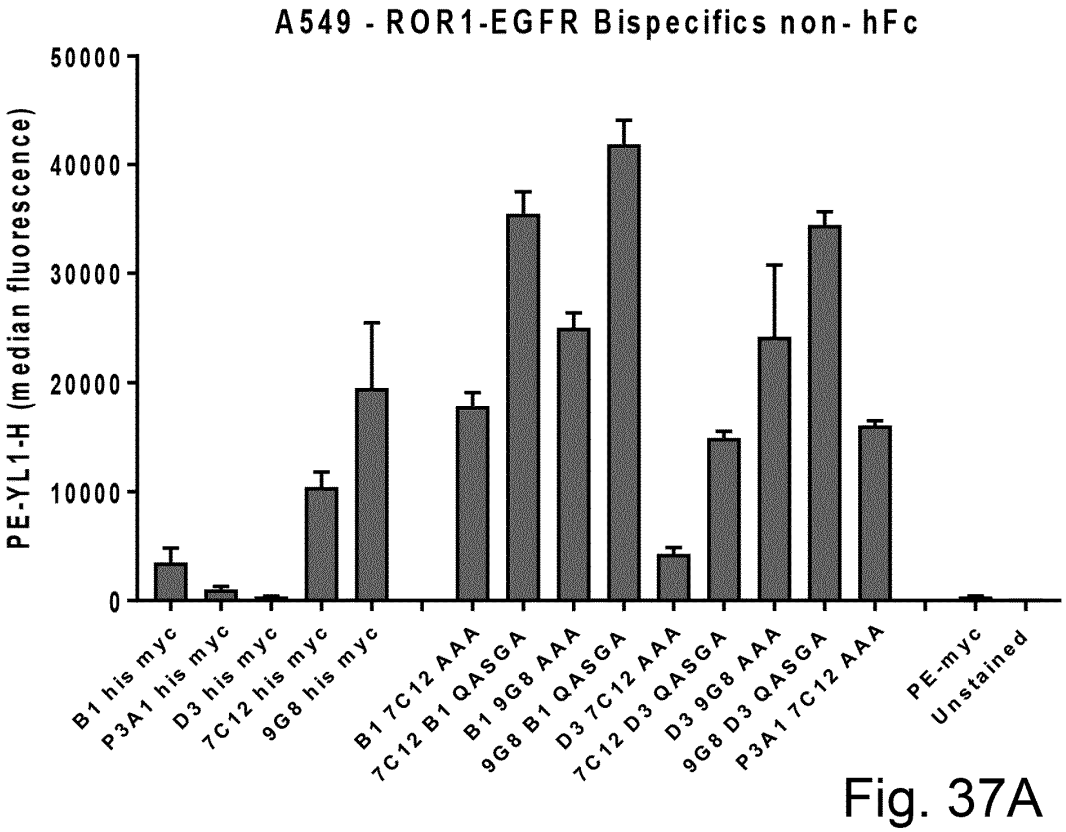


Fig. 37A

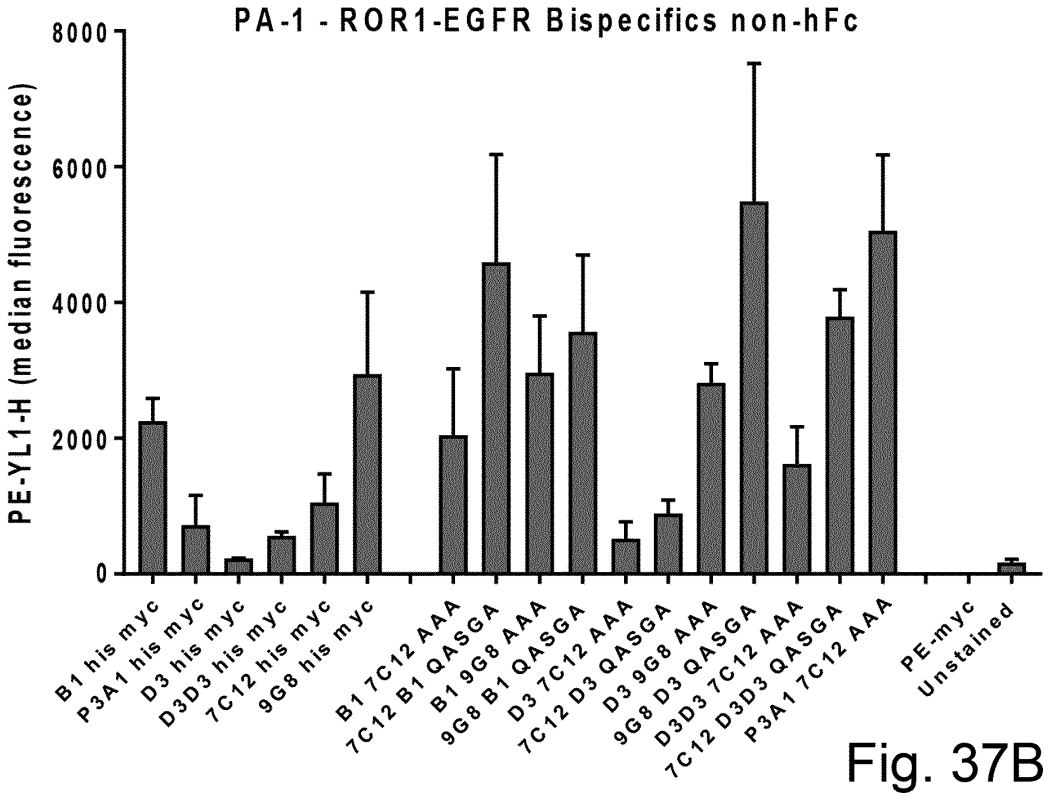


Fig. 37B

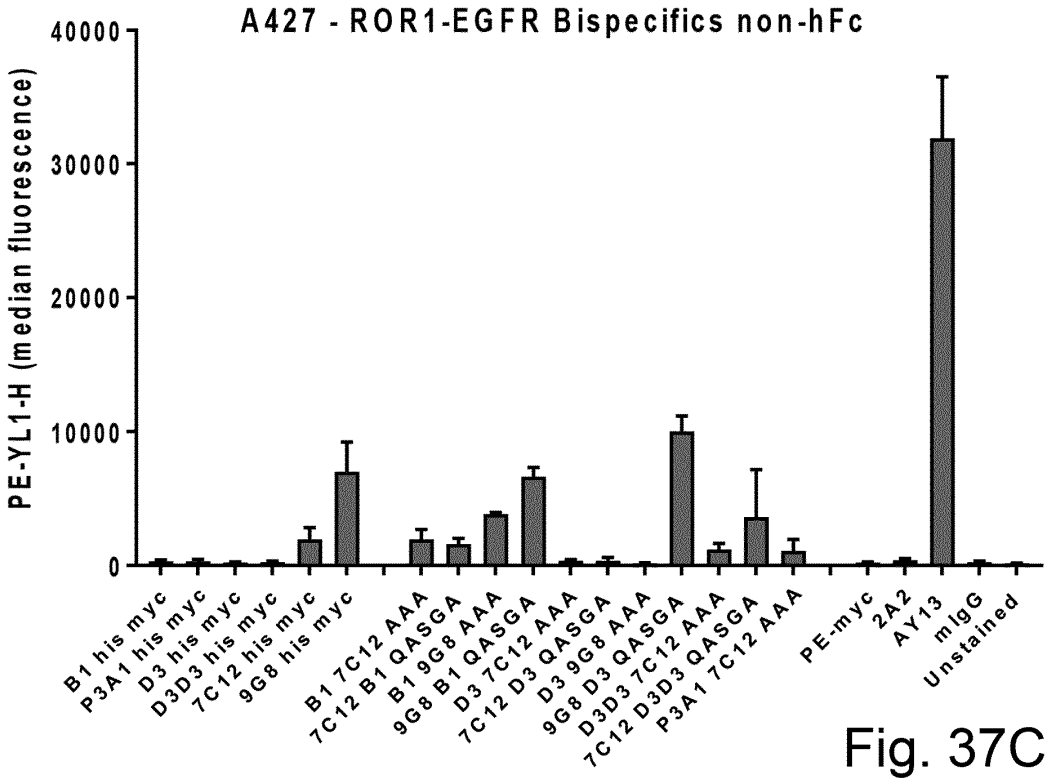


Fig. 37C

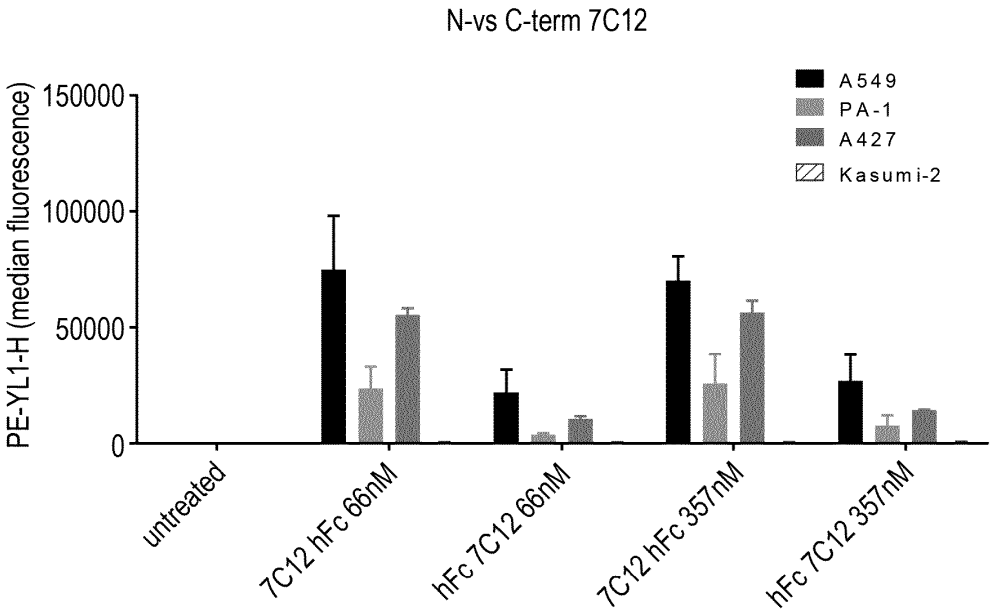

N-vs C-term 7C12
PE-YL1-H (median fluorescence)
150000
100000
50000
0
A549
PA-1
A427
Kasumi-2
untreated
7C12 hFc 66nM
hFc 7C12 66nM
7C12 hFc 357nM
hFc 7C12 357nM

Fig. 38

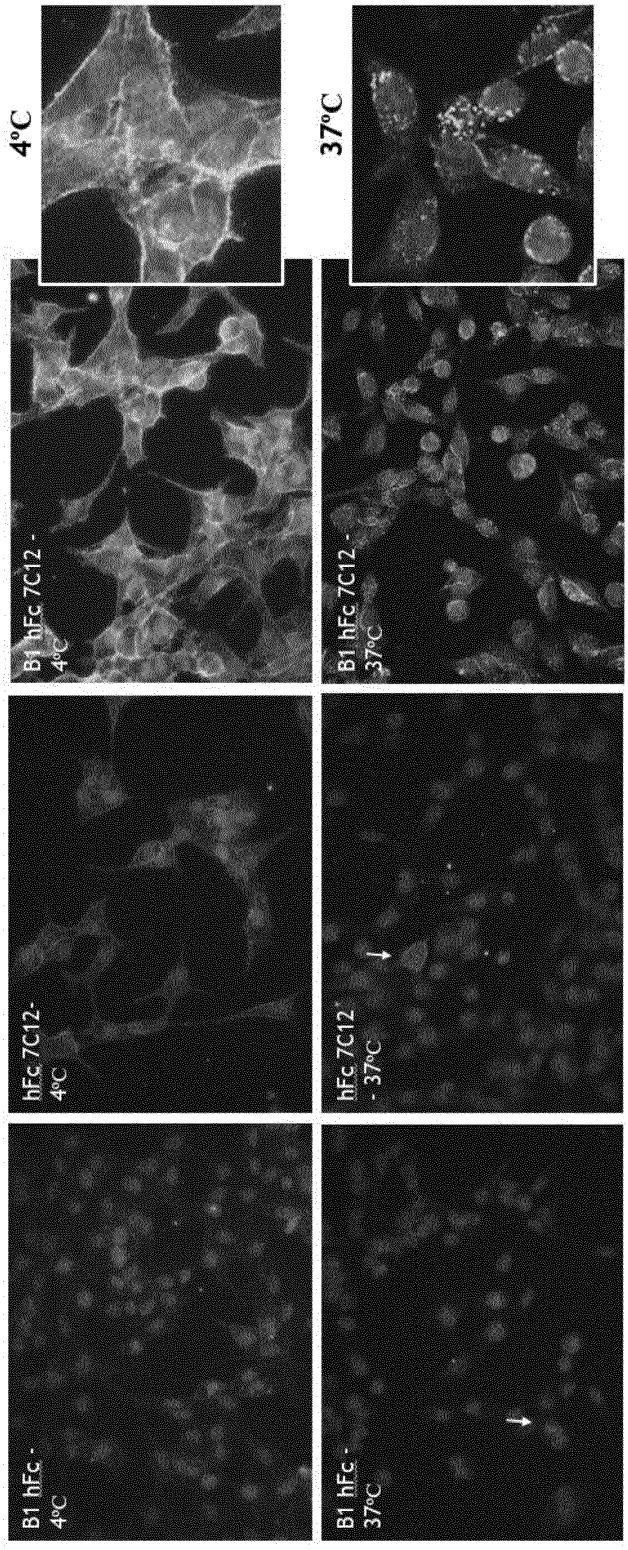

4°C
37°C
B1 hFc 7C12 - 4°C
B1 hFc 7C12 - 37°C
hFc 7C12 - 4°C
hFc 7C12 - 37°C
B1 hFc - 4°C
B1 hFc - 37°C

Fig. 39

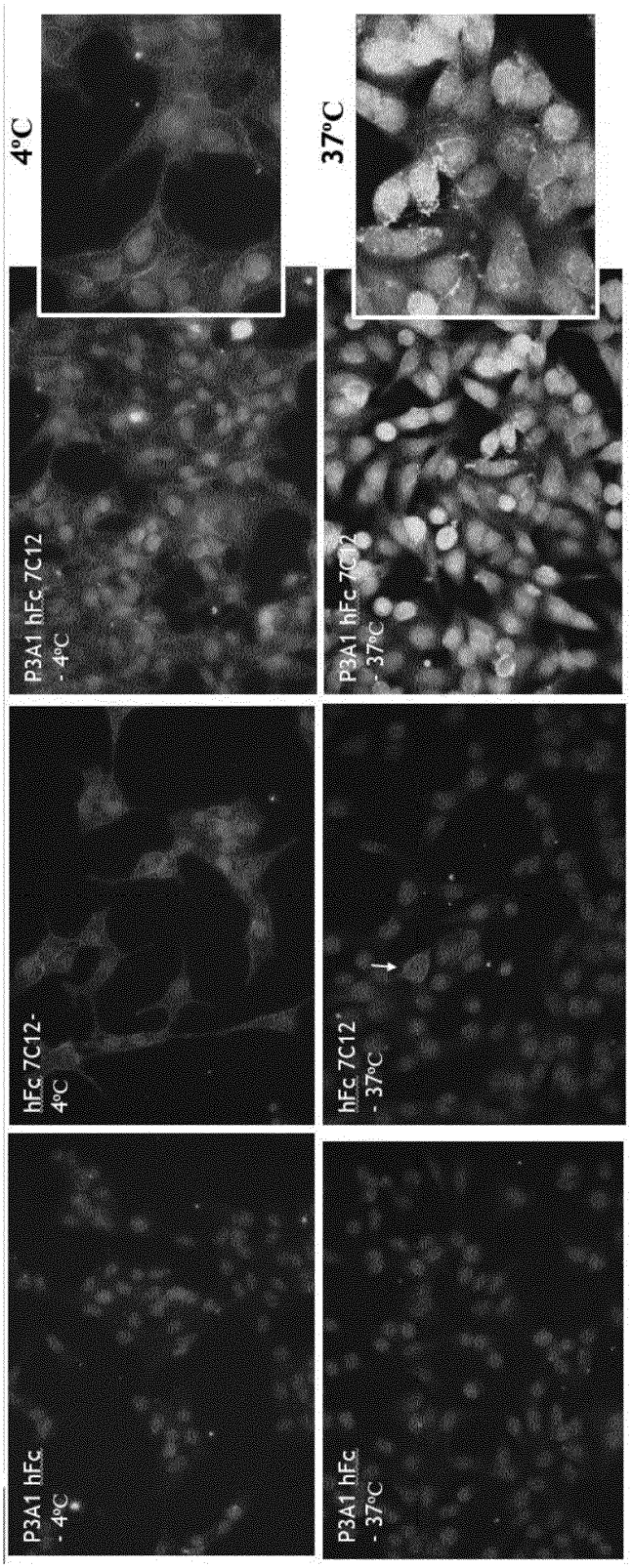

4°C
37°C
P3A1 hFc 7C12
- 4°C
P3A1 hFc 7C12
- 37°C
hFc 7C12-
4°C
hFc 7C12
- 37°C
P3A1 hFc
- 4°C
P3A1 hFc
- 37°C

Fig. 40

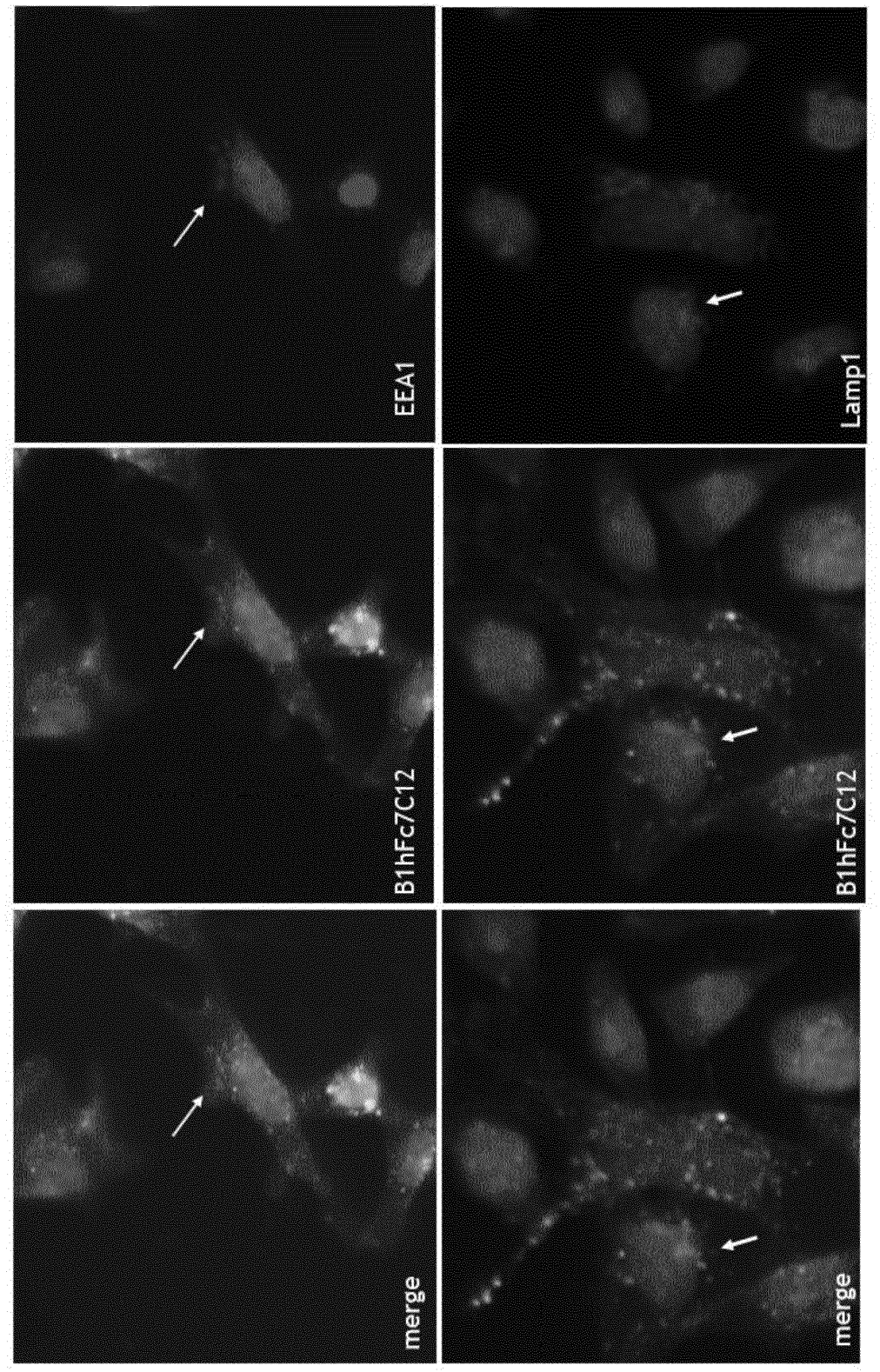


Fig. 41

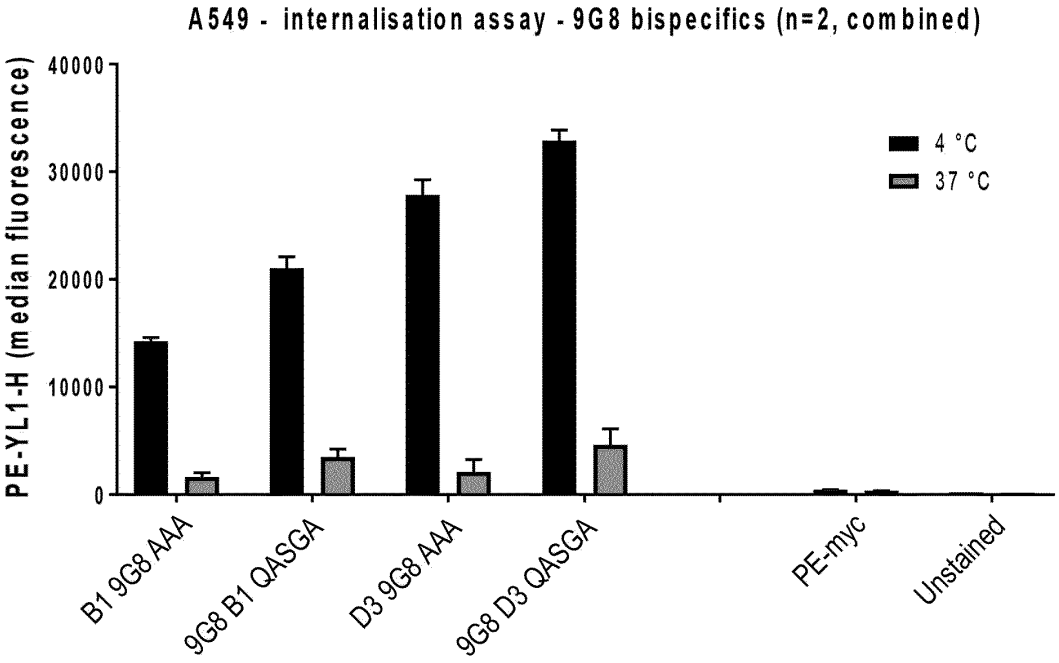


Fig. 42A

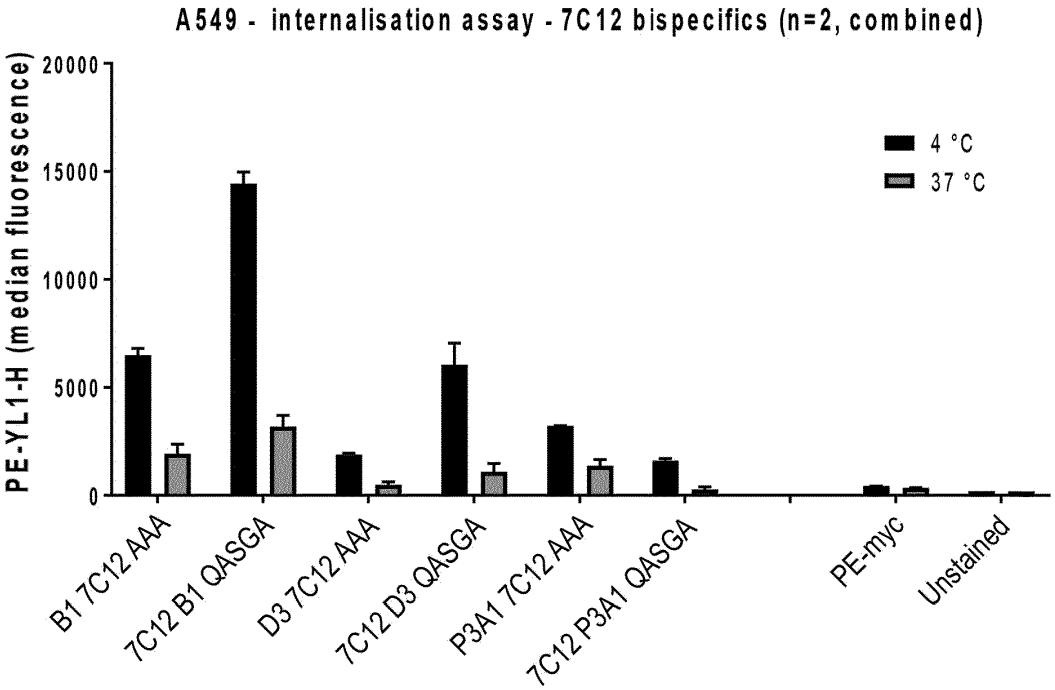


Fig. 42B

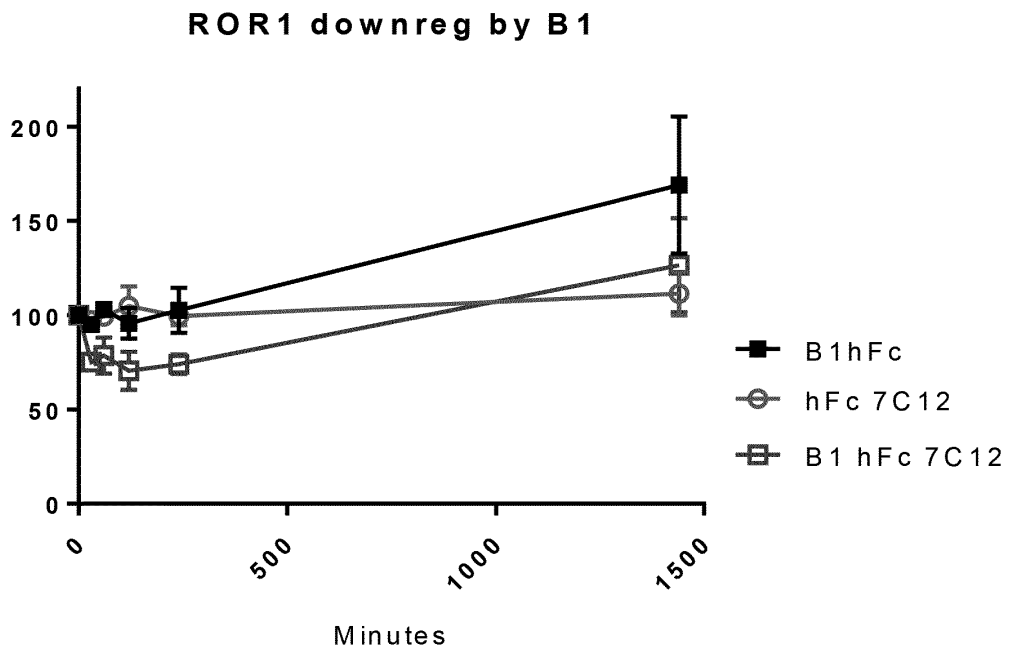


Fig. 43A

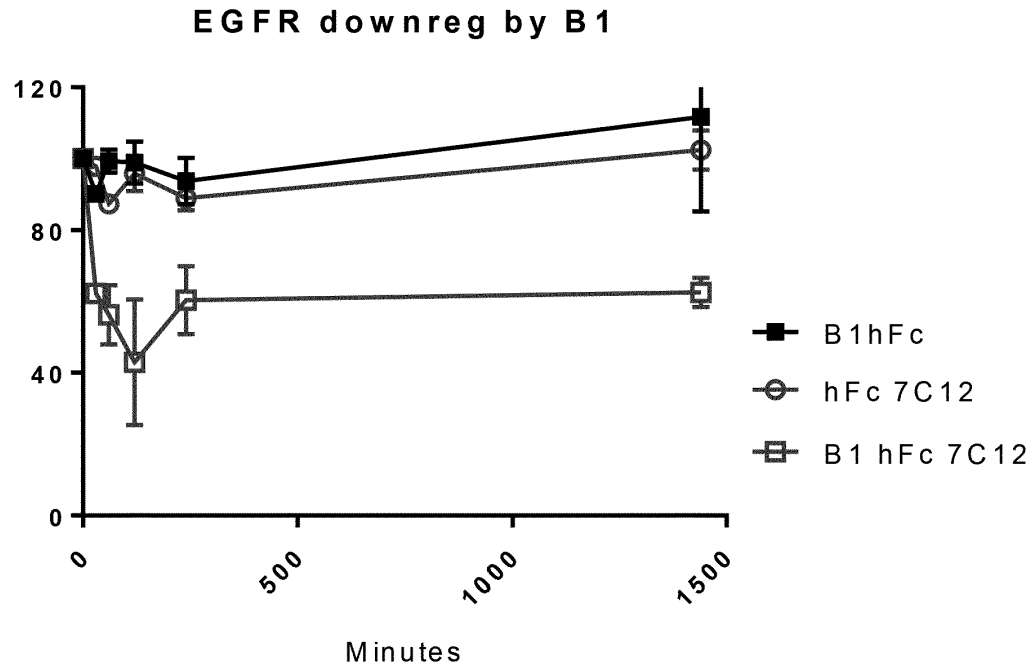


Fig. 43B

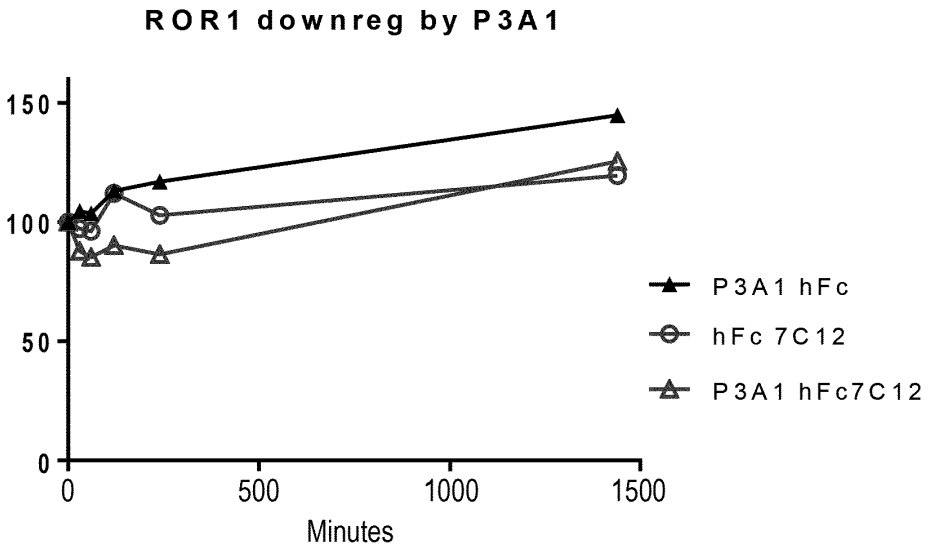


Fig. 44A

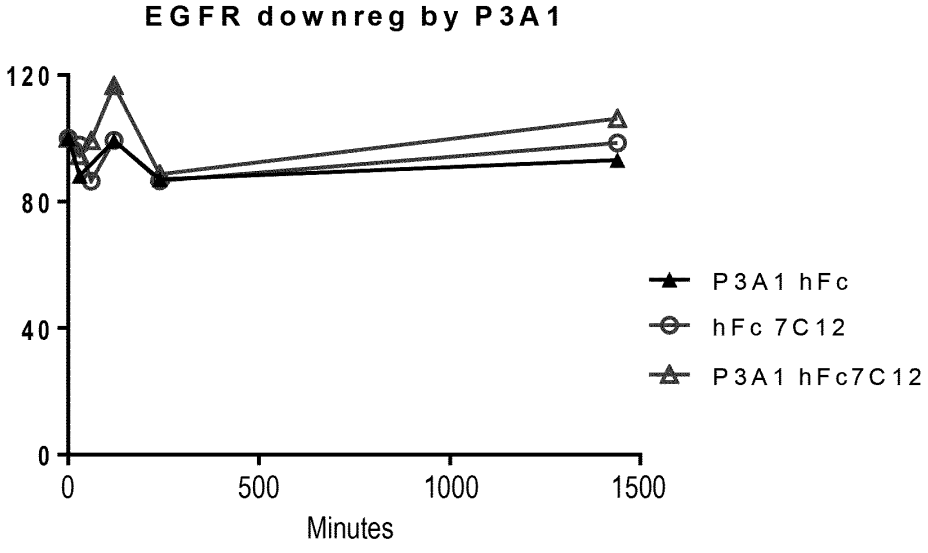


Fig. 44B

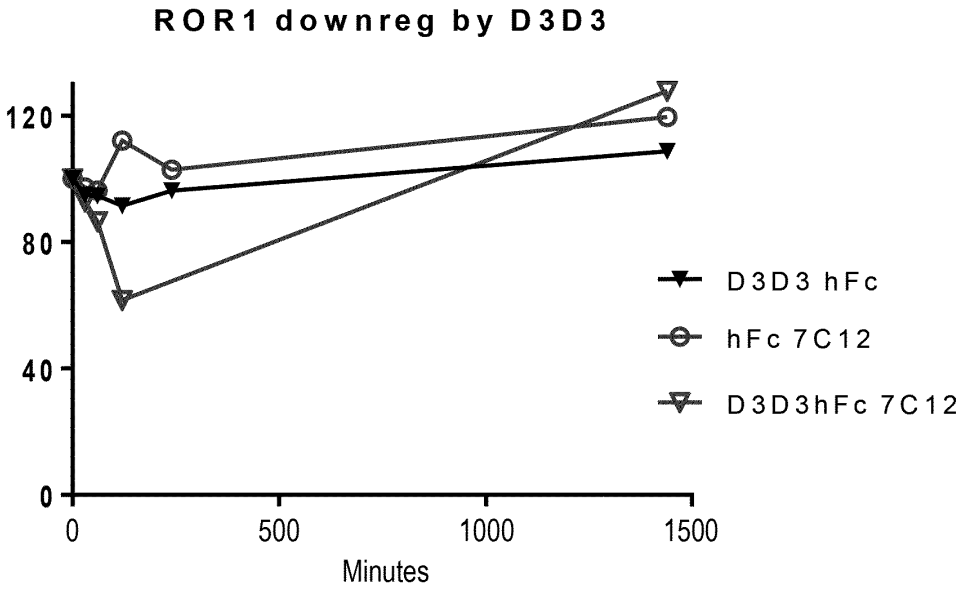


Fig. 45A

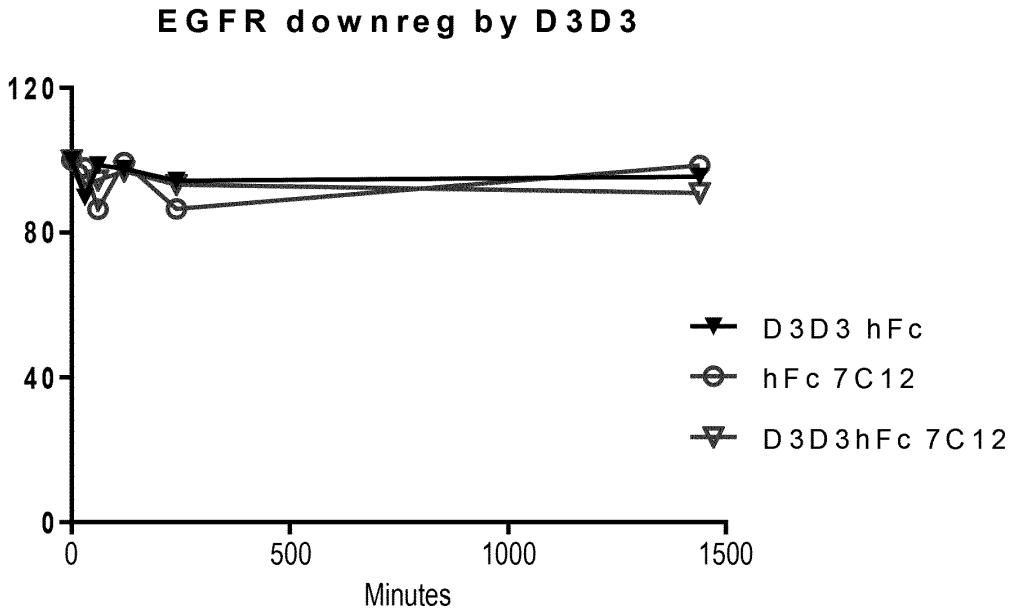


Fig. 45B

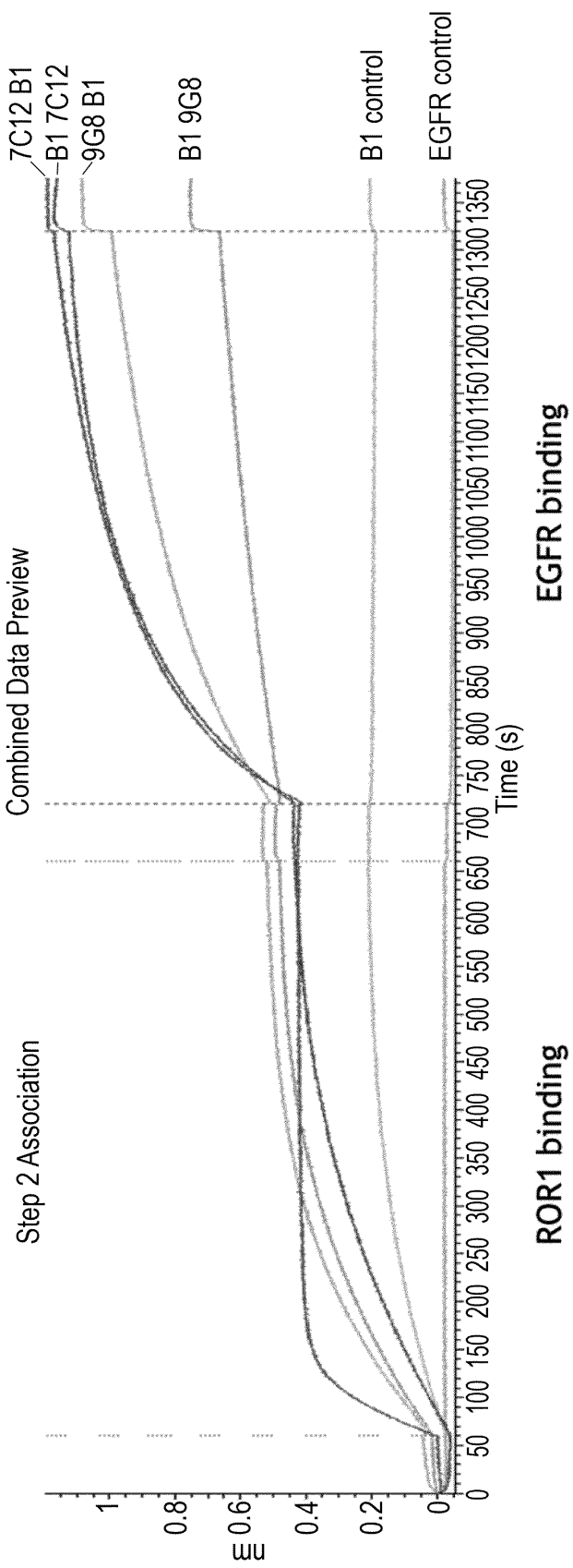

7C12 B1
B1 7C12
9G8 B1
B1 9G8
B1 control
EGFR control
Combined Data Preview
Step 2 Association
nm
Time (s)
ROR1 binding
EGFR binding

Fig. 46