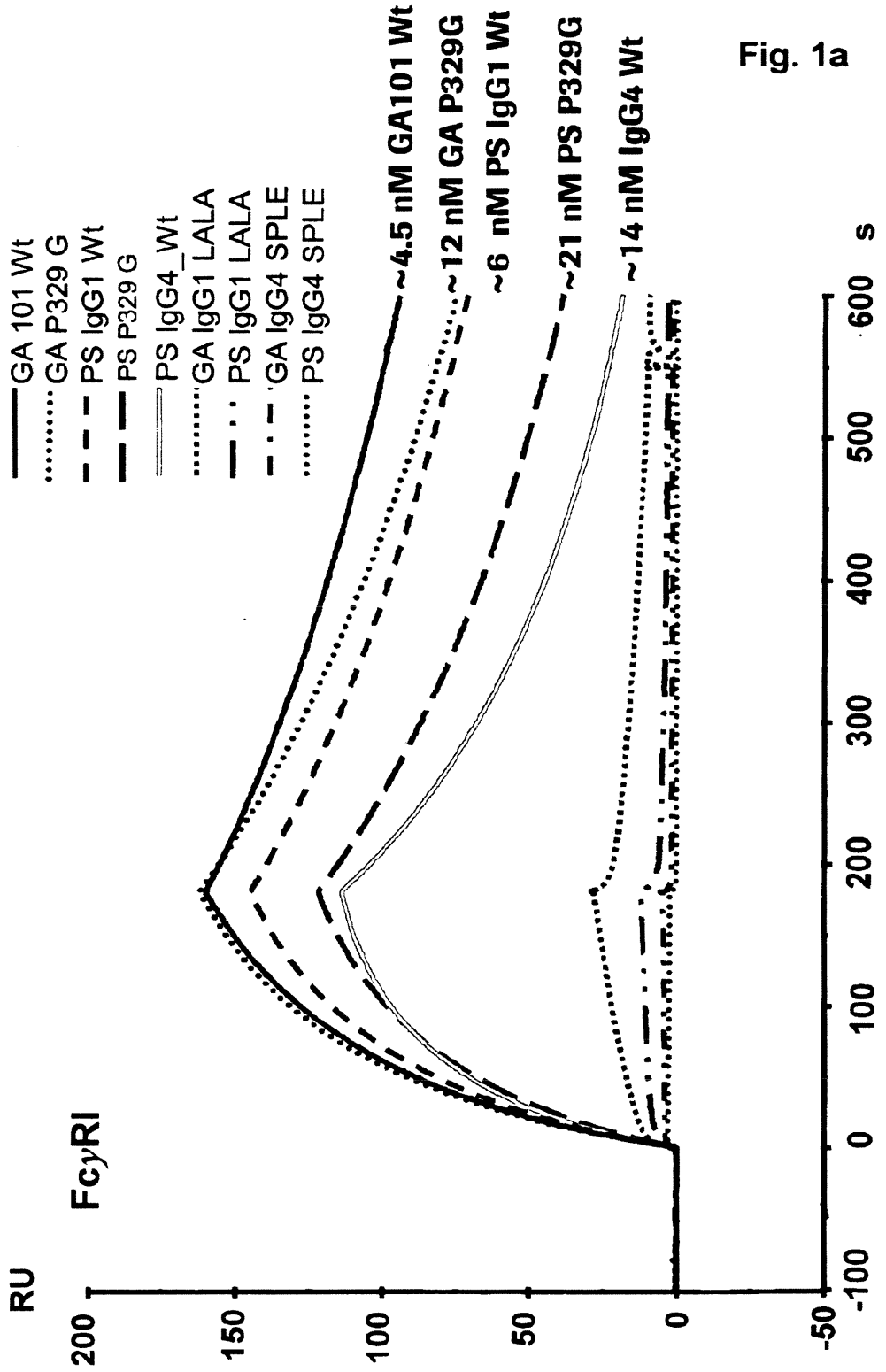

RU
FcγRI
200
150
100
50
0
-50
-100
0
100
200
300
400
500
600
s
GA 101 Wt
GA P329 G
PS IgG1 Wt
PS P329 G
PS IgG4_Wt
GA IgG1 LALA
PS IgG1 LALA
GA IgG4 SPLE
PS IgG4 SPLE
~4.5 nM GA101 Wt
~12 nM GA P329G
~6 nM PS IgG1 Wt
~21 nM PS P329G
~14 nM IgG4 Wt

Fig. 1a

Fig. 1b

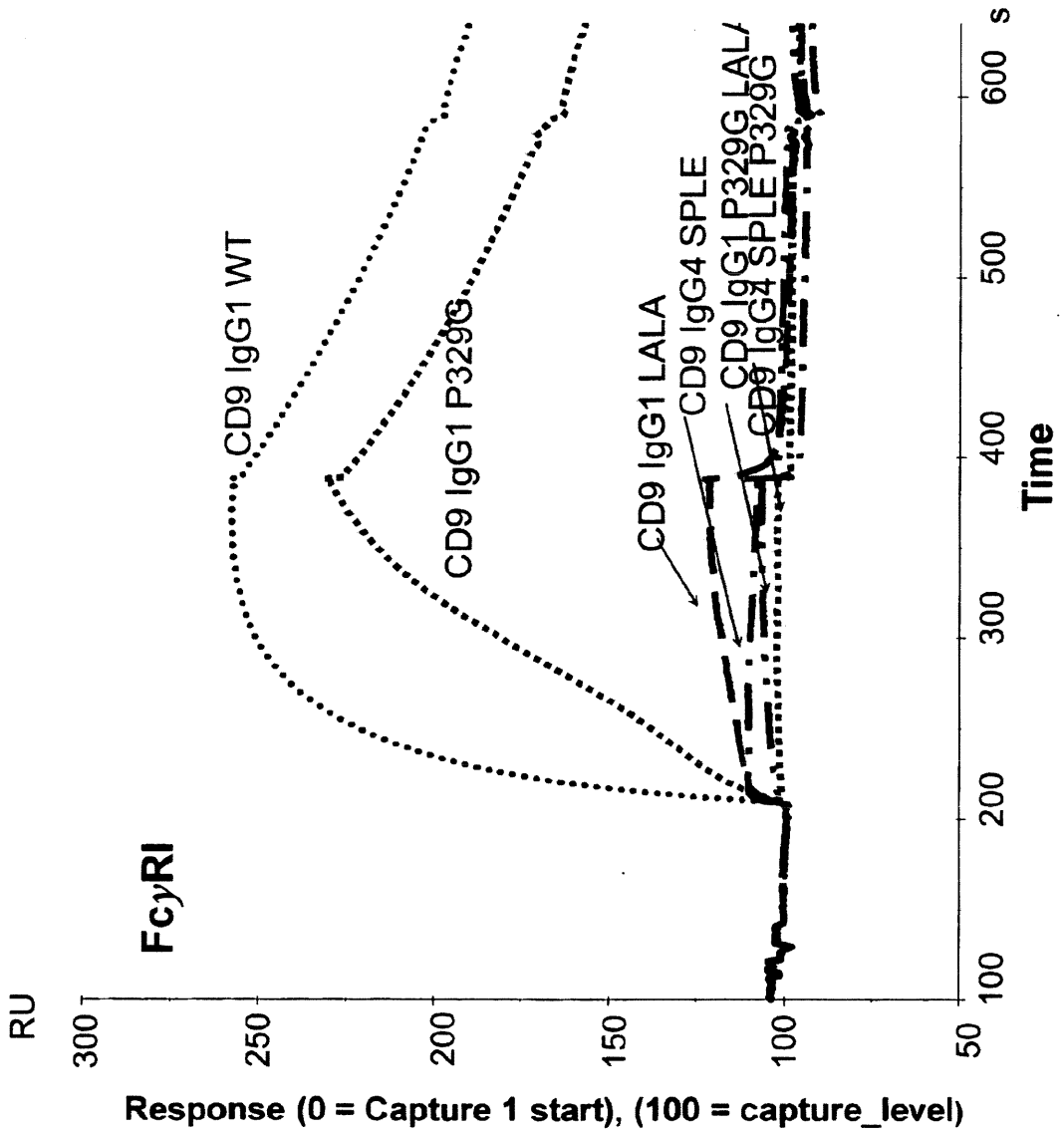

FcγRI
RU
300
250
200
150
100
50
100
200
300
400
500
600
s
Time
CD9 IgG1 WT
CD9 IgG1 P329G
CD9 IgG1 LALA
CD9 IgG4 SPLE
CD9 IgG1 P329G LALA
CD9 IgG4 SPLE P329G
Response (0 = Capture 1 start), (100 = capture_level)

Fig. 1c

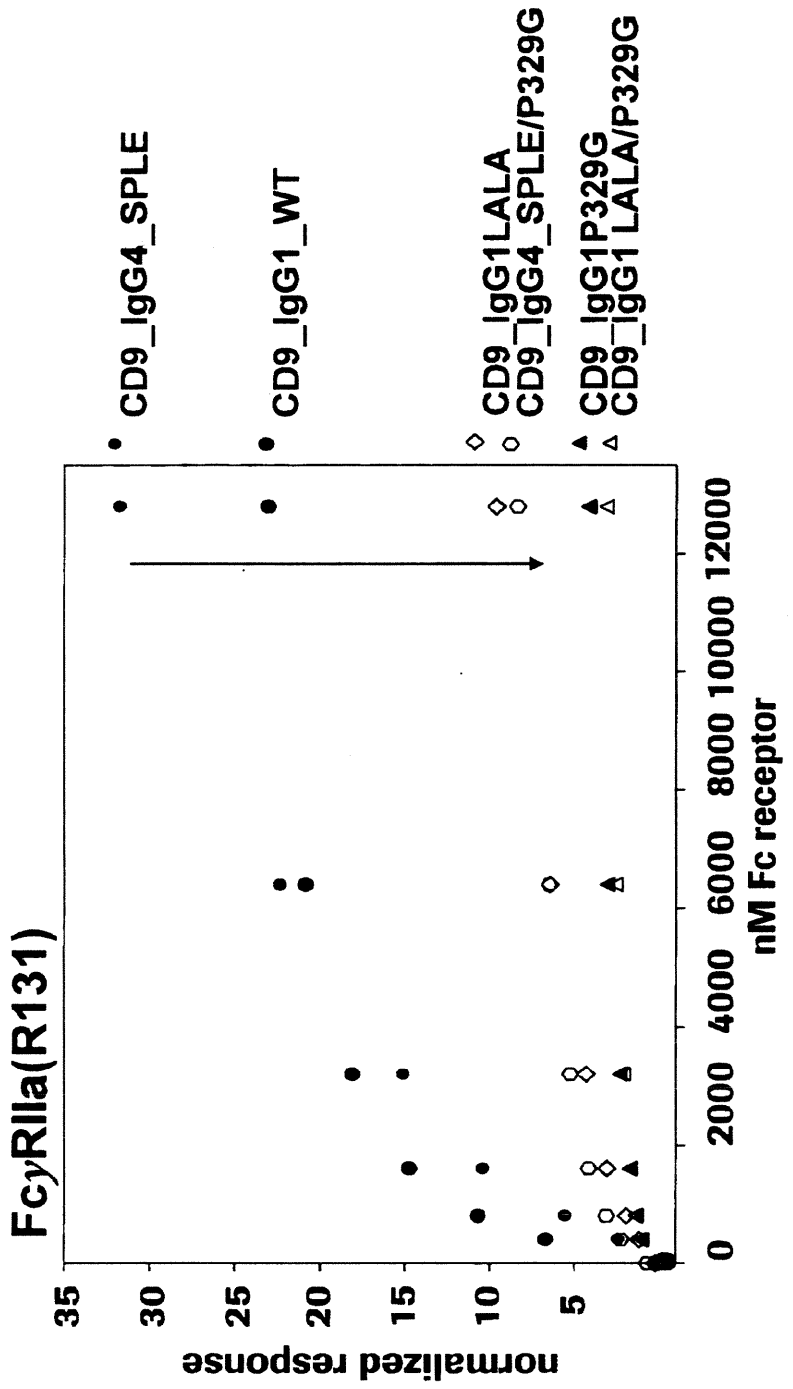

FcγRIIa(R131)
normalized response
nM Fc receptor
0
2000
4000
6000
8000
10000
12000
5
10
15
20
25
30
35
CD9_IgG4_SPLE
CD9_IgG1_WT
CD9_IgG1LALA
CD9_IgG4_SPLE/P329G
CD9_IgG1P329G
CD9_IgG1 LALA/P329G

Fig. 1d

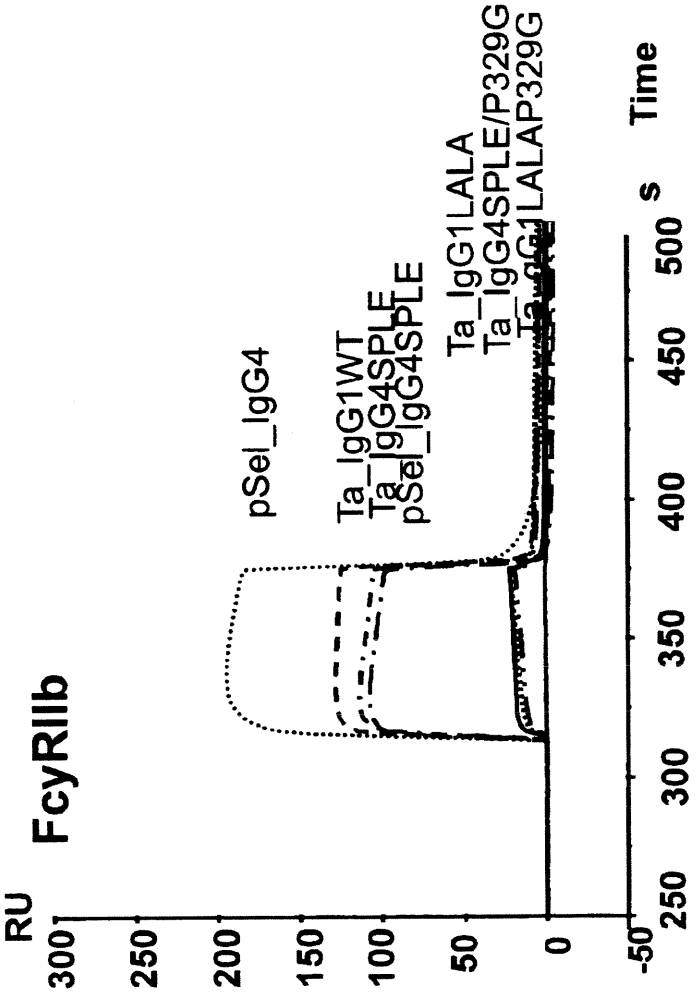

FcyRIIb
RU
300
250
200
150
100
50
0
-50
250
300
350
400
450
500
s
Time
pSel_IgG4
Ta_IgG1WT
Ta_IgG4SPLE
pSel_IgG4SPLE
Ta_IgG1LALA
Ta_IgG4SPLE/P329G
Ta_IgG1LALAP329G

Fig. 1e

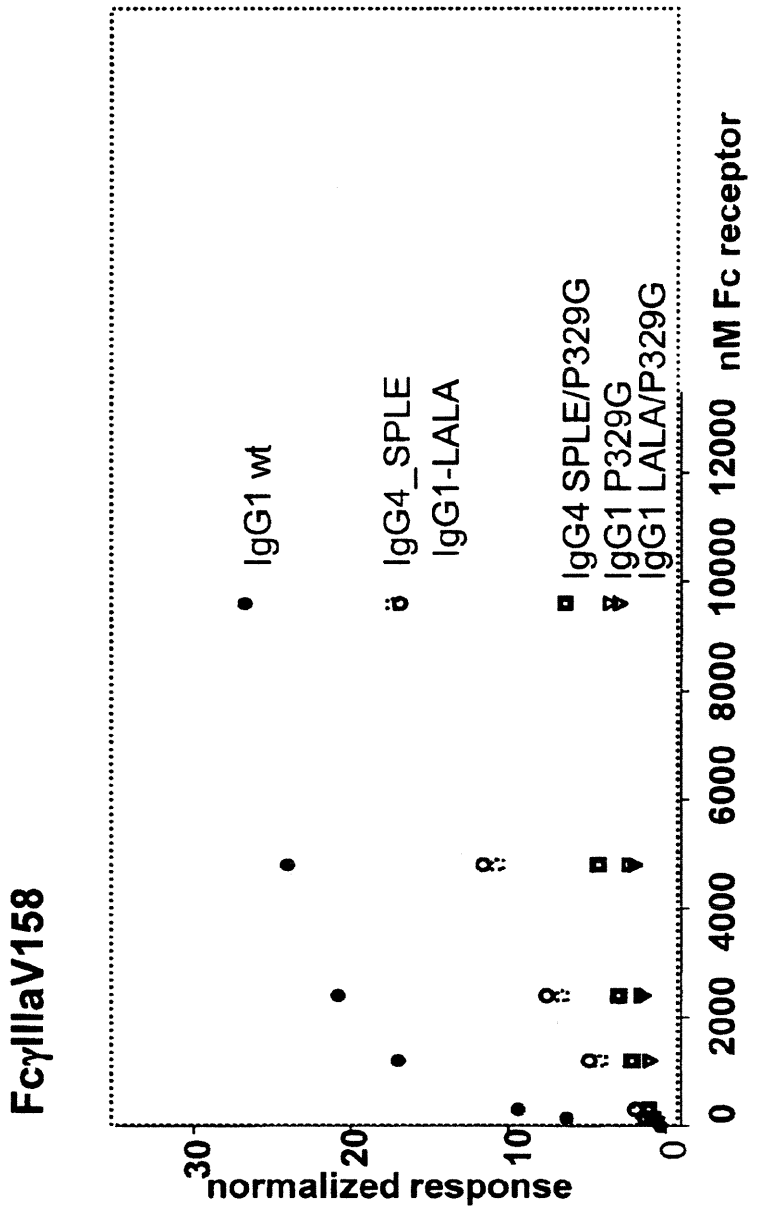
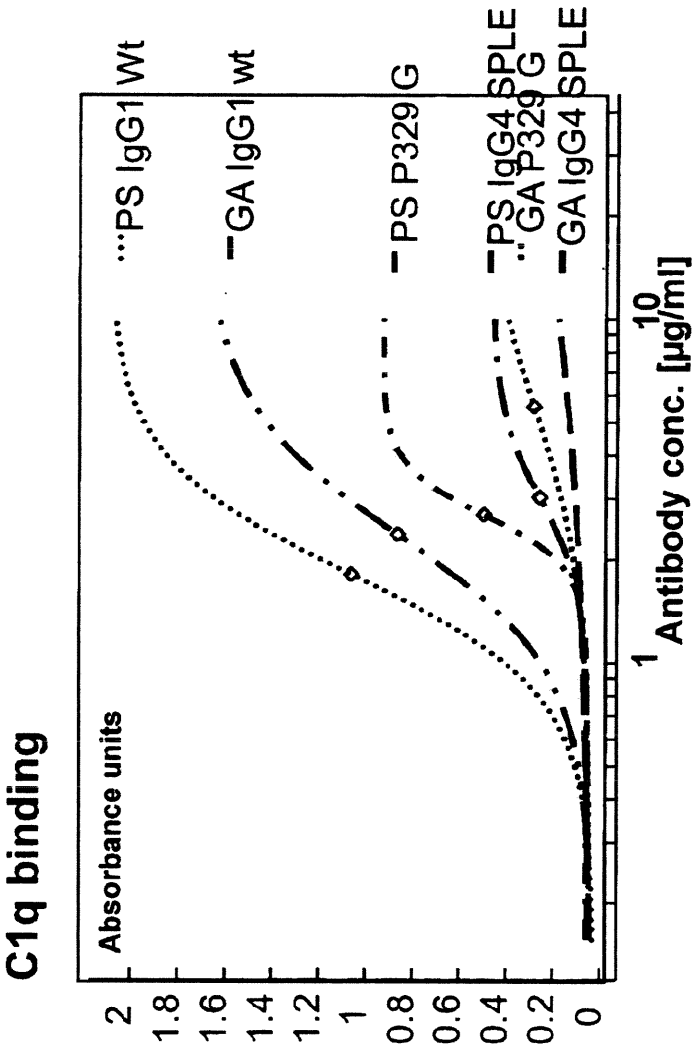

FcγIIIaV158
normalized response
30
20
10
0
0
2000
4000
6000
8000
10000
12000
nM Fc receptor
IgG1 wt
IgG4_SPLE
IgG1-LALA
IgG4 SPLE/P329G
IgG1 P329G
IgG1 LALA/P329G

Fig. 2


C1q binding
Absorbance units
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
10
Antibody conc. [µg/ml]
PS IgG1 Wt
GA IgG1 wt
PS P329G
PS IgG4 SPLE
GA P329G
GA IgG4 SPLE

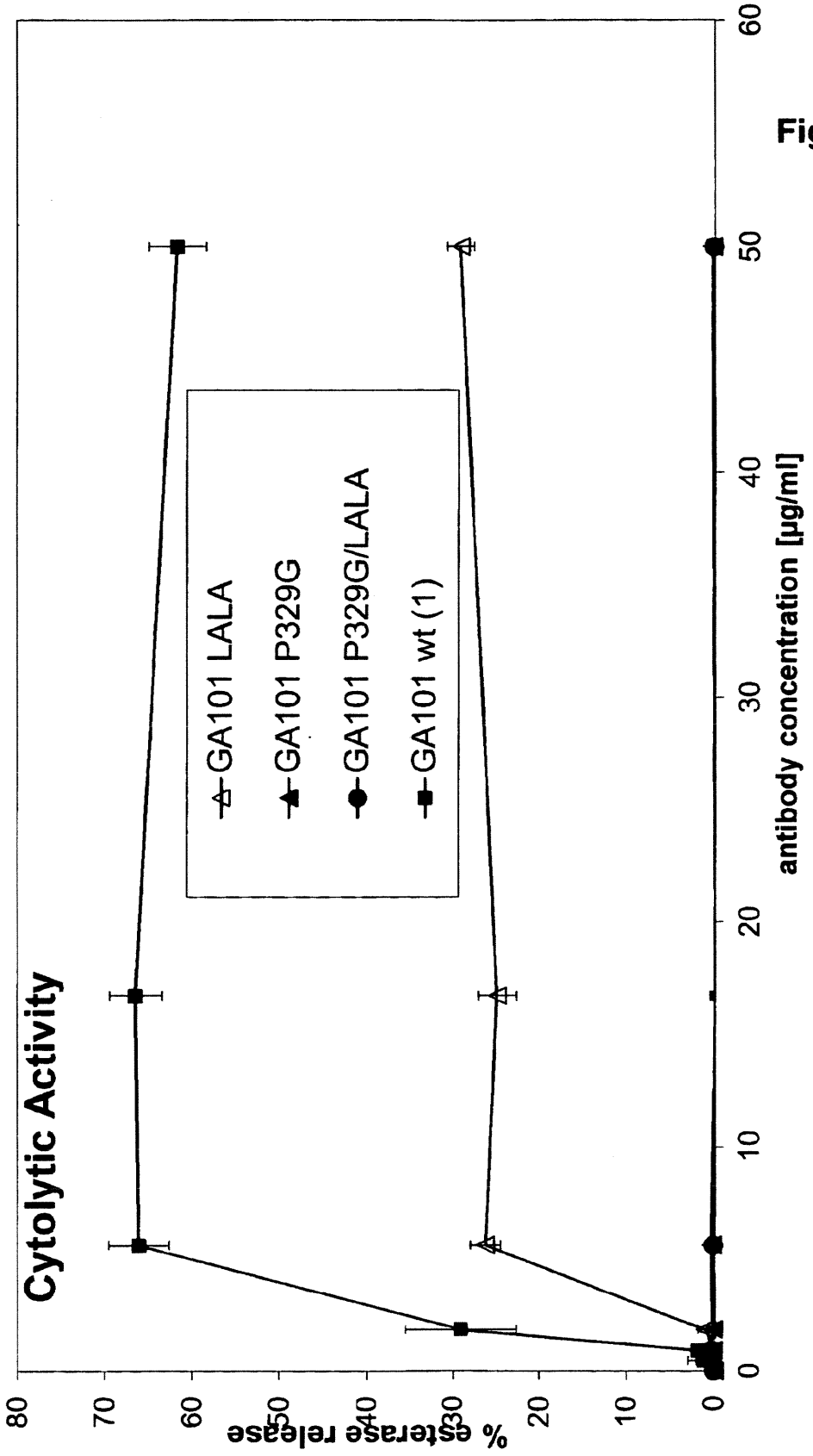

Cytolytic Activity
% esterase release
antibody concentration [µg/ml]
GA101 LALA
GA101 P329G
GA101 P329G/LALA
GA101 wt (1)
0
10
20
30
40
50
60
70
80

Fig. 3a

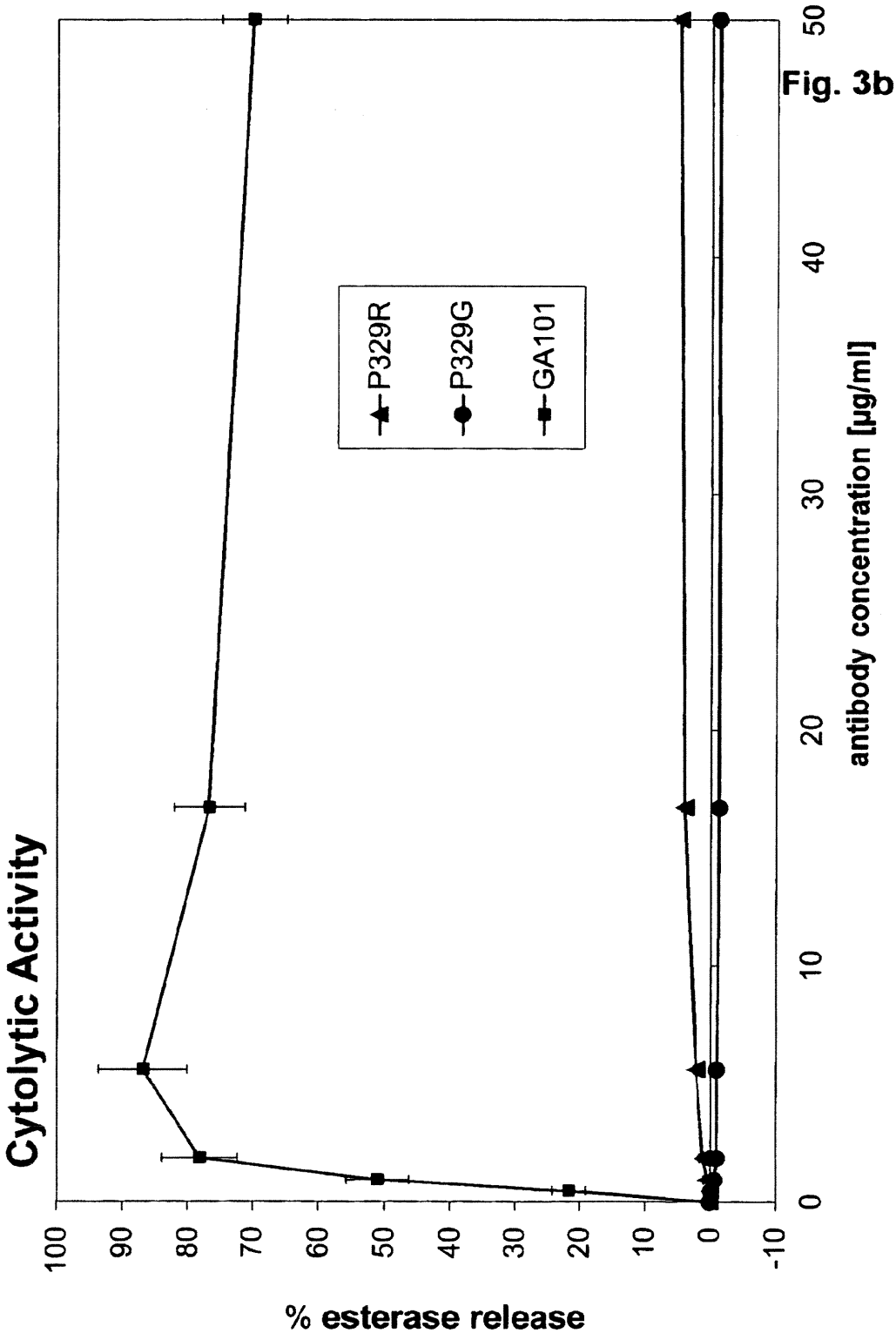

Cytolytic Activity
% esterase release
antibody concentration [µg/ml]
P329R
P329G
GA101
100
90
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50

Fig. 3b

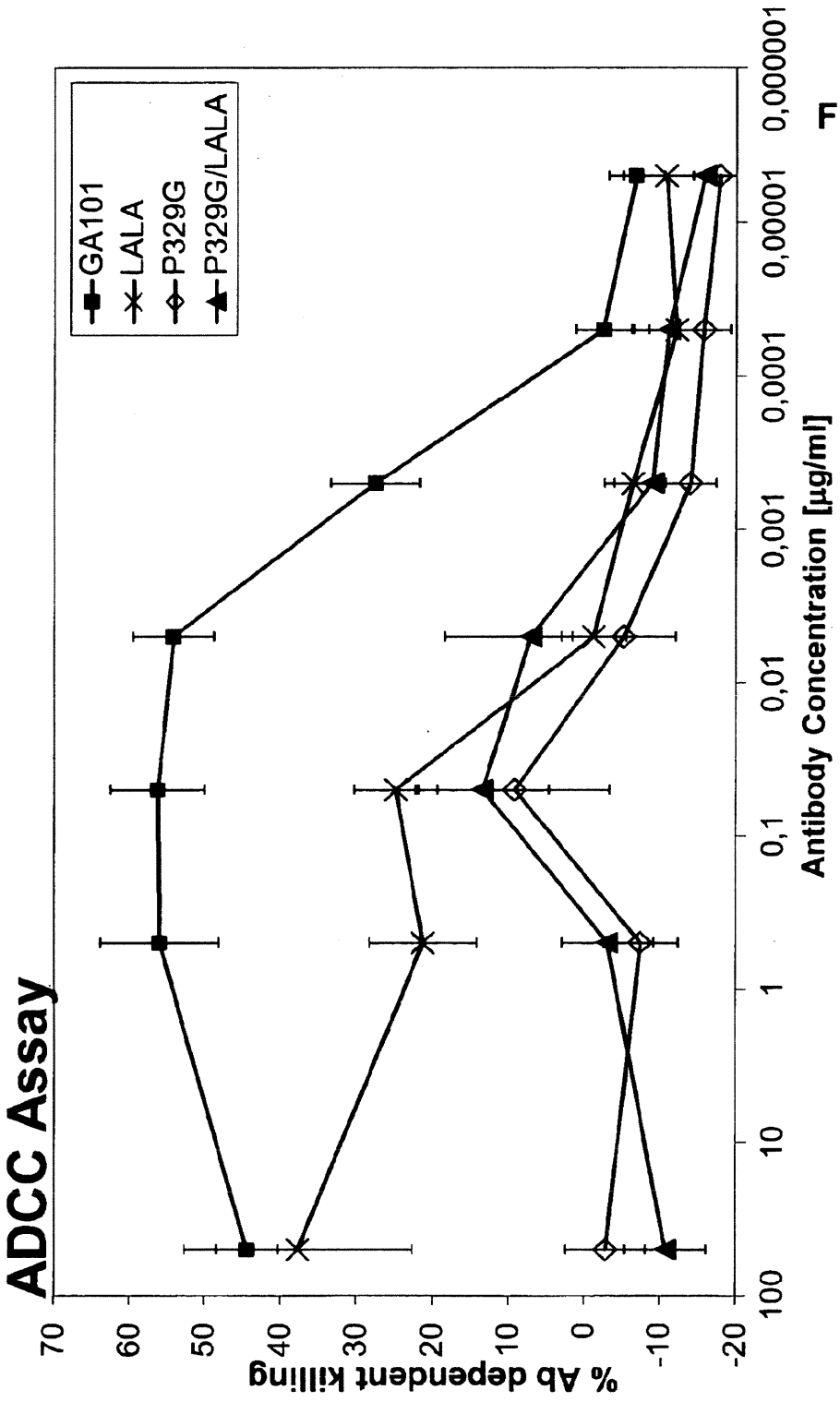

ADCC Assay
GA101
LALA
P329G
P329G/LALA
% Ab dependent killing
70
60
50
40
30
20
10
0
-10
-20
100
10
1
0,1
0,01
0,001
0,0001
0,00001
0,000001
Antibody Concentration [µg/ml]

Fig. 4a

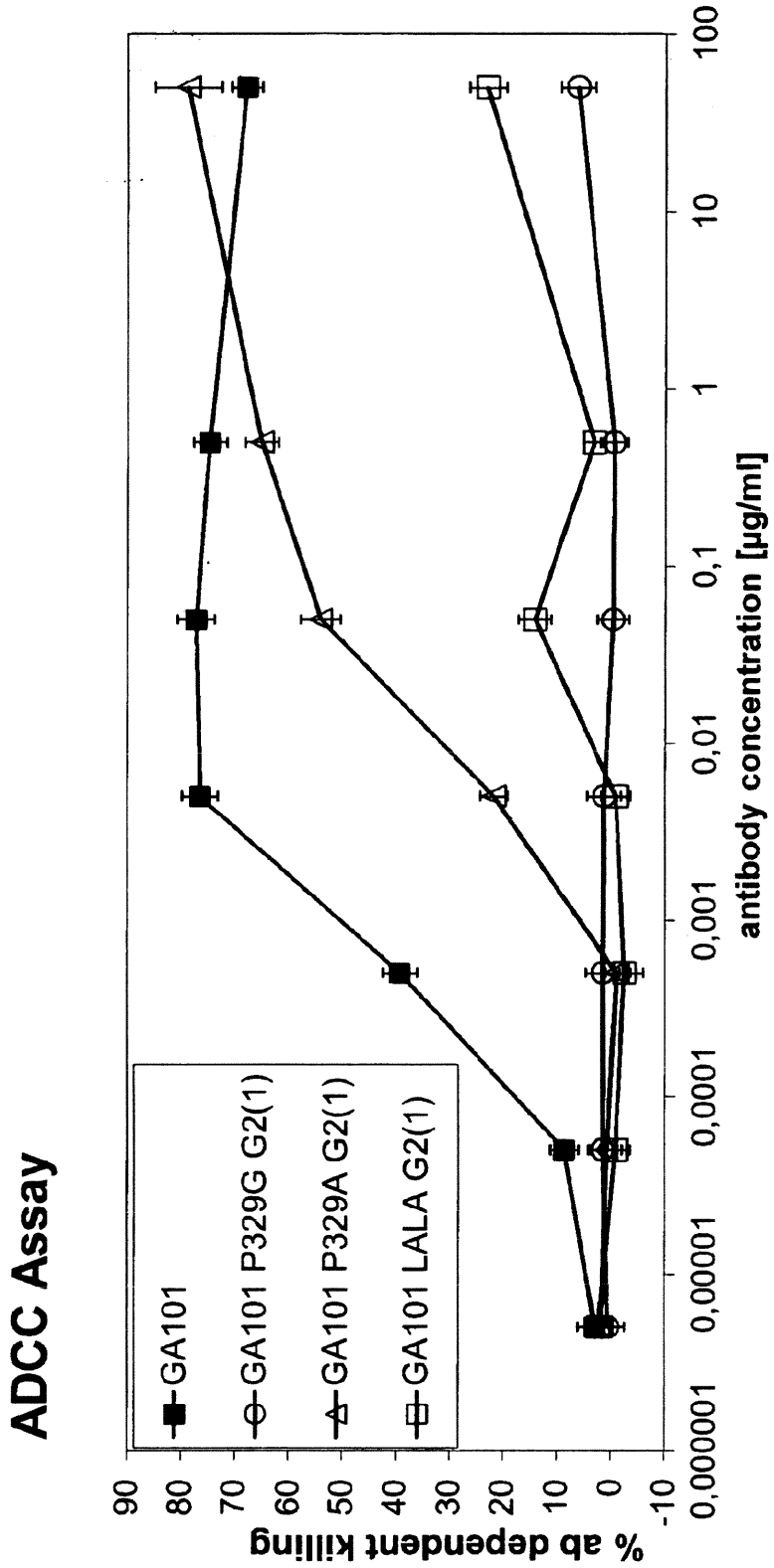

ADCC Assay
% ab dependent killing
90
80
70
60
50
40
30
20
10
0
-10
0,000001
0,00001
0,0001
0,001
0,01
0,1
1
10
100
antibody concentration [µg/ml]
GA101
GA101 P329G G2(1)
GA101 P329A G2(1)
GA101 LALA G2(1)

Fig. 4b

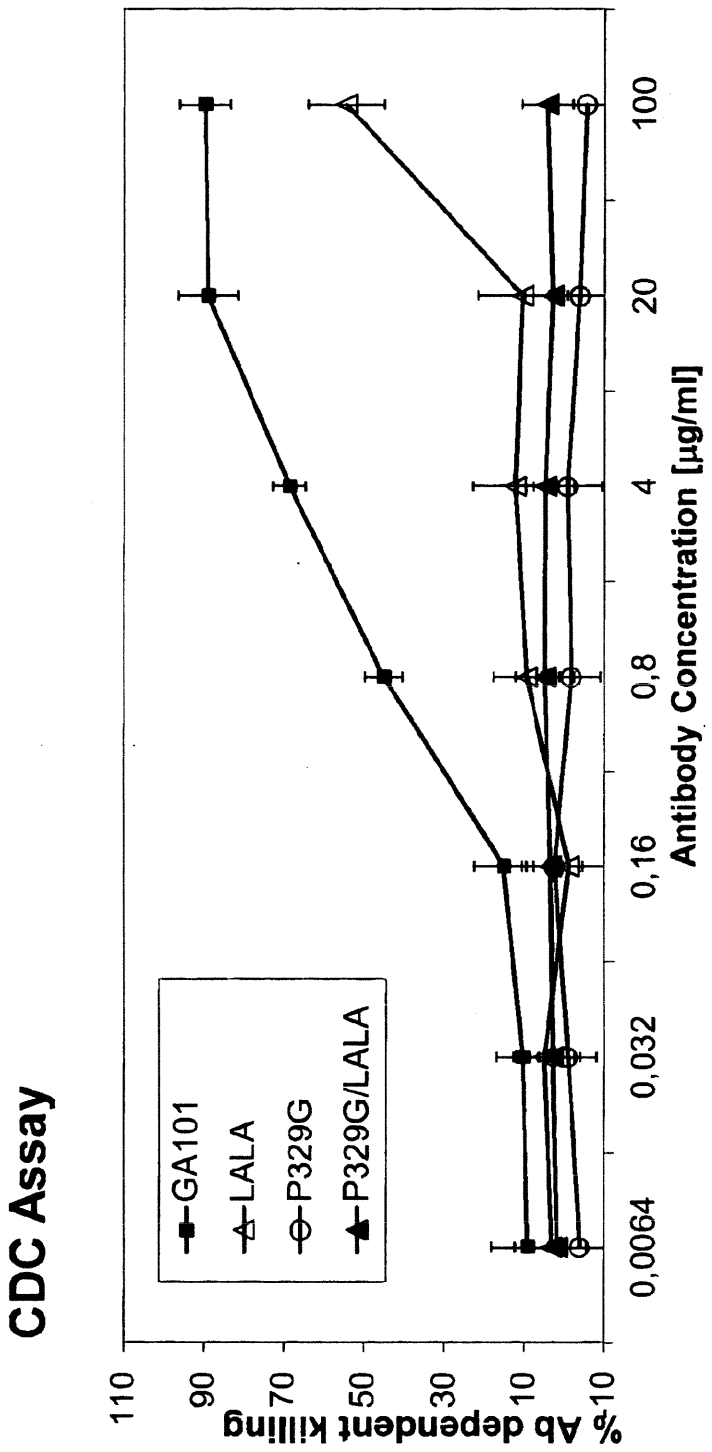

CDC Assay
% Ab dependent killing
110
90
70
50
30
10
-10
0,0064
0,032
0,16
0,8
4
20
100
Antibody Concentration [µg/ml]
GA101
LALA
P329G
P329G/LALA

Fig. 5a

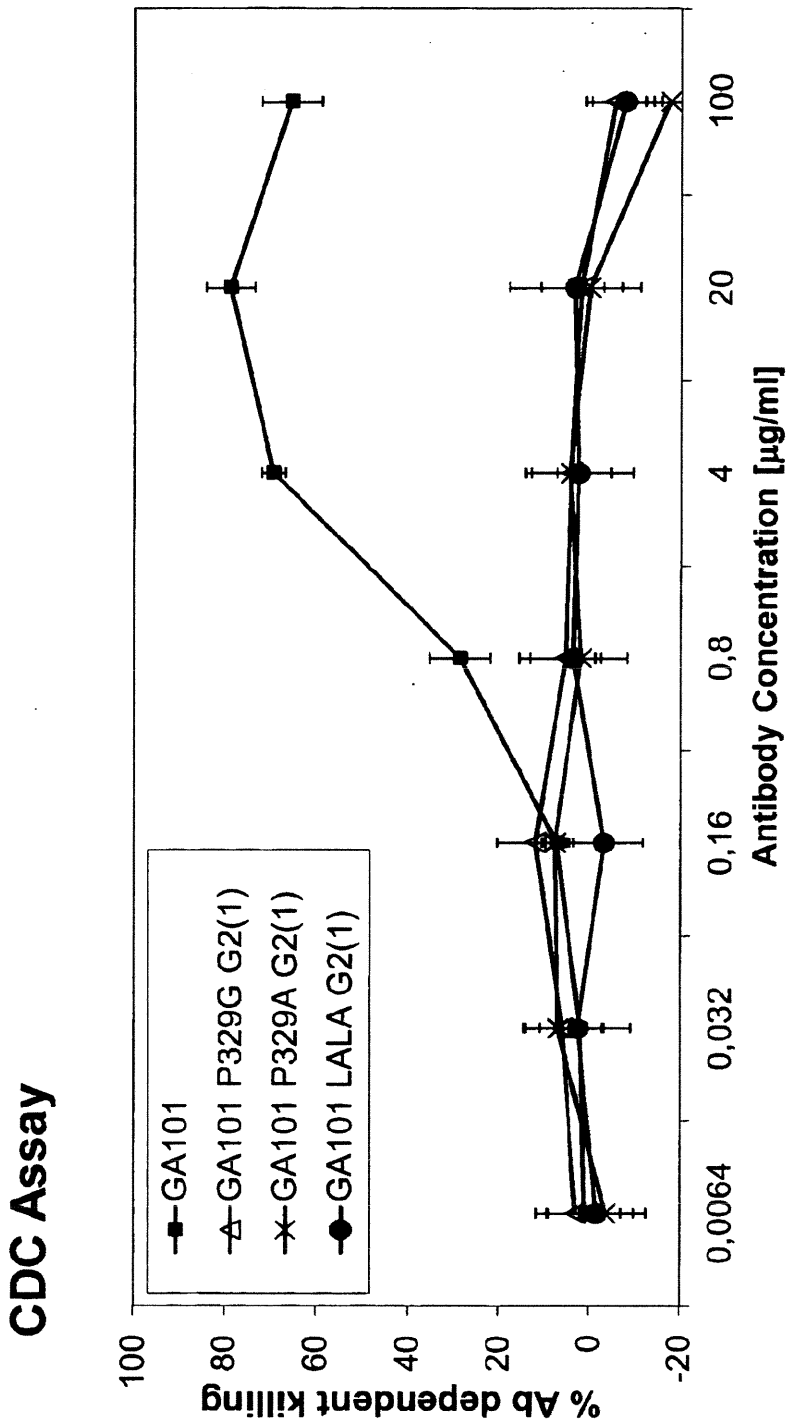

CDC Assay
% Ab dependent killing
100
80
60
40
20
0
-20
0,0064
0,032
0,16
0,8
4
20
100
Antibody Concentration [µg/ml]
GA101
GA101 P329G G2(1)
GA101 P329A G2(1)
GA101 LALA G2(1)

Fig. 5b

Fig. 6a

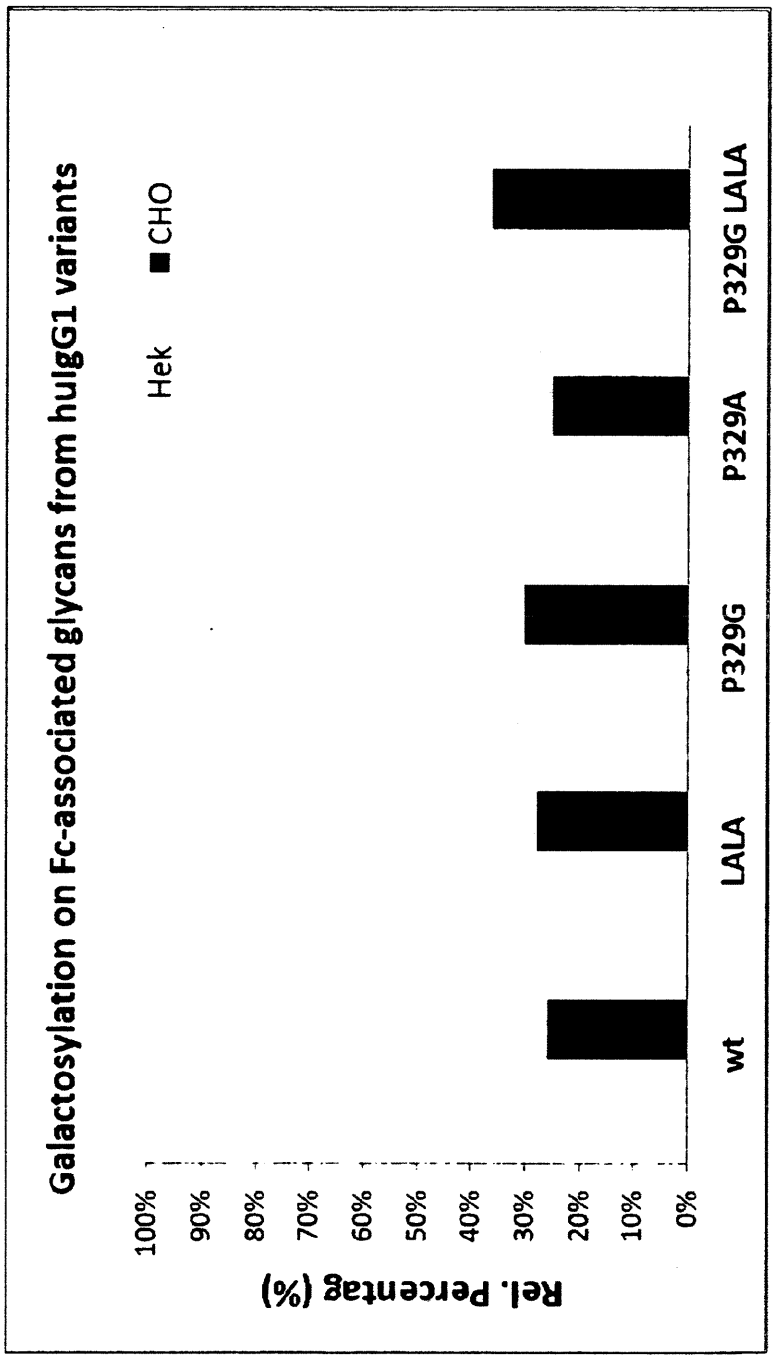
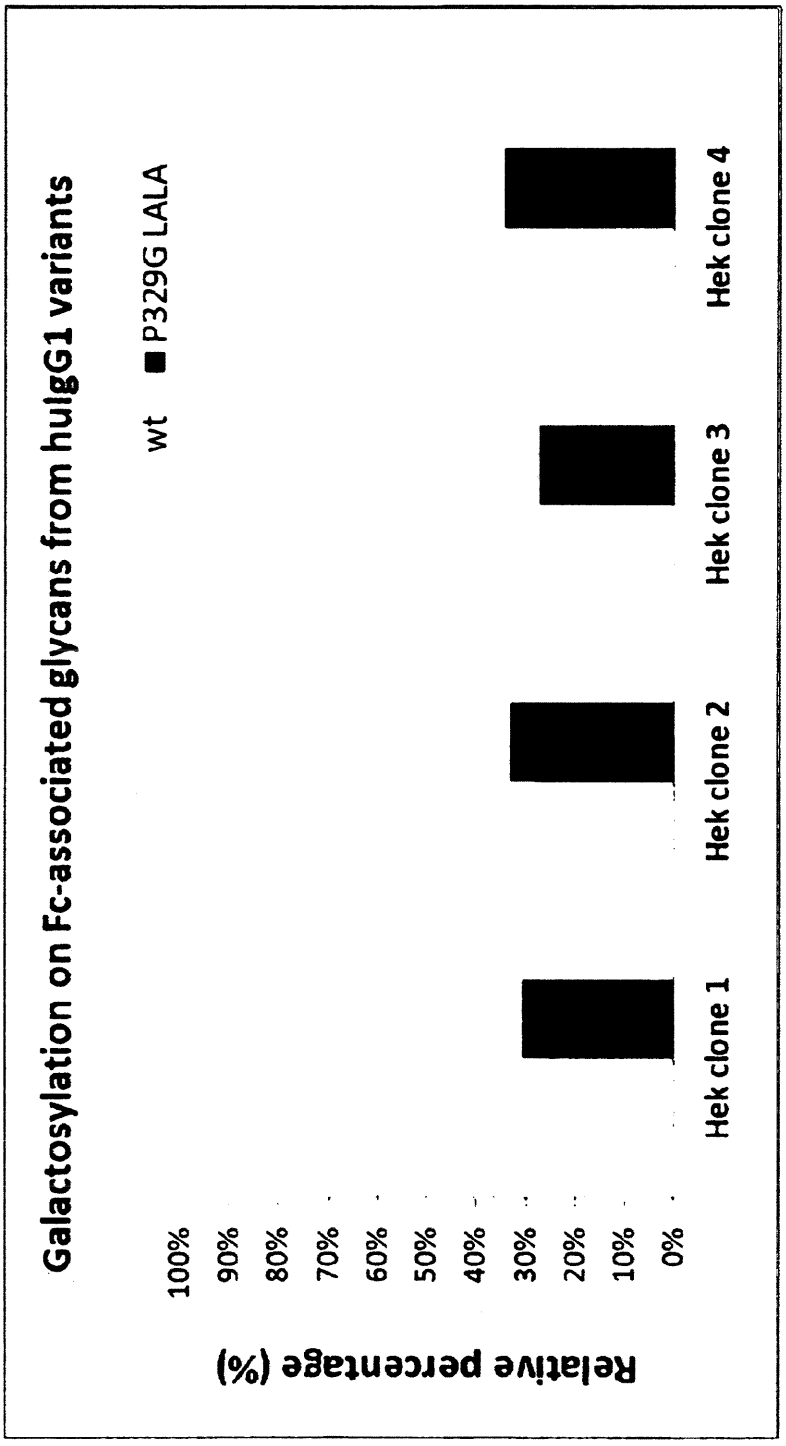

Galactosylation on Fc-associated glycans from huIgG1 variants
Hek
CHO
Rel. Percentag (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
wt
LALA
P329G
P329A
P329G LALA

Fig. 6b


Galactosylation on Fc-associated glycans from huIgG1 variants
wt
P329G LALA
Relative percentage (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Hek clone 1
Hek clone 2
Hek clone 3
Hek clone 4

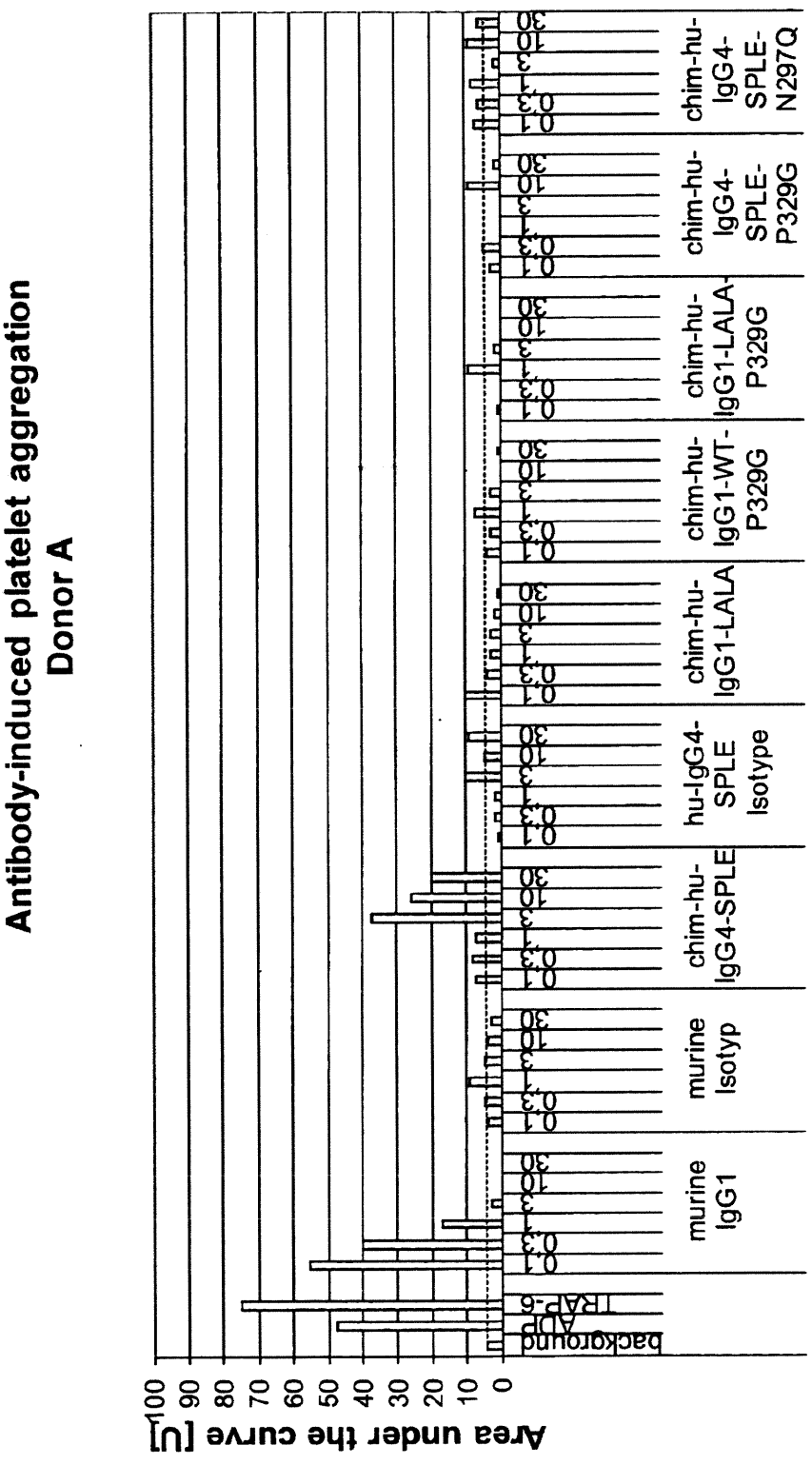

Antibody-induced platelet aggregation
Donor A
Area under the curve [U]
100
90
80
70
60
50
40
30
20
10
0
background
ADP
TRAP-6
murine IgG1
murine Isotyp
chim-hu-IgG4-SPLE
hu-IgG4-SPLE Isotype
chim-hu-IgG1-LALA
chim-hu-IgG1-WT-P329G
chim-hu-IgG1-LALA-P329G
chim-hu-IgG4-SPLE-P329G
chim-hu-IgG4-SPLE-N297Q
0,1
0,3
1
3
10
30

Fig. 7a

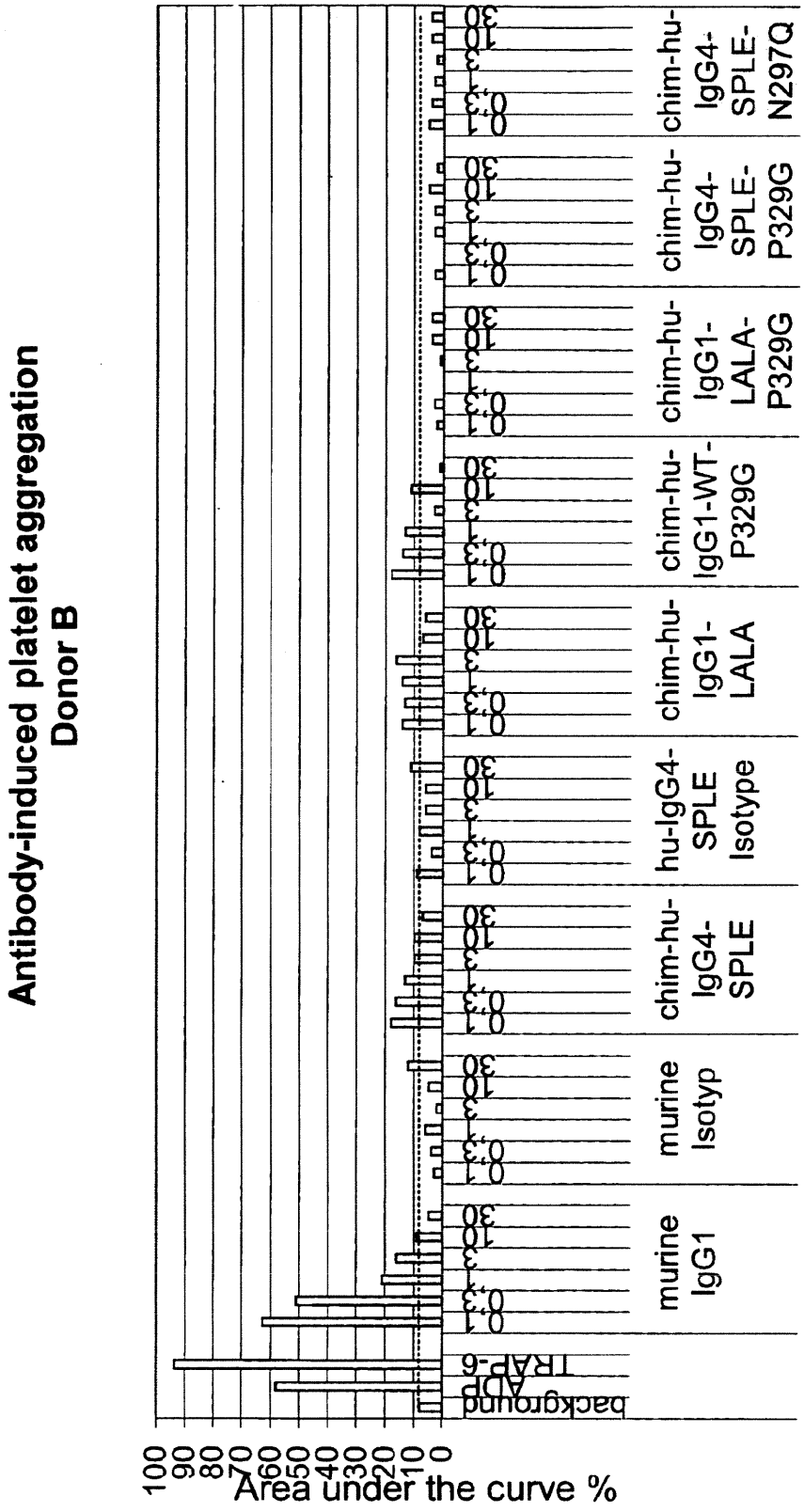

Antibody-induced platelet aggregation
Donor B
Area under the curve %
100
90
80
70
60
50
40
30
20
10
0
background
ADP
TRAP-6
murine IgG1
murine Isotyp
chim-hu-IgG4-SPLE
hu-IgG4-SPLE Isotype
chim-hu-IgG1-LALA
chim-hu-IgG1-WT-P329G
chim-hu-IgG1-LALA-P329G
chim-hu-IgG4-SPLE-P329G
chim-hu-IgG4-SPLE-N297Q
0,1
0,3
1
3
10
30

Fig. 7b