Fig. 1A

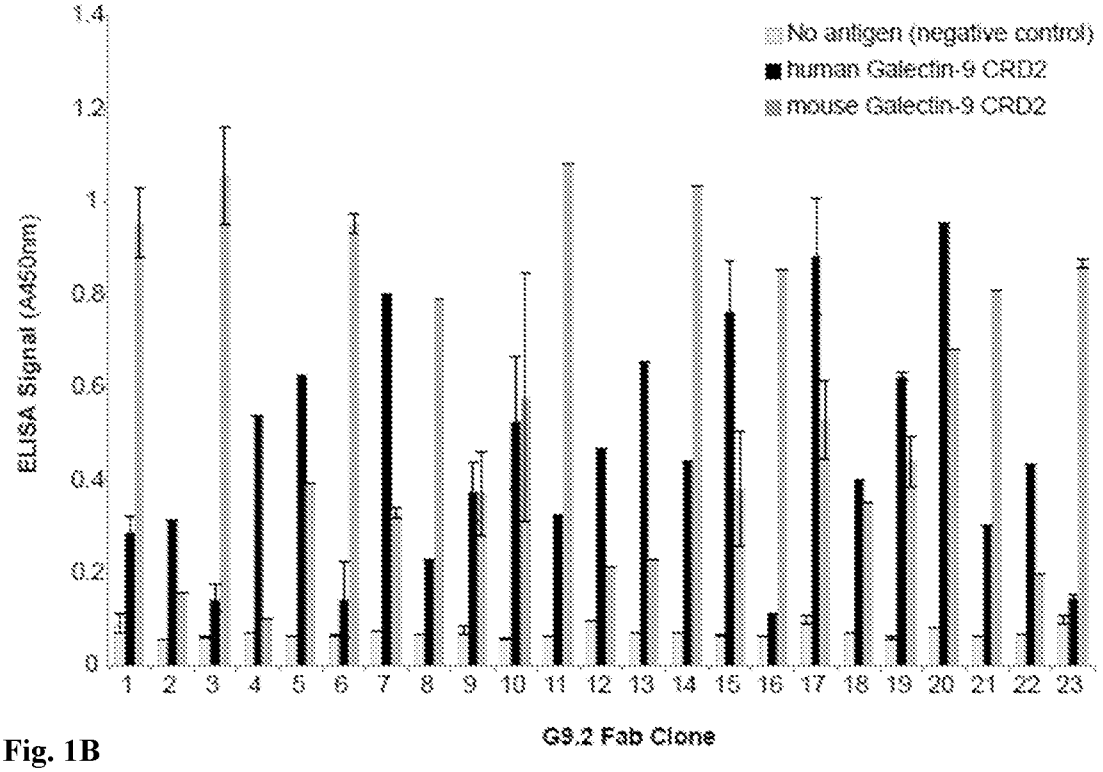

No antigen (negative control)
human Galectin-9 CRD2
mouse Galectin-9 CRD2
ELISA Signal (A450nm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
G9.2 Fab Clone

Fig. 1B

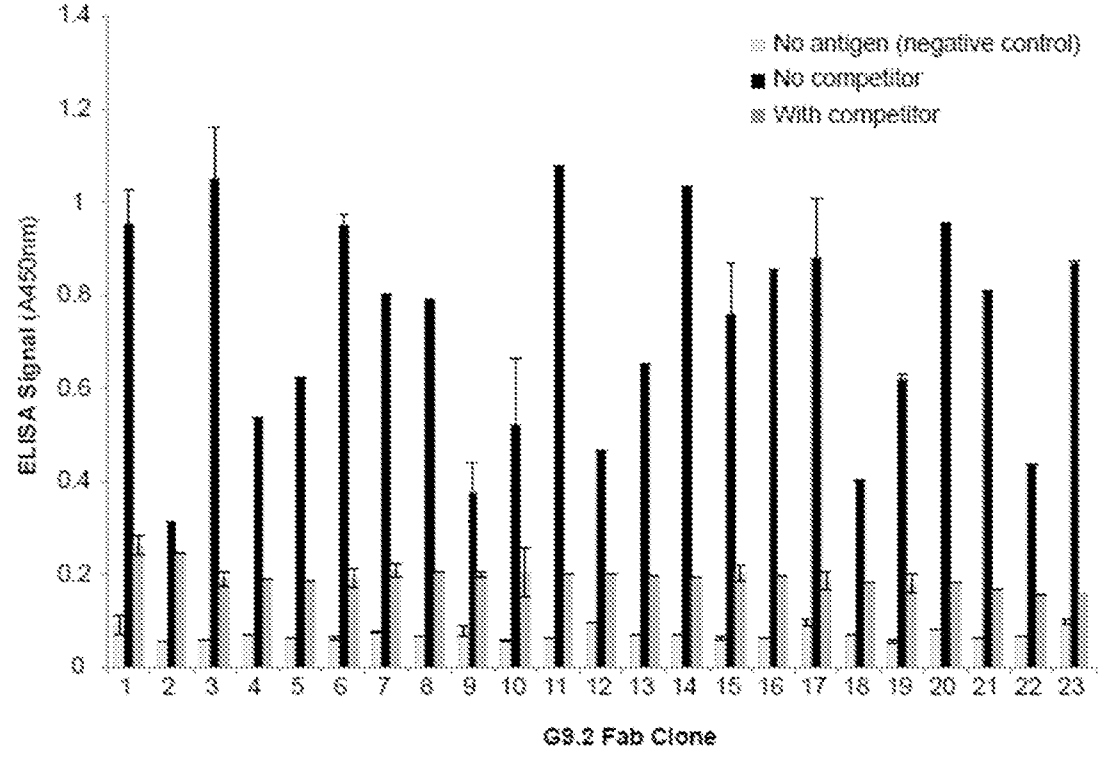

No antigen (negative control)
No competitor
With competitor
ELISA Signal (A450nm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
G9.2 Fab Clone

Fig. 2A

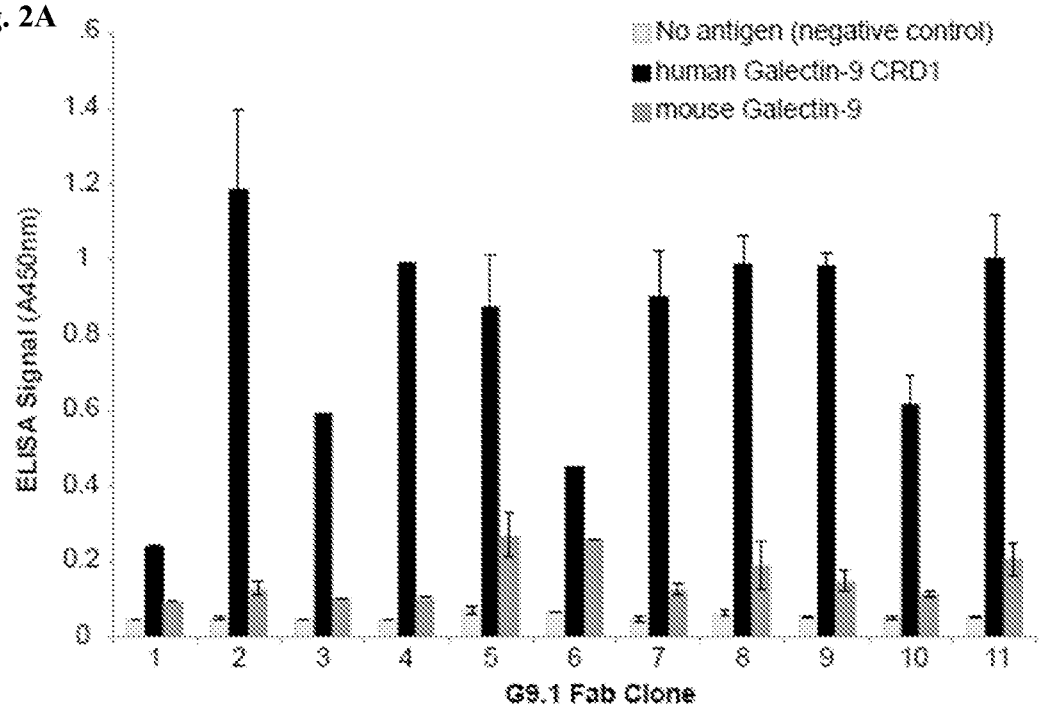

No antigen (negative control)
human Galectin-9 CRD1
mouse Galectin-9
ELISA Signal (A450nm)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1
2
3
4
5
6
7
8
9
10
11
G9.1 Fab Clone

Fig. 2B

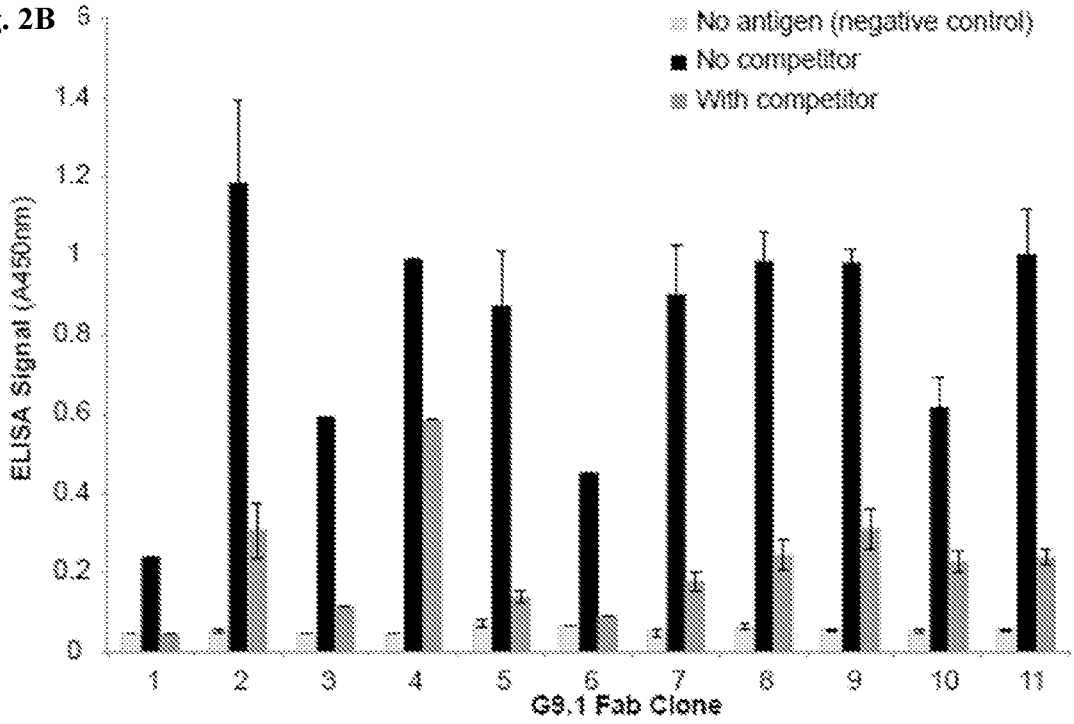

No antigen (negative control)
No competitor
With competitor
ELISA Signal (A450nm)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1
2
3
4
5
6
7
8
9
10
11
G9.1 Fab Clone

Fig. 3A

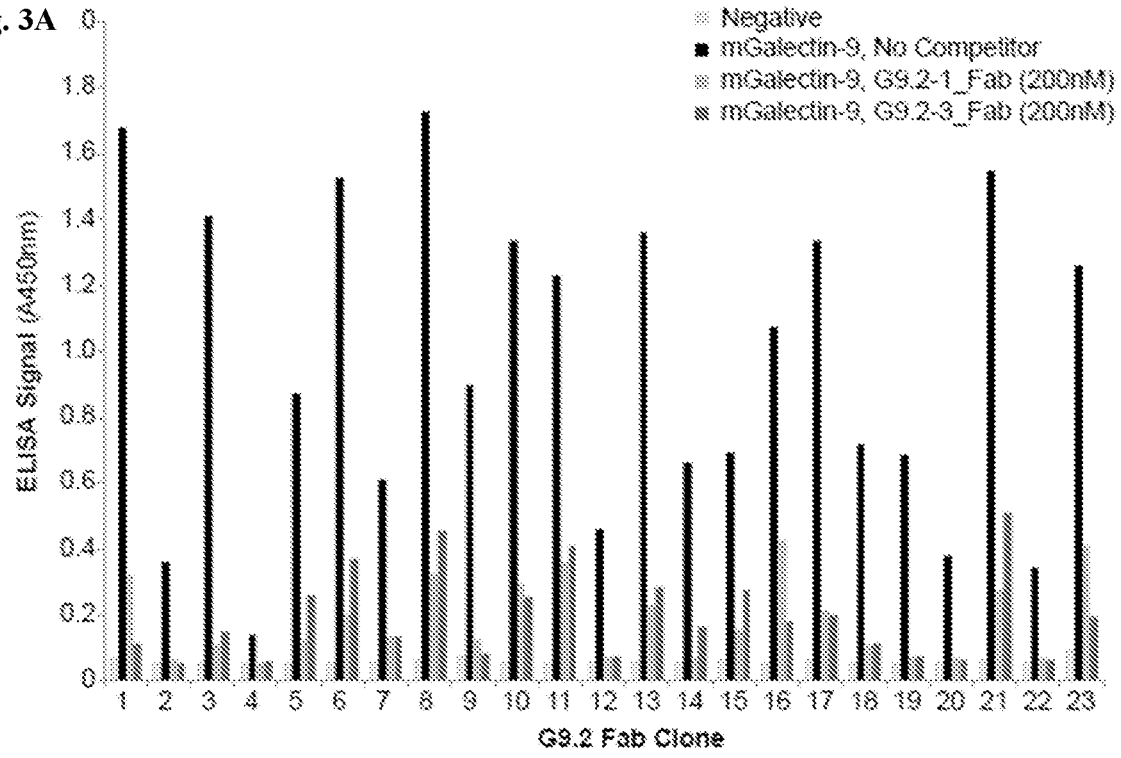

Negative
mGalectin-9, No Competitor
mGalectin-9, G9.2-1_Fab (200nM)
mGalectin-9, G9.2-3_Fab (200nM)
ELISA Signal (A450nm)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
G9.2 Fab Clone

Fig. 3B

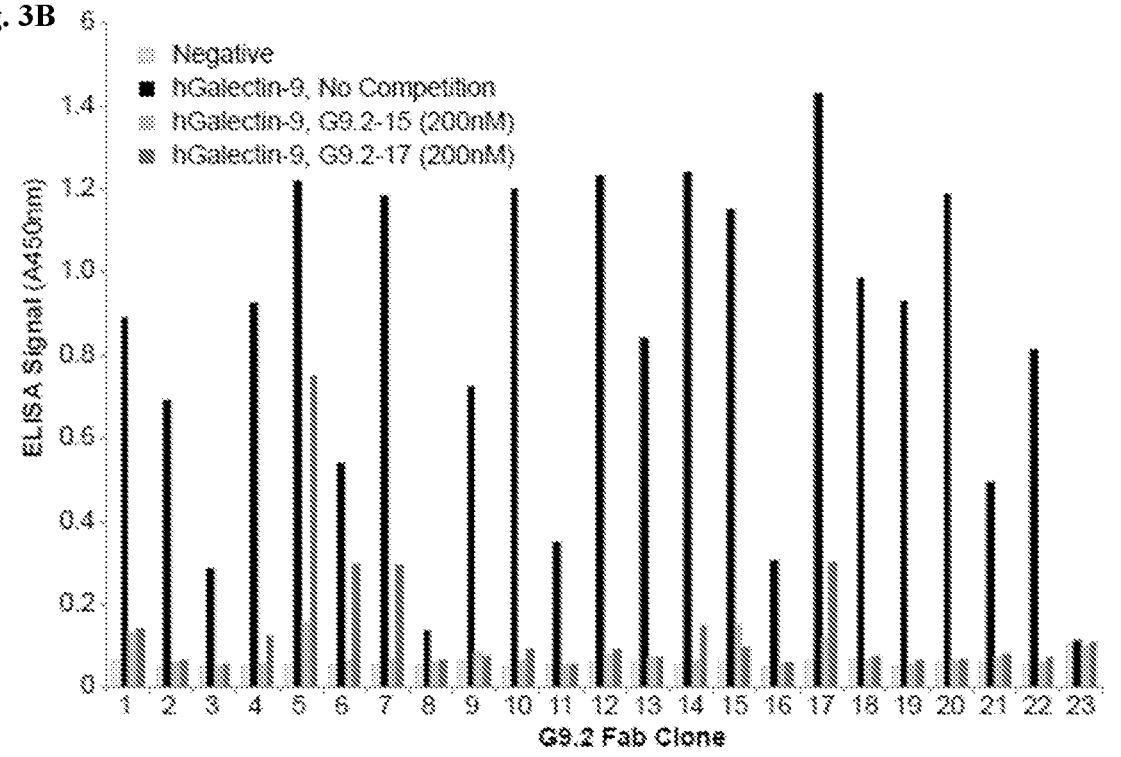
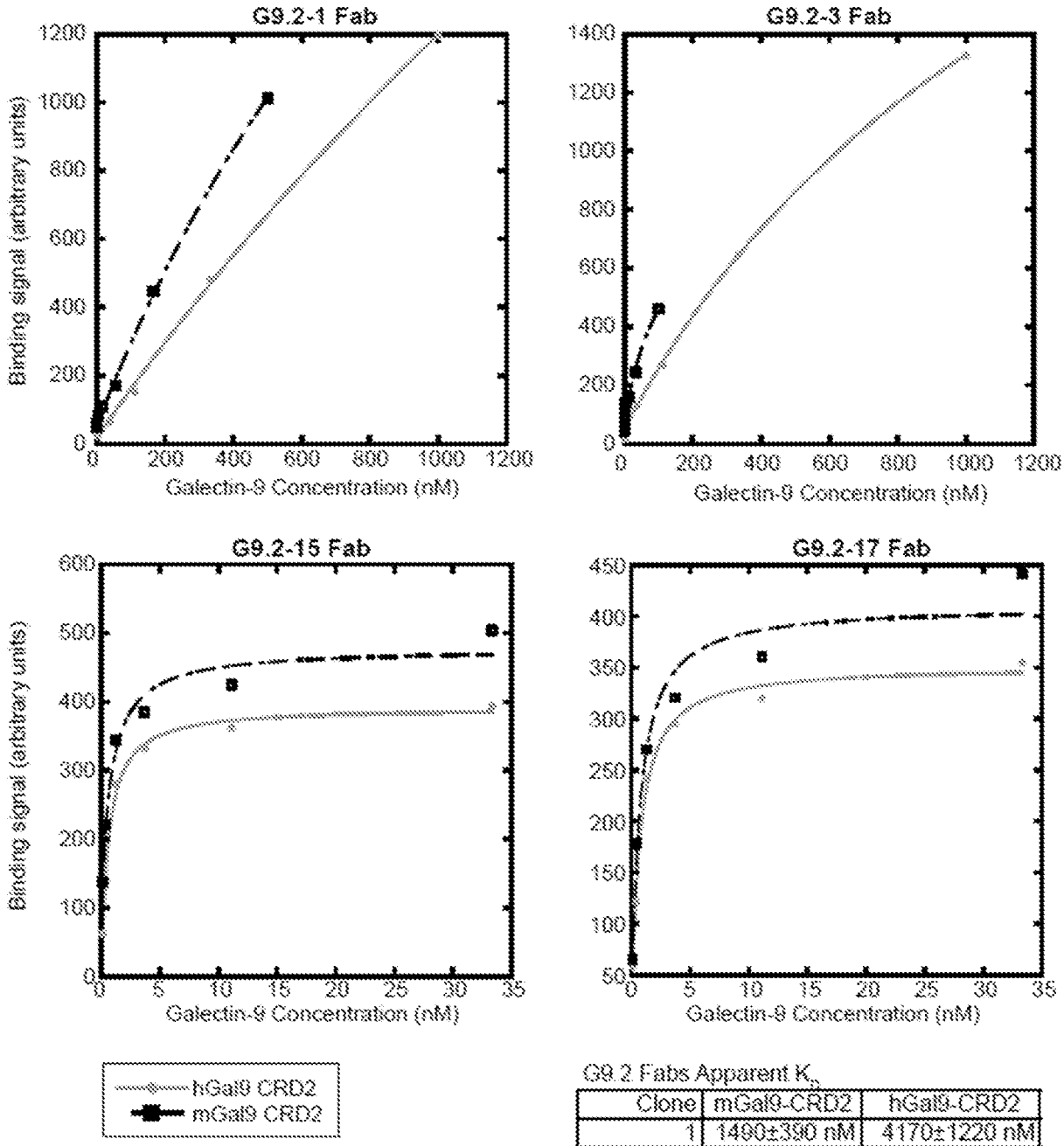

Negative
hGalectin-9, No Competition
hGalectin-9, G9.2-15 (200nM)
hGalectin-9, G9.2-17 (200nM)
ELISA Signal (A450nm)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
G9.2 Fab Clone

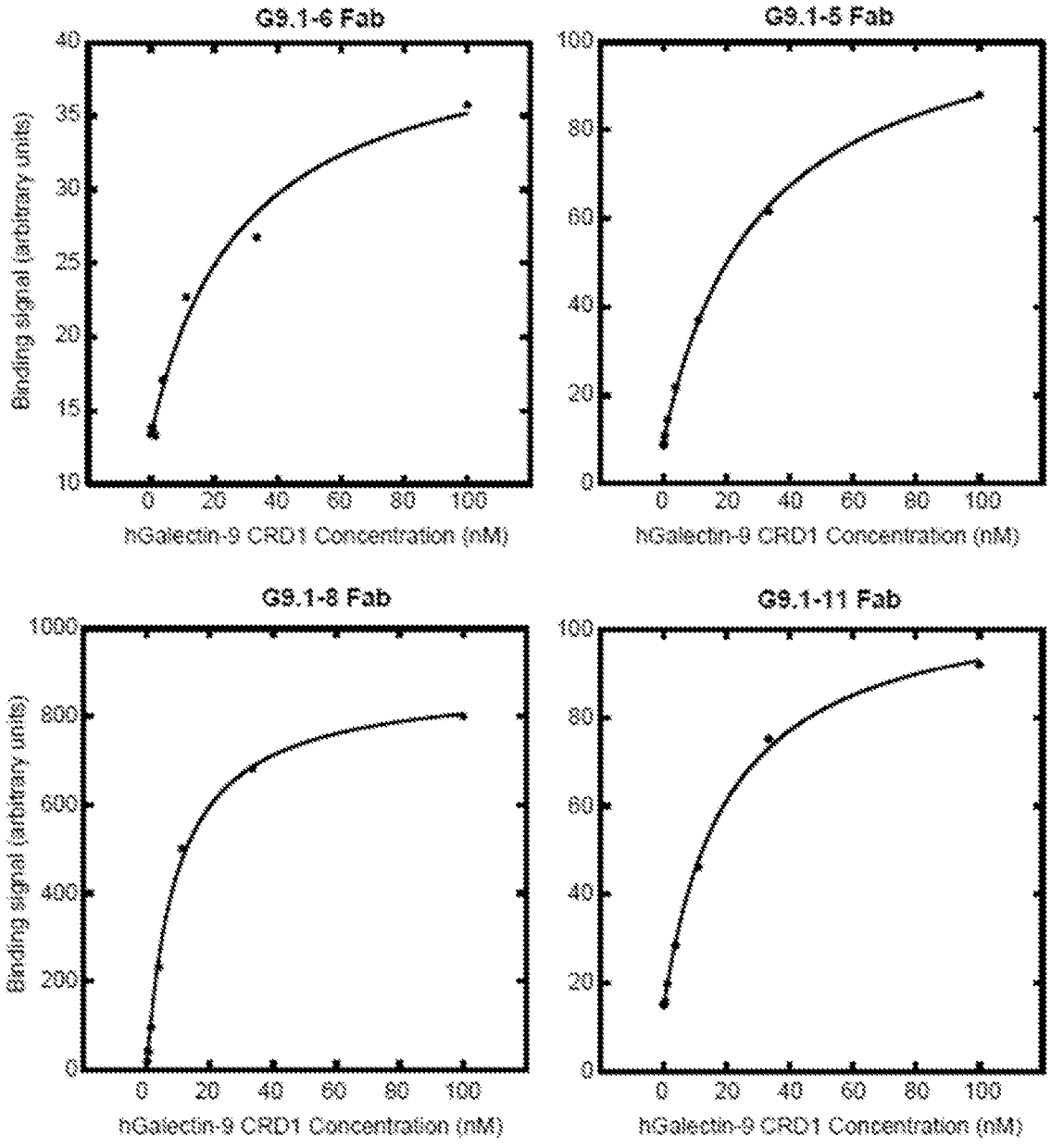
Fig. 4



G9.2 Fabs Apparent K_D

Clone	mGal9-CRD2	hGal9-CRD2
1	1400±390 nM	4170±1220 nM
3	122±89 nM	1380±210nM
15	0.85±0.16 nM	0.71±0.39 nM
17	0.55±0.14 nM	0.76±0.39 nM

Fig. 5



G9.1 Fabs Apparent Kd	
Clone	hGal9-2
5	30.4±2.1 nM
6	29.2±8.5 nM
8	9.8±1.0 nM
11	20.4±1.7 nM

Fig. 6

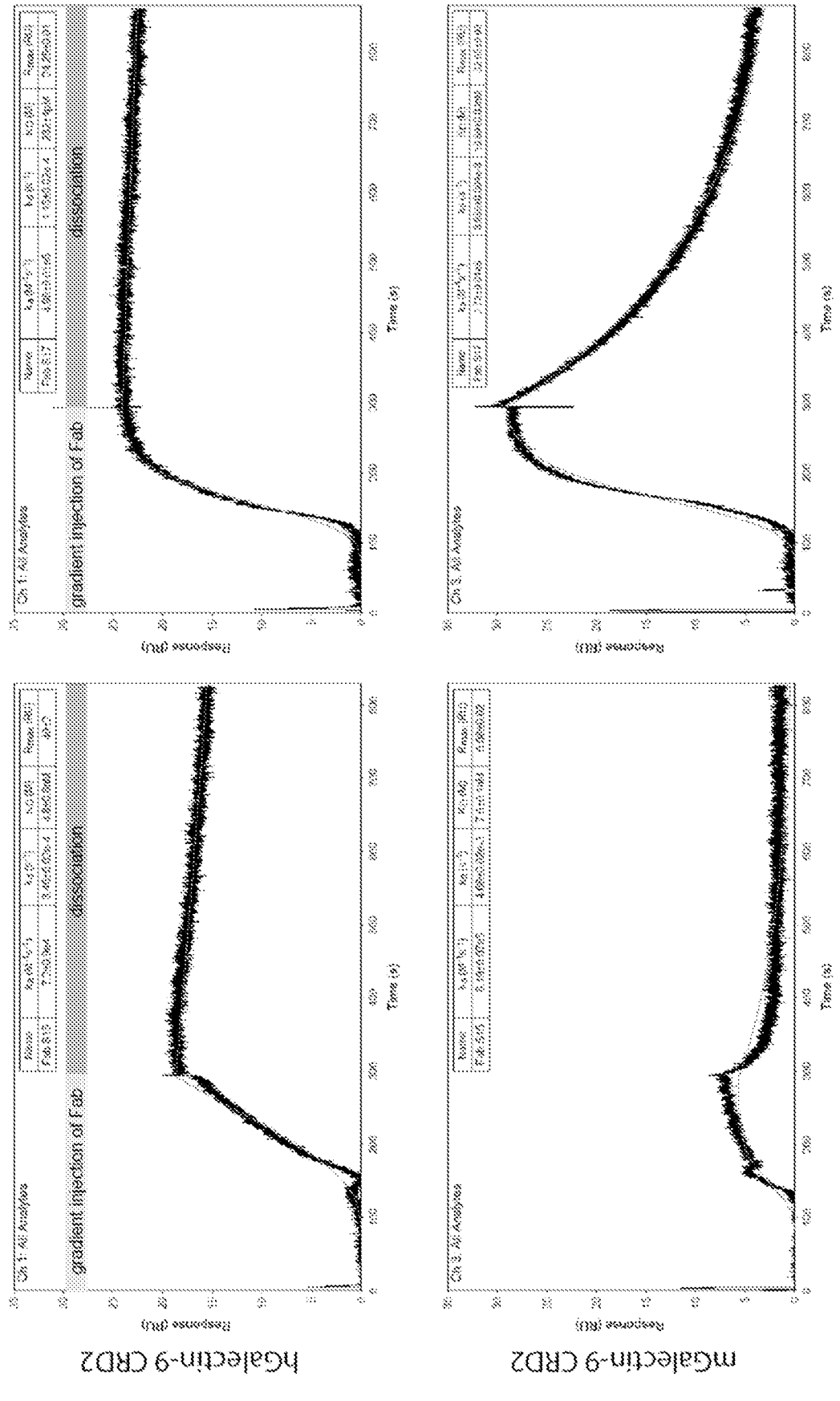

Fab G9.2-15
Fab G9.2-17
hGalectin-9 CRD2
mGalectin-9 CRD2
gradient injection of Fab
dissociation
Time (s)

Fig. 7

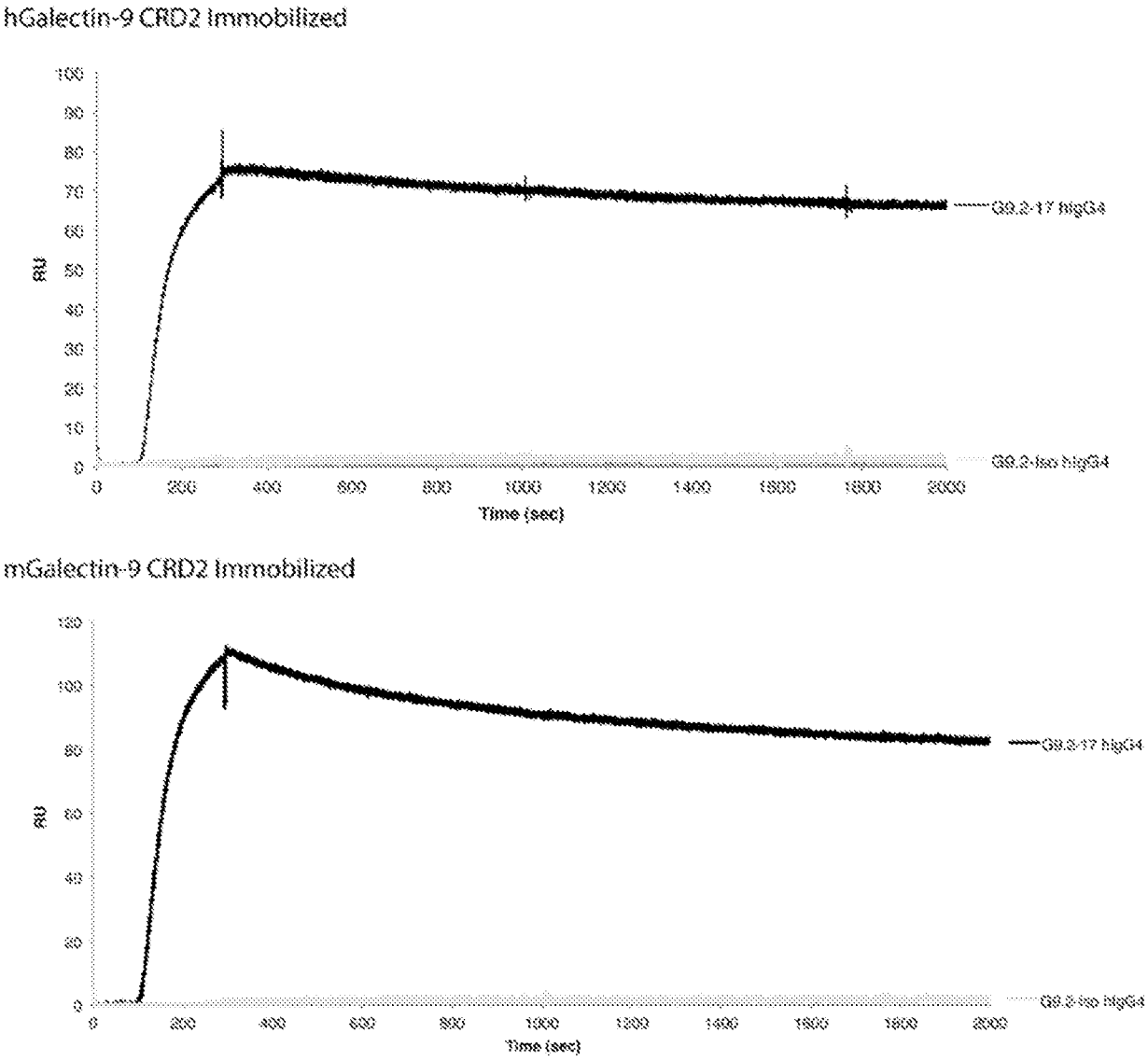

hGalectin-9 CRD2 Immobilized
RU
Time (sec)
G9.2-17 hIgG4
G9.2-Iso hIgG4
mGalectin-9 CRD2 Immobilized
RU
Time (sec)
G9.2-17 hIgG4
G9.2-Iso hIgG4

Fig. 8

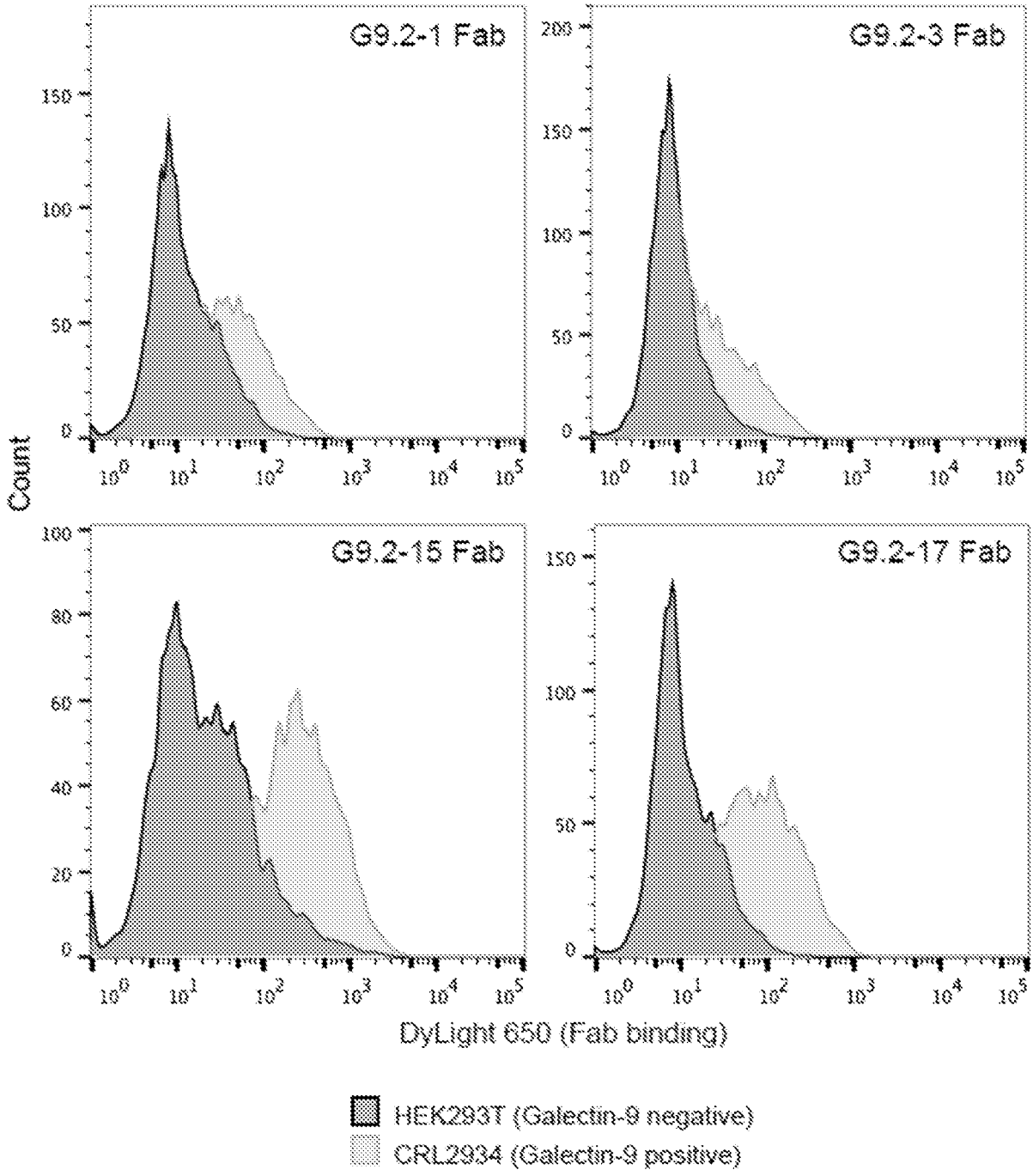

G9.2-1 Fab
G9.2-3 Fab
G9.2-15 Fab
G9.2-17 Fab
Count
DyLight 650 (Fab binding)
HEK293T (Galectin-9 negative)
CRL2934 (Galectin-9 positive)

Fig. 9

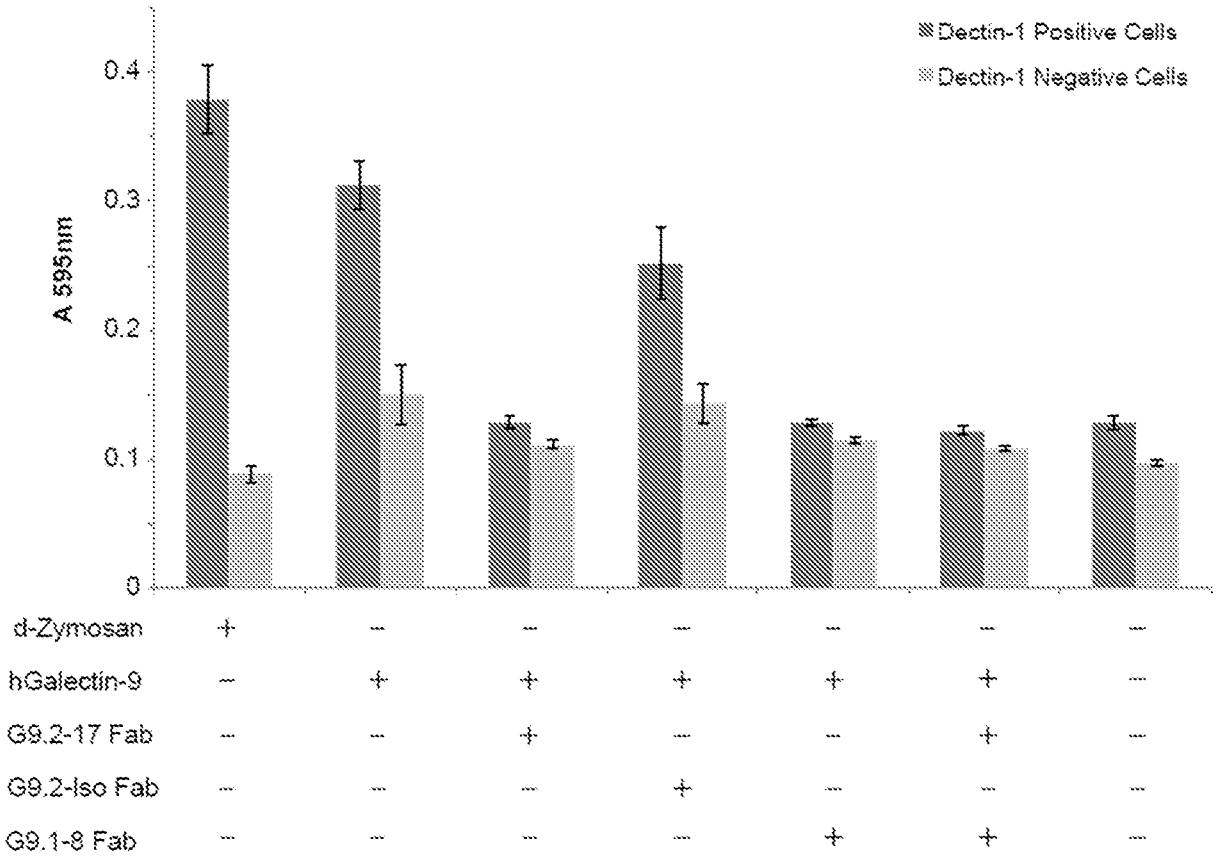

Dectin-1 Positive Cells
Dectin-1 Negative Cells
A 595nm
0.4
0.3
0.2
0.1
0
d-Zymosan + – – – – – –
hGalectin-9 – + + + + + –
G9.2-17 Fab – – + – – + –
G9.2-Iso Fab – – – + – – –
G9.1-8 Fab – – – – + + –

Fig. 10A

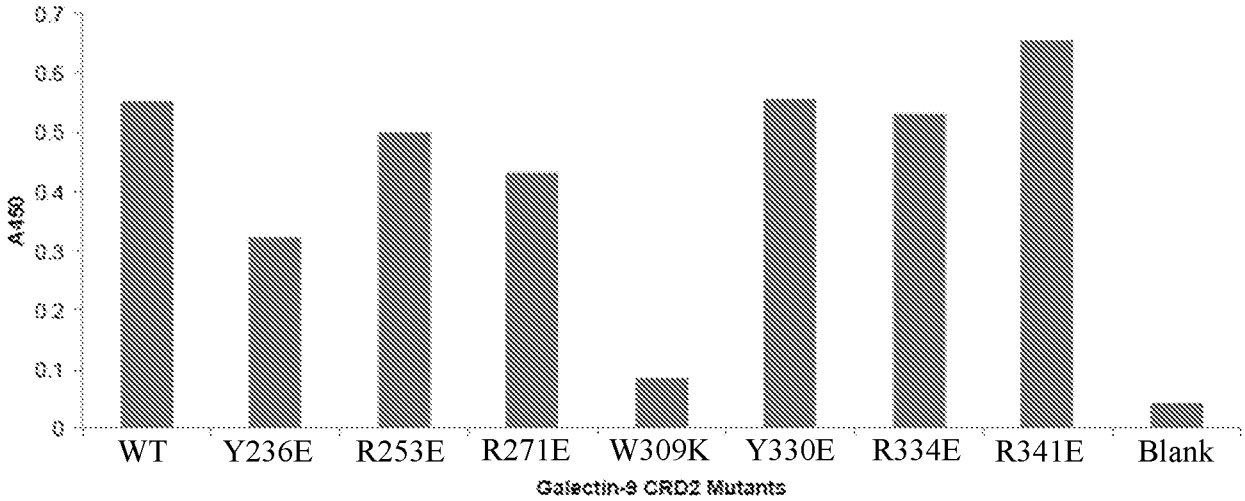


Fig. 10B

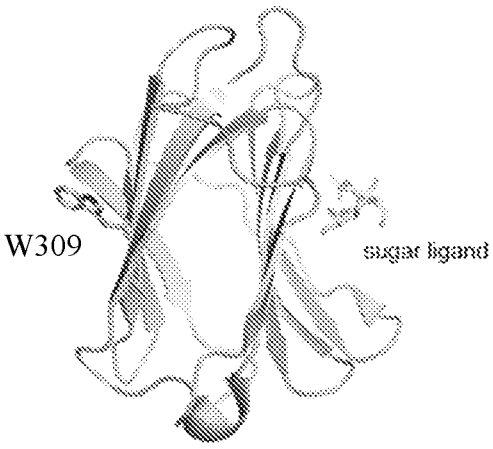


Fig. 11

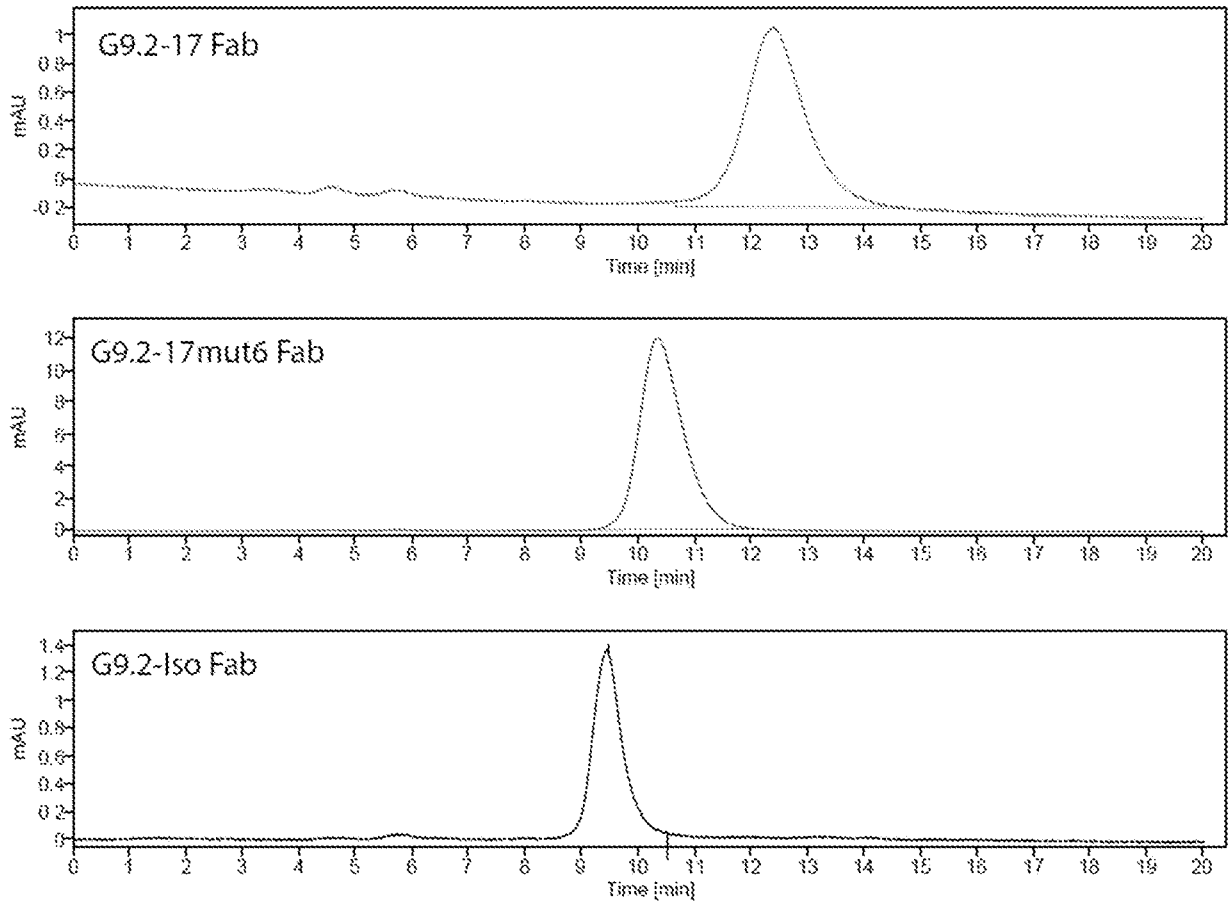

G9.2-17 Fab
mAU
Time [min]
G9.2-17mut6 Fab
mAU
Time [min]
G9.2-Iso Fab
mAU
Time [min]

Fig. 12

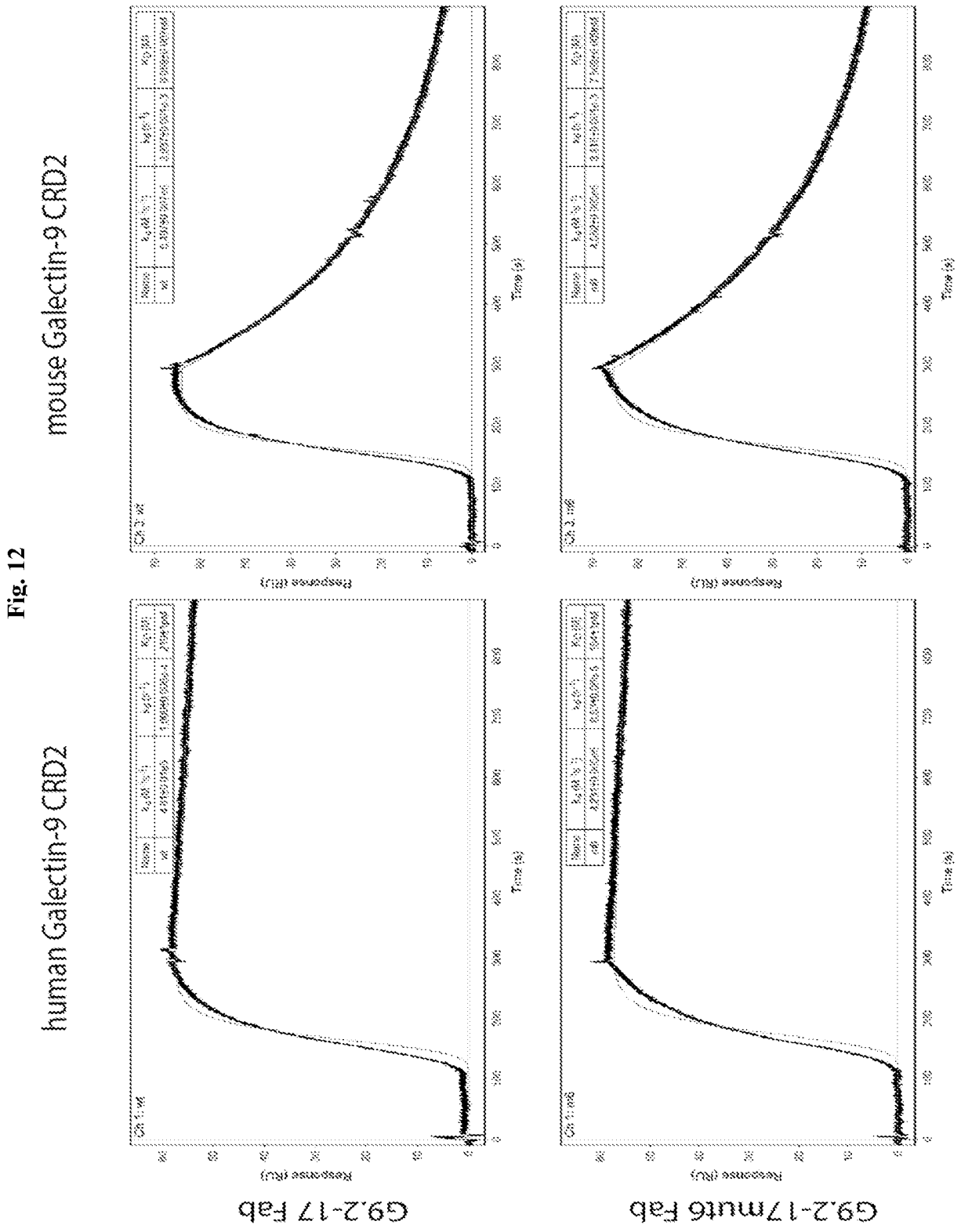

human Galectin-9 CRD2
mouse Galectin-9 CRD2
G9.2-17 Fab
G9.2-17mut6 Fab
Response (RU)
Time (s)

Fig. 13

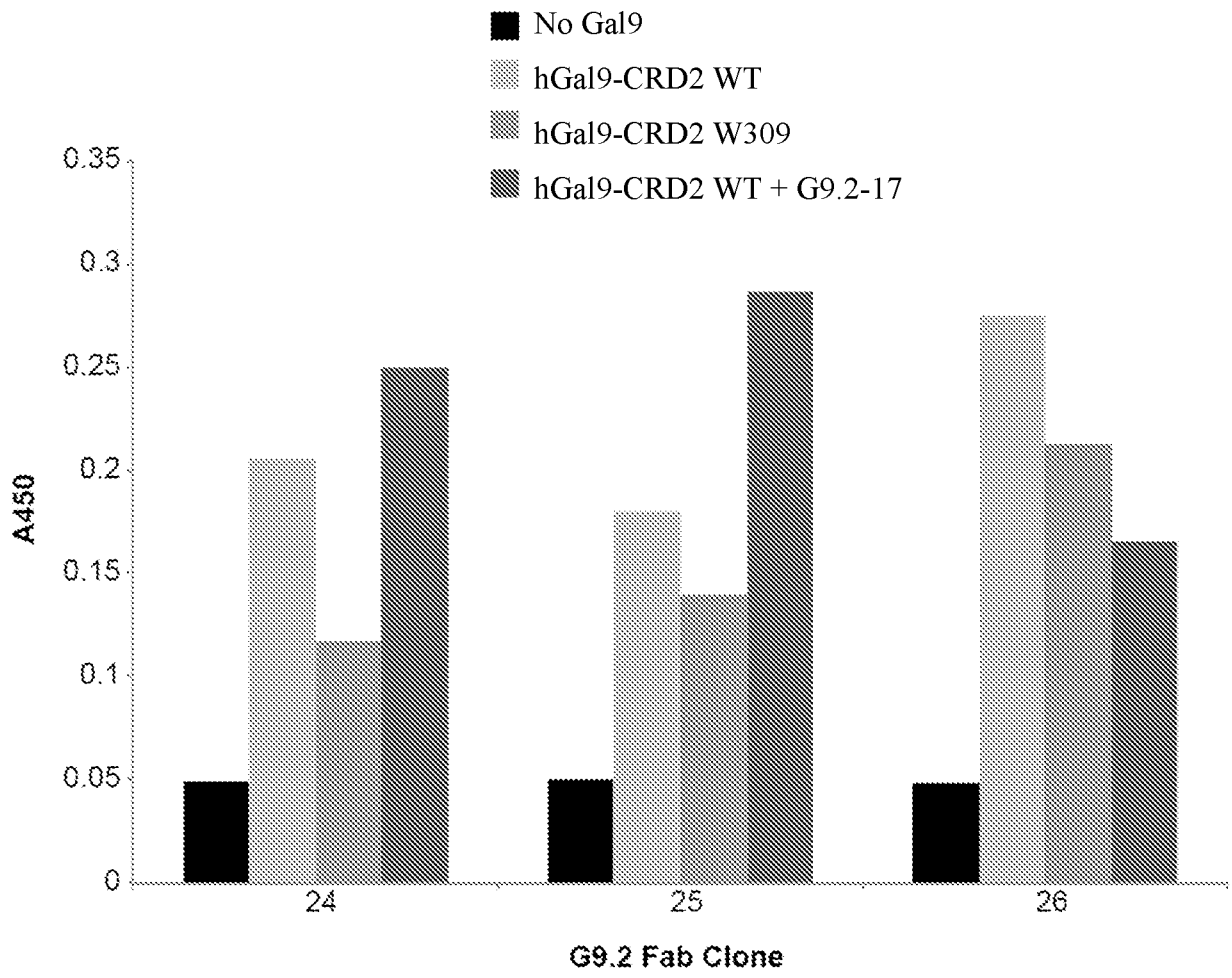

No Gal9
hGal9-CRD2 WT
hGal9-CRD2 W309
hGal9-CRD2 WT + G9.2-17
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
A450
24
25
26
G9.2 Fab Clone

Fig. 14

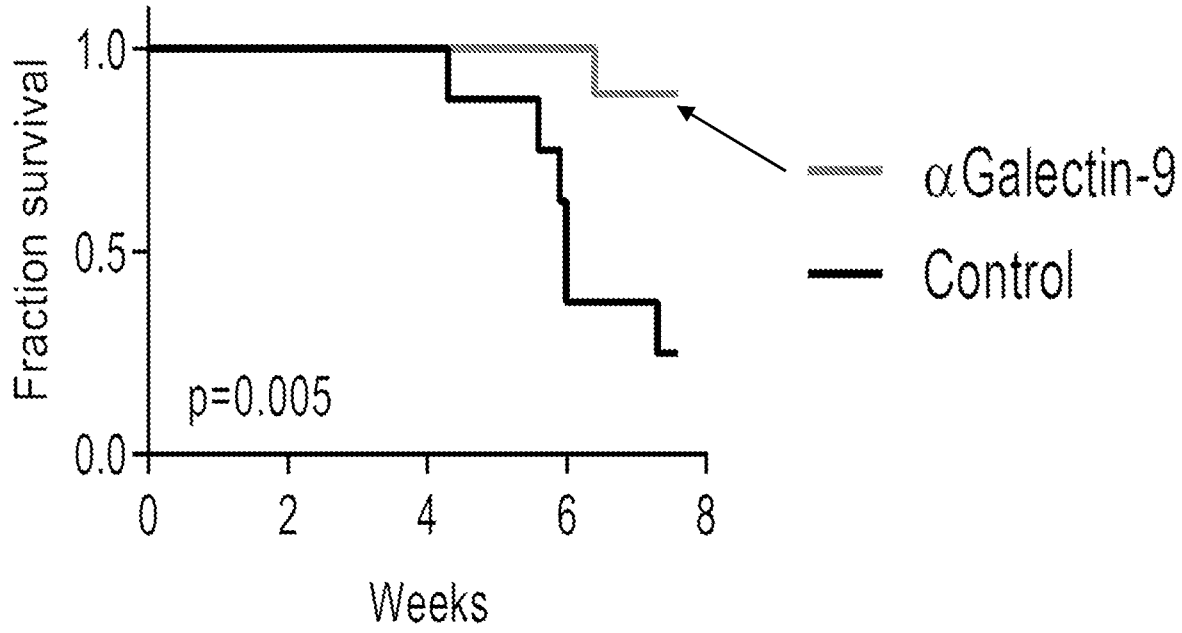

Fraction survival
1.0
0.5
0.0
p=0.005
0
2
4
6
8
Weeks
αGalectin-9
Control

Fig. 15

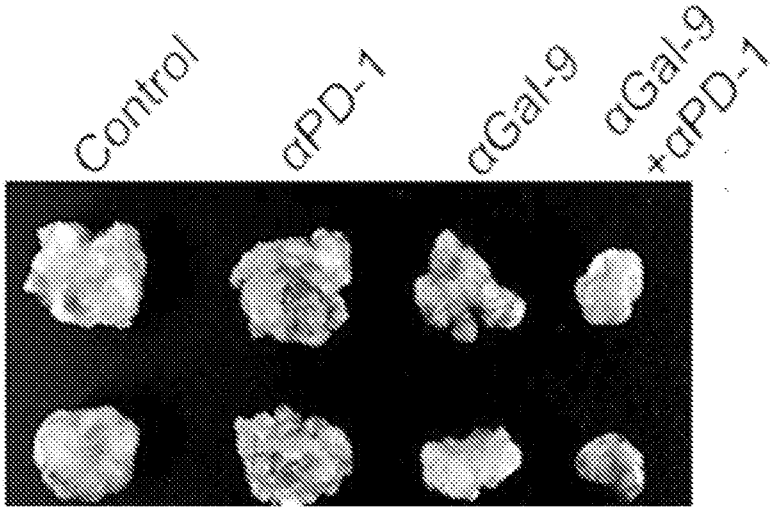

Control
αPD-1
αGal-9
αGal-9
+αPD-1

Fig. 16

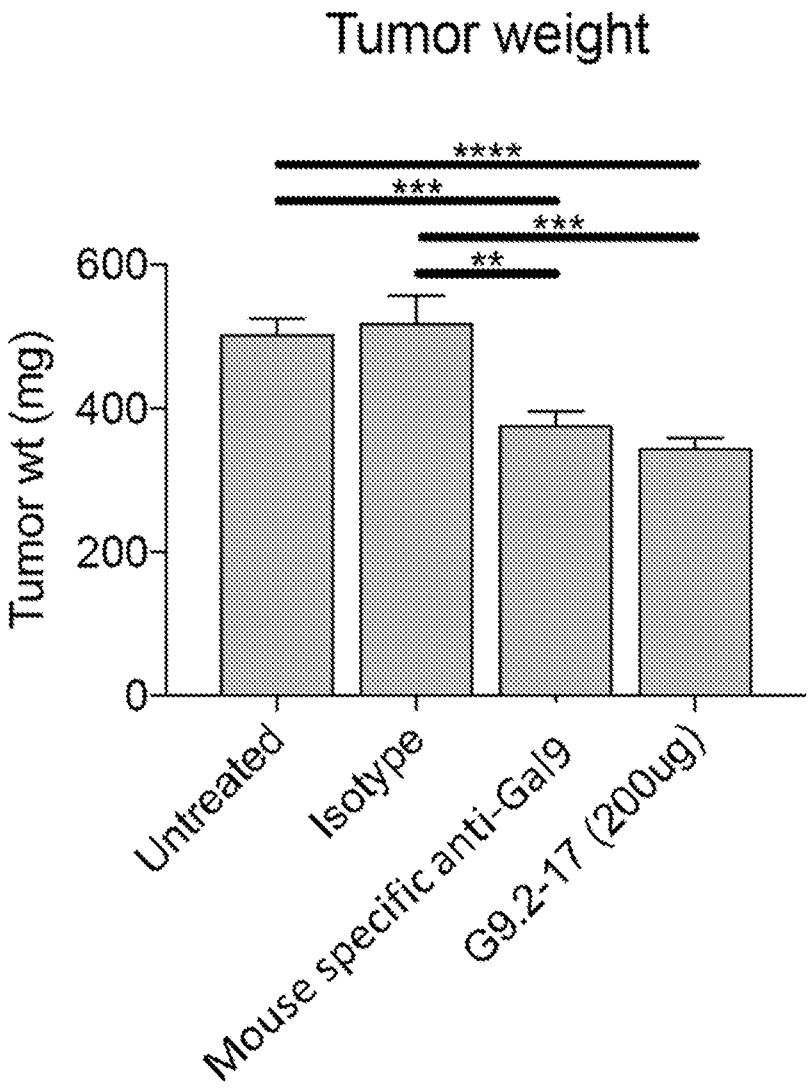

Tumor weight
****
***
***
**
600
400
200
0
Tumor wt (mg)
Untreated
Isotype
Mouse specific anti-Gal9
G9.2-17 (200ug)

Fig. 17

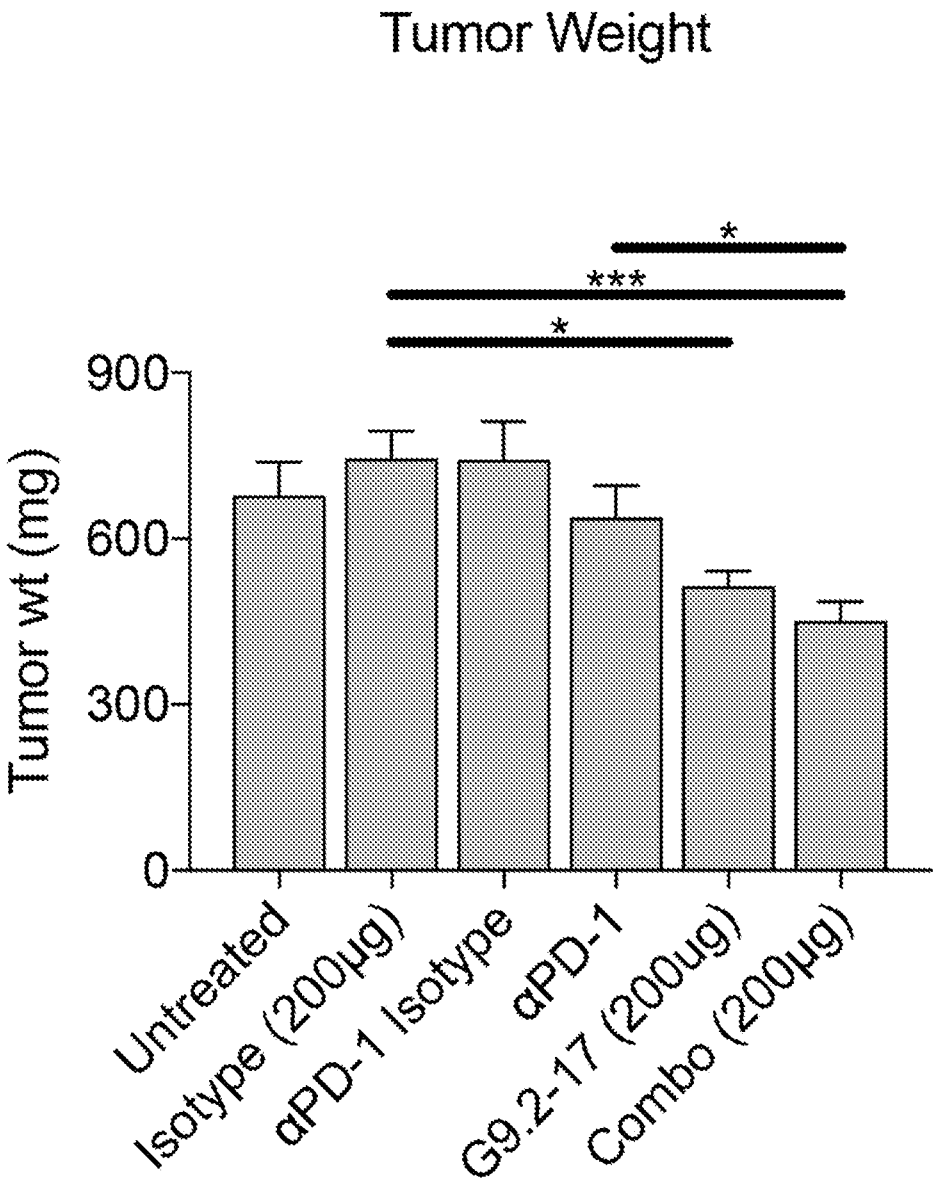

Tumor Weight
*
***
*
900
600
300
0
Tumor wt (mg)
Untreated
Isotype (200µg)
αPD-1 Isotype
αPD-1
G9.2-17 (200ug)
Combo (200µg)

Fig. 18A

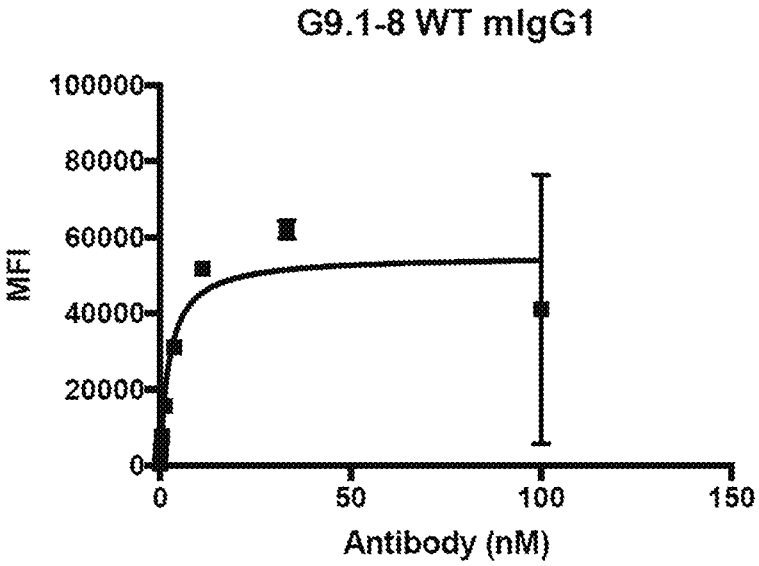


Fig. 18B

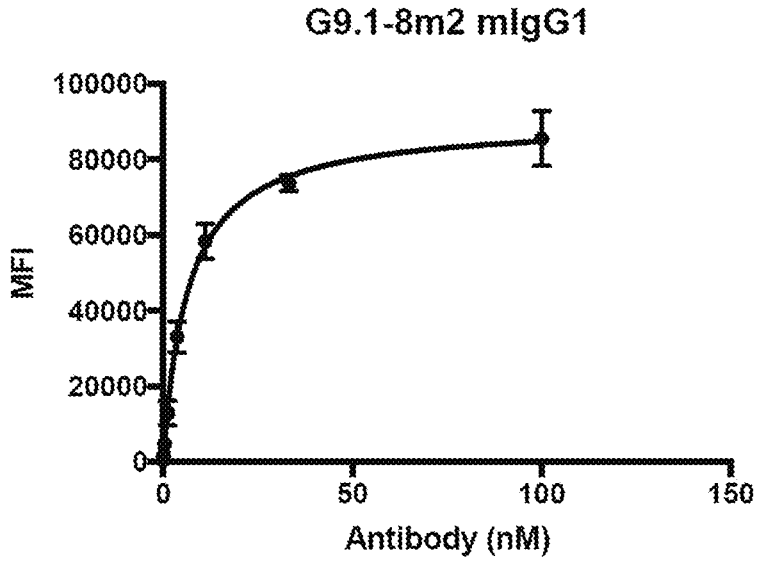


Fig. 18C

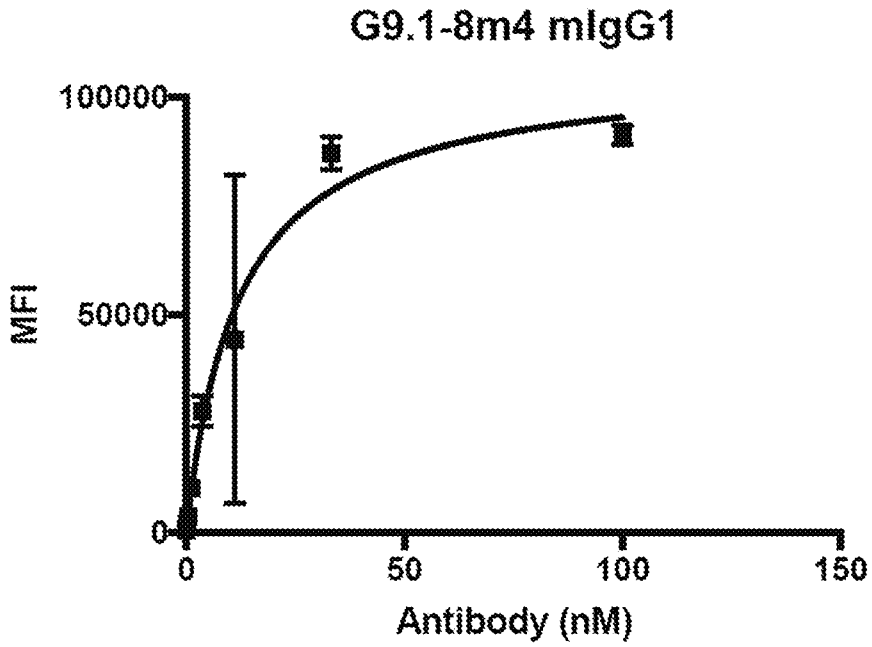


Fig. 19A

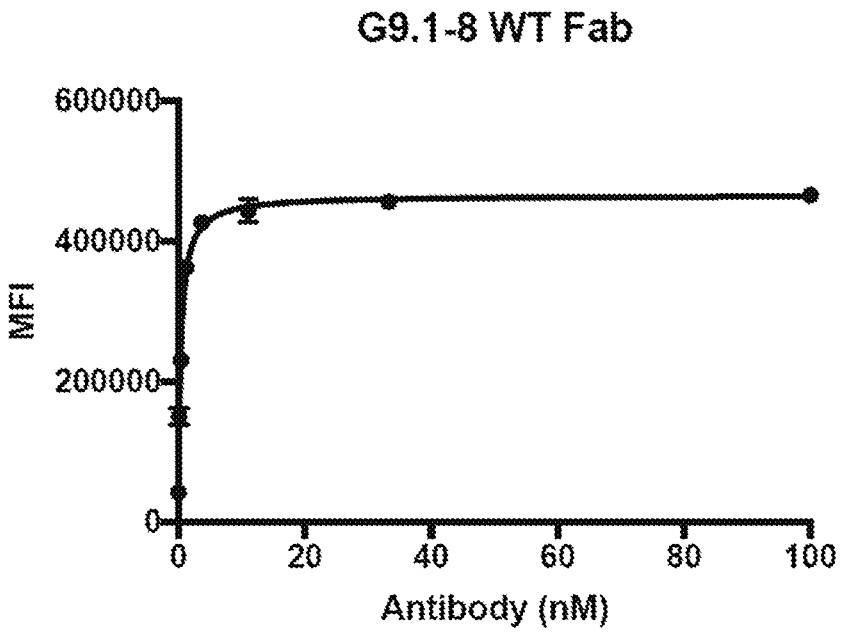


Fig. 19B

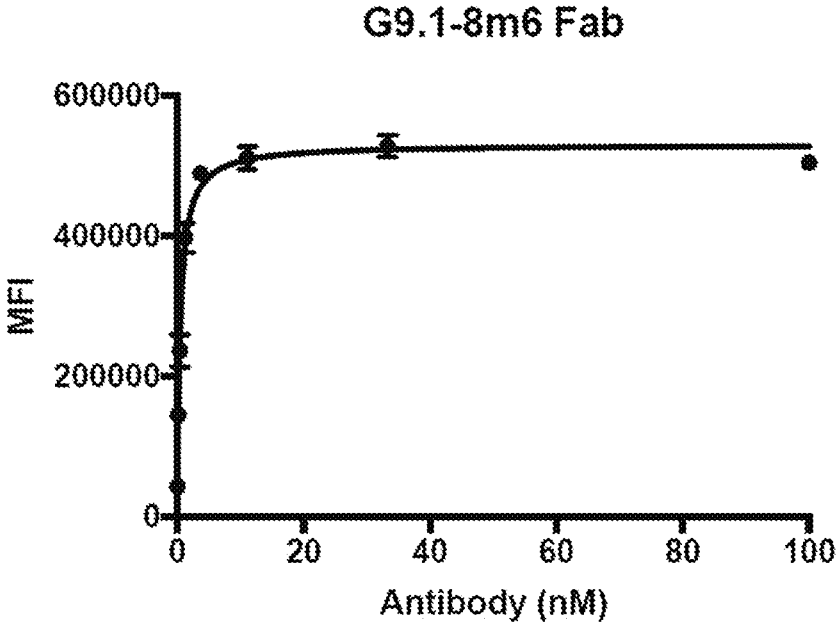


Fig. 19C

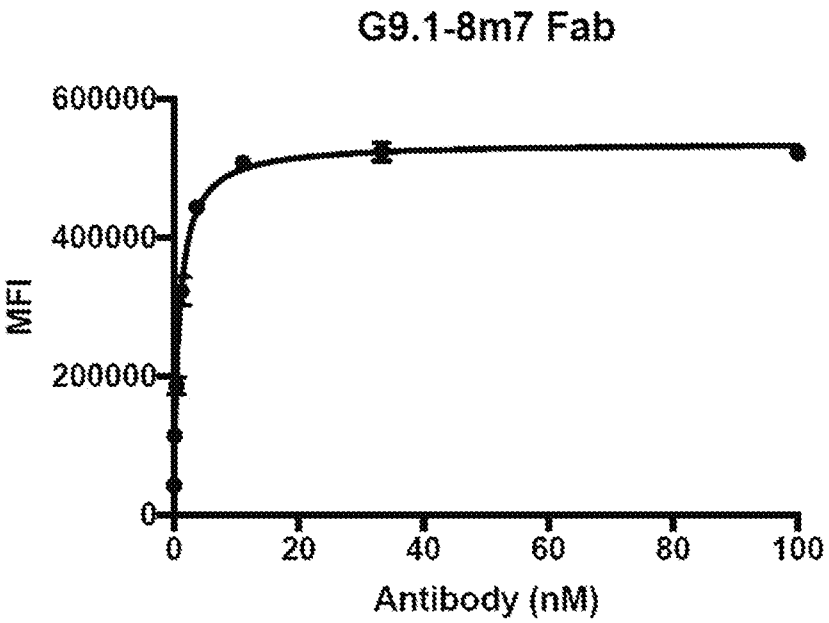


Fig. 19D

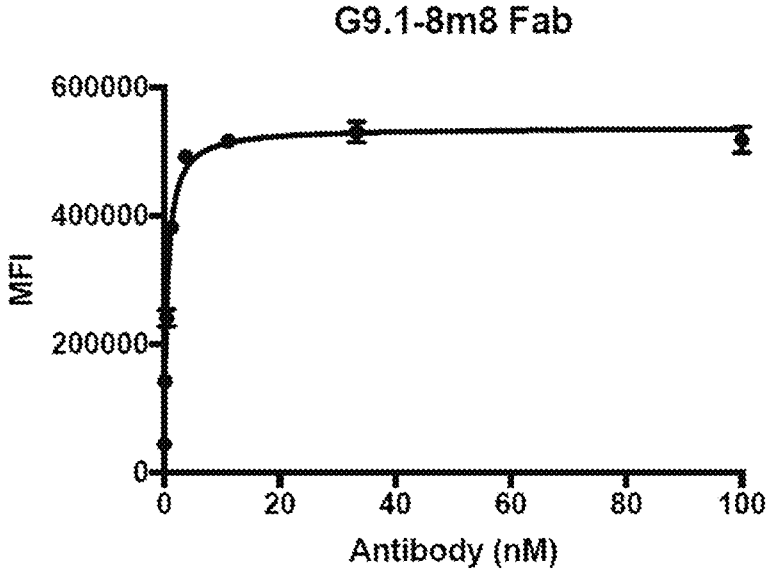


Fig. 19E

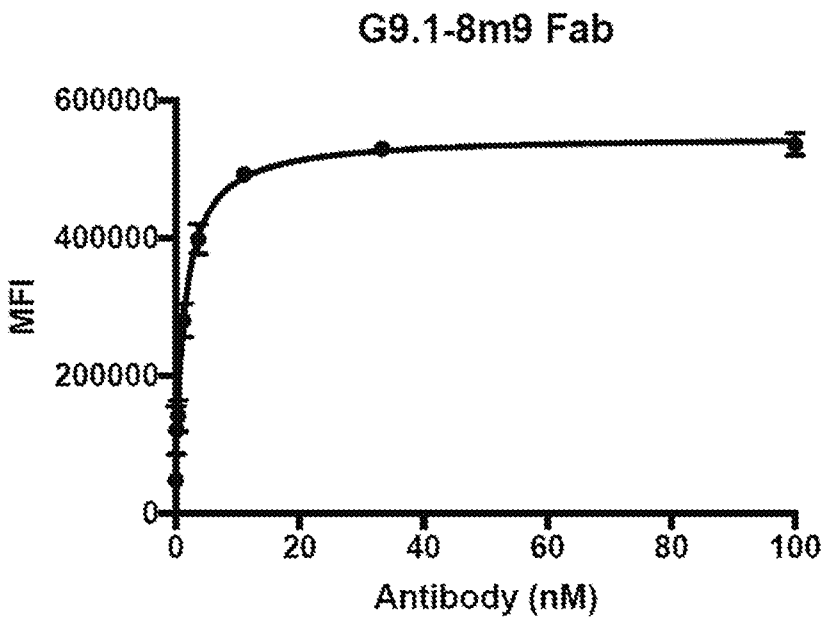


Fig. 19F

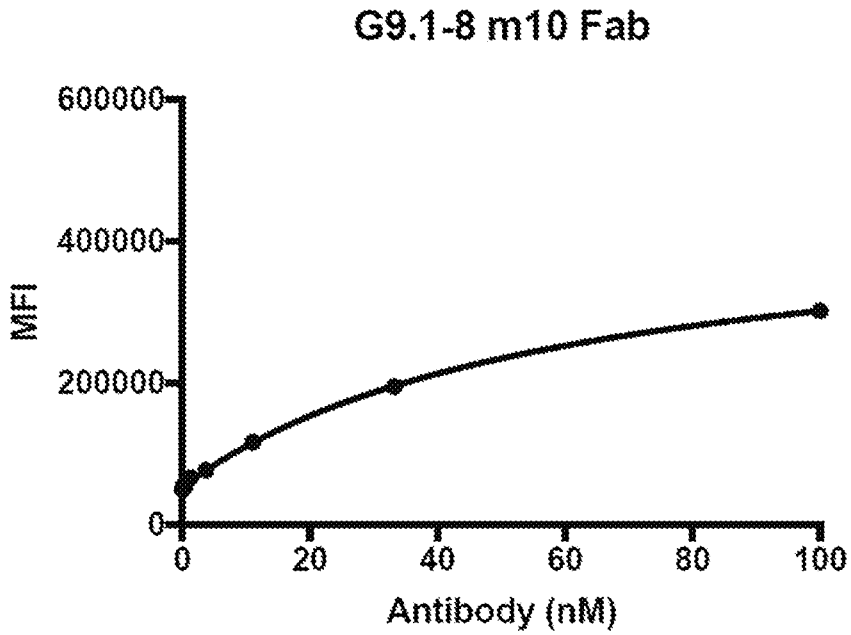


Fig. 19G

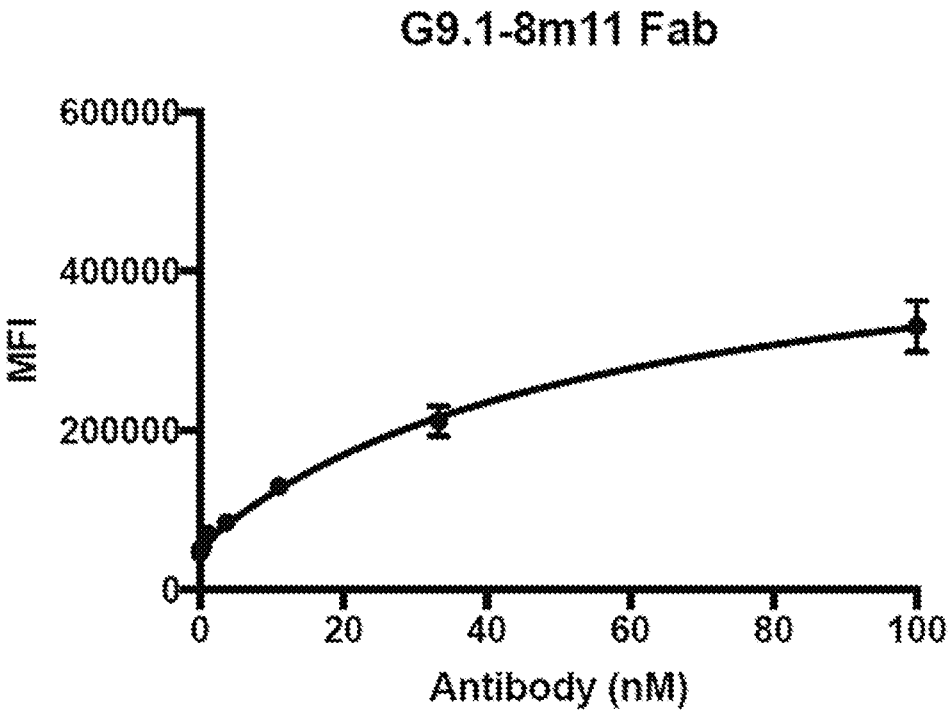

G9.1-8m11 Fab
600000
400000
200000
0
MFI
0
20
40
60
80
100
Antibody (nM)

Fig. 20A

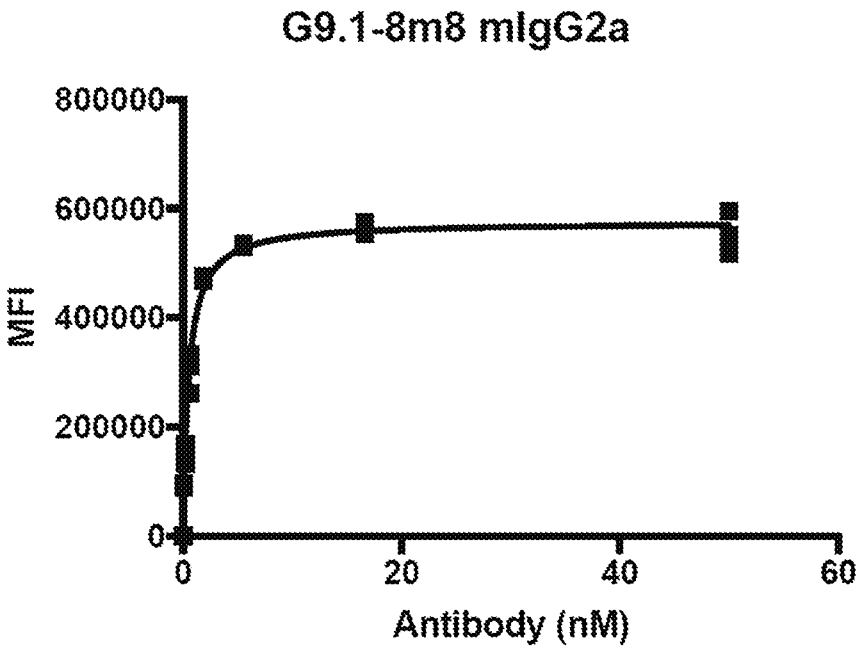


Fig. 20B

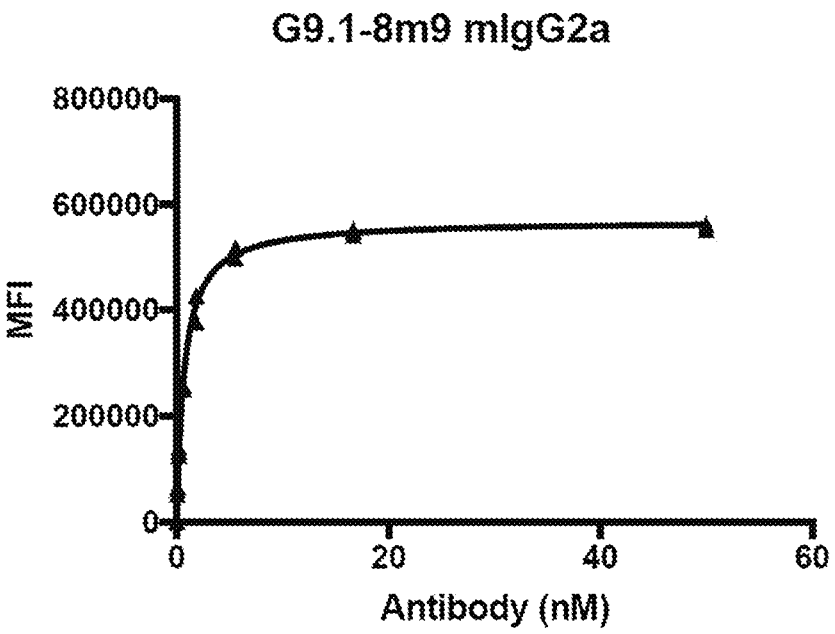


Fig. 20C

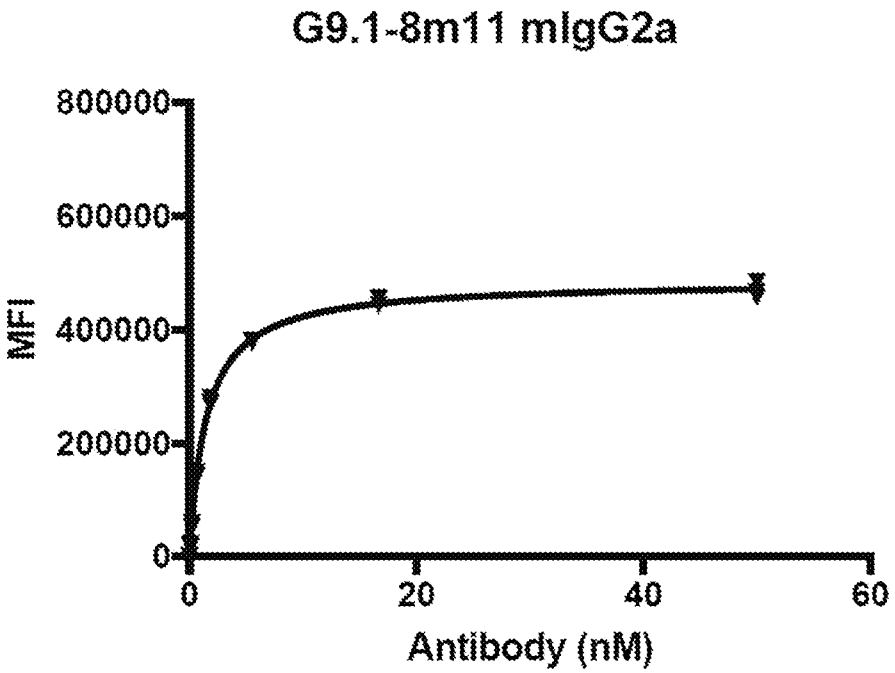

G9.1-8m11 mIgG2a
800000
600000
400000
200000
0
MFI
0
20
40
60
Antibody (nM)

Fig. 21A

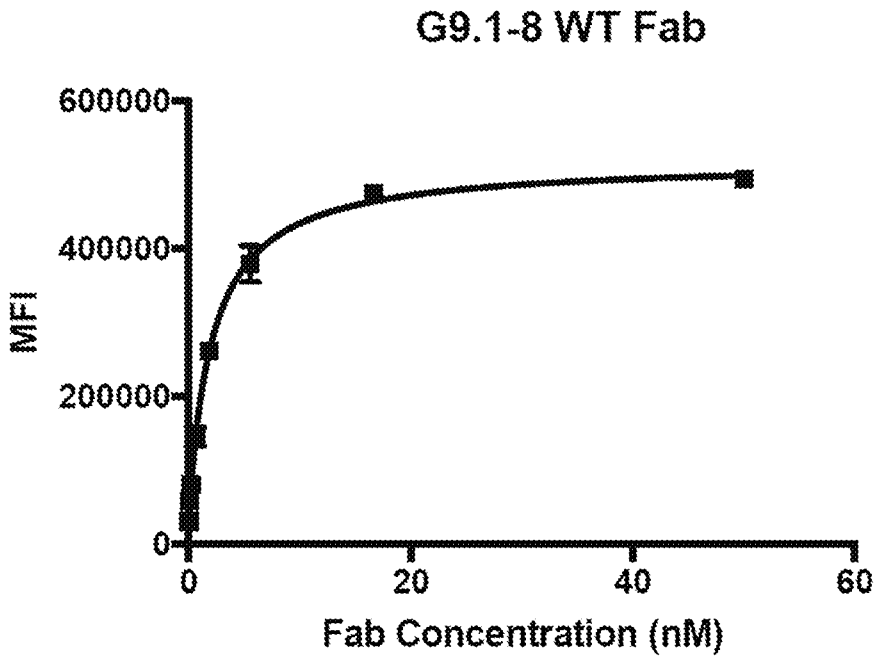


Fig. 21B

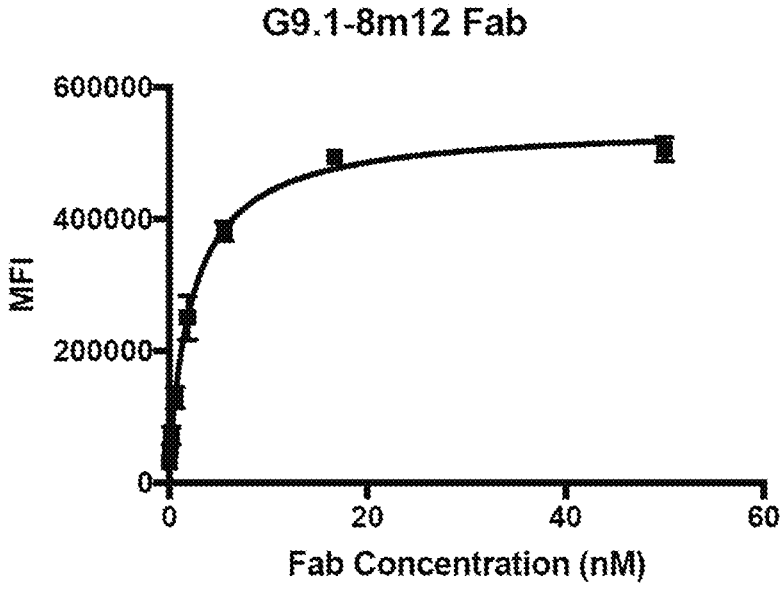


Fig. 21C

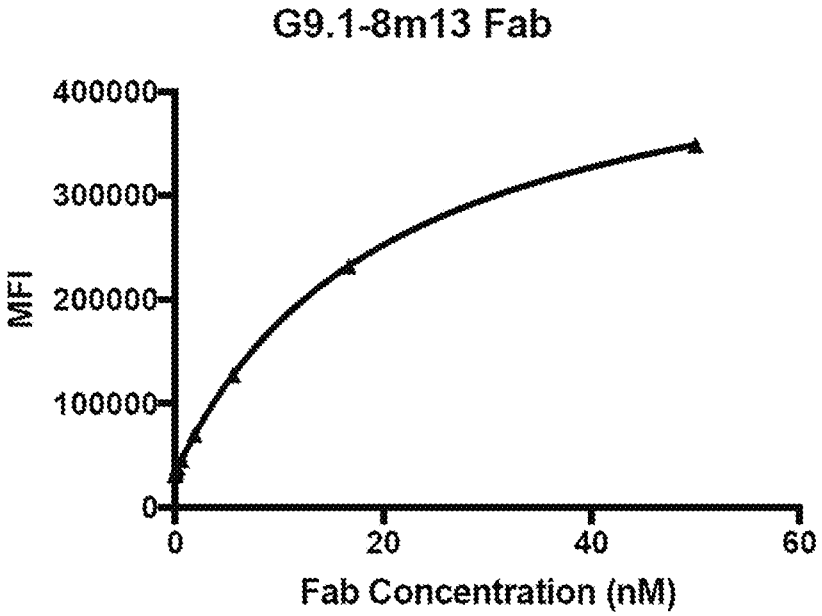


Fig. 21D

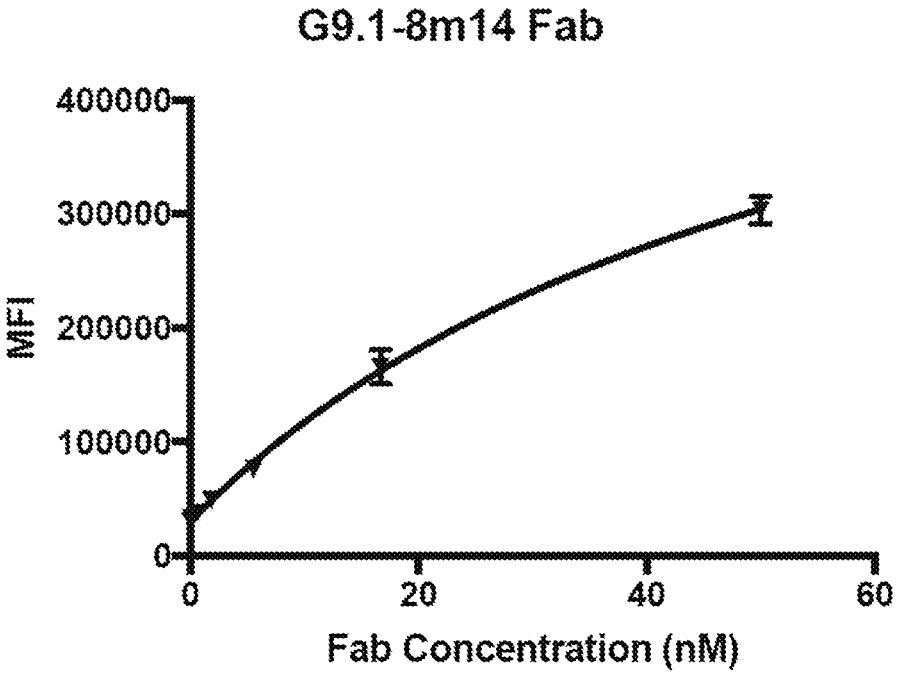


Fig. 22A

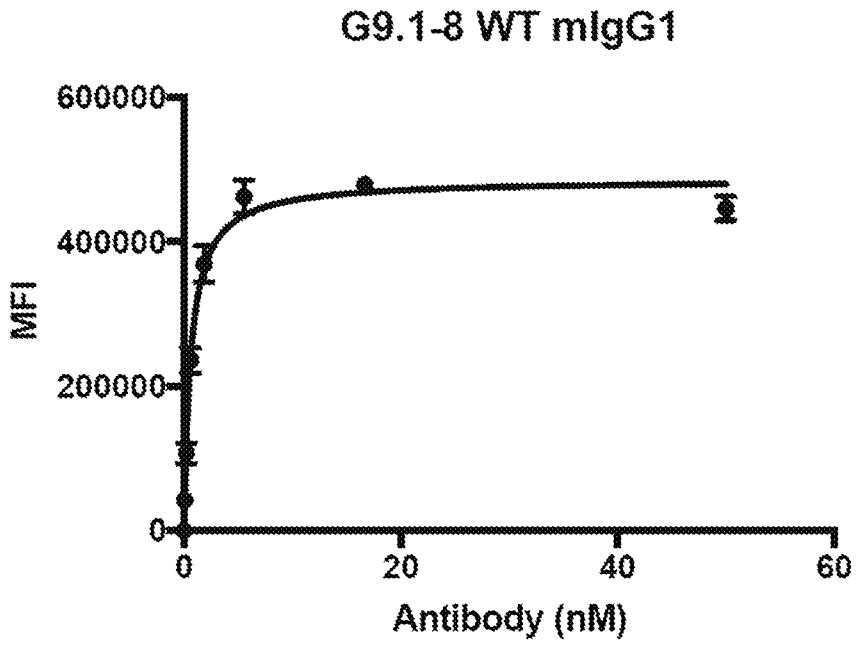


Fig. 22B

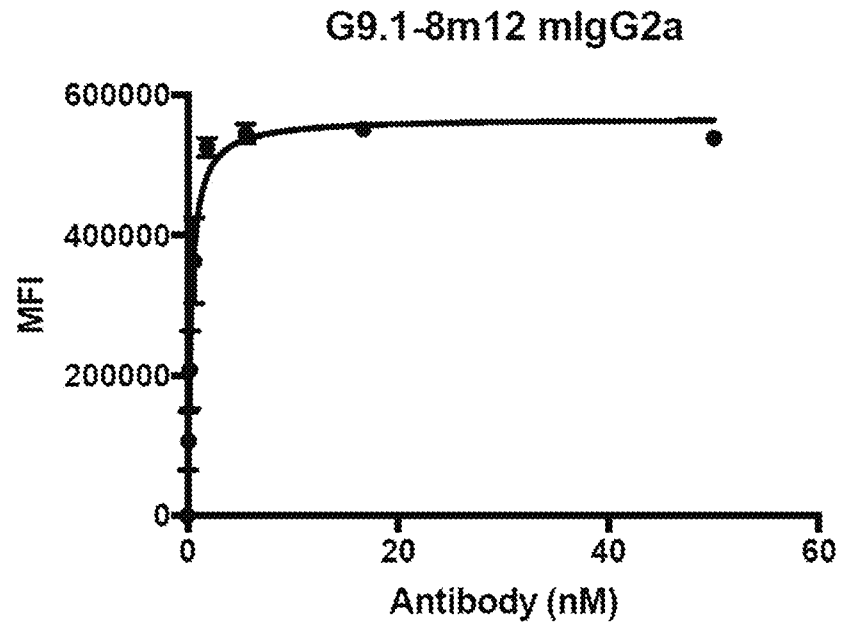


Fig. 22C

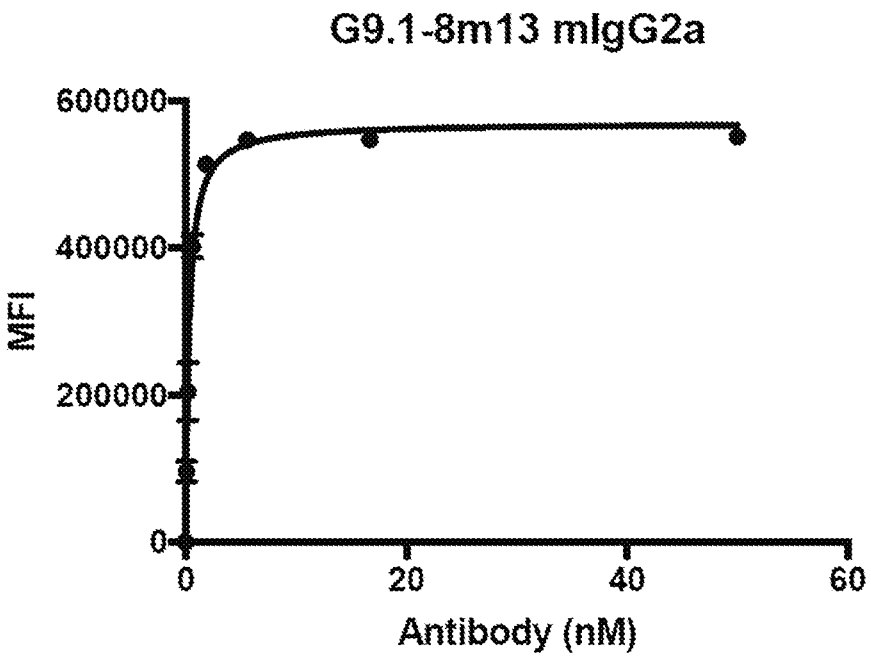


Fig. 22D

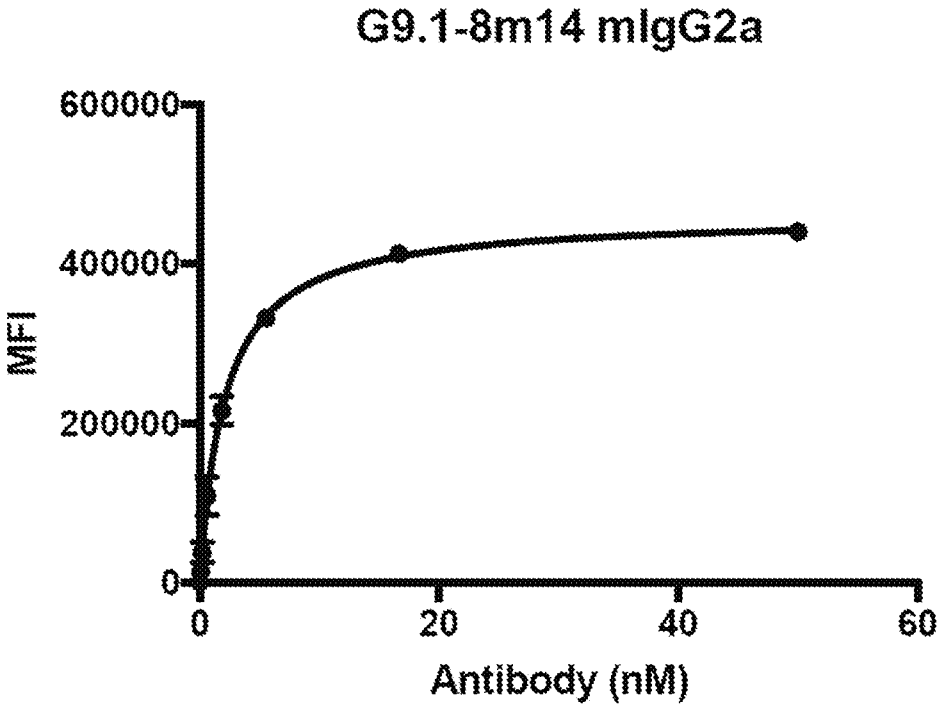


Fig. 23

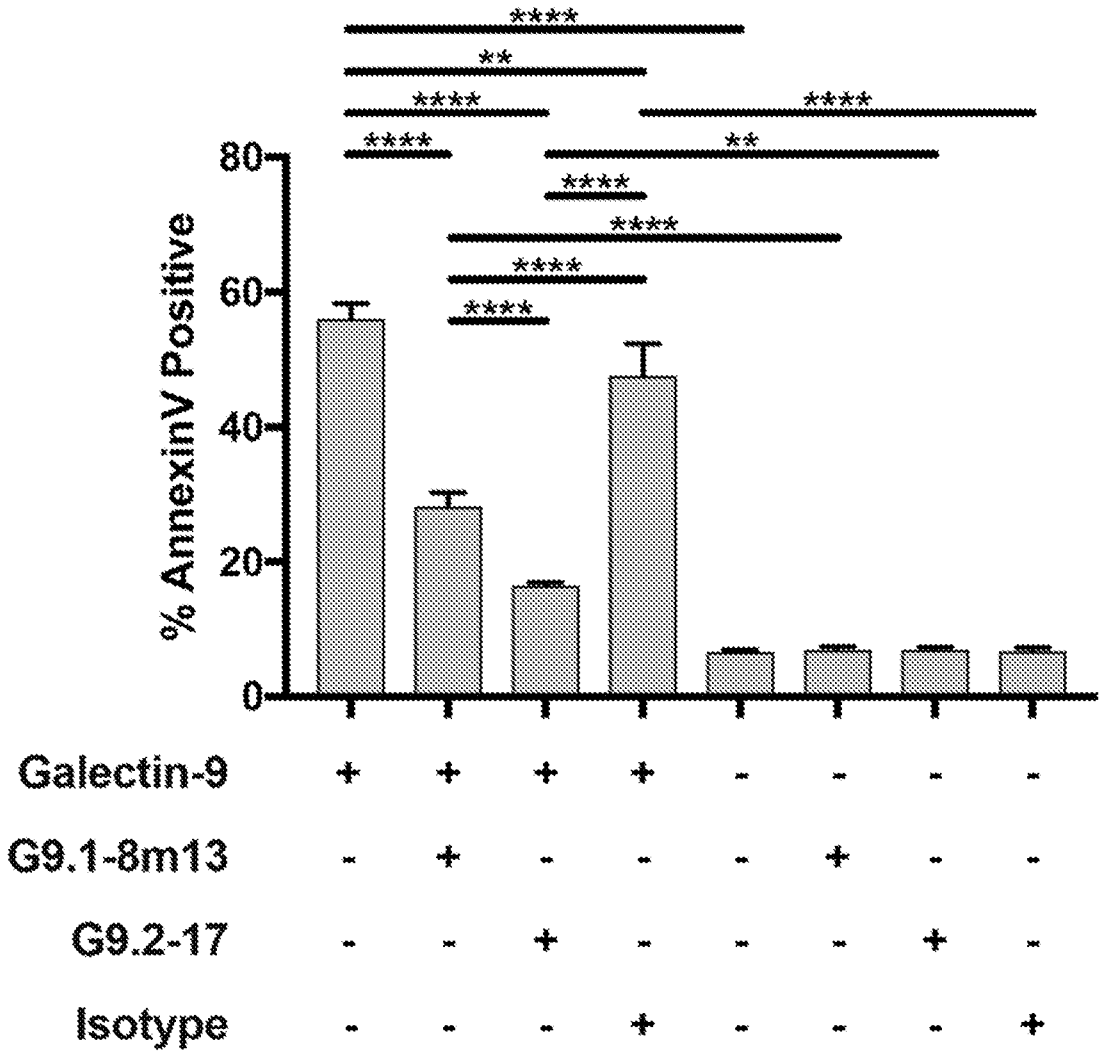

****
**
****
****
****
**
****
****
****
****
80
60
40
20
0
% AnnexinV Positive
Galectin-9 + + + + - - - -
G9.1-8m13 - + - - - + - -
G9.2-17 - - + - - - + -
Isotype - - - + - - - +

Fig. 24

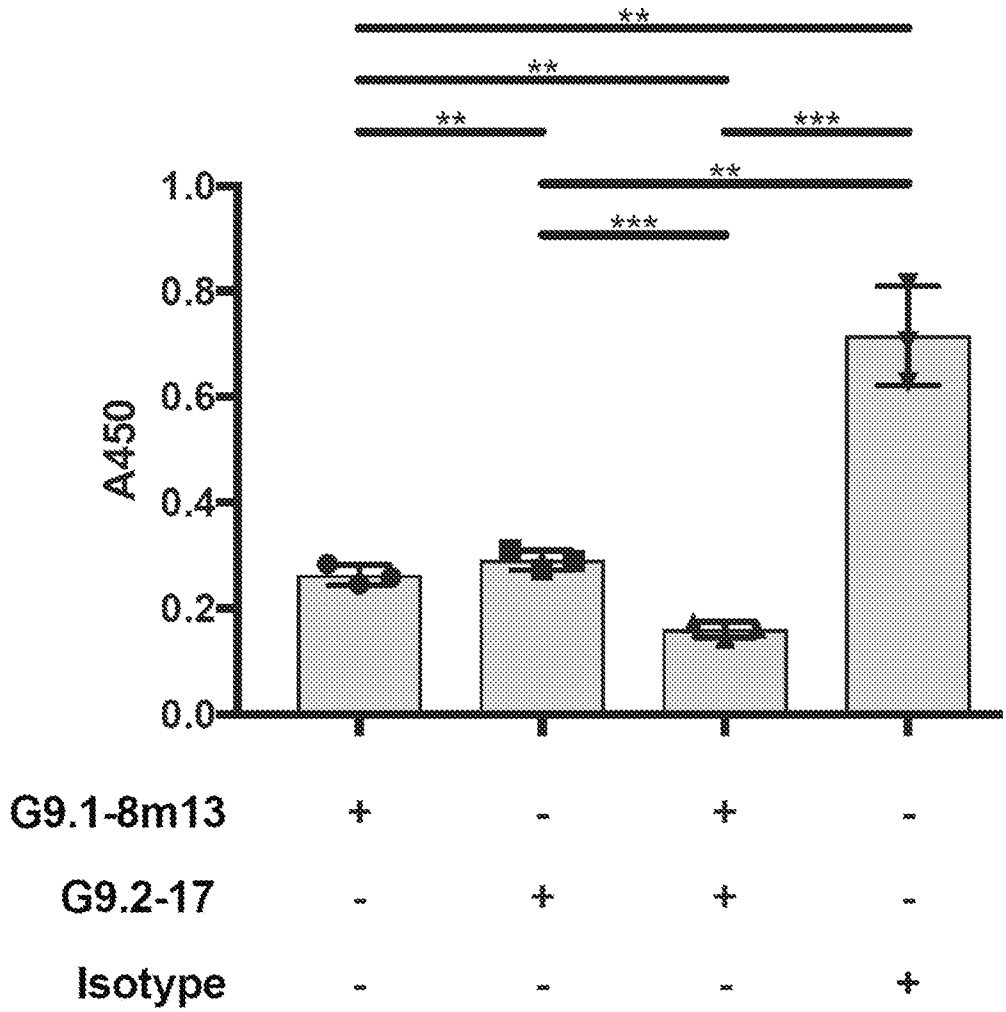


Fig. 25

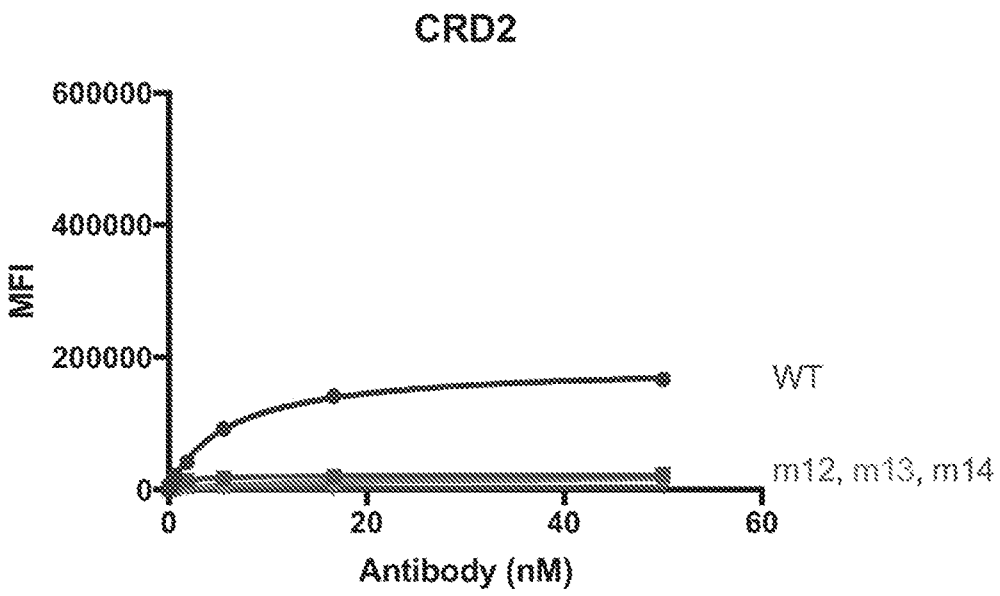

CRD2
600000
400000
200000
0
MFI
WT
m12, m13, m14
0
20
40
60
Antibody (nM)

Fig. 26A

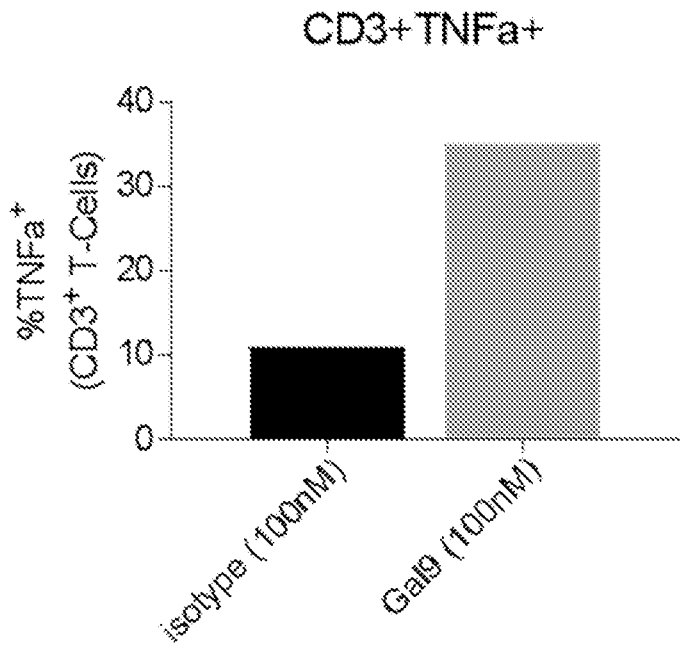


Fig. 26B

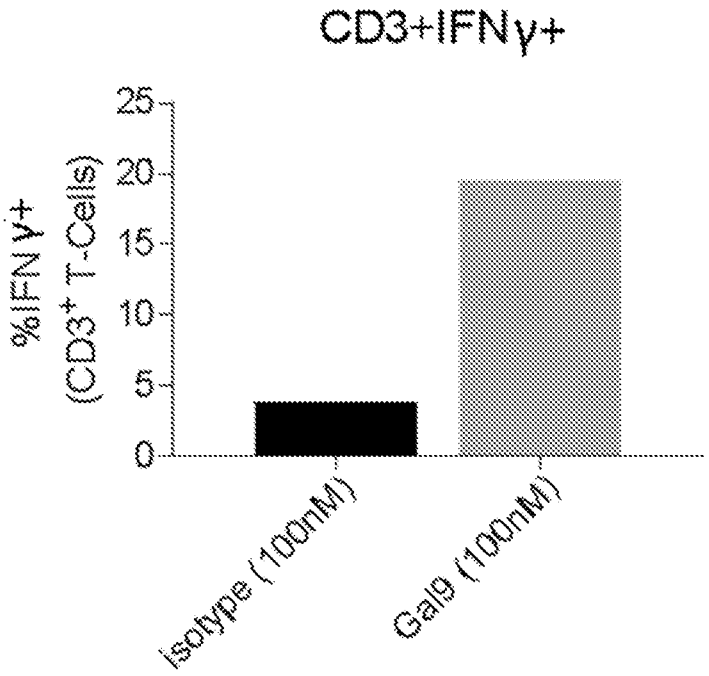


Fig. 27A

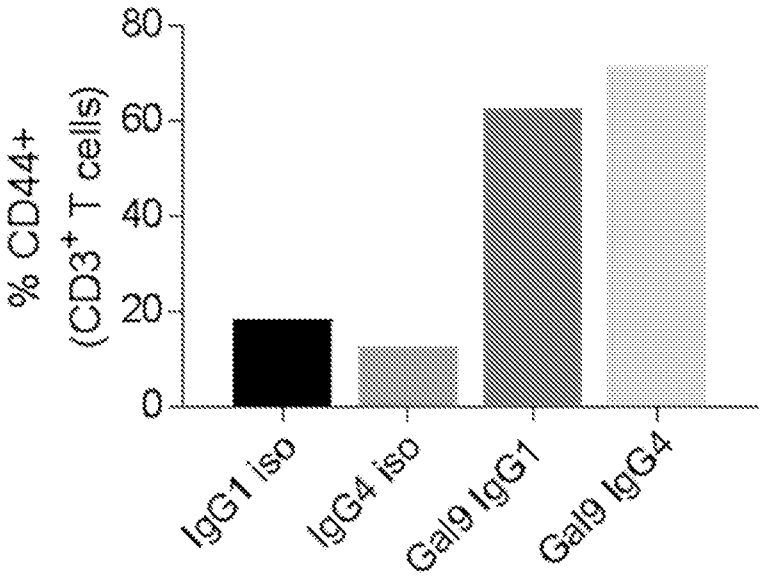


Fig. 27B

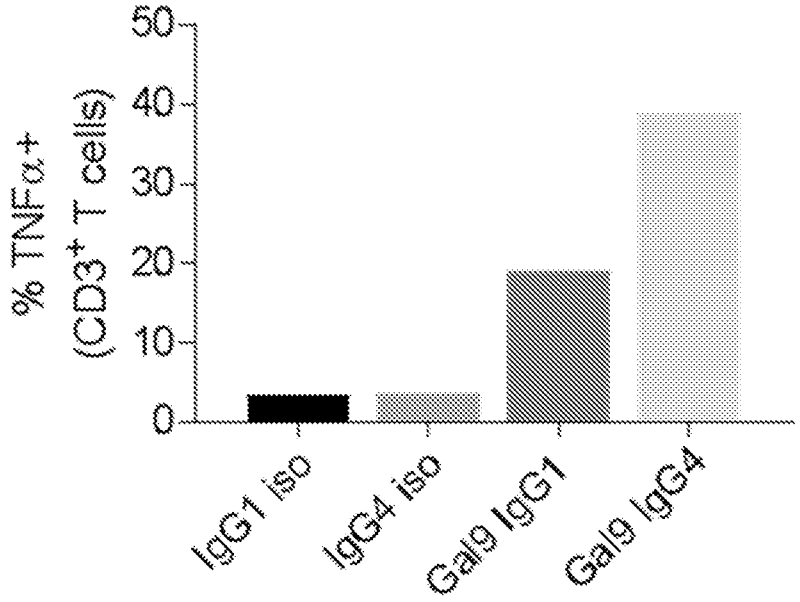


Fig. 27C

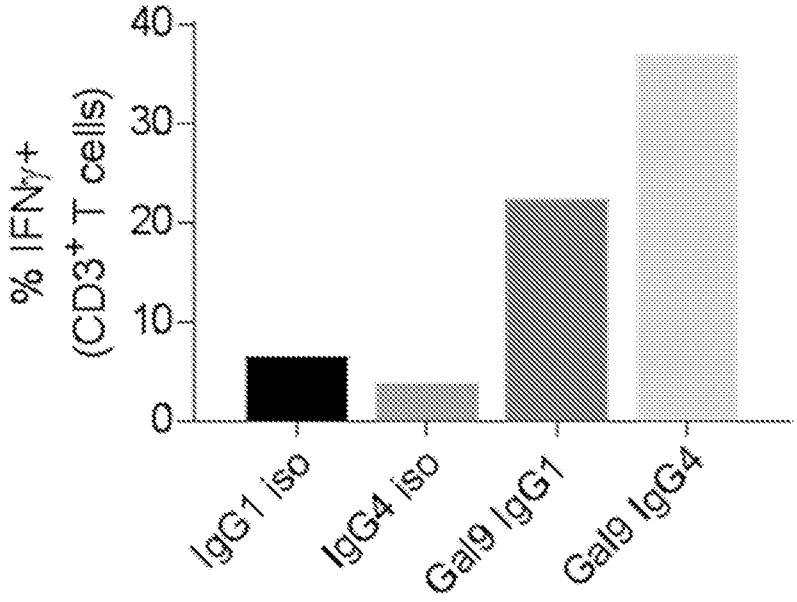

40
30
20
10
0
% IFNγ+
(CD3+ T cells)
IgG1 iso
IgG4 iso
Gal9 IgG1
Gal9 IgG4

Fig. 28A

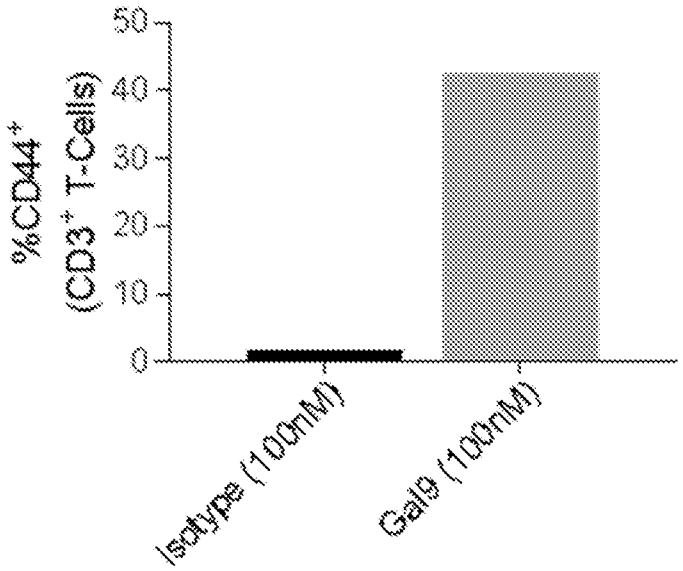


Fig. 28B

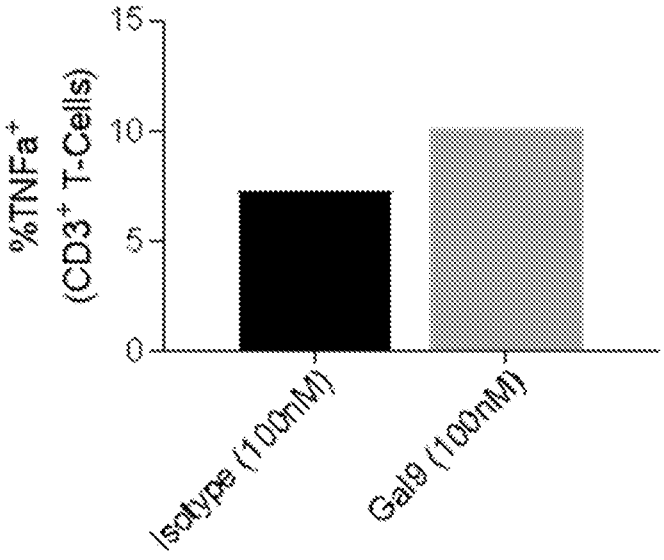


Fig. 28C

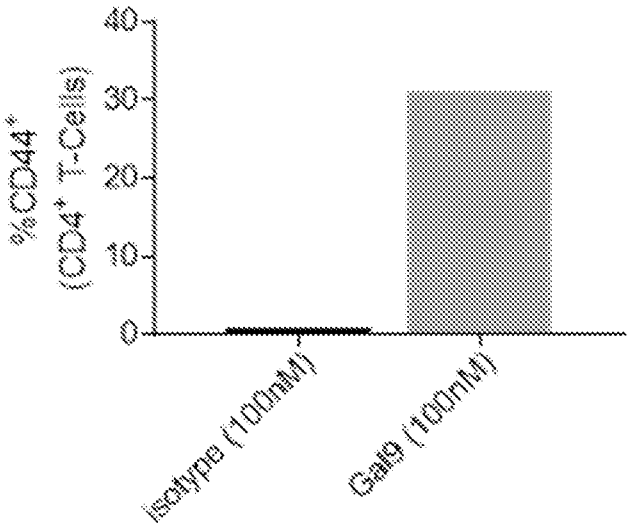


Fig. 28D

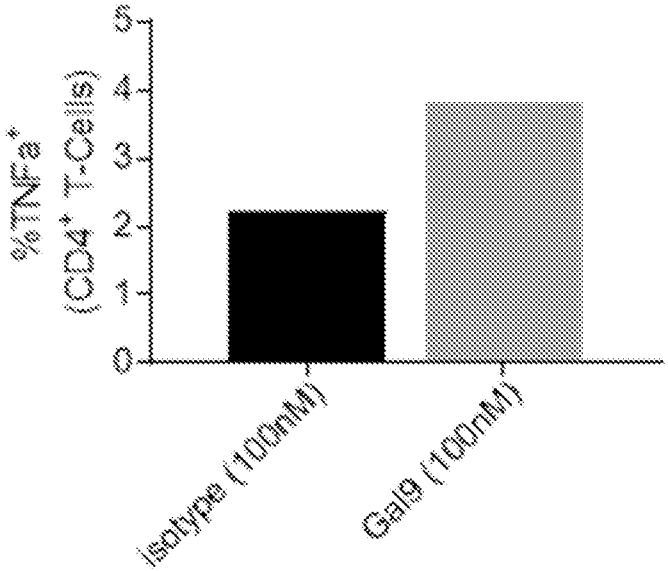


Fig. 28E

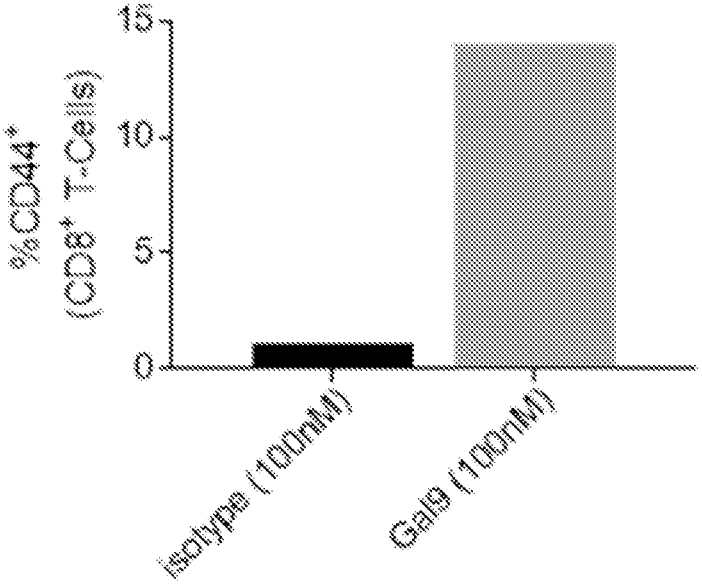


Fig. 28F

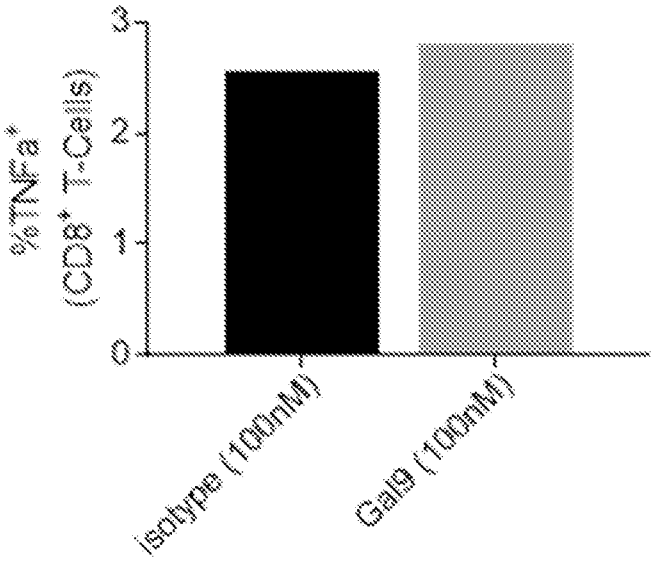


Fig. 29A

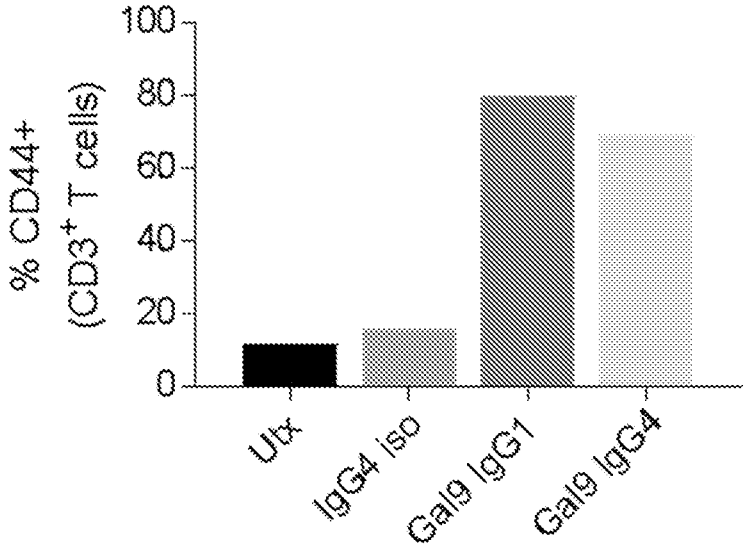


Fig. 29B

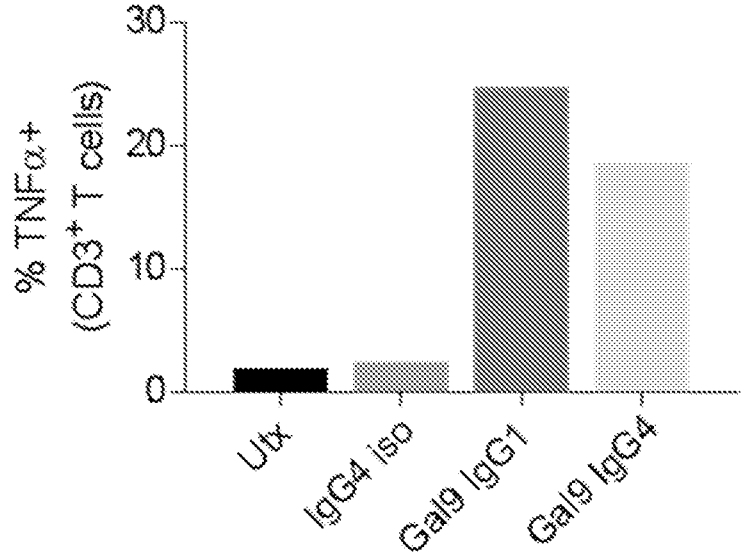


Fig. 29C

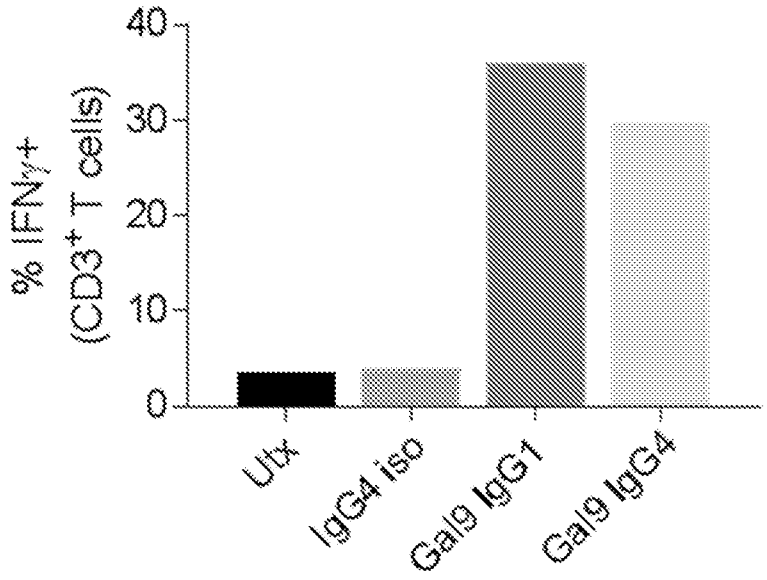


Fig. 30

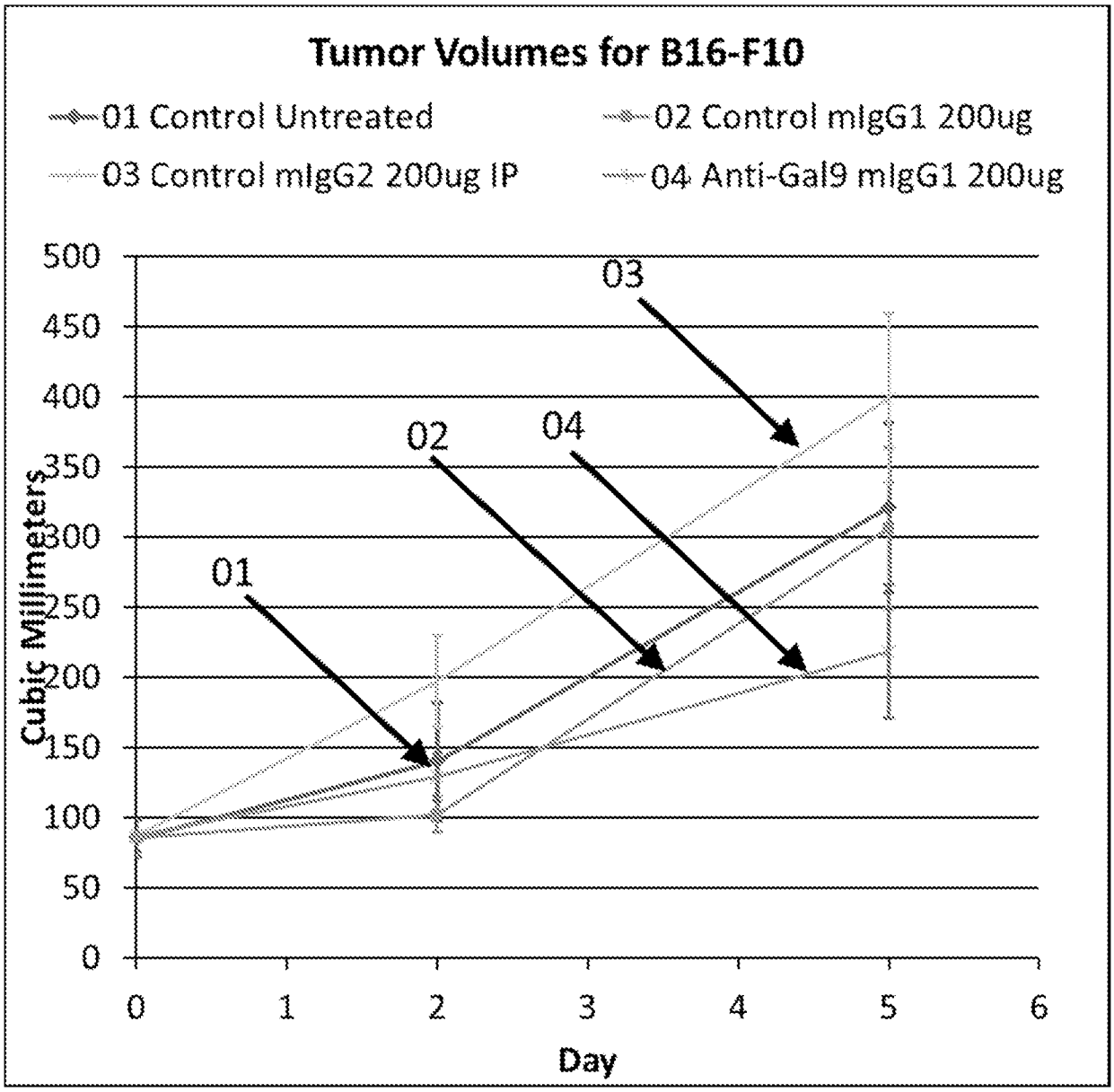

Tumor Volumes for B16-F10
01 Control Untreated
02 Control mIgG1 200ug
03 Control mIgG2 200ug IP
04 Anti-Gal9 mIgG1 200ug
Cubic Millimeters
500
450
400
350
300
250
200
150
100
50
0
0
1
2
3
4
5
6
Day
01
02
03
04

Fig. 31

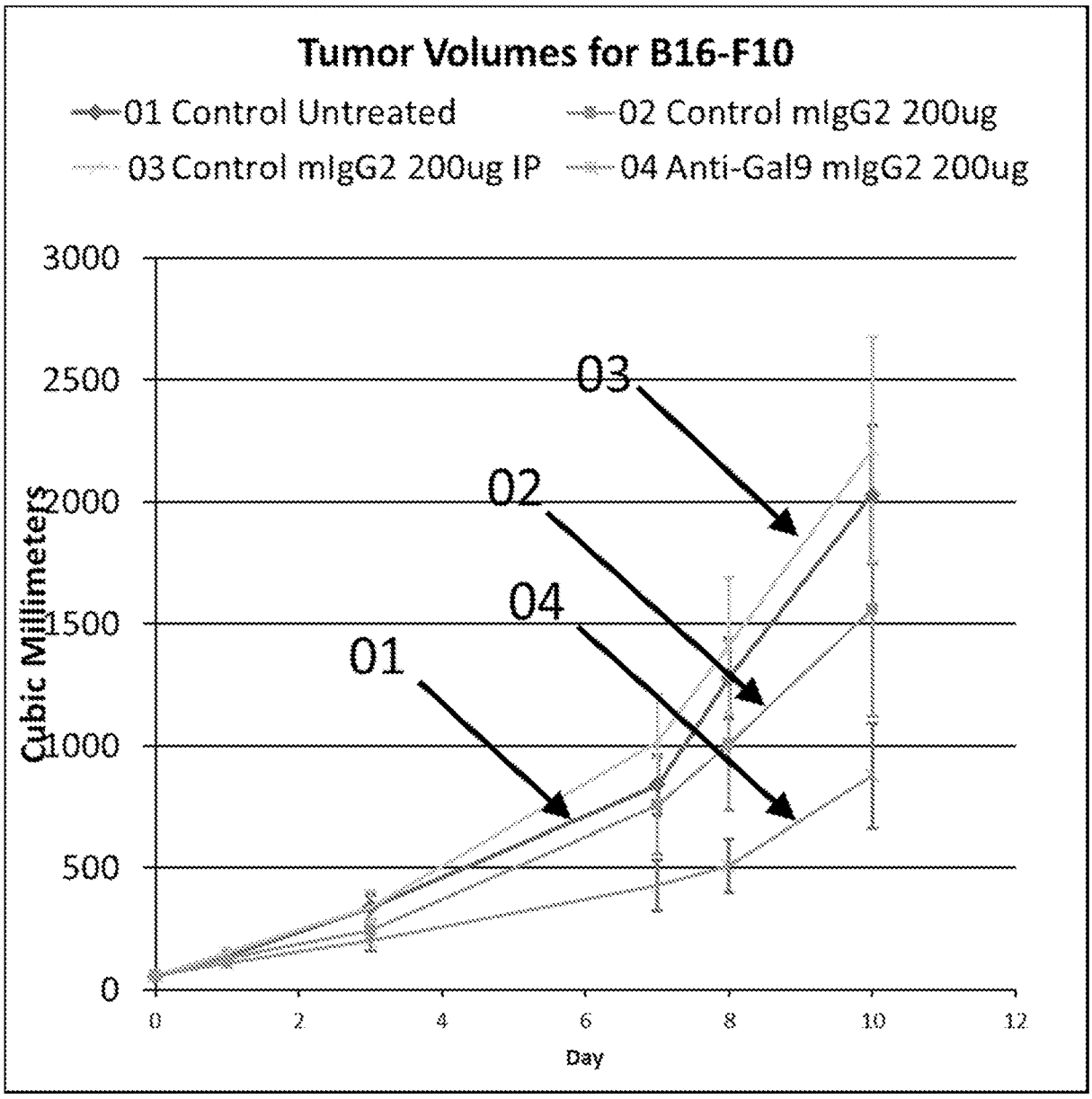

Tumor Volumes for B16-F10
01 Control Untreated
02 Control mIgG2 200ug
03 Control mIgG2 200ug IP
04 Anti-Gal9 mIgG2 200ug
Cubic Millimeters
3000
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
Day
03
02
04
01

Fig. 32

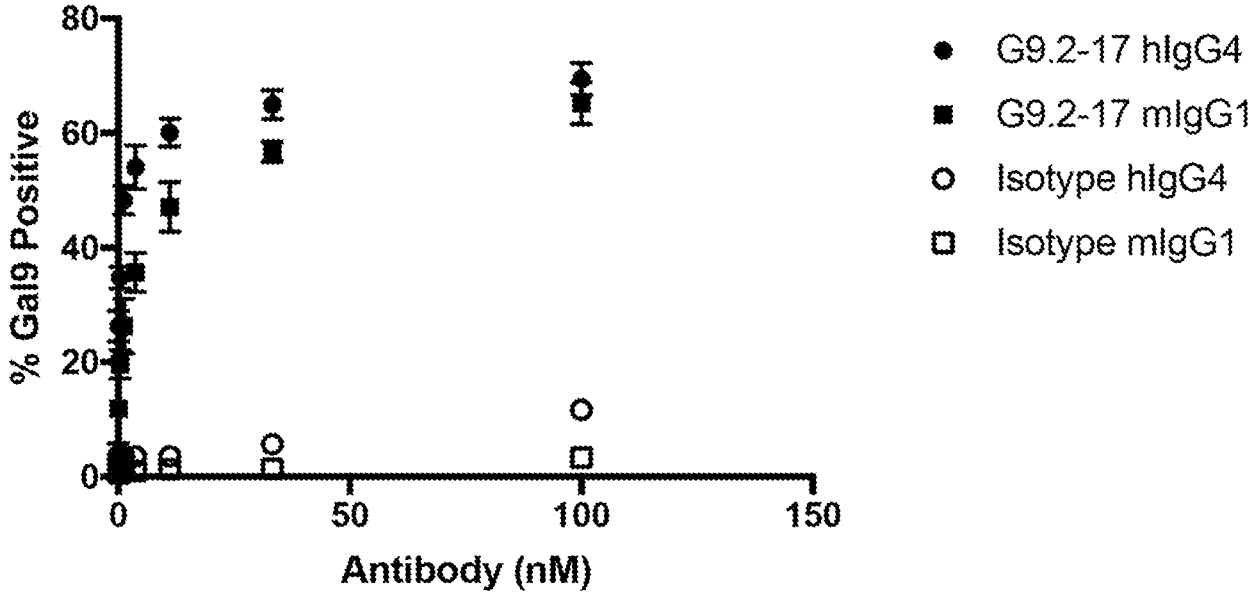

80
60
40
20
0
% Gal9 Positive
0
50
100
150
Antibody (nM)
G9.2-17 hIgG4
G9.2-17 mIgG1
Isotype hIgG4
Isotype mIgG1

Fig. 33A

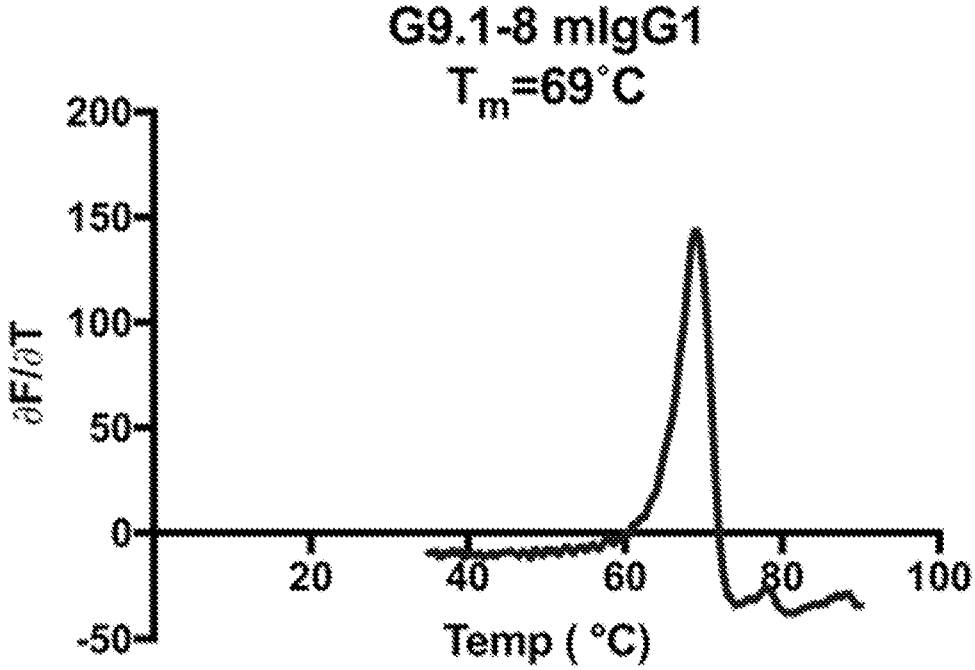


Fig. 33B

