ROR1 mAbs

V_κ

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
	1 23		35 49		57 88		98 107
ERR1-324	EIVLTQSPGTLSLSPGERATLSC	QASQSVSGWMELA	WYQQKPGQAPRLLIY	RASILTS	GVPSRFSGSGSGTDFTLTISWLQSEDFAVYYC	LGSYWSGSYRAA	FGGGTKLEIK
ERR1-TOP40	DIVLTQSPSSLSASVGDRVTITC	QASQSISSYLA	WYQQKPGKAPKLLIY	GASTLAS	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC	LGSYNYPHRTA	FGQGTKLEIK

V_λ

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
	1 23		35 49		57 88		98 107
XBR1-402	SYELTQPPSVSVSPGQTARITC	EGNNIGSKAVH--	WYQQKPGLAPGLLIY	DDSDRPS------	GVPERFSGSNSGNTATLTISRVEAGDEADYYC	QVWDSSAVV--	FGGGTQLTVLG
ERR1-301	SYELTQPPSVSVSPGQTARITC	GGNSIGSKAVH--	WYQQKPGLAPGLLIY	DDSDRPS------	GVPERFSGSNSGNTATLTISRAQAGDEADYYC	QLWDSSAVAYV	FGGGTQLTVLS
ERR1-306	QPVLTQPSSVSAAPGQKVTIS	TLSSAHSTYTID-	WYQQLPGTAPKLMS	LKSDGSYTKGD	GVPDRFSGSSSGADR--KYLIISGVQAEDEADYYC	GTDYSGSYV--	FGGGTQLTVLG
ERR1-316	SYELTQPPSVSVSPGQTARITC	GGNSIGSKAVH--	WYQQKPGLAPGLLIY	GDDERPS------	GVPDRFSGSNSGNTATLTISGAQAGDEADYYC	QLWDGSAYV--	FGGGTQLTVLG
ERR1-403	SYELTQLPSVSVSLGQTARITC	GGNSIGSKAVH--	WYQQKPGLAPGLLIY	DDDERPS------	GVPARFSGSNSGNTATLTISGAQAGDEADYYC	QLWDSSTGAYA	FGGGTQLTVLG
ERR1-TOP22	SYELTQPPSVSVSLGQTARITC	GGNSIGSKAVH--	WYQQKPGLAPGLLIY	DDDERPS------	GVPERFSGSNSGNTATLTISGAQAGDEADYYC	QLWDSSAYV--	FGGGTQLTVLG
ERR1-TOP43	SYELTQPPSVSVSLGQTARITC	GGNNIGSKAVH--	WYQQKPGLAPGLLIY	MDDERPS------	GVPERFSGSNSGNTATLTISGAQAGDEADYYC	QLWDSSMGAYV	FGGGTQLTVLG
ERR1-TOP54	SYELTQLPSVSVSPGQTARITC	GGSSIGSKAVH--	WYQQKPGLAPGLLIY	DDSERPS------	GVPERFSGSNSGNTATLTISGAQAGDEADYYC	QLWDSSAWAFV	FGGGTQLTVLG

V_H

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
	1 30		36 49		66 94		103 113
XBR1-402	QEQLVESGGGLFTPGTPLTLTCTASGFDIS	SYYMS	WVRQAPGKGLEWIG	AIGISGRAYYASWAK-	RSTTTRNTWLKT-VTLKMTSLTTADTATYFCAR	DRPTYGMDL----------	WGPGTLVTVSS
ERR1-301	QEQLEESGGRLVTPGSSLTLTCTASGFTIS	TYEMS	WVRQAPGKGLEWIG	SIYAGSGTTYYASWVNG	RFTISSWTTQWT-VSLQMMSLTVADTATYFCAR	DSPSYGMDL----------	WGPGTLVTVSS
XBR1-306	QEQLVESGGGLVQPGGSLKLSCAASGFDLS	WYGVS	WVRQAPGKGLEWIG	YIDPTDVTTYYASWVNG	RFSISRDNSQNT-VSLQLNSLTTADTATYFCAR	WTGYADYGGNSWFDL	WGQGTLVTISS
ERR1-316	QEQLEESGGRLVTPGTPLTLTCTASGFPLS	SYTVS	WVRQAPGKGLEWIG	AIGTGGSAYYASWAK-	RSTTTRNTMLST-VTLKMTSLTAADTATYFCAR	DRPWYGMDL----------	WGPGTLVTVSS
ERR1-324	QS-LEESGGRLVTPGGSLTLTCTVSGFSLS	RMGMT	WVRQAPGKGLEWIG	LITSGGNTYYATWAKG-	RFTISKT---SSTVDLKMTSPTTEDTATYFCAR	GTWSSDI----------	WGPGTLVTISS
ERR1-403	QEQLEESGGRLVTPGGSLKLSCKASGFDFS	GWYMT	WVRQAPGKGLEWIG	YIGTTGGTTYYASWVNG	RFTISS---MAQMTVRLQMSSLTAADTATYFCVR	GSDYDL----------	WGPGTLVTISS
ERR1-409	QEQLVESGGRLVTPGGSLTLTCTASGFSIS	SYIMG	WVRQAPGKGLEWIG	TIYGGSSTWYASWVNG	RFTISKT---S-TSVDLKITSPTTEDTATYFCAR	SYGWTTTEPYGGI----	WGPGTLVTVSS
ERR1-TOP4	QS-VEESGGRLVTPGTPLTLTCTASGIDLS	RYAIM	WVRQAPGKGLEWIG	VISGSGTTYYASWAKG-	RFTISKT---S-TTVDLKITSPTTEDTATYFCAR	GSWTDSLFGTGTLVTVSS	WGQGTLVTVSS
ERR1-TOP15	QEQLVESGGRLVTPGGSLTLTCTASGFSFS	SYWMS	WVRQAPGKGLEWIG	AIYVDSGAYYASWAKG	RFTISKT---SSTVDLKMTSLTTEDTAIYICAR	DGWSSGV----------	WGRGLLVTISS
ERR1-TOP22	QS-LEESGGRLVTPEPLTLTCTVSGFSLS	SYGVG	WVRQAPGKGLEWIG	TISSGGTTYYASWAKG-	RFTISKT---S-TTVDLKMTSLTTEDTATYFCAR	DLTTDMGL----------	WGPGTLVTVSS
ERR1-TOP40	QEQLEESGGRLVTPEETLTLTCTVSGFSLS	WYTMS	WVRQAPGKGLEYIG	YISSGGSTYYASWAKG-	RPTISKT---SSTVDLKITSPTTEDTATYFCAR	LVVSGSTDAL----------	WGPGTLVTVSS
ERR1-TOP43	QS-LEESGGRLVTPEETLTLTCTVSGFSLS	SYWMS	WVRQAPGKGLEWIG	AIYGSGWTYYATWAKG-	RFTISKT---S-TTVDLKITSPTTEDTATYFCAR	DWSTATFDL----------	WGPGTLVTISS
ERR1-TOP54	QS-LEESGGRLVTPGTPLTLTCTASGFSLS	SYTMS	WVRQAPGKGLEWIG	TIYGGSGTTYYATWAKG	RFTISKT---S-TTVDLKITSPTTEDTATYFCAR	DPGLSTGGL----------	WGPGTLVTISS

FIG. 1

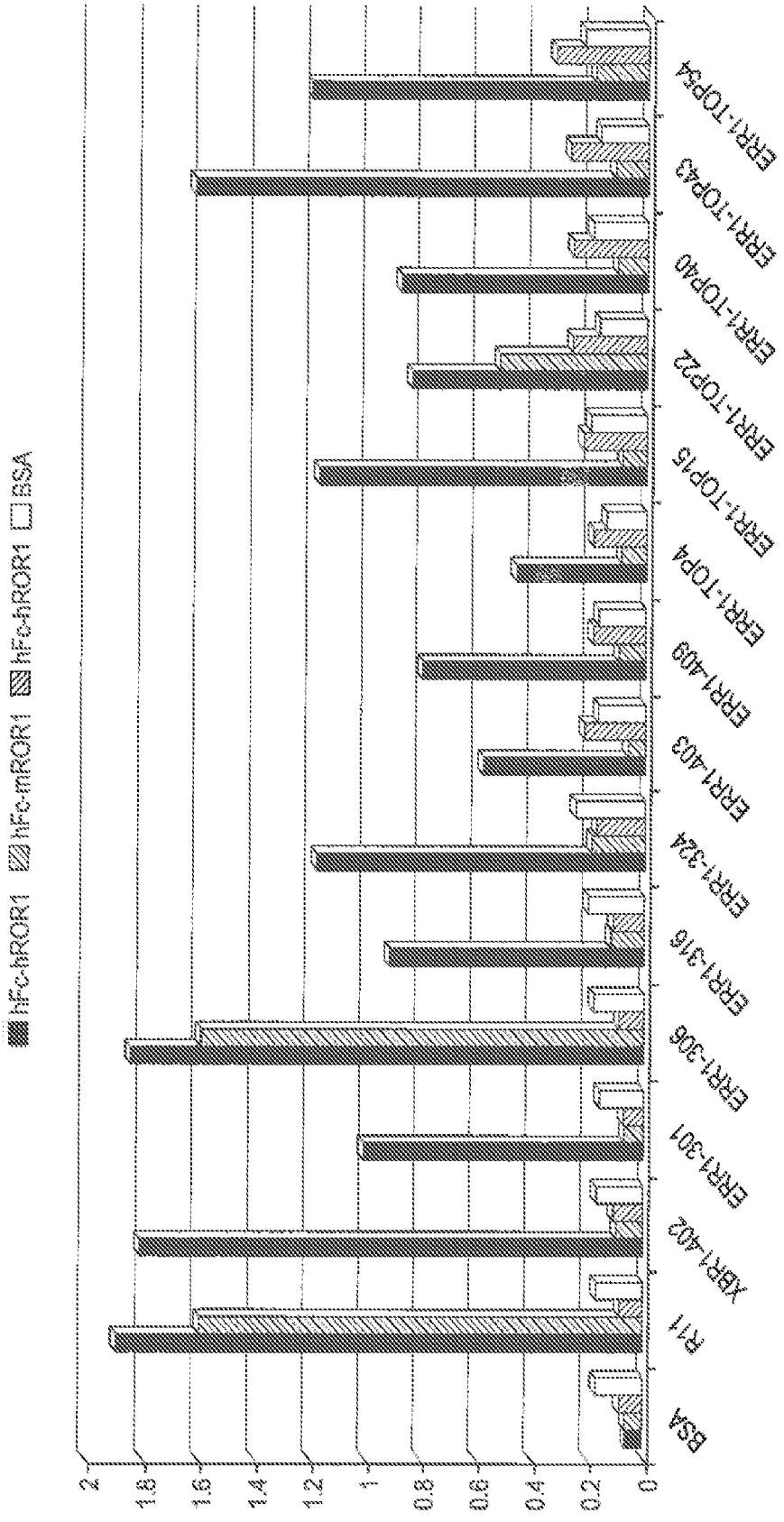

hFc-hROR1
hFc-mROR1
hFc-hROR1
BSA
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
BSA
R1
XBR1-402
ERR1-301
ERR1-306
ERR1-316
ERR1-324
ERR1-403
ERR1-409
ERR1-TOP4
ERR1-TOP15
ERR1-TOP22
ERR1-TOP40
ERR1-TOP43
ERR1-TOP54

FIG. 2

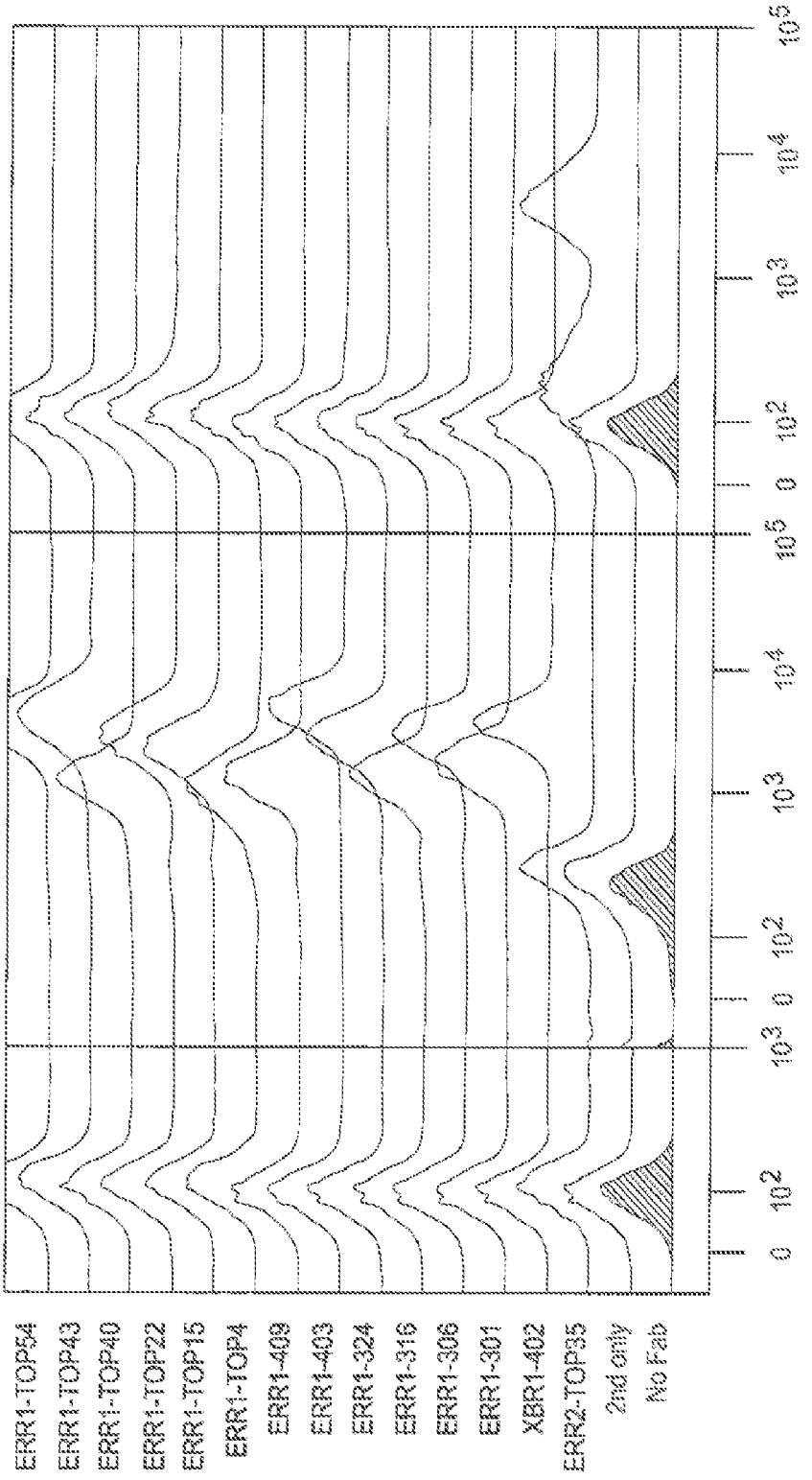

Fabs
63-12
63-12 / hROR1
63-12 / hROR2
ERR1-TOP54
ERR1-TOP43
ERR1-TOP40
ERR1-TOP22
ERR1-TOP15
ERR1-TOP4
ERR1-409
ERR1-403
ERR1-324
ERR1-316
ERR1-306
ERR1-301
XBR1-402
ERR2-TOP35
2nd only
No Fab
0
10^2
10^3
0
10^2
10^3
10^4
10^5
0
10^2
10^3
10^4
10^5

FIG. 3

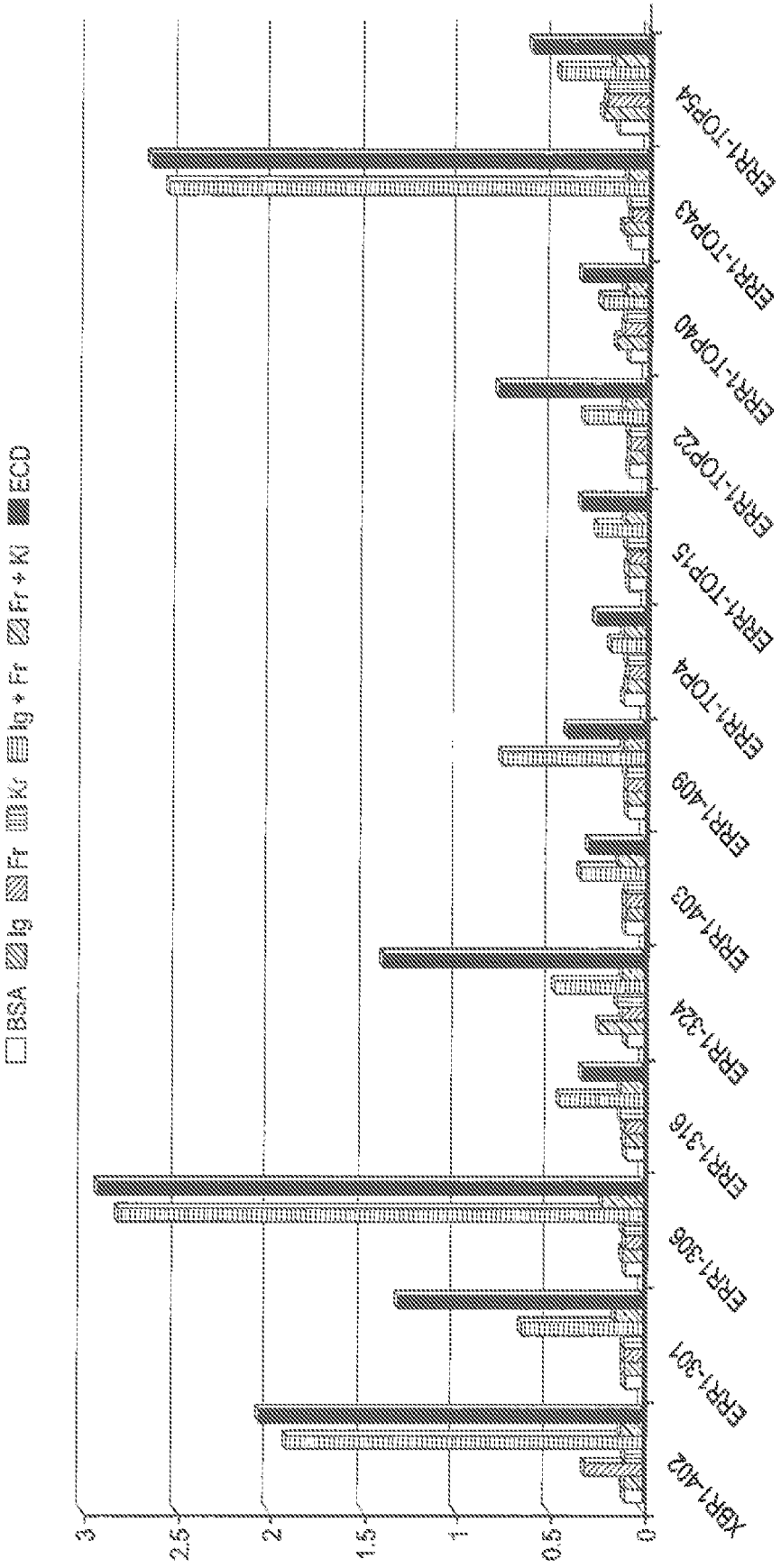

BSA
Ig
Fr
Kr
Ig + Fr
Fr + Kr
ECD
3
2.5
2
1.5
1
0.5
0
XBR1-402
ERR1-301
ERR1-306
ERR1-316
ERR1-324
ERR1-403
ERR1-409
ERR1-TOP4
ERR1-TOP15
ERR1-TOP22
ERR1-TOP40
ERR1-TOP43
ERR1-TOP54

FIG. 4

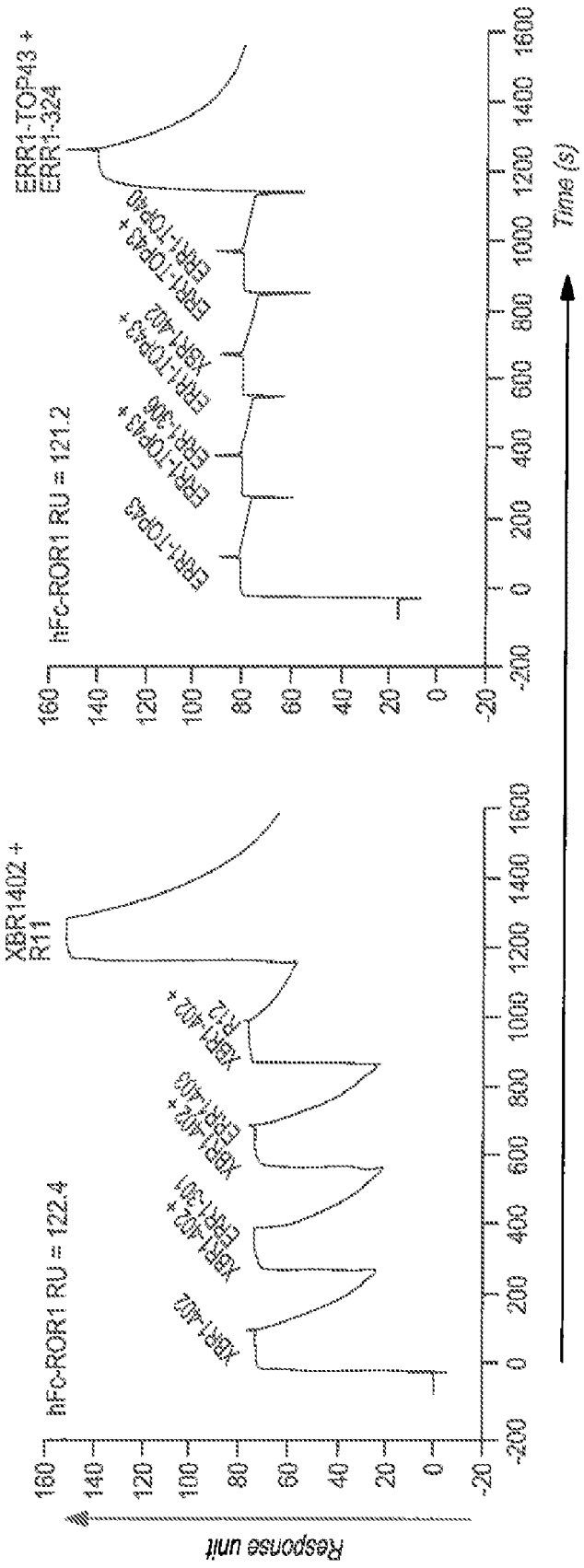
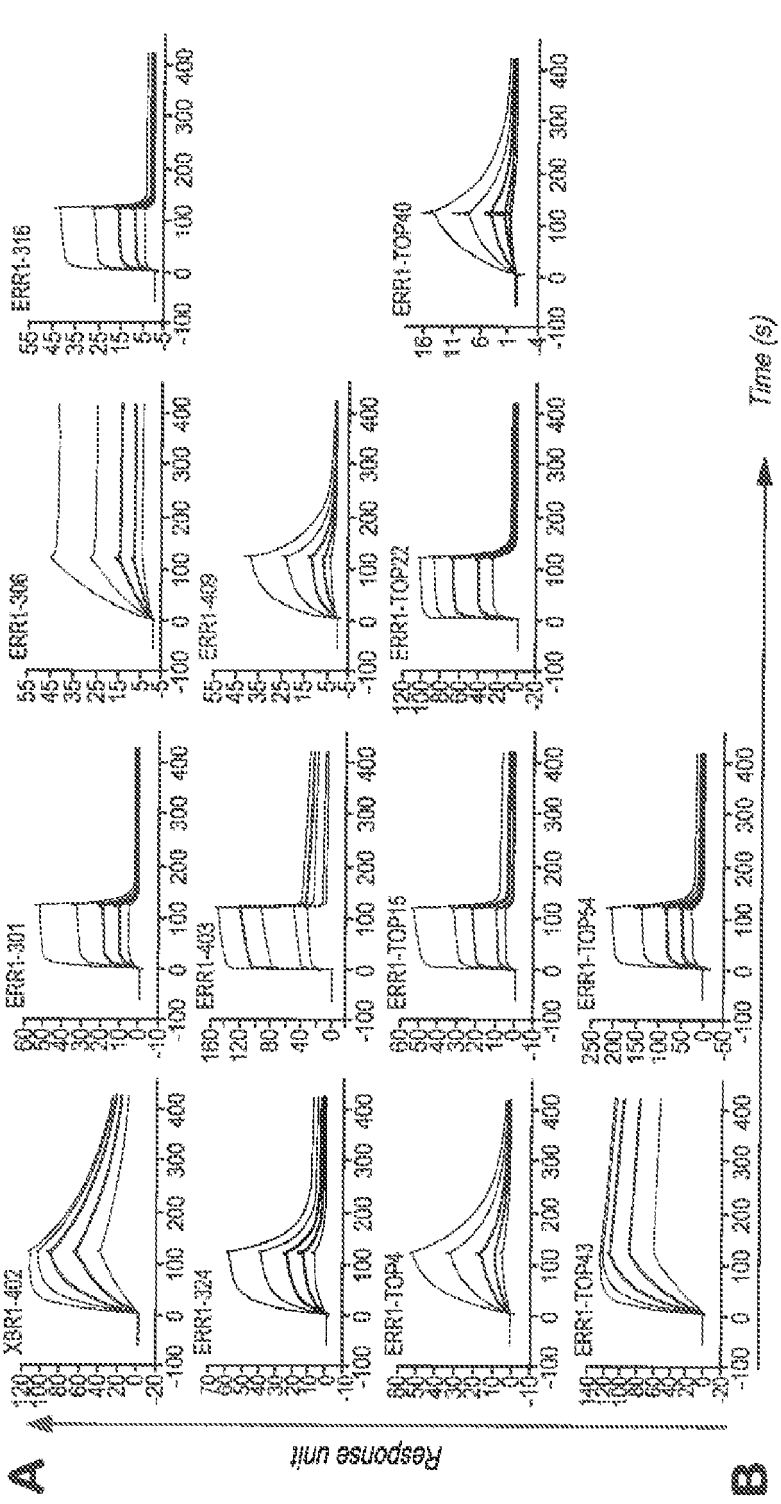

hFc-ROR1 RU = 122.4
XBR1402 + R11
XBR1-402
XBR1-402 + ERR1-324
XBR1-402 + ERR1-LO6
XBR1-402 + ERR1-3qh
XBR1-402 + R12
hFc-ROR1 RU = 121.2
ERR1-TOP43 + ERR1-324
ERR1-TOP43
ERR1-TOP43 + ERR1-3qh
ERR1-TOP43 + XBR1-402
ERR1-TOP43 + ERR1-LO6
ERR1-TOP43 + ERR1-TOP40
Response unit
Time (s)

FIG. 5



clone	XBR1-402	ERR1-301	ERR1-306	ERR1-316	ERR1-324	ERR1-403	ERR1-409	ERR1-TOP4	ERR1-TOP15	ERR1-TOP22	ERR1-TOP40	ERR1-TOP43	ERR1-TOP54
immobilization RU	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000
hFc-R0R1 RU	203.8	203	169.4	198.7	179.3	326.4	186.8	172.2	167.9	209.2	184.9	290.3	77.8
Fab. Con.[b] (nM)	100	100	500	100	200	1000	200	200	200	500	500	200	200
k_{on} ($10^4 M^{-1}s^{-1}$)	111	84.9	1.51	110	11.5	3.08	5.75	24.7	46.9	73.7	3.18	45.1	47.5
K_{off} (10^{-3} s^{-1})	6.43	100	0.146	132.6	12.51	1.28	16.16	59.24	65.36	81.26	26.19	0.501	68.85
K_d (nM)	5.81	118	9.66	121	106	41.6	281	240	139	110	636	1.11	145
Chi^2	0.616	0.922	0.137	1.85	4.64	16.7	0.302	0.403	9	14.9	0.14	1.17	54.7

FIG. 6

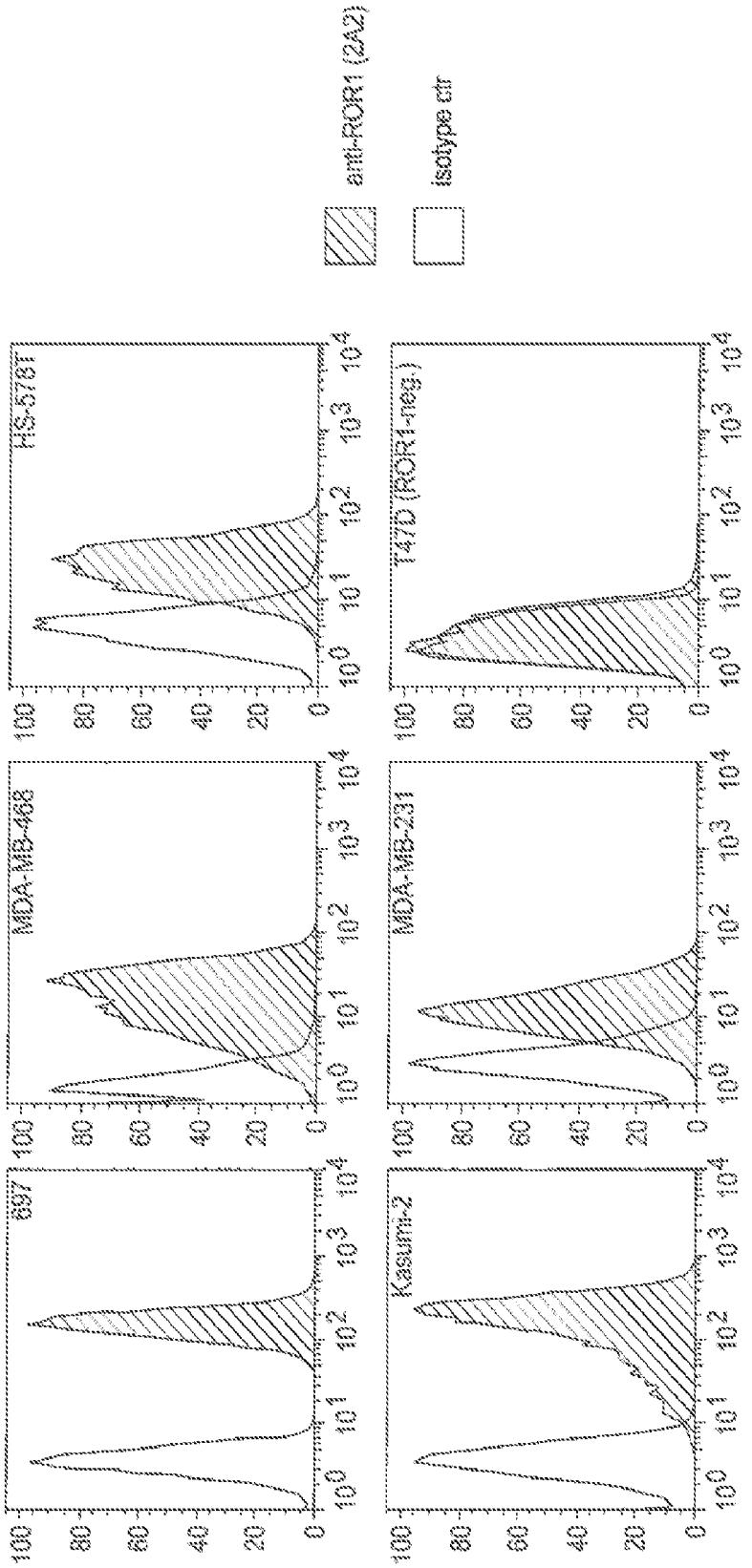

anti-ROR1 (2A2)
isotype ctr
HS-578T
MDA-MB-468
697
T47D (ROR1-neg.)
MDA-MB-231
Kasumi-2

FIG. 7

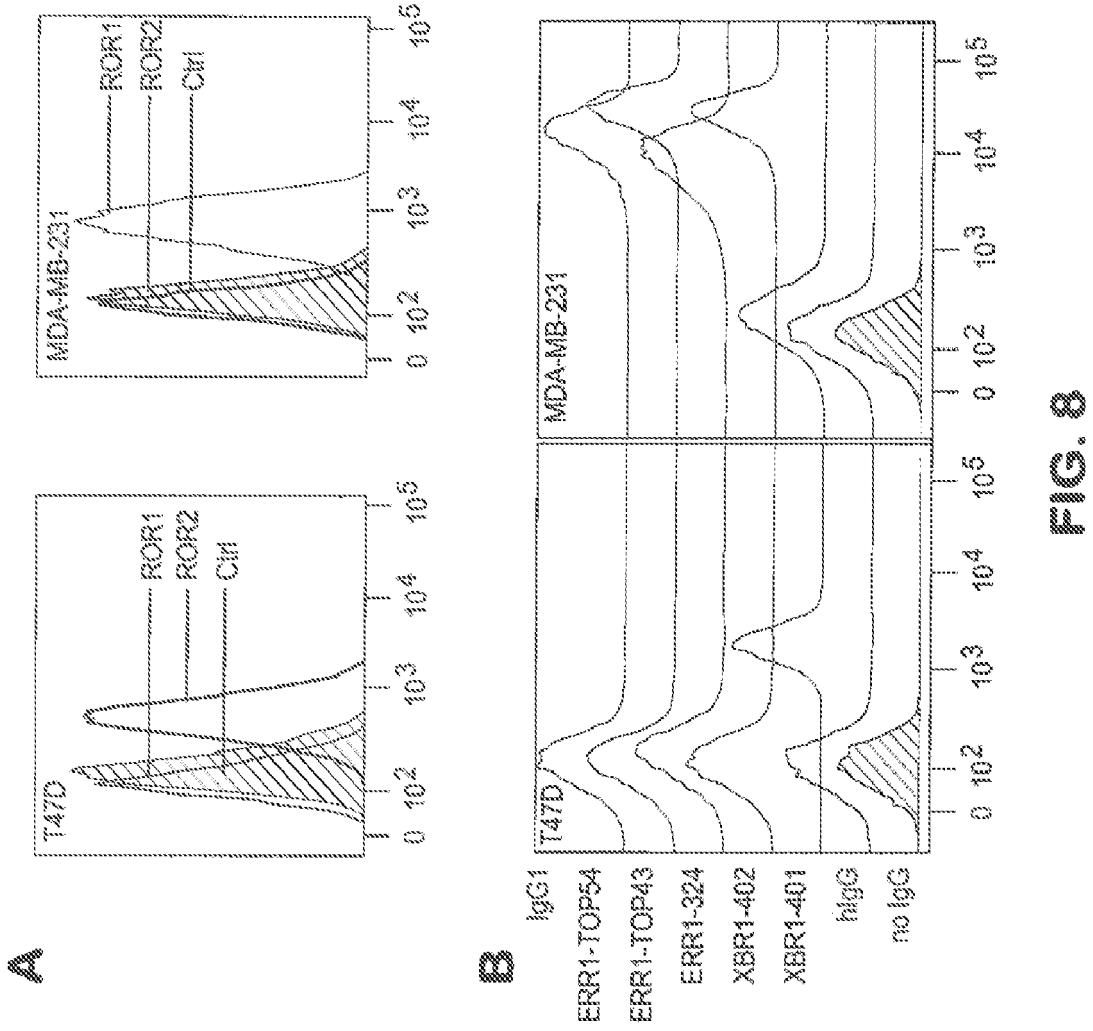

A
T47D
MDA-MB-231
ROR1
ROR2
Ctrl
0
10^2
10^3
10^4
10^5
B
IgG1
ERR1-TOP54
ERR1-TOP43
ERR1-324
XBR1-402
XBR1-401
hIgG
no IgG

FIG. 8

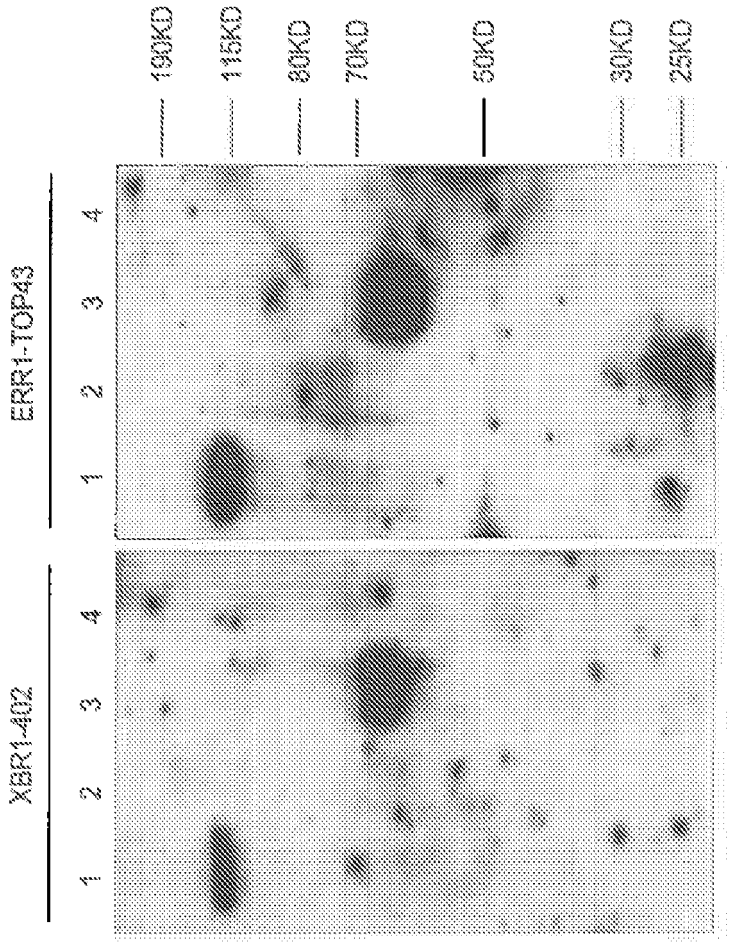

190KD
115KD
80KD
70KD
50KD
30KD
25KD
ERR1-TDP43
XBR1-402
1
2
3
4
Lane 1: K562-hROR1
Lane 2: K562
Lane 3: hROR1-ECD-HIS
Lane 4: hROR2-ECD-HIS

FIG. 9

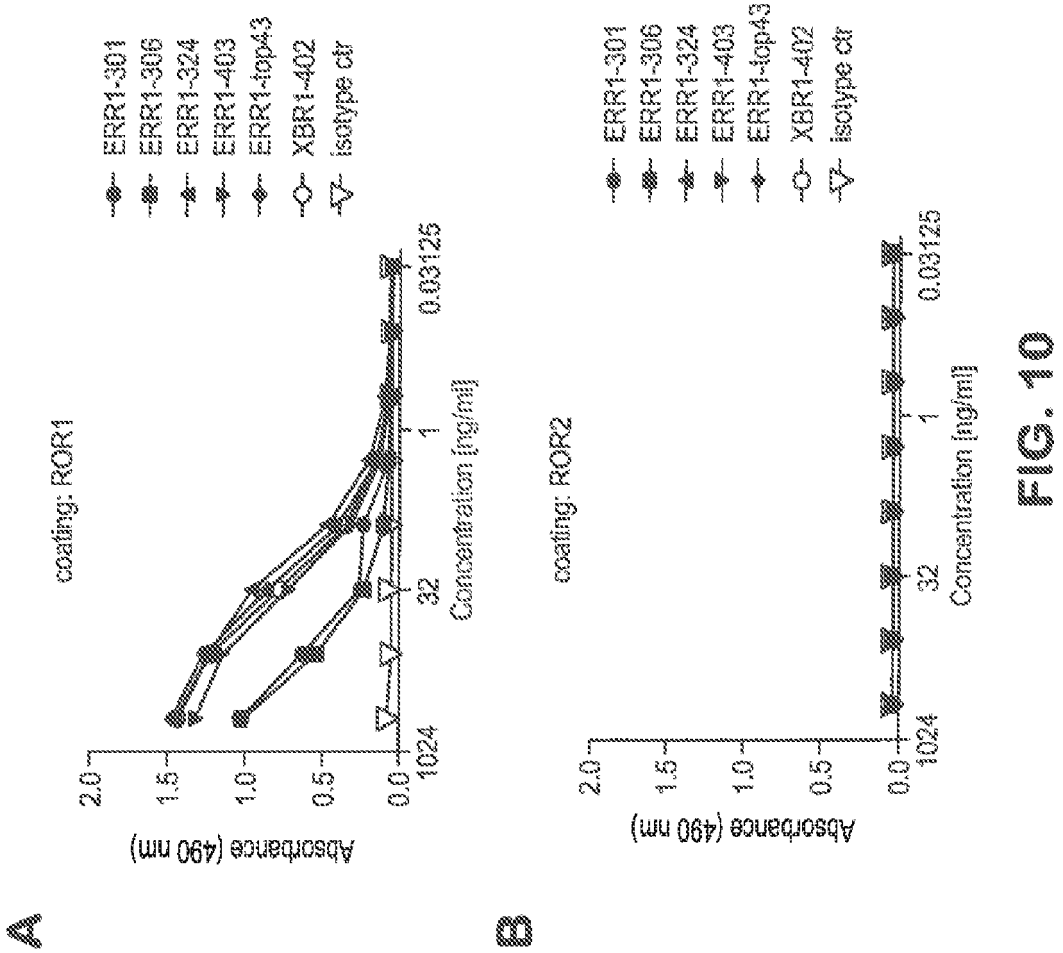

A
coating: ROR1
ERR1-301
ERR1-306
ERR1-324
ERR1-403
ERR1-top43
XBR1-402
isotype ctr
Absorbance (490 nm)
2.0
1.5
1.0
0.5
0.0
1024
32
1
0.03125
Concentration [ng/ml]
B
coating: ROR2
ERR1-301
ERR1-306
ERR1-324
ERR1-403
ERR1-top43
XBR1-402
isotype ctr
Absorbance (490 nm)
2.0
1.5
1.0
0.5
0.0
1024
32
1
0.03125
Concentration [ng/ml]

FIG. 10

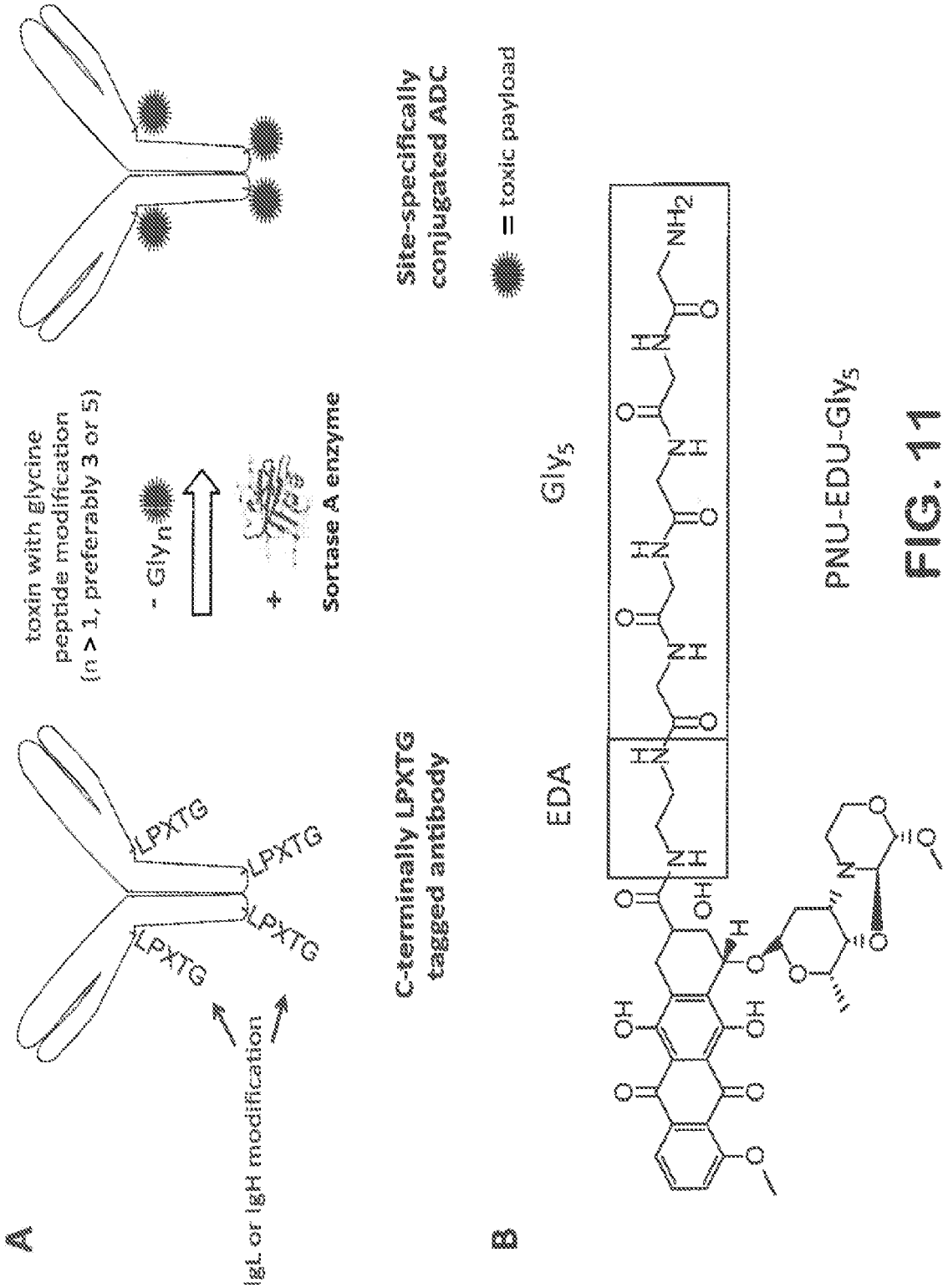
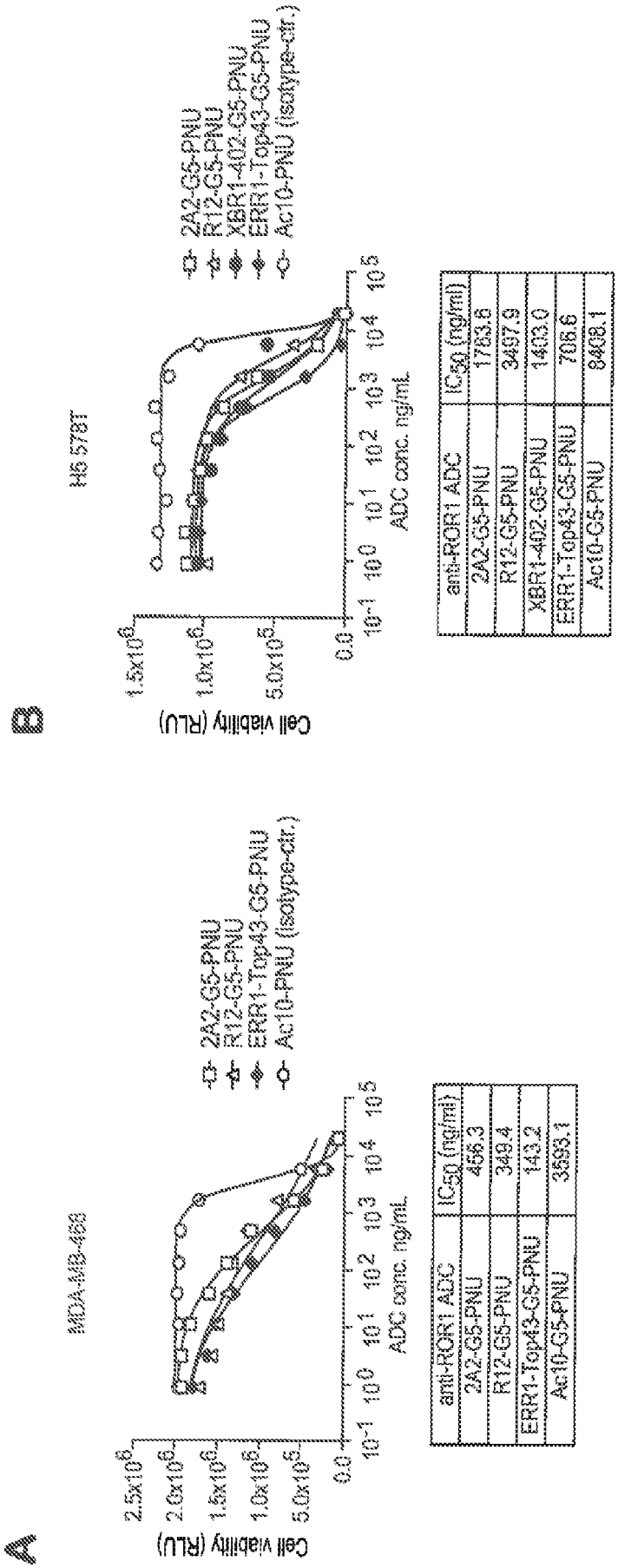

A
toxin with glycine peptide modification (n > 1, preferably 3 or 5)
- Gly_n
+
Sortase A enzyme
Site-specifically conjugated ADC
LPXTG
LPXTG
LPXTG
LPXTG
IgL or IgH modification
C-terminally LPXTG tagged antibody
= toxic payload
B
EDA
Gly_5
PNU-EDU-Gly_5

FIG. 11

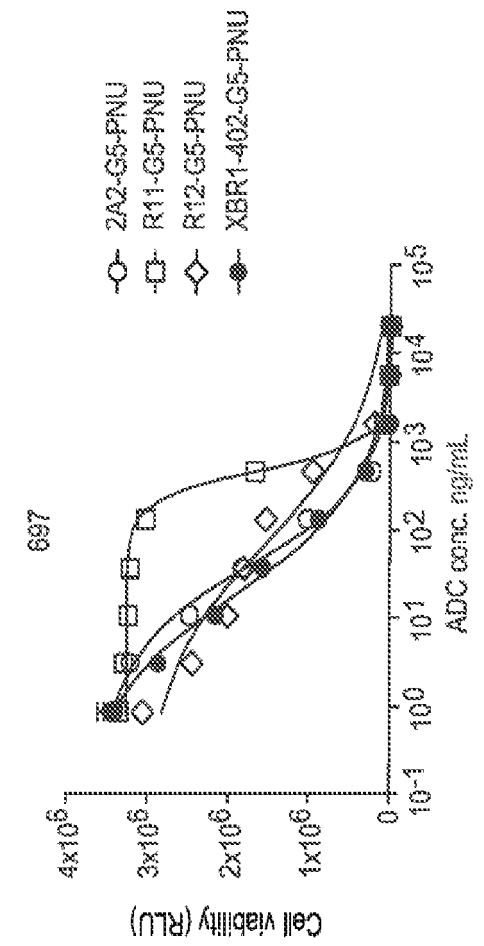


anti-ROR1 ADC	IC50 (ng/ml)
2A2-G5-PNU	456.3
R12-G5-PNU	349.4
ERR1-Top43-G5-PNU	143.2
Ac10-G5-PNU	3593.1

anti-ROR1 ADC	IC50 (ng/ml)
2A2-G5-PNU	1763.6
R12-G5-PNU	3497.9
XBR1-402-G5-PNU	1403.0
ERR1-Top43-G5-PNU	706.6
Ac10-G5-PNU	8408.1

FIG. 12

A



B

anti-ROR1 ADC	IC_{50} (ng/ml)
2A2	38.0
R11	469.4
R12	72.1
XBR1-402	17.6

FIG. 13

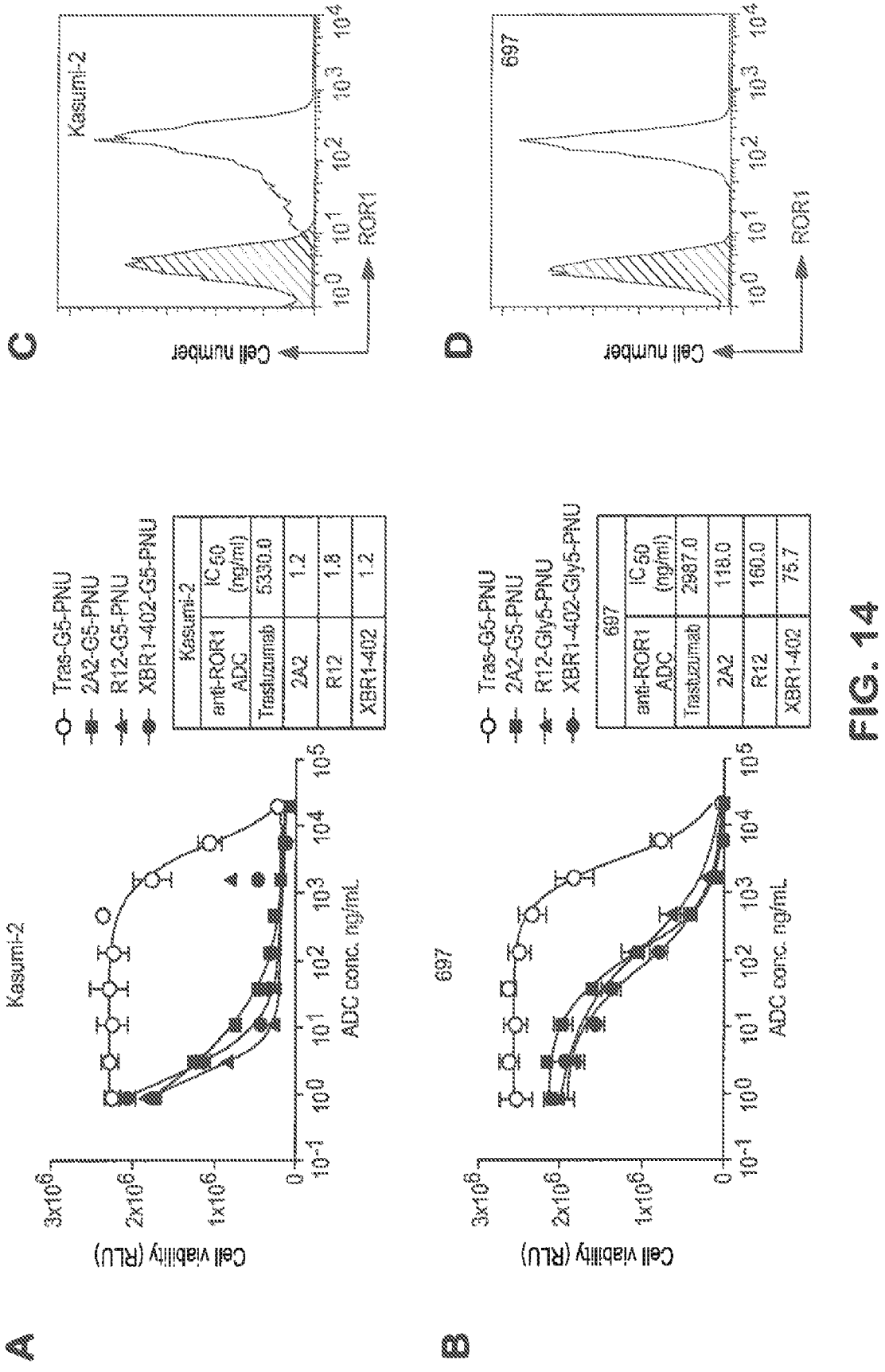

A
Kasumi-2
Tras-G5-PNU
2A2-G5-PNU
R12-G5-PNU
XBR1-402-G5-PNU
Cell viability (RLU)
ADC conc. ng/mL
Kasumi-2
anti-ROR1 ADC
IC 50 (ng/ml)
Trastuzumab 5330.0
2A2 1.2
R12 1.8
XBR1-402 1.2
B
697
Tras-G5-PNU
2A2-G5-PNU
R12-Gly5-PNU
XBR1-402-Gly5-PNU
Cell viability (RLU)
ADC conc. ng/mL
697
anti-ROR1 ADC
IC 50 (ng/ml)
Trastuzumab 2987.0
2A2 118.0
R12 160.0
XBR1-402 75.7
C
Kasumi-2
Cell number
ROR1
D
697
Cell number
ROR1

FIG. 14

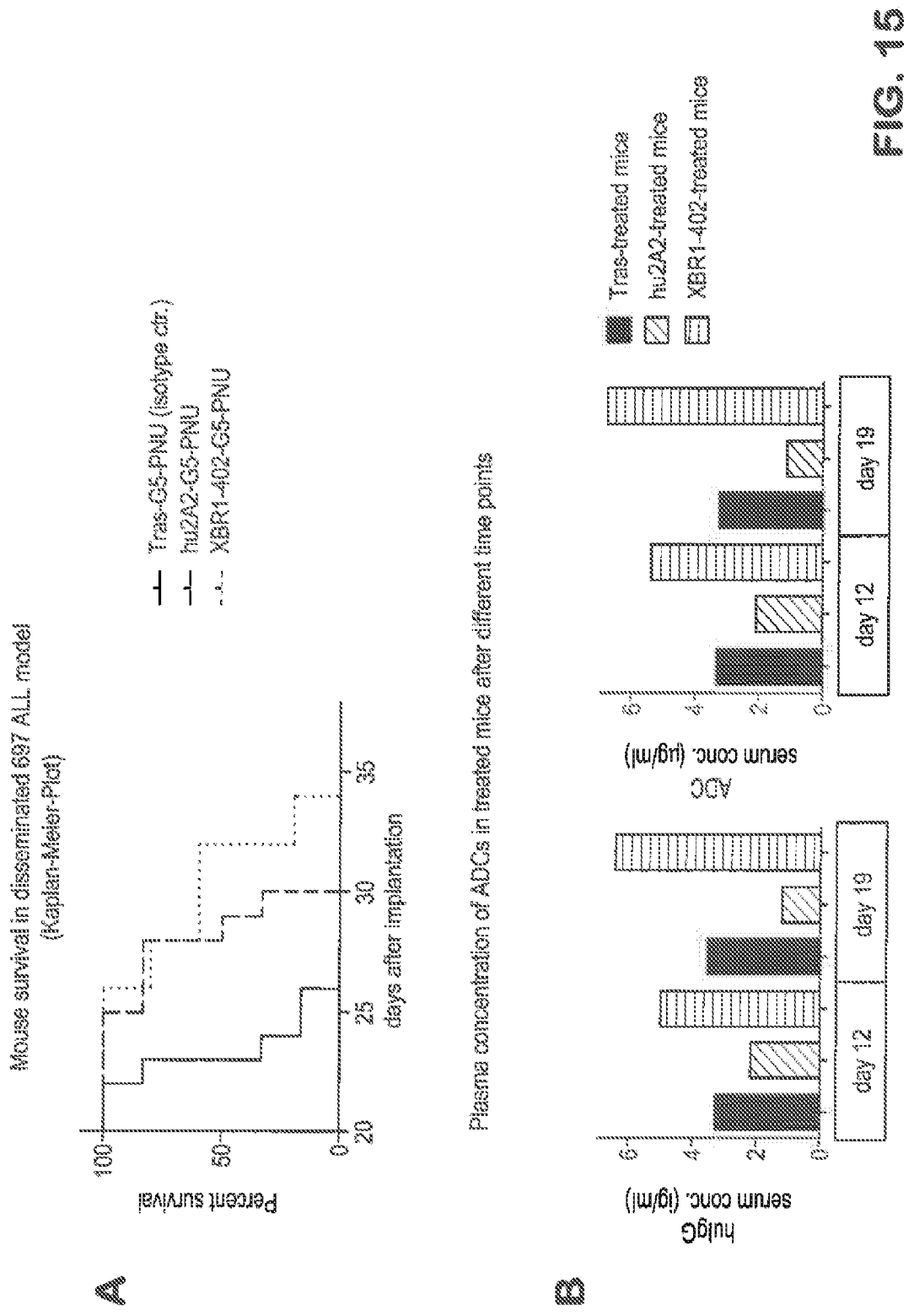

A
Mouse survival in disseminated 697 ALL model
(Kaplan-Meier-Plot)
Tras-G5-PNU (isotype ctr.)
hu2A2-G5-PNU
XBR1-402-G5-PNU
Percent survival
100
50
0
20
25
30
35
days after implantation
B
Plasma concentration of ADCs in treated mice after different time points
Tras-treated mice
hu2A2-treated mice
XBR1-402-treated mice
huIgG
serum conc. (µg/ml)
6
4
2
0
day 12
day 19
ADC
serum conc. (µg/ml)
6
4
2
0
day 12
day 19

FIG. 15

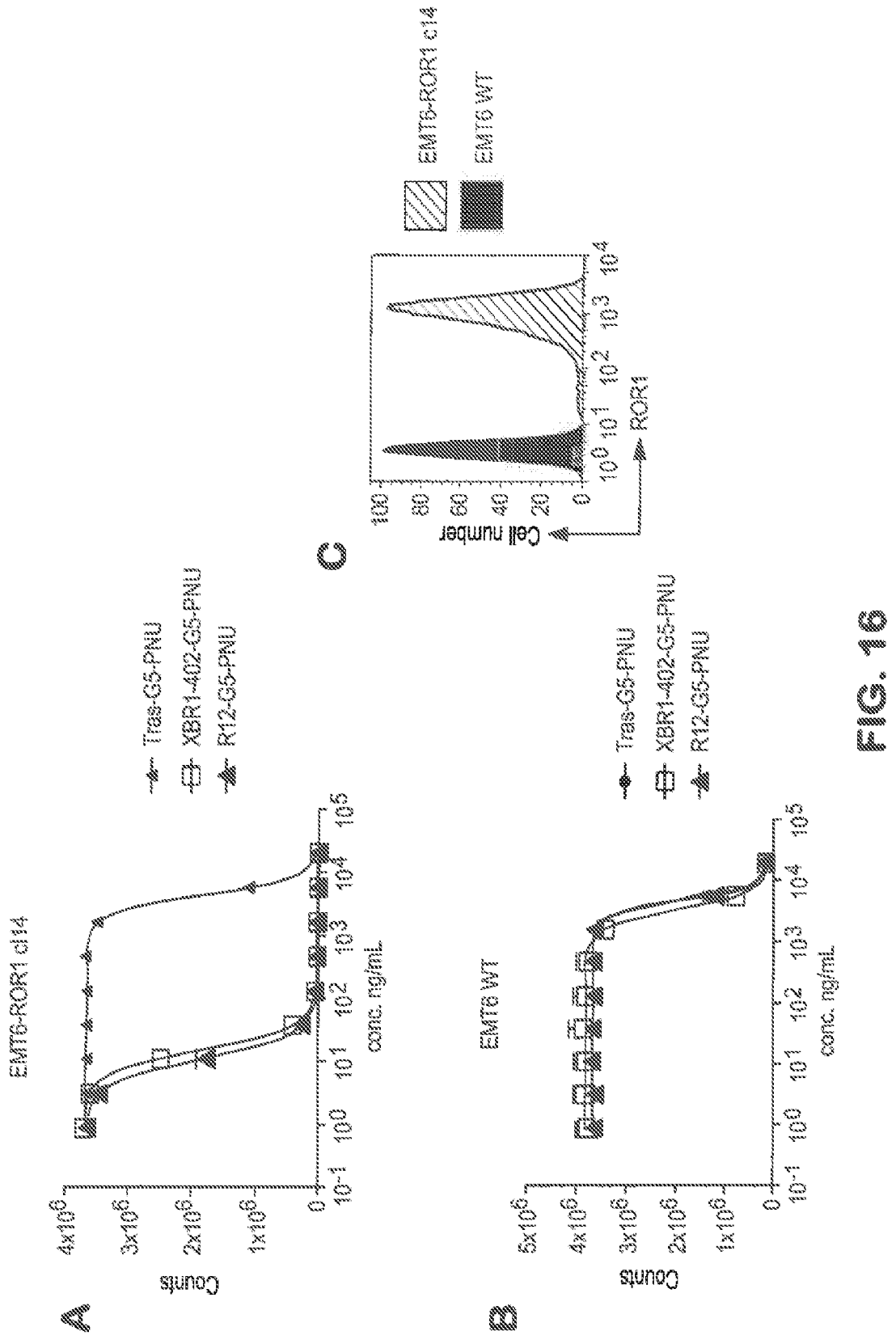

A
EMT6-ROR1 cl14
Counts
conc. ng/mL
Tras-G5-PNU
XBR1-402-G5-PNU
R12-G5-PNU
B
EMT6 WT
Counts
conc. ng/mL
Tras-G5-PNU
XBR1-402-G5-PNU
R12-G5-PNU
C
EMT6-ROR1 c14
EMT6 WT
Cell number
ROR1

FIG. 16

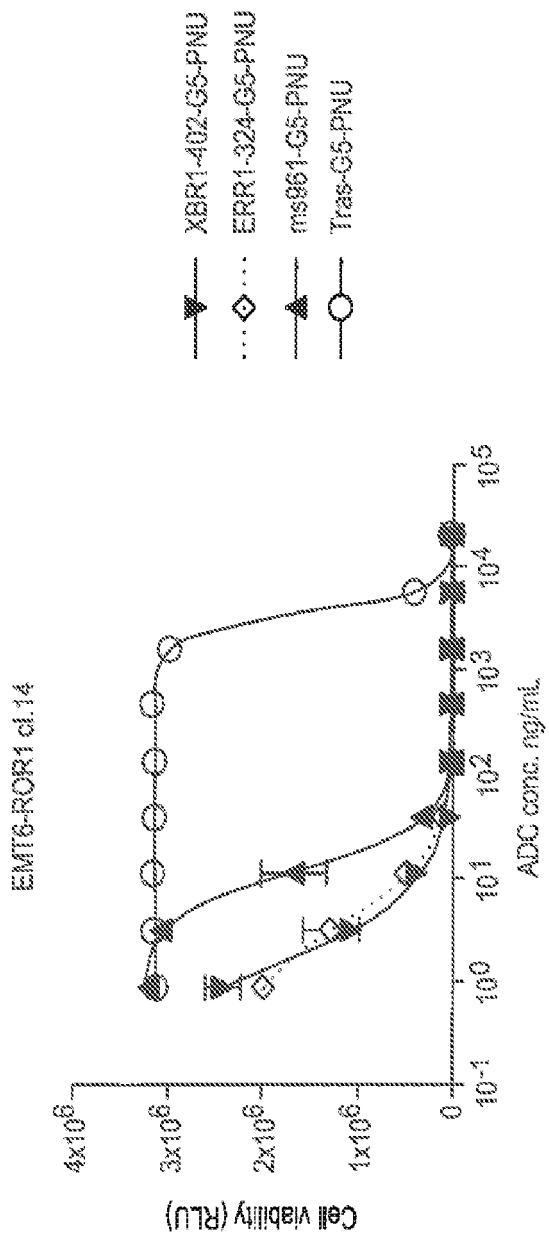

XBR1-402-G5-PNU
ERR1-324-G5-PNU
ms861-G5-PNU
Tras-G5-PNU
EMT6-ROR1 cl.14
Cell viability (RLU)
4x10^6
3x10^6
2x10^6
1x10^6
0
10^-1
10^0
10^1
10^2
10^3
10^4
10^5
ADC conc. ng/mL

FIG. 17

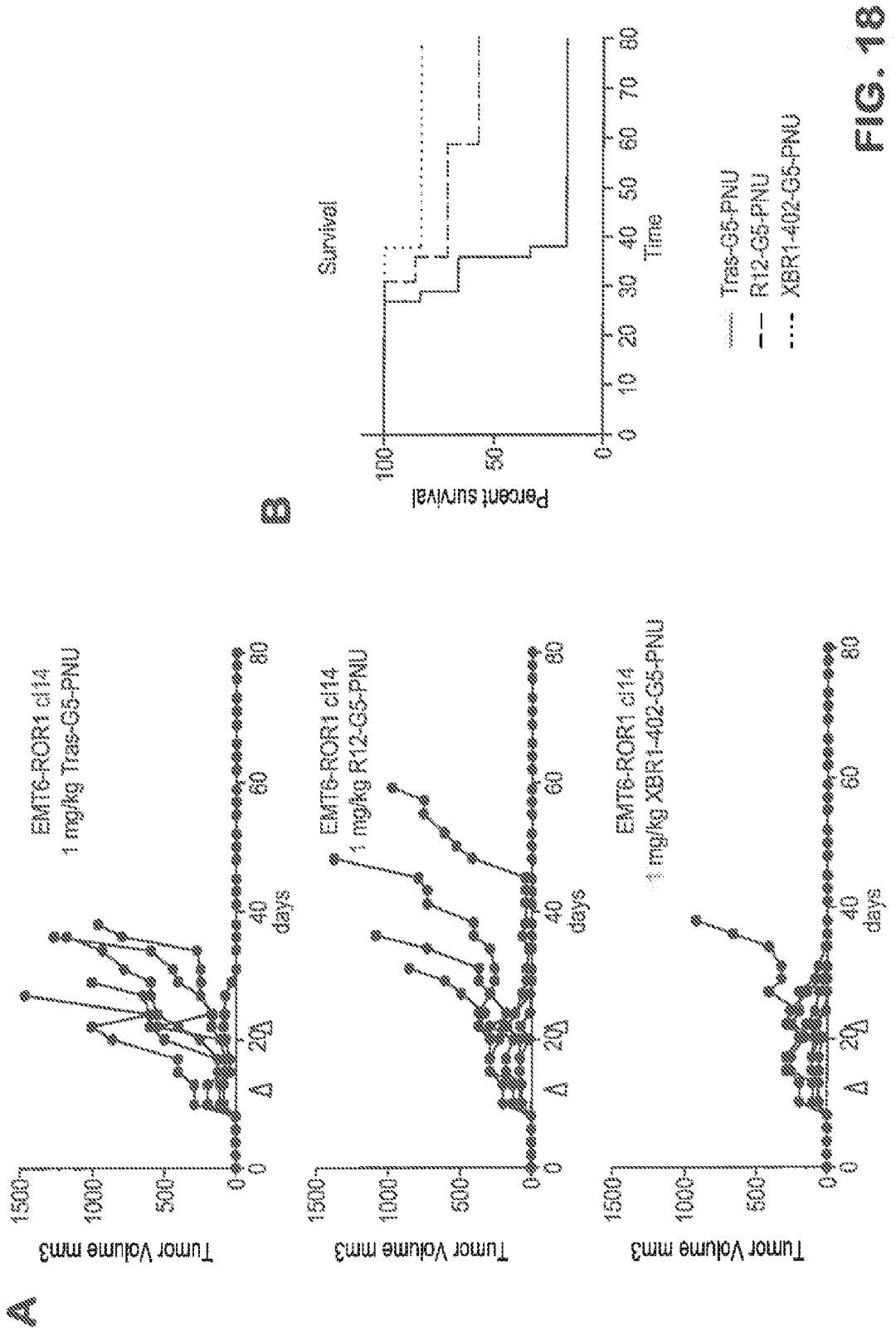

A
EMT6-ROR1 cl14
1 mg/kg Tras-G5-PNU
EMT6-ROR1 cl14
1 mg/kg R12-G5-PNU
EMT6-ROR1 cl14
1 mg/kg XBR1-402-G5-PNU
Tumor Volume mm3
1500
1000
500
0
0
20
40
60
days
B
Survival
Percent survival
100
50
0
0
10
20
30
40
50
60
70
80
Time
Tras-G5-PNU
R12-G5-PNU
XBR1-402-G5-PNU

FIG. 18

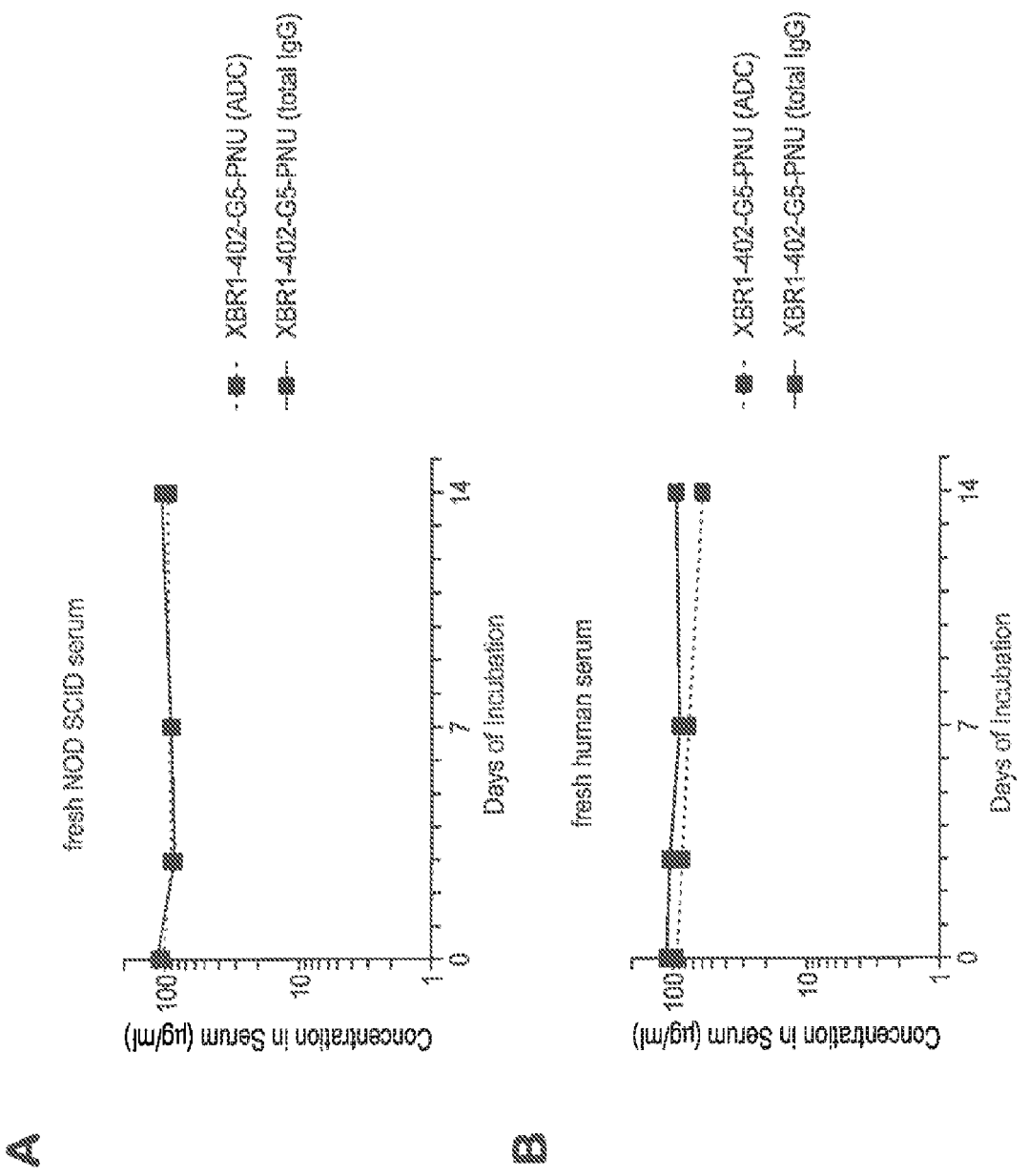

A
fresh NOD SCID serum
Concentration in Serum (µg/ml)
100
10
1
0
7
14
Days of incubation
XBR1-402-G5-PNU (ADC)
XBR1-402-G5-PNU (total IgG)
B
fresh human serum
Concentration in Serum (µg/ml)
100
10
1
0
7
14
Days of incubation
XBR1-402-G5-PNU (ADC)
XBR1-402-G5-PNU (total IgG)

FIG. 19

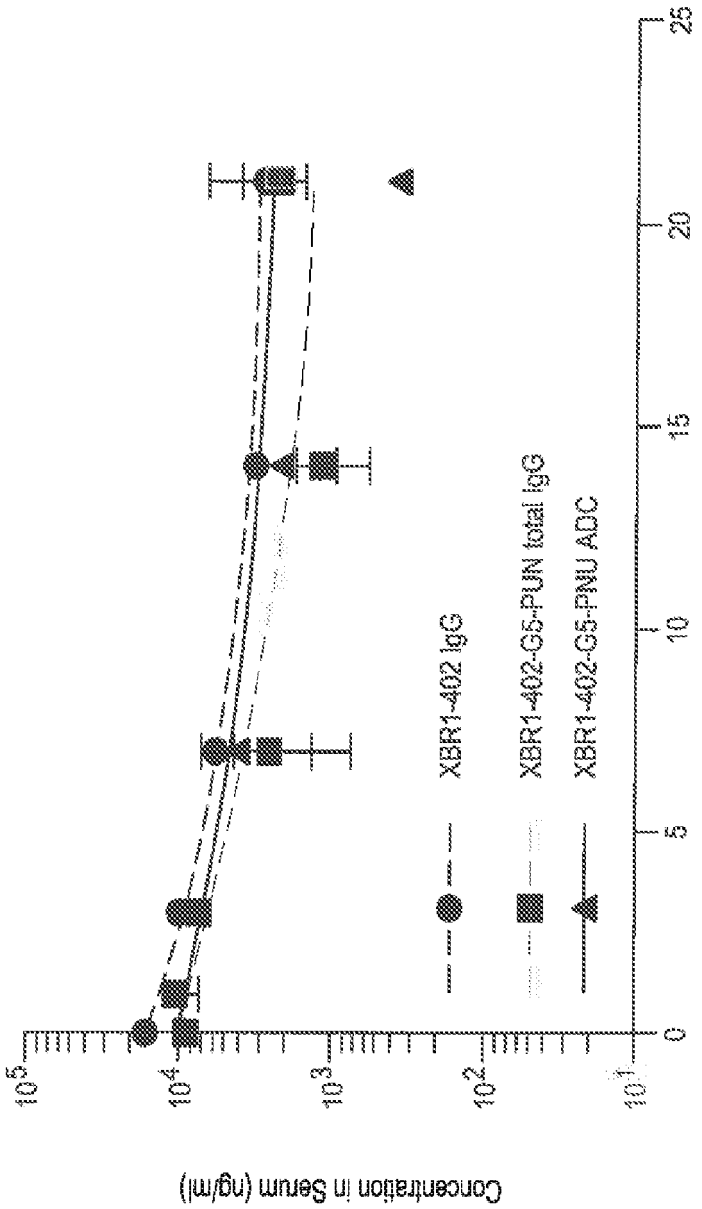

Concentration in Serum (ng/ml)
10^5
10^4
10^3
10^2
10^1
0
5
10
15
20
25
Days
XBR1-402 IgG
XBR1-402-G5-PUN total IgG
XBR1-402-G5-PNU ADC

FIG. 20

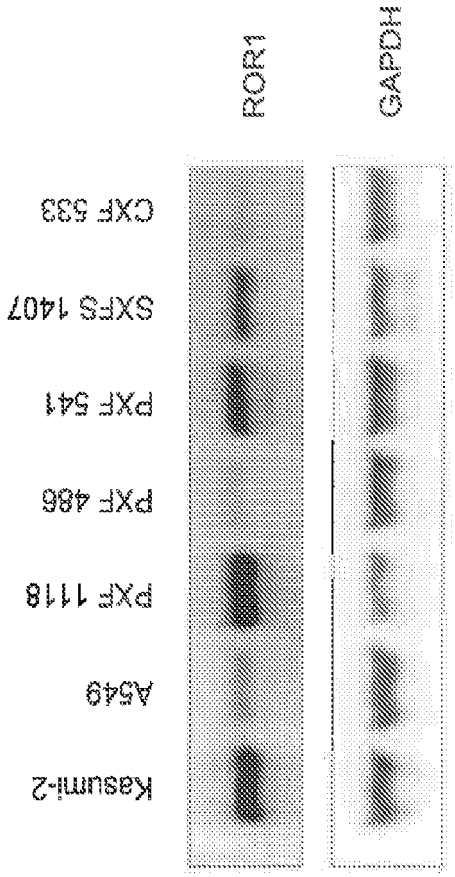

ROR1
GAPDH
CXF 533
SXFS 1407
PXF 541
PXF 486
PXF 1118
A549
Kasumi-2

FIG. 21

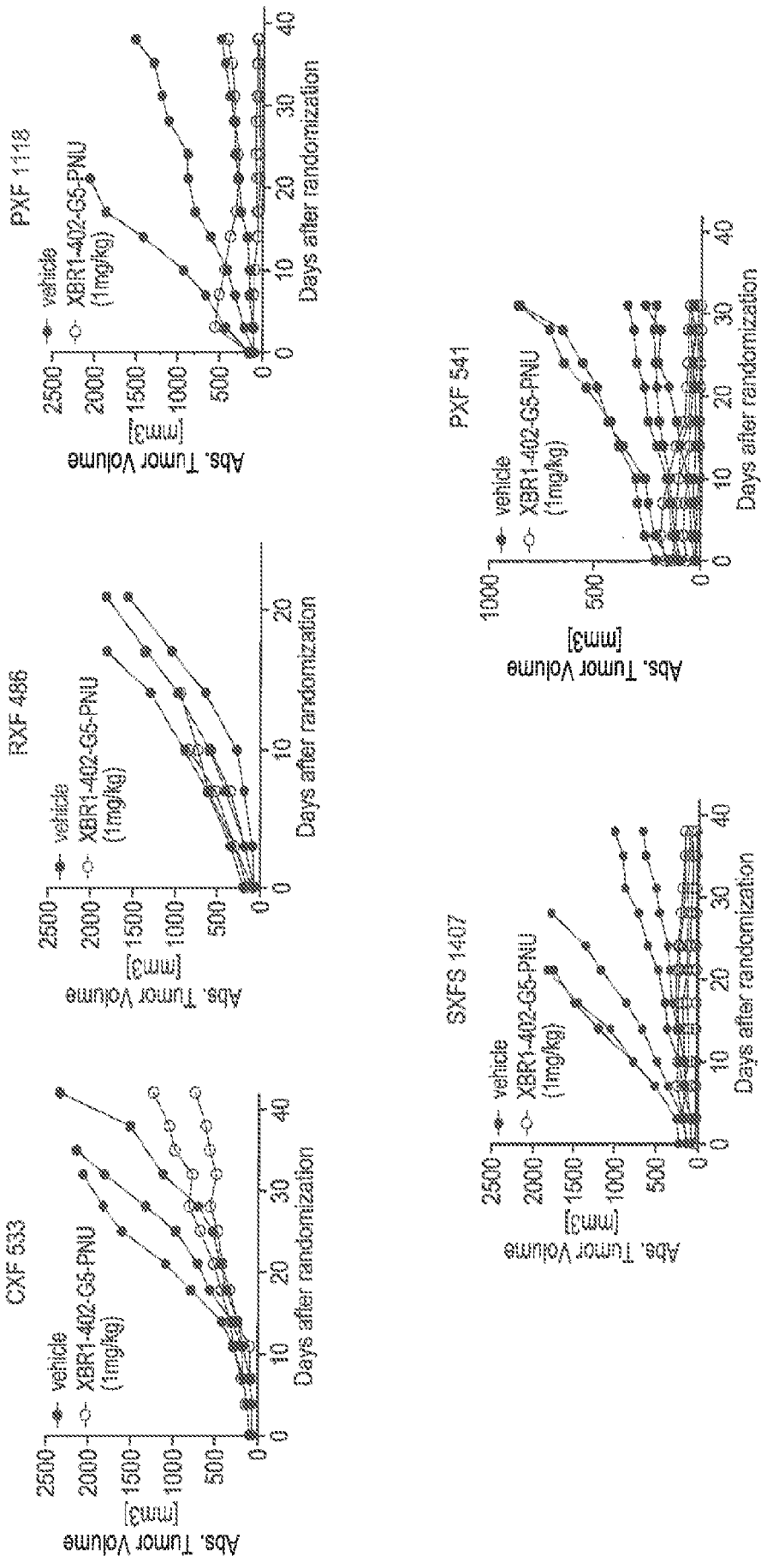

CXF 533
RXF 486
PXF 1118
SXFS 1407
PXF 541
vehicle
XBR1-402-G5-PNU (1mg/kg)
Abs. Tumor Volume [mm3]
Days after randomization

FIG. 22

ROR1 mAbs

V_λ

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
	1 22	24 34	36 46	50 56	66 87	97	107
XBR1-402	SYELTQPPSVSVAPGQTARITC	GGNNIGSKAVH	WYQQKPGQAPVLVIY	DDSDRPS	GIPERFSGSNSGNTATLTISRVEAGDEADYYC	QVWDSSAIV	FGGGTKLTVLG
huXBR1-402-3	SYELTQPSVSVAPGQTARITC	GGNNIGSKAVH	WYQQKPGQAPVLVIY	DDSDRPS	GIPERFSGSNSGNTATLTISRVEAGDEADYYC	QVWDSSAIV	FGGGTKLTVLG
huXBR1-402-8	SYELTQPPSVSVAPGKTARITC	GGNNIGSKAVH	WYQQKPGQAPVLVIY	DDSDRPS	GIPERFSGSNSGNTATLTISRVEAGDEADYYC	QVWDSSAIV	FGGGTKLTVLG
huXBR1-402-15	SYELTQPASVSVAPGQTARITC	GGNNIGSKAVH	WYQQKPGQAPVLVIY	DDSDRPS	GIPERFSGSNSGNTATLTISRVEAGDEADYYC	QVWDSSAIV	FGGGTKLTVLG
huXBR1-402-17	SYELTQPPSVSVAPGKTARITC	GGNNIGSKAVH	WYQQKPGQAPVLVIY	DDSDRPS	GIPERFSGSNSGNTATLTISRVEAGDEADYYC	QVWDSSAIV	FGGGTKLTVLG
huXBR1-402-19	SYELTQPASVSVAPGKTARITC	GGNNIGSKAVH	WYQQKPGQAPVLVIY	DDSDRPS	GIPERFSGSNSGNTATLTISRVEAGDEADYYC	QVWDSSAIV	FGGGTKLTVLG
huXBR1-402-26	SYELTQPPSVSVAPGQTARITC	GGNNIGSKAVH	WYQQKPGQAPVLVIY	DDSDRPS	GIPERFSGSNSGNTATLTISRVEAGDEADYYC	QVWDSSAIV	FGGGTKLTVLG

V_H

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
	1 30	36	49	50 66	97	107	117
XBR1-402	QVQLVESGGGLVKPGGSLRLSCAASGFDIS	SYWMS	WVRQAPGKGLEWVG	AIGISGMAYYASWAKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	DRPYGMDL	WGQGTLVTVSS
huXBR1-402-3	QVQLVESGGGLVKPGGSLRLSCAASGFDIS	SYWMS	WVRQAPGKGLEWVS	AIGISGMAYYASWAKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	DRPYGMDL	WGQGTLVTVSS
huXBR1-402-8	QVQLVESGGGLVKPGGSLRLSCAASGFDIS	SYWMS	WVRQAPGKGLEWVG	AIGISGMAYYASWAKG	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	DRPYGMDL	WGQGTLVTVSS
huXBR1-402-15	QVQLQESGPGLVKPSETLSLTCTVSGFDIS	SYWMS	WIRQPPGKGLEWIG	AIGISGMAYYASWAKS	RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR	DRPYGMDL	WGQGTLVTVSS
huXBR1-402-17	QVQLVESGGGLVKPGGSLRLSCAASGFDIS	SYWMS	WVRQAPGKGLEWVG	AIGISGMAYYASWAKS	RVTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	DRPYGMDL	WGQGTLVTVSS
huXBR1-402-19	QVQLQESGPGLVKPSETLSLTCTVSGFDIS	SYWMS	WIRQPPGKGLEWIG	AIGISGMAYYASWAKS	RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR	DRPYGMDL	WGQGTLVTVSS
huXBR1-402-26	QVQLVESGGGLVKPGGSLRLSCAASGFDIS	SYWMS	WVRQAPGKGLEWVG	AIGISGMAYYASWAKG	RVTISRDTSKNQFSLKLSSVTAADTAVYYCAR	DRPYGMDL	WGQGTLVTVSS

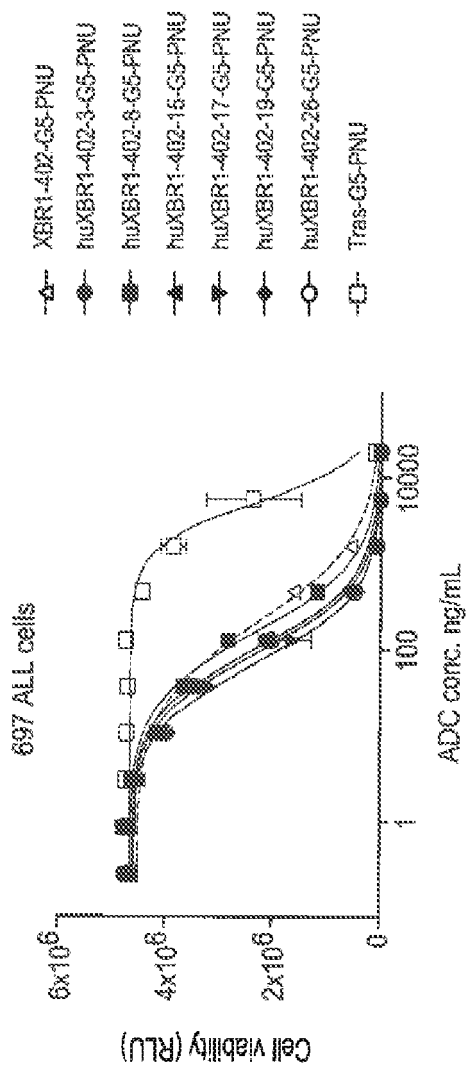
FIG. 23

ADC clone	T20 score HC	T20 score LC	K_{on} (10^{-4} M^{-1} s^{-1})	K_{off} (10^{-3} s^{-1})	K_D (nM)
XBR1-402*1	60.64	78.85	116	5.91	5.1
XBR1-402*1	60.64	78.85	110	5.56	5.1
huXBR1-402-3	80.34	88.26	119	3.75	3.2
huXBR1-402-8	81.20	82.75	129	2.76	2.1
huXBR1-402-15	81.20	91.15	111	3.94	3.5
huXBR1-402-17	80.34	91.15	96	3.22	3.4
huXBR1-402-19	80.30	91.15	111	3.32	3.0
huXBR1-402-26	80.30	86.56	105	3.28	3.1

*1: two independent measurements

FIG. 24

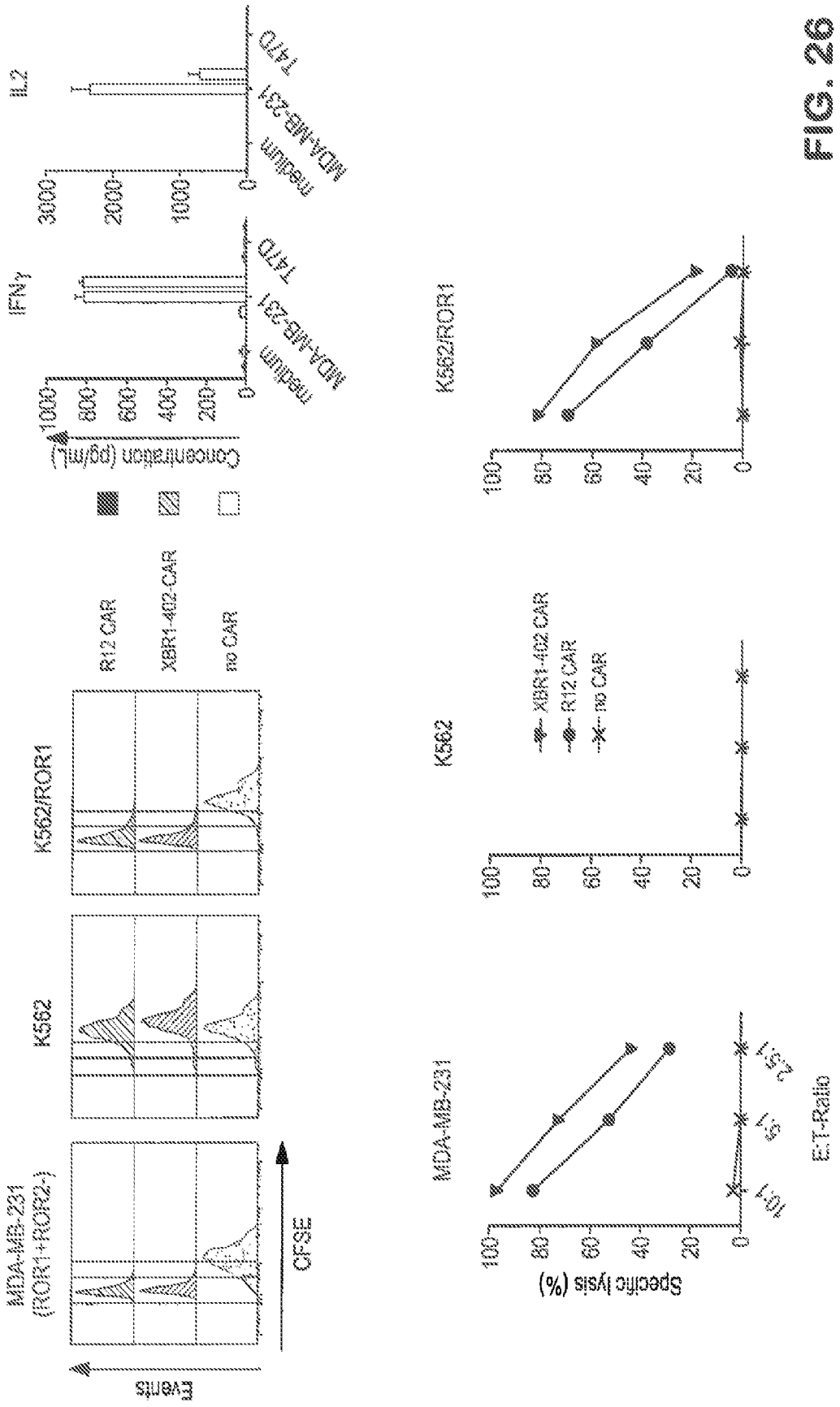
A



B

ADC clone	IC_{50}
XBR1-402-G5-PNU	211.6
huXBR1-402-3-G5-PNU	98.1
huXBR1-402-8-G5-PNU	164.2
huXBR1-402-15-G5-PNU	105.6
huXBR1-402-17-G5-PNU	70.6
huXBR1-402-19-G5-PNU	91.7
huXBR1-402-26-G5-PNU	93.9
Trastuzumab-G5-PNU	5214

FIG. 25


MDA-MB-231 (ROR1+ROR2-)
K562
K562/ROR1
R12 CAR
XBR1-402-CAR
no CAR
CFSE
Events
IFNγ
IL2
Concentration (pg/mL)
medium
MDA-MB-231
T47D
Specific lysis (%)
E:T-Ratio
FIG. 26

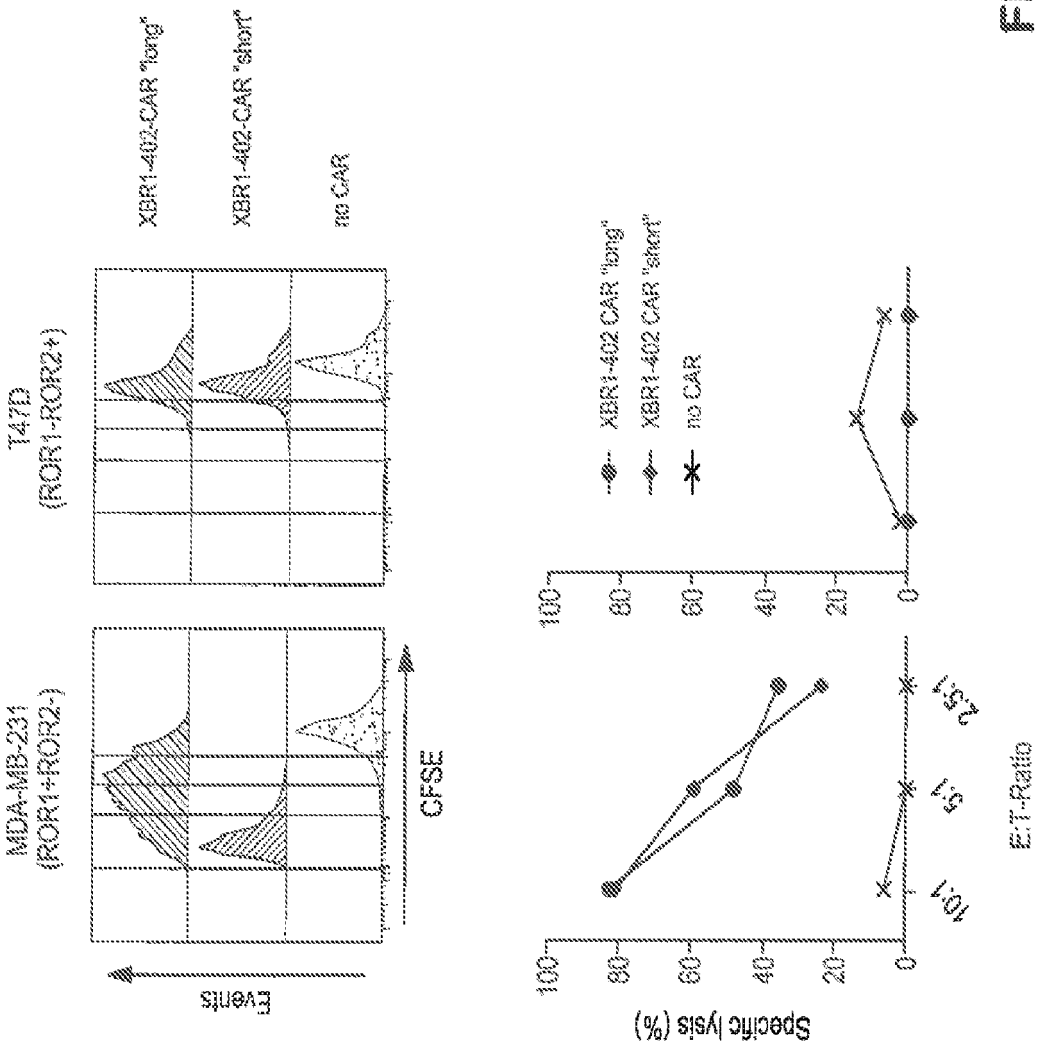

MDA-MB-231
(ROR1+ROR2-)
T47D
(ROR1+ROR2+)
XBR1-402-CAR "long"
XBR1-402-CAR "short"
no CAR
CFSE
Events
XBR1-402 CAR "long"
XBR1-402 CAR "short"
no CAR
Specific lysis (%)
E:T-Ratio
10:1
5:1
2.5:1

FIG. 27

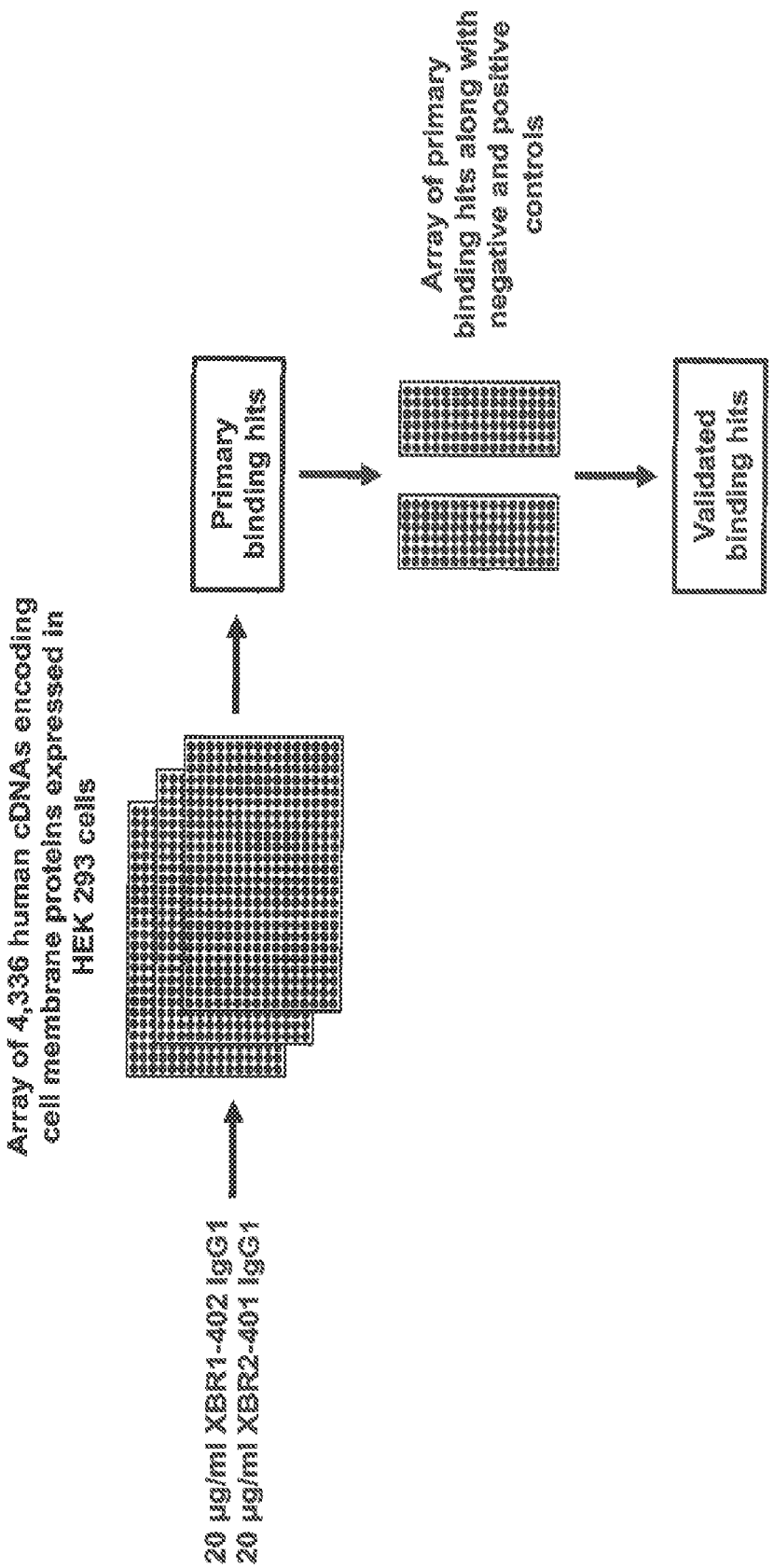

20 µg/ml XBR1-402 IgG1
20 µg/ml XBR2-401 IgG1
Array of 4,336 human cDNAs encoding cell membrane proteins expressed in HEK 293 cells
Primary binding hits
Array of primary binding hits along with negative and positive controls
Validated binding hits

FIG. 28

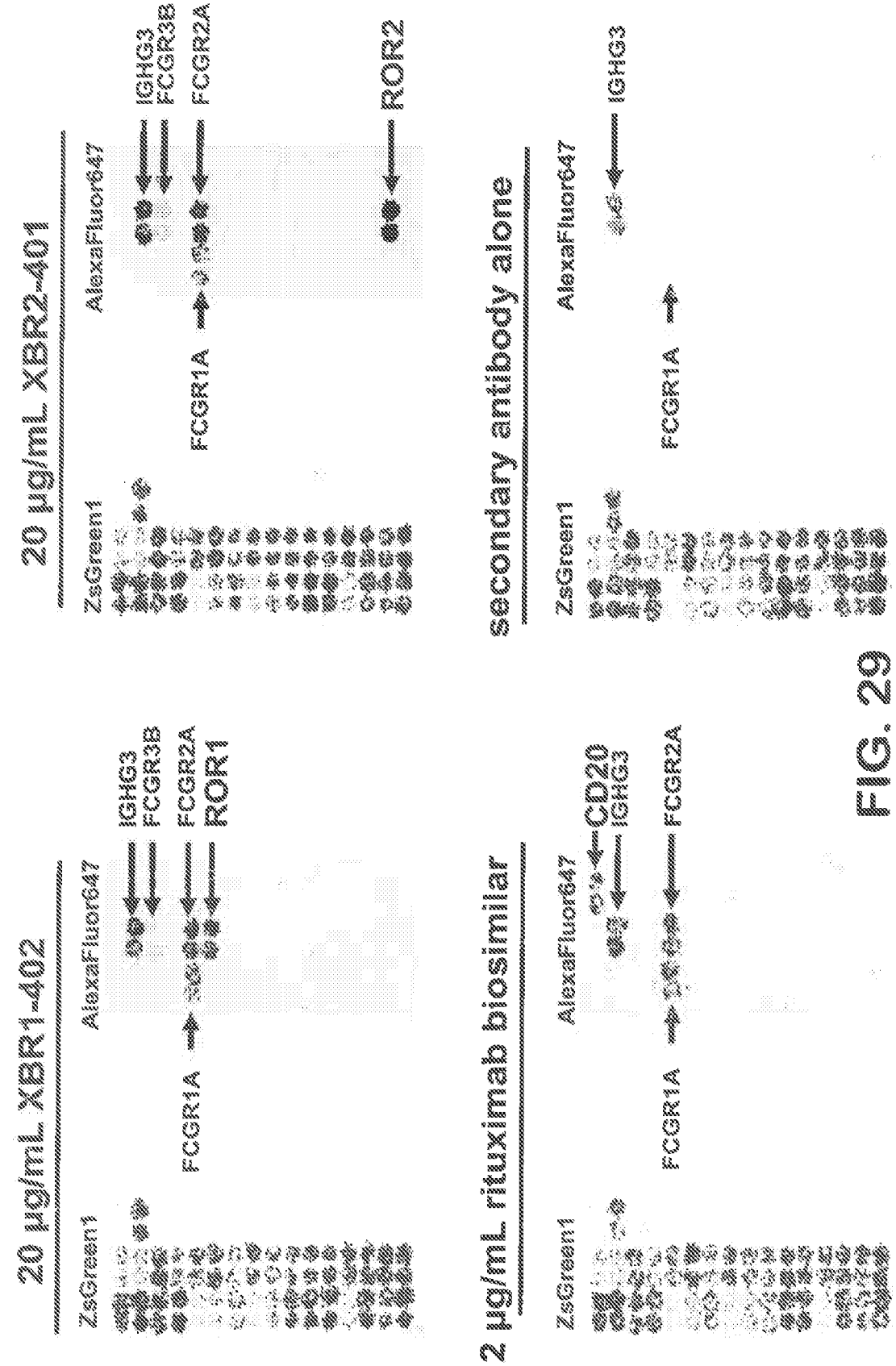

20 µg/mL XBR2-401
ZsGreen1
AlexaFluor647
IGHG3
FCGR3B
FCGR2A
ROR2
FCGR1A
20 µg/mL XBR1-402
ZsGreen1
AlexaFluor647
IGHG3
FCGR3B
FCGR2A
ROR1
FCGR1A
secondary antibody alone
ZsGreen1
AlexaFluor647
IGHG3
FCGR1A
2 µg/mL rituximab biosimilar
ZsGreen1
AlexaFluor647
CD20
IGHG3
FCGR2A
FCGR1A

FIG. 29