Human BCMA

MLQMAGQCSQ NEYFDSLLHA CIPCQLRCSS NTPPLTCQRY CNASVTNSVK
GTNAILWTCL GLSLIISLAV FVLMFLLRKI SSEPLKDEFK NTGSGLLGMA
NIDLEKSRTG DEIILPRGLE YTVEECTCED CIKSKPKVDS DHCFPLPAME
EGATILVTTK TNDYCKSLPA ALSATEIEKS ISAR

(SEQ ID NO:285)

Cynomolgus (Macaca fascicularis) BCMA

MLQMARQCSQ NEYFDSLLHD CKPCQLRCSS TPPLTCQRYC NASMTNSVKG
MNAILWTCLG LSLIISLAVF VLTFLLRKMS SEPLKDEFKN TGSGLLGMAN
IDLEKGRTGD EIVLPRGLEY TVEECTCEDC IKNKPKVDSD HCFPLPAMEE
GATILVTTKT NDYCNSLSAA LSVTEIEKSI SAR

(SEQ ID NO:286)

Fig. 1

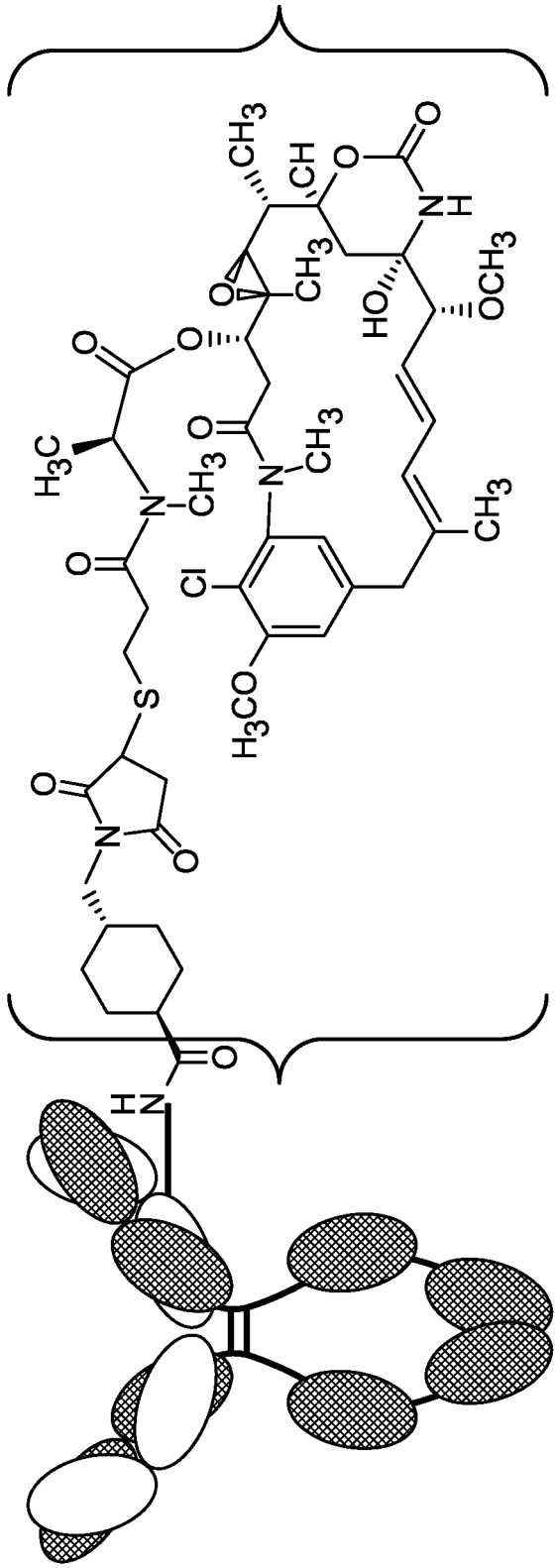


Fig. 2

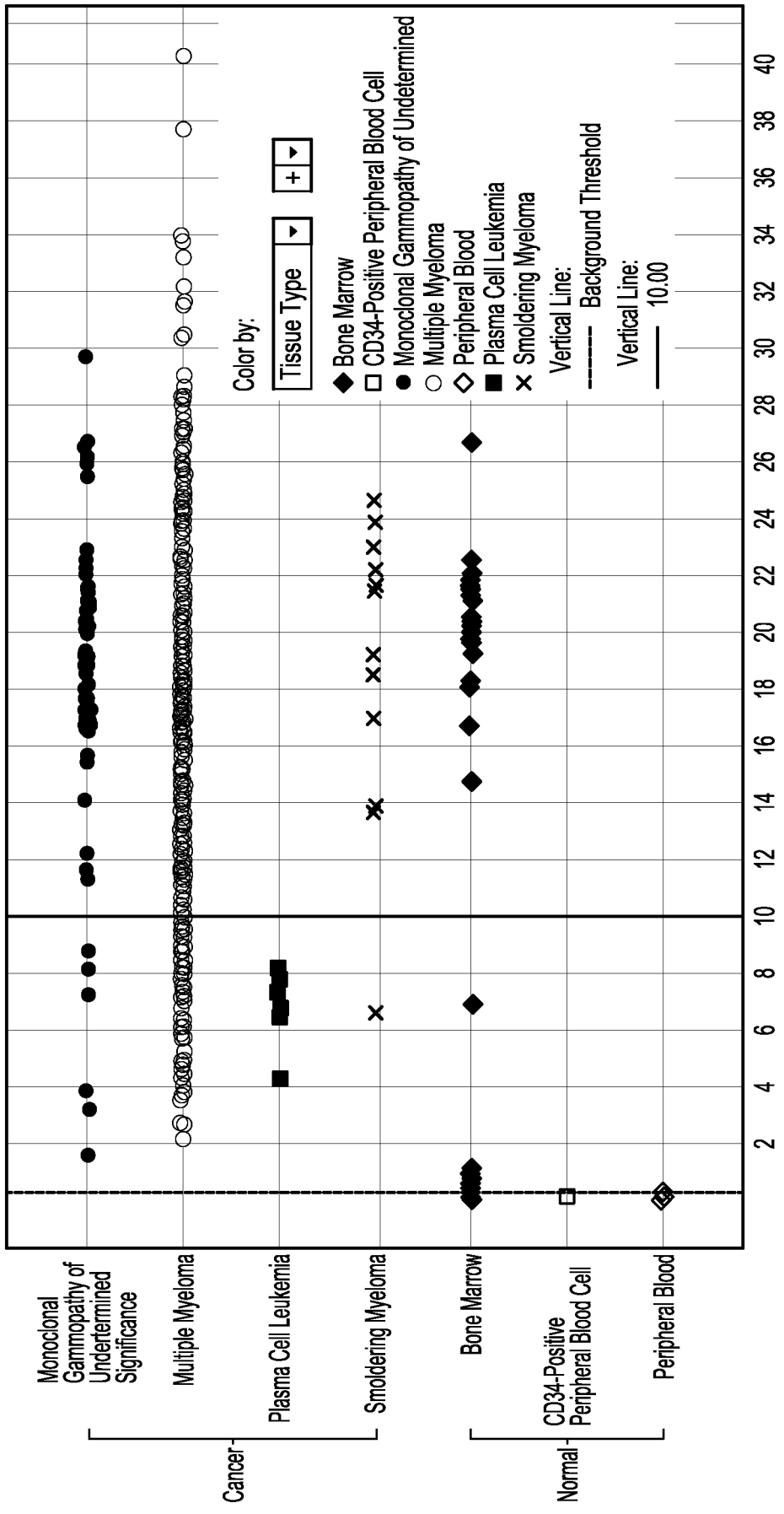

Color by:
Tissue Type
Bone Marrow
CD34-Positive Peripheral Blood Cell
Monoclonal Gammopathy of Undetermined
Multiple Myeloma
Peripheral Blood
Plasma Cell Leukemia
Smoldering Myeloma
Vertical Line:
Background Threshold
Vertical Line:
10.00
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Monoclonal Gammopathy of Undetermined Significance
Multiple Myeloma
Plasma Cell Leukemia
Smoldering Myeloma
Bone Marrow
CD34-Positive Peripheral Blood Cell
Peripheral Blood
Cancer
Normal

Fig. 3

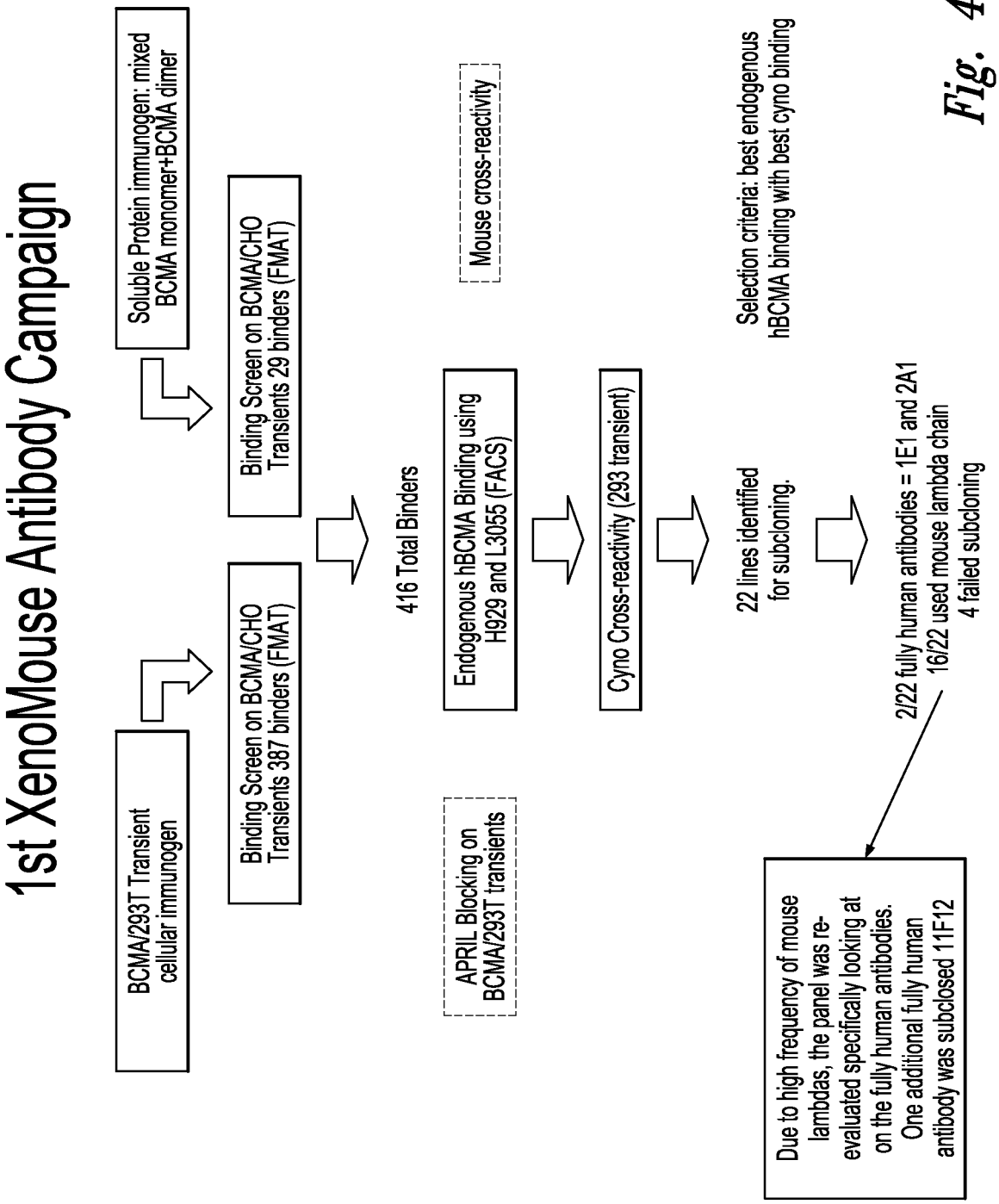

1st XenoMouse Antibody Campaign
BCMA/293T Transient cellular immunogen
Soluble Protein immunogen: mixed BCMA monomer+BCMA dimer
Binding Screen on BCMA/CHO Transients 387 binders (FMAT)
Binding Screen on BCMA/CHO Transients 29 binders (FMAT)
416 Total Binders
APRIL Blocking on BCMA/293T transients
Endogenous hBCMA Binding using H929 and L3055 (FACS)
Mouse cross-reactivity
Cyno Cross-reactivity (293 transient)
22 lines identified for subcloning.
Selection criteria: best endogenous hBCMA binding with best cyno binding
2/22 fully human antibodies = 1E1 and 2A1
16/22 used mouse lambda chain
4 failed subcloning
Due to high frequency of mouse lambdas, the panel was re-evaluated specifically looking at on the fully human antibodies. One additional fully human antibody was subclosed 11F12

Fig. 4A

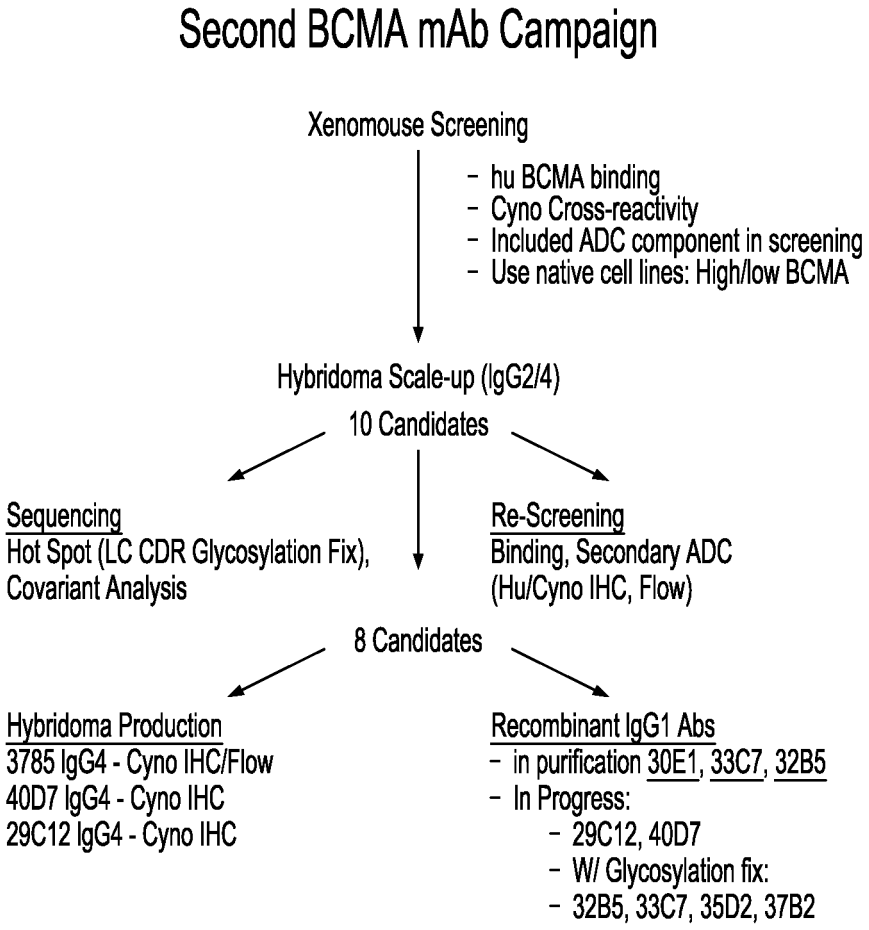

Second BCMA mAb Campaign
Xenomouse Screening
- hu BCMA binding
- Cyno Cross-reactivity
- Included ADC component in screening
- Use native cell lines: High/low BCMA
Hybridoma Scale-up (IgG2/4)
10 Candidates
Sequencing
Hot Spot (LC CDR Glycosylation Fix),
Covariant Analysis
Re-Screening
Binding, Secondary ADC
(Hu/Cyno IHC, Flow)
8 Candidates
Hybridoma Production
3785 IgG4 - Cyno IHC/Flow
40D7 IgG4 - Cyno IHC
29C12 IgG4 - Cyno IHC
Recombinant IgG1 Abs
- in purification 30E1, 33C7, 32B5
- In Progress:
- 29C12, 40D7
- W/ Glycosylation fix:
- 32B5, 33C7, 35D2, 37B2

Fig. 4B

mAB	Avg % inhibition	H929 FACS	L3055 FACS	MoBCMA binding on 293T transients by FMAT	FACS binding BCMA/293 transients (background subtracted): HuBCMA	FACS binding BCMA/293 transients (background subtracted): MoBCMA	FACS binding BCMA/293 transients (background subtracted): CynoBCMA	CynoBCMA binding on 293T transients FMAT	FACS binding BCMA/293 transients (background subtracted): CynoBCMA	Immunogen
19F11	16%	84.9	-	-	-	-	-	Y	165.8	A
20C10	20%	57.4	-	-	-	-	-	Y	139.2	A
1C1	24%	-	-	-	-	-	-	Y	50.8	A
1E1	72%	-	-	-	-	-	-	Y	91.1	A
2A1	89%	-	47.8	Y	128.2	4.4	70.7	Y	210.2	B
16B2	66%	48.0	-	Y	131.8	31.6	54.4	Y	143.1	B
7B9	78%	58.5	-	Y	144.9	29.0	57.4	Y	138.2	B
11C9	69%	56.1	-	Y	118.1	19.4	33.7	Y	113.8	B
3H9	72%	60.5	-	Y	126.8	12.9	30.1	Y	85.8	B
9F10	81%	49.9	-	Y	114	11.2	17.9	Y	64.3	B
12H11	38%	75.1	-	Y	144.7	17.6	18.3	Y	55.7	B
18H9	71%	60.0	-	Y	115.6	11.8	14.3	Y	48.4	B
5A7	34%	40.6	-	Y	106.3	11.4	18.9	Y	46.4	B

Fig. 5A

mAB	Avg % inhibition	H929 FACS	L3055 FACS	MoBCMA binding on 293T transients by FMAT	FACS binding BCMA/293 transients (background subtracted): HuBCMA	FACS binding BCMA/293 transients (background subtracted): MoBCMA	FACS binding BCMA/293 transients (background subtracted): CynoBCMA	CynoBCMA binding on 293T transients FMAT	FACS binding BCMA/293 transients (background subtracted): CynoBCMA	Immunogen
3F8	16%	54.6	-	Y	106.5	16.1	16.3	Y	46.4	B
13B10	40%	38.4	-	Y	135.0	24.0	19.5	Y	43.0	B
3G1	65%	61.3	-	Y	101.0	7.7	8.9	Y	35.3	B
7F9	29%	40.6	-	Y	77.7	7.7	14.3	Y	35.1	B
13B3	1%	56.5	-	Y	98.5	14.6	19.2	Y	33.5	B
3D1	28%	31.0	-	Y	63.2	7.9	12.4	Y	31.9	B
8C3	20%	34.8	-	Y	66.0	7.2	13.0	Y	31.2	B
6B9	52%	49.7	-	Y	101.5	11.2	12.1	Y	29.7	B
4A9	73%	63.5	-	Y	146.2	31.4	7.4	Y	28.6	B

Fig. 5B

		FACS Binding (MFI)						APRIL Blocking			
Antibody ID	hu BCMA	Cyno BCMA	Positive for Cyno Binding	hu BAFFR	hu TACI	BCMA (Mol. detect)	BAFFR/ TACI/ Mol.	% Inhibition n=1	% Inhibition n=2	Avg. % Inhibition	CynoBCMA O/N FACS Binding (MFI)
30D5	160451	205088	Y	-1733	2455	-4468	N	78%	101%	90%	166.0
35D2	129445	198499	Y	-119	506	-6991	N	57%	82%	70%	151.8
39B3	146555	198505	Y	-2949	371	-749	N	76%	105%	90%	137.7
35A4	114426	101450	Y	-495	592	689	N	42%	34%	38%	129.8
30E1	108585	78469	Y	-935	555	-2342	N	31%	46%	38%	129.7
32H3	97721	51189	Y	-942	1356	-1228	N	20%	47%	33%	121.6
39G2	161545	235326	Y	-507	562	1083	N	70%	95%	82%	115.8
32H4	141081	213465	Y	-324	687	-4532	N	50%	62%	56%	111.1
33H7	99708	160681	Y	-104	15	-422	N	41%	58%	49%	110.6
29C12	82565	97267	Y	-3102	256	-5194	N	30%	62%	46%	109.8
33C7	132175	238007	Y	-251	1887	347	N	68%	88%	78%	109.1
31D5	142124	183962	Y	436	1151	1249	N	54%	81%	68%	108.7
32B5	137058	256791	Y	-1093	274	-5762	N	87%	116%	101%	108.5
32G12	152554	265751	Y	-975	514	-2904	N	83%	107%	95%	108.0
37B2	83678	135996	Y	-351	1039	-1063	N	37%	58%	48%	104.1
35A8	77459	89438	Y	-363	522	-1237	N	35%	NA	35%	101.6
32B11	44741	68112	Y	407	1853	-2817	N	20%	38%	29%	99.6
31D11	94187	137457	Y	278	220	-1013	N	44%	53%	49%	96.3

Fig. 5C-1

			FACS Binding (MFI)					APRIL Blocking			
Antibody ID	hu BCMA	Cyno BCMA	Positive for Cyno Binding	hu BAFFR	hu TACI	BCMA (Mol. detect)	BAFFR/ TACI/ Mol.	% Inhibition n=1	% Inhibition n=2	Avg. % Inhibition	CynoBCMA O/N FACS Binding (MFI)
30G7	103535	42884	Y	-189	469	-920	N	39%	58%	49%	91.2
30E2	37196	37828	Y	-77	409	322	N	19%	26%	22%	90.8
30B1	32881	35359	Y	-292	301	-432	N	9%	27%	18%	85.0
40D7	36313	39335	Y	-444	1386	-989	N	23%	25%	24%	72.7
31A11	80147	26528	Y	-1907	172	-9413	N	28%	33%	31%	71.5
32G10	25705	31341	Y	-258	255	-1126	N	13%	36%	24%	61.7
43H8	30040	2555	Y	641	355	-881	N	53%	60%	56%	56.0
29G5	117875	119979	Y	-11685	331	-736	N	45%	71%	58%	56.0
38D5	43575	59724	Y	-2394	769	-15667	N	26%	40%	33%	55.7
33D4	89926	19377	Y	723	261	-1060	N	19%	30%	24%	55.6
37E1	2329	3323	Y	-308	297	-4598	N	11%	22%	17%	31.4
33E7	139459	5242	Y	-269	506	-673	N	58%	90%	74%	30.3
33C12	66304	2702	Y	-943	296	-543	N	26%	23%	24%	18.84
29G8	48959	4249	Y	-1229	1577	-1753	N	25%	51%	38%	16.36
39H2	153669	7699	Y	-42	-565	965	N	82%	110%	96%	15.39
31C6	96065	3525	Y	-927	325	80	N	35%	63%	49%	11.51
32E5	79989	6279	Y	-2861	1186	-2252	N	26%	54%	40%	10.68
30H2	144412	1743	Y	-570	168	897	N	61%	86%	73%	9.21
35F8	11736	1867	Y	484	2441	-1416	N	19%	31%	25%	7.64
38C6	62160	2579	Y	-2415	997	-556	N	59%	65%	62%	7.3
35D4	63542	5405	Y	-91	1057	-4007	N	33%	51%	42%	6.37
39D4	22286	4349	Y	-50	521	-2895	N	33%	66%	49%	6.23
32G2	10256	2508	Y	-779	3213	-5196	N	18%	33%	26%	5.93

Fig. 5C-2

			FACS Binding (MFI)					APRIL Blocking			
Antibody ID	hu BCMA	Cyno BCMA	Positive for Cyno Binding	hu BAFFR	hu TACI	BCMA (Mol. detect)	BAFFR/ TACI/ Mol.	% Inhibition n=1	% Inhibition n=2	Avg. % Inhibition	CynoBCMA O/N FACS Binding (MFI)
32E6	42268	1691	Y	-95	1889	-5121	N	28%	35%	32%	5.23
31G6	154878	5854	Y	-617	1011	1100	N	81%	96%	88%	4.15
33E3	27393	5095	Y	-185	213	-913	N	18%	22%	20%	3.85
29B8	44354	221350	Y	877	351	-1430	Y	86%	116%	101%	129.39
29H10	45527	27640	Y	23	3099	76790	Y	23%	32%	26%	45.11
29D11	25215	12933	Y	6165	71	1392	Y	22%	30%	26%	63.29
29D12	178813	153427	Y	1704	1680	-2258	Y	52%	84%	68%	174.22
30H1	58514	1958	Y	703	-1046	7860	Y	26%	51%	39%	11.25
30G2	104029	67738	Y	2514	538	-2742	Y	29%	46%	37%	107.87
30H3	20195	1729	Y	-1173	318	34788	Y	29%	37%	33%	47.09
30G5	29027	16087	Y	670	250	53331	Y	22%	46%	34%	73.54
30D9	29633	1755	Y	1661	346	-4298	Y	22%	28%	25%	18.8
30C11	113056	15274	Y	-2168	673	137874	Y	54%	71%	63%	82.48
31F4	136023	265326	Y	405	1804	1586	Y	73%	97%	85%	134.1
31C5	28190	30427	Y	2597	527	-7931	Y	32%	50%	41%	63.91
31F6	202340	186148	Y	3861	-939	-3147	Y	83%	119%	101%	123.82
31E11	118670	12507	Y	-1810	154	2106	Y	45%	57%	51%	12.88
31H12	140883	151966	Y	1733	27	-1527	Y	50%	68%	59%	98.06
32A2	28887	12885	Y	2625	3856	-1125	Y	25%	38%	31%	5.02
32G5	103178	2220	Y	1370	292	-11836	Y	20%	14%	17%	3.08
32E11	46680	26201	Y	794	347	87883	Y	35%	59%	47%	67.49
33B3	35322	29608	Y	1177	991	-6849	Y	38%	47%	42%	76.81
33D3	180320	13901	Y	-329	385	1633	Y	66%	85%	76%	6.08

Fig. 5C-3

			FACS Binding (MFI)					APRIL Blocking			
Antibody ID	hu BCMA	Cyno BCMA	Positive for Cyno Binding	hu BAFFR	hu TACI	BCMA (Mol. detect)	BAFFR/ TACI/ Mol.	% Inhibition n=1	% Inhibition n=2	Avg. % Inhibition	CynoBCMA O/N FACS Binding (MFI)
33E6	12309	8142	Y	-180	1760	27038	Y	22%	23%	22%	40.75
33D8	78697	132778	Y	1244	521	-1694	Y	35%	50%	43%	76.74
34E2	189614	245880	Y	2504	585	-1544	Y	79%	102%	90%	93.41
34G5	115973	113760	Y	-413	814	176225	Y	65%	76%	70%	101.52
34C11	120846	139430	Y	-311	338	6785	Y	46%	57%	52%	110.11
34E11	81112	3874	Y	9	-103	2942	Y	62%	71%	66%	10.58
35B3	33122	18880	Y	-380	489	52894	Y	24%	39%	32%	40.28
36C4	115182	53740	Y	-180	675	113439	Y	53%	62%	58%	72.77
36E12	58750	82422	Y	31153	328	925	Y	44%	59%	51%	142.83
37B3	93055	75554	Y	-273	752	117310	Y	53%	64%	58%	68.84
37C3	118160	65297	Y	-249	428	153353	Y	61%	80%	70%	114.89
37D3	108195	2283	Y	-236	390	3005	Y	83%	104%	93%	14.11
37H5	20302	11408	Y	192	26	33378	Y	30%	45%	37%	61.3
37F7	142820	233237	Y	1042	1392	1452	Y	69%	99%	84%	113.09
38A2	80016	58239	Y	-230	-1680	113711	Y	63%	76%	70%	120.85
38G10	94432	91159	Y	50982	372	-2987	Y	45%	48%	46%	91.66
38A12	86795	2384	Y	3035	1045	1784	Y	49%	53%	51%	8.77
39E1	48465	26900	Y	32	2132	67970	Y	31%	48%	39%	53.17
35F8	22218	4466	Y	-115	103	2858	Y	19%	25%	22%	18.75
38C6	102974	37705	Y	20079	1543	-1315	Y	54%	47%	51%	34.22
35D4	24920	9255	Y	-71	1658	17137	Y	29%	33%	31%	25.88
39D4	56284	6167	Y	-1599	9530	397	Y	59%	84%	71%	300.6
32G2	31677	5170	Y	-1275	7837	-856	Y	28%	46%	37%	205

Fig. 5C-4

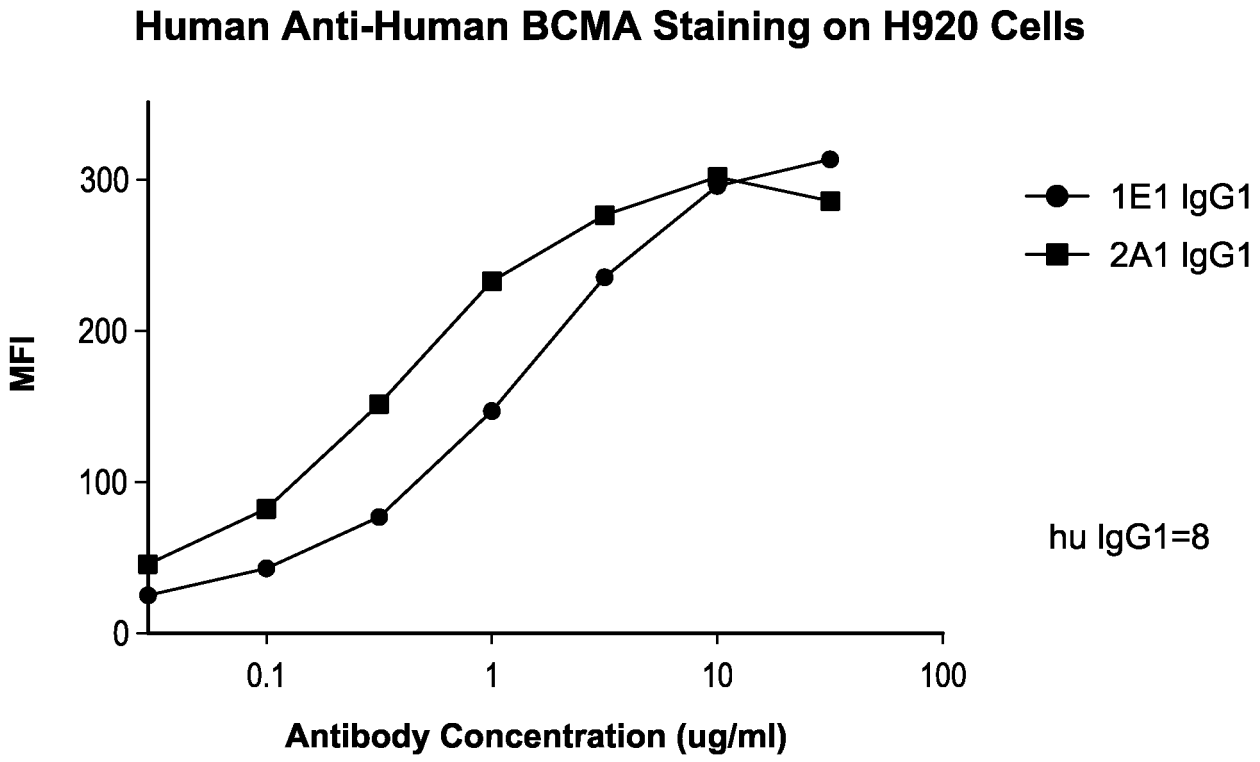

Human Anti-Human BCMA Staining on H920 Cells
MFI
300
200
100
0
0.1
1
10
100
Antibody Concentration (ug/ml)
1E1 IgG1
2A1 IgG1
hu IgG1=8

Fig. 6

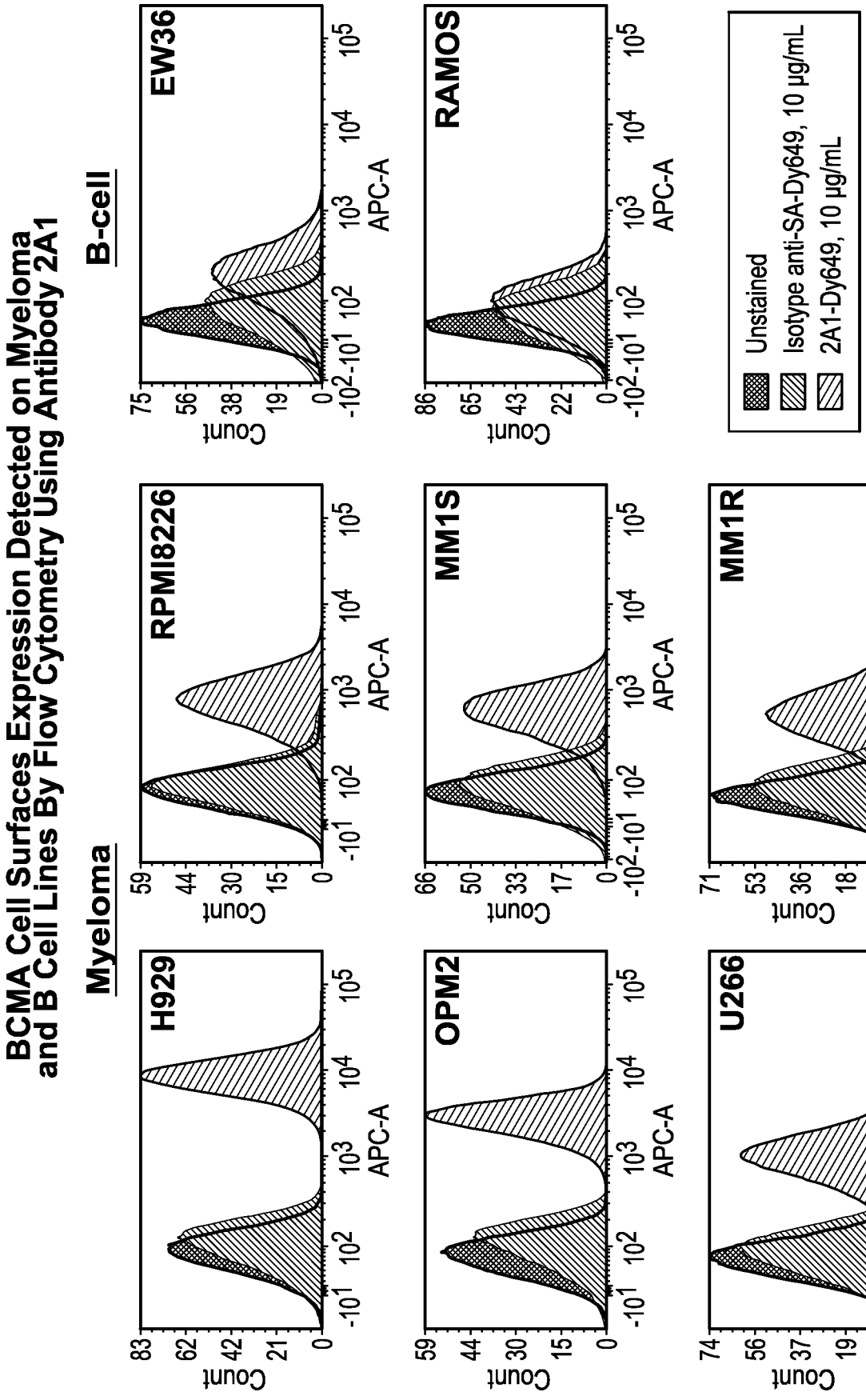

BCMA Cell Surfaces Expression Detected on Myeloma and B Cell Lines By Flow Cytometry Using Antibody 2A1
Myeloma
B-cell
H929
RPMI8226
EW36
OPM2
MM1S
RAMOS
U266
MM1R
Count
APC-A
Unstained
Isotype anti-SA-Dy649, 10 µg/mL
2A1-Dy649, 10 µg/mL

Fig. 7

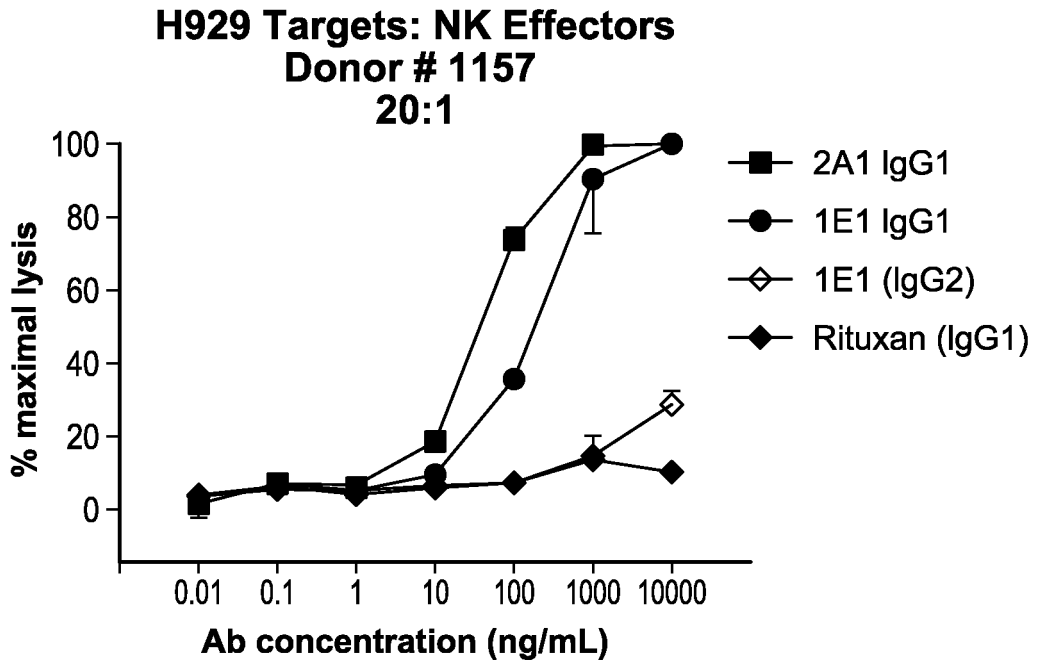

H929 Targets: NK Effectors
Donor # 1157
20:1
% maximal lysis
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
10000
Ab concentration (ng/mL)
2A1 IgG1
1E1 IgG1
1E1 (IgG2)
Rituxan (IgG1)

Fig. 8

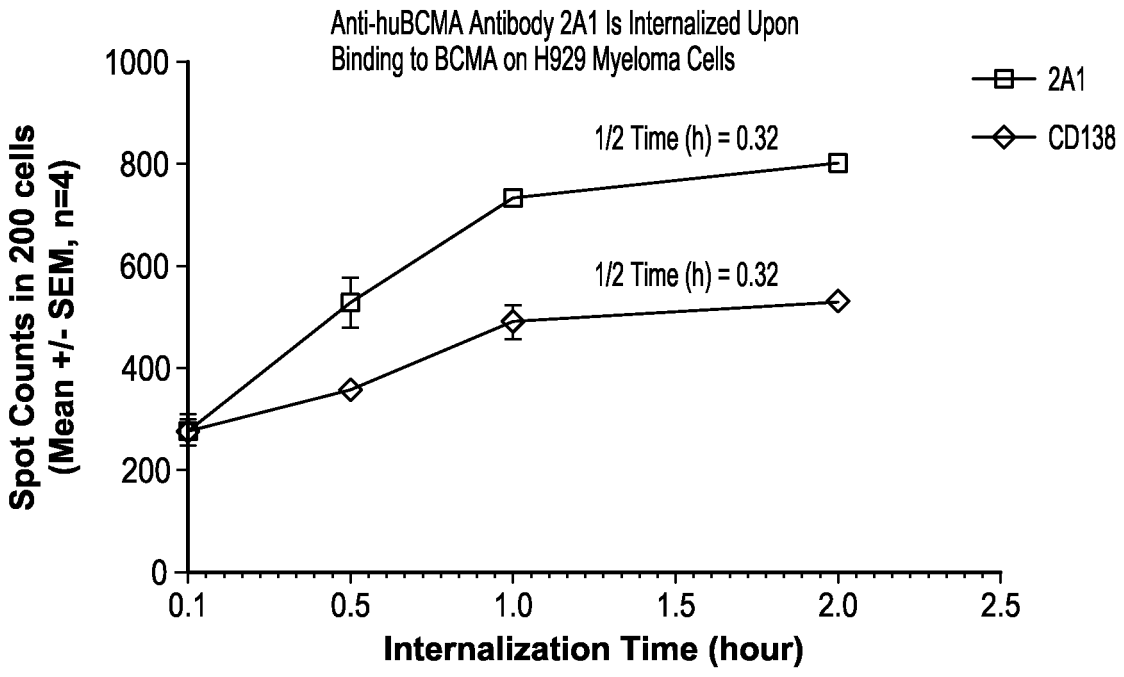

Anti-huBCMA Antibody 2A1 Is Internalized Upon
Binding to BCMA on H929 Myeloma Cells
Spot Counts in 200 cells
(Mean +/- SEM, n=4)
1000
800
600
400
200
0
0.1
0.5
1.0
1.5
2.0
2.5
Internalization Time (hour)
1/2 Time (h) = 0.32
1/2 Time (h) = 0.32
2A1
CD138

Fig. 9

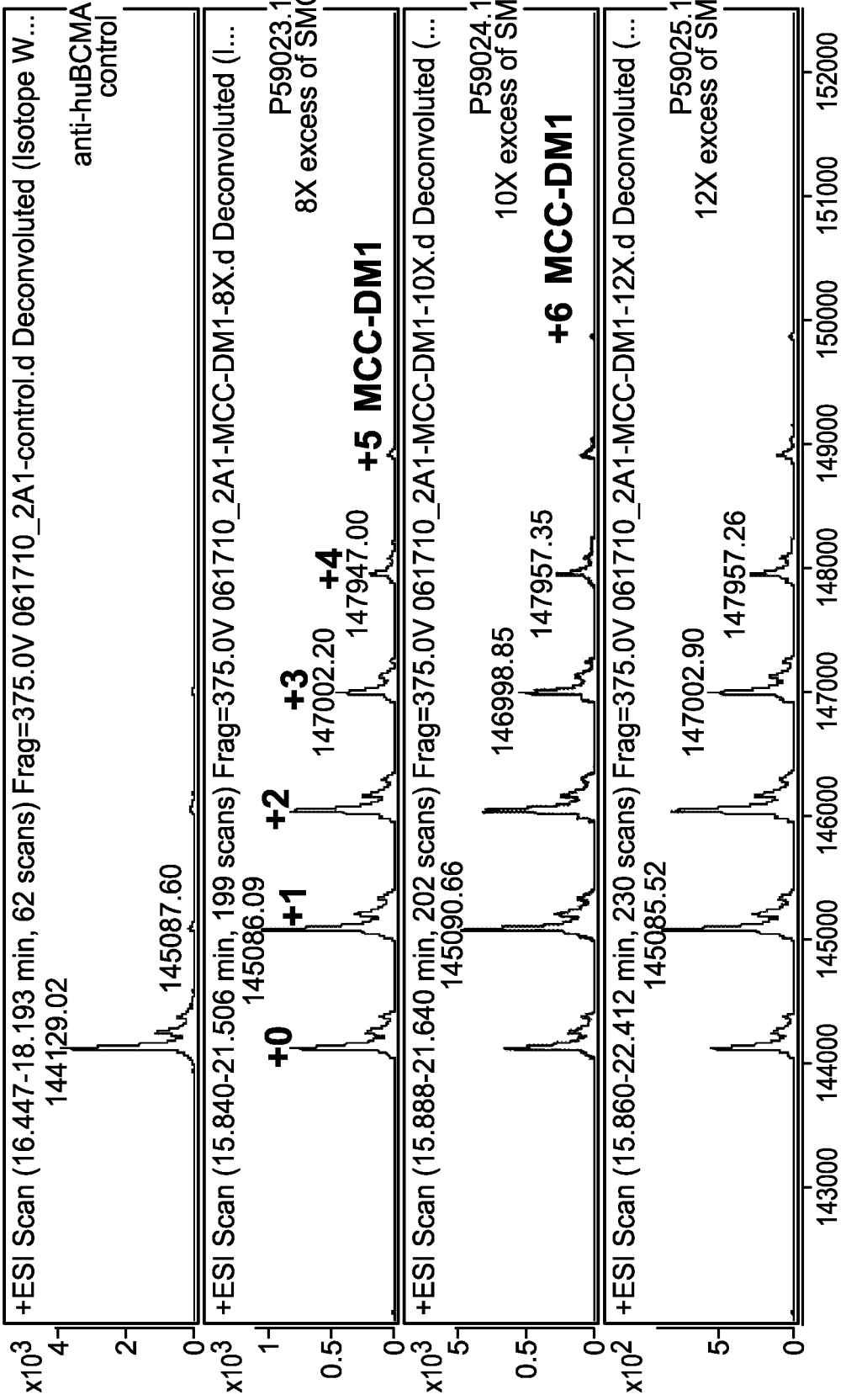

Mass Spec Analysis of anti-huBCMA 2A1-MCC-DM1
+ESI Scan (16.447-18.193 min, 62 scans) Frag=375.0V 061710_2A1-control.d Deconvoluted (Isotope W...
anti-huBCMA 2A1 control
144129.02
145087.60
+ESI Scan (15.840-21.506 min, 199 scans) Frag=375.0V 061710_2A1-MCC-DM1-8X.d Deconvoluted (I...
P59023.1
8X excess of SMCC-DM1
145086.09
+0
+1
+2
147002.20
+3
147947.00
+4
+5 MCC-DM1
+ESI Scan (15.888-21.640 min, 202 scans) Frag=375.0V 061710_2A1-MCC-DM1-10X.d Deconvoluted (...
P59024.1
10X excess of SMCC-DM1
145090.66
146998.85
147957.35
+6 MCC-DM1
+ESI Scan (15.860-22.412 min, 230 scans) Frag=375.0V 061710_2A1-MCC-DM1-12X.d Deconvoluted (...
P59025.1
12X excess of SMCC-DM1
145085.52
147002.90
147957.26
x10^3
x10^2
143000
144000
145000
146000
147000
148000
149000
150000
151000
152000

Fig. 10

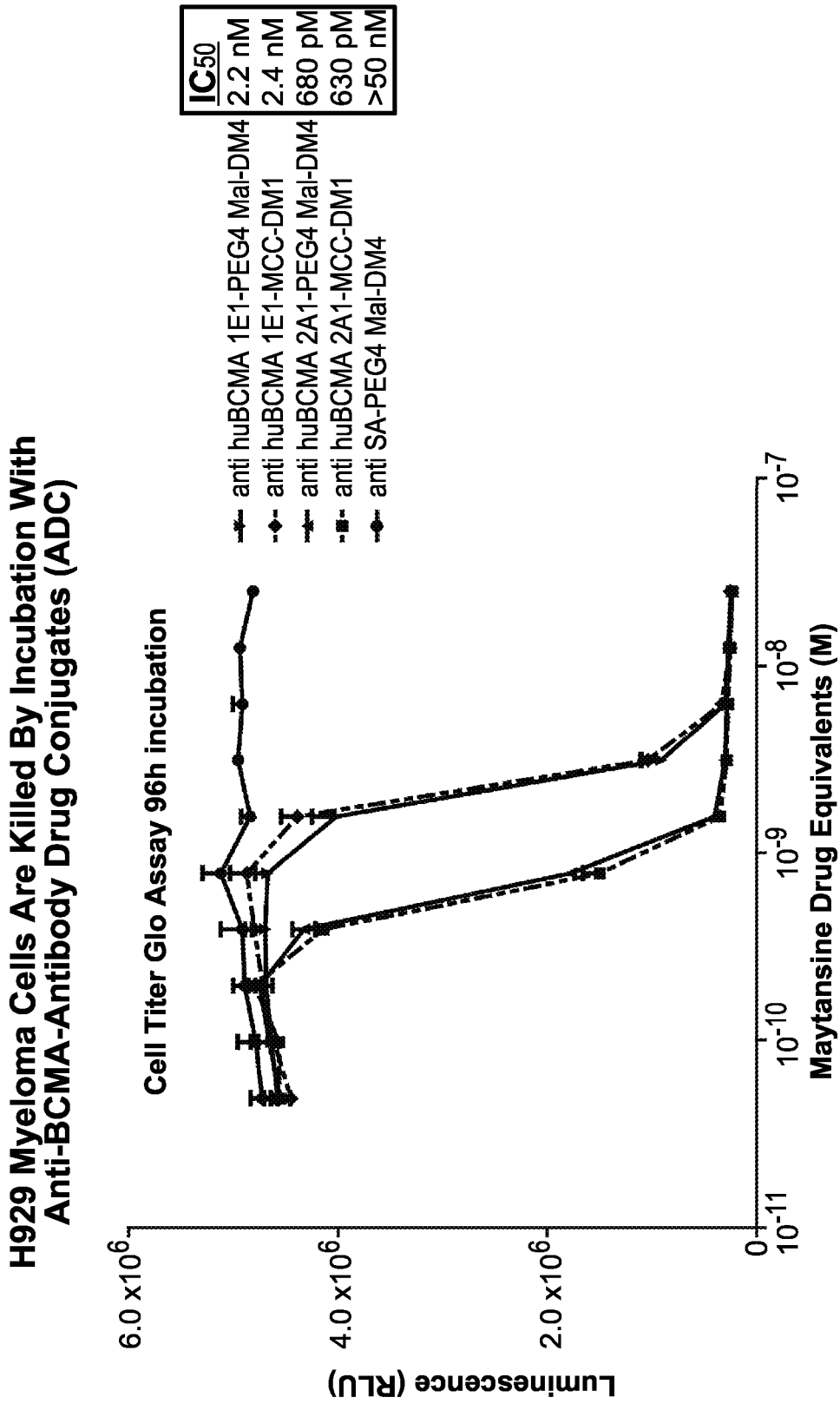

H929 Myeloma Cells Are Killed By Incubation With Anti-BCMA-Antibody Drug Conjugates (ADC)
Cell Titer Glo Assay 96h incubation
IC50
anti huBCMA 1E1-PEG4 Mal-DM4 2.2 nM
anti huBCMA 1E1-MCC-DM1 2.4 nM
anti huBCMA 2A1-PEG4 Mal-DM4 680 pM
anti huBCMA 2A1-MCC-DM1 630 pM
anti SA-PEG4 Mal-DM4 >50 nM
Luminescence (RLU)
6.0 x10^6
4.0 x10^6
2.0 x10^6
0
10^-11
10^-10
10^-9
10^-8
10^-7
Maytansine Drug Equivalents (M)

Fig. 11

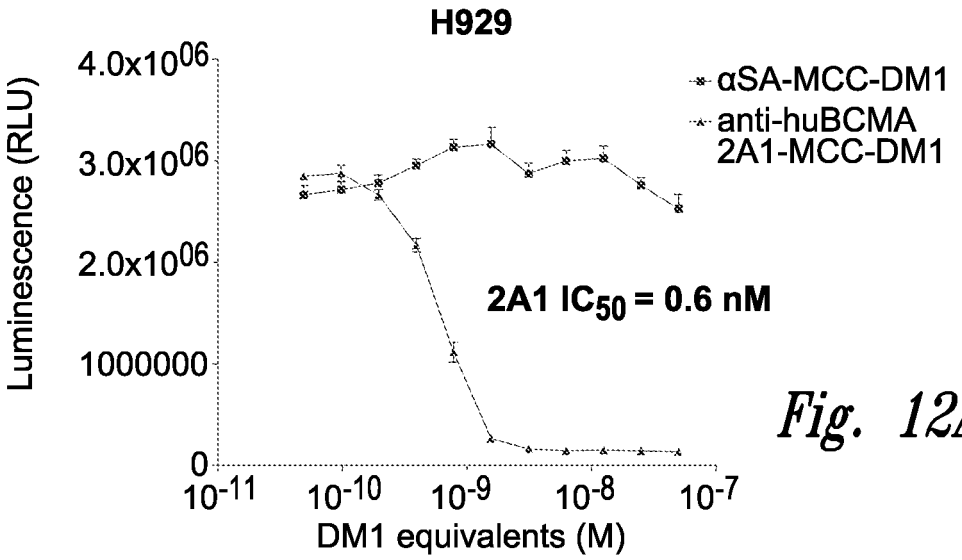


Fig. 12A

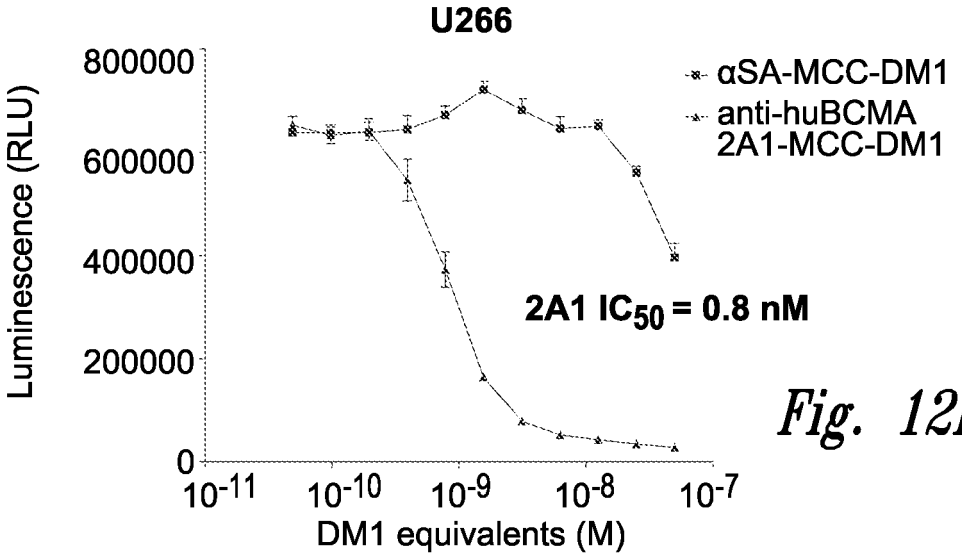


Fig. 12B

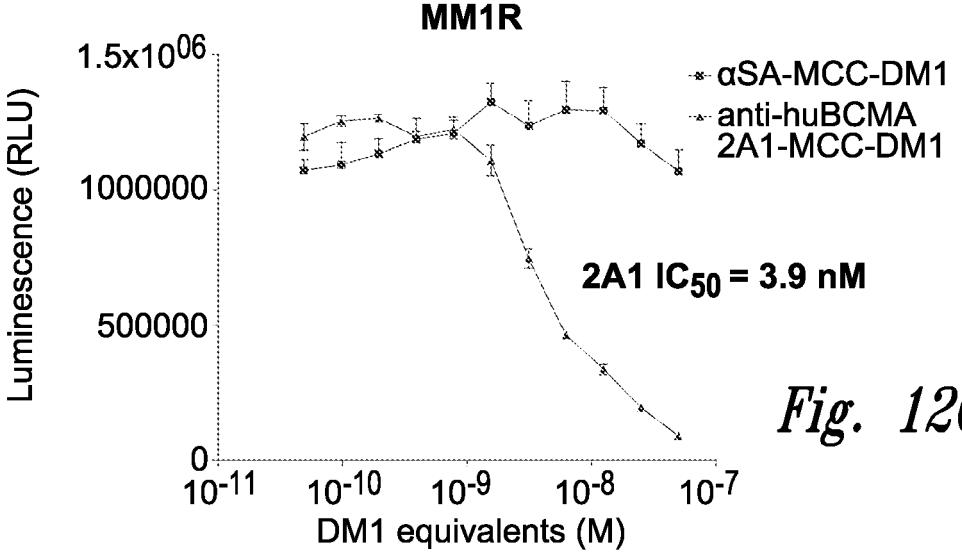


Fig. 12C

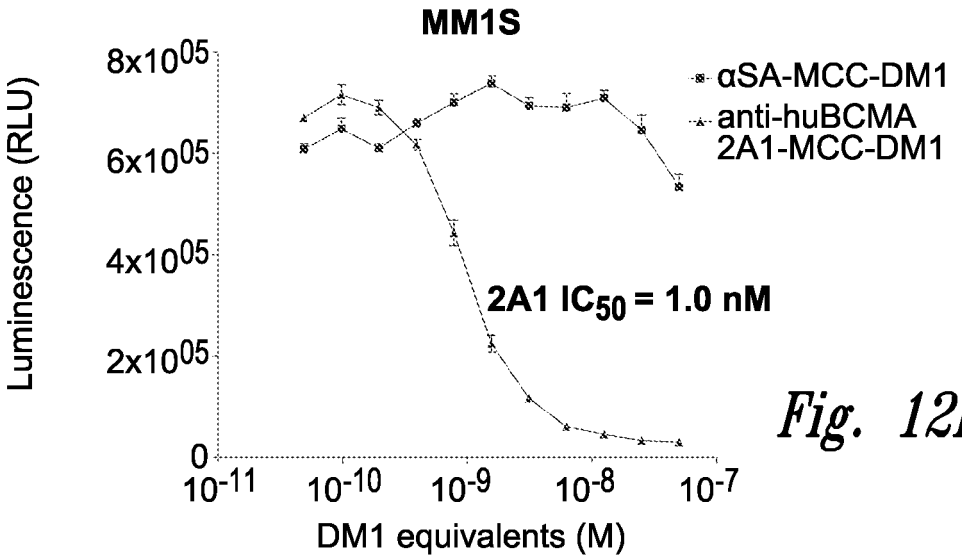


Fig. 12D

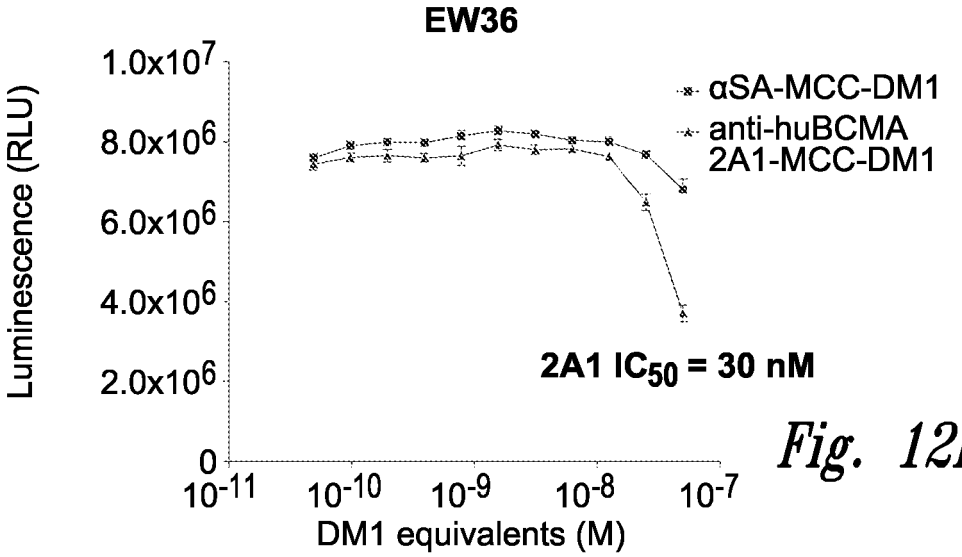


Fig. 12E

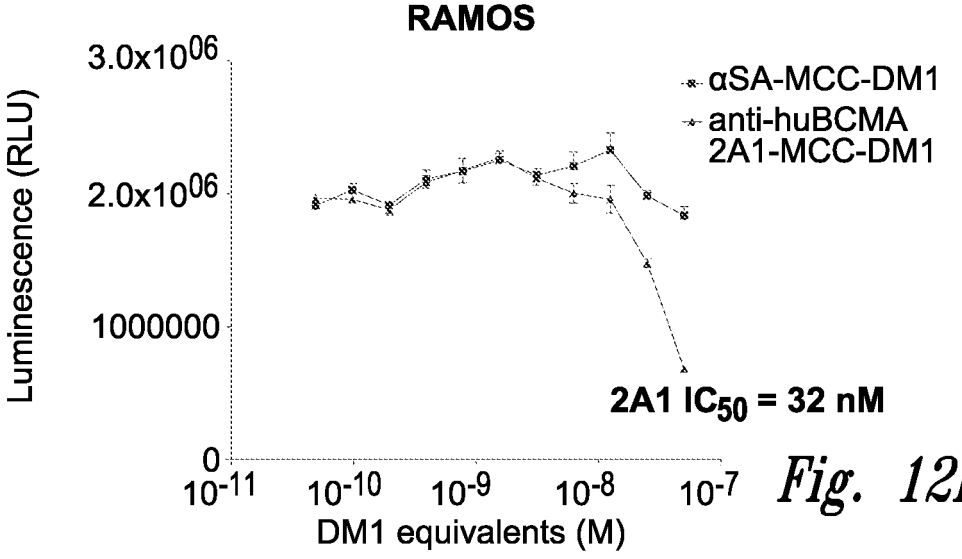
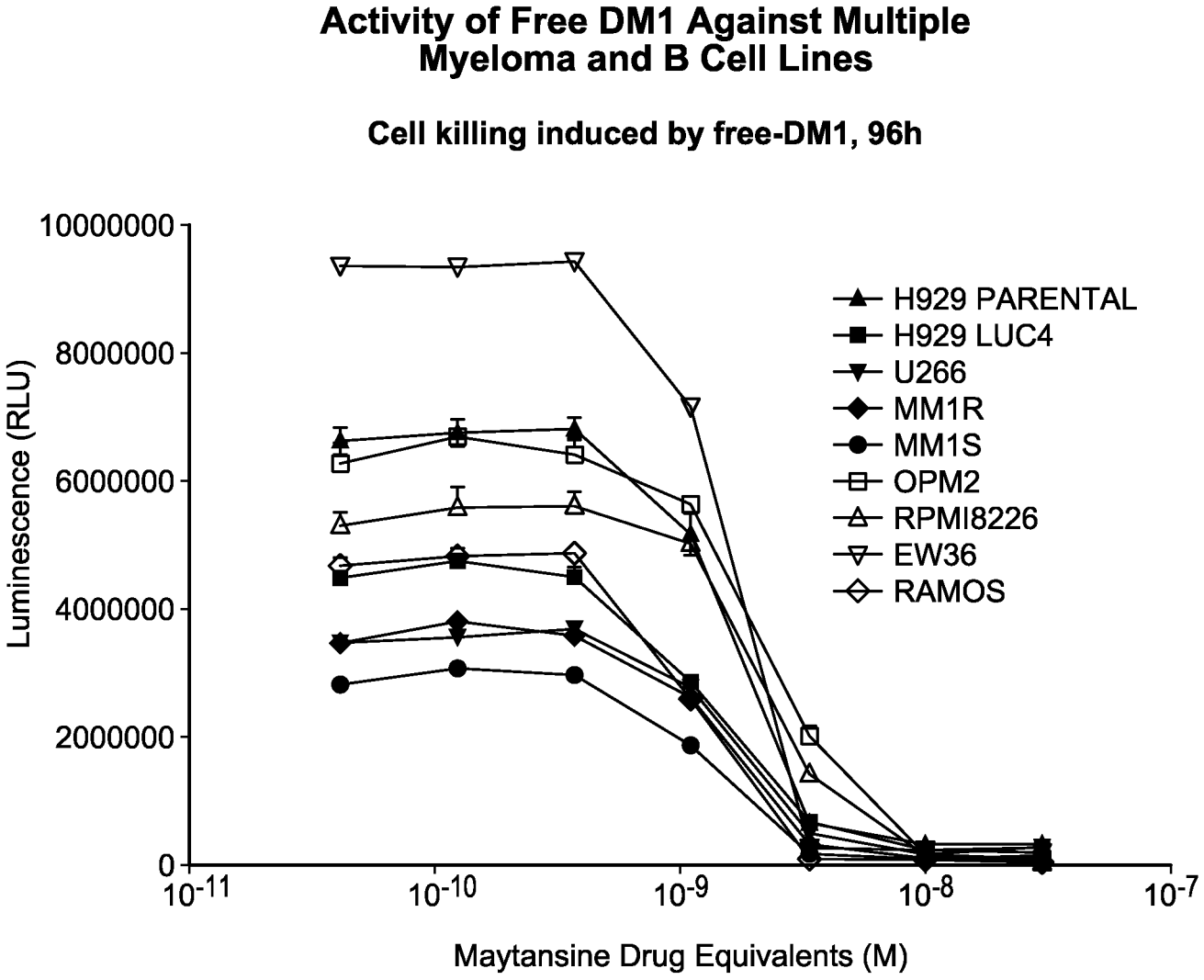


Fig. 12F



	H929 PARENTAL	H929 LUC4	U266	MM1R	MM1S	OPM2	RPMI8226	EW36	RAMOS
IC50	1.489e-009	1.301e-009	1.577e-009	1.431e-009	1.231e-009	2.405e-009	2.350e-009	1.325e-009	~ 1.121e-009

Fig. 13

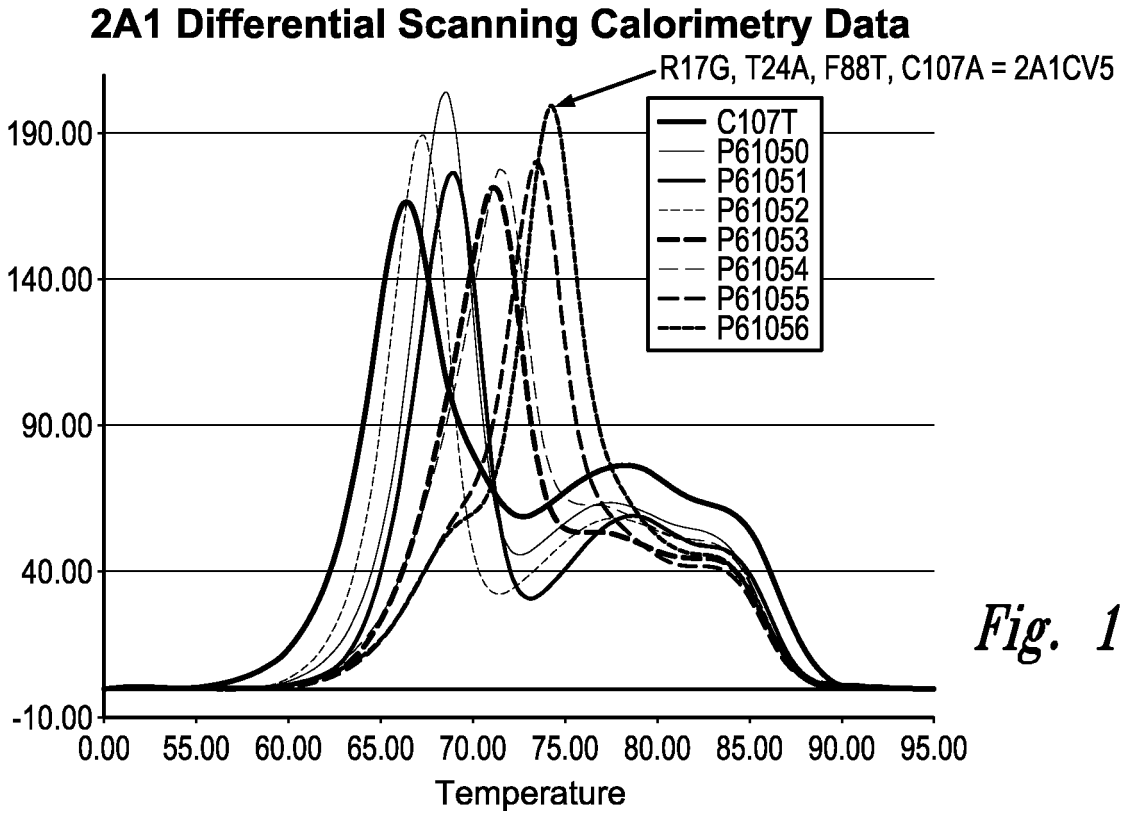


Fig. 14A

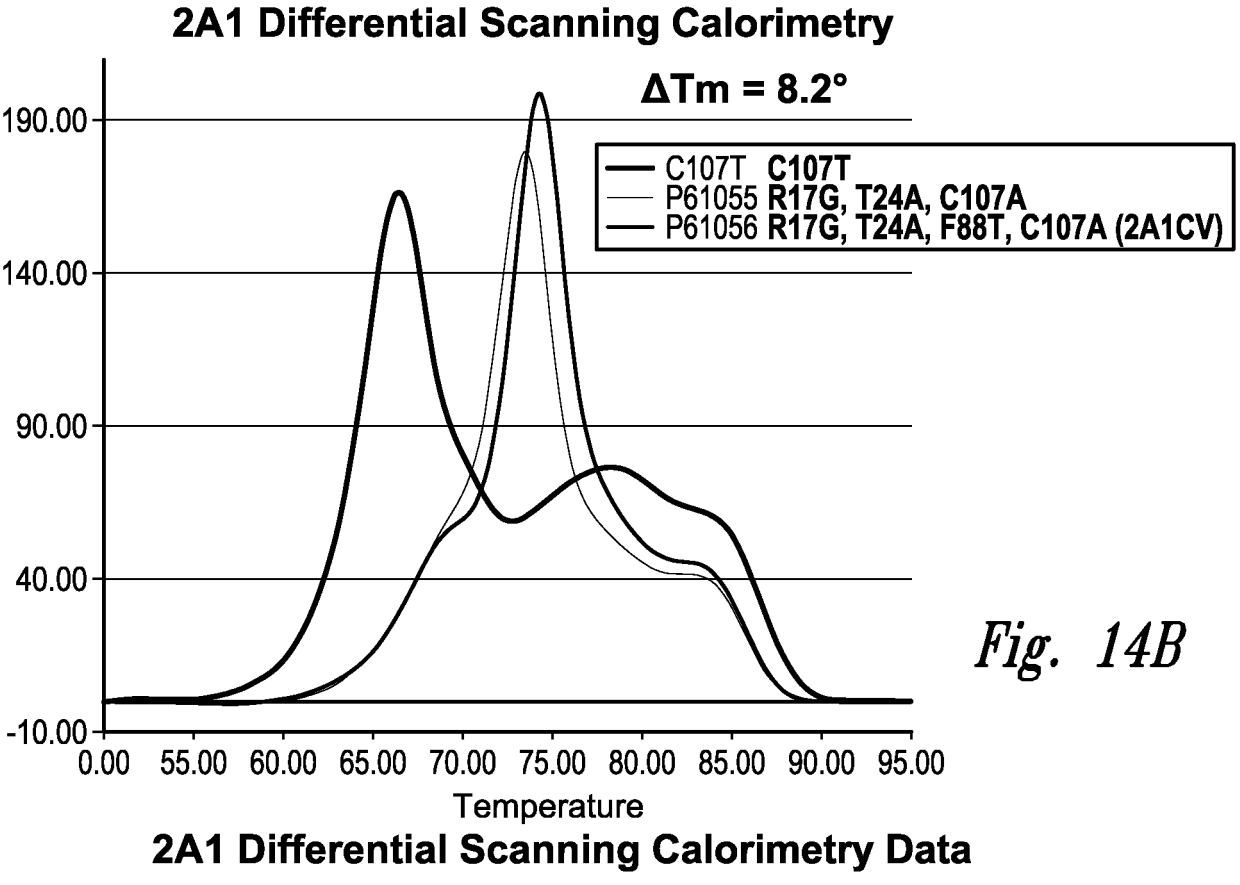


Fig. 14B

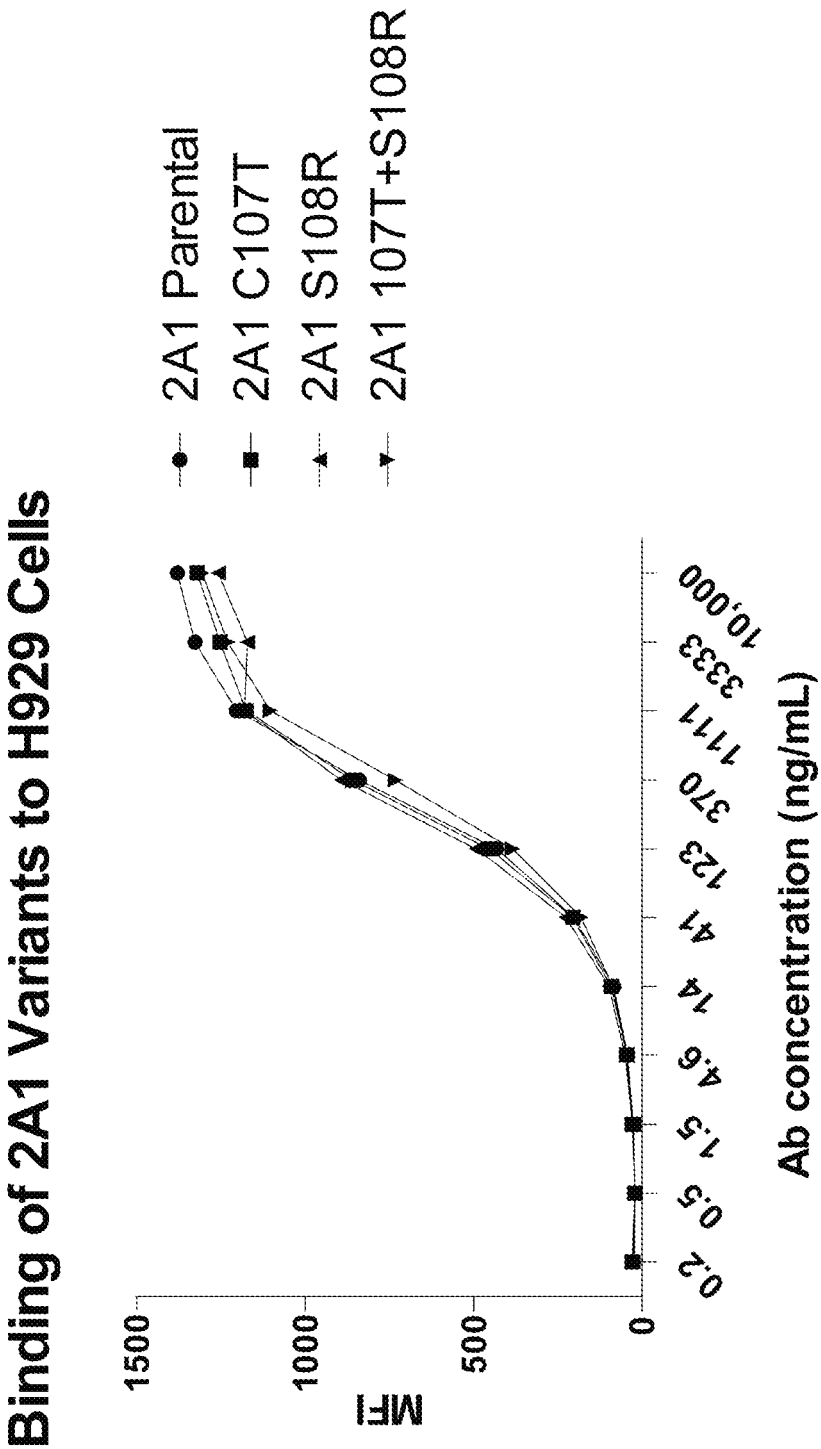

Binding of 2A1 Variants to H929 Cells
2A1 Parental
2A1 C107T
2A1 S108R
2A1 107T+S108R
MFI
1500
1000
500
0
0.2
0.5
1.5
4.6
14
41
123
370
1111
3333
10,000
Ab concentration (ng/mL)

Fig. 15

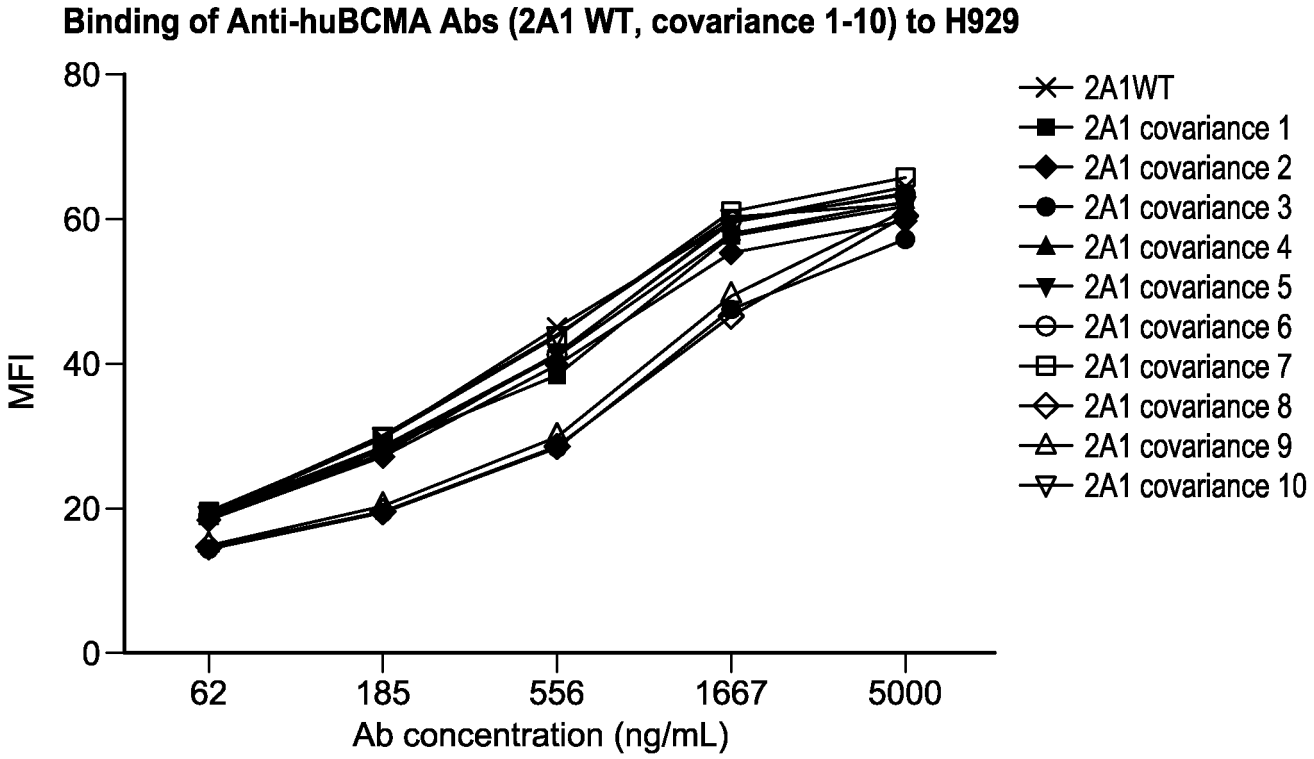


Fig. 16A

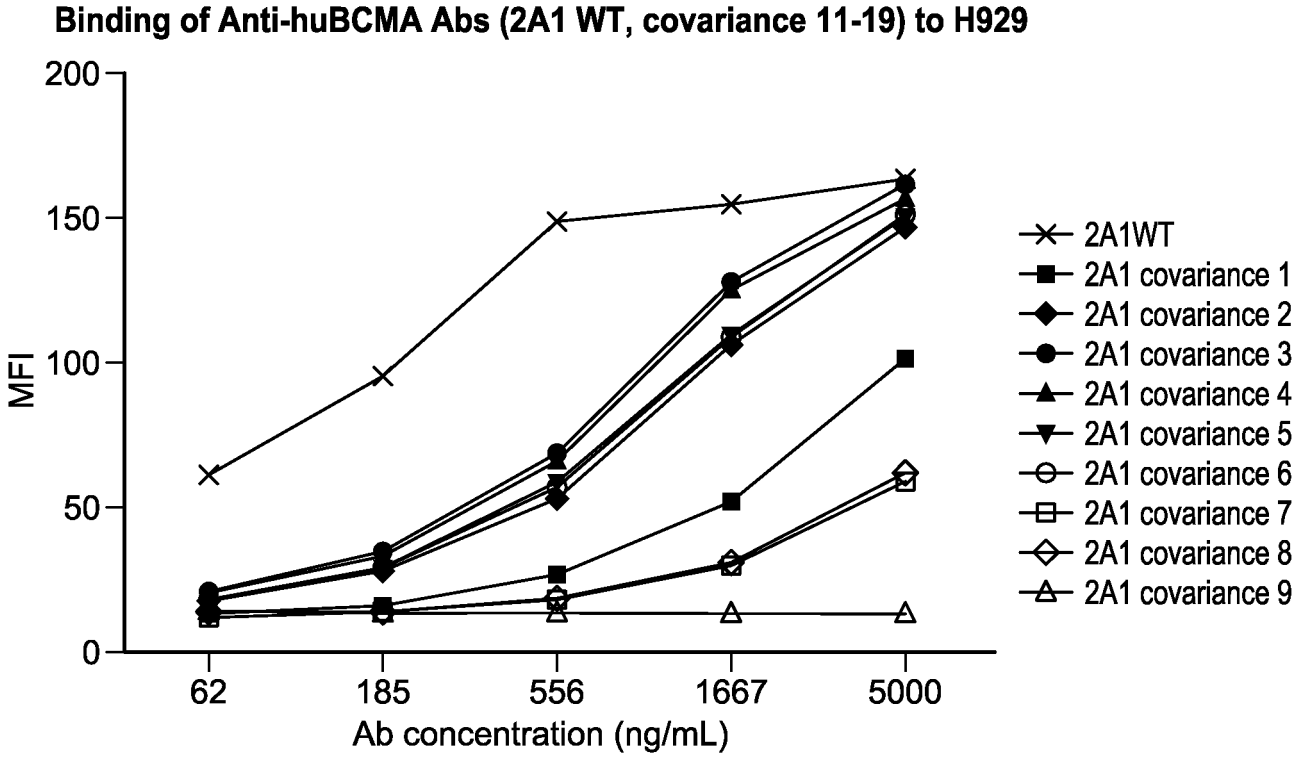


Fig. 16B

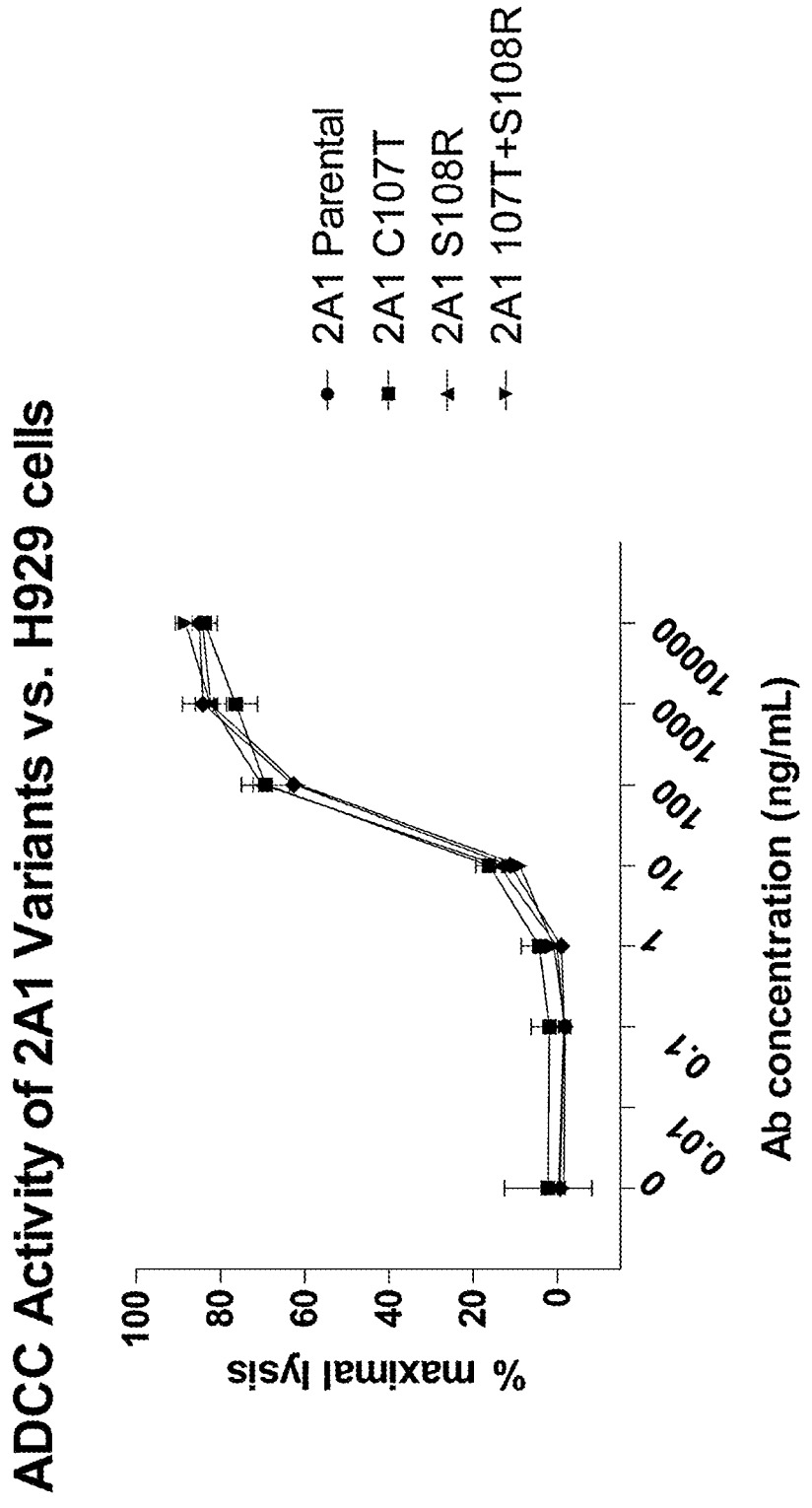

ADCC Activity of 2A1 Variants vs. H929 cells
2A1 Parental
2A1 C107T
2A1 S108R
2A1 107T+S108R
% maximal lysis
0
20
40
60
80
100
Ab concentration (ng/mL)
0
0.01
0.1
1
10
100
1000
10000

Fig. 17

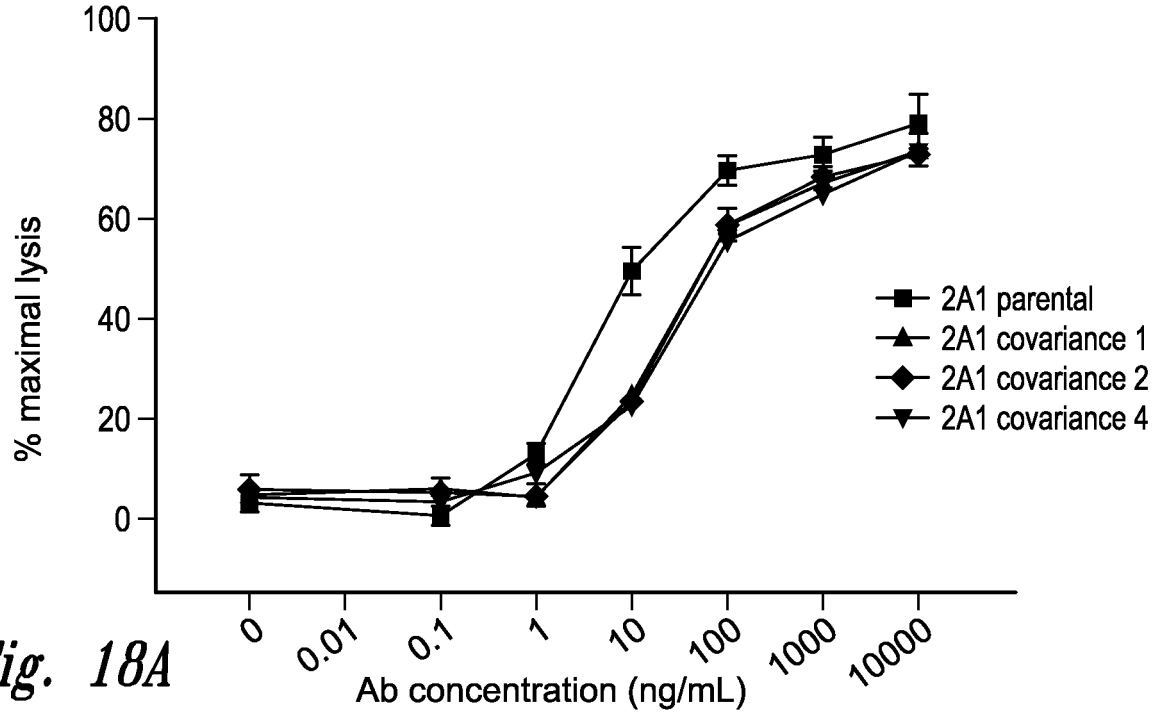


Fig. 18A

	2A1 parental	2A1 covariance 1	2A1 covariance 2	2A1 covariance 4
EC50	5.504	23.78	24.07	27.99

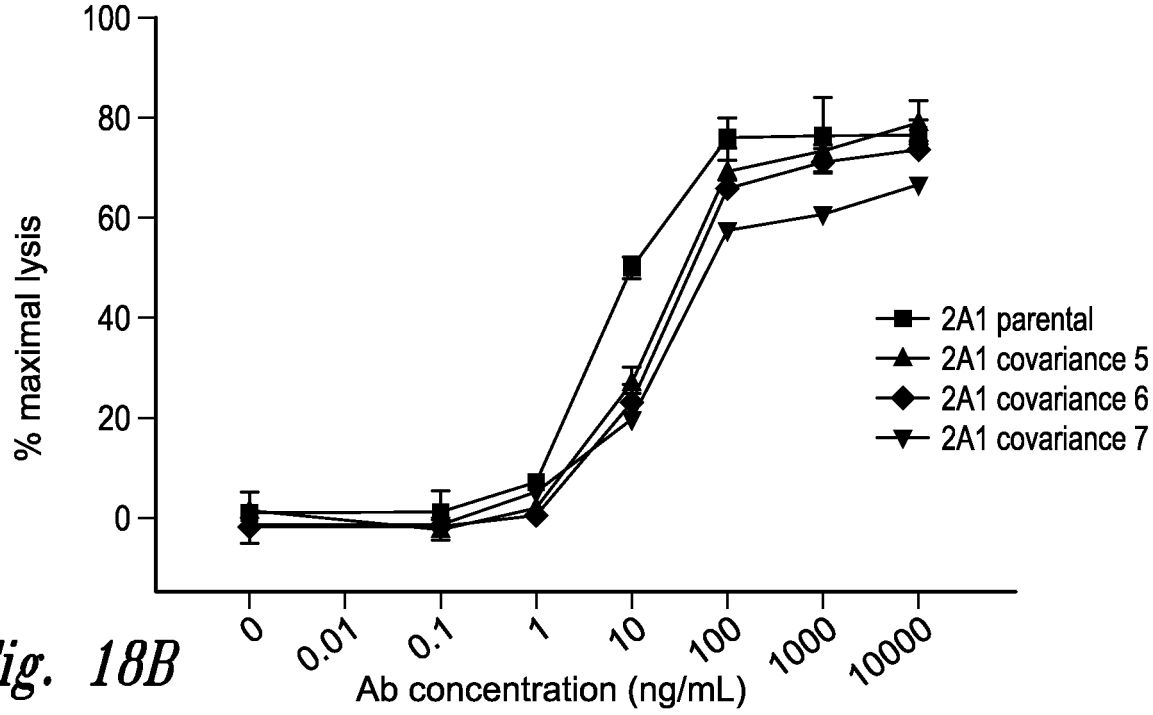


Fig. 18B

	2A1 parental	2A1 covariance 5	2A1 covariance 6	2A1 covariance 7
EC50	6.307	16.04	16.89	19.14

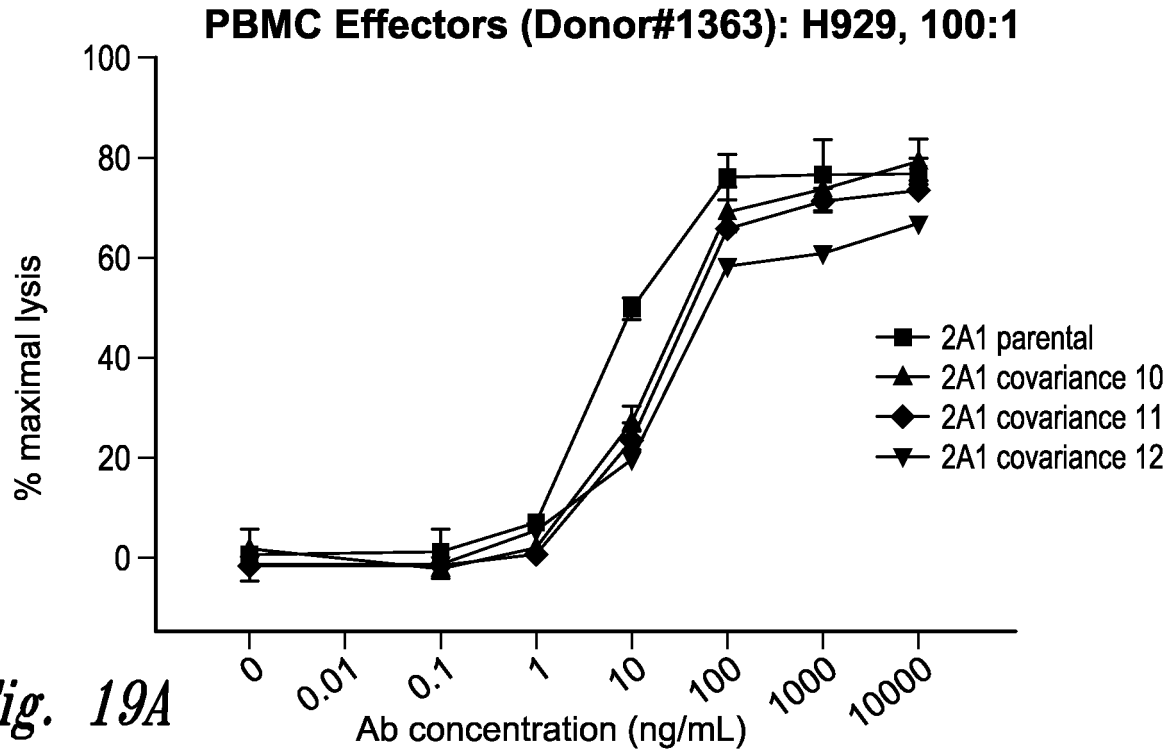


Fig. 19A

	2A1 parental	2A1 covariance 10	2A1 covariance 11	2A1 covariance 12
EC50	7.538	26.23	364.4	110.9

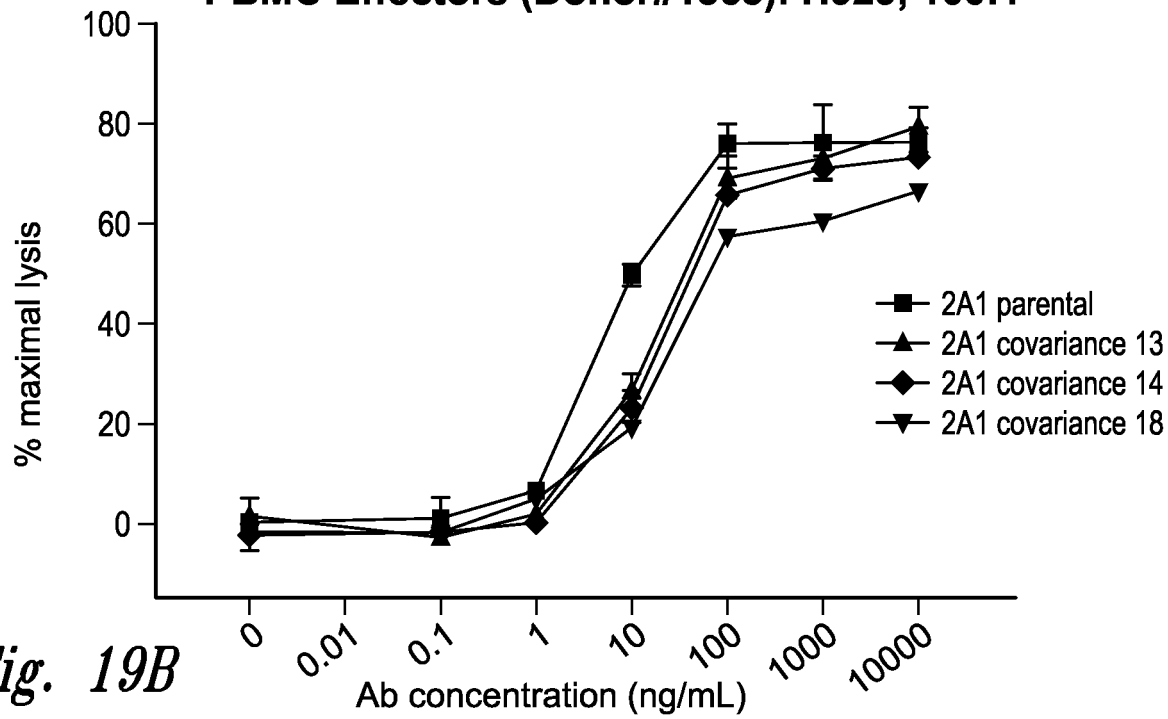
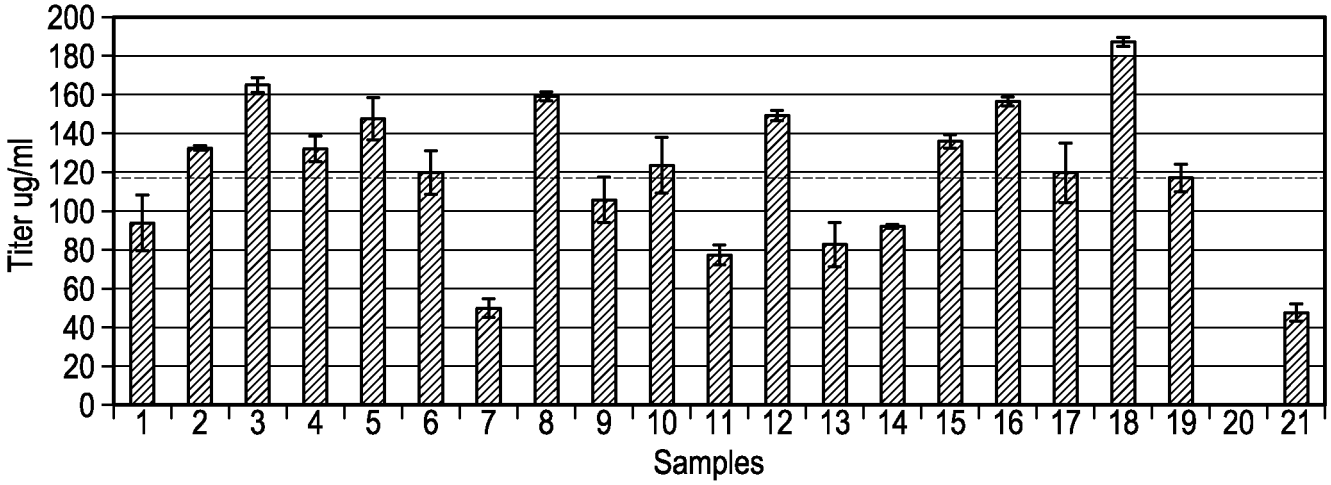


Fig. 19B

	2A1 parental	2A1 covariance 13	2A1 covariance 14	2A1 covariance 15
EC50	5.240	32.73	41.67	359.0

24-well Expression titers



LMR construct entries:				
#		HC#	Alias	LC#
1	A01	C51893	pTT5:aBCMA:VK_2A1_C107T_IgG1	C27648
2	B01	C58114	VK1_12A1_R16G	C27648
3	C01	C58115	VK1_2A1_F37V	C27648
4	D01	C58116	VK1_2A1_D76N	C27648
5	A02	C58117	VK1_2A1_F80T	C27648
6	B02	C58118	VK_2A1_T99A	C27648
7	C01	C58119	VK1_2A1_S100K	C27648
8	D02	C58120	VK1_2A1_F44V,F88T	C27648
9	A03	C58121	VK1_2A1_F44V,T107A	C27648
10	B03	C58122	VK1_2A1_T24A,F88T	C27648
11	C03	C58123	VK1_2A1_F44V,T017A,S108K	C27648
12	D03	C58124	VK1_2A1_T24A,F44V,F88T	C27648
13	A04	C58125	VK1_2A1_F44V,F88T,T107A	C27648
14	B04	C58126	VK1_2A1_T24A,F44V,F88T,T107A	C27648
15	C04	C58127	VK1_2A1_K14Q+T24A+G32S+F44V+F88T+T98A+T107A	C27648
16	D01	C58127	VK1_2A1_K14Q+T24A+G32S+F44V+F88T+T98A+T107A	C58130
17	A05	C58128	VK1_2A1_K14Q+R17G+T24A+G32S+F44V+G56S+D84N+F88T+T98A+T107A	C27648
18	B05	C58128	VK1_2A1_K14Q+R17G+T24A+G32S+F44V+G56S+D84N+F88T+T98A+T107A	C58130
19	C05	C58129	VK1_2A1_K14Q+R17G+T24A+G32S+F44V+G56S+D84N+F88T+T98A+T107A+S108K	C58130
20	pTT5			
21	anti DNP			

Fig. 20

Binding of Anti-huBCMA Abs (2A1 C107T, covariance 1-7) to H929

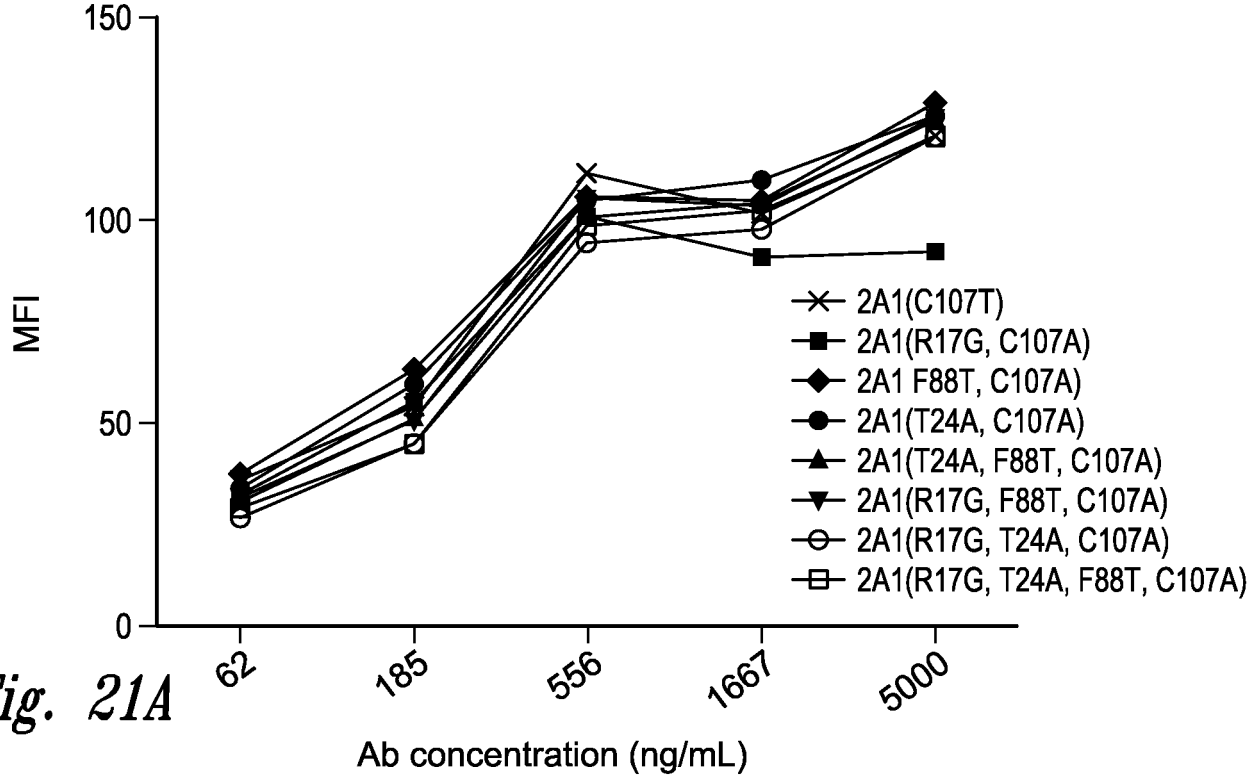


Fig. 21A

Binding of Anti-huBCMA Abs (2A1 C107T, covariance 1-7) to JD38

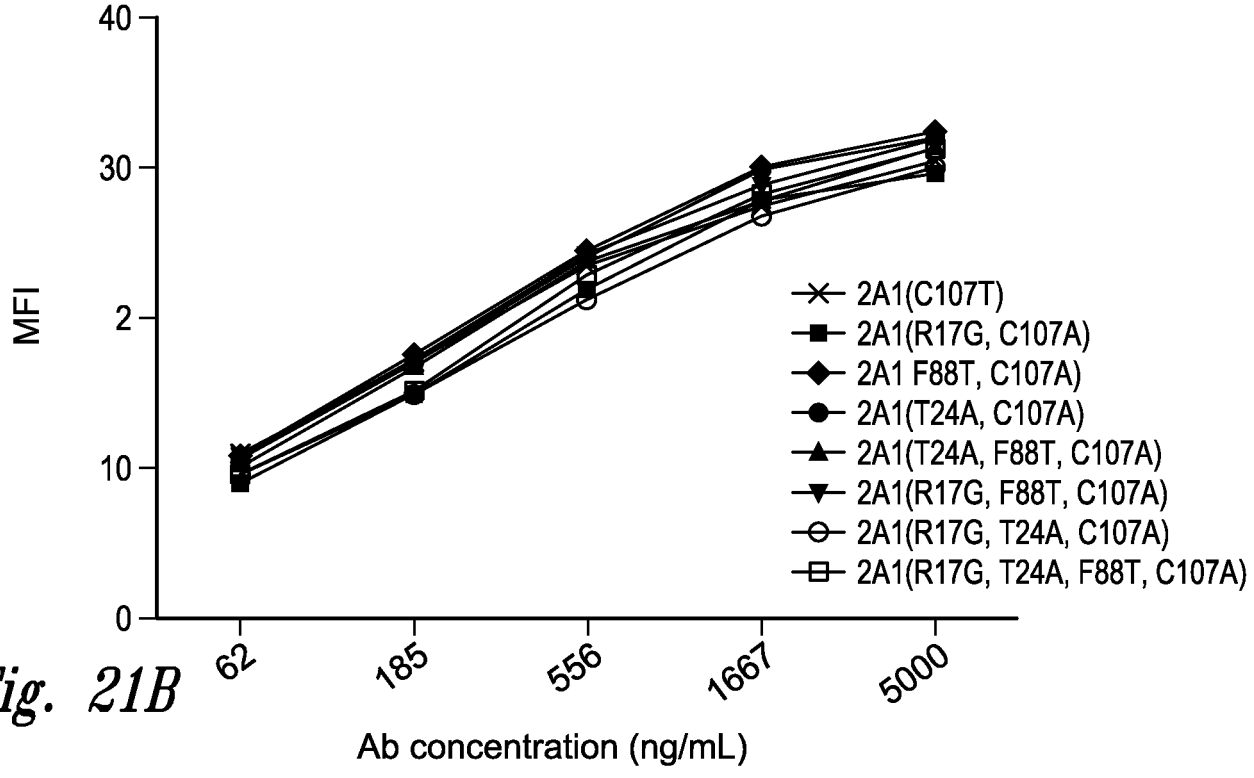


Fig. 21B

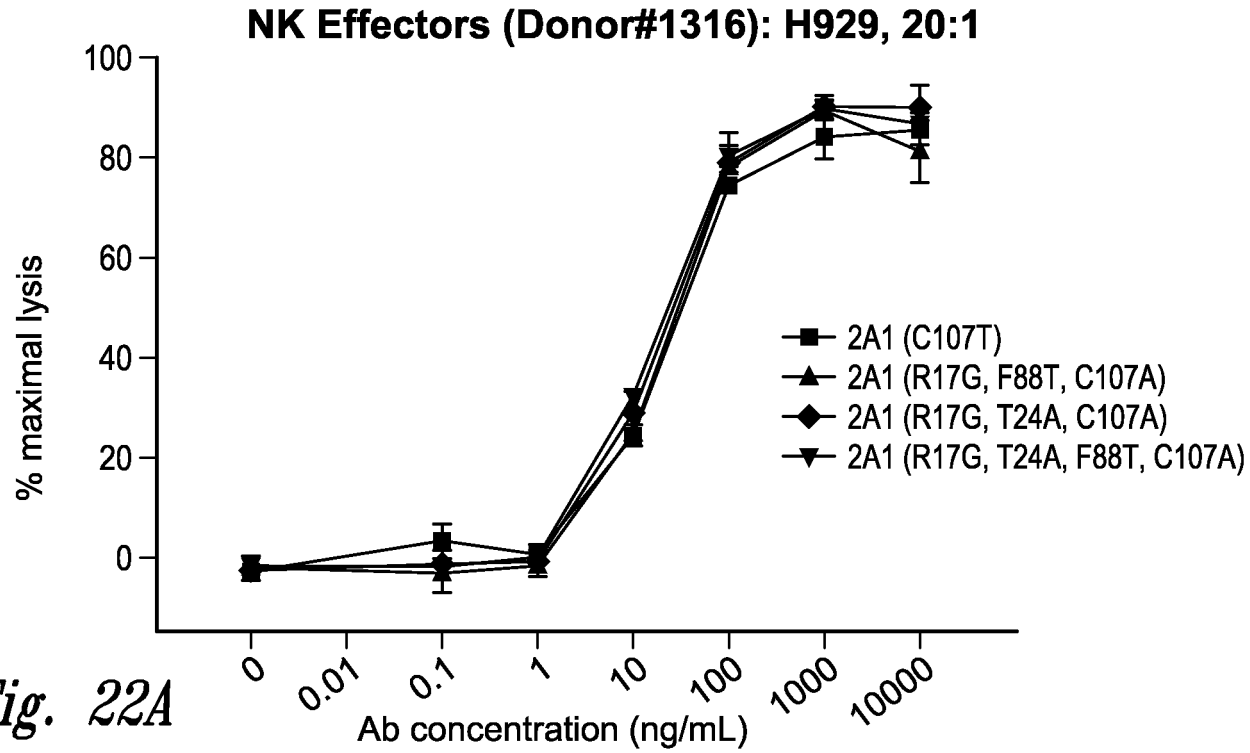


Fig. 22A

	2A1 (C107T)	2A1 (R17G, F88T, C107A)	2A1 (R17G, T24A, C107A)	2A1 (R17G, T24A, F88T, C107A)
EC50	20.73	16.49	17.65	14.74

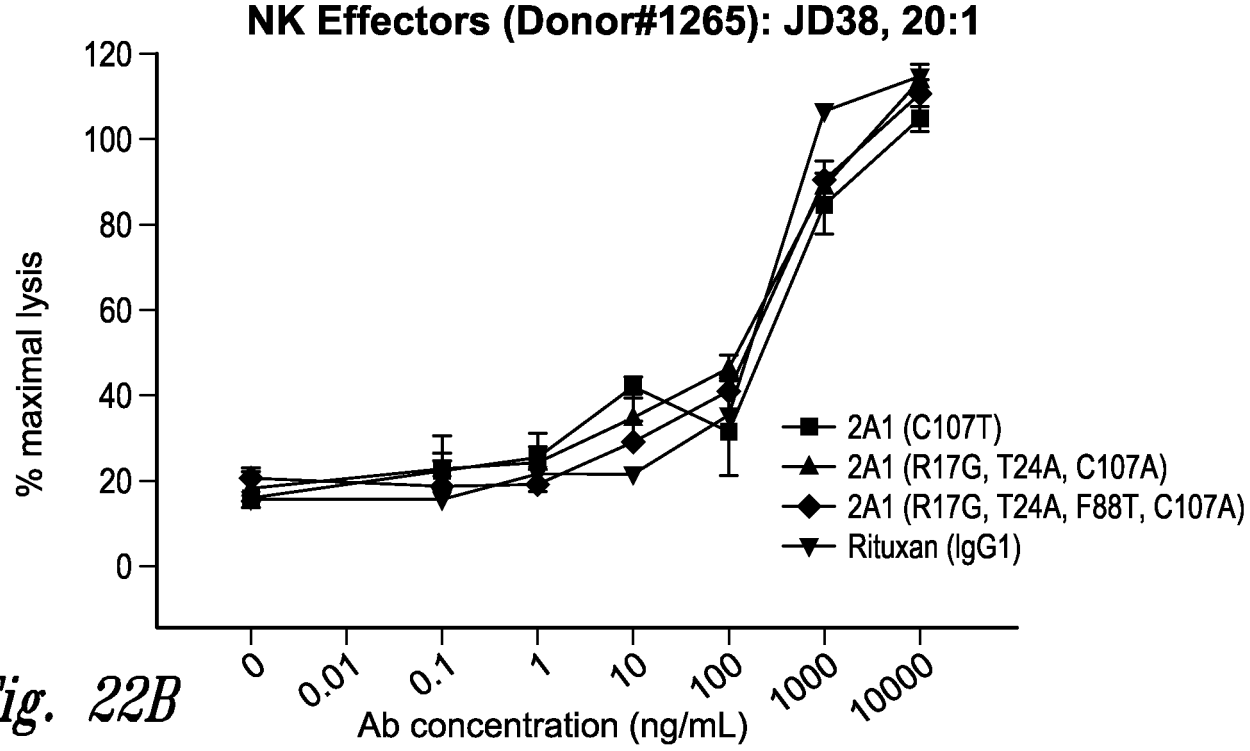


Fig. 22B

	2A1 (C107T)	2A1 (R17G, T24A, C107A)	2A1 (R17G, T24A, F88T, C107A)	Rituxan
EC50	516.1	524.9	341.14	248.5

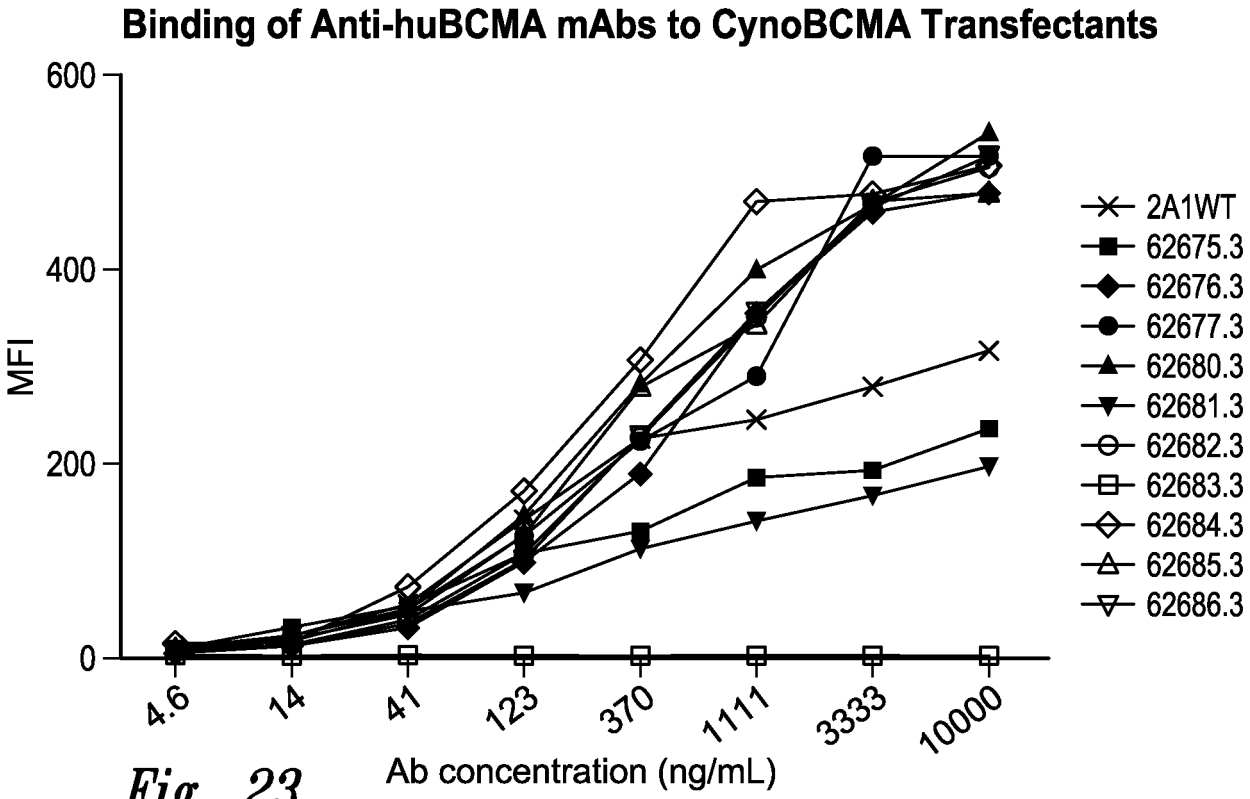

Binding of Anti-huBCMA mAbs to CynoBCMA Transfectants
MFI
600
400
200
0
4.6
14
41
123
370
1111
3333
10000
Ab concentration (ng/mL)
2A1WT
62675.3
62676.3
62677.3
62680.3
62681.3
62682.3
62683.3
62684.3
62685.3
62686.3

Fig. 23

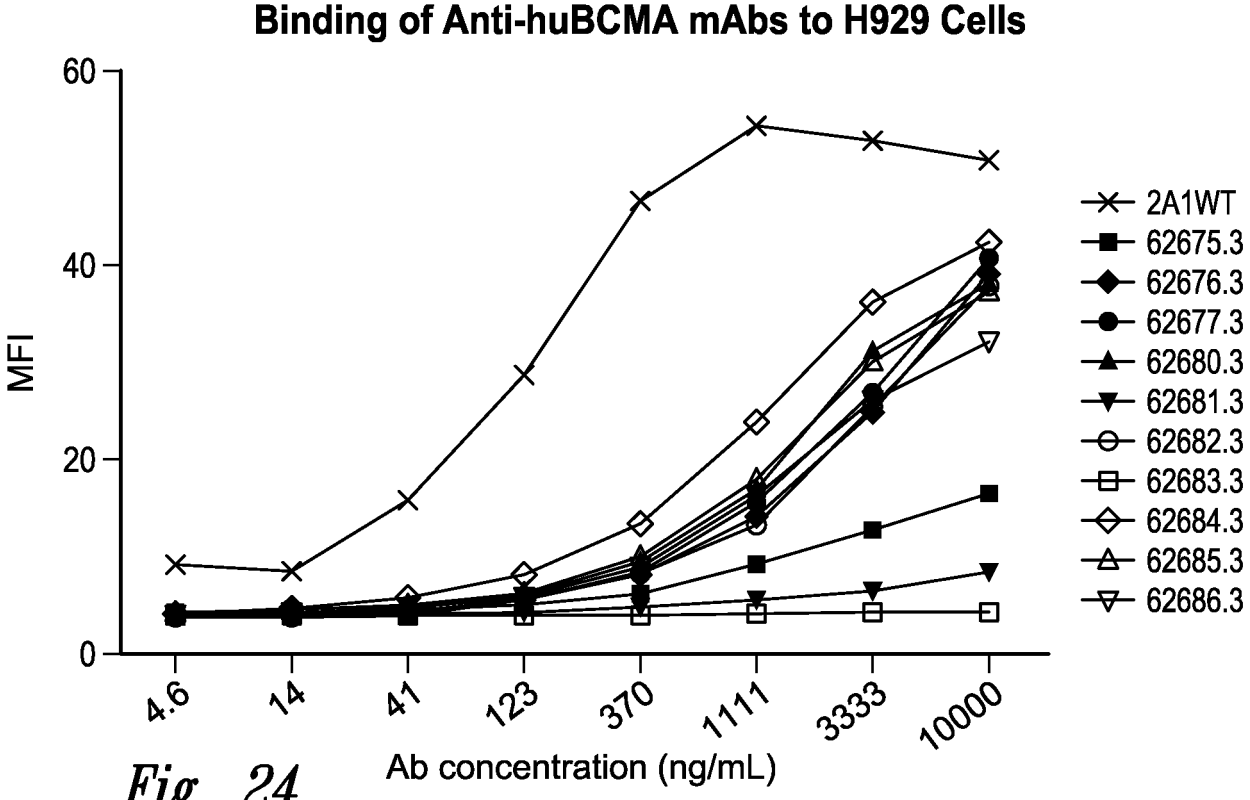

Binding of Anti-huBCMA mAbs to H929 Cells
MFI
60
40
20
0
4.6
14
41
123
370
1111
3333
10000
Ab concentration (ng/mL)
2A1WT
62675.3
62676.3
62677.3
62680.3
62681.3
62682.3
62683.3
62684.3
62685.3
62686.3

Fig. 24

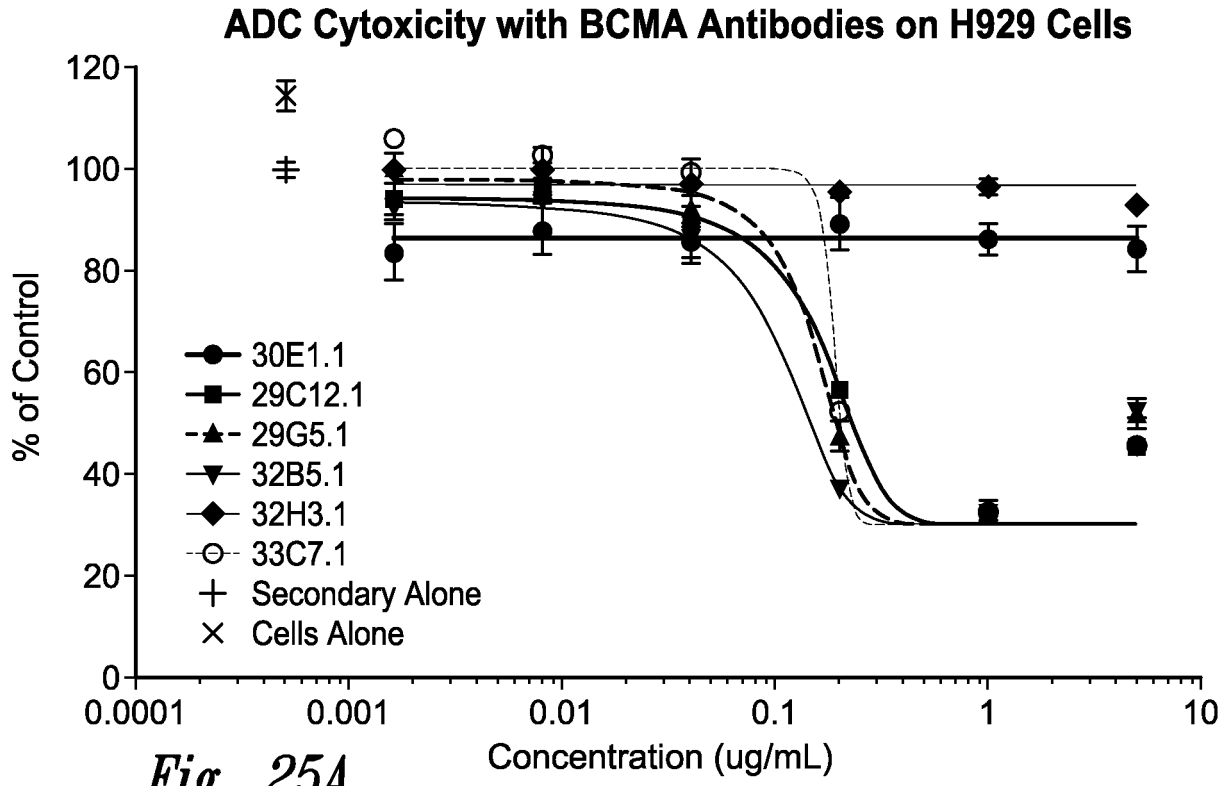


Fig. 25A

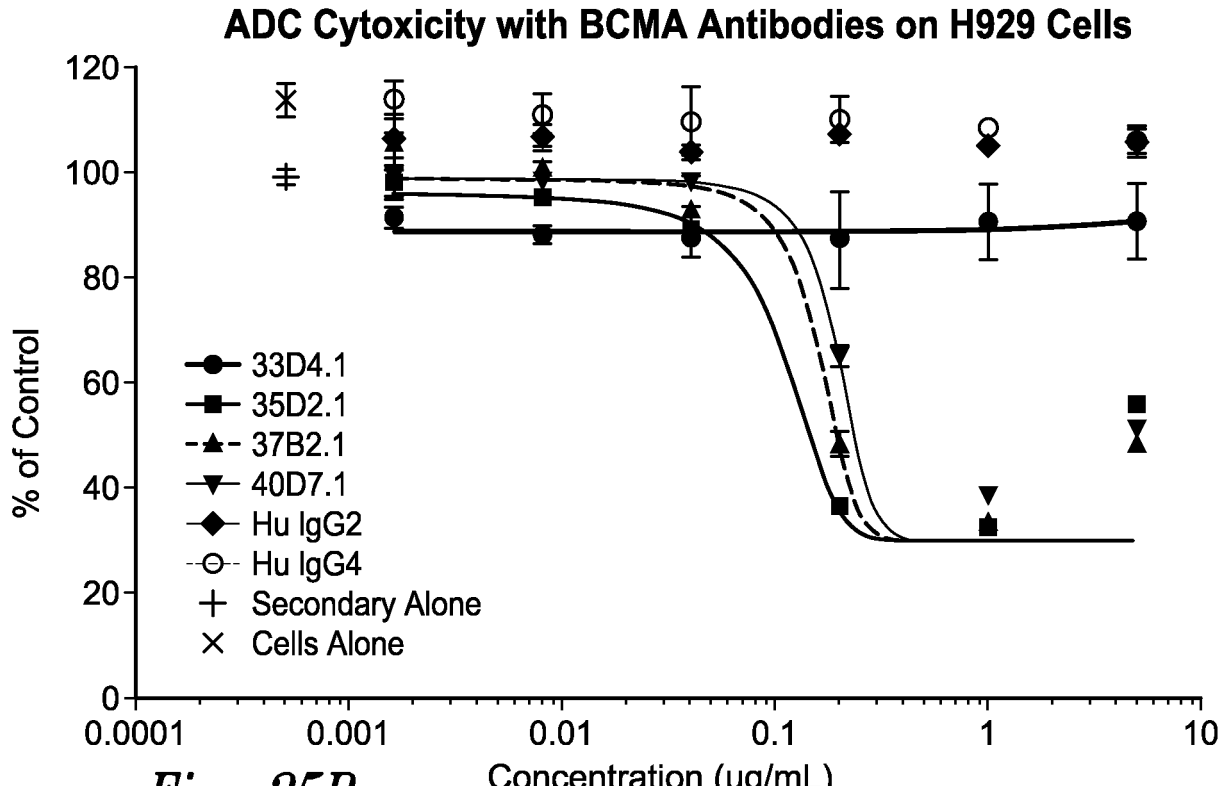


Fig. 25B

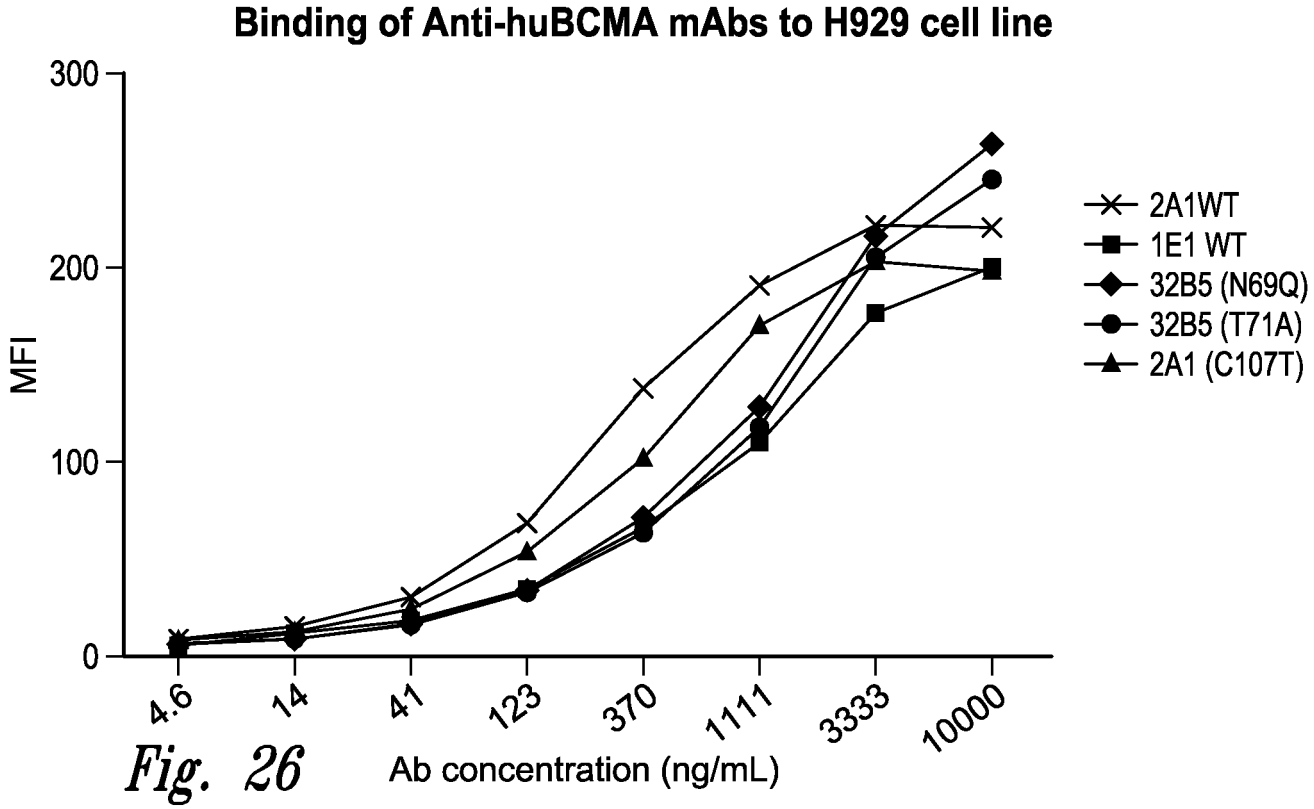

Binding of Anti-huBCMA mAbs to H929 cell line
MFI
300
200
100
0
4.6
14
41
123
370
1111
3333
10000
Ab concentration (ng/mL)
2A1WT
1E1 WT
32B5 (N69Q)
32B5 (T71A)
2A1 (C107T)

Fig. 26

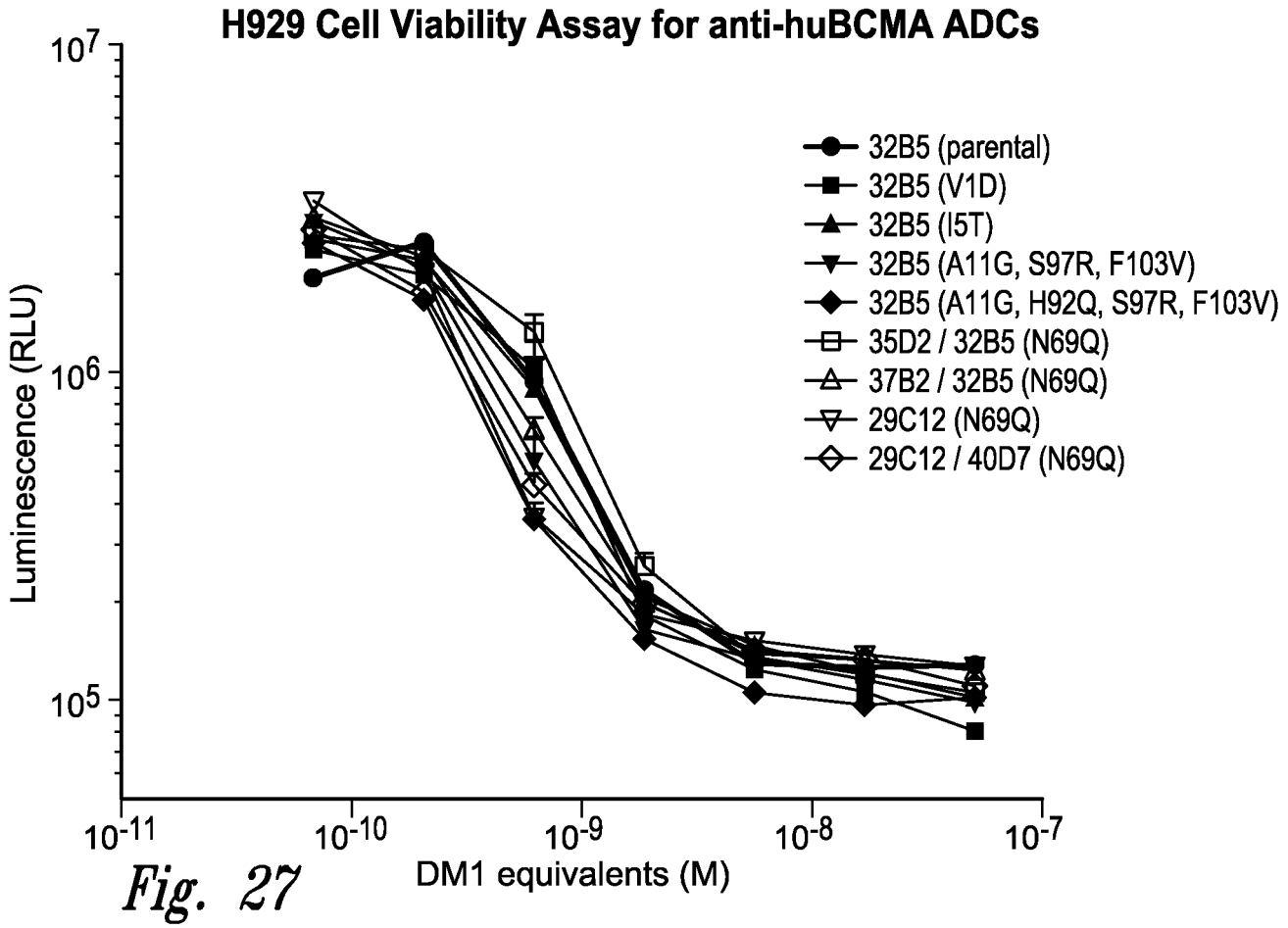

H929 Cell Viability Assay for anti-huBCMA ADCs
Luminescence (RLU)
10^7
10^6
10^5
10^-11
10^-10
10^-9
10^-8
10^-7
DM1 equivalents (M)
32B5 (parental)
32B5 (V1D)
32B5 (I5T)
32B5 (A11G, S97R, F103V)
32B5 (A11G, H92Q, S97R, F103V)
35D2 / 32B5 (N69Q)
37B2 / 32B5 (N69Q)
29C12 (N69Q)
29C12 / 40D7 (N69Q)

Fig. 27

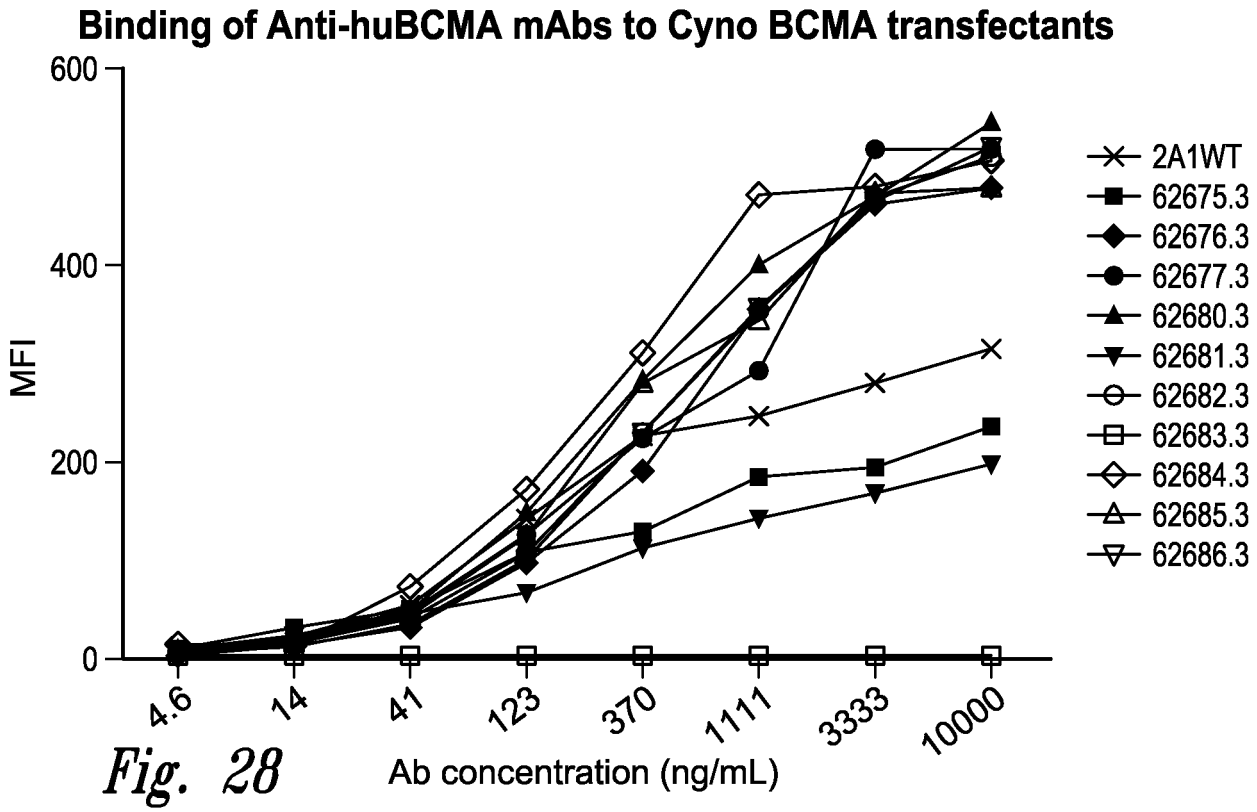

Binding of Anti-huBCMA mAbs to Cyno BCMA transfectants
MFI
600
400
200
0
4.6
14
41
123
370
1111
3333
10000
Ab concentration (ng/mL)
2A1WT
62675.3
62676.3
62677.3
62680.3
62681.3
62682.3
62683.3
62684.3
62685.3
62686.3

Fig. 28

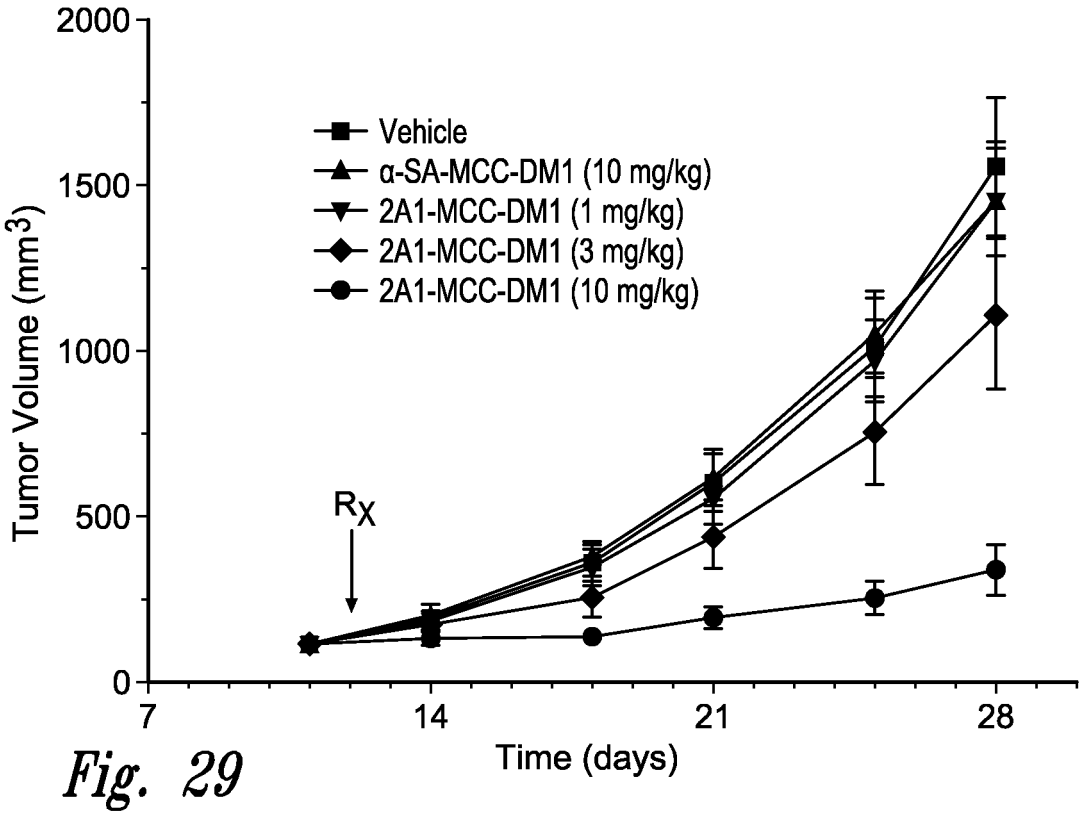

Tumor Volume (mm3)
2000
1500
1000
500
0
7
14
21
28
Time (days)
Rx
Vehicle
α-SA-MCC-DM1 (10 mg/kg)
2A1-MCC-DM1 (1 mg/kg)
2A1-MCC-DM1 (3 mg/kg)
2A1-MCC-DM1 (10 mg/kg)

Fig. 29

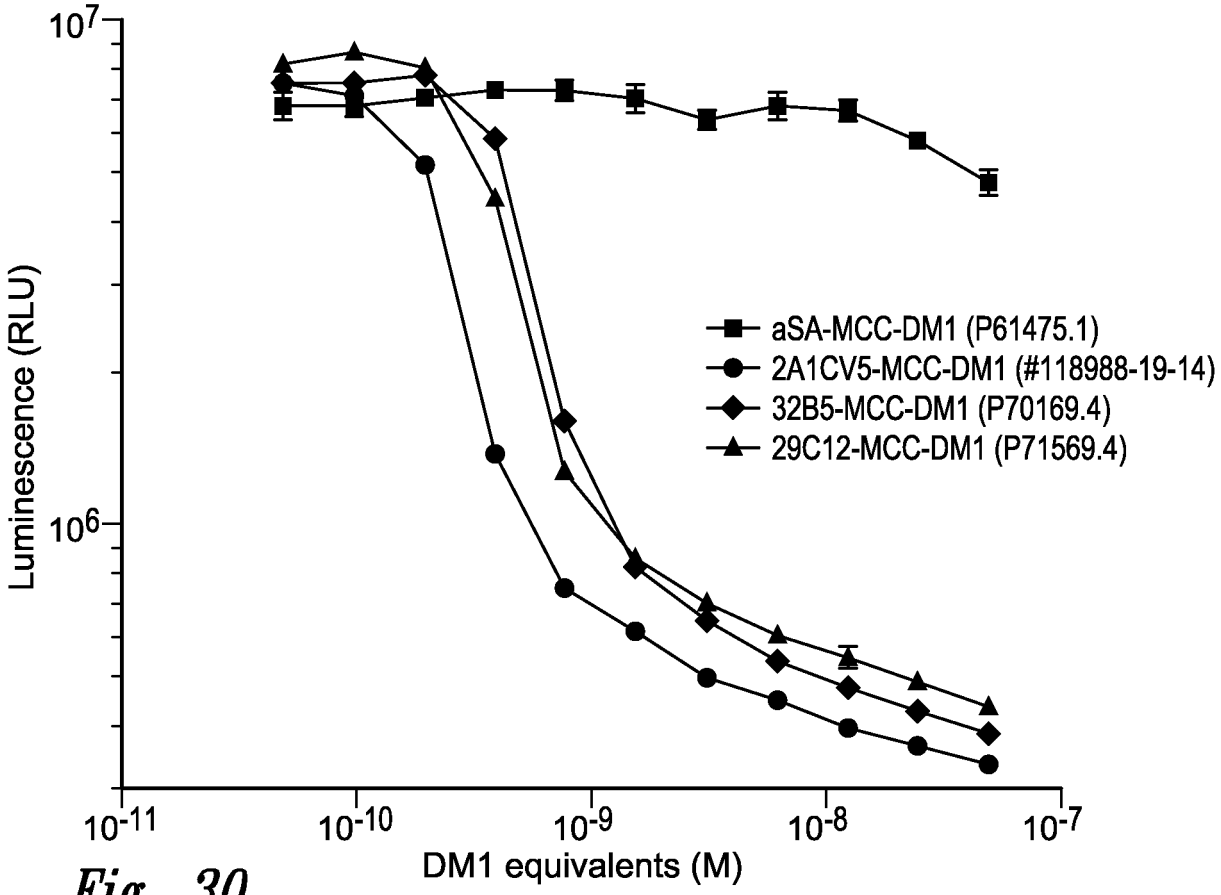


Fig. 30

Human Anti-human BCMA-2A1CV5-MCC-DM1 Mediated Receptor Internalization in H929 Cells

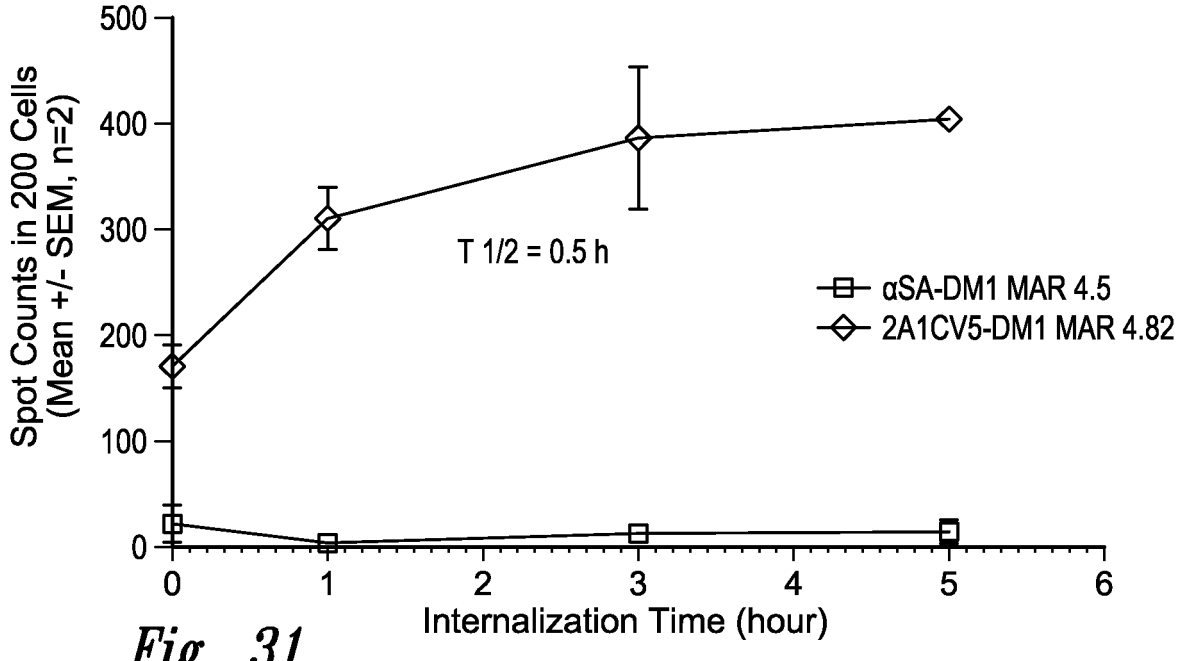


Fig. 31

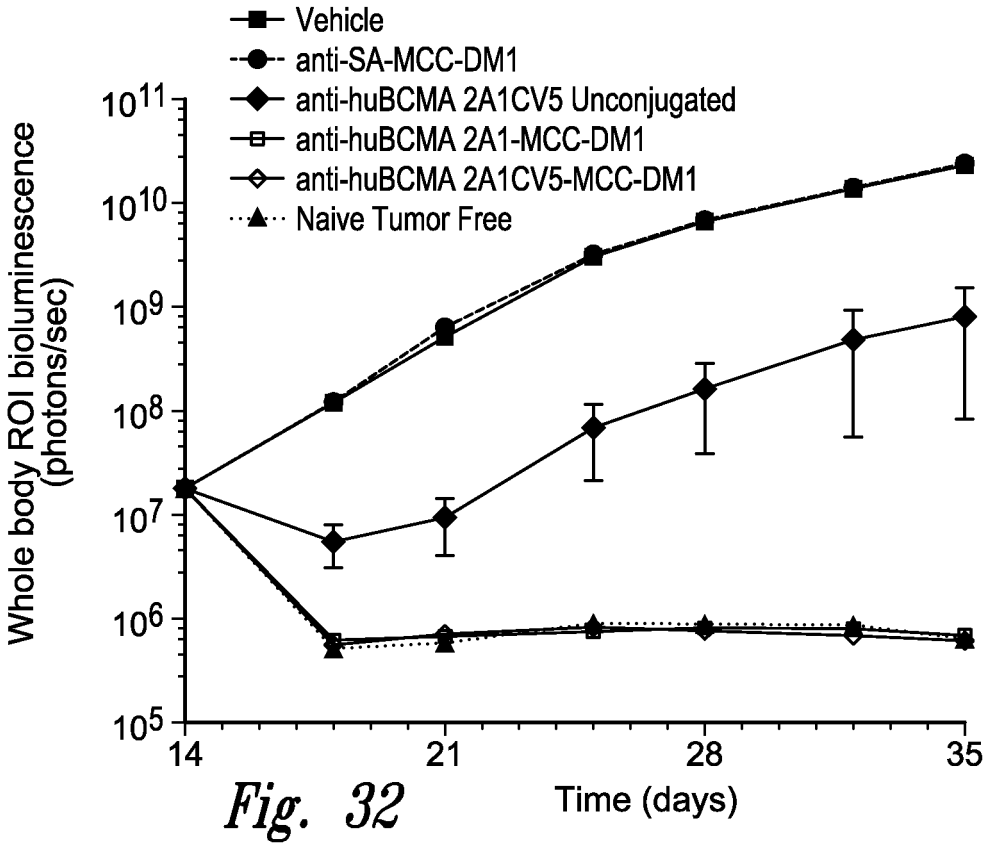

Vehicle
anti-SA-MCC-DM1
anti-huBCMA 2A1CV5 Unconjugated
anti-huBCMA 2A1-MCC-DM1
anti-huBCMA 2A1CV5-MCC-DM1
Naive Tumor Free
Whole body ROI bioluminescence (photons/sec)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
14
21
28
35
Time (days)

Fig. 32

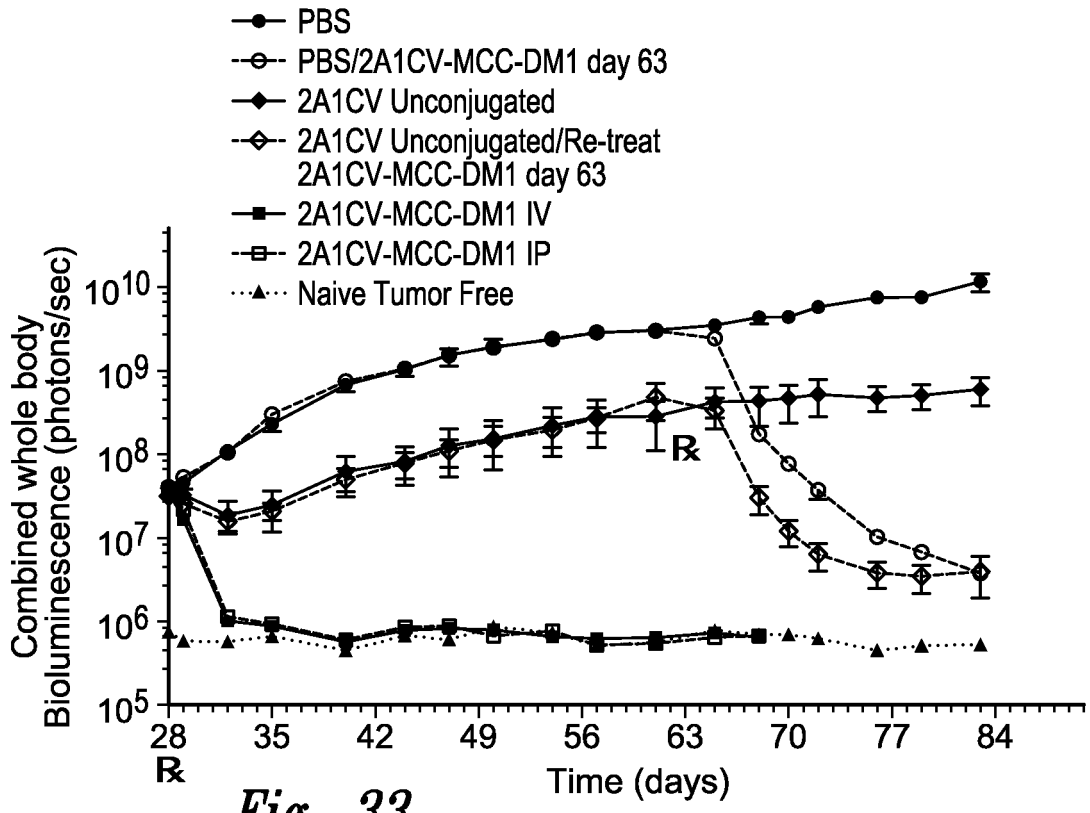

PBS
PBS/2A1CV-MCC-DM1 day 63
2A1CV Unconjugated
2A1CV Unconjugated/Re-treat
2A1CV-MCC-DM1 day 63
2A1CV-MCC-DM1 IV
2A1CV-MCC-DM1 IP
Naive Tumor Free
Combined whole body Bioluminescence (photons/sec)
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
Rx
Rx
28
35
42
49
56
63
70
77
84
Time (days)

Fig. 33

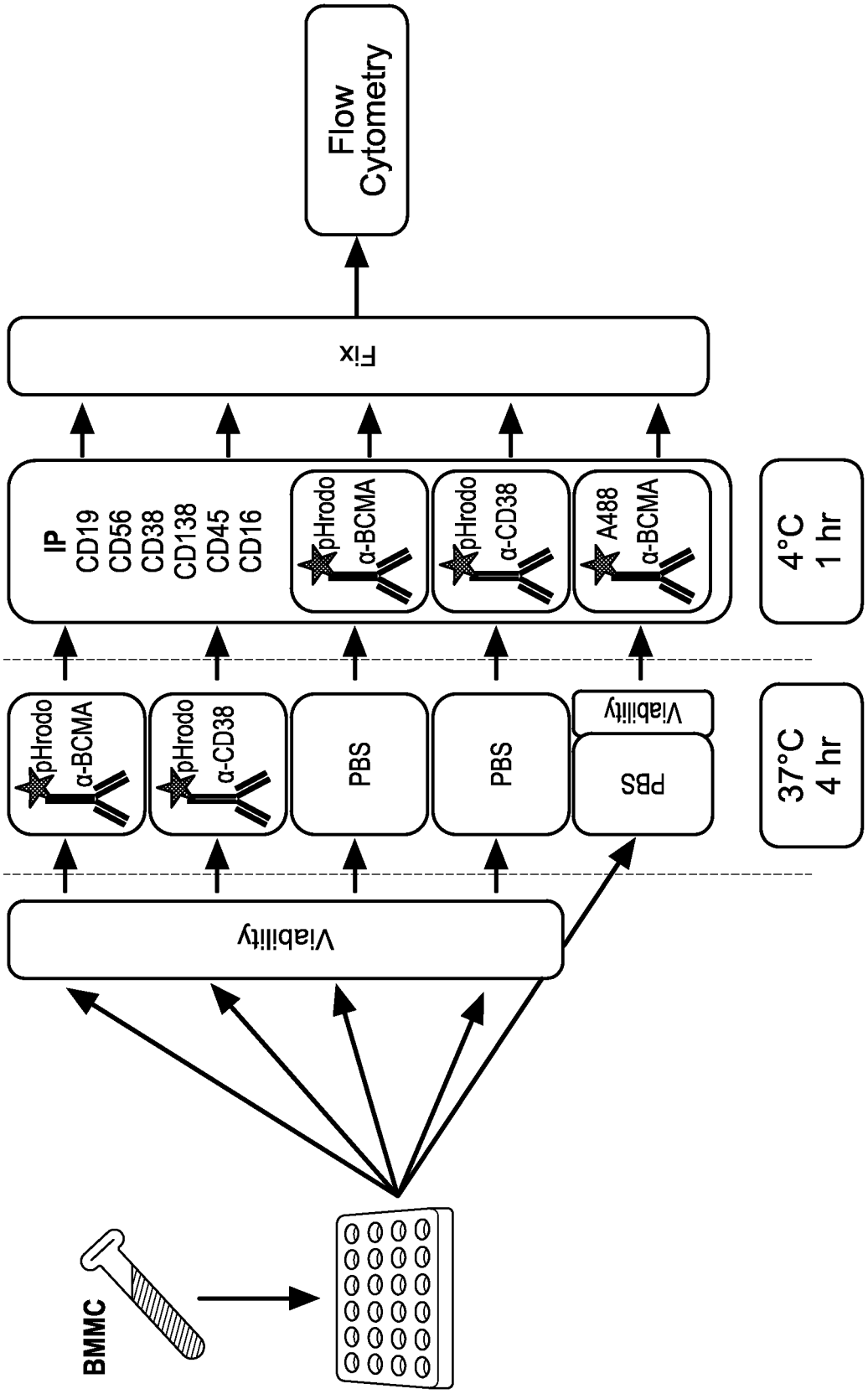

BMMC
Viability
pHrodo α-BCMA
pHrodo α-CD38
PBS
PBS
Viability
PBS
37°C 4 hr
IP CD19 CD56 CD38 CD138 CD45 CD16
pHrodo α-BCMA
pHrodo α-CD38
A488 α-BCMA
4°C 1 hr
Fix
Flow Cytometry

Fig. 34A

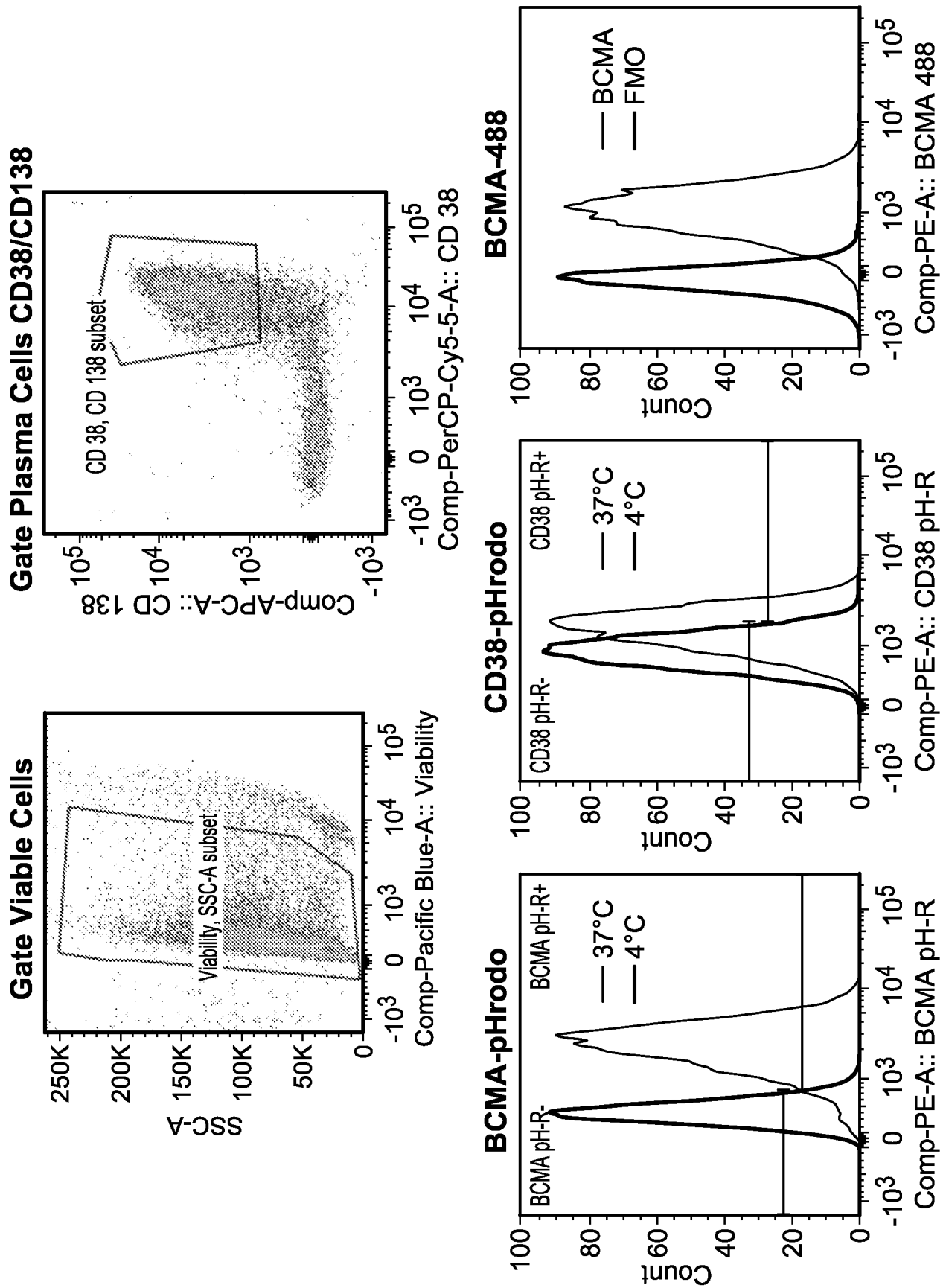

Gate Viable Cells
SSC-A
Viability, SSC-A subset
Comp-Pacific Blue-A:: Viability
Gate Plasma Cells CD38/CD138
Comp-APC-A:: CD 138
CD 38, CD 138 subset
Comp-PerCP-Cy5-5-A:: CD 38
BCMA-pHrodo
BCMA pH-R-
BCMA pH-R+
37°C
4°C
Count
Comp-PE-A:: BCMA pH-R
CD38-pHrodo
CD38 pH-R-
CD38 pH-R+
Comp-PE-A:: CD38 pH-R
BCMA-488
BCMA
FMO
Comp-PE-A:: BCMA 488

Fig. 34B

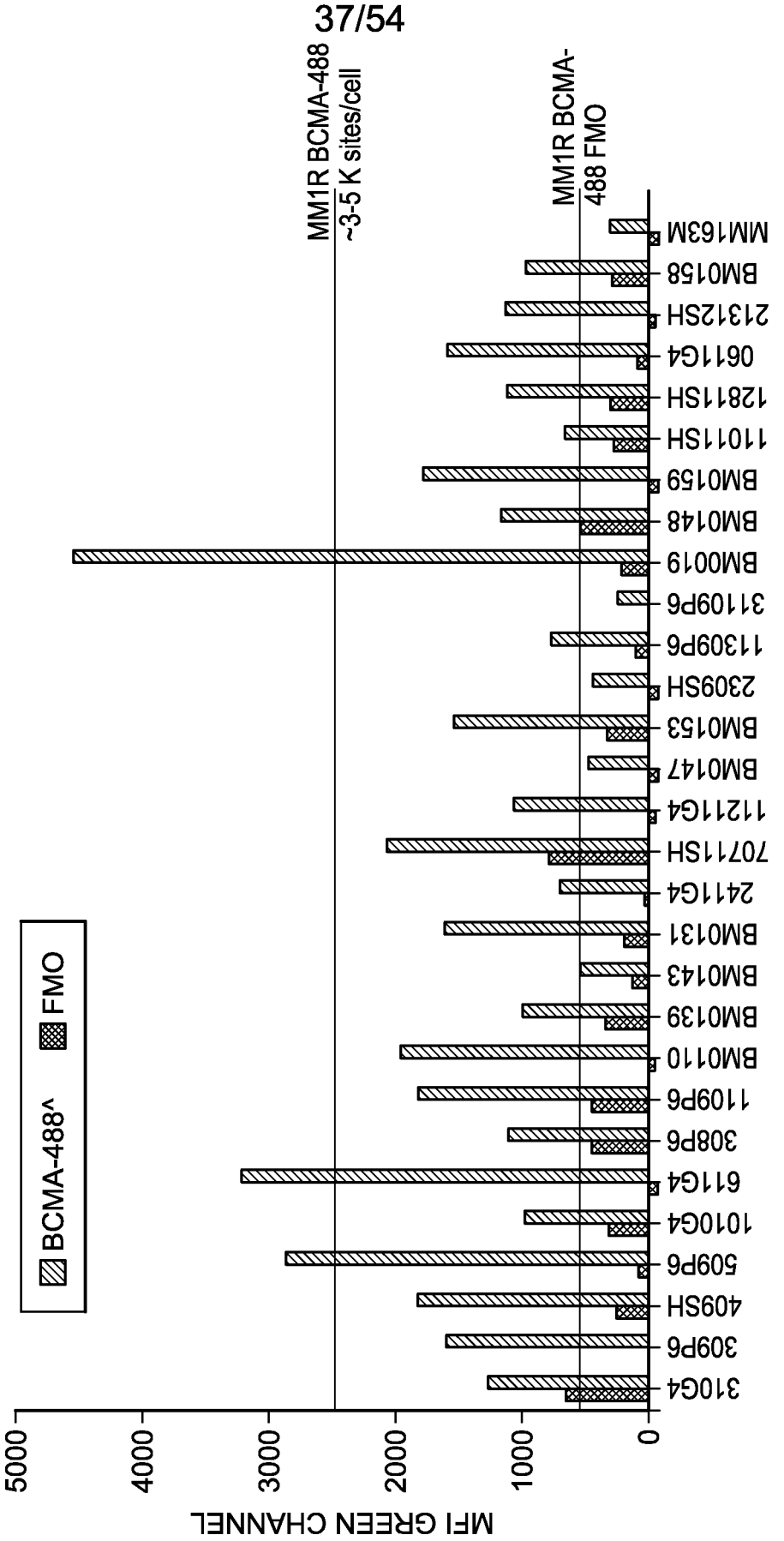

MM1R BCMA-488
~3-5 K sites/cell
MM1R BCMA-488 FMO
BCMA-488^
FMO
MFI GREEN CHANNEL
0
1000
2000
3000
4000
5000
MM163M
BM0158
21312SH
0611G4
12811SH
11011SH
BM0159
BM0148
BM0019
3110P6
1130P6
230SH
BM0153
BM0147
1121 1G4
7071 1SH
2411G4
BM0131
BM0143
BM0139
BM0110
1109P6
308P6
611G4
1010G4
509P6
409SH
309P6
310G4

Fig. 35

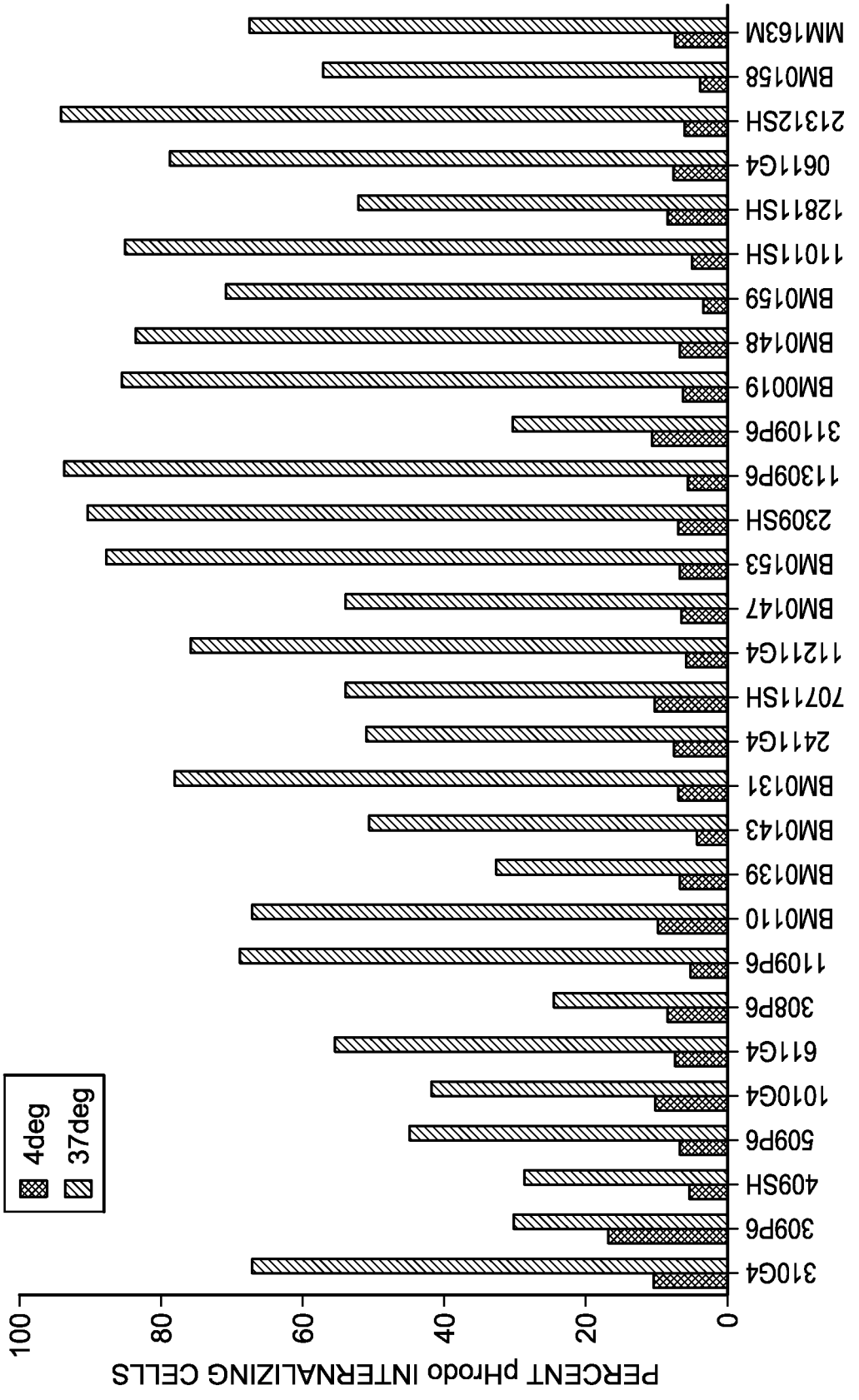

PERCENT pHrodo INTERNALIZING CELLS
0
20
40
60
80
100
4deg
37deg
310G4
309P6
409SH
509P6
1010G4
611G4
308P6
1109P6
BM0110
BM0139
BM0143
BM0131
2411G4
707115H
1211G4
BM0147
BM0153
2309SH
1130 9P6
3110 9P6
BM0019
BM0148
BM0159
1101 1SH
1281 1SH
0611G4
213 12SH
BM0158
MM163M

Fig. 36

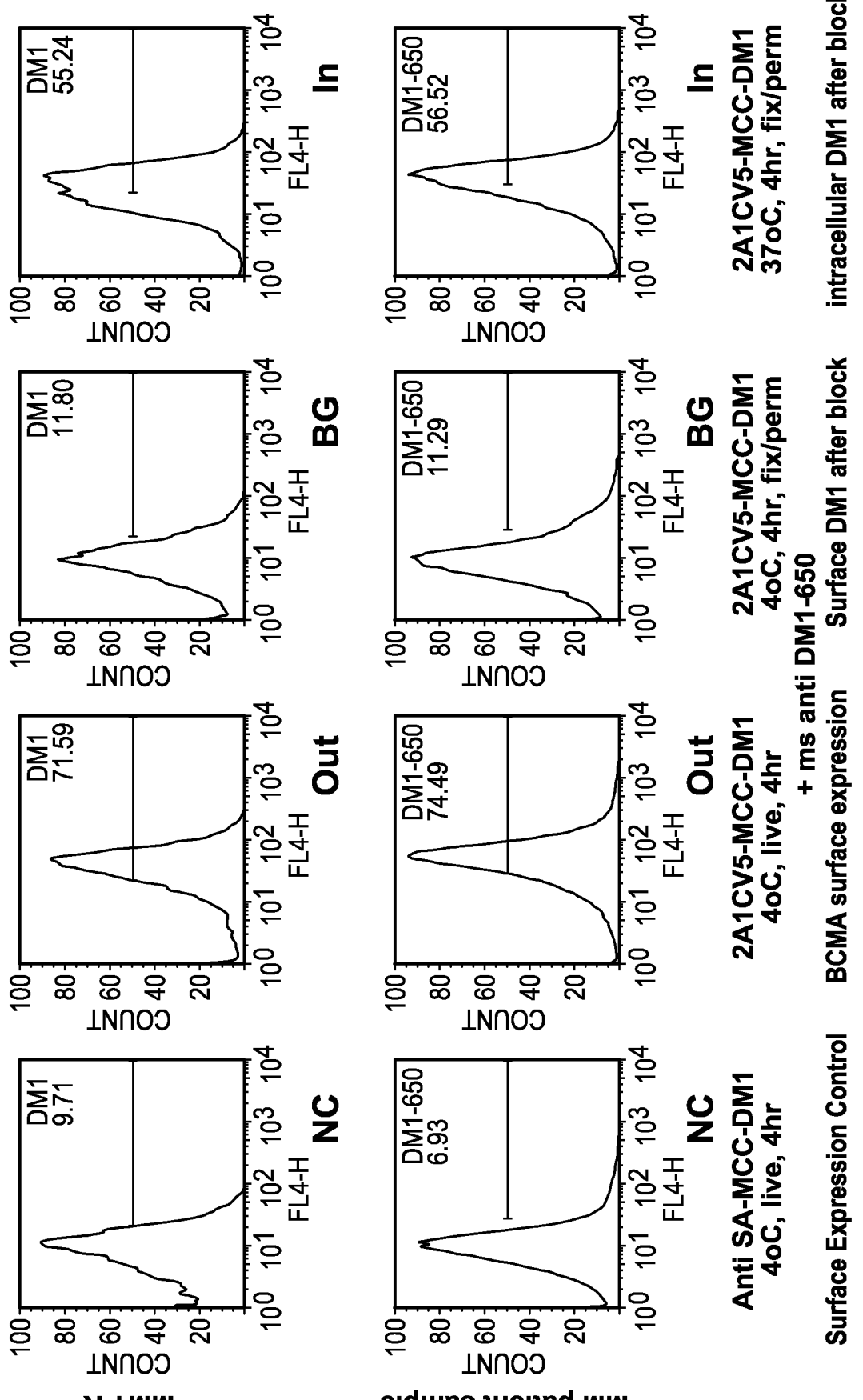

MM1-R
MM patient sample
NC
Out
BG
In
DM1 9.71
DM1 71.59
DM1 11.80
DM1 55.24
DM1-650 6.93
DM1-650 74.49
DM1-650 11.29
DM1-650 56.52
COUNT
FL4-H
Anti SA-MCC-DM1 4oC, live, 4hr
2A1CV5-MCC-DM1 4oC, live, 4hr
2A1CV5-MCC-DM1 4oC, 4hr, fix/perm
2A1CV5-MCC-DM1 37oC, 4hr, fix/perm
+ ms anti DM1-650
Surface Expression Control
BCMA surface expression
Surface DM1 after block
intracellular DM1 after block

Fig. 37

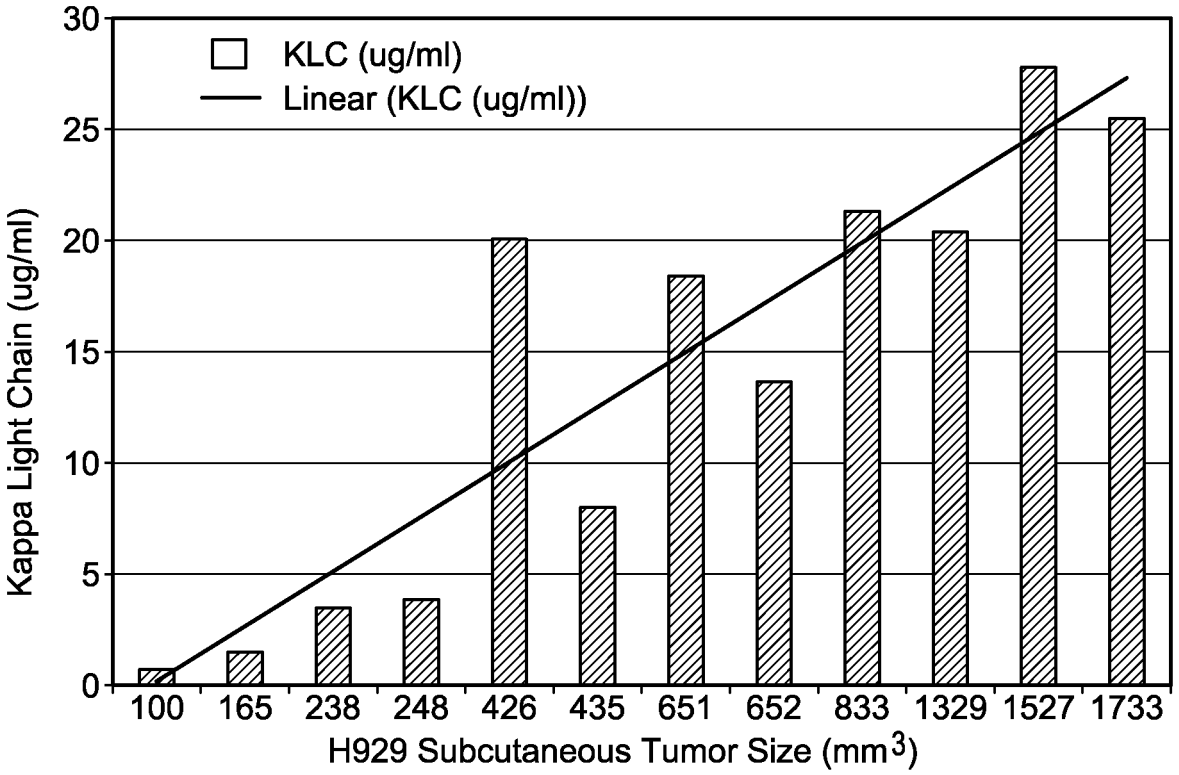

KLC (ug/ml)
Linear (KLC (ug/ml))
Kappa Light Chain (ug/ml)
0
5
10
15
20
25
30
100
165
238
248
426
435
651
652
833
1329
1527
1733
H929 Subcutaneous Tumor Size (mm3)

Fig. 38

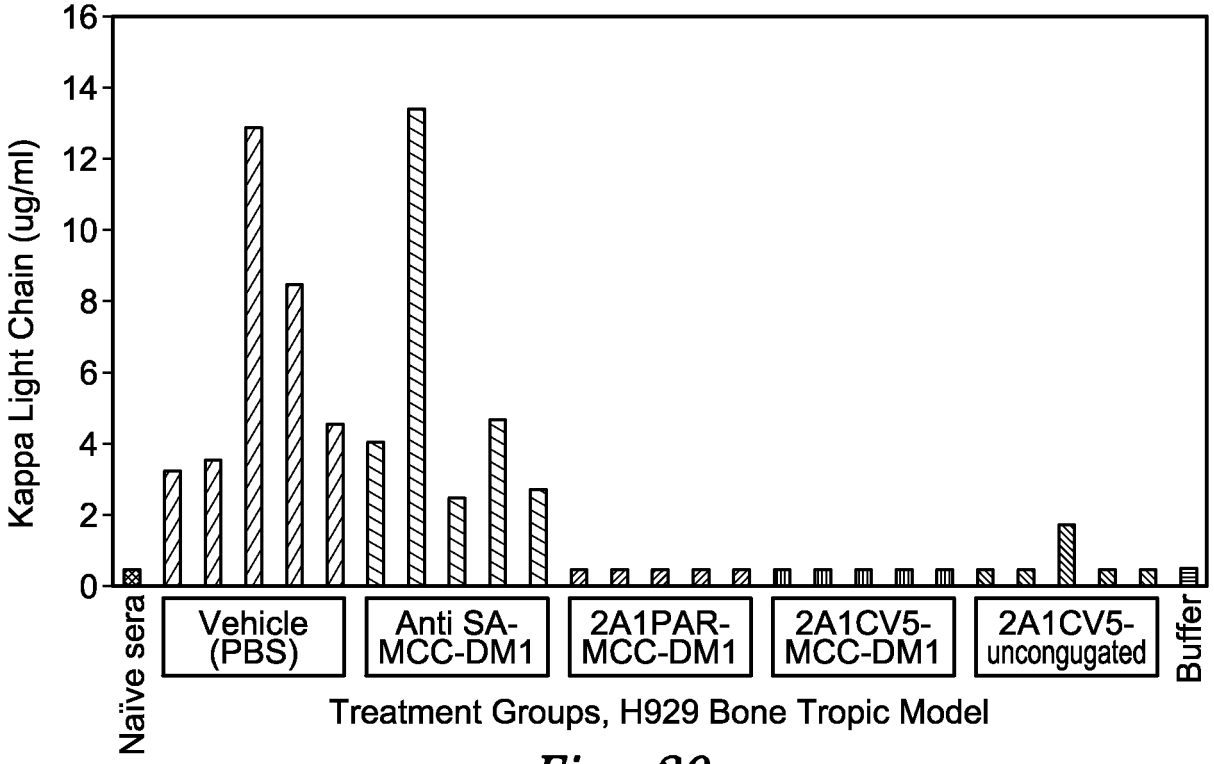

Kappa Light Chain (ug/ml)
0
2
4
6
8
10
12
14
16
Naïve sera
Vehicle (PBS)
Anti SA-MCC-DM1
2A1PAR-MCC-DM1
2A1CV5-MCC-DM1
2A1CV5-uncongugated
Buffer
Treatment Groups, H929 Bone Tropic Model

Fig. 39

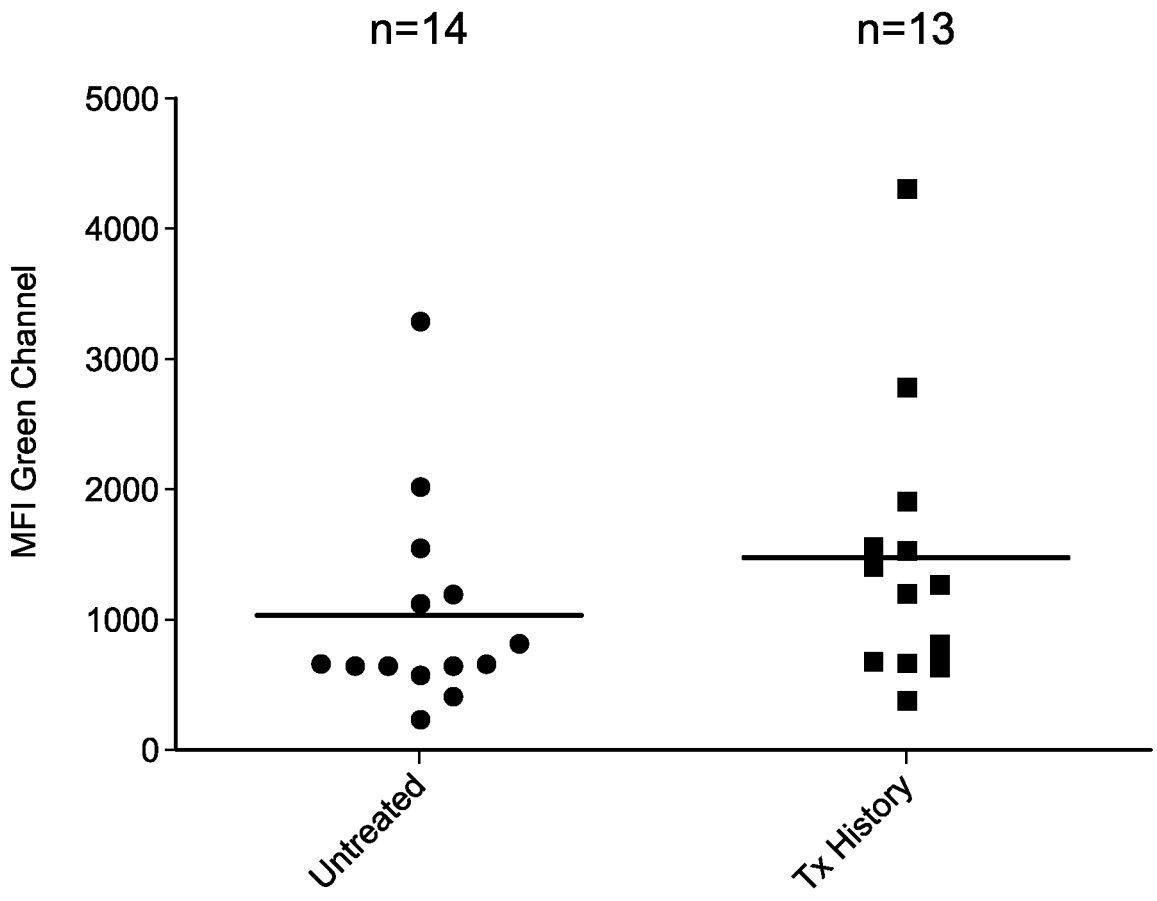

n=14
n=13
5000
4000
3000
2000
1000
0
MFI Green Channel
Untreated
Tx History

Fig. 40

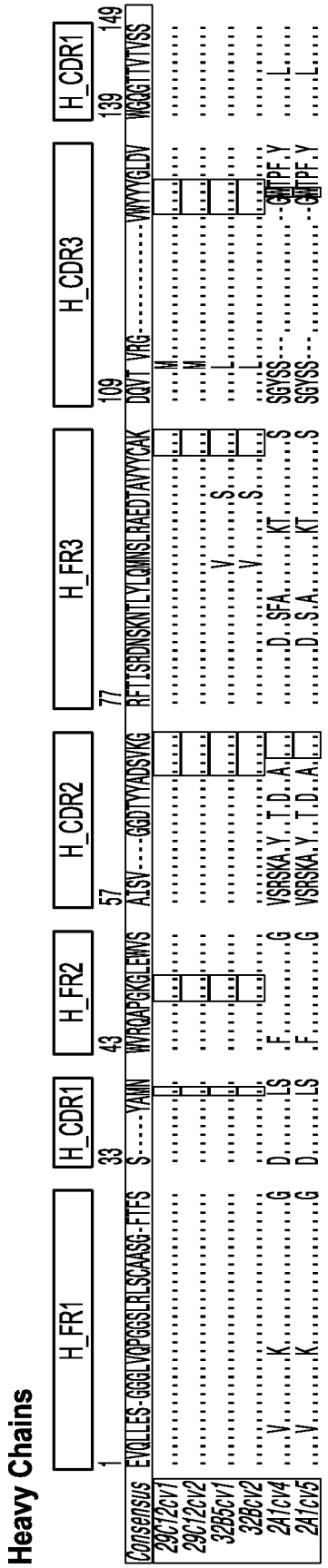


Fig. 41A

Fig. 41B

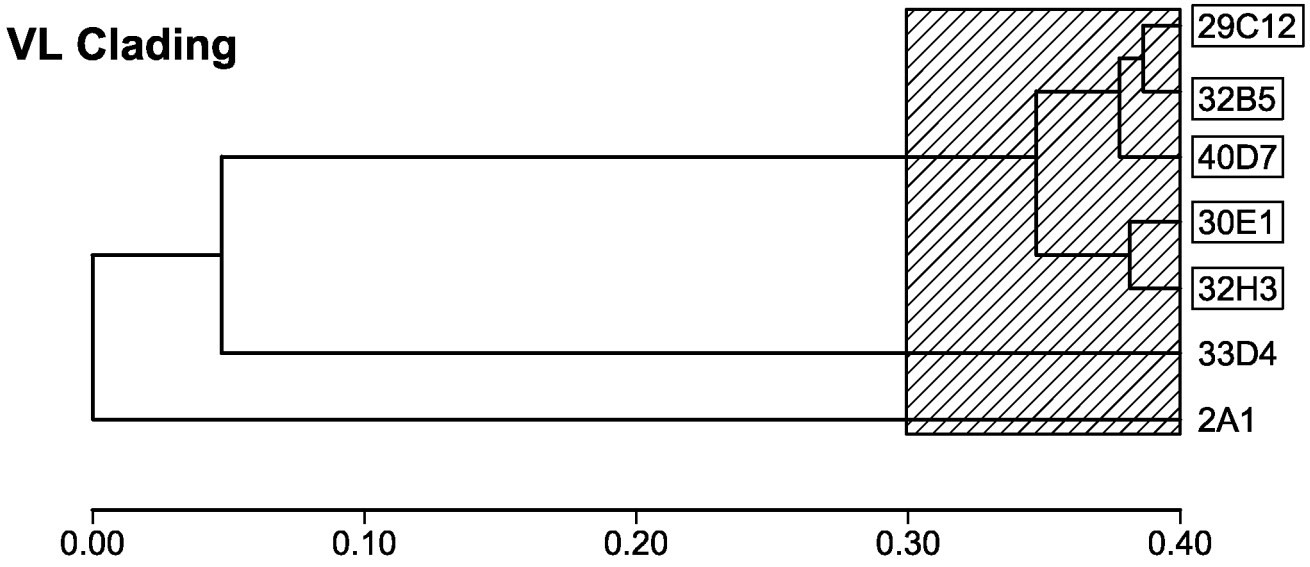


Fig. 42A

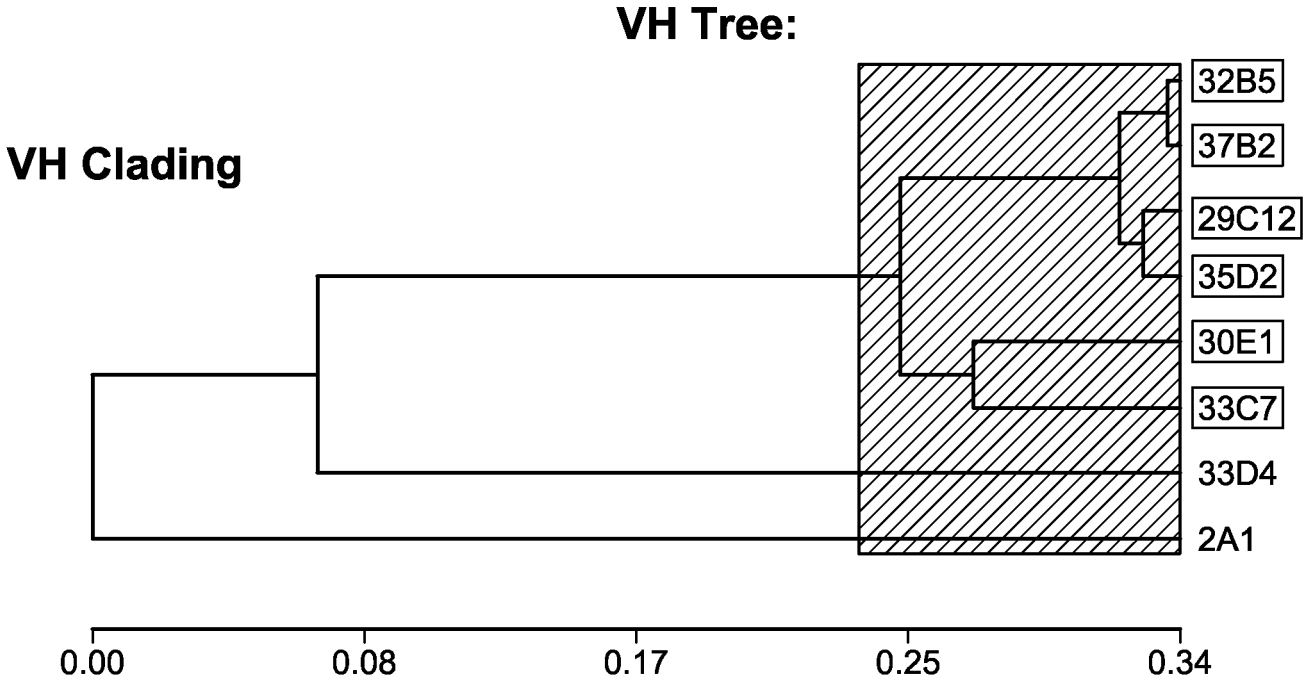


Fig. 42B

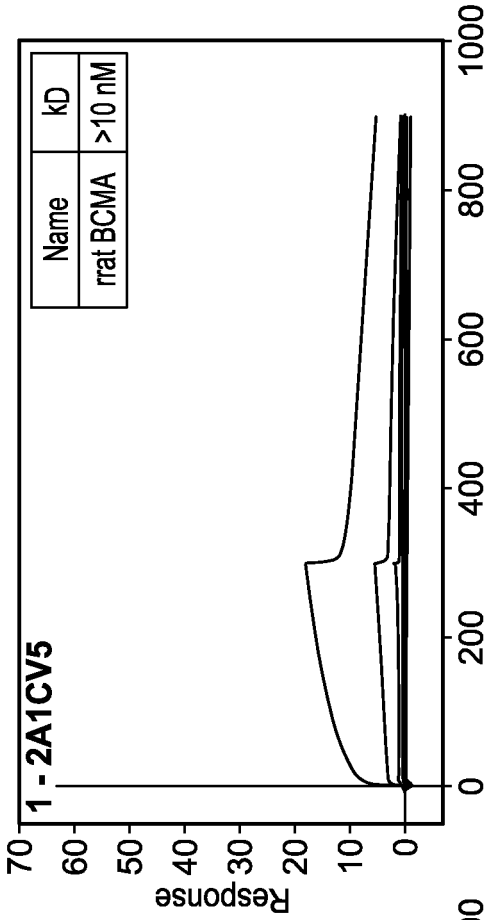


Fig. 43A

Fig. 43B

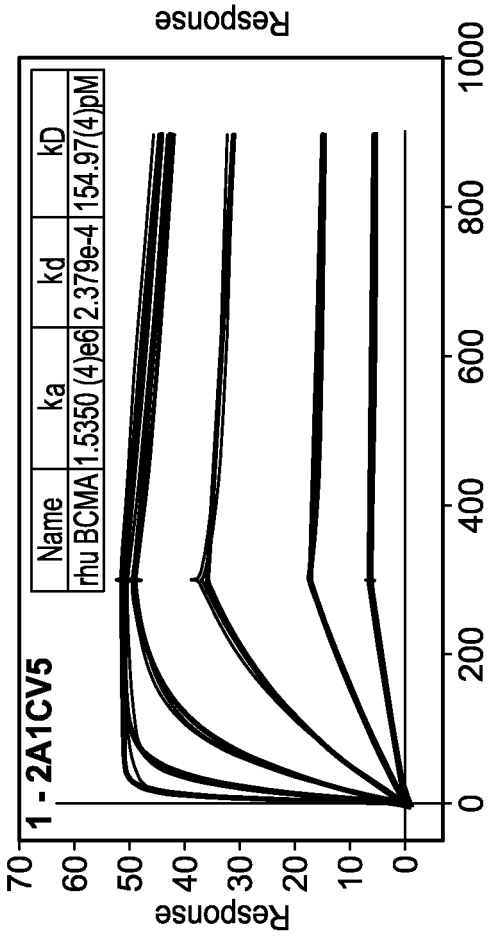


Fig. 43C

Fig. 43D

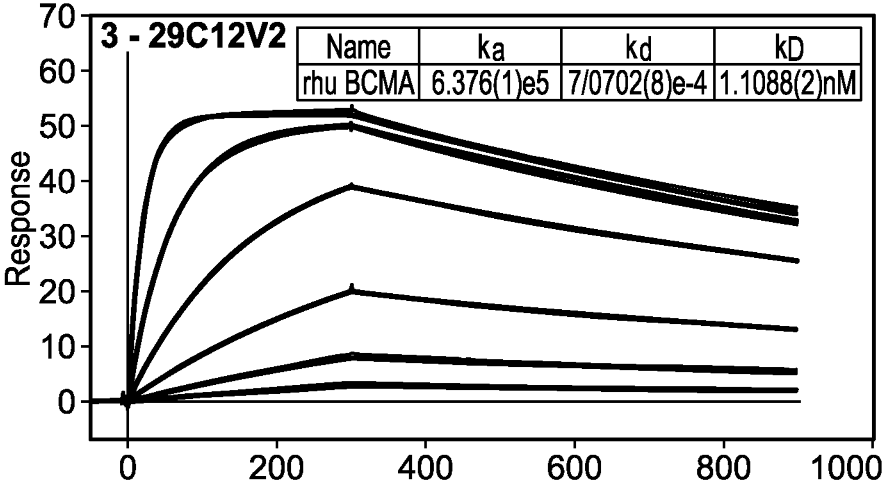


Fig. 43E

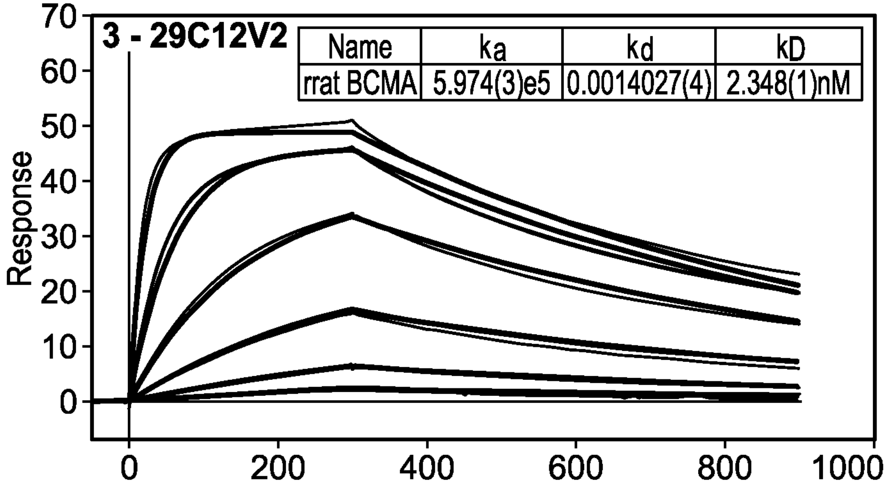


Fig. 43F

```
                  1                                                      50
huBCMA_2_51       LQMAGQCSQN EYFDSLLHAC IPCQLRCSSN TPPLTCQRYC NASVTNSVKG ~~~
CynoBCMA_2_51     LQM RQ SQN          D K  Q    S. T  LT  R   NA M N  K  M~~
ratBCMA_2_49      ~~~ QR FHS          A K  R    N. .  AP  P   DP M S  R  TYT
                     *  *   ******** *  ** ****    **  ** **    *  * ** *
```

Fig. 44

rhuBCMA (6xHis::Sumo::huBCMA(aa 2-51)::GGS::G3::Avi) fusion protein:

MGSSHHHHHHGSGLVPRGSAS MSDSEVNQEAKPEVKP ETHINLKVSDGSSEIFFKIK KTTPLRRLMEAFAKRQGKEM
DSLRFLYDGIRIQADQTPED LDMEDNDIIEAHREQIGG **AGQCSQNEYFDSLLHACIPCQL**
RCSSNTPPLTCQRYCNASVT NSVKGTNAGGGSGGGLNDIF EAQKIEWHE (SEQ ID NO:350)

GSSHHHHHHGSGLVPRGSAS MSDSEVNQEAKPEVKPEVKP ETHINLKVSDGSSEIFFKIK KTTPLRRLMEAFAKRQGKEM
DSLRFLYDGIRIQADQTPED LDMEDNDIIEAHREQIGG **AGQCSQNEYFDSLLHACIPCQL**
RCSSNTPPLTCQRYC (SEQ ID NO:354)

GSSHHHHHHGSGLVPRGSAS MSDSEVNQEAKPEVKP ETHINLKVSDGSSEIFFKIK KTTPLRRLMEAFAKRQGKEM
DSLRFLYDGIRIQADQTPED LDMEDNDIIEAHREQIGG **AGQCSQNEYFDSLLHACIPCQL R** (SEQ ID NO:355)

GSSHHHHHHGSGLVPRGSAS MSDSEVNQEAKPEVKPEVKP ETHINLKVSDGSSEIFFKIK KTTPLRRLMEAFAKRQGKEM
DSLRFLYDGIRIQADQTPED LDMEDNDIIEAHREQIGG **AGQCSQNEYFDSLLHA** (SEQ ID NO:356)

Fig. 45

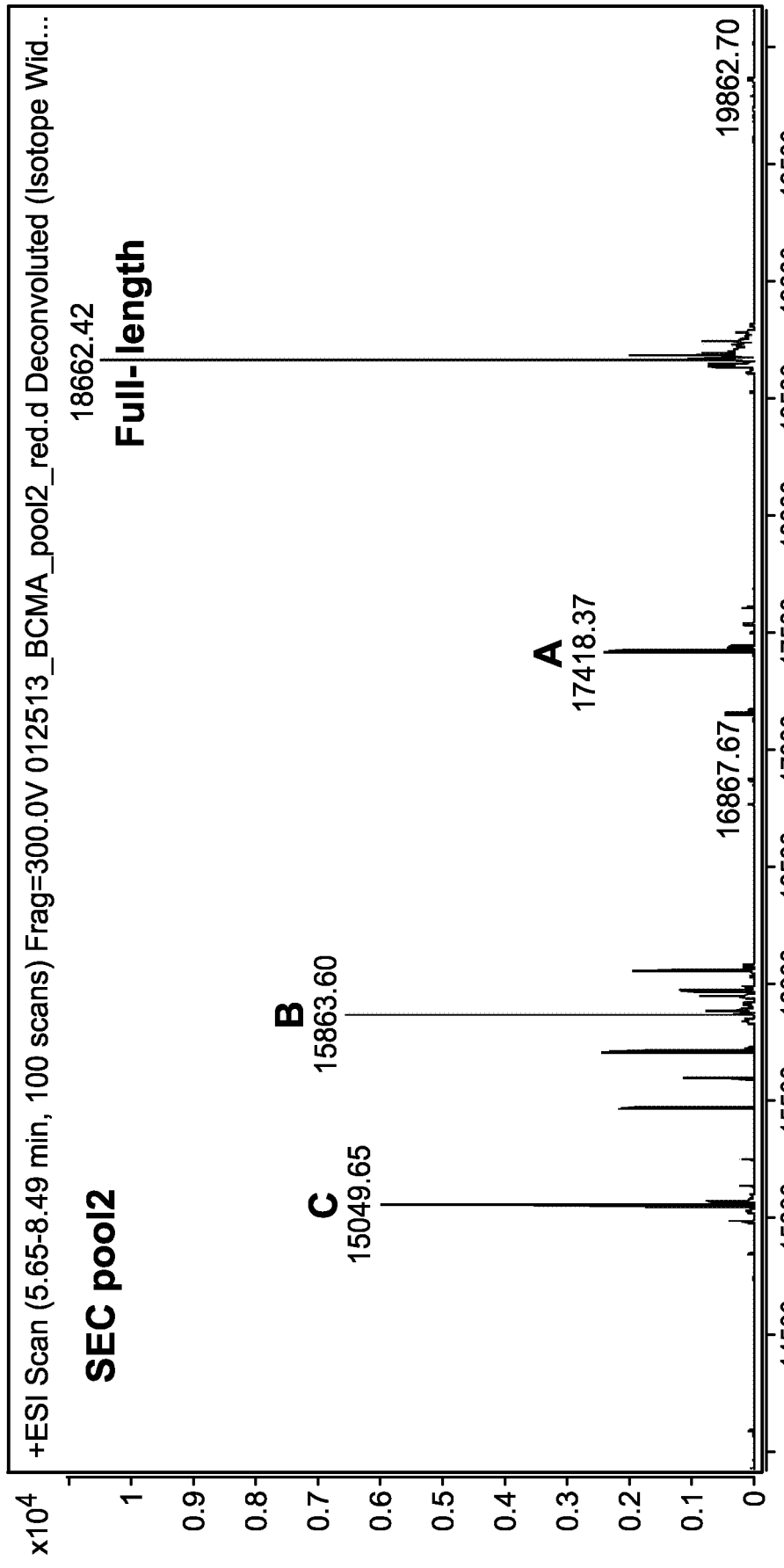
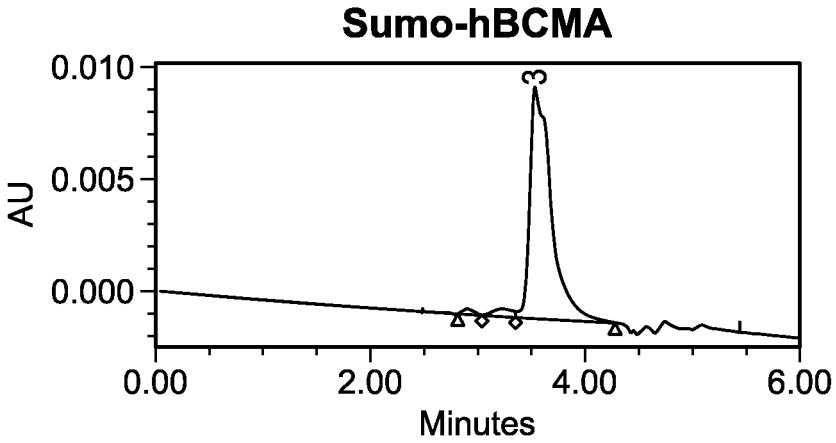

+ESI Scan (5.65-8.49 min, 100 scans) Frag=300.0V 012513_BCMA_pool2_red.d Deconvoluted (Isotope Wid...
SEC pool2
18662.42
Full-length
19862.70
A
17418.37
16867.67
B
15863.60
C
15049.65
x10^4
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
14500
15000
15500
16000
16500
17000
17500
18000
18500
19000
19500

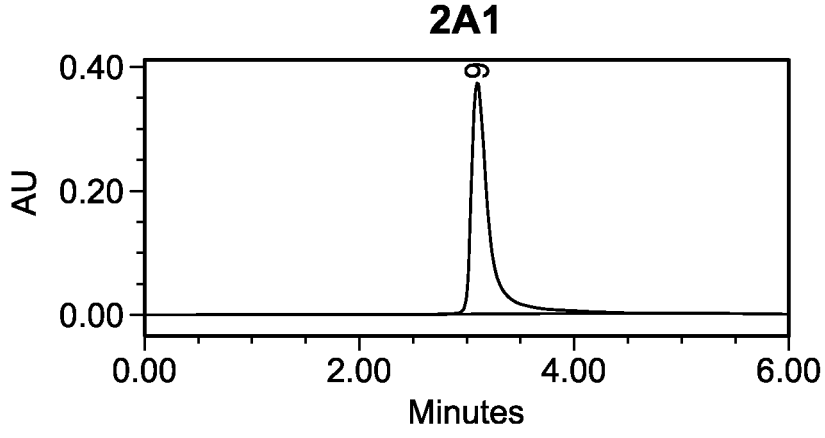
Fig. 46



GPC Results

	Retention Time (min)		MP	% Area
1	2.904		150360	1.06
2	3.222		73427	2.86
3	3.535	*	37159	96.08

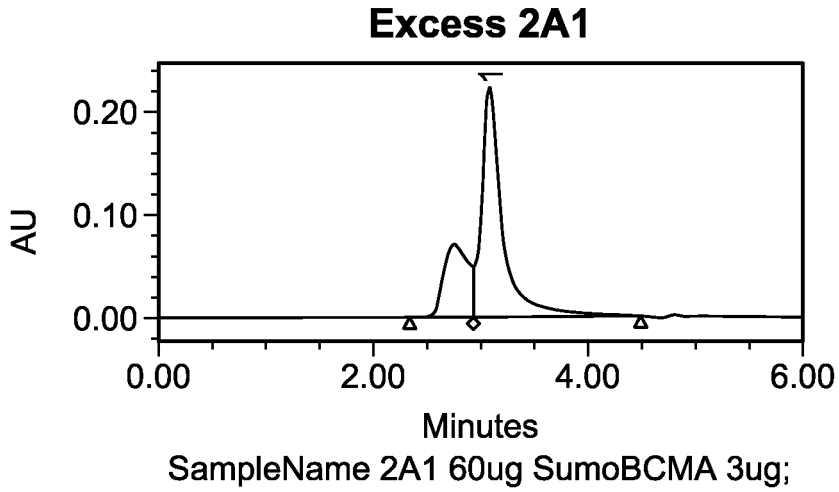
Fig. 47A



GPC Results

	Retention Time (min)		MP	% Area
1	2.559		341160	0.11
2	2.739		221715	0.05
3	3.094	*	97601	99.84

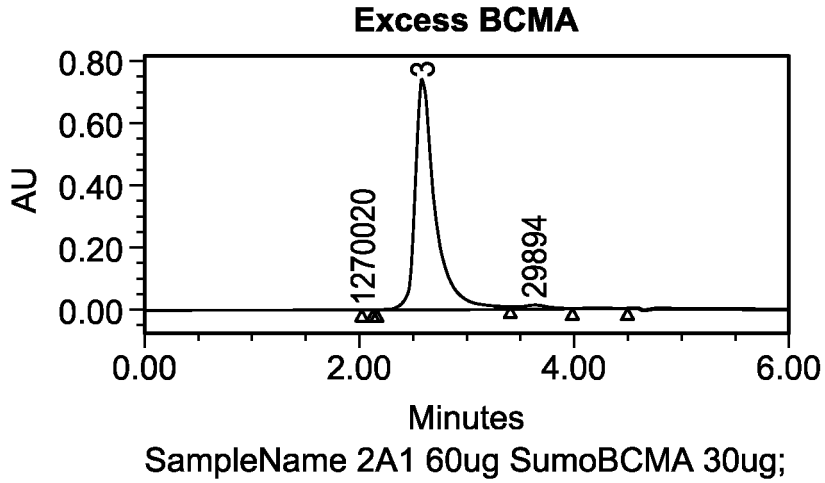
Fig. 47B



GPC Results

	Retention Time (min)	MP	% Area
1	2.748	216815	26.75
2 *	3.078	101155	73.25

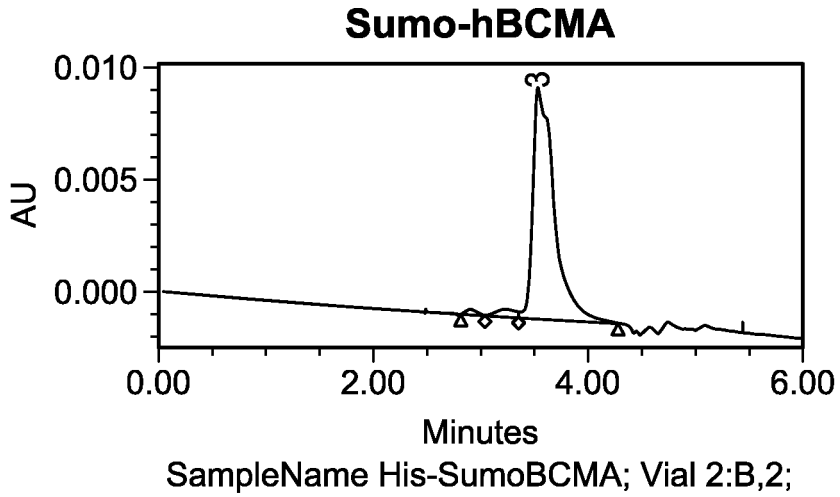
Fig. 47C



GPC Results

	Retention Time (min)	MP	% Area
1	2.034	1270020	0.00
2 *	2.584	321613	98.71
3	3.635	29694	1.29

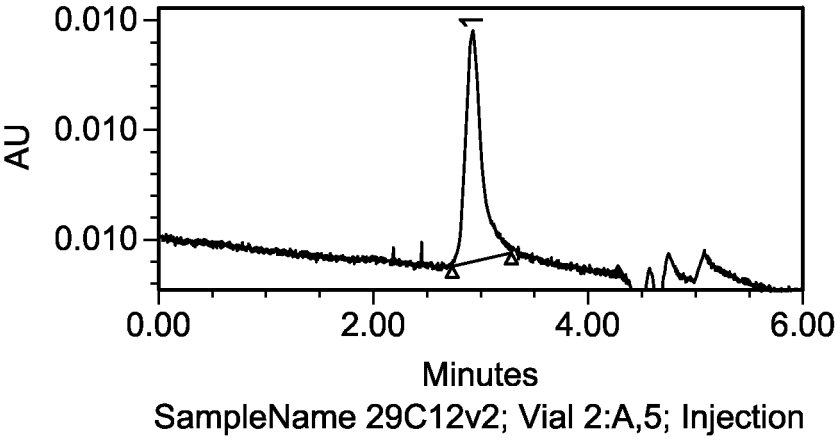
Fig. 47D



GPC Results

	Retention Time (min)	MP	% Area
1	2.904	150360	1.06
2	3.222	73427	2.86
3 *	3.535	37159	96.08

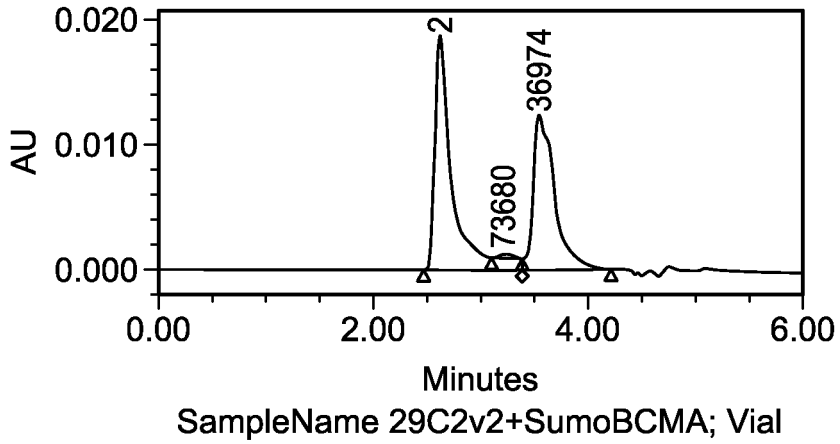
Fig. 48A



GPC Results

	Retention Time (min)	MP	% Area
1 *	2.920	144903	100.00

Fig. 48B



GPC Results

	Retention Time (min)	MP	% Area
1 *	2.617	296869	55.25
2	3.221	73680	0.85
3	3.537	36974	43.91

Fig. 48C

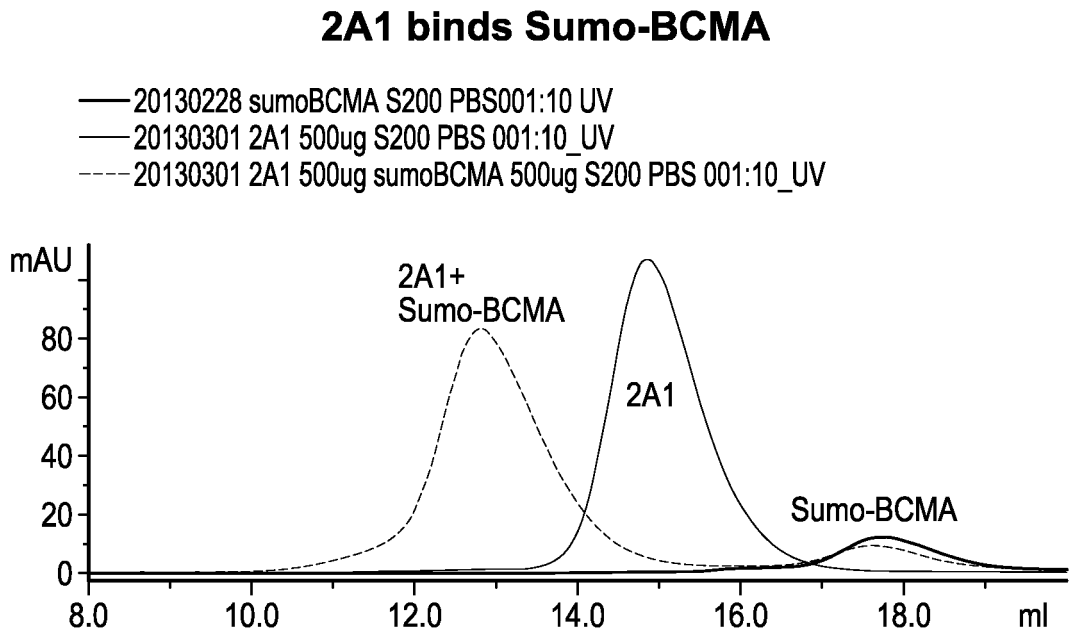


Fig. 49A

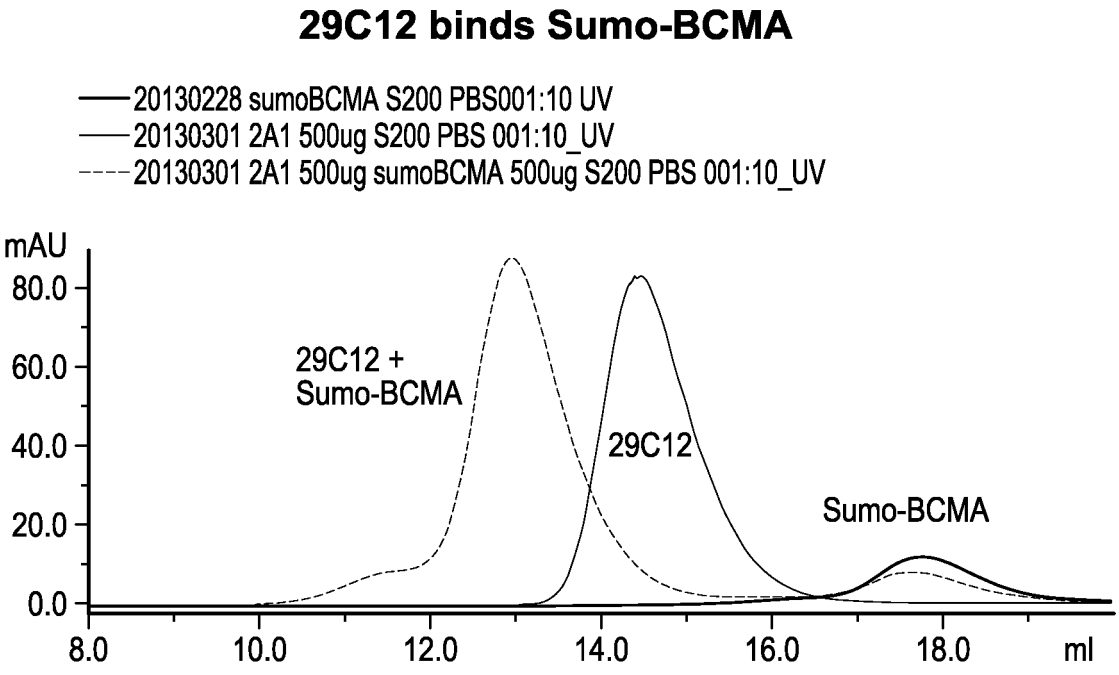


Fig. 49B

Control Proteins Run on SEC

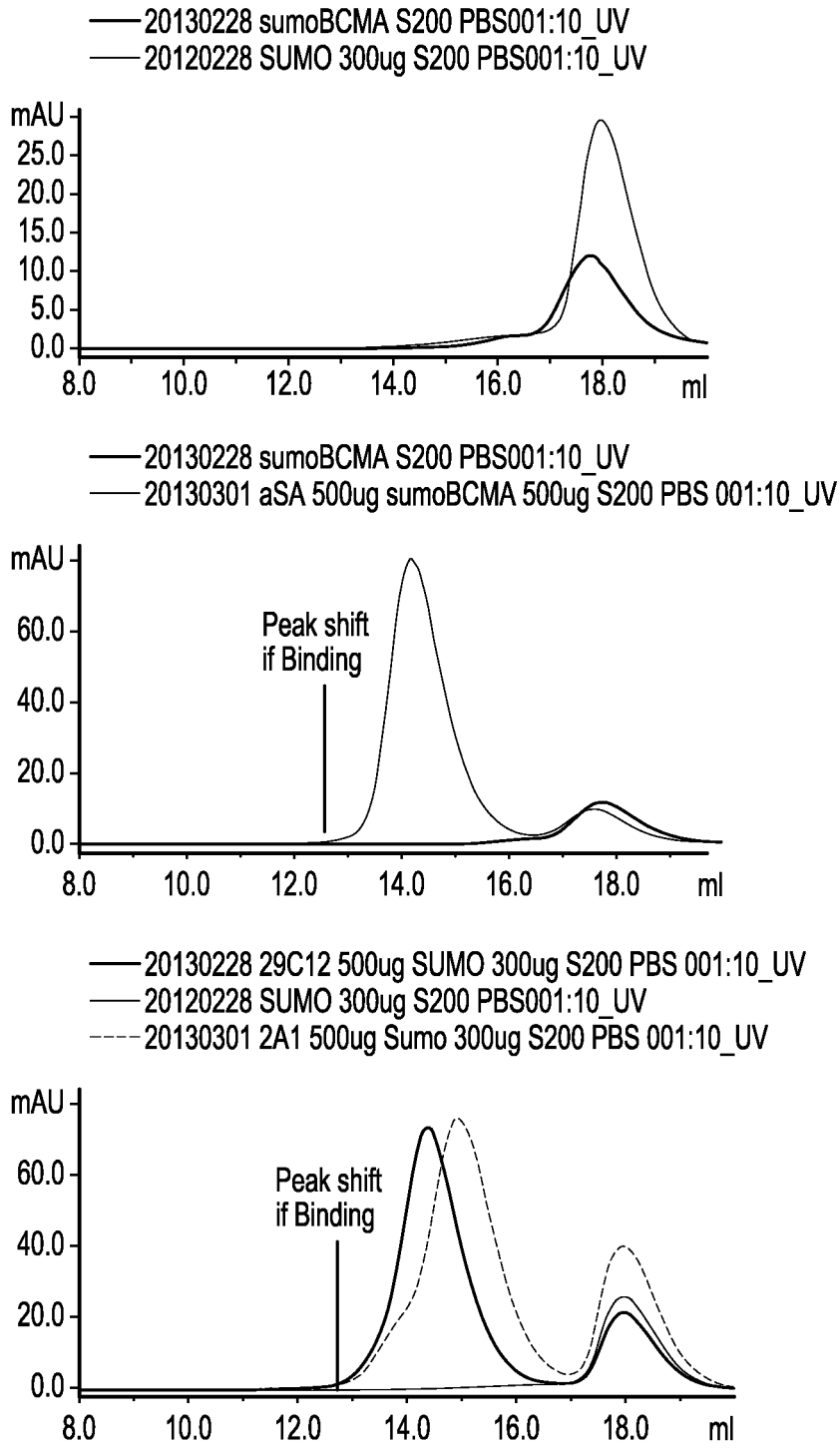


Fig. 49C

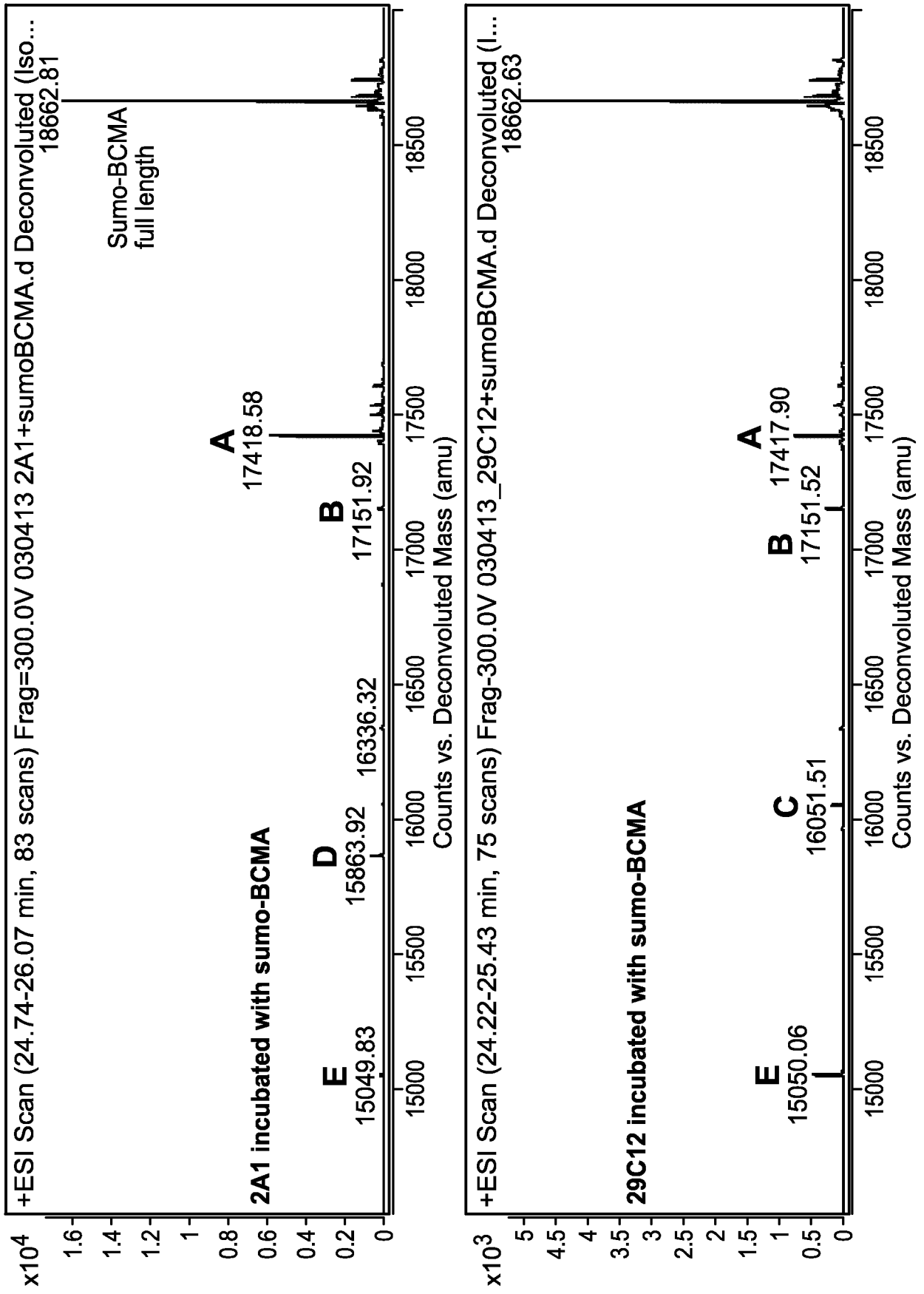

+ESI Scan (24.74-26.07 min, 83 scans) Frag=300.0V 030413 2A1+sumoBCMA.d Deconvoluted (Iso...
18662.81
Sumo-BCMA
full length
A
17418.58
B
17151.92
D
15863.92
16336.32
E
15049.83
2A1 incubated with sumo-BCMA
x10^4
Counts vs. Deconvoluted Mass (amu)
+ESI Scan (24.22-25.43 min, 75 scans) Frag-300.0V 030413_29C12+sumoBCMA.d Deconvoluted (I...
18662.63
A
17417.90
B
17151.52
C
16051.51
E
15050.06
29C12 incubated with sumo-BCMA
x10^3
Counts vs. Deconvoluted Mass (amu)

Fig. 50

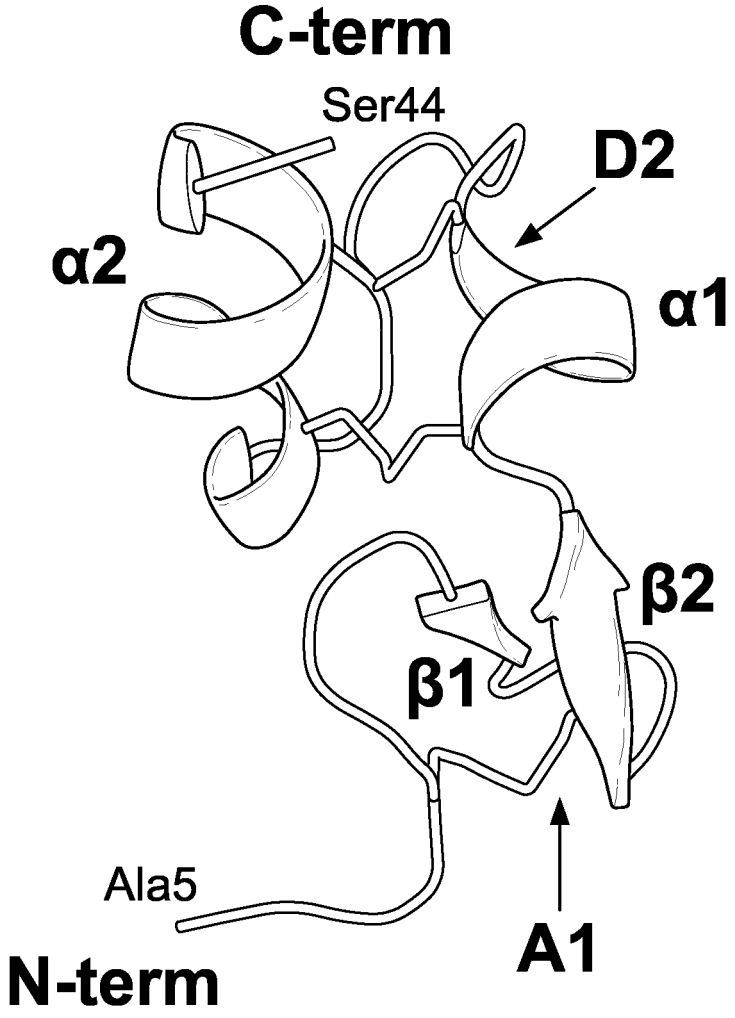


Fig. 51