	Ab#	Control 3	Bin 6 MBA001	Bin 7 MBN005	Bin 1 MBN001	Bin 3 MBN004	Bin 2 MBN002	Bin 5 MBV002	PBST
	Control 3	-0.2098	0.9789	0.7824	0.8244	-0.0582	0.8321	0.7855	-0.3099
Bin 6	MBA001	0.852	-0.3265	0.5705	0.6645	1.0393	1.4165	0.6581	-0.4274
Bin 7	MBN005	0.9697	1.0029	-0.3881	0.8364	1.1846	1.6827	0.7306	-0.4427
Bin 1	MBN001	1.0304	1.0149	0.7758	-0.2185	1.1801	1.3824	0.7127	-0.2724
Bin 3	MBN004	-0.1677	0.9315	0.8456	0.7465	-0.0564	0.8324	0.7526	-0.2672
Bin 2	MBN002	0.1891	0.6704	0.609	0.5325	0.2701	0.1461	0.5274	-0.053
Bin 5	MBV002	1.1007	1.0253	0.8381	0.9074	1.2824	1.6045	0.0042	-0.0717

FIG. 1A

	CEACAM5	CEACAM1	CEACAM6
Bin1	+	-	-
Bin2	+	-	-
Bin3	+	-	-
Bin4	+	+	-
Bin5	+	-	-
Bin6	+	-	+
Bin7	+	+	-
Bin8	+	+	-

FIG. 1B

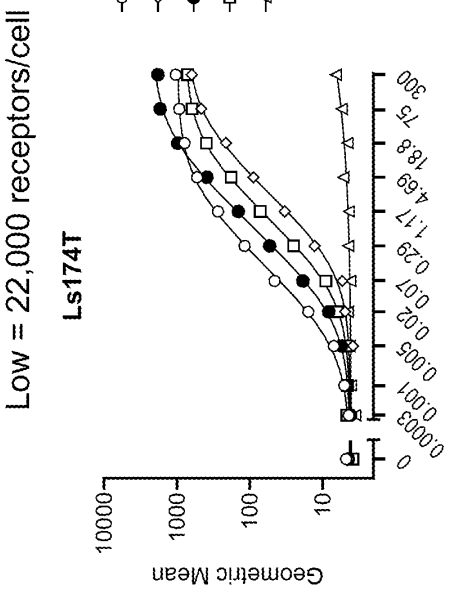


FIG. 2A

FIG. 2B

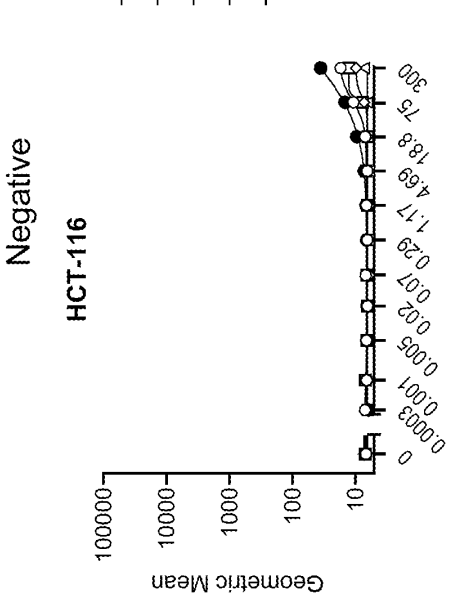


FIG. 2C

FIG. 2D

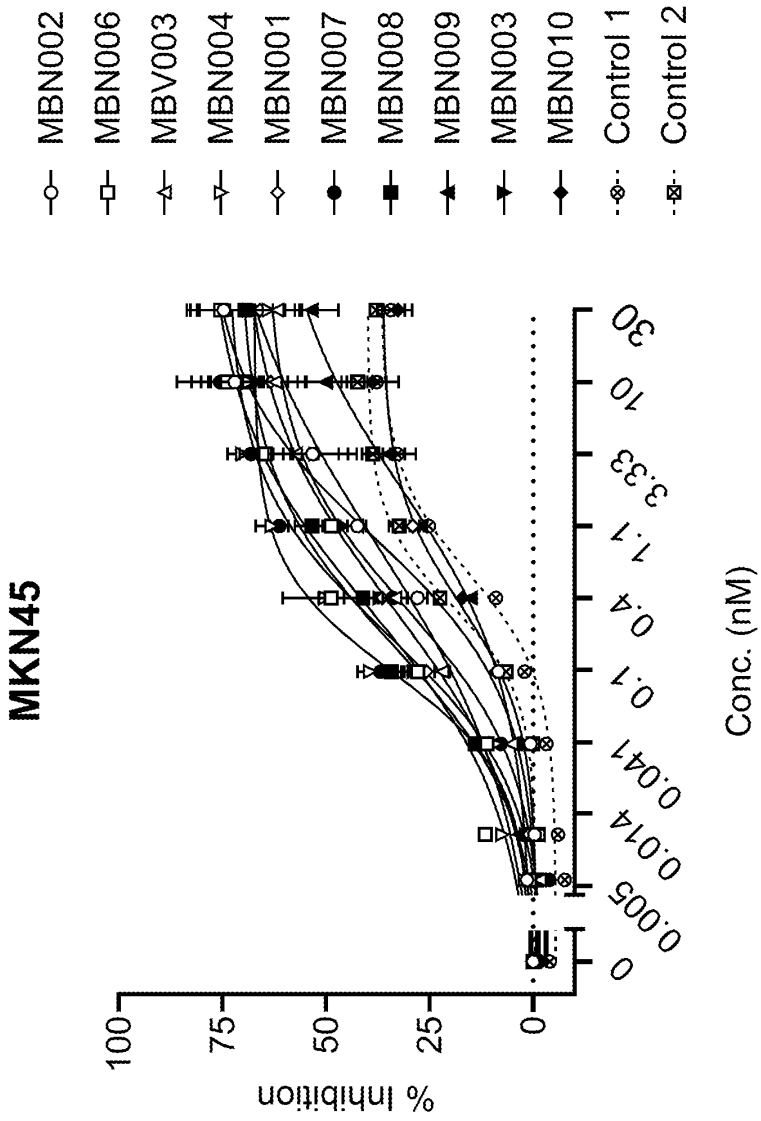

MBN002
MBN006
MBV003
MBN004
MBN001
MBN007
MBN008
MBN009
MBN003
MBN010
Control 1
Control 2
MKN45
% Inhibition
100
75
50
25
0
0
0.005
0.014
0.041
0.1
0.4
1.1
3.33
10
30
Conc. (nM)

FIG. 3A

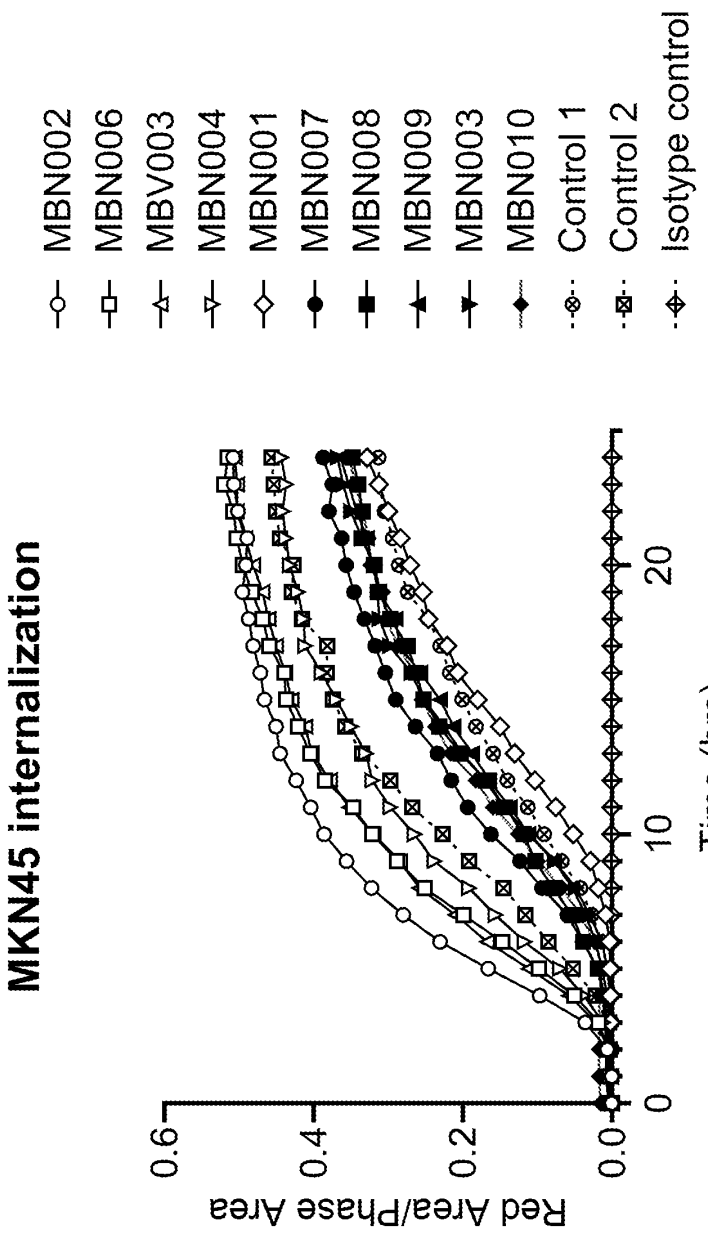

MKN45 internalization
MBN002
MBN006
MBV003
MBN004
MBN001
MBN007
MBN008
MBN009
MBN003
MBN010
Control 1
Control 2
Isotype control
Red Area/Phase Area
0.0
0.2
0.4
0.6
0
10
20
Time (hrs)

FIG. 3B

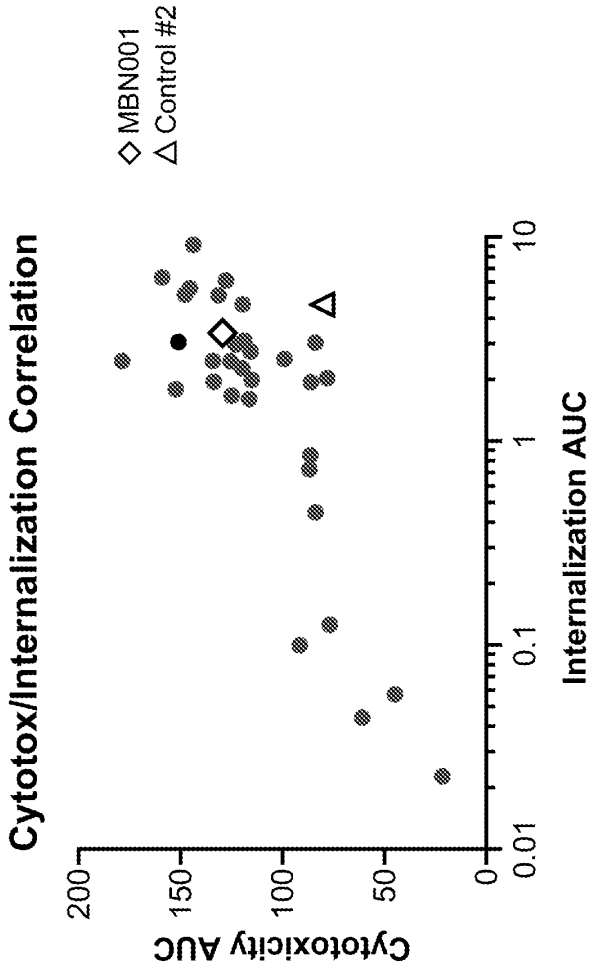

Cytotox/Internalization Correlation
◇ MBN001
△ Control #2
Cytotoxicity AUC
200
150
100
50
0
0.01
0.1
1
10
Internalization AUC

FIG. 3C

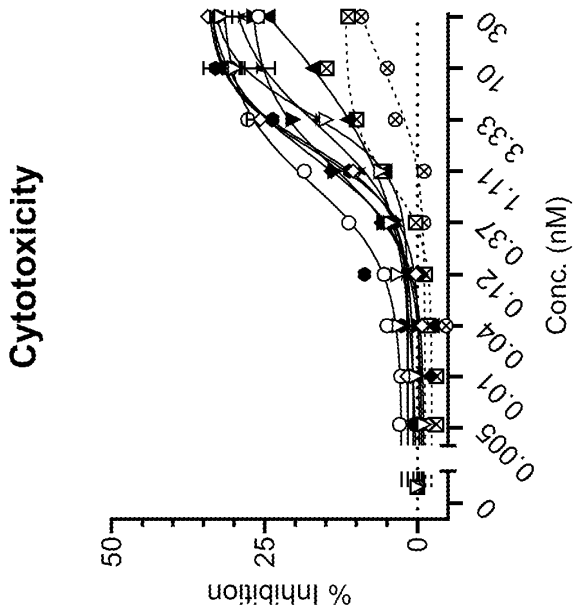

Cytotoxicity
% Inhibition
50
25
0
0
0.005
0.01
0.04
0.12
0.37
1.11
3.33
10
30
Conc. (nM)
Isotype Control
Control #2
MBN002
MBN006
MBV003
MBN001
MBN007
MBN008
MBN009
MBN003

FIG. 4A

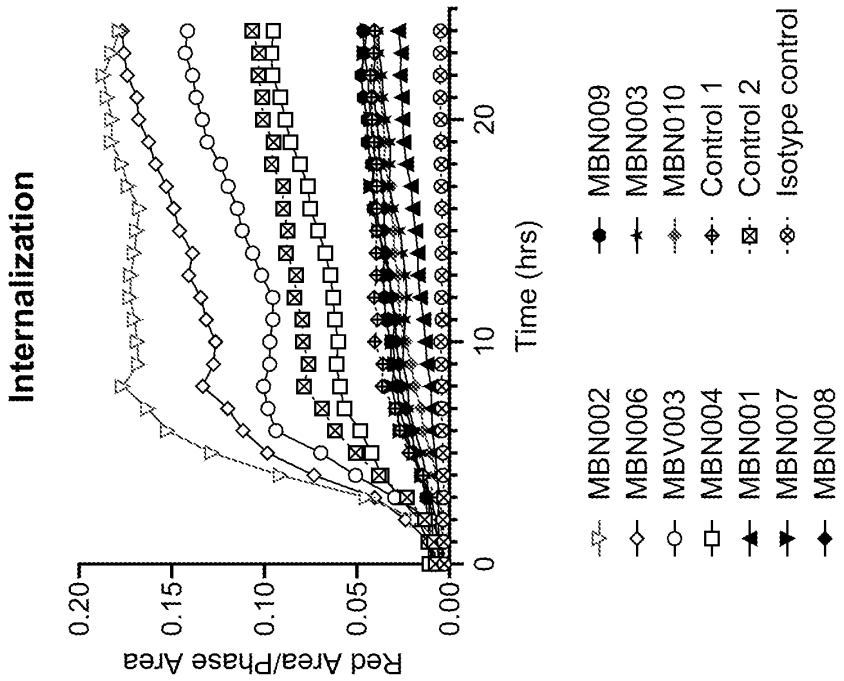

Internalization
Red Area/Phase Area
0.20
0.15
0.10
0.05
0.00
0
10
20
Time (hrs)
MBN002
MBN006
MBV003
MBN004
MBN001
MBN007
MBN008
MBN009
MBN003
MBN010
Control 1
Control 2
Isotype control

FIG. 4B

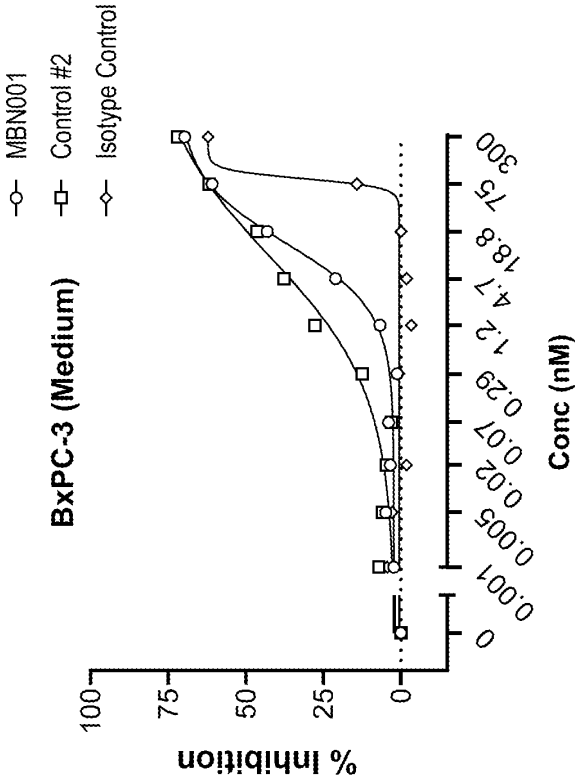


FIG. 5B

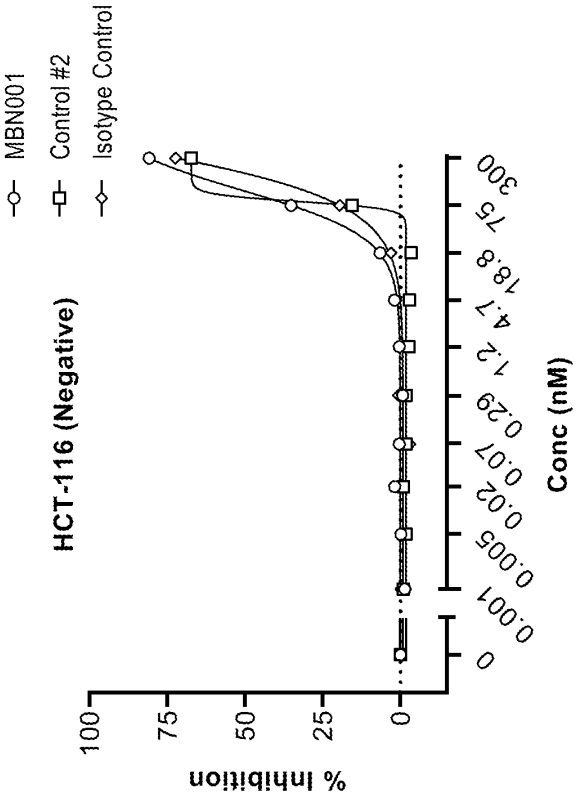


FIG. 5A

HCT-116	IC50	Amax
MBN001	116	80.83
Control #1	84.32	67.24
MBN011	525.7	72.33

FIG. 5C

BxPC3	IC50	Amax
MBN001	13.3	69.63
Control #1	9.272	71.92
MBN011	88.23	62.19

FIG. 5D

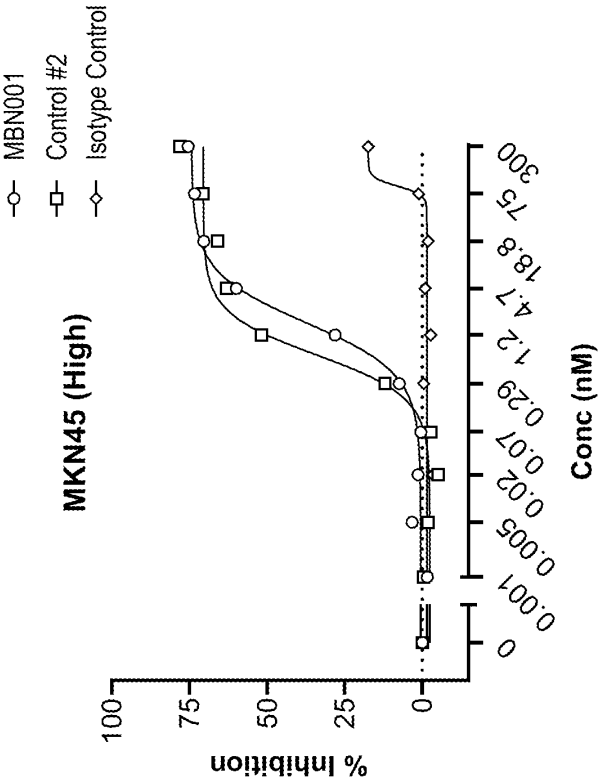


FIG. 5F

MKN45	IC50	Amax
MBN001	1.724	75.46
Control #1	0.6708	78.16
MBN011	94.21	17.39

FIG. 5H

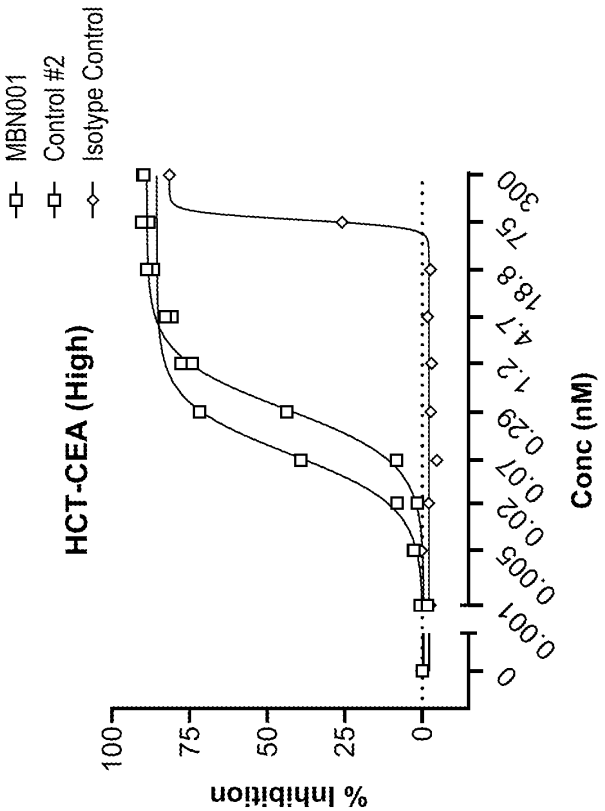


FIG. 5E

HCT-CEA	IC50	Amax
MBN001	0.3165	90.51
Control #1	0.08458	90.16
MBN011	81.1	81.52

FIG. 5G

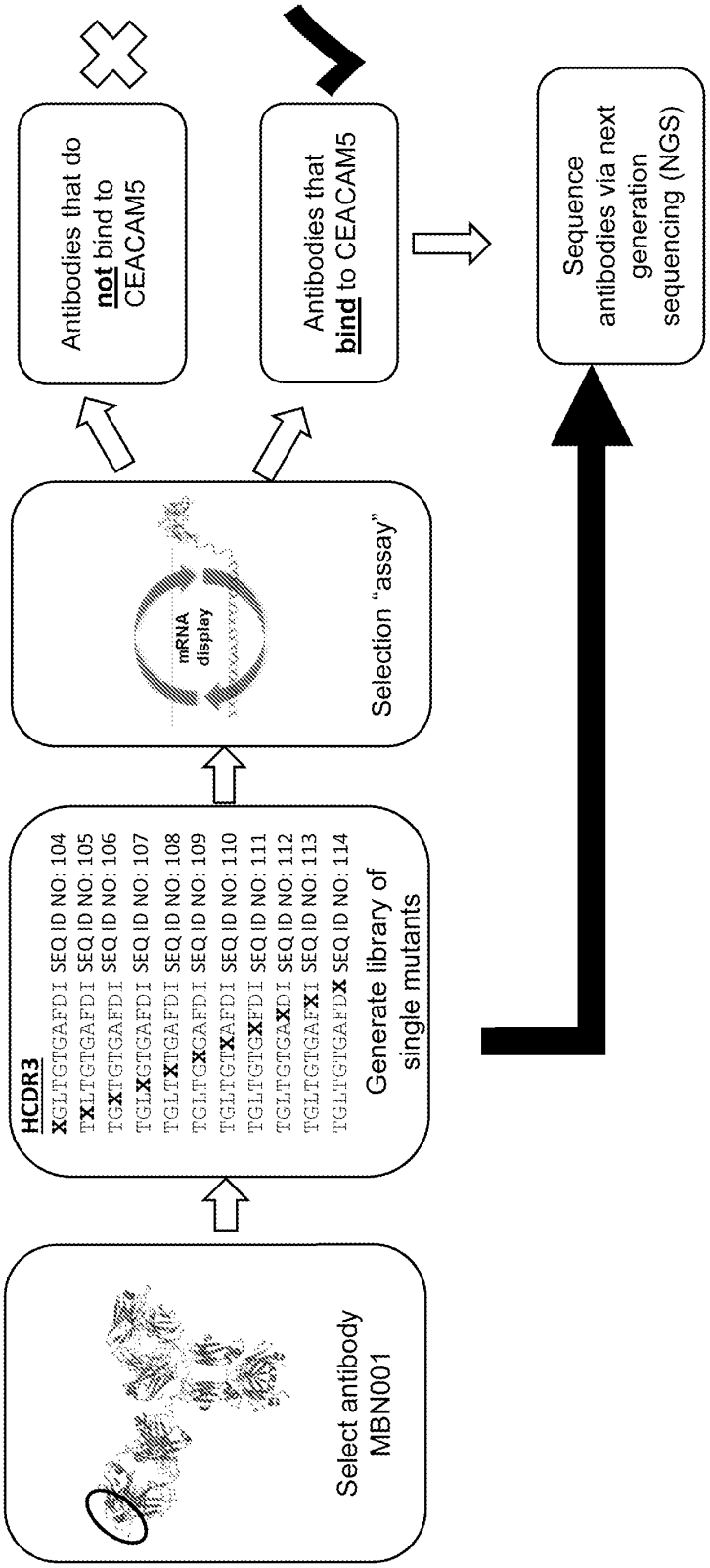
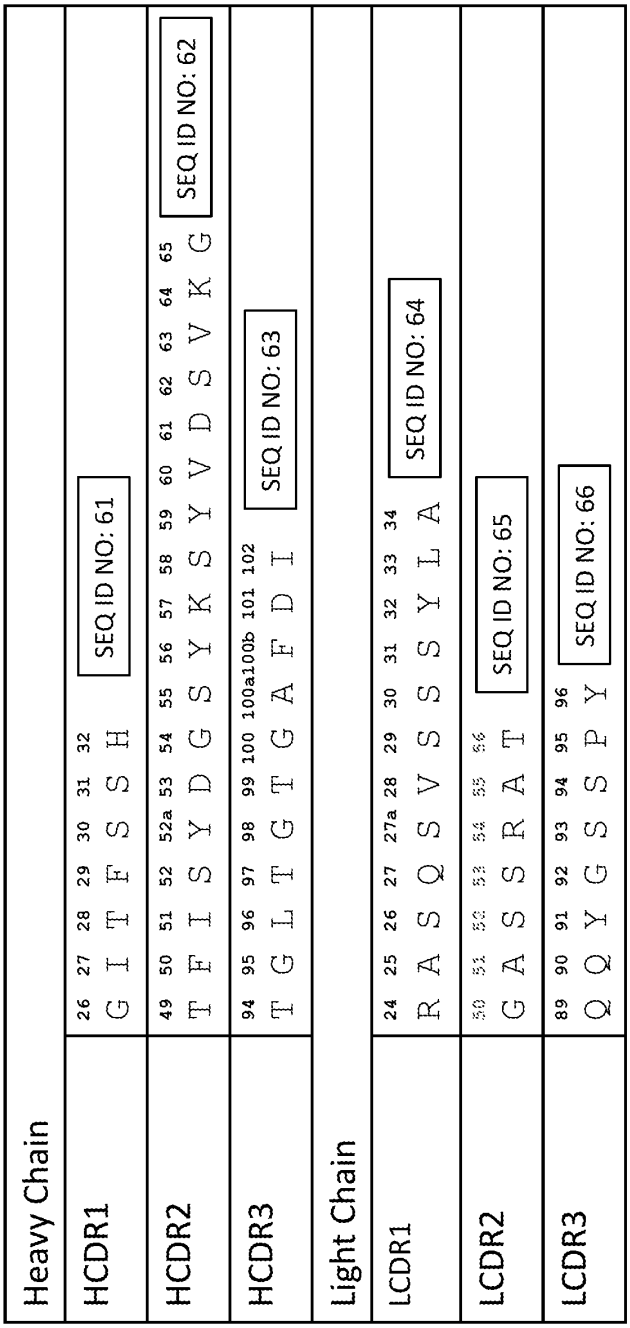

Select antibody MBN001
HCDR3
XGLTGTGAFDI SEQ ID NO: 104
TXLTGTGAFDI SEQ ID NO: 105
TGXTGTGAFDI SEQ ID NO: 106
TGLXGTGAFDI SEQ ID NO: 107
TGLTXTGAFDI SEQ ID NO: 108
TGLTGXGAFDI SEQ ID NO: 109
TGLTGTXAFDI SEQ ID NO: 110
TGLTGTGXFDI SEQ ID NO: 111
TGLTGTGAXDI SEQ ID NO: 112
TGLTGTGAFXI SEQ ID NO: 113
TGLTGTGAFDX SEQ ID NO: 114
Generate library of single mutants
mRNA display
Selection "assay"
Antibodies that do not bind to CEACAM5
Antibodies that bind to CEACAM5
Sequence antibodies via next generation sequencing (NGS)

FIG. 6A



Mutations to germline VH: T7S, S40A, A68T, P84A

FIG. 6B

MBN001 HCDR1
Human CEACAM5

Germline	_	F	_	_	_	_	Y
Parental	G	I	T	F	S	S	H
	26	27	28	29	30	31	32
Normalized Enrichment Ratio							
*	0.00	0.00	0.00	0.00	0.00	0.51	0.00
P	0.06	0.07	0.28	0.00	0.33	0.07	0.45
G	1.00	0.04	1.32	0.03	0.53	0.16	0.02
A	0.46	0.08	0.92	0.00	0.24	0.38	0.00
C	0.35	0.00	1.45	0.16	0.36	0.76	0.00
S	0.26	0.00	1.11	0.03	1.00	1.00	0.09
T	0.67	0.05	1.00	0.00	0.43	0.22	0.09
V	0.49	0.28	0.79	0.12	0.24	0.29	0.12
L	0.18	1.25	**3.86**	0.02	0.25	0.75	0.50
I	0.17	1.00	**3.29**	0.07	0.45	**2.68**	0.17
M	0.09	0.27	**3.97**	0.00	0.42	1.67	0.08
K	0.62	0.15	0.32	0.20	0.00	0.42	0.05
R	0.44	0.09	**3.55**	0.03	0.18	0.35	0.10
H	0.34	0.00	**2.55**	0.00	0.00	**5.51**	1.00
F	0.09	0.09	**4.12**	1.00	0.00	**8.08**	0.16
Y	0.40	0.00	**8.19**	1.24	0.52	**15.50**	0.59
W	0.20	0.09	0.00	0.25	0.42	**14.41**	0.00
N	0.51	0.08	1.91	0.00	1.24	0.94	0.68
D	0.44	0.00	**3.73**	0.00	**4.06**	**2.70**	0.17
E	0.67	0.00	1.64	0.13	0.86	**8.62**	0.08
Q	0.54	0.00	1.10	0.00	0.40	1.52	0.25

SEQ ID NO: 61

Legend

Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

FIG. 6C

MBN001 HCDR1
Cynomolgus CEACAM5

Germline	_	F	_	_	_	_	Y
Parental	G	I	T	F	S	S	H
	26	27	28	29	30	31	32
Normalized Enrichment Ratio							
*	0.19	0.00	0.00	0.00	0.00	0.76	0.00
P	0.00	0.16	0.44	0.00	0.00	0.26	0.66
G	1.00	0.10	0.97	0.19	0.72	0.35	0.22
A	0.43	0.17	0.52	0.31	0.00	0.29	0.06
C	0.23	0.17	**2.44**	0.19	**2.14**	0.43	0.17
S	0.70	0.10	1.88	0.10	1.00	1.00	0.08
T	0.53	0.00	1.00	0.44	0.74	1.27	0.08
V	0.40	0.21	1.74	0.30	0.58	0.89	0.12
L	0.33	1.19	**3.59**	0.22	0.56	0.58	0.52
I	0.35	1.00	**8.73**	0.29	0.19	1.86	0.38
M	0.48	0.27	**3.30**	0.28	0.35	0.67	0.29
K	0.57	0.14	0.41	0.00	0.35	0.66	0.11
R	0.44	0.16	1.63	0.06	0.57	0.52	0.19
H	0.50	0.13	**4.28**	0.00	0.27	**3.48**	1.00
F	0.14	0.19	**32.19**	1.00	0.00	**4.46**	0.29
Y	0.58	0.00	**48.51**	0.85	0.00	**7.19**	0.69
W	0.28	0.11	1.29	0.38	**2.71**	**17.45**	0.15
N	0.71	0.13	**6.49**	0.00	0.00	0.91	0.66
D	0.33	0.00	1.51	0.00	1.27	1.07	0.26
E	0.57	0.11	0.69	0.00	0.50	**3.23**	0.31
Q	0.36	0.30	1.51	0.00	0.00	0.63	0.46

SEQ ID NO: 61

Legend

Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

FIG. 6D

MBN001 HCDR2
Human CEACAM5

SEQ ID NO: 62

Germline	A	V	_	_	_	_	_	_	N	_	Y	_	A	_	_	_	_	_
Parental	T	F	I	S	Y	D	G	S	Y	K	S	Y	V	D	S	V	K	G
	49	50	51	52	52a	53	54	55	56	57	58	59	60	61	62	63	64	65
Normalized Enrichment Ratio																		
*	0.00	0.00	0.00	0.00	0.24	0.40	0.00	0.00	0.23	0.20	0.60	0.52	0.18	0.15	0.00	0.00	0.35	0.00
P	0.27	0.06	0.06	0.40	0.07	0.16	0.00	0.13	0.00	**2.15**	0.39	0.25	0.49	1.59	0.92	0.19	0.43	0.88
G	0.55	0.27	0.69	0.08	0.00	0.12	1.00	0.06	0.08	0.44	1.21	0.81	0.35	0.86	1.06	0.67	0.43	1.00
A	0.97	0.19	**3.85**	0.24	0.00	0.08	1.80	0.08	0.05	0.90	0.57	1.10	0.50	0.76	0.71	0.76	0.32	0.78
C	0.70	0.53	0.44	0.07	0.13	0.09	1.31	0.05	0.09	1.28	1.27	0.90	0.67	1.05	1.06	1.33	0.24	1.16
S	0.84	0.16	**3.59**	1.00	0.06	0.10	1.90	1.00	0.17	0.73	1.00	1.14	0.76	0.91	1.00	0.59	0.39	1.41
T	1.00	0.14	**1.94**	**2.58**	0.00	0.11	0.59	0.56	0.07	1.54	**4.02**	1.12	0.74	0.68	1.34	**2.29**	0.24	1.14
V	1.19	0.35	1.75	1.39	0.05	0.09	0.15	0.06	0.43	**2.41**	1.21	0.94	1.00	1.03	1.06	1.00	0.20	1.00
L	1.26	0.33	0.85	0.09	0.05	0.14	0.26	0.07	1.27	**3.34**	**7.72**	1.57	1.41	0.88	0.85	0.62	0.30	0.80
I	1.39	0.24	1.00	0.11	0.13	0.20	0.07	0.05	**3.76**	**3.85**	**4.67**	1.34	1.47	0.66	0.79	1.03	0.41	0.68
M	0.87	1.40	0.69	0.00	0.11	0.17	0.58	0.08	**3.55**	1.99	**5.92**	1.62	1.25	0.62	1.14	0.54	0.45	0.79
K	0.34	0.00	0.19	0.00	0.00	0.11	0.24	0.00	0.00	1.00	**4.57**	0.52	0.37	0.79	0.66	0.60	1.00	1.05
R	0.35	0.06	0.05	0.03	0.00	0.06	0.41	0.10	0.00	1.13	1.15	1.40	0.38	0.75	0.96	0.42	0.94	0.86
H	0.33	0.00	0.00	0.37	0.31	0.11	0.32	0.00	0.31	1.59	0.60	1.75	0.48	0.48	1.41	0.66	0.56	1.30
F	0.99	1.00	0.19	0.15	0.41	0.09	0.19	0.07	0.39	1.22	0.96	1.42	0.68	0.89	0.79	0.62	0.18	1.78
Y	1.02	0.87	0.25	0.10	1.00	0.25	0.39	0.17	1.00	1.81	0.12	1.00	0.69	0.84	0.91	0.70	0.51	1.59
W	0.35	0.39	0.06	0.06	0.18	0.09	0.35	0.11	0.79	1.39	1.22	1.07	0.76	1.37	0.81	0.51	0.38	**2.74**
N	**2.00**	0.06	0.16	0.00	0.09	0.14	0.24	0.11	0.09	0.97	0.68	0.61	0.36	0.88	0.62	0.16	0.33	1.50
D	1.02	0.17	1.09	0.00	0.14	1.00	0.32	0.06	0.00	0.70	0.05	0.61	0.57	1.00	1.00	0.46	0.20	1.09
E	0.96	0.57	0.93	0.00	0.00	0.15	1.06	0.00	0.00	1.45	0.00	0.70	0.83	1.33	0.66	0.45	0.28	0.60
Q	0.48	0.21	0.11	0.00	0.00	0.21	1.97	0.00	0.57	0.21	**9.58**	1.91	0.46	0.72	0.76	0.34	0.46	0.62

Legend

Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

FIG. 6E

MBN001 HCDR2
Cynomolgus CEACAM5

SEQ ID NO: 62

Germline	A	V	_	_	_	_	_	_	N	_	Y	_	A	_	_	_	_	_
Parental	T	F	I	S	Y	D	G	S	Y	K	S	Y	V	D	S	V	K	G
	49	50	51	52	52a	53	54	55	56	57	58	59	60	61	62	63	64	65
Normalized Enrichment Ratio																		
*	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.59	0.54	1.38	0.49	0.00	0.15	0.00	0.00	0.51	0.00
P	0.58	0.32	0.11	0.00	0.12	0.32	0.16	0.23	0.40	1.50	1.42	0.24	0.86	0.88	1.15	0.79	0.78	1.01
G	0.49	0.18	1.22	0.38	0.07	0.27	1.00	0.19	0.10	0.43	1.02	0.75	0.31	1.03	0.77	0.62	0.61	1.00
A	0.93	0.22	**4.51**	0.26	0.00	0.18	1.75	0.19	0.06	0.66	1.27	1.29	1.05	1.65	1.04	0.95	0.60	0.96
C	0.68	0.44	0.45	0.17	0.13	0.23	1.31	0.09	0.38	1.67	0.95	1.58	0.71	**2.01**	0.84	1.01	0.24	1.16
S	0.94	0.19	**6.18**	1.00	0.21	0.33	**2.87**	1.00	0.17	0.54	1.00	0.46	0.62	0.95	1.00	0.63	0.56	1.09
T	1.00	0.22	1.59	**2.34**	0.35	0.37	0.48	0.73	0.43	1.05	**9.46**	1.18	0.57	1.07	1.10	**2.71**	0.62	1.44
V	1.11	0.31	1.27	0.81	0.12	0.15	0.41	0.19	0.38	**2.23**	1.05	1.14	1.00	1.14	1.26	1.00	0.36	0.91
L	1.06	0.45	1.12	0.35	0.12	0.43	0.54	0.20	1.39	**3.52**	**20.15**	1.00	1.13	1.26	0.79	0.66	0.33	0.86
I	0.98	0.77	1.00	0.21	0.00	0.28	0.30	0.19	**13.42**	**6.98**	**4.97**	1.27	1.64	0.77	1.19	1.25	0.23	1.02
M	0.96	0.87	1.00	0.28	0.12	0.47	0.53	0.10	**11.09**	**1.87**	**10.49**	1.45	0.40	0.57	0.62	0.63	0.35	1.04
K	0.70	0.00	0.00	0.00	0.20	0.25	1.02	0.00	0.00	1.00	**3.54**	0.50	0.53	0.83	0.80	0.60	1.00	0.90
R	0.47	0.20	0.36	0.25	0.11	0.14	0.37	0.18	0.13	1.19	1.13	1.07	0.25	0.85	0.67	0.35	1.00	1.15
H	1.01	0.22	0.00	0.00	0.58	0.00	0.82	0.11	0.58	0.91	0.32	1.33	0.44	1.21	1.21	0.60	0.70	0.61
F	1.57	1.00	0.58	0.34	0.47	0.31	0.41	0.26	0.37	0.84	0.97	1.89	0.62	0.70	0.79	0.73	0.40	1.00
Y	1.33	0.53	0.47	0.23	1.00	0.33	0.29	0.23	1.00	1.40	0.26	1.00	0.46	1.08	0.80	0.66	0.86	1.07
W	0.28	0.72	0.24	0.35	0.39	0.18	0.48	0.17	1.07	1.05	1.03	0.59	0.80	1.47	0.71	0.53	0.34	**2.12**
N	1.39	0.22	0.38	0.11	0.07	0.34	0.46	0.27	0.18	0.89	0.54	0.76	0.35	1.40	0.36	0.78	0.60	1.09
D	1.11	0.15	0.95	0.08	0.34	1.00	0.38	0.14	0.13	0.49	0.26	0.34	0.70	1.00	0.78	0.57	0.22	0.79
E	0.50	0.40	0.79	0.11	0.14	0.33	0.92	0.06	0.29	1.07	0.12	0.28	0.75	1.57	0.48	0.62	0.31	0.54
Q	0.63	0.00	0.85	0.00	0.00	0.52	1.43	0.25	0.00	0.46	**11.91**	1.06	0.23	0.90	0.98	0.39	0.24	1.14

Legend

Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

FIG. 6F

MBN001 HCDR3
Human CEACAM5

SEQ ID NO: 63

Germline	_	_	_	_	_	_	_	_	_	_	_
Parental	T	G	L	T	G	T	G	A	F	D	I
	94	95	96	97	98	99	100	100a	100b	101	102
Normalized Enrichment Ratio											
*	0.00	ND	ND	0.00	0.00	ND	0.00	0.00	0.00	0.00	0.00
P	0.14	0.00	0.00	0.00	0.00	0.11	1.78	0.07	0.00	0.00	0.00
G	0.00	1.00	0.05	0.05	1.00	0.00	1.00	0.15	0.18	0.27	0.08
A	0.14	0.07	0.13	0.00	0.00	0.29	0.19	1.00	0.63	0.05	0.00
C	0.00	0.11	1.40	0.19	0.00	0.07	0.84	0.08	1.23	0.17	0.00
S	0.05	0.03	0.03	0.04	0.03	0.06	0.22	0.21	**2.59**	0.14	0.09
T	1.00	0.06	0.00	1.00	0.00	1.00	0.10	0.04	1.64	0.00	0.00
V	0.03	0.00	0.13	0.11	0.07	0.14	0.59	0.08	**2.64**	0.21	1.24
L	0.04	0.08	1.00	0.03	0.00	0.15	**2.49**	0.06	**3.16**	0.00	0.10
I	0.09	0.00	0.00	0.22	0.08	0.26	1.16	0.13	1.28	0.00	1.00
M	0.07	0.00	0.36	0.10	0.00	0.00	0.31	0.00	**3.07**	0.00	0.16
K	0.00	0.00	0.00	0.00	0.32	0.00	0.00	0.00	**3.26**	0.00	0.19
R	0.00	0.04	0.00	0.00	0.00	0.00	0.12	0.09	0.00	0.00	0.00
H	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.25	**2.74**	0.14	0.00
F	0.06	0.05	0.11	0.06	0.00	0.00	0.00	0.09	1.00	0.12	0.15
Y	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.10	**4.01**	0.00	0.00
W	0.09	0.21	0.00	0.00	0.17	0.04	0.29	0.18	0.64	0.08	0.00
N	0.24	0.13	0.25	0.14	0.00	0.14	**3.68**	0.00	**7.04**	0.12	0.00
D	0.08	0.00	0.04	0.00	0.06	0.00	0.66	0.04	1.15	1.00	0.00
E	0.06	0.11	0.08	0.00	0.00	0.20	0.12	0.00	0.11	0.40	0.00
Q	0.00	0.13	0.34	0.00	0.12	0.00	0.26	0.00	1.55	0.00	0.30

Legend

Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

ND= Enrichment ratios could not be determined because NGS counts in the starting library were too low

FIG. 6G

MBN001 HCDR3
Cynomolgus CEACAM5

SEQ ID NO: 63

Germline	_	_	_	_	_	_	_	_	_	_	_
Parental	T	G	L	T	G	T	G	A	F	D	I
	94	95	96	97	98	99	100	100a	100b	101	102
Normalized Enrichment Ratio											
*	0.00	ND	ND	0.00	0.00	ND	0.00	0.00	0.00	0.00	0.00
P	0.16	0.15	0.15	0.00	0.19	0.11	0.62	0.00	0.00	0.00	0.00
G	0.26	1.00	0.25	0.06	1.00	0.12	1.00	0.22	0.07	0.33	0.11
A	0.07	0.00	0.19	0.14	0.08	0.35	0.26	1.00	1.03	0.08	0.33
C	0.16	0.20	**2.58**	0.00	0.11	0.00	0.40	0.27	1.94	0.43	0.33
S	0.11	0.23	0.18	0.22	0.18	0.28	0.46	0.28	**2.16**	0.68	0.17
T	1.00	0.20	0.30	1.00	0.00	1.00	0.00	0.08	0.90	0.38	0.00
V	0.04	0.14	0.25	0.04	0.09	0.23	0.48	0.15	**3.11**	0.27	1.10
L	0.13	0.14	1.00	0.13	0.10	0.11	0.96	0.15	**5.23**	0.00	0.11
I	0.00	0.21	0.26	0.21	0.33	0.14	0.27	0.12	1.30	0.00	1.00
M	0.14	0.16	0.44	0.19	0.00	0.00	0.24	0.11	**5.08**	0.00	0.00
K	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.15	**3.43**	0.00	0.00
R	0.11	0.15	0.19	0.06	0.00	0.19	0.11	0.24	0.23	0.00	0.15
H	0.00	0.00	0.00	0.23	0.00	0.36	0.00	0.67	**3.10**	0.00	0.00
F	0.24	0.14	0.21	0.34	0.10	0.35	0.27	0.08	1.00	0.00	0.51
Y	0.22	0.14	0.17	0.17	0.21	0.32	0.38	0.28	**4.49**	0.18	0.19
W	0.24	0.12	0.12	0.18	0.39	0.07	0.17	0.20	0.50	0.25	0.50
N	0.32	0.35	0.00	0.25	0.19	0.22	**19.17**	0.00	**22.72**	0.00	0.21
D	0.26	0.38	0.00	0.00	0.00	0.00	0.92	0.28	1.64	1.00	0.00
E	0.00	0.13	0.22	0.00	0.00	0.00	0.22	0.09	0.28	0.37	0.00
Q	0.25	0.39	0.98	0.00	0.00	0.00	0.14	0.00	0.78	0.00	0.00

ND= Enrichment ratios could not be determined because NGS counts in the starting library were too low

Legend

Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

FIG. 6H

MBN001 LCDR1
Human CEACAM5

SEQ ID NO: 64

Germline	--	--	--	--	--	--	--	--	--	--	--	--
Parental	R	A	S	Q	S	V	S	S	S	Y	L	A
	24	25	26	27	27a	28	29	30	31	32	33	34
Normalized Enrichment Ratio												
*	0.21	ND	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	ND
P	0.47	**2.56**	0.77	1.23	0.68	**2.27**	0.47	1.79	**2.20**	**2.20**	0.00	0.00
G	1.19	0.70	1.25	0.54	0.97	0.35	1.61	0.40	0.90	**2.68**	**2.01**	0.39
A	0.94	1.00	1.20	0.56	0.83	0.44	0.90	1.19	1.16	0.53	**2.20**	1.00
C	1.03	**3.03**	0.75	0.82	1.01	**2.44**	1.19	0.14	1.70	0.10	0.80	0.71
S	1.04	0.86	1.00	0.59	1.00	0.75	1.00	1.00	1.00	0.61	1.82	0.72
T	0.75	1.58	0.55	1.01	0.66	**2.00**	0.28	0.49	1.26	0.57	0.86	0.22
V	0.73	1.38	0.79	1.11	0.77	1.00	0.37	0.87	**2.48**	0.64	1.45	0.00
L	0.82	1.98	1.12	0.65	0.61	1.62	0.72	0.30	1.81	0.09	1.00	0.00
I	1.05	1.55	1.13	1.20	0.59	1.66	0.48	0.57	**6.43**	0.84	**2.32**	0.00
M	0.78	1.99	1.40	0.73	0.52	**2.73**	0.41	0.19	1.54	0.95	1.88	0.00
K	0.94	0.29	1.12	0.90	0.85	0.55	0.19	0.22	1.15	0.08	0.57	0.00
R	1.00	0.12	0.97	0.55	0.95	0.24	0.10	0.37	0.61	0.13	0.75	0.00
H	0.72	0.34	1.57	0.64	1.32	0.44	0.57	0.00	0.73	0.75	0.34	0.00
F	1.31	1.42	1.04	0.56	1.39	**2.23**	0.39	0.11	0.75	0.91	1.67	0.00
Y	1.13	0.37	1.46	0.59	1.55	0.63	0.56	0.27	1.02	1.00	1.85	0.00
W	1.10	0.31	0.87	0.43	0.82	0.49	0.46	0.08	**6.16**	0.89	1.96	0.30
N	0.98	0.57	0.75	0.52	0.51	0.36	0.66	0.27	0.00	0.84	0.52	0.00
D	0.62	0.50	1.11	0.63	0.68	0.69	1.12	0.55	0.31	0.76	1.19	0.00
E	1.05	0.28	1.48	1.14	0.64	0.26	1.53	0.26	0.88	0.37	1.74	0.00
Q	0.73	0.83	1.49	1.00	0.95	0.75	0.80	0.19	ND	0.33	0.84	ND

ND= Enrichment ratios could not be determined because NGS counts in the starting library were too low

Legend

Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

FIG. 6I

MBN001 LCDR1
Cynomolgus CEACAM5

SEQ ID NO: 64

Germline												
Parental	R	A	S	Q	S	V	S	S	S	Y	L	A
	24	25	26	27	27a	28	29	30	31	32	33	34
Normalized Enrichment Ratio												
*	0.00	ND	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.00	0.00	ND
P	0.89	**3.13**	0.61	0.98	1.03	0.77	0.59	**2.12**	1.78	1.84	0.00	0.00
G	0.82	0.53	1.27	0.50	0.71	0.14	2.54	0.08	0.00	1.33	1.15	0.79
A	0.63	1.00	0.92	0.49	1.19	0.49	0.41	1.92	1.60	0.25	1.39	1.00
C	1.23	**2.85**	0.85	0.54	1.20	1.22	0.86	0.26	1.52	0.73	0.24	1.01
S	0.68	1.34	1.00	0.62	1.00	0.44	1.00	1.00	1.00	0.79	0.61	0.52
T	0.50	1.95	0.82	0.89	0.60	**2.11**	0.27	0.81	1.31	0.57	0.34	0.50
V	0.63	1.38	0.87	1.23	0.60	1.00	0.13	0.99	**3.15**	0.91	1.81	0.25
L	0.65	**3.20**	0.51	0.62	0.62	1.41	0.45	0.11	1.79	0.05	1.00	0.11
I	0.99	**2.09**	1.03	1.12	0.88	1.77	0.15	0.91	**10.62**	1.24	**3.41**	0.00
M	0.66	**2.77**	1.52	1.08	1.24	**2.69**	0.00	0.25	1.56	1.05	**2.86**	0.53
K	0.45	0.40	1.71	0.82	0.00	0.76	0.00	0.00	**2.62**	0.00	0.63	0.00
R	1.00	0.44	0.89	0.80	0.96	0.36	0.60	0.13	0.76	0.00	0.52	0.00
H	0.38	0.62	0.46	0.64	1.17	0.29	0.36	0.00	1.13	0.48	0.26	0.00
F	1.49	1.80	1.14	0.44	1.47	**2.20**	0.34	0.00	0.24	0.77	1.65	0.00
Y	0.59	0.70	0.98	0.78	**3.51**	0.53	0.11	0.21	0.71	1.00	**2.11**	0.58
W	0.73	0.52	0.74	0.87	**4.29**	0.36	0.67	0.61	**12.32**	1.65	**2.76**	0.26
N	**1.95**	0.73	0.84	0.55	0.54	0.35	0.68	0.00	0.00	0.59	0.63	0.00
D	0.63	0.24	0.80	0.41	0.35	0.00	1.18	0.00	0.18	0.74	0.00	0.00
E	1.12	0.65	0.84	0.50	0.37	0.00	0.87	0.35	0.00	0.00	1.09	0.00
Q	0.62	0.45	1.70	1.00	0.73	0.00	0.00	0.39	ND	0.68	1.06	ND

ND= Enrichment ratios could not be determined because NGS counts in the starting library were too low

Legend

Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

FIG. 6J

MBN001 LCDR2
Human CEACAM5

Germline	_	_	_	_	_	_	_
Parental	G	A	S	S	R	A	T
	50	51	52	53	54	55	56
Normalized Enrichment Ratio							
*	ND	ND	0.00	0.00	0.00	0.00	0.00
P	ND	**2.98**	0.16	0.59	0.53	0.62	1.52
G	1.00	**3.07**	0.52	0.55	0.47	0.82	0.37
A	**4.16**	1.00	0.85	1.19	0.53	1.00	0.32
C	0.91	**2.36**	0.86	0.64	0.50	0.19	0.50
S	**2.43**	0.91	1.00	1.00	0.49	0.74	0.50
T	0.79	1.80	0.73	1.64	0.74	0.45	1.00
V	1.92	0.24	0.96	1.50	0.79	0.48	0.76
L	0.14	0.76	1.04	0.04	1.10	**2.21**	0.55
I	0.35	0.00	0.56	0.44	0.87	0.49	0.56
M	**4.11**	0.67	0.38	0.60	1.22	1.67	0.59
K	0.00	0.27	0.81	0.00	0.83	0.22	0.22
R	0.71	0.47	0.66	0.07	1.00	0.29	0.70
H	1.58	1.20	0.43	1.71	1.32	**2.25**	0.44
F	**7.10**	0.23	1.37	**2.52**	0.83	0.31	0.59
Y	**6.97**	0.25	**2.35**	**4.04**	1.10	1.57	0.51
W	**8.12**	1.61	1.66	5.71	0.80	0.62	0.97
N	0.80	1.08	0.64	1.19	0.62	1.60	1.20
D	0.00	0.42	0.65	0.09	0.32	**2.76**	0.66
E	0.00	0.47	0.62	0.42	0.53	0.19	0.34
Q	0.91	0.53	**2.41**	0.88	0.79	1.13	0.66

SEQ ID NO: 65

Legend

Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

ND= Enrichment ratios could not be determined because NGS counts in the starting library were too low

FIG. 6K

MBN001 LCDR2
Cynomolgus CEACAM5

Legend	
Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

Germline	_	_	_	_	_	_	_
Parental	G	A	S	S	R	A	T
	50	51	52	53	54	55	56
Normalized Enrichment Ratio							
*	ND	ND	1.46	0.00	0.00	0.36	0.00
P	ND	**2.92**	0.49	0.52	1.19	0.79	**2.79**
G	1.00	**2.79**	0.28	0.31	0.40	0.76	0.74
A	**2.75**	1.00	1.03	1.84	0.40	1.00	0.85
C	**4.63**	**3.14**	1.16	0.20	0.53	0.43	**2.08**
S	**4.64**	1.66	1.00	1.00	0.13	0.74	0.74
T	0.26	1.61	0.19	1.06	1.03	0.65	1.00
V	**4.74**	0.00	1.01	0.32	0.44	0.42	0.45
L	0.55	0.77	1.05	0.00	1.08	**2.67**	0.92
I	0.28	0.00	1.22	0.00	0.57	0.42	0.51
M	**12.30**	0.67	1.06	0.22	0.79	**10.37**	0.21
K	0.00	1.12	0.30	0.21	0.78	0.00	1.18
R	0.62	0.85	0.11	0.17	1.00	0.18	1.07
H	0.59	**2.45**	0.00	1.13	0.44	0.92	0.90
F	**21.52**	0.22	**3.24**	1.58	0.61	**2.90**	0.67
Y	**32.14**	0.00	**4.09**	1.85	0.19	**7.46**	1.48
W	**45.22**	1.20	**2.13**	**10.33**	0.91	0.36	**3.29**
N	0.00	0.21	0.26	0.55	0.81	**8.97**	**2.10**
D	0.16	0.00	0.00	0.10	0.49	**5.65**	0.16
E	0.44	0.14	0.64	0.21	0.15	0.24	0.48
Q	0.35	0.51	**3.50**	1.37	1.05	0.49	0.00

SEQ ID NO: 65

ND= Enrichment ratios could not be determined because NGS counts in the starting library were too low

FIG. 6L

MBN001 LCDR3
Human CEACAM5

Germline	--	--	--	--	--	--	--	--
Parental	Q	Q	Y	G	S	S	P	Y
	89	90	91	92	93	94	95	96
Normalized Enrichment Ratio								
*	0.00	0.49	0.44	0.53	ND	ND	0.00	0.00
P	0.38	0.10	0.00	0.07	**7.51**	0.80	1.00	1.98
G	1.74	0.09	1.49	1.00	0.20	0.77	0.14	1.27
A	1.04	0.00	1.42	**1.97**	0.91	0.85	1.02	1.10
C	0.00	0.00	1.52	**2.47**	1.90	1.77	1.12	1.68
S	**2.56**	0.07	**3.34**	1.74	1.00	1.00	0.48	**3.11**
T	0.35	0.00	**2.76**	0.98	1.85	1.19	1.44	**3.02**
V	0.03	0.10	0.62	**2.63**	**2.47**	1.49	**2.39**	**2.52**
L	0.43	0.17	0.08	**4.75**	**4.94**	**3.46**	1.71	0.85
I	0.00	0.12	0.11	**3.43**	**3.51**	**3.58**	1.75	**3.12**
M	**2.23**	0.23	0.25	**3.49**	**2.43**	1.59	**2.09**	**5.58**
K	0.95	0.00	0.00	**4.04**	0.72	0.46	**3.10**	0.00
R	0.22	0.10	0.15	**2.66**	**3.39**	1.32	**2.13**	0.08
H	**3.22**	0.16	0.27	1.16	**2.01**	1.64	**2.16**	**2.46**
F	**4.29**	0.15	0.55	**3.53**	**3.04**	**3.06**	1.66	0.29
Y	**2.50**	0.10	1.00	**3.87**	**2.72**	**3.35**	1.53	1.00
W	0.26	0.16	0.10	**3.65**	1.37	1.14	1.78	**2.97**
N	**2.68**	0.00	**3.24**	1.35	1.53	1.69	1.22	**3.56**
D	**2.70**	0.09	1.60	0.94	0.44	0.29	0.20	**3.19**
E	1.32	0.27	0.31	1.94	0.49	0.00	1.31	1.46
Q	1.00	1.00	1.92	1.45	**2.71**	1.79	**3.79**	**4.11**

SEQ ID NO: 66

ND= Enrichment ratios could not be determined because NGS counts in the starting library were too low

Legend

Favorable	>2
Neutral	} 0.5 to 2
Unfavorable	<0.5

FIG. 6M

MBN001 LCDR3
Cynomolgus CEACAM5

Legend	
Favorable	>2
Neutral	0.5 to 2
Unfavorable	<0.5

Germline	_	_	_	_	_	_	_	_
Parental	Q	Q	Y	G	S	S	P	Y
	89	90	91	92	93	94	95	96
Normalized Enrichment Ratio								
*	0.00	0.00	0.51	0.68	ND	ND	0.00	0.00
P	0.65	0.00	0.00	0.07	**43.51**	0.47	1.00	1.17
G	1.24	0.17	**4.40**	1.00	0.32	0.54	0.23	0.42
A	1.46	0.07	1.07	1.09	0.59	0.79	0.77	0.73
C	0.00	0.19	**2.61**	1.47	**3.50**	**2.72**	0.61	0.51
S	**2.03**	0.10	**3.81**	1.07	1.00	1.00	0.32	0.87
T	0.00	0.00	**2.68**	0.63	**5.66**	1.01	1.05	1.42
V	0.25	0.00	0.36	1.61	**8.10**	1.69	0.85	0.89
L	0.26	0.05	0.00	**6.63**	**16.69**	**8.11**	1.04	0.49
I	0.00	0.25	0.00	**3.92**	**15.44**	**2.52**	**2.19**	1.67
M	0.73	0.00	0.35	**2.97**	**4.31**	**2.02**	1.46	**2.10**
K	0.51	0.00	0.00	**29.40**	**3.63**	0.00	**3.58**	0.20
R	0.08	0.05	0.35	**9.73**	**33.98**	1.08	1.36	0.10
H	**4.56**	0.00	0.62	0.40	**6.28**	1.25	0.64	0.00
F	1.14	0.00	0.39	1.82	**3.58**	**5.30**	1.12	0.42
Y	0.77	0.00	1.00	2.44	**6.25**	**2.80**	0.36	1.00
W	0.20	0.00	0.13	**3.08**	**2.67**	1.09	1.19	1.79
N	0.80	0.00	**20.59**	**2.10**	**2.75**	0.47	1.19	**3.42**
D	**2.77**	0.48	1.14	0.22	0.00	0.00	0.21	**2.32**
E	1.49	0.27	0.00	0.41	0.17	0.00	0.60	**2.34**
Q	1.00	1.00	**2.27**	1.06	4.12	**2.68**	0.88	1.90

SEQ ID NO: 66

ND= Enrichment ratios could not be determined because NGS counts in the starting library were too low

FIG. 6N

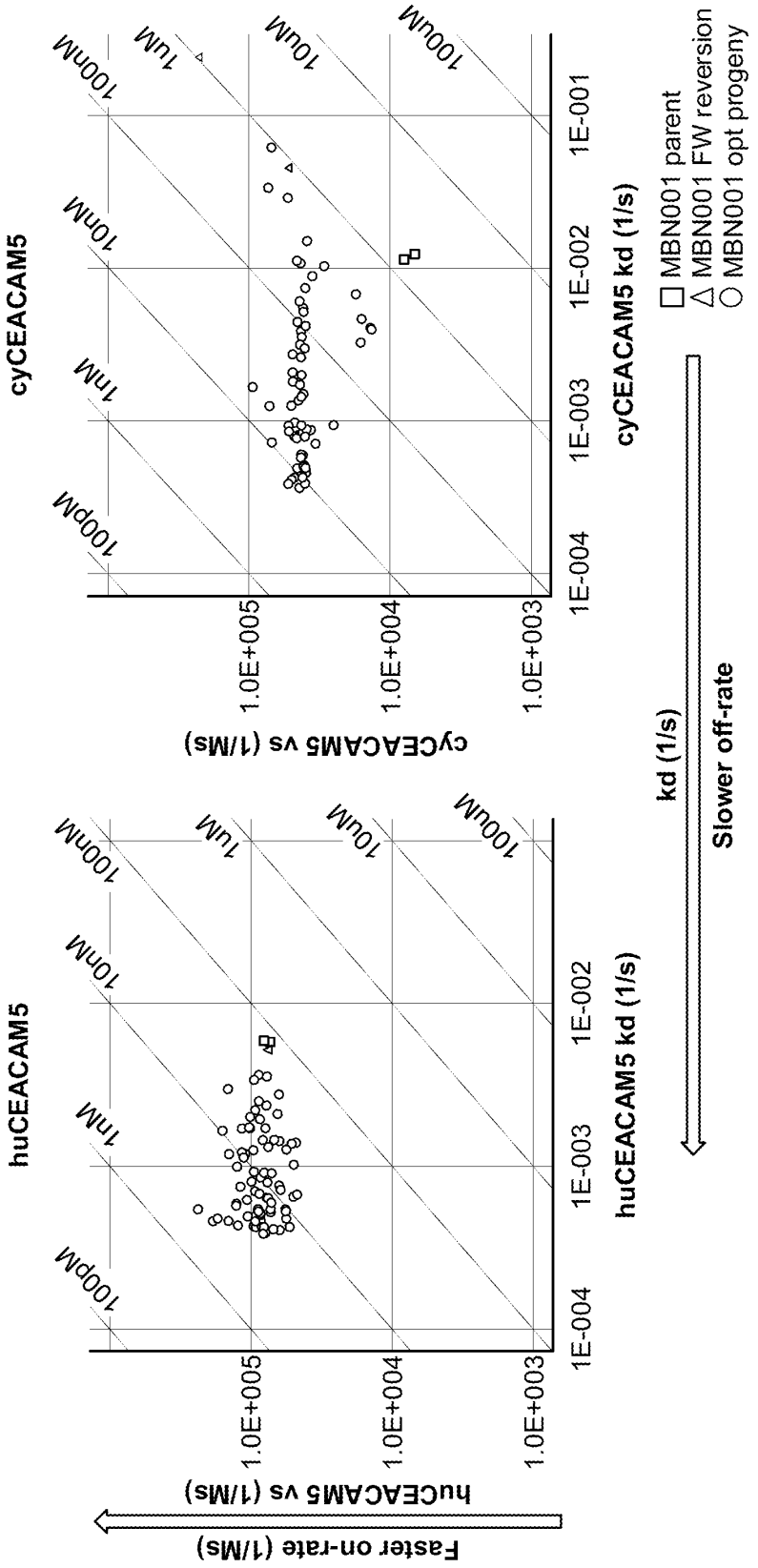

cyCEACAM5
huCEACAM5
cyCEACAM5 vs (1/Ms)
huCEACAM5 vs (1/Ms)
Faster on-rate (1/Ms)
1.0E+005
1.0E+004
1.0E+003
100pM
1nM
10nM
100nM
1uM
10uM
100uM
1E-004
1E-003
1E-002
1E-001
cyCEACAM5 kd (1/s)
huCEACAM5 kd (1/s)
kd (1/s)
Slower off-rate
MBN001 parent
MBN001 FW reversion
MBN001 opt progeny

FIG. 7A

FIG. 7B

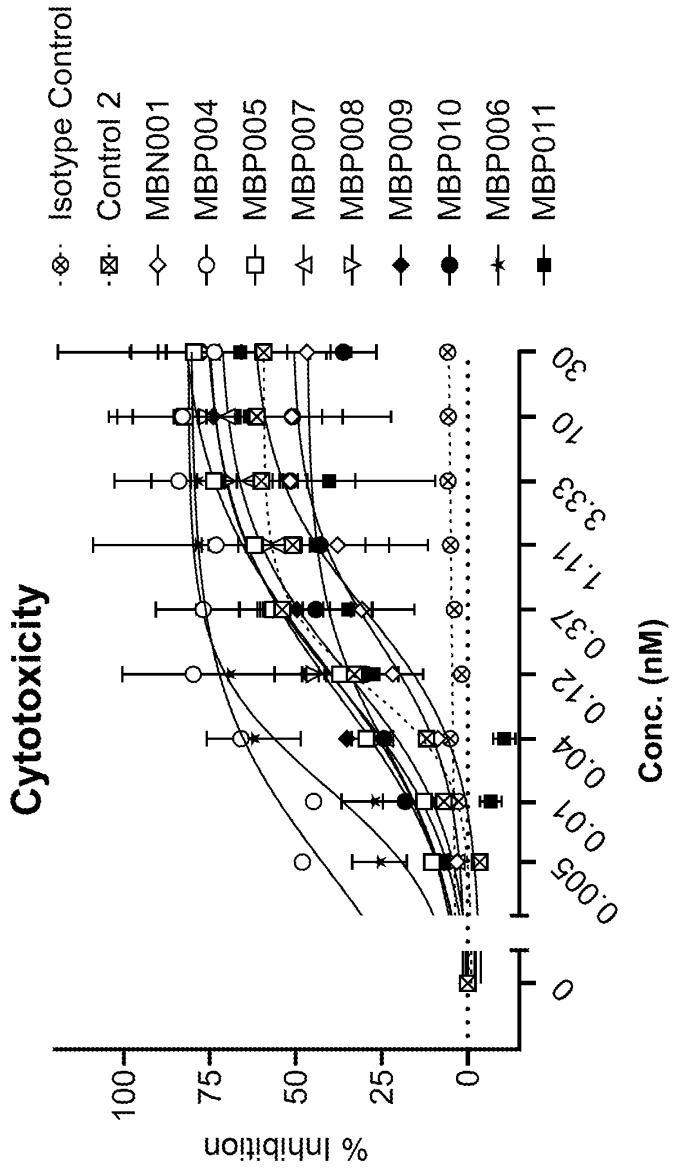

Cytotoxicity
% Inhibition
100
75
50
25
0
0
0.005
0.01
0.04
0.12
0.37
1.11
3.33
10
30
Conc. (nM)
Isotype Control
Control 2
MBN001
MBP004
MBP005
MBP007
MBP008
MBP009
MBP010
MBP006
MBP011

FIG. 8A

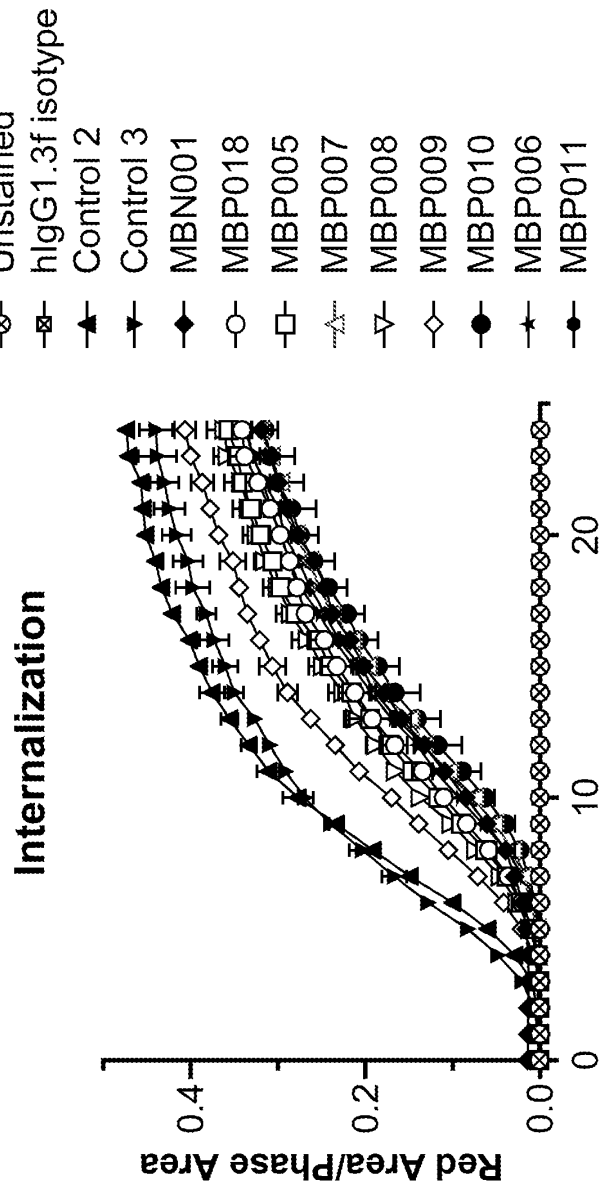

Unstained
hIgG1.3f isotype
Control 2
Control 3
MBN001
MBP018
MBP005
MBP007
MBP008
MBP009
MBP010
MBP006
MBP011
Internalization
Red Area/Phase Area
0.0
0.2
0.4
0
10
20
Time (hrs)

FIG. 8B

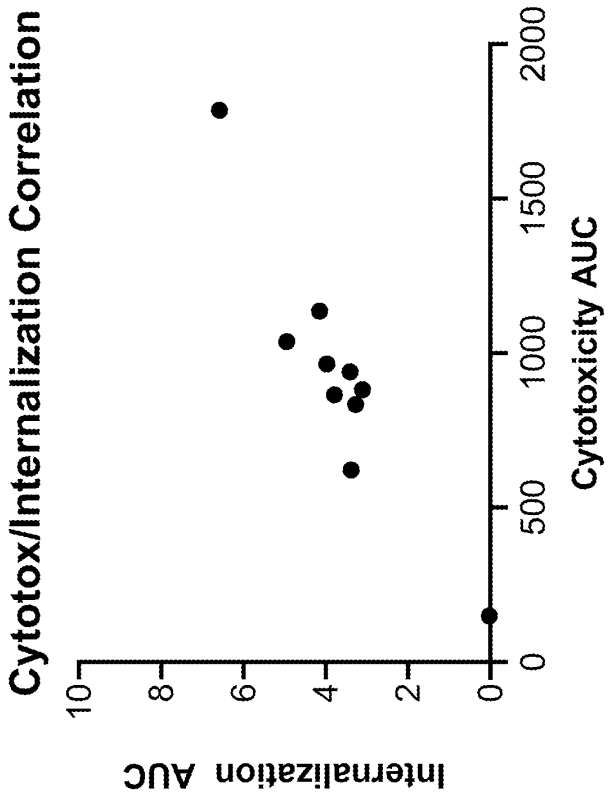

Cytotox/Internalization Correlation
Internalization AUC
0
2
4
6
8
10
0
500
1000
1500
2000
Cytotoxicity AUC

FIG. 8C

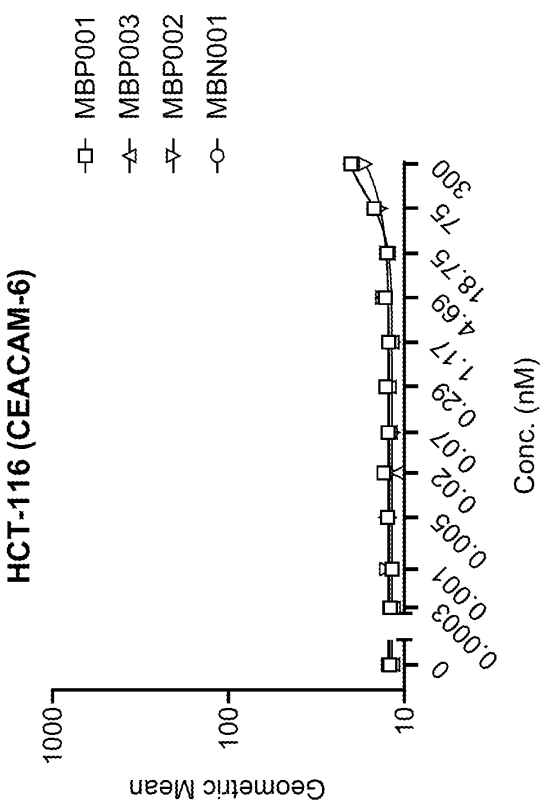

HCT-116 (CEACAM-6)
MBP001
MBP003
MBP002
MBN001
Geometric Mean
1000
100
10
Conc. (nM)
0
0.0003
0.001
0.005
0.02
0.07
0.29
1.17
4.69
18.75
75
300

FIG. 9B

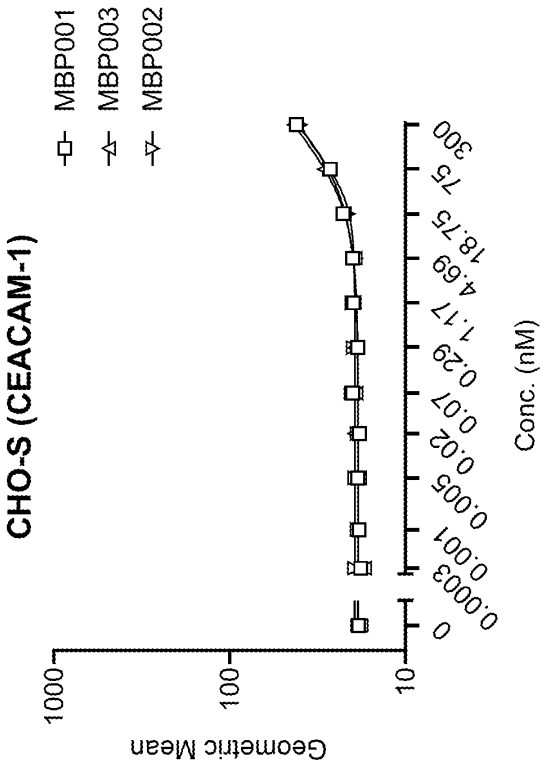

CHO-S (CEACAM-1)
MBP001
MBP003
MBP002
Geometric Mean
1000
100
10
Conc. (nM)
0
0.0003
0.001
0.005
0.02
0.07
0.29
1.17
4.69
18.75
75
300

FIG. 9A

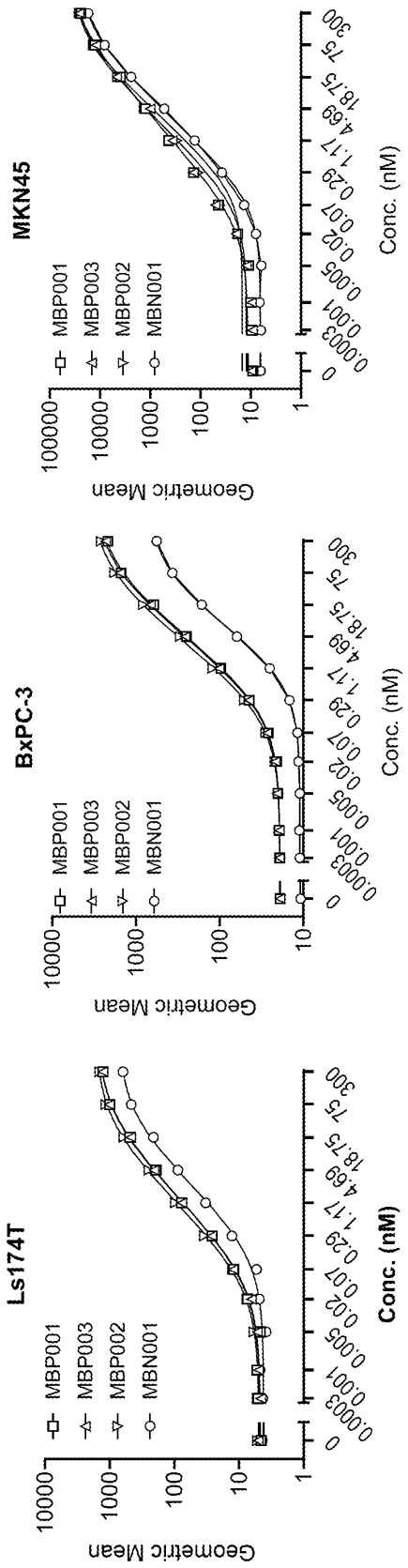


FIG. 10A

FIG. 10B

FIG. 10C

Clone name	LS174T EC50 (nM)	BxPC3 EC50 (nM)	MKN45 EC50 (nM)
MBN001	1.7	1.8	2.2
MBP003	1.7	1.9	2.4
MBP001	1.7	1.9	2.0
MBP002	1.5	1.9	2.1
MBN003	1.2	1.3	1.6

FIG. 10D

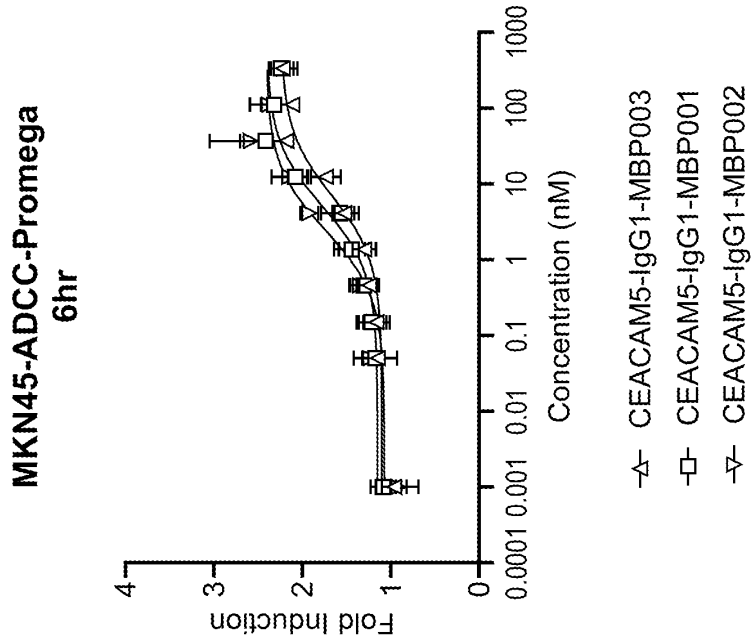


FIG. 11B

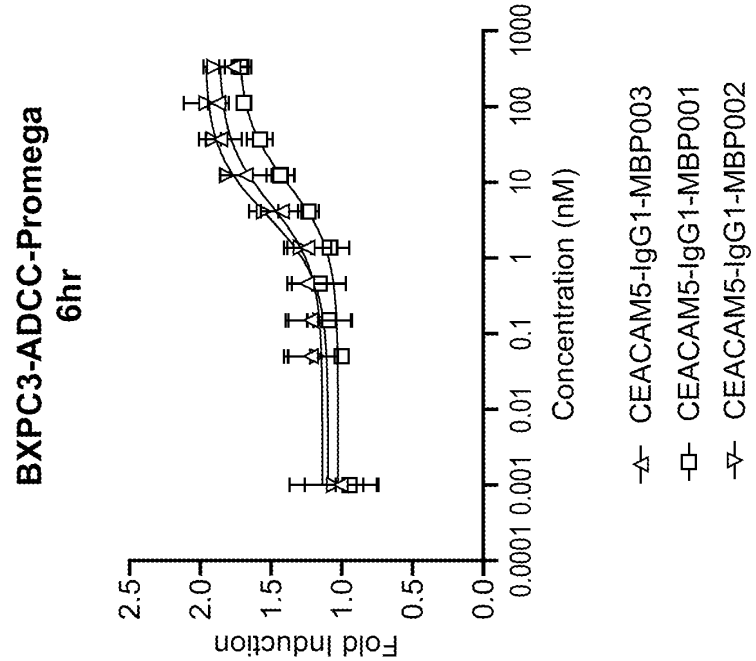


FIG. 11A

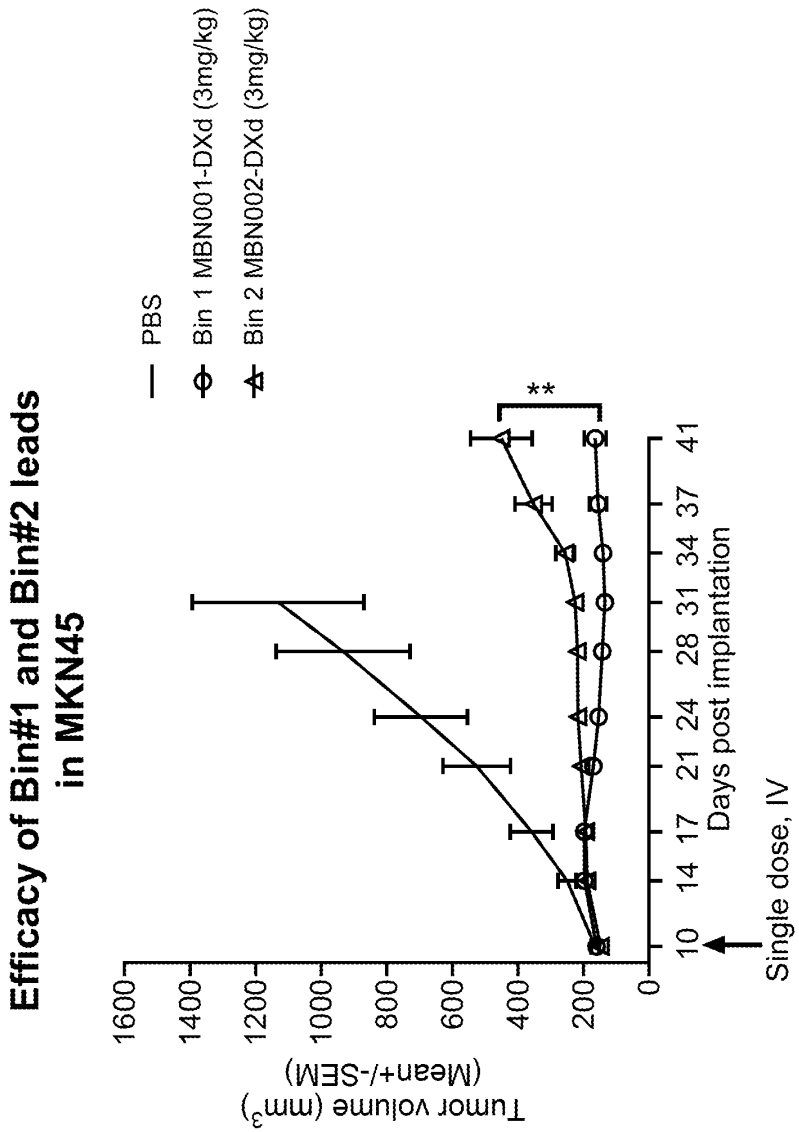

Efficacy of Bin#1 and Bin#2 leads in MKN45
PBS
Bin 1 MBN001-DXd (3mg/kg)
Bin 2 MBN002-DXd (3mg/kg)
Tumor volume (mm3) (Mean+/-SEM)
1600
1400
1200
1000
800
600
400
200
0
**
10 14 17 21 24 28 31 34 37 41
Days post implantation
Single dose, IV

FIG. 12

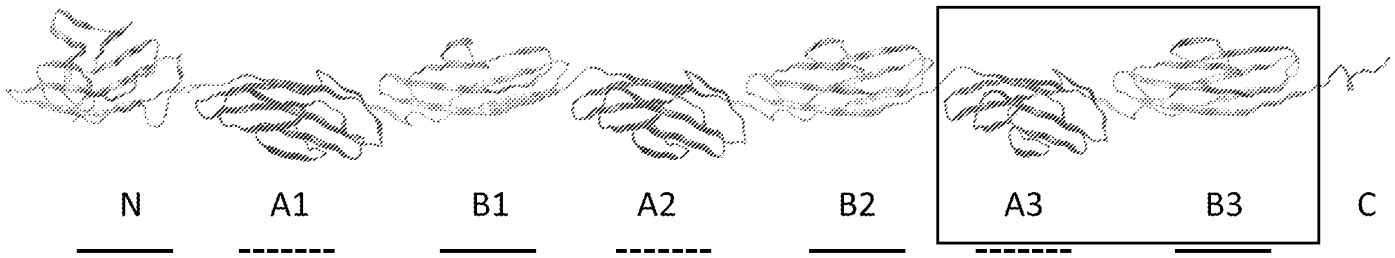


FIG. 13A

>sp|P06731|CEAM5_HUMAN

KLTIESTPFNVAEGKEVLLLVHNLPQHLFGYSWYKGERVDGNRQIIGYVIGTQQATPGPAYSGREIIYPNASLLI N

QNIIQNDTGFYTLHVIKSDLVNEEATGQFRVYPELPKPSISSNNSKPVEDKDAVAFTCEPETQDATYLWWVNNQS A1

LPVSPRLQLSNGNRTLTLFNVTRNDTASYKCETQNPVSARRSDSVILNVLYGPDAPTISPLNTSYRSGENLNLSC B1

HAASNPPAQYSWFVNGTFQQSTQELFIPNITVNNSGSYTCQAHNSDTGLNRTTVTTITVYAEPPKPFITSNNSNP A2

VEDEDAVALTCEPEIQNTTYLWWVNNQSLPVSPRLQLSNDNRTLTLLSVTRNDVGPYECGIQNELSVDHSDPVIL

NVLYGPDDPTISPSYTYYRPGVNLSLSCHAASNPPAQYSWLIDGNIQQHTQELFISNITEKNSGLYTCQANNSAS B2

GHSRTTVKTITVSAELPKPSISSNNSKPVEDKDAVAFTCEPEAQNTTYLWWVNGQSLPVSPRLQLSNGNRTLTLF A3

NVTRNDARAYVCGIQNSVSANRSDPVTLDVLYGPDTPIISPPDSSYLSGANLNLSCHSASNPSPQYSWRINGIPQ B3

QHTQVLFIAKITPNNNGTYACFVSNLATGRNNSIVKSITVSASGTSPGLSAGATVGIMIGVLVGVALI C

(SEQ ID NO: 103)

FIG. 13B

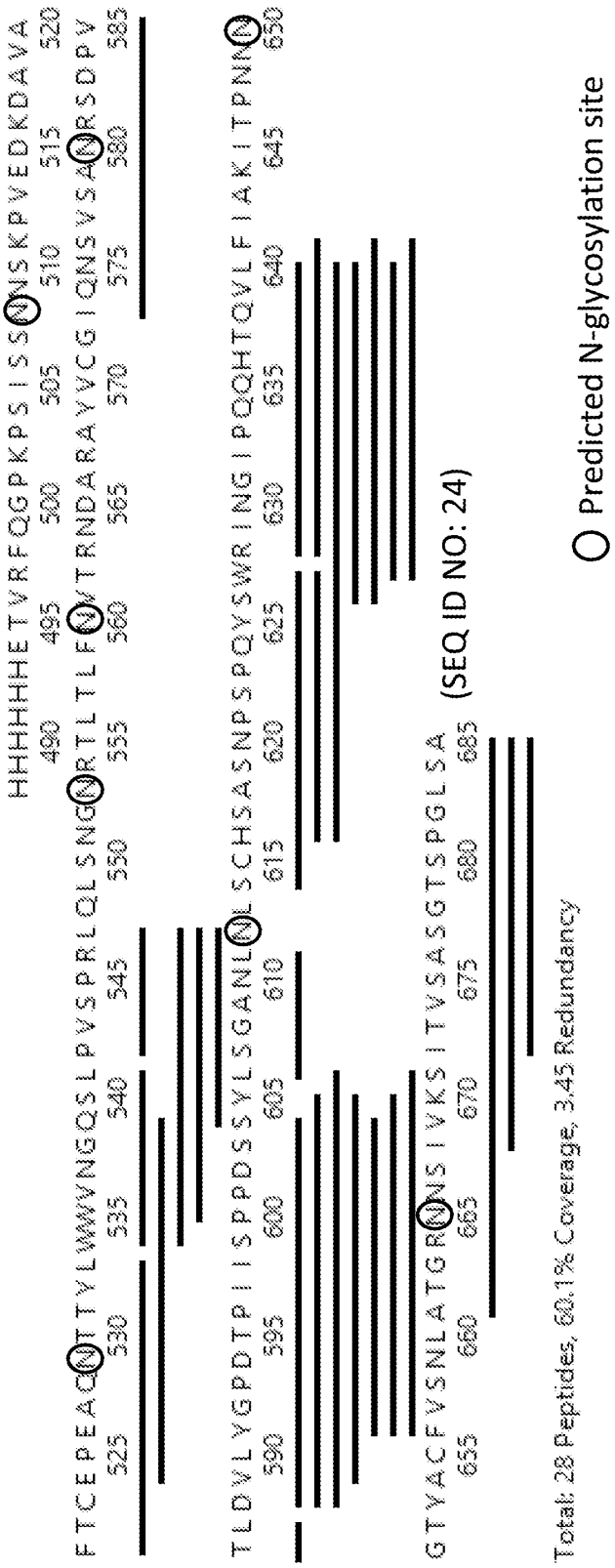

HHHHHHETVRFQGPKPSISSNSKPVEDKDAVA
490 495 500 505 510 515 520
FTCEPEACNTTYLWWVNGQSLPVSPRLQLSNGNRTLTLFNVTRNDARAYVCGIQNSVSANRSDPV
525 530 535 540 545 550 555 560 565 570 575 580 585
TLDVLYGPDTPIISPPDSSYLSGANLNLSCHSASNPSPQYSWRINGIPQQHTQVLFIAKITPNNN
590 595 600 605 610 615 620 625 630 635 640 645 650
GTYACFVSNLATGRNNSIVKSITVSASGTSPGLSA
655 660 665 670 675 680 685
Total: 28 Peptides, 60.1% Coverage, 3.45 Redundancy
(SEQ ID NO: 24)
Predicted N-glycosylation site

FIG. 13C

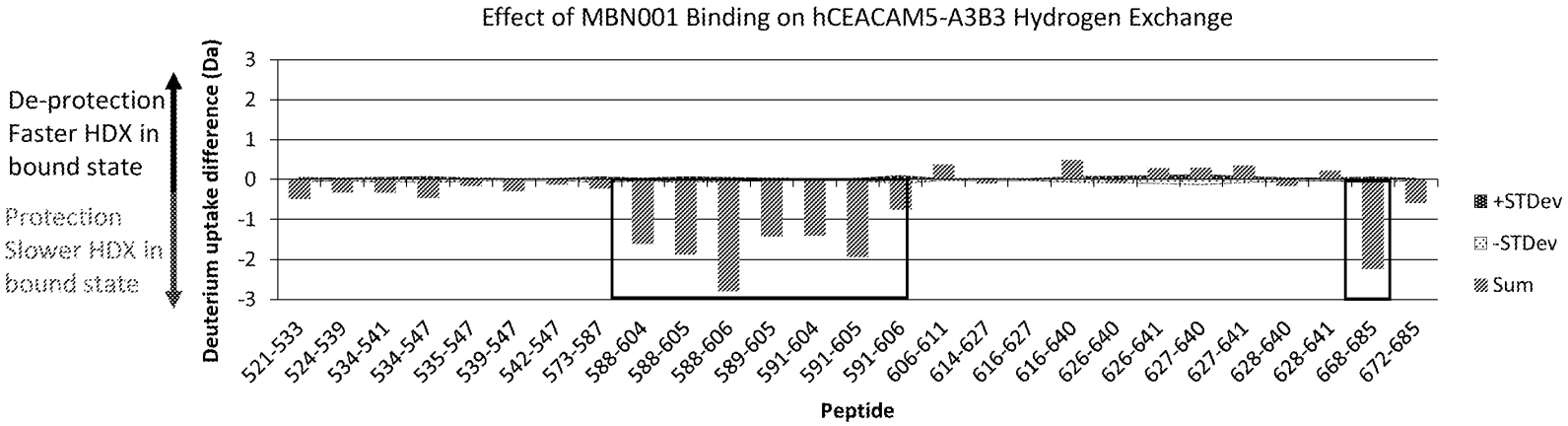


FIG. 13D

hCEACAM5 A3-B3-CT:
*HHHHHHETVRFQGPKPSISSNNSKPVEDKDAVA*FTCEPEAQNTTYLWWVNG*QSLPVSPRLQLSNGNRTLTLFNVTRNDA*
*R*AYVCGIQNSVSANRSDPVTL*DV***LYG***P***DT***P***IIS***PP***DSSYL***SGANL*NL*SCHSASNPSPQYSWRINGIPQQHTQVLF*IAKITPNN*
*NGTYACFVSNL*ATGRNNSIV***KSITVSASGTS***P***GLSA*** (SEQ ID NO: 24)

FIG. 13E

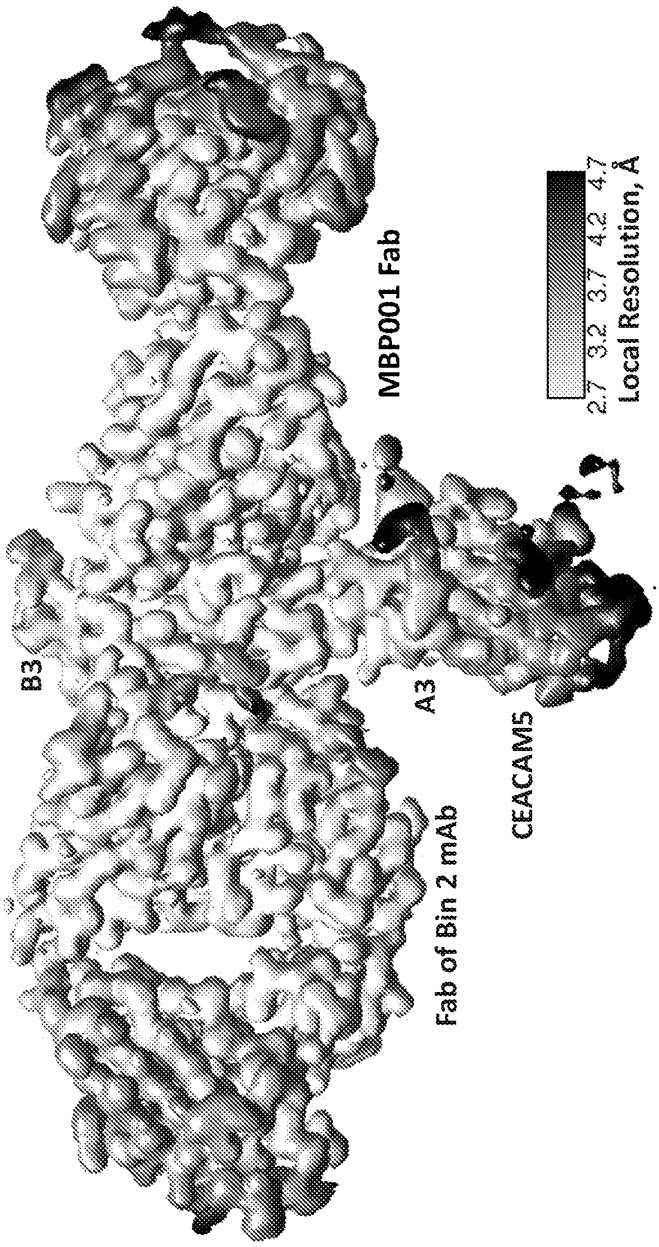

MBP001 Fab
B3
A3
CEACAM5
Fab of Bin 2 mAb
2.7 3.2 3.7 4.2 4.7
Local Resolution, Å

FIG. 14A

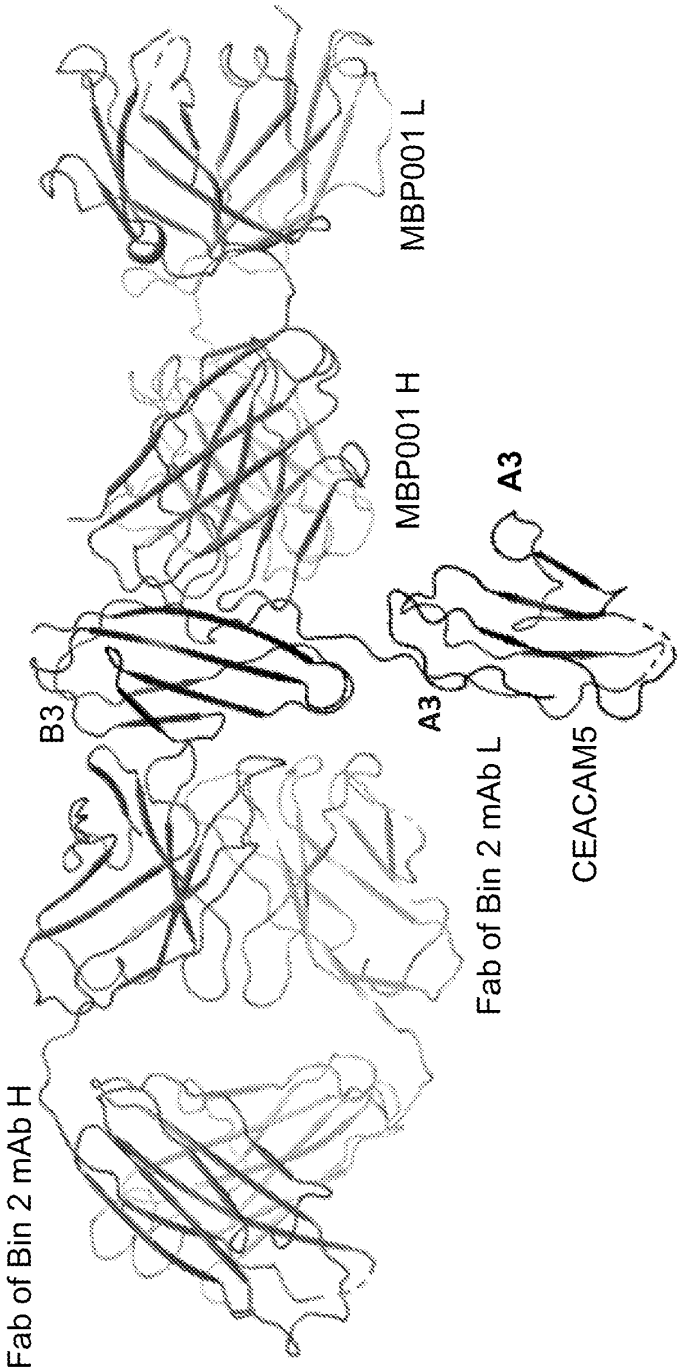
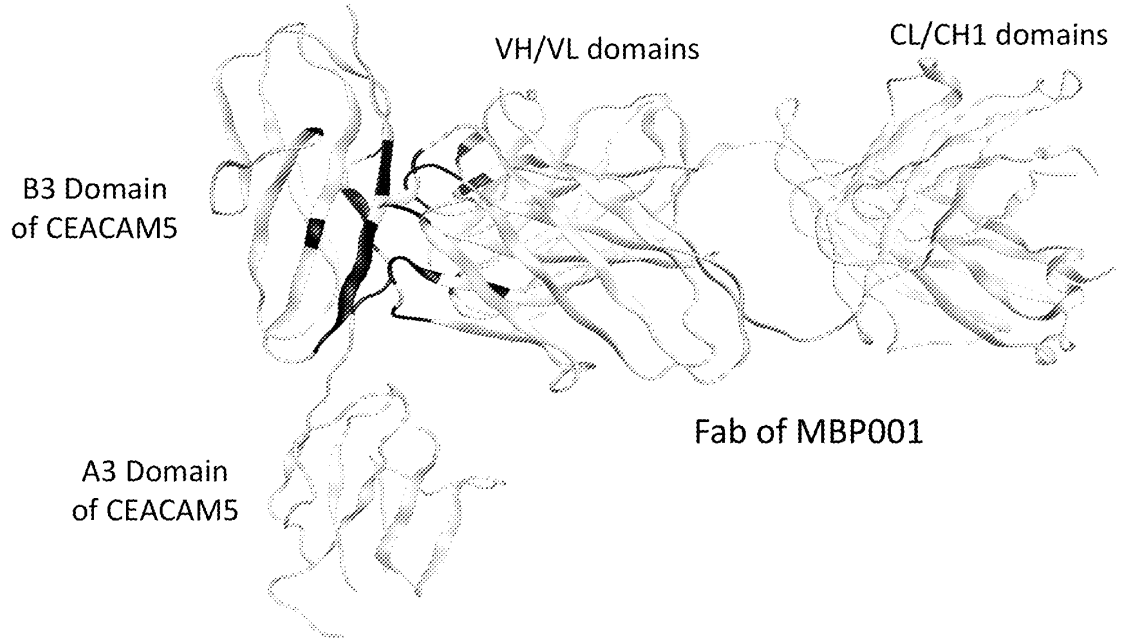

MBP001 L
MBP001 H
A3
A3
B3
Fab of Bin 2 mAb L
CEACAM5
Fab of Bin 2 mAb H

FIG. 14B



6xHis-A3-B3 huCEACAM5 (MBP001 epitope residues bolded)
HHHHHHETVRFQGPKPSISSNNSKPVEDKDAVAFTCEPEAQNTTYLWWVNGQSLPVSPRLQLSNGNRTLTLFNVTRNDARAYVCGIQNSVSANRSDPVTLDVLYG**PDT**P**II**SP**P**DSSY
LSGANLNLSCHSASNPSPQYSWRI**N**GIPQQHTQVLFIAKITPNNNGTYAC**F**VSNLAT**GRNNS**I**VK**SITVSASGTSPGLSA (SEQ ID NO: 24)

MBP001 VL (paratope residues bolded; CDRs underlined)
EIVLTQSPGTLSLSPGERATLSCRASQSVSS**S**YLAWYQQKPGQAPRLLI**Y**GAS**S**RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY**G**SSPYTFGQGTKLEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 46)

MBP001 VH (paratope residues bolded; CDRs underlined)
QVQLVESGGGVVQPGRSLRLSCAASGI**Y**FS**SH**GMHWVRQAPGKGLEWVT**F**I**SYD**G**SY**KSYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATG**LTGT**GAFDIWGQGTMV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCGG (SEQ ID NO:
100)

FIG. 14C