

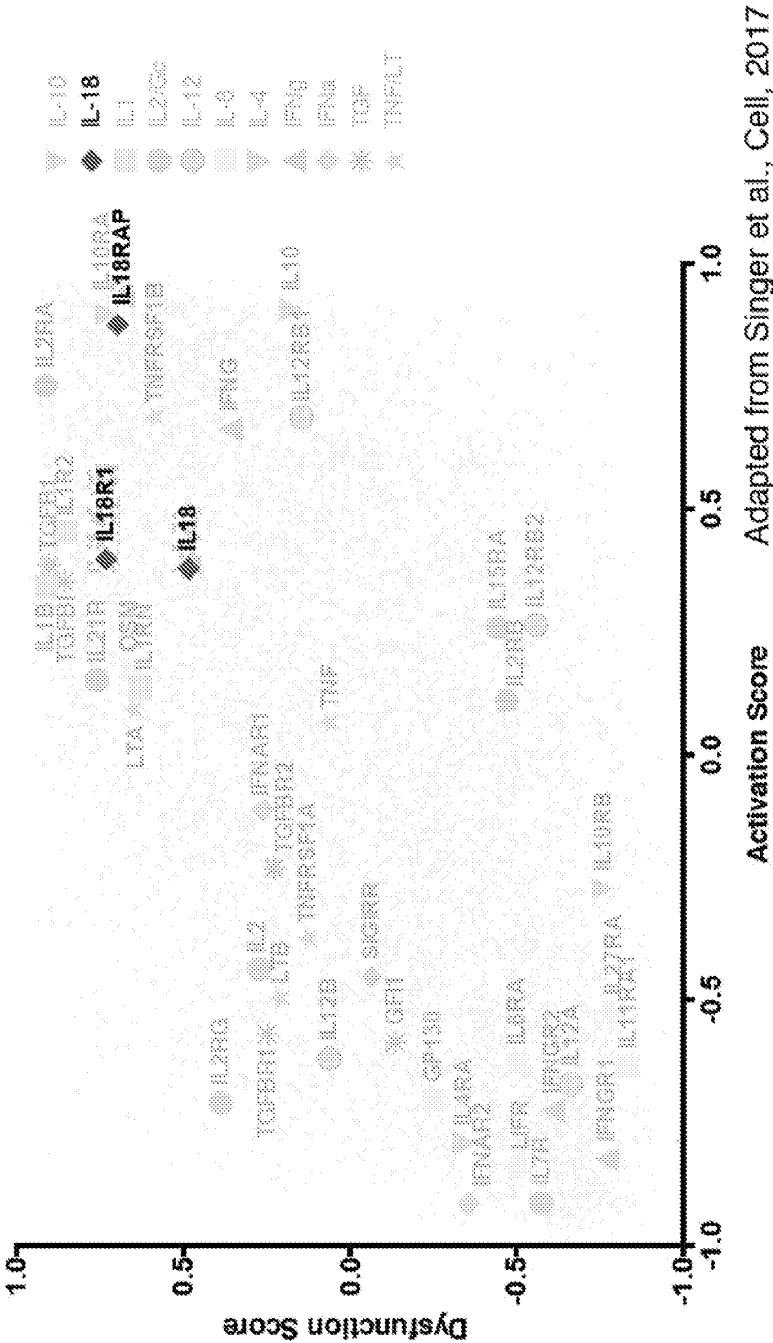


FIG. 1A

Adapted from Singer et al., Cell, 2017

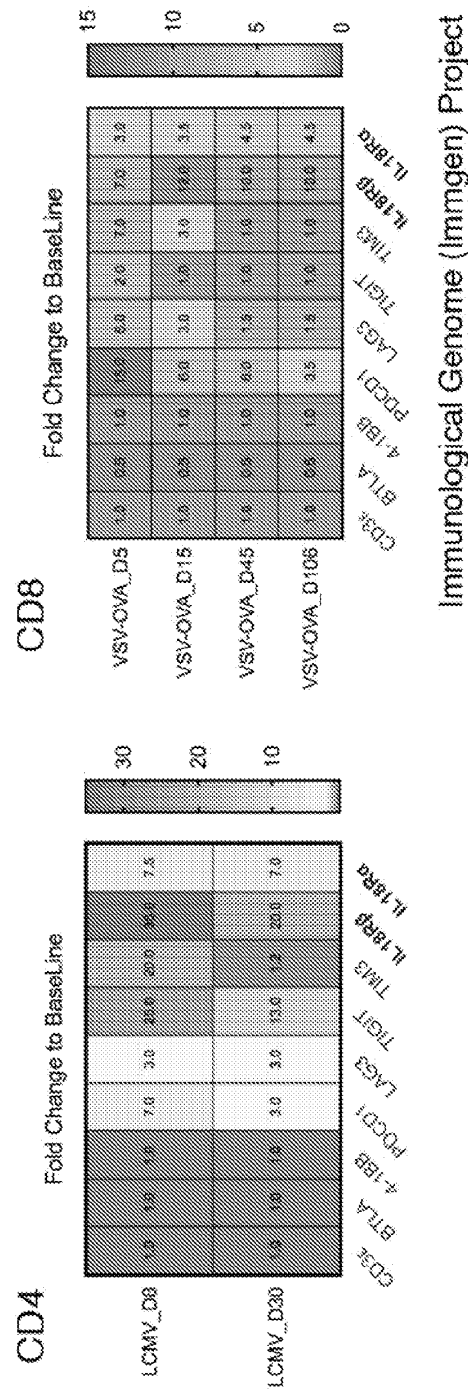
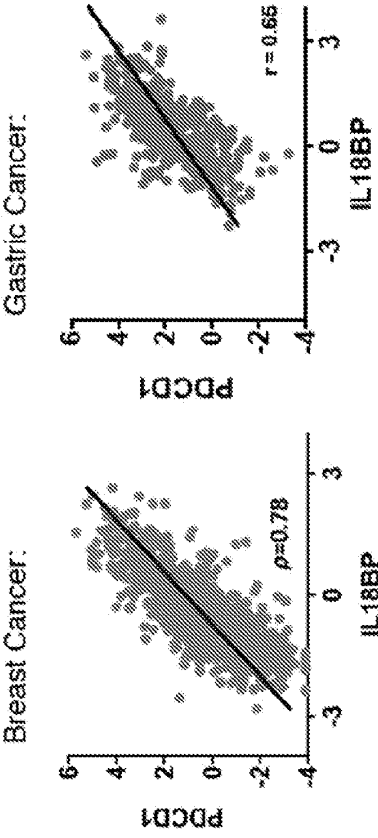
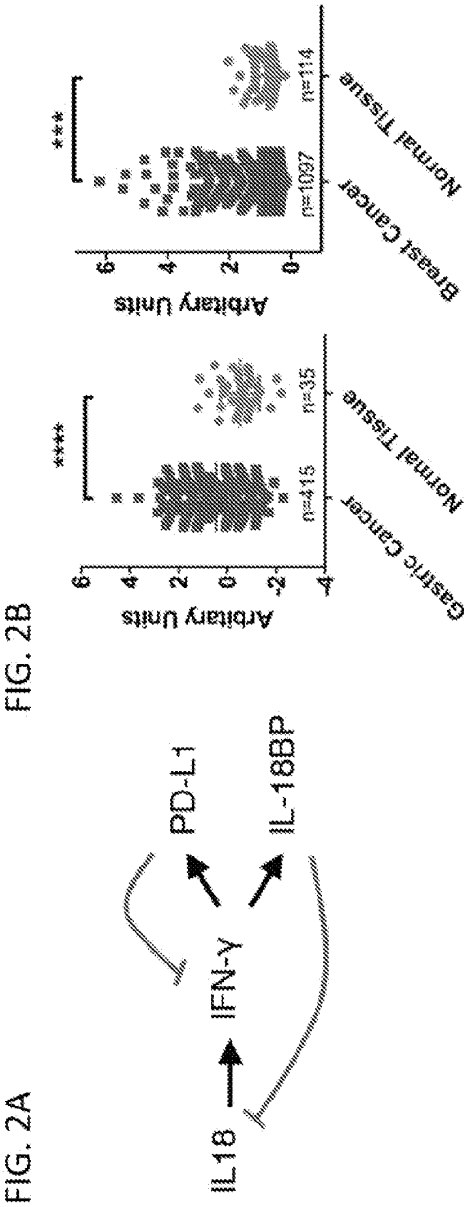


FIG. 1B



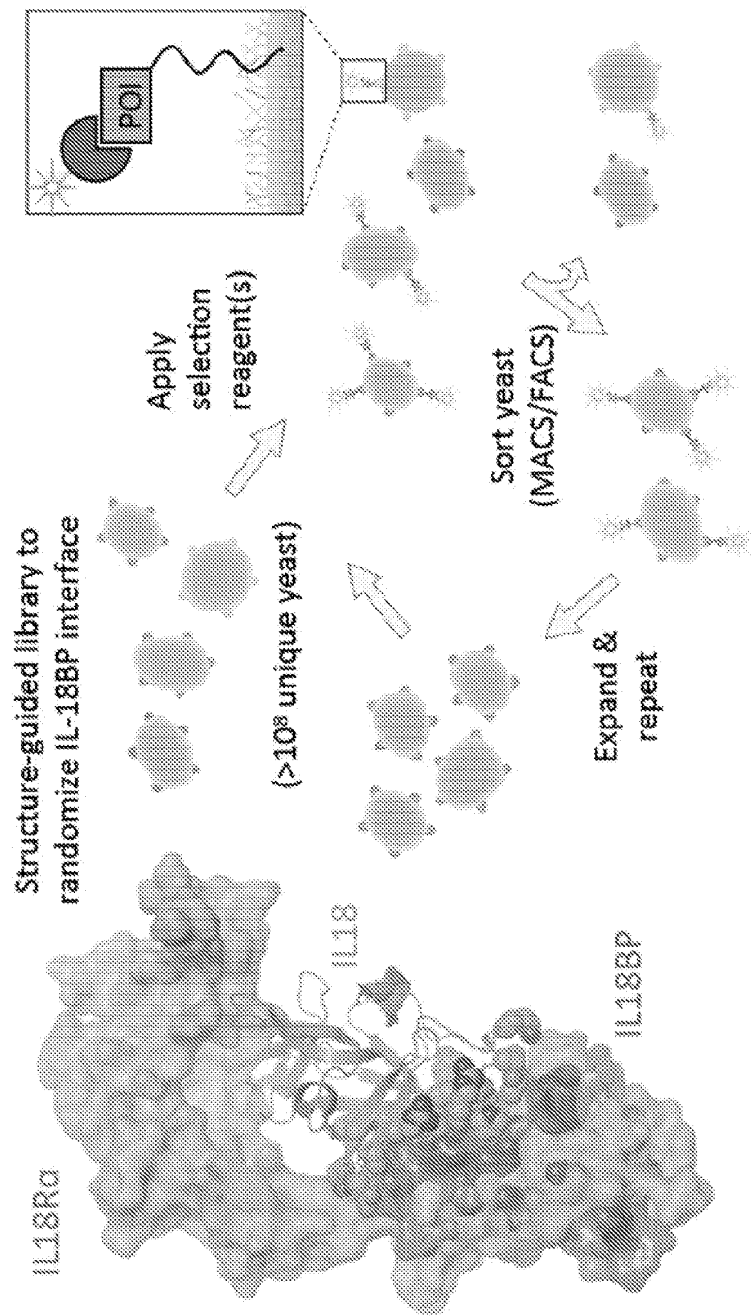


FIG. 3A

FIG. 3B

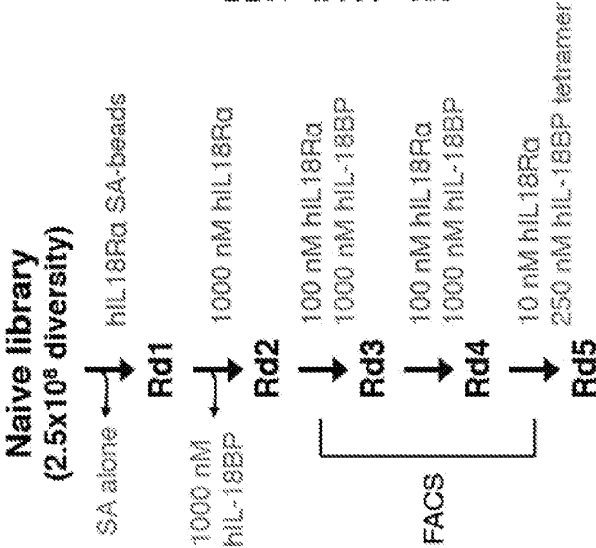
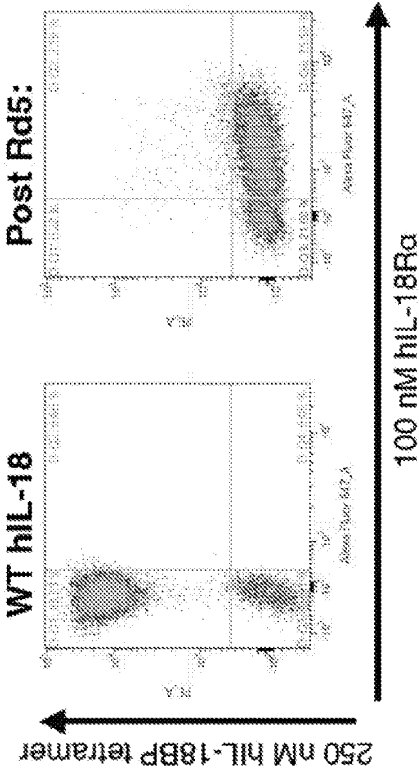


FIG. 3C



A

Human DR-IL-18 variants sequence summary

WT hIL-18	1	5	8	51	53	55	59	60	77	103	105	110	111	153	155	SEQ ID NO:
	Y	L	K	M	K	S	G	M	E	Q	S	D	N	V	N	
hC4			Q	T		K	T	K			R	H	H	I	K	38
hA8			R	K		K	A	Q			D	K	H	I		39
hD6			R	Q		K	A	K			D	K	H	I		40
hH12				T	R			K			D	N	H	T		41
hB11		H		K		K	A	Q			D	N	H		K	42
hC3		I		T		R		K		E	D	H	H	I		43
hC2		I		T		K		K			D	H	H	T	H	44
hG10		I		T		K	A	K			R	H	H	I	K	45
hG1		I		T		K		K			D		Y	I	K	46
hF1		Y	R	T	R			K			D	E	H		K	47
hD2	H	Y		T	R			K			D	H			K	48
hA1	R			T	R		A	K			D	Q	H	A	K	49
hB3	R		R	T	R			R		K	N	K	Y		H	50
hB4	R			D	R			Q		K	R	N	H		K	51
hH3	R		R	N	R			K		K	D	K	H			52
hC5	R		R	T	R		T	K			K	N	H		K	53
hH4	R		R	T	R		A	K		E	D	Q	H	I	K	54
hE1	R		R	T	R		T	K			N	H	D		H	55
hG2	R		R	T	R		A	K		E	D	Q	H	I	K	56
hB9	R	H		T	R			K		E	N	K	Y			57
hE12	R	Y		T	R		T	K	D		D	K	H			58
hCS1				T		K	A	K			D	K	H	I		34
hCS2	R			T				K			D	K	H			35
hCS3				T				K			D	K	H			36
hCS4	R			T	R			K			N	K	Y			37

Selected Variants

Consensus Variants

FIG. 4

FIG. 5A

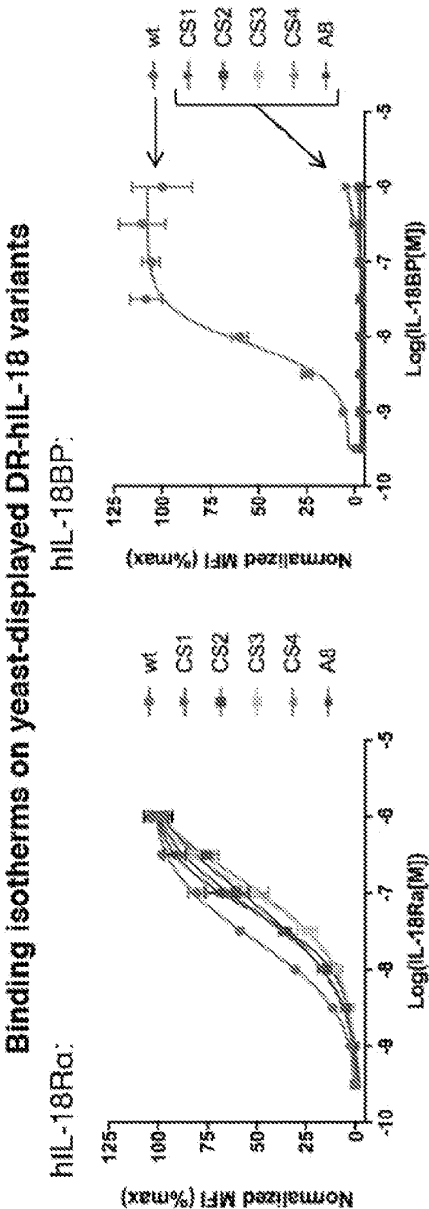


FIG. 5B

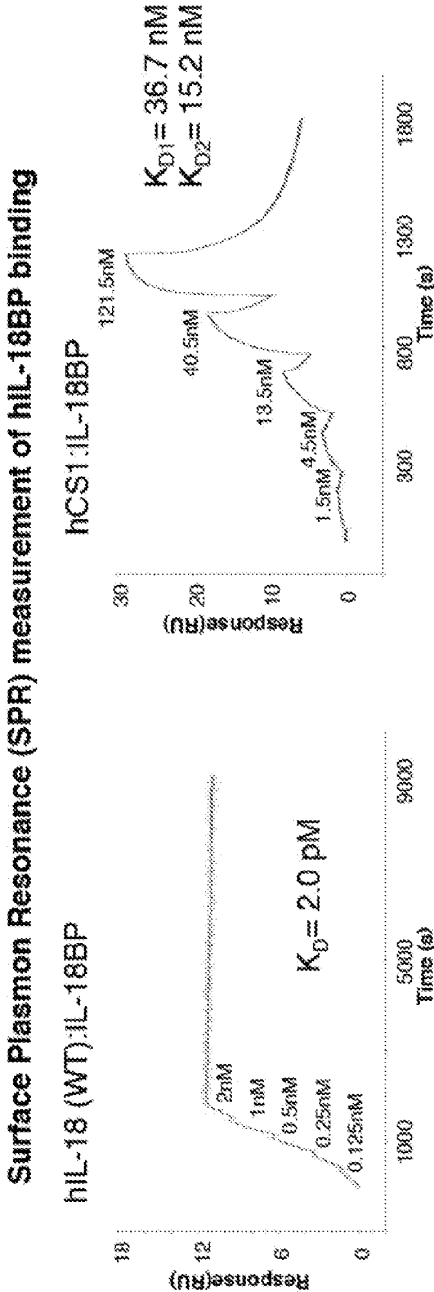


FIG. 6A

hIL-18Ra binding assays on-yeast

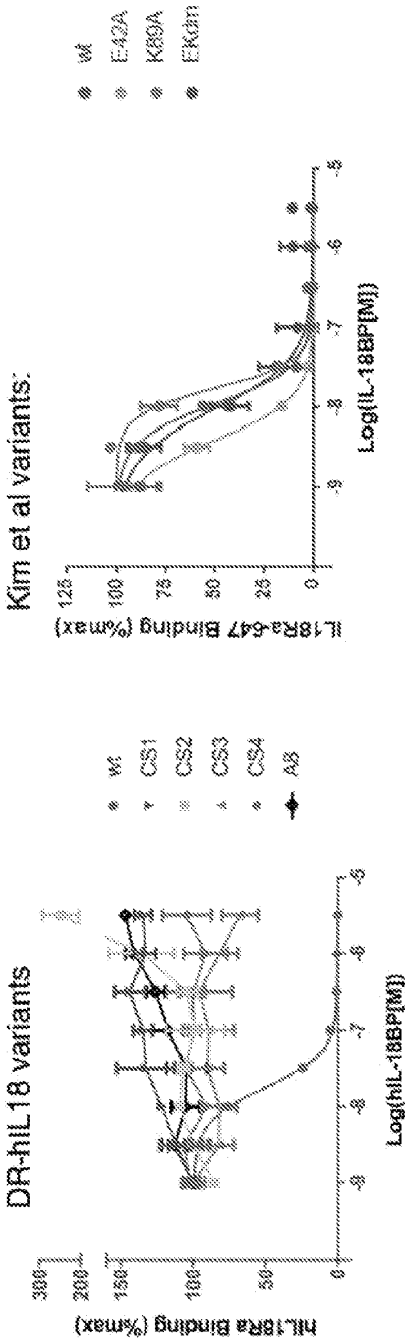
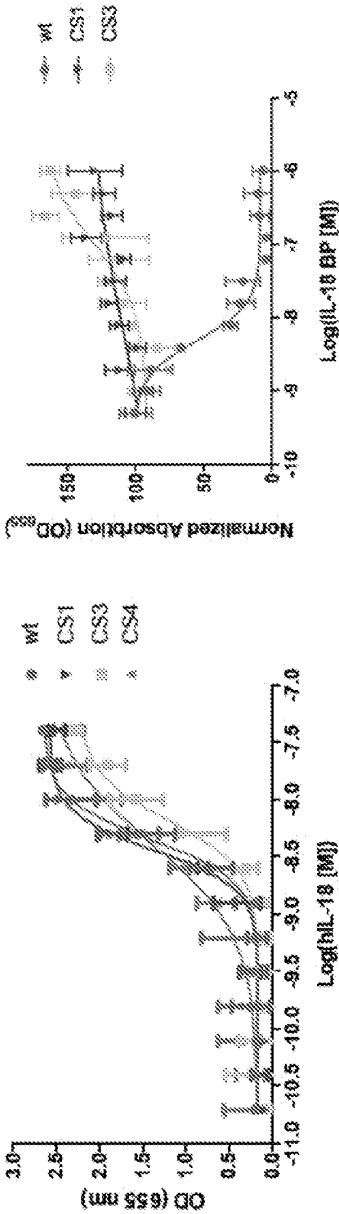
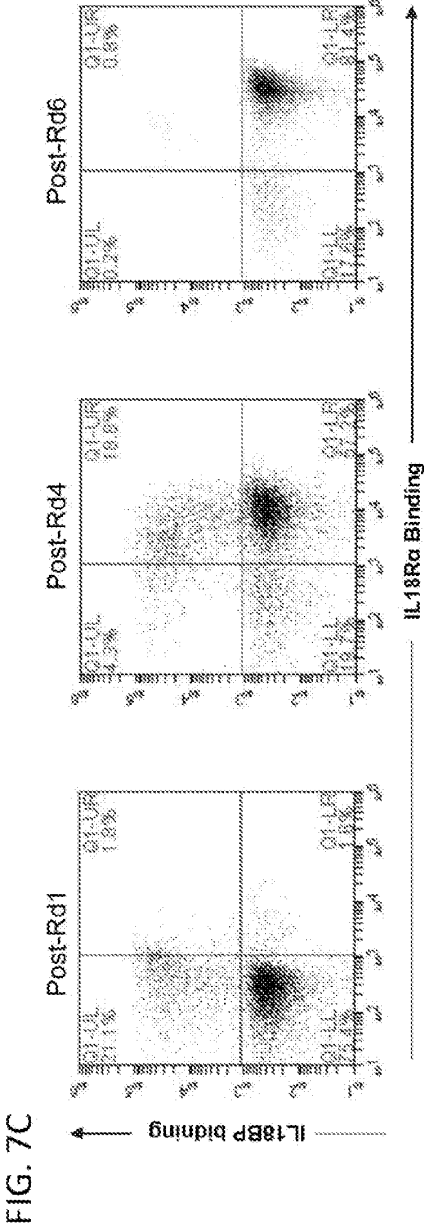
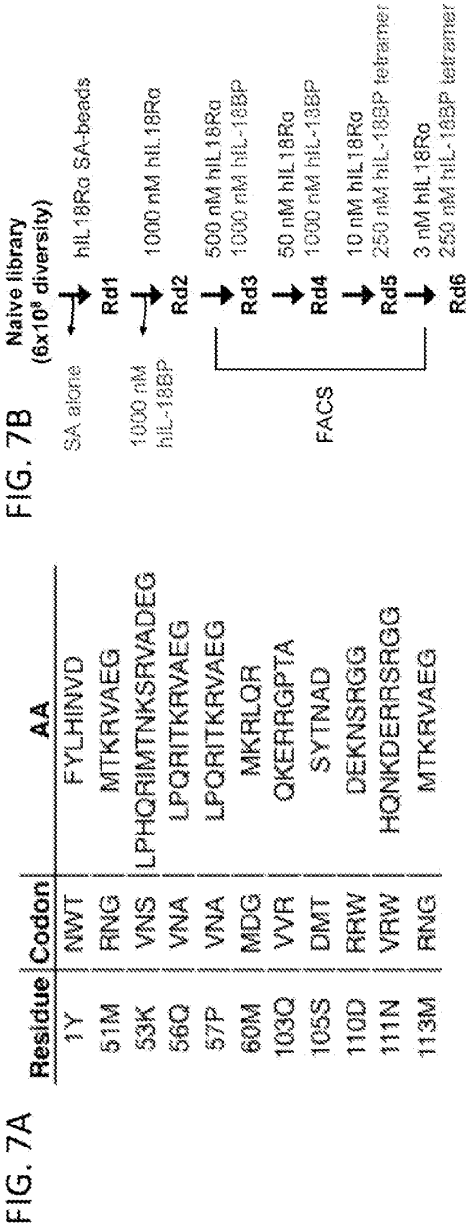


FIG. 6B

HEK Blue IL-18 signaling assay





WT hIL-18		51	53	56	57	60	103	105	110	111	113	SEQ ID NO:
		M	K	Q	P	M	Q	S	D	N	M	
Round 5 variants	5-18	E		E	L	R	P	A	N	R	V	73
	5-29	K		A	G	L	E	D	S	S	R	74
	5-8	K	G	A	A	L			K	R		75
	5-6	K	G	R	G	L	E	D	N	S	R	76
	5-26	K	G	V		L	A	A	S	R	T	77
	5-20	K	S	G	A	L	A	A	G	R	T	78
	5-2	K	S	K	A		A	D	S	S	R	79
	5-9	K	S	L	A	L		D	S	R		80
	5-42	K	S	R	A	L		N	G	R		81
	5-17	K	S	R	A	L	A		G	R	T	192
	5-41	K	S	R	A	L	A	D	S	G	R	193
	5-1	K	T	R		L	E	D	S	S	K	84
	5-33	K	T	R	A		E	D	N	D	R	85
	5-21	R		G	K	L	R		S	R	V	86
Round 6 variants	6-31	K	G	G	A	L	E	D	S	G	V	87
	6-20	K	G	R				A	N	R		88
	6-12	K	S	L	A	L		D	S	R		89
	6-27	K	S	R	A	L	A		G	R	T	90
	6-29	K	S	R	A	L		N	G	R		91

FIG. 8

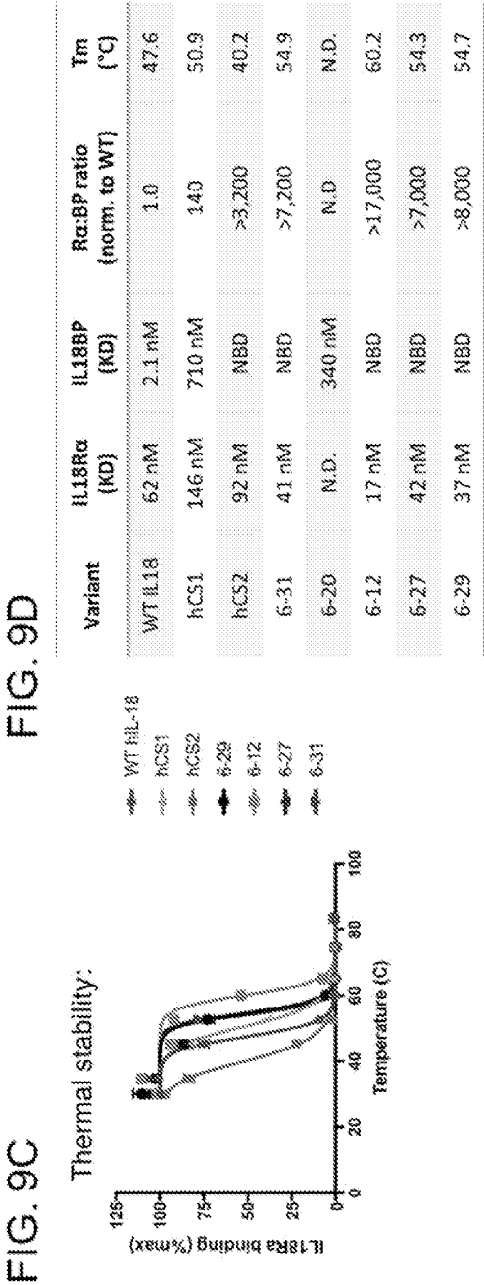
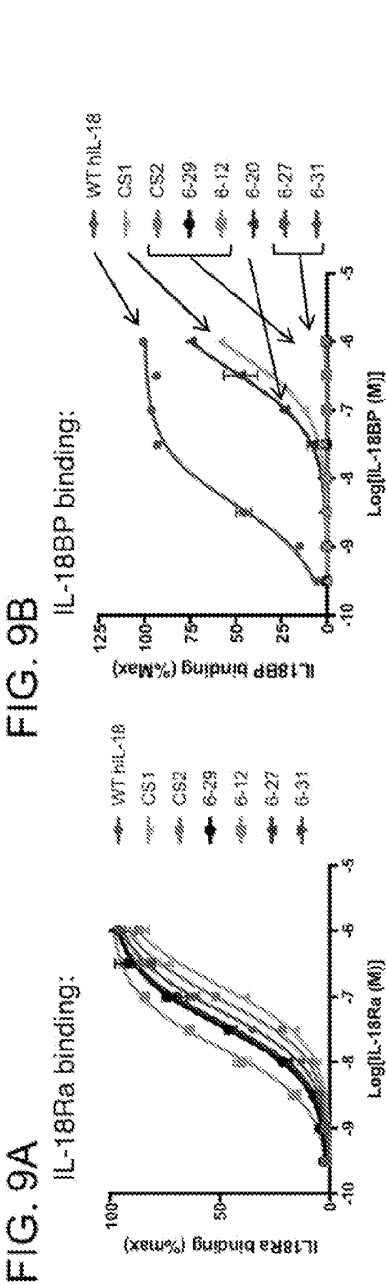


FIG. 10A

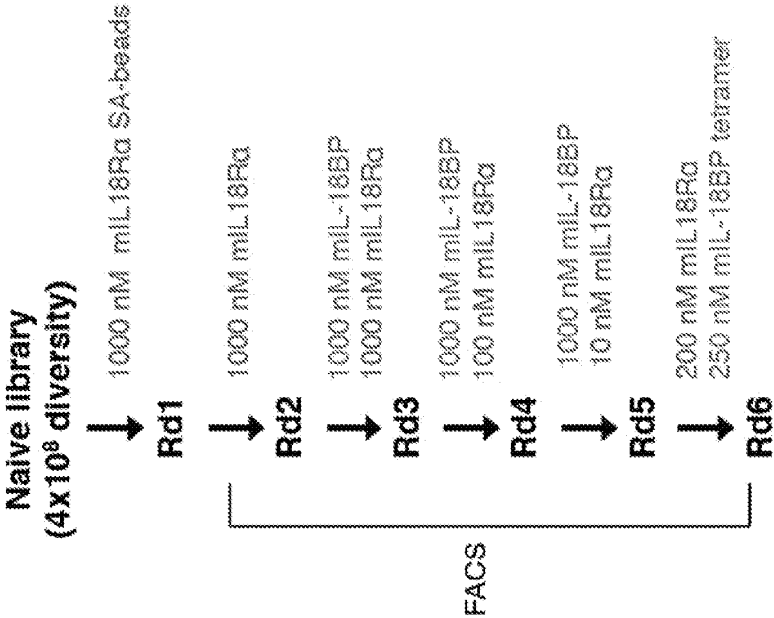


FIG. 10B

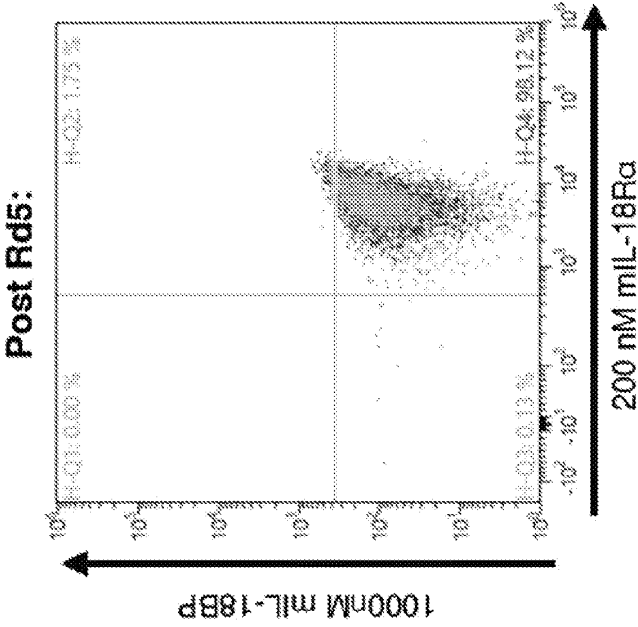


FIG. 10C

C

Murine DR-IL-18 variants sequence summary

mIL-18	1	50	51	52	54	55	56	57	58	59	104	109	151	SEQ ID NO:
	N	M	Y	K	S	E	V	R	G	L	R	N	L	
mC1		A		V	R	R	L			K	K			62
mA12		S		S	K	H	M			K	L			63
mE8		V		T	G	R	R			K	K	D		64
mC10	H	A		G		H	M			K	Q		V	65
mB7	H	A		G		N	A	G		R	K			66
mB1	H	G		A		D	A	A		K	S		V	67
mD1	H	G		S	R	G	S	K		K	Q			68
mH7	Y			A	R	R	A			K	K	D	V	69
mA7	Y	A		G	N	R				K				70
mE1	Y	G		G			R	G		R		D		71
mH3	Y	T	R	T	G	G	Q	K		V		D		72
mCS1		G		A		R				K				60
mCS2	H	A		G		R	A			K				61

Selected Variants

Consensus Variants

FIG. 11A

Binding isotherms on yeast-displayed cytokines

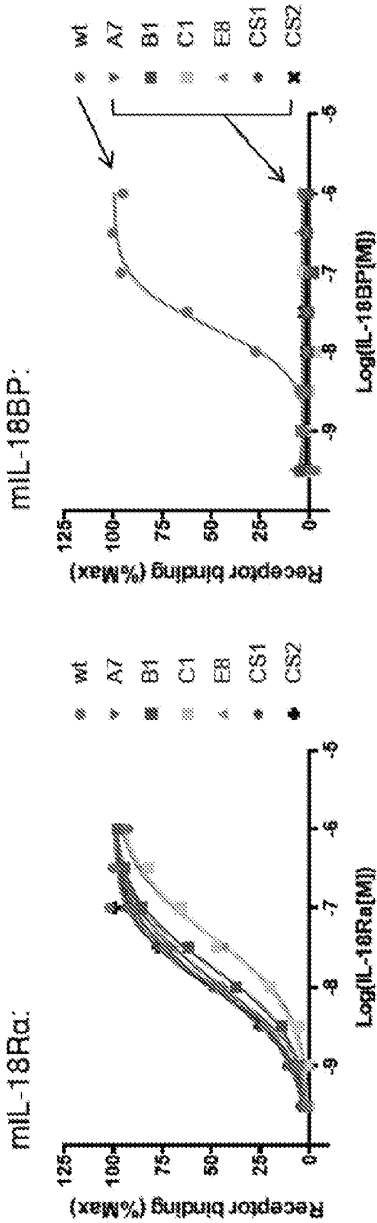


FIG. 11B

Surface Plasmon Resonance (SPR) measurement of mIL-18BP binding

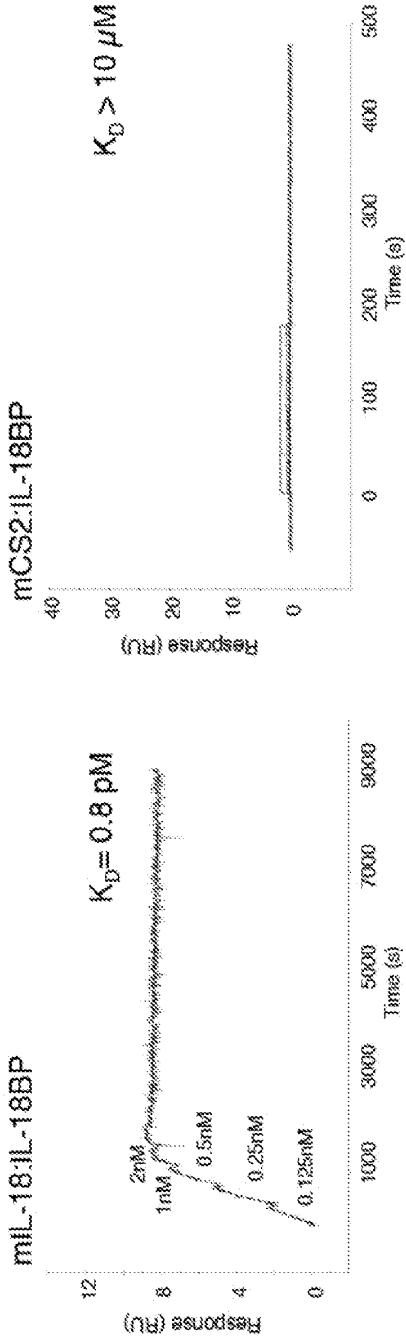


FIG. 12A

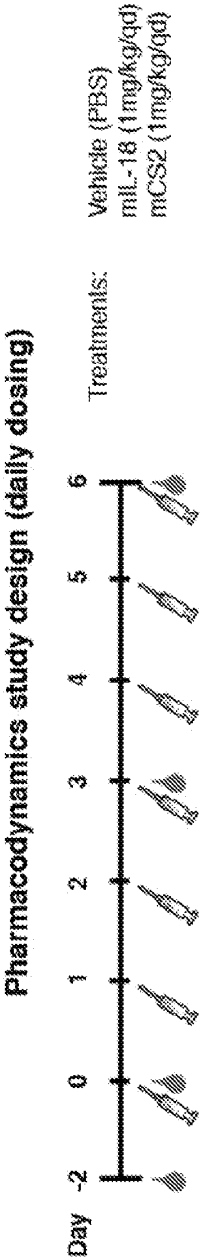
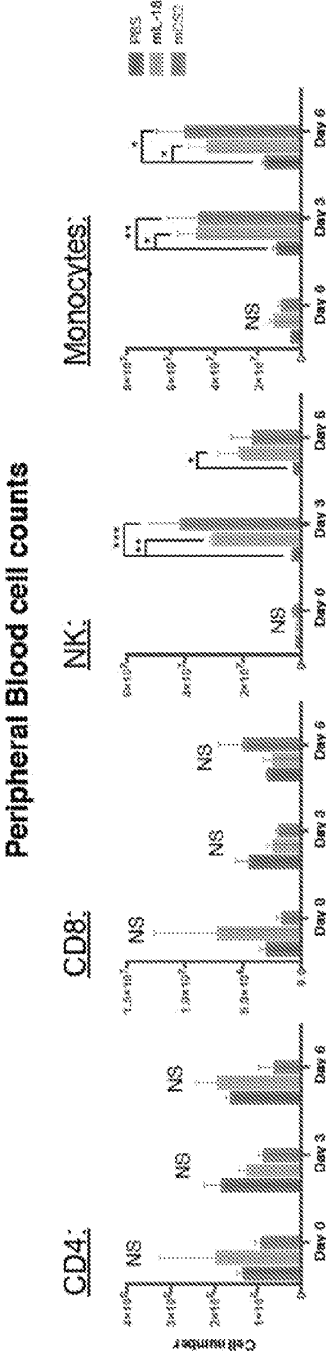
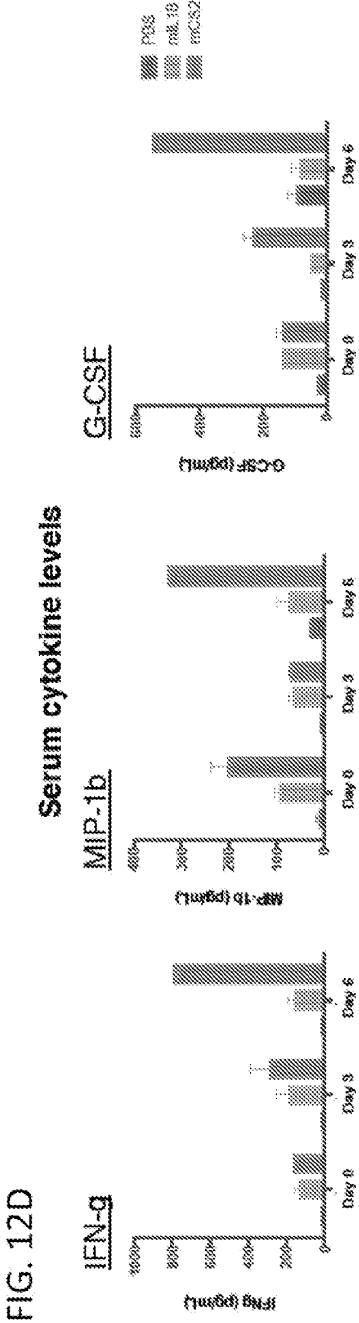
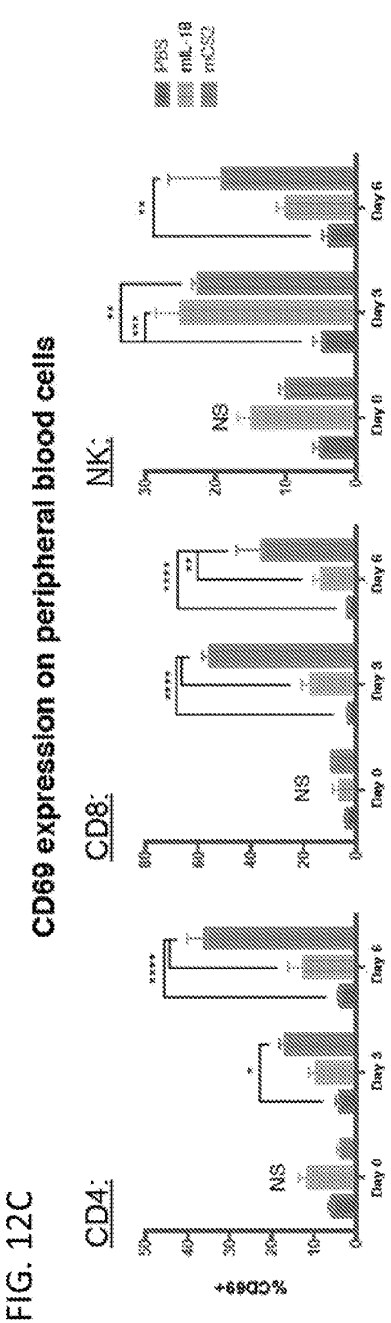


FIG. 12B





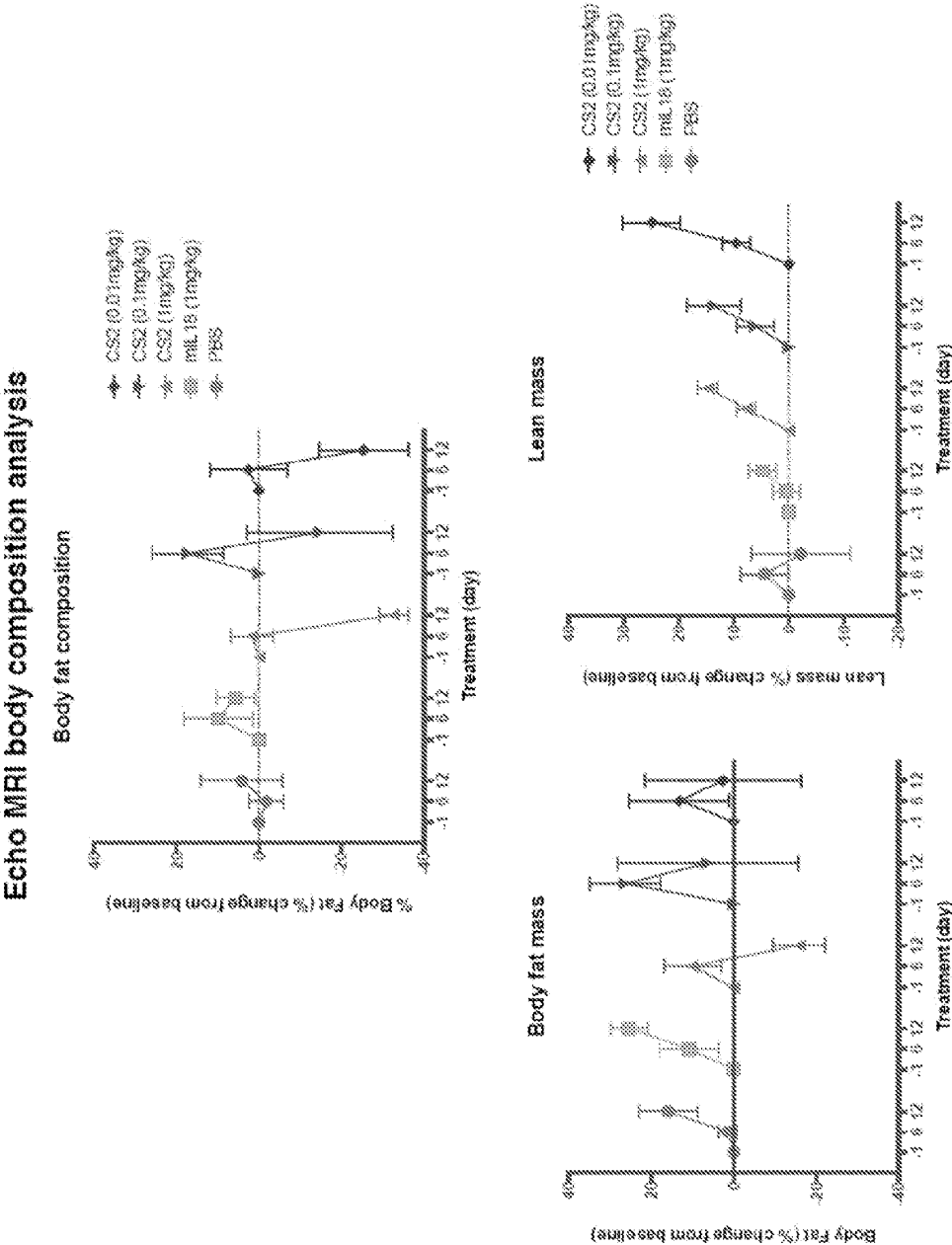


FIG. 13

Yummer1.7 melanoma treatment model-Tumor Growth

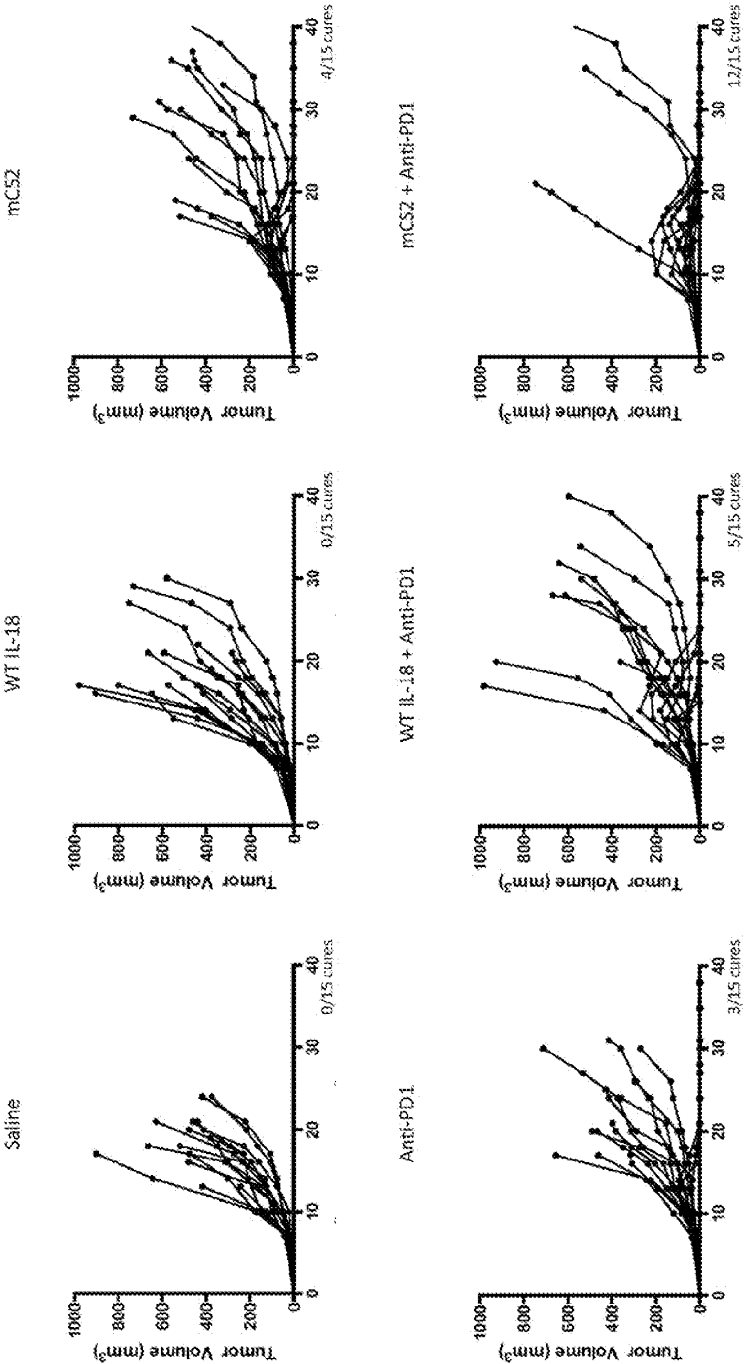


FIG. 14A

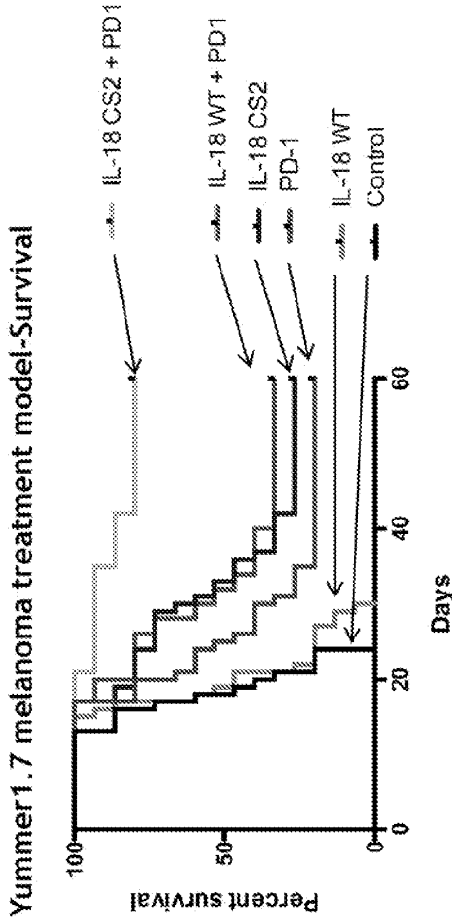


FIG. 14B

FIG. 15A

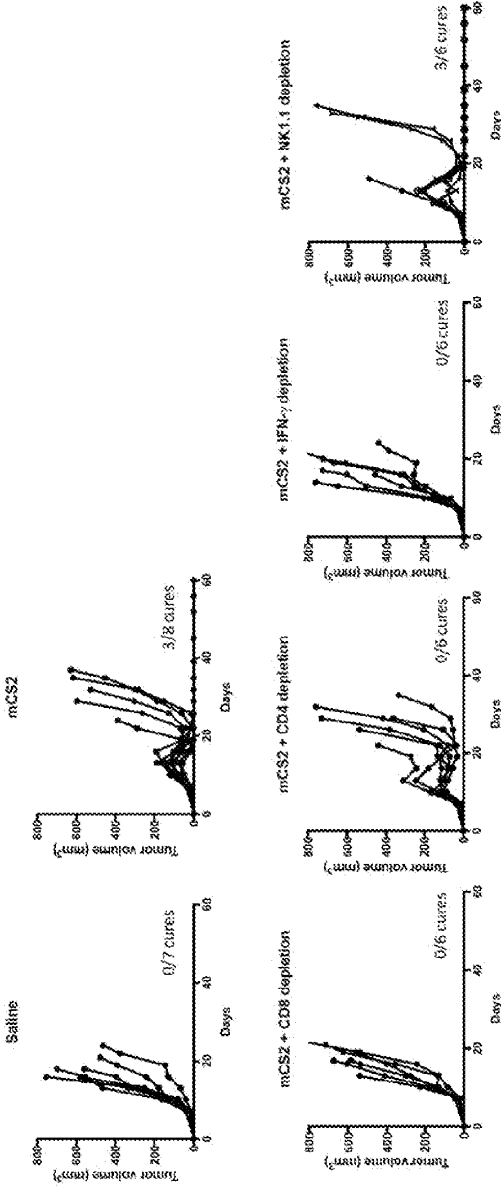
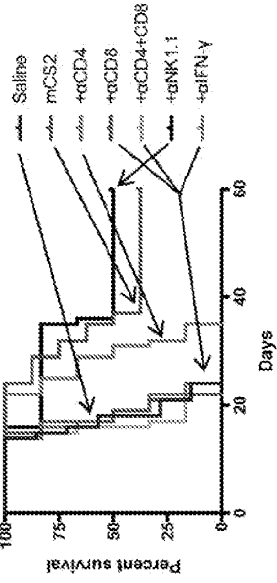


FIG. 15B



MC38 tumor treatment model- tumor growth

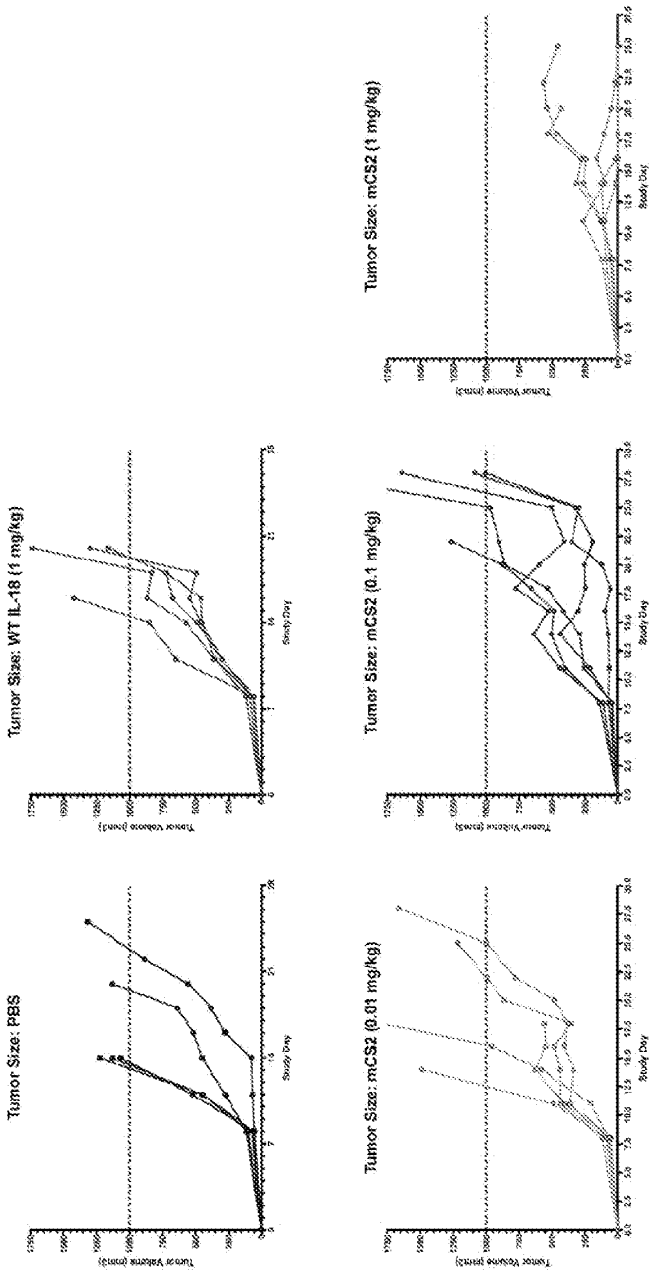


FIG. 16

Expanded MC38 treatment cohorts-Tumor Growth

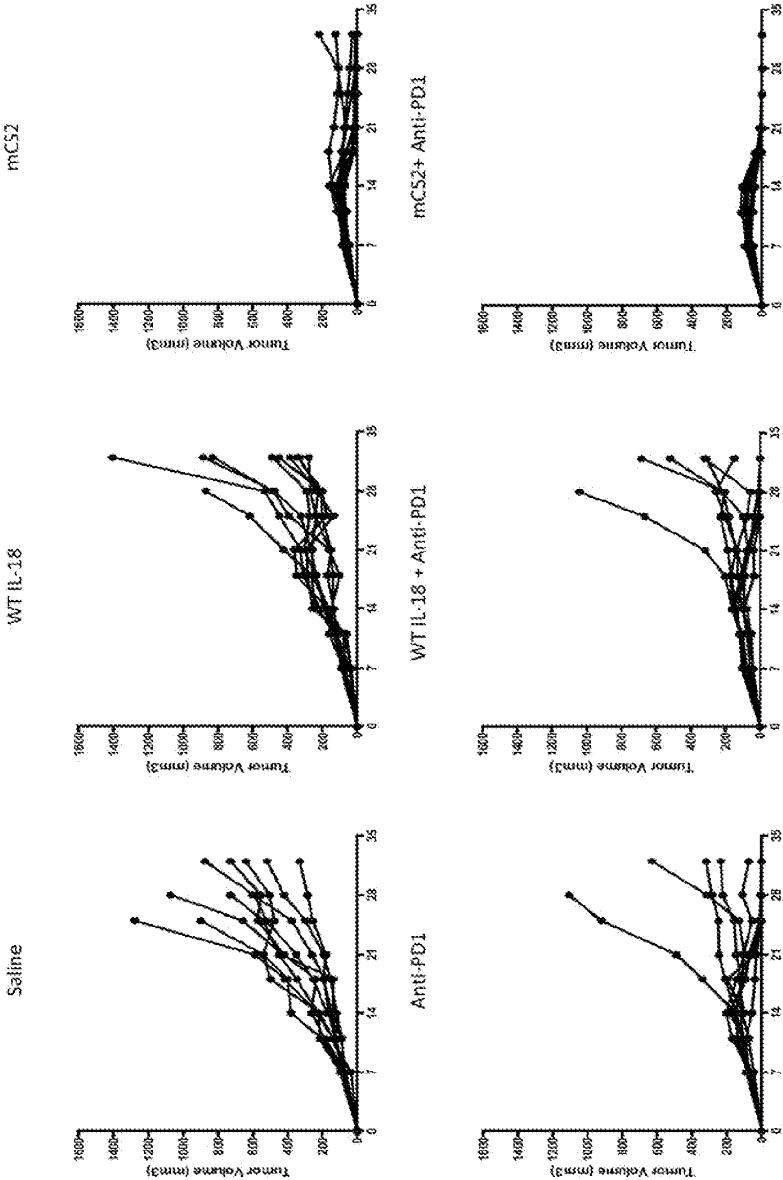


FIG. 17

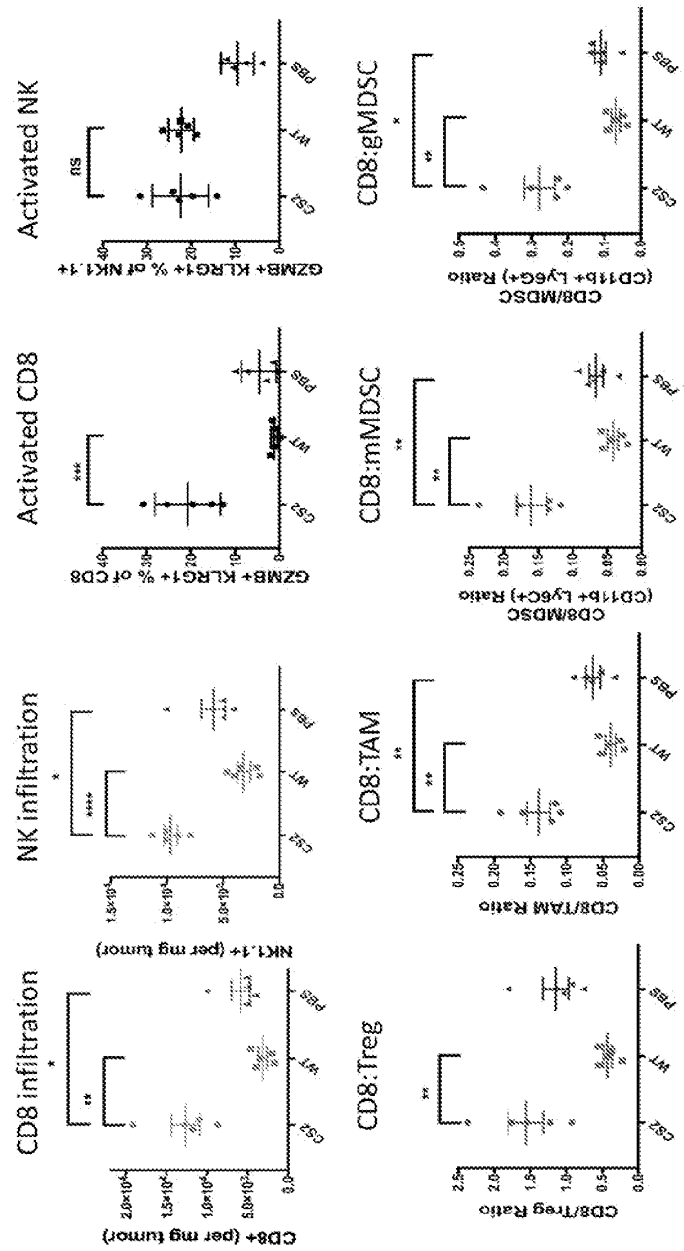


FIG. 18A

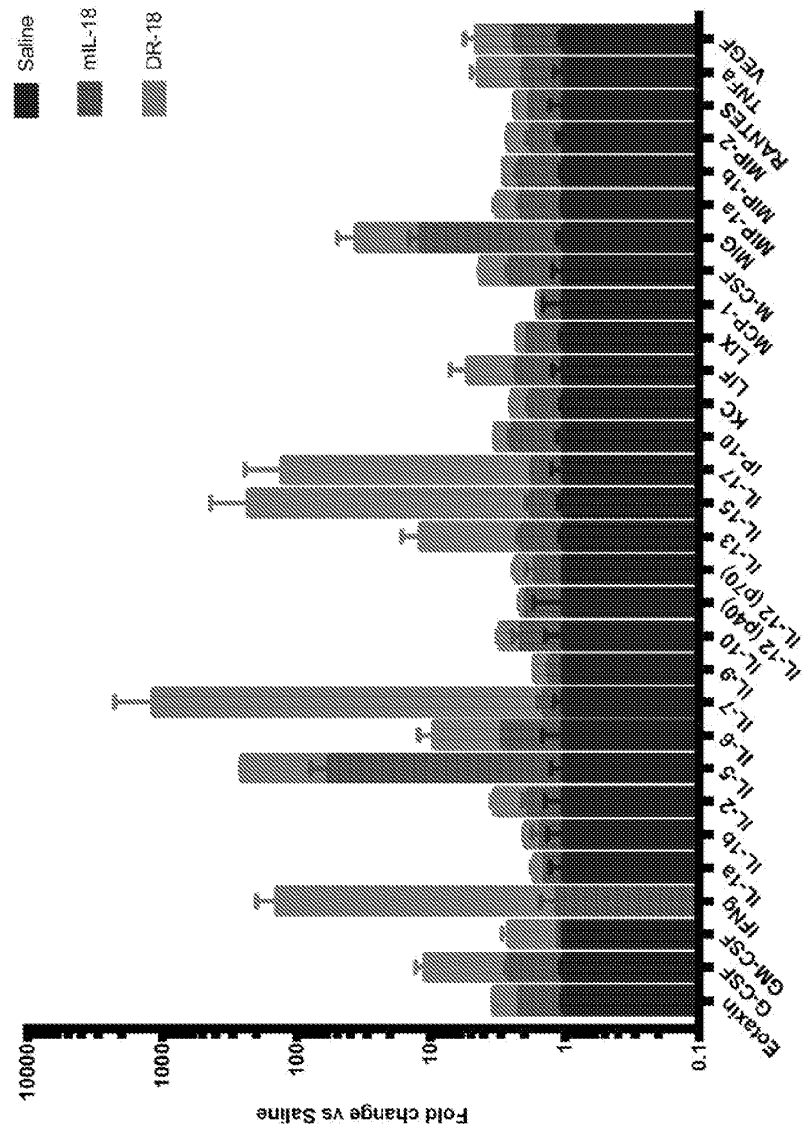


FIG. 18B

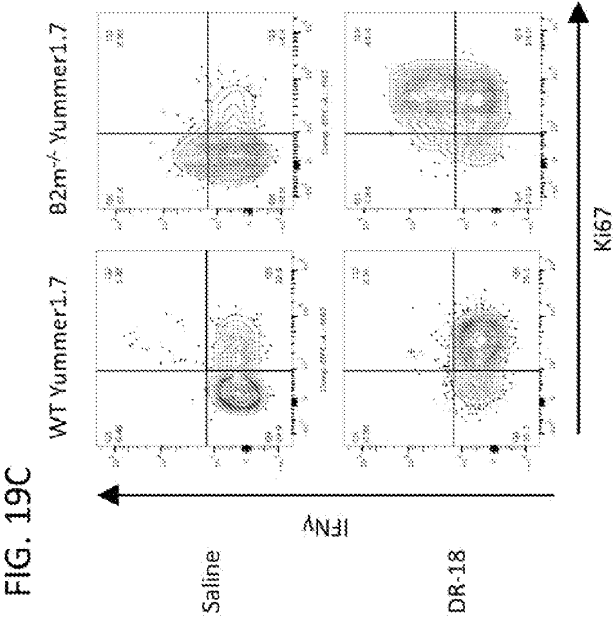
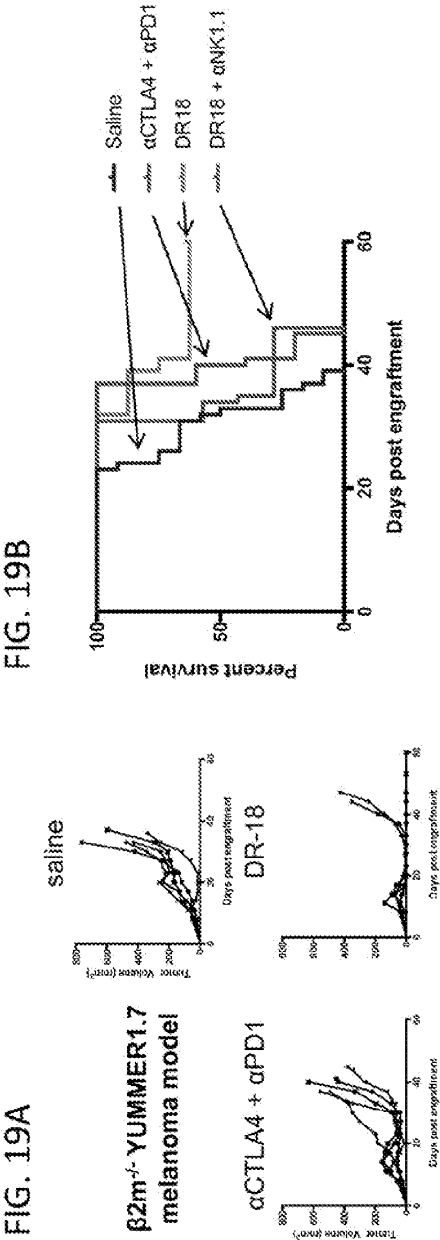


FIG. 20A

Residue	Amino Acids	Codon
Y1	YELHVD	BWT
L5	LFYHRC	YDT
D17	DA,GRHP	SVT
E31	EAG,TKR	RVA
T34	TA,EX	RMA
D35	DA,SY	KMT
S36	S,N,KR	ARW
D37	DA,Y,S	SMT
D40	DA,Y,S	KMT
N41	N,K,S,R	ARW
M51	M,I,L,F	WTS
M60	M,I,L,F	WTS
Q103	Q,I,L,K	MWA
H109	H,D,A,P	SMT
M113	M,I,L,F	WTS
D132	DA,Y,S,V,F	RHY

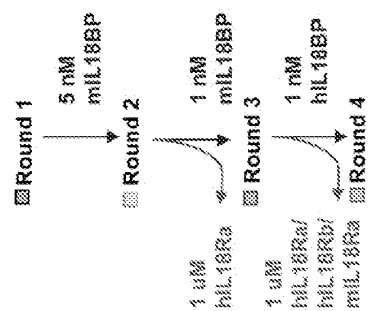
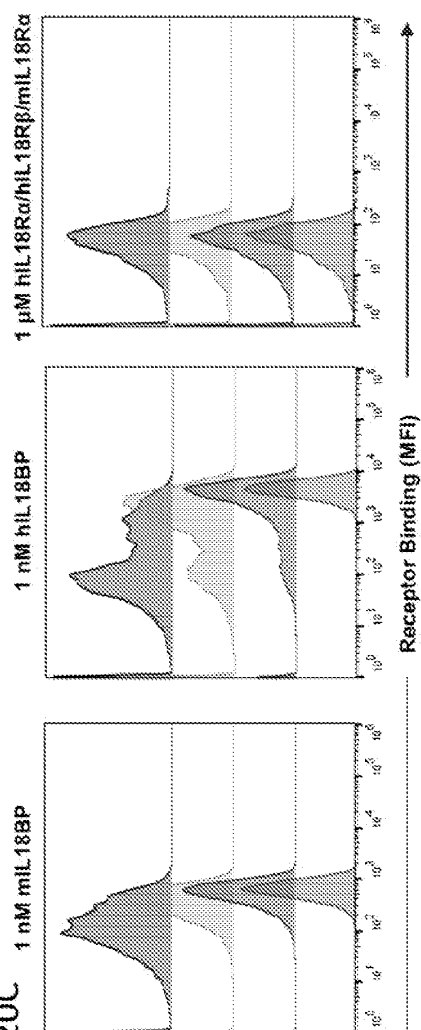


FIG. 20C



WT hIL-18		SEQ ID NO:																
		1	5	17	31	34	35	36	37	40	41	51	56	60	103	109	113	131
hd2D-5D02	V	L	D	A	A	S	N	P	Y	K				L	L	A	L	109
hd2D-5D08				A	K	A	K	A	S		F			F	L	A	I	105
hd2D-5A02			A	T		A	K	P	S	S	L			L	L	P	I	122
hd2D-5B02			G	A		S	K	R			L			F	I		I	120
hd2D-5B10			G	G		A	K	H	A	R	F			I	I	A	F	117
hd2D-5C03			G	K	E	A	N		S	S	I			L	I	A		115
hd2D-5C08			G	T		A		H	S		L			L	L	F	F	112
hd2D-5E08			G	T	E	S		P			F			L	L	I	I	101
hd2D-5C04			G	T	E	S	R	L		L	L			L	L	A	F	114
hd2D-5C05			G	T	E	Y		L	A		H			L	L	D	L	113
hd2D-5D05			G	T	K	S	N	L	S		F			I	I	D	I	107
hd2D-5F04			R			Y	K				F			F	I	A	I	97
hd2D-5F11	D			A			N	P	S		F			I	L	P		93
hd2D-5B11	D			A	K	A	N	A	A					L	L		F	116
hd2D-5E03	D			A	K	S	N	V	A					L	I	P	L	102
hd2D-5B05	F			A	A	S	N	H	A		L			L	I			119
hd2D-5B06	F	F	G	T	E	S	K			R	F			L	L	L	L	118
hd2D-5F06	H		G	T	E	S	K	P		S				I	I	F	F	96
hd2D-5C09	H		G	A	A	S	N	P	S		F			F	I			111
hd2D-5D10	H		G	A	E	S	N	R	A		F			F	I	P		104
hd2D-5F10	H		G	A	E	S	N			S				L	L			94
hd2D-5E10	H		G	A	E	S	N				F			F	L		I	100
hd2D-5F02	H		G	G	E	A		P			F			I	L			98
hd2D-5E02	H		G	G	E	A		P	A	K	I			F	L	A	L	103
hd2D-5F12	H		G	K	A	A		H			F			I	L	F	F	92
hd2D-5D03	H		G	R	A	A			A	S	I			F	L			108
hd2D-5F01	H		G	T	A	S	N		Y		F			L	L	F	I	99
hd2D-5C10	H		G	T	A	S	N			K	F			L	L	P	F	110
hd2D-5D06	H		H	T	A		N	A			F			L	L	A	I	106
hd2D-5F08	L		G	A	S			P	S	R	F			L	I	D		95
hd2D-5A09	L	H	G	G	E	S		P	Y		F			L	I		I	121
hd2D-CS1			G	A		S					F			L	L			123
hd2D-CS2			G	A	E	S								L	L			124
hd2D-CS3			G	A	E	S					F			L	L			125

FIG. 21

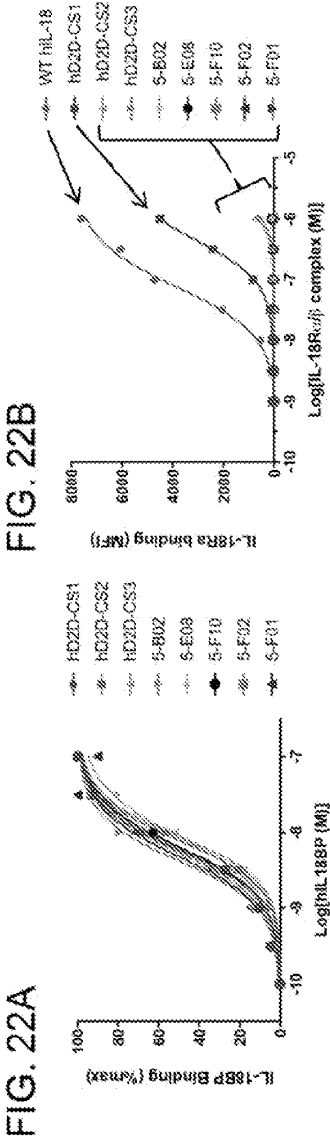


FIG. 22C

Variant	IL18Ra (KD)	IL18BP (KD)	BP:Ra ratio (norm. to WT)
WT IL18	62 nM	2.1 nM	1
HD2D-CS1	430 nM	5.9 nM	2.5
HD2D-CS2	21 μM	7.9 nM	90
HD2D-CS3	9.7 μM	8.9 nM	37
5-B02	NBD	4.2 nM	>170
5-E08	NBD	8.8 nM	>81
5-F10	NBD	6.4 nM	>110
5-F02	NBD	5.4 nM	>130
5-F01	NBD	4.8 nM	>150

	1	5	17	30	33	34	35	36	50	102	104	108	109	111	129	130	SEQ ID NO:
WT mIL-18	N	L	D	E	T	D	I	D	M	Q	R	H	N	M	D	D	
mD2D-A5	Y	Y	Q	A	G	Y	T	V		L	E		R	L		E	126
mD2D-A6	D			A	G	S		A	F	L	A	D		I	A	T	127
mD2D-A7	Y		G	R	A		T	V	F	I	P	A	S		A	G	128
mD2D-A8	H			K	E	Y	T	V		I	A	D	R	I		N	129
mD2D-A9	Y		A	A	A		K	G		L	P	D	T		F	G	130
mD2D-A11	Y		E	A	G		R	H		I	P	A	S	L			131
mD2D-A12	H			R	G	A		G	F	I	P	D	S	L	V		132
mD2D-B4	H		S	T	G	S		V		I	G	D		I		R	133
mD2D-B7	Y		S	R	E		T	P	F	I		D	S	L	F	E	134
mD2D-B11	H		A	G		A	T	V	F	I	P	D	S	L		N	135
mD2D-B12			N	K	E	Y	T	L	F	I	P	D		L	Y	E	136
mD2D-C1	Y		G	A	E	A	T	R	F	I	G	A				G	137
mD2D-C3			G	A	R	A		L	F	L	G	D		L		R	138
mD2D-C5	Y		A	A	E	A	T	A	F	I	G	A	S			G	139
mD2D-C6	L		G	A	G	A	T	L		L	P	D	T		A	S	140
mD2D-C9			G		A	Y	T	V	F	I	G	D	S		Y		141
mD2D-C10	D			K	E	S	K	P	F	L	A	A	S	L	A	N	142
mD2D-C11	L		G	A	G		K	V		I	P	D		L		E	143
mD2D-D1	Y	H	Q	R	A	A	T	R		L	G	D					144
mD2D-D9			Q	T	E	S		G	F	L	A	A		L		S	145
mD2D-D12	F	H	G	G	G		R	V		I	A	D	S	I		G	146
mD2D-E3	V	H	G	K		Y				L	A	D	T		A	Q	147
mD2D-E4			G	A		A	T	R		I	Q	A		I	F	R	148
mD2D-E5	D		G	G	A	Y		G	F	I	A		S	I	S	G	149
mD2D-E7	Y			R	G	S		A		I	P	A	T	L		G	150
mD2D-E8	Y		E	T	E	A		G	F	I	G	D	R			G	151
mD2D-F9	F		N		E	Y	R	L		L	P	A	S	L	S		152
mD2D-E10			N	A	E		R	L		L	G	D				H	153
mD2D-E11	Y		A	R	G	Y		L	L	L	P	D	T	I		N	154
mD2D-E12	Y			G	A		T	A	F	I	P	D	S		A		155
mD2D-F3	D		G		A	Y		A	F	I	P	D	S	I	A		156
mD2D-F4			E	R	K	Y		L	F	L	G	D			Y	G	157
mD2D-F5	D		E	T	A	Y		L	F	I	A	D	S	L	T		158
mD2D-F7	D		N	K	E	S	T	A		L	G	A	S	L	A	G	159
mD2D-F8	H		E	A	E	A		G	F	I	G	D	T	L		G	160
mD2D-F9	I		E	K	R	Y		V	F	I	E	A	S	L		E	161
mD2D-G1	Y		A	T	G	Y	T	L	L	I	P			I		R	162
mD2D-G7			N	R	A	S	T	A		I	G			I			163
mD2D-G9	D		G		K		R	A	F	L	A		S			E	164
mD2D-H7			E	A			A	A	L	P	D	I			Y	G	165
mD2D-E1	Y		E	A			T	L	F	L	G	D				T	166
mD2D-A10	H		G	K	K	Y		V		L	A		S	I		S	169
mD2D-F12	Y		G		K	A	K	A	F	I	P	A	S			G	171
mD2D-E2	L		G	G	S			P	F	I	H	A	T			N	174
mD2D-C4	Y		S	T	A	Y	T	V	F	I	A	D	S	L		N	176
mD2D-C2	Y		G	T	G	A	R	V	F	L	P	D		L	S	G	178
mD2D-A2	D		G	G	K	A	T	G	F	I	A	A		L	A	G	180
mD2D-A1	D		S	R	G	S		H	F	L	A	A		L		G	182
mD2D-D4	Y		E	K	K		K	L	F	L	G	D		L	F	G	184
mD2D-A3	Y		G	A	A	S	T	H	F	L	G	A		I			186
mD2D-B9	Y		S	G	K	Y		V	F	L	G	D	T		S	G	190

FIG. 23

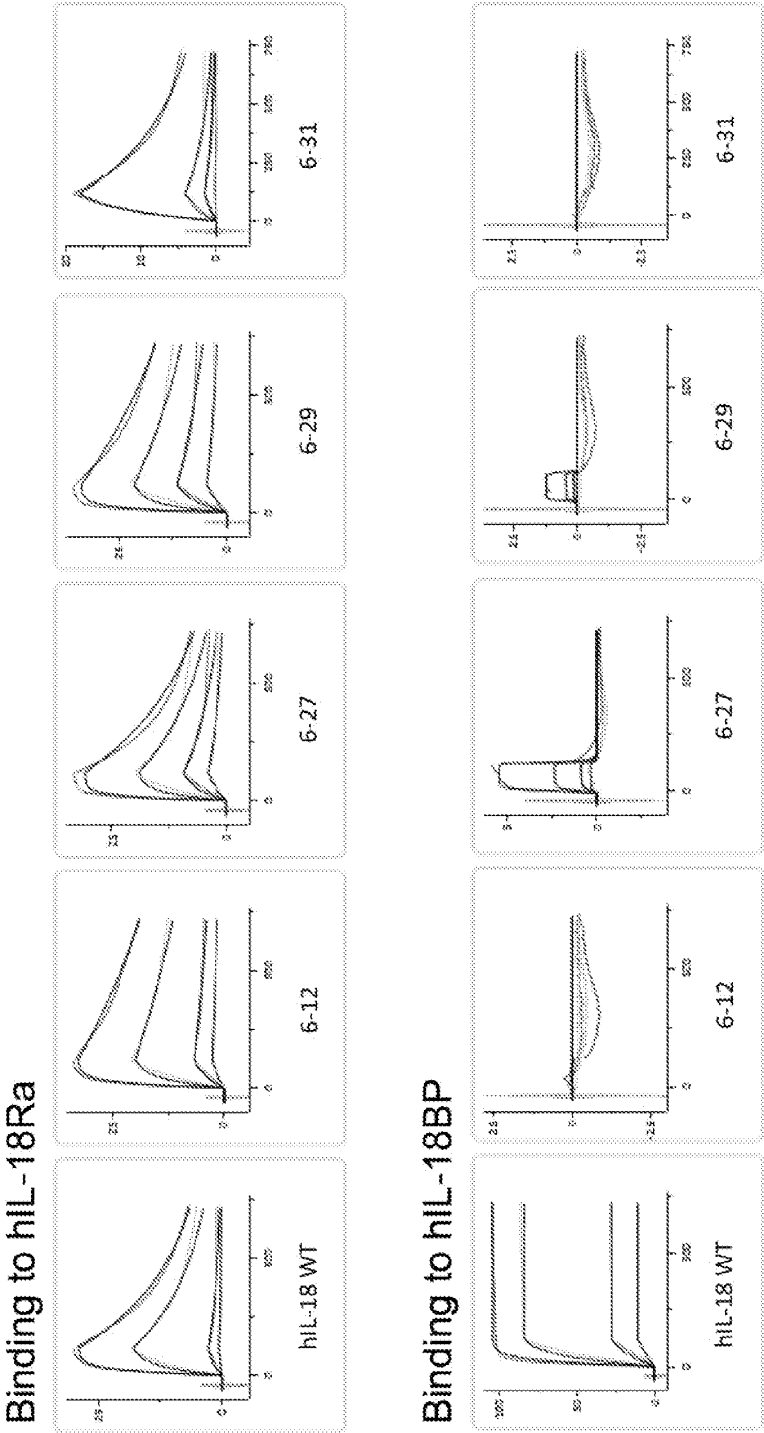


FIG. 24

FIG. 25A

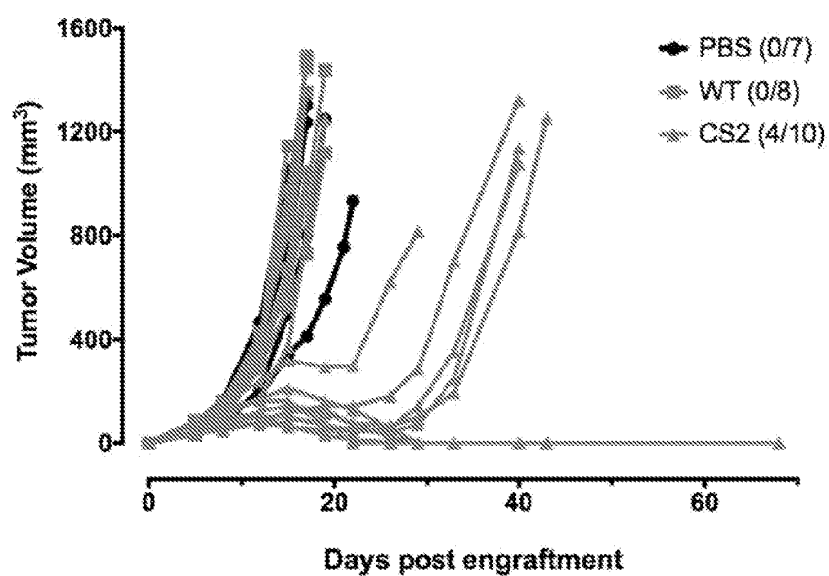


FIG. 25B

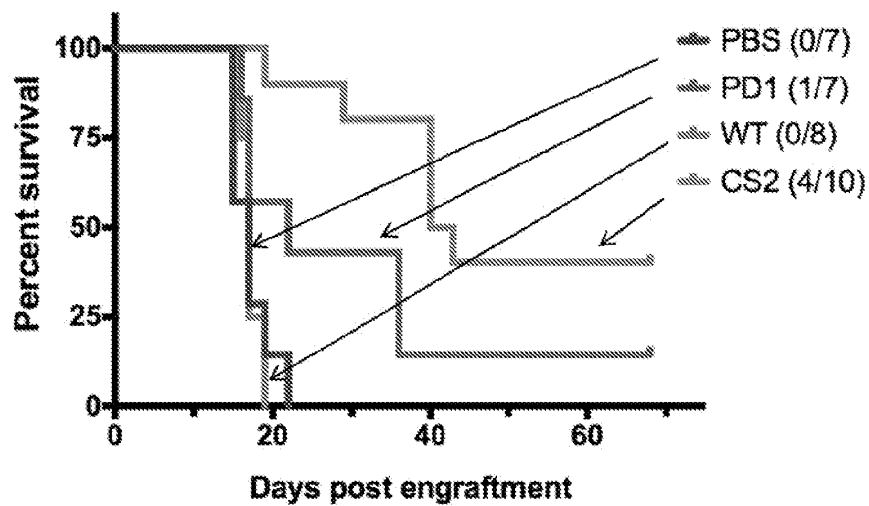


FIG. 26A

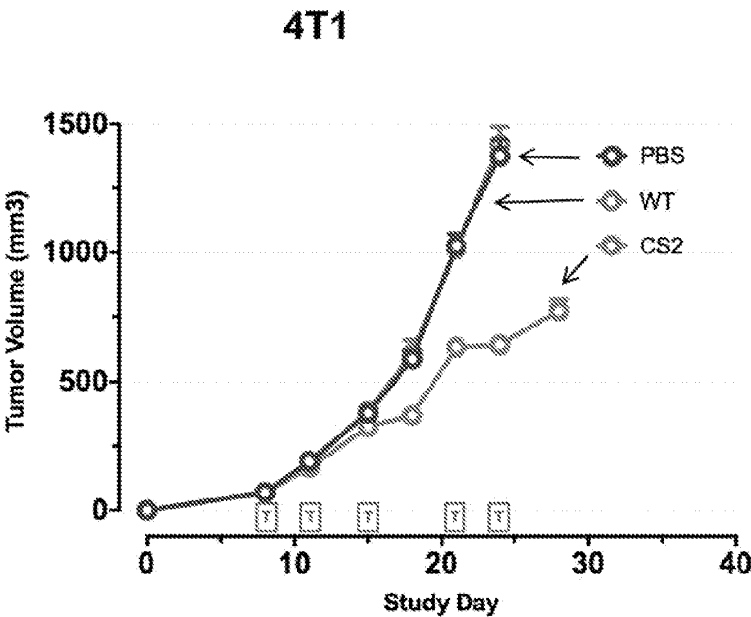


FIG. 26B

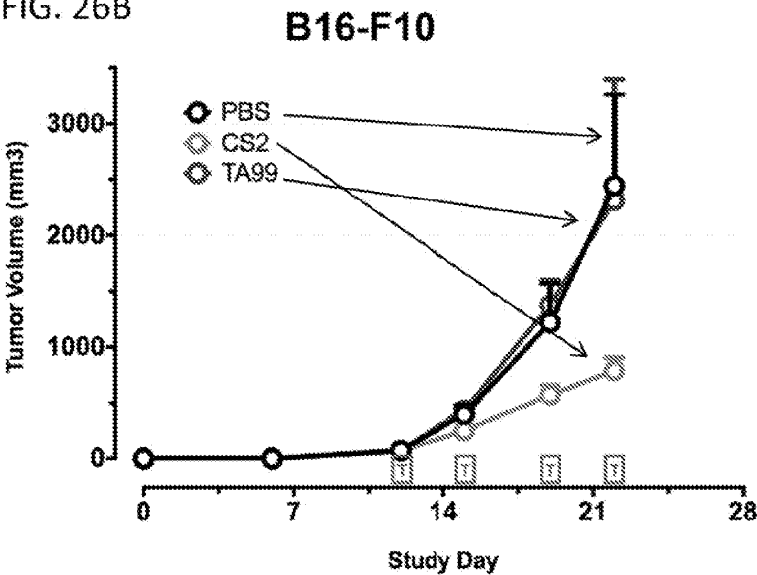


FIG. 27A

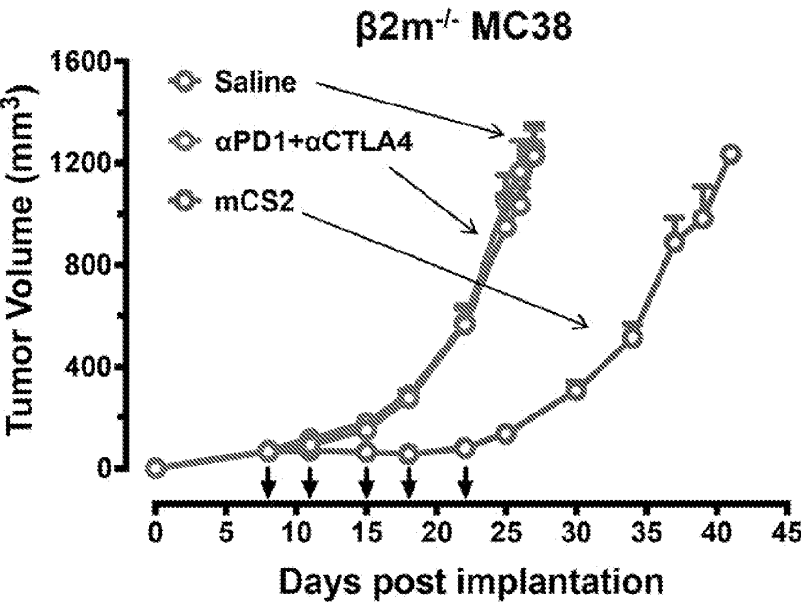


FIG. 27B

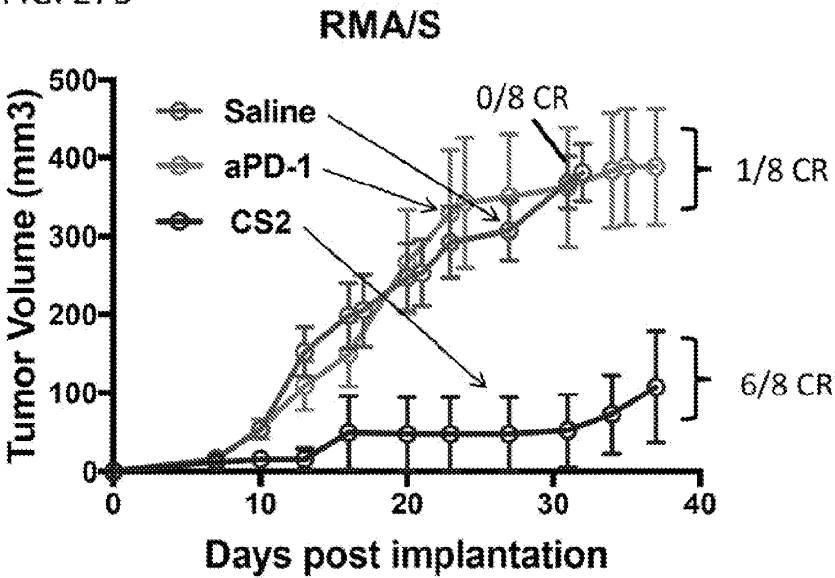
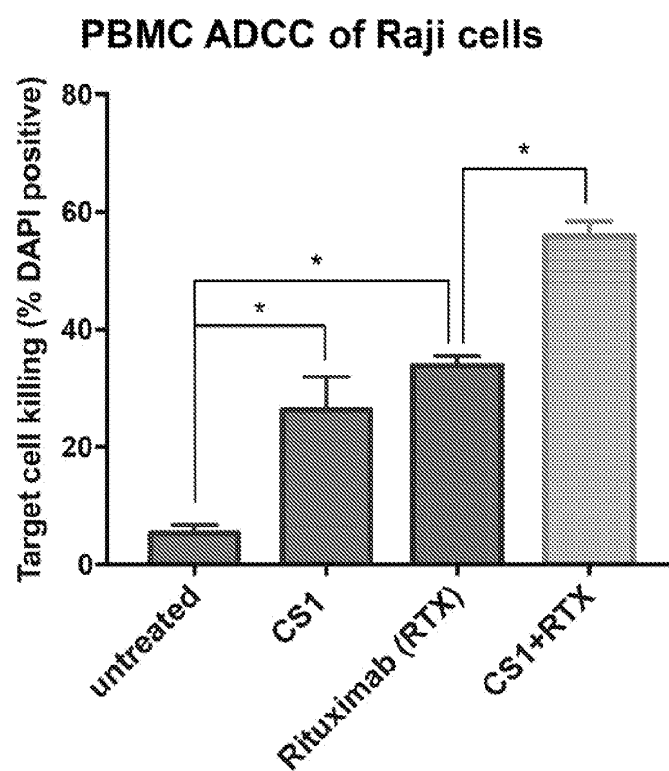


FIG. 28



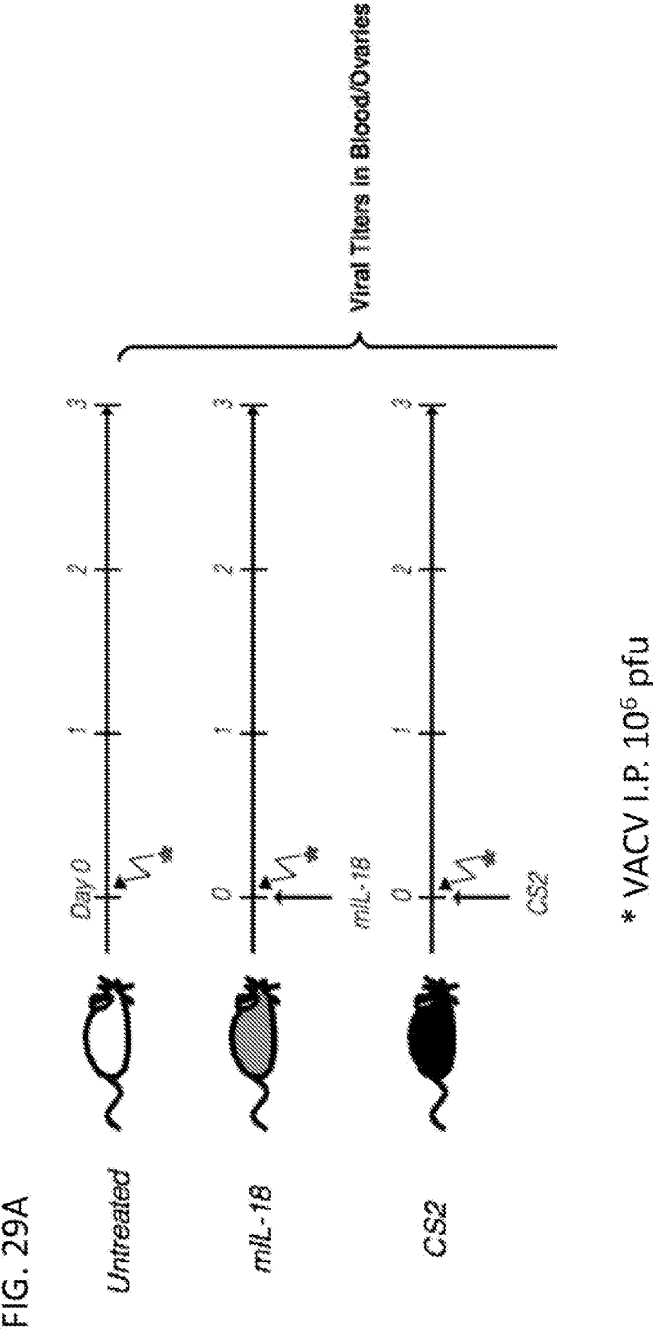
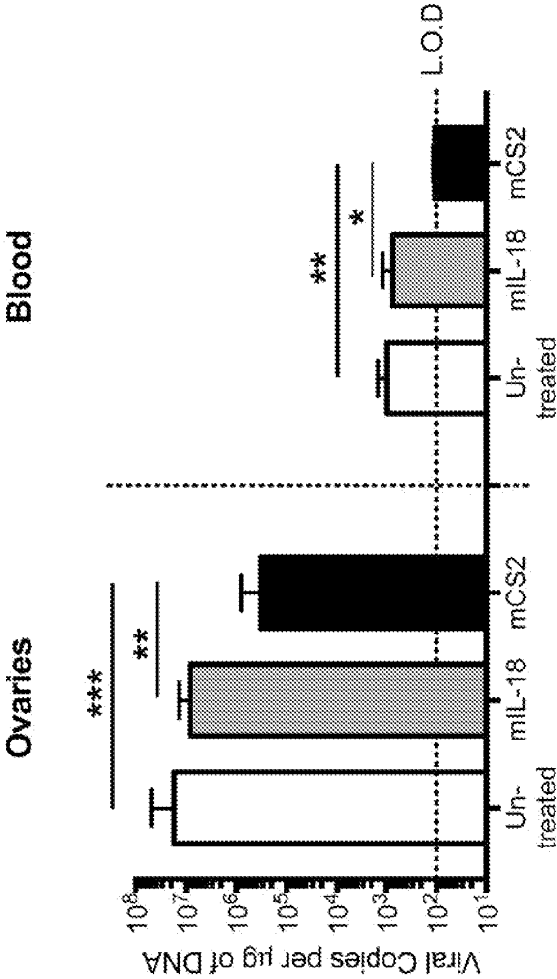
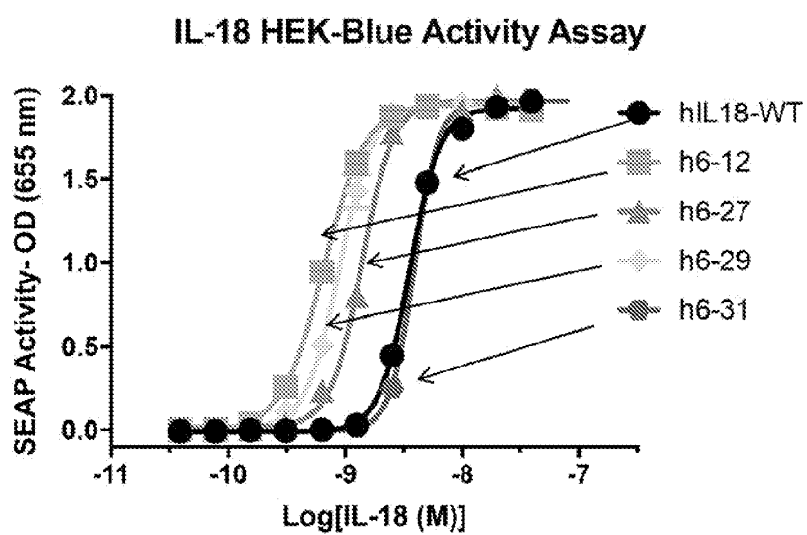


FIG. 29B



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FIG. 30



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FIG. 31

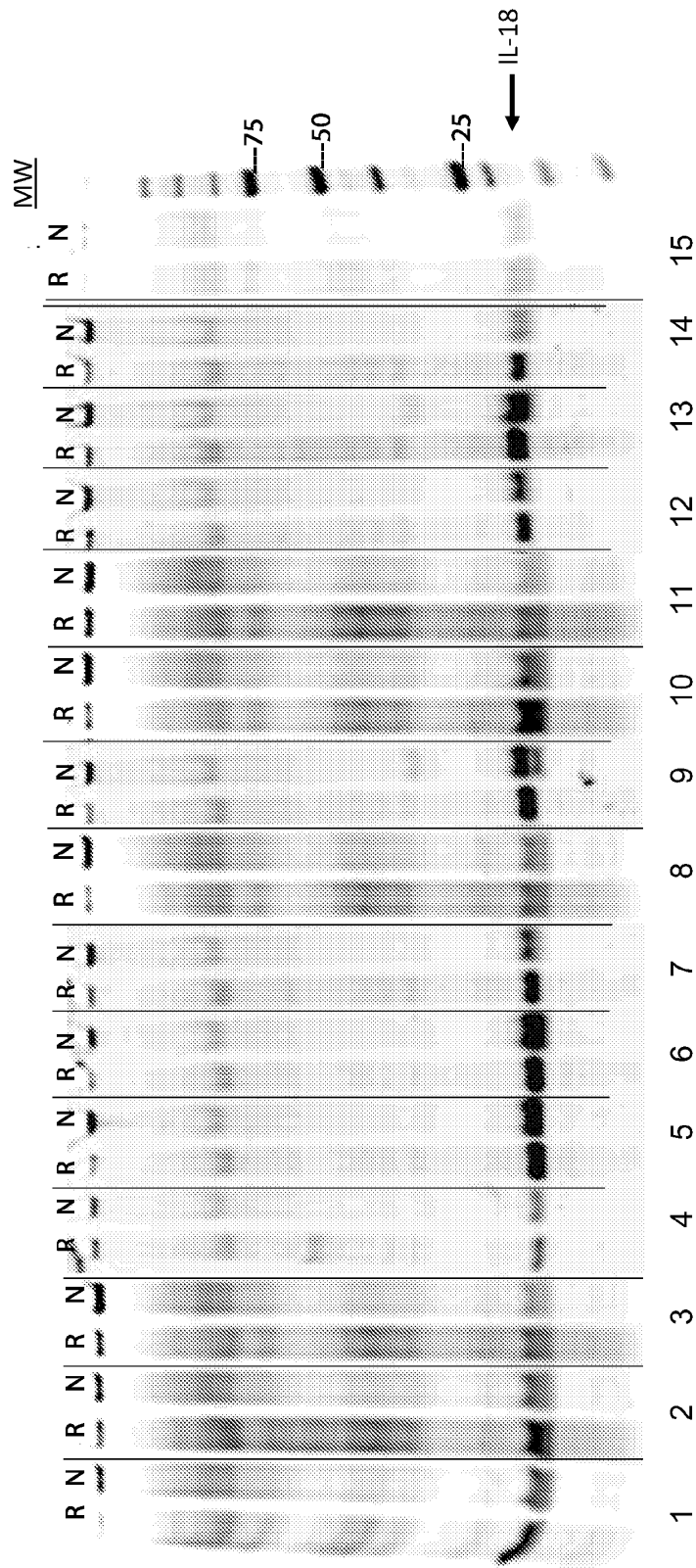


FIG. 32

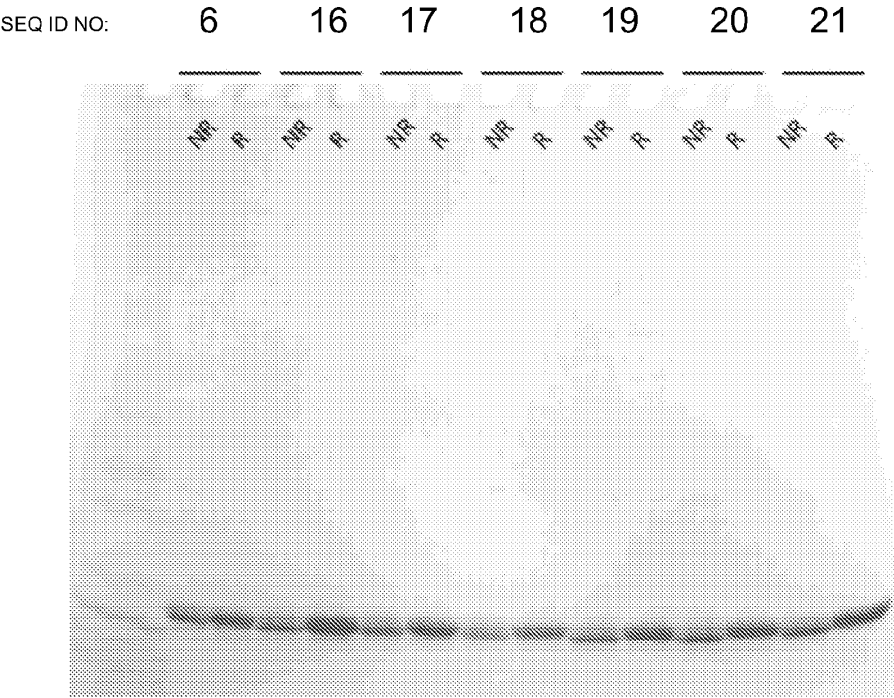
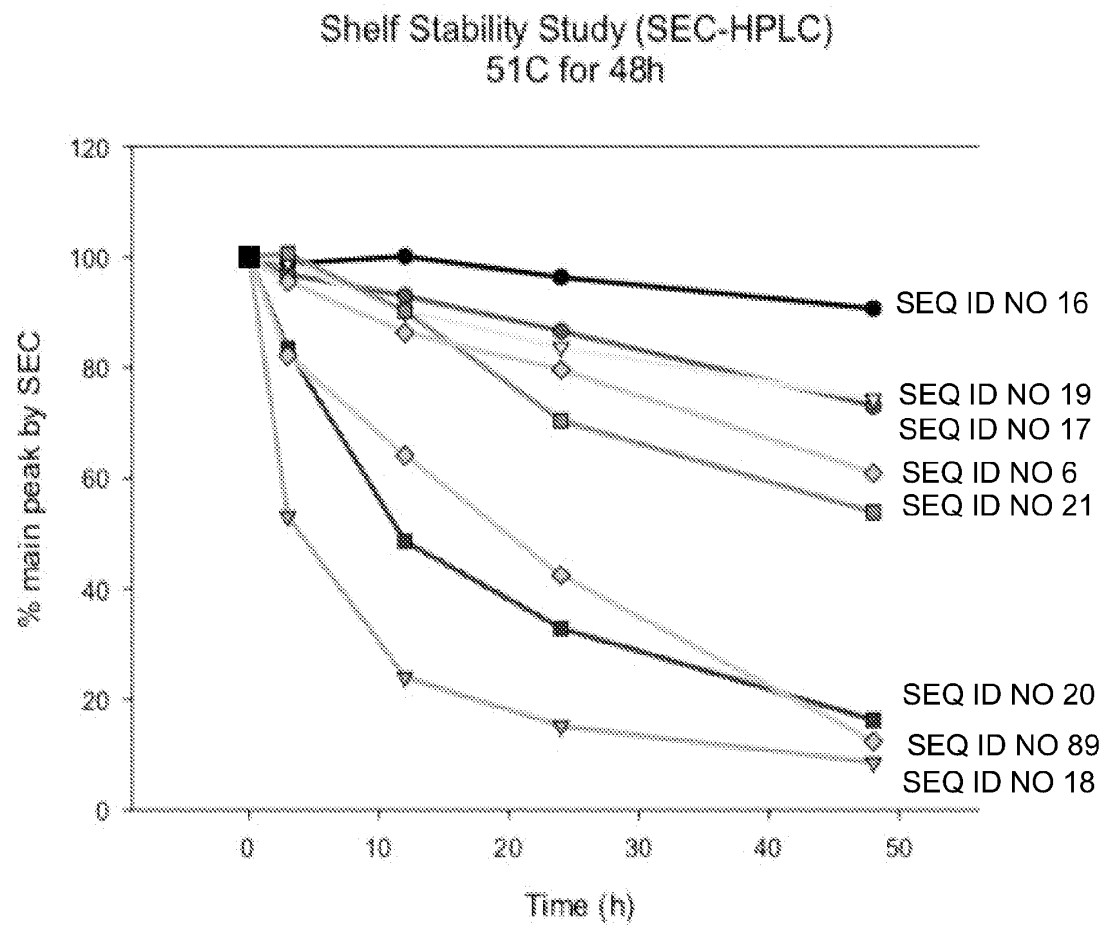


FIG. 33



Preparation #1

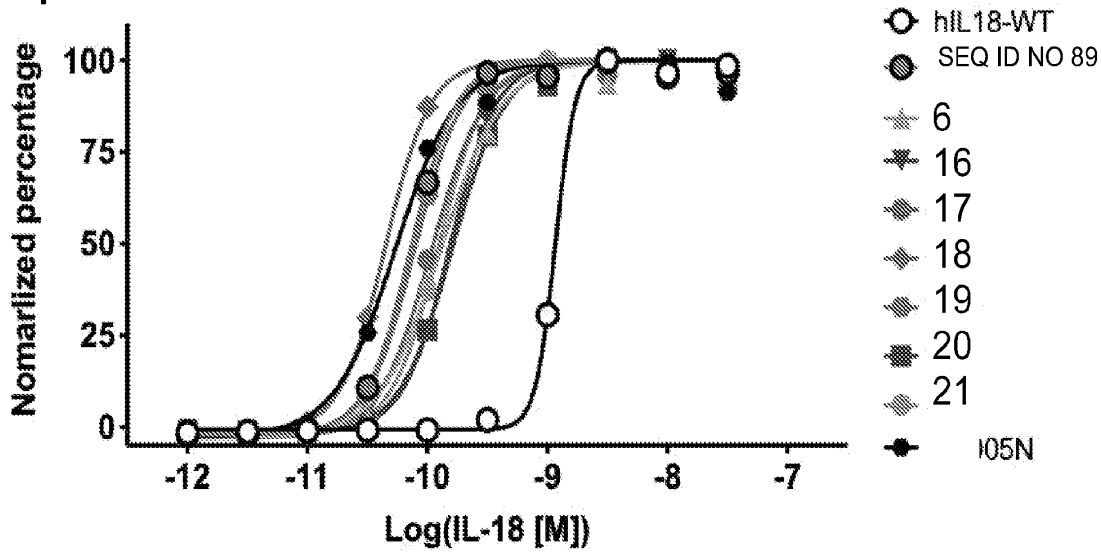
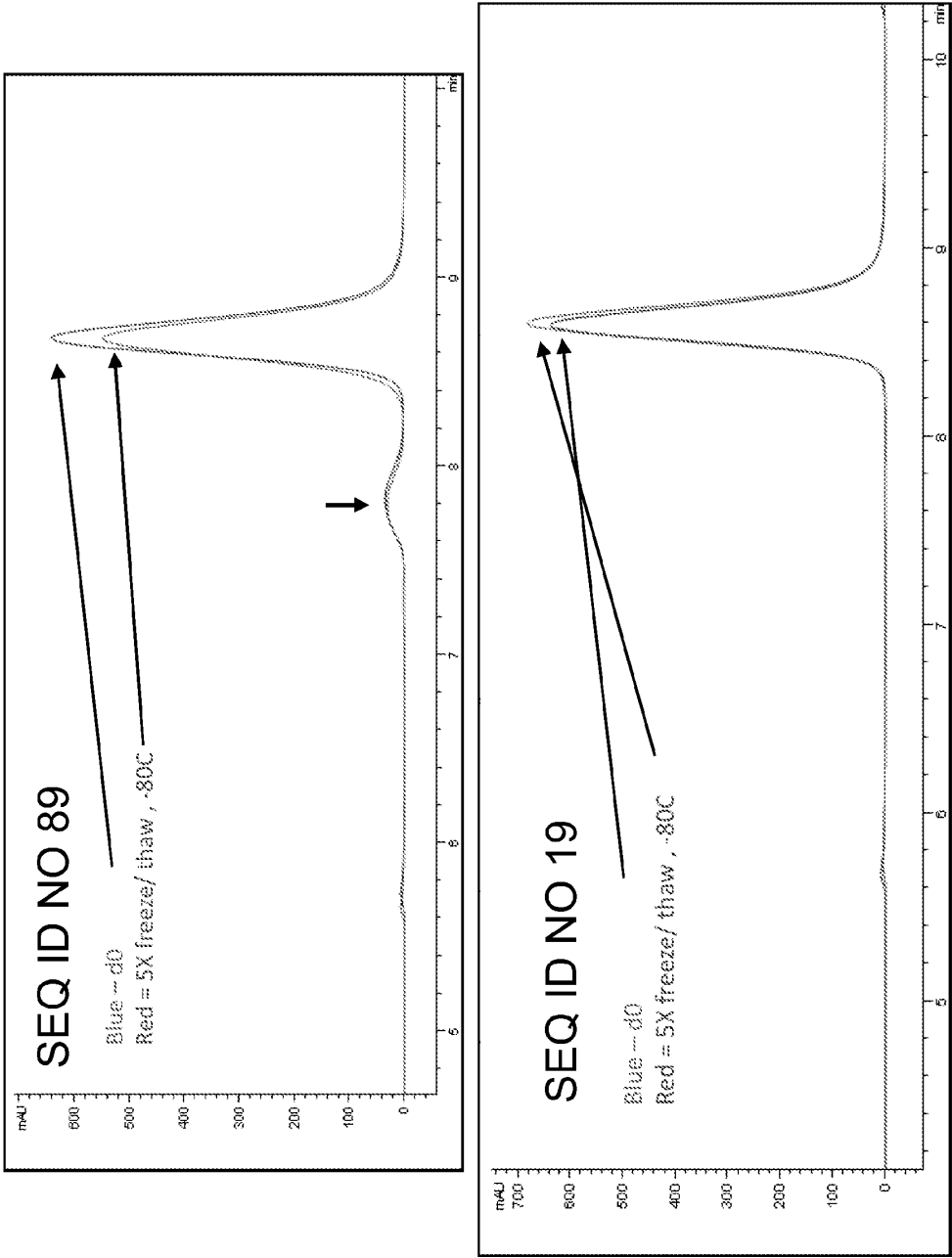


FIG. 35



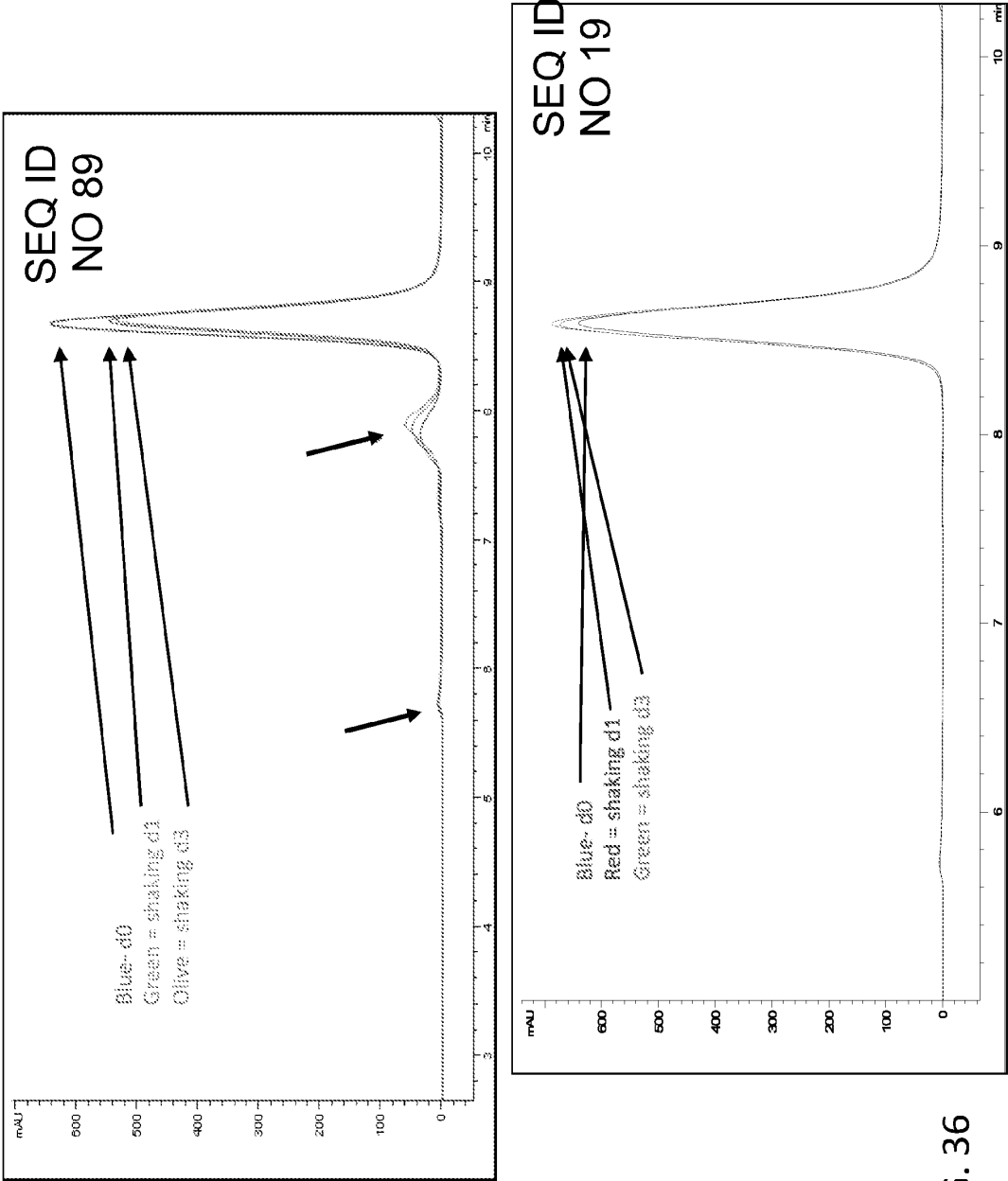


FIG. 36

FIG. 37A

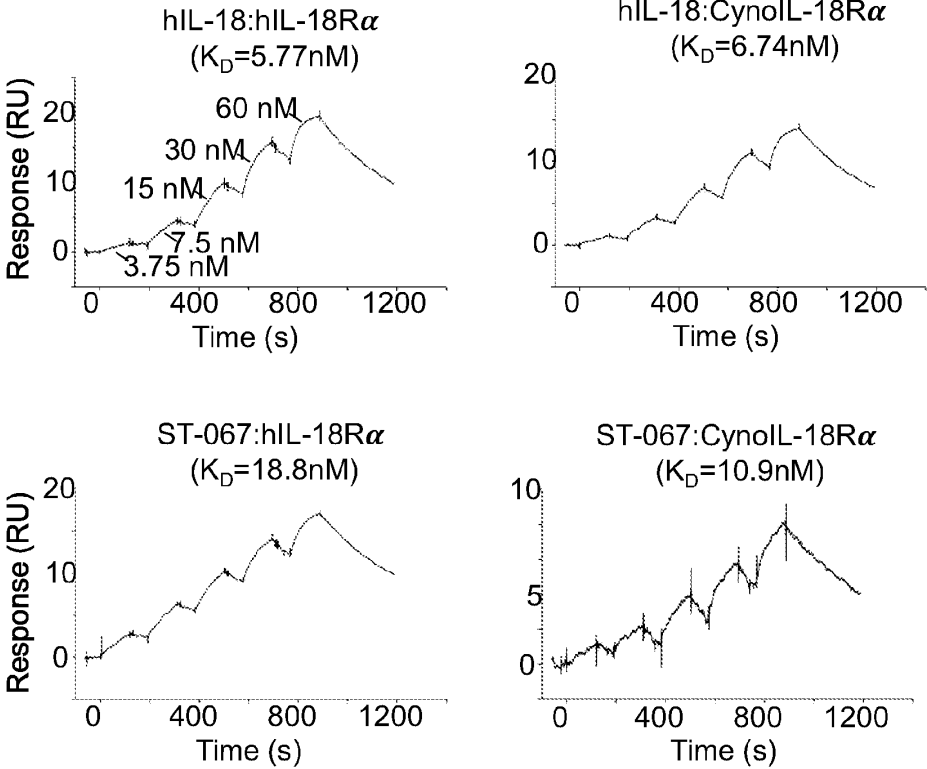


FIG. 37B

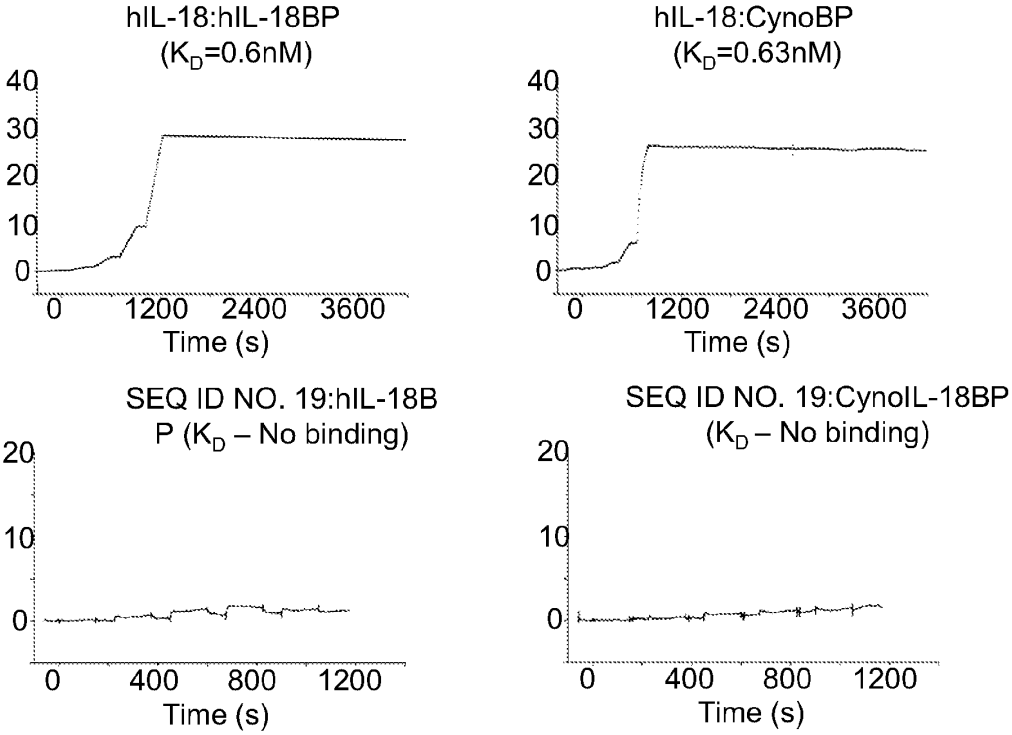


FIG. 38

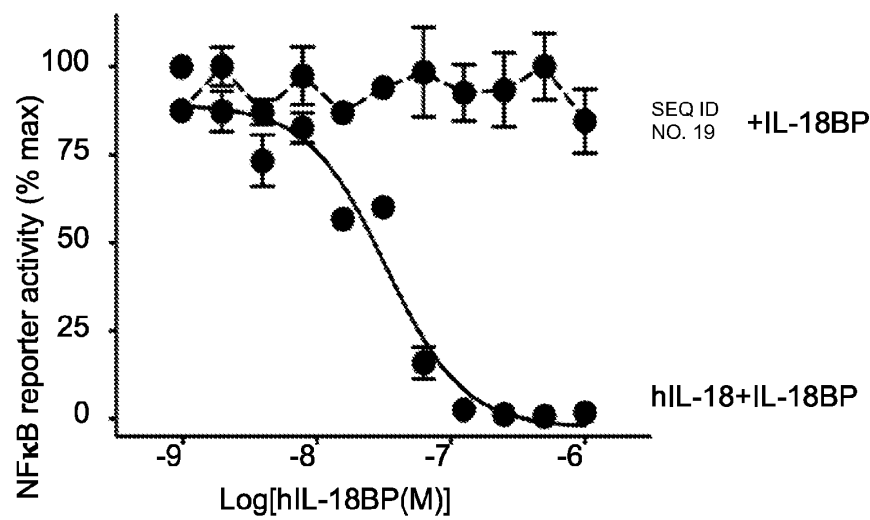


FIG. 39

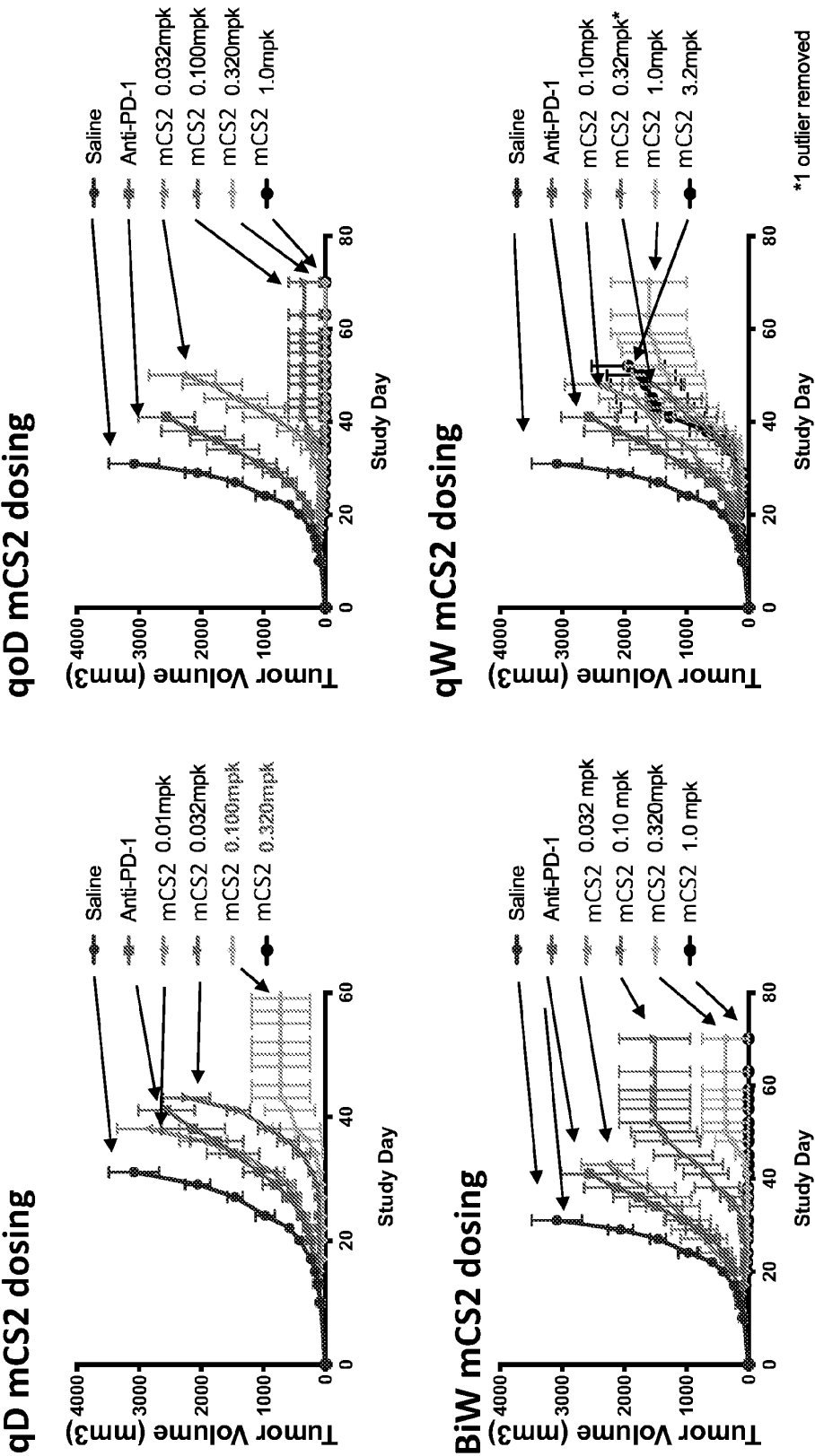
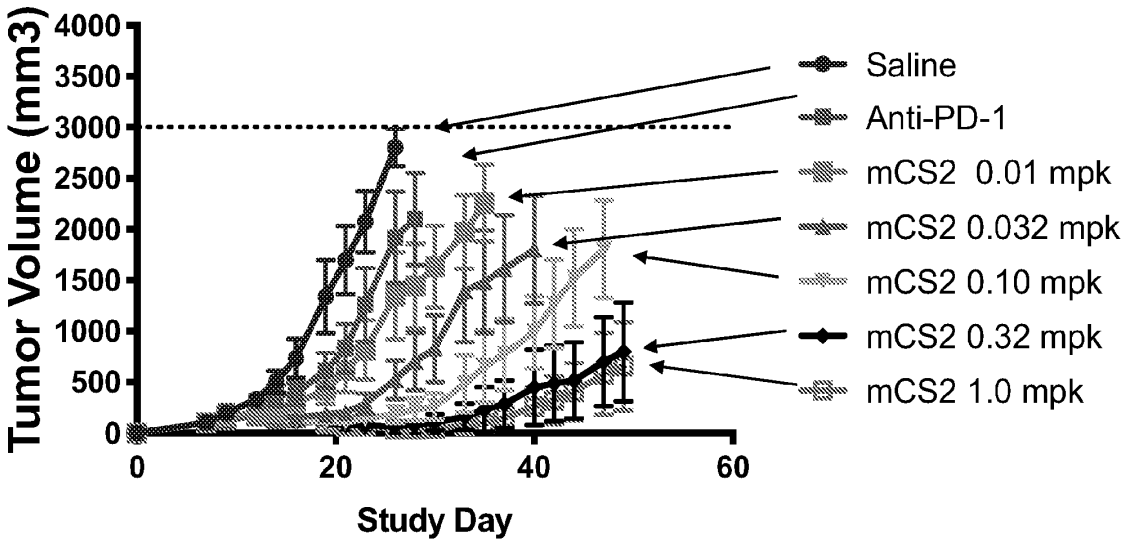


FIG. 40

qW mCS2 dosing



q2W mCS2 dosing

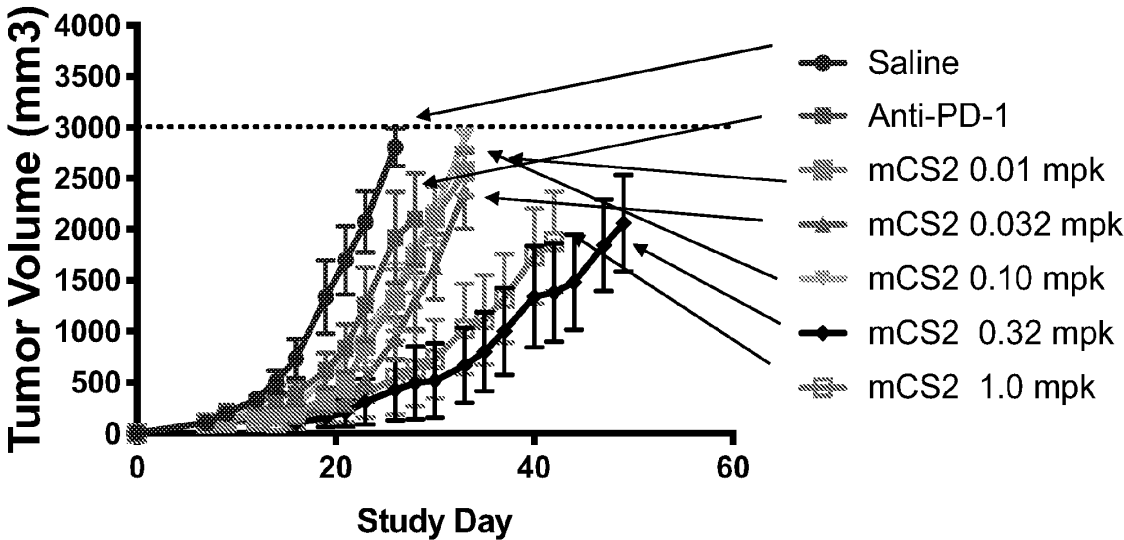


FIG. 41A

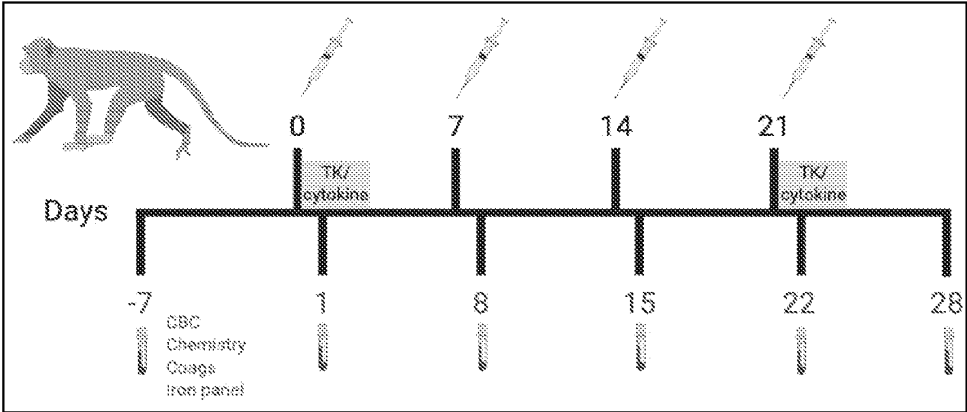


FIG. 41B

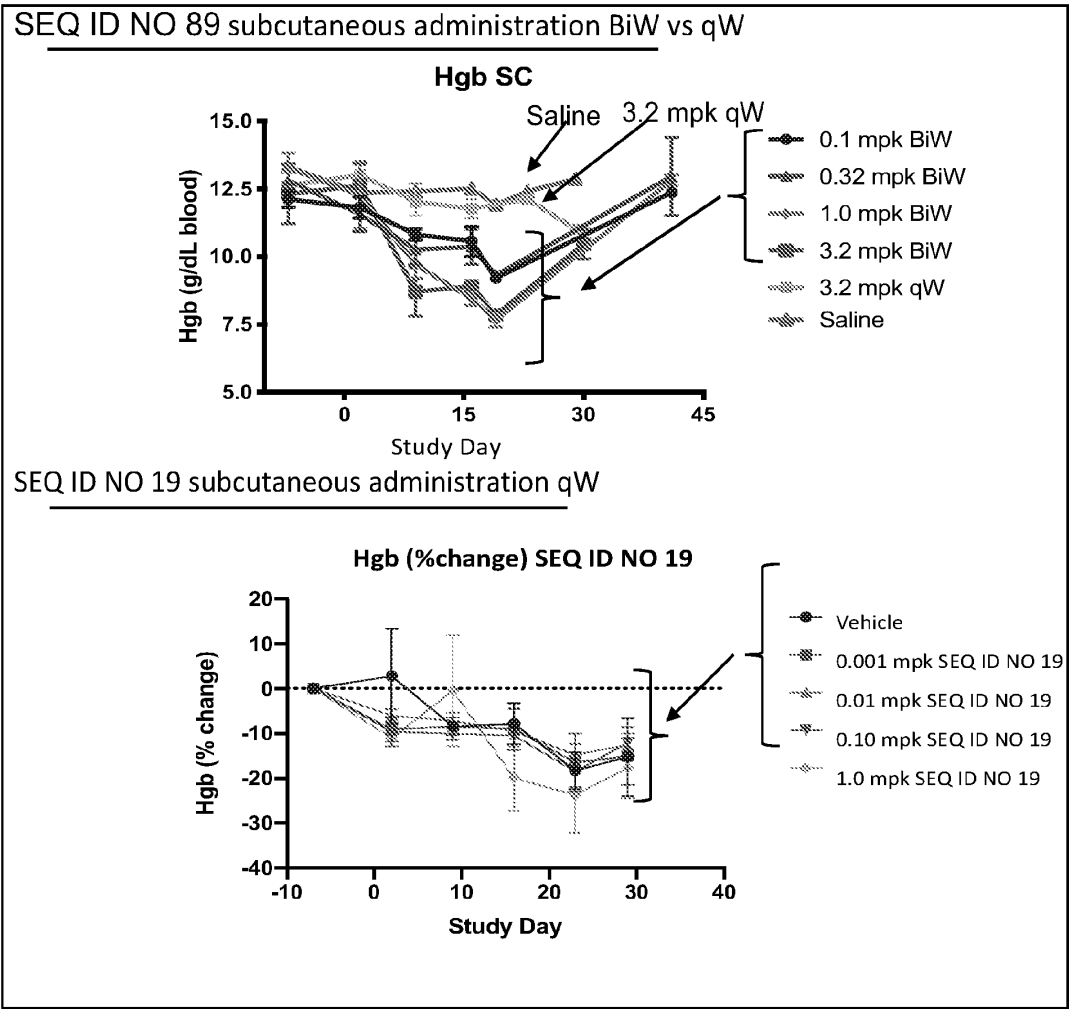


FIG. 42A

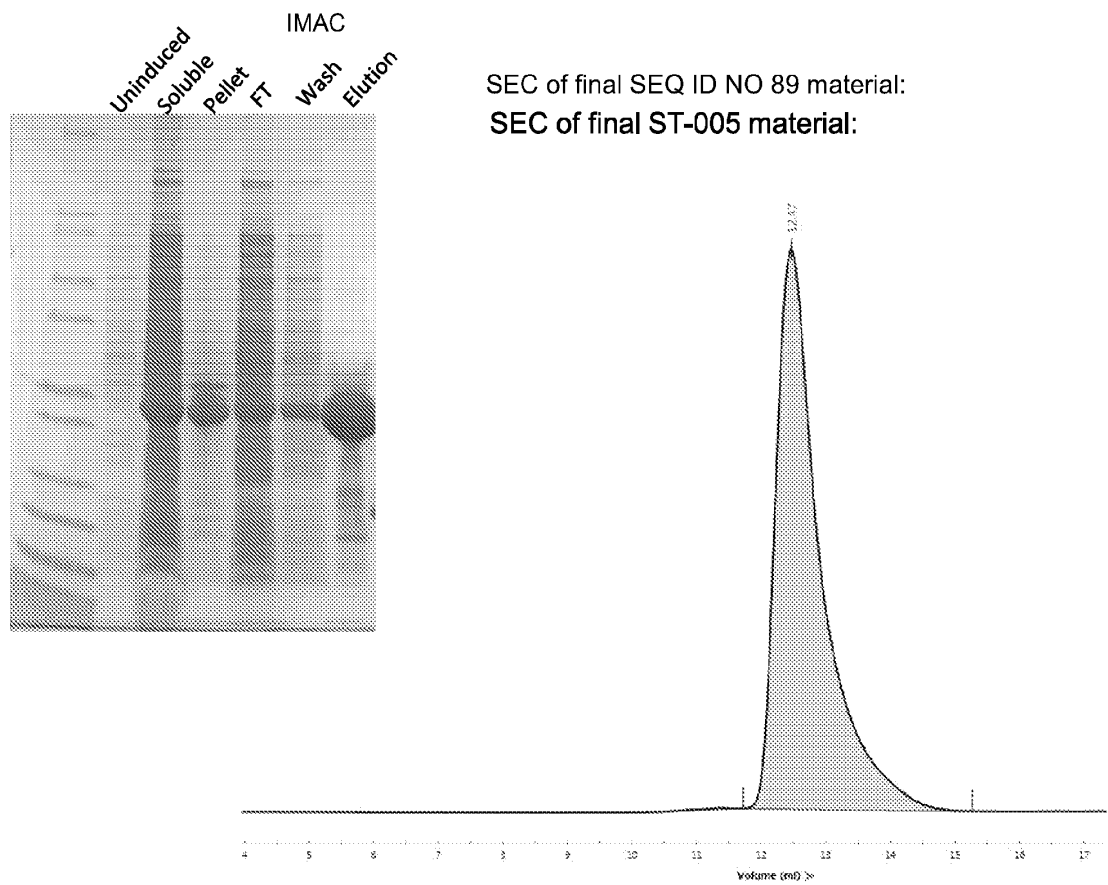


FIG. 42B

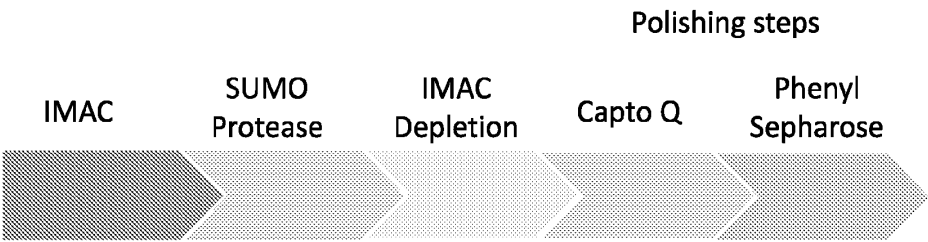
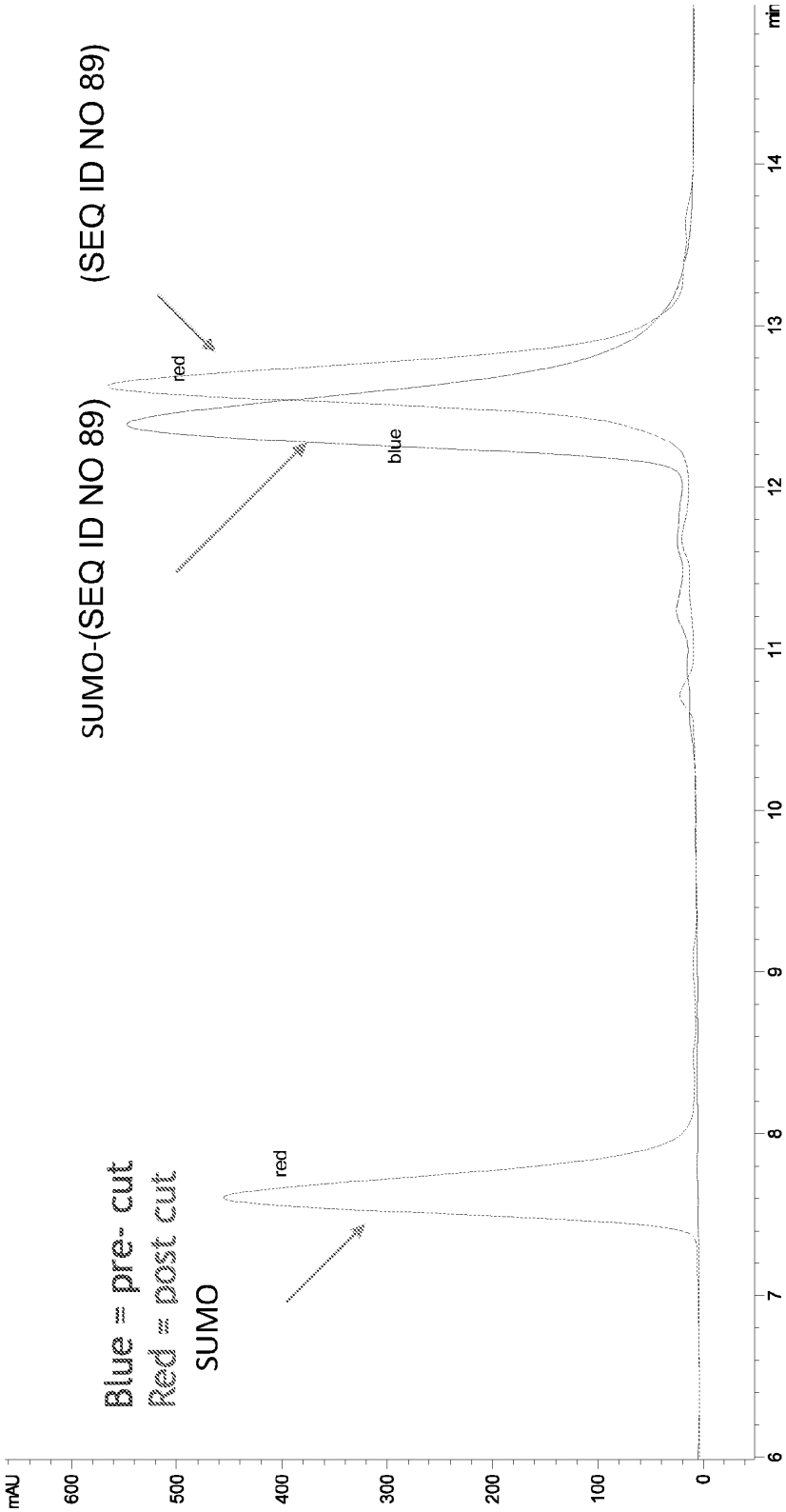
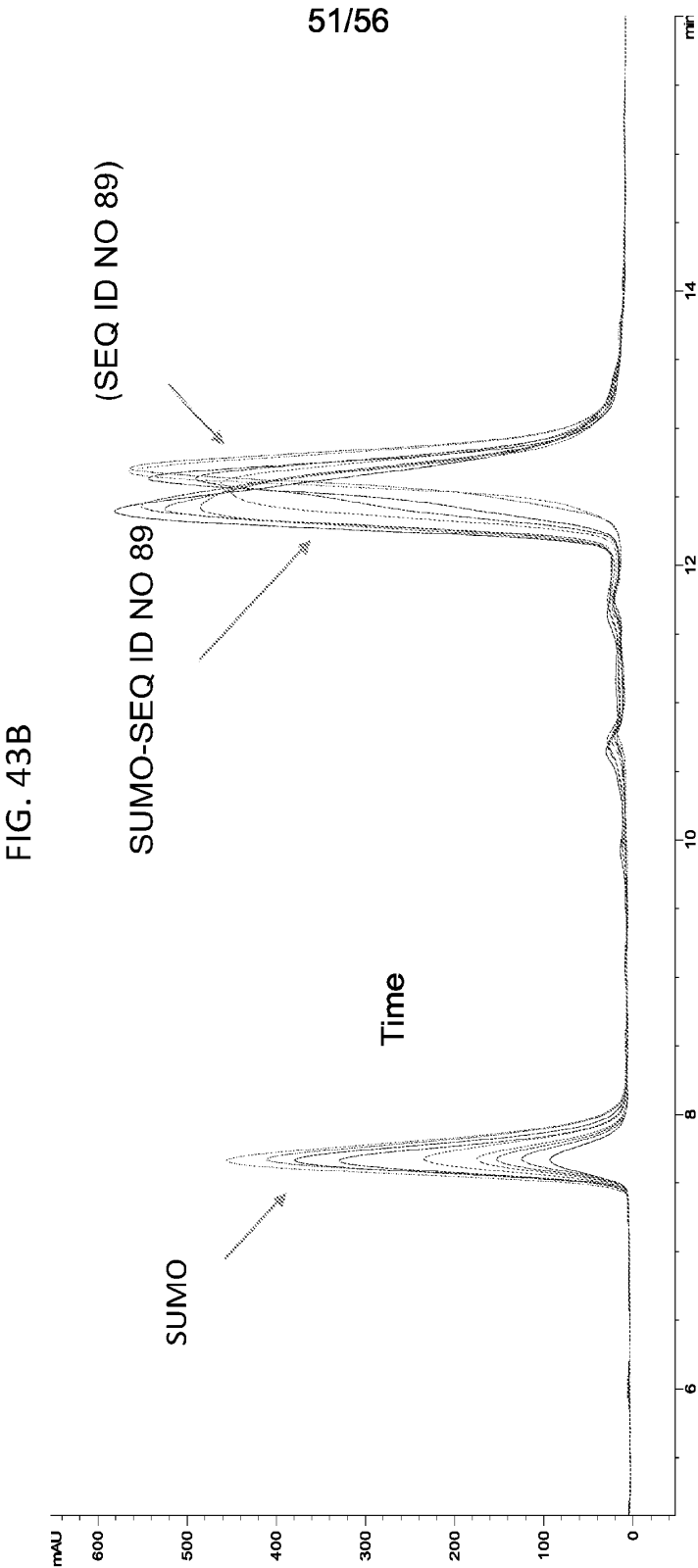
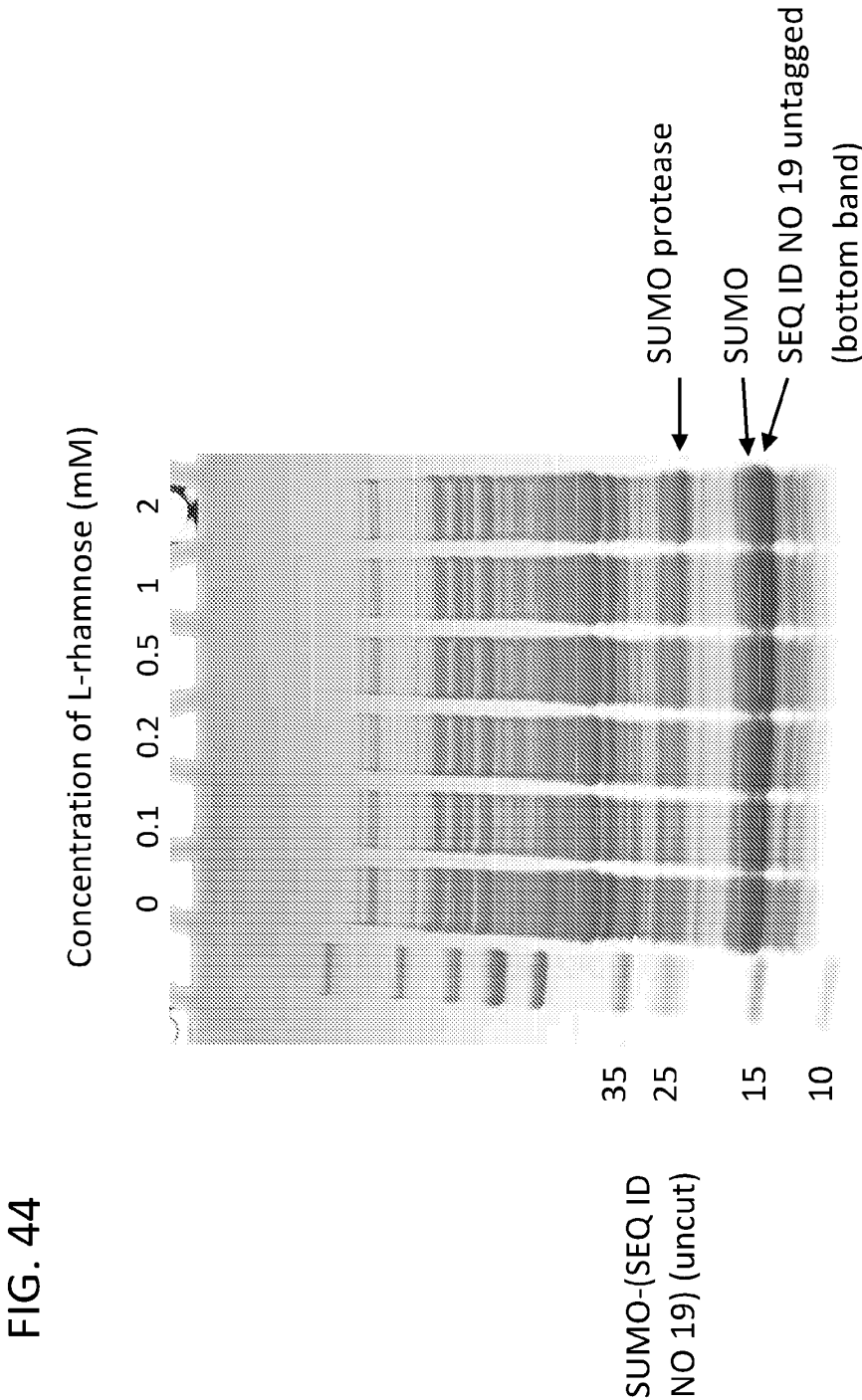


FIG. 43A







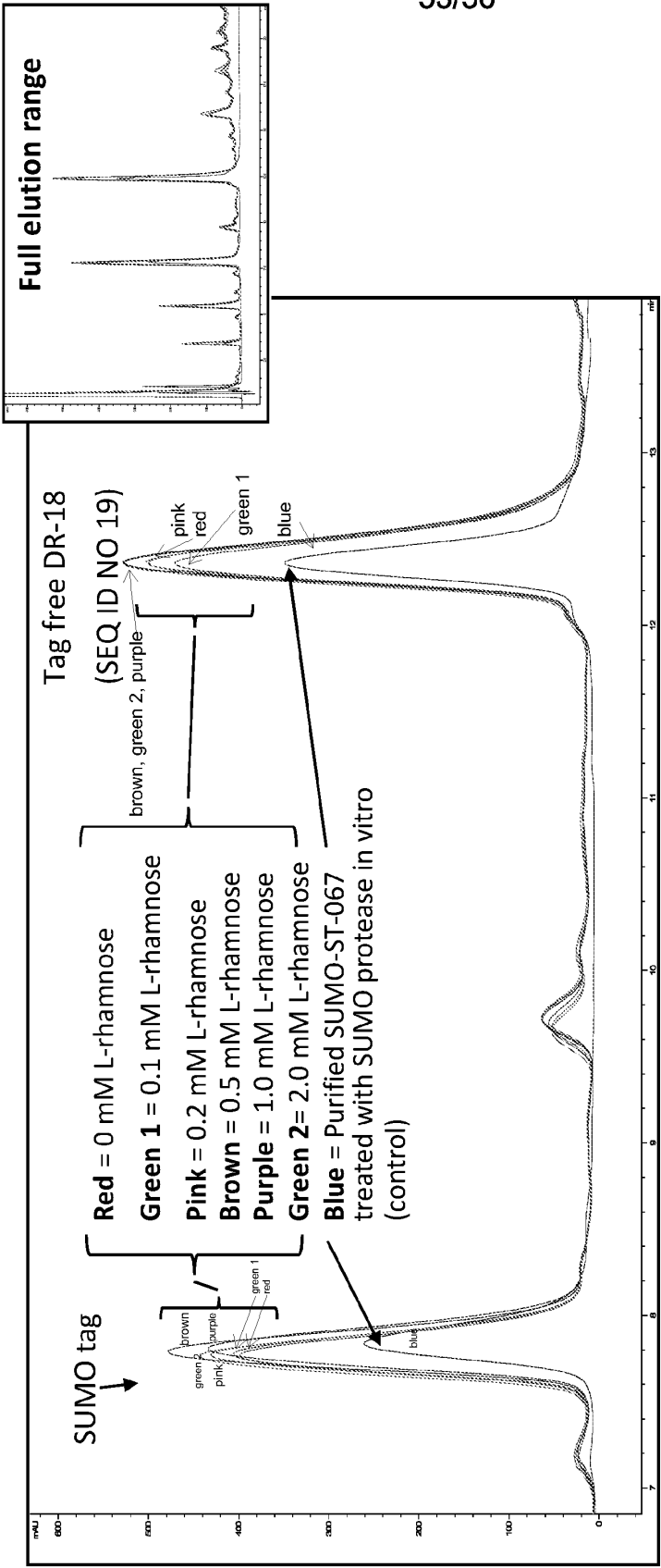


FIG. 45

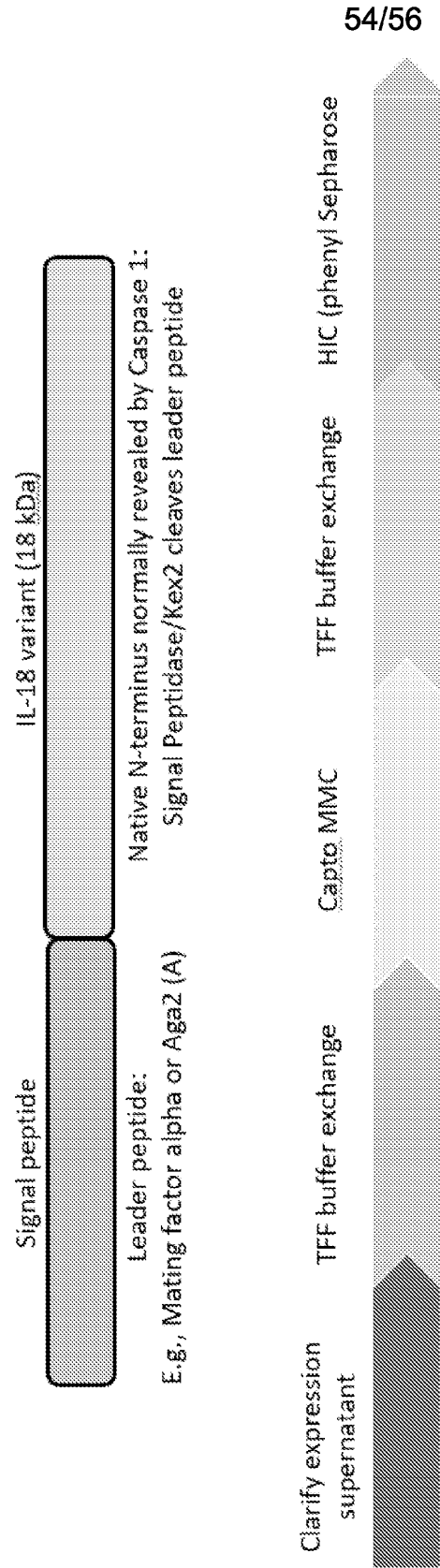
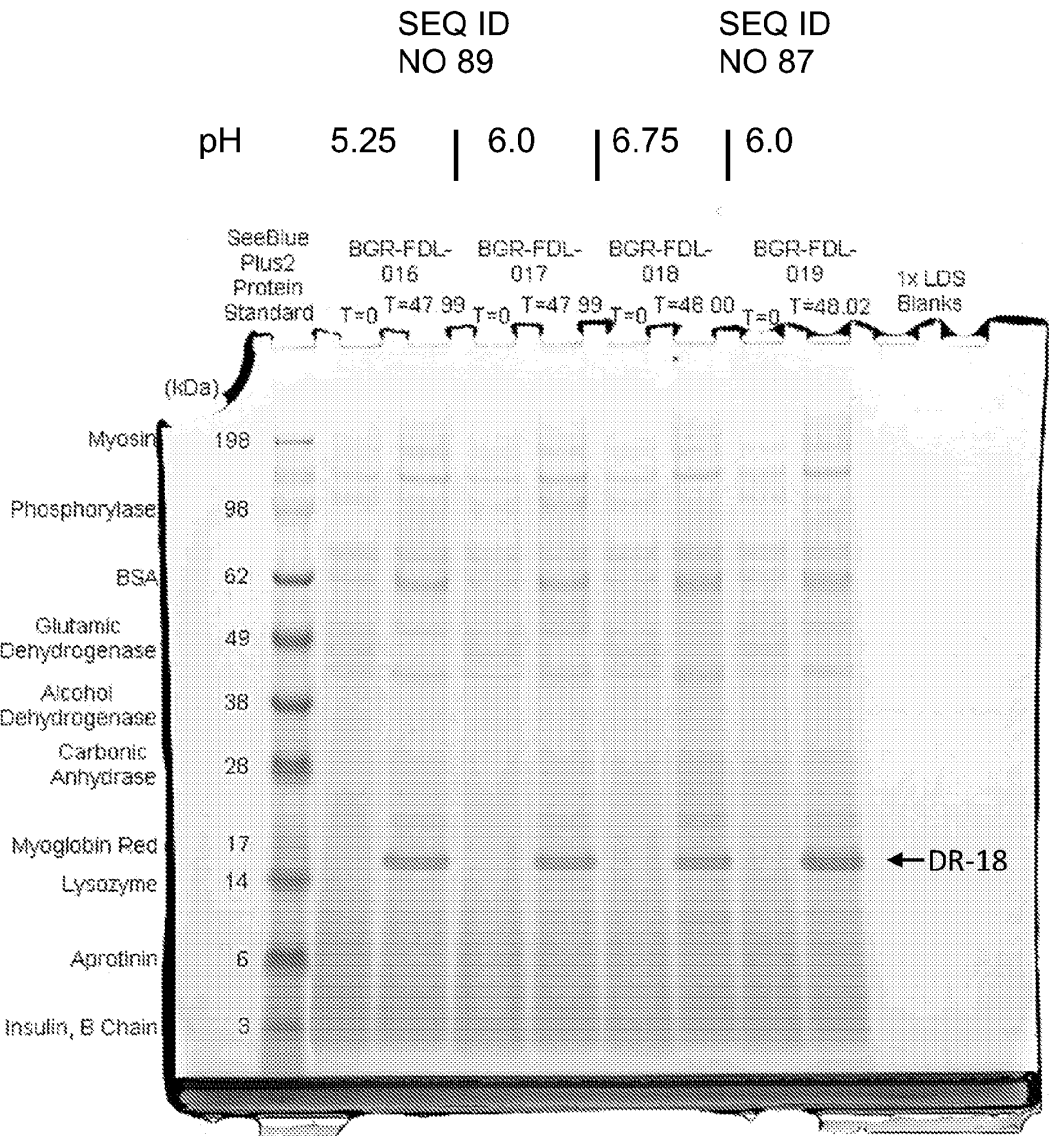


FIG. 46

FIG. 47



BGR-FDL-(016-9) Reduced SDS-PAGE, 4-12% Bis-Tris gel, 1x MES running buffer.
T= Induction hours. 10 microliters per lane. Coomassie Stained. 07Dec2018 Notebook 486-114.

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FIG. 48

