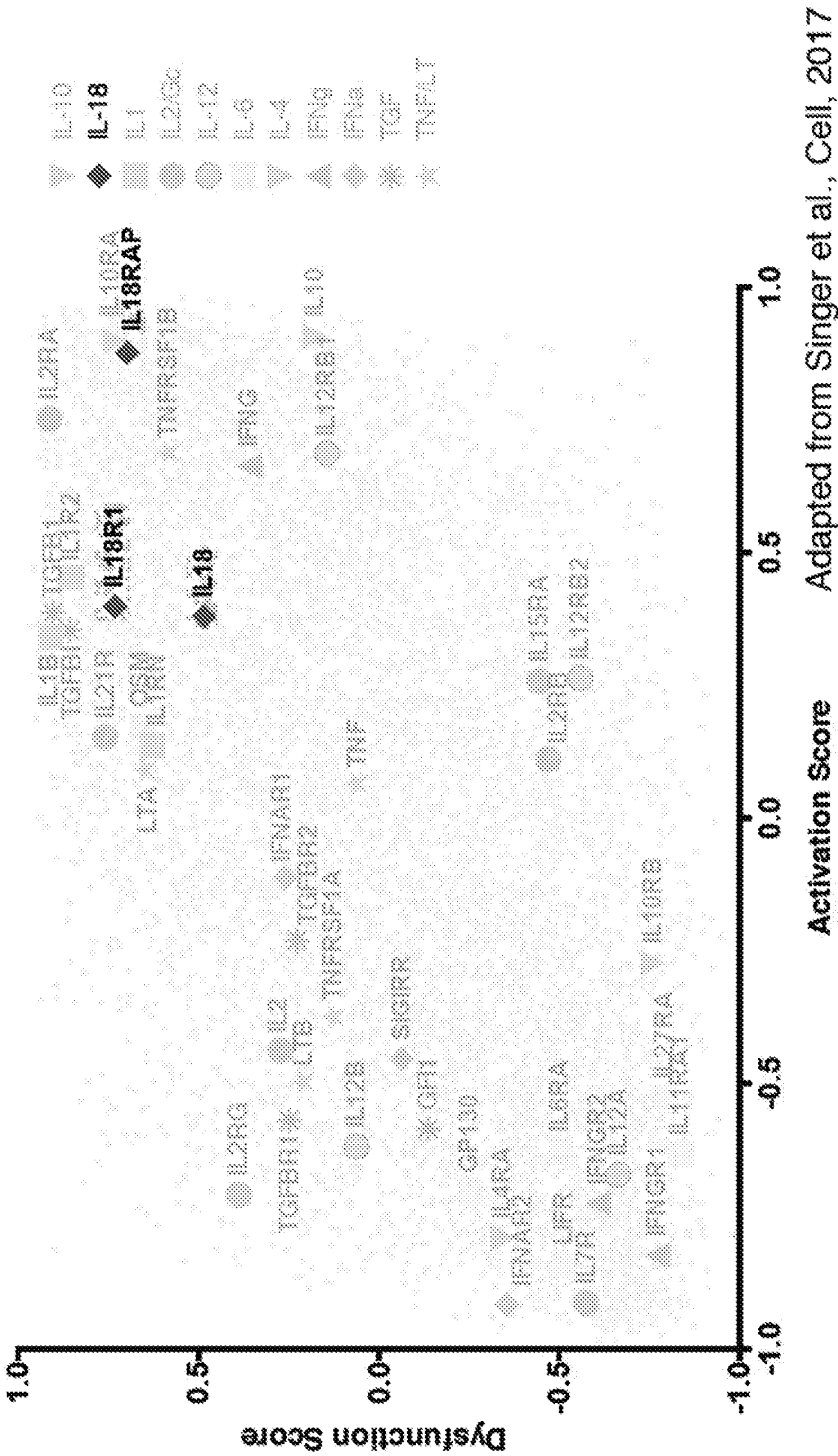




Adapted from Singer et al., Cell, 2017

FIG. 1A

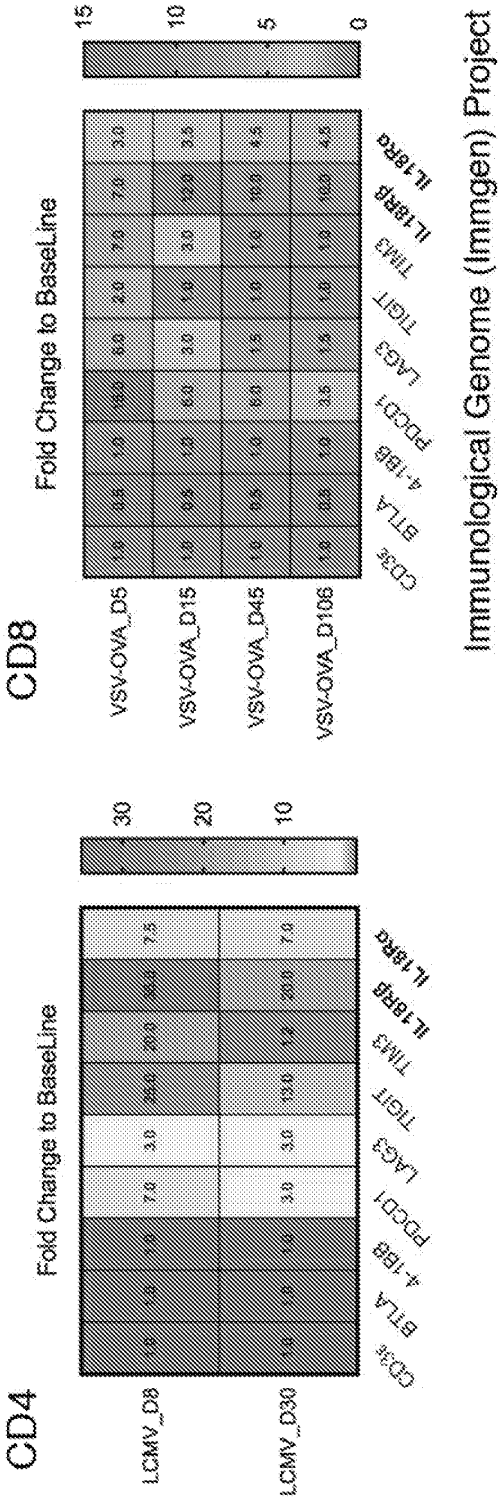
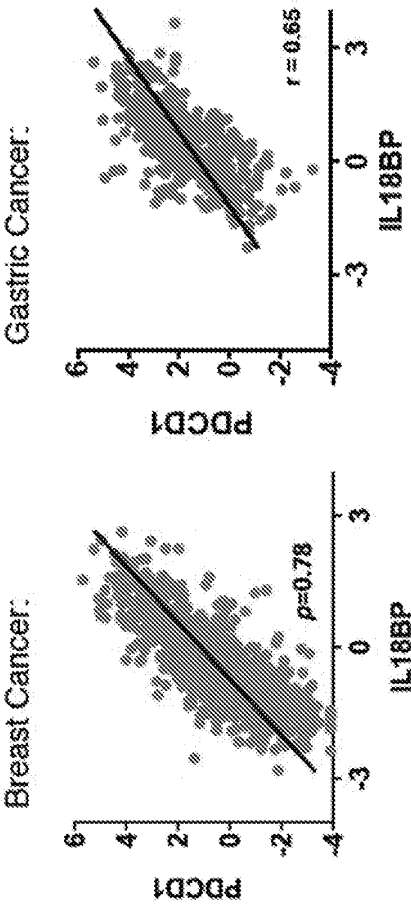
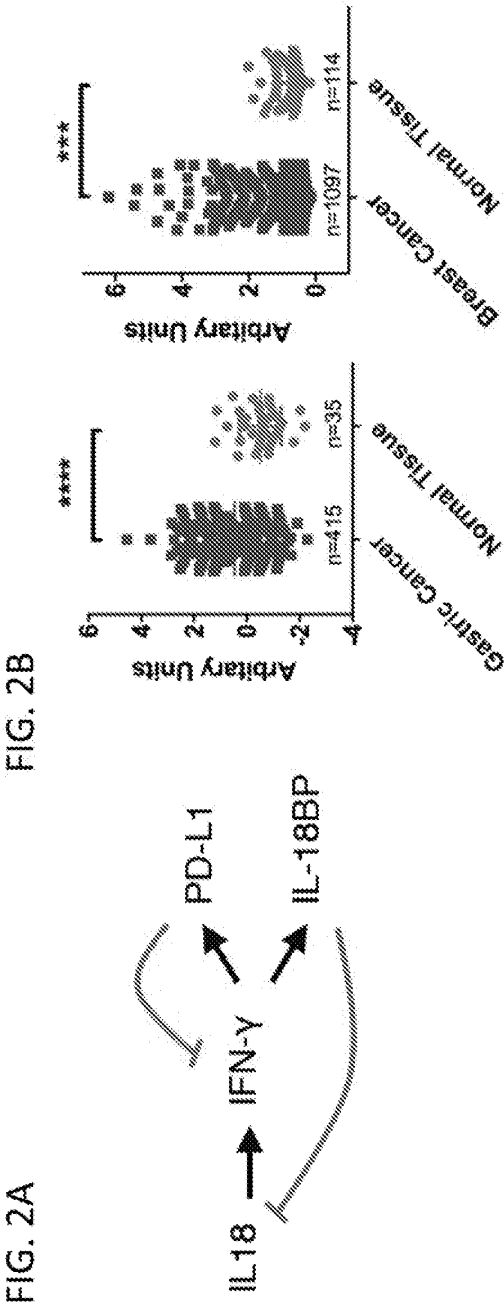


FIG. 1B



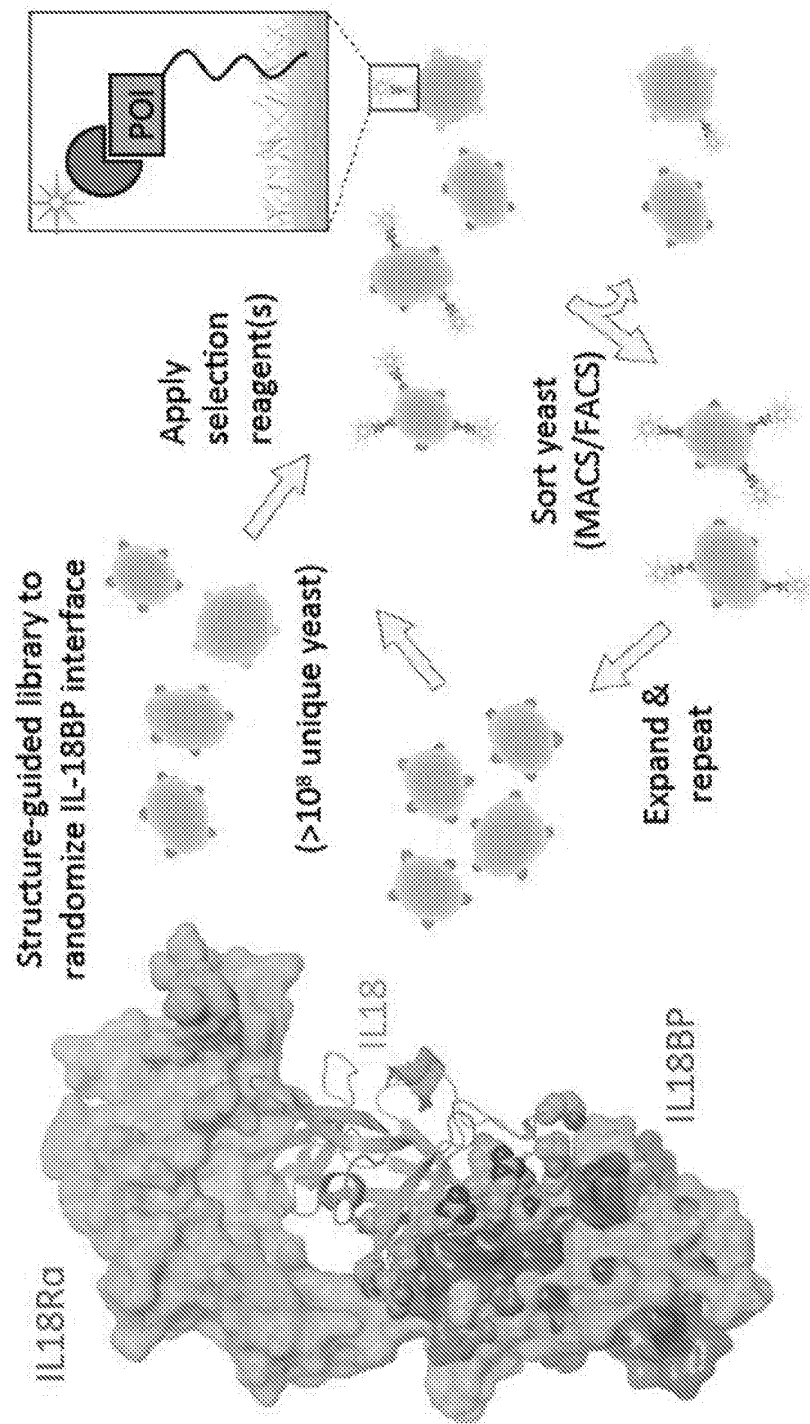


FIG. 3A

FIG. 3B

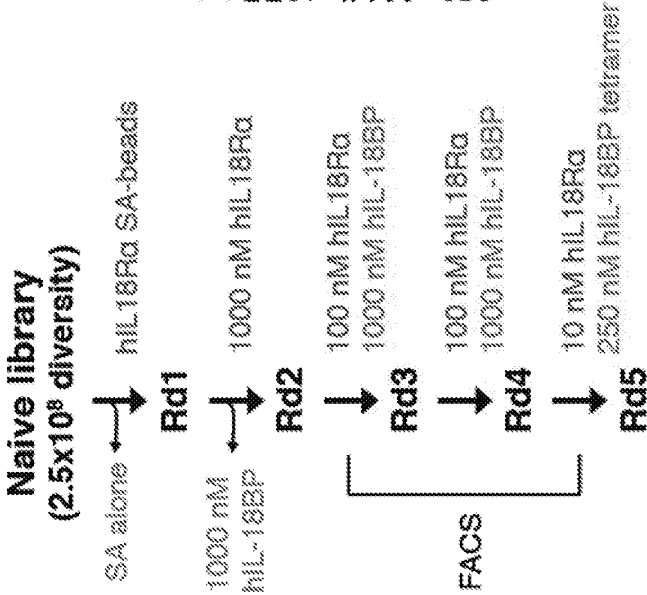
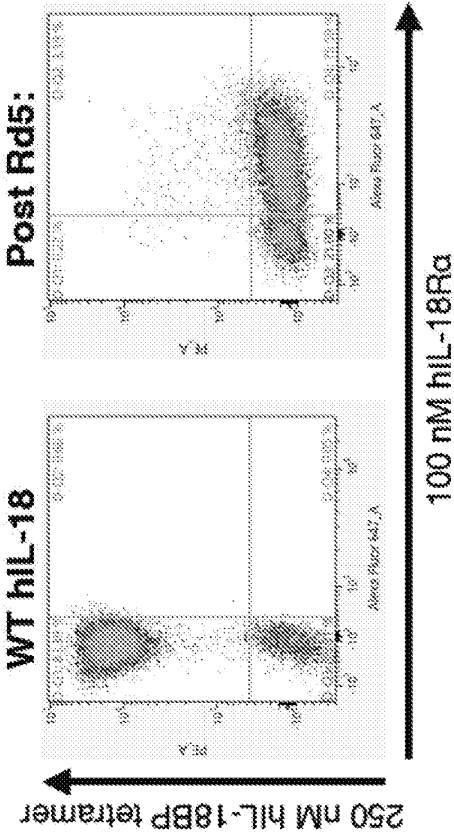


FIG. 3C



Human DR-IL-18 variants sequence summary

	1	5	8	51	53	55	59	60	77	103	105	110	111	153	155
WT hIL-18	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
hA8			R	K		K	A	Q			D	K	H	I	
hD6			R	D		K	A	K			D	K	H	I	
hH12		H		T	R		A	Q		E	D	N	H	T	K
hB11		I		K		K		K			D	H	H	I	
hC3		I		T		R		K			D	K	H	T	H
hC2		I		T		K	A	K			D	H	H	I	K
hG10		I		T		K		K			D	H	H	I	K
hG1		I		T		K		K			D	H	H	I	K
hF1		Y		T	R			K			D	H	H	I	K
hD2	H	Y	R	T	R			K			D	H	H	I	K
hA1	R			T	R		A	K		K	D	H	H	A	K
hB3	R		R	T	R			K		K	D	H	H		K
hB4	R		R	D	R			Q		K	N	N	H		K
hH3	R		R	N	R			K		K	D	K	H		K
hC5	R		R	T	R		T	K			D	K	H		K
hH4	R		R	T	R		A	K		E	D	N	H	I	K
hE1	R		R	T	R		T	K		E	D	N	H	I	K
hG2	R		R	T	R		A	K		E	D	N	H	I	K
hB9	R	H		T	R			K		E	D	N	H	I	K
hE12	R	Y		T	R		T	K	D		D	K	H		
hCS1				T		K	A	K			D	K	H	I	
hCS2				T		K		K			D	K	H		
hCS3	R			T	R			K			D	K	H		
hCS4	R			T	R			K			N	K	H		

Selected Variants

Consensus Variants

FIG. 4

FIG. 5A

Binding isotherms on yeast-displayed DR-hiL-18 variants

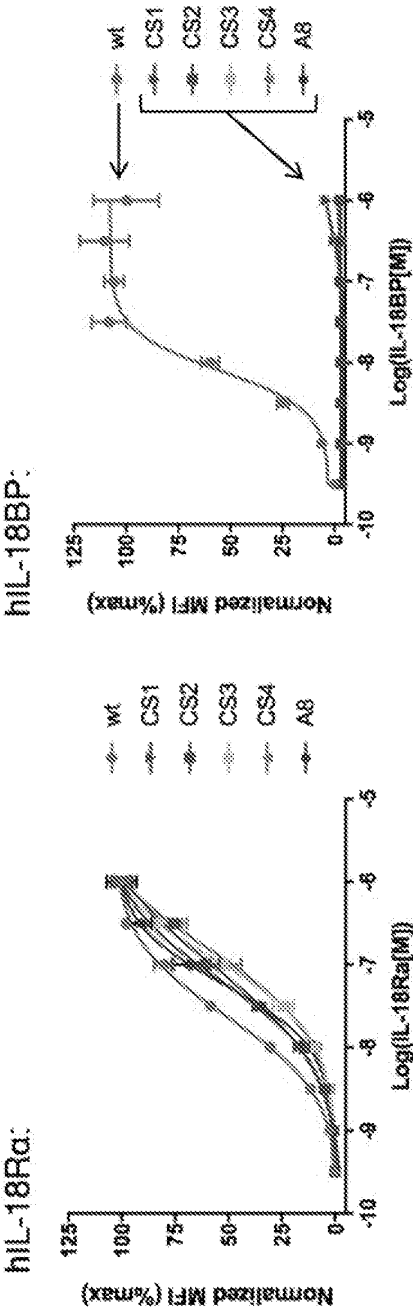


FIG. 5B

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

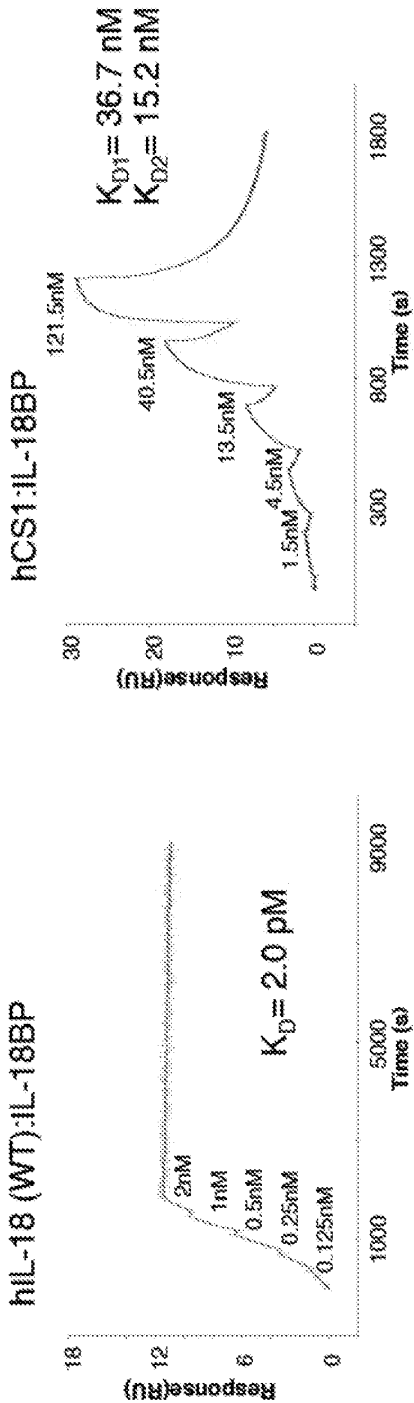


FIG. 6A

hIL-18Ra binding assays on-yeast

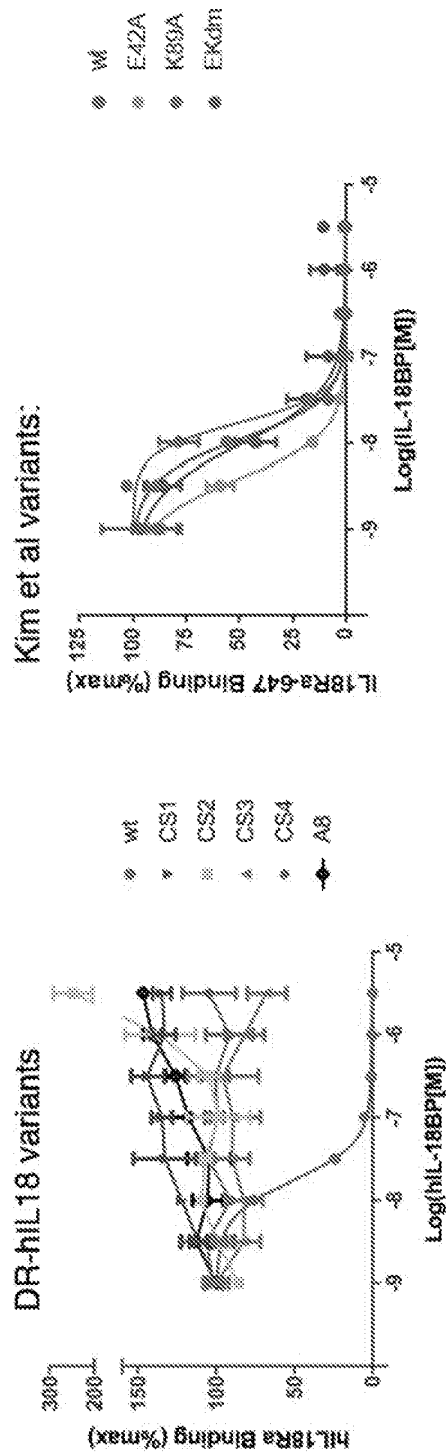
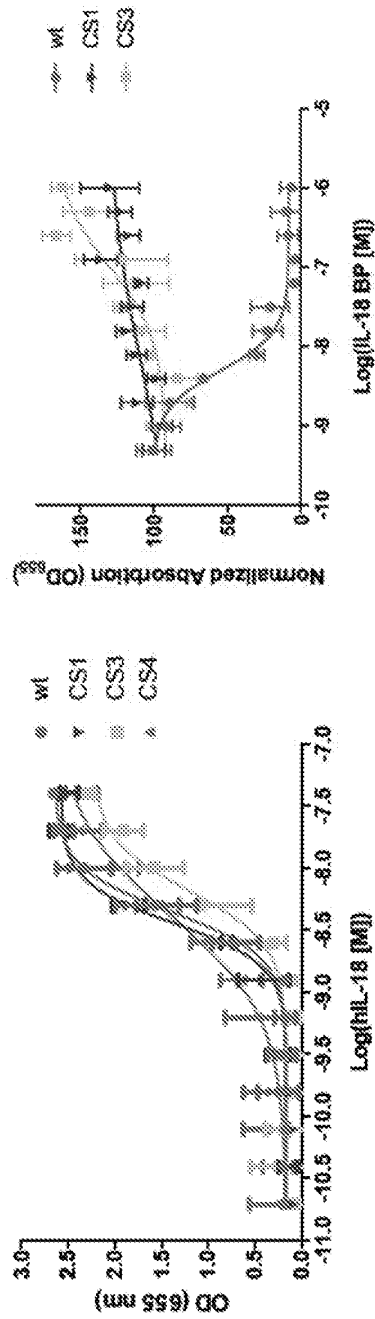
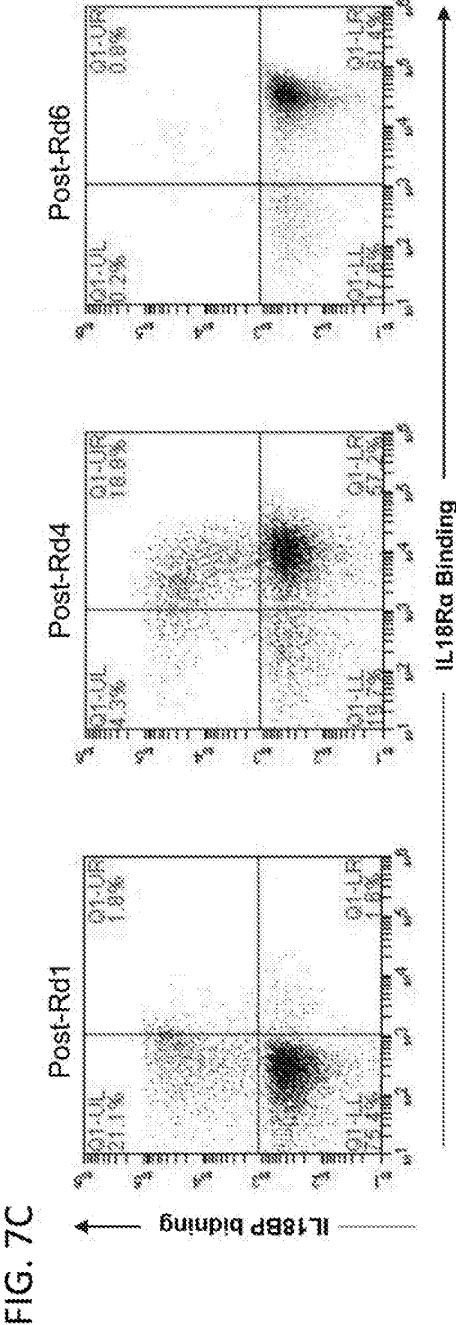
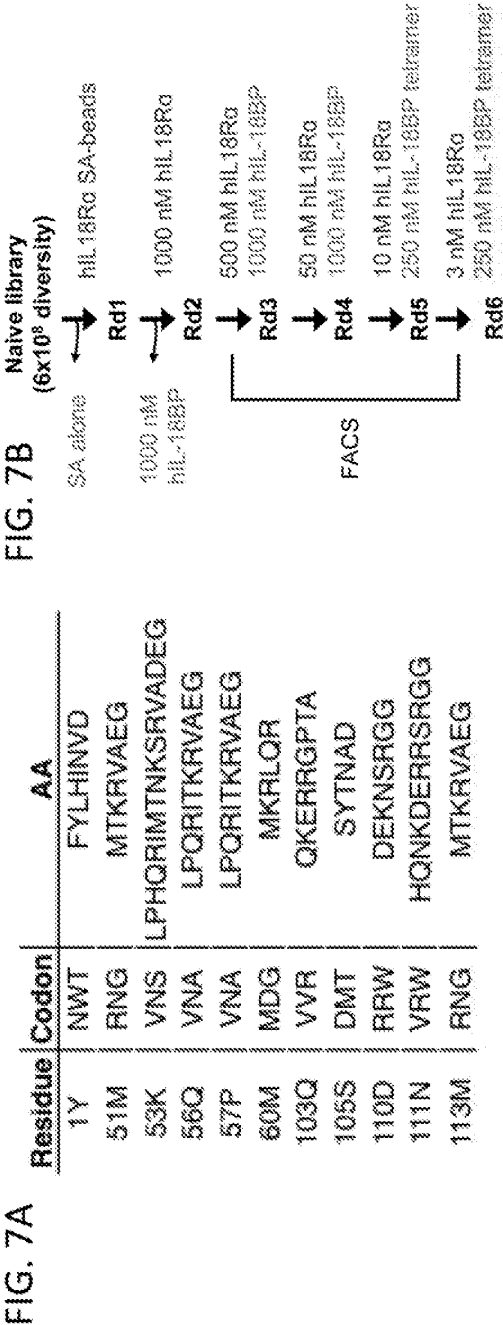


FIG. 6B

HEK Blue IL-18 signaling assay





WT hIL-18	51	53	56	57	60	103	105	110	111	113
	M	K	Q	P	M	Q	S	D	N	M
5-18	E		E	L	R	P	A	N	R	V
5-29	K		A	G	L	E	D	S	S	R
5-8	K	G	A	A	L			K	R	
5-6	K	G	R	G	L	E	D	N	S	R
5-26	K	G	V		L	A	A	S	R	T
5-20	K	S	G	A	L	A	A	G	R	T
5-2	K	S	K	A		A	D	S	S	R
5-9	K	S	L	A	L		D	S	R	
5-42	K	S	R	A	L		N	G	R	
5-17	K	S	R	A	L	A		G	R	T
5-41	K	S	R	A	L	A	D	S	G	R
5-1	K	T	R		L	E	D	S	S	K
5-33	K	T	R	A		E	D	N	D	R
5-21	R		G	K	L	R		S	R	V
6-31	K	G	G	A	L	E	D	S	G	V
6-20	K	G	R				A	N	R	
6-12	K	S	L	A	L		D	S	R	
6-27	K	S	R	A	L	A		G	R	T
6-29	K	S	R	A	L		N	G	R	

Round 5 variants

Round 6 variants

FIG. 8

FIG. 9A

IL-18Ra binding:

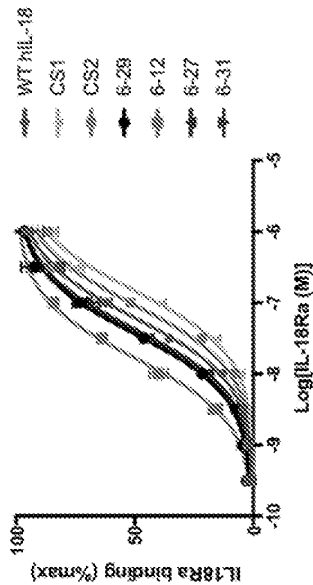


FIG. 9B

IL-18BP binding:

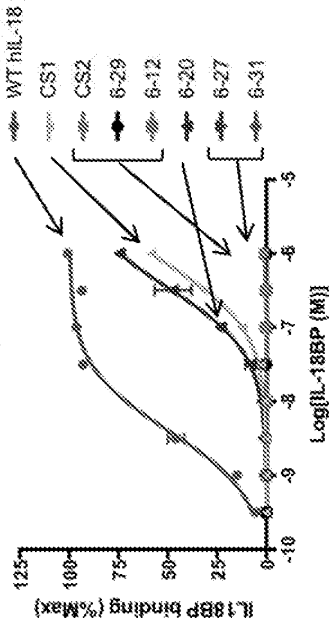


FIG. 9C

Thermal stability:

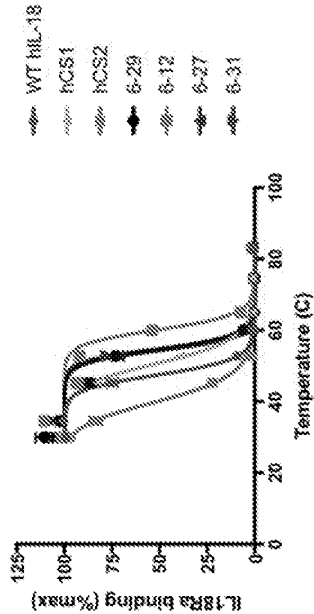


FIG. 9D

Variant	IL18Ra (KD)	IL18BP (KD)	Ra:BP ratio (norm. to WT)	Tm (°C)
WT IL18	62 nM	2.1 nM	1.0	47.6
hCS1	146 nM	710 nM	140	50.9
hCS2	92 nM	NBD	>3,200	40.2
6-31	41 nM	NBD	>7,200	54.9
6-20	N.D.	340 nM	N.D.	N.D.
6-12	17 nM	NBD	>17,000	60.2
6-27	42 nM	NBD	>7,000	54.3
6-29	37 nM	NBD	>8,000	54.7

FIG. 10B

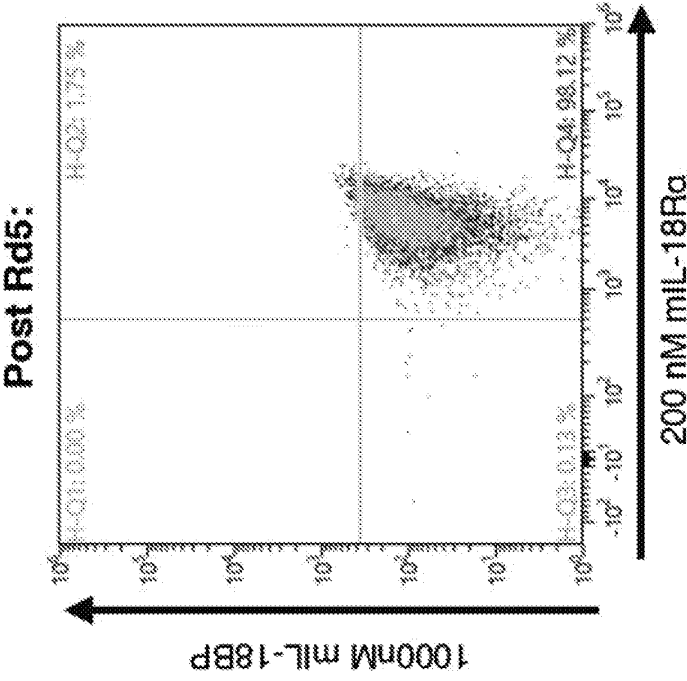


FIG. 10A

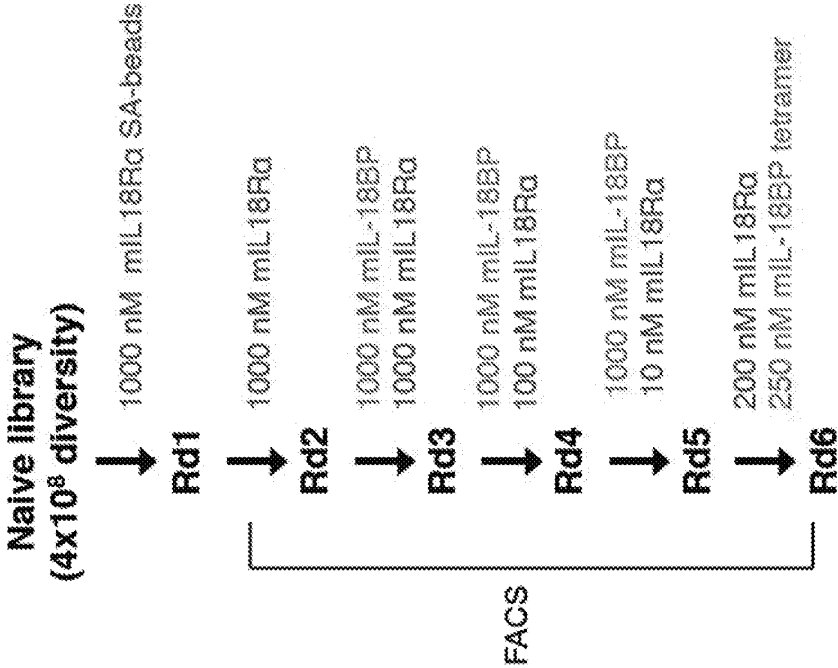


FIG. 10C

Murine DR-IL-18 variants sequence summary

	1	50	51	52	54	55	56	57	58	59	104	109	151
mIL-18	N	M	Y	K	S	E	V	R	G	L	R	N	L
mC1		A		V	R	R	L			K	K		
mA12		S		S	K	H	M			K	L		
mE8		V		T	G	R	R			K	K	D	
mC10	H	A		G		H	M			K	Q		V
mB7	H	A		G		N	A	G		R	K		
mB1	H	G		A		D	A		A	K	S		V
mD1	H	G		S	R	G	S	K		K	Q		
mH7	Y			A	R	R	A			K	K	D	V
mA7	Y	A		G	N	R				K			
mE1	Y	G		G		R	R	G		R		D	
mH3	Y	T	R	T	G	G	Q	K		V		D	
mCS1		G		A		R				K			
mCS2	H	A		G		R	A			K			

Selected Variants

Consensus Variants

FIG. 11A

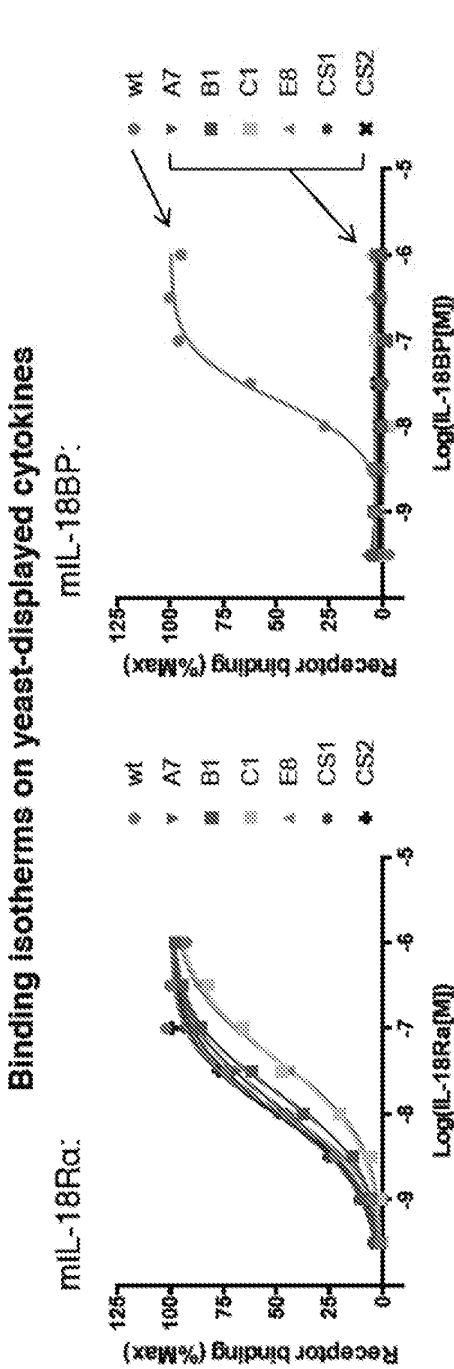


FIG. 11B

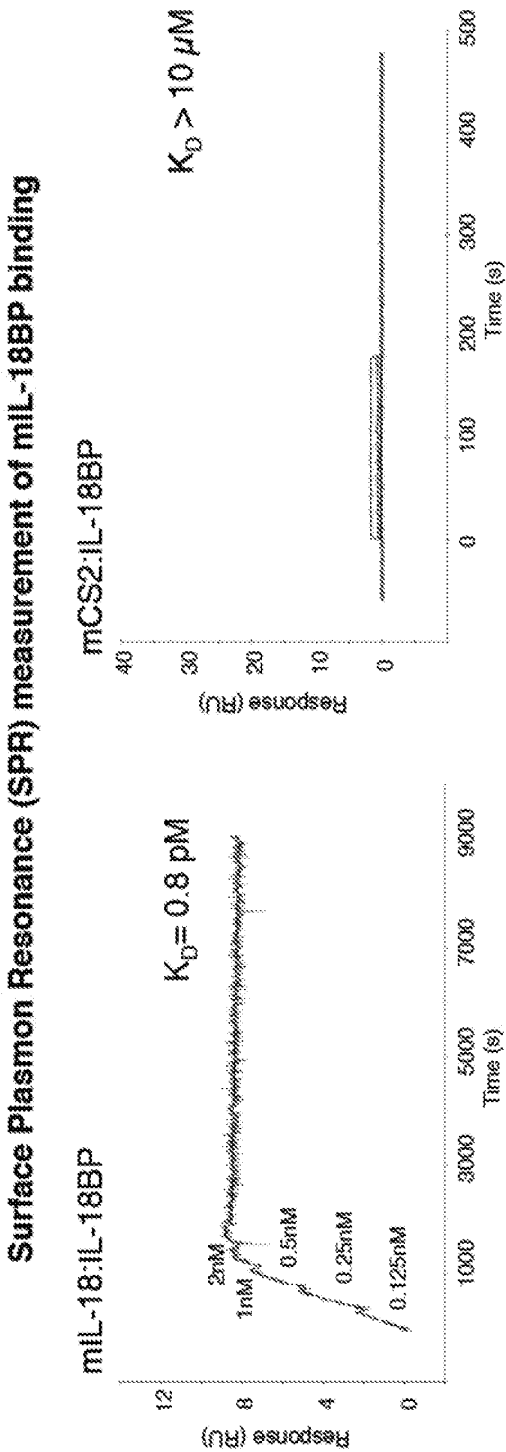


FIG. 12A

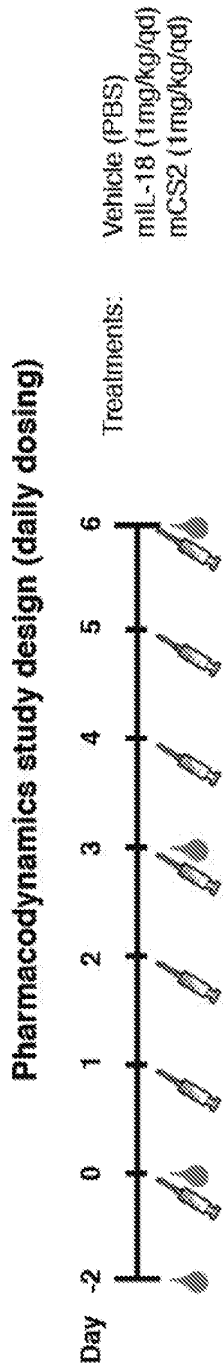
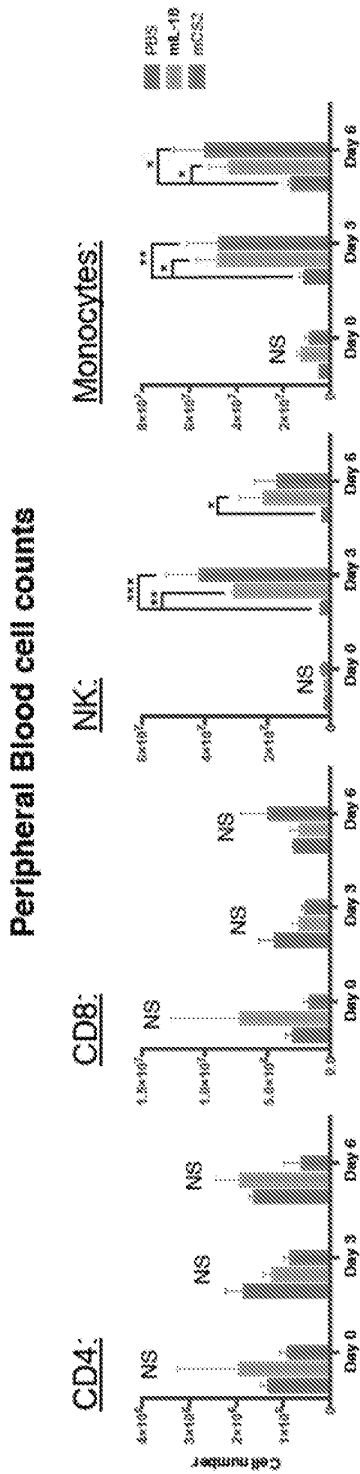
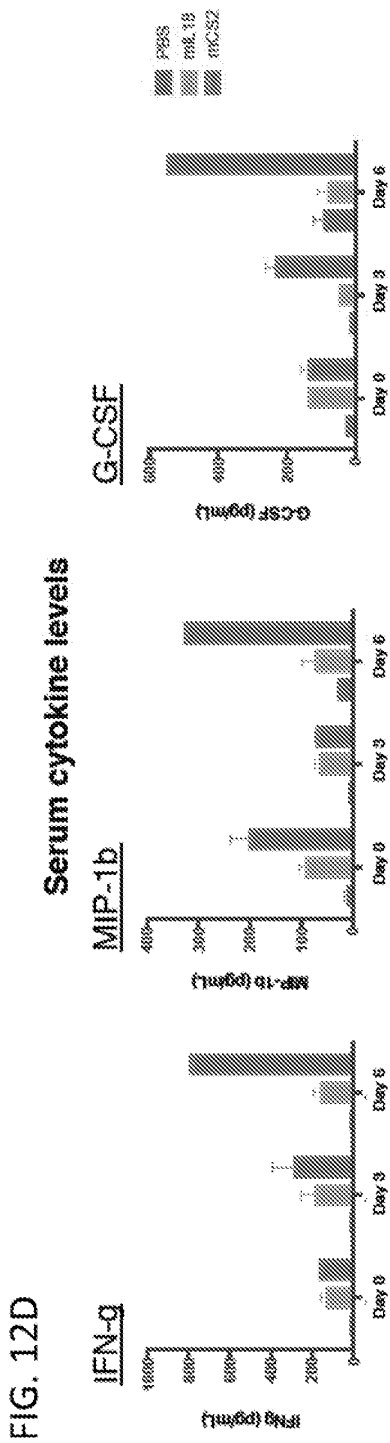
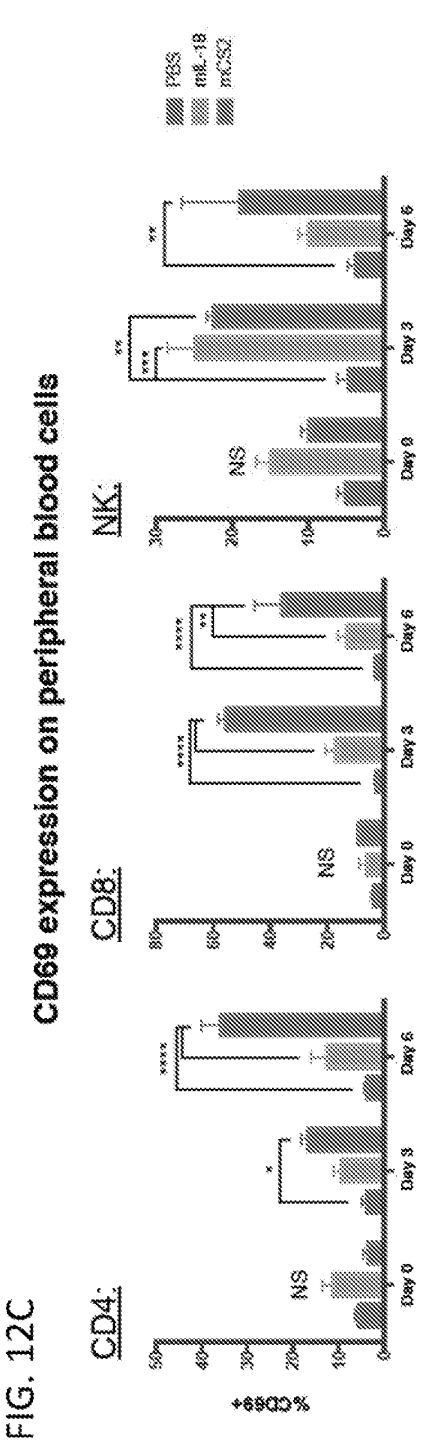


FIG. 12B





Echo MRI body composition analysis

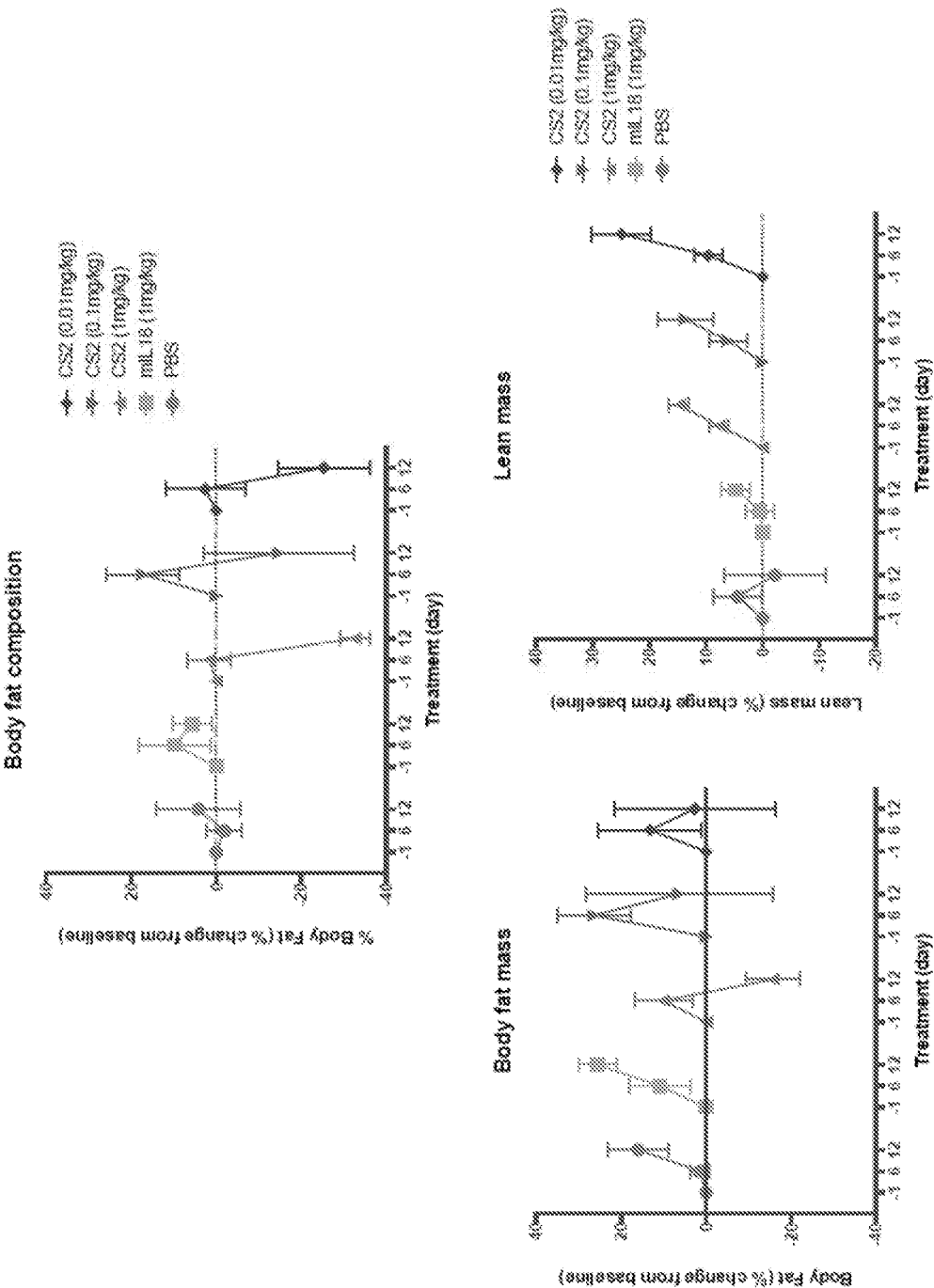


FIG. 13

18/37

Yumme1.7 melanoma treatment model-Tumor Growth

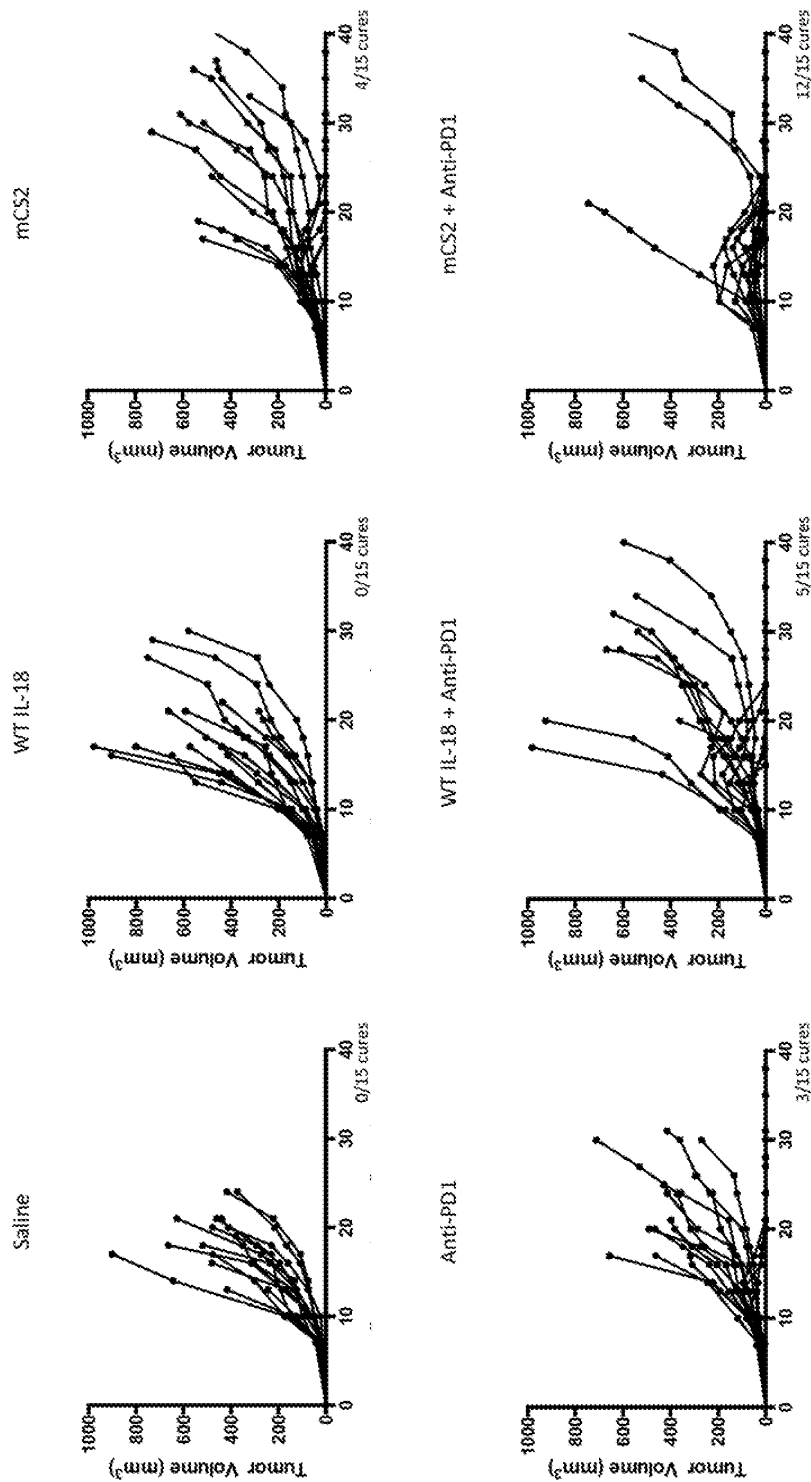


FIG. 14A

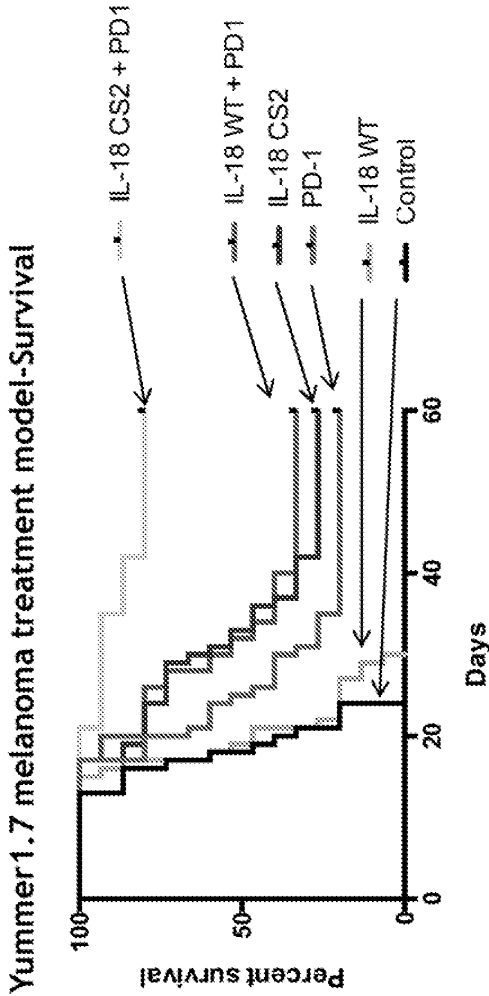


FIG. 14B

FIG. 15A

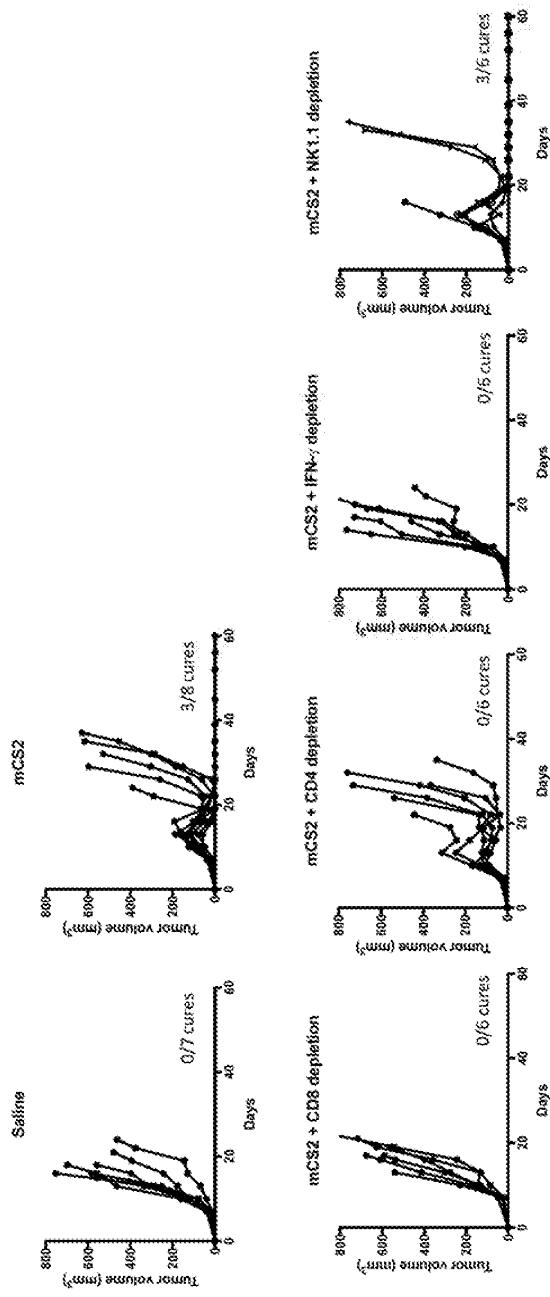
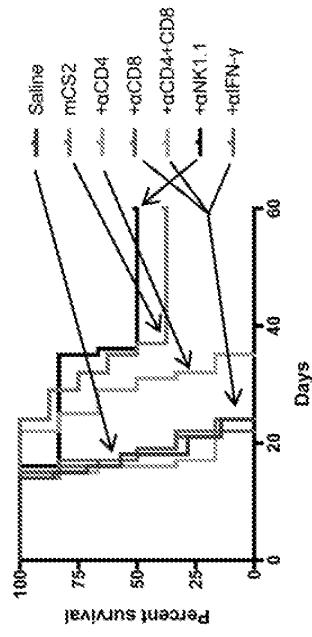


FIG. 15B



MC38 tumor treatment model- tumor growth

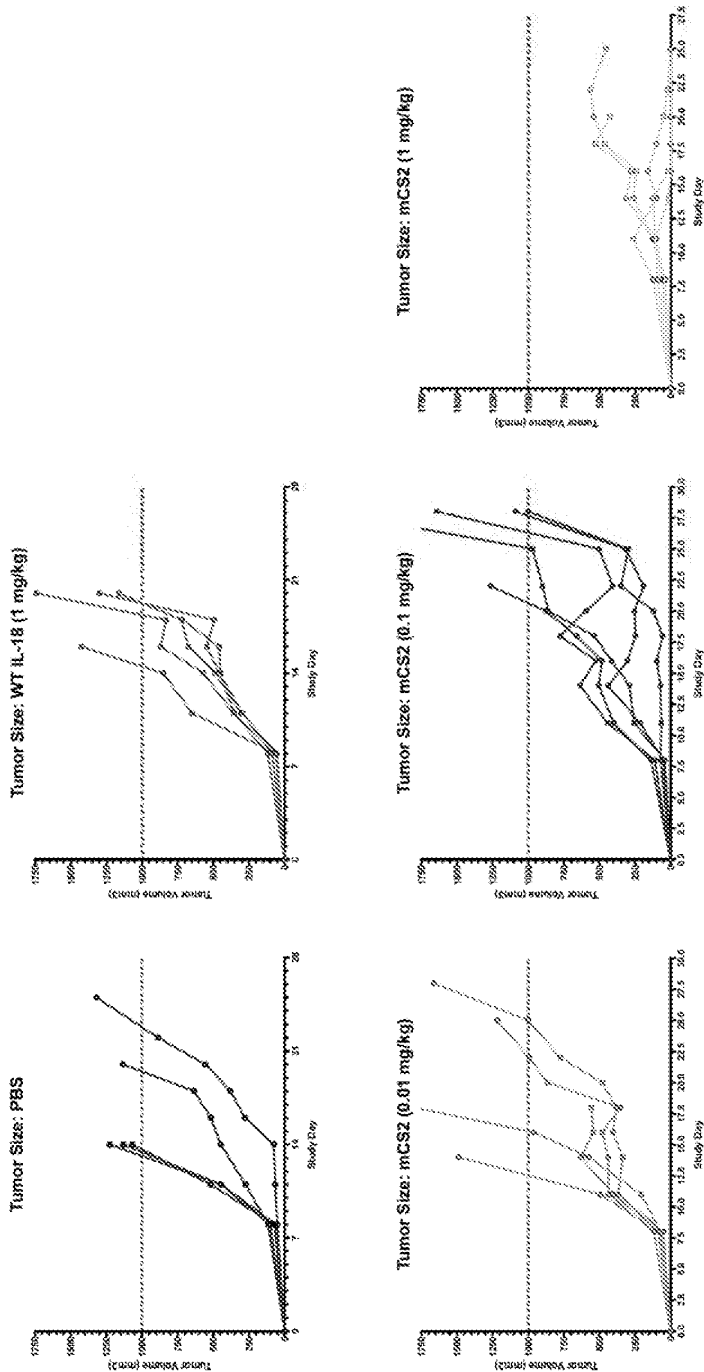


FIG. 16

Expanded MC38 treatment cohorts-Tumor Growth

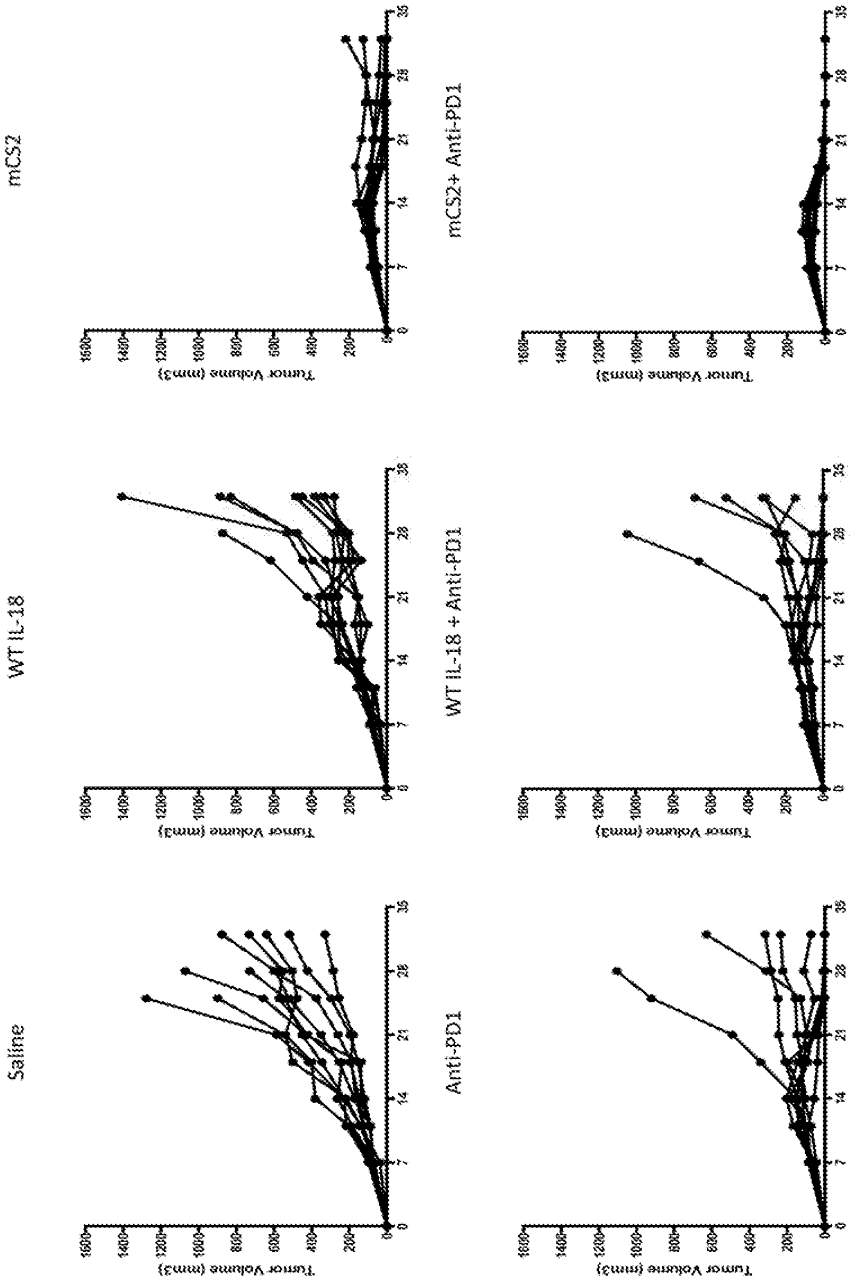


FIG. 17

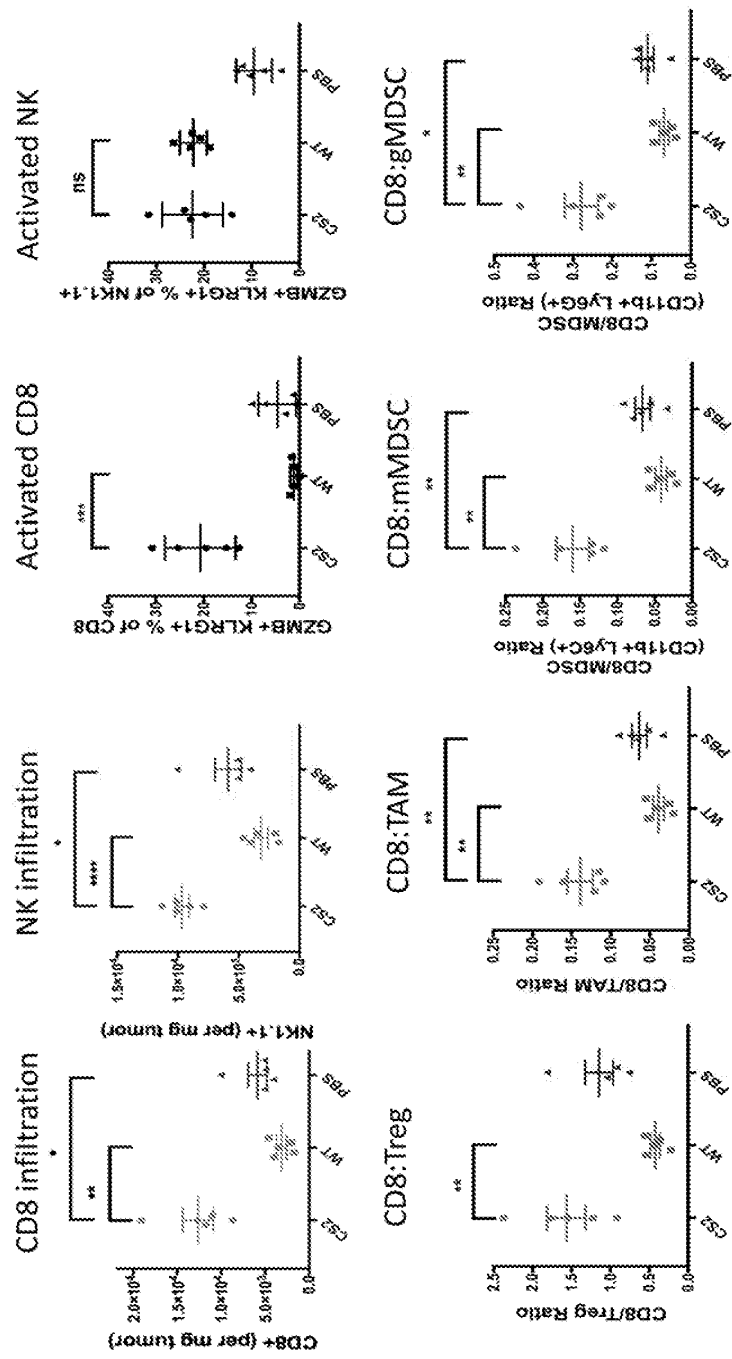


FIG. 18A

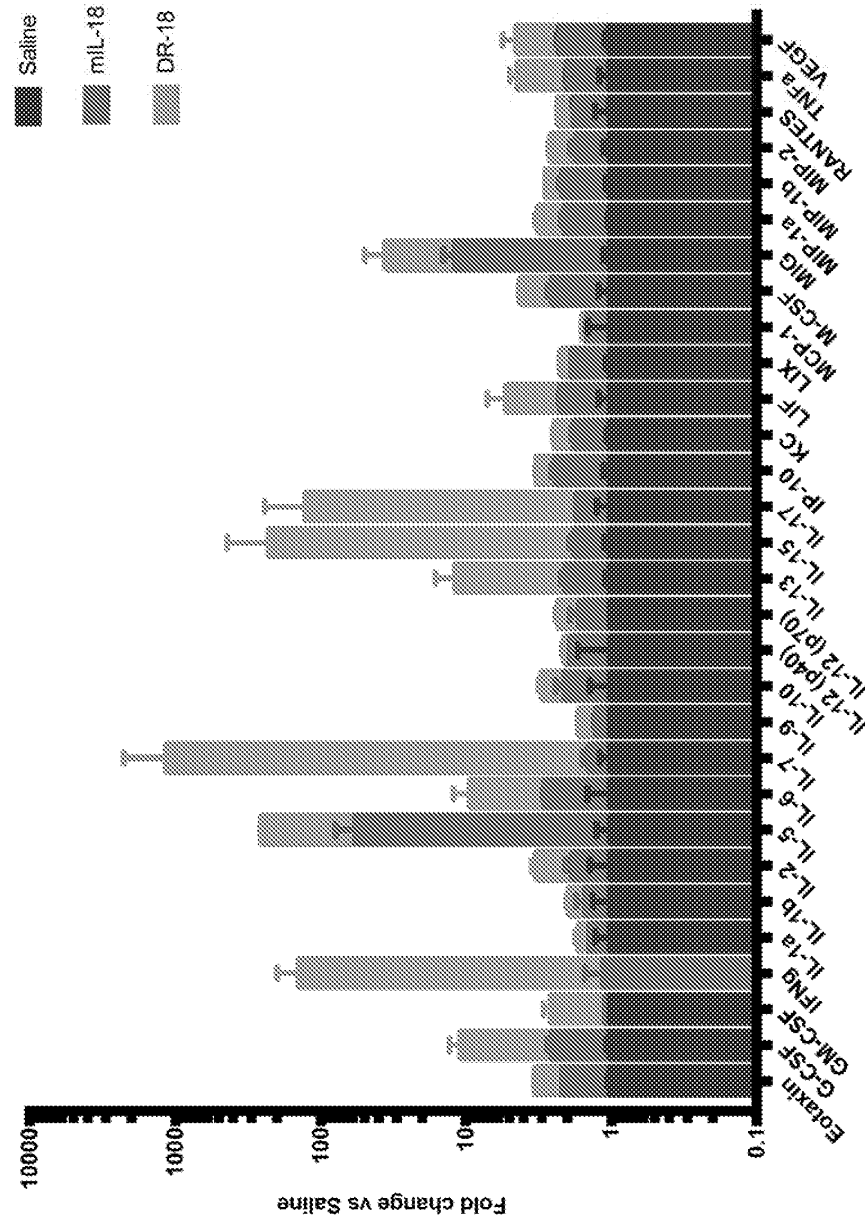


FIG. 18B

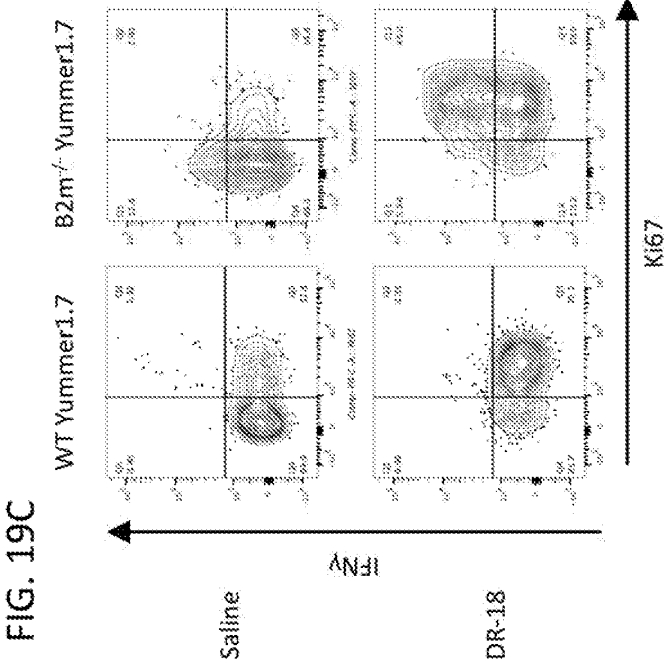
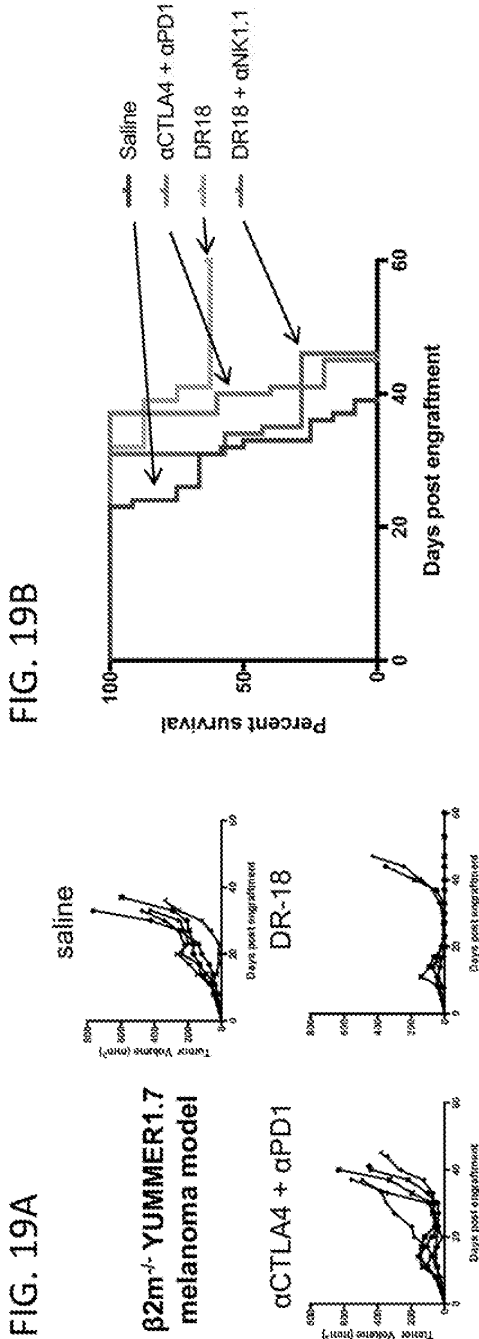


FIG. 20B

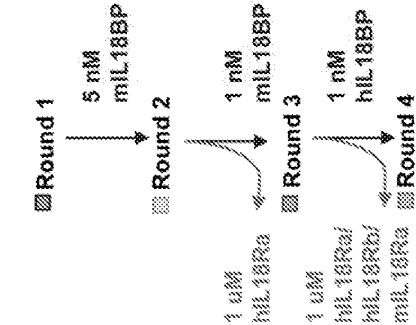
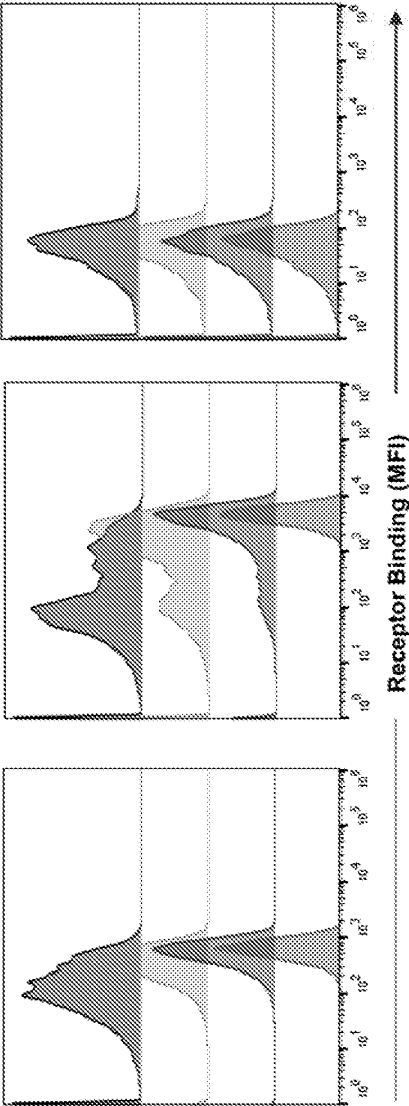


FIG. 20A

Residue	Amino Acids	Codon
Y1	YELHVD	BWT
L5	LFY,H,R,C	YDT
D17	DAG,R,H,P	SVT
E31	EAG,T,K,R	RVA
T34	TAE,K	RMA
D35	DA,S,Y	KMT
S36	SN,K,R	ARW
D37	DA,Y,S	SNT
D40	DA,Y,S	KMT
N41	NK,S,R	ARW
M51	M,I,L,F	WTS
M60	M,I,L,F	WTS
Q103	QL,I,K	MWA
H109	H,D,A,P	SMT
M113	M,I,L,F	WTS
D132	DA,Y,S,V,F	KHY

FIG. 20C



	1	5	17	31	34	35	36	37	40	41	51	56	60	103	109	113	131
WT hIL-18	Y	L	D	E	T	D	S	D	D	N	M	Q	M	Q	H	M	R
hd2D-5D02				A	A	S	N	P	Y	K			L	L	A	L	
hd2D-5D08				A	K	A	K	A	S		F		F	L	A	I	
hd2D-5A02			A	T		A	K	P	S	S	L		L	L	P	I	
hd2D-5B02			G	A		S	K	R			L		F	I		I	
hd2D-5B10			G	G		A	K	H	A	R	F			I	A	F	
hd2D-5C03			G	K	E	A	N		S	S	I		L	I	A		
hd2D-5C08			G	T		A		H	S	S	L		L	L		F	S
hd2D-5E08			G	T	E	S		P			F		L	L		I	S
hd2D-5C04			G	T	E	S		L	A		L	H	L	L	A	F	S
hd2D-5C05			G	T	E	Y	R	L	S		F		L	L	D	L	
hd2D-5D05			G	T	K	S	N	L	S		F		I	I	D	I	
hd2D-5F04			R	T	Y	K	Y	K			F		F	I	A	I	
hd2D-5F11	D		A			N	N	P	S		F		I	L	P		
hd2D-5B11	D		A	K	A	A	N	A	A				L	L		F	
hd2D-5E03	D		A	K	S	N	N	V	A				L	I	P	L	
hd2D-5B05	F		A	A	S	N	N	H	A		L		L	I			
hd2D-5B06	F	F	G	T	E	S	K	P		R	F		L	L		L	
hd2D-5F06	H		G	T	E	S	K	P		S			I	I		F	
hd2D-5C09	H		G	A		S	N	P	S	R	F			I		F	
hd2D-5D10	H		G	A	A	S	N	R	A		F		F	I	P	F	
hd2D-5F10	H		G	A	E	A	N	P			F		L	L			
hd2D-5E10	H		G	A	E	S	N	R		S	F		F	L		I	
hd2D-5F02	H		G	G	E	A		P			F			I	A	L	S
hd2D-5E02	H		G	G	E	A		P	A	K	I		F	L	A	F	
hd2D-5F12	H		G	G	E	A		P	A		F			I	A	F	
hd2D-5D03	H		G	R	A	A		H			F		F	L		I	
hd2D-5F01	H		G	T	A	S				K	I		L	L	P	F	
hd2D-5C10	H		G	T	A	S	N	H	Y		F		L	L	A	I	
hd2D-5D06	H		H	T	A	S	N	A			F		L	L	A		
hd2D-5F08	L		G	A	S		N	P	S	R	F		L	I	D	I	
hd2D-5A09	L	H	G	G	E	S		P	Y		F		L	I			
hd2D-CS1			G	A		S					F		L	L			
hd2D-CS2			G	A		S					F		L	L			
hd2D-CS3			G	A	E	S					F		L	L			

FIG. 21

FIG. 22A

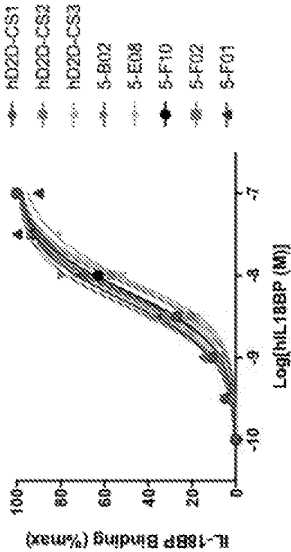


FIG. 22B

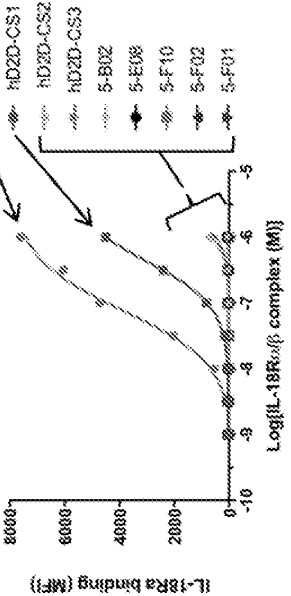


FIG. 22C

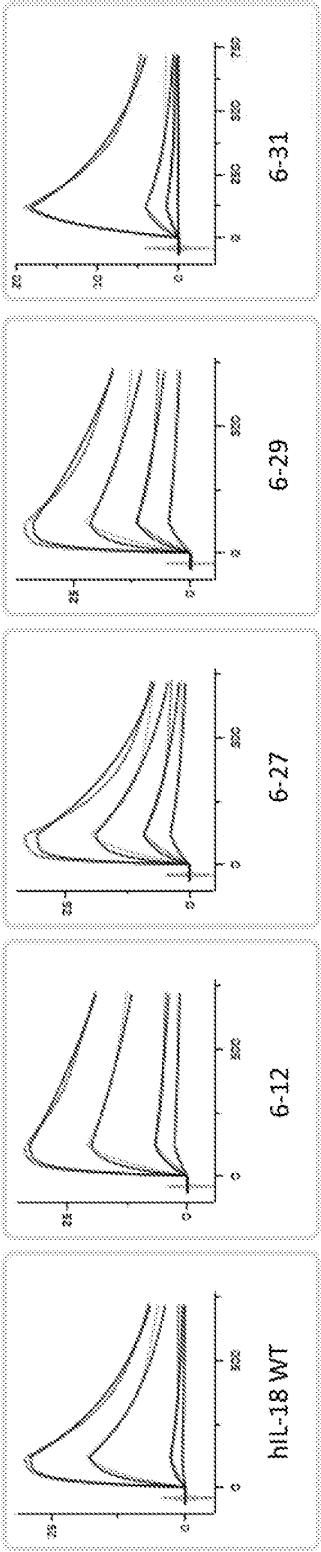
Variant	IL18Ra (nM)	IL18BP (nM)	BP:Ra ratio (norm. to WT)
WT IL18	62 nM	2.1 nM	1
hD2D-CS1	430 nM	5.9 nM	2.5
hD2D-CS2	21 μM	7.9 nM	90
hD2D-CS3	9.7 μM	8.9 nM	37
5-B02	NBD	4.2 nM	>170
5-E08	NBD	8.8 nM	>81
5-F10	NBD	6.4 nM	>110
5-F02	NBD	5.4 nM	>130
5-F01	NBD	4.8 nM	>150

29/37

WT mL-18	1	5	17	30	33	34	35	36	50	102	104	108	109	111	129	130
	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
mD2D-A6	D			A	G	S		A	F	L	A	D		I	A	T
mD2D-A7	Y		G	R	A		T	V	F	I	P	A	S		A	G
mD2D-A8	H			K	E	Y	T	V		I	A	D	R	I		N
mD2D-A9	Y		A	A	A		K	G		L	P	D	T		F	G
mD2D-A11	Y		E	A	G		R	H		I	P	A	S	L		
mD2D-A12	H			R	G	A		G	F	I	P	D	S	L	V	
mD2D-B4	H		S	T	G	S		V		I	G	D		I		R
mD2D-B7	Y		S	R	E		T	P	F	I		D	S	L	F	E
mD2D-B11	H		A	G		A	T	V	F	I	P	D	S	L		N
mD2D-B12			N	K	E	Y	T	L	F	I	P	D		L	Y	E
mD2D-C1	Y		G	A	E	A	T	R	F	I	G	A				G
mD2D-C3			G	A	R	A		L	F	L	G	D		L		R
mD2D-C5	Y		A	A	E	A	T	A	F	I	G	A	S			G
mD2D-C6	L		G	A	G	A	T	L		L	P	D	T		A	S
mD2D-C9			G		A	Y	T	V	F	I	G	D	S		Y	
mD2D-C10	D			K	E	S	K	P	F	L	A	A	S	L	A	N
mD2D-C11	L		G	A	G		K	V		I	P	D		L		E
mD2D-D1	Y	H	Q	R	A	A	T	R		L	G	D				
mD2D-D9			Q	T	E	S		G	F	L	A	A		L		S
mD2D-D12	F	H	G	G	G		R	V		I	A	D	S	I		G
mD2D-E3	V	H	G	K		Y				L	A	D	T		A	Q
mD2D-E4			G	A		A	T	R		I	Q	A		I	F	R
mD2D-E5	D		G	G	A	Y		G	F	I	A		S	I	S	G
mD2D-E7	Y			R	G	S		A		I	P	A	T	L		G
mD2D-E8	Y		E	T	E	A		G	F	I	G	D	R			G
mD2D-E9	F		N		E	Y	R	L		L	P	A	S	L	S	
mD2D-E10			N	A	E		R	L		L	G	D				H
mD2D-E11	Y		A	R	G	Y		L	L	L	P	D	T	I		N
mD2D-E12	Y			G	A		T	A	F	I	P	D	S		A	
mD2D-F3	D		G		A	Y		A	F	I	P	D	S	I	A	
mD2D-F4			E	R	K	Y		L	F	L	G	D			Y	G
mD2D-F5	D		E	T	A	Y		L	F	I	A	D	S	L		T
mD2D-F7	D		N	K	E	S	T	A		L	G	A	S	L	A	G
mD2D-F8	H		E	A	E	A		G	F	I	G	D	T	L		G
mD2D-F9	I		E	K	R	Y		V	F	I	E	A	S	L		E
mD2D-G1	Y		A	T	G	Y	T	L	L	I	P			I		R
mD2D-G7			N	R	A	S	T	A		I	G			I		
mD2D-G9	D		G		K		R	A	F	L	A		S			E
mD2D-H7			E	A				A		L	P	D	I		Y	G
mD2D-I1	Y		E	A			T	L	F	L	G	D				T
mD2D-A10	H		G	K	K	Y		V		L	A		S	I		S
mD2D-F12	Y		G		K	A	K	A	F	I	P	A	S			G
mD2D-E2	L		G	G	G	S		P	F	I	H	A	T			N
mD2D-C4	Y		S	T	A	Y	T	V	F	I	A	D	S	L		N
mD2D-C2	Y		G	T	G	A	R	V	F	L	P	D		L	S	G
mD2D-A2	D		G	G	K	A	T	G	F	I	A	A		L	A	G
mD2D-A1	D		S	R	G	S		H	F	L	A	A		L		G
mD2D-D4	Y		E	K	K		K	L	F	L	G	D		L	F	G
mD2D-A3	Y		G	A	A	S	T	H	F	L	G	A		I		
mD2D-B9	Y		S	G	K	Y		V	F	L	G	D	T		S	G

FIG. 23

Binding to hIL-18Ra



Binding to hIL-18BP

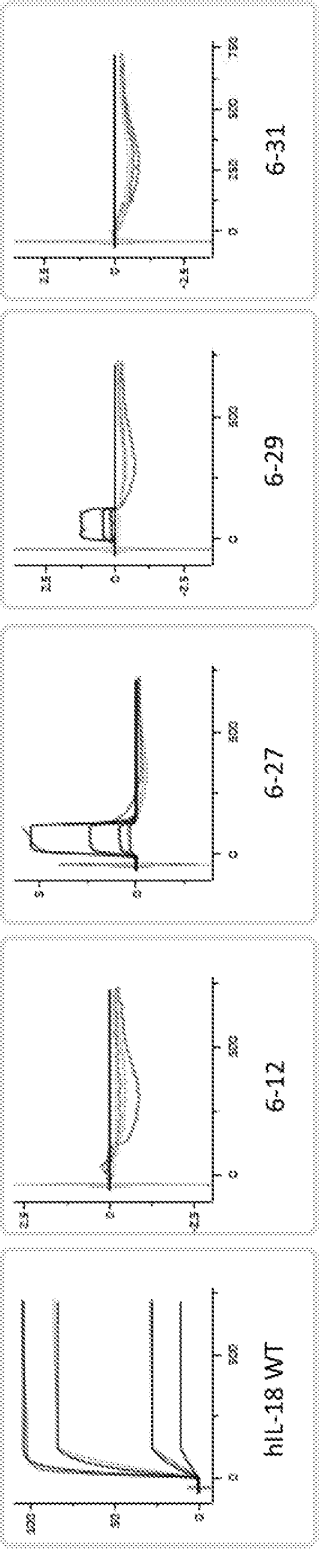


FIG. 24

31/37

FIG. 25A

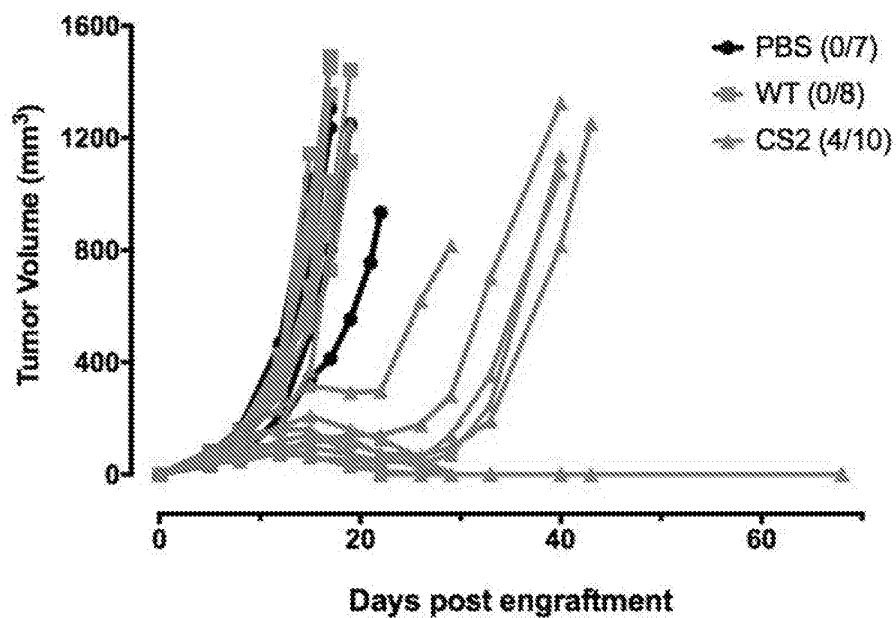


FIG. 25B

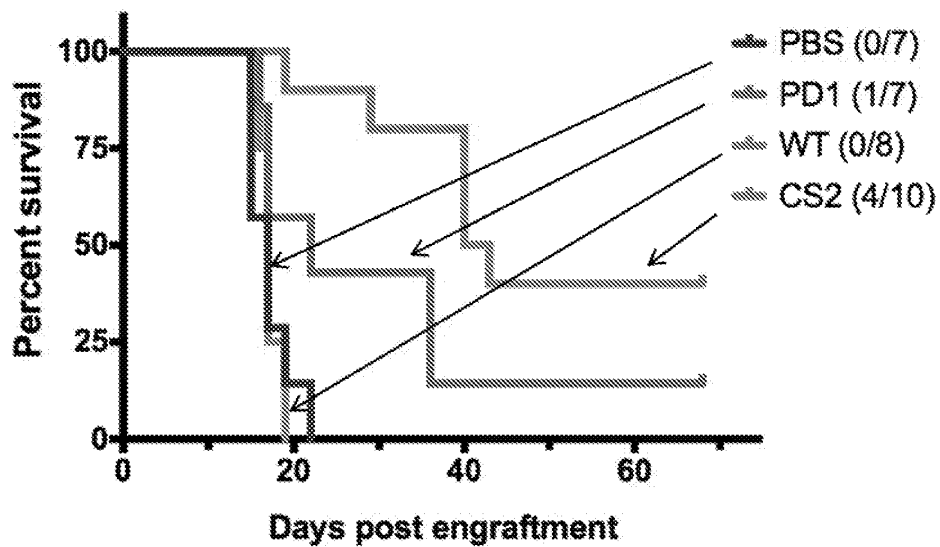


FIG. 26A

4T1

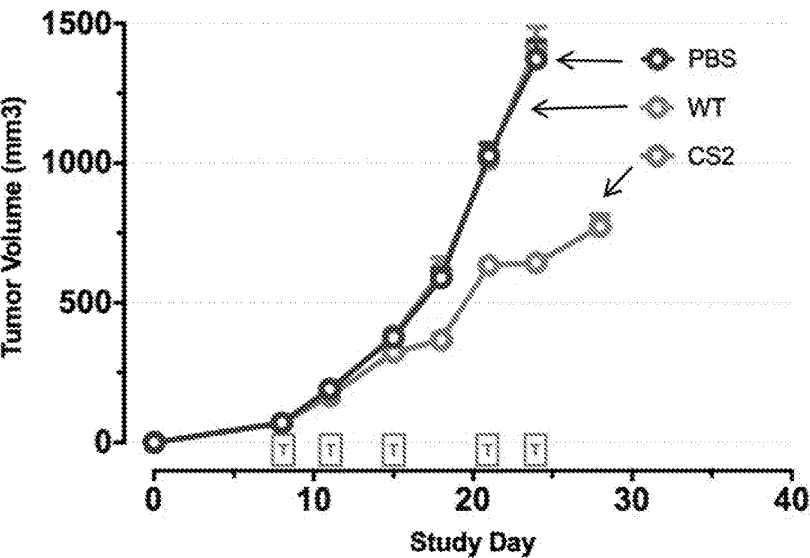
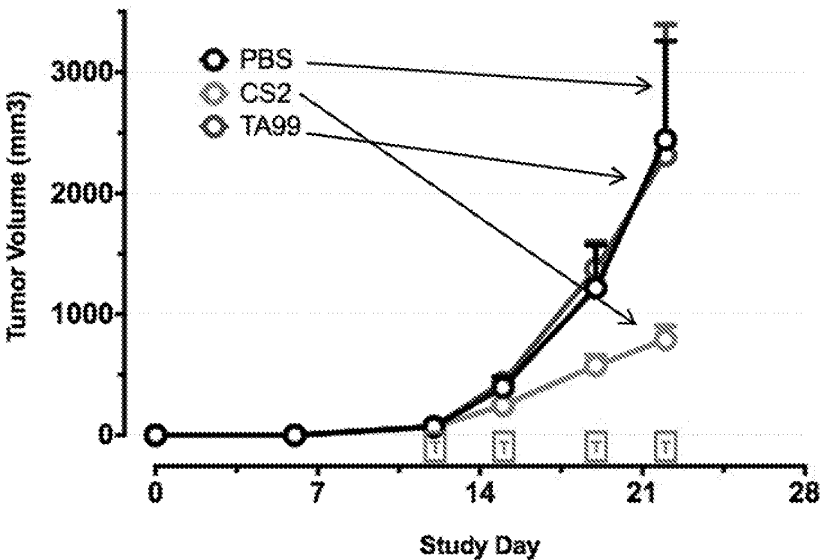


FIG. 26B

B16-F10



33/37

FIG. 27A

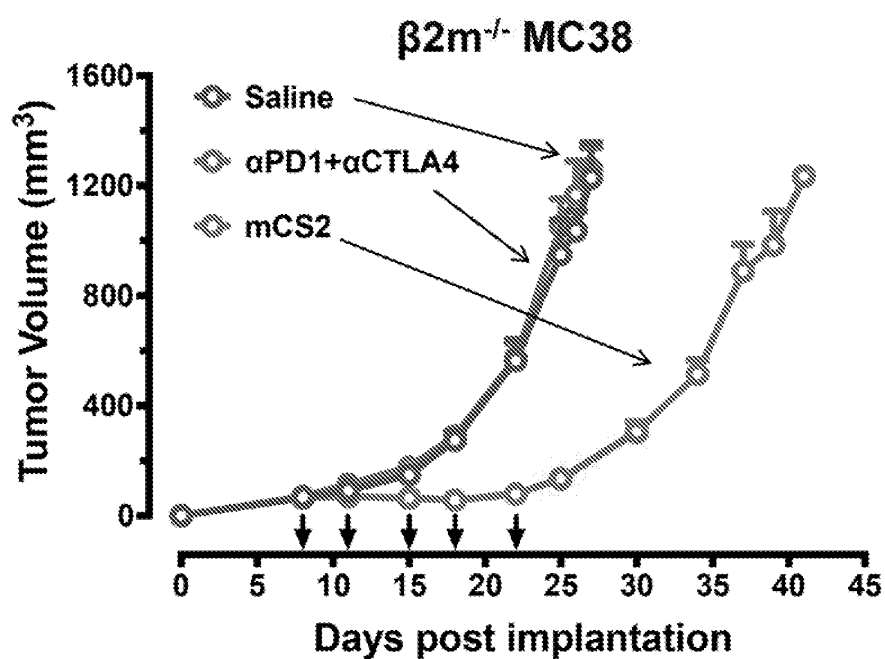
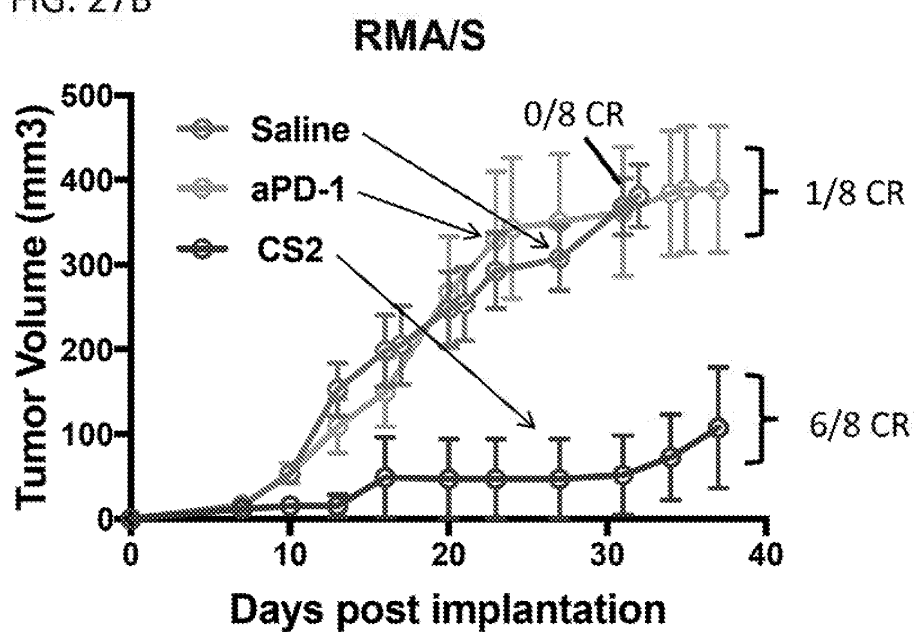
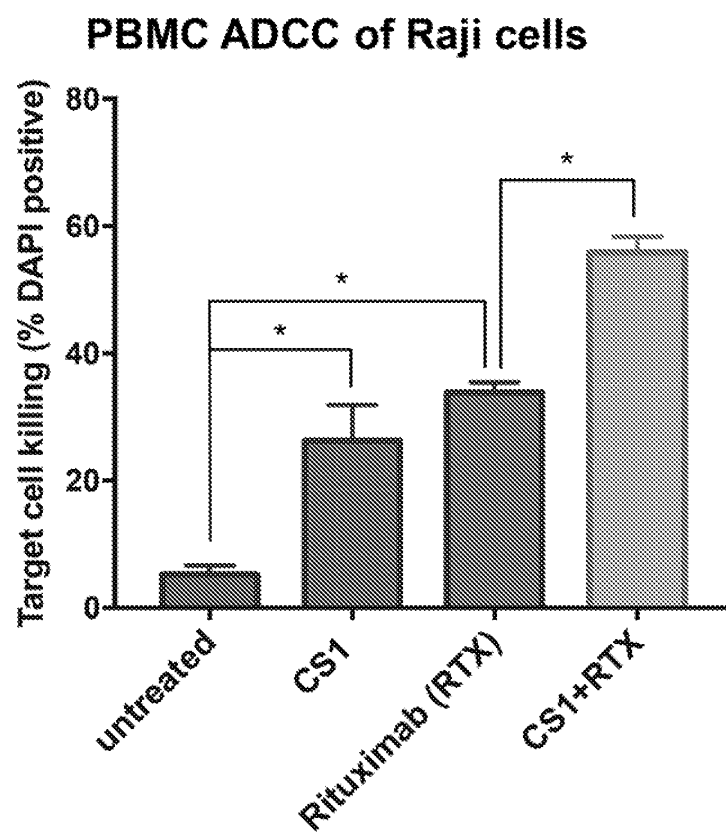


FIG. 27B



34/37

FIG. 28



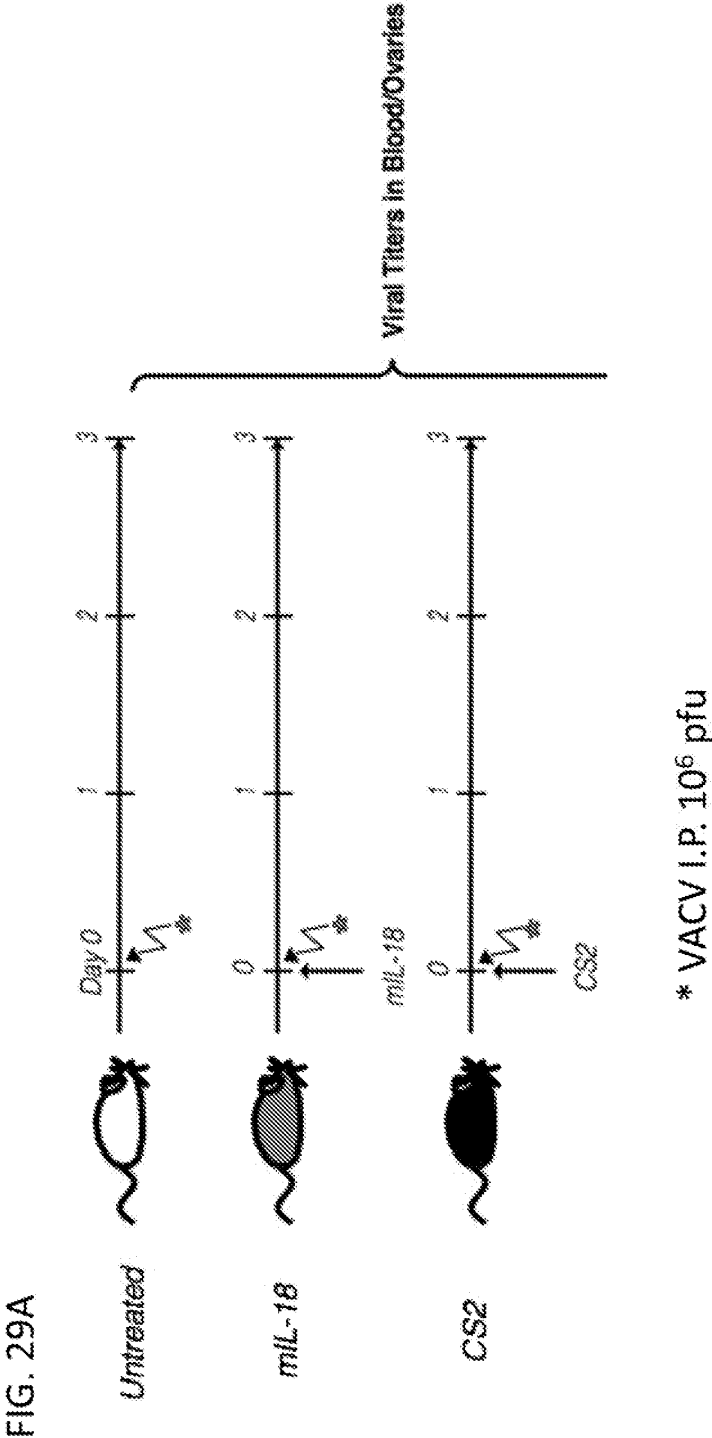


FIG. 29B

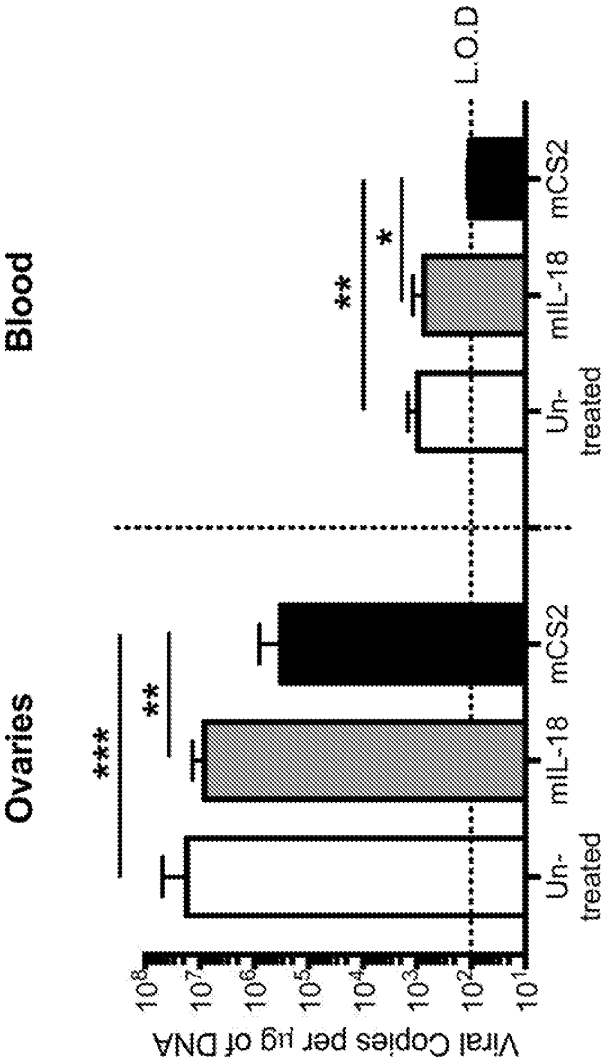


FIG. 30A

