

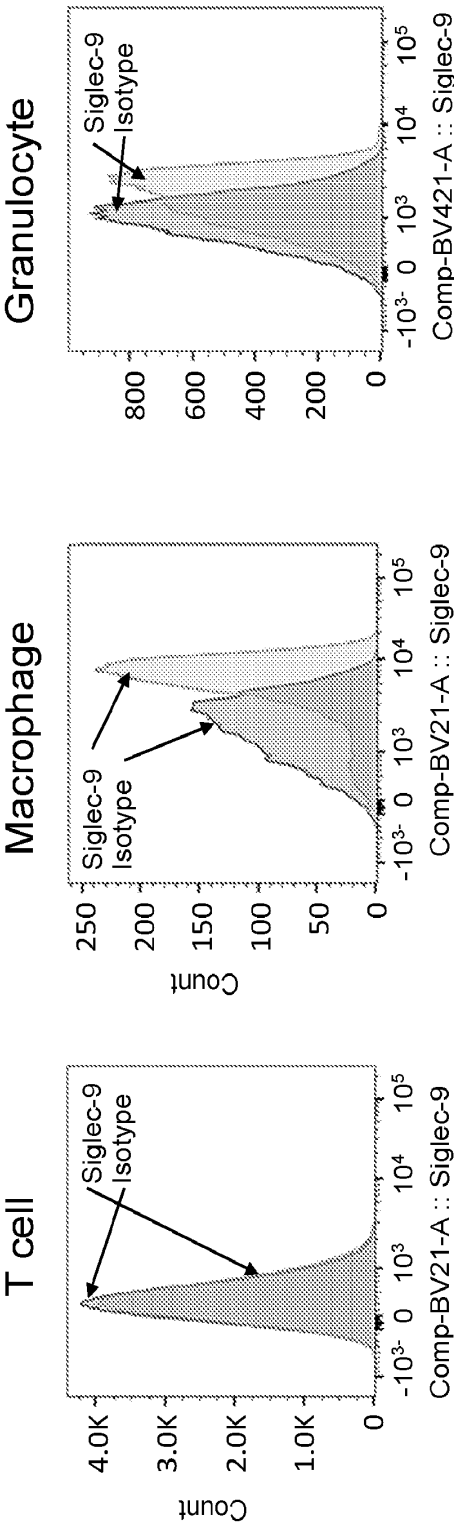


FIG. 1

>Human siglec 9
MLLLPLLWGRERAEGQTSKLLTMQSSVTVQEGLCVHVPCSFSPSHGWIYPGPVVHGYWFREGANTDQDAPVATNNPARAVWEETRDREHLLGDPHTKNCTLSIR
DARRSDAGRYFFERMEKGSIKWNYKHHRLSVNVTALTHRPNNILIPGTLESGCPQNLTCVSPWACEQGTTPPMISWIGTSVSPIDPSTTRSSVLTLLIPQPDHGTSLTCQ
VTFPGASVTTNKTVHINVSYPPQNL TMTVFQGDGTSTVLGNGSSLSLPEGQSLRLVCAVDASNPAPRLSLSWRGLTLCPSQPSNPGVLELPWVHLRDAAEFTCR
AQNPLGSQQVYLVNYSIQSKATSGVTQGVVGGAGATALLVFLSFCVIFVWVRSCRKKKSARPAAGVGDGTIEDANAVRGSA SQGPLTEPWAEDSPPDQPPPASARSSVGE
GELQYASLSFQMVKPWDSRGQEATDIEYSEIKIHR (SEQ ID NO: 1)

FIG. 2

Variant	Mutations	Stability change Kcal/mol	Hydrophobic patch Å ²	Positive patch Å ²	Negative patch Å ²	pI	Net charge
S9.1 (wt)	1:W38W,1:I39I,1:Y40Y,1:P41P - parental	0	410	330	40	9.42	4.85
S9.2	1:W38D,1:I39I,1:Y40E,1:P41G	-0.86	220	270	40	8.68	3.21
S9.3	1:W38S,1:I39I,1:Y40E,1:P41T	-0.59	220	270	40	9.22	3.91
S9.4	1:W38S,1:I39I,1:Y40E,1:P41P	-0.58	280	270	40	9.22	3.9
S9.5	1:W38D,1:I39I,1:Y40E,1:P41P	-0.52	220	270	40	8.68	3.18
S9.6	1:W38Y,1:I39Q,1:Y40E,1:P41S	-0.38	220	270	40	9.07	3.92
S9.7	1:W38T,1:I39H,1:Y40E,1:P41T	-0.04	220	270	40	9.2	3.99
S9.8	1:L23T,1:H26S,1:H80Y,1:L82E	-0.44	430	280	40	9.16	3.38
S9.9	1:L23T,1:H26T,1:H80Y,1:L82D	-0.3	430	280	90	9.14	3.38
S9.10	1:S35D,1:W38T,1:I39I,1:Y40Y	-2.06	270	270	40	9.11	3.9
S9.11	1:S35D,1:W38E,1:I39I,1:Y40Y	-1.81	270	270	40	8.63	2.97
S9.12	1:S35S,1:W38S,1:I39H,1:Y40H	-1.32	220	270	40	9.47	4.95
S9.13	1:S35D,1:W38Q,1:I39H,1:Y40E	-1.04	220	270	40	8.56	3.15
S9.14	1:S35T,1:W38S,1:I39H,1:Y40E	-1.04	220	270	40	9.22	3.95
S9.15	1:S35D,1:W38E,1:I39T,1:Y40Y	-1.02	220	270	40	8.6	2.98
S9.16	1:S35N,1:W38T,1:I39E,1:Y40Y	-0.97	220	270	40	9.09	3.87
S9.17	1:S35H,1:W38T,1:I39T,1:Y40T	-0.96	270	280	40	9.45	5.01
S9.18	1:S35H,1:W38S,1:I39T,1:Y40T	-0.95	270	280	40	9.47	4.97
S9.19	1:S35S,1:W38G,1:I39T,1:Y40E	-0.94	280	270	40	9.16	3.89
S9.20	1:S8D,1:K9Y,1:L10T,1:W116E	-0.69	330	180	40	7.77	0.65
S9.21	1:S8D,1:K9Y,1:L10Q,1:W116N	-0.48	330	170	40	8.26	2.35
S9.22	1:S8E,1:K9Y,1:L10T,1:W116E	-0.41	390	190	40	7.72	1.39
S9.26	1:S35V,1:H36H,1:G37G,1:W38Q,1:I39I,1:Y40D,1:P41S,1:G42D	-2.12	220	270	100	8.63	2.99
S9.27	1:S35V,1:H36H,1:G37S,1:W38Q,1:I39I,1:Y40D,1:P41S,1:G42D	-1.73	220	270	130	8.63	2.99
S9.28	1:S35V,1:H36D,1:G37S,1:W38Q,1:I39I,1:Y40D,1:P41S,1:G42D	-1.53	220	270	80	7.97	1.98
S9.29	1:S35S,1:H36D,1:G37S,1:W38Q,1:I39I,1:Y40D,1:P41S,1:G42D	-1.17	220	270	80	7.97	1.99
S9.30	1:S35V,1:H36D,1:G37G,1:W38Q,1:I39I,1:Y40D,1:P41S,1:G42D	-1.02	220	270	90	7.97	1.98

FIG. 3

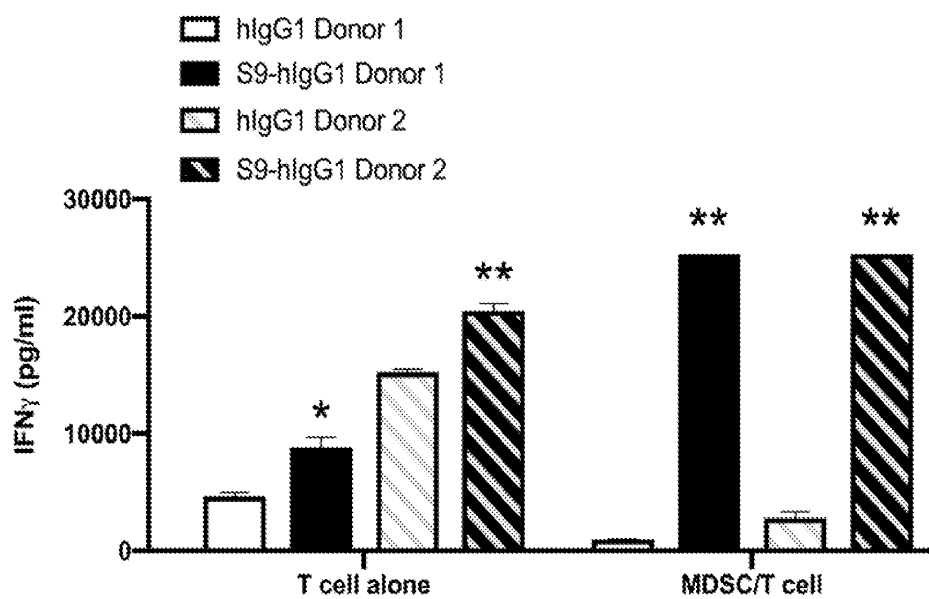


FIG. 4

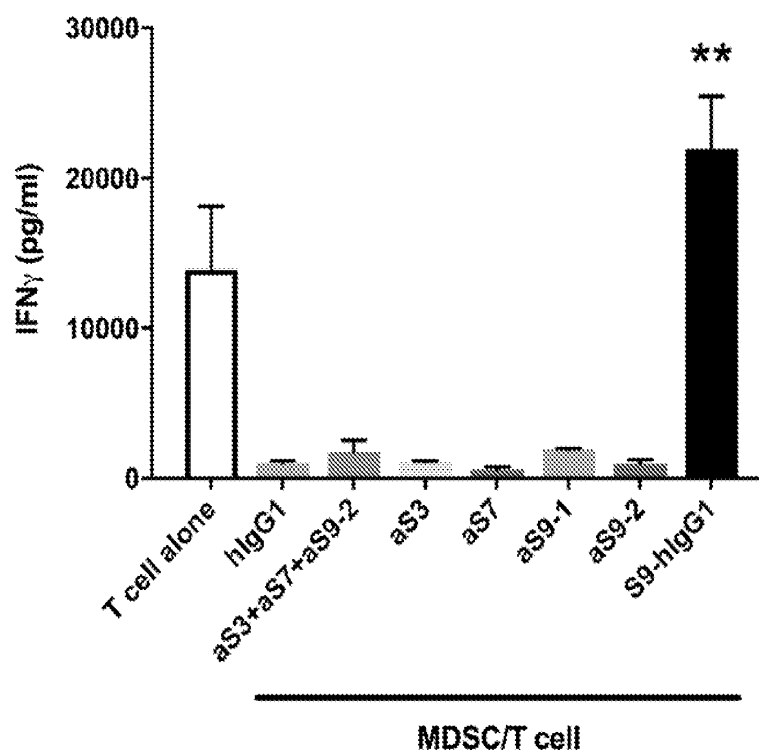


FIG. 5

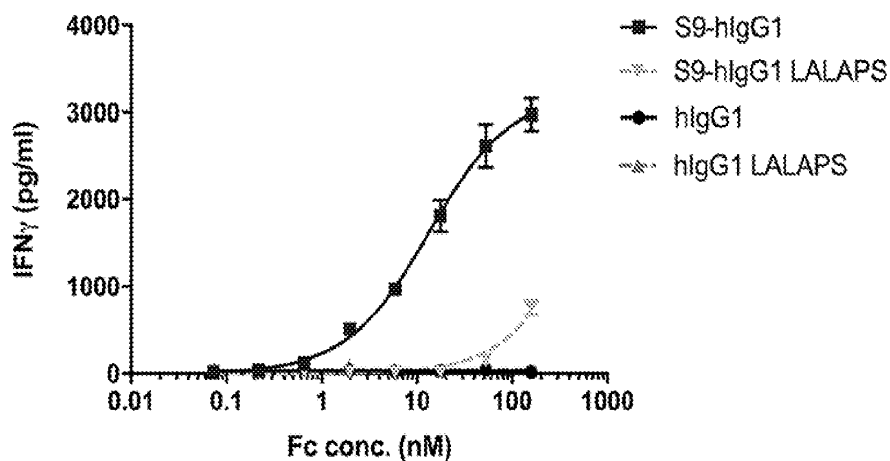


FIG. 6

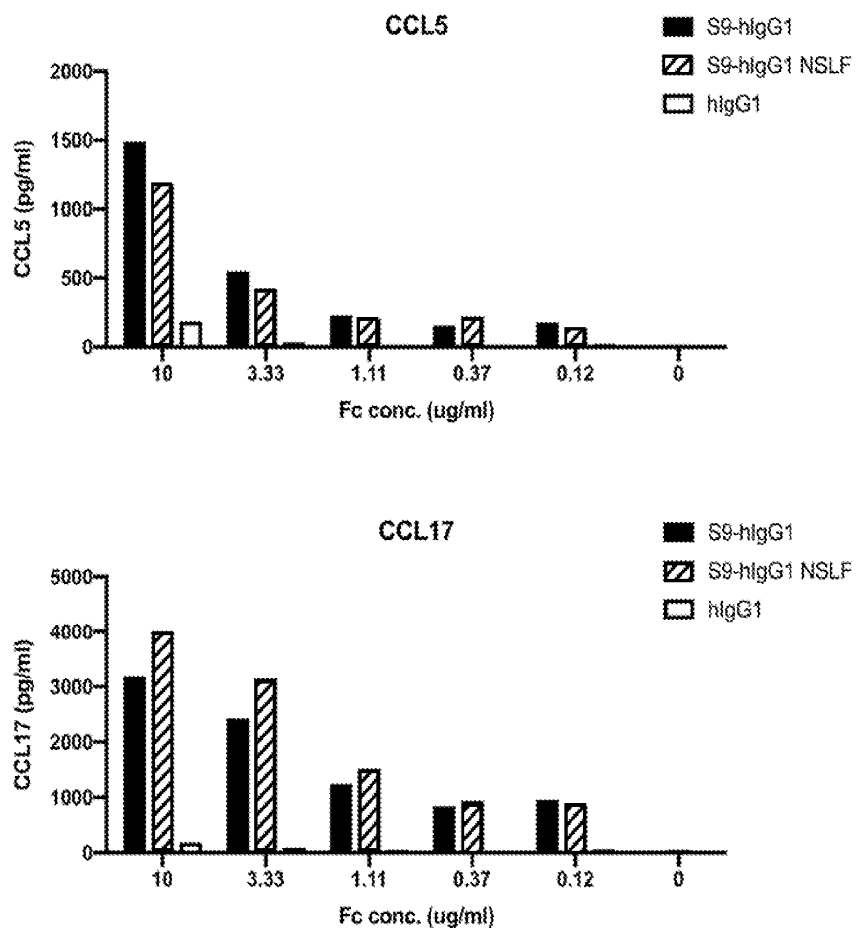


FIG. 7

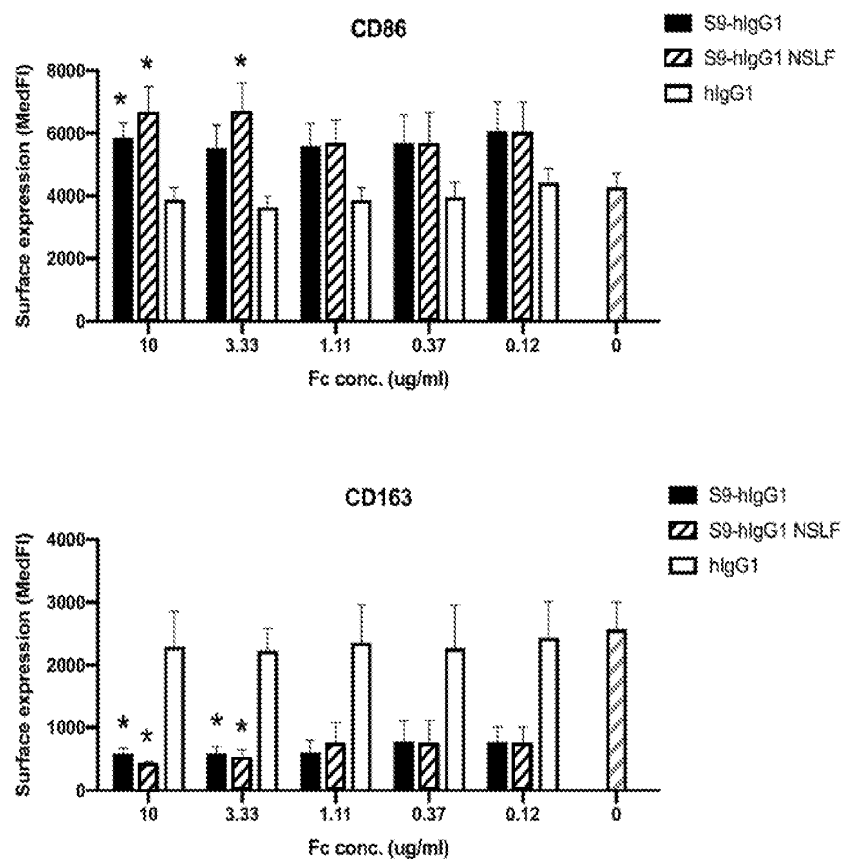


FIG. 8

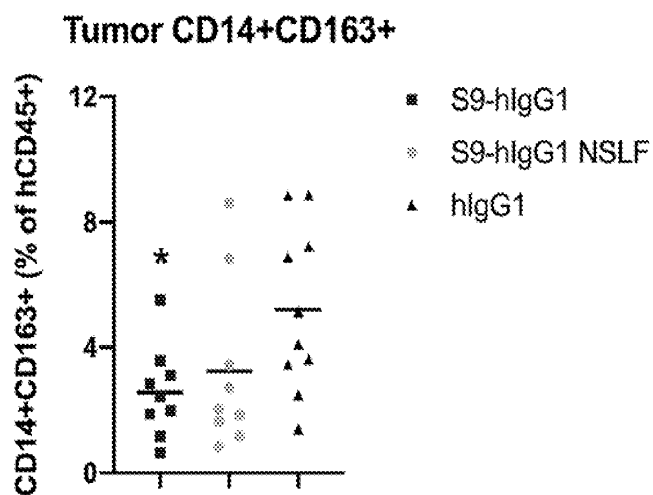


FIG. 9A

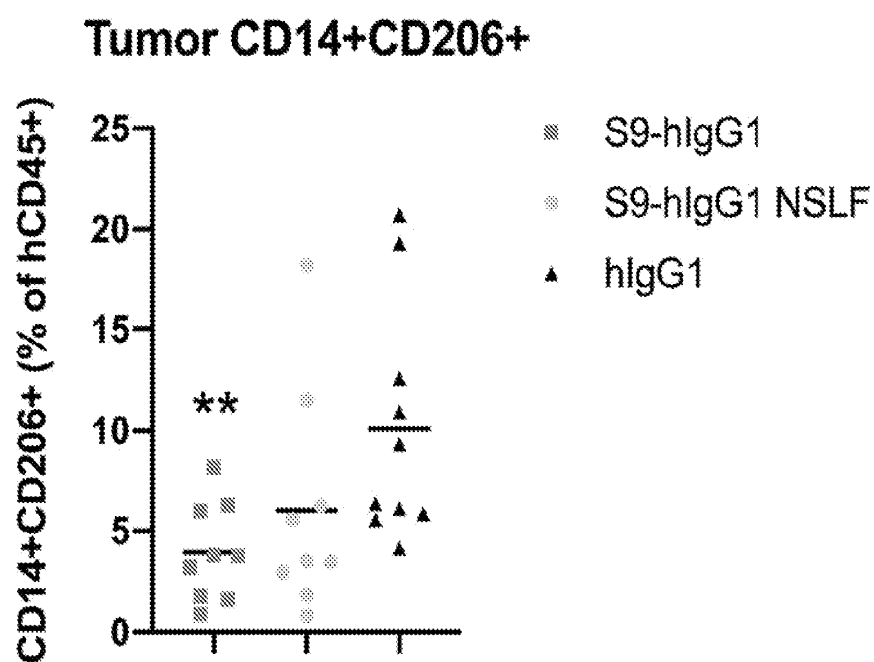


FIG. 9B

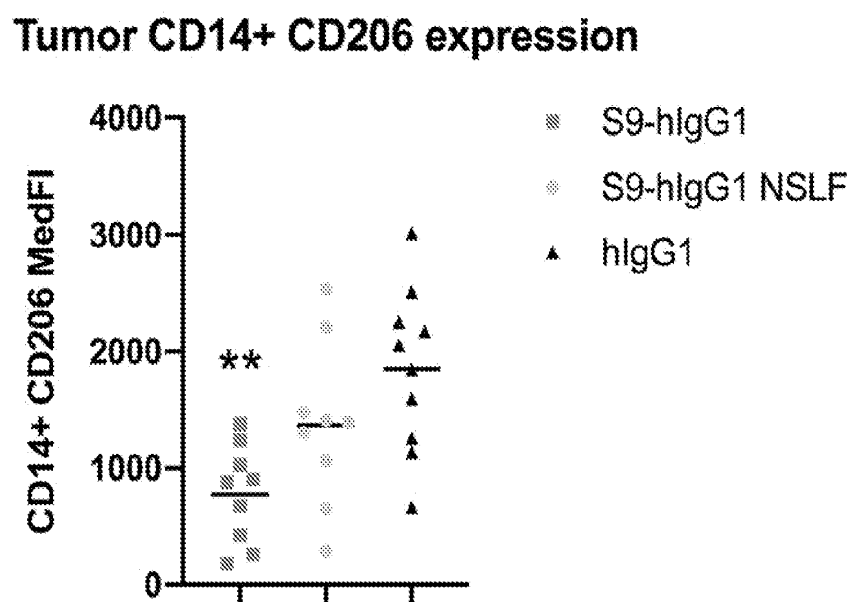


FIG. 9C

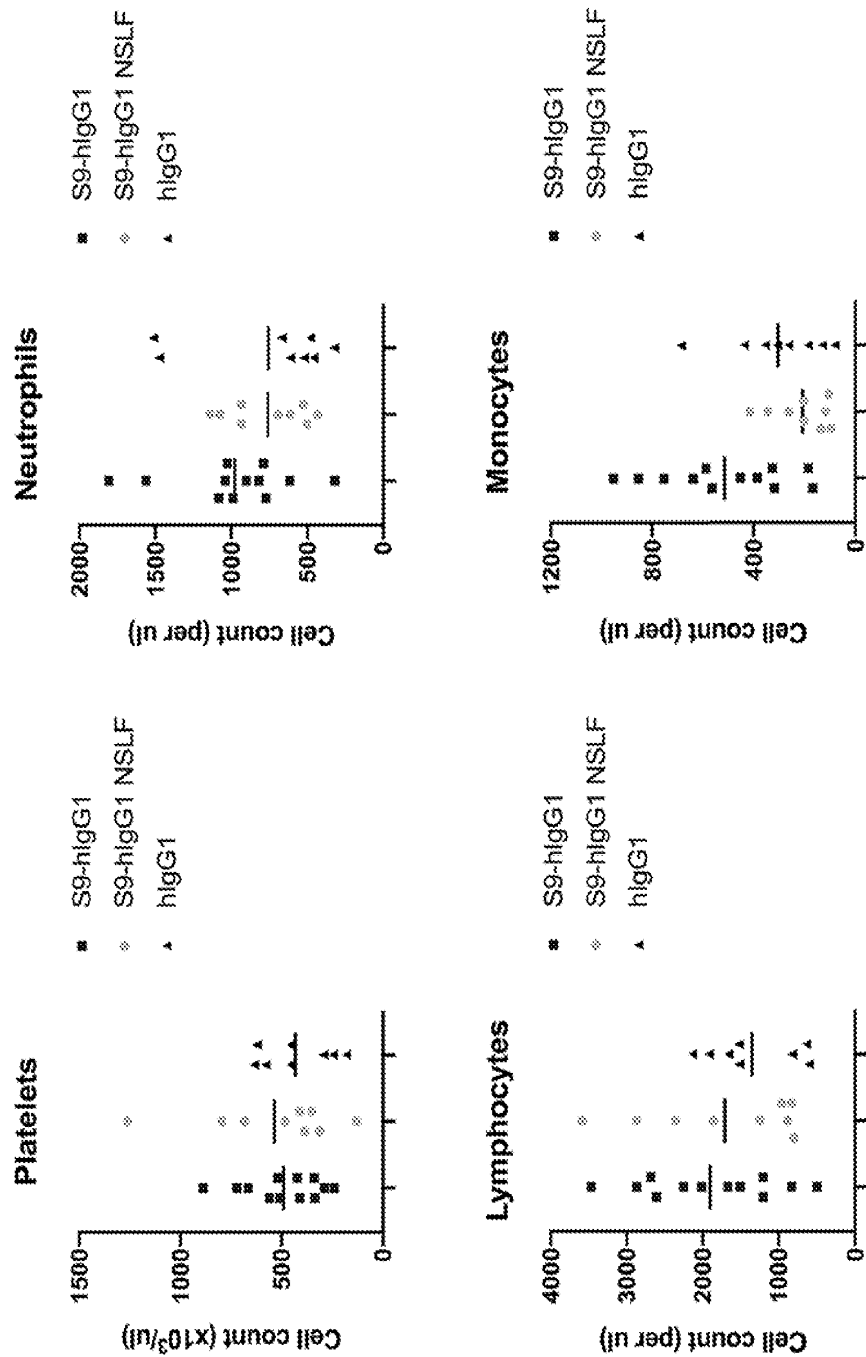


FIG. 10

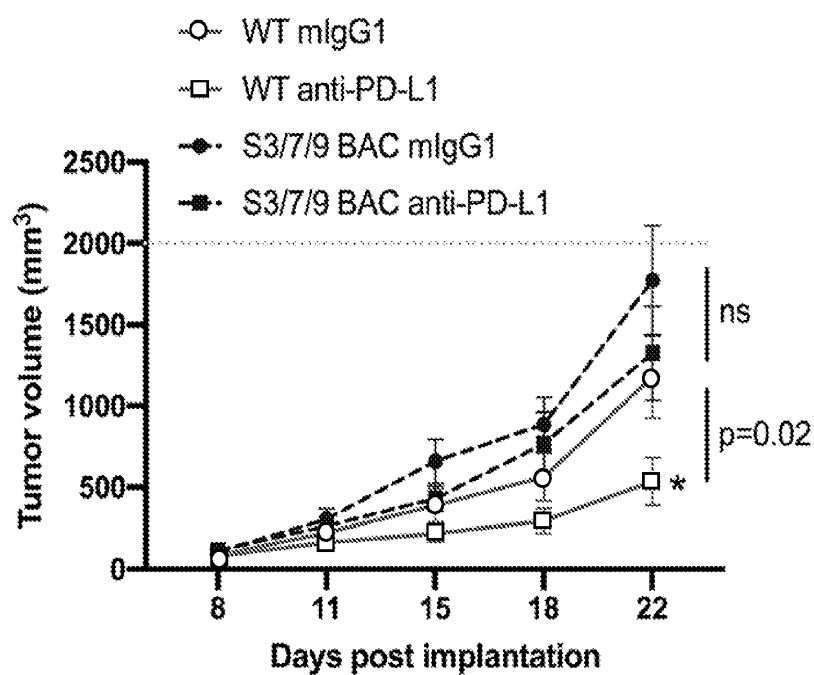


FIG. 11

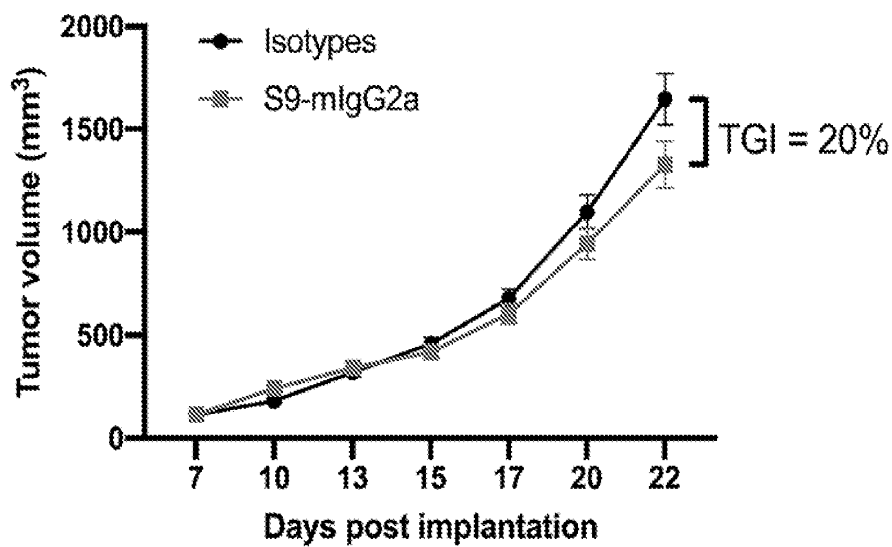


FIG. 12

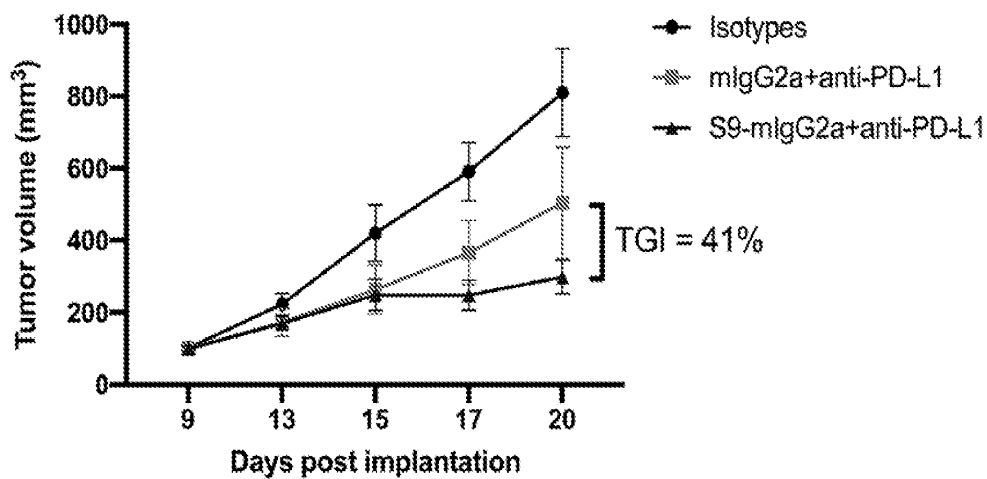


FIG. 13

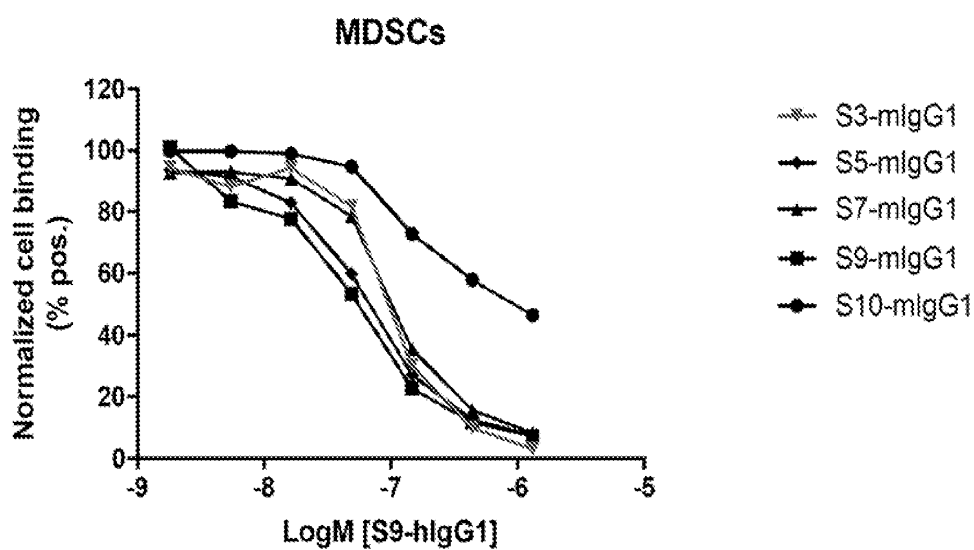
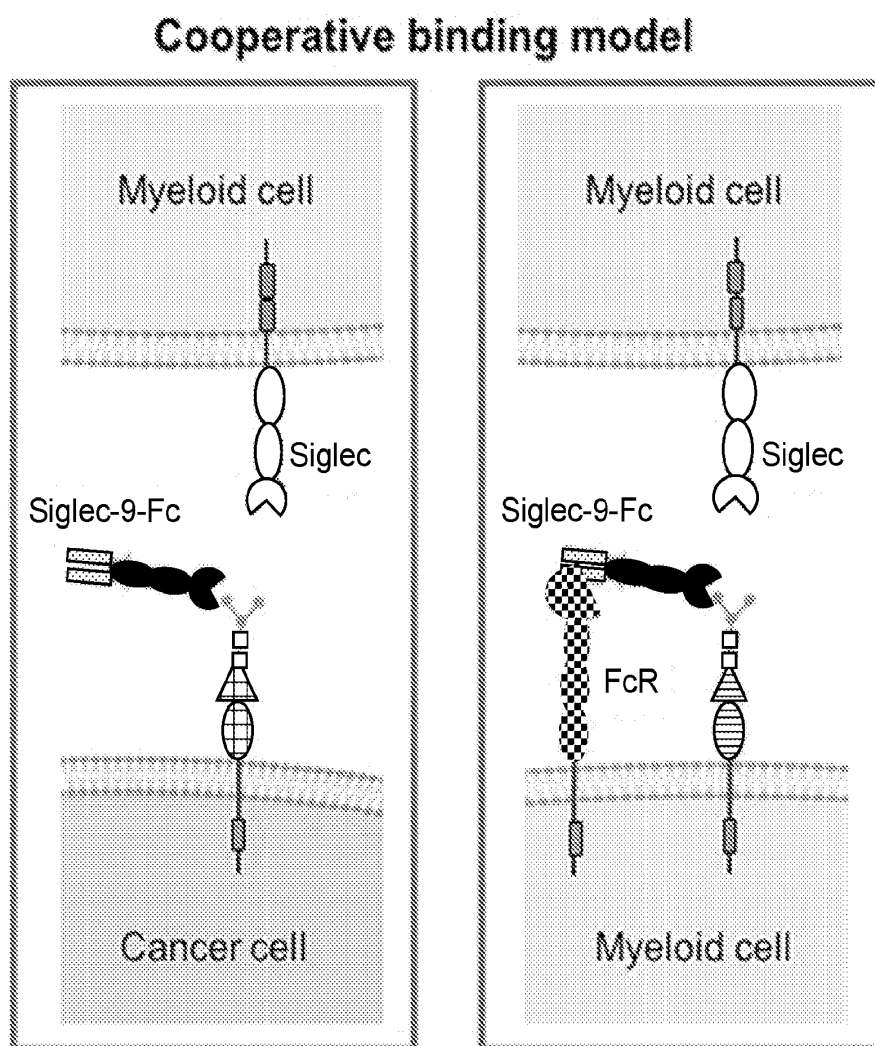


FIG. 14

**FIG. 15**

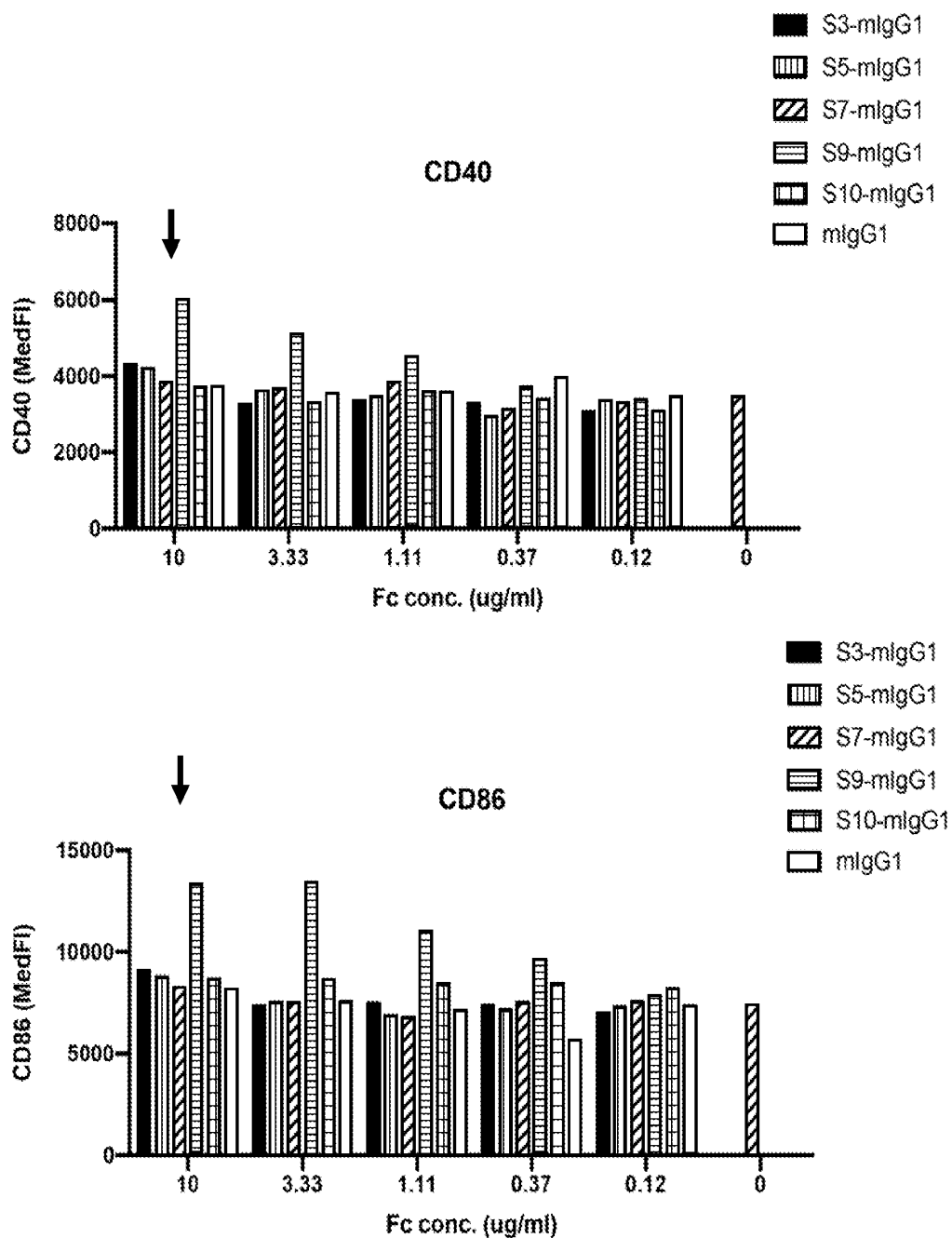


FIG. 16A

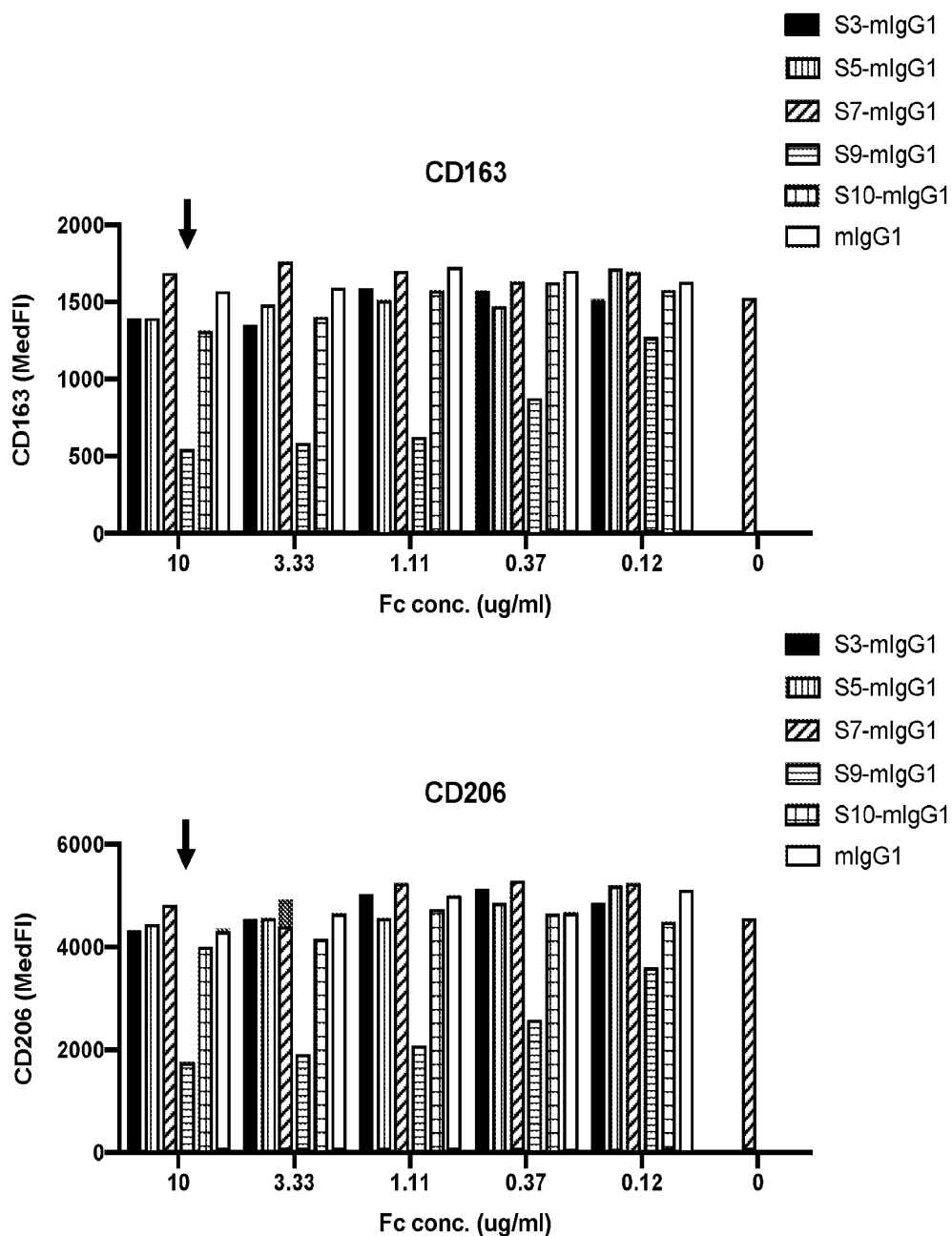


FIG. 16B

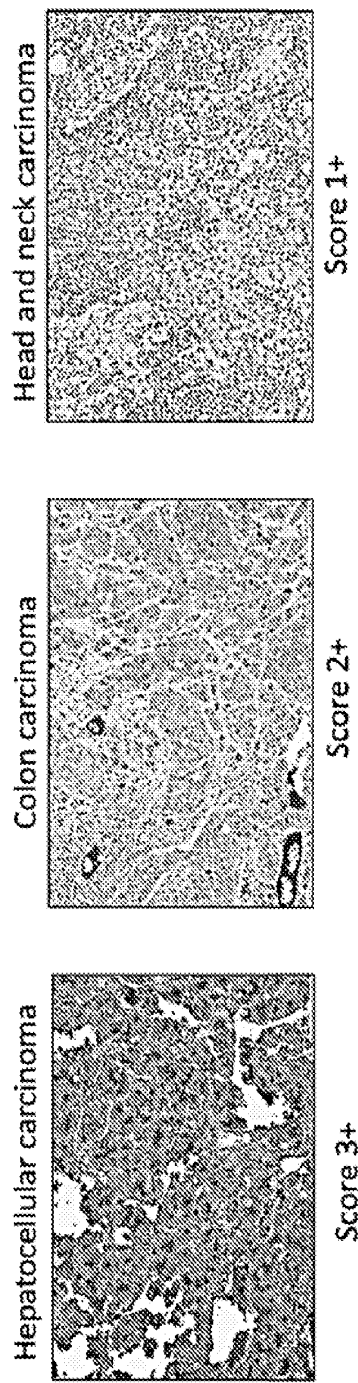


FIG. 17

Variant	A375 binding MFI	MDSC CD86 MFI	MDSC CD163 MFI	Yield (mg)	DSF Tm1	DSF Tm2	Percent Monomer
S9.1	12470	45480	1297	17.1	59.7	69	92.4
S9.2	7517	26778	2270	24.8	62.7	70.5	96.7
S9.3	5807	33120	2252	53.8	60.7	70.5	97.6
S9.4	7958	35921	2114	25	61.6	70.1	97.7
S9.5	5160	NA	NA	17.9	61.2	71.1	98.2
S9.6	7673	NA	NA	15.5	60.5	68.6	97.8
S9.7	8119	32708	2227	11.1	60.6	70.7	97.7
S9.8	7061	NA	NA	13.7	56.7	67.3	69.4
S9.9	5877	40290	1734	44.5	57.5	72.4	73.9
S9.10	6668	40555	1926	10.6	60.6	67.8	97.4
S9.11	4042	24547	2066	46.9	58.1	69.5	98.4
S9.12	12207	NA	NA	18.5	59.5	68.6	80.1
S9.13	2702	26302	1984	29.1	61.7	67.2	97.7
S9.14	7719	33470	2025	16.9	61.5	69.5	94.9
S9.15	4359	NA	NA	17.5	61.5	67.9	98.7
S9.16	7260	30178	2099	23.8	63	69	94.8
S9.17	10546	33215	2088	22.7	62.5	69.5	92.5
S9.18	11575	39959	2153	22.5	59.7	66.6	92.9
S9.19	6195	29171	2222	42.5	61	68.3	99.1
S9.20	572	22413	2028	35.6	58.4	70.3	94.9
S9.21	875	NA	NA	5.5	61.8	70.1	77.2
S9.22	436	NA	NA	33.2	58.8	71.4	96.5
S9.23	5416	28651	2230	31	62.2	69.8	97.8
S9.24	4886	28290	2037	47.5	60.8	70	98.1
S9.25	2902	NA	NA	41.2	62	71.9	97.9
S9.26	5969	20251	2021	47.4	61.8	71.5	98
S9.27	5360	21196	1970	49.2	61.6	71.6	98.1
S9.28	2437	18270	2240	50.9	62.2	71.9	97.9
S9.29	1828	NA	NA	55.1	62.3	70.6	97.6
S9.30	2106	19547	2321	52.3	62.2	72.3	

FIG. 18

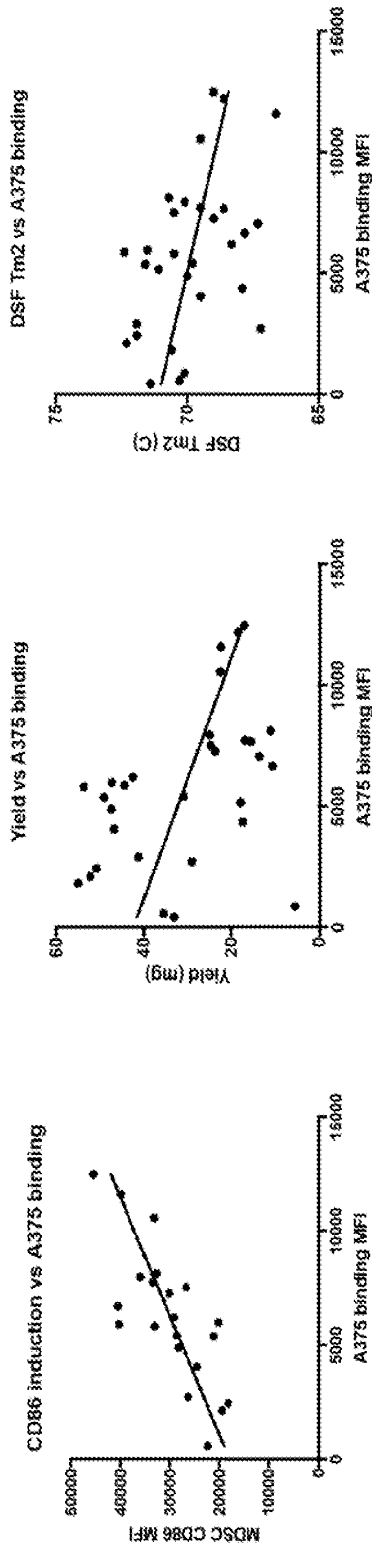


FIG. 19A

FIG. 19B

FIG. 19C

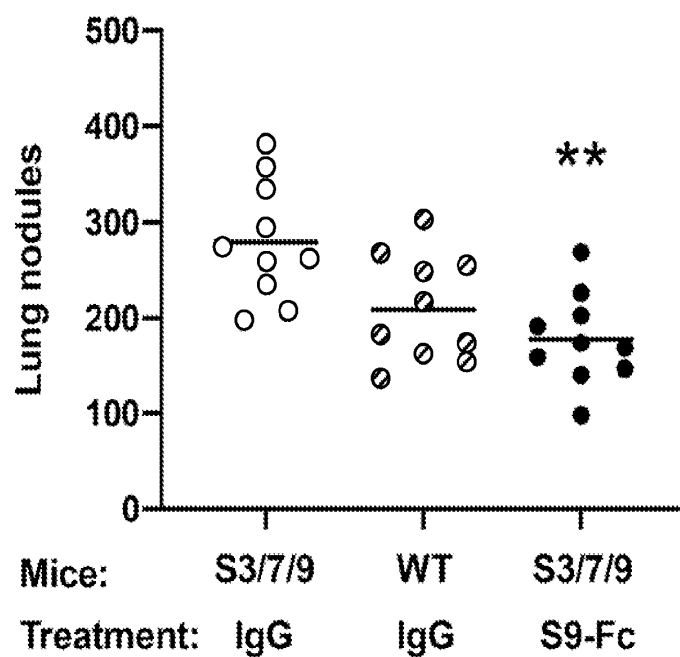


FIG. 20

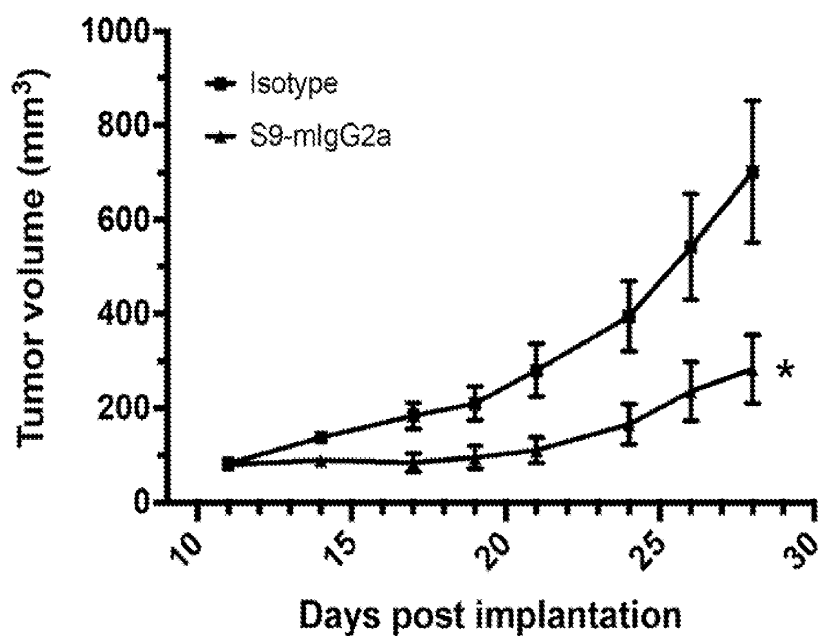


FIG. 21

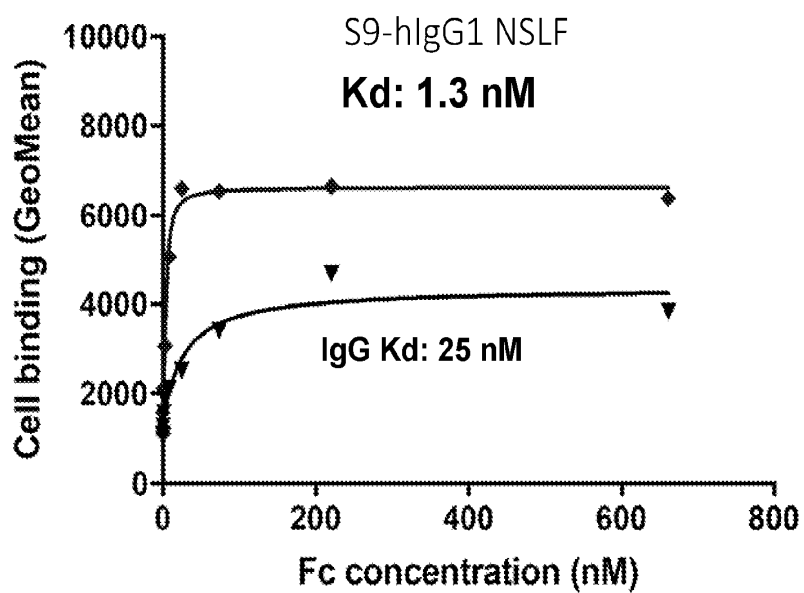


FIG. 22A

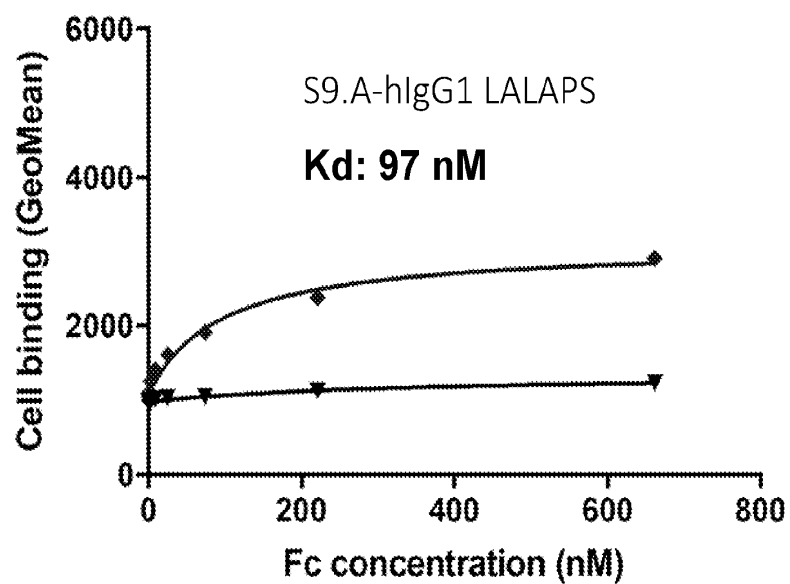
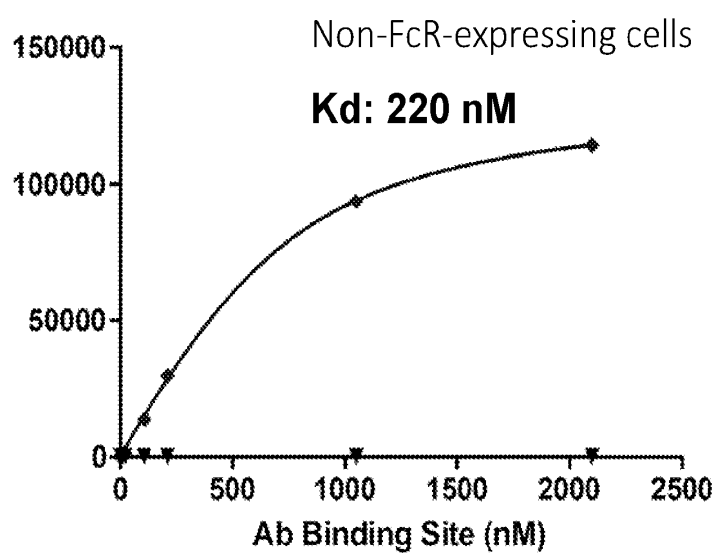


FIG. 22B

*FIG. 22C*

Sialic acid moiety	SIGLEC												
	3	5	7	9	10	15							
Neu5Ac-a-2,8-Neu5Ac-a-2,3-Gal-β-1,4-GlcNAc-β-1,3-Gal-β-1,4-Glc-Sp5													
Neu5Ac-α-2,8-Neu5Ac-α-2,3-Gal-β-1,4-Glc-Sp5													
Neu5Ac-a-2,3-Gal-β-1,3-GalNAc-β-1,3-Gal-α-1,3-Gal-β-1,4-Glc-Sp5													
Neu5Ac-α-2,3Gal-β-1,4-(6S)GlcNAc-β-Sp													
Neu5Ac-α-2,8-Neu5Ac-α-2,3-(GalNAc-b-1,4-)Gal-β-1,4-Glc-Sp5													
Neu5Ac-α-2,8-Kdn-α-2,3-Gal-β-1,4-Glc-Sp5													
Neu5Ac-a-2,3-Gal-β-1,4-GlcNAc-β-1,3-Gal-β-1,4-Glc-Sp5													
Neu5Ac-α-2,8-Neu5Gc-α-2,3-(GalNAc-b-1,4-)Gal-β-1,4-Glc-Sp5													
Neu5Ac-a-2,3-Gal-β-1,4-GlcNAc-β-1,3-Gal-β-1,4-GlcNAc-β-1,3-Gal-β-1,4-Glc-Sp5													
Neu5Ac-a-2,3-Gal-β-1,4-(Fuc-α-1,3-)GlcNAc-β-1,3-Gal-β-1,4-Glc-Sp5													
Neu5Ac-α-2,3-Gal-β-1,4-Glc-Sp5													
Neu5Ac-α-2,6-Gal-β-1,4-Glc-β-Sp													
Neu5Ac-α-2,6-(Neu5Ac-α-2,3)-Gal-β-1,3-GalNAc-β-Sp													
Neu5Ac-α-2,8-Neu5Ac-α-2,3-(Gal-b-1,3-GalNAc-b-1,4-)Gal-β-1,4-Glc-Sp5													
Neu5Ac-α-2,6-Gal-β-1,3-GlcNAc-β-Sp													
GalNAc-β-1,4-(Neu5Ac-α-2,8-Neu5A-α-2,3)-Gal-β-1,4-Glc-β-[GD2]-Sp													
Neu5Ac-α-2,3-Gal-β-1,4-Glc-β-Sp													
Neu5Ac-α-2,8-Neu5Ac-α-2,3-Gal β-1,4-Glc-β-Sp													
Neu5Ac-a-2,3-Gal-β-1,3-GlcNAc-β-1,3-Gal-β-1,4-Glc-Sp5													
Neu5Ac-α-2,3-Gal-β-1,4-(Fuc-α-1,3)-GlcNAc-β-[Sialyl Lewis X]-Sp													
Kdn-α-2,8-Neu5Ac-α-2,3-Gal-β-1,4-Glc-Sp5													

FIG. 23

Sialic acid moiety	SIGLEC										
	3	5	7	9	10	15					
Neu5Ac- α -2,3-Gal- β -1,3-GlcNAc- β -Sp											
Neu5Ac- α -2,6-Gal- β -1,3-Neu5Ac- α -2,6)-GalNAc- β -Sp											
Kdn- α -2,8-Neu5Ac- α -2,3-(GalNAc-b-1,4)-Gal- β -1,4-Glc-Sp5											
Neu5Ac-a-2,3-Gal- β -1,3-GalNAc- β -1,3-Gal- α -1,4-Gal- β -1,4-Glc-Sp5											
Neu5Ac- α -2,3-Gal- β -Sp1											
Neu5Ac-a-2,8-Neu5Ac-a-2,6-Gal-b-1,4-Glc-Sp5											
Neu5Ac- α -2,8-Neu5Ac- α -2,8-Neu5Ac- α -2,3-Gal- β -1,4-Glc- β -Sp3											
Neu5Ac-a-2,3-GalNAc-b-1,4)-Gal-b-1,4-Glc-Sp5											
Neu5Ac- α -2,3-Gal- β -1,3-Neu5Ac- α -2,6)-GalNAc- β -Sp											
GalNAc- β -1,4-(Neu5Ac- α -2,3)-Gal- β -1,4-Glc- β - [GM2]-Sp											
Neu5Ac- α -2,6-Gal- β -1,3-GalNAc- β -Sp											
Neu5Ac- α -2,3-Gal- β -1,4-Fuc- α -1,3)-Glc- β - [3-Sialyl-3-fucosyllactose/ F-SL]-Sp1											
Gal- β -1,3-(Neu5Ac- α -2,6)-GalNAc- β -Sp											
Neu5Gc- α -2,8-Neu5Ac- α -2,3-(Gal-b-1,3-GalNAc-b-1,4)-Gal- β -1,4-Glc-Sp5											
Neu5Ac-a-2,3-(Gal-b-1,3-GalNAc-b-1,4)-Gal-b-1,4-Glc-Sp5											
Neu5Ac- α -2,3-Gal- β -1,3-(Fuc- α -1,4)-GlcNAc- β - [Sialyl Lewis A]-Sp											
Gal- β -1,3-GalNAc- β -1,4-(Neu5Gc- α -2,3)-Gal- β -1,4-Glc- β -Sp1											
Gal- β -1,3-GalNAc- β -1,4-(Neu5Ac- α -2,3)-Gal- β -1,4-Glc- β -Sp1											

Darker shading indicates higher binding

FIG. 23 (cont.)

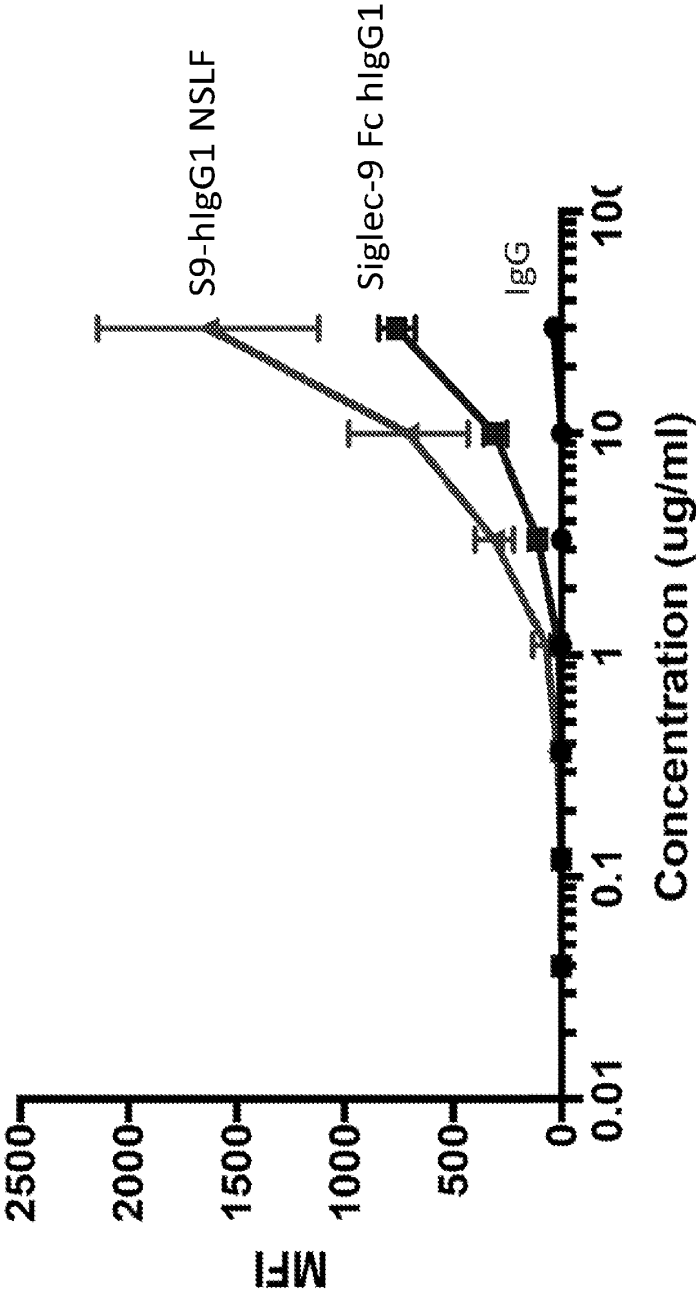


FIG. 24A

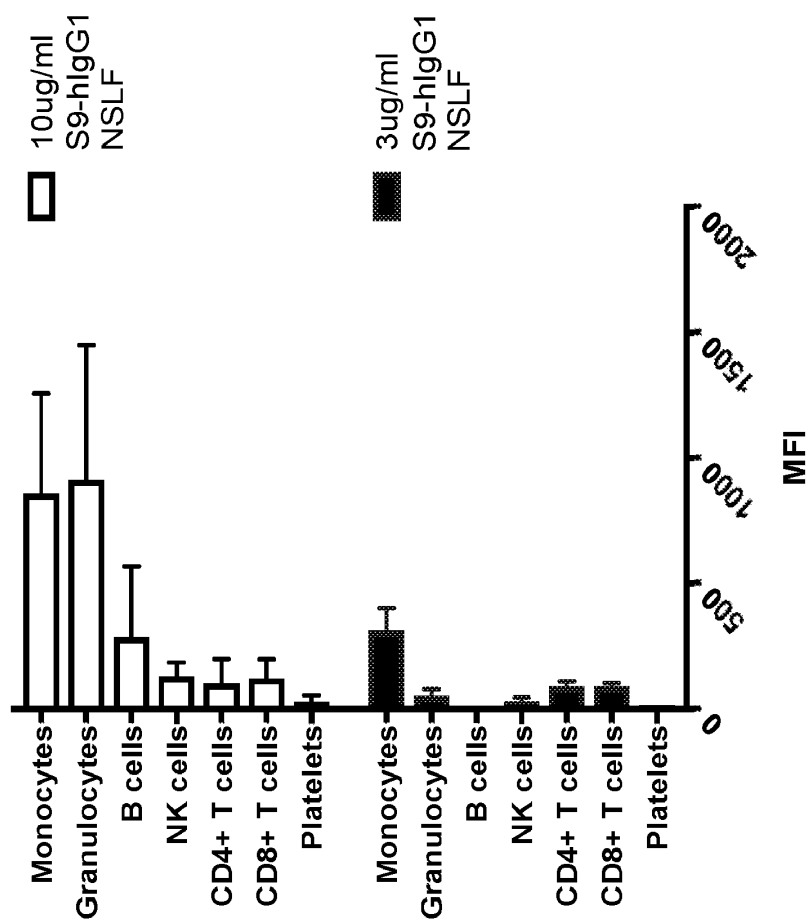


FIG. 24B

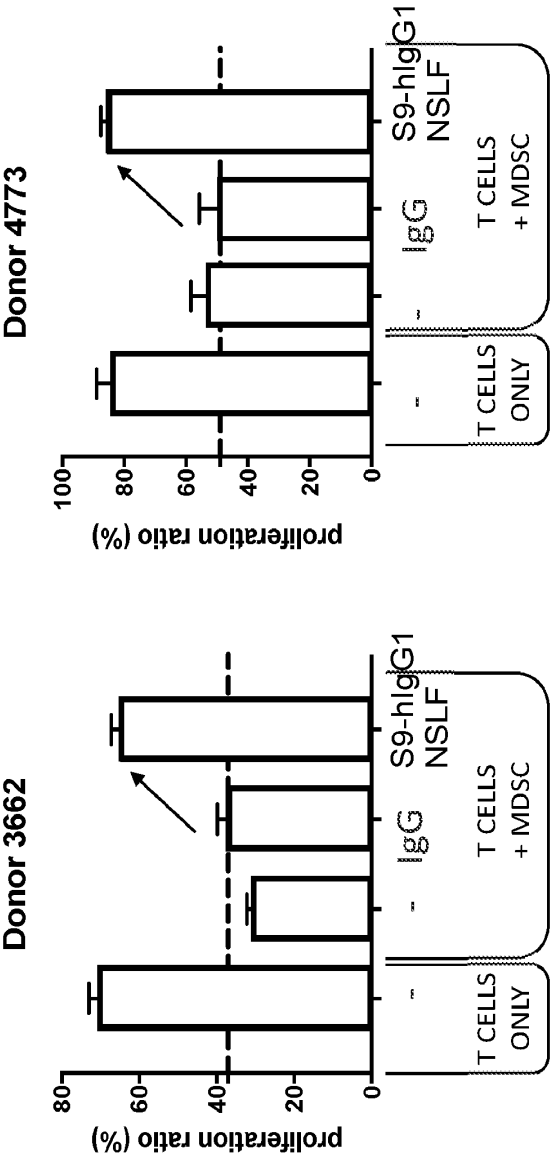
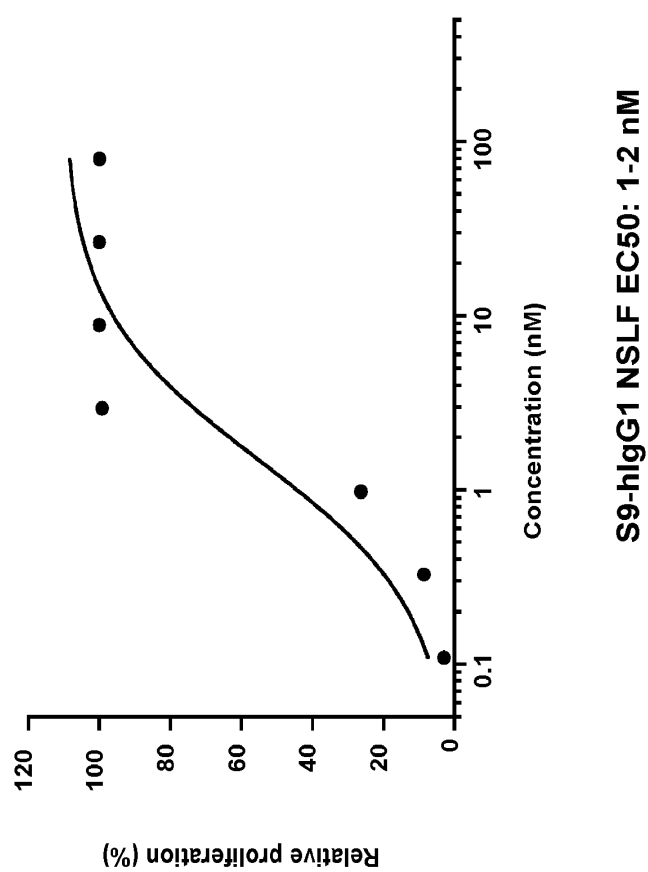


FIG. 25A

**FIG. 25B**

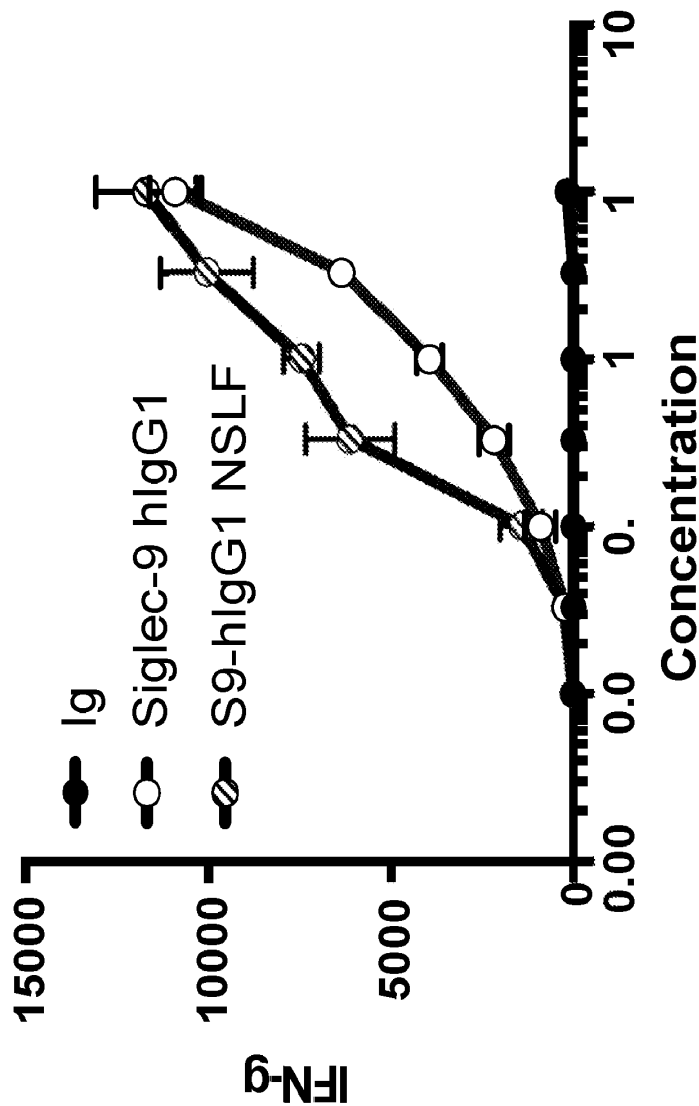


FIG. 26

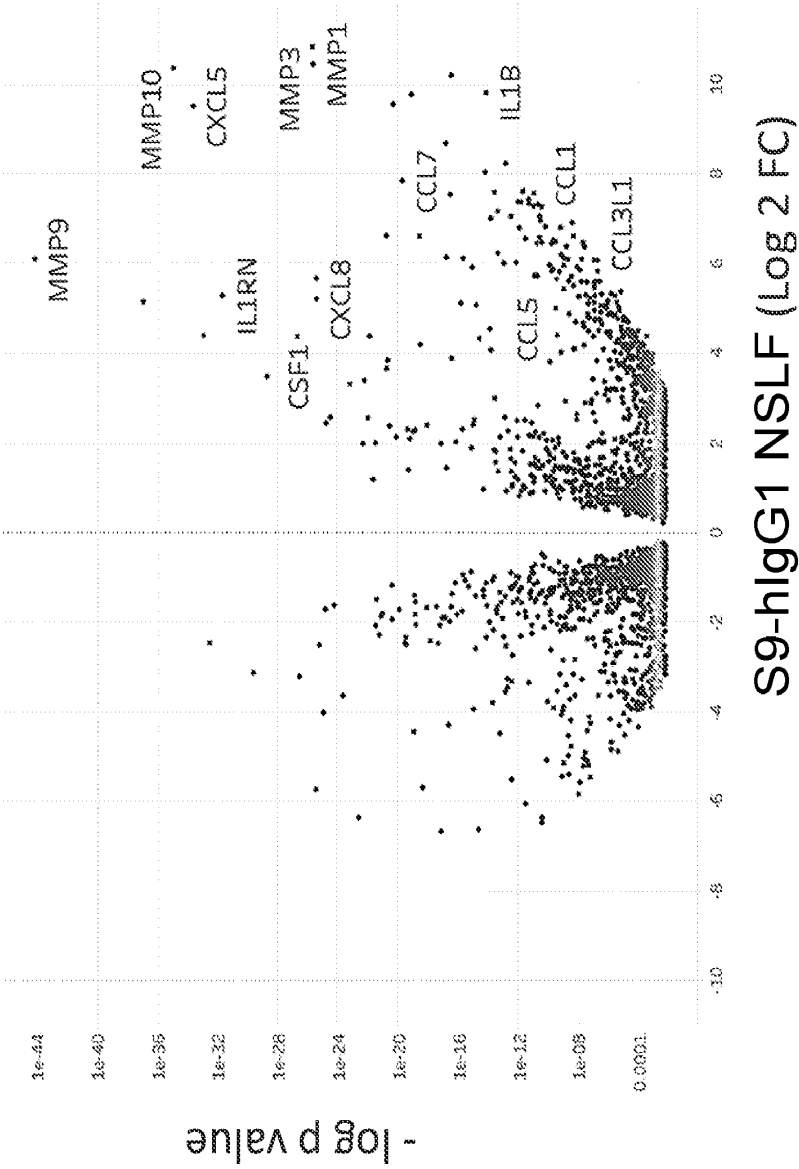


FIG. 27

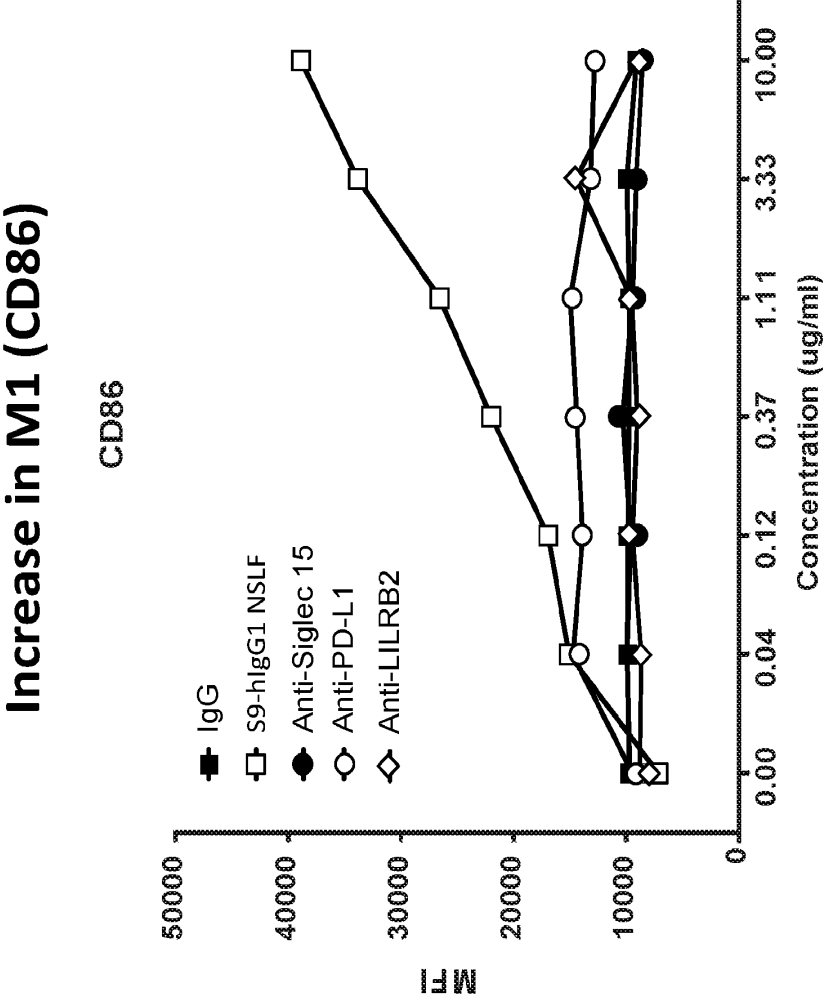


FIG. 28A

Decrease in M2 (CD206)

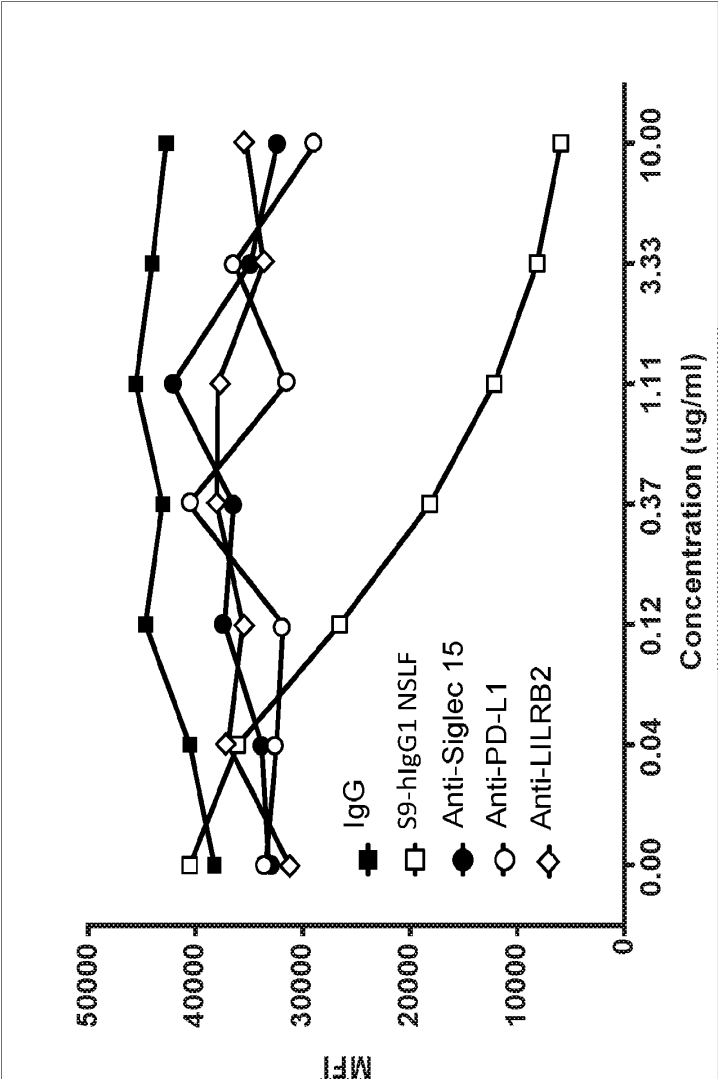


FIG. 28B

E0771 Breast Cancer

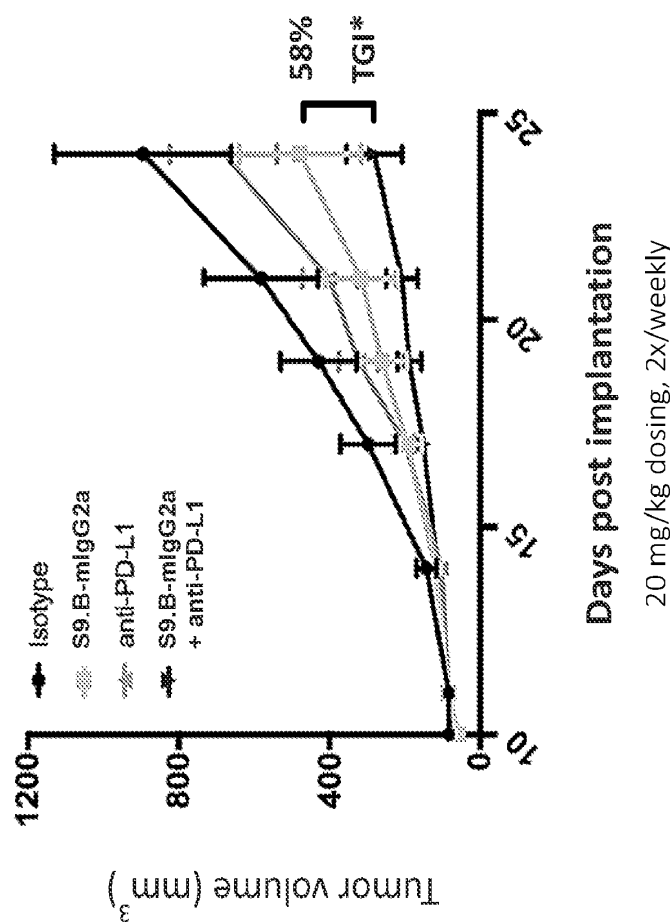
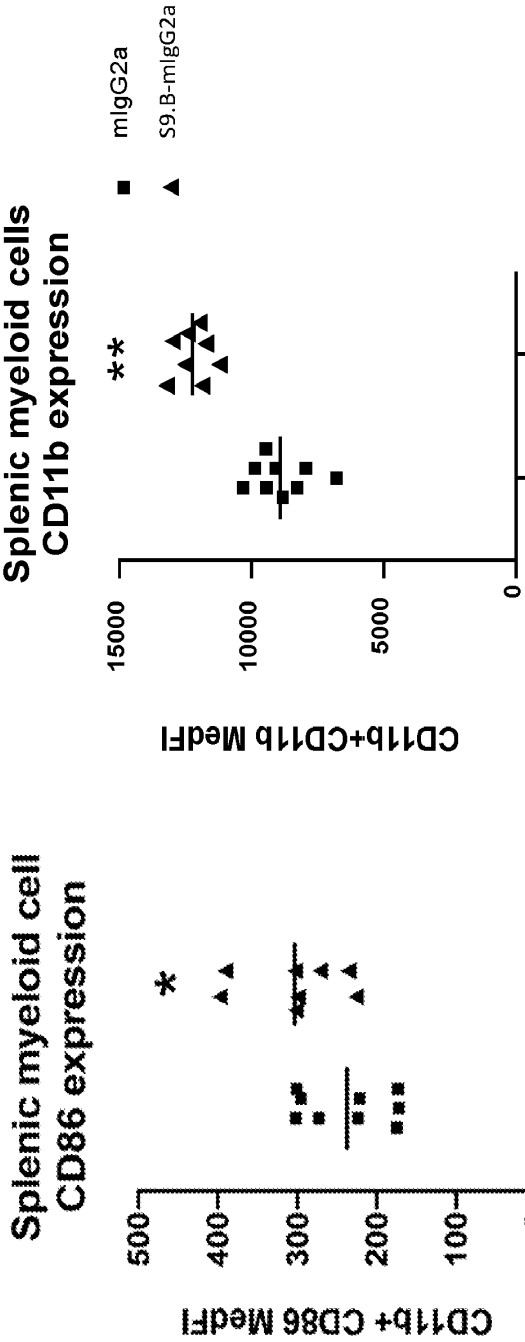


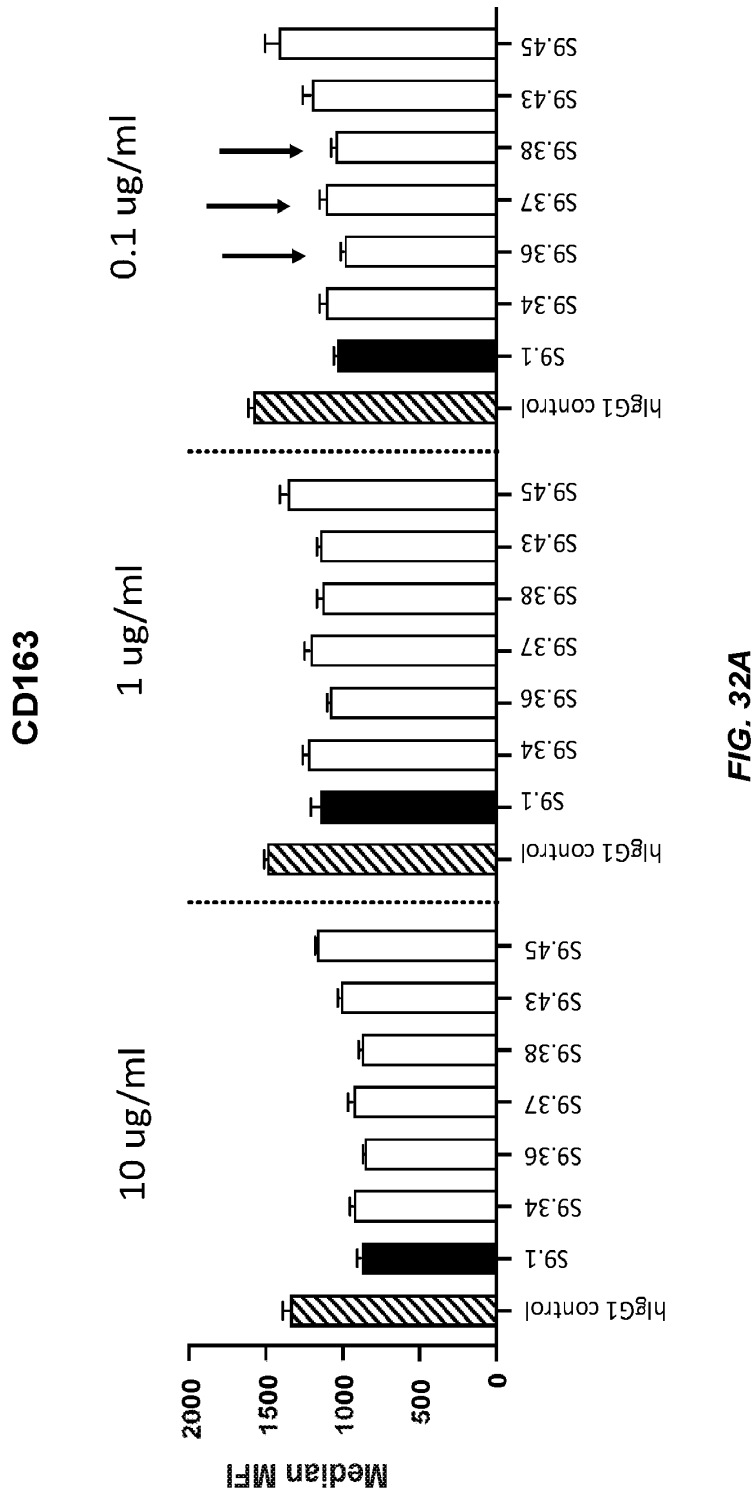
FIG. 29



Variant	SEQ ID NO:	Name	Yield (mg)	A375 binding at 28 ug/ml (MedFI)	MDSC CD86 at 10 ug/ml (MedFI)	MDSC CD163 at 10 ug/ml (MedFI)	MDSC CD11b at 10 ug/ml (MedFI)
S9.1	48	WT hlgG1	53.4	64385	17963	1433	9295
S9.32	171	W38T	52.7	53113	18819	1713	8903
S9.33	172	W38E	69.0	52649	13180	2087	8089
S9.34	173	W38S	77.3	52804	18886	1633	8863
S9.35	174	W38A	40.8	69502	18180	1487	8953
S9.36	175	W38R	42.3	66284	19364	1273	9028
S9.37	176	W38Q	43.2	47014	18231	1470	9013
S9.38	177	W38K	42.9	56372	19669	1267	9149
S9.39	178	W38S_Y40T	31.0	37418	13981	1891	8292
S9.10	57	S35D_W38T	46.0	38417	13752	1846	7627
S9.41	179	L_ER_R	35.1	136272	11883	1920	6786
S9.42	180	D_SI_R	35.2	66444	9139	1852	6571
S9.43	181	L_KI_R	35.4	154017	16242	1560	7066
S9.44	182	T_EI_E	33.8	23450	8981	2128	7162
S9.45	183	S_QI_R	37.3	215774	8987	1754	6762
S9.47	184	R_SS_I_T	6.8	9260	8817	1920	6636
S9.48	185	R_DS_I_T	9.9	8049	9443	2014	6306
S9.49	186	G_VT_Q_I_T	39.2	58239	18384	1551	7947

Signal sequences cleaved during production)

FIG. 31



CD206

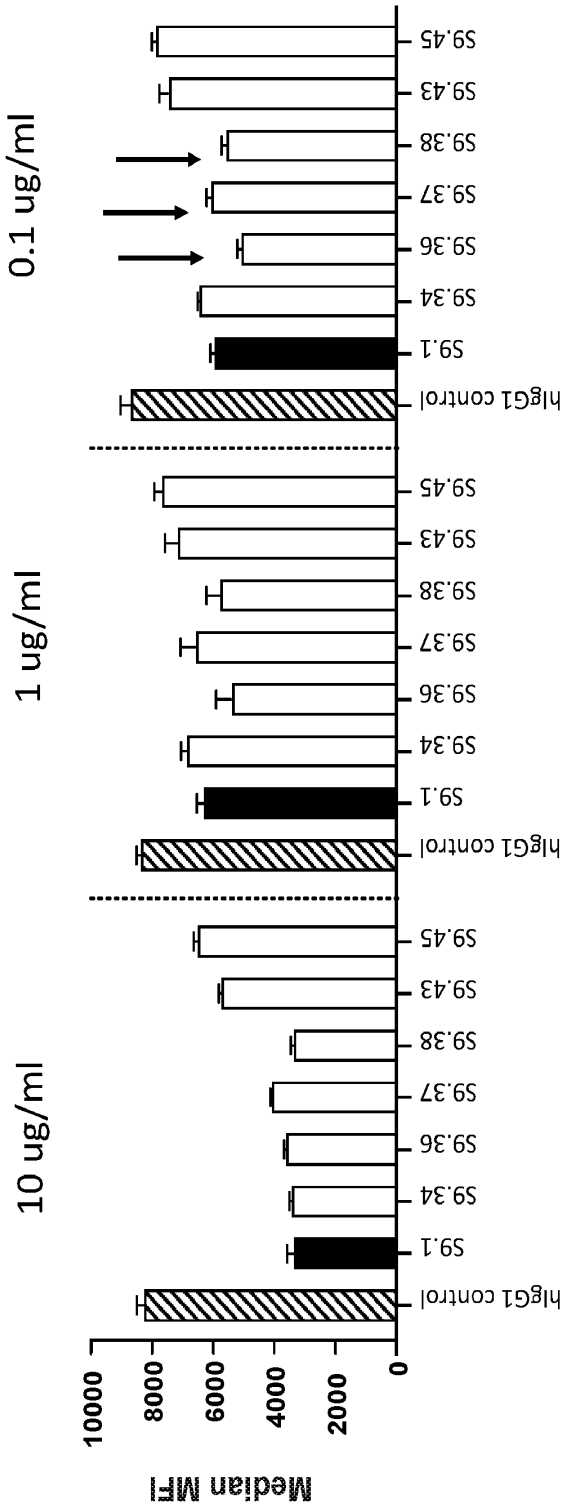


FIG. 32B

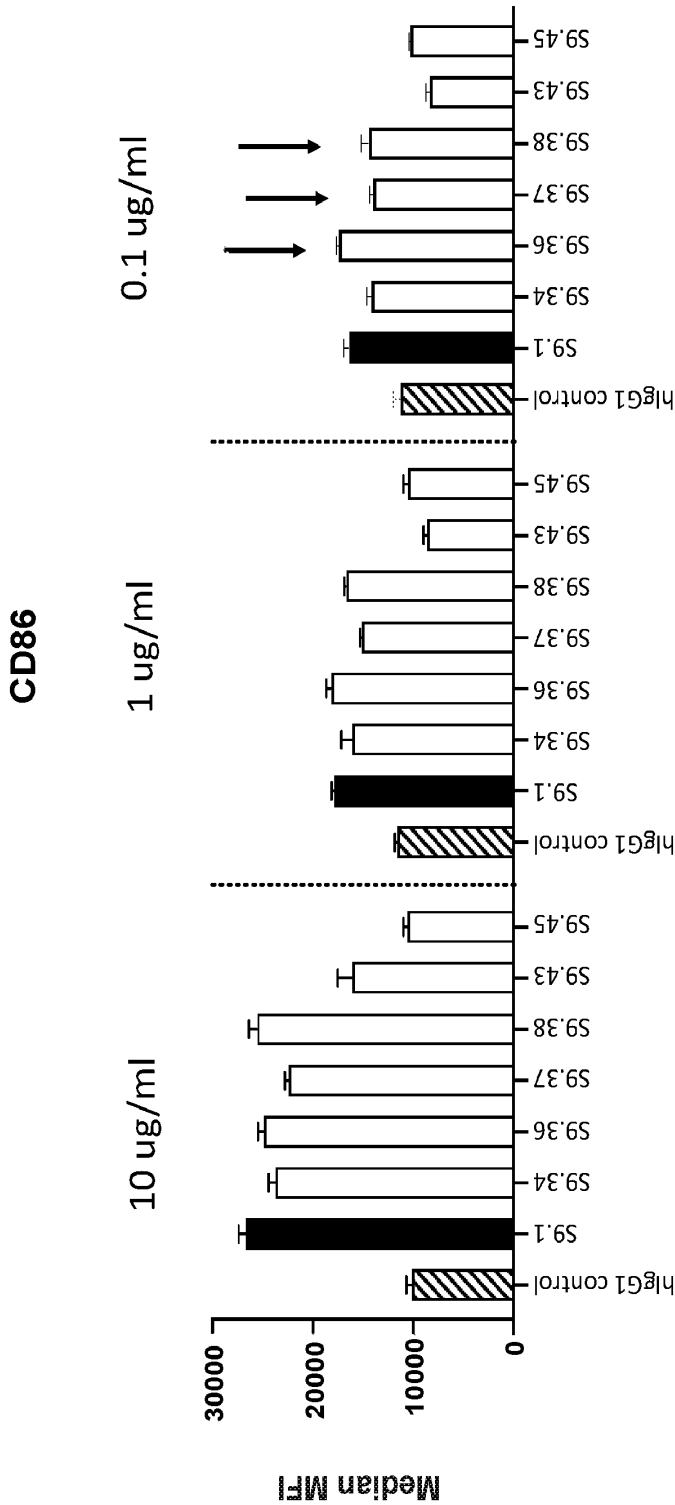


FIG. 32C

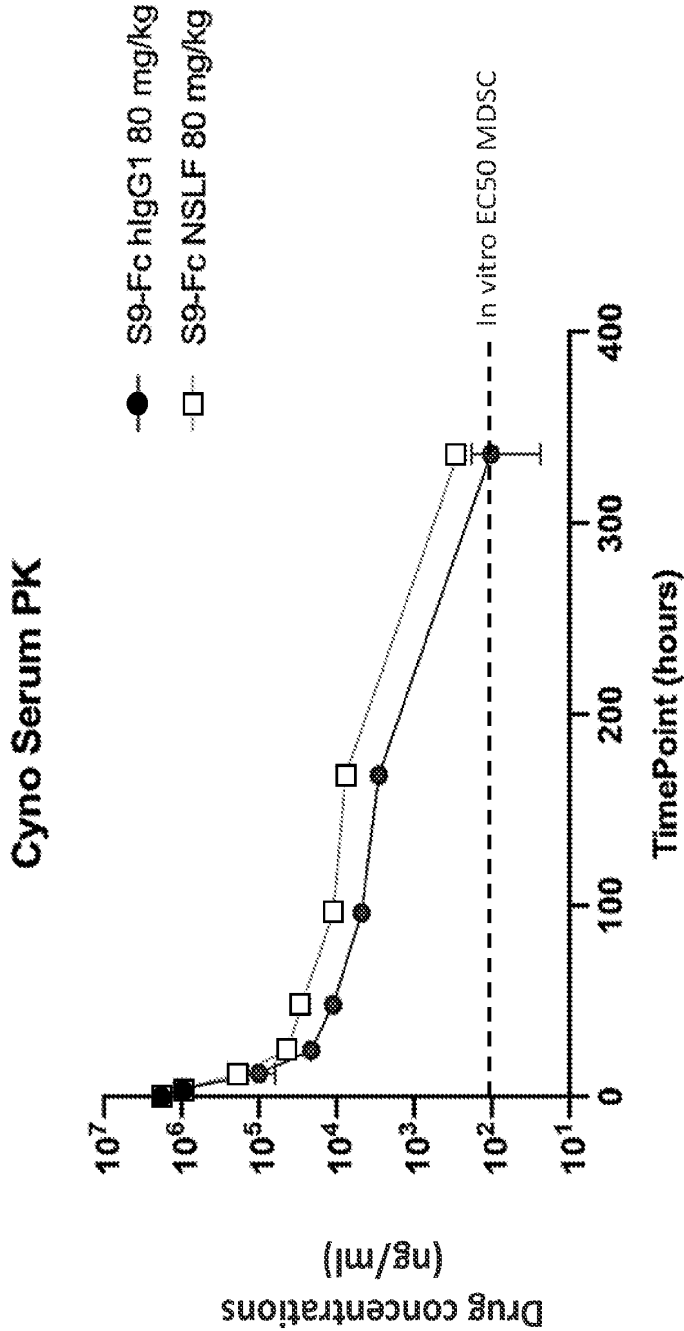


FIG. 33

Variant (SEQ ID NO)	Dose (mg/kg)	Animal #	C ₀ (ng/mL)	C _{max} (ng/mL)	AUC _{0-t} (ng*hr/mL)	AUC _{0-inf} (ng*hr/mL)	CL (mL/hr/kg)	t _{max} (hr)	t _{1/2} (hr)	V _{ss} (mL/kg)
S9.1 (SEQ ID NO:48)	10	Mean	9970	6660	58000	61400	164	1	29	3950
		SD	978	646	5110	5540	15.5	NA	6.01	836
S9.36 (SEQ ID NO:175)	10	Mean	7560	5050	50800	53300	190	1	27.6	5290
		SD	1630	973	9620	7280	24.5	NA	4.16	1190
S9.37 (SEQ ID NO:176)	10	Mean	24200	15300	130000	136000	75.5	1	29.4	1920
		SD	8540	4440	15300	24100	11.8	NA	8.34	222
S9.38 (SEQ ID NO:177)	10	Mean	16000	10000	72800	77200	131	1	32.1	3070
		SD	3730	2110	9890	8660	14.9	NA	11.4	982
S9.39 (SEQ ID NO:178)	10	Mean	9340	6160	41900	42400	242	1	30	3290
		SD	2710	1470	6750	6730	43.9	NA	9.49	902

(Signal sequences cleaved during production)

FIG. 34