

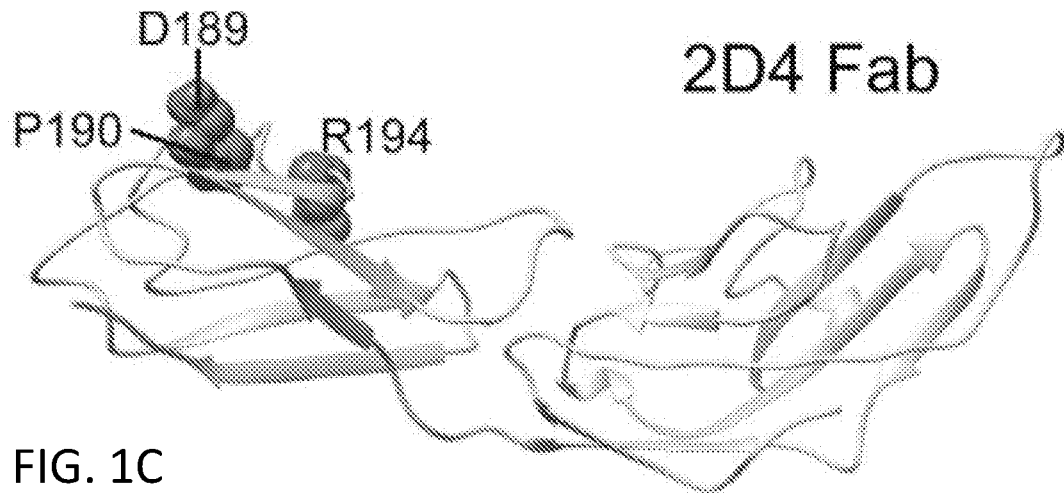
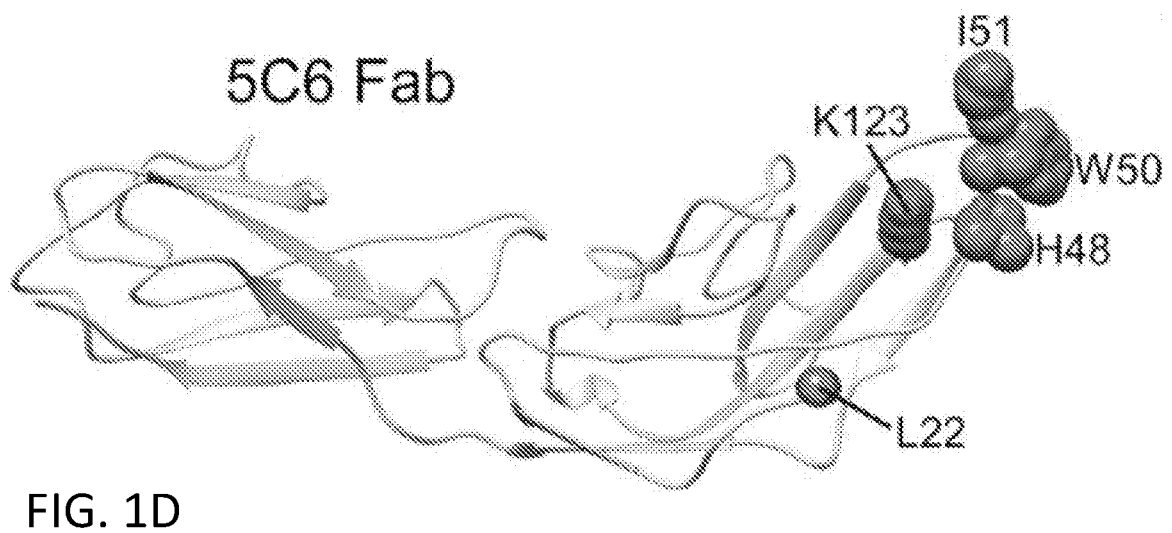
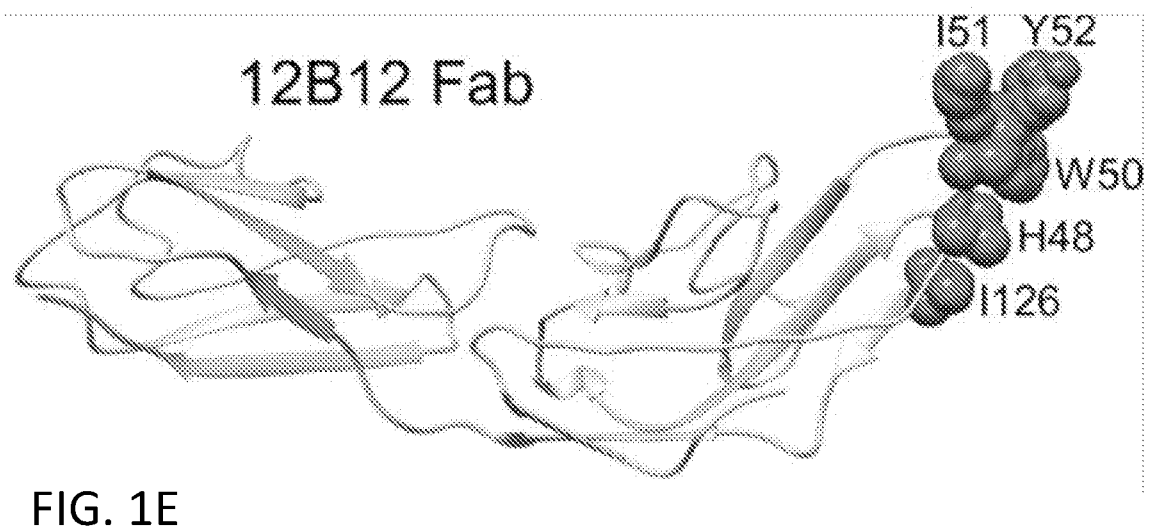
Human Siglec-9	--MLLLLLLPLLWGRERAEGQTS---KLLTMQSSVTQEGLCVHVPCSFYSYPS---HGWI	51
Chimpanzee Siglec-9	--MLLLLLLPLLWGRERAEGQTS---NLLTMQSSVMVQEGLCVHVPCSFYSYPS---RGWI	51
Green monkey Siglec-9	-MLLLLLLPLLWGRERVEGQRNNRKDYPLTMQGSATVQEGLCVHVLCFSYSYPR---FGWI	56
Rhesus macaque Siglec-9	MLLLLLLPLLWGRERVEGQRNNQKNYPLTMQESVTVQQGLCVHVLCFSYSYPW---YGWI	57
Mouse Siglec-9	-MLLLLLLPLLWGIKGVGQNP-QEVFTLNVERKVVVQEGLCVLPVPCNFSYLKKRLTDWT	58
	:***** : .*** *.:. . **:* ** * *.* ** *	
Human Siglec-9	YPGPVVHGYWFREGANTDQDAPVATNNPARAVWEETRDRFHLLGDPHTKNCTLSIRDARR	111
Chimpanzee Siglec-9	YPGPVVHGYWFREGANTDQDAPVATNNPARAVREETRDRFHLLGDPHTKNCTLSIRDARR	111
Green monkey Siglec-9	TSDP-VHGYWFQAGADTDRDTPVATNNPARAREETRDRFHLLGDPQTKNCTLSIRDARS	115
Rhesus macaque Siglec-9	SSDP-VHGYWFRAGARTDRDAPVATNNPARAVREDTRDRFHLLGDPQTTNCTLSIRDARS	116
Mouse Siglec-9	DSDP-VHGFYREGTDRKDSIVATNPIRKAVKETRNRFLLGDPWRNDCSLNIREIRK	117
	* **:*.: * : :*: ***** * . :*:**.* ** . :*:**.* :	
Human Siglec-9	SDAGRYFFRMEKGSIKWNYKHHRLSVNVTALTHRPNILIPGTLES GCPQNLTC SVPWACE	171
Chimpanzee Siglec-9	SDAGRYFFRVETGNIKWNYKHHRLSVNVTALTHRPNILIPGTLES GCPQNLTC SVPWACE	171
Green monkey Siglec-9	SDAGTYFFRVETGKTWNYKYVLLSVRVTALTHRPNILIPGTLES GCPQNLTC SVPWACE	175
Rhesus macaque Siglec-9	SDAGKYFFRVETGKTWNYKYAPLSVHVTALTHRPNILIPGTLES GCPQNLTC SVPWACE	176
Mouse Siglec-9	KDAGLYFFRLERGKTKYNMWDKMTLVVTALTNTQIILLPETLEAGHPSNLTC SVPWDGCG	177
	.*** ***: * . *.:* :.: ***** .*:**.* ***: * * ***** *	
Human Siglec-9	QGTPPMISWIGTSVSPLDPSTTRSSVLTILIPQPDHGTSLTCQVTFPGASVTNKTIVHLN	231
Chimpanzee Siglec-9	QGTPPMISWIGTSVSPLDPSTTHSSVLTILIPQPDHGTSLTCQVTFPGASVTNKTIVHLN	231
Green monkey Siglec-9	QGTPPMISWMGTSVSPLDPSTTRSSVLTILIPQPDHGTSLTCQVTFPGASVTNKTIVHLN	235
Rhesus macaque Siglec-9	QGTAPMISWMGTSVSPLDPSTTRSSVLTILIPQPDHGTSLTCQVTFPGASVTNKTIVHLN	236
Mouse Siglec-9	WTAPPIFS WTGT SVSPLSTNTTGSSVLTITIPQPDHGTSLTCQVTLPGTNVSTRMTIRLN	237
	: *:** ***** * . ** *****: *****.* ***:**.* *:** *	
Human Siglec-9	VSYPPQNLMTVFQDGTSTVTLGNSSLSLPEGQSLRLVCAVDAVDSNPPARLSLSWRG	291
Chimpanzee Siglec-9	VSYPPQNLMTVFQDGTSTVTLGNSSLSLPEGQSLRLVCAVDAVDSNPPARLSLSWRG	291
Green monkey Siglec-9	VSYPPQNLMTVFQNGTSTVTLGNSSLSLPEGQSLRLVCAV---DSNPPARLSLSWRG	292
Rhesus macaque Siglec-9	VSYPPQNLMTVFQNDTSTVTLGNSSSVSVEGQSLRLVCAV---DSNPPARLSLSWRG	293
Mouse Siglec-9	VSYAPKNTVTIYQGADSVSTIKNSSSLPISEGQSLRLICST---DSYPPANLSWSWLN	294
	*** *:***:*. ** :* *****: * * *****:.. ** ***. ** *	
Human Siglec-9	LTLCPSPQSPNPGVLELPWVHLRDAAEFTCRAQNPLGSSQVVLNVSLQSKAT-SGVTQGVV	350
Chimpanzee Siglec-9	LTLCPSPQSPNPGVLELPWVHLRDEEFTCRAQNPLGSSQVVLNVSLQSKAT-SGVTQGVV	350
Green monkey Siglec-9	LTLCPSPSPGVLELPVHLRDEEFTCRAQNLLGSSQVVLNVSLQSKAT-SGLTQGVV	351
Rhesus macaque Siglec-9	LTLCPSPQSPNPGVLELPVHLRDEEFTCRAQNLLGSSQVVLNVSLQSKAT-SGLTQGVV	352
Mouse Siglec-9	LTLCPSKLSKPGLELFPVHLKHGGVYTCQAQHALGSHLSLSLPQSSATLSEMMGTTF	354
	*****: *.**:* ***: . **:*. *****: *.** **.* * : ..	
Human Siglec-9	GGAGATALVFLSFCVIFVVVRSRCKKSARPAAGVGDTGIEDANAVRGASQGPLTEPWAE	410
Chimpanzee Siglec-9	GGAGATALVFLSFCVIFVVVRSRCKKSARPAAGVGDTGIEDANAVRGASQGPLTEPCAE	410
Green monkey Siglec-9	G-AGATALVFLSFCVIFVVVRSRCKKSARPVAGVGDTGIEDANAVRGASQGPLTEPWAE	410
Rhesus macaque Siglec-9	G-AGATALVFLSFCVIFVVVRSRCKKSARPVAGVGDTGIEDANAVRGASQGPLTEPWAE	411
Mouse Siglec-9	VGSGVTALLFLSVCILLAVRSYRRKPARPAVVAP-----HPDALKVSQNPVLESQAD	409
	:*.***:***.***:..*** *:* ***. . . :*: .** *.* *:	
Human Siglec-9	DSPPDQPPASARSSVGEGELQYASLSFQMVKPDWSRGQEATDTEYSEIKIHR-----	463
Chimpanzee Siglec-9	DSPPDQPPASARSSVGEGELQYASLSFQMVKPRDLRGQEATDTEYSEIKIHR-----	463
Green monkey Siglec-9	DSPPDQPPASARSSVGEEELQYASLSFQTVKPRDLQGQEATNTEYSEIKIHK-----	463
Rhesus macaque Siglec-9	DSPPDQPPASARSSVGEEELQYASLSFQTVKPRDLQGQEATNTEYSEIKIHK-----	464
Mouse Siglec-9	DSSEPLPSILEAAPSTEEIHYATLSFHEMKPMNLWGQDQDTTEYSEIKFPQRTAWP	467
	** * * * * *:***: ** : ** : * *****: :	

FIG. 1A

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Binding Reactivity (% WT)				
Mutation	2D4 Fab High Stringency	12B12 Fab	5C6 Fab	MAb 1139
L22A	94.7 (23)	91.2 (2)	6.6 (4)	100.6 (18)
H48A	147.5 (21)	2.6 (3)	0.7 (2)	118.7 (30)
W50A	99.8 (39)	2.2 (3)	7.1 (7)	68.7 (29)
I51A	103.1 (8)	5.8 (6)	3.2 (3)	75.6 (18)
Y52A	144.3 (10)	1.5 (2)	58.8 (39)	108.3 (8)
K123A	142.0 (8)	27.6 (8)	4.5 (0)	87.3 (31)
I126A	163.6 (7)	9.1 (2)	75.8 (2)	127.4 (24)
D189A	24.3 (8)	115.0 (10)	56.3 (9)	114.9 (3)
P190A	25.9 (9)	146.0 (13)	48.3 (37)	124.6 (29)
R194A	2.6 (2)	174.3 (3)	85.1 (67)	134.0 (26)
C213A	20.3 (15)	72.1 (10)	46.1 (9)	76.7 (3)

FIG. 1B



Oligosaccharide	R1	R2	R3	Relative Recognition by Human Siglec										
				1	2	3	4	5	6	7	8	9	10	11
	◆ = Sia	○ = Gal	■ = GlcNAc											
	□ = GalNAc	▲ = Fuc												
N-Glycan	+		+	-	++	++	-	-	-	-	-	+	+	-
O-Glycan	+			++	-	+	+	++	-	-	+	++	++	+
Glycolipid	+		+	+	-	+	-	-	-	++	+	+	-	++
◆α6○β4■β-R1				++	-	+	+	-	-	-	+	+	+	-
◆α3○β4■β-R1				+	-	+	-	-	-	+	+	+	-	+
◆α8◆α3○β4■β-R1				++	-	+	+	-	-	-	+	+	+	-
◆α3○β3■β-R1				+	-	+	-	-	-	-	+	+	+	-
◆α3○β4■β-R1					-	+	-	-	-	-	+	+	+	-
◆α3○β3□β-R2/α-R3				+	-	+	+	-	-	-	-	+	+	-
◆α3○β3□β-R2/α-R3				?	?	?	++	-	-	+	-	+	?	?
◆α6◆				+	-	+	+	-	-	-	-	+	+	-
◆β-R2/α-R3				+	+	+	-	-	+	+	+	+	+	-

FIG. 2

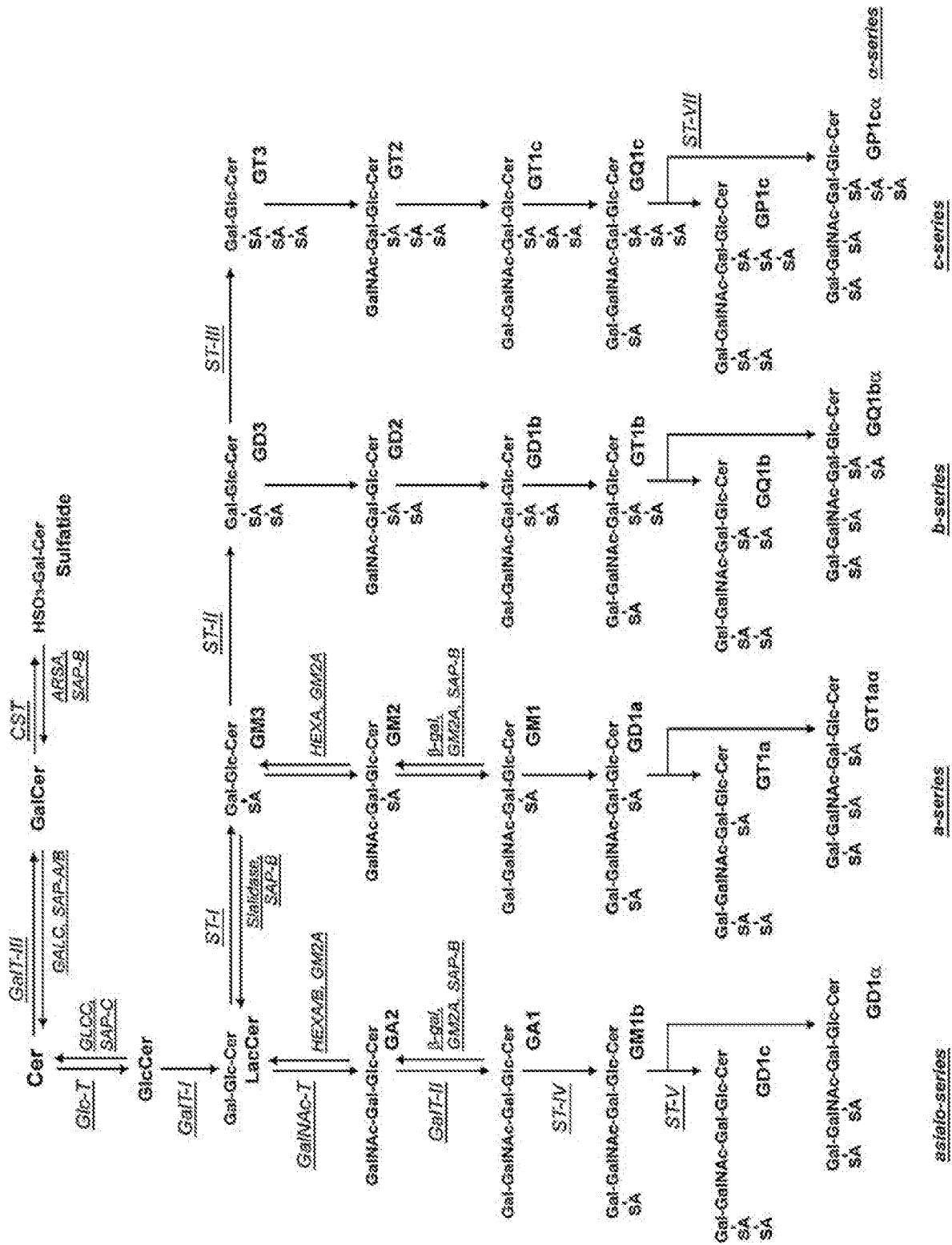


FIG. 3

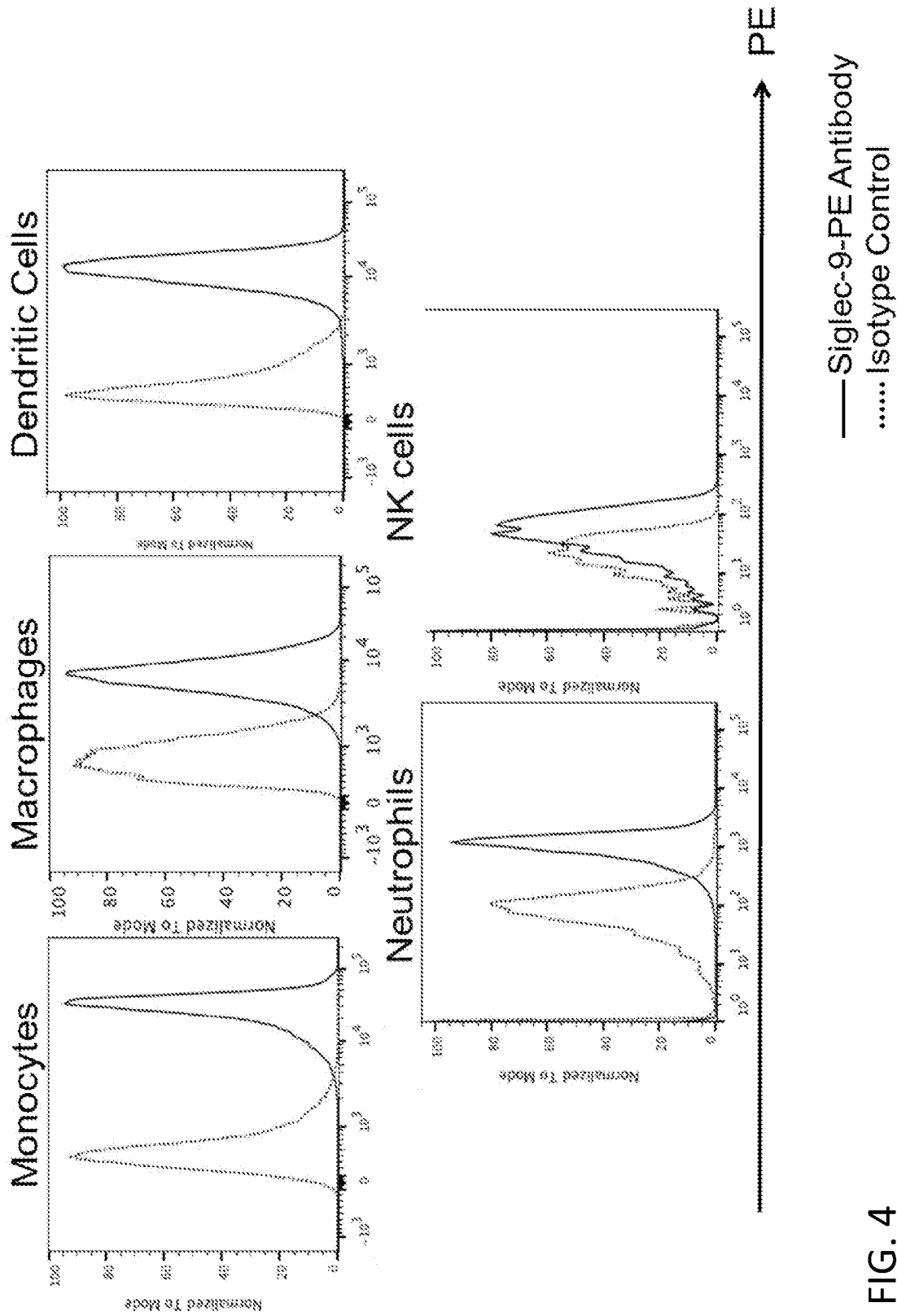
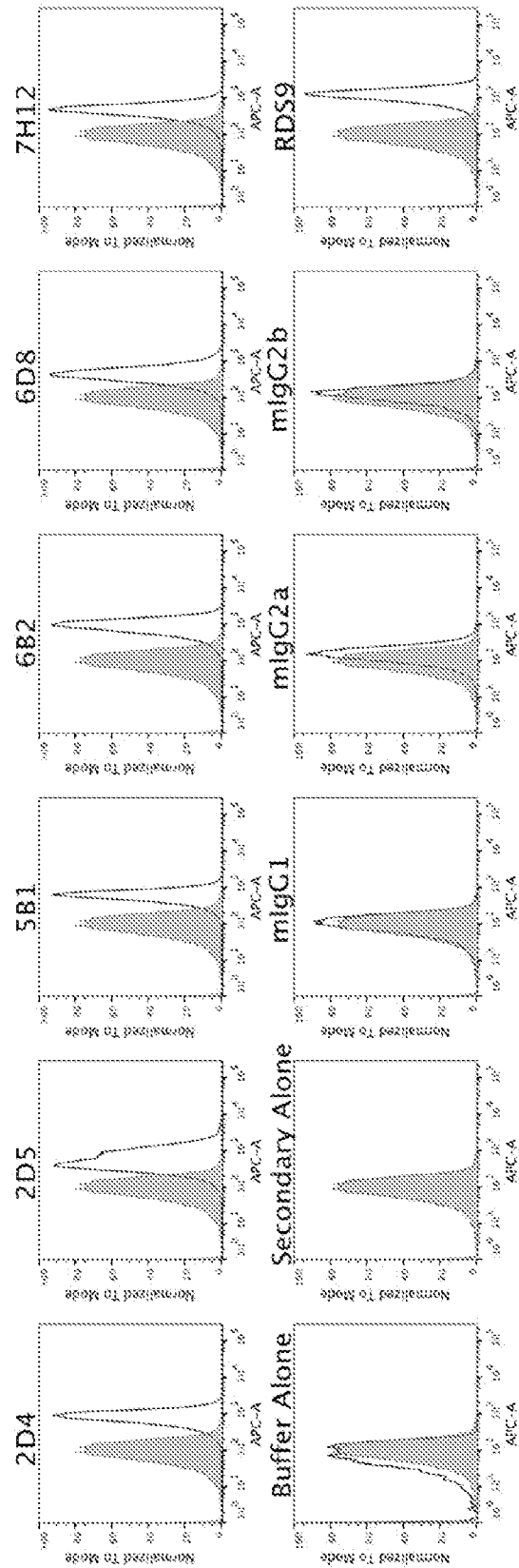


FIG. 4



— Siglec-9-Antibody
■ Isotype

FIG. 5

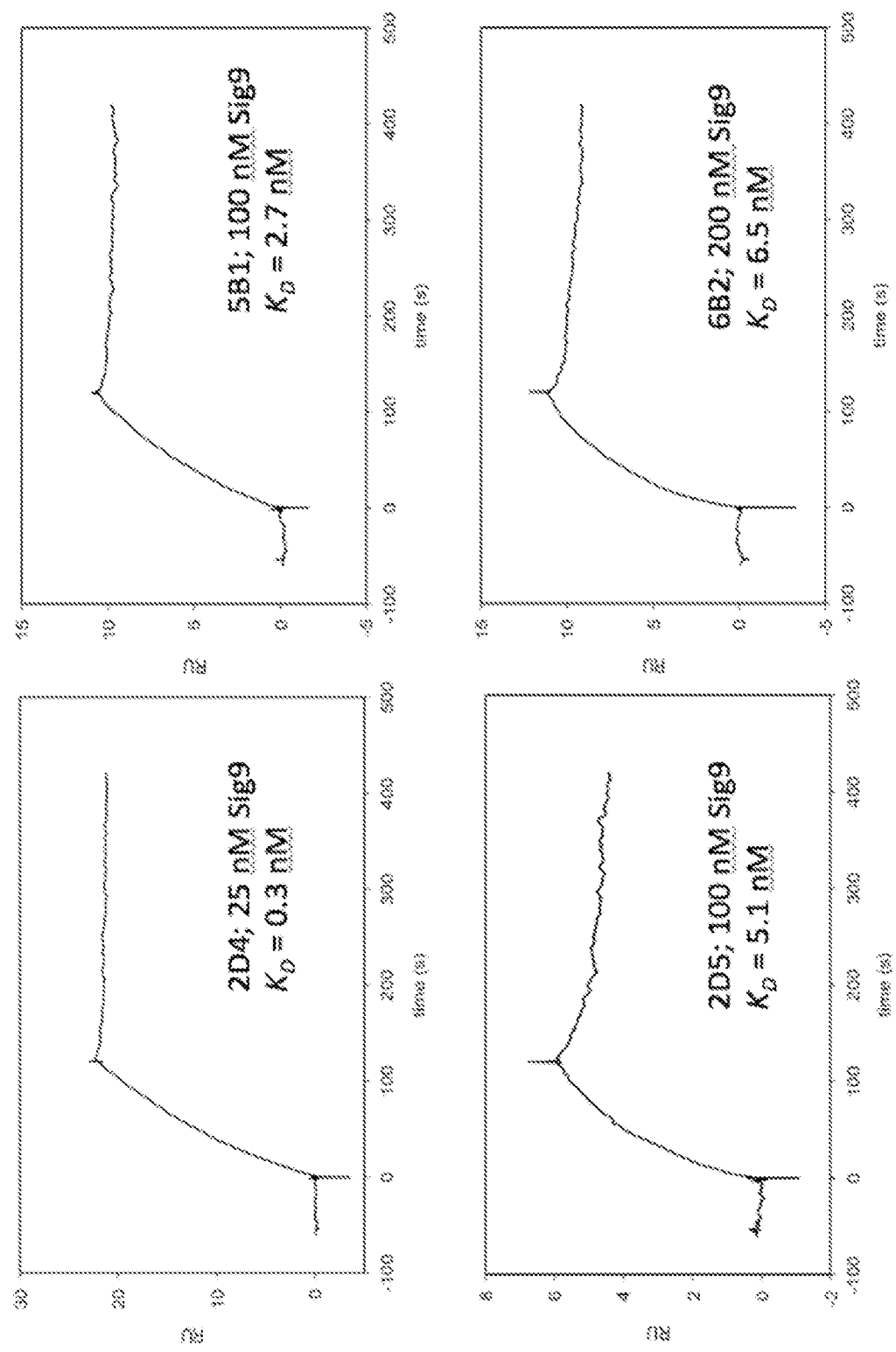


FIG. 6A

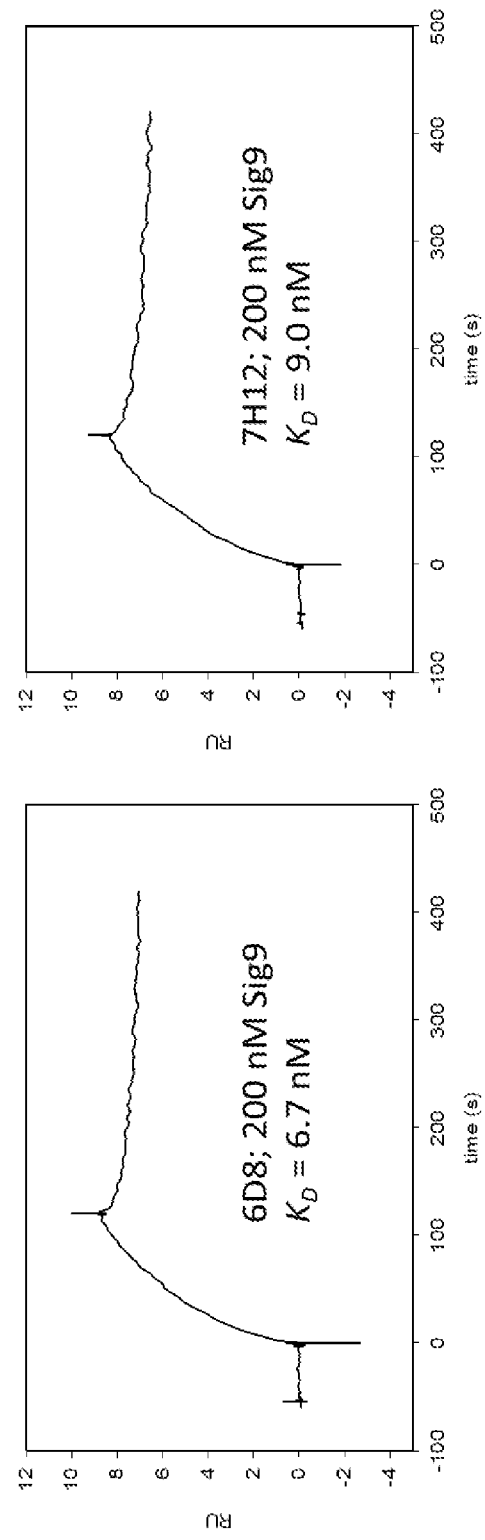
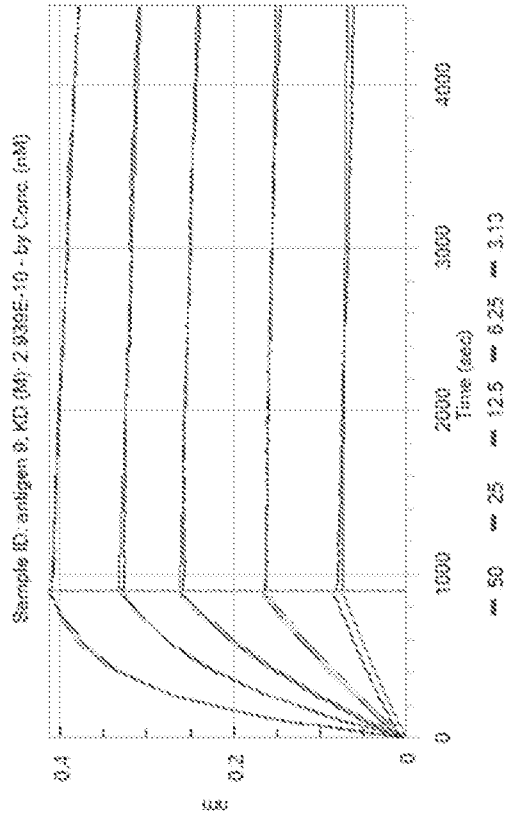
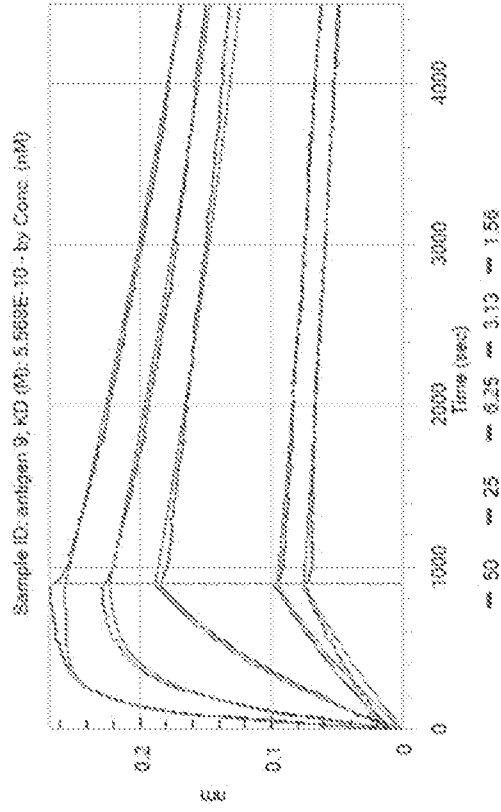


FIG. 6A (Contd.)

2D4



5C6



12B12

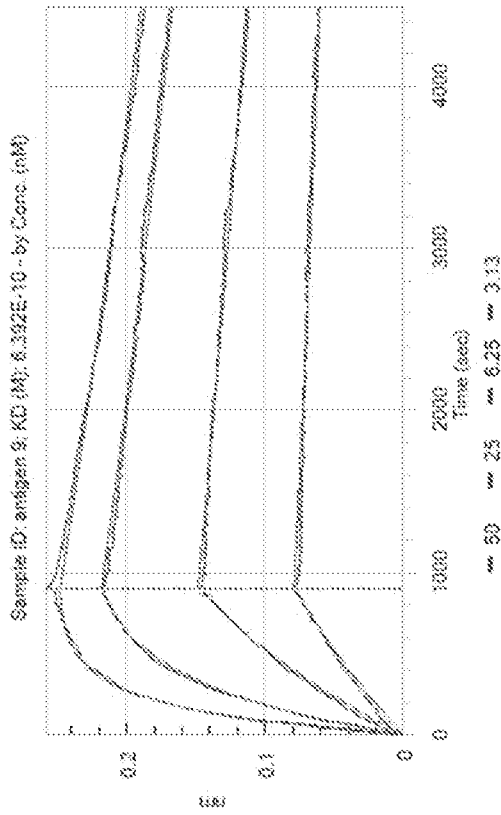


FIG. 6B

Potential humanized sequence based on IMGT ICKV2-30*01 acceptor framework (AbM CDR definition)
ICKV2-30*01 DVVMTQSP¹SLPVTLCQPASISCRSSQSLVYSDGNTYLNWFQQRPGQSPRRLLIYKVSNRDS
GVPDFRFGSGSGTDFTLKISRVEAEDVGVIYCMQGTWPP
Joining region IMGT J00242|ICKJ2*01|YTFGQGTKLEIK

seq	10	20	30	40	50	60
AbM	10	20	30abcde	40	50	
	b b b	p p	b b b	b b i	i i i b b i	i
5C6.3	DVVM	TQSP	SLPVTLCQ	PASISCRSSQ	SLVYSDGNTYLN	WFQQRPGQSPRRLLIYKVSNRDS
	*	*	***	** **	** **	**
2-30*01	DVVM	TQSP	SLPVTLCQ	PASISCRSSQ	SLVYSDGNTYLN	WFQQRPGQSPRRLLIYKVSNRDS
h5C6.3-L1	DVVM	TQSP	SLPVTLCQ	PASISCRSSQ	SLVHSGNGNTYLH	WFQQRPGQSPRRLLIYKVSNRDS
h5C6.3-L2	DVVM	TQSP	SLPVTLCQ	PASISCRSSQ	SLVHSGNGNTYLH	WFQQRPGQSPRRLLIYKVSNRDS
h5C6.3-L3	DVVM	TQSP	SLPVTLCQ	PASISCRSSQ	SLVHSGNGNTYLH	WFQQRPGQSPRRLLIYKVSNRDS

seq	70	80	90	100	110
AbM	60	70	80	90	100
	b	b	b b b	i b i b i	b b b
5C6.3	GVPDFRFGSGSGTDFTLKISRVEAEDVGVIYCMQGTWPP	FGAGTKLEIK			
	*	*	*	*	*
2-30*01	GVPDFRFGSGSGTDFTLKISRVEAEDVGVIYCMQGTWPP	FGAGTKLEIK			
h5C6.3-L1	GVPDFRFGSGSGTDFTLKISRVEAEDVGVIYCMQGTWPP	FGAGTKLEIK			
h5C6.3-L2	GVPDFRFGSGSGTDFTLKISRVEAEDVGVIYCMQGTWPP	FGAGTKLEIK			
h5C6.3-L3	GVPDFRFGSGSGTDFTLKISRVEAEDVGVIYCMQGTWPP	FGAGTKLEIK			

deamidation substitutions: Q/S/A/D
@ isocaspate formation substitutions: S/Q/A

h5C6.3-L1 is a "CDR-swap" with no changes to human framework. Subsequent sequences alter framework changes shown in red (compared to sequence above it).

FIG. 6C

Potential humanised sequence based on IMGT IGHV1-18*01 acceptor framework (AbM CDR definition)

IGHV1-18*01 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWSAYNGNTN

YAQKLQGRVTMTTDTSTSTAYMELRSLSRSDDTAVYYCAR

Joining region IMGT J00256||IGHJ4*01|YFDYWGQGLTVTVSS

seq	10	20	30	40	50		
AbM	10	20	30	40	50 a		
	b b b	p b b b b	b b b	b i i i i b b	b		
5C6.3	EVQLQSGPELVKPGASVSRISCKAS	GYTFTDYNIH	WVKQSQGKSLEWIG	YIYPYNGDTG			
	* * * * *	**	* * * *	*			
1-18*01	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTSYGIS	WVRQAPGQGLEWMG	WISAYNGNTN			
h5C6.3-H1	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTDYNIH	WVRQAPGQGLEWMG	YIYPYNGDTG			
h5C6.3-H2	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTDYNIH	WVRQAPGQGLEWMG	YIYPYNGDTG			
h5C6.3-H3	QVQLVQSGAEVKKPGASVKISCKAS	GYTFTDYNIH	WVRQAPGQGLEWIG	YIYPYNGDTG			
h5C6.3-H4	QVQLVQSGAEVKKPGASVKISCKAS	GYTFTDYNIH	WVRQAPGQGLEWIG	YIYPYNGDTG			
		#		§			
		V					
seq	60	70	80	90	100	110	120
AbM	60	70	80 abc	90		110	
	i b b b b x	b b b b	b i b i b b		i b b b		
5C6.3	YNQKFPQNKATLTVDNSSSTAYMELRSLSRSDDTAVYYCAN	GNLYLYYYIAMDY	WGQGLTVTVSS				
	* * * * *	* * * *	*				
1-18*01	YAQKLQGRVTMTTDTSTAYMELRSLSRSDDTAVYYCAR						
h5C6.3-H1	YAQKLQGRVTMTTDTSTAYMELRSLSRSDDTAVYYCAR	GNLYLYYYIAMDY	WGQGLTVTVSS				
h5C6.3-H2	YAQKLQGRVTMTTDTSTAYMELRSLSRSDDTAVYYCAR	GNLYLYYYIAMDY	WGQGLTVTVSS				
h5C6.3-H3	YAQKFGKRAATMTVDNSTSTAYMELRSLSRSDDTAVYYCAN	GNLYLYYYIAMDY	WGQGLTVTVSS				
h5C6.3-H4	YAQKFGKRAATMTVDNSTSTAYMELRSLSRSDDTAVYYCAN	GNLYLYYYIAMDY	WGQGLTVTVSS				
		N			#		

deamidation substitutions: Q/S/A/D
§ isocaspate substitutions: Q/S/A/H

h5C6.3-H1 is a "CDR-swap" with no changes to human framework. Subsequent sequences alter framework changes shown in red (compared to sequence above it).

FIG. 6D

Potential humanized sequence based on IMGT IGV1-39*01 acceptor framework (AbM CDR definition)
IGKV1-39*01 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASLSQS

GVPSRFGSGSGTDFLTITSSLSQPEDFATYYCQQSYSTPP

Joining region IMGT J00243|IGKJ2*01|YTFGQGTKLEIK

seq	10	20	30	40	50	60
AbM	10	20	30abcd	40	50	60
	b b b	p p	b b b	b b b	i i i	i
12B12.2	DIVLTQSPASLA	VSIGQRATIS	RASENVDSYGISFMH	WYQQKPGQPPKLLIY	RASNLES	
	**	*	*	*	*	*
1-39*01	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASLSQS		
h12B12.2-L1	DIQMTQSPSSLSASVGDRVTITC	RASENVDSYGISFMH	WYQQKPGKAPKLLIY	RASNLES		
h12B12.2-L2	DIQMTQSPSSLSASVGDRVTITC	RASENVDSYGISFMH	WYQQKPGKAPKLLIY	RASNLES		
h12B12.2-L3	DIQMTQSPSSLSASVGDRATITC	RASENVDSYGISFMH	WYQQKPGKAPKLLIY	RASNLES		
	V	#	P	#		
seq	70	80	90	100	110	
AbM	60	70	80	90	100	110
	b b	b b b	b b b	i b i	b b b	
12B12.2	GIPARFSGSGSKTDFLTINPVEADVA	FATYYCQQSNEDPWT	FGGGTKLEIK			
	*	*	*	*	*	*
1-39*01	GVPSRFGSGSGTDFLTITSSLSQPEDFATYYC	QQSYSTPP	FGGGTKLEIK			
h12B12.2-L1	GVPSRFGSGSGTDFLTITSSLSQPEDFATYYC	QQSNEDPWT	FGGGTKLEIK			
h12B12.2-L2	GVPSRFGSGSGTDFLTITSSLSQPEDFATYYC	QQSNEDPWT	FGGGTKLEIK			
h12B12.2-L3	GIPSRFGSGSGTDFLTITSSVQPEDFATYYC	QQSNEDPWT	FGGGTKLEIK			
	V	#	@			

deamidation substitutions: Q/S/A/D
@ tryptophan oxidation: F/Y/H

h12B12.2-L1 is a "CDR-swap" with no changes to human framework. Subsequent sequences alter framework changes shown in red (compared to sequence above it).

FIG. 6E

Potential humanized sequence based on IMGT IGHV3-23*04 acceptor framework (AbM CDR definition)

IGHV3-23*04 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTY

YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYICAK

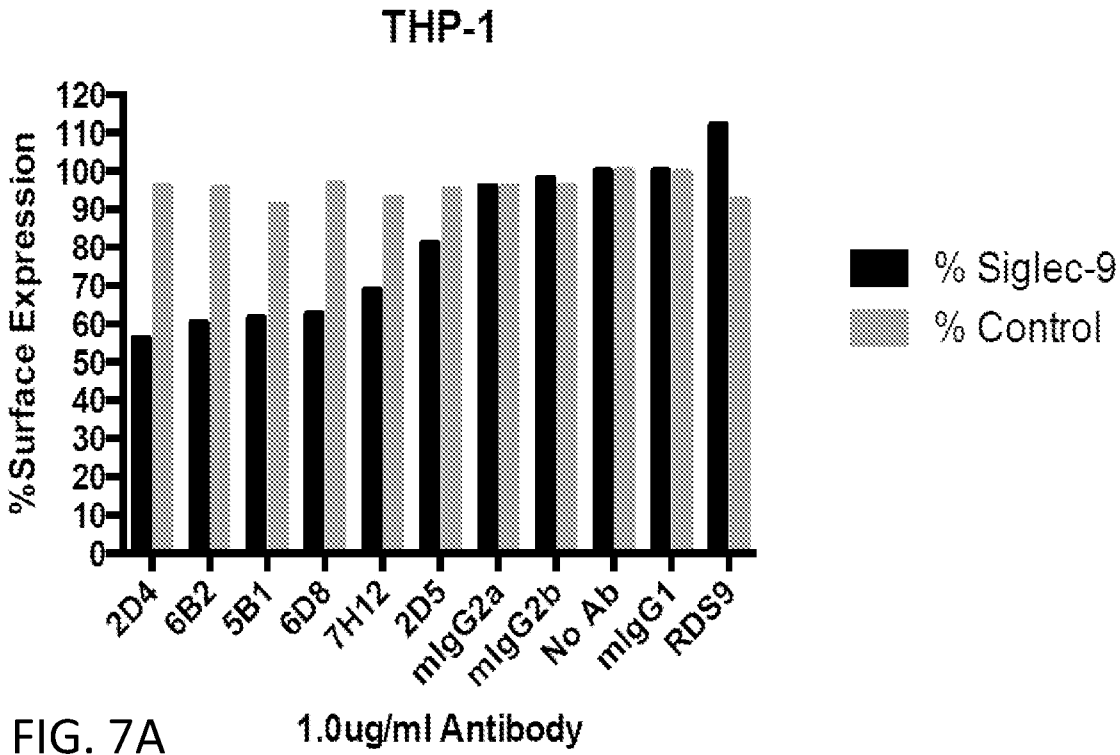
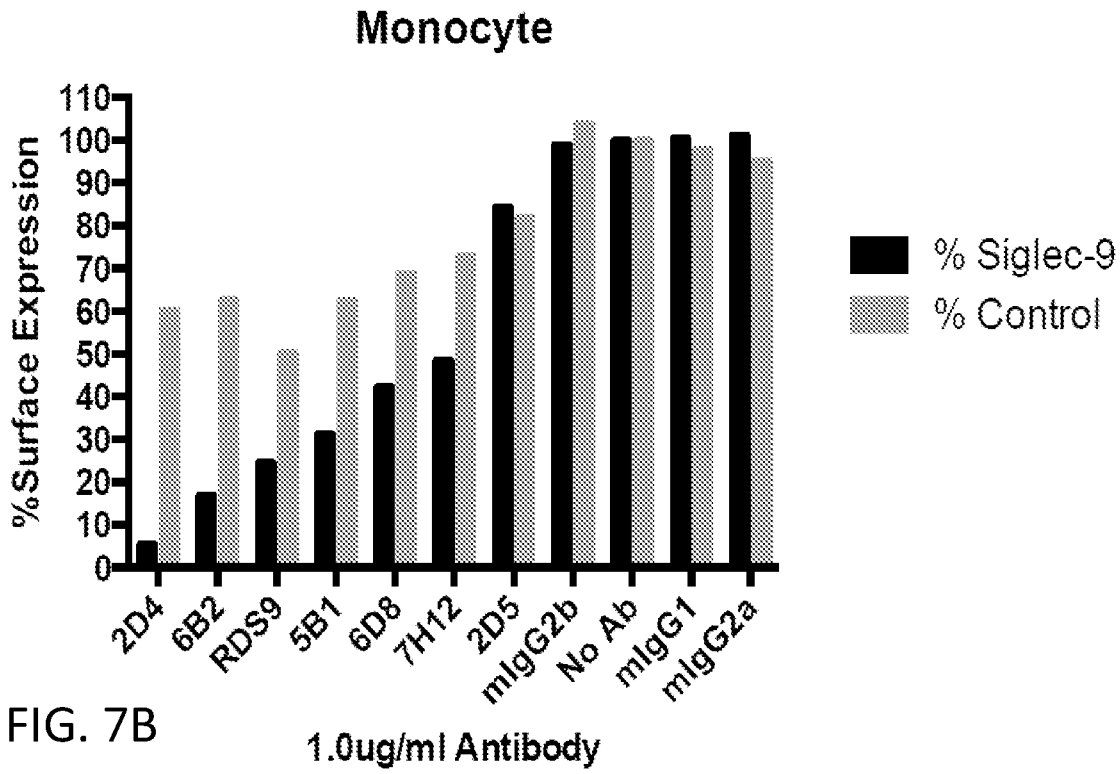
Joining region IMGT J00256|IGHJ4*01|YFDYWGQGLVTVSS

seq	10	20	30	40	50		
AbM	10	20	30	40	50 a		
	b b b	p b b b b	b b b	b i i	i i b b b		
12B12.2	EVQLVESGGGLVQPGGSLKLSAAS	GFTFSNYGMS	WVRQILDKRLELVA	TINSNGGRTY			
	*		*** *	* *			
3-23*04	EVQLVESGGGLVQPGGSLRLSCAAS	GFTFSSYAMS	WVRQAPGKGLEWVS	AISGSGGSTY			
h12B12.2-H1	EVQLVESGGGLVQPGGSLRLSCAAS	GFTFSNYGMS	WVRQAPGKGLEWVS	TINSNGGRTY			
h12B12.2-H2	EVQLVESGGGLVQPGGSLRLSCAAS	GFTFSNYGMS	WVRQAPGKGLELVA	TINSNGGRTY	#		
					#		
seq	60	70	80	90	100	110	120
AbM	60	70	80 abc	90		110	
	i b b b b x	b b b b	b i b i b	b b b			
12B12.2	YFDSVKGRFTISRDN AKNTLYLQMSSLRSEDTAIYICVT	YIRYDSYAMDY	WGQGLTVTVSS				
	*	*	*	*	*	*	
3-23*04	YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYICAK						
h12B12.2-H1	YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYICAK	YIRYDSYAMDY	WGQGLTVTVSS				
h12B12.2-H2	YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYICAK	YIRYDSYAMDY	WGQGLTVTVSS				

deamidation substitutions: Q/S/A/D
@ isoaspartate substitutions: Q/S/A

h12B12.2-H1 is a "CDR-swap" with no changes to human framework. Subsequent sequences alter framework changes shown in red (compared to sequence above it).

FIG. 6F



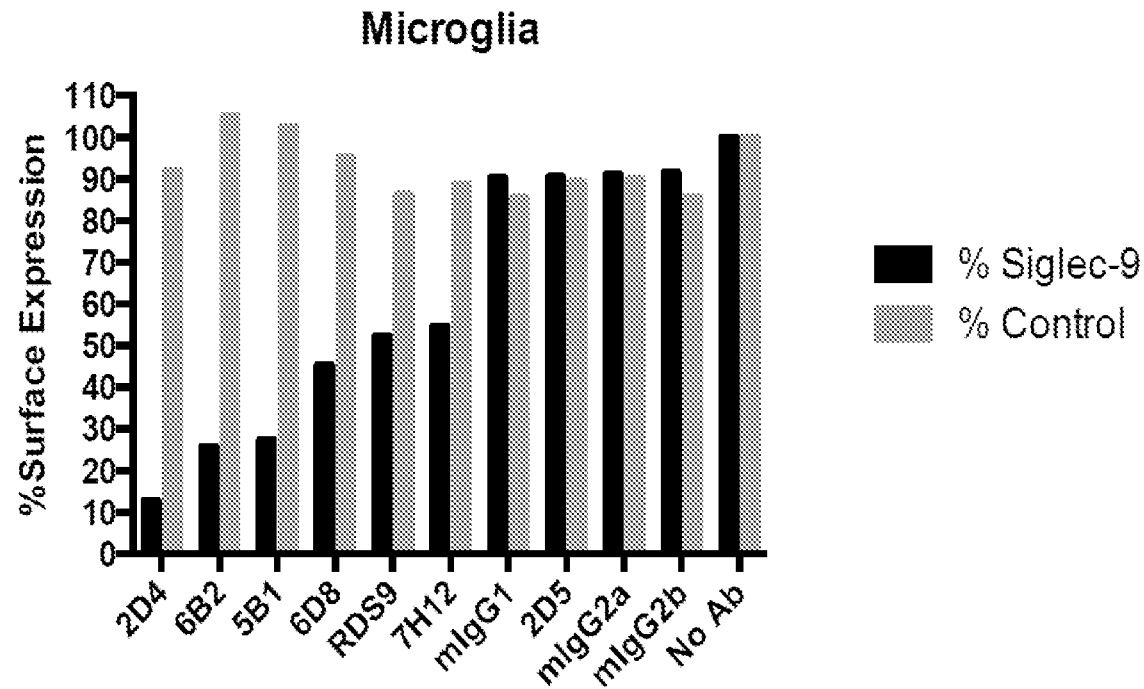


FIG. 7C 1.0ug/ml Antibody

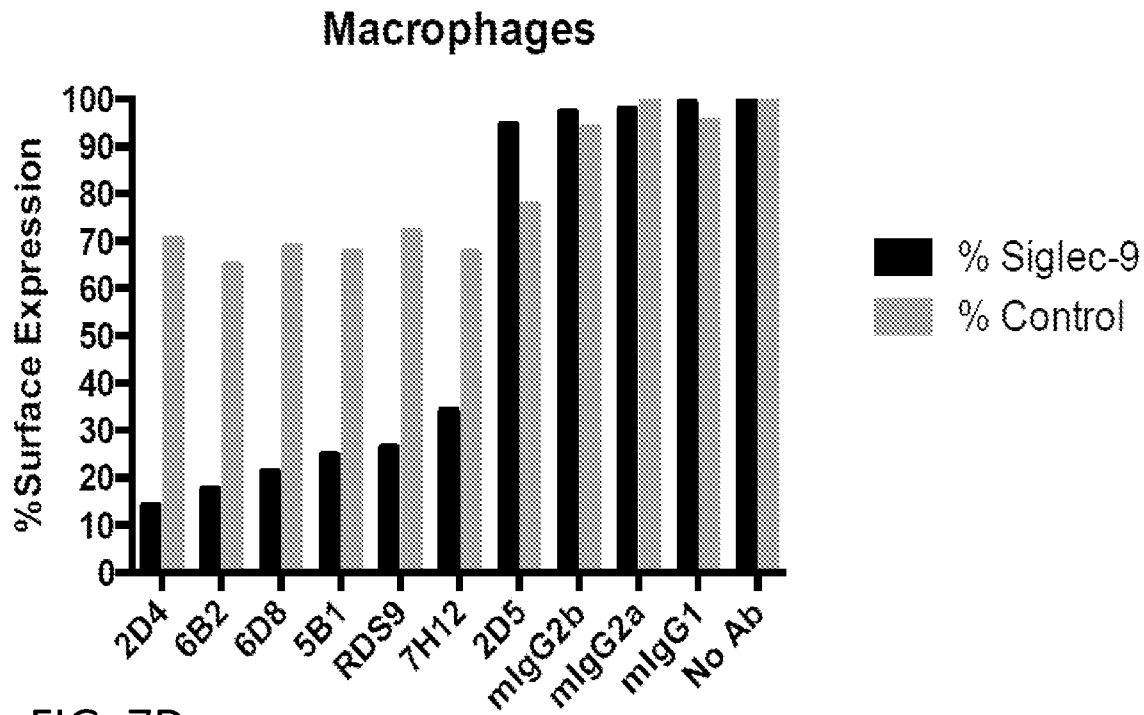


FIG. 7D 1.0ug/ml Antibody

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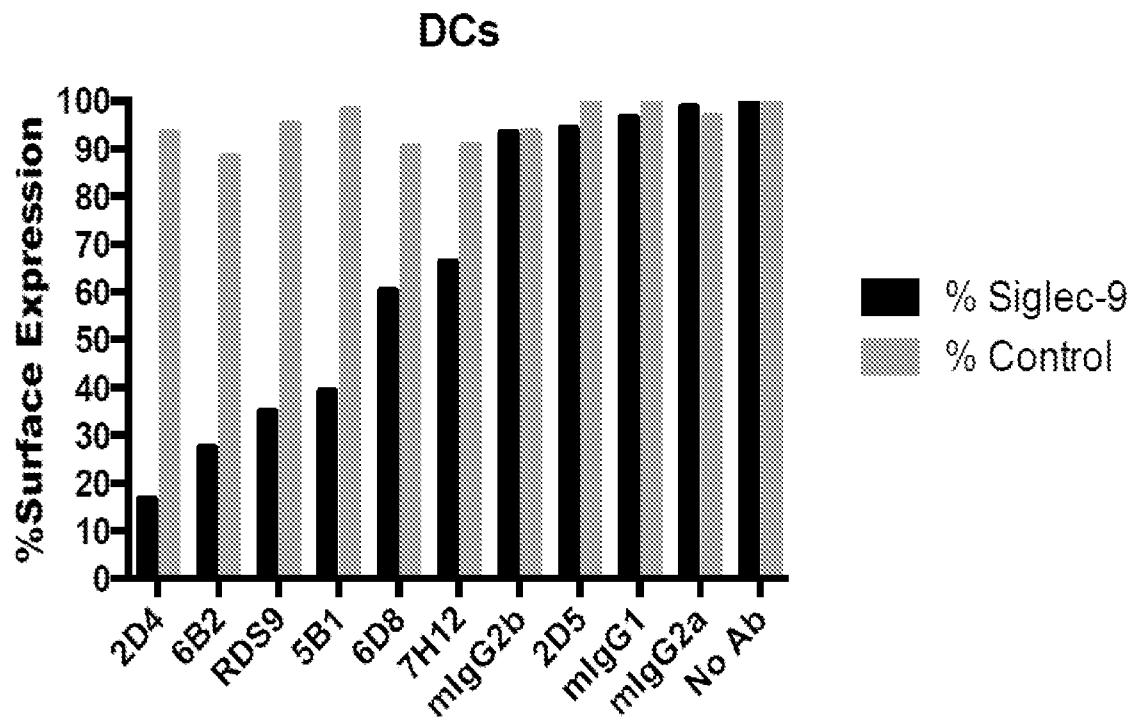


FIG. 7E 1.0ug/ml Antibody

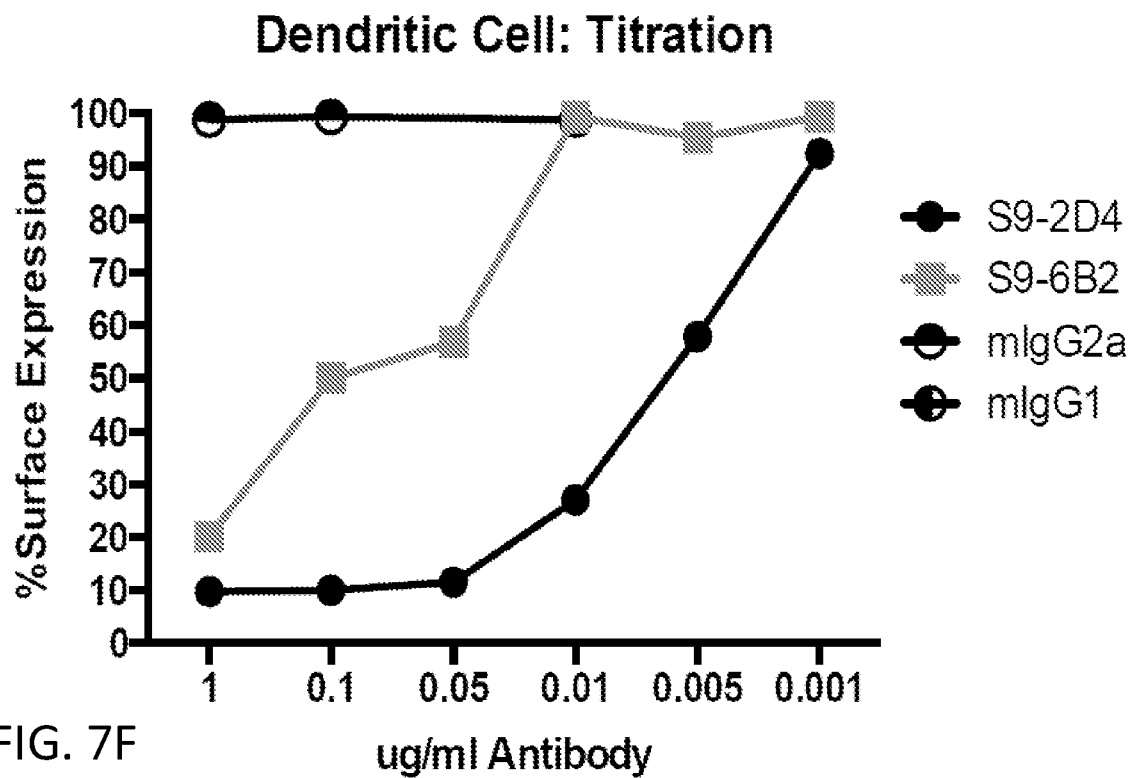


FIG. 7F

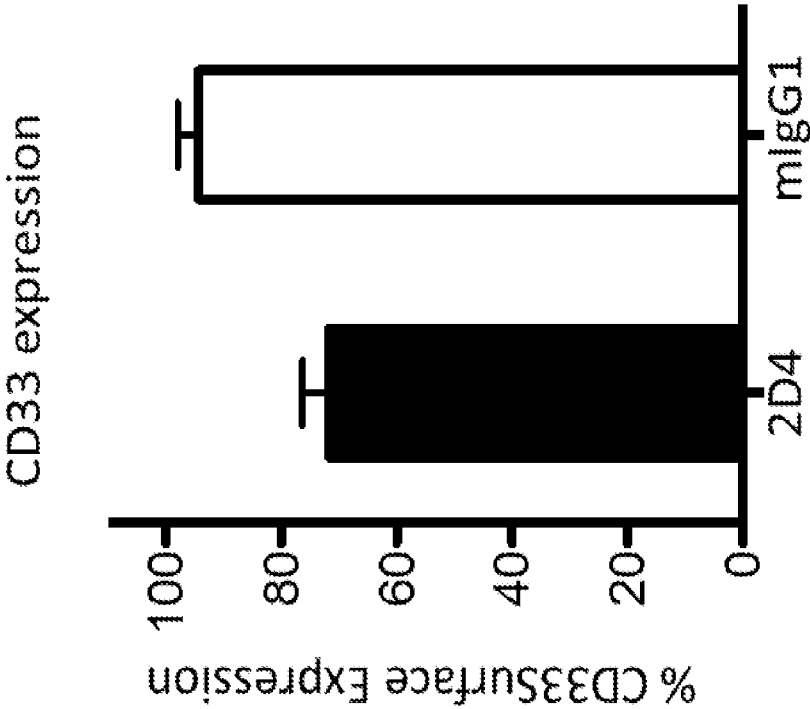


FIG. 7H

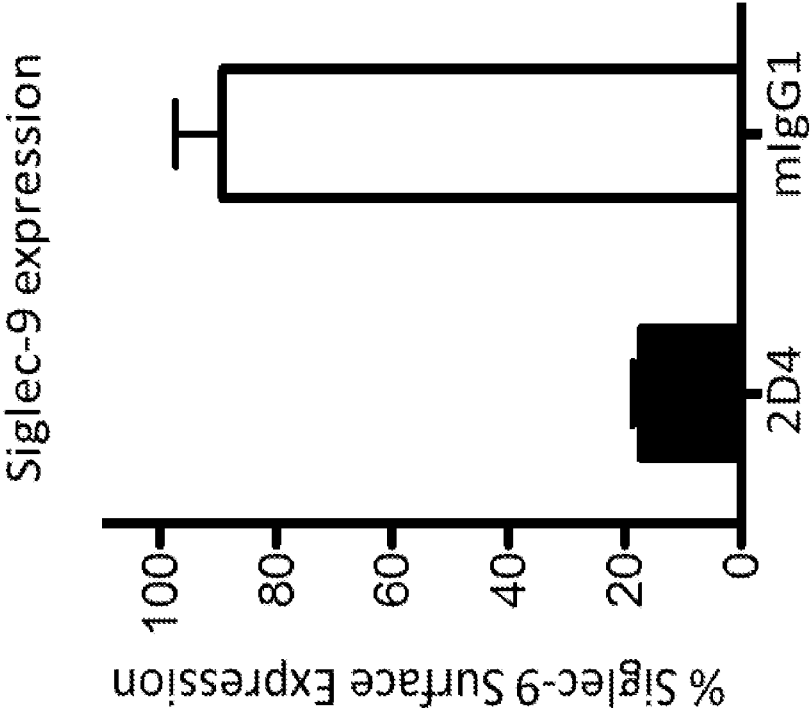


FIG. 7G

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Siglec-9 Surface Expression

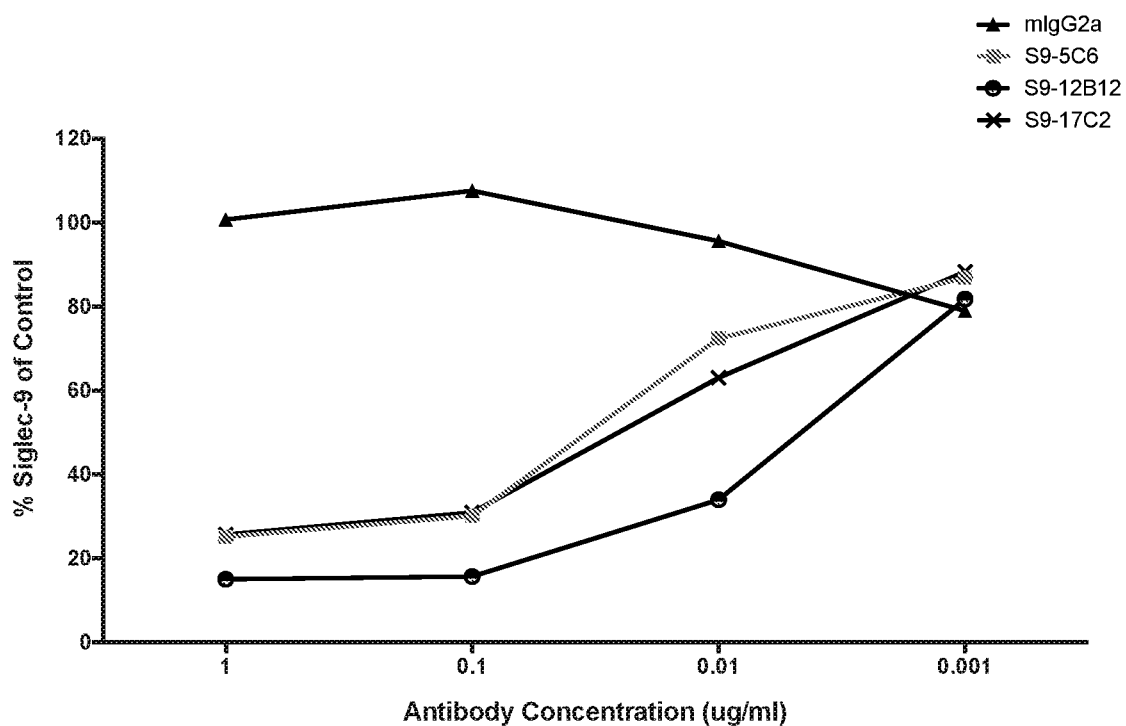


FIG. 7I

CD11b Control Expression

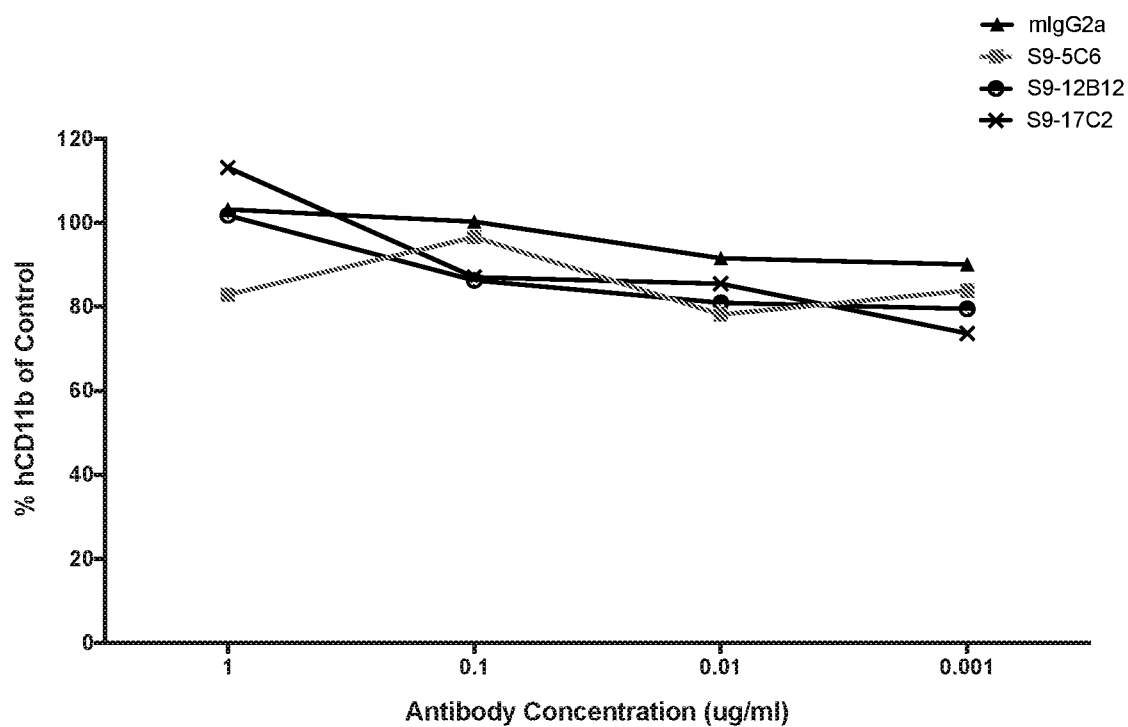


FIG. 7J

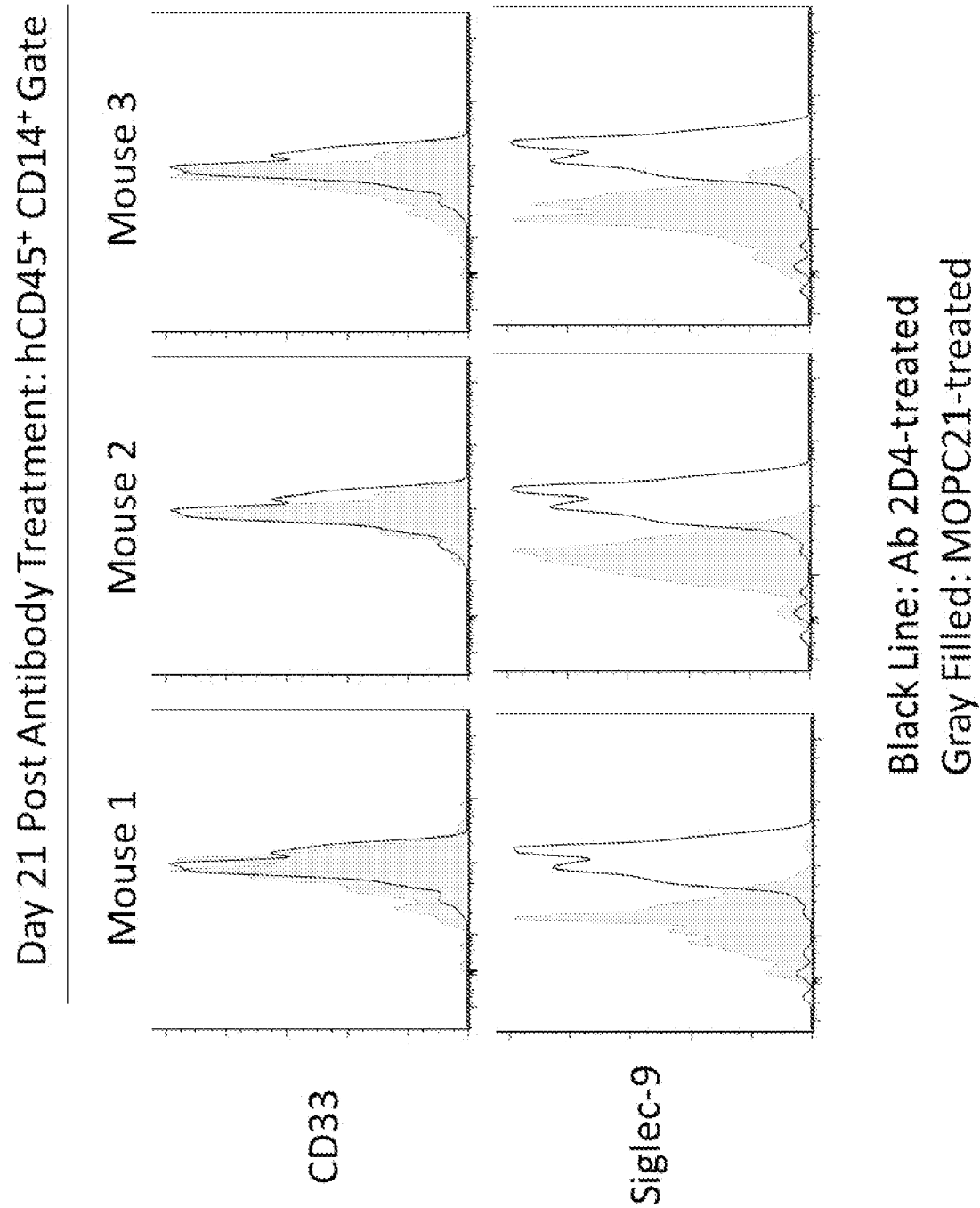


FIG. 7K

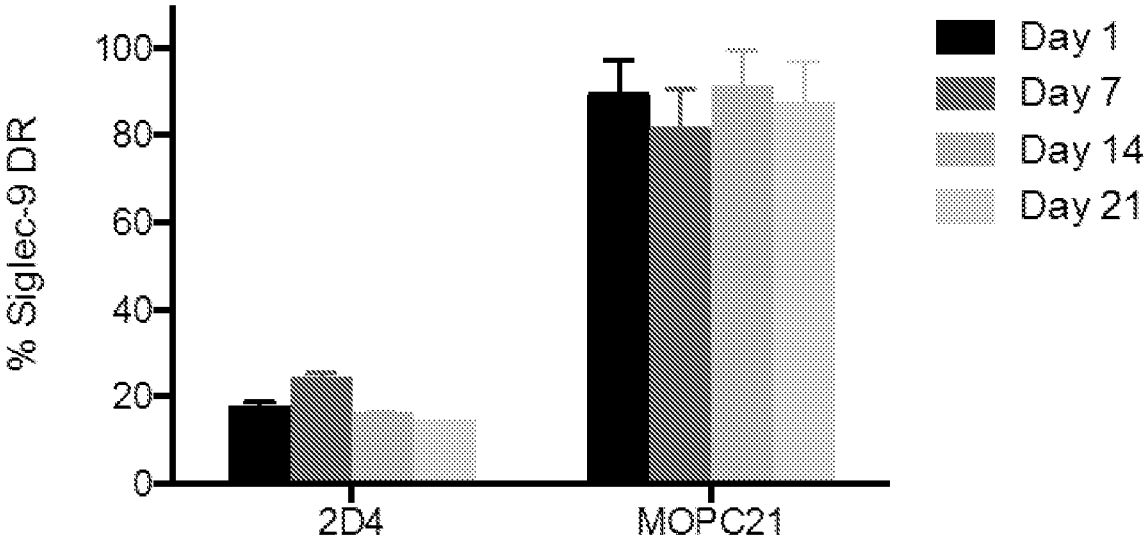


FIG. 7L

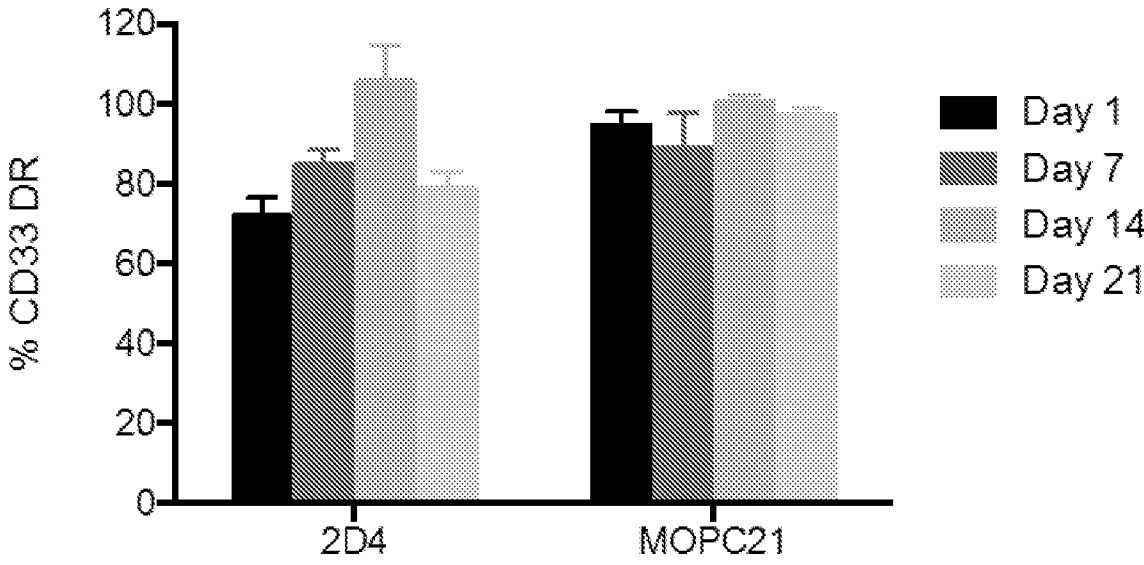
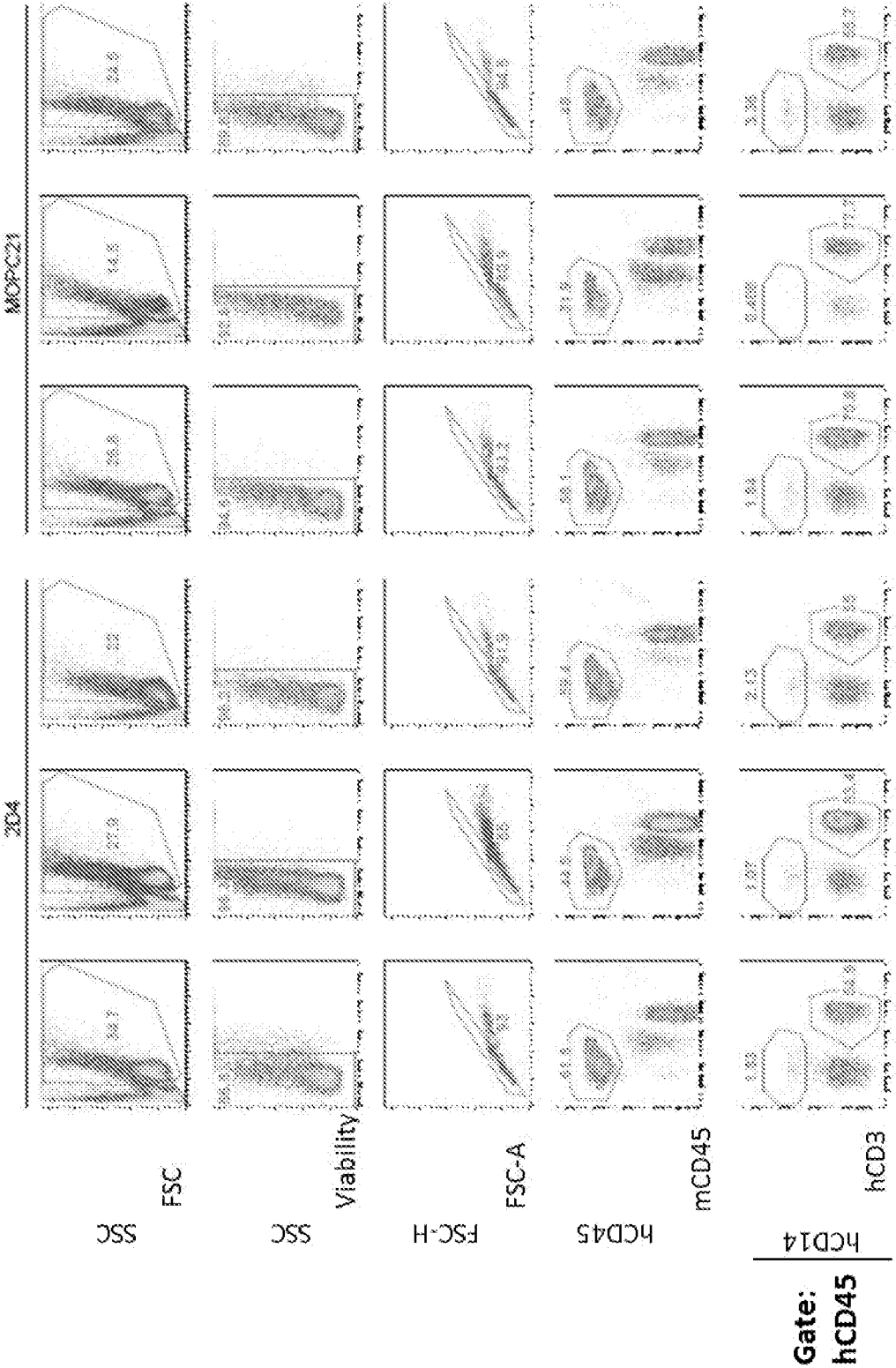


FIG. 7M



Flow Plots from Day 21

FIG. 7N

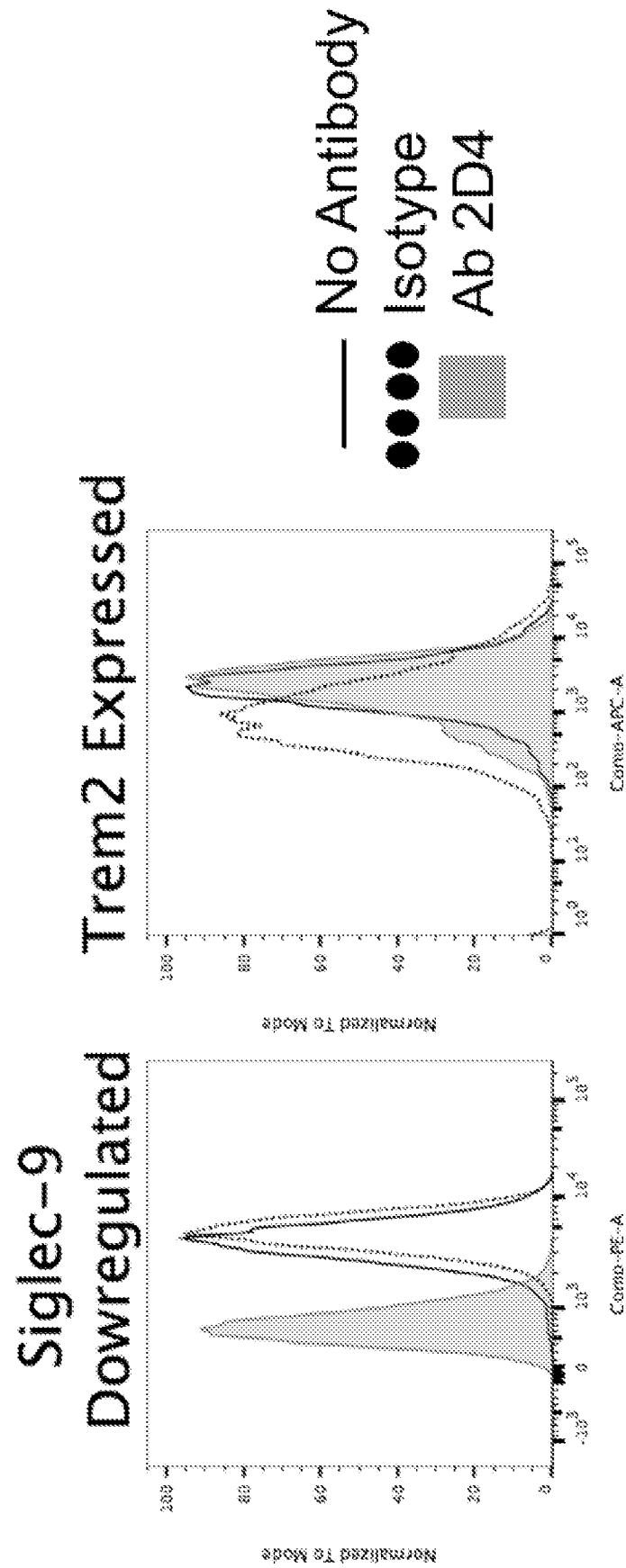


FIG. 70

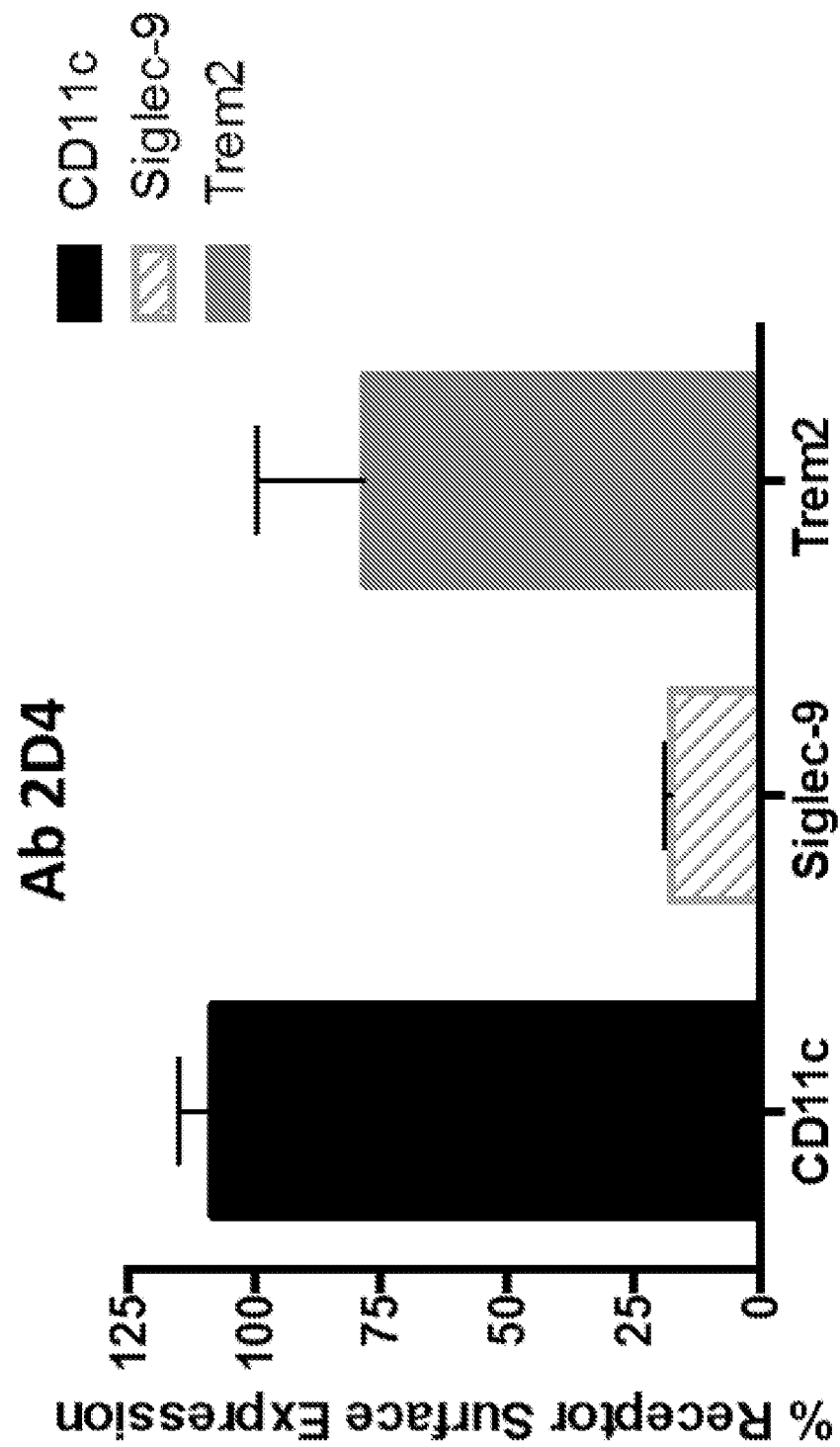
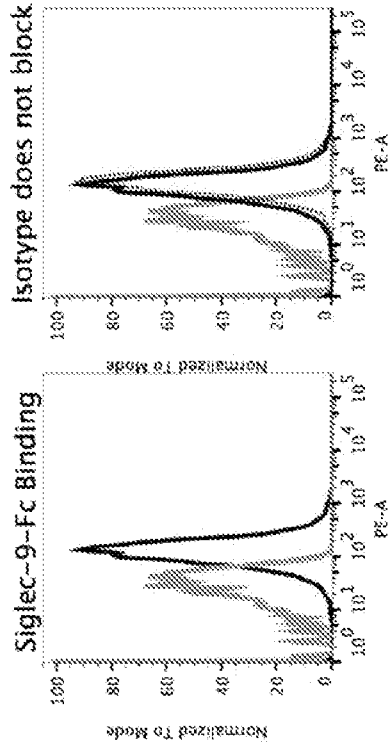


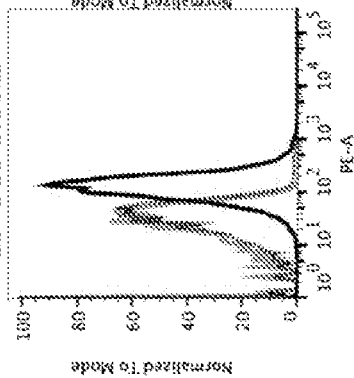
FIG. 7P

FIG. 7Q

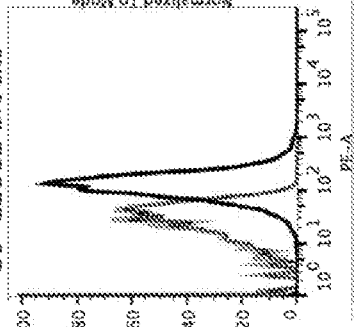
Legend:
Black **Solid** line: S9-Fc Alone
Grey line: Secondary Alone
Black Dotted line: + Indicated mAb



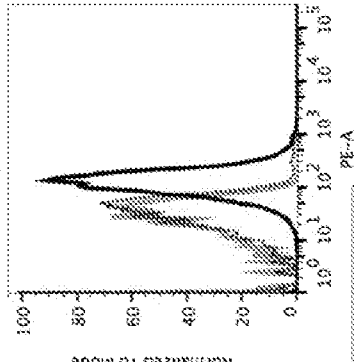
S9-5C6 blocks



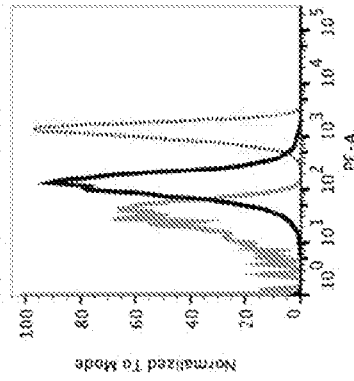
S9-12B12 blocks



S9-17C2 blocks



S9-2D4 enhances binding



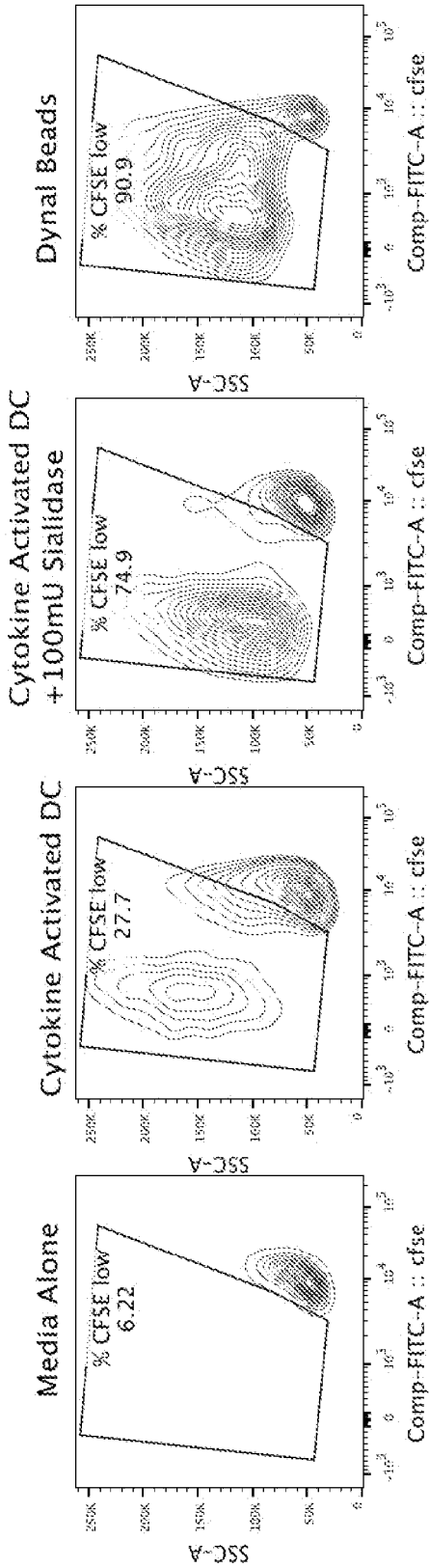


FIG. 8

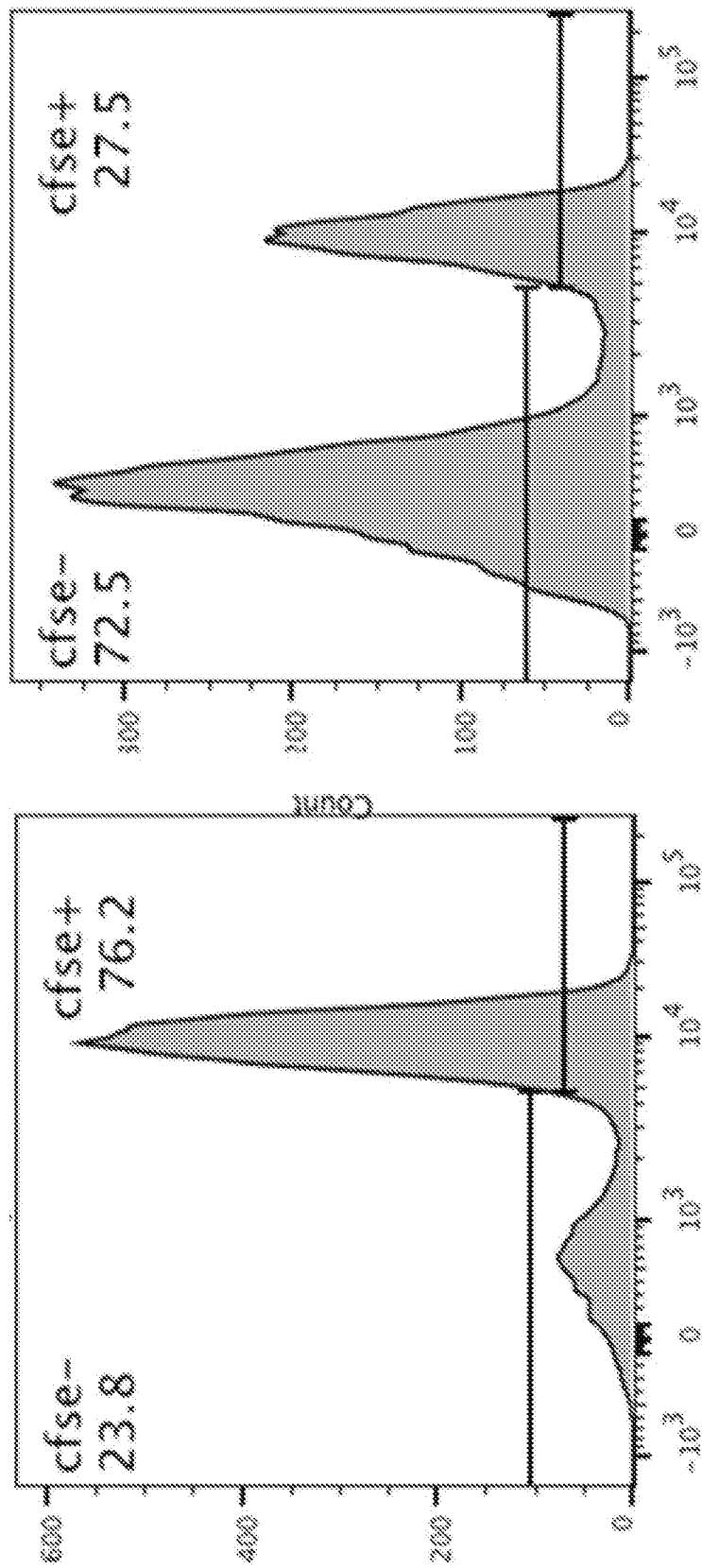


FIG. 9

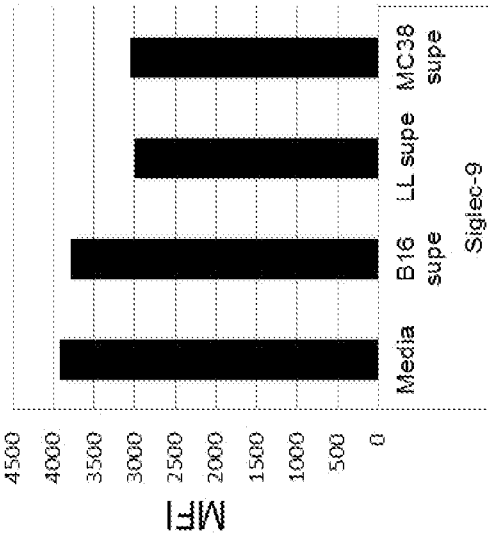


FIG. 10A

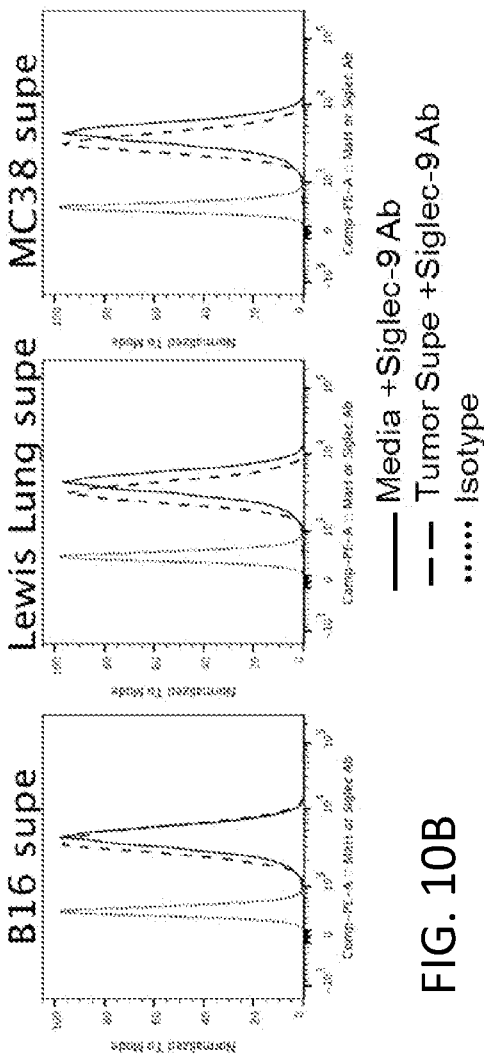


FIG. 10B

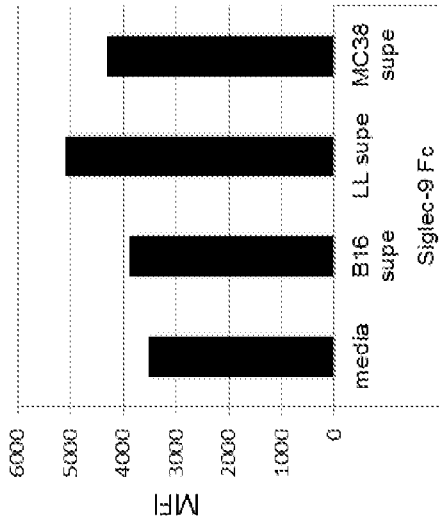


FIG. 10C

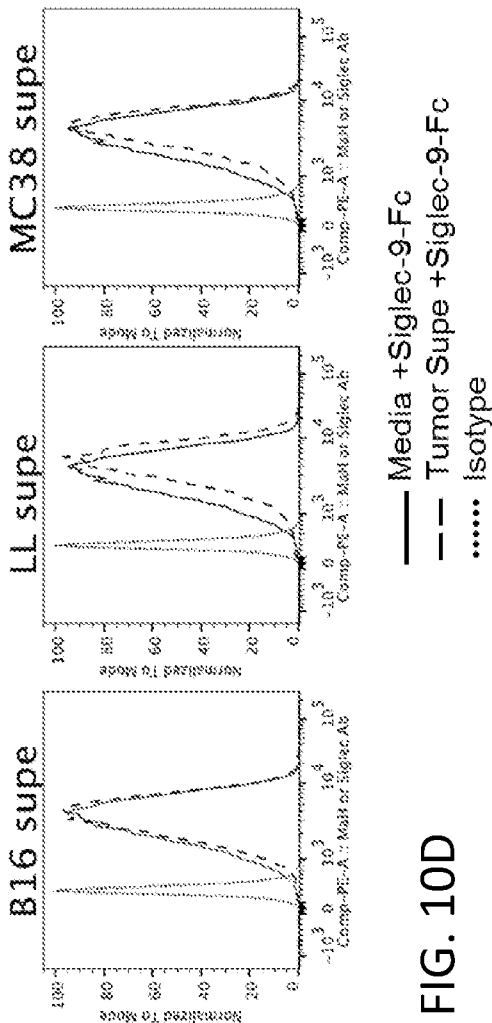


FIG. 10D

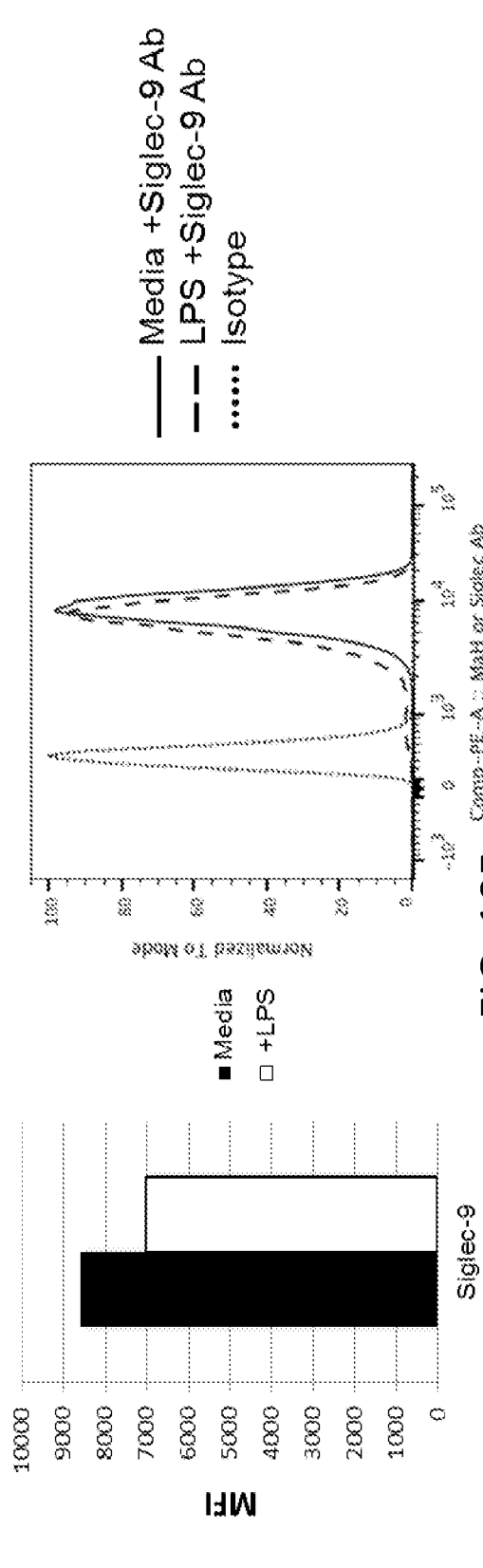


FIG. 10E

FIG. 10F

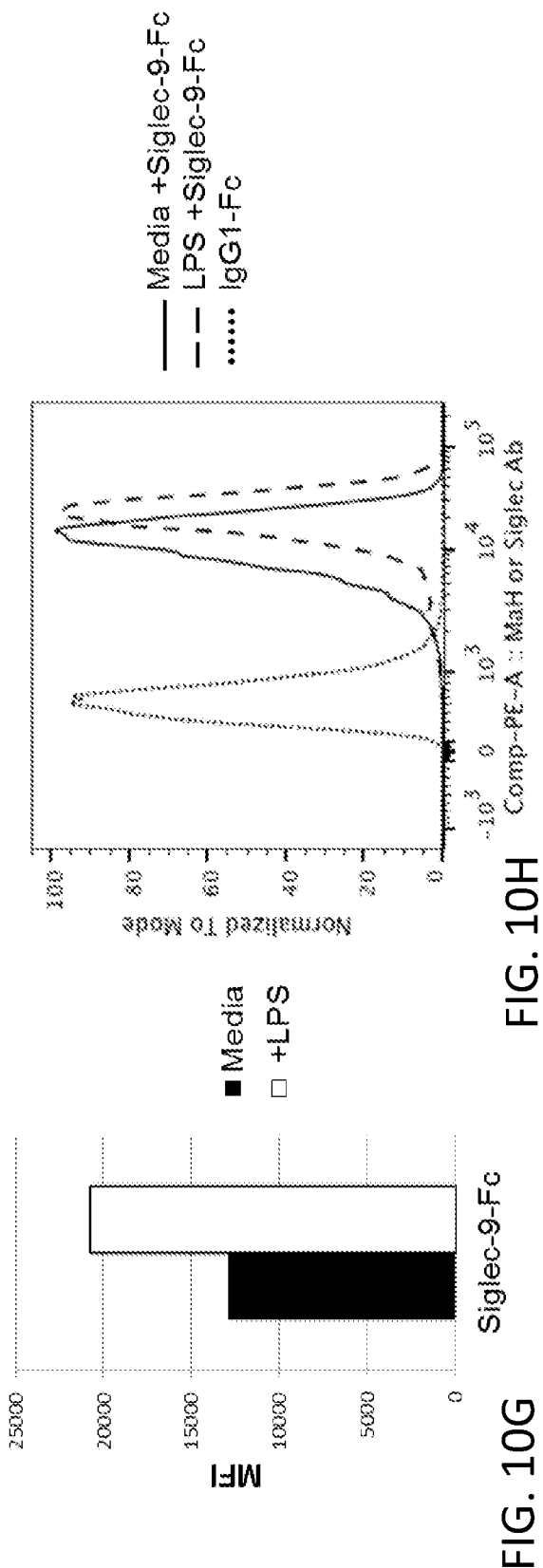


FIG. 10G

FIG. 10H

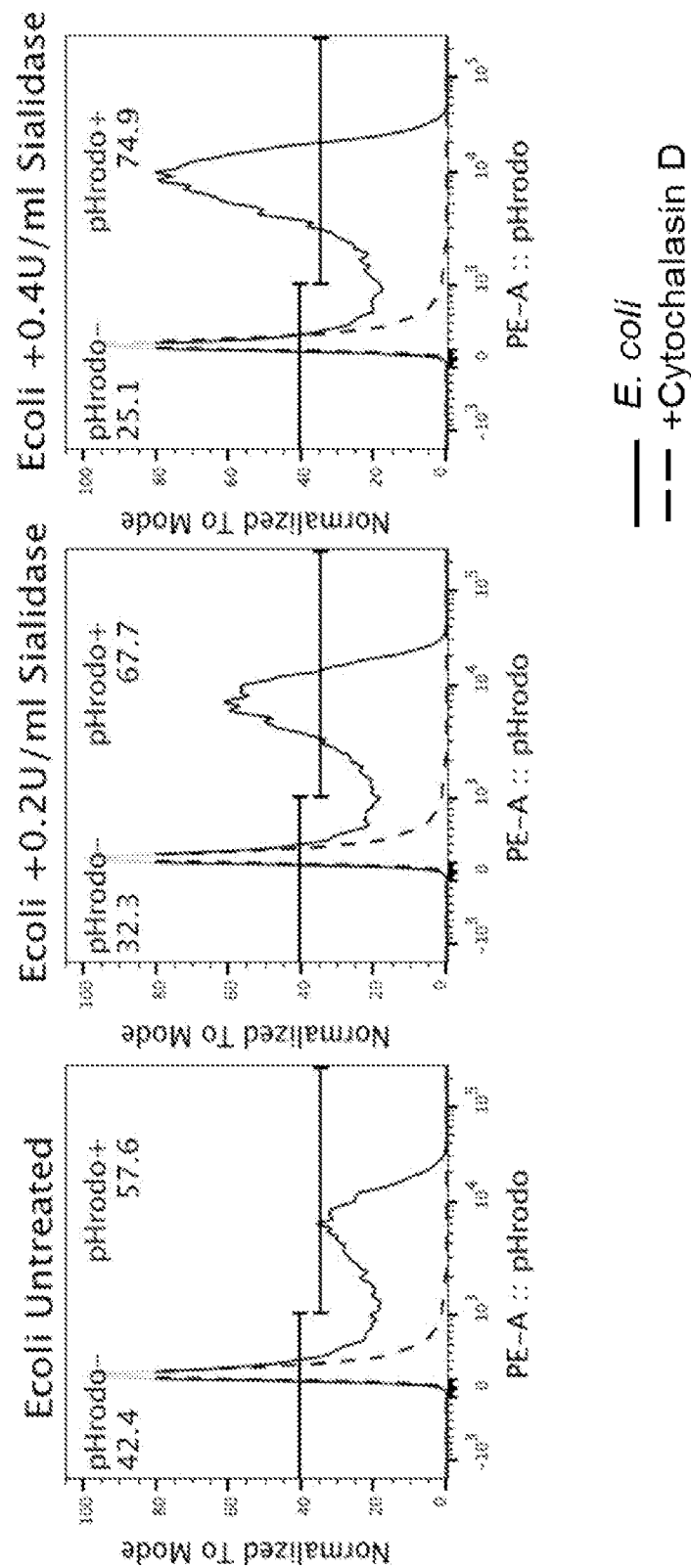


FIG. 11

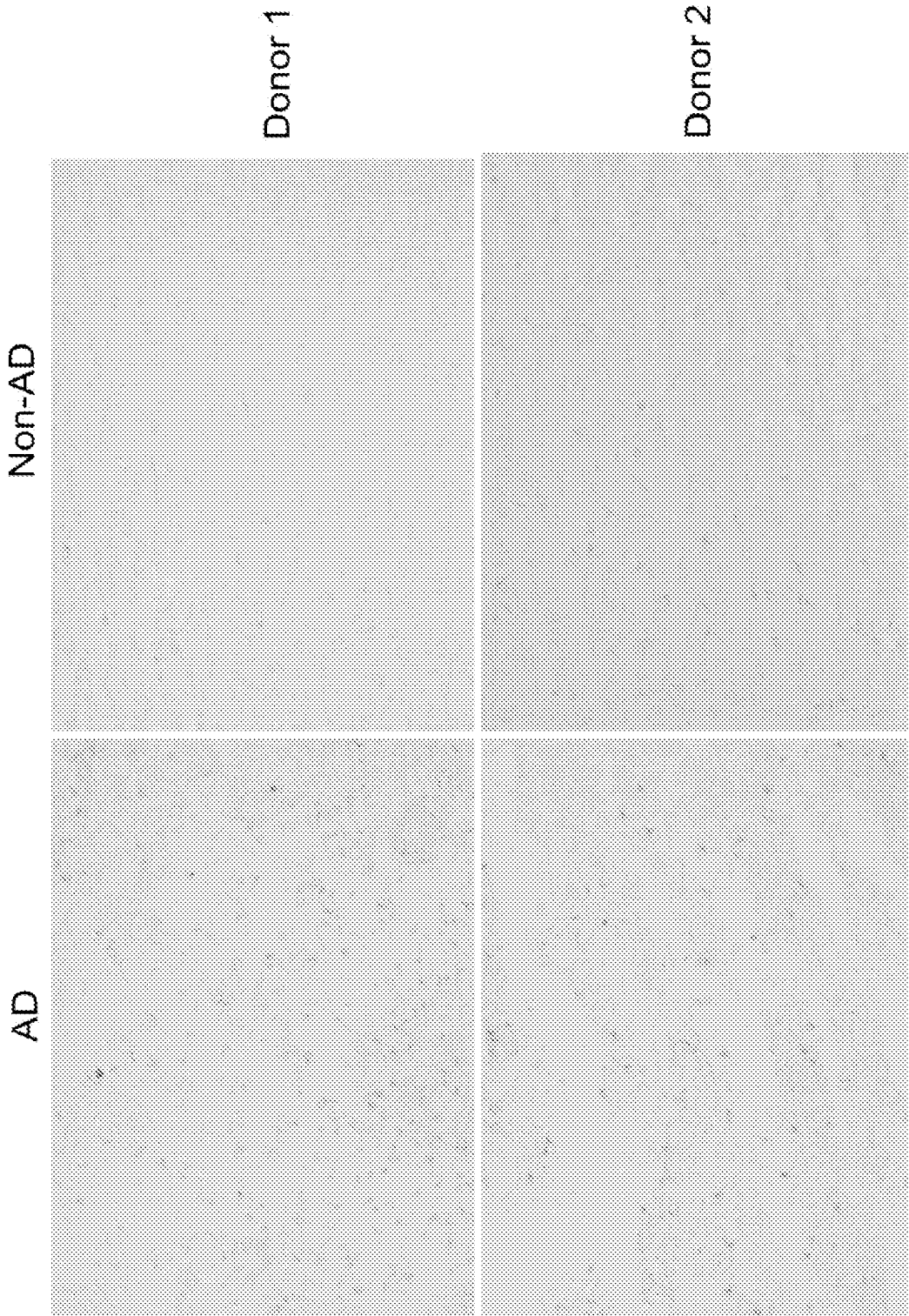


FIG. 12

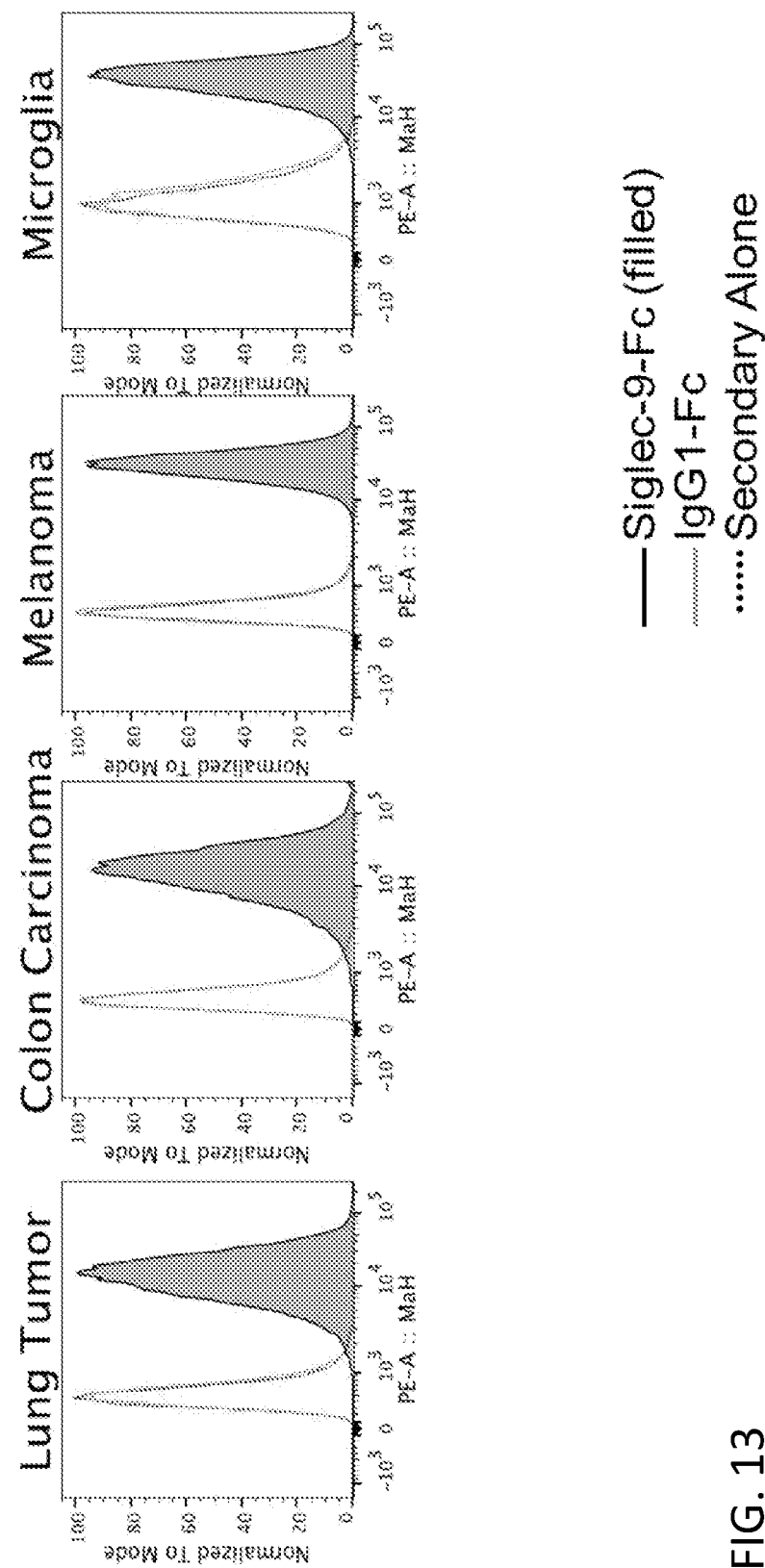


FIG. 13

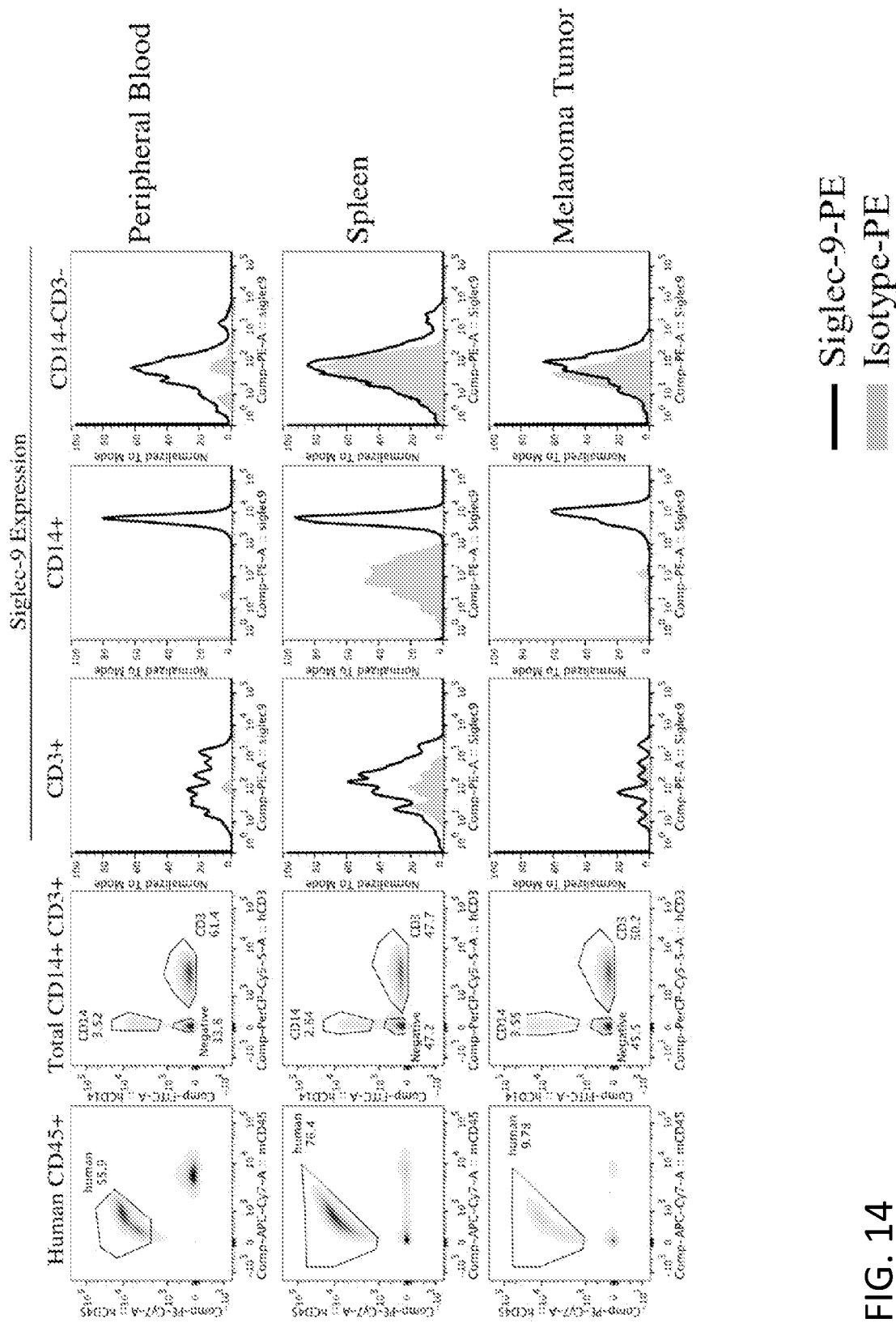


FIG. 14

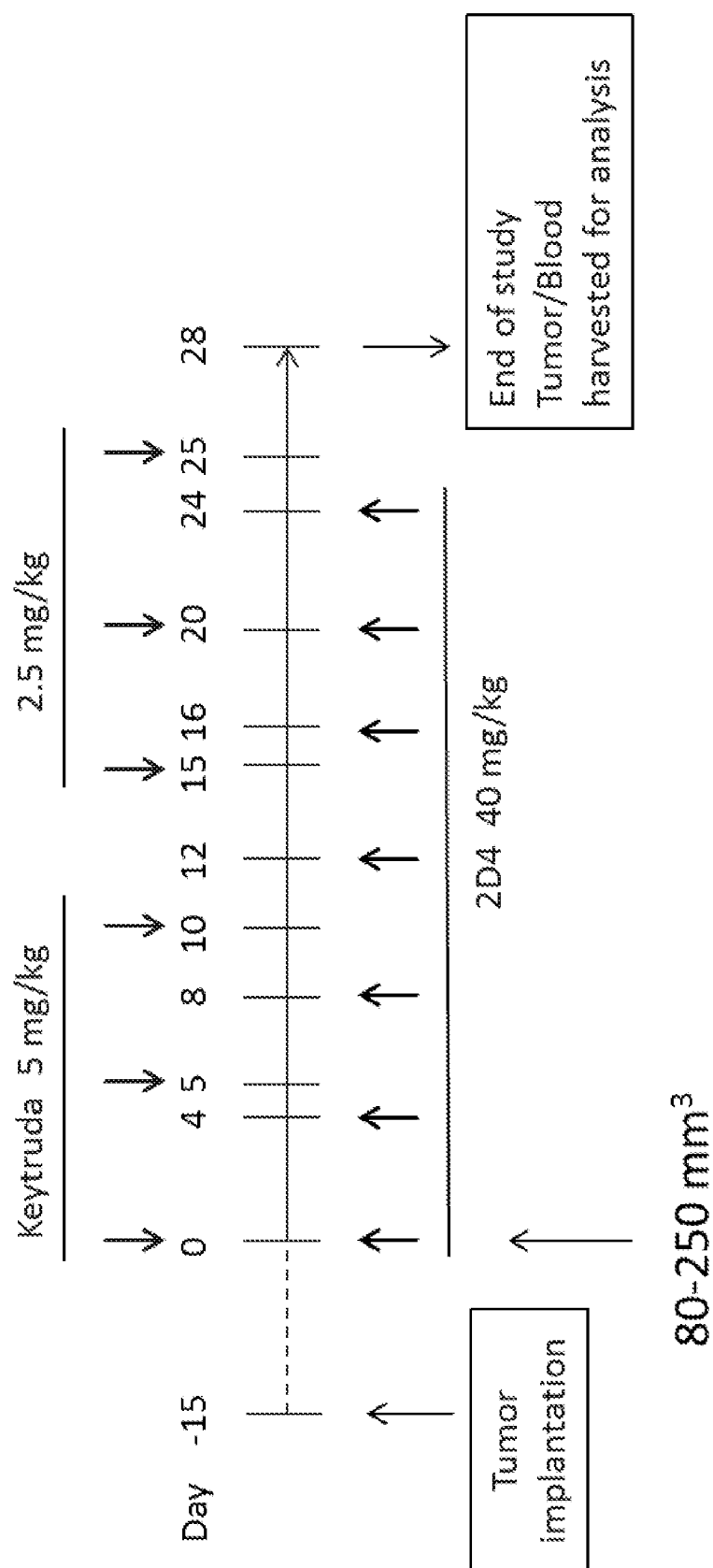


FIG. 15A

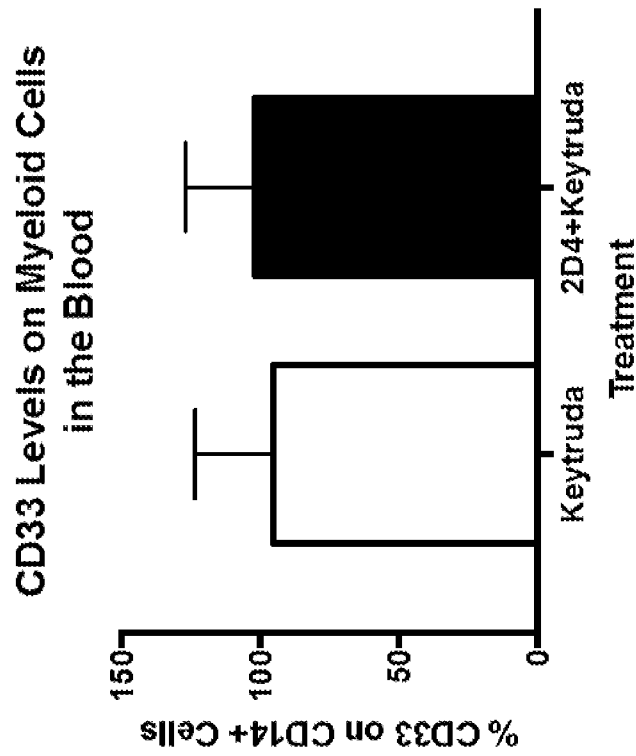


FIG. 15C

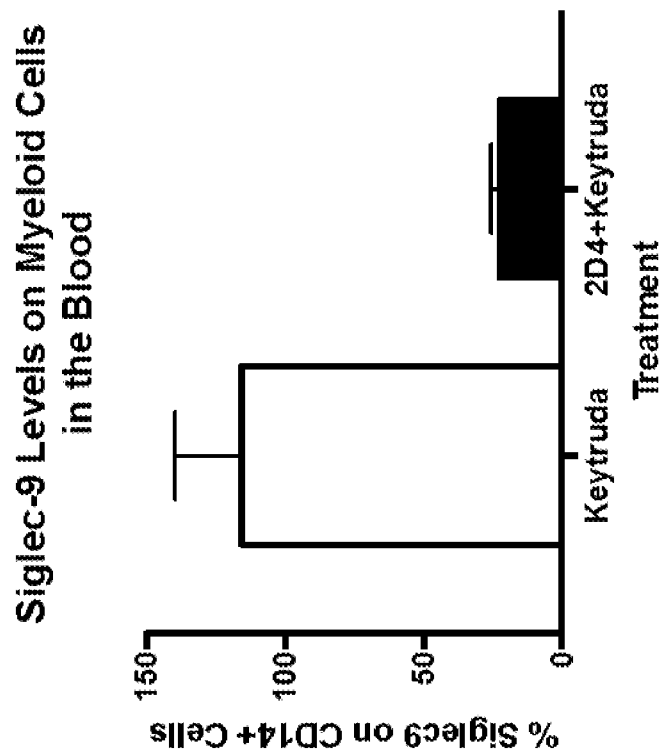


FIG. 15B

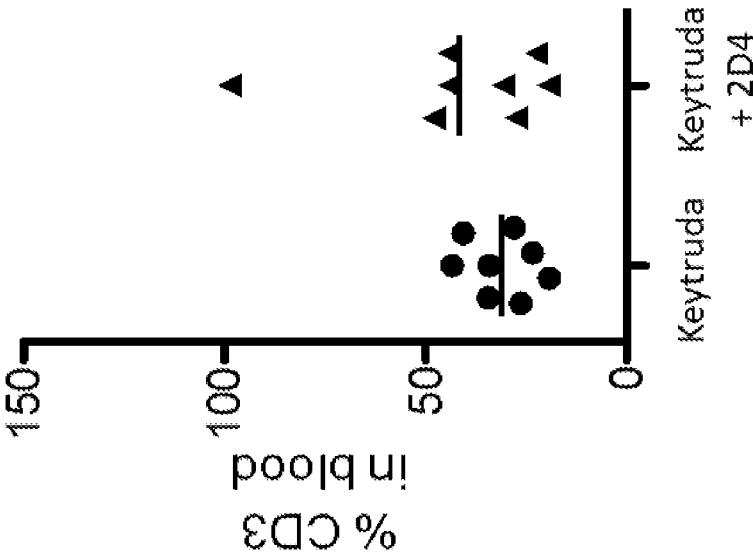


FIG. 15E

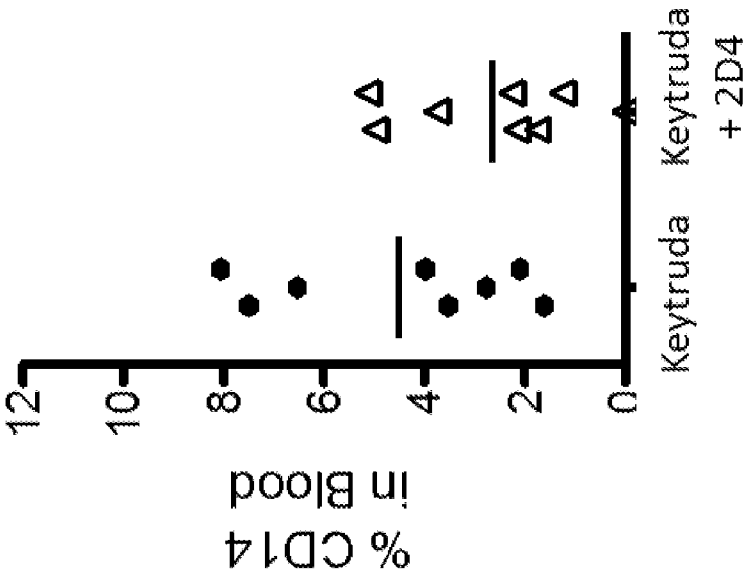


FIG. 15D

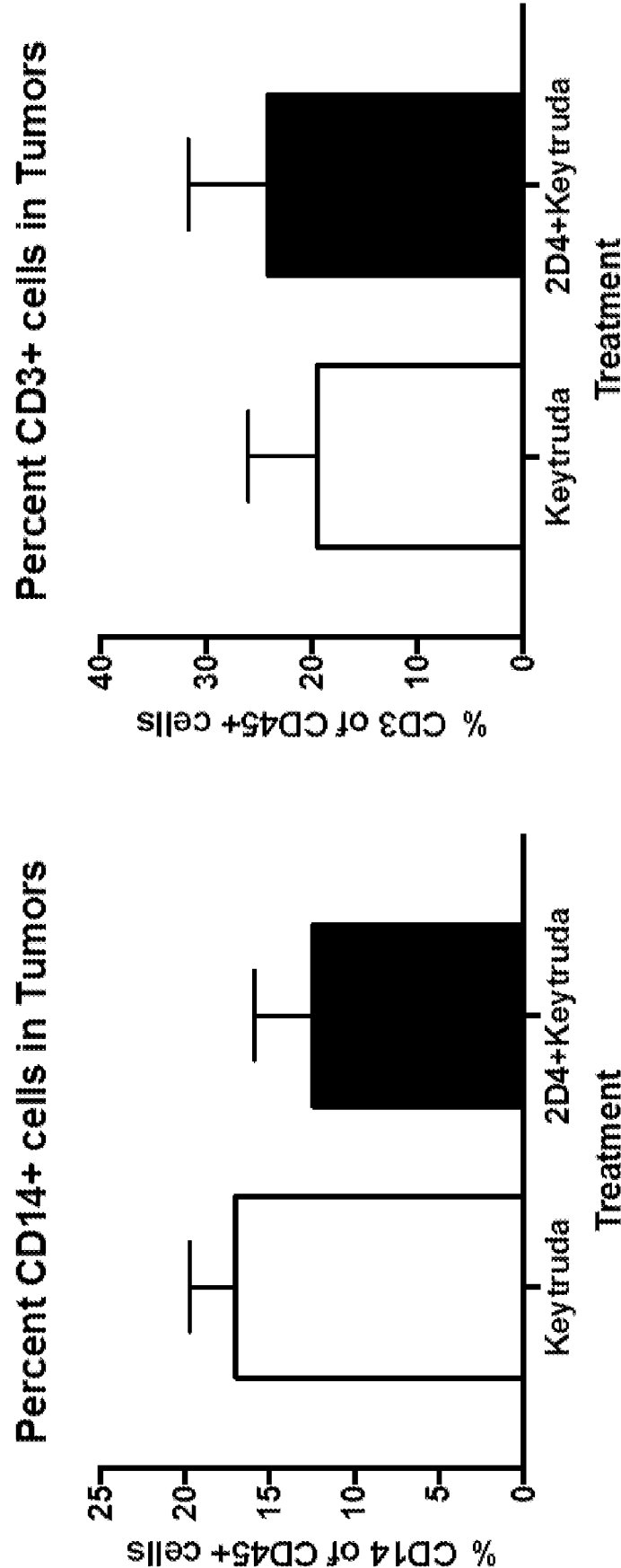


FIG. 15G

FIG. 15F

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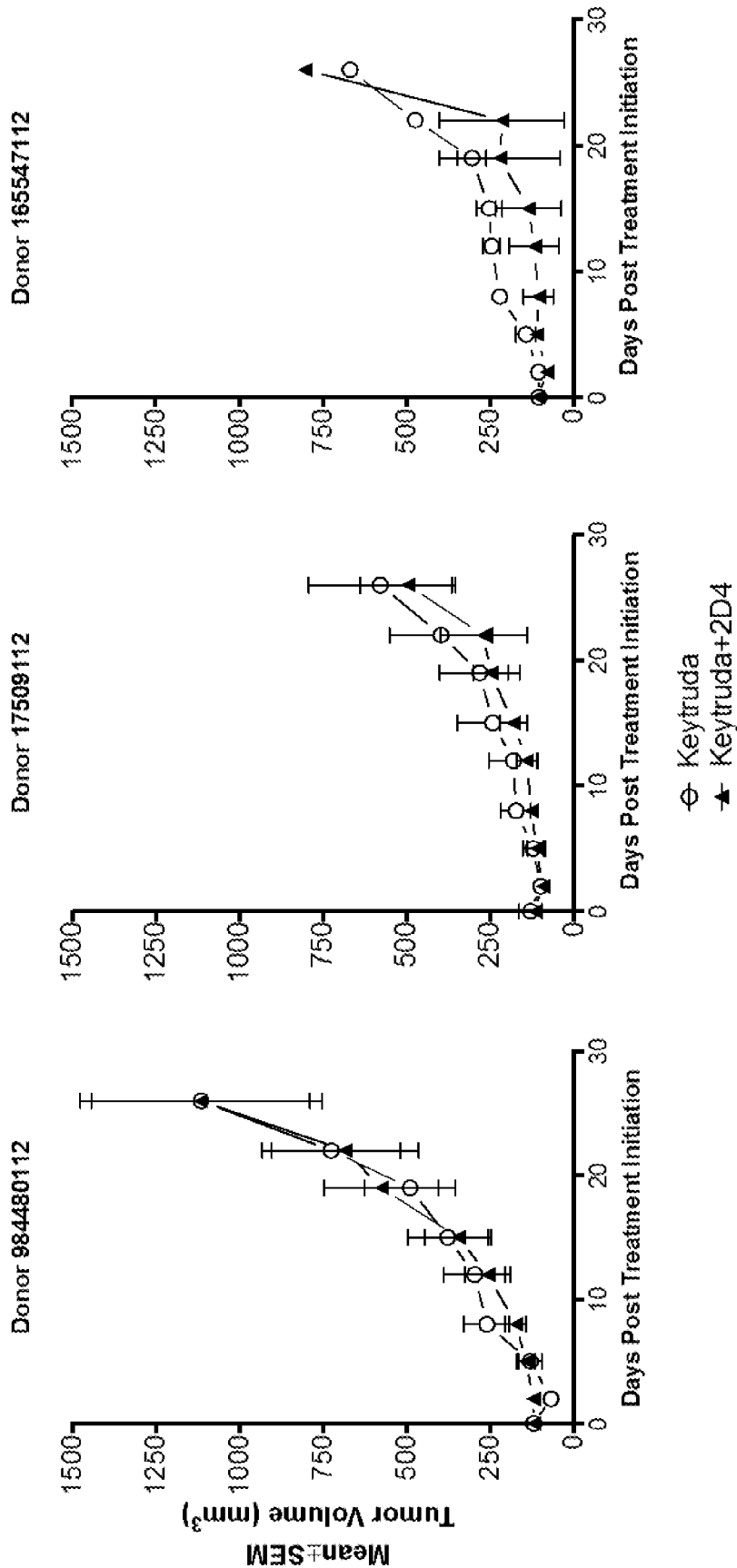


FIG. 15H

FIG. 15I

FIG. 15J