

Figure 1A

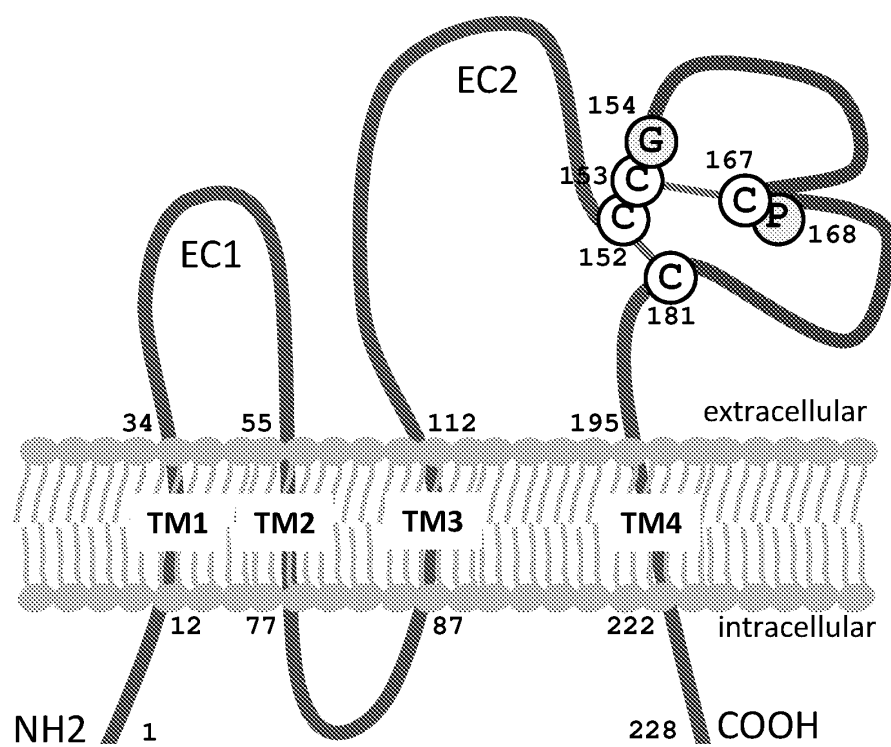


Figure 1B

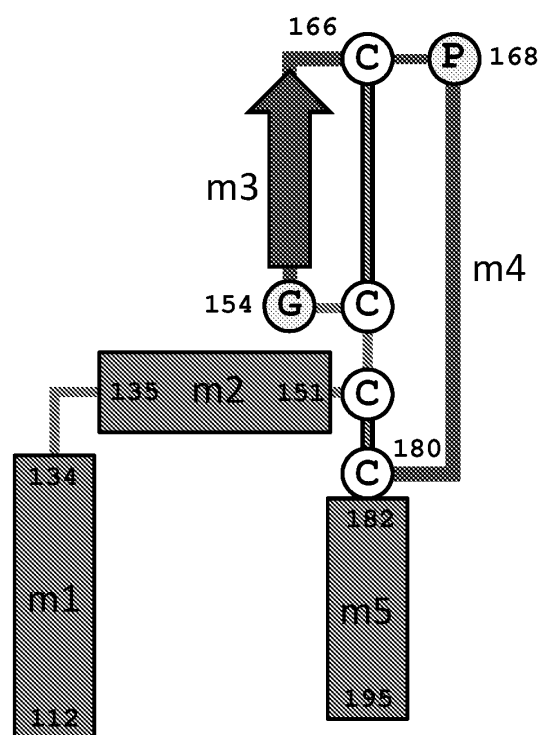


Figure 2

CD9 sequence (UNIPROT)

10	20	30	40	50
MPVKGGTKCI	KYLLFGFNFI	FWLAGIAVLA	IGLWLRFD SQ	TKSIFEQETN
60	70	80	90	100
NNSSFYTG V	YILIGAGALM	MLVGFLGCCG	AVQESQCMLG	LFFGFLLVIF
110	120	130	140	150
AIEIAAAIWG	YSHKDEVIKE	VQEFYKDTYN	KLTKDEPQR	ETLKAIHYAL
160	170	180	190	200
NCCGLAGGVE	QFISDICEPKK	DVLETFTVKS	CPDAIKEVFD	NKFHIIIGAVG
210	220			
IGIAVVMIEG	MIFSMILCCA	IRRNREMV		

Figure 3

NB: CDR numbering according to Kabat et al (1991)

AT14-012 (2H15-1A10)**Isotype IgG, Subtype IgG3, Light chain kappa****Variable region heavy chain**

Recombined from gene segments:

IGHV3-9*01

IGHD3-3*01

IGHJ4*02

AMINO ACID:

FW1 EVQVVESGGGLVQPGRSLRLSCAASGFTFD

CDR1 DYAMH

FW2 WVRQAPGKGLEWVS

CDR2 GISWNSGSIVYADSVKG

FW3 RFTISRDNAKNSLYLQLNSLRAEDTAFYYCAK

CDR3 AVSGYYPYFDY

FW4 WGQGILVTVSS

NUCLEOTIDE:

FW1 gaa gtg cag gtg gtg gag tct ggg gga ggc ttg gta cag cct ggc agg tcc ctg aga ctc tcc tgt
gca gcc tct gga ttc acc ttt gat

CDR1 gat tat gcc atg cac

FW2 tgg gtc cgg caa gct cca ggg aag ggc ctg gag tgg gtc tca

CDR2 ggt att agt tgg aat agt ggt agc ata gtc tat gcg gac tct gtg aag ggc

FW3 cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg caa ctg aac agt ctg aga ggt
gag gac acg gcc ttc tat tac tgt gca aaa

CDR3 gcc gtg agt ggt tat tat ccc tac ttt gac tac

FW4 tgg ggc cag gga att ttg gtc acc gtc tcc tca

Variable region light chain

Recombined from gene segments:

IGKV4-1*01

IGKJ5*01

AMINO ACID:

FW1 DIVMTQSPDLSLVSLGERATINC

CDR1 KSSQSVLYSSNNKNYL

FW2 WYQQKPGQPPKLLIY

CDR2 WASTRES

FW3 GVPDRFSGSGSGTDFTLTISLQAEDVAVYYC

CDR3 QQYYTTP

FW4 STFGQGTRLEIK

NUCLEOTIDE:

FW1 gac atc gtg atg acc cag tct cca gac tcc ctg tct gtg tct ctg ggc gag agg gcc acc atc aac
tgc

CDR1 aag tcc agc cag agt gtt tta tac agc tcc aac aat aag aac tac tta ggt

FW2 tgg tac cag cag aaa cca gga cag cct cct aag ctg ctc att tac

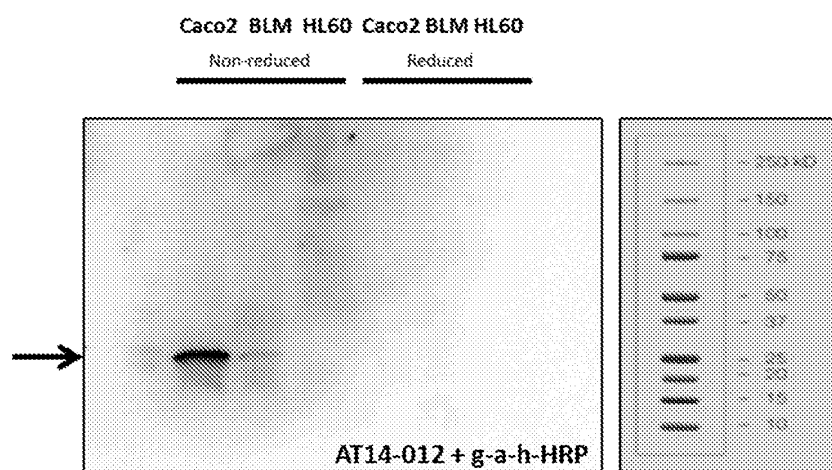
CDR2 tgg gca tct acc cgg gaa tcc

FW3 ggg gtc cct gac cga ttc agt ggc agc ggg tct ggg aca gat ttc act ctc acc atc agc agc ctg
cag gct gaa gat gtg gca gtt tat tac tgt

CDR3 cag caa tat tat act act cct

FW4 tcc acc ttc ggc caa ggg aca cga ctg gag att aaa

Figure 4A



TritonX114 lysis (50 ug of lysate on gel)
AT14-012 @ 0.5 ug/ml (1 hr)
Goat-anti-human-IgG-HRP 1:10.000 (0.5 hr)

Figure 4B

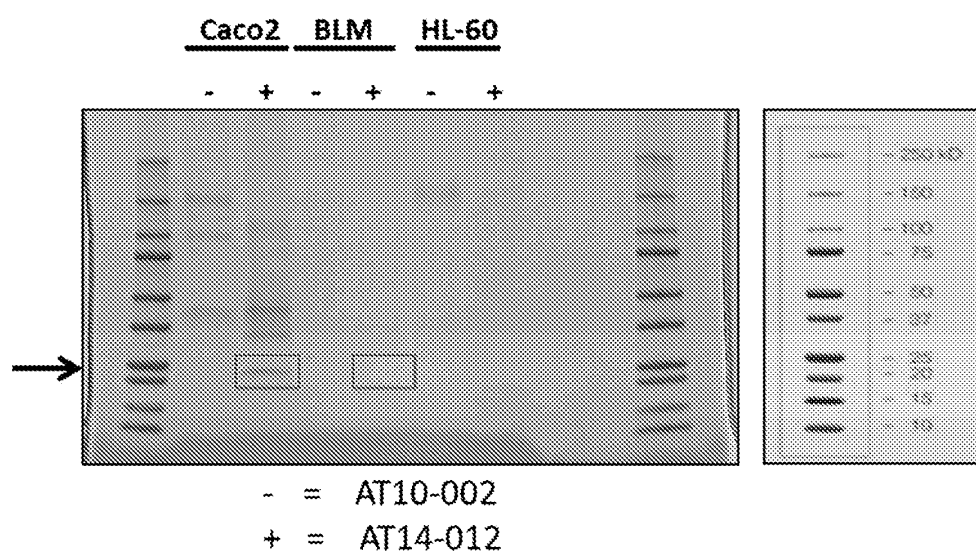


Figure 4C

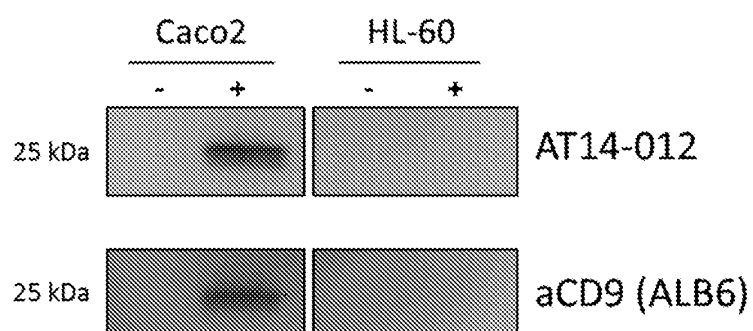


Figure 5A

Epitope mapping (FACs):
CD9/CD81 hybrid mutants EC1 – EC2 – EC2m1 – EC2m2 – EC2m3 – EC2m4 – EC2m5

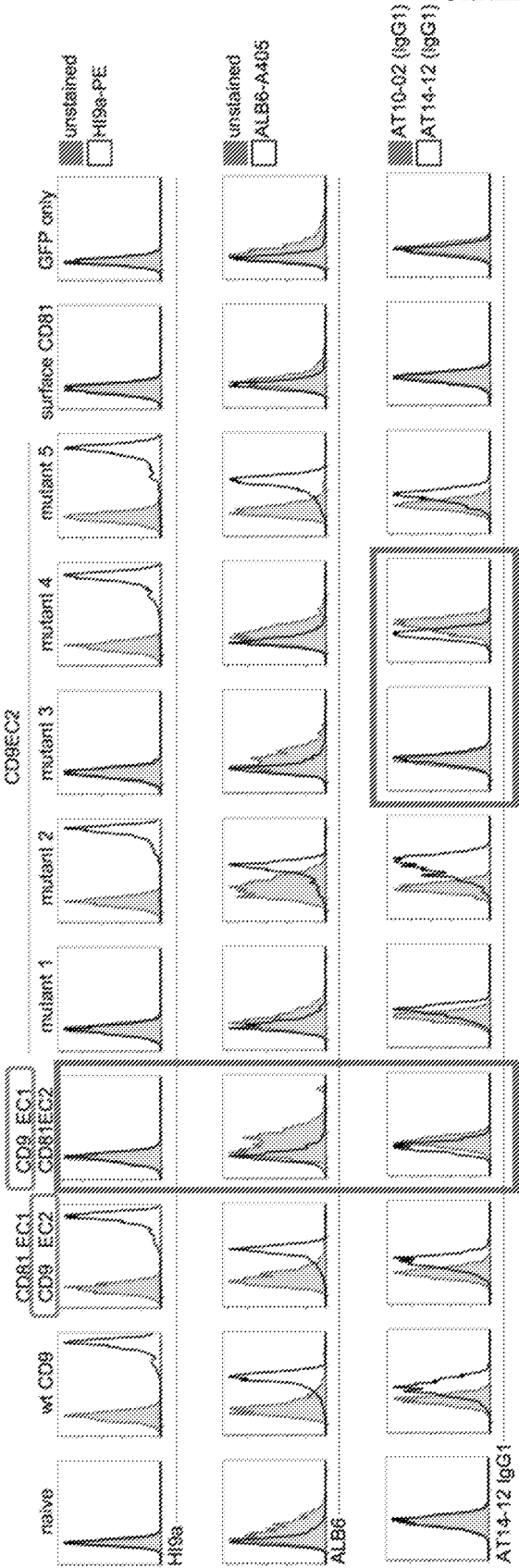


Figure 5B

Epitope mapping (FACs):
CD9 EC2m4 alanine mutants

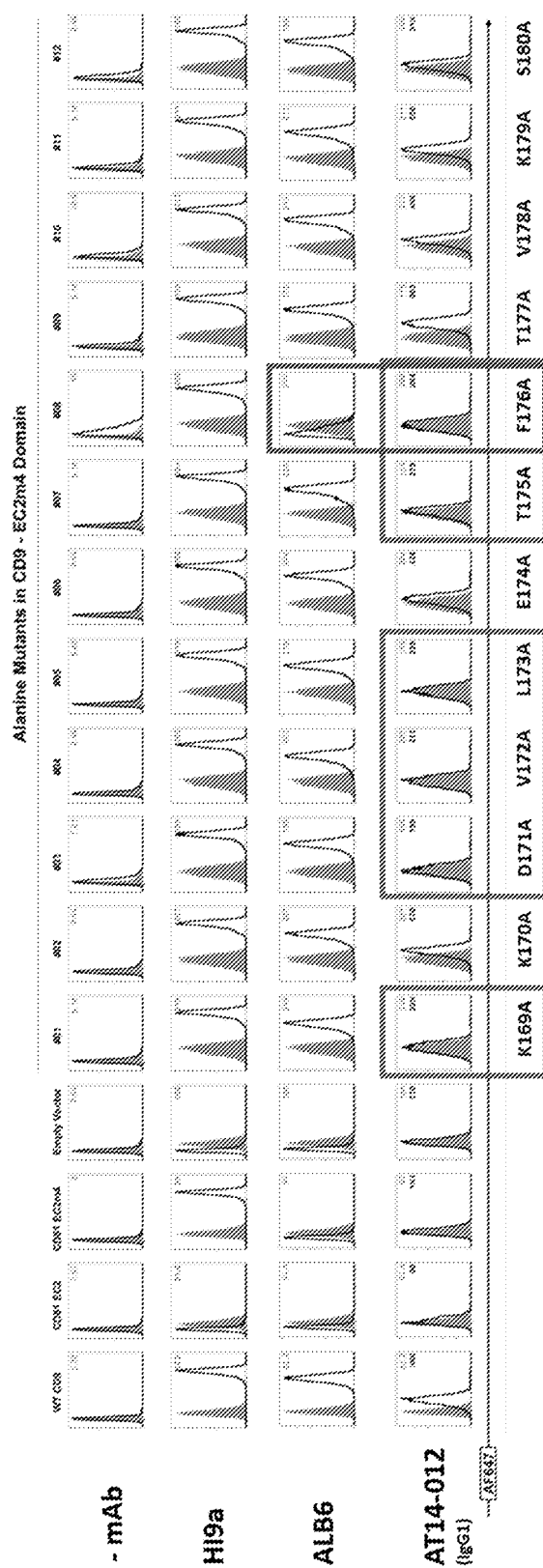


Figure 6A

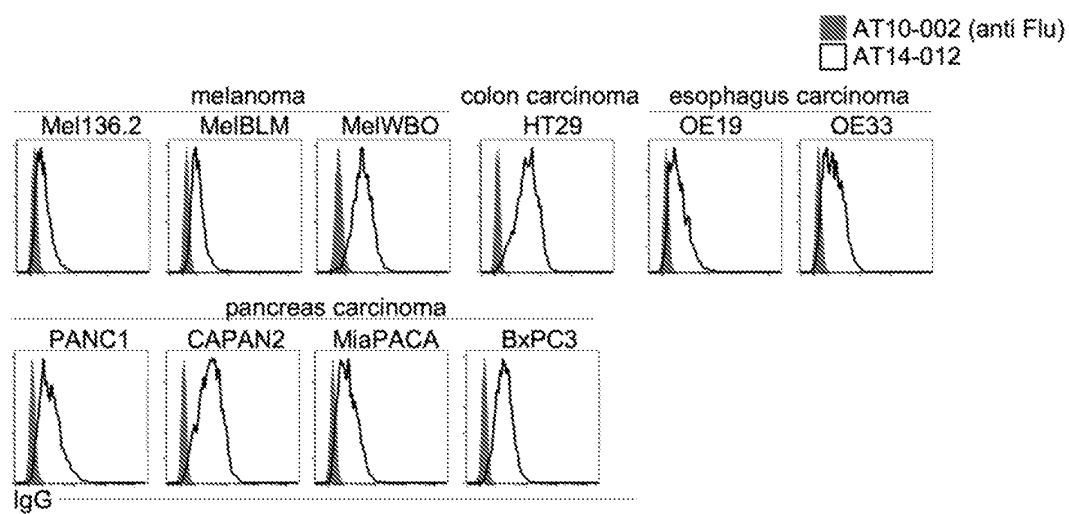


Figure 6B

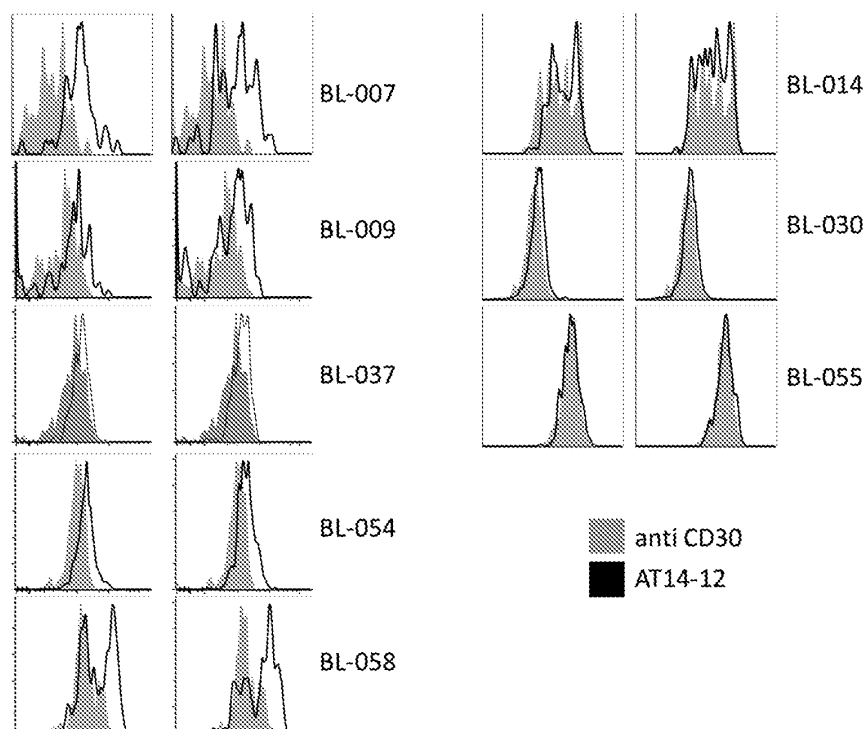


Figure 6C

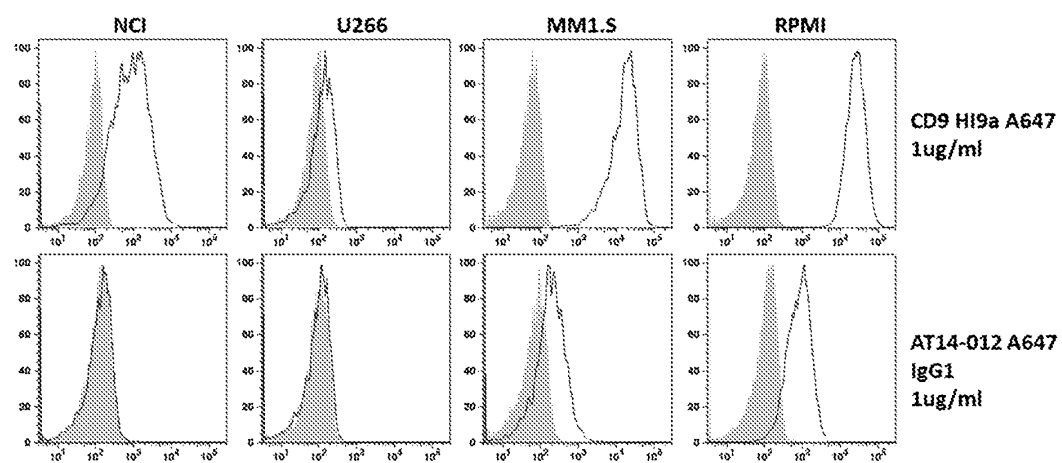


Figure 7A

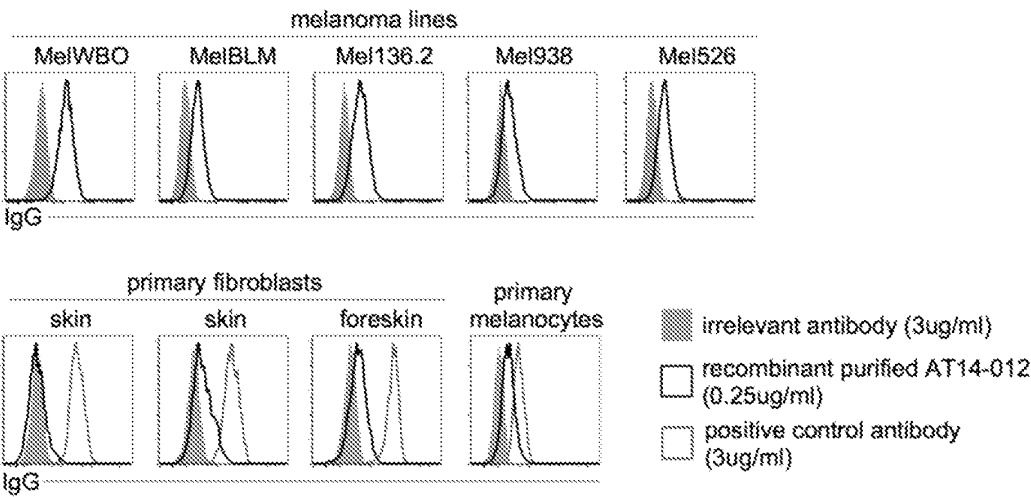


Figure 7B

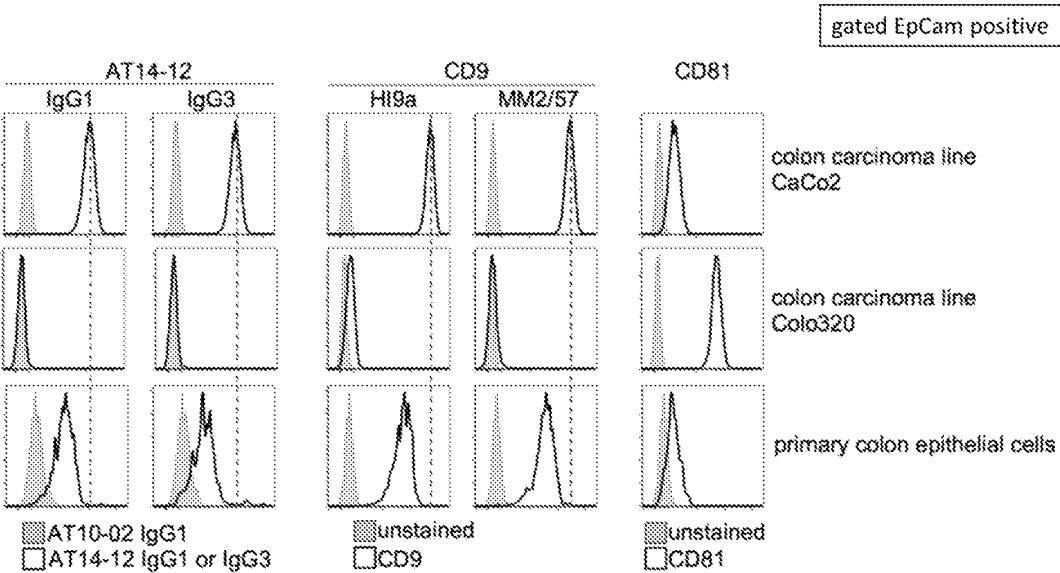


Figure 7C

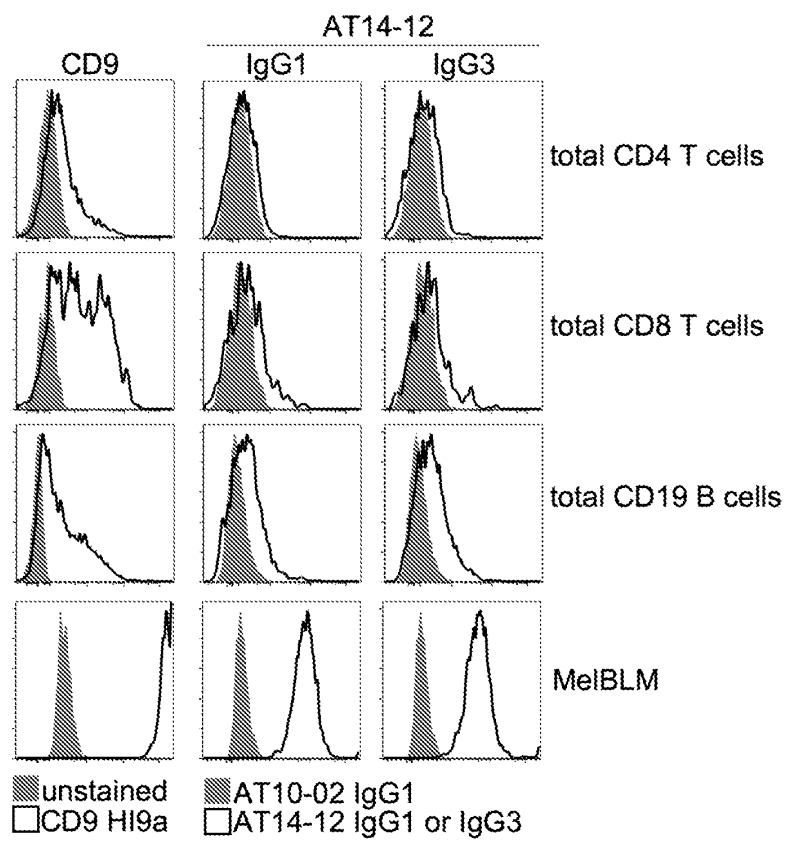


Figure 8A

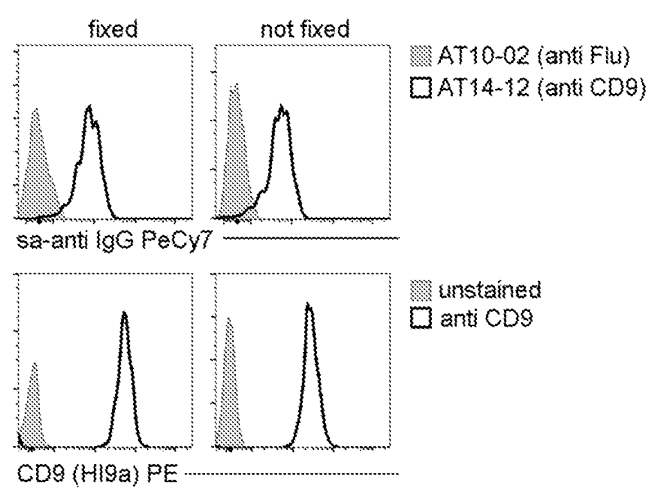


Figure 8B

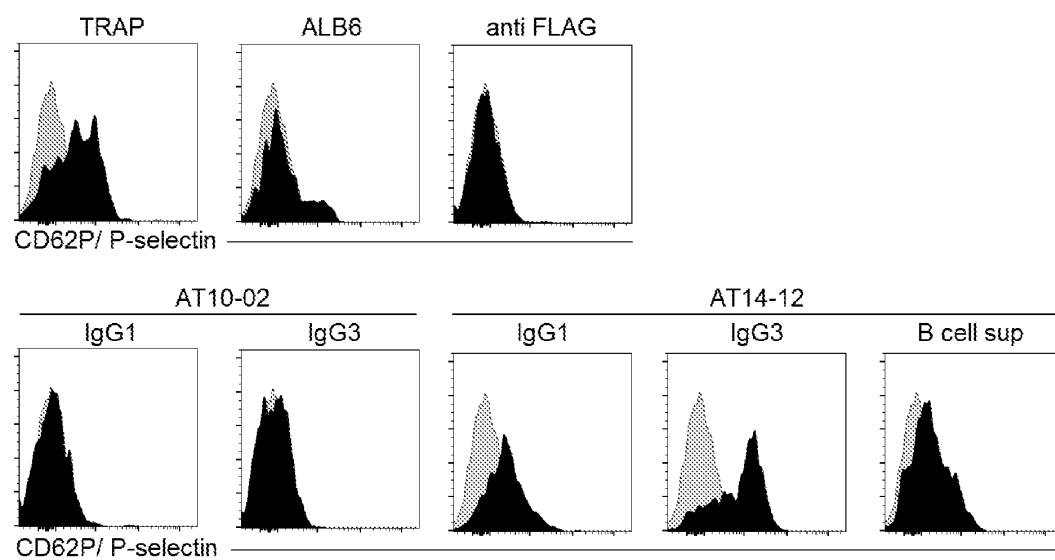


Figure 8C

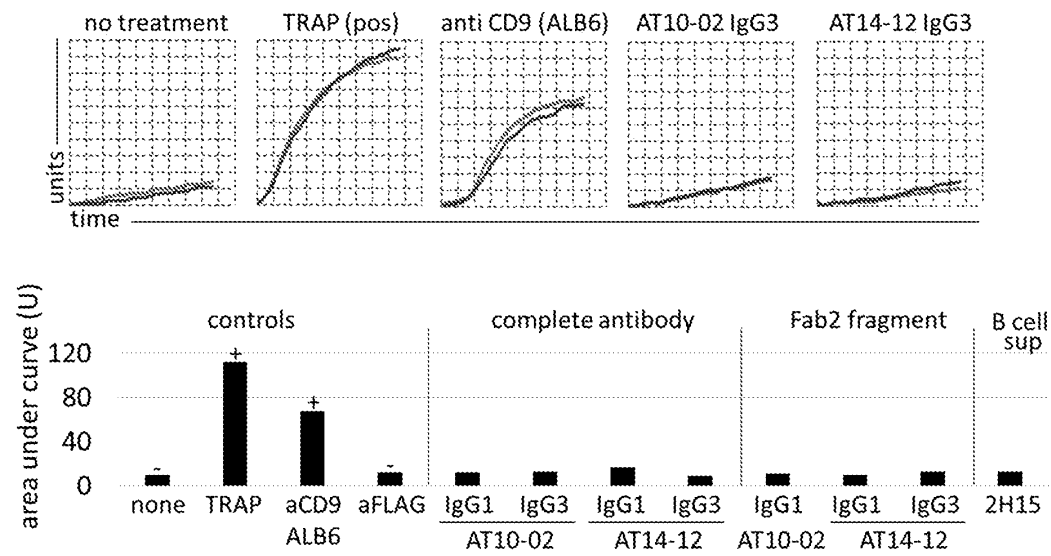


Figure 9A

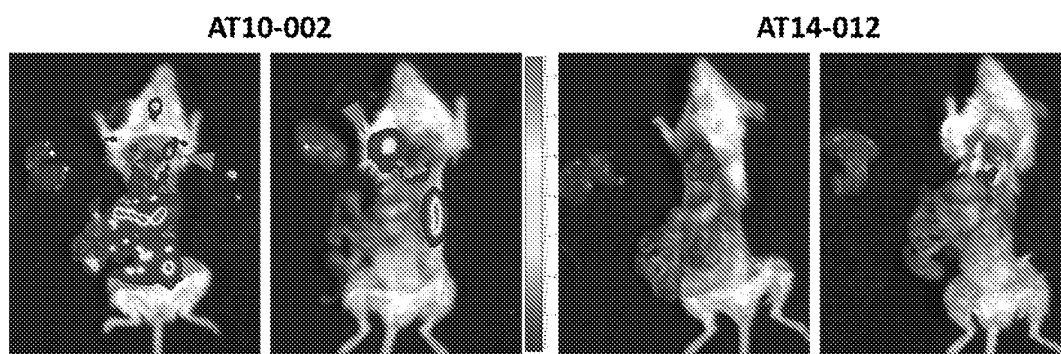


Figure 9B

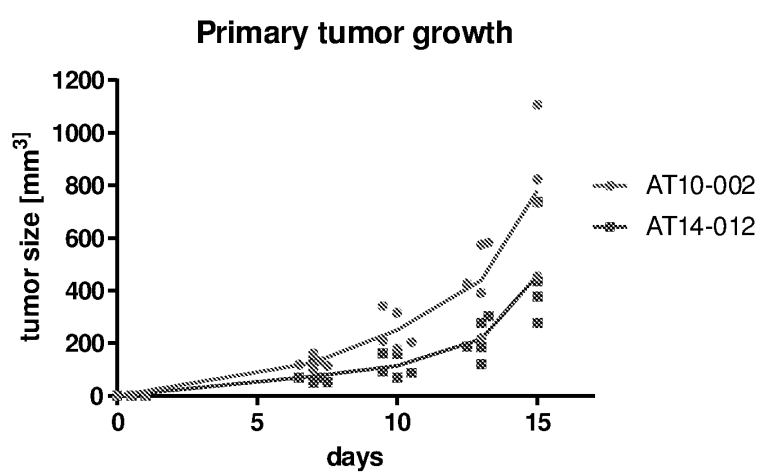


Figure 9C

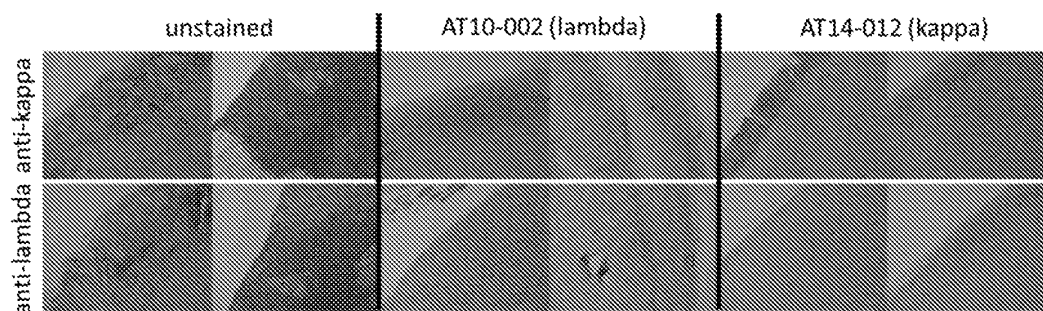


Figure 9D

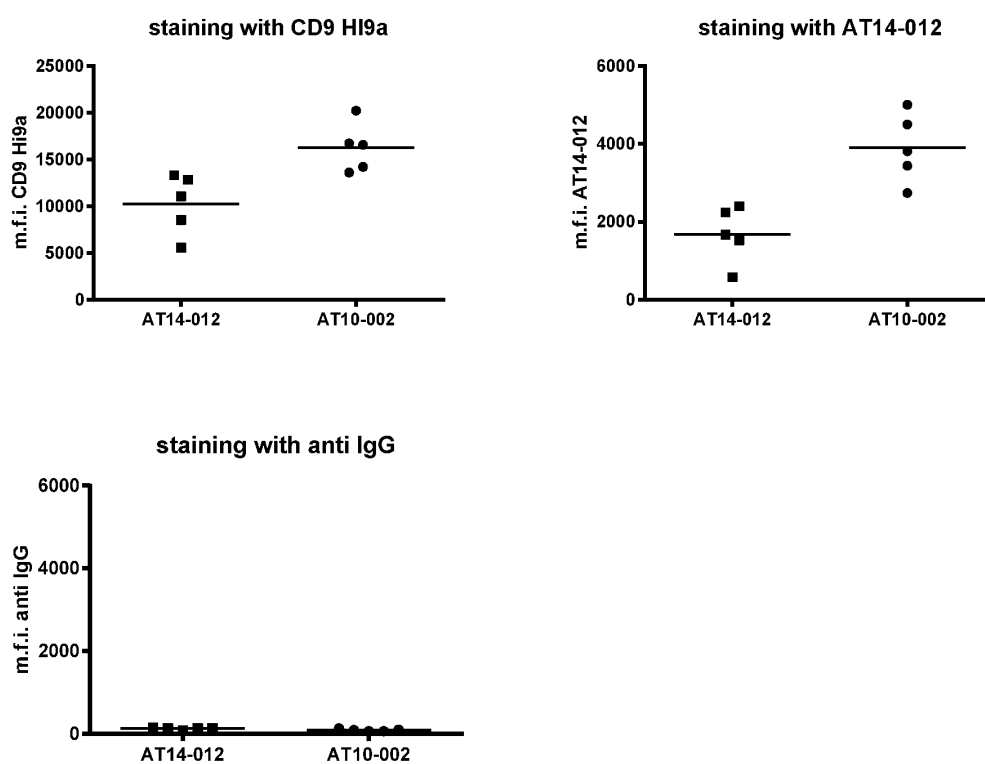


Figure 9E-G

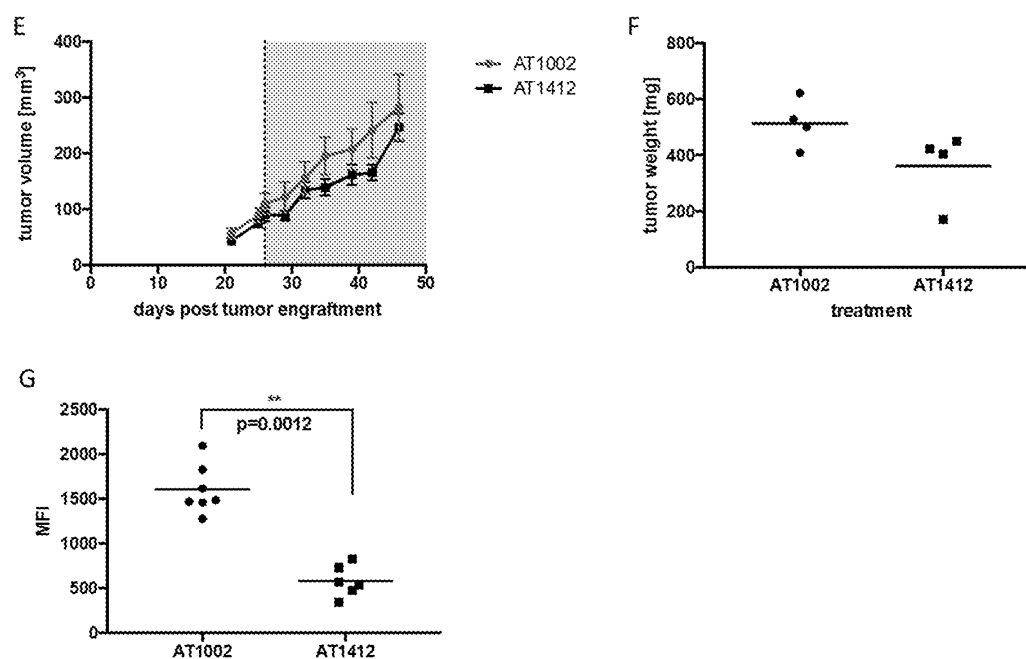


Figure 10A

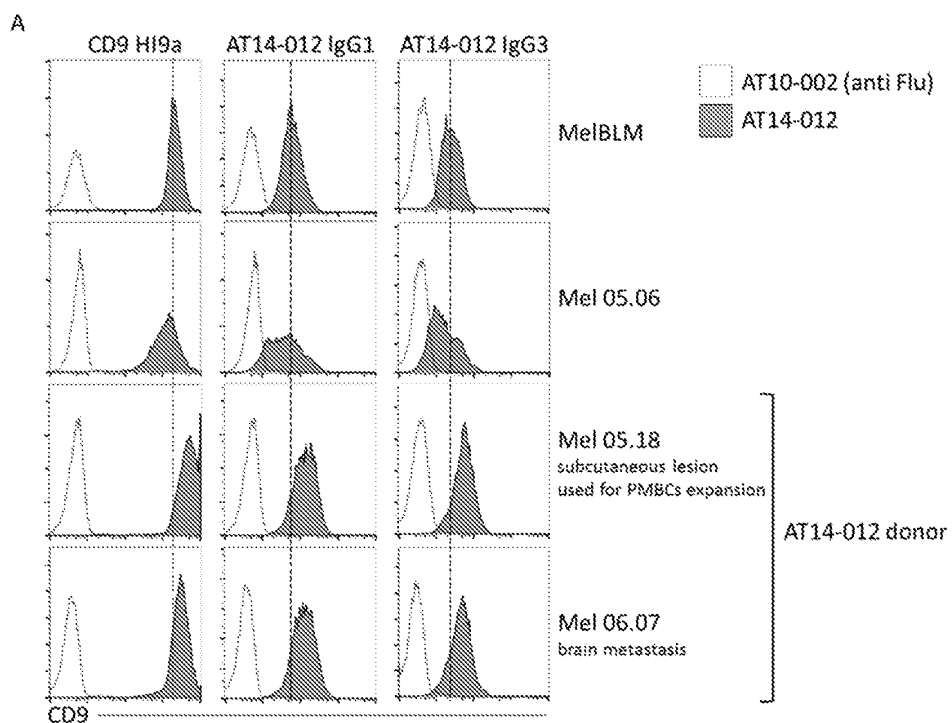


Figure 10B

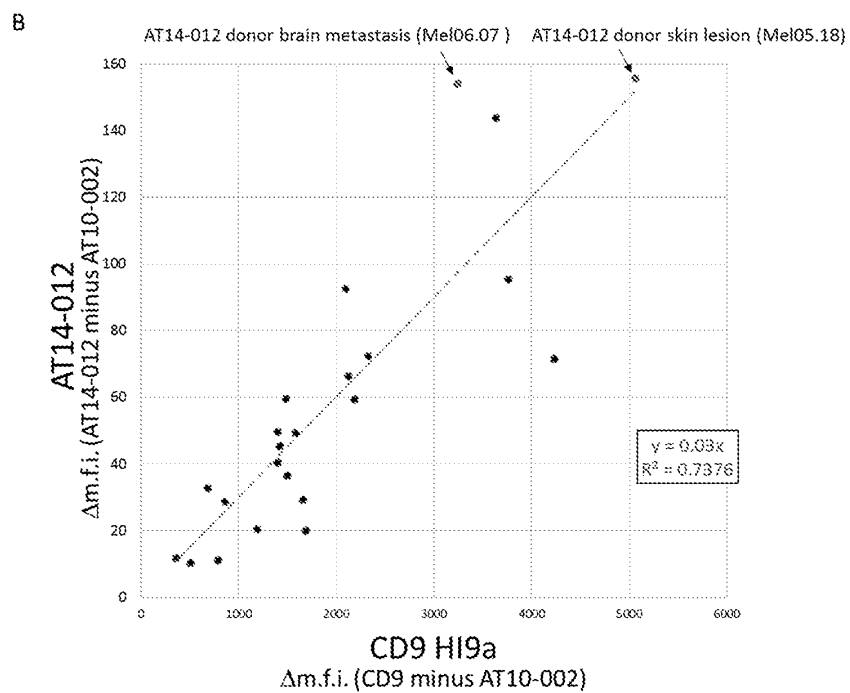


Figure 10C

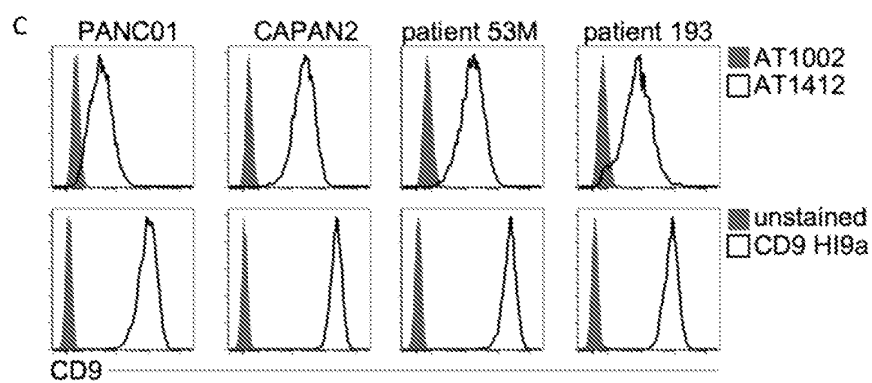


Figure 11A

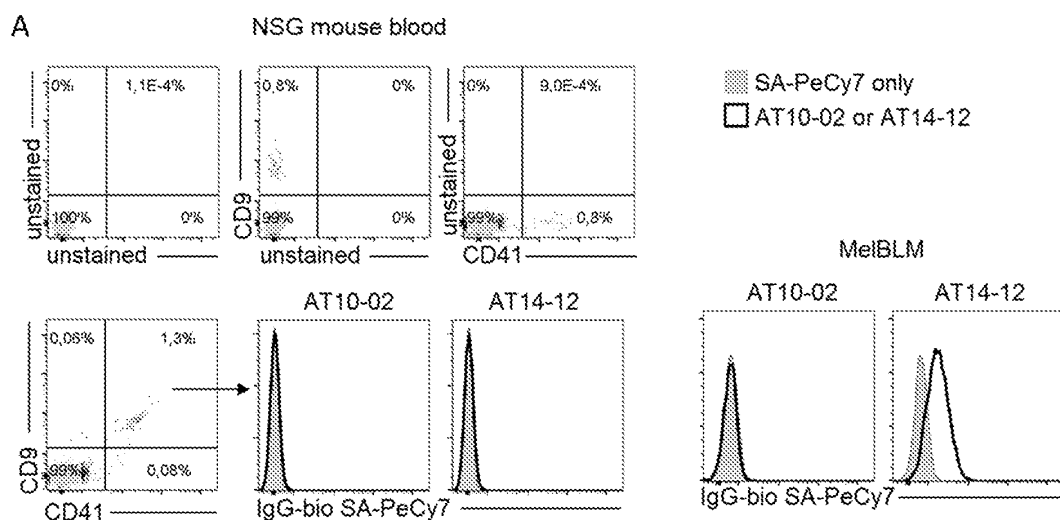


Figure 11B

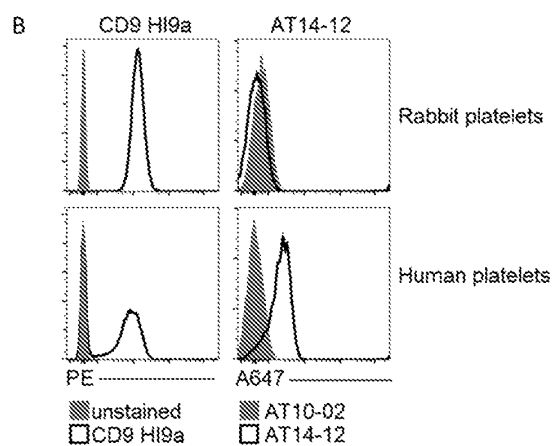


Figure 11C

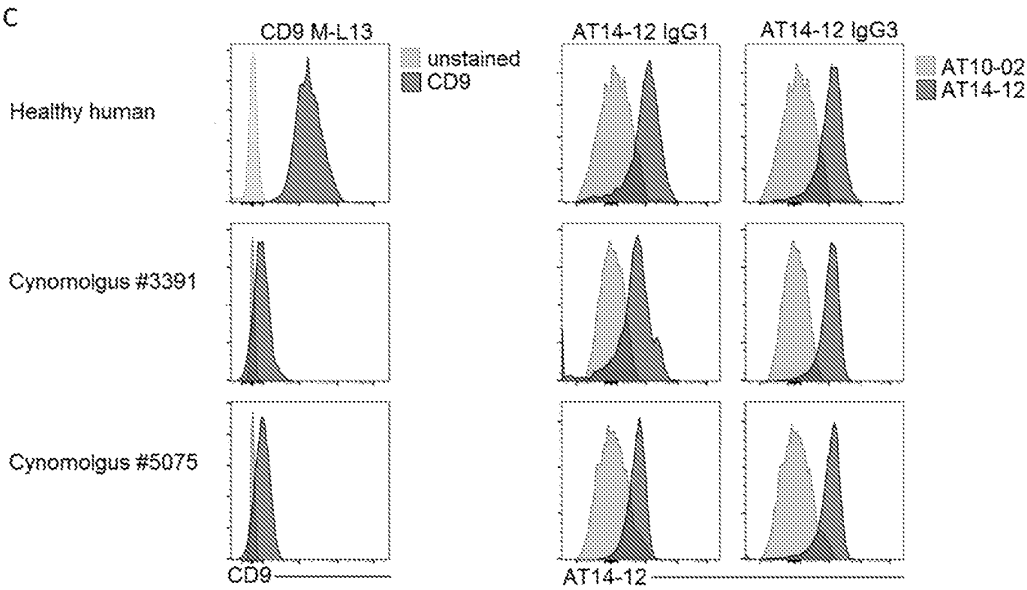


Figure 12A

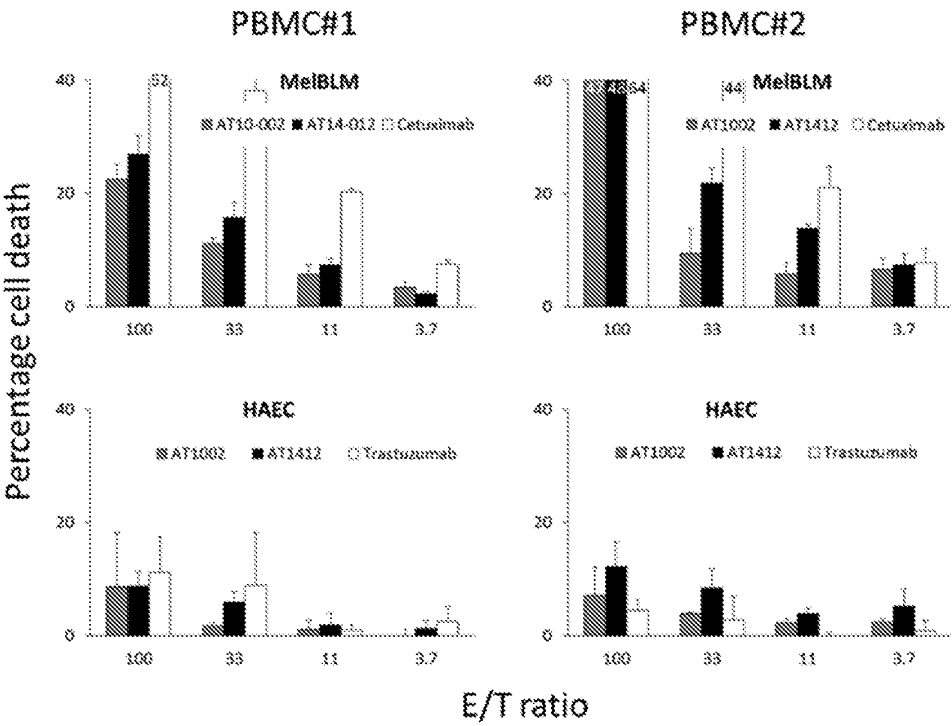


Figure 12B

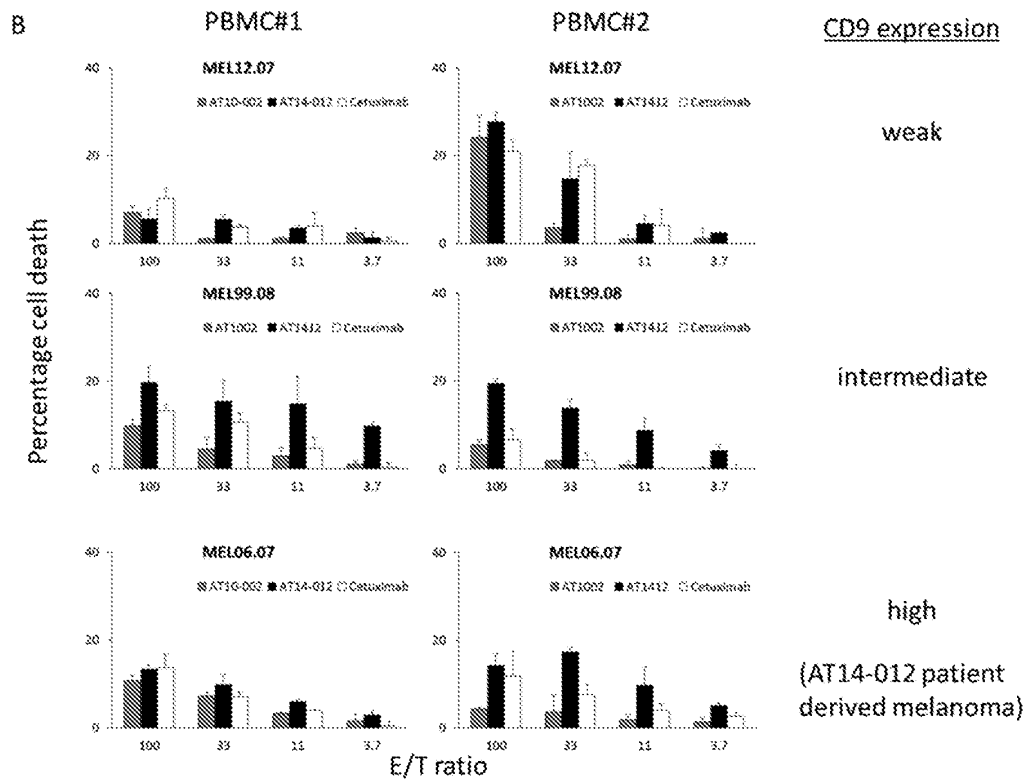


Figure 13

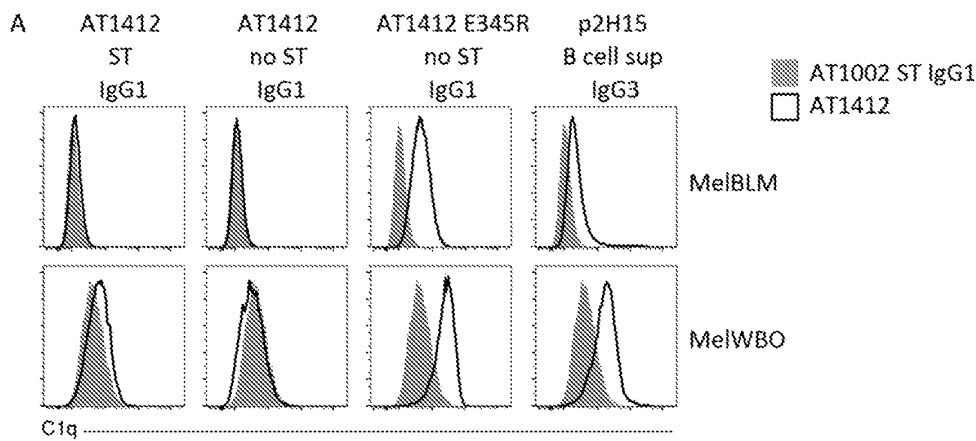


Figure 13B

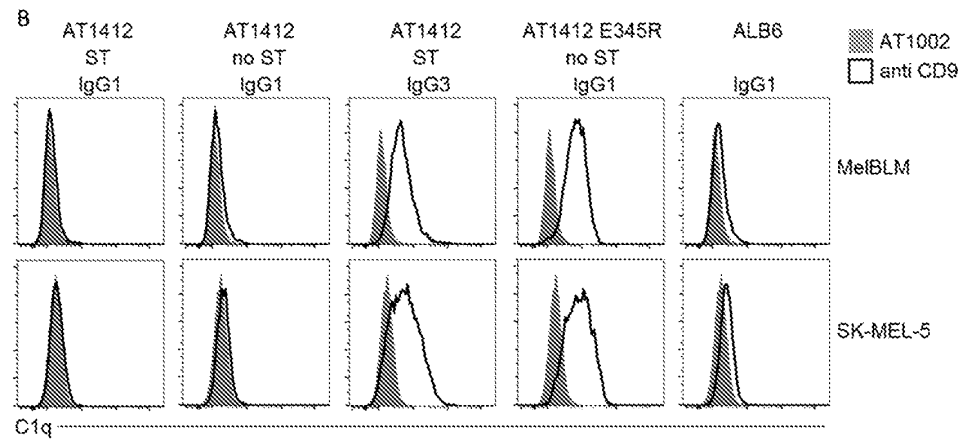


Figure 13C

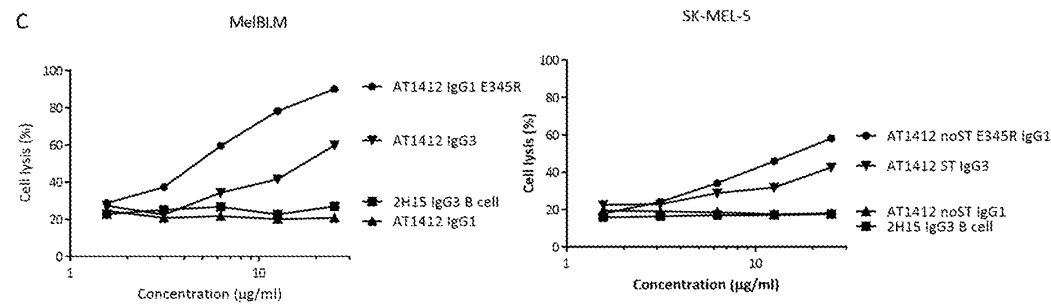


Figure 13D

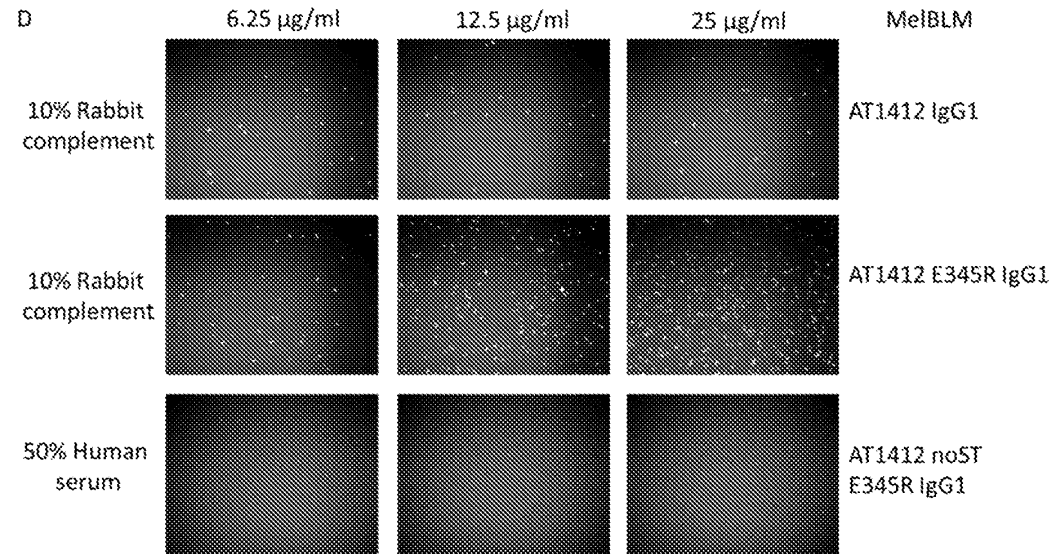


Figure 13E

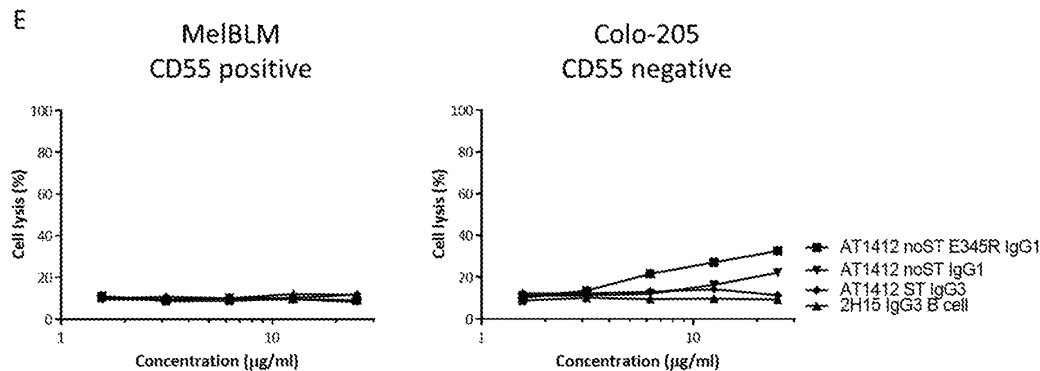


Figure 14A

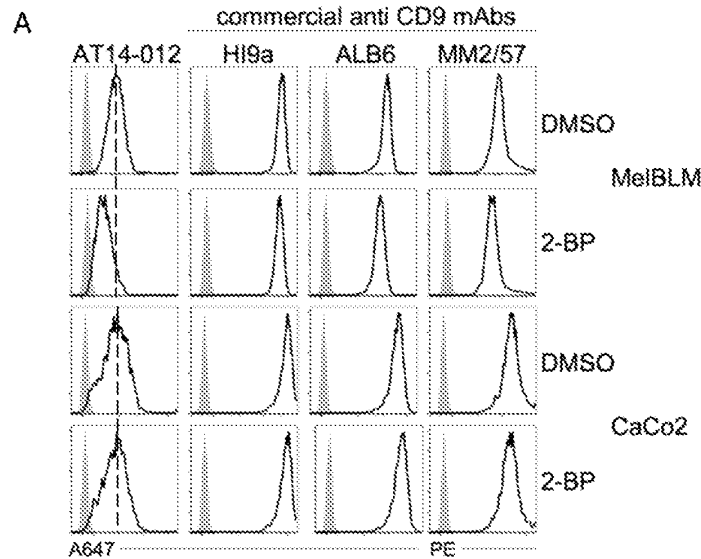


Figure 14B

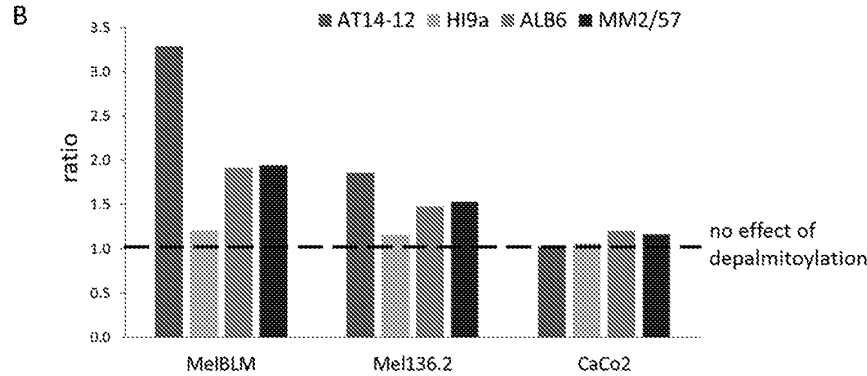
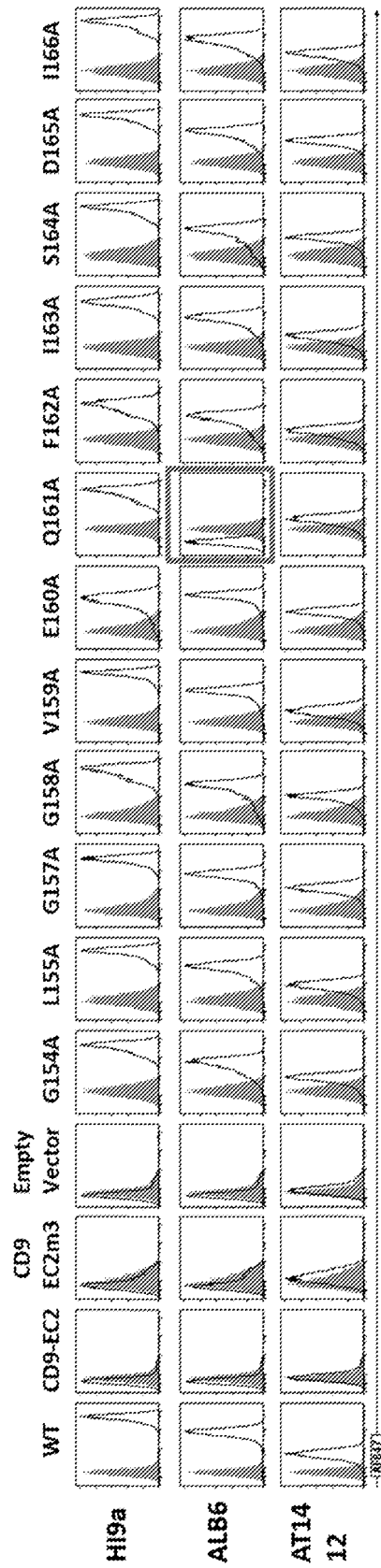


Figure 15

WO 2017/119811

PCT/NL2017/050003



153 154 155 156 157 158 159 160 161 162 163 164 165 166 167
ALB6 C G L A G G V E Q Q F I S D I C
AT1412 C G L A G G V E Q Q F I S D I C

Figure 16A

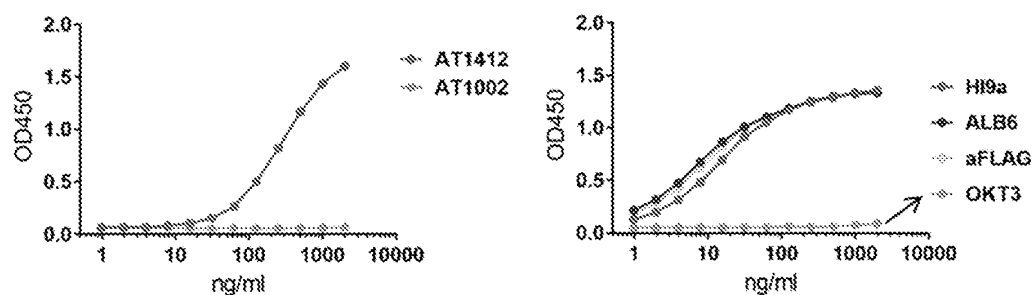


Figure 16B

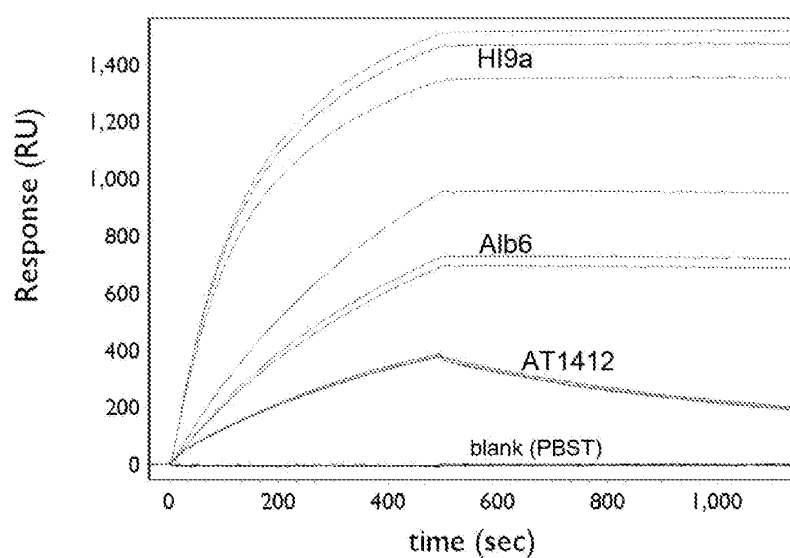


Figure 16C

Antibody:	k_a	k_d	K_D
AT1412	1.62 ($\pm$ 0.52)	71.0 ($\pm$ 20.6)	44,300 ($\pm$ 1,500)
HI9a	43.0 ($\pm$ 2.1)	0.1 *	2.33 ($\pm$ 0.11)
ALB6	15.1 ($\pm$ 1.3)	2.16 ($\pm$ 0.30)	145 ($\pm$ 8)

Figure 17A

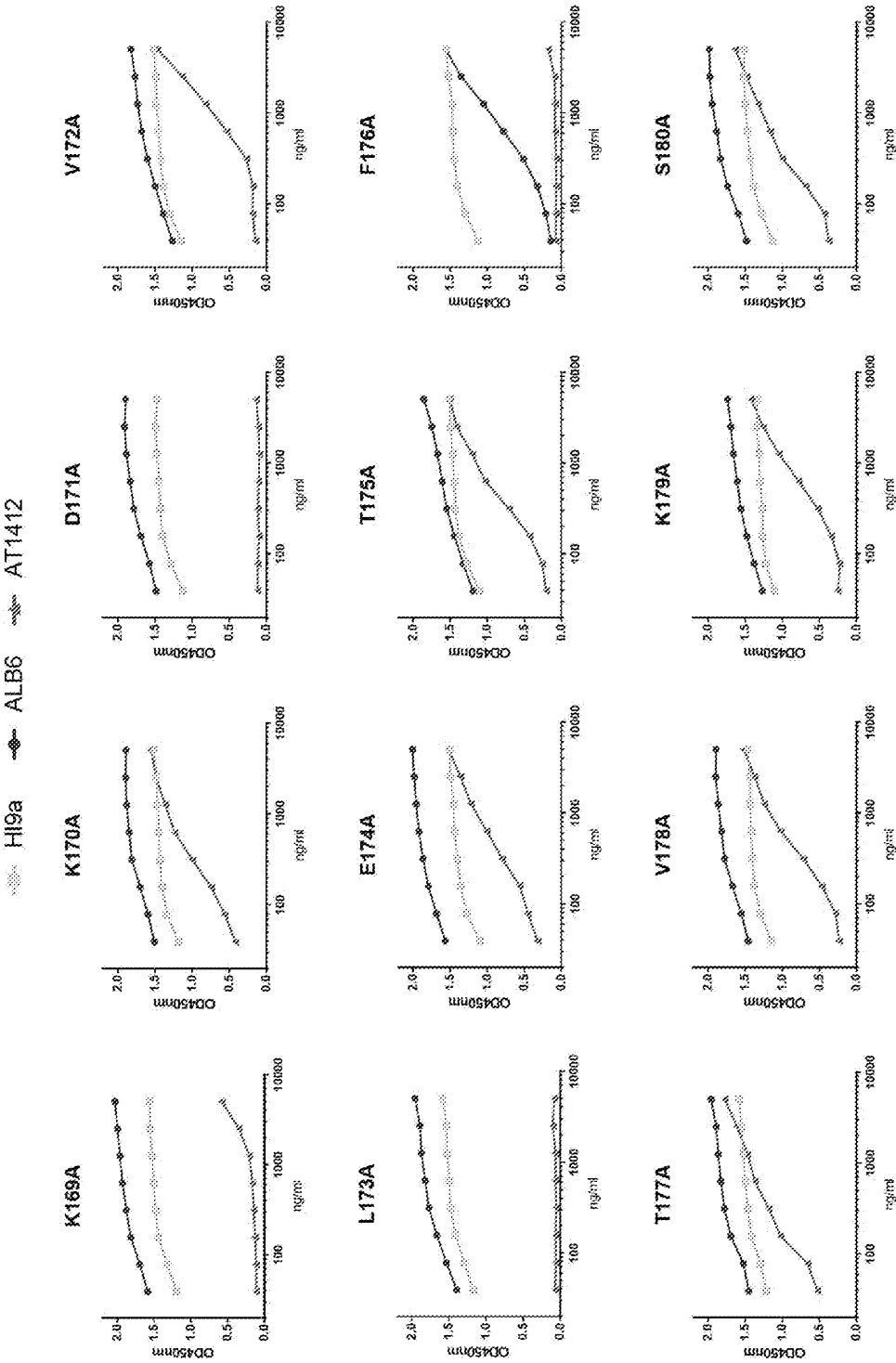


Figure 17B

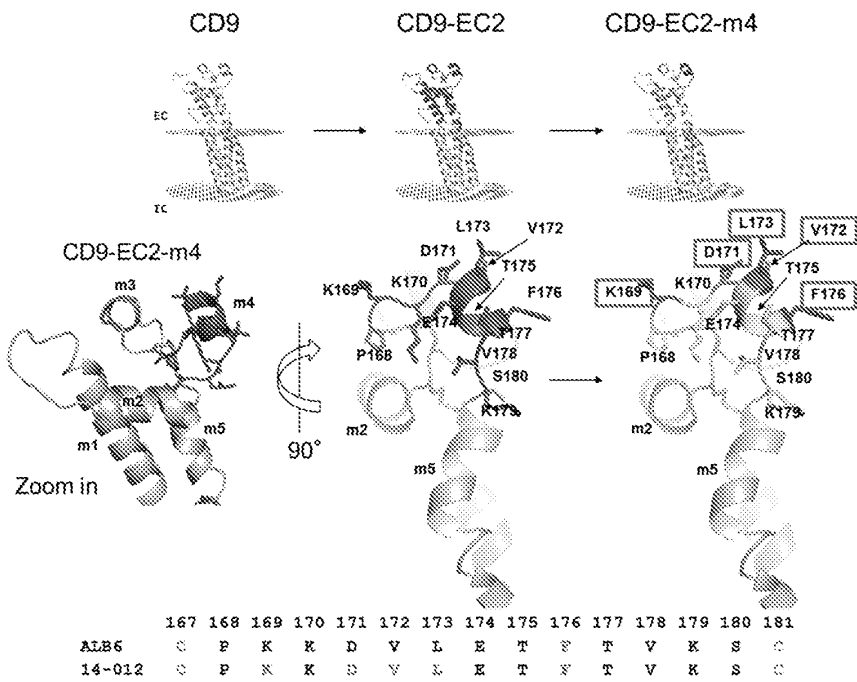


Figure 17C

	23	33	43	53	63	73	83
Human	LAGIAVLAIG	LWLRFDSTQK	SIFEQETNNN	NSSFYTGVIYI	LIGAGALMML	VGFLGCCGAV	QESQCMLGLF
Gibbon							
Gorilla							
Orangutan							
Chimpansee							
Macaque							
Baboon							
Rabbit			DK.....				
Mouse			--.....	H.....			

	93	103	113	123	133	143	153
Human	FGFLLVIFAI	EIAAAIWGYS	HKDEVIKEVQ	EFYKDTYNKL	KTKDEPQRET	LKAIHYALNC	CGLAGGVEQF
Gibbon							
Gorilla					L.....		
Orangutan							V.....
Chimpansee							
Macaque						D.....	
Baboon						D.....	A.....
Rabbit	S.....	T.....			N.....	D.....	M.....
Mouse		V.....T.....	L.....	Q.....	RS.....	M.....D.....	I.....PL.....

	163	173	183	193	203	213	223
Human	ISDICEP	KETTVKSCP	DAIKEVFDNK	FHIIGAVGIG	IAVVMIFGMI	FSMILCCAIR	RNREMV
Gibbon							
Gorilla							
Orangutan							
Chimpansee							
Macaque		I.....					
Baboon		I.....P.....	A.....				
Rabbit		S.....Q.....	E.....	V.....			S.....
Mouse	T.....	S.....Q.....P.....	E.....S.....N.....				S.....

Figure 18A

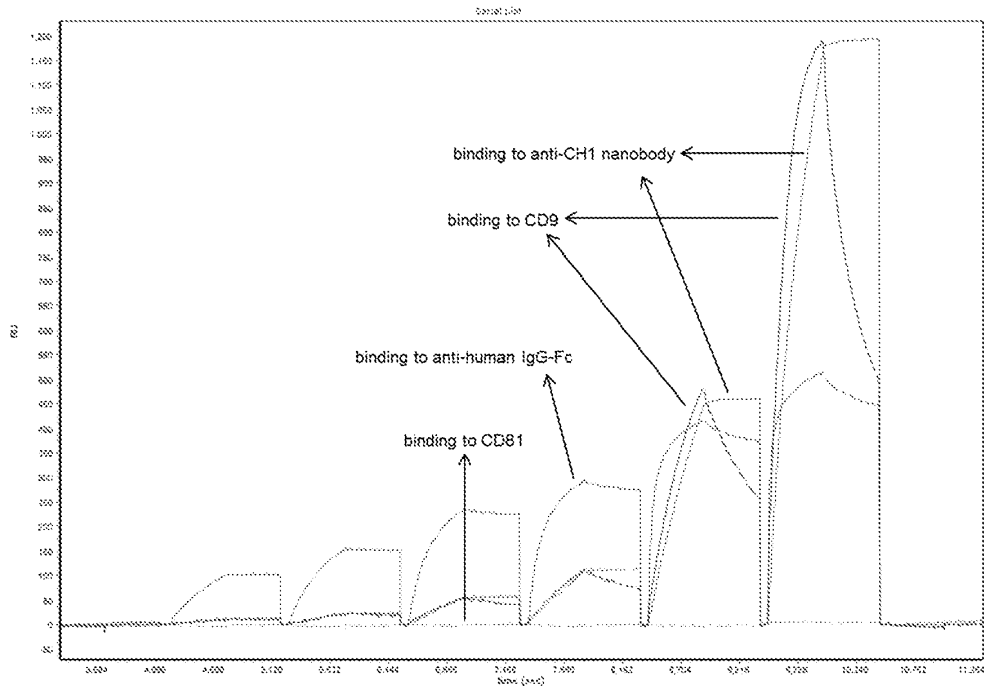


Figure 18B

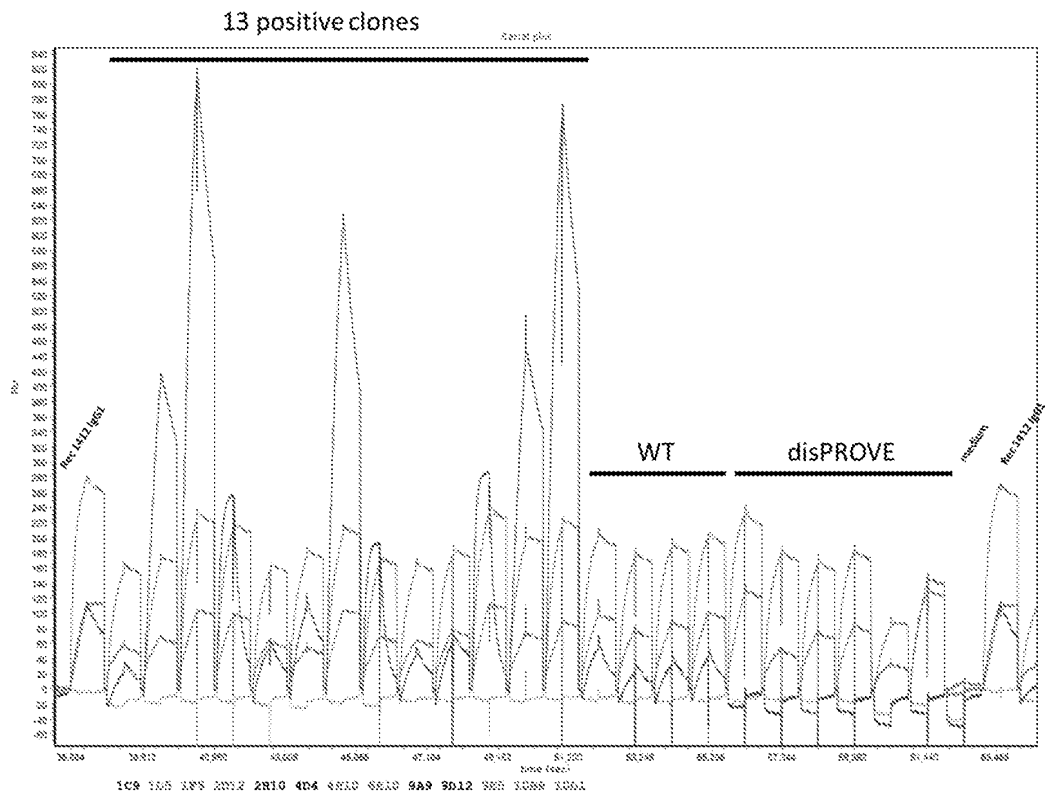


Figure 18C

Group 1: faster association (ka), slower dissociation (kd)				Mutations:	
	ka	kd	KD	Heavy chain	Light chain
1D5	281.8E+3	6.41E-04	2.3E-9	H40Y	-
1F5	232.8E+3	8.36E-04	3.8E-9	Y112F	-
4H10	190.3E+3	1.06E-03	6.1E-9	H40Y	L120V
10B9	267.6E+3	6.67E-04	2.5E-9	H40Y - A45V (+silent)	-
10D1	230.9E+3	9.04E-04	3.9E-9	H40Y	-
Group 2: faster association (ka), faster dissociation (kd)				Mutations:	
	ka	kd	KD	Heavy chain	Light chain
2D12	896.5E+3	4.6E-3	5.2E-9	D116H	-
4D4	99.5E+3	1.6E-3	15.9E-9	-	T66I - L94P
6E10	498.0E+3	3.1E-3	10.5E-9	T29N	-
9E5	925.4E+3	6.1E-3	6.7E-9	D116H	S28N
Group 3: no clear difference				Mutations:	
	ka	kd	KD	Heavy chain	Light chain
1C9	52.7E+3	2.2E-3	41.9E-9	Q3P	- (+silent)
2H10	60.7E+3	2.1E-3	33.9E-9	A24T	-
9A9	48.4E+3	2.1E-3	43.4E-9	G27R	-
9D12	42.7E+3	1.1E-3	26.0E-9	S64N - V66I	-
2H15 similar				Mutations:	
	ka	kd	KD	Heavy chain	Light chain
1G2	33.4E+3	2.4E-3	73.0E-9	-	-
1G3	33.4E+3	2.4E-3	73.0E-9	-	-
1G4	33.4E+3	2.4E-3	73.0E-9	-	-
1G5	33.4E+3	2.4E-3	73.0E-9	A96G (+silent)	-
disPROVE				Mutations:	
	ka	kd	KD	Heavy chain	Light chain
1E3	-	-	-	V108L	-
1E4	-	-	-	Y37C - (+silent) - A45V - K85R - G110A - G121A	-
1E5	-	-	-	Y117C	-
1F12	-	-	-	S62T	-
2A3	-	-	-	(+silent) - G110D	- (+silent)
5B1	-	-	-	G55S - (+silent) - A96V - T125I	-

Figure 19A

	CDR1										CDR2				
	1	12	20	26	40	45	50	55	67						
1412-WT	EVQVVESGGG	LVQPGRSLRL	SCAASGFTFD	DYAMHWVRQA	PGKGLEWVSG	ISWNSGSIVY									
H40Y															
Y112F															
H40Y-Y112F															
D116H															
T29N				N											
D116H-T29N				N											
H40Y-Y112F-D116H-T29N				N											
G110D															

	CDR3														
	70	80	90	100	104	120	128								
1412-WT	ADSVKGRFTI	SRDNAKNSLY	LQLNSLRAED	TAFYYCAKAV	SGYYPYFDYW	GQGILVTVSS									
H40Y															
Y112F															
H40Y-Y112F															
D116H						H									
T29N															
D116H-T29N						H									
H40Y-Y112F-D116H-T29N						H									
G110D															

Figure 19B

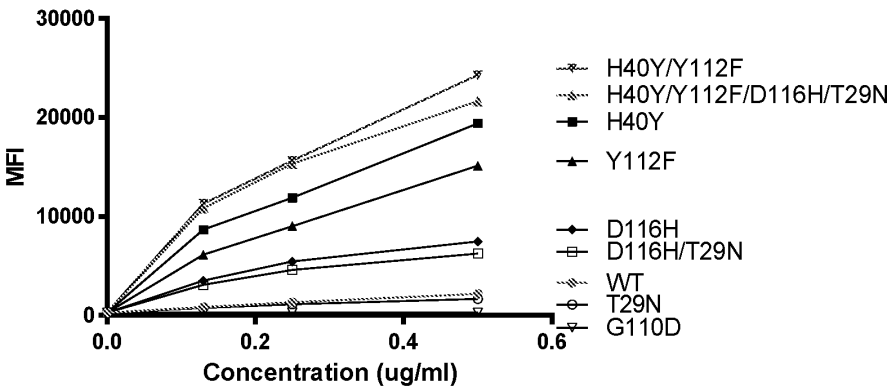
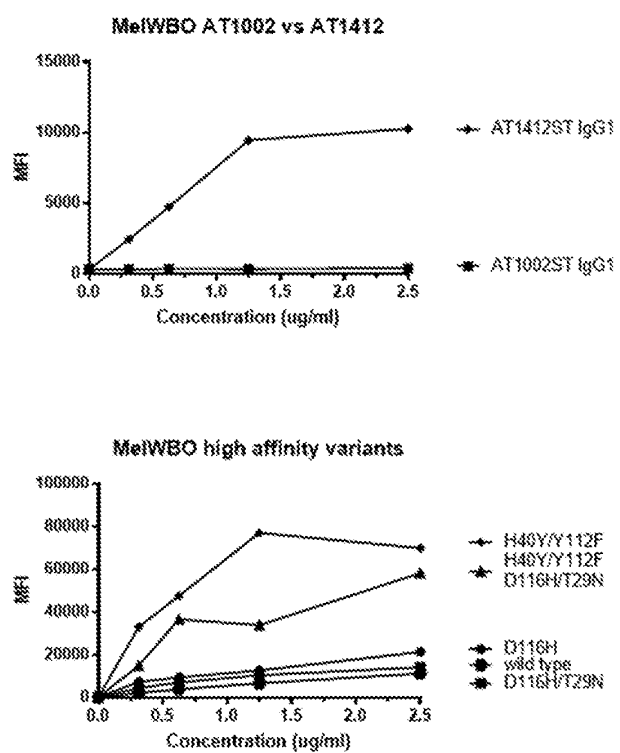


Figure 19C



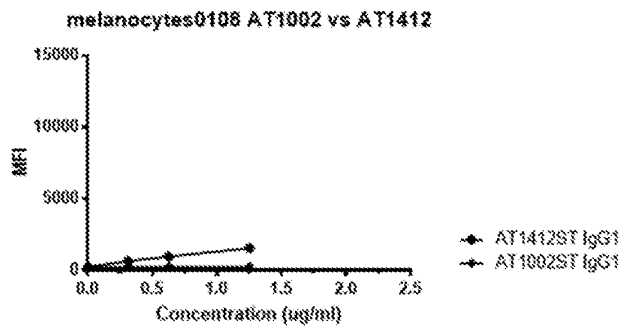
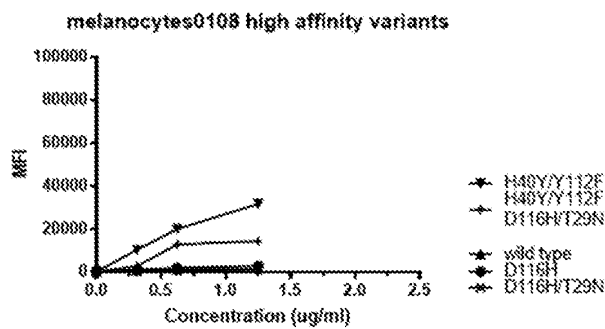
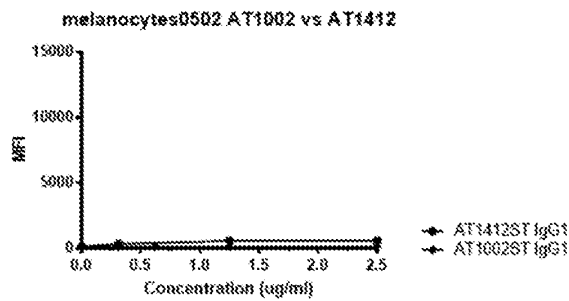
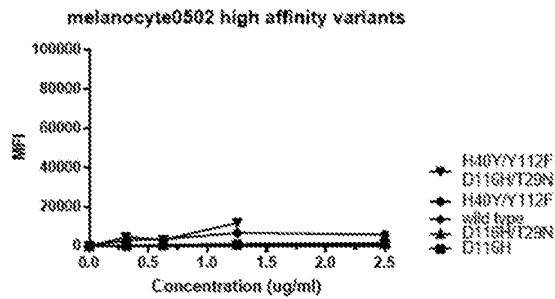


Figure 19C continued

Figure 19D

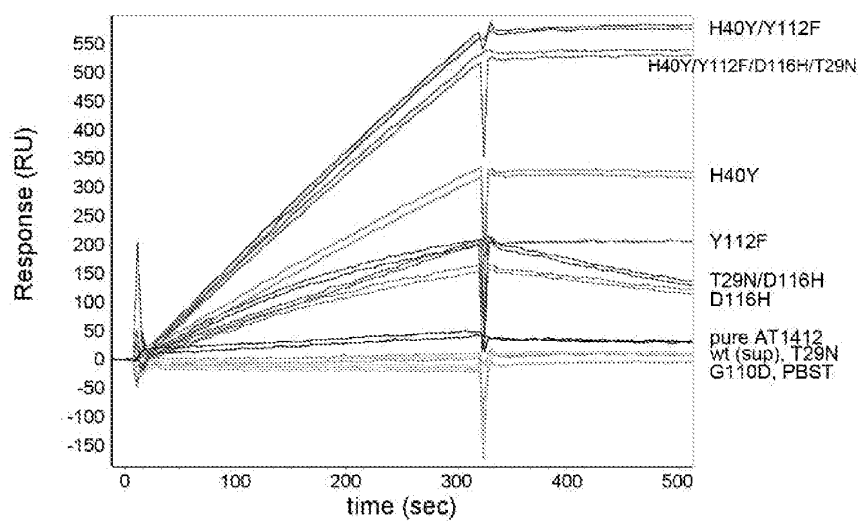


Figure 19E

	k_{on}	k_{off}	K_D
Purified AT1412	1.66 (± 0.22)	80.8 (± 1.1)	49,500 ($\pm 7,500$)
AT1412 ^a	*	*	*
H40Y ^a	37.2 (± 1.3)	20.2 (± 1.1)	543 (± 11)
Y112F ^a	18.5 (± 1.2)	20.3 (± 2.1)	1,090 (± 40)
H40Y/Y112F ^a	16.4 (± 1.6)	3.69 (± 1.31)	219 (± 58)
D116H ^a	47.0 (± 0.8)	217 (± 3)	4,620 (± 140)
T29N ^a	2.59 (± 0.41)	126 (± 10)	50,100 ($\pm 12,100$)
D116/T29N ^a	70.8 (± 1.1)	297 (± 1)	4,190 (± 80)
H40Y/Y112F/D116H/T29N ^a	15.3 (± 1.6)	6.92 (± 0.31)	455 (± 27)
G110D ^a	-	-	-

Figure 20

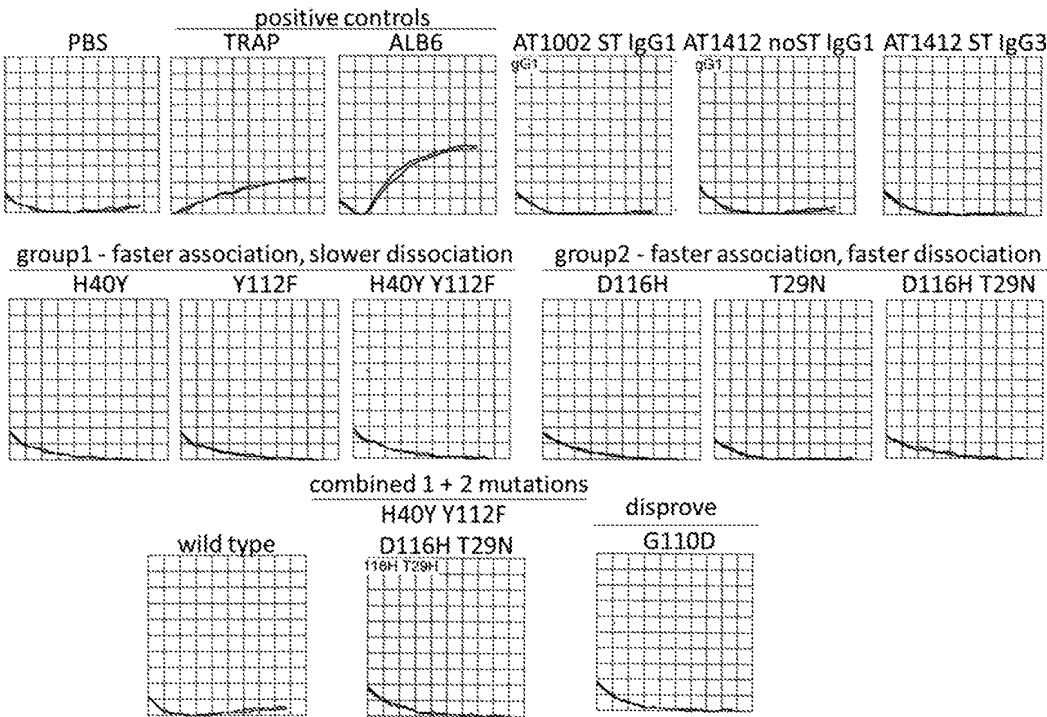


Figure 21

CH1		Hinge exons 1-4				CH2	CH3	
G3m(b*)	S SL	+	+	+	+	PRYL T	VSNMKQIRF	IGHG3*01,*05,*10, AJ390236
G3m(b*)	S SL	+	~	~	+	PRYL T	VSNMKQIRF	IGHG3*04, X89549
G3m(b*)	S SL	+	+	+	+	PRYV T	VSNMKQIRF	IGHG3*09, AJ390242
G3m(b*)	S SL	+	+	+	+	PRFL T	VSNMKQIRF	IGHG3*11, AJ390247
G3m(b*)	S SL	+	~	+	+	PRFL T	VSNMKQIRF	IGHG3*12, AJ390252
G3m(b*)	S SL	+	+	+	+	PRYL T	VSKMKQIRF	IGHG3*06,*07, AJ390237
G3m(b*)	S SL	+	+	+	+	PRYL T	VNNMKQIRF	IGHG3*08, AJ390241
G3m(c3*)	S SL	+	+	+	+	PRYL T	VSKMKQIRF	IGHG3*13, AJ390244
G3m(c3c5*)	S SL	+	~	+	+	PRYL T	VSNVREVRF	IGHG3*03, X16110
G3m(g*)	S SL	+	+	+	+	LRYL T	VNNMKQIRY	IGHG3*14, AJ390254
G3m(g*)	S SL	+	+	+	+	LRYL T	VNNMKQIRY	IGHG3*15, AJ390260
G3m(g*)	S SL	+	+	+	+	LRYL A	VNNMKQIRY	IGHG3*16, AJ390283
G3m(s*)	S NF	+	~	+	+	PRYL T	MSFVKQIHV	IGHG3*17, AJ390272
G3m(st*)	Y SL	+	~	+	+	PWYL T	MSFVKQIHV	IGHG3*18, AJ390276
G3m(st*)	S SL	+	~	+	+	PWYL T	MSKVKQIHV	IGHG3*19, AJ390279 ^(71, 74)

Figure 22A

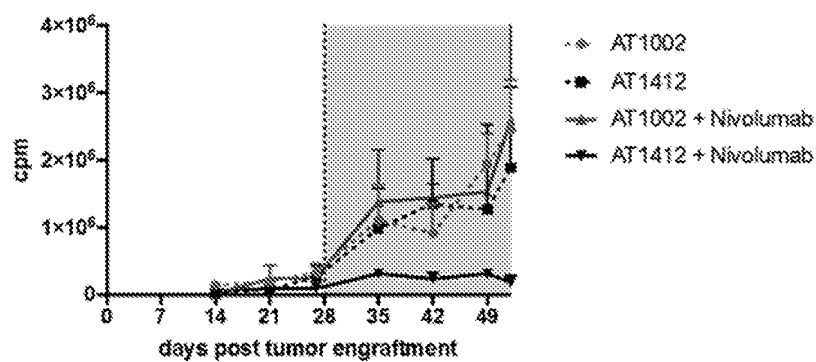


Figure 22B

