

Figure 1A

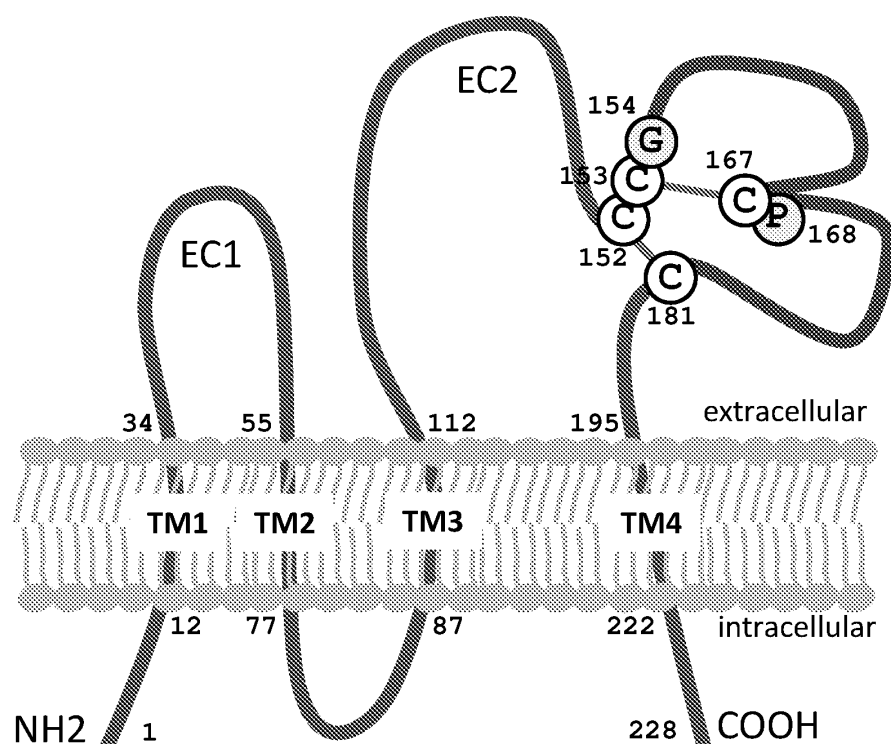


Figure 1B

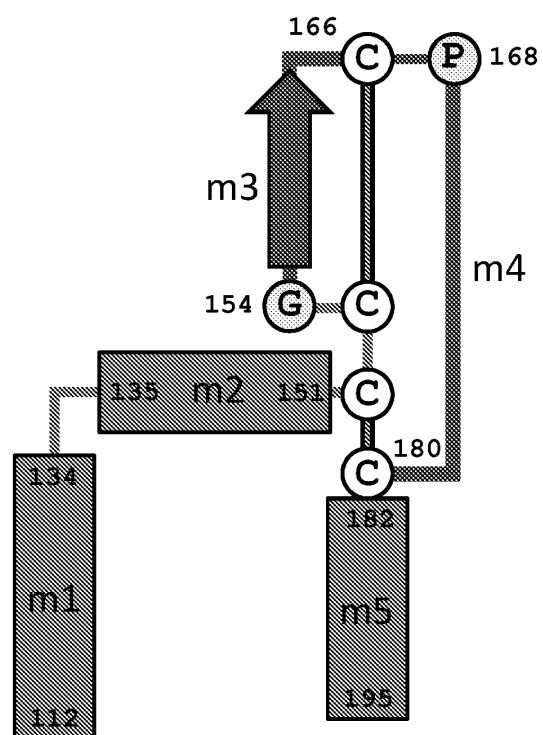


Figure 2

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 gct  
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

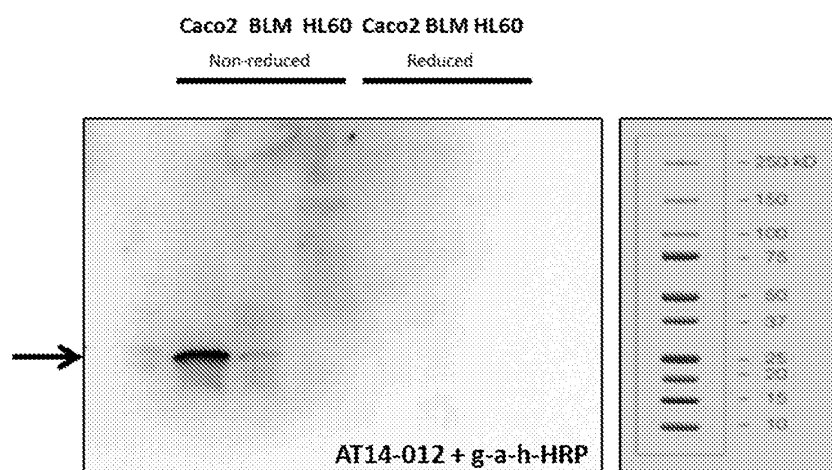
CDR2 tgg gca tct acc cgg gaa tcc

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

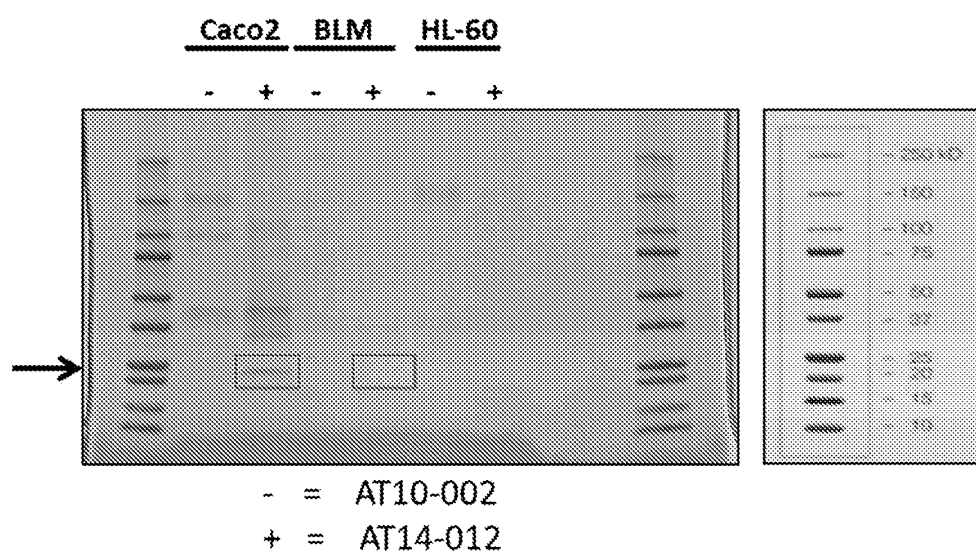


Figure 4C

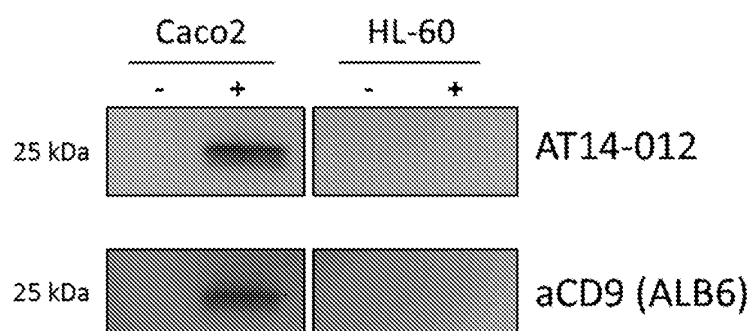


Figure 5A

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

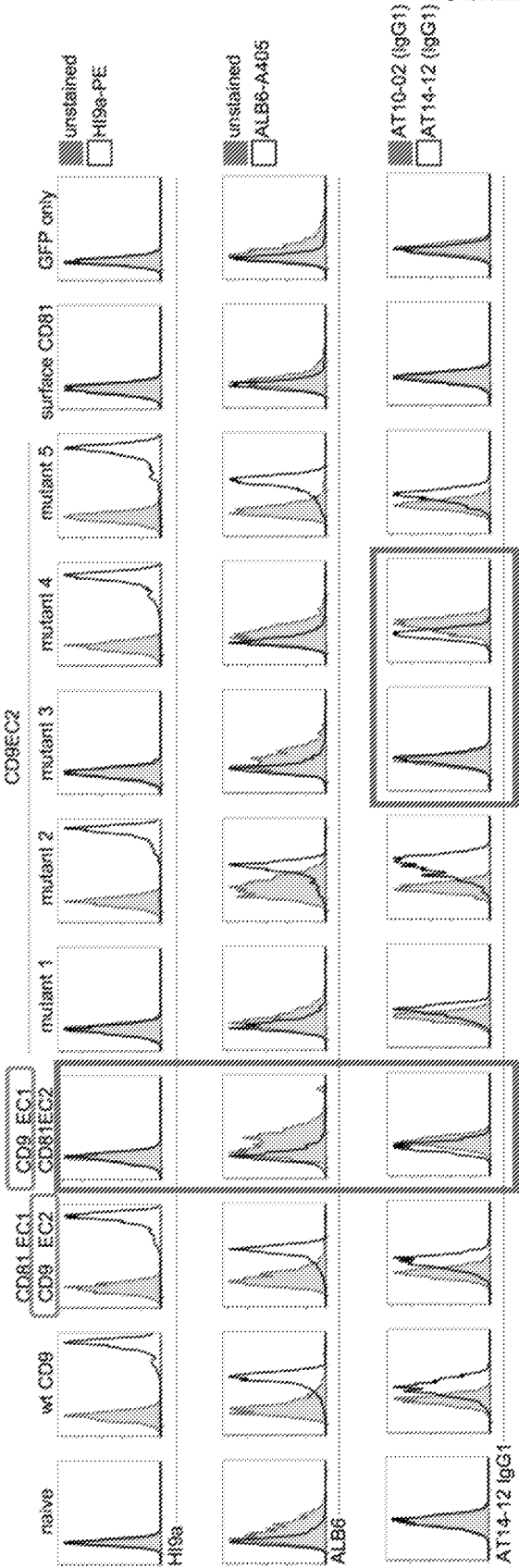


Figure 5B

Epitope mapping (FACs):  
CD9 EC2m4 alanine mutants

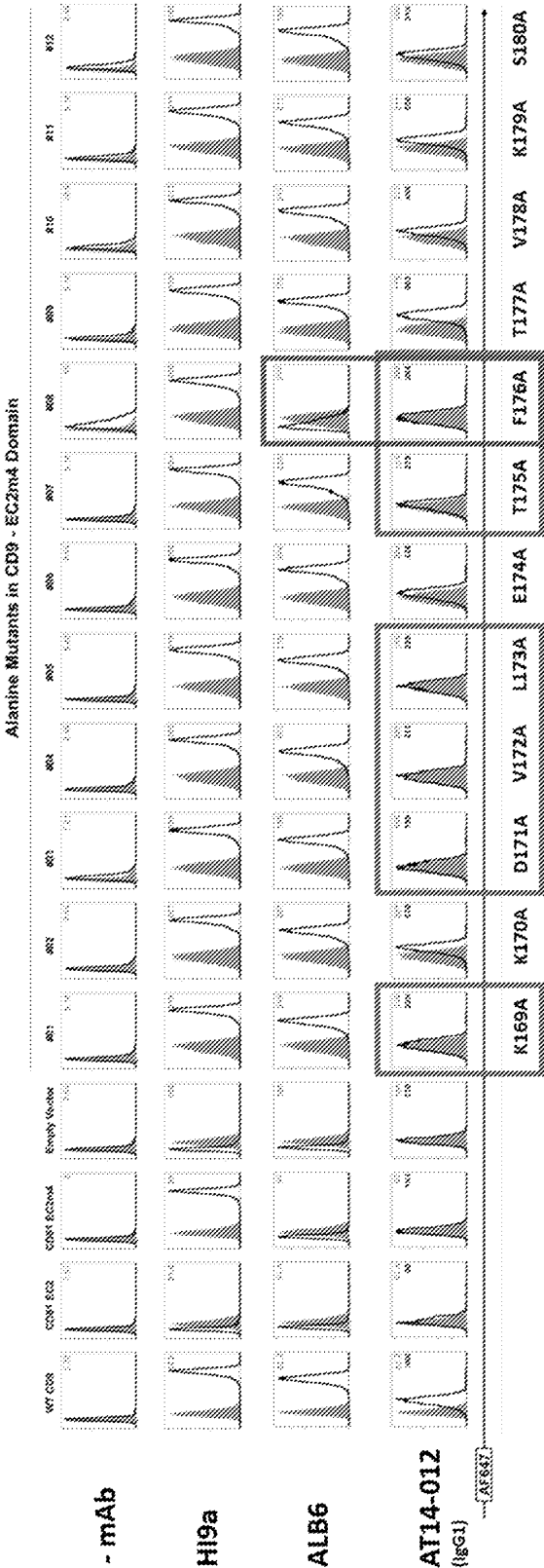


Figure 6A

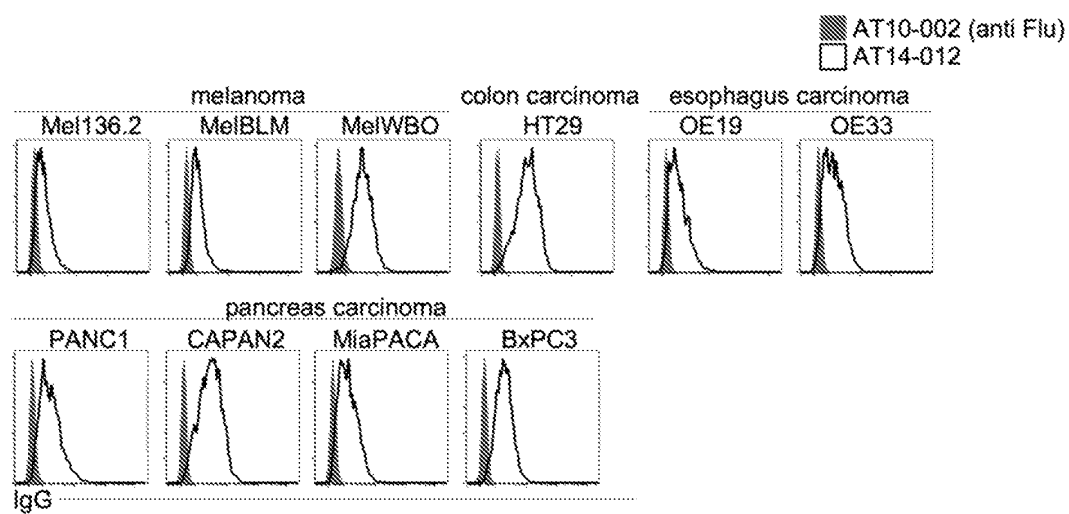


Figure 6B

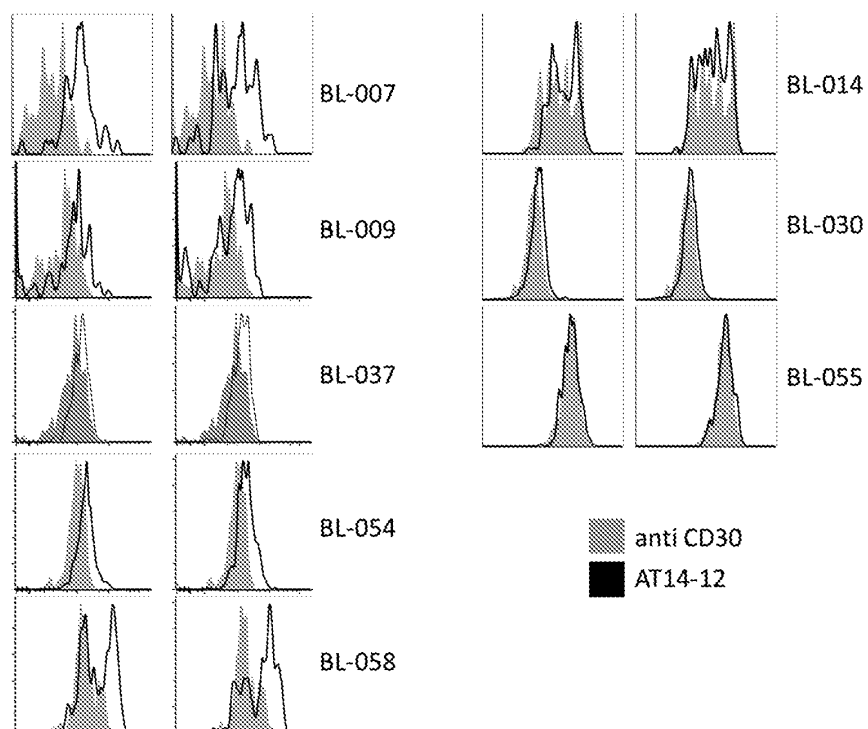


Figure 6C

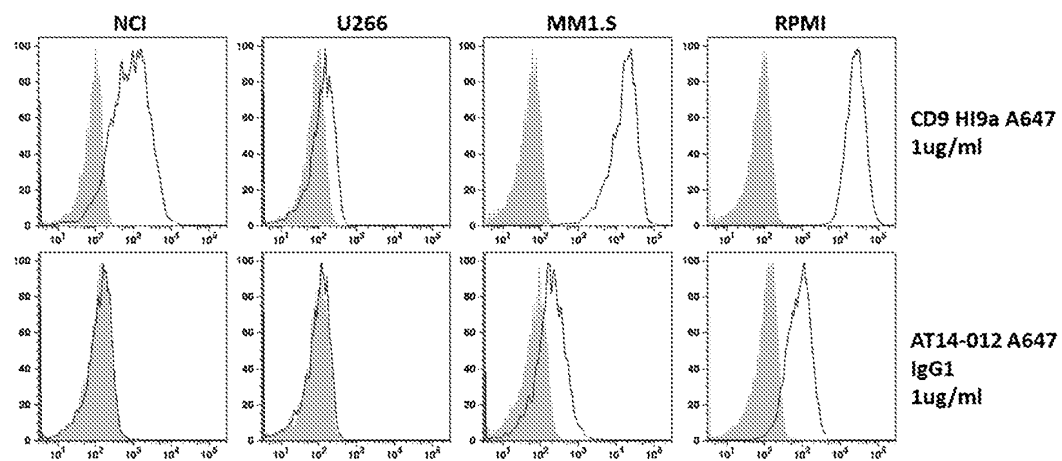


Figure 7A

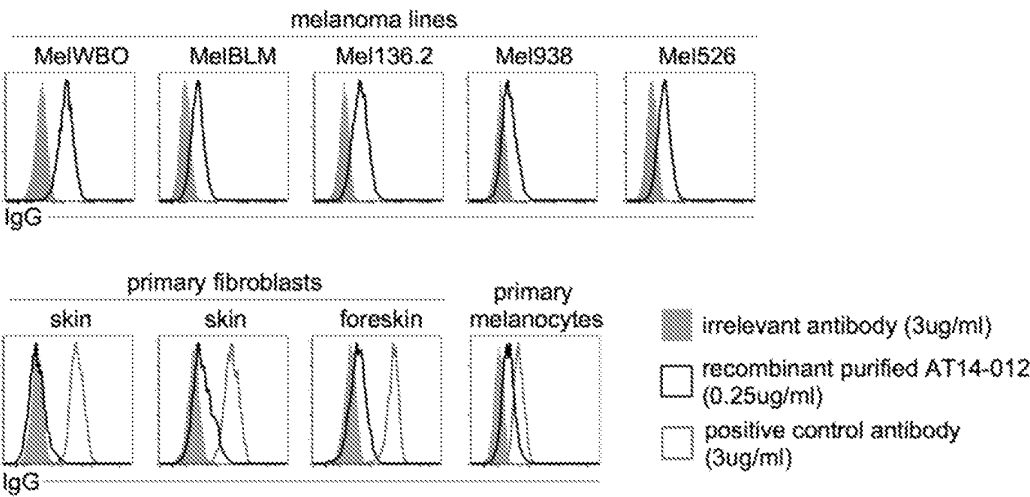


Figure 7B

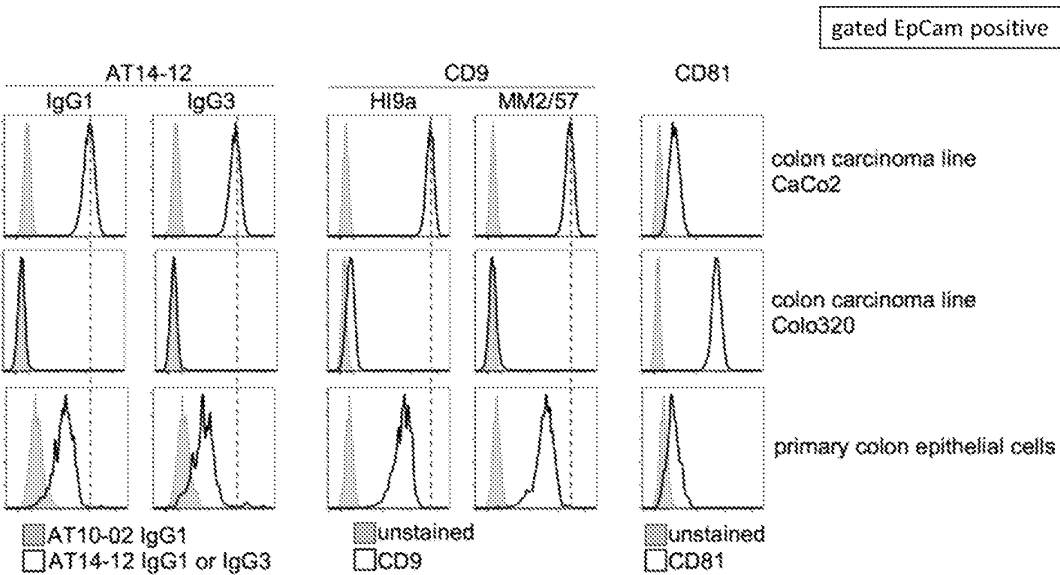


Figure 7C

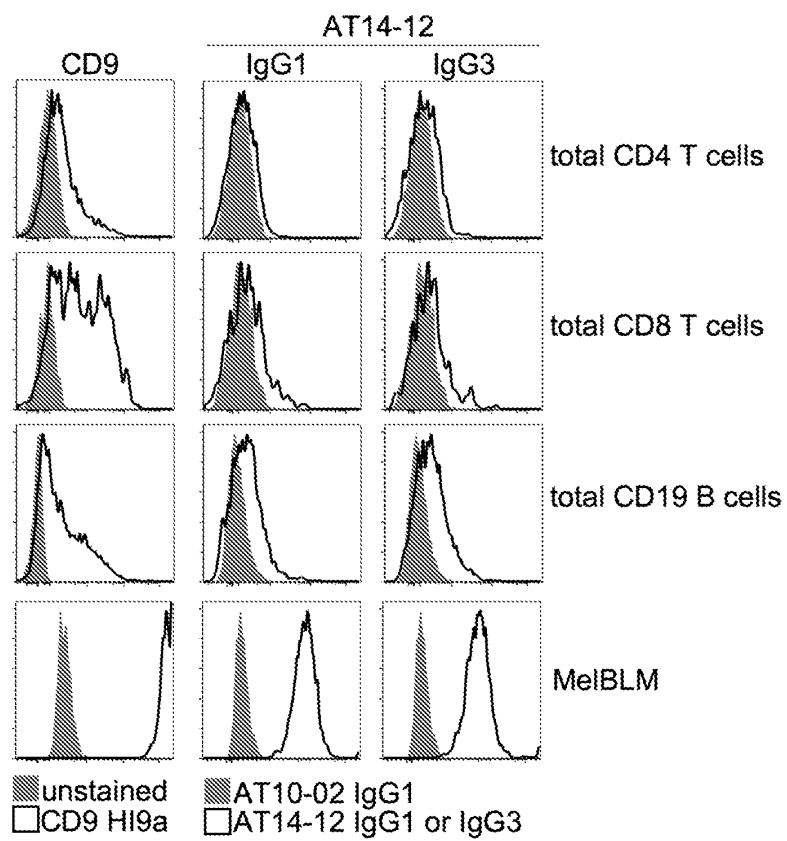


Figure 8A

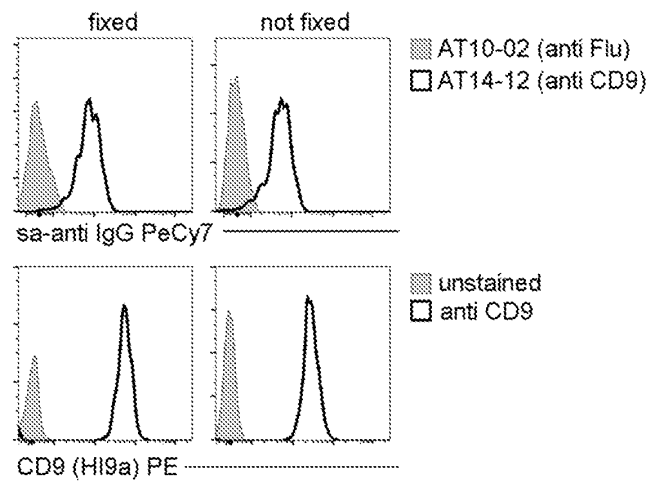


Figure 8B

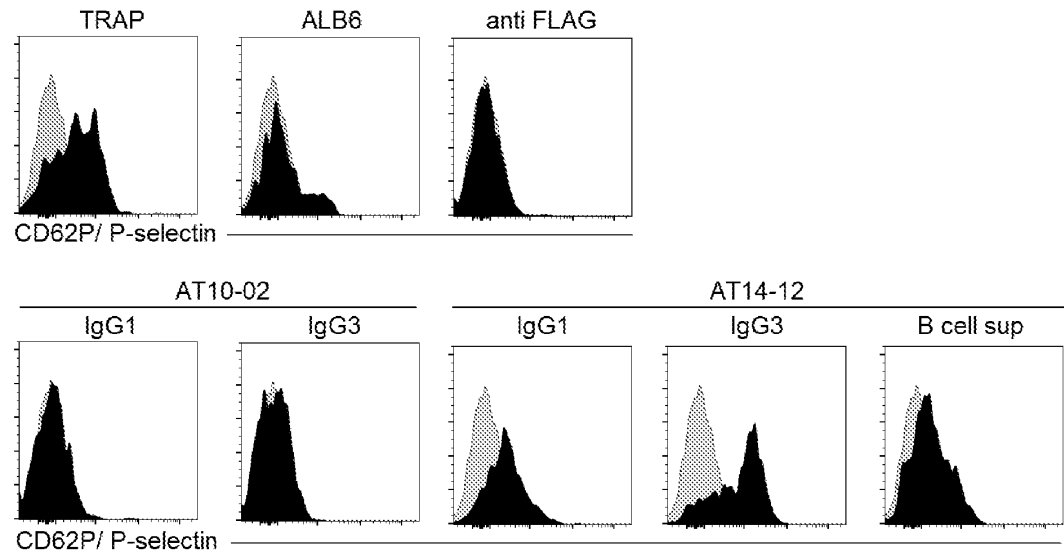


Figure 8C

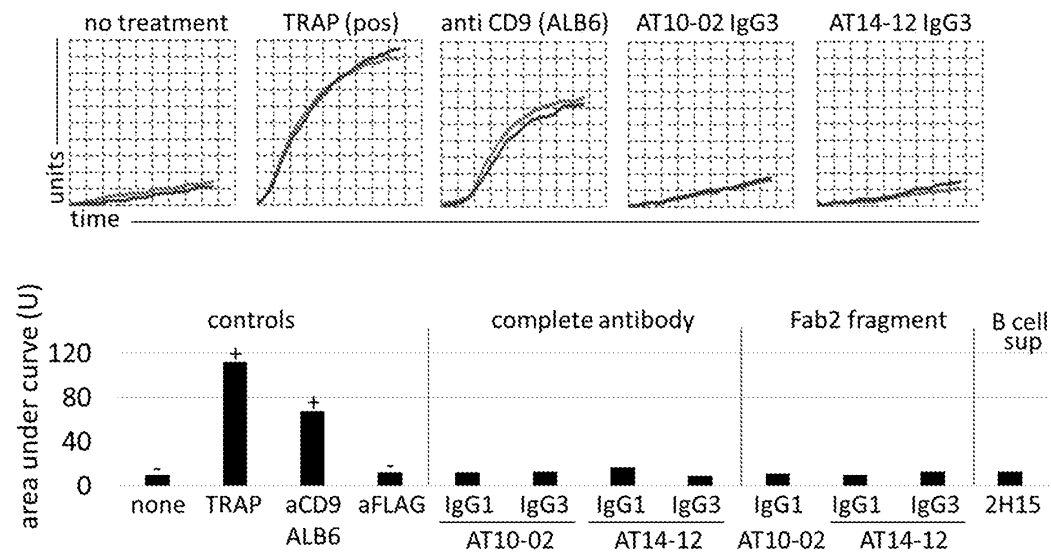


Figure 9A

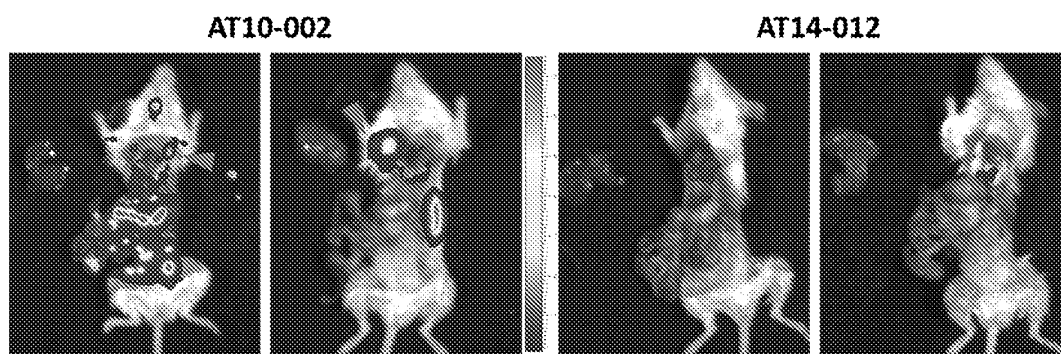


Figure 9B

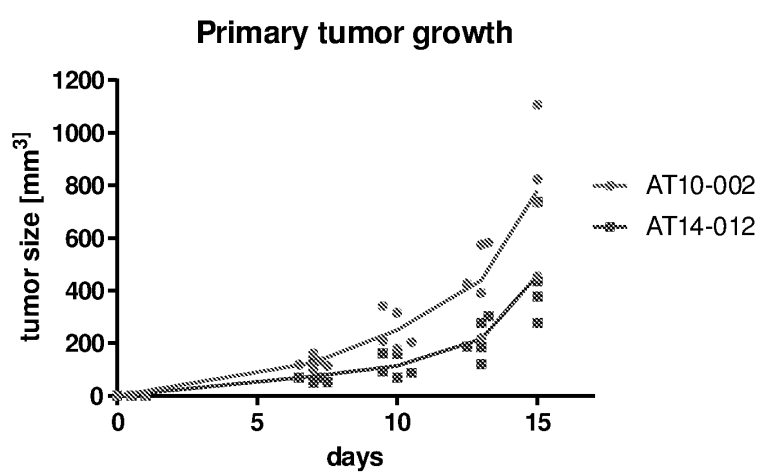


Figure 9C

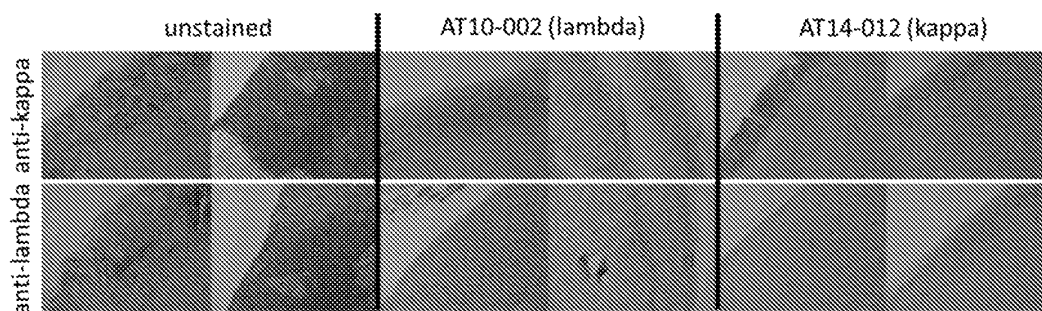


Figure 9D

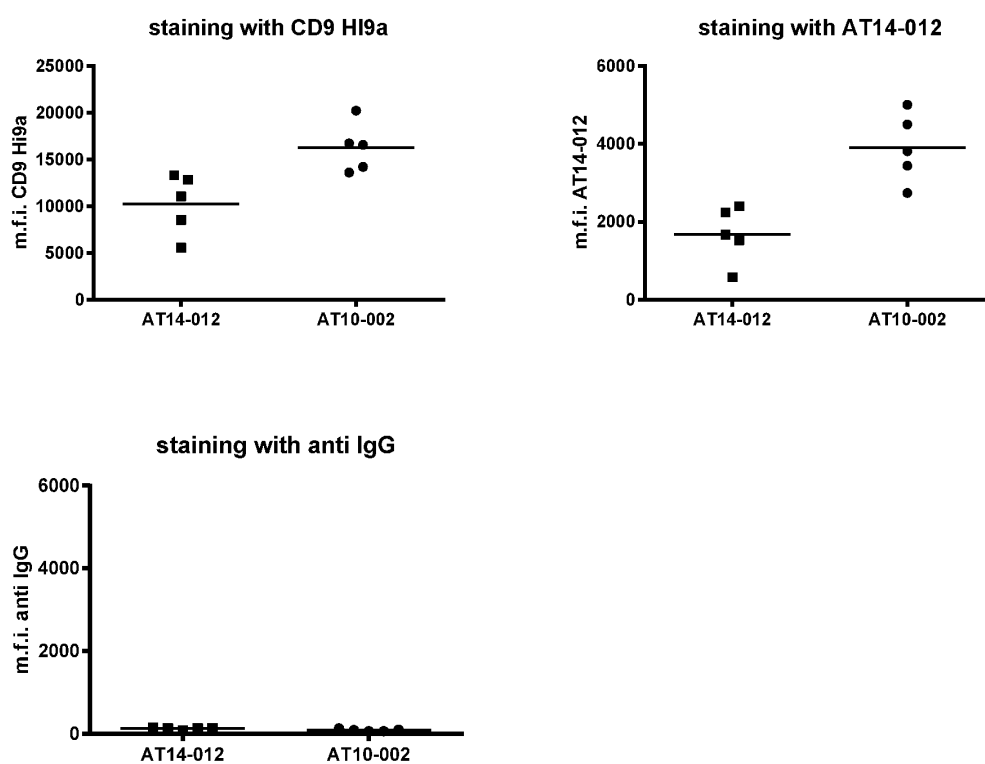


Figure 9E-G

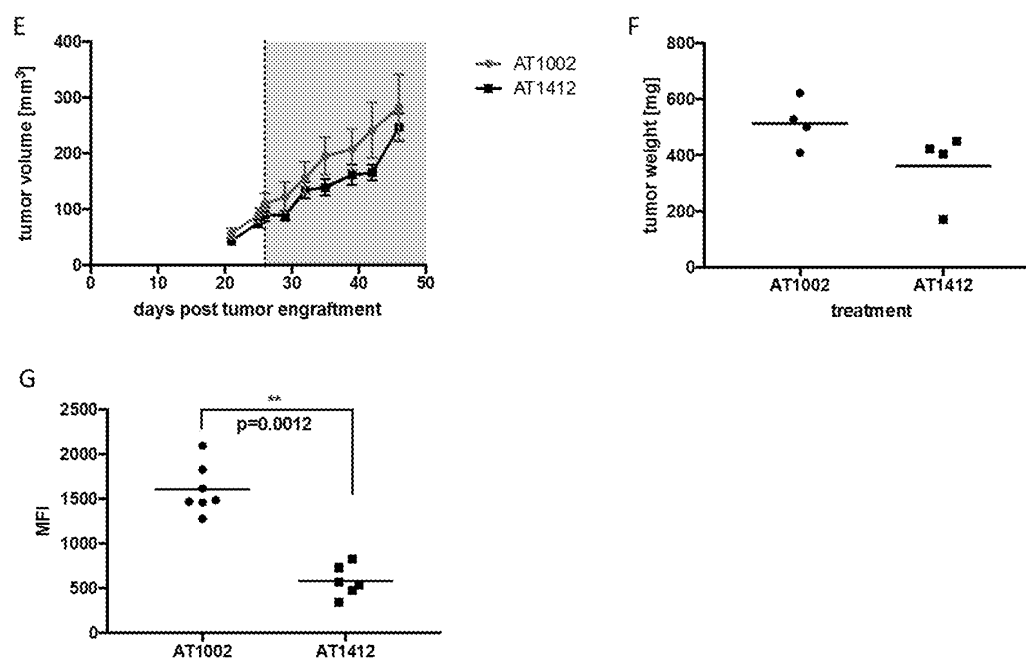


Figure 10A

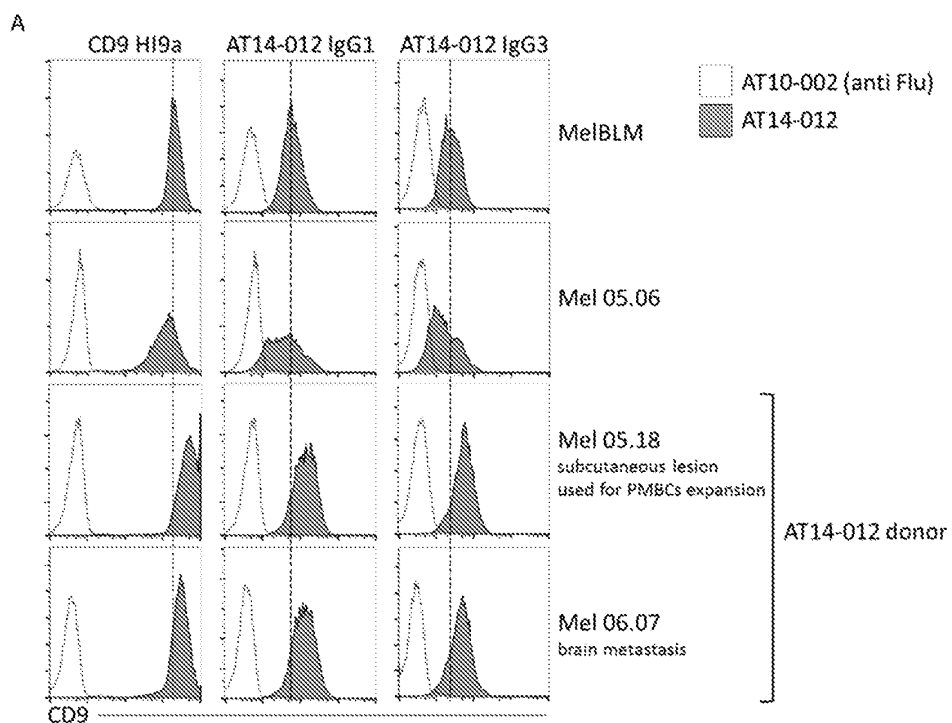


Figure 10B

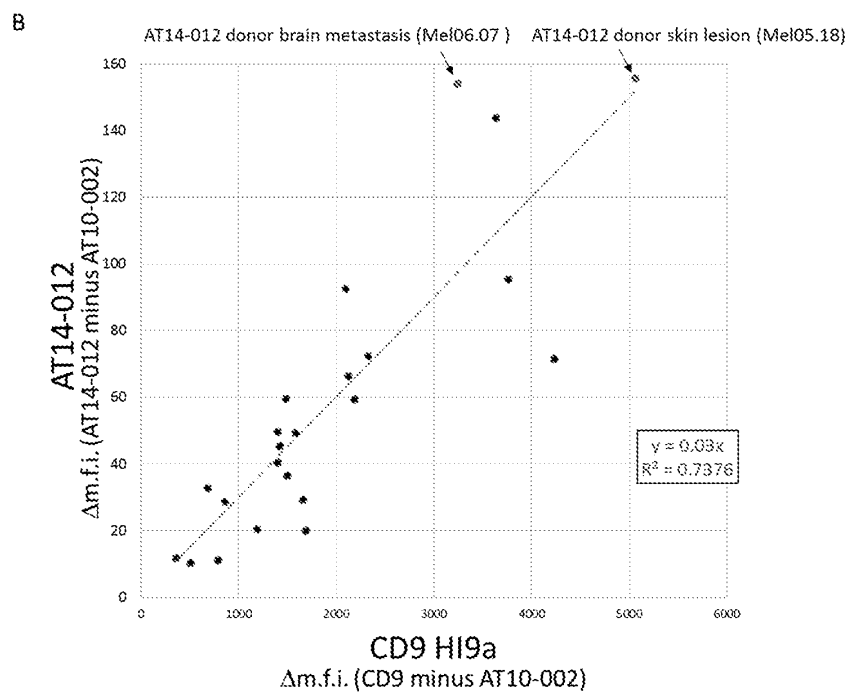


Figure 10C

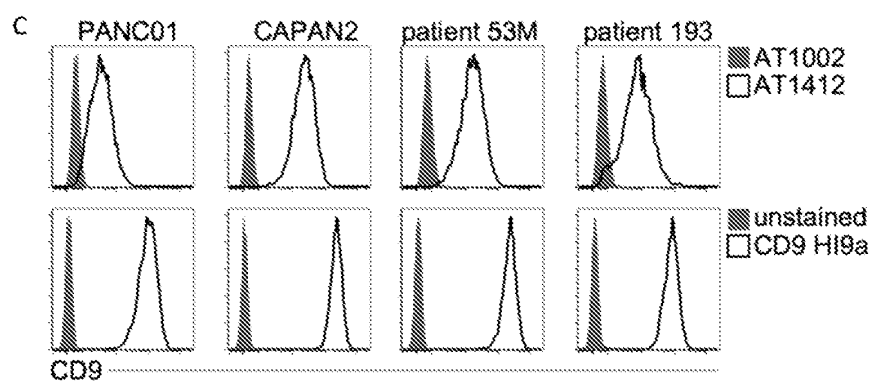


Figure 11A

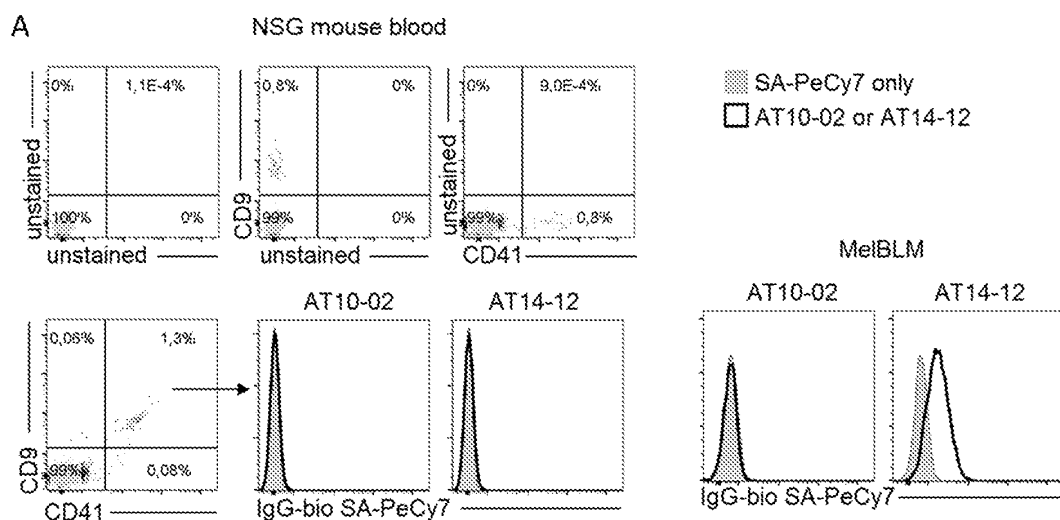


Figure 11B

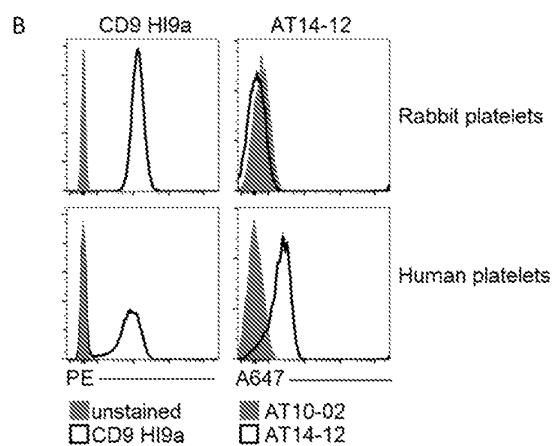


Figure 11C

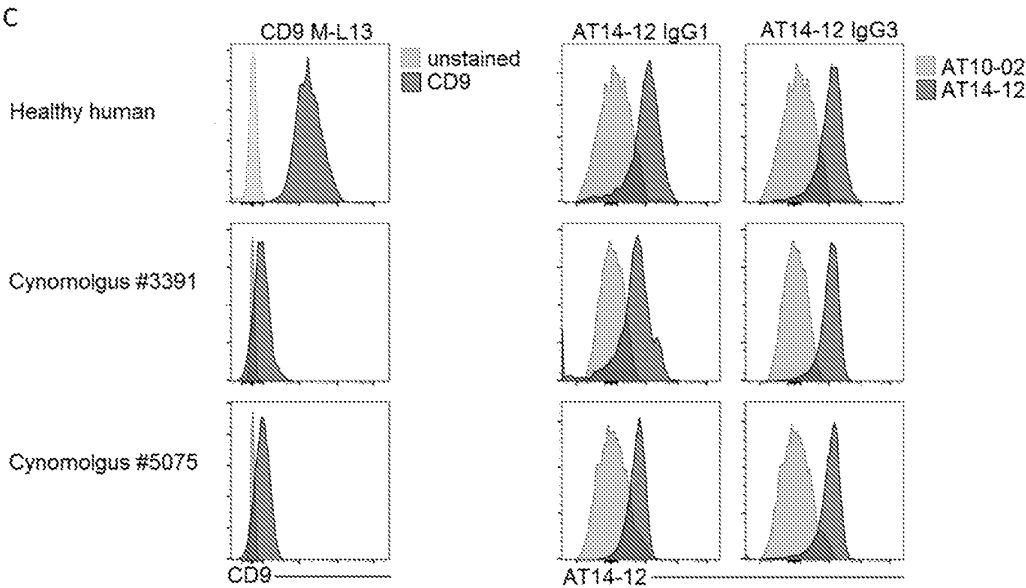


Figure 12A

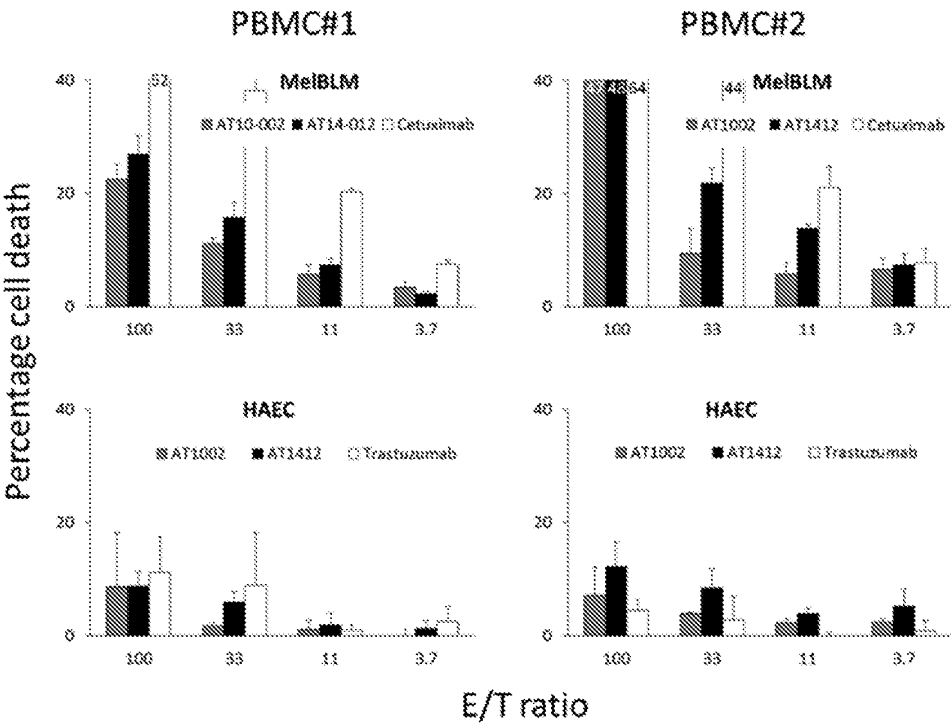


Figure 12B

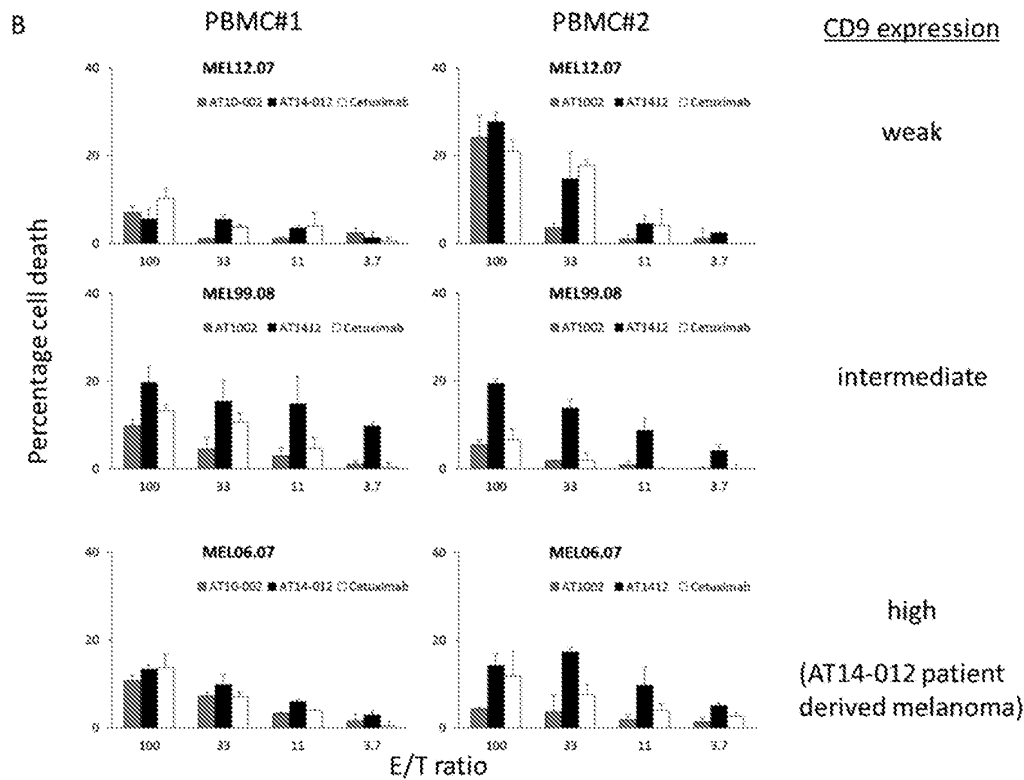


Figure 13

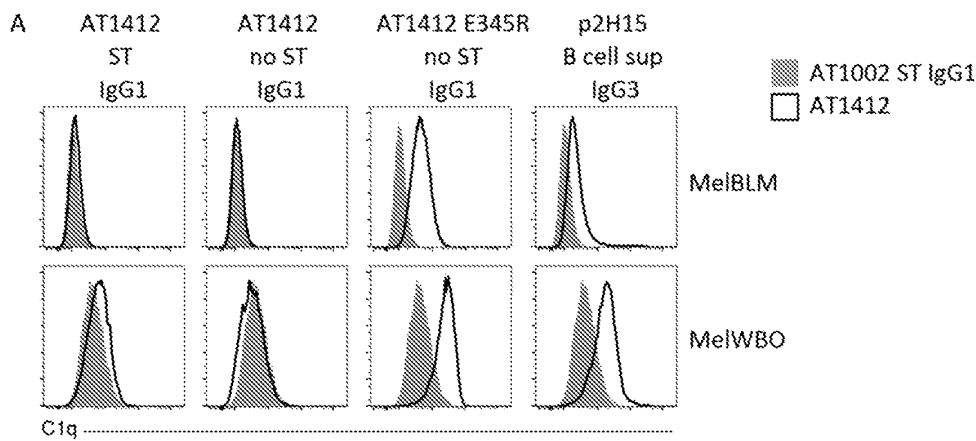


Figure 13B

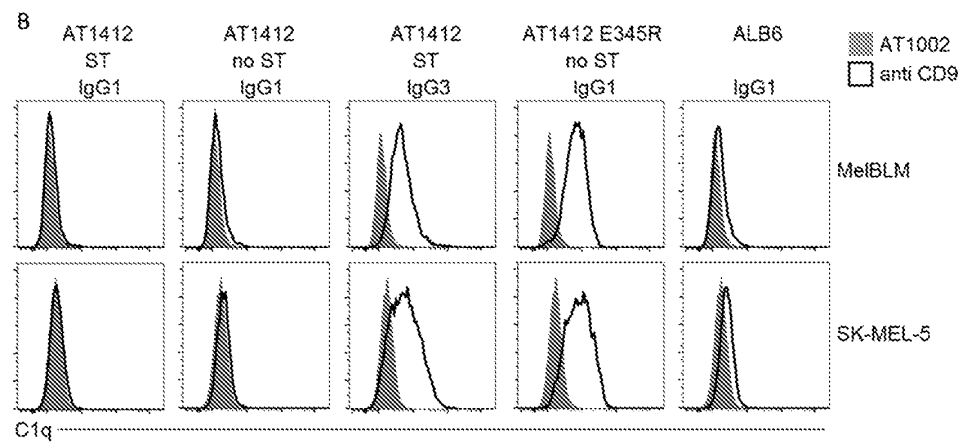


Figure 13C

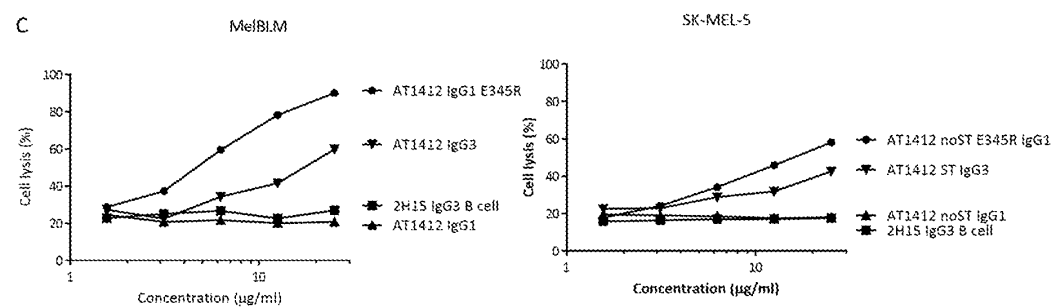


Figure 13D

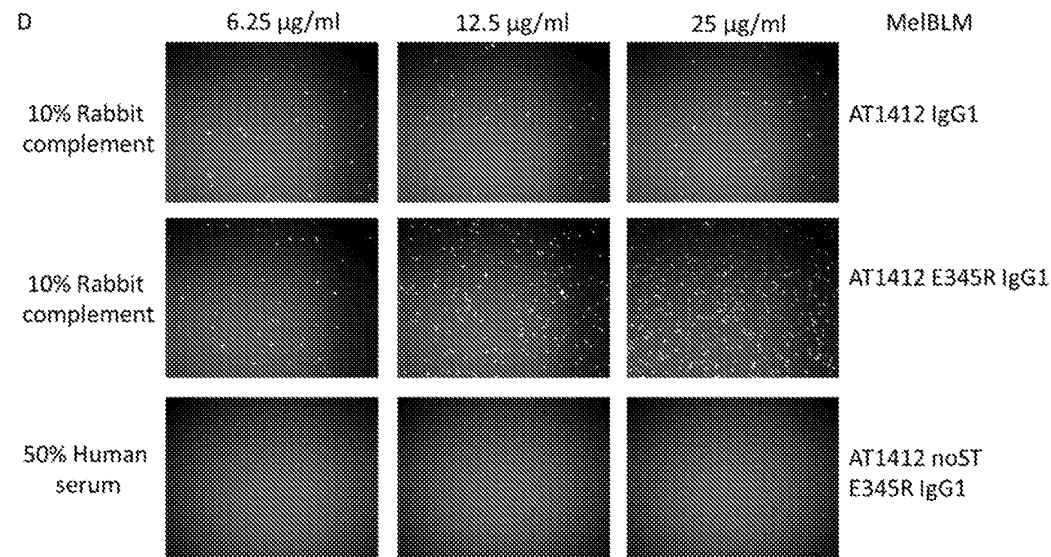


Figure 13E

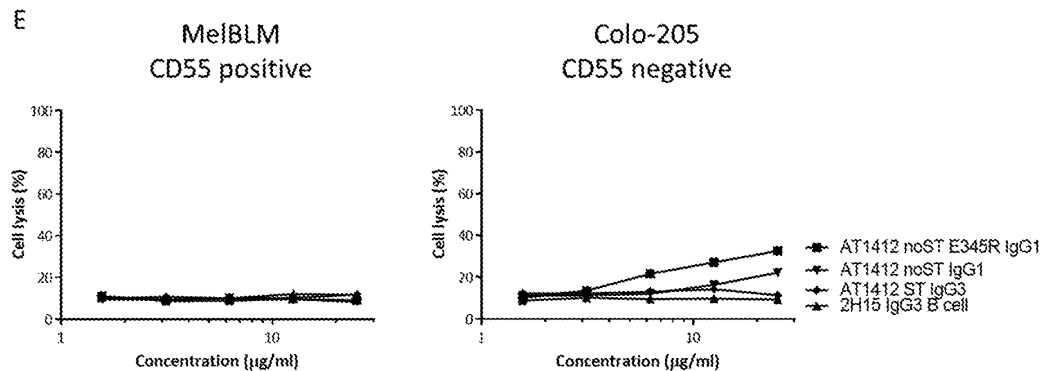


Figure 14A

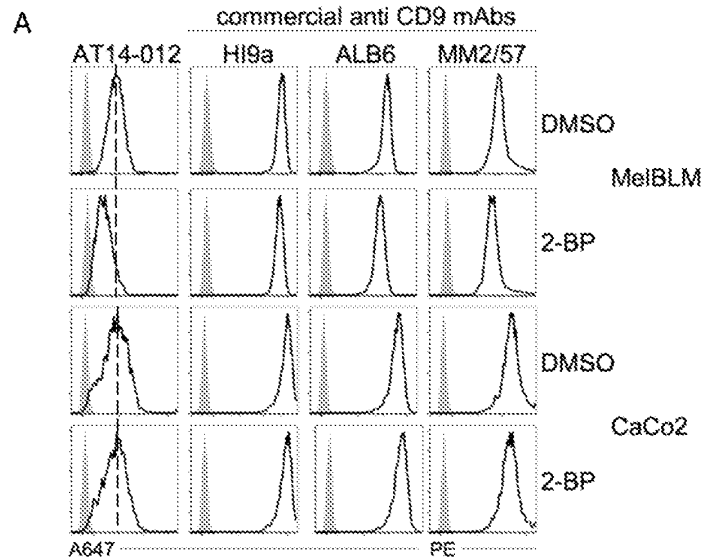


Figure 14B

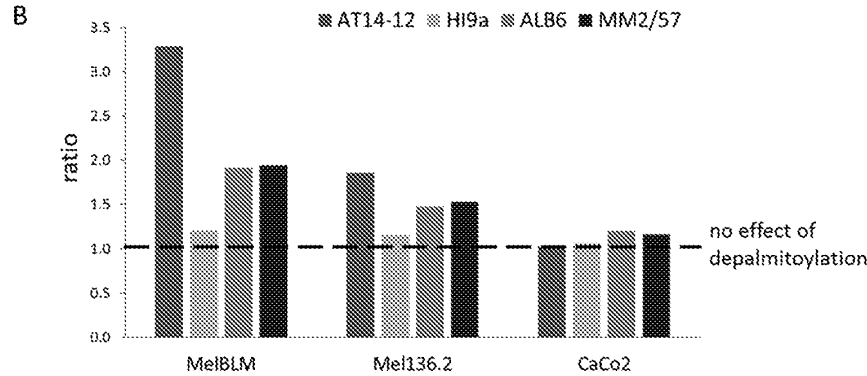
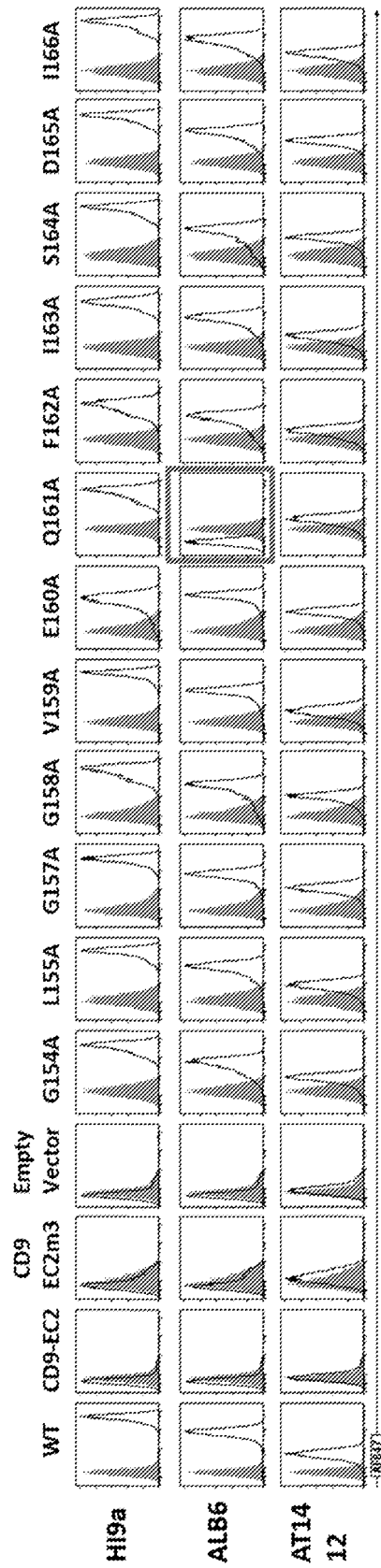


Figure 15

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 F I S D I C  
AT1412 C G L A G G V E Q F I S D I C

Figure 16A

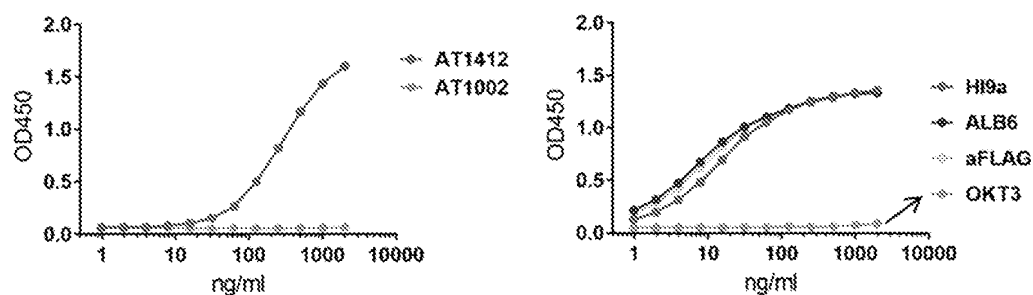


Figure 16B

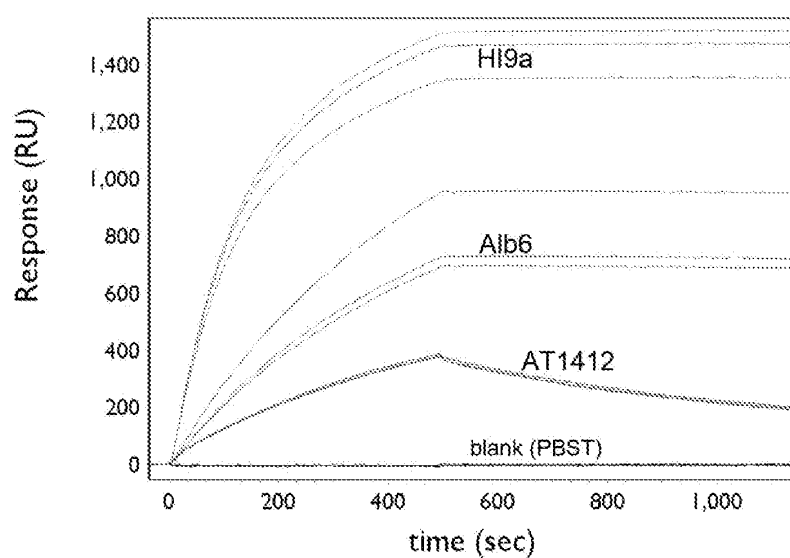


Figure 16C

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

Figure 17A

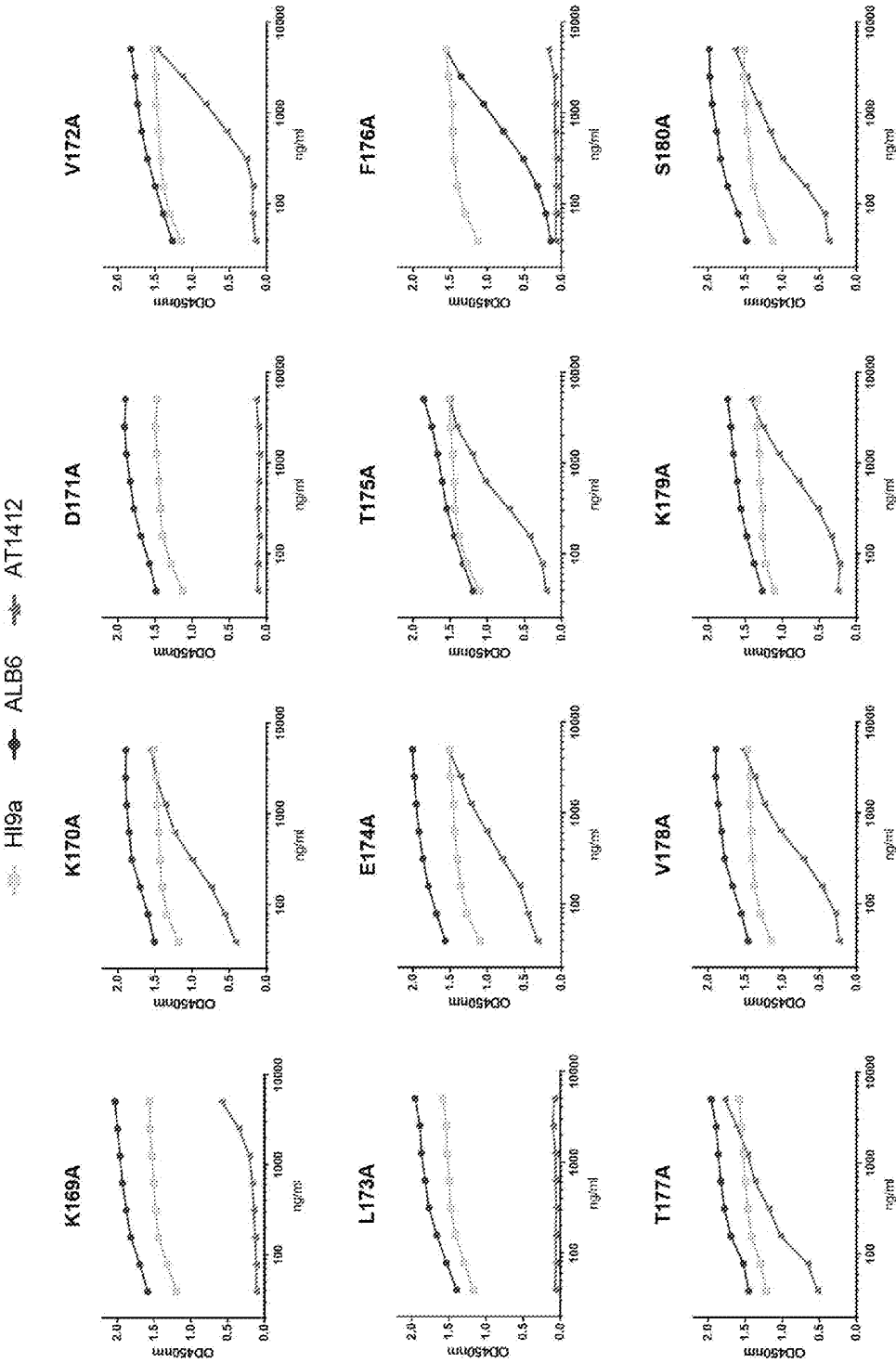


Figure 17B

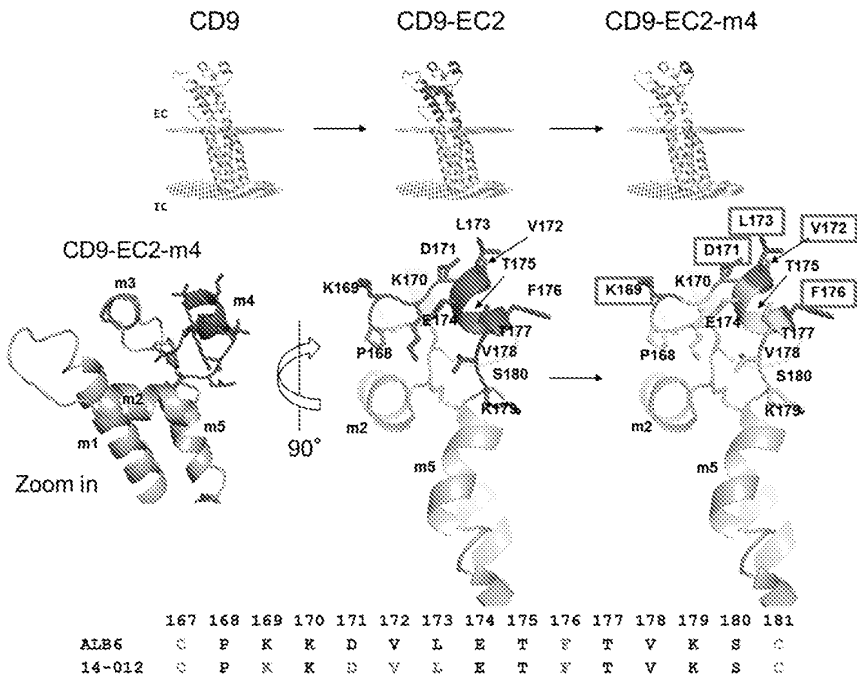


Figure 17C

|            |            |            |              |             |            |            |            |
|------------|------------|------------|--------------|-------------|------------|------------|------------|
|            | 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 K   | ET TVKSCP               | 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

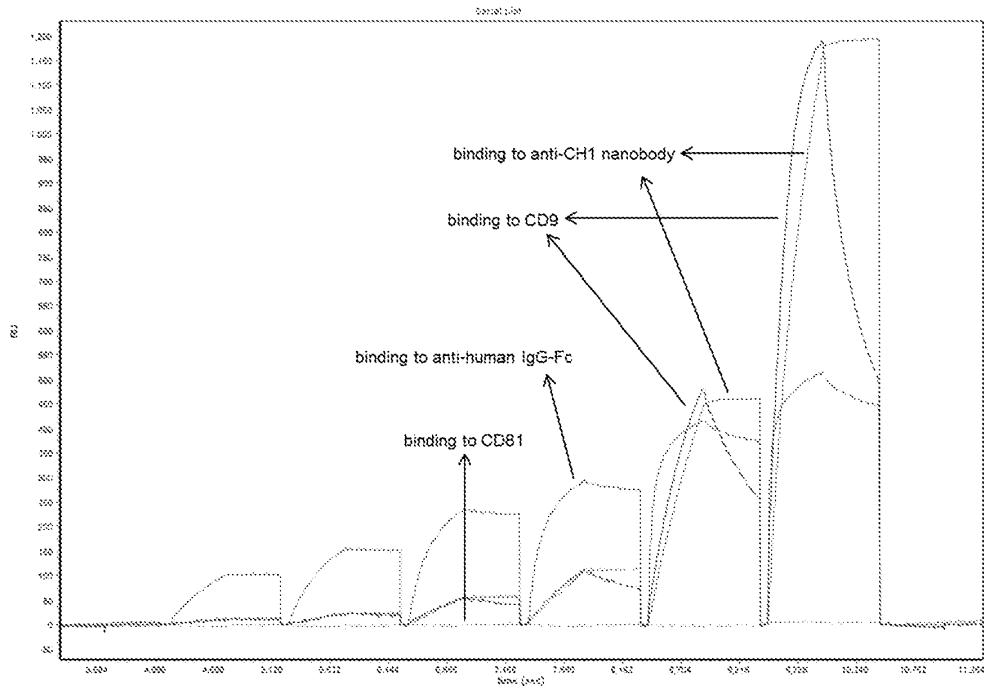


Figure 18B

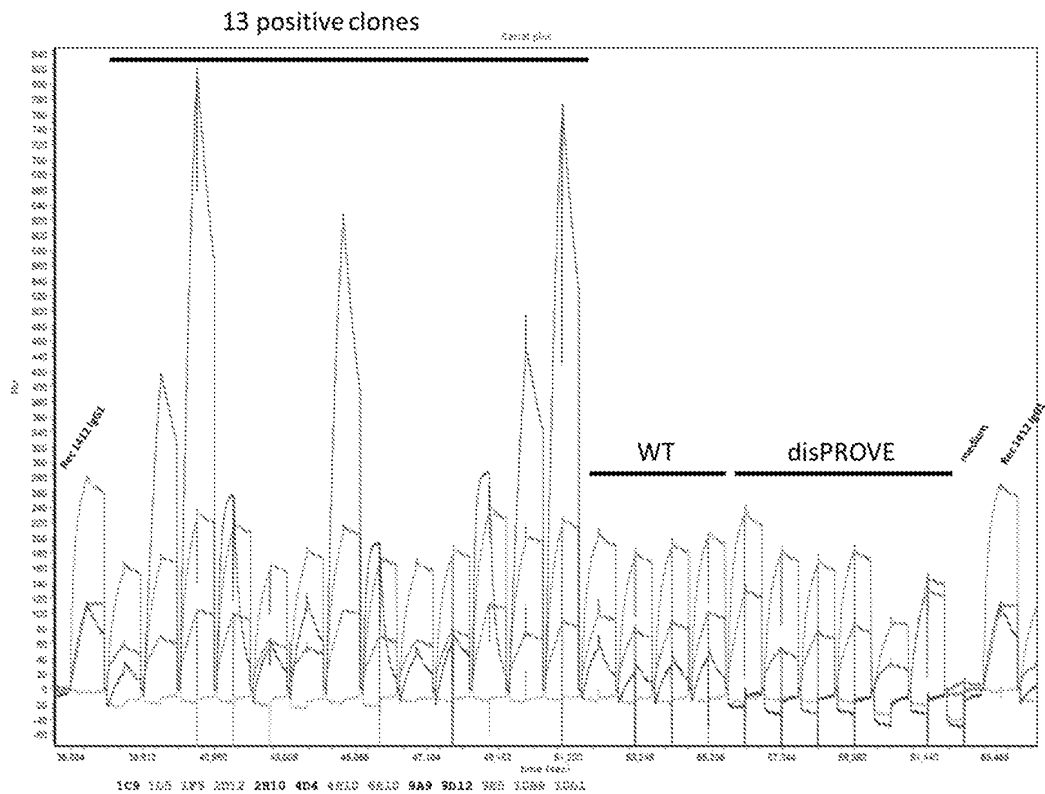


Figure 18C

| 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               | EVQV  | VESGGG | LVQP  | GRSLRL | SCAAS | GF    | TFD   | DYAM  | HWVRQA | PGK   | GLEW  | VSG   | ISWN  | SGSIVY |       |       |       |       |       |       |
| 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               | ADSV  | KGRFTI | SRDNA | KNSLY | LQLNS | LRAED | TAFYY | CAKAV | SGYYP | YFDYW | GQGIL | VTVSS |       |       |       |       |       |       |       |       |
| H40Y                  | ..... | .....  | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Y112F                 | ..... | .....  | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| H40Y-Y112F            | ..... | .....  | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| D116H                 | ..... | .....  | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| T29N                  | ..... | .....  | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| D116H-T29N            | ..... | .....  | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| H40Y-Y112F-D116H-T29N | ..... | .....  | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| G110D                 | ..... | .....  | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |

Figure 19B

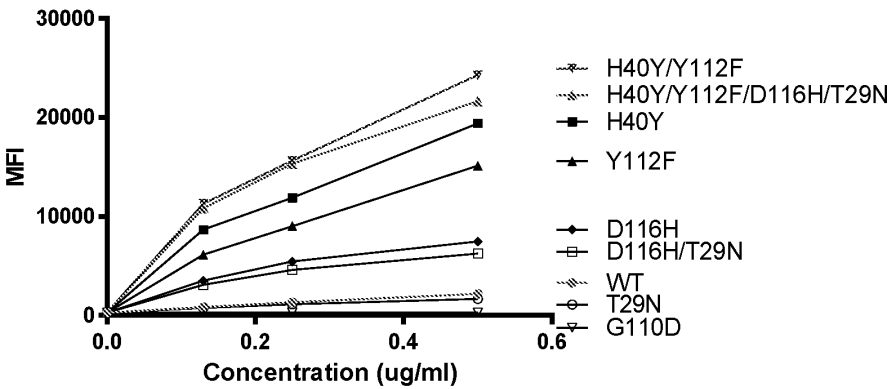
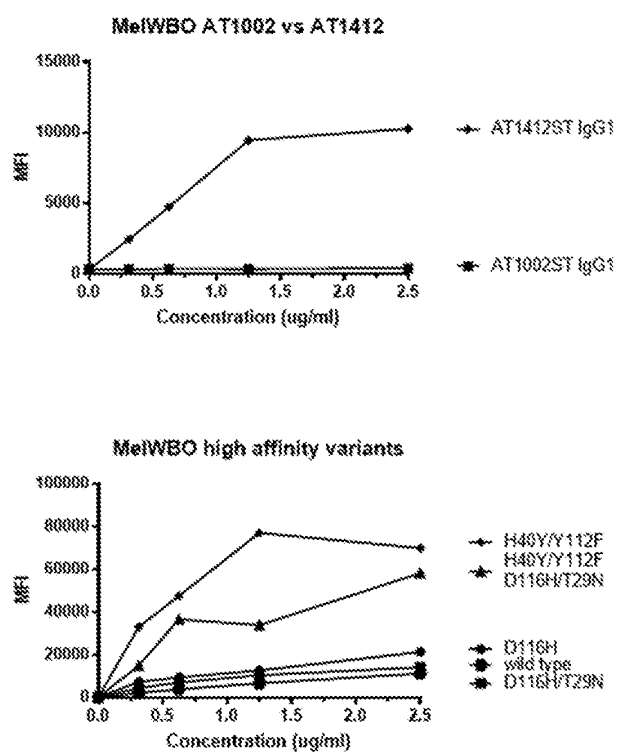


Figure 19C



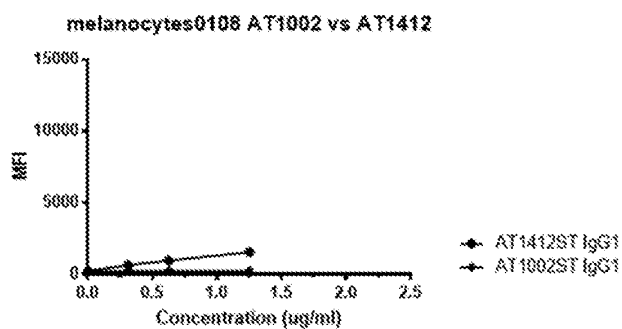
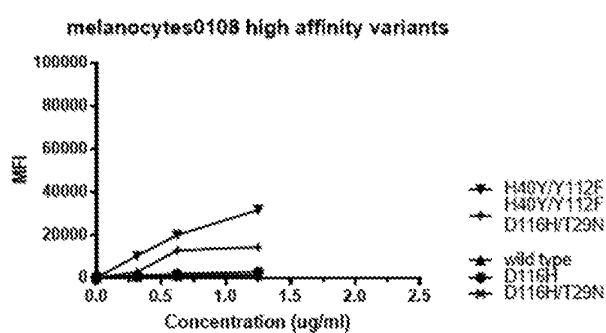
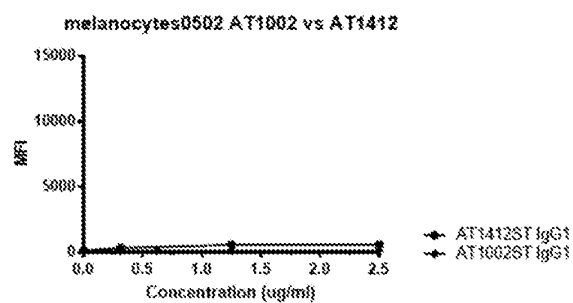
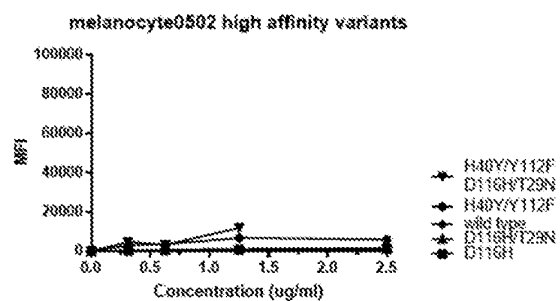


Figure 19C continued

Figure 19D

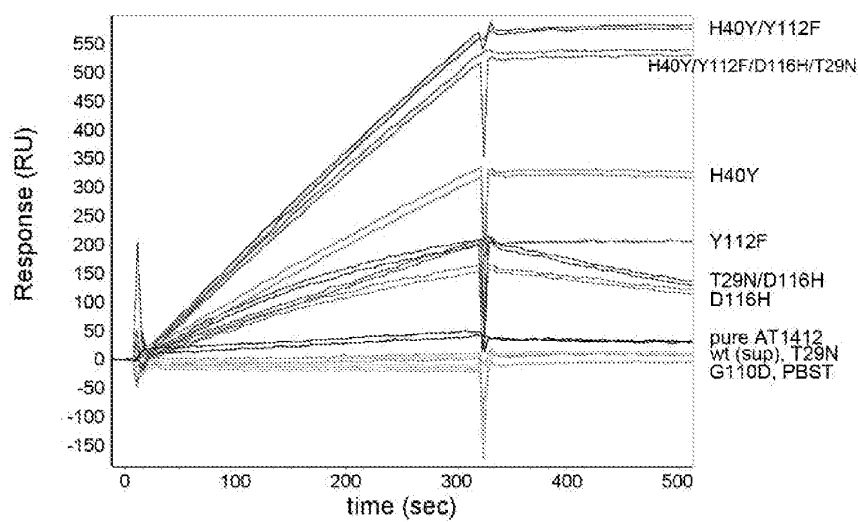


Figure 19E

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

Figure 20

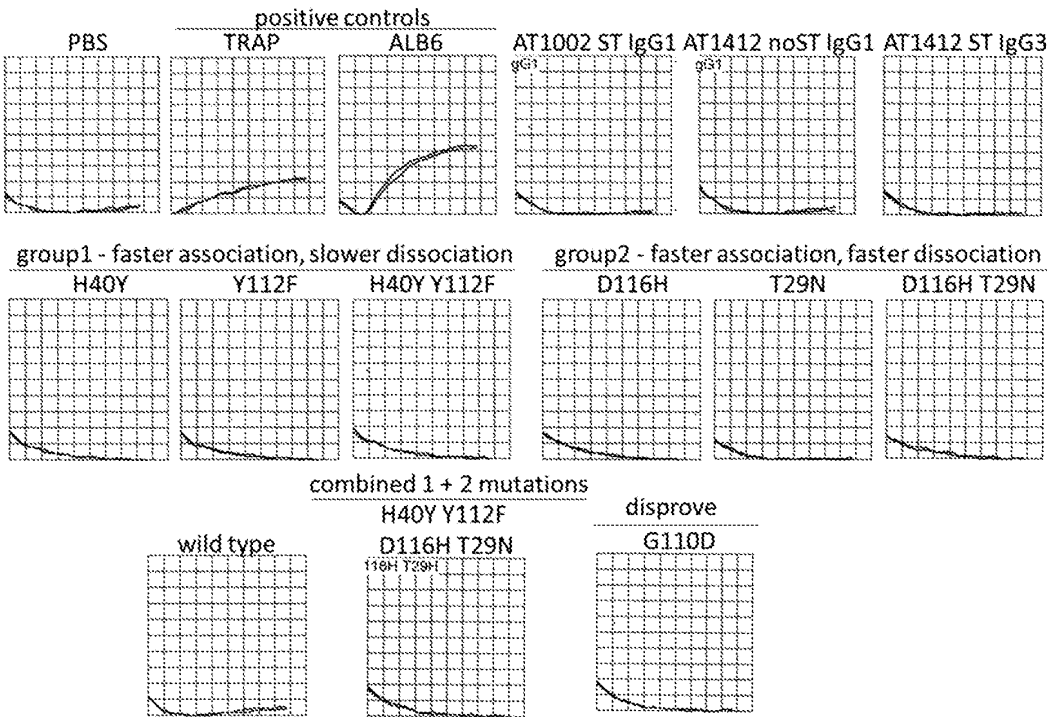


Figure 21

| 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 NP | +               | ~ | + | + | 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 <sup>(71, 74)</sup> |

Figure 22A

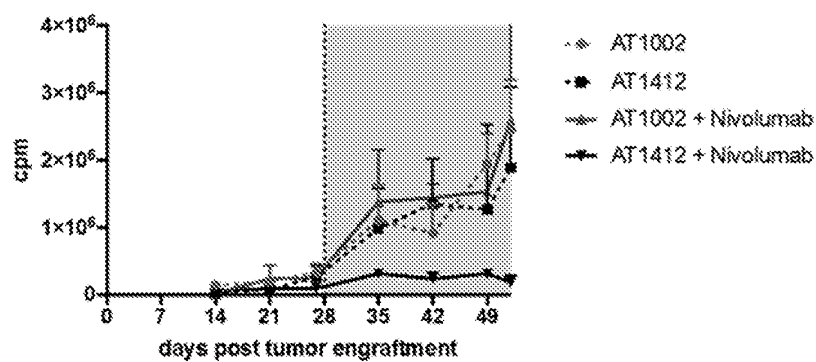


Figure 22B

