

FIGURE 1

Anti-CD28 VH1 Sequences

```

10      20      30      40      50      60      70      80      90     100
GAGGTGAAGCTGCAGCAGCTCAGGACCTGGCCTGGTGAAGCCCTCAGAGACCCTGTCCTCACTTGTACTGTCTCTGGGTTTTCATTAAAGCGACIATGGTG
E V K L Q Q S G P G L V K P S E T L S L T C T V S G F S L S D Y G

10      20      30      40      50      60      70      80      90     100
TTCAITGGGTTGCGCCAGGCTCCAGGAAGGGAGCTGGAGTGGTGGAGTAAATATGGGCTGGTGGAGCCACGAATTATATTCGGCTCTCATGTCCAGAAA
V H W V R Q A P G K G L E W L G V I W A G G C T N Y N S A L M S R K

10      20      30      40      50      60      70      80      90     100
GACCATCAAGCAAGACACTCCAGAGCCCAAGTTTCTTAAATGAACAGTCTGACAGCTGCTGACACAGCCGCTGTATTACTGTGCCAGAGATAAGGGA
T I S K D N S K S Q V F L K W N S L T A A D T A V Y Y C A R D K G

10      20      30      40      50      60      70      80      90     100
TACTCCTATTACTATTCTATGGACTACTGGGGCCCAAGCCACCCCTGGTCACCGTCTCCTCA
Y S Y Y Y S M D Y W G Q G T L V T V S S
100 A B C D

```

CDR definitions and protein sequence numbering according to Kabat. CDR nucleotide and protein sequences are highlighted in red

FIGURE 2

Anti-CD28 VH2 Sequences

```

10      20      30      40      50      60      70      80      90     100
GAGGTGAAGCTGCAGCAGTCCAGGACCTGGCCCTGGTGAAGCCCTCAGAGACCCCTGTCCCTCAGCTTGTACTGTCTCTGGGTTTTTCATTAAACCGACTATGGTG
E V K L Q Q S G P G L V K P S E T L S L T C T V S G F S L S D Y G
10      20      30      40      50      60      70      80      90     100
TTCATTGGGTTGCGCCAGGCTCCAGGAAGGGACTGGAGTGGCTGGTGGGAGTAATATGGGCTGGTGGAGGACGCAATTAATTCGGCTCTCATGTCCAGAAA
V H W V R Q A P G K G L E W L G V I W A G G G T N Y N S A L M S R K
10      20      30      40      50      60      70      80      90     100
GACCATCAGCAAGACAACCTCCAGAGCCCAAGTTCCTTAAATAATGAGCAGTGTGACAGCTGCTGACACAGCCGCTGTATTACTGTGCCACAGAGATAAGGGA
T I S K D N S K S Q V S L K M S S V T A A D T A V Y Y C A R D K G
10      20      30      40      50      60      70      80      90     100
TACTCCTATTACTATTCTATGGACTACTGGGCGCAAGCCCTGGTACCCGTCTCTCA
Y S Y Y S M D Y W G Q T L V T V S S
100 A B C D

```

CDR definitions and protein sequence numbering according to Kabat. CDR nucleotide and protein sequences are highlighted in red

FIGURE 3

Anti-CD28 VH3 Sequences

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10      20      30      40      50      60      70      80      90     100
GAGGTGAAGCTGCAGCAGTCCAGGACCTGGCTGGTGAAGCCCTCAGAGACCCCTGTCCTCACTTGTACTGTCTCTGGGTTTTTCATTAAAGCGACTATGGTG
E V K L Q Q S G P G L V K P S E T L S L T C T V S G F S L S D Y G
10      20      30
110     120     130     140     150     160     170     180     190     200
TTCATTGGGTTCCGCCAGGCTCCAGGAAGCGGACTGGAGTGGCTGGGAGTAATATGGGCTGGTGGAGGCACGAATTATAATTCGGCTCTCATGTCCAGAGT
V H W V R Q A P G K G L E W L G V I W A G G G T N Y N S A L M S R V
40      50      60
210     220     230     240     250     260     270     280     290     300
GACCATCAGCAAGACAACCTCCAAGAGCCCAAGTTTCCTTAAACTGAGCAGTGTGACAGCTGCTGACACAGCCGTGTATTACTGTGCCAGAGATAAGGGA
T I S K D N S K S Q V S L K L S S V T A A D T A V Y Y C A R D K G
70      80      82      90
310     320     330     340     350     360
TACTCCTATTACTATTCTATGGACTACTGGGGCCCAAGGCACCCCTGGTCACCGTCTCCTCA
Y S Y Y Y S M D Y W G G Q G T L V T V S S
100 A B C D
110

```

CDR definitions and protein sequence numbering according to Kabat. CDR nucleotide and protein sequences are highlighted in red.

FIGURE 4

Anti-CD28 VK1 Sequences

```

10      20      30      40      50      60      70      80      90      100
GACATCGAGCTCACTCAGTCTCCAGATTCTTTGGCTGTGTCTCTAGGGGAGAGAGCCACCATCAACTGCAGAGCCAGTGAAGTGTGTAATATTATGTC
D I E L T Q S P D S L A V S L G E R A T I N C R A S E S V E Y Y V
10      20      30      40      50      60      70      80      90      100
CAAGTTTAATGCAGTGGTACCCAGCAGAGAGCCAGGACAGCCACCAACTCTCTATCTTTGCTGCAACGTAAGTCTGGGGTCCCTGACACAGGTTTA
T S L M Q W Y Q Q K P G Q P K L L I F A A S N V E S G V P D R F
30      40      50      60      70      80      90      100      110
TGGCAGTGGGTCTGGGACAAACTTCACCCCTACCCATCTCTCTCTGCGAGGAGGAGGATGTTGCAATGTAATTTCTGTCAGCAAGTAGGAAGGTTCTTAA
G S G S G T N F T L T I S S L Q E E D V A M Y F C Q Q S R K V P Y
70      80      90      100      110
ACGTTGCGAGGGGGGACCAAGGTGGAATAAAA
T F G G G T K V E I K
110

```

CDR definitions and protein sequence numbering according to Kabat. CDR nucleotide and protein sequences are highlighted in red

FIGURE 5

Anti-CD28 VK2 Sequences

```

10      20      30      40      50      60      70      80      90      100
GACATCGAGCTCACATCAGTCTCCAGATTCTTTGGCTGTCTCTAGGGGAGAGAGCCCACTCAACTGCAGAGCCCACTGAGAGTGTGAAATATGTCA
D I E L T Q S P D S L A V S L G E R A T I N C R A S E S V E Y Y V
10      20      27 A B C D

110     120     130     140     150     160     170     180     190     200
CAAGTTTAATGCAGTGGTACCAGCAGAGAGCCAGGACAGCCCACTCTCTCATCTTTGCTGCATCCCAAGTAGAATCTGGGGTCCCTGACAGGTTTAG
T S L M Q W Y Q Q K P G Q P P K L L I F A A S N V E S G V P D R F S
30      40      50      60

210     220     230     240     250     260     270     280     290     300
TGCAGTGGGTCTGGGACAACTTCACCCCTCACCATCTCTCTCTGCGAGCCGAGGATGTTGCAATGTATTTCTGTCAAGCAAGTAGGAAGGTTCCCTTAC
G S G S G T N F T L T I S S L Q A E D V A M Y F C Q Q S R K V P Y
70      80      90

310     320     330
ACGTTCCGGAGGGGGACCAAGCTGGAATAAAA
T F G G G T K V E I K
110

```

CDR definitions and protein sequence numbering according to Kabat. CDR nucleotide and protein sequences are highlighted in red

FIGURE 6

Anti-CD28 VK3 Sequences

```

10      20      30      40      50      60      70      80      90     100
GACATCGAGCTCAGTCAGTCTCCAGATTCTTTGGCTGTGCTCTAGGGGAGAGAGAGCCCACTCAACTGCAGAGCCCACTGAGAGTGTGTTGAATATTATGTCA
D I E L T Q S P D S L A V S L G E R A T I N C R A S E S V E Y Y V
10
110     120     130     140     150     160     170     180     190     200
CAAGTTTAATGCAGTGGTACCAGCAGAGAGCCAGGACAGCCACCAACTCCTCATCTTTGCTGCATCCAACTAGAGATCTGGGCTCCCTGACAGGTTTAG
T S L M Q W Y Q Q K P G Q P P K L L I F A A S N V E S G V P D R F S
30      40      50      60
210     220     230     240     250     260     270     280     290     300
TGGCAGTGGGTCTGGGACAGACTTCACCCCTCACCATCTCTCTCTGCGAGCCGAGGATGTTGCAATGTATTTCTGTCAGCAAAGTAGGAAGGTTCTCTTAC
G S G S G T D F T L T I S S L Q A E D V A M Y F C Q Q S R K V P Y
70      80      90
310     320     330
ACGTTGCGAGGGGGGACCAAGGTGGAATAAAA
T F G G G T K V E I K
110
```

CDR definitions and protein sequence numbering according to Kabat. CDR nucleotide and protein sequences are highlighted in red

FIGURE 7

Modified Constant Heavy:

```

10      20      30      40      50      60      70      80      90     100
GTTCCACCAAGGGCCCATCCGTCTTCCCTGGCGCCCTCCAGGAGCACAGCCGCCCTGGCTGGCTGGTCAAGGACTACTTCC
A S T K G P S V F P L A P C S R S T S E S T A A L G C L V K D Y F
120
110      120      130      140      150      160      170      180      190     200
CCGAACCGGTGACGGTGTGGAACCTCAGCGCCCTGACCAAGCGCGGTGCACACCTTCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAG
P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S
150      154 156 157 162      159 171      180 182
CGTGGTGACCGTGCCCTCCAGCAGCTTGGCCACGAGACCTACACCTGCAATGTAGATCACAAAGCCAGCAACACCAAGGTGGACAAGAGTTGAGTCC
V V T V P S S S L G T K T Y T C N V D H K P S N T K V D K R V E S
190      200 203 205      210      220      223 226
210      220      230      240      250      260      270      280      290     300
CGTGGTGACCGTGCCCTCCAGCAGCTTGGCCACGAGACCTACACCTGCAATGTAGATCACAAAGCCAGCAACACCAAGGTGGACAAGAGTTGAGTCC
V V T V P S S S L G T K T Y T C N V D H K P S N T K V D K R V E S
190      200 203 205      210      220      223 226
310      320      330      340      350      360      370      380      390     400
AAATATGGTCCCCCATGCCACCATGCCAGCACCTGAGTTCGAGGGGGGACCATCAGTCTTCTGTTCCTCCCGCCAAACCCCAAGGACACTCTCATGATCT
K Y G P P C P C P A P E F E G G P S V F L F P P K P K D T L M I
228 232 233 235 237 239 240      250      260
410      420      430      440      450      460      470      480      490     500
CCCGGACCCCTGAGGTACAGTCCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAGTTCAGTGGATGGCGTGGAGGTGCATAATGC
S R T P E V T C V V V D V S Q E D P E V Q F N W Y V D G V E V H N A
270      280      290      292 295 296 299 300

```

FIGURE 7 (continued)

510 520 530 540 550 560 570 580 590 600
 CAAGACAAGCCCGGAGGAGCAGTTCAACAGCCACGTACCGTGTGGTCAGCGTCTCTCACCCTCTGCAACAGGACTGGCTGAACGGCAAGGAGTACAAAG
 K T K P R E E Q F N S T Y R V V S V L T V L H Q D W L N G K E Y K
 310 314 317 320 330

610 620 630 640 650 660 670 680 690 700
 TGCAGGTCTCCAAAGAGGCTCCCGTCCCTCCATCGAGAAACCATCTCCAAAGCCAGCGCCGAGAGCCACAGGTGTACACCTGCCCCCAT
 C K V S N K G L P S S I E K T I S K A K G Q P R E P Q V Y T L P P
 340 350 354 357 360 363 370

710 720 730 740 750 760 770 780 790 800
 CCCAGGAGGAGTACCAAGAACCCAGGTACGCTGACCTGCCTGGTCAAGGCTTCTACCCAGCGGACATCGCCGTGGAGTGGGAGACCAATGGGCAGCC
 S Q E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P
 378 381 390 400 402 405 408 410 411 414

810 820 830 840 850 860 870 880 890 900
 GGAGAACAACTACAAGACCACCGCTCCCGTGGTGGACTCCGACGGCTCCTTCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG
 E N N Y K T T P P V L D S D G S F F L Y S R L T V D K S R W Q E G
 420 428 430 433 440 450

910 920 930 940 950 960 970 980
 AATGTCTTCATGCTCCGTGATGCATGAGGCTCTGCACACCACTACACACAGAGAGCCTCTGCCCTGTCTCTGGGTAAATGA
 N V F S C S V M H E A L H N H Y T Q K S L C L S L G K .
 460 470

Protein sequence numbering according to Kabat. Mutations are highlighted red.

FIGURE 8

Constant κ Light:

```

10      20      30      40      50      60      70      80      90     100
CGAACTGTGGCTGCACCATCTGTCTTTCATCTTCCGGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCCTCTGTTGTGTGCTGCTGAATAACTTCTATC
R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L N N F Y
110
110     120     130     140     150     160     170     180     190     200
CCAGAGAGGCCCAAGTACAGTGGAGGTGGATAAGGCCCTCCCAATCGGGTAACCTCCAGGAGAGTGTCAACAGCAGGACAGCAAGGACAGCACCTACAG
P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S
150
210     220     230     240     250     260     270     280     290     300
CCTCAGCAGCACCCCTGACGCTGAGCAAGCAGACTACGAGAAACACAAAGTCTACGCCCTGCCAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG
L S S T L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K
180
310     320
AGCTTCACAGGGGAGAGTGTAG
S F N R G E C .
200

```

Protein sequence numbering according to Kabat.

FIGURE 9

Potential Non-Antigen Binding Humanised Variant

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10      20      30      40      50      60      70      80      90     100
GAGGTGAAGCTGCAGCAGTCAAGGACCTGGCCCTGGTGAAGCCCTCAGAGACCCCTGTCCTCACTTGTACGTCTCTCGGTTTACATTTCAGCGACTATGGTG
E V K L Q Q S G P G L V K P S E T L S L T C T V S G F T F S D Y G

10      20      30      40      50      60      70      80      90     100
TTCATTGGATTGCCAGCCTCCAGAAAGGGACTGGAGTGGATCGGAGTAAATATGGGCTGGTGGAGGCACGAATTATAATTCGGCTCTCATGTCCAGAGT
V H W I R Q P P G K G L E W I G V I W A G G T N Y N S A L M S R V

210     220     230     240     250     260     270     280     290     300
GACCATCAGCGTGGACACCTCCAAGAACCAATTTTCCTTAAACTGAGCAGTGTGACAGCTGCTGACACAGCCGGTGTATTACTGTGCCAGAGATAAGGSA
T I S V D T S K N Q F S L K L S S V T A A D T A V Y Y C A R D K G

70      80      90      100      110
TACTCCGCTGCCGCTTCTATGGACTACTGGGGCCAAAGGCACCCCTGGTCACCGTCTCCTCA
Y S A A A S M D Y W G Q G T L V T V S S
100 A B C D 110

```

CDR definitions and protein sequence numbering according to Kabat. CDR nucleotide and protein sequences are highlighted in red

FIGURE 10

Gln Val Gln Leu Thr Arg Glu Gly Ser Gly Ser His Ser Met Arg Tyr
 1 5 10 15
 Phe Phe Thr Ser Val Ser Arg Pro Gly Arg Gly Glu Pro Arg Phe Ile
 20 25 30
 Ala Val Gly Tyr Val Asp Asp Thr Gln Phe Val Arg Phe Asp Ser Asp
 35 40 45
 Ala Ala Ser Gln Arg Met Glu Pro Arg Ala Pro Trp Ile Glu Gln Glu
 50 55 60
 Gly Pro Glu Tyr Trp Asp Gly Glu Thr Arg Lys Val Lys Ala His Ser
 65 70 75 80
 Gln Thr His Arg Val Asp Leu Gly Thr Leu Arg Gly Tyr Tyr Asn Gln
 85 90 95
 Ser Glu Ala Gly Ser His Thr Val Gln Arg Met Tyr Gly Cys Asp Val
 100 105 110
 Gly Ser Asp Trp Arg Phe Leu Arg Gly Tyr His Gln Tyr Ala Tyr Asp
 115 120 125
 Gly Lys Asp Tyr Ile Ala Leu Lys Glu Asp Leu Arg Ser Trp Thr Ala
 130 135 140
 Ala Asp Met Ala Ala Gln Thr Thr Lys His Lys Trp Glu Ala Ala His
 145 150 155 160
 Val Ala Glu Gln Leu Arg Ala Tyr Leu Glu Gly Thr Cys Val Glu Trp
 165 170 175
 Leu Arg Arg Tyr Leu Glu Asn Gly Lys Glu Thr Leu Gln Arg Thr Asp
 180 185 190
 Ala Pro Lys Thr His Met Thr His His Ala Val Ser Asp His Glu Ala
 195 200 205
 Thr Leu Arg Cys Trp Ala Leu Ser Phe Tyr Pro Ala Glu Ile Thr Leu
 210 215 220

FIGURE 10, continued

Thr Trp Gln Arg Asp Gly Glu Asp Gln Thr Gln Asp Thr Glu Leu Val
 225 230 235 240
 Glu Thr Arg Pro Ala Gly Asp Gly Thr Phe Gln Lys Trp Ala Ala Val
 245 250 255
 Val Val Pro Ser Gly Gln Glu Gln Arg Tyr Thr Cys His Val Gln His
 260 265 270
 Glu Gly Leu Pro Lys Pro Leu Thr Trp Ala Arg Glu Val Ser Glu Val
 275 280 285
 Lys Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Glu Thr Leu
 290 295 300
 Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Ser Asp Tyr Gly Val
 305 310 315 320
 His Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Gly Val
 325 330 335
 Ile Trp Ala Gly Gly Gly Thr Asn Tyr Asn Ser Ala Leu Met Ser Arg
 340 345 350
 Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Lys Leu
 355 360 365
 Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
 370 375 380
 Lys Gly Tyr Ser Ala Ala Ala Ser Met Asp Tyr Trp Gly Gln Gly Thr
 385 390 395 400
 Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
 405 410 415
 Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly
 420 425 430
 Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn

FIGURE 10, continued

13/39

435	440	445
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln		
450	455	460
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser		
465	470	475
Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser		
485	490	495
Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys		
500	505	510
Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val Phe Leu		
515	520	525
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu		
530	535	540
Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln		
545	550	555
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys		
565	570	575
Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu		
580	585	590
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys		
595	600	605
Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys		
610	615	620
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser		
625	630	635
Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys		
645	650	655

FIGURE 10, continued

14/39

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
660 665 670

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
675 680 685

Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln
690 695 700

Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
705 710 715 720

His Tyr Thr Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys
725 730

FIGURE 11

Asp Ile Glu Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Glu Ser Val Glu Tyr Tyr
 20 25 30
 Val Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45
 Lys Leu Leu Ile Phe Ala Ala Ser Asn Val Glu Ser Gly Val Pro Asp
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 65 70 75 80
 Ser Leu Gln Ala Glu Asp Val Ala Met Tyr Phe Cys Gln Gln Ser Arg
 85 90 95
 Lys Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
 100 105 110
 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160
 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175
 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 180 185 190
 His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 195 200 205
 Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

FIGURE 12

Glu Val Lys Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1 5 10 15
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Asp Tyr
 20 25 30
 Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45
 Gly Val Ile Trp Ala Gly Gly Gly Thr Asn Tyr Asn Ser Ala Leu Met
 50 55 60
 Ser Arg Lys Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
 65 70 75 80
 Lys Met Asn Ser Leu Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Arg Asp Lys Gly Tyr Ser Tyr Tyr Tyr Ser Met Asp Tyr Trp Gly Gln
 100 105 110
 Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
 115 120 125
 Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
 130 135 140
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
 145 150 155 160
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
 165 170 175
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
 180 185 190
 Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
 195 200 205
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
 210 215 220

FIGURE 12, continued

Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly Gly Pro Ser Val
 225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
 260 265 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 275 280 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
 290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 305 310 315 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
 325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 340 345 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
 355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
 405 410 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys
 435 440 445

FIGURE 13

Glu Val Lys Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1 5 10 15
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Asp Tyr
 20 25 30
 Gly Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45
 Gly Val Ile Trp Ala Gly Gly Gly Thr Asn Tyr Asn Ser Ala Leu Met
 50 55 60
 Ser Arg Lys Thr Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Ser Leu
 65 70 75 80
 Lys Met Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Arg Asp Lys Gly Tyr Ser Tyr Tyr Tyr Ser Met Asp Tyr Trp Gly Gln
 100 105 110
 Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
 115 120 125
 Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
 130 135 140
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
 145 150 155 160
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
 165 170 175
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
 180 185 190
 Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
 195 200 205

FIGURE 13, continued

Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Ser	Lys	Tyr	Gly	Pro	210	215	220	
Pro	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Phe	Glu	Gly	Gly	Pro	Ser	Val	225	230	235	240
Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	245	250	255	
Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu	260	265	270	
Val	Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	275	280	285	
Thr	Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	290	295	300	
Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	305	310	315	320
Cys	Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	325	330	335	
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	340	345	350	
Pro	Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	355	360	365	
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	370	375	380	
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	385	390	395	400
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	405	410	415	

FIGURE 13, continued

20/39

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu

420

425

430

His Asn His Tyr Thr Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys

435

440

445

FIGURE 14

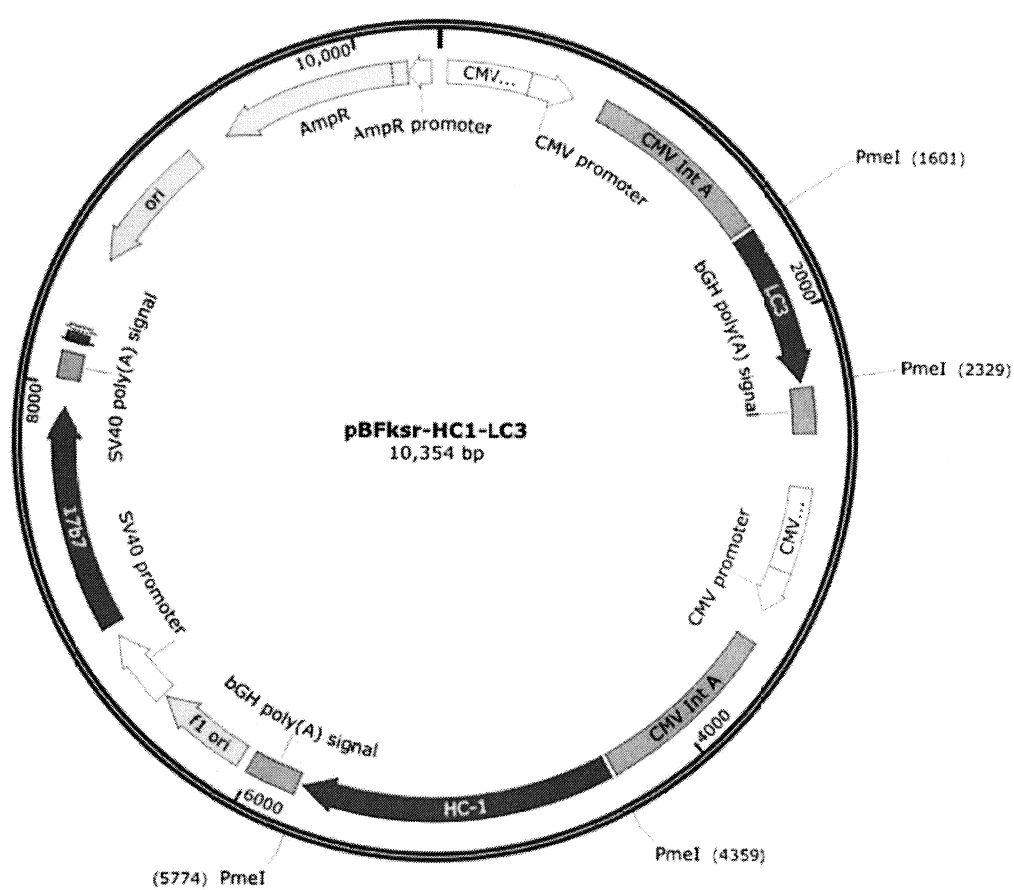


FIGURE 15

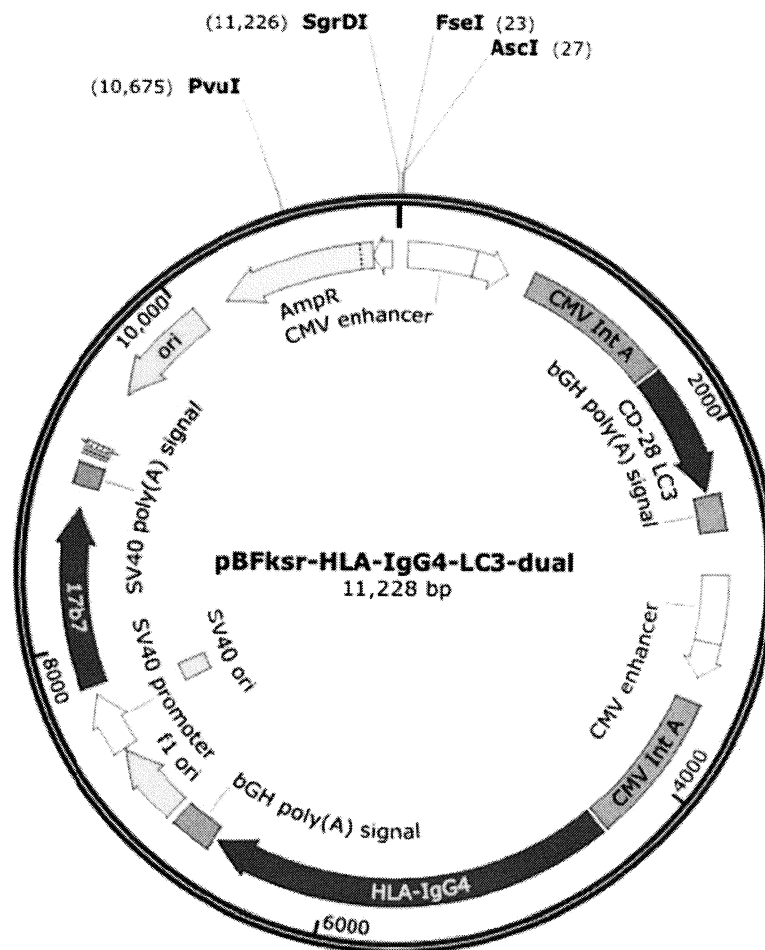


FIGURE 16

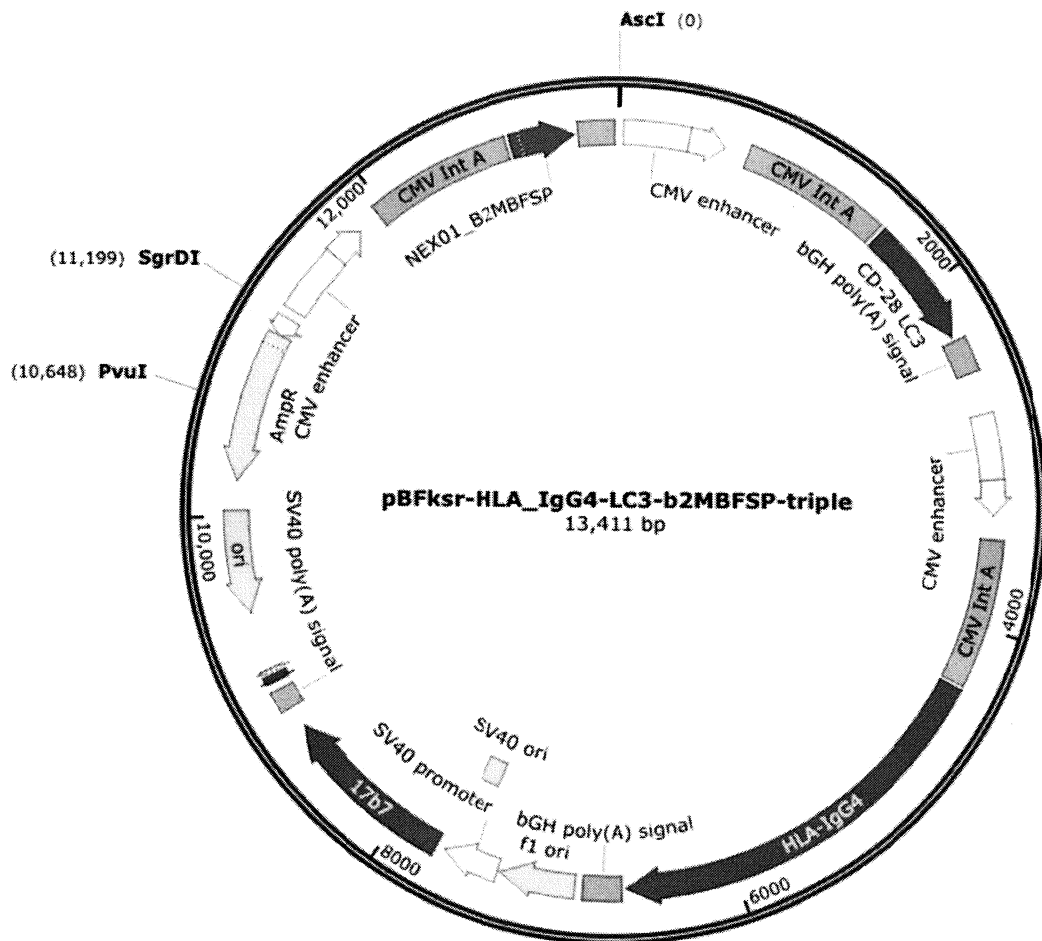


FIGURE 17

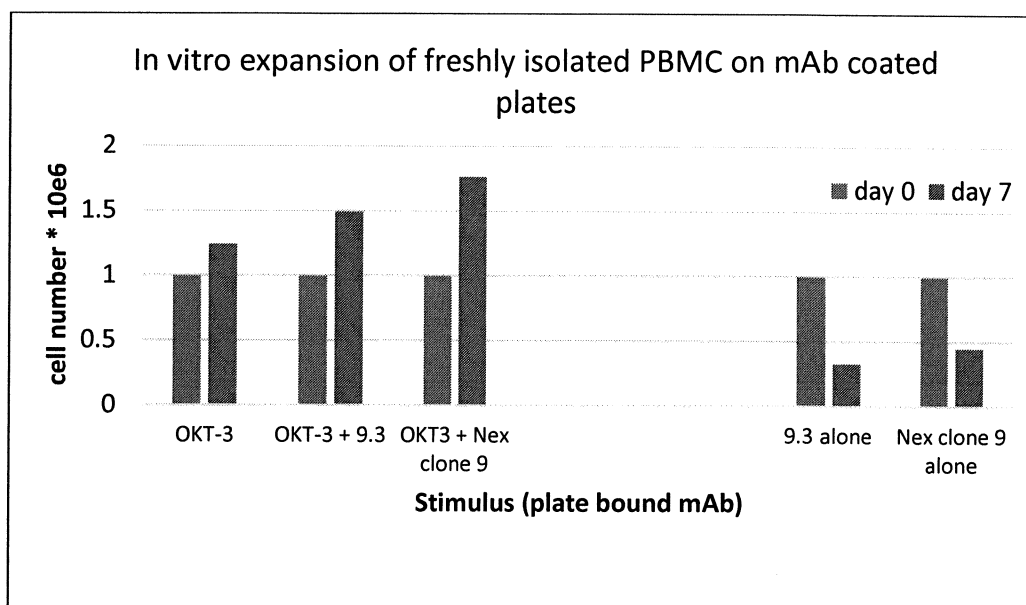
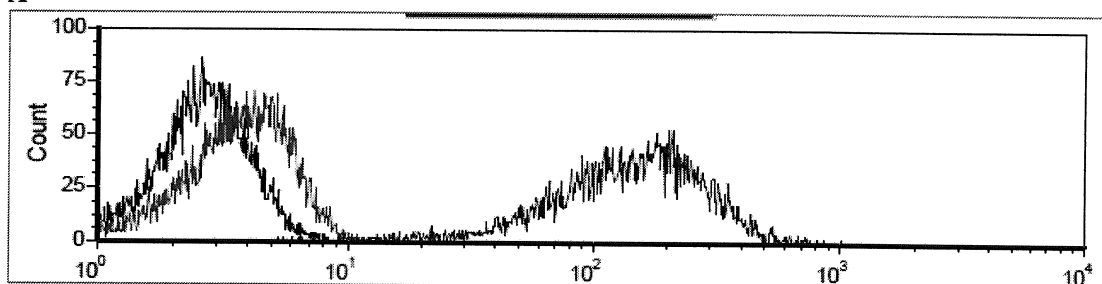


FIGURE 18

A



B

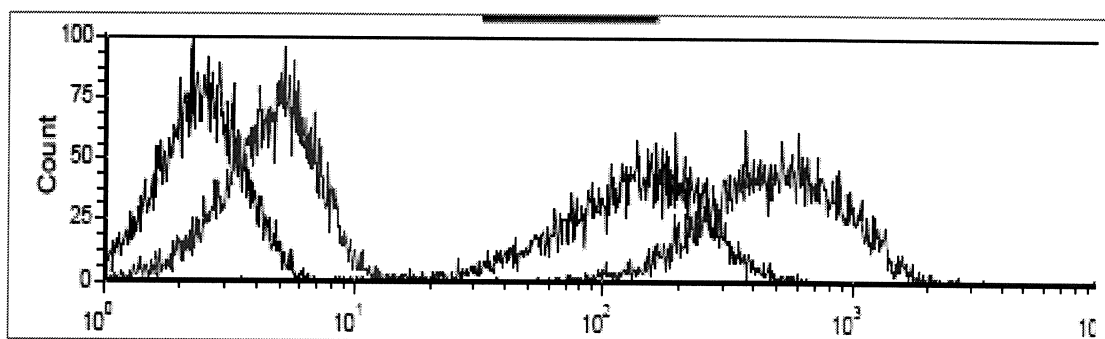
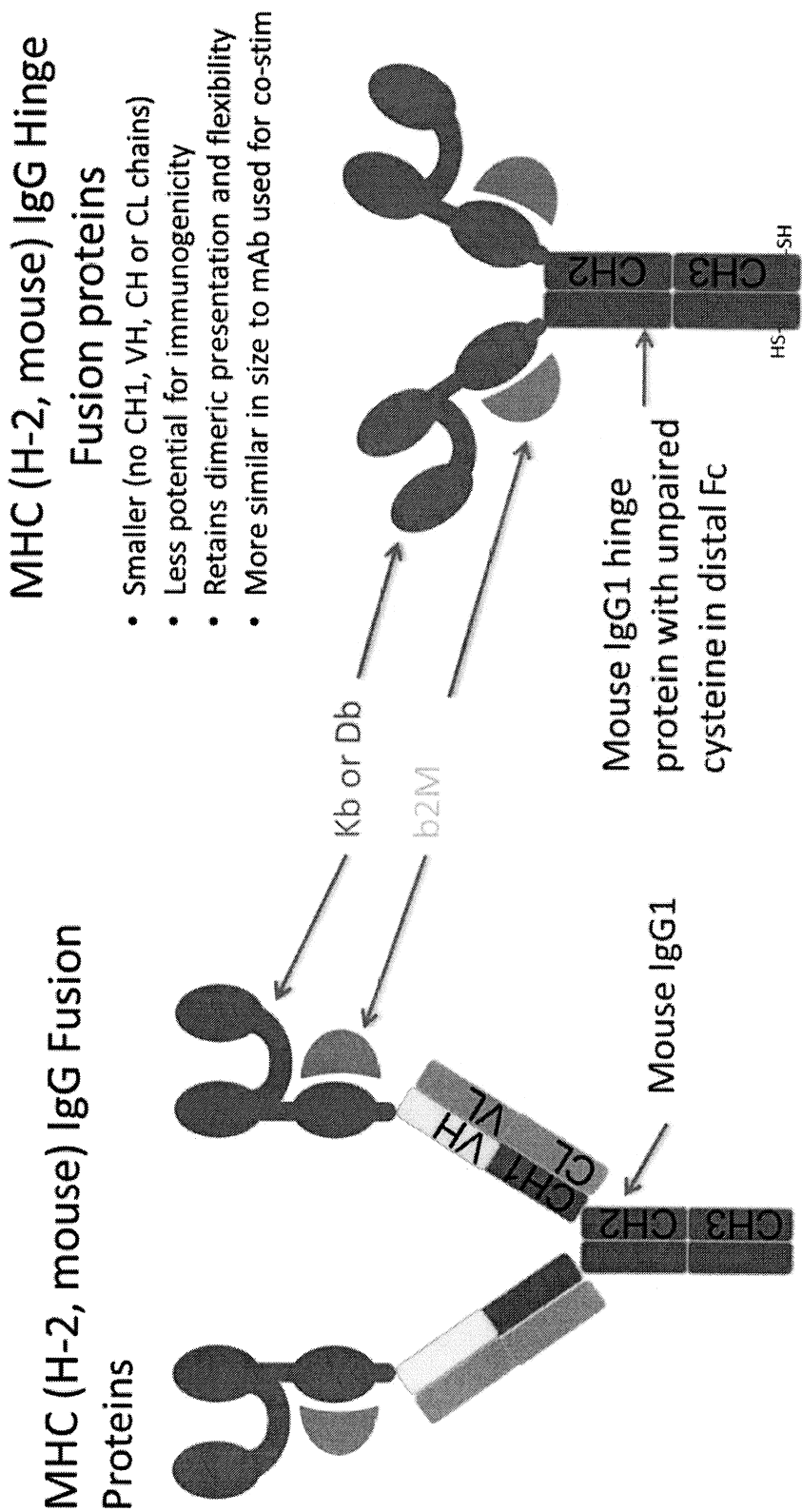


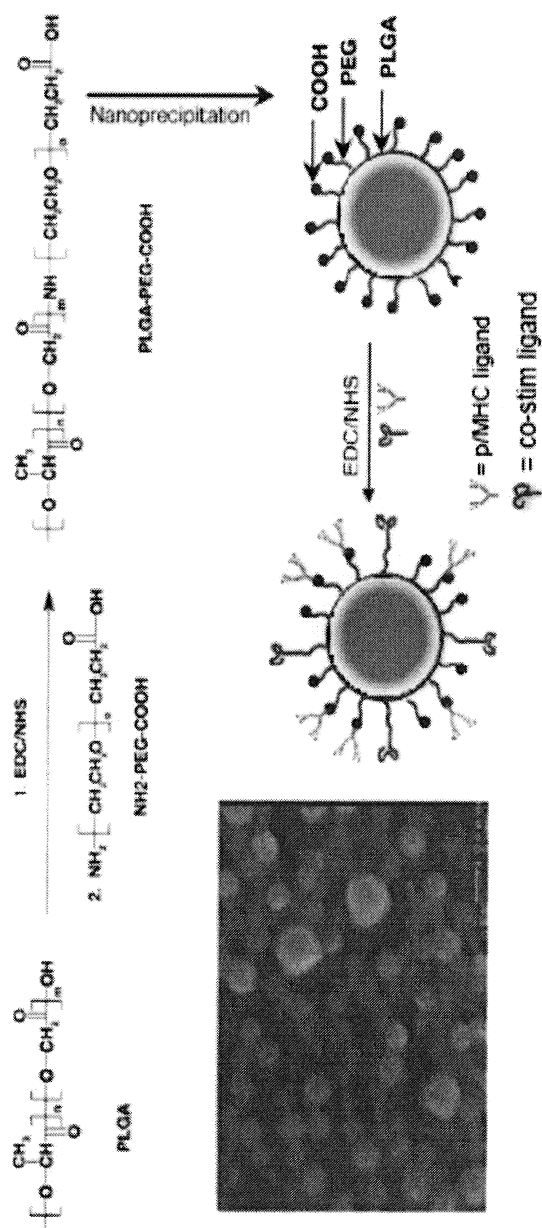
FIGURE 19



Used in v1.0 aAPC

Used in v2.0 aAPC

FIGURE 20



Batch	Composition	Ratio	Size	ζ (mV)	% Ligand	Active	Stable
PLGA	PLGA 40K	1.0	200 nm	-30 mV	H2-Ig 20% α -CD28 20%	+	N
63A	PLGA-PEG-COOH 40K/3K PLGA-mPEG 17K/3K	0.2 0.8	115 nm	-5 mV	H2-Ig > 50% α -CD28 > 50%	++	Y

- PLGA-PEG is biocompatible and recognized by FDA as safe for use in humans
- PEG provides “stealth” layer to prevent removal from circulation by phagocytic cells for better PK/PD
- Nano-size (<200 nm) allows distribution to target tissues (lymph nodes, spleen, tumor sites)
- Polymer composition provides long elimination half-life and infrequent dosing

FIGURE 21

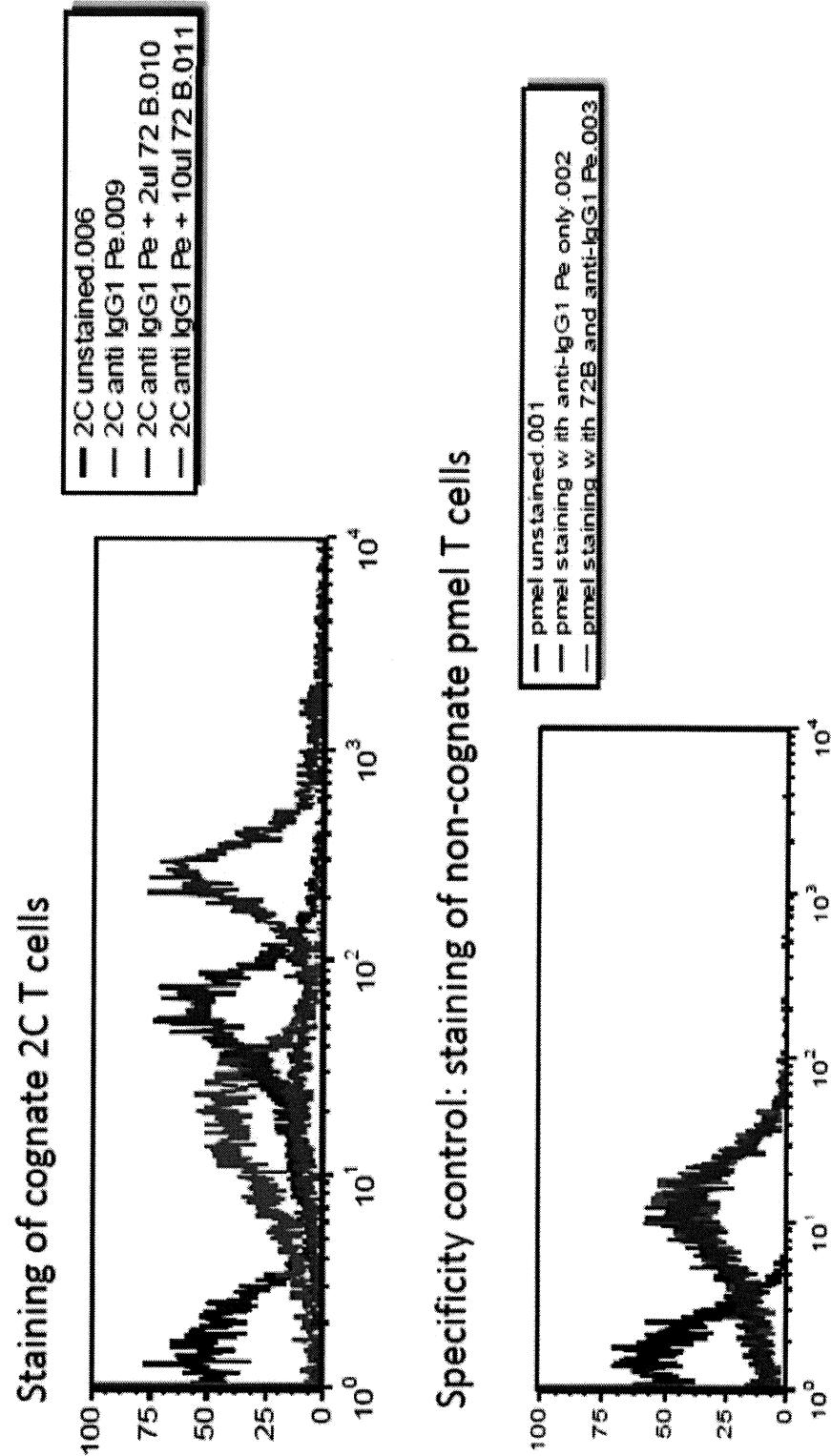
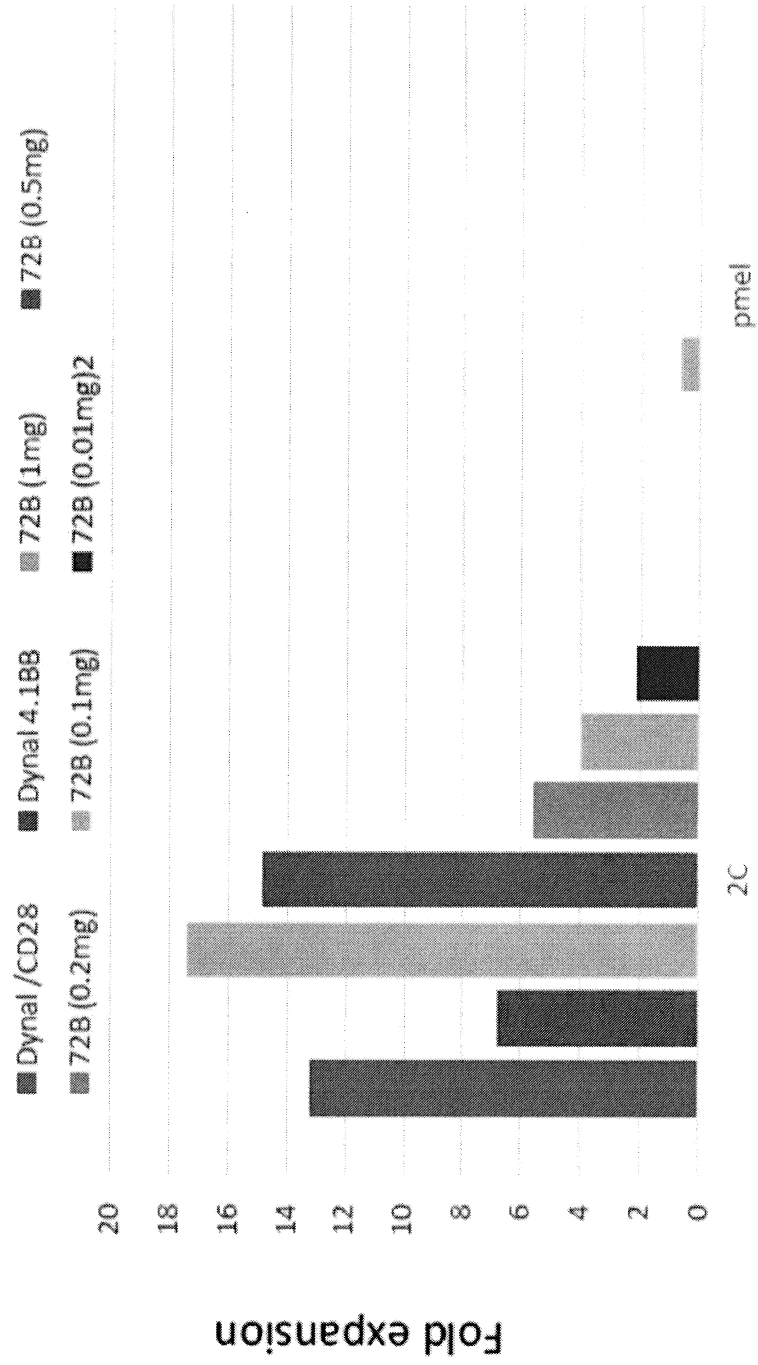


FIGURE 22



Batch 72B nano-aAPC at indicated dose per 10e6 2C T cells (day 5)

FIGURE 23

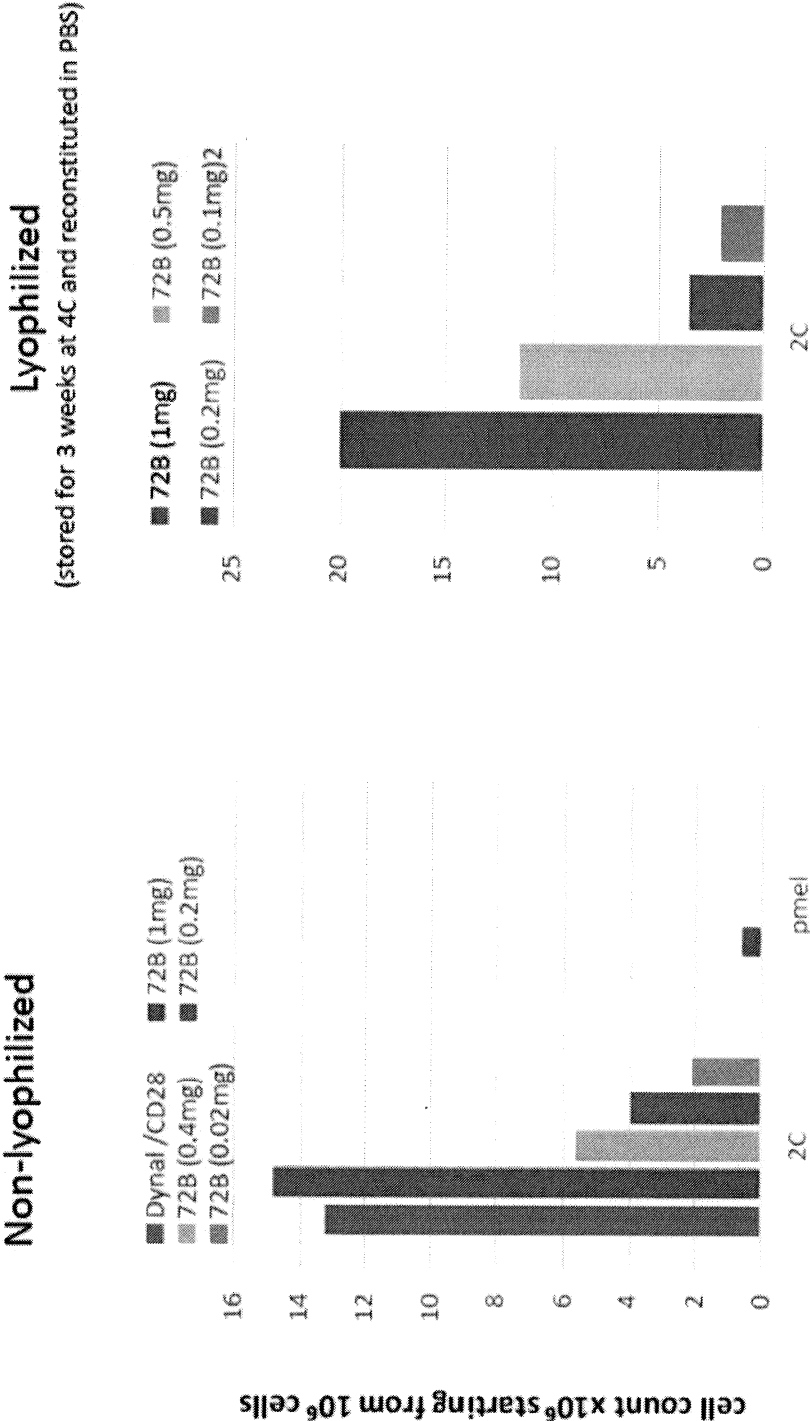
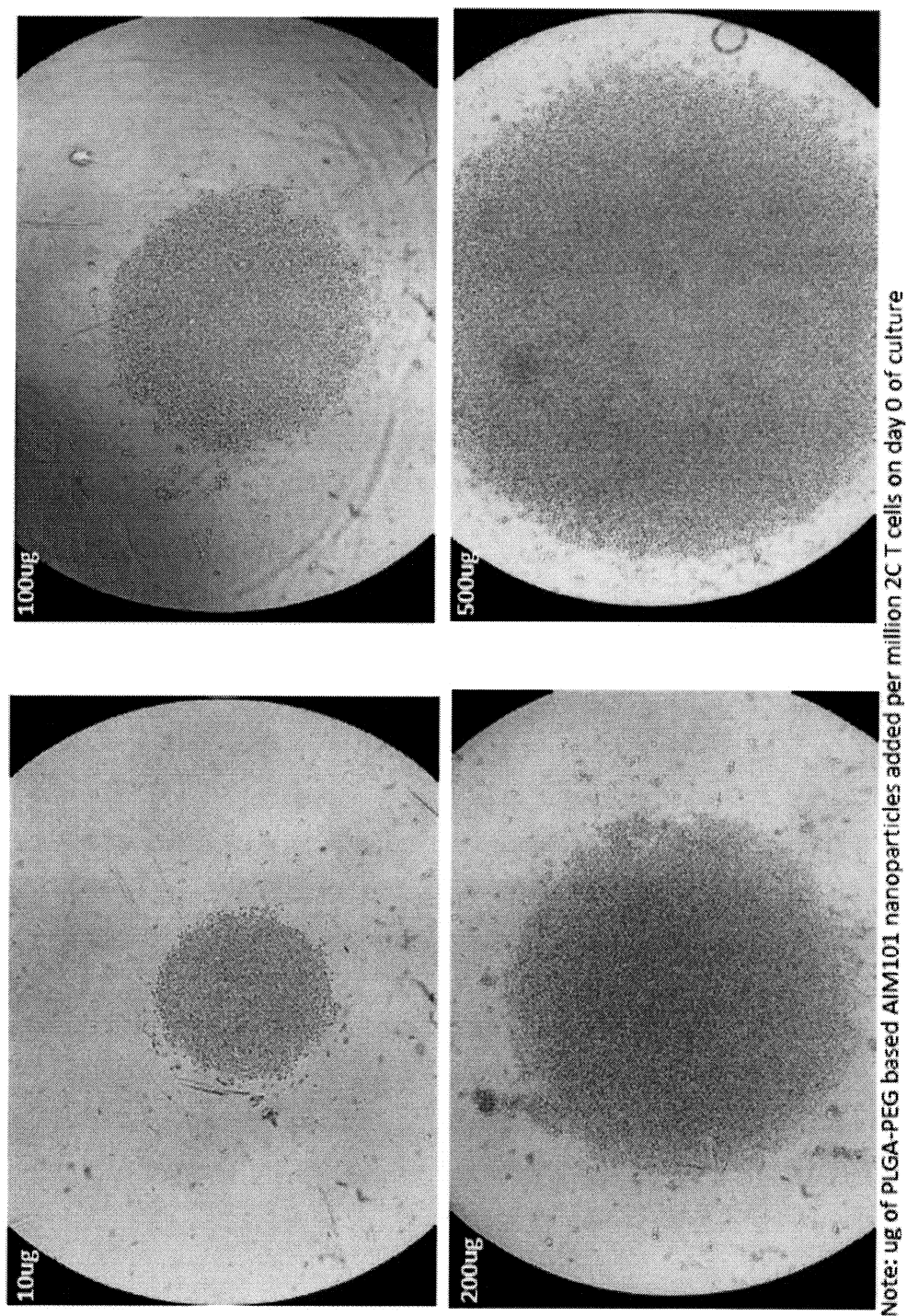


FIGURE 24



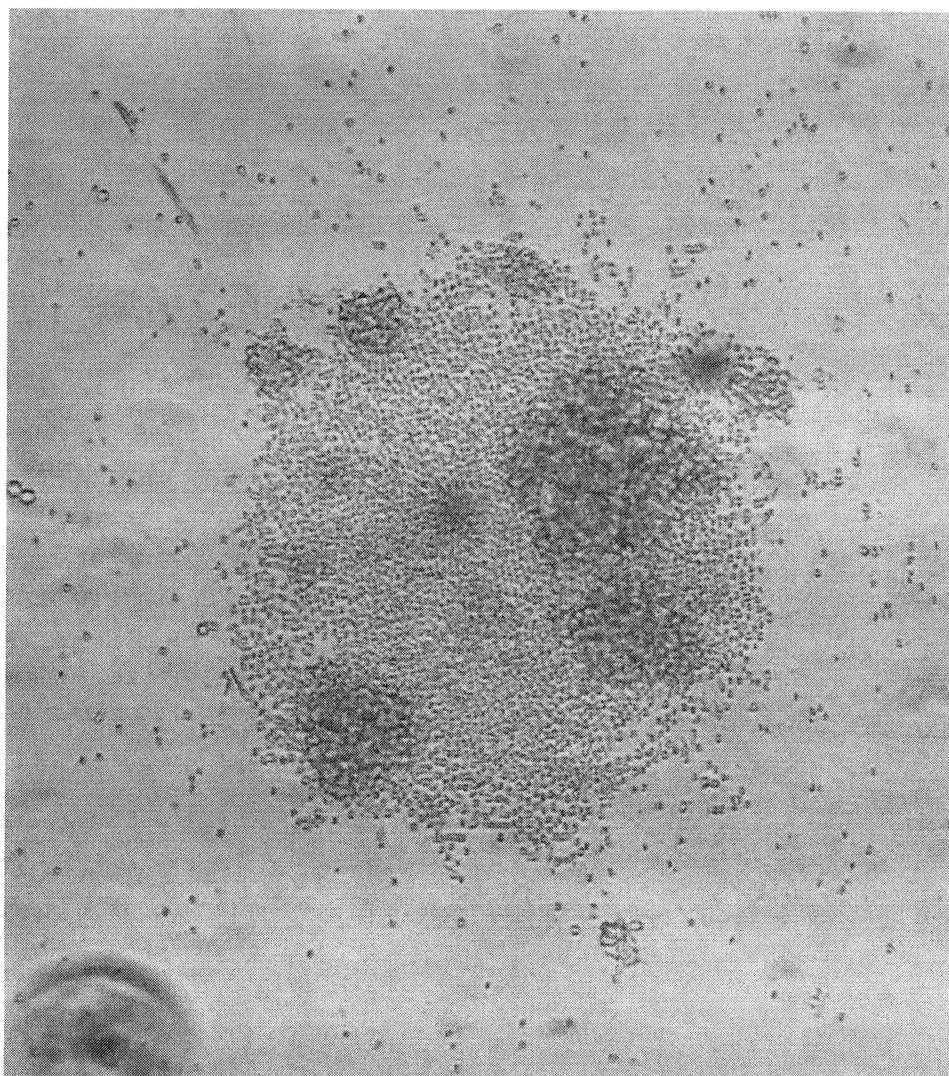


FIGURE 25

FIGURE 26

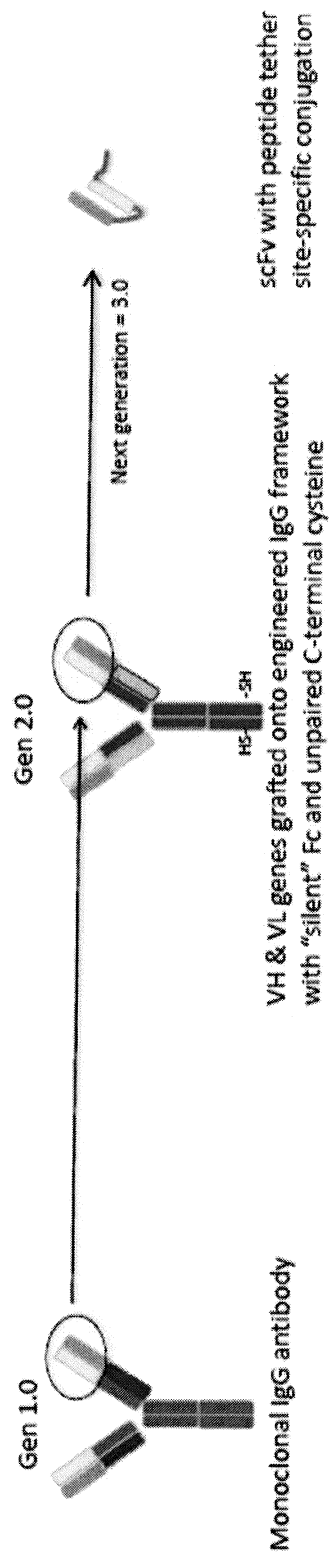
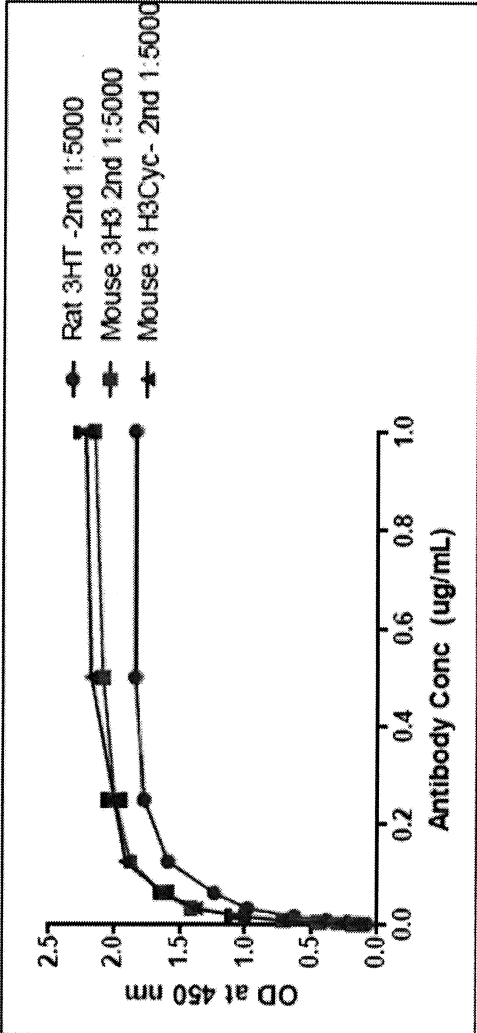


FIGURE 27

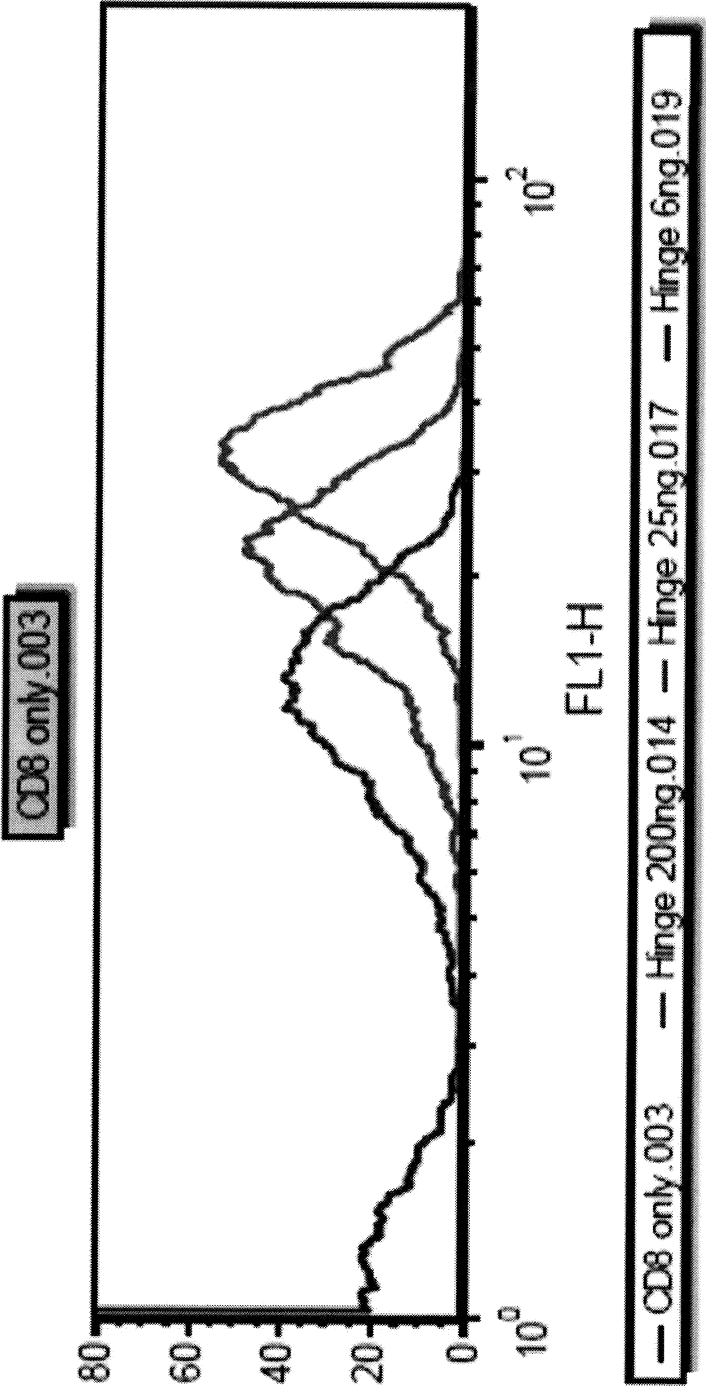


FIGURE 28

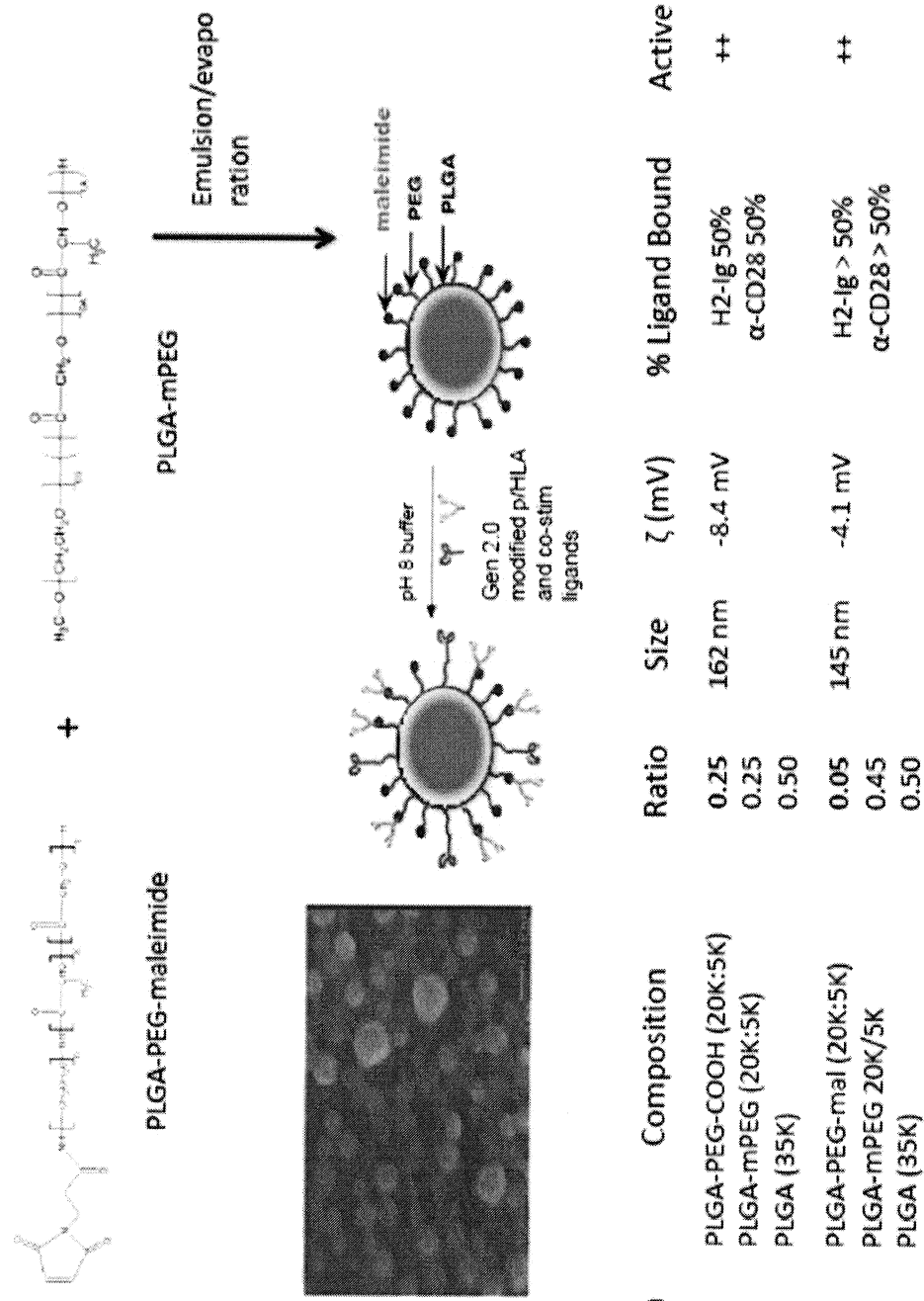
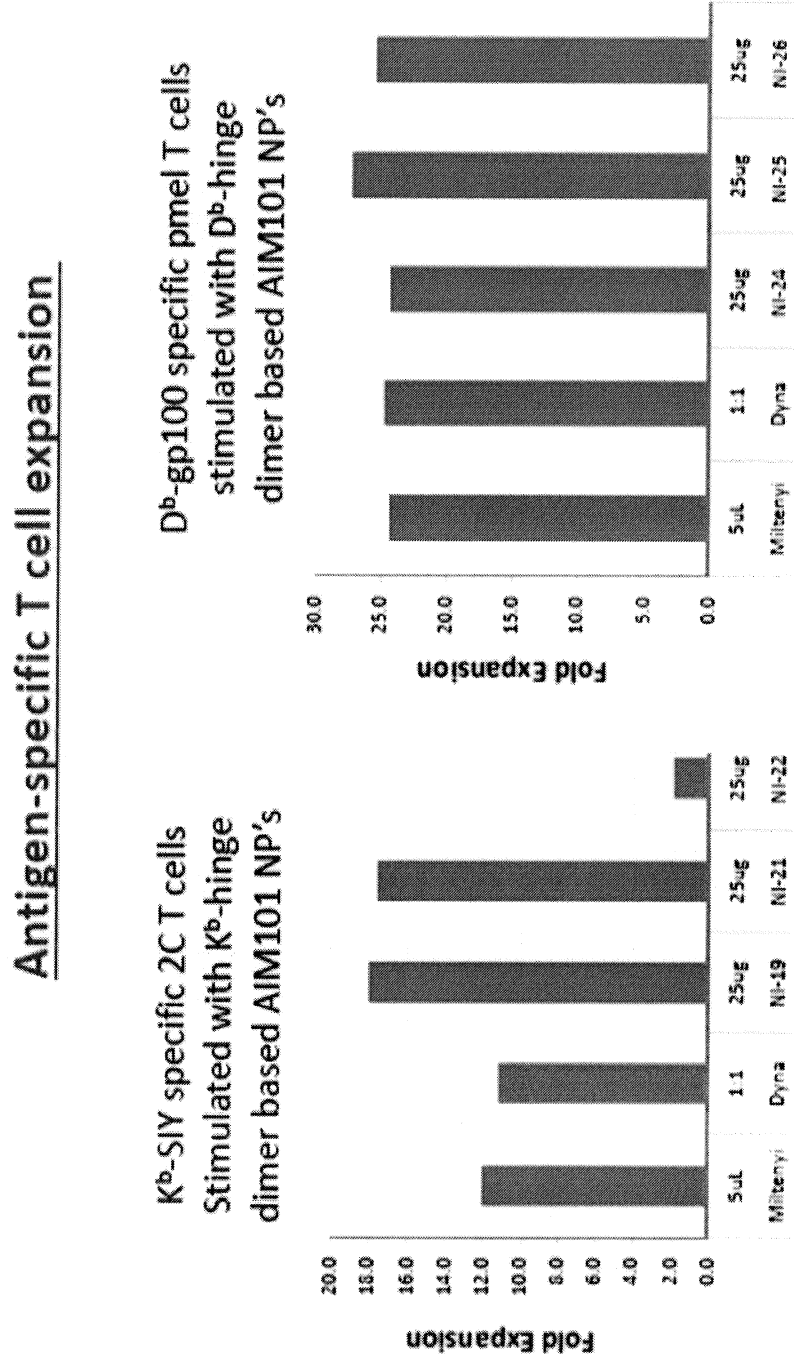


FIGURE 29



Stimulus per 10e6 T cells

FIGURE 30

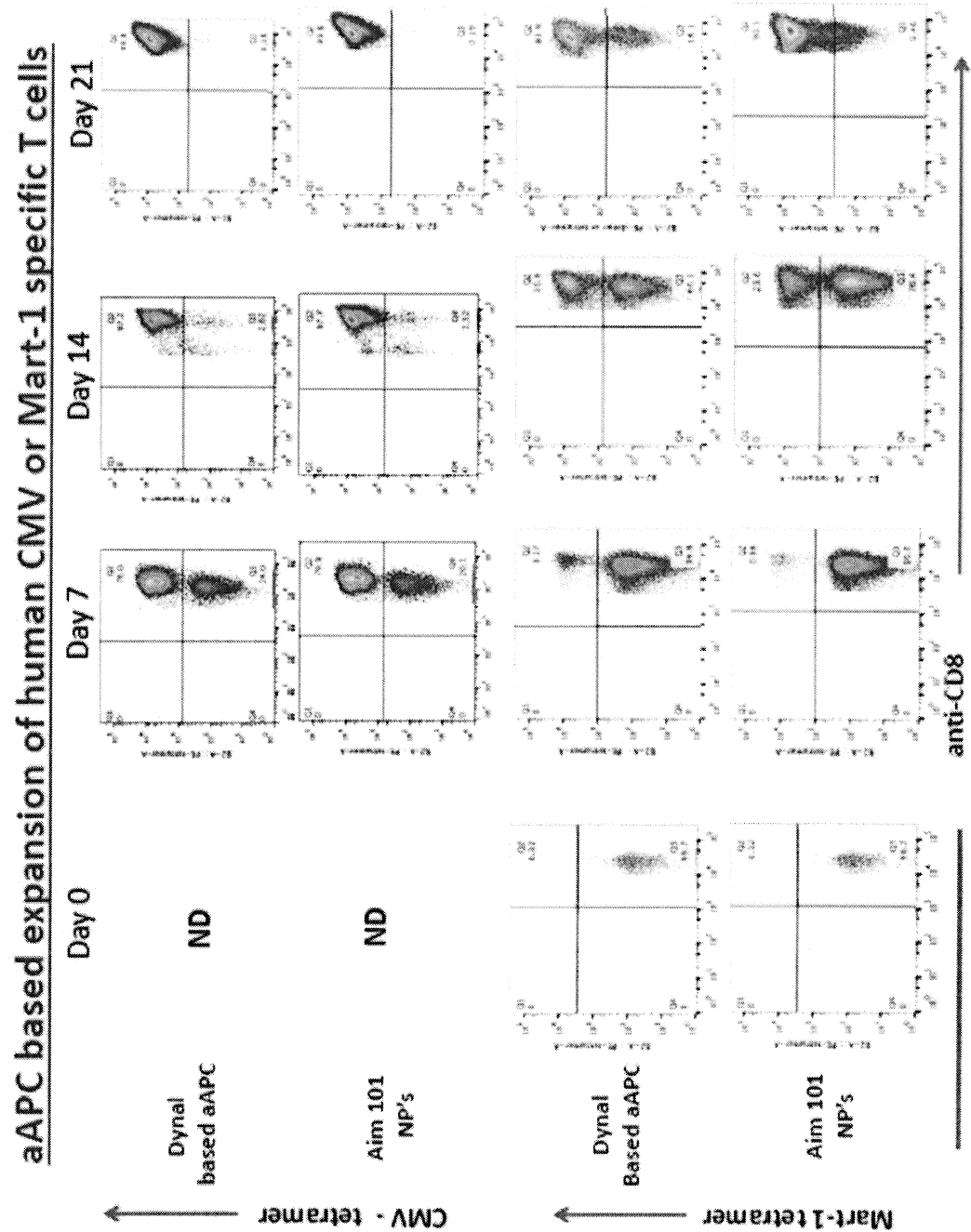


FIGURE 31

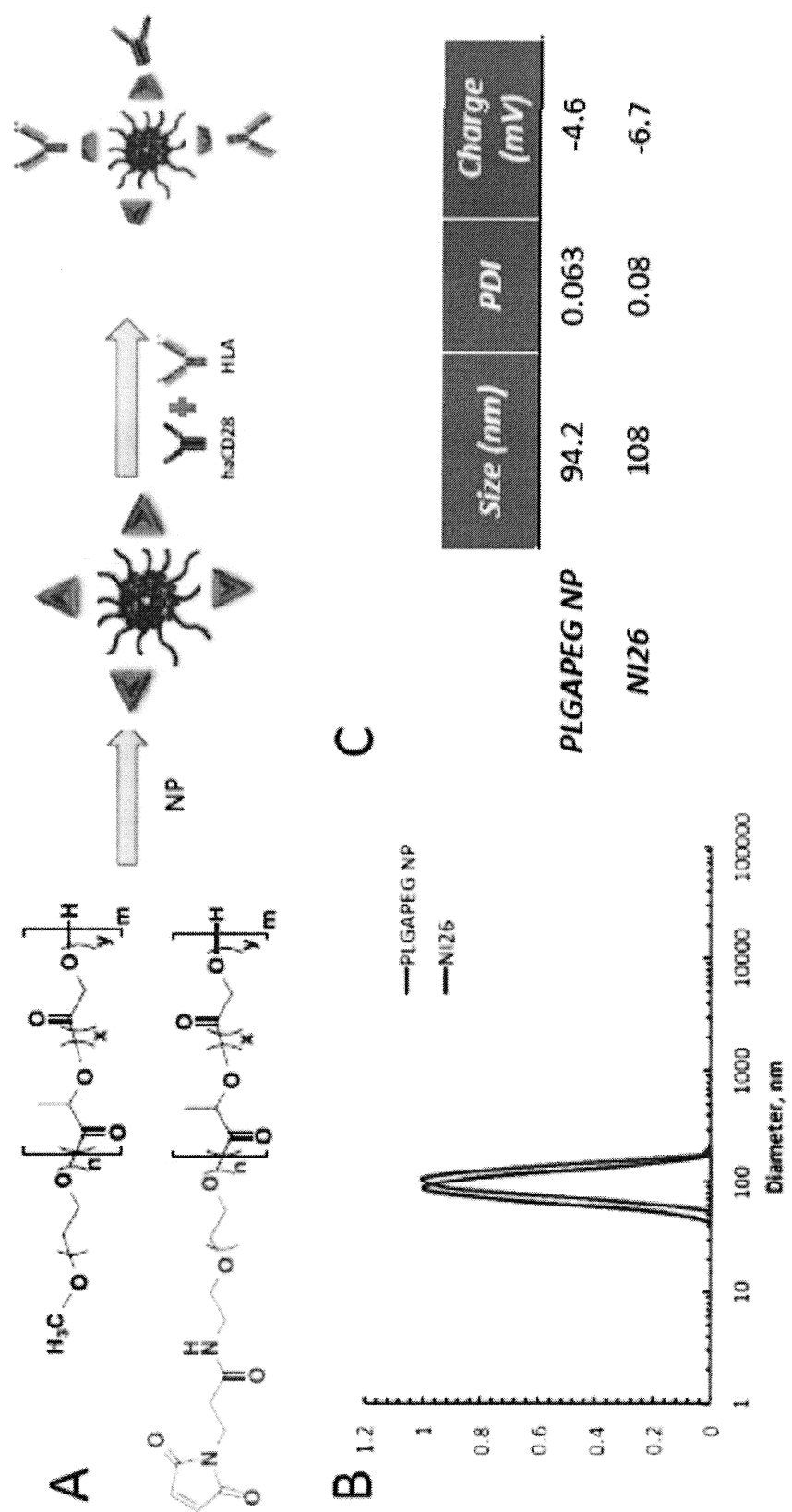


FIGURE 32

Batch	Maleimide content (%w/w)	Average size (nm)	ξ (mV)	μ g Prot input/mg NP	Coupling efficiency, %	# ligands/NP
NI19	5	91.8	-8.7	51.5	41.6	45.8
NI20	25	95.9	-4.9	51.5	95.8	120.2
NI21	55	98	-3.1	51.5	93.5	125.2
NI22	55	102	-8.6	51.5	79.1	119
NI23*	0	68	-9.2	51.5	-----	-----
NI24	1	80.9	-2.5	51.5	22.9	17.3
NI25	10	92.9	-5.8	51.5	67.2	76.8
NI26	10	108	-6.7	51.5	59.6	106.8

*Batch NI23 was used to determine the amount of protein that absorbs nonspecifically to nanoparticles. The amount of protein absorbed nonspecifically was found $\leq 3\%$.