Anti-CD70 2H5 VH Regions

V segment: 3-30.3
D segment:undetermined
J segment: JH4b

```
    Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
1   CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                    CDR1
                                                    ~~~~~~~~~~~~~~~~~~
    R   L   S   C   A   A   S   G   F   T   F   S   S   Y   I   M   H   W
55  AGA CTC TCC TGT GCA GCC TCT GGA TTT ACC TTC AGT AGC TAT ATT ATG CAC TGG

                                                        CDR2
                                                        ~~~~~~~~~~~~~~~~~~
    V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   S   Y   D
109 GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATA TCA TAT GAT

                    CDR2
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    G   R   N   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163 GGA AGA AAC AAA TAC TAC GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA

    D   N   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D
217 GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC

                                        CDR3
                                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    T   A   V   Y   Y   C   A   R   D   T   D   G   Y   D   F   D   Y   W
271 ACG GCT GTG TAT TAC TGT GCG AGA GAT ACG GAT GGC TAC GAT TTT GAC TAC TGG
                                                    |->
                                                    JH4b
    G   Q   G   T   L   V   T   V   S   S
325 GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Fig. 1A

Anti-CD70 2H5 VK Regions

```
V segment:     L6
J segment:     JK4

     E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
  1  GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                   CDR1
                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
 55  GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                CDR2
                                                          ~~~~~~~~~~~~~~~~~~~
     Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109  CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

     CDR2
    ~~~~~~~~
     A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163  GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                    CDR3
                                                                  ~~~~~~~
     L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217  CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

            CDR3
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     R   T   N   W   P   L   T   F   G   G   G   T   K   V   E   I   K
271  CGT ACC AAC TGG CCG CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
                     |
                     |→ JK4
```

Fig. 1B

Anti-CD70 10B4 VH Regions

V segment: 3-30.3
D segment: 4-11
J segment: JH4b

```
      Q   I   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
  1  CAA ATA CAA CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                          CDR1
                                                      ~~~~~~~~~~~~~~~~~~
      R   L   S   C   A   A   S   G   F   T   F   G   Y   Y   A   M   H   W
 55  AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC GGT TAC TAT GCT ATG CAC TGG

                                                              CDR2
                                                          ~~~~~~~~~~~~~~~~~~
      V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   S   Y   D
109  GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATA TCA TAT GAT

                    CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      G   S   I   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163  GGA AGC ATT AAA TAC TAC GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA

      D   N   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D
217  GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC

                                                  CDR3
                                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      T   A   V   Y   Y   C   A   R   E   G   P   Y   S   N   Y   L   D   Y
271  ACG GCT GTG TAT TAC TGT GCG AGA GAG GGC CCT TAC AGT AAC TAC CTT GAC TAC
                                                          |
                                                          |→ JH4b

      W   G   Q   G   T   L   V   T   V   S   S
325  TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Fig. 2A

Anti-CD70 10B4 VK Regions

```
V segment:     L18
J segment:     JK3

     A   I   Q   L   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
  1  GCC ATC CAG TTG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA

                                   CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     V   T   I   T   C   R   A   S   Q   G   I   S   S   A   L   A   W   Y
 55  GTC ACC ATC ACT TGC CGG GCA AGT CAG GGC ATT AGC AGT GCT TTA GCC TGG TAT

                                                                CDR2
                                                        ~~~~~~~~~~~~~~~~~~~
     Q   Q   K   P   G   K   A   P   K   F   L   I   Y   D   A   S   S   L
109  CAG CAG AAA CCA GGG AAA GCT CCT AAG TTC TTG ATC TAT GAT GCC TCC AGT TTG

     CDR2
     ~~~~~~~
     E   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163  GAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                    CDR3
                                                                    ~~~~~~~
     L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217  CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGT CAA CAG

             CDR3
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     F   N   S   Y   P   F   T   F   G   P   G   T   K   V   D   I   K
271  TTT AAT AGT TAC CCA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA
                     |
                     ---->
                     JK3
```

Fig. 2B

Anti-CD70 8B5 VH Regions

V segment: 3-33
D segment: 3-10
J segment: JH4b

```
     Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
  1  CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                         CDR1
                                                     ~~~~~~~~~~~~~~~~~~
     R   L   S   C   A   T   S   G   F   T   F   S   D   Y   G   M   H   W
 55  AGA CTC TCC TGT GCG ACG TCT GGA TTC ACC TTC AGT GAC TAT GGC ATG CAC TGG

                                                             CDR2
                                                         ~~~~~~~~~~~~~~~~~~
     V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109  GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATA TGG TAT GAT

                    CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     G   S   N   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163  GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA

     D   N   S   K   K   T   L   S   L   Q   M   N   S   L   R   A   E   D
217  GAC AAT TCC AAG AAA ACG CTG TCT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                                CDR3
                                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     T   A   V   Y   Y   C   A   R   D   S   I   M   V   R   G   D   Y   W
271  ACG GCT GTG TAT TAC TGT GCG AGA GAT TCT ATT ATG GTT CGG GGG GAC TAC TGG
                                                                 |
                                                                 |→ JH4b

     G   Q   G   T   L   V   T   V   S   S
325  GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Fig. 3A

Anti-CD70 8B5 VK Regions

V segment: L15
J segment: JK4

```
     D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
  1 GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTA GGA GAC AGA

                                   CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     V   T   I   T   C   R   A   S   Q   G   I   S   S   W   L   A   W   Y
 55 GTC ACC ATC ACT TGT CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT

                                                                CDR2
                                                          ~~~~~~~~~~~~~~~~~~~
     Q   Q   K   P   E   K   A   P   K   S   L   I   Y   A   A   S   S   L
109 CAG CAG AAA CCA GAG AAA GCC CCT AAG TCC CTG ATC TAT GCT GCA TCC AGT TTG

     CDR2
    ~~~~~~~
     Q   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163 CAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                    CDR3
                                                                  ~~~~~~~
     L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217 CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGC CAA CAG

            CDR3
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~
     Y   N   S   Y   P   L   T   F   G   G   G   T   K   V   E   I   K
271 TAT AAT AGT TAC CCG CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
                    |
                    →
                    JK4
```

Fig. 3B

Anti-CD70 18E7 VH Regions

V segment: 3-33
D segment: 3-10
J segment: JH4b

```
     Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
  1  CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                         CDR1
                                                     ~~~~~~~~~~~~~~~~~~
     R   L   S   C   A   A   S   G   F   T   F   S   D   H   G   M   H   W
 55  AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGC GAC CAT GGC ATG CAC TGG

                                                             CDR2
                                                         ~~~~~~~~~~~~~~~~~~
     V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109  GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATA TGG TAT GAT

                 CDR2
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     G   S   N   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R
163  GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA

     D   N   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D
217  GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                         CDR3
                                 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     T   A   V   Y   Y   C   A   R   D   S   I   M   V   R   G   D   Y   W
271  ACG GCT GTG TAT TAC TGT GCG AGA GAT TCT ATT ATG GTT CGG GGG GAC TAC TGG
                                                                 |
                                                                 →
                                                                 JH4b

     G   Q   G   T   L   V   T   V   S   S
325  GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Fig. 4A

Anti-CD70 18E7 VK Regions

V segment: L15
J segment: JK4

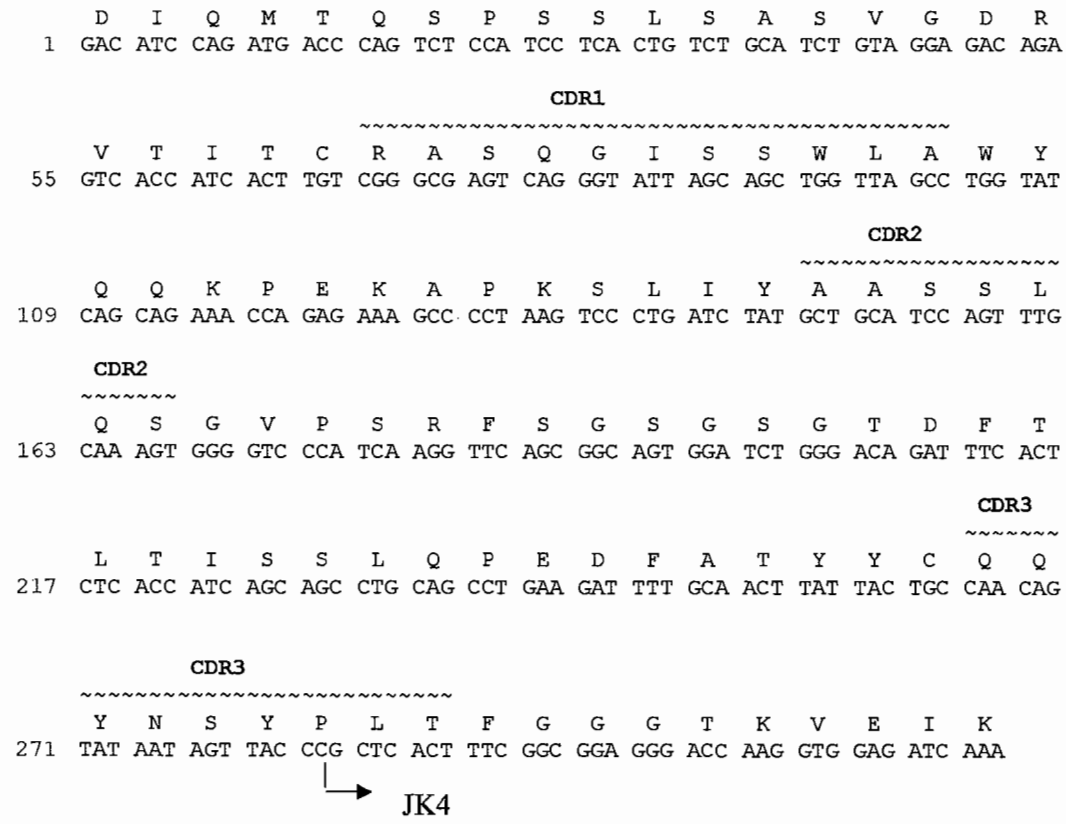


Fig. 4B

Anti-CD70 69A7 VH

V segment: 4-61
D segment:4-23
J segment: JH4b

```
         Q   V   Q   L   Q   E   S   G   P   G   L   V   K   P   S   E   T   L
1       CAG GTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GAG ACC CTG

                                                                CDR1
                                                        ~~~~~~~~~~~~~~~~~~~~~~~
         S   L   T   C   T   V   S   G   G   S   V   S   S   D   Y   Y   Y   W
55      TCC CTC ACC TGC ACT GTC TCT GGT GGC TCC GTC AGC AGT GAT TAT TAC TAC TGG

        CDR1                                                            CDR2
        ~~~                                                          ~~~~~~~~~~~
         S   W   I   R   Q   P   P   G   K   G   L   E   W   L   G   Y   I   Y
109     AGC TGG ATC CGG CAG CCC CCA GGG AAG GGA CTG GAG TGG CTT GGG TAT ATC TAT

                        CDR2
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
         Y   S   G   S   T   N   Y   N   P   S   L   K   S   R   V   T   I   S
163     TAC AGT GGG AGC ACC AAC TAC AAC CCC TCC CTC AAG AGT CGA GTC ACC ATA TCA

         V   D   T   S   K   N   Q   F   S   L   K   L   R   S   V   T   T   A
217     GTA GAC ACG TCC AAG AAC CAG TTC TCC CTG AAG CTG AGG TCT GTG ACC ACT GCG

                                                        CDR3
                                                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
         D   T   A   V   Y   Y   C   A   R   G   D   G   D   Y   G   G   N   C
271     GAC ACG GCC GTG TAT TAC TGT GCG AGA GGG GAT GGG GAC TAC GGT GGT AAC TGT

          CDR3
        ~~~~~~~~~~~
         F   D   Y   W   G   Q   G   T   L   V   T   V   S   S
325     TTT GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Fig. 5A

Anti-CD70 69A7 VK

V segment: L6
J segment: JK4

```
          E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1       GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                    CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55      GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                CDR2
                                                        ~~~~~~~~~~~~~~~~~~~
          Q   Q   K   P   G   Q   A   P   R   L   L   I   F   D   A   S   N   R
109     CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TTT GAT GCA TCC AAC AGG

          CDR2
        ~~~~~~~
          A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163     GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                        CDR3
                                                                      ~~~~~~~
          L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217     CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAA

                CDR3
        ~~~~~~~~~~~~~~~~~~~~~~~~~~
          R   S   N   W   P   L   T   F   G   G   G   T   K   V   E   I   K
271     CGT AGC AAC TGG CCG CTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
```

Fig. 5B

Anti- CD70 1F4 VH Regions

V segment: 3-23
D segment: 4-4
J segment: JH4b

```
   E   V   Q   L   L   E   S   G   G   G   L   V   Q   P   G   G   S   L
 1 GAG GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG GGG TCC CTG

                                                       CDR 1
                                                       ~~~~~~~~~~~~~~~~~~
   R   L   S   C   A   A   S   G   F   T   F   S   I   Y   A   M   S   W
55 AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGC ATC TAT GCC ATG AGC TGG

                                                           CDR 2
                                                           ~~~~~~~~~~~~~~~~~~
   V   R   Q   A   P   G   K   G   L   E   W   V   S   A   I   S   D   S
109 GTC CGC CAG GCT CCA GGG AAG GGG CTG GAG TGG GTC TCA GCT ATT AGT GAT AGT

                 CDR 2
   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   G   G   R   T   Y   F   A   D   S   V   R   G   R   F   T   I   S   R
163 GGT GGT CGC ACA TAC TTC GCA GAC TCC GTG AGG GGC CGG TTC ACC ATC TCC AGA

   D   N   S   K   N   T   L   S   L   Q   M   N   S   L   R   A   E   D
217 GAC AAT TCC AAG AAC ACG CTG TCT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                           CDR 3
                                           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   T   A   V   Y   Y   C   A   K   V   D   Y   S   N   Y   L   F   F   D
271 ACG GCC GTA TAT TAC TGT GCG AAG GTC GAC TAC AGT AAC TAC CTA TTC TTT GAC

   ~~~
   Y   W   G   Q   G   T   L   V   T   V   S   S
325 TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

Fig. 6A

Anti- CD70 1F4 VK Regions

V segment: A27
J segment: JK2

```
   E   I   V   L   T   Q   S   P   G   T   L   S   L   S   P   G   E   R
 1 GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                             CDR 1
                           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   A   T   L   S   C   R   A   S   Q   S   I   S   S   S   Y   L   A   W
55 GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT ATT AGC AGC AGC TAC TTA GCC TGG

                                                           CDR 2
                                                         ~~~~~~~~~~~~~~~~
   Y   Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   G   A   S   S
109 TAC CAG CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GGT GCA TCC AGC

     CDR 2
   ~~~~~~~~~~~
   R   A   T   G   I   P   D   R   F   S   G   S   G   S   G   T   D   F
163 AGG GCC ACT GGC ATC CCA GAC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC

                                                                   CDR
                                                                   ~~~
   T   L   T   I   S   R   L   E   P   E   D   F   A   V   Y   Y   C   Q
217 ACT CTC ACC ATC AGC AGA CTG GAG CCT GAA GAT TTT GCA GTG TAT TAC TGT CAG

           CDR 3
   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   Q   Y   G   S   S   P   Y   T   F   G   Q   G   T   K   L   E   I   K
271 CAG TAT GGT AGC TCA CCG TAC ACT TTT GGC CAG GGG ACC AAG CTG GAG ATC AAA
```

Fig. 6B

Anti-CD70 2H5 and 10B4 V_H Regions

```
                                                
```

Anti-CD70 8B5 and 18E7 V_H regions

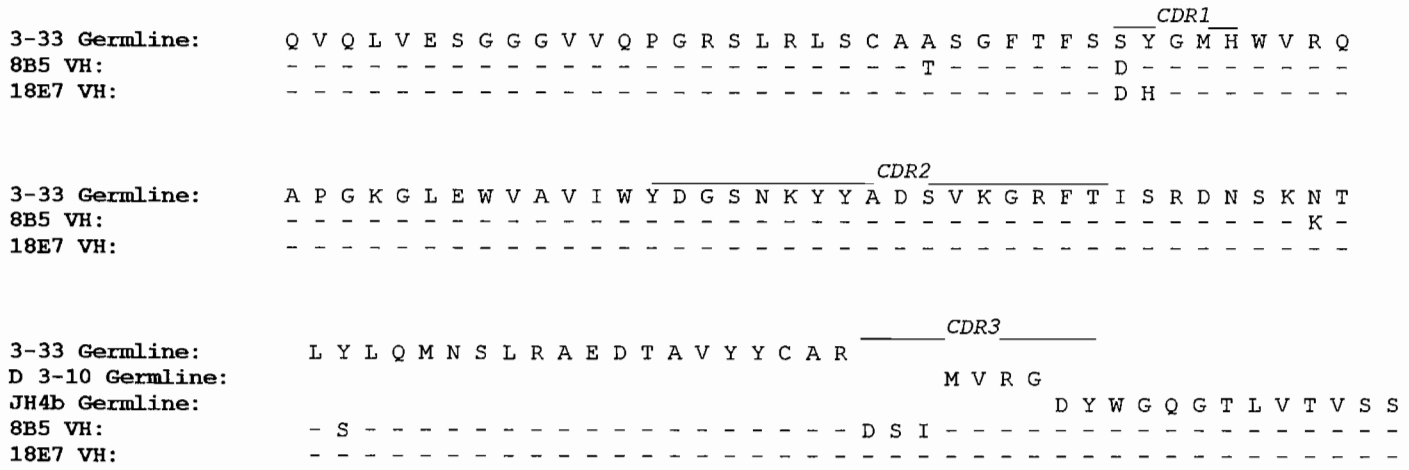


Fig. 8

Anti-CD70 69A7 VH region

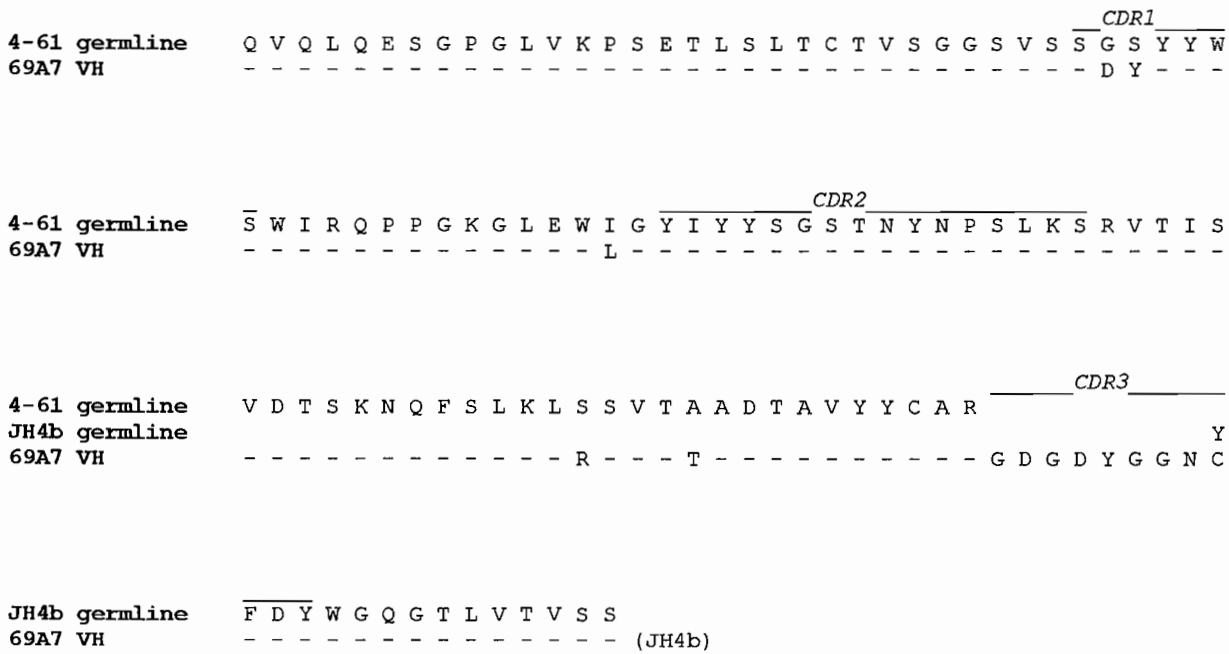


Fig. 9

Anti- CD70 1F4 VH Region

```
3-23 germline       E V Q L L E S G G G L V Q P G G S L R
1F4  VH             - - - - - - - - - - - - - - - - - - -

                                        _CDR1____
3-23 germline       L S C A A S G F T F S S Y A M S W V R
1F4  VH             - - - - - - - - - - - I - - - - - - -

                                        _CDR2_________
3-23 germline       Q A P G K G L E W V S A I S G S G G S
1F4  VH             - - - - - - - - - - - - - - D - - - R

                    ________________
3-23 germline       T Y Y A D S V K G R F T I S R D N S K
1F4  VH             - - F - - - - R - - - - - - - - - - -

3-23 germline       N T L Y L Q M N S L R A E D T A V Y Y
1F4  VH             - - - S - - - - - - - - - - - - - - -

                          _CDR3_____________
3-23 germline       C A K
4-4  germline       . . . . D Y S N Y . . . . . . . . . .
JH4b germline                           F D Y W G Q G T
1F4  VH             - - - V - - - - - L F - - - - - - - -

JH4b germline       L V T V S S
1F4  VH             - - - - - -
```

Fig. 10

Anti-CD70 mAb1 2H5 VK Region

```
                                                                  ________CDR1________
L6 Germline:  E I V L T Q S P A T L S L S P G E R A T L S C R A S Q S V S S Y L A
2H5 VK #1:    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                 ____CDR2______
L6 Germline:  W Y Q Q K P G Q A P R L L I Y D A S N R A T G I P A R F S G S G S G
2H5 VK #1:    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                 ____CDR3_________
L6 Germline:  T D F T L T I S S L E P E D F A V Y Y C Q Q R S N W
JK4 Germline:                                                         L T F G G G T
2H5 VK #1:    - - - - - - - - - - - - - - - - - - - - - - - - T - - P - - - - - - -

JK4 Germline: K V E I K
2H5 VK #1:    - - - - - (JK4)
```

Fig. 11

Anti-CD70 mAb3 10B4 VK Regions

```
                                                                                                  CDR1
L18 Germline:    A I Q L T Q S P S S L S A S V G D R V T I T C R A S Q G I S S A L A
10B4 VK:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                    CDR2
L18 Germline:    W Y Q Q K P G K A P K L L I Y D A S S L E S G V P S R F S G S G S G
10B4 VK:         - - - - - - - - - - - F - - - - - - - - - - - - - - - - - - - - - - -

                                                                  CDR3
L18 Germline:    T D F T L T I S S L Q P E D F A T Y Y C Q Q F N S Y P
JK3 Germline:                                                           F T F G P G T
10B4 VK:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

JK3 Germline:    K V D I K
10B4 VK:         - - - - - (JK3)
```

Fig. 12

Anti-CD70 8B5 and 18E7 VK Regions

```
                                                                                  ______CDR1___________
L15 Germline:      D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q G I S S W L A
8B5    VK:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
18E7 VK:           - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -


                                                    ____CDR2_____
L15 Germline:      W Y Q Q K P E K A P K S L I Y A A S S L Q S G V P S R F S G S G S G
8B5    VK:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
18E7 VK #1:        - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -


                                                                  _______CDR3_______
L15 Germline:      T D F T L T I S S L Q P E D F A T Y Y C Q Q Y N S Y P
JK4 Germline:                                                                L T F G G G
8B5    VK:         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
18E7 VK #1:        - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -


JK4 Germline:      T K V E I K
8B5    VK:         - - - - - -  (JK4)
18E7 VK #1:        - - - - - -  (JK4)
```

Fig. 13

Anti-CD70 69A7 VK region

```
                                                                                  CDR1
L6 germline     E I V L T Q S P A T L S L S P G E R A T L S C R A S Q S V S S
69A7 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                CDR2
L6 germline     Y L A W Y Q Q K P G Q A P R L L I Y D A S N R A T G I P A R F
69A7 VK         - - - - - - - - - - - - - - - - - F - - - - - - - - - - - - -

                                                                                CDR3
L6 germline     S G S G S G T D F T L T I S S L E P E D F A V Y Y C Q Q R S N
69A7 VK         - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

L6 germline     W P
JK4 germline        L T F G G G T K V E I K
69A7 VK         - - - - - - - - - - - - - -     (JK4)
```

Fig. 14

Anti- CD70 1F4 VK Region

```
A27 germline        E I V L T Q S P G T L S L S P G E R
1F4 VK              - - - - - - - - - - - - - - - - -

                        CDR1
A27 germline        A T L S C R A S Q S V S S S Y L A W
1F4 VK              - - - - - - - - - - I - - - - - - -

                                                CDR 2
A27 germline        Y Q Q K P G Q A P R L L I Y G A S S
1F4 VK              - - - - - - - - - - - - - - - - - -

A27 germline        R A T G I P D R F S G S G S G T D F
1F4 VK              - - - - - - - - - - - - - - - - - -

A27 germline        T L T I S R L E P E D F A V Y Y C Q
1F4 VK              - - - - - - - - - - - - - - - - - -

                    CDR3
A27 germline        Q Y G S S P
JK2 germline                  Y T F G Q G T K L E I K
1F4 VK              - - - - - - - - - - - - - - - - - -
```

Fig. 15

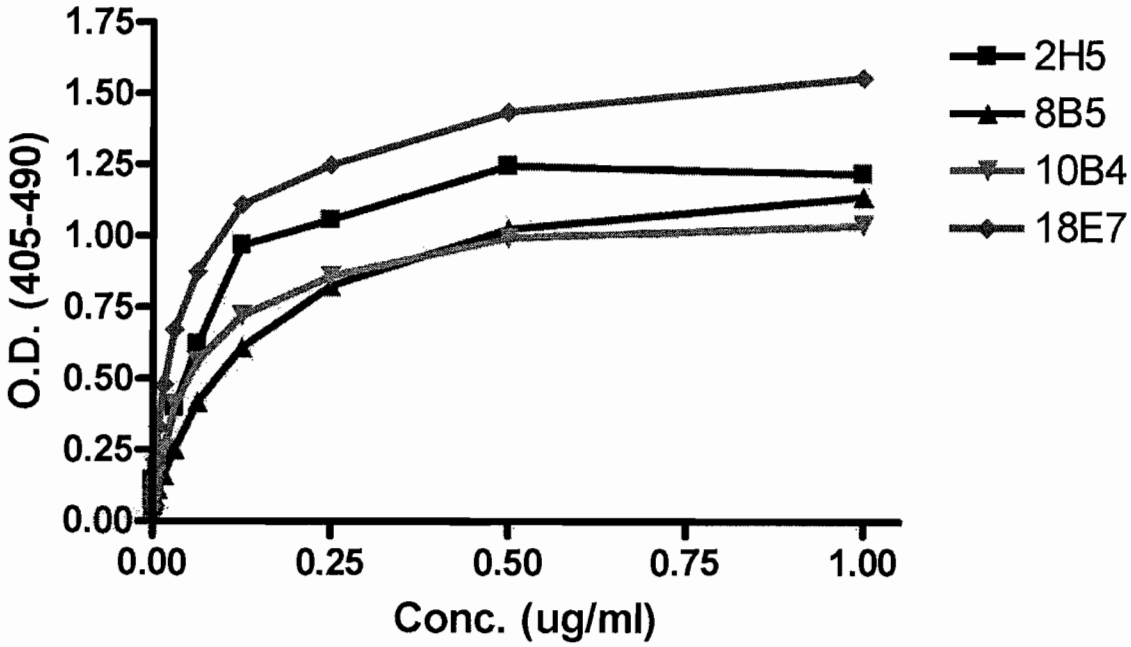

1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
O.D. (405-490)
0.00
0.25
0.50
0.75
1.00
Conc. (ug/ml)
2H5
8B5
10B4
18E7

Fig. 16

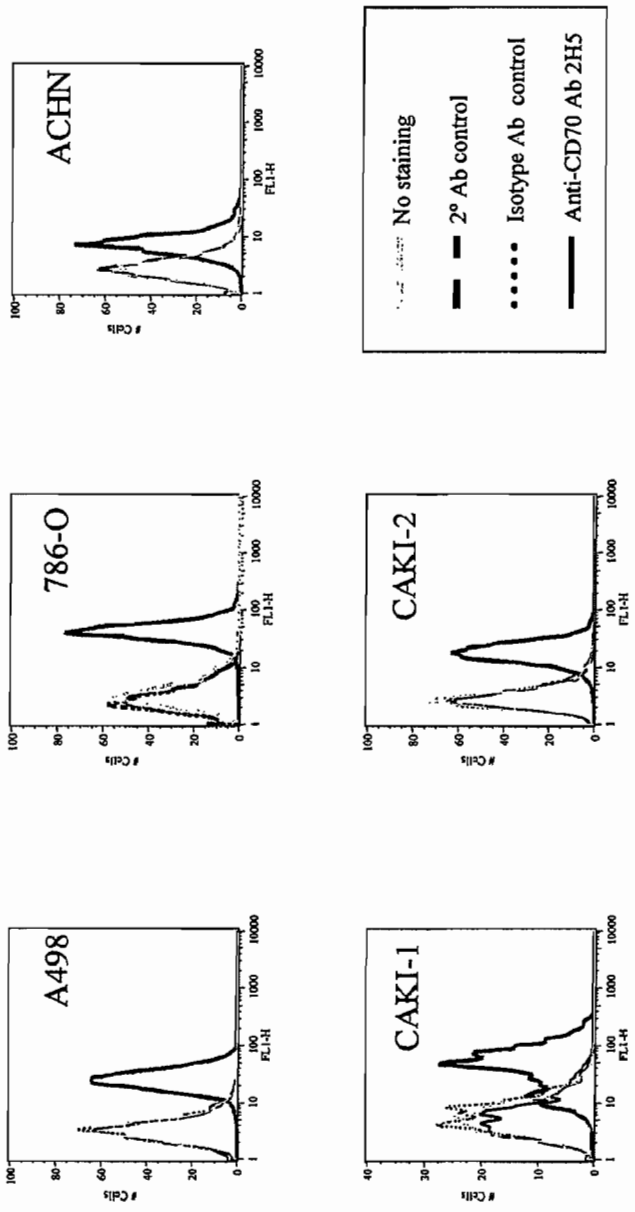

ACHN
786-O
CAKI-2
A498
CAKI-1
No staining
2° Ab control
Isotype Ab control
Anti-CD70 Ab 2H5
Cells
FL1-H

Fig. 17

786-O FACS Titration

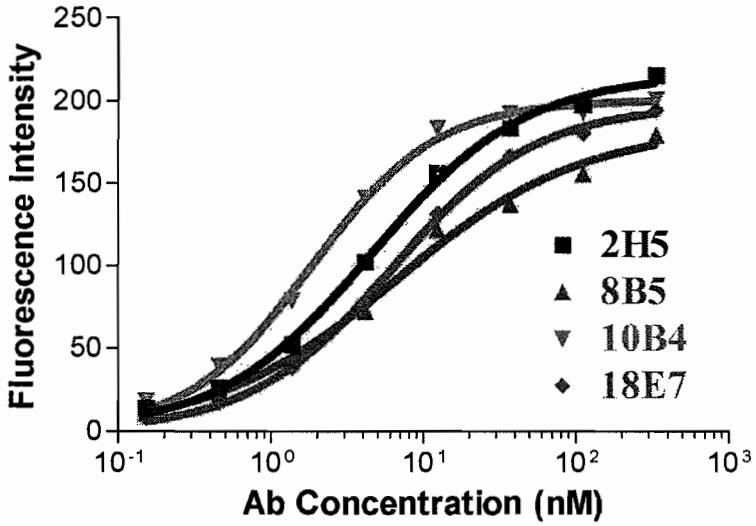


Fig. 18A

A498 FACS Titration

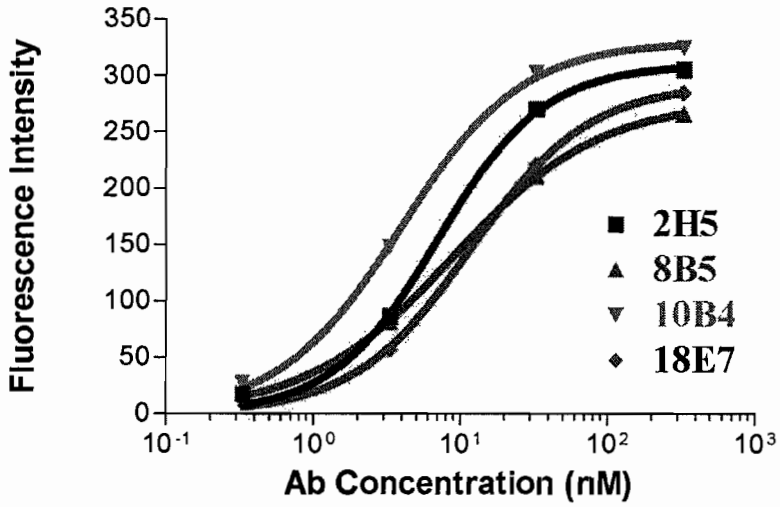


Fig. 18B

69A7 binds CD70+ human renal cancer cell line (786-O)

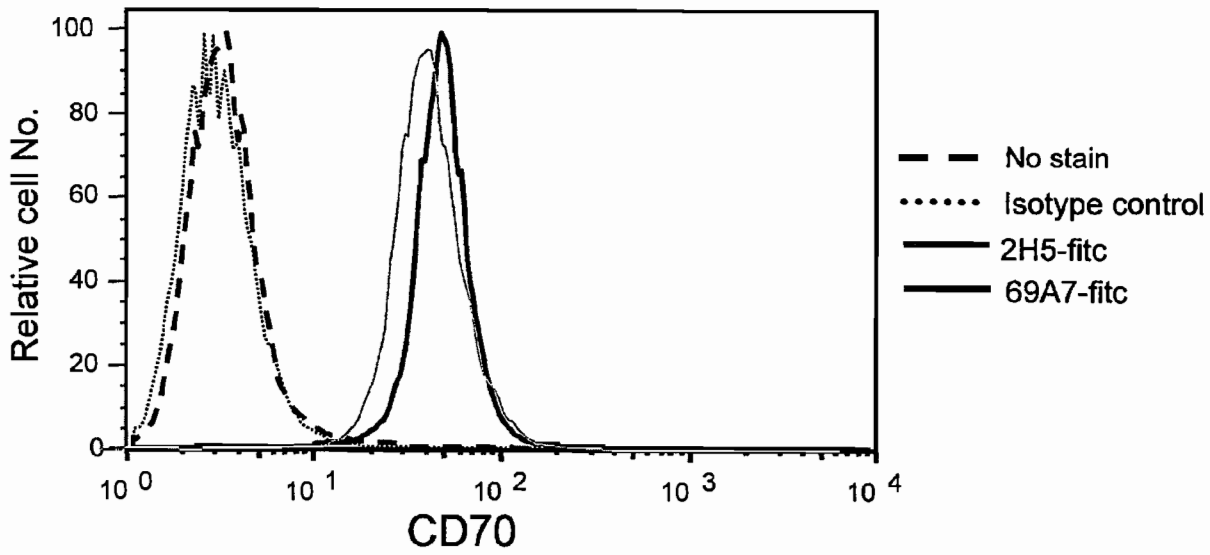
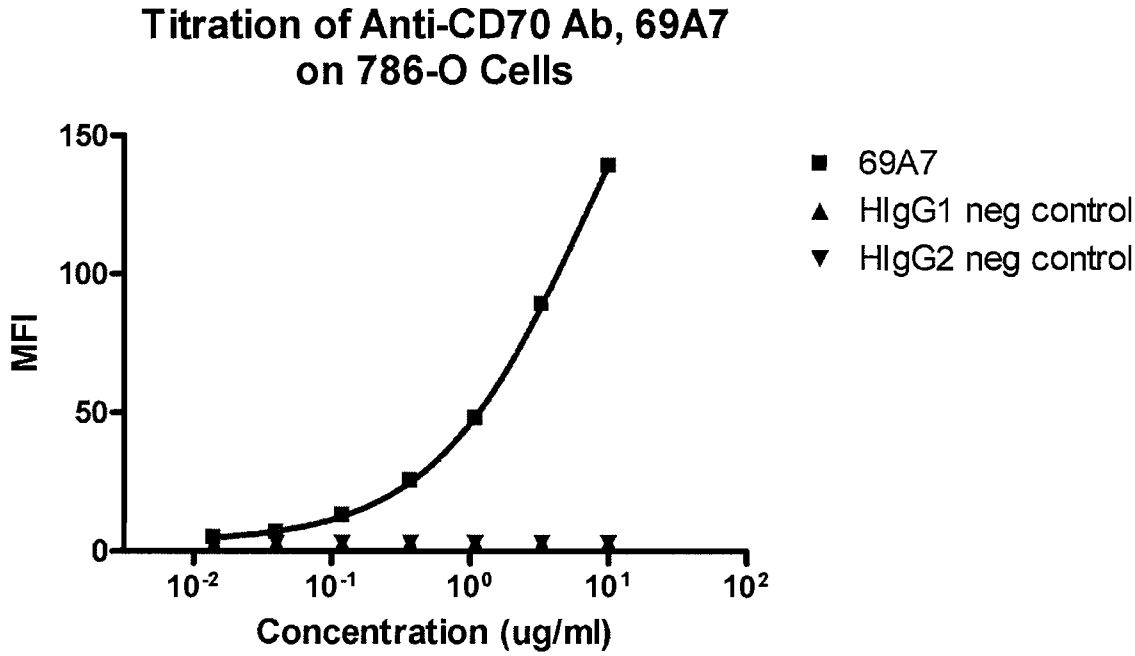


Fig. 18C



	69A7.2.1.1
EC50	6.927

Fig. 18D

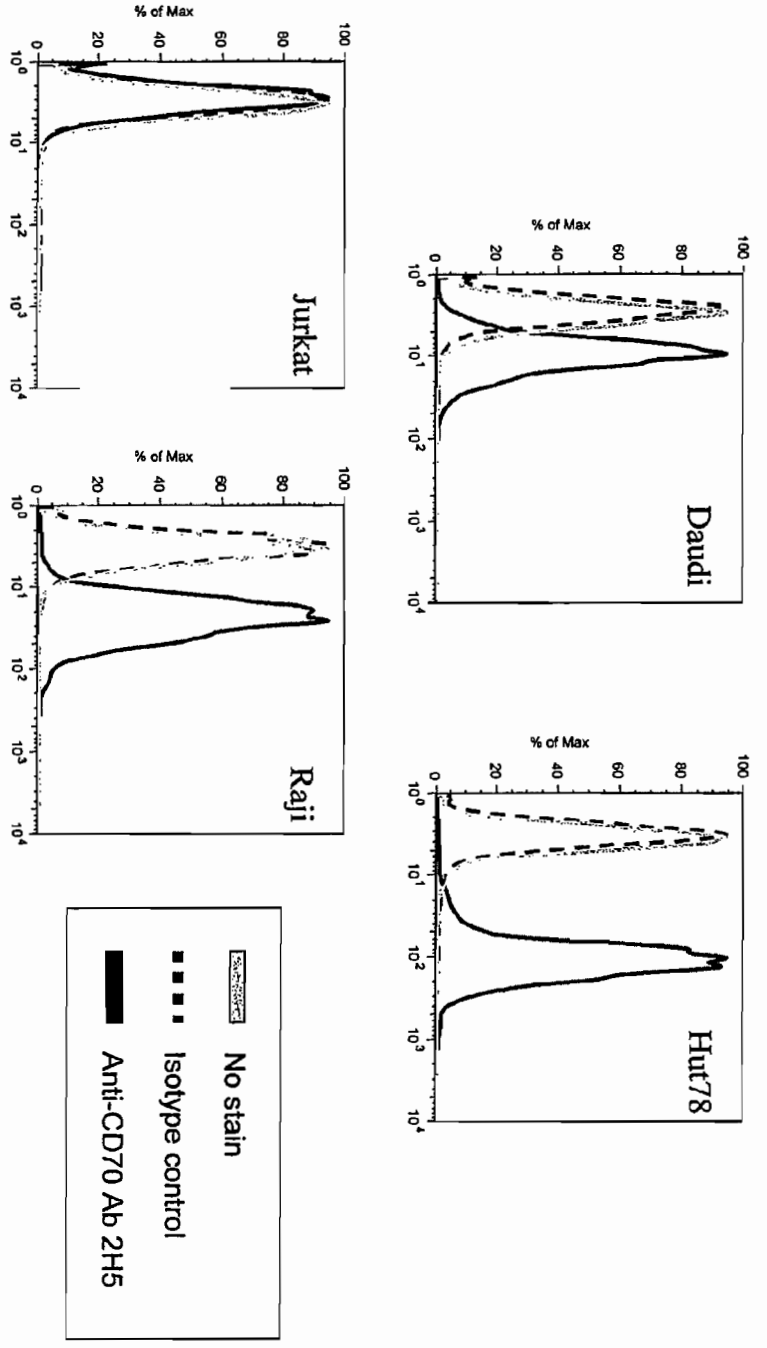

Daudi
Hut78
Jurkat
Raji
% of Max
No stain
Isotype control
Anti-CD70 Ab 2H5

Fig. 19

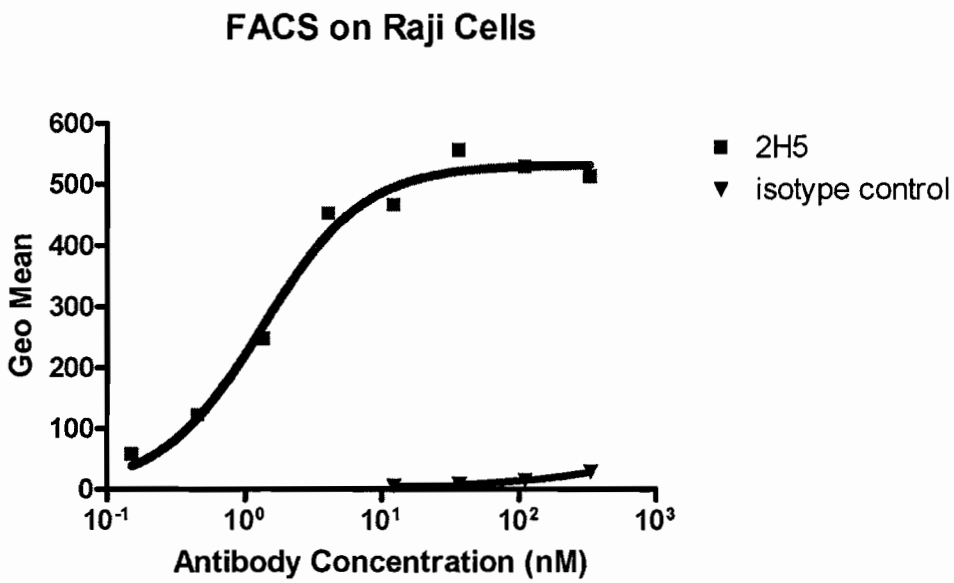


Fig. 20A

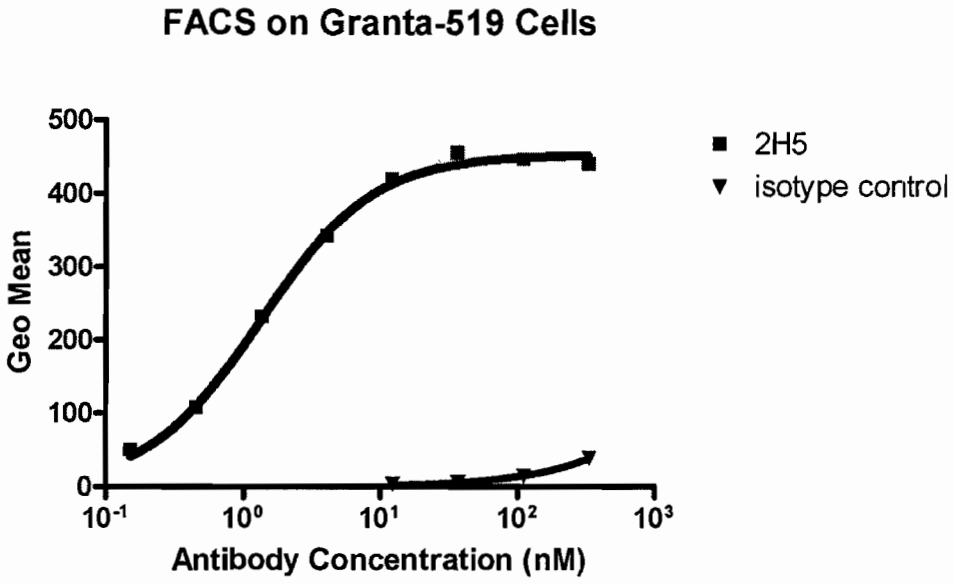


Fig. 20B

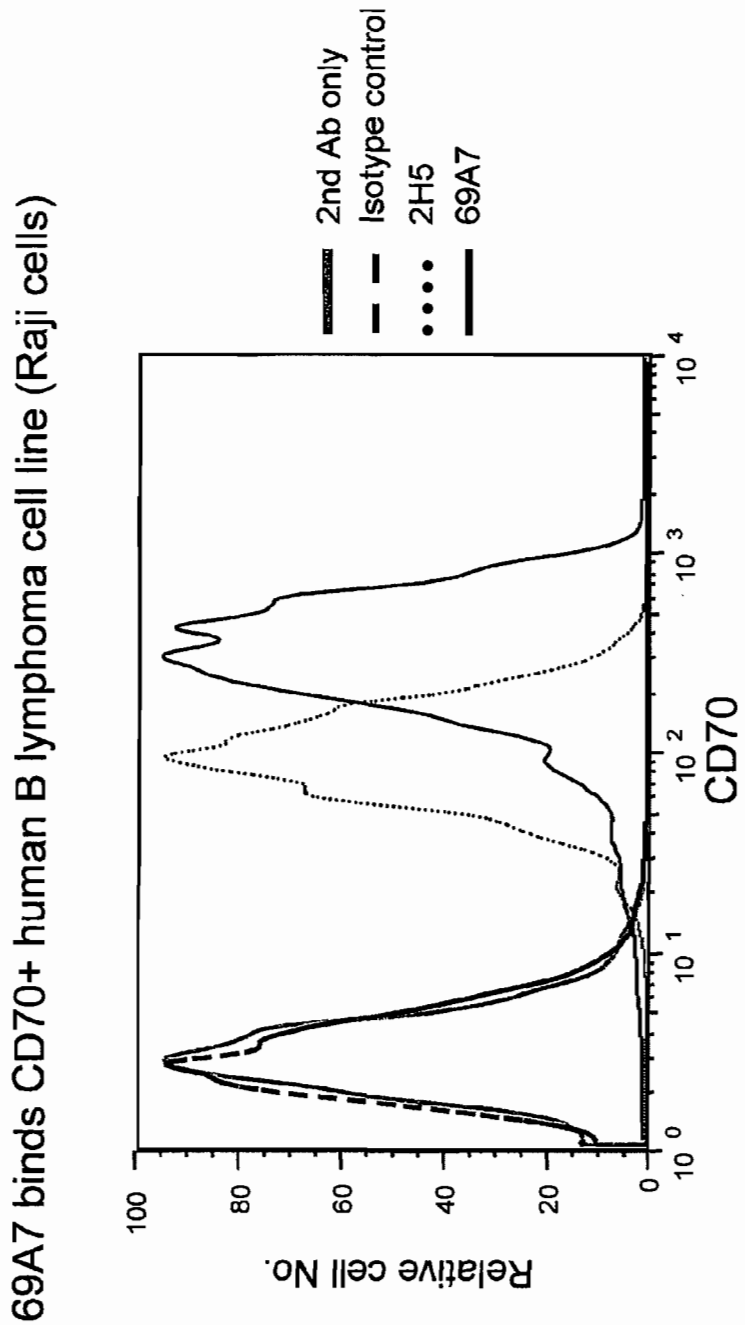

69A7 binds CD70+ human B lymphoma cell line (Raji cells)
2nd Ab only
Isotype control
2H5
69A7
Relative cell No.
100
80
60
40
20
0
10 0
10 1
10 2
10 3
10 4
CD70

Fig. 20C

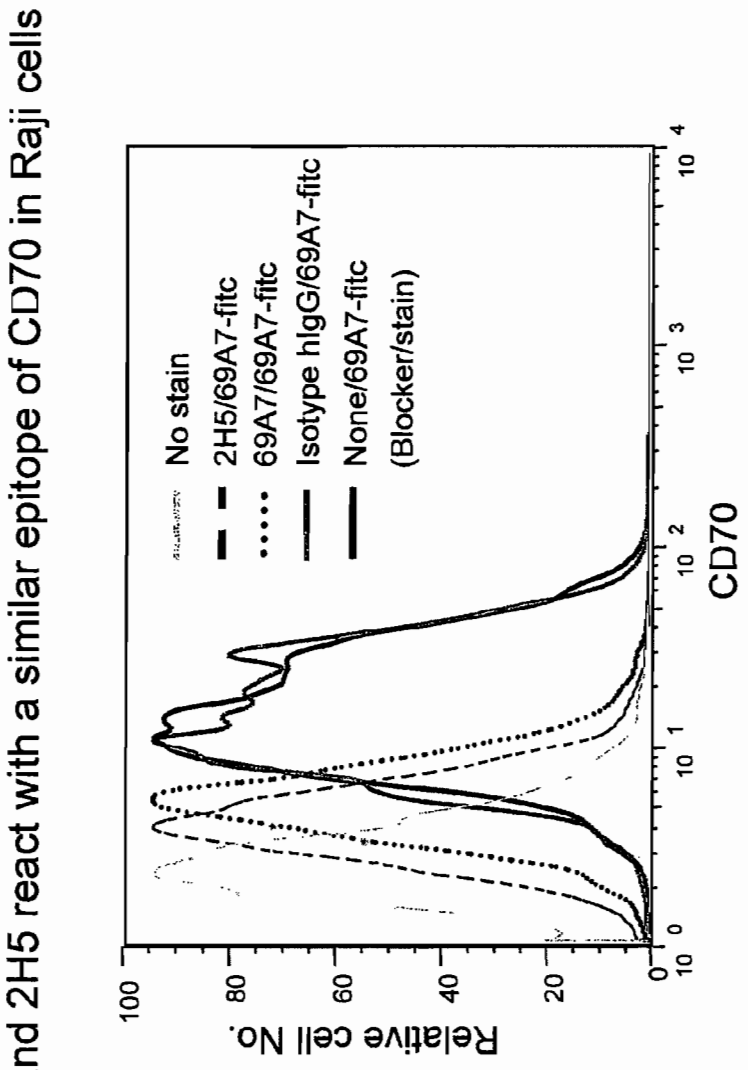

69A7 and 2H5 react with a similar epitope of CD70 in Raji cells
Relative cell No.
100
80
60
40
20
0
10 0
10 1
10 2
10 3
10 4
CD70
No stain
2H5/69A7-fitc
69A7/69A7-fitc
Isotype hIgG/69A7-fitc
None/69A7-fitc
(Blocker/stain)

Fig. 20D

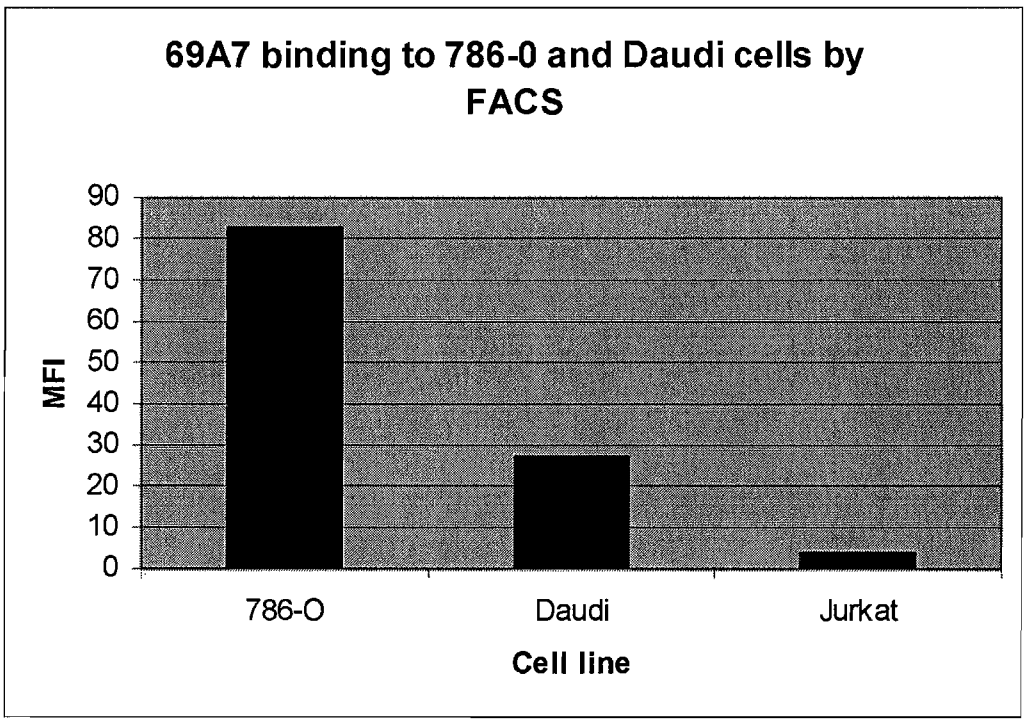

69A7 binding to 786-0 and Daudi cells by FACS
MFI
90
80
70
60
50
40
30
20
10
0
786-O
Daudi
Jurkat
Cell line

Fig. 20E

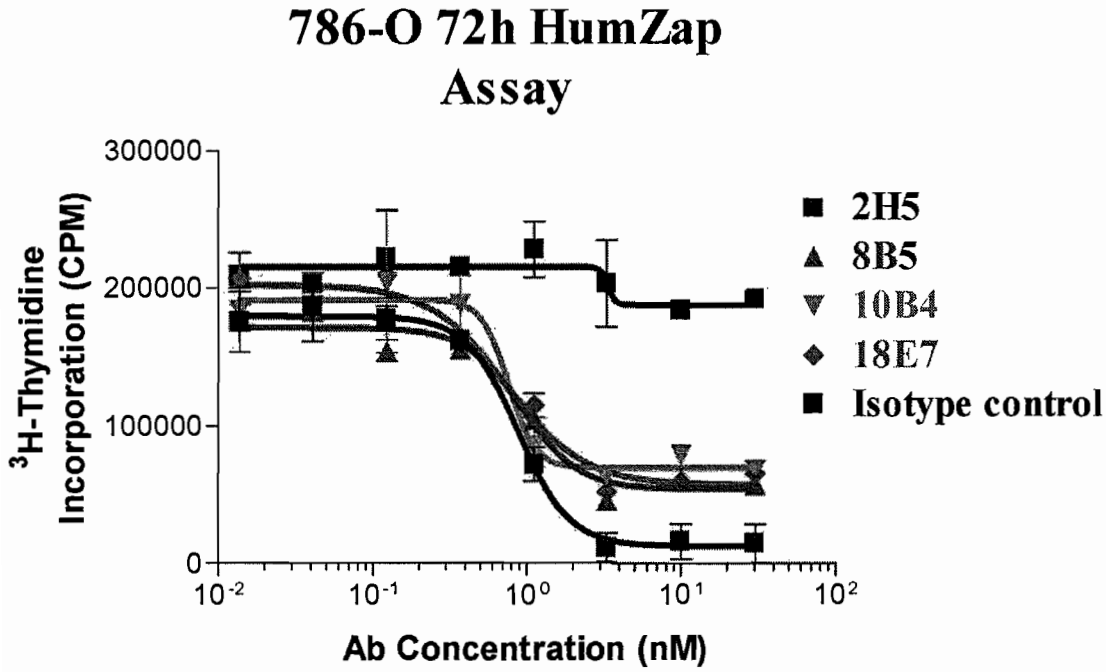

786-O 72h HumZap
Assay
300000
200000
100000
0
^{3}H-Thymidine
Incorporation (CPM)
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
Ab Concentration (nM)
2H5
8B5
10B4
18E7
Isotype control

Fig. 21

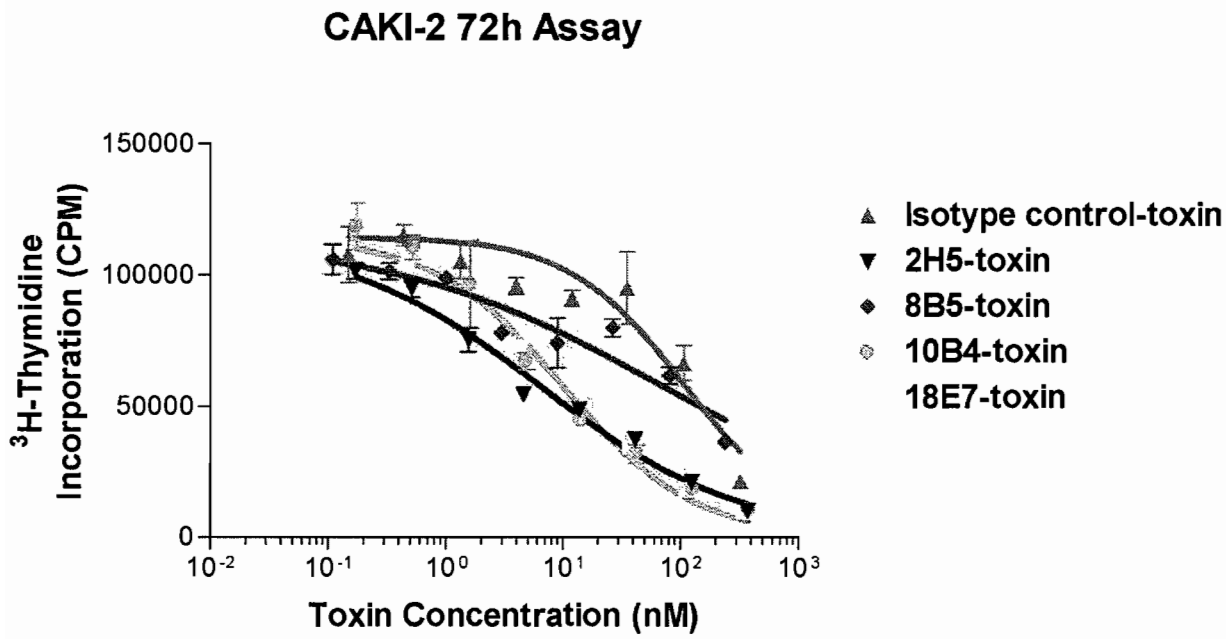


Fig. 22A

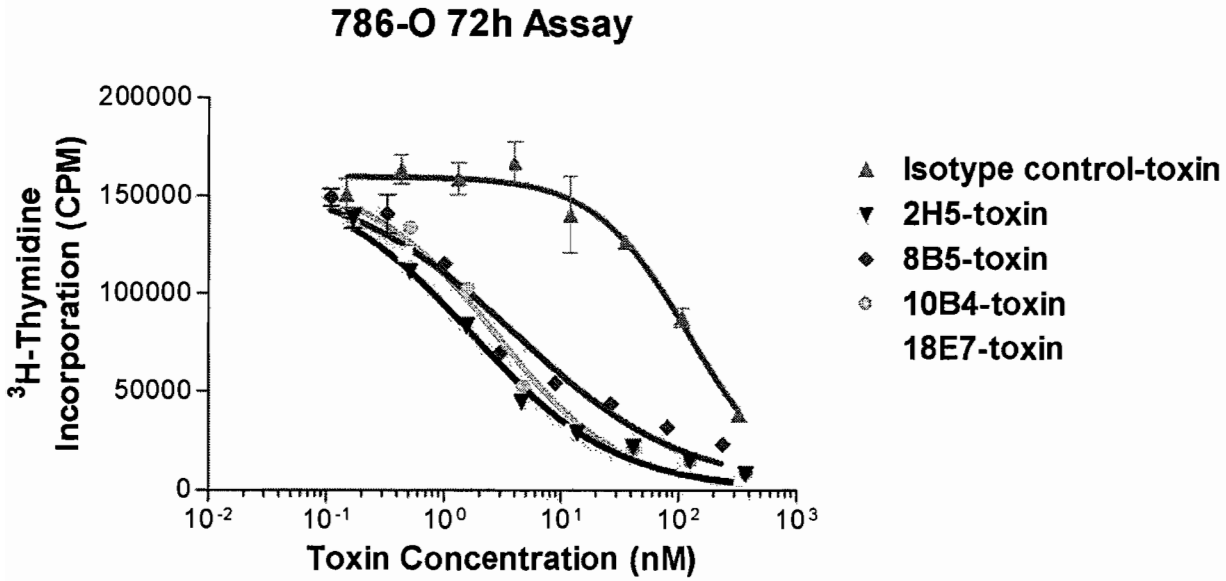


Fig. 22B

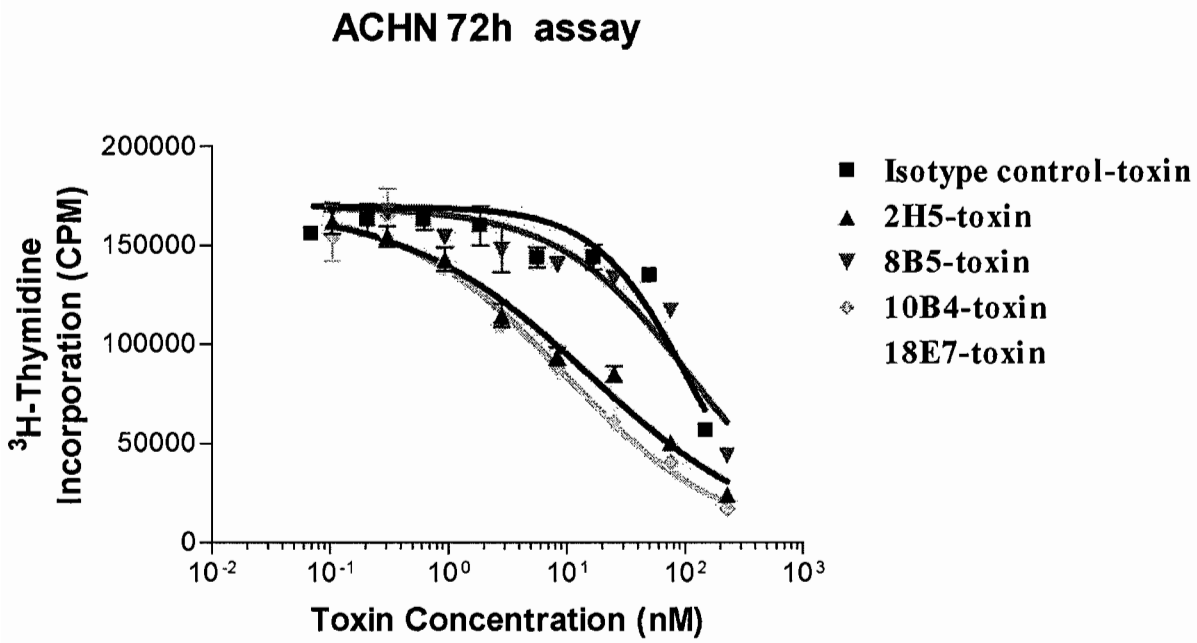

ACHN 72h assay
^{3}H-Thymidine Incorporation (CPM)
200000
150000
100000
50000
0
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
Toxin Concentration (nM)
Isotype control-toxin
2H5-toxin
8B5-toxin
10B4-toxin
18E7-toxin

Fig. 22C

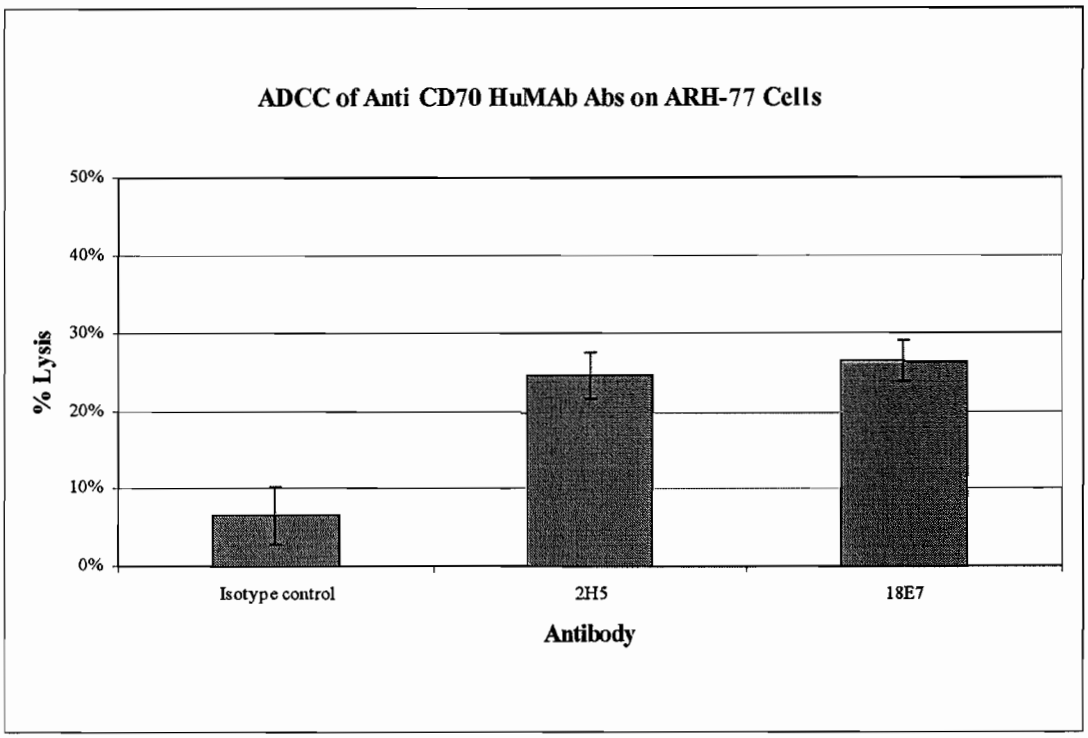


Fig. 23A

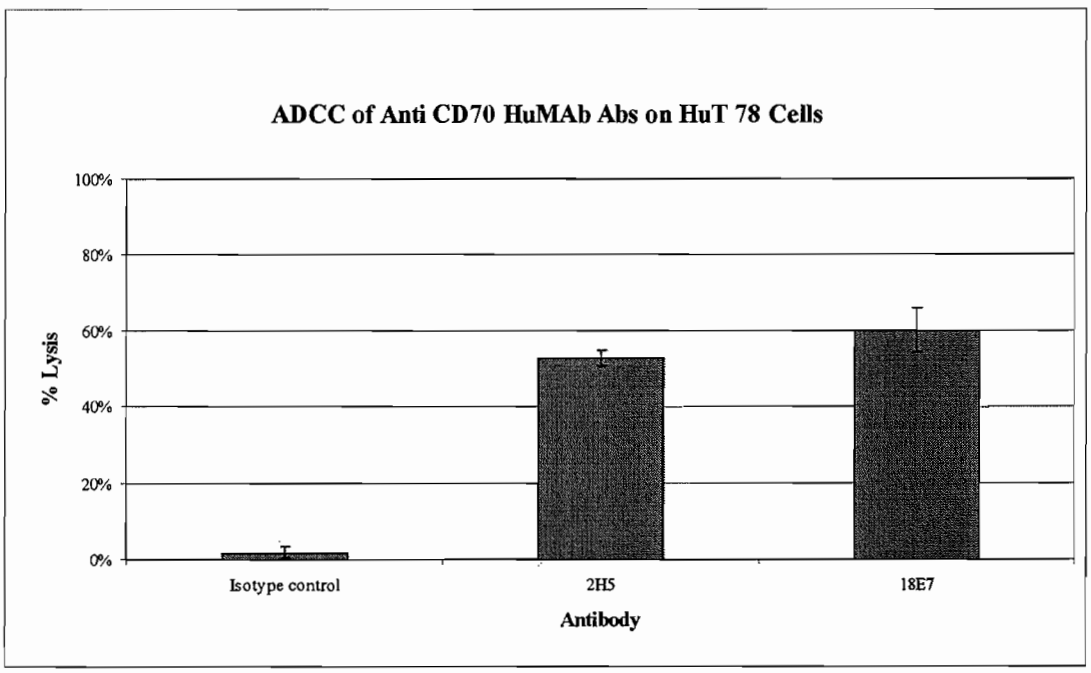


Fig. 23B

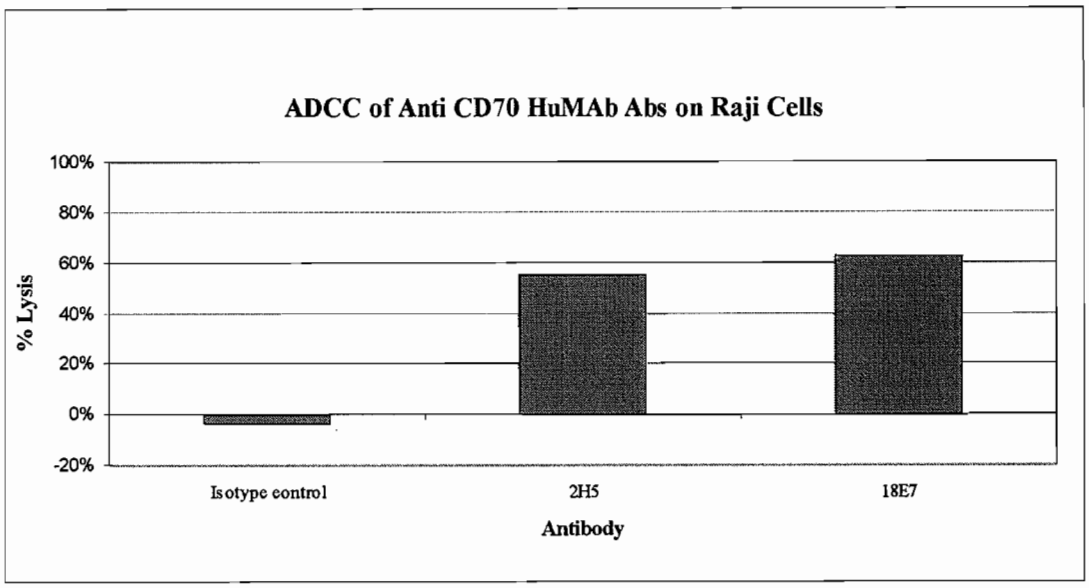


Fig. 23C

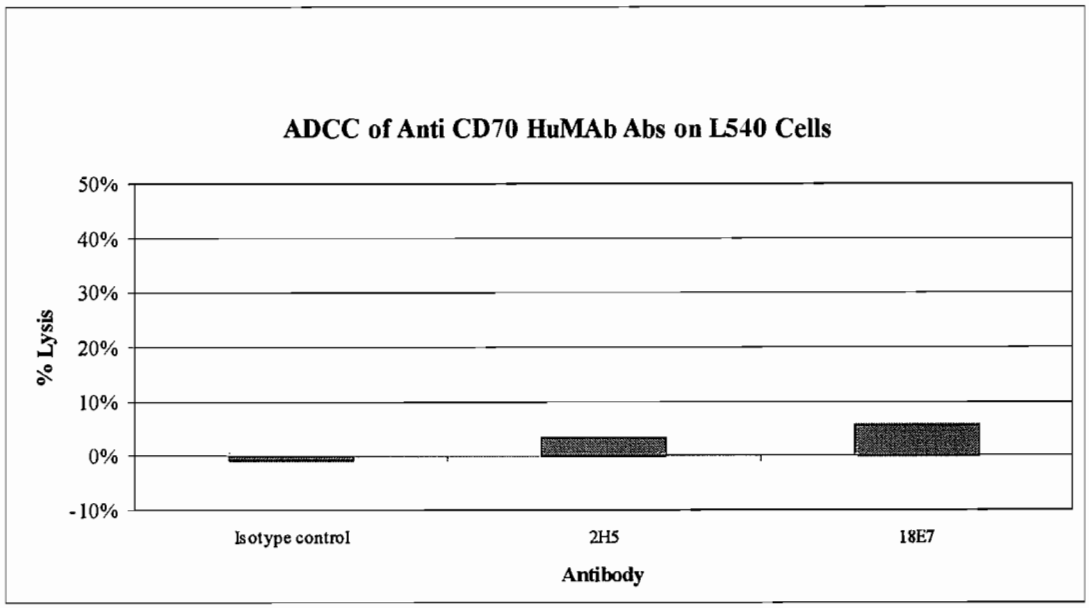


Fig. 23D

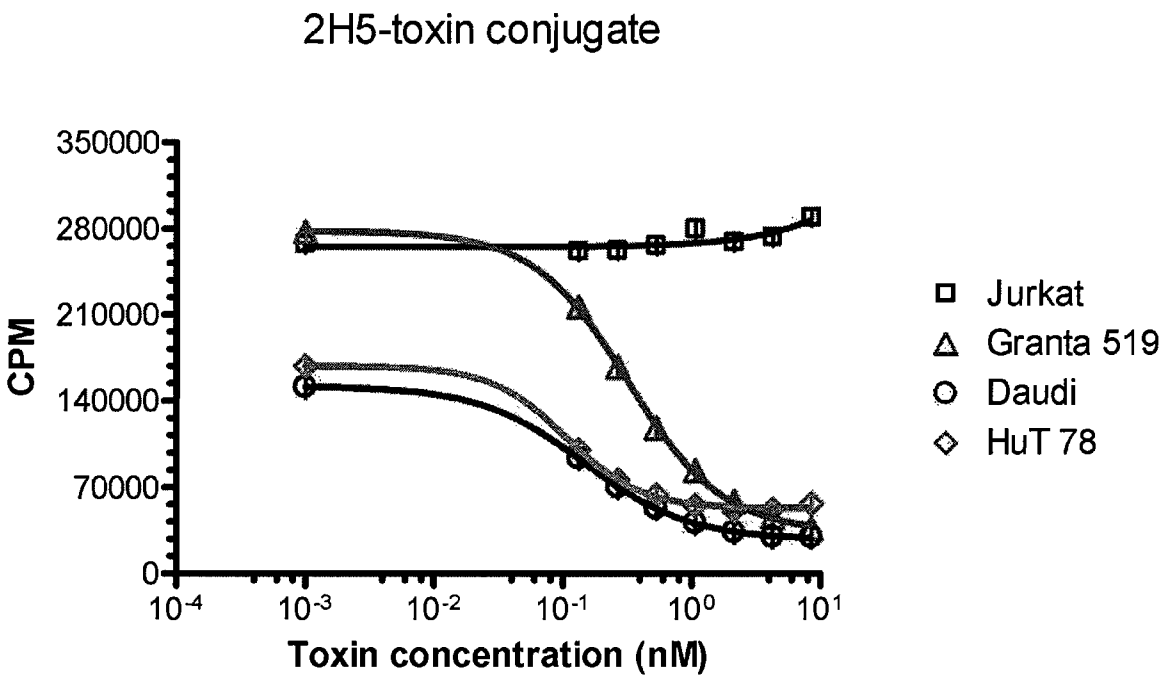

2H5-toxin conjugate
350000
280000
210000
140000
70000
0
CPM
10-4
10-3
10-2
10-1
100
101
Toxin concentration (nM)
Jurkat
Granta 519
Daudi
HuT 78

Fig. 24

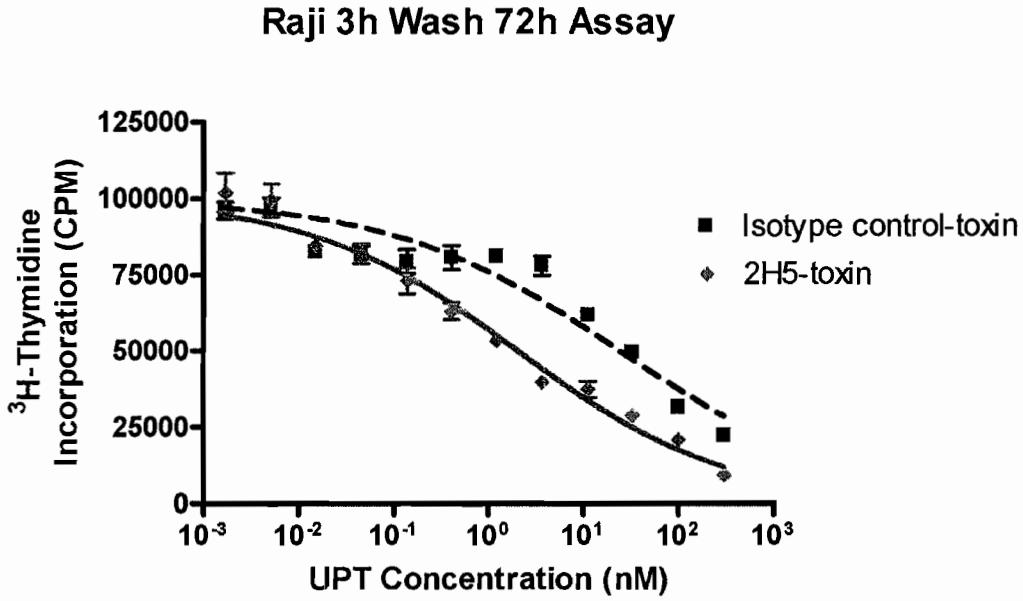


Fig. 25A

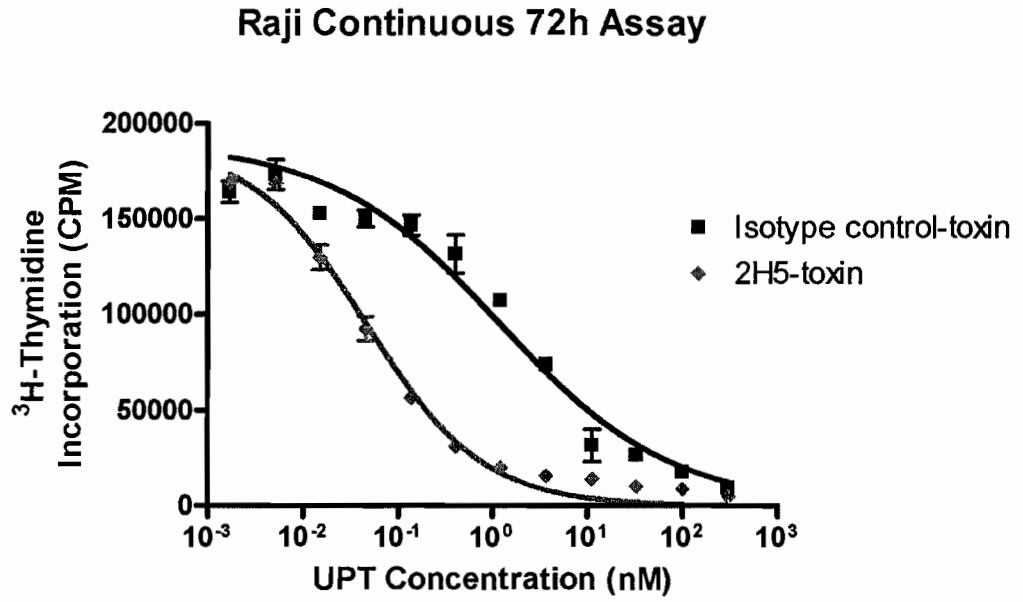


Fig. 25B

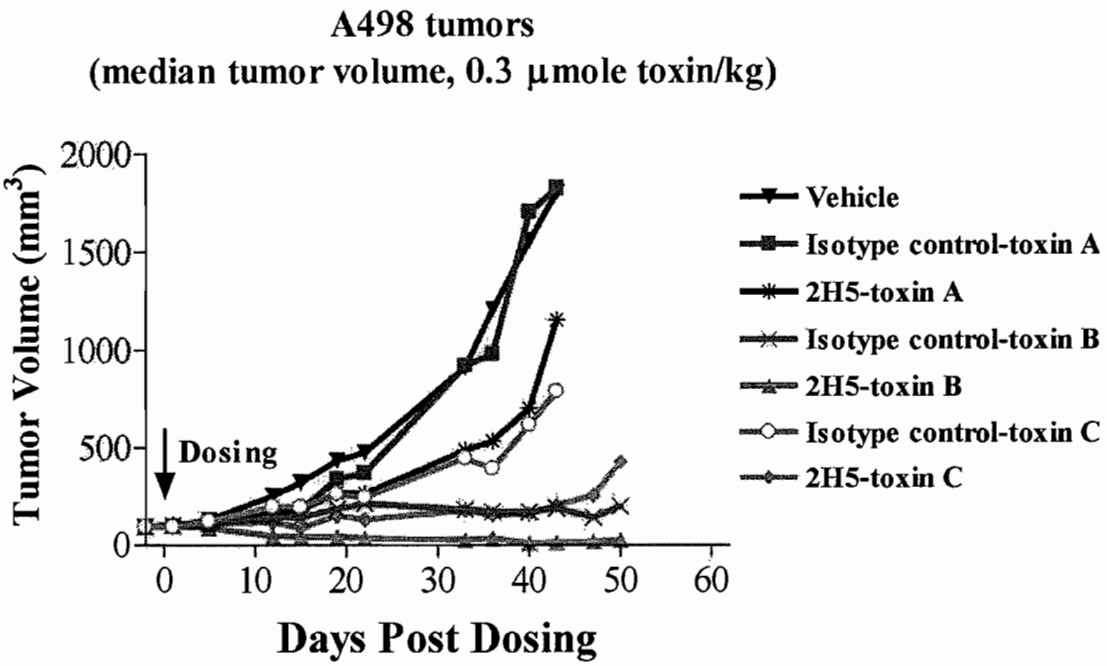


Fig. 26A

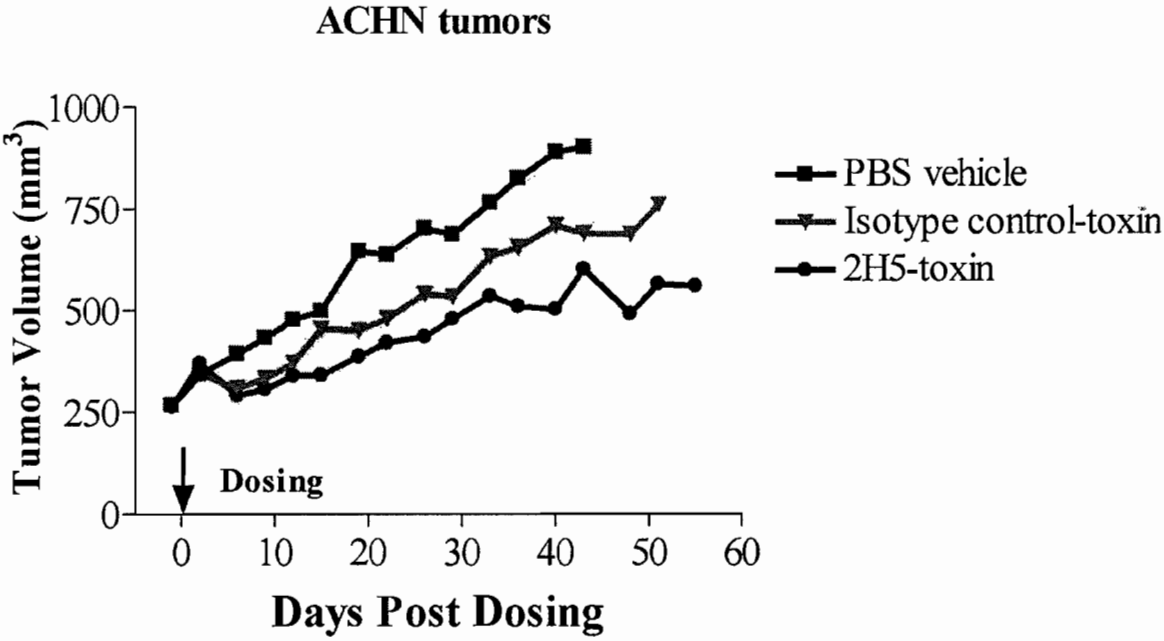


Fig. 26B

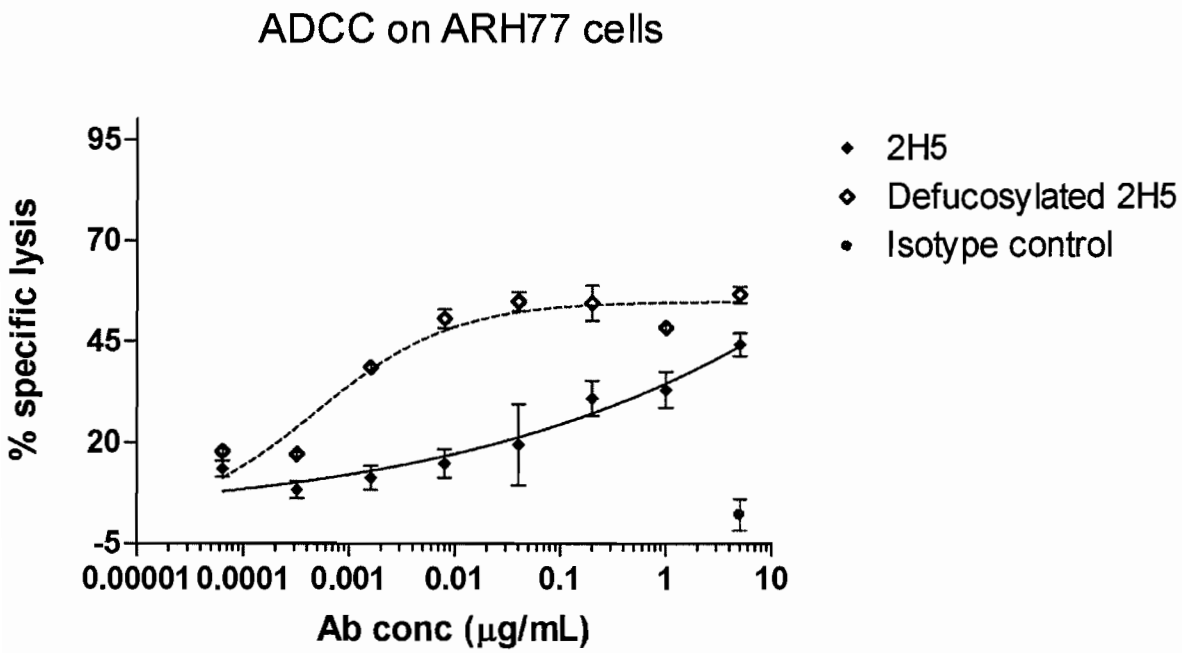


Fig. 27A

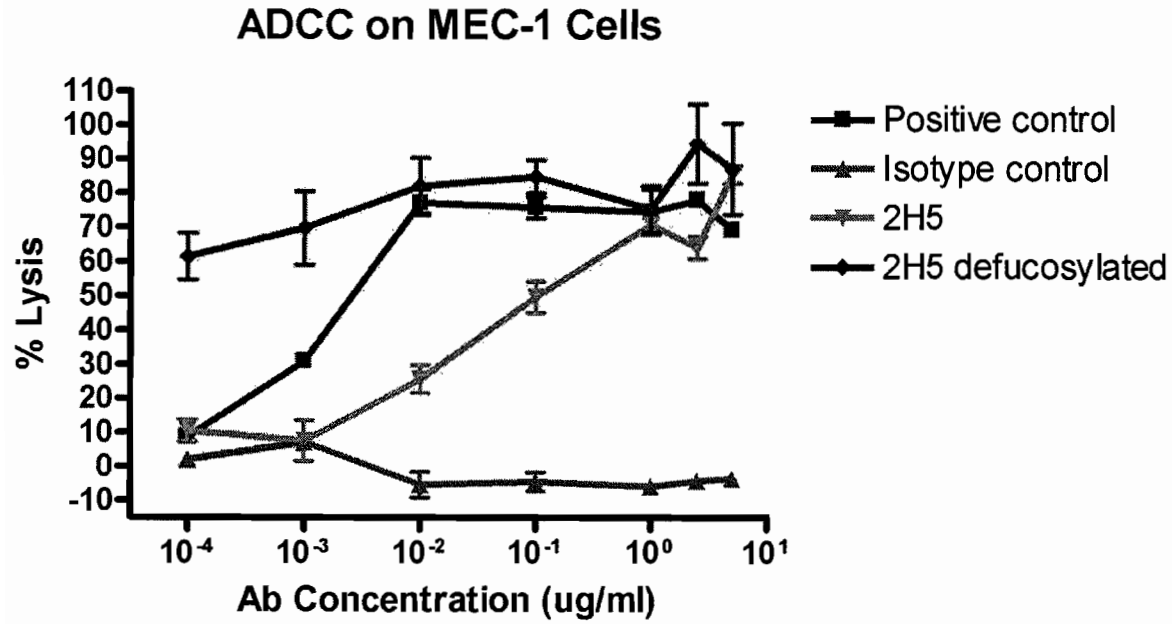


Fig. 27B

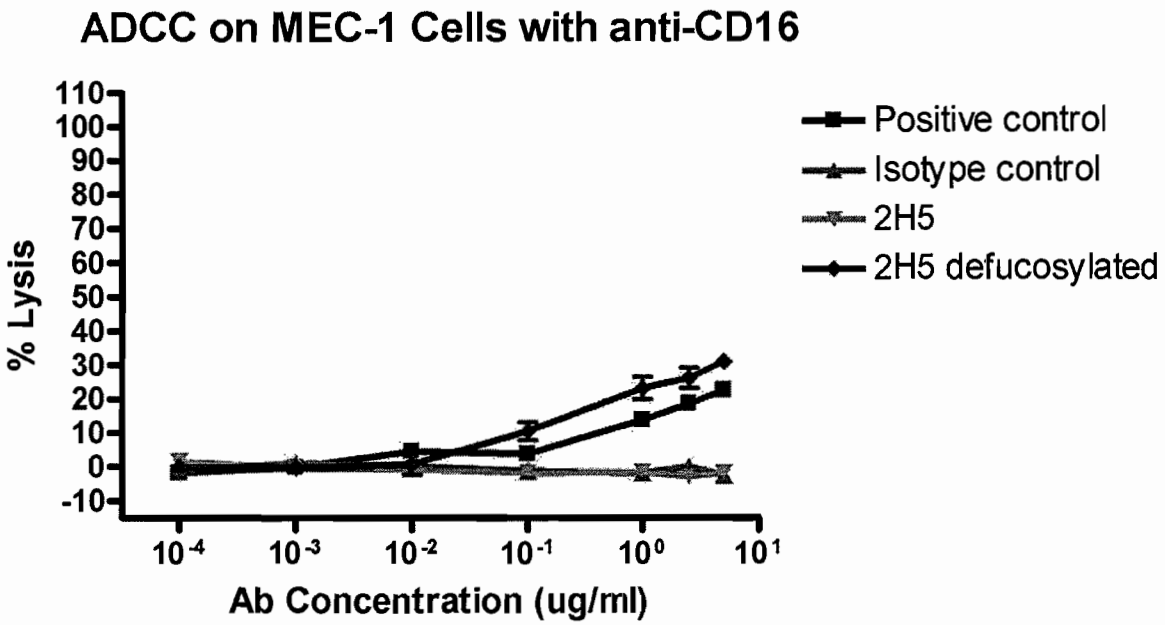


Fig. 27C

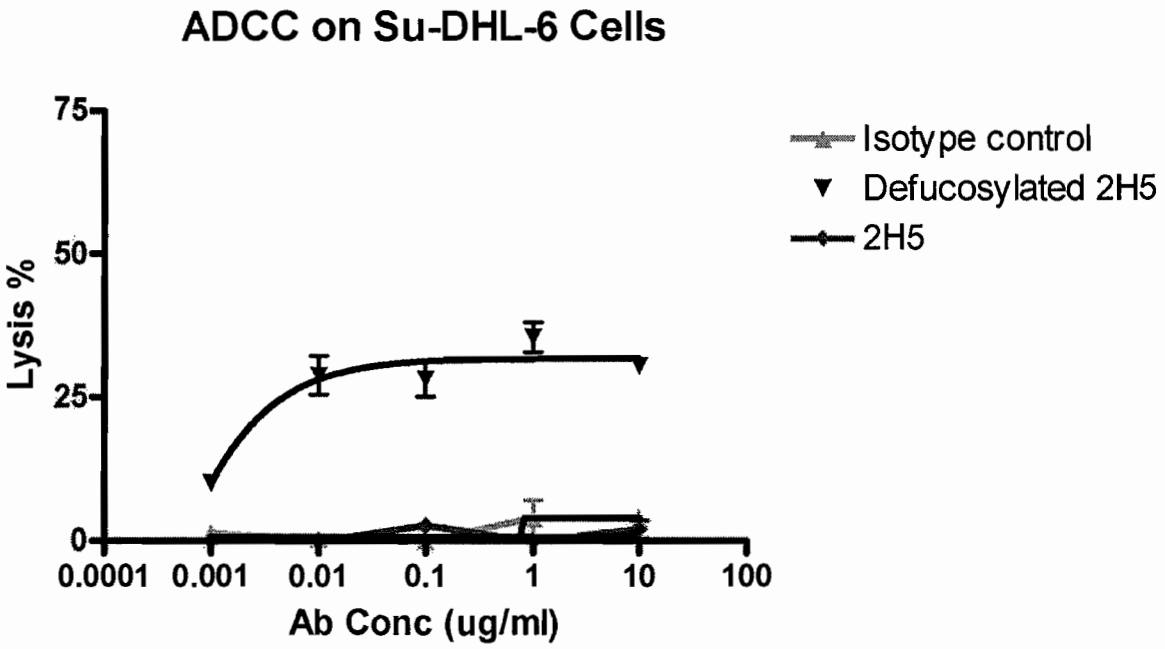


Fig. 27D

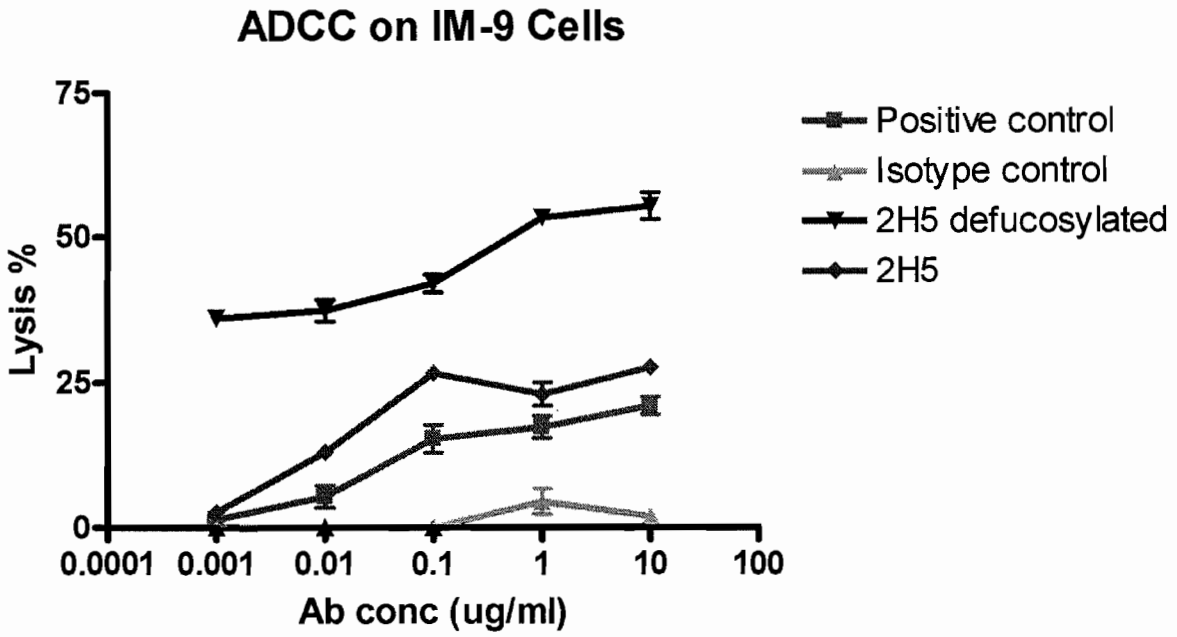


Fig. 27E

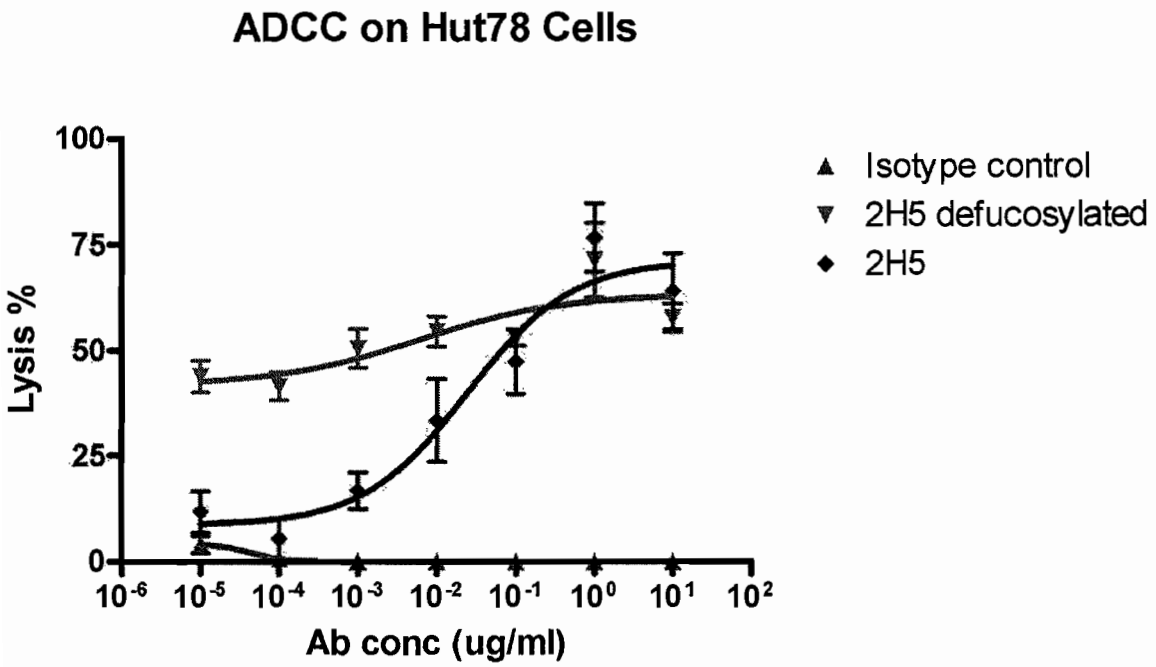


Fig. 27F

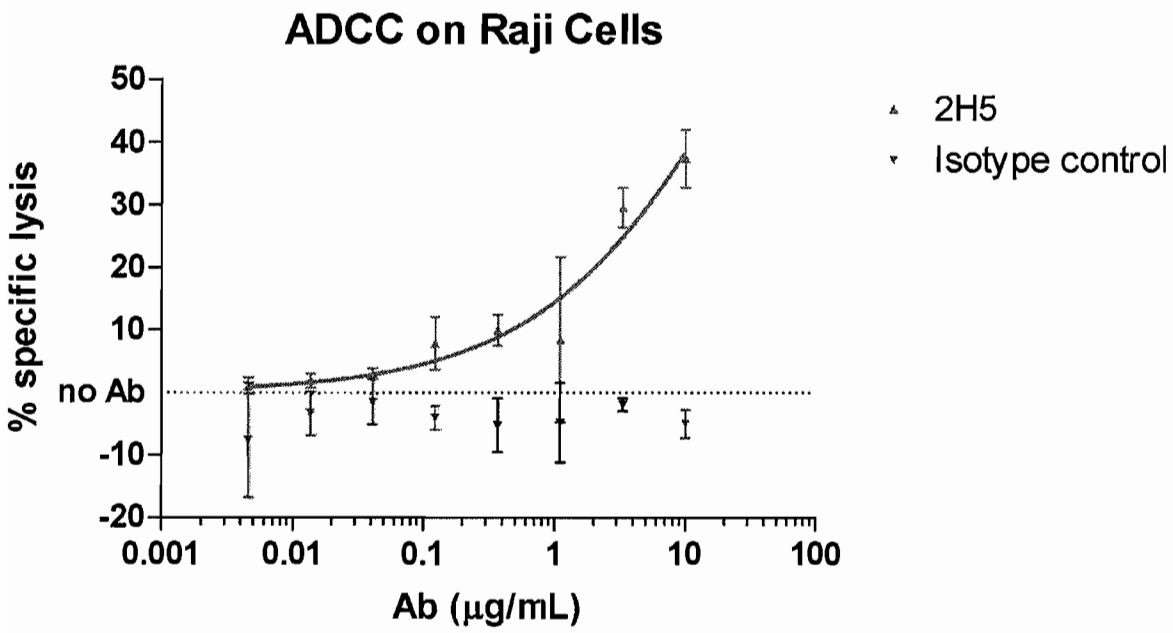

ADCC on Raji Cells
% specific lysis
50
40
30
20
10
no Ab
-10
-20
0.001
0.01
0.1
1
10
100
Ab (µg/mL)
2H5
Isotype control

Fig. 28

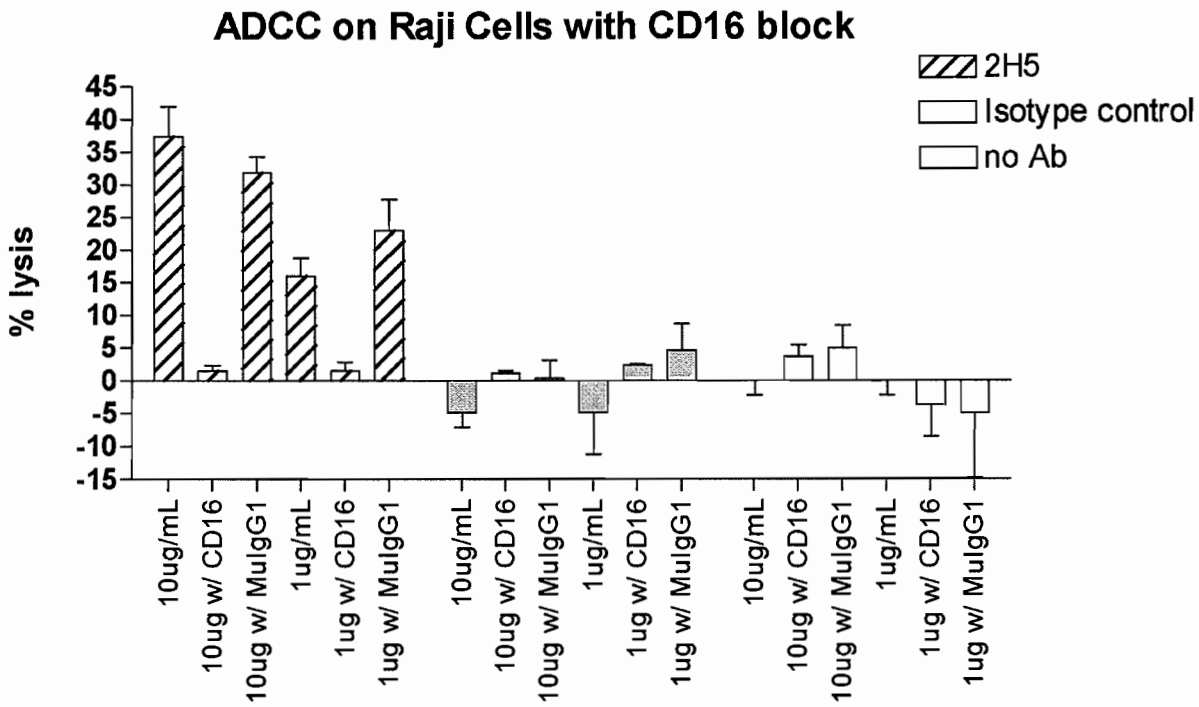

ADCC on Raji Cells with CD16 block
2H5
Isotype control
no Ab
% lysis
45
40
35
30
25
20
15
10
5
0
-5
-10
-15
10ug/mL
10ug w/ CD16
10ug w/ MuIgG1
1ug/mL
1ug w/ CD16
1ug w/ MuIgG1
10ug/mL
10ug w/ CD16
10ug w/ MuIgG1
1ug/mL
1ug w/ CD16
1ug w/ MuIgG1
10ug/mL
10ug w/ CD16
10ug w/ MuIgG1
1ug/mL
1ug w/ CD16
1ug w/ MuIgG1

Fig. 29

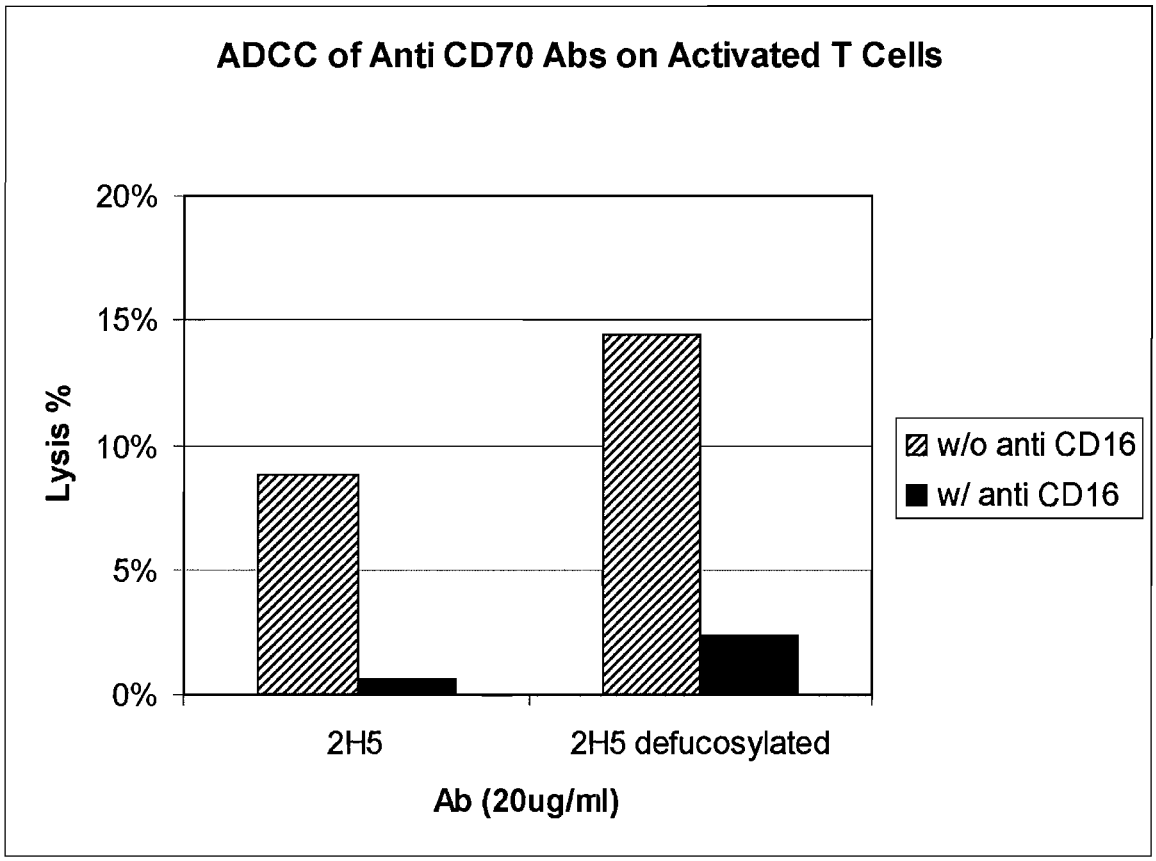

ADCC of Anti CD70 Abs on Activated T Cells
20%
15%
10%
5%
0%
Lysis %
2H5
2H5 defucosylated
Ab (20ug/ml)
w/o anti CD16
w/ anti CD16

Fig. 30

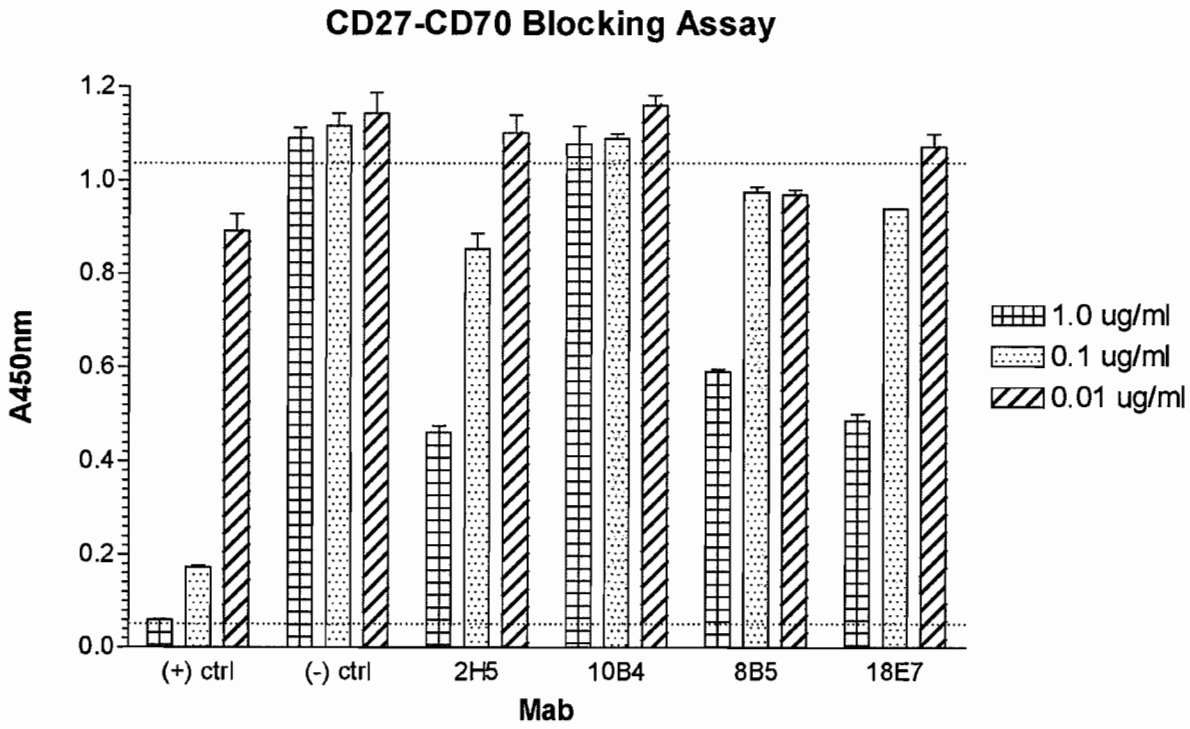

CD27-CD70 Blocking Assay
A450nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
(+) ctrl
(-) ctrl
2H5
10B4
8B5
18E7
Mab
1.0 ug/ml
0.1 ug/ml
0.01 ug/ml

Fig. 31

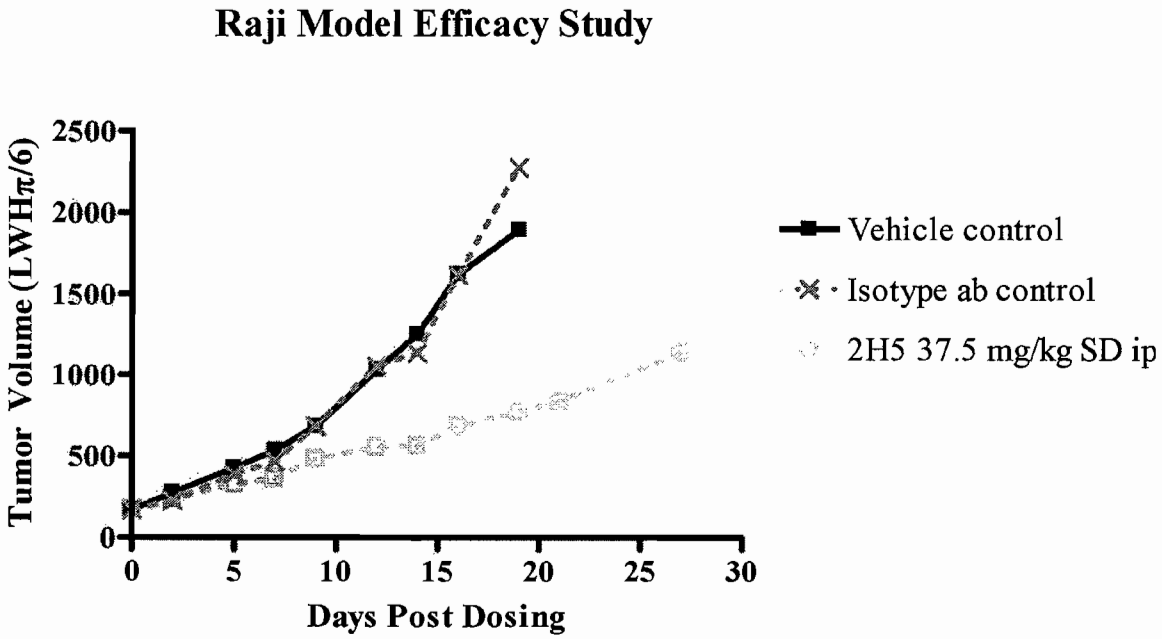


Fig. 32A

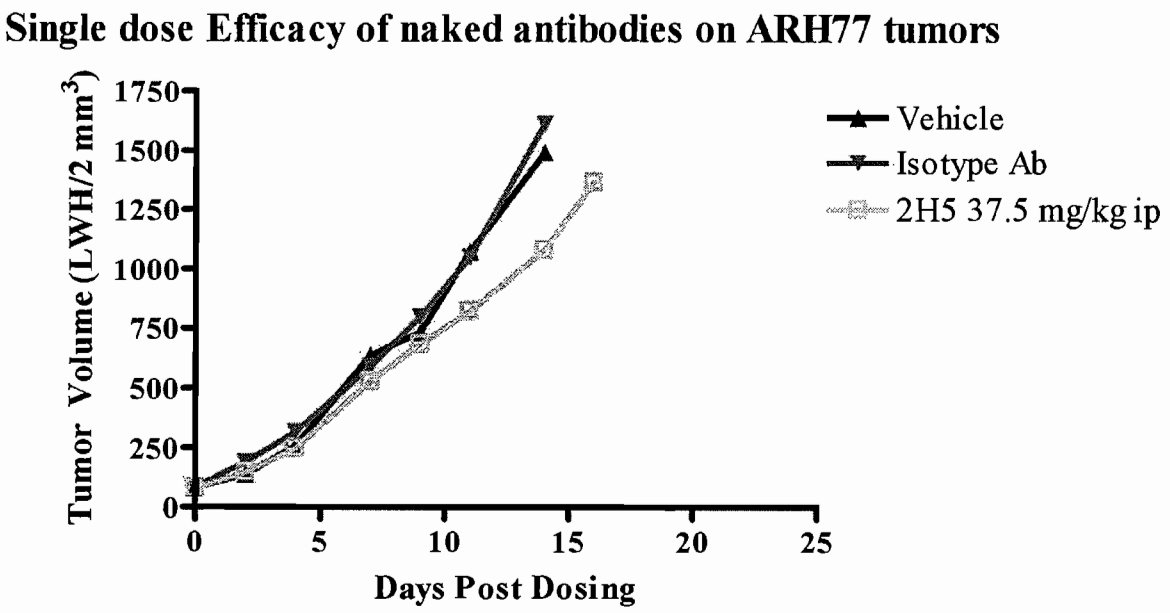


Fig. 32B

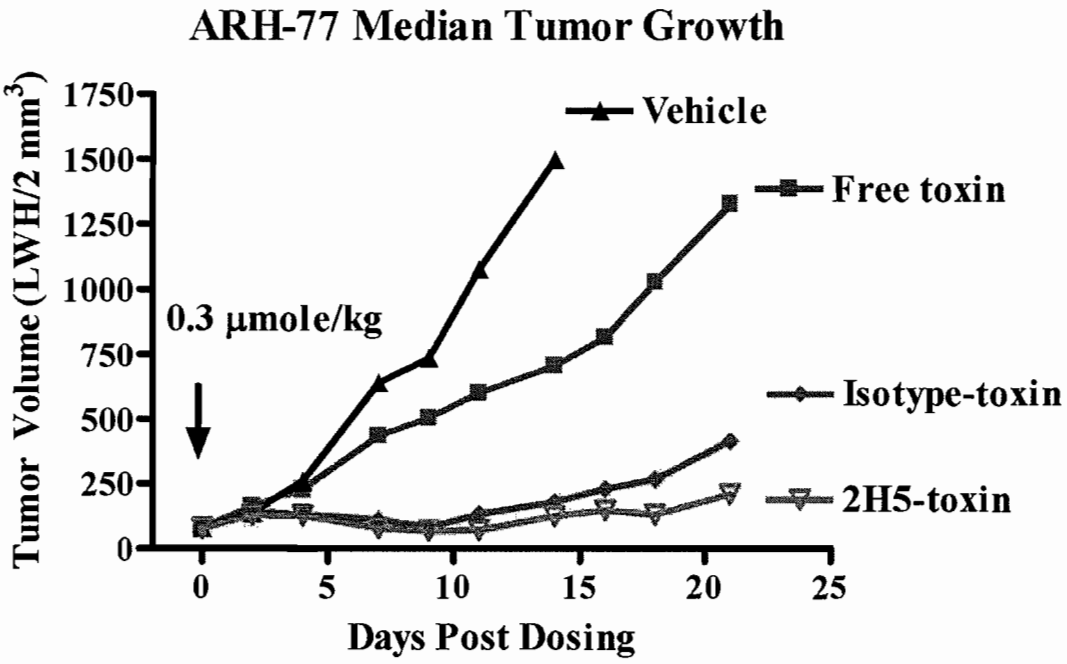


Fig. 33A

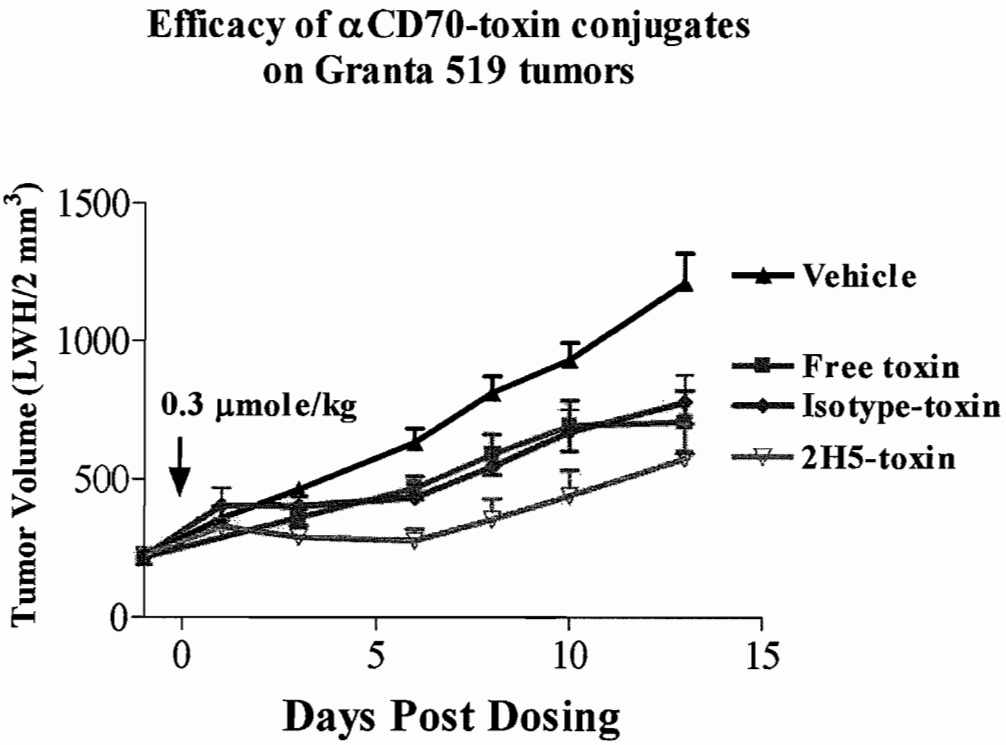


Fig. 33B

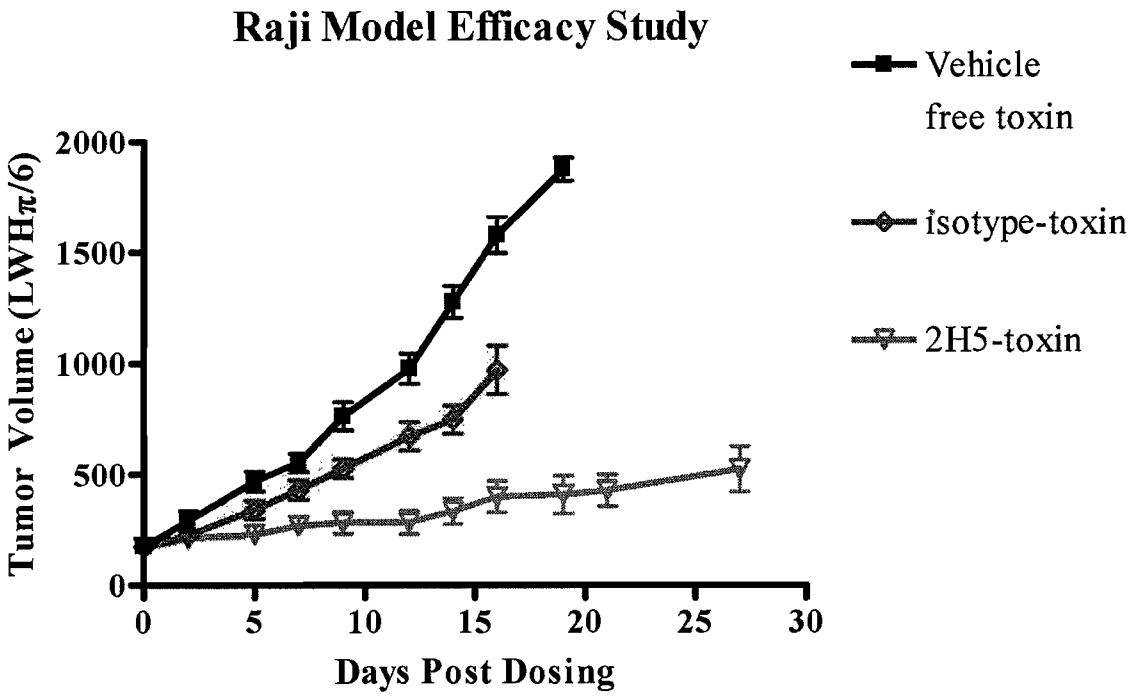

Raji Model Efficacy Study
Vehicle free toxin
isotype-toxin
2H5-toxin
Tumor Volume (LWHπ/6)
2000
1500
1000
500
0
0
5
10
15
20
25
30
Days Post Dosing

Fig. 33C

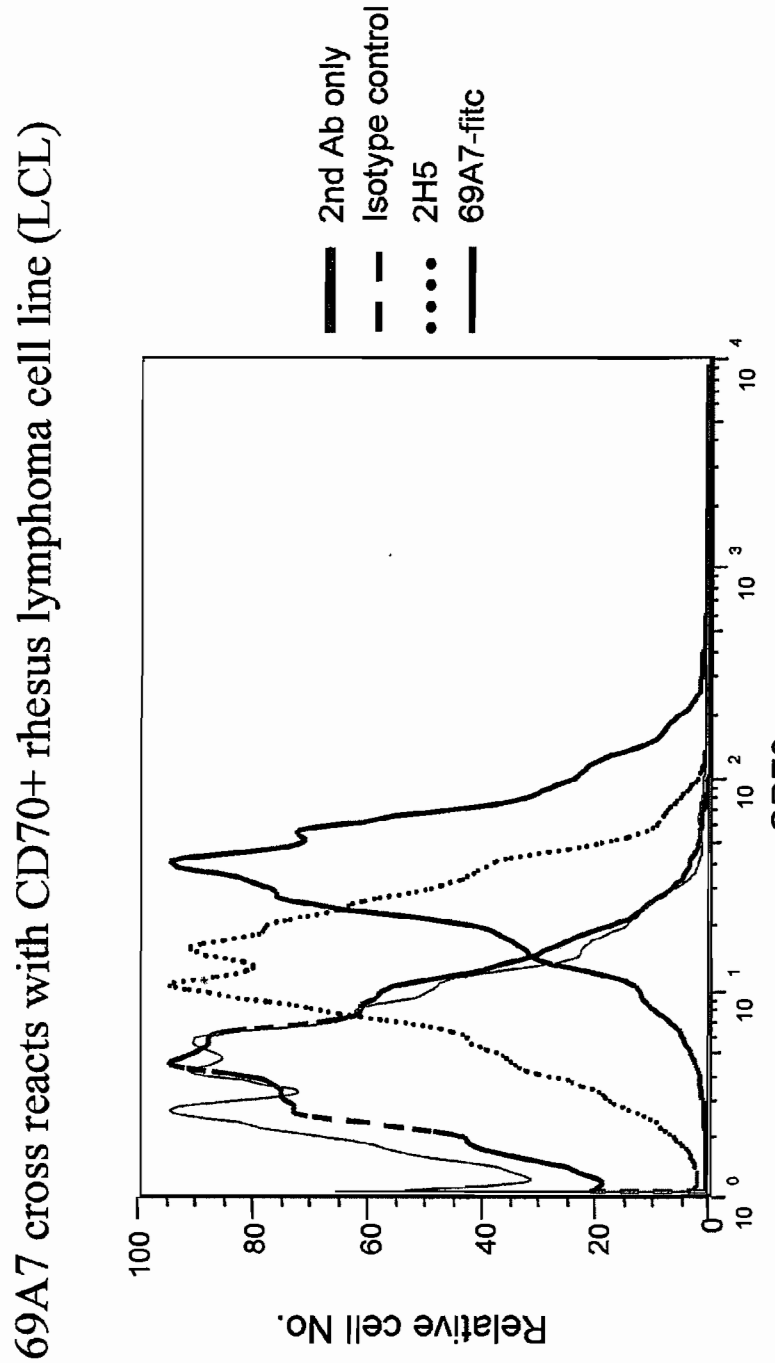

69A7 cross reacts with CD70+ rhesus lymphoma cell line (LCL)
2nd Ab only
Isotype control
2H5
69A7-fitc
Relative cell No.
100
80
60
40
20
0
10 0
10 1
10 2
10 3
10 4
CD70

Fig. 34

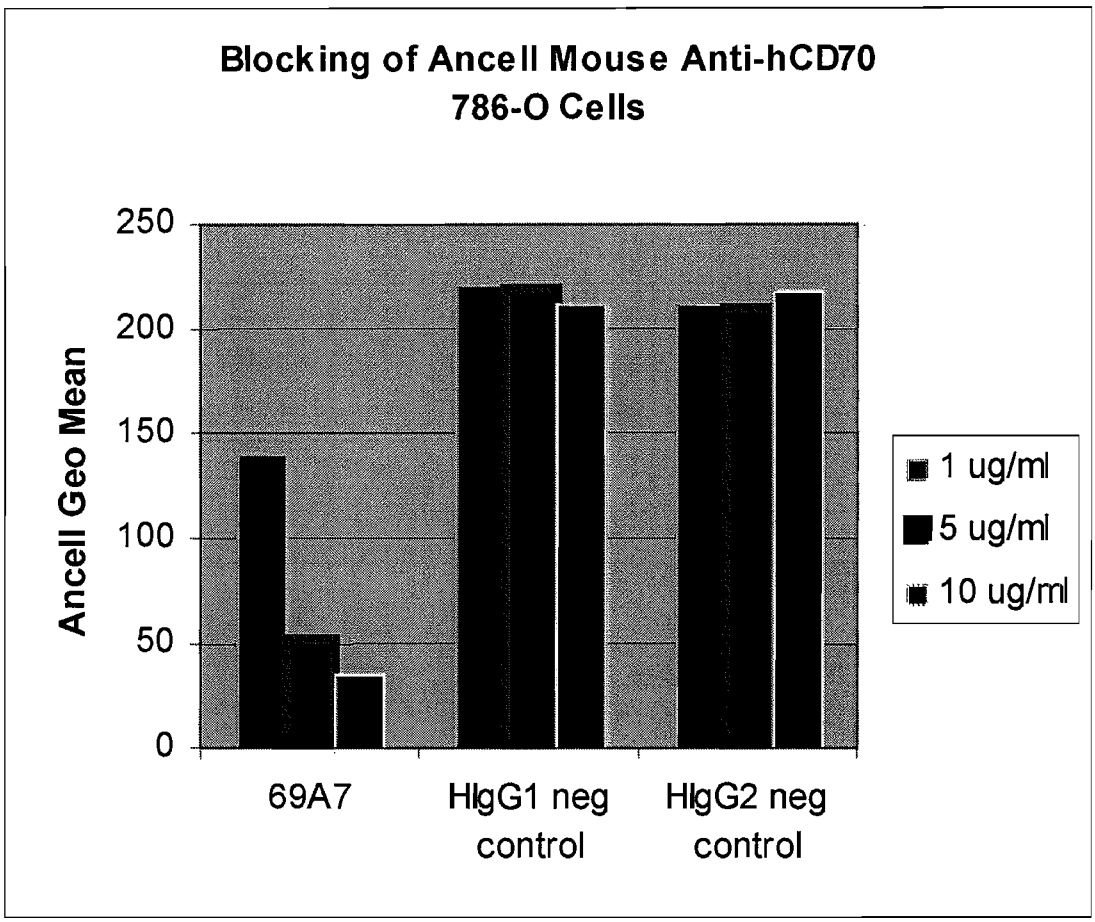

Blocking of Ancell Mouse Anti-hCD70
786-O Cells
Ancell Geo Mean
250
200
150
100
50
0
69A7
HIgG1 neg control
HIgG2 neg control
1 ug/ml
5 ug/ml
10 ug/ml

Fig. 35

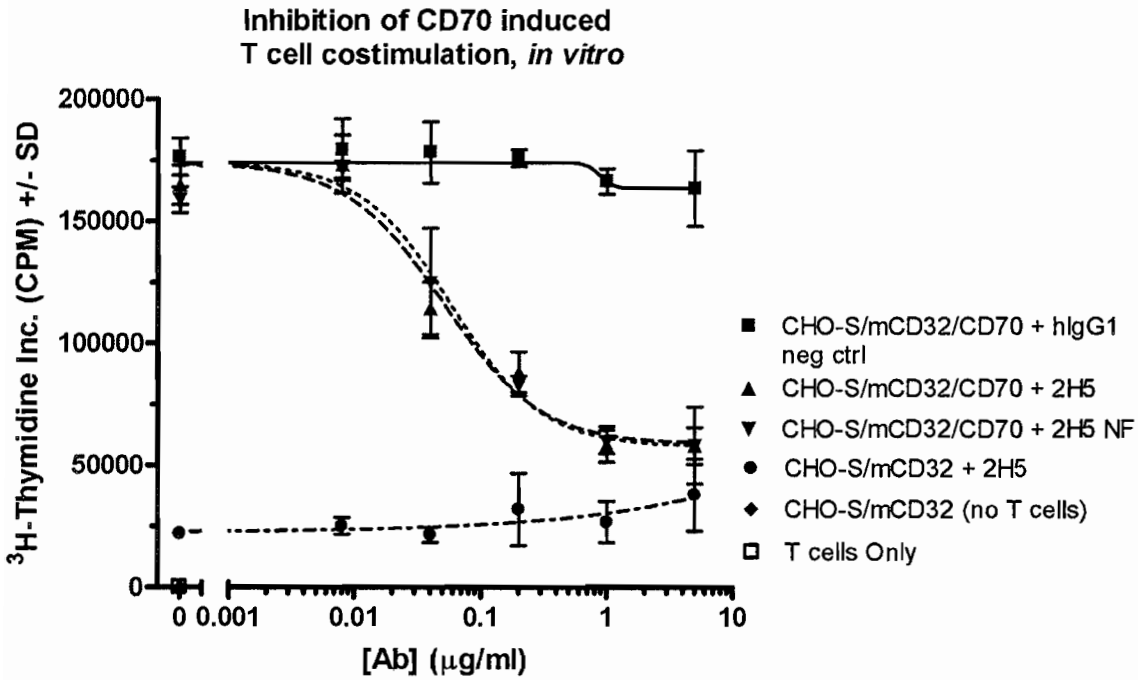

Inhibition of CD70 induced
T cell costimulation, in vitro
^{3}H-Thymidine Inc. (CPM) +/- SD
200000
150000
100000
50000
0
0 0.001 0.01 0.1 1 10
[Ab] (μg/ml)
CHO-S/mCD32/CD70 + hIgG1 neg ctrl
CHO-S/mCD32/CD70 + 2H5
CHO-S/mCD32/CD70 + 2H5 NF
CHO-S/mCD32 + 2H5
CHO-S/mCD32 (no T cells)
T cells Only

Fig. 36A

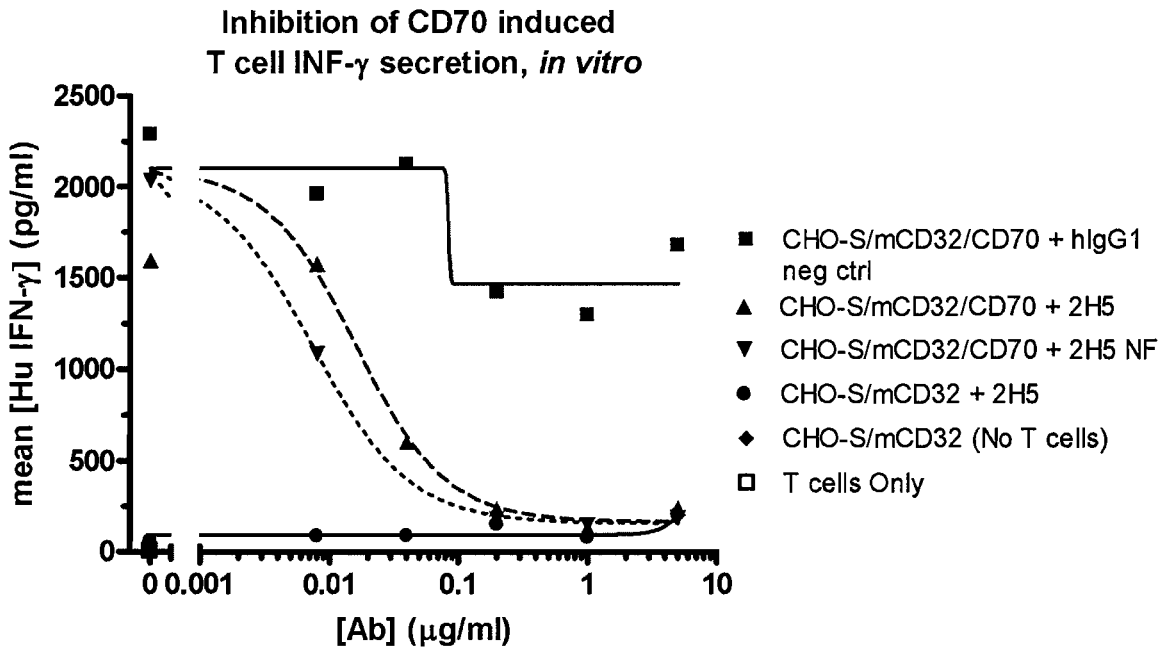

Inhibition of CD70 induced
T cell INF-γ secretion, *in vitro*
mean [Hu IFN-γ] (pg/ml)
2500
2000
1500
1000
500
0
0 0.001
0.01
0.1
1
10
[Ab] (μg/ml)
CHO-S/mCD32/CD70 + hIgG1 neg ctrl
CHO-S/mCD32/CD70 + 2H5
CHO-S/mCD32/CD70 + 2H5 NF
CHO-S/mCD32 + 2H5
CHO-S/mCD32 (No T cells)
T cells Only

Fig. 36B

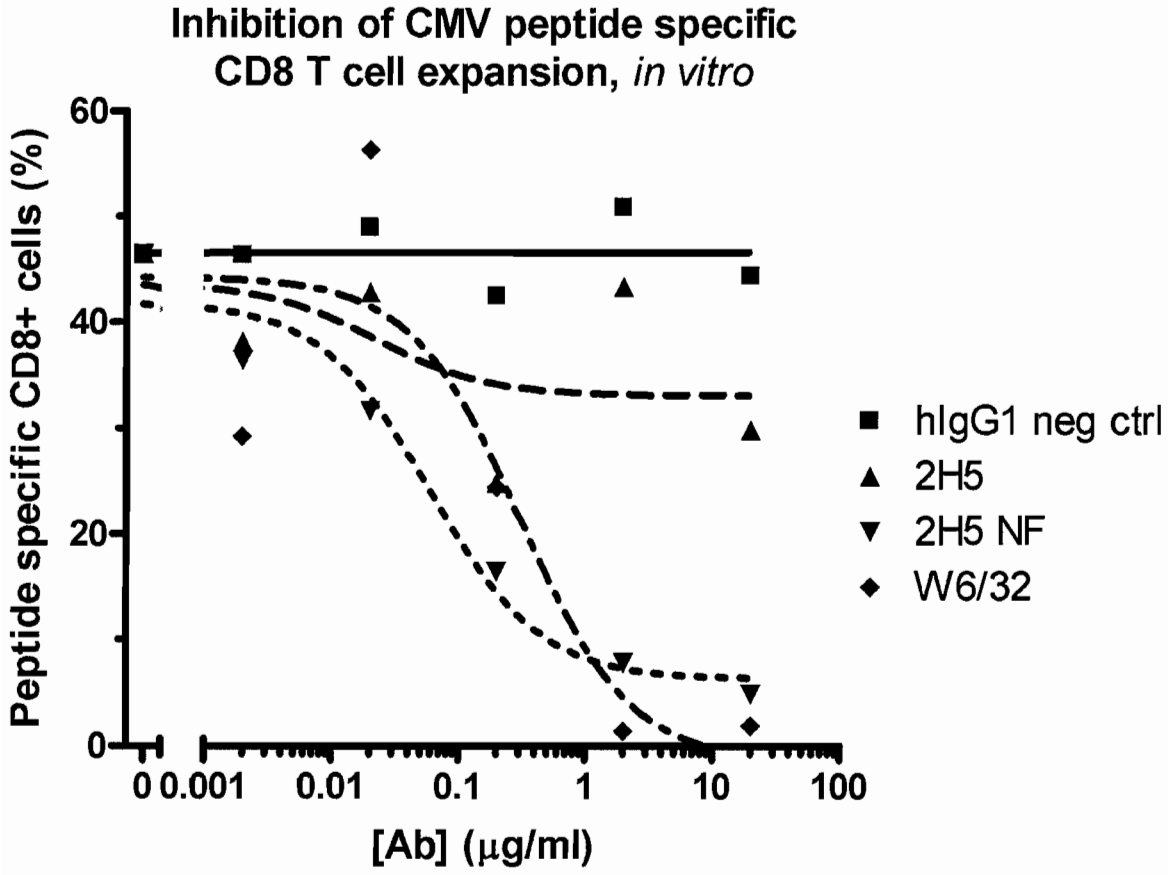

Inhibition of CMV peptide specific
CD8 T cell expansion, in vitro
Peptide specific CD8+ cells (%)
60
40
20
0
0 0.001 0.01 0.1 1 10 100
[Ab] (µg/ml)
hIgG1 neg ctrl
2H5
2H5 NF
W6/32

Fig. 37A

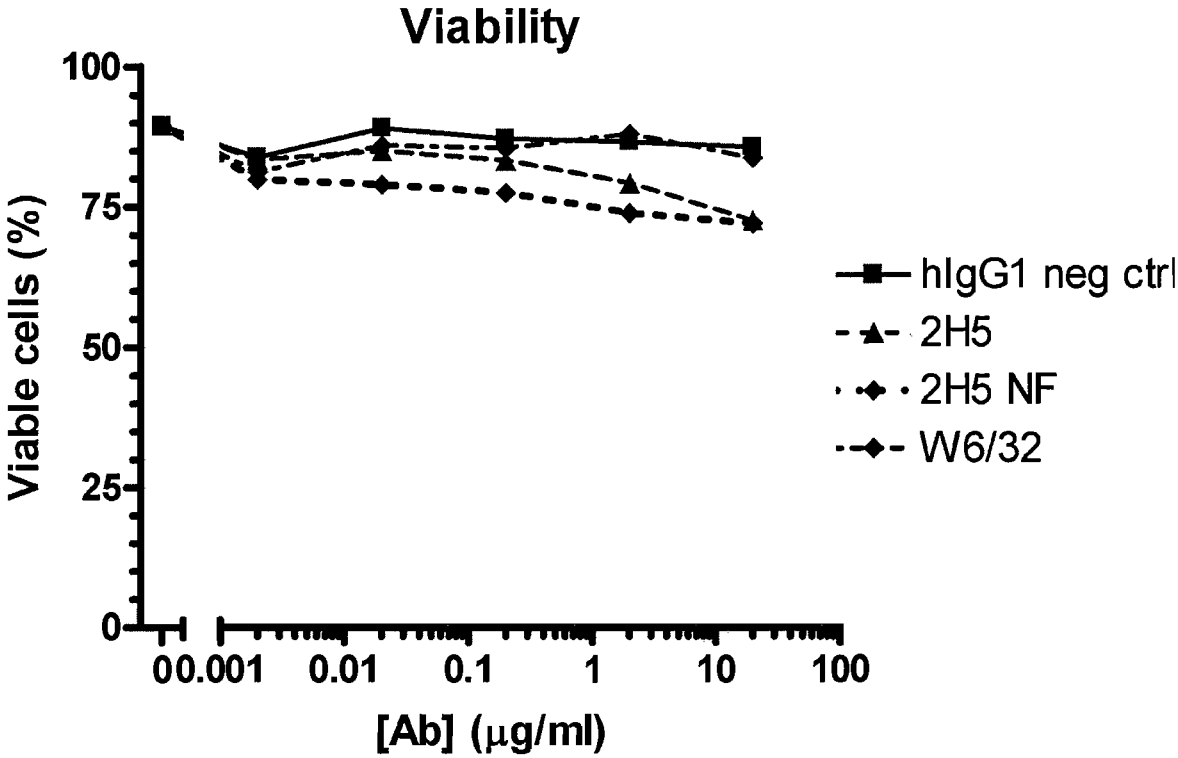

Viability
100
75
50
25
0
Viable cells (%)
00.001
0.01
0.1
1
10
100
[Ab] (µg/ml)
hIgG1 neg ctrl
2H5
2H5 NF
W6/32

Fig. 37B

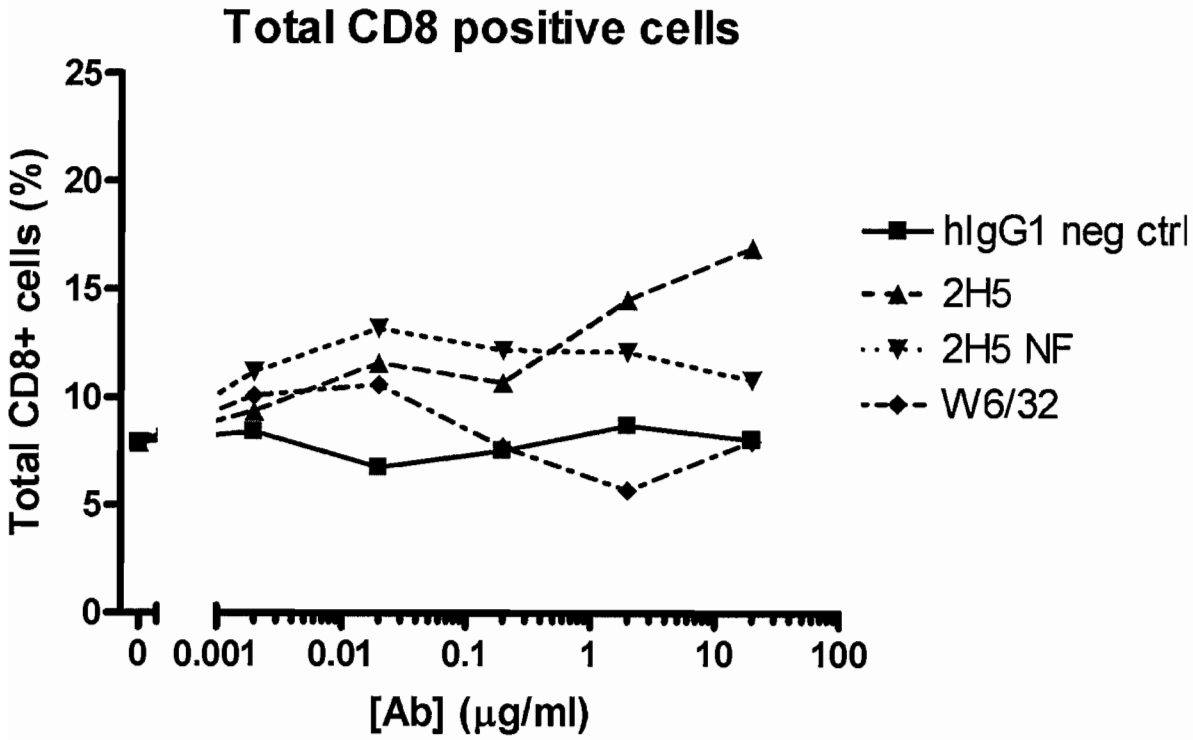

Total CD8 positive cells
Total CD8+ cells (%)
25
20
15
10
5
0
0
0.001
0.01
0.1
1
10
100
[Ab] (µg/ml)
hIgG1 neg ctrl
2H5
2H5 NF
W6/32

Fig. 37C

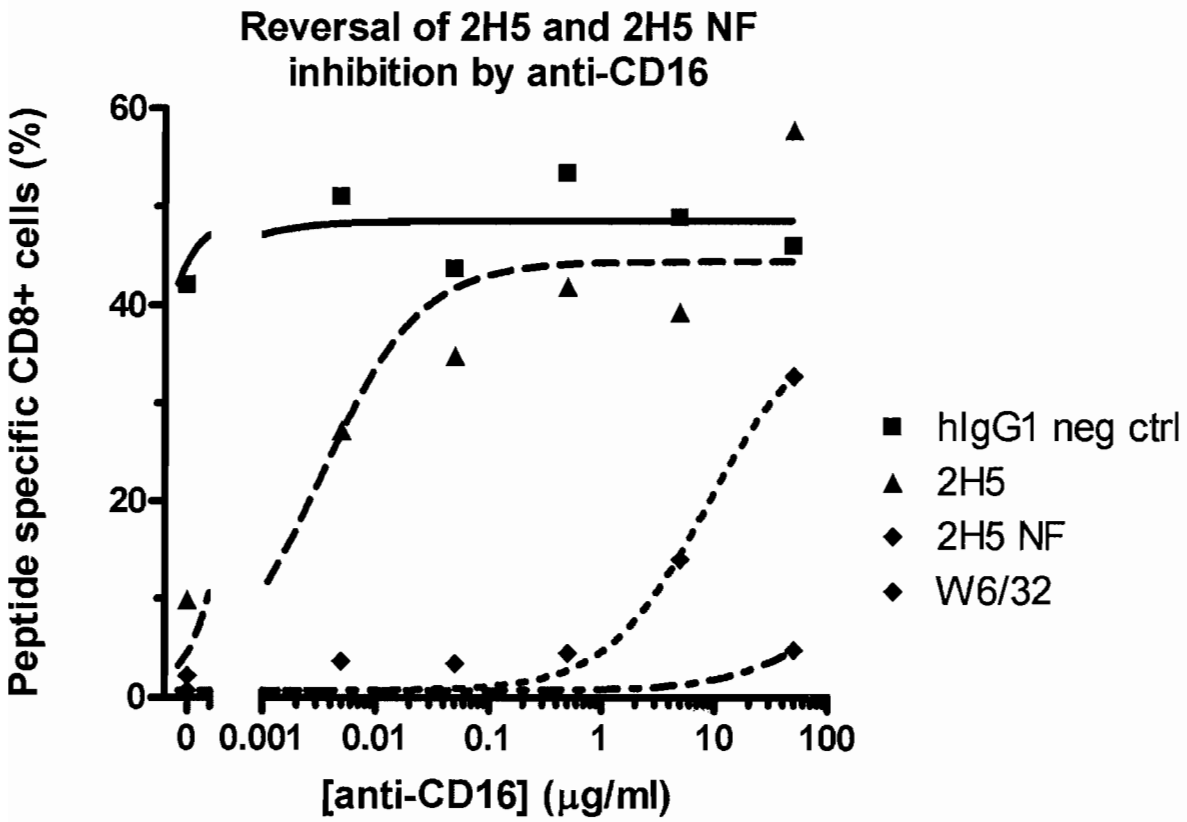

Reversal of 2H5 and 2H5 NF inhibition by anti-CD16
Peptide specific CD8+ cells (%)
60
40
20
0
0
0.001
0.01
0.1
1
10
100
[anti-CD16] (µg/ml)
hIgG1 neg ctrl
2H5
2H5 NF
W6/32

Fig. 38

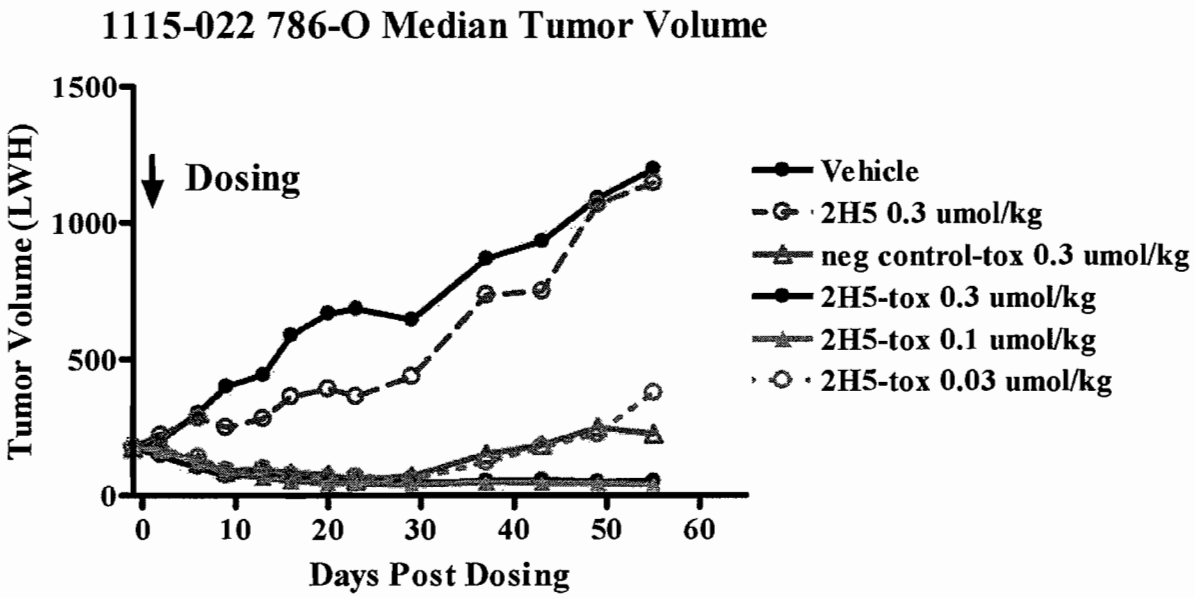


Fig. 39A

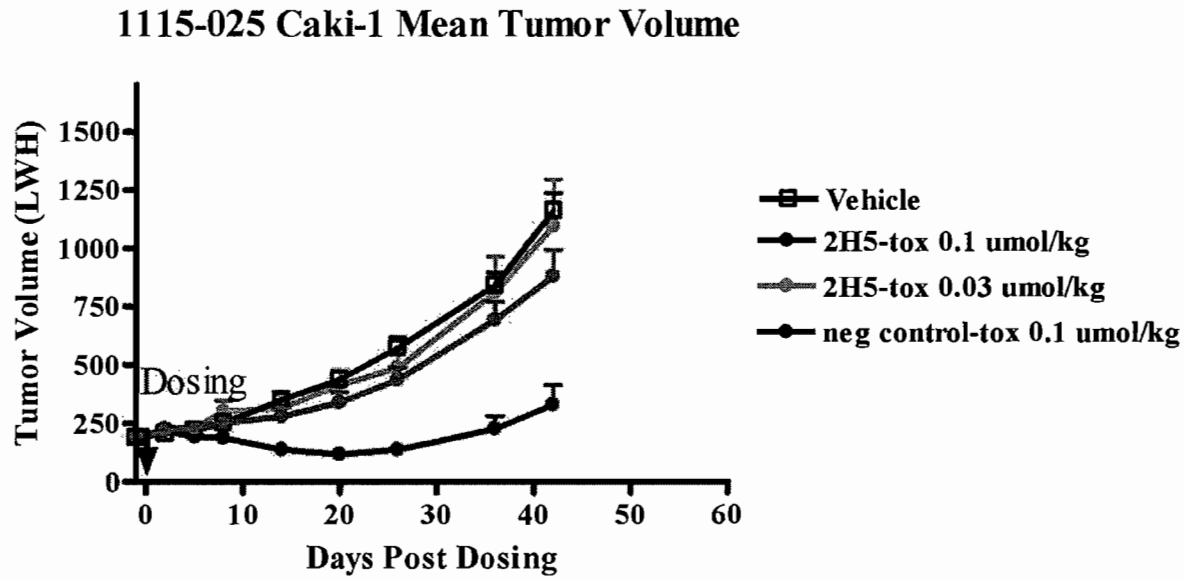


Fig. 39B

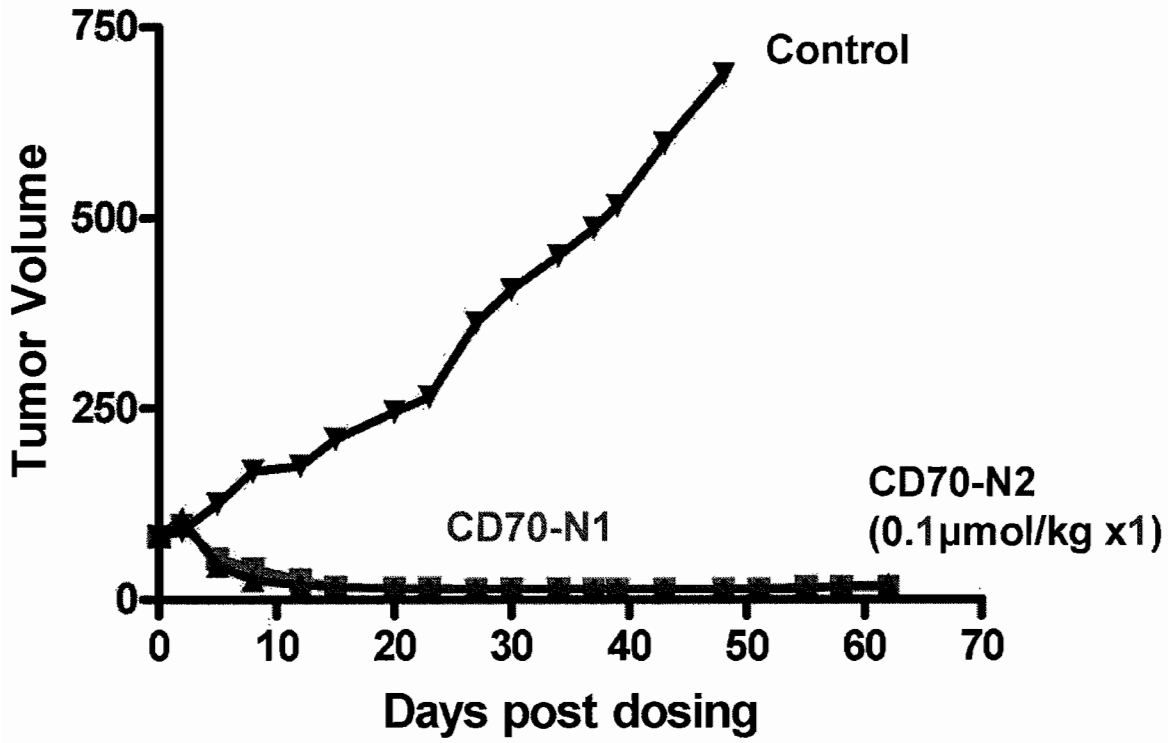

750
500
250
0
Tumor Volume
Control
CD70-N1
CD70-N2
(0.1µmol/kg x1)
0
10
20
30
40
50
60
70
Days post dosing

Fig. 40

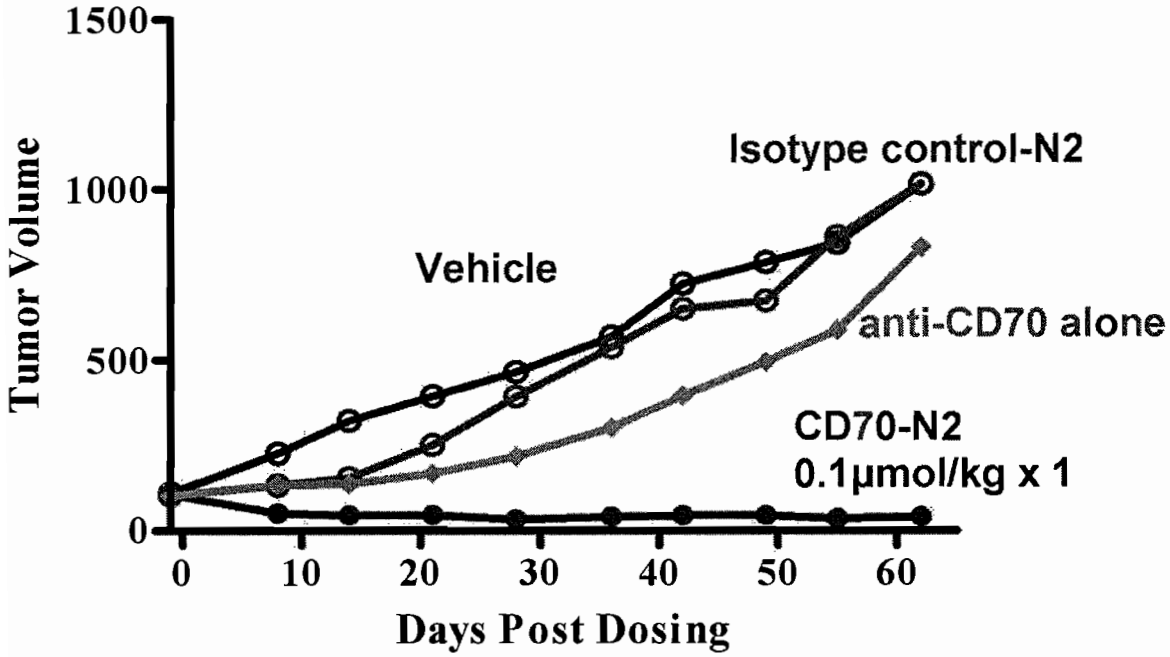

1500
1000
500
0
Tumor Volume
0
10
20
30
40
50
60
Days Post Dosing
Isotype control-N2
Vehicle
anti-CD70 alone
CD70-N2
0.1μmol/kg x 1

Fig. 41

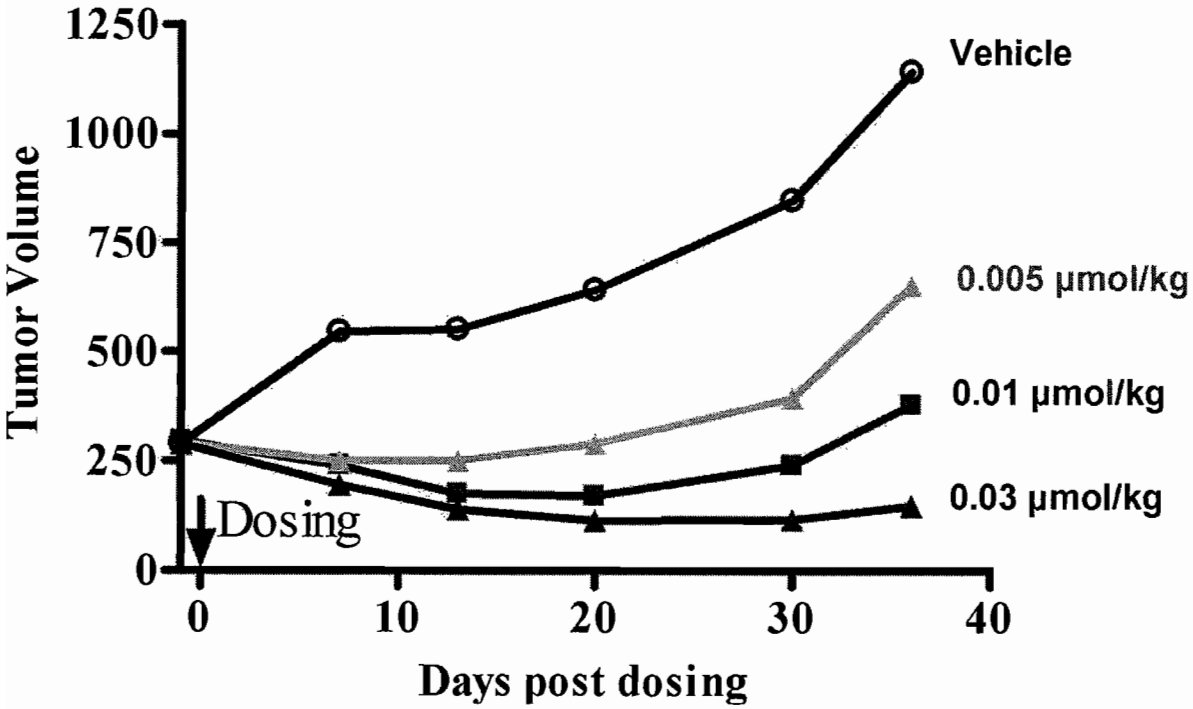

1250
1000
750
500
250
0
Tumor Volume
Dosing
0
10
20
30
40
Days post dosing
Vehicle
0.005 µmol/kg
0.01 µmol/kg
0.03 µmol/kg

Fig. 42

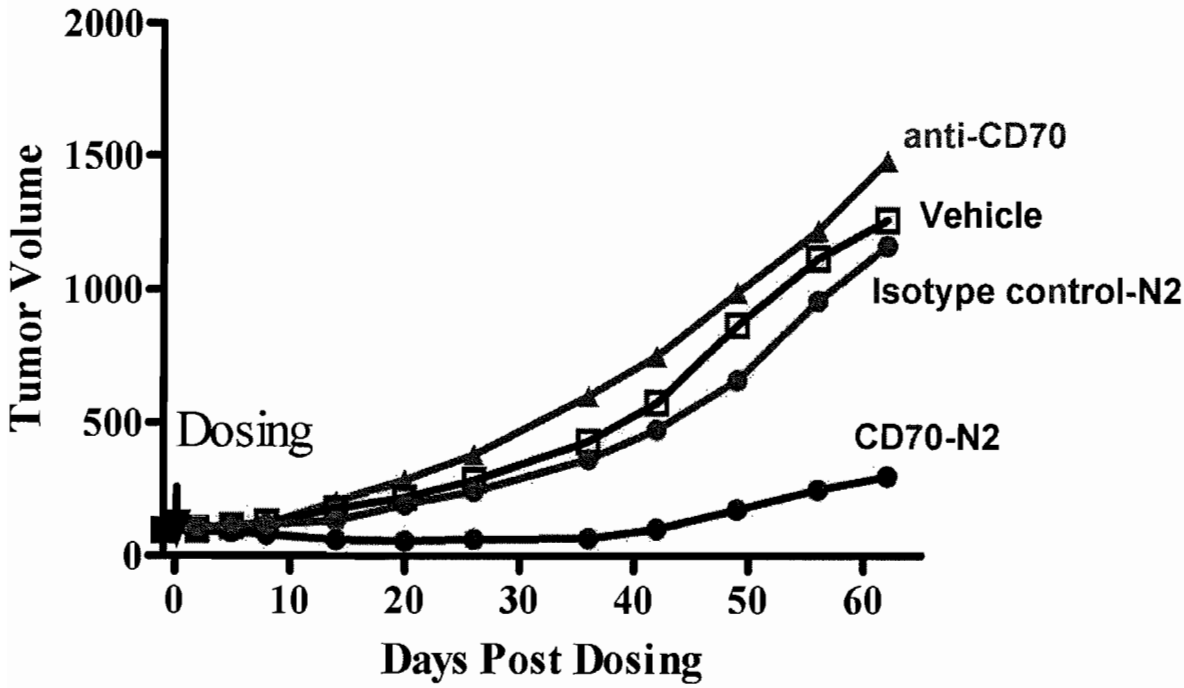

2000
1500
1000
500
0
Tumor Volume
Dosing
anti-CD70
Vehicle
Isotype control-N2
CD70-N2
0
10
20
30
40
50
60
Days Post Dosing

Fig. 43

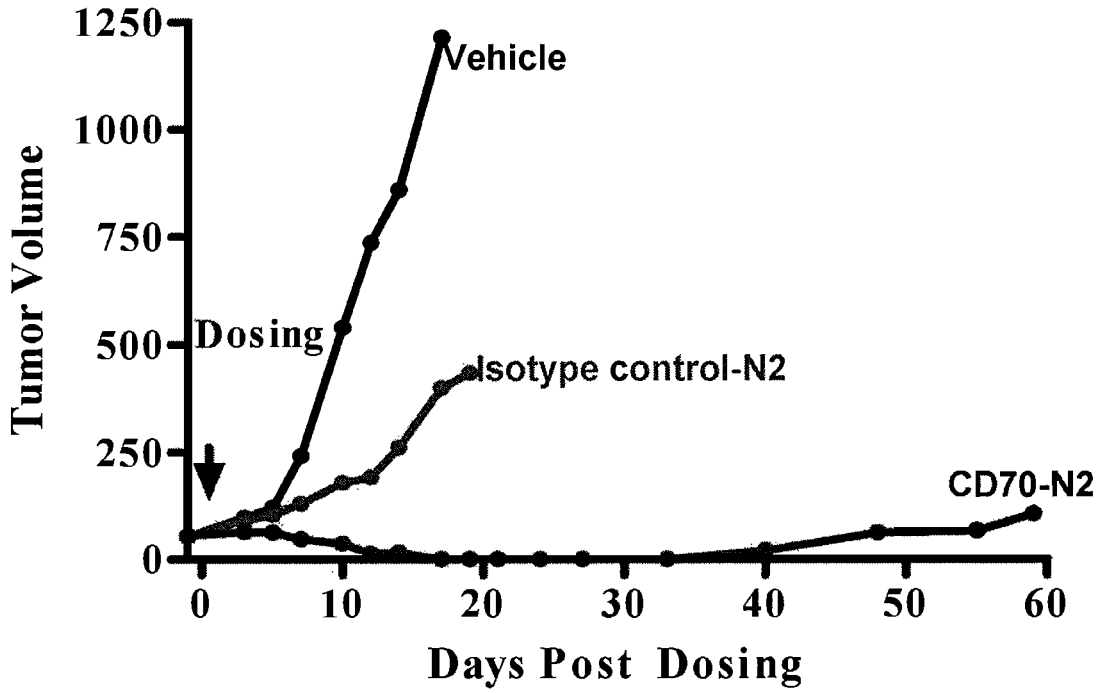

1250
1000
750
500
250
0
Tumor Volume
Dosing
Vehicle
Isotype control-N2
CD70-N2
0
10
20
30
40
50
60
Days Post Dosing

Fig. 44

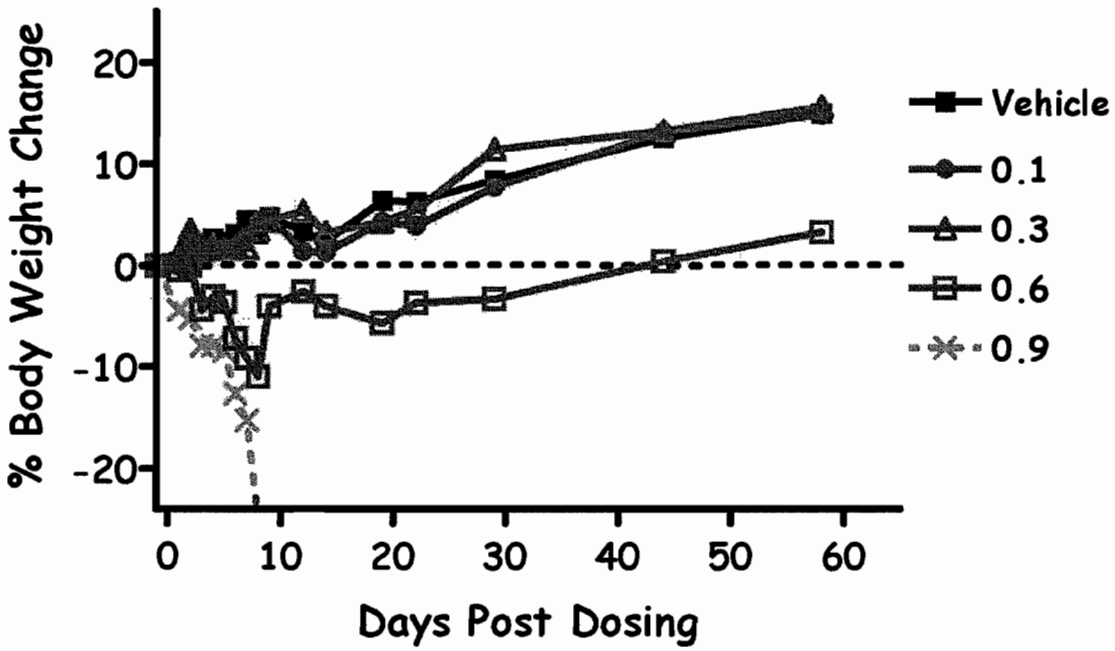

% Body Weight Change
20
10
0
-10
-20
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
0.1
0.3
0.6
0.9

Fig. 45

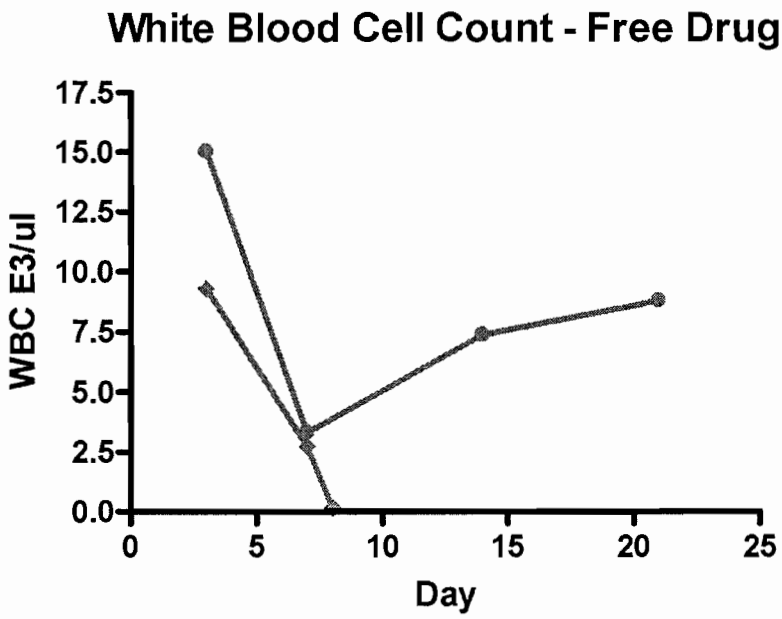


Fig. 46A

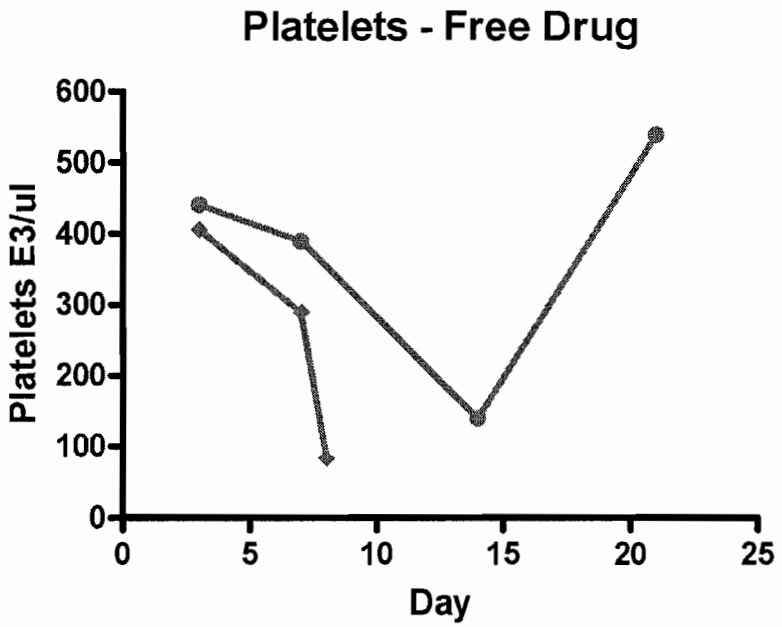


Fig. 46B

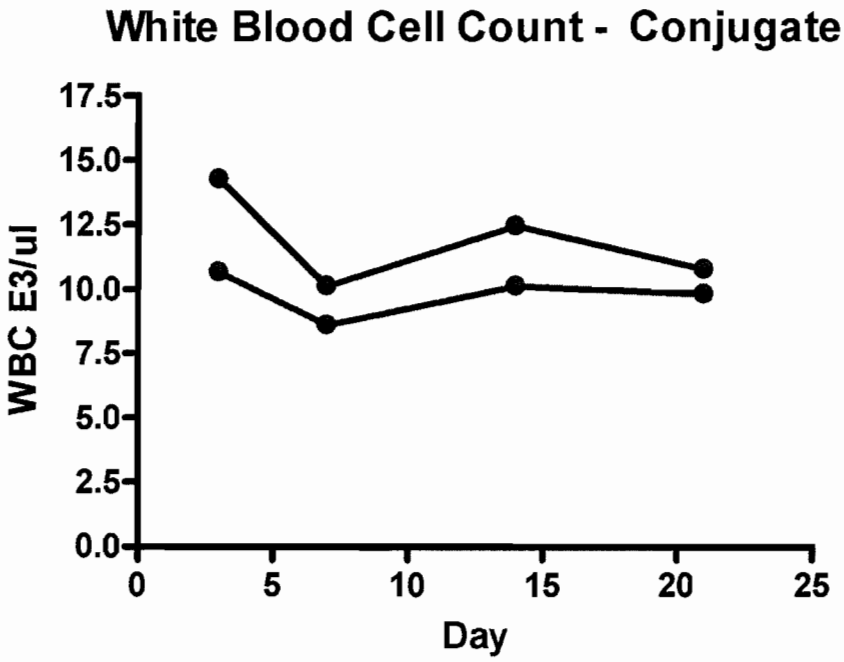


Fig. 46C

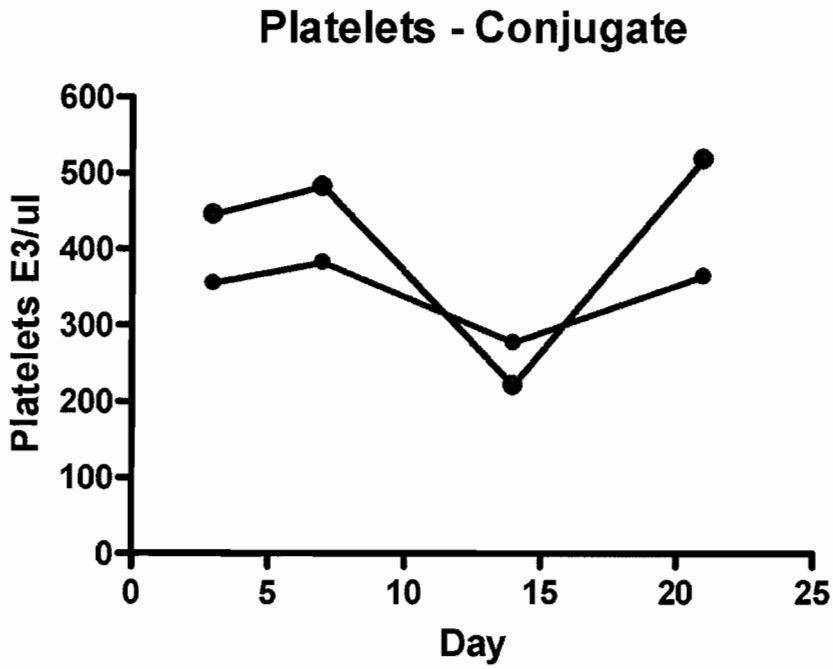


Fig. 46D

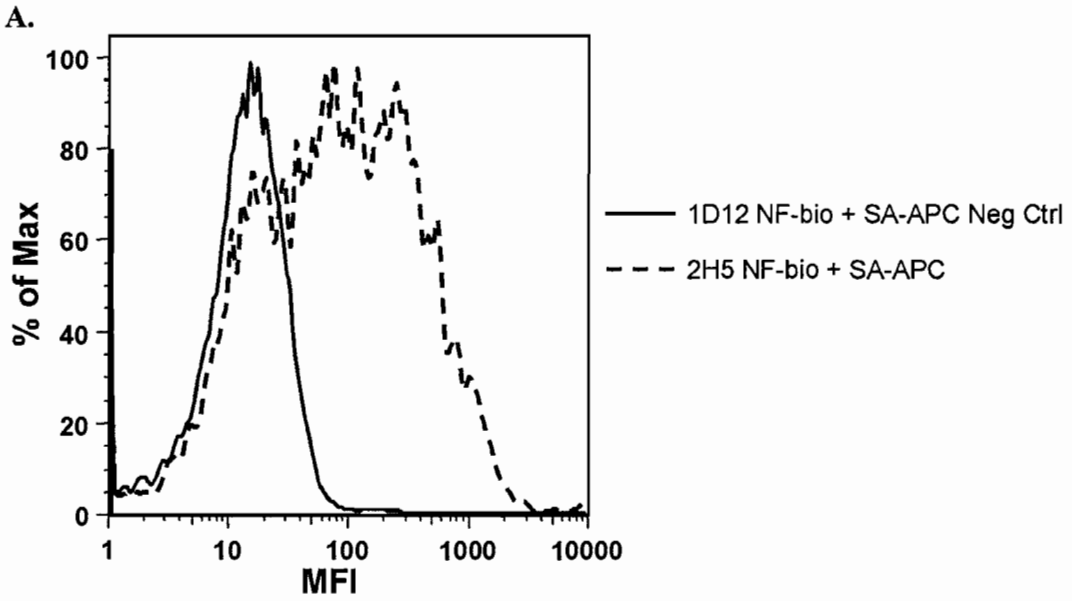

A.
100
80
60
40
20
0
% of Max
1
10
100
1000
10000
MFI
1D12 NF-bio + SA-APC Neg Ctrl
2H5 NF-bio + SA-APC

Fig. 47A

B.

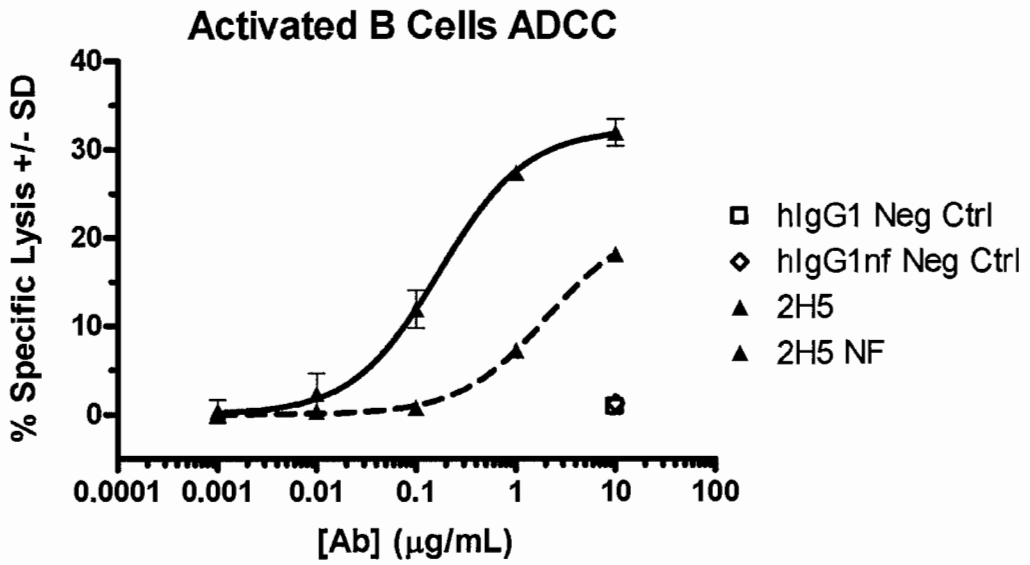

Activated B Cells ADCC
% Specific Lysis +/- SD
40
30
20
10
0
0.0001
0.001
0.01
0.1
1
10
100
[Ab] (µg/mL)
hIgG1 Neg Ctrl
hIgG1nf Neg Ctrl
2H5
2H5 NF

Fig. 47B

C.

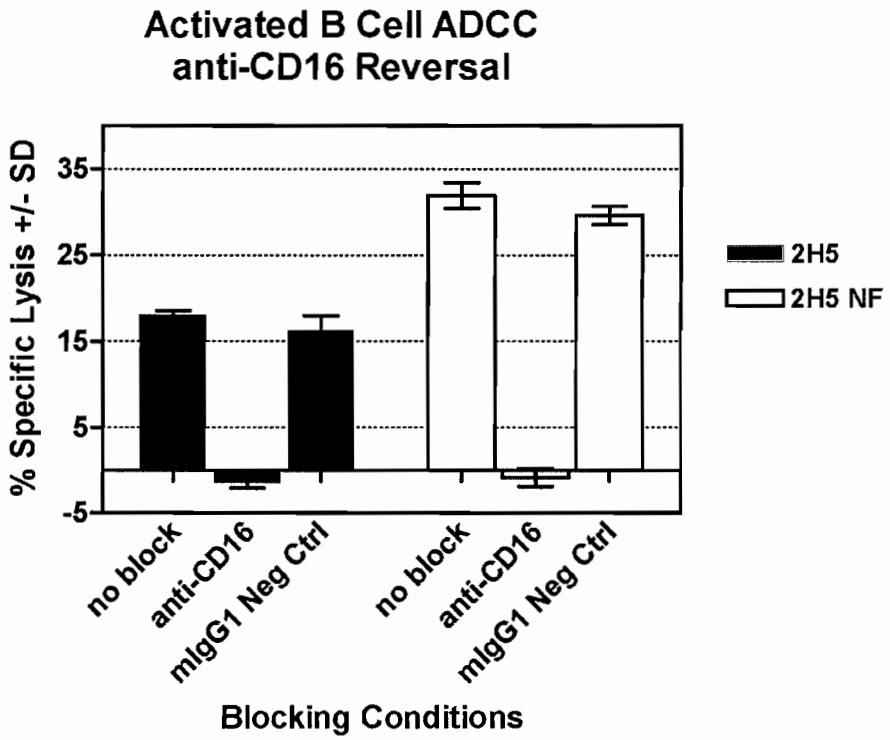

Activated B Cell ADCC
anti-CD16 Reversal
% Specific Lysis +/- SD
35
25
15
5
-5
no block
anti-CD16
mIgG1 Neg Ctrl
no block
anti-CD16
mIgG1 Neg Ctrl
Blocking Conditions
2H5
2H5 NF

Fig. 47C

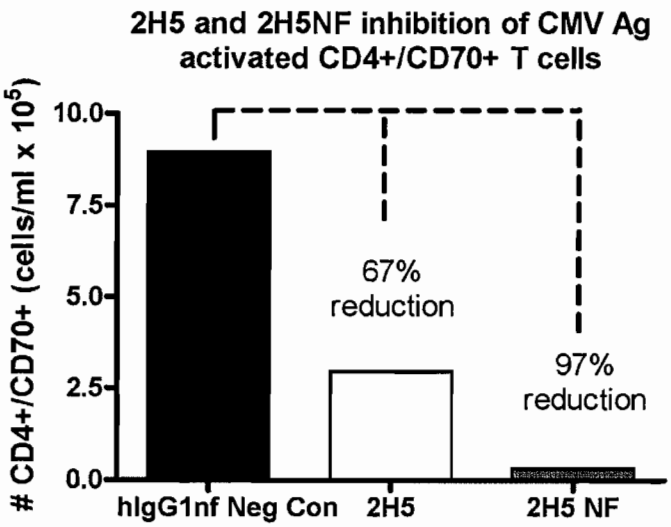

2H5 and 2H5NF inhibition of CMV Ag activated CD4+/CD70+ T cells
CD4+/CD70+ (cells/ml x 10^5)
10.0
7.5
5.0
2.5
0.0
67% reduction
97% reduction
hIgG1nf Neg Con
2H5
2H5 NF

Fig. 48

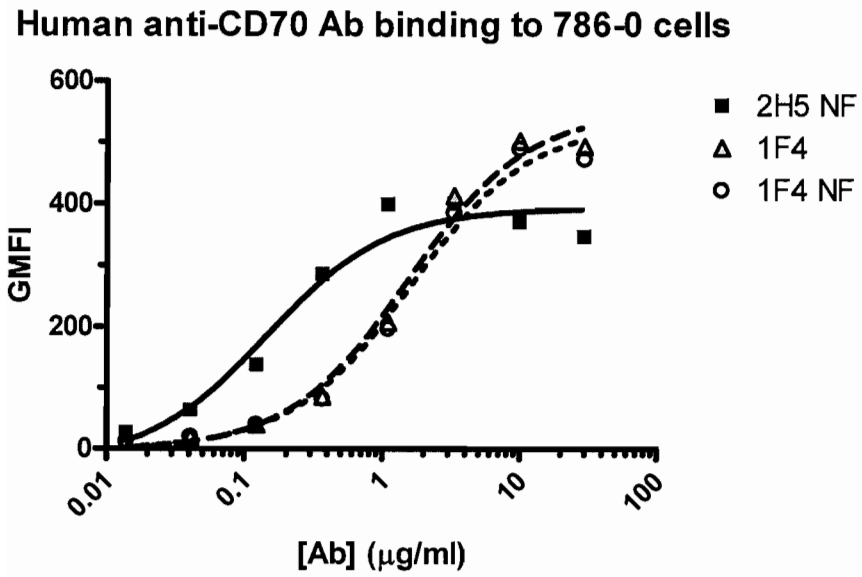

Human anti-CD70 Ab binding to 786-0 cells
600
400
200
0
GMFI
0.01
0.1
1
10
100
[Ab] (μg/ml)
2H5 NF
1F4
1F4 NF

Fig. 49

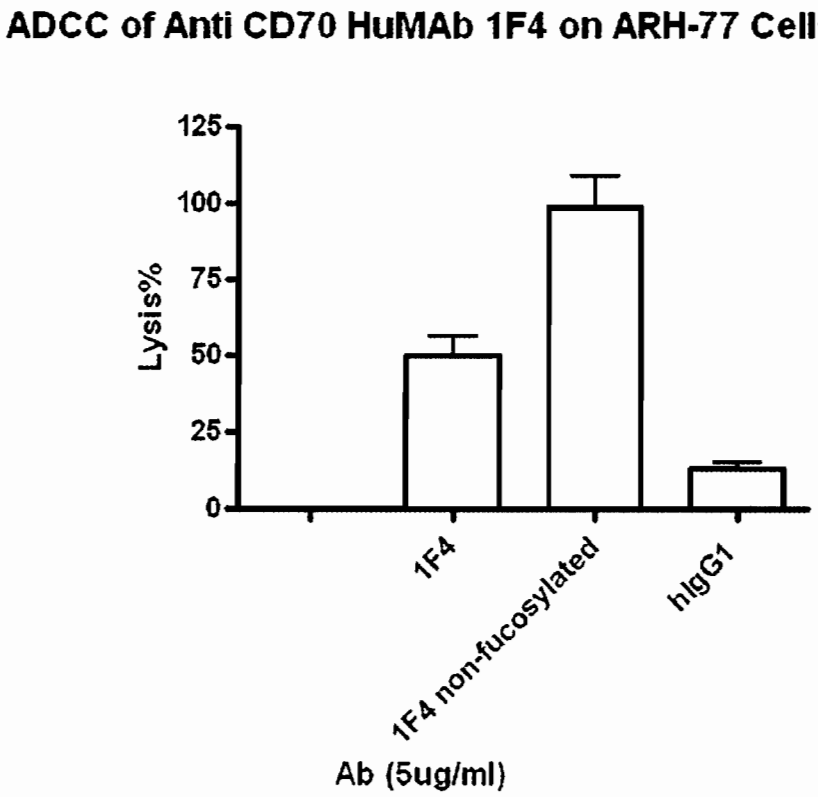

ADCC of Anti CD70 HuMAb 1F4 on ARH-77 Cells
125
100
75
50
25
0
Lysis%
1F4
1F4 non-fucosylated
hIgG1
Ab (5ug/ml)

Fig. 50

Figure 51

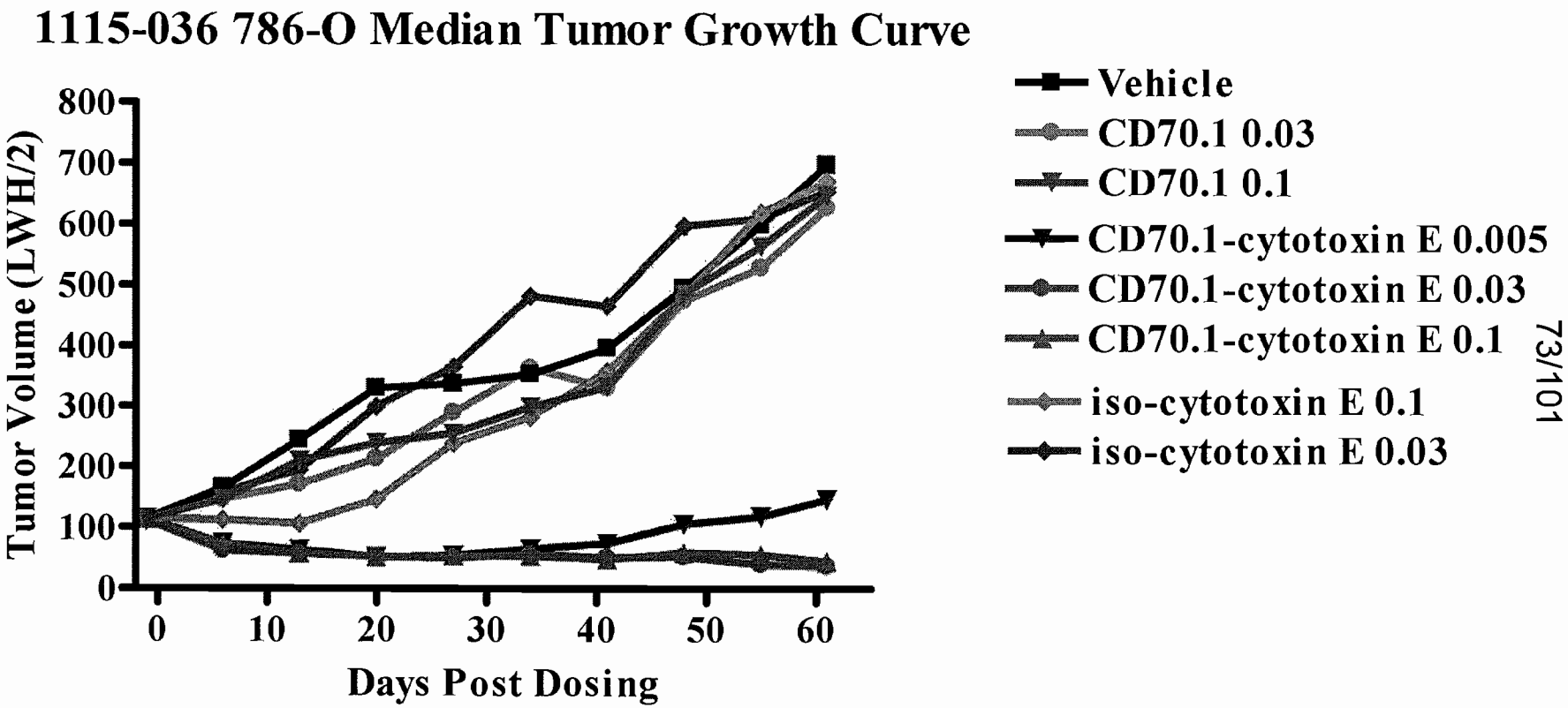

1115-036 786-O Median Tumor Growth Curve
Tumor Volume (LWH/2)
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
CD70.1 0.03
CD70.1 0.1
CD70.1-cytotoxin E 0.005
CD70.1-cytotoxin E 0.03
CD70.1-cytotoxin E 0.1
iso-cytotoxin E 0.1
iso-cytotoxin E 0.03

Figure 52

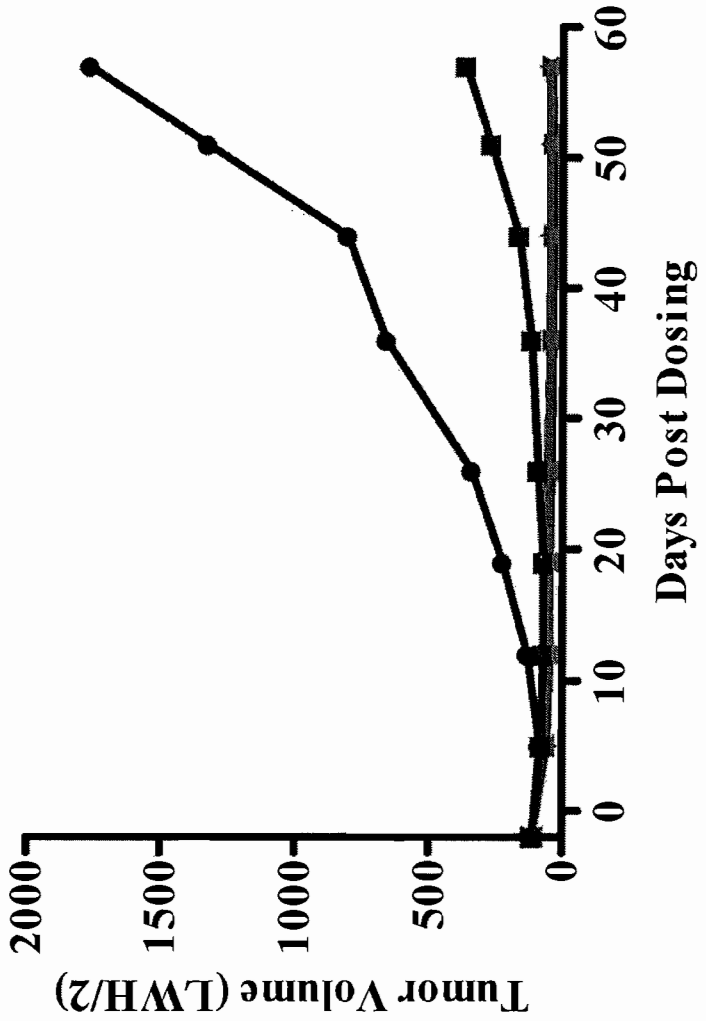

1115-044 A498 Median Tumor Volume
Tumor Volume (LWH/2)
2000
1500
1000
500
0
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
CD70.1-cytotoxin E SD 0.3
CD70.1-cytotoxin E SD 0.1
CD70.1-cytotoxin E SD 0.03

Figure 53

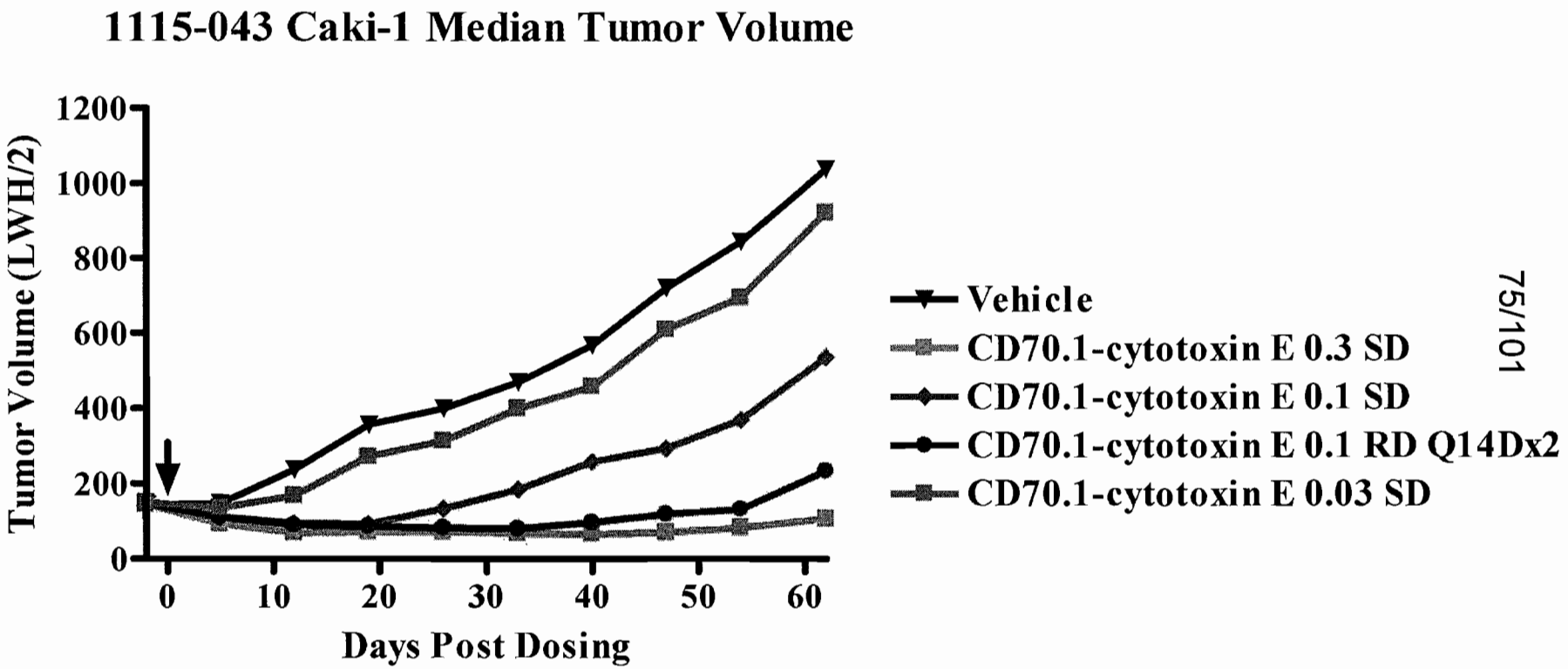
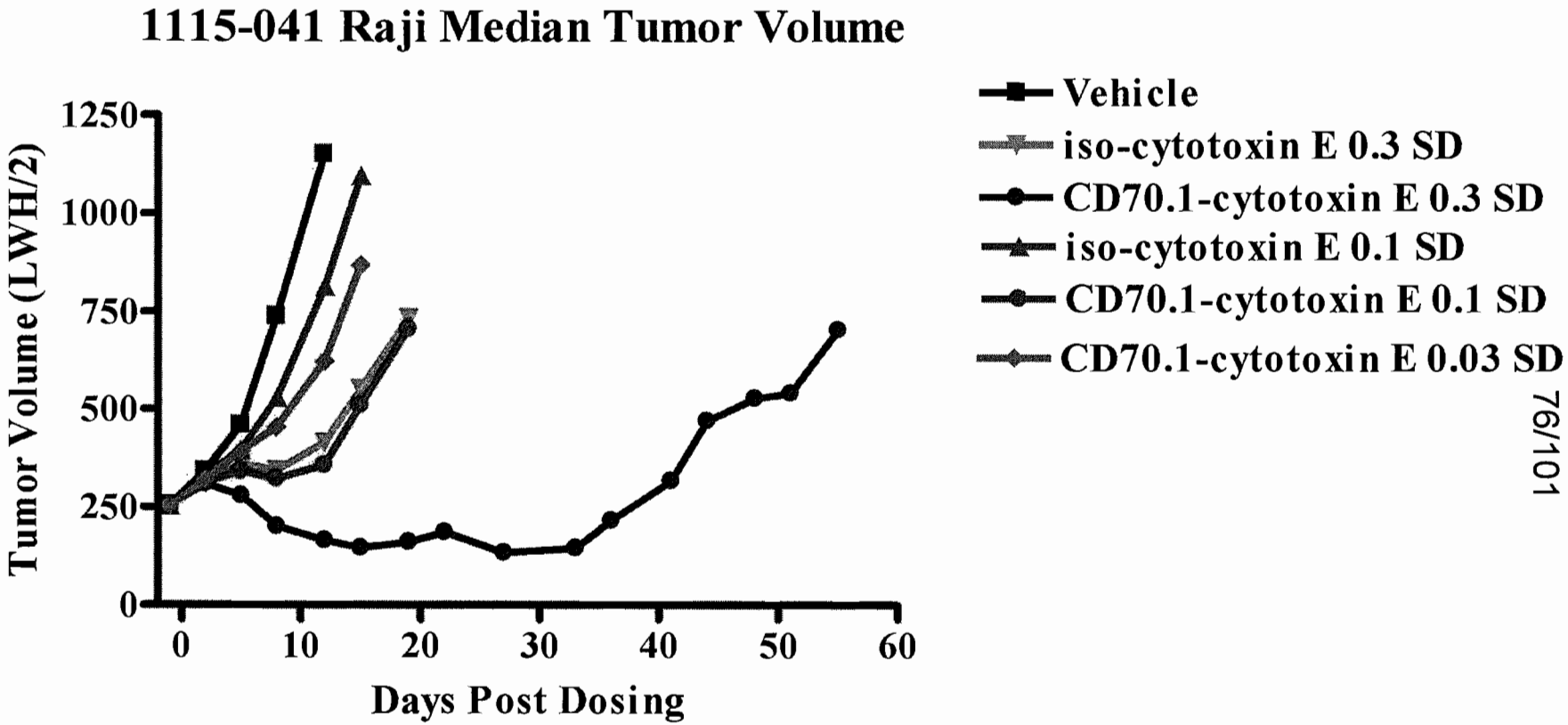
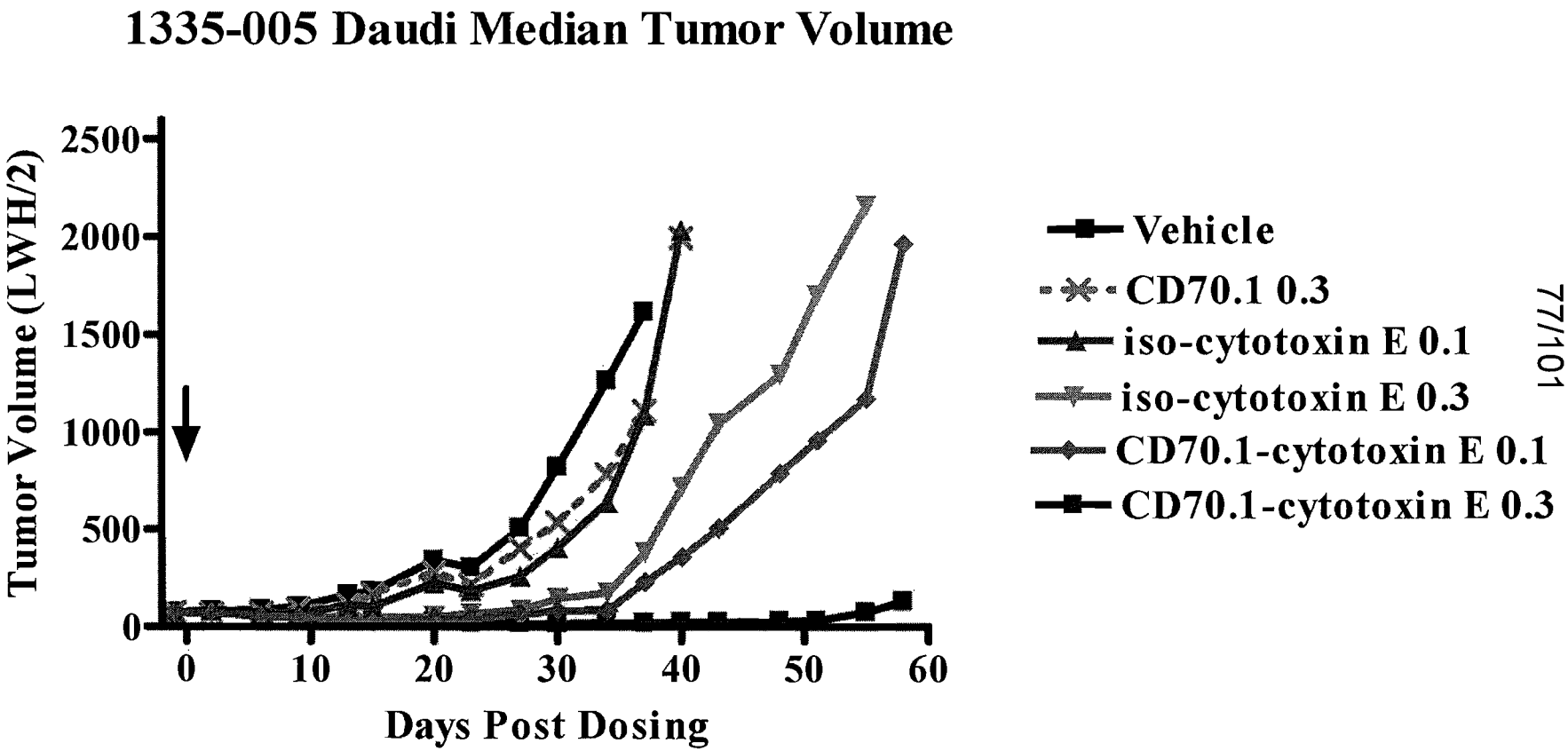

1115-043 Caki-1 Median Tumor Volume
Tumor Volume (LWH/2)
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
CD70.1-cytotoxin E 0.3 SD
CD70.1-cytotoxin E 0.1 SD
CD70.1-cytotoxin E 0.1 RD Q14Dx2
CD70.1-cytotoxin E 0.03 SD

Figure 54



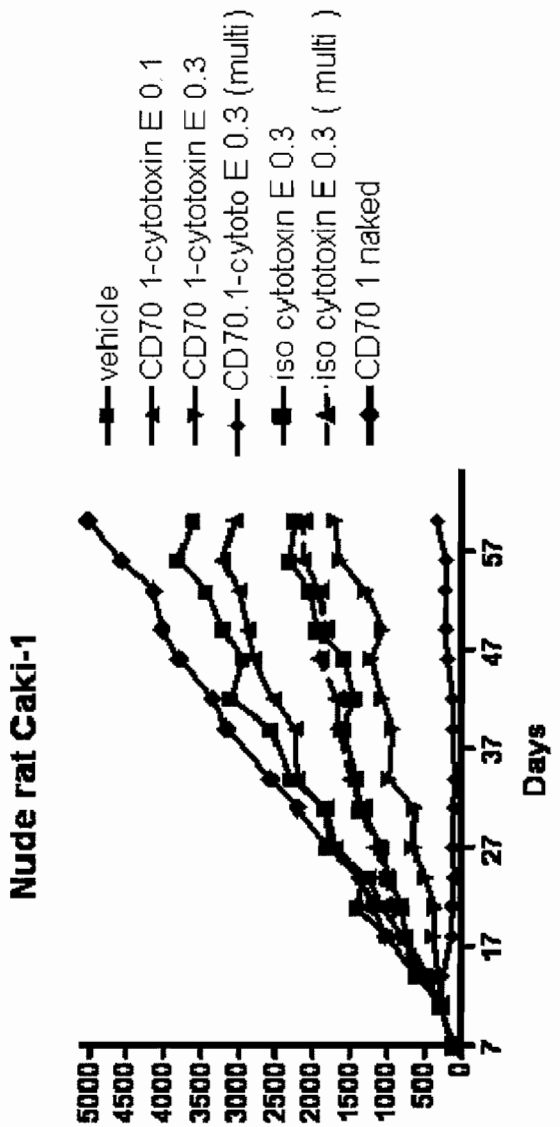
Carol Soderberg, Jerry Jiang, Orville Cortez and Chin Pan

Figure 55



77/101

Figure 56


Nude rat Caki-1
vehicle
CD70 1-cytotoxin E 0.1
CD70 1-cytotoxin E 0.3
CD70.1-cytoto E 0.3 (multi)
iso cytotoxin E 0.3
iso cytotoxin E 0.3 (multi)
CD70 1 naked
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
7
17
27
37
47
57
Days

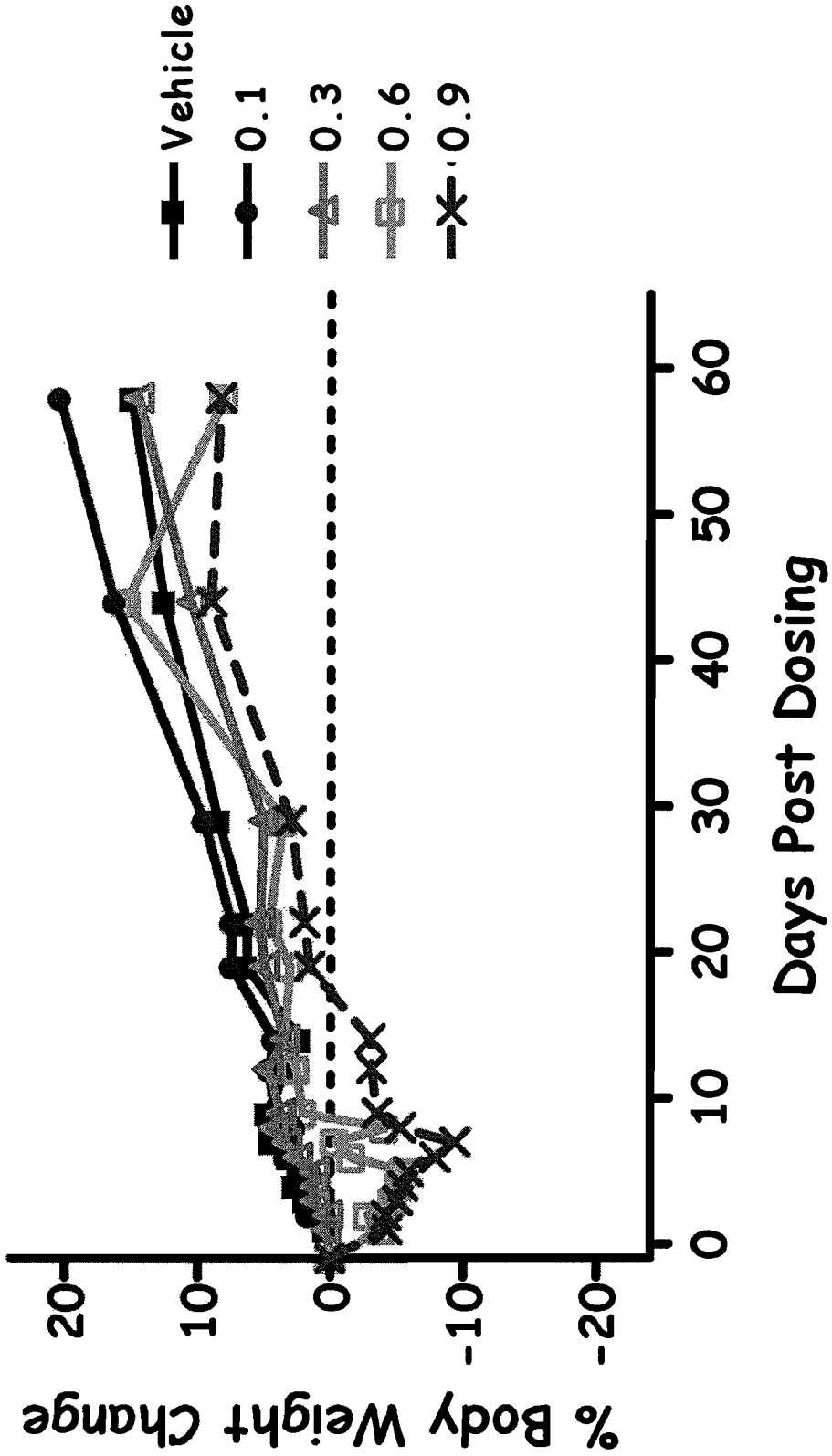
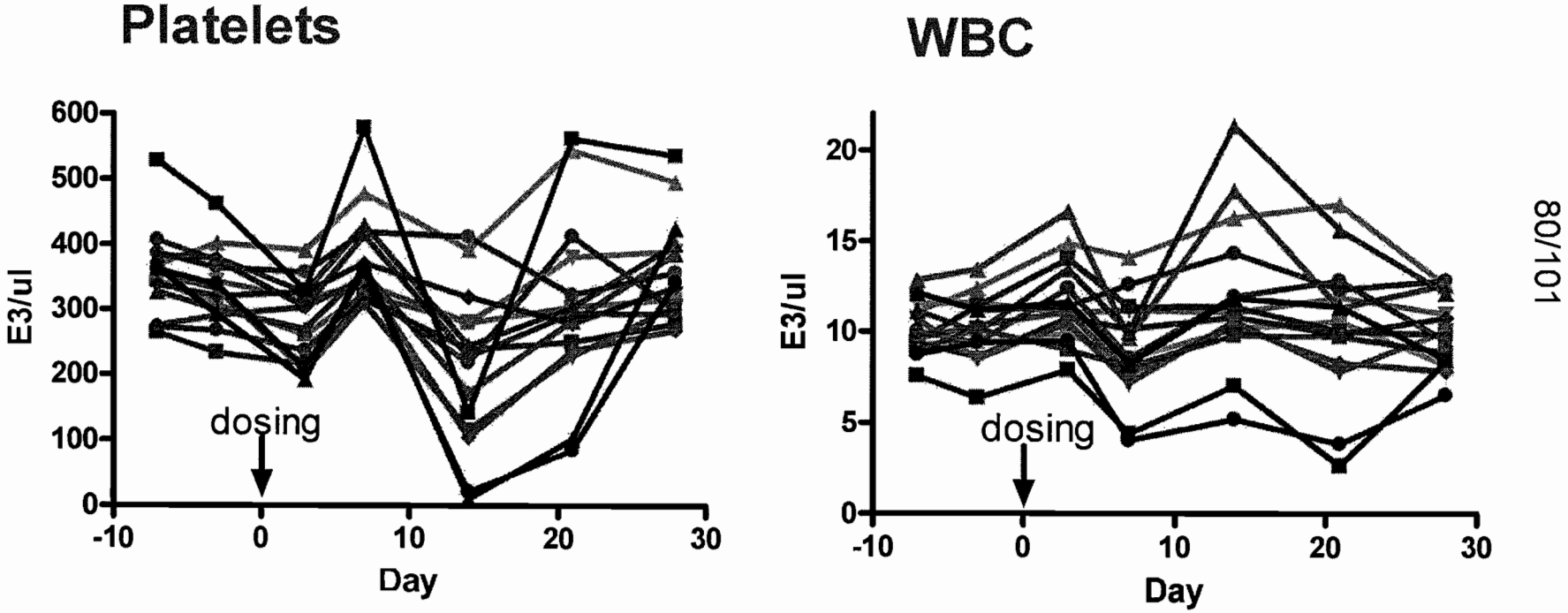

Vehicle
0.1
0.3
0.6
0.9
% Body Weight Change
20
10
0
-10
-20
0
10
20
30
40
50
60
Days Post Dosing

Figure 57

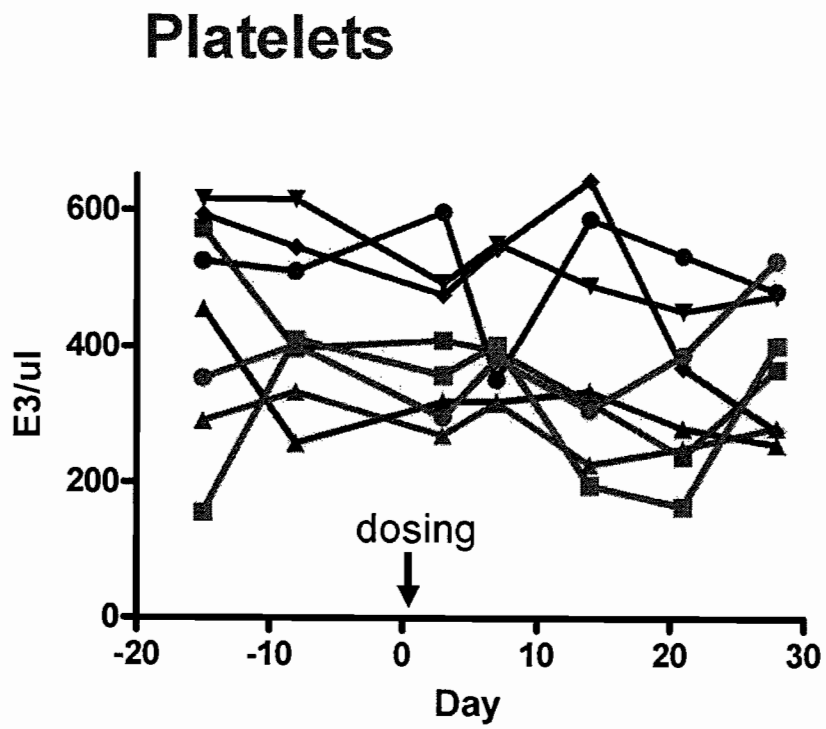
Figure 58

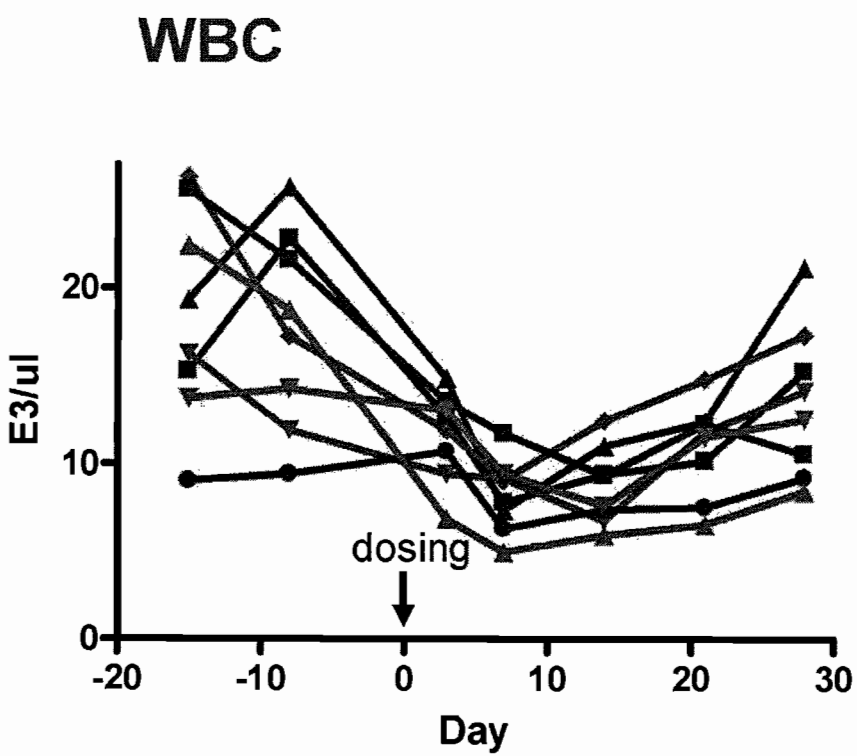


0.1 μmol/kg **0.3 μmol/kg** **0.6 μmol/kg – MTD (91.5mg/kg ab)**

0.2 μmol/kg **0.4 μmol/kg**

Figure 59





0.2 μmol/kg 0.6 μmol/kg

0.4 μmol/kg 0.8 μmol/kg

Figure 60

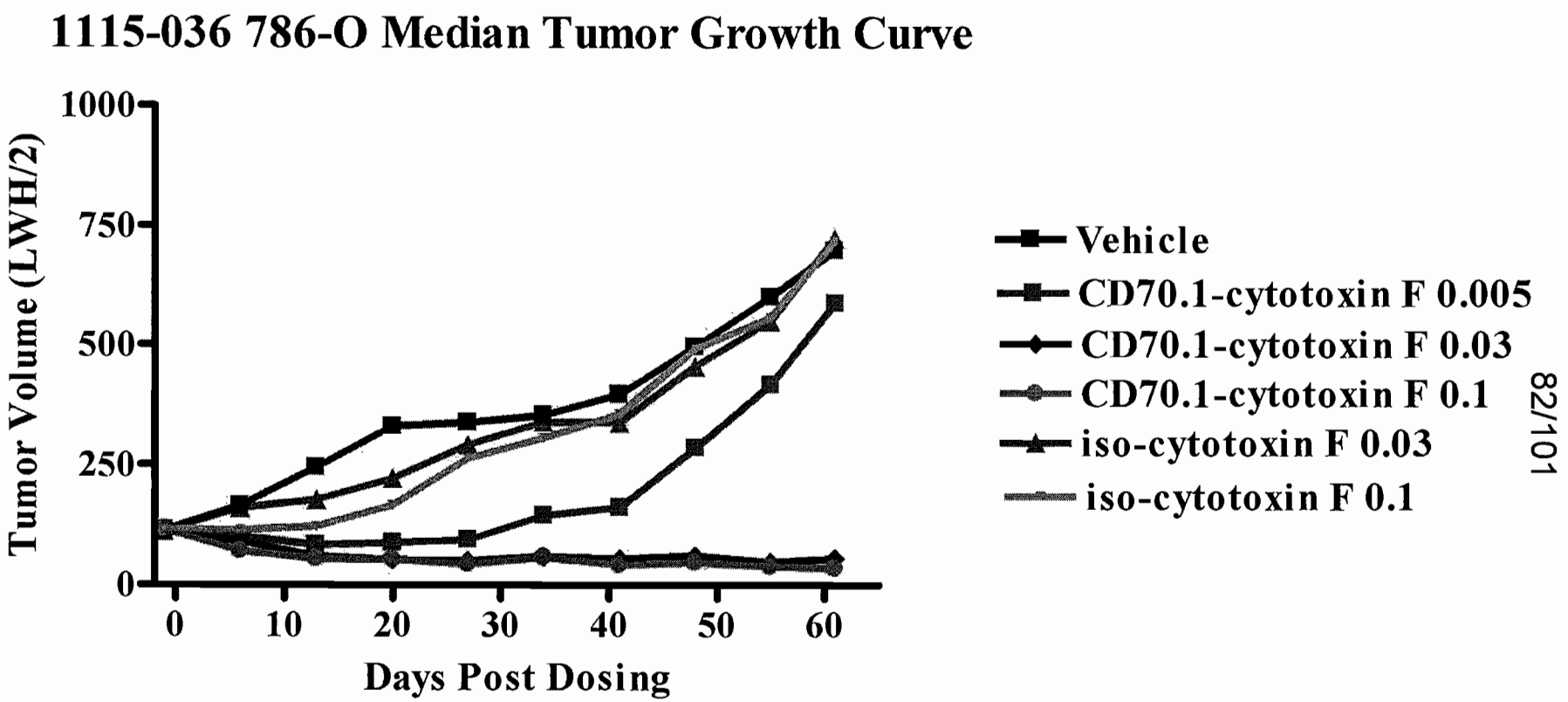

1115-036 786-O Median Tumor Growth Curve
Tumor Volume (LWH/2)
1000
750
500
250
0
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
CD70.1-cytotoxin F 0.005
CD70.1-cytotoxin F 0.03
CD70.1-cytotoxin F 0.1
iso-cytotoxin F 0.03
iso-cytotoxin F 0.1

Figure 61

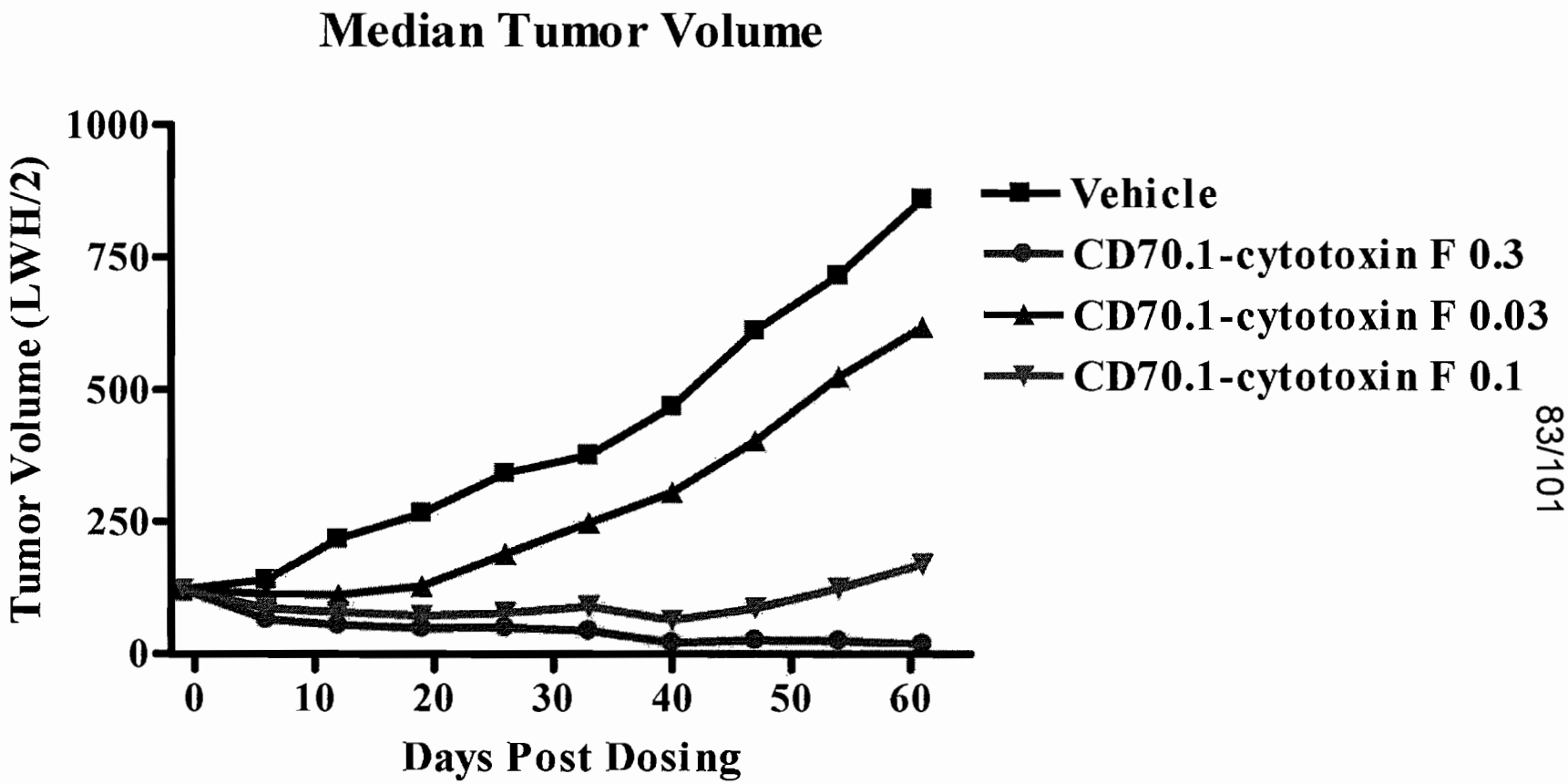

Median Tumor Volume
Tumor Volume (LWH/2)
1000
750
500
250
0
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
CD70.1-cytotoxin F 0.3
CD70.1-cytotoxin F 0.03
CD70.1-cytotoxin F 0.1

Figure 62

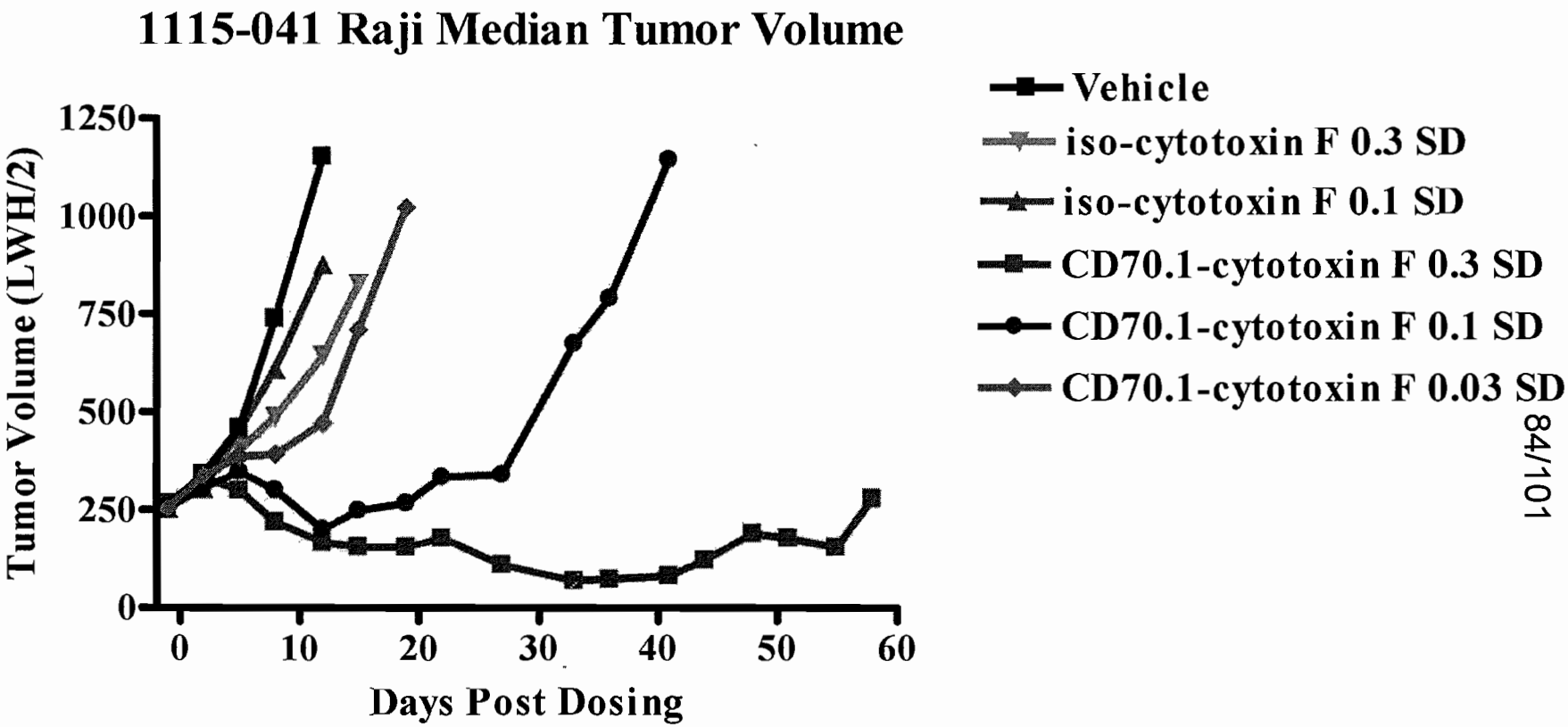

1115-041 Raji Median Tumor Volume
Tumor Volume (LWH/2)
1250
1000
750
500
250
0
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
iso-cytotoxin F 0.3 SD
iso-cytotoxin F 0.1 SD
CD70.1-cytotoxin F 0.3 SD
CD70.1-cytotoxin F 0.1 SD
CD70.1-cytotoxin F 0.03 SD

Figure 63

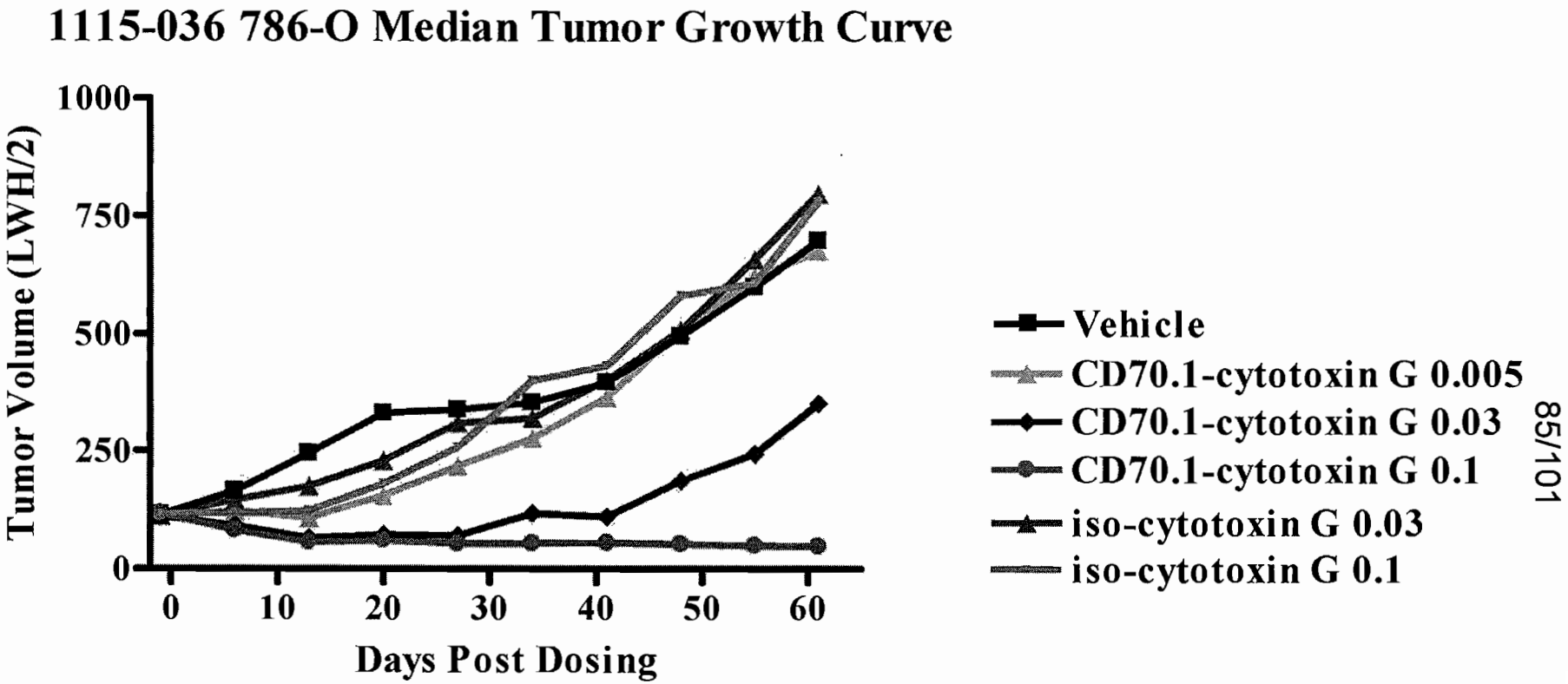

1115-036 786-O Median Tumor Growth Curve
Tumor Volume (LWH/2)
1000
750
500
250
0
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
CD70.1-cytotoxin G 0.005
CD70.1-cytotoxin G 0.03
CD70.1-cytotoxin G 0.1
iso-cytotoxin G 0.03
iso-cytotoxin G 0.1

Figure 64

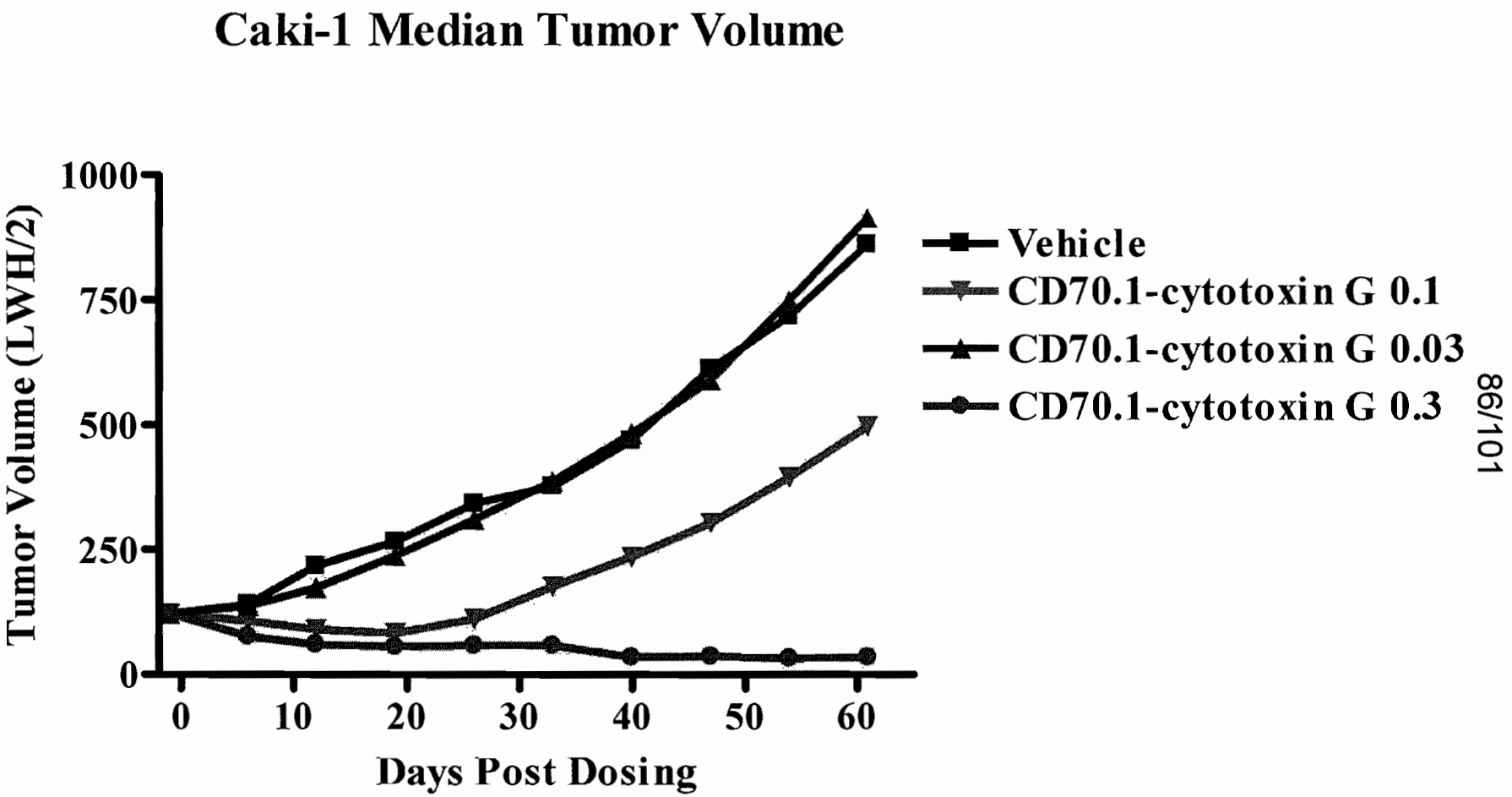

Caki-1 Median Tumor Volume
Tumor Volume (LWH/2)
1000
750
500
250
0
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
CD70.1-cytotoxin G 0.1
CD70.1-cytotoxin G 0.03
CD70.1-cytotoxin G 0.3

Figure 65

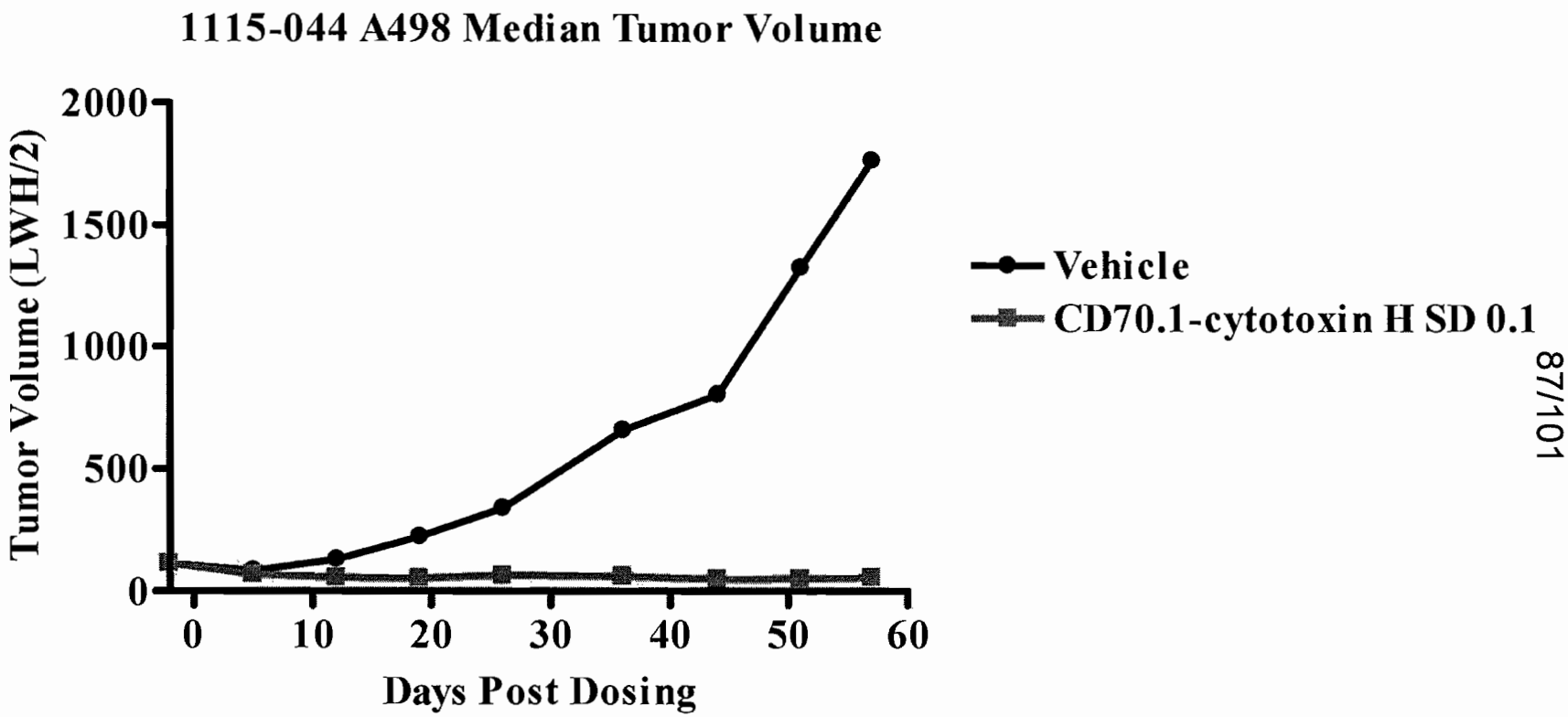

1115-044 A498 Median Tumor Volume
Tumor Volume (LWH/2)
2000
1500
1000
500
0
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
CD70.1-cytotoxin H SD 0.1

Figure 66

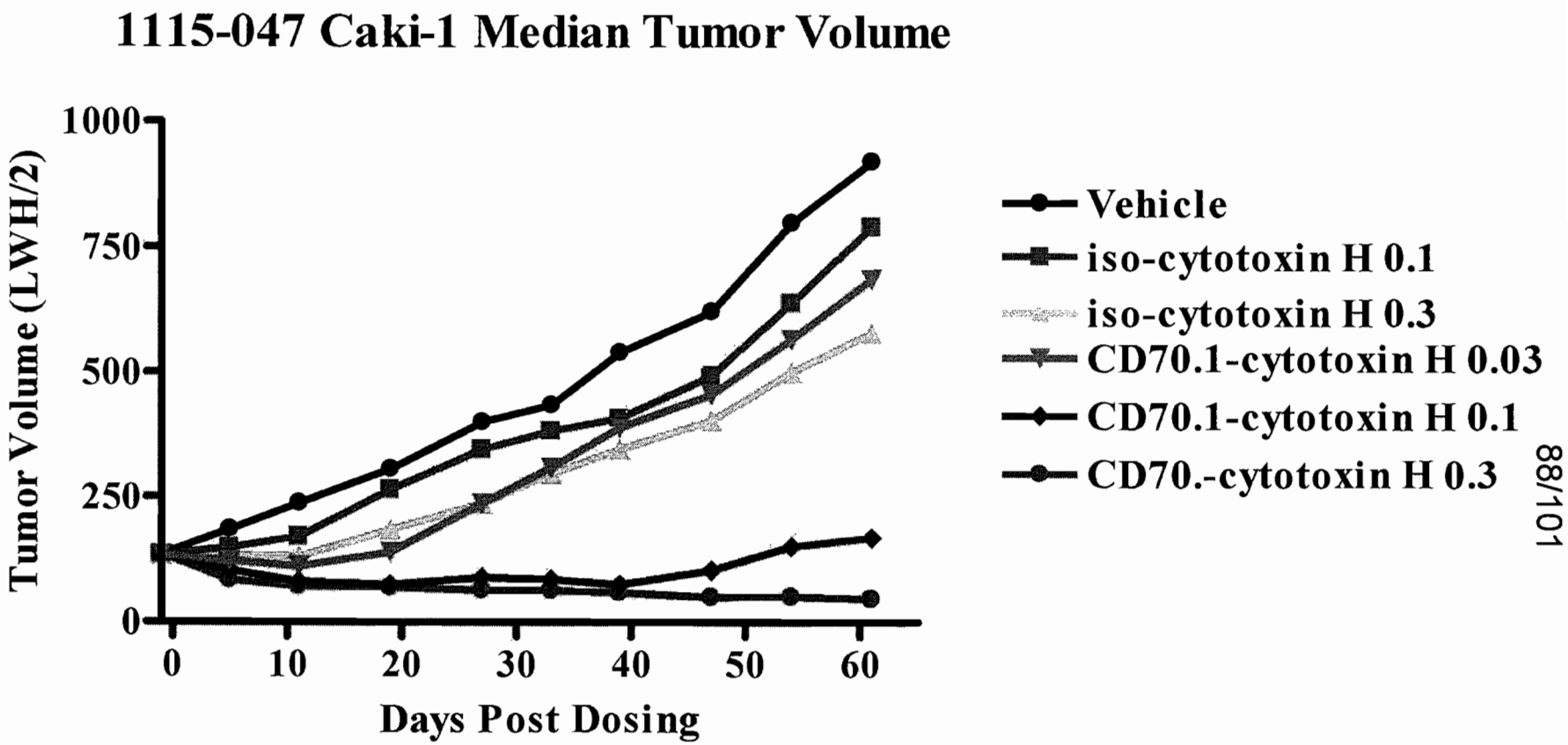

1115-047 Caki-1 Median Tumor Volume
Tumor Volume (LWH/2)
1000
750
500
250
0
0
10
20
30
40
50
60
Days Post Dosing
Vehicle
iso-cytotoxin H 0.1
iso-cytotoxin H 0.3
CD70.1-cytotoxin H 0.03
CD70.1-cytotoxin H 0.1
CD70.-cytotoxin H 0.3

Figure 67

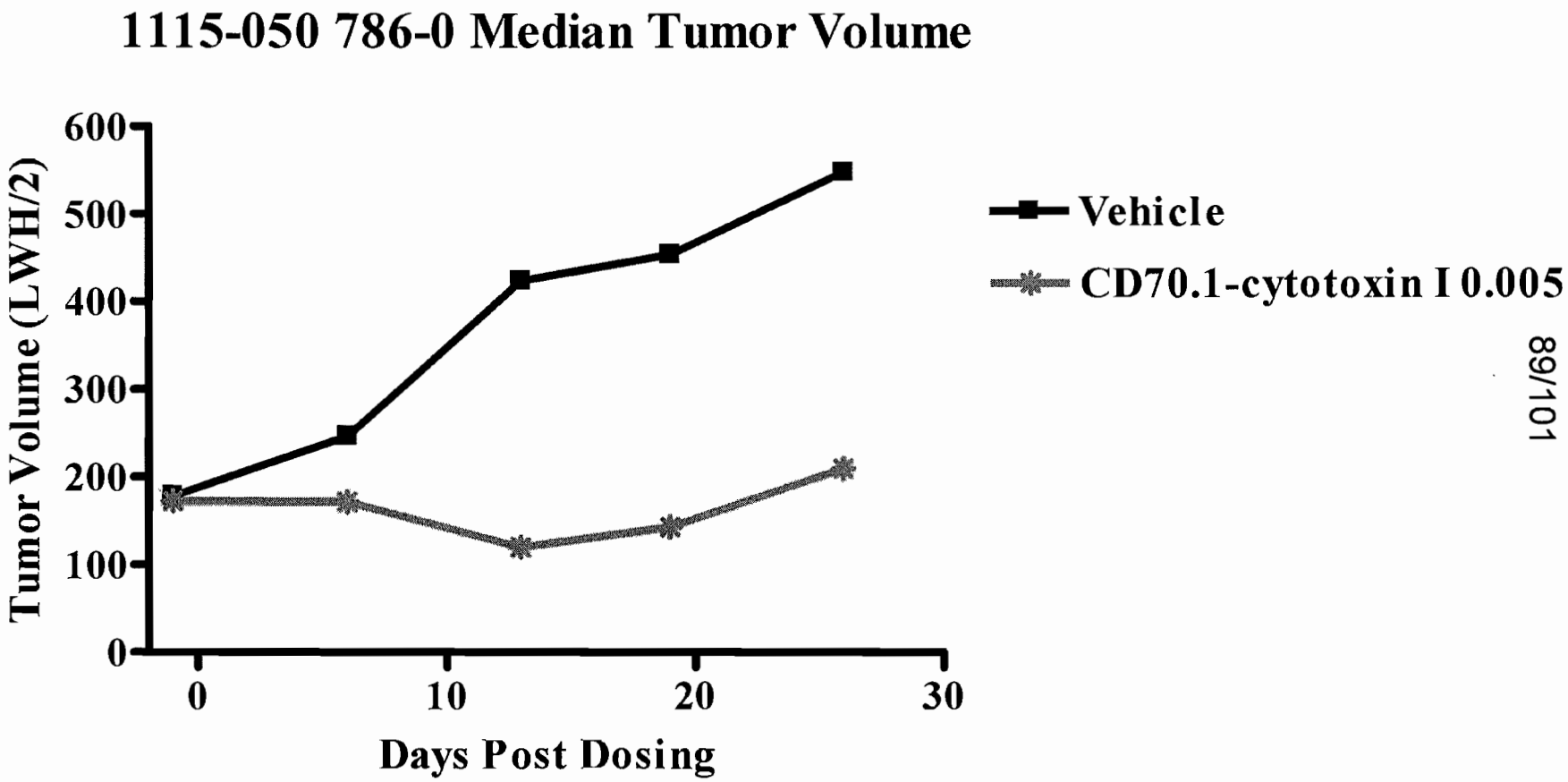

1115-050 786-0 Median Tumor Volume
Tumor Volume (LWH/2)
600
500
400
300
200
100
0
0
10
20
30
Days Post Dosing
Vehicle
CD70.1-cytotoxin I 0.005

Figure 68

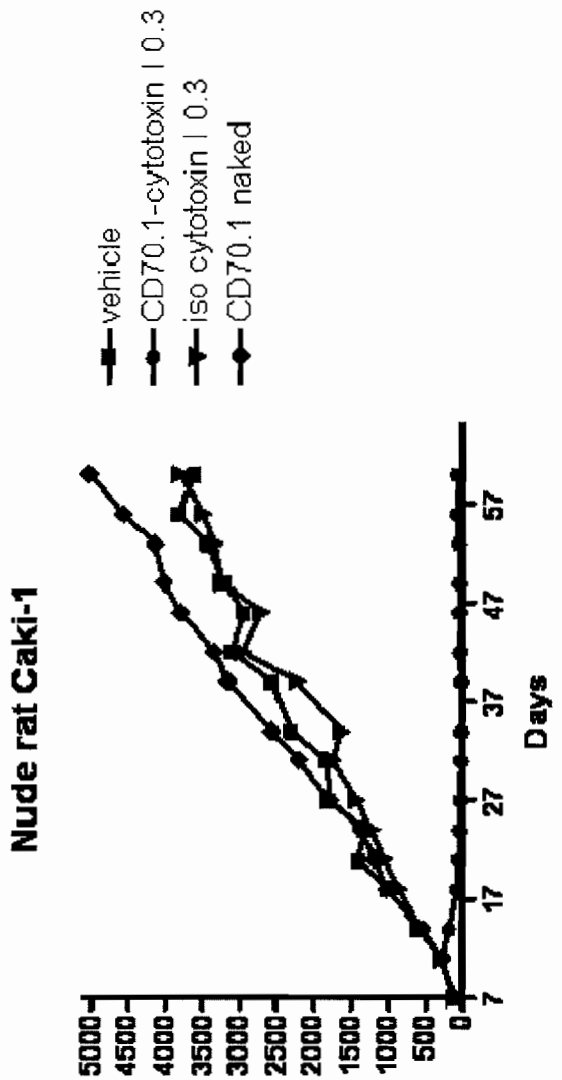
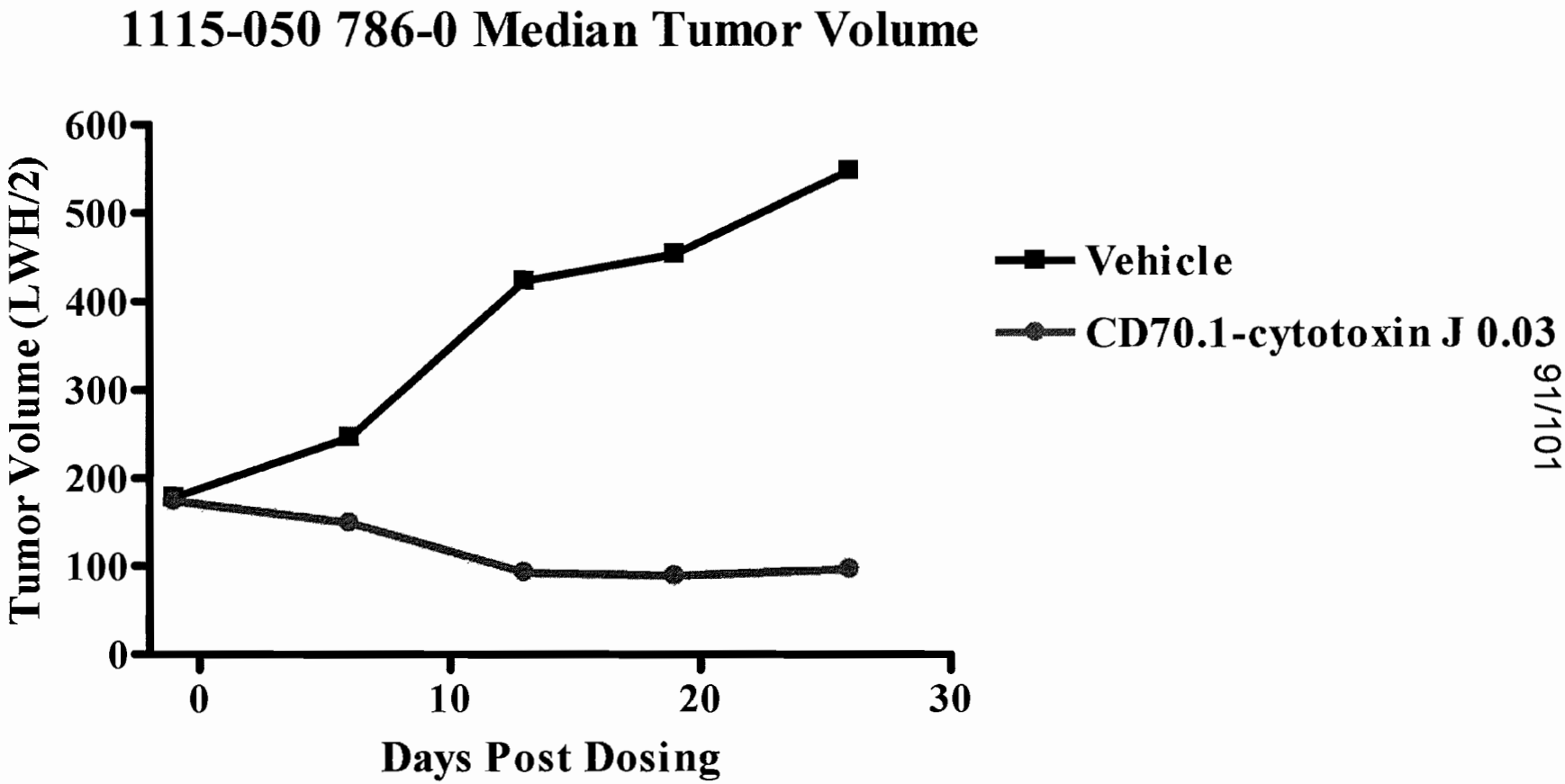

Nude rat Caki-1
vehicle
CD70.1-cytotoxin I 0.3
iso cytotoxin I 0.3
CD70.1 naked
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
7
17
27
37
47
57
Days

Figure 69


1115-050 786-0 Median Tumor Volume
Tumor Volume (LWH/2)
600
500
400
300
200
100
0
0
10
20
30
Days Post Dosing
Vehicle
CD70.1-cytotoxin J 0.03

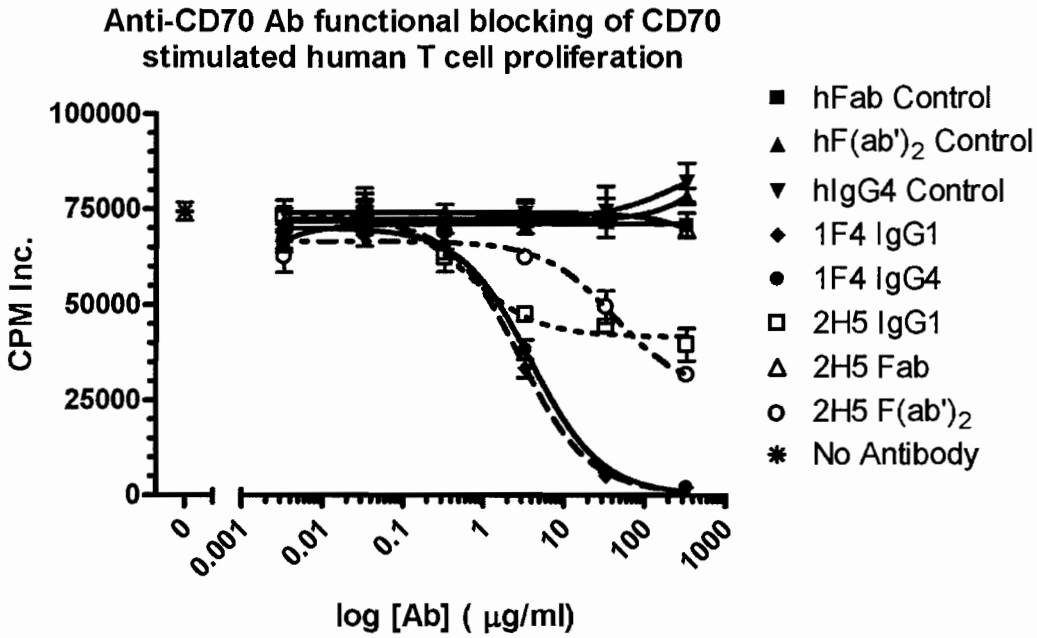
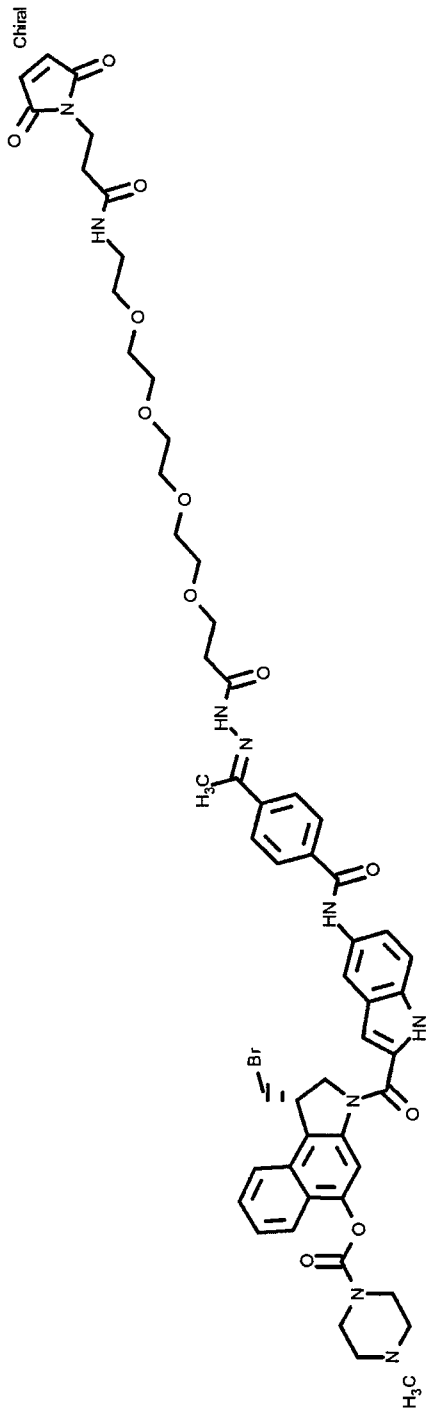

Anti-CD70 Ab functional blocking of CD70 stimulated human T cell proliferation
CPM Inc.
100000
75000
50000
25000
0
0
0.001
0.01
0.1
1
10
100
1000
log [Ab] (μg/ml)
hFab Control
hF(ab')2 Control
hIgG4 Control
1F4 IgG1
1F4 IgG4
2H5 IgG1
2H5 Fab
2H5 F(ab')2
No Antibody

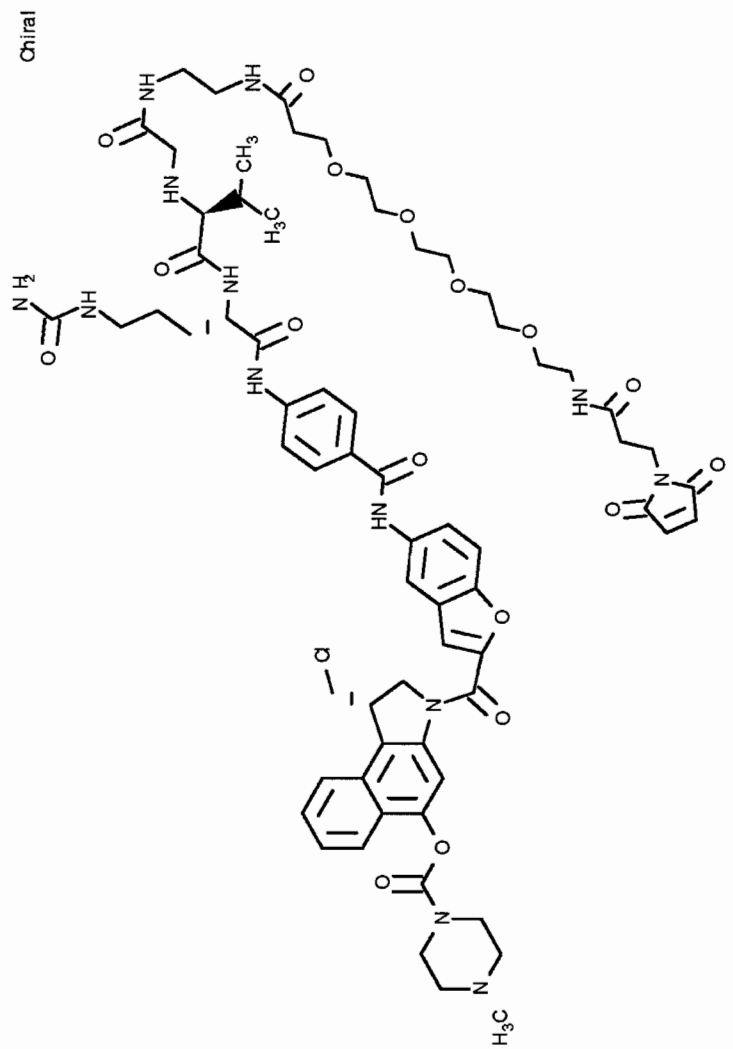
Fig. 70

Figure 71



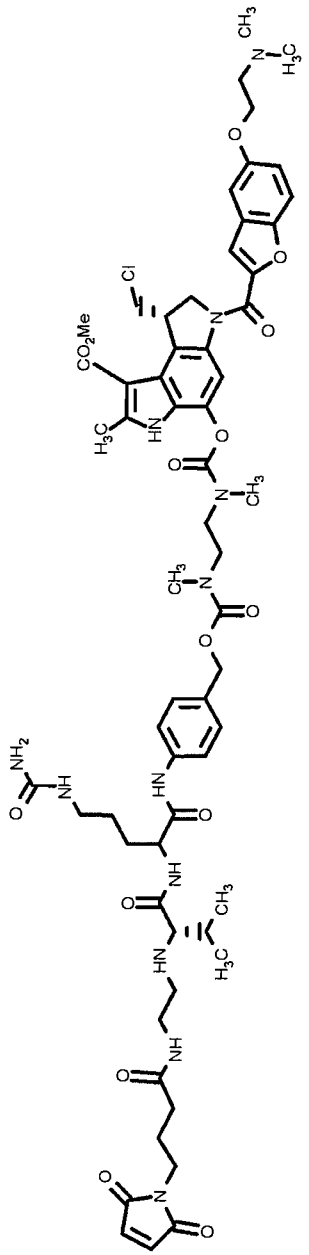
Cytotoxin B

Figure 72



Cytotoxin C

Figure 73



Cytotoxin D

Cytotoxin E

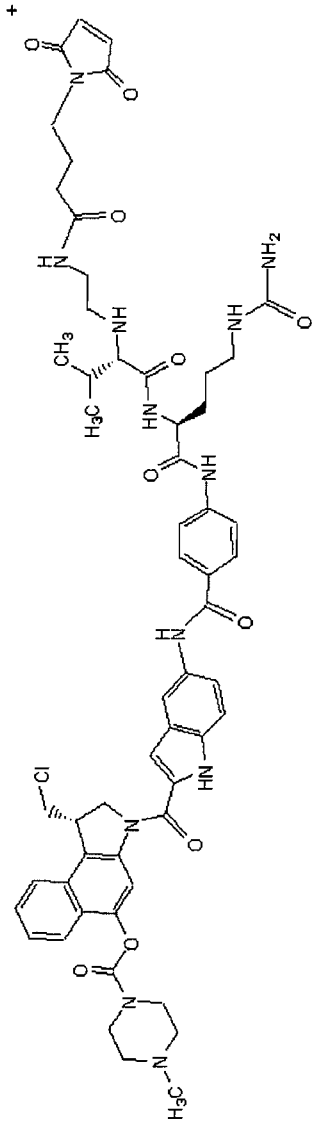


Figure 74

Cytotoxin F

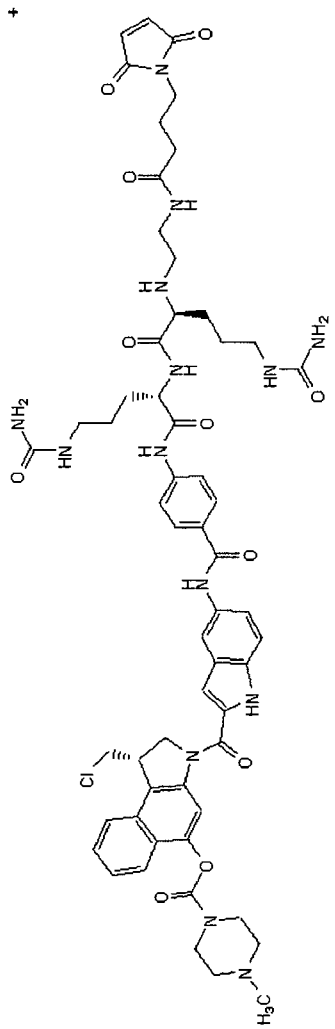


Figure 75

Cytotoxin G

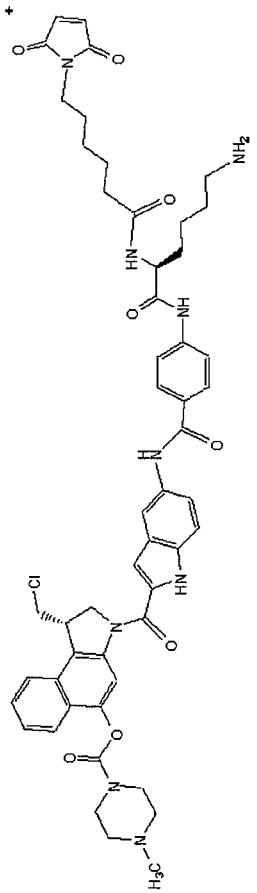


Figure 76

Cytotoxin H

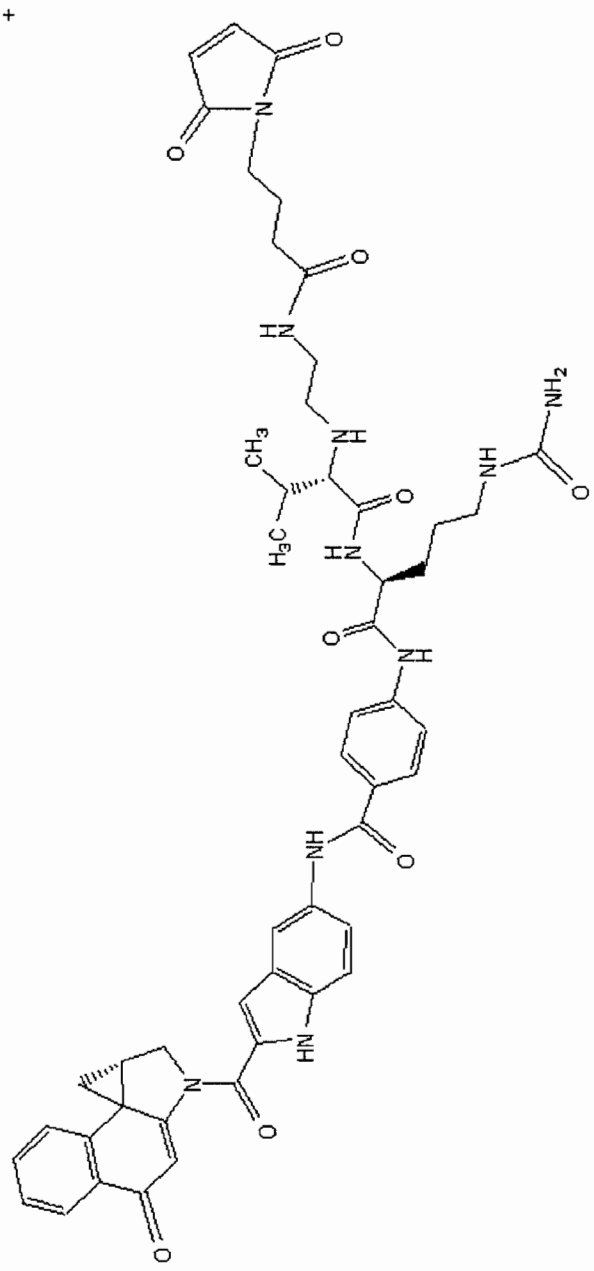


Figure 77

Cytotoxin I

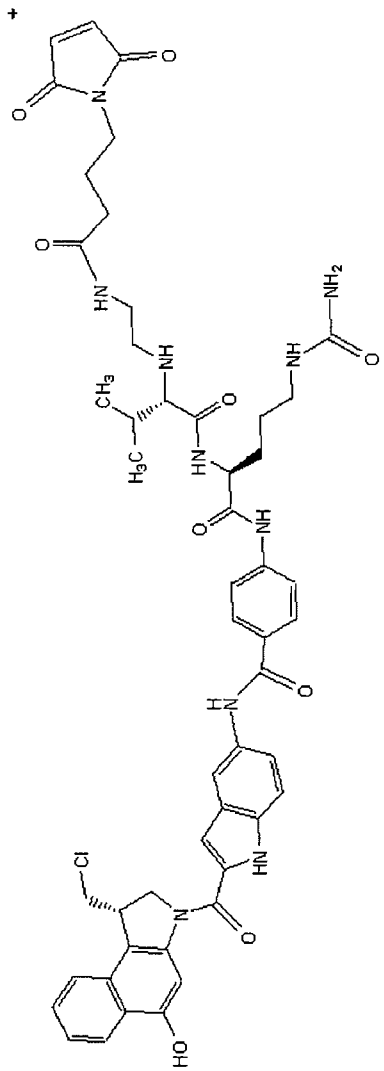


Figure 78

Cytotoxin J

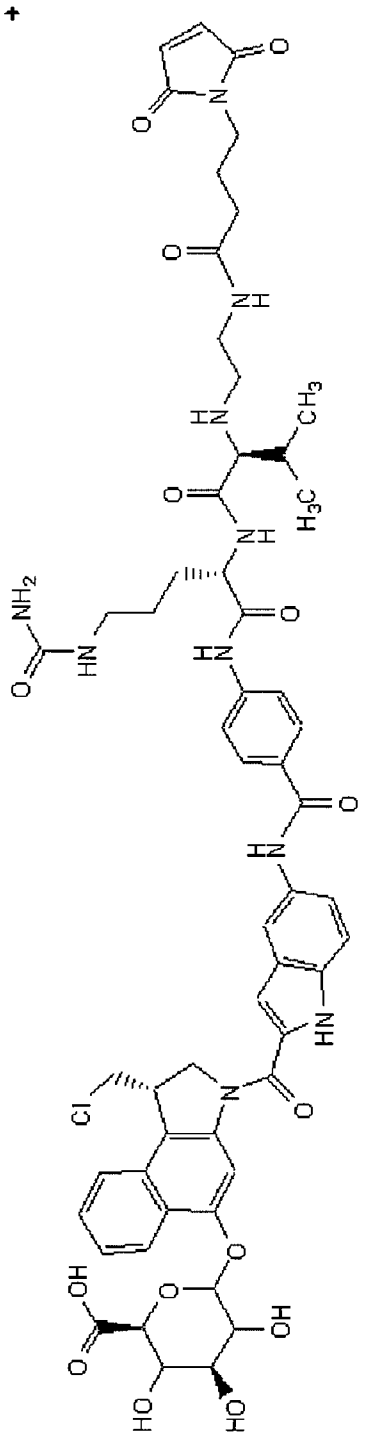


Figure 79