FIG. 1A

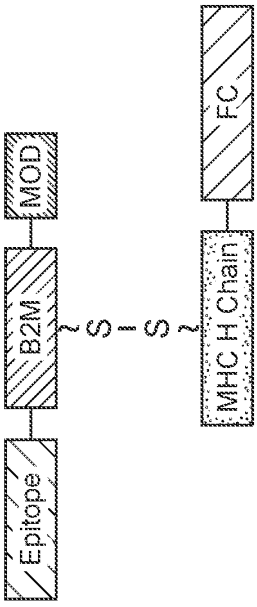


FIG. 1B

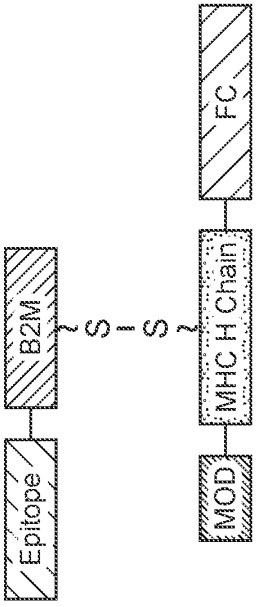


FIG. 1C

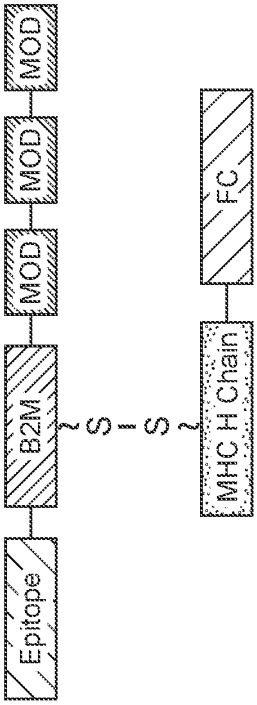


FIG. 1D

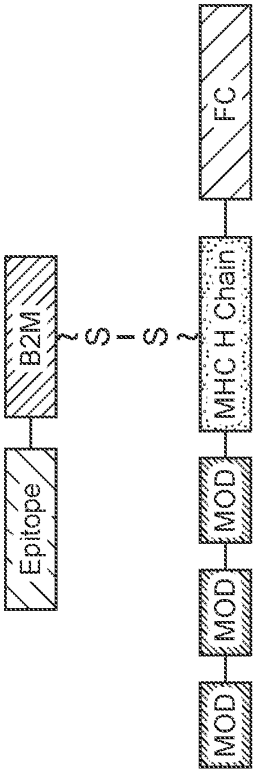


FIG. 2A

IL2 – *Homo sapiens*

APTSSSTKKT QLQL**EH**LLL**D** LQMILNGINN YKNPKLTRML T**F**KF**Y**MPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFC**Q**SIIS TLT (SEQ ID NO:1)

FIG. 2B

IL2 (F42X) (SEQ ID NO:38)

APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML T**X**KFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2C

IL2 (D20X) (SEQ ID NO:39)

APTSSSTKKT QLQLEHLLL**X** LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2D

IL2 (E15X) (SEQ ID NO:40)

APTSSSTKKT QLQL**X**HLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2E

IL2 (H16X) (SEQ ID NO:41)

APTSSSTKKT QLQLEXLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2F

IL2 (Y45X) (SEQ ID NO:42)

APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFXMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2G

IL2 (Q126X) (SEQ ID NO:43)

APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCXSIIS TLT

FIG. 2H

IL2 (F42X; H16X) (SEQ ID NO:44)

APTSSSTKKT QLQLEXLLLD LQMILNGINN YKNPKLTRML TXKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2I

IL2 (F42X; D20X) (SEQ ID NO:45)

APTSSSTKKT QLQLEHLLLX LQMILNGINN YKNPKLTRML TXKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2J

IL2 (F42X; D20X; E15X) (SEQ ID NO:46)

APTSSSTKKT QLQLXHLLLX LQMILNGINN YKNPKLTRML TXKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2K

IL2 (F42X; D20X; H16X) (SEQ ID NO:47)

APTSSSTKKT QLQLEXLLLX LQMILNGINN YKNPKLTRML TXKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2L

IL2 (F42X; D20X; Q126X) (SEQ ID NO:48)

APTSSSTKKT QLQLEHLLLX LQMILNGINN YKNPKLTRML TXKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCXSIIS TLT

FIG. 2M

IL2 (F42X; D20X; Y45X) (SEQ ID NO:49)

APTSSSTKKT QLQLEHLLLX LQMILNGINN YKNPKLTRML TXKFXMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2N

IL2 (F42X; D20X; Y45X; H16X) (SEQ ID NO:50)

APTSSSTKKT QLQLEXLLLX LQMILNGINN YKNPKLTRML TXKFXMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

FIG. 2O

IL2 (F42X; D20X; Y45X; Q126X) (SEQ ID NO:51)

APTSSSTKKT QLQLEHLLLX LQMILNGINN YKNPKLTRML TXKFXMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCXSIIS TLT

FIG. 2P

IL2 (F42X; D20X; Y45X; H16X; Q126X) (SEQ ID NO:52)

APTSSSTKKT QLQLEXLLLX LQMILNGINN YKNPKLTRML TXKFXMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCXSIIS TLT

FIG. 2Q

IL2 – (F42X, H16X, Q126X) (SEQ ID NO:53)

APTSSSTKKT QLQLEXLLLD LQMILNGINN YKNPKLTRML TXKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCXSIIS TLT

FIG. 3A
IL2R-alpha chain (SEQ ID NO:54)
Homo sapiens

```
  1 MDSYLLMWGL LTFIMVPGCQ AELCDDDPPE IPHATFKAMA YKEGTMLNCE CKRGFRRIKS
 61 GSLYMLCTGN SSHSSWDNQC QCTSSATRNT TKQVTPQPEE QKERKTTEMQ SPMQPVDQAS
121 LPGHCREPPP WENEATERIY HFVVGQMVYY QCVQGYRALH RGPAESVCKM THGKTRWTQP
181 QLICTGEMET SQFPGEEKPQ ASPEGRPESE TSCLVTTTDF QIQTEMAATM ETSIFTTEYQ
241 VAVAGCVFLL ISVLLLSGLT WQRRQRKSRR TI
```

Mature = amino acids 22-272

FIG. 3B
IL2R-beta chain (SEQ ID NO:55)
Homo sapiens

```
  1 MAAPALSWRL PLLILLLPLA TSWASAAVNG TSQFTCFYNS RANISCVWSQ DGALQDTSCQ
 61 VHAWPDRRRW NQTCELLPVS QASWACNLIL GAPDSQKLTT VDIVTLRVLC REGVRWRVMA
121 IQDFKPFENL RLMAPISLQV VHVETHRCNI SWEISQASHY FERHLEFEAR TLSPGHTWEE
181 APLLTLKQKQ EWICLETLTP DTQYEFQVRV KPLQGEFTTW SPWSQPLAFR TKPAALGKDT
241 IPWLGHLLVG LSGAFGFIIL VYLLINCRNT GPWLKKVLKC NTPDPSKFFS QLSSEHGGDV
301 QKWLSSPFPS SSFSPGGLAP EISPLEVLER DKVTQLLLQQ DKVPEPASLS SNHSLTSCFT
361 NQGYFFFHLP DALEIEACQV YFTYDPYSEE DPDEGVAGAP TGSSPQPLQP LSGEDDAYCT
421 FPSRDDLLLF SPSLLGGPSP PSTAPGGSGA GEERMPPSLQ ERVPRDWDPQ PLGPPTPGVP
481 DLVDFQPPPE LVLREAGEEV PDAGPREGVS FPWSRPPGQG EFRALNARLP LNTDAYLSLQ
541 ELQGQDPTHL V
```

Mature = amino acids 27-551

FIG. 3C
IL2R-gamma chain (SEQ ID NO:56)
Homo sapiens

```
  1 MLKPSLPFTS LLFLQLPLLG VGLNTTILTP NGNEDTTADF FLTTMPTDSL SVSTLPLPEV
 61 QCFVFNVEYM NCTWNSSSEP QPTNLTLHYW YKNSDNDKVQ KCSHYLFSEE ITSGCQLQKK
121 EIHLYQTFVV QLQDPREPRR QATQMLKLQN LVIPWAPENL TLHKLSESQL ELNWNNRFLN
181 HCLEHLVQYR TDWDHSWTEQ SVDYRHKFSL PSVDGQKRYT FRVRSRFNPL CGSAQHWSEW
241 SHPIHWGSNT SKENPFLFAL EAVVISVGSM GLIISLLCVY FWLERTMPRI PTLKNLEDLV
301 TEYHGNFSAW SGVSKGLAES LQPDYSERLC LVSEIPPKGG ALGEGPGASP CNQHSPYWAP
361 PCYTLKPET
```

Mature = amino acids 23-369

Figure 4A
GenBank 3S7G_A (SEQ ID NO:57)
Homo sapiens **IgG1** Fc
227 aa

```
  1 dkthtcppcp apellggpsv flfppkpkdt lmisrtpevt cvvvdvshed pevkfnwyvd
 61 gvevhnaktk preeqynsty rvvsvltvlh qdwlngkeyk ckvsnkalpa piektiskak
121 gqprepqvyt lppsrdeltk nqvsltclvk gfypsdiave wesngqpenn ykttppvlds
181 dgsfflyskl tvdksrwqqg nvfscsvmhe alhnhytqks lslspgk
```

GenBank AAN76044 (SEQ ID NO:58)
Homo sapiens **IgG2** Fc (amino acids 99-325)
227 aa

```
  1 stkgpsvfpl apcsrstses taalgclvkd yfpepvtvsw nsgaltsgvh tfpavlqssg
 61 lyslssvvtv pssnfgtqty tcnvdhkpsn tkvdktverk ccvecppcpa ppvagpsvfl
121 fppkpkdtlm isrtpevtcv vvdvshedpe vqfnwyvdgv evhnaktkpr eeqfnstfrv
181 vsvltvvhqd wlngkeykck vsnkglpapi ektisktkgq prepqvytlp psreemtknq
241 vsltclvkgf ypsdiavewe sngqpennyk ttppmldsdg sfflyskltv dksrwqqgnv
301 fscsvmheal hnhytqksls lspgk
```

GenBank AAW65947 (SEQ ID NO:59)
Homo sapiens **IgG3** Fc (amino acids 19-246)
238 aa

```
  1 hkpsntkvdk rvelktplgd tthtcppcpa pellggpsvf lfppkpkdtl misrtpevtc
 61 vvvdvshedp evkfnwyvdg vevhnaktkp reeqynstyr vvsvltvlhq dwlngkeykc
121 kvsnkalpap iektiskakg qprepqvytl ppsrdeltkn qvsltclvkg fypsdiavew
181 esngqpenny kttppvldsd gsfflysklt vdksrwqqgn vfscsvmhea lhnhytqksl
241 slspgk
```

Figure 4B

GenBank AAA52770 (SEQ ID NO:60)
Homo sapiens **IgD** Fc (amino acids 162-383)
222 aa

```
  1 ptkapdvfpi isgcrhpkdn spvvlaclit gyhptsvtvt wymgtqsqpq rtfpeiqrrd
 61 syymtssqls tplqqwrqge ykcvvqhtas kskkeifrwp espkaqassv ptaqpqaegs
121 lakattapat trntgrggee kkkekekeeq eeretktpec pshtqplgvy lltpavqdlw
181 lrdkatftcf vvgsdlkdah ltwevagkvp tggveeglle rhsngsqsqh srltlprslw
241 nagtsvtctl nhpslppqrl malrepaaqa pvklslnlla ssdppeaasw llcevsgfsp
301 pnillmwled qrevntsgfa parpppqprs ttfwawsvlr vpappspqpa tytcvvshed
361 srtllnasrs levsyvtdhg pmk
```

GenBank 0308221A (SEQ ID NO:61)
Homo sapiens **IgM** Fc
276 aa

```
  1 vtstltikzs dwlgesmftc rvdhrgltfq qnassmcvpd qdtairvfai ppsfasiflt
 61 kstkltclvt dlttybsvti swtreengav kthtnisesh pnatfsavge asicedbdws
121 gerftctvth tdlpsplkqt isrpkgvalh rpbvyllppa rzzlnlresa titclvtgfs
181 padvfvewmq rgeplspqky vtsapmpepq apgryfahsi ltvseeewnt ggtytcvvah
241 ealpnrvter tvdkstgkpt lynvslvmsd tagtcy
```

Figure 4C

GenBank P01876 (SEQ ID NO:62)
Homo sapiens **IgA** Fc (amino acids 120-353)
234 aa

```
  1 asptspkvfp lslcstqpdg nvviaclvqg ffpqeplsvt wsesgqgvta rnfppsqdas
 61 gdlyttssql tlpatqclag ksvtchvkhy tnpsqdvtvp cpvpstpptp spstpptpsp
121 scchprlslh rpaledlllg seanltctlt glrdasgvtf twtpssgksa vqgpperdlc
181 gcysvssvlp gcaepwnhgk tftctaaype sktpltatls ksgntfrpev hllpppseel
241 alnelvtltc largfspkdv lvrwlqgsqe lprekyltwa srqepsqgtt tfavtsilrv
301 aaedwkkgdt fscmvgheal plaftqktid rlagkpthvn vsvvmaevdg tcy
```

GenBank 1F6A_B (SEQ ID NO:63)
Homo sapiens **IgE** Fc (amino acids 6-222)
212 aa

```
  1 adpcdsnprg vsaylsrpsp fdlfirkspt itclvvdlap skgtvnltws rasgkpvnhs
 61 trkeekqrng tltvtstlpv gtrdwieget yqcrvthphl pralmrsttk tsgpraapev
121 yafatpewpg srdkrtlacl iqnfmpedis vqwlhnevql pdarhsttqp rktkgsgffv
181 fsrlevtrae weqkdeficr avheaaspsq tvqravsvnp gk
```

GenBank P01861 (SEQ ID NO:64)
Homo sapiens **IgG4** Fc (amino acids 100-327)
228 aa

```
  1 astkgpsvfp lapcsrstse staalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss
 61 glyslssvvt vpssslgtkt ytcnvdhkps ntkvdkrves kygppcpscp apeflggpsv
121 flfppkpkdt lmisrtpevt cvvvdvsqed pevqfnwyvd gvevhnaktk preeqfnsty
181 rvvsvltvlh qdwlngkeyk ckvsnkglps siektiskak gqprepqvyt lppsqeemtk
241 nqvsltclvk gfypsdiave wesngqpenn ykttppvlds dgsfflysrl tvdksrwqeg
301 nvfscsvmhe alhnhytqks lslslgk
```

Figure 5A
Homo sapiens (SEQ ID NO:65)
GenBank NP_001229687
HLA-A
Amino acids 25-365

```
  1 mavmaprtll lllsgalalt qtwagshsmr yfftsvsrpg rgeprfiavg yvddtqfvrf
 61 dsdaasqkme prapwieqeg peywdqetrn mkahsqtdra nlgtlrgyyn qsedgshtiq
121 imygcdvgpd grflrgyrqd aydgkdyial nedlrswtaa dmaaqitkrk weavhaaeqr
181 rvylegrcvd glrrylengk etlqrtdppk thmthhpisd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrpa gdgtfqkwaa vvvpsgeeqr ytchvqhegl pkpltlrwel
301 ssqptipivg iiaglvllga vitgavvaav mwrrkssdrk ggsytqaass dsaqgsdvsl
361 tackv
```

Figure 5B
Homo sapiens (SEQ ID NO:66)
GenBank NP_005505
HLA-B
Amino acids 25-362

```
  1 mlvmaprtvl lllsaalalt etwagshsmr yfytsvsrpg rgeprfisvg yvddtqfvrf
 61 dsdaaspree prapwieqeg peywdrntqi ykaqaqtdre slrnlrgyyn qseagshtlq
121 smygcdvgpd grllrghdqy aydgkdyial nedlrswtaa dtaaqitqrk weaareaeqr
181 raylegecve wlrrylengk dkleradppk thvthhpisd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrpa gdrtfqkwaa vvvpsgeeqr ytchvqhegl pkpltlrwep
301 ssqstvpivg ivaglavlav vvigavvaav mcrrkssggk ggsysqaacs dsaqgsdvsl
361 ta
```

Figure 5C

Homo sapiens

GenBank NP_001229971 (SEQ ID NO:67)

HLA-C

Amino acids 25-366

```
  1 mrvmaprall lllsgglalt etwacshsmr yfdtavsrpg rgeprfisvg yvddtqfvrf
 61 dsdaasprge prapwveqeg peywdretqn ykrqaqadrv slrnlrgyyn qsedgshtlq
121 rmygcdlgpd grllrgydqs aydgkdyial nedlrswtaa dtaaqitqrk leaaraaeql
181 raylegtcve wlrrylengk etlqraeppk thvthhplsd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrpa gdgtfqkwaa vvvpsgqeqr ytchmqhegl qepltlswep
301 ssqptipimg ivaglavlvv lavlgavvta mmcrrkssgg kggscsqaac snsaqgsdes
361 litcka
```

FIG. 6

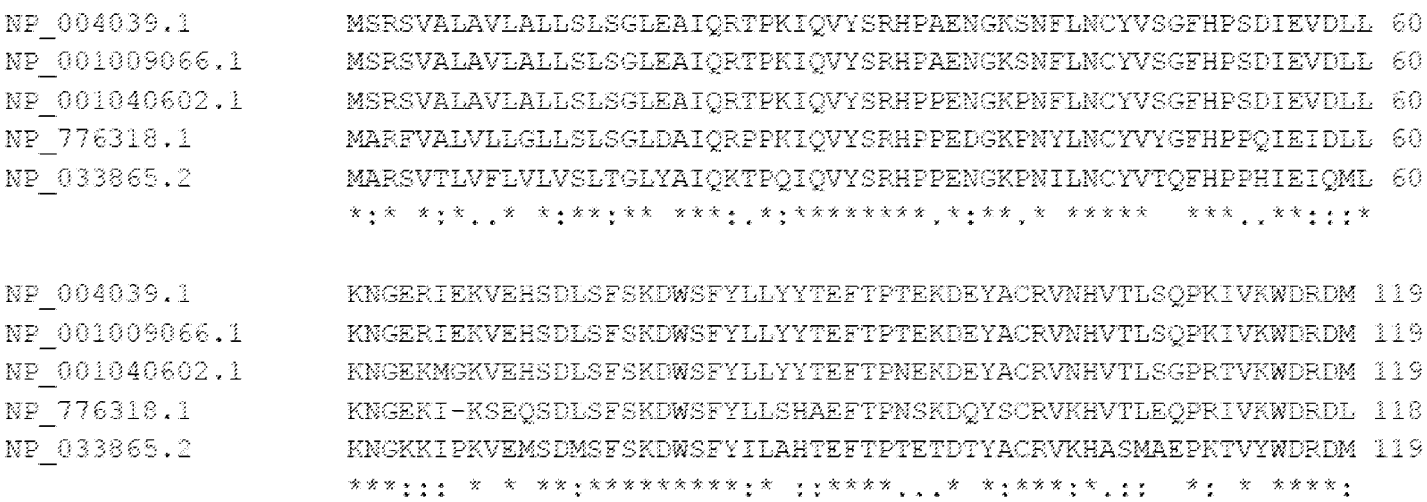

NP_004039.1 MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL 60
NP_001009066.1 MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL 60
NP_001040602.1 MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPPENGKPNFLNCYVSGFHPSDIEVDLL 60
NP_776318.1 MARFVALVLLGLLSLSGLDAIQRPPKIQVYSRHPPEDGKPNYLNCYVYGFHPFQIEIDLL 60
NP_033865.2 MARSVTLVFLVLVSLTGLYAIQKTPQIQVYSRHPPENGKPNILNCYVTQFHPPHIEIQML 60
NP_004039.1 KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM 119
NP_001009066.1 KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM 119
NP_001040602.1 KNGEKMGKVEHSDLSFSKDWSFYLLYYTEFTPNEKDEYACRVNHVTLSGPRTVKWDRDM 119
NP_776318.1 KNGEKI-KSEQSDLSFSKDWSFYLLSHAEFTPNSKDQYSCRVKHVTLEQPRIVKWDRDL 118
NP_033865.2 KNGKKIPKVEMSDMSFSKDWSFYILAHTEFTPTETDTYACRVKHASMAEPKTVYWDRDM 119

FIG. 7A

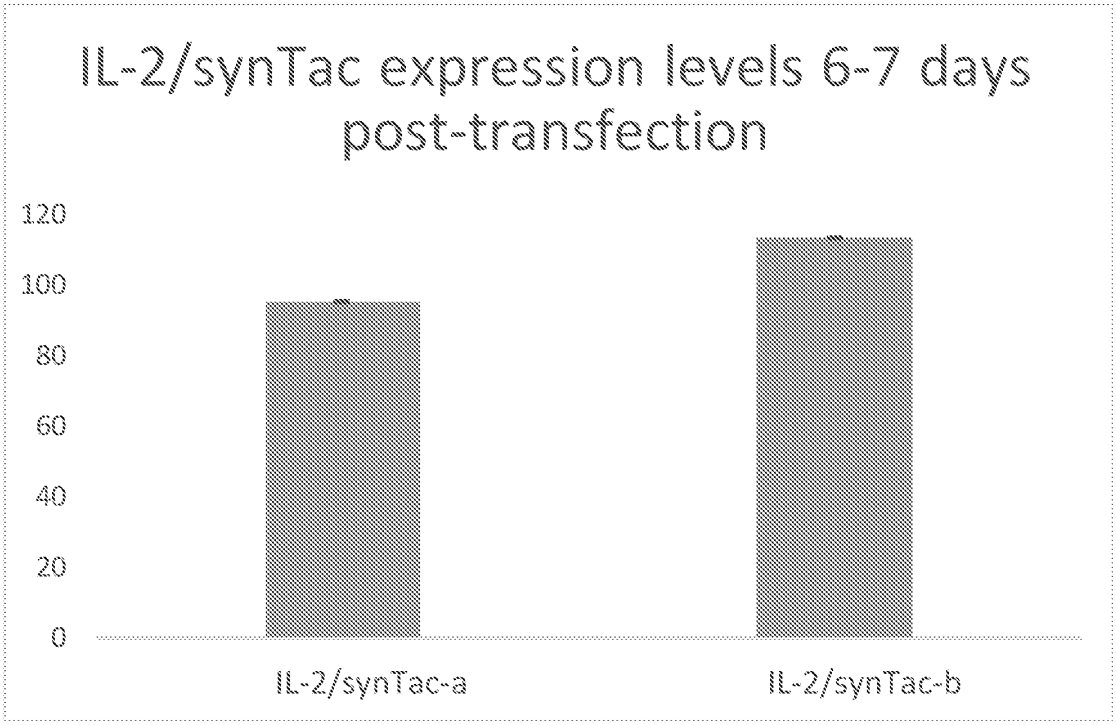

IL-2/synTac expression levels 6-7 days post-transfection
120
100
80
60
40
20
0
IL-2/synTac-a
IL-2/synTac-b

FIG. 7B

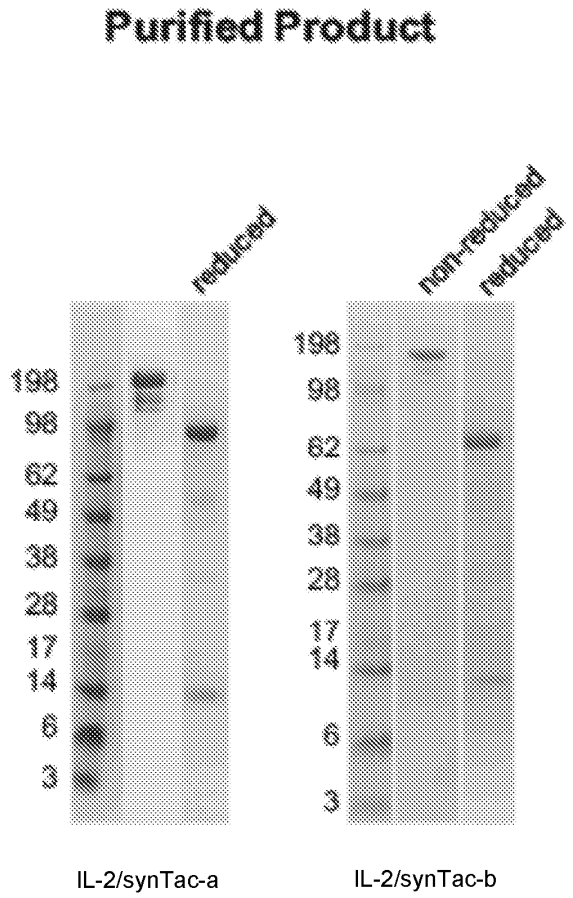

Purified Product
reduced
non-reduced
reduced
198
98
62
49
38
28
17
14
6
3
IL-2/synTac-a
IL-2/synTac-b

FIG. 8A

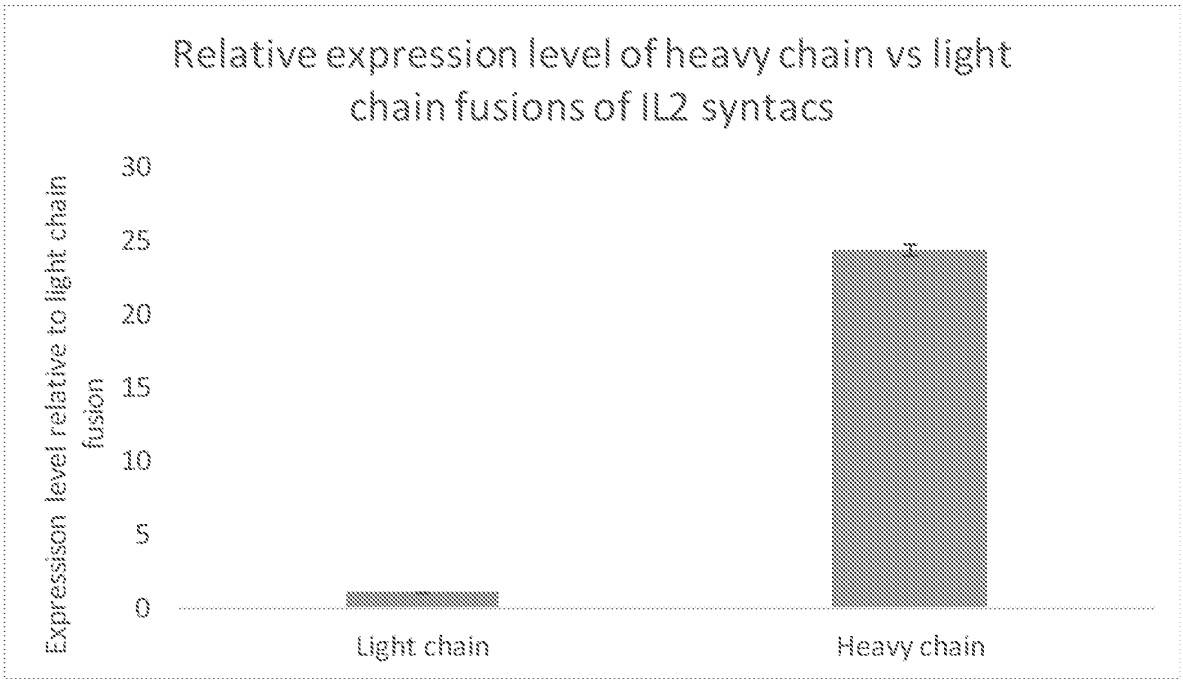

Relative expression level of heavy chain vs light chain fusions of IL2 syntacs
Expression level relative to light chain fusion
30
25
20
15
10
5
0
Light chain
Heavy chain

FIG. 8B

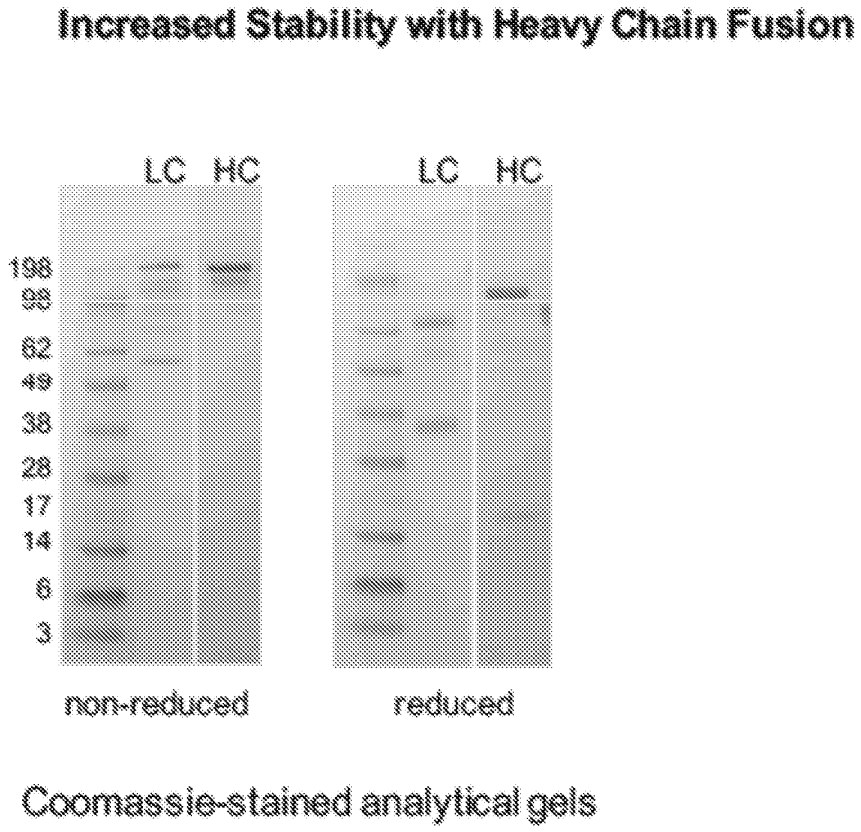

Increased Stability with Heavy Chain Fusion
LC
HC
LC
HC
198
98
62
49
38
28
17
14
6
3
non-reduced
reduced
Coomassie-stained analytical gels

FIG. 9

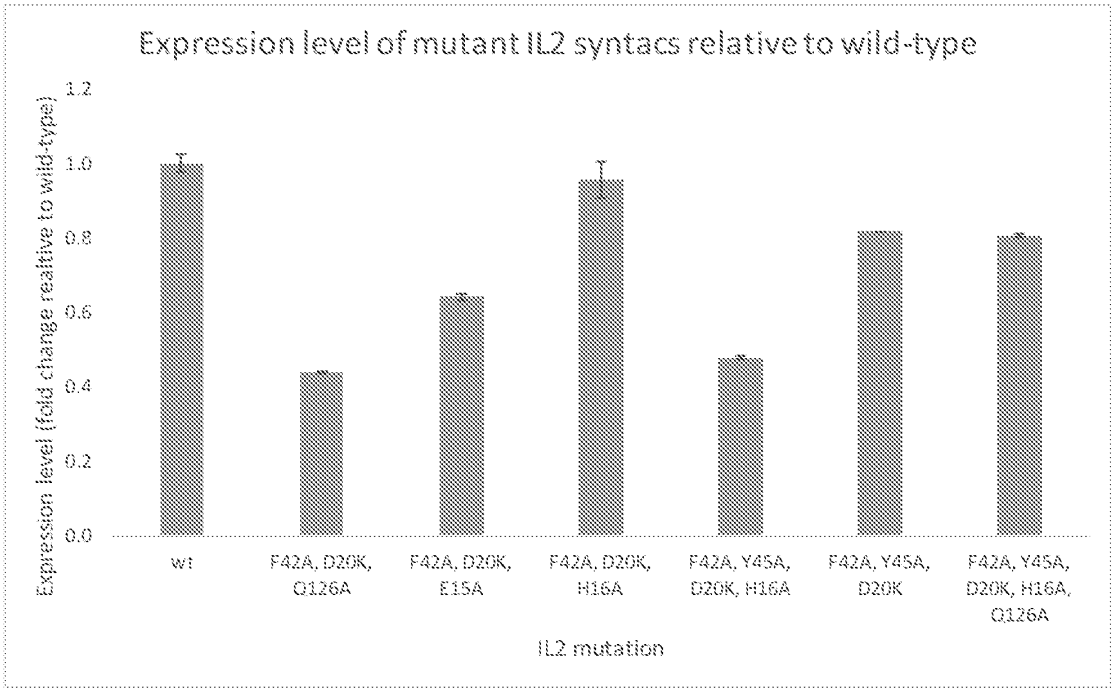

Expression level of mutant IL2 syntacs relative to wild-type
Expression level (fold change realtive to wild-type)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
wt
F42A, D20K, Q126A
F42A, D20K, E15A
F42A, D20K, H16A
F42A, Y45A, D20K, H16A
F42A, Y45A, D20K
F42A, Y45A, D20K, H16A, Q126A
IL2 mutation

FIG. 10

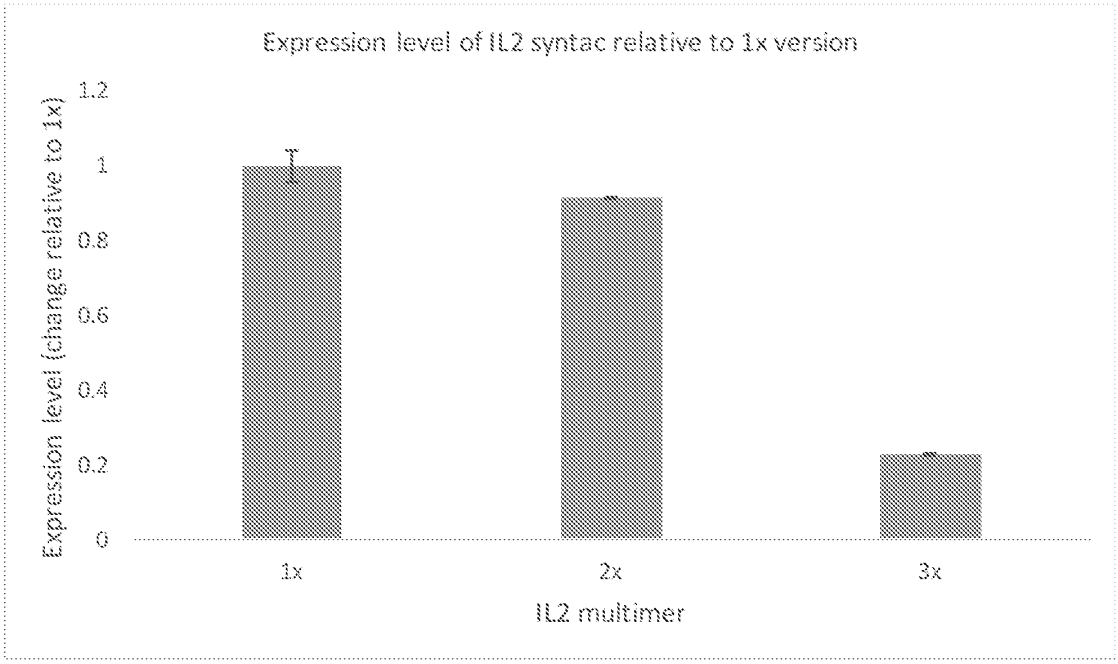

Expression level of IL2 syntac relative to 1x version
Expression level (change relative to 1x)
1.2
1
0.8
0.6
0.4
0.2
0
1x
2x
3x
IL2 multimer

FIG. 11

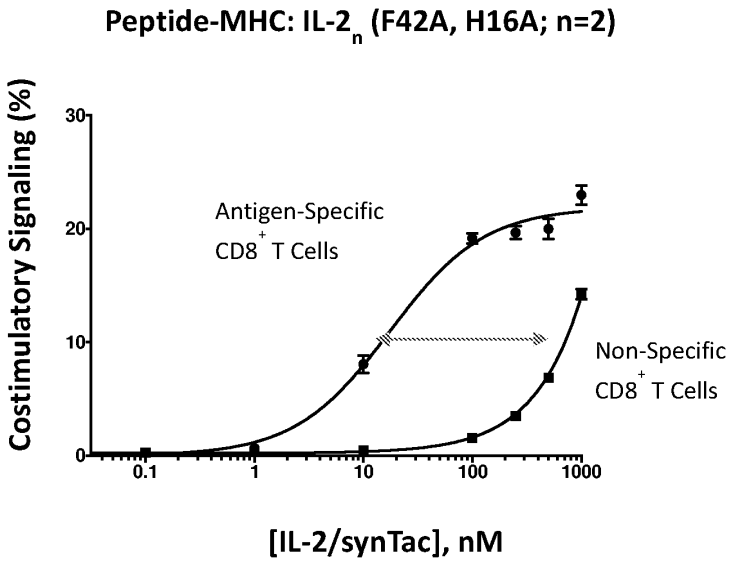

Peptide-MHC: IL-2n (F42A, H16A; n=2)
Costimulatory Signaling (%)
30
20
10
0
0.1
1
10
100
1000
Antigen-Specific
CD8+ T Cells
Non-Specific
CD8+ T Cells
[IL-2/synTac], nM

FIG. 12

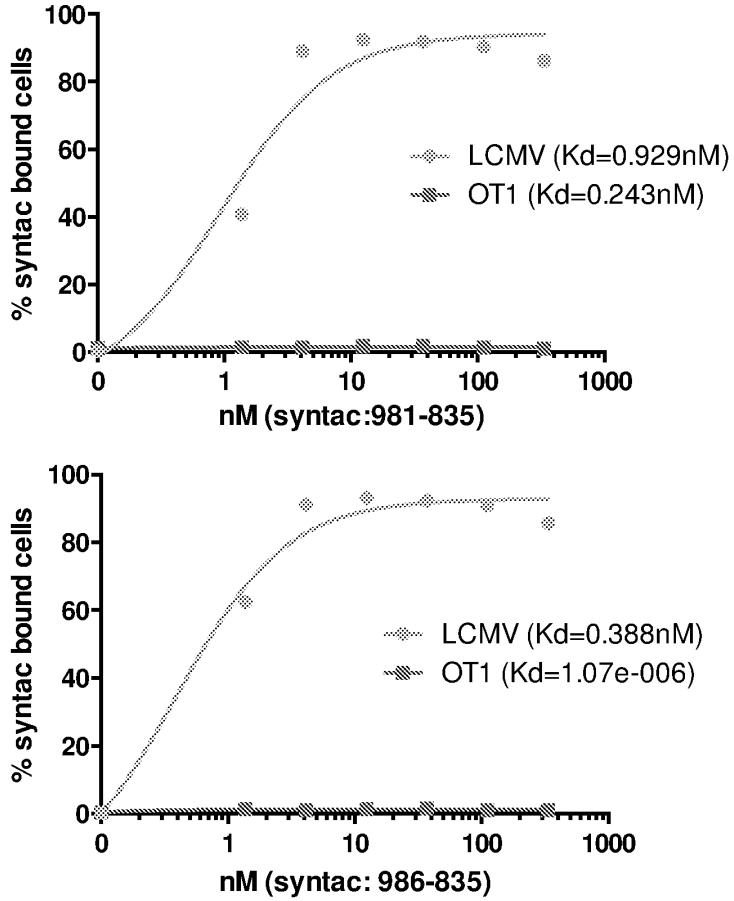

% syntac bound cells
100
80
60
40
20
0
0
1
10
100
1000
nM (syntac:981-835)
LCMV (Kd=0.929nM)
OT1 (Kd=0.243nM)
% syntac bound cells
100
80
60
40
20
0
0
1
10
100
1000
nM (syntac: 986-835)
LCMV (Kd=0.388nM)
OT1 (Kd=1.07e-006)

FIG. 13

Number of IL-2 Repeats versus Mutations

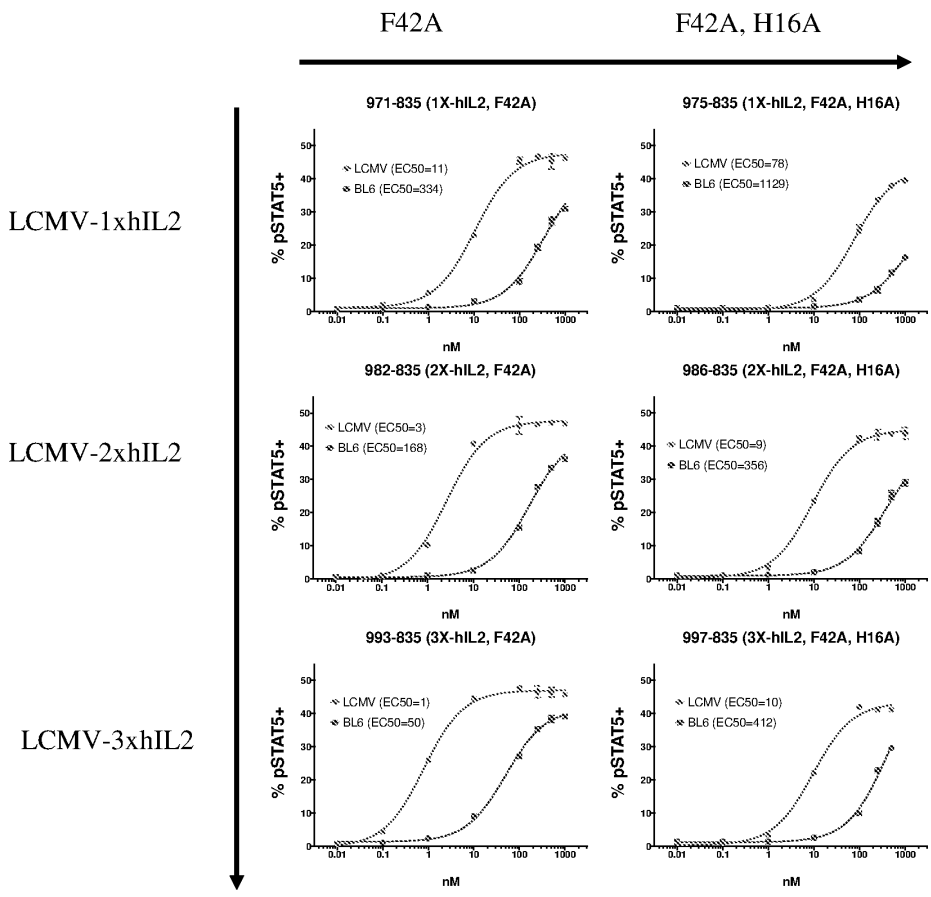
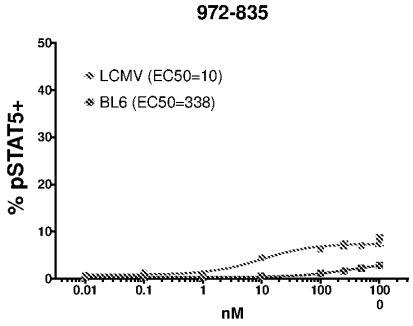


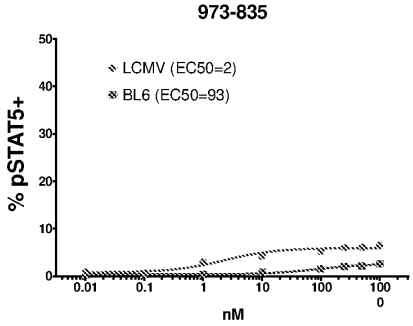
FIG. 14A

One copy of IL-2

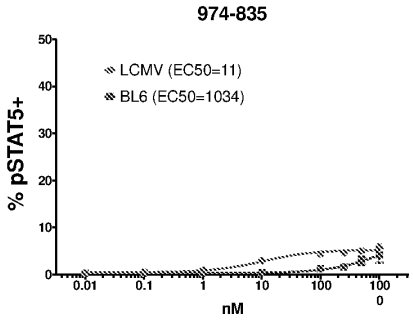
Mutation: F42A, D20K



Mutation: F42A, D20K, Q126A



Mutation: F42A, D20K, E15A



Mutation: F42A, D20K, H16A

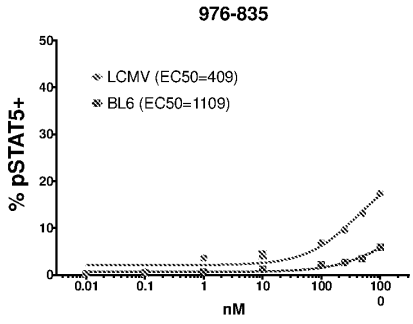
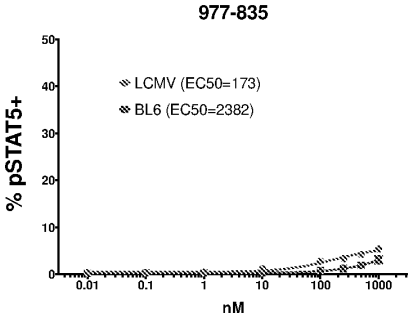


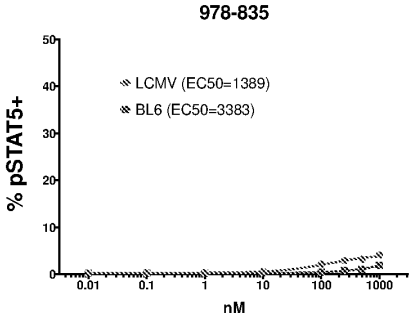
FIG. 14B

One copy of IL-2

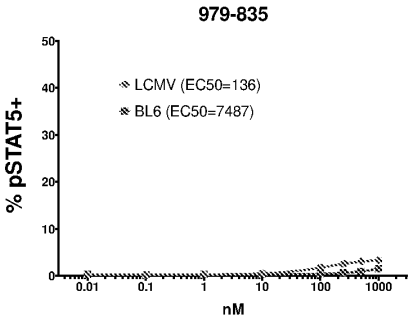
Mutation: F42A, Y45A, D20K, H16A



Mutation: F42A, Y45A, D20K



Mutation: F42A, Y45A, D20K, H16A, Q126A



Mutation: F42A, Y45A, D20K, Q126A

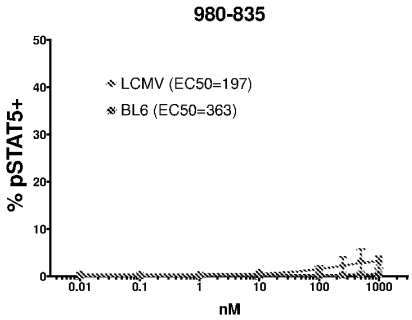
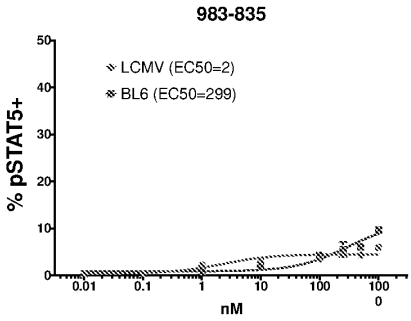


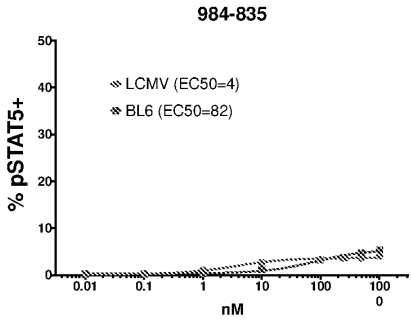
FIG. 14C

Two copies of IL-2

Mutation: F42A, D20K



Mutation: F42A, D20K, Q126A



Mutation: F42A, D20K, H16A

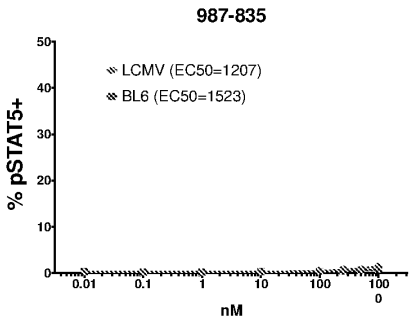
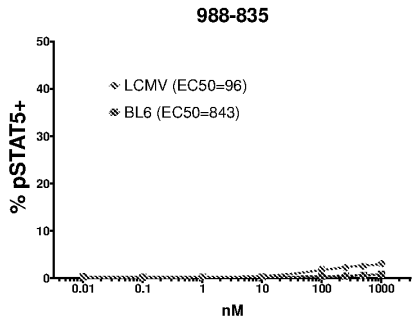


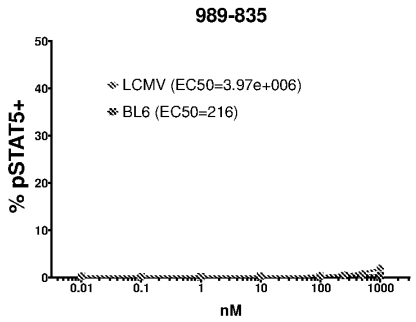
FIG. 14D

Two copies of IL-2

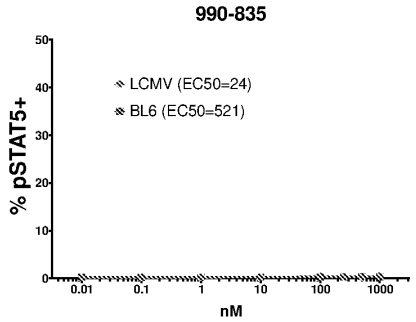
Mutation: F42A, Y45A, D20K, H16A



Mutation: F42A, Y45A, D20K



Mutation: F42A, Y45A, D20K, H16A, Q126A



Mutation: F42A, Y45A, D20K, Q126A

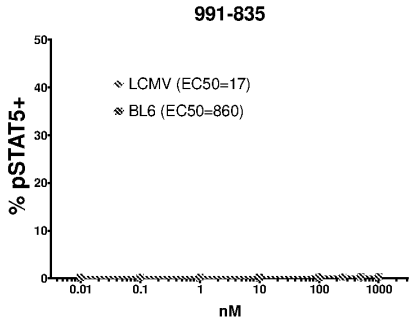
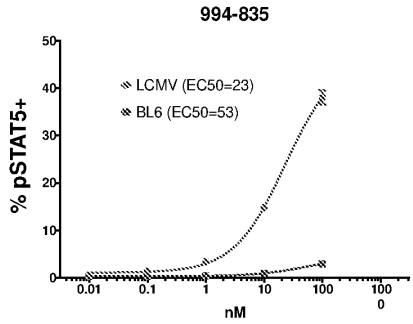


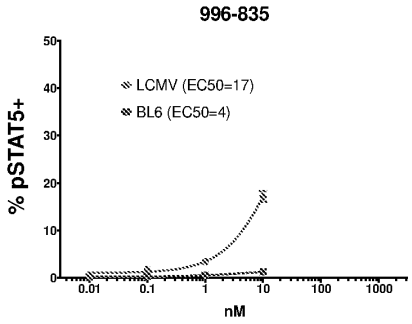
FIG. 14E

Three copies of IL-2

Mutation: F42A, D20K



Mutation: F42A, D20K, E15A



Mutation: F42A, D20K, H16A

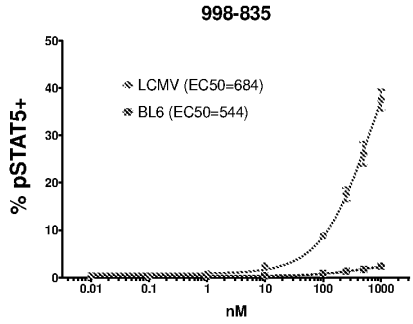
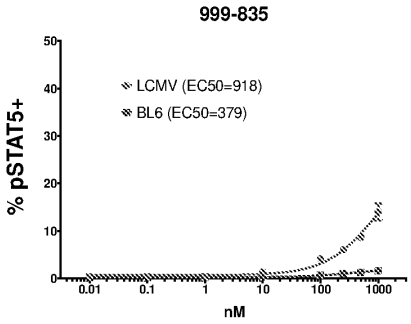


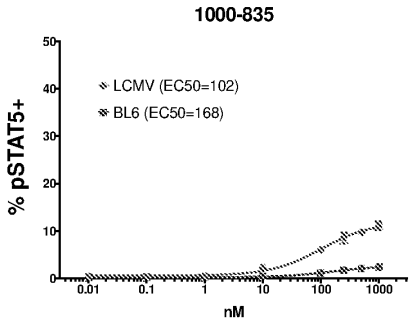
FIG. 14F

Three copies of IL-2

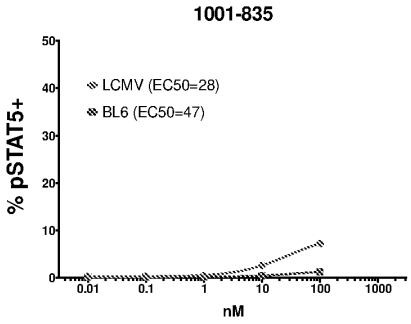
Mutation: F42A, Y45A, D20K, H16A



Mutation: F42A, Y45A, D20K



Mutation: F42A, Y45A, D20K, H16A, Q126A



Mutation: F42A, Y45A, D20K, Q126A

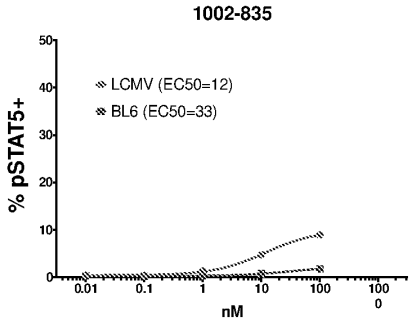


FIG. 15

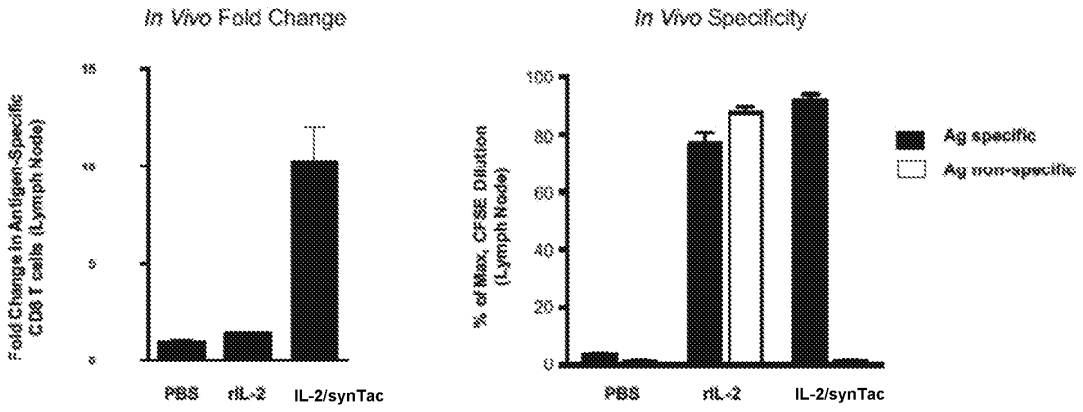

In Vivo Fold Change
Fold Change in Antigen-Specific CD8 T cells (Lymph Node)
15
10
5
0
PBS
rIL-2
IL-2/synTac
In Vivo Specificity
% of Max, CFSE Dilution (Lymph Node)
100
80
60
40
20
0
PBS
rIL-2
IL-2/synTac
Ag specific
Ag non-specific

FIG. 16A

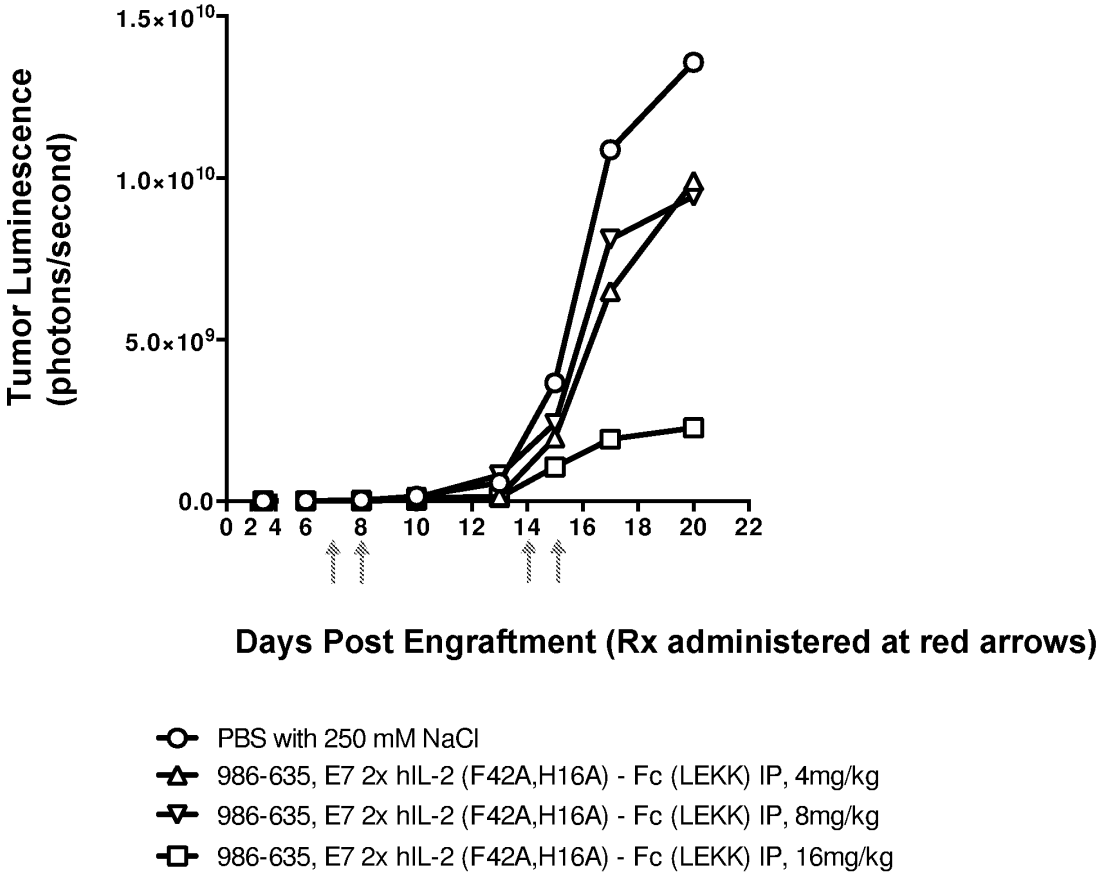

Tumor Luminescence (photons/second)
1.5×10^10
1.0×10^10
5.0×10^9
0.0
0 2 4 6 8 10 12 14 16 18 20 22
Days Post Engraftment (Rx administered at red arrows)
PBS with 250 mM NaCl
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 4mg/kg
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 8mg/kg
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 16mg/kg

FIG. 16B

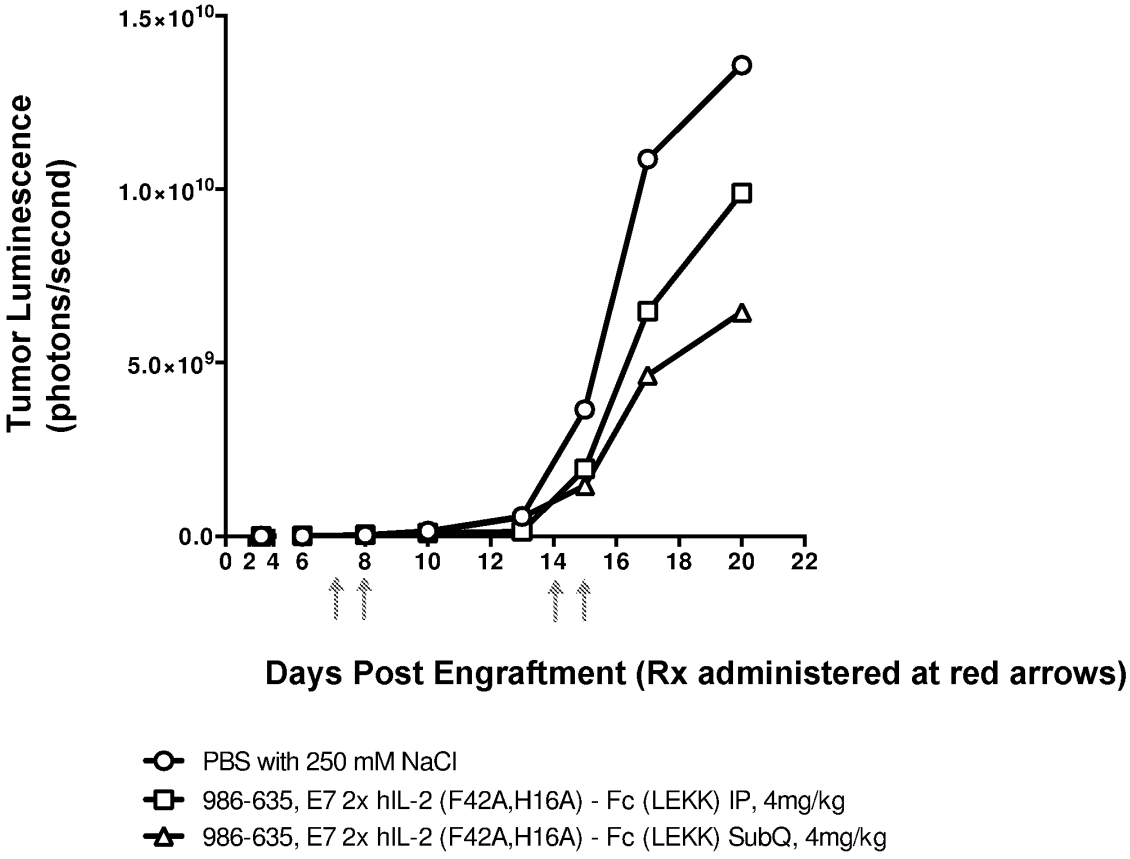

1.5×10^10
1.0×10^10
5.0×10^9
0.0
Tumor Luminescence (photons/second)
0 2 4 6 8 10 12 14 16 18 20 22
Days Post Engraftment (Rx administered at red arrows)
PBS with 250 mM NaCl
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 4mg/kg
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) SubQ, 4mg/kg

FIG. 17A

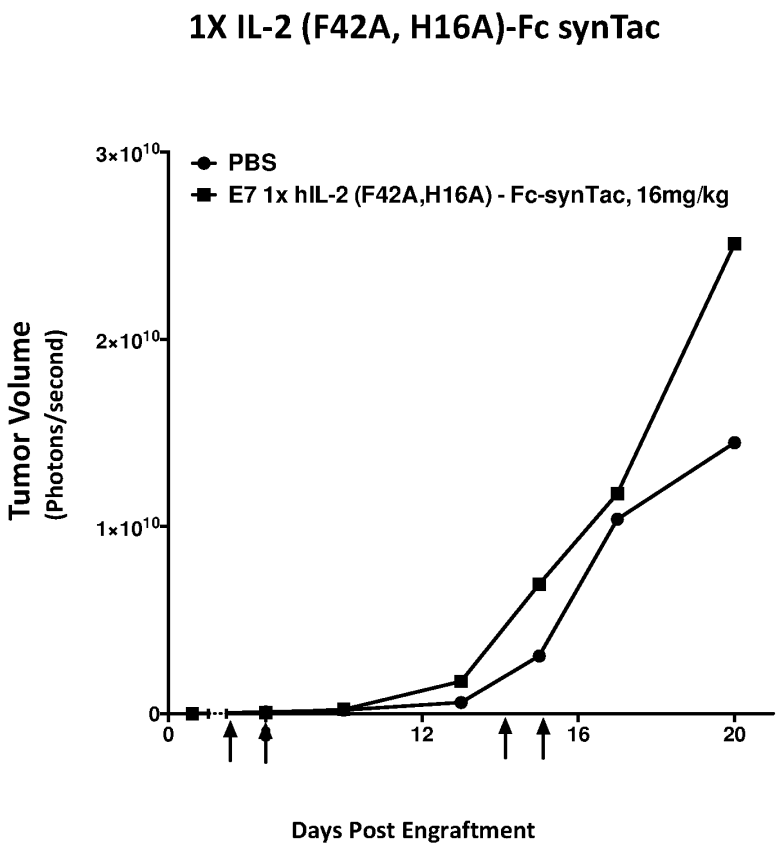

1X IL-2 (F42A, H16A)-Fc synTac
PBS
E7 1x hIL-2 (F42A,H16A) - Fc-synTac, 16mg/kg
Tumor Volume
(Photons/second)
3×10^10
2×10^10
1×10^10
0
0
12
16
20
Days Post Engraftment

FIG. 17B

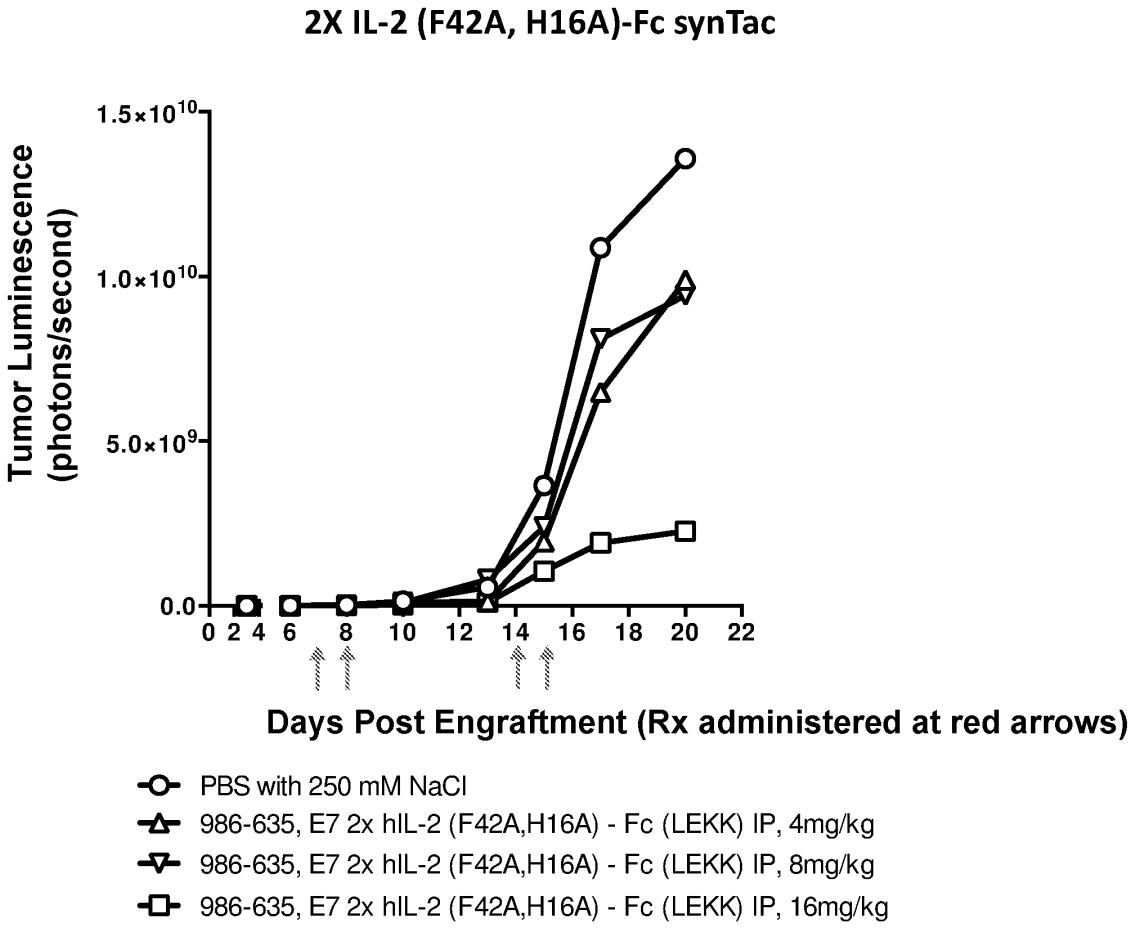

2X IL-2 (F42A, H16A)-Fc synTac
Tumor Luminescence (photons/second)
1.5×10^10
1.0×10^10
5.0×10^9
0.0
0 2 4 6 8 10 12 14 16 18 20 22
Days Post Engraftment (Rx administered at red arrows)
PBS with 250 mM NaCl
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 4mg/kg
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 8mg/kg
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 16mg/kg

FIG. 18

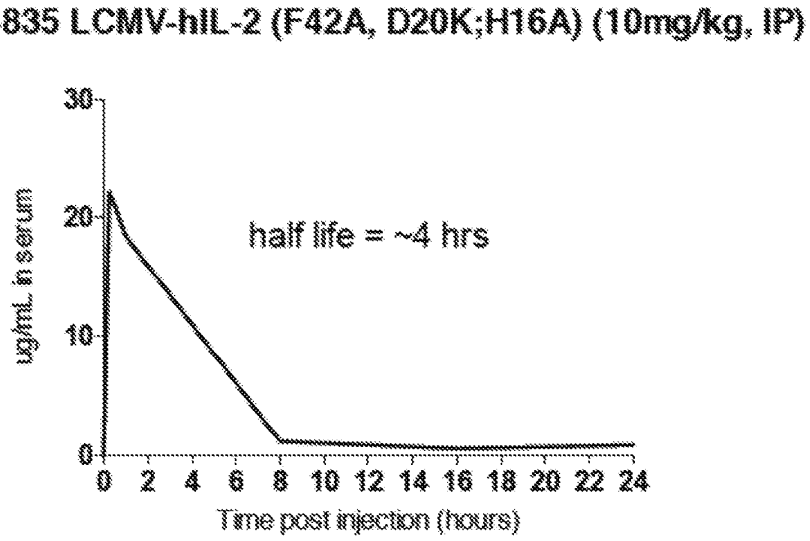
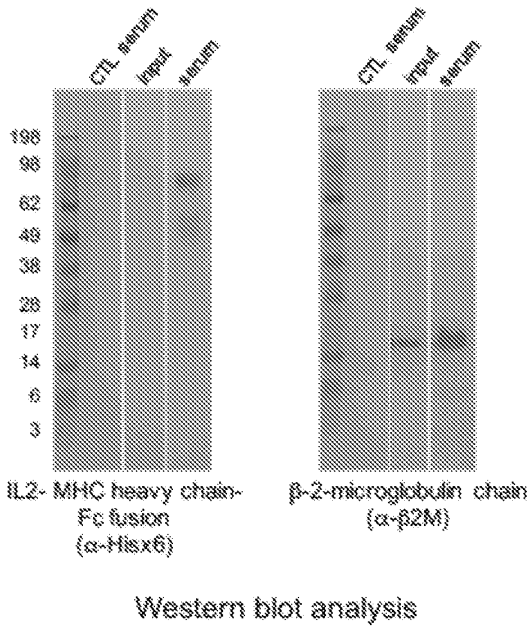

976-835 LCMV-hIL-2 (F42A, D20K;H16A) (10mg/kg, IP)
ug/mL in serum
30
20
10
0
half life = ~4 hrs
0 2 4 6 8 10 12 14 16 18 20 22 24
Time post injection (hours)

FIG. 19



Western blot analysis

FIG. 20

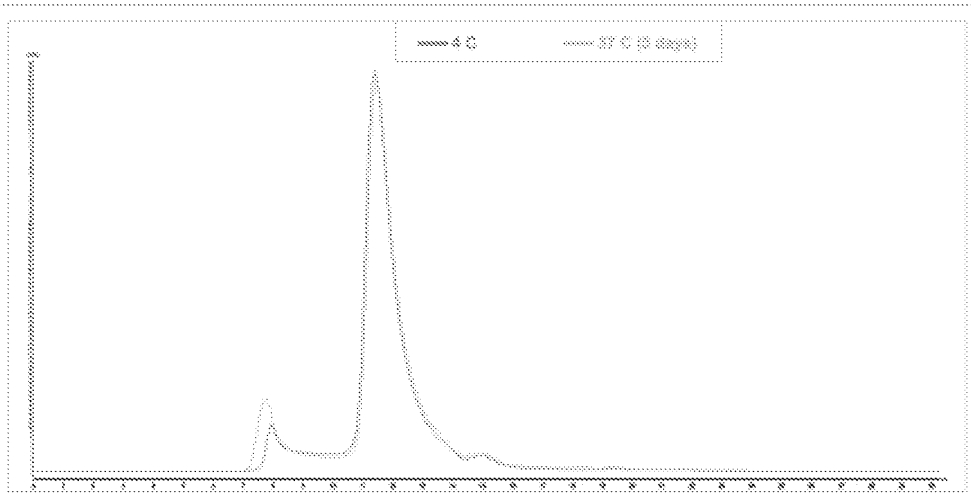


FIG. 21

CUE101-N297A with leader peptide (SEQ ID NO:68)

*MYRMQLLSCIALSLALVTNS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRML**
TAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGGGS**APTSSST**
KKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLE
EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQS
IISTLTGGGGSGGGGSGGGGSGGGGSGSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFV
RFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGAYNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAE
QLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRW
EAAAGG**DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK**
FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Human IL2 Leader sequence – italics

IL-2 (H16A/F42A) – bold (with H16 and F42 underlined)

(G4S)4 **Linkers – single underlined**

MHC H chain Y84A; A236C– double underlined (with Y84A and A236C in bold)

AAAGG linker – single underlined

Human IgG1 Fc; N297A – (bold and underlined, with N297A unbolded)

FIG. 22

CUE101-N297A without leader peptide (SEQ ID NO:69)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRML
TAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGGGS**APTSSST**
KKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLE
EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQS
IISTLTGGGGSGGGGSGGGGSGGGGSGSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFV
RFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGAYNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAE
QLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRW
EAAAGG **DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK**
FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

IL-2 (H16A/F42A) – bold (with H16 and F42 underlined)

(G4S)4 **Linkers – single underlined**

MHC H chain Y84A; A236C– double underlined (with Y84A and A236C in bold)

AAAGG linker – single underlined

Human IgG1 Fc; N297A – (bold and underlined, with N297A unbolded)

FIG. 23A

CUE101-N297A (SEQ ID NO:70)

1360:

*ATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACAAACAGT***GCACCTACTTC**
AAGTTCTACAAAGAAAACACAGCTACAACTGGAGGCA**TTACTGCTGGATTTACAGATGATTTTGAATG**
GAATTAATAATTACAAGAATCCCAAACTCACCAGGATGCTCACAGCA**AAGTTTTACATGCCCAAGAAG**
GCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTT
AGCTCAAAGCAAAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGA
ACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTC
TGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTGACTGGAGGCGGAGGATCTGGTGGTG
GAGGTTCTGGTGGTGGGGGATCTGGAGGCGGAGGATCT**GCACCTACTTCAAGTTCTACAAAGAAAACA**
CAGCTACAACTGGAGGCA**TTACTGCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAAT**
CCCAAACTCACCAGGATGCTCACAGCA**AAGTTTTACATGCCCAAGAAGGCCACAGAACTGAAACATCTT**
CAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCA
CTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAA
CATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGAACAGATGGATTACCTTTT
GTCAAAGCATCATCTCAACACTGACTGGAGGCGGAGGATCTGGTGGTGGAGGTTCTGGTGGTGGGGGA
TCTGGAGGCGGAGGATCTGGCTCTCACTCCATGAGGTATTTCTTCACATCCGTGTCCCGGCCCGGCCGCG
GGGAGCCCCGCTTCATCGCAGTGGGCTACGTGGACGACACGCAGTTCGTGCGGTTCGACAGCGACGCCG
CGAGCCAGAGGATGGAGCCGCGGGCGCCGTGGATAGAGCAGGAGGGTCCGGAGTATTGGGACGGGGA
GACACGGAAAGTGAAGGCCCACTCACAGACTCACCGAGTGGACCTGGGGACCCTGCGCGGC**GCC**TACA
ACCAGAGCGAGGCCGGTTCTCACACCGTCCAGAGGATGTATGGCTGCGACGTGGGGTCGGACTGGCGC
TTCCTCCGCGGGTACCACCAGTACGCCTACGACGGCAAGGATTACATCGCCCTGAAAGAGGACCTGCGCT
CTTGGACCGCGGCGGACATGGCAGCTCAGACCACCAAGCACAAGTGGGAGGCGGCCCATGTGGCGGAG
CAGTTGAGAGCCTACCTGGAGGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAGGA
GACGCTGCAGCGCACGGACGCCCCCAAAACGCATATGACTCACCACGCTGTCTCTGACCATGAAGCCACC
CTGAGGTGCTGGGCCCTGAGCTTCTACCCTGCGGAGATCACACTGACCTGGCAGCGGGATGGGGAGGA
CCAGACCCAGGACACGGAGCTCGTGGAGACCAGGCCT**TGC**GGGGATGGAACCTTCCAGAAGTGGGCGG
CTGTGGTGGTGCCTTCTGGACAGGAGCAGAGATACACCTGCCATGTGCAGCATGAGGGTTTGCCCAAGC
CCCTCACCCTGAGATGGGAGGCAGCTGCGGGTGGC**GACAAAACTCACACATGCCCACCGTGCCCAGCA**
CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC
GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCA**AGCAC**
GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA
AGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA
GAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTG
CCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC
AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCAC
TACACGCAGAAGTCCCTCTCCCTGTCTCCGGGTAAATAGTGA

FIG. 23B

Human IL2 Leader sequence – italics
Human IL2; H16A=GCA; F42A=GCA – bold (with GCA underlined)
(G4S)4 linker – single underlined
Human A0201; Y84A=**GCC**; A236C=**TGC**
AAAGG linker – single underlined
Human IgG1 Fc; N297A= GCA; AGG to **AGA** (still R) and AGC to **TCC** (still S) – (bold and underlined, with GCA italicized)
Stop codons (TAGTGA)

FIG. 24

CUE101-LALA with leader peptide (SEQ ID NO:71)

*MYRMQLLSCIALSLALVTNS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRML**
TAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGGGS**APTSSST**
KKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLE
EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQS
IISTLTGGGGSGGGGSGGGGSGGGGSGSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFV
RFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**A**YNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAE
QLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRW
EAAAGG**DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK**
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Leader peptide – italics
IL-2 (H16A/F42A) – bold (with H16 and F42 underlined)
(G4S)4 **Linkers – single underlined**
MHC H chain Y84A; A236C– double underlined (with Y84A and A236C in bold)
AAAGG linker – single underlined
Human IgG1 Fc; L234A; L235A – (bold and underlined, with L234A and L235A unbolded)

FIG. 25

CUE101-LALA without leader peptide (SEQ ID NO:72)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRML
TAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGGGSAPTSSST
KKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLE
EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQS
IISTLTGGGGSGGGGSGGGGSGGGGSGSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFV
RFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGAYNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAE
QLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRW
EAAAGGDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

IL-2 (H16A/F42A) – bold (with H16 and F42 underlined)

(G4S)4 **Linkers – single underlined**

MHC H chain Y84A; A236C– double underlined (with Y84A and A236C in bold)

AAAGG linker – single underlined

Human IgG1 Fc; L234A; L235A – (bold and underlined, with L234A and L235A unbolded)

FIG. 26A (SEQ ID NO:73)

CUE101-LALA: nucleotide sequence encoding CUE101-LALA with leader peptide

*ATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACAAACAGT***GCACCTACTTC**
AAGTTCTACAAAGAAAACACAGCTACAACTGGAGGCA**TTACTGCTGGATTTACAGATGATTTTGAATG**
GAATTAATAATTACAAGAATCCCAAACTCACCAGGATGCTCACAGCA**AAGTTTTACATGCCCAAGAAG**
GCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTT
AGCTCAAAGCAAAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGA
ACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTC
TGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTGACTGGAGGCGGAGGATCTGGTGGTG
GAGGTTCTGGTGGTGGGGGATCTGGAGGCGGAGGATCT**GCACCTACTTCAAGTTCTACAAAGAAAACA**
CAGCTACAACTGGAGGCA**TTACTGCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAAT**
CCCAAACTCACCAGGATGCTCACAGCA**AAGTTTTACATGCCCAAGAAGGCCACAGAACTGAAACATCTT**
CAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCA
CTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAA
CATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGAACAGATGGATTACCTTTT
GTCAAAGCATCATCTCAACACTGACTGGAGGCGGAGGATCTGGTGGTGGAGGTTCTGGTGGTGGGGGA
TCTGGAGGCGGAGGATCTGGCTCTCACTCCATGAGGTATTTCTTCACATCCGTGTCCCGGCCCGGCCGCG
GGGAGCCCCGCTTCATCGCAGTGGGCTACGTGGACGACACGCAGTTCGTGCGGTTCGACAGCGACGCCG
CGAGCCAGAGGATGGAGCCGCGGGCGCCGTGGATAGAGCAGGAGGGTCCGGAGTATTGGGACGGGGA
GACACGGAAAGTGAAGGCCCACTCACAGACTCACCGAGTGGACCTGGGGACCCTGCGCGGC**GCC**TACA
ACCAGAGCGAGGCCGGTTCTCACACCGTCCAGAGGATGTATGGCTGCGACGTGGGGTCGGACTGGCGC
TTCCTCCGCGGGTACCACCAGTACGCCTACGACGGCAAGGATTACATCGCCCTGAAAGAGGACCTGCGCT
CTTGGACCGCGGCGGACATGGCAGCTCAGACCACCAAGCACAAGTGGGAGGCGGCCCATGTGGCGGAG
CAGTTGAGAGCCTACCTGGAGGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAGGA
GACGCTGCAGCGCACGGACGCCCCCAAAACGCATATGACTCACCACGCTGTCTCTGACCATGAAGCCACC
CTGAGGTGCTGGGCCCTGAGCTTCTACCCTGCGGAGATCACACTGACCTGGCAGCGGGATGGGGAGGA
CCAGACCCAGGACACGGAGCTCGTGGAGACCAGGCCT**TGC**GGGGATGGAACCTTCCAGAAGTGGGCGG
CTGTGGTGGTGCCTTCTGGACAGGAGCAGAGATACACCTGCCATGTGCAGCATGAGGGTTTGCCCAAGC
CCCTCACCCTGAGATGGGAGGCAGCTGCGGGTGGC**GACAAAACTCACACATGCCCACCGTGCCCAGCA**
CCTGAAGCCGCC**GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC**
GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC**AGCAC**
GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA
AGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA
GAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTG
CCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC
AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCAC
TACACGCAGAAGTCCCTCTCCCTGTCTCCGGGTAAATAGTGA

FIG. 26B

Human IL2 Leader sequence – italics
Human IL2; H16A=GCA; F42A=GCA – bold (with GCA underlined)
(G4S)4 linker – single underlined
Human A0201; Y84A=**GCC**; A236C=**TGC** – double underlined (with GCC and TGC in bold)
AAAGG linker – single underlined
Human IgG1 Fc; L234A, L235A = GCCGCC
N297= AAC; AGG to **AGA** (still R) and AGC to **TCC** (still S) – (bold and underlined, with GCCGCC italicized)
Stop codons (TAGTGA)

FIG. 27 (SEQ ID NO:74)

CUE101-TM with leader peptide

*MYRMQLLSCIALSLALVTNS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRML**
TAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGGGS**APTSSST**
KKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLE
EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQS
IISTLTGGGGSGGGGSGGGGSGGGGSGSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFV
RFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**A**YNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAE
QLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRW
EAAAGG**DKTHTCPPCPAPE**FE**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK**
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Leader peptide – italics
IL-2 (H16A/F42A) – bold (with H16 and F42 underlined)
(G4S)4 **Linkers – single underlined**
MHC H chain Y84A; A236C– double underlined (with Y84A and A236C in bold)
AAAGG linker – single underlined
Human IgG1 Fc; L234F; L235E; P331S – (bold and underlined, with L234F, L235E, and P331S unbolded)

FIG. 28
CUE101-TM without leader peptide (SEQ ID NO:75)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRML
TAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE
TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGGGSAPTSSST
KKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLE
EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQS
IISTLTGGGGSGGGGSGGGGSGGGGSGSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFV
RFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGAYNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAE
QLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRW
EAAAGG DKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

IL-2 (H16A/F42A) – bold (with H16 and F42 underlined)
(G4S)4 **Linkers – single underlined**
MHC H chain Y84A; A236C– double underlined (with Y84A and A236C in bold)
AAAGG linker – single underlined
Human IgG1 Fc; L234F; L235E; P331S – (bold and underlined, with L234F, L235E, and P331S unbolded)

FIG. 29A (SEQ ID NO:76)

CUE101-TM: nucleotide sequence encoding CUE101-TM with leader sequence

*ATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACAAACAGT***GCACCTACTTC**
AAGTTCTACAAAGAAAACACAGCTACAACTGGAGGCA**TTACTGCTGGATTTACAGATGATTTTGAATG**
GAATTAATAATTACAAGAATCCCAAACTCACCAGGATGCTCACAGCA**AAGTTTTACATGCCCAAGAAG**
GCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTT
AGCTCAAAGCAAAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGA
ACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTC
TGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTGACTGGAGGCGGAGGATCTGGTGGTG
GAGGTTCTGGTGGTGGGGGATCTGGAGGCGGAGGATCT**GCACCTACTTCAAGTTCTACAAAGAAAACA**
CAGCTACAACTGGAGGCA**TTACTGCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAAT**
CCCAAACTCACCAGGATGCTCACAGCA**AAGTTTTACATGCCCAAGAAGGCCACAGAACTGAAACATCTT**
CAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCA
CTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAA
CATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGAACAGATGGATTACCTTTT
GTCAAAGCATCATCTCAACACTGACTGGAGGCGGAGGATCTGGTGGTGGAGGTTCTGGTGGTGGGGGA
TCTGGAGGCGGAGGATCTGGCTCTCACTCCATGAGGTATTTCTTCACATCCGTGTCCCGGCCCGGCCGCG
GGGAGCCCCGCTTCATCGCAGTGGGCTACGTGGACGACACGCAGTTCGTGCGGTTCGACAGCGACGCCG
CGAGCCAGAGGATGGAGCCGCGGGCGCCGTGGATAGAGCAGGAGGGTCCGGAGTATTGGGACGGGGA
GACACGGAAAGTGAAGGCCCACTCACAGACTCACCGAGTGGACCTGGGGACCCTGCGCGGC**GCC**TACA
ACCAGAGCGAGGCCGGTTCTCACACCGTCCAGAGGATGTATGGCTGCGACGTGGGGTCGGACTGGCGC
TTCCTCCGCGGGTACCACCAGTACGCCTACGACGGCAAGGATTACATCGCCCTGAAAGAGGACCTGCGCT
CTTGGACCGCGGCGGACATGGCAGCTCAGACCACCAAGCACAAGTGGGAGGCGGCCCATGTGGCGGAG
CAGTTGAGAGCCTACCTGGAGGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAGGA
GACGCTGCAGCGCACGGACGCCCCCAAAACGCATATGACTCACCACGCTGTCTCTGACCATGAAGCCACC
CTGAGGTGCTGGGCCCTGAGCTTCTACCCTGCGGAGATCACACTGACCTGGCAGCGGGATGGGGAGGA
CCAGACCCAGGACACGGAGCTCGTGGAGACCAGGCCT**TGC**GGGGATGGAACCTTCCAGAAGTGGGCGG
CTGTGGTGGTGCCTTCTGGACAGGAGCAGAGATACACCTGCCATGTGCAGCATGAGGGTTTGCCCAAGC
CCCTCACCCTGAGATGGGAGGCAGCTGCGGGTGGC**GACAAAACTCACACATGCCCACCGTGCCCAGCA**
CCTGAATTCGAG**GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC**
GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC**AGCAC**
GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA
AGGTCTCCAACAAAGCCCTCCCAGCCAGC**ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA**
GAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTG
CCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC
AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCAC
TACACGCAGAAGTCCCTCTCCCTGTCTCCGGGTAAATAGTGA

FIG. 29B

Human IL2 Leader sequence – italics
Human IL2; H16A=GCA; F42A=GCA – bold (with GCA underlined)
(G4S)4 linker – single underlined
Human A0201; Y84A=**GCC**; A236C=**TGC** – double underlined (with GCC and TGC in bold)
AAAGG linker – single underlined
Human IgG1 Fc; L234F=TTC; L235E=GAG; P331S=AGC
N297= AAC; AGG to **AGA** (still R) and AGC to **TCC** (still S) – (bold and underlined, with TTC, GAG, AAC, and AGC italicized)
Stop codons (TAGTGA)

FIG. 30
1274: (SEQ ID NO:77)
*MSRSVALAVLALLSLSGLEA***YMLDLQPETT**GGGGSGGGGSGGGGSIQRTPKIQVYS**C**HPA
ENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEK
DEYACRVNHVTLSQPKIVKWDRDM

Human β2M leader sequence -- italics
E7(11-20) – bold and underlined
(G4S)3 linker – single underlined
Human β2M; R12C– double underlined (R12C bolded)

FIG. 31

1274 without leader peptide (SEQ ID NO:78)

YMLDLQPETTGGGGSGGGGSGGGGSIQYMLDLQPETTGGGGSGGGGSGGGGSIQRTPKIQVYSCH
PAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEY
ACRVNHVTLSQPKIVKWDRDMRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGEPI
EKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM

E7(11-20) – bold and underlined (YMLDLQPETT; SEQ ID NO:13)
(G4S)3 linker – single underlined (GGGGSGGGGSGGGGS ;SEQ ID NO:89)
Human β2M; R12C – double underlined

FIG. 32 (SEQ ID NO:79)
1274 nucleotide sequence encoding 1274 with leader peptide

*ATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCTGGAGGCC***TACATGCTCGA**
TTTGCAGCCCGAAACGACGGGTGGAGGTGGTTCTGGAGGAGGCGGTTCGGGCGGAGGTGGTAGTATC
CAGCGTACTCCAAAGATTCAGGTTTACTCA**TGC**CATCCAGCAGAGAATGGAAAGTCAAATTTCCTGAATT
GCTATGTGTCTGGGTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAATGGAGAGAGAATTGAAAA
AGTGGAGCATTCAGACTTGTCTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTATTATACTGAATTCACCCC
CACTGAAAAAGATGAGTATGCCTGCCGTGTGAACCACGTGACTTTGTCACAGCCCAAGATAGTTAAGTG
GGATCGAGACATGTAGTGA

Human β2M leader sequence -- italics
E7(11-20) – bold and underlined
(G4S)3 linker – single underlined
Human β2M; R12C=TGC -- double underlined (TGC in bold)
Stop codons TAGTGA

FIG. 33A

WT Human IgG1 Fc Sequence: (SEQ ID NO:80)

DKTHTCPPCPAPE**LL**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA**P**IEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FIG. 33B

Human IgG1 Fc Mutant: L234F/L235E/P331S (Triple Mutant "TM") (SEQ ID NO:81)

DKTHTCPPCPAPE**FE**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA**S**IEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FIG. 33C

Human IgG1 Fc Mutant: N297A (SEQ ID NO:82)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FIG. 33D

Human IgG1 Fc Mutant: L234A/L235A ("LALA") (SEQ ID NO:83)

DKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Residue numbered according to EU index (Kabat Numbering)

FIG. 34A

B2M R12C (SEQ ID NO:17)

IQRTPKIQVYSCHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLL
YYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM

FIG. 34B

IL-2 (H16A; F42A) (SEQ ID NO:84)

APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKP
LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIIS
TLT

FIG. 34C

Class I MHC-H chain A0201 (Y84A; A236C) (SEQ ID NO:19)

GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETR
KVKAHSQTHRVDLGTLRGAYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDL
RSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVS
DHEATLRCWALSFYPAEITLTWQRDGEDQTQDTELVETRPCGDGTFQKWAAVVVPSGQEQRYTCH
VQHEGLPKPLTLRWE

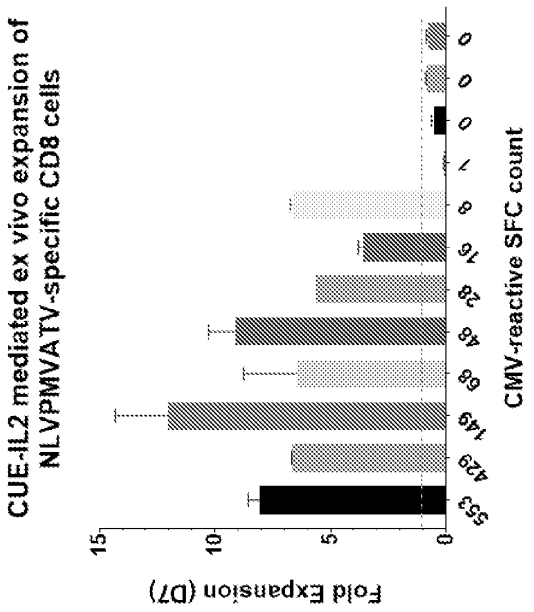

CUE-IL2 mediated ex vivo expansion of NLVPMVATV-specific CD8 cells
Fold Expansion (D7)
0
5
10
15
553
429
149
69
46
28
15
8
7
0
0
0
CMV-reactive SFC count

FIG. 35

FIG. 36

Amino Acid	Syntac Number	Expression level (mg/L)	Receptor binding by Octet (KD in nM)			EC50 for cell binding, nM		EC50 for pSTAT5, nM		
			αβγ	βγ	β	LCMV	Black6	LCMV	Black6	B6/LCMV
H	982	77	14.9			0.25	>100	5.019	57.05	11
A	986	89	16.5			0.22	>100	20.09	543.8	27
R	1284	81	ND			0.29	>100	>1000	>1000	ND
N	1285	50	9.0			0.48	>100	35.19	1041	30
D	1286	31	12.3			0.28	>100	0.8365	15.71	25
C	1287	37	7.6			0.50	>100	59.98	1152	19
Q	1288	66	13.8			0.23	>100	21.64	530.1	24
E	1289	66	12.7			0.31	>100	3.899	117.2	30
G	1290									
I	1291	61	8.4			0.31	>100	>1000	>1000	ND
L	1292	50	12.5			0.36	>100	>1000	>1000	ND
K	1293	74	ND			0.27	>100	>1000	>1000	ND
M	1294	81	19.2			0.41	>100	34.88	674.5	19
F	1295	32	18.6			0.46	>100	4.864	69.1	14
P	1296	2								
S	1297	77	21.2			0.28	>100	14.42	113.4	8
T	1298	123	29.5			0.31	>100	29.66	>1000	>34
W	1299	24	18.6			0.59	>100	70.95	>1000	>14
Y	1300	31	29.4			0.49	>100	16.82	188	11
V	1301	87	7.8			0.28	>100	230.4	>1000	>4

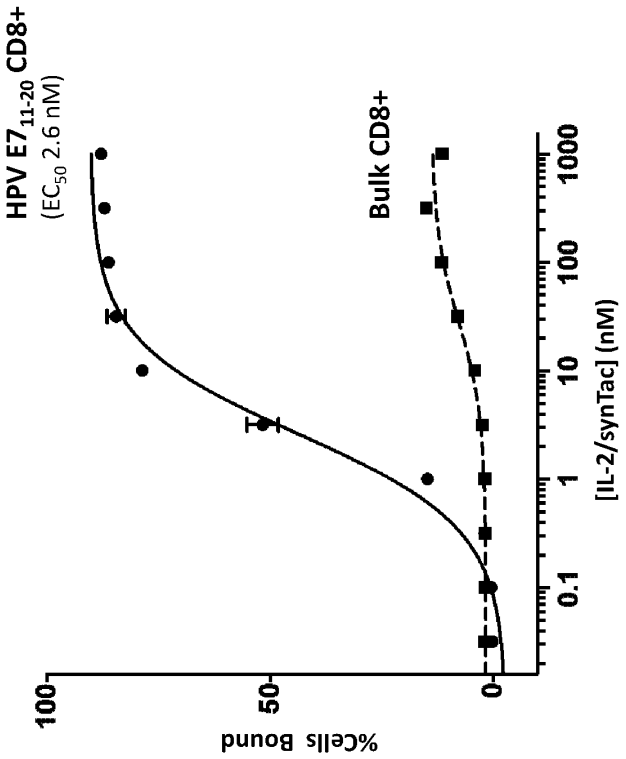

HPV E7 11-20 CD8+
(EC50 2.6 nM)
Bulk CD8+
%Cells Bound
100
50
0
0.1
1
10
100
1000
[IL-2/synTac] (nM)

FIG. 37

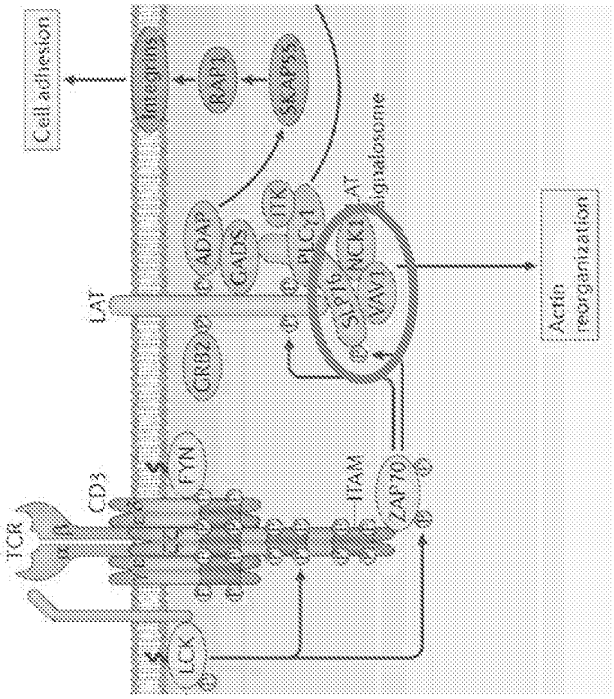
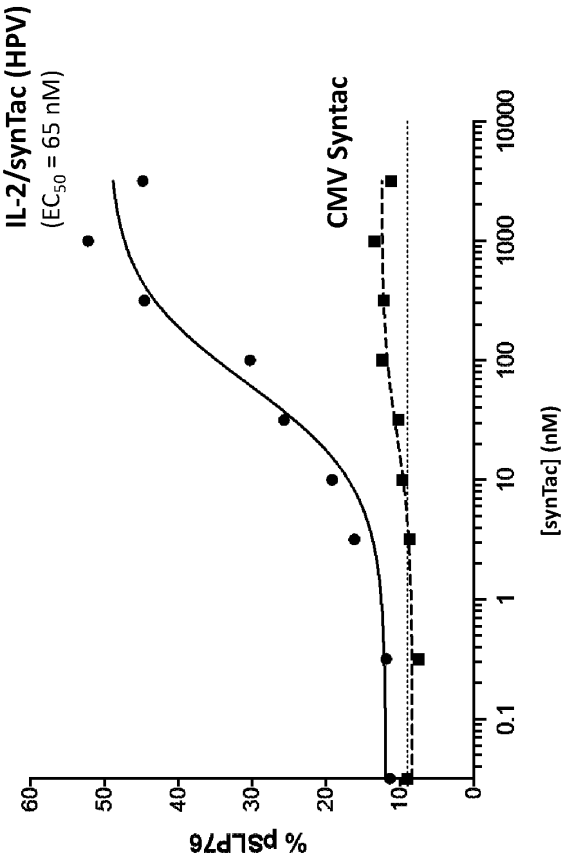

Cell adhesion
Integrins
LAT
ADAP
CRK2
CRKL
CAS
GRAP
SKAP55
ITK
PLCγ1
SLP76
VAV1
LAT signalosome
Actin reorganization
TCR
CD3
FYN
LCK
ITAM
ZAP70

FIG. 38


IL-2/synTac (HPV)
(EC50 = 65 nM)
CMV Syntac
% pSLP76
[synTac] (nM)
60
50
40
30
20
10
0
0.1
1
10
100
1000
10000

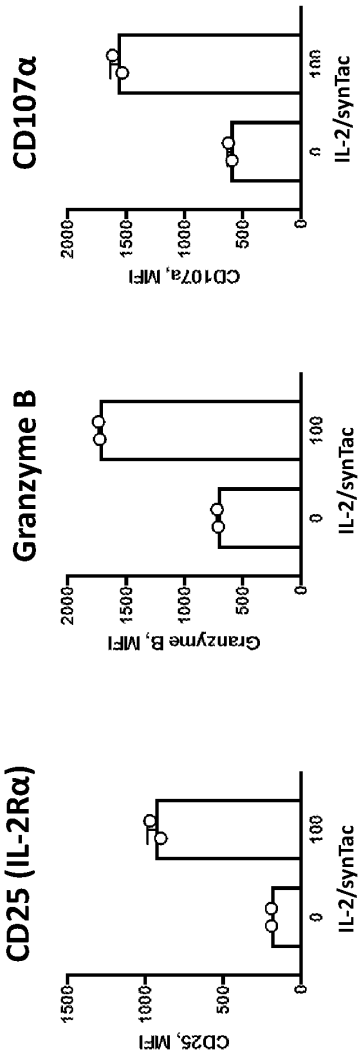

CD107a
CD107a, MFI
2000
1500
1000
500
0
0
100
IL-2/synTac
Granzyme B
Granzyme B, MFI
2000
1500
1000
500
0
0
100
IL-2/synTac
CD25 (IL-2Rα)
CD25, MFI
1500
1000
500
0
0
100
IL-2/synTac

FIG. 39

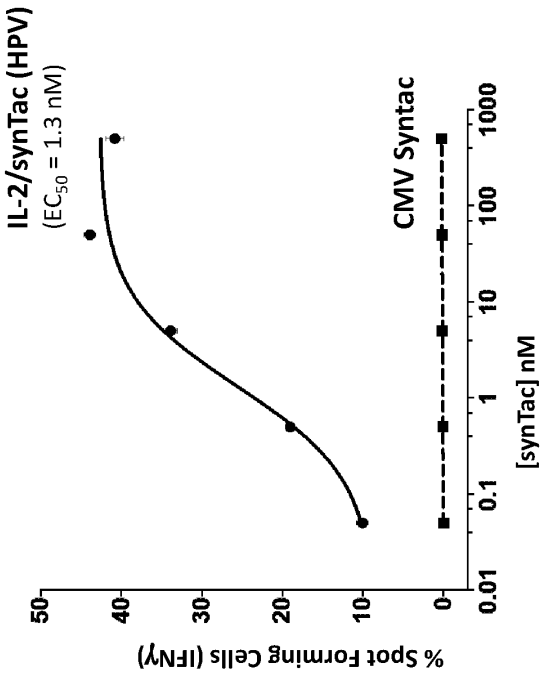

IL-2/synTac (HPV)
(EC50 = 1.3 nM)
CMV Syntac
% Spot Forming Cells (IFNγ)
50
40
30
20
10
0
0.01
0.1
1
10
100
1000
[synTac] nM

FIG. 40