FIG. 1A

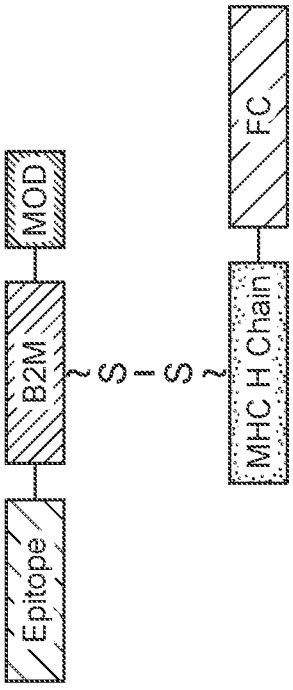



FIG. 1B

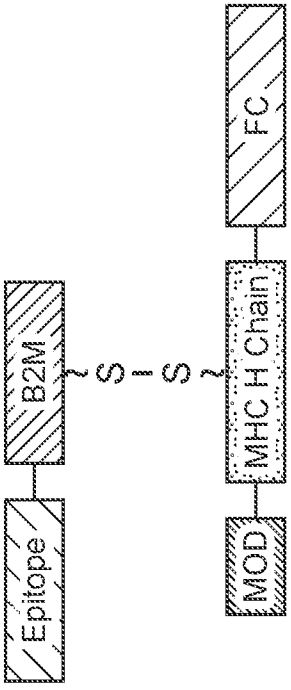



FIG. 1C

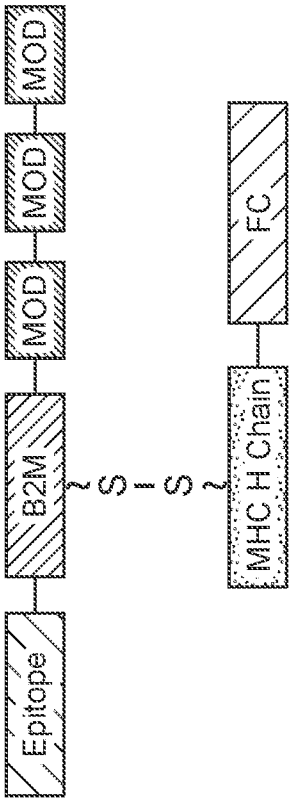



FIG. 1D

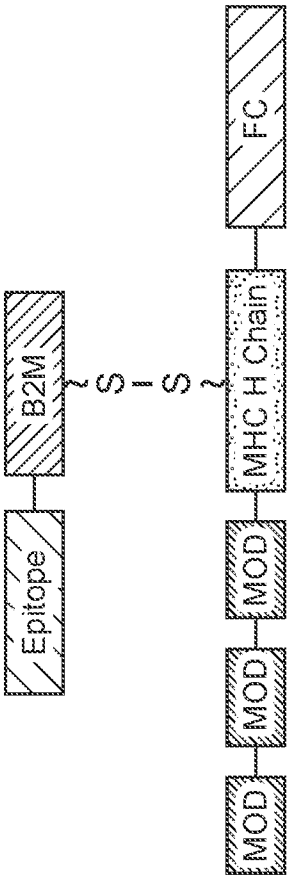


**FIG. 2A**

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 QLQLE**X**LLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFCQSIIS TLT

**FIG. 2F**

IL2 (Y45X) (SEQ ID NO:42)

APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKF**X**MPKKA 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

WITFC**X**SIIS TLT

**FIG. 2H**

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

APTSSSTKKT QLQLE**X**LLLD LQMILNGINN YKNPKLTRML T**X**KFYMPKKA 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 QLQLE**X**LLLD LQMILNGINN YKNPKLTRML T**X**KFYMPKKA TELKHLQCLE

EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE TTFMCEYADE TATIVEFLNR

WITFC**X**SIIS 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**

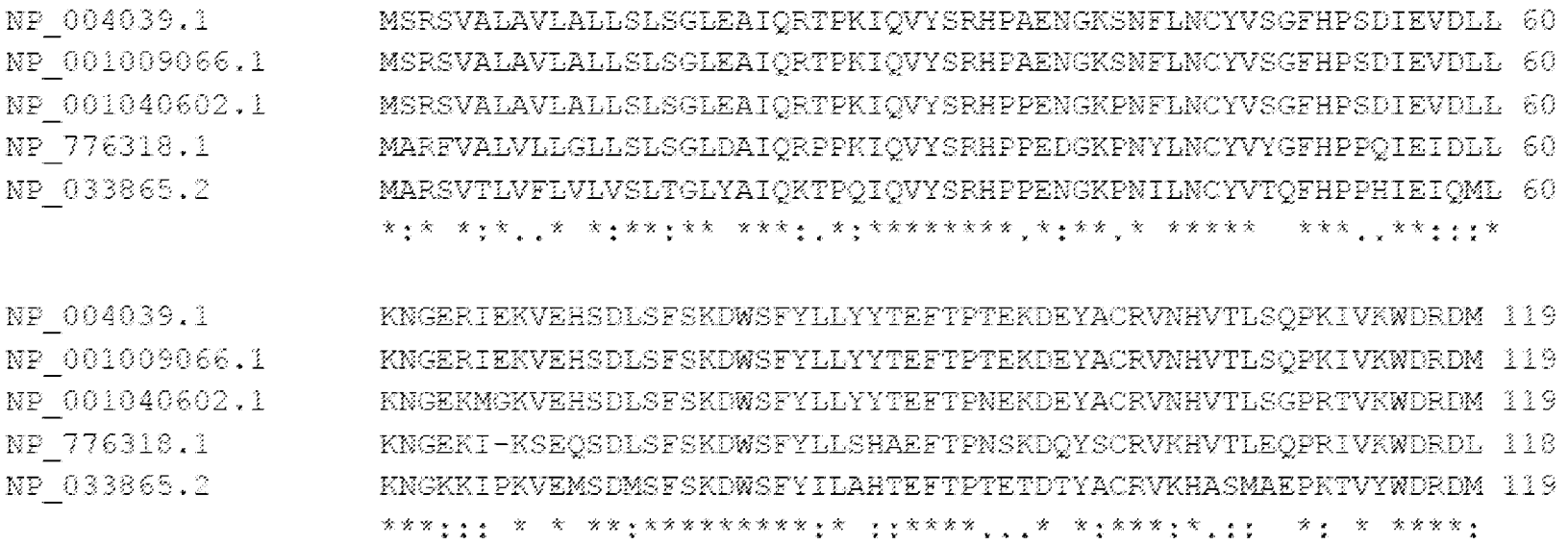

NP_004039.1 MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL 60
NP_001009066.1 MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL 60
NP_001040602.1 MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPPENGKPNFLNCYVSGFHPSDIEVDLL 60
NP_776318.1 MARFVALVLLGLLSLSGLDAIQRPPKIQVYSRHPPEDGKPNYLNCYVYGFHPPQIEIDLL 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**

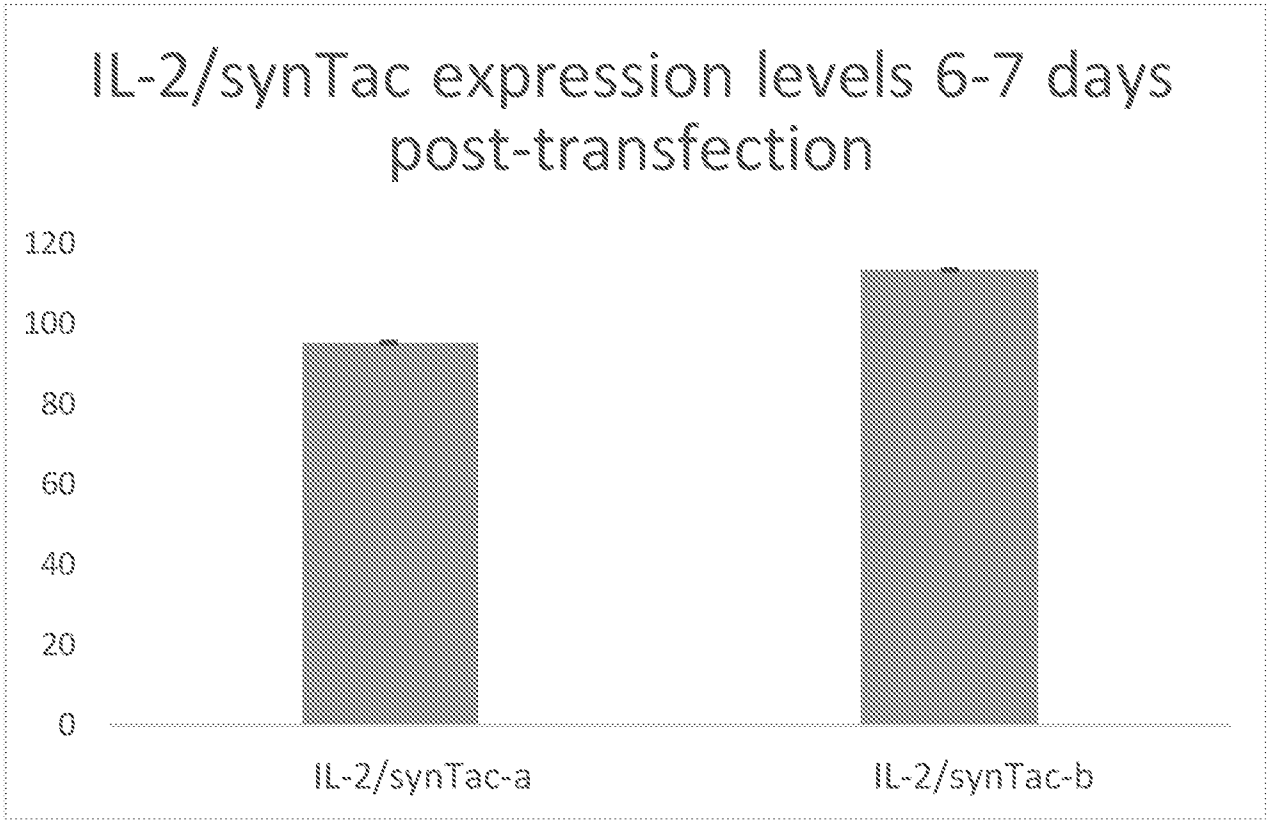

IL-2/synTac expression levels 6-7 days post-transfection
120
100
80
60
40
20
0
IL-2/synTac-a
IL-2/synTac-b

FIG. 7B

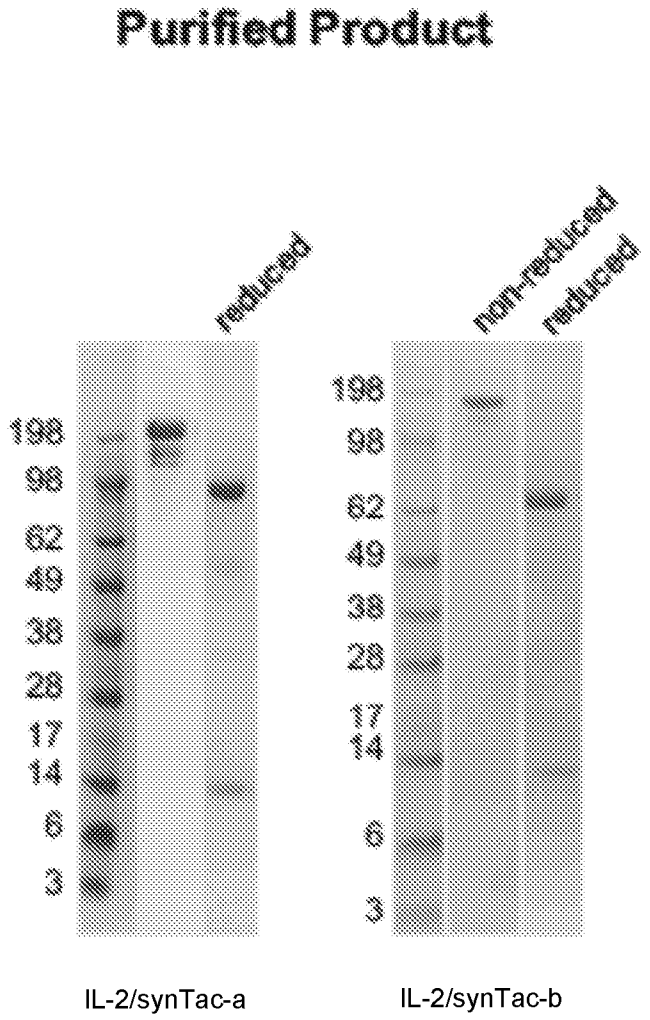

Purified Product
reduced
non-reduced
reduced
198
98
62
49
38
28
17
14
6
3
IL-2/synTac-a
IL-2/synTac-b

**FIG. 8A**

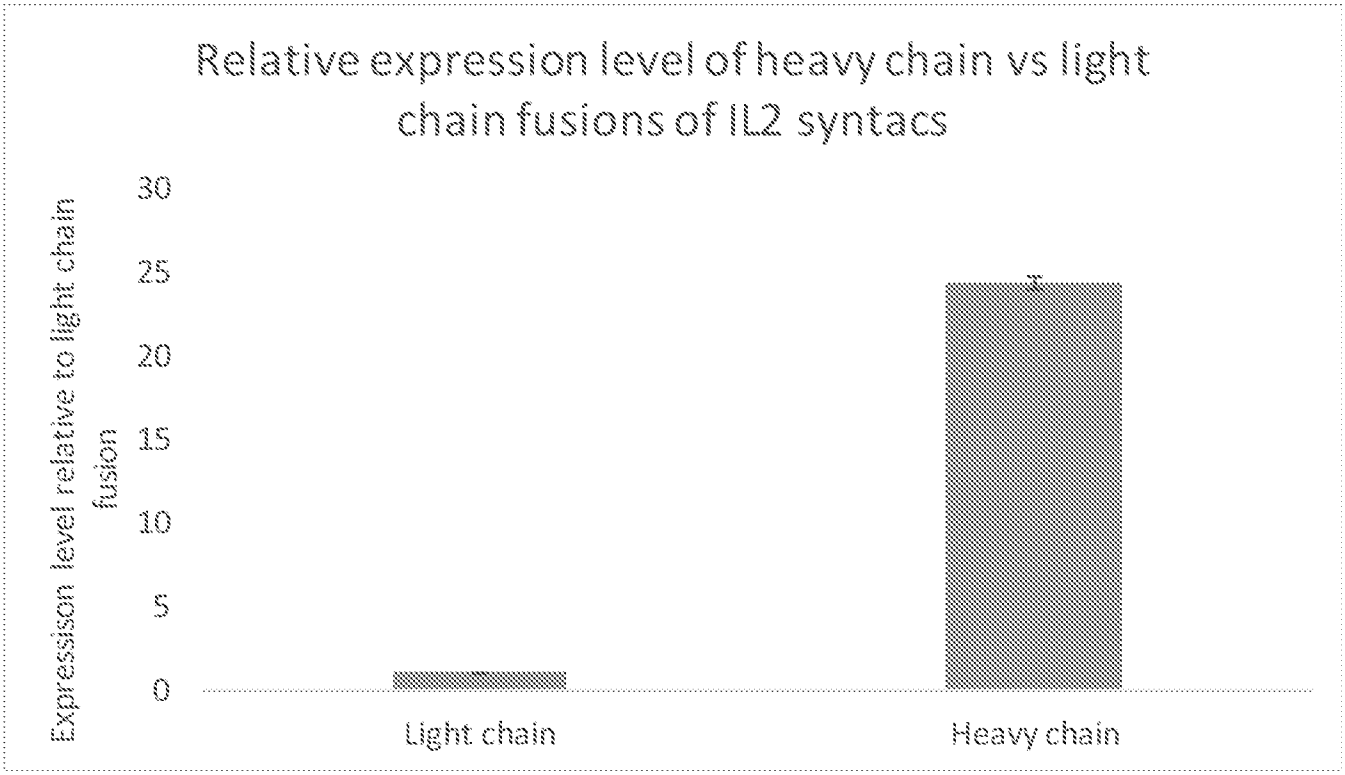

Relative expression level of heavy chain vs light chain fusions of IL2 syntacs
Expressison level relative to light chain fusion
30
25
20
15
10
5
0
Light chain
Heavy chain

FIG. 8B

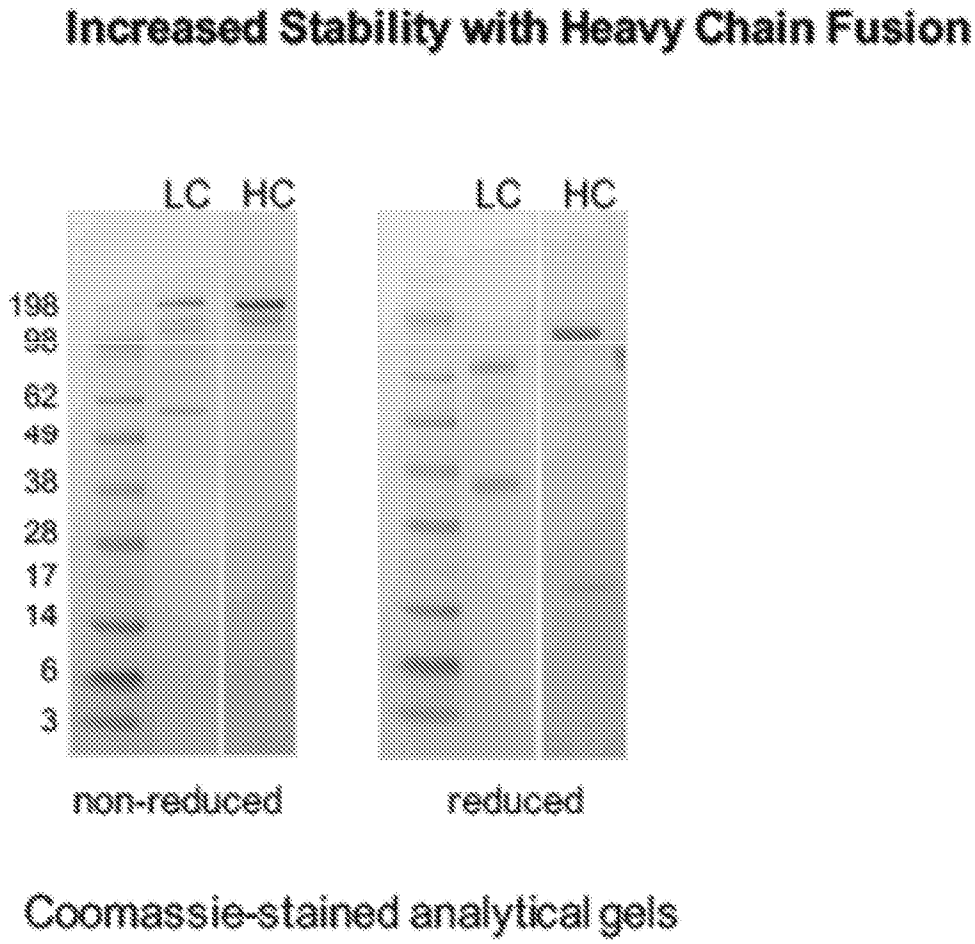

Increased Stability with Heavy Chain Fusion
LC
HC
LC
HC
198
98
62
49
38
28
17
14
6
3
non-reduced
reduced
Coomassie-stained analytical gels

**FIG. 9**

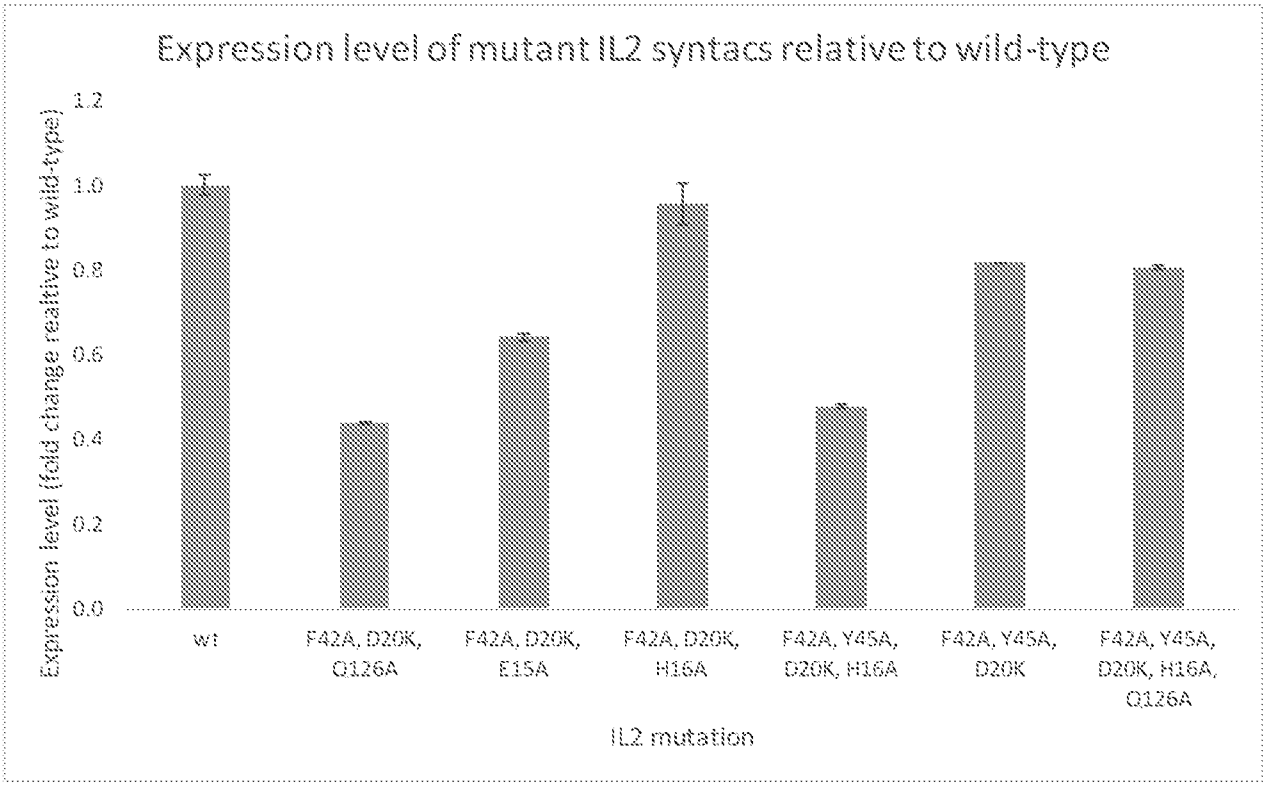

Expression level of mutant IL2 syntacs relative to wild-type
Expression level (fold change realtive to wild-type)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
wt
F42A, D20K, Q126A
F42A, D20K, E15A
F42A, D20K, H16A
F42A, Y45A, D20K, H16A
F42A, Y45A, D20K
F42A, Y45A, D20K, H16A, Q126A
IL2 mutation

**FIG. 10**

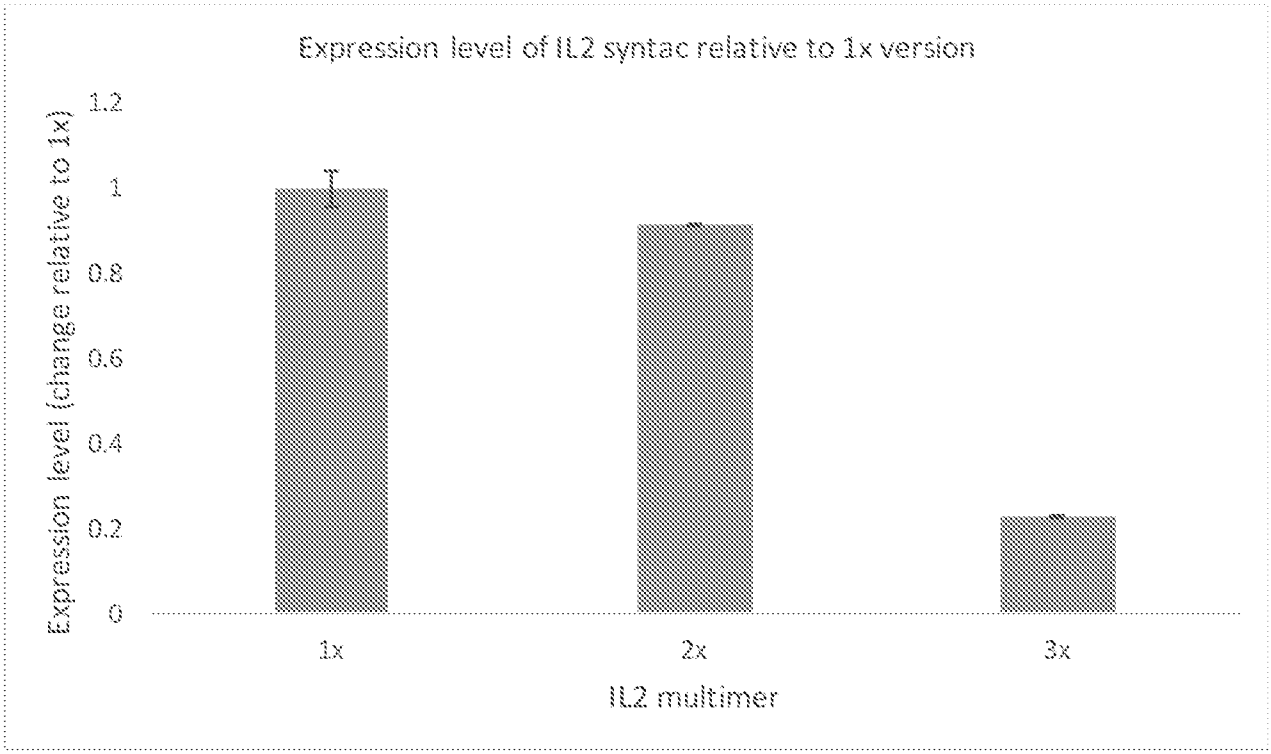

Expression level of IL2 syntac relative to 1x version
Expression level (change relative to 1x)
1.2
1
0.8
0.6
0.4
0.2
0
1x
2x
3x
IL2 multimer

FIG. 11

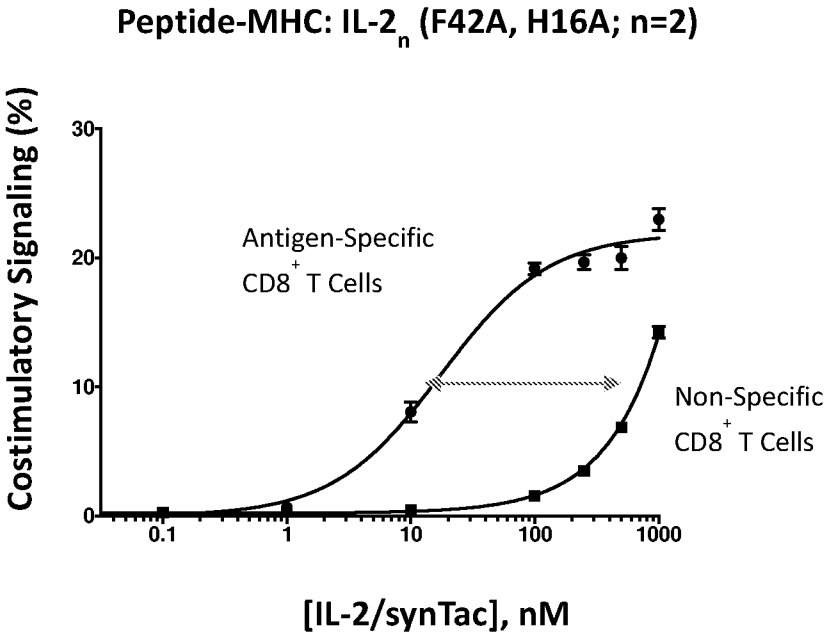

Peptide-MHC: IL-2n (F42A, H16A; n=2)
Costimulatory Signaling (%)
30
20
10
0
0.1
1
10
100
1000
Antigen-Specific
CD8+ T Cells
Non-Specific
CD8+ T Cells
[IL-2/synTac], nM

FIG. 12

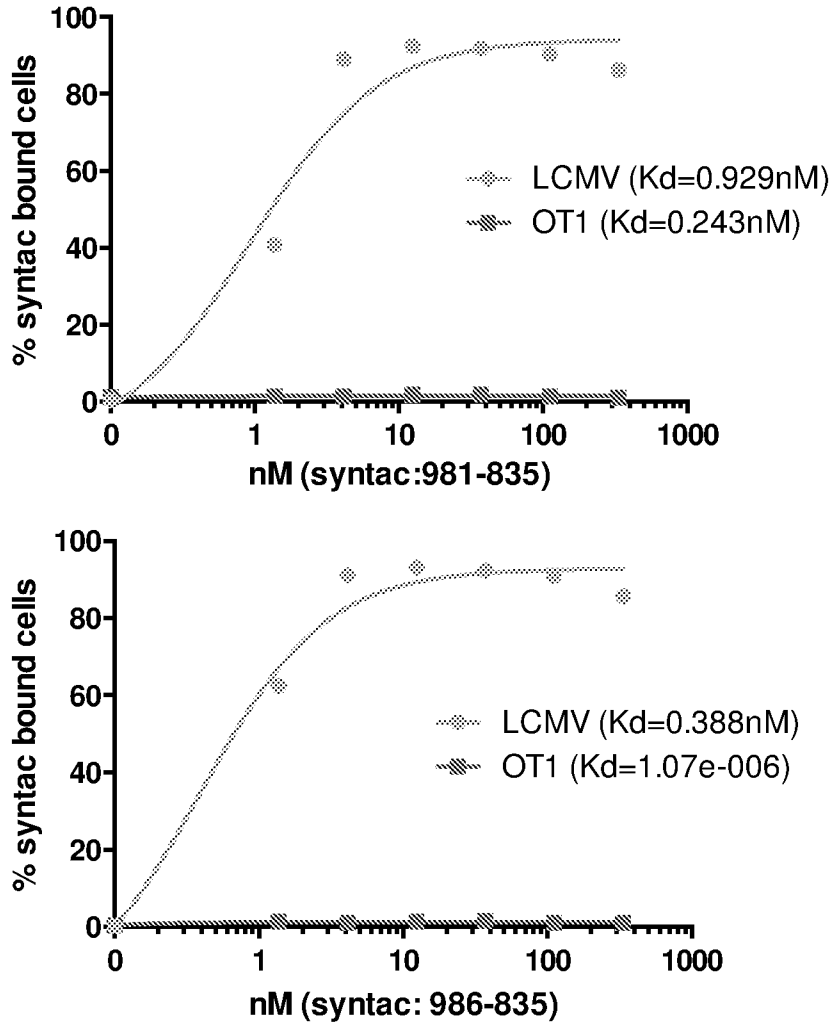

% syntac bound cells
100
80
60
40
20
0
0
1
10
100
1000
nM (syntac:981-835)
LCMV (Kd=0.929nM)
OT1 (Kd=0.243nM)
% syntac bound cells
100
80
60
40
20
0
0
1
10
100
1000
nM (syntac: 986-835)
LCMV (Kd=0.388nM)
OT1 (Kd=1.07e-006)

**FIG. 13**

Number of IL-2 Repeats versus Mutations

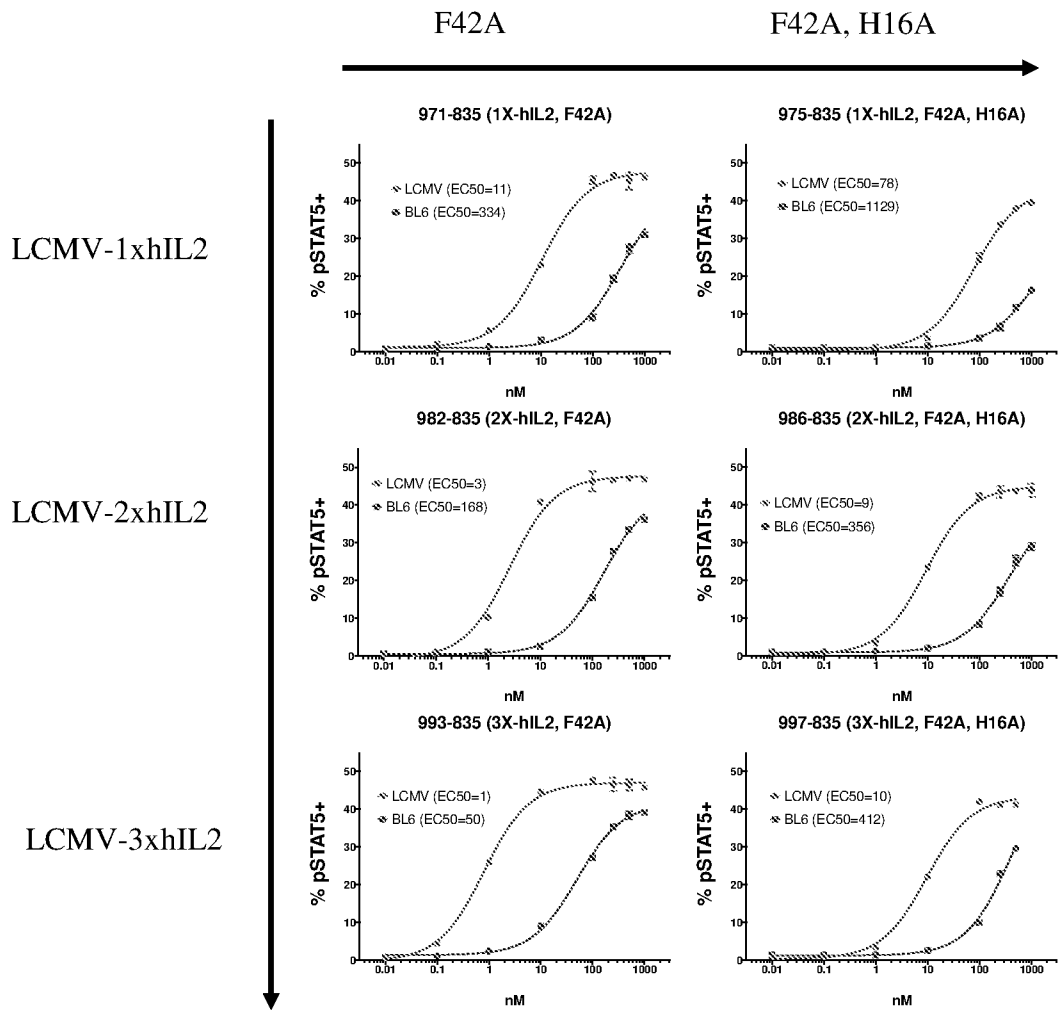
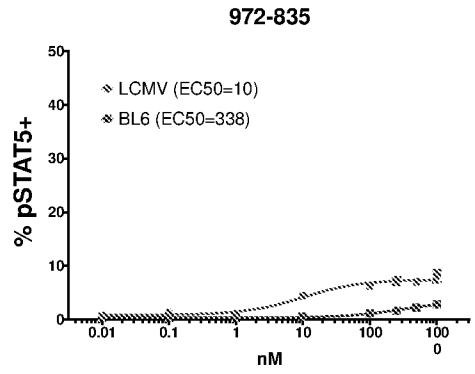


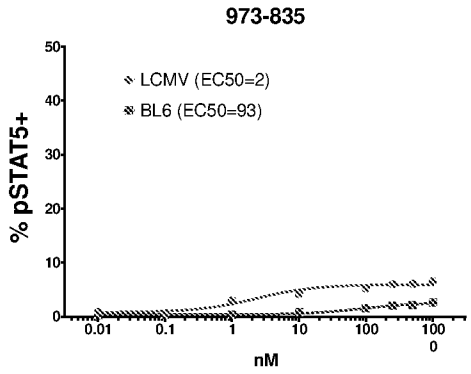
**FIG. 14A**

One copy of IL-2

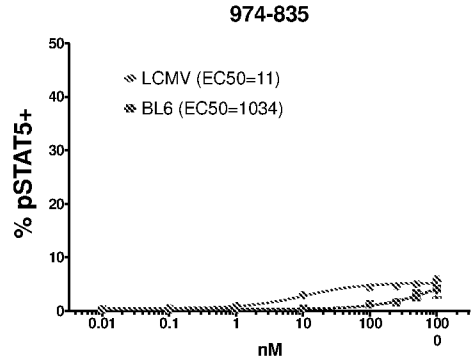
Mutation: F42A, D20K




Mutation: F42A, D20K, Q126A




Mutation: F42A, D20K, E15A




Mutation: F42A, D20K, H16A

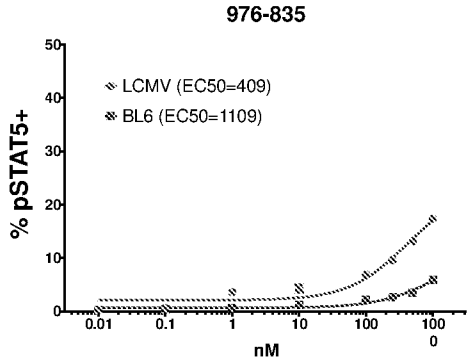
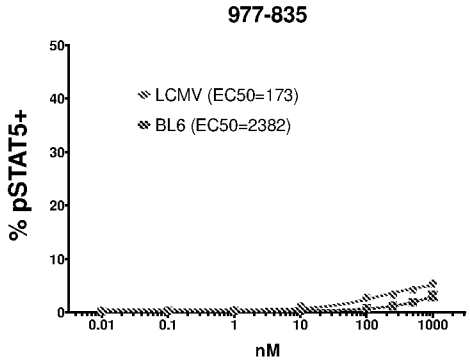


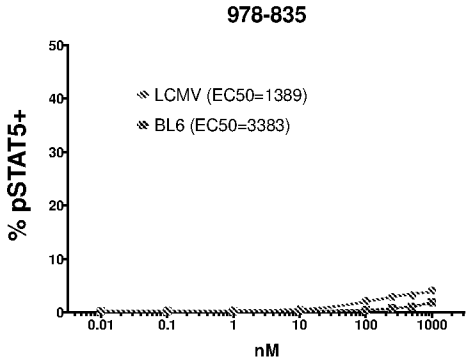
**FIG. 14B**

One copy of IL-2

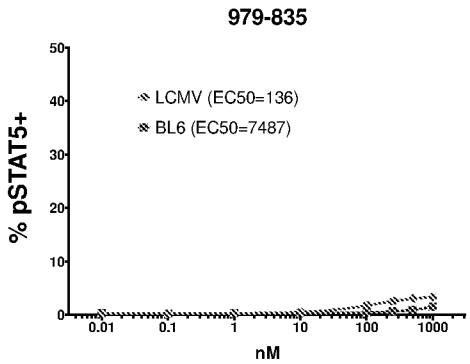
Mutation: F42A, Y45A, D20K, H16A




Mutation: F42A, Y45A, D20K




Mutation: F42A, Y45A, D20K, H16A, Q126A




Mutation: F42A, Y45A, D20K, Q126A

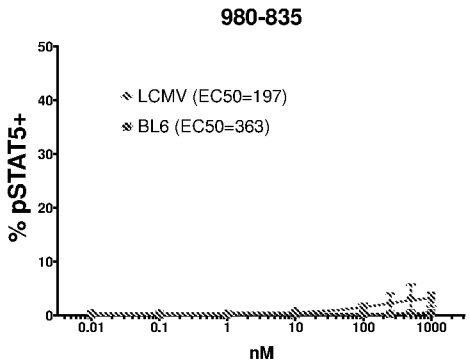
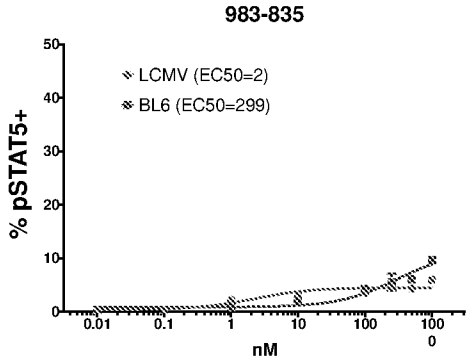


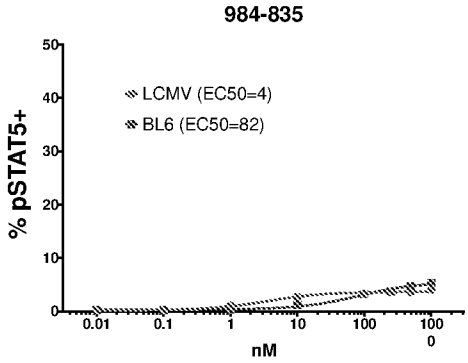
**FIG. 14C**

Two copies of IL-2

Mutation: F42A, D20K




Mutation: F42A, D20K, Q126A




Mutation: F42A, D20K, H16A

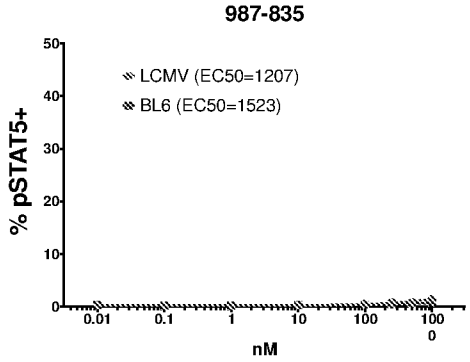
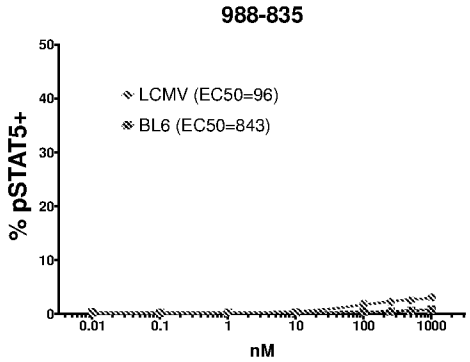


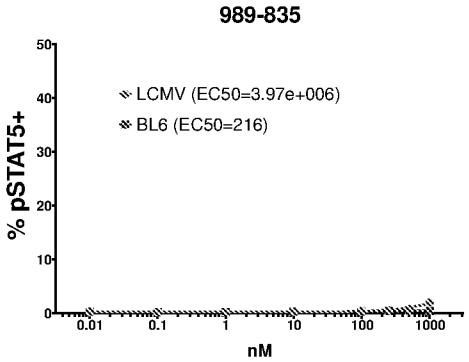
**FIG. 14D**

Two copies of IL-2

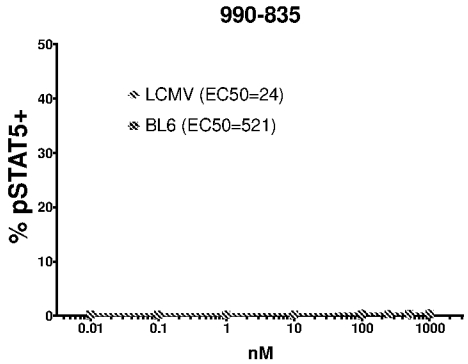
Mutation: F42A, Y45A, D20K, H16A




Mutation: F42A, Y45A, D20K




Mutation: F42A, Y45A, D20K, H16A, Q126A




Mutation: F42A, Y45A, D20K, Q126A

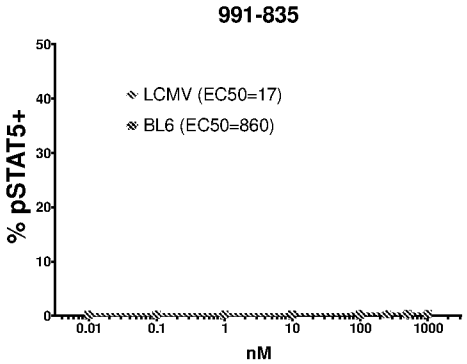
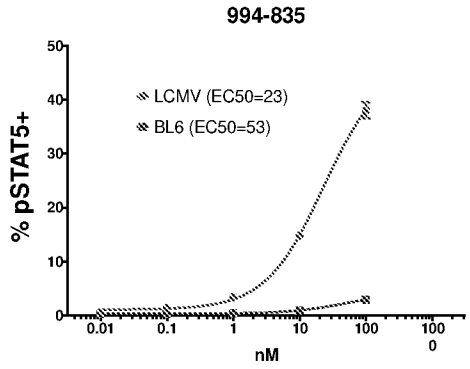


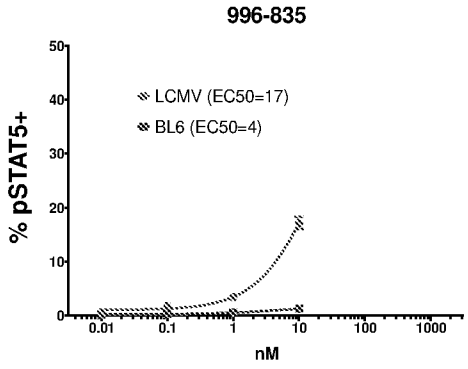
**FIG. 14E**

Three copies of IL-2

Mutation: F42A, D20K




Mutation: F42A, D20K, E15A




Mutation: F42A, D20K, H16A

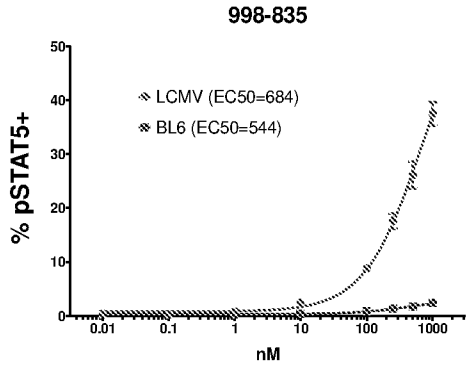
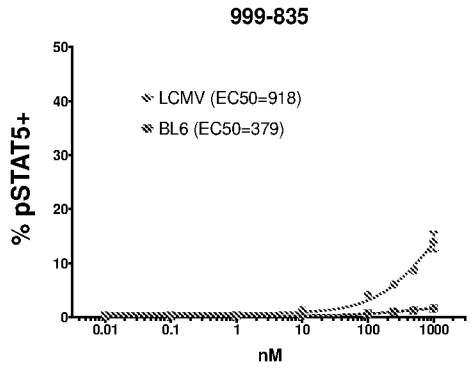


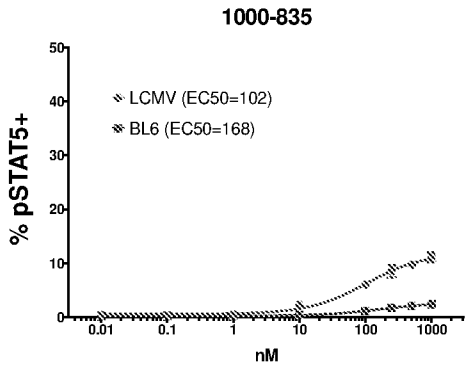
**FIG. 14F**

Three copies of IL-2

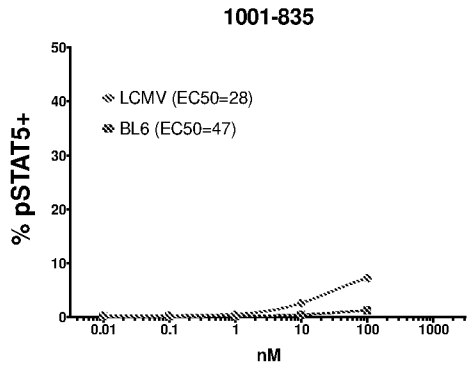
Mutation: F42A, Y45A, D20K, H16A




Mutation: F42A, Y45A, D20K




Mutation: F42A, Y45A, D20K, H16A, Q126A




Mutation: F42A, Y45A, D20K, Q126A

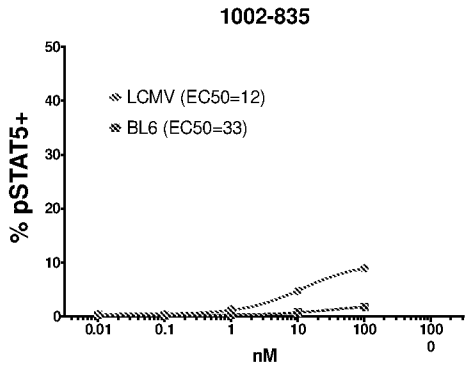


FIG. 15

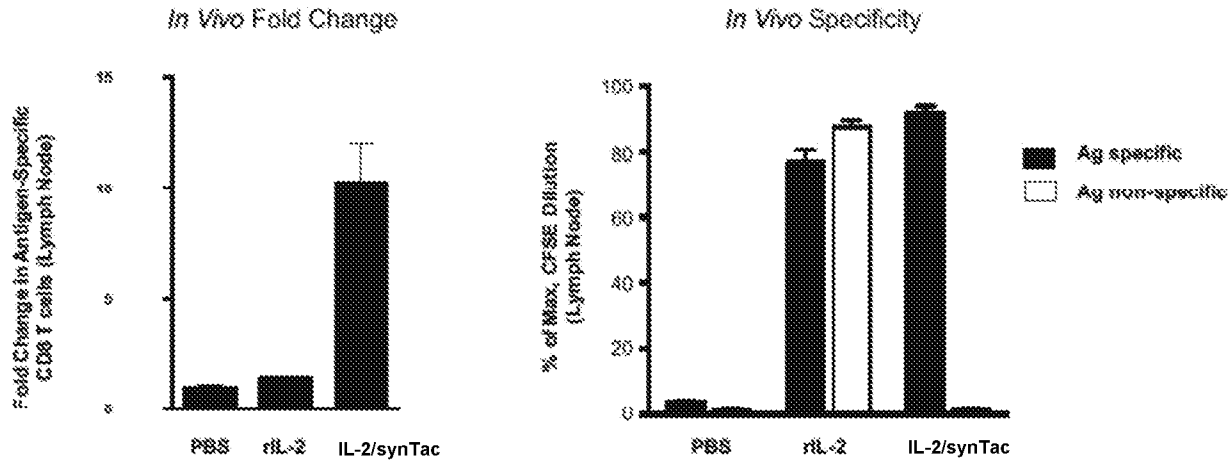

In Vivo Fold Change
Fold Change in Antigen-Specific CD8 T cells (Lymph Node)
PBS
rIL-2
IL-2/synTac
In Vivo Specificity
% of Max, CFSE Dilution (Lymph Node)
100
80
60
40
20
0
PBS
rIL-2
IL-2/synTac
Ag specific
Ag non-specific

FIG. 16A

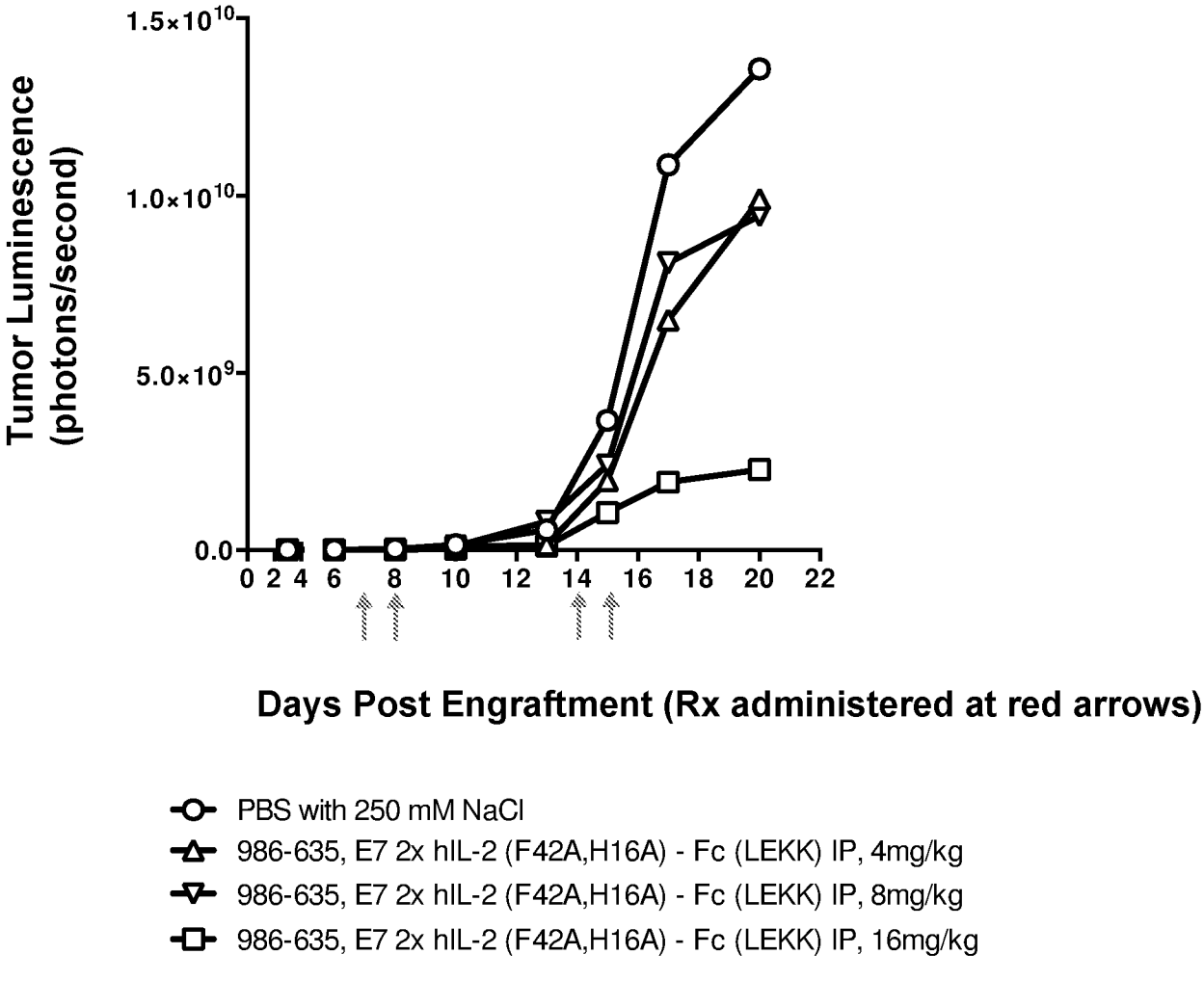

Tumor Luminescence (photons/second)
1.5×10^10
1.0×10^10
5.0×10^9
0.0
0 2 4 6 8 10 12 14 16 18 20 22
Days Post Engraftment (Rx administered at red arrows)
PBS with 250 mM NaCl
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 4mg/kg
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 8mg/kg
986-635, E7 2x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

FIG. 16B

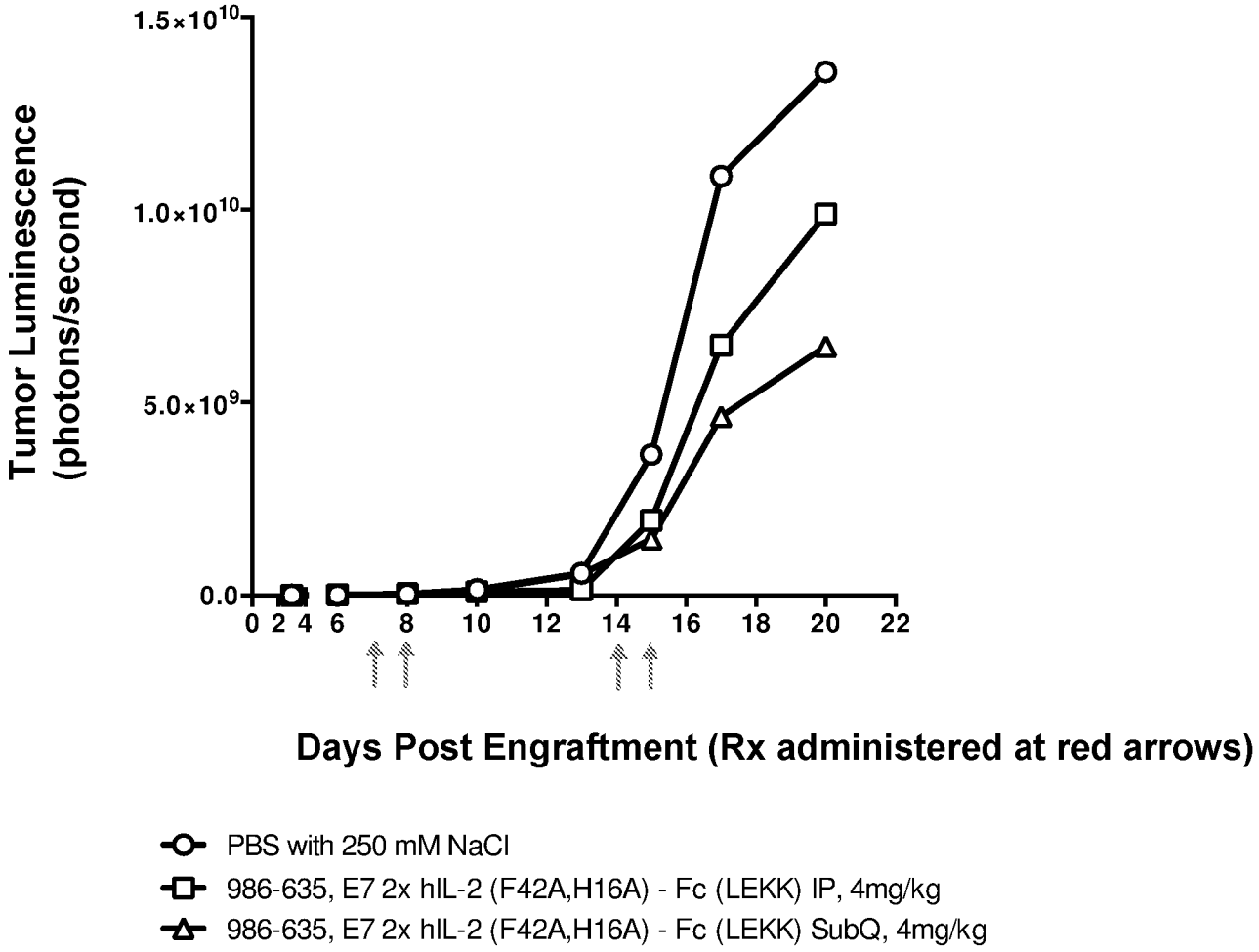

Tumor Luminescence (photons/second)
1.5×10^10
1.0×10^10
5.0×10^9
0.0
0 2 4 6 8 10 12 14 16 18 20 22
Days Post Engraftment (Rx administered at red arrows)
PBS with 250 mM NaCl
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 4mg/kg
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) SubQ, 4mg/kg

FIG. 17A

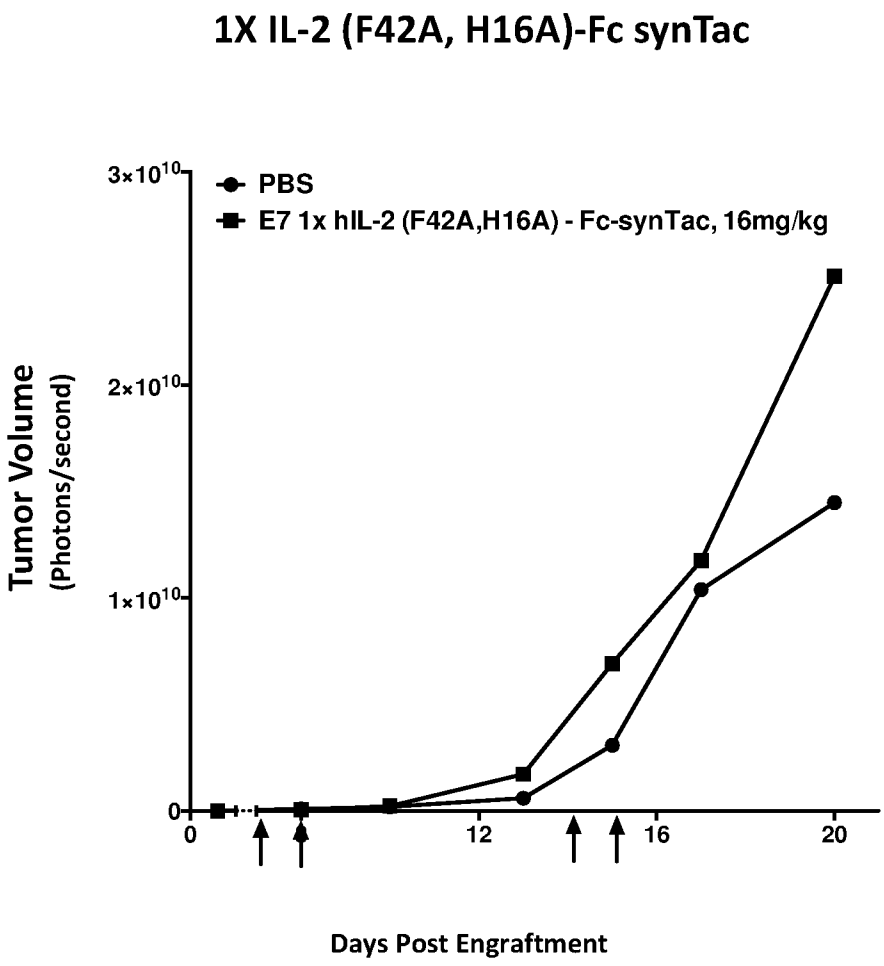

1X IL-2 (F42A, H16A)-Fc synTac
PBS
E7 1x hIL-2 (F42A,H16A) - Fc-synTac, 16mg/kg
Tumor Volume
(Photons/second)
3×10^10
2×10^10
1×10^10
0
0
12
16
20
Days Post Engraftment

FIG. 17B

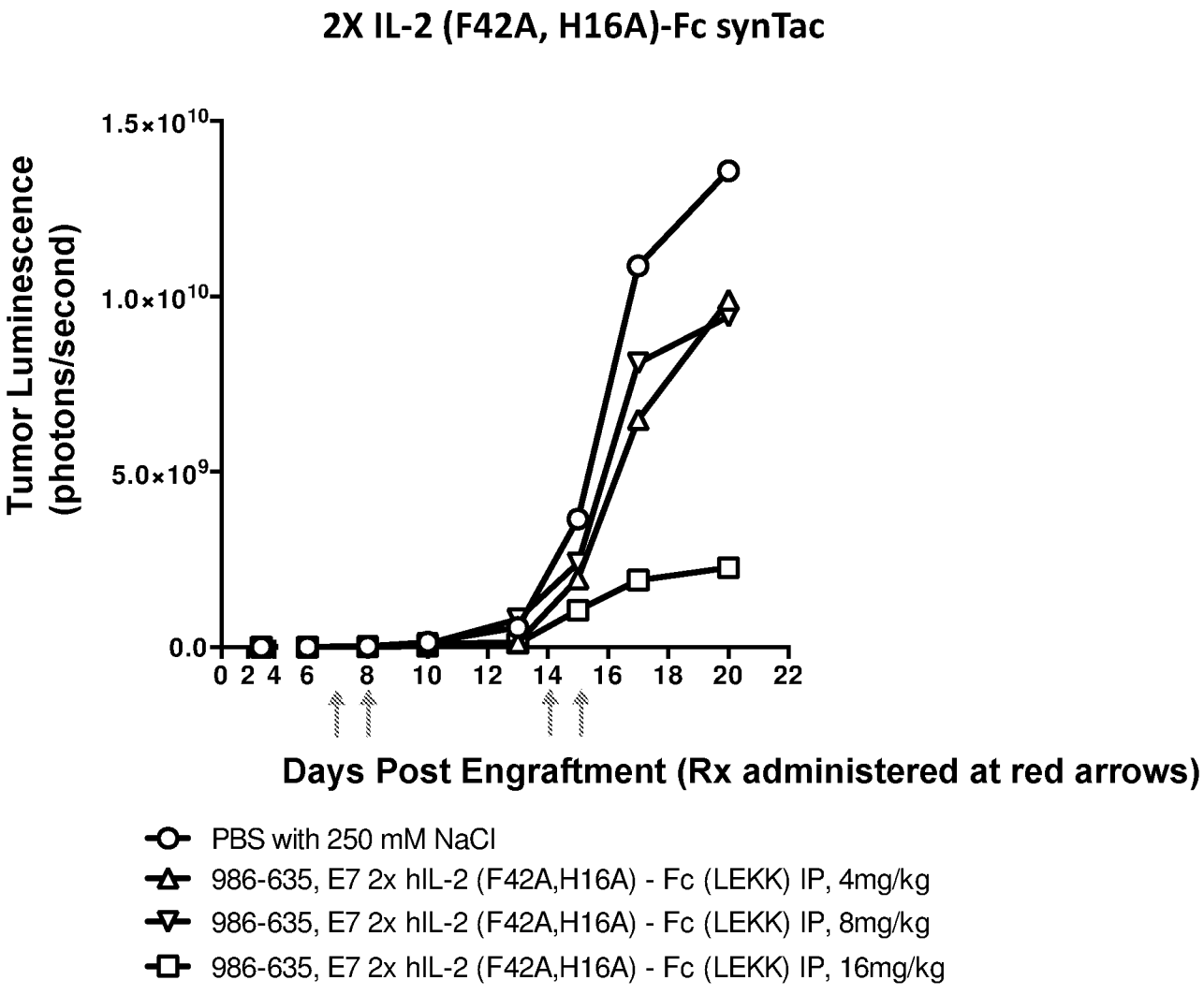

2X IL-2 (F42A, H16A)-Fc synTac
Tumor Luminescence (photons/second)
1.5×10^10
1.0×10^10
5.0×10^9
0.0
0 2 4 6 8 10 12 14 16 18 20 22
Days Post Engraftment (Rx administered at red arrows)
PBS with 250 mM NaCl
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK) IP, 4mg/kg
986-635, E7 2x hIL-2 (F42A,H16A) - Fc (LEKK)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

FIG. 18

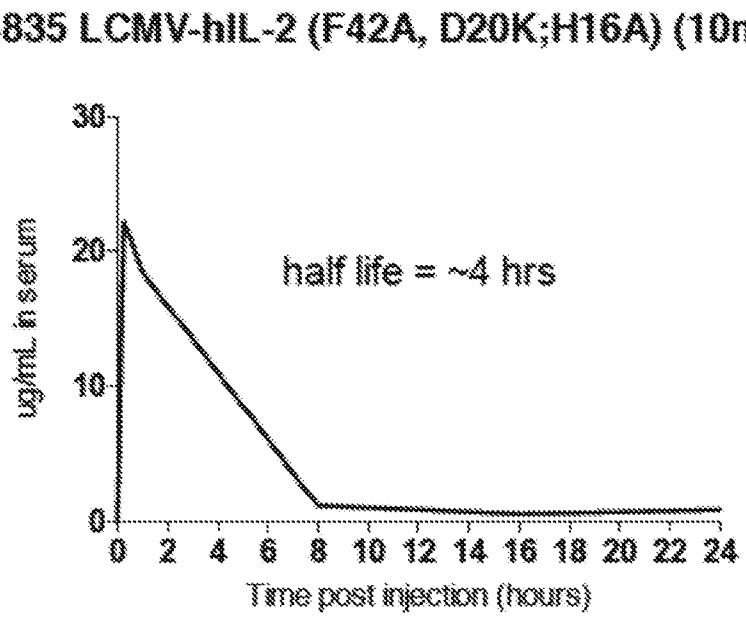
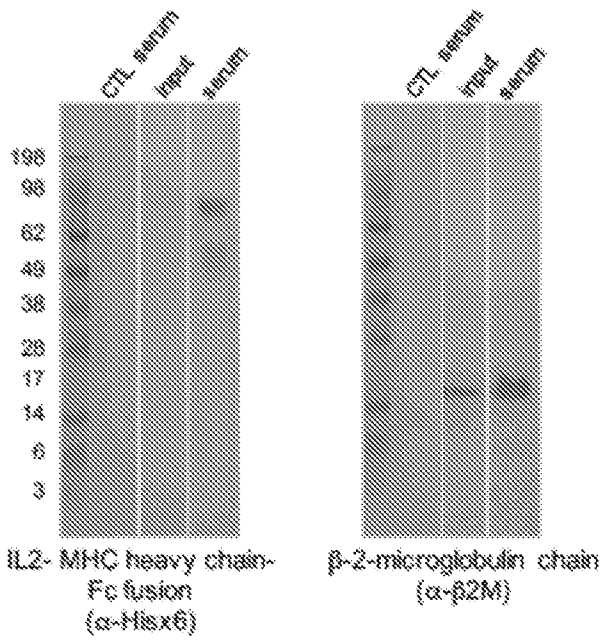

976-835 LCMV-hIL-2 (F42A, D20K;H16A) (10mg/kg, IP)
ug/mL in serum
30
20
10
0
half life = ~4 hrs
0 2 4 6 8 10 12 14 16 18 20 22 24
Time post injection (hours)

**FIG. 19**




Western blot analysis

**FIG. 20**

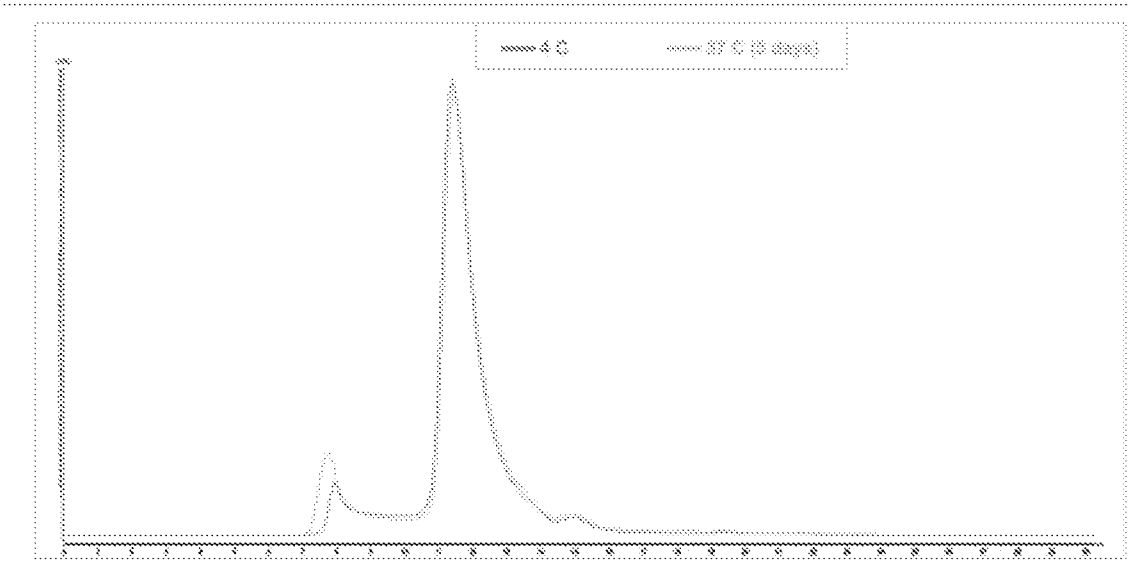


**FIG. 21**

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

*MYRMQLLSCIALSLALVTNS***APTSSSTKKTQLQLEALLLDLQMILNGINNYKNPKLTRML**
**TAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE**
**TTFMCEYADETATIVEFLNRWITFCQSIISTLT**GGGGSGGGGSGGGGSGGGGS**APTSSST**
**KKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLE**
**EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQS**
**IISTLT**GGGGSGGGGSGGGGSGGGGSGS**HSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFV**
**RFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**AYNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAE
QLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRW
EAAAGG**DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK**
**FNWYVDGVEVHNAKTKPREEQYA**STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
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**
**TTFMCEYADETATIVEFLNRWITFCQSIISTLT**GGGGSGGGGSGGGGSGGGGS**APTSSST**
**KKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLE**
**EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQS**
**IISTLT**GGGGSGGGGSGGGGSGGGGSGS**HSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFV**
**RFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**AYNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAE
QLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRW
EAAAGG **DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK**
**FNWYVDGVEVHNAKTKPREEQYA**STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
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**
**AAGTTCTACAAAGAAAACACAGCTACAACTGGAG**GCA**TTACTGCTGGATTTACAGATGATTTTGAATG**
**GAATTAATAATTACAAGAATCCCAAACTCACCAGGATGCTCACA**GCA**AAGTTTTACATGCCCAAGAAG**
**GCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTT**
**AGCTCAAAGCAAAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGA**
**ACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTC**
**TGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTGACT**GGAGGCGGAGGATCTGGTGGTG
GAGGTTCTGGTGGTGGGGGATCTGGAGGCGGAGGATCT**GCACCTACTTCAAGTTCTACAAAGAAAACA**
**CAGCTACAACTGGAG**GCA**TTACTGCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAAT**
**CCCAAACTCACCAGGATGCTCACA**GCA**AAGTTTTACATGCCCAAGAAGGCCACAGAACTGAAACATCTT**
**CAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCA**
**CTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAA**
**CATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGAACAGATGGATTACCTTTT**
**GTCAAAGCATCATCTCAACACTGACT**GGAGGCGGAGGATCTGGTGGTGGAGGTTCTGGTGGTGGGGGA
TCTGGAGGCGGAGGATCTGGCTCTCACTCCATGAGGTATTTCTTCACATCCGTGTCCCGGCCCGGCCGCG
GGGAGCCCCGCTTCATCGCAGTGGGCTACGTGGACGACACGCAGTTCGTGCGGTTCGACAGCGACGCCG
CGAGCCAGAGGATGGAGCCGCGGGCGCCGTGGATAGAGCAGGAGGGTCCGGAGTATTGGGACGGGGA
GACACGGAAAGTGAAGGCCCACTCACAGACTCACCGAGTGGACCTGGGGACCCTGCGCGGC**GCC**TACA
ACCAGAGCGAGGCCGGTTCTCACACCGTCCAGAGGATGTATGGCTGCGACGTGGGGTCGGACTGGCGC
TTCCTCCGCGGGTACCACCAGTACGCCTACGACGGCAAGGATTACATCGCCCTGAAAGAGGACCTGCGCT
CTTGGACCGCGGCGGACATGGCAGCTCAGACCACCAAGCACAAGTGGGAGGCGGCCCATGTGGCGGAG
CAGTTGAGAGCCTACCTGGAGGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAGGA
GACGCTGCAGCGCACGGACGCCCCCAAAACGCATATGACTCACCACGCTGTCTCTGACCATGAAGCCACC
CTGAGGTGCTGGGCCCTGAGCTTCTACCCTGCGGAGATCACACTGACCTGGCAGCGGGATGGGGAGGA
CCAGACCCAGGACACGGAGCTCGTGGAGACCAGGCCT**TGC**GGGGATGGAACCTTCCAGAAGTGGGCGG
CTGTGGTGGTGCCTTCTGGACAGGAGCAGAGATACACCTGCCATGTGCAGCATGAGGGTTTGCCCAAGC
CCCTCACCCTGAGATGGGAGGCAGCTGCGGGTGGC**GACAAAACTCACACATGCCCACCGTGCCCAGCA**
**CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC**
**GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG**
**GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC**GCA**AGCAC**
**GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA**
**AGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA**
**GAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTG**
**CCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC**
**AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG**
**ACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCAC**
**TACACGCAGAAGTCCCTCTCCCTGTCTCCGGGTAAA**TAGTGA

**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**
**TTFMCEYADETATIVEFLNRWITFCQSIISTLT**GGGGSGGGGSGGGGSGGGGS**APTSSST**
**KKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLE**
**EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQS**
**IISTLT**GGGGSGGGGSGGGGSGGGGSGSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFV
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
TTFMCEYADETATIVEFLNRWITFCQSIISTLTGGGGSGGGGSGGGGSGGGGSGGGGSAPTSSST
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**
**AAGTTCTACAAAGAAAACACAGCTACAACTGGAG**GCA**TTACTGCTGGATTTACAGATGATTTTGAATG**
**GAATTAATAATTACAAGAATCCCAAACTCACCAGGATGCTCACA**GCA**AAGTTTTACATGCCCAAGAAG**
**GCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTT**
**AGCTCAAAGCAAAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGA**
**ACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTC**
**TGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTGACT**GGAGGCGGAGGATCTGGTGGTG
GAGGTTCTGGTGGTGGGGGATCTGGAGGCGGAGGATCT**GCACCTACTTCAAGTTCTACAAAGAAAACA**
**CAGCTACAACTGGAG**GCA**TTACTGCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAAT**
**CCCAAACTCACCAGGATGCTCACA**GCA**AAGTTTTACATGCCCAAGAAGGCCACAGAACTGAAACATCTT**
**CAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCA**
**CTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAA**
**CATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGAACAGATGGATTACCTTTT**
**GTCAAAGCATCATCTCAACACTGACT**GGAGGCGGAGGATCTGGTGGTGGAGGTTCTGGTGGTGGGGGA
TCTGGAGGCGGAGGATCTGGCTCTCACTCCATGAGGTATTTCTTCACATCCGTGTCCCGGCCCGGCCGCG
GGGAGCCCCGCTTCATCGCAGTGGGCTACGTGGACGACACGCAGTTCGTGCGGTTCGACAGCGACGCCG
CGAGCCAGAGGATGGAGCCGCGGGCGCCGTGGATAGAGCAGGAGGGTCCGGAGTATTGGGACGGGGA
GACACGGAAAGTGAAGGCCCACTCACAGACTCACCGAGTGGACCTGGGGACCCTGCGCGGC**GCC**TACA
ACCAGAGCGAGGCCGGTTCTCACACCGTCCAGAGGATGTATGGCTGCGACGTGGGGTCGGACTGGCGC
TTCCTCCGCGGGTACCACCAGTACGCCTACGACGGCAAGGATTACATCGCCCTGAAAGAGGACCTGCGCT
CTTGGACCGCGGCGGACATGGCAGCTCAGACCACCAAGCACAAGTGGGAGGCGGCCCATGTGGCGGAG
CAGTTGAGAGCCTACCTGGAGGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAGGA
GACGCTGCAGCGCACGGACGCCCCCAAAACGCATATGACTCACCACGCTGTCTCTGACCATGAAGCCACC
CTGAGGTGCTGGGCCCTGAGCTTCTACCCTGCGGAGATCACACTGACCTGGCAGCGGGATGGGGAGGA
CCAGACCCAGGACACGGAGCTCGTGGAGACCAGGCCT**TGC**GGGGATGGAACCTTCCAGAAGTGGGCGG
CTGTGGTGGTGCCTTCTGGACAGGAGCAGAGATACACCTGCCATGTGCAGCATGAGGGTTTGCCCAAGC
CCCTCACCCTGAGATGGGAGGCAGCTGCGGGTGGC**GACAAAACTCACACATGCCCACCGTGCCCAGCA**
**CCTGAA**GCCGCC**GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC**
**GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG**
**GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC**AAC**AGCAC**
**GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA**
**AGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA**
**GAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTG**
**CCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC**
**AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG**
**ACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCAC**
**TACACGCAGAAGTCCCTCTCCCTGTCTCCGGGTAAA**TAGTGA

**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**
**TTFMCEYADETATIVEFLNRWITFCQSIISTLT**GGGGSGGGGSGGGGSGGGGS**APTSSST**
**KKTQLQLEALLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLE**
**EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQS**
**IISTLT**GGGGSGGGGSGGGGSGGGGSGSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFV
RFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRG**A**YNQSEAGSHT
VQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAE
QLRAYLEGTCVEWLRRYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFYPAEIT
LTWQRDGEDQTQDTELVETRP**C**GDGTFQKWAAVVVPSGQEQRYTCHVQHEGLPKPLTLRW
EAAAGG**DKTHTCPPCPAPE**FEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
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**
**AAGTTCTACAAAGAAAACACAGCTACAACTGGAG**GCA**TTACTGCTGGATTTACAGATGATTTTGAATG**
**GAATTAATAATTACAAGAATCCCAAACTCACCAGGATGCTCACA**GCA**AAGTTTTACATGCCCAAGAAG**
**GCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTT**
**AGCTCAAAGCAAAAACTTTCACTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGA**
**ACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTC**
**TGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTGACT**GGAGGCGGAGGATCTGGTGGTG
GAGGTTCTGGTGGTGGGGGATCTGGAGGCGGAGGATCT**GCACCTACTTCAAGTTCTACAAAGAAAACA**
**CAGCTACAACTGGAG**GCA**TTACTGCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAAT**
**CCCAAACTCACCAGGATGCTCACA**GCA**AAGTTTTACATGCCCAAGAAGGCCACAGAACTGAAACATCTT**
**CAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCA**
**CTTAAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAA**
**CATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGAACAGATGGATTACCTTTT**
**GTCAAAGCATCATCTCAACACTGACT**GGAGGCGGAGGATCTGGTGGTGGAGGTTCTGGTGGTGGGGGA
TCTGGAGGCGGAGGATCTGGCTCTCACTCCATGAGGTATTTCTTCACATCCGTGTCCCGGCCCGGCCGCG
GGGAGCCCCGCTTCATCGCAGTGGGCTACGTGGACGACACGCAGTTCGTGCGGTTCGACAGCGACGCCG
CGAGCCAGAGGATGGAGCCGCGGGCGCCGTGGATAGAGCAGGAGGGTCCGGAGTATTGGGACGGGGA
GACACGGAAAGTGAAGGCCCACTCACAGACTCACCGAGTGGACCTGGGGACCCTGCGCGGC**GCC**TACA
ACCAGAGCGAGGCCGGTTCTCACACCGTCCAGAGGATGTATGGCTGCGACGTGGGGTCGGACTGGCGC
TTCCTCCGCGGGTACCACCAGTACGCCTACGACGGCAAGGATTACATCGCCCTGAAAGAGGACCTGCGCT
CTTGGACCGCGGCGGACATGGCAGCTCAGACCACCAAGCACAAGTGGGAGGCGGCCCATGTGGCGGAG
CAGTTGAGAGCCTACCTGGAGGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGGAGAACGGGAAGGA
GACGCTGCAGCGCACGGACGCCCCCAAAACGCATATGACTCACCACGCTGTCTCTGACCATGAAGCCACC
CTGAGGTGCTGGGCCCTGAGCTTCTACCCTGCGGAGATCACACTGACCTGGCAGCGGGATGGGGAGGA
CCAGACCCAGGACACGGAGCTCGTGGAGACCAGGCCT**TGC**GGGGATGGAACCTTCCAGAAGTGGGCGG
CTGTGGTGGTGCCTTCTGGACAGGAGCAGAGATACACCTGCCATGTGCAGCATGAGGGTTTGCCCAAGC
CCCTCACCCTGAGATGGGAGGCAGCTGCGGGTGGC**GACAAAACTCACACATGCCCACCGTGCCCAGCA**
**CCTGAA**TTCGAG**GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC**
**GGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG**
**GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC**AAC**AGCAC**
**GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA**
**AGGTCTCCAACAAAGCCCTCCCAGCC**AGC**ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA**
**GAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTG**
**CCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC**
**AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG**
**ACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCAC**
**TACACGCAGAAGTCCCTCTCCCTGTCTCCGGGTAAA**TAGTGA

**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**
**TTTGCAGCCCGAAACGACG**GGTGGAGGTGGTTCTGGAGGAGGCGGTTCGGGCGGAGGTGGTAGTATC
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

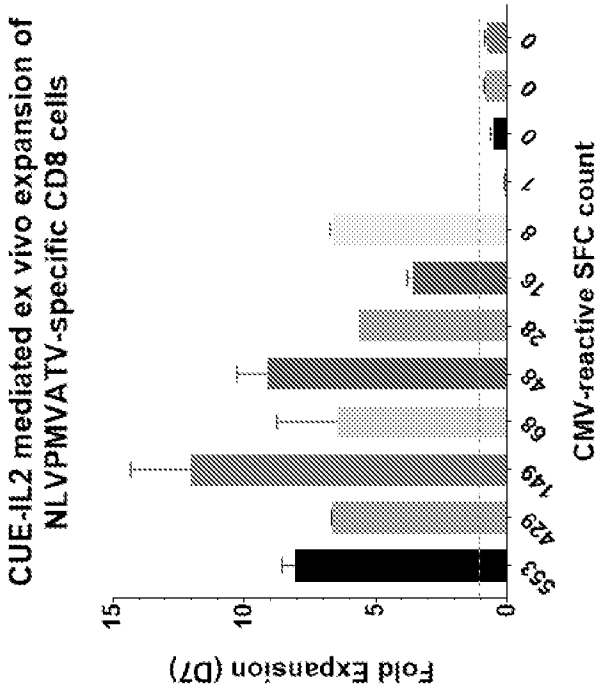

CUE-IL2 mediated ex vivo expansion of NLVPMVATV-specific CD8 cells
Fold Expansion (D7)
15
10
5
0
553
429
149
69
49
29
15
8
7
0
0
0
CMV-reactive SFC count

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 | 0.6365 | 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 | 19.6 | | | 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 | 70.96 | >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 |

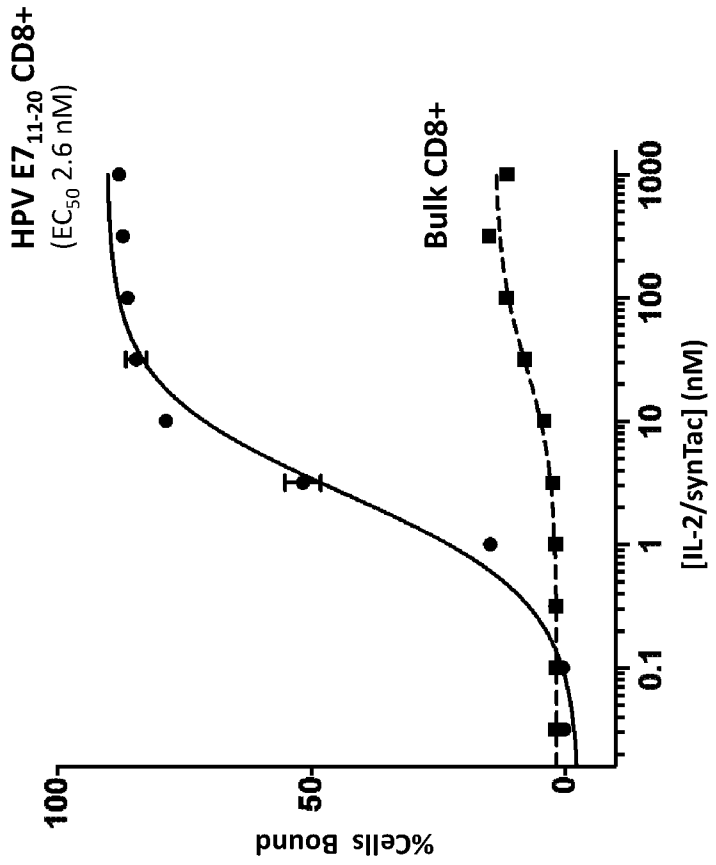

HPV E7 11-20 CD8+
(EC50 2.6 nM)
Bulk CD8+
%Cells Bound
100
50
0
0.1
1
10
100
1000
[IL-2/synTac] (nM)

FIG. 37

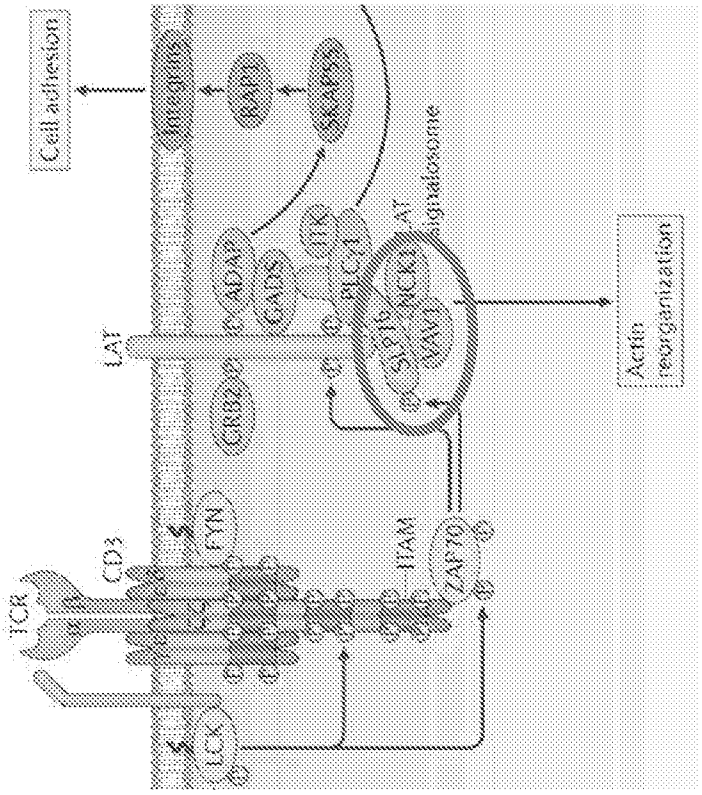
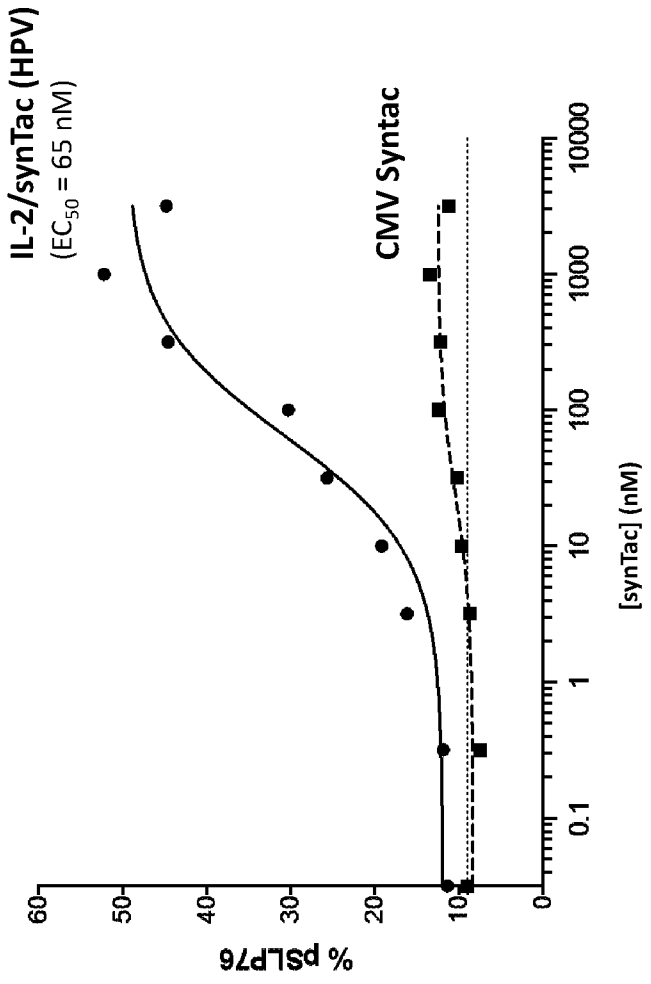

Cell adhesion
Integrins
RAP1
GRAP58
LAT
ADAP
GADS
ITK
PLCγ1
SLP76
NCK1
WAS
Signalosome
Actin reorganization
CRKL
FYN
TCR
CD3
LCK
HAM
ZAP70

FIG. 38


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

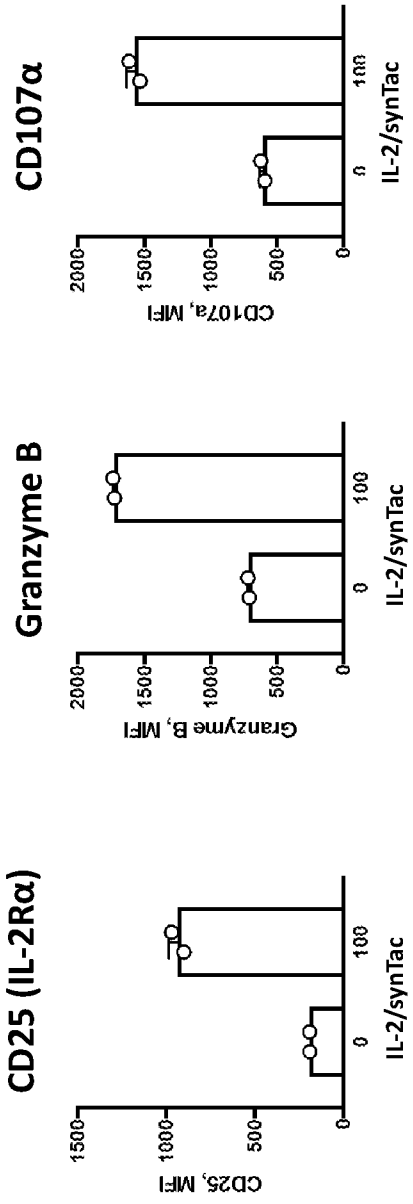

CD107α
CD107a, MFI
2000
1500
1000
500
0
0
100
IL-2/synTac
Granzyme B
Granzyme B, MFI
2000
1500
1000
500
0
0
100
IL-2/synTac
CD25 (IL-2Rα)
CD25, MFI
1500
1000
500
0
0
100
IL-2/synTac

FIG. 39

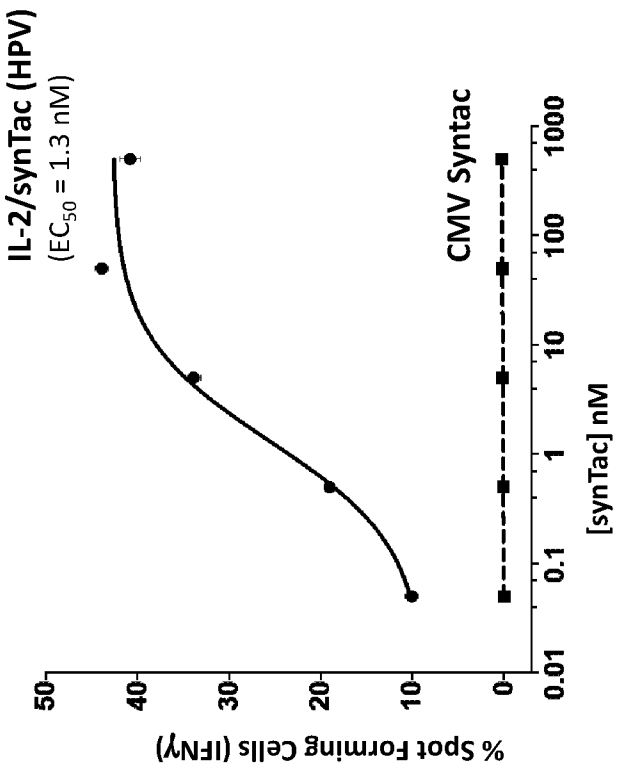

IL-2/synTac (HPV)
($EC_{50}$ = 1.3 nM)
CMV Syntac
% Spot Forming Cells (IFNγ)
50
40
30
20
10
0
0.01
0.1
1
10
100
1000
[synTac] nM

FIG. 40