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(54) **RATIONAL EVOLUTION OF CYTOKINES FOR HIGHER STABILITY, THE CYTOKINES AND ENCODING NUCLEIC ACID MOLECULES**

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(57) **ABSTRACT**

Compositions of modified cytokines and uses thereof generated using processes and systems for the high throughput directed evolution of peptides and proteins, particularly cytokines that act in complex biological settings, are provided. Also provided are modified cytokines formulated for oral delivery and uses thereof to treat diseases and conditions mediated by cytokines.

The "Percent Accepted Mutation" (PAM250) matrix

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	2	-2	0	0	-2	0	0	1	-1	-1	-2	-1	-1	-3	1	1	1	-6	-3	0
R	-2	6	0	-1	-4	1	-1	-3	2	-2	-3	3	0	-4	0	0	-1	2	-4	-2
N	0	0	2	2	-4	1	1	0	2	-2	-3	1	-2	-3	0	1	0	-4	-2	-2
D	0	-1	2	4	-5	2	3	1	1	-2	-4	0	-3	-6	-1	0	0	-7	-4	-2
C	-2	-4	-4	-5	12	-5	-5	-3	-3	-2	-6	-5	-5	-4	-3	0	-2	-8	0	-2
Q	0	1	1	2	-5	4	2	-1	3	-2	-2	1	-1	-5	0	-1	-1	-5	-4	-2
E	0	-1	1	3	-5	2	4	0	1	-2	-3	0	-2	-5	-1	0	0	-7	-4	-2
G	1	-3	0	1	-3	-1	0	5	-2	-3	-4	-2	-3	-5	0	1	0	-7	-5	-1
H	-1	2	2	1	-3	3	1	-2	6	-2	-2	0	-2	-2	0	-1	-1	-3	0	-2
I	-1	-2	-2	-2	-2	-2	-2	-3	-2	5	2	-2	2	1	-2	-1	0	-5	-1	4
L	-2	-3	-3	-4	-6	-2	-3	-4	-2	2	6	-3	4	2	-3	-3	-2	-2	-1	2
K	-1	3	1	0	-5	1	0	-2	0	-2	-3	5	0	-5	-1	0	0	-3	-4	-2
M	-1	0	-2	-3	-5	-1	-2	-3	-2	2	4	0	6	0	-2	-2	-1	-4	-2	2
F	-3	-4	-3	-6	-4	-5	-5	-5	-2	1	2	-5	0	9	-5	-3	-3	0	7	-1
P	1	0	0	-1	-3	0	-1	0	0	-2	-3	-1	-2	-5	6	1	0	-6	-5	-1
S	1	0	1	0	0	-1	0	1	-1	-1	-3	0	-2	-3	1	2	1	-2	-3	-1
T	1	-1	0	0	-2	-1	0	0	-1	0	-2	0	-1	-3	0	1	3	-5	-3	0
W	-6	2	-4	-7	-8	-5	-7	-7	-3	-5	-2	-3	-4	0	-6	-2	-5	17	0	-6
Y	-3	-4	-2	-4	0	-4	-4	-5	0	-1	-1	-4	-2	7	-5	-3	-3	0	10	-2
V	0	-2	-2	-2	-2	-2	-2	-1	-2	4	2	-2	2	-1	-1	-1	0	-6	-2	4

Amino acid sequence of human mature IFNα-2b

```
                1         10         20         30         40         50
                .          .          .          .          .          .
IFNα-2b         CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKA

               51         60         70         80         90        100
                .          .          .          .          .          .
IFNα-2b         ETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVI

              101        110        120        130        140        150
                .          .          .          .          .          .
IFNα-2b         QGVGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRS

              151        160
                .          .
IFNα-2b         FSLSTNLQESLRSKE
```

FIG.1A

Three dimensional structure of INFα-2b showing candidate LEADs

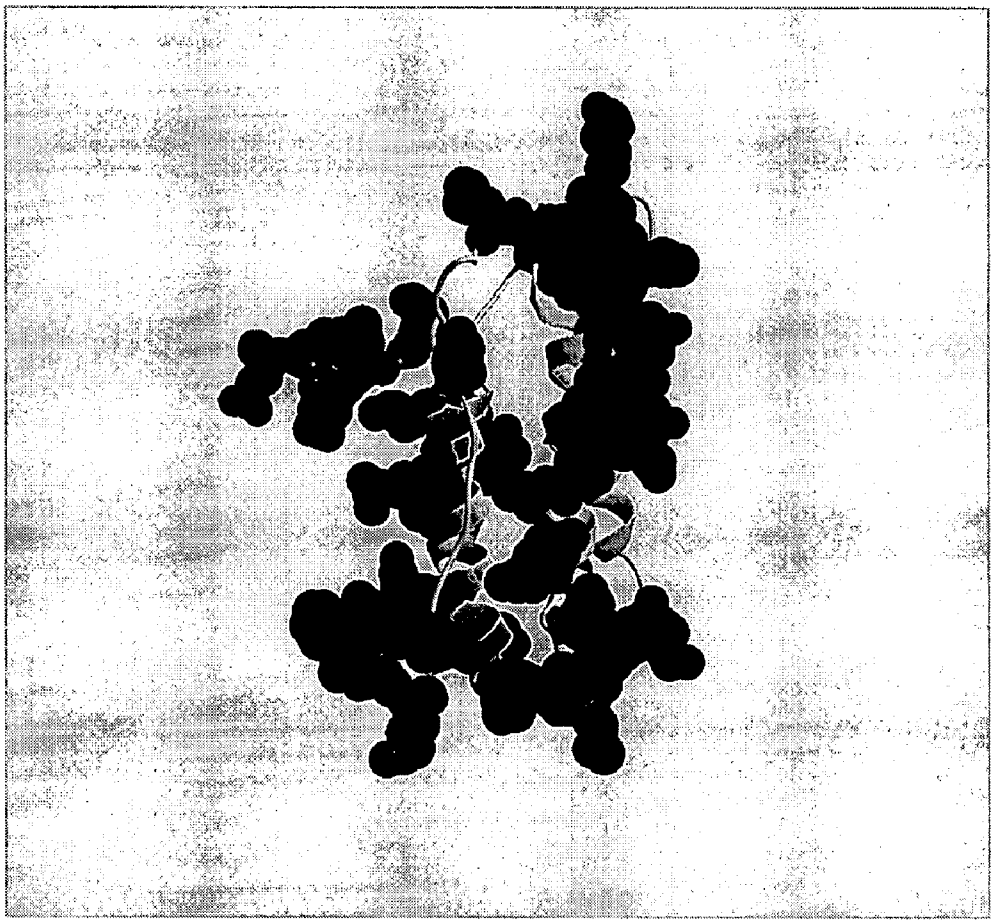

FIG.1B

The "Percent Accepted Mutation" (PAM250) matrix

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	2	-2	0	0	-2	0	0	1	-1	-1	-2	-1	-1	-3	1	1	1	-6	-3	0
R	-2	6	0	-1	-4	1	-1	-3	2	-2	-3	3	0	-4	0	0	-1	2	-4	-2
N	0	0	2	2	-4	1	1	0	2	-2	-3	1	-2	-3	0	1	0	-4	-2	-2
D	0	-1	2	4	-5	2	3	1	1	-2	-4	0	-3	-6	-1	0	0	-7	-4	-2
C	-2	-4	-4	-5	12	-5	-5	-3	-3	-2	-6	-5	-5	-4	-3	0	-2	-8	0	-2
Q	0	1	1	2	-5	4	2	-1	3	-2	-2	1	-1	-5	0	-1	-1	-5	-4	-2
E	0	-1	1	3	-5	2	4	0	1	-2	-3	0	-2	-5	-1	0	0	-7	-4	-2
G	1	-3	0	1	-3	-1	0	5	-2	-3	-4	-2	-3	-5	0	1	0	-7	-5	-1
H	-1	2	2	1	-3	3	1	-2	6	-2	-2	0	-2	-2	0	-1	-1	-3	0	-2
I	-1	-2	-2	-2	-2	-2	-2	-3	-2	5	2	-2	2	1	-2	-1	0	-5	-1	4
L	-2	-3	-3	-4	-6	-2	-3	-4	-2	2	6	-3	4	2	-3	-3	-2	-2	-1	2
K	-1	3	1	0	-5	1	0	-2	0	-2	-3	5	0	-5	-1	0	0	-3	-4	-2
M	-1	0	-2	-3	-5	-1	-2	-3	-2	2	4	0	6	0	-2	-2	-1	-4	-2	2
F	-3	-4	-3	-6	-4	-5	-5	-5	-2	1	2	-5	0	9	-5	-3	-3	0	7	-1
P	1	0	0	-1	-3	0	-1	0	0	-2	-3	-1	-2	-5	6	1	0	-6	-5	-1
S	1	0	1	0	0	-1	0	1	-1	-1	-3	0	-2	-3	1	2	1	-2	-3	-1
T	1	-1	0	0	-2	-1	0	0	-1	0	-2	0	-1	-3	0	1	3	-5	-3	0
W	-6	2	-4	-7	-8	-5	-7	-7	-3	-5	-2	-3	-4	0	-6	-2	-5	17	0	-6
Y	-3	-4	-2	-4	0	-4	-4	-5	0	-1	-1	-4	-2	7	-5	-3	-3	0	10	-2
V	0	-2	-2	-2	-2	-2	-2	-1	-2	4	2	-2	2	-1	-1	-1	0	-6	-2	4

FIG.2

Scores from PAM250, given to residue substitutions to protect human INF α–2b against proteolysis

	R	D	E	L	K	M	F	P	W	Y
A	-2	0	0	-2	-1	-1	-3	1	-6	-3
N	0	2	1	-3	1	-2	-3	0	-4	-2
C	-4	-5	-5	-6	-5	-5	-4	-3	-8	0
Q	1	2	2	-2	1	-1	-5	0	-5	-4
G	-3	1	0	-4	-2	-3	-5	0	-7	-5
H	2	1	1	-2	0	-2	-2	0	-3	0
I	-2	-2	-2	2	-2	2	1	-2	-5	-1
S	0	0	0	-3	0	-2	-3	1	-2	-3
T	-1	0	0	-2	0	-1	-3	0	-5	-3
V	-2	-2	-2	2	-2	2	-1	-1	-6	-2

FIG.3

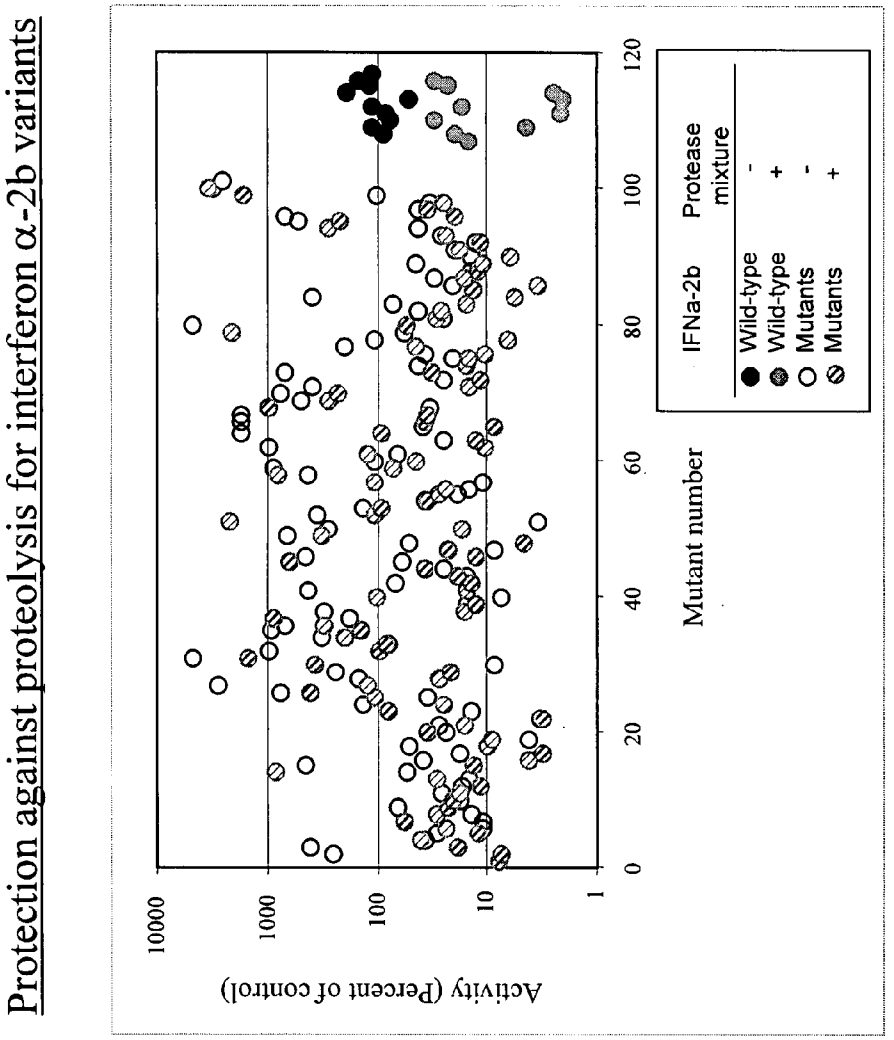
Protection against proteolysis for interferon α-2b variants
Activity (Percent of control)
10000
1000
100
10
1
0
20
40
60
80
100
120
Mutant number
IFNa-2b
Protease mixture
Wild-type -
Wild-type +
Mutants -
Mutants +

FIG.4A

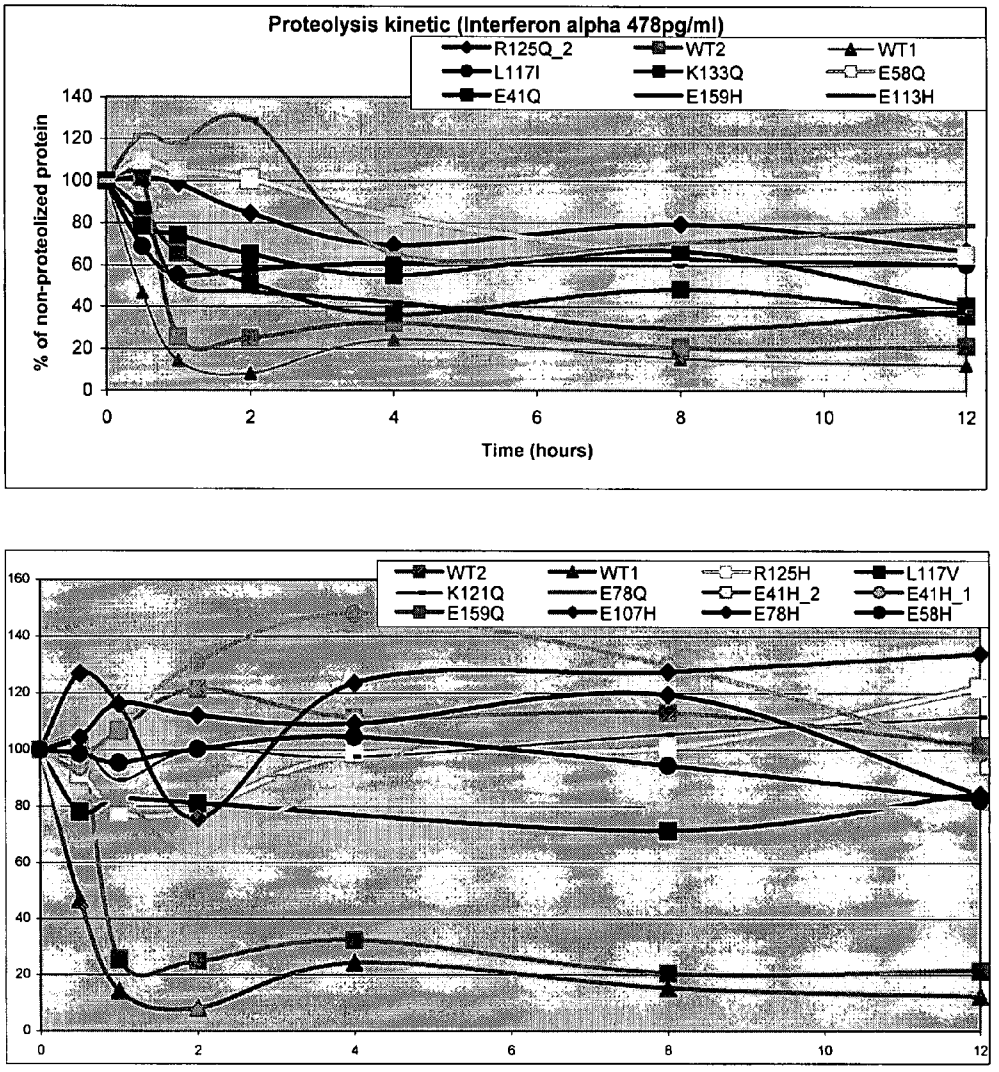
Proteolysis kinetic (Interferon alpha 478pg/ml)
R125Q_2
WT2
WT1
L117I
K133Q
E58Q
E41Q
E159H
E113H
% of non-proteolized protein
Time (hours)
WT2
WT1
R125H
L117V
K121Q
E78Q
E41H_2
E41H_1
E159Q
E107H
E78H
E58H

FIG.4B

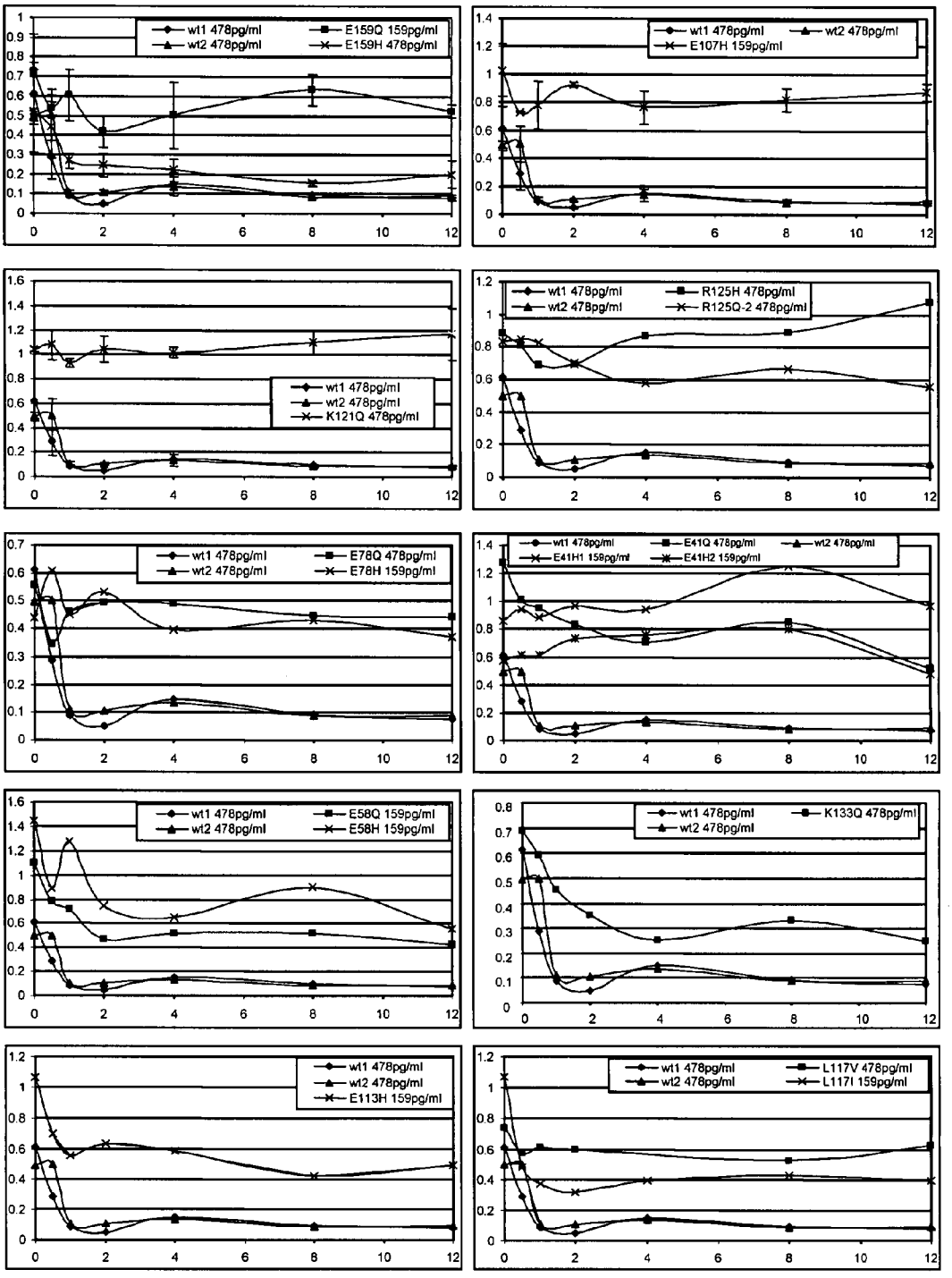
wt1 478pg/ml
wt2 478pg/ml
E159Q 159pg/ml
E159H 478pg/ml
wt1 478pg/ml
wt2 478pg/ml
E107H 159pg/ml
wt1 478pg/ml
wt2 478pg/ml
K121Q 478pg/ml
wt1 478pg/ml
wt2 478pg/ml
R125H 478pg/ml
R125Q-2 478pg/ml
wt1 478pg/ml
wt2 478pg/ml
E78Q 478pg/ml
E78H 159pg/ml
wt1 478pg/ml
wt2 478pg/ml
E41Q 478pg/ml
E41H1 159pg/ml
E41H2 159pg/ml
wt1 478pg/ml
wt2 478pg/ml
E58Q 159pg/ml
E58H 159pg/ml
wt1 478pg/ml
wt2 478pg/ml
K133Q 478pg/ml
wt1 478pg/ml
wt2 478pg/ml
E113H 159pg/ml
wt1 478pg/ml
wt2 478pg/ml
L117V 478pg/ml
L117I 159pg/ml

FIG. 4C

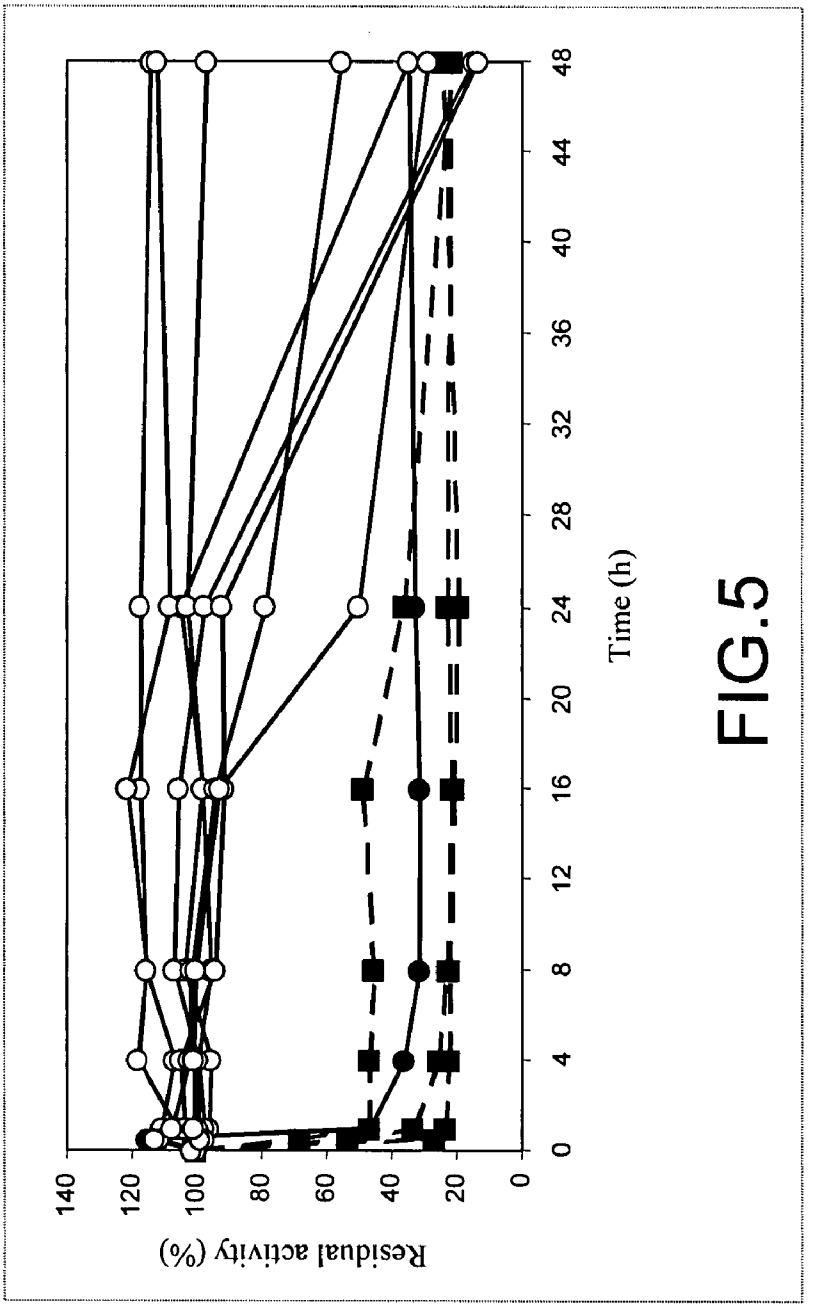
Treatment with chymotrypsin
Residual activity (%)
140
120
100
80
60
40
20
0
0
4
8
12
16
20
24
28
32
36
40
44
48
Time (h)

FIG.5

Treatment with chymotrypsin

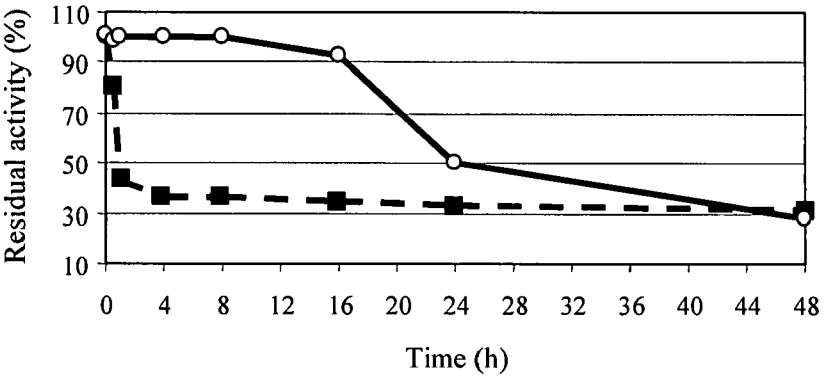

FIG.6A

Treatment with protease mixture

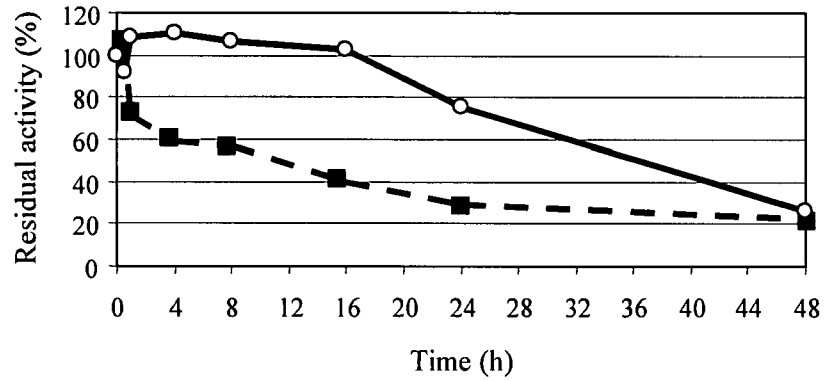

FIG.6B

Treatment with blood lysate

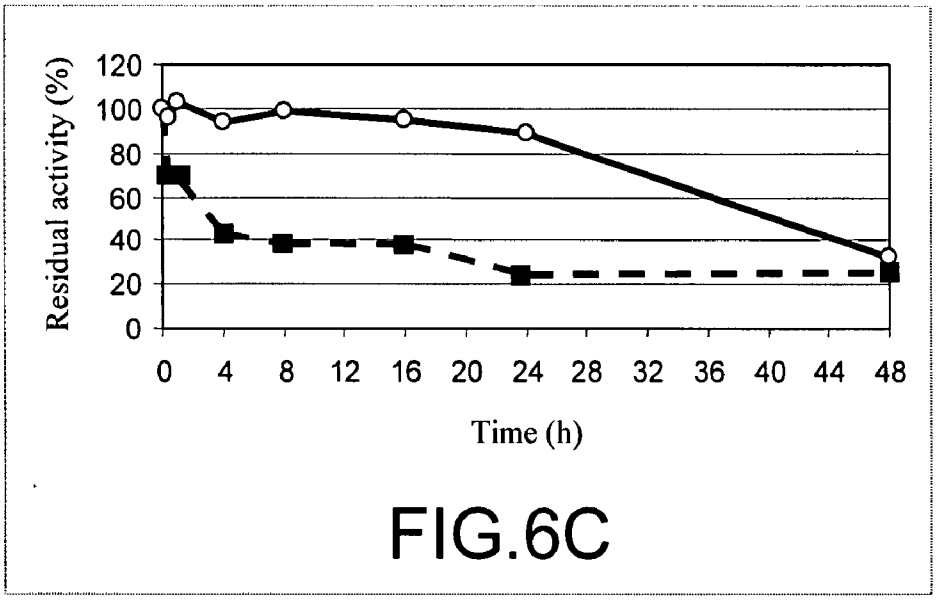

FIG.6C

Treatment with serum

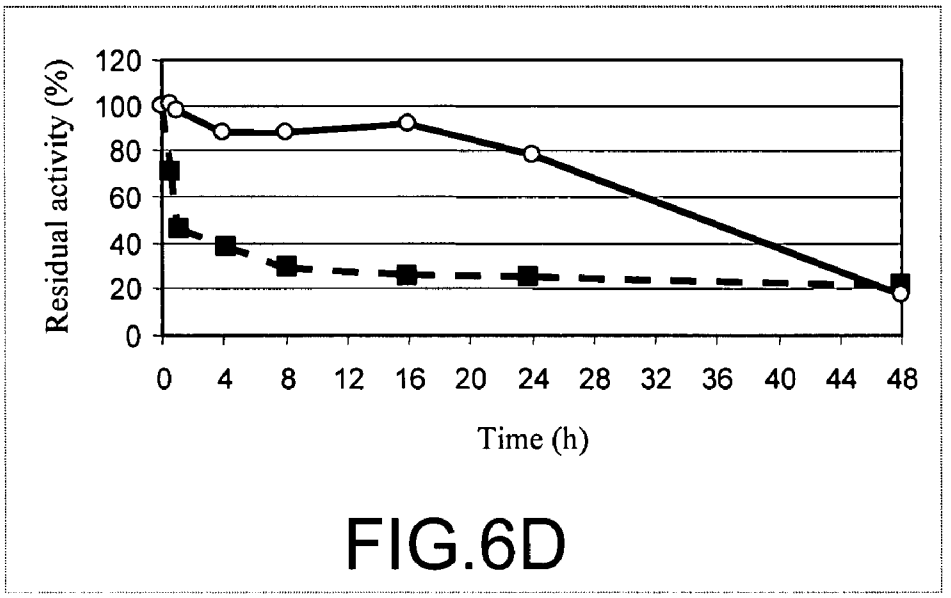

FIG.6D

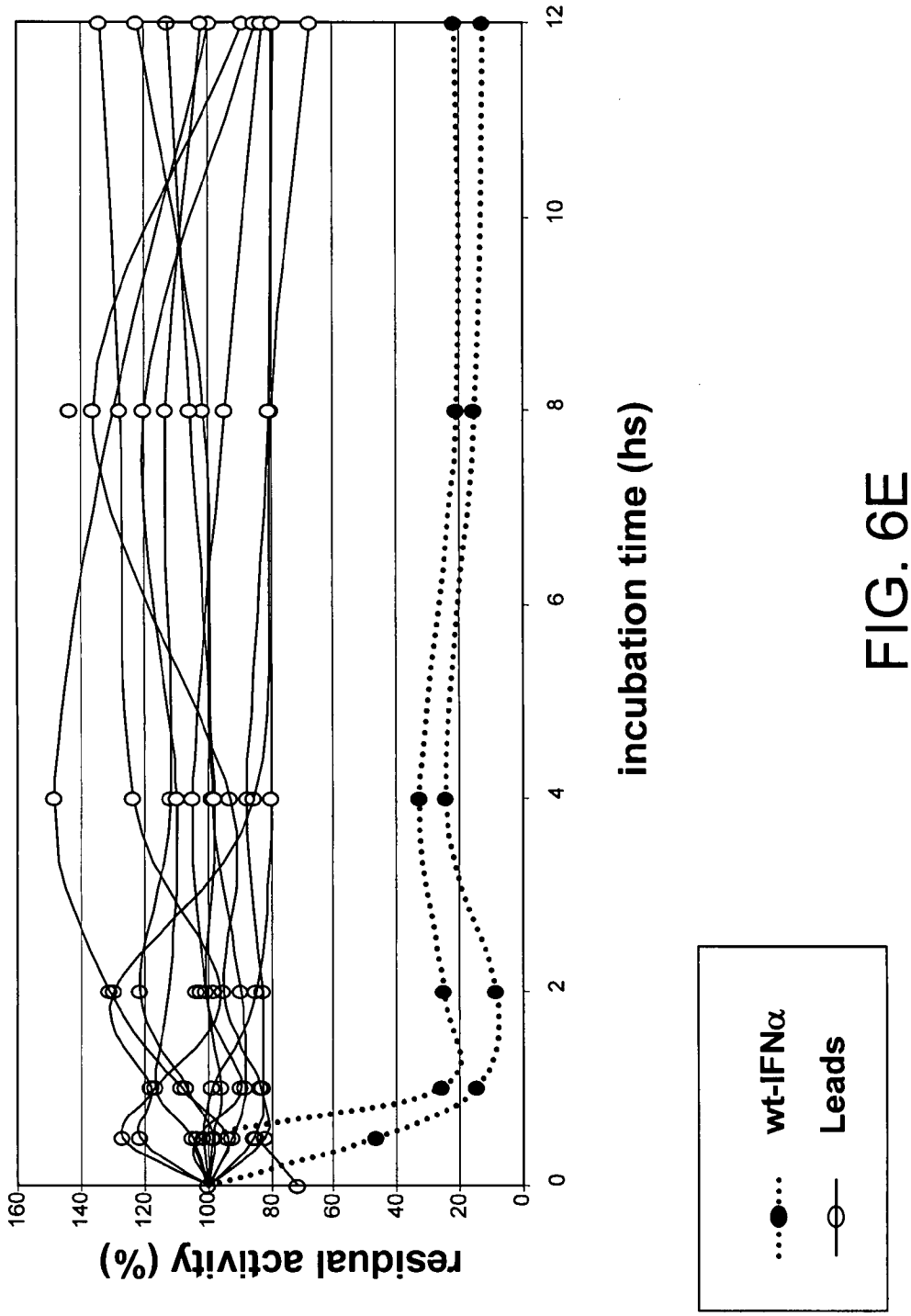
residual activity (%)
160
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
incubation time (hs)
wt-IFNα
Leads

FIG. 6E

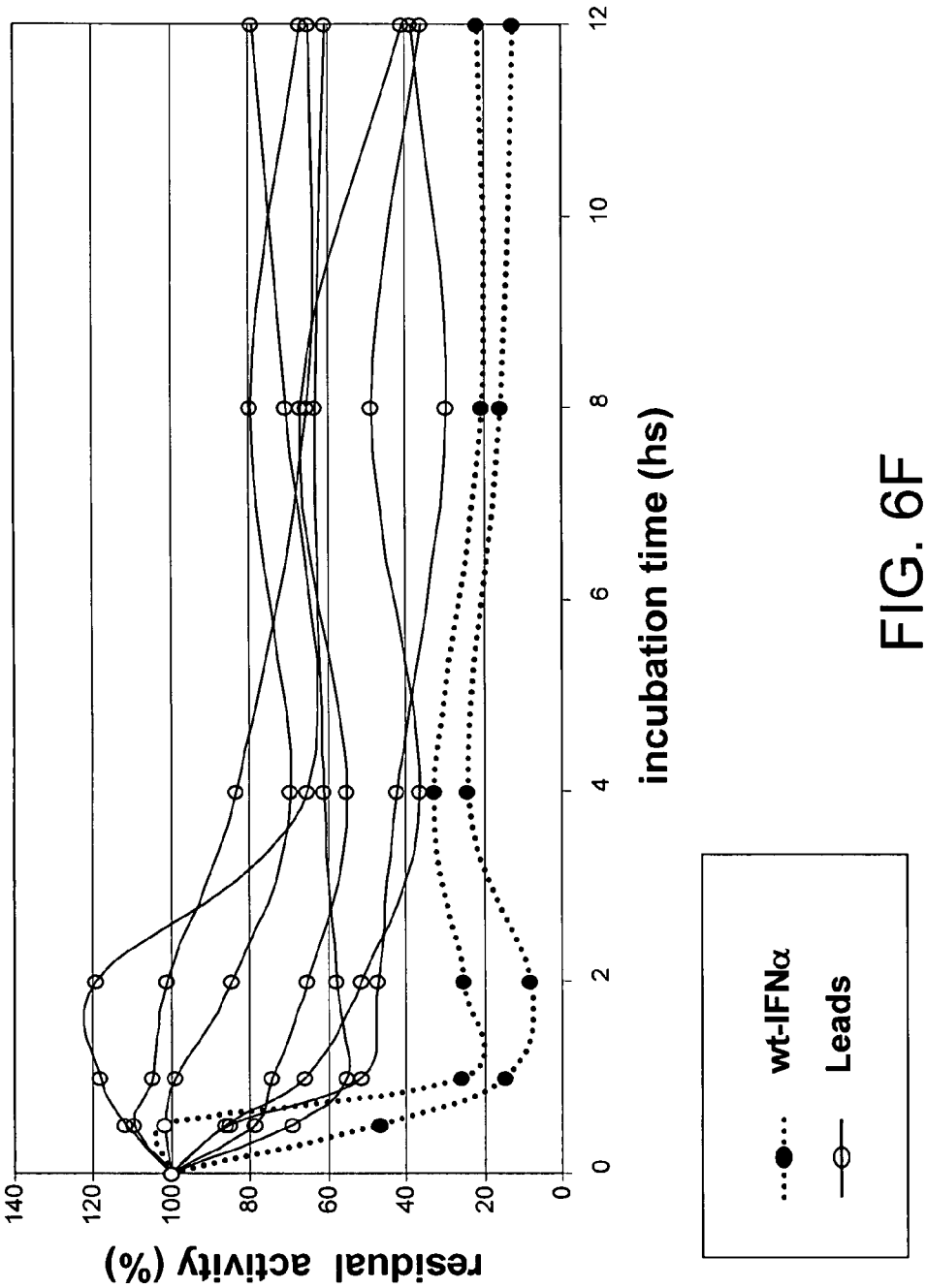
residual activity (%)
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
incubation time (hs)
wt-IFNα
Leads

FIG. 6F

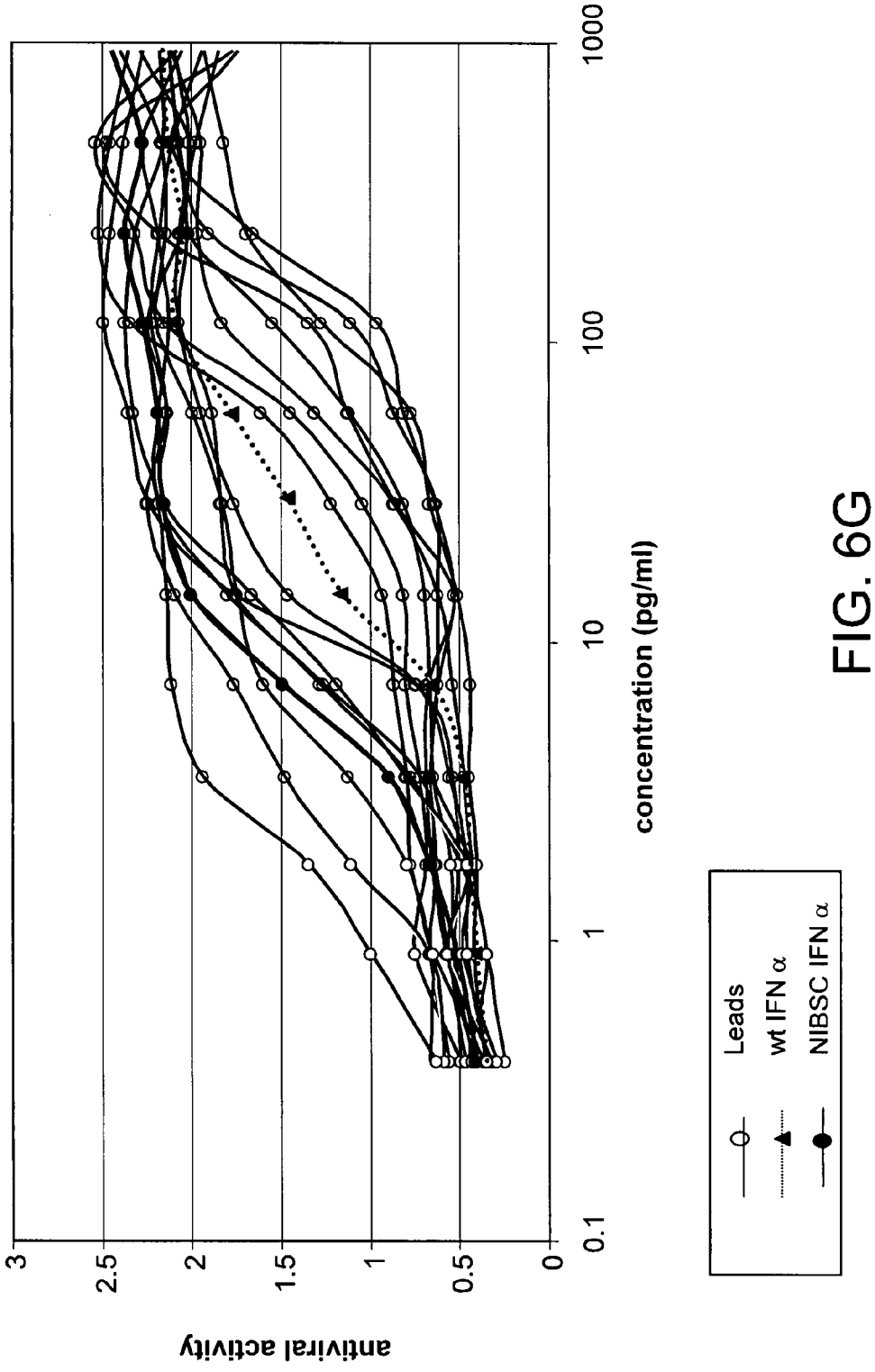
antiviral activity
3
2.5
2
1.5
1
0.5
0
0.1
1
10
100
1000
concentration (pg/ml)
Leads
wt IFN α
NIBSC IFN α

FIG. 6G

Potency (antiproliferation) – IFNα leads

	Potency (10^8 U/mg)
WT	1,70
Lead 13	1,60
Lead 9	1,90
Lead 8	2,05
Lead 2	3,70
Lead 16	1,60
Lead 4	0,50
Lead 5	0,65
Lead 15	3,20
Lead 10	0,50
Lead 12	1,50
Lead 11	nd
Lead 6	1,20
Lead 1	2,95
Lead 7	1,60
Lead 3	2,25
Lead 14	nd

FIG. 6H

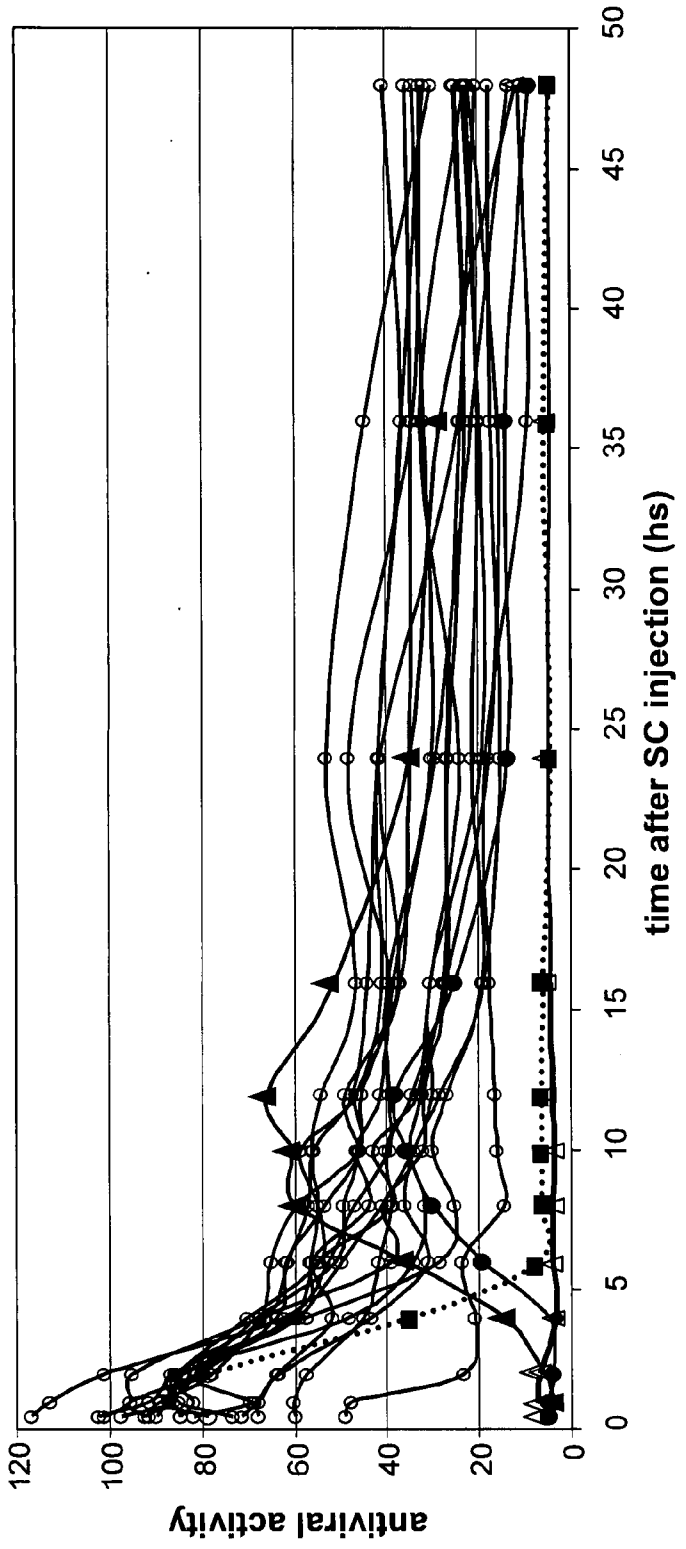
antiviral activity
0
20
40
60
80
100
120
time after SC injection (hs)
0
5
10
15
20
25
30
35
40
45
50
Reference to Figures 6I, 6K-N
Leads
wt IFN α
vehicle
Pegasys 36 µg / ml
Pegasys 18 µg / ml

FIG. 6I

FIGURE 6J
IFN-α LEADS

IFN-α LEAD	SEQ ID N°	Mutation(s)
1	983	K121Q / P109A
2	987	E159H / Y89H
3	124	E159Q
4	90	E58H
5	89	E58Q
6	979	E41H / Y89H / N45D
7	103	L117I
8	986	R125H / M111V
9	96	E107H
10	101	E113H
11	87	E41Q
12	107	R125Q
13	985	L117V / A139G
14	980	E41Q / D94G
15	93	E78H
16	984	K133Q / K121Q / P109A / G102R

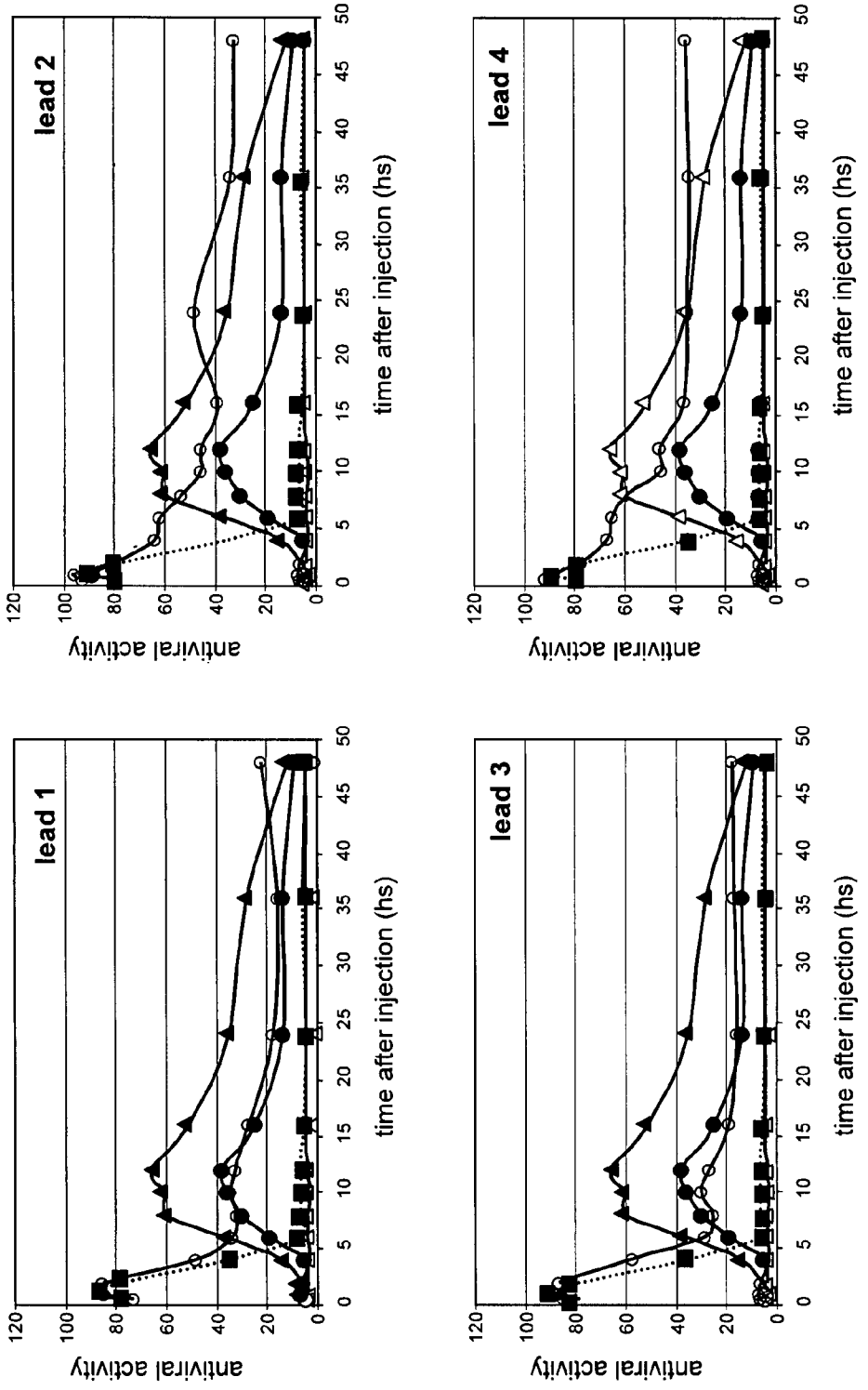
lead 2
lead 4
lead 1
lead 3
antiviral activity
time after injection (hs)
0
20
40
60
80
100
120
0
5
10
15
20
25
30
35
40
45
50

FIG. 6K

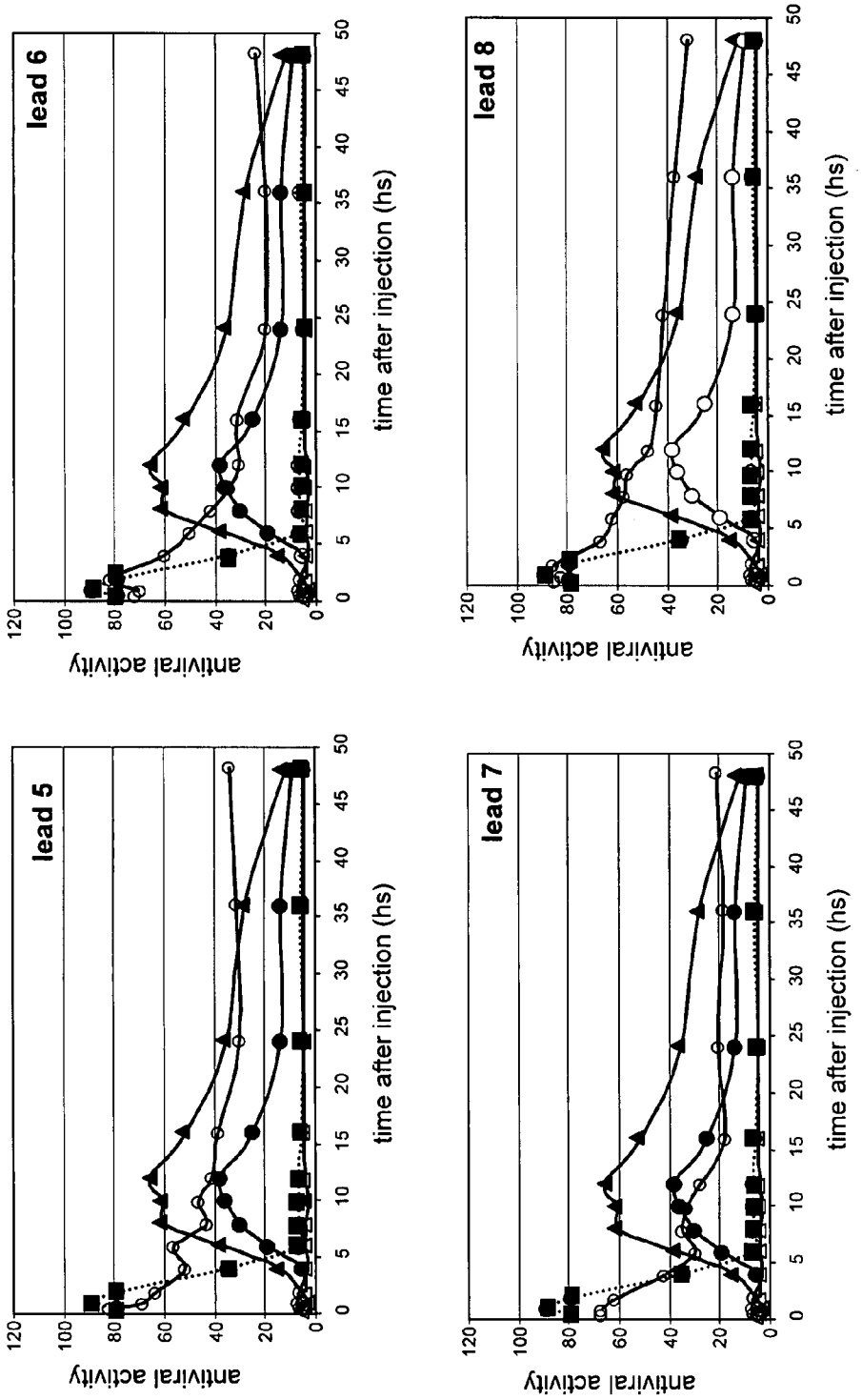
lead 6
lead 8
lead 5
lead 7
antiviral activity
time after injection (hs)
0
20
40
60
80
100
120
0
5
10
15
20
25
30
35
40
45
50

FIG. 6L

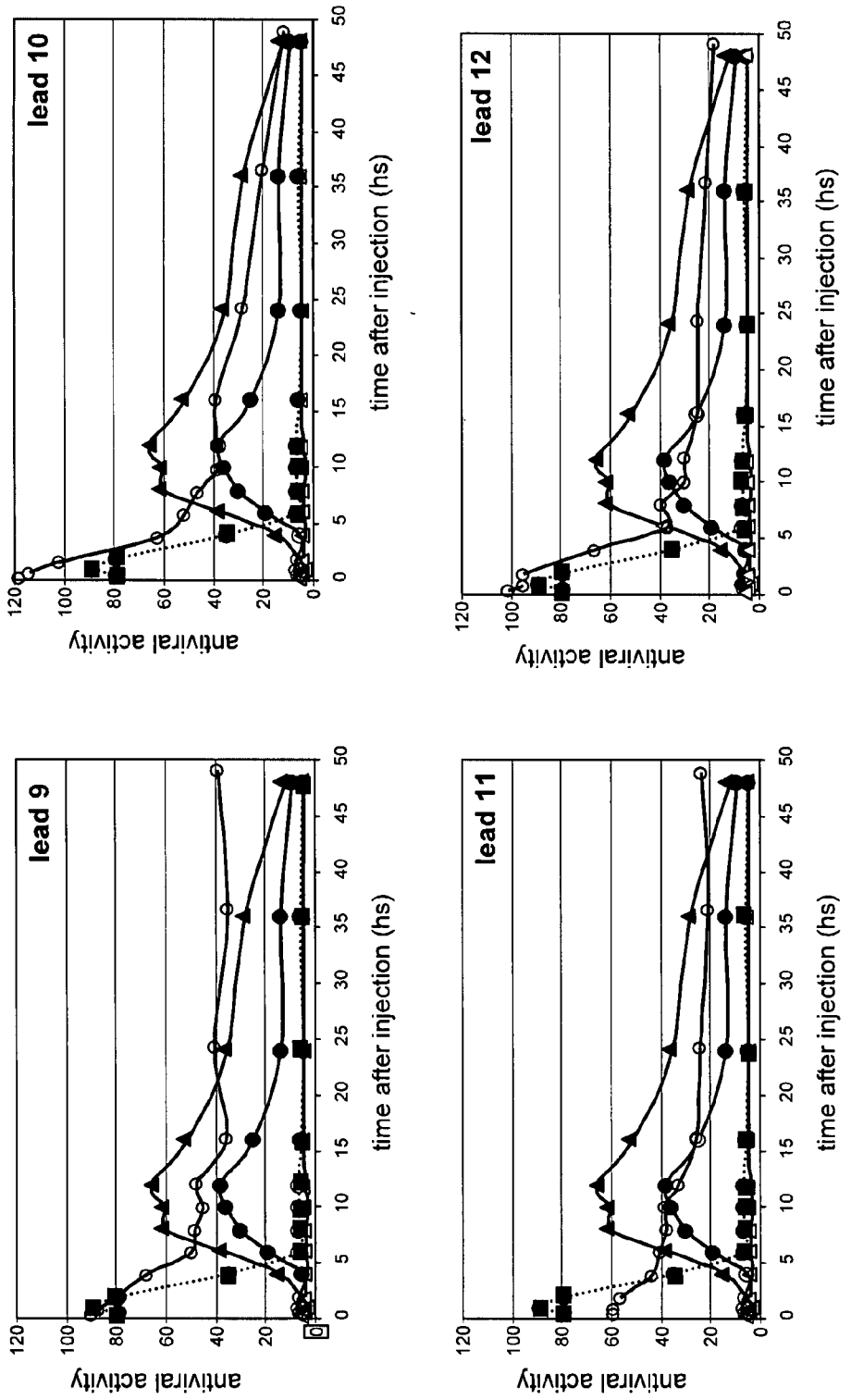
lead 10
lead 12
lead 9
lead 11
antiviral activity
time after injection (hs)
0
20
40
60
80
100
120
0
5
10
15
20
25
30
35
40
45
50

FIG. 6M

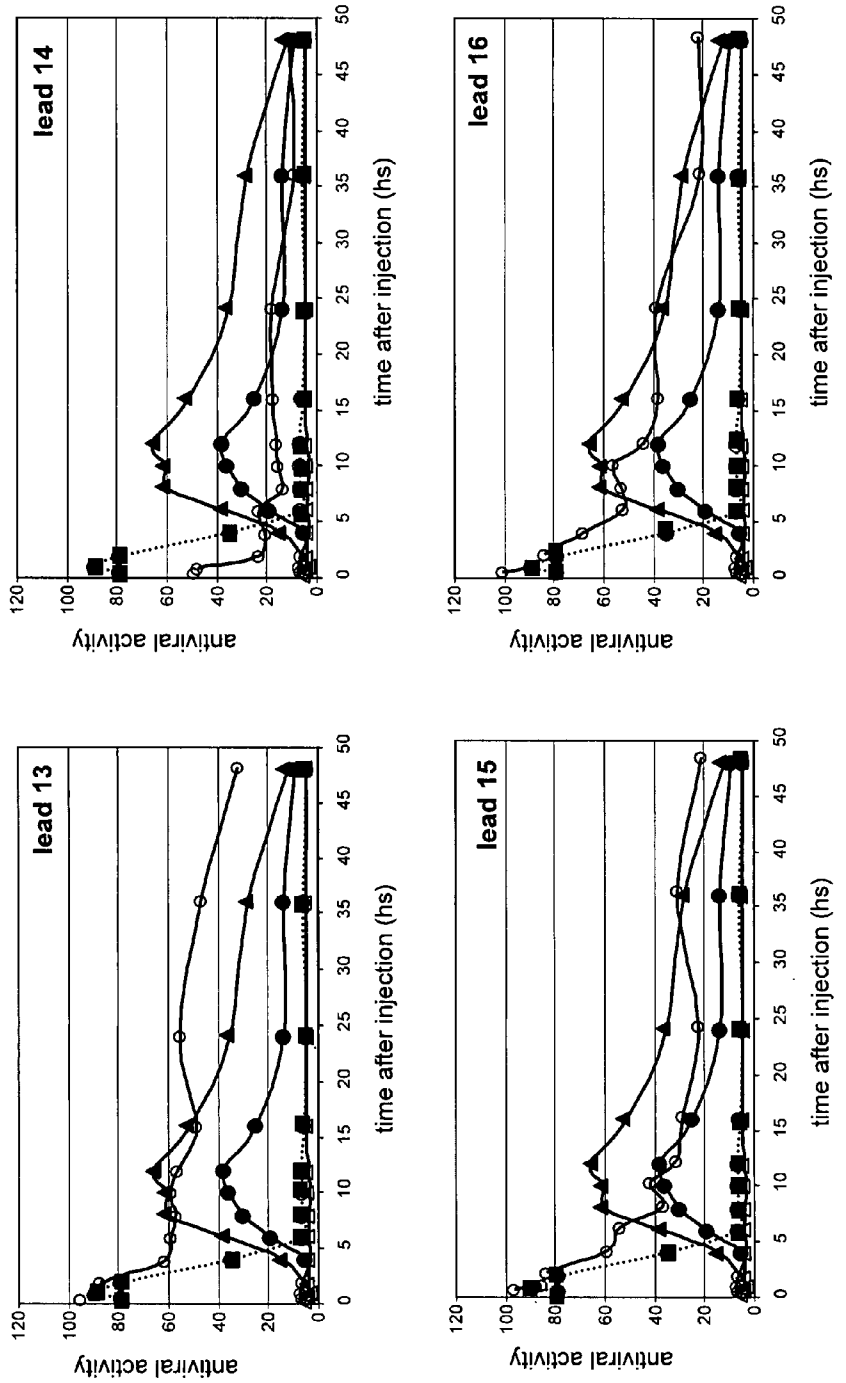
lead 14
lead 16
lead 13
lead 15
antiviral activity
time after injection (hs)
0
20
40
60
80
100
120
0
5
10
15
20
25
30
35
40
45
50

FIG. 6N

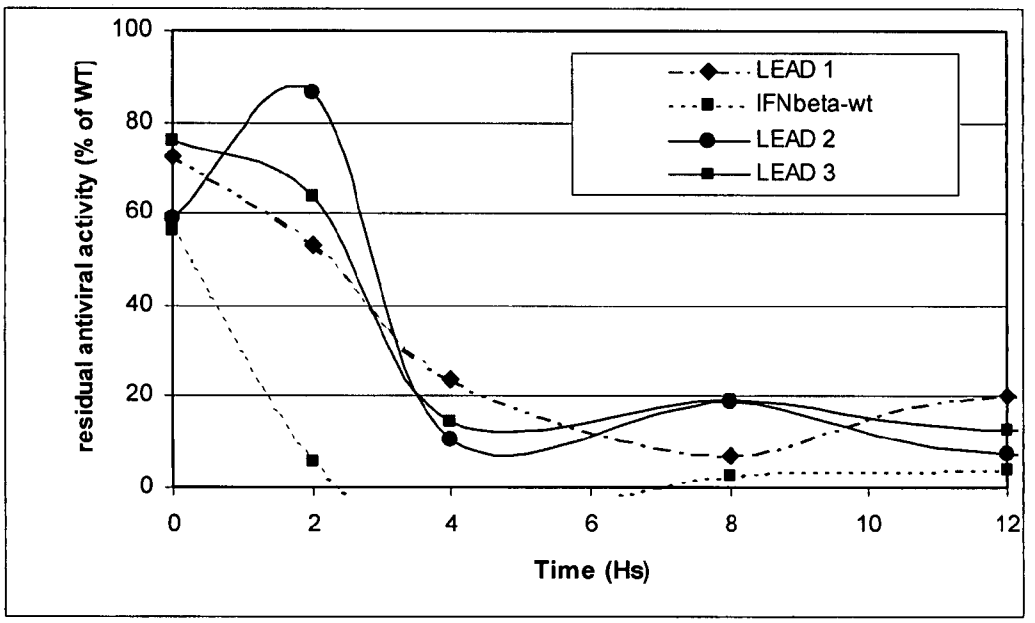
100
80
60
40
20
0
residual antiviral activity (% of WT)
0
2
4
6
8
10
12
Time (Hs)
LEAD 1
IFNbeta-wt
LEAD 2
LEAD 3

FIG 6O

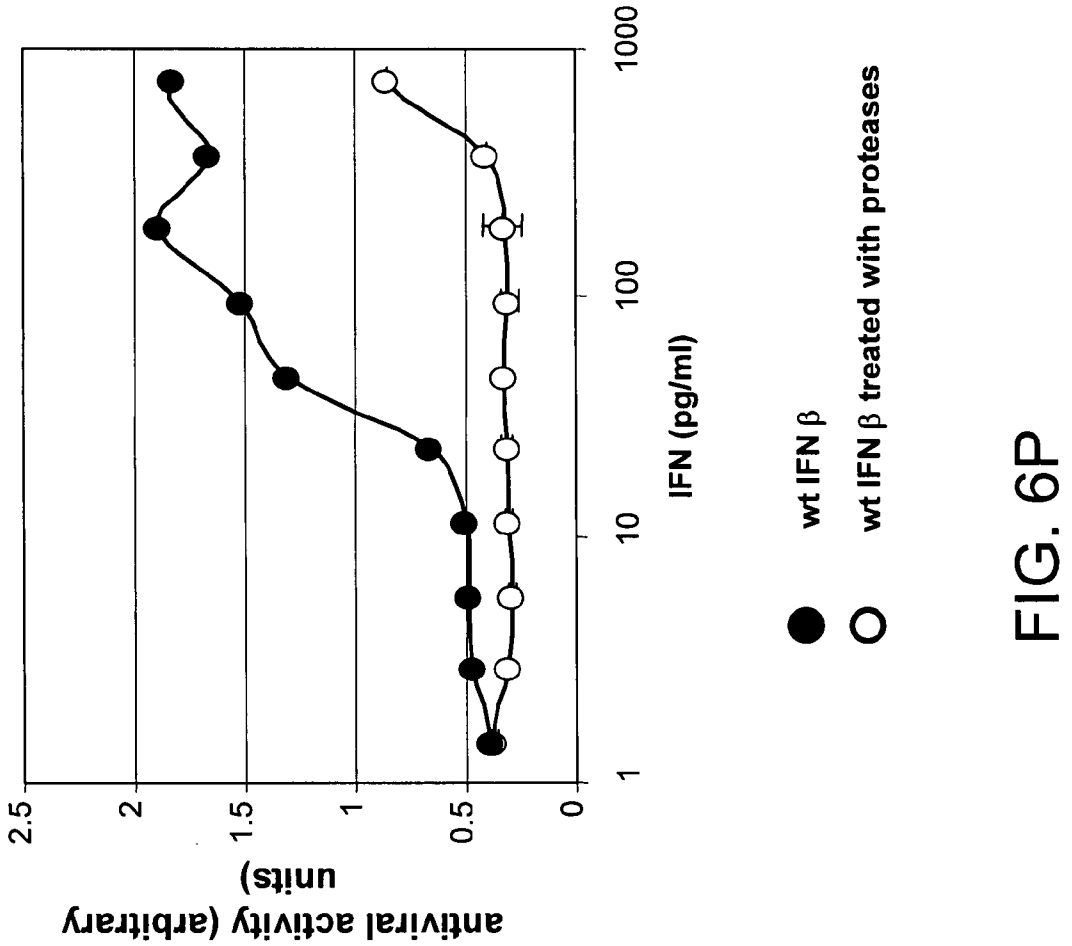
antiviral activity (arbitrary units)
2.5
2
1.5
1
0.5
0
1
10
100
1000
IFN (pg/ml)
wt IFN β
wt IFN β treated with proteases

FIG. 6P

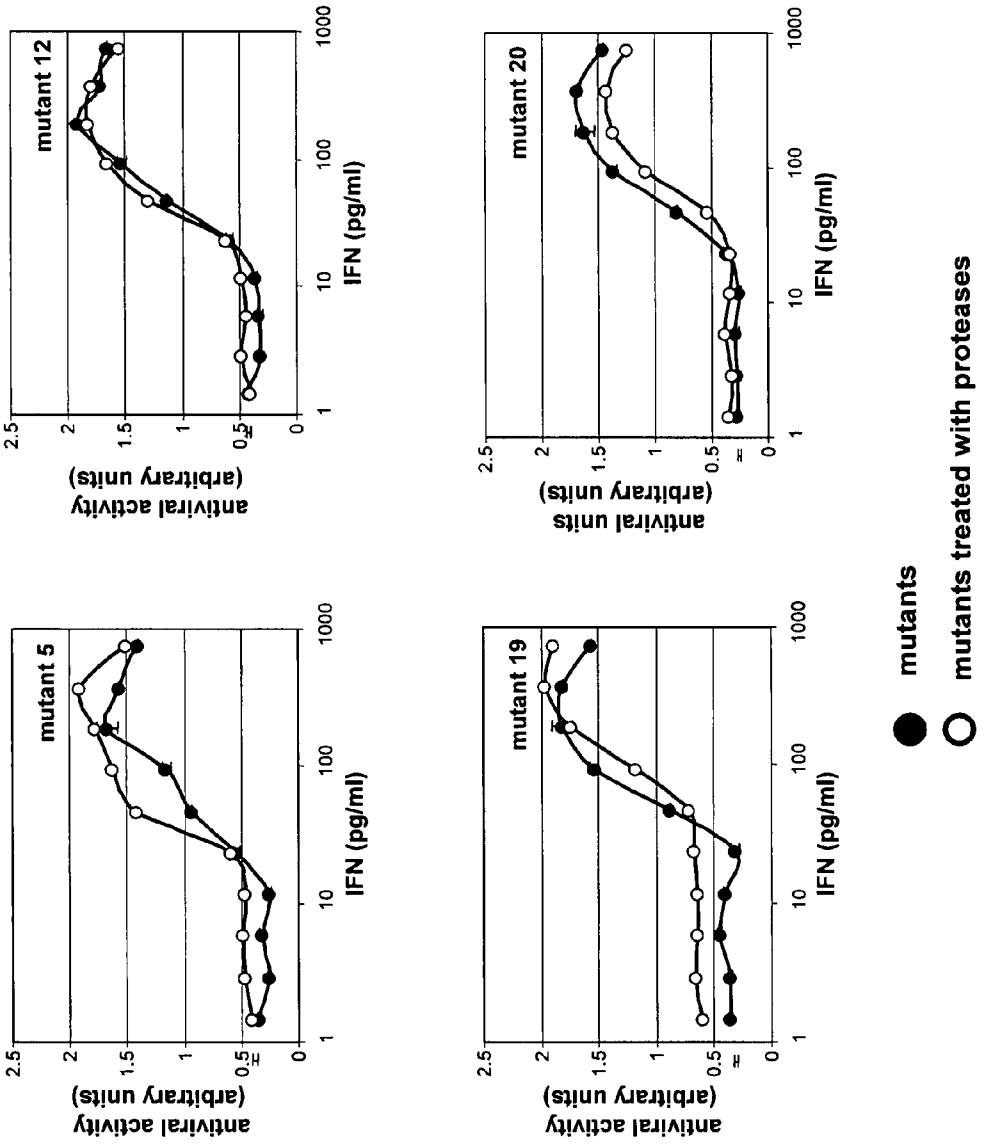
mutant 12
mutant 20
mutant 5
mutant 19
antiviral activity (arbitrary units)
IFN (pg/ml)
0
0.5
1
1.5
2
2.5
1
10
100
1000
mutants
mutants treated with proteases

FIG. 6Q

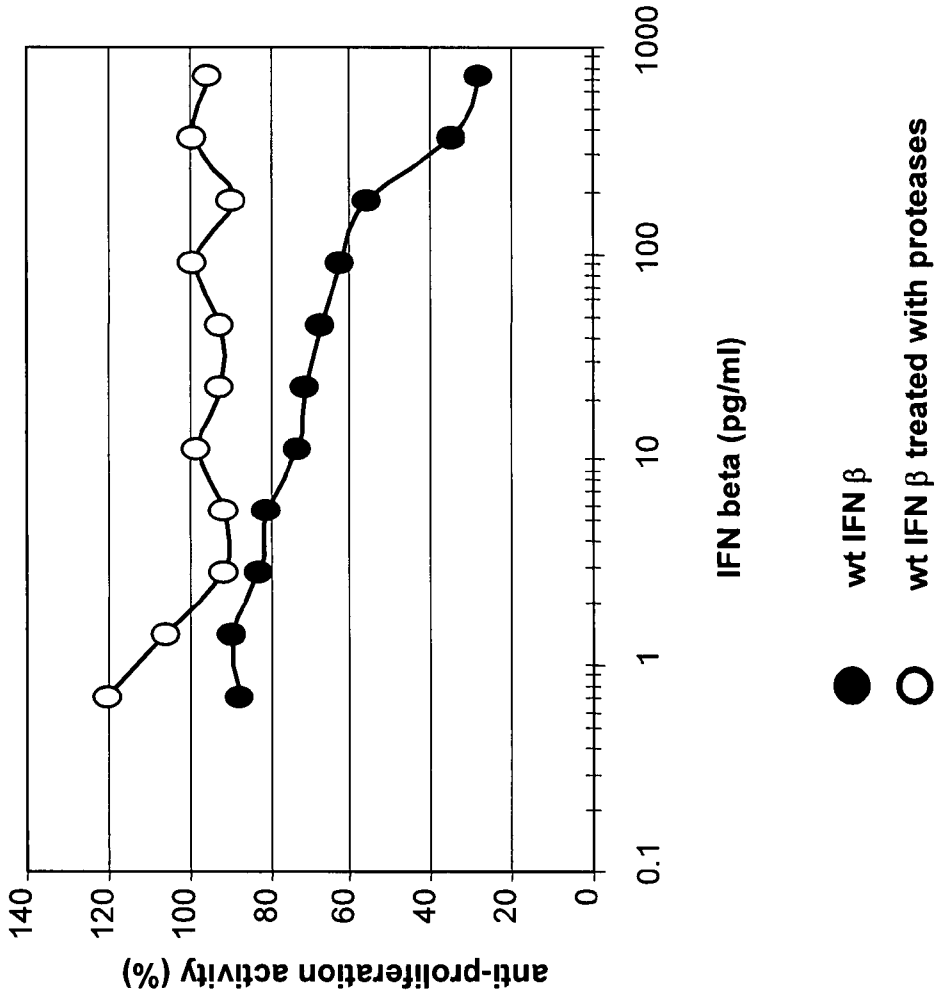
anti-proliferation activity (%)
140
120
100
80
60
40
20
0
0.1
1
10
100
1000
IFN beta (pg/ml)
wt IFN β
wt IFN β treated with proteases

FIG. 6R

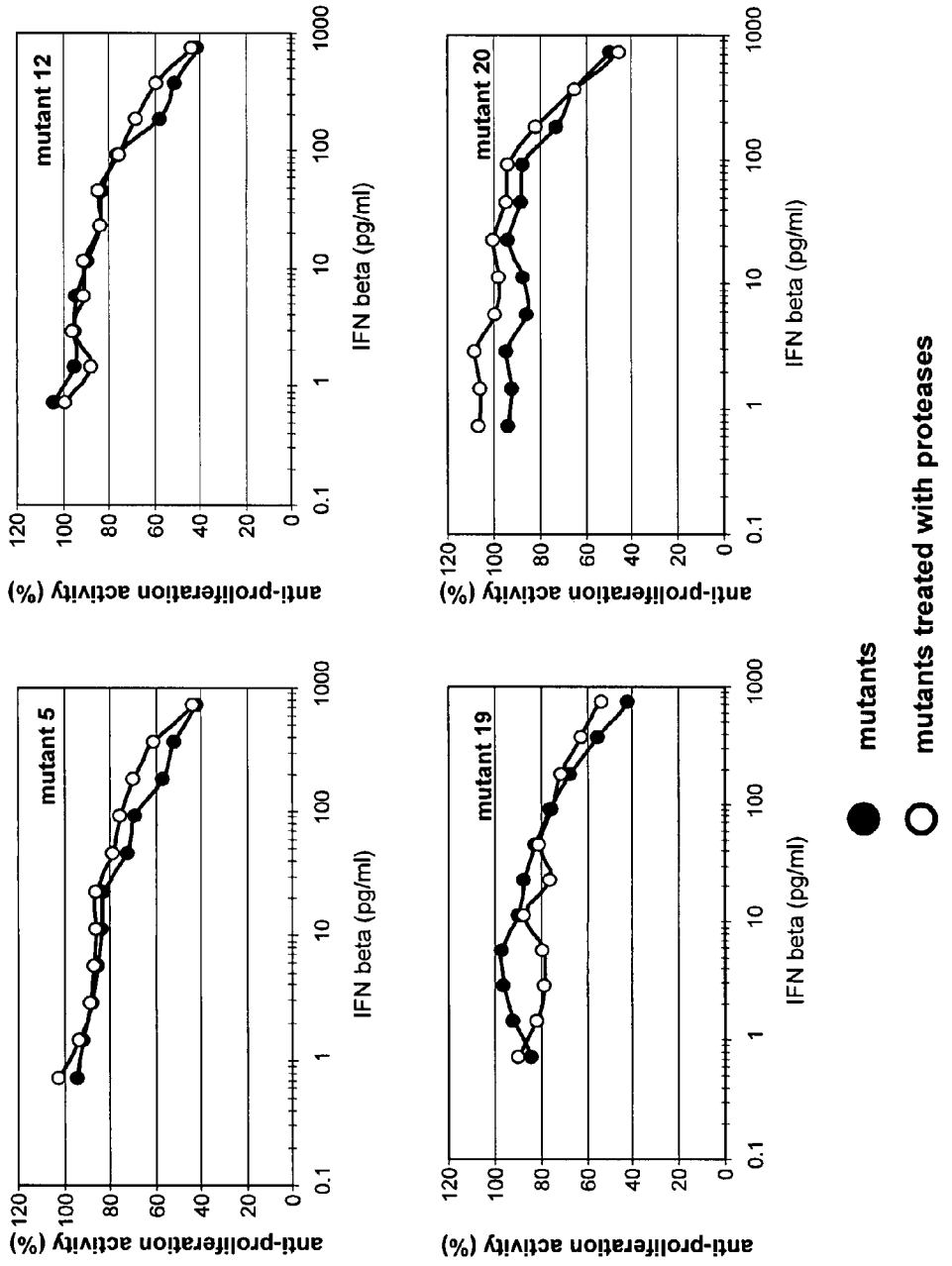
mutant 12
mutant 20
mutant 5
mutant 19
anti-proliferation activity (%)
IFN beta (pg/ml)
0
20
40
60
80
100
120
0.1
1
10
100
1000
mutants
mutants treated with proteases

FIG. 6S

Summary – IFNα leads

	Potency (AV) (10^8 U/mg)	Potency (AP) (10^8 U/mg)	AUC (arbitrary units)
WT	1,6	1,7	16,5
Pegasys			33,0
Pegasys			77,0
Lead 13	0,4	1,6	129,7
Lead 9	1,2	1,9	109,0
Lead 8	1,0	2,1	107,0
Lead 2	2,1	3,7	105,0
Lead 16	0,8	1,6	101,6
Lead 4	4,3	0,5	100,0
Lead 5	1,2	0,7	88,6
Lead 15	1,7	3,2	88,0
Lead 10	5,5	0,5	85,6
Lead 12	1,4	1,5	77,0
Lead 11	28,5	nd	69,0
Lead 6	1,7	1,2	64,2
Lead 1	1,9	3,0	58,5
Lead 7	1,7	1,6	56,5
Lead 3	1,7	2,3	54,6
Lead 14	0,9	nd	25,0

FIG. 6T

IFNα LEADS– Area under the curve (AUC)

	AUC (arbitrary units)	protein injected (µg/ml*)	IFN units injected / ml ($x10^6$)
WT	16,5	2,5	2,0
Pegasys	33,0	18,0	
Pegasys	77,0	36,0	
Lead 13	129,7	10,3	2,0
Lead 9	109,0	3,5	2,0
Lead 8	107,0	4,2	2,0
Lead 2	105,0	2,0	2,0
Lead 16	101,6	5,4	2,0
Lead 4	100,0	1,0	2,0
Lead 5	88,6	3,6	2,0
Lead 15	88,0	2,4	2,0
Lead 10	85,6	1,0	2,0
Lead 12	77,0	3,0	2,0
Lead 11	69,0	0,2	2,0
Lead 6	64,2	3,4	2,0
Lead 1	58,5	2,1	2,0
Lead 7	56,5	2,4	2,0
Lead 3	54,6	2,5	2,0
Lead 14	25,0	2,0	2,0

FIG. 6U

Interferon α-2b structure in "space filling" representation

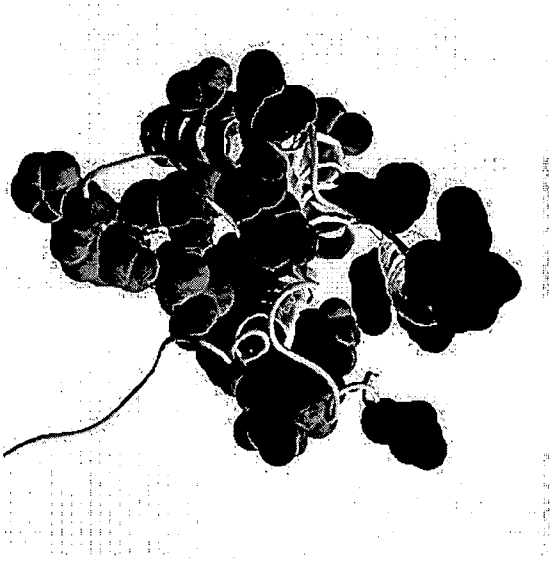

FIG.7A

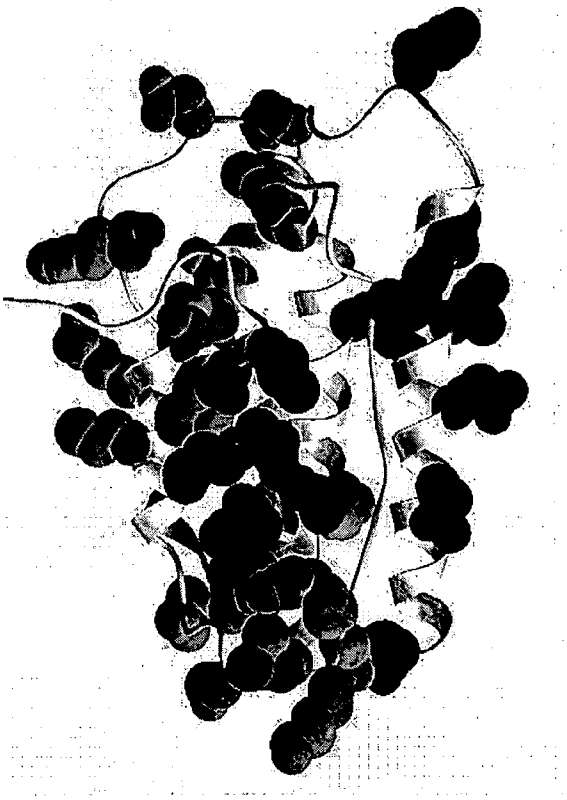

FIG.7B

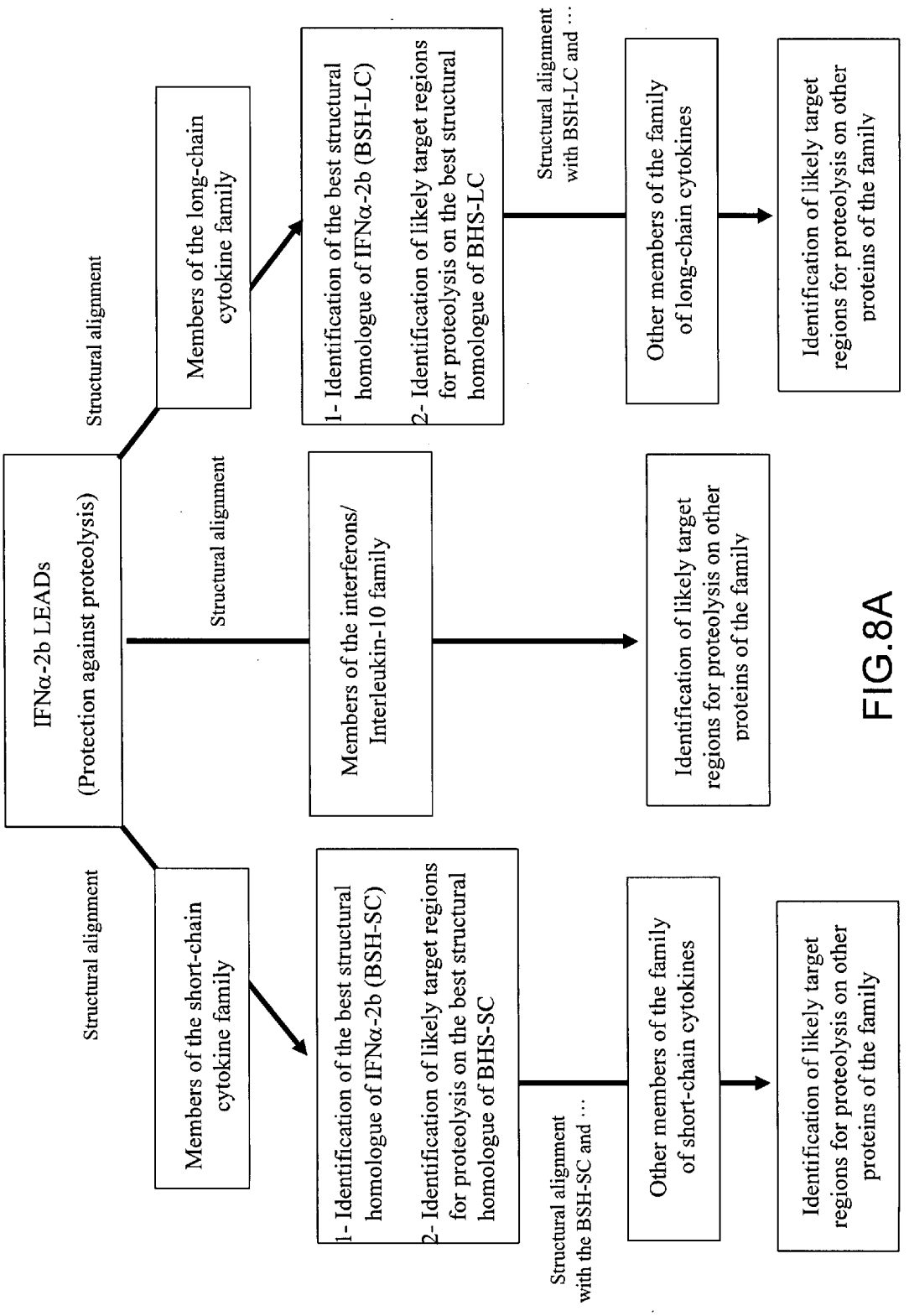
IFNα-2b LEADs
(Protection against proteolysis)
Structural alignment
Members of the short-chain cytokine family
1- Identification of the best structural homologue of IFNα-2b (BSH-SC)
2- Identification of likely target regions for proteolysis on the best structural homologue of BHS-SC
Structural alignment with the BSH-SC and ···
Other members of the family of short-chain cytokines
Identification of likely target regions for proteolysis on other proteins of the family
Structural alignment
Members of the interferons/ Interleukin-10 family
Identification of likely target regions for proteolysis on other proteins of the family
Structural alignment
Members of the long-chain cytokine family
1- Identification of the best structural homologue of IFNα-2b (BSH-LC)
2- Identification of likely target regions for proteolysis on the best structural homologue of BHS-LC
Structural alignment with BSH-LC and ···
Other members of the family of long-chain cytokines
Identification of likely target regions for proteolysis on other proteins of the family

FIG.8A

Structural super-imposition of interferon α-2b (1RH2) and interferon β (1AU1)

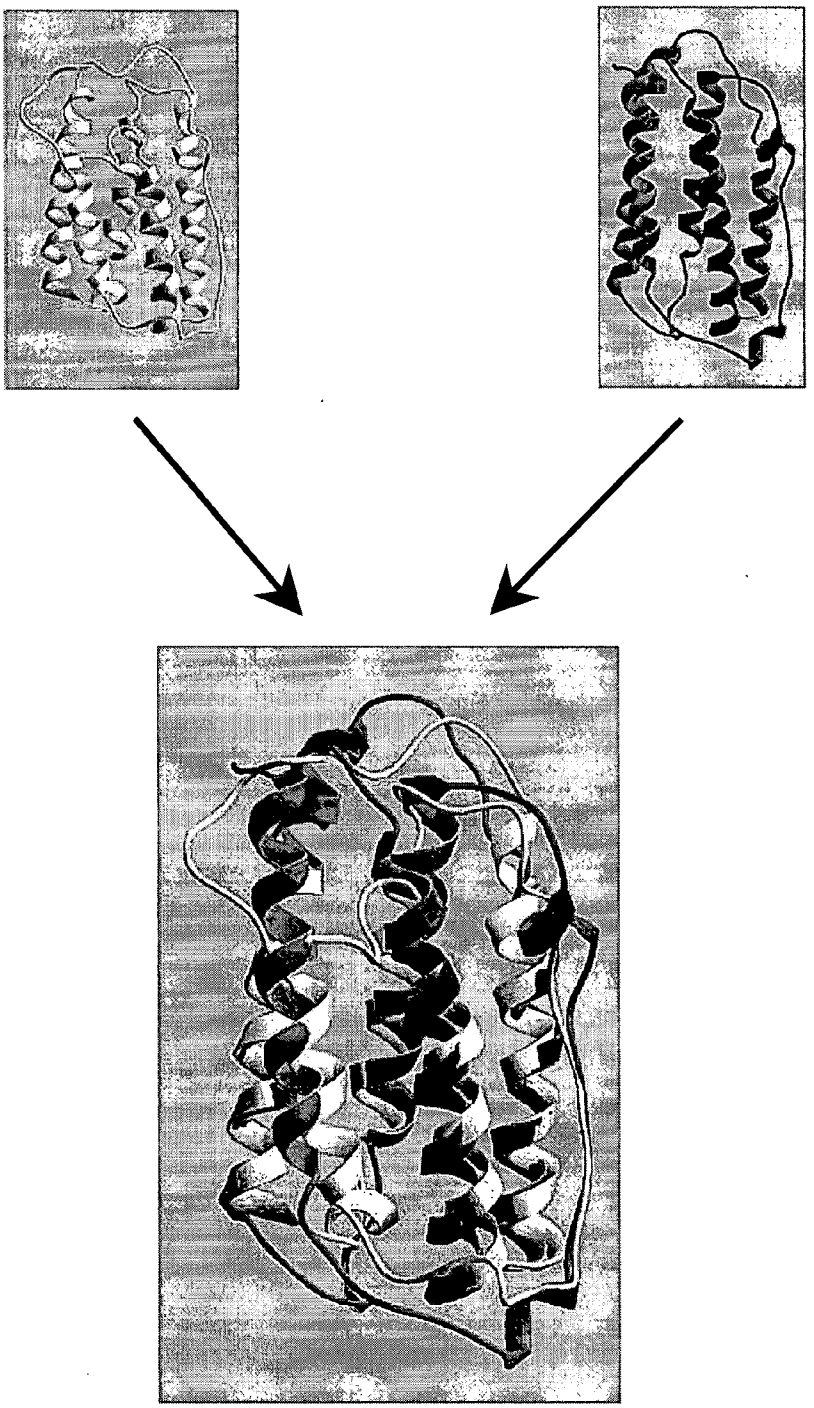

FIG.8B

Structural super-imposition of interferon α-2b (1RH2) and erythropoietin (1BUY)

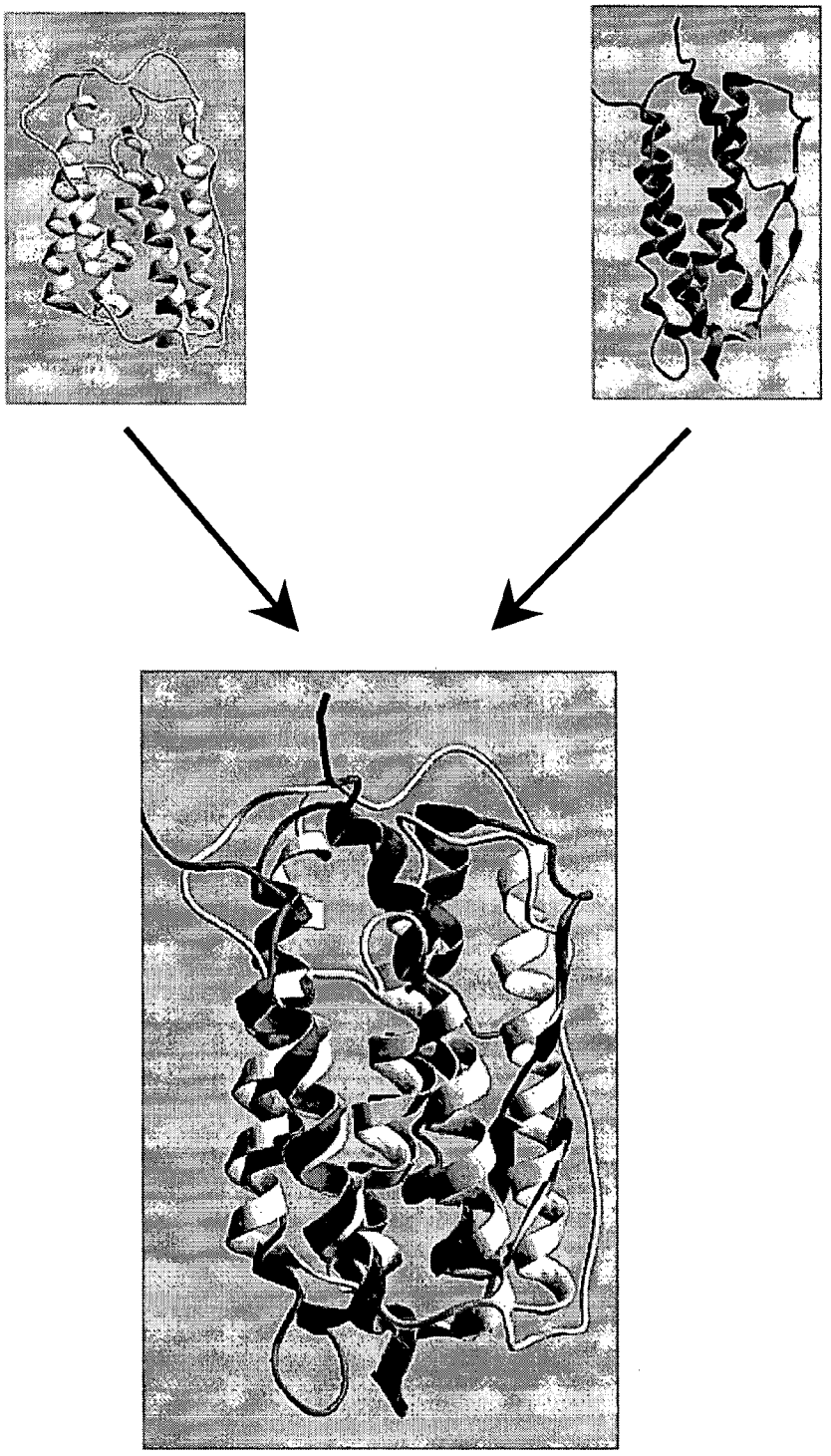

FIG.8C

Structural super-imposition of interferon α-2b (1RH2) and granulocyte-colony stimulating factor (1CD9)

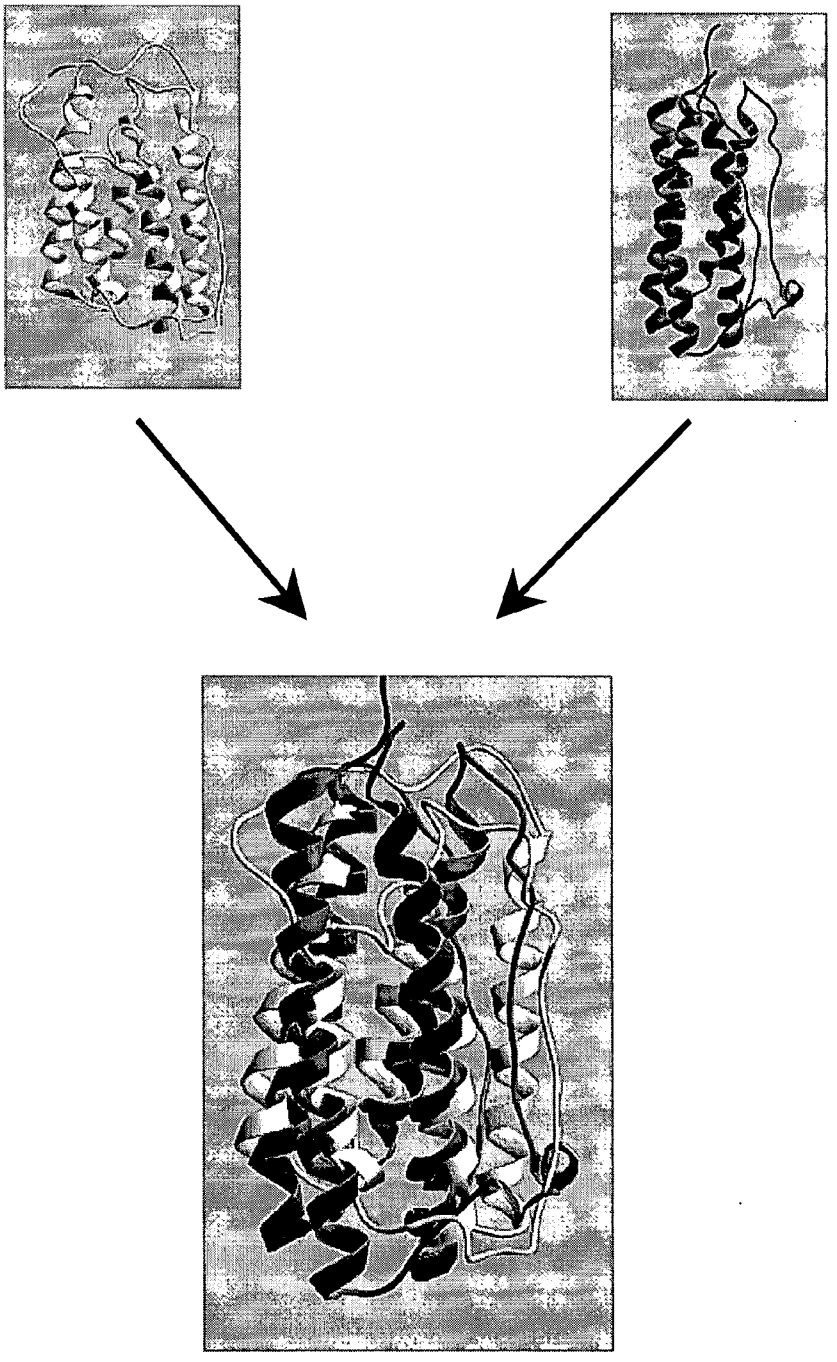

FIG.8D

Cytokine regions susceptible to protease attack identified by structural alignment with Lead mutants of IFN α-2b

IFN-α2b CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQG

VGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE

Exemplary protein of the interferons/interleukin-10 family

IFN-β MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEK

LEKEDFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN

Exemplary protein of the short-chain cytokines family

EPO APPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSL

TTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDR

Exemplary protein of the long-chain cytokines family

G-CSF TPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKLVSECATYKLCHPEELVLLGHSLGIPWAPLSSCPSQALQLAGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQL

DVADFATTIWQQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRHLAQP

FIG. 9

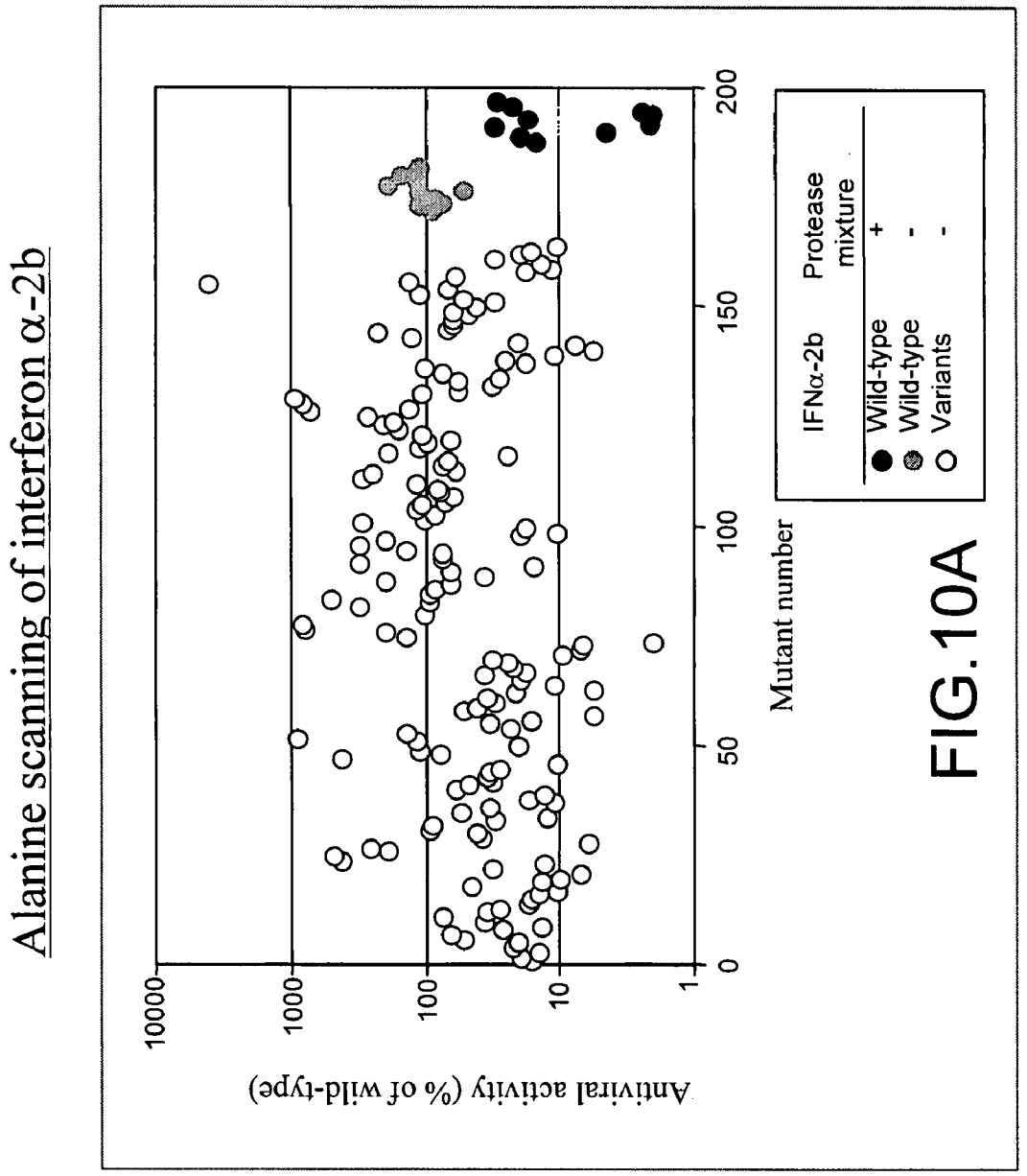
Alanine scanning of interferon α-2b
Antiviral activity (% of wild-type)
10000
1000
100
10
1
0
50
100
150
200
Mutant number
IFNα-2b
Protease mixture
Wild-type +
Wild-type -
Variants -

FIG.10A

Cell proliferation assay for alanine scanning of interferon α-2b

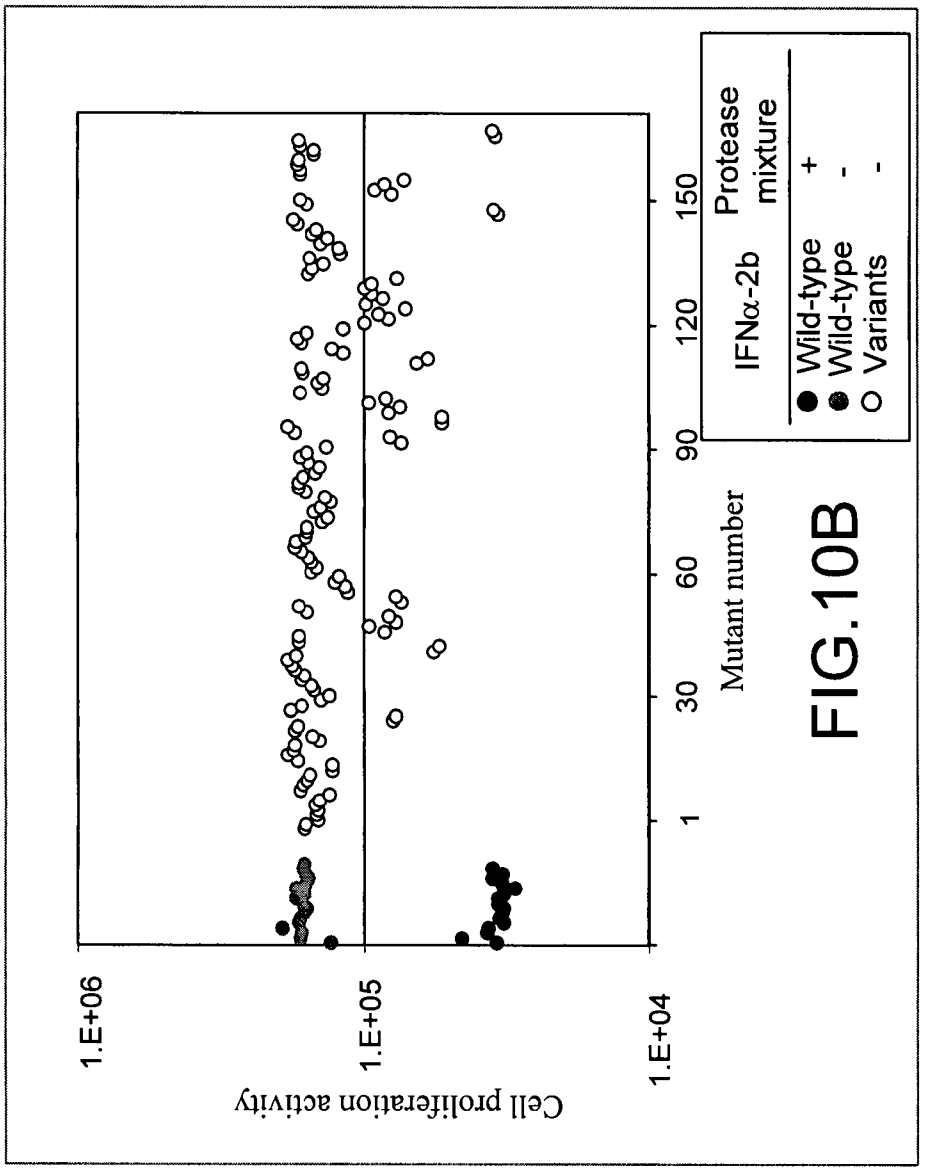

FIG.10B

Correlation between antiviral and cell proliferation activities for alanine scanning of interferon α-2b

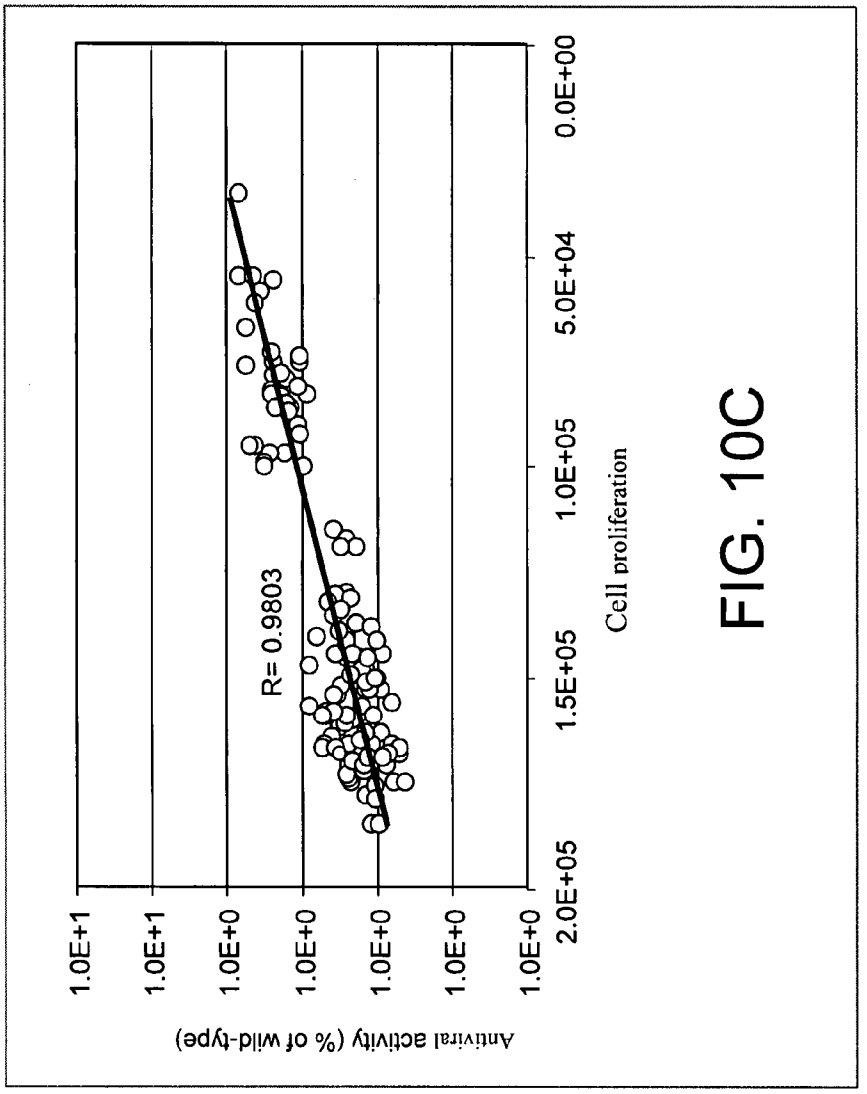

FIG. 10C

Glycosylation of interferon α-2b

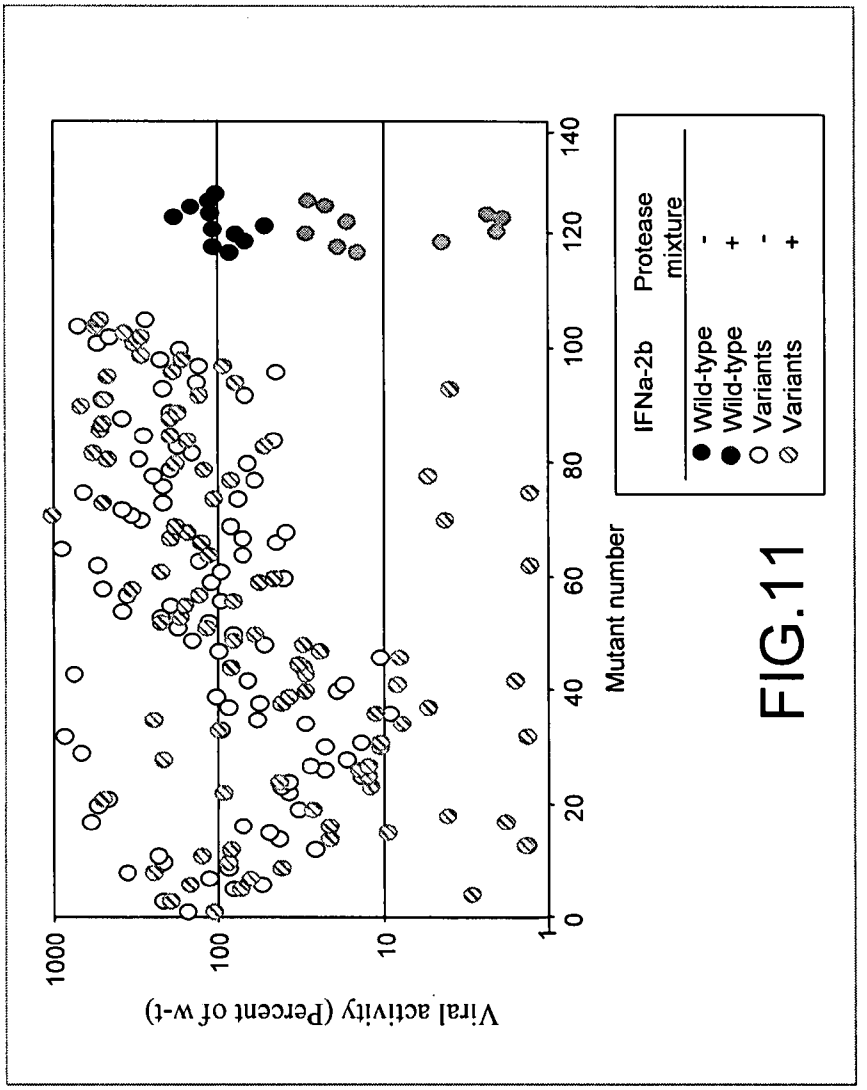

FIG.11

Interferon-beta

Protection against proteolysis

Sequence:

MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTI

YEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMS

SLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN

Exposed residues:

----------D--E--KQLQQ-QK-------

----Q--FA--RQD-SS-G-NET----------EKEDF-R--L--

SLH-KR--GR-LH--KAKE----------Y-RN

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	D39Q	16.	D73Q	31.	F111I	46.	L130I
2.	D39N	17.	D73N	32.	F111V	47.	K134Q
3.	E42Q	18.	E81Q	33.	R113H	48.	K134N
4.	E42N	19.	E81N	34.	R113Q	49.	K136Q
5.	E42H	20.	E81H	35.	L116V	50.	K136N
6.	K45Q	21.	E107Q	36.	L116I	51.	E137Q
7.	K45N	22.	E107N	37.	L120V	52.	E137N
8.	L47V	23.	E107H	38.	L120I	53.	E137H
9.	L47I	24.	K108Q	39.	K123Q	54.	Y163H
10.	K52Q	25.	K108N	40.	K123N	55.	Y163I
11.	K52N	26.	E109Q	41.	R124H	56.	R165H
12.	F67I	27.	E109N	42.	R124Q	57.	R165Q
13.	F67V	28.	E109H	43.	R128H		
14.	R71H	29.	D110Q	44.	R128Q		
15.	R71Q	30.	D110N	45.	L130V		

FIG. 12A

Interferon-gamma

Protection against proteolysis

Sequence:

CYCQDPYVKEAENLKKYFNAGHSDVADNGTLFLGILKNWKEESDRKIMQSQIVSFYFKL

FKNFKDDQSIQKSVETIKEDMNVKFFNSNKKKRDDFEKLTN

Exposed residues:

----------T--L---KN-KEE----------K-

-KN-KDDQS----------

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. L33V
2. L33I
3. K37Q
4. K37N
5. K40Q
6. K40N
7. E41Q
8. E41N
9. E41H
10. E42Q
11. E42N
12. E42H
13. K58Q
14. K58N
15. K61Q
16. K61N
17. K64Q
18. K64N
19. D65Q
20. D65N
21. D66Q

FIG. 12B

Interleukin-10

Protection against proteolysis

Sequence:

SPGQGTQSENSCTHFPGNLPNMLRDLRDAFSRVKTFFQMKDQLDNLLLKESLLEDFKGY

LGCQALSEMIQFYLEEVMPQAENQDPDIKAHVNSLGENLKT

Exposed residues:

----------KESLLEDFKGY

L------EM-QFY-EEV-PQ-ENQDPD----------K-

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	K49Q	18.	K57N	35.	E75Q
2.	K49N	19.	Y59H	36.	E75N
3.	E50Q	20.	Y59I	37.	E75H
4.	E50N	21.	L60V	38.	P78S
5.	E50H	22.	L60I	39.	P78A
6.	L52V	23.	E67Q	40.	E81Q
7.	L52I	24.	E67N	41.	E81N
8.	L53V	25.	E67H	42.	E81H
9.	L53I	26.	M68V	43.	D84Q
10.	E54Q	27.	M68I	44.	D84N
11.	E54N	28.	F71I	45.	P85S
12.	E54H	29.	F71V	46.	P85A
13.	D55Q	30.	Y72H	47.	D86Q
14.	D55N	31.	Y72I	48.	D86N
15.	F56I	32.	E74Q	49.	K88Q
16.	F56V	33.	E74N	50.	K88N
17.	K57Q	34.	E74H		

FIG. 12C

Ciliary neurotrophic factor

Protection against proteolysis

Sequence:

DSADGMPVASTDQWSELTEAERLQENLQAYRTFHVLLARLLEDQQVHFTPTEGDFHQAI

HTLLLQVAAFAYQIEELMILLEYKIPRNEADGMPINVGDGGLFEKKLWGLKVLQELSQW

TVRSIHDLRFISSHQTGIPA

Exposed residues:

-------VASTDQWSELT---------Q---T-HVL-AR--E--QVH--PTEGD-----

----------EYKIPRNE-DGMPINVGDG-L----------

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	D62Q	16.	E92H	31.	P135S
2.	D62N	17.	P100S	32.	P135A
3.	W64S	18.	P100A	33.	R136H
4.	W64H	19.	E102Q	34.	R136Q
5.	E66Q	20.	E102N	35.	E138Q
6.	E66N	21.	E102H	36.	E138N
7.	E66H	22.	D104Q	37.	E138H
8.	L67V	23.	D104N	38.	D140Q
9.	L67I	24.	E131Q	39.	D140N
10.	L86V	25.	E131N	40.	P143S
11.	L86I	26.	E131H	41.	P143A
12.	R89H	27.	Y132H	42.	D148Q
13.	R89Q	28.	Y132I	43.	D148N
14.	E92Q	29.	K133Q	44.	L151V
15.	E92N	30.	K133N	45.	L151I

FIG. 12D

Granulocyte-colony stimulating factor

Protection against proteolysis

Sequence:

VLLGHSLGIPWAPLSSCPSQALQLAGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTL

QLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSY

RVLRHLAQP

Exposed residues:

----------W-P-SS-PSQALQ---------S--F------Q--E---PE--------

------------------G-APALQPTQ-AM-A-ASAF----------

R--RH--QP-

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',

'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	W61S	12.	E96N	23.	P135S
2.	W61H	13.	E96H	24.	P135A
3.	P63S	14.	P100S	25.	F147I
4.	P63A	15.	P100A	26.	F147V
5.	P68S	16.	E101Q	27.	R169H
6.	P68A	17.	E101N	28.	R169Q
7.	L72V	18.	E101H	29.	R172H
8.	L72I	19.	P131S	30.	R172Q
9.	F86I	20.	P131A	31.	P177S
10.	F86V	21.	L133V	32.	P177A
11.	E96Q	22.	L133I		

FIG. 12E

Human growth hormone

Protection against proteolysis

Sequence:

SLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSN

VYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRK

DMDKVETFLRIVQCRSVEGSCGF

Expose residues:

-----ES-PT-SNREE--------------------E--QF-RS--AN-L--------

-------------------EDG-PRT-Q--KQTY-KFD---------------------

--------------RS-EGSCG-

Proteases: ['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	E56Q	17.	F92I	33.	K140N
2.	E56N	18.	F92V	34.	Y143H
3.	E56H	19.	R94H	35.	Y143I
4.	P59S	20.	R94Q	36.	K145Q
5.	P59A	21.	L101V	37.	K145N
6.	R64H	22.	L101I	38.	F146I
7.	R64Q	23.	E129Q	39.	F146V
8.	E65Q	24.	E129N	40.	D147Q
9.	E65N	25.	E129H	41.	D147N
10.	E65H	26.	D130Q	42.	R183H
11.	E66Q	27.	D130N	43.	R183Q
12.	E66N	28.	P133S	44.	E186Q
13.	E66H	29.	P133A	45.	E186N
14.	E88Q	30.	R134H	46.	E186H
15.	E88N	31.	R134Q		
16.	E88H	32.	K140Q		

FIG. 12F

Interleukin-12

Protection against proteolysis

Sequence:

DITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDL

KMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYK

TKIKLCILLHAFRIRAVTIDRVMSYLNAS

Exposed residues:

-----KT--VE----LELTKNES-LNSRETSF-TNGSCLA-RK----------E--

KM--VE-KT-N---LM-PKR-----------------------------------

--------------------R--S--NAS-

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	K56Q	15.	E72Q	29.	R92H	43.	K117Q
2.	K56N	16.	E72N	30.	R92Q	44.	K117N
3.	E61Q	17.	E72H	31.	K93Q	45.	L124V
4.	E61N	18.	L75V	32.	K93N	46.	L124I
5.	E61H	19.	L75I	33.	E107Q	47.	M125V
6.	L66V	20.	R78H	34.	E107N	48.	M125I
7.	L66I	21.	R78Q	35.	E107H	49.	P127S
8.	E67Q	22.	E79Q	36.	K110Q	50.	P127A
9.	E67N	23.	E79N	37.	K110N	51.	K128Q
10.	E67H	24.	E79H	38.	M111V	52.	K128N
11.	L68V	25.	F82I	39.	M111I	53.	R129H
12.	L68I	26.	F82V	40.	E115Q	54.	R129Q
13.	K70Q	27.	L89V	41.	E115N	55.	R189H
14.	K70N	28.	L89I	42.	E115H	56.	R189Q

FIG. 12G

Interleukin-6

Protection against proteolysis

Sequence:

SSKEALAENNLNLPKMAEKDGCFQSGFNEETCLVKIITGLLEFEVYLEYLQNRFESSEE

QARAVQMSTKVLIQFLQKKAKNLDAITTPDPTTNASLLTKLQAQNQWLQDMTTHLILRS

FKEFLQSSLRALRQM

Exposed residues:

----------PKMAEK---FQSGF----------T---E-----E--QNR-ES-E-

----------DA-TTPDPTT-AS--TK-QAQNQW----------

---------R--RQM

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',

'Staphylococcal P.']

Exclusion list:['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	P64S	16.	E92Q	31.	D133N
2.	P64A	17.	E92N	32.	P138S
3.	K65Q	18.	E92H	33.	P138A
4.	K65N	19.	E98Q	34.	D139Q
5.	M66V	20.	E98N	35.	D139N
6.	M66I	21.	E98H	36.	P140S
7.	E68Q	22.	R103H	37.	P140A
8.	E68N	23.	R103Q	38.	K149Q
9.	E68H	24.	E105Q	39.	K149N
10.	K69Q	25.	E105N	40.	W156S
11.	K69N	26.	E105H	41.	W156H
12.	F73I	27.	E108Q	42.	R178H
13.	F73V	28.	E108N	43.	R178Q
14.	F77I	29.	E108H	44.	R181H
15.	F77V	30.	D133Q	45.	R181Q

FIG. 12H

Leptin

Protection against proteolysis

Sequence:

VPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVTGLDFIPGLHPILTLSKMDQTLA

VYQQILTSMPSRNVIQISNDLENLRDLLHVLAFSKSCHLPWASGLETLDSLGGVLEASG

YSTEVVALSRLQGSLQDMLWQLDLSPGC

Exposed residues:

----------P--H-IL----------

----------SCH-PW-SGLETLDS--GV-----

----------DLS-GC

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. P43S
2. P43A
3. L49V
4. L49I
5. P99S
6. P99A
7. W100S
8. W100H
9. L104V
10. L104I
11. E105Q
12. E105N
13. E105H
14. L107V
15. L107I
16. D108Q
17. D108N
18. D141Q
19. D141N
20. L142V
21. L142I

FIG. 12I

Leukemia inhibitory factor

Protection against proteolysis

Sequence:

PFPNNLDKLCGPNVTDFPPFHANGTEKAKLVELYRIVVYLGTSLGNITRDQKILNPSAL

SLHSKLNATADILRGLLSNVLCRLCSKYHVGHVDVTYGPDTSGKDVFQKKKLGCQLLGK

YKQIIAVLAQAF

Exposed residues:

----------PFHAN-T---------R------T------R--KIL-PS-

LS----------YH-GHVDVTYGPD-SGKDVF----------

----------Q---

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',

'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	P69S	12.	L104I	23.	P148S
2.	P69A	13.	P106S	24.	P148A
3.	F70I	14.	P106A	25.	D149Q
4.	F70V	15.	L109V	26.	D149N
5.	R85H	16.	L109I	27.	K153Q
6.	R85Q	17.	Y137H	28.	K153N
7.	R99H	18.	Y137I	29.	D154Q
8.	R99Q	19.	D143Q	30.	D154N
9.	K102Q	20.	D143N	31.	F156I
10.	K102N	21.	Y146H	32.	F156V
11.	L104V	22.	Y146I		

FIG. 12J

Oncostatin M

Protection against proteolysis

Sequence:

ERPGAFPSEETLRGLGRRGFLQTLNATLGCVLHRLADLEQRLPKAQDLERSGLNIEDLE

KLQMARPNILGLRNNIYCMAQLLDNSDTAEPTKAGRGASQP

Exposed residues:

-------SEET-RGLG--------NA---C--HR-AD-EQR--KAQD-ERSGLNIE---

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	E59Q	12.	R84Q	23.	D97N
2.	E59N	13.	D87Q	24.	E99Q
3.	E59H	14.	D87N	25.	E99N
4.	E60Q	15.	E89Q	26.	E99H
5.	E60N	16.	E89N	27.	R100H
6.	E60H	17.	E89H	28.	R100Q
7.	R63H	18.	R91H	29.	L103V
8.	R63Q	19.	R91Q	30.	L103I
9.	L65V	20.	K94Q	31.	E106Q
10.	L65I	21.	K94N	32.	E106N
11.	R84H	22.	D97Q	33.	E106H

FIG. 12K

Erythropoietin

Protection against proteolysis

Sequence:

APPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQ

AVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKEA

ISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDR

Exposed residues:

----------N-T--DTKVNFYA-KR-EV---

--------A--SE--LR-QA--VNSSQ----------

ISPPDA-SAAPLR-IT----------RTGDR

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	D43Q	14.	E55N	27.	L130V
2.	D43N	15.	E55H	28.	L130I
3.	K45Q	16.	E72Q	29.	R131H
4.	K45N	17.	E72N	30.	R131Q
5.	F48I	18.	E72H	31.	R162H
6.	F48V	19.	L75V	32.	R162Q
7.	Y49H	20.	L75I	33.	D165Q
8.	Y49I	21.	R76H	34.	D165N
9.	K52Q	22.	R76Q	35.	P121S
10.	K52N	23.	D123Q	36.	P121A
11.	R53H	24.	D123N	37.	P122S
12.	R53Q	25.	P129S	38.	P122A
13.	E55Q	26.	P129A		

FIG. 12L

Flt3 ligand

Protection against proteolysis

Sequence:

TQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRLVLAQRWMER

LKTVAGSKMQGLLERVNTEIHFVTKCAFQPPPSCLRFVQTN

Exposed residues:

TQD----------T--S--QD-EL----------R--ER

-KTV-G----------A-QPPPSC-RFV---

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	D3Q	15.	R59H
2.	D3N	16.	R59Q
3.	D40Q	17.	K61Q
4.	D40N	18.	K61N
5.	E42Q	19.	P89S
6.	E42N	20.	P89A
7.	E42H	21.	P90S
8.	L43V	22.	P90A
9.	L43I	23.	P91S
10.	R55H	24.	P91A
11.	R55Q	25.	R95H
12.	E58Q	26.	R95Q
13.	E58N	27.	F96I
14.	E58H	28.	F96V

FIG. 12M

Granulocyte-macrophage colony-stimulating factor

Protection against proteolysis

Sequence:

APARSPSPSTQPWEHVNAIQEARRLLNLSRDTAAEMNETVEVISEMFDLQEPTCLQTRL

ELYKQGLRGSLTKLKGPLTMMASHYKQHCPPTPETSCATQIITFESFKENLKDFLLVIP

FDCWEPVQE

Exposed residues:

----------ET-E--SEM-DLQE--------

E--KQ--R----------PETSCATQI-T----------

FD--EP---

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase',

'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	E38Q	14.	L49V	27.	P92A
2.	E38N	15.	L49I	28.	E93Q
3.	E38H	16.	E51Q	29.	E93N
4.	E41Q	17.	E51N	30.	E93H
5.	E41N	18.	E51H	31.	F119I
6.	E41H	19.	E60Q	32.	F119V
7.	E45Q	20.	E60N	33.	D120Q
8.	E45N	21.	E60H	34.	D120N
9.	E45H	22.	K63Q	35.	E123Q
10.	M46V	23.	K63N	36.	E123N
11.	M46I	24.	R67H	37.	E123H
12.	D48Q	25.	R67Q	38.	P124S
13.	D48N	26.	P92S	39.	P124A

FIG. 12N

Interleukin-13

Protection against proteolysis

Sequence:

GPVPPSTALRELIEELVNITQNQKAPLCNGSMVWSINLTAGMYCAALESLINVSGCSAI

EKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLFREGRFN

Exposed residues:

------------------------------M-WS-NLTAG------E--INVSG----

----------------AGQFSSLHVRDTK------------------REGRFN

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	M32V	11.	F79V	21.	R107Q
2.	M32I	12.	L82V	22.	E108Q
3.	W34S	13.	L82I	23.	E108N
4.	W34H	14.	R85H	24.	E108H
5.	L38V	15.	R85Q	25.	R110H
6.	L38I	16.	D86Q	26.	R110Q
7.	E48Q	17.	D86N	27.	F111I
8.	E48N	18.	K88Q	28.	F111V
9.	E48H	19.	K88N		
10.	F79I	20.	R107H		

FIG. 120

Interleukin-2

Protection against proteolysis

Sequence:

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCL

EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFL

NRWITFCQSIISTLT

Exposed residues:

----------K-Y--KKATEL---Q--

EE--KP-EE--NL----------ETTFM-EYADET-T-----

----------STLT

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	K43Q	13.	L53I	25.	E68Q	37.	E106Q
2.	K43N	14.	E60Q	26.	E68N	38.	E106N
3.	Y45H	15.	E60N	27.	E68H	39.	E106H
4.	Y45I	16.	E60H	28.	L72V	40.	Y107H
5.	K48Q	17.	E61Q	29.	L72I	41.	Y107I
6.	K48N	18.	E61N	30.	E100Q	42.	D109Q
7.	K49Q	19.	E61H	31.	E100N	43.	D109N
8.	K49N	20.	P65S	32.	E100H	44.	E110Q
9.	E52Q	21.	P65A	33.	F103I	45.	E110N
10.	E52N	22.	E67Q	34.	F103V	46.	E110H
11.	E52H	23.	E67N	35.	M104V	47.	L132V
12.	L53V	24.	E67H	36.	M104I	48.	L132I

FIG. 12P

Interleukin-3

Protection against proteolysis

Sequence:

APMTQTTPLKTSWVNCSNMIDEIITHLKQPPLPLLDFNNLNGEDQDILMENNLRRPNLE

AFNRAVKSLQNASAIESILKNLLPCLPLATAAPTRHPIHIKDGDWNEFRRKLTFYLKTL

ENAQAQQTTLSLAIF

Exposed residues:

----------F-N-NGE-QD----------E

---R--KS-Q----------HP-H-KD-D----------

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. F37I
2. F37V
3. E43Q
4. E43N
5. E43H
6. D46Q
7. D46N
8. E59Q
9. E59N
10. E59H
11. R63H
12. R63Q
13. K66Q
14. K66N
15. P96S
16. P96A
17. K100Q
18. K100N
19. D101Q
20. D101N
21. D103Q
22. D103N

FIG. 12Q

Interleukin-4

Protection against proteolysis

Sequence:

HKCDITLQEIIKTLNSLTEQKTLCTELTVTDIFAASKNTTEKETFCRAATVLRQFYSHH

EKDTRCLGATAQQFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQSTLENFLERLKT

IMREKYSKCSS

Exposed residues:

-------------------------E-T-----AASKNTT------------RQ--SH-

EK-TR-L--------------------------------SCPVKEANQ------------

-------KCSS

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1. E26Q
2. E26N
3. E26H
4. K37Q
5. K37N
6. R53H
7. R53Q
8. E60Q
9. E60N
10. E60H
11. K61Q
12. K61N
13. R64H
14. R64Q
15. L66V
16. L66I
17. P100S
18. P100A
19. K102Q
20. K102N
21. E103Q
22. E103N
23. E103H
24. K126Q
25. K126N

FIG. 12R

Interleukin-5

Protection against proteolysis

Sequence:

IPTEIPTSALVKETLALLSTHRTLLIANETLRIPVPVHKNHQLCTEEIFQGIGTLESQT

VQGGTVERLFKNLSLIKKYIDGQKKKCGEERRRVNQFLDYLQEFLGVMNTEWIIES

Exposed residues:

------------------------------R-P--V-K------EE--Q--GT-ESQ-

------------------------KK-GEER-----------E-----NTEW----

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	R32H	13.	E56Q	25.	E89H
2.	R32Q	14.	E56N	26.	R90H
3.	P34S	15.	E56H	27.	R90Q
4.	P34A	16.	K84Q	28.	E102Q
5.	K39Q	17.	K84N	29.	E102N
6.	K39N	18.	K85Q	30.	E102H
7.	E46Q	19.	K85N	31.	E110Q
8.	E46N	20.	E88Q	32.	E110N
9.	E46H	21.	E88N	33.	E110H
10.	E47Q	22.	E88H	34.	W111S
11.	E47N	23.	E89Q	35.	W111H
12.	E47H	24.	E89N		

FIG. 12S

Stem cell factor

Protection against proteolysis

Sequence:

EGICRNRVTNNVKDVTKLVANLPKDYMITLKYVPGMDVLPSHCWISEMVVQLSDSLTDL

LDKFSNISEGLSNYSIIDKLVNIVDDLVECVKENSSKDLKKSFKSPEPRLFTPEEFFRI

FNRSIDAFKDFVVASETSDCVVS

Exposed residues:

----------M-T-K--P--DV----------V---D--TD-

-DKFSN----------SK-LKKSFKS-EPRL---------

----------ASETSDCVVS

Proteases:

['Trypsin', 'Endoproteinase Asp-N', 'Chymotrypsin', 'Proline endopeptidase', 'Staphylococcal P.']

Exclusion list:

['B', 'Z', 'X', '*', 'K', 'R', 'D', 'F', 'W', 'Y', 'M', 'L', 'P', 'E']

Substitutions:

1.	M27V	16.	K62N	31.	E106Q
2.	M27I	17.	F63I	32.	E106N
3.	K31Q	18.	F63V	33.	E106H
4.	K31N	19.	K96Q	34.	P107S
5.	P34S	20.	K96N	35.	P107A
6.	P34A	21.	L98V	36.	R108H
7.	D37Q	22.	L98I	37.	R108Q
8.	D37N	23.	K99Q	38.	L109V
9.	D54Q	24.	K99N	39.	L109I
10.	D54N	25.	K100Q	40.	E134Q
11.	D58Q	26.	K100N	41.	E134N
12.	D58N	27.	F102I	42.	E134H
13.	D61Q	28.	F102V	43.	D137Q
14.	D61N	29.	K103Q	44.	D137N
15.	K62Q	30.	K103N		

FIG. 12T

RATIONAL EVOLUTION OF CYTOKINES FOR HIGHER STABILITY, THE CYTOKINES AND ENCODING NUCLEIC ACID MOLECULES

RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 10/658,834, entitled, "RATIONAL EVOLUTION OF CYTOKINES FOR HIGHER STABILITY, THE CYTOKINES AND ENCODING NUCLEIC ACID MOLECULES," filed Sep. 8, 2003, which claims the benefit of priority under 35 U.S.C. 119(e) to U.S. provisional application Ser. No. 60/457,135, entitled "RATIONAL EVOLUTION OF CYTOKINES FOR HIGHER STABILITY, ENCODING NUCLEIC ACID MOLECULES AND RELATED APPLICATIONS," filed Mar. 21, 2003, and U.S. provisional application Ser. No. 60/409,898, entitled "RATIONAL EVOLUTION OF CYTOKINES FOR HIGHER STABILITY, ENCODING NUCLEIC ACID MOLECULES AND RELATED APPLICATIONS," filed Sep. 9, 2002, each to Rene Gantier, Thierry Guyon, Manuel Vega and Lila Drittanti.

[0002] This application is related to U.S. application Ser. No. 11/176,830, filed Jul. 6, 2005, which is also a continuation of U.S. application Ser. No. 10/658,834, filed Sep. 8, 2003. This application is also related to PCT Application No. PCT/IB03/004347, entitled, "RATIONAL EVOLUTION OF CYTOKINES FOR HIGHER STABILITY, THE CYTOKINES AND ENCODING NUCLEIC ACID MOLECULES," to Rene Gantier, Thierry Guyon, Manuel Vega and Lila Drittanti. This application also is related to U.S. application Ser. No. 10/658,355, filed Sep. 8, 2003, entitled "RATIONAL DIRECTED PROTEIN EVOLUTION USING TWO-DIMENSIONAL RATIONAL MUTAGENESIS SCANNING," and to U.S. provisional application Ser. No. 60/457,063, entitled "RATIONAL DIRECTED PROTEIN EVOLUTION USING TWO-DIMENSIONAL RATIONAL MUTAGENESIS SCANNING," filed Mar. 21, 2003, and to U.S. provisional application Ser. No. 60/410,258, entitled "RATIONAL DIRECTED PROTEIN EVOLUTION USING TWO-DIMENSIONAL RATIONAL MUTAGENESIS SCANNING," filed Sep. 9, 2002, each to Rene Gantier, Thierry Guyon, Hugo Cruz Ramos, Manuel Vega and Lila Drittanti. This application also is related to co-pending U.S. application Ser. No. 10/022,249, filed Dec. 17, 2001, entitled "HIGH THROUGHPUT DIRECTED EVOLUTION BY RATIONAL MUTAGENESIS," to Manuel Vega and Lila Drittanti.

[0003] The subject matter of each of the above-noted applications, international applications and provisional applications is incorporated by reference in its entirety.

Incorporation by Reference of Sequence Listing Provided on Compact Discs

[0004] An electronic version on compact disc (CD-R) of the Sequence Listing is filed herewith in duplicate (labeled Copy # 1 and Copy # 2), the contents of which are incorporated by reference in their entirety. The computer-readable file on each of the aforementioned compact discs, created on Feb. 1, 2007 is identical, 1,843 kilobytes in size, and titled 922CSEQ.001.txt.

FIELD OF INVENTION

[0005] Modified cytokine proteins having selected modified properties compared to the unmodified or wild type proteins, and nucleic acid molecules encoding these proteins are provided. The proteins can be used for treatment and diagnosis.

BACKGROUND

[0006] The delivery of therapeutic proteins for clinical use is a major challenge to pharmaceutical science. Once in the blood stream, these proteins are constantly eliminated from circulation within a short time by different physiological processes, involving metabolism as well as clearance using normal pathways for protein elimination, such as (glomerular) filtration in the kidneys or proteolysis in blood. The latter is often the limiting process affecting the half-life of proteins used as therapeutic agents in per-oral administration and either intravenous or intramuscular injection. The problems associated with these routes of administration of proteins are well known and various strategies have been used in attempts to solve them.

[0007] A protein family, which has been the focus of much clinical work, and efforts to improve its administration and bio-assimilation, is the cytokine family, including the interferon family. Interferon molecules are grouped in the heterogeneous family of cytokines, originally identified on the basis of their ability to induce cellular resistance to viral infections (Diaz et al., *J Interferon Cytokine Res.,* 16:179-180, 1996). Type I interferons, referred to as interferons α/β, include many members of the interferon α family (interferon α1, α2, ω and τ) as well as interferon β. The type II interferon γ is different from type I in its particular mechanisms that regulate its production. Whereas the production of interferons α/β is most efficiently induced in many types of cells upon viral infection, interferon-γ is produced mainly in cells of hemopoietic system, such as T-cells or natural killer cells, upon stimulation by antigens or cytokines, respectively. These two interferon systems are functionally non-redundant in the antiviral defense host.

[0008] Interferon α, hereinafter "interferon alpha-2b," or "interferon α-2b" or "IFNα-2b," used interchangeably, has a broad spectrum of biological effects, including antiviral effects. Antiviral effects include antiproliferative and immuno-modulatory actions (Stark et al., *Annu. Rev. Biochem.,* 67: 227-264, 1998). As well as eliciting strong antiviral activities in target cells, interferons α/β also activate effector cells of the innate immune system such as natural killer cells and macrophages (Pestka et al., *Annu. Rev. Biochem.,* 56: 727-777, 1987; Biron et al., *Annu. Rev. Immunol.,* 17:189-220, 1999). As part of its immuno-modulatory action, interferon type I protects T-lymphocytes from apoptosis (Scheel-Toeller et al., *Eur. J. Immunol.,* 29:2603-2612, 1999; Marrack et al., *J. Exp. Med.,* 189:521-530, 1999) and growth enhancing factors (Robert et al., *Hematol. Oncol.,* 4:113-120, 1986; Morikawa et al., *J. Immunol.,* 139:761-766, 1987). The biological effects of interferons α/β are initiated upon binding to the IFN type I receptor, which results in activation of several downstream effector molecules (Hibbert and Foster, *J. Interferon Cytokine Res.,* 19:309-318, 1999).

[0009] Interferons as well as many cytokines are important therapeutics. Since naturally occurring variants have not evolved as therapeutics, they often have undesirable side-effects as well as the above-noted problems of short-half life, administration and bioavailability. Hence, there is a need to

improve properties of cytokines, including interferons, for use as therapeutic agents. Therefore, among the objects herein, it is an object to provide cytokines that have improved therapeutic properties.

SUMMARY

[0010] Provided herein are methods for directed evolution of families of proteins and resulting families of modified proteins. A family, such as the cytokine protein family, is initially identified. A property or phenotype for modification, such as resistance to proteolysis for increased stability in blood, is selected for modification. A representative member or members of the family, such as members of the interferon α family, such as IFNα-2b or IFNα-2a, or interferon β family, is (are) selected. It is modified using any directed evolution method and protein(s) with a desired phenotype are screened and identified. In addition, the 3-dimensional structure of the protein can be mapped to topologically and spatially identify the loci that are modified to achieve the phenotypic change. 3-dimensional structures of other members of the family are generated or obtained and compared with the modified family member. Loci in the other family members that correspond on the protein to those modified in the original protein are identified and modified. The resulting proteins can be tested to confirm that they exhibit the modified phenotype.

[0011] Provided herein are methods for generating modified cytokines based on structural homology (3D scanning). These methods are based on the spatial and topological structure; they are not based on their underlying sequences of amino acid residues. The methods are used for identification of target sites for mutagenesis, particularly in families of target proteins. The targets are identified through comparison of patterns of protein backbone folding between and among structurally related proteins. The methods are exemplified herein for cytokines. Families of the modified cytokines also are provided herein.

[0012] Any protein known or otherwise available to those of skill in the art is suitable for modification, such as optimization or improvement of a selected property, using the directed evolution methods provided herein, including cytokines (e.g., IFNα, including IFNα-2b and IFNα-2a, and IFNβ) or any other proteins that have already been mutated or optimized.

[0013] Provided herein are modified cytokines that exhibit increased resistance to proteolysis as assessed in vivo or in vitro. Typically the increase in resistance is a least 5%, generally 8%, 10% or more. The modified cytokines provided herein include those designed by 3D scanning using the interferon α's that were modified based upon 2D scanning methods herein.

[0014] Also provided herein are modified (mutant) cytokine proteins, such as variants of IFNβ and IFNα, including IFNα-2b and IFNα-2a proteins and IFNβ proteins, that have altered, particularly, improved therapeutic properties, including higher stability compared to the unmodified forms. In particular, exemplary modified cytokines provided herein have increased stability, which, for example, improves their use as therapeutics. Among the modified cytokines provided herein are those that exhibit increased resistance to proteolysis compared to the unmodified cytokine. In particular, such resistance is at least 10%, 20%, 30%, 40%, 50%, 70%, 100% or more resistant to proteolysis compared to the unmodified cytokine. Also provided are cytokines that have increased anti-proliferative and/or antiviral activity and/or resistance to proteolysis compared to an unmodified cytokine.

[0015] Exemplary of the modified cytokines provided herein are modified interferons that exhibit higher stability compared to unmodified forms. Such modified interferons can be used for treating conditions in humans that are responsive to treatment with interferons, such, but are not limited to, as viral infections, cancer or tumors, undesired cell proliferation and for immuno-modulation.

[0016] Exemplary of proteins that can be modified by the 2D and 3D scanning methods provided herein are cytokines from the interferons/interleukin-10 family. This family includes, for example, interleukin-10 (IL-10; SEQ ID NO:200, interferon beta (IFNβ; SEQ ID NO: 196), interferon alpha-2a (IFNα-2a; SEQ ID NO: 182), interferon alpha-2b (IFNα-2b; SEQ ID NO:1), and interferon gamma (IFN-γ; SEQ ID NO: 199). The long-chain cytokine protein family includes, among others, granulocyte colony stimulating factor (G-CSF; SEQ ID NO: 210), leukemia inhibitory factor (LIF; SEQ ID NO: 213), growth hormone (hGH; SEQ ID NO: 216), ciliary neurotrophic factor (CNTF; SEQ ID NO: 212), leptin (SEQ ID NO: 211), oncostatin M (SEQ ID NO: 214), interleukin-6 (IL-6; SEQ ID NO: 217) and interleukin-12 (IL-12; SEQ ID NO: 215). The short-chain cytokine protein family includes, among others, erythropoietin (EPO; SEQ ID NO: 201), granulocyte-macrophage colony stimulating factor (GM-CSF; SEQ ID NO: 202), interleukin-2 (IL-2; SEQ ID NO: 204), interleukin-3 (IL-3; SEQ ID NO: 205), interleukin-4 (IL-4; SEQ ID NO: 207), interleukin-5 (IL-5; SEQ ID NO: 208), interleukin-13 (IL-13; SEQ ID NO: 209), Flt3 ligand (SEQ ID NO: 203) and stem cell factor (SCF; SEQ ID NO: 206). Modified forms of each that have increased resistance to proteolysis are provided. They were generated by comparison among the 3D-structures to identify residues that improve resistance to proteolysis.

[0017] Pharmaceutical compositions containing each modified cytokine and uses and methods of treatment are provided.

[0018] The modified cytokines have use as therapeutics. Each cytokine has improved biological and or therapeutic activity, compared to the know activity of the unmodified cytokine. Accordingly, uses of the cytokines for treatment of cytokine-mediated diseases and diseases for which immunotherapy is employed are provided. Methods of treatment using the modified cytokines for diseases also are provided. Each cytokine has a known therapeutic use, and such use is contemplated herein. Cytokines provided herein have improved properties, such as increased bioavailability, improved stability, particularly in vivo, and/or greater efficacy.

BRIEF DESCRIPTION OF THE FIGURES

[0019] FIG. **1**(A) displays the sequence of the mature IFNα-2b (SEQ ID NO: 1). Residues targeted by a mixture of proteases, including α-chymotrypsin (F, L, M, W, and Y), endoproteinase Arg-C(R), endoproteinase Asp-N (D), endoproteinase Glu-C (E), endoproteinase Lys-C (K), and trypsin (K, and R), are underlined and in bold lettering.

[0020] FIG. **1**(B) displays the structure of IFNα-2b obtained from the NMR structure of IFNα-2a (PDB code 1ITF) in ribbon representation. Surface residues exposed to the action of the proteases considered in FIG. **1**A are in space filling representation.

[0021] FIG. **2** depicts the "Percent Accepted Mutation" (PAM250) matrix Values given to identical residues are shown in gray squares. Highest values in the matrix are shown in black squares and correspond to the highest occurrence of substitution between two residues.

[0022] FIG. **3** presents the scores obtained from PAM250 analysis for the amino acid substitutions (replacing amino acids on the vertical axis; amino acid position on the horizontal axis) aimed at introducing resistance to proteolysis into the IFNα-2b at the protease target sequences. The two best replacing residues for each target amino acid according to the highest substitution scores are shown in black rectangles.

[0023] FIGS. **4**(A)-**4**(C) provide graphs of experiments indicating the levels of protection against in vitro proteolysis for IFNα-2b variants produced in mammalian cells. In FIGS. **4**(B) and **4**(C), the vertical axis indicates the relative level of non-proteolyzed protein and the horizontal axis indicates time in hours.

[0024] FIG. **5** displays the characterization of several IFNα-2b variants, produced in mammalian cells, treated with α-chymotrypsin.

[0025] FIG. **6**(A) shows the characterization of the E113H IFNα-2b variant when treated with α-chymotrypsin. The percent of residual (anti-viral) activity for the variant (black line and white circles) after treatment with α-chymotrypsin was compared to the treated wild-type IFNα-2b (dashed line and black squares). For this experiment, the E113H IFNα-2b variant was produced in mammalian cells.

[0026] FIG. **6**(B) shows the characterization of the E113H IFNα-2b variant treated with a mixture of proteases. The percent of residual (anti-viral) activity for the variant (black line and white circles) after treatment with protease mixture was compared to the treated wild-type IFNα-2b (dashed line and black squares). For this experiment, the E113H IFNα-2b variant was produced in mammalian cells.

[0027] FIG. **6**(C) presents the characterization of the E113H IFNα-2b variant treated with blood lysate. The percent of residual (anti-viral) activity for the variant (black line and white circles) after treatment with blood lysate was compared to the treated wild-type IFNα-2b (dashed line and black squares). For this experiment, the E113H IFNα-2b variant was produced in mammalian cells.

[0028] FIG. **6**(D) presents the characterization of the E113H IFNα-2b variant treated with serum. The percent of residual (anti-viral) activity for the variant (black line and white circles) after treatment with serum was compared to the treated wild-type IFNα-2b (dashed line and black squares). For this experiment, the E113H IFNα-2b variant was produced in mammalian cells.

[0029] FIGS. **6**(E) and **6**(F) provide graphs indicating the levels of protection against in vitro proteolysis for IFNα-2b variants produced in bacteria. In FIGS. **6**(E) and **6**(F), the vertical axis indicates the relative level of non-proteolyzed protein and the horizontal axis indicates time in hours. The percent of residual (anti-viral) activity for the variants (gray circles with continuous lines) after treatment were compared to the treated wild-type IFNα-2b (solid circles with dashed lines).

[0030] FIG. **6**(G) provides graphs indicating the in vitro potency for antiviral activity, for IFNα-2b variants produced in bacteria. The vertical axis indicates the level of antiviral activity and the horizontal axis indicates concentration of the variants at which each level of activity is achieved. The activity for the variants (continuous line with gray circles) was compared to that of the wild-type IFNα-2b (black triangles with dashed lines). The potency for each variant was calculated from the graphs as the concentration at the inflection point of the respective curves. FIG. **6**(T) shows the value of potency obtained for each variant tested compared to the wild type IFNα.

[0031] FIG. **6**(H) provides the in vitro potency for anti-proliferation activity, for IFNα-2b variants produced in bacteria. The activity for the variants was compared to that of the wild-type IFNα-2b in serial dilution experiments where the anti-proliferation activity was measured for a number of dilutions for each variant. Potency was calculated from the graphs as the concentration at the inflection point of the respective curves. The figure shows the value of potency obtained for each variant tested and in comparison to the wild type IFNα.

[0032] FIGS. **6**(I) to **6**(N) provide graphs indicating the pharmacokinetics in mice following subcutaneous injection of IFNα-2b variants produced in bacteria. The vertical axis indicates the level of antiviral activity in blood and the horizontal axis indicates the time after injection at which the level of antiviral activity is determined. The pharmacokinetics of the variants (in gray solid circles with gray continuous lines) was compared to that of the wild-type IFNα-2b (in black with dashed lines) and of a pegylated derivative (Pegasys, Roche) (36 μg/ml open triangles with continuous black lines; and 18 μg/ml open circles with continuous black lines); and vehicle (gray solid triangles with continuous gray lines. The Area Under the Curve (AUC) for each variant was calculated from the graphs and is shown in **6**(U).

[0033] FIG. **6**(O) provides graphs indicating the levels of protection against in vitro proteolysis for IFNβ variants produced in mammalian cells. FIG. **6**(N), the vertical axis indicates the relative level of non-proteolyzed protein and the horizontal axis indicates time in hours. The percent of residual (anti-viral) activity for the variants after treatment were compared to the treated wild-type IFNβ.

[0034] FIGS. **6**(P) to **6**(S) provide graphs indicating the in vitro potency for either antiviral activity (**6**(P) and **6**(Q)) or anti-proliferative activity (**6**(R) and **6**(S), for a number of IFNβ variants produced in mammalian cells. The vertical axis indicates the level of (antiviral or anti-proliferation) activity and the horizontal axis indicates the concentration of the variants at which each level of activity is achieved. The activity for the variants (**6**(Q) and (**6**(S)) was compared to that of the wild-type IFNβ (**6**(P) and (**6**(R)). The activity obtained with either no previous treatment or by treating the variants with proteases prior to the activity test is shown.

[0035] FIG. **6**(T) provides a comparison of antiviral activity (potency), anti-proliferation activity (potency), number of mutations present and AUC (from PK) for a number of IFNα-2b and in comparison with the wild-type IFNα-2b.

[0036] FIG. 6(U) provides IFN units injected and protein injected (μg/ml) for the data in FIG. 6(T).

[0037] FIG. 7(A) depicts a top view ribbon representation of IFNα-2b structure obtained from the NMR structure of IFNα-2a (PDB code 1ITF). Residues represented in "space filling" define (1) the "receptor binding region" based on either our "alanine scanning" analysis or on studies by Piehler et al., *J. Biol. Chem.,* 275:40425-40433, 2000, and Roisman et al., *Proc. Natl. Acad. Sci. USA,* 98:13231-13236, 2001 (in light-gray and dark-gray, respectively), and (2) replacing residues (LEADs) for resistance to proteolysis (in black).

[0038] FIG. 7(B) depicts a side view ribbon representation of IFNα-2b structure. Residue representation is as in FIG. 7A.

[0039] FIG. 8(A) schematizes the identification of homologous amino acid positions between a number of cytokines and the LEAD mutants of IFNα-2b using 3-dimensional scanning (also referred to herein as based on "structure-based homology" methods or "structural homology" methods).

[0040] FIG. 8(B) illustrates a structural overlapping between human interferon α-2b obtained from the NMR structure of IFNα-2a (PDB code 1ITF) and human interferon β (PDB code 1AU1) using Swiss PDB Viewer.

[0041] FIG. 8(C) illustrates a structural overlapping between human interferon α-2b obtained from the NMR structure of IFNα-2a (PDB code 1ITF) and erythropoietin (PDB code 1BUY) using Swiss PDB Viewer.

[0042] FIG. 8(D) illustrates a structural overlapping between human interferon α-2b obtained from the NMR structure of IFNα-2a (PDB code 1ITF) and granulocyte-colony stimulating factor (PDB code 1CD9) using Swiss PDB Viewer.

[0043] FIG. 9 illustrates a structural alignment of a number of cytokines and interferon α-2b sequences (SEQ ID NO: 1 (IFN-α2b); SEQ ID NO: 196 (IFN-β); SEQ ID NO: 201 (EPO); and SEQ ID NO: 210 (G-CSF)). Bold underlined residues define the region on each cytokine sequence that based on structural homology comparison corresponds to the structurally-related mutations found on the LEADs for protease resistance of IFNα-2b.

[0044] FIG. 10(A) shows the antiviral activity of interferon α-2b mutants generated by alanine-scanning analysis used for protein redesign. Plotted symbols for wild type and variants of interferon α-2b are indicated in the inset.

[0045] FIG. 10(B) displays cell proliferation after treatment with interferon α-2b mutants obtained by alanine-scanning analysis. Plotted symbols for wild type and variants of interferon α-2b are indicated in the inset.

[0046] FIG. 10(C) displays the correlation between the antiviral activity and cell proliferation activity of interferon α-2b mutants obtained by alanine-scanning analysis.

[0047] FIG. 11 Candidate glycosylation sites for interferon α-2b stabilization and redesign thereof.

[0048] FIG. 12 (A) shows a representative number of the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of interferon β (corresponding to SEQ ID Nos: 233-289, 989-1015, and 1016-1302) compared to the wild-type sequence (SEQ ID NO: 196), based on 3D-scanning (structural homology method), including PAM250 analysis.

[0049] FIG. 12 (B) displays the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of interferon gamma (corresponding to SEQ ID Nos: 290-311) compared to residues 1-100 of the wild-type sequence (SEQ ID NO: 199), based on structural homology and PAM250 analysis.

[0050] FIG. 12 (C) shows the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of interleukin-10 (corresponding to SEQ ID Nos: 312-361) compared to residues 1-100 of the wild-type sequence (SEQ ID NO: 200), based on structural homology and PAM250 analysis.

[0051] FIG. 12 (D) displays the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of ciliary neurotrophic factor (corresponding to SEQ ID Nos: 684-728) compared to residues 51-188 of the wild-type sequence (SEQ ID NO: 212), based on structural homology and PAM250 analysis.

[0052] FIG. 12(E) shows the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of granulocyte-colony stimulating factor (corresponding to SEQ ID Nos: 631-662) compared to residues 51-177 of the wild-type sequence (SEQ ID NO: 210), based on structural homology and PAM250 analysis.

[0053] FIG. 12 (F) displays the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of human growth hormone (corresponding to SEQ ID Nos: 850-895) compared to residues 51-191 of the wild-type sequence (SEQ ID NO: 216), based on structural homology and PAM250 analysis.

[0054] FIG. 12 (G) shows the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of interleukin-12 (corresponding to SEQ ID Nos: 794-849) compared to residues 51-197 of the wild-type sequence (SEQ ID NO: 215), based on structural homology and PAM250 analysis.

[0055] FIG. 12 (H) displays the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of interleukin-6 (corresponding to SEQ ID Nos: 896-939) compared to residues 51-183 of the wild-type sequence (SEQ ID NO: 217), based on structural homology and PAM250 analysis.

[0056] FIG. 12 (I) shows the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of leptin (corresponding to SEQ ID Nos: 663-683) compared to the wild-type sequence (SEQ ID NO: 211), based on structural homology and PAM250 analysis.

[0057] FIG. 12 (J) displays the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of leukemia inhibitory factor (corresponding to SEQ ID Nos: 729-760) compared to residues 51-180 of the wild-type sequence (SEQ ID NO: 213), based on structural homology and PAM250 analysis.

[0058] FIG. 12 (K) shows the is-HIT residue positions and type of replacing amino acids selected to generate modified

protein sequences of oncostatin M (corresponding to SEQ ID Nos: 761-793) compared to residues 51-150 of the wild-type sequence (SEQ ID NO: 214), based on structural homology and PAM250 analysis.

[0059] FIG. **12** (L) displays the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of erythropoietin (corresponding to SEQ ID Nos: 940-977) compared to the wild-type sequence (SEQ ID NO: 201), based on structural homology and PAM250 analysis.

[0060] FIG. **12** (M) shows the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of Flt3 ligand (corresponding to SEQ ID Nos: 401-428) compared to residues 1-100 of the wild-type sequence (SEQ ID NO: 203), based on structural homology and PAM250 analysis.

[0061] FIG. **12** (N) displays the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of granulocyte-macrophage colony-stimulating factor (corresponding to SEQ ID Nos: 362-400) compared to the wild-type sequence (SEQ ID NO: 202), based on structural homology and PAM250 analysis.

[0062] FIG. **12** (O) shows the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of interleukin-13 (corresponding to SEQ ID Nos: 603-630) compared to the wild-type sequence (SEQ ID NO: 209), based on structural homology and PAM250 analysis.

[0063] FIG. **12** (P) displays the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of interleukin-2 (corresponding to SEQ ID Nos: 429-476) compared to the wild-type sequence (SEQ ID NO: 204), based on structural homology and PAM250 analysis.

[0064] FIG. **12** (Q) shows the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of interleukin-3 (corresponding to SEQ ID Nos: 477-498) compared to the wild-type sequence (SEQ ID NO: 205), based on structural homology and PAM250 analysis.

[0065] FIG. **12** (R) displays the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of interleukin-4 (corresponding to SEQ ID Nos: 543-567) compared to the wild-type sequence (SEQ ID NO: 207), based on structural homology and PAM250 analysis.

[0066] FIG. **12** (S) shows the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of interleukin-5 (corresponding to SEQ ID Nos: 568-602) compared to the wild-type sequence (SEQ ID NO: 208), based on structural homology and PAM250 analysis.

[0067] FIG. **12** (T) displays the is-HIT residue positions and type of replacing amino acids selected to generate modified protein sequences of stem cell factor (corresponding to SEQ ID Nos: 499-542) compared to residues 1-141 of the wild-type sequence (SEQ ID NO: 206), based on structural homology and PAM250 analysis.

DETAILED DESCRIPTION

[0103] J. Uses of the Mutant IFNα and IFNβ Genes and Cytokines in Therapeutic Methods

[0104] 1. Fusion Proteins

[0105] 2. Nucleic Acid Molecules for Expression

[0106] 3. Formulation of Optimized Cytokines and Methods of Treatment

[0107] K. Examples

A. Definitions

[0108] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. All patents, patent applications, published applications and publications, Genbank sequences, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there is a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

[0109] As used herein, biological activity of a protein refers to any activity manifested by the protein in vivo.

[0110] As used herein, "a directed evolution method" refers to methods that "adapt" either natural proteins, synthetic proteins or protein domains to work in new or existing natural or artificial chemical or biological environments and/or to elicit new functions and/or to increase or decrease a given activity, and/or to modulate a given feature. Exemplary directed evolution methods include pure random mutagenesis methods; restricted random mutagenesis methods; and non-restricted rational mutagenesis methods, such as the rational directed evolution method described in copending U.S. application Ser. No. 10/022,249; and the 2-dimensional rational scanning method provided herein.

[0111] As used herein, two dimensional rational mutagenesis scanning (2D scanning) refers to the processes provided herein in which two dimensions of a particular protein sequence are scanned: (1) one dimension is to identify specific amino acid residues along the protein sequence to replace with different amino acids, referred to as is-HIT target positions, and (2) the second dimension is the amino acid type selected for replacing the particular is-HIT target, referred to as the replacing amino acid.

[0112] As used herein, in silico refers to research and experiments performed using a computer. In silico methods include, but are not limited to, molecular modeling studies, and biomolecular docking experiments.

[0113] As used herein, "is-HIT" refers to an in silico identified amino acid position along a target protein sequence that has been identified based on i) the particular protein properties to be evolved, ii) the protein's amino acid sequence, and/or iii) the known properties of the individual amino acids. These is-HIT loci on the protein sequence are identified without use of experimental biological methods. For example, once the protein feature(s) to be optimized is (are) selected, diverse sources of information or previous knowledge (i.e., protein primary, secondary or tertiary structures, literature, patents) are exploited to determine those amino acid positions that may be amenable to improved protein fitness by replacement with a different amino acid. This step utilizes protein analysis "in silico." All possible candidate amino acid positions along a target protein's primary sequence that might be involved in the feature being evolved are referred to herein as "in silico HITs" ("is-HITs"). The collection (library), of all is-HITs identified during this step represents the first dimension (target residue position) of the two-dimensional scanning methods provided herein.

[0114] As used herein, "amenable to providing the evolved predetermined property or activity," in the context of identifying is-HITs, refers to an amino acid position on a protein that is contemplated, based on in silico analysis, to possess properties or features that when replaced would result in the desired activity being evolved. The phrase "amenable to providing the evolved predetermined property or activity," in the context of identifying replacement amino acids, refers to a particular amino acid type that is contemplated, based on in silico analysis, to possess properties or features that when used to replace the original amino acid in the unmodified starting protein would result in the desired activity being evolved.

[0115] As used herein, high-throughput screening (HTS) refers to processes that test a large number of samples, such as samples of test proteins or cells containing nucleic acids encoding the proteins of interest to identify structures of interest or the identify test compounds that interact with the variant proteins or cells containing them. HTS operations are amenable to automation and are typically computerized to handle sample preparation, assay procedures and the subsequent processing of large volumes of data.

[0116] As used herein, the term "restricted," when used in the context of the identification of is-HIT amino acid positions along the protein sequence selected for amino acid replacement and/or the identification of replacing amino acids, means that fewer than all amino acids on the protein-backbone are selected for amino acid replacement; and/or fewer than all of the remaining 19 amino acids available to replace the original amino acid present in the unmodified starting protein are selected for replacement. In particular embodiments of the methods provided herein, the is-HIT amino acid positions are restricted, such that fewer than all amino acids on the protein-backbone are selected for amino acid replacement. In other embodiments, the replacing amino acids are restricted, such that fewer than all of the remaining 19 amino acids available to replace the native amino acid present in the unmodified starting protein are selected as replacing amino acids. In a particular embodiment, both of the scans to identify is-HIT amino acid positions and the replacing amino acids are restricted, such that fewer than all amino acids on the protein-backbone are selected for amino acid replacement and fewer than all of the remaining 19 amino acids available to replace the native amino acid are selected for replacement.

[0117] As used herein, "candidate LEADs," are mutant proteins that are contemplated as potentially having an alteration in any attribute, chemical, physical or biological property in which such alteration is sought. In the methods herein, candidate LEADs are generally generated by systematically replacing is-HITS loci in a protein or a domain

thereof with typically a restricted subset, or all, of the remaining 19 amino acids, such as obtained using PAM analysis. Candidate LEADs can be generated by other methods known to those of skill in the art tested by the high throughput methods herein.

[0118] As used herein, "LEADs" are "candidate LEADs" whose activity has been demonstrated to be optimized or improved for the particular attribute, chemical, physical or biological property. For purposes herein a "LEAD" typically has activity with respect to the function of interest that differs by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or more from the unmodified and/or wild type (native) protein. In certain embodiments, the change in activity is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein. In other embodiments, the change in activity is not more than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, of the activity of the unmodified target protein. In yet other embodiments, the change in activity is at least about 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times, 400 times, 500 times, 600 times, 700 times, 800 times, 900 times, 1000 times, or more greater than the activity of the unmodified target protein. The desired alteration, which can be either an increase or a reduction in activity, will depend upon the function or property of interest (e.g., +10%, ±20%, etc.). The LEADs may be further optimized by replacement of a plurality (2 or more) of "is-HIT" target positions on the same protein molecule to generate "super-LEADs."

[0119] As used herein, the term "super-LEAD" refers to protein mutants (variants) obtained by combining the single mutations present in two or more of the LEAD molecules into a single protein molecule. Accordingly, in the context of the modified proteins provided herein, the phrase "proteins comprising one or more single amino acid replacements" encompasses any combination of two or more of the mutations described herein for a respective protein. For example, the modified proteins provided herein having one or more single amino acid replacements can have can have any combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more of the amino acid replacements at the disclosed replacement positions. The collection of super-LEAD mutant molecules is generated, tested and phenotypically characterized one-by-one in addressable arrays. Super-LEAD mutant molecules are such that each molecule contains a variable number and type of LEAD mutations. Those molecules displaying further improved fitness for the particular feature being evolved, are referred to as super-LEADs. Super-LEADs can be generated by other methods known to those of skill in the art and tested by the high throughput methods herein. For purposes herein a super-LEAD typically has activity with respect to the function of interest that differs from the improved activity of a LEAD by a desired amount, such as at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or more from at least one of the LEAD mutants from which it is derived. As with LEADs, the change in the activity for super-LEADs is dependent upon the activity that is being "evolved." The desired alteration, which can be either an increase or a reduction in activity, will depend upon the function or property of interest.

[0120] As used herein, a recitation that modified protein has more antiviral activity (or other activity) than antiproliferative activity (or another activity) compared to the unmodified cytokine, is comparing the absolute value of the change in each activity compared to wild type.

[0121] As used herein, the phrase "altered loci" refers to the is-HIT amino acid positions in the LEADs or super-LEADs that are replaced with different replacing amino acids, resulting in the desired altered phenotype or activity.

[0122] As used herein, an exposed residue presents more than 15% of its surface exposed to the solvent.

[0123] As used herein, the phrase "structural homology" refers to the degree of coincidence in space between two or more protein backbones. Protein backbones that adopt the same protein structure, fold and show similarity upon three-dimensional structural superposition in space can be considered structurally homologous. Structural homology is not based on sequence homology, but rather on three-dimension homology. Two amino acids in two different proteins said to be homologous based on structural homology between those proteins, do not necessarily need to be in sequence-based homologous regions. For example, protein backbones that have a root mean squared (RMS) deviation of less than 3.5, 3.0, 2.5, 2.0, 1.7 or 1.5 angstroms (Å) at a given space position or defined region between each other can be considered to be structurally homologous in that region, and are referred to herein as having a "high coincidence" between their backbones. It is contemplated herein that substantially equivalent (e.g., "structurally related") amino acid positions that are located on two or more different protein sequences that share a certain degree of structural homology will have comparable functional tasks; also referred to herein as "structurally homologous loci." These two amino acids than can be said to be "structurally similar" or "structurally related" with each other, even if their precise primary linear positions on the amino acid sequences, when these sequences are aligned, do not match with each other. Amino acids that are "structurally related" can be far away from each other in the primary protein sequences, when these sequences are aligned following the rules of classical sequence homology.

[0124] As used herein, a structural homolog is a protein that is generated by structural homology.

[0125] As used herein, the phrase "unmodified target protein,""unmodified protein" or "unmodified cytokine," or grammatical variations thereof, refers to a starting protein that is selected for modification using the methods provided herein. The starting unmodified target protein can be the naturally occurring, wild type form of a protein. In addition, the starting unmodified target protein may have previously been altered or mutated, such that it differs from the native wild type isoform, but is nonetheless referred to herein as a starting unmodified target protein relative to the subsequently modified proteins produced herein. Thus, existing proteins known in the art that have previously been modified to have a desired increase or decrease in a particular biological activity compared to an unmodified reference protein can be selected and used herein as the starting "unmodified target protein." For example, a protein that has been modified from its native form by one or more single amino acid changes and possesses either an increase or decrease in a desired activity, such as resistance to proteolysis, can be

utilized with the methods provided herein as the starting unmodified target protein for further modification of either the same or a different biological activity.

[0126] Likewise, existing proteins known in the art that have previously been modified to have a desired increase or decrease in a particular biological activity compared to an unmodified reference protein can be selected and used herein for identification of structurally homologous loci on other structurally homologous target proteins. For example, a protein that has been modified by one or more single amino acid changes and possesses either an increase or decrease in a desired activity, such as resistance to proteolysis, can be utilized with the methods provided herein to identify on structurally homologous target proteins, corresponding structurally homologous loci that can be replaced with suitable replacing amino acids and tested for either an increase or decrease in the desired biological activity.

[0127] As used herein, the phrase "only one amino acid replacement occurs on each target protein" refers to the modification of a target protein, such that it differs from the unmodified form of the target protein by a single amino acid change. For example, in one embodiment, mutagenesis is performed by the replacement of a single amino acid residue at only one is-HIT target position on the protein backbone (e.g., "one-by-one" in addressable arrays), such that each individual mutant generated is the single product of each single mutagenesis reaction. The single amino acid replacement mutagenesis reactions are repeated for each of the replacing amino acids selected at each of the is-HIT target positions. Thus, a plurality of mutant protein molecules are produced, whereby each mutant protein contains a single amino acid replacement at only one of the is-HIT target positions.

[0128] As used herein, the phrase "pseudo-wild type," in the context of single or multiple amino acid replacements, are those amino acids that, while different from the original, such as native, amino acid at a given amino acid position, can replace the native one at that position without introducing any measurable change in a particular protein activity. A population of sets of nucleic acid molecules encoding a collection of mutant molecules is generated and phenotypically characterized such that proteins with amino acid sequences different from the original amino acid, but that still elicit substantially the same level (i.e., at least 10%, 50%, 70%, 90%, 95%, 100%, depending upon the protein) and type of desired activity as the original protein are selected.

[0129] As used herein, biological and pharmacological activity includes any activity of a biological pharmaceutical agent and includes, but is not limited to, resistance to proteolysis, biological efficiency, transduction efficiency, gene/transgene expression, differential gene expression and induction activity, titer, progeny productivity, toxicity, cytotoxicity, immunogenicity, cell proliferation and/or differentiation activity, anti-viral activity, morphogenetic activity, teratogenetic activity, pathogenetic activity, therapeutic activity, tumor suppressor activity, ontogenetic activity, oncogenetic activity, enzymatic activity, pharmacological activity, cell/tissue tropism and delivery.

[0130] As used herein, a "small region" on a polypeptide is relative term depending upon the size of the polypeptide, but typically refers to a region that is less than about 10%, 15%, 25% of the protein. A large region is greater than about 10%, 15% or 25% of the protein.

[0131] As used herein, "output signal" refers to parameters that can be followed over time and, if desired, quantified. For example, when a recombinant protein is introduced into a cell, the cell containing the recombinant protein undergoes a number of changes. Any such change that can be monitored and used to assess the transformation or transfection, is an output signal, and the cell is referred to as a reporter cell; the encoding nucleic acid is referred to as a reporter gene, and the construct that includes the encoding nucleic acid is a reporter construct. Output signals include, but are not limited to, enzyme activity, fluorescence, luminescence, amount of product produced and other such signals. Output signals include expression of a gene or gene product, including heterologous genes (transgenes) inserted into the plasmid virus. Output signals are a function of time ("t") and are related to the amount of protein used in the composition. For higher concentrations of protein, the output signal can be higher or lower. For any particular concentration, the output signal increases as a function of time until a plateau is reached. Output signals can also measure the interaction between cells, expressing heterologous genes, and biological agents

[0132] As used herein, the activity of an IFNα-2b or IFNα-2a protein refers to any biological activity that can be assessed. In particular, herein, the activity assessed for the IFNα-2b or IFNα-2a proteins is resistance to proteolysis, antiviral activity and cell proliferation activity.

[0133] As used herein, the Hill equation is a mathematical model that relates the concentration of a drug (i.e., test compound or substance) to the response measured

$$y = \frac{y_{max}[D]^x}{[D]^n + [D_{50}]^n}$$

where y is the variable measured, such as a response, signal, y_{max} is the maximal response achievable, [D] is the molar concentration of a drug, [D50] is the concentration that produces a 50% maximal response to the drug, n is the slope parameter, which is 1 if the drug binds to a single site and with no cooperativity between or among sites. A Hill plot is $\log_{10}$ of the ratio of ligand-occupied receptor to free receptor vs. log [D] (M). The slope is n, where a slope of greater than 1 indicates cooperativity among binding sites, and a slope of less than 1 can indicate heterogeneity of binding. This general equation has been employed for assessing interactions in complex biological systems (see, published International PCT application No. WO 01/44809 based on PCT No. PCT/FR00/03503, see also, the EXAMPLES).

[0134] As used herein, in the Hill-based analysis (published International PCT application No. WO 01/44809 based on PCT No. PCT/FR00/03503), the parameters, π, κ, τ, ϵ, η, θ, are as follows:

[0135] π is the potency of the biological agent acting on the assay (cell-based) system;

[0136] κ is the constant of resistance of the assay system to elicit a response to a biological agent;

[0137] ϵ is the global efficiency of the process or reaction triggered by the biological agent on the assay system;

[0138] τ is the apparent titer of the biological agent;

[0139] θ is the absolute titer of the biological agent; and

[0140] η is the heterogeneity of the biological process or reaction.

[0141] In particular, as used herein, the parameters π (potency) or κ (constant of resistance) are used to respectively assess the potency of a test agent to produce a response in an assay system and the resistance of the assay system to respond to the agent.

[0142] As used herein, ϵ (efficiency), is the slope at the inflexion point of the Hill curve (or, in general, of any other sigmoidal or linear approximation), to assess the efficiency of the global reaction (the biological agent and the assay system taken together) to elicit the biological or pharmacological response.

[0143] As used herein, τ (apparent titer) is used to measure the limiting dilution or the apparent titer of the biological agent.

[0144] As used herein, θ (absolute titer), is used to measure the absolute limiting dilution or titer of the biological agent.

[0145] As used herein, η (heterogeneity) measures the existence of discontinuous phases along the global reaction, which is reflected by an abrupt change in the value of the Hill coefficient or in the constant of resistance.

[0146] As used herein, a population of sets of nucleic acid molecules encoding a collection (library) of mutants refers to a collection of plasmids or other vehicles that carry (encode) the gene variants, such that individual plasmids or other individual vehicles carry individual gene variants. Each element (member) of the collection is physically separated from the others, such as individually in an appropriate addressable array, and has been generated as the single product of an independent mutagenesis reaction. When a collection (library) of such proteins is contemplated, it will be so-stated.

[0147] As used herein, a "reporter cell" is the cell that "reports," i.e., undergoes the change, in response to a condition, such as, for example, exposure to a protein or a virus or to a change it its external or internal environment.

[0148] As used herein, "reporter" or "reporter moiety" refers to any moiety that allows for the detection of a molecule of interest, such as a protein expressed by a cell. Reporter moieties include, but are not limited to, for example, fluorescent proteins, such as red, blue and green fluorescent proteins; LacZ and other detectable proteins and gene products. For expression in cells, nucleic acid encoding the reporter moiety can be expressed as a fusion protein with a protein of interest or under to the control of a promoter of interest.

[0149] As used herein, phenotype refers to the physical, physiological or other manifestation of a genotype (a sequence of a gene). In methods herein, phenotypes that result from alteration of a genotype are assessed.

[0150] As used herein, "activity" means in the largest sense of the term any change in a system (either biological, chemical or physical system) of any nature (changes in the amount of product in an enzymatic reaction, changes in cell proliferation, in immunogenicity, in toxicity) caused by a protein or protein mutant when they interact with that system. In addition, the term "activity,""higher activity" or "lower activity" as used herein in reference to resistance to proteases, proteolysis, incubation with serum or with blood, means the ratio or residual biological (antiviral) activity between "after" protease/blood or serum treatment and "before" protease/blood or serum treatment.

[0151] As used herein, activity refers to the function or property to be evolved. An active site refers to a site(s) responsible or that participates in conferring the activity or function. The activity or active site evolved (the function or property and the site conferring or participating in conferring the activity) can have nothing to do with natural activities of a protein. For example, it could be an "active site" for conferring immunogenicity (immunogenic sites or epitopes) on a protein.

[0152] As used herein, treatment means any manner in which the symptoms of a condition, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the modified cytokines and compositions provided herein.

[0153] As used herein, cytokine-mediated or cytokine-involved diseases refer to diseases in which cytokines potentiate, cause or are involved in the disease process or to diseases in which administration of a cytokine is ameliorative of a disease or symptoms thereof. Cytokines can be used in immunotherapeutic therapies or protocols.

[0154] As used herein, the amino acids, which occur in the various amino acid sequences appearing herein, are identified according to their known, three-letter or one-letter abbreviations (see, Table 1). The nucleotides, which occur in the various nucleic acid fragments, are designated with the standard single-letter designations used routinely in the art.

[0155] As used herein, amino acid residue refers to an amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are presumed to be in the "L" isomeric form. Residues in the "D" isomeric form, which are so-designated, can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in *J. Biol. Chem.,* 243:3552-3559, 1969, and adopted at 37 C.F.R. §§ 1.821-1.822, abbreviations for amino acid residues are shown in Table 1:

TABLE 1

Table of Correspondence

SYMBOL		
1-Letter	3-Letter	AMINO ACID
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine

TABLE 1-continued

Table of Correspondence

SYMBOL		
1-Letter	3-Letter	AMINO ACID
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
B	Asx	Asn and/or Asp
C	Cys	cysteine
X	Xaa	Unknown or other

[0156] It should be noted that all amino acid residue sequences represented herein by formulae have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence (Table 1) and modified and unusual amino acids, such as those referred to in 37 C.F.R. §§ 1.821-1.822, and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or to an amino-terminal group such as NH2 or to a carboxyl-terminal group such as COOH.

[0157] As used herein, nucleic acids include DNA, RNA and analogs thereof, including protein nucleic acids (PNA) and mixtures thereof. Nucleic acids can be single or double stranded. When referring to probes or primers, optionally labeled, with a detectable label, such as a fluorescent or radiolabel, single-stranded molecules are contemplated. Such molecules are typically of a length such that they are statistically unique of low copy number (typically less than 5, generally less than 3) for probing or priming a library. Generally a probe or primer contains at least 14, 16 or 30 contiguous of sequence complementary to or identical a gene of interest. Probes and primers can be 10, 14, 16, 20, 30, 50, 100 or more nucleic acid bases long.

[0158] Therefore, as used herein, the term "identity" represents a comparison between a test and a reference polypeptide or polynucleotide. For example, a test polypeptide can be defined as any polypeptide that is 90% or more identical to a reference polypeptide.

[0159] As used herein, "corresponding structurally-related" positions on two or more proteins, such as the IFNα-2b protein and other cytokines, refers those amino acid positions determined based upon structural homology to maximize tri-dimensional overlapping between proteins.

[0160] As used herein, the term at least "90% identical to" refers to percent identities from 90 to 100% relative to the reference polypeptides. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polypeptide length of 100 amino acids are compared. No more than 10% (i.e., 10 out of 100) amino acids in the test polypeptide differ from that of the reference polypeptides. Similar comparisons can be made between a test and reference polynucleotides. Such differences can be represented as point mutations randomly distributed over the entire length of an amino acid sequence or they can be clustered in one or more locations of varying length up to the maximum allowable, e.g., 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, or deletions.

[0161] As used herein, the phrase "sequence-related proteins" refers to proteins that have at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% amino acid identity or homology with each other.

[0162] As used herein, families of non-related proteins or "sequence-non-related proteins" refers to proteins that have less than 50%, less than 40%, less than 0%, less than 20% amino acid identity or homology with each other.

[0163] As used herein, it also is understood that the terms "substantially identical" or "similar" varies with the context as understood by those skilled in the relevant art.

[0164] As used herein, heterologous or foreign nucleic acid, such as DNA and RNA, are used interchangeably and refer to DNA or RNA that does not occur naturally as part of the genome in which it is present or which is found in a location or locations in the genome that differ from that in which it occurs in nature. Heterologous nucleic acid is generally not endogenous to the cell into which it is introduced, but has been obtained from another cell or prepared synthetically. Generally, although not necessarily, such nucleic acid encodes RNA and proteins that are not normally produced by the cell in which it is expressed. Heterologous DNA herein encompasses any DNA or RNA that one of skill in the art would recognize or consider as heterologous or foreign to the cell in which it is expressed. Heterologous DNA and RNA can also encode RNA or proteins that mediate or alter expression of endogenous DNA by affecting transcription, translation, or other regulatable biochemical processes. Examples of heterologous nucleic acid include, but are not limited to, nucleic acid that encodes traceable marker proteins, such as a protein that confers drug resistance, nucleic acid that encodes therapeutically effective substances, such as anti-cancer agents, enzymes and hormones, and DNA that encodes other types of proteins, such as antibodies.

[0165] Hence, herein heterologous DNA or foreign DNA, includes a DNA molecule not present in the exact orientation and position as the counterpart DNA molecule found in the genome. It can also refer to a DNA molecule from another organism or species (i.e., exogenous).

[0166] As used herein, a therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of disease.

[0167] As used herein, isolated with reference to a nucleic acid molecule or polypeptide or other biomolecule means that the nucleic acid or polypeptide has separated from the genetic environment from which the polypeptide or nucleic

acid were obtained. It can also mean altered from the natural state. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated," as the term is employed herein. Thus, a polypeptide or polynucleotide produced and/or contained within a recombinant host cell is considered isolated. Also intended as an "isolated polypeptide" or an "isolated polynucleotide" are polypeptides or polynucleotides that have been purified, partially or substantially, from a recombinant host cell or from a native source. For example, a recombinantly produced version of a compound can be substantially purified by the one-step method described in Smith et al., *Gene,* 67:31-40, 1988. The terms isolated and purified are sometimes used interchangeably.

[0168] Thus, by "isolated" is meant that the nucleic is free of the coding sequences of those genes that, in the naturally-occurring genome of the organism (if any) immediately flank the gene encoding the nucleic acid of interest. Isolated DNA can be single-stranded or double-stranded, and can be genomic DNA, cDNA, recombinant hybrid DNA, or synthetic DNA. It can be identical to a starting DNA sequence, or can differ from such sequence by the deletion, addition, or substitution of one or more nucleotides.

[0169] Isolated or purified as it refers to preparations made from biological cells or hosts means any cell extract containing the indicated DNA or protein including a crude extract of the DNA or protein of interest. For example, in the case of a protein, a purified preparation can be obtained following an individual technique or a series of preparative or biochemical techniques and the DNA or protein of interest can be present at various degrees of purity in these preparations. The procedures can include for example, but are not limited to, ammonium sulfate fractionation, gel filtration, ion exchange change chromatography, affinity chromatography, density gradient centrifugation and electrophoresis.

[0170] A preparation of DNA or protein that is "substantially pure" or "isolated" should be understood to mean a preparation free from naturally occurring materials with which such DNA or protein is normally associated in nature. "Essentially pure" should be understood to mean a "highly" purified preparation that contains at least 95% of the DNA or protein of interest.

[0171] A cell extract that contains the DNA or protein of interest should be understood to mean a homogenate preparation or cell-free preparation obtained from cells that express the protein or contain the DNA of interest. The term "cell extract" is intended to include culture media, especially spent culture media from which the cells have been removed.

[0172] As used herein, "a targeting agent" refers to any molecule that can bind another target-molecule, such as an antibody, receptor, or ligand.

[0173] As used herein, receptor refers to a biologically active molecule that specifically binds to (or with) other molecules. The term "receptor protein" can be used to more specifically indicate the proteinaceous nature of a specific receptor.

[0174] As used herein, recombinant refers to any progeny formed as the result of genetic engineering.

[0175] As used herein, a promoter region refers to the portion of DNA of a gene that controls transcription of the DNA to which it is operatively linked. The promoter region includes specific sequences of DNA that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to as the promoter. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of the RNA polymerase. These sequences can be cis acting or can be responsive to trans acting factors. Promoters, depending upon the nature of the regulation, can be constitutive or regulated.

[0176] As used herein, the phrase "operatively linked" generally means the sequences or segments have been covalently joined into one piece of DNA, whether in single or double stranded form, whereby control or regulatory sequences on one segment control or permit expression or replication or other such control of other segments. The two segments are not necessarily contiguous. For gene expression a DNA sequence and a regulatory sequence(s) are connected in such a way to control or permit gene expression when the appropriate molecular, e.g., transcriptional activator proteins, are bound to the regulatory sequence(s).

[0177] As used herein, production by recombinant means by using recombinant DNA methods means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA, including cloning expression of genes and methods, such as gene shuffling and phage display with screening for desired specificities.

[0178] As used herein, a splice variant refers to a variant produced by differential processing of a primary transcript of genomic DNA that results in more than one type of mRNA.

[0179] As used herein, a composition refers to any mixture of two or more products or compounds. It can be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

[0180] As used herein, a combination refers to any association between two or more items.

[0181] As used herein, substantially identical to a product means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place of the product.

[0182] As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of exemplary vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Exemplary vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids" which refer generally to circular double stranded DNA loops which, in their vector form are not bound to the chromosome. "Plasmid" and "vector" are used interchangeably as the plasmid is the most commonly used form of vector. Other such other forms of expression vectors that serve equivalent functions and that become known in the art subsequently hereto.

[0183] As used herein, vector also is used interchangeable with "virus vector" or "viral vector. In this case, which will be clear from the context, the "vector" is not self-replicating. Viral vectors are engineered viruses that are operatively linked to exogenous genes to transfer (as vehicles or shuttles) the exogenous genes into cells.

[0184] As used herein, transduction refers to the process of gene transfer into and expression in mammalian and other cells mediated by viruses. Transfection refers to the process when mediated by plasmids.

[0185] As used herein, transformation refers to the process of gene transfer into and expression in bacterial cells mediated by plasmids.

[0186] As used herein, "allele," which is used interchangeably herein with "allelic variant" refers to alternative forms of a gene or portions thereof. Alleles occupy the same locus or position on homologous chromosomes. When a subject has two identical alleles of a gene, the subject is said to be homozygous for the gene or allele. When a subject has two different alleles of a gene, the subject is said to be heterozygous for the gene. Alleles of a specific gene can differ from each other in a single nucleotide, or several nucleotides, and can include substitutions, deletions, and insertions of nucleotides. An allele of a gene also can be a form of a gene containing a mutation.

[0187] As used herein, the term "gene" or "recombinant gene" refers to a nucleic acid molecule comprising an open reading frame and including at least one exon and (optionally) an intron sequence. A gene can be either RNA or DNA. Genes can include regions preceding and following the coding region (leader and trailer).

[0188] As used herein, "intron" refers to a DNA sequence present in a given gene which is spliced out during mRNA maturation.

[0189] As used herein, "nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO:" refers to the nucleotide sequence of the complementary strand of a nucleic acid strand having the particular SEQ ID NO:. The term "complementary strand" is used herein interchangeably with the term "complement." The complement of a nucleic acid strand can be the complement of a coding strand or the complement of a non-coding strand. When referring to double stranded nucleic acids, the complement of a nucleic acid having a particular SEQ ID NO: refers to the complementary strand of the strand set forth in the particular SEQ ID NO: or to any nucleic acid having the nucleotide sequence of the complementary strand of the particular SEQ ID NO:. When referring to a single stranded nucleic acid having a nucleotide sequence corresponding to a particular SEQ ID NO:, the complement of this nucleic acid is a nucleic acid having a nucleotide sequence which is complementary to that of the particular SEQ ID NO:.

[0190] As used herein, the term "coding sequence" refers to that portion of a gene that encodes an amino acid sequence of a protein.

[0191] As used herein, the term "sense strand" refers to that strand of a double-stranded nucleic acid molecule that has the sequence of the mRNA that encodes the amino acid sequence encoded by the double-stranded nucleic acid molecule.

[0192] As used herein, the term "antisense strand" refers to that strand of a double-stranded nucleic acid molecule that is the complement of the sequence of the mRNA that encodes the amino acid sequence encoded by the double-stranded nucleic acid molecule.

[0193] As used herein, an "array" refers to a collection of elements, such as nucleic acid molecules, containing three or more members. An addressable array is one in which the members of the array are identifiable, typically by position on a solid phase support or by virtue of an identifiable or detectable label, such as by color, fluorescence, electronic signal (i.e., RF, microwave or other frequency that does not substantially alter the interaction of the molecules of interest), bar code or other symbology, chemical or other such label. In certain embodiments, the members of the array are immobilized to discrete identifiable loci on the surface of a solid phase or directly or indirectly linked to or otherwise associated with the identifiable label, such as affixed to a microsphere or other particulate support (herein referred to as beads) and suspended in solution or spread out on a surface.

[0194] As used herein, a "support" (also referred to as a matrix support, a matrix, an insoluble support or solid support) refers to any solid or semisolid or insoluble support to which a molecule of interest, typically a biological molecule, organic molecule or biospecific ligand is linked or contacted. Such materials include any materials that are used as affinity matrices or supports for chemical and biological molecule syntheses and analyses, such as, but are not limited to: polystyrene, polycarbonate, polypropylene, nylon, glass, dextran, chitin, sand, pumice, agarose, polysaccharides, dendrimers, buckyballs, polyacryl-amide, silicon, rubber, and other materials used as supports for solid phase syntheses, affinity separations and purifications, hybridization reactions, immunoassays and other such applications. The matrix herein can be particulate or can be in the form of a continuous surface, such as a microtiter dish or well, a glass slide, a silicon chip, a nitrocellulose sheet, nylon mesh, or other such materials. When particulate, typically the particles have at least one dimension in the 5-10 mm range or smaller. Such particles, referred collectively herein as "beads," are often, but not necessarily, spherical. Such reference, however, does not constrain the geometry of the matrix, which can be any shape, including random shapes, needles, fibers, and elongated. Roughly spherical "beads," particularly microspheres that can be used in the liquid phase, also are contemplated. The "beads" can include additional components, such as magnetic or paramagnetic particles (see, e.g., Dynabeads (Dynal, Oslo, Norway)) for separation using magnets, as long as the additional components do not interfere with the methods and analyses herein.

[0195] As used herein, a "matrix" or "support particles" refers to matrix materials that are in the form of discrete particles. The particles have any shape and dimensions, but typically have at least one dimension that is 100 mm or less, 50 mm or less, 10 mm or less, 1 mm or less, 100 μm or less, 50 μm or less and typically have a size that is 100 mm^3 or less, 50 mm^3 or less, 10 mm^3 or less, and 1 mm^3 or less, 100 μm^3 or less and can be order of cubic microns. Such particles are collectively called "beads."

[0196] As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless

indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, *Biochem.,* 11:942-944 (1972)).

B. Directed Evolution

[0197] To date, there have been three general approaches described for protein directed evolution based on mutagenesis.

[0198] 1. Pure Random Mutagenesis

[0199] Random mutagenesis methodology requires that the amino acids in the starting protein sequence are replaced by all (or a group) of the 20 amino acids. Either single or multiple replacements at different amino acid positions are generated on the same molecule, at the same time. The random mutagenesis method relies on a direct search for fitness improvement based on random amino acid replacement and sequence changes at multiple amino acid positions. In this approach neither the amino acid position (first dimension) nor the amino acid type (second dimension) are restricted; and everything possible is generated and tested. Multiple replacements can randomly happen at the same time on the same molecule. For example, random mutagenesis methods are widely used to develop antibodies with higher affinity for its ligand, by the generation of random-sequence libraries of antibody molecules, followed by expression and screening using filamentous phages.

[0200] 2. Restricted Random Mutagenesis

[0201] Restricted random mutagenesis methods introduce either all of the 20 amino acids or DNA-biased residues. The bias is based on the sequence of the DNA and not on that of the protein, in a stochastic or semi-stochastic manner, respectively, within restricted or predefined regions of the protein, known in advance to be involved in the biological activity being "evolved." This method relies on a direct search for fitness improvement based on random amino acid replacement and sequence changes at either restricted or multiple amino acid positions. In this approach the scanning can be restricted to selected amino acid positions and/or amino acid types, while material changes continue to be random in position and type. For example, the amino acid position can be restricted by prior selection of the target region to be mutated (selection of target region is based upon prior knowledge on protein structure/function); while the amino acid type is not primarily restricted as replacing amino acids are stochastically or at most "semi-stochastically" chosen. As an example, this method is used to optimize known binding sites on proteins, including hormone-receptor systems and antibody-epitope systems.

[0202] 3. Non-Restricted Rational Mutagenesis

[0203] Rational mutagenesis is a two-step process and is described in co-pending U.S. application Ser. No. 10/022,249. Briefly, the first step requires amino acid scanning where all and each of the amino acids in the starting protein sequence are replaced by a third amino acid of reference (e.g., alanine). Only a single amino acid is replaced on each protein molecule at a time. A collection of protein molecules having a single amino acid replacement is generated such that molecules differ from each other by the amino acid position at which the replacement has taken place. Mutant DNA molecules are designed, generated by mutagenesis and cloned individually, such as in addressable arrays, such that they are physically separated from each other and such that each one is the single product of an independent mutagenesis reaction.

[0204] Mutant protein molecules derived from the collection of mutant nucleic acid molecules also are physically separated from each other, such as by formatting in addressable arrays. Activity assessment on each protein molecule allows for the identification of those amino acid positions that result in a drop in activity when replaced, thus indicating the involvement of that particular amino acid position in the protein's biological activity and/or conformation that leads to fitness of the particular feature being evolved. Those amino acid positions are referred to as HITs. At the second step, a new collection of molecules is generated such that each molecule differs from each of the others by the amino acid present at the individual HIT positions identified in step 1. All 20 amino acids (19 remaining) are introduced at each of the HIT positions identified in step 1; while each individual molecule contains, in principle, one and only one amino acid replacement. Mutant DNA molecules are designed, generated by mutagenesis and cloned individually, such as in addressable arrays, such that they are physically separated from each other and such that each one is the single product of an independent mutagenesis reaction. Mutant protein molecules derived from the collection of mutant DNA molecules also are physically separated from each other, such as by formatting in addressable arrays.

[0205] Activity assessment then is individually performed on each individual mutant molecule. The newly generated mutants that lead to a desired alteration (such as an improvement) in a protein activity are referred to as LEADs. This method permits an indirect search for activity alteration, such as improvement, based on one rational amino acid replacement and sequence change at a single amino acid position at a time, in search of a new, unpredicted amino acid sequence at some unpredicted regions along a protein to produce a protein that exhibits a desired activity or altered activity, such as better performance than the starting protein.

[0206] In this approach, neither the amino acid position nor the replacing amino acid type are restricted. Full length protein scanning is performed during the first step to identify HIT positions, and then all 20 amino acids are tested at each of the HIT positions, to identify LEAD sequences; while, as a starting point, only one amino acid at a time is replaced on each molecule. The selection of the target region (HITs and surrounding amino acids) for the second step is based upon experimental data on activity obtained in the first step. Thus, no prior knowledge of protein structure and/or function is necessary. Using this approach, LEAD sequences have been found on proteins that are located at regions of the protein not previously known to be involved in the particular biological activity being optimized; thus emphasizing the power of this approach to discover unpredictable regions (HITs) as targets for fitness improvement.

C. 2-Dimensional Rational Scanning (2D Scanning)

[0207] The 2-Dimensional rational scanning (or "2-dimensional scanning") methods for protein rational evolution provided herein (see, also copending U.S. application Ser. No. 10/658,355, filed Sep. 8, 2003, based on U.S. provisional application Ser. Nos. 60/457,063 and 60/410,258) are

based on scanning over two dimensions. The first dimension scanned is amino acid position along the protein sequence to identify is-HIT target positions, and the second dimension is the amino acid type selected for replacing a particular is-HIT amino acid position. An advantage of the 2-dimensional scanning methods provided herein is that at least one, and typically both, of the amino acid position scan and/or the replacing amino acid scan can be restricted such that fewer than all amino acids on the protein-backbone are selected for amino acid replacement; and/or fewer than all of the remaining 19 amino acids available to replace an original, such as native, amino acid are selected for replacement.

[0208] In particular embodiments, based on i) the particular protein properties to be evolved, ii) the protein's amino acid sequence, and iii) the known properties of the individual amino acids, a number of target positions along the protein sequence are selected, in silico, as "is-HIT target positions." This number of is-HIT target positions is as large as possible such that all reasonably possible target positions for the particular feature being evolved are included. In particular, embodiments where a restricted number of is-HIT target positions are selected for replacement, the amino acids selected to replace the is-HIT target positions on the particular protein being optimized can be either all of the remaining 19 amino acids or, more frequently, a more restricted group comprising selected amino acids that are contemplated to have the desired effect on protein activity. In another embodiment, so long as a restricted number of replacing amino acids are used, all of the amino acid positions along the protein backbone can be selected as is-HIT target positions for amino acid replacement. Mutagenesis then is performed by the replacement of single amino acid residues at specific is-HIT target positions on the protein backbone (e.g., "one-by-one," such as in addressable arrays), such that each individual mutant generated is the single product of each single mutagenesis reaction. Mutant DNA molecules are designed, generated by mutagenesis and cloned individually, such as in addressable arrays, such that they are physically separated from each other and that each one is the single product of an independent mutagenesis reaction. Mutant protein molecules derived from the collection of mutant DNA molecules also are physically separated from each other, such as by formatting in addressable arrays. Thus, a plurality of mutant protein molecules are produced. Each mutant protein contains a single amino acid replacement at only one of the is-HIT target positions. Activity assessment is then individually performed on each individual protein mutant molecule, following protein expression and measurement of the appropriate activity. An example of practice of this method is shown in the Example in which mutant IFNα molecules and IFNβ molecules are produced.

[0209] The newly generated proteins that lead to altered, typically improvement, in a target protein activity are referred to as LEADs. This method relies on an indirect search for protein improvement for a particular activity, such as increased resistance to proteolysis, based on a rational amino acid replacement and sequence change at single or, in another embodiment, a limited number of amino acid positions at a time. As a result, optimized proteins that have new amino acid sequences at some regions along the protein that perform better (at a particular target activity or other property) than the starting protein are identified and isolated.

[0210] 1. Identifying in-silico HITs

[0211] Provided herein is a method for directed evolution that includes identifying and selecting (using in silico analysis) specific amino acids and amino acid positions (referred to herein as is-HITs) along the protein sequence that are contemplated to be directly or indirectly involved in the feature being evolved. As noted, the 2-dimensional scanning methods provided include the following two-steps. The first step is an in silico search of a target protein's amino acid sequence to identify all possible amino acid positions that potentially can be targets for the activity being evolved. This is effected, for example, by assessing the effect of amino acid residues on the property(ies) to be altered on the protein, using any known standard software. The particulars of the in silico analysis is a function of the property to be modified. For example, in the example herein, a property that is altered resistance of the protein to proteolysis. To determine amino acid residues that are potential targets as is-HITs, in this example, all possible target residues for proteases were first identified. The 3-dimensional structure of the protein was then considered in order to identify surface residues. Comparison of exposed residues with proteolytically cleavable residues yields residues that are targets for change.

[0212] Once identified, these amino acid positions or target sequences are referred to as "is-HITs" (in silico HITs). In silico HITs are defined as those amino acid positions (or target positions) that potentially are involved in the "evolving" feature, such as increased resistance to proteolysis. In one embodiment, the discrimination of the is-HITs among all the amino acid positions in a protein sequence is made based on i) the amino acid type at each position in addition to, whenever available but not necessarily, ii) the information on the protein secondary or tertiary structure. In silico HITs constitute a collection of mutant molecules such that all possible amino acids, amino acid positions or target sequences potentially involved in the evolving feature are represented. No strong theoretical discrimination among amino acids or amino acid positions is made at this stage.

[0213] In silico HIT positions are spread over the full length of the protein sequence. In one embodiment, only a single is-HIT amino acid at a time is replaced on the target protein. In another embodiment, a limited number of is-HIT amino acids are replaced at the same time on the same target protein molecule. The selection of target regions (is-HITs and surrounding amino acids) for the second step is based upon rational assumptions and predictions. No prior knowledge of protein structure/function is necessary. Hence, the 2-dimensional scanning methodology provided herein does not require any previous knowledge of the 3-dimensional conformational structure of the protein.

[0214] Any protein known or otherwise available to those of skill in the art is suitable for modification using the directed evolution methods provided herein, including cytokines (e.g., IFNα-2b) or any other proteins that have previously been mutated or optimized.

[0215] A variety of parameters can be analyzed to determine whether or not a particular amino acid on a protein might be involved in the evolving feature. For example, the information provided by crystal structures of proteins can be rationally exploited in order to perform a computer-assisted (in silico) analysis towards the prediction of variants with

desired features. In a particular embodiment, a limited number of initial premises (typically no more than 2) are used to determine the in silico HITs. In other embodiments, the number of premises used to determine the in silico hits can range from 1 to 10 premises, including no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, but are typically no more than 2 premises. It is important to the methods provided herein that the number of initial premises be kept to a minimum, so as to maintain the number of potential is-HITs at a maximum (here is where the methods provided are not limited by too much prediction based on theoretical assumptions). When two premises are employed, the first condition is typically the amino acid type itself, which is directly linked to the nature of the evolving feature. For example, if the goal were to change the optimum pH for an enzyme, then the replacing amino acids selected at this step for the replacement of the original sequence would be only those with a certain pKa value. The second premise is typically related to the specific position of those amino acids along the protein structure. For example, some amino acids might be discarded if they are not expected to be exposed enough to the solvent, even when they might have appropriate pKa values.

[0216] During the first step of identification of is-HITs according to the methods provided herein, each individual amino acid along the protein sequence is considered individually to assess whether it is a candidate for is-HIT. This search is done one-by-one and the decision on whether the amino acid is considered to be a candidate for a is-HIT is based on (1) the amino acid type itself; (2) the position on the amino acid sequence and protein structure if known; and (3) the predicted interaction between that amino acid and its neighbors in sequence and space.

[0217] Using the 3D-scanning methods provided herein, once one protein within a family of proteins (e.g., IFNα-2b within the cytokine family) is optimized using the methods provided herein for generating LEAD mutants, is-HITs can be identified on other or all proteins within a particular family by identifying the corresponding amino acid positions therein using structural homology analysis (based upon comparisons of the 3-D structures of the family members with original protein to identify corresponding residues for replacement) as described hereinafter. The is-HITs on family identified in this manner then can be subjected to the next step of identifying replacing amino acids and further assayed to obtain LEADs or super-LEADs as described herein.

[0218] 2. Identifying Replacing Amino Acids

[0219] Once the is-HITs target positions are selected, the next step is identifying those amino acids that will replace the original, such as native, amino acid at each is-HIT position to alter the activity level for the particular feature being evolved. The set of replacing amino acids to be used to replace the original, such as native, amino acid at each is-HIT position can be different and specific for the particular is-HIT position. The choice of the replacing amino acids takes into account the need to preserve the physicochemical properties such as hydrophobicity, charge and polarity, of essential (e.g., catalytic, binding, etc.) residues. The number of replacing amino acids, of the remaining 19 non-native (or non-original) amino acids, that can be used to replace a particular is-HIT target position ranges from 1 up to about 19, from 1 up to about 15, from 1 up to about 10, from 1 up to about 9, from 1 up to about 8, from 1 up to about 7, from 1 up to about 6, from 1 up to about 5, from 1 up to about 4, from 1 up to about 3, or from 1 to 2 amino acid replacements.

[0220] Numerous methods of selecting replacing amino acids (also referred to herein as "replacement amino acids") are well known in the art. Protein chemists determined that certain amino acid substitutions commonly occur in related proteins from different species. As the protein still functions with these substitutions, the substituted amino acids are compatible with protein structure and function. Often, these substitutions are to a chemically similar amino acid, but other types of changes, although relatively rare, can also occur.

[0221] Knowing the types of changes that are most and least common in a large number of proteins can assist with predicting alignments and amino acid substitutions for any set of protein sequences. Amino acid substitution matrices are used for this purpose.

[0222] In amino acid substitution matrices, amino acids are listed across the top of a matrix and down the side, and each matrix position is filled with a score that reflects how often one amino acid would have been paired with the other in an alignment of related protein sequences. The probability of changing amino acid A into amino acid B is assumed to be identical to the reverse probability of changing B into A. This assumption is made because, for any two sequences, the ancestor amino acid in the phylogenetic tree is usually not known. Additionally, the likelihood of replacement should depend on the product of the frequency of occurrence of the two amino acids and on their chemical and physical similarities. A prediction of this model is that amino acid frequencies will not change over evolutionary time (Dayhoff et al., *Atlas of Protein Sequence and Structure,* 5(3):345-352, 1978). Below are several exemplary amino acid substitution matrices, including, but not limited to block substitution matrix (BLOSUM), Jones, Gonnet, Fitch, Feng, McLachlan, Grantham, Miyata, Rao, Risler, Johnson and percent accepted mutation (PAM). Any such method known to those of skill in the art can be employed.

[0223] a. Percent Accepted Mutation (PAM)

[0224] Dayhoff and coworkers developed a model of protein evolution that resulted in the development of a set of widely used replacement matrices (Dayhoff et al., *Atlas of Protein Sequence and Structure,* 5(3):345-352, 1978) termed percent accepted mutation matrices (PAM). In deriving these matrices, each change in the current amino acid at a particular site is assumed to be independent of previous mutational events at that site. Thus, the probability of change of any amino acid A to amino acid B is the same, regardless of the previous changes at that site and also regardless of the position of amino acid A in a protein sequence.

[0225] In the Dayhoff approach, replacement rates are derived from alignments of protein sequences that are at least 85% identical; this constraint ensures that the likelihood of a particular mutation being the result of a set of successive mutations is low. Because these changes are observed in closely related proteins, they represent amino acid substitutions that do not significantly change the function of the protein. Hence, they are called "accepted mutations," as defined as amino acid changes that are accepted by natural selection.

[0226] i. PAM Analysis

[0227] In particular embodiments of the methods provided herein, "Percent Accepted Mutation" (PAM; Dayhoff et al., *Atlas of Protein Sequence and Structure,* 5(3):345-352, 1978 FIG. 2) PAM values are used to select an appropriate group of replacement amino acids. PAM matrices were originally developed to produce alignments between protein sequences based evolutionary distances. Because, in a family of proteins or homologous (related) sequences, identical or similar amino acids (85% similarity) are shared, conservative substitutions for, or allowed point mutations of the corresponding amino acid residues can be determined throughout an aligned reference sequence. Conservative substitutions of a residue in a reference sequence are those substitutions that are physically and functionally similar to the corresponding reference residues, e.g., that have a similar size, shape, electric charge, chemical properties, including the ability to form bonds such as covalent and hydrogen bonds. Particularly suitable conservative amino acid substitutions are those that show the highest scores and fulfill the PAM matrix criteria in the form of "accepted point mutations." For example, by comparing a family of scoring matrices, Dayhoff et al., *Atlas of Protein Sequence and Structure,* 5(3):345-352, 1978, found a consistently higher score significance when using PAM250 matrix to analyze a variety of proteins, known to be distantly related.

[0228] ii. PAM 250

[0229] In a particular embodiment, the PAM250 matrix set forth in FIG. **2** is used for determining the replacing amino acids based on similarity criteria. The PAM250 matrix uses data obtained directly from natural evolution to facilitate the selection of replacing amino acids for the is-HITs to generate conservative mutations without much affecting the overall protein function. By using the PAM250 matrix, candidate replacing amino acids are identified from related proteins from different organisms.

[0230] b. Jones et al. and Gonnet et al.

[0231] This method (see, e.g., Jones et al., *Comput. Appl. Biosci.,* 8:275-282, 1992 and Gonnet et al., *Science,* 256:1433-1445, 1992) uses much of the same methodology as Dayhoff (see below), but with modern databases. The matrix of Jones et al., is extracted from Release 15.0 of the SWISS-PROT protein sequence database. Point mutations totaling 59,160 from 16,130 protein sequences were used to calculate a PAM250 (see below) matrix.

[0232] The matrix published by Gonnet et al., *Science,* 256:1433-1445, 1992, was built from a sequence database of 8,344,353 amino acid residues. Each sequence was compared against the entire database, such that 1.7×10^6 subsequent matches resulted for the significant alignments. These matches were then used to generate a matrix with a PAM distance of 250.

[0233] c. Fitch and Feng et al.

[0234] Fitch, *J. Mol. Evol.,* 16(1): 9-16, 1966, used an exchange matrix that contained for each pair (A, B) of amino acid types the minimum number of nucleotides that must be changed to encode amino acid A instead of amino acid B. Feng et al., *J. Mol. Evol.,* 21: 112-125, 1985, used an enhanced version of Fitch, *J. Mol. Evol.,* 16(1): 9-16, 1966, to build a Structure-Genetic matrix. In addition to considering the minimum number of base changes required to encode amino acid B instead of A, this method also considers the structural similarity of the amino acids.

[0235] d. McLachlan, Grantham and Miyata

[0236] McLachlan, *J. Mol. Biol.,* 61:409-424 1971, used 16 protein families, each with 2 to 14 members. The 89 sequences were aligned and the pairwise exchange frequency, observed in 9280 substitutions, was used to generate an exchange matrix with values varying from 0 to 9.

[0237] Grantham, *Science,* 185:862-864, 1974, considers composition, polarity and molecular volume of amino acid side-chains, properties that were highly correlated to the relative substitution frequencies tabulated by McLachlan, *J. Mol. Biol.,* 61:409-424, 1971, to build the matrix.

[0238] Miyata, *J. Mol. Evol.,* 12:219-236, 1979, uses the volume and polarity values of amino acids published by Grantham, *Science,* 185:862-864, 1974. For every amino acid type pair, the difference for both properties was calculated and divided by the standard deviation of all the differences. The square root of the sum of both values is then used in the matrix.

[0239] e. Rao

[0240] Rao, *J. Pept. Protein Res.,* 29:276-281, 1987, employs five amino acid properties to create a matrix; namely, alpha-helical, beta-strand and reverse-turn propensities as well as polarity and hydrophobicity. The standardized properties were summed and the matrix rescaled to the same average as that for PAM (Dayhoff et al., *Atlas of Protein Sequence and Structure,* 5(3):345-352, 1978).

[0241] f. Risler et al.

[0242] Risler et al., *J. Mol. Biol.,* 204:1019-1029, 1988, aligned 32 three-dimensional structures from 11 protein families by rigid-body superposition of the backbone topology. Only substitutions were considered where at least three adjacent and equivalent main-chain Cα atom pairs in the compared structures were each not more than 1.2 Å apart. A total of 2860 substitutions were considered and used to build a matrix based on χ^2 distance calculations.

[0243] g. Johnson et al.

[0244] Johnson et al., *J. Mol. Biol.,* 233:716-738, 1993, derived their matrix from the tertiary structural alignment of 65 families in a database of 235 structures created with the method of Sali et al., *J. Mol. Biol.,* 212:403-428, 1990. Their examination of the substitutions was based on the expected and observed ratios of occurrences and the final matrix values were taken as log 10 of the ratios.

[0245] h. Block Substitution Matrix (BLOSUM)

[0246] One empirical approach (Henikoff et al., *Proc. Natl. Acad. Sci. USA,* 89:10915-10919, 1992) uses local, ungapped alignments of distantly related sequences to derive the blocks amino acid substitution matrix (BLOSUM) series of matrices. The matrix values are based on the observed amino acid substitutions in a larger set of about 2000 conserved amino acid patterns, termed blocks. These blocks act as signatures of families of related proteins. Matrices of this series are identified by a number after the matrix (e.g., BLOSUM50), which refers to the minimum percentage identity of the blocks of multiple aligned amino acids used

to construct the matrix. It is noteworthy that these matrices are directly calculated without extrapolations, and are analogous to transition probability matrices P(T) for different values of T, estimated without reference to any rate matrix Q.

[0247] The outcome of these two steps set forth above, which is performed in silico is that: (1) the amino acid positions that will be the target for mutagenesis are identified; these positions are referred to as is-HITs; (2) the replacing amino acids for the original, such as native, amino acids at the is-HITs are identified, to provide a collection of candidate LEAD mutant molecules that are expected to perform different from the native one. These are assayed for a desired optimized (or improved or altered) biological activity.

[0248] 3. Physical Construction of Mutant Proteins and Biological Assays

[0249] Once is-HITs are selected as set forth above, replacing amino acids are introduced. Mutant proteins typically are prepared using recombinant DNA methods and assessed in appropriate biological assays for the particular biological activity (feature) optimized (see, e.g., Example 1). An exemplary method of preparing the mutant proteins is by mutagenesis of the original, such as native, gene using methods well known in the art. Mutant molecules are generated one-by-one, such as in addressable arrays, such that each individual mutant generated is the single product of each single and independent mutagenesis reaction. Individual mutagenesis reactions are conducted separately, such as in addressable arrays where they are physically separated from each other. Once a population of sets of nucleic acid molecules encoding the respective mutant proteins is prepared, each is separately introduced one-by-one into appropriate cells for the production of the corresponding mutant proteins. This can also be performed, for example, in addressable arrays where each set of nucleic acid molecules encoding a respective mutant protein is introduced into cells confined to a discrete location, such as in a well of a multi-well microtiter plate. Each individual mutant protein is individually phenotypically characterized and performance is quantitatively assessed using assays appropriate for the feature being optimized (i.e., feature being evolved). Again, this step can be performed in addressable arrays. Those mutants displaying a desired increased or decreased performance compared to the original, such as native molecules are identified and designated LEADs. From the beginning of the process of generating the mutant DNA molecules up through the readout and analysis of the performance results, each candidate LEAD mutant is generated, produced and analyzed individually, such as from its own address in an addressable array. The process is amenable to automation.

D. 2-Dimensional Scanning of Proteins for Increased Resistance to Proteolysis

[0250] The methods of 2-dimensional scanning permit preparation of proteins modified for a selected trait, activity or other phenotype. Among modifications of interest for therapeutic proteins are those that increase protection against protease digestion while maintaining the requisite biological activity. Such changes are useful for producing longer-lasting therapeutic proteins.

[0251] The delivery of stable peptide and protein drugs to patients is a major challenge for the pharmaceutical industry. These types of drugs in the human body are constantly eliminated or taken out of circulation by different physiological processes including internalization, glomerular filtration and proteolysis. The latter is often the limiting process affecting the half-life of proteins used as therapeutic agents in per-oral administration and either intravenous or intramuscular injections.

[0252] The 2-dimensional scanning process for protein evolution is used to effectively improve protein resistance to proteases and thus increase protein half-life in vitro and, ultimately in vivo. As noted, the methods provided herein for designing and generating highly stable, longer lasting proteins, or proteins having a longer half-life include: i) identifying some or all possible target sites on the protein sequence that are susceptible to digestion by one or more specific proteases (these sites are referred to herein as is-HITs); ii) identifying appropriate replacing amino acids, specific for each is-HIT, such that upon replacement of one or more of the original, such as native, amino acids at that specific is-HIT, they can be expected to increase the is-HIT's resistance to digestion by protease while at the same time, maintaining or improving the requisite biological activity of the protein (these proteins with replaced amino acids are the "candidate LEADs"); iii) systematically introducing the specific replacing amino acids (candidate LEADs) at every specific is-HIT target position to generate a collection containing the corresponding mutant candidate lead molecules. Mutants are generated, produced and phenotypically characterized one-by-one, such as in addressable arrays, such that each mutant molecule contains initially an amino acid replacement at only one is-HIT site.

[0253] In particular embodiments, such as in subsequent rounds, mutant molecules also can be generated that contain one or more amino acids at one or more is-HIT sites that have been replaced by candidate LEAD amino acids. Those mutant proteins carrying one or more mutations at one or more is-HITs, and that display improved protease resistance are called LEADs (one mutation at one is-HIT) and super-LEADs (mutations at more than one is-HIT).

[0254] The first step of the process takes into consideration existing knowledge from different domains:

[0255] (1) About the galenic and the delivery environment (tissue, organ or corporal fluid) of the particular therapeutic protein in order to establish a list of proteases more likely to be found in that environment. For example, a therapeutic protein in per-oral application is likely to encounter typical proteases of the luminal gastrointestinal tract. In contrast, if this protein were injected in the blood circulation, serum proteases would be implicated in the proteolysis. Based on the specific list of proteases involved, the complete list of all amino acid sequences that potentially could be targeted by the proteases in the list is determined.

[0256] (2) Since protease mixtures in the body are quite complex in composition, almost all the residues in any target protein potentially are targeted for proteolysis (FIG. **1**A). Nevertheless, proteins form specific tri-dimensional structures where residues are more or less exposed to the environment and protease action. It can be assumed that those residues constituting the core of a protein are inaccessible to proteases, while those more "exposed" to the environment

are better targets for proteases. The probability for every specific amino acid to be "exposed" and then to be accessible to proteases can be taken into account to reduce the number of is-HIT. Consequently, the methods herein consider the analysis with respect to solvent "exposure" or "accessibility" for each individual amino acid in the protein sequence. Solvent accessibility of residues can alternatively be estimated, regardless of any previous knowledge of specific protein structural data, by using an algorithm derived from empirical amino acid probabilities of accessibility, which is expressed in the following equation (Boger et al., *Reports of the Sixth International Congress in Immunology*, p. 250, 1986):

$$A(i) = \left[\prod_{j=1}^{6} \_\delta\_{i+4-j} \right] * [0.62]^{-6}.$$

Briefly, these are fractional probabilities (δ_(i)) determined for an amino acid (i) found on the surface of a protein, which are based upon structural data from a set of several proteins. It is thus possible to calculate the solvent accessibility (A) of an amino acid (A(i)) at sequence position (i−2 to i+3, onto a sliding window of length equal to 6) that is within an average surface accessible to solvent of >20 square angstroms ($Å^2$).

[0257] The protease accessible target amino acids along the protein sequence, i.e., the amino acids to be replaced, are thus identified and are referred to herein as in silico HITs (is-HITs).

[0258] Amino acids at the is-HITs then are replaced by residues that render the sequence less vulnerable (by a factor, for example, of 1%, 10%, 20%, 30%, 40%, 50%, . . . 100% depending upon the protein) or invulnerable (substantially no detectable digestion within a set time period) to protease digestion, while at the same time maintain a biological activity or activities of interest of the protein. The choice of the replacing amino acids is complicated by (1) the broad target specificity of certain proteases and (2) the need to preserve the physicochemical properties such as hydrophobicity, charge and polarity, of essential (e.g., catalytic, binding and/or other activities depending upon the protein) residues. For use in the methods herein, the "Percent Accepted Mutation" values (PAM values; see, Dayhoff et al., *Atlas of Protein Sequence and Structure,* 5(3):345-352, 1978), FIG. 2) can be used as described herein. PAM values, originally developed to produce alignments between protein sequences, are available in the form of probability matrices, which reflect an evolutionary distance. Since, in a family of proteins or homologous (related) sequences, identical or similar amino acids (85% similarity) are shared, conservative substitutions for, or "allowed point mutations" of the corresponding amino acid residues can be determined throughout an aligned reference sequence. As noted, conservative substitutions of a residue in a reference sequence are those substitutions that are physically and functionally similar to the corresponding reference residues e.g., that have a similar size, shape, electric charge, chemical properties, including the ability to form bonds such as covalent and hydrogen bonds. For example, conservative substitutions can be those that exhibit the highest scores and fulfill the PAM matrix criteria in the form of "accepted point mutations."

[0259] By comparing a family of scoring matrices, Dayhoff et al., *Atlas of Protein Sequence and Structure,* 5(3):345-352, 1978), found consistently higher score significance when using PAM250 matrix to analyze a variety of proteins, known to be distantly related. For methods herein, the PAM250 matrix was selected for use. The PAM250 matrix is used, by learning directly from natural evolution, to find replacing amino acids for the is-HITs to generate conservative mutations without affecting the protein function. By using PAM250, candidate replacing amino acids are identified from related proteins from different organisms.

[0260] An exemplary class of proteins that can be optimized according to the methods provided herein are the cytokines. For example, 2D-scanning methods provided herein can be used to modify the following cytokines to increase their stability as assessed by an increased resistance to proteolysis resulting in an increased protein half-life in the bloodstream or any other desired biological activity of the selected protein. Exemplary cytokines, include, but are not limited to: interleukin-10 (IL-10; SEQ ID NO: 200), interferon beta (IFN-β; SEQ ID NO: 196), interferon alpha-2a (IFNα-2a; SEQ ID NO: 182), interferon alpha-2b (IFNα-2b; SEQ ID NO: 1), and interferon gamma (IFN-γ; SEQ ID NO: 199), granulocyte colony stimulating factor (G-CSF; SEQ ID NO: 210), leukemia inhibitory factor (LIF; SEQ ID NO: 213), growth hormone (hGH; SEQ ID NO: 216), ciliary neurotrophic factor (CNTF; SEQ ID NO: 212), leptin (SEQ ID NO: 211), oncostatin M (SEQ ID NO: 214), interleukin-6 (IL-6; SEQ ID NO: 217), interleukin-12 (IL-12; SEQ ID NO: 215), erythropoietin (EPO; SEQ ID NO: 201), granulocyte-macrophage colony stimulating factor (GM-CSF; SEQ ID NO: 202), interleukin-2 (IL-2; SEQ ID NO: 204), interleukin-3 (IL-3; SEQ ID NO: 205), interleukin-4 (IL-4; SEQ ID NO: 207), interleukin-5 (IL-5; SEQ ID NO: 208), interleukin-13 (IL-13; SEQ ID NO: 209), Flt3 ligand (SEQ ID NO: 203) and stem cell factor (SCF; SEQ ID NO: 206).

[0261] Accordingly, provided herein are modified cytokines that exhibit increased resistance to proteolysis compared to the unmodified cytokine. The modified cytokines can be selected from among a member of the interferons/interleukin-10 protein family, a member of the long-chain cytokine family; and a member of the short-chain cytokine family. In particular embodiments, the modified cytokines provided herein are selected from among: interleukin-10 (IL-10), interferon beta (IFNβ), interferon alpha-2a (IFNα-2a), interferon alpha-2b (IFNα-2b), and interferon gamma (IFN-γ), granulocyte colony stimulating factor (G-CSF), leukemia inhibitory factor (LIF), human growth hormone (hGH), ciliary neurotrophic factor (CNTF), leptin, oncostatin M, interleukin-6 (IL-6) and interleukin-12 (IL-12), erythropoietin (EPO), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-13 (IL-13), Flt3 ligand and stem cell factor (SCF). In one embodiment, the modified cytokine is an interferon, including modified interferon α-2b (IFNα-2b).

E. Rational Evolution of IFNα-2b for Increased Resistance to Proteolysis

[0262] IFNα-2b is used for a variety of applications. Typically it is used for treatment of type B and C chronic hepatitis. Additional indications include, but are not limited to, melanomas, herpes infections, Kaposi sarcomas and

some leukemia and lymphoma cases. Patients receiving interferon are subject to frequent repeat applications of the drug. Since such frequent injections generate uncomfortable physiological as well as undesirable psychological reactions in patients, increasing the half-life of interferons and thus decreasing the necessary frequency of interferon injections, would be extremely useful to the medical community. For example, after injection of native human IFNα-2b injection in mice, as a model system, its presence can be detected in the serum between 3 and 10 hours with a half-life of only around 4 hours. The IFNα-2b completely disappears to undetectable levels by 18-24 hours after injection. Provided herein are mutant variants of the IFNα-2b protein that display altered properties including: (a) highly improved stability as assessed by resistance to proteases in vitro and by pharmacokinetics studies in mice; and (b) at least comparable biological activity as assessed by antiviral and antiproliferative action compared to both the unmodified and wild type native IFNα-2b protein and to at least one pegylated derivative of the wild type native IFNα. As a result, the IFNα-2b mutant proteins provided herein confer a higher half-life and at least comparable antiviral and antiproliferation activity (sufficient for a therapeutic effect) with respect to the native sequence and to the pegylated derivatives molecules currently being used for the clinical treatment of hepatitis C infection. See FIGS. **6**(A)-**6**(N), **6**(T) and **6**(U). Thus, the optimized IFNα-2b protein mutants that possess increased resistance to proteolysis and/or glomerular filtration provided herein result in a decrease in the frequency of injections needed to maintain a sufficient drug level in serum, leading to i) higher comfort and acceptance by patients, ii) lower doses necessary to achieve comparable biological effects, and iii) as a consequence of (ii), an attenuation of the (dose-dependent) secondary effects observed in humans.

[0263] In particular embodiments, the half-life of the IFNα-2b and IFNα-2a mutants provided herein is increased by an amount selected from at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 150%, at least 200%, at least 250%, at least 300%, at least 350%, at least 400%, at least 450%, at least 500% or more, when compared to the half-life of native human IFNα-2b and IFNα-2a in either human blood, human serum or an in vitro mixture containing one or more proteases. In other embodiments, the half-life of the IFNα-2b and IFNα-2a mutants provided herein is increased by an amount selected from at least 6 times, 7 times, 8 times, 9 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times, 400 times, 500 times, 600 times, 700 times, 800 times, 900 times, 1000 times, or more, when compared to the half-life of native human IFNα-2b and IFNα-2a in either human blood, human serum or an in vitro mixture containing one or more proteases.

[0264] Two methodologies were used herein to increase the stability of IFNα-2b by amino acid replacement: i) amino acid replacement that leads to higher resistance to proteases by direct destruction of the protease target residue or sequence, while either maintaining or improving the requisite biological activity (e.g., antiviral activity, antiproliferation activity), and/or ii) amino acid replacement that leads to a different pattern of N-glycosylation, thus decreasing both glomerular filtration and sensitivity to proteases, while either improving or maintaining the requisite biological activity (e.g., antiviral activity, antiproliferation activity).

[0265] The 2D-scanning methods provided herein were used to identify the amino acid changes on IFNα-2b that lead to an increase in stability when challenged either with proteases, human blood lysate or human serum. Increasing protein stability to proteases, human blood lysate or human serum, and/or increasing the molecular size is contemplated herein to provide a longer in vivo half-life for the particular protein molecules, and thus to a reduction in the frequency of necessary injections into patients. The biological activities that were measured for the IFNα-2b molecules are i) their capacity to inhibit virus replication when added to permissive cells previously infected with the appropriate virus, and ii) their capacity to stimulate cell proliferation when added to the appropriate cells. Prior to the measurement of biological activity, IFNα-2b molecules were challenged with proteases, human blood lysate or human serum during different incubation times. The biological activity measured, corresponds then to the residual biological activity following exposure to the protease-containing mixtures.

[0266] As set forth above, provided herein are methods for the development of IFNα-2b and IFNα-2a molecules that, while maintaining the requisite biological activity intact, have been rendered less susceptible to digestion by blood proteases and therefore display a longer half-life in blood circulation. In this particular example, the method used included the following specific steps as set forth in Example 2:

[0267] 1) Identifying some or all possible target sites on the protein sequence that are susceptible to digestion by one or more specific proteases (these sites are the is-HITs) and

[0268] 2) Identifying appropriate replacing amino acids, specific for each is-HIT, such that if used to replace one or more of the original amino acids at that specific is-HIT, they can be expected to increase the is-HIT's resistance to digestion by protease while at the same time, keeping the biological activity of the protein unchanged (these replacing amino acids are the "candidate LEADs").

[0269] As set forth in Example 2, the 3-dimensional structure of IFNα-2b obtained from the NMR structure of IFNα-2a (PDB code 1ITF) was used to select only those residues exposed to solvent from a list of residues along the IFNα-2b and IFNα-2a sequence which can be recognized as a substrate for different enzymes present in the serum. Residue 1 corresponds to the first residue of the mature peptide IFNα-2b (SEQ ID NO:1) encoded by nucleotides 580-1074 of sequence accession No. J00207. Using this approach, the following 42 amino acid target positions were identified as is-HITs on IFNα-2b or IFNα-2a, which numbering is that of the mature protein (SEQ ID NO:1 or SEQ ID NO:182, respectively): L3, P4, R12, R13, M16, R22, K23 or R23, F27, L30, K31, R33, E41, K49, E58, K70, E78, K83, Y89, E96, E107, P109, L110, M111, E113, L117, R120, K121, R125, L128, K131, E132, K133, K134, Y135, P137, M148, R149, E159, L161, R162, K164, and E165. Each of these positions was replaced by residues defined as compatible by the substitution matrix PAM250 while at the same time not generating any new substrates for proteases. For these 42 is-HITs, the residue substitutions determined by PAM250 analysis were as follows:

[0270] R to H, Q

[0271] E to H, Q

[0272] K to Q, T

[0273] L to V, I

[0274] M to I, V

[0275] P to A, S

[0276] Y to I, H.

[0277] 1. Modified IFNα-2b Proteins with Single Amino Acid Substitutions (is-HITS)

[0278] Among the mutant proteins provided herein, are mutant IFNα-2b proteins that have increased resistance proteolysis compared to the unmodified, typically wild-type, protein. The mutant IFNα-2b proteins include those selected from among proteins containing more single amino acid replacements in SEQ ID NO:1, corresponding to: L by V at position 3; L by I at position 3; P by S at position 4; P by A at position 4; R by H at position 12; R by Q at position 12; R by H at position 13; R by Q at position 13; M by V at position 16; M by I at position 16; R by H at position 22; R by Q at position 22; R by H at position 23; R by Q at position 23; F by I at position 27; F by V at position 27; L by V at position 30; L by I at position 30; K by Q at position 31; K by T at position 31; R by H at position 33; R by Q at position 33; E by Q at position 41; E by H at position 41; K by Q at position 49; K by T at position 49; E by Q at position 58; E by H at position 58; K by Q at position 70; K by T at position 70; E by Q at position 78; E by H at position 78; K by Q at position 83; K by T at position 83; Y by H at position 89; Y by I at position 89; E by Q at position 96; E by H at position 96; E by Q at position 107; E by H at position 107; P by S at position 109; P by A at position 109; L by V at position 110; L by I at position 110; M by V at position 111; M by I at position 111; E by Q at position 113; E by H at position 113; L by V at position 117; L by I at position 117; R by H at position 120; R by Q at position 120; K by Q at position 121; K by T at position 121; R by H at position 125; R by Q at position 125; L by V at position 128; L by I at position 128; K by Q at position 131; K by T at position 131; E by Q at position 132; E by H at position 132; K by Q at position 133; K by T at position 133; K by Q at position 134; K by T at position 134; Y by H at position 135; Y by I at position 135; P by S at position 137; P by A at position 137; M by V at position 148; M by I at position 148; R by H at position 149; R by Q at position 149; E by Q at position 159; E by H at position 159; L by V at position 161; L by I at position 161; R by H at position 162; R by Q at position 162; K by Q at position 164; K by T at position 164; E by Q at position 165; and E by H at position 165.

[0279] 2. LEAD Identification

[0280] Next the specific replacing amino acids (candidate LEADs) are systematically introduced at every specific is-HIT position to generate a collection containing the corresponding mutant IFNα-2b DNA molecules, as set forth in Example 2. The mutant DNA molecules were used to produce the corresponding mutant IFNα-2b protein molecules by transformation or transfection into the appropriate cells. These protein mutants were assayed for (i) protection against proteolysis, (ii) antiviral and antiproliferation activity in vitro, (iii) pharmacokinetics in mice. Of particular interest are mutations that increase these activities of the IFNα-2b mutant proteins compared to unmodified wild type IFNα-2b protein and to pegylated derivates of the wild type protein. Based on the results obtained from these assays, each individual IFNα-2b variant was assigned a specific activity. Those variant proteins displaying the highest stability and/or resistance to proteolysis were selected as LEADs. The candidate LEADs that possessed at least as much residual antiviral activity following protease treatment as the control, native IFNα-2b, before protease treatment were selected as LEADs. The results are set forth in Table 2 of Example 2.

[0281] Using this method, the following mutants selected as LEADs are provided herein and correspond to the group of proteins containing one or more single amino acid replacements in SEQ ID NO:1, corresponding to: F by V at position 27; R by H at position 33; E by Q at position 41; E by H at position 41; E by Q at position 58; E by H at position 58; E by Q at position 78; E by H at position 78; Y by H at position 89; E by Q at position 107; E by H at position 107; P by A at position 109; L by V at position 110; M by V at position 111; E by Q at position 113; E by H at position 113; L by V at position 117; L by I at position 117; K by Q at position 121; K by T at position 121; R by H at position 125; R by Q at position 125; K by Q at position 133; K by T at position 133; and E by Q at position 159; E by H at position 159. Among these are mutations that can have multiple effects. For example, among mutations described herein, are mutations that result in an increase of the IFNα-2b activity as assessed by detecting the requisite biological activity.

[0282] Also provided are IFNα-2b proteins that contain a plurality of mutations based on the LEADs (see, e.g., Tables 6 and 7, EXAMPLE 5, which lists candidate LEADs and LEAD sites), are generated. These IFNα-2b proteins have activity that is further optimized. Examples of such proteins are described in the EXAMPLES. Other combinations of mutations can be prepared and tested as described herein to identify other LEADs of interest, particularly those that have further increased IFNα-2b antiviral activity or further increased resistance to proteolysis.

[0283] Also provided herein are modified IFNα-2b or IFNα-2a cytokines selected from among proteins comprising one or more single amino acid replacements in SEQ ID NOS:1 or 182, corresponding to the replacement of: N by D at position 45; D by G at position 94; G by R at position 102; A by G at position 139; or any combination thereof. These particular proteins have also been found herein to have increased resistance to proteolysis.

[0284] In another embodiment, IFNα-2b and IFNα-2a proteins that contain a plurality of mutations based on the LEADs (see Tables in the EXAMPLES, listing the candidate LEADs and LEAD sites), are produced to produce IFNα-2b and IFNα-2a proteins that have activity that is further optimized. Examples of such proteins are described herein. Other combinations of mutations can be prepared and tested as described herein to identify other LEADs of interest, particularly those that have further increased IFNα-2b and IFNα-2a antiviral activity or further increased resistance to proteolysis.

[0285] 3. N-glycosylation Site Addition

[0286] In additional embodiments, N-glycosylation sites can be added to increase resistance to proteolysis while maintaining or improving the requisite biological activity.

Exemplary N-glycosylation mutants containing duo-amino acid replacements corresponding to the N-X-S or N-X-T consensus sequences are set forth in Example 3. Accordingly, provided herein are IFNα-2b and IFNα-2a mutant proteins having an increased resistance to proteolysis compared to unmodified IFNα-2b and IFNα-2a, selected from among proteins comprising one or more sets of duo-amino acid replacements in SEQ ID NO:1, corresponding to:

D by N at position 2 and P by S at position 4;

D by N at position 2 and P by T at position 4;

L by N at position 3 and Q by S at position 5;

L by N at position 3 and Q by T at position 5;

P by N at position 4 and T by S at position 6;

P by N at position 4 and T by T at position 6;

Q by N at position 5 and H by S at position 7;

Q by N at position 5 and H by T at position 7;

T by N at position 6 and S by S at position 8;

T by N at position 6 and S by T at position 8;

H by N at position 7 and L by S at position 9;

H by N at position 7 and L by T at position 9;

S by N at position 8 and G by S at position 10;

S by N at position 8 and G by T at position 10;

L by N at position 9 and S by S at position 11;

L by N at position 9 and S by T at position 11;

M by N at position 21 and K by S at position 23;

M by N at position 21 and K by T at position 23;

R by N at position 22 and I by S at position 24;

R by N at position 22 and I by T at position 24;

K or R by N at position 23 and S by S at position 25;

K or R by N at position 23 and S by T at position 25;

I by N at position 24 and L by S at position 26;

I by N at position 24 and L by T at position 26;

S by N at position 25 and F by S at position 27;

S by N at position 25 and F by T at position 27;

L by N at position 26 and S by S at position 28;

L by N at position 26 and S by T at position 28;

S by N at position 28 and L by S at position 30;

S by N at position 28 and L by T at position 30;

L by N at position 30 and D by S at position 32;

L by N at position 30 and D by T at position 32;

K by N at position 31 and R by S at position 33;

K by N at position 31 and R by T at position 33;

D by N at position 32 and H by S at position 34;

D by N at position 32 and H by T at position 34;

R by N at position 33 and D by S at position 35;

R by N at position 33 and D by T at position 35;

H by N at position 34 and F by S at position 36;

H by N at position 34 and F by T at position 36;

D by N at position 35 and G by S at position 37;

D by N at position 35 and G by T at position 37;

F by N at position 36 and F by S at position 38;

F by N at position 36 and F by T at position 38;

G by N at position 37 and P by S at position 39;

G by N at position 37 and P by T at position 39;

F by N at position 38 and Q by S at position 40;

F by N at position 38 and Q by T at position 40;

P by N at position 39 and E by S at position 41;

P by N at position 39 and E by T at position 41;

Q by N at position 40 and E by S at position 42;

Q by N at position 40 and E by T at position 42;

E by N at position 41 and F by S at position 43;

E by N at position 41 and F by T at position 43;

E by N at position 42 and G by S at position 44;

E by N at position 42 and G by T at position 44;

F by N at position 43 and N by S at position 45;

F by N at position 43 and N by T at position 45;

G by N at position 44 and Q by S at position 46;

G by N at position 44 and Q by T at position 46;

N by N at position 45 and F by S at position 47;

N by N at position 45 and F by T at position 47;

Q by N at position 46 and Q by S at position 48;

Q by N at position 46 and Q by T at position 48;

F by N at position 47 and K by S at position 49;

F by N at position 47 and K by T at position 49;

Q by N at position 48 and A by S at position 50;

Q by N at position 48 and A by T at position 50;

K by N at position 49 and E by S at position 51;

K by N at position 49 and E by T at position 51;

A by N at position 50 and T by S at position 52;

A by N at position 50 and T by T at position 52;

S by N at position 68 and K by S at position 70;

S by N at position 68 and K by T at position 70;

K by N at position 70 and S by S at position 72;

K by N at position 70 and S by T at position 72;

A by N at position 75 and D by S at position 77;

A by N at position 75 and D by T at position 77;

D by N at position 77 and T by S at position 79;

D by N at position 77 and T by T at position 79;

I by N at position 100 and G by S at position 102;

I by N at position 100 and G by T at position 102;

Q by N at position 101 and V by S at position 103;

Q by N at position 101 and V by T at position 103;

G by N at position 102 and G by S at position 104;

G by N at position 102 and G by T at position 104;

V by N at position 103 and V by S at position 105;

V by N at position 103 and V by T at position 105;

G by N at position 104 and T by S at position 106;

G by N at position 104 and T by T at position 106;

V by N at position 105 and E by S at position 107;

V by N at position 105 and E by T at position 107;

T by N at position 106 and T by S at position 108;

T by N at position 106 and T by T at position 108;

E by N at position 107 and P by S at position 109;

E by N at position 107 and P by T at position 109;

T by N at position 108 and I by S at position 110;

T by N at position 108 and I by T at position 110;

K by N at position 134 and S by S at position 136;

K by N at position 134 and S by T at position 136;

S by N at position 154 and N by S at position 156;

S by N at position 154 and N by T at position 156;

T by N at position 155 and L by S at position 157;

T by N at position 155 and L by T at position 157;

N by N at position 156 and Q by S at position 158;

N by N at position 156 and Q by T at position 158;

L by N at position 157 and E by S at position 159;

L by N at position 157 and E by T at position 159;

Q by N at position 158 and S by S at position 160;

Q by N at position 158 and S by T at position 160;

E by N at position 159 and L by S at position 161;

E by N at position 159 and L by T at position 161;

S by N at position 160 and R by S at position 162;

S by N at position 160 and R by T at position 162;

L by N at position 161 and S by S at position 163;

L by N at position 161 and S by T at position 163;

R by N at position 162 and K by S at position 164;

R by N at position 162 and K by T at position 164;

S by N at position 163 and E by S at position 165; and

S by N at position 163 and E by T at position 165,

[0287] where residue 1 corresponds to residue 1 of the mature IFNα-2b or IFNα-2a protein set forth in SEQ ID NO:1 or SEQ ID NO:182, respectively. In particular embodiments, the IFNα-2b or IFNα-2a mutant protein has increased resistance to proteolysis compared to unmodified IFNα-2b or IFNα-2a, and is selected from among proteins comprising one or more sets of duo-amino acid replacements in SEQ ID NO:1, corresponding to:

Q by N at position 5 and H by S at position 7;

P by N at position 39 and E by S at position 41;

P by N at position 39 and E by T at position 41;

Q by N at position 40 and E by S at position 42;

Q by N at position 40 and E by T at position 42;

E by N at position 41 and F by S at position 43;

E by N at position 41 and F by T at position 43;

F by N at position 43 and N by S at position 45;

G by N at position 44 and Q by T at position 46;

N by N at position 45 and F by S at position 47;

N by N at position 45 and F by T at position 47;

Q by N at position 46 and Q by S at position 48;

F by N at position 47 and K by S at position 49;

F by N at position 47 and K by T at position 49;

I by N at position 100 and G by S at position 102;

I by N at position 100 and G by T at position 102;

V by N at position 105 and E by S at position 107;

V by N at position 105 and E by T at position 107;

T by N at position 106 and T by S at position 108;

T by N at position 106 and T by T at position 108;

E by N at position 107 and P by S at position 109;

E by N at position 107 and P by T at position 109;

L by N at position 157 and E by S at position 159;

L by N at position 157 and E by T at position 159;

E by N at position 159 and L by S at position 161; and

E by N at position 159 and L by T at position 161.

F. Protein Redesign

[0288] Provided herein are methods for designing and generating new versions of native or modified cytokines, such as IFNα-2b and IFNα-2a. Using these methods, the redesigned cytokine maintains either sufficient, typically equal or improved levels of a selected phenotype, such as a biological activity, of the original protein, while at the same time its amino acid sequence is changed by replacement of up to: at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 12%, at least 14%, at least 16%, at least 18%, at least 20%, at least 30%, at least 40% up to 50% or more of its native amino acids by the appropriate pseudo-wild type amino acids. Pseudo-wild type amino acids are those amino acids such that when they replace an original, such as native, amino acid at a given position on the protein sequence, the resulting protein displays substantially the same levels of biological activity (or sufficient activity for its therapeutic or other use) compared to the original, such as native, protein. In other embodiments, pseudo-wild type

amino acids are those amino acids such that when they replace an original, such as native, amino acid at a given position on the protein sequence, the resulting protein displays the same phenotype, such as levels of biological activity, compared to an original, typically a native, protein. Pseudo-wild type amino acids and the appropriate replacing positions can be detected and identified by any analytical or predictive means; such as for example, by performing an alanine-scanning. Any other amino acid, particularly another amino acid that has a neutral effect on structure, such as Gly or Ser, also can be used for the scan. All those replacements of original, such as native, amino acids by Ala that do not lead to the generation of a HIT (a protein that has lost the desired biological activity), have either led to the generation of a LEAD (a protein with increased biological activity); or the replacement by Ala will be a neutral replacement, i.e., the resulting protein will display comparable levels of biological activity compared to the original, such as native, protein. The methods provided herein for protein redesign of cytokines, such as IFNα-2b and IFNα-2a, are intended to design and generate "artificial" (versus naturally existing) proteins, such that they consist of amino acid sequences not existing in nature, but that display biological activities characteristic of the original, such as native, protein. These redesigned proteins are contemplated herein to be useful for avoiding potential side effects that might otherwise exist in other forms of cytokines in treatment of disease. Other uses of redesigned proteins provided herein are to establish cross-talk between pathways triggered by different proteins; to facilitate structural biology by generating mutants that can be crystallized while maintaining activity; and to destroy an activity of a protein without changing a second activity or multiple additional activities.

[0289] In one embodiment, a method for obtaining redesigned proteins includes i) identifying some or all possible target sites on the protein sequence that are susceptible to amino acid replacement without losing protein activity (protein activity in a largest sense of the term: enzymatic, binding, hormone, etc.) (These sites are the pseudo-wild type, ψ-wt sites); ii) identifying appropriate replacing amino acids (ψ-wt amino acids), specific for each ψ-wt site, such that if used to replace the native amino acids at that specific ψ-wt site, they can be expected to generate a protein with comparable biological activity compared to the original, such as native, protein, thus keeping the biological activity of the protein substantially unchanged; iii) systematically introducing the specific ψ-wt amino acids at every specific ψ-wt position so as to generate a collection containing the corresponding mutant molecules. Mutants are generated, produced and phenotypically characterized one-by-one, in addressable arrays, such that each mutant molecule contains initially amino acid replacements at only one ψ-wt site. In subsequent rounds mutant molecules also can be generated such that they contain one or more ψ-wt amino acids at one or more ψ-wt sites. Those mutant proteins carrying several mutations at a number of ψ-wt sites, and that display comparable or improved biological activity are called redesigned proteins or ψ-wt proteins. In particular embodiments, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, at least 25%, or more of the amino acid residue positions on a particular cytokine, such as IFNα-2b and IFNα-2a are replaced with an appropriate pseudo-wild type amino acid.

[0290] The first step is an amino acid scan over the full length of the protein. At this step, each and every one of the amino acids in the protein sequence is replaced by a selected reference amino acid, such as alanine. This permits the identification of "redesign-HIT" positions, i.e., positions that are sensitive to amino acid replacement. All of the other positions that are not redesign-HIT positions (i.e., those at which the replacement of the original, such as native, amino acid by the replacing amino acid, for example Ala, does not lead to a drop in protein fitness or biological activity) are referred to herein as "pseudo-wild type" positions. When the replacing amino acid, for example Ala, replaces the original, such as native, amino acid at a non-HIT position, then the replacement is neutral, in terms of protein activity, and the replacing amino acid is said to be a pseudo-wild type amino acid at that position. Pseudo-wild type positions appear to be less sensitive than redesign-HIT positions since they tolerate the amino acid replacement without affecting the protein activity that is being either maintained or improved. Amino acid replacement at the pseudo-wild type positions, result in a non-change in the protein fitness (e.g., possess substantially the same biological activity), while at the same time to a divergence in the resulting protein sequence compared to the original, such as native, sequence.

[0291] To first identify those amino acid positions on the IFNα-2b and IFNα-2a protein that are involved or not involved in IFNα-2b and IFNα-2a protein activity, such as binding activity of IFNα-2b and IFNα-2a to its receptor, an Ala-scan was performed on the IFNα-2b sequence as set forth in Example 4. For this purpose, each amino acid in the IFNα-2b protein sequence was individually changed to Alanine. Any other amino acid, particularly another amino acid that has a neutral effect on structure, such as Gly or Ser, also can be used. Each resulting mutant IFNα-2b protein was then expressed and the activity of the interferon molecule was then assayed. These particular amino acid positions, referred to herein as HITs would in principle not be suitable targets for amino acid replacement to increase protein stability, because of their involvement in the recognition of IFN-receptor or in the downstream pathways involved in IFN activity. For the Ala-scanning, the biological activity measured for the IFNα-2b molecules was: i) their capacity to inhibit virus replication when added to permissive cells previously infected with the appropriate virus and, ii) their capacity to stimulate cell proliferation when added to the appropriate cells. The relative activity of each individual mutant compared to the native protein is indicated in FIG. **10**A through C. HITs are those mutants that produce a decrease in the activity of the protein (in the example: all the mutants with activities below about 30% of the native activity.

[0292] In addition, the alanine-scan was used to identify the amino acid residues on IFNα-2b that when replaced with alanine correspond to "pseudo-wild type" activity, i.e., those that can be replaced by alanine without leading to a decrease in biological activity. Knowledge of these amino acids is useful for the re-design of the IFNα-2b and IFNα-2a proteins. The results are set forth in Table 5, and include pseudo-wild type amino acid positions of IFNα-2b corresponding to SEQ ID NO:1, amino acid residues: 9, 10, 17, 20, 24, 25, 35, 37, 41, 52, 54, 56, 57, 58, 60, 63, 64, 65, 76, 89, and 90.

[0293] Accordingly, provided herein are IFNα-2b and IFNα-2a mutant proteins comprising one or more pseudo-wild type mutations at amino acid positions of IFNα-2b or IFNα-2a corresponding to SEQ ID NO: 1 or SEQ ID NO:182, respectively, amino acid residues: 9, 10, 17, 20, 24, 25, 35, 37, 41, 52, 54, 56, 57, 58, 60, 63, 64, 65, 76, 89, and 90. The mutations can be either one or more of insertions, deletions and/or replacements of the native amino acid residue(s). In one embodiment, the pseudo-wild type replacements are mutations with alanine at each position. In another embodiment, the pseudo-wild type replacements are one or more mutations in SEQ ID NO:1 corresponding to:

[0294] L by A at position 9, L by A at position 17,

[0295] Q by A at position 20, I by A at position 24,

[0296] S by A at position 25, D by A at position 35,

[0297] G by A at position 37, E by A at position 41,

[0298] T by A at position 52, P by A at position 54,

[0299] L by A at position 56, H by A at position 57,

[0300] E by A at position 58, I by A at position 60,

[0301] I by A at position 63, F by A at position 64,

[0302] N by A at position 65, W by A at position 76,

[0303] Y by A at position 89, and Q by A at position 90.

[0304] In addition, the IFNα-2b alanine scan revealed the following redesign-HITs having decreased antiviral activity at amino acid positions of IFNα-2b corresponding to SEQ ID NO:1, amino acid residues: 2, 7, 8, 11, 13, 15, 16, 23, 26, 28, 29, 30, 31, 32, 33, 53, 69, 91, 93, 98, and 101. Accordingly, in particular embodiments where it is desired to decrease the anti-viral activity of IFNα-2b or IFNα-2a, either one or more of insertions, deletions and/or replacements of the native amino acid residue(s) can be carried out at one or more of amino acid positions of IFNα-2b or IFNα-2a corresponding to SEQ ID NO:1, amino acid residues: 2, 7, 8, 11, 13, 15, 16, 23, 26, 28, 29, 30, 31, 32, 33, 53, 69, 91, 93, 98, and 101.

[0305] Each of the redesign mutations set forth above can be combined with one or more of the IFNα-2b or IFNα-2a candidate LEAD mutations or one or more of the IFNα-2b or IFNα-2a LEAD mutants provided herein.

G. 3D-Scanning and its Use for Modifying Cytokines

[0306] Also provided herein is a method of structural homology analysis for comparing proteins regardless of their underlying amino acid sequences. For a subset of proteins families, such as the family of human cytokines, this information is rationally exploited to produce modified proteins. This method of structural homology analysis can be applied to proteins that are evolved by any method, including the 2D scanning method described herein. When used with the 2D method in which a particular phenotype, activity or characteristic of a protein is modified by 2D analysis, the method is referred to as 3D-scanning.

[0307] The use of "structural homology" analysis in combination with the directed evolution methods provided herein provides a powerful technique for identifying and producing various new protein mutants, such as cytokines, having desired biological activities, such as increased resistance to proteolysis. For example, the analysis of the "structural homology" between an optimized mutant version of a given protein and "structurally homologous" proteins allows identification of the corresponding structurally related or structurally similar amino acid positions (also referred to herein as "structurally homologous loci") on other proteins. This permits identification of mutant versions of the latter that have a desired optimized feature(s) (biological activity, phenotype) in a simple, rapid and predictive manner (regardless of amino acid sequence and sequence homology). Once a mutant version of a protein is developed, then, by applying the rules of structural homology, the corresponding structurally related amino acid positions (and replacing amino acids) on other "structurally homologous" proteins readily are identified, thus allowing a rapid and predictive discovery of the appropriate mutant versions for the new proteins.

[0308] 3-dimensionally structurally equivalent or similar amino acid positions that are located on two or more different protein sequences that share a certain degree of structural homology, have comparable functional tasks (activities and phenotypes). These two amino acids that occupy substantially equivalent 3-dimensional structural space within their respective proteins than can be said to be "structurally similar" or "structurally related" with each other, even if their precise positions on the amino acid sequences, when these sequences are aligned, do not match with each other. The two amino acids also are said to occupy "structurally homologous loci."“Structural homology" does not take into account the underlying amino acid sequence and solely compares 3-dimensional structures of proteins. Thus, two proteins can be said to have some degree of structural homology whenever they share conformational regions or domains showing comparable structures or shapes with 3-dimensional overlapping in space. Two proteins can be said to have a higher degree of structural homology whenever they share a higher amount of conformational regions or domains showing comparable structures or shapes with 3-dimensional overlapping in space. Amino acids positions on one or more proteins that are "structurally homologous" can be relatively far way from each other in the protein sequences, when these sequences are aligned following the rules of primary sequence homology. Thus, when two or more protein backbones are determined to be structurally homologous, the amino acid residues that are coincident upon three-dimensional structural superposition are referred to as "structurally similar" or "structurally related" amino acid residues in structurally homologous proteins (also referred to as "structurally homologous loci"). Structurally similar amino acid residues are located in substantially equivalent spatial positions in structurally homologous proteins.

[0309] For example, for proteins of average size (approximately 180 residues), two structures with a similar fold will usually display rms deviations not exceeding 3 to 4 angstroms. For example, structurally similar or structurally related amino acid residues can have backbone positions less than 3.5, 3.0, 2.5, 2.0, 1.7 or 1.5 angstrom from each other upon protein superposition. RMS deviation calculations and protein superposition can be carried out using any of a number of methods known in the art. For example, protein superposition and RMS deviation calculations can be carried out using all peptide backbone atoms (e.g., N, C, C(C═O), O and CA (when present)). As another example, protein

superposition can be carried out using just one or any combination of peptide backbone atoms, such as, for example, N, C, C(C═O), O and CA (when present). In addition, one skilled in the art will recognize that protein superposition and RMS deviation calculations generally can be performed on only a subset of the entire protein structure. For example, if the protein superposition is carried out using one protein that has many more amino acid residues than another protein, protein superposition can be carried out on the subset (e.g., a domain) of the larger protein that adopts a structure similar to the smaller protein. Similarly, only portions of other proteins can be suitable for superimposition. For example, if the position of the C-terminal residues from two structurally homologous proteins differ significantly, the C-terminal residues can be omitted from the structural superposition or RMS deviation calculations.

[0310] Accordingly, provided herein are methods of rational evolution of proteins based on the identification of potential target sites for mutagenesis (is-HITs) through comparison of patterns of protein backbone folding between structurally related proteins, irrespective of the underlying sequences of the compared proteins. Once the structurally related amino acid positions are identified on the new protein, then suitable amino acid replacement criteria, such as PAM analysis, can be employed to identify candidate LEADs for construction and screening as described herein.

[0311] For example, analysis of "structural homology" between and among a number of related cytokines was used to identify on various members of the cytokine family, other than interferon alpha, those amino acid positions and residues that are structurally similar or structurally related to those found in the IFNα-2b mutants provided herein that have been optimized for improved stability. The resulting modified cytokines are provided. This method can be applied to any desired phenotype using any protein, such as a cytokine, as the starting material to which an evolution procedure, such as the rational directed evolution procedure of U.S. application Ser. No. 10/022,249 or the 2-dimensional scanning method provided herein, is applied. The structurally corresponding residues are then altered on members of the family to produce additional cytokines with similar phenotypic alterations.

[0312] 1. Homology

[0313] Typically, homology between proteins is compared at the level of their amino acid sequences, based on the percent or level of coincidence of individual amino acids, amino acid per amino acid, when sequences are aligned starting from a reference, generally the residue encoded by the start codon. For example, two proteins are said to be "homologous" or to bear some degree of homology whenever their respective amino acid sequences show a certain degree of matching upon alignment comparison. Comparative molecular biology is primarily based on this approach. From the degree of homology or coincidence between amino acid sequences, conclusions can be made on the evolutionary distance between or among two or more protein sequences and biological systems.

[0314] The concept of "convergent evolution" is applied to describe the phenomena by which phylogenetically unrelated organisms or biological systems have evolved to share features related to their anatomy, physiology and structure as a response to common forces, constraints, and evolutionary demands from the surrounding environment and living organisms. Alternatively, "divergent evolution," is applied to describe the phenomena by which strongly phylogenetically related organisms or biological systems have evolved to diverge from identity or similarity as a response to divergent forces, constraints, and evolutionary demands from the surrounding environment and living organisms.

[0315] In the typical traditional analysis of homologous proteins there are two conceptual biases corresponding to: i) "convergent evolution," and ii) "divergent evolution." Whenever the aligned amino acid sequences of two proteins do not match well with each other, these proteins are considered "not related" or "less related" with each other and have different phylogenetic origins. There is no (or low) homology between these proteins and their respective genes are not homologous (or show little homology). If these two "non-homologous" proteins under study share some common functional features (e.g., interaction with other specific molecules, activity), they are determined to have arisen by "convergent evolution," i.e., by evolution of their non-homologous amino acid sequences, in such a way that they end up generating functionally "related" structures.

[0316] On the other hand, whenever the aligned amino acid sequences of two proteins do match with each other to a certain degree, these proteins are considered to be "related" and to share a common phylogenetic origin. A given degree of homology is assigned between these two proteins and their respective genes likewise share a corresponding degree of homology. During the evolution of their initial highly homologous amino acid sequence, enough changes can be accumulated in such a way that they end up generating "less-related" sequences and less related function. The divergence from perfect matching between these two "homologous" proteins under study is said come from "divergent evolution."

[0317] 2. 3D-Scanning (Structural Homology) Methods

[0318] Structural homology refers to homology between the topology and three-dimensional structure of two proteins. Structural homology is not necessarily related to "convergent evolution" or to "divergent evolution," nor is it related to the underlying amino acid sequence. Rather, structural homology is likely driven (through natural evolution) by the need of a protein to fit specific conformational demands imposed by its environment. Particular structurally homologous "spots" or "loci" would not be allowed to structurally diverge from the original structure, even when its own underlying sequence does diverge. This structural homology is exploited herein to identify loci for mutation.

[0319] Within the amino acid sequence of a protein resides the appropriate biochemical and structural signals to achieve a specific spatial folding in either an independent or a chaperon-assisted manner. Indeed, this specific spatial folding ultimately determines protein traits and activity. Proteins interact with other proteins and molecules in general through their specific topologies and spatial conformations. In principle, these interactions are not based solely on the precise amino acid sequence underlying the involved topology or conformation. If protein traits, activity (behavior and phenotypes) and interactions rely on protein topology and conformation, then evolutionary forces and constraints acting on proteins can be expected to act on topology and conformation. Proteins sharing similar functions will share

comparable characteristics in their topology and conformation, despite the underlying amino acid sequences that create those topologies and conformations.

[0320] 3. Application of the 3D-Scanning Methods to Cytokines

[0321] The method based on structural homology, including the 3D-scanning method provided herein can be applied to any related proteins. For exemplary purposes herein it is applied to cytokines. In exemplary embodiments, methods for altering phenotypes of members of families of cytokines by altering one member such as by employing the 2-dimensional rational scanning method are provided. As provided herein, other members of these cytokine families then can be similarly modified by identifying and changing structurally homologous residues to similarly alter the phenotypes of such proteins.

[0322] In an exemplary embodiment herein, IFNα-2b mutants with increased resistance to proteolysis are generated by the 2-dimensional rational scanning method; IFNβ mutants also were generated. The corresponding residues on members of cytokine families that possess structural homology to IFNα-2b were identified and the identified residues on the other cytokines were similarly modified to produce cytokines with increased resistance to proteolysis. Hence also provided herein are cytokine mutants that display increased resistance to proteolysis and/or glomerular filtration containing one or more amino acid replacements.

[0323] Provided herein are mutant (modified) cytokines that display altered features and properties, such as a resistance to proteolysis. Methods for producing such modified cytokines also are provided.

[0324] Also provided herein is a method of structural homology analysis for comparing proteins regardless their underlying amino acid sequences. For a subset of proteins families, such as the family of human cytokines, this information is rationally exploited herein. Human cytokines all share a common helix bundle fold, which is used to structurally define the 4-helical cytokine superfamily in the structural classification of the protein database SCOP© (Structural Classification of Proteins; see, e.g., Murzin et al., *J. Mol. Biol.,* 247:536-540, 1995 and "scop.mrc-lmb.cam.ac.uk/scop/"). This superfamily includes three different families: 1) the interferons/interleukin-10 protein family (SEQ ID NOS: 1 and 182-200); 2) the long-chain cytokine family (SEQ ID NOS: 210-217); and 3) the short-chain cytokine family (SEQ ID NOS: 201-209).

[0325] For example, a distinct feature of cytokines from the interferons/interleukin-10 family is an additional (fifth) helix. This family includes interleukin-10 (IL-10; SEQ ID NO:200, interferon beta (IFNβ; SEQ ID NO: 196), interferon alpha-2a (IFNα-2a; SEQ ID NO: 182), interferon alpha-2b (IFNα-2b; SEQ ID NO:1), and interferon gamma (IFN-γ; SEQ ID NO: 199). The long-chain cytokine protein family includes, among others, granulocyte colony stimulating factor (G-CSF; SEQ ID NO: 210), leukemia inhibitory factor (LIF; SEQ ID NO: 213), growth hormone (hGH; SEQ ID NO: 216), ciliary neurotrophic factor (CNTF; SEQ ID NO: 212), leptin (SEQ ID NO: 211), oncostatin M (SEQ ID NO: 214), interleukin-6 (IL-6; SEQ ID NO: 217) and interleukin-12 (IL-12; SEQ ID NO: 215). The short-chain cytokine protein family includes, among others, erythropoietin (EPO; SEQ ID NO: 201), granulocyte-macrophage colony stimulating factor (GM-CSF; SEQ ID NO: 202), interleukin-2 (IL-2; SEQ ID NO: 204), interleukin-3 (IL-3; SEQ ID NO: 205), interleukin-4 (IL-4; SEQ ID NO: 207), interleukin-5 (IL-5; SEQ ID NO: 208), interleukin-13 (IL-13; SEQ ID NO: 209), Flt3 ligand (SEQ ID NO: 203) and stem cell factor (SCF; SEQ ID NO: 206).

[0326] Although the degree of similarity among the underlying amino acid sequences of these cytokines does not appear high, their corresponding 3-dimensional structures present a high level of similarity (see, e.g., FIGS. **8**B through D). Effectively, the best structural similarity is obtained between two 3-dimensional protein structures of the same family in the 4-helical cytokine superfamily.

[0327] The methods provided herein for producing mutant cytokines are exemplified with reference to production of cytokines that display a substantially equivalent increase in resistance to proteolysis relative to the optimized IFNα-2b mutants. It is understood that this method can be applied to other families of proteins and for other phenotypes.

[0328] In one embodiment, proteins of the 4-helical cytokine superfamily are provided herein that are structurally homologous IFNα-2b LEAD mutants set forth herein. For example, by virtue of the knowledge of the 3-dimensional structural amino acid positions within the LEAD IFNα-2b mutants provided herein that confer higher resistance to a challenge with either proteases or blood lysate or serum, while maintaining or improving the requisite biological activity, the corresponding structurally related (e.g., structurally similar) amino acid residues on a variety of other cytokines are identified (FIG. **9**).

[0329] Numerous methods are well known in the art for identifying structurally related amino acid positions with 3-dimensionally structurally homologous proteins. Exemplary methods include, but are not limited to: CATH (Class, Architecture, Topology and Homologous superfamily) which is a hierarchical classification of protein domain structures based on four different levels (Orengo et al., *Structure,* 5(8):1093-1108 (1997)); CE (Combinatorial Extension of the optimal path), which is a method that calculates pairwise structure alignments (Shindyalov et al., *Protein Engineering,* 11(9):739-747 (1998)); FSSP (Fold classification based on Structure-Structure alignment of Proteins), which is a database based on the complete comparison of all 3-dimensional protein structures that currently reside in the Protein Data Bank (PDB) (Holm et al., *Science,* 273:595-602 (1996)); SCOP® (Structural Classification of Proteins), which provides a descriptive database based on the structural and evolutionary relationships between all proteins whose structure is known (Murzin et al., *J. Mol. Biol.,* 247:536-540 (1995)); and VAST (Vector Alignment Search Tool), which compares newly determined 3-dimensional protein structure coordinates to those found in the MMDB/PDB database (Gibrat et al., *Current Opinion in Structural Biology,* 6:377-385 (1995)).

[0330] In an exemplary embodiment, the step-by-step process including the use of a program referred to as TOP (see FIG. 8A and Lu, G., *J. Appl. Cryst.,* 33:176-189 (2000)); publicly available, for example, at bioinfo1.mbfys.lu.se/TOP is used for protein structure comparison. This program runs two steps for each protein structure comparison. In the first step topology of secondary

structure in the two structures is compared. The program uses two points to represent each secondary structure element (alpha helices or beta strands) then systematically searches all the possible super-positions of these elements in 3-dimensional space (defined as the root mean square deviation—rmsd, the angle between the two lines formed by the two points and the line-line distance). The program searches to determine whether additional secondary structure elements can fit by the same superposition operation. If secondary structures that can fit each other exceed a given number, the program identifies the two structures as similar. The program gives as an output a comparison score called "Structural Diversity" that considers the distance between matched α-carbon atoms and the number of matched residues. The lower the "Structural Diversity" score, the more the two structures are similar. In various embodiments herein, the Structural Diversity scores range from 0 up to about 67.

[0331] In the exemplified embodiment, all the cytokines were first structurally aligned against the IFNα-2b structure. For the proteins within the same family as IFNα-2b (e.g., the interferons/interleukin-10 cytokine family), this alignment was directly used to identify the structurally related is-HIT target amino acid positions and/or regions corresponding to the structurally homologous positions and/or regions on IFNα-2b where LEAD mutants were found (FIG. **8**B). For the other cytokines, the protein of the family (either long- or short-chain cytokines) with the best 3-dimensional structural alignment with IFNα-2b was selected using the lowest "Structural Diversity" score as the representative for that family. From the short-chain cytokine protein family, erythropoietin (EPO; see FIG. **8**C) was identified as the best structural homologue of IFNα-2b (rmsd=1.9 angstroms; number of aligned residues=62; Structural Diversity=13.8). From the long-chain cytokine protein family, granulocyte-colony stimulating factor (G-CSF; see FIG. **8**D) was identified as the best structural homologue of IFNα-2b (rmsd=1.7 angstroms; number of aligned residues=77; Structural Diversity=7.8).

[0332] Next, the amino acid positions and/or regions corresponding to the LEAD mutant regions on IFNα-2b were identified on these two proteins. These two best structural homologues of IFNα-2b (e.g., EPO and G-CSF; see FIGS. **12**L and **12**E, respectively) were structurally aligned to each of the other cytokines within their respective cytokine protein families. As a result, protein regions likely to be targets for serum protease resistance were identified on all cytokines (see FIGS. **12**A through T). Amino acids in these target regions were then checked for their exposure to the solvent and their susceptibility to be protease substrate. Exposed and substrate residues are then subjected to PAM250 analysis as set forth above, so that a group of non-substrate and functionally conservative amino acid residues are selected as replacements. The results of the above structural homology analysis for each of the cytokines provided herein are set forth in FIGS. **12**A through T.

[0333] Accordingly, provided herein are modified cytokines that exhibit greater resistance to proteolysis compared to the unmodified cytokine protein, comprising one or more amino acid replacements at one or more target positions on the cytokine corresponding to a structurally-related modified amino acid position within the 3-dimensional structure of an IFNα-2b modified protein provided herein. The resistance to proteolysis can be measured by mixing it with a protease in vitro, incubation with blood or incubation with serum. Also provided herein are cytokine structural homologues of an IFNα-2b modified protein provided herein, comprising one or more amino acid replacements in the cytokine structural homologue at positions corresponding to the 3-dimensional-structurally-similar modified positions within the 3-dimensional structure of the modified IFNα-2b. In one embodiment, the cytokine homologue has increased resistance to proteolysis compared to its unmodified and/or wild type cytokine counterpart. Resistance to proteolysis can be measured by mixture with a protease in vitro, incubation with blood, or incubation with serum.

[0334] a. Structurally Homologous Interferon Mutants

[0335] Also provided herein are modified cytokines or cytokine structural homologues of IFNα-2b that are IFNα cytokines. These IFNα cytokines include, but are not limited to, IFNα-2a, IFNα-c, IFNα-2c, IFNα-d, IFNα-5, IFNα-6, IFNα-4, IFNα-4-b, IFNα-I, IFNα-J, IFNα-H, IFNα-F, IFNα-8 and IFNα-consensus cytokine (see, SEQ ID No. 232). Accordingly, among the modified IFNα cytokines provided herein are those with one or more amino acid replacements at one or more target positions in either IFNα-2a, IFNα-c, IFNα-2c, IFNα-d, IFNα-5, IFNα-6, IFNα-4, IFNα-4-b, IFNα-I, IFNα-J, IFNα-H, IFNα-F, IFNα-8, or IFNα-consensus cytokine corresponding to a structurally-related modified amino acid position within the 3-dimensional structure of the IFNα-2b modified proteins provided herein. The replacements lead to greater resistance to proteases, as assessed by incubation with a protease or a with a blood lysate or by incubation with serum, compared to the unmodified IFNα-2a.

[0336] In particular embodiments, the modified IFNα cytokines are selected from among:

[0337] the modified IFNα-2a that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 182, corresponding to amino acid positions: 41, 58, 78, 107, 117, 125, 133 and 159;

[0338] the modified IFNα-c that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 183, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

[0339] the modified IFNα-2c cytokine that is human and is selected from among cytokines comprising one or more single amino acid replacements in SEQ ID NO: 185, corresponding to amino acid positions: 41, 58, 78, 107, 117, 125, 133 and 159;

[0340] the IFNα-d modified protein that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 186, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

[0341] the IFNα-5 modified protein that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 187, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

[0342] the IFNα-6 modified protein that is human and is selected from among proteins comprising one or more single

amino acid replacements in SEQ ID NO: 188, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

[0343] the IFNα-4 modified protein that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 189, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

[0344] the IFNα-4-b modified protein that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 190, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

[0345] the IFNα-I modified protein that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 191, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

[0346] the IFNα-J modified protein that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 192, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

[0347] the IFNα-H modified protein that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 193, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

[0348] the IFNα-F modified protein that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 194, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160;

[0349] the IFNα-8 modified protein that is human and is selected from among proteins comprising one or more single amino acid replacements in SEQ ID NO: 195, corresponding to amino acid positions: 41, 59, 79, 108, 118, 126, 134 and 160; and

[0350] the IFNα-consensus modified protein that is human and is selected from among proteins that contain one or more single amino acid replacements in SEQ ID NO: 232, corresponding to amino acid positions: 41, 58, 78, 107, 117, 125, 133 and 159.

[0351] b. Structurally Homologous Cytokine Mutants

[0352] As set forth above, provided herein are modified cytokines that contain one or more amino acid replacements at one or more target positions in either interleukin-10 (IL-10), interferon beta (IFNβ), IFNβ-1, IFNβ-2a, interferon gamma (IFN-γ), granulocyte colony stimulating factor (G-CSF), and human erythropoietin (EPO); corresponding to a structurally-related modified amino acid position within the 3-dimensional structure of the IFNα-2b modified proteins provided herein. The replacements lead to greater resistance to proteases, as assessed by incubation with a protease or a with a blood lysate or by incubation with serum, compared to the unmodified cytokine.

[0353] Also provided herein are modified cytokines that contain one or more amino acid replacements at one or more target positions in either granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-13 (IL-13), Flt3 ligand and stem cell factor (SCF); corresponding to a structurally-related modified amino acid position within the 3-dimensional structure of the human EPO modified proteins provided herein. The replacements lead to greater resistance to proteases, as assessed by incubation with a protease or a with a blood lysate or by incubation with serum, compared to the unmodified cytokine.

[0354] Also provided herein are modified cytokines that contain one or more amino acid replacements at one or more target positions in either interleukin-10 (IL-10), interferon beta (IFNβ), interferon gamma (IFN-γ), human granulocyte colony stimulating factor (G-CSF), leukemia inhibitory factor (LIF), human growth hormone (hGH), ciliary neurotrophic factor (CNTF), leptin, oncostatin M, interleukin-6 (IL-6) and interleukin-12 (IL-12); corresponding to a structurally-related modified amino acid position within the 3-dimensional structure of the human G-CSF modified proteins provided herein. The replacements lead to greater resistance to proteases, as assessed by incubation with a protease or a with a blood lysate or by incubation with serum, compared to the unmodified cytokine.

[0355] In particular embodiments, the modified cytokines are selected from the following:

[0356] A modified IFNβ cytokine, comprising mutations at one or more amino acid residues of IFNβ corresponding to SEQ ID NO: 196: 39, 42, 45, 47, 52, 67, 71, 73, 81, 107, 108, 109, 110, 111, 113, 116, 120, 123, 124, 128, 130, 134, 136, 137, 163 and 165. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In particular embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 196 set forth in FIG. **12**A corresponding to SEQ ID NOS: 233-289, where the first amino acid indicated is substituted by the second at the position indicated for all of the substitutions set forth in FIG. **12**A through T.

[0357] A modified IFN-gamma cytokine, comprising mutations at one or more amino acid residues of IFN-gamma corresponding to SEQ ID NO:199: 33, 37, 40, 41, 42, 58, 61, 64, 65 and 66. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In particular embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO:199 set forth in FIG. **12**B corresponding to SEQ ID NOS: 290-311.

[0358] A modified IL-10 cytokine, comprising mutations at one or more amino acid residues of IL-10 corresponding to SEQ ID NO:200: 49, 50, 52, 53, 54, 55, 56, 57, 59, 60, 67, 68, 71, 72, 74, 75, 78, 81, 84, 85, 86, and 88. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, replacements are selected from among amino acid substitutions in SEQ ID NO:200 set forth in FIG. **12**C corresponding to SEQ ID NOS: 312-361.

[0359] A modified erythropoietin cytokine, comprising mutations at one or more amino acid residues of erythropoietin corresponding to SEQ ID NO:201: 43, 45, 48, 49, 52, 53, 55, 72, 75, 76, 123, 129, 130, 131, 162, and 165. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodi-

ments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 201 set forth in FIG. **12**L corresponding to SEQ ID NOS: 940-977.

[**0360**] A modified GM-CSF cytokine, comprising mutations at one or more amino acid residues of GM-CSF corresponding to SEQ ID NO: 202: 38, 41, 45, 46, 48, 49, 51, 60, 63, 67, 92, 93, 119, 120, 123, and 124. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 202 set forth in FIG. **12**N corresponding to SEQ ID NOS: 362-400.

[**0361**] A modified Flt3 ligand cytokine, comprising mutations at one or more amino acid residues of Flt3 ligand corresponding to SEQ ID NO: 203: 3, 40, 42, 43, 55, 58, 59, 61, 89, 90, 91, 95, and 96. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 203 set forth in FIG. **12**M corresponding to SEQ ID NOS: 401-428.

[**0362**] A modified IL-2 cytokine, comprising mutations at one or more amino acid residues of IL-2 corresponding to SEQ ID NO: 204 at positions 43, 45, 48, 49, 52, 53, 60, 61, 65, 67, 68, 72, 100, 103, 104, 106, 107, 109, 110, and 132. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 204 set forth in FIG. **12**P and SEQ ID NOS: 429-476.

[**0363**] A modified IL-3 cytokine, comprising mutations at one or more amino acid residues of IL-3 corresponding to SEQ ID NO: 205: 37, 43, 46, 59, 63, 66, 96, 100, 101, and 103. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO:205 set forth in FIG. **12**Q corresponding to SEQ ID NOS: 477-498.

[**0364**] A modified SCF cytokine, comprising mutations at one or more amino acid residues of SCF corresponding to SEQ ID NO: 206: 27, 31, 34, 37, 54, 58, 61, 62, 63, 96, 98, 99, 100, 102, 103, 106, 107, 108, 109, 134, and 137. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 206 set forth in FIG. **12**T corresponding to SEQ ID NOS: 499-542.

[**0365**] A modified IL-4 cytokine, comprising mutations at one or more amino acid residues of IL-4 corresponding to SEQ ID NO: 207: 26, 37, 53, 60, 61, 64, 66, 100, 102, 103, and 126. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 207 set forth in FIG. **12**R corresponding to SEQ ID NOS: 543-567.

[**0366**] A modified IL-5 cytokine, comprising mutations at one or more amino acid residues of IL-5 corresponding to SEQ ID NO: 208: 32, 34, 39, 46, 47, 56, 84, 85, 88, 89, 90, 102, 110, and 111. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 208 set forth in FIG. **12**S corresponding to SEQ ID NOS: 568-602.

[**0367**] A modified IL-13 cytokine, comprising mutations at one or more amino acid residues of IL-13 corresponding to SEQ ID NO: 209: 32, 34, 38, 48, 79, 82, 85, 86, 88, 107, 108, 110, and 111. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 209 set forth in FIG. **12**O corresponding to SEQ ID NOS: 603-630.

[**0368**] A modified G-CSF cytokine, comprising mutations at one or more amino acid residues of G-CSF corresponding to SEQ ID NO: 210: 61, 63, 68, 72, 86, 96, 100, 101, 131, 133, 135, 147, 169, 172, and 177. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 210 set forth in FIG. **12**E corresponding to SEQ ID NOS: 631-662.

[**0369**] A modified leptin cytokine, comprising mutations at one or more amino acid residues of leptin corresponding to SEQ ID NO: 211: 43, 49, 99, 100, 104, 105, 107, 108, 141 and 142. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 211 set forth in FIG. **12**I corresponding to SEQ ID NOS: 663-683.

[**0370**] A modified CNTF cytokine, comprising mutations at one or more amino acid residues of CNTF corresponding to SEQ ID NO: 212: 62, 64, 66, 67, 86, 89, 92, 100, 102, 104, 131, 132, 133, 135, 136, 138, 140, 143, 148, and 151. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 212 set forth in FIG. **12**D corresponding to SEQ ID NOS: 684-728.

[**0371**] A modified LIF cytokine, comprising mutations at one or more amino acid residues of LIF corresponding to SEQ ID NO: 213: 69, 70, 85, 99, 102, 104, 106, 109, 137, 143, 146, 148, 149, 153, 154, and 156. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 213 set forth in FIG. **12**J corresponding to SEQ ID NOS: 729-760.

[**0372**] A modified oncostatin M cytokine, comprising mutations at one or more amino acid residues of oncostatin M corresponding to SEQ ID NO: 214: 59, 60, 63, 65, 84, 87, 89, 91, 94, 97, 99, 100, 103, and 106. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 214 set forth in FIG. **12**K corresponding to SEQ ID NOS: 761-793.

[**0373**] A modified IL-12 cytokine, comprising mutations at one or more amino acid residues of IL-12 corresponding to SEQ ID NO: 215: 56, 61, 66, 67, 68, 70, 72, 75, 78, 79, 82, 89, 92, 93, 107, 110, 111, 115, 117, 124, 125, 127, 128, 129, and 189. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from

among amino acid substitutions in SEQ ID NO: 215 set forth in FIG. **12**G corresponding to SEQ ID NOS: 794-849.

[**0374**] A modified hGH cytokine, comprising mutations at one or more amino acid residues of hGH corresponding to SEQ ID NO: 216: 56, 59, 64, 65, 66, 88, 92, 94, 101, 129, 130, 133, 134, 140, 143, 145, 146, 147, 183, and 186. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 216 set forth in FIG. **12**F corresponding to SEQ ID NOS: 850-895.

[**0375**] A modified IL-6 cytokine, comprising mutations at one or more amino acid residues of IL-6 corresponding to SEQ ID NO: 217: 64, 65, 66, 68, 69, 75, 77, 92, 98, 103, 105, 108, 133, 138, 139, 140, 149, 156, 178, and 181. The mutations include insertions, deletions and replacements of the native amino acid residue(s). In exemplary embodiments, the replacements are selected from among amino acid substitutions in SEQ ID NO: 217 set forth in FIG. **12**H corresponding to SEQ ID NOS: 896-939.

[**0376**] In certain embodiments, the modified cytokines provided herein possess increased stability compared to the unmodified cytokine. Stability can be assessed by any in vitro or in vivo method, such as by measuring residual inhibition of viral replication or to stimulation of cell proliferation in appropriate cells, after incubation with either mixtures of proteases, individual proteases, blood lysate or serum.

[**0377**] In other embodiments, the modified cytokines provided herein possess decreased stability compared to the unmodified cytokine. Stability can be assessed by any in vitro or in vivo method, such as by measuring residual inhibition of viral replication or to stimulation of cell proliferation in appropriate cells, after incubation with either mixtures of proteases, individual proteases, blood lysate or serum.

[**0378**] In other embodiments, the modified cytokines provided herein possess increased activity compared to the unmodified cytokine. Stability can be assessed by any in vitro or in vivo method, such as by measuring residual inhibition of viral replication or to stimulation of cell proliferation in appropriate cells, after incubation with either mixtures of proteases, individual proteases, blood lysate or serum.

H. Rational Evolution of IFNβ for Increased Resistance to Proteolysis and/or Higher Conformational Stability

[**0379**] Treatment with interferon β (IFNβ) is a well established therapy. Typically it is used for treatment of multiple sclerosis (MS). Patients receiving interferon β are subject to frequent repeat applications of the drug. The instability of IFNβ in the blood stream and under the storage conditions is well known. Hence it would be useful to increasing stability (half-life) of IFNβ in serum and also in vitro would improve it as a drug.

[**0380**] The 2D-scanning method and the 3D-scanning method (using structural homology) provided herein (see, copending U.S. application Ser. No. 10/658,355, filed Sep. 8, 2003, based on U.S. provisional application Ser. Nos. 60/457,063 and 60/410,258) were each applied to interferon β. Provided herein are mutant variants of the IFNβ protein that display improved stability as assessed by resistance to proteases (thereby possessing increased protein half-life) and at least comparable biological activity as assessed by antiviral or antiproliferation activity compared to the unmodified and wild type native IFNβ protein (SEQ ID NO: 196). The IFNβ mutant proteins provided herein confer a higher half-life and at least comparable biological activity with respect to the native sequence. Thus, the optimized IFNβ protein mutants provided herein that possess increased resistance to proteolysis result in a decrease in the frequency of injections needed to maintain a sufficient drug level in serum, thus leading to, for example: i) higher comfort and acceptance by patients, ii) lower doses necessary to achieve comparable biological effects, and iii) as a consequence of (ii), likely attenuation of any secondary effects.

[**0381**] In exemplary embodiments, the half-life of the IFNβ mutants provided herein is increased by an amount selected from at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 150%, at least 200%, at least 250%, at least 300%, at least 350%, at least 400%, at least 450%, at least 500% or more, when compared to the half-life of native human IFNβ in either human blood, human serum or an in vitro mixture containing one or more proteases. In other embodiments, the half-life of the IFNβ mutants provided herein is increased by an amount selected from at least 6 times, 7 times, 8 times, 9 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times, 400 times, 500 times, 600 times, 700 times, 800 times, 900 times, 1000 times, or more, when compared to the half-life of native human IFNβ in either human blood, human serum or an in vitro mixture containing one or more proteases.

[**0382**] Two approaches were used herein to increase the stability of IFNβ by amino acid replacement: i) Resistance to proteases: amino acid replacement that leads to higher resistance to proteases by direct destruction of the protease target residue or sequence, while either maintaining or improving the requisite biological activity (e.g., antiviral and anti-proliferation activity), and/or ii) conformational stability: amino acid replacement that leads to an increase in conformational stability (i.e. half-life at room temperature or at 37° C.), while either improving or maintaining the requisite biological activity (e.g., antiviral and anti-proliferation activity).

[**0383**] Two methodologies were used to address the improvements described above:

[**0384**] (a) 2D-scanning methods were used to identify amino acid changes that lead to improvement in protease resistance and to improvement in conformational stability, and (b) 3D-scanning, which employs structural homology methods also were used to identify amino acid changes that lead to improvement in protease resistance. The 2D-scanning and 3D-scanning methods each were used to identify the amino acid changes on IFNβ that lead to an increase in stability when challenged either with proteases, human blood lysate or human serum. Increasing protein stability to proteases, human blood lysate or human serum is contemplated herein to provide a longer in vivo half-life for the particular protein molecules, and thus a reduction in the frequency of necessary injections into patients. The biologi-

cal activities that have been measured for the IFNβ molecules are i) their capacity to inhibit virus replication when added to permissive cells previously infected with the appropriate virus, and ii) their capacity to stimulate cell proliferation when added to the appropriate cells. Prior to the measurement of biological activity, IFNβ molecules were challenged with proteases, human blood lysate or human serum during different incubation times. The biological activity measured, corresponds then to the residual biological activity following exposure to the proteolytic mixtures.

[0385] As set forth above, provided herein are methods for the generating IFNβ molecules (or any target protein, particularly cytokines) that, while maintaining a requisite biological activity without substantial change (sufficient for therapeutic application(s)), have been rendered less susceptible to digestion by blood proteases and therefore display a longer half-life in blood circulation. In this particular example, the method used included the following specific steps as exemplified in the Examples:

For the improvement of resistance to proteases, by 2D-scanning, the method included:

[0386] 1) Identifying some or all possible target sites on the protein sequence that are susceptible to digestion by one or more specific proteases (these sites are the is-HITs); and

[0387] 2) Identifying appropriate replacing amino acids, specific for each is-HIT, such that if used to replace one or more of the original amino acids at that specific is-HIT, they can be expected to increase the is-HIT's resistance to digestion by protease while at the same time, keeping the biological activity of the protein unchanged (these replacing amino acids are the candidate LEADs).

For the improvement of resistance to proteases, by 3D-scanning (structural homology):

[0388] 1) Identifying some or all possible target sites (is-HITS) on the protein sequence that display an acceptable degree of structural homology around the amino acid positions mutated in the LEAD molecules previously obtained for IFNα using 2D-scanning, and that are susceptible to digestion by one or more specific proteases; and

[0389] 2) Identifying appropriate replacing amino acids, specific for each is-HIT, such that if used to replace one or more of the original amino acids at that specific is-HIT, they can be expected to increase the is-HIT's resistance to digestion by protease while at the same time, keeping the biological activity of the protein unchanged (these replacing amino acids are the candidate LEADs).

[0390] For the improvement of conformational stability, by 2D-scanning, as provided herein:

[0391] 1) Identifying some or all possible target sites on the protein sequence that are susceptible to being directly involved in the intramolecular flexibility and conformational change (these sites are the is-HITs); and

[0392] 2) Identifying appropriate replacing amino acids, specific for each is-HIT, such that if used to replace one or more of the original amino acids at that specific is-HIT, they can be expected to increase the thermal stability of the molecule while at the same time, keeping the biological activity of the protein unchanged (these replacing amino acids are the candidate LEADs). See FIGS. **6**(O)-**6**(S) and FIG. **8**(A).

[0393] Using the 2D-scanning and 3D-scanning methods and the 3-dimensional structure of IFNβ, the following amino acid target positions were identified as is-HITs on IFNβ, which numbering is that of the mature protein (SEQ ID NO:196):

[0394] By 3D-scanning (see, SEQ ID Nos: 234-289, 989-1015): D by Q at position 39, D by H at position 39, D by G at position 39, E by Q at position 42, E by H at position 42, K by Q at position 45, K by T at position 45, K by S at position 45, K by H at position 45, L by V at position 47, L by I at position 47, L by T at position 47, L by Q at position 47, L by H at position 47, L by A at position 47, K by Q at position 52, K by T at position 52, K by S at position 52, K by H at position 52, F by I at position 67, F by V at position 67, R by H at position 71, R by Q at position 71, D by H at position 73, D by G at position 73, D by Q at position 73, E by Q at position 81, E by H at position 81, E by Q at position 107, E by H at position 107, K by Q at position 108, K by T at position 108, K by S at position 108, K by H at position 108, E by Q at position 109, E by H at position 109, D by Q at position 110, D by H at position 110, D by G at position 110, F by I at position 111, F by V at position 111, R by H at position 113, R by Q at position 113, L by V at position 116, L by I at position 116, L by T at position 116, L by Q at position 116, L by H at position 116, L by A at position 116, L by V at position 120, L by I at position 120, L by T at position 120, L by Q at position 120, L by H at position 120, L by A at position 120, K by Q at position 123, K by T at position 123, K by S at position 123, K by H at position 123, R by H at position 124, R by Q at position 124, R by H at position 128, R by Q at position 128, L by V at position 130, L by I at position 130, L by T at position 130, L by Q at position 130, L by H at position 130, L by A at position 130, K by Q at position 134, K by T at position 134, K by S at position 134, K by H at position 134, K by Q at position 136, K by T at position 136, K by S at position 136, K by H at position 136, E by Q at position 137, E by H at position 137, Y by H at position 163, Y by I at position 163, R by H at position 165, R by Q at position 165.

[0395] By 2D-scanning (see SEQ ID Nos: 1016-1302): M by V at position 1, M by I at position 1, M by T at position 1, M by Q at position 1, M by A at position 1, L by V at position 5, L by I at position 5, L by T at position 5, L by Q at position 5, L by H at position 5, L by A at position 5, F by I at position 8, F by V at position 8, L by V at position 9, L by I at position 9, L by T at position 9, L by Q at position 9, L by H at position 9, L by A at position 9, R by H at position 11, R by Q at position 11, F by I at position 15, F by V at position 15, K by Q at position 19, K by T at position 19, K by S at position 19, K by H at position 19, W by S at position 22, W by H at position 22, N by H at position 25, N by S at position 25, N by Q at position 25, R by H position 27, R by Q position 27, L by V at position 28, L by I at position 28, L by T at position 28, L by Q at position 28, L by H at position 28, L by A at position 28, E by Q at position 29, E by H at position 29, Y by H at position 30, Y by I at position 30, L by V at position 32, L by I at position 32, L by T at position 32, L by Q at position 32, L by H at position 32, L by A at position 32, K by Q at position 33, K by T at position 33, K by S at position 33, K by H at position 33, R by H at position 35, R by Q at position 35, M by V at

position 36, M by I at position 36, M by T at position 36, M by Q at position 36, M by A at position 36, D by Q at position 39, D by H at position 39, D by G at position 39, E by Q at position 42, E by H at position 42, K by Q at position 45, K by T at position 45, K by S at position 45, K by H at position 45, L by V at position 47, L by I at position 47, L by T at position 47, L by, Q at position 47, L by H at position 47, L by A at position 47, K by Q at position 52, K by T at position 52, K by S at position 52, K by H at position 52, F by I at position 67, F by V at position 67, R by H at position 71, R by Q at position 71, D by Q at position 73, D by H at position 73, D by G at position 73, E by Q at position 81, E by H at position 81, E by Q at position 85, E by H at position 85, Y by H at position 92, Y by I at position 92, K by Q at position 99, K by T at position 99, K by S at position 99, K by H at position 99, E by Q at position 103, E by H at position 103, E by Q at position 104, E by H at position 104, K by Q at position 105, K by T at position 105, K by S at position 105, K by H at position 105, E by Q at position 107, E by H at position 107, K by Q at position 108, K by T at position 108, K by S at position 108, K by H at position 108, E by Q at position 109, E by H at position 109, D by Q at position 110, D by H at position 110, D by G at position 110, F by I at position 111, F by V at position 111, R by H at position 113, R by Q at position 113, L by V at position 116, L by I at position 116, L by T at position 116, L by Q at position 116, L by H at position 116, L by A at position 1116, L by V at position 120, L by I at position 120, L by T at position 120, L by Q at position 120, L by H at position 120, L by A at position 120, K by Q at position 123, K by T at position 123, K by S at position 123, K by H at position 123, R by H at position 124, R by Q at position 124, R by H at position 128, R by Q at position 128, L by V at position 130, L by I at position 130, L by T at position 130, L by Q at position 130, L by H at position 130, L by A at position 130, K by Q at position 134, K by T at position 134, K by S at position 134, K by H at position 134, K by Q at position 136, K by T at position 136, K by S at position 136, K by H at position 136, E by Q at position 137, E by H at position 137, Y by H at position 138, Y by I at position 138, R by H at position 152, R by Q at position 152, Y by H at position 155, Y by I at position 155, R by H at position 159, R by Q at position 159, Y by H at position 163, Y by I at position 163, R by H at position 165, R by Q at position 165, M by D at position 1, M by E at position 1, M by K at position 1, M by N at position 1, M by R at position 1, M by S at position 1, L by D at position 5, L by E at position 5, L by K at position 5, L by N at position 5, L by R at position 5, L by S at position 5, L by D at position 6, L by E at position 6, L by K at position 6, L by N at position 6, L by R at position 6, L by S at position 6, L by Q at position 6, L by T at position 6, F by E at position 8, F by K at position 8, F by R at position 8, F by D at position 8, L by D at position 9, L by E at position 9, L by K at position 9, L by N at position 9, L by R at position 9, L by S at position 9, Q by D at position 10, Q by E at position 10, Q by K at position 10, Q by N at position 10, Q by R at position 10, Q by S at position 10, Q by T at position 10, S by D at position 12, S by E at position 12, S by K at position 12, S by R at position 12, S by D at position 13, S by E at position 13, S by K at position 13, S by R at position 13, S by N at position 13, S by Q at position 13, S by T at position 13, N by D at position 14, N by E at position 14, N by K at position 14, N by Q at position 14, N by R at position 14, N by S at position 14, N by T at position 14, F by D at position 15, F by E at position 15, F by K at position 15, F by R at position 15, Q by D at position 16, Q by E at position 16, Q by K at position 16, Q by N at position 16, Q by R at position 16, Q by S at position 16, Q by T at position 16, C by D at position 17, C by E at position 17, C by K at position 17, C by N at position 17, C by Q at position 17, C by R at position 17, C by S at position 17, C by T at position 17, L by N at position 20, L by Q at position 20, L by R at position 20, L by S at position 20, L by T at position 20, L by D at position 20, L by E at position 20, L by K at position 20, W by D at position 22, W by E at position 22, W by K at position 22, W by R at position 22, Q by D at position 23, Q by E at position 23, Q by K at position 23, Q by R at position 23, L by D at position 24, L by E at position 24, L by K at position 24, L by R at position 24, W by D at position 79, W by E at position 79, W by K at position 79, W by R at position 79, N by D at position 80, N by E at position 80, N by K at position 80, N by R at position 80, T by D at position 82, T by E at position 82, T by K at position 82, T by R at position 82, I by D at position 83, I by E at position 83, I by K at position 83, I by R at position 83, I by N at position 83, I by Q at position 83, I by S at position 83, I by T at position 83, N by D at position 86, N by E at position 86, N by K at position 86, N by R at position 86, N by Q at position 86, N by S at position 86, N by T at position 86, L by D at position 87, L by E at position 87, L by K at position 87, L by R at position 87, L by N at position 87, L by Q at position 87, L by S at position 87, L by T at position 87, A by D at position 89, A by E at position 89, A by K at position 89, A by R at position 89, N by D at position 90, N by E at position 90, N by K at position 90, N by Q at position 90, N by R at position 90, N by S at position 90, N by T at position 90, V by D at position 91, V by E at position 91, V by K at position 91, V by N at position 91, V by Q at position 91, V by R at position 91, V by S at position 91, V by T at position 91, Q by D at position 94, Q by E at position 94, Q by Q at position 94, Q by N at position 94, Q by R at position 94, Q by S at position 94, Q by T at position 94, I by D at position 95, I by E at position 95, I by K at position 95, I by N at position 95, I by Q at position 95, I by R at position 95, I by S at position 95, I by T at position 95, H by D at position 97, H by E at position 97, H by K at position 97, H by N at position 97, H by Q at position 97, H by R at position 97, H by S at position 97, H by T at position 97, L by D at position 98, L by E at position 98, L by K at position 98, L by N at position 98, L by Q at position 98, L by R at position 98, L by S at position 98, L by T at position 98, V by D at position 101, V by E at position 101, V by K at position 101, V by N at position 101, V by Q at position 101, V by R at position 101, V by S at position 101, V by T at position 101, M by C at position 1, L by C at position 6, Q by C at position 10, S by C at position 13, Q by C at position 16, L by C at position 17, V by C at position 101, L by C at position 98, H by C at position 97, Q by C at position 94, V by C at position 91, N by C at position 90.

SEQ ID NO.	Mutant
SEQ ID No 1016	(M1V)
SEQ ID No 1017	(M1I)
SEQ ID No 1018	(M1T)
SEQ ID No 1019	(M1A)
SEQ ID No 1020	(L5V)

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SEQ ID NO.	Mutant
SEQ ID No 1021	(L5I)
SEQ ID No 1022	(L5T)
SEQ ID No 1023	(L5Q)
SEQ ID No 1024	(L5H)
SEQ ID No 1025	(L5A)
SEQ ID No 1026	(F8I)
SEQ ID No 1027	(F8V)
SEQ ID No 1028	(L9V)
SEQ ID No 1029	(L9I)
SEQ ID No 1030	(L9T)
SEQ ID No 1031	(L9Q)
SEQ ID No 1032	(L9H)
SEQ ID No 1033	(L9A)
SEQ ID No 1034	(R11H)
SEQ ID No 1035	(R11Q)
SEQ ID No 1036	(F15I)
SEQ ID No 1037	(F15V)
SEQ ID No 1038	(K19Q)
SEQ ID No 1039	(K19T)
SEQ ID No 1040	(K19S)
SEQ ID No 1041	(K19H)
SEQ ID No 1042	(W22S)
SEQ ID No 1043	(W22H)
SEQ ID No 1044	(N25H)
SEQ ID No 1045	(N25S)
SEQ ID No 1046	(N25Q)
SEQ ID No 1047	(R27H)
SEQ ID No 1048	(R27Q)
SEQ ID No 1049	(L28V)
SEQ ID No 1050	(L28I)
SEQ ID No 1051	(L28T)
SEQ ID No 1052	(L28Q)
SEQ ID No 1053	(L28H)
SEQ ID No 1054	(L28A)
SEQ ID No 1055	(E29Q)
SEQ ID No 1056	(E29H)
SEQ ID No 1057	(Y30H)
SEQ ID No 1058	(Y30I)
SEQ ID No 1059	(L32V)
SEQ ID No 1060	(L32I)
SEQ ID No 1061	(L32T)
SEQ ID No 1062	(L32Q)
SEQ ID No 1063	(L32H)
SEQ ID No 1064	(L32A)
SEQ ID No 1065	(M1Q)
SEQ ID No 1066	(K33Q)
SEQ ID No 1067	(K33T)
SEQ ID No 1068	(K33S)
SEQ ID No 1069	(K33H)
SEQ ID No 1070	(R35H)
SEQ ID No 1071	(R35Q)
SEQ ID No 1072	(M36V)
SEQ ID No 1073	(M36I)
SEQ ID No 1074	(M36T)
SEQ ID No 1075	(M36Q)
SEQ ID No 1076	(M36A)
SEQ ID No 1077	(E85Q)
SEQ ID No 1078	(E85H)
SEQ ID No 1079	(Y92H)
SEQ ID No 1080	(Y92I)
SEQ ID No 1081	(K99Q)
SEQ ID No 1082	(K99T)
SEQ ID No 1083	(K99S)
SEQ ID No 1084	(K99H)
SEQ ID No 1085	(E103Q)
SEQ ID No 1086	(E103H)
SEQ ID No 1087	(E104Q)
SEQ ID No 1088	(E104H)
SEQ ID No 1089	(K105Q)
SEQ ID No 1090	(K105T)
SEQ ID No 1091	(K105S)
SEQ ID No 1092	(K105H)
SEQ ID No 1093	(Y138H)
SEQ ID No 1094	(Y138I)

-continued

SEQ ID NO.	Mutant
SEQ ID No 1095	(R152H)
SEQ ID No 1096	(R152Q)
SEQ ID No 1097	(Y155H)
SEQ ID No 1098	(Y155I)
SEQ ID No 1099	(R159H)
SEQ ID No 1100	(R159Q)
SEQ ID No 1101	(M1D)
SEQ ID No 1102	(M1E)
SEQ ID No 1103	(M1K)
SEQ ID No 1104	(M1N)
SEQ ID No 1105	(M1R)
SEQ ID No 1106	(M1S)
SEQ ID No 1107	(L5D)
SEQ ID No 1108	(L5E)
SEQ ID No 1109	(L5K)
SEQ ID No 1110	(L5R)
SEQ ID No 1111	(L5N)
SEQ ID No 1112	(L5S)
SEQ ID No 1113	(L6D)
SEQ ID No 1114	(L6E)
SEQ ID No 1115	(L6K)
SEQ ID No 1116	(L6N)
SEQ ID No 1117	(L6Q)
SEQ ID No 1118	(L6R)
SEQ ID No 1119	(L6S)
SEQ ID No 1120	(L6T)
SEQ ID No 1121	(F8D)
SEQ ID No 1122	(F8E)
SEQ ID No 1123	(F8K)
SEQ ID No 1124	(F8R)
SEQ ID No 1125	(L9D)
SEQ ID No 1126	(L9E)
SEQ ID No 1127	(L9K)
SEQ ID No 1128	(L9N)
SEQ ID No 1129	(L9R)
SEQ ID No 1130	(L9S)
SEQ ID No 1131	(Q10D)
SEQ ID No 1132	(Q10E)
SEQ ID No 1133	(Q10K)
SEQ ID No 1134	(Q10N)
SEQ ID No 1135	(Q10R)
SEQ ID No 1136	(Q10S)
SEQ ID No 1137	(Q10T)
SEQ ID No 1138	(S12D)
SEQ ID No 1139	(S12E)
SEQ ID No 1140	(S12K)
SEQ ID No 1141	(S12R)
SEQ ID No 1142	(S13D)
SEQ ID No 1143	(S13E)
SEQ ID No 1144	(S13K)
SEQ ID No 1145	(S13N)
SEQ ID No 1146	(S13Q)
SEQ ID No 1147	(S13R)
SEQ ID No 1148	(S13T)
SEQ ID No 1149	(N14D)
SEQ ID No 1150	(N14E)
SEQ ID No 1151	(N14K)
SEQ ID No 1152	(N14Q)
SEQ ID No 1153	(N14R)
SEQ ID No 1154	(N14S)
SEQ ID No 1155	(N14T)
SEQ ID No 1156	(F15D)
SEQ ID No 1157	(F15E)
SEQ ID No 1158	(F15K)
SEQ ID No 1159	(F15R)
SEQ ID No 1160	(Q16D)
SEQ ID No 1161	(Q16E)
SEQ ID No 1162	(Q16K)
SEQ ID No 1163	(Q16N)
SEQ ID No 1164	(Q16R)
SEQ ID No 1165	(Q16S)
SEQ ID No 1166	(Q16T)
SEQ ID No 1167	(C17D)
SEQ ID No 1168	(C17E)

-continued

SEQ ID NO.	Mutant
SEQ ID No 1169	(C17K)
SEQ ID No 1170	(C17N)
SEQ ID No 1171	(C17Q)
SEQ ID No 1172	(C17R)
SEQ ID No 1173	(C17S)
SEQ ID No 1174	(C17T)
SEQ ID No 1175	(L20N)
SEQ ID No 1176	(L20Q)
SEQ ID No 1177	(L20R)
SEQ ID No 1178	(L20S)
SEQ ID No 1179	(L20T)
SEQ ID No 1180	(L20D)
SEQ ID No 1181	(L20E)
SEQ ID No 1182	(L20K)
SEQ ID No 1183	(W22D)
SEQ ID No 1184	(W22E)
SEQ ID No 1185	(W22K)
SEQ ID No 1186	(W22R)
SEQ ID No 1187	(Q23D)
SEQ ID No 1188	(Q23E)
SEQ ID No 1189	(Q23K)
SEQ ID No 1190	(Q23R)
SEQ ID No 1191	(L24D)
SEQ ID No 1192	(L24E)
SEQ ID No 1193	(L24K)
SEQ ID No 1194	(L24R)
SEQ ID No 1195	(G78D)
SEQ ID No 1196	(G78E)
SEQ ID No 1197	(G78K)
SEQ ID No 1198	(G78R)
SEQ ID No 1199	(W79D)
SEQ ID No 1200	(W79E)
SEQ ID No 1201	(W79K)
SEQ ID No 1202	(W79R)
SEQ ID No 1203	(N80D)
SEQ ID No 1204	(N80E)
SEQ ID No 1205	(N80K)
SEQ ID No 1206	(N80R)
SEQ ID No 1207	(T82D)
SEQ ID No 1208	(T82E)
SEQ ID No 1209	(T82K)
SEQ ID No 1210	(T82R)
SEQ ID No 1211	(I83D)
SEQ ID No 1212	(I83E)
SEQ ID No 1213	(I83K)
SEQ ID No 1214	(I83R)
SEQ ID No 1215	(I83N)
SEQ ID No 1216	(I83Q)
SEQ ID No 1217	(I83S)
SEQ ID No 1218	(I83T)
SEQ ID No 1219	(N86D)
SEQ ID No 1220	(N86E)
SEQ ID No 1221	(N86K)
SEQ ID No 1222	(N86R)
SEQ ID No 1223	(N86Q)
SEQ ID No 1224	(N86S)
SEQ ID No 1225	(N86T)
SEQ ID No 1226	(L87D)
SEQ ID No 1227	(L87E)
SEQ ID No 1228	(L87K)
SEQ ID No 1229	(L87R)
SEQ ID No 1230	(L87N)
SEQ ID No 1231	(L87Q)
SEQ ID No 1232	(L87S)
SEQ ID No 1233	(L87T)
SEQ ID No 1234	(A89D)
SEQ ID No 1235	(A89E)
SEQ ID No 1236	(A89K)
SEQ ID No 1237	(A89R)
SEQ ID No 1238	(N90D)
SEQ ID No 1239	(N90E)
SEQ ID No 1240	(N90K)
SEQ ID No 1241	(N90Q)
SEQ ID No 1242	(N90R)

-continued

SEQ ID NO.	Mutant
SEQ ID No 1243	(N90S)
SEQ ID No 1244	(N90T)
SEQ ID No 1245	(V91D)
SEQ ID No 1246	(V91E)
SEQ ID No 1247	(V91K)
SEQ ID No 1248	(V91N)
SEQ ID No 1249	(V91Q)
SEQ ID No 1250	(V91R)
SEQ ID No 1251	(V91S)
SEQ ID No 1252	(V91T)
SEQ ID No 1253	(Q94D)
SEQ ID No 1254	(Q94E)
SEQ ID No 1255	(Q94K)
SEQ ID No 1256	(Q94N)
SEQ ID No 1257	(Q94R)
SEQ ID No 1258	(Q94S)
SEQ ID No 1259	(Q94T)
SEQ ID No 1260	(I95D)
SEQ ID No 1261	(I95E)
SEQ ID No 1262	(I95K)
SEQ ID No 1263	(I95N)
SEQ ID No 1264	(I95Q)
SEQ ID No 1265	(I95R)
SEQ ID No 1266	(I95S)
SEQ ID No 1267	(I95T)
SEQ ID No 1268	(H97D)
SEQ ID No 1269	(H97E)
SEQ ID No 1270	(H97K)
SEQ ID No 1271	(H97N)
SEQ ID No 1272	(H97Q)
SEQ ID No 1273	(H97R)
SEQ ID No 1274	(H97S)
SEQ ID No 1275	(H97T)
SEQ ID No 1276	(L98D)
SEQ ID No 1277	(L98E)
SEQ ID No 1278	(L98K)
SEQ ID No 1279	(L98N)
SEQ ID No 1280	(L98Q)
SEQ ID No 1281	(L98R)
SEQ ID No 1282	(L98S)
SEQ ID No 1283	(L98T)
SEQ ID No 1284	(V101D)
SEQ ID No 1285	(V101E)
SEQ ID No 1286	(V101K)
SEQ ID No 1287	(V101N)
SEQ ID No 1288	(V101Q)
SEQ ID No 1289	(V101R)
SEQ ID No 1290	(V101S)
SEQ ID No 1291	(V101T)
SEQ ID No 1292	(M1C)
SEQ ID No 1293	(V101C)
SEQ ID No 1294	(L6C)
SEQ ID No 1295	(L98C)
SEQ ID No 1296	(Q10C)
SEQ ID No 1297	(H97C)
SEQ ID No 1298	(S13C)
SEQ ID No 1299	(Q94C)
SEQ ID No 1300	(Q16C)
SEQ ID No 1301	(N90C)
SEQ ID No 1302	(V91C)

I. Super-LEADs and Additive Directional Mutagenesis (ADM)

[0396] Also provided herein are super-LEAD mutant proteins comprising a combination of single amino acid mutations present in two or more of the respective LEAD mutant proteins. Thus, the super-LEAD mutant proteins have two of more of the single amino acid mutations derived from two or more of the respective LEAD mutant proteins. As described herein, LEAD mutant proteins provided herein are

defined as mutants whose performance or fitness has been optimized with respect to the native protein. LEADs typically contain one single mutation relative to its respective native protein. This mutation represents an appropriate amino acid replacement that takes place at one is-HIT position. Further super-LEAD mutant proteins are created such that they carry on the same protein molecule, more than one LEAD mutation, each at a different is-HIT position. Once the LEAD mutant proteins have been identified using the 2D-scanning methods provided herein, super-LEADs can be generated by combining two or more individual LEAD mutant mutations using methods well-known in the art, such as recombination, mutagenesis and DNA shuffling, and by methods, such as additive directional mutagenesis and Multi-Overlapped Primer Extensions, provided herein.

[0397] 1. Additive Directional Mutagenesis

[0398] Also provided herein are methods for assembling on a single mutant protein multiple mutations present on the individual LEAD molecules, so as to generate super-LEAD mutant proteins. This method is referred to herein as "Additive Directional Mutagenesis" (ADM). ADM is a repetitive multi-step process where at each step after the creation of the first LEAD mutant protein a new LEAD mutation is added onto the previous LEAD mutant protein to create successive super-LEAD mutant proteins. ADM is not based on genetic recombination mechanisms, nor on shuffling methodologies; instead it is a simple one-mutation-at-a-time process, repeated as many times as necessary until the total number of desired mutations is introduced on the same molecule. To avoid the exponentially increasing number of all possible combinations that can be generated by putting together on the same molecule a given number of single mutations, a method is provided herein that, although it does not cover all the combinatorial possible space, still captures a big part of the combinatorial potential. The word "combinatorial" is used here in its mathematical meaning (i.e., subsets of a group of elements, containing some of the elements in any possible order) and not in the molecular biological or directed evolution meaning (i.e., generating pools, or mixtures, or collections of molecules by randomly mixing their constitutive elements).

[0399] A population of sets of nucleic acid molecules encoding a collection of new super-LEAD mutant molecules is generated, tested and phenotypically characterized one-by-one in addressable arrays. super-LEAD mutant molecules are such that each molecule contains a variable number and type of LEAD mutations. Those molecules displaying further improved fitness for the particular feature being evolved, are referred to as super-LEADs. Super-LEADs may be generated by other methods known to those of skill in the art and tested by the high throughput methods herein. For purposes herein a super-LEAD typically has activity with respect to the function or biological activity of interest that differs from the improved activity of a LEAD by a desired amount, such as at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or more from at least one of the LEAD mutants from which it is derived. In yet other embodiments, the change in activity is at least about 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, 100 times, 200 times, 300 times, 400 times, 500 times, 600 times, 700 times, 800 times, 900 times, 1000 times, or more greater than at least one of the LEAD molecules from which it is derived. As with LEADs, the change in the activity for super-LEADs is dependent upon the activity that is being "evolved." The desired alteration, which can be either an increase or a reduction in activity, will depend upon the function or property of interest.

[0400] In one embodiment provided herein, the ADM method employs a number of repetitive steps, such that at each step a new mutation is added on a given molecule. Although numerous different ways are possible for combining each LEAD mutation onto a super-LEAD protein, an exemplary way the new mutations (e.g., mutation 1 (m1), mutation 2 (m2), mutation 3 (m3), mutation 4 (m4), mutation 5 (m5), mutation n (mn)) can be added corresponds to the following diagram:

m1
m1 + m2
m1 + m2 + m3
m1 + m2 + m3 + m4
m1 + m2 + m3 + m4 + m5
m1 + m2 + m3 + m4 + m5 + . . . + mn
m1 + m2 + m4
m1 + m2 + m4 + m5
m1 + m2 + m4 + m5 + . . . + mn
m1 + m2 + m5
m1 + m2 + m5 + . . . + mn
m2
m2 + m3
m2 + m3 + m4
m2 + m3 + m4 + m5
m2 + m3 + m4 + m5 + . . . + mn
m2 + m4
m2 + m4 + m5
m2 + m4 + m5 + . . . + mn
m2 + m5
m2 + m5 + . . . + mn
. . . , etc . . .

[0401] 2. Multi-Overlapped Primer Extensions

[0402] In another embodiment, provided herein is a method for the rational evolution of proteins using oligonucleotide-mediated mutagenesis referred to as "multi overlapped primer extensions." This method can be used for the rational combination of mutant LEADs to form super-LEADS. This method allows the simultaneous introduction of several mutations throughout a small protein or protein-region of known sequence. Overlapping oligonucleotides of typically around 70 bases in length (since longer oligonucleotides lead to increased error) are designed from the DNA sequence (gene) encoding the mutant LEAD proteins in such a way that they overlap with each other on a region of typically around 20 bases. These overlapping oligonucleotides (including or not point mutations) act as both template and primers in a first step of PCR (using a proofreading polymerase, e.g., Pfu DNA polymerase, to avoid unexpected mutations) to create small amounts of full-length gene. The full-length gene resulting from the first PCR is then selectively amplified in a second step of PCR using flanking primers, each one tagged with a restriction site in order to facilitate subsequent cloning. One multi overlapped extension process yields a full-length (multi-mutated) nucleic acid molecule encoding a candidate super-LEAD protein having multiple mutations therein derived from LEAD mutant proteins.

[0403] Although typically about 70 bases are used to create the overlapping oligonucleotides, the length of additional overlapping oligonucleotides for use herein can range from about 30 bases up to about 100 bases, from about 40 bases up to about 90 bases, from about 50 bases up to about 80 bases, from about 60 bases up to about 75 bases, and from about 65 bases up to about 75 bases. As set forth above, typically about 70 bases are used herein.

[0404] Likewise, although typically the overlapping region of the overlapping oligonucleotides is about 20 bases, the length of other overlapping regions for use herein can range from about 5 bases up to about 40 bases, from about 10 bases up to about 35 bases, from about 15 bases up to about 35 bases, from about 15 bases up to about 25 bases, from about 16 bases up to about 24 bases, from about 17 bases up to about 23 bases, from about 18 bases up to about 22 bases, and from about 19 bases up to about 21 bases. As set forth above, typically about 20 bases are used herein for the overlapping region.

J. Uses of the Mutant IFNα and IFNβ Genes and Cytokines in Therapeutic Methods

[0405] The optimized cytokines provided herein, such as the IFNα-2b and IFNβ proteins and other modified cytokines, are intended for use in various therapeutic as well as diagnostic methods. These include all methods for which the unmodified proteins are used. By virtue of their improved phenotypes and activities, the proteins provided herein should exhibit improvement in the corresponding in vivo phenotype.

[0406] In particular, the optimized cytokines, such as the IFNα-2b and IFNβ proteins, are intended for use in therapeutic methods in which cytokines have been used for treatment. Such methods include, but are not limited to, methods of treatment of infectious diseases, allergies, microbial diseases, pregnancy related diseases, bacterial diseases, heart diseases, viral diseases, histological diseases, genetic diseases, blood related diseases, fungal diseases, adrenal diseases, cancers, liver diseases, autoimmune diseases, growth disorders, diabetes, neurodegenerative diseases, including multiple sclerosis, Parkinson's disease and Alzheimer's disease.

[0407] 1. Fusion Proteins

[0408] Fusion proteins containing a targeting agent and mutant IFNα, including IFNα-2b and IFNα-2a, and IFNβ mutant proteins, or cytokine protein also are provided. Pharmaceutical compositions containing such fusion proteins formulated for administration by a suitable route are provided. Fusion proteins are formed by linking in any order the mutant protein and an agent, such as an antibody or fragment thereof, growth factor, receptor, ligand and other such agent for directing the mutant protein to a targeted cell or tissue. Linkage can be effected directly or indirectly via a linker. The fusion proteins can be produced recombinantly or chemically by chemical linkage, such as via heterobifunctional agents or thiol linkages or other such linkages. The fusion proteins can contain additional components, such as *E. coli* maltose binding protein (MBP) that aid in uptake of the protein by cells (see, International PCT application No. WO 01/32711).

[0409] 2. Nucleic Acid Molecules for Expression

[0410] Nucleic acid molecules encoding the mutant cytokines including the mutant IFNβ proteins and IFN a proteins, such as the IFNα-2b and IFNα-2a proteins, provided herein, or the fusion protein operably linked to a promoter, such as an inducible promoter for expression in mammalian cells also are provided. Such promoters include, but are not limited to, CMV and SV40 promoters; adenovirus promoters, such as the E2 gene promoter, which is responsive to the HPV E7 oncoprotein; a PV promoter, such as the PBV p89 promoter that is responsive to the PV E2 protein; and other promoters that are activated by the HIV or PV or oncogenes.

[0411] The mutant cytokines including the mutant interferons (IFNα's and IFNβ's) proteins provided herein, also can be delivered to the cells in gene transfer vectors. The transfer vectors also can encode additional other therapeutic agent(s) for treatment of the disease or disorder, such cancer or HIV infection, for which the cytokine is administered.

[0412] 3. Formulation of Optimized Cytokines and Methods of Treatment

[0413] Pharmaceutical compositions containing an optimized cytokine produced herein, such as IFNα-2b, IFNα-2a and IFNβ, fusion proteins or encoding nucleic acid molecules can be formulated in any conventional manner by mixing a selected amount of an optimized cytokine with one or more physiologically acceptable carriers or excipients. Selection of the carrier or excipient depends upon the mode of administration (i.e., systemic, local, topical or any other mode) and disorder treated. The pharmaceutical compositions provided herein can be formulated for single dosage administration. The concentrations of the compounds in the formulations are effective for delivery of an amount, upon administration, that is effective for the intended treatment. Typically, the compositions are formulated for single dosage administration. To formulate a composition, the weight fraction of a compound or mixture thereof is dissolved, suspended, dispersed or otherwise mixed in a selected vehicle at an effective concentration such that the treated condition is relieved or ameliorated. Pharmaceutical carriers or vehicles suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration.

[0414] In addition, the compounds may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients. Liposomal suspensions, including tissue-targeted liposomes, may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be prepared as described in U.S. Pat. No. 4,522,811.

[0415] The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the compounds in known in vitro and in vivo systems, such as the assays provided herein. The active compounds can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid, semi-liquid or solid form and are formulated in a manner suitable for each route of administration.

[0416] The optimized cytokine and physiologically acceptable salts and solvates can be formulated for administration by inhalation (either through the mouth or the nose) or for oral, buccal, parenteral or rectal administration. For administration by inhalation, the optimized cytokine can be delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluorethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of a therapeutic compound and a suitable powder base such as lactose or starch.

[0417] For oral administration, the pharmaceutical compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets can be coated by methods well known in the art. Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

[0418] Preparations for oral administration can be suitably formulated to give controlled release of the active compound. For buccal administration the compositions can take the form of tablets or lozenges formulated in conventional manner.

[0419] The optimized cytokine can be formulated for parenteral administration by injection e.g., by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form e.g., in ampules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder-lyophilized form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0420] In addition to the formulations described previously, the optimized cytokine also can be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the therapeutic compounds can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0421] The active agents can be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Such solutions, particularly those intended for ophthalmic use, can be formulated as 0.01%-10% isotonic solutions, pH about 5-7, with appropriate salts. The compounds can be formulated as aerosols for topical application, such as by inhalation (see, e.g., U.S. Pat. Nos. 4,044,126, 4,414,209, and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment inflammatory diseases, particularly asthma).

[0422] The concentration of active compound in the drug composition will depend on absorption, inactivation and excretion rates of the active compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art. For example, the amount that is delivered is sufficient to treat the symptoms of hypertension.

[0423] The compositions, if desired, can be presented in a package, in kit or a dispenser device, that can contain one or more unit dosage forms containing the active ingredient. The package, for example, contains metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration. The compositions containing the active agents can be packaged as articles of manufacture containing packaging material, an agent provided herein, and a label that indicates the disorder for which the agent is provided.

[0424] Methods of treatment of cytokine-mediated or cytokine-involved diseases and immunotherapeutic methods are provided. The modified cytokines can be used in any method of treatment for which the unmodified cytokine is used. Hence the modified cytokines can be used for treatment of all disorders noted herein for the respective cytokines and for those known to those of skill in the art for each of the others, such as immunotherapeutic treatment (interleukins) and red blood cell expansion and stem cell expansion. The following table summarizes exemplary uses in addition to those noted herein of exemplary modified cytokines provided herein:

Cytokine	Exemplary Uses, Diseases and Treatment
IL-10	anti-inflammatory treatment of chronic liver injury and disease; myeloma
Interferon-gamma	Interstitial/idiopathic pulmonary fibrosis; adjunctive immunotherapy for immunosuppressed patients
Granulocyte colony stimulating factor	Crohn's disease; cardiac disease; acquired and congenital neutropenias; asthma
Leukemia inhibitory factor	myocardial infarction; multiple sclerosis; prevention of axonal atrophy; olfactory epithelium replacement stimulation
Human growth hormone	growth hormone deficiency; acromegaly
Ciliary neurotrophic factor	retinal degeneration treatments; neurodegenerative diseases such as Huntington's; auditory degenerative diseases
Leptin	obesity; pancreatitis; endometriosis
Oncostatin M	chronic inflammatory diseases; rheumatoid arthritis; multiple sclerosis; tissue damage suppression

-continued

Cytokine	Exemplary Uses, Diseases and Treatment
Interleukin-6	Protection from liver injury; Crohn's disease; hematopoietic associated diseases
Interleukin-12	coxsackievirus treatment; neuroblastoma; melanoma, renal cell carcinoma; mucosal immunity induction
Erythropoietin	hypoxia; myocardial ischemia; anemia with renal failure and cancer treatments
Granulocyte-macrophage colony stimulating factor	stimulate antigen presenting cells; anti-tumor activity for leukemia, melanoma, and breast, liver and renal cell carcinomas; adjunctive immunotherapy for immunosuppressed patients; autoimmune disease
Interleukin-2	immune reactivation after chemotherapy; melanoma; colon carcinoma
Interleukin-3	leukemia cell targeting; motor neuropathy; amyotrophic lateral sclerosis; asthma
Interleukin-4	allergic asthma; lupus
Interleukin-5	treatment for parasites; asthma; allergic diseases accompanied by eosinophilia
Interleukin-13	intracellular infections; B-cell cancers; asthma
Flt3 ligand	prostate cancer; myeloid leukemia; engraftment of allogenic hematopoietic stem cells
Stem cell factor	hepatic injury; asthma; hematopoietic engraftment

[0425] Treatment can be effected by any suitable route of administration using suitable formulations. If necessary, a particular dosage and duration and treatment protocol can be empirically determined or extrapolated.

K. Examples

[0426] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention. The specific methods exemplified can be practiced with other species. The examples are intended to exemplify generic processes.

Example 1

[0427] This example describes a plurality of chronological steps including steps from (i) to (viii):

[0428] (i) cloning of IFNα cDNA in a mammalian cell expression plasmid (section A.1)

[0429] (ii) generation of a collection of targeted mutants on the IFNα cDNA in the mammalian cell expression plasmid (section B)

[0430] (iii) production of IFNα mutants in mammalian cells (section C.1)

[0431] (iv) screening and partial in vitro characterization of IFNα mutants produced in mammalian cells in search of lead mutants (section D)

[0432] (v) cloning of the lead mutants into a bacterial cell expression plasmid (section A.2)

[0433] (vi) expression of lead mutants in bacterial cells (section C.2)

[0434] (vii) in vitro characterization of lead mutants produced in bacteria (section D)

[0435] (viii) in vivo characterization of lead mutants produced in bacteria (section E).

A. Cloning of IFNα-2b Encoding cDNA

[0436] A.1. Cloning of IFNα-2b cDNA in a Mammalian Cell Expression Plasmid

[0437] The IFNα-2b cDNA was first cloned into a mammalian expression vector, prior to the generation of the selected mutations. A collection of mutants was then generated such that each individual mutant was created and processed individually, physically separated from each other and in addressable arrays. The mammalian expression vector pSSV9 CMV 0.3 pA was engineered as follows:

[0438] The pSSV9 CMV 0.3 pA was cut by PvuII and religated (this step gets rid of the ITR functions), prior to the introduction of a new EcoRI restriction site by Quickchange mutagenesis (Stratagene). The oligonucleotides primers were:

```
EcoRI forward primer
                                                    (SEQ ID NO:218)
5'-GCCTGTATGATTTATTGGATGTTGGAATTCC-CTGATGCGGTATTTT

CTCCTTACG-3'

EcoRI reverse primer
                                                    (SEQ ID NO:219)
5'-CGTAAGGAGAAAATACCGCATCAGGGAATT-CCAACATCCAATAAAT

CATACAGGC-3'.
```

[0439] The construct sequence was confirmed by using the following oligonucleotides:

```
Seq ClaI forward primer:
                                                    (SEQ ID NO:220)
5'-CTGATTATCAACCGGGGTACATATGATTGAC-ATGC-3'

Seq XmnI reverse primer

                                                    (SEQ ID NO:221)
5'-TACGGGATAATACCGCGCCACATAGCAGAA-C-3'.
```

[0440] Then, the XmnI-ClaI fragment containing the newly introduced EcoRI site was cloned into pSSV9 CMV 0.3 pA (SSV9 is a clone containing the entire adeno-associated virus (AAV) genome inserted into the PvuII site of plasmid pEMBL (see, Du et al. (1996) *Gene Ther* 3:254-261)) to replace the corresponding wild-type fragment and produce construct pSSV9-2EcoRI.

[0441] The DNA sequence of the IFNα-2b cDNA, which was inserted into the mammalian vector pDG6 (ATCC accession No. 53169), was confirmed using a pair of internal primers. The sequences of the IFNα-2b-related oligonucleotides for sequencing follow:

```
                                                    (SEQ ID NO:222)
Seq forward primer:   5'-CCTGATGAAGGAGGACTC-3'

                                                    (SEQ ID NO:223)
Seq reverse primer:   5'-CCAAGCAGCAGATGAGTC-3'.
```

[0442] Since the beginning of the IFNα-2b encoding cDNA (the signal peptide encoding sequence) is absent in

pDG6, it was added using the oligonucleotide (see below) to the amplified gene. First, the IFNα-2b cDNA was amplified by PCR using pDG6 as template using the following oligonucleotides as primers:

IFNα-2b 5' primer
(SEQ ID NO:224)
5'-TCAGCTGCAAGTCAAGCTGCTCTGTGGGCTG-3'

IFNα-2b 3' primer
(SEQ ID NO:225)
5'-GCTCTAGATCATTCCTTACTTCTTAAACTTTC-TTGCAAGTTTGTTG

AC-3'

[0443] The PCR product was then used in an overlapping PCR using the following oligonucleotide sequences, having Hind III or XbaI restriction sites (underlined) or the DNA sequence missing in pDG6 (underlined):

IFNα-2b HindIII primer
(SEQ ID NO:226)
5'-CCCAAGCTTATGGCCTTGACCTTTGCTTTACT-GGTG-3'

IFNα-2b XbaI primer
(SEQ ID NO:227)
5'-GCTCTAGATCATTCCTTACTTCTTAAACTTTC-TTGCAAGTTTGTTG

AC-3'

IFNα-2b 80bp 5' primer
(SEQ ID NO:228)
5'-CCCAAGCTT**ATGGCCTTGACCTTTGCTTTA-**CTGGTGGCCCTCCTGG

TGCTCAGCTGCAAGTCAAGCTGCTCTGTGGGCTG-3'.

[0444] The entire IFNα-2b cDNA was cloned into the pTOPO-TA vector (Invitrogen). After checking gene sequence by automatic DNA sequencing, the HindIII-XbaI fragment containing the gene of interest was subcloned into the corresponding sites of pSSV9-2EcoRI to produce pAAV-EcoRI-IFNalpha-2b (pNB-AAV-IFN alpha-2b).

[0445] A.2 Cloning of the IFN α-2b Leads in an *E. coli* Expression Plasmid

[0446] A.2.1 Characterization of the Bacterial Cells

[0447] BL21-CodonPlus(DE3)-RP® competent *Escherichia coli* cells are derived from Stratagene's high-performance BL21-Gold competent cells. These cells enable efficient high-level expression of heterologous proteins in *E. coli*. Efficient production of heterologous proteins in *E. coli* is frequently limited by the rarity, in *E. coli*, of certain tRNAs that are abundant in the organisms from which the heterologous proteins are derived. Availability of tRNAs allows high-level expression of many heterologous recombinant genes in BL21-Codon Plus cells that are poorly expressed in conventional BL21 strains. BL21-CodonPLus(DE3)-RP cells contain a ColE1-compatible, pACYC-based plasmid containing extra copies of the argU and proL tRNA genes.

[0448] A.2.2 Cloning of wild-type IFN α

[0449] To express IFNα-2b in *E. coli* cDNA encoding the mature form of IFNα-2b was finally cloned into the plasmid pET-11 (Novagen). Briefly, this cDNA fragment was amplified by PCR using the primers SEQ ID Nos. 1306 and 1305, respectively:

FOR-IFNA-5'
AACATATGTGTGATCTGCCTCAAACCCACAGCCTGGGTAGC 3'

REV-IFNA-5'
AAGGATCCTCATTCCTTACTTCTTAAACTTTCTTGCAAGTTTGTTG 3',

from pSSV9-EcoRI-IFN α-2b (see above), which contains full-length IFN-2 alpha cDNA as a matrix, using Herculase DNA-polymerase (Stratagene). The PCR fragment was subcloned into pTOPO-TA vector (Invitrogen) yielding pTOPO—IFN α-2b. The sequence was verified by sequencing. pET11 IFN α-2b was prepared by insertion of the NdeI-Bam HI (Biolabs) fragment from pTOPO—IFN α-2b into the NdeI-Bam HI sites of pET 11. The DNA sequence of the resulting pET 11-IFN α-2b construct was verified by sequencing and the plasmid was used for IFN α-2b expression in *E. coli.*

[0450] A.2.3 Cloning of IFN α-2b Mutants from the Mammalian Expression Plasmid into the *E. coli* Expression Plasmid

[0451] Lead mutants of Interferon alpha were first generated in the pSSV9-IFNα-EcoRI plasmid. With the only exception of E159H and E159Q, all mutants were amplified using the primers below. Primers contained NdeI (in Forward) and BamHI (in Reverse) restriction sites:

FOR-IFNA-5'
(SEQ ID No. 1306
AACATATGTGTGATCTGCCTCAAACCCACAGCCTGGGTAGC 3';
and

REV-IFNA-5'
(SEQ ID No. 1305)
AAGGATCCTCATTCCTTACTTCTTAAACTTTCTTGCAAGTTTGTTG 3'.

[0452] Mutants E159H and E159Q were amplified using the following primers on reverse side (primer forward was the same than described above):

REV-IFNA-E159H-5'
AAGGATCCTCATTCCTTACTTCTTAAACTGTGTTGCAAGTTTGTTG 3'

SEQ ID No. 1304 above;
and

REV-IFNA-E159Q-5'
AAGGATCCTCATTCCTTACTTCTTAAACTCTGTTGCAAGTTTGTTG 3'

SEQ ID No. 1305.

Mutants were amplified with Pfu Turbo Polymerase (Stratagene). PCR products were cloned into pTOPO plasmid (Zero Blunt TOPO PCR cloning kit, Invitrogen). The presence of the desired mutations was checked by automatic sequencing. The NdeI+BamHI fragment of the pTOPO—IFNα positive clones was then cloned into NdeI+BamHI sites of the pET11 plasmid.

B. Construction of a Collection of IFNα-2b Mutants in a Mammalian expression plasmid

[0453] A series of mutagenic primers was designed to generate the appropriate site-specific mutations in the IFNα-

2b cDNA. Mutagenesis reactions were performed with the Chameleon mutagenesis kit (Stratagene) using pNB-AAV-IFNα-2b as the template. Each individual mutagenesis reaction was designed to generate one single mutant protein. Each individual mutagenesis reaction contains one and only one mutagenic primer. For each reaction, 25 pmoles of each (phosphorylated) mutagenic primer were mixed with 0.25 pmoles of template, 25 pmoles of selection primer (introducing a new restriction site), and 2 μl of 10× mutagenesis buffer (100 mM Tris-acetate pH 7.5; 100 mM MgOAc; 500 mM KOAc pH 7.5) into each well of 96 well-plates. To allow DNA annealing, PCR plates were incubated at 98° C. during 5 min and immediately placed 5 min on ice, before incubating at room temperature during 30 min. Elongation and ligation reactions were allowed by addition of 7 μl of nucleotide mix (2.86 mM each nucleotide; 1.43× mutagenesis buffer) and 3 μl of a freshly prepared enzyme mixture of dilution buffer (20 mM Tris HCl pH7.5; 10 mM KCl; 10 mM β-mercaptoethanol; 1 mM DTT; 0.1 mM EDTA; 50% glycerol), native T7 DNA polymerase (0.025 U/μl), and T4 DNA ligase (1 U/μl) in a ratio of 1:10, respectively. Reactions were incubated at 37° C. for 1 h before inactivation of T4 DNA ligase at 72° C. during 15 min. In order to eliminate the parental plasmid, 30 μl of a mixture containing 1× enzyme buffer and 10 U of restriction enzyme was added to the mutagenic reactions followed by incubation at 37° C. for at least 3 hours. Next, 90 μl aliquots of XLmutS competent cells (Stratagene) containing 25 mM β-mercaptoethanol were place in ice-chilled deep-well plates. Then, plates were incubated on ice for 10 min with gentle vortex every 2 min. Transformation of competent cells was performed by adding aliquots of the restriction reactions (1/10 of reaction volume) and incubating on ice for 30 min. A heat pulse was performed in a 42° C. water bath for 45 s, followed by incubation on ice for 2 minutes. Preheated SOC medium (0.45 ml) was added to each well and plates were incubated at 37° C. for 1 h with shaking. In order to enrich for mutated plasmids, 1 ml of 2×YT broth medium supplemented with 100 μg/ml ampicillin was added to each transformation mixture followed by overnight incubation at 37° C. with shaking. Plasmid DNA isolation was performed by alkaline lysis using Nucleospin Multi-96 Plus Plasmid Kit (Macherey-Nagel) according to the manufacturer's instructions. Selection of mutated plasmids was performed by digesting 500 μg of plasmid preparation with 10 U of selection endonuclease in an overnight incubation at 37° C. A fraction of the digested reactions (1/10 of the total volume) was transformed into 40 μl of Epicurian coli XL1-Blue competent cells (Stratagene) supplemented with 25 mM β-mercaptoethanol.

[0454] Transformation was performed was as described above. Transformants were selected on LB-ampicillin agar plates incubated overnight at 37° C. Isolated colonies were picked up and grown overnight at 37° C. into deep-well plates. Four clones per reaction were screened by endonuclease digestion of a new restriction site introduced by the selection primer. Finally, each mutation that was introduced to produce this collection of candidate LEAD IFNα-2b mutant plasmids encoding the proteins set forth in Table 2 of Example 2 was confirmed by automatic DNA sequencing.

C. Production of IFNα-2b Mutants

[0455] C.1 In Mammalian Cells

[0456] IFNα-2b mutants were produced in 293 human embryo kidney (HEK) cells (obtained from ATCC), using Dulbecco's modified Eagle's medium supplemented with glucose (4.5 g/L; Gibco-BRL) and fetal bovine serum (10%, Hyclone). Cells were transiently transfected with the plasmids encoding the IFNα-2b mutants as follows: 0.6×10^5 cells were seeded into 6 well-plates and grown for 36 h before transfection. Confluent cells at about 70%, were supplemented with 2.5 μg of plasmid (IFNα-2b mutants) and 10 mM poly-ethylene-imine (25 KDa PEI, Sigma-Aldrich). After gently shaking, cells were incubated for 16 h. Then, the culture medium was changed with 1 ml of fresh medium supplemented with 1% of serum. IFNα-2b was measured on culture supernatants obtained 40 h after transfection and stored in aliquots at −80° C. until use.

[0457] Supernatants containing IFNα-2b from transfected cells were screened following sequential biological assays as follows. Normalization of IFNα-2b concentration from culture supernatants was performed by enzyme-linked immunoabsorbent assay (ELISA) using a commercial kit (R & D) and following the manufacturer's instructions. This assay includes plates coated with an IFNα-2b monoclonal antibody that can be developed by coupling a secondary antibody conjugated to the horseradish peroxidase (HRP). IFNα-2b concentrations on samples containing (i) wild type IFNα-2b produced under comparable conditions as the mutants, (ii) the IFNα-2b mutants and (iii) control samples (produced from cells expressing GFP) were estimated by using an international reference standard provided by the NIBSC, UK.

[0458] C.2 In Bacteria

[0459] A volume of 200 ml of culture medium (LB/Ampicillin/Chloramphenicol) was inoculated with 5 ml of pre-culture BL21-pCodon+-pET-IFN α-2b muta overnight at 37° C. with constant shaking (225 rpm). The production of IFN α-2b was induced by the addition of 50 μl of 2M IPTG at $DO_{600\ nm}$~0.6.

[0460] The culture was continued for 3 additional hours and was centrifuged at 4° C. and 5000 g for 15 minutes. The supernatant (culture medium) was discarded and bacteria were lysed in 8 ml of lysis buffer by thermal shock (freezing-thawing: 37° C.-15 min; −80° C.-10 min; 37° C.-15 min; −80° C.-10 min; 37° C.-15 min). After centrifugation (10000 g, 15 min, 4° C.), the supernatant (soluble proteins fraction) was discarded, and the precipitated material (insoluble protein fraction containing the IFN a −2b protein as inclusion bodies) was purified.

[0461] C.3 Pre-Purification of IFN α-2b as Inclusion Bodies in *E. coli*

[0462] C.3.1 Washing of Inclusion Bodies by Sonication

[0463] Pellets containing the inclusion bodies were suspended in 10 ml of buffer and sonicated (80 watts) on ice, 1 second "on," 1 second "off" for a total of 4 min. Suspensions were then centrifuged (4° C., 10000 g, 15 min), and supernatants were recovered. Pellets were resuspended in 10 ml of buffer for a new sonication/centrifugation cycle. Triton X-100 was then eliminated by two additional cycles of sonication/centrifugation with buffer. Pellets containing the inclusion bodies were recovered and dissolved. The washed supernatants were stored at 4° C.

[0464] C.3.2 Solubilization of Inclusion Bodies by Denaturation

[0465] Once washed, the inclusion bodies were solubilized in buffer at a concentration estimated in 0.3 mg/ml measuring the OD280 (considering the coefficient of molar extinction of IFN α-2b). Solubilization was carried out overnight at 4° C., under shaking.

[0466] C.3.3 Renaturation of IFN α-2b by Dialysis of GdnHCl

[0467] Samples contained 1 mg of protein at 0.3 mg/ml (5 ml in total) in buffer. The GdnHCl (Guanidium hydrochloride) present in the samples was eliminated by dialysis (minimum membrane cut=10 kDa) overnight at 4° C. against buffer (1 litre) (final concentration of GdnHCl: 43 mM). Next, samples were further dialysed against 1 litre of buffer during 2.5 h. This step was repeated two additional times. After dialysis, very little precipitate was visible.

D. Screening and In Vitro Characterization of IFN α-2b Mutants

[0468] Two activities were measured directly on IFN samples: antiviral and antiproliferation activities. Dose (concentration)—response (activity) experiments for antiviral or antiproliferation activity permitted calculation of the "potency" for antiviral and antiproliferation activities, respectively. Antiviral and antiproliferation activities also were measured after incubation with proteolytic samples, such as specific proteases, mixtures of selected proteases, human serum or human blood. Assessment of activity following incubation with proteolytic samples allowed to determine the residual (antiviral or antiproliferation) activity and the respective kinetics of half-life upon exposure to proteases.

[0469] D.1. Antiviral activity

[0470] IFNα-2b protects cells against viral infection by a complex mechanism devoted to create an unfavorable environment for viral proliferation. Cellular antiviral response due to IFNα-2b (IFN anti-viral assay) was assessed using an interferon-sensitive HeLa cell line (ATCC accession no. CCL-2) treated with the encephalomyocarditis virus (EMCV). The assessment of either the virus-induced cytopathic effects (CPE) or the amount of EMCV mRNA in extracts of infected cells by RT-PCR was used to determine IFNα activity in samples.

[0471] D.1.1 Antiviral activity—measure by RT-PCR

[0472] Confluent cells were trypsinized and plated at density 2×10^4 cells/well in DMEM 5% SVF medium (Day 0). Cells were incubated with IFN α-2b (at a concentration of 500 U/ml) to get 500 pg/ml and 150 pg/well (100 μl of IFN solution), during 24 h at 37° C. prior to be challenged with EMCV (1/1000 dilution; MOI 100). After an incubation of 16 h, when virus-induced CPE was near maximum in untreated cells, the number of EMCV particles in each well was determined by RT-PCR quantification of EMCV mRNA, using lysates of infected cells. RNA from cell extracts was purified after a DNAse/proteinase K treatment (Applied Biosystems). The CPE was evaluated using both Uptibleu (Interchim) and MTS (Promega) methods, which are based on detecting bio-reductions produced by the metabolic activity of cells in a fluorometric and colorimetric manner, respectively. In order to produce a standard curve for EMCV quantification, a 22 bp DNA fragment of the capsid protein-cDNA was amplified by PCR and cloned into pTOPO-TA vector (Invitrogen). Next, RT-PCR quantification of known amounts of pTOPO-TA-EMCV capsid gene was performed using the One-step RT-PCR kit (Applied Biosystems) and the following EMCV-related (cloning) oligonucleotides and probe:

```
EMCV forward primer
                                        (SEQ ID NO:229)
5'-CCCCTACATTGAGGCATCCA-3'

EMCV reverse primer
                                        (SEQ ID NO:230)
5'-CAGGAGCAGGACAAGGTCACT-3'

EMCV probe
                                        (SEQ ID NO:231)
5'-(FAM)CAGCCGTCAAGACCCAACCGCT(TAMRA)-3'.
```

[0473] D.1.2 Antiviral activity—measure by CPE

[0474] Antiviral activity of IFN α-2b was determined by the capacity of the cytokine to protect HeLa cells against EMC (mouse encephalomyocarditis) virus-induced cytopathic effects. The day before, HeLa cells (2×10^5 cells/ml) were seeded in flat-bottomed 96-well plates containing 100 μl/well of Dulbecco's MEM-Glutamaxl-sodium pyruvate medium supplemented with 5% SVF and 0.2% of gentamicin. Cells were growth at 37° C. in an atmosphere of 5% CO_2 for 24 hours.

[0475] Two-fold serial dilutions of interferon samples were made with MEM complete media into 96-Deep-Well plates with final concentration ranging from 1600 to 0.6 pg/ml. The medium was aspirated from each well and 100 μl of interferon dilutions were added to HeLa cells. Each interferon sample dilution was assessed in triplicate. The two last rows of the plates were filled with 100 μl of medium without interferon dilution samples in order to serve as controls for cells with and without virus.

[0476] After 24 hours of growth, a 1/1000 EMC virus dilution solution was placed in each well except for the cell control row. Plates were returned to the CO_2 incubator for 48 hours. Then, the medium was aspirated and the cells were stained for 1 hour with 100 μl of Blue staining solution to determine the proportion of intact cells. Plates were washed in a distilled water bath. The cell bound dye was extracted using 100 μl of ethylene-glycol mono-ethyl-ether (Sigma). The absorbance of the dye was measured using an Elisa plate reader (Spectramax). The antiviral activity of IFN α-2b samples (expressed as number of IU/mg of proteins) was determined as the concentration needed for 50% protection of the cells against EMC virus-induced cytopathic effects. For proteolysis experiments, each point of for the kinetic measurements was assessed at 500 and 166 pg/ml in triplicate.

[0477] D.2 Antiproliferation activity

[0478] Anti-proliferative activity of interferonβ-2b was determined by the capacity of the cytokine to inhibit proliferation of Daudi cells. Daudi cells (1×10^4 cells) Were seeded in flat-bottomed 96-well plates containing 50 μl/well of RPMI 1640 medium supplemented with 10% SVF, 1× glutamine and 1 ml of gentamicin. No cell was added to the

last row ("H" row) of the flat-bottomed 96-well plates in order to evaluate background absorbance of culture medium.

[0479] At the same time, two-fold serial dilutions of interferon samples were made with RPMI 1640 complete media into 96-Deep-Well plates with final concentration ranging from 6000 to 2.9 pg/ml. Interferon dilutions (50 μl) were added to each well containing 50 μl of Daudi cells. The total volume in each well should now be 100 μl. Each interferon sample dilution was assessed in triplicate. Each well of the "G" row of the plates was filled with 50 μl of RPMI 1640 complete media in order to be used as positive control. The plates are incubated for 72 hours at 37° C. in a humidified, 5% CO_2 atmosphere.

[0480] After 72 hours of growth, 20 μl of Cell titer 96 Aqueous one solution reagent (Promega) was added to each well and incubated 1H30 at 37° C. in an atmosphere of 5% CO_2. To measure the amount of colored soluble formazan produced by cellular reduction of the MTS, the absorbance of the dye was measured using an Elisa plate reader (Spectramax) at 490 nm.

[0481] The corrected absorbances ("H" row background value subtracted) obtained at 490 nm were plotted versus concentration of cytokine. The ED50 value was calculated by determining the X-axis value corresponding to one-half the difference between the maximum and minimum absorbance values. (ED50=the concentration of cytokine necessary to give one-half the maximum response).

[0482] D.3 Treatment of IFN α-2b with Proteolytic Preparations

[0483] Mutants were treated with proteases in order to identify resistant molecules. The resistance of the mutant IFN α-2b molecules compared to wild-type IFN α-2b against enzymatic cleavage (30 min, 25° C.) by a mixture of proteases (containing 1.5 μg of each of the following proteases (1% wt/wt, Sigma): α-chymotrypsin, carboxypeptidase, endoproteinase Arg-C, endoproteinase Asp-N, endoproteinase Glu-C, endoproteinase Lys-C, and trypsin) was determined. At the end of the incubation time, 10 μl of anti-proteases complete, mini EDTA free, Roche (one tablet was dissolved in 10 ml of DMEM and then diluted to 1/1000) was added to each reaction in order to inhibit protease activity. Treated samples were then used to determine residual antiviral or antiproliferation activities.

[0484] D.4 Protease Resistance—Kinetic Analysis

[0485] The percent of residual IFN α-2b activity over time of exposure to proteases was evaluated by a kinetic study using either (a) 15 pg of chymotrypsin (10% wt/wt), (b) a lysate of human blood at dilution 1/100, (c) 1.5 pg of protease mixture, or (d) human serum. Incubation times were: 0 h, 0.5 h, 1 h, 4 h, 8 h, 16 h, 24 h and 48 h. Briefly, 20 μl of each proteolytic sample (proteases, serum, blood) was added to 100 μl of IFN α-2b at 1500 pg/ml (500 U/ml) and incubated for variable times, as indicated. At the appropriate time points, 10 μl of anti-proteases mixture, mini EDTA free, Roche (one tablet was dissolved in 10 ml of DMEM and then diluted to 1/500) was added to each well in order to stop proteolysis reactions. Biological activity assays were then performed as described for each sample in order to determine the residual activity at each time point.

[0486] D.5 Performance

[0487] The various biological activities, protease resistance and potency of each individual mutant were analyzed using a mathematical model and algorithm (NautScan™; described in French Patent No. 9915884; (published as International PCT application No. WO 01/44809 based on PCT no PCT/FROO/03503). Data was processed using a Hill equation-based model that uses key feature indicators of the performance of each individual mutant. Mutants were ranked based on the values of their individual performance and those on the top of the ranking list were selected as leads.

E. Pharmacokinetics of Selected Lead Mutants in Mice

[0488] IFNα-2b mutants selected on the basis of their overall performance in vitro, were tested for pharmacokinetics in mice in order to have an indication of their half-life in blood in vivo. Mice were treated by subcutaneous (SC) injection with aliquots of each of a number of selected lead mutants. Blood was collected at increasing time points between 0.5 and 48 hours after injection. Immediately after collection, 20 ml of anti-protease solution were added to each blood sample. Serum was obtained for further analysis. Residual IFN-α activity in blood was determined using the tests described in the precedent sections for in vitro characterization. Wild-type IFN a (that had been produced in bacteria under comparable conditions as the lead mutants) as well as a pegylated derivative of IFN α, Pegasys (Roche), also were tested for pharmacokinetics in the same experiments.

Example 2

[0489] This example demonstrates the 2-dimensional (2D)scanning of IFNα-2b for increased resistance to proteolysis. For results, see FIGS. **6**(A)-**6**(N), **6**(T) and **6**(U).

[0490] A) Identifying Some or all Possible Target Sites on the Protein Sequence that are Susceptible to Digestion by One or More Specific Proteases (these Sites are the is-HITs).

[0491] Because IFNα-2b is administered as a therapeutic protein in the blood stream, a set of proteases was identified that were expected to broadly mimic the protease contents in serum. From that list of proteases, a list of the corresponding target amino acids was identified (shown in parenthesis) as follows: α-chymotrypsin (F, L, M, W, and Y), endoproteinase Arg-C(R), endoproteinase Asp-N (D), endoproteinase Glu-C (E), endoproteinase Lys-C (K), and trypsin (K and R) Carboxypeptidase Y, which cleaves non-specifically from the carboxy-terminal ends of proteins, was also included in the protease mixture. The distribution of the target amino acids over the protein sequence spreads over the complete length of the protein, suggesting that the protein is potentially sensitive to protease digestion all over its sequence (FIG. **1**A). In order to restrict the number of is-HITs to a lower number of candidate positions, the 3-dimensional structure of the IFNα-2b molecule (PDB code 1RH2) was used to identify and select only those residues exposed on the surface, while discarding from the candidate list those which remain buried in the structure, and therefore stay less susceptible to proteolysis (FIG. **1**B).

[0492] B) Identifying Appropriate Replacing Amino Acids, Specific for Each is-HIT, Such that if Used to Replace One or More of the Original, Such as Native, Amino Acids at that Specific is-HIT, they can be Expected to Increase the

is-HIT Amino Acid Position's Resistance to Digestion by Protease while at the Same Time, Maintaining or Improving the Requisite Biological Activity of the Protein (these Replacing Amino Acids are the Candidate LEADS).

[0493] To select the candidate replacing amino acids for each is-HIT position, PAM250 matrix based analysis was used (FIG. **2**). In one embodiment, the two highest values in PAM250 matrix, corresponding to the highest occurrence of substitutions between residues ("conservative substitutions" or "accepted point mutations"), were chosen (FIG. **3**). Whenever only a conservative substitution was available for a given high value of the PAM250, the following higher value was selected and the totality of conservative substitutions for this value was considered. The replacement of amino acids that are exposed on the surface by cysteine residues (as shown in FIG. **3**, while replacing Y by H or I) was explicitly avoided, since this change would potentially lead to the formation of intermolecular disulfide bonds.

[0494] Thus, based on the nature of the challenging proteases, and on evolutionary considerations as well as protein structural analysis, a strategy was defined for the rational design of human IFNα-2b mutants having increased resistance to proteolysis which could produce therapeutic proteins having a longer half-life. By using the algorithm PROTEOL (see, e.g., infobiogen.fr), a list of residues along the IFNα-2b sequence was established, which can be recognized as a substrate for different enzymes present in the serum. Because the number of residues in this particular list was high, the 3-dimensional structure of IFNα-2b obtained from the NMR structure of IFNα-2a (PDB code 1ITF) was used to select only those residues exposed to the solvent. Using this approach, 42 positions were identified, which numbering is that of the mature protein (SEQ ID NO:1): L3, P4, R12, R13, M16, R22, R23, F27, L30, K31, R33, E41, K49, E58, K70, E78, K83, Y89, E96, E107, P109, L110, M111, E113, L117, R120, K121, R125, L128, K131, E132, K133, K134, Y135, P137, M148, R149, E159, L161, R162, K164, and E165. Each of these positions was replaced by amino acid residues, such that they are defined as compatible by the substitution matrix PAM250 while at the same time the replacement amino acids do not generate new sites for proteases.

[0495] The list of performed residue substitutions as determined by PAM250 analysis is as follows:

[0496] R to H, Q

[0497] E to H, Q

[0498] K to Q, T

[0499] L to V, I

[0500] M to I, V

[0501] P to A, S

[0502] Y to I, H.

[0503] C) Systematically Introducing the Specific Replacing Amino Acids (candidate LEADS) at Every Specific is-HIT Position to Generate a Collection Containing the Corresponding Mutant Molecules.

[0504] The individual IFNα-2b mutants are generated, produced and phenotypically characterized one-by-one, in addressable arrays as set forth in Example 1, such that each mutant molecule contains initially amino acid replacements at only one is-HIT site. LEAD positions were obtained in IFNα-2b variants after a screening for protection against proteases, and comparing protease-untreated and protease-treated variant preparations with the corresponding conditions for the wild-type IFNα-2b. The percent of residual (anti-viral) activity for the IFNα-2b E113H variant after treatment with chymotrypsin, protease mixture, blood lysate or serum was compared to the treated wild-type IFNα-2b. Selected IFNα-2b LEADs are shown in Table 2.

[0505] A top and side view of IFNα-2b structure in ribbon representation (obtained from NMR structure of IFNα-2a, PDB code 1ITF) depict residues in "space filling" defining (1) the "receptor binding region" as deduced either by "alanine scanning" data and studies by Piehler et al., *J. Biol. Chem.,* 275:40425-40433, 2000, and Roisman et al., *Proc. Natl. Acad. Sci. USA,* 98:13231-13236, 2001, and (2) replacing residues (LEADs) for resistance to proteolysis.

TABLE 2

Selected LEADs of IFNα-2b following protease resistance

Mutant	SEQ ID No.	Proteolysis protection	IFN antiviral activity
F27V	83	Pseudo wt	Pseudo wt
R33H	86	Pseudo wt	Pseudo wt
E41Q	87	Increased	Increased
E41H	88	Pseudo wt	Increased
E58Q	89	Increased	Pseudo wt
E58H	90	Increased	Increased
E78Q	92	Increased	Increased
E78H	93	Increased	Increased
Y89H	1303	Pseudo wt	Pseudo wt
E107Q	95	Increased	Pseudo wt
E107H	96	Increased	Pseudo wt
P109A	97	Pseudo wt	Pseudo wt
L110V	98	Pseudo wt	Pseudo wt
M111V	978	Pseudo wt	Pseudo wt
E113H	101	Increased	Pseudo wt
L117V	102	Increased	Pseudo wt
L117I	103	Increased	Pseudo wt
K121Q	104	Increased	Pseudo wt
R125H	106	Increased	Increased
R125Q	107	Increased	Increased
K133Q	114	Increased	Increased
E159H	125	Increased	Pseudo wt
E159Q	124	Increased	Pseudo wt

Example 3

[0506] Stabilization of IFNα-2b by Creation of N-Glycosylation Sites

[0507] The creation of N-glycosylation sites on the protein was a second strategy that was used to stabilize IFNα-2b. Natural human IFNα-2b contains a unique O-glycosylation site at position 129 (the numbering corresponds to the mature protein; SEQ ID NO:1), however, no N-glycosylation sites are found in this sequence. N-glycosylation sites are defined by the N-X-S or N-X-T consensus sequences. Glycosylation has been found to play a role in protein stability. For example, glycosylation has been found to increase bioavailability via higher metabolic stability and reduced clearance. In order to generate more stable IFNα-2b variants, the N-glycosylation consensus sequences indicated above were introduced in the IFNα-2b sequence by mutagenesis. Variants of IFNα-2b carrying new glycosylation sites were assessed as previously described.

[0508] The structure of IFNα-2b is characterized by a helix bundle composed of 5 helices (A, B, C, D and E) connected with each other by a series of loops (a large AB loop and three shorter BC, CD, DE loops). The helices are joined together by two disulfide bridges between residues 1/98 and 29/138 of SEQ ID NO:1. The loops are contemplated herein to represent preferential sites for glycosylation given their exposure. Therefore, N-glycosylation sites (N-X-S or N-X-T) were created in each of the loop sequences (Table 3). Selected LEADs and pseudo wild-type IFNα-2b mutants after screening for addition of glycosylation sites are shown in Table 4.

TABLE 3

In silico HITs for addition of glycosylation sites on IFNα-2b

Codon No.	SEQ ID No.	N-X-S	SEQ ID No.	N-X-T
c2-4		D2N/P4S		D2N/P4T
c3-5		L3N/Q5S		L3N/Q5T
c4-6		P4N/T6S		P4N/T6T
c5-7	127	Q5N/H7S	128	Q5N/H7T
c6-8	129	T6N/S8S		T6N/S8T
c7-9		H7N/L9S		H7N/L9T
c8-10	130	S8N/G10S	131	S8N/G10T
c9-11		L9N/S11S		L9N/S11T
c10-12	132	M21N/R23S		M21N/R23T
c22-24		R22N/I24S		R22N/I24T
c23-25		R23N/S25S	133	R23N/S25T
c24-26	134	I24N/L26S		I24N/L26T
c25-27	135	S25N/F27S	136	S25N/F27T
c26-28	137	L26N/S28S	138	L26N/S28T
c28-30		S28N/L30S		S28N/L30T
c30-32	139	L30N/D32S		L30N/D32T
c31-33		K31N/R33S		K31N/R33T
c32-34		D32N/H34S		D32N/H34T
c33-35	140	R33N/D35S	141	R33N/D35T
c34-36	142	H34N/F36S	143	H34N/F36T
c35-37	144	D35N/G37S		D35N/G37T
c36-38	145	F36N/F38S	146	F36N/F38T
c37-39		G37N/P39S	147	G37N/P39T
c38-40	148	F38N/Q40S	149	F38N/Q40T
c39-41	150	P39N/E41S	151	P39N/E41T
c40-42	152	Q40N/E42S	153	Q40N/E42T
c41-43		E41N/F43S	155	E41N/F43T
c42-44		E42N/G44S		E42N/G44T
c43-45		F43N/N45S		F43N/N45T
c44-46	156	G44N/Q46S	157	G44N/Q46T
c45-47	158	N45N/F47S	159	N45N/F47T
c46-48	160	Q46N/Q48S	161	Q46N/Q48T
c47-49	162	F47N/K49S	163	F47N/K49T
c48-50		Q48N/A50S		Q48N/A50T
c49-51	164	K49N/E51S		K49N/E51T
c50-52		A50N/T52S		A50N/T52T
c68-70		S68N/K70S		S68N/K70T
c70-72		K70N/S72S		K70N/S72T
c75-77	165	A75N/D77S		A75N/D77T
c77-79		D77N/T79S		D77N/T79T
C100-102	166	I100N/G102S	167	I100N/G102T
C101-103		Q101N/V103S		Q101N/V103T
C102-104		G102N/G104S		G102N/G104T
C103-105	168	V103N/V105S	169	V103N/V105T
C104-106		G104N/T106S	170	G104N/T106T
C105-107	171	V105N/E107S		V105N/E107T
C10--108	172	T106N/T108S	173	T106N/T108T
C107-109	174	E107N/P109S	175	E107N/P109T
C108-110		T108N/I110S		T108N/I110T
C134-136		K134N/S136S	176	K134N/S136T
C154-156		S154N/N156S		S154N/N156T
C155-157		T155N/L157S		T155N/L157T
C156-158		N156N/Q158S		N156N/Q158T
C157-159	177	L157N/E159S	178	L157N/E159T
C158-160		Q158N/S160S	179	Q158N/S160T
C159-161	180	E159N/L161S	181	E159N/L161T

TABLE 3-continued

In silico HITs for addition of glycosylation sites on IFNα-2b

Codon No.	SEQ ID No.	N-X-S	SEQ ID No.	N-X-T
C160-162		S160N/R162S		S160N/R162T
C161-163		L161N/S163S		L161N/S163T
C162-164		R162N/K164S		R162N/K164T
C163-165		S163N/E165S		S163N/E165T

[0509]

TABLE 4

Selected LEADs and pseudo wild-type IFNα-2b mutants after screening for addition of glycosylation sites

Mutant	SEQ ID No.	Proteolysis protection	IFN antiviral activity
Q5N/H7S	127	Increased	Pseudo wt
Q5N/H7T	128	ND*	ND
P39N/E41S	150	Increased	Pseudo wt
P39N/E41T	151	Increased	Pseudo wt
Q40N/E42S	152	Increased	Pseudo wt
Q40N/E42T	153	Increased	Pseudo wt
E41N/F43S	154	Increased	Pseudo wt
E41N/F43T	155	Increased	Pseudo wt
F43N/N45S		Increased	Pseudo wt
F43N/N45T		ND	ND
G44N/Q46S	156	ND	ND
G44N/Q46T	157	Increased	Pseudo wt
N45N/F47S	158	Increased	Pseudo wt
N45N/F47T	159	Increased	Pseudo wt
Q46N/Q48S	160	Increased	Pseudo wt
Q46N/Q48T	161	ND	ND
F47N/K49S	162	Increased	Pseudo wt
F47N/K49T	163	Increased	Pseudo wt
I100N/G102S	166	Pseudo wt	Increased
I100N/G102T	167	Pseudo wt	Increased
V105N/E107S	171	Pseudo wt	Increased
V105N/E107T		Pseudo wt	Increased
T106N/T108S	172	Pseudo wt	Increased
T106N/T108T	173	Pseudo wt	Increased
E107N/P109S	174	Pseudo wt	Increased
E107N/P109T	175	Pseudo wt	Increased
L157N/E159S	177	Pseudo wt	Increased
L157N/E159T	178	Pseudo wt	Increased
E159N/L161S	180	Pseudo wt	Increased
E159N/L161T	181	Pseudo wt	Increased

*ND, not determined

Example 4

Redesign of Interferon α-2b Proteins

[0510] The use of the protein redesign approach provided herein permits the generation of proteins such that they maintain requisite levels and types of biological activity compared to the native protein while their underlying amino acid sequences have been significantly changed by amino acid replacement. To first identify those amino acid positions on the IFNα-2b protein that are involved or not involved IFNα-2b protein activity, such as binding activity of IFNα-2b to its receptor, an Ala-scan was performed on the IFNα-2b sequence. For this purpose, each amino acid in the IFNα-2b protein sequence was individually changed into Alanine. Any other amino acid, particularly another amino acid that has a neutral effect on structure, such as Gly or Ser,

also can be used. Each resulting mutant IFNα-2b protein was then expressed and the antiviral activity of the individual mutants was assayed. The particular amino acid positions that are sensitive to replacement by Ala, referred to herein as HITs would in principle not be suitable targets for amino acid replacement to increase protein stability, because of their involvement in the activity of the molecule. For the Ala-scanning, the biological activity measured for the IFNα-2b molecules was: i) their capacity to inhibit virus replication when added to permissive cells previously infected with the appropriate virus and, ii) their capacity to stimulate cell proliferation when added to the appropriate cells. The relative activity of each individual mutant compared to the native protein was assayed. HITS are those mutants that produce a decrease in the activity of the protein (e.g., in this example, all the mutants with activities below about 30% of the native activity).

[0511] In addition, to identify the HIT positions, the Alanine-scan was used to identify the amino acid residues on IFNα-2b that when replaced with alanine lead to a 'pseudo-wild type' activity, i.e., those that can be replaced by alanine without leading to a decrease in biological activity.

[0512] A collection of mutant molecules was generated and phenotypically characterized such that IFNα-2b proteins with amino acid sequences different from the native ones but that still elicit the same level and type of activity as the native protein were selected. HITs and pseudo wild-type amino acid positions are shown in Table 5.

TABLE 5

HITs and pseudo wild-type positions to IFNα-2b redesign

Mutants	SEQ ID No.	HITs (viral activity)	Pseudo wt (viral activity)
D2A	2	Decreased	
P4A	3		Pseudo wt
Q5A	4		Pseudo wt
T6A	5		Pseudo wt
H7A	6	Decreased	
S8A	7	Decreased	
L9A	8		Pseudo wt
G10A	9		Pseudo wt
S11A	10	Decreased	
R12A	11	Decreased	
R13A	12	Decreased	
T14A	13	Decreased	
L15A	14	Decreased	
M16A	15	Decreased	
L17A	16		Pseudo wt
Q20A	17		Pseudo wt
R23A	18	Decreased	
I24A	19		Pseudo wt
S25A	20		Pseudo wt
L26A	21	Decreased	
S28A	22	Decreased	
C29A	23	Decreased	
L30A	24	Decreased	
K31A	25	Decreased	
D32A	26	Decreased	
R33A	27	Decreased	
D35A	28		Pseudo wt
G37A	29		Pseudo wt
G39A	30		Pseudo wt
E41A	31		Pseudo wt
E42A	32		Pseudo wt
F43A	33	Decreased	
N45A	34	Decreased	

TABLE 5-continued

HITs and pseudo wild-type positions to IFNα-2b redesign

Mutants	SEQ ID No.	HITs (viral activity)	Pseudo wt (viral activity)
F47A	35	Decreased	
E51A	36		Pseudo wt
T52A	37		Pseudo wt
I53A	38	Decreased	
P54A	39		Pseudo wt
V55A	40		Pseudo wt
L56A	41		Pseudo wt
H57A	42		Pseudo wt
E58A	43		Pseudo wt
M59A	44	Decreased	
I60A	45		Pseudo wt
I63A	46		Pseudo wt
F64A	47		Pseudo wt
N65A	48		Pseudo wt
L66A	49	Decreased	
F67A	50	Decreased	
T69A	51	Decreased	
K70A	52	Decreased	
D71A	53	Decreased	
S72A	54	Decreased	
W76A	55		Pseudo wt
D77A	56		Pseudo wt
E78A	57		Pseudo wt
L81A	58		Pseudo wt
D82A	59	Decreased	
K83A	60	Decreased	
F84A	61	Decreased	
Y85A	62		Pseudo wt
Y89A	63		Pseudo wt
Q90A	64		Pseudo wt
Q91A	65	Decreased	
N93A	66	Decreased	
D94A	67	Decreased	
C98A	68	Decreased	
V99A	69	Decreased	
Q101A		Decreased	
G104A	70		Pseudo wt
L110A	71		Pseudo wt
S115A	72		Pseudo wt
Y122A	73	Decreased	
W140A	74	Decreased	
E146A	75		Pseudo wt

Example 5

Super LEADS of Interferon α-2b Protein by Additive Directional Mutagenesis

[0513] The use of an additive directional mutagenesis approach provided a method for the assembly of multiple mutations previously present on the individual LEAD molecules in a single mutant protein thereby generating super-LEAD mutant proteins. In this method, a collection of nucleic acid molecules encoding a library of new mutant molecules is generated, tested and phenotypically characterized one-by-one in addressable arrays. Super-LEAD mutant molecules are such that each molecule contains a variable number and type of LEAD mutations

[0514] Using the LEADs obtained in Example 2, six series of mutant molecules were generated with more than one mutation per molecule as shown in Table 6. Some Super-LEAD mutant molecules were phenotypically characterized and the results are shown in Table 7. As shown in the table not all SuperLEADS have improved activity compared with the original Leads; some showed decreased activity of some type.

TABLE 6

Schema of LEADs position for SuperLEADS generation

Series 1
m1 = E41H
m1 + m2 = E41H + Y89H
Series 2
m1 = E58Q
m1 + m2 = E58Q + F27V
Series 3
m1 = R125H
m1 + m2 = R125H + M111V
Series 4
m1 = E159H
m1 + m2 = E159H + Y89H
Series 5
m1 = K121Q
m1 + m2 = K121Q + P109A
m1 + m2 + m3 = K121Q + P109A + K133Q
Series 6
m1 = E78H
m1 + m2 = E78H + R33H
m1 + m2 + m3 = E78H + R33H + E58H
m1 + m2 + m3 + m4 = E78H + R33H + E58H + L11OV

[0515]

TABLE 7

SuperLEADs of IFNα-2b multiple mutants

Mutant	SEQ ID No.	Proteolysis protection	IFN antiviral activity
E41H	88	Pseudo wt	Increased
Y89H	1303	Pseudo wt	Pseudo wt
E41H/Y89H/N45D	979	Increased	Increased
E58Q	89	Increased	Pseudo wt
F27V	83	Pseudo wt	Pseudo wt
E58Q/F27V	981	Increased	Pseudo wt
R125H	106	Increased	Increased
M111V	978	Pseudo wt	Pseudo wt
R125H/M111V	986	Increased	Increased
E159H	125		
Y89H	1303		
E159H/Y89H	987		
K121Q	104	Increased	Pseudo wt
P109A	97	Pseudo wt	Pseudo wt
K133Q	114	Increased	Increased
K121Q/P109A	983	Increased	Pseudo wt
K121Q/P109A/ K133Q/G102R	984	Increased	Increased
E78H	93	Increased	Increased
R33H	86	Pseudo wt	Pseudo wt
E58H	90	Increased	Increased
L110V	98	Pseudo wt	Pseudo wt
E78H/R33H/ E58H/L110V	982	Decreased	Decreased

Four mutants with mutations in addition to those selected by the rational mutagenesis were generated in the *E. coli* MutS strain and were detected by sequencing. The mutants were the following: E41Q/D94G; L117V/A139G; E41H/Y89H/N45D; and K121Q/P109A/K133Q/G102.

Example 6

[0516] Cloning of IFN β in pNAUT, a Mammalian Cell Expression Plasmid

[0517] The cDNA encoding IFN β (see, SEQ ID No. 196) was cloned into a mammalian expression vector, prior to the generation of the selected mutations (see, FIGS. **6**(O)-**6**(S) and **8**(A). A collected of predesigned, targeted mutants was then generated such that each individual mutant was created and processed individually, physically separated form each other and in addressable arrays. The mammalian expression vector pSSV9 CMV 0.3 pA (see, Example 1) was engineered as follows:

[0518] The pSSV9 CMV 0.3 pA was cut by PvuII and religated (this step gets rid of the ITR functions), prior to the introduction of a new EcoRI restriction site by Quickchange mutagenesis (Stratagene). The oligonucleotide sequences used, follow:

```
EcoRI forward primer:
                                                    (SEQ ID NO:218)
5'-GCCTGTATGATTTATTGGATGTTGGAATTCCCTGATGCGGTATTTTC

TCCTTACG-3'

EcoRI reverse prime:
                                                    (SEQ ID NO:219)
5'-CGTAAGGAGAAAATACCGCATCAGGGAATTCCAACATCCAATAAATC

ATACAGGC-3'
```

[0519] The construct sequence was confirmed by using the following oligonucleotides:

```
Seq ClaI forward primer:
                                                    (SEQ ID NO: 220)
5'-CTGATTATCAACCGGGGTACATATGATTGACATGC-3'

Seq XmnI reverse primer
                                                    (SEQ ID NO: 221)
5'-TACGGGATAATACCGCGCCACATAGCAGAAC-3'.
```

[0520] Then, the XmnI-ClaI fragment containing the newly introduced EcoRI site was cloned into pSSV9 CMV 0.3 pA to replace the corresponding wild-type fragment and produce construct pSSV9-2EcoRI.

[0521] The IFN β-cDNA was obtained from the pIFNβ1 (ATCC) construct. The sequence of the IFN β-cDNA was confirmed by sequencing using the primers below:

```
Seq forward primer:
5'-CCTGATGAAGGAGGACTC-3'     (SEQ ID NO:222)

Seq reverse primer:
5'-CCAAGCAGCAGATGAGTC-3'.    (SEQ ID NO:223)
```

[0522] The verified IFN β-encoding cDNA first was cloned into the pTOPO-TA vector (Invitrogen). After checking of the cDNA sequence by automatic DNA sequencing, the HindIII-XbaI fragment containing the IFN cDNA was subcloned into the corresponding sites of pSSV9-2EcoRI, leading to the construct pAAV-EcoRI-IFNbeta (pNB-AAV-IFN beta) Finally the fragment PvuII of plasmid pNB-AAV-IFN beta was subcloned in PvuII site of pUC 18 leading the final construct pUC-CMVIFNbetapA called pNAUT-IFN-beta.

Production and Normalization of IFNβ in Mammalian Cells

[0523] IFN β was produced in CHO Chinese Hamster Ovarian cells (obtained from ATCC), using Dulbecco's

modified Eagle's medium supplemented with glucose (4.5 g/L; Gibco-BRL) and fetal bovine serum (5%, Hyclone). Cells were transiently transfected as follows: 0.6×10^5 cells were seeded into 6 well plates and grown for 24 h before transfection. Confluent cells at about 70%, were supplemented with 1.0 μg of plasmid (from the library of IFN β mutants) by Lipofectamine Plus reagent (Invitrogen). After gently shaking, cells were incubated for 24 h with 1 ml of culture medium supplemented with 1% of serum. IFN β was obtained from culture supernatants 24 h after transfection and stored in aliquots at −80° C. until use.

[0524] Preparations of IFN β produced from transfected cells were screened following sequential biological assays as follows. Normalization of IFN β concentration from culture supernatants was performed by ELISA. IFN β concentrations from wild type, and mutant samples were estimated by using an international reference standard provided by the NIBSC, UK.

Screening and In Vitro Characterization of IFN β Mutants

[0525] Two activities were measured directly on IFN samples: antiviral and antiproliferation activities. Dose (concentration)—response (activity) experiments for antiviral or antiproliferation activity allowed for the calculation of the "potency" for antiviral and antiproliferation activities, respectively. Antiviral and antiproliferation activities also were measured after incubation with proteolytic samples such as specific proteases, mixtures of selected proteases, human serum or human blood. Assessment of activity following incubation with proteolytic samples allowed to determine the residual (antiviral or antiproliferation) activity and the respective kinetics of half-life upon exposure to proteases.

Antiviral Activity—Measured by Cytopathic Effects (CPE)

[0526] Antiviral activity of IFN β was determined by the capacity of the cytokine to protect HeLa cells against EMC (mouse encephalomyocarditis) virus-induced cytopathic effects. The day before, HeLa cells (2×10^5 cells/ml) were seeded in flat-bottomed 96-well plates containing 100 μl/well of Dulbecco's MEM-Glutamaxl-sodium pyruvate medium supplemented with 5% SVF and 0.2% of gentamicin. Cells were growth at 37° C. in an atmosphere of 5% CO_2 for 24 hours.

[0527] Two-fold serial dilutions of interferon samples were made with MEM complete media into 96-Deep-Well plates with final concentration ranging from 1600 to 0.6 pg/ml. The medium was aspirated from each well and 100 μl of interferon dilutions were added to HeLa cells. Each interferon sample dilution was assessed in triplicate. The two last rows of the plates were filled with 100 μl of medium without interferon dilution samples in order to serve as controls for cells with and without virus.

[0528] After 24 hours of growth, a 1/1000 EMC virus dilution solution was placed in each well, except for the cell control row. Plates were returned to the CO_2 incubator for 48 hours. Then, the medium was aspirated and the cells were stained for 1 hour with 100 μl of Blue staining solution to determine the proportion of intact cells. Plates were washed in a distilled water bath. The cell bound dye was extracted using 100 μl of ethylene-glycol mono-ethyl-ether (Sigma). The absorbance of the dye was measured using an Elisa plate reader (Spectramax). The antiviral activity of IFN β samples (expressed as number of IU/mg of proteins) was determined as the concentration needed for 50% protection of the cells against EMC virus-induced cytopathic effects. For proteolysis experiments, each point of the kinetic was assessed at 800 and 400 pg/ml in triplicate.

Anti-Proliferative Activity

[0529] Anti-proliferative activity of IFN β was determined by assessing the capacity of the cytokine to inhibit proliferation of Daudi cells. Daudi cells (1×10^4 cells) were seeded in flat-bottomed 96-well plates containing 50 μl/well of RPMI 1640 medium supplemented with 10% SVF, 1× glutamine and 1 ml of gentamicin. No cell was added to the last row ("H" row) of the flat-bottomed 96-well plates in order to evaluate background absorbance of culture medium.

[0530] At the same time, two-fold serial dilutions of interferon samples were made with RPMI 1640 complete media into 96-Deep-Well plates with final concentration ranging from 6000 to 2.9 pg/ml. Interferon dilutions (50 μl) were added to each well containing 50 μl of Daudi cells. The total volume in each well should now be 100 μl. Each interferon sample dilution was assessed in triplicate. Each well of the "G" row of the plates was filled with 50 μl of RPMI 1640 complete media in order to be used as positive control. The plates were incubated for 72 hours at 37° C. in a humidified, 5% CO_2 atmosphere.

[0531] After 72 hours of growth, 20 μl of Cell titer 96 Aqueous one solution reagent (Promega) was added to each well and incubated 1H30 at 37° C. in an atmosphere of 5% CO_2. To measure the amount of colored soluble formazan produced by cellular reduction of the MTS, the absorbance of the dye was measured using an Elisa plate reader (Spectramax) at 490 nm.

[0532] The corrected absorbances ("H" row background value subtracted) obtained at 490 nm were plotted versus concentration of cytokine. The ED50 value was calculated by determining the X-axis value corresponding to one-half the difference between the maximum and minimum absorbance values. (ED50=the concentration of cytokine necessary to give one-half the maximum response).

[0533] Treatment of IFN β with Proteolytic Preparations

[0534] Mutants were treated with proteases in order to identify resistant molecules. The resistance of the mutant IFN β molecules compared to wild-type IFN β against enzymatic cleavage (120 min, 25° C.) by a mixture of proteases (containing 1.5 pg of each of the following proteases (1% wt/wt, Sigma): α-chymotrypsin, carboxypeptidase, endoproteinase Arg-C, endoproteinase Asp-N, endoproteinase Glu-C, endoproteinase Lys-C, and trypsin) was determined. At the end of the incubation time, 10 μl of anti-proteases complete, mini EDTA free, Roche (one tablet was dissolved in 10 ml of DMEM and then diluted to 1/1000) was added to each reaction in order to inhibit protease activity. Treated samples were then used to determine residual antiviral or antiproliferation activities.

Protease Resistance—Kinetic Analysis

[0535] The percent of residual IFN β activity over time of exposure to proteases was evaluated by a kinetic study using 1.5 pg of protease mixture. Incubation times were: 0 h, 0.5 h, 2 h, 4 h, 8 h, 12 h, 24 h and 48 h. Briefly, 20 μl of each proteolytic sample (proteases, serum, blood) was added to

100 μl of IFN β at 400 and 800 pg/ml and incubated for variable times, as indicated. At the appropriate time points, 10 μl of anti-proteases mixture, mini EDTA free, Roche (one tablet was dissolved in 10 ml of DMEM and then diluted to 1/500) was added to each well in order to stop proteolysis reactions. Biological activity assays were then performed as described for each sample in order to determine the residual activity at each time point.

Performance

[0536] The various biological activities, protease resistance and potency of each individual mutant were analyzed using a mathematical model and algorithm (NautScan™; Fr. Patent No. 9915884; see, also published International PCT application No. WO 01/44809 based on PCT no PCT/FR00/03503). Data was processed using a Hill equation-based model that uses key feature indicators of the performance of each individual mutant. Mutants were ranked based on the values of their individual performance and those on the top of the ranking list were selected as leads.

[0537] Using the 2D-scanning and 3D-scanning methods described above in addition to the 3-dimensional structure of IFNβ, the following amino acid target positions were identified as is-HITs on IFNβ, which numbering is that of the mature protein (SEQ ID NO:196):

[0538] By 3D-scanning (see, SEQ ID Nos. 234-289, 989-1015): D by Q at position 39, D by H at position 39, D by G at position 39, E by Q at position 42, E by H at position 42, K by Q at position 45, K by T at position 45, K by S at position 45, K by H at position 45, L by V at position 47, L by I at position 47, L by T at position 47, L by Q at position 47, L by H at position 47, L by A at position 47, K by Q at position 52, K by T at position 52, K by S at position 52, K by H at position 52, F by I at position 67, F by V at position 67, R by H at position 71, R by Q at position 71, D by H at position 73, D by G at position 73, D by Q at position 73, E by Q at position 81, E by H at position 81, E by Q at position 107, E by H at position 107, K by Q at position 108, K by T at position 108, K by S at position 108, K by H at position 108, E by Q at position 109, E by H at position 109, D by Q at position 110, D by H at position 110, D by G at position 110, F by I at position 111, F by V at position 111, R by H at position 113, R by Q at position 113, L by V at position 116, L by I at position 116, L by T at position 116, L by Q at position 116, L by H at position 116, L by A at position 116, L by V at position 120, L by I at position 120, L by T at position 120, L by Q at position 120, L by H at position 120, L by A at position 120, K by Q at position 123, K by T at position 123, K by S at position 123, K by H at position 123, R by H at position 124, R by Q at position 124, R by H at position 128, R by Q at position 128, L by V at position 130, L by I at position 130, L by T at position 130, L by Q at position 130, L by H at position 130, L by A at position 130, K by Q at position 134, K by T at position 134, K by S at position 134, K by H at position 134, K by Q at position 136, K by T at position 136, K by S at position 136, K by H at position 136, E by Q at position 137, E by H at position 137, Y by H at position 163, Y by I at position 163, R by H at position 165, R by Q at position 165.

[0539] By 2D-scanning (see, SEQ ID Nos.1016-1302, and table above): M by V at position 1, M by I at position 1, M by T at position 1, M by Q at position 1, M by A at position 1, L by V at position 5, L by I at position 5, L by T at position 5, L by Q at position 5, L by H at position 5, L by A at position 5, F by I at position 8, F by V at position 8, L by V at position 9, L by I at position 9, L by T at position 9, L by Q at position 9, L by H at position 9, L by A at position 9, R by H at position 11, R by Q at position 11, F by I at position 15, F by V at position 15, K by Q at position 19, K by T at position 19, K by S at position 19, K by H at position 19, W by S at position 22, W by H at position 22, N by H at position 25, N by S at position 25, N by Q at position 25, R by H position 27, R by Q position 27, L by V at position 28, L by I at position 28, L by T at position 28, L by Q at position 28, L by H at position 28, L by A at position 28, E by Q at position 29, E by H at position 29, Y by H at position 30, Y by I at position 30, L by V at position 32, L by I at position 32, L by T at position 32, L by Q at position 32, L by H at position 32, L by A at position 32, K by Q at position 33, K by T at position 33, K by S at position 33, K by H at position 33, R by H at position 35, R by Q at position 35, M by V at position 36, M by I at position 36, M by T at position 36, M by Q at position 36, M by A at position 36, D by Q at position 39, D by H at position 39, D by G at position 39, E by Q at position 42, E by H at position 42, K by Q at position 45, K by T at position 45, K by S at position 45, K by H at position 45, L by V at position 47, L by I at position 47, L by T at position 47, L by, Q at position 47, L by H at position 47, L by A at position 47, K by Q at position 52, K by T at position 52, K by S at position 52, K by H at position 52, F by I at position 67, F by V at position 67, R by H at position 71, R by Q at position 71, D by Q at position 73, D by H at position 73, D by G at position 73, E by Q at position 81, E by H at position 81, E by Q at position 85, E by H at position 85, Y by H at position 92, Y by I at position 92, K by Q at position 99, K by T at position 99, K by S at position 99, K by H at position 99, E by Q at position 103, E by H at position 103, E by Q at position 104, E by H at position 104, K by Q at position 105, K by T at position 105, K by S at position 105, K by H at position 105, E by Q at position 107, E by H at position 107, K by Q at position 108, K by T at position 108, K by S at position 108, K by H at position 108, E by Q at position 109, E by H at position 109, D by Q at position 110, D by H at position 110, D by G at position 110, F by I at position 111, F by V at position 111, R by H at position 113, R by Q at position 113, L by V at position 116, L by I at position 116, L by T at position 116, L by Q at position 116, L by H at position 116, L by A at position 116, L by V at position 120, L by I at position 120, L by T at position 120, L by Q at position 120, L by H at position 120, L by A at position 120, K by Q at position 123, K by T at position 123, K by S at position 123, K by H at position 123, R by H at position 124, R by Q at position 124, R by H at position 128, R by Q at position 128, L by V at position 130, L by I at position 130, L by T at position 130, L by Q at position 130, L by H at position 130, L by A at position 130, K by Q at position 134, K by T at position 134, K by S at position 134, K by H at position 134, K by Q at position 136, K by T at position 136, K by S at position 136, K by H at position 136, E by Q at position 137, E by H at position 137, Y by H at position 138, Y by I at position 138, R by H at position 152, R by Q at position 152, Y by H at position 155, Y by I at position 155, R by H at position 159, R by Q at position 159, Y by H at position 163, Y by I at position 163, R by H at position 165, R by Q at position 165, M by D at position 1, M by E at position 1, M by K at position 1, M by N at position 1, M by R at position 1, M

by S at position 1, L by D at position 5, L by E at position 5, L by K at position 5, L by N at position 5, L by R at position 5, L by S at position 5, L by D at position 6, L by E at position 6, L by K at position 6, L by N at position 6, L by R at position 6, L by S at position 6, L by Q at position 6, L by T at position 6, F by E at position 8, F by K at position 8, F by R at position 8, F by D at position 8, L by D at position 9, L by E at position 9, L by K at position 9, L by N at position 9, L by R at position 9, L by S at position 9, Q by D at position 10, Q by E at position 10, Q by K at position 10, Q by N at position 10, Q by R at position 10, Q by S at position 10, Q by T at position 10, S by D at position 12, S by E at position 12, S by K at position 12, S by R at position 12, S by D at position 13, S by E at position 13, S by K at position 13, S by R at position 13, S by N at position 13, S by Q at position 13, S by T at position 13, N by D at position 14, N by E at position 14, N by K at position 14, N by Q at position 14, N by R at position 14, N by S at position 14, N by T at position 14, F by D at position 15, F by E at position 15, F by K at position 15, F by R at position 15, Q by D at position 16, Q by E at position 16, Q by K at position 16, Q by N at position 16, Q by R at position 16, Q by S at position 16, Q by T at position 16, C by D at position 17, C by E at position 17, C by K at position 17, C by N at position 17, C by Q at position 17, C by R at position 17, C by S at position 17, C by T at position 17, L by N at position 20, L by Q at position 20, L by R at position 20, L by S at position 20, L by T at position 20, L by D at position 20, L by E at position 20, L by K at position 20, W by D at position 22, W by E at position 22, W by K at position 22, W by R at position 22, Q by D at position 23, Q by E at position 23, Q by K at position 23, Q by R at position 23, L by D at position 24, L by E at position 24, L by K at position 24, L by R at position 24, W by D at position 79, W by E at position 79, W by K at position 79, W by R at position 79, N by D at position 80, N by E at position 80, N by K at position 80, N by R at position 80, T by D at position 82, T by E at position 82, T by K at position 82, T by R at position 82, I by D at position 83, I by E at position 83, I by K at position 83, I by R at position 83, I by N at position 83, I by Q at position 83, I by S at position 83, I by T at position 83, N by D at position 86, N by E at position 86, N by K at position 86, N by R at position 86, N by Q at position 86, N by S at position 86, N by T at position 86, L by D at position 87, L by E at position 87, L by K at position 87, L by R at position 87, L by N at position 87, L by Q at position 87, L by S at position 87, L by T at position 87, A by D at position 89, A by E at position 89, A by K at position 89, A by R at position 89, N by D at position 90, N by E at position 90, N by K at position 90, N by Q at position 90, N by R at position 90, N by S at position 90, N by T at position 90, V by D at position 91, V by E at position 91, V by K at position 91, V by N at position 91, V by Q at position 91, V by R at position 91, V by S at position 91, V by T at position 91, Q by D at position 94, Q by E at position 94, Q by Q at position 94, Q by N at position 94, Q by R at position 94, Q by S at position 94, Q by T at position 94, I by D at position 95, I by E at position 95, I by K at position 95, I by N at position 95, I by Q at position 95, I by R at position 95, I by S at position 95, I by T at position 95, H by D at position 97, H by E at position 97, H by K at position 97, H by N at position 97, H by Q at position 97, H by R at position 97, H by S at position 97, H by T at position 97, L by D at position 98, L by E at position 98, L by K at position 98, L by N at position 98, L by Q at position 98, L by R at position 98, L by S at position 98, L by T at position 98, V by D at position 101, V by E at position 101, V by K at position 101, V by N at position 101, V by Q at position 101, V by R at position 101, V by S at position 101, V by T at position 101, M by C at position 1, L by C at position 6, Q by C at position 10, S by C at position 13, Q by C at position 16, L by C at position 17, V by C at position 101, L by C at position 98, H by C at position 97, Q by C at position 94, V by C at position 91, N by C at position 90.

[0540] Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 1306

<210> SEQ ID NO 1
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

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Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80
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Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 2
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: D2A Mutant IFN-alpha 2b

<400> SEQUENCE: 2

Cys Ala Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 3
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P4A Mutant IFN-alpha 2b

<400> SEQUENCE: 3

Cys Asp Leu Ala Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
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            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 4
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q5A Mutant IFN-alpha 2b

<400> SEQUENCE: 4

Cys Asp Leu Pro Ala Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 5
<211> LENGTH: 165
<212> TYPE: PRT
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: T6A Mutant IFN-alpha 2b

<400> SEQUENCE: 5

Cys Asp Leu Pro Gln Ala His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 6
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: H7A Mutant IFN-alpha 2b

<400> SEQUENCE: 6

Cys Asp Leu Pro Gln Thr Ala Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

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Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 7
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: S8A Mutant IFN-alpha 2b

<400> SEQUENCE: 7

Cys Asp Leu Pro Gln Thr His Ala Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 8
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L9A Mutant IFN-alpha 2b

<400> SEQUENCE: 8

Cys Asp Leu Pro Gln Thr His Ser Ala Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95
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```
Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
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<210> SEQ ID NO 9
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: G10A Mutant IFN-alpha 2b

<400> SEQUENCE: 9

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Cys Asp Leu Pro Gln Thr His Ser Leu Ala Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
```

<210> SEQ ID NO 10
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: S11A Mutant IFN-alpha 2b

<400> SEQUENCE: 10

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Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ala Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
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        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 11
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R12A Mutant IFN-alpha 2b

<400> SEQUENCE: 11

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Ala Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 12
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R13A Mutant IFN-alpha 2b
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<400> SEQUENCE: 12

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Ala Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 13
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: T14A Mutant IFN-alpha 2b

<400> SEQUENCE: 13

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Ala Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

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Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 14
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L15A Mutant IFN-alpha 2b

<400> SEQUENCE: 14

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Ala Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 15
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: M16A Mutant IFN-alpha 2b

<400> SEQUENCE: 15

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Ala
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

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Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 16
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L17A Mutant IFN-alpha 2b

<400> SEQUENCE: 16

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Ala Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 17
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q20A Mutant IFN-alpha 2b

<400> SEQUENCE: 17

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Ala Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
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    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 18
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R23A Mutant IFN-alpha 2b

<400> SEQUENCE: 18

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Ala Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 19
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: I24A Mutant IFN-alpha 2b

<400> SEQUENCE: 19
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Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ala Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 20
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: S25A Mutant IFN-alpha 2b

<400> SEQUENCE: 20

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ala Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
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<210> SEQ ID NO 21
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L26A Mutant IFN-alpha 2b

<400> SEQUENCE: 21

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Ala Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 22
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: S28A Mutant IFN-alpha 2b

<400> SEQUENCE: 22

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ala Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

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Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 23
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: C29A Mutant IFN-alpha 2b

<400> SEQUENCE: 23

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Ala Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 24
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L30A Mutant IFN-alpha 2b

<400> SEQUENCE: 24

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Ala Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
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65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 25
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K31A Mutant IFN-alpha 2b

<400> SEQUENCE: 25

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Ala Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 26
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: D32A Mutant IFN-alpha 2b

<400> SEQUENCE: 26

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15
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Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Ala
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 27
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R33A Mutant IFN-alpha 2b

<400> SEQUENCE: 27

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Ala His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 28
<211> LENGTH: 165
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: D35A Mutant IFN-alpha 2b

<400> SEQUENCE: 28

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Ala Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 29
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: G37A Mutant IFN-alpha 2b

<400> SEQUENCE: 29

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Ala Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

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Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 30
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P39A Mutant IFN-alpha 2b

<400> SEQUENCE: 30

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Ala Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 31
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E41A Mutant IFN-alpha 2b

<400> SEQUENCE: 31

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Ala Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
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                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 32
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E42A Mutant IFN-alpha 2b

<400> SEQUENCE: 32

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Ala Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 33
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F43A Mutant IFN-alpha 2b

<400> SEQUENCE: 33

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30
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Arg His Asp Phe Gly Phe Pro Gln Glu Glu Ala Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 34
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: N45A Mutant IFN-alpha 2b

<400> SEQUENCE: 34

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Ala Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 35
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: F47A Mutant IFN-alpha 2b

<400> SEQUENCE: 35

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Ala Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 36
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E51A Mutant IFN-alpha 2b

<400> SEQUENCE: 36

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Ala Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

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```
Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 37
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: T52A Mutant IFN-alpha 2b

<400> SEQUENCE: 37

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Ala Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 38
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: I53A Mutant IFN-alpha 2b

<400> SEQUENCE: 38

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ala Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
```

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```
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
```

<210> SEQ ID NO 39
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P54A Mutant IFN-alpha 2b

<400> SEQUENCE: 39

```
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Ala Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
```

<210> SEQ ID NO 40
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: V55A Mutant IFN-alpha 2b

<400> SEQUENCE: 40

```
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45
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```
Lys Ala Glu Thr Ile Pro Ala Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 41
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L56A Mutant IFN-alpha 2b

<400> SEQUENCE: 41

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Ala His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 42
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: H57A Mutant IFN-alpha 2b

<400> SEQUENCE: 42
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```
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu Ala Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 43
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E58A Mutant IFN-alpha 2b

<400> SEQUENCE: 43

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Ala Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
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<210> SEQ ID NO 44
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: M59A Mutant IFN-alpha 2b

<400> SEQUENCE: 44

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Ala Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 45
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: I60A Mutant IFN-alpha 2b

<400> SEQUENCE: 45

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ala Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu

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```
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 46
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: I63A Mutant IFN-alpha 2b

<400> SEQUENCE: 46

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ala Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 47
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F64A Mutant IFN-alpha 2b

<400> SEQUENCE: 47

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Ala
    50                  55                  60
```

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```
Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 48
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: N65A Mutant IFN-alpha 2b

<400> SEQUENCE: 48

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Ala Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 49
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L66A Mutant IFN-alpha 2b

<400> SEQUENCE: 49

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15
```

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```
Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Ala Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 50
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F67A Mutant IFN-alpha 2b

<400> SEQUENCE: 50

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Ala Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 51
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<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: T69A Mutant IFN-alpha 2b

<400> SEQUENCE: 51

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Ala Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 52
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K70A Mutant IFN-alpha 2b

<400> SEQUENCE: 52

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Ala Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg

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    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 53
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: D71A Mutant IFN-alpha 2b

<400> SEQUENCE: 53

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Ala Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 54
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: S72A Mutant IFN-alpha 2b

<400> SEQUENCE: 54

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ala Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80
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Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 55
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: W76A Mutant IFN-alpha 2b

<400> SEQUENCE: 55

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Ala Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 56
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: D77A Mutant IFN-alpha 2b

<400> SEQUENCE: 56

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30
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Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Ala Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 57
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E78A Mutant IFN-alpha 2b

<400> SEQUENCE: 57

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Ala Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 58
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: L81A Mutant IFN-alpha 2b

<400> SEQUENCE: 58

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Ala Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 59
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: D82A Mutant IFN-alpha 2b

<400> SEQUENCE: 59

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Ala Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser

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145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 60
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K83A Mutant IFN-alpha 2b

<400> SEQUENCE: 60

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Ala Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 61
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F84A Mutant IFN-alpha 2b

<400> SEQUENCE: 61

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Ala Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

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Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 62
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Y85A Mutant IFN-alpha 2b

<400> SEQUENCE: 62

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Ala Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 63
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Y89A Mutant IFN-alpha 2b

<400> SEQUENCE: 63

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45
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Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Ala Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 64
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q90A Mutant IFN-alpha 2b

<400> SEQUENCE: 64

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Ala Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 65
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q91A Mutant IFN-alpha 2b
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<400> SEQUENCE: 65

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Ala Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 66
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: N93A Mutant IFN-alpha 2b

<400> SEQUENCE: 66

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Ala Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu

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165

<210> SEQ ID NO 67
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: D94A Mutant IFN-alpha 2b

<400> SEQUENCE: 67

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Ala Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 68
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: C98A Mutant IFN-alpha 2b

<400> SEQUENCE: 68

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Ala Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

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Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 69
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: V99A Mutant IFN-alpha 2b

<400> SEQUENCE: 69

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Ala Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 70
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: G104A Mutant IFN-alpha 2b

<400> SEQUENCE: 70

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60
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Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Ala Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 71
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L110A Mutant IFN-alpha 2b

<400> SEQUENCE: 71

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Ala Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 72
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: S115A Mutant IFN-alpha 2b

<400> SEQUENCE: 72

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
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 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ala Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 73
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Y122A Mutant IFN-alpha 2b

<400> SEQUENCE: 73

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Ala Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
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<210> SEQ ID NO 74
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: W140A Mutant IFN-alpha 2b

<400> SEQUENCE: 74

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Ala Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 75
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E146A Mutant IFN-alpha 2b

<400> SEQUENCE: 75

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

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Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Ala Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 76
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L3V Mutant IFN-alpha 2b

<400> SEQUENCE: 76

Cys Asp Val Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 77
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R12H Mutant IFN-alpha 2b

<400> SEQUENCE: 77

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser His Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80
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Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 78
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R13H Mutant IFN-alpha 2b

<400> SEQUENCE: 78

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg His Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 79
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: M16V Mutant IFN-alpha 2b

<400> SEQUENCE: 79

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Val
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp

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            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 80
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: M16I Mutant IFN-alpha 2b

<400> SEQUENCE: 80

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Ile
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 81
<211> LENGTH: 165
<212> TYPE: PRT
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R22H Mutant IFN-alpha 2b

<400> SEQUENCE: 81

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met His Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 82
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F27I Mutant IFN-alpha 2b

<400> SEQUENCE: 82

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Ile Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

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Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 83
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F27V Mutant IFN-alpha 2b

<400> SEQUENCE: 83

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Val Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 84
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L30I Mutant IFN-alpha 2b

<400> SEQUENCE: 84

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Ile Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95
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Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 85
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K31Q Mutant IFN-alpha 2b

<400> SEQUENCE: 85

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Gln Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 86
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R33H Mutant IFN-alpha 2b

<400> SEQUENCE: 86

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

His His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
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        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 87
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E41Q Mutant IFN-alpha 2b

<400> SEQUENCE: 87

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Gln Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 88
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E41H Mutant IFN-alpha 2b
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<400> SEQUENCE: 88

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln His Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 89
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E58Q Mutant IFN-alpha 2b

<400> SEQUENCE: 89

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Gln Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

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Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 90
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E58H Mutant IFN-alpha 2b

<400> SEQUENCE: 90

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His His Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 91
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K70T Mutant IFN-alpha 2b

<400> SEQUENCE: 91

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Thr Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

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Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 92
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E78Q Mutant IFN-alpha 2b

<400> SEQUENCE: 92

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Gln Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 93
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E78H Mutant IFN-alpha 2b

<400> SEQUENCE: 93

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
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    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp His Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 94
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Y89I Mutant IFN-alpha 2b

<400> SEQUENCE: 94

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Ile Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 95
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E107Q Mutant IFN-alpha 2b

<400> SEQUENCE: 95
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Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Gln Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 96
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E107H Mutant IFN-alpha 2b

<400> SEQUENCE: 96

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr His Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
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<210> SEQ ID NO 97
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P109A Mutant IFN-alpha 2b

<400> SEQUENCE: 97

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Ala Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 98
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L110V Mutant IFN-alpha 2b

<400> SEQUENCE: 98

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Val Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

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Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 99
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: M111I Mutant IFN-alpha 2b

<400> SEQUENCE: 99

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Ile Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 100
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E113Q Mutant IFN-alpha 2b

<400> SEQUENCE: 100

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu

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65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Gln Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 101
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E113H Mutant IFN-alpha 2b

<400> SEQUENCE: 101

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

His Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 102
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L117V Mutant IFN-alpha 2b

<400> SEQUENCE: 102

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15
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Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Val Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 103
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L117I Mutant IFN-alpha 2b

<400> SEQUENCE: 103

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Ile Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 104
<211> LENGTH: 165
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K121Q Mutant IFN-alpha 2b

<400> SEQUENCE: 104

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Gln Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 105
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K121T Mutant IFN-alpha 2b

<400> SEQUENCE: 105

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Thr Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

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Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 106
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R125H Mutant IFN-alpha 2b

<400> SEQUENCE: 106

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln His Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 107
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R125Q Mutant IFN-alpha 2b

<400> SEQUENCE: 107

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
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                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Gln Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 108
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L128V Mutant IFN-alpha 2b

<400> SEQUENCE: 108

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Val
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 109
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L128I Mutant IFN-alpha 2b

<400> SEQUENCE: 109

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30
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```
Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Ile
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 110
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K131Q Mutant IFN-alpha 2b

<400> SEQUENCE: 110

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Gln Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 111
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<223> OTHER INFORMATION: K131T Mutant IFN-alpha 2b

<400> SEQUENCE: 111

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 112
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E132Q Mutant IFN-alpha 2b

<400> SEQUENCE: 112

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Gln Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

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Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 113
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E132H Mutant IFN-alpha 2b

<400> SEQUENCE: 113

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys His Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 114
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K133Q Mutant IFN-alpha 2b

<400> SEQUENCE: 114

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys

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            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Gln Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 115
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K133T Mutant IFN-alpha 2b

<400> SEQUENCE: 115

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Thr Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 116
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K134Q Mutant IFN-alpha 2b

<400> SEQUENCE: 116

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45
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Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Gln Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 117
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Y135H Mutant IFN-alpha 2b

<400> SEQUENCE: 117

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys His Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 118
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Y135I Mutant IFN-alpha 2b

<400> SEQUENCE: 118
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Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Ile Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 119
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P137A Mutant IFN-alpha 2b

<400> SEQUENCE: 119

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Ala Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
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<210> SEQ ID NO 120
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: M148V Mutant IFN-alpha 2b

<400> SEQUENCE: 120

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Val Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 121
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: M148I Mutant IFN-alpha 2b

<400> SEQUENCE: 121

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu

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        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Ile Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 122
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R149H Mutant IFN-alpha 2b

<400> SEQUENCE: 122

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met His Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 123
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R149Q Mutant IFN-alpha 2b

<400> SEQUENCE: 123

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60
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Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Gln Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 124
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E159Q Mutant IFN-alpha 2b

<400> SEQUENCE: 124

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Gln Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 125
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E159H Mutant IFN-alpha 2b

<400> SEQUENCE: 125

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15
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Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln His Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 126
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P4S Mutant IFN-alpha 2b

<400> SEQUENCE: 126

Cys Asp Leu Ser Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 127

-continued

<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q5N/H7S Mutant IFN-alpha 2b

<400> SEQUENCE: 127

Cys Asp Leu Pro Asn Thr Ser Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 128
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q5N/H7T Mutant IFN-alpha 2b

<400> SEQUENCE: 128

Cys Asp Leu Pro Asn Thr Thr Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg

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    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 129
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: T6N/S8S Mutant IFN-alpha 2b

<400> SEQUENCE: 129

Cys Asp Leu Pro Gln Asn His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 130
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: S8N/G10S Mutant IFN-alpha 2b

<400> SEQUENCE: 130

Cys Asp Leu Pro Gln Thr His Asn Leu Ser Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80
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```
Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 131
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: S8N/G10T Mutant IFN-alpha 2b

<400> SEQUENCE: 131

Cys Asp Leu Pro Gln Thr His Asn Leu Thr Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 132
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: M21N/R23S Mutant IFN-alpha 2b

<400> SEQUENCE: 132

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Asn Arg Ser Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30
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Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 133
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R23N/S25T Mutant IFN-alpha 2b

<400> SEQUENCE: 133

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Asn Ile Thr Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 134
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: I24N/L26S Mutant IFN-alpha 2b

<400> SEQUENCE: 134

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Asn Ser Ser Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 135
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: S25N/F27S Mutant IFN-alpha 2b

<400> SEQUENCE: 135

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Asn Leu Ser Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser

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145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 136
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: S25N/F27T Mutant IFN-alpha 2b

<400> SEQUENCE: 136

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Asn Leu Thr Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 137
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L26N/S28S Mutant IFN-alpha 2b

<400> SEQUENCE: 137

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Asn Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

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Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 138
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L26N/S28T Mutant IFN-alpha 2b

<400> SEQUENCE: 138

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Asn Phe Thr Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 139
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L30N/D32S Mutant IFN-alpha 2b

<400> SEQUENCE: 139

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Asn Lys Ser
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45
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```
Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 140
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R33N/D35S Mutant IFN-alpha 2b

<400> SEQUENCE: 140

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Asn His Ser Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 141
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R33N/D35T Mutant IFN-alpha 2b
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<400> SEQUENCE: 141

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Asn His Thr Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 142
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: H34N/F36S Mutant IFN-alpha 2b

<400> SEQUENCE: 142

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg Asn Asp Ser Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu

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165

<210> SEQ ID NO 143
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: H34N/F36T Mutant IFN-alpha 2b

<400> SEQUENCE: 143

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg Asn Asp Thr Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 144
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: D35N/G37S Mutant IFN-alpha 2b

<400> SEQUENCE: 144

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asn Phe Ser Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

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```
Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
```

<210> SEQ ID NO 145
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F36N/F38S Mutant IFN-alpha 2b

<400> SEQUENCE: 145

```
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Asn Gly Ser Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
```

<210> SEQ ID NO 146
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F36N/F38T Mutant IFN-alpha 2b

<400> SEQUENCE: 146

```
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Asn Gly Thr Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60
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```
Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 147
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: G37N/P39T Mutant IFN-alpha 2b

<400> SEQUENCE: 147

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Asn Phe Thr Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 148
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F38N/Q40S Mutant IFN-alpha 2b

<400> SEQUENCE: 148

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
```

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```
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Asn Pro Ser Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 149
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F38N/Q40T Mutant IFN-alpha 2b

<400> SEQUENCE: 149

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Asn Pro Thr Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
```

-continued

<210> SEQ ID NO 150
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P39N/E41S Mutant IFN-alpha 2b

<400> SEQUENCE: 150

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Asn Gln Ser Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 151
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: P39N/E41T Mutant IFN-alpha 2b

<400> SEQUENCE: 151

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Asn Gln Thr Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

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```
Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 152
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q40N/E42S Mutant IFN-alpha 2b

<400> SEQUENCE: 152

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Asn Glu Ser Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 153
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q40N/E42T Mutant IFN-alpha 2b

<400> SEQUENCE: 153

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Asn Glu Thr Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80
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Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 154
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E41N/F43S Mutant IFN-alpha 2b

<400> SEQUENCE: 154

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Asn Glu Ser Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 155
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E41N/F43T Mutant IFN-alpha 2b

<400> SEQUENCE: 155

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
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            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Asn Glu Thr Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 156
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: G44N/Q46S Mutant IFN-alpha 2b

<400> SEQUENCE: 156

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asn Asn Ser Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 157
<211> LENGTH: 165
<212> TYPE: PRT
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: G44N/Q46T Mutant IFN-alpha 2b

<400> SEQUENCE: 157

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asn Asn Thr Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 158
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: N45N/F47S Mutant IFN-alpha 2b

<400> SEQUENCE: 158

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Ser Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

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Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 159
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: N45N/F47T Mutant IFN-alpha 2b

<400> SEQUENCE: 159

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Thr Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 160
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q46N/Q48S Mutant IFN-alpha 2b

<400> SEQUENCE: 160

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Asn Phe Ser
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95
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Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 161
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q46N/Q48T Mutant IFN-alpha 2b

<400> SEQUENCE: 161

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Asn Phe Thr
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 162
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F47N/K49S Mutant IFN-alpha 2b

<400> SEQUENCE: 162

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Asn Gln

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        35                  40                  45

Ser Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 163
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: F47N/K49T Mutant IFN-alpha 2b

<400> SEQUENCE: 163

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Asn Gln
        35                  40                  45

Thr Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 164
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K49N/E51S Mutant IFN-alpha 2b
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<400> SEQUENCE: 164

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Asn Ala Ser Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 165
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: A75N/D77S Mutant IFN-alpha 2b

<400> SEQUENCE: 165

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Asn Trp Ser Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

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Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 166
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: I100N/G102S Mutant IFN-alpha 2b

<400> SEQUENCE: 166

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Asn Gln Ser Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145 150 155 160

Leu Arg Ser Lys Glu
165

<210> SEQ ID NO 167
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: I100N/G102T Mutant IFN-alpha 2b

<400> SEQUENCE: 167

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
35 40 45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65 70 75 80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Asn Gln Thr Val Gly Val Thr Glu Thr Pro Leu Met Lys
100 105 110

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Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 168
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: V103N/V105S Mutant IFN-alpha 2b

<400> SEQUENCE: 168

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Asn Gly Ser Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 169
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: V103N/V105T Mutant IFN-alpha 2b

<400> SEQUENCE: 169

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
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    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Asn Gly Thr Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 170
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: G104N/T106T Mutant IFN-alpha 2b

<400> SEQUENCE: 170

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Asn Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 171
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: V105N/E107S Mutant IFN-alpha 2b

<400> SEQUENCE: 171
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Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Asn Thr Ser Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 172
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: T106N/T108S Mutant IFN-alpha 2b

<400> SEQUENCE: 172

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Asn Glu Ser Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
```

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<210> SEQ ID NO 173
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: T106N/T108T Mutant IFN-alpha 2b

<400> SEQUENCE: 173

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Asn Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145   150   155   160

Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 174
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E107N/P109S Mutant IFN-alpha 2b

<400> SEQUENCE: 174

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Asn Thr Ser Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

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```
Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
```

<210> SEQ ID NO 175
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E107N/P109T Mutant IFN-alpha 2b

<400> SEQUENCE: 175

```
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Asn Thr Thr Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165
```

<210> SEQ ID NO 176
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: K134N/S136T Mutant IFN-alpha 2b

<400> SEQUENCE: 176

```
Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
```

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```
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Asn Tyr Thr Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 177
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L157N/E159S Mutant IFN-alpha 2b

<400> SEQUENCE: 177

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Asn Gln Ser Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 178
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: L157N/E159T Mutant IFN-alpha 2b

<400> SEQUENCE: 178

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15
```

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```
Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Asn Gln Thr Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 179
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Q158N/S160T Mutant IFN-alpha 2b

<400> SEQUENCE: 179

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Asn Glu Thr
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 180
<211> LENGTH: 165
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E159N/L161S Mutant IFN-alpha 2b

<400> SEQUENCE: 180

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Asn Ser
145   150   155   160

Ser Arg Ser Lys Glu
                165

<210> SEQ ID NO 181
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E159N/L161T Mutant IFN-alpha 2b

<400> SEQUENCE: 181

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35   40   45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50   55   60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65   70   75   80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85   90   95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100   105   110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115   120   125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130   135   140

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```
Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Asn Ser
145                 150                 155                 160

Thr Arg Ser Lys Glu
                165


<210> SEQ ID NO 182
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA23805
<309> DATABASE ENTRY DATE: 1994-12-17

<400> SEQUENCE: 182

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met

 1               5                  10                  15
Leu Leu Ala Gln Met Arg Lys Ile Ser Leu Phe Ser Cys Leu Lys Asp

            20                  25                  30
Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln

        35                  40                  45
Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe

    50                  55                  60
Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu

65                  70                  75                  80
Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu

                85                  90                  95
Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys

            100                 105                 110
Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu

        115                 120                 125
Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg

    130                 135                 140
Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser

145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 183
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank P01566
<309> DATABASE ENTRY DATE: 1986-07-21

<400> SEQUENCE: 183

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile

 1               5                  10                  15
Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp

            20                  25                  30
Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe

        35                  40                  45
Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr

    50                  55                  60
Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln Ser
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65                  70                  75                  80
Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu

                85                  90                  95
Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met

            100                 105                 110
Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr

        115                 120                 125
Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp Glu Val Val

    130                 135                 140
Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln Lys

145                 150                 155                 160

Arg Leu Arg Arg Lys Asp
                165


<210> SEQ ID NO 184
<211> LENGTH: 174
<212> TYPE: PRT
<213> ORGANISM: Marmota monax
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAL76913
<309> DATABASE ENTRY DATE: 2002-02-12

<400> SEQUENCE: 184

Cys Asp Leu Pro Gln Ile His Asn Leu Gly Leu Glu Thr Ser Glu Glu
 1               5                  10                  15

Asn Glu Glu Gly Ala Leu Thr Leu Leu Glu Lys Met Arg Arg Ile Pro
            20                  25                  30

Ile Phe Ser Cys Leu Asn Tyr Arg Lys Asp Phe Ala Phe Pro Gln Glu
        35                  40                  45

Gln Leu Glu Gly Glu Gln Val Gln Lys Ala Gln Ala Val Ala Val Leu
    50                  55                  60

His Gln Met Thr Gln Gln Ile Leu Asn Leu Phe Ser Thr Gln Lys Ala
65                  70                  75                  80

Phe Ala Ala Trp Asp Lys Thr Leu Leu Asp Thr Phe Leu Ser Gly Leu
                85                  90                  95

Tyr Gln Leu Leu Asp Asp Leu Lys Ala Cys Gly Ser Lys Gln Val Gly
            100                 105                 110

Val Glu Glu Ala Val Arg Lys Tyr Phe His Arg Ile Thr Val Tyr Leu
        115                 120                 125

Lys Glu Lys Lys Tyr Leu Pro Cys Ala Trp Glu Val Val Arg Thr Glu
    130                 135                 140

Ile Met Lys Ser Phe Ser Leu Ser Val Asn Leu Tyr Glu Arg Leu Arg
145                 150                 155                 160

Ser Met Glu Gly Asp Leu Val Gln Gln Gly Asn Ala Ser His
                165                 170


<210> SEQ ID NO 185
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 185

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met
 1               5                  10                  15
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```
Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg Arg Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln
        35                  40                  45

Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile Phe
    50                  55                  60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu
65                  70                  75                  80

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
                85                  90                  95

Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys
            100                 105                 110

Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
        115                 120                 125

Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
    130                 135                 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser
145                 150                 155                 160

Leu Arg Ser Lys Glu
                165


<210> SEQ ID NO 186
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAB59403
<309> DATABASE ENTRY DATE: 1994-11-15

<400> SEQUENCE: 186

Cys Asp Leu Pro Glu Thr His Ser Leu Asp Asn Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Leu Ala Gln Met Ser Arg Ile Ser Pro Ser Ser Cys Leu Met Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
        35                  40                  45

Gln Lys Ala Pro Ala Ile Ser Val Leu His Glu Leu Ile Gln Gln Ile
    50                  55                  60

Phe Asn Leu Phe Thr Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Asp
65                  70                  75                  80

Leu Leu Asp Lys Phe Cys Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
                85                  90                  95

Glu Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met
            100                 105                 110

Asn Ala Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr
        115                 120                 125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
    130                 135                 140

Arg Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu
145                 150                 155                 160

Arg Leu Arg Arg Lys Glu
                165


<210> SEQ ID NO 187
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<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA26702
<309> DATABASE ENTRY DATE: 1995-03-30

<400> SEQUENCE: 187

Cys Asp Leu Pro Gln Thr His Ser Leu Ser Asn Arg Arg Thr Leu Met
 1   5   10   15

Ile Met Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
        35   40   45

Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
    50   55   60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Asp Glu Thr
65   70   75   80

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
                85   90   95

Glu Ala Cys Met Met Gln Glu Val Gly Val Glu Asp Thr Pro Leu Met
            100   105   110

Asn Val Asp Ser Ile Leu Thr Val Arg Lys Tyr Phe Gln Arg Ile Thr
        115   120   125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
    130   135   140

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ala Asn Leu Gln Glu
145   150   155   160

Arg Leu Arg Arg Lys Glu
                165

<210> SEQ ID NO 188
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA26704
<309> DATABASE ENTRY DATE: 1995-03-30

<400> SEQUENCE: 188

Cys Asp Leu Pro Gln Thr His Ser Leu Gly His Arg Arg Thr Met Met
 1   5   10   15

Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp
            20   25   30

Arg His Asp Phe Arg Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
        35   40   45

Gln Lys Ala Glu Ala Ile Ser Val Leu His Glu Val Ile Gln Gln Thr
    50   55   60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Val Ala Trp Asp Glu Arg
65   70   75   80

Leu Leu Asp Lys Leu Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
                85   90   95

Glu Ala Cys Val Met Gln Glu Val Trp Val Gly Gly Thr Pro Leu Met
            100   105   110

Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
        115   120   125

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```
Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
    130                 135                 140

Arg Ala Glu Ile Met Arg Ser Phe Ser Ser Ser Arg Asn Leu Gln Glu
145                 150                 155                 160

Arg Leu Arg Arg Lys Glu
                165


<210> SEQ ID NO 189
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank NP_066546
<309> DATABASE ENTRY DATE: 2000-11-02

<400> SEQUENCE: 189

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile
 1               5                  10                  15

Leu Leu Ala Gln Met Gly Arg Ile Ser His Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Glu Glu Glu Phe Asp Gly His Gln Phe
        35                  40                  45

Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
    50                  55                  60

Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln Ser
65                  70                  75                  80

Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
                85                  90                  95

Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met
            100                 105                 110

Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
        115                 120                 125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
    130                 135                 140

Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln Lys
145                 150                 155                 160

Arg Leu Arg Arg Lys Asp
                165


<210> SEQ ID NO 190
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA26701
<309> DATABASE ENTRY DATE: 1995-03-30

<400> SEQUENCE: 190

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile
 1               5                  10                  15

Leu Leu Ala Gln Met Gly Arg Ile Ser His Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Phe Pro Glu Glu Glu Phe Asp Gly His Gln Phe
        35                  40                  45

Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
    50                  55                  60
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```
Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln Ser
65                  70                  75                  80

Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
                85                  90                  95

Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met
            100                 105                 110

Asn Val Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
        115                 120                 125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
    130                 135                 140

Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln Lys
145                 150                 155                 160

Arg Leu Arg Arg Lys Asp
                165


<210> SEQ ID NO 191
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAA52725
<309> DATABASE ENTRY DATE: 1994-11-08

<400> SEQUENCE: 191

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile
 1               5                  10                  15

Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Gly Leu Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
        35                  40                  45

Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
    50                  55                  60

Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln Ser
65                  70                  75                  80

Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asn Leu
                85                  90                  95

Glu Ala Cys Val Ile Gln Glu Val Gly Met Glu Glu Thr Pro Leu Met
            100                 105                 110

Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
        115                 120                 125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
    130                 135                 140

Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln Lys
145                 150                 155                 160

Ile Leu Arg Arg Lys Asp
                165


<210> SEQ ID NO 192
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA23792
<309> DATABASE ENTRY DATE: 1994-08-04

<400> SEQUENCE: 192
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Cys Asp Leu Pro Gln Thr His Ser Leu Arg Asn Arg Arg Ala Leu Ile
 1               5                  10                  15

Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Glu Phe Arg Phe Pro Glu Glu Glu Phe Asp Gly His Gln Phe
        35                  40                  45

Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
    50                  55                  60

Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln Ser
65                  70                  75                  80

Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
                85                  90                  95

Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met
            100                 105                 110

Asn Glu Asp Phe Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
        115                 120                 125

Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
    130                 135                 140

Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser Thr Asn Leu Lys Lys
145                 150                 155                 160

Gly Leu Arg Arg Lys Asp
                165


<210> SEQ ID NO 193
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA23794
<309> DATABASE ENTRY DATE: 1994-12-17

<400> SEQUENCE: 193

Cys Asn Leu Ser Gln Thr His Ser Leu Asn Asn Arg Arg Thr Leu Met
 1               5                  10                  15

Leu Met Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp
            20                  25                  30

Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
        35                  40                  45

Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Met Gln Gln Thr
    50                  55                  60

Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser Ala Ala Trp Asp Glu Thr
65                  70                  75                  80

Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe Gln Gln Met Asn Asp Leu
                85                  90                  95

Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met
            100                 105                 110

Asn Glu Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile Thr
        115                 120                 125

Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
    130                 135                 140

Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln Lys
145                 150                 155                 160

Arg Leu Arg Arg Lys Asp
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165

<210> SEQ ID NO 194
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAA52718
<309> DATABASE ENTRY DATE: 1994-11-08

<400> SEQUENCE: 194

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile
1 5 10 15

Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
35 40 45

Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
50 55 60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Glu Gln Ser
65 70 75 80

Leu Leu Glu Lys Phe Ser Thr Glu Leu Asn Gln Gln Leu Asn Asp Leu
85 90 95

Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met
100 105 110

Asn Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile Thr
115 120 125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
130 135 140

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Lys Ile Phe Gln Glu
145 150 155 160

Arg Leu Arg Arg Lys Glu
165

<210> SEQ ID NO 195
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA26903
<309> DATABASE ENTRY DATE: 1995-03-30

<400> SEQUENCE: 195

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Asp Lys Gln Phe
35 40 45

Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr
50 55 60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Leu Asp Glu Thr
65 70 75 80

Leu Leu Asp Glu Phe Tyr Ile Glu Leu Asp Gln Gln Leu Asn Asp Leu
85 90 95

Glu Ser Cys Val Met Gln Glu Val Gly Val Ile Glu Ser Pro Leu Met

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            100                 105                 110

Tyr Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
        115                 120                 125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Ser Cys Ala Trp Glu Val Val
    130                 135                 140

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ile Asn Leu Gln Lys
145                 150                 155                 160

Arg Leu Lys Ser Lys Glu
                165
```

<210> SEQ ID NO 196
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAC41702
<309> DATABASE ENTRY DATE: 1995-01-01

<400> SEQUENCE: 196

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 197
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA23795
<309> DATABASE ENTRY DATE: 1993-09-12

<400> SEQUENCE: 197

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
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        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 198
<211> LENGTH: 183
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA00839
<309> DATABASE ENTRY DATE: 1993-12-03

<400> SEQUENCE: 198

Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His Arg Gln
 1               5                  10                  15

Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr Ile Leu
            20                  25                  30

Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser Asn Met
        35                  40                  45

Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro
    50                  55                  60

Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu
65                  70                  75                  80

Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr
                85                  90                  95

Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg
            100                 105                 110

Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys
        115                 120                 125

Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala
    130                 135                 140

Ser Leu Leu Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp Met
145                 150                 155                 160

Thr Thr His Leu Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser Ser
                165                 170                 175

Leu Arg Ala Leu Arg Gln Met
            180


<210> SEQ ID NO 199
<211> LENGTH: 146
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA31639
<309> DATABASE ENTRY DATE: 1994-11-15

<400> SEQUENCE: 199

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln
145

<210> SEQ ID NO 200
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank NP_000563
<309> DATABASE ENTRY DATE: 2000-10-31

<400> SEQUENCE: 200

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1   5   10   15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20   25   30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35   40   45

Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50   55   60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65   70   75   80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85   90   95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100   105   110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115   120   125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130   135   140

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Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
145                 150                 155                 160


<210> SEQ ID NO 201
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAA52400
<309> DATABASE ENTRY DATE: 1994-11-08

<400> SEQUENCE: 201

Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu
 1               5                  10                  15

Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His
            20                  25                  30

Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe
        35                  40                  45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp
    50                  55                  60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu
65                  70                  75                  80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp
                85                  90                  95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu
            100                 105                 110

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
        115                 120                 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val
    130                 135                 140

Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala
145                 150                 155                 160

Cys Arg Thr Gly Asp Arg
                165


<210> SEQ ID NO 202
<211> LENGTH: 127
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAA98768
<309> DATABASE ENTRY DATE: 1996-05-02

<400> SEQUENCE: 202

Ala Pro Ala Arg Ser Pro Ser Pro Ser Thr Gln Pro Trp Glu His Val
 1               5                  10                  15

Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser Arg Asp Thr
            20                  25                  30

Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu Met Phe Asp
        35                  40                  45

Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys Gln
    50                  55                  60

Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu Thr Met Met
65                  70                  75                  80

Ala Ser His Tyr Lys Gln His Cys Pro Pro Thr Pro Glu Thr Ser Cys
                85                  90                  95
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Ala Thr Gln Ile Ile Thr Phe Glu Ser Phe Lys Glu Asn Leu Lys Asp
            100                 105                 110

Phe Leu Leu Val Ile Pro Phe Asp Cys Trp Glu Pro Val Gln Glu
        115                 120                 125


<210> SEQ ID NO 203
<211> LENGTH: 209
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAA19825
<309> DATABASE ENTRY DATE: 1994-07-19

<400> SEQUENCE: 203

Thr Gln Asp Cys Ser Phe Gln His Ser Pro Ile Ser Ser Asp Phe Ala
 1               5                   10                  15

Val Lys Ile Arg Glu Leu Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val
            20                  25                  30

Thr Val Ala Ser Asn Leu Gln Asp Glu Glu Leu Cys Gly Gly Leu Trp
        35                  40                  45

Arg Leu Val Leu Ala Gln Arg Trp Met Glu Arg Leu Lys Thr Val Ala
    50                  55                  60

Gly Ser Lys Met Gln Gly Leu Leu Glu Arg Val Asn Thr Glu Ile His
65                  70                  75                  80

Phe Val Thr Lys Cys Ala Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe
                85                  90                  95

Val Gln Thr Asn Ile Ser Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu
            100                 105                 110

Val Ala Leu Lys Pro Trp Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu
        115                 120                 125

Glu Leu Gln Cys Gln Pro Asp Ser Ser Thr Leu Pro Pro Pro Trp Ser
    130                 135                 140

Pro Arg Pro Leu Glu Ala Thr Ala Pro Thr Ala Pro Gln Pro Pro Leu
145                 150                 155                 160

Leu Leu Leu Leu Leu Leu Pro Val Gly Leu Leu Leu Leu Ala Ala Ala
                165                 170                 175

Trp Cys Leu His Trp Gln Arg Thr Arg Arg Arg Thr Pro Arg Pro Gly
            180                 185                 190

Glu Gln Val Pro Pro Val Pro Ser Pro Gln Asp Leu Leu Leu Val Glu
        195                 200                 205

His


<210> SEQ ID NO 204
<211> LENGTH: 133
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAD48509
<309> DATABASE ENTRY DATE: 1999-08-11

<400> SEQUENCE: 204

Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
 1               5                   10                  15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
            20                  25                  30

Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys
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        35                  40                  45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
    50                  55                  60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
65                  70                  75                  80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
                85                  90                  95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
            100                 105                 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
        115                 120                 125

Ile Ser Thr Leu Thr
    130


<210> SEQ ID NO 205
<211> LENGTH: 133
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAA59146
<309> DATABASE ENTRY DATE: 1995-01-06

<400> SEQUENCE: 205

Ala Pro Met Thr Gln Thr Thr Pro Leu Lys Thr Ser Trp Val Asn Cys
 1               5                  10                  15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu
            20                  25                  30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu
        35                  40                  45

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
    50                  55                  60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn
65                  70                  75                  80

Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro
                85                  90                  95

Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr
            100                 105                 110

Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu
        115                 120                 125

Ser Leu Ala Ile Phe
    130


<210> SEQ ID NO 206
<211> LENGTH: 248
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAA85450
<309> DATABASE ENTRY DATE: 1996-01-19

<400> SEQUENCE: 206

Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val Thr
 1               5                  10                  15

Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys Tyr
            20                  25                  30

Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu Met
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        35                  40                  45

Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe Ser
    50                  55                  60

Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu Val
65                  70                  75                  80

Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser Lys
                85                  90                  95

Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr Pro
            100                 105                 110

Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys Asp
        115                 120                 125

Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr Leu
    130                 135                 140

Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met Leu
145                 150                 155                 160

Pro Pro Val Ala Ala Ser Ser Leu Arg Asn Asp Ser Ser Ser Ser Asn
                165                 170                 175

Arg Lys Ala Lys Asn Pro Pro Gly Asp Ser Ser Leu His Trp Ala Ala
            180                 185                 190

Met Ala Leu Pro Ala Leu Phe Ser Leu Ile Ile Gly Phe Ala Phe Gly
        195                 200                 205

Ala Leu Tyr Trp Lys Lys Arg Gln Pro Ser Leu Thr Arg Ala Val Glu
    210                 215                 220

Asn Ile Gln Ile Asn Glu Glu Asp Asn Glu Ile Ser Met Leu Gln Glu
225                 230                 235                 240

Lys Glu Arg Glu Phe Gln Glu Val
                245


<210> SEQ ID NO 207
<211> LENGTH: 129
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAA59149
<309> DATABASE ENTRY DATE: 1995-01-06

<400> SEQUENCE: 207

His Lys Cys Asp Ile Thr Leu Gln Glu Ile Ile Lys Thr Leu Asn Ser
 1               5                  10                  15

Leu Thr Glu Gln Lys Thr Leu Cys Thr Glu Leu Thr Val Thr Asp Ile
            20                  25                  30

Phe Ala Ala Ser Lys Asn Thr Thr Glu Lys Glu Thr Phe Cys Arg Ala
        35                  40                  45

Ala Thr Val Leu Arg Gln Phe Tyr Ser His His Glu Lys Asp Thr Arg
    50                  55                  60

Cys Leu Gly Ala Thr Ala Gln Gln Phe His Arg His Lys Gln Leu Ile
65                  70                  75                  80

Arg Phe Leu Lys Arg Leu Asp Arg Asn Leu Trp Gly Leu Ala Gly Leu
                85                  90                  95

Asn Ser Cys Pro Val Lys Glu Ala Asn Gln Ser Thr Leu Glu Asn Phe
            100                 105                 110

Leu Glu Arg Leu Lys Thr Ile Met Arg Glu Lys Tyr Ser Lys Cys Ser
        115                 120                 125
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Ser

<210> SEQ ID NO 208
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA28390
<309> DATABASE ENTRY DATE: 1995-03-21

<400> SEQUENCE: 208

Ile Pro Thr Glu Ile Pro Thr Ser Ala Leu Val Lys Glu Thr Leu Ala
 1   5   10   15

Leu Leu Ser Thr His Arg Thr Leu Leu Ile Ala Asn Glu Thr Leu Arg
            20   25   30

Ile Pro Val Pro Val His Lys Asn His Gln Leu Cys Thr Glu Glu Ile
        35   40   45

Phe Gln Gly Ile Gly Thr Leu Glu Ser Gln Thr Val Gln Gly Gly Thr
    50   55   60

Val Glu Arg Leu Phe Lys Asn Leu Ser Leu Ile Lys Lys Tyr Ile Asp
65   70   75   80

Gly Gln Lys Lys Lys Cys Gly Glu Glu Arg Arg Arg Val Asn Gln Phe
                85   90   95

Leu Asp Tyr Leu Gln Glu Phe Leu Gly Val Met Asn Thr Glu Trp Ile
            100   105   110

Ile Glu Ser
        115

<210> SEQ ID NO 209
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank NP_002179
<309> DATABASE ENTRY DATE: 2000-10-31

<400> SEQUENCE: 209

Gly Pro Val Pro Pro Ser Thr Ala Leu Arg Glu Leu Ile Glu Glu Leu
 1   5   10   15

Val Asn Ile Thr Gln Asn Gln Lys Ala Pro Leu Cys Asn Gly Ser Met
            20   25   30

Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala Leu Glu
        35   40   45

Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr Gln Arg
    50   55   60

Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln Phe Ser
65   70   75   80

Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe Val Lys
                85   90   95

Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly Arg Phe Asn
            100   105   110

<210> SEQ ID NO 210
<211> LENGTH: 177
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA27168

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<309> DATABASE ENTRY DATE: 1995-03-21

<400> SEQUENCE: 210

```
Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1               5                  10                  15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
            20                  25                  30

Glu Lys Leu Val Ser Glu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu
        35                  40                  45

Glu Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu
    50                  55                  60

Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln
65                  70                  75                  80

Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu
                85                  90                  95

Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp
            100                 105                 110

Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly
        115                 120                 125

Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala
    130                 135                 140

Ser Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu
145                 150                 155                 160

Gln Ser Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln
                165                 170                 175

Pro
```

<210> SEQ ID NO 211
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAA60470
<309> DATABASE ENTRY DATE: 1995-01-13

<400> SEQUENCE: 211

```
Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
 1               5                  10                  15

Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Gln Ser Val Ser Ser
            20                  25                  30

Lys Gln Lys Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro Ile
        35                  40                  45

Leu Thr Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile
    50                  55                  60

Leu Thr Ser Met Pro Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu
65                  70                  75                  80

Glu Asn Leu Arg Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys
                85                  90                  95

His Leu Pro Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly
            100                 105                 110

Val Leu Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg
        115                 120                 125

Leu Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
    130                 135                 140
```

-continued

Gly Cys
145

<210> SEQ ID NO 212
<211> LENGTH: 200
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank NP_000605
<309> DATABASE ENTRY DATE: 2002-07-17

<400> SEQUENCE: 212

Met Ala Phe Thr Glu His Ser Pro Leu Thr Pro His Arg Arg Asp Leu
 1   5   10   15

Cys Ser Arg Ser Ile Trp Leu Ala Arg Lys Ile Arg Ser Asp Leu Thr
            20   25   30

Ala Leu Thr Glu Ser Tyr Val Lys His Gln Gly Leu Asn Lys Asn Ile
        35   40   45

Asn Leu Asp Ser Ala Asp Gly Met Pro Val Ala Ser Thr Asp Gln Trp
    50   55   60

Ser Glu Leu Thr Glu Ala Glu Arg Leu Gln Glu Asn Leu Gln Ala Tyr
65   70   75   80

Arg Thr Phe His Val Leu Leu Ala Arg Leu Leu Glu Asp Gln Gln Val
                85   90   95

His Phe Thr Pro Thr Glu Gly Asp Phe His Gln Ala Ile His Thr Leu
            100   105   110

Leu Leu Gln Val Ala Ala Phe Ala Tyr Gln Ile Glu Glu Leu Met Ile
        115   120   125

Leu Leu Glu Tyr Lys Ile Pro Arg Asn Glu Ala Asp Gly Met Pro Ile
    130   135   140

Asn Val Gly Asp Gly Gly Leu Phe Glu Lys Lys Leu Trp Gly Leu Lys
145   150   155   160

Val Leu Gln Glu Leu Ser Gln Trp Thr Val Arg Ser Ile His Asp Leu
                165   170   175

Arg Phe Ile Ser Ser His Gln Thr Gly Ile Pro Ala Arg Gly Ser His
            180   185   190

Tyr Ile Ala Asn Asn Lys Lys Met
        195   200

<210> SEQ ID NO 213
<211> LENGTH: 180
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank CAA32147
<309> DATABASE ENTRY DATE: 1995-03-22

<400> SEQUENCE: 213

Ser Pro Leu Pro Ile Thr Pro Val Asn Ala Thr Cys Ala Ile Arg His
 1   5   10   15

Pro Cys His Asn Asn Leu Met Asn Gln Ile Arg Ser Gln Leu Ala Gln
            20   25   30

Leu Asn Gly Ser Ala Asn Ala Leu Phe Ile Leu Tyr Tyr Thr Ala Gln
        35   40   45

Gly Glu Pro Phe Pro Asn Asn Leu Asp Lys Leu Cys Gly Pro Asn Val
    50   55   60

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Thr Asp Phe Pro Pro Phe His Ala Asn Gly Thr Glu Lys Ala Lys Leu
65                  70                  75                  80

Val Glu Leu Tyr Arg Ile Val Val Tyr Leu Gly Thr Ser Leu Gly Asn
                85                  90                  95

Ile Thr Arg Asp Gln Lys Ile Leu Asn Pro Ser Ala Leu Ser Leu His
            100                 105                 110

Ser Lys Leu Asn Ala Thr Ala Asp Ile Leu Arg Gly Leu Leu Ser Asn
        115                 120                 125

Val Leu Cys Arg Leu Cys Ser Lys Tyr His Val Gly His Val Asp Val
    130                 135                 140

Thr Tyr Gly Pro Asp Thr Ser Gly Lys Asp Val Phe Gln Lys Lys Lys
145                 150                 155                 160

Leu Gly Cys Gln Leu Leu Gly Lys Tyr Lys Gln Ile Ile Ala Val Leu
                165                 170                 175

Ala Gln Ala Phe
            180


<210> SEQ ID NO 214
<211> LENGTH: 227
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAA36388
<309> DATABASE ENTRY DATE: 1993-04-27

<400> SEQUENCE: 214

Ala Ala Ile Gly Ser Cys Ser Lys Glu Tyr Arg Val Leu Leu Gly Gln
 1               5                  10                  15

Leu Gln Lys Gln Thr Asp Leu Met Gln Asp Thr Ser Arg Leu Leu Asp
            20                  25                  30

Pro Tyr Ile Arg Ile Gln Gly Leu Asp Val Pro Lys Leu Arg Glu His
        35                  40                  45

Cys Arg Glu Arg Pro Gly Ala Phe Pro Ser Glu Glu Thr Leu Arg Gly
    50                  55                  60

Leu Gly Arg Arg Gly Phe Leu Gln Thr Leu Asn Ala Thr Leu Gly Cys
65                  70                  75                  80

Val Leu His Arg Leu Ala Asp Leu Glu Gln Arg Leu Pro Lys Ala Gln
                85                  90                  95

Asp Leu Glu Arg Ser Gly Leu Asn Ile Glu Asp Leu Glu Lys Leu Gln
            100                 105                 110

Met Ala Arg Pro Asn Ile Leu Gly Leu Arg Asn Asn Ile Tyr Cys Met
        115                 120                 125

Ala Gln Leu Leu Asp Asn Ser Asp Thr Ala Glu Pro Thr Lys Ala Gly
    130                 135                 140

Arg Gly Ala Ser Gln Pro Pro Thr Pro Thr Pro Ala Ser Asp Ala Phe
145                 150                 155                 160

Gln Arg Lys Leu Glu Gly Cys Arg Phe Leu His Gly Tyr His Arg Phe
                165                 170                 175

Met His Ser Val Gly Arg Val Phe Ser Lys Trp Gly Glu Ser Pro Asn
            180                 185                 190

Arg Ser Arg Arg His Ser Pro His Gln Ala Leu Arg Lys Gly Val Arg
        195                 200                 205

Arg Thr Arg Pro Ser Arg Lys Gly Lys Arg Leu Met Thr Arg Gly Gln
```

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210 215 220

Leu Pro Arg
225

<210> SEQ ID NO 215
<211> LENGTH: 197
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AF101062
<309> DATABASE ENTRY DATE: 1999-03-03

<400> SEQUENCE: 215

Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly Met Phe Pro Cys Leu
1 5 10 15

His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn Met Leu Gln Lys
20 25 30

Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu Glu Ile Asp
35 40 45

His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu Ala Cys Leu
50 55 60

Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser Arg Glu Thr
65 70 75 80

Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys Thr Ser Phe
85 90 95

Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu Lys Met Tyr
100 105 110

Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met Asp Pro Lys
115 120 125

Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile Asp Glu Leu
130 135 140

Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln Lys Ser Ser
145 150 155 160

Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu Cys Ile Leu
165 170 175

Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg Val Met Ser
180 185 190

Tyr Leu Asn Ala Ser
195

<210> SEQ ID NO 216
<211> LENGTH: 191
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 216

Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg
1 5 10 15

Ala His Arg Leu His Gln Leu Ala Phe Asp Thr Tyr Gln Glu Phe Glu
20 25 30

Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro
35 40 45

Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg
50 55 60

Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu

-continued

65 70 75 80

Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val
85 90 95

Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp
100 105 110

Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu
115 120 125

Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser
130 135 140

Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr
145 150 155 160

Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe
165 170 175

Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
180 185 190

<210> SEQ ID NO 217
<211> LENGTH: 183
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: Genbank AAD13886
<309> DATABASE ENTRY DATE: 1993-06-28

<400> SEQUENCE: 217

Val Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His Arg Gln
1 5 10 15

Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr Ile Leu
20 25 30

Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser Asn Met
35 40 45

Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro
50 55 60

Lys Met Ala Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu
65 70 75 80

Thr Cys Leu Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr
85 90 95

Leu Glu Tyr Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg
100 105 110

Ala Val Gln Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys
115 120 125

Ala Lys Asn Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala
130 135 140

Ser Leu Leu Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Gln Asp Met
145 150 155 160

Thr Thr His Leu Ile Leu Arg Ser Phe Lys Glu Phe Leu Gln Ser Ser
165 170 175

Leu Arg Ala Leu Arg Gln Met
180

<210> SEQ ID NO 218
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: EcoRI Forward Primer

<400> SEQUENCE: 218

gcctgtatga tttattggat gttggaattc cctgatgcgg tattttctcc ttacg 55

<210> SEQ ID NO 219
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EcoRI Reverse Primer

<400> SEQUENCE: 219

cgtaaggaga aaataccgca tcagggaatt ccaacatcca ataaatcata caggc 55

<210> SEQ ID NO 220
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Seq ClaI Forward Primer

<400> SEQUENCE: 220

ctgattatca accgcggtac atatgattga catgc 35

<210> SEQ ID NO 221
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Seq ClaI Reverse Primer

<400> SEQUENCE: 221

tacgggataa taccgcgcca catagcagaa c 31

<210> SEQ ID NO 222
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Seq Forward Primer

<400> SEQUENCE: 222

cctgatgaag gaggactc 18

<210> SEQ ID NO 223
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Seq Reverse Primer

<400> SEQUENCE: 223

ccaagcagca gatgagtc 18

<210> SEQ ID NO 224
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IFN alpha-2b 5' Primer

<400> SEQUENCE: 224

-continued

tcagctgcaa gtcaagctgc tctgtgggct g 31

<210> SEQ ID NO 225
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IFN alpha-2b 3' Primer

<400> SEQUENCE: 225

gctctagatc attccttact tcttaaactt tcttgcaagt ttgttgac 48

<210> SEQ ID NO 226
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IFN alpha-2b HindIII Primer

<400> SEQUENCE: 226

cccaagctta tggccttgac ctttgcttta ctggtg 36

<210> SEQ ID NO 227
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IFN alpha-2b XbaI Primer

<400> SEQUENCE: 227

gctctagatc attccttact tcttaaactt tcttgcaagt ttgttgac 48

<210> SEQ ID NO 228
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: IFN alpha-2b 80 bp 5' Primer

<400> SEQUENCE: 228

cccaagctta tggccttgac ctttgcttta ctggtggccc tcctggtgct cagctgcaag 60

tcaagctgct ctgtgggctg 80

<210> SEQ ID NO 229
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EMCV Forward Primer

<400> SEQUENCE: 229

cccctacatt gaggcatcca 20

<210> SEQ ID NO 230
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EMCV Reverse Primer

<400> SEQUENCE: 230

caggagcagg acaaggtcac t 21

-continued

<210> SEQ ID NO 231
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EMCV Probe
<220> FEATURE:
<223> OTHER INFORMATION: FAM attached to 5' end of sequence
<220> FEATURE:
<223> OTHER INFORMATION: TAMR A attached to 3' end of sequence

<400> SEQUENCE: 231

cagccgtcaa gacccaaccg ct 22

<210> SEQ ID NO 232
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Interferon alpha consensus sequence

<400> SEQUENCE: 232

Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu Ile
1 5 10 15

Leu Leu Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp
20 25 30

Arg His Asp Phe Gly Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe Gln
35 40 45

Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr Phe
50 55 60

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Ser Leu
65 70 75 80

Leu Glu Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
85 90 95

Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu Met Asn
100 105 110

Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile Thr Leu
115 120 125

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
130 135 140

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Arg
145 150 155 160

Leu Arg Arg Lys Glu
165

<210> SEQ ID NO 233
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 233

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Gln Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45

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```
Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 234
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 234

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Gln Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 235
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 235

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15
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-continued

```
Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe His Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 236
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 236

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                   10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Gly Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 237
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
```

-continued

<400> SEQUENCE: 237

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Gln Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145 150 155 160

Thr Gly Tyr Leu Arg Asn
165

<210> SEQ ID NO 238
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 238

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro His Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145 150 155 160

Thr Gly Tyr Leu Arg Asn
165

-continued

<210> SEQ ID NO 239
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 239

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1   5   10   15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20   25   30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Gln Gln Leu Gln
        35   40   45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50   55   60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65   70   75   80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85   90   95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100   105   110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115   120   125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130   135   140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145   150   155   160

Thr Gly Tyr Leu Arg Asn
                165

<210> SEQ ID NO 240
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 240

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1   5   10   15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20   25   30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Thr Gln Leu Gln
        35   40   45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50   55   60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65   70   75   80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85   90   95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100   105   110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115   120   125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130   135   140

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```
Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 241
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 241

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Ser Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 242
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 242

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile His Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110
```

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```
Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 243
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 243

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Val Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 244
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 244

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Ile Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80
```

-continued

```
Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 245
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 245

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Thr Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 246
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 246

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Gln Gln
        35                  40                  45
```

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```
Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 247
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 247

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln His Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 248
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 248

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15
```

-continued

```
Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Ala Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 249
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 249

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Gln Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 250
<211> LENGTH: 166
<212> TYPE: PRT
```

-continued

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 250

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1   5   10   15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20   25   30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35   40   45

Gln Phe Gln Thr Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50   55   60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65   70   75   80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85   90   95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100   105   110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115   120   125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130   135   140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145   150   155   160

Thr Gly Tyr Leu Arg Asn
                165

<210> SEQ ID NO 251
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 251

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1   5   10   15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20   25   30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35   40   45

Gln Phe Gln Ser Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50   55   60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65   70   75   80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85   90   95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100   105   110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115   120   125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130   135   140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145   150   155   160

Thr Gly Tyr Leu Arg Asn

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               165


<210> SEQ ID NO 252
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 252

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln His Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 253
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 253

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Ile Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
```

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```
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 254
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 254

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Val Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 255
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 255

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe His Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
```

-continued

```
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 256
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 256

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Gln Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 257
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 257

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Gln Ser Ser Ser Thr Gly Trp Asn
```

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```
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 258
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 258

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln His Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 259
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 259

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
```

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```
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Gly Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 260
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 260

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Gln Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 261
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 261

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
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 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

His Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 262
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 262

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Gln Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 263
<211> LENGTH: 166
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-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 263

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1   5   10   15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20   25   30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35   40   45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50   55   60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65   70   75   80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85   90   95

His Leu Lys Thr Val Leu Glu Glu Lys Leu His Lys Glu Asp Phe Thr
            100   105   110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115   120   125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130   135   140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145   150   155   160

Thr Gly Tyr Leu Arg Asn
                165

<210> SEQ ID NO 264
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 264

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1   5   10   15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20   25   30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35   40   45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50   55   60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65   70   75   80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85   90   95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Gln Glu Asp Phe Thr
            100   105   110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115   120   125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130   135   140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145   150   155   160

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Thr Gly Tyr Leu Arg Asn
165

<210> SEQ ID NO 265
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 265

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Thr Glu Asp Phe Thr
100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
130 135 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145 150 155 160

Thr Gly Tyr Leu Arg Asn
165

<210> SEQ ID NO 266
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 266

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
20 25 30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
35 40 45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
50 55 60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65 70 75 80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
85 90 95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Ser Glu Asp Phe Thr
100 105 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
115 120 125

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```
Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 267
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 267

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu His Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 268
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 268

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95
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```
His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Gln Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 269
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 269

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys His Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 270
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 270

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60
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```
Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Gln Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 271
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 271

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu His Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 272
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 272

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30
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```
Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Gly Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 273
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 273

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Ile Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 274
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 274
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```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Val Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 275
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 275

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

His Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 276
```

-continued

<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 276

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1   5   10   15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20   25   30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35   40   45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50   55   60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65   70   75   80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85   90   95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100   105   110

Gln Gly Lys Leu Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115   120   125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130   135   140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145   150   155   160

Thr Gly Tyr Leu Arg Asn
                165

<210> SEQ ID NO 277
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 277

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1   5   10   15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20   25   30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35   40   45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50   55   60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65   70   75   80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85   90   95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100   105   110

Arg Gly Lys Val Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115   120   125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130   135   140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145   150   155   160

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```
Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 278
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 278

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Ile Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 279
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 279

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Thr Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125
```

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```
Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 280
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 280

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Gln Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 281
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 281

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95
```

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```
His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys His Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 282
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 282

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Ala Met Ser Ser Leu His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

<210> SEQ ID NO 283
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 283

```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60
```

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```
Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Val His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 284
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 284

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Ile His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 285
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 285

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30
```

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```
Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Thr His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 286
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 286

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Gln His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 287
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 287
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```
Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser His His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165


<210> SEQ ID NO 288
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 288

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1               5                  10                  15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20                  25                  30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35                  40                  45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50                  55                  60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65                  70                  75                  80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85                  90                  95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100                 105                 110

Arg Gly Lys Leu Met Ser Ser Ala His Leu Lys Arg Tyr Tyr Gly Arg
        115                 120                 125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130                 135                 140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145                 150                 155                 160

Thr Gly Tyr Leu Arg Asn
                165
```

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<210> SEQ ID NO 289
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 289

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
 1   5   10   15

Cys Gln Lys Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu
            20   25   30

Lys Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys Gln Leu Gln
        35   40   45

Gln Phe Gln Lys Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln
    50   55   60

Asn Ile Phe Ala Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn
65   70   75   80

Glu Thr Ile Val Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn
                85   90   95

His Leu Lys Thr Val Leu Glu Glu Lys Leu Glu Lys Glu Asp Phe Thr
            100   105   110

Arg Gly Lys Leu Met Ser Ser Leu His Leu Gln Arg Tyr Tyr Gly Arg
        115   120   125

Ile Leu His Tyr Leu Lys Ala Lys Glu Tyr Ser His Cys Ala Trp Thr
    130   135   140

Ile Val Arg Val Glu Ile Leu Arg Asn Phe Tyr Phe Ile Asn Arg Leu
145   150   155   160

Thr Gly Tyr Leu Arg Asn
                165

<210> SEQ ID NO 290
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 290

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Val Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln

-continued

145

<210> SEQ ID NO 291
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 291

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Ile Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln
145

<210> SEQ ID NO 292
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 292

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Gln Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln

-continued

145

<210> SEQ ID NO 293
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 293

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Asn Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln
145

<210> SEQ ID NO 294
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 294

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Gln Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln

-continued

145

<210> SEQ ID NO 295
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 295

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
1 5 10 15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
20 25 30

Leu Gly Ile Leu Lys Asn Trp Asn Glu Glu Ser Asp Arg Lys Ile Met
35 40 45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
50 55 60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65 70 75 80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
85 90 95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
100 105 110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
115 120 125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
130 135 140

Ser Gln
145

<210> SEQ ID NO 296
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 296

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
1 5 10 15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
20 25 30

Leu Gly Ile Leu Lys Asn Trp Lys Gln Glu Ser Asp Arg Lys Ile Met
35 40 45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
50 55 60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65 70 75 80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
85 90 95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
100 105 110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
115 120 125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
130 135 140

Ser Gln

-continued

145

<210> SEQ ID NO 297
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 297

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Asn Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln
145

<210> SEQ ID NO 298
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 298

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys His Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln

-continued

145

<210> SEQ ID NO 299
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 299

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Gln Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln
145

<210> SEQ ID NO 300
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 300

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Asn Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln

-continued

145

<210> SEQ ID NO 301
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 301

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu His Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln
145

<210> SEQ ID NO 302
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 302

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Gln Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln

-continued

145

<210> SEQ ID NO 303
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 303

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Asn Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln
145

<210> SEQ ID NO 304
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 304

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Gln Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln

-continued

145

<210> SEQ ID NO 305
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 305

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Asn Asn Phe Lys
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln
145

<210> SEQ ID NO 306
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 306

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Gln
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln

-continued

145

<210> SEQ ID NO 307
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 307

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Asn
    50   55   60

Asp Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln
145

<210> SEQ ID NO 308
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 308

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Gln Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln

-continued

145

<210> SEQ ID NO 309
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 309

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asn Asp Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln
145

<210> SEQ ID NO 310
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 310

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
 1   5   10   15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
            20   25   30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
        35   40   45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
    50   55   60

Asp Gln Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65   70   75   80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
                85   90   95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
            100   105   110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
        115   120   125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
    130   135   140

Ser Gln

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145

<210> SEQ ID NO 311
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 311

Cys Tyr Cys Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys
1 5 10 15

Tyr Phe Asn Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe
20 25 30

Leu Gly Ile Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met
35 40 45

Gln Ser Gln Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys
50 55 60

Asp Asn Gln Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met
65 70 75 80

Asn Val Lys Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu
85 90 95

Lys Leu Thr Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala
100 105 110

Ile His Glu Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys
115 120 125

Thr Gly Lys Arg Lys Arg Ser Gln Met Leu Phe Gln Gly Arg Arg Ala
130 135 140

Ser Gln
145

<210> SEQ ID NO 312
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 312

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
1 5 10 15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
20 25 30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
35 40 45

Gln Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
50 55 60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65 70 75 80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
85 90 95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
100 105 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
115 120 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
130 135 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn

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145                 150                 155                 160


<210> SEQ ID NO 313
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 313

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Asn Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
145                 150                 155                 160


<210> SEQ ID NO 314
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 314

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Lys Gln Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
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145                 150                 155                 160


<210> SEQ ID NO 315
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 315

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Lys Asn Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
145                 150                 155                 160


<210> SEQ ID NO 316
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 316

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Lys His Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
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145                 150                 155                 160


<210> SEQ ID NO 317
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 317

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Lys Glu Ser Val Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
145                 150                 155                 160


<210> SEQ ID NO 318
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 318

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Lys Glu Ser Ile Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
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145                 150                 155                 160


<210> SEQ ID NO 319
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 319

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Lys Glu Ser Leu Val Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
145                 150                 155                 160


<210> SEQ ID NO 320
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 320

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Lys Glu Ser Leu Ile Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
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145 150 155 160

<210> SEQ ID NO 321
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 321

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
1 5 10 15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
20 25 30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
35 40 45

Lys Glu Ser Leu Leu Gln Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
50 55 60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65 70 75 80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
85 90 95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
100 105 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
115 120 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
130 135 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
145 150 155 160

<210> SEQ ID NO 322
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 322

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
1 5 10 15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
20 25 30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
35 40 45

Lys Glu Ser Leu Leu Asn Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
50 55 60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65 70 75 80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
85 90 95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
100 105 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
115 120 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
130 135 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn

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145                 150                 155                 160


<210> SEQ ID NO 323
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 323

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Lys Glu Ser Leu Leu His Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
145                 150                 155                 160


<210> SEQ ID NO 324
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 324

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Lys Glu Ser Leu Leu Glu Gln Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
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-continued

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145                 150                 155                 160


<210> SEQ ID NO 325
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 325

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20                  25                  30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35                  40                  45

Lys Glu Ser Leu Leu Glu Asn Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
145                 150                 155                 160


<210> SEQ ID NO 326
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 326

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
 1               5                  10                  15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser A0
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What is claimed is:

1. A modified granulocyte colony stimulating factor (G-CSF) cytokine, comprising one or more amino acid replacements in its sequence of amino acid residues, wherein:

the modified G-CSF cytokine exhibits increased resistance to proteolysis compared to the unmodified G-CSF cytokine that does not comprise the one or more amino acid replacements;

the one or more amino acid replacements and positions thereof are selected from among replacement of: W61S, W61H, P63S, P63A, P68A, L72V, L721, F86I, F86V, E96N, E96H, P100S, P100A, E101N, E101H, P131S, P131A, L133V, L1331, P135S, P135A, F1471, F147V, R169H, R169Q, R172H, R172Q, P177S and P177A; and

the one or more amino acid replacements occur in a mature G-CSF cytokine having the sequence set forth in SEQ ID NO: 210 or in a sequence-related G-CSF cytokine at corresponding amino acid position(s) relative to SEQ ID NO: 210.

2. The modified G-CSF cytokine of claim 1, wherein the unmodified G-CSF cytokine contains the amino acids residues having the sequence set forth in SEQ ID NO: 210.

3. The G-CSF cytokine of claim 1 that comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more of the amino acid replacements in its sequence of amino acid residues.

4. The G-CSF cytokine of claim 1 that comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acid replacements in its sequence of amino acid residues.

5. The G-CSF cytokine of claim 1, further comprising one or more additional amino acid replacements in its sequence of amino acids, wherein:

the one or more amino acid replacements and positions thereof are selected from among replacement of: W61S, W61H, P63S, P63A, P68S, P68A, L72V, L721, F861, F86V, E96Q, E96N, E96H, P100S, P100A, E101Q, E101N, E101H, P131S, P131A, L133V, L1331, P135S, P135A, F147I, F147V, R169H, R169Q, R172H, R172Q, P177S and P177A; and

the one or more amino acid replacements occur in a mature G-CSF cytokine having the sequence set forth in SEQ ID NO: 210 or in a sequence-related G-CSF cytokine at corresponding amino acid position(s) relative to SEQ ID NO: 210.

6. The G-CSF cytokine of claim 1, wherein only the primary amino acid sequence is modified, and the G-CSF cytokine exhibits increased resistance to proteolysis.

7. A G-CSF cytokine of claim 1, wherein the cytokine comprises the sequence of amino acids set forth in any of SEQ ID NOS: 631, 632, 633, 634, 636, 637, 638, 639, 640, 642, 643, 644, 645, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661 or 662.

8. The G-CSF cytokine of claim 1 that exhibits increased stability compared to the unmodified G-CSF cytokine.

9. The G-CSF cytokine of claim 8, wherein the G-CSF cytokine exhibits increased stability to proteases, human blood lysate or human serum.

10. The G-CSF cytokine of claim 1 that exhibits increased protein half-life in vitro or in vivo compared to the unmodified G-CSF cytokine.

11. The G-CSF cytokine of claim 1 that exhibits increased resistance to proteolysis by a protease of the gastrointestinal tract.

12. The G-CSF cytokine of claim 1 that exhibits increased resistance to proteolysis by a protease in the serum.

13. The G-CSF cytokine of claim 1, wherein increased resistance to proteolysis is due to replacement of one or more amino acids at target positions in an unmodified G-CSF cytokine that increase resistance of the G-CSF cytokine to digestion by a protease.

14. The G-CSF cytokine of claim 1 that exhibits increased biological activity compared to the unmodified G-CSF cytokine.

15. The G-CSF cytokine of claim 1 that exhibits decreased biological activity compared to the unmodified G-CSF cytokine.

16. A nucleic acid molecule encoding a modified G-CSF cytokine of claim 1.

17. A vector, comprising a nucleic acid molecule of claim 16.

18. A eukaryotic cell, comprising the nucleic acid molecule of claim 16.

19. A eukaryotic cell, comprising the vector of claim 17.

20. A collection of nucleic acid molecules, comprising a plurality of the molecules of claim 16.

21. A collection of nucleic acid molecules, comprising a plurality of the vectors of claim 17.

22. A method for expression of a modified G-CSF cytokine, comprising:

introducing a nucleic acid of claim 16 into a host; and

culturing the cell under conditions, whereby the encoded modified G-CSF cytokine is expressed.

23. The method of claim 22, further comprising isolating the modified G-CSF cytokine.

24. The method of claim 22, wherein the host cell is a eukaryotic cell or a bacterial cell.

25. A pharmaceutical composition, comprising a G-CSF cytokine of claim 1.

26. The pharmaceutical composition of claim 25, further comprising a pharmaceutically acceptable carrier or excipient.

27. The pharmaceutical composition of claim 26, wherein the pharmaceutically acceptable carrier or excipient is selected from among a binding agent, a filler, a lubricant, a disintegrant, and a wetting agent.

28. The pharmaceutical composition of claim 25, further comprising a pharmaceutically acceptable additive.

29. The pharmaceutical composition of claim 28, wherein the pharmaceutically acceptable additive is selected from among a suspending agent, an emulsifying agent, a non-aqueous vehicle, and a preservative.

30. The pharmaceutical composition of claim 25, wherein the composition is in the form of a liquid, a solution, a suspension, an aerosol, a tablet, a lozenge or a capsule.

31. The pharmaceutical composition of claim 25, formulated for oral, parenteral, intravenous, intradermal, subcutaneous, buccal, inhalation, intramuscular, rectal or topical administration.

32. The pharmaceutical composition of claim 31, formulated for oral administration.

33. The pharmaceutical composition of claim 32, wherein the pharmaceutical composition is formulated for oral administration to the mouth or gastrointestinal tract.

34. The pharmaceutical composition of claim 25, wherein the pharmaceutical composition is formulated for controlled-release of the G-CSF cytokine.

35. A pharmaceutical composition formulated for oral administration, comprising a G-CSF cytokine that contains one or more amino acid modification(s), whereby the G-CSF cytokine exhibits increased protease resistance compared to a G-CSF cytokine that does not contain the modification(s).

36. The pharmaceutical composition of claim 35, wherein the modified G-CSF cytokine has been modified by an insertion, a deletion and/or a replacement of one or more amino acid residues, whereby the cytokine is rendered resistant to proteolysis.

37. The pharmaceutical composition of claim 35, wherein:

the modified G-CSF cytokine comprises one or more amino acid replacements at one or more amino acid target positions in the unmodified cytokine.

38. The pharmaceutical composition of claim 37, wherein:

the modified G-CSF cytokine comprises one or more amino acid replacements selected from among replacement of: W61S, W61H, P63S, P63A, P68S, P68A, L72V, L721, F861, F86V, E96Q, E96N, E96H, P100S, P100A, E101Q, E101N, E101H, P131S, P131A, L133V, L1331, P135S, P135A, F1471, F147V, R169H, R169Q, R172H, R172Q, P177S and P177A; and

the one or more amino acid replacements occur in a mature G-CSF cytokine having the sequence set forth

in SEQ ID NO: 210 or in a sequence-related G-CSF cytokine at corresponding amino acid position(s) relative to SEQ ID NO: 210.

39. The pharmaceutical composition of claim 35, further comprising a pharmaceutically acceptable carrier or excipient.

40. The pharmaceutical composition of claim 39, wherein the pharmaceutically acceptable carrier or excipient is selected from among a binding agent, a filler, a lubricant, a disintegrant, and a wetting agent.

41. The pharmaceutical composition of claim 35, further comprising a pharmaceutically acceptable additive.

42. The pharmaceutical composition of claim 41, wherein the pharmaceutically acceptable additive is selected from among a suspending agent, an emulsifying agent, a non-aqueous vehicle and a preservative.

43. The pharmaceutical composition of claim 35, wherein the composition is in the form of a liquid, a solution, a suspension, an aerosol, a tablet, a lozenge or a capsule.

44. The pharmaceutical composition of claim 35, wherein the pharmaceutical composition is formulated for controlled-release of the G-CSF cytokine.

45. The pharmaceutical composition of claim 35, wherein the G-CSF cytokine has been modified by removing proteolytic digestion sites in the G-CSF cytokine.

46. The pharmaceutical composition of claim 35, wherein the G-CSF cytokine has an increased half-life compared to the unmodified G-CSF cytokine.

47. The pharmaceutical composition of claim 35, wherein the G-CSF cytokine exhibits increased resistance to proteolysis by a protease of the gastrointestinal tract.

48. The pharmaceutical composition of claim 35, wherein the G-CSF cytokine exhibits increased biological activity compared to the unmodified G-CSF cytokine.

49. The pharmaceutical composition of claim 35, wherein the G-CSF cytokine exhibits decreased biological activity compared to the unmodified G-CSF cytokine.

50. A pharmaceutical composition, comprising a nucleic acid molecule of claim 16.

51. A method, comprising treating a subject by administering the pharmaceutical composition of claim 25, wherein the subject has a disease or condition that is treated by administration of a G-CSF cytokine.

52. The method of claim 51, wherein in the disease or condition is selected from among Crohn's disease, cardiac disease, acquired and congenital neutropenias and asthma.

53. A method, comprising treating a subject by orally administering the pharmaceutical composition of claim 35, wherein the subject has a disease or condition that is treated by administration of a G-CSF cytokine.

54. The method of claim 53, wherein in the disease or condition is selected from among Crohn's disease, cardiac disease, acquired and congenital neutropenias and asthma.

55. A method, comprising treating a subject by administering the pharmaceutical composition of claim 50, wherein the subject has a disease or condition that is treated by administration of a G-CSF cytokine.

56. The method of claim 55, wherein in the disease or condition is selected from among Crohn's disease, cardiac disease, acquired and congenital neutropenias and asthma.

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