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(54) EXPRESSED LIGAND - VASCULAR INTERCELLULAR SIGNALLING MOLECULE

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Related U.S. Application Data

(60) Division of application No. 09/709,188, filed on Nov. 9, 2000, now Pat. No. 6,441,137, which is a continuation of application No. 08/740,223, filed on Oct. 25, 1996, now Pat. No. 6,265,564.

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(57) **ABSTRACT**

The present invention provides for a modified TIE-2 ligand which has been altered by addition, deletion or substitution of one or more amino acids, or by way of tagging, with for example, the Fc portion of human IgG-1, but which retains its ability to bind the TIE-2 receptor. The invention further provides for a modified TIE-2 ligand which is a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first. In a specific embodiment, the invention further provides for a chimeric TIE ligand comprising at least a portion of TIE-2 Ligand-1 and a portion of TIE-2 Ligand-2. In addition the present invention provides for isolated nucleic acid molecule encoding the modified TIE-2 ligands described. The invention also provides for therapeutic compositions as well as a method of blocking blood vessel growth, a method of promoting neovascularization, a method of promoting the growth or differentiation of a cell expressing the TIE receptor, a method of blocking the growth or differentiation of a cell expressing the TIE receptor and a method of attenuating or preventing tumor growth in a human.

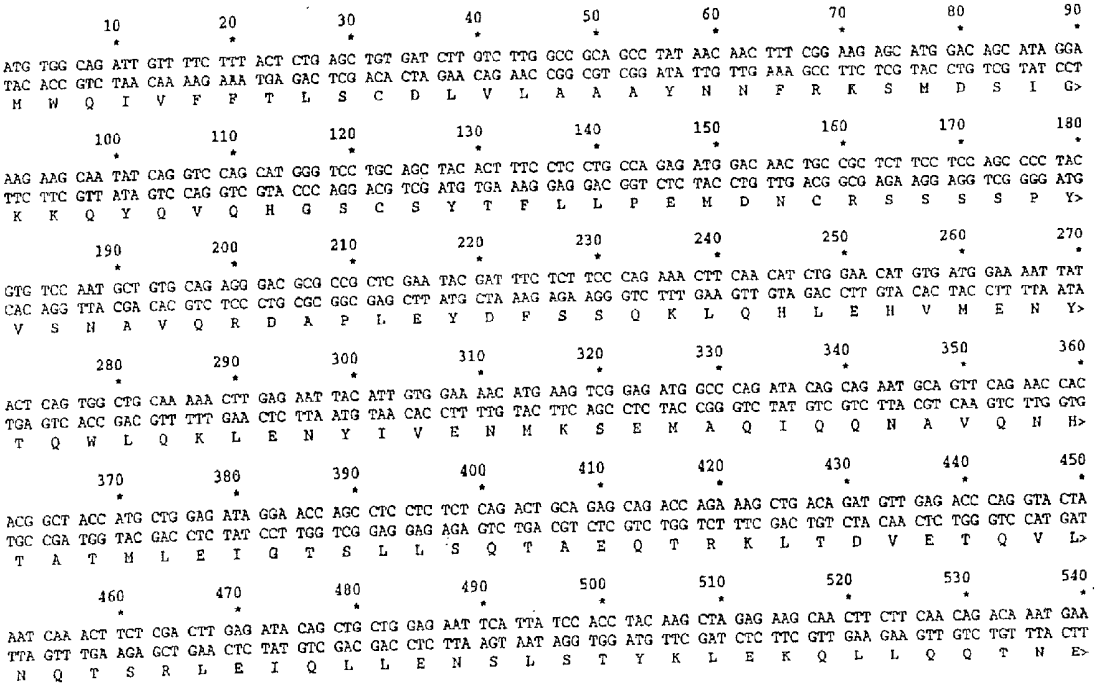
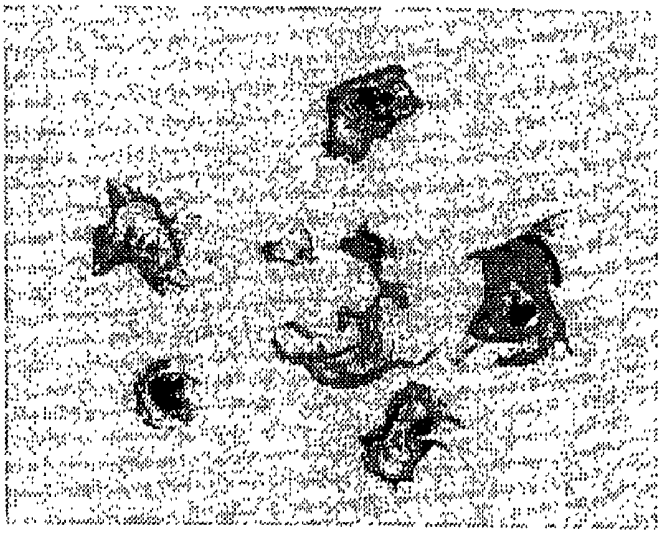


Fig. 1 A

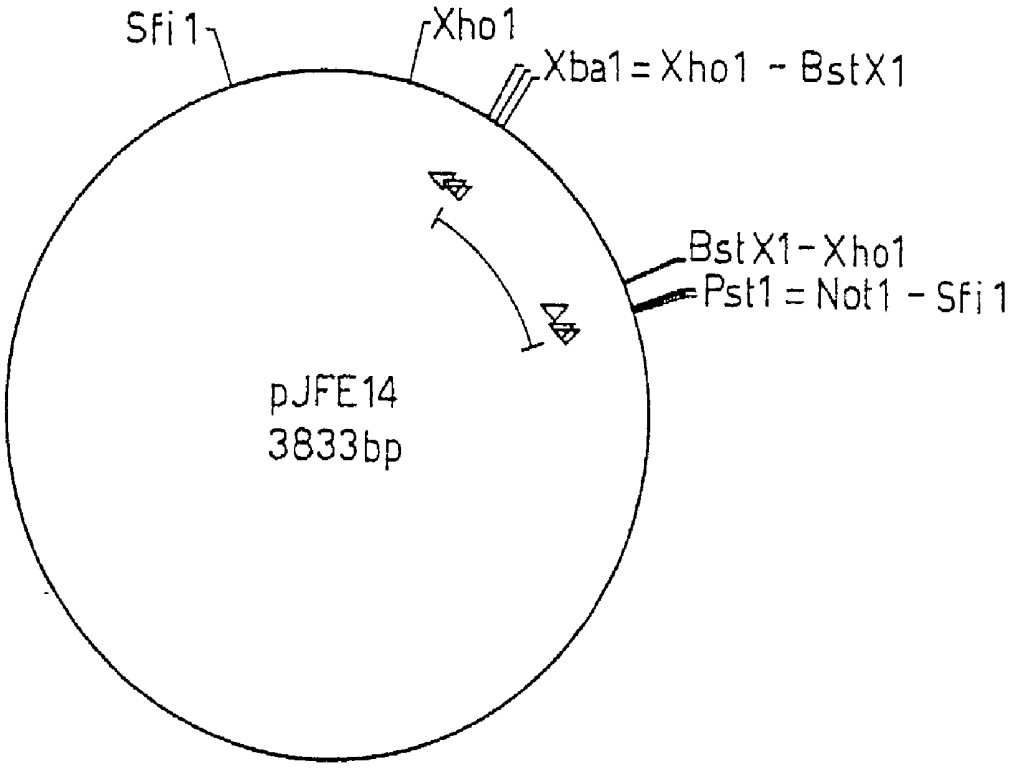
r EHK-1 ecto/h IgG1 Fc
Gelfoam (6ug)

Fig. 1 B



r TIE-2 ecto/h IgG1 Fc
Gelfoam (6ug)

Fig. 2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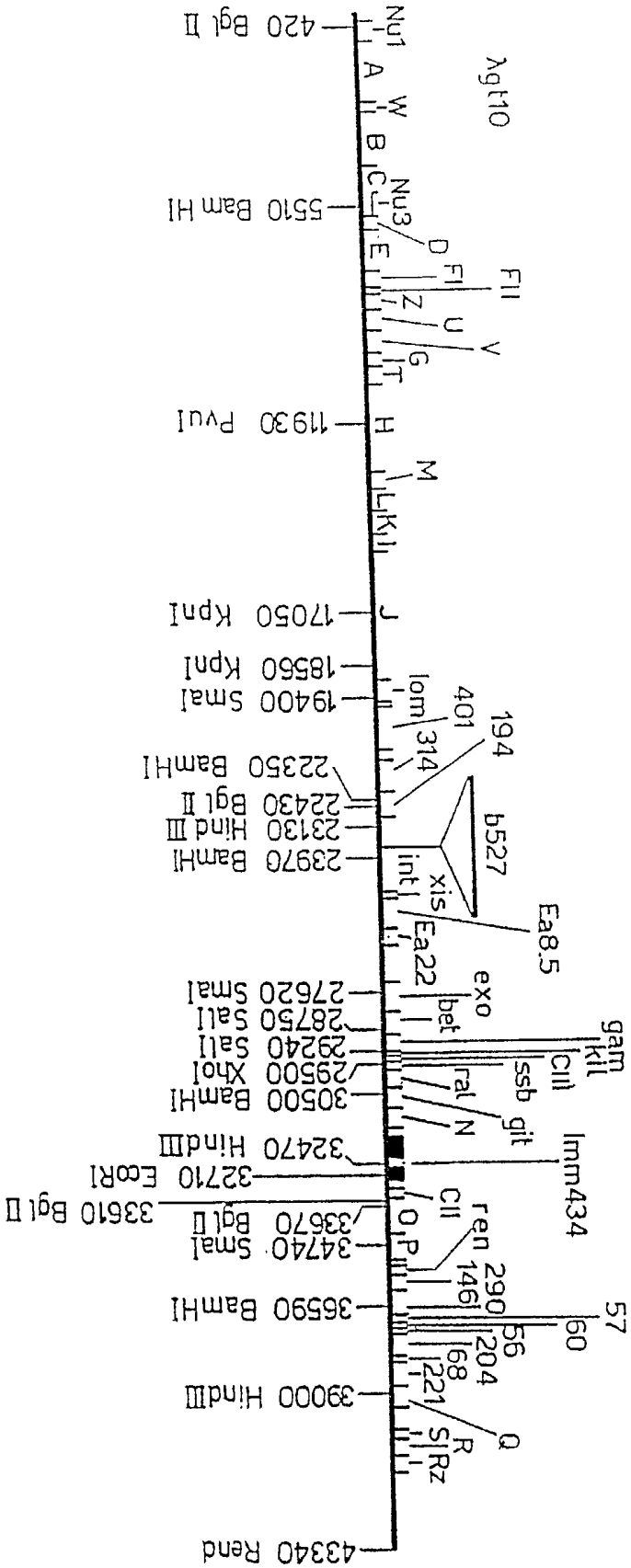
Xho1
Xba1 = Xho1 - BstX1
BstX1 - Xho1
Pst1 = Not1 - Sfi 1
pJFE14
3833bp

Fig. 3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11930 PvuI
17050 KpnI
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22430 Bgl II
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23970 BamHI
27620 SmaI
28750 SalI
29240 SalI
29500 XhoI
30500 BamHI
32470 HindIII
32710 EcoRI
33610 Bgl II
33670 Bgl II
34740 SmaI
36590 BamHI
39000 HindIII
43340 Rend
Nu1
A
W
B
C
Nu3
D
E
FI
FII
Z
U
V
G
T
H
M
LKJ
J
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b527
int
xis
Ea8.5
Ea22
exo
bet
gam
kil
CIII
ssb
ral
N
git
Imm434
CII
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O
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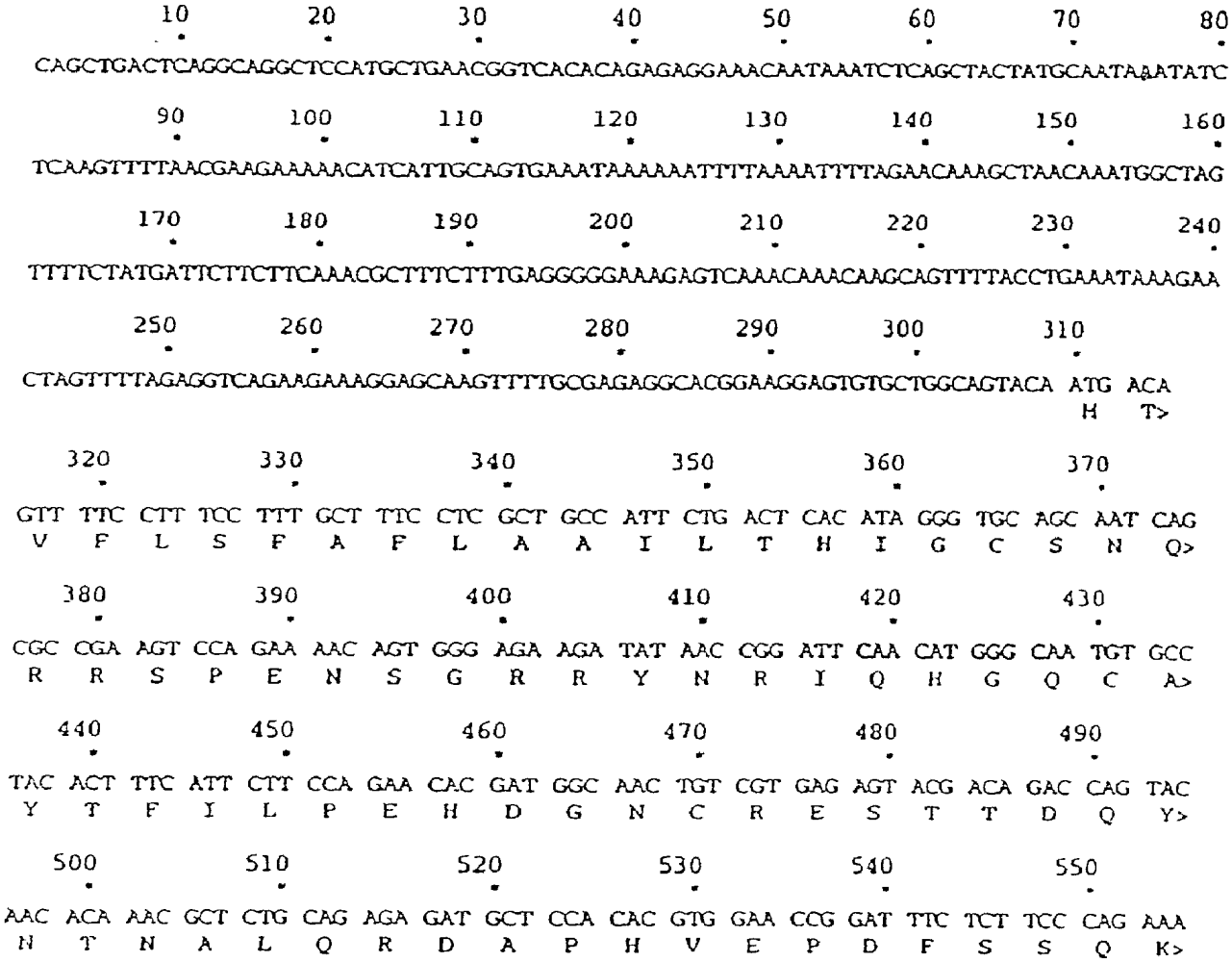
146
290
57
60
56
204
68
221
S
R
Rz
Q

Fig. 4 A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M T>
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V F L S F A F L A A I L T H I G C S N Q>
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Y T F I L P E H D G N C R E S T T D Q Y>
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N T N A L Q R D A P H V E P D F S S Q K>
M T>

Fig. 4 B

```
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    .          .          .          .          .          .
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 L   Q   H   L   E   H   V   M   E   N   Y   T   Q   W   L   Q   K   L   E   N>

   620        630        640        650        660        670
    .          .          .          .          .          .
TAC ATT GTG GAA AAC ATG AAG TCG GAG ATG GCC CAG ATA CAG CAG AAT GCA GTT CAG AAC
 Y   I   V   E   N   M   K   S   E   M   A   Q   I   Q   Q   N   A   V   Q   N>

   680        690        700        710        720        730
    .          .          .          .          .          .
CAC ACG GCT ACC ATG CTG GAG ATA GGA ACC AGC CTC CTC TCT CAG ACT GCA GAG CAG ACC
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   740        750        760        770        780        790
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 L   L   E   N   S   L   S   T   Y   K   L   E   K   Q   L   L   Q   Q   T   N>

   860        870        880        890        900        910
    .          .          .          .          .          .
GAA ATC TTG AAG ATC CAT GAA AAA AAC AGT TTA TTA GAA CAT AAA ATC TTA GAA ATG GAA
 E   I   L   K   I   H   E   K   N   S   L   L   E   H   K   I   L   E   M   E>

   920        930        940        950        960        970
    .          .          .          .          .          .
GGA AAA CAC AAG GAA GAG TTG GAC ACC TTA AAG GAA GAG AAA GAG AAC CTT CAA GGC TTG
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    .          .          .          .          .          .
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 V   T   R   Q   T   Y   I   I   Q   E   L   E   K   Q   L   N   R   A   T   T>

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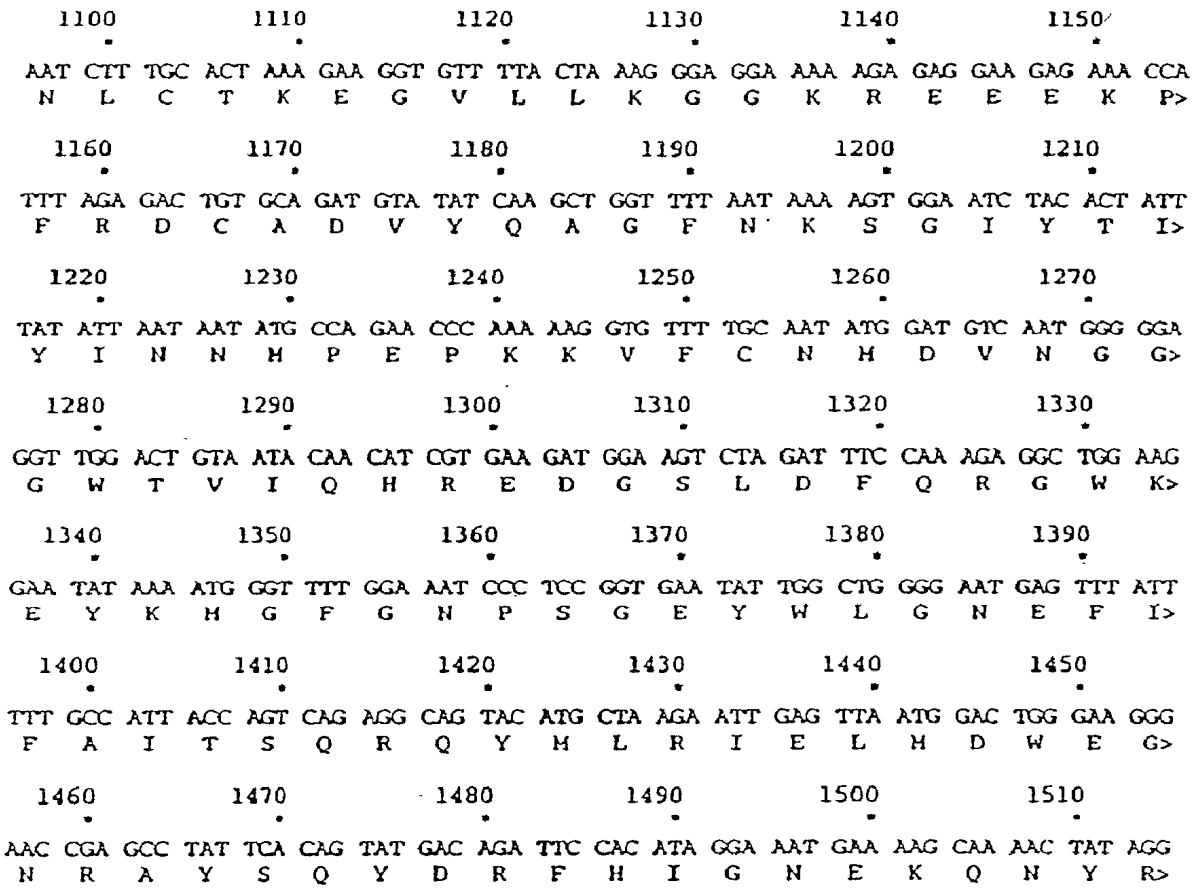
Fig. 4 C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F R D C A D V Y Q A G F N K S G I Y T I>
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Fig. 4 D

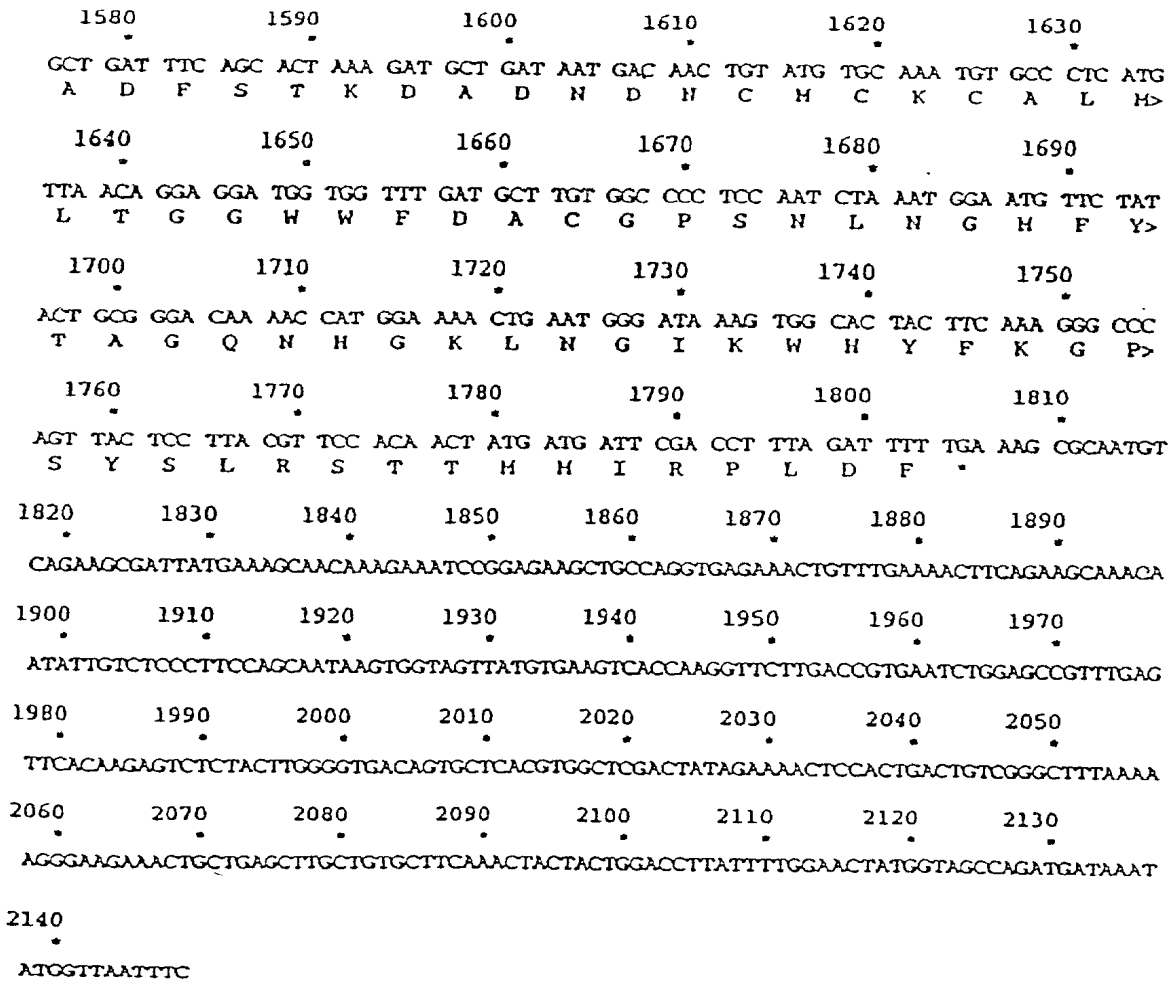
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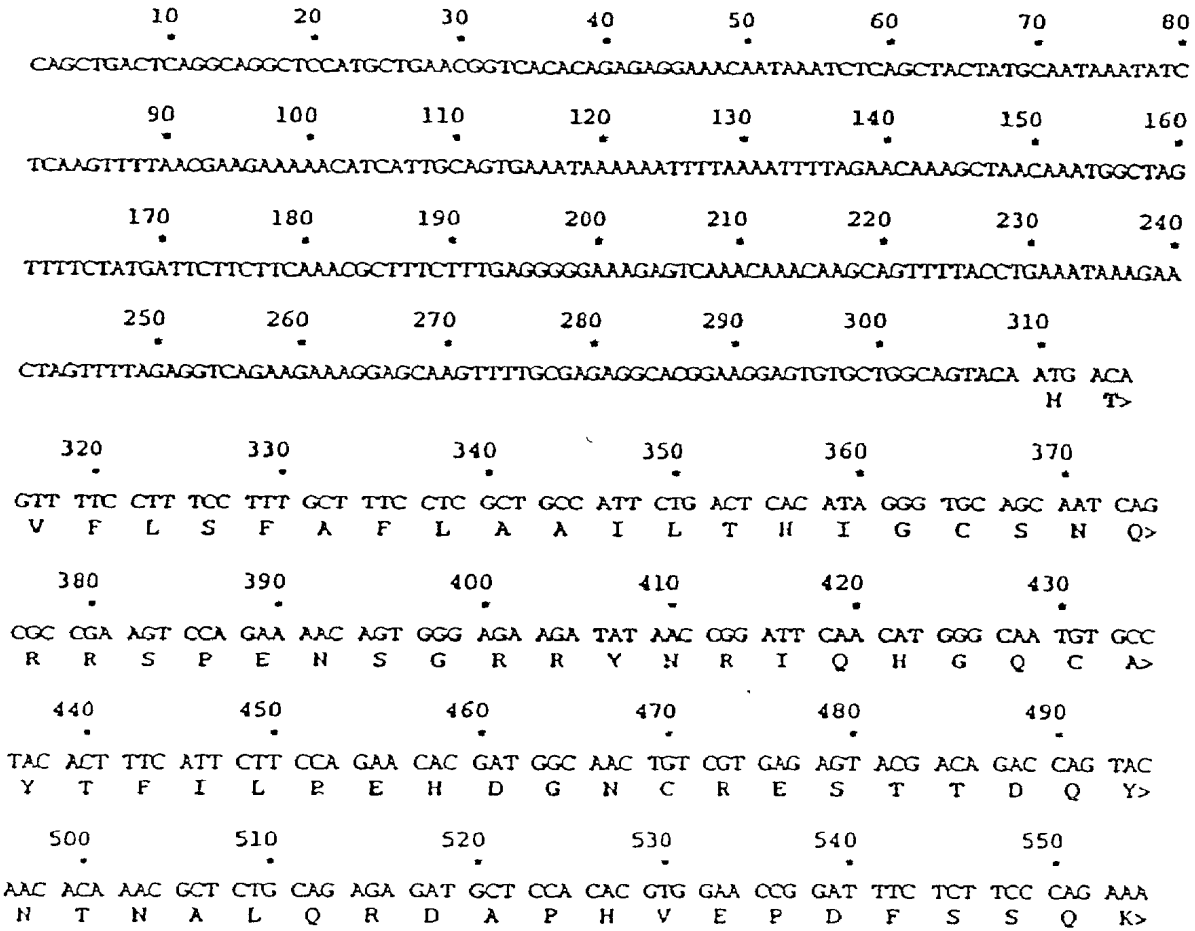
Fig. 5 A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V F L S F A F L A A I L T H I G C S N Q>
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Y T F I L P E H D G N C R E S T T D Q Y>
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Fig. 5B

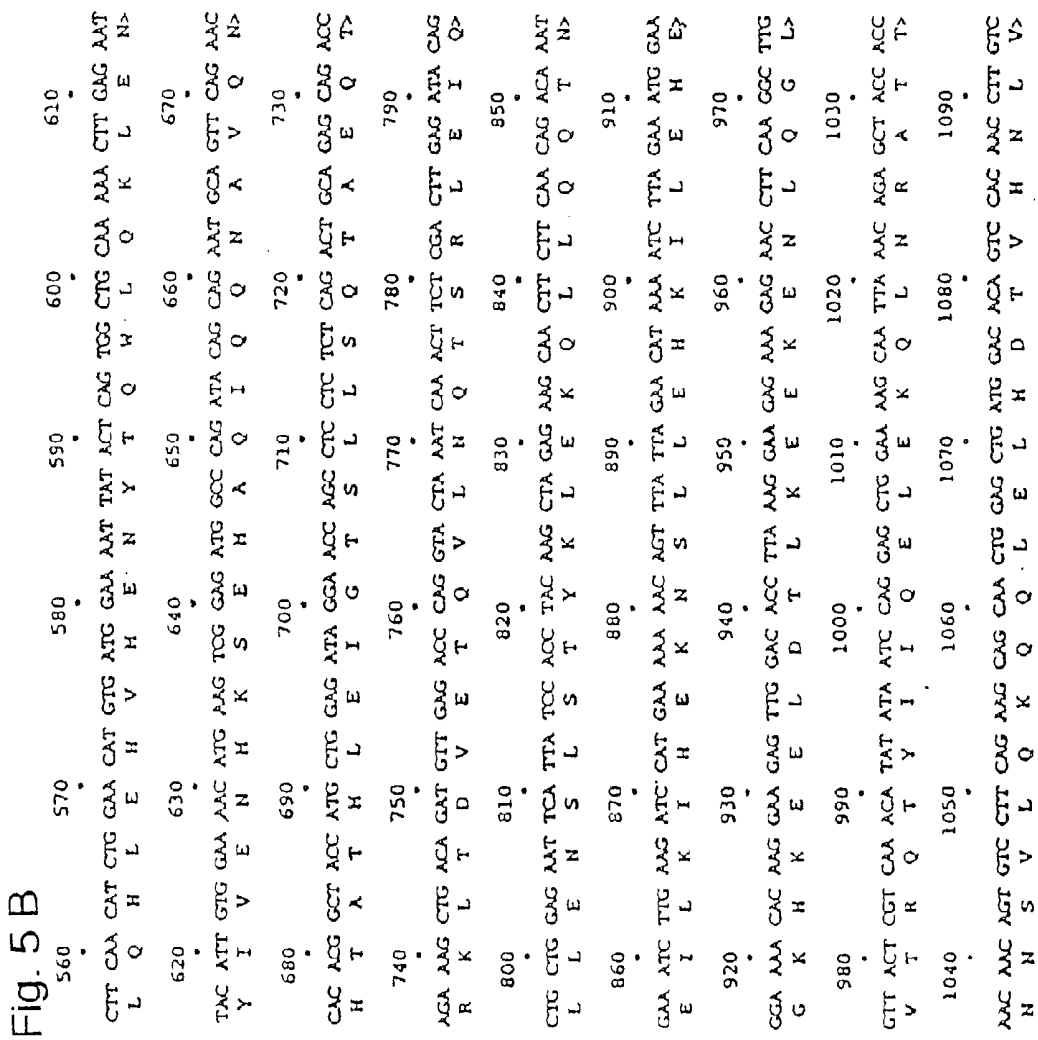

Fig. 5C

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1160        1170        1180        1190        1200        1210
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 R   D   C   A   D   V   Y   Q   A   G   F   N   K   S   G   I   Y   T   I   Y>

1220        1230        1240        1250        1260        1270
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 I   N   N   M   P   E   P   K   K   V   F   C   N   M   D   V   N   G   G   G>

1280        1290        1300        1310        1320        1330
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 W   T   V   I   Q   H   R   E   D   G   S   L   D   F   Q   R   G   W   K   E>

1340        1350        1360        1370        1380        1390
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 Y   K   M   G   F   G   N   P   S   G   E   Y   W   L   G   N   E   F   I   F>

1400        1410        1420        1430        1440        1450
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1460        1470        1480        1490        1500        1510
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1520        1530        1540        1550        1560        1570
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Fig. 5D

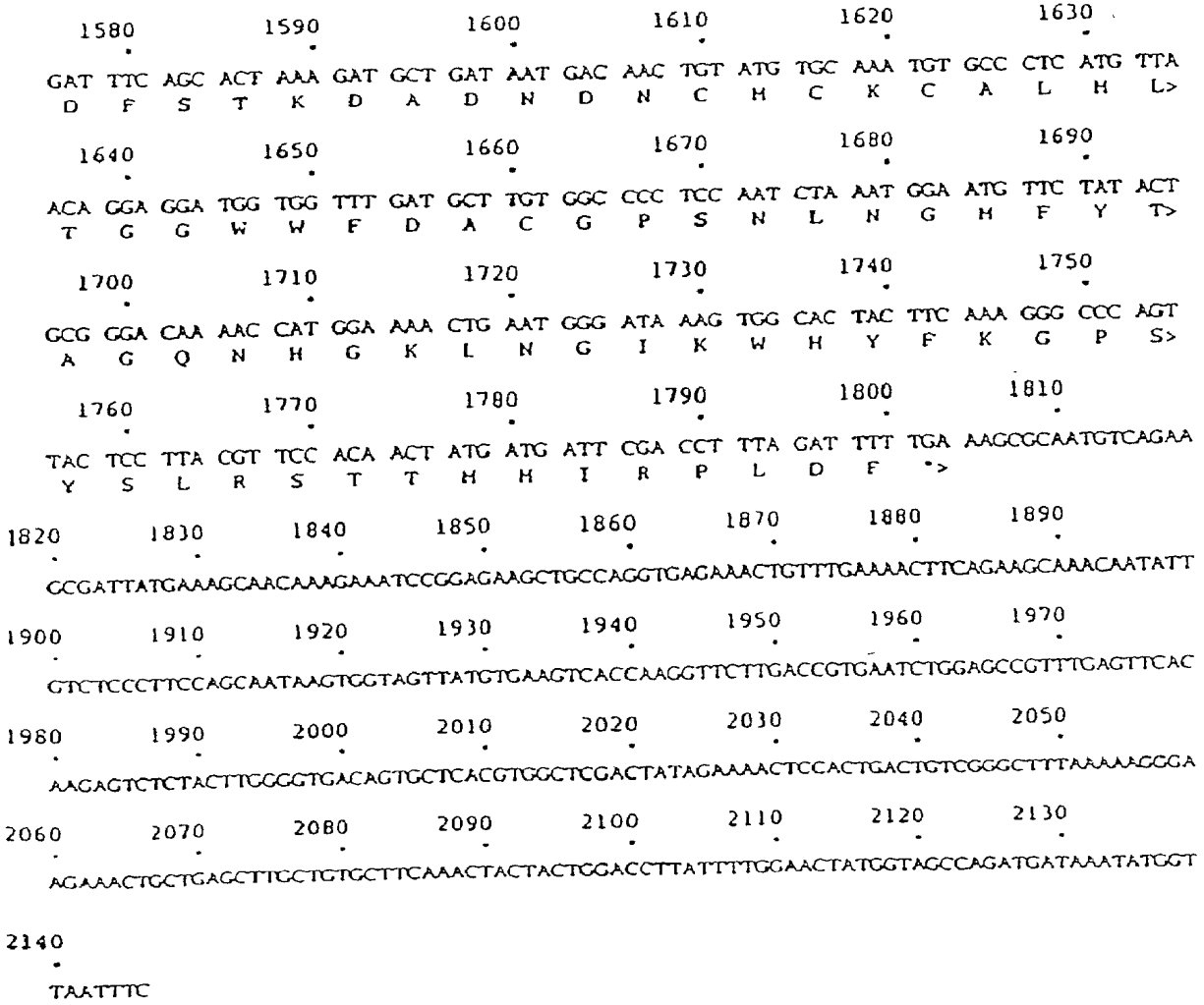
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Fig. 6 A

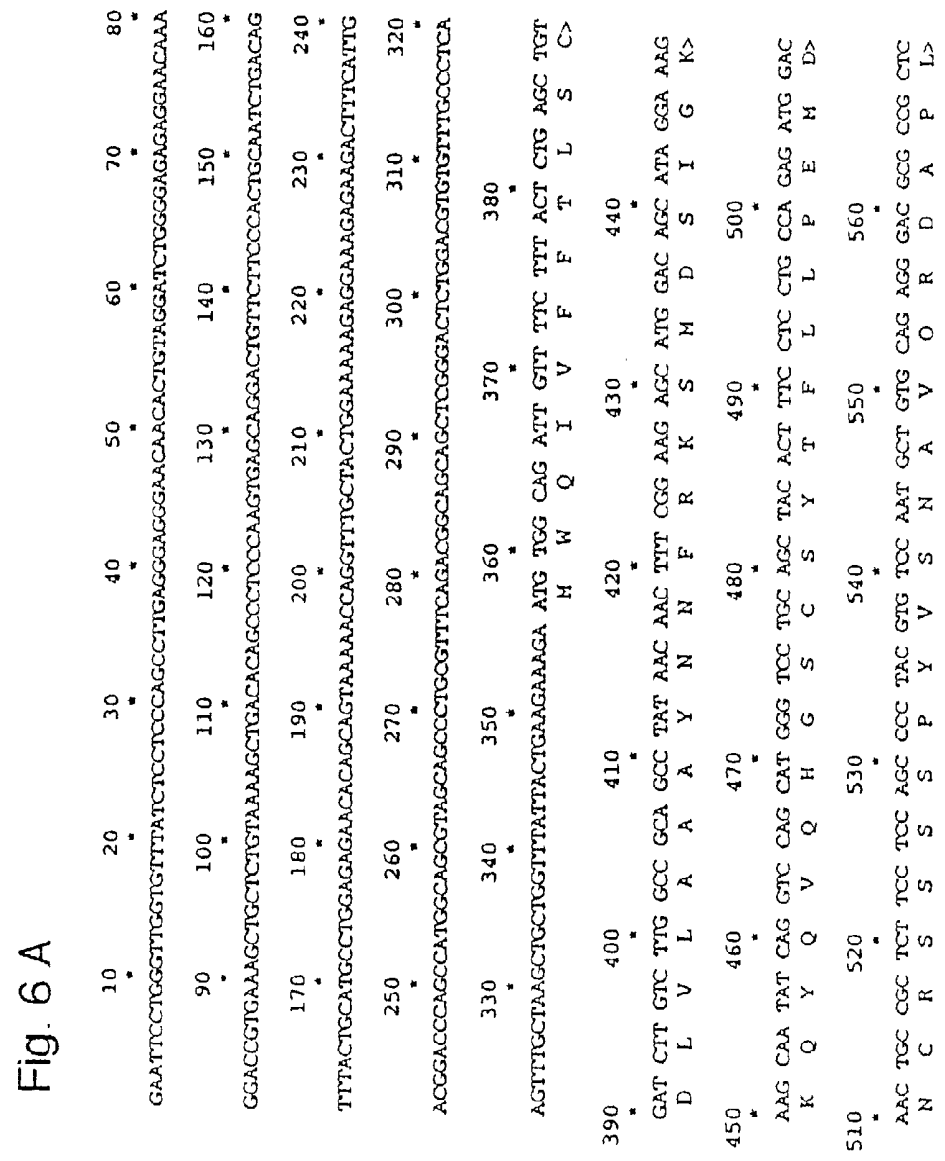
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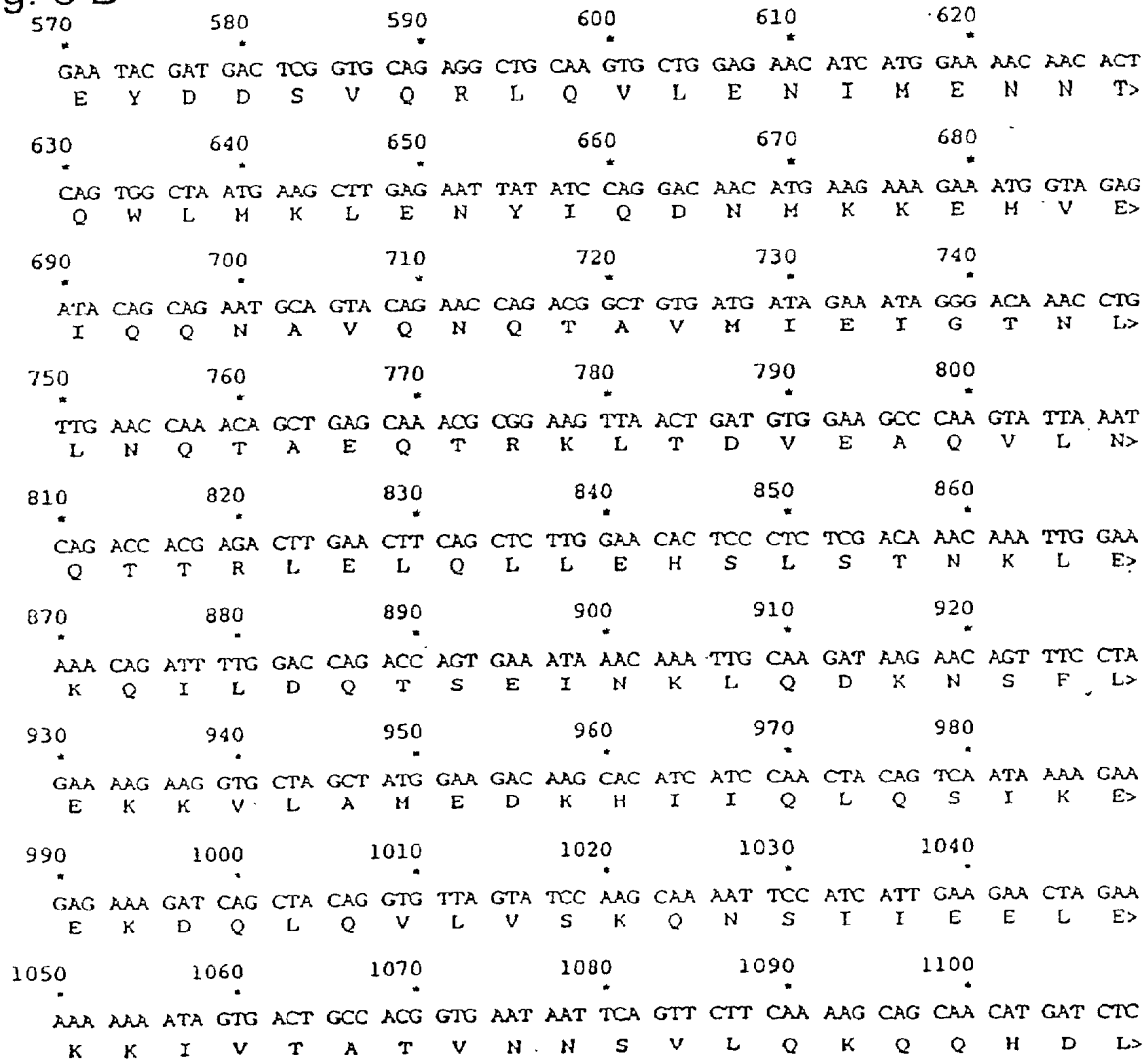
Fig. 6 B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I Q Q N A V Q N Q T A V M I E I G T N L>
750 760 770 780 790 800
TTG AAC CAA ACA GCT GAG CAA ACG CGG AAG TTA ACT GAT GTG GAA GCC CAA GTA TTA AAT
L N Q T A E Q T R K L T D V E A Q V L N>
810 820 830 840 850 860
CAG ACC ACG AGA CTT GAA CTT CAG CTC TTG GAA CAC TCC CTC TCG ACA AAC AAA TTG GAA
Q T T R L E L Q L L E H S L S T N K L E>
870 880 890 900 910 920
AAA CAG ATT TTG GAC CAG ACC AGT GAA ATA AAC AAA TTG CAA GAT AAG AAC AGT TTC CTA
K Q I L D Q T S E I N K L Q D K N S F L>
930 940 950 960 970 980
GAA AAG AAG GTG CTA GCT ATG GAA GAC AAG CAC ATC ATC CAA CTA CAG TCA ATA AAA GAA
E K K V L A M E D K H I I Q L Q S I K E>
990 1000 1010 1020 1030 1040
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E K D Q L Q V L V S K Q N S I I E E L E>
1050 1060 1070 1080 1090 1100
AAA AAA ATA GTG ACT GCC ACG GTG AAT AAT TCA GTT CTT CAA AAG CAG CAA CAT GAT CTC
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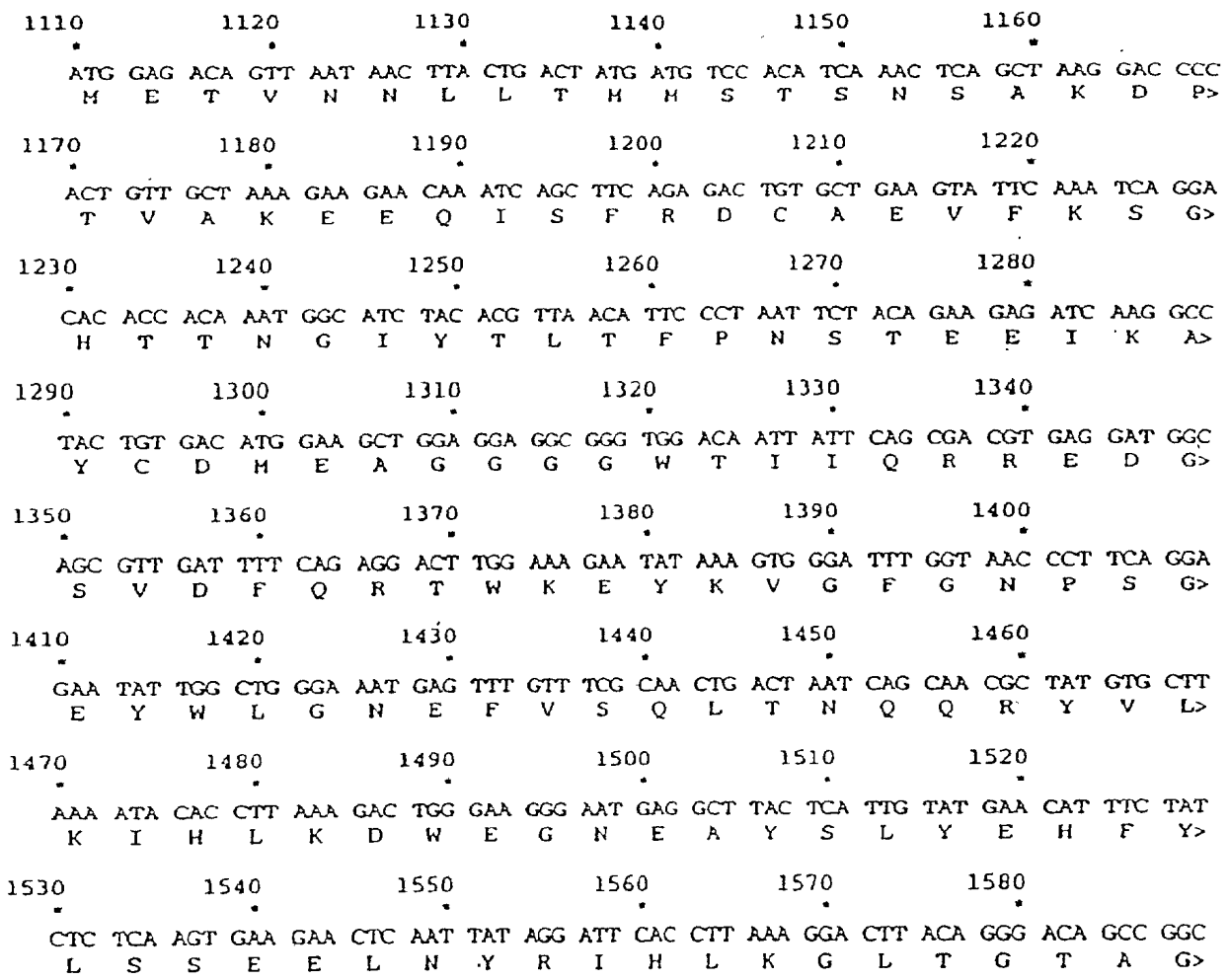
Fig. 6 C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ACT GTT GCT AAA GAA GAA CAA ATC AGC TTC AGA GAC TGT GCT GAA GTA TTC AAA TCA GGA
T V A K E E Q I S F R D C A E V F K S G>
1230 1240 1250 1260 1270 1280
CAC ACC ACA AAT GGC ATC TAC ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC
H T T N G I Y T L T F P N S T E E I K A>
1290 1300 1310 1320 1330 1340
TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT GGC
Y C D M E A G G G G W T I I Q R R E D G>
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S V D F Q R T W K E Y K V G F G N P S G>
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GAA TAT TGG CTG GGA AAT GAG TTT GTT TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT
E Y W L G N E F V S Q L T N Q Q R Y V L>
1470 1480 1490 1500 1510 1520
AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG TAT GAA CAT TTC TAT
K I H L K D W E G N E A Y S L Y E H F Y>
1530 1540 1550 1560 1570 1580
CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC
L S S E E L N Y R I H L K G L T G T A G>
ATG GAG ACA GTT AAT AAC TTA CTG ACT ATG ATG TCC ACA TCA AAC TCA GCT AAG GAC CCC
M E T V N N L L T M M S T S N S A K D P>
ACT GTT GCT AAA GAA GAA CAA ATC AGC TTC AGA GAC TGT GCT GAA GTA TTC AAA TCA GGA
T V A K E E Q I S F R D C A E V F K S G>
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H T T N G I Y T L T F P N S T E E I K A>
TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT GGC
Y C D M E A G G G G W T I I Q R R E D G>
AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA
S V D F Q R T W K E Y K V G F G N P S G>
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E Y W L G N E F V S Q L T N Q Q R Y V L>
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K I H L K D W E G N E A Y S L Y E H F Y>
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L S S E E L N Y R I H L K G L T G T A G>
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M E T V N N L L T M M S T S N S A K D P>
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T V A K E E Q I S F R D C A E V F K S G>
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H T T N G I Y T L T F P N S T E E I K A>
TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT GAG GAT GGC
Y C D M E A G G G G W T I I Q R R E D G>
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S V D F Q R T W K E Y K V G F G N P S G>
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E Y W L G N E F V S Q L T N Q Q R Y V L>
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K I H L K D W E G N E A Y S L Y E H F Y>
CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC
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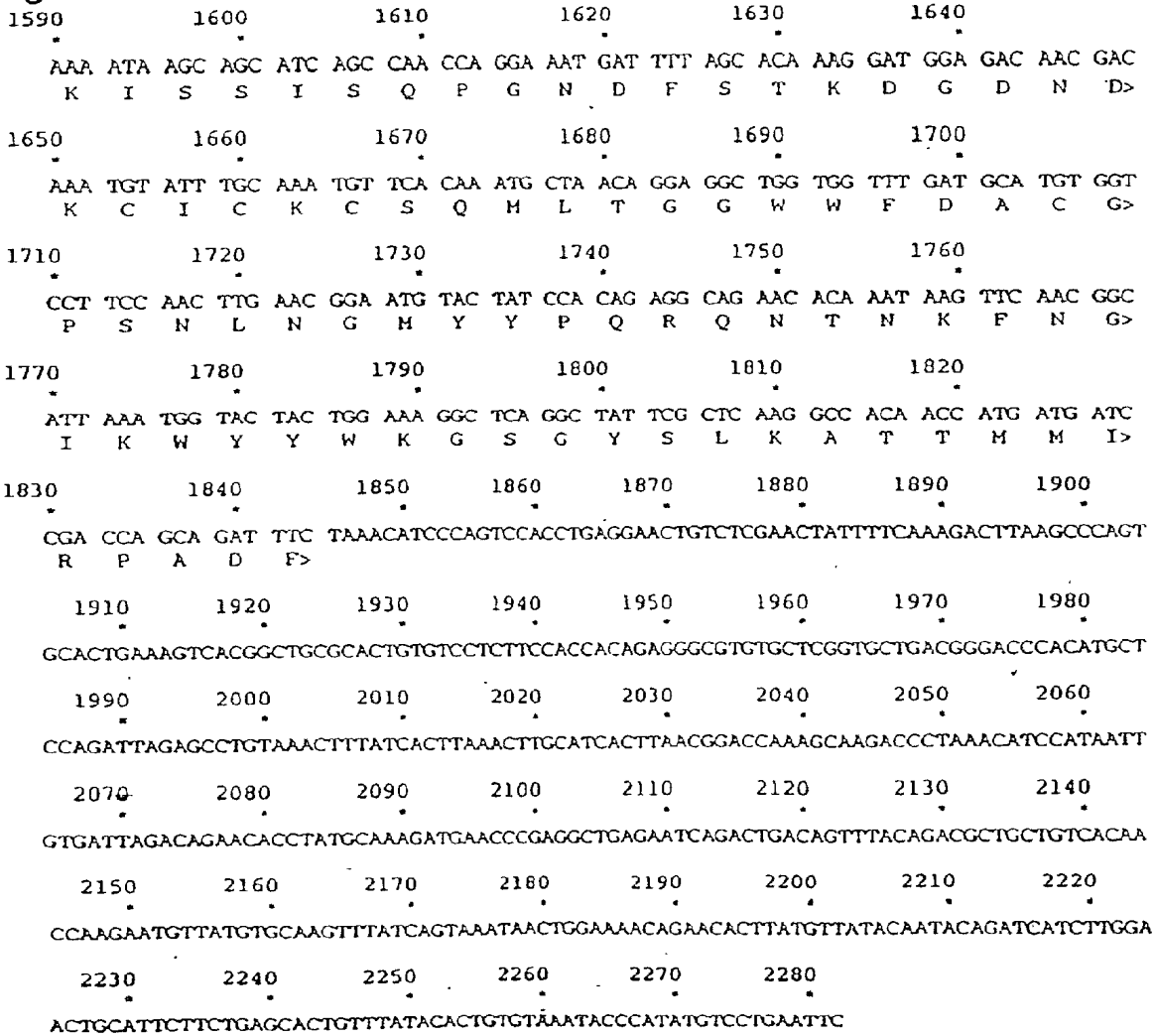
Fig. 6 D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1650 1660 1670 1680 1690 1700
AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG TTT GAT GCA TGT GGT
K C I C K C S Q M L T G G W W F D A C G>
1710 1720 1730 1740 1750 1760
CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC
P S N L N G M Y Y P Q R Q N T N K F N G>
1770 1780 1790 1800 1810 1820
ATT AAA TGG TAC TAC TGG AAA GGC TCA GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC
I K W Y Y W K G S G Y S L K A T T M M I>
1830 1840 1850 1860 1870 1880 1890 1900
CGA CCA GCA GAT TTC TAAACATCCCAGTCCACCTGAGGAACTGTCTCGAACTATTTTCAAAGACTTAAGCCCAGT
R P A D F>
1910 1920 1930 1940 1950 1960 1970 1980
GCACTGAAAGTCACGGCTGCGCACTGTGTCCTCTTCCACCACAGAGGGCGTGTGCTCGGTGCTGACGGGACCCACATGCT
1990 2000 2010 2020 2030 2040 2050 2060
CCAGATTAGAGCCTGTAAACTTTATCACTTAAACTTGCATCACTTAACGGACCAAAGCAAGACCCTAAACATCCATAATT
2070 2080 2090 2100 2110 2120 2130 2140
GTGATTAGACAGAACACCTATGCAAAGATGAACCCGAGGCTGAGAATCAGACTGACAGTTTACAGACGCTGCTGTCACAA
2150 2160 2170 2180 2190 2200 2210 2220
CCAAGAATGTTATGTGCAAGTTTATCAGTAAATAACTGGAAAACAGAACACTTATGTTATACAATACAGATCATCTTGGA
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Fig. 7

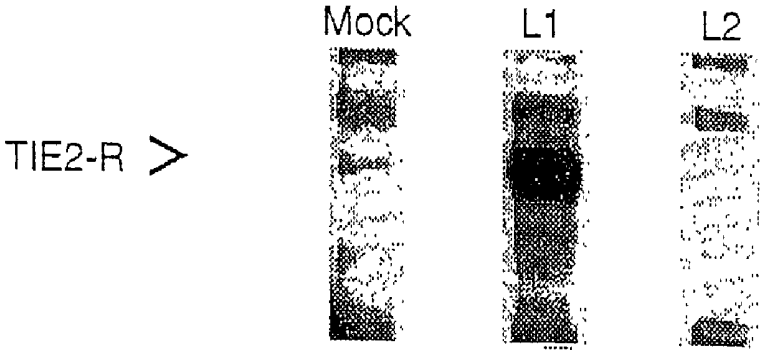


Fig. 8

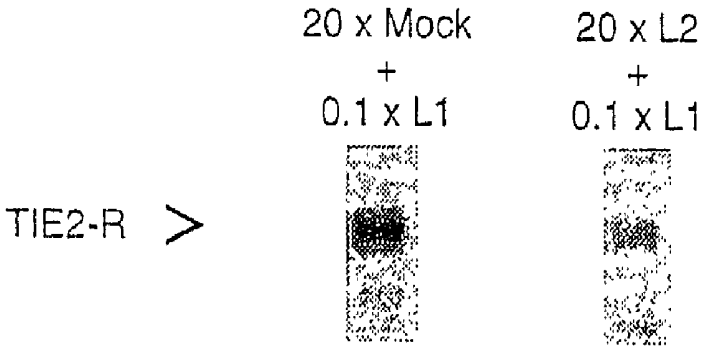


Fig. 9

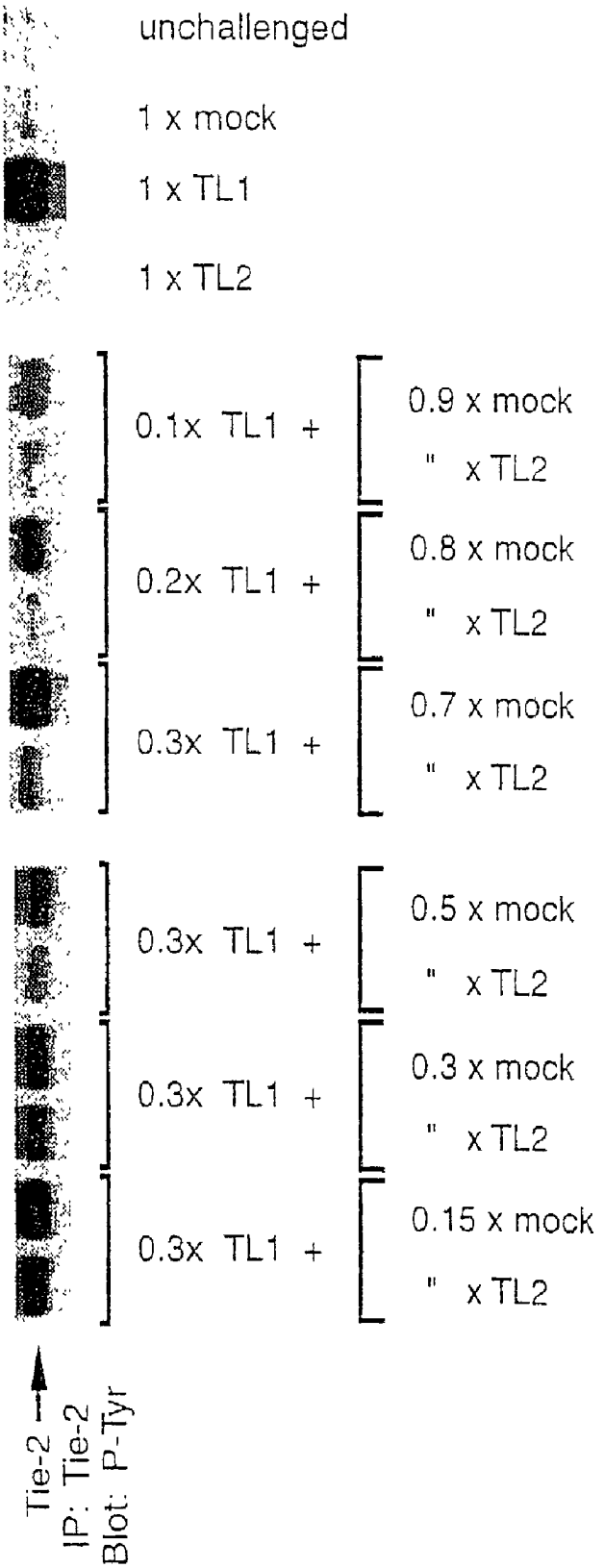

unchallenged
1 x mock
1 x TL1
1 x TL2
0.1x TL1 +
0.9 x mock
" x TL2
0.2x TL1 +
0.8 x mock
" x TL2
0.3x TL1 +
0.7 x mock
" x TL2
0.3x TL1 +
0.5 x mock
" x TL2
0.3x TL1 +
0.3 x mock
" x TL2
0.3x TL1 +
0.15 x mock
" x TL2
Tie-2
IP: Tie-2
Blot: P-Tyr

Fig. 10 A

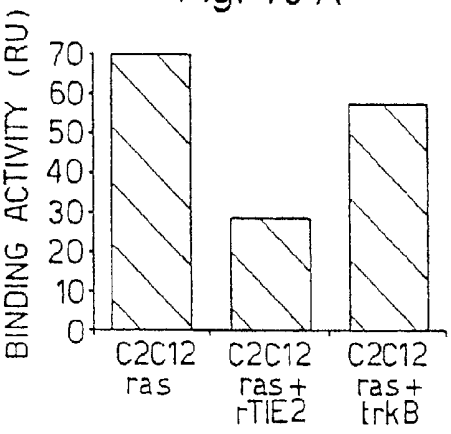


Fig. 10 B

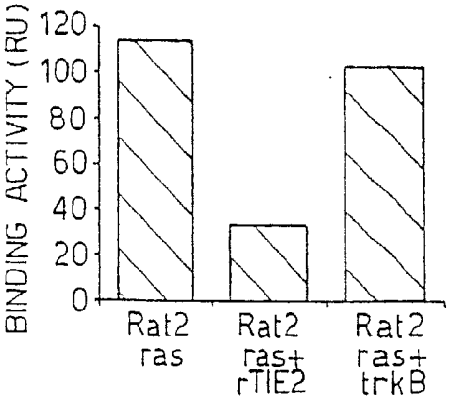


Fig. 10 C

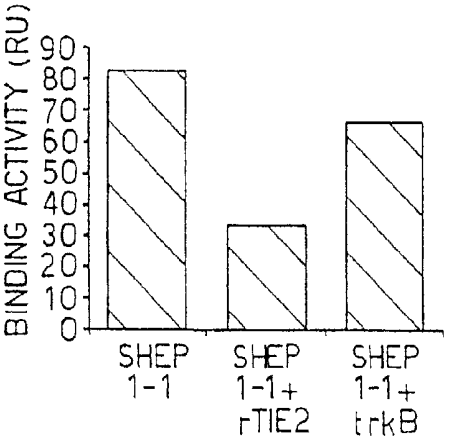


Fig. 10 D

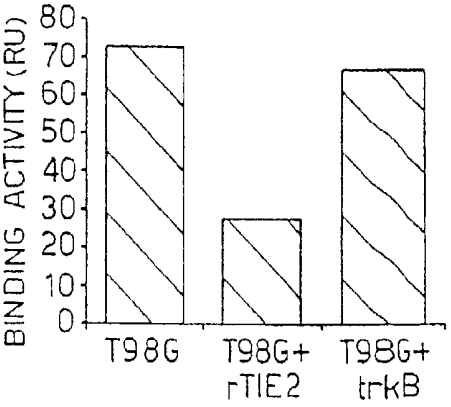


Fig. 11 A

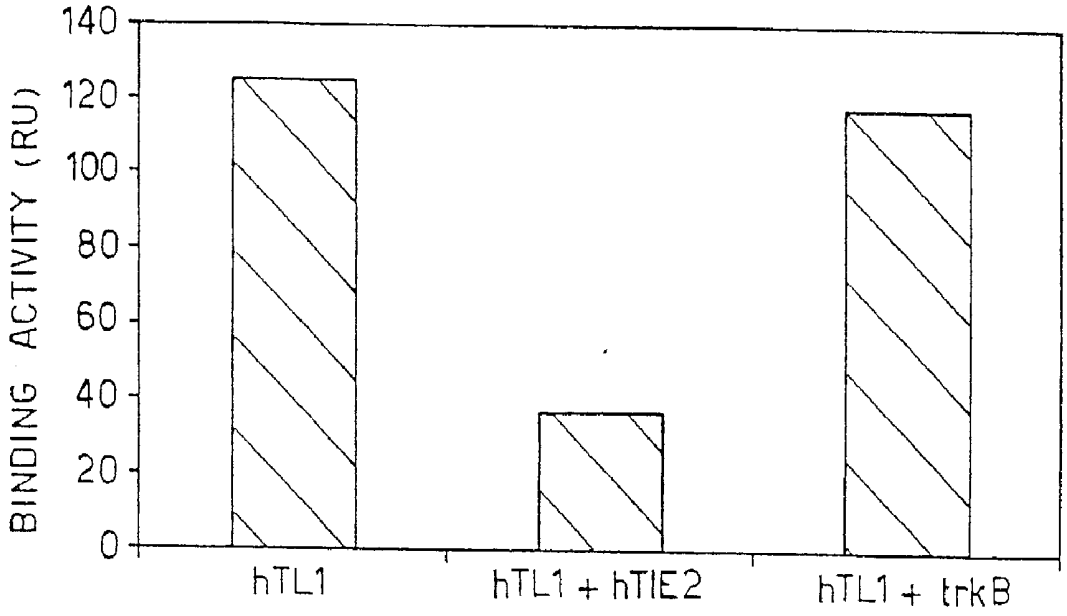


Fig. 11 B

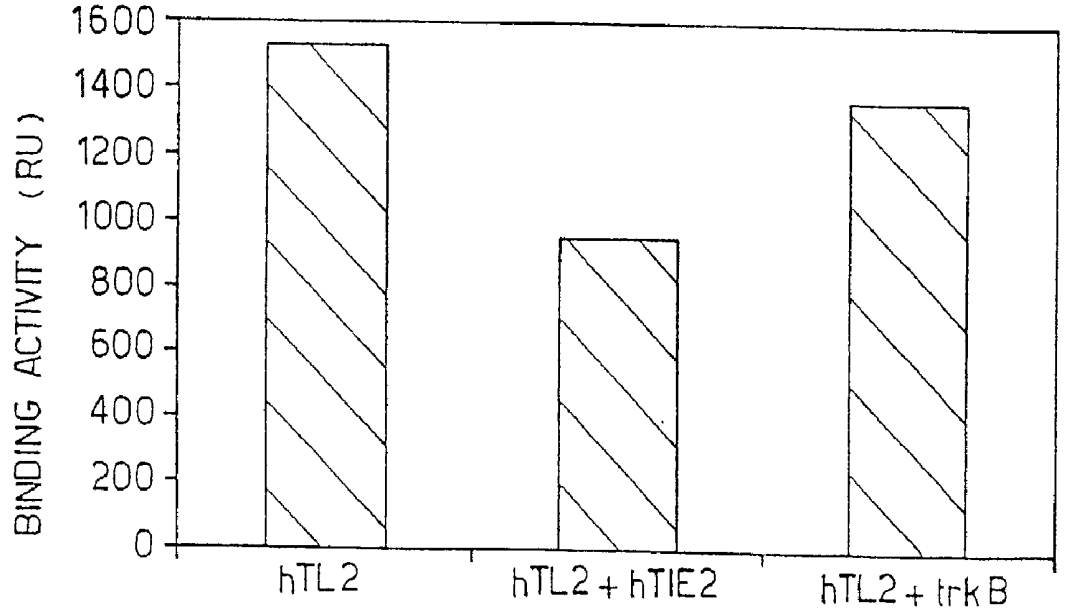


Fig. 12

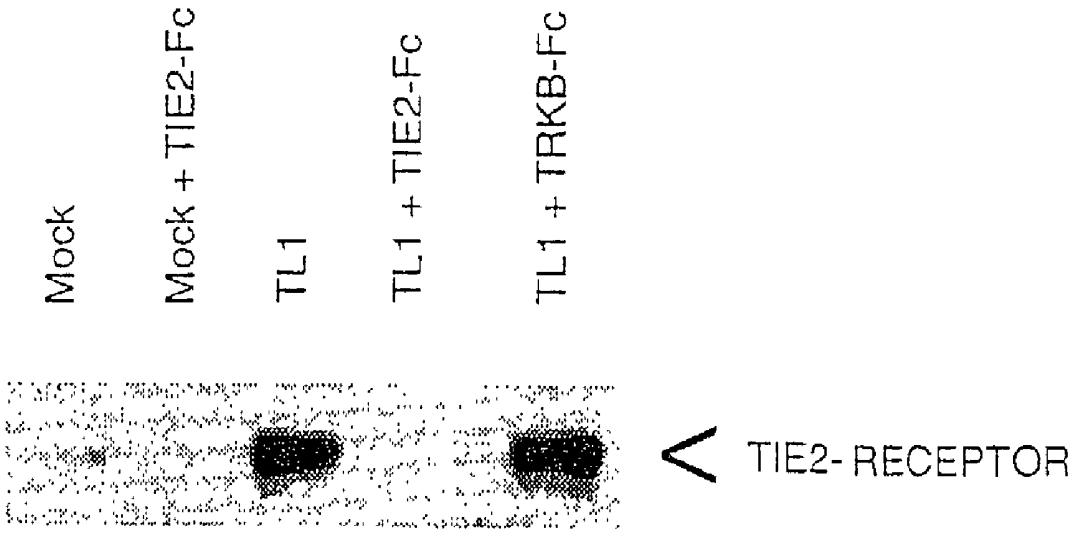
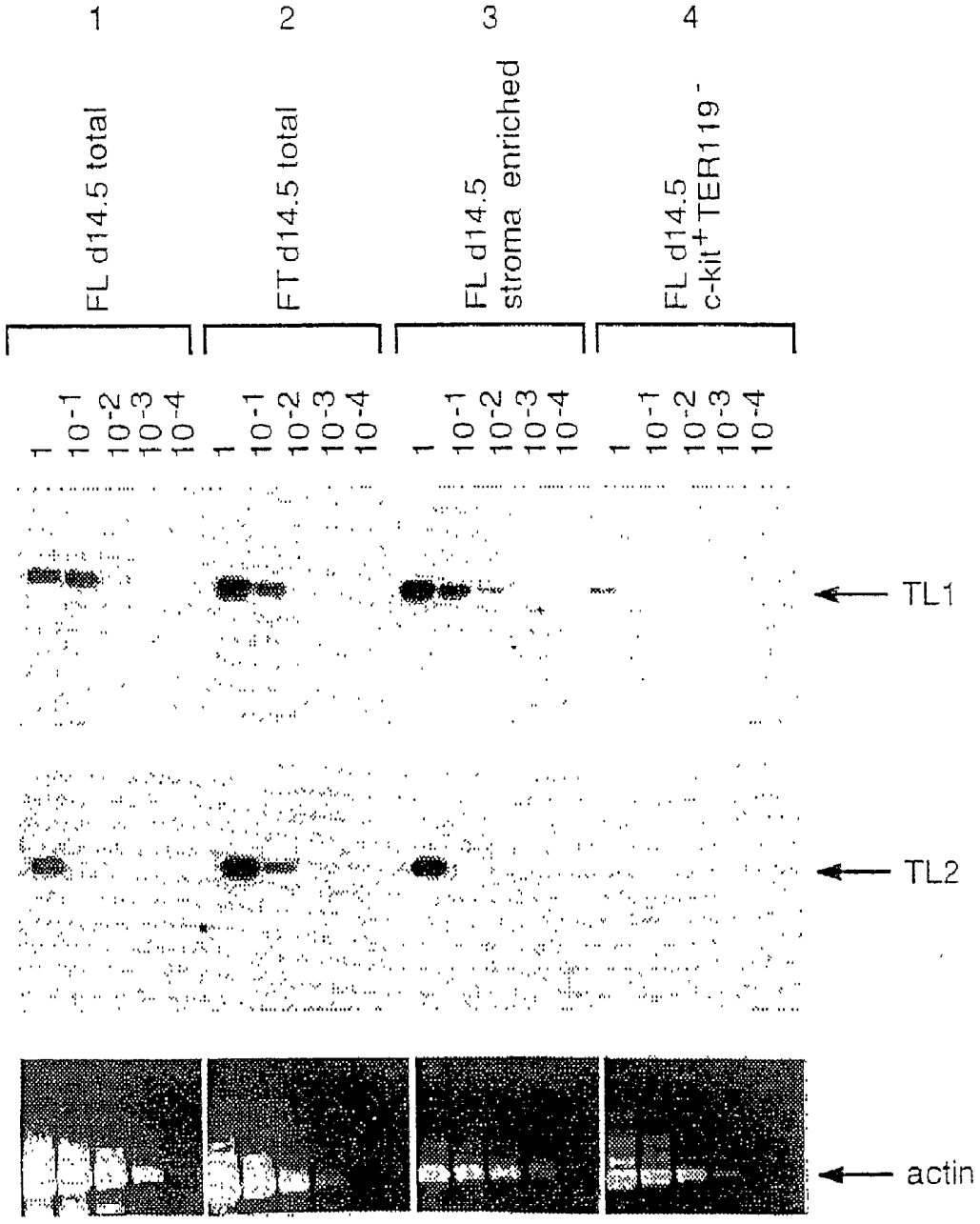
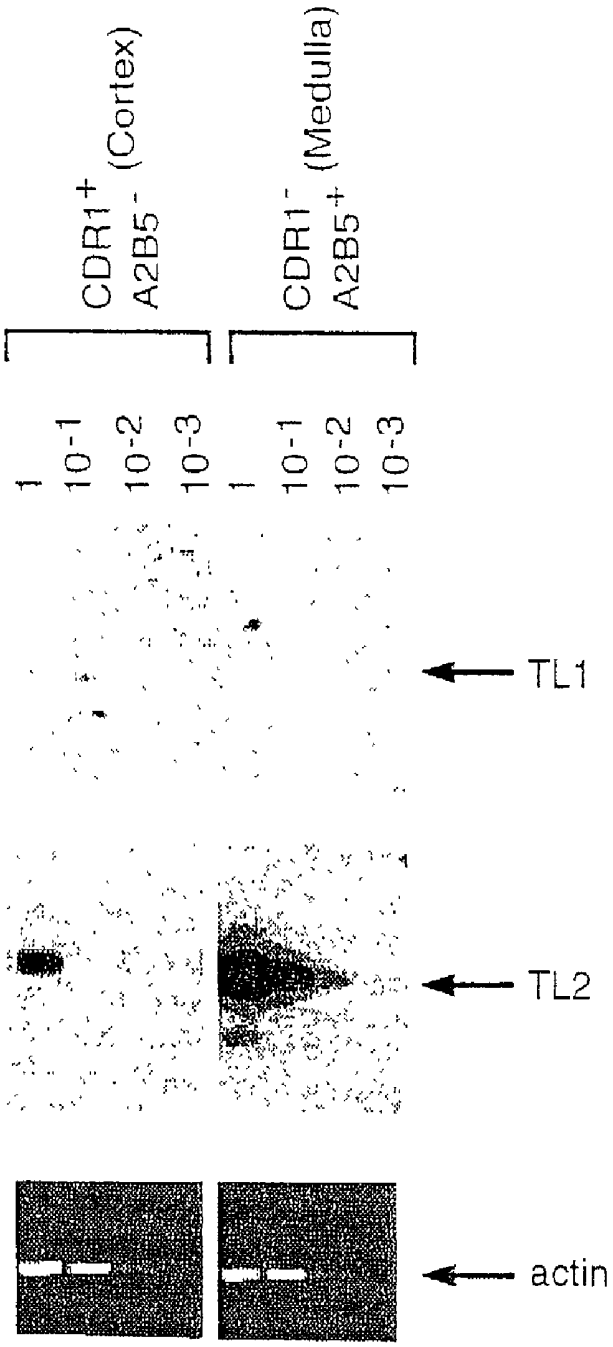


Fig. 13



FL: Fetal Liver

Fig. 14



Fetal Thymus E17.5

$CDR1^+$: Cortical stromal cells

$A2B5^+$: Medulla stromal cells

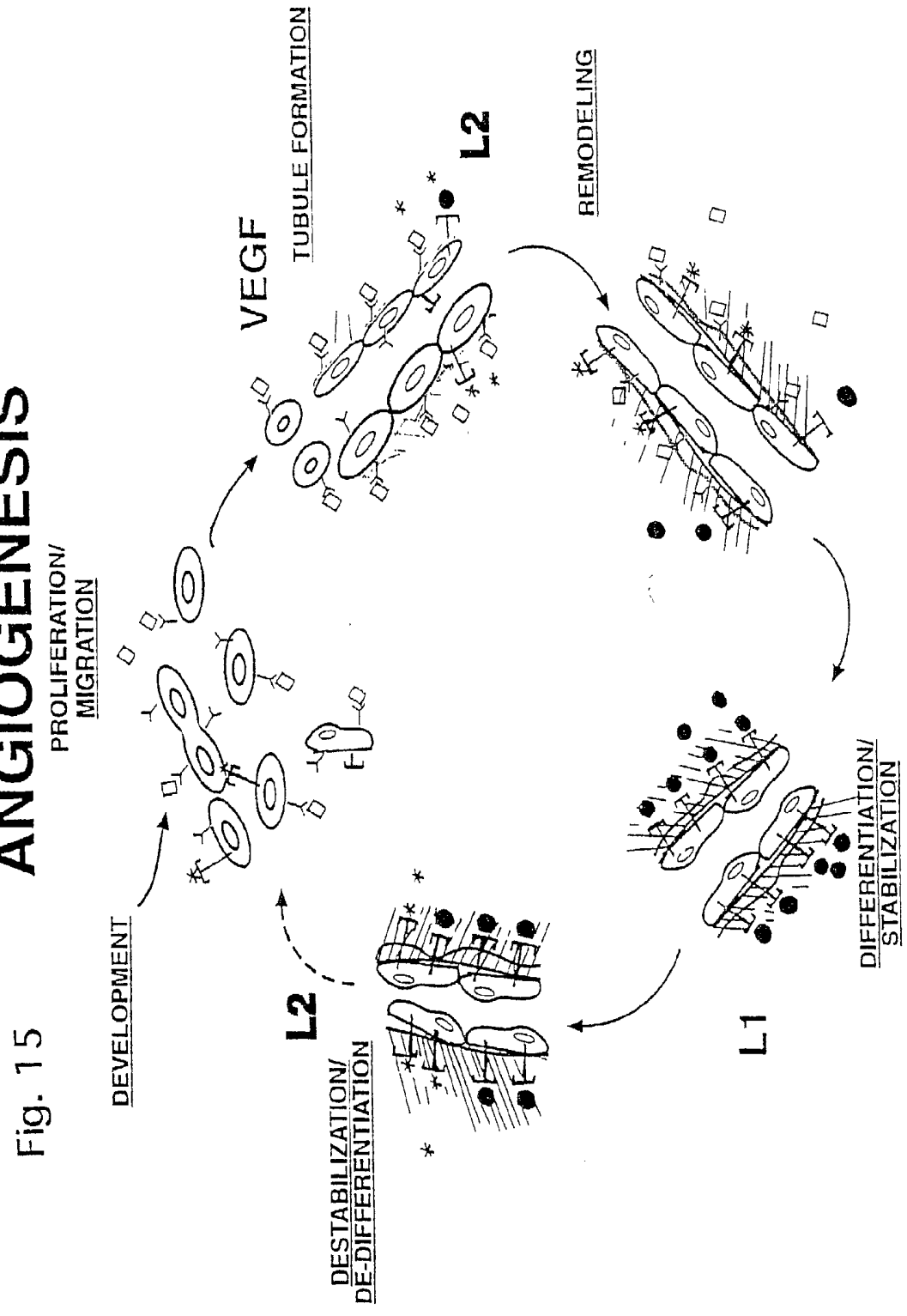

ANGIOGENESIS
PROLIFERATION/
MIGRATION
VEGF
TUBULE FORMATION
L2
REMODELING
DEVELOPMENT
L2
DESTABILIZATION/
DE-DIFFERENTIATION
L1
DIFFERENTIATION/
STABILIZATION

Fig. 15

Fig. 16

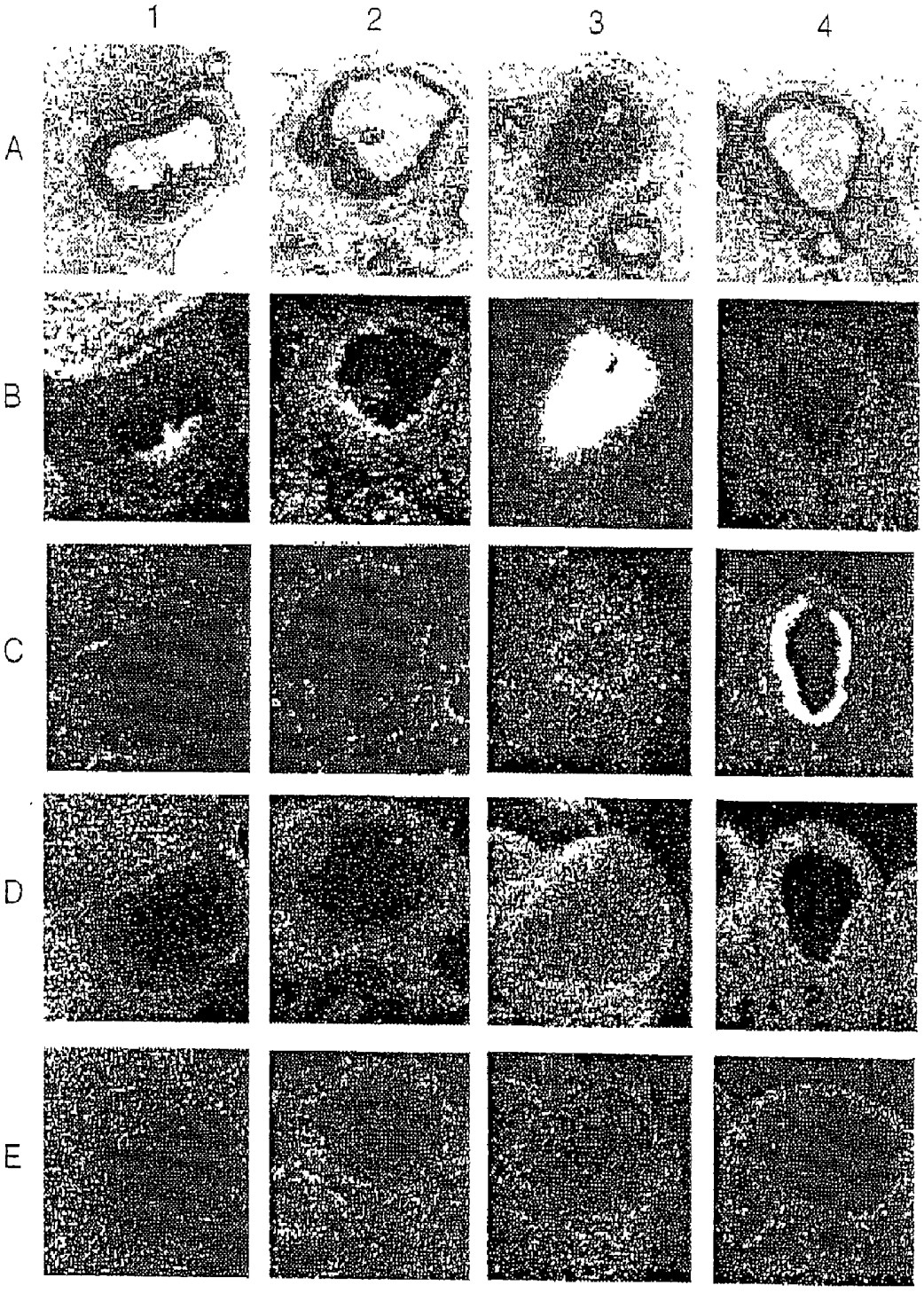
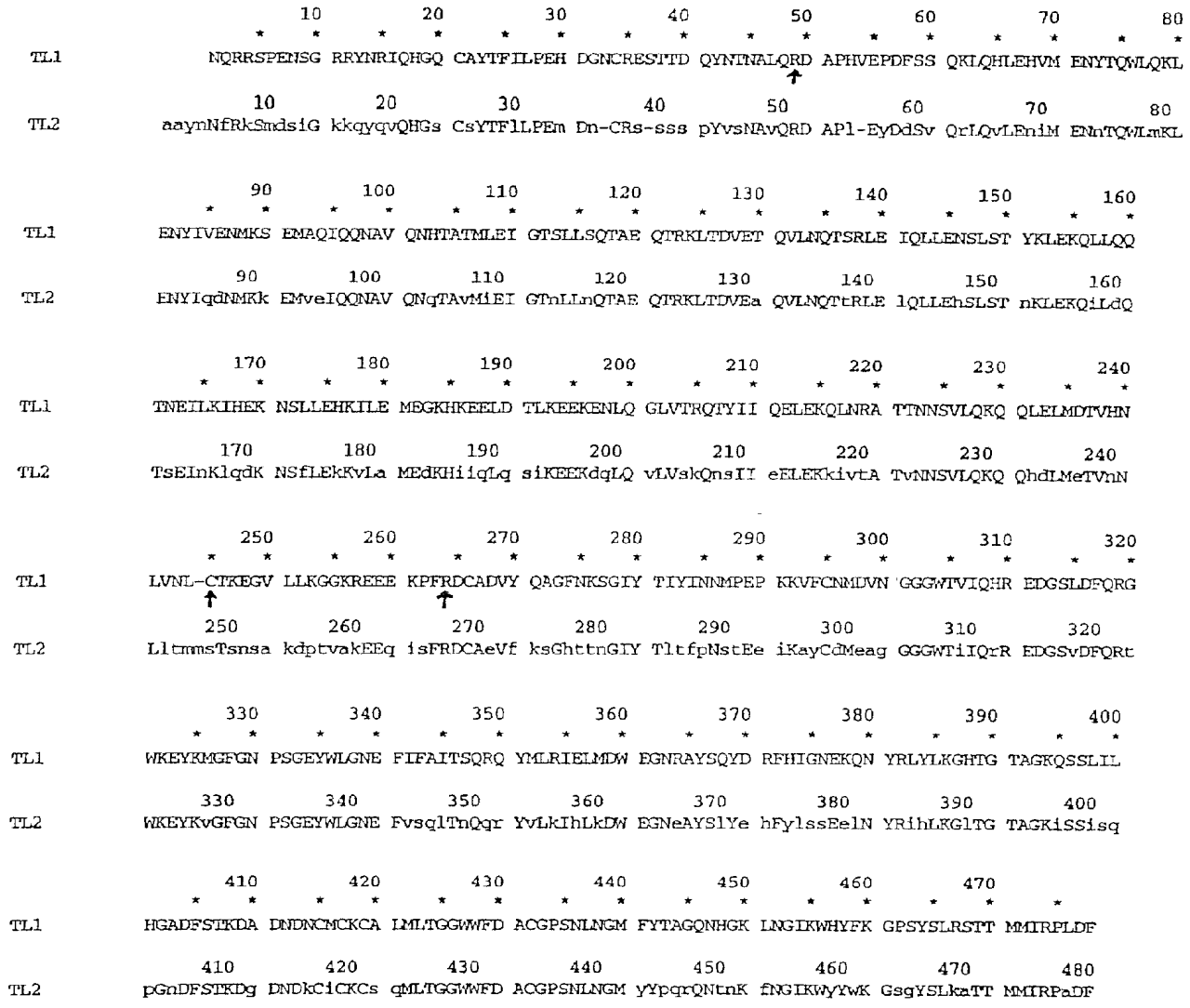


Fig. 17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TL2 aaynNfRkSmdsiG kkqyqvQHGs CsYTFlLPEm Dn-CRs-sss pYvsNAvQRD APl-EyDdSv QrLQvLEniM ENnTQWLmKL
90 100 110 120 130 140 150 160
TL1 ENYIVENMKS EMAQIQQNAV QNHTATMLEI GTSLLSQTAE QTRKLTDVET QVLNQTSRLE IQLLENSLST YKLEKQLLQQ
TL2 ENYIqdNMKk EMveIQQNAV QNqTAvMiEI GTnLLnQTAE QTRKLTDVEa QVLNQTtRLE lQLLEhSLST nKLEKQiLdQ
170 180 190 200 210 220 230 240
TL1 TNEILKIHEK NSLLEHKILE MDGKHKEELD TLKEEKENLQ GLVTRQTYII QELEKQLNRA TTNNSVLQKQ QLELMDIVHN
TL2 TsEInKlqdK NSfLEkKvLa MEdKHiiqLq siKEEKdqLQ vLVskQnsII eELEKkivtA TvNNSVLQKQ QhdLMeTVnN
250 260 270 280 290 300 310 320
TL1 LVNL-CTKEGV LLKGGKREEE KPFRDCADVY QAGFNKSGIY TIYINNMPEP KKVFCNMDVN GGGWTVIQHR EDGSLDFQRG
TL2 LltmmsTsnsa kdptvakEEq isFRDCAeVf ksGhttnGIY TltfpNstEe iKayCdMeag GGGWTiIQrR EDGSvDFQRt
330 340 350 360 370 380 390 400
TL1 WKEYKMGFGN PSGEYWLGNE FIFAITSQRQ YMLRIELMDW EGNRAYSQYD RFHIGNEKQN YRLYLKGHTG TAGKQSSLIL
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410 420 430 440 450 460 470
TL1 HGADFSTKDA DNDNCMCKCA LMLTGGWWFD ACGPSNLNGM FYTAGQNHGK LNGIKWHYFK GPSYSLRSTT MMIRPLDF
TL2 pGnDFSTKDg DNDkCiCKCs qMLTGGWWFD ACGPSNLNGM yYpqrQNtnK fNGIKWyYwK GsgYSLkaTT MMIRPaDF

Fig. 18

COVALENT MULTIMERIC STRUCTURE OF
TL1 AND TL2 AND THEIR INTERCONVERSION
BY THE MUTATION OF ONE CYSTEINE

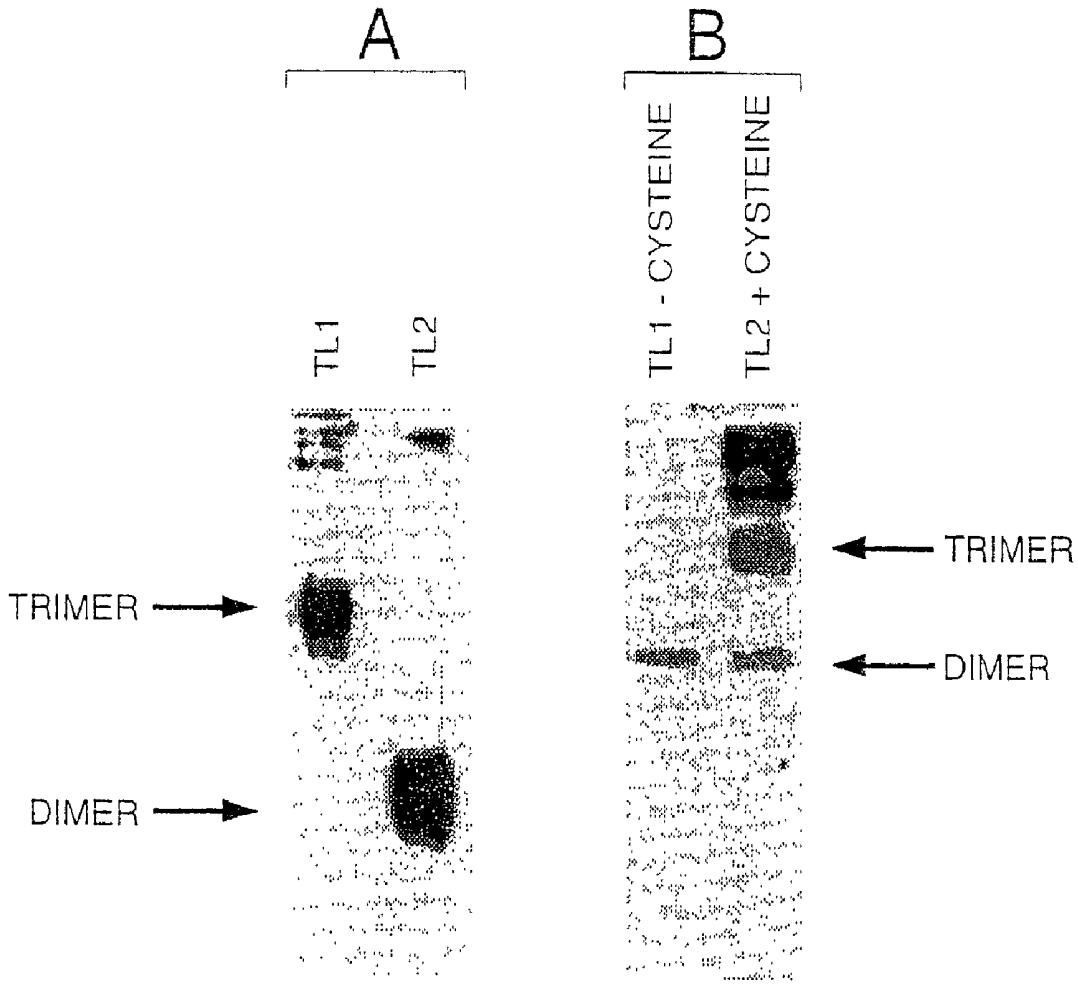
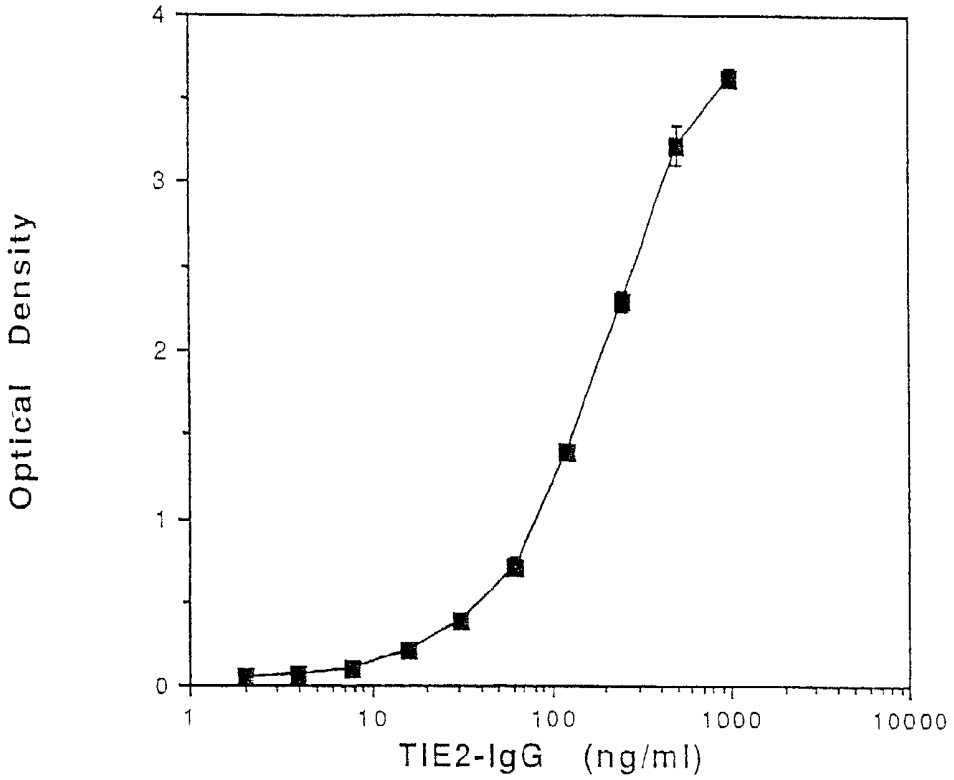


Fig. 19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4
3
2
1
0
1
10
100
1000
10000
TIE2-IgG (ng/ml)

Fig. 20

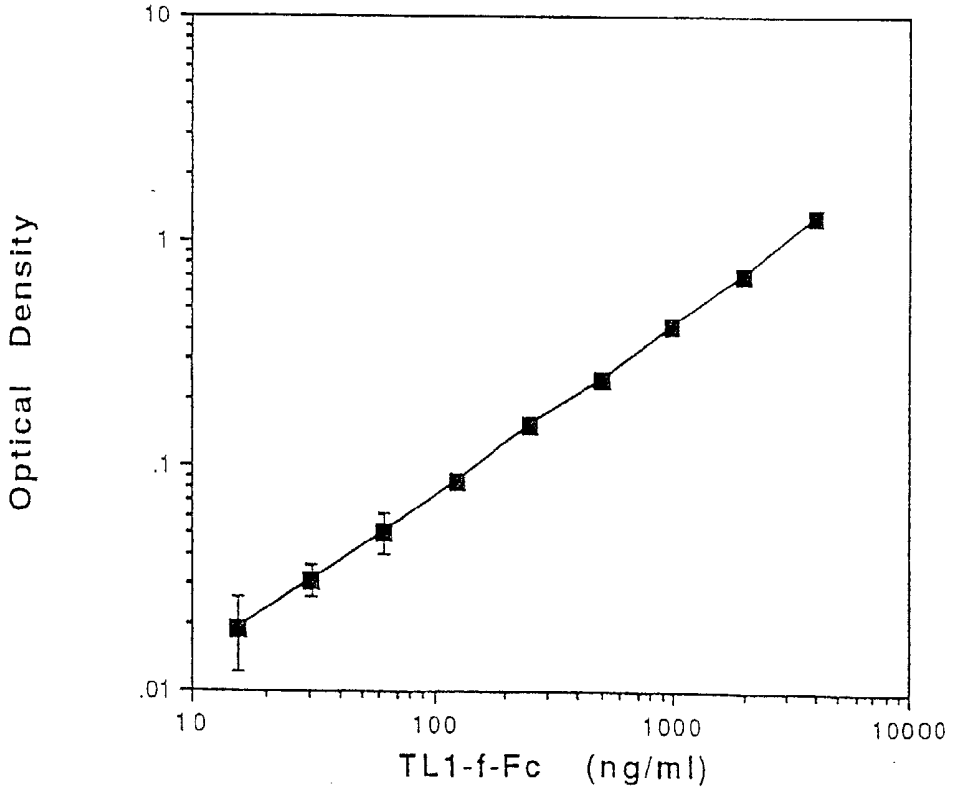
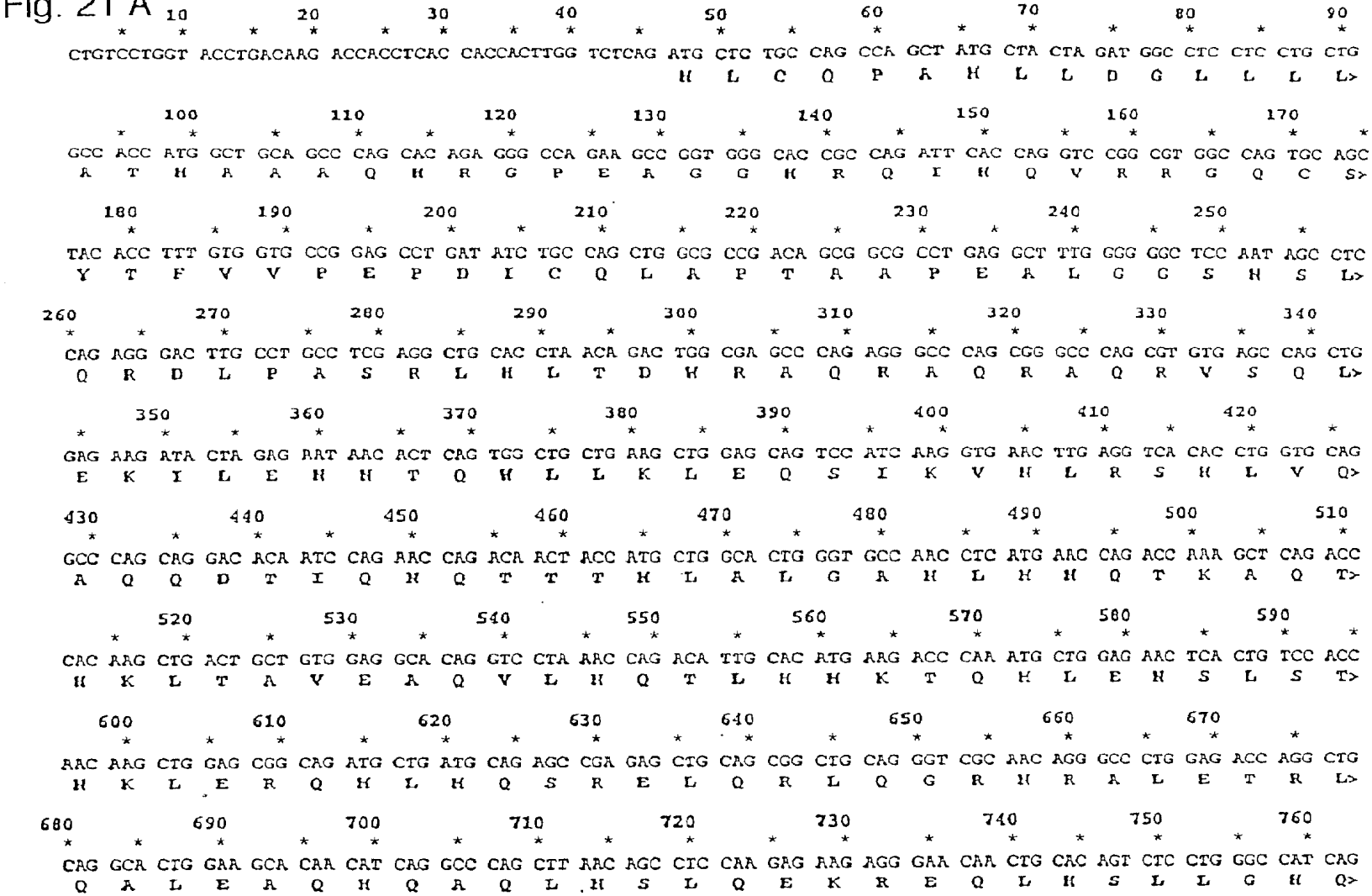

Optical Density
10
1
.1
.01
10
100
1000
10000
TL1-f-Fc (ng/ml)

Fig. 21 A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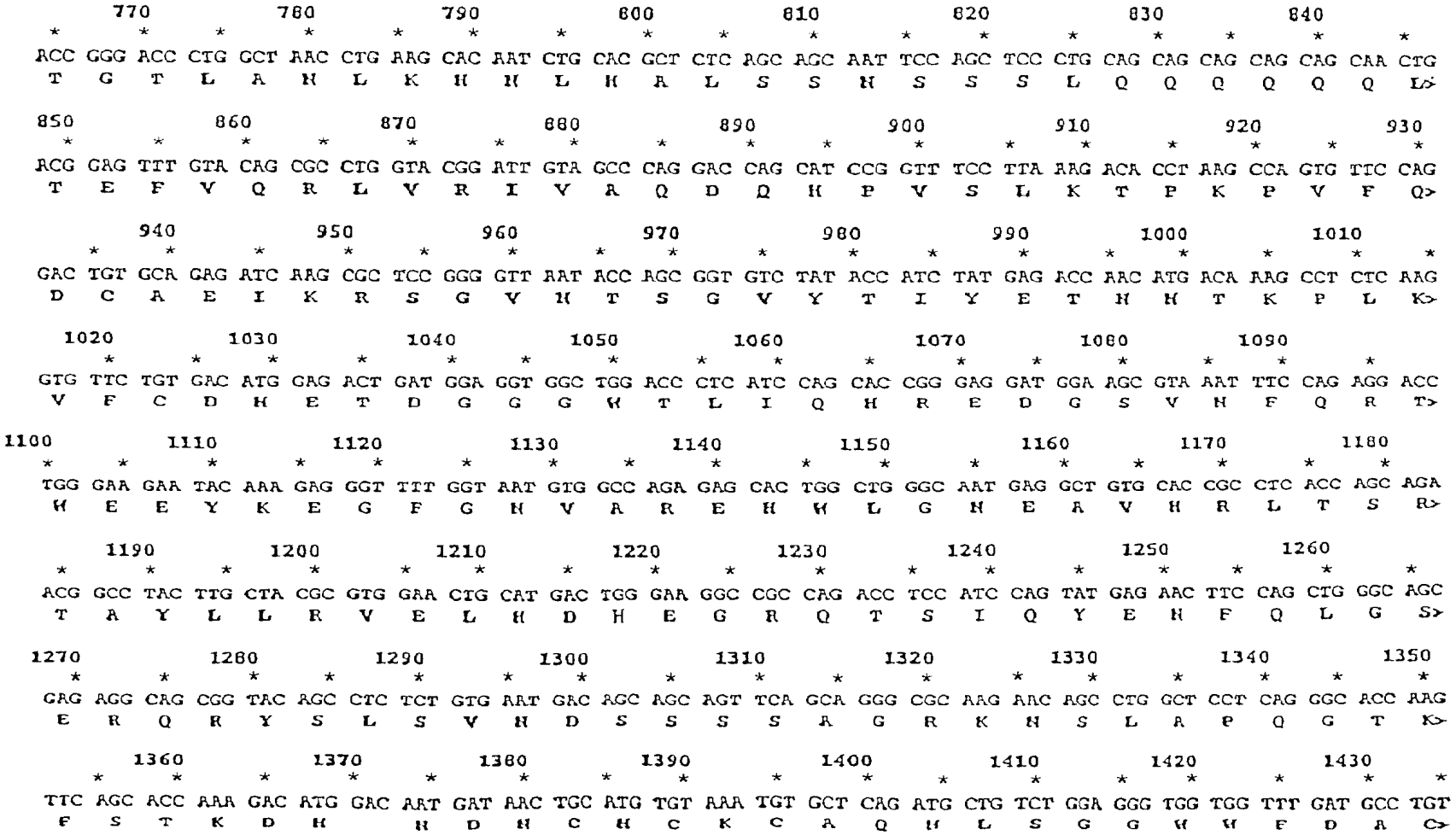
M L C Q P A M L L D G L L L L>
100 110 120 130 140 150 160 170
GCC ACC ATG GCT GCA GCC CAG CAC AGA GGG CCA GAA GCC GGT GGG CAC CGC CAG ATT CAC CAG GTC CGG CGT GGC CAG TGC AGC
A T M A A A Q H R G P E A G G H R Q I H Q V R R G Q C S>
180 190 200 210 220 230 240 250
TAC ACC TTT GTG GTG CCG GAG CCT GAT ATC TGC CAG CTG GCG CCG ACA GCG GCG CCT GAG GCT TTG GGG GGC TCC AAT AGC CTC
Y T F V V P E P D I C Q L A P T A A P E A L G G S N S L>
260 270 280 290 300 310 320 330 340
CAG AGG GAC TTG CCT GCC TCG AGG CTG CAC CTA ACA GAC TGG CGA GCC CAG AGG GCC CAG CGG GCC CAG CGT GTG AGC CAG CTG
Q R D L P A S R L H L T D W R A Q R A Q R A Q R V S Q L>
350 360 370 380 390 400 410 420
GAG AAG ATA CTA GAG AAT AAC ACT CAG TGG CTG CTG AAG CTG GAG CAG TCC ATC AAG GTG AAC TTG AGG TCA CAC CTG GTG CAG
E K I L E N N T Q W L L K L E Q S I K V N L R S H L V Q>
430 440 450 460 470 480 490 500 510
GCC CAG CAG GAC ACA ATC CAG AAC CAG ACA ACT ACC ATG CTG GCA CTG GGT GCC AAC CTC ATG AAC CAG ACC AAA GCT CAG ACC
A Q Q D T I Q N Q T T T M L A L G A N L M N Q T K A Q T>
520 530 540 550 560 570 580 590
CAC AAG CTG ACT GCT GTG GAG GCA CAG GTC CTA AAC CAG ACA TTG CAC ATG AAG ACC CAA ATG CTG GAG AAC TCA CTG TCC ACC
H K L T A V E A Q V L N Q T L H M K T Q M L E N S L S T>
600 610 620 630 640 650 660 670
AAC AAG CTG GAG CGG CAG ATG CTG ATG CAG AGC CGA GAG CTG CAG CGG CTG CAG GGT CGC AAC AGG GCC CTG GAG ACC AGG CTG
N K L E R Q M L M Q S R E L Q R L Q G R N R A L E T R L>
680 690 700 710 720 730 740 750 760
CAG GCA CTG GAA GCA CAA CAT CAG GCC CAG CTT AAC AGC CTC CAA GAG AAG AGG GAA CAA CTG CAC AGT CTC CTG GGC CAT CAG
Q A L E A Q H Q A Q L N S L Q E K R E Q L H S L L G H Q>

Fig. 21 B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T G T L A N L K H N L H A L S S N S S S L Q Q Q Q Q Q L>
850 860 870 880 890 900 910 920 930
ACG GAG TTT GTA CAG CGC CTG GTA CGG ATT GTA GCC CAG GAC CAG CAT CCG GTT TCC TTA AAG ACA CCT AAG CCA GTG TTC CAG
T E F V Q R L V R I V A Q D Q H P V S L K T P K P V F Q>
940 950 960 970 980 990 1000 1010
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D C A E I K R S G V N T S G V Y T I Y E T N M T K P L K>
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V F C D M E T D G G G W T L I Q H R E D G S V N F Q R T>
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W E E Y K E G F G N V A R E H W L G N E A V H R L T S R>
1190 1200 1210 1220 1230 1240 1250 1260
ACG GCC TAC TTG CTA CGC GTG GAA CTG CAT GAC TGG GAA GGC CGC CAG ACC TCC ATC CAG TAT GAG AAC TTC CAG CTG GGC AGC
T A Y L L R V E L H D W E G R Q T S I Q Y E N F Q L G S>
1270 1280 1290 1300 1310 1320 1330 1340 1350
GAG AGG CAG CGG TAC AGC CTC TCT GTG AAT GAC AGC AGC AGT TCA GCA GGG CGC AAG AAC AGC CTG GCT CCT CAG GGC ACC AAG
E R Q R Y S L S V N D S S S S A G R K N S L A P Q G T K>
1360 1370 1380 1390 1400 1410 1420 1430
TTC AGC ACC AAA GAC ATG GAC AAT GAT AAC TGC ATG TGT AAA TGT GCT CAG ATG CTG TCT GGA GGG TGG TGG TTT GAT GCC TGT
F S T K D M D N D N C M C K C A Q M L S G G W W F D A C>
.

Fig. 21 C

```
   1440          1450          1460          1470          1480          1490          1500          1510
     *             *             *             *             *             *             *             *
GGC CTC TCC AAC CTC AAT GGC ATC TAC TAT TCA GTT CAT CAG CAC TTG CAC AAG ATC AAT GGC ATC CGC TGG CAC TAC TTC CGA
 G   L   S   N   L   N   G   I   Y   Y   S   V   H   Q   H   L   H   K   I   N   G   I   R   W   H   Y   F   R>

1520          1530          1540          1550          1560          1570          1580          1590          1600
   *             *             *             *             *             *             *             *             *
GGC CCC AGC TAC TCA CTG CAC GGC ACA CGC ATG ATG CTG AGG CCA ATG GGT GCC TGA CACA CAGCCCTGCA GAGACTGATG
 G   P   S   Y   S   L   H   G   T   R   H   H   L   R   P   H   G   A   *>                    .

      1610          1620          1630          1640          1650          1660          1670          1680          1690          1700
        *             *             *             *             *             *             *             *             *             *
CCGTAGGAGG ATTCTCAACC CAGGTGACTC TGTGCACGCT GGGCCCTGCC CAGAAATCAG TGCCCAGGGC TCATCTTGAC ATTCTGGAAC ATCGGAACCA

      1710          1720          1730          1740          1750          1760          1770          1780          1790          1800
        *             *             *             *             *             *             *             *             *             *
GCTTACCTTG CCCCTGAATT ACAAGAATTC ACCTGCCTCC CTGTTGCCCT CTAATTGTGA AATTGCTGGG TGCTTGAAGG CACCTGCCTC TGTTGGAACC

      1810          1820          1830          1840
        *             *             *             *
ATACTCTTTC CCCCTCCTGC TGCATGCCCG GGAATCCCTG CCATGAACT
```

Fig. 22 A

```
                    10         20         30         40         50         60         70         80
mTL3          MLLDGLLLLA TMAAAQHRGP EAGGHRQIHQ VRRGQCSYTF VVPEPDICQL APTAAPEALG GSNSLQRDLP ASRLHIADWR
hTL1.         af.aai.thi -gcsn.r.s. .ns.r-rynr iqh...a... il..h-dg-n cresttdq-y nt.a....a- ---p.-v--e>
chTL1.        af.aa..ahi -gctt.r... ..s.r-rfnr iqh...t... il..q-dg-n cresttdq-y nt.a....a- ---p.-v-e->
mTL1.   mtvflsfaffaailthigcsn.r.n. .n..r.-ynr iqh...a... il..h.-gn- cres.t.qy- nt.a....a. ----.v-e-->
mTL2. mwqiifltfgwd.v.. saysnfrksv dst.r..-y. .qn.p..... ll..t.s.r- -ssss.-ym- -..av...a. ---.dy-.-->
hTL2  mwqivfftlscd.v.. aaynnfrksm dsi.kk.-y. .qh.s..... ll..m.n.r- -ssss.-yv- -..av...a. ---.ey-.-->

                    90        100        110        120        130        140        150        160
mTL3          AQRAQRAQRV SQLEKILENN TQWLLKLEQS IKVHLRSHLV QAQQDTIQNQ TTTMLALGAN LMNQTKAQTH KLTAVEAQVL
hTL1.         pdf--ss.kl qh..hvm..y ....q...ny .ve.mk.ema .i..nav..h .a...ei.ts .ls..ae..r ...d..t...>
chTL1.        qdf--sf.kl qh..hvm..y ....q...sy .ve.mk.em. .l..nav..h .a...ei.ts .ls..ae..r ...d..t...>
mTL1.         pdfs--s.kl qh..hvm..y ....q...ny .ve.mk.ema .i..nav..h .a...ei.ts .ls..ae..r ...d..t...>
mTL2.         -dsv..l.-. --..n..... ....m...ny .qd.mkkem. ei..nvv... .av.iei.ts .l...a...r ...d......>
hTL2.         -dsv..l.-. --..n.m... ....m...ny .qd.mkkem. ei..nav... .av.iei.t. .l...ae..r ...d......>

                    170        180        190        200        210        220        230        240
mTL3          NQTLHMKTQM LENSLSTNKL ERQMLMQSRE LQRLQGRNRA LETRLQALEA QHQAQLNSLQ EKREQLHSLL GHQTGTLANL
hTL1.         ...srlei.l .......y.. .k.l.q.tn. ilkihek.sl ..hkilem.g k.kee.dt.k .ek.n.qg.v tr..yiiqe.>
chTL1         ...srlei.l .......y.. .k.l.q.tn. ilkihek.sl ..hkilem.e r.keemdt.k .ek.n.q..v tr.syiiqe.>
mTL1.         ...srlei.l .......y.. .k.l.q.tn. ilkihek.sl ..hkilem.g k.kee.dt.k .ek.n.qg.v sr..fiiqe.>
mTL2.         ...trlel.l .qh.i..... .k.i.d.ts. ink..nk.sf ..qkvldm.g k.se..q.mk .qkde.qv.v sk.ssvide.>
hTL2.         ...trlel.l ..h..... .k.i.d.ts. ink..dk.sf ..kkvl.m.d k.ii..q.ik .ekd..qv.v sk.nsiiee.>

                    250        260        270        280        290        300        310        320
mTL3          KHNLHALSSN SSSLQQQQQQ LTEFVQRLVR IV---AQ-DQHP--V--S L-KTPKPVFQD CAEIKRSGVN TSGVYTIYET NMTKPLKVFC
hTL1.         ekq.nratt. n.v..k..le .mdt.hn..n lc---tkevllk--g--g k-reeekp.r. ..dvyqa.f. k..i....in ..pe.k....>
chTL1.        ekq.nkatt. n.v..k..le .mdt.ht.it lc---sk-egvllkn--a k-.eeekp.r. ..dvyq..f. k.......in .vsd.k....>
mTL1.         ekq.sratn. n.i..k..le .mdt.hn..s lc---tk-egvl--lkgg k-reeekp.r. ..dvyqa.f. k..i....fn ..pe.k....>
mTL2.         ekk.vtatv. n.l..k..hd .m.t.ns.lt mmss-pn-skss--.--a ir.eeqtt.r. ....fk..lt ...i..ltfp .s.eei.ay.>
hTL2.         ekkivtatv. n.v..k..hd .m.t.nn.lt mmstsns-akd.--t--v a-.eeqis.r. ...vfk..ht .n.i..ltfp .s.eei.ay.>
```

Fig. 22 B

```
                330       340       350       360       370       380       390       400
mTL3      DMETDGGGWT LIQHREDGSV NFQRTWEEYK EGFGNVAREH WLGNEAVHRL TSRTAYLLRV ELHDWEGRQT SIQYENFQLG
hTL1.     n.dvn..... v........l d...g.k... m....psg.y .....fifai ..qrq.m..i ..m....nra ys..dr.hi.>
chTL1.    n..vn..... v........l d..kg.k... m...spsg.. .....fifai ..qrq.s..i ..m....nra ys..dr.hi.>
mTL1.     n.dvn..... v........l d...g.k... m....psg.y .....fifai ..qrq.m..i ..m....nra ys..dr.hi.>
mTL2.     ..dvg..... v......... d.....k... .....plg.y .....f.sq. .gqhr.v.ki q.k....nea hsl.dh.y.a>
hTL2.     ...ag..... i..r...... d.....k... v....psg.y .....f.sq. .nqqr.v.ki h.k....nea ysl..h.y.s>

                410       420       430       440       450       460       470       480
mTL3      SERQRYSLSV NDSSSSAGRK NSLAPQGTKP STKDMDNDNC MCKCAQMLSG GWWFDACGLS NLNGIYYSVH QHLHKINGIR
hTL1.     n.k.n.r.yl kghtgt..kq s..ilh.ad. ....a..... .....l..t. ........p. ....mf.tag .nhg.l...k>
chTL1.    n.k.n.r.yl kgh.gt..kq s..ilh.ae. ....a..... .....l..t. ........p. ....mf..ag .nhg.l...k>
mTL1.     n.k.n.r.yl kghtgt..kq s..ilh.ad. ....a..... .....l..t. ........p. ....mf.tag .nhg.l...k>
mTL2.     g.esn.rihl tgltgt.aki s.isqp.sd. ....s...k. i...s..... ........p. ....q..pqk .ntn.f...k>
hTL2.     ..eln.rihl kgltgt..ki s.isqp.nd. ....g...k. i...s...t. ........p. ....m..pqr .ntn.f...k>

                490       500
mTL3      WHYFRGPSYS IHGTRMMLRP MGA*
hTL1.     ....k..... .rs.t..i.. ldf
chTL1.    ....k..r.. .rs.t..i.. ldf>
mTL1.     ....k..... .rs.t..i.. ldf>
mTL2.     .y.wk.sg.. .ka.t..i.. adf>
hTL2.     .v.wk.sg.. .ka.t..i.. adf>
```

Fig. 23 A

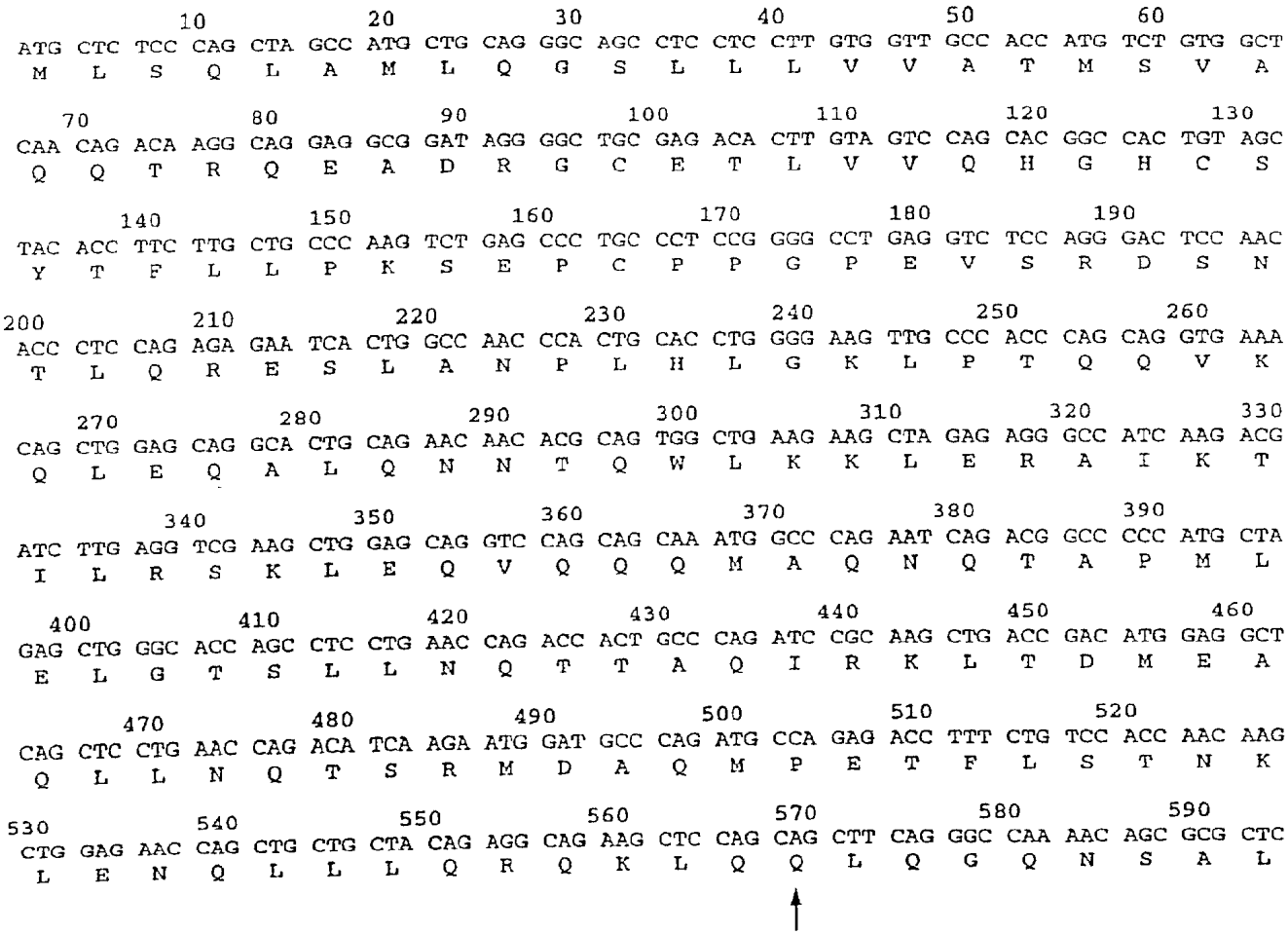

10 20 30 40 50 60
ATG CTC TCC CAG CTA GCC ATG CTG CAG GGC AGC CTC CTC CTT GTG GTT GCC ACC ATG TCT GTG GCT
M L S Q L A M L Q G S L L L V V A T M S V A
70 80 90 100 110 120 130
CAA CAG ACA AGG CAG GAG GCG GAT AGG GGC TGC GAG ACA CTT GTA GTC CAG CAC GGC CAC TGT AGC
Q Q T R Q E A D R G C E T L V V Q H G H C S
140 150 160 170 180 190
TAC ACC TTC TTG CTG CCC AAG TCT GAG CCC TGC CCT CCG GGG CCT GAG GTC TCC AGG GAC TCC AAC
Y T F L L P K S E P C P P G P E V S R D S N
200 210 220 230 240 250 260
ACC CTC CAG AGA GAA TCA CTG GCC AAC CCA CTG CAC CTG GGG AAG TTG CCC ACC CAG CAG GTG AAA
T L Q R E S L A N P L H L G K L P T Q Q V K
270 280 290 300 310 320 330
CAG CTG GAG CAG GCA CTG CAG AAC AAC ACG CAG TGG CTG AAG AAG CTA GAG AGG GCC ATC AAG ACG
Q L E Q A L Q N N T Q W L K K L E R A I K T
340 350 360 370 380 390
ATC TTG AGG TCG AAG CTG GAG CAG GTC CAG CAG CAA ATG GCC CAG AAT CAG ACG GCC CCC ATG CTA
I L R S K L E Q V Q Q Q M A Q N Q T A P M L
400 410 420 430 440 450 460
GAG CTG GGC ACC AGC CTC CTG AAC CAG ACC ACT GCC CAG ATC CGC AAG CTG ACC GAC ATG GAG GCT
E L G T S L L N Q T T A Q I R K L T D M E A
470 480 490 500 510 520
CAG CTC CTG AAC CAG ACA TCA AGA ATG GAT GCC CAG ATG CCA GAG ACC TTT CTG TCC ACC AAC AAG
Q L L N Q T S R M D A Q M P E T F L S T N K
530 540 550 560 570 580 590
CTG GAG AAC CAG CTG CTG CTA CAG AGG CAG AAG CTC CAG CAG CTT CAG GGC CAA AAC AGC GCG CTC
L E N Q L L L Q R Q K L Q Q L Q G Q N S A L

Fig. 23 B

```
      600         610         620         630         640         650         660
GAG AAG CGG TTG CAG GCC CTG GAG ACC AAG CAG CAG GAG GAG CTG GCC AGC ATC CTC AGC AAG AAG
 E   K   R   L   Q   A   L   E   T   K   Q   Q   E   E   L   A   S   I   L   S   K   K

          670         680         690         700         710         720
GCG AAG CTG CTG AAC ACG CTG AGC CGC CAG AGC GCC GCC CTC ACC AAC ATC GAG CGC GGC CTG CGC
 A   K   L   L   N   T   L   S   R   Q   S   A   A   L   T   N   I   E   R   G   L   R

  730         740         750         760         770         780         790
GGT GTC AGG CAC AAC TCC AGC CTC CTG CAG GAC CAG CAG CAC AGC CTG CGC CAG CTG CTG GTG TTG
 G   V   R   H   N   S   S   L   L   Q   D   Q   Q   H   S   L   R   Q   L   L   V   L

        800         810         820         830         840         850
TTG CGG CAC CTG GTG CAA GAA AGG GCT AAC GCC TCG GCC CCG GCC TTC ATA ATG GCA GGT GAG CAG
 L   R   H   L   V   Q   E   R   A   N   A   S   A   P   A   F   I   M   A   G   E   Q

860         870         880         890         900         910         920
GTG TTC CAG GAC TGT GCA GAG ATC CAG CGC TCT GGG GCC AGT GCC AGT GGT GTC TAC ACC ATC CAG
 V   F   Q   D   C   A   E   I   Q   R   S   G   A   S   A   S   G   V   Y   T   I   Q

    930         940         950         960         970         980         990
GTG TCC AAT GCA ACG AAG CCC AGG AAG GTG TTC TGT GAC CTG CAG AGC AGT GGA GGC AGG TGG ACC
 V   S   N   A   T   K   P   R   K   V   F   C   D   L   Q   S   S   G   G   R   W   T

         1000        1010        1020        1030        1040        1050
CTC ATC CAG CGC CGT GAG AAT GGC ACC GTG AAT TTT CAG CGG AAC TGG AAG GAT TAC AAA CAG GGC
 L   I   Q   R   R   E   N   G   T   V   N   F   Q   R   N   W   K   D   Y   K   Q   G

 1060        1070        1080        1090        1100        1110        1120
TTC GGA GAC CCA GCT GGG GAG CAC TGG CTG GGC AAT GAA GTG GTG CAC CAG CTC ACC AGA AGG GCA
 F   G   D   P   A   G   E   H   W   L   G   N   E   V   V   H   Q   L   T   R   R   A

      1130        1140        1150        1160        1170        1180
GCC TAC TCT CTG CGT GTG GAG CTG CAA GAC TGG GAA GGC CAC GAG GCC TAT GCC CAG TAC GAA CAT
 A   Y   S   L   R   V   E   L   Q   D   W   E   G   H   E   A   Y   A   Q   Y   E   H
```

Fig. 23 C

```
1190          1200          1210          1220          1230          1240          1250
 TTC CAC CTG GGC AGT GAG AAC CAG CTA TAC AGG CTT TCT GTG GTC GGG TAC AGC GGC TCA GCA GGG
  F   H   L   G   S   E   N   Q   L   Y   R   L   S   V   V   G   Y   S   G   S   A   G

   1260          1270          1280          1290          1300          1310          1320
 CGC CAG AGC AGC CTG GTC CTG CAG AAC ACC AGC TTT AGC ACC CTT GAC TCA GAC AAC GAC CAC TGT
  R   Q   S   S   L   V   L   Q   N   T   S   F   S   T   L   D   S   D   N   D   H   C

          1330          1340          1350          1360          1370          1380
 CTC TGC AAG TGT GCC CAG GTG ATG TCT GGA GGG TGG TGG TTT GAC GCC TGT GGC CTG TCA AAC CTC
  L   C   K   C   A   Q   V   M   S   G   G   W   W   F   D   A   C   G   L   S   N   L

 1390          1400          1410          1420          1430          1440          1450
 AAC GGC GTC TAC TAC CAC GCT CCC GAC AAC AAG TAC AAG ATG GAC GGC ATC CGC TGG CAC TAC TTC
  N   G   V   Y   Y   H   A   P   D   N   K   Y   K   M   D   G   I   R   W   H   Y   F

       1460          1470          1480          1490          1500          1510
 AAG GGC CCC AGC TAC TCA CTG CGT GCC TCT CGC ATG ATG ATA CGG CCT TTG GAC ATC TAA
  K   G   P   S   Y   S   L   R   A   S   R   M   M   I   R   P   L   D   I   *
```

Fig. 24 A

```
                10                  20                  30                  40                  50                  60                  70                  80                  90
                 *                   *                   *                   *                   *                   *                   *                   *                   *
ATG ACA GTT TTC CTT TCC TTT GCT TTC CTC GCT GCC ATT CTG ACT CAC ATA GGG TGC AGC AAT CAG CGC CGA AGT CCA GAA AAC AGT GGG
TAC TGT CAA AAG GAA AGG AAA CGA AAG GAG CGA CGG TAA GAC TGA GTG TAT CCC ACG TCG TTA GTC GCG GCT TCA GGT CTT TTG TCA CCC
 M   T   V   F   L   S   F   A   F   L   A   A   I   L   T   H   I   G   C   S   N   Q   R   R   S   P   E   N   S   G>

               100                 110                 120                 130                 140                 150                 160                 170                 180
                 *                   *                   *                   *                   *                   *                   *                   *                   *
AGA AGA TAT AAC CGG ATT CAA CAT GGG CAA TGT GCC TAC ACT TTC ATT CTT CCA GAA CAC GAT GGC AAC TGT CGT GAG AGT ACG ACA GAC
TCT TCT ATA TTG GCC TAA GTT GTA CCC GTT ACA CGG ATG TGA AAG TAA GAA GGT CTT GTG CTA CCG TTG ACA GCA CTC TCA TGC TGT CTG
 R   R   Y   N   R   I   Q   H   G   Q   C   A   Y   T   F   I   L   P   E   H   D   G   N   C   R   E   S   T   T   D>

               190                 200                 210                 220                 230                 240                 250                 260                 270
                 *                   *                   *                   *                   *                   *                   *                   *                   *
CAG TAC AAC ACA AAC GCT CTG CAG AGA GAT GCT CCA CAC GTG GAA CCG GAT TTC TCT TCC CAG AAA CTT CAA CAT CTG GAA CAT GTG ATG
GTC ATG TTG TGT TTG CGA GAC GTC TCT CTA CGA GGT GTG CAC CTT GGC CTA AAG AGA AGG GTC TTT GAA GTT GTA GAC CTT GTA CAC TAC
 Q   Y   N   T   N   A   L   Q   R   D   A   P   H   V   E   P   D   F   S   S   Q   K   L   Q   H   L   E   H   V   M>

               280                 290                 300                 310                 320                 330                 340                 350                 360
                 *                   *                   *                   *                   *                   *                   *                   *                   *
GAA AAT TAT ACT CAG TGG CTG CAA AAA CTT GAG AAT TAC ATT GTG GAA AAC ATG AAG TCG GAG ATG GCC CAG ATA CAG CAG AAT GCA GTT
CTT TTA ATA TGA GTC ACC GAC GTT TTT GAA CTC TTA ATG TAA CAC CTT TTG TAC TTC AGC CTC TAC CGG GTC TAT GTC GTC TTA CGT CAA
 E   N   Y   T   Q   W   L   Q   K   L   E   N   Y   I   V   E   N   M   K   S   E   M   A   Q   I   Q   Q   N   A   V>

               370                 380                 390                 400                 410                 420                 430                 440                 450
                 *                   *                   *                   *                   *                   *                   *                   *                   *
CAG AAC CAC ACG GCT ACC ATG CTG GAG ATA GGA ACC AGC CTC CTC TCT CAG ACT GCA GAG CAG ACC AGA AAG CTG ACA GAT GTT GAG ACC
GTC TTG GTG TGC CGA TGG TAC GAC CTC TAT CCT TGG TCG GAG GAG AGA GTC TGA CGT CTC GTC TGG TCT TTC GAC TGT CTA CAA CTC TGG
 Q   N   H   T   A   T   M   L   E   I   G   T   S   L   L   S   Q   T   A   E   Q   T   R   K   L   T   D   V   E   T>

               460                 470                 480                 490                 500                 510                 520                 530                 540
                 *                   *                   *                   *                   *                   *                   *                   *                   *
CAG GTA CTA AAT CAA ACT TCT CGA CTT GAG ATA CAG CTG CTG GAG AAT TCA TTA TCC ACC TAC AAG CTA GAG AAG CAA CTT CTT CAA CAG
GTC CAT GAT TTA GTT TGA AGA GCT GAA CTC TAT GTC GAC GAC CTC TTA AGT AAT AGG TGG ATG TTC GAT CTC TTC GTT GAA GAA GTT GTC
 Q   V   L   N   Q   T   S   R   L   E   I   Q   L   L   E   N   S   L   S   T   Y   K   L   E   K   Q   L   L   Q   Q>
```

Fig. 24 B

```
          550         560         570         580         590         600         610         620         630
           *           *           *           *           *           *           *           *           *
ACA AAT GAA ATC TTG AAG ATC CAT GAA AAA AAC AGT TTA TTA GAA CAT AAA ATC TTA GAA ATG GAA GGA AAA CAC AAG GAA GAG TTG GAC
TGT TTA CTT TAG AAC TTC TAG GTA CTT TTT TTG TCA AAT AAT CTT GTA TTT TAG AAT CTT TAC CTT CCT TTT GTG TTC CTT CTC AAC CTG
 T   N   E   I   L   K   I   H   E   K   N   S   L   L   E   H   K   I   L   E   M   E   G   K   H   K   E   E   L   D>

          640         650         660         670         680         690         700         710         720
           *           *           *           *           *           *           *           *           *
ACC TTA AAG GAA GAG AAA GAG AAC CTT CAA GGC TTG GTT ACT CGT CAA ACA TAT ATA ATC CAG GAG CTG GAA AAG CAA TTA AAC AGA GCT
TGG AAT TTC CTT CTC TTT CTC TTG GAA GTT CCG AAC CAA TGA GCA GTT TGT ATA TAT TAG GTC CTC GAC CTT TTC GTT AAT TTG TCT CGA
 T   L   K   E   E   K   E   N   L   Q   G   L   V   T   R   Q   T   Y   I   I   Q   E   L   E   K   Q   L   N   R   A>

          730         740         750         760         770         780         790         800         810
           *           *           *           *           *           *           *           *           *
ACC ACC AAC AAC AGT GTC CTT CAG AAG CAG CAA CTG GAG CTG ATG GAC ACA GTC CAC AAC CTT GTC AAT CTT TGC ACT AAA GAA GGT GTT
TGG TGG TTG TTG TCA CAG GAA GTC TTC GTC GTT GAC CTC GAC TAC CTG TGT CAG GTG TTG GAA CAG TTA GAA ACG TGA TTT CTT CCA CAA
 T   T   N   N   S   V   L   Q   K   Q   Q   L   E   L   M   D   T   V   H   N   L   V   N   L   C   T   K   E   G   V>

          820         830         840         850         860         870         880         890         900
           *           *           *           *           *           *           *           *           *
TTA CTA AAG GGA GGA AAA AGA GAG GAA GAG AAA CCA TTT AGA GAC TGT GCT GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC ATC TAC
AAT GAT TTC CCT CCT TTT TCT CTC CTT CTC TTT GGT AAA TCT CTG ACA CGA CTT CAT AAG TTT AGT CCT GTG TGG TGT TTA CCG TAG ATG
 L   L   K   G   G   K   R   E   E   E   K   P   F   R   D   C   A   E   V   F   K   S   G   H   T   T   N   G   I   Y>

          910         920         930         940         950         960         970         980         990
           *           *           *           *           *           *           *           *           *
ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA CGT
TGC AAT TGT AAG GGA TTA AGA TGT CTT CTC TAG TTC CGG ATG ACA CTG TAC CTT CGA CCT CCT CCG CCC ACC TGT TAA TAA GTC GCT GCA
 T   L   T   F   P   N   S   T   E   E   I   K   A   Y   C   D   M   E   A   G   G   G   G   W   T   I   I   Q   R   R>

         1000        1010        1020        1030        1040        1050        1060        1070        1080
           *           *           *           *           *           *           *           *           *
GAG GAT GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA AAT GAG
CTC CTA CCG TCG CAA CTA AAA GTC TCC TGA ACC TTT CTT ATA TTT CAC CCT AAA CCA TTG GGA AGT CCT CTT ATA ACC GAC CCT TTA CTC
 E   D   G   S   V   D   F   Q   R   T   W   K   E   Y   K   V   G   F   G   N   P   S   G   E   Y   W   L   G   N   E>

         1090        1100        1110        1120        1130        1140        1150        1160        1170
           *           *           *           *           *           *           *           *           *
TTT GTT TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG TAT GAA
AAA CAA AGC GTT GAC TGA TTA GTC GTT GCG ATA CAC GAA TTT TAT GTG GAA TTT CTG ACC CTT CCC TTA CTC CGA ATG AGT AAC ATA CTT
 F   V   S   ?   L   T   N   Q   Q   R   Y   V   L   K   I   H   L   K   D   W   E   G   N   E   A   Y   S   L   Y   E>
```

Fig. 24 C

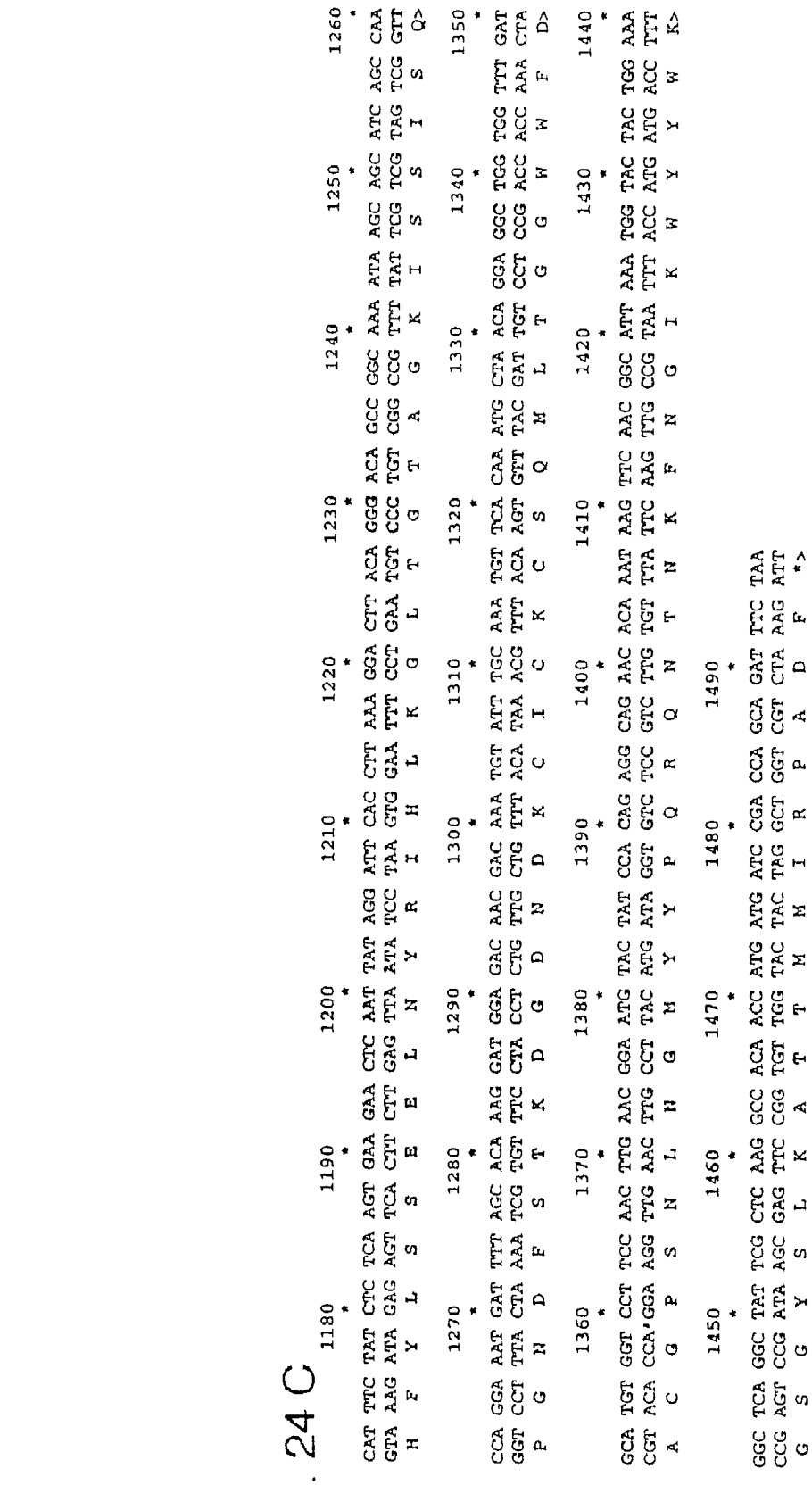

1180
1190
1200
1210
1220
1230
1240
1250
1260
CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC AGC CAA
GTA AAG ATA GAG AGT TCA CTT CTT GAG TTA ATA TCC TAA GTG GAA TTT CCT GAA TGT CCC TGT CGG CCG TTT TAT TCG TCG TAG TCG GTT
H F Y L S S E E L N Y R I H L K G L T G T A G K I S S I S Q>
1270
1280
1290
1300
1310
1320
1330
1340
1350
CCA GGA AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG TTT GAT
GGT CCT TTA CTA AAA TCG TGT TTC CTA CCT CTG TTG CTG TTT ACA TAA ACG TTT ACA AGT GTT TAC GAT TGT CCT CCG ACC ACC AAA CTA
P G N D F S T K D G D N D K C I C K C S Q M L T G G W W F D>
1360
1370
1380
1390
1400
1410
1420
1430
1440
GCA TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC TGG AAA
CGT ACA CCA GGA AGG TTG AAC TTG CCT TAC ATG ATA GGT GTC TCC GTC TTG TGT TTA TTC AAG TTG CCG TAA TTT ACC ATG ATG ACC TTT
A C G P S N L K A T T M M I R P A D F *>
1450
1460
1470
1480
1490
GGC TCA GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC CGA CCA GCA GAT TTC TAA
CCG AGT CCG ATA AGC GAG TTC CGG TGT TGG TAC TAC TAG GCT GGT CGT CTA AAG ATT
G S G Y S L K A T T M M I R P A D F *>

Fig. 25 A

```
          10          20          30          40          50          60          70          80          90
           *           *           *           *           *           *           *           *           *
ATG TGG CAG ATT GTT TTC TTT ACT CTG AGC TGT GAT CTT GTC TTG GCC GCA GCC TAT AAC AAC TTT CGG AAG AGC ATG GAC AGC ATA GGA
TAC ACC GTC TAA CAA AAG AAA TGA GAC TCG ACA CTA GAA CAG AAC CGG CGT CGG ATA TTG TTG AAA GCC TTC TCG TAC CTG TCG TAT CCT
 M   W   Q   I   V   F   F   T   L   S   C   D   L   V   L   A   A   A   Y   N   N   F   R   K   S   M   D   S   I   G>

         100         110         120         130         140         150         160         170         180
           *           *           *           *           *           *           *           *           *
AAG AAG CAA TAT CAG GTC CAG CAT GGG TCC TGC AGC TAC ACT TTC CTC CTG CCA GAG ATG GAC AAC TGC CGC TCT TCC TCC AGC CCC TAC
TTC TTC GTT ATA GTC CAG GTC GTA CCC AGG ACG TCG ATG TGA AAG GAG GAC GGT CTC TAC CTG TTG ACG GCG AGA AGG AGG TCG GGG ATG
 K   K   Q   Y   Q   V   Q   H   G   S   C   S   Y   T   F   L   L   P   E   M   D   N   C   R   S   S   S   S   P   Y>

         190         200         210         220         230         240         250         260         270
           *           *           *           *           *           *           *           *           *
GTG TCC AAT GCT GTG CAG AGG GAC GCG CCG CTC GAA TAC GAT GAC TCG GTG CAG AGG CTG CAA GTG CTG GAG AAC ATC ATG GAA AAC AAC
CAC AGG TTA CGA CAC GTC TCC CTG CGC GGC GAG CTT ATG CTA CTG AGC CAC GTC TCC GAC GTT CAC GAC CTC TTG TAG TAC CTT TTG TTG
 V   S   N   A   V   Q   R   D   A   P   L   E   Y   D   D   S   V   Q   R   L   Q   V   L   E   N   I   M   E   N   N>

         280         290         300         310         320         330         340         350         360
           *           *           *           *           *           *           *           *           *
ACT CAG TGG CTA ATG AAG CTT GAG AAT TAT ATC CAG GAC AAC ATG AAG AAA GAA ATG GTA GAG ATA CAG CAG AAT GCA GTA CAG AAC CAG
TGA GTC ACC GAT TAC TTC GAA CTC TTA ATA TAG GTC CTG TTG TAC TTC TTT CTT TAC CAT CTC TAT GTC GTC TTA CGT CAT GTC TTG GTC
 T   Q   W   L   M   K   L   E   N   Y   I   Q   D   N   M   K   K   E   M   V   E   I   Q   Q   N   A   V   Q   N   Q>

         370         380         390         400         410         420         430         440         450
           *           *           *           *           *           *           *           *           *
ACG GCT GTG ATG ATA GAA ATA GGG ACA AAC CTG TTG AAC CAA ACA GCT GAG CAA ACG CGG AAG TTA ACT GAT GTG GAA GCC CAA GTA TTA
TGC CGA CAC TAC TAT CTT TAT CCC TGT TTG GAC AAC TTG GTT TGT CGA CTC GTT TGC GCC TTC AAT TGA CTA CAC CTT CGG GTT CAT AAT
 T   A   V   M   I   E   I   G   T   N   L   L   N   Q   T   A   E   Q   T   R   K   L   T   D   V   E   A   Q   V   L>

         460         470         480         490         500         510         520         530         540
           *           *           *           *           *           *           *           *           *
AAT CAG ACC ACG AGA CTT GAA CTT CAG CTC TTG GAA CAC TCC CTC TCG ACA AAC AAA TTG GAA AAA CAG ATT TTG GAC CAG ACC AGT GAA
TTA GTC TGG TGC TCT GAA CTT GAA GTC GAG AAC CTT GTG AGG GAG AGC TGT TTG TTT AAC CTT TTT GTC TAA AAC CTG GTC TGG TCA CTT
 N   Q   T   T   R   L   E   L   Q   L   L   E   H   S   L   S   T   N   K   L   E   K   Q   I   L   D   Q   T   S   E>
```

Fig. 25 B

```
          550         560         570         580         590         600         610         620         630
           *           *           *           *           *           *           *           *           *
ATA AAC AAA TTG CAA GAT AAG AAC AGT TTC CTA GAA AAG AAG GTG CTA GCT ATG GAA GAC AAG CAC ATC ATC CAA CTA CAG TCA ATA AAA
TAT TTG TTT AAC GTT CTA TTC TTG TCA AGG GAT CTT TTC TTC CAC GAT CGA TAC CTT CTG TTC GTG TAG TAG GTT GAT GTC AGT TAT TTT
 I   N   K   L   Q   D   K   N   S   F   L   E   K   K   V   L   A   M   E   D   K   H   I   I   Q   L   Q   S   I   K>

          640         650         660         670         680         690         700         710         720
           *           *           *           *           *           *           *           *           *
GAA GAG AAA GAT CAG CTA CAG GTG TTA GTA TCC AAG CAA AAT TCC ATC ATT GAA GAA CTA GAA AAA AAA ATA GTG ACT GCC ACG GTG AAT
CTT CTC TTT CTA GTC GAT GTC CAC AAT CAT AGG TTC GTT TTA AGG TAG TAA CTT CTT GAT CTT TTT TTT TAT CAC TGA CGG TGC CAC TTA
 E   E   K   D   Q   L   Q   V   L   V   S   K   Q   N   S   I   I   E   E   L   E   K   K   I   V   T   A   T   V   N>

          730         740         750         760         770         780         790         800         810
           *           *           *           *           *           *           *           *           *
AAT TCA GTT CTT CAA AAG CAG CAA CAT GAT CTC ATG GAG ACA GTT AAT AAC TTA CTG ACT ATG ATG TCC ACA TCA AAC TCA GCT AAG GAC
TTA AGT CAA GAA GTT TTC GTC GTT GTA CTA GAG TAC CTC TGT CAA TTA TTG AAT GAC TGA TAC TAC AGG TGT AGT TTG AGT CGA TTC CTG
 N   S   V   L   Q   K   Q   Q   H   D   L   M   E   T   V   N   N   L   L   T   M   M   S   T   S   N   S   A   K   D>

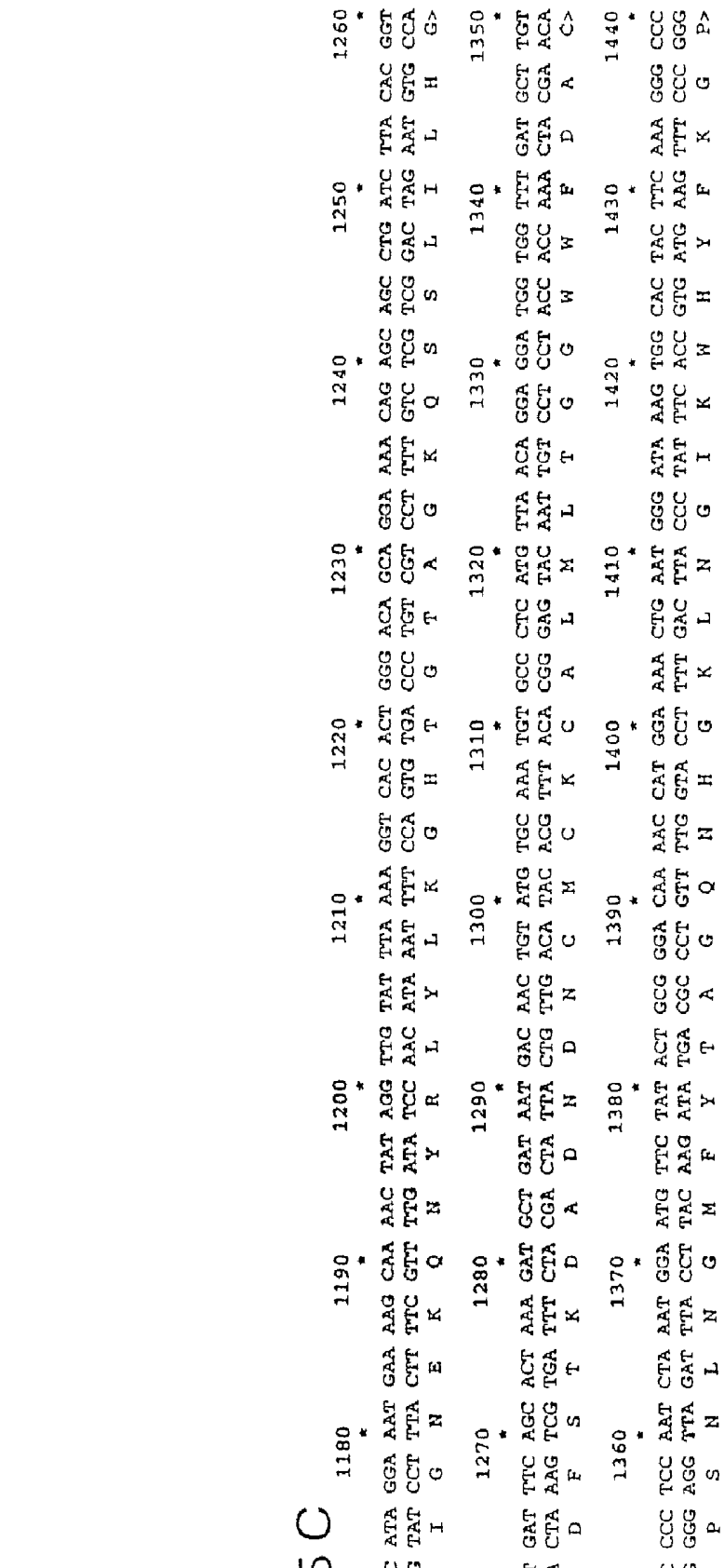
          820         830         840         850         860         870         880         890         900
           *           *           *           *           *           *           *           *           *
CCC ACT GTT GCT AAA GAA GAA CAA ATC AGC TTC AGA GAC TGT GCA GAT GTA TAT CAA GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT
GGG TGA CAA CGA TTT CTT CTT GTT TAG TCG AAG TCT CTG ACA CGT CTA CAT ATA GTT CGA CCA AAA TTA TTT TCA CCT TAG ATG TGA TAA
 P   T   V   A   K   E   E   Q   I   S   F   R   D   C   A   D   V   Y   Q   A   G   F   N   K   S   G   I   Y   T   I>

          910         920         930         940         950         960         970         980         990
           *           *           *           *           *           *           *           *           *
TAT ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT GTC AAT GGG GGA GGT TGG ACT GTA ATA CAA CAT CGT GAA GAT
ATA TAA TTA TTA TAC GGT CTT GGG TTT TTC CAC AAA ACG TTA TAC CTA CAG TTA CCC CCT CCA ACC TGA CAT TAT GTT GTA GCA CTT CTA
 Y   I   N   N   M   P   E   P   K   K   V   F   C   N   M   D   V   N   G   G   G   W   T   V   I   Q   H   R   E   D>

         1000        1010        1020        1030        1040        1050        1060        1070        1080
           *           *           *           *           *           *           *           *           *
GGA AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA TAT AAA ATG GGT TTT GGA AAT CCC TCC GGT GAA TAT TGG CTG GGG AAT GAG TTT ATT
CCT TCA GAT CTA AAG GTT TCT CCG ACC TTC CTT ATA TTT TAC CCA AAA CCT TTA GGG AGG CCA CTT ATA ACC GAC CCC TTA CTC AAA TAA
 G   S   L   D   F   Q   R   G   W   K   E   Y   K   M   G   F   G   N   P   S   G   E   Y   W   L   G   N   E   F   I>

         1090        1100        1110        1120        1130        1140        1150        1160        1170
           *           *           *           *           *           *           *           *           *
TTT GCC ATT ACC AGT CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG GAC TGG GAA GGG AAC CGA GCC TAT TCA CAG TAT GAC AGA TTC
AAA CGG TAA TGG TCA GTC TCC GTC ATG TAC GAT TCT TAA CTC AAT TAC CTG ACC CTT CCC TTG GCT CGG ATA AGT GTC ATA CTG TCT AAG
 F   A   I   T   S   Q   R   Q   Y   M   L   R   I   E   L   M   D   W   E   G   N   R   A   Y   S   Q   Y   D   R   F>
```

Fig. 25 C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CAC ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG ATC TTA CAC GGT
GTG TAT CCT TTA CTT TTC GTT TTG ATA TCC AAC ATA AAT TTT CCA GTG TGA CCC TGT CGT CCT TTT GTC TCG TCG GAC TAG AAT GTG CCA
H I G N E K Q N Y R L Y L K G H T G T A G K Q S S L I L H G>
1270 1280 1290 1300 1310 1320 1330 1340 1350
GCT GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC AAC TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG TTT GAT GCT TGT
CGA CTA AAG TCG TGA TTT CTA CGA CTA TTA CTG TTG ACA TAC ACG TTT ACA CGG GAG TAC AAT TGT CCT CCT ACC ACC AAA CTA CGA ACA
A D F S T K D A D N D N C M C K C A L M L T G G W W F D A C>
1360 1370 1380 1390 1400 1410 1420 1430 1440
GGC CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA AAC CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCC
CCG GGG AGG TTA GAT TTA CCT TAC AAG ATA TGA CGC CCT GTT TTG GTA CCT TTT GAC TTA CCC TAT TTC ACC GTG ATG AAG TTT CCC GGG
G P S N L N G M F Y T A G Q N H G K L N G I K W H Y F K G P>
1450 1460 1470 1480 1490
AGT TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT TGA
TCA ATG AGG AAT GCA AGG TGT TGA TAC TAC TAA GCT GGA AAT CTA AAA ACT
S Y S L R S T T M M I R P L D F *>

Fig. 26 A

```
          10                  20                  30                  40                  50                  60                  70                  80                  90
          *                   *                   *                   *                   *                   *                   *                   *                   *
ATG ACA GTT TTC CTT TCC TTT GCT TTC CTC GCT GCC ATT CTG ACT CAC ATA GGG TGC AGC AAT CAG CGC CGA AGT CCA GAA AAC AGT GGG
TAC TGT CAA AAG GAA AGG AAA CGA AAG GAG CGA CGG TAA GAC TGA GTG TAT CCC ACG TCG TTA GTC GCG GCT TCA GGT CTT TTG TCA CCC
 M   T   V   F   L   S   F   A   F   L   A   A   I   L   T   H   I   G   C   S   N   Q   R   R   S   P   E   N   S   G>

         100                 110                 120                 130                 140                 150                 160                 170                 180
          *                   *                   *                   *                   *                   *                   *                   *                   *
AGA AGA TAT AAC CGG ATT CAA CAT GGG CAA TGT GCC TAC ACT TTC ATT CTT CCA GAA CAC GAT GGC AAC TGT CGT GAG AGT ACG ACA GAC
TCT TCT ATA TTG GCC TAA GTT GTA CCC GTT ACA CGG ATG TGA AAG TAA GAA GGT CTT GTG CTA CCG TTG ACA GCA CTC TCA TGC TGT CTG
 R   R   Y   N   R   I   Q   H   G   Q   C   A   Y   T   F   I   L   P   E   H   D   G   N   C   R   E   S   T   T   D>

         190                 200                 210                 220                 230                 240                 250                 260                 270
          *                   *                   *                   *                   *                   *                   *                   *                   *
CAG TAC AAC ACA AAC GCT CTG CAG AGA GAT GCT CCA CAC GTG GAA CCG GAT GAC TCG GTG CAG AGG CTG CAA GTG CTG GAG AAC ATC ATG
GTC ATG TTG TGT TTG CGA GAC GTC TCT CTA CGA GGT GTG CAC CTT GGC CTA CTG AGC CAC GTC TCC GAC GTT CAC GAC CTC TTG TAG TAC
 Q   Y   N   T   N   A   L   Q   R   D   A   P   H   V   E   P   D   D   S   V   Q   R   L   Q   V   L   E   N   I   M>

         280                 290                 300                 310                 320                 330                 340                 350                 360
          *                   *                   *                   *                   *                   *                   *                   *                   *
GAA AAC AAC ACT CAG TGG CTA ATG AAG CTT GAG AAT TAT ATC CAG GAC AAC ATG AAG AAA GAA ATG GTA GAG ATA CAG CAG AAT GCA GTA
CTT TTG TTG TGA GTC ACC GAT TAC TTC GAA CTC TTA ATA TAG GTC CTG TTG TAC TTC TTT CTT TAC CAT CTC TAT GTC GTC TTA CGT CAT
 E   N   N   T   Q   W   L   M   K   L   E   N   Y   I   Q   D   N   M   K   K   E   M   V   E   I   Q   Q   N   A   V>

         370                 380                 390                 400                 410                 420                 430                 440                 450
          *                   *                   *                   *                   *                   *                   *                   *                   *
CAG AAC CAG ACG GCT GTG ATG ATA GAA ATA GGG ACA AAC CTG TTG AAC CAA ACA GCT GAG CAA ACG CGG AAG TTA ACT GAT GTG GAA GCC
GTC TTG GTC TGC CGA CAC TAC TAT CTT TAT CCC TGT TTG GAC AAC TTG GTT TGT CGA CTC GTT TGC GCC TTC AAT TGA CTA CAC CTT CGG
 Q   N   Q   T   A   V   M   I   E   I   G   T   N   L   L   N   Q   T   A   E   Q   T   R   K   L   T   D   V   E   A>

         460                 470                 480                 490                 500                 510                 520                 530                 540
          *                   *                   *                   *                   *                   *                   *                   *                   *
CAA GTA TTA AAT CAG ACC ACG AGA CTT GAA CTT CAG CTC TTG GAA CAC TCC CTC TCG ACA AAC AAA TTG GAA AAA CAG ATT TTG GAC CAG
GTT CAT AAT TTA GTC TGG TGC TCT GAA CTT GAA GTC GAG AAC CTT GTG AGG GAG AGC TGT TTG TTT AAC CTT TTT GTC TAA AAC CTG GTC
 Q   V   L   N   Q   T   T   R   L   E   L   Q   L   L   E   H   S   L   S   T   N   K   L   E   K   Q   I   L   D   Q>
```

Fig. 26 B

```
          550         560         570         580         590         600         610         620         630
           *           *           *           *           *           *           *           *           *
ACC AGT GAA ATA AAC AAA TTG CAA GAT AAG AAC AGT TTC CTA GAA AAG AAG GTG CTA GCT ATG GAA GAC AAG CAC ATC ATC CAA CTA CAG
TGG TCA CTT TAT TTG TTT AAC GTT CTA TTC TTG TCA AAG GAT CTT TTC TTC CAC GAT CGA TAC CTT CTG TTC GTG TAG TAG GTT GAT GTC
 T   S   E   I   N   K   L   Q   D   K   N   S   F   L   E   K   K   V   L   A   M   E   D   K   H   I   I   Q   L   Q>

          640         650         660         670         680         690         700         710         720
           *           *           *           *           *           *           *           *           *
TCA ATA AAA GAA GAG AAA GAT CAG CTA CAG GTG TTA GTA TCC AAG CAA AAT TCC ATC ATT GAA GAA CTA GAA AAA AAA ATA GTG ACT GCC
AGT TAT TTT CTT CTC TTT CTA GTC GAT GTC CAC AAT CAT AGG TTC GTT TTA AGG TAG TAA CTT CTT GAT CTT TTT TTT TAT CAC TGA CGG
 S   I   K   E   E   K   D   Q   L   Q   V   L   V   S   K   Q   N   S   I   I   E   E   L   E   K   K   I   V   T   A>

          730         740         750         760         770         780         790         800         810
           *           *           *           *           *           *           *           *           *
ACG GTG AAT AAT TCA GTT CTT CAA AAG CAG CAA CAT GAT CTC ATG GAG ACA GTT AAT AAC TTA CTG ACT ATG ATG TCC ACA TCA AAC TCA
TGC CAC TTA TTA AGT CAA GAA GTT TTC GTC GTT GTA CTA GAG TAC CTC TGT CAA TTA TTG AAT GAC TGA TAC TAC AGG TGT AGT TTG AGT
 T   V   N   N   S   V   L   Q   K   Q   Q   H   D   L   M   E   T   V   N   N   L   L   T   M   M   S   T   S   N   S>

          820         830         840         850         860         870         880         890         900
           *           *           *           *           *           *           *           *           *
GCT AAG GAC CCC ACT GTT GCT AAA GAA GAA CAA ATC AGC TTC AGA GAC TGT GCT GAA GTA TTC AAA TCA GGA CAC ACC ACA AAT GGC ATC
CGA TTC CTG GGG TGA CAA CGA TTT CTT CTT GTT TAG TCG AAG TCT CTG ACA CGA CTT CAT AAG TTT AGT CCT GTG TGG TGT TTA CCG TAG
 A   K   D   P   T   V   A   K   E   E   Q   I   S   F   R   D   C   A   E   V   F   K   S   G   H   T   T   N   G   I>

          910         920         930         940         950         960         970         980         990
           *           *           *           *           *           *           *           *           *
TAC ACG TTA ACA TTC CCT AAT TCT ACA GAA GAG ATC AAG GCC TAC TGT GAC ATG GAA GCT GGA GGA GGC GGG TGG ACA ATT ATT CAG CGA
ATG TGC AAT TGT AAG GGA TTA AGA TGT CTT CTC TAG TTC CGG ATG ACA CTG TAC CTT CGA CCT CCT CCG CCC ACC TGT TAA TAA GTC GCT
 Y   T   L   T   F   P   N   S   T   E   E   I   K   A   Y   C   D   M   E   A   G   G   G   G   W   T   I   I   Q   R>

         1000        1010        1020        1030        1040        1050        1060        1070        1080
           *           *           *           *           *           *           *           *           *
CGT GAG GAT GGC AGC GTT GAT TTT CAG AGG ACT TGG AAA GAA TAT AAA GTG GGA TTT GGT AAC CCT TCA GGA GAA TAT TGG CTG GGA AAT
GCA CTC CTA CCG TCG CAA CTA AAA GTC TCC TGA ACC TTT CTT ATA TTT CAC CCT AAA CCA TTG GGA AGT CCT CTT ATA ACC GAC CCT TTA
 R   E   D   G   S   V   D   F   Q   R   T   W   K   E   Y   K   V   G   F   G   N   P   S   G   E   Y   W   L   G   N>
```

Fig. 26 C

```
          1090        1100        1110        1120        1130        1140        1150        1160        1170
             *           *           *           *           *           *           *           *           *
GAG TTT GTT TCG CAA CTG ACT AAT CAG CAA CGC TAT GTG CTT AAA ATA CAC CTT AAA GAC TGG GAA GGG AAT GAG GCT TAC TCA TTG TAT
CTC AAA CAA AGC GTT GAC TGA TTA GTC GTT GCG ATA CAC GAA TTT TAT GTG GAA TTT CTG ACC CTT CCC TTA CTC CGA ATG AGT AAC ATA
 E   F   V   S   Q   L   T   N   Q   Q   R   Y   V   L   K   I   H   L   K   D   W   E   G   N   E   A   Y   S   L   Y>

          1180        1190        1200        1210        1220        1230        1240        1250        1260
             *           *           *           *           *           *           *           *           *
GAA CAT TTC TAT CTC TCA AGT GAA GAA CTC AAT TAT AGG ATT CAC CTT AAA GGA CTT ACA GGG ACA GCC GGC AAA ATA AGC AGC ATC AGC
CTT GTA AAG ATA GAG AGT TCA CTT CTT GAG TTA ATA TCC TAA GTG GAA TTT CCT GAA TGT CCC TGT CGG CCG TTT TAT TCG TCG TAG TCG
 E   H   F   Y   L   S   S   E   E   L   N   Y   R   I   H   L   K   G   L   T   G   T   A   G   K   I   S   S   I   S>

          1270        1280        1290        1300        1310        1320        1330        1340        1350
             *           *           *           *           *           *           *           *           *
CAA CCA GGA AAT GAT TTT AGC ACA AAG GAT GGA GAC AAC GAC AAA TGT ATT TGC AAA TGT TCA CAA ATG CTA ACA GGA GGC TGG TGG TTT
GTT GGT CCT TTA CTA AAA TCG TGT TTC CTA CCT CTG TTG CTG TTT ACA TAA ACG TTT ACA AGT GTT TAC GAT TGT CCT CCG ACC ACC AAA
 Q   P   G   N   D   F   S   T   K   D   G   D   N   D   K   C   I   C   K   C   S   Q   M   L   T   G   G   W   W   F>

          1360        1370        1380        1390        1400        1410        1420        1430        1440
             *           *           *           *           *           *           *           *           *
GAT GCA TGT GGT CCT TCC AAC TTG AAC GGA ATG TAC TAT CCA CAG AGG CAG AAC ACA AAT AAG TTC AAC GGC ATT AAA TGG TAC TAC TGG
CTA CGT ACA CCA GGA AGG TTG AAC TTG CCT TAC ATG ATA GGT GTC TCC GTC TTG TGT TTA TTC AAG TTG CCG TAA TTT ACC ATG ATG ACC
 D   A   C   G   P   S   N   L   N   G   M   Y   Y   P   Q   R   Q   N   T   N   K   F   N   G   I   K   W   Y   Y   W>

          1450        1460        1470        1480        1490        1500
             *           *           *           *           *           *
AAA GGC TCA GGC TAT TCG CTC AAG GCC ACA ACC ATG ATG ATC CGA CCA GCA GAT TTC TAA
TTT CCG AGT CCG ATA AGC GAG TTC CGG TGT TGG TAC TAC TAG GCT GGT CGT CTA AAG ATT
 K   G   S   G   Y   S   L   K   A   T   T   M   M   I   R   P   A   D   F   *>
```

Fig. 27 A

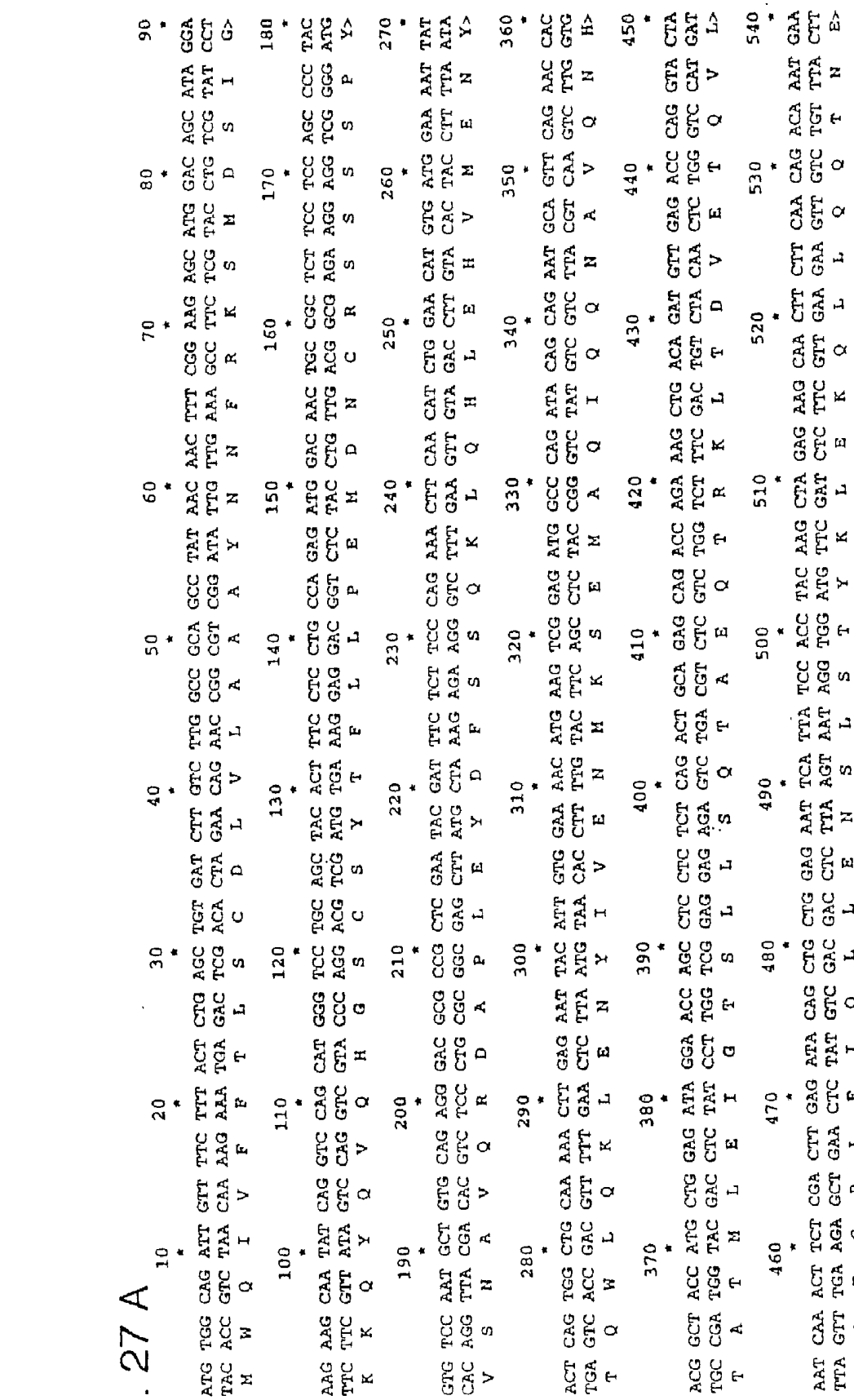


Fig. 27 B

```
          550         560         570         580         590         600         610         620         630
           *           *           *           *           *           *           *           *           *
ATC TTG AAG ATC CAT GAA AAA AAC AGT TTA TTA GAA CAT AAA ATC TTA GAA ATG GAA GGA AAA CAC AAG GAA GAG TTG GAC ACC TTA AAG
TAG AAC TTC TAG GTA CTT TTT TTG TCA AAT AAT CTT GTA TTT TAG AAT CTT TAC CTT CCT TTT GTG TTC CTT CTC AAC CTG TGG AAT TTC
 I   L   K   I   H   E   K   N   S   L   L   E   H   K   I   L   E   M   E   G   K   H   K   E   E   L   D   T   L   K>

          640         650         660         670         680         690         700         710         720
           *           *           *           *           *           *           *           *           *
GAA GAG AAA GAG AAC CTT CAA GGC TTG GTT ACT CGT CAA ACA TAT ATA ATC CAG GAG CTG GAA AAG CAA TTA AAC AGA GCT ACC ACC AAC
CTT CTC TTT CTC TTG GAA GTT CCG AAC CAA TGA GCA GTT TGT ATA TAT TAG GTC CTC GAC CTT TTC GTT AAT TTG TCT CGA TGG TGG TTG
 E   E   K   E   N   L   Q   G   L   V   T   R   Q   T   Y   I   I   Q   E   L   E   K   Q   L   N   R   A   T   T   N>

          730         740         750         760         770         780         790         800         810
           *           *           *           *           *           *           *           *           *
AAC AGT GTC CTT CAG AAG CAG CAA CTG GAG CTG ATG GAC ACA GTC CAC AAC CTT GTC AAT CTT TGC ACT AAA GAA GGT GTT TTA CTA AAG
TTG TCA CAG GAA GTC TTC GTC GTT GAC CTC GAC TAC CTG TGT CAG GTG TTG GAA CAG TTA GAA ACG TGA TTT CTT CCA CAA AAT GAT TTC
 N   S   V   L   Q   K   Q   Q   L   E   L   M   D   T   V   H   N   L   V   N   L   C   T   K   E   G   V   L   L   K>

          820         830         840         850         860         870         880         890         900
           *           *           *           *           *           *           *           *           *
GGA GGA AAA AGA GAG GAA GAG AAA CCA TTT AGA GAC TGT GCA GAT GTA TAT CAA GCT GGT TTT AAT AAA AGT GGA ATC TAC ACT ATT TAT
CCT CCT TTT TCT CTC CTT CTC TTT GGT AAA TCT CTG ACA CGT CTA CAT ATA GTT CGA CCA AAA TTA TTT TCA CCT TAG ATG TGA TAA ATA
 G   G   K   R   E   E   E   K   P   F   R   D   C   A   D   V   Y   Q   A   G   F   N   K   S   G   I   Y   T   I   Y>

          910         920         930         940         950         960         970         980         990
           *           *           *           *           *           *           *           *           *
ATT AAT AAT ATG CCA GAA CCC AAA AAG GTG TTT TGC AAT ATG GAT GTC AAT GGG GGA GGT TGG ACT GTA ATA CAA CAT CGT GAA GAT GGA
TAA TTA TTA TAC GGT CTT GGG TTT TTC CAC AAA ACG TTA TAC CTA CAG TTA CCC CCT CCA ACC TGA CAT TAT GTT GTA GCA CTT CTA CCT
 I   N   N   M   P   E   P   K   K   V   F   C   N   M   D   V   N   G   G   G   W   T   V   I   Q   H   R   E   D   G>

          1000        1010        1020        1030        1040        1050        1060        1070        1080
           *           *           *           *           *           *           *           *           *
AGT CTA GAT TTC CAA AGA GGC TGG AAG GAA TAT AAA ATG GGT TTT GGA AAT CCC TCC GGT GAA TAT TGG CTG GGG AAT GAG TTT ATT TTT
TCA GAT CTA AAG GTT TCT CCG ACC TTC CTT ATA TTT TAC CCA AAA CCT TTA GGG AGG CCA CTT ATA ACC GAC CCC TTA CTC AAA TAA AAA
 S   L   D   F   Q   R   G   W   K   E   Y   K   M   G   F   G   N   P   S   G   E   Y   W   L   G   N   E   F   I   F>
```

Fig. 27 C

```
          1090          1100          1110          1120          1130          1140          1150          1160          1170
GCC ATT ACC AGT CAG AGG CAG TAC ATG CTA AGA ATT GAG TTA ATG GAC TGG GAA GGG AAC CGA GCC TAT TCA CAG TAT GAC AGA TTC CAC
CGG TAA TGG TCA GTC TCC GTC ATG TAC GAT TCT TAA CTC AAT TAC CTG ACC CTT CCC TTG GCT CGG ATA AGT GTC ATA CTG TCT AAG GTG
 A   I   T   S   Q   R   Q   Y   M   L   R   I   E   L   M   D   W   E   G   N   R   A   Y   S   Q   Y   D   R   F   H>

          1180          1190          1200          1210          1220          1230          1240          1250          1260
ATA GGA AAT GAA AAG CAA AAC TAT AGG TTG TAT TTA AAA GGT CAC ACT GGG ACA GCA GGA AAA CAG AGC AGC CTG ATC TTA CAC GGT GCT
TAT CCT TTA CTT TTC GTT TTG ATA TCC AAC ATA AAT TTT CCA GTG TGA CCC TGT CGT CCT TTT GTC TCG TCG GAC TAG AAT GTG CCA CGA
 I   G   N   E   K   Q   N   Y   R   L   Y   L   K   G   H   T   G   T   A   G   K   Q   S   S   L   I   L   H   G   A>

          1270          1280          1290          1300          1310          1320          1330          1340          1350
GAT TTC AGC ACT AAA GAT GCT GAT AAT GAC AAC TGT ATG TGC AAA TGT GCC CTC ATG TTA ACA GGA GGA TGG TGG TTT GAT GCT TGT GGC
CTA AAG TCG TGA TTT CTA CGA CTA TTA CTG TTG ACA TAC ACG TTT ACA CGG GAG TAC AAT TGT CCT CCT ACC ACC AAA CTA CGA ACA CCG
 D   F   S   T   K   D   A   D   N   D   N   C   M   C   K   C   A   L   M   L   T   G   G   W   W   F   D   A   C   G>

          1360          1370          1380          1390          1400          1410          1420          1430          1440
CCC TCC AAT CTA AAT GGA ATG TTC TAT ACT GCG GGA CAA AAC CAT GGA AAA CTG AAT GGG ATA AAG TGG CAC TAC TTC AAA GGG CCC AGT
GGG AGG TTA GAT TTA CCT TAC AAG ATA TGA CGC CCT GTT TTG GTA CCT TTT GAC TTA CCC TAT TTC ACC GTG ATG AAG TTT CCC GGG TCA
 P   S   N   L   N   G   M   F   Y   T   A   G   Q   N   H   G   K   L   N   G   I   K   W   H   Y   F   K   G   P   S>

          1450          1460          1470          1480
TAC TCC TTA CGT TCC ACA ACT ATG ATG ATT CGA CCT TTA GAT TTT TGA
ATG AGG AAT GCA AGG TGT TGA TAC TAC TAA GCT GGA AAT CTA AAA ACT
 Y   S   L   R   S   T   T   M   M   I   R   P   L   D   F   *>
```

EXPRESSED LIGAND - VASCULAR INTERCELLULAR SIGNALLING MOLECULE

[0001] This application claims the priority of U.S. Provisional application 60/022,999 filed Aug. 2, 1996. Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

INTRODUCTION

[0002] The present invention relates generally to the field of genetic engineering and more particularly to genes for receptor tyrosine kinases and their cognate ligands, their insertion into recombinant DNA vectors, and the production of the encoded proteins in recipient strains of microorganisms and recipient eukaryotic cells. More specifically, the present invention is directed to a novel modified TIE-2 ligand that binds the TIE-2 receptor, as well as to methods of making and using the modified ligand. The invention further provides a nucleic acid sequence encoding the modified ligand, and methods for the generation of nucleic acid encoding the modified ligand and the gene product. The modified TIE-2 ligand, as well as nucleic acid encoding it, may be useful in the diagnosis and treatment of certain diseases involving endothelial cells and associated TIE receptors, such as neoplastic diseases involving tumor angiogenesis, wound healing, thromboembolic diseases, atherosclerosis and inflammatory diseases. In addition, the modified ligand may be used to promote the proliferation and/or differentiation of hematopoietic stem cells.

[0003] More generally, the receptor activating modified TIE-2 ligands described herein may be used to promote the growth, survival, migration, and/or differentiation and/or stabilization or destabilization of cells expressing TIE receptor. Biologically active modified TIE-2 ligand may be used for the in vitro maintenance of TIE receptor expressing cells in culture. Cells and tissues expressing TIE receptor include, for example, cardiac and vascular endothelial cells, lens epithelium and heart epicardium and early hematopoietic cells. Alternatively, such human ligand may be used to support cells which are engineered to express TIE receptor. Further, modified TIE-2 ligand and its cognate receptor may be used in assay systems to identify further agonists or antagonists of the receptor.

BACKGROUND OF THE INVENTION

[0004] The cellular behavior responsible for the development, maintenance, and repair of differentiated cells and tissues is regulated, in large part, by intercellular signals conveyed via growth factors and similar ligands and their receptors. The receptors are located on the cell surface of responding cells and they bind peptides or polypeptides known as growth factors as well as other hormone-like ligands. The results of this interaction are rapid biochemical changes in the responding cells, as well as a rapid and a long-term readjustment of cellular gene expression. Several receptors associated with various cell surfaces may bind specific growth factors.

[0005] The phosphorylation of tyrosine residues in proteins by tyrosine kinases is one of the key modes by which signals are transduced across the plasma membrane. Several currently known protein tyrosine kinase genes encode transmembrane receptors for polypeptide growth factors and hormones such as epidermal growth factor (EGF), insulin, insulin-like growth factor-I (IGF-I), platelet derived growth factors (PDGF-A and -B), and fibroblast growth factors (FGFs). (Heldin et al., Cell Regulation, 1: 555-566 (1990); Ullrich, et al., Cell, 61: 243-54 (1990)). In each instance, these growth factors exert their action by binding to the extracellular portion of their cognate receptors, which leads to activation of the intrinsic tyrosine kinase present on the cytoplasmic portion of the receptor. Growth factor receptors of endothelial cells are of particular interest due to the possible involvement of growth factors in several important physiological and pathological processes, such as vasculogenesis, angiogenesis, atherosclerosis, and inflammatory diseases. (Folkman, et al. Science, 235: 442-447 (1987)). Also, the receptors of several hematopoietic growth factors are tyrosine kinases; these include c-fms, which is the colony stimulating factor 1 receptor, Sherr, et al., Cell, 41: 665-676 (1985), and c-kit, a primitive hematopoietic growth factor receptor reported in Huang, et al., Cell, 63: 225-33 (1990).

[0006] The receptor tyrosine kinases have been divided into evolutionary subfamilies based on the characteristic structure of their ectodomains. (Ullrich, et al. Cell, 61: 243-54 (1990)). Such subfamilies include, EGF receptor-like kinase (subclass I) and insulin receptor-like kinase (subclass II), each of which contains repeated homologous cysteine-rich sequences in their extracellular domains. A single cysteine-rich region is also found in the extracellular domains of the eph-like kinases. Hirai, et al., Science, 238: 1717-1720 (1987); Lindberg, et al. Mol. Cell. Biol., 10: 6316-24 (1990); Lhotak, et al., Mol. Cell. Biol. 11: 2496-2502 (1991). PDGF receptors as well as c-fms and c-kit receptor tyrosine kinases may be grouped into subclass III; while the FGF receptors form subclass IV. Typical for the members of both of these subclasses are extracellular folding units stabilized by intrachain disulfide bonds. These so-called immunoglobulin (Ig)-like folds are found in the proteins of the immunoglobulin superfamily which contains a wide variety of other cell surface receptors having either cell-bound or soluble ligands. Williams, et al., Ann. Rev. Immunol., 6: 381-405 (1988).

[0007] Receptor tyrosine kinases differ in their specificity and affinity. In general, receptor tyrosine kinases are glycoproteins which consist of (1) an extracellular domain capable of binding the specific growth factor(s);

[0008] (2) a transmembrane domain which usually is an alpha-helical portion of the protein; (3) a juxtamembrane domain where the receptor may be regulated by, e.g., protein phosphorylation; (4) a tyrosine kinase domain which is the enzymatic component of the receptor; and (5) a carboxyterminal tail which in many receptors is involved in recognition and binding of the substrates for the tyrosine kinase.

[0009] Processes such as alternative exon splicing and alternative choice of gene promoter or polyadenylation sites have been reported to be capable of producing several distinct polypeptides from the same gene. These polypeptides may or may not contain the various domains listed above. As a consequence, some extracellular domains may be expressed as separate, secreted proteins and some forms of the receptors may lack the tyrosine kinase domain and contain only the extracellular domain inserted in the plasma membrane via the transmembrane domain plus a short carboxyl terminal tail.

[0010] A gene encoding an endothelial cell transmembrane tyrosine kinase, originally identified by RT-PCR as an unknown tyrosine kinase-homologous cDNA fragment from human leukemia cells, was described by Partanen, et al., Proc. Nati. Acad. Sci. USA, 87: 8913-8917 (1990). This gene and its encoded protein are called "TIE" which is an abbreviation for "tyrosine kinase with Ig and EGF homology domains." Partanen, et al. Mol. Cell. Biol. 12: 1698-1707 (1992).

[0011] It has been reported that tie mRNA is present in all human fetal and mouse embryonic tissues. Upon inspection, tie message has been localized to the cardiac and vascular endothelial cells. Specifically, tie mRNA has been localized to the endothelia of blood vessels and endocardium of 9.5 to 18.5 day old mouse embryos. Enhanced tie expression was shown during neovascularization associated with developing ovarian follicles and granulation tissue in skin wounds. Korhonen, et al. Blood 80: 2548-2555 (1992). Thus the TIEs have been suggested to play a role in angiogenesis, which is important for developing treatments for solid tumors and several other angiogenesis-dependent diseases such as diabetic retinopathy, psoriasis, atherosclerosis and arthritis.

[0012] Two structurally related rat TIE receptor proteins have been reported to be encoded by distinct genes with related profiles of expression. One gene, termed tie-1, is the rat homolog of human tie. Maisonpierre, et al., Oncogene 8: 1631-1637 (1993). The other gene, tie-2, may be the rat homolog of the murine tek gene, which, like tie, has been reported to be expressed in the mouse exclusively in endothelial cells and their presumptive progenitors. Dumont, et al. Oncogene 8: 1293-1301 (1993). The human homolog of tie-2 is described in Ziegler, U.S. Pat. No. 5,447,860 which issued on Sep. 5, 1995 (wherein it is referred to as "ork"), which is incorporated in its entirety herein.

[0013] Both genes were found to be widely expressed in endothelial cells of embryonic and postnatal tissues. Significant levels of tie-2 transcripts were also present in other embryonic cell populations, including lens epithelium, heart epicardium and regions of mesenchyme. Maisonpierre, et al., Oncogene 8: 1631-1637 (1993).

[0014] The predominant expression of the TIE receptor in vascular endothelia suggests that TIE plays a role in the development and maintenance of the vascular system. This could include roles in endothelial cell determination, proliferation, differentiation and cell migration and patterning into vascular elements. Analyses of mouse embryos deficient in TIE-2 illustrate its importance in angiogenesis, particularly for vascular network formation in endothelial cells. Sato, T. N., et al., Nature 376:70-74 (1995). In the mature vascular system, the TIEs could function in endothelial cell survival, maintenance and response to pathogenic influences.

[0015] The TIE receptors are also expressed in primitive hematopoietic stem cells, B cells and a subset of megakaryocytic cells, thus suggesting the role of ligands which bind these receptors in early hematopoiesis, in the differentiation and/or proliferation of B cells, and in the megakaryocytic differentiation pathway. Iwama, et al. Biochem. Biophys. Research Communications 195:301-309 (1993); Hashiyama, et al. Blood 87:93-101 (1996), Batard, et al. Blood 87:2212-2220 (1996).

SUMMARY OF THE INVENTION

[0016] The present invention provides for a composition comprising a modified TIE-2 ligand substantially free of other proteins. As used herein, modified TIE-2 ligand refers to a ligand of the TIE family of ligands, whose representatives comprise ligands TL1, TL2, TL3 and TL4 as described herein, which has been altered by addition, deletion or substitution of one or more amino acids, or by way of tagging, with for example, the Fc portion of human IgG-1, but which retains its ability to bind the TIE-2 receptor. Modified TIE-2 ligand also includes a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first. By way of non-limiting example, the first TIE-2 ligand is TL1 and the second TIE-2 ligand is TL2. The invention envisions other combinations using additional TIE-2 ligand family members. For example, other combinations for creating a chimeric TIE-2 ligand are possible, including but not limited to those combinations wherein the first ligand is selected from the group consisting of TL1, TL2, TL3 and TL4, and the second ligand, different from the first ligand, is selected from the group consisting of TL1, TL2, TL3 and TL4.

[0017] The invention also provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand. In one embodiment, the isolated nucleic acid molecule encodes a TIE-2 ligand of the TIE family of ligands, whose representatives comprise ligands TL1, TL2, TL3 and TL4 as described herein, which has been altered by addition, deletion or substitution of one or more amino acids, or by way of tagging, with for example, the Fc portion of human IgG-1, but which retains its ability to bind the TIE-2 receptor. In another embodiment, the isolated nucleic acid molecule encodes a modified TIE-2 ligand which is a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first. By way of non-limiting example, the first TIE-2 ligand is TL1 and the second TIE-2 ligand is TL2. The invention envisions other combinations using additional TIE-2 ligand family members. For example, other combinations are possible, including but not limited to those combinations wherein the isolated nucleic acid molecule encodes a modified TIE-2 ligand which is a chimeric TIE-2 ligand comprising a portion of a first ligand selected from the group consisting of TL1, TL2, TL3 and TL4, and a portion of a second ligand, different from the first ligand, selected from the group consisting of TL1, TL2, TL3 and TL4.

[0018] The isolated nucleic acid may be DNA, cDNA or RNA. The invention also provides for a vector comprising an isolated nucleic acid molecule encoding a modified TIE-2 ligand. The invention further provides for a host-vector system for the production in a suitable host cell of a polypeptide having the biological activity of a modified TIE-2 ligand. The suitable host cell may be bacterial, yeast, insect or mammalian. The invention also provides for a method of producing a polypeptide having the biological activity of a modified TIE-2 ligand which comprises growing cells of the host-vector system under conditions permitting production of the polypeptide and recovering the polypeptide so produced.

[0019] The invention herein described of an isolated nucleic acid molecule encoding a modified TIE-2 ligand

further provides for the development of the ligand as a therapeutic for the treatment of patients suffering from disorders involving cells, tissues or organs which express the TIE-2 receptor. The present invention also provides for an antibody which specifically binds such a therapeutic molecule. The antibody may be monoclonal or polyclonal. The invention also provides for a method of using such a monoclonal or polyclonal antibody to measure the amount of the therapeutic molecule in a sample taken from a patient for purposes of monitoring the course of therapy.

[0020] The present invention also provides for an antibody which specifically binds a modified TIE-2 ligand as described herein. The antibody may be monoclonal or polyclonal. Thus the invention further provides for therapeutic compositions comprising an antibody which specifically binds a modified TIE-2 ligand, in a pharmaceutically acceptable vehicle. The invention also provides for a method of blocking blood vessel growth in a mammal by administering an effective amount of a therapeutic composition comprising an antibody which specifically binds a receptor activating modified TIE-2 ligand as described herein, in a pharmaceutically acceptable vehicle.

[0021] The invention further provides for therapeutic compositions comprising a modified TIE-2 ligand as described herein, in a pharmaceutically acceptable vehicle. The invention also provides for a method of promoting neovascularization in a patient by administering an effective amount of a therapeutic composition comprising a receptor activating modified TIE-2 ligand as described herein, in a pharmaceutically acceptable vehicle. In one embodiment, the method may be used to promote wound healing. In another embodiment, the method may be used to treat ischemia. In yet another embodiment, a receptor activating modified TIE-2 ligand as described herein is used, alone or in combination with other hematopoietic factors, to promote the proliferation or differentiation of hematopoietic stem cells, B cells or megakaryocytic cells.

[0022] Alternatively, the invention provides that a modified TIE-2 ligand may be conjugated to a cytotoxic agent and a therapeutic composition prepared therefrom. The invention further provides for a receptorbody which specifically binds a modified TIE-2 ligand. The invention further provides for therapeutic compositions comprising a receptorbody which specifically binds a modified TIE-2 ligand in a pharmaceutically acceptable vehicle. The invention also provides for a method of blocking blood vessel growth in a mammal by administering an effective amount of a therapeutic composition comprising a receptorbody which specifically binds a modified TIE-2 ligand in a pharmaceutically acceptable vehicle.

[0023] The invention also provides for a TIE-2 receptor antagonist as well as a method of inhibiting TIE-2 biological activity in a mammal comprising administering to the mammal an effective amount of a TIE-2 antagonist. According to the invention, the antagonist may be a modified TIE-2 ligand as described herein which binds to, but does not activate, the TIE-2 receptor.

BRIEF DESCRIPTION OF THE FIGURES

[0024] FIGS. 1A and 1B—TIE-2 receptorbody (TIE-2 RB) inhibits the development of blood vessels in the embryonic chicken chorioallantoic membrane (CAM). A single piece of resorbable gelatin foam (Gelfoam) soaked with 6 μg of RB was inserted immediately under the CAM of 1-day chick embryos. After 3 further days of incubation, 4 day old embryos and surrounding CAM were removed and examined. **FIG. 1**A: embryos treated with EHK-1 RB (rEHK-1 ecto/hlgG1 Fc) were viable and possessed normally developed blood vessels in their surrounding CAM. **FIG. 1**B: all embryos treated with TIE-2 RB (r TIE-2 ecto/hlgG1 Fc) were dead, diminished in size and were almost completely devoid of surrounding blood vessels.

[0025] **FIG. 2**—Vector pJFE14.

[0026] **FIG. 3**—Restriction map of λgt10.

[0027] **FIG. 4**—Nucleic acid and deduced amino acid (single letter code) sequences of human TIE-2 ligand 1 from clone λgt10 encoding htie-2 ligand 1.

[0028] **FIG. 5**—Nucleic acid and deduced amino acid (single letter code) sequences of human TIE-2 ligand 1 from T98G clone.

[0029] **FIG. 6**—Nucleic acid and deduced amino acid (single letter code) sequences of human TIE-2 ligand 2 from clone pBluescript KS encoding human TIE 2 ligand 2.

[0030] **FIG. 7**—Western blot showing activation of TIE-2 receptor by TIE-2 ligand 1 (Lane L1) but not by TIE-2 ligand 2 (Lane L2) or control (Mock).

[0031] **FIG. 8**—Western blot showing that prior treatment of HAEC cells with excess TIE-2 ligand 2 (Lane 2) antagonizes the subsequent ability of dilute TIE-2 ligand 1 to activate the TIE-2 receptor (TIE2-R) as compared with prior treatment of HAEC cells with MOCK medium (Lane 1).

[0032] **FIG. 9**—Western blot demonstrating the ability of TL2 to competitively inhibit TL1 activation of the TIE-2 receptor using the human cell hybrid line, EA.hy926.

[0033] **FIG. 10**—Histogram representation of binding to rat TIE-2 IgG immobilized surface by TIE-2 ligand in C2C12 ras, Rat2 ras, SHEP, and T98G concentrated (10×) conditioned medium. Rat TIE-2 (rTIE2) specific binding is demonstrated by the significant reduction in the binding activity in the presence of 25 μg/ml soluble rat TIE-2 RB as compared to a minor reduction in the presence of soluble trkB RB.

[0034] **FIG. 11**—Binding of recombinant human TIE-2 ligand 1 (hTL1) and human TIE-2 ligand 2 (hTL2), in COS cell supernatants, to a human TIE-2 receptorbody (RB) immobilized surface. Human TIE-2-specific binding was determined by incubating the samples with 25 μg/ml of either soluble human TIE-2 RB or trkB RB; significant reduction in the binding activity is observed only for the samples incubated with human TIE-2 RB.

[0035] **FIG. 12**—Western blot showing that TIE-2 receptorbody (denoted TIE-2 RB or, as here, TIE2-Fc) blocks the activation of TIE-2 receptors by TIE-2 ligand 1 (TL1) in HUVEC cells, whereas an unrelated receptorbody (TRKB-Fc) does not block this activation.

[0036] **FIG. 13**—Agarose gels showing serial dilutions [undiluted (1) to 10^{-4}] of the TL1 and TL2 RT-PCR products obtained from E14.5 mouse fetal liver (Lanes 1—total, Lanes 3—stromal enriched, and Lanes 4—c-kit^{+}TER119 hematopoietic precursor cells) and E14.5 mouse fetal thymus (Lanes 2—total).

[0037] **FIG. 14**—Agarose gels showing serial dilutions [undiluted (1) to 10^{-3}] of the TL1 and TL2 RT-PCR products obtained from E17.5 mouse fetal thymus cortical stromal cells (Lanes 1—CDR1+/A2B5−) and medullary stromal cells (Lane CDR1−/A2B5+).

[0038] **FIG. 15**—A schematic representation of the hypothesized role of the TIE-2/TIE ligands in angiogenesis. TL1 is represented by (•), TL2 is represented by (*), TIE-2 is represented by (T), VEGF is represented by ([]), and flk-1 (a VEGF receptor) is represented by (Y).

[0039] **FIG. 16**—In situ hybridization slides showing the temporal expression pattern of TIE-2, TL1, TL2, and VEGF during angiogenesis associated with follicular development and corpus luteum formation in the ovary of a rat that was treated with pregnant mare serum. Column 1: Early pre-ovulatory follicle; Column 2: pre-ovulatory follicle; Column 3: early corpus luteum; and Column 4: atretic follicle; Row A: bright field; Row B: VEGF; Row C: TL2; Row D: TL1 and Row E: TIE-2 receptor.

[0040] **FIG. 17**—Comparison of amino acid sequences of mature TL1 protein and mature TL2 protein. The TL1 sequence is the same as that set forth in **FIG. 4**, except that the putative leader sequence has been removed. Similarly, the TL2 sequence is the same as that set forth in **FIG. 6**, except that the putative leader sequence has been removed. Arrows indicate residues Arg49, Cys245 and Arg264 of TL1, which correspond to the residues at amino acid positions 69, 265 and 284, respectively, of TL1 as set forth in **FIG. 4**.

[0041] **FIG. 18**—Western blot of the covalent multimeric structure of TL1 and TL2 (Panel A) and the interconversion of TL1 and TL2 by the mutation of one cysteine (Panel B).

[0042] **FIG. 19**—A typical curve of TIE-2-IgG binding to immobilized TL1 in a quantitative cell-free binding assay.

[0043] **FIG. 20**—A typical curve showing TIE-2 ligand 1 ligandbody comprising the fibrinogen-like domain of the ligand bound to the Fc domain of IgG (TL1-fFc) binding to immobilized TIE-2 ectodomain in a quantitative cell-free binding assay.

[0044] **FIG. 21**—Nucleotide and deduced amino acid (single letter code) sequences of TIE ligand-3. The coding sequence starts at position 47. The fibrinogen-like domain starts at position 929.

[0045] **FIG. 22**—Comparison of Amino Acid Sequences of TIE Ligand Family Members. mTL3=mouse TIE ligand-3; hTL1=human TIE-2 ligandl; chTL1=chicken TIE-2 ligand1; mTL1=mouse TIE-2 ligand 1; mTL2=mouse TIE-2 ligand 2; hTL2=human TIE-2 ligand 2. The boxed regions indicate conserved regions of homology among the family members.

[0046] **FIG. 23**—Nucleotide and deduced amino acid (single letter code) sequences of TIE ligand-4. Arrow indicates nucleotide position 569.

[0047] **FIG. 24**—Nucleotide and deduced amino acid (single letter code) sequences of chimeric TIE ligand designated 1N1C2F (chimera 1). The putative leader sequence is encoded by nucleotides 1-60.

[0048] **FIG. 25**—Nucleotide and deduced amino acid (single letter code) sequences of chimeric TIE ligand designated 2N2C1F (chimera 2). The putative leader sequence is encoded by nucleotides 1-48.

[0049] **FIG. 26**—Nucleotide and deduced amino acid (single letter code) sequences of chimeric TIE ligand designated 1N2C2F (chimera 3). The putative leader sequence is encoded by nucleotides 1-60.

[0050] **FIG. 27**—Nucleotide and deduced amino acid (single letter code) sequences of chimeric TIE ligand designated 2N1C1F (chimera 4). The putative leader sequence is encoded by nucleotides 1-48.

DETAILED DESCRIPTION OF THE INVENTION

[0051] As described in greater detail below, applicants have created novel modified TIE-2 ligands that bind the TIE-2 receptor. The present invention provides for a composition comprising a modified TIE-2 ligand substantially free of other proteins. As used herein, modified TIE-2 ligand refers to a ligand of the TIE family of ligands, whose representatives comprise ligands TL1, TL2, TL3 and TL4 as described herein, which has been altered by addition, deletion or substitution of one or more amino acids, or by way of tagging, with for example, the Fc portion of human IgG-1, but which retains its ability to bind the TIE-2 receptor. Modified TIE-2 ligand also includes a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first. By way of non-limiting example, the first TIE-2 ligand is TL1 and the second TIE-2 ligand is TL2. The invention envisions other combinations using additional TIE-2 ligand family members. For example, other combinations for creating a chimeric TIE-2 ligand are possible,)including but not limited to those combinations wherein the first ligand is selected from the group consisting of TL1, TL2, TL3 and TL4, and the second ligand, different from the first ligand, is selected from the group consisting of TL1, TL2, TL3 and TL4.

[0052] The invention also provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand. In one embodiment, the isolated nucleic acid molecule encodes a TIE-2 ligand of the TIE family of ligands, whose representatives comprise ligands TL1, TL2, TL3 and TL4 as described herein, which has been altered by addition, deletion or substitution of one or more amino acids, or by way of tagging, with for example, the Fc portion of human IgG-1, but which retains its ability to bind the TIE-2 receptor. In another embodiment, the isolated nucleic acid molecule encodes a modified TIE-2 ligand which is a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first. By way of non-limiting example, the first TIE-2 ligand is TL1 and the second TIE-2 ligand is TL2. The invention envisions other combinations using additional TIE-2 ligand family members. For example, other combinations are possible, including but not limited to those combinations wherein the isolated nucleic acid molecule encodes a modified TIE-2 ligand which is a chimeric TIE-2 ligand comprising a portion of a first ligand selected from the group consisting of TL1, TL2, TL3 and TL4, and a portion of a second ligand, different from the first ligand, selected from the group consisting of TL1, TL2, TL3 and TL4.

[0053] The present invention comprises the modified TIE-2 ligands and their amino acid sequences, as well as functionally equivalent variants thereof, as well as proteins or peptides comprising substitutions, deletions or insertional mutants of the described sequences, which bind TIE-2 receptor and act as agonists or antagonists thereof. Such variants include those in which amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid(s) of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the class of nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

[0054] Also included within the scope of the invention are proteins or fragments or derivatives thereof which exhibit the same or similar biological activity as the modified TIE-2 ligands described herein, and derivatives which are differentially modified during or after translation, eg., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Functionally equivalent molecules also include molecules that contain modifications, including N-terminal modifications, which result from expression in a particular recombinant host, such as, for example, N-terminal methylation which occurs in certain bacterial (e.g. *E. coli*) expression systems.

[0055] The present invention also encompasses the nucleotide sequences that encode the proteins described herein as modified TIE-2 ligands, as well as host cells, including yeast, bacteria, viruses, and mammalian cells, which are genetically engineered to produce the proteins, by e.g. transfection, transduction, infection, electroporation, or microinjection of nucleic acid encoding the modified TIE-2 ligands described herein in a suitable expression vector. The present invention also encompasses introduction of the nucleic acid encoding modified TIE-2 ligands through gene therapy techniques such as is described, for example, in Finkel and Epstein FASEB J. 9:843-851 (1995); Guzman, et al. PNAS (USA) 91:10732-10736 (1994).

[0056] One skilled in the art will also recognize that the present invention encompasses DNA and RNA sequences that hybridize to a modified TIE-2 ligand encoding nucleotide sequence, under conditions of moderate stringency, as defined in, for example, Sambrook, et al. Molecular Cloning: A Laboratory Manual, 2 ed. Vol. 1, pp. 101-104, Cold Spring Harbor Laboratory Press (1989). Thus, a nucleic acid molecule contemplated by the invention includes one having a nucleotide sequence deduced from an amino acid sequence of a modified TIE-2 ligand prepared as described herein, as well as a molecule having a sequence of nucleotides that hybridizes to such a nucleotide sequence, and also a nucleotide sequence which is degenerate of the above sequences as a result of the genetic code, but which encodes a ligand that binds TIE-2 receptor and which has an amino acid sequence and other primary, secondary and tertiary characteristics that are sufficiently duplicative of a modified TIE-2 ligand described herein so as to confer on the molecule the same biological activity as the modified TIE-2 ligand described herein.

[0057] The present invention provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds and activates TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 2. The invention also provides for such a nucleic acid molecule, with a further modification such that the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 2.

[0058] The present invention also provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds and activates TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 2 and which is further modified to encode a different amino acid instead of the cysteine residue encoded by nucleotides 784-787 as set forth in **FIG. 27**. A serine residue is preferably substituted for the cysteine residue. In another embodiment, the nucleic acid molecule is further modified to encode a different amino acid instead of the arginine residue encoded by nucleotides 199-201 as set forth in **FIG. 27**. A serine residue is preferably substituted for the arginine residue.

[0059] The present invention also provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds and activates TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 which is modified to encode a different amino acid instead of the cysteine residue at amino acid position 245. A serine residue is preferably substituted for the cysteine residue.

[0060] The invention further provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 1 is deleted. The invention also provides for such a nucleic acid molecule further modified so that the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is deleted and the portion encoding the fibrinogen-like domain is fused in-frame to a nucleotide sequence encoding a human immunoglobulin gamma-1 constant region (IgG1 Fc).

[0061] The invention further provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 2 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 2 is deleted. The invention also provides for such a nucleic acid molecule further modified so that the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 2 is deleted and the portion encoding the fibrinogen-like domain is fused in-frame to a nucleotide sequence encoding a human immunoglobulin gamma-1 constant region (IgG1 Fc).

[0062] The invention further provides for an isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the fibrinogen-like domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the fibrinogen-like domain of TIE-2 ligand 2. The invention also provides for such a nucleic acid molecule further modified so that the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 2.

[0063] The invention further provides for a modified TIE-2 ligand encoded by any of nucleic acid molecules of the invention.

[0064] The present invention also provides for a chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first, wherein the first and second TIE-2 ligands are selected from the group consisting of TIE-2 Ligand-1, TIE-2 Ligand-2, TIE Ligand-3 and TIE Ligand-4. Preferably, the chimeric TIE ligand comprises at least a portion of TIE-2 Ligand-1 and a portion of TIE-2 Ligand-2.

[0065] The invention also provides a nucleic acid molecule that encodes a chimeric TIE ligand as set forth in **FIG. 24, 25, 26,** or **27**. The invention also provides a chimeric TIE ligand as set forth in **FIG. 24, 25, 26,** or **27**. The invention further provides a chimeric TIE ligand as set forth in **FIG. 27**, modified to have a different amino acid instead of the cysteine residue encoded by nucleotides 784-787.

[0066] Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors encoding a modified TIE-2 ligand using appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinations (genetic recombination). Expression of a nucleic acid sequence encoding a modified TIE-2 ligand or peptide fragments thereof may be regulated by a second nucleic acid sequence which is operably linked to the a modified TIE-2 ligand encoding sequence such that the modified TIE-2 ligand protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a modified TIE-2 ligand described herein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the ligand include, but are not limited to the long terminal repeat as described in Squinto et al., (Cell 65:1-20 (1991)); the SV40 early promoter region (Bernoist and Chambon, Nature 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat, the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., Cell 22:787-797 (1980)), the herpes thymidine kinase promoter (Wagner et al., Proc. Nati. Acad. Sci. U.S.A. 78:144-1445 (1981)), the adenovirus promoter, the regulatory sequences of the metallothionein gene (Brinster et al., Nature 296:39-42 (1982)); prokaryotic expression vectors such as the P-lactamase promoter (Villa-Kamaroff, et al., Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731 (1978)), or the tac promoter (DeBoer, et al., Proc. Natl. Acad. Sci. U.S.A. 80:21-25 (1983)), see also "Useful proteins from recombinant bacteria" in Scientific American, 242:74-94 (1980); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals; elastase I gene control region which is active in pancreatic acinar cells (Swift et al., Cell 38:639-646 (1984); Ornitz et al., Cold Spring Harbor Symp. Quant. Biol. 50:399-409 (1986); MacDonald, Hepatology 7:425-515 (1987); insulin gene control region which is active in pancreatic beta cells [Hanahan, Nature 315:115-122 (1985)]; immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58); alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al, 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocytes in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378). The invention further encompasses the production of antisense compounds which are capable of specifically hybridizing with a sequence of RNA encoding a modified TIE-2 ligand to modulate its expression. Ecker, U.S. Pat. No. 5,166,195, issued Nov. 24, 1992.

[0067] Thus, according to the invention, expression vectors capable of being replicated in a bacterial or eukaryotic host comprising a nucleic acid encoding a modified TIE-2 ligand as described herein, are used to transfect a host and thereby direct expression of such nucleic acid to produce a modified TIE-2 ligand, which may then be recovered in a biologically active form. As used herein, a biologically active form includes a form capable of binding to TIE receptor and causing a biological response such as a differentiated function or influencing the phenotype of the cell expressing the receptor. Such biologically active forms could, for example, induce phosphorylation of the tyrosine kinase domain of TIE receptor. Alternatively, the biological activity may be an effect as an antagonist to the TIE receptor. In alternative embodiments, the active form of a modified TIE-2 ligand is one that can recognize TIE receptor and thereby act as a targeting agent for the receptor for use in both diagnostics and therapeutics. In accordance with such embodiments, the active form need not confer upon any TIE expressing cell any change in phenotype.

[0068] Expression vectors containing the gene inserts can be identified by four general approaches: (a) DNA-DNA hybridization, (b) presence or absence of "marker" gene functions, (c) expression of inserted sequences and (d) PCR detection. In the first approach, the presence of a foreign

gene inserted in an expression vector can be detected by DNA-DNA hybridization using probes comprising sequences that are homologous to an inserted modified TIE-2 ligand encoding gene. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. For example, if a nucleic acid encoding a modified TIE-2 ligand is inserted within the marker gene sequence of the vector, recombinants containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of a modified TIE-2 ligand gene product, for example, by binding of the ligand to TIE receptor or a portion thereof which may be tagged with, for example, a detectable antibody or portion thereof or by binding to antibodies produced against the modified TIE-2 ligand protein or a portion thereof. Cells of the present invention may transiently or, preferably, constitutively and permanently express a modified TIE-2 ligand as described herein. In the fourth approach, DNA nucleotide primers can be prepared corresponding to a tie specific DNA sequence. These primers could then be used to PCR a tie gene fragment. (PCR Protocols: A Guide To Methods and Applications, Edited by Michael A. Innis et al., Academic Press (1 990)).

[**0069**] The recombinant ligand may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. Preferably, the ligand is secreted into the culture medium from which it is recovered. Alternatively, the ligand may be recovered from cells either as soluble proteins or as inclusion bodies, from which it may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis in accordance with well known methodology. In order to further purify the ligand, affinity chromatography, conventional ion exchange chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

[**0070**] In additional embodiments of the invention, as described in greater detail in the Examples, a modified TIE-2 ligand encoding gene may be used to inactivate or "knock out" an endogenous gene by homologous recombination, and thereby create a TIE ligand deficient cell, tissue, or animal. For example, and not by way of limitation, the recombinant TIE ligand-4 encoding gene may be engineered to contain an insertional mutation, for example the neo gene, which would inactivate the native TIE ligand-4 encoding gene. Such a construct, under the control of a suitable promoter, may be introduced into a cell, such as an embryonic stem cell, by a technique such as transfection, transduction, or injection. Cells containing the construct may then be selected by G418 resistance. Cells which lack an intact TIE ligand-4 encoding gene may then be identified, e.g. by Southern blotting, PCR detection, Northern blotting or assay of expression. Cells lacking an intact TIE ligand-4 encoding gene may then be fused to early embryo cells to generate transgenic animals deficient in such ligand. Such an animal may be used to define specific in vivo processes, normally dependent upon the ligand.

[**0071**] The present invention also provides for antibodies to a modified TIE-2 ligand described herein which are useful for detection of the ligand in, for example, diagnostic applications. For preparation of monoclonal antibodies directed toward a modified TIE-2 ligand, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc. pp. 77-96) and the like are within the scope of the present invention.

[**0072**] The monoclonal antibodies may be human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:7308-7312; Kozbor et al., 1983, Immunology Today 4:72-79; Olsson et al., 1982, Meth. Enzymol. 92:3-16). Chimeric antibody molecules may be prepared containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851, Takeda et al., 1985, Nature 314:452).

[**0073**] Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of a modified TIE-2 ligand described herein. For the production of antibody, various host animals, including but not limited to rabbits, mice and rats can be immunized by injection with a modified TIE-2 ligand, or a fragment or derivative thereof. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum.*

[**0074**] A molecular clone of an antibody to a selected a modified TIE-2 ligand epitope can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, or antigen binding region thereof.

[**0075**] The present invention provides for antibody molecules as well as fragments of such antibody molecules. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Antibody molecules may be purified by known techniques, es, immunoabsorption or immunoaffinity chroma-

tography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof.

[0076] The present invention further encompasses an immunoassay for measuring the amount of a modified TIE-2 ligand in a biological sample by

[0077] a) contacting the biological sample with at least one antibody which specifically binds a modified TIE-2 ligand so that the antibody forms a complex with any modified TIE-2 ligand present in the sample; and

[0078] b) measuring the amount of the complex and thereby measuring the amount of the modified TIE-2 ligand in the biological sample.

[0079] The invention further encompasses an assay for measuring the amount of TIE receptor in a biological sample by

[0080] a) contacting the biological sample with at least one ligand of the invention so that the ligand forms a complex with the TIE receptor; and

[0081] b) measuring the amount of the complex and thereby measuring the amount of the TIE receptor in the biological sample.

[0082] The present invention also provides for the utilization of a modified TIE-2 ligand which activates the TIE-2 receptor as described herein, to support the survival and/or growth and/or migration and/or differentiation of TIE-2 receptor expressing cells. Thus, the ligand may be used as a supplement to support, for example, endothelial cells in culture.

[0083] Further, the creation by applicants of a modified TIE-2 ligand for the TIE-2 receptor enables the utilization of assay systems useful for the identification of agonists or antagonists of the TIE-2 receptor. Such assay systems would be useful in identifying molecules capable of promoting or inhibiting angiogenesis. For example, in one embodiment, antagonists of the TIE-2 receptor may be identified as test molecules that are capable of interfering with the interaction of the TIE-2 receptor with a modified TIE-2 ligand that binds the TIE-2 receptor. Such antagonists are identified by their ability to 1) block the binding of a biologically active modified TIE-2 ligand to the receptor as measured, for example, using BIAcore biosensor technology (BIAcore; Pharmacia Biosensor, Piscataway, N.J.); or 2) block the ability of a biologically active modified TIE-2 ligand to cause a biological response. Such biological responses include, but are not limited to, phosphorylation of the TIE receptor or downstream components of the TIE signal transduction pathway, or survival, growth or differentiation of TIE receptor bearing cells.

[0084] In one embodiment, cells engineered to express the TIE receptor may be dependent for growth on the addition of a modified TIE-2 ligand. Such cells provide useful assay systems for identifying additional agonists of the TIE receptor, or antagonists capable of interfering with the activity of the modified TIE-2 ligand on such cells. Alternatively, autocrine cells, engineered to be capable of co-expressing both a modified TIE-2 ligand and receptor, may provide useful systems for assaying potential agonists or antagonists.

[0085] Therefore, the present invention provides for introduction of a TIE-2 receptor into cells that do not normally express this receptor, thus allowing these cells to exhibit profound and easily distinguishable responses to a ligand which binds this receptor. The type of response elicited depends on the cell utilized, and not the specific receptor introduced into the cell. Appropriate cell lines can be chosen to yield a response of the greatest utility for assaying, as well as discovering, molecules that can act on tyrosine kinase receptors. The molecules may be any type of molecule, including but not limited to peptide and non-peptide molecules, that will act in systems to be described in a receptor specific manner.

[0086] One of the more useful systems to be exploited involves the introduction of a TIE receptor (or a chimeric receptor comprising the extracellular domain of another receptor tyrosine kinase such as, for example, trkC and the intracellular domain of a TIE receptor) into a fibroblast cell line (e.g., NIH3T3 cells) thus such a receptor which does not normally mediate proliferative or other responses can, following introduction into fibroblasts, nonetheless be assayed by a variety of well established methods to quantitate effects of fibroblast growth factors (e.g. thymidine incorporation or other types of proliferation assays; see van Zoelen, 1990, "The Use of Biological Assays For Detection Of Polypeptide Growth Factors" in Progress Factor Research, Vol. 2, pp. 131-152; Zhan and M. Goldfarb, 1986, Mol. Cell. Biol., Vol. 6, pp. 3541-3544). These assays have the added advantage that any preparation can be assayed both on the cell line having the introduced receptor as well as the parental cell line lacking the receptor; only specific effects on the cell line with the receptor would be judged as being mediated through the introduced receptor. Such cells may be further engineered to express a modified TIE-2 ligand, thus creating an autocrine system useful for assaying for molecules that act as antagonists/agonists of this interaction. Thus, the present invention provides for host cells comprising nucleic acid encoding a modified TIE-2 ligand and nucleic acid encoding TIE receptor.

[0087] The TIE receptor/modified TIE-2 ligand interaction also provides a useful system for identifying small molecule agonists or antagonists of the TIE receptor. For example, fragments, mutants or derivatives of a modified TIE-2 ligand may be identified that bind TIE receptor but do not induce any other biological activity. Alternatively, the characterization of a modified TIE-2 ligand enables the further characterization of active portions of the molecule. Further, the identification of a ligand enables the determination of the X-ray crystal structure of the receptor/ligand complex, thus enabling identification of the binding site on the receptor. Knowledge of the binding site will provide useful insight into the rational design of novel agonists and antagonists.

[0088] The specific binding of a test molecule to TIE receptor may be measured in a number of ways. For example, the actual binding of test molecule to cells expressing TIE may be detected or measured, by detecting or measuring (i) test molecule bound to the surface of intact cells; (ii) test molecule cross-linked to TIE protein in cell lysates; or (iii) test molecule bound to TIE in vitro. The specific interaction between test molecule and TIE may be evaluated by using reagents that demonstrate the unique properties of that interaction.

[0089] As a specific, nonlimiting example, the methods of the invention may be used as follows. Consider a case in which a modified TIE-2 ligand in a sample is to be measured. Varying dilutions of the sample (the test molecule), in parallel with a negative control (NC) containing no modified TIE-2 ligand activity, and a positive control (PC) containing a known amount of a modified TIE-2 ligand, may be exposed to cells that express TIE in the presence of a detectably labeled modified TIE-2 ligand (in this example, radioiodinated ligand). The amount of modified TIE-2 ligand in the test sample may be evaluated by determining the amount of ^{125}I-labeled modified TIE-2 ligand that binds to the controls and in each of the dilutions, and then comparing the sample values to a standard curve. The more modified TIE-2 ligand in the sample, the less ^{125}I-ligand that will bind to TIE.

[0090] The amount of ^{125}I-ligand bound may be determined by measuring the amount of radioactivity per cell, or by cross-linking a modified TIE-2 ligand to cell surface proteins using DSS, as described in Meakin and Shooter, 1991, Neuron 6:153-163, and detecting the amount of labeled protein in cell extracts using, for example, SDS polyacrylamide gel electrophoresis, which may reveal a labeled protein having a size corresponding to TIE receptor/modified TIE-2 ligand. The specific test molecule/TIE interaction may further be tested by adding to the assays various dilutions of an unlabeled control ligand that does not bind the TIE receptor and therefore should have no substantial effect on the competition between labeled modified TIE-2 ligand and test molecule for TIE binding. Alternatively, a molecule known to be able to disrupt TIE receptor/modified TIE-2 ligand binding, such as, but not limited to, anti-TIE antibody, or TIE receptorbody as described herein, may be expected to interfere with the competition between ^{125}I-modified TIE-2 ligand and test molecule for TIE receptor binding.

[0091] Detectably labeled modified TIE-2 ligand includes, but is not limited to, a modified TIE-2 ligand linked covalently or noncovalently to a radioactive substance, a fluorescent substance, a substance that has enzymatic activity, a substance that may serve as a substrate for an enzyme (enzymes and substrates associated with calorimetrically detectable reactions are preferred) or to a substance that can be recognized by an antibody molecule that is preferably a detectably labeled antibody molecule.

[0092] Alternatively, the specific binding of test molecule to TIE may be measured by evaluating the secondary biological effects of a modified TIE-2 ligand/TIE receptor binding, including, but not limited to, cell growth and/or differentiation or immediate early gene expression or phosphorylation of TIE. For example, the ability of the test molecule to induce differentiation can be tested in cells that lack tie and in comparable cells that express tie; differentiation in tie-expressing cells but not in comparable cells that lack tie would be indicative of a specific test molecule/TIE interaction. A similar analysis could be performed by detecting immediate early gene (e.g. fos and jun) induction in tie-minus and tie-plus cells, or by detecting phosphorylation of TIE using standard phosphorylation assays known in the art. Such analysis might be useful in identifying agonists or antagonists that do not competitively bind to TIE.

[0093] Similarly, the present invention provides for a method of identifying a molecule that has the biological activity of a modified TIE-2 ligand comprising (i) exposing a cell that expresses tie to a test molecule and (ii) detecting the specific binding of the test molecule to TIE receptor, in which specific binding to TIE positively correlates with TIE-like activity. Specific binding may be detected by either assaying for direct binding or the secondary biological effects of binding, as discussed supra. Such a method may be particularly useful in identifying new members of the TIE ligand family or, in the pharmaceutical industry, in screening a large array of peptide and non-peptide molecules (e.g., peptidomimetics) for TIE associated biological activity. In a preferred, specific, nonlimiting embodiment of the invention, a large grid of culture wells may be prepared that contain, in alternate rows, PC12 (or fibroblasts, see infra) cells that are either tie-minus or engineered to be tie-plus. A variety of test molecules may then be added such that each column of the grid, or a portion thereof, contains a different test molecule. Each well could then be scored for the presence or absence of growth and/or differentiation. An extremely large number of test molecules could be screened for such activity in this manner.

[0094] In additional embodiments, the invention provides for methods of detecting or measuring TIE ligand-like activity or identifying a molecule as having such activity comprising (i) exposing a test molecule to a TIE receptor protein in vitro under conditions that permit binding to occur and (ii) detecting binding of the test molecule to the TIE receptor protein, in which binding of test molecule to TIE receptor correlates with TIE ligand-like activity. According to such methods, the TIE receptor may or may not be substantially purified, may be affixed to a solid support (e.g. as an affinity column or as an ELISA assay), or may be incorporated into an artificial membrane. Binding of test molecule to TIE receptor may be evaluated by any method known in the art. In preferred embodiments, the binding of test molecule may be detected or measured by evaluating its ability to compete with detectably labeled known TIE ligands for TIE receptor binding.

[0095] The present invention also provides for a method of detecting the ability of a test molecule to function as an antagonist of TIE ligand-like activity comprising detecting the ability of the molecule to inhibit an effect of TIE ligand binding to TIE receptor on a cell that expresses the receptor. Such an antagonist may or may not interfere with TIE receptor/modified TIE-2 ligand binding. Effects of a modified TIE-2 ligand binding to TIE receptor are preferably biological or biochemical effects, including, but not limited to, cell survival or proliferation, cell transformation, immediate early gene induction, or TIE phosphorylation.

[0096] The invention further provides for both a method of identifying antibodies or other molecules capable of neutralizing the ligand or blocking binding to the receptor, as well as the molecules identified by the method. By way of nonlimiting example, the method may be performed via an assay which is conceptually similar to an ELISA assay. For example, TIE receptorbody may be bound to a solid support, such as a plastic multiwell plate. As a control, a known amount of a modified TIE-2 ligand which has been Myc-tagged may then be introduced to the well and any tagged modified TIE-2 ligand which binds the receptorbody may then be identified by means of a reporter antibody directed against the Myc-tag. This assay system may then be used to screen test samples for molecules which are capable of i)

binding to the tagged ligand or ii) binding to the receptorbody and thereby blocking binding to the receptorbody by the tagged ligand. For example, a test sample containing a putative molecule of interest together with a known amount of tagged ligand may be introduced to the well and the amount of tagged ligand which binds to the receptorbody may be measured. By comparing the amount of bound tagged ligand in the test sample to the amount in the control, samples containing molecules which are capable of blocking ligand binding to the receptor may be identified. The molecules of interest thus identified may be isolated using methods well known to one of skill in the art.

[0097] Once a blocker of ligand binding is found, one of skill in the art would know to perform secondary assays to determine whether the blocker is binding to the receptor or to the ligand, as well as assays to determine if the blocker molecule can neutralize the biological activity of the ligand. For example, by using a binding assay which employs BlAcore biosensor technology (or the equivalent), in which either TIE receptorbody or a modified TIE-2 ligand or ligandbody is covalently attached to a solid support (e.g. carboxymethyl dextran on a gold surface), one of skill in the art would be able to determine if the blocker molecule is binding specifically to the ligand, ligandbody or to the receptorbody. To determine if the blocker molecule can neutralize the biological activity of the ligand, one of skill in the art could perform a phosphorylation assay (see Example 5) or alternatively, a functional bioassay, such as a survival assay, by using primary cultures of, for example, endothelial cells. Alternatively, a blocker molecule which binds to the receptorbody could be an agonist and one of skill in the art would know to how to determine this by performing an appropriate assay for identifying additional agonists of the TIE receptor.

[0098] In addition, the invention further contemplates compositions wherein the TIE ligand is the receptor binding domain of a TIE-2 ligand described herein, For example, TIE-2 ligand 1 contains a "coiled coil" domain (beginning at the 5' end and extending to the nucleotide at about position 1160 of **FIG. 4** and about position 1157 of **FIG. 5**) and a fibrinogen-like domain (which is encoded by the nucleotide sequence of **FIG. 4** beginning at about position 1161 and about position 1158 of **FIG. 5**). The fibrinogen-like domain of TIE-2 ligand 2 is believed to begin on or around the same amino acid sequence as in ligand 1 (FRDCA) which is encoded by nucleotides beginning around 1197 of **FIG. 6**. The fibrinogen-like domain of TIE ligand-3 is believed to begin on or around the amino acid sequence which is encoded by nucleotides beginning around position 929 as set forth in **FIG. 21**. Multimerization of the coiled coil domains during production of the ligand hampers purification. As described in Example 19, Applicants have discovered, however, that the fibrinogen-like domain comprises the TIE-2 receptor binding domain. The monomeric forms of the fibrinogen-like domain do not, however, appear to bind the receptor. Studies utilizing myc-tagged fibrinogen-like domain, which has been "clustered" using anti-myc antibodies, do bind the TIE-2 receptor. [Methods of production of clustered ligands and ligandbodies are described in Davis, et al. Science 266:816-819 (1994)]. Based on these finding, applicants produced "ligandbodies" which comprise the fibrinogen-like domain of the TIE-2 ligands coupled to the Fc domain of IgG ("fFc's"). These ligandbodies, which form dimers, efficiently bind the TIE-2 receptor. Accordingly, the present invention contemplates the production of modified TIE ligandbodies which may be used as targeting agents, in diagnostics or in therapeutic applications, such as targeting agents for tumors and/or associated vasculature wherein a TIE antagonist is indicated.

[0099] The invention herein further provides for the development of the ligand, a fragment or derivative thereof, or another molecule which is a receptor agonist or antagonist, as a therapeutic for the treatment of patients suffering from disorders involving cells, tissues or organs which express the TIE receptor. Such molecules may be used in a method of treatment of the human or animal body, or in a method of diagnosis.

[0100] Because TIE receptor has been identified in association with endothelial cells and, as demonstrated herein, blocking of TIE-2 ligand 1 appears to prevent vascularization, applicants expect that a modified TIE-2 ligand described herein may be useful for the induction of vascularization in diseases or disorders where such vascularization is indicated. Such diseases or disorders would include wound healing, ischaemia and diabetes. The ligands may be tested in animal models and used therapeutically as described for other agents, such as vascular endothelial growth factor (VEGF), another endothelial cell-specific factor that is angiogenic. Ferrara, et al. U.S. Pat. No. 5,332,671 issued Jul. 26, 1994. The Ferrara reference, as well as other studies, describe in vitro and in vivo studies that may be used to demonstrate the effect of an angiogenic factor in enhancing blood flow to ischemic myocardium, enhancing wound healing, and in other therapeutic settings wherein neoangiogenesis is desired. [see Sudo, et al. European Patent Application 0 550 296 A2 published Jul. 7, 1993; Banai, et al. Circulation 89:2183-2189 (1994); Unger, et al. Am. J. Physiol. 266:H1588-H1595 (1994); Lazarous, et al. Circulation 91:145-153 (1995)]. According to the invention, a modified TIE-2 ligand may be used alone or in combination with one or more additional pharmaceutically active compounds such as, for example, VEGF or basic fibroblast growth factor (bFGF), as well as cytokines, neurotrophins, etc.

[0101] Conversely, antagonists of the TIE receptor, such as modified TIE-2 ligands which bind but do not activate the receptor as described herein, receptorbodies as described herein in Examples 2 and 3, and TIE-2 ligand 2 as described in Example 9, would be useful to prevent or attenuate vascularization, thus preventing or attenuating, for example, tumor growth. These agents may be used alone or in combination with other compositions, such as anti-VEGF antibodies, that have been shown to be useful in treating conditions in which the therapeutic intent is to block angiogenesis. Applicants expect that a modified TIE-2 ligand described herein may also be used in combination with agents, such as cytokine antagonists such as IL-6 antagonists, that are known to block inflammation.

[0102] For example, applicants have determined that TIE ligands are expressed in cells within, or closely associated with, tumors. For example, TIE-2 ligand 2 appears to be tightly associated with tumor endothelial cells. Accordingly, it and other TIE antagonists may also be useful in preventing or attenuating, for example, tumor growth. In addition, TIE ligands or ligandbodies may be useful for the delivery of toxins to a receptor bearing cell. Alternatively, other mol-

ecules, such as growth factors, cytokines or nutrients, may be delivered to a TIE receptor bearing cell via TIE ligands or ligandbodies. TIE ligands or ligandbodies such as modified TIE-2 ligand described herein may also be used as diagnostic reagents for TIE receptor, to detect the receptor in vivo or in vitro. Where the TIE receptor is associated with a disease state, TIE ligands or ligandbodies such as a modified TIE-2 ligand may be useful as diagnostic reagents for detecting the disease by, for example, tissue staining or whole body imaging. Such reagents include radioisotopes, flurochromes, dyes, enzymes and biotin. Such diagnostics or targeting agents may be prepared as described in Alitalo, et al. WO 95/26364 published Oct. 5, 1995 and Burrows, F. and P. Thorpe, PNAS (USA) 90:8996-9000 (1993) which is incorporated herein in its entirety.

[0103] In other embodiments, the TIE ligands, a receptor activating modified TIE-2 ligand described herein are used as hematopoietic factors. A variety of hematopoietic factors and their receptors are involved in the proliferation and/or differentiation and/or migration of the various cells types contained within blood. Because the TIE receptors are expressed in early hematopoietic cells, the TIE ligands are expected to play a comparable role in the proliferation or differentiation or migration of these cells. Thus, for example, TIE containing compositions may be prepared, assayed, examined in in vitro and in vivo biological systems and used therapeutically as described in any of the following: Sousa, U.S. Pat. No. 4,810,643, Lee, et al., Proc. Natl. Acad. Sci. USA 82:4360-4364 (1985) Wong, et al. Science, 228:810-814 (1985); Yokota, et al. Proc. Natl. Acad. Sci (USA) 81:1070 (1984); Bosselman, et al. WO 9105795 published May 2, 1991 entitled "Stem Cell Factor" and Kirkness, et al. WO 95/19985 published Jul. 27, 1995 entitled "Haemopoietic Maturation Factor". Accordingly, receptor activating modified TIE-2 ligand may be used to diagnose or treat conditions in which normal hematopoiesis is suppressed, including, but not limited to anemia, thrombocytopenia, leukopenia and granulocytopenia. In a preferred embodiment, receptor activating modified TIE-2 ligand may be used to stimulate differentiation of blood cell precursors in situations where a patient has a disease, such as acquired immune deficiency syndrome (AIDS) which has caused a reduction in normal blood cell levels, or in clinical settings in which enhancement of hematopoietic populations is desired, such as in conjunction with bone marrow transplant, or in the treatment of aplasia or myelosuppression caused by radiation, chemical treatment or chemotherapy.

[0104] The receptor activating modified TIE-2 ligands of the present invention may be used alone, or in combination with another pharmaceutically active agent such as, for example, ctyokines, neurotrophins, interleukins, etc. In a preferred embodiment, the ligands may be used in conjunction with any of a number of the above referenced factors which are known to induce stem cell or other hematopoietic precursor proliferation, or factors acting on later cells in the hematopoietic pathway, including, but not limited to, hemopoietic maturation factor, thrombopoietin, stem cell factor, erythropoietin, G-CSF, GM-CSF, etc.

[0105] In an alternative embodiment, TIE receptor antagonists are used to diagnose or treat patients in which the desired result is inhibition of a hematopoietic pathway, such as for the treatment of myeloproliferative or other proliferative disorders of blood forming organs such as thrombocythemias, polycythemias and leukemias. In such embodiments, treatment may comprise use of a therapeutically effective amount of the a modified TIE-2 ligand, TIE antibody, TIE receptorbody, a conjugate of a modified TIE-2 ligand, or a ligandbody or fFC as described herein.

[0106] The present invention also provides for pharmaceutical compositions comprising a modified TIE-2 ligand or ligandbodies described herein, peptide fragments thereof, or derivatives in a pharmacologically acceptable vehicle. The modified TIE-2 ligand proteins, peptide fragments, or derivatives may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, including, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

[0107] The present invention also provides for an antibody which specifically binds such a therapeutic molecule. The antibody may be monoclonal or polyclonal. The invention also provides for a method of using such a monoclonal or polyclonal antibody to measure the amount of the therapeutic molecule in a sample taken from a patient for purposes of monitoring the course of therapy.

[0108] The invention further provides for a therapeutic composition comprising a modified TIE-2 ligand or ligandbody and a cytotoxic agent conjugated thereto. In one embodiment, the cytotoxic agent may be a radioisotope or toxin.

[0109] The invention also provides for an antibody which specifically binds a modified TIE-2 ligand. The antibody may be monoclonal or polyclonal. The invention further provides for a method of purifying a modified TIE-2 ligand comprising:

[0110] a) coupling at least one TIE binding substrate to a solid matrix;

[0111] b) incubating the substrate of a) with a cell lysate so that the substrate forms a complex with any modified TIE-2 ligand in the cell lysate;

[0112] c) washing the solid matrix; and

[0113] d) eluting the modified TIE-2 ligand from the coupled substrate.

[0114] The substrate may be any substance that specifically binds the modified TIE-2 ligand. In one embodiment, the substrate is selected from the group consisting of anti-modified TIE-2 ligand antibody, TIE receptor and TIE receptorbody. The invention further provides for a receptorbody which specifically binds a modified TIE-2 ligand, as well as a therapeutic composition comprising the receptorbody in a pharmaceutically acceptable vehicle, and a method of blocking blood vessel growth in a human comprising administering an effective amount of the therapeutic composition.

[0115] The invention also provides for a therapeutic composition comprising a receptor activating modified TIE-2 ligand or ligandbody in a pharmaceutically acceptable vehicle, as well as a method of promoting neovascularization in a patient comprising administering to the patient an effective amount of the therapeutic composition.

[0116] In addition, the present invention provides for a method for identifying a cell which expresses TIE receptor which comprises contacting a cell with a detectably labeled modified TIE-2 ligand or ligandbody, under conditions permitting binding of the detectably labeled ligand to the TIE receptor and determining whether the detectably labeled ligand is bound to the TIE receptor, thereby identifying the cell as one which expresses TIE receptor. The present invention also provides for a therapeutic composition comprising a modified TIE-2 ligand or ligandbody and a cytotoxic agent conjugated thereto. The cytotoxic agent may be a radioisotope or toxin.

[0117] The invention also provides a method of detecting expression of a modified TIE-2 ligand by a cell which comprises obtaining mRNA from the cell, contacting the mRNA so obtained with a labeled nucleic acid molecule encoding a modified TIE-2 ligand, under hybridizing conditions, determining the presence of mRNA hybridized to the labeled molecule, and thereby detecting the expression of a modified TIE-2 ligand in the cell.

[0118] The invention further provides a method of detecting expression of a modified TIE-2 ligand in tissue sections which comprises contacting the tissue sections with a labeled nucleic acid molecule encoding a modified TIE-2 ligand, under hybridizing conditions, determining the presence of mRNA hybridized to the labelled molecule, and thereby detecting the expression of a modified TIE-2 ligand in tissue sections.

EXAMPLE 1

Identification of the ABAE Cell Line as Reporter Cells for the TIE-2 Receptor

[0119] Adult BAE cells are registered in the European Cell Culture Repository, under ECACC#92010601. (See PNAS 75:2621 (1978)). Northern (RNA) analyses revealed moderate levels of tie-2 transcripts in the ABAE (Adult Bovine Arterial Endothelial) cell line, consistent with in situ hybridization results that demonstrated almost exclusive localization of tie-2 RNAs to vascular endothelial cells. We therefore examined ABAE cell lysates for the presence of TIE-2 protein, as well as the extent to which this TIE-2 protein is tyrosine-phosphorylated under normal versus serum-deprived growth conditions. ABAE cell lysates were harvested and subjected to immunoprecipitation, followed by Western blot analyses of immunoprecipitated proteins with TIE-2 specific and phosphotyrosine-specific antisera. Omission or inclusion of TIE-2 peptides as specific blocking molecules during TIE-2 immunoprecipitation allowed unambiguous identification of TIE-2 as a moderately detectable protein of ~150 kD whose steady-state phosphotyrosine levels diminish to near undetectable levels by prior serum-starvation of the cells.

[0120] Culture of ABAE cells and harvest of cell lysates was done as follows. Low-passage-number ABAE cells were plated as a monolayer at a density of 2×10^6 cells/150 mm plastic petri plate (Falcon) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine calf serum (10% BCS), 2 mM L-glutamine (Q) and 1% each of penicillin and streptomycin (P-S) in an atmosphere of 5% CO_2. Prior to harvest of cell lysates, cells were serum-starved for 24 hours in DMEM/Q/P-S, followed by aspiration of the medium and rinsing of the plates with ice-cold phosphate buffered saline (PBS) supplemented with sodium orthovanadate, sodium fluoride and sodium benzamidine. Cells were lysed in a small volume of this rinse buffer that had been supplemented with 1% NP40 detergent and the protease inhibitors PMSF and aprotinin. Insoluble debris was removed from the cell lysates by centrifugation at 14,000×G for 10 minutes, at 4° C. and the supernatants were subjected to immunoprecipitation with antisera specific for TIE-2 receptor, with or without the presence of blocking peptides added to ~20 μg/ml lysate. Immunoprecipitated proteins were resolved by PAGE (7.5% Laemmli gel), and then electro-transferred to PVDF membrane and incubated either with various TIE-2- or phosphotyrosine-specific antisera. TIE-2 protein was visualized by incubation of the membrane with HRP-linked secondary antisera followed by treatment with ECL reagent (Amersham).

EXAMPLE 2

Cloning and Expression of TIE-2 Receptorbody for Affinity-based Study of TIE-2 Ligand Interactions

[0121] An expression construct was created that would yield a secreted protein consisting of the entire extracellular portion of the rat TIE-2 receptor fused to the human immunoglobulin gamma-1 constant region (IgG1 Fc). This fusion protein is called a TIE-2 "receptorbody" (RB), and would be normally expected to exist as a dimer in solution based on formation of disulfide linkages between individual IgG1 Fc tails. The Fc portion of the TIE-2 RB was prepared as follows. A DNA fragment encoding the Fc portion of human IgG1 that spans from the hinge region to the carboxy-terminus of the protein, was amplified from human placental cDNA by PCR with oligonucleotides corresponding to the published sequence of human IgG1; the resulting DNA fragment was cloned in a plasmid vector. Appropriate DNA restriction fragments from a plasmid encoding the full-length TIE-2 receptor and from the human IgG1 Fc plasmid were ligated on either side of a short PCR-derived fragment that was designed so as to fuse, in-frame, the TIE-2 and human IgG1 Fc protein-coding sequences. Thus, the resulting TIE-2 ectodomain-Fc fusion protein precisely substituted the IgG1 Fc in place of the region spanning the TIE-2 transmembrane and cytoplasmic domains. An alternative method of preparing RBs is described in Goodwin, et. al. Cell 73:447-456 (1993).

[0122] Milligram quantities of TIE-2 RB were obtained by cloning the TIE-2 RB DNA fragment into the pVL1393 baculovirus vector and subsequently infecting the *Spodoptera frugiperda* SF-21AE insect cell line. Alternatively, the cell line SF-9 (ATCC Accession No. CRL-1711) or the cell line BTI-TN-5b1-4 may be used. DNA encoding the TIE-2 RB was cloned as an Eco RI-NotI fragment into the baculovirus transfer plasmid pVL1393. Plasmid DNA purified by cesium chloride density gradient centrifugation was recombined into viral DNA by mixing 3 μg of plasmid DNA with 0.5 μg of Baculo-Gold DNA (Pharminigen), followed by introduction into liposomes using 30 μg Lipofectin (GIBCO-BRL). DNA-liposome mixtures were added to SF-21AE cells (2×10^6 cells/60 mm dish) in TMN-FH medium (Modified Grace's Insect Cell Medium (GIBCO-BRL) for 5 hours at 27° C., followed by incubation at 27° C. for 5 days in TMN-FH medium supplemented with 5%

fetal calf serum. Tissue culture medium was harvested for plaque purification of recombinant viruses, which was carried out using methods previously described (O'Reilly, D. R., L. K. Miller, and V. A. Luckow, *Baculovirus Expression Vectors—A Laboratory Manual.* 1992, New York: W. H. Freeman) except that the agarose overlay contained 125 μg/mL X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; GIBCO-BRL). After 5 days of incubation at 27° C., non-recombinant plaques were scored by positive chromogenic reaction to the X-gal substrate, and their positions marked. Recombinant plaques were then visualized by addition of a second overlay containing 100 μg/mL MTT (3-[4, 5-dimethylthiazol-2-yl]2,5,diphenyltetrazolium bromide; Sigma). Putative recombinant virus plaques were picked by plug aspiration, and purified by multiple rounds of plaque isolation to assure homogeneity. Virus stocks were generated by serial, low-multiplicity passage of plaque-purified virus. Low passage stocks of one virus clone (vTIE-2 receptorbody) were produced.

[0123] SF-21AE cells were cultured in serum free medium (SF-900 II, Gibco BRL) containing 1×antibiotic/antimycotic solution (Gibco BRL) and 25 mg/L Gentamycin (Gibco BRL). Pluronic F-68 was added as a surfactant to a final concentration of 1 g/L. Cultures (4 L) were raised in a bioreactor (Artisan Cell Station System) for at least three days prior to infection. Cells were grown at 27° C., with gassing to 50% dissolved oxygen, at a gas flow rate of 80 mL/min (aeration at a sparge ring). Agitation was by means of a marine impeller at a rate of 100 rpm. Cells were harvested in mid-logarithmic growth phase (~2×10^6 cells/mL), concentrated by centrifugation, and infected with 5 plaque forming units of vTIE-2 receptorbody per cell. Cells and inoculum were brought to 400 mL with fresh medium, and virus was adsorbed for 2 hours at 27° C. in a spinner flask. The culture was then resuspended in a final volume of 8 L with fresh serum-free medium, and the cells incubated in the bioreactor using the previously described conditions.

[0124] Culture medium from vTIE-2 receptorbody-infected SF21AE cells were collected by centrifugation (500× g, 10 minutes) at 72 hours post-infection. Cell supernatants were brought to pH 8 with NaOH. EDTA was added to a final concentration of 10 mM and the supernatant pH was readjusted to 8. Supernatants were filtered (0.45 μm, Millipore) and loaded on a protein A column (protein A sepharose 4 fast flow or HiTrap protein A, both from Pharmacia). The column was washed with PBS containing 0.5 M NaCl until the absorbance at 280 nm decreased to baseline. The column was washed in PBS and eluted with 0.5 M acetic acid. Column fractions were immediately neutralized by eluting into tubes containing 1 M Tris pH 9. The peak fractions containing the TIE-2 receptorbody were pooled and dialyzed versus PBS.

EXAMPLE 3

Demonstration that TIE-2 has a Critical Role in Development of the Vasculature

[0125] Insight into the function of TIE-2 was gained by introduction of "excess" soluble TIE-2 receptorbody (TIE-2 RB) into a developing system. The potential ability of TIE-2 RB to bind, and thereby neutralize, available TIE-2 ligand could result in an observable disruption of normal vascular development and characterization of the ligand. To examine whether TIE-2 RB could be used to disrupt vascular development in early chick embryos, small pieces of a biologically resorbable foam were soaked with TIE-2 RB and inserted immediately beneath the chorioallantoic membrane at positions just lateral to the primitive embryo.

[0126] Early chicken embryos develop atop the yolk from a small disk of cells that is covered by the chorioallantoic membrane (CAM). The endothelial cells that will come to line the vasculature in the embryo arise from both extra- and intra-embryonic cell sources. Extra-embryonically-derived endothelial cells, which provide the major source of endothelial cells in the embryo, originate from accretions of mesenchyme that are situated laterally around the embryo-proper, just underneath the CAM. As these mesenchyme cells mature, they give rise to a common progenitor of both the endothelial and hematopoietic cell lineages, termed the hemangioblast. In turn, the hemangioblast gives rise to a mixed population of angioblasts (the endothelial cell progenitor) and hematoblasts (the pluripotential hematopoietic precursor). Formation of rudiments of the circulatory system begins when endothelial cell progeny segregate to form a one-cell-thick vesicle that surrounds the primitive blood cells. Proliferation and migration of these cellular components eventually produces a vast network of blood-filled microvessels under the CAM that will ultimately invade the embryo to join with limited, intra-embryonically-derived vascular elements.

[0127] Newly fertilized chicken eggs obtained from Spafas, Inc. (Boston, Mass.) were incubated at 99.5° F., 55% relative humidity. At about 24 hrs. of development, the egg shell was wiped down with 70% ethanol and a dentist's drill was used to make a 1.5 cm. hole in the blunt apex of each egg. The shell membrane was removed to reveal an air space directly above the embryo. Small rectangular pieces of sterile Gelfoam (Upjohn) were cut with a scalpel and soaked in equal concentrations of either TIE-2- or EHK-1 receptorbody. EHK-1 receptorbody was made as set forth in Example 2 using the EHK-1 extracellular domain instead of the TIE-2 extracellular domain (Maisonpierre et al., Oncogene 8:3277-3288 (1993). Each Gelfoam piece absorbed approximately 6 μg of protein in 30 μl. Sterile watchmakers forceps were used to make a small tear in the CAM at a position several millimeters lateral to the primitive embryo. The majority of the piece of RB-soaked Gelfoam was inserted under the CAM and the egg shell was sealed over with a piece of adhesive tape. Other similarly-staged eggs were treated in parallel with RB of the unrelated, neuronally expressed receptor tyrosine kinase, EHK-1 (Maisonpierre et al., Oncogene 8:3277-3288 (1993). Development was allowed to proceed for 4 days and then the embryos were examined by visual inspection. Embryos were removed by carefully breaking the shells in dishes of warmed PBS and carefully cutting away the embryo with surrounding CAM. Of 12 eggs treated with each RB, 6 TIE-2 RB and 5 EHK-1 RB treated embryos had developed beyond the stage observed at the start of the experiment. A dramatic difference was seen between these developed embryos, as shown in **FIGS. 1A and 1B**. Those treated with EHK-1 RB appeared to have developed relatively normally. Four out of five EHK-1 embryos were viable as judged by the presence of a beating heart. Furthermore, the extra-embryonic vasculature, which is visually obvious due to the presence of red blood cells, was profuse and extended several centimeters laterally under the CAM. By contrast, those treated with

TIE-2 RB were severely stunted, ranging from 2-5 mm. in diameter, as compared with more than 10 mm in diameter for the EHK-1 RB embryos. All of the TIE-2 RB treated embryos were dead and their CAMs were devoid of blood vessels. The ability of TIE-2 RB to block vascular development in the chicken demonstrates that TIE-2 ligand is necessary for development of the vasculature.

EXAMPLE 4

Identification of a TIE-2-Specific Binding Activity in Conditioned Medium from the ras Oncogene-transformed C2C12 Mouse Myoblast Cell Line

[0128] Screening of ten-fold-concentrated cell-conditioned media (10×CCM) from various cell lines for the presence of soluble, TIE-2-specific binding activity (BIAcore; Pharmacia Biosensor, Piscataway, N.J.) revealed binding activity in serum-free medium from oncogenic-ras-transformed C2C12 cells (C2C12-ras), RAT 2-ras (which is a ras transformed fibroblast cell line), human glioblastoma T98G and the human neuroblastoma cell line known as SHEP-1.

[0129] The C2C12-ras 10×CCM originated from a stably transfected line of C2C12 myoblasts that was oncogenically transformed by transfection with the T-24 mutant of H-ras by standard calcium phosphate-based methods. An SV40 based neomycin-resistance expression plasmid was physically linked with the ras expression plasmid in order to permit selection of transfected clones. Resulting G418-resistant ras-C2C12 cells were routinely maintained as a monolayer on plastic dishes in DMEM/glutamine/penicillin-streptomycin supplemented with 10% fetal calf serum (FCS). Serum-free C2C12-ras 10×CCM was made by plating the cells at 60% confluence in a serum free defined media for 12 hours. [Zhan and Goldfarb, Mol. Cell. Biol. 6: 3541-3544 (1986)); Zhan, et al. Oncogene 1: 369-376 (1987)]. The medium was discarded and replaced with fresh DMEM/Q/P-S for 24 hours. This medium was harvested and cells were re-fed fresh DMEM/Q/P-S, which was also harvested after a further 24 hours. These CCM were supplemented with the protease inhibitors PMSF (1 mM) and aprotinin (10 μg/ml), and ten-fold concentrated on sterile size-exclusion membranes (Amicon). TIE-2-binding activity could be neutralized by incubation of the medium with an excess of TIE-2 RB, but not by incubation with EHK-1 RB, prior to BlAcore analysis.

[0130] Binding activity of the 10×CCM was measured using biosensor technology (BIAcore; Pharmacia Biosensor, Piscataway, N.J.) which monitors biomolecular interactions in real-time via surface plasmon resonance. Purified TIE-2 RB was covalently coupled through primary amines to the carboxymethyl dextran layer of a CM5 research grade sensor chip (Pharmacia Biosensor; Piscataway, N.J.). The sensor chip surface was activated using a mixture of N-hydroxysuccinimide (NHS) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide (EDC), followed by immobilization of TIE-2 RB (25 μg/mL, pH 4.5) and deactivation of unreacted sites with 1.0 M ethanolamine (pH 8.5). A negative control surface of the EHK-1 receptorbody was prepared in a similar manner.

[0131] The running buffer used in the system was HBS (10 mM Hepes, 3.4 mM EDTA, 150 mM NaCl, 0.005% P20 surfactant, pH 7.4). The 10×CCM samples were centrifuged for 15 min at 4° C. and further clarified using a sterile, low protein-binding 0.45 μm filter (Millipore; Bedford, Mass.). Dextran (2 mg/ml) and P20 surfactant (0.005%) were added to each CCM sample. Aliquots of 40 μL were injected across the immobilized surface (either TIE-2 or EHK-1) at a flow rate of 5 μL/min and the receptor binding was monitored for 8 min. The binding activity (resonance units, RU) was measured as the difference between a baseline value determined 30 s prior to the sample injection and a measurement taken at 30 s post-injection. Regeneration of the surface was accomplished with one 12-μL pulse of 3 M $MgCl_2$.

[0132] The instrument noise level is 20 RU; therefore, any binding activity with a signal above 20 RU may be interpreted as a real interaction with the receptor. For C2C12-ras conditioned media, the binding activities were in the range 60-90 RU for the TIE-2 RB immobilized surface. For the same samples assayed on a EHK-1 RB immobilized surface, the measured activities were less than 35 RU. Specific binding to the TIE-2 receptorbody was evaluated by incubating the samples with an excess of either soluble TIE-2 or EHK-1 RB prior to assaying the binding activity. The addition of soluble EHK-1 RB had no effect on the TIE-2 binding activity of any of the samples, while in the presence of soluble TIE-2 binding to the surface is two-thirds less than that measured in the absence of TIE-2. A repeat assay using >50×concentrated C2C12-ras CCM resulted in a four-fold enhancement over background of the TIE-2 specific binding signal.

EXAMPLE 5

C2C12-ras CCM Contains an Activity that Induces Tyrosine Phosphorylation of TIE-2 Receptor

[0133] C2C12-ras 10×CCM was examined for its ability to induce tyrosine phosphorylation of TIE-2 in ABAE cells. Serum-starved ABAE cells were briefly incubated with C2C12-ras CCM, lysed and subjected to immunoprecipitation and Western analyses as described above. Stimulation of serum-starved ABAE cells with serum-free C2C12-ras 1oX CCM was done as follows. The medium of ABAE cells starved as described above was removed and replaced with either defined medium or 10×CCM that had been pre-warmed to 37° C. After 10 minutes, the media were removed and the cells were twice rinsed on ice with an excess of chilled PBS supplemented with orthovanadate/NaF/benzamidine. Cell lysis and TIE-2-specific immunoprecipitation was done as described above.

[0134] ABAE cells incubated for 10 minutes with defined medium showed no induction of TIE-2 tyrosine phosphorylation, whereas incubation with C2C12-ras CCM stimulated at least a 100×increase in TIE-2 phosphorylation. This activity was almost totally depleted by pre-incubation of the C2C12-ras 10×CCM for 90 minutes at room temperature with 13 μg of TIE-2 RB coupled to protein G-Sepharose beads. Medium incubated with protein G Sepharose alone was not depleted of this phosphorylating activity.

EXAMPLE 6

Expression Cloning of TIE-2 Ligand

[0135] COS-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine

serum (FBS), 1% each of penicillin and streptomycin (P/S) and 2 mM glutamine in an atmosphere of 5% CO_2. The mouse myoblast C2C12 ras cell line was cultured in Eagle's minimal essential medium (EMEM) with 10% FBS, (P/S) and 2 mM glutamine. Full length mouse TIE-2 ligand cDNA clones were obtained by screening a C2C12 ras cDNA library in the pJFE14 vector expressed in COS cells. This vector, as shown in **FIG. 2**, is a modified version of the vector pSR_α (Takebe, et al. 1988, Mol. Cell. Biol. 8:466-472). The library was created using the two BSTX1 restriction sites in the pJFE14 vector.

[**0136**] COS-7 cells were transiently transfected with either the pJFE14 library or control vector by the DEAE-dextran transfection protocol. Briefly, COS-7 cells were plated at a density of 1.0×10^6 cells/100 mm plate 24 hours prior to transfection. For transfection, the cells were cultured in serum-free DMEM containing 400 μg/ml of DEAE-dextran, 1 μM chloroquine, and 2 mM glutamine, and 1 μg of the appropriate DNA for 3-4 hours at 37° C. in an atmosphere of 5% CO_2. The transfection media was aspirated and replaced with PBS with 10% DMSO for 2-3 min. Following this DMSO "shock", the COS-7 cells were placed into DMEM with 10% FBS, 1% each of penicillin and streptomycin, and 2 mM glutamine for 48 hours.

[**0137**] Because the TIE-2 ligand is secreted it was necessary to permeabilize the cells to detect binding of the receptorbody probe to the ligand. Two days after transfection the cells were rinsed with PBS and then incubated with PBS containing 1.8% formaldehyde for 15-30 min. at room temperature. Cells were then washed with PBS and incubated for 15 min. with PBS containing 0.1% Triton X-100 and 10% Bovine Calf Serum to permeabilize the cells and block non-specific binding sites.

[**0138**] The screening was conducted by direct localization of staining using a TIE-2 receptorbody (RB), which consisted of the extracellular domain of TIE-2 fused to the IgG1 constant region. This receptorbody was prepared as set forth in Example 2. A 100 mm dish of transfected, fixed and permeabilized COS cells was probed by incubating them for 30 min with TIE-2 RB. The cells were then washed twice with PBS and incubated for an additional 30 min with PBS/10% Bovine Calf Serum/anti-human IgG-alkaline phosphatase conjugate. After three PBS washes, cells were incubated in alkaline-phosphatase substrate for 30-60 min. The dish was then inspected microscopically for the presence of stained cells. For each stained cell, a small area of cells including the stained cell was scraped from the dish using a plastic pipette tip and plasmid DNA was then rescued and used to electroporate bacterial cells. Single bacterial colonies resulting from the electroporation were picked and plasmid DNA prepared from these colonies was used to transfect COS-7 cells which were probed for TIE-2 ligand expression as evidenced by binding to TIE-2 receptorbodies. This allowed identification of single clones coding for TIE-2 ligand. Confirmation of TIE-2 ligand expression was obtained by phosphorylation of the TIE-2 receptor using the method set forth in Example 5. A plasmid clone encoding the TIE-2 ligand was deposited with the ATCC on Oct. 7, 1994 and designated as "pJFE14 encoding TIE-2 ligand" under ATCC Accession No. 75910.

EXAMPLE 7

Isolation and Sequencing of Full Length cDNA Clone Encoding Human TIE-2 Ligand

[**0139**] A human fetal lung cDNA library in lambda gt-10 (see **FIG. 3**) was obtained from Clontech Laboratories, Inc. (Palo Alto, Calif.). Plaques were plated at a density of 1.25×10^6/ 20×20 cm plate, and replica filters taken following standard procedures (Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., page 8.46, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

[**0140**] Isolation of human tie-2 ligand clones was carried out as follows. A 2.2 kb Xhol fragment from the deposited tie-2 ligand clone (ATCC NO. 75910—see Example 6 above) was labeled by random priming to a specific activity of approximately 5×10^8 cpm/ng. Hybridization was carried out at 65° C. in hybridization solution containing 0.5 mg/ml salmon sperm DNA. The filters were washed at 65° C. in 2×SSC, 0.1% SDS and exposed to Kodak XAR-5 film overnight at −70° C. Positive phage were plaque purified. High titre phage lysates of pure phage were used for isolation of DNA via a Qiagen column using standard techniques (Qiagen, Inc., Chatsworth, Calif., 1995 catalog, page 36). Phage DNA was digested with EcoRI to release the cloned cDNA fragment for subsequent subdloning. A lambda phage vector containing human tie-2 ligand DNA was deposited with the ATCC on Oct. 26, 1994 under the designation λgt10 encoding htie-2 ligand 1 (ATCC Accession No. 75928). Phage DNA may be subjected directly to DNA sequence analysis by the dideoxy chain termination method (Sanger, et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74: 5463-5467).

[**0141**] Subcloning of the human tie-2 ligand DNA into a mammalian expression vector may be accomplished as follows. The clone λgt10 encoding htie-2 ligand 1 contains an EcoRI site located 490 base pairs downstream from the start of the coding sequence for the human TIE-2 ligand. The coding region may be excised using unique restriction sites upstream and downstream of the initiator and stop codons respectively. For example, an Spel site, located 70 bp 5' to the initiator codon, and a Bpu1102i (also known as Blpl) site, located 265 bp 3' to the stop codon, may be used to excise the complete coding region. This may then be subcloned into the pJFE14 cloning vector, using the Xbal (compatible to the Spel overhang) and the Pstl sites (the Pstl and Bpu1102i sites are both made blunt ended).

[**0142**] The coding region from the clone λgt10 encoding htie-2 ligand 1 was sequenced using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, Calif.). The nucleotide and deduced amino acid sequence of human TIE-2 ligand from the clone λgt10 encoding htie-2 ligand 1 is shown in **FIG. 4**.

[**0143**] In addition, full length human tie-2 ligand cDNA clones were obtained by screening a human glioblastoma T98G cDNA library in the pJFE14 vector. Clones encoding human TIE-2 ligand were identified by DNA hybridization using a 2.2 kb Xhol fragment from the deposited tie-2 ligand clone (ATCC NO. 75910) as a probe (see Example 6 above). The, coding region was sequenced using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, Calif.). This

sequence was nearly identical to that of clone λgt10 encoding htie-2 ligand 1. As shown in **FIG. 4**, the clone λgt10 encoding htie-2 ligand 1 contains an additional glycine residue which is encoded by nucleotides 1114-1116. The coding sequence of the T98G clone does not contain this glycine residue but otherwise is identical to the coding sequence of the clone λgt10 encoding htie-2 ligand 1. **FIG. 5** sets forth the nucleotide and deduced amino acid sequence of human TIE-2 ligand from the T98G clone.

EXAMPLE 8

Isolation and Sequencing of Second Full Length cDNA Clone a Encoding Human TIE-2 Ligand

[0144] A human fetal lung cDNA library in lambda gt-10 (see **FIG. 3**) was obtained from Clontech Laboratories, Inc. (Palo Alto, Calif.). Plaques were plated at a density of 1.25×10^6/20×20 cm plate, and replica filters taken following standard procedures (Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., page 8.46, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). Duplicate filters were screened at low stringency (2×SSC, 55° C.) with probes made to the human TIE-2 ligand 1 sequence. One of the duplicate filters was probed with a 5' probe, encoding amino acids 25-265 of human TIE-2 ligand 1 as set forth in **FIG. 4**. The second duplicate filter was probed with a 3' probe, encoding amino acids 282-498 of human TIE-2 ligand 1 sequence (see **FIG. 4**). Both probes were hybridized at 55° C. in hybridization solution containing 0.5 mg/ml salmon sperm DNA. Filters were washed in 2×SSC at 55° C. and exposed overnight to X-ray film. In addition, duplicate filters were also hybridized at normal stringency (2×SSC, 65° C.) to the full length coding probe of mouse TIE-2 ligand 1 (F3-15, Xhol insert). Three positive clones were picked that fulfilled the following criteria: i. hybridization had not been seen to the full length (mouse) probe at normal stringency, and ii. hybridization was seen at low stringency to both 5' and 3' probes. EcoRI digestion of phage DNA obtained from these clones indicated two independent clones with insert sizes of approximately 2.2 kb and approximately 1.8 kb. The 2.2 kb EcoRI insert was subcloned into the EcoRI sites of both pBluescript KS (Stratagene) and a mammalian expression vector suitable for use in COS cells. Two orientations were identified for the mammalian expression vector. The 2.2 kb insert in pBluescript KS was deposited with the ATCC on Dec. 9, 1994 and designated as pBluescript KS encoding human TIE 2 ligand 2. The start site of the TIE-2 ligand 2 coding sequence is approximately 355 base pairs downstream of the pBluescript EcoRI site.

[0145] COS-7 cells were transiently transfected with either the expression vector or control vector by the DEAE-dextran transfection protocol. Briefly, COS-7 cells were plated at a density of 1.0×10^6 cells/100 mm plate 24 hours prior to transfection. For transfection, the cells were cultured in serum-free DMEM containing 400 μg/ml of DEAE-dextran, 1 μM chloroquine, and 2 mM glutamine, and 1 μg of the appropriate DNA for 3-4 hours at 37° C. in an atmosphere of 5% CO_2. The transfection media was aspirated and replaced with phosphate-buffered saline with 10% DMSO for 2-3 min. Following this DMSO "shock", the COS-7 cells were placed into DMEM with 10% FBS, 1% each of penicillin and streptomycin, and 2 mM glutamine for 48 hours.

[0146] Because the TIE-2 ligand is secreted it was necessary to permeabilize the cells to detect binding of the receptorbody probe to the ligand. Transfected COS-7 cells were plated at a density of 1.0×10^6 cells/100 mm plate. The cells were rinsed with PBS and then incubated with PBS containing 1.8% formaldehyde for 15-30 min. at room temperature. Cells were then washed with PBS and incubated for 15 min. with PBS containing 0.1% Triton X-100 and 10% Bovine Calf Serum to permeabilize the cells and block non-specific binding sites. The screening was conducted by direct localization of staining using a TIE-2 receptorbody, which consisted of the extracellular domain of TIE-2 fused to the IgGi constant region. This receptorbody was prepared as set forth in Example 2. Transfected COS cells were probed by incubating them for 30 min with TIE-2 receptorbody. The cells were then washed twice with PBS, fixed with methanol, and then incubated for an additional 30 min with PBS/10% Bovine Calf Serum/anti-human IgG-alkaline phosphatase conjugate. After three PBS washes, cells were incubated in alkaline-phosphatase substrate for 30-60 min. The dish was then inspected microscopically for the presence of stained cells. Cells expressing one orientation of the clone, but not the other orientation, were seen to bind the TIE-2 receptorbody.

[0147] One of skill in the art will readily see that the described methods may be used to further identify other related members of the TIE ligand family.

[0148] The coding region from the clone pBluescript KS encoding human TIE-2 ligand 2 was sequenced using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, Calif.). The nucleotide and deduced amino acid sequence of human TIE-2 ligand from the clone pBluescript KS encoding human TIE-2 ligand 2 is shown in **FIG. 6**.

EXAMPLE 9

TIE-2 Ligand 2 is a Receptor Antagonist

[0149] Conditioned media from COS cells expressing either TIE-2 ligand 2 (TL2) or TIE-2 ligand 1 (TL1) were compared for their ability to activate TIE-2 receptors naturally present in human endothelial cell lines.

[0150] Lipofectamine reagent (GIBCO-BRL, Inc.) and recommended protocols were used to transfect COS-7 cells with either the pJFE14 expression vector alone, pJFE14 vector containing the human TIE-2 ligand 1 oDNA, or with a pMT21 expression vector (Kaufman, R. J., 1985, Proc. Natl. Acad. Sci. USA 82: 689-693) containing the human TIE-2 ligand 2 cDNA. COS media containing secreted ligands were harvested after three days and concentrated 20-fold by diafiltration (DIAFLO ultrafiltration membranes, Amicon, Inc.). The quantity of active TIE-2 ligand 1 and TIE-2 ligand 2 present in these media was determined and expressed as the amount (in resonance units, R.U.) of TIE-2 receptor specific binding activity measured by a BIAcore binding assay.

[0151] Northern (RNA) analyses revealed significant levels of TIE-2 transcripts in HAEC (Human Aortic Endothelial Cell) human primary endothelial cells (Clonetics, Inc.). Therefore, these cells were used to examine whether TIE-2 receptor is tyrosine-phosphorylated when exposed to COS media containing the TIE-2 ligands. HAEC cells were

maintained in a complete endothelial cell growth medium (Clonetics, Inc.) that contained 5% fetal bovine serum, soluble bovine brain extract, 10 ng/ml human EGF, 1 mg/ml hydrocortisone, 50 mg/ml gentamicin and 50 ng/ml amphotericin-B. Assessment of whether TL1 and TL2 could activate TIE-2 receptor in the HAEC cells was done as follows. Semi-confluent HAEC cells were serum-starved for two hours in high-glucose Dulbecco's MEM with added L-glutamine and penicillin-streptomycin at 37° C. followed by replacement of the starvation medium with ligand-containing conditioned COS media for 7 minutes at 37° C. in a 5% CO2 incubator. The cells were subsequently lysed and TIE-2 receptor protein was recovered by immunoprecipitation of the lysates with TIE-2 peptide antiserum, followed by Western blotting with antiphosphotyrosine antiserum, exactly as described in example 1. The results are shown in **FIG. 7**. Phosphotyrosine levels on the TIE-2 receptor (TIE-2-R) were induced by treatment of HEAC cells with TIE-2 ligand 1 (Lane L1) but not by TIE-2 ligand 2 (Lane L2) conditioned COS media. MOCK is conditioned media from COS transfected with JFE14 empty vector.

[0152] Evidence that both TL1 and TL2 specifically bind to the TIE-2 receptor was demonstrated by using a BIAcore to assay the TIE-2 receptor specific binding activities in transfected COS media and by immunostaining of TL1- and TL2-expressing COS cells with TIE-2 receptorbodies.

[0153] Because TL2 did not activate the TIE-2 receptor, applicants set out to determine whether TL2 might be capable of serving as an antagonist of TL1 activity. HAEC phosphorylation assays were performed in which cells were first incubated with an "excess" of TL2, followed by addition of dilute TL1. It was reascned that prior occupancy of TIE-2 receptor due to high levels of TL2 might prevent subsequent stimulation of the receptor following exposure to TL1 present at a limiting concentration.

[0154] Semi-confluent HAEC cells were serum-starved as described above and then incubated for 3 min., at 37° C. with 1-2 ml. of 20×COS/JFE14-TL2 conditioned medium. Control plates were treated with 20×COS/JFE14-only medium (MOCK). The plates were removed from the incubator and various dilutions of COS/JFE14-TL1 medium were then added, followed by further incubation of the plates for 5-7 min. at 37° C. Cells were subsequently rinsed, lysed and TIE-2-specific tyrosine phosphorylation in the lysates was examined by receptor immunoprecipitation and Western blotting, as described above. TL1 dilutions were made using 20×COS/JFE14-TL1 medium diluted to 2×, 0.5×, 0.1×, or 0.02× by addition of 20×COS/JFE14-alone medium. An assay of the initial 20×TL1 and 20×TL2 COS media using BIAcore biosensor technology indicated that they contained similar amounts of TIE-2-specific binding activities, i.e., 445 R.U. and 511 R.U. for TL1 and TL2, respectively. The results of the antiphosphotyrosine Western blot, shown in **FIG. 8**, indicate that when compared to prior treatment of HAEC cells with MOCK medium (lane 1), prior treatment of HAEC cells with excess TIE-2 ligand 2 (lane 2) antagonizes the subsequent ability of dilute TIE-2 ligand 1 to activate the TIE-2 receptor (TIE-2-R).

[0155] The ability of TL2 to competitively inhibit TL1 activation of the TIE-2-R was further demonstrated using the human cell hybrid line, EA.hy926 (see Example 21 for detailed description of this cell line and its maintenance). Experiments were performed in which unconcentrated COS cell media containing TL1 were mixed at varying dilutions with either MOCK- or TL2-conditioned media and placed on serum-starved EA.hy926 cell monolayers for 5 minutes at 37° C. The media were then removed, the cells were harvested by lysis and TIE-2-specific tyrosine phosphorylation was examined by Western blots, as described above. **FIG. 9** shows an experiment which contains three groups of treatments, as viewed from left to right. As shown in the four lanes at the left, treatment of the EA.hy926 cells with 1×COS-TL1 alone robustly activated the endogenous TIE-2-R in these cells, whereas 1×TL2 COS medium was inactive. However, mixture of TL1 with either MOCK or TL2 demonstrated that TL2 can block the activity of TL1 in a dose-dependent fashion. In the central three pairs of lanes the ratio of TL2 (or MOCK) was decreased while the amount of TL1 in the mixture was correspondingly increased from 0.1×to 0.3×. At any of these mixture ratios the TL1:TL2 lanes showed a reduced level of TIE-2-R phosphorylation compared to that of the corresponding TL1:MOCK lanes. When the amount TL1 was held steady and the amount of TL2 (or MOCK) was decreased, however (shown in the three pairs of lanes at the right), a point was reached at which the TL2 in the sample was too dilute to effectively inhibit TL1 activity. The relative amount of each ligand present in these conditioned COS media could be estimated from their binding units as measured by the BIAcore assay and from Western blots of the COS media with ligand-specific antibodies. Consequently, we can infer that only a few-fold molar excess of TL2 is required to effectively block the activity of TL1 in vitro. This is significant because we have observed distinct examples in vivo (see Example 17 and **FIG. 16**) where TL2 mRNAs achieve considerable abundance relative to those of TL1. Thus, TL2 may be serving an important physiological role in effectively blocking signaling by the TIE-2-R at these sites.

[0156] Taken together these data confirm that, unlike TL1, TL2 is unable to stimulate endogenously expressed TIE-2-R on endothelial cells. Furthermore, at a few fold molar excess TL2 can block TL1 stimulation of the TIE-2 receptor, indicating that TL2 is a naturally occurring TIE-2 receptor antagonist.

EXAMPLE 10

Identification of TIE-2-Specific Binding Activity in Conditioned Medium and COS Cell Supernatants

[0157] Binding activity of 10×CCM from the cell lines C2C12-ras, Rat2 ras, SHEP, and T98G, or COS cell supernatants after transfection with either human TIE-2 ligand 1 (hTL1) or human TIE-2 ligand 2 (hTL2) was measured using biosensor technology (BIAcore; Pharmacia Biosensor, Piscataway, N.J.) which monitors biomolecular interactions in real-time via surface plasmon resonance (SPR). Purified rat or human TIE-2 RB was covalently coupled through primary amines to the carboxymethyl dextran layer of a CM5 research grade sensor chip (Pharmacia Biosensor; Piscataway, N.J.). The sensor chip surface was activated using a mixture of N-hydroxysuccinimide (NHS) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide (EDC), followed by immobilization of TIE-2 RB (25 μg/mL, pH 4.5) and deactivation of unreacted sites with 1.0 M ethanolamine (pH 8.5). In general, 9000-10000 RU of each receptorbody was coupled to the sensor chip.

[0158] The running buffer used in the system was HBS (10 mM Hepes, 150 mM NaCl, 0.005% P20 surfactant, pH 7.4). The samples were centrifuged for 15 min at 4° C. and further clarified using a sterile, low protein-binding 0.45 μm filter (Millipore; Bedford, Mass.). Dextran (2 mg/ml) and P20 surfactant (0.005%) were added to each sample. Aliquots of 40 μL were injected across the immobilized surface (either rat or human TIE-2) at a flow rate of 5 μL/min and the receptor binding was monitored for 8 min. The binding activity (resonance units, RU) was measured as the difference between a baseline value determined 30 s prior to the sample injection and a measurement taken at 30 s post-injection. Regeneration of the surface was accomplished with one 15-μL pulse of 3 M $MgCl_2$.

[0159] The CCM samples (C2C12-ras, Rat2-ras, SHEP, T98G) were tested on the rat TIE-2 RB immobilized surface, while the recombinant hTL1 and hTL2 were tested on the human TIE-2 RB immobilized surface. In each case, specific binding to the TIE-2 receptorbody was evaluated by incubating the samples with 25 μg/ml of either soluble TIE-2 (rat or human) RB or trkB RB prior to assaying the binding activity. As shown in **FIGS. 10 and 11**, the addition of soluble trkB RB causes a slight decrease in the TIE-2 binding activity, while the addition of soluble TIE-2 RB significantly reduces the binding activity as compared to that measured in the absence of TIE-2 RB.

EXAMPLE 11

TIE-2 RB Specifically Blocks Activation of the TIE-2 Receptor by TIE-2 Ligand 1

[0160] The applicants sought to determine whether soluble TIE-2 RB can serve as a competitive inhibitor to block activation of TIE-2 receptor by TIE-2 ligand 1 (TL1). To do this, TL1-containing COS media were preincubated with either TIE-2- or TrkB-RB and then compared for their ability to activate TIE-2 receptors naturally present in a human endothelial cell line.

[0161] Conditioned COS media were generated from COS-7 cells transfected with either the pJFE14 expression vector alone (MOCK), or pJFE14 vector containing the human TIE-2 ligand 1 cDNA (TL1) and harvested as described in Example 9 hereinabove, with the exception that the media were sterile filtered but not concentrated. The quantity of TL1 was determined and expressed as the amount (in resonance units, R.U.) of TIE-2 receptor-specific binding activity measured by BIAcore binding assay.

[0162] Northern (RNA) analyses revealed significant levels of tie-2 transcripts in HUVEC (Human Umbilical Vein Endothelial Cell) human primary endothelial cells (Clonetics, Inc.). Therefore, these cells were used to examine whether TIE-2 receptor can be tyrosine-phosphorylated when exposed in the presence of TIE-2- or TrkB-RBs to COS media containing TL1. HUVEC cells were maintained at 37° C., 5% CO_2 in a complete endothelial cell growth medium (Clonetics, Inc.) that contained 5% fetal bovine serum, soluble bovine brain extract with 10 μg/ml heparin, 10 ng/ml human EGF, 1 ug/ml hydrocortisone, 50 μg/ml gentamicin and 50 ng/ml amphotericin-B. Assessment of whether TL1 could activate TIE-2 receptor in the HUVEC cells was done as follows. Confluent dishes of HUVEC cells were serum-starved for two-to-four hours in low-glucose Dulbecco's MEM at 37° C., 5% CO_2, followed by 10 minute incubation in starvation medium that included 0.1 mM sodium orthovanadate, a potent inhibitor of phosphotyrosine phosphatases. Meanwhile, conditioned COS media were preincubated 30 min. at room temperature with either TIE-2- or TrkB-RB added to 50 μg/ml. The starvation medium was then removed from the HUVEC dishes and incubated with the RB-containing COS media for 7 minutes at 37° C. HUVEC cells were subsequently lysed and TIE-2 receptor protein was recovered by immunoprecipitation with TIE-2 peptide antiserum, followed by Western blotting with an anti-phosphotyrosine antibody, as described in Example 1.

[0163] The results are shown in **FIG. 12**. Phosphotyrosine levels on the TIE-2 receptor were induced by treatment of HUVEC cells with TIE-2 ligand 1 (TL1) relative to that seen with control medium (MOCK) and this induction is specifically blocked by prior incubation with TIE-2-RB (TIE-2-Fc) but not by incubation with TrkB-RB (TrkB-Fc). These data indicate that soluble TIE-2 RB can serve as a selective inhibitor to block activation of TIE-2 receptor by TIE-2 ligand 1.

EXAMPLE 12

Construction of TIE-2 Ligandbodies

[0164] An expression construct was created that would yield a secreted protein consisting of the entire coding sequence of human TIE-2 ligand 1 (TL1) or TIE-2 ligand 2 (TL2) fused to the human immunoglobulin gamma-1 constant region (IgG1 Fc). These fusion proteins are called TIE-2 "ligandbodies" (TL1-Fc or TL2-Fc). The Fc portion of TL1-Fc and TL2-Fc was prepared as follows. A DNA fragment encoding the Fc portion of human IgG1 that spans from the hinge region to the carboxy-terminus of the protein, was amplified from human placental cDNA by PCR with oligonucleotides corresponding to the published sequence of human IgG1; the resulting DNA fragment was cloned in a plasmid vector. Appropriate DNA restriction fragments from a plasmid encoding full-length TL1 or TL2 and from the human IgG1 Fc plasmid were ligated on either side of a short PCR-derived fragment that was designed so as to fuse, in-frame, TL1 or TL2 with human IgG1 Fc protein-coding sequences.

[0165] Milligram quantities of TL2-Fc were obtained by cloning the TL2-Fc DNA fragment into the pVL1393 baculovirus vector and subsequently infecting the Spodoptera frugiperda SF-21AE insect cell line. Alternatively, the cell line SF-9 (ATCC Accession No. CRL-1711) or the cell line BTI-TN-5b1-4 may be used. DNA encoding the TL2-Fc was cloned as an Eco RI-NotI fragment into the baculovirus transfer plasmid pVL1393. Plasmid DNA was recombined into viral DNA by mixing 3 μg of plasmid DNA with 0.5 μg of Baculo-Gold DNA (Pharminigen), followed by introduction into liposomes using 30 μg Lipofectin (GIBCO-BRL). DNA-liposome mixtures were added to SF-21AE cells (2×106 cells/60 mm dish) in TMN-FH medium (Modified Grace's Insect Cell Medium (GIBCO-BRL) for 5 hours at 27° C., followed by incubation at 27° C. for 5 days in TMN-FH medium supplemented with 5% fetal calf serum. Tissue culture medium was harvested for plaque purification of recombinant viruses, which was carried out using methods previously described (O'Reilly, D. R., L. K. Miller, and V. A. Luckow, Baculovirus Expression Vectors—A Labora-

tory Manual. 1992, New York: W. H. Freeman) except that the agarose overlay contained 125 mg/mL X-gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside; GIBCO-BRL). After 5 days of incubation at 27° C., non-recombinant plaques were scored by positive chromogenic reaction to the X-gal substrate, and their positions marked. Recombinant plaques were then visualized by addition of a second overlay containing 100 mg/mL MTT (3-[4,5-dimethylthiazol-2-yl] 2,5,diphenyltetrazolium bromide; Sigma). Putative recombinant virus plaques were picked by plug aspiration, and purified by multiple rounds of plaque isolation to assure homogeneity. Virus stocks were generated by serial, low-multiplicity passage of plaque-purified virus. Low passage stocks of one virus clone (vTL2-Fc Clone #7) were produced.

[0166] SF-21AE cells were cultured in serum-free medium (SF-900 II, Gibco BRL) containing 1×antibiotic/antimycotic solution (Gibco BRL) and 25 mg/L Gentamycin (Gibco BRL). Pluronic F-68 was added as a surfactant to a final concentration of 1 g/L. Cultures (4 L) were raised in a bioreactor (Artisan Cell Station System) for at least three days prior to infection. Cells were grown at 27° C., with gassing to 50% dissolved oxygen, at a gas flow rate of 80 mL/min (aeration at a sparge ring). Agitation was by means of a marine impeller at a rate of 100 rpm. Cells were harvested in mid-logarithmic growth phase (~2×10 6 cells/mL), concentrated by centrifugation, and infected with 5 plaque forming units of vTL2-Fc per cell. Cells and inoculum were brought to 400 mL with fresh medium, and virus was adsorbed for 2 hours at 27° C. in a spinner flask. The culture was then resuspended in a final volume of 8 L with fresh serum-free medium, and the cells incubated in the bioreactor using the previously described conditions.

[0167] Culture medium from vTL2-Fc-infected SF21AE cells were collected by centrifugation (500×g, 10 minutes) at 72 hours post-infection. Cell supernatants were brought to pH 8 with NaOH. EDTA was added to a final concentration of 10 mM and the supernatant pH was readjusted to 8. Supernatants were filtered (0.45 μm, Millipore) and loaded on a protein A column (protein A sepharose 4 fast flow or HiTrap protein A, both from Pharmacia). The column was washed with PBS containing 0.5 M NaCl until the absorbance at 280 nm decreased to baseline. The column was washed in PBS and eluted with 0.5 M acetic acid. Column fractions were immediately neutralized by eluting into tubes containing 1 M Tris pH 9. The peak fractions containing the TL2-Fc were pooled and dialyzed versus PBS.

EXAMPLE 13

Expression of TIE-1, TIE-2, TL1, and TL2 in Renal Cell Carcinoma

[0168] In situ hybridization experiments were performed on human renal cell carcinoma tumor tissue using TIE-1, TIE-2, TL1, and TL2 cDNA probes. TIE-2, TIE-1, TL1, and TL2 expression were all up-regulated in the tumor vasculature. Ligand expression appeared to be localized to either the vascular endothelial cells (TL2) or very near the vascular endothelial cells in the mesenchyme (TL1). VEGF has been shown to be dramatically up-regulated in this tumor tissue. Brown, et al. Am. J. Pathol. 143:1255-1262 (1993).

EXAMPLE 14

Expression of TIE-1, TIE-2, TL1, and TL2 in Wound Healing

[0169] In situ hybridization experiments were performed on cross-sectional tissue slices obtained from a rat cutaneous wound model using TIE-1, TIE-2, TL1, and TL2 cDNA probes. The wound healing model involves pressing a small cork bore against the skin of a rat and removing a small, cylindrical plug of skin. As healing begins at the base of the wound, a vertical slice of tissue is taken and used for in situ hybridization. In the tested tissue sample, TL1 and TL2 appeared to be slightly up-regulated by four days post-injury. In contrast to the slightly up-regulated expression of TL1 and TL2 in this tissue, VEGF expression, which may precede TL1 and TL2 expression, is dramatically up-regulated.

EXAMPLE 15

Expression of TIE Ligands in Fetal Liver and Thymus

[0170] Reverse transcription-PCR (RT-PCR) was performed on mouse E14.5 fetal liver and mouse E17.5 fetal thymus. Agarose gel electrophoresis of the RT-PCR products revealed that in the mouse fetal liver, TIE-2 ligand 1 (TL1) RNA is enriched in the stromal region, but is absent in c-kit$^+$TER119 hematopoietic precursor cells. In this same tissue, TIE-2 ligand 2 (TL2) RNA is enriched in the stromal cells, but absent in the hematopoietic precursor cells (**FIG. 13**). In the mouse fetal thymus, TL2 is enriched in the stromal cells (**FIG. 14**).

EXAMPLE 16

The TIE Receptor/Ligand System in Angiogenesis

[0171] Although the TIE-2/TIE ligand system appears to play an important role in endothelial cell biology, it has not been shown to play a significant, active role in the early to intermediate stages of vascularization (f angioblast or endothelial cell proliferation and migration, tubule formation, and other early stage events in vascular modeling). In contrast to the receptors and factors known to mediate these aspects of vascular development, the temporally late pattern of expression of TIE-2 and TL1 in the course of vascularization suggests that this system plays a distinct role in the latter stages vascular development, including the structural and functional differentiation and stabilization of new blood vessels. The pattern of expression of TIE-2/TL1 also is consistent with a continuing role in the maintenance of the structural integrity and/or physiological characteristics of an established vasculature.

[0172] TIE Ligand 2 (TL2) appears to be a competitive inhibitor of TL1. The spatiotemporal characteristics of TL2 expression suggest that this single inhibitory molecule may play multiple, context-dependent roles essential to appropriate vascular development or remodeling (e.g. de-stabilization/de-differentiation of mature endothelial cells allowing the formation of new vessels from existing vasculature, inhibition of inappropriate blood vessel formation, and regression/involution of mature blood vessels). **FIG. 15** is a schematic representation of the hypothesized role of the TIE-2/TIE ligands in angiogenesis. In this figure TL1 is

represented by (•), TL2 is represented by (*), TIE-2 is represented by (T), VEGF is represented by ([]), and flk-1 (a VEGF receptor) is represented by (Y).

EXAMPLE 17

Expression of TIE Ligands in the Female Reproductive System: Expression in the Ovary

[0173] Preliminary observations made in experiments examining the expression of the TIE receptors and ligands in the female reproductive system are consistent with the hypothesis the TL1 plays a role in neovascularization which temporally follows that of VEGF. The pattern of TL2 expression is also consistent with an antagonism of the action of TL1, and a specific role in vascular regression. To verify this, expression of relevant mRNAs can be examined following experimental induction of follicular and luteal development so that their temporal relation to various aspects of neovascularization/vascular regression can be more clearly defined (e g in conjunction with endothelial cell staining, vascular fills). Angiogenesis associated with follicular development and corpus luteum formation in staged ovaries of mature, female rats or following induced ovulation in pre-pubertal animals was followed using in situ hybridization. **FIG. 16** contains photographs of in situ hybridization slides showing the temporal expression pattern of TIE-2, TL1, TL2, and VEGF during the ovarian cycle [Column 1: Early pre-ovulatory follicle; Column 2: pre-ovulatory follicle; Column 3: early corpus luteum; and Column 4: atretic follicle; Row A:bright field; Row B:VEGF; Row C: TL2;

[0174] Row D: TL1 and Row E: TIE-2 receptor]. These studies revealed that VEGF, TL1 and TL2 are expressed in a temporally and spatially coordinate fashion with respect to the development and regression of vasculature in the ovary, specifically with respect to the establishment of the vascular system which is generated in the course of the conversion of an ovarian follicle to a corpus luteum (CL).

[0175] Briefly, VEGF expression increases in the follicular granule layer prior to its vascularization during the process of luteinization. During the process of CL formation, highest levels of VEGF expression are apparent in the center of the developing CL in the vicinity of luteinizing cells which are not yet vascularized. VEGF levels remain moderately high and are diffusely distributed in the developed CL. In contrast, noticeably enhanced expression of TIE-2 ligand 1 occurs only late in process of CL formation, after a primary vascular plexus has been established. Later, TL1 expression is apparent throughout the CL at which time the definitive capillary network of the CL has been established.

[0176] TL2 exhibits a more complex pattern of expression than either VEGF or TL1. In the developing CL, TL2 is expressed at highest levels at the front of the developing capillary plexus between the central avascular region of the CL where VEGF expression is highest, and the most peripheral portion of the CL where TL1 expression is dominant and where the luteinization process is complete and the vascular system is most mature. TL2 also appears to be expressed at high levels in the follicular layer of large follicles which are undergoing atresia. While TL1 is also apparent in atretic follicles, VEGF is not expressed.

[0177] The pattern of expression described above is most consistent with a role for VEGF in the initiation of angiogenesis, with TL1 acting late in this process-for example in modeling and/or stabilization of the definitive vascular network. In contrast, TL2 is present both in areas of active expansion of a newly forming vascular network (during CL formation), and in regions which fail to establish a new vasculature and vascular regression is in progress (atretic follicles). This suggests a more dynamic and complex role for TL2, possibly involving destabilization of existing vasculature (necessary for regression) or developing vasculature (necessary for the dynamic modeling of newly forming vessels).

EXAMPLE 18

A Receptorbody Binding Assay and a Ligand Binding and Competition Assay

[0178] A quantitative cell-free binding assay with two alternate formats has been developed for detecting either TIE-2 receptorbody binding or ligand binding and competition. In the receptorbody binding version of the assay, TIE-2 ligands (purified or partially purified; either TL1 or TL2) are coated onto an ELISA plate. Receptorbody at varying concentrations is then added, which binds to the immobilized ligand in a dose-dependent manner. At the end of 2 hours, excess receptorbody is washed away, then the amount bound to the plate is reported using a specific anti-human Fc antibody which is alkaline phosphatase tagged. Excess reporter antibody is washed away, then the AP reaction is developed using a colored substrate. The assay is quantitated using a spectrophotometer. **FIG. 19** shows a typical TIE-2-IgG binding curve. This assay has been used to evaluate the integrity of TIE-2-IgG after injection into rats and mice. The assay can also be used in this format as a ligand competition assay, in which purified or partially-purified TIE ligands compete with immobilized ligand for receptorbody. In the ligand binding and competition version of the binding assay, TIE-2 ectodomain is coated onto the ELISA plate. The Fc-tagged fibrinogen-like domain fragments of the TIE ligands (TL1-fFc and TL2-fFc) then bind to the ectodomain, and can be detected using the same anti-human Fc antibody as described above. **FIG. 20** shows an example of TL1-fFc binding to TIE-2 ectodomain. This version of the assay can also be used to quantitate levels of TL1-fFc in serum or other samples. If untagged ligand (again, either purified or unpurified) is added at the same time as the TL1-fFc, then a competition is set up between tagged ligand fragment and full-length ligand. The full-length ligand can displace the Fc-tagged fragment, and a competition curve is generated.

EXAMPLE 19

EA.hy926 Cell Line can be Used as a Reporter Cell Line for TIE Ligand Activity

[0179] EA.hy926 is a cell hybrid line that was established by fusion of HUVEC with the human lung carcinoma-derived line, A549 [Edgell, et al. Proc. Natl. Acad. Sci. (USA) 80, 3734-3737 (1983). EA.hy926 cells have been found to express significant levels of TIE-2 receptor protein with low basal phosphotyrosine levels. The density at which EA.hy926 cells are passaged prior to their use for receptor assays, as well as their degree of confluency at the time of assay, can affect TIE-2 receptor abundance and relative inducibility in response to treatment with ligand. By adopt-

ing the following regimen for growing these cells the EA.hy926 cell line can be used as a dependable system for assay of TIE-2 ligand activities.

[0180] EA.hy926 cells are seeded at 1.5×10^6 cells in T-75 flasks (Falconware) and re-fed every other day with high-glucose Dulbecco's MEM, 10% fetal bovine serum, L-glutamine, penicillin-streptomycin, and 1×hypoxanthine-aminopterin-thymidine (HAT, Gibco/BRL). After three to four days of growth, the cells are passaged once again at 1.5×10^6 cells per T-75 flask and cultured an additional three to four days. For phosphorylation assays, cells prepared as described above were serum-starved by replacement of the culture medium with high-glucose DMEM and incubation for 2-3 hours at 37° C. This medium was aspirated from the flask and samples of conditioned media or purified ligand were added to the flask in a total volume of 1.5 ml followed by incubation at 37° C. for 5 minutes. Flasks were removed from the incubator and placed on a bed of ice. The medium was removed and replaced with 1.25 ml Lysis Buffer containing 1% nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS in 20 mM Tris, pH 7.6, 150 mM NaCl, 50 mM NaF, 1 mM sodium orthovanadate, 5 mM benzamidine, and 1 mM EDTA containing the protease inhibitors PMSF, aprotinin, and leupeptin. After 10 minutes on ice to allow membrane solubilization, plates were scraped and cell lysates were clarified by microcentrifugation at top speed for 10 minutes at 4° C. TIE-2 receptor was immunoprecipitated from the clarified supernatant by incubation in the cold with an anti-TIE-2 polyclonal antiserum and Protein G-conjugated Sepharose beads. The beads were washed three times with cold cell lysis buffer and boiled 5 minutes in Laemmli sample buffer, which was then loaded on 7.5% SDS-polyacrylamide gels. Resolved proteins were electrotransferred to PVDF (Lamblia-P) membrane and then subjected to Western blot analysis using anti-phosphotyrosine antibody and the ECL reagent. Subsequent comparison of total TIE-2 protein levels on the same blots was done by stripping the anti-phosphotyrosine antibody and reincubating with a polyclonal antiserum specific to the ectodomain of TIE-2.

EXAMPLE 20

Isolation and Sequencing of Full Length cDNA Clone Encoding Mammalian TIE Ligand-3

[0181] TIE ligand-3 (TL3) was cloned from a mouse BAC genomic library (Research Genetics) by hybridizing library duplicates, with either mouse TL1 or mouse TL2 probes corresponding to the entire coding sequence of those genes. Each copy of the library was hybridized using phosphate buffer at 55° C. overnight. After hybridization, the filters were washed using 2×SSC, 0.1% SDS at 60° C., followed by exposure of X ray film to the filters. Strong hybridization signals were identified corresponding to mouse TL1 and mouse TL2. In addition, signals were identified which weakly hybridized to both mouse TL1 and mouse TL2. DNA corresponding to these clones was purified, then digested with restriction enzymes, and two fragments which hybridized to the original probes were subdloned into a bacterial plasmid and sequenced. The sequence of the fragments contained two exons with homology to both mouse TL1 and mouse TL2. Primers specific for these sequences were used as PCR primers to identify tissues containing transcripts corresponding to TL3. A PCR band corresponding to TL3 was identified in a mouse uterus cDNA library in lambda gt-11. (Clontech Laboratories, Inc., Palo Alto, Calif.).

[0182] Plaques were plated at a density of 1.25×10^6/20×20 cm plate and replica filters taken following standard procedures (Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., page 8.46, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). Duplicate filters were screened at "normal" stringency (2×SSC, 65° C.) with a 200 bp PCR radioactive probe made to the mouse TL3 sequence. Hybridization was at 65° C. in a solution containing 0.5 mg/ml salmon sperm DNA. Filters were washed in 2×SSC at 65° C. and exposed for 6 hours to X-ray film. Two positive clones that hybridized in duplicate were picked. EcoRI digestion of phage DNA obtained from these clones indicated two independent clones with insert sizes of approximately 1.2 kb and approximately 2.2 kb. The 2.2 kb EcoRI insert was subcloned into the EcoRI site of pBluescript KS (Stratagene). Sequence analysis showed that the longer clone was lacking an initiator methionine and signal peptide but otherwise encoded a probe homologous to both mouse TL1 and mouse TL2.

[0183] Two TL3-specific PCR primers were then synthesised as follows:

[0184] US2: cctctgggctogccagtttgttagg

[0185] US1: ccagctggcagatatcagg

[0186] The following PCR reactions were performed using expression libraries derived from the mouse cell lines C2C12ras and MG87. In the primary PCR reaction, the specific primer US2 was used in conjunction with vector-specific oligos to allow amplification in either orientation. PCR was in a total volume of 100 ml using 35 cycles of 94° C., 1 min; 42° C. or 48° C. for 1 min; 72° C., 1 min. The secondary PCR reaction included the second specific primer, US1, which is contained within the primary PCR product, in conjunction with the same vector oligos. The secondary reactions were for 30 cycles, using the same temperatures and times as previous. PCR products were gel isolated and submitted for sequence analysis. On the basis of sequences obtained from a total of four independent PCR reactions using two different cDNA libraries, the 5' end of the TL3 sequence was deduced. Northern analysis revealed moderate to low levels of mouse TL3 transcript in mouse placenta. The expression of mouse TL3 consisted of a transcript of approximately 3 kb. The full length TL3 coding sequence is set forth in **FIG. 21**.

[0187] The mouse TL3 sequence may then be used to obtain a human clone containing the coding sequence of human TL3 by hybridizing either a human genomic or cDNA library with a probe corresponding to mouse TL3 as has been described previously, for example, in Example 8 supra.

EXAMPLE 21

Isolation of Full Length Genomic Clone Encoding Human TIE Ligand-4

[0188] TIE ligand-4 (TL4) was cloned from a mouse BAC genomic library (BAC HUMAN (II), Genome Systems Inc.) by hybridizing library duplicates, with either a human TL1 radioactive probe corresponding to the entire fibrinogen coding sequence of TL1 (nucleotides 1153 to 1806 of **FIG.**

4) or a mouse TL3 radioactive probe corresponding to a segment of186 nucleotides from the fibrinogen region of mouse TL3 (nucleotides 1307 to 1492 of **FIG. 21**). Each probe was labeled by PCR using exact oligonucleotides and standard PCR conditions, except that dCTP was replaced by P^{32}dCTP. The PCR mixture was then passed through a gel filtration column to separate the probe from free P^{32} dCTP. Each copy of the library was hybridized using phosphate buffer, and radiactive probe at 55° C. overnight using standard hybridization conditions. After hybridization, the filters were washed using 2×SSC, 0.1% SDS at 55° C., followed by exposure of X ray film. Strong hybridization signals were observed corresponding to human TL1. In addition, signals were identified which weakly hybridized to both human TL1 and mouse TL3. DNA corresponding to these clones was purified using standard procedures, then digested with restriction enzymes, and one fragment which hybridized to the original probes was subcloned into a bacterial plasmid and sequenced. The sequence of the fragments contained one exon with homology to both human TL1 and mouse TL3 and other members of the TIE ligand family. Primers specific for these sequences may be used as PCR primers to identify tissues containing transcripts corresponding to TL4.

[**0189**] The complete sequence of human TL4 may be obtained by sequencing the full BAC clone contained in the deposited bacterial cells. Exons may be identified by homology to known members of the TIE-ligand family such as TL1, TL2 and TL3. The full coding sequence of TL4 may then be determined by splicing together the exons from the TL4 genomic clone which, in turn, may be used to produce the TL4 protein. Alternatively, the exons may be used as probes to obtain a full length cDNA clone, which may then be used to produce the TL4 protein. Exons may also be identified from the BAC clone sequence by homology to protein domains such as fibrinogen domains, coiled coil domains, or protein signals such as signal peptide sequences. Missing exons from the BAC clone m,ay be obtained by identification of contiguous BAC clones, for example, by using the ends of the deposited BAC clone as probes to screen a human genomic library such as the one used herein, by using the exon sequence contained in the BAC clone to screen a cDNA library, or by performing either 5' or 3' RACE procedure using oligonucleotide primers based on the TL4 exon sequences.

[**0190**] Identification of Additional TIE Ligand Family Members

[**0191**] The novel TIE ligand-4 sequence may be used in a rational search for additional members of the TIE ligand family using an approach that takes advantage of the existence of conserved segments of strong homology between the known family members. For example, an alignment of the amino acid sequences of the TIE ligands shows several regions of conserved sequence (see boxed regions of **FIG. 22**). Degenerate oligonucleotides essentially based on these boxes in combination with either previously known or novel TIE ligand homology segments may be used to identify new TIE ligands.

[**0192**] The highly conserved regions among TL1, TL2 and TL3 may be used in designing degenerate oligonucleotide primers with which to prime PCR reactions using cDNAs. cDNA templates may be generated by reverse transcription of tissue RNAs using oligo d(T) or other appropriate primers. Aliquots of the PCR reactions may then be subjected to electrophoresis on an agarose gel. Resulting amplified DNA fragments may be cloned by insertion into plasmids, sequenced and the DNA sequences compared with those of all known TIE ligands.

[**0193**] Size-selected amplified DNA fragments from these PCR reactions may be cloned into plasmids, introduced into *E. coli* by electroporation, and transformants plated on selective agar. Bacterial colonies from PCR transformation may be analyzed by sequencing of plasmid DNAs that are purified by standard plasmid procedures.

[**0194**] Cloned fragments containing a segment of a novel TIE ligand may be used as hybridization probes to obtain full length cDNA clones from a cDNA library. For example, the human TL4 genomic sequence may be used to obtain a human cDNA clone containing the complete coding sequence of human TL4 by hybridizing a human cDNA library with a probe corresponding to human TL4 as has been described previously.

EXAMPLE 22

Cloning of the Full Coding Sequence of hTL4

[**0195**] Both 5' and 3' coding sequence from the genomic human TL-4 clone encoding human TIE ligand-4 (hTL-4 ATCC Accession No. 98095) was obtained by restriction enzyme digestion, Southern blotting and hybridization of the hTL-4 clone to coding sequences from mouse TL3, followed by subcloning and sequencing the hybridizing fragments. Coding sequences corresponding to the N-terminal and C-terminal amino acids of hTL4 were used to design PCR primers (shown below), which in turn were used for PCR amplification of TL4 from human ovary cDNA. A PCR band was identified as corresponding to human TL4 by DNA sequencing using the ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, Calif.). The PCR band was then subcdoned into vector pCR-script and several plasmid clones were analyzed by sequencing. The complete human TL4 coding sequence was then compiled and is shown in **FIG. 23**. In another embodiment of the invention, the nucleotide at position 569 is changed from A to G, resulting in an amino acid change from Q to R.

[**0196**] The PCR primers used as described above were designed as follows:

[**0197**] hTL4atg 5'-gcatgctatctcgagccaccATGCTCTC-CCAGCTAGCCATGCTGCAG-3'

[**0198**] hTL4not 5'-gtgtcgacgcggccgctctagatca-gacTTAGATGTCCAAAGGCCGTATCATCAT-3'

[**0199**] Lowercase letters indicate "tail" sequences added to the PCR primers to facilitate cloning of the amplified PCR fragments.

EXAMPLE 23

Construction and Characterization of Modified TIE Ligands

[**0200**] A genetic analysis of TIE-2 ligand-1 and TIE-2 ligand-2 (TL1 and TL2) was undertaken to gain insight into a number of their observed properties. Although TL1 and

TL2 share similar structural homology, they exhibit different physical and biological properties. The most prominent feature that distinguishes the two ligands is that although they both bind to the TIE-2 receptor, TL1 is an agonist while TL2 is an antagonist. Under non-reducing electrophoretic conditions both proteins exhibit covalent, multimeric structures. TL1 is produced as a mixture of disulfide cross-linked multimers, primarily trimers and higher order species, without any dimeric species. But TL2 is produced almost exclusively as a dimeric species. Also, while TL2 is produced well in most expression systems, TL1 is expressed poorly and is difficult to produce in large quantities. Finally, production and purification conditions also appear to predispose TL1 to inactivation by proteolytic cleavage at a site near the amino terminus.

[0201] To study these differences, several modified ligands were constructed as follows.

[0202] 23.1. Cysteine substitution—Investigations into what factors might be contributing to the different physical and biological properties of the two molecules revealed the presence in TL1 of a cysteine residue (CYS 265 in **FIG. 4**; CYS 245 in **FIG. 17**) preceding the fibrinogen-like domain in TL1 but absent in TL2—i.e., there was no corresponding cysteine residue in TL2. The CYS265 residue in TL1 is encoded by TGC and is located at about nucleotides 1102-1104 (see **FIG. 4**) at the approximate junction between the coiled-coil and fibrinogen-like domains. Because cysteine residues are generally involved in disulfide bond formation, the presence of which can contribute to both the tertiary structure and biological properties of a molecule, it was thought that perhaps the presence of the CYS265 residue in TL1 might be at least partially responsible for the different properties of the two molecules.

[0203] To test this hypothesis, an expression plasmid was constructed which contained a mutation in TL1 in which the CYS (residue 265 in **FIG. 4**; residue 245 in **FIG. 17**) was replaced with an amino acid (serine) which does not form disulfide bonds. In addition to this TL1/CYS^- mutant, a second expression plasmid was constructed which mutated the approximately corresponding position in TL2 (Met247 in **FIG. 17**) so that this residue was now a cysteine. Both non-mutated and mutated expression plasmids of TL1 and TL2 were transiently transfected into COS7 cells, cell supernatants containing the recombinant proteins were harvested, and samples were subjected to both reducing and non-reducing SDS/PAGE electrophoresis and subsequent Western blotting.

[0204] **FIG. 18** shows the Western blots under non-reducing conditions of both non-mutated and mutated TL1 and TL2 proteins, revealing that the TL1/CYS^- mutant runs as a dimer much like TL2 and that the TL2/CYS+mutant is able to form a trimer, as well as higher-order multimers, more like TL1. When the two mutant proteins were tested for their ability to induce phosphorylation in TIE-2 expressing cells, the TL1/CYS^- mutant was able to activate the TIE-2 receptor, whereas the TL2/CYS^+ mutant was not.

[0205] Thus, when the cysteine residue (residue 265 in **FIG. 4**; residue 245 in **FIG. 17**) of TL1 was genetically altered to a serine, it was found that the covalent structure of TL1 became similar to that of TL2, i.e., primarily dimeric. The modified TL1 molecule still behaved as an agonist, thus the trimeric and/or higher order multimeric structure was not the determining factor giving TL1 the ability to activate. Although the removal of the cysteine did make a molecule with more desirable properties, it did not improve the production level of TL1.

[0206] 23.2. Domain deletions—The nucleotide sequences encoding TL1 and TL2 share a genetic structure that can be divided into three domains, based on the amino acid sequences of the mature proteins. The last approximately 215 amino acid residues of each mature protein contains six cysteines and bears strong resemblance to a domain of fibrinogen. This region was thus denoted the "fibrinogen-like" domain or "F-domain." A central region of the mature protein containing approximately 205 residues had a high probability of assuming a "coiled-coil" structure and was denoted the "coiled-coil" domain or "C-domain." The amino-terminal approximately 55 residues of the mature protein contained two cysteines and had a low probability of having a coiled-coil structure. This region was designated the "N-terminal" domain or "N-domain." The modified ligands described herein are designated using a terminology wherein N=N-terminal domain, C=coiled-coil domain, F=fibrinogen-like domain and the numbers 1 and 2 refer to TL1 and TL2 respectively. Thus 1N indicates the N-terminal domain from TL1, 2F indicates the fibrinogen-like domain of TL2, and so forth.

[0207] In order to test whether the fibrinogen-like domain (F-domain) of the TIE-2 ligands contained TIE-2 activating activity, expression plasmids were constructed which deleted the coiled-coil and N-terminal domains, leaving only that portion of the DNA sequence encoding the F-domain (for TL1, beginning in **FIG. 4** at about nucleotide 1159, amino acid residue ARG284; for TL2, corresponding to about nucleotide 1200 in **FIG. 6**, amino acid residue 282). This mutant construct was then transiently transfected into COS cells. The supernatant containing the recombinant protein was harvested. The TL1/F-domain mutant was tested for its ability to bind the TIE-2 receptor. The results showed that, as a monomer, the TL1/F-domain mutant was not able to bind TIE-2 at a detectable level.

[0208] But when the TL1/F-domain monomer was myc-tagged and subsequently clustered with an antibody directed against the myc tag, it exhibited detectable binding to TIE-2. However, the antibody-clustered TL1/F-domain mutant was not able to induce phosphorylation in a TIE-2 expressing cell line.

[0209] Thus it was determined that the F-domain of the TIE-2 ligands is involved in binding the receptor but that a truncation consisting of just the F-domain alone is not sufficient for receptor binding. This raised the possibility that the coiled-coil domain was responsible for holding together several fibrinogen-like domains, which might be essential for receptor binding. In an attempt to confirm this hypothesis, the F-domain was fused with the Fc section of human antibody IgGl. Because Fc sections dimerize upon expression by mammalian cells, these recombinant proteins mimicked the theoretical configuration of the F-domains were the native ligands to dimerize. This F-domain-Fc construct bound but failed to activate the receptor. Apparently, multimerization caused by other regions of the ligands is necessary to enable the ligands to bind the TIE receptor. In addition, some other factor outside of the F-domain must contribute to phosphorylation of the receptor.

[0210] Mutants were then constructed which were missing the fibrinogen-like domain, and therefore contained only the N-terminal and coiled-coil domains. They were not capable of binding to the receptor. To assess the role of the N-terminal domain in receptor binding and activation, the ligands were truncated to just their C- and F-domains and tagged with a FLAG tag at the N-terminus, creating constructs termed FLAG-1C1F and FLAG-2C2F. Although these molecules stained robustly in COS7 cells transfected transiently to express the TIE receptor, they failed to respond in a phosphorylation assay. Thus the N-domain does contain an essential factor for receptor activation although, as disclosed infra, the ability of chimeric molecule 2N2C1F to activate the receptor shows that even the N-domain of an inactive ligand can fill that role.

[0211] The differences in behavior between the myc-tagged F-domain truncation and the Fc-tagged F-domain truncation described previously suggested that the TIE ligands can only bind in dimeric or higher multimeric forms. Indeed, non-reducing SDS-PAGE showed that the TIE ligands exist naturally in dimeric, trimeric, and multimeric forms. That the FLAG-1C1F and FLAG-2C2F truncations can bind to the TIE-2 receptor without dimerization by a synthetic tag (such as Fc), whereas the F truncations cannot, suggests that the C-region is at least partly responsible for the aggregation of the F-domains.

[0212] 23.3. Swapping Constructs (chimeras)

[0213] Applicants had noted that the level of production of TL1 in COS7 cells was approximately tenfold lower than production of TL2. Therefore, chimeras of TL1 and TL2 were constructed in an attempt to explain this difference and also to further characterize the agonist activity of TL1 as compared to the antagonist activity of TL2.

[0214] Four chimeras were constructed in which either the N-terminal domain or the fibrinogen domain was exchanged between TL1 and TL2 and were designated using the terminology described previously such that, for example, 1N1C2F refers to a chimera having the N-terminal and coiled-coil domains of TL1, together with the fibrinogen-like domain from TL2.

[0215] The four chimeras were constructed as follows:

[0216] chimera 1—1N1C2F

[0217] chimera 2—2N2C1F

[0218] chimera 3—1N2C2F

[0219] chimera 4—2N1C1F

[0220] The nucleotide and amino acid sequences of chimeras 1-4 are shown in FIGS. **24-27** respectively.

[0221] Each chimera was inserted into a separate expression vector pJFE14. The chimeras were then transfected into COS7 cells, along with the empty pJFE14 vector, native TL1, and native TL2 as controls, and the culture supernatants were collected.

[0222] In order to determine how the swapping affected the level of expression of the ligands, a 1:5 dilution and a 1:50 dilution of the COS7 supernatants were dot-blotted onto nitrocellulose. Three ligands that contained the TL1 N-domain (i.e. native TL1, 1N2C2F and 1N1C2F) were then probed with a rabbit antibody specific to the N-terminus of TL1. Three ligands containing the TL2 N-domain, (i.e. native TL2, 2N1C1F and 2N2C1F) were probed with a rabbit antibody specific for the N-terminus of TL2. The results demonstrated that the COS7 cells were expressing any molecule containing the N-domain of TL2 at roughly ten times the level of any molecule containing the TL1 N-domain, regardless of the makeup of the rest of the protein. The conclusion was that the N-domain must principally control the level of expression of the ligand.

[0223] The next question addressed was the chimeras' ability or inability to activate the TIE-2 receptor. EAhy926 cells were challenged with the four chimeras, as well as TL1 as a positive control for phosphorylation and TL2 or an empty pJFE14-transfected COS7 cell supernatant as negative controls for phosphorylation. The cells were lysed, and the TIE-2 receptor was immunoprecipitated out of the cell lysate and run on an SDS-PAGE. The samples were Western blotted and probed with an anti-phosphotyrosine antibody to detect any receptors that had been phosphorylated. Surprisingly, only the constructs containing the TL1 fibrinogen-like domain (2N1C1F and 2N2C1F) could phosphorylate the TIE-2 receptor. Thus, although the N-terminal region of TL1 is essential for activation, it can be replaced by the N-terminal region of TL2, i.e., the information that determines whether the ligand is an agonist or an antagonist is actually contained in the fibrinogen-like domain. Thus it was determined that the F-domain, in addition to binding the TIE-2 receptor, is responsible for the phosphorylation activity of TL1. Further, when TL2, an otherwise inactive molecule, was altered by replacing its F-domain with the TL1 F-domain, the altered TL2 acted as an agonist.

[0224] The 2N1C1F construct was somewhat more potent, however. The signal caused by chimera 2N1C1F appeared slightly stronger than that of chimera 2N2C1F, leading to speculation that the C-domain of TL1, though not crucial for phosphorylation, might enhance the potency of TL1. However, since the samples used for the phosphorylation assay were not normalized in terms of the concentration of ligand, it was possible that a stronger phosphorylation signal only indicated the presence of more ligand. The phosphorylation assay was therefore repeated with varying amounts of ligand to determine whether the active chimeras displayed different potencies. The concentration of ligand in the COS7 supernatants of ligand transfections was determined through BIAcore biosenser technology according to methods previously described (Stitt, T. N., et al. (1995) Cell 80: 661-670). BIAcore measured the binding activity of a supernatant to the TIE-2 receptor in arbitrary units called resonance units (RU). Fairly good correlation between RU's and ligand concentration has been generally observed, with 400 RU of activity corresponding to about 1 μg of protein per mL of supernatant. Samples were diluted to concentrations of 100 RU, 20 RU, and 5 RU each and the phosphorylation assay was repeated. The results demonstrated that chimera 2N2C1F was clearly more potent than either the native TL1 or chimera 1N1C2F at the same concentrations.

[0225] Another interesting aspect of these exchange constructs is in their levels of expression. Each of the four chimeras was tested for its level of production in COS cells, its ability to bind to TIE2, and its ability to phosphorylate TIE2. The results of these experiments showed that chimeras 1 and 3 were produced at levels comparable to TL1, whereas chimeras 2 and 4 were produced at levels comparable to

TL2. Thus a high level of protein production was correlated with the TL2 N-terminal domain. Additionally, when tested on endothelial EAhy926 cells, chimeras 2 and 4 were active, whereas 1 and 3 were not. Thus activity (phosphorylation of the receptor) correlates with the TL1 fibrinogen-like domain. Chimeras 2 and 4 therefore each had the desirable properties of high production levels as well as agonist activity.

[0226] 23.4. Proteolytic resistant constructs—Based on the observation that a large fraction of TL1 preparations was often proteolytically cleaved near the N-terminus, it was proposed that an arginine residue located at position 49 of the mature protein (see **FIG. 17**) was a candidate cleavage site that might be involved in the regulation of the protein's activity in vivo, and that replacing the arginine with a serine (R49→S) might increase the stability of the protein without necessarily affecting its activity. Such a mutant of TL1 was constructed and was found to be about as active as the native TL1 but did not exhibit resistance to proteolytic cleavage.

[0227] 23.5. Combination mutants—The most potent of the chimeric constructs, 2N1C1F, was additionally altered so that the cysteine encoded by nucleotides 784-787 as shown in **FIG. 27** was converted to a serine. This molecule (denoted 2N1C1F (C246S)) was expressed well, potently activated the receptor, was resistant to proteolytic cleavage and was primarily dimeric, rather than higher-order multimeric. Thus the 2N domain appeared to confer protease resistance on the molecule. Finally, this molecule was further altered to eliminate the potentially protease sensitive site encoded by nucleotides 199-201 as shown in **FIG. 27**, to give a molecule (denoted 2N1C1F (R51->S,C246->S)) which was expected to be activating, well expressed, dimeric, and protease resistant.

[0228] Table 1 summarizes the modified TIE-2 ligand constructs that were made and characterizes each of them in terms of ability to bind the TIE-2 receptor, ability to activate the TIE-2 receptor, the type of structure formed (monomer, dimer, etc.) and their relative production levels. Unmodified TL1 (plain) and TL2 (striped) are shown with the three domains as boxes. Thus striped boxes indicate domains from TL2. The cysteine located at position 245 of the mature TL1 protein is indicated by a "C." An "X" through the "C" indicates that that cysteine residue was substituted for by another amino acid as in, for example, the TL1 CYS-mutant. Similarly, an "X" through the "R" in the last construct indicates the substitution for an Arg residue at position 49 of the mature TL1 protein. The "C." is present in one modified TL2 construct showing the TL2 CYS^+ mutant. Constructs having Fc tails or flag tagging are also indicated.

[0229] Based upon the teachings herein, one of skill in the art can readily see that further constructs may be made in order to create additional modified and chimeric TIE-2 ligands which have altered properties. For example, one may create a construct comprised of the N-terminal domain of TL2 and the F-domain of TL1 fused with the Fc section of human antibody IgGl. This construct would be expected to bind and activate the TIE-2 receptor. Similarly, other constructs may be created using the teachings herein and are therefore considered to be within the scope of this invention.

[0230] 23.6. Materials and Methods—Construction of Chimeras

[0231] Swapping constructs were inserted into a pJFE14 vector in which the Xbal site was changed to an AscI site. This vector was then digested with AscI and NotI yielding an AscI-NotI backbone. DNA fragments for the chimeras were generated by PCR using appropriate oligonucleotides.

[0232] The FLAG-1C1F and FLAG-2C2F inserts were subdloned into a pMT21 vector backbone that had been digested with EcoRI and NotI. The "CF" truncations were obtained through PCR, and the FLAG tag and a preceding trypsin signalling sequence were constructed by annealing synthetic oligonucleotides.

[0233] Transfections

[0234] All constructs were transfected transiently into COS7 cells using either DEAE-Dextran or LipofectAMINE according to standard protocols. Cell cultures were harvested 3 days after the transfection and spun down at 1000 rpm for 1 minute, and the supernatants were transferred to fresh tubes and stored at −20° C.

[0235] Staining of FLAG-1C1F-Transfected and FLAG-2C2F-Transfected Cells 6-well dishes of COS7 cells were transfected transiently with the TIE-2 receptor. The COS7 supernatant from various ligand tansfections was incubated on the cells for 30 minutes, followed by two washes with Phosphate Buffered Saline (PBS) without magnesium or calcium. The cells were fixed in −20° C. methanol for 3 minutes, washed once with PBS, and incubated with anti-FLAG M2 antibody (IBI;1:3000 dilution) in PBS/10% Bovine Calf Serum (BCS) for 30 minutes. The cells were washed once with PBS and incubated with goat anti-mouse IgG Alkaline Phosphatase (AP) conjugated antibody (Promega;1:1000) in PBS/10% BCS. The cells were washed twice with PBS and incubated with the phosphate substrate, BCIP/NBT, with 1 mM levamisole.

[0236] Phosphorylation Assays

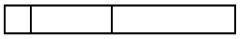
[0237] Dilution of COS7 supernatants for the dose response study was done in the supernatants of COS7 cells transfected with the empty vector pJFE14. EA cells that naturally express the TIE-2 receptor were starved for >2 hours in serum-free medium, followed by challenge with the appropriate COS7 supernatant for 10 minutes at 37° C. in an atmosphere of 5% CO2. The cells were then rinsed in ice-cold PBS and lysed with 1% NP40 lysis buffer containing protease inhibitors (10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM PMSF) followed by immunoprecipitation with an antibody specific for the TIE-2 receptor. Samples were then subjected to immunoblot analysis, using anti pTyr antibodies.

[0238] Dot Blots

[0239] Samples were applied to a nitrocellulose membrane, which was blocked and probed with the appropriate antibodies.

TABLE 1

MUTATION ANALYSIS OF TIE LIGANDS



	N	COILED-COIL	FIBRINOGEN-LIKE	TIE2 Binding	TIE2 Activation	Multimeric Structure	Production Levels
TL1		c		+	+	HIGHER ORDER	LOW
TL2				+	–	DIMER	HIGH
		X		+	+	DIMER	LOW
		c		+	–	HIGHER ORDER	HIGH
		c		–	N.D.	N.D.	LOW
				–	N.D.	N.D.	HIGH
				–	–	MONOMER	HIGH
				–	–	MONOMER	HIGH
			Fc	+	–	DIMER	HIGH
			Fc	+	–	DIMER	HIGH
		c	Fc	+	+	HIGHER ORDER	LOW
			Fc	+	–	HIGHER ORDER	LOW
flag-		c		+	+	N.D.	LOW
flag-				+	–	N.D.	HIGH
		c		+	–	N.D.	HIGH
				+	–	N.D.	HIGH
		c		+	–	N.D.	LOW
				+	+	N.D.	HIGH*
				+	–	N.D.	LOW
		c		+	+**	N.D.	HIGH
		X		+	+**	DIMER	HIGH
	X	c		+	+	N.D.	LOW

*HIGHEST PRODUCTION OF RU
**MOST POTENTLY ACTIVATING
N.D. = NOT DETERMINED

Deposits

[0240] The following have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852 in accordance with the Budapest Treaty. A plasmid clone encoding a TIE-2 ligand was deposited with the ATCC on Oct. 7, 1994 and designated as "pJFE14 encoding TIE-2 ligand" under ATCC Accession No. 75910. Recombinant *Autographa californica* baculovirus encoding TIE-2 receptorbody was deposited with the ATCC on Oct. 7, 1994 and designated as "vTIE-2 receptorbody" under ATCC Accession No. VR2484. A lambda phage vector containing human tie-2 ligand DNA was deposited with the ATCC on Oct. 26, 1994 and designated as "λgt10 encoding htie-2 ligand 1" under ATCC Accession No. 75928. A plasmid clone encoding a second TIE-2 ligand was deposited with the ATCC on Dec. 9, 1994 and designated as "pBluescript KS encoding human TIE 2 ligand 2" under ATCC Accession No. 75963. *E. coli* strain DH10B containing plasmid pBeLoBac11 with a human TL-4 gene insert encoding human TIE ligand-4 was deposited with the ATCC on Jul. 2, 1996 and designated as "hTL-4" under ATCC Accession No. 98095.

[0241] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

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aacgctttct ttgaggggga aagagtcaaa caaacaagca gttttacctg aaataaagaa    240

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ggcagtaca atg aca gtt ttc ctt tcc ttt gct ttc ctc gct gcc att ctg    351
          Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu
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act cac ata ggg tgc agc aat cag cgc cga agt cca gaa aac agt ggg      399
Thr His Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly
15                  20                  25                  30

aga aga tat aac cgg att caa cat ggg caa tgt gcc tac act ttc att      447
Arg Arg Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile
                35                  40                  45

ctt cca gaa cac gat ggc aac tgt cgt gag agt acg aca gac cag tac      495
Leu Pro Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr
            50                  55                  60

aac aca aac gct ctg cag aga gat gct cca cac gtg gaa ccg gat ttc      543
Asn Thr Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe
        65                  70                  75

tct tcc cag aaa ctt caa cat ctg gaa cat gtg atg gaa aat tat act      591
Ser Ser Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr
    80                  85                  90

cag tgg ctg caa aaa ctt gag aat tac att gtg gaa aac atg aag tcg      639
Gln Trp Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser
95                  100                 105                 110

gag atg gcc cag ata cag cag aat gca gtt cag aac cac acg gct acc      687
Glu Met Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr
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                115                 120                 125

atg ctg gag ata gga acc agc ctc ctc tct cag act gca gag cag acc      735
Met Leu Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr
            130                 135                 140

aga aag ctg aca gat gtt gag acc cag gta cta aat caa act tct cga      783
Arg Lys Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg
        145                 150                 155

ctt gag ata cag ctg ctg gag aat tca tta tcc acc tac aag cta gag      831
Leu Glu Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu
    160                 165                 170

aag caa ctt ctt caa cag aca aat gaa atc ttg aag atc cat gaa aaa      879
Lys Gln Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys
175                 180                 185                 190

aac agt tta tta gaa cat aaa atc tta gaa atg gaa gga aaa cac aag      927
Asn Ser Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys
                195                 200                 205

gaa gag ttg gac acc tta aag gaa gag aaa gag aac ctt caa ggc ttg      975
Glu Glu Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu
            210                 215                 220

gtt act cgt caa aca tat ata atc cag gag ctg gaa aag caa tta aac     1023
Val Thr Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn
        225                 230                 235

aga gct acc acc aac aac agt gtc ctt cag aag cag caa ctg gag ctg     1071
Arg Ala Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu
    240                 245                 250

atg gac aca gtc cac aac ctt gtc aat ctt tgc act aaa gaa ggt gtt     1119
Met Asp Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val
255                 260                 265                 270

tta cta aag gga gga aaa aga gag gaa gag aaa cca ttt aga gac tgt     1167
Leu Leu Lys Gly Gly Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys
                275                 280                 285

gca gat gta tat caa gct ggt ttt aat aaa agt gga atc tac act att     1215
Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile
            290                 295                 300

tat att aat aat atg cca gaa ccc aaa aag gtg ttt tgc aat atg gat     1263
Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp
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gtc aat ggg gga ggt tgg act gta ata caa cat cgt gaa gat gga agt     1311
Val Asn Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser
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cta gat ttc caa aga ggc tgg aag gaa tat aaa atg ggt ttt gga aat     1359
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ccc tcc ggt gaa tat tgg ctg ggg aat gag ttt att ttt gcc att acc     1407
Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr
                355                 360                 365

agt cag agg cag tac atg cta aga att gag tta atg gac tgg gaa ggg     1455
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            370                 375                 380

aac cga gcc tat tca cag tat gac aga ttc cac ata gga aat gaa aag     1503
Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys
        385                 390                 395

caa aac tat agg ttg tat tta aaa ggt cac act ggg aca gca gga aaa     1551
Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys
    400                 405                 410

cag agc agc ctg atc tta cac ggt gct gat ttc agc act aaa gat gct     1599
Gln Ser Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala
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Asp Asn Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly
                435                 440                 445

tgg tgg ttt gat gct tgt ggc ccc tcc aat cta aat gga atg ttc tat     1695
Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr
            450                 455                 460

act gcg gga caa aac cat gga aaa ctg aat ggg ata aag tgg cac tac     1743
Thr Ala Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr
        465                 470                 475

ttc aaa ggg ccc agt tac tcc tta cgt tcc aca act atg atg att cga     1791
Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg
    480                 485                 490

cct tta gat ttt tgaaagcgca atgtcagaag cgattatgaa agcaacaaag         1843
Pro Leu Asp Phe
495

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Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu
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Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln
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225                 230                 235                 240

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Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu
            260                 265                 270

Lys Gly Gly Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp
        275                 280                 285

Val Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile
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Asn Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn
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Gly Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp
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Phe Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser
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Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln
        355                 360                 365

Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg
    370                 375                 380

Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn
385                 390                 395                 400

Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser
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Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn
            420                 425                 430

Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp
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Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala
    450                 455                 460

Gly Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys
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Thr His Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly
15                  20                  25                  30

aga aga tat aac cgg att caa cat ggg caa tgt gcc tac act ttc att      447
Arg Arg Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile
                35                  40                  45

ctt cca gaa cac gat ggc aac tgt cgt gag agt acg aca gac cag tac      495
Leu Pro Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr
            50                  55                  60

aac aca aac gct ctg cag aga gat gct cca cac gtg gaa ccg gat ttc      543
Asn Thr Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe
        65                  70                  75

tct tcc cag aaa ctt caa cat ctg gaa cat gtg atg gaa aat tat act      591
Ser Ser Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr
    80                  85                  90

cag tgg ctg caa aaa ctt gag aat tac att gtg gaa aac atg aag tcg      639
Gln Trp Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser
95                  100                 105                 110

gag atg gcc cag ata cag cag aat gca gtt cag aac cac acg gct acc      687
Glu Met Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr
                115                 120                 125

atg ctg gag ata gga acc agc ctc ctc tct cag act gca gag cag acc      735
Met Leu Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr
            130                 135                 140

aga aag ctg aca gat gtt gag acc cag gta cta aat caa act tct cga      783
Arg Lys Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg
        145                 150                 155

ctt gag ata cag ctg ctg gag aat tca tta tcc acc tac aag cta gag      831
Leu Glu Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu
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175                 180                 185                 190

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                195                 200                 205

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Val Thr Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn
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            290                 295                 300

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Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser
                355                 360                 365

cag agg cag tac atg cta aga att gag tta atg gac tgg gaa ggg aac     1455
Gln Arg Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn
            370                 375                 380

cga gcc tat tca cag tat gac aga ttc cac ata gga aat gaa aag caa     1503
Arg Ala Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln
        385                 390                 395

aac tat agg ttg tat tta aaa ggt cac act ggg aca gca gga aaa cag     1551
Asn Tyr Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln
    400                 405                 410

agc agc ctg atc tta cac ggt gct gat ttc agc act aaa gat gct gat     1599
Ser Ser Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp
415                 420                 425                 430

aat gac aac tgt atg tgc aaa tgt gcc ctc atg tta aca gga gga tgg     1647
Asn Asp Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp
                435                 440                 445

tgg ttt gat gct tgt ggc ccc tcc aat cta aat gga atg ttc tat act     1695
Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr
            450                 455                 460

gcg gga caa aac cat cga aaa ctg aat ggg ata aag tgg cac tac ttc     1743
Ala Gly Gln Asn His Arg Lys Leu Asn Gly Ile Lys Trp His Tyr Phe
        465                 470                 475

aaa ggg ccc agt tac tcc tta cgt tcc aca act atg atg att cga cct     1791
Lys Gly Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro
    480                 485                 490

tta gat ttt tga aagcgcaatg tcagaagcga ttatgaaagc aacaaagaaa         1843
Leu Asp Phe
495

tccggagaag ctgccaggtg agaaactgtt tgaaaacttc agaagcaaac aatattgtct   1903

cccttccacc aataagtggt agttatgtga agtcaccaag gttcttgacc gtgaatctgg   1963

agccgtttga gttcacaaga gtctctactt ggggtgacag tgctcacgtg gctcgactat   2023

agaaaactcc actgactgtc gggctttaaa aagggaagaa actgctgagc ttgctgtgct   2083

tcaaactact actggacctt attttggaac tatggtagcc agatgataaa tatggttaat   2143

ttc                                                                 2146


<210> SEQ ID NO 4
<211> LENGTH: 497
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<400> SEQUENCE: 4

```
Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
1               5                  10                  15

Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
            20                  25                  30

Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
        35                  40                  45

Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
    50                  55                  60

Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser
65                  70                  75                  80

Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp
                85                  90                  95

Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met
            100                 105                 110

Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu
        115                 120                 125

Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys
    130                 135                 140

Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu
145                 150                 155                 160

Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln
                165                 170                 175

Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser
            180                 185                 190

Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu
        195                 200                 205

Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr
    210                 215                 220

Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala
225                 230                 235                 240

Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp
                245                 250                 255

Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Val Leu Leu Lys
            260                 265                 270

Gly Gly Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val
        275                 280                 285

Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn
    290                 295                 300

Asn Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly
305                 310                 315                 320

Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe
                325                 330                 335

Gln Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly
            340                 345                 350

Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg
        355                 360                 365

Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala
    370                 375                 380

Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr
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385                 390                 395                 400

Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser
                405                 410                 415

Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp
            420                 425                 430

Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe
        435                 440                 445

Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly
    450                 455                 460

Gln Asn His Arg Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly
465                 470                 475                 480

Pro Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp
                485                 490                 495

Phe
```

<210> SEQ ID NO 5
<211> LENGTH: 2282
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (357)..(1844)
<223> OTHER INFORMATION:

<400> SEQUENCE: 5

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gaattcctgg gttggtgttt atctcctccc agccttgagg gagggaacaa cactgtagga     60

tctggggaga gaggaacaaa ggaccgtgaa agctgctctg taaaagctga cacagccctc    120

ccaagtgagc aggactgttc ttcccactgc aatctgacag tttactgcat gcctggagag    180

aacacagcag taaaaaccag gtttgctact ggaaaaagag gaaagagaag actttcattg    240

acggacccag ccatggcagc gtagcagccc tgcgtttcag acggcagcag ctcgggactc    300

tggacgtgtg tttgccctca agtttgctaa gctgctggtt tattactgaa gaaaga atg    359
                                                                 Met
                                                                 1

tgg cag att gtt ttc ttt act ctg agc tgt gat ctt gtc ttg gcc gca      407
Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala Ala
            5                  10                  15

gcc tat aac aac ttt cgg aag agc atg gac agc ata gga aag aag caa      455
Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys Gln
        20                  25                  30

tat cag gtc cag cat ggg tcc tgc agc tac act ttc ctc ctg cca gag      503
Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro Glu
    35                  40                  45

atg gac aac tgc cgc tct tcc tcc agc ccc tac gtg tcc aat gct gtg      551
Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala Val
50                  55                  60                  65

cag agg gac gcg ccg ctc gaa tac gat gac tcg gtg cag agg ctg caa      599
Gln Arg Asp Ala Pro Leu Glu Tyr Asp Ser Val Gln Arg Leu Gln
                70                  75                  80

gtg ctg gag aac atc atg gaa aac aac act cag tgg cta atg aag ctt      647
Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys Leu
            85                  90                  95

gag aat tat atc cag gac aac atg aag aaa gaa atg gta gag ata cag      695
Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile Gln
        100                 105                 110
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cag aat gca gta cag aac cag acg gct gtg atg ata gaa ata ggg aca      743
Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly Thr
    115                 120                 125

aac ctg ttg aac caa aca gct gag caa acg cgg aag tta act gat gtg      791
Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp Val
130                 135                 140                 145

gaa gcc caa gta tta aat cag acc acg aga ctt gaa ctt cag ctc ttg      839
Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu Leu
                150                 155                 160

gaa cac tcc ctc tcg aca aac aaa ttg gaa aaa cag att ttg gac cag      887
Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp Gln
            165                 170                 175

acc agt gaa ata aac aaa ttg caa gat aag aac agt ttc cta gaa aag      935
Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu Lys
        180                 185                 190

aag gtg cta gct atg gaa gac aag cac atc atc caa cta cag tca ata      983
Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser Ile
    195                 200                 205

aaa gaa gag aaa gat cag cta cag gtg tta gta tcc aag caa aat tcc     1031
Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn Ser
210                 215                 220                 225

atc att gaa gaa cta gaa aaa aaa ata gtg act gcc acg gtg aat aat     1079
Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala Thr Val Asn Asn
                230                 235                 240

tca gtt ctt caa aag cag caa cat gat ctc atg gag aca gtt aat aac     1127
Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu Thr Val Asn Asn
            245                 250                 255

tta ctg act atg atg tcc aca tca aac tca gct aag gac ccc act gtt     1175
Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr Val
        260                 265                 270

gct aaa gaa gaa caa atc agc ttc aga gac tgt gct gaa gta ttc aaa     1223
Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Glu Val Phe Lys
    275                 280                 285

tca gga cac acc aca aat ggc atc tac acg tta aca ttc cct aat tct     1271
Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro Asn Ser
290                 295                 300                 305

aca gaa gag atc aag gcc tac tgt gac atg gaa gct gga gga ggc ggg     1319
Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly Gly Gly
                310                 315                 320

tgg aca att att cag cga cgt gag gat ggc agc gtt gat ttt cag agg     1367
Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp Phe Gln Arg
            325                 330                 335

act tgg aaa gaa tat aaa gtg gga ttt ggt aac cct tca gga gaa tat     1415
Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu Tyr
        340                 345                 350

tgg ctg gga aat gag ttt gtt tcg caa ctg act aat cag caa cgc tat     1463
Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg Tyr
    355                 360                 365

gtg ctt aaa ata cac ctt aaa gac tgg gaa ggg aat gag gct tac tca     1511
Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr Ser
370                 375                 380                 385

ttg tat gaa cat ttc tat ctc tca agt gaa gaa ctc aat tat agg att     1559
Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg Ile
                390                 395                 400

cac ctt aaa gga ctt aca ggg aca gcc ggc aaa ata agc agc atc agc     1607
His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile Ser
            405                 410                 415
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caa cca gga aat gat ttt agc aca aag gat gga gac aac gac aaa tgt     1655
Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys Cys
        420                 425                 430

att tgc aaa tgt tca caa atg cta aca gga ggc tgg tgg ttt gat gca     1703
Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp Ala
    435                 440                 445

tgt ggt cct tcc aac ttg aac gga atg tac tat cca cag agg cag aac     1751
Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln Asn
450                 455                 460                 465

aca aat aag ttc aac ggc att aaa tgg tac tac tgg aaa ggc tca ggc     1799
Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser Gly
                470                 475                 480

tat tcg ctc aag gcc aca acc atg atg atc cga cca gca gat ttc         1844
Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe
            485                 490                 495

taaacatccc agtccacctg aggaactgtc tcgaactatt ttcaaagact taagcccagt   1904

gcactgaaag tcacggctgc gcactgtgtc ctcttccacc acagagggcg tgtgctcggt   1964

gctgacggga cccacatgct ccagattaga gcctgtaaac tttatcactt aaacttgcat   2024

cacttaacgg accaaagcaa gaccctaaac atccataatt gtgattagac agaacaccta   2084

tgcaaagatg aacccgaggc tgagaatcag actgacagtt tacagacgct gctgtcacaa   2144

ccaagaatgt tatgtgcaag tttatcagta aataactgga aaacagaaca cttatgttat   2204

acaatacaga tcatcttgga actgcattct tctgagcact gtttatacac tgtgtaaata   2264

cccatatgtc ctgaattc                                                2282


<210> SEQ ID NO 6
<211> LENGTH: 496
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala
1               5                   10                  15

Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
            20                  25                  30

Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro
        35                  40                  45

Glu Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala
    50                  55                  60

Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Asp Ser Val Gln Arg Leu
65                  70                  75                  80

Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys
                85                  90                  95

Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile
            100                 105                 110

Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly
        115                 120                 125

Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
    130                 135                 140

Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
145                 150                 155                 160

Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp
                165                 170                 175
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Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu
            180                 185                 190

Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser
        195                 200                 205

Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn
    210                 215                 220

Ser Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala Thr Val Asn
225                 230                 235                 240

Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu Thr Val Asn
                245                 250                 255

Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr
            260                 265                 270

Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Glu Val Phe
        275                 280                 285

Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro Asn
    290                 295                 300

Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly Gly
305                 310                 315                 320

Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp Phe Gln
                325                 330                 335

Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu
            340                 345                 350

Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg
        355                 360                 365

Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr
    370                 375                 380

Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg
385                 390                 395                 400

Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile
                405                 410                 415

Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys
            420                 425                 430

Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp
        435                 440                 445

Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln
    450                 455                 460

Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser
465                 470                 475                 480

Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe
                485                 490                 495


<210> SEQ ID NO 7
<211> LENGTH: 478
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg Tyr Asn Arg Ile
1               5                   10                  15

Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro Glu His Asp Gly
            20                  25                  30

Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr Asn Ala Leu Gln
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        35                  40                  45

Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser Gln Lys Leu Gln
    50                  55                  60

His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp Leu Gln Lys Leu
65                  70                  75                  80

Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met Ala Gln Ile Gln
                85                  90                  95

Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu Glu Ile Gly Thr
            100                 105                 110

Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp Val
        115                 120                 125

Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu Ile Gln Leu Leu
    130                 135                 140

Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln Leu Leu Gln Gln
145                 150                 155                 160

Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser Leu Leu Glu His
                165                 170                 175

Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu Leu Asp Thr Leu
            180                 185                 190

Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr Arg Gln Thr Tyr
        195                 200                 205

Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala Thr Thr Asn Asn
    210                 215                 220

Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp Thr Val His Asn
225                 230                 235                 240

Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu Lys Gly Gly Lys
                245                 250                 255

Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val Tyr Gln Ala
            260                 265                 270

Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn Met Pro
        275                 280                 285

Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly Gly Trp
    290                 295                 300

Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg Gly
305                 310                 315                 320

Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp
                325                 330                 335

Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr Met
            340                 345                 350

Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln
        355                 360                 365

Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr
    370                 375                 380

Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu
385                 390                 395                 400

His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys Met
                405                 410                 415

Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys
            420                 425                 430

Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn His
        435                 440                 445
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Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser Tyr
    450                 455                 460

Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe
465                 470                 475


<210> SEQ ID NO 8
<211> LENGTH: 480
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
1               5                   10                  15

Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro
            20                  25                  30

Glu Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala
        35                  40                  45

Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Asp Ser Val Gln Arg Leu
    50                  55                  60

Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys
65                  70                  75                  80

Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile
                85                  90                  95

Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly
            100                 105                 110

Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
        115                 120                 125

Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
    130                 135                 140

Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp
145                 150                 155                 160

Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu
                165                 170                 175

Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser
            180                 185                 190

Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn
        195                 200                 205

Ser Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala Thr Val Asn
    210                 215                 220

Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu Thr Val Asn
225                 230                 235                 240

Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr
                245                 250                 255

Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Glu Val Phe
            260                 265                 270

Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe Pro Asn
        275                 280                 285

Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly Gly Gly
    290                 295                 300

Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp Phe Gln
305                 310                 315                 320

Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser Gly Glu
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                325                 330                 335

Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln Gln Arg
            340                 345                 350

Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr
        355                 360                 365

Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn Tyr Arg
    370                 375                 380

Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser Ser Ile
385                 390                 395                 400

Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys
                405                 410                 415

Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp Phe Asp
            420                 425                 430

Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln Arg Gln
        435                 440                 445

Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser
    450                 455                 460

Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe
465                 470                 475                 480


<210> SEQ ID NO 9
<211> LENGTH: 1849
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (47)..(1573)
<223> OTHER INFORMATION: The fibrinogen-like domain starts at position
      929.

<400> SEQUENCE: 9

ctgtcctggt acctgacaag accacctcac caccacttgg tctcag atg ctc tgc        55
                                                    Met Leu Cys
                                                    1

cag cca gct atg cta cta gat ggc ctc ctc ctg ctg gcc acc atg gct      103
Gln Pro Ala Met Leu Leu Asp Gly Leu Leu Leu Leu Ala Thr Met Ala
    5                   10                  15

gca gcc cag cac aga ggg cca gaa gcc ggt ggg cac cgc cag att cac      151
Ala Ala Gln His Arg Gly Pro Glu Ala Gly Gly His Arg Gln Ile His
20                  25                  30                  35

cag gtc cgg cgt ggc cag tgc agc tac acc ttt gtg gtg ccg gag cct      199
Gln Val Arg Arg Gly Gln Cys Ser Tyr Thr Phe Val Val Pro Glu Pro
                40                  45                  50

gat atc tgc cag ctg gcg ccg aca gcg gcg cct gag gct ttg ggg ggc      247
Asp Ile Cys Gln Leu Ala Pro Thr Ala Ala Pro Glu Ala Leu Gly Gly
            55                  60                  65

tcc aat agc ctc cag agg gac ttg cct gcc tcg agg ctg cac cta aca      295
Ser Asn Ser Leu Gln Arg Asp Leu Pro Ala Ser Arg Leu His Leu Thr
        70                  75                  80

gac tgg cga gcc cag agg gcc cag cgg gcc cag cgt gtg agc cag ctg      343
Asp Trp Arg Ala Gln Arg Ala Gln Arg Ala Gln Arg Val Ser Gln Leu
    85                  90                  95

gag aag ata cta gag aat aac act cag tgg ctg ctg aag ctg gag cag      391
Glu Lys Ile Leu Glu Asn Asn Thr Gln Trp Leu Leu Lys Leu Glu Gln
100                 105                 110                 115

tcc atc aag gtg aac ttg agg tca cac ctg gtg cag gcc cag cag gac      439
Ser Ile Lys Val Asn Leu Arg Ser His Leu Val Gln Ala Gln Gln Asp
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               120                 125                 130

aca atc cag aac cag aca act acc atg ctg gca ctg ggt gcc aac ctc      487
Thr Ile Gln Asn Gln Thr Thr Thr Met Leu Ala Leu Gly Ala Asn Leu
           135                 140                 145

atg aac cag acc aaa gct cag acc cac aag ctg act gct gtg gag gca      535
Met Asn Gln Thr Lys Ala Gln Thr His Lys Leu Thr Ala Val Glu Ala
       150                 155                 160

cag gtc cta aac cag aca ttg cac atg aag acc caa atg ctg gag aac      583
Gln Val Leu Asn Gln Thr Leu His Met Lys Thr Gln Met Leu Glu Asn
   165                 170                 175

tca ctg tcc acc aac aag ctg gag cgg cag atg ctg atg cag agc cga      631
Ser Leu Ser Thr Asn Lys Leu Glu Arg Gln Met Leu Met Gln Ser Arg
180                 185                 190                 195

gag ctg cag cgg ctg cag ggt cgc aac agg gcc ctg gag acc agg ctg      679
Glu Leu Gln Arg Leu Gln Gly Arg Asn Arg Ala Leu Glu Thr Arg Leu
               200                 205                 210

cag gca ctg gaa gca caa cat cag gcc cag ctt aac agc ctc caa gag      727
Gln Ala Leu Glu Ala Gln His Gln Ala Gln Leu Asn Ser Leu Gln Glu
           215                 220                 225

aag agg gaa caa ctg cac agt ctc ctg ggc cat cag acc ggg acc ctg      775
Lys Arg Glu Gln Leu His Ser Leu Leu Gly His Gln Thr Gly Thr Leu
       230                 235                 240

gct aac ctg aag cac aat ctg cac gct ctc agc agc aat tcc agc tcc      823
Ala Asn Leu Lys His Asn Leu His Ala Leu Ser Ser Asn Ser Ser Ser
   245                 250                 255

ctg cag cag cag cag cag caa ctg acg gag ttt gta cag cgc ctg gta      871
Leu Gln Gln Gln Gln Gln Gln Leu Thr Glu Phe Val Gln Arg Leu Val
260                 265                 270                 275

cgg att gta gcc cag gac cag cat ccg gtt tcc tta aag aca cct aag      919
Arg Ile Val Ala Gln Asp Gln His Pro Val Ser Leu Lys Thr Pro Lys
               280                 285                 290

cca gtg ttc cag gac tgt gca gag atc aag cgc tcc ggg gtt aat acc      967
Pro Val Phe Gln Asp Cys Ala Glu Ile Lys Arg Ser Gly Val Asn Thr
           295                 300                 305

agc ggt gtc tat acc atc tat gag acc aac atg aca aag cct ctc aag     1015
Ser Gly Val Tyr Thr Ile Tyr Glu Thr Asn Met Thr Lys Pro Leu Lys
       310                 315                 320

gtg ttc tgt gac atg gag act gat gga ggt ggc tgg acc ctc atc cag     1063
Val Phe Cys Asp Met Glu Thr Asp Gly Gly Gly Trp Thr Leu Ile Gln
   325                 330                 335

cac cgg gag gat gga agc gta aat ttc cag agg acc tgg gaa gaa tac     1111
His Arg Glu Asp Gly Ser Val Asn Phe Gln Arg Thr Trp Glu Glu Tyr
340                 345                 350                 355

aaa gag ggt ttt ggt aat gtg gcc aga gag cac tgg ctg ggc aat gag     1159
Lys Glu Gly Phe Gly Asn Val Ala Arg Glu His Trp Leu Gly Asn Glu
               360                 365                 370

gct gtg cac cgc ctc acc agc aga acg gcc tac ttg cta cgc gtg gaa     1207
Ala Val His Arg Leu Thr Ser Arg Thr Ala Tyr Leu Leu Arg Val Glu
           375                 380                 385

ctg cat gac tgg gaa ggc cgc cag acc tcc atc cag tat gag aac ttc     1255
Leu His Asp Trp Glu Gly Arg Gln Thr Ser Ile Gln Tyr Glu Asn Phe
       390                 395                 400

cag ctg ggc agc gag agg cag cgg tac agc ctc tct gtg aat gac agc     1303
Gln Leu Gly Ser Glu Arg Gln Arg Tyr Ser Leu Ser Val Asn Asp Ser
   405                 410                 415

agc agt tca gca ggg cgc aag aac agc ctg gct cct cag ggc acc aag     1351
Ser Ser Ser Ala Gly Arg Lys Asn Ser Leu Ala Pro Gln Gly Thr Lys
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420                 425                 430                 435

ttc agc acc aaa gac atg gac aat gat aac tgc atg tgt aaa tgt gct     1399
Phe Ser Thr Lys Asp Met Asp Asn Asp Asn Cys Met Cys Lys Cys Ala
                440                 445                 450

cag atg ctg tct gga ggg tgg tgg ttt gat gcc tgt ggc ctc tcc aac     1447
Gln Met Leu Ser Gly Gly Trp Trp Phe Asp Ala Cys Gly Leu Ser Asn
            455                 460                 465

ctc aat ggc atc tac tat tca gtt cat cag cac ttg cac aag atc aat     1495
Leu Asn Gly Ile Tyr Tyr Ser Val His Gln His Leu His Lys Ile Asn
        470                 475                 480

ggc atc cgc tgg cac tac ttc cga ggc ccc agc tac tca ctg cac ggc     1543
Gly Ile Arg Trp His Tyr Phe Arg Gly Pro Ser Tyr Ser Leu His Gly
    485                 490                 495

aca cgc atg atg ctg agg cca atg ggt gcc tgacacacag ccctgcagag       1593
Thr Arg Met Met Leu Arg Pro Met Gly Ala
500                 505

actgatgccg taggaggatt ctcaacccag gtgactctgt gcacgctggg ccctgcccag   1653

aaatcagtgc ccagggctca tcttgacatt ctggaacatc ggaaccagct taccttgccc   1713

ctgaattaca agaattcacc tgcctccctg ttgccctcta attgtgaaat tgctgggtgc   1773

ttgaaggcac ctgcctctgt tggaaccata ctctttcccc ctcctgctgc atgcccggga   1833

atccctgcca tgaact                                                   1849


<210> SEQ ID NO 10
<211> LENGTH: 509
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Met Leu Cys Gln Pro Ala Met Leu Leu Asp Gly Leu Leu Leu Leu Ala
1               5                   10                  15

Thr Met Ala Ala Ala Gln His Arg Gly Pro Glu Ala Gly Gly His Arg
            20                  25                  30

Gln Ile His Gln Val Arg Arg Gly Gln Cys Ser Tyr Thr Phe Val Val
        35                  40                  45

Pro Glu Pro Asp Ile Cys Gln Leu Ala Pro Thr Ala Ala Pro Glu Ala
    50                  55                  60

Leu Gly Gly Ser Asn Ser Leu Gln Arg Asp Leu Pro Ala Ser Arg Leu
65                  70                  75                  80

His Leu Thr Asp Trp Arg Ala Gln Arg Ala Gln Arg Ala Gln Arg Val
                85                  90                  95

Ser Gln Leu Glu Lys Ile Leu Glu Asn Asn Thr Gln Trp Leu Leu Lys
            100                 105                 110

Leu Glu Gln Ser Ile Lys Val Asn Leu Arg Ser His Leu Val Gln Ala
        115                 120                 125

Gln Gln Asp Thr Ile Gln Asn Gln Thr Thr Thr Met Leu Ala Leu Gly
    130                 135                 140

Ala Asn Leu Met Asn Gln Thr Lys Ala Gln Thr His Lys Leu Thr Ala
145                 150                 155                 160

Val Glu Ala Gln Val Leu Asn Gln Thr Leu His Met Lys Thr Gln Met
                165                 170                 175

Leu Glu Asn Ser Leu Ser Thr Asn Lys Leu Glu Arg Gln Met Leu Met
            180                 185                 190
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Gln Ser Arg Glu Leu Gln Arg Leu Gln Gly Arg Asn Arg Ala Leu Glu
        195                 200                 205

Thr Arg Leu Gln Ala Leu Glu Ala Gln His Gln Ala Gln Leu Asn Ser
    210                 215                 220

Leu Gln Glu Lys Arg Glu Gln Leu His Ser Leu Leu Gly His Gln Thr
225                 230                 235                 240

Gly Thr Leu Ala Asn Leu Lys His Asn Leu His Ala Leu Ser Ser Asn
                245                 250                 255

Ser Ser Ser Leu Gln Gln Gln Gln Gln Gln Leu Thr Glu Phe Val Gln
            260                 265                 270

Arg Leu Val Arg Ile Val Ala Gln Asp Gln His Pro Val Ser Leu Lys
        275                 280                 285

Thr Pro Lys Pro Val Phe Gln Asp Cys Ala Glu Ile Lys Arg Ser Gly
    290                 295                 300

Val Asn Thr Ser Gly Val Tyr Thr Ile Tyr Glu Thr Asn Met Thr Lys
305                 310                 315                 320

Pro Leu Lys Val Phe Cys Asp Met Glu Thr Asp Gly Gly Gly Trp Thr
                325                 330                 335

Leu Ile Gln His Arg Glu Asp Gly Ser Val Asn Phe Gln Arg Thr Trp
            340                 345                 350

Glu Glu Tyr Lys Glu Gly Phe Gly Asn Val Ala Arg Glu His Trp Leu
        355                 360                 365

Gly Asn Glu Ala Val His Arg Leu Thr Ser Arg Thr Ala Tyr Leu Leu
    370                 375                 380

Arg Val Glu Leu His Asp Trp Glu Gly Arg Gln Thr Ser Ile Gln Tyr
385                 390                 395                 400

Glu Asn Phe Gln Leu Gly Ser Glu Arg Gln Arg Tyr Ser Leu Ser Val
                405                 410                 415

Asn Asp Ser Ser Ser Ser Ala Gly Arg Lys Asn Ser Leu Ala Pro Gln
            420                 425                 430

Gly Thr Lys Phe Ser Thr Lys Asp Met Asp Asn Asp Asn Cys Met Cys
        435                 440                 445

Lys Cys Ala Gln Met Leu Ser Gly Gly Trp Trp Phe Asp Ala Cys Gly
    450                 455                 460

Leu Ser Asn Leu Asn Gly Ile Tyr Tyr Ser Val His Gln His Leu His
465                 470                 475                 480

Lys Ile Asn Gly Ile Arg Trp His Tyr Phe Arg Gly Pro Ser Tyr Ser
                485                 490                 495

Leu His Gly Thr Arg Met Met Leu Arg Pro Met Gly Ala
            500                 505


<210> SEQ ID NO 11
<211> LENGTH: 503
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Met Leu Leu Asp Gly Leu Leu Leu Leu Ala Thr Met Ala Ala Ala Gln
1               5                   10                  15

His Arg Gly Pro Glu Ala Gly Gly His Arg Gln Ile His Gln Val Arg
            20                  25                  30

Arg Gly Gln Cys Ser Tyr Thr Phe Val Val Pro Glu Pro Asp Ile Cys
        35                  40                  45
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Gln Leu Ala Pro Thr Ala Ala Pro Glu Ala Leu Gly Gly Ser Asn Ser
    50                  55                  60

Leu Gln Arg Asp Leu Pro Ala Ser Arg Leu His Leu Thr Asp Trp Arg
65                  70                  75                  80

Ala Gln Arg Ala Gln Arg Ala Gln Arg Val Ser Gln Leu Glu Lys Ile
                85                  90                  95

Leu Glu Asn Asn Thr Gln Trp Leu Leu Lys Leu Glu Gln Ser Ile Lys
            100                 105                 110

Val Asn Leu Arg Ser His Leu Val Gln Ala Gln Gln Asp Thr Ile Gln
        115                 120                 125

Asn Gln Thr Thr Thr Met Leu Ala Leu Gly Ala Asn Leu Met Asn Gln
    130                 135                 140

Thr Lys Ala Gln Thr His Lys Leu Thr Ala Val Glu Ala Gln Val Leu
145                 150                 155                 160

Asn Gln Thr Leu His Met Lys Thr Gln Met Leu Glu Asn Ser Leu Ser
                165                 170                 175

Thr Asn Lys Leu Glu Arg Gln Met Leu Met Gln Ser Arg Glu Leu Gln
            180                 185                 190

Arg Leu Gln Gly Arg Asn Arg Ala Leu Glu Thr Arg Leu Gln Ala Leu
        195                 200                 205

Glu Ala Gln His Gln Ala Gln Leu Asn Ser Leu Gln Glu Lys Arg Glu
    210                 215                 220

Gln Leu His Ser Leu Leu Gly His Gln Thr Gly Thr Leu Ala Asn Leu
225                 230                 235                 240

Lys His Asn Leu His Ala Leu Ser Ser Asn Ser Ser Ser Leu Gln Gln
                245                 250                 255

Gln Gln Gln Gln Leu Thr Glu Phe Val Gln Arg Leu Val Arg Ile Val
            260                 265                 270

Ala Gln Asp Gln His Pro Val Ser Leu Lys Thr Pro Lys Pro Val Phe
        275                 280                 285

Gln Asp Cys Ala Glu Ile Lys Arg Ser Gly Val Asn Thr Ser Gly Val
    290                 295                 300

Tyr Thr Ile Tyr Glu Thr Asn Met Thr Lys Pro Leu Lys Val Phe Cys
305                 310                 315                 320

Asp Met Glu Thr Asp Gly Gly Gly Trp Thr Leu Ile Gln His Arg Glu
                325                 330                 335

Asp Gly Ser Val Asn Phe Gln Arg Thr Trp Glu Glu Tyr Lys Glu Gly
            340                 345                 350

Phe Gly Asn Val Ala Arg Glu His Trp Leu Gly Asn Glu Ala Val His
        355                 360                 365

Arg Leu Thr Ser Arg Thr Ala Tyr Leu Leu Arg Val Glu Leu His Asp
    370                 375                 380

Trp Glu Gly Arg Gln Thr Ser Ile Gln Tyr Glu Asn Phe Gln Leu Gly
385                 390                 395                 400

Ser Glu Arg Gln Arg Tyr Ser Leu Ser Val Asn Asp Ser Ser Ser Ser
                405                 410                 415

Ala Gly Arg Lys Asn Ser Leu Ala Pro Gln Gly Thr Lys Phe Ser Thr
            420                 425                 430

Lys Asp Met Asp Asn Asp Asn Cys Met Cys Lys Cys Ala Gln Met Leu
        435                 440                 445
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Ser Gly Gly Trp Trp Phe Asp Ala Cys Gly Leu Ser Asn Leu Asn Gly
    450                 455                 460

Ile Tyr Tyr Ser Val His Gln His Leu His Lys Ile Asn Gly Ile Arg
465                 470                 475                 480

Trp His Tyr Phe Arg Gly Pro Ser Tyr Ser Ile His Gly Thr Arg Met
                485                 490                 495

Met Leu Arg Pro Met Gly Ala
            500


<210> SEQ ID NO 12
<211> LENGTH: 490
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Ala Phe Leu Ala Ala Ile Leu Thr His Ile Gly Cys Ser Asn Gln Arg
1               5                   10                  15

Arg Ser Pro Glu Asn Ser Gly Arg Arg Tyr Asn Arg Ile Gln His Gly
            20                  25                  30

Gln Cys Ala Tyr Thr Phe Ile Leu Pro Glu His Asp Gly Asn Cys Arg
        35                  40                  45

Glu Ser Thr Thr Asp Gln Tyr Asn Thr Asn Ala Leu Gln Arg Asp Ala
    50                  55                  60

Pro His Val Glu Pro Asp Phe Ser Ser Gln Lys Leu Gln His Leu Glu
65                  70                  75                  80

His Val Met Glu Asn Tyr Thr Gln Trp Leu Gln Lys Leu Glu Asn Tyr
                85                  90                  95

Ile Val Glu Asn Met Lys Ser Glu Met Ala Gln Ile Gln Gln Asn Ala
            100                 105                 110

Val Gln Asn His Thr Ala Thr Met Leu Glu Ile Gly Thr Ser Leu Leu
        115                 120                 125

Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp Val Glu Thr Gln
    130                 135                 140

Val Leu Asn Gln Thr Ser Arg Leu Glu Ile Gln Leu Leu Glu Asn Ser
145                 150                 155                 160

Leu Ser Thr Tyr Lys Leu Glu Lys Gln Leu Leu Gln Gln Thr Asn Glu
                165                 170                 175

Ile Leu Lys Ile His Glu Lys Asn Ser Leu Leu Glu His Lys Ile Leu
            180                 185                 190

Glu Met Glu Gly Lys His Lys Glu Glu Leu Asp Thr Leu Lys Glu Glu
        195                 200                 205

Lys Glu Asn Leu Gln Gly Leu Val Thr Arg Gln Thr Tyr Ile Ile Gln
    210                 215                 220

Glu Leu Glu Lys Gln Leu Asn Arg Ala Thr Thr Asn Asn Ser Val Leu
225                 230                 235                 240

Gln Lys Gln Gln Leu Glu Leu Met Asp Thr Val His Asn Leu Val Asn
                245                 250                 255

Leu Cys Thr Lys Glu Val Leu Leu Lys Gly Gly Lys Arg Glu Glu Glu
            260                 265                 270

Lys Pro Phe Arg Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn Lys
        275                 280                 285

Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn Met Pro Glu Pro Lys Lys
    290                 295                 300
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Val Phe Cys Asn Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile Gln
305                 310                 315                 320

His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg Gly Trp Lys Glu Tyr
                325                 330                 335

Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr Trp Leu Gly Asn Glu
            340                 345                 350

Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr Met Leu Arg Ile Glu
        355                 360                 365

Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg Phe
    370                 375                 380

His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly His
385                 390                 395                 400

Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala Asp
                405                 410                 415

Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys Met Cys Lys Cys Ala
            420                 425                 430

Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser Asn
        435                 440                 445

Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn His Gly Lys Leu Asn
    450                 455                 460

Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser Tyr Ser Ile Arg Ser
465                 470                 475                 480

Thr Thr Met Met Ile Arg Pro Leu Asp Phe
                485                 490


<210> SEQ ID NO 13
<211> LENGTH: 491
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Ala Phe Leu Ala Ala Ile Leu Ala His Ile Gly Cys Thr Thr Gln Arg
1               5                   10                  15

Arg Ser Pro Glu Asn Ser Gly Arg Arg Phe Asn Arg Ile Gln His Gly
            20                  25                  30

Gln Cys Thr Tyr Thr Phe Ile Leu Pro Glu Gln Asp Gly Asn Cys Arg
        35                  40                  45

Glu Ser Thr Thr Asp Gln Tyr Asn Thr Asn Ala Leu Gln Arg Asp Ala
    50                  55                  60

Pro His Val Glu Gln Asp Phe Ser Phe Gln Lys Leu Gln His Leu Glu
65                  70                  75                  80

His Val Met Glu Asn Tyr Thr Gln Trp Leu Gln Lys Leu Glu Ser Tyr
                85                  90                  95

Ile Val Glu Asn Met Lys Ser Glu Met Ala Gln Leu Gln Gln Asn Ala
            100                 105                 110

Val Gln Asn His Thr Ala Thr Met Leu Glu Ile Gly Thr Ser Leu Leu
        115                 120                 125

Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp Val Glu Thr Gln
    130                 135                 140

Val Leu Asn Gln Thr Ser Arg Leu Glu Ile Gln Leu Leu Glu Asn Ser
145                 150                 155                 160

Leu Ser Thr Tyr Lys Leu Glu Lys Gln Leu Leu Gln Gln Thr Asn Glu
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                165                 170                 175

Ile Leu Lys Ile His Glu Lys Asn Ser Leu Leu Glu His Lys Ile Leu
            180                 185                 190

Glu Met Glu Glu Arg His Lys Glu Glu Met Asp Thr Leu Lys Glu Glu
        195                 200                 205

Lys Glu Asn Leu Gln Gly Leu Val Thr Arg Gln Ser Tyr Ile Ile Gln
    210                 215                 220

Glu Leu Glu Lys Gln Leu Asn Lys Ala Thr Thr Asn Asn Ser Val Leu
225                 230                 235                 240

Gln Lys Gln Gln Leu Glu Leu Met Asp Thr Val His Thr Leu Ile Thr
                245                 250                 255

Leu Cys Ser Lys Glu Gly Val Leu Leu Lys Asn Ala Lys Arg Glu Glu
            260                 265                 270

Glu Lys Pro Phe Arg Asp Cys Ala Asp Val Tyr Gln Ala Gly Phe Asn
        275                 280                 285

Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn Val Ser Asp Pro Lys
    290                 295                 300

Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly Gly Trp Thr Val Ile
305                 310                 315                 320

Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln Lys Gly Trp Lys Glu
                325                 330                 335

Tyr Lys Met Gly Phe Gly Ser Pro Ser Gly Glu Tyr Trp Leu Gly Asn
            340                 345                 350

Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr Ser Leu Arg Ile
        355                 360                 365

Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser Gln Tyr Asp Arg
    370                 375                 380

Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu Tyr Leu Lys Gly
385                 390                 395                 400

His Ser Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile Leu His Gly Ala
                405                 410                 415

Glu Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys Met Cys Lys Cys
            420                 425                 430

Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala Cys Gly Pro Ser
        435                 440                 445

Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn His Gly Lys Leu
    450                 455                 460

Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Arg Tyr Ser Ile Arg
465                 470                 475                 480

Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe
                485                 490


<210> SEQ ID NO 14
<211> LENGTH: 497
<212> TYPE: PRT
<213> ORGANISM: Mus sp.

<400> SEQUENCE: 14

Met Thr Val Phe Leu Ser Phe Ala Phe Phe Ala Ala Ile Leu Thr His
1               5                   10                  15

Ile Gly Cys Ser Asn Gln Arg Arg Asn Pro Glu Asn Ser Gly Arg Arg
            20                  25                  30
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Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
        35                  40                  45

Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
    50                  55                  60

Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser
65                  70                  75                  80

Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp
                85                  90                  95

Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met
            100                 105                 110

Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu
        115                 120                 125

Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys
    130                 135                 140

Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu
145                 150                 155                 160

Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln
                165                 170                 175

Leu Leu Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser Leu
            180                 185                 190

Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu Met
        195                 200                 205

Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Ser Arg
    210                 215                 220

Gln Ser Phe Ile Ile Gln Glu Leu Glu Lys Gln Leu Ser Arg Ala Thr
225                 230                 235                 240

Asn Asn Asn Ser Ile Leu Gln Lys Gln Gln Leu Glu Leu Met Asp Thr
                245                 250                 255

Val His Asn Leu Ile Ser Leu Cys Thr Lys Glu Gly Val Leu Leu Lys
            260                 265                 270

Gly Gly Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val
        275                 280                 285

Tyr Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Phe Asn
    290                 295                 300

Asn Val Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly
305                 310                 315                 320

Gly Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe
                325                 330                 335

Gln Lys Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Ser Pro Ser Gly
            340                 345                 350

Glu Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg
        355                 360                 365

Gln Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala
    370                 375                 380

Tyr Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr
385                 390                 395                 400

Arg Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser
                405                 410                 415

Leu Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp
            420                 425                 430

Asn Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe
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        435                 440                 445

Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly
    450                 455                 460

Gln Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly
465                 470                 475                 480

Pro Arg Tyr Ser Ile Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp
                485                 490                 495

Phe


<210> SEQ ID NO 15
<211> LENGTH: 496
<212> TYPE: PRT
<213> ORGANISM: Mus sp.

<400> SEQUENCE: 15

Met Trp Gln Ile Ile Phe Leu Thr Phe Gly Trp Asp Ala Val Leu Thr
1               5                   10                  15

Ser Ala Tyr Ser Asn Phe Arg Lys Ser Val Asp Ser Thr Gly Arg Arg
            20                  25                  30

Arg Tyr Arg Ile Gln Asn Gly Pro Cys Ala Tyr Thr Phe Leu Leu Pro
        35                  40                  45

Glu Thr Asp Ser Gly Arg Ser Ser Ser Ser Thr Tyr Met Thr Asn Ala
    50                  55                  60

Val Gln Arg Asp Ala Pro Pro Asp Tyr Glu Asp Ser Val Gln Ser Leu
65                  70                  75                  80

Gln Leu Leu Glu Asn Val Met Glu Asn Tyr Thr Gln Trp Leu Met Lys
                85                  90                  95

Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Ala Glu Ile
            100                 105                 110

Gln Gln Asn Val Val Gln Asn His Thr Ala Val Met Ile Glu Ile Gly
        115                 120                 125

Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
    130                 135                 140

Val Glu Thr Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
145                 150                 155                 160

Leu Gln His Ser Ile Ser Thr Tyr Lys Leu Glu Lys Gln Ile Leu Asp
                165                 170                 175

Gln Thr Ser Glu Ile Asn Lys Ile His Asn Lys Asn Ser Phe Leu Glu
            180                 185                 190

Gln Lys Val Leu Asp Met Glu Gly Lys His Ser Glu Glu Met Gln Thr
        195                 200                 205

Met Lys Glu Gln Lys Asp Glu Leu Gln Val Leu Val Ser Lys Gln Ser
    210                 215                 220

Ser Val Ile Asp Glu Leu Glu Lys Lys Leu Val Thr Ala Thr Val Asn
225                 230                 235                 240

Asn Ser Leu Leu Gln Lys Gln Gln His Asp Leu Met Asp Thr Val Asn
                245                 250                 255

Ser Leu Leu Thr Met Met Ser Ser Pro Asn Ser Lys Ser Ser Leu Ala
            260                 265                 270

Ile Arg Arg Glu Glu Gln Thr Thr Phe Arg Asp Cys Ala Asp Val Phe
        275                 280                 285

Lys Ala Gly Leu Thr Lys Ser Gly Ile Tyr Thr Leu Thr Phe Pro Asn
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    290                 295                 300

Ser Pro Glu Glu Ile Lys Ala Tyr Cys Asn Met Asp Val Gly Gly Gly
305                 310                 315                 320

Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln
                325                 330                 335

Lys Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Leu Gly Glu
            340                 345                 350

Tyr Trp Leu Gly Asn Glu Phe Ile Ser Gln Ile Thr Gly Gln His Arg
        355                 360                 365

Tyr Val Leu Lys Ile Gln Leu Lys Asp Trp Glu Gly Asn Glu Ala His
    370                 375                 380

Ser Leu Tyr Asp His Phe Tyr Ile Ala Gly Glu Glu Ser Asn Tyr Arg
385                 390                 395                 400

Ile His Leu Thr Gly Leu Thr Gly Thr Ala Ala Lys Ile Ser Ser Ile
                405                 410                 415

Ser Gln Pro Gly Ser Asp Phe Ser Thr Lys Asp Ser Asp Asn Asp Lys
            420                 425                 430

Cys Ile Cys Lys Cys Ser Leu Met Leu Thr Gly Gly Trp Trp Phe Asp
        435                 440                 445

Ala Cys Gly Pro Ser Asn Leu Asn Gly Gln Phe Tyr Pro Gln Lys Gln
    450                 455                 460

Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser
465                 470                 475                 480

Gly Tyr Ser Ile Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe
                485                 490                 495


<210> SEQ ID NO 16
<211> LENGTH: 496
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Ala Val Leu Thr
1               5                   10                  15

Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
            20                  25                  30

Arg Tyr Arg Ile Gln His Gly Ser Cys Ala Tyr Thr Phe Leu Leu Pro
        35                  40                  45

Glu Met Asp Asn Gly Arg Ser Ser Ser Ser Thr Tyr Val Thr Asn Ala
    50                  55                  60

Val Gln Arg Asp Ala Pro Pro Glu Tyr Glu Asp Ser Val Gln Ser Leu
65                  70                  75                  80

Gln Leu Leu Glu Asn Val Met Glu Asn Tyr Thr Gln Trp Leu Met Lys
                85                  90                  95

Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Ala Glu Ile
            100                 105                 110

Gln Gln Asn Ala Val Gln Asn His Thr Ala Val Met Ile Glu Ile Gly
        115                 120                 125

Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
    130                 135                 140

Val Glu Thr Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
145                 150                 155                 160
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Leu Gln His Ser Ile Ser Thr Tyr Lys Leu Glu Lys Gln Ile Leu Asp
                165                 170                 175

Gln Thr Ser Glu Ile Asn Lys Ile His Asp Lys Asn Ser Phe Leu Glu
            180                 185                 190

Lys Lys Val Leu Asp Met Glu Asp Lys His Ile Ile Glu Met Gln Thr
        195                 200                 205

Ile Lys Glu Glu Lys Asp Glu Leu Gln Val Leu Val Ser Lys Gln Asn
    210                 215                 220

Ser Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala Thr Val Asn
225                 230                 235                 240

Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Asp Thr Val Asn
                245                 250                 255

Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Ser Thr
            260                 265                 270

Val Ala Arg Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Asp Val Phe
        275                 280                 285

Lys Ala Gly His Thr Lys Asn Gly Ile Tyr Thr Leu Thr Phe Pro Asn
    290                 295                 300

Ser Pro Glu Glu Ile Lys Ala Tyr Cys Asn Met Asp Ala Gly Gly Gly
305                 310                 315                 320

Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Leu Asp Phe Gln
                325                 330                 335

Lys Gly Trp Lys Glu Tyr Lys Val Gly Phe Gly Ser Pro Ser Gly Glu
            340                 345                 350

Tyr Trp Leu Gly Asn Glu Phe Ile Ser Gln Ile Thr Asn Gln Gln Arg
        355                 360                 365

Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu Ala Tyr
    370                 375                 380

Ser Leu Tyr Asp His Phe Tyr Ile Ser Gly Glu Glu Leu Asn Tyr Arg
385                 390                 395                 400

Ile His Leu Lys Gly Leu Thr Gly Thr Ala Ala Lys Ile Ser Ser Ile
                405                 410                 415

Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn Asp Lys
            420                 425                 430

Cys Ile Cys Lys Cys Ser Leu Met Leu Thr Gly Gly Trp Trp Phe Asp
        435                 440                 445

Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Pro Gln Arg Gln
    450                 455                 460

Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys Gly Ser
465                 470                 475                 480

Gly Tyr Ser Ile Lys Ala Thr Thr Met Met Ile Arg Pro Ala Asp Phe
                485                 490                 495


<210> SEQ ID NO 17
<211> LENGTH: 1512
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1512)
<223> OTHER INFORMATION:

<400> SEQUENCE: 17

atg ctc tcc cag cta gcc atg ctg cag ggc agc ctc ctc ctt gtg gtt       48
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```
Met Leu Ser Gln Leu Ala Met Leu Gln Gly Ser Leu Leu Leu Val Val
1               5                  10                  15

gcc acc atg tct gtg gct caa cag aca agg cag gag gcg gat agg ggc       96
Ala Thr Met Ser Val Ala Gln Gln Thr Arg Gln Glu Ala Asp Arg Gly
            20                  25                  30

tgc gag aca ctt gta gtc cag cac ggc cac tgt agc tac acc ttc ttg      144
Cys Glu Thr Leu Val Val Gln His Gly His Cys Ser Tyr Thr Phe Leu
        35                  40                  45

ctg ccc aag tct gag ccc tgc cct ccg ggg cct gag gtc tcc agg gac      192
Leu Pro Lys Ser Glu Pro Cys Pro Pro Gly Pro Glu Val Ser Arg Asp
    50                  55                  60

tcc aac acc ctc cag aga gaa tca ctg gcc aac cca ctg cac ctg ggg      240
Ser Asn Thr Leu Gln Arg Glu Ser Leu Ala Asn Pro Leu His Leu Gly
65                  70                  75                  80

aag ttg ccc acc cag cag gtg aaa cag ctg gag cag gca ctg cag aac      288
Lys Leu Pro Thr Gln Gln Val Lys Gln Leu Glu Gln Ala Leu Gln Asn
                85                  90                  95

aac acg cag tgg ctg aag aag cta gag agg gcc atc aag acg atc ttg      336
Asn Thr Gln Trp Leu Lys Lys Leu Glu Arg Ala Ile Lys Thr Ile Leu
            100                 105                 110

agg tcg aag ctg gag cag gtc cag cag caa atg gcc cag aat cag acg      384
Arg Ser Lys Leu Glu Gln Val Gln Gln Gln Met Ala Gln Asn Gln Thr
        115                 120                 125

gcc ccc atg cta gag ctg ggc acc agc ctc ctg aac cag acc act gcc      432
Ala Pro Met Leu Glu Leu Gly Thr Ser Leu Leu Asn Gln Thr Thr Ala
    130                 135                 140

cag atc cgc aag ctg acc gac atg gag gct cag ctc ctg aac cag aca      480
Gln Ile Arg Lys Leu Thr Asp Met Glu Ala Gln Leu Leu Asn Gln Thr
145                 150                 155                 160

tca aga atg gat gcc cag atg cca gag acc ttt ctg tcc acc aac aag      528
Ser Arg Met Asp Ala Gln Met Pro Glu Thr Phe Leu Ser Thr Asn Lys
                165                 170                 175

ctg gag aac cag ctg ctg cta cag agg cag aag ctc cag cag ctt cag      576
Leu Glu Asn Gln Leu Leu Leu Gln Arg Gln Lys Leu Gln Gln Leu Gln
            180                 185                 190

ggc caa aac agc gcg ctc gag aag cgg ttg cag gcc ctg gag acc aag      624
Gly Gln Asn Ser Ala Leu Glu Lys Arg Leu Gln Ala Leu Glu Thr Lys
        195                 200                 205

cag cag gag gag ctg gcc agc atc ctc agc aag aag gcg aag ctg ctg      672
Gln Gln Glu Glu Leu Ala Ser Ile Leu Ser Lys Lys Ala Lys Leu Leu
    210                 215                 220

aac acg ctg agc cgc cag agc gcc gcc ctc acc aac atc gag cgc ggc      720
Asn Thr Leu Ser Arg Gln Ser Ala Ala Leu Thr Asn Ile Glu Arg Gly
225                 230                 235                 240

ctg cgc ggt gtc agg cac aac tcc agc ctc ctg cag gac cag cag cac      768
Leu Arg Gly Val Arg His Asn Ser Ser Leu Leu Gln Asp Gln Gln His
                245                 250                 255

agc ctg cgc cag ctg ctg gtg ttg ttg cgg cac ctg gtg caa gaa agg      816
Ser Leu Arg Gln Leu Leu Val Leu Leu Arg His Leu Val Gln Glu Arg
            260                 265                 270

gct aac gcc tcg gcc ccg gcc ttc ata atg gca ggt gag cag gtg ttc      864
Ala Asn Ala Ser Ala Pro Ala Phe Ile Met Ala Gly Glu Gln Val Phe
        275                 280                 285

cag gac tgt gca gag atc cag cgc tct ggg gcc agt gcc agt ggt gtc      912
Gln Asp Cys Ala Glu Ile Gln Arg Ser Gly Ala Ser Ala Ser Gly Val
    290                 295                 300

tac acc atc cag gtg tcc aat gca acg aag ccc agg aag gtg ttc tgt      960
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Tyr Thr Ile Gln Val Ser Asn Ala Thr Lys Pro Arg Lys Val Phe Cys
305                 310                 315                 320

gac ctg cag agc agt gga ggc agg tgg acc ctc atc cag cgc cgt gag     1008
Asp Leu Gln Ser Ser Gly Gly Arg Trp Thr Leu Ile Gln Arg Arg Glu
                325                 330                 335

aat ggc acc gtg aat ttt cag cgg aac tgg aag gat tac aaa cag ggc     1056
Asn Gly Thr Val Asn Phe Gln Arg Asn Trp Lys Asp Tyr Lys Gln Gly
            340                 345                 350

ttc gga gac cca gct ggg gag cac tgg ctg ggc aat gaa gtg gtg cac     1104
Phe Gly Asp Pro Ala Gly Glu His Trp Leu Gly Asn Glu Val Val His
        355                 360                 365

cag ctc acc aga agg gca gcc tac tct ctg cgt gtg gag ctg caa gac     1152
Gln Leu Thr Arg Arg Ala Ala Tyr Ser Leu Arg Val Glu Leu Gln Asp
    370                 375                 380

tgg gaa ggc cac gag gcc tat gcc cag tac gaa cat ttc cac ctg ggc     1200
Trp Glu Gly His Glu Ala Tyr Ala Gln Tyr Glu His Phe His Leu Gly
385                 390                 395                 400

agt gag aac cag cta tac agg ctt tct gtg gtc ggg tac agc ggc tca     1248
Ser Glu Asn Gln Leu Tyr Arg Leu Ser Val Val Gly Tyr Ser Gly Ser
                405                 410                 415

gca ggg cgc cag agc agc ctg gtc ctg cag aac acc agc ttt agc acc     1296
Ala Gly Arg Gln Ser Ser Leu Val Leu Gln Asn Thr Ser Phe Ser Thr
            420                 425                 430

ctt gac tca gac aac gac cac tgt ctc tgc aag tgt gcc cag gtg atg     1344
Leu Asp Ser Asp Asn Asp His Cys Leu Cys Lys Cys Ala Gln Val Met
        435                 440                 445

tct gga ggg tgg tgg ttt gac gcc tgt ggc ctg tca aac ctc aac ggc     1392
Ser Gly Gly Trp Trp Phe Asp Ala Cys Gly Leu Ser Asn Leu Asn Gly
    450                 455                 460

gtc tac tac cac gct ccc gac aac aag tac aag atg gac ggc atc cgc     1440
Val Tyr Tyr His Ala Pro Asp Asn Lys Tyr Lys Met Asp Gly Ile Arg
465                 470                 475                 480

tgg cac tac ttc aag ggc ccc agc tac tca ctg cgt gcc tct cgc atg     1488
Trp His Tyr Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ala Ser Arg Met
                485                 490                 495

atg ata cgg cct ttg gac atc taa                                    1512
Met Ile Arg Pro Leu Asp Ile
            500
```

<210> SEQ ID NO 18
<211> LENGTH: 503
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

```
Met Leu Ser Gln Leu Ala Met Leu Gln Gly Ser Leu Leu Leu Val Val
1               5                   10                  15

Ala Thr Met Ser Val Ala Gln Gln Thr Arg Gln Glu Ala Asp Arg Gly
            20                  25                  30

Cys Glu Thr Leu Val Val Gln His Gly His Cys Ser Tyr Thr Phe Leu
        35                  40                  45

Leu Pro Lys Ser Glu Pro Cys Pro Pro Gly Pro Glu Val Ser Arg Asp
    50                  55                  60

Ser Asn Thr Leu Gln Arg Glu Ser Leu Ala Asn Pro Leu His Leu Gly
65                  70                  75                  80

Lys Leu Pro Thr Gln Gln Val Lys Gln Leu Glu Gln Ala Leu Gln Asn
                85                  90                  95
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Asn Thr Gln Trp Leu Lys Lys Leu Glu Arg Ala Ile Lys Thr Ile Leu
            100                 105                 110

Arg Ser Lys Leu Glu Gln Val Gln Gln Gln Met Ala Gln Asn Gln Thr
        115                 120                 125

Ala Pro Met Leu Glu Leu Gly Thr Ser Leu Leu Asn Gln Thr Thr Ala
    130                 135                 140

Gln Ile Arg Lys Leu Thr Asp Met Glu Ala Gln Leu Leu Asn Gln Thr
145                 150                 155                 160

Ser Arg Met Asp Ala Gln Met Pro Glu Thr Phe Leu Ser Thr Asn Lys
                165                 170                 175

Leu Glu Asn Gln Leu Leu Leu Gln Arg Gln Lys Leu Gln Gln Leu Gln
            180                 185                 190

Gly Gln Asn Ser Ala Leu Glu Lys Arg Leu Gln Ala Leu Glu Thr Lys
        195                 200                 205

Gln Gln Glu Glu Leu Ala Ser Ile Leu Ser Lys Lys Ala Lys Leu Leu
    210                 215                 220

Asn Thr Leu Ser Arg Gln Ser Ala Ala Leu Thr Asn Ile Glu Arg Gly
225                 230                 235                 240

Leu Arg Gly Val Arg His Asn Ser Ser Leu Leu Gln Asp Gln Gln His
                245                 250                 255

Ser Leu Arg Gln Leu Leu Val Leu Leu Arg His Leu Val Gln Glu Arg
            260                 265                 270

Ala Asn Ala Ser Ala Pro Ala Phe Ile Met Ala Gly Glu Gln Val Phe
        275                 280                 285

Gln Asp Cys Ala Glu Ile Gln Arg Ser Gly Ala Ser Ala Ser Gly Val
    290                 295                 300

Tyr Thr Ile Gln Val Ser Asn Ala Thr Lys Pro Arg Lys Val Phe Cys
305                 310                 315                 320

Asp Leu Gln Ser Ser Gly Gly Arg Trp Thr Leu Ile Gln Arg Arg Glu
                325                 330                 335

Asn Gly Thr Val Asn Phe Gln Arg Asn Trp Lys Asp Tyr Lys Gln Gly
            340                 345                 350

Phe Gly Asp Pro Ala Gly Glu His Trp Leu Gly Asn Glu Val Val His
        355                 360                 365

Gln Leu Thr Arg Arg Ala Ala Tyr Ser Leu Arg Val Glu Leu Gln Asp
    370                 375                 380

Trp Glu Gly His Glu Ala Tyr Ala Gln Tyr Glu His Phe His Leu Gly
385                 390                 395                 400

Ser Glu Asn Gln Leu Tyr Arg Leu Ser Val Val Gly Tyr Ser Gly Ser
                405                 410                 415

Ala Gly Arg Gln Ser Ser Leu Val Leu Gln Asn Thr Ser Phe Ser Thr
            420                 425                 430

Leu Asp Ser Asp Asn Asp His Cys Leu Cys Lys Cys Ala Gln Val Met
        435                 440                 445

Ser Gly Gly Trp Trp Phe Asp Ala Cys Gly Leu Ser Asn Leu Asn Gly
    450                 455                 460

Val Tyr Tyr His Ala Pro Asp Asn Lys Tyr Lys Met Asp Gly Ile Arg
465                 470                 475                 480

Trp His Tyr Phe Lys Gly Pro Ser Tyr Ser Leu Arg Ala Ser Arg Met
                485                 490                 495
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Met Ile Arg Pro Leu Asp Ile
            500


<210> SEQ ID NO 19
<211> LENGTH: 1497
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Chimeric
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1494)
<223> OTHER INFORMATION: 1N1C2F (chimera 1)
<220> FEATURE:
<223> OTHER INFORMATION: Putative leader sequence is encoded by
      nucleotides 1-60

<400> SEQUENCE: 19

atg aca gtt ttc ctt tcc ttt gct ttc ctc gct gcc att ctg act cac       48
Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
1               5                   10                  15

ata ggg tgc agc aat cag cgc cga agt cca gaa aac agt ggg aga aga       96
Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
            20                  25                  30

tat aac cgg att caa cat ggg caa tgt gcc tac act ttc att ctt cca      144
Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
        35                  40                  45

gaa cac gat ggc aac tgt cgt gag agt acg aca gac cag tac aac aca      192
Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
    50                  55                  60

aac gct ctg cag aga gat gct cca cac gtg gaa ccg gat ttc tct tcc      240
Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser
65                  70                  75                  80

cag aaa ctt caa cat ctg gaa cat gtg atg gaa aat tat act cag tgg      288
Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp
                85                  90                  95

ctg caa aaa ctt gag aat tac att gtg gaa aac atg aag tcg gag atg      336
Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met
            100                 105                 110

gcc cag ata cag cag aat gca gtt cag aac cac acg gct acc atg ctg      384
Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu
        115                 120                 125

gag ata gga acc agc ctc ctc tct cag act gca gag cag acc aga aag      432
Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys
    130                 135                 140

ctg aca gat gtt gag acc cag gta cta aat caa act tct cga ctt gag      480
Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu
145                 150                 155                 160

ata cag ctg ctg gag aat tca tta tcc acc tac aag cta gag aag caa      528
Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln
                165                 170                 175

ctt ctt caa cag aca aat gaa atc ttg aag atc cat gaa aaa aac agt      576
Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser
            180                 185                 190

tta tta gaa cat aaa atc tta gaa atg gaa gga aaa cac aag gaa gag      624
Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu
        195                 200                 205

ttg gac acc tta aag gaa gag aaa gag aac ctt caa ggc ttg gtt act      672
Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr
    210                 215                 220

cgt caa aca tat ata atc cag gag ctg gaa aag caa tta aac aga gct      720
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Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala
225                 230                 235                 240

acc acc aac aac agt gtc ctt cag aag cag caa ctg gag ctg atg gac      768
Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp
                245                 250                 255

aca gtc cac aac ctt gtc aat ctt tgc act aaa gaa ggt gtt tta cta      816
Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu
            260                 265                 270

aag gga gga aaa aga gag gaa gag aaa cca ttt aga gac tgt gct gaa      864
Lys Gly Gly Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Glu
        275                 280                 285

gta ttc aaa tca gga cac acc aca aat ggc atc tac acg tta aca ttc      912
Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe
    290                 295                 300

cct aat tct aca gaa gag atc aag gcc tac tgt gac atg gaa gct gga      960
Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly
305                 310                 315                 320

gga ggc ggg tgg aca att att cag cga cgt gag gat ggc agc gtt gat     1008
Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp
                325                 330                 335

ttt cag agg act tgg aaa gaa tat aaa gtg gga ttt ggt aac cct tca     1056
Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser
            340                 345                 350

gga gaa tat tgg ctg gga aat gag ttt gtt tcg caa ctg act aat cag     1104
Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln
        355                 360                 365

caa cgc tat gtg ctt aaa ata cac ctt aaa gac tgg gaa ggg aat gag     1152
Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu
    370                 375                 380

gct tac tca ttg tat gaa cat ttc tat ctc tca agt gaa gaa ctc aat     1200
Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn
385                 390                 395                 400

tat agg att cac ctt aaa gga ctt aca ggg aca gcc ggc aaa ata agc     1248
Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser
                405                 410                 415

agc atc agc caa cca gga aat gat ttt agc aca aag gat gga gac aac     1296
Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn
            420                 425                 430

gac aaa tgt att tgc aaa tgt tca caa atg cta aca gga ggc tgg tgg     1344
Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp
        435                 440                 445

ttt gat gca tgt ggt cct tcc aac ttg aac gga atg tac tat cca cag     1392
Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln
    450                 455                 460

agg cag aac aca aat aag ttc aac ggc att aaa tgg tac tac tgg aaa     1440
Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys
465                 470                 475                 480

ggc tca ggc tat tcg ctc aag gcc aca acc atg atg atc cga cca gca     1488
Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala
                485                 490                 495

gat ttc taa                                                        1497
Asp Phe
```

<210> SEQ ID NO 20
<211> LENGTH: 498
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Chimeric
<220> FEATURE:
<223> OTHER INFORMATION: 1N1C2F (chimera 1)

<400> SEQUENCE: 20

Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
1 5 10 15

Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
20 25 30

Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
35 40 45

Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
50 55 60

Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Phe Ser Ser
65 70 75 80

Gln Lys Leu Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp
85 90 95

Leu Gln Lys Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met
100 105 110

Ala Gln Ile Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu
115 120 125

Glu Ile Gly Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys
130 135 140

Leu Thr Asp Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu
145 150 155 160

Ile Gln Leu Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln
165 170 175

Leu Leu Gln Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser
180 185 190

Leu Leu Glu His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu
195 200 205

Leu Asp Thr Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr
210 215 220

Arg Gln Thr Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala
225 230 235 240

Thr Thr Asn Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp
245 250 255

Thr Val His Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu
260 265 270

Lys Gly Gly Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Glu
275 280 285

Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr Phe
290 295 300

Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala Gly
305 310 315 320

Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val Asp
325 330 335

Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro Ser
340 345 350

Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn Gln
355 360 365

Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn Glu

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    370                 375                 380

Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu Asn
385                 390                 395                 400

Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile Ser
                405                 410                 415

Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp Asn
            420                 425                 430

Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp Trp
        435                 440                 445

Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro Gln
    450                 455                 460

Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp Lys
465                 470                 475                 480

Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro Ala
                485                 490                 495

Asp Phe


<210> SEQ ID NO 21
<211> LENGTH: 1491
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Chimeric
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1491)
<223> OTHER INFORMATION: 2N2C1F (chimera 2)
<220> FEATURE:
<223> OTHER INFORMATION: Putative leader sequence is encoded by
      nucleotides 1-48

<400> SEQUENCE: 21

atg tgg cag att gtt ttc ttt act ctg agc tgt gat ctt gtc ttg gcc       48
Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala
1               5                   10                  15

gca gcc tat aac aac ttt cgg aag agc atg gac agc ata gga aag aag       96
Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
            20                  25                  30

caa tat cag gtc cag cat ggg tcc tgc agc tac act ttc ctc ctg cca      144
Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro
        35                  40                  45

gag atg gac aac tgc cgc tct tcc tcc agc ccc tac gtg tcc aat gct      192
Glu Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala
    50                  55                  60

gtg cag agg gac gcg ccg ctc gaa tac gat gac tcg gtg cag agg ctg      240
Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Asp Ser Val Gln Arg Leu
65                  70                  75                  80

caa gtg ctg gag aac atc atg gaa aac aac act cag tgg cta atg aag      288
Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys
                85                  90                  95

ctt gag aat tat atc cag gac aac atg aag aaa gaa atg gta gag ata      336
Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile
            100                 105                 110

cag cag aat gca gta cag aac cag acg gct gtg atg ata gaa ata ggg      384
Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly
        115                 120                 125

aca aac ctg ttg aac caa aca gct gag caa acg cgg aag tta act gat      432
Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
    130                 135                 140
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gtg gaa gcc caa gta tta aat cag acc acg aga ctt gaa ctt cag ctc      480
Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
145                 150                 155                 160

ttg gaa cac tcc ctc tcg aca aac aaa ttg gaa aaa cag att ttg gac      528
Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp
                165                 170                 175

cag acc agt gaa ata aac aaa ttg caa gat aag aac agt ttc cta gaa      576
Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu
            180                 185                 190

aag aag gtg cta gct atg gaa gac aag cac atc atc caa cta cag tca      624
Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser
        195                 200                 205

ata aaa gaa gag aaa gat cag cta cag gtg tta gta tcc aag caa aat      672
Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn
    210                 215                 220

tcc atc att gaa gaa cta gaa aaa aaa ata gtg act gcc acg gtg aat      720
Ser Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala Thr Val Asn
225                 230                 235                 240

aat tca gtt ctt caa aag cag caa cat gat ctc atg gag aca gtt aat      768
Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu Thr Val Asn
                245                 250                 255

aac tta ctg act atg atg tcc aca tca aac tca gct aag gac ccc act      816
Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr
            260                 265                 270

gtt gct aaa gaa gaa caa atc agc ttc aga gac tgt gca gat gta tat      864
Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Asp Val Tyr
        275                 280                 285

caa gct ggt ttt aat aaa agt gga atc tac act att tat att aat aat      912
Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn
    290                 295                 300

atg cca gaa ccc aaa aag gtg ttt tgc aat atg gat gtc aat ggg gga      960
Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly
305                 310                 315                 320

ggt tgg act gta ata caa cat cgt gaa gat gga agt cta gat ttc caa     1008
Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln
                325                 330                 335

aga ggc tgg aag gaa tat aaa atg ggt ttt gga aat ccc tcc ggt gaa     1056
Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu
            340                 345                 350

tat tgg ctg ggg aat gag ttt att ttt gcc att acc agt cag agg cag     1104
Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln
        355                 360                 365

tac atg cta aga att gag tta atg gac tgg gaa ggg aac cga gcc tat     1152
Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr
    370                 375                 380

tca cag tat gac aga ttc cac ata gga aat gaa aag caa aac tat agg     1200
Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg
385                 390                 395                 400

ttg tat tta aaa ggt cac act ggg aca gca gga aaa cag agc agc ctg     1248
Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu
                405                 410                 415

atc tta cac ggt gct gat ttc agc act aaa gat gct gat aat gac aac     1296
Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn
            420                 425                 430

tgt atg tgc aaa tgt gcc ctc atg tta aca gga gga tgg tgg ttt gat     1344
Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp
        435                 440                 445
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gct tgt ggc ccc tcc aat cta aat gga atg ttc tat act gcg gga caa     1392
Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln
    450                 455                 460

aac cat gga aaa ctg aat ggg ata aag tgg cac tac ttc aaa ggg ccc     1440
Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro
465                 470                 475                 480

agt tac tcc tta cgt tcc aca act atg atg att cga cct tta gat ttt     1488
Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe
                485                 490                 495

tga                                                                1491


<210> SEQ ID NO 22
<211> LENGTH: 496
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Chimeric
<220> FEATURE:
<223> OTHER INFORMATION: 2N2C1F (chimera 2)

<400> SEQUENCE: 22

Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala
1               5                   10                  15

Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
            20                  25                  30

Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro
        35                  40                  45

Glu Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala
    50                  55                  60

Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Asp Ser Val Gln Arg Leu
65                  70                  75                  80

Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp Leu Met Lys
                85                  90                  95

Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met Val Glu Ile
            100                 105                 110

Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile Glu Ile Gly
        115                 120                 125

Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
    130                 135                 140

Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu Leu Gln Leu
145                 150                 155                 160

Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln Ile Leu Asp
                165                 170                 175

Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser Phe Leu Glu
            180                 185                 190

Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln Leu Gln Ser
        195                 200                 205

Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser Lys Gln Asn
    210                 215                 220

Ser Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala Thr Val Asn
225                 230                 235                 240

Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu Thr Val Asn
                245                 250                 255

Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys Asp Pro Thr
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            260                 265                 270

Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala Asp Val Tyr
        275                 280                 285

Gln Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn
    290                 295                 300

Met Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly
305                 310                 315                 320

Gly Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln
                325                 330                 335

Arg Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu
            340                 345                 350

Tyr Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln
        355                 360                 365

Tyr Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr
    370                 375                 380

Ser Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg
385                 390                 395                 400

Leu Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu
                405                 410                 415

Ile Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn
            420                 425                 430

Cys Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp
        435                 440                 445

Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln
    450                 455                 460

Asn His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro
465                 470                 475                 480

Ser Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe
                485                 490                 495


<210> SEQ ID NO 23
<211> LENGTH: 1500
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Chimeric
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1497)
<223> OTHER INFORMATION: 1N2C2F (chimera 3)
<220> FEATURE:
<223> OTHER INFORMATION: Putative leader sequence is encoded by
      nucleotides 1-60

<400> SEQUENCE: 23

atg aca gtt ttc ctt tcc ttt gct ttc ctc gct gcc att ctg act cac       48
Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
1               5                   10                  15

ata ggg tgc agc aat cag cgc cga agt cca gaa aac agt ggg aga aga       96
Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
            20                  25                  30

tat aac cgg att caa cat ggg caa tgt gcc tac act ttc att ctt cca      144
Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
        35                  40                  45

gaa cac gat ggc aac tgt cgt gag agt acg aca gac cag tac aac aca      192
Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
    50                  55                  60
```

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aac gct ctg cag aga gat gct cca cac gtg gaa ccg gat gac tcg gtg      240
Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Asp Ser Val
65                  70                  75                  80

cag agg ctg caa gtg ctg gag aac atc atg gaa aac aac act cag tgg      288
Gln Arg Leu Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp
                85                  90                  95

cta atg aag ctt gag aat tat atc cag gac aac atg aag aaa gaa atg      336
Leu Met Lys Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met
            100                 105                 110

gta gag ata cag cag aat gca gta cag aac cag acg gct gtg atg ata      384
Val Glu Ile Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile
        115                 120                 125

gaa ata ggg aca aac ctg ttg aac caa aca gct gag caa acg cgg aag      432
Glu Ile Gly Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys
    130                 135                 140

tta act gat gtg gaa gcc caa gta tta aat cag acc acg aga ctt gaa      480
Leu Thr Asp Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu
145                 150                 155                 160

ctt cag ctc ttg gaa cac tcc ctc tcg aca aac aaa ttg gaa aaa cag      528
Leu Gln Leu Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln
                165                 170                 175

att ttg gac cag acc agt gaa ata aac aaa ttg caa gat aag aac agt      576
Ile Leu Asp Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser
            180                 185                 190

ttc cta gaa aag aag gtg cta gct atg gaa gac aag cac atc atc caa      624
Phe Leu Glu Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln
        195                 200                 205

cta cag tca ata aaa gaa gag aaa gat cag cta cag gtg tta gta tcc      672
Leu Gln Ser Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser
    210                 215                 220

aag caa aat tcc atc att gaa gaa cta gaa aaa aaa ata gtg act gcc      720
Lys Gln Asn Ser Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala
225                 230                 235                 240

acg gtg aat aat tca gtt ctt caa aag cag caa cat gat ctc atg gag      768
Thr Val Asn Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu
                245                 250                 255

aca gtt aat aac tta ctg act atg atg tcc aca tca aac tca gct aag      816
Thr Val Asn Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys
            260                 265                 270

gac ccc act gtt gct aaa gaa gaa caa atc agc ttc aga gac tgt gct      864
Asp Pro Thr Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala
        275                 280                 285

gaa gta ttc aaa tca gga cac acc aca aat ggc atc tac acg tta aca      912
Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr
    290                 295                 300

ttc cct aat tct aca gaa gag atc aag gcc tac tgt gac atg gaa gct      960
Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala
305                 310                 315                 320

gga gga ggc ggg tgg aca att att cag cga cgt gag gat ggc agc gtt     1008
Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val
                325                 330                 335

gat ttt cag agg act tgg aaa gaa tat aaa gtg gga ttt ggt aac cct     1056
Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro
            340                 345                 350

tca gga gaa tat tgg ctg gga aat gag ttt gtt tcg caa ctg act aat     1104
Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn
        355                 360                 365
```

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```
cag caa cgc tat gtg ctt aaa ata cac ctt aaa gac tgg gaa ggg aat     1152
Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn
    370                 375                 380

gag gct tac tca ttg tat gaa cat ttc tat ctc tca agt gaa gaa ctc     1200
Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu
385                 390                 395                 400

aat tat agg att cac ctt aaa gga ctt aca ggg aca gcc ggc aaa ata     1248
Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile
                405                 410                 415

agc agc atc agc caa cca gga aat gat ttt agc aca aag gat gga gac     1296
Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp
            420                 425                 430

aac gac aaa tgt att tgc aaa tgt tca caa atg cta aca gga ggc tgg     1344
Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp
        435                 440                 445

tgg ttt gat gca tgt ggt cct tcc aac ttg aac gga atg tac tat cca     1392
Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro
    450                 455                 460

cag agg cag aac aca aat aag ttc aac ggc att aaa tgg tac tac tgg     1440
Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp
465                 470                 475                 480

aaa ggc tca ggc tat tcg ctc aag gcc aca acc atg atg atc cga cca     1488
Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro
                485                 490                 495

gca gat ttc taa                                                    1500
Ala Asp Phe
```

<210> SEQ ID NO 24
<211> LENGTH: 499
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Chimeric
<220> FEATURE:
<223> OTHER INFORMATION: 1N2C2F (chimera 3)

<400> SEQUENCE: 24

```
Met Thr Val Phe Leu Ser Phe Ala Phe Leu Ala Ala Ile Leu Thr His
1               5                   10                  15

Ile Gly Cys Ser Asn Gln Arg Arg Ser Pro Glu Asn Ser Gly Arg Arg
            20                  25                  30

Tyr Asn Arg Ile Gln His Gly Gln Cys Ala Tyr Thr Phe Ile Leu Pro
        35                  40                  45

Glu His Asp Gly Asn Cys Arg Glu Ser Thr Thr Asp Gln Tyr Asn Thr
    50                  55                  60

Asn Ala Leu Gln Arg Asp Ala Pro His Val Glu Pro Asp Asp Ser Val
65                  70                  75                  80

Gln Arg Leu Gln Val Leu Glu Asn Ile Met Glu Asn Asn Thr Gln Trp
                85                  90                  95

Leu Met Lys Leu Glu Asn Tyr Ile Gln Asp Asn Met Lys Lys Glu Met
            100                 105                 110

Val Glu Ile Gln Gln Asn Ala Val Gln Asn Gln Thr Ala Val Met Ile
        115                 120                 125

Glu Ile Gly Thr Asn Leu Leu Asn Gln Thr Ala Glu Gln Thr Arg Lys
    130                 135                 140

Leu Thr Asp Val Glu Ala Gln Val Leu Asn Gln Thr Thr Arg Leu Glu
145                 150                 155                 160
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Leu Gln Leu Leu Glu His Ser Leu Ser Thr Asn Lys Leu Glu Lys Gln
                165                 170                 175

Ile Leu Asp Gln Thr Ser Glu Ile Asn Lys Leu Gln Asp Lys Asn Ser
            180                 185                 190

Phe Leu Glu Lys Lys Val Leu Ala Met Glu Asp Lys His Ile Ile Gln
        195                 200                 205

Leu Gln Ser Ile Lys Glu Glu Lys Asp Gln Leu Gln Val Leu Val Ser
    210                 215                 220

Lys Gln Asn Ser Ile Ile Glu Glu Leu Glu Lys Lys Ile Val Thr Ala
225                 230                 235                 240

Thr Val Asn Asn Ser Val Leu Gln Lys Gln Gln His Asp Leu Met Glu
                245                 250                 255

Thr Val Asn Asn Leu Leu Thr Met Met Ser Thr Ser Asn Ser Ala Lys
            260                 265                 270

Asp Pro Thr Val Ala Lys Glu Glu Gln Ile Ser Phe Arg Asp Cys Ala
        275                 280                 285

Glu Val Phe Lys Ser Gly His Thr Thr Asn Gly Ile Tyr Thr Leu Thr
    290                 295                 300

Phe Pro Asn Ser Thr Glu Glu Ile Lys Ala Tyr Cys Asp Met Glu Ala
305                 310                 315                 320

Gly Gly Gly Gly Trp Thr Ile Ile Gln Arg Arg Glu Asp Gly Ser Val
                325                 330                 335

Asp Phe Gln Arg Thr Trp Lys Glu Tyr Lys Val Gly Phe Gly Asn Pro
            340                 345                 350

Ser Gly Glu Tyr Trp Leu Gly Asn Glu Phe Val Ser Gln Leu Thr Asn
        355                 360                 365

Gln Gln Arg Tyr Val Leu Lys Ile His Leu Lys Asp Trp Glu Gly Asn
    370                 375                 380

Glu Ala Tyr Ser Leu Tyr Glu His Phe Tyr Leu Ser Ser Glu Glu Leu
385                 390                 395                 400

Asn Tyr Arg Ile His Leu Lys Gly Leu Thr Gly Thr Ala Gly Lys Ile
                405                 410                 415

Ser Ser Ile Ser Gln Pro Gly Asn Asp Phe Ser Thr Lys Asp Gly Asp
            420                 425                 430

Asn Asp Lys Cys Ile Cys Lys Cys Ser Gln Met Leu Thr Gly Gly Trp
        435                 440                 445

Trp Phe Asp Ala Cys Gly Pro Ser Asn Leu Asn Gly Met Tyr Tyr Pro
    450                 455                 460

Gln Arg Gln Asn Thr Asn Lys Phe Asn Gly Ile Lys Trp Tyr Tyr Trp
465                 470                 475                 480

Lys Gly Ser Gly Tyr Ser Leu Lys Ala Thr Thr Met Met Ile Arg Pro
                485                 490                 495

Ala Asp Phe
```

<210> SEQ ID NO 25
<211> LENGTH: 1488
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Chimeric
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1485)
<223> OTHER INFORMATION: 2N1C1F (chimera 4)

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<220> FEATURE:
<223> OTHER INFORMATION: Putative leader sequence is encoded by nucleotides 1-48

<400> SEQUENCE: 25

```
atg tgg cag att gtt ttc ttt act ctg agc tgt gat ctt gtc ttg gcc       48
Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala
1               5                   10                  15

gca gcc tat aac aac ttt cgg aag agc atg gac agc ata gga aag aag       96
Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
            20                  25                  30

caa tat cag gtc cag cat ggg tcc tgc agc tac act ttc ctc ctg cca      144
Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro
        35                  40                  45

gag atg gac aac tgc cgc tct tcc tcc agc ccc tac gtg tcc aat gct      192
Glu Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala
    50                  55                  60

gtg cag agg gac gcg ccg ctc gaa tac gat ttc tct tcc cag aaa ctt      240
Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Phe Ser Ser Gln Lys Leu
65                  70                  75                  80

caa cat ctg gaa cat gtg atg gaa aat tat act cag tgg ctg caa aaa      288
Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp Leu Gln Lys
                85                  90                  95

ctt gag aat tac att gtg gaa aac atg aag tcg gag atg gcc cag ata      336
Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met Ala Gln Ile
            100                 105                 110

cag cag aat gca gtt cag aac cac acg gct acc atg ctg gag ata gga      384
Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu Glu Ile Gly
        115                 120                 125

acc agc ctc ctc tct cag act gca gag cag acc aga aag ctg aca gat      432
Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
    130                 135                 140

gtt gag acc cag gta cta aat caa act tct cga ctt gag ata cag ctg      480
Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu Ile Gln Leu
145                 150                 155                 160

ctg gag aat tca tta tcc acc tac aag cta gag aag caa ctt ctt caa      528
Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln Leu Leu Gln
                165                 170                 175

cag aca aat gaa atc ttg aag atc cat gaa aaa aac agt tta tta gaa      576
Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser Leu Leu Glu
            180                 185                 190

cat aaa atc tta gaa atg gaa gga aaa cac aag gaa gag ttg gac acc      624
His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu Leu Asp Thr
        195                 200                 205

tta aag gaa gag aaa gag aac ctt caa ggc ttg gtt act cgt caa aca      672
Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr Arg Gln Thr
    210                 215                 220

tat ata atc cag gag ctg gaa aag caa tta aac aga gct acc acc aac      720
Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala Thr Thr Asn
225                 230                 235                 240

aac agt gtc ctt cag aag cag caa ctg gag ctg atg gac aca gtc cac      768
Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp Thr Val His
                245                 250                 255

aac ctt gtc aat ctt tgc act aaa gaa ggt gtt tta cta aag gga gga      816
Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu Lys Gly Gly
            260                 265                 270

aaa aga gag gaa gag aaa cca ttt aga gac tgt gca gat gta tat caa      864
Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val Tyr Gln
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        275                 280                 285

gct ggt ttt aat aaa agt gga atc tac act att tat att aat aat atg      912
Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn Met
    290                 295                 300

cca gaa ccc aaa aag gtg ttt tgc aat atg gat gtc aat ggg gga ggt      960
Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly Gly
305                 310                 315                 320

tgg act gta ata caa cat cgt gaa gat gga agt cta gat ttc caa aga     1008
Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg
                325                 330                 335

ggc tgg aag gaa tat aaa atg ggt ttt gga aat ccc tcc ggt gaa tat     1056
Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr
            340                 345                 350

tgg ctg ggg aat gag ttt att ttt gcc att acc agt cag agg cag tac     1104
Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr
        355                 360                 365

atg cta aga att gag tta atg gac tgg gaa ggg aac cga gcc tat tca     1152
Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser
    370                 375                 380

cag tat gac aga ttc cac ata gga aat gaa aag caa aac tat agg ttg     1200
Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu
385                 390                 395                 400

tat tta aaa ggt cac act ggg aca gca gga aaa cag agc agc ctg atc     1248
Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile
                405                 410                 415

tta cac ggt gct gat ttc agc act aaa gat gct gat aat gac aac tgt     1296
Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys
            420                 425                 430

atg tgc aaa tgt gcc ctc atg tta aca gga gga tgg tgg ttt gat gct     1344
Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala
        435                 440                 445

tgt ggc ccc tcc aat cta aat gga atg ttc tat act gcg gga caa aac     1392
Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn
    450                 455                 460

cat gga aaa ctg aat ggg ata aag tgg cac tac ttc aaa ggg ccc agt     1440
His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser
465                 470                 475                 480

tac tcc tta cgt tcc aca act atg atg att cga cct tta gat ttt tga     1488
Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe
                485                 490                 495


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Met Trp Gln Ile Val Phe Phe Thr Leu Ser Cys Asp Leu Val Leu Ala
1               5                   10                  15

Ala Ala Tyr Asn Asn Phe Arg Lys Ser Met Asp Ser Ile Gly Lys Lys
            20                  25                  30

Gln Tyr Gln Val Gln His Gly Ser Cys Ser Tyr Thr Phe Leu Leu Pro
        35                  40                  45

Glu Met Asp Asn Cys Arg Ser Ser Ser Ser Pro Tyr Val Ser Asn Ala
```

-continued

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    50                  55                  60

Val Gln Arg Asp Ala Pro Leu Glu Tyr Asp Phe Ser Ser Gln Lys Leu
65                  70                  75                  80

Gln His Leu Glu His Val Met Glu Asn Tyr Thr Gln Trp Leu Gln Lys
                85                  90                  95

Leu Glu Asn Tyr Ile Val Glu Asn Met Lys Ser Glu Met Ala Gln Ile
            100                 105                 110

Gln Gln Asn Ala Val Gln Asn His Thr Ala Thr Met Leu Glu Ile Gly
        115                 120                 125

Thr Ser Leu Leu Ser Gln Thr Ala Glu Gln Thr Arg Lys Leu Thr Asp
    130                 135                 140

Val Glu Thr Gln Val Leu Asn Gln Thr Ser Arg Leu Glu Ile Gln Leu
145                 150                 155                 160

Leu Glu Asn Ser Leu Ser Thr Tyr Lys Leu Glu Lys Gln Leu Leu Gln
                165                 170                 175

Gln Thr Asn Glu Ile Leu Lys Ile His Glu Lys Asn Ser Leu Leu Glu
            180                 185                 190

His Lys Ile Leu Glu Met Glu Gly Lys His Lys Glu Glu Leu Asp Thr
        195                 200                 205

Leu Lys Glu Glu Lys Glu Asn Leu Gln Gly Leu Val Thr Arg Gln Thr
    210                 215                 220

Tyr Ile Ile Gln Glu Leu Glu Lys Gln Leu Asn Arg Ala Thr Thr Asn
225                 230                 235                 240

Asn Ser Val Leu Gln Lys Gln Gln Leu Glu Leu Met Asp Thr Val His
                245                 250                 255

Asn Leu Val Asn Leu Cys Thr Lys Glu Gly Val Leu Leu Lys Gly Gly
            260                 265                 270

Lys Arg Glu Glu Glu Lys Pro Phe Arg Asp Cys Ala Asp Val Tyr Gln
        275                 280                 285

Ala Gly Phe Asn Lys Ser Gly Ile Tyr Thr Ile Tyr Ile Asn Asn Met
    290                 295                 300

Pro Glu Pro Lys Lys Val Phe Cys Asn Met Asp Val Asn Gly Gly Gly
305                 310                 315                 320

Trp Thr Val Ile Gln His Arg Glu Asp Gly Ser Leu Asp Phe Gln Arg
                325                 330                 335

Gly Trp Lys Glu Tyr Lys Met Gly Phe Gly Asn Pro Ser Gly Glu Tyr
            340                 345                 350

Trp Leu Gly Asn Glu Phe Ile Phe Ala Ile Thr Ser Gln Arg Gln Tyr
        355                 360                 365

Met Leu Arg Ile Glu Leu Met Asp Trp Glu Gly Asn Arg Ala Tyr Ser
    370                 375                 380

Gln Tyr Asp Arg Phe His Ile Gly Asn Glu Lys Gln Asn Tyr Arg Leu
385                 390                 395                 400

Tyr Leu Lys Gly His Thr Gly Thr Ala Gly Lys Gln Ser Ser Leu Ile
                405                 410                 415

Leu His Gly Ala Asp Phe Ser Thr Lys Asp Ala Asp Asn Asp Asn Cys
            420                 425                 430

Met Cys Lys Cys Ala Leu Met Leu Thr Gly Gly Trp Trp Phe Asp Ala
        435                 440                 445

Cys Gly Pro Ser Asn Leu Asn Gly Met Phe Tyr Thr Ala Gly Gln Asn
    450                 455                 460
```

-continued

```
His Gly Lys Leu Asn Gly Ile Lys Trp His Tyr Phe Lys Gly Pro Ser
465                 470                 475                 480

Tyr Ser Leu Arg Ser Thr Thr Met Met Ile Arg Pro Leu Asp Phe
                485                 490                 495


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cctctgggctcgccagtttgttagg                                            25


<210> SEQ ID NO 30
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ccagctggcagatatcagg                                                  19
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What is claimed is:

1. An isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds and activates TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 2.

2. The nucleic acid molecule of claim 1, wherein the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 2.

3. The nucleic acid molecule of claim 1, which is modified to encode a different amino acid instead of the cysteine residue encoded by nucleotides 784-787 as set forth in **FIG. 27**.

4. The nucleic acid molecule of claim 3, wherein a serine residue is substituted for the cysteine residue.

5. The nucleic acid molecule of claim 3, which is further modified to encode a different amino acid instead of the arginine residue encoded by nucleotides 199-201 as set forth in **FIG. 27**.

6. The nucleic acid molecule of claim 5, wherein a serine residue is substituted for the arginine residue.

7. An isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds and activates TIE-2 receptor com-

prising a nucleotide sequence encoding TIE-2 ligand 1 which is modified to encode a different amino acid instead of the cysteine residue at amino acid position 245.

8. The nucleic acid molecule of claim 7, wherein a serine residue is substituted for the cysteine residue.

9. A modified TIE-2 ligand encoded by a nucleic acid molecule of claim 1, **2**, **3**, **4**, **5**, **6**, **7**, or **8**.

10. An isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 1 is deleted.

11. The nucleic acid molecule of claim 10, wherein the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is deleted and the portion encoding the fibrinogen-like domain is fused in-frame to a nucleotide sequence encoding a human immunoglobulin gamma-1 constant region (IgG1 Fc).

12. An isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 2 wherein the portion of the nucleotide sequence that encodes the N-terminal domain of TIE-2 ligand 2 is deleted.

13. The nucleic acid molecule of claim 12, wherein the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 2 is deleted and the portion encoding the fibrinogen-like domain is fused in-frame to a nucleotide sequence encoding a human immunoglobulin gamma-1 constant region (IgG1 Fc).

14. An isolated nucleic acid molecule encoding a modified TIE-2 ligand that binds but does not activate TIE-2 receptor comprising a nucleotide sequence encoding TIE-2 ligand 1 wherein the portion of the nucleotide sequence that encodes the fibrinogen-like domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the fibrinogen-like domain of TIE-2 ligand 2.

15. The nucleic acid molecule of claim 14, wherein the portion of the nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 1 is replaced by a nucleotide sequence that encodes the coiled-coil domain of TIE-2 ligand 2.

16. A modified TIE-2 ligand encoded by a nucleic acid molecule of claim 10, **11**, **12**, **13**, **14** or **15**.

17. A chimeric TIE-2 ligand comprising at least a portion of a first TIE-2 ligand and a portion of a second TIE-2 ligand which is different from the first, wherein the first and second TIE-2 ligands are selected from the group consisting of TIE-2 Ligand-1, TIE-2 Ligand-2, TIE Ligand-3 and TIE Ligand-4.

18. A chimeric TIE ligand of claim 17, comprising at least a portion of TIE-2 Ligand-1 and a portion of TIE-2 Ligand-2.

19. A chimeric TIE ligand according to claim 18, as set forth in **FIG. 24**.

20. A chimeric TIE ligand according to claim 18, as set forth in **FIG. 25**.

21. A chimeric TIE ligand according to claim 18, as set forth in **FIG. 26**.

22. A chimeric TIE ligand according to claim 18, as set forth in **FIG. 27**.

23. A chimeric TIE ligand as set forth in **FIG. 27**, modified to have a different amino acid instead of the cysteine residue encoded by nucleotides 784-787.

24. A nucleic acid molecule of claim 1, as set forth in **FIG. 27**.

25. A nucleic acid molecule of claim 2, as set forth in **FIG. 25**.

26. A nucleic acid molecule of claim 14, as set forth in **FIG. 24**.

27. A nucleic acid molecule of claim 15, as set forth in **FIG. 26**.

* * * * *

专利名称(译)	表达配体 - 血管细胞间信号分子		
公开(公告)号	US20030092891A1	公开(公告)日	2003-05-15
申请号	US10/225060	申请日	2002-08-21
[标]申请(专利权)人(译)	DAVIS SAMUEL YANCOPOULOS GEORGEĐ		
申请(专利权)人(译)	DAVIS SAMUEL YANCOPOULOS GEORGE D.		
当前申请(专利权)人(译)	DAVIS SAMUEL YANCOPOULOS GEORGE D.		
[标]发明人	DAVIS SAMUEL YANCOPOULOS GEORGE D		
发明人	DAVIS, SAMUEL YANCOPOULOS, GEORGE D.		
IPC分类号	G01N33/53 A61K38/00 A61K38/18 A61K45/00 A61K48/00 A61K51/00 A61P7/00 A61P7/02 A61P9/00 A61P17/02 A61P27/00 A61P29/00 A61P35/00 C07K14/515 C07K14/71 C07K16/22 C07K19/00 C12N1/19 C12N1/21 C12N5/02 C12N5/10 C12N15/09 C12N15/12 C12N15/62 C12N15/63 C12N15/85 C12P21/02 C12P21/08 C07K14/705 C07H21/04 C12N5/06		
CPC分类号	A01K2207/15 C12N15/8509 A01K2217/05 A01K2217/075 A01K2227/105 A01K2227/30 A01K2267/01 A01K2267/03 A01K2267/0331 A01K2267/0375 A01K2267/0381 A61K38/00 C07K14/515 C07K14/71 C07K2319/00 C07K2319/02 A01K2217/00 A61P17/02 A61P27/00 A61P29/00		
优先权	PCT/US1997/013557 1997-08-01 WO 60/022999 1996-08-02 US		
其他公开文献	US6825008		
外部链接	Espacenet USPTO		

摘要(译)

本发明提供了修饰的TIE-2配体，其通过添加，缺失或取代一个或多个氨基酸或通过标记，例如人IgG-1的Fc部分而改变，但保留了它结合TIE-2受体的能力。本发明进一步提供了修饰的TIE-2配体，其是嵌合TIE-2配体，其包含至少一部分第一TIE-2配体和一部分第二TIE-2配体，其不同于第一配体。在一个具体实施方案中，本发明还提供嵌合TIE配体，其包含至少一部分TIE-2配体-1和一部分TIE-2配体-2。此外，本发明提供了编码所述修饰的TIE-2配体的分离的核酸分子。本发明还提供治疗组合物以及阻断血管生长的方法，促进新血管形成的方法，促进表达TIE受体的细胞生长或分化的方法，阻断生长或分化的方法。表达TIE受体的细胞和减轻或预防人体肿瘤生长的方法。