



(72) REVEL, MICHEL, IL

(72) ABRAMOVITCH, CAROLINA, IL

(72) CHEBATH, JUDITH E., IL

(71) YEDA RESEARCH AND DEVELOPMENT CO. LTD., IL

(51) Int.Cl.⁶ C12N 15/12, A61K 31/80, C07K 14/715, C12N 15/63,
C07K 16/28

(30) 1997/01/15 (60/035,636) US

(54) **NOUVELLES PROTEINES LIANT LE RECEPTEUR 1 D'IFN,
ADN CODANT CES PROTEINES ET PROCEDE POUR
MODULER LA REPONSE CELLULAIRE AUX INTERFERONS**

(54) **NOVEL IFN RECEPTOR 1 BINDING PROTEINS, DNA
ENCODING THEM, AND METHODS OF MODULATING
CELLULAR RESPONSE TO INTERFERONS**

(57) L'invention porte sur de nouvelles protéines IR1B1 et IR1B4 qui ont été isolées. Ces protéines se lient au récepteur IFNAR1 d'IFN de type I et interviennent dans la réponse cellulaire aux IFNs. De l'ADN à orientation sens ou anti-sens codant pour ces protéines peut être administré pour augmenter ou inhiber la réponse cellulaire aux IFNs. Les anticorps de ces protéines peuvent être utilisés pour l'isolation des nouvelles protéine ou pour l'immunodétection de ces dernières.

(57) Novel proteins IR1B1 and IR1B4 have been isolated which bind to the type I IFN receptor IFNAR1 and function in the cellular response to IFNs. DNA encoding such proteins in either the sense or anti-sense orientation can be administered to either enhance or inhibit the cellular response to IFNs. Antibodies to the proteins can be used for isolation of the new protein or for immunodetection thereof.

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, 15/63, A61K 31/80, C07K 14/715, 16/28	A1	(11) International Publication Number: WO 98/31796 (43) International Publication Date: 23 July 1998 (23.07.98)
(21) International Application Number: PCT/US98/00671 (22) International Filing Date: 15 January 1998 (15.01.98) (30) Priority Data: 60/035,636 15 January 1997 (15.01.97) US (71) Applicant (for all designated States except US): YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; P.O. Box 95, 76100 Rehovot (IL). (71) Applicant (for MW only): MCINNIS, Patricia, A. [US/US]; Apartment #203, 2325 42nd Street, N.W., Washington, DC 20007 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): REVEL, Michel [IL/IL]; Weizmann Institute of Science, Beit Brazil 5, 76100 Rehovot (IL). ABRAMOVITCH, Carolina [IL/IL]; Hasayfan Street 32, Yavne (IL). CHEBATH, Judith, E. [IL/IL]; Rehov Miller 13, 76100 Rehovot (IL). (74) Agent: BROWDY, Roger, L.; Browdy and Neimark, P.L.L.C., Suite 300, 419 Seventh Street N.W., Washington, DC 20004 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: NOVEL IFN RECEPTOR 1 BINDING PROTEINS, DNA ENCODING THEM, AND METHODS OF MODULATING CELLULAR RESPONSE TO INTERFERONS (57) Abstract Novel proteins IR1B1 and IR1B4 have been isolated which bind to the type I IFN receptor IFNAR1 and function in the cellular response to IFNs. DNA encoding such proteins in either the sense or anti-sense orientation can be administered to either enhance or inhibit the cellular response to IFNs. Antibodies to the proteins can be used for isolation of the new protein or for immunodetection thereof.		

NOVEL IFN RECEPTOR 1 BINDING PROTEINS, DNA ENCODING THEM,
AND METHODS OF MODULATING CELLULAR RESPONSE TO INTERFERONS

5

FIELD OF THE INVENTION

The present invention relates generally to the molecular mechanisms of interferon action and, more specifically, to novel interferon receptor 1-binding proteins, recombinant DNA molecules encoding them, and
10 methods for modulating cellular response to interferon.

BACKGROUND OF THE INVENTION

Type I interferons (IFN- α and - β subtypes) produce pleiotropic effects on cells, such as inhibition of virus
15 replication (antiviral effect), inhibition of cell proliferation (antitumoral effects), and modulation of immune cell activities (immunoregulatory effects). These multiple effects of interferons (IFNs) are correlated with morphological and biochemical modifications of cells (Revel,
20 1984, for review).

Interferons exert their activities through species-specific receptors. For type I IFNs, two transmembranal receptor chains have been identified: IFNAR1 (Uze et al, 1990) and IFNAR2-2 (or IFNAR2-c, Domanski et al,
25 1995). Transduction of the signal generated by IFN- α, β, ω involves protein tyrosine kinases of the Janus kinases (Jak) family and transcription factors of the Stat family (Darnell et al, 1994). Proteins of the Jak-Stat pathways are activated by binding to the intracytoplasmic (IC) domains of
30 the IFNAR1 and IFNAR2 receptor chains. Among the proteins binding to the IFNAR1 IC domain are tyk2 and Stat2 (Abramovich et al, 1994). Stat2 would then recruit Stat1 to form the IFN-induced ISGF3 transcription complex which activates IFN-induced genes (Leung et al, 1995).
35 Transcription complexes containing Stat3 are also induced by IFN- β (Harroch et al, 1994) and an IFN-dependent binding of Stat3 to IFNAR1-IC was observed (Yang et al, 1996). Protein-tyrosine phosphatase PTP1C and D reversibly associate with IFNAR1 upon IFN addition (David et al,
40 1995a). In addition, two serine/threonine protein kinases, the 48 kDa ERK2 MAP kinase (David et al, 1995b) and the cAMP

WO 98/31796

PCT/US98/00671

activated protein kinase A (David et al, 1996) bind to the isolated membrane-proximal 50 residues of IFNAR1-IC.

Therefore, the type I IFN receptor IC domains serve as docking sites for multiple proteins which serve to generate
5 and regulate the biological effects of IFNs on cells.

Two-hybrid screening in yeast is a potent method for identifying new proteins which bind to defined polypeptide sequences (Fields and Song, 1989). Briefly, the two-hybrid screen is performed by transfecting yeast cells
10 with (a) a plasmid DNA in which the defined polypeptide (bait) is fused to the DNA-binding domain of the Gal4 transcription factor, and (b) a cDNA library fused to the activation domain of Gal4 in a pACT plasmid. Yeast cells transfected with a cDNA that encodes for a protein which
15 binds to the polypeptide bait will then reconstitute the Gal4 activity. The presence of such a protein which binds the polypeptide bait is revealed by expression of an enzymatic activity, such as β -galactosidase, from a GAL1-lacZ construct that is preferably introduced into the yeast
20 genome. From yeast clones which are positive in this test, it is possible to isolate the pACT plasmid, to determine the nucleotide sequence of its insert and to identify the protein which it encodes. This method has allowed the identification of novel proteins which interact with the IC
25 domain of cytokine receptors (Boldin et al, 1995).

Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date
30 of any document is based on the information available to applicants at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

35 The present invention relates to two novel human proteins, herein designated IR1B1 and IR1B4, which have been identified to be IFN Receptor 1 (IFNAR1) binding proteins,

WO 98/31796

PCT/US98/00671

and to the DNA encoding these two proteins. Each of IR1B1 and IR1B4 proteins interacts with the intracytoplasmic (IC) domain of IFNAR1 and mediates the cellular responses to interferon.

5 The present invention is directed to a recombinant DNA molecule containing a nucleotide sequence encoding either the IR1B1 or IR1B4 proteins, or fragments thereof, as well as the proteins encoded thereby. In the recombinant DNA molecules, the nucleotide sequence encoding the IR1B1 or
10 IR1B4 protein, or fragments thereof, is operably linked to a promoter in either a sense orientation or an anti-sense orientation.

 By administering the recombinant DNA molecule containing a promoter operably linked to the nucleotide
15 sequence encoding a novel IFNAR1 binding protein in the sense orientation directly into tumors, the response to exogenous interferon therapy in the treatment of cancer is enhanced.

 Furthermore, the present invention also relates to
20 a method of prolonging tissue graft survival by introducing the recombinant molecule containing a promoter operably-linked to the nucleotide sequence encoding a novel IFNAR1 binding protein, or fragment thereof, in the anti-sense orientation into the graft tissue prior to grafting to the
25 patient.

 Thus, the present invention also relates to pharmaceutical compositions containing such DNA, RNA or protein and therapeutic methods for using same.

 The present invention also relates to antibodies
30 specific to the novel proteins of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

 Figure 1 shows the interaction of IR1B1 with the IFNAR1-IC domain as measured by the two-hybrid genetic
35 interaction analysis in yeast. In the boxed lower portion of the figure, the cDNA insert in pACT as combined with various "baits" are indicated.

WO 98/31796

PCT/US98/00671

Figure 2 shows the interaction of IR1B4 with the IFNAR1-IC domain as measured by the two-hybrid genetic interaction analysis in yeast. In the boxed lower portion of the figure, the cDNA insert in pACT as combined with various "baits" are indicated.

Figure 3 shows the nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequence of IR1B1.

Figure 4 shows the homology and alignment of the amino acid sequence of IR1B1 (SEQ ID NO:2) with the amino acid sequences of two calcium-binding proteins, calcineurin B (abbreviated CALB; SEQ ID NO:3) and caltractin (abbreviated CATR; SEQ ID NO:4). Identical amino acids in IR1B1 and CALB or between CALB and CATR are shown by the symbol "|" therebetween. Identity between IR1B1 and CATR, but not with CALB, is shown by the symbol ":" therebetween. Regions shown in bold type are the calcium binding helix-loop-helix EF-hand domains.

Figure 5 shows Northern blots of IR1B1 mRNA and 18S rRNA (lower line) in human myeloma U266S cells hybridized to IR1B1 cDNA and the rapid and transient induction of IR1B1 upon treatment of the cells with either IFN- α 8 or IFN- β for 2 hrs. or 18 hrs. The first line is a control without IFN treatment after 2 hrs.

Figures 6A and 6B are SDS-PAGE lanes showing the *in vitro* interaction of IR1B4 with the isolated IFNAR1-IC domain (Fig. 6A) and with cell extracts from human U266S and U266R cell membranes (Fig. 6B). In Fig. 6A, the [³⁵S]methionine-labeled translation products with or without flag-IR1B4 *in vitro* transcripts were either immunoprecipitated (10 μ l) with anti-flag M2 beads (lanes 1 and 4), or reacted (50 μ l) with glutathione beads coupled to GST fused to the 100 amino acid long IFNAR1-IC domain (lanes 2 and 5) or coupled to GST alone (lanes 3 and 6). After overnight incubation at 4°C (final volume 100 μ l), the beads were washed and SDS-eluted proteins boiled in reducing conditions before SDS-PAGE. In Fig. 6B, U266S (lane 1) or U266R cells (lane 2) were extracted with Brij buffer and

antiproteases (Abramovich et al., 1994) and 0.35 ml (10^7 cells) was incubated with 75 μ l of [^{35} S]methionine-labeled translation products of flag-IR1B4 transcripts overnight at 4°C. Anti-IFNAR1 mAb R3 immobilized on protein G beads (25 μ l) was added for 2.5 hr, washed in Brij buffer, and SDS-eluted, boiled and reduced proteins analyzed by SDS-PAGE. A control with anti-flag M2 beads as above was run (lane 3). The dried gels were visualized in a Phosphor-Imager. In the first three lanes of Fig. 6A, no IR1B4 mRNA was added to the *in vitro* translation reaction. In the second three lanes of Fig. 6A, mRNA encoding IR1B4 protein fused to the flag protein was translated in an *in vitro* system.

Figure 7 shows the nucleotide (SEQ ID NO:7) and deduced amino acid sequence (SEQ ID NO:8) of IR1B4.

Figure 8 shows the amino acid alignment of IR1B4 (SEQ ID NO:8) and PRMT1 (SEQ ID NO:9) and their differences.

Figure 9 shows the amino acid alignment of IR1B4 and HCP-1 (SEQ ID NO:10) and their differences.

Figure 10 shows a methyltransferase assay. Extract of U266S cells were reacted with beads coated with Protein A and anti-IFNAR1 antibody (lane 1) or with Protein A alone (lane 2). Methyltransferase activity was measured by labeling of histones with ^{14} C(methyl)-S-adenosyl methionine and analyzing radioactivity in the histone band by electrophoresis on SDS-PAGE.

Figure 11 shows an assay of protein-arginine methyltransferase activity in U266S cells. In lane 1, the protein-arginine methyltransferase activity of human U266S cells was measured by methylation of peptide R1, having the sequence of SEQ ID NO:11. In lane 2 an anti-sense oligonucleotide of SEQ ID NO:12, complementary to the sequence of nucleotides 12-33 around the initiation codon of IR1B4 cDNA, was added. In lane 3 the corresponding sense oligonucleotide was added. It is seen that the anti-sense oligonucleotide substantially inhibits the protein-arginine methyltransferase activity while the control sense oligonucleotide has little effect.

WO 98/31796

PCT/US98/00671

Figure 12 is a graph showing the growth inhibition of human U266S cells in response to IFN- β treatment in the presence or absence of the anti-sense oligonucleotide used in Fig. 11 (AS-1), the corresponding sense oligonucleotide (S-3), and another anti-sense oligonucleotide directed to the middle of IR1B4 cDNA (AS-2). Cell density was quantitated by a color test with Alamar Blue (see Example 7) and the reduction in cell density was calculated in percent of control wells untreated, and plotted as growth inhibition.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the discovery of two novel human proteins which interact with the intracytoplasmic domain (IC) of the IFNAR1 chain of the interferon type 1 (IFN- α , β or ω) receptor and are designated herein as IFN Receptor Binding protein 1 (IR1B1) and IFN Receptor Binding Protein 4 (IR1B4). The interaction of these two novel proteins with IFNAR1 was demonstrated with a two-hybrid genetic test in yeast where transfection of the yeast reporter strain SFY526 (Bartel et al., 1993) with IR1B1 or IR1B4 cDNA fused to the Gal4 activation domain resulted in β -galactosidase activity only when the IFNAR1-IC domain (fused to the Gal4 DNA-binding domain) was used as bait.

The sequence of IR1B1 cDNA encodes a 191 amino acid polypeptide. Computer searches of sequence databases revealed that IR1B1 is a novel protein which shows marked homology, e.g., calcium binding sites (E-F handles), to the calcium binding proteins, calcineurin β and caltractin. Calcineurin β (Guerini et al., 1989) is a 19 kDa subunit of a serine/threonine phosphatase which plays a key role in activating the translocation of transcription factor NFAT to the nucleus of T-lymphocytes, and which is inhibited by immunosuppressive drugs such as cyclosporin. Caltractin (Lee and Huang, 1993), a 21 kDa protein, is a cytoskeleton-associated protein found in centrosomes, and is involved in

the movement of chromosomes during mitosis, and more generally in microtubule organization centers. Thus, the novel IR1B1 protein is a new member of the calcineurin and caltractin family of calcium-regulated proteins.

5 The gene for IR1B1 was surprisingly found to be rapidly activated in human cells by IFN treatment. Thus, this is the first example of a calcium-binding protein which is induced by IFN. Since calcium ions regulate cell morphology, adhesion and division, modulation of IR1B1
10 activity in cells could affect the response of normal and malignant cells to IFN. The role of IR1B1 in mediating the action of IFN in cells is supported by the interaction of IR1B1 with the IC-domain of an IFN receptor chain.

 While IR1B4, like IR1B1, was found to be a novel
15 protein as determined by computer searches of sequence databases, it was also found that IR1B4 has sequence homology to enzymes which utilize S-adenosyl methionine for methylating arginine residues in proteins and are designated as protein arginine methyltransferases (PRMT1; Kagan and
20 Clarke, 1994; Lin et al., 1996). IR1B4 was found to bind directly to the IC-domain of IFNAR1 *in vitro*, and the constitutive association of PRMT activity with the IFNAR chain of the IFN- α , β receptor isolated from human cells was demonstrated by methylation of histones. When anti-sense
25 oligodeoxynucleotides from the IR1B4 cDNA was added to human cell cultures, depletion of PRMT activity in the cell culture was observed. Human myeloma cells that were treated in this manner showed a much reduced response to IFN as measured by growth-inhibition. Therefore, IR1B4/PRMT is
30 involved in the pathway by which the IFN receptor causes growth-inhibition in tumor cells and is also involved in other functions of the IFN receptor. Known substrates of PRMT include a number of RNA and DNA binding proteins, and in particular heterologous nuclear ribonucleoproteins
35 (hnRNPs). The hnRNPs are involved in mRNA transport from the nucleus to the cytoplasm, alternative splicing of pre-mRNA, and post-transcriptional controls (Liu and Dreyfuss,

1995). Accordingly, the novel human IR1B4/PRMT cDNA and protein, which were discovered by its association with the IFN receptor, can be used to modify the response of human or animal cells to IFN.

5 A recombinant DNA molecule according to the present invention contains a nucleotide sequence that encodes the IR1B1 or IR1B4 protein, or a fragment thereof, and can be used either to increase the cellular response to IFN by increasing expression of IR1B1 or IR1B4 cDNA or to
10 decrease the cellular response to IFN by decreasing the expression of IR1B1 or IR1B4 proteins with anti-sense RNA molecules.

 The increased *in vivo* expression of IR1B1 or IR1B4 cDNA would be useful in cancer therapy where the increased
15 cellular response to IFN would result in a decrease in malignant cell growth and an enhanced response to exogenous IFN therapy. To obtain increased *in vivo* expression of IR1B1 and IR1B4 at the target location for increased cellular response to IFN, expression vectors containing
20 IR1B1 or IR1B4 cDNA operably-linked in a sense orientation to a strong constitutive promoter can be injected directly at the target location, such as into brain tumors or metastatic tumor nodules (e.g., melanoma or breast cancer).

 Conversely, the decreased *in vivo* expression of
25 IR1B1 or IR1B4 proteins would be useful in prolonging the survival of tissue grafts as the rejection of these grafts in the host is mediated by the histocompatibility antigens (MHC class I) whose synthesis depends on the IFN stimulus. When the cDNA of IR1B1 or IR1B4, or fragments thereof,
30 carried on a suitable vector and operably-linked in an anti-sense orientation to a promoter, is introduced into cells of the tissue to be grafted, the expression of anti-sense RNA leads to the degradation of IR1B1 or IR1B4 mRNA (or sense RNA for IR1B1/IR1B4) and a consequent decrease in the
35 cellular levels of IR1B1 or IR1B4 protein.

 Anti-sense RNA is transcribed from an upstream promoter operably-linked to a coding sequence oriented in

the anti-sense direction, i.e., opposite the normal or sense direction of the DNA and its transcribed sense RNA (mRNA). The expression of anti-sense RNA complementary to the sense RNA is a powerful way of regulating the biological function of RNA molecules. Through the formation of a stable duplex between sense RNA and anti-sense RNA, the normal or sense RNA transcript is rendered inactive and untranslatable.

Recombinant DNA molecules, as embodiments of the present invention, contain the cDNA of IR1B1 or IR1B4, or fragments thereof, operably-linked to a promoter in either a sense or anti-sense orientation. The term "promoter" is meant to comprehend a double-stranded DNA or RNA sequence which is capable of binding RNA polymerase and promoting the transcription of an "operably linked" nucleic acid sequence. Thus, a DNA sequence would be operably linked to a promoter sequence if the promoter is capable of effecting the transcription of the DNA sequence, regardless of the orientation of the DNA sequence.

The types of promoters used to control transcription may be any of those which are functional in the host/target cells. Examples of promoters functional in mammalian cells include the SV40 early promoter, adenovirus major late promoter, herpes simplex (HSV) thymidine kinase promoter, rous sarcoma (RSV) LTR promoter, human cytomegalovirus (CMV) immediate early promoter, mouse mammary tumor virus (MMTV) LTR promoter, interferon β promoter, heat shock protein 70 (hsp70) promoter, as well as many others well known in the art.

A promoter operably linked to IR1B1 or IR1B4 cDNA in the sense orientation for expression of IR1B1 or IR1B4 protein is preferably a strong constitutive promoter. This allows for a high level of IR1B1 or IR1B4 regardless of the presence of endogenous cellular mechanisms for regulating the expression of IR1B1 or IR1B4.

Likewise, the promoter, which is operably linked to IR1B1 or IR1B4 cDNA in the anti-sense orientation, is preferably a strong promoter, such as the promoter present

in the Epstein-Barr Virus (EBV) regulating region which allows for high levels of anti-sense RNA expression (Deiss and Kimchi, 1991).

5 The anti-sense sequence is preferably only expressible in the host/target cells, which are preferably human cells and the expressed anti-sense RNA should be stable (i.e., does not undergo rapid degradation). The anti-sense RNA should only specifically hybridize to the sense mRNA expressed in host/target cells, and form a stable
10 double-stranded RNA molecule that is essentially non-translatable. In other words, the anti-sense RNA expressed in host/target cells prevents the expressed sense mRNA from being translated into IR1B1 or IR1B4 proteins. The vector-borne anti-sense sequence may carry either the entire IR1B1
15 or IR1B4 cDNA sequence or merely a portion thereof, as long as the anti-sense portion is capable of hybridizing to sense mRNA and preventing its translation into IR1B1 or IR1B4 protein. Accordingly, an "anti-sense" sequence as used throughout the specification and claims is defined as the
20 entire anti-sense sequence or a portion thereof which is capable of being expressed in transformed/transfected cells, and which is also capable of specifically hybridizing to "sense" IR1B1 or IR1B4 mRNA to form a nontranslatable double-stranded RNA molecule.

25 The anti-sense sequence need not hybridize to the entire length of the IR1B1 or IR1B4 mRNA. Instead, it may hybridize to selected regions, such as the 5'-untranslated non-coding sequence, the coding sequence, or the 3'-untranslated sequence of the "sense" mRNA. Preferably, the
30 anti-sense sequence hybridizes to the 5'-coding sequence and/or 5'-non-coding region, such as at cap and initiation codon sites, since it has been observed it has been observed with many examples of anti-sense oligonucleotides that targeting the initiation codon is more effective, whereas
35 targeting internal sequences within the coding region is not as effective (Wickstrom, 1991). The effectiveness of an anti-sense sequence in preventing translation of IR1B4 sense

mRNA can easily be tested in an assay for protein-arginine methyltransferase activity in U266S cells as described in Example 7. In view of the size of the mammalian genome, the anti-sense IR1B1 or IR1B4 sequence is preferably at least 5 17, more preferably at least 30 base pairs in length. However, shorter sequences may still be useful, i.e., they either fortuitously do not hybridize to other mammalian sequences, or such "cross-hybridization" does not interfere with the metabolism of the cell in a manner and to a degree 10 which prevents the accomplishment of the objects of this invention.

Both the preferred hybridization target and the preferred anti-sense sequence length are readily determined by systematic experiment. Standard methods such as 15 described in Ausubel et al, eds. Current Protocols in Molecular Biology, Greene Publishing Assoc., New York, N.Y., 1987-1996, and Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989) can be used to systematically 20 remove an increasingly larger portion of the anti-sense sequence from the vector. Besides the full length anti-sense sequence, a series of staggered deletions may be generated, preferably at the 5'-end of the anti-sense sequence. This creates a set of truncated anti-sense 25 sequences that still remain complementary to preferably the 5'-end of the sense mRNA and as a result, still forms an RNA molecule that is double-stranded at the 5'-end of the sense mRNA (complements the 3'-end of an anti-sense RNA) and remains non-translatable. Moreover, anti-sense 30 oligonucleotides, such as oligonucleotide AS-1 (SEQ ID NO:12), can be readily synthesized chemically and introduced onto a vector in operable linkage with a promoter for use in decreasing the *in vivo* cellular expression of IR1B1 or IR1B4 protein.

35 The vectors of the present invention may be any suitable eukaryotic or prokaryotic vector normally used for transfecting mammalian cells, such as episomal, replicable,

or chromosomally integratable vectors well-known in the art. A particularly preferred vector for the expression of IR1B1 or IR1B4 anti-sense RNA is the episomal plasmid containing the Epstein-Barr Virus regulatory region (Deiss and Kimchi,
5 1991) to serve as the promoter that is operably-linked to IR1B1 or IR1B4 cDNA arranged in an anti-sense orientation relative to this regulatory region. The use of anti-sense vectors and oligonucleotide phosphorothioates are addressed in Annals of the New York of Sciences: Gene Therapy for
10 Neoplastic Diseases, eds. B.E. Huber and J.S. Lazo, Vol. 716, 1994 (e.g. Milligan et al., pp. 228-241).

According to the present invention, the survival of tissues or organs grafted to a patient in need of such a graft can be prolonged by decreasing the cellular response
15 to IFN. Rejection of graft tissue is mediated by the histocompatibility antigens, with the synthesis of these MHC class I antigens being dependent on IFN stimulus. Thus, a decrease in cellular response to IFN stimulus will prolong the survival of graft tissue. The method for prolonging
20 tissue graft survival according to the present invention involves introducing into cells of a tissue or organ to be grafted to a patient a recombinant DNA molecule containing a IR1B1 or IR1B4 cDNA sequence, or fragment thereof, operably linked to a promoter in the anti-sense orientation, whereby
25 anti-sense IR1B1 or IR1B4 RNA is expressed in such transfected/

transformed cells. The recombinant DNA molecule can be introduced into the cells of a tissue or organ in any manner well-known in the art to be suitable for this purpose.
30 Following the introduction of the recombinant DNA molecule into cells of the tissue or organ, the tissue or organ can be grafted to the patient in need of such a graft.

A pharmaceutical composition containing a recombinant DNA molecule, which is an expression vector and
35 which carries IR1B1 or IR1B4 cDNA operably linked to a promoter in a sense orientation, can be injected directly into tumors, e.g., brain tumors and metastatic tumor

nodules, to make the cells within these tumors more responsive to exogenous IFN therapy as a treatment for cancer. The enhanced cellular response to exogenous IFN therapy would lead to an inhibition of malignant cell growth.

Gene transfer *in vivo* or *ex vivo* is well-reported, i.e., in Annals of the New York Academy of Sciences: Gene Therapy for Neoplastic Diseases, Vol. 716, 1994; see, for example, "Direct Gene Transfer for the Understanding and Treatment of Human Disease" by G.E. Plautz on pages 144-153, and "Mechanisms of Action of the p53 Tumor suppressor and Prospects for Cancer Gene Therapy by Reconstitution of p53 Function" by Roemer et al., on pages 265-282. Methods of inserting recombinant DNA molecules into cells of a tissue or organ to be grafted or of a tumor include adenovirus, retrovirus, adenovirus-associated virus (AAV) vectors, as well as direct DNA injection or oligonucleotide-liposome injection. Clinical trials where retroviral vectors are injected into brain tumors or where adenovirus is used to infect upper respiratory tract cells of a patient with cystic fibrosis are well-known.

Pharmaceutical compositions comprising the recombinant DNA molecule encoding IR1B1 or IR1B4 cDNA, or a fragment thereof, either in a sense or anti-sense orientation with respect to an operably linked promoter, is intended to include all compositions where the recombinant DNA molecule is contained in an amount effective for achieving its intended purpose. In addition, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers or excipients which stabilize the recombinant DNA molecule or facilitate its administration.

Another embodiment of the present invention is directed to molecules which include the antigen-binding portion of an antibody specific for IFNAR1-binding proteins IR1B1 or IR1B4, or fragments thereof, for use in diagnostics, such as immunodetection methods to assay for

WO 98/31796

PCT/US98/00671

the level of IR1B1 or IR1B4 proteins in tumor tissue obtained from biopsies or for use in affinity chromatography purification of the protein.

The term "antibody" is meant to include polyclonal
5 antibodies, monoclonal antibodies (mAbs), chimeric
antibodies, anti-idiotypic (anti-Id) antibodies, single-
chain antibodies, and recombinantly produced humanized
antibodies, as well as active fractions thereof provided by
any known technique, such as, but not limited to enzymatic
10 cleavage, peptide synthesis or recombinant techniques.

Polyclonal antibodies are heterogeneous
populations of antibody molecules derived from the sera of
animals immunized with an antigen. A monoclonal antibody
contains a substantially homogeneous population of
15 antibodies specific to antigens, which population contains
substantially similar epitope binding sites. MAbs may be
obtained by methods known to those skilled in the art. See,
for example Kohler and Milstein, *Nature* 256:495-497 (1975);
U.S. Patent No. 4,376,110; Ausubel et al, eds., *supra*,
20 Harlow and Lane ANTIBODIES: A LABORATORY MANUAL Cold Spring
Harbor Laboratory (1988); and Colligan et al., eds., CURRENT
PROTOCOLS IN IMMUNOLOGY, Greene Publishing Assoc. and Wiley
Interscience, N.Y., (1992, 1993), the contents of which
references are incorporated entirely herein by reference.
25 Such antibodies may be of any immunoglobulin class including
IgG, IgM, IgE, IgA, GILD and any subclass thereof. A
hybridoma producing a mAb of the present invention may be
cultivated *in vitro*, *in situ* or *in vivo*. Production of high
titers of mAbs *in vivo* or *in situ* makes this the presently
30 preferred method of production.

Chimeric antibodies are molecules different
portions of which are derived from different animal species,
such as those having the variable region derived from a
murine mAb and a human immunoglobulin constant region.
35 Chimeric antibodies are primarily used to reduce
immunogenicity in application and to increase yields in
production, for example, where murine mAbs have higher

WO 98/31796

PCT/US98/00671

yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric mAbs are used. Chimeric antibodies and methods for their production are known in the art (Cabilly et al, *Proc. Natl. Acad. Sci. USA* 81:3273-3277
5 (1984); Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Boulianne et al., *Nature* 312:643-646 (1984); Cabilly et al., European Patent Application 125023 (published November 14, 1984); Neuberger et al., *Nature* 314:268-270 (1985); Taniguchi et al., European Patent
10 Application 171496 (published February 19, 1985); Morrison et al., European Patent Application 173494 (published March 5, 1986); Neuberger et al., PCT Application WO 8601533, (published March 13, 1986); Kudo et al., European Patent Application 184187 (published June 11, 1986); Morrison et
15 al., European Patent Application 173494 (published March 5, 1986); Sahagan et al., *J. Immunol.* 137:1066-1074 (1986); Robinson et al., International Patent Publication, WO 9702671 (published 7 May 1987); Liu et al., *Proc. Natl. Acad. Sci. USA* 84:3439-3443 (1987); Sun et al., *Proc. Natl.*
20 *Acad. Sci. USA* 84:214-218 (1987); Better et al., *Science* 240:1041- 1043 (1988); and Harlow and Lane, *ANTIBODIES: A LABORATORY MANUAL*, *supra*.

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally
25 associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type (e.g., mouse strain) as the source of the mAb with the mAb to which an anti-Id is being prepared. The immunized animal will recognize and respond
30 to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody). See, for example, U.S. patent No. 4,699,880.

The anti-Id antibody may also be used as an
35 "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original

mAb which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

5 It should be understood that antibodies of the present invention may be intact antibodies, such as monoclonal antibodies, but that it is the epitope binding site of the antibody which provides the desired function. Thus, besides the intact antibody, proteolytic fragments
10 thereof such as the Fab or F(ab')₂ fragments can be used. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)).
15 Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments).

 Furthermore, the DNA encoding the variable region of the antibody can be inserted into other antibodies to
20 produce chimeric antibodies (see, for example, U.S. Patent 4,816,567) or into T-cell receptors to produce T-cells with the same broad specificity (see Eshhar, Z. et al., *Br. J. Cancer Suppl.*, 10:27-9, 1990; Gross, G. et al., *Proc. Natl. Acad. Sci. USA*, 86:10024-8, 1989). Single chain antibodies
25 can also be produced and used. Single chain antibodies can be single chain composite polypeptides having antigen binding capabilities and comprising a pair of amino acid sequences homologous or analogous to the variable regions of an immunoglobulin light and heavy chain (linked V_H-V_L or
30 single chain F_V). Both V_H and V_L may copy natural monoclonal antibody sequences or one or both of the chains may comprise a CDR-FR construct of the type described in U.S. Patent 5,091,513. The separate polypeptides analogous to the variable regions of the light and heavy chains are held together
35 by a polypeptide linker. Methods of production of such single antibodies, particularly where the DNA encoding the polypeptide structures of the V_H and V_L chains are known, may

be accomplished in accordance with the methods described, for example, in U.S. Patents 4,946,778, 5,091,513 and 5,096,815.

Thus, the term "a molecule which includes the antigen-binding portion of an antibody" is intended to include not only intact immunoglobulin molecules of any isotype and generated by any animal cell line or microorganism, but also the reactive fraction thereof including, but not limited to, the Fab fragment, the Fab' fragment, the F(ab')₂ fragment, the variable portion of the heavy and/or light chains thereof, and chimeric or single-chain antibodies incorporating such reactive fraction, as well as any other type of molecule or cell in which such antibody reactive fraction has been physically inserted, such as a chimeric T-cell receptor or a T-cell having such a receptor, or molecules developed to deliver therapeutic moieties by means of a portion of the molecule containing such a reactive fraction.

Having now fully described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration and is not intended to be limiting of the present invention.

Example 1: Two Human Proteins, IR1B1 and IR1B4, Bind to the IFN Receptor

A cDNA fragment encoding the entire IFNAR1-IC domain amplified by PCR using a BamHI-sense primer (5'ctgaggatccAAAGTCTTCTTGAGATGCATC (SEQ ID NO:5)) and an EcoRI anti-sense primer (5'tgacgaattcctaTCATACAAAGTC (SEQ ID NO:6)), was cloned in a BlueScript vector (BS-SK⁺, Stratagene). The BamHI-SalI fragment from this BS-IFNAR1-IC was introduced in the pGBT₁₀ vector (CloneTech) and fused in-phase after the Gal4 DNA binding domain (pGBT₁₀-IFNAR1-IC) for two-hybrid screening. The two-hybrid screening method (Fields and Song, 1989) was carried out with the modified procedure of Durfee et al (1993) using the pACT plasmid cDNA library from human Epstein-Barr Virus (EBV)-transformed B-

WO 98/31796

PCT/US98/00671

lymphocytes to cotransform yeast reporter strain Y153 with pGBT₁₀-IFNAR1-IC. The yeast Y153 strain has two reporter genes under the control of GAL1 Upstream Activating Sequences (UAS) which are transcribed only if the activity of the Gal4 transcription factor is reconstituted. This requires that the fusion protein encoded by the pACT plasmid which was introduced into this particular yeast clone have affinity for the IFNAR1-IC domain from the pGBT₁₀ plasmid. One of the reporter genes is GAL1 His3, which allows for growth in a medium lacking histidine; the other reporter gene is GAL-LacZ, which provides β -galactosidase activity. In addition, the pACT plasmids have the Leu2 gene and the pGBT₁₀ plasmid has the TRP1 gene which allows for growth in a medium lacking leucine and tryptophan, respectively.

Colonies were selected in synthetic medium SC minus Trp, Leu, His in the presence of 25 mM 3-aminotriazole (which further selects for histidine prototrophy). The growing colonies were then tested for β -galactosidase activity using the X-gal filter assay (Breedon and Naysmith, 1985).

Nine positive yeast clones were obtained and their pACT plasmids were recovered by transfection into *E. coli* HB101 and selection for leu⁺ transformants. For each yeast DNA, two such *E. coli* HB101 clones were isolated. Partial DNA sequencing of the pACT plasmids from these *E. coli* clones showed that they fell into two groups of cDNA sequences which were designated IR1B1 and IR1B4. The pACT plasmids of the IR1B1 and IR1B4 groups were subjected to specificity tests by cotransformation of the SFY526 yeast reporter strain (Bartel et al, 1993) with pAS plasmids harboring lamin, cdk2 and p53 or other control inserts (CloneTech). Colonies which grew in SC -trp, -leu were tested for β -galactosidase expression. From the specifically positive pACT plasmids, inserts were excised with XhoI, cloned into BS-KS (Stratagene) and subjected to sequencing from T7 and T3 promoters using the DyeDeoxy Terminator Cycle Sequencing Kit in a 373A DNA Sequencer (Applied Biosystems).

Figure 1 shows the results for pACT clone IR1B1 co-transfected into yeast SFY526 with different pAS or pGBT₁₀ plasmid baits. Yeast cells grew in the selective SC medium -trp, -leu in streaks 1 to 9 of the filter. Staining by X-gal reagent (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) was positive only in streaks 2 and 4. As indicated in Fig. 1, streak 4 is a control yeast with an active lacZ gene. Streak 2 is the combination of IR1B1 and IFNAR1-IC fusion proteins. IR1B1 alone (streak 9), or any other combination besides IR1B1 and IFNAR1-IC, did not exhibit β -galactosidase activity. Therefore, IR1B1 is specifically able to combine with the IC domain of the IFNAR1 IFN receptor chain.

Similarly, Figure 2 shows the results for pACT clone IR1B4 co-transfected into yeast SFY526 with different pAS or pGBT₁₀ plasmid baits. Yeast cells grew in SC medium -trp, leu in streaks 1 to 8 of the filter and staining by X-gal reagent was positive only in streaks 3 and 7. As indicated in the lower boxed portion of Fig. 2, streak 7 is a control yeast with an active lacZ gene. Streak 3 is the combination of IR1B4 and IFNAR1-IC fusion proteins. Like the results obtained with IR1B1, IR1B4 alone (streak 1), or any other combination besides IR1B4 and IFNAR1-IC, did not exhibit β -galactosidase activity. Therefore, IR1B4 is also specifically able to combine with the IC domain of the IFNAR1 IFN receptor chain.

Example 2: IR1B1 Protein Sequence Shows Calcium-Binding EF Hand Sites.

The cDNA insert of the pACT-IR1B1 plasmids was excised with restriction enzyme XhoI, cloned into a Bluescript BS-KS vector and subjected to sequencing from T7 and T3 promoters using the DyeDeoxy Terminator Cycle Sequencing kit in a 373A DNA sequencer (Applied Biosystems). The longest plasmid had a sequence of 830 nucleotides (Fig. 3) following the Gal4 Activation domain and linker sequence of the pACT plasmid and an open reading frame of 191 amino acids was found therein (Fig. 3). An online search of the

protein databases was performed using the BlastP algorithm (Altschul et al, 1990) as well as the Bioaccelerator Alignment (Henikoff and Henikoff, 1992). The highest scores were obtained for caltractin (CATR_HUMAN, accession Swiss Protein SW New P41208) with 62.1 % similarity and 32.4% identity from amino acids 52 to 173, and for calcineurin B (CALB_NAEGR, accession Swiss Protein P42322; CALB_HUMAN, accession P06705) with 59.8% similarity and 32.5% identity from amino acids 50 to 171.

Figure 4 shows the alignment of IR1B1 with human calcineurin B (CALB) and caltractin (CATR). The calcium binding, helix-loop-helix EF-hand domains are shown in bold and underlined characters. IR1B1 has two EF-hand sites but the first two EF-hand domains are not conserved. IR1B1 shows homology to both calcineurin B (represented by vertical lines in Fig. 4) and caltractin (represented by colons in Fig. 4). However, IR1B1 is clearly a novel and different human protein which has not been previously identified.

Example 3: IR1B1 is an IFN-Induced Gene Product

Human myeloma U266S cells (about 3×10^6 cells in 5 ml suspension cultures) were treated with recombinant IFN- α 8 (2×10^8 IU/mg from bacteria) or with recombinant IFN- β (3×10^8 IU/mg from CHO cells) at 750 IU/ml for 2 hours or for 18 hours. After treatment with IFN, the cells were collected and extracted with Tri-reagent (Molecular Research Center, Cincinnati, Ohio), which is a product containing guanidinium thiocyanate and phenol. The extracted RNA was ethanol precipitated, denatured with formaldehyde, analyzed by electrophoresis in formaldehyde-agarose gels ($10 \mu\text{g}$ RNA/slot), and blotted on GeneScreen Plus (Dupont, New England Nuclear, Billerica, MA). The Northern blot was reacted with 10^6 cpm of IR1B1 cDNA labeled with the Rediprime kit (Amersham, UK) using ^{32}P -dCTP and random priming.

Figure 5 shows that the IR1B1 cDNA hybridized to a 1.1 kb RNA. The amount of IR1B1 mRNA was markedly increased

2 hours after IFN- β treatment of U266S cells. However, at 18 hours after IFN treatment, the IR1B1 mRNA had disappeared from the cells, indicating that the induction is both rapid and transient. Many IFN-induced mRNAs continue to
5 accumulate in the cells for over 24 hours after IFN treatment (Revel and Chebath, 1986).

It was verified that the same amount of RNA was present in each lane. As shown on the lower part of Fig. 5, hybridization of the same U266S (rich in IFN receptor) RNA
10 to an 18S ribosomal cDNA probe reveals the same amount of 18S rRNA in each lane (only the part of the blot where 18S rRNA runs is shown). In another experiment using 1,200 U/ml of IFN for induction, IR1B1 mRNA was also observed with IFN- α 8 at 2 hours, but not at 30 minutes (not shown).

15 The IR1B1 mRNA was found to have the same 1.1 kb size in different human cells (U266, Daudi and THP-1 cells). It is notable that this size is close to that of caltractin mRNA but not to that of calcineurin B mRNA (2.5 kb). The small size of the mRNA is consistent with IR1B1 being a
20 small protein of about 20 kDa.

Example 4: IR1B4 Protein Binds to IFNAR1 in vitro

The binding of IR1B4 to the IC-domain of IFNAR1 was tested by synthesizing the IR1B4 protein with a protein
25 tag (flag sequence) using *in vitro* translation in reticulocyte lysates and reacting this protein with a recombinant IFNAR1-IC fusion protein in *E. coli*. The pACT-IR1B4 DNA from Example 1, cut with XhoI and filled-in by Klenow enzyme, was cloned in the PECE-flag expression vector
30 (Ellis et al., 1986) cut with EcoRI and filled-in. The NotI-BamHI fragment containing the in-frame flag-IR1B4 fusion was recloned in BS-SK cut with NotI-BamHI and downstream from the T3 promoters. The sequence of the flag fusion was verified by sequencing from the T3 promoter. In
35 *vitro* transcription (Promega kit) was done with T3 polymerase and 1 μ g of BamHI-linearized BS-flag-IR1B4 DNA. *In vitro* translation was carried out in rabbit reticulocyte

lysates (Promega kit) with [³⁵S]methionine (Amersham) and 5 µg of RNA transcripts for 1h at 30°C. The products were RNase treated before use. The GST-IFNAR1-IC fusion protein was prepared by cloning the BamHI-EcoRI, insert of BS-
5 IFNAR1-IC (see above) into the same sites of pGEX2 (Pharmacia Biotech). GST and GST-IFNAR1-IC were expressed in *E.coli* and recovered bound to glutathionine-agarose beads (Sigma).

Anti-flag M2 agarose beads were from Kodak
10 Scientific Imaging Systems. Monoclonal antibodies IFNAR3 to the α-component of the IFN receptor (IFNAR1) were a kind gift of Dr. O. Colamonici (Colamonici et al., 1990) and were used at 1:100 dilution. Rabbit antibodies to the C-terminal peptide of IFNAR1-IC (Ab 631) were prepared and used for
15 immunoprecipitation of IFNAR1 from Brij extracts (0.75 ml) of 2 x 10⁷ human myeloma U266S and U266R cells with antiproteases previously detailed (Ambrovich et al., 1994) except that protein G beads (Pharmacia) were used with mAb IFNAR3 SDS-PAGE and analysis in a Fujix BAS1000 Phosphor-
20 Imager were as before (Harroch et al., 1994).

It was first verified that a protein product of about 32 kDa is obtained when the translation products were immunoprecipitated by anti-flag antibodies (Figs. 6A and 6B). In Fig. 6A and 6B, whenever the use of anti-flag
25 antibodies is noted (by + sign), it means that the radioactive translation product of the IR1B4-flag fusion mRNA (*in vitro* transcribed from the corresponding DNA construct) was reacted with anti-flag M2 antibody bound to agarose beads (product of Kodak Scientific Imaging Systems).
30 The translated protein which contains IR1B4 fused to the flag amino acid sequence was bound to these anti-flag antibody beads and after centrifuging down the beads, the protein was eluted with SDS buffer and applied onto SDS-PAGE. These reactions serve as a control to demonstrate
35 that the expected fused protein is present.

Beads of Glutathione-Sepharose (Sigma), to which the Glutathione S-transferase (GST) fused to IFNAR1-IC was

bound, were added to the reticulocyte lysate translation reaction. The beads were centrifuged and washed and the proteins bound to GST beads were released by sodium dodecyl sulfate (SDS 1%) and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE). The 32 kDa protein labeled by ³⁵S-methionine was observed to be bound to GST-IFNAR1-IC but not to GST alone (Fig. 6A). This demonstrates that IR1B4 directly binds to the isolated IFNAR1-IC peptide region.

To verify that IR1B4 interacts with the IFNAR1 protein as present in human cell membranes, detergent extracts of human myeloma U266 cells were mixed with the ³⁵S-methionine labeled translation products of IR1B4 mRNA from reticulocyte lysates. The IFNAR1 protein was immunoprecipitated by a monoclonal antibody IFNaR3 specific to the ectodomain of IFNAR1 (from Colamonici et al., 1990). Analysis by SDS-PAGE showed the presence of the 32 kDa IR1B4-flag band (Fig. 6B) when the detergent extracts originated from U266S (rich in IFN receptor), but not when originating from U266R cells - a mutant IFN- α , β -resistant derivative cell line from U266 deficient in IFN receptors (Abramovich et al., 1994). The 32 kDa band similarly was seen when U266S extracts were reacted with Ab 631 against the C-terminal peptide of IFNAR1, and IFNAR1 was precipitated by anti-flag when Cos-7 cells were transfected by flg-IR1B4 and human IFNAR1 cDNAs. These results demonstrated that IR1B4 binds to intact IFNAR1 from human cells in a specific manner.

Example 5: IR1B4 cDNA and Protein Sequences

The nucleotide sequence of the IR1B4 cDNA has an open reading frame encoding a 361 amino-acid long protein (Fig. 7). This human cDNA recognized a 1.5 kb constitutively expressed poly-A⁺ mRNA in various human cells including U266 myeloma cells. An online search of the protein databases was performed using the BlastP algorithm (Altschul et al., 1990) as well as the Bioaccelerator Alignment (Henikoff and Henikoff, 1992), and it was found that IR1B4 is a unique member of the protein-arginine

methytransferase family. The rat PRMT1 cDNA described by Lin et al. (1996, Genbank sequence I.D. 1390024; Accession U60882) is only 81.4% homologous when analyzed by the ALIGN computer program. At the amino acid level (Fig. 8), the
5 human IR1B4/PRMT differs clearly in its amino terminus from PRMT1, with the first 19 amino acids being completely different. N-terminal sequencing of IR1B4 alone would not have provided any indication that IR1B4 is homologous to PRMT1. Another human protein which has been described, HCP-
10 1 (Nikawa et al., 1996; Genbank accession D66904) was also found to have homology to IR1B4. However, HCP-1 has a different amino acid sequence from residues 147-175 (Fig. 9). HCP-1 was originally identified based on its ability to complement the ire15 mutation in yeast and its enzymatic
15 function was not previously identified (Nikawa et al., 1996). Therefore, IR1B4 is a novel human protein.

Example 6: IR1B4 Protein Bound to IFNAR1-IC has Methytransferase Activity

20 Methytransferase activity could be co-immunoprecipitated from human cell extracts with the IFNAR1 receptor. Brij-detergent extracts of U266S cells were reacted overnight at 4°C with or without anti-IFNAR1
25 antibody Ab 631 (Abramovich et al., 1994). Protein A beads (40 µl of a 50% of IPA-400 fast flow, Repligen) were added for 1 hour. The beads were washed and incubated in 0.1 ml of 25 mM Tris-HCl, pH 7.5,
1 mM EDTA, 1 mM EGTA, 50 µM (0.25 µCi) ¹⁴C-(methyl)-S-
30 adenosyl-methionine (Amersham), and 100 µg histones (Type IIA from calf thymus, Sigma) for 30 min. at 30°C. The *in vitro* methylation of histones was carried out under the conditions described by Lin et al. (1996). The radioactivity in the histone band was analyzed after SDS-
35 PAGE (15% acrylamide) and exposure in the Phosphor-imager. A ¹⁴C-methyl labeling of the histones was observed with the beads that were coated with anti-IFNAR1, but not with those in the control reaction (Fig. 10). Therefore, protein

methy1-transferase activity is constitutively associated with the IFN receptor chain of these human cells. A similar enzyme activity was recovered when IFNAR1 was immunoprecipitated five minutes after addition of IFN- β to the U266S cells.

Example 7: Involvement of IR1B4/PRMT1 in IFN Action

An anti-sense oligodeoxynucleotide phosphorothioate (Stein et al., 1989) complementary to the sequence of nucleotides 12-33 around the initiation codon of IR1B4 cDNA (AS-1, anti-sense sequence 5'-GGCTACAAAATTCTCCATGATG-3'; SEQ ID NO:12) was synthesized chemically. The oligonucleotides were added to U266S cells seeded in 96-well microplates (8000 cells/well/0.2 ml RPMI, 10% FCS) at a final concentration of 10 μ M on day 0 and re-added at 5 μ M on day 2. IFN- β was added at 64 or 125 IU/ml on day 0. After 3 days of culture, 20 μ l of Alamar Blue, a colorimetric cell density indicator based on oxido-reduction (BioSource, Camarillo, CA), was added to each well and incubation continued for 6-7 h. Color was measured in a microplate ELISA reader (test filter 530 nm, reference filter 630 nm) with multiple reading of duplicate wells. Correlation of the growth curves by live cell number and by OD was verified. To measure methyltransferase, cells from pooled wells were lysed by freeze-thawing in 25 μ l/well of 25 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM EGTA, 40 μ g/ml leupeptin and aprotinin, 20 μ g/ml pepstatin, 1 μ M phenylmethylsulfonyl fluoride (PMSF). Reactions were in 50 μ l with 25 μ l of cell extracts, 100 μ M peptide R1 (Najbauer et al., 1993; obtained from Genosys, Cambridge, UK), 3 μ Ci of [^{3} H] (methyl)S-adenosylmethionine (Amersham, 73 Ci/mmol) for 30 min at 30°C. After electrophoresis in SDS-polyacrylamide (16%) gel, fixation in 50% methanol, 10% acetic acid and treatment by Amplify® (Amersham), autoradiography was carried out for 8 days. This AS-1 anti-sense DNA was able to strongly reduce the protein-arginine methyltransferase activity in U266S cells as

measured by incorporation of tritiated-methyl groups to the R1 peptide substrate (Fig. 11), and was used to investigate the role that this enzyme may play in IFN action. The growth-inhibitory activity of IFN was chosen because it can
5 be most directly quantified on cells and because an interaction of rat PRMT1 with growth-related gene products has been observed (Lin et al., 1996). Addition of the antisense-1 oligonucleotide AS-1, which is complementary to the sequence around the initiation codon of IR1B4/PRMT cDNA,
10 reduced the growth inhibitory effect of IFN- β on human myeloma U266S cells (Fig. 12). This means that, in the presence of anti-sense AS-1, the IFN-treated cells exhibited a higher growth (excluding any toxic effect of phosphorothioates). The growth in the absence of IFN was
15 not significantly affected. The sense oligonucleotide S-3 corresponding to the same cDNA region had only a small effect (S-3, Fig. 12) as compared to antisense-1. Sense S-3 also had only a slight inhibitory effect on the level of enzyme activity (Fig. 11). Another anti-sense
20 phosphorothioate oligonucleotide AS-2 (SEQ ID NO:13), directed to the middle of the cDNA and complementary to nucleotides 572-592 of SEQ ID NO:7, had almost no effect (Fig. 12). The up to 5 fold reduction in the growth inhibitory effect of IFN- β on myeloma cells, which were
25 rendered partially deficient in PRMT activity by antisense-1 oligonucleotide demonstrates that the association of the IR1B4/PRMT enzyme with the IC domain of the IFNAR1 receptor is functionally significant for IFN action on cells.

These experiments also demonstrate that the IR1B4
30 protein methylates peptide substrates of the PRMT class of enzymes, such as the R1 peptide Gly-Gly-Phe-Gly-Gly-Arg-Gly-Gly-Phe-Gly (SEQ ID NO:11; Najbauer et al., 1993), which was used in the experiment illustrated in Fig. 11. Methylation of proteins on arginine residues next to glycine residues
35 (e.g., as in the above peptide) could be a type of protein modification which, like phosphorylation, serves to transduce signals into the cell. The hnRNP group of

WO 98/31796

PCT/US98/00671

proteins is a target for PRMT enzymes, and since these proteins affect mRNA processing, splicing, transport and stability (Liu and Dreyfuss, 1995), their methylation may play a role in post-transcriptional controls of gene
5 expression. The IR1B4/PRMT protein, discovered here as binding to a chain of the IFN receptor, could mediate changes in gene expression in response to IFN. Other protein substrates may become methylated through the IFN receptor, including other components of the IFN receptor
10 complex and transcription factors. Lin et al. (1996) have observed that the binding of rat PRMT1 to growth factor-induced proteins activates PRMT1 and modifies its substrate specificity, possibly by removal of some inhibitory proteins associated with PRMT1 in the cytoplasm of cells. A similar
15 activation of IR1B4 bound to the IFNAR1 chain of the IFN receptor can be expected.

Conclusions

A new protein IR1B1 is described which interacts
20 with the intracytoplasmic domain of the IFNAR1 chain of the type I interferon receptor. This protein is induced very rapidly and transiently following IFN treatment of human cells. IR1B1 is characterized by the presence of helix-loop-helix EF-handle sites which are the hallmark of
25 calcium-binding proteins. Calcium ion fluxes have been implicated in the mechanism of action of IFNs, and in particular for the initial cell responses and changes in cell morphology and in cytoskeleton organization (Tamm et al, 1987). Calcium ion-activated enzymes could produce
30 second messengers, such as diacyl-glycerol, in response to IFNs. Furthermore, calmodulin-like proteins regulate a number of protein kinases and these pathways have been observed to function in IFN-treated cells (Tamm et al, 1987). It is likely that the IFN receptor binding protein
35 IR1B1 is involved in such Ca^{++} -dependent effects of IFNs on cells.

The two-hybrid screening for proteins interacting with the IFNAR1-IC domain also identified another protein IR1B4, which turned out to be a member of the protein-arginine methyl transferase family of enzymes (PRMT1; Lin et al, 1996). This enzyme is known to methylate a number of RNA and DNA binding proteins, in particular heterologous nuclear ribonucleoproteins (hnRNPs). The hnRNPs are involved in mRNA transport from nucleus to cytoplasm, alternative splicing of pre-mRNA, and post-transcriptional controls (Liu and Dreyfuss, 1995). The IR1B1 and IR1B4/PRMT1 proteins which dock onto the IFNAR1-IC domain reveal novel signaling mechanisms of IFNs that exist besides the known Jak-Stat pathways described by Darnell et al (1994).

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the

WO 98/31796

PCT/US98/00671

entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

References

- 5 Abramovich, C., Shulman, L.M, Ratovitski, E., Harroch, S.,
Tovey, M, Eid, P. and Revel, M. (1994) Differential
tyrosine phosphorylation of the IFNAR chain of the type
I Interferon receptor and of an associated surface
protein in response to IFN- α and IFN- β . EMBO J.,
13:5871-5877.
- 10 Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and
Lipman, D.J. (1990) Basic local alignment research
tool. J. Mol. Biol., 215:403-410.
- 15 Barter, P.L., Chien, C.T., Sternglanz, R. and Fields, S.
(1993) Elimination of false positives that arise in
using the two-hybrid system. BioTechniques, 14:920-
924.
- 20 Boldin, M.P. Varfolomeev, E.E. Pancer, Z. Mett, I.L. Camonis
J.H. and Wallach D. (1995) A novel protein that
interacts with the death domain of Fas/AP01 contains a
sequence motif related to the death domain. J Biol
Chem, 270:7795-7798 .
- 25 Breeden, L. and Naysmith, K., (1995) Regulation of the yeast
HO gene. Cold Spring Harbor Symp. Quant. Biol.,
50:643-650.
- 30 Colamonici, O.R., D'Allessandro, F., Diaz, M.O., Gregory,
S.a., Necker, L.M. and Nordan, R. (1990)
Characterization of three monoclonal antibodies that
recognize the Interferon- α 2 receptor. Proc. Natl. Acad.
Sci. USA 87, 7230-7234.
- 35 Darnell, J.E., Kerr, I.M. and Stark, G.R. (1994) Jak-Stat
pathways and transcriptional activation in response to
IFNs and other extracellular signaling proteins.
Science, 264:1415-1421.
- 40 David, M., Chen, H.E., Goelz, S., Larner, A.C. and Neel,
B.G. (1995a) Differential regulation of the alpha/beta
Interferon-stimulated Jak/Stat pathway by the SH2
domain-containing tyrosine phosphatase SHPTP1. Mol.
Cell. Biol., 15:7050-7058.
- 45 David, M., Petricoin, E. III, Benjamin, C., Pine, R., Weber,
M.J. and Larner, A.C. (1995b) Requirement for MAP
kinase (ERK2) activity in Interferon α - and Interferon
 β -stimulated gene expression through Stat proteins.
Science, 269:1721-1723.
- 50 David, M., Petricoin, E. III. And Larner, A.C. (1996)
Activation of Protein kinase A inhibits Interferon
induction of the Jak/Stat pathway in U266 cells. J.
Biol. Chem., 271:4585-4588.
- 55

- Deiss, L.P. and Kimchi, A. (199) A genetic tool used to identify thioredoxin as a mediator of a growth inhibitory signal. *Science* 252, 117-20.
- 5 Domanski, P., Witte, M., Kellum, M., Rubinstein, M., Hackett, R., Pitha, P. And Colamonici, O.R. (1995) Cloning and expression of a long form of the beta subunit of the Interferon alpha beta receptor that is required for signaling. *J. Biol. Chem.*, 270:21606-
10 21611.
- Durfee, T., Becherer, K, Chen, P.-L., Yeh, S-H, Yang, Y., Kilburn, A.E., Lee, W.-H. and Elledge, S. (1993). The retinoblastoma protein associates with the protein
15 phosphatase type 1 catalytic subunit. *Genes & Devpt.*, 7:555-569.
- Ellis, L., Clauser, E., Morgan, D.O., Edery, M., Roth, R.A. and Rutter, W.J. (1986) Replacement of insulin receptor
20 tyrosine residues 1162 and 1163 compromises insulin -stimulated kinase activity and uptake of 2 -deoxyglucose. *Cell*, 45, 721-731.
- Fields, S. and Song, O. (1989). A novel genetic system to
25 detect protein-protein interactions. *Nature*, 340:245 -246.
- Guerini, D. et al (1989). *DNA* 8:675-682.
- 30 Harroch, S., Revel, M. and Chebath, J. (1994). Interleukin -6 signaling via four transcription factors binding palindromic enhancers of different genes. *J. Biol. Chem.*, 269:26191-26195.
- 35 Henikoff, S. and Henikoff, J.G. (1992). *Proc. Natl. Acad. Sci. USA*, 89:10915-10919.
- Kagan, R.M. and Clarke, S. (1994) Widespread occurrence of three sequence motifs in diverse S-adenosyl
40 methionine-dependent methyltransferases suggests a common structure for these enzymes. *Arch. Biochem. Biophys.*, 310, 417-427.
- Lee, V.D. and Huang, B. (1993). *Proc. Natl. Acad. Sci. USA*
45 90:11039-11043.
- Leung, S., Qureshi, S.A., Kerr, I.M., Darnell, J.E. and Stark, G.R. (1995). Role of Stat2 in the alpha
50 Interferon signaling pathway. *Mol. Cell. Biol.*, 15:1312-1317.
- Lin, W.-J., Gary, J.D., Yang, M.C., Clarke, S. and Herschman, H.R. (1996). The mammalian immediate-early TIS21 protein and the leukemia-associated BTG1 protein
55 interact with a Protein-arginine Methyltransferase. *J. Biol. Chem.*, 271:15034-15044.

- Liu, Q. And Dreyfuss, G. (1995). In vivo and in vitro arginine methylation of RNA-binding proteins. Mol. Cell. Biol., 15:2800-2808.
- 5 Najbauer, J., Johnson, B.A., Young, A.L. and Asward, D.W. (1993) Peptides with sequences similar to glycine arginine rich motifs in proteins interacting with RNA are efficiently recognized by methyltransferases modifying arginine in numerous proteins. J. Biol. Chem., 268, 10501-10509.
- 10
- Nikawa, J.-I., Nakano, H. and Ohi, N. (1996) Structural and functional conservation of human yeast HCPI genes which can suppress the growth defect of the *Saccharomyces cerevisiae* ire15 mutant. Gene, 171, 107-111.
- 15
- Revel, M. (1984). The Interferon system in man: nature of the Interferon molecules and mode of action. In Becker, I. (ed.), Antiviral Drugs and Interferon. The molecular basis of their activity. Martinus Nijhoff Publ., Boston, pp 357-433.
- 20
- Revel, M. and Chebath, J. (1986) Interferon-activated genes. Trends Biochem. Sci., 11:166-170.
- 25
- Stein, C.A., Subasinghi, C., Shinozuka, K. and Cohen, J.S. (1989) Physicochemical properties of phosphorothionate oligodeoxynucleotides. Nucleic Acids Res., 16, 3209-3221.
- 30
- Tamm, I., Lin, S.L., Pfeffer, L.M. and Sehgal, P.B. (1987). Interferons α and β as cellular regulatory molecules. In Gresser, I. (ed.), Interferon 9, Acad. Press, London, pp 14-74.
- 35
- Uze, G., Lutfalla, G. and Gresser, I. (1990). Genetic transfer of a functional human Interferon α receptor into mouse cells: cloning and expression of its cDNA. Cell, 60:225-234.
- 40
- Wickstrom, E. (1991). In: Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS, pp. 7-24, Wiley-Liss, New York.
- 45
- Yang, C.H., Shi, W, Basu, L., Murti, A., Constantinescu, S.N., Blatt, L., Croze, E., Mullersman, J.E. and Pfeffer, L.M. (1996). Direct association of Stat3 with the TFNAR-1 chain of the human type I Interferon receptor. J. Biol. Chem., 271:8057-8061.
- 50

WO 98/31796

PCT/US98/00671

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: REVEL, Michael
CHEBATH, Judith
ABRAMOVICH, Carolina
- (ii) TITLE OF INVENTION: NOVEL IFN RECEPTOR I BINDING PROTEIN,
DNA ENCODING THEM, AND METHODS OF MODULATING CELLULAR
RESPONSE TO INTERFERONS
- (iii) NUMBER OF SEQUENCES: 13
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: BROWDY AND NEIMARK
 - (B) STREET: 419 Seventh Street, N.W., Suite 300
 - (C) CITY: Washington
 - (D) STATE: D.C.
 - (E) COUNTRY: USA
 - (F) ZIP: 20004
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US
 - (B) FILING DATE:
- (vi) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 60/035,636
 - (B) FILING DATE: 15-JAN-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: BROWDY, Roger L.
 - (B) REGISTRATION NUMBER: 25,618
 - (C) REFERENCE/DOCKET NUMBER: REVEL=14 PCT
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 202-628-5197
 - (B) TELEFAX: 202-737-3528

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 830 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 43..615
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGTCTCGAGG CGAGTTGGCG GAGCTGTGCG CGCGGCGGGG CG ATG GGG GGC TCG
Met Gly Gly Ser

54

1

WO 98/31796

PCT/US98/00671

GGC Gly 5	AGT Ser	CGC Arg	CTG Leu	TCC Ser	AAG Lys 10	GAG Glu	CTG Leu	CTG Leu	GCC Ala	GAG Glu 15	TAC Tyr	CAG Gln	GAC Asp	TTG Leu	ACG Thr 20	102		
TTC Phe	CTG Leu	ACG Thr	AAG Lys	CAG Gln 25	GAG Glu	ATC Ile	CTC Leu	CTA Leu	GCC Ala 30	CAC His	AGG Arg	CGG Arg	TTT Phe	TGT Cys 35	GAG Glu	150		
CTG Leu	CTT Leu	CCC Pro	CAG Gln 40	GAG Glu	CAG Gln	CGG Arg	AGC Ser	GTG Val 45	GAG Glu	TCG Ser	TCA Ser	CTT Leu	CGG Arg 50	GCA Ala	CAA Gln	198		
GTG Val	CCC Pro	TTC Phe 55	GAG Glu	CAG Gln	ATT Ile	CTC Leu	AGC Ser 60	CTT Leu	CCA Pro	GAG Glu	CTC Leu	AAG Lys 65	GCC Ala	AAC Asn	CCC Pro	246		
TTC Phe 70	AAG Lys	GAG Glu	CGA Arg	ATC Ile	TGC Cys	AGG Arg 75	GTC Val	TTC Phe	TCC Ser	ACA Thr	TCC Ser 80	CCA Pro	GCC Ala	AAA Lys	GAC Asp	294		
AGC Ser 85	CTT Leu	AGC Ser	TTT Phe	GAG Glu	GAC Asp 90	TTC Phe	CTG Leu	GAT Asp	CTC Leu	CTC Leu 95	AGT Ser	GTG Val	TTC Phe	AGT Ser	GAC Asp 100	342		
ACA Thr	GCC Ala	ACG Thr	CCA Pro	GAC Asp 105	ATC Ile	AAG Lys	TCC Ser	CAT His	TAT Tyr 110	GCC Ala	TTC Phe	CGC Arg	ATC Ile	TTT Phe 115	GAC Asp	390		
TTT Phe	GAT Asp	GAT Asp	GAC Asp 120	GGA Gly	ACC Thr	TTG Leu	AAC Asn	AGA Arg 125	GAA Glu	GAC Asp	CTG Leu	AGC Ser	CGG Arg 130	CTG Leu	GTG Val	438		
AAC Asn	TGC Cys	CTC Leu 135	ACG Thr	GGA Gly	GAG Glu	GGC Gly 140	GAG Glu	GAC Asp	ACA Thr	CGG Arg	CTT Leu	AGT Ser 145	GCG Ala	TCT Ser	GAG Glu	486		
ATG Met 150	AAG Lys	CAG Gln	CTC Leu	ATC Ile	GAC Asp	TAC Tyr 155	ATC Ile	CTG Leu	GAA Glu	GAG Glu	TCT Ser 160	GAC Asp	ATT Ile	GAC Asp	AGG Arg	534		
GAT Asp 165	GGA Gly	ACC Thr	ATC Ile	AAC Asn	CTC Leu 170	TCT Ser	GAG Glu	TTC Phe	CAG Gln	CAC His 175	GTC Val	ATC Ile	TCC Ser	CGT Arg	TCT Ser 180	582		
CCA Pro	GAC Asp	TTT Phe	GCC Ala	AGC Ser 185	TCC Ser	TTT Phe	AAG Lys	ATT Ile	GTC Val 190	CTG Leu	TGACAGCAGC				CCCAGCGTGT		635	
GTCCTGGCAC			CCTGTCCAAG			AACCTTTCTA			CTGCTGAGCT			GTGGCCAAGG			TCAAGCCTGT			695
GTTGCCAGTG			CGGGCCAAGC			TGGCCCAGCC			TGGAGCTGGC			GCTGTGCAGC			CTCACCCCGG			755
GCAGGGGCGG			CCCTCGTTGT			CAGGGCCTCT			CCTCACTGCT			GTTGTCATTG			CTCCGTTTGT			815
GGGCCTTCGT			GGCCA															830

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 191 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

WO 98/31796

PCT/US98/00671

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Gly Gly Ser Gly Ser Arg Leu Ser Lys Glu Leu Leu Ala Glu Tyr
 1              5              10              15
Gln Asp Leu Thr Phe Leu Thr Lys Gln Glu Ile Leu Leu Ala His Arg
              20              25              30
Arg Phe Cys Glu Leu Leu Pro Gln Glu Gln Arg Ser Val Glu Ser Ser
              35              40              45
Leu Arg Ala Gln Val Pro Phe Glu Gln Ile Leu Ser Leu Pro Glu Leu
              50              55              60
Lys Ala Asn Pro Phe Lys Glu Arg Ile Cys Arg Val Phe Ser Thr Ser
 65              70              75              80
Pro Ala Lys Asp Ser Leu Ser Phe Glu Asp Phe Leu Asp Leu Leu Ser
              85              90              95
Val Phe Ser Asp Thr Ala Thr Pro Asp Ile Lys Ser His Tyr Ala Phe
              100              105              110
Arg Ile Phe Asp Phe Asp Asp Asp Gly Thr Leu Asn Arg Glu Asp Leu
              115              120              125
Ser Arg Leu Val Asn Cys Leu Thr Gly Glu Gly Glu Asp Thr Arg Leu
              130              135              140
Ser Ala Ser Glu Met Lys Gln Leu Ile Asp Tyr Ile Leu Glu Glu Ser
145              150              155              160
Asp Ile Asp Arg Asp Gly Thr Ile Asn Leu Ser Glu Phe Gln His Val
              165              170              175
Ile Ser Arg Ser Pro Asp Phe Ala Ser Ser Phe Lys Ile Val Leu
              180              185              190

```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 170 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Gly Asn Glu Ala Ser Tyr Pro Leu Glu Met Cys Ser His Phe Asp
 1              5              10              15
Ala Asp Glu Ile Lys Arg Leu Gly Lys Arg Phe Lys Lys Leu Asp Leu
              20              25              30
Asp Asn Ser Gly Ser Leu Ser Val Glu Glu Phe Met Ser Leu Pro Glu
              35              40              45
Leu Gln Gln Asn Pro Leu Val Gln Arg Val Ile Asp Ile Phe Asp Thr
              50              55              60
Asp Gly Asn Gly Glu Val Asp Phe Lys Glu Phe Ile Glu Gly Val Ser
 65              70              75              80
Gln Phe Ser Val Lys Gly Asp Lys Glu Gln Lys Leu Arg Phe Ala Phe
              85              90              95

```

WO 98/31796

PCT/US98/00671

Arg Ile Tyr Asp Met Asp Lys Asp Gly Tyr Ile Ser Asn Gly Glu Leu
 100 105 110
 Phe Gln Val Leu Lys Met Met Val Gly Asn Asn Leu Lys Asp Thr Gln
 115 120 125
 Leu Gln Gln Ile Val Asp Lys Thr Ile Ile Asn Ala Asp Lys Asp Gly
 130 135 140
 Asp Gly Arg Ile Ser Phe Glu Glu Phe Cys Ala Val Val Gly Gly Leu
 145 150 155 160
 Asp Ile His Lys Lys Met Val Val Asp Val
 165 170

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 172 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Ser Asn Phe Lys Lys Ala Asn Met Ala Ser Ser Ser Gln Arg
 1 5 10 15
 Lys Arg Met Ser Pro Lys Pro Glu Leu Thr Glu Glu Gln Lys Gln Glu
 20 25 30
 Ile Arg Glu Ala Phe Asp Leu Phe Asp Ala Asp Gly Thr Gly Thr Ile
 35 40 45
 Asp Val Lys Glu Leu Lys Val Ala Met Arg Ala Leu Gly Phe Glu Pro
 50 55 60
 Lys Lys Glu Glu Ile Lys Lys Met Ile Ser Glu Ile Asp Lys Glu Gly
 65 70 75 80
 Thr Gly Lys Met Asn Phe Gly Asp Phe Leu Thr Val Met Thr Gln Lys
 85 90 95
 Met Ser Glu Lys Asp Thr Lys Glu Glu Ile Leu Lys Ala Phe Lys Leu
 100 105 110
 Phe Asp Asp Asp Glu Thr Gly Lys Ile Ser Phe Lys Asn Leu Lys Arg
 115 120 125
 Val Ala Lys Glu Leu Gly Glu Asn Leu Thr Asp Glu Glu Leu Gln Glu
 130 135 140
 Met Ile Asp Glu Ala Asp Arg Asp Gly Asp Gly Glu Val Ser Glu Gln
 145 150 155 160
 Glu Phe Leu Arg Ile Met Lys Lys Thr Ser Leu Tyr
 165 170

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

WO 98/31796

PCT/US98/00671

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTGAGGATCC AAAGTCTTCT TGAGATGCAT C

31

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGACGAATTC CTATCATACA AAGTC

25

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1308 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 16..1098

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCCGCGAACT GCATC ATG GAG AAT TTT GTA GCC ACC TTG GCT AAT GGG ATG	51
Met Glu Asn Phe Val Ala Thr Leu Ala Asn Gly Met	
195 200	
AGC CTC CAG CCG CCT CTT GAA GAA GTG TCC TGT GGC CAG GCG GAA AGC	99
Ser Leu Gln Pro Pro Leu Glu Glu Val Ser Cys Gly Gln Ala Glu Ser	
205 210 215	
AGT GAG AAG CCC AAC GCT GAG GAC ATG ACA TCC AAA GAT TAC TAC TTT	147
Ser Glu Lys Pro Asn Ala Glu Asp Met Thr Ser Lys Asp Tyr Tyr Phe	
220 225 230 235	
GAC TCC TAC GCA CAC TTT GGC ATC CAC GAG GAG ATG CTG AAG GAC GAG	195
Asp Ser Tyr Ala His Phe Gly Ile His Glu Glu Met Leu Lys Asp Glu	
240 245 250	
GTG CGC ACC CTC ACT TAC CGC AAC TCC ATG TTT CAT AAC CGG CAC CTC	243
Val Arg Thr Leu Thr Tyr Arg Asn Ser Met Phe His Asn Arg His Leu	
255 260 265	
TTC AAG GAC AAG GTG GTG CTG GAC GTC GGC TCG GGC ACC GGC ATC CTC	291
Phe Lys Asp Lys Val Val Leu Asp Val Gly Ser Gly Thr Gly Ile Leu	
270 275 280	
TGC ATG TTT GCT GCC AAG GCC GGG GCC CGC AAG GTC ATC GGG ATC GAG	339
Cys Met Phe Ala Ala Lys Ala Gly Ala Arg Lys Val Ile Gly Ile Glu	
285 290 295	
TGT TCC AGT ATC TCT GAT TAT GCG GTG AAG ATC GTC AAA GCC AAC AAG	387
Cys Ser Ser Ile Ser Asp Tyr Ala Val Lys Ile Val Lys Ala Asn Lys	
300 305 310 315	

WO 98/31796

PCT/US98/00671

TTA	GAC	CAC	GTG	GTG	ACC	ATC	ATC	AAG	GGG	AAG	GTG	GAG	GAG	GTG	GAG	435
Leu	Asp	His	Val	Val	Thr	Ile	Ile	Lys	Gly	Lys	Val	Glu	Glu	Val	Glu	
			320						325					330		
CTC	CCA	GTG	GAG	AAG	GTG	GAC	ATC	ATC	ATC	AGC	GAG	TGG	ATG	GGC	TAC	483
Leu	Pro	Val	Glu	Lys	Val	Asp	Ile	Ile	Ile	Ser	Glu	Trp	Met	Gly	Tyr	
			335					340					345			
TGC	CTC	TTC	TAC	GAG	TCC	ATG	CTC	AAC	ACC	GTG	CTC	TAT	GCC	CGG	GAC	531
Cys	Leu	Phe	Tyr	Glu	Ser	Met	Leu	Asn	Thr	Val	Leu	Tyr	Ala	Arg	Asp	
		350					355					360				
AAG	TGG	CTG	GCG	CCC	GAT	GGC	CTC	ATC	TTC	CCA	GAC	CGG	GCC	ACG	CTG	579
Lys	Trp	Leu	Ala	Pro	Asp	Gly	Leu	Ile	Phe	Pro	Asp	Arg	Ala	Thr	Leu	
	365					370					375					
TAT	GTG	ACG	GCC	ATC	GAG	GAC	CGC	CAG	TAC	AAA	GAC	TAC	AAG	ATC	CAC	627
Tyr	Val	Thr	Ala	Ile	Glu	Asp	Arg	Gln	Tyr	Lys	Asp	Tyr	Lys	Ile	His	
380					385					390					395	
TGG	TGG	GAG	AAC	GTG	TAT	GGC	TTC	GAC	ATG	TCT	TGC	ATC	AAA	GAT	GTG	675
Trp	Trp	Glu	Asn	Val	Tyr	Gly	Phe	Asp	Met	Ser	Cys	Ile	Lys	Asp	Val	
			400					405						410		
GCC	ATT	AAG	GAG	CCC	CTA	GTG	GAT	GTC	GTG	GAC	CCC	AAA	CAG	CTG	GTC	723
Ala	Ile	Lys	Glu	Pro	Leu	Val	Asp	Val	Val	Asp	Pro	Lys	Gln	Leu	Val	
			415					420					425			
ACC	AAC	GCC	TGC	CTC	ATA	AAG	GAG	GTG	GAC	ATC	TAT	ACC	GTC	AAG	GTG	771
Thr	Asn	Ala	Cys	Leu	Ile	Lys	Glu	Val	Asp	Ile	Tyr	Thr	Val	Lys	Val	
		430					435					440				
GAA	GAC	CTG	ACC	TTC	ACC	TCC	CCG	TTC	TGC	CTG	CAA	GTG	AAG	CGG	AAT	819
Glu	Asp	Leu	Thr	Phe	Thr	Ser	Pro	Phe	Cys	Leu	Gln	Val	Lys	Arg	Asn	
	445					450					455					
GAC	TAC	GTG	CAC	GCC	CTG	GTG	GCC	TAC	TTC	AAC	ATC	GAG	TTC	ACA	CGC	867
Asp	Tyr	Val	His	Ala	Leu	Val	Ala	Tyr	Phe	Asn	Ile	Glu	Phe	Thr	Arg	
460					465					470					475	
TGC	CAC	AAG	AGG	ACC	GGC	TTC	TCC	ACC	AGC	CCC	GAG	TCC	CCG	TAC	ACG	915
Cys	His	Lys	Arg	Thr	Gly	Phe	Ser	Thr	Ser	Pro	Glu	Ser	Pro	Tyr	Thr	
				480					485					490		
CAC	TGG	AAG	CAG	ACG	GTG	TTC	TAC	ATG	GAG	GAC	TAC	CTG	ACC	GTG	AAG	963
His	Trp	Lys	Gln	Thr	Val	Phe	Tyr	Met	Glu	Asp	Tyr	Leu	Thr	Val	Lys	
			495					500					505			
ACG	GGC	GAG	GAG	ATC	TTC	GGC	ACC	ATC	GGC	ATG	CGG	CCC	AAC	GCC	AAG	1011
Thr	Gly	Glu	Glu	Ile	Phe	Gly	Thr	Ile	Gly	Met	Arg	Pro	Asn	Ala	Lys	
		510					515					520				
AAC	AAC	CGG	GAC	CTG	GAC	TTC	ACC	ATC	GAC	CTG	GAC	TTC	AAG	GGC	CAG	1059
Asn	Asn	Arg	Asp	Leu	Asp	Phe	Thr	Ile	Asp	Leu	Asp	Phe	Lys	Gly	Gln	
	525					530					535					
CTG	TGC	GAG	CTG	TCC	TGC	TCC	ACC	GAC	TAC	CGG	ATG	CGC	TGAGGCCCCG			1108
Leu	Cys	Glu	Leu	Ser	Cys	Ser	Thr	Asp	Tyr	Arg	Met	Arg				
540					545					550						
CTCTCCCGCC	CTGCACGAGC	CCAGGGGCTG	AGCGTTCCTA	GGCGGTTTCG	GGGCTCCCCC											1168
TTCCTCTCCC	TCCCTCCCGC	AGAAGGGGGT	TTTAGGGGCC	TGGGCTGGGG	GGATGGGGAG											1228
GGCACATTGG	GACTGTGTTT	TTCATAAATT	ATGTTTTTAT	ATGGTTGCAT	TTAATGCCAA											1288
TAAATCCTCA	GCTGGGGAAA															1308

WO 98/31796

PCT/US98/00671

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 361 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Glu	Asn	Phe	Val	Ala	Thr	Leu	Ala	Asn	Gly	Met	Ser	Leu	Gln	Pro	1	5	10	15
Pro	Leu	Glu	Glu	Val	Ser	Cys	Gly	Gln	Ala	Glu	Ser	Ser	Glu	Lys	Pro	20	25	30	
Asn	Ala	Glu	Asp	Met	Thr	Ser	Lys	Asp	Tyr	Tyr	Phe	Asp	Ser	Tyr	Ala	35	40	45	
His	Phe	Gly	Ile	His	Glu	Glu	Met	Leu	Lys	Asp	Glu	Val	Arg	Thr	Leu	50	55	60	
Thr	Tyr	Arg	Asn	Ser	Met	Phe	His	Asn	Arg	His	Leu	Phe	Lys	Asp	Lys	65	70	75	80
Val	Val	Leu	Asp	Val	Gly	Ser	Gly	Thr	Gly	Ile	Leu	Cys	Met	Phe	Ala	85	90	95	
Ala	Lys	Ala	Gly	Ala	Arg	Lys	Val	Ile	Gly	Ile	Glu	Cys	Ser	Ser	Ile	100	105	110	
Ser	Asp	Tyr	Ala	Val	Lys	Ile	Val	Lys	Ala	Asn	Lys	Leu	Asp	His	Val	115	120	125	
Val	Thr	Ile	Ile	Lys	Gly	Lys	Val	Glu	Glu	Val	Glu	Leu	Pro	Val	Glu	130	135	140	
Lys	Val	Asp	Ile	Ile	Ile	Ser	Glu	Trp	Met	Gly	Tyr	Cys	Leu	Phe	Tyr	145	150	155	160
Glu	Ser	Met	Leu	Asn	Thr	Val	Leu	Tyr	Ala	Arg	Asp	Lys	Trp	Leu	Ala	165	170	175	
Pro	Asp	Gly	Leu	Ile	Phe	Pro	Asp	Arg	Ala	Thr	Leu	Tyr	Val	Thr	Ala	180	185	190	
Ile	Glu	Asp	Arg	Gln	Tyr	Lys	Asp	Tyr	Lys	Ile	His	Trp	Trp	Glu	Asn	195	200	205	
Val	Tyr	Gly	Phe	Asp	Met	Ser	Cys	Ile	Lys	Asp	Val	Ala	Ile	Lys	Glu	210	215	220	
Pro	Leu	Val	Asp	Val	Val	Asp	Pro	Lys	Gln	Leu	Val	Thr	Asn	Ala	Cys	225	230	235	240
Leu	Ile	Lys	Glu	Val	Asp	Ile	Tyr	Thr	Val	Lys	Val	Glu	Asp	Leu	Thr	245	250	255	
Phe	Thr	Ser	Pro	Phe	Cys	Leu	Gln	Val	Lys	Arg	Asn	Asp	Tyr	Val	His	260	265	270	
Ala	Leu	Val	Ala	Tyr	Phe	Asn	Ile	Glu	Phe	Thr	Arg	Cys	His	Lys	Arg	275	280	285	

WO 98/31796

PCT/US98/00671

Thr Gly Phe Ser Thr Ser Pro Glu Ser Pro Tyr Thr His Trp Lys Gln
 290 295 300

Thr Val Phe Tyr Met Glu Asp Tyr Leu Thr Val Lys Thr Gly Glu Glu
 305 310 315 320

Ile Phe Gly Thr Ile Gly Met Arg Pro Asn Ala Lys Asn Asn Arg Asp
 325 330 335

Leu Asp Phe Thr Ile Asp Leu Asp Phe Lys Gly Gln Leu Cys Glu Leu
 340 345 350

Ser Cys Ser Thr Asp Tyr Arg Met Arg
 355 360

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 353 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ala Ala Ala Glu Ala Ala Asn Cys Ile Met Glu Val Ser Cys Gly
 1 5 10 15

Gln Ala Glu Ser Ser Glu Lys Pro Asn Ala Glu Asp Met Thr Ser Lys
 20 25 30

Asp Tyr Tyr Phe Asp Ser Tyr Ala His Phe Gly Ile His Glu Glu Met
 35 40 45

Leu Lys Asp Glu Val Arg Thr Leu Thr Tyr Arg Asn Ser Met Phe His
 50 55 60

Asn Arg His Leu Phe Lys Asp Lys Val Val Leu Asp Val Gly Ser Gly
 65 70 75 80

Thr Gly Ile Leu Cys Met Phe Ala Ala Lys Ala Gly Ala Arg Lys Val
 85 90 95

Ile Gly Ile Glu Cys Ser Ser Ile Ser Asp Tyr Ala Val Lys Ile Val
 100 105 110

Lys Ala Asn Lys Leu Asp His Val Val Thr Ile Ile Lys Gly Lys Val
 115 120 125

Glu Glu Val Glu Leu Pro Val Glu Lys Val Asp Ile Ile Ile Ser Glu
 130 135 140

Trp Met Gly Tyr Cys Leu Phe Tyr Glu Ser Met Leu Asn Thr Val Leu
 145 150 155 160

His Ala Arg Asp Lys Trp Leu Ala Pro Asp Gly Leu Ile Phe Pro Asp
 165 170 175

Arg Ala Thr Leu Tyr Val Thr Ala Ile Glu Asp Arg Gln Tyr Lys Asp
 180 185 190

Tyr Lys Ile His Trp Trp Glu Asn Val Tyr Gly Phe Asp Met Ser Cys
 195 200 205

WO 98/31796

PCT/US98/00671

Ile Lys Asp Val Ala Ile Lys Glu Pro Leu Val Asp Val Val Asp Pro
 210 215 220
 Lys Gln Leu Val Thr Asn Ala Cys Leu Ile Lys Glu Val Asp Ile Tyr
 225 230 235 240
 Thr Val Lys Val Glu Asp Leu Thr Phe Thr Ser Pro Phe Cys Leu Gln
 245 250 255
 Val Lys Arg Asn Asp Tyr Val His Ala Leu Val Ala Tyr Phe Asn Ile
 260 265 270
 Glu Phe Thr Arg Cys His Lys Arg Thr Gly Phe Ser Thr Ser Pro Glu
 275 280 285
 Ser Pro Tyr Thr His Trp Lys Gln Thr Val Phe Tyr Met Glu Asp Tyr
 290 295 300
 Leu Thr Val Lys Thr Gly Glu Glu Ile Phe Gly Thr Ile Gly Met Arg
 305 310 315 320
 Pro Asn Ala Lys Asn Asn Arg Asp Leu Asp Phe Thr Ile Asp Leu Asp
 325 330 335
 Phe Lys Gly Gln Leu Cys Glu Leu Ser Cys Ser Thr Asp Tyr Arg Met
 340 345 350
 Arg

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 360 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Glu Asn Phe Val Ala Thr Leu Ala Asn Gly Met Ser Leu Gln Pro
 1 5 10 15
 Pro Leu Glu Glu Val Ser Cys Gly Gln Ala Glu Ser Ser Glu Lys Pro
 20 25 30
 Asn Ala Glu Asp Met Thr Ser Lys Asp Tyr Tyr Phe Asp Ser Tyr Ala
 35 40 45
 His Phe Gly Ile His Glu Glu Met Leu Lys Asp Glu Val Arg Thr Leu
 50 55 60
 Thr Tyr Arg Asn Ser Met Phe His Asn Arg His Leu Phe Lys Asp Lys
 65 70 75 80
 Val Val Leu Asp Val Gly Ser Gly Thr Gly Ile Leu Cys Met Phe Ala
 85 90 95
 Ala Lys Ala Gly Ala Arg Lys Val Ile Gly Ile Val Cys Ser Ser Ile
 100 105 110
 Ser Asp Tyr Ala Val Lys Ile Val Lys Ala Asn Lys Leu Asp His Val
 115 120 125

WO 98/31796

PCT/US98/00671

Val	Thr	Ile	Ile	Lys	Gly	Lys	Val	Glu	Glu	Val	Glu	Leu	Pro	Val	Glu				
130						135					140								
Lys	Val	Ala	Ser	Ser	Ser	Ala	Ser	Gly	Trp	Ala	Thr	Ala	Ser	Ser	Thr				
145					150					155					160				
Ser	Pro	Cys	Ser	Thr	Pro	Cys	Ser	Met	Pro	Gly	Thr	Ser	Val	Ala	Pro				
				165					170					175					
Asp	Gly	Leu	Ile	Phe	Pro	Asp	Arg	Ala	Thr	Leu	Tyr	Val	Thr	Ala	Ile				
			180					185					190						
Glu	Asp	Arg	Gln	Tyr	Lys	Asp	Tyr	Lys	Ile	His	Trp	Trp	Glu	Asn	Val				
		195					200					205							
Tyr	Gly	Phe	Asp	Met	Ser	Cys	Ile	Lys	Asp	Val	Ala	Ile	Lys	Glu	Pro				
	210					215					220								
Leu	Val	Asp	Val	Val	Asp	Pro	Lys	Gln	Leu	Val	Thr	Asn	Ala	Cys	Leu				
225					230					235					240				
Ile	Lys	Glu	Val	Asp	Ile	Tyr	Thr	Val	Lys	Val	Glu	Asp	Leu	Thr	Phe				
				245					250					255					
Thr	Ser	Pro	Phe	Cys	Leu	Gln	Val	Lys	Arg	Asn	Asp	Tyr	Val	His	Ala				
			260					265					270						
Leu	Val	Ala	Tyr	Phe	Asn	Ile	Glu	Phe	Thr	Arg	Cys	His	Lys	Arg	Thr				
		275					280					285							
Gly	Phe	Ser	Thr	Ser	Pro	Glu	Ser	Pro	Tyr	Thr	His	Trp	Lys	Gln	Thr				
	290					295					300								
Val	Phe	Tyr	Met	Glu	Asp	Tyr	Leu	Thr	Val	Lys	Thr	Gly	Glu	Glu	Ile				
305					310					315					320				
Phe	Gly	Thr	Ile	Gly	Met	Arg	Pro	Asn	Ala	Lys	Asn	Asn	Arg	Asp	Leu				
				325					330					335					
Asp	Phe	Thr	Ile	Asp	Leu	Asp	Phe	Lys	Gly	Gln	Leu	Cys	Glu	Leu	Ser				
			340					345					350						
Cys	Ser	Thr	Asp	Tyr	Arg	Met	Arg												
		355					360												

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly	Gly	Phe	Gly	Gly	Arg	Gly	Gly	Phe	Gly
1			5					10	

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

WO 98/31796

PCT/US98/00671

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGCTACAAAA TTCTCCATGA TG

22

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGGCCGTCAC ATACAGCGTG G

21

WHAT IS CLAIMED IS:

1. A recombinant DNA molecule comprising a nucleotide sequence encoding an IFNAR1 receptor binding protein capable of being induced by IFN- α or IFN- β and having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8.
2. A recombinant DNA molecule in accordance with claim 1, further comprising a promoter operably-linked to said sequence encoding an IFNAR1 receptor-binding protein in a sense orientation such that said promoter is capable of expressing said protein when the recombinant DNA molecule is in an appropriate expression host.
3. A recombinant DNA molecule according to claim 2, wherein said promoter is a strong constitutive promoter in human cells.
4. A recombinant DNA molecule in accordance with claim 1, wherein said nucleotide sequence is SEQ ID NO:1 or SEQ ID NO:7.
5. A recombinant DNA or RNA molecule comprising a DNA molecule comprising the nucleotide sequence SEQ ID NO:1 or SEQ ID NO:7, in either sense or anti-sense orientation, or a fragment thereof capable of hybridizing to mRNA encoding an interferon inducible IFNAR1 receptor binding protein and thereby preventing its expression, or an RNA molecule of a sequence corresponding to that of said DNA molecule.
6. A recombinant DNA or RNA molecule in accordance with claim 5, comprising an RNA molecule.
7. A recombinant DNA or RNA molecule in accordance with claim 5, wherein said sequence is in the sense orientation.
8. A recombinant DNA or RNA molecule in accordance with claim 5, wherein said sequence is in the anti-sense orientation.
9. A recombinant DNA or RNA molecule in accordance with claim 8, comprising a recombinant DNA molecule further comprising a promoter operably linked to said sequence in an anti-sense orientation such that said

promoter is capable of expressing an anti-sense RNA complementary to a whole or part of a sense RNA encoding an interferon-induced IFNAR1 receptor binding protein.

10. A recombinant DNA molecule according to claim 9, wherein said nucleotide sequence is a segment from the 5'-end of SEQ ID NO:1 or SEQ ID NO:7.

11. A recombinant DNA molecule according to any one of claims 2, 9 and 10, wherein said promoter is an interferon-inducible promoter.

12. A recombinant DNA molecule according to any one of claims 1-5 and 7-10, which is an expression vector.

13. A host cell capable of expressing an anti-sense RNA complementary to a sense RNA encoding an interferon-inducible IFNAR1 receptor binding protein, which cell includes an expression vector in accordance with claim 12.

14. A method of prolonging tissue graft survival, comprising the steps of:

introducing an expression vector comprising the recombinant DNA molecule of any one of claims 8-10 into cells of a tissue or organ to be grafted to a patient in need thereof; and

grafting the tissue or organ to the patient.

15. A method of enhancing response to exogenous interferon therapy in treating cancer, comprising the steps of:

administering a pharmaceutical composition comprising an expression vector comprising the recombinant DNA molecule of claim 3 or claim 4, and a pharmaceutically acceptable excipient directly into a tumor; and

subsequently administering exogenous interferon therapy.

16. An IFNAR1-binding protein having the sequence of SEQ ID NO:2 or SEQ ID NO:8.

17. A molecule comprising the antigen binding portion of an antibody specific for an IFNAR1-binding protein in accordance with claim 16.

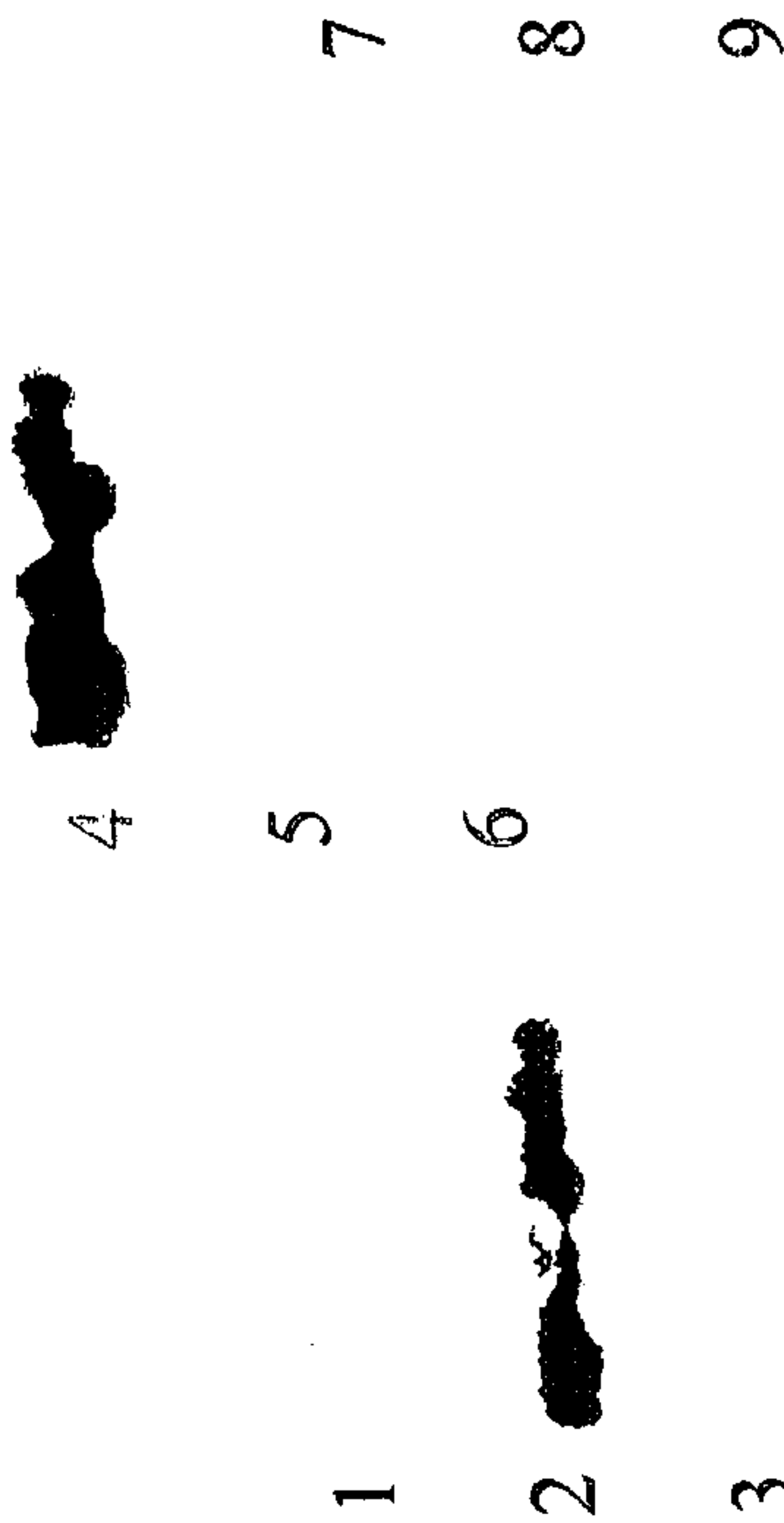
18. A molecule according to claim 17, consisting of a monoclonal antibody.

WO 98/31796

PCT/US98/00671

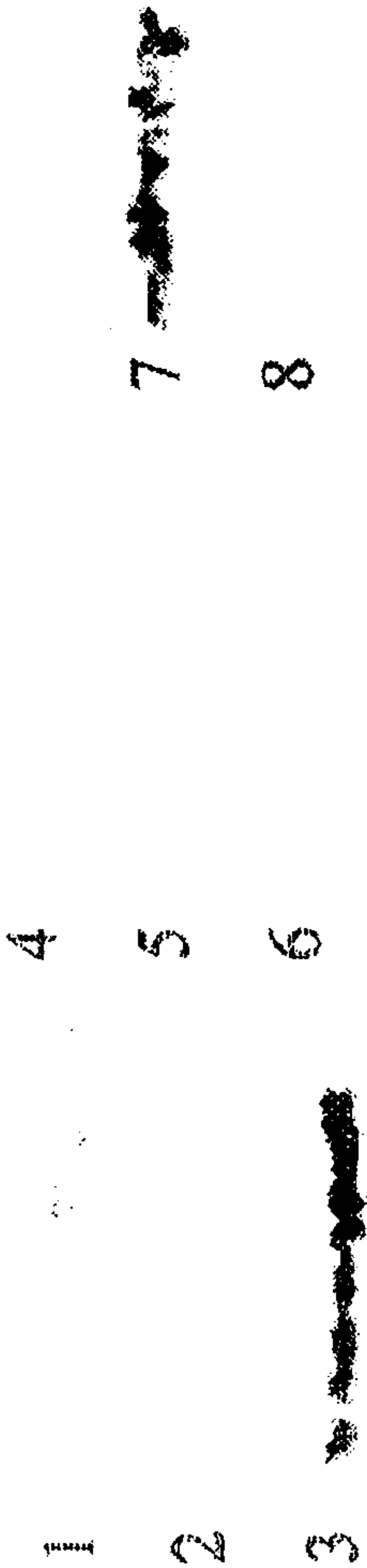
19. A method for providing an IFNAR1 receptor binding protein comprising placing a DNA in accordance with claim 1 into an appropriate expression host in a manner whereby expression of said protein can be obtained upon culturing of said host, and culturing said host so as to obtain expression of said protein.

FIG. 1



Number	pACT	pAS or pGBT10	Number	pACT	pAS or pGBT10
1	IR1B1	laminin	5	IR1B1	rev
2	IR1B1	IFNARI-IC	6	IR1B1	cdk
3	vector	IFNARI-IC	7	IR1B1	tat
4	-lacZ control -		8	IR1B1	p53
			9	IR1B1	vector

FIG. 2



Number	pACT	pAS or pGBT10	Number	pACT	pAS or pGBT10
1	IRIB4	vector	5	IRIB4	tat
2	IRIB4	p53	6	IRIB4	rev
3	IRIB4	IFNARI-IC	7	-	lacZ control -
4	IRIB4	cdk	8	IRIB4	lamin

WO 98/31796

PCT/US98/00671

3 / 13

FIG. 3

CGT CTC GAG GCG AGT TGG CGG AGC TGT GCG CGC GGC GGG GCG ATG GGG GGC TCG GGC AGT	60
MET GLY GLY SER GLY SER	
CGC CTG TCC AAG GAG CTG CTG GCC GAG TAC CAG GAC TTG ACG TTC CTG ACG AAG CAG GAG	120
ARG LEU SER LYS GLU LEU LEU ALA GLU TYR GLN ASP LEU THR PHE LEU THR LYS GLN GLU	
ATC CTC CTA GCC CAC AGG CGG TTT TGT GAG CTG CTT CCC CAG GAG CAG CGG AGC GTG GAG	180
ILE LEU LEU ALA HIS ARG ARG PHE CYS GLU LEU LEU PRO GLN GLU GLN ARG SER VAL GLU	
TCG TCA CTT CGG GCA CAA GTG CCC TTC GAG CAG ATT CTC AGC CTT CCA GAG CTC AAG GCC	240
SER SER LEU ARG ALA GLN VAL PRO PHE GLU GLN ILE LEU SER LEU PRO GLU LEU LYS ALA	
AAC CCC TTC AAG GAG CGA ATC TGC AGG GTC TTC TCC ACA TCC CCA GCC AAA GAC AGC CTT	300
ASN PRO PHE LYS GLU ARG ILE CYS ARG VAL PHE SER THR SER PRO ALA LYS ASP SER LEU	
AGC TTT GAG GAC TTC CTG GAT CTC CTC AGT GTG TTC AGT GAC ACA GCC ACG CCA GAC ATC	360
SER PHE GLU ASP PHE LEU ASP LEU LEU SER VAL PHE SER ASP THR ALA THR PRO ASP ILE	
AAG TCC CAT TAT GCC TTC CGC ATC TTT GAC TTT GAT GAT GAC GGA ACC TTG AAC AGA GAA	420
LYS SER HIS TYR ALA PHE ARG ILE PHE ASP PHE ASP ASP ASP GLY THR LEU ASN ARG GLU	
GAC CTG AGC CGG CTG GTG AAC TGC CTC ACG GGA GAG GGC GAG GAC ACA CGG CTT AGT GCG	480
ASP LEU SER ARG LEU VAL ASN CYS LEU THR GLY GLU GLY GLU ASP THR ARG LEU SER ALA	
TCT GAG ATG AAG CAG CTC ATC GAC TAC ATC CTG GAA GAG TCT GAC ATT GAC AGG GAT GGA	540
SER GLU MET LYS GLN LEU ILE ASP TYR ILE LEU GLU GLU SER ASP ILE ASP ARG ASP GLY	
ACC ATC AAC CTC TCT GAG TTC CAG CAC GTC ATC TCC CGT TCT CCA GAC TTT GCC AGC TCC	600
THR ILE ASN LEU SER GLU PHE GLN HIS VAL ILE SER ARG SER PRO ASP PHE ALA SER SER	
TTT AAG ATT GTC CTG TGA CAG CAG CCC CAG CGT GTG TCC TGG CAC CCT GTC CAA GAA CCT	660
PHE LYS ILE VAL LEU	
TTC TAC TGC TGA GCT GTG GCC AAG GTC AAG CCT GTG TTG CCA GTG CGG GCC AAG CTG GCC	720
CAG CCT GGA GCT GGC GCT GTG CAG CCT CAC CCC GGG CAG GGG CGG CCC TCG TTG TCA GGG	780
CCT CTC CTC ACT GCT GTT GTC ATT GCT CCG TTT GTG GGC CTT CGT GGC CA	830

SUBSTITUTE SHEET (RULE 26)

WO 98/31796

PCT/US98/00671

4 / 13

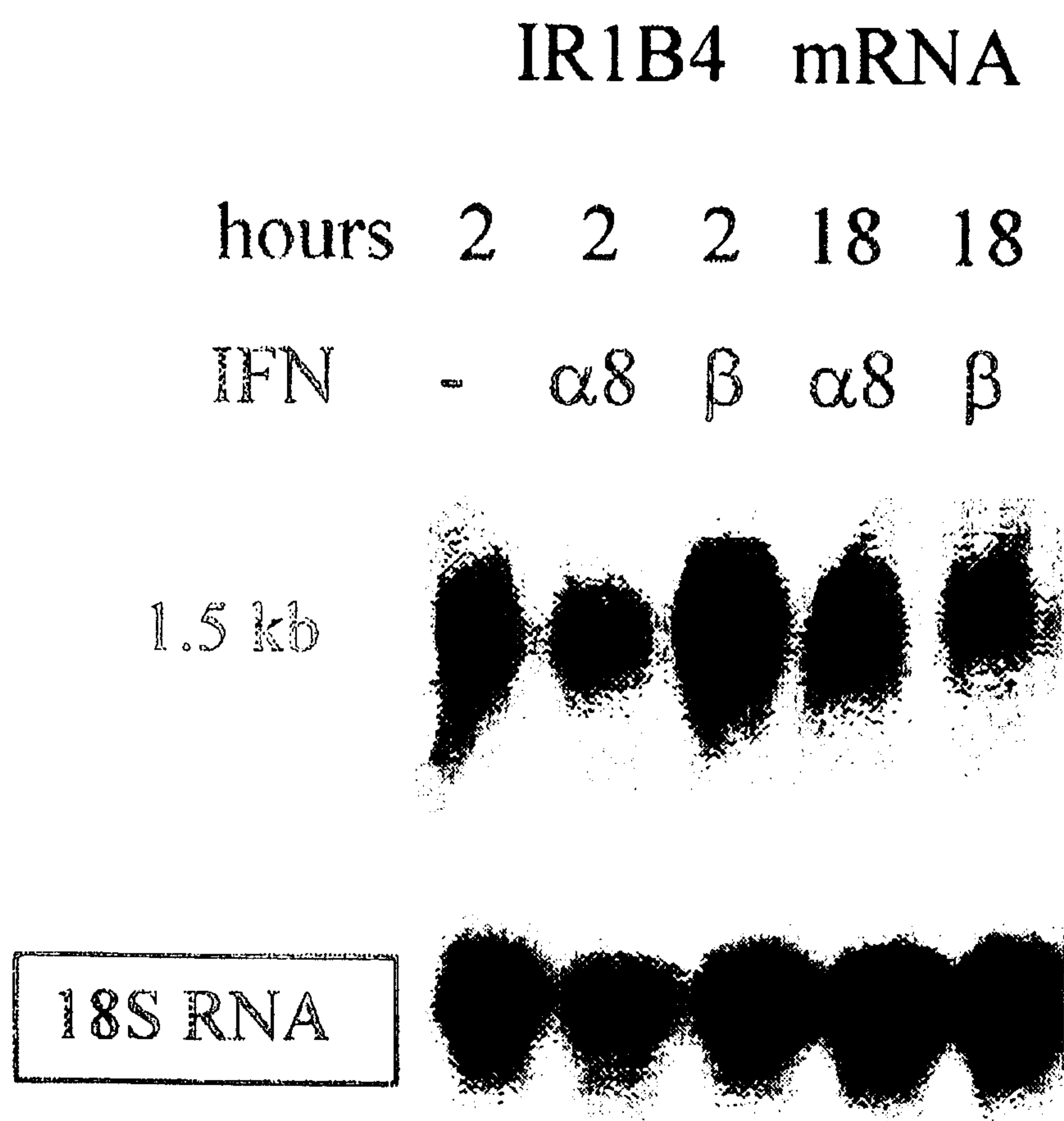
FIG. 4

Alignment of IR1B1 with Calcineurin B (Calb) and Caltractin (Catr):

IR1B1		MRLSKELLEAYQDLTELTKQEILLAH
CALB		GNEASYPLEMCSHFDA
CATR		MASNFKKANMASSSQRKRMSPKPELTE
IR1B1	26	RRFCELLPQEQRSSVESSLRAQVPFEQILSLPELKANPFKERICRVFSTSP
		: : :
CALB	17	DEIKRLGKRFKKLDLDNSGSLSVVEEF.MSLPELQQNPL...VQRVIDIF.
CATR	28	EQKQEIREAFDLFDADGTGTIDVKELKVAMRALGFEPKKEEIKKMISEI.
IR1B1	76	AKDSLSEFEDFLDLLSVFSDTAT.PDIKS..HYAFRIFDFDDDGTNLNREDLS
		: : :
CALB	62	DTDGNGEVDFKEFIEGVSQFSVKGDKEQKLRFARIYDMDKDGYSNGELF
CATR	76	DKEGTGKMNFGDFTVMTQKMSEKDTKEEILKAFKLFDDDETGKISFKNLK
IR1B1	124	RLVNCLTGEGEDTRLSASEMKQLIDYILEESDIDRDGTINLSEFQHVI
		:
CALB	113	QVLKMMVGNNLKDQQLQIYDKTIIN....ADKGDGRISFEEFCVV
CATR	128	RVAKEL.GENLTDEELQEMIDE.....ADRDGDGEVSEQEFLRIM
IR1B1	172	SRSPDFASSFKILV 185
CALB	157	GGLDIHKKMVVDV 169
CATR	167	KKTSLY 172

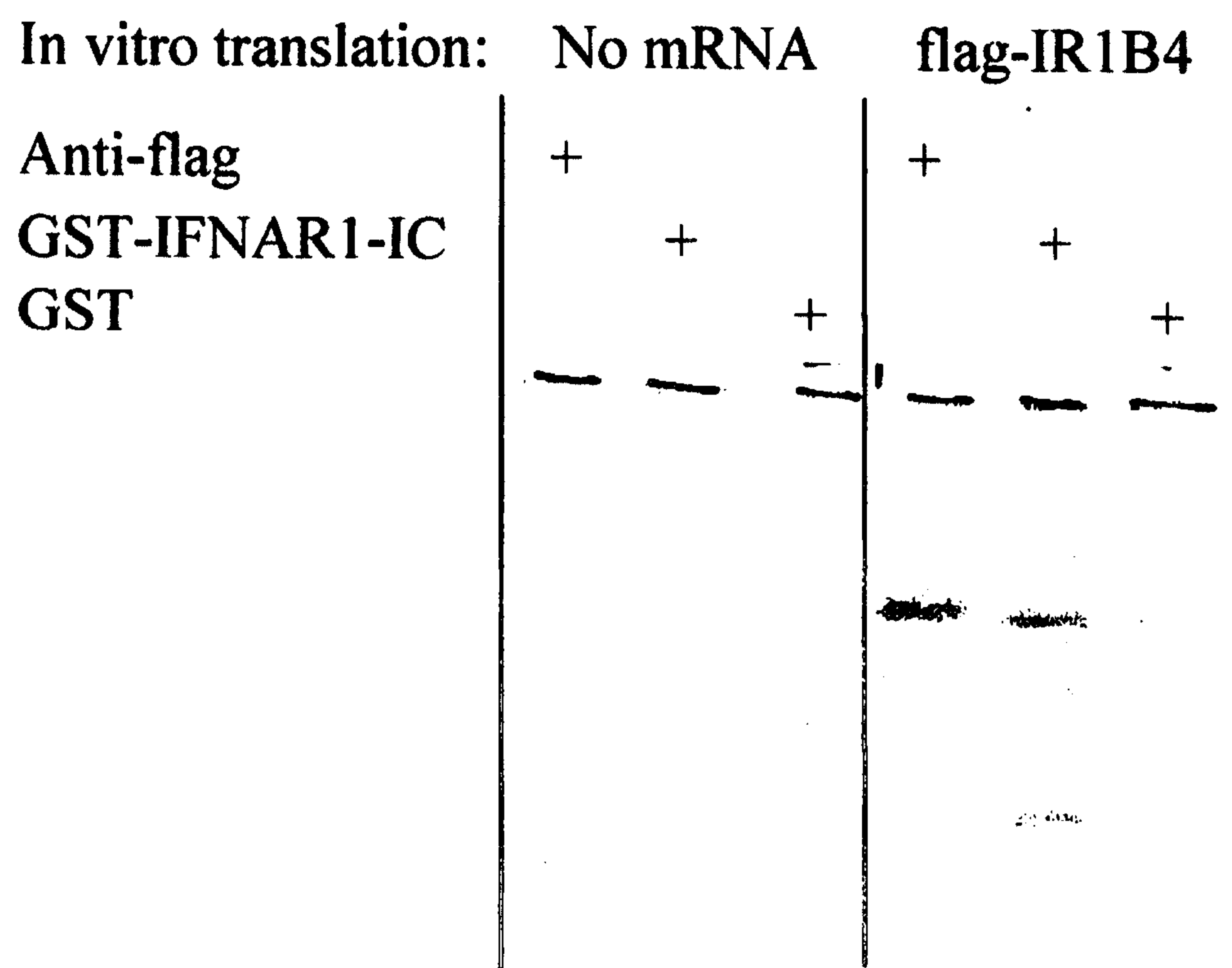
5 / 13

FIG. 5



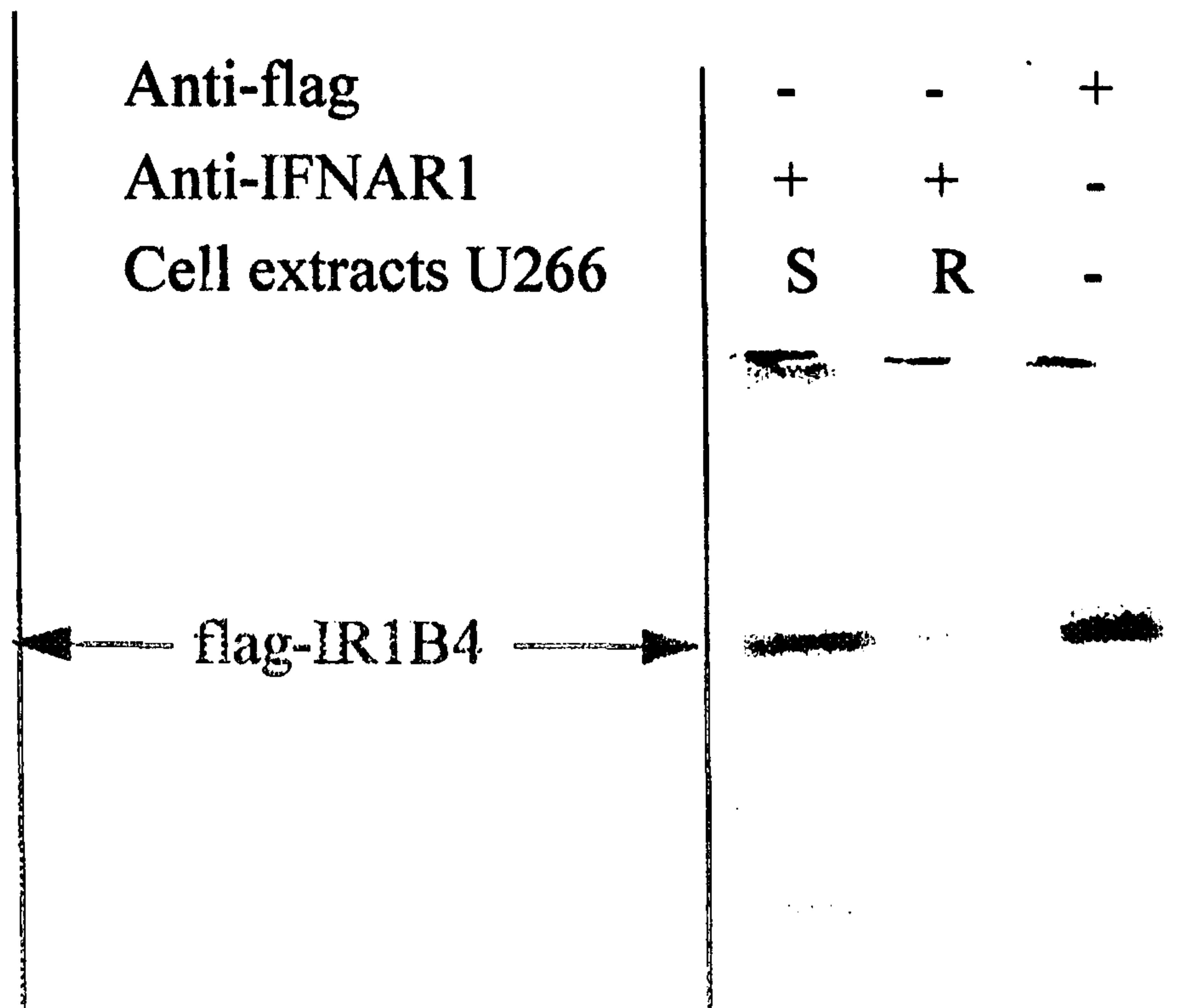
6 / 13

FIG. 6A



7 / 13

FIG. 6B



WO 98/31796

PCT/US98/00671

8 / 13

FIG. 7

1	GCC	GCG	AAC	TGC	ATC	ATG	GAG	AAT	TTT	GTA	GCC	ACC	TTG	GCT	42
						M	E	N	F	V	A	T	L	A	
43	AAT	GGG	ATG	AGC	CTC	CAG	CCG	CCT	CTT	GAA	GAA	GTG	TCC	TGT	84
	N	G	M	S	L	Q	P	P	L	E	E	V	S	C	
85	GGC	CAG	GCG	GAA	AGC	AGT	GAG	AAG	CCC	AAC	GCT	GAG	GAC	ATG	126
	G	Q	A	E	S	S	E	K	P	N	A	E	D	M	
127	ACA	TCC	AAA	GAT	TAC	TAC	TTT	GAC	TCC	TAC	GCA	CAC	TTT	GGC	168
	T	S	K	D	Y	Y	F	D	S	Y	A	H	F	G	
169	ATC	CAC	GAG	GAG	ATG	CTG	AAG	GAC	GAG	GTG	CGC	ACC	CTC	ACT	210
	I	H	E	E	M	L	K	D	E	V	R	T	L	T	
211	TAC	CGC	AAC	TCC	ATG	TTT	CAT	AAC	CGG	CAC	CTC	TTC	AAG	GAC	252
	Y	R	N	S	M	F	H	N	R	H	L	F	K	D	
253	AAG	GTG	GTG	CTG	GAC	GTC	GGC	TCG	GGC	ACC	GGC	ATC	CTC	TGC	294
	K	V	V	L	D	V	G	S	G	T	G	I	L	C	
295	ATG	TTT	GCT	GCC	AAG	GCC	GGG	GCC	CGC	AAG	GTC	ATC	GGG	ATC	336
	M	F	A	A	K	A	G	A	R	K	V	I	G	I	
337	GAG	TGT	TCC	AGT	ATC	TCT	GAT	TAT	GCG	GTG	AAG	ATC	GTC	AAA	378
	E	C	S	S	I	S	D	Y	A	V	K	I	V	K	
379	GCC	AAC	AAG	TTA	GAC	CAC	GTG	GTG	ACC	ATC	ATC	AAG	GGG	AAG	420
	A	N	K	L	D	H	V	V	T	I	I	K	G	K	
421	GTG	GAG	GAG	GTG	GAG	CTC	CCA	GTG	GAG	AAG	GTG	GAC	ATC	ATC	462
	V	E	E	V	E	L	P	V	E	K	V	D	I	I	
463	ATC	AGC	GAG	TGG	ATG	GGC	TAC	TGC	CTC	TTC	TAC	GAG	TCC	ATG	504
	I	S	E	W	M	G	Y	C	L	F	Y	E	S	M	
505	CTC	AAC	ACC	GTG	CTC	TAT	GCC	CGG	GAC	AAG	TGG	CTG	GCG	CCC	546
	L	N	T	V	L	Y	A	R	D	K	W	L	A	P	
547	GAT	GGC	CTC	ATC	TTC	CCA	GAC	CGG	GCC	ACG	CTG	TAT	GTG	ACG	588
	D	G	L	I	F	P	D	R	A	T	L	Y	V	T	
589	GCC	ATC	GAG	GAC	CGG	CAG	TAC	AAA	GAC	TAC	AAG	ATC	CAC	TGG	630
	A	I	E	D	R	Q	Y	K	D	Y	K	I	H	W	
631	TGG	GAG	AAC	GTG	TAT	GGC	TTC	GAC	ATG	TCT	TGC	ATC	AAA	GAT	672
	W	E	N	V	Y	G	F	D	M	S	C	I	K	D	
673	GTG	GCC	ATT	AAG	GAG	CCC	CTA	GTG	GAT	GTC	GTG	GAC	CCC	AAA	714
	V	A	I	K	E	P	L	V	D	V	V	D	P	K	
715	CAG	CTG	GTC	ACC	AAC	GCC	TGC	CTC	ATA	AAG	GAG	GTG	GAC	ATC	756
	Q	L	V	T	N	A	C	L	I	K	E	V	D	I	
757	TAT	ACC	GTC	AAG	GTG	GAA	GAC	CTG	ACC	TTC	ACC	TCC	CCG	TTC	798
	Y	T	V	K	V	E	D	L	T	F	T	S	P	F	
799	TGC	CTG	CAA	GTG	AAG	CGG	AAT	GAC	TAC	GTG	CAC	GCC	CTG	GTG	840
	C	L	Q	V	K	R	N	D	Y	V	H	A	L	V	
841	GCC	TAC	TTC	AAC	ATC	GAG	TTC	ACA	CGC	TGC	CAC	AAG	AGG	ACC	882
	A	Y	F	N	I	E	F	T	R	C	H	K	R	T	
883	GGC	TTC	TCC	ACC	AGC	CCC	GAG	TCC	CCG	TAC	ACG	CAC	TGG	AAG	924
	G	F	S	T	S	P	E	S	P	Y	T	H	W	K	
925	CAG	ACG	GTG	TTC	TAC	ATG	GAG	GAC	TAC	CTG	ACC	GTG	AAG	ACG	966
	Q	T	V	F	Y	M	E	D	Y	L	T	V	K	T	
967	GGC	GAG	GAG	ATC	TTC	GGC	ACC	ATC	GGC	ATG	CGG	CCC	AAC	GCC	1008
	G	E	E	I	F	G	T	I	G	M	R	P	N	A	
1009	AAG	AAC	AAC	CGG	GAC	CTG	GAC	TTC	ACC	ATC	GAC	CTG	GAC	TTC	1050
	K	N	N	R	D	L	D	F	T	I	D	L	D	F	
1051	AAG	GGC	CAG	CTG	TGC	GAG	CTG	TCC	TGC	TCC	ACC	GAC	TAC	CGG	1092
	K	G	Q	L	C	E	L	S	C	S	T	D	Y	R	
1093	ATG	CGC	TGA	GGC	CCG	GCT	CTC	CCG	CCC	TGC	ACG	AGC	CCA	GGG	1134
	M	R													
1135	GCT	GAG	CGT	TCC	TAG	GCG	GTT	TCG	GGG	CTC	CCC	CTT	CCT	CTC	1176
1177	CCT	CCC	TCC	CGC	AGA	AGG	GGG	TTT	TAG	GGG	CCT	GGG	CTG	GGG	1218
1219	GGA	TGG	GGA	GGG	CAC	ATT	GGG	ACT	GTG	TTT	TTC	ATA	AAT	TAT	1260
1261	GTT	TTT	ATA	TGG	TTG	CAT	TTA	ATG	CCA	ATA	AAT	CCT	CAG	CTG	1302
1303	GGG	AAA													1308

SUBSTITUTE SHEET (RULE 26)

FIG. 8

	10	20	30	40	50	60
IR1B4	MENFVATLANGMGLQPPL EEVSCGQAESSEKPN AEDMTSKDYYFDSYAHFGIHEEMLKDE					
PRMT1	MA-----AAEAANCIMEVSCGQAESSEKPN AEDMTSKDYYFDSYAHFGIHEEMLKDE					
	10	20	30	40	50	
	70	80	90	100	110	120
IR1B4	VRTLT YRNSMFHNRHLFKDKV VLDVGSGTGILCMFAAKAGARKVIGIECSSISDYAVKIV					
PRMT1	VRTLT YRNSMFHNRHLFKDKV VLDVGSGTGILCMFAAKAGARKVIGIECSSISDYAVKIV					
	60	70	80	90	100	110
	130	140	150	160	170	180
IR1B4	KANKLDHVVTIIKGKVEEVELPVEKVDII ISEWMGYCLFYESMLNTVLYARDKWLAPDGL					
PRMT1	KANKLDHVVTIIKGKVEEVELPVEKVDII ISEWMGYCLFYESMLNTVLHARDKWLAPDGL					
	120	130	140	150	160	170
	190	200	210	220	230	240
IR1B4	IFPDRATLYVTAIEDRQYKDYKIHWWENVYGFDMSCIKDVAIKEPLVDVDPKQLVTNAC					
PRMT1	IFPDRATLYVTAIEDRQYKDYKIHWWENVYGFDMSCIKDVAIKEPLVDVDPKQLVTNAC					
	180	190	200	210	220	230
	250	260	270	280	290	300
IR1B4	LIKEVDIYTVKVEDLTFTSPFCLQVKRNDYVHALVAYFNIEFTRCHKRTGFSTSPESPYT					
PRMT1	LIKEVDIYTVKVEDLTFTSPFCLQVKRNDYVHALVAYFNIEFTRCHKRTGFSTSPESPYT					
	240	250	260	270	280	290
	310	320	330	340	350	360
IR1B4	HWKQTVFYMEDYLT VKTGEEIFGTIGMRPN AKNNRDLDF TIDLDFK GQLCELS CSTDYRMR					
PRMT1	HWKQTVFYMEDYLT VKTGEEIFGTIGMRPN AKNNRDLDF TIDLDFK GQLCELS CSTDYRMR					
	300	310	320	330	340	350

WO 98/31796

PCT/US98/00671

10 / 13

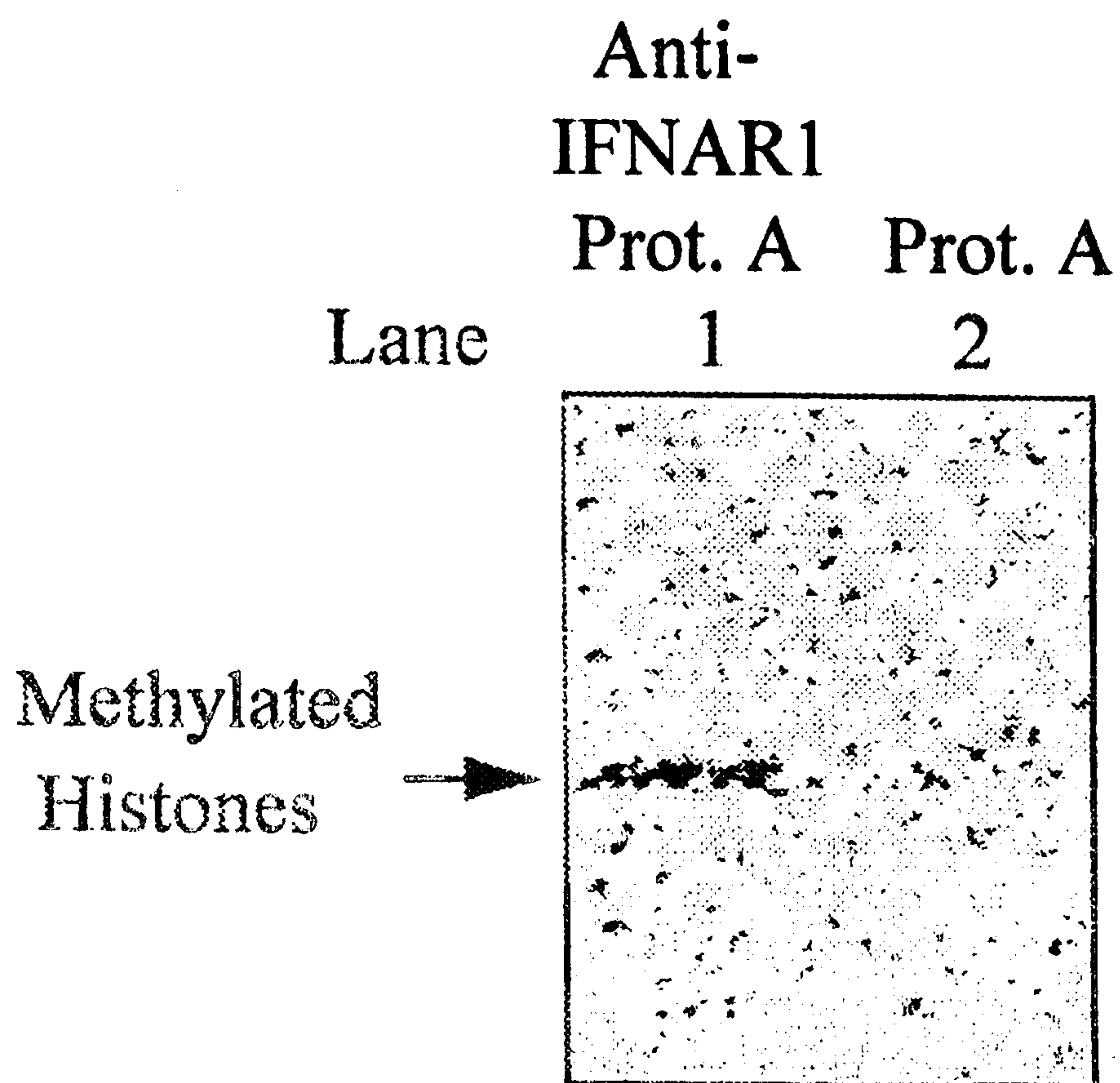
FIG. 9

	10	20	30	40	50	60	
IR1B4	MENFVATLANGMGLQPPLEEVSCGQAESSEKPNAEDMTSKDYYFDSYAHFGIHEEMLKDE						
	::						
HCP-1	MENFVATLANGMGLQPPLEEVSCGQAESSEKPNAEDMTSKDYYFDSYAHFGIHEEMLKDE						
	10	20	30	40	50		
	70	80	90	100	110	120	
IR1B4	VRTLTyrNSMFHNRHLFKDKVVLdVGSGTGILCMFAAKAGARKVIGIECSSISDYAVKIV						
	::						
HCP-1	VRTLTyrNSMFHNRHLFKDKVVLdVGSGTGILCMFAAKAGARKVIGIECSSISDYAVKIV						
	70	80	90	100	110	120	
	130	140	150	160	170	120	
IR1B4	KANKLDHVVTIIKGKVEEVELPVEKVDIII-SEWMGYCLFYESMLNTVLYARDKWLPDg						
	::						
HCP-1	KANKLDHVVTIIKGKVEEVELPVEKVDIII-SEWMGYCLFYESMLNTVLYARDKWLPDg						
	130	140	150	160	170	120	
	180	190	200	210	220	230	
IR1B4	LIFPD RATLYVTAIEDRQYKDYKIHWVENYGFDMSCIKDVAIKEPLVDVDPKQLVTNA						
	::						
HCP-1	LIFPD RATLYVTAIEDRQYKDYKIHWVENYGFDMSCIKDVAIKEPLVDVDPKQLVTNA						
	180	190	200	210	220	230	
	240	250	260	270	280	290	
IR1B4	CLIKEVDIYTVKVEDLTFTSPFCLQVKRNDYVHALVAYFNIEFTRCHKRTGFSTSPESPY						
	::						
HCP-1	CLIKEVDIYTVKVEDLTFTSPFCLQVKRNDYVHALVAYFNIEFTRCHKRTGFSTSPESPY						
	240	250	260	270	280	290	
	300	310	320	330	340	350	360
IR1B4	THWKQTVFYMEDYLTvKTGEEIFGTIGMRPNakNNRDLdFTIDLDFKGQLCELSCSTDYRM						
	::						
HCP-1	THWKQTVFYMEDYLTvKTGEEIFGTIGMRPNakNNRDLdFTIDLDFKGQLCELSCSTDYRM						
	300	310	320	330	340	350	360

SUBSTITUTE SHEET (RULE 26)

11 / 13

FIG. 10



WO 98/31796

PCT/US98/00671

12 / 13

FIG. 11

Oligonucleotide: None Anti sense Sense

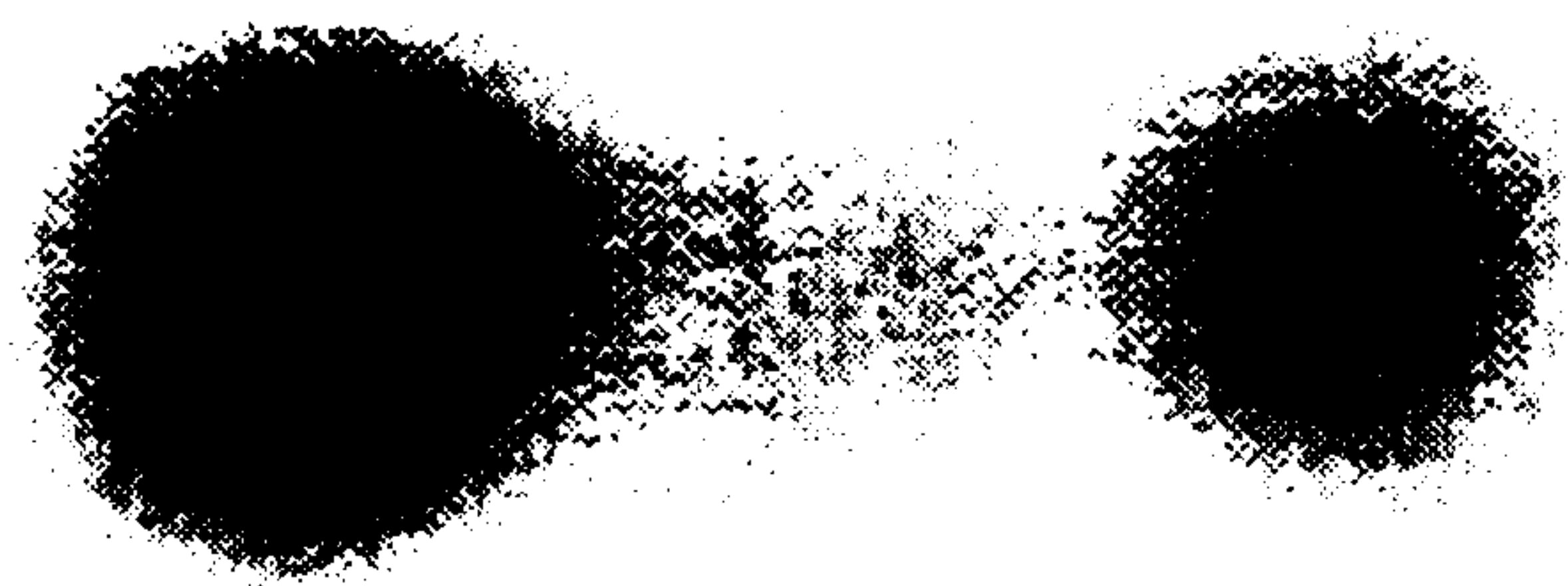
Lane

1

2

3

Peptide R1



13 / 13

FIG. 12

