Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2575800 C 2018/06/05

(11)(21) **2 575 800**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) **Date de dépôt PCT/PCT Filing Date:** 2005/08/26
(87) **Date publication PCT/PCT Publication Date:** 2006/03/02
(45) **Date de délivrance/Issue Date:** 2018/06/05
(85) **Entrée phase nationale/National Entry:** 2007/02/01
(86) **N° demande PCT/PCT Application No.:** EP 2005/009247
(87) **N° publication PCT/PCT Publication No.:** 2006/021453
(30) **Priorité/Priority:** 2004/08/27 (EP04020455.4)

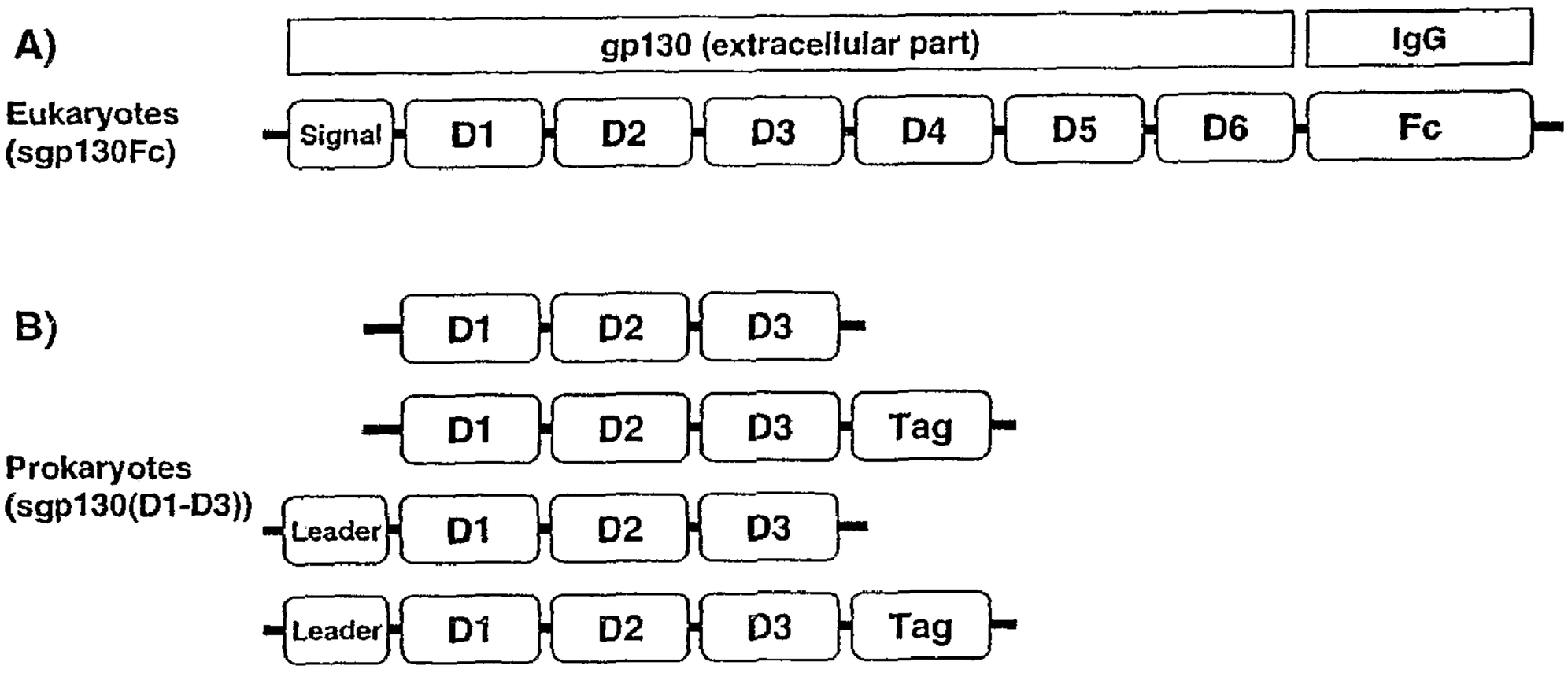
(51) **Cl.Int./Int.Cl.** ***C12N 15/67*** (2006.01),
C07K 14/715 (2006.01)

(72) **Inventeurs/Inventors:**
SEEGERT, DIRK, DE;
WAETZIG, GEORG, DE;
RAHAUS, NIKOLAUS, DE;
DAECKE, JESSICA, DE;
ROSE-JOHN, STEFAN, DE

(73) **Propriétaire/Owner:**
CONARIS RESEARCH INSTITUTE AG, DE

(74) **Agent:** SMART & BIGGAR

(54) **Titre : SEQUENCES NUCLEOTIDIQUES OPTIMISEES CODANT SGP 130**
(54) **Title: OPTIMIZED NUCLEOTIDE SEQUENCES ENCODING SGP130**



(57) **Abrégé/Abstract:**
Described are codon optimized sgpl30 encoding nucleic acid molecules as well as a method for the highly efficient recombinant production of sgpl30 in mammalian cells or bacteria using a nucleic acid molecule of the invention.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 March 2006 (02.03.2006)

PCT

(10) International Publication Number
WO 2006/021453 A3

(51) International Patent Classification:
C12N 15/67 (2006.01) *C07K 14/715* (2006.01)

(21) International Application Number:
PCT/EP2005/009247

(22) International Filing Date: 26 August 2005 (26.08.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
04020455.4 27 August 2004 (27.08.2004) EP

(71) Applicant *(for all designated States except US)*: **CONARIS RESEARCH INSTITUTE AG** [DE/DE]; Schauenburgerstrasse 116, 24118 Kiel (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **SEEGERT, Dirk** [DE/DE]; Freesenberg 148, 24161 Altenholz (DE). **WÄTZIG, Georg** [DE/DE]; Heiderstrasse 19, 24106 Kiel (DE). **RAHAUS, Nikolaus** [DE/DE]; Brammerkamp 12, 24159 Kiel (DE). **DAECKE, Jessica** [DE/DE]; Jungmannstrasse, 24118 Kiel (DE). **ROSE-JOHN, Stefan** [DE/DE]; Theodor-Heuss-Weg 6, 24211 Schellhorn (DE).

(74) Agent: **SCHÜSSLER, Andrea**; Huber & Schüssler, Truderinger Strasse 246, 81825 Munich (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

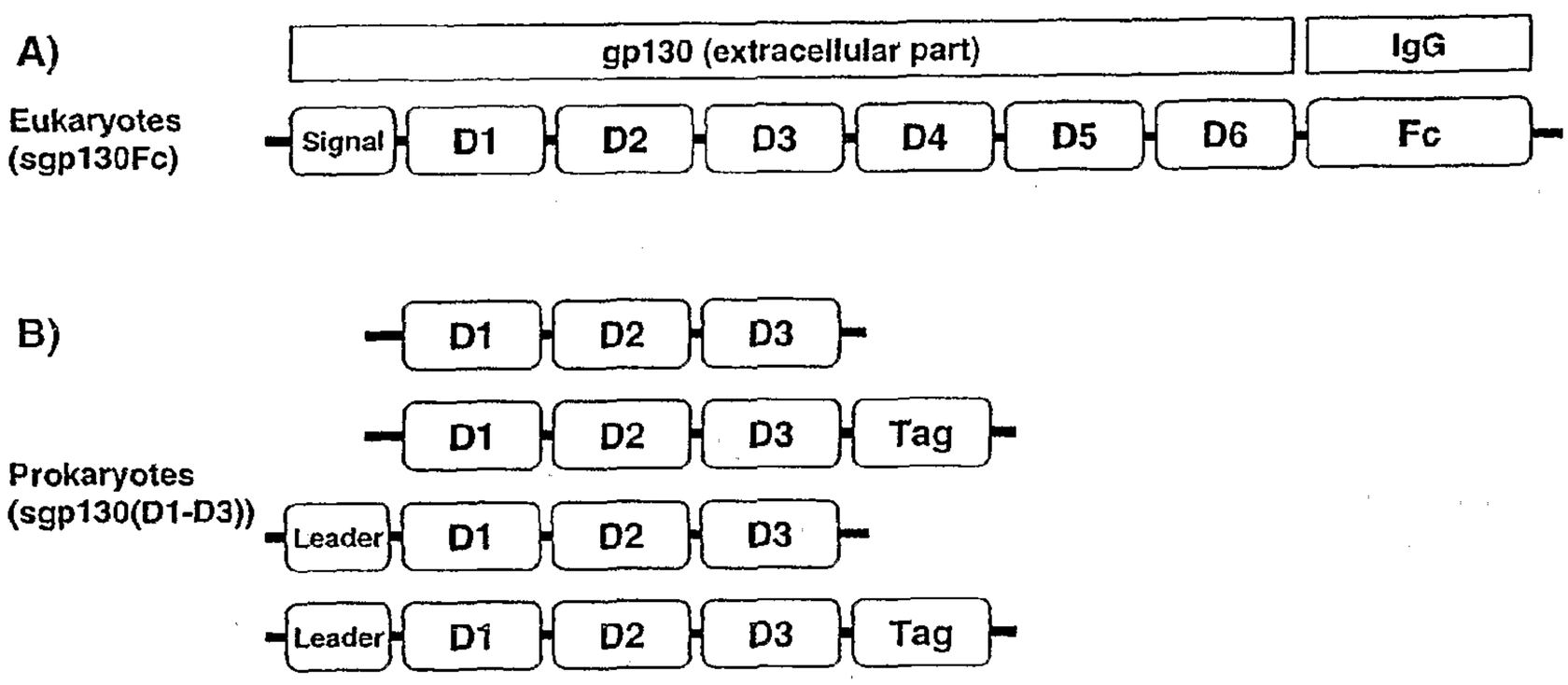
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
22 June 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: OPTIMIZED NUCLEOTIDE SEQUENCES ENCODING SGP 130



(57) Abstract: Described are codon optimized sgpl30 encoding nucleic acid molecules as well as a method for the highly efficient recombinant production of sgpl30 in mammalian cells or bacteria using a nucleic acid molecule of the invention.

WO 2006/021453 A3

Optimized nucleotide sequences encoding sgp130

The present invention relates to codon optimized sgp130 encoding nucleic acid molecules as well as a method for the highly efficient recombinant production of sgp130 in mammalian cells or bacteria using a nucleic acid molecule of the invention.

For the treatment of various diseases such as Crohn's disease etc. the specific blocking of IL-6 responses dependent on soluble IL-6R might be desirable for treatment. It was found that a soluble gp130-dimer, in particular an IgG-Fc fusion protein or a PEGylated version of spg130, efficiently inhibits the anti-apoptotic effect of sIL-6R from LPMC from Crohn's disease (CD) patients and that, thus, said compound is useful for the treatment of said disease and related diseases like, e.g., colitis or rheumatoid arthritis. Unfortunately, so far the recombinant production of sgp130 is difficult in particular due to the fact that only low amounts of protein can be obtained

Thus, the technical problem underlying the present invention was to provide means allowing to improve the efficiency of recombinant production of sgp130Fc or sgp130(D1-D3).

During the experiments leading to the present invention it was found that by use of particular codon optimized versions of the DNA encoding sgp130Fc the yields of recombinant protein can be increased at least 10- to 20-fold compared to the unmodified version of the DNA. In case of the prokaryotic sgp130(D1-D39 version, the optimization of the DNA led to the reduction of undesired shorter side products.

Summary of the invention

In one aspect of the invention, there is provided a nucleic acid molecule encoding sgp130 comprising (a) the nucleic acid sequence as depicted in SEQ ID No. 7 (sgp130(D1-3)_opt) or (b) a fragment thereof which maintains the ability to inhibit the activity of the agonistic complex Il-6/sIl-6R.

In another aspect, there is provided a nucleic acid molecule encoding sgp130 comprising (a) the nucleic acid sequence as depicted in SEQ ID No. 12 (sgp130Fc_opt), wherein at least 80% of the codons altered in the nucleic acid sequence of SEQ ID No. 12 vs. the wild type sequence are present, or (b) a fragment thereof which encodes a polypeptide that comprises extracellular domains D1 to D3 of sgp130, wherein the encoded polypeptide inhibits the activity of the agonistic complex IL-6/sIL-6R.

In another aspect of the invention, there is provided an expression vector containing a nucleic acid molecule of the present invention.

In another aspect of the invention, there is provided a host cell containing an expression vector of the present invention.

In another aspect of the invention, there is provided a method of producing a sgp130 polypeptide comprising culturing a host cell of the present invention and recovering the polypeptide from said host cell or the culture.

Brief description of the drawings

Figure 1: Schematic presentation of the constructs

Grey shedding marks the parts of the protein which have been optimized. **(A)** Eukaryotic construct comprising a signal peptide, six extracellular gp130 domains and the IgG-Fc part. **(B)** Variations of the sgp130 protein expressed in prokaryotic cells. sgp130 can be expressed with or without N-terminal leader sequence and/or C-terminal Tag for purification purposes.

Figure 2: sgp130(D1-D3) (nucleotide sequence and amino acid sequence) for expression in bacterial cells

An alignment of the nucleotide sequence with optimized codons (sgp130(D1-D3)_opt) vs. the original sequence (sgp130(D1-D3)_wt) is shown.

Figure 3: sgp130(Fc) (nucleotide sequence and amino acid sequence) for expression in mammalian cells

An alignment of the nucleotide sequence with optimized codons (sgp130Fc_opt) vs. the original sequence (sgp130Fc_wt) is shown.

Figure 4: Detection of sgp130Fc after transient transfection of HEK293 cells (A) or CHO cells (B) with wildtype or optimized (opt) sgp130Fc expression plasmids

The position of sgp130Fc is indicated by arrows (◂). Wildtype and optimized sgp130Fc expression was detected in two independent transfection experiments each. The different sizes of the protein (left panel) result from the leader sequence which has been partially cleaved off after secretion into the medium. The right panel represents the results derived from

whole cell extracts from CHO cells (sgp130Fc with leader sequence).

Figure 5: Detection of RNA transcribed from transfected plasmid DNA (sgp130Fc; neomycin resistance gene (NeoR)) by gene-specific RT-PCR

HEK293 cells were transfected with an expression plasmid encoding either wildtype or optimized sgp130Fc. Transfection of the empty vector (mock) or non-transfected cells (control) served as negative controls. β-actin was amplified from total RNA to demonstrate the use of equal amounts of RNA in each single experiment.

Figure 6: Expression of sgp130(D1-D3) in BL21(DE3)pLys bacteria

The cDNA encoding sgp130(D1-D3) was cloned into the expression plasmid pET22b (Invitrogen, Carlsbad, CA, USA) which in addition encodes a leading pelB sequence and a C-terminal His-tag. sgp130(D1-D3) was detected by western blot with a His-specific antibody and marked with an arrow (◀).

Figure 7: Comparison and selection of different optimized sgp130Fc sequences

The wt cDNA encoding sgp130Fc was optimized using different optimization algorithms ("Java Codon Adaption Tool" (JCat, available at http://www.prodoric.de/JCat/ and "UpGene" Gao et al. (2004), Biotechnol. Proc. 20(2): 443-8) and the resulting expression constructs were transfected into HEK293 cells. The expression of sgp130Fc was determined on RNA level by RT-PCR and on protein level by western blot as described earlier. The figure represents an example of the comparison of two different optimized sgp130Fc sequences (Opt1 and Opt2) with a sgp130Fc sequence optimized according to the present invention (Opt CONARIS).

Figure 8: Alignment of different optimized sgp130Fc cDNA sequences and comparison with the unmodified wild type (wt) sequence

The shaded regions are showing a few examples of codons which either have been left unmodified (grey) as compared to the wild type (wt) or have been changed (partly in different ways) by the algorithms.

Figure 9: The table indicates the numbers of base pairs which are different between two compared sequences

sgp130Fc sequence optimized according to the present invention (opt_CON), JCat (opt_JCat) or UpGene (opt_Upgene).

Thus, the present invention relates to a nucleic acid molecule encoding sgp130 comprising the nucleic acid sequence (a) as depicted in Figure 2 (sgp130(D1-3)_opt) or Figure 3 (sgp130Fc_opt) or (b) a fragment or analogue thereof which maintains the codon usage pattern thereof.

The letter "s" of sgp130 means "soluble". The term "soluble" as used herein refers to a gp130 molecule lacking the intracellular domain and the transmembrane domain. The domains utilized in sgp130(D1-D3)_opt consist of the first three extracellular domains D1-D3 of gp130.

The term "fragment" as used herein refers to sgp130 fragments which comprise the entire or smaller parts of the optimized cDNA encoding the extracellular domain of gp130. Preferably, such fragments show a biological activity of the full length molecule, e. g. maintain the ability to inhibit the activity of the agonistic complex IL-6/sIL-6R. For the expression in bacteria such fragment also comprises sgp130 without the eukaryotic secretory leader sequence (MLTLQTWVVQALFIFLTTESTG,

Pos. 1 to 22). Moreover, a prokaryotic secretory leader sequence, e. g. *pelB* or *OmpA* could be cloned in front of the sgp130 sequence or parts of it and could be derived from the respective suitable expression plasmid, e. g. pET22b (Merck Biosciences GmbH, Bad Soden, Germany), pASK-IBA2, pASK-IBA12 (IBA, Goettingen, Germany). In addition the sgp130 protein can be expressed with or without Tag for purification purposes, e. g. His_6, Strep, Myc or others.

The term "analogue" as used herein refers to a nucleic acid molecule which encodes the same amino acid sequence but which, through the redundancy of the genetic code, has a different nucleotide sequence. The term "codon usage pattern" as used herein refers to the average frequencies in the nucleotide sequence, e.g., highly expressed mammalian genes. Codon usage patterns for mammals, including humans can be found in the literature; see, e.g., Nakamura et al., Nucleic Acids Research 1996, 24:214-5. In the nucleic acid molecules of the present invention, the codon usage pattern is altered to more closely represent the codon bias of the host organism, e.g. a mammalian cell.

Alternatively, the present invention relates to a nucleic acid molecule, wherein at least 80%, preferably at least 90%, more preferably at least 95% and, most preferably at least 98% of the codons altered in the nucleic acid sequence of Figure 2 or 3 vs. the wild type sequence are present.

In a preferred embodiment, the nucleic acid molecule of the present invention is a DNA molecule.

The present invention includes expression vectors that comprise the nucleic acid molecules of the invention. The expression vectors can be constructed according to methods well known to the person skilled in the art; see, e.g.,

Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. The "control elements" or "regulatory sequences" used for recombinant expression are those non-translated regions of the vector-enhancers, promoters, 5' and 3' untranslated regions which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilised, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. Promoters and other expression regulation signals may be selected to be compatible with the host cell for which expression is designed. For example, mammalian promoters include the metallothionein promoter, which can be induced in response to heavy metals such as cadmium, and the ß-actin 30 promoter. Viral promoters such as the SV40 large T antigen promoter, human cytomegalovirus (CMV) immediate early (IE) promoter, rous sarcoma virus LTR promoter, adenovirus promoter, or a HPV promoter, particularly the HPV upstream regulatory region (URR) may also be used. All these promoters are well described and readily available in the art.

In mammalian host cells, a number of viral-based expression systems may be utilised. In cases where an adenovirus is used as an expression vector, sequences encoding the polypeptide(s) of the present invention may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the antibody in infected host cells (Logan, J. and Shenk, T. (1984) Proc. Natl. Acad. Sci. 81:3655-3659). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV)

enhancer, may be used to increase expression in mammalian host cells.

Further examples of suitable viral vectors, include herpes simplex viral vectors, vaccinia or alpha-virus vectors and retroviruses, including lentiviruses and adeno-associated viruses. Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the nucleic acid molecules of the invention into the host genome, although such recombination is not preferred. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient expression. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding the spg130 polypeptides, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6 to 10 M are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

Specific initiation signals may also be used to achieve more efficient translation. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the sgp130, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in case where only a coding sequence for a fragment is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert.

Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed polypeptide chains in the desired fashion. Post-translational processing which cleaves a "prepro" form of the polypeptide may also be used to facilitate correct insertion, folding and/or function. Different mammalian host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, 293, COS-7 and W138), are available from the American Type Culture Collection (ATCC; Bethesda, Md.) and may be chosen to ensure the correct modification and processing of the foreign polypeptide chains.

For long-term, high-yield production of sgp130, stable expression in mammalian cells is preferred. For example, cell lines which stably express sgp130Fc may be transfected using expression vectors which may contain viral origins of replication and/or endogenous expression elements and one or more selectable marker genes on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

After the introduction of the recombinant vector(s), the host cells are grown in a selective medium, which selects for the growth of vector-containing cells. Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. et al. (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. et al. (1980) Cell 22:817-23) genes which can be employed in tk.sup.- or aprt.sup.- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilise indole in place of tryptophan, or hisD, which allows cells to utilise histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. et al. (1995) Methods Mol. Biol. 55:121-131).

The person skilled also knows vectors and host cells for bacterial expression, e.g. bacteriophage, plasmid, or cosmid DNA expression vectors. Vectors suitable for use in the present invention include, but are not limited to the pSKK expression vector for expression in bacteria. Depending on the

vector system and host utilised, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript.RTM. phagemid (Stratagene, LaJolla, Calif.) or pSport1.TM. plasmid (Gibco BRL) and the like may be used.

Purification of the recombinant sgp130 is carried out by any one of the methods known for this purpose, i.e., any conventional procedure involving extraction, precipitation, chromatography, electrophoresis, or the like. A further purification procedure that may be used is affinity chromatography using monoclonal antibodies which bind the target polypeptide or a Tag fused to it, e.g., His, Strep or Myc, and which are produced and immobilized on a gel matrix contained within a column. Impure preparations containing the recombinant sgp130 are passed through the column. The sgp130 will be bound to the column by the specific antibody while the impurities will pass through. After washing the polypeptide is eluted from the gel by a change in pH or ionic strength and can then, if desired, dimerized and/or PEGylated.

Accordingly, the present invention also relates to a method of producing the sgp130 of the present invention, comprising culturing a host cell transformed with a nucleic acid molecule of the invention and recovering the sgp130 polypeptide from said host cell or the culture.

The sgp130 polypeptide produced from a nucleic acid molecule of the present invention is useful in the treatment and/or prevention of all the pathologies, in which the activity of the agonistic complex IL-6/slL6R must be inhibited, e.g., for the treatment/prevention of bone resorption, hypercalcemia,

cachexia, tumours, autoimmune diseases such as Crohn's disease and bacterial or viral infections.

The below examples explain the invention in more detail.

Example 1: Material and Methods

(A) Construct and transfection

The cDNA encoding either wildtype or optimized sgp130Fc was cloned into the expression plasmid pDEST40 (Invitrogen, Carlsbad, CA, USA) according to standard procedures. The wildtype sequence was derived from an expression plasmid which has been described in Jostock et al., Eur. J. Biochem. 268 (2001), 160-7 (Figure 1; upper panel). The construct was sequence verified. 3 x 10^5 HEK293 cells were transiently transfected with 1 μg of plasmid and 3 μl of Fugene (Roche Diagnostics, Mannheim, Germany) in 3 ml of medium according to the manufacturer's manual. The cells were subsequently incubated for 24 h at 37°C and supernatants and cells were harvested for further preparations of either total proteins or RNA, respectively.

One set of cells was transfected with the empty vector (mock), another set of cells was left untransfected (control). Both sets served as negative controls.

(B) Protein extraction and western blot

The sgp130Fc protein was precipitated from the cell supernatants by adding 20 μl of Protein-A/G-Plus Agarose (Santa Cruz, CA, USA). The slurry was incubated overnight at 4°C and finally centrifuged. Bound proteins were extracted by boiling the agarose pellet in SDS sample buffer for 5 minutes at 100°C. In parallel the cells were scraped from the plates

using a rubber policeman, harvested in 100 µl of PBS and centrifuged. All protein samples were separated on a standard acrylamide gel, transferred to a PDVF-membrane by semi-dry blotting and stained with a gp130-specific antibody (Hölzel Diagnostika, Köln, Germany). 50 ng of recombinant sgp130 served as positive control (sgp130).

(C) RNA extraction

Total RNA was extracted from the cell pellets using a RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. The following primers were used to determine RNA transcribed from the transfected plasmid DNA by RT-PCR: sgp130Fc_f: 5'-ATGAGGTGTGAGTGGGATGG-3'; sgp130Fc_r: 5'-ACCTTGCACTTGTACTCCTTGC-3'; neomycin resistance gene NeoR_s: 5'-GATGCCTGCTTGCCGAATATC-3'; NeoR_r: 5'-CGCCAAGCTCTTCAGCAATATC-3'. Total RNA was initially reverse transcribed and the cDNA was amplified by 30 cycles of 30 seconds at 95°C followed by 2 minutes at 57°C and a final elongation step of 5 minutes at 72°C. Expected amplicon sizes: gp130: 1.712 bp, NeoR: 133 bp. The amplification of NeoR was performed to document an equal transfection efficiency of the plasmid. In addition, β-actin was amplified to demonstrated the use of equal amounts of total RNA in each experiment.

Example 2: Highly efficient recombinant production of sgp130Fc in HEK293 cells

Figure 4 demonstrates that in comparison to the wildtype expression plasmid the production of sgp130Fc was increased at least 10 to 20-fold in HEK293 cells transfected with the optimized sgp130Fc expression plasmid. On the RNA level (Figure 5) a similar increase of sgp130Fc expression was detected with the optimized construct. This elevation of sgp130Fc RNA amounts was not due to a different transfection

efficiency as shown by equal amounts of RNA encoded by the neomycin resistance gene which was also located on the expression plasmid.

The results indicate that the significant increase of sgp130Fc production after optimization of the cDNA sequence is partially based on an improved codon usage during translation but is mainly derived from the elevation of the corresponding RNA levels. This might be due to a more efficient transcription or a higher stability of the RNA.

Example 3: Highly efficient recombinant production of sgp130(D1-D3) in bacteria

(A) Constructs and transformation

The cDNA encoding either wildtype or optimized sgp130(D1-D3) was cloned into the prokaryotic expression plasmid pET22b (Merck Biosciences GmbH, Bad Soden, Germany) according to standard procedures. The D1-D3 fragment was amplified afore from the pSVL-sgp130Fc plasmid described in Jostock et al. 2001. The construct was sequence verified and transformed into BL21(DE3)pLys bacteria (Invitrogen, Carlsbad, CA, USA).

(B) Protein expression and western blot

10 ml of bacterial suspension were diluted at 1:100 with LB-medium and grown at 30°C overnight until the $OD_{600\ nm}$ reached a value of 0.3 (250 rpm). Protein expression was induced by the addition of 0.3 mM of IPTG (Isopropyl-beta-D-thiogalactopyranoside) (Qiagen, Hilden, Germany) and further incubation of the cells overnight at 25°C. The cells were pelleted by centrifugation at 4°C and 4600 rpm for 30 minutes and the pellet was resuspended in 1 ml PBS (PAA Laboratories GmbH, Cölbe, Germany). Disruption of the cells was performed

by sonication (3 x 30 sec, 10% cycle, 20% power) with a Bandelin Sonoplus HD 2070 sonicator. Insoluble material was pelleted at 13.000 rpm and 4°C for 30 min and the pellet was resuspended in 1 ml of urea buffer (50 mM NaH_2PO_4, 8 M urea, pH8). An aliquot was diluted at 1:100 and subsequently analyzed by SDS PAGE according to standard protocols. His-tagged target proteins were detected with an anti-PentaHis antibody (Qiagen, Hilden, Germany).

(C) Results

Whereas the wildtype sequence generated a second shorter form of sgp130(D1-D3) (Figure 6, left lane) this by-product was not observed with the optimized cDNA (Figure 6, right lane). This unwanted variation of sgp130(D1-D3) is generated by further alternative transcriptional and translational start sides which have been eliminated by codon modifications in the optimized cDNA sequence. Subsequently the efficiency to produce the desired protein with the right size was increased at least at a factor of 3-fold.

Example 4: Identification of the best optimized sgp130Fc cDNA sequence

The wt sgp130Fc sequence was optimized using different optimization algorithms. The resulting sequences were synthesized, cloned into respective expression vectors and transfected into certain expression cells. The best sgp130Fc sequence was identified by detection of sgp130Fc expression on the RNA and the protein level. Figure 7 shows an example of these sets of experiments and demonstrates that although three different optimized sequences were used only one sequence (namely "Opt CONARIS"), i.e., a sequence optimized according

to the present invention, significantly increased the expression of sgp130Fc protein by HEK293 cells. Figure 8 represents an alignment of the same sequences. Although in all cases the optimization approach was based on an optimal codon usage in eukaryotes, the figure clearly demonstrates that the prediction for the optimal codon at a certain position is often completely different. Figure 9 summarizes the findings of these alignments by showing the number of different base pairs between each of the sequences.

These results clearly demonstrate that the computer aided prediction of cDNA sequences for optimal protein expression in a certain organism has only an extremely limited value. The development of best optimized sequences makes it necessary to choose individual research approaches and must be accompanied by high innovative technologies and inventive power.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 94022-5seq04-12-12v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

<110> Conaris Research Institute AG

<120> Optimized nucleotide sequences encoding sgp130

<130> 94022-5

<140> 2,575,800
<141> 2005-08-26

<150> EP 04020455.4
<151> 2004-08-27

<160> 13

<170> PatentIn version 3.2

<210> 1
<211> 22
<212> PRT
<213> Artificial sequence

<220>
<223> secretory leader sequence of sgp130

<400> 1

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

Thr Thr Glu Ser Thr Gly
20

<210> 2
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 2
atgaggtgtg agtgggatgg 20

<210> 3
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 3
accttgcact tgtactcctt gc 22

<210> 4
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 4
gatgcctgct tgccgaatat c 21

<210> 5
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 5
cgccaagctc ttcagcaata tc 22

<210> 6
<211> 918
<212> DNA
<213> sgp130(d1-D3)_wt

<400> 6
atggaactgc tggacccgtg cggttatatc agcccggaaa gcccagttgt tcaactgcat 60

agcaatttca ccgcggtttg tgtgctgaaa gagaagtgta tggattattt ccacgtcaac 120

gcgaactaca ttgtgtggaa aaccaaccat ttcaccatcc cgaaagaaca gtataccatc 180

attaaccgta ccgcgagcag cgttaccttt accgatatcg cgagcctgaa cattcaactg 240

acctgcaaca ttctgacctt cggtcaactg gaacagaatg tttatggcat caccatcatt 300

agcggcctgc cgccggaaaa accgaaaaat ctgagctgca ttgtgaacga aggcaaaaaa 360

atgcgctgcg aatgggatgg tggtcgcgaa acccacctgg aaaccaactt caccctgaaa 420

agcgaatggg cgacccacaa atttgcggat tgcaaagcga aacgcgatac cccgacctct 480

tgcaccgttg attacagcac cgtgtacttc gtgaacattg aagtgtgggt ggaagcggaa 540

aacgccctgg gtaaagtcac cagcgatcat atcaactttg atccggtgta caaagtgaaa 600

ccgaatccgc cgcataatct gagcgtgatc aacagcgaag aactgagcag catcctgaaa 660

ctgacctgga ccaacccgag cattaagagc gttatcatcc tgaaatacaa cattcagtat 720

cgcaccaaag atgccagcac ctggagccag attccgccgg aagataccgc gagcacccgt 780

agcagcttca ccgttcagga tctgaaaccg tttaccgaat atgtgtttcg cattcgctgt 840

atgaaagaag atggtaaagg ctactggagc gattggagcg aagaagcgag cggtatcacc 900

tatgaagatc gtccgtga 918

<210> 7
<211> 918
<212> DNA
<213> Artificial sequence

<220>
<223> sgp130(D1-D3)_opt

<400> 7
atggaacttc tagatccatg tggttatatc agtcctgaat ctccagttgt acaacttcat 60

tctaatttca ctgcagtttg tgtgctaaag gaaaaatgta tggattattt tcatgtaaat 120

gctaattaca ttgtctggaa aacaaaccat tttactattc ctaaggagca atatactatc 180

ataaacagaa cagcatccag tgtcaccttt acagatatag cttcattaaa tattcagctc 240

acttgcaaca ttcttacatt cggacagctt gaacagaatg tttatggaat cacaataatt 300

tcgggcttgc ctccagaaaa acctaaaaat ttgagttgca ttgtgaacga ggggaagaaa 360

atgaggtgtg agtgggatgg tggaagggaa acacacttgg agacaaactt cactttaaaa 420

tctgaatggg caacacacaa gtttgctgat tgcaaagcaa aacgtgacac ccccacctca 480

tgcactgttg attattctac tgtgtatttt gtcaacattg aagtctgggt agaagcagag 540

aatgcccttg ggaaggttac atcagatcat atcaattttg atcctgtata taaagtgaag 600

cccaatccgc cacataattt atcagtgatc aactcagagg aactgtctag tatcttaaaa 660

ttgacatgga ccaacccaag tattaagagt gttataatac taaaatataa cattcaatat 720

aggaccaaag atgcctcaac ttggagccag attcctcctg aagacacagc atccacccga 780

tcttcattca ctgtccaaga ccttaaacct tttacagaat atgtgtttag gattcgctgt 840

atgaaggaag atggtaaggg atactggagt gactggagtg aagaagcaag tgggatcacc 900

tatgaagata gaccatga 918

<210> 8
<211> 305
<212> PRT
<213> Artificial sequence

<220>
<223> sgp130(D1-D3)_wt

<400> 8

Met Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser Pro Glu Ser Pro Val
1 5 10 15

Val Gln Leu His Ser Asn Phe Thr Ala Val Cys Val Leu Lys Glu Lys
20 25 30

Cys Met Asp Tyr Phe His Val Asn Ala Asn Tyr Ile Val Trp Lys Thr
35 40 45

Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr Ile Ile Asn Arg Thr
50 55 60

Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser Leu Asn Ile Gln Leu
65 70 75 80

Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu Gln Asn Val Tyr Gly
85 90 95

Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu Lys Pro Lys Asn Leu Ser
100 105 110

Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys Glu Trp Asp Gly Gly
115 120 125

Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu Lys Ser Glu Trp Ala
130 135 140

Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg Asp Thr Pro Thr Ser
145 150 155 160

Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val Asn Ile Glu Val Trp
165 170 175

Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr Ser Asp His Ile Asn
180 185 190

Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro Pro His Asn Leu Ser
195 200 205

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

Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys Tyr Asn Ile Gln Tyr
225 230 235 240

Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile Pro Pro Glu Asp Thr
245 250 255

Ala Ser Thr Arg Ser Ser Phe Thr Val Gln Asp Leu Lys Pro Phe Thr
260 265 270

Glu Tyr Val Phe Arg Ile Arg Cys Met Lys Glu Asp Gly Lys Gly Tyr
275 280 285

Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly Ile Thr Tyr Glu Asp Arg
290 295 300

Pro
305

<210> 9
<211> 305
<212> PRT
<213> Artificial sequence

<220>

<223> sgp130(D-D3)_opt

<400> 9

```
Met Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser Pro Glu Ser Pro Val
1               5                  10                  15

Val Gln Leu His Ser Asn Phe Thr Ala Val Cys Val Leu Lys Glu Lys
            20                  25                  30

Cys Met Asp Tyr Phe His Val Asn Ala Asn Tyr Ile Val Trp Lys Thr
        35                  40                  45

Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr Ile Ile Asn Arg Thr
    50                  55                  60

Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser Leu Asn Ile Gln Leu
65                  70                  75                  80

Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu Gln Asn Val Tyr Gly
                85                  90                  95

Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu Lys Pro Lys Asn Leu Ser
            100                 105                 110

Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys Glu Trp Asp Gly Gly
        115                 120                 125

Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu Lys Ser Glu Trp Ala
    130                 135                 140

Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg Asp Thr Pro Thr Ser
145                 150                 155                 160

Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val Asn Ile Glu Val Trp
                165                 170                 175

Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr Ser Asp His Ile Asn
            180                 185                 190

Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro Pro His Asn Leu Ser
        195                 200                 205
```

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

Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys Tyr Asn Ile Gln Tyr
225 230 235 240

Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile Pro Pro Glu Asp Thr
245 250 255

Ala Ser Thr Arg Ser Ser Phe Thr Val Gln Asp Leu Lys Pro Phe Thr
260 265 270

Glu Tyr Val Phe Arg Ile Arg Cys Met Lys Glu Asp Gly Lys Gly Tyr
275 280 285

Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly Ile Thr Tyr Glu Asp Arg
290 295 300

Pro
305

<210> 10
<211> 2511
<212> DNA
<213> artificial

<220>
<223> sgp130Fc_wt

<400> 10
atgttgacgt tgcagacttg gctagtgcaa gccttgttta ttttcctcac cactgaatct 60

acaggtgaac ttctagatcc atgtggttat atcagtcctg aatctccagt tgtacaactt 120

cattctaatt tcactgcagt ttgtgtgcta aaggaaaaat gtatggatta ttttcatgta 180

aatgctaatt acattgtctg gaaaacaaac cattttacta ttcctaagga gcaatatact 240

atcataaaca gaacagcatc cagtgtcacc tttacagata tagcttcatt aaatattcag 300

ctcacttgca acattcttac attcggacag cttgaacaga atgtttatgg aatcacaata 360

atttcgggct tgcctccaga aaaacctaaa aatttgagtt gcattgtgaa cgaggggaag 420

aaaatgaggt gtgagtggga tggtggaagg gaaacacact tggagacaaa cttcacttta 480

aaatctgaat gggcaacaca caagtttgct gattgcaaag caaaacgtga caccccacc 540

tcatgcactg ttgattattc tactgtgtat tttgtcaaca ttgaagtctg ggtagaagca 600

gagaatgccc ttgggaaggt tacatcagat catatcaatt ttgatcctgt atataaagtg 660

aagcccaatc cgccacataa tttatcagtg atcaactcag aggaactgtc tagtatctta 720

aaattgacat ggaccaaccc aagtattaag agtgttataa tactaaaata taacattcaa 780

tataggacca aagatgcctc aacttggagc cagattcctc ctgaagacac agcatccacc 840

cgatcttcat tcactgtcca agaccttaaa ccttttacag aatatgtgtt taggattcgc 900

tgtatgaagg aagatggtaa gggatactgg agtgactgga gtgaagaagc aagtgggatc 960

acctatgaag atagaccatc taaagcacca agtttctggt ataaaataga tccatcccat 1020

actcaaggct acagaactgt acaactcgtg tggaagacat tgcctccttt tgaagccaat 1080

ggaaaaatct tggattatga agtgactctc acaagatgga aatcacattt acaaaattac 1140

acagttaatg ccacaaaact gacagtaaat ctcacaaatg atcgctatct agcaacccta 1200

acagtaagaa atcttgttgg caaatcagat gcagctgttt taactatccc tgcctgtgac 1260

tttcaagcta ctcaccctgt aatggatctt aaagcattcc ccaaagataa catgctttgg 1320

gtggaatgga ctactccaag ggaatctgta aagaaatata tacttgagtg gtgtgtgtta 1380

tcagataaag caccctgtat cacagactgg caacaagaag atggtaccgt gcatcgcacc 1440

tatttaagag ggaacttagc agagagcaaa tgctatttga taacagttac tccagtatat 1500

gctgatggac caggaagccc tgaatccata aaggcatacc ttaaacaagc tccaccttcc 1560

aaaggaccta ctgttcggac aaaaaaagta gggaaaaacg aagctgtctt agagtgggac 1620

caacttcctg ttgatgttca gaatggattt atcagaaatt atactatatt ttatagaacc 1680

atcattggaa atgaaactgc tgtgaatgtg gattcttccc acacagaata tacattgtcc 1740

tctttgacta gtgacacatt gtacatggta cgaatggcag catacacaga tgaaggtggg 1800

aaggatggtc cagaattcag atcttgtgac aaaactcaca catgcccacc gtgcccagca 1860

cctgaagccg agggcgcgcc gtcagtcttc ctcttccccc caaaacccaa ggacaccctc 1920

atgatctccc ggacccctga ggtcacatgc gtggtggtgg acgtgagcca cgaagaccct 1980

gaggtcaagt tcaactggta cgtggacggc gtggaggtgc ataatgccaa gacaaagccg 2040

cgggaggagc agtacaacag cacgtaccgt gtggtcagcg tcctcaccgt cctgcaccag 2100

gactggctga atggcaagga gtacaagtgc aaggtctcca acaaagccct cccagccccc 2160

atcgagaaaa ccatctccaa agccaaaggg cagccccgag aaccacaggt gtacaccctg 2220

cccccatccc gggaggagat gaccaagaac caggtcagcc tgacctgcct ggtcaaaggc 2280

ttctatccca gcgacatcgc cgtggagtgg gagagcaatg ggcagccgga gaacaactac 2340

aagaccacgc ctcccgtgct ggactccgac ggctccttct tcctctatag caagctcacc 2400

gtggacaaga gcaggtggca gcaggggaac gtcttctcat gctccgtgat gcatgaggct 2460

ctgcacaacc actacacgca gaagagcctc tccctgtctc cgggtaaatg a 2511

<210> 11
<211> 835
<212> PRT
<213> artificial

<220>
<223> sgp130Fc_wt

<400> 11

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

Thr Thr Glu Ser Thr Gly Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser
20 25 30

Pro Glu Ser Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val Cys
35 40 45

Val Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn Tyr
50 55 60

Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr
65 70 75 80

Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser
85 90 95

Leu Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu
100 105 110

Gln Asn Tyr Tyr Gly Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu Lys
115 120 125

```
Pro Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys
    130                 135                 140

Glu Trp Asp Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu
145                 150                 155                 160

Lys Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg
                165                 170                 175

Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val
            180                 185                 190

Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr
        195                 200                 205

Ser Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro
    210                 215                 220

Pro His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile Leu
225                 230                 235                 240

Lys Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys
                245                 250                 255

Tyr Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile
            260                 265                 270

Pro Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln Asp
        275                 280                 285

Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys Met Lys Glu
    290                 295                 300

Asp Gly Lys Gly Val Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly Ile
305                 310                 315                 320

Thr Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser Phe Trp Tyr Lys Ile
                325                 330                 335

Asp Pro Ser His Thr Gln Gly Tyr Arg Thr Val Gln Leu Val Trp Lys
            340                 345                 350
```

Thr Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu Val
355 360 365

Thr Leu Thr Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn Ala
370 375 380

Thr Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr Leu
385 390 395 400

Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val Leu Thr Ile
405 410 415

Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val Met Asp Leu Lys Ala
420 425 430

Phe Pro Lys Asp Asn Met Leu Trp Val Glu Trp Thr Thr Pro Arg Glu
435 440 445

Ser Val Lys Lys Tyr Ile Leu Glu Trp Cys Val Leu Ser Asp Lys Ala
450 455 460

Pro Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr
465 470 475 480

Tyr Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr Val
485 490 495

Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys Ala
500 505 510

Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr Lys
515 520 525

Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro Val
530 535 540

Asp Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr Ile Phe Tyr Arg Thr
545 550 555 560

Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr Glu
565 570 575

Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met
580 585 590

Ala Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Arg Ser
595 600 605

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu
610 615 620

Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
625 630 635 640

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
645 650 655

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
660 665 670

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
675 680 685

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
690 695 700

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
705 710 715 720

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
725 730 735

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
740 745 750

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
755 760 765

Glu Trp Glu Ser Asn Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
770 775 780

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val

785 790 795 800

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
805 810 815

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
820 825 830

Pro Gly Lys
835

<210> 12
<211> 2511
<212> DNA
<213> artificial

<220>
<223> sgp130Fc_opt

<400> 12
atgctgacac tgcagacatg gctggtgcag gccctgttta tctttctgac caccgagtct 60

acaggagagc tgctggatcc ttgcggctat atctcccctg agtctcctgt ggtgcagctg 120

cattctaact tcaccgccgt gtgtgtgctg aaggaaaagt gcatggacta cttccacgtg 180

aacgccaact acatcgtgtg gaaaaccaac cacttcacca tccccaagga gcagtacacc 240

atcatcaacc ggaccgcttc ttctgtgacc ttcaccgata tcgcctccct gaatatccag 300

ctgacctgca acatcctgac ctttggacag ctggagcaga atgtgtacgg catcaccatc 360

atctctggcc tgcctccaga gaagcctaag aacctgtcct gcatcgtgaa tgagggcaag 420

aagatgaggt gtgagtggga tggcggcaga gagacacatc tggagaccaa cttcaccctg 480

aagtctgagt gggccaccca caagtttgcc gactgcaagg ccaagagaga taccctacc 540

tcttgcaccg tggactactc caccgtgtac ttcgtgaaca tcgaggtgtg ggtggaggct 600

gagaatgctc tgggcaaggt gacctctgac cacatcaact tcgaccccgt gtacaaggtg 660

aagcctaacc ctcctcacaa cctgtccgtg atcaactctg aggagctgtc ctctatcctg 720

aagctgacct ggaccaaccc ttccatcaag tccgtgatca tcctgaagta caacatccag 780

tacaggacca aggatgcttc tacctggtct cagatccctc ctgaggatac cgcttccacc 840

agatccagct tcacagtgca ggacctgaag ccttttaccg agtacgtgtt caggatccgg 900

tgcatgaagg aggatggcaa gggctattgg tctgactggt ctgaggaggc ttctggcatc 960

acctacgagg acagaccttc taaggcccct agcttctggt acaagatcga cccttctcac 1020

acccagggct atagaacagt gcagctggtg tggaaaaccc tgcctccatt cgaggctaat 1080

ggcaagatcc tggactatga ggtgaccctg accagatgga agtctcacct gcagaactac 1140

accgtgaacg ctaccaagct gaccgtgaac ctgaccaacg atagatacct ggctaccctg 1200

accgtgagaa atctggtggg caagtctgat gctgctgtgc tgaccatccc tgcctgtgat 1260

tttcaggcta cccaccctgt gatggatctg aaggccttcc ccaaggataa catgctgtgg 1320

gtggagtgga caacacctag agagtccgtg aagaagtaca tcctggagtg gtgcgtgctg 1380

tctgataagg ccccttgcat cacagattgg cagcaggagg atggcaccgt gcatagaacc 1440

tacctgagag gcaatctggc cgagtctaag tgctatctga tcaccgtgac ccctgtgtat 1500

gctgatggac ctggctctcc tgagtctatc aaggcctacc tgaagcaggc tcctccatct 1560

aagggaccta ccgtgaggac aaagaaggtg ggcaagaacg aggctgtgct ggagtgggat 1620

cagctgcctg tggatgtgca gaacggcttc atccggaact acaccatctt ctaccggacc 1680

atcatcggca atgagaccgc cgtgaacgtg gattcttccc acaccgagta cacactgtcc 1740

tctctgacct ctgacaccct gtacatggtg agaatggccg cttataccga tgagggcggc 1800

aaggatggac ctgagttcag atcctgcgac aagacccaca cctgtcctcc ttgtcctgct 1860

cctgaggctg agggcgctcc ttctgtgttt ctgttccccc caaagcctaa ggataccctg 1920

atgatctcca gaacccctga ggtgacatgt gtggtggtgg atgtgtctca tgaggacccc 1980

gaggtgaagt tcaactggta cgtggatggc gtggaggtgc acaatgctaa gaccaagcct 2040

agggaggagc agtacaactc cacctacaga gtggtgtctg tgctgacagt gctgcatcag 2100

gattggctga acggcaagga gtacaagtgc aaggtgtcca acaaggctct gcctgctcct 2160

atcgaaaaga ccatctccaa ggctaaggga cagcctagag agcctcaggt gtacacactg 2220

cctccatcta gggaggagat gaccaagaat caggtgtccc tgacctgtct ggtgaagggc 2280

ttctaccctt ctgatatcgc tgtggagtgg gagtctaatg gccagcccga gaacaattac 2340

aagaccaccc ctcctgtgct ggattctgac ggctccttct tcctgtactc caaactgacc 2400

gtggacaagt ctagatggca gcagggcaac gtgttctctt gttccgtgat gcacgaggct 2460

ctgcacaatc actataccca gaagtccctg tctctgtctc ctggcaagtg a 2511

<210> 13
<211> 835
<212> PRT
<213> artificial

<220>
<223> sgp130Fc_opt

<400> 13

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

Thr Thr Glu Ser Thr Gly Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser
20 25 30

Pro Glu Ser Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val Cys
35 40 45

Val Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn Tyr
50 55 60

Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr
65 70 75 80

Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser
85 90 95

Leu Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu
100 105 110

Gln Asn Tyr Tyr Gly Ile Thr Ile Ile Ser Gly Leu Pro Pro Glu Lys
115 120 125

Pro Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys
130 135 140

Glu Trp Asp Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu
145 150 155 160

Lys Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg
165 170 175

Asp Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val

180 185 190

Asn Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr
195 200 205

Ser Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro
210 215 220

Pro His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile Leu
225 230 235 240

Lys Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys
245 250 255

Tyr Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile
260 265 270

Pro Pro Glu Asp Thr Ala Ser Thr Arg Ser Ser Phe Thr Val Gln Asp
275 280 285

Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile Arg Cys Met Lys Glu
290 295 300

Asp Gly Lys Gly Val Trp Ser Asp Trp Ser Glu Glu Ala Ser Gly Ile
305 310 315 320

Thr Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser Phe Trp Tyr Lys Ile
325 330 335

Asp Pro Ser His Thr Gln Gly Tyr Arg Thr Val Gln Leu Val Trp Lys
340 345 350

Thr Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile Leu Asp Tyr Glu Val
355 360 365

Thr Leu Thr Arg Trp Lys Ser His Leu Gln Asn Tyr Thr Val Asn Ala
370 375 380

Thr Lys Leu Thr Val Asn Leu Thr Asn Asp Arg Tyr Leu Ala Thr Leu
385 390 395 400

Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala Ala Val Leu Thr Ile
405 410 415

Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val Met Asp Leu Lys Ala
420 425 430

Phe Pro Lys Asp Asn Met Leu Trp Val Glu Trp Thr Thr Pro Arg Glu
435 440 445

Ser Val Lys Lys Tyr Ile Leu Glu Trp Cys Val Leu Ser Asp Lys Ala
450 455 460

Pro Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr
465 470 475 480

Tyr Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Ile Thr Val
485 490 495

Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys Ala
500 505 510

Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr Lys
515 520 525

Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro Val
530 535 540

Asp Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr Ile Phe Tyr Arg Thr
545 550 555 560

Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr Glu
565 570 575

Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met
580 585 590

Ala Ala Tyr Thr Asp Glu Gly Gly Lys Asp Gly Pro Glu Phe Arg Ser
595 600 605

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu
610 615 620

```
Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
625                 630                 635                 640

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
                645                 650                 655

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
            660                 665                 670

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
        675                 680                 685

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
    690                 695                 700

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
705                 710                 715                 720

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
                725                 730                 735

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
            740                 745                 750

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
        755                 760                 765

Glu Trp Glu Ser Asn Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
    770                 775                 780

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
785                 790                 795                 800

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                805                 810                 815

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
            820                 825                 830

Pro Gly Lys
        835
```

Claims

1. A nucleic acid molecule encoding sgp130 comprising (a) the nucleic acid sequence as depicted in SEQ ID No. 12 (sgp130Fc_opt), wherein at least 80% of the codons altered in the nucleic acid sequence of SEQ ID No. 12 vs. the wild type sequence are present, or (b) a fragment thereof which encodes a polypeptide that comprises extracellular domains D1 to D3 of sgp130, wherein the encoded polypeptide inhibits the activity of the agonistic complex IL-6/sIL-6R.

2. The nucleic acid molecule of claim 1 which is a DNA molecule.

3. An expression vector containing a nucleic acid molecule of claim 1 or 2.

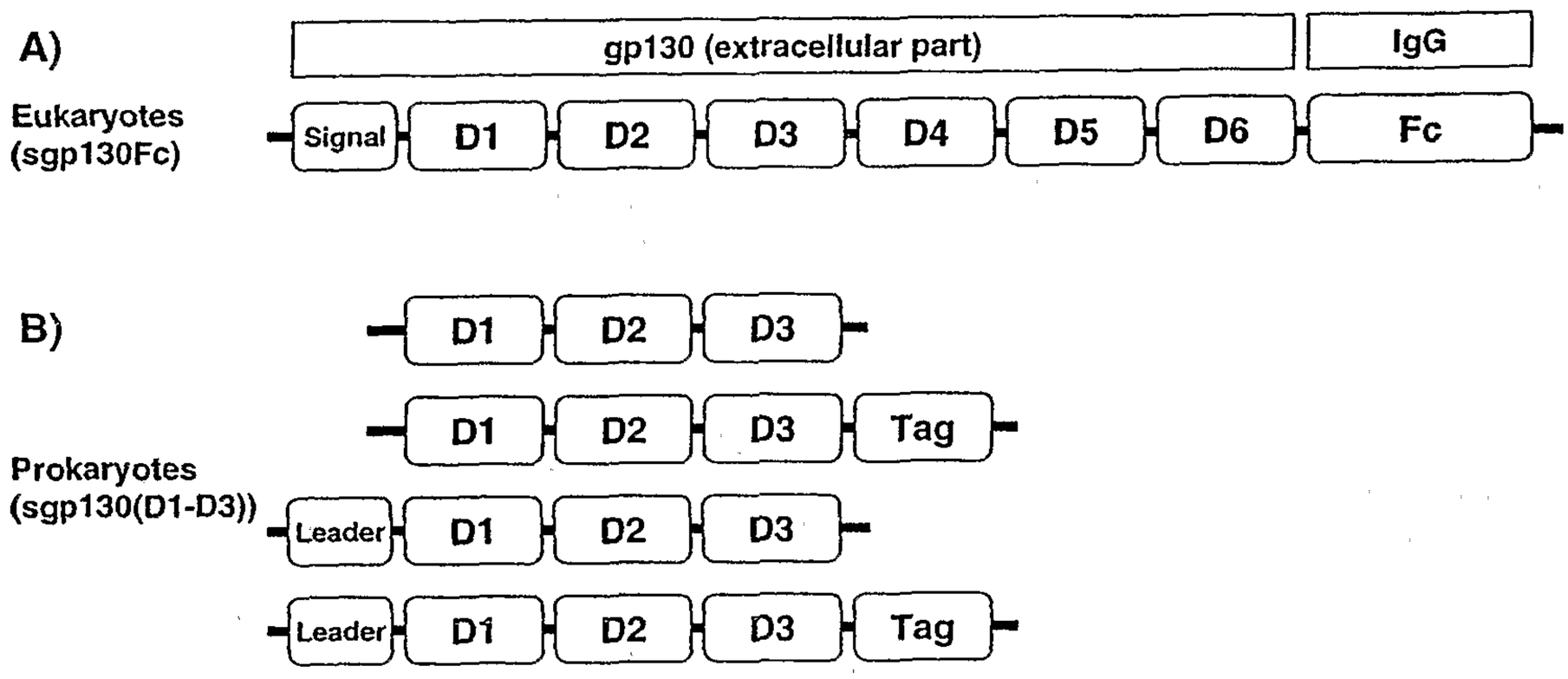
4. A host cell containing an expression vector of claim 3.

5. The host cell of claim 4 which is a mammalian host cell.

6. The host cell of claim 5 which is a CHO or HEK293 cell.

7. A method of producing a sgp130 polypeptide comprising culturing a host cell of any one of claims 4 to 6 and recovering the polypeptide from said host cell or the culture.

Figure 1


A)
gp130 (extracellular part)
IgG
Eukaryotes
(sgp130Fc)
Signal
D1
D2
D3
D4
D5
D6
Fc
B)
D1
D2
D3
Prokaryotes
(sgp130(D1-D3))
Tag
Leader
D1
D2
D3
Leader
D1
D2
D3
Tag

```
sgp130(D1-D3)_wt      #1   ATGGAACTTC TAGATCCATG TGGTTATATC AGTCCTGAAT CTCCAGTTGT ACAACTTCAT TCTAATTTCA CTGCAGTTTG TGTGCTAAAG GAAAAATGTA TGGATTATTT
                           M  E  L  L  D  P  C  G  Y  I  S  P  E  S  P  V  V  Q  L  H  S  N  F  T  A  V  C  V  L  K  E  K  C  M  D  Y  F
sgp130(D1-D3)_opt     #1   ATGGAACTGC TGGACCCGTG CGGTTATATC AGCCCGGAAA GCCCAGTTGT TCAACTGCAT AGCAATTTCA CCGCGGTTTG TGTGCTGAAA GAGAAGTGTA TGGATTATTT
                           M  E  L  L  D  P  C  G  Y  I  S  P  E  S  P  V  V  Q  L  H  S  N  F  T  A  V  C  V  L  K  E  K  C  M  D  Y  F

sgp130(D1-D3)_wt      #111  TCATGTAAAT GCTAATTACA TTGTCTGGAA AACAAACCAT TTTACTATTC CTAAGGAGCA ATATACTATC ATAAACAGAA CAGCATCCAG TGTCACCTTT ACAGATATAG
                           H  V  N  A  N  Y  I  V  W  K  T  N  H  F  T  I  P  K  E  Q  Y  T  I  I  N  R  T  A  S  S  V  T  F  T  D  I  A
sgp130(D1-D3)_opt     #111  CCACGTCAAC GCGAACTACA TTGTGTGGAA AACCAACCAT TTCACCATCC CGAAAGAACA GTATACCATC ATTAACCGTA CCGCGAGCAG CGTTACCTTT ACCGATATCG
                           H  V  N  A  N  Y  I  V  W  K  T  N  H  F  T  I  P  K  E  Q  Y  T  I  I  N  R  T  A  S  S  V  T  F  T  D  I  A

sgp130(D1-D3)_wt      #221  CTTCATTAAA TATTCAGCTC ACTTGCAACA TTCTTACATT CGGACAGCTT GAACAGAATG TTTATGGAAT CACAATAATT TCGGGCTTGC CTCCAGAAAA ACCTAAAAAT
                           S  L  N  I  Q  L  T  C  N  I  L  T  F  G  Q  L  E  Q  N  V  Y  G  I  T  I  I  S  G  L  P  P  E  K  P  K  N
sgp130(D1-D3)_opt     #221  CGAGCCTGAA CATTCAACTG ACCTGCAACA TTCTGACCTT CGGTCAACTG GAACAGAATG TTTATGGCAT CACCATCATT AGCGGCCTGC CGCCGGAAAA ACCGAAAAAT
                           S  L  N  I  Q  L  T  C  N  I  L  T  F  G  Q  L  E  Q  N  V  Y  G  I  T  I  I  S  G  L  P  P  E  K  P  K  N

sgp130(D1-D3)_wt      #331  TTGAGTTGCA TTGTGAACGA GGGGAAGAAA ATGAGGTGTG AGTGGGATGG TGGAAGGGAA ACACACTTGG AGACAAACTT CACTTTAAAA TCTGAATGGG CAACACACAA
                           L  S  C  I  V  N  E  G  K  K  M  R  C  E  W  D  G  G  R  E  T  H  L  E  T  N  F  T  L  K  S  E  W  A  T  H  K
sgp130(D1-D3)_opt     #331  CTGAGCTGCA TTGTGAACGA AGGCAAAAAA ATGCGCTGCG AATGGGATGG TGGTCGCGAA ACCCACCTGG AAACCAACTT CACCCTGAAA AGCGAATGGG CGACCCACAA
                           L  S  C  I  V  N  E  G  K  K  M  R  C  E  W  D  G  G  R  E  T  H  L  E  T  N  F  T  L  K  S  E  W  A  T  H  K
```

Figure 2

CA 02575800 2014-05-05

```
sgp130(D1-D3)_wt      #441  GTTTGCTGAT TGCAAAGCAA AACGTGACAC CCCCACCTCA TGCACTGTTG ATTATTCTAC TGTGTATTTT GTCAACATTG AAGTCTGGGT AGAAGCAGAG AATGCCCTTG
                            F  A  D  C  K  A  K  R  D  T  P  T  S  C  T  V  D  Y  S  T  V  Y  F  V  N  I  E  V  W  V  E  A  E  N  A  L  G
sgp130(D1-D3)_opt     #441  ATTTGCGGAT TGCAAAGCGA AACGCGATAC CCCGACCTCT TGCACCGTTG ATTACAGCAC CGTGTACTTC GTGAACATTG AAGTGTGGGT GGAAGCGGAA AACGCCCTGG
                            F  A  D  C  K  A  K  R  D  T  P  T  S  C  T  V  D  Y  S  T  V  Y  F  V  N  I  E  V  W  V  E  A  E  N  A  L  G

sgp130(D1-D3)_wt      #551  GGAAGGTTAC ATCAGATCAT ATCAATTTTG ATCCTGTATA TAAAGTGAAG CCCAATCCGC CACATAATTT ATCAGTGATC AACTCAGAGG AACTGTCTAG TATCTTAAAA
                            K  V  T  S  D  H  I  N  F  D  P  V  Y  K  V  K  P  N  P  P  H  N  L  S  V  I  N  S  E  E  L  S  S  I  L  K
sgp130(D1-D3)_opt     #551  GTAAAGTCAC CAGCGATCAT ATCAACTTTG ATCCGGTGTA CAAAGTGAAA CCGAATCCGC CGCATAATCT GAGCGTGATC AACAGCGAAG AACTGAGCAG CATCCTGAAA
                            K  V  T  S  D  H  I  N  F  D  P  V  Y  K  V  K  P  N  P  P  H  N  L  S  V  I  N  S  E  E  L  S  S  I  L  K

sgp130(D1-D3)_wt      #661  TTGACATGGA CCAACCCAAG TATTAAGAGT GTTATAATAC TAAAATATAA CATTCAATAT AGGACCAAAG ATGCCTCAAC TTGGAGCCAG ATTCCTCCTG AAGACACAGC
                            L  T  W  T  N  P  S  I  K  S  V  I  I  L  K  Y  N  I  Q  Y  R  T  K  D  A  S  T  W  S  Q  I  P  P  E  D  T  A
sgp130(D1-D3)_opt     #661  CTGACCTGGA CCAACCCGAG CATTAAGAGC GTTATCATCC TGAAATACAA CATTCAGTAT CGCACCAAAG ATGCCAGCAC CTGGAGCCAG ATTCCGCCGG AAGATACCGC
                            L  T  W  T  N  P  S  I  K  S  V  I  I  L  K  Y  N  I  Q  Y  R  T  K  D  A  S  T  W  S  Q  I  P  P  E  D  T  A

sgp130(D1-D3)_wt      #771  ATCCACCCGA TCTTCATTCA CTGTCCAAGA CCTTAAACCT TTTACAGAAT ATGTGTTTAG GATTCGCTGT ATGAAGGAAG ATGGTAAGGG ATACTGGAGT GACTGGAGTG
                            S  T  R  S  S  F  T  V  Q  D  L  K  P  F  T  E  Y  V  F  R  I  R  C  M  K  E  D  G  K  G  Y  W  S  D  W  S  E
sgp130(D1-D3)_opt     #771  GAGCACCCGT AGCAGCTTCA CCGTTCAGGA TCTGAAACCG TTTACCGAAT ATGTGTTTCG CATTCGCTGT ATGAAAGAAG ATGGTAAAGG CTACTGGAGC GATTGGAGCG
                            S  T  R  S  S  F  T  V  Q  D  L  K  P  F  T  E  Y  V  F  R  I  R  C  M  K  E  D  G  K  G  Y  W  S  D  W  S  E
```

```
sgp130(D1-D3)_wt      #881  AAGAAGCAAG TGGGATCACC TATGAAGATA GACCATGA
                              E  A  S  G  I  T  Y  E  D  R  P  .
sgp130(D1-D3)_opt     #881  AAGAAGCGAG CGGTATCACC TATGAAGATC GTCCGTGA
                              E  A  S  G  I  T  Y  E  D  R  P  .
```

```
sgp130Fc_wt      #1   ATGTTGACGT TGCAGACTTG GCTAGTGCAA GCCTTGTTTA TTTTCCTCAC CACTGAATCT ACAGGTGAAA TTCTAGATCC ATGTGGTTAT ATCAGTCCTG AATCTCCAGT TGTACAACTT
                      M  L  T  L  Q  T  W  L  V  Q  A  L  F  I  F  L  T  T  E  S  T  G  E  L  D  P  C  G  Y  I  S  P  E  S  P  V  V  Q  L

sgp130Fc_opt     #1   ATGCTGACAC TGCAGACATG GCTGGTGCAG GCCCTGTTTA TCTTTCTGAC CACCGAGTCT ACAGGAGAGC TGCTGGATCC TTGCGGCTAT ATCTCCCCTG AGTCTCCTGT GGTGCAGCTG
                      M  L  T  L  Q  T  W  L  V  Q  A  L  F  I  F  L  T  T  E  S  T  G  E  L  L  D  P  C  G  Y  I  S  P  E  S  P  V  V  Q  L

sgp130Fc_wt      #121  CATTCTAATT TCACTGCAGT TTGTGTGCTA AAGGAAAAAT GTATGGATTA TTTTCATGTA AATGCTAATT ACATTGTCTG GAAAACAAAC CATTTTACTA TTCCTAAGGA GCAATATACT
                      H  S  N  F  T  A  V  C  V  L  K  E  K  C  M  D  Y  F  H  V  N  A  N  Y  I  V  W  K  T  N  H  F  T  I  P  K  E  Q  Y  T

sgp130Fc_opt     #121  CATTCTAACT TCACCGCCGT GTGTGTGCTG AAGGAAAAGT GCATGGACTA CTTCCACGTG AACGCCAACT ACATCGTGTG GAAAACCAAC CACTTCACCA TCCCTAAGGA GCAGTACACC
                      H  S  N  F  T  A  V  C  V  L  K  E  K  C  M  D  Y  F  H  V  N  A  N  Y  I  V  W  K  T  N  H  F  T  I  P  K  E  Q  Y  T

sgp130Fc_wt      #241  ATCATAAACA GAACAGCATC CAGTGTCACC TTTACAGATA TAGCTTCATT AAATATTCAG CTCACTTGCA ACATTCTTAC ATTCGGACAG CTTGAACAGA ATGTTTATGG AATCACAATA
                      I  I  N  R  T  A  S  S  V  T  F  T  D  I  A  S  L  N  I  Q  L  T  C  N  I  L  T  F  G  Q  L  E  Q  N  V  Y  G  I  T  I

sgp130Fc_opt     #241  ATCATCAACC GGACCGCTTC TTCTGTGACC TTCACCGATA TCGCCTCCCT GAATATCCAG CTGACCTGCA ACATCCTGAC CTTTGGACAG CTGGAGCAGA ATGTGTACGG CATCACCATC
                      I  I  N  R  T  A  S  S  V  T  F  T  D  I  A  S  L  N  I  Q  L  T  C  N  I  L  T  F  G  Q  L  E  Q  N  V  Y  G  I  T  I

sgp130Fc_wt      #361  ATTTCGGGCT TGCCTCCAGA AAAACCTAAA AATTTGAGTT GCATTGTGAA CGAGGGGAAG AAAATGAGGT GTGAGTGGGA TGGTGGAAGG GAAACACACT TGGAGACAAA CTTCACTTTA
                      I  S  G  L  P  P  E  K  P  K  N  L  S  C  I  V  N  E  G  K  K  M  R  C  E  W  D  G  G  R  E  T  H  L  E  T  N  F  T  L

sgp130Fc_opt     #361  ATCTCTGGCC TGCCTCCAGA GAAGCCTAAG AACCTGTCCT GCATCGTGAA TGAGGGCAAG AAGATGAGGT GTGAGTGGGA TGGCGGCAGA GAGACACATC TGGAGACCAA CTTCACCCTG
                      I  S  G  L  P  P  E  K  P  K  N  L  S  C  I  V  N  E  G  K  K  M  R  C  E  W  D  G  G  R  E  T  H  L  E  T  N  F  T  L
```

Figure 3

CA 02757800 2014-05-05

```
sgp130Fc_wt    #481  AAATCTGAAT GGGCAACACA CAAGTTTGCT GATTGCAAAG CAAAACGTGA CACCCCCACC TCATGCACTG TTGATTATTC TACTGTGTAT TTTGTCAACA TTGAAGTCTG GGTAGAAGCA
                     K  S  E  W  A  T  H  K  F  A  D  C  K  A  K  R  D  T  P  T  S  C  T  V  D  Y  S  T  V  Y  F  V  N  I  E  V  W  V  E  A
sgp130Fc_opt   #481  AAGTCTGAGT GGGCCACCCA CAAGTTTGCC GACTGCAAGG CCAAGAGAGA TACCCCTACC TCTTGCACCG TGGACTACTC CACCGTGTAC TTCGTGAACA TCGAGGTGTG GGTGGAGGCT
                     K  S  E  W  A  T  H  K  F  A  D  C  K  A  K  R  D  T  P  T  S  C  T  V  D  Y  S  T  V  Y  F  V  N  I  E  V  W  V  E  A

sgp130Fc_wt    #601  GAGAATGCCC TTGGGAAGGT TACATCAGAT CATATCAATT TTGATCCTGT ATATAAAGTG AAGCCCAATC CGCCACATAA TTTATCAGTG ATCAACTCAG AGGAACTGTC TAGTATCTTA
                     E  N  A  L  G  K  V  T  S  D  H  I  N  F  D  P  V  Y  K  V  K  P  N  P  P  H  N  L  S  V  I  N  S  E  E  L  S  S  I  L
sgp130Fc_opt   #601  GAGAATGCTC TGGGCAAGGT GACCTCTGAC CACATCAACT TCGACCCCGT GTACAAGGTG AAGCCTAACC CTCCTCACAA CCTGTCCGTG ATCAACTCTG AGGAGCTGTC CTCTATCCTG
                     E  N  A  L  G  K  V  T  S  D  H  I  N  F  D  P  V  Y  K  V  K  P  N  P  P  H  N  L  S  V  I  N  S  E  E  L  S  S  I  L

sgp130Fc_wt    #721  AAATTGACAT GGACCAACCC AAGTATTAAG AGTGTTATAA TACTAAAATA TAACATTCAA TATAGGACCA AAGATGCCTC AACTTGGAGC CAGATTCCTC CTGAAGACAC AGCATCCACC
                     K  L  T  W  T  N  P  S  I  K  S  V  I  I  L  K  Y  N  I  Q  Y  R  T  K  D  A  S  T  W  S  Q  I  P  P  E  D  T  A  S  T
sgp130Fc_opt   #721  AAGCTGACCT GGACCAACCC TTCCATCAAG TCCGTGATCA TCCTGAAGTA CAACATCCAG TACAGGACCA AGGATGCTTC TACCTGGTCT CAGATCCCTC CTGAGGATAC CGCTTCCACC
                     K  L  T  W  T  N  P  S  I  K  S  V  I  I  L  K  Y  N  I  Q  Y  R  T  K  D  A  S  T  W  S  Q  I  P  P  E  D  T  A  S  T

sgp130Fc_wt    #841  CGATCTTCAT TCACTGTCCA AGACCTTAAA CCTTTTACAG AATATGTGTT TAGGATTCGC TGTATGAAGG AAGATGGTAA GGGATACTGG AGTGACTGGA GTGAAGAAGC AAGTGGGATC
                     R  S  S  F  T  V  Q  D  L  K  P  F  T  E  Y  V  F  R  I  R  C  M  K  E  D  G  K  G  Y  W  S  D  W  S  E  E  A  S  G  I
sgp130Fc_opt   #841  AGATCCAGCT TCACAGTGCA GGACCTGAAG CCTTTTACCG AGTACGTGTT CAGGATCCGG TGCATGAAGG AGGATGGCAA GGGCTATTGG TCTGACTGGT CTGAGGAGGC TTCTGGCATC
                     R  S  S  F  T  V  Q  D  L  K  P  F  T  E  Y  V  F  R  I  R  C  M  K  E  D  G  K  G  Y  W  S  D  W  S  E  E  A  S  G  I
```

```
sgp130Fc_wt    #961   ACCTATGAAG ATAGACCATC TAAAGCACCA AGTTTCTGGT ATAAAATAGA TCCATCCCAT ACTCAAGGCT ACAGAACTGT ACAACTCGTG TGGAAGACAT TGCCTCCTTT TGAAGCCAAT
                      T Y E D R P S K A P S F W Y K I D P S H T Q G Y R T V Q L V W K T L P P F E A N
sgp130Fc_opt   #961   ACCTACGAGG ACAGACCTTC TAAGGCCCCT AGCTTCTGGT ACAAGATCGA CCCTTCTCAC ACCCAGGGCT ATAGAACAGT GCAGCTGGTG TGGAAAACCC TGCCTCCATT CGAGGCTAAT
                      T Y E D R P S K A P S F W Y K I D P S H T Q G Y R T V Q L V W K T L P P F E A N

sgp130Fc_wt    #1081  GGAAAAATCT TGGATTATGA AGTGACTCTC ACAAGATGGA AATCACATTT ACAAAATTAC ACAGTTAATG CCACAAAACT GACAGTAAAT CTCACAAATG ATCGCTATCT AGCAACCCTA
                      G K I L D Y E V T L T R W K S H L Q N Y T V N A T K L T V N L T N D R Y L A T L
sgp130Fc_opt   #1081  GGCAAGATCC TGGACTATGA GGTGACCCTG ACCAGATGGA AGTCTCACCT GCAGAACTAC ACCGTGAACG CTACCAAGCT GACCGTGAAC CTGACCAACG ATAGATACCT GGCTACCCTG
                      G K I L D Y E V T L T R W K S H L Q N Y T V N A T K L T V N L T N D R Y L A T L

sgp130Fc_wt    #1201  ACAGTAAGAA ATCTTGTTGG CAAATCAGAT GCAGCTGTTT TAACTATCCC TGCCTGTGAC TTTCAAGCTA CTCACCCTGT AATGGATCTT AAAGCATTCC CCAAAGATAA CATGCTTTGG
                      T V R N L V G K S D A A V L T I P A C D F Q A T H P V M D L K A F P K D N M L W
sgp130Fc_opt   #1201  ACCGTGAGAA ATCTGGTGGG CAAGTCTGAT GCTGCTGTGC TGACCATCCC TGCCTGTGAT TTTCAGGCTA CCCACCCTGT GATGGATCTG AAGGCCTTCC CCAAGGATAA CATGCTGTGG
                      T V R N L V G K S D A A V L T I P A C D F Q A T H P V M D L K A F P K D N M L W

sgp130Fc_wt    #1321  GTGGAATGGA CTACTCCAAG GGAATCTGTA AAGAAATATA TACTTGAGTG GTGTGTGTTA TCAGATAAAG CACCCTGTAT CACAGACTGG CAACAAGAAG ATGGTACCGT GCATCGCACC
                      V E W T T P R E S V K K Y I L E W C V L S D K A P C I T D W Q Q E D G T V H R T
sgp130Fc_opt   #1321  GTGGAGTGGA CAACACCTAG AGAGTCCGTG AAGAAGTACA TCCTGGAGTG GTGCGTGCTG TCTGATAAGG CCCCTTGCAT CACAGATTGG CAGCAGGAGG ATGGCACCGT GCATAGAACC
                      V E W T T P R E S V K K Y I L E W C V L S D K A P C I T D W Q Q E D G T V H R T
```

```
sgp130Fc_wt    #1441  TATTTAAGAG GGAACTTAGC AGAGAGCAAA TGCTATTTGA TAACAGTTAC TCCAGTATAT GCTGATGGAC CAGGAAGCCC TGAATCCATA AAGGCATACC TTAAACAAGC TCCACCTTCC
                      Y  L  R  G  N  L  A  E  S  K  C  Y  L  I  T  V  T  P  V  Y  A  D  G  P  G  S  P  E  S  I  K  A  Y  L  K  Q  A  P  P  S
sgp130Fc_opt   #1441  TACCTGAGAG GCAATCTGGC CGAGTCTAAG TGCTATCTGA TCACCGTGAC CCCTGTGTAT GCTGATGGAC CTGGCTCTCC TGAGTCTATC AAGGCCTACC TGAAGCAGGC TCCTCCATCT
                      Y  L  R  G  N  L  A  E  S  K  C  Y  L  I  T  V  T  P  V  Y  A  D  G  P  G  S  P  E  S  I  K  A  Y  L  K  Q  A  P  P  S

sgp130Fc_wt    #1561  AAAGGACCTA CTGTTCGGAC AAAAAAAGTA GGGAAAAACG AAGCTGTCTT AGAGTGGGAC CAACTTCCTG TTGATGTTCA GAATGGATTT ATCAGAAATT ATACTATATT TTATAGAACC
                      K  G  P  T  V  R  T  K  K  V  G  K  N  E  A  V  L  E  W  D  Q  L  P  V  D  V  Q  N  G  F  I  R  N  Y  T  I  F  Y  R  T
sgp130Fc_opt   #1561  AAGGGACCTA CCGTGAGGAC AAAGAAGGTG GGCAAGAACG AGGCTGTGCT GGAGTGGGAT CAGCTGCCTG TGGATGTGCA GAACGGCTTC ATCCGGAACT ACACCATCTT CTACCGGACC
                      K  G  P  T  V  R  T  K  K  V  G  K  N  E  A  V  L  E  W  D  Q  L  P  V  D  V  Q  N  G  F  I  R  N  Y  T  I  F  Y  R  T

sgp130Fc_wt    #1681  ATCATTGGAA ATGAAACTGC TGTGAATGTG GATTCTTCCC ACACAGAATA TACATTGTCC TCTTTGACTA GTGACACATT GTACATGGTA CGAATGGCAG CATACACAGA TGAAGGTGGG
                      I  I  G  N  E  T  A  V  N  V  D  S  S  H  T  E  Y  T  L  S  S  L  T  S  D  T  L  Y  M  V  R  M  A  A  Y  T  D  E  G  G
sgp130Fc_opt   #1681  ATCATCGGCA ATGAGACCGC CGTGAACGTG GATTCTTCCC ACACCGAGTA CACACTGTCC TCTCTGACCT CTGACACCCT GTACATGGTG AGAATGGCCG CTTATACCGA TGAGGGCGGC
                      I  I  G  N  E  T  A  V  N  V  D  S  S  H  T  E  Y  T  L  S  S  L  T  S  D  T  L  Y  M  V  R  M  A  A  Y  T  D  E  G  G

sgp130Fc_wt    #1801  AAGGATGGTC CAGAATTCAG ATCTTGTGAC AAAACTCACA CATGCCCACC GTGCCCAGCA CCTGAAGCCG AGGGCGCGCC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA GGACACCCTC
                      K  D  G  P  E  F  R  S  C  D  K  T  H  T  C  P  P  C  P  A  P  E  A  E  G  A  P  S  V  F  L  F  P  P  K  P  K  D  T  L
sgp130Fc_opt   #1801  AAGGATGGAC CTGAGTTCAG ATCCTGCGAC AAGACCCACA CCTGTCCTCC TTGTCCTGCT CCTGAGGCTG AGGGCGCTCC TTCTGTGTTT CTGTTCCCCC CAAAGCCTAA GGATACCCTG
                      K  D  G  P  E  F  R  S  C  D  K  T  H  T  C  P  P  C  P  A  P  E  A  E  G  A  P  S  V  F  L  F  P  P  K  P  K  D  T  L
```

```
sgp130Fc_wt    #1921  ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG
                      M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  T  K  P
sgp130Fc_opt   #1921  ATGATCTCCA GAACCCCTGA GGTGACATGT GTGGTGGTGG ATGTGTCTCA TGAGGACCCC GAGGTGAAGT TCAACTGGTA CGTGGATGGC GTGGAGGTGC ACAATGCTAA GACCAAGCCT
                      M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  T  K  P

sgp130Fc_wt    #2041  CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC
                      R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
sgp130Fc_opt   #2041  AGGGAGGAGC AGTACAACTC CACCTACAGA GTGGTGTCTG TGCTGACAGT GCTGCATCAG GATTGGCTGA ACGGCAAGGA GTACAAGTGC AAGGTGTCCA ACAAGGCTCT GCCTGCTCCT
                      R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P

sgp130Fc_wt    #2161  ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGAGGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC
                      I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G
sgp130Fc_opt   #2161  ATCGAAAAGA CCATCTCCAA GGCTAAGGGA CAGCCTAGAG AGCCTCAGGT GTACACACTG CCTCCATCTA GGGAGGAGAT GACCAAGAAT CAGGTGTCCC TGACCTGTCT GGTGAAGGGC
                      I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G

sgp130Fc_wt    #2281  TTCTATCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTATAG CAAGCTCACC
                      F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T
sgp130Fc_opt   #2281  TTCTACCCTT CTGATATCGC TGTGGAGTGG GAGTCTAATG GCCAGCCCGA GAACAATTAC AAGACCACCC CTCCTGTGCT GGATTCTGAC GGCTCCTTCT TCCTGTACTC CAAACTGACC
                      F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T
```

```
sgp130Fc_wt    #2401  GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGTAAATG A
                      V  D  K  S   R  W  Q   Q  G  N   V  F  S  C   S  V  M   H  E  A   L  H  N  H   Y  T  Q   K  S  L   S  L  S  P   G  K  .
sgp130Fc_opt   #2401  GTGGACAAGT CTAGATGGCA GCAGGGCAAC GTGTTCTCTT GTTCCGTGAT GCACGAGGCT CTGCACAATC ACTATACCCA GAAGTCCCTG TCTCTGTCTC CTGGCAAGTG A
                      V  D  K  S   R  W  Q   Q  G  N   V  F  S  C   S  V  M   H  E  A   L  H  N  H   Y  T  Q   K  S  L   S  L  S  P   G  K  .
```

Figure 4

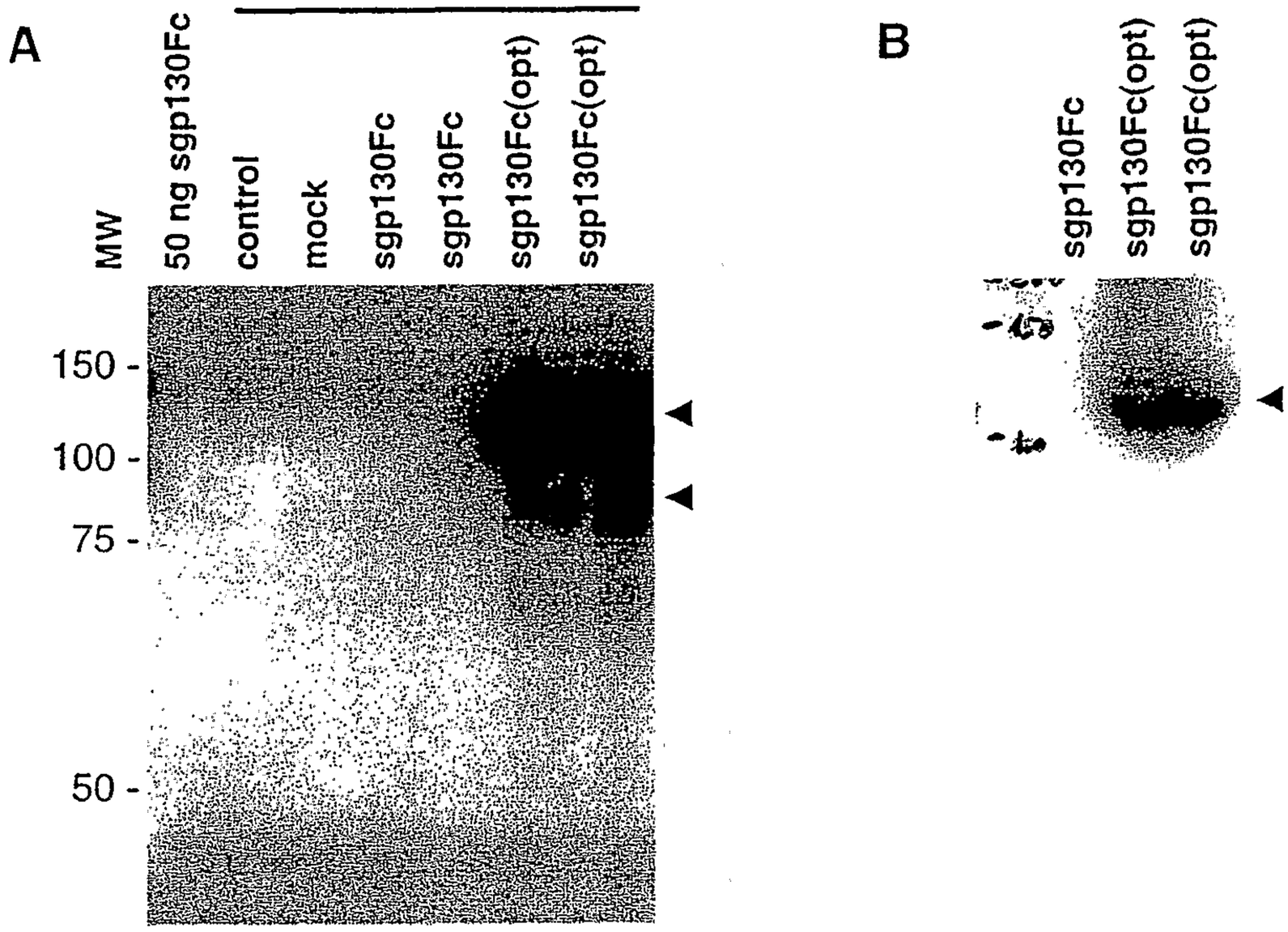
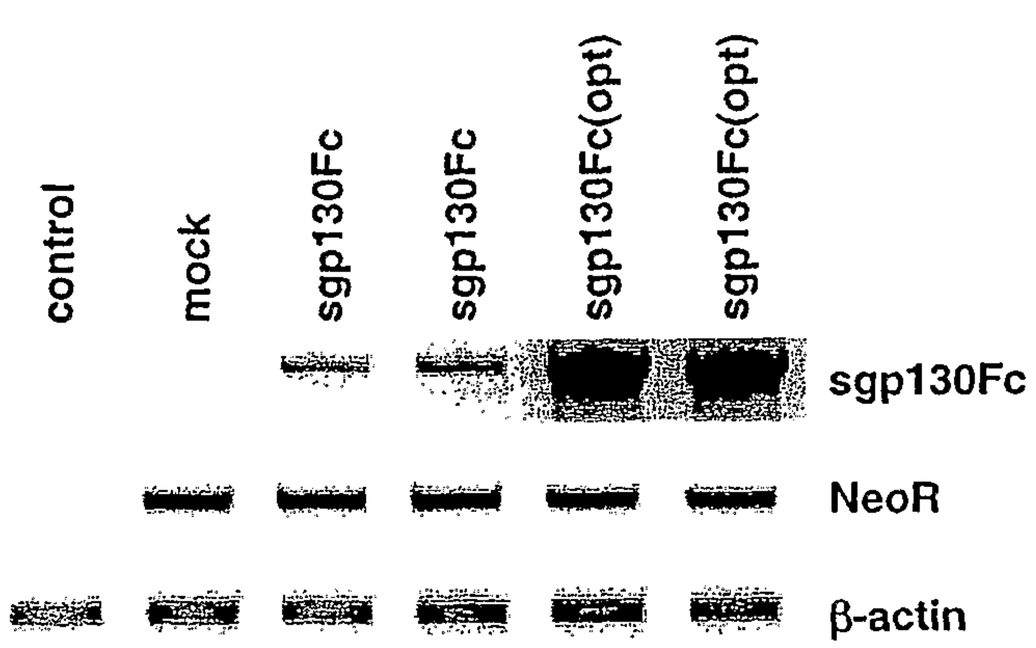

Supernatants/IP
A
MW
50 ng sgp130Fc
control
mock
sgp130Fc
sgp130Fc
sgp130Fc(opt)
sgp130Fc(opt)
150 -
100 -
75 -
50 -
B
sgp130Fc
sgp130Fc(opt)
sgp130Fc(opt)

Figure 5


control
mock
sgp130Fc
sgp130Fc
sgp130Fc(opt)
sgp130Fc(opt)
sgp130Fc
NeoR
β-actin

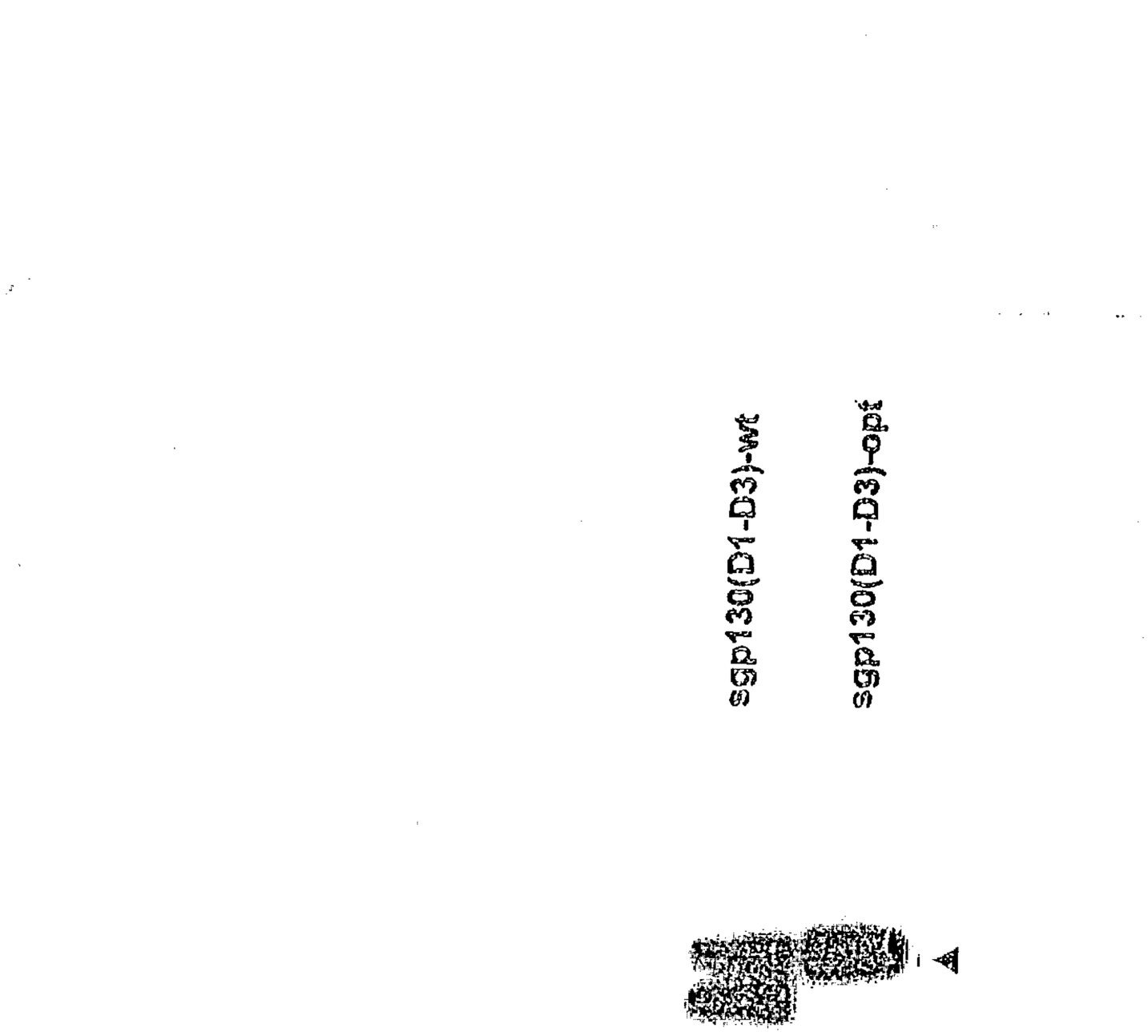


Figure 6

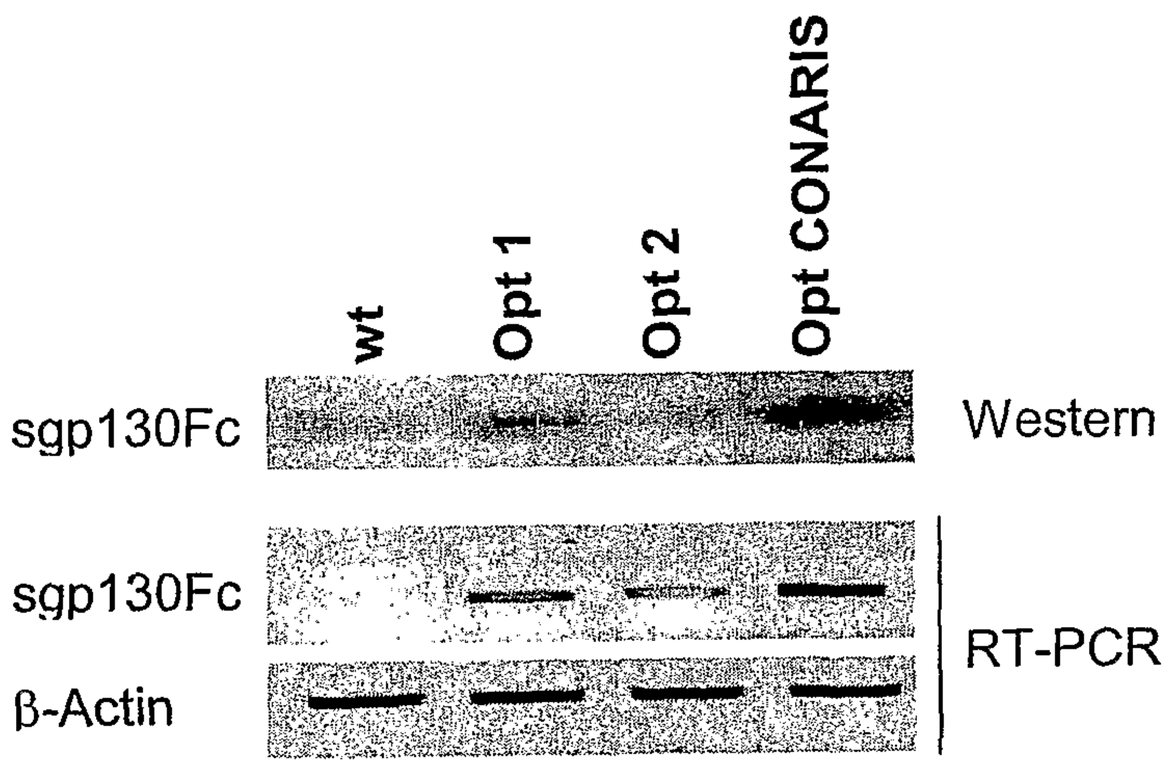

wt
Opt 1
Opt 2
Opt CONARIS
sgp130Fc
Western
sgp130Fc
β-Actin
RT-PCR

Figure 7

Figure 8

```
>sgp130Fc_wt          #1      ATGTTCACGT TGCAGACTTG GCTAGTGCAA GCCTTGTTTA TTTTCCTCAC CACTGAATCT ACAGGTGAAC TTCTAGATCC ATGTGGTTAT ATCAGTCCTG AATCTCCAGT
>sgp130Fc_opt_CON     #1      ATGCTGACAC TGCAGACATG GCTGGTGCAG GCCCTGTTTA TCTTTCTGAC CACCGAGTCT ACAGGAGAGC TGCTGGATCC TTGCGGCTAT ATCTCCCCTG AGTCTCCTGT
>sgp130Fc_opt_JCat    #1      ATGCTGACCC TGCAGACCTG GCTGGTGCAG GCCCTGTTCA TCTTCCTGAC CACCGAGAGC ACCGGCGAGC TGCTGGACCC CTGCGGCTAC ATCAGCCCCG AGAGCCCCGT
>sgp130Fc_opt_Upgene  #1      ATGCTGACAC TCCAGACGTG GCTCGTGCAG GCACTATTCA TTTTGCTCAC TACGGAGTCT ACAGGCGAGC TGCTGGACCC CTGCGGCTAT ATTTCCCCCG AGTCTCCCGT

                      #1      ATGCTGACAC TGCAGACNTG GCTGGTGCAG GCCCTGTTYA TYTTCCTSAC CACCGAGTCT ACAGGCGAGC TGCTGGAYCC CTGCGGCTAT ATCWSCCCYG AGTCTCCCGT

>sgp130Fc_wt          #111     TGTACAACTT CATTCTAATT TCACTGCAGT TTGTGTGCTA AAGGAAAAAT GTATGGATTA TTTTCATGTA AATGCTAATT ACATTGTCTG GAAAACAAAC CATTTTACTA
>sgp130Fc_opt_CON     #111     GGTGCAGCTG CATTCTAACT TCACCGCCGT GTGTGTGCTG AAGGAAAAGT GCATGGACTA CTTCCACGTG AACGCCAACT ACATCGTGTG GAAAACCAAC CACTTCACCA
>sgp130Fc_opt_JCat    #111     GGTGCAGCTG CACAGCAACT TCACCGCCGT GTGCGTGCTG AAGGAGAAGT GCATGGACTA CTTCCACGTG AACGCCAACT ACATCGTGTG GAAGACCAAC CACTTCACCA
>sgp130Fc_opt_Upgene  #111     GGTGCAGCTG CACTCGAATT TCACGGCCGT ATGCGTCCTC AAGGAGAAGT GCATGGATTA CTTTCACGTG AATGCAAATT ATATCGTTTG GAAAACGAAC CACTTTACCA

                      #111     GGTGCAGCTG CAYTCTAAYT TCACCGCCGT GTGYGTGCTG AAGGARAAGT GCATGGAYTA CTTYCACGTG AAYGCCAAYT ACATCGTGTG GAAAACCAAC CACTTYACCA

>sgp130Fc_wt          #221     TTCCTAAGGA GCAATATACT ATCATAAACA GAACAGCATC CAGTGTCACC TTTACAGATA TAGCTTCATT AAATATTCAG CTCACTTGCA ACATTCTTAC ATTCGGACAG
>sgp130Fc_opt_CON     #221     TCCCCAAGGA GCAGTACACC ATCATCAACC GGACCGCTTC TTCTGTGACC TTCACCGATA TCGCCTCCCT GAATATCCAG CTGACCTGCA ACATCCTGAC CTTTGGACAG
>sgp130Fc_opt_JCat    #221     TCCCCAAGGA GCAGTACACC ATCATCAACC GCACCGCCAG CAGCGTGACC TTCACCGACA TCGCCAGCCT GAACATCCAG CTGACCTGCA ACATCCTGAC CTTCGGCCAG
>sgp130Fc_opt_Upgene  #221     TCCCCAAGGA ACAGTACACA ATCATCAACC GCACAGCATC GAGTGTGACC TTCACCGACA TTGCGTCCCT CAATATCCAG CTGACCTGCA ACATCCTGAC ATTTGGGCAG

                      #221     TCCCCAAGGA GCAGTACACC ATCATCAACC GCACMGCATC CAGTGTGACC TTCACCGAYA TCGCCTCCCT GAATATCCAG CTGACCTGCA ACATCCTGAC MTTYGGACAG

>sgp130Fc_wt          #331     CTTGAACAGA ATGTTTATGG AATCACAATA ATTTCGGGCT TGCCTCCAGA AAAACCTAAA AATTTGAGTT GCATTGTGAA CGAGGGGAAG AAAATGAGGT GTGAGTGGGA
>sgp130Fc_opt_CON     #331     CTGGAGCAGA ATGTGTACGG CATCACCATC ATCTCTGGCC TGCCTCCAGA GAAGCCTAAG AACCTGTCCT GCATCGTGAA TGAGGGCAAG AAGATGAGGT GTGAGTGGGA
>sgp130Fc_opt_JCat    #331     CTGGAGCAGA ACGTGTACGG CATCACCATC ATCAGCGGCC TGCCCCCCGA GAAGCCCAAG AACCTGAGCT GCATCGTGAA CGAGGGCAAG AAGATGCGCT GCGAGTGGGA
>sgp130Fc_opt_Upgene  #331     CTGGAGCAGA ACGTTTACGG CATCACTATC ATCTCGGGGC TCCCCCCTGA GAAGCCAAAG AACCTCTCCT GTATCGTTAA CGAGGGAAAG AAGATGAGGT GCGAGTGGGA

                      #331     CTGGAGCAGA AYGTKTACGG CATCACCATC ATCTCGGGCC TGCCYCCAGA GAAGCCTAAG AACCTGWSCT GCATCGTGAA CGAGGGCAAG AAGATGAGGT GYGAGTGGGA

>sgp130Fc_wt          #441     TGGTGGAAGG GAAACACACT TGGAGACAAA CTTCACTTTA AAATCTGAAT GGGCAACACA CAAGTTTGCT GATTGCAAAG CAAAACGTGA CACCCCCACC TCATGCACTG
>sgp130Fc_opt_CON     #441     TGGCGGCAGA GAGACACATC TGGAGACCAA CTTCACCCTG AAGTCTGAGT GGGCCACCCA CAAGTTTGCC GACTGCAAGG CCAAGAGAGA TACCCCTACC TCTTGCACCG
>sgp130Fc_opt_JCat    #441     CGGCGGCCGC GAGACCCACC TGGAGACCAA CTTCACCCTG AAGAGCGAGT GGGCCACCCA CAAGTTCGCC GACTGCAAGG CCAAGCGCGA CACCCCCACC AGCTGCACCG
>sgp130Fc_opt_Upgene  #441     TGGGGGCCGG GAGACCCACC TGGAGACGAA CTTCACCCTG AAGTCGGAGT GGGCGACGCA CAAGTTTGCG GATTGCAAGG CCAAAAGGGA TACACCCACC AGCTGTACTG

                      #441     TGGCGGCMGG GAGACMCACC TGGAGACCAA CTTCACCCTG AAGTCTGAGT GGGCCACCCA CAAGTTTGCC GAYTGCAAGG CCAARMGNGA YACCCCCACC WSCTGCACYG
```

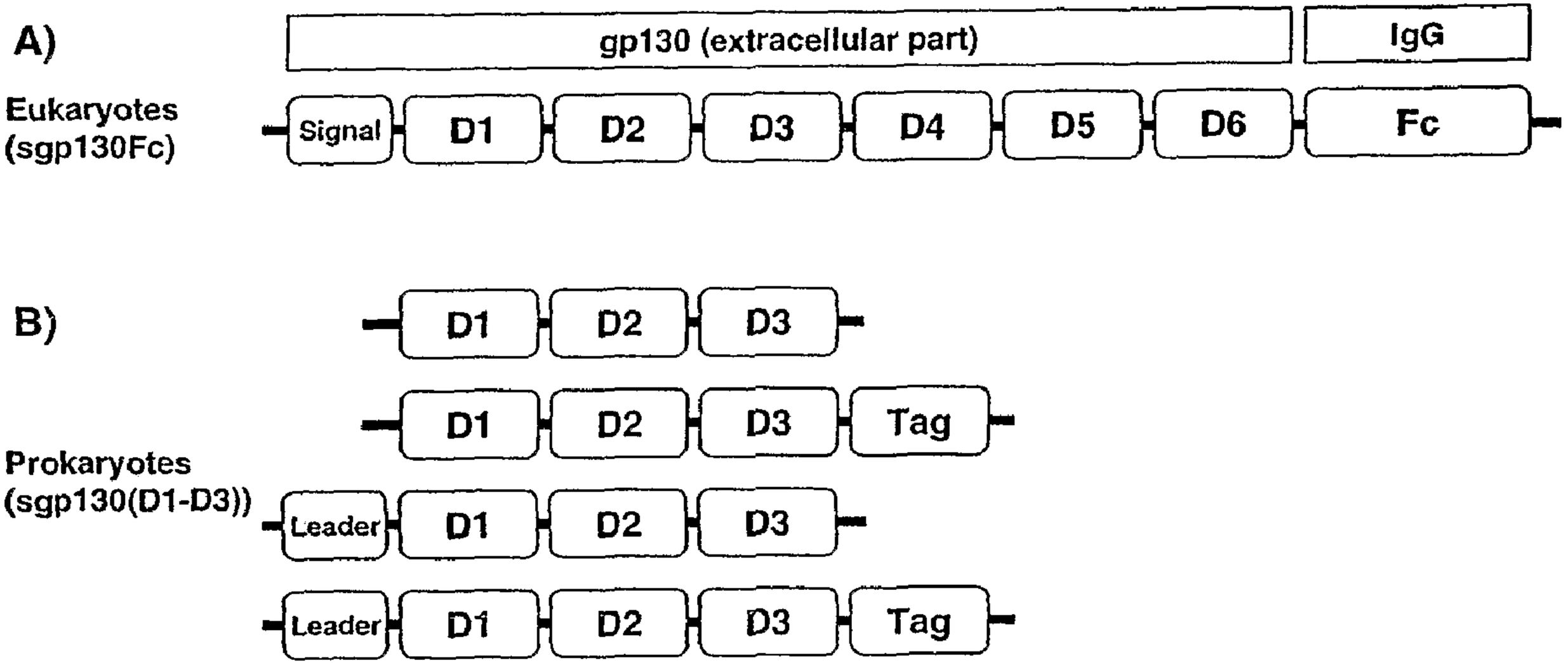
```
>sgp130Fc_wt          #551    TTGATTATTC TACTGTGTAT TTTGTCAACA TTGAAGTCTG GGTAGAAGCA GAGAATGCCC TTGGGAAGGT TACATCAGAT CATATCAATT TTGATCCTGT ATATAAAGTG
>sgp130Fc_opt_CON     #551    TGGACTACTC CACCGTGTAC TTCGTGAACA TCGAGGTGTG GGTGGAGGCT GAGAATGCTC TGGGCAAGGT GACCTCTGAC CACATCAACT TCGACCCCGT GTACAAGGTG
>sgp130Fc_opt_JCat    #551    TGGACTACAG CACCGTGTAC TTCGTGAACA TCGAGGTGTG GGTGGAGGCC GAGAACGCCC TGGGCAAGGT GACCAGCGAC CACATCAACT TCGACCCCGT GTACAAGGTG
>sgp130Fc_opt_Upgene  #551    TCGACTACTC AACGGTTTAC TTTGTCAACA TAGAGGTTTG GGTGGAGGCG GAGAACGCCC TGGGCAAGGT GACCTCCGAC CACATCAATT TCGATCCCGT GTATAAAGTG
                              ....................................................................................................................
                      #551    TGGACTACTC CACCGTGTAC TTYGTSAACA TCGAGGTGTG GGTGGAGGCN GAGAAYGCCC TGGGCAAGGT GACCTCCGAC CACATCAAYT TCGAYCCCGT GTAYAARGTG
                               *  *  *** *  *  *  *   *  *     *  *  *     *  *  *     *  *   *  *     *  **** *   *    *   *  *  *   *  *  *

>sgp130Fc_wt          #661    AAGCCCAATC CGCCACATAA TTTATCAGTG ATCAACTCAG AGGAACTGTC TAGTATCTTA AAATTGACAT GGACCAACCC AAGTATTAAG AGTGTTATAA TACTAAAATA
>sgp130Fc_opt_CON     #661    AAGCCTAACC CTCCTCACAA CCTGTCCGTG ATCAACTCTG AGGAGCTGTC CTCTATCCTG AAGCTGACCT GGACCAACCC TTCCATCAAG TCCGTGATCA TCCTGAAGTA
>sgp130Fc_opt_JCat    #661    AAGCCCAACC CCCCCCACAA CCTGAGCGTG ATCAACAGCG AGGAGCTGAG CAGCATCCTG AAGCTGACCT GGACCAACCC CAGCATCAAG AGCGTGATCA TCCTGAAGTA
>sgp130Fc_opt_Upgene  #661    AAACCTAACC CGCCCCACAA TCTCAGTGTA ATTAACTCCG AGGAGCTCAG TTCTATCCTT AAGTTGACTT GGACCAACCC TTCCATCAAG AGCGTAATCA TCCTGAAGTA
                              ....................................................................................................................
                      #661      *  *  *   *  *  *   ** ****  *   *   ***     *  *** ****   * *   **    *           ****  *    ***  *  *   *  *  *

>sgp130Fc_wt          #771    TAACATTCAA TATAGGACCA AAGATGCCTC AACTTGGAGC CAGATTCCTC CTGAAGACAC AGCATCCACC CGATCTTCAT TCACTGTCCA AGACCTTAAA CCTTTTACAG
>sgp130Fc_opt_CON     #771    CAACATCCAG TACAGGACCA AGGATGCTTC TACCTGGTCT CAGATCCCTC CTGAGGATAC CGCTTCCACC AGATCCAGCT TCACAGTGCA GGACCTGAAG CCTTTTACCG
>sgp130Fc_opt_JCat    #771    CAACATCCAG TACCGCACCA AGGACGCCAG CACCTGGAGC CAGATCCCCC CCGAGGACAC CGCCAGCACC CGCAGCAGCT TCACCGTGCA GGACCTGAAG CCCTTCACCG
>sgp130Fc_opt_Upgene  #771    CAACATTCAG TACCGTACTA AGGACGCGTC CACATGGAGC CAGATCCCCC CTGAGGATAC CGCATCCACC CGGTCGAGTT TTACCGTGCA GGACCTGAAA CCCTTCACCG
                              ....................................................................................................................
                      #771    CAACATYCAG TACMGGACCA AGGAYGCCTC CACCTGGAGC CAGATCCCYC CTGAGGAYAC CGCATCCACC CGATCCAGCT TCACCGTGCA GGACCTGAAR CCYTTYACCG
                              *     *  *   ** *  *   *  *  *** *  *   ***     *  *   *  *  *  *  ***    * *******   *  *  *   *    *  *   *  *  *

>sgp130Fc_wt          #881    AATATGTGTT TAGGATTCGC TGTATGAAGG AAGATGGTAA GGGATACTGG AGTGACTGGA GTGAAGAAGC AAGTGGGATC ACCTATGAAG ATAGACCATC TAAAGCACCA
>sgp130Fc_opt_CON     #881    AGTACGTGTT CAGGATCCGG TGCATGAAGG AGGATGGCAA GGGCTATTGG TCTGACTGGT CTGAGGAGGC TTCTGGCATC ACCTACGAGG ACAGACCTTC TAAGGCCCCT
>sgp130Fc_opt_JCat    #881    AGTACGTGTT CCGCATCCGC TGCATGAAGG AGGACGGCAA GGGCTACTGG AGCGACTGGA GCGAGGAGGC CAGCGGCATC ACCTACGAGG ACCGCCCCAG CAAGGCCCCC
>sgp130Fc_opt_Upgene  #881    AGTACGTGTT CCGCATCCGG TGCATGAAAG AGGACGGGAA GGGGTACTGG TCCGATTGGT CCGAGGAGGC CAGTGGCATT ACCTATGAAG ACCGGCCTAG TAAGGCCCCC
                              ....................................................................................................................
                      #881    AGTACGTGTT CMGSATCCGS TGCATGAAGG AGGAYGGCAA GGGCTACTGG WSYGACTGGW SYGAGGAGGC CAGTGGCATC ACCTAYGARG ACMGACCTWS TAAGGCCCCC
                               *  *      ** *  *  *   *     *   *  *  *  *     *  *    ***  *   * **  *  *   ****  *  *      *  *   ** *  *** *  *  *  *

>sgp130Fc_wt          #991    AGTTTCTGGT ATAAAATAGA TCCATCCCAT ACTCAAGGCT ACAGAACTGT ACAACTCGTG TGGAAGACAT TGCCTCCTTT TGAAGCCAAT GGAAAAATCT TGGATTATGA
>sgp130Fc_opt_CON     #991    AGCTTCTGGT ACAAGATCGA CCCTTCTCAC ACCCAGGGCT ATAGAACAGT GCAGCTGGTG TGGAAAACCC TGCCTCCATT CGAGGCTAAT GGCAAGATCC TGGACTATGA
>sgp130Fc_opt_JCat    #991    AGCTTCTGGT ACAAGATCGA CCCCAGCCAC ACCCAGGGCT ACCGCACCGT GCAGCTGGTG TGGAAGACCC TGCCCCCCTT CGAGGCCAAC GGCAAGATCC TGGACTACGA
>sgp130Fc_opt_Upgene  #991    TCCTTCTGGT ATAAAATCGA CCCGTCCCAC ACCCAGGGCT ACCGCACCGT GCAGTTGGTG TGGAAGACGC TGCCGCCATT CGAGGCCAAC GGGAAGATTC TGGACTACGA
                              ....................................................................................................................
                      #991    AGCTTCTGGT AYAARATCGA CCCNTCCCAC ACCCAGGGCT ACMGMACCGT GCAGCTGGTG TGGAAGACCC TGCCTCCATT CGAGGCCAAY GGCAAGATCC TGGACTAYGA
                              ***          *  *  *   *  ****  *   *  *     ** *  *   *  ** *        *  **     *  *   *  *  *  *   *  *  **     *  *

>sgp130Fc_wt          #1101   AGTGACTCTC ACAAGATGGA AATCACATTT ACAAAATTAC ACAGTTAATG CCACAAAACT GACAGTAAAT CTCACAAATG ATCGCTATCT AGCAACCCTA ACAGTAAGAA
>sgp130Fc_opt_CON     #1101   GGTGACCCTG ACCAGATGGA AGTCTCACCT GCAGAACTAC ACCGTGAACG CTACCAAGCT GACCGTGAAC CTGACCAACG ATAGATACCT GGCTACCCTG ACCGTGAGAA
>sgp130Fc_opt_JCat    #1101   GGTGACCCTG ACCCGCTGGA AGAGCCACCT GCAGAACTAC ACCGTGAACG CCACCAAGCT GACCGTGAAC CTGACCAACG ACCGCTACCT GGCCACCCTG ACCGTGCGCA
>sgp130Fc_opt_Upgene  #1101   GGTCACCCTG ACTCGCTGGA AATCCCACCT CCAGAATTAT ACCGTGAACG CCACGAAGCT CACCGTTAAC CTGACGAACG ACCGCTATCT GGCCACCCTG ACCGTGCGCA
                              ....................................................................................................................
                      #1101   GGTGACCCTG ACCMGMTGGA ARTCCCACCT GCAGAAYTAC ACCGTGAACG CCACCAAGCT GACCGTGAAC CTGACCAACG AYCGCTAYCT GGCCACCCTG ACCGTGMGMA
                              *  *  *  *   ** *     ****  **  *  *  *  *  *  *  *   *  *  *   *  *  *  *  *   *  *  *   ** *  *   *  *     *   *  ** *
```

```
>sgp130Fc_wt          #1211   ATCTTGTTGG CAAATCAGAT GCAGCTGTTT TAACTATCCC TGCCTGTGAC TTTCAAGCTA CTCACCCTGT AATGGATCTT AAAGCATTCC CCAAAGATAA CATGCTTTGG
>sgp130Fc_opt_CON     #1211   ATCTGGTGGG CAAGTCTGAT GCTGCTGTGC TGACCATCCC TGCCTGTGAT TTTCAGGCTA CCCACCCTGT GATGGATCTG AAGGCCTTCC CCAAGGATAA CATGCTGTGG
>sgp130Fc_opt_JCat    #1211   ACCTGGTGGG CAAGAGCGAC GCCGCCGTGC TGACCATCCC CGCCTGCGAC TTCCAGGCCA CCCACCCCGT GATGGACCTG AAGGCCTTCC CCAAGGACAA CATGCTGTGG
>sgp130Fc_opt_Upgene  #1211   ACCTGGTGGG CAAGTCCGAC GCTGCAGTGC TGACCATCCC GGCATGTGAC TTCCAGGCGA CACACCCCGT GATGGATCTG AAGGCGTTTC CTAAGGATAA TATGCTGTGG
                      ....................................................................................................
                      #1211   AYCTGGTGGG CAAGTCCGAY GCTGCTGTGC TGACCATCCC TGCCTGTGAC TTYCAGGCTA CCCACCCYGT GATGGATCTG AAGGCCTTCC CCAAGGATAA CATGCTGTGG
                               *  *  *     **** *   *  *  ** *  *     * * * *   * * *   *     *  *    * *   * * *  * * *  *     *

>sgp130Fc_wt          #1321   GTGGAATGGA CTACTCCAAG GGAATCTGTA AAGAAATATA TACTTGAGTG GTGTGTGTTA TCAGATAAAG CACCCTGTAT CACAGACTGG CAACAAGAAG ATGGTACCGT
>sgp130Fc_opt_CON     #1321   GTGGAGTGGA CAACACCTAG AGAGTCCGTG AAGAAGTACA TCCTGGAGTG GTGCGTGCTG TCTGATAAGG CCCCTTGCAT CACAGATTGG CAGCAGGAGG ATGGCACCGT
>sgp130Fc_opt_JCat    #1321   GTGGAGTGGA CCACCCCCCG CGAGAGCGTG AAGAAGTACA TCCTGGAGTG GTGCGTGCTG AGCGACAAGG CCCCCTGCAT CACCGACTGG CAGCAGGAGG ACGGCACCGT
>sgp130Fc_opt_Upgene  #1321   GTGGAGTGGA CGACCCCCCG AGAGTCCGTG AAGAAATACA TTCTGGAGTG GTGCGTGCTG AGCGACAAGG CCCCTTGCAT CACCGATTGG CAACAAGAGG ACGGTACCGT
                      ....................................................................................................
                      #1321   GTGGAGTGGA CNACCCCCMG AGAGTCCGTG AAGAARTACA TCCTGGAGTG GTGCGTGCTG WSCGAYAAGG CCCCYTGCAT CACMGAYTGG CARCARGAGG AYGGYACCGT
                                 *     *  *  **  *  ****  *      *  *   *  *        *   * * ***  *  *   *  *  *     *  *     *  *  *   *  *

>sgp130Fc_wt          #1431   GCATCGCACC TATTTAAGAG GGAACTTAGC AGAGAGCAAA TGCTATTTGA TAACAGTTAC TCCAGTATAT GCTGATGGAC CAGGAAGCCC TGAATCCATA AAGGCATACC
>sgp130Fc_opt_CON     #1431   GCATAGAACC TACCTGAGAG GCAATCTGGC CGAGTCTAAG TGCTATCTGA TCACCGTGAC CCCTGTGTAT GCTGATGGAC CTGGCTCTCC TGAGTCTATC AAGGCCTACC
>sgp130Fc_opt_JCat    #1431   GCACCGCACC TACCTGCGCG GCAACCTGGC CGAGAGCAAG TGCTACCTGA TCACCGTGAC CCCCGTGTAC GCCGACGGCC CCGGCAGCCC CGAGAGCATC AAGGCCTACC
>sgp130Fc_opt_Upgene  #1431   CCACCGAACC TACCTGAGGG GGAACCTCGC TGAGTCCAAG TGCTACCTAA TCACGGTGAC CCCAGTGTAC GCCGATGGTC CAGGGTCCCC TGAGTCCATC AAGGCCTACC
                      ....................................................................................................
                      #1431   GCAYCGMACC TACCTGAGAG GSAACCTGGC CGAGWSCAAG TGCTAYCTGA TCACCGTGAC CCCAGTGTAY GCYGATGGAC CAGGCWSCCC TGAGTCCATC AAGGCCTACC
                              *  ** *     ** ** *   *  ** *   *  *** *     ** *   *  *  *   *  *  *  *   *  *  *   *  ****   *  **** *     *

>sgp130Fc_wt          #1541   TTAAACAAGC TCCACCTTCC AAAGGACCTA CTGTTCGGAC AAAAAAAGTA GGGAAAAACG AAGCTGTCTT AGAGTGGGAC CAACTTCCTG TTGATGTTCA GAATGGATTT
>sgp130Fc_opt_CON     #1541   TGAAGCAGGC TCCTCCATCT AAGGGACCTA CCGTGAGGAC AAAGAAGGTG GGCAAGAACG AGGCTGTGCT GGAGTGGGAT CAGCTGCCTG TGGATGTGCA GAACGGCTTC
>sgp130Fc_opt_JCat    #1541   TGAAGCAGGC CCCCCCCAGC AAGGGCCCCA CCGTGCGCAC CAAGAAGGTG GGCAAGAACG AGGCCGTGCT GGAGTGGGAC CAGCTGCCCG TGGACGTGCA GAACGGCTTC
>sgp130Fc_opt_Upgene  #1541   TCAAGCAGGC CCCACCTTCG AAGGGCCCTA CAGTGAGAAC CAAGAAGGTC GGGAAGAACG AGGCGGTGCT GGAATGGGAC CAGCTGCCAG TGGACGTGCA GAATGGCTTC
                      ....................................................................................................
                      #1541   TGAAGCAGGC YCCACCTTCC AAGGGMCCTA CCGTGMGGAC MAAGAAGGTG GGSAAGAACG AGGCTGTGCT GGAGTGGGAC CAGCTGCCTG TGGAYGTGCA GAAYGGCTTC
                               *  *  *   *  *  ****   *  *  *   *  ** *   *  *  *  *   *  *     *  *  **  *  *     *   *  *  *   *  *  *     *  *  *

>sgp130Fc_wt          #1651   ATCAGAAATT ATACTATATT TTATAGAACC ATCATTGGAA ATGAAACTGC TGTGAATGTG GATTCTTCCC ACACAGAATA TACATTGTCC TCTTTGACTA GTGACACATT
>sgp130Fc_opt_CON     #1651   ATCCGGAACT ACACCATCTT CTACCGGACC ATCATCGGCA ATGAGACCGC CGTGAACGTG GATTCTTCCC ACACCGAGTA CACACTGTCC TCTCTGACCT CTGACACCCT
>sgp130Fc_opt_JCat    #1651   ATCCGCAACT ACACCATCTT CTACCGCACC ATCATCGGCA ACGAGACCGC CGTGAACGTG GACAGCAGCC ACACCGAGTA CACCCTGAGC AGCCTGACCA GCGACACCCT
>sgp130Fc_opt_Upgene  #1651   ATCCGAAATT ACACCATCTT CTACCGGACC ATCATCGGCA ATGAGACCGC CGTCAACGTA GACTCCTCCC ACACCGAGTA CACGCTGTCC TCACTGACTA GCGACACCCT
                      ....................................................................................................
                      #1651   ATCCGAAAYT ACACCATCTT CTACCGGACC ATCATCGGCA ATGAGACCGC CGTGAACGTG GAYTCYTCCC ACACCGAGTA CACACTGTCC TCTCTGACYA GYGACACCCT
                                 * *  *   * *  *   * *** *         * *   *  *  *   * * *  *   ******      *  *   *  **  **  ****    ** **     **

>sgp130Fc_wt          #1761   GTACATGGTA CGAATGGCAG CATACACAGA TGAAGGTGGG AAGGATGGTC CAGAATTCAG ATCTTGTGAC AAAACTCACA CATGCCCACC GTGCCCAGCA CCTGAAGCCG
>sgp130Fc_opt_CON     #1761   GTACATGGTG AGAATGGCCG CTTATACCGA TGAGGGCGGC AAGGATGGAC CTGAGTTCAG ATCCTGCGAC AAGACCCACA CCTGTCCTCC TTGTCCTGCT CCTGAGGCTG
>sgp130Fc_opt_JCat    #1761   GTACATGGTG CGCATGGCCG CCTACACCGA CGAGGGCGGC AAGGACGGCC CCGAGTTCCG CAGCTGCGAC AAGACCCACA CCTGCCCCCC CTGCCCCGCC CCCGAGGCCG
>sgp130Fc_opt_Upgene  #1761   GTATATGGTT CGCATGGCTG CTTACACCGA TGAAGGCGGG AAAGACGGCC CTGAGTTCAG GTCCTGTGAC AAGACTCATA CGTGCCCCCC ATGTCCTGCC CCGGAAGCCG
                      ....................................................................................................
                      #1761   GTACATGGTG CGMATGGCCG CTTACACCGA TGARGGCGGS AAGGAYGGCC CTGAGTTCAG ATCCTGYGAC AAGACYCACA CCTGCCCCCC NTGYCCTGCC CCTGARGCCG
                                 *     * * *     *   *  *  *   *  *  *  *   *  *  *   *  *   *  **** *     *  *  *   *  *  *   *  *  *  *   *  *  *
```

```
>sgp130Fc_wt          #1871   AGGGCGCGCC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT
>sgp130Fc_opt_CON     #1871   AGGGCGCTCC TTCTGTGTTT CTGTTCCCCC CAAAGCCTAA GGATACCCTG ATGATCTCCA GAACCCCTGA GGTGACATGT GTGGTGGTGG ATGTGTCTCA TGAGGACCCC
>sgp130Fc_opt_JCat    #1871   AGGGCGGCCC CAGCGTGTTC CTGTTCCCCC CCAAGCCCAA GGACACCCTG ATGATCAGCC GCACCCCCGA GGTGACCTGC GTGGTGGTGG ACGTGAGCCA CGAGGACCCC
>sgp130Fc_opt_Upgene  #1871   AGGGGGGTCC CTCCGTGTTC CTCTTCCCCC CCAAGCCGAA AGACACCCTC ATGATCTCAC GGACCCCCGA GGTGACATGC GTGGTGGTGG ATGTGTCACA CGAGGACCCC
                               ..................................................................................................................
                      #1871   AGGGCGSTCC CTCCGTGTTC CTSTTCCCCC CMAAGCCCAA GGACACCCTS ATGATCTCCC GGACCCCYGA GGTGACATGC GTGGTGGTGG AYGTGWSCCA CGAGGACCCC
                                  * **   **** *  *   *        *  *  *   *  *     *       **** *     *     *  *  *              *   ***   *  *     *

>sgp130Fc_wt          #1981   GAGGTCAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
>sgp130Fc_opt_CON     #1981   GAGGTGAAGT TCAACTGGTA CGTGGATGGC GTGGAGGTGC ACAATGCTAA GACCAAGCCT AGGGAGGAGC AGTACAACTC CACCTACAGA GTGGTGTCTG TGCTGACAGT
>sgp130Fc_opt_JCat    #1981   GAGGTGAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ACAACGCCAA GACCAAGCCC CGCGAGGAGC AGTACAACAG CACCTACCGC GTGGTGAGCG TGCTGACCGT
>sgp130Fc_opt_Upgene  #1981   GAAGTCAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTCC ACAATGCCAA GACCAAGCCA CGGGAGGAGC AGTACAATTC CACCTACAGA GTAGTCAGTG TGCTGACCGT
                               ..................................................................................................................
                      #1981   GAGGTSAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ACAATGCCAA GACCAAGCCN CGGGAGGAGC AGTACAACWS CACCTACMGA GTGGTSAGYG TGCTGACCGT
                                * *                   *          *  * * *    *    * * *              ***    *  * *   *  ****   * * *

>sgp130Fc_wt          #2091   CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG
>sgp130Fc_opt_CON     #2091   GCTGCATCAG GATTGGCTGA ACGGCAAGGA GTACAAGTGC AAGGTGTCCA ACAAGGCTCT GCCTGCTCCT ATCGAAAAGA CCATCTCCAA GGCTAAGGGA CAGCCTAGAG
>sgp130Fc_opt_JCat    #2091   GCTGCACCAG GACTGGCTGA ACGGCAAGGA GTACAAGTGC AAGGTGAGCA ACAAGGCCCT GCCCGCCCCC ATCGAGAAGA CCATCAGCAA GGCCAAGGGC CAGCCCCGCG
>sgp130Fc_opt_Upgene  #2091   CCTCCACCAG GACTGGCTCA ACGGCAAAGA GTACAAGTGC AAGGTGTCCA ACAAGGCCCT GCCAGCCCCG ATCGAGAAAA CCATTTCCAA GGCCAAGGGT CAGCCCAGGG
                               ..................................................................................................................
                      #2091   SCTGCACCAG GACTGGCTGA ACGGCAAGGA GTACAAGTGC AAGGTGTCCA ACAAGGCCCT GCCAGCCCCC ATCGAGAARA CCATCTCCAA GGCCAAGGGN CAGCCCMGAG
                               * * *     *    *  *   *              ***     * *  * * * * *    * *     ***   * * * *     ** *

>sgp130Fc_wt          #2201   AACCACAGGT GTACACCCTG CCCCCATCCC GGGAGGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG
>sgp130Fc_opt_CON     #2201   AGCCTCAGGT GTACACACTG CCTCCATCTA GGGAGGAGAT GACCAAGAAT CAGGTGTCCC TGACCTGTCT GGTGAAGGGC TTCTACCCTT CTGATATCGC TGTGGAGTGG
>sgp130Fc_opt_JCat    #2201   AGCCCCAGGT GTACACCCTG CCCCCCAGCC GCGAGGAGAT GACCAAGAAC CAGGTGAGCC TGACCTGCCT GGTGAAGGGC TTCTACCCCA GCGACATCGC CGTGGAGTGG
>sgp130Fc_opt_Upgene  #2201   AGCCTCAGGT CTACACGCTG CCTCCGTCCA GAGAGGAAAT GACCAAGAAT CAGGTGTCGC TGACTTGCCT CGTGAAAGGG TTCTACCCCA GCGACATCGC TGTGGAGTGG
                               ..................................................................................................................
                      #2201   AGCCTCAGGT GTACACCCTG CCYCCATCCM GGGAGGAGAT GACCAAGAAY CAGGTGWSCC TGACCTGCCT GGTGAARGGC TTCTACCCCA GCGACATCGC YGTGGAGTGG
                               *  *     *    *     *  *****  *    *           *     ****     *  *   *  *  *  *      *  ** **  *      *

>sgp130Fc_wt          #2311   GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTATAG CAAGCTCACC GTGGACAAGA GCAGGTGGCA
>sgp130Fc_opt_CON     #2311   GAGTCTAATG GCCAGCCCGA GAACAATTAC AAGACCACCC CTCCTGTGCT GGATTCTGAC GGCTCCTTCT TCCTGTACTC CAAACTGACC GTGGACAAGT CTAGATGGCA
>sgp130Fc_opt_JCat    #2311   GAGAGCAACG GCCAGCCCGA GAACAACTAC AAGACCACCC CCCCCGTGCT GGACAGCGAC GGCAGCTTCT TCCTGTACAG CAAGCTGACC GTGGACAAGA GCCGCTGGCA
>sgp130Fc_opt_Upgene  #2311   GAGTCGAACG GGCAGCCTGA GAATAATTAT AAGACAACCC CCCCCGTGCT GGACTCCGAC GGCTCCTTTT TCCTCTACTC TAAGCTGACC GTGGACAAGA GTAGGTGGCA
                               ..................................................................................................................
                      #2311   GAGWSCAAYG GSCAGCCCGA GAACAAYTAC AAGACCACCC CYCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTSTACWS CAAGCTGACC GTGGACAAGA GYAGGTGGCA
                                 ***  *   *     *     *  *  *     *  *   *  *       ****      **   *     *  *** *  *  *            * *** *

>sgp130Fc_wt          2421    GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGTAAATG A
>sgp130Fc_opt_CON     2421    GCAGGGCAAC GTGTTCTCTT GTTCCGTGAT GCACGAGGCT CTGCACAATC ACTATACCCA GAAGTCCCTG TCTCTGTCTC CTGGCAAGTG A
>sgp130Fc_opt_JCat    2421    GCAGGGCAAC GTGTTCAGCT GCAGCGTGAT GCACGAGGCC CTGCACAACC ACTACACCCA GAAGAGCCTG AGCCTGAGCC CCGGCAAGTA A
>sgp130Fc_opt_Upgene  2421    GCAGGGGAAC GTCTTCTCTT GTTCGGTCAT GCACGAGGCC CTCCACAACC ATTATACTCA GAAGTCCCTG TCGCTCAGTC CCGGTAAATG A
                               ..................................................................................................
                      #2421    GCAGGGSAAC GTSTTCTCTT GYTCCGTGAT GCACGAGGCY CTGCACAACC ACTAYACCCA GAAGWSCCTG TCCCTGWSTC CCGGYAARTG A
                                     *     *   ***   ****  *     *    *   *    *   *  *  *      **   * ***  ****   *  *  * *
```

	wt	opt_Con	opt_JCat	opt_UpGene
wt	-	561	626	588
opt_Con	561	-	383	405
opt_JCat	626	.383	-	404
opt_UpGene	588	405	404	-

Figure 9


A)
gp130 (extracellular part)
IgG
Eukaryotes
(sgp130Fc)
Signal
D1
D2
D3
D4
D5
D6
Fc
B)
D1
D2
D3
D1
D2
D3
Tag
Prokaryotes
(sgp130(D1-D3))
Leader
D1
D2
D3
Leader
D1
D2
D3
Tag