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(54) Title: NOVEL CYTOKINE ZCYTOR17 LIGAND

(57) Abrégé/Abstract:
The present invention relates to zcytor171ig polynucleotide, polypeptide and anti-zcytor17 antibody molecules. The zcytor171ig is a novel cytokine. The polypeptides may be used within methods for stimulating the immune system, and proliferation and/or development of hematopoietic cells in vitro and in vivo. The present invention also includes methods for producing the protein, uses therefor and antibodies thereto.

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(54) Title: NOVEL CYTOKINE ZCYTOR17 LIGAND

(57) Abstract: The present invention relates to zcytor171ig polynucleotide, polypeptide and anti-zcytor17 antibody molecules. The zcytor171ig is a novel cytokine. The polypeptides may be used within methods for stimulating the immune system, and proliferation and/or development of hematopoietic cells *in vitro* and *in vivo*. The present invention also includes methods for producing the protein, uses therefor and antibodies thereto.

NOVEL CYTOKINE ZCYTOR17 LIGAND

REFERENCE TO RELATED APPLICATIONS

The present application is related to U.S. Provisional Application Serial No. 60/435,315 filed December 19, 2002, U.S. Provisional Application Serial No. 60/375,323, filed April 25, 2002, and U.S. Provisional Application Serial No. 60/350,325, filed January 18, 2002. Under 35 U.S.C. § 119(e)(1), this application claims benefit of said Provisional Applications.

BACKGROUND OF THE INVENTION

Proliferation and differentiation of cells of multicellular organisms are controlled by hormones and polypeptide growth factors. These diffusable molecules allow cells to communicate with each other and act in concert to form cells, tissues and organs, and to repair damaged tissue. Examples of hormones and growth factors include the steroid hormones (e.g. estrogen, testosterone), parathyroid hormone, follicle stimulating hormone, the interleukins, platelet derived growth factor (PDGF), epidermal growth factor (EGF), granulocyte-macrophage colony stimulating factor (GM-CSF), erythropoietin (EPO) and calcitonin.

Hormones and growth factors influence cellular metabolism by binding to receptors. Receptors may be integral membrane proteins that are linked to signaling pathways within the cell, such as second messenger systems. Other classes of receptors are soluble molecules, such as the transcription factors.

Cytokines generally stimulate proliferation or differentiation of cells of the hematopoietic lineage or participate in the immune and inflammatory response mechanisms of the body. Examples of cytokines which affect hematopoiesis are erythropoietin (EPO), which stimulates the development of red blood cells; thrombopoietin (TPO), which stimulates development of cells of the megakaryocyte lineage; and granulocyte-colony stimulating factor (G-CSF), which stimulates

development of neutrophils. These cytokines are useful in restoring normal blood cell levels in patients suffering from anemia, thrombocytopenia, and neutropenia or receiving chemotherapy for cancer.

The interleukins are a family of cytokines that mediate immunological responses, including inflammation. The interleukins mediate a variety of inflammatory pathologies. Central to an immune response are T cells, which produce many cytokines and adaptive immunity to antigens. Cytokines produced by T cells have been classified as type 1 and type 2 (Kelso, A. Immun. Cell Biol. 76:300-317, 1998). Type 1 cytokines include IL-2, IFN-γ, LT-α, and are involved in inflammatory responses, viral immunity, intracellular parasite immunity and allograft rejection. Type 2 cytokines include IL-4, IL-5, IL-6, IL-10 and IL-13, and are involved in humoral responses, helminth immunity and allergic response. Shared cytokines between Type 1 and 2 include IL-3, GM-CSF and TNF-α. There is some evidence to suggest that Type 1 and Type 2 producing T cell populations preferentially migrate into different types of inflamed tissue.

Mature T cells may be activated, i.e., by an antigen or other stimulus, to produce, for example, cytokines, biochemical signaling molecules, or receptors that further influence the fate of the T cell population.

B cells can be activated via receptors on their cell surface including B cell receptor and other accessory molecules to perform accessory cell functions, such as production of cytokines.

Monocytes/macrophages and T-cells can be activated by receptors on their cell surface and play a central role in the immune response by presenting antigen to lymphocytes and also act as accessory cells to lymphocytes by secreting numerous cytokines.

Natural killer (NK) cells have a common progenitor cell with T cells and B cells, and play a role in immune surveillance. NK cells, which comprise up to 15% of blood lymphocytes, do not express antigen receptors, and therefore do not use MHC recognition as requirement for binding to a target cell. NK cells are involved in the recognition and killing of certain tumor cells and virally infected cells. *In vivo*, NK cells are believed to require activation, however, *in vitro*, NK cells have been shown to kill some types of tumor cells without activation.

The demonstrated *in vivo* activities of the cytokine family illustrate the enormous clinical potential of, and need for, other cytokines, cytokine agonists, and cytokine antagonists. The present invention addresses these needs by providing a new cytokine that stimulates cells of the hematopoietic cell lineage, as well as related compositions and methods.

The present invention provides such polypeptides for these and other uses that should be apparent to those skilled in the art from the teachings herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an illustration of a multiple alignment of human zcytor17lig (SEQ ID NO:2) (zcytor17lig), mouse zcytor17lig (SEQ ID NO:11) (mzcytor17lig), mouse IL-3 (mIL-3) (SEQ ID NO:100), and human IL-3 (hIL-3) (SEQ ID NO:102).

Figure 2 is an illustration of a multiple alignment of human zcytor17lig (SEQ ID NO:2) (zcytor17lig), and mouse zcytor17lig (SEQ ID NO:11) (mzcytor17lig).

Figure 3 is a Hopp/Woods hydrophilicity plot of human zcytor17lig (SEQ ID NO:2).

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention in detail, it may be helpful to the understanding thereof to define the following terms:

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al., Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952-4, 1985), substance P, Flag™ peptide (Hopp et al., Biotechnology 6:1204-10, 1988), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., Protein Expression and Purification 2: 95-107, 1991. DNAs

encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of $<10^9\ M^{-1}$.

The term "complements of a polynucleotide molecule" denotes a polynucleotide molecule having a complementary base sequence and reverse orientation as compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a polynucleotide that has a contiguous stretch of identical or complementary sequence to another polynucleotide. Contiguous sequences are said to "overlap" a given stretch of polynucleotide sequence either in their entirety or along a partial stretch of the polynucleotide. For example,

representative contigs to the polynucleotide sequence 5'-ATGGCTTAGCTT-3' are 5'-TAGCTTgagtct-3' and 3'-gtcgacTACCGA-5'.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

The term "expression vector" is used to denote a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments include promoter and terminator sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, etc. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

The term "isolated", when applied to a polynucleotide, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, Nature 316:774-78, 1985).

An "isolated" polypeptide or protein is a polypeptide or protein that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin. It is preferred to provide the polypeptides in a highly purified form, i.e., greater than 95% pure, more preferably greater than 99% pure. When used in this context, the term "isolated" does not exclude

the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

The term "neoplastic", when referring to cells, indicates cells undergoing new and abnormal proliferation, particularly in a tissue where in the proliferation is uncontrolled and progressive, resulting in a neoplasm. The neoplastic cells can be either malignant, i.e., invasive and metastatic, or benign.

The term "operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g., transcription initiates in the promoter and proceeds through the coding segment to the terminator.

The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. Sizes of polynucleotides are expressed as base pairs (abbreviated "bp"), nucleotides ("nt"), or kilobases ("kb"). Where the context allows, the latter two terms may describe polynucleotides that are single-stranded or double-stranded. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term "base pairs". It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide may differ slightly in length and that the ends thereof may be staggered as a result of enzymatic cleavage; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides".

The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule (i.e., a ligand) and mediates the effect of the ligand on the cell. Membrane-bound receptors are characterized by a multi-peptide structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. Binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell. This interaction in turn leads to an alteration in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. In general, receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor).

The term "secretory signal sequence" denotes a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

Molecular weights and lengths of polymers determined by imprecise analytical methods (e.g., gel electrophoresis) will be understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

All references cited herein are incorporated by reference in their entirety.

The present invention is based in part upon the discovery of a novel DNA sequence that encodes a protein having the structure of a four-helical-bundle cytokine. Through processes of cloning, and proliferation assays described in detail herein, a polynucleotide sequence encoding a novel ligand polypeptide has been identified that is a ligand with high specificity for the receptor zcytor17 (SEQ ID NO:5) and at least one additional subunit comprising OncostatinM receptor beta (OSMRbeta) (SEQ ID NO:7) and WSX-1 (SEQ ID NO:9). This polypeptide ligand, designated zcytor17lig, was isolated from a cDNA library generated from activated human peripheral blood cells (hPBCs), which were selected for CD3. CD3 is a cell surface marker unique to cells of lymphoid origin, particularly T cells.

In the examples which follow, a cell line that is dependent on the OSMRbeta and zcytor17 receptor linked pathway or dependent on the OSMRbeta and WSX-1 and the zcytor17 receptor-linked pathway for survival and growth in the absence of other growth factors was used to screen for a source of the cDNA encoding the zcytor17lig. The preferred growth factor-dependent cell line that was used for transfection and expression of zcytor17 receptor was BaF3 (Palacios and Steinmetz, Cell 41: 727-734, 1985; Mathey-Prevot et al., Mol. Cell. Biol. 6: 4133-4135, 1986). However, other growth factor-dependent cell lines, such as FDC-P1 (Hapel et al., Blood

64: 786-790, 1984), and MO7e (Kiss et al., Leukemia 7: 235-240, 1993) are suitable for this purpose.

The amino acid sequence for the OSMR, WSX-1 and zcytor17 receptors indicated that the encoded receptors belonged to the Class I cytokine receptor subfamily that includes, but is not limited to, the receptors for IL-2, IL-4, IL-7, Lif, IL-12, IL-15, EPO, TPO, GM-CSF and G-CSF (for a review see, Cosman, "The Hematopoietin Receptor Superfamily" in Cytokine 5(2): 95-106, 1993). The zcytor17 receptor is fully described in commonly-owned PCT Patent Application No. US01/20484 (WIPO publication No. WO 02/00721), and WSX-1 is fully described in US Patent No. 5,925,735. Analysis of the tissue distribution of the mRNA of the zcytor17 receptor revealed expression in activated CD4+ and CD8+ T-cell subsets, CD14+ monocytes, and weaker expression in CD19+ B-cells. Moreover, the mRNA was present in both resting or activated monocytic cell lines THP-1 (ATCC No. TIB-202), U937 (ATCC No. CRL-1593.2) and HL60 (ATCC No. CCL-240).

The expression of WSX-1 is strongest in thymus, spleen, PBL, and lymph node, as well as increased expression observed for activated T-cells. The tissue distribution for OSMRbeta is described as very broad. The tissue distribution of these three receptors suggests that a target for the predicted Zcytor17lig is hematopoietic lineage cells, in particular T-cells, monocytes/macrophages and lymphoid progenitor cells and lymphoid cells. Other known four-helical-bundle cytokines that act on lymphoid cells include IL-2, IL-4, IL-7, and IL-15. For a review of four-helical-bundle cytokines, see, Nicola et al., Advances in Protein Chemistry 52:1-65, 1999 and Kelso, A., Immunol. Cell Biol. 76:300-317, 1998.

Conditioned media (CM) from CD3+ selected, PMA/Ionomycin-stimulated human peripheral blood cells supported the growth of BaF3 cells that expressed the zcytor17 receptor, OSMRbeta and WSX-1 receptor and were otherwise dependent on IL-3. Conditioned medias from cells that were not: 1) PMA/Ionomycin-stimulated; or were not: 2) CD3 selected (with or without PMA/Ionomycin stimulation) did not support the growth of Baf3 cells expressing zcytor17, OSMRbeta and WSX-1 (BaF3/zcytor17/WSX-1/OSMRbeta) receptor-expressing cells. Control experiments demonstrated that this proliferative activity was not attributable to other known growth

factors, and that the ability of such conditioned media to stimulate proliferation of zcytor17/WSX-1/OSMRbeta receptor-expressing cells could be neutralized by a soluble form of the zcytor17 receptor.

Conditioned-media from CD3+ selected cells activated with PMA/Ionomycin also supported growth of BaF3 cells that expressed the zcytor17 receptor and OSMRbeta receptor (zcytor17/OSMRbeta), while BaF3 cells expressing only zcytor17 receptor and WSX-1 receptor (zcytor17/WSX-1), or containing only the OSMRbeta receptor, were not stimulated by this conditioned-media.

Proliferation of zcytor17/WSX-1/OSMRbeta receptor-expressing BaF3 cells exposed to CM from CD3+ selected, PMA/Ionomycin-stimulated human peripheral blood cells were identified by visual inspection of the cultures and/or by proliferation assay. Many suitable proliferation assays are known in the art, and include assays for reduction of a dye such as AlamarBlue™ (AccuMed International, Inc. Westlake, Ohio), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Mosman, J. Immunol. Meth. 65: 55-63, 1983); 3,(4,5 dimethyl thiazol-2yl)-5-3-carboxymethoxyphenyl-2H-tetrazolium; 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide; and cyanoditolyl-tetrazolium chloride (which are commercially available from Polysciences, Inc., Warrington, PA); mitogenesis assays, such as measurement of incorporation of ^{3}H-thymidine; dye exclusion assays using, for example, naphthalene black or trypan blue; dye uptake using diacetyl fluorescein; and chromium release. See, in general, Freshney, Culture of Animal Cells: A Manual of Basic Technique, 3rd ed., Wiley-Liss, 1994, which is incorporated herein by reference.

A cDNA library was prepared from CD3+ selected, PMA- and Ionomycin-stimulated primary human peripheral blood cells. The CD3+ selected, PMA- and Ionomycin-stimulated human peripheral blood cells cDNA library was divided into pools containing multiple cDNA molecules and was transfected into a host cell line, for example, BHK 570 cells (ATCC Accession No. 10314). The transfected host cells were cultured in a medium that did not contain exogenous growth factors (e.g., 5% FBS) and conditioned medium was collected. The conditioned media were assayed for the ability to stimulate proliferation of BaF3 cells transfected with the

zcytor17, WSX-1, and OSMRbeta receptors. CDNA pools producing conditioned medium that stimulated BaF3/zcytor17/WSX-1/OSMRbeta receptor cells were identified. This pooled plasmid cDNA was electroporated into *E. coli.* CDNA was isolated from single colonies and transfected individually into BHK 570 cells. Positive clones were identified by a positive result in the BaF3/zcytor17/WSX-1/OSMRbeta receptor proliferation assay, and the activity was confirmed by neutralization of proliferation using the soluble zcytor17 receptor.

A positive clone was isolated, and sequence analysis revealed that the polynucleotide sequence contained within the plasmid DNA was novel. The secretory signal sequence is comprised of amino acid residues 1 (Met) to 23 (Ala), and the mature polypeptide is comprised of amino acid residues 24 (Ser) to 164 (Thr) (as shown in SEQ ID NO:2). Further N-terminal sequencing analysis of purified zcytor17lig from 293T cells showed an N-terminus at residue 27 (Leu) as shown in SEQ ID NO:2, with the mature polypeptide comprised of amino acid residues 27 (Leu) to 164 (Thr) (as shown in SEQ ID NO:2).

In general, cytokines are predicted to have a four-alpha helix structure, with helices A, C and D being most important in ligand-receptor interactions, and are more highly conserved among members of the family. Referring to the human zcytor17lig amino acid sequence shown in SEQ ID NO:2, alignment of human zcytor17lig, human IL-3, and human cytokine amino acid sequences it is predicted that zcytor17lig helix A is defined by amino acid residues 38-52; helix B by amino acid residues 83-98; helix C by amino acid residues 104-117; and helix D by amino acid residues 137-152; as shown in SEQ ID NO:2. Structural analysis suggests that the A/B loop is long, the B/C loop is short and the C/D loop is long. This loop structure results in an up-up-down-down helical organization. Based on 4-helix bundle structure, the cysteine residues within zcytor17lig that are conserved correspond to amino acid residues 72, 133, and 147 of SEQ ID NO:2; and 74, 137, and 151 of SEQ ID NO:11 described herein. Consistent cysteine placement is further confirmation of the four-helical-bundle structure. Also highly conserved in the zcytor17lig is the Glu residue as shown in SEQ ID NO:2 at residue 43.

Moreover, the predicted amino acid sequence of murine zcytor17lig shows 31% identity to the predicted human protein over the entire length of the sequences (SEQ ID NO:2 and SEQ ID NO:11). Based on comparison between sequences of human and murine zcytor17lig conserved residues were found in the regions predicted to encode alpha helices C and D. The corresponding polynucleotides encoding the human zcytor17lig polypeptide regions, domains, motifs, residues and sequences described herein are as shown in SEQ ID NO:1.

While helix D is relatively conserved between human and murine zcytor17lig, helix C is the most conserved. While both species have predominant acidic amino acids in this region, the differences may account for species specificity in interaction between zcytor17lig and its receptor, zcytor17 comprising monomeric, heterodimeric (e.g., zcytor17/OSMRbeta, WSX-1/OSMRbeta, zcytor17/WSX-1) or multimeric (e.g., zcytor17/OSMRbeta/WSX-1) receptors. Loop A/B and helix B of zcytor17lig are marginally conserved, and helix C through Loop C/D into helix D is most conserved between species; conservation through this region suggests that it is functionally significant. The D helices of human and murine zcytor17lig are also conserved. Zcytor17 receptor antagonists may be designed through mutations within zcytor17lig helix D. These may include truncation of the protein from residue Thr156 (SEQ ID NO:2), or conservation of residues that confer binding of the ligand to the receptor, but diminish signaling activity.

Four-helical bundle cytokines are also grouped by the length of their component helices. "Long-helix" form cytokines generally consist of between 24-30 residue helices, and include IL-6, ciliary neutrotrophic factor (CNTF), leukemia inhibitory factor (LIF) and human growth hormone (hGH). "Short-helix" form cytokines generally consist of between 18-21 residue helices and include IL-2, IL-4 and GM-CSF. Zcytor17lig is believed to be a new member of the short-helix form cytokine group. Studies using CNTF and IL-6 demonstrated that a CNTF helix can be exchanged for the equivalent helix in IL-6, conferring CTNF-binding properties to the chimera. Thus, it appears that functional domains of four-helical cytokines are determined on the basis of structural homology, irrespective of sequence identity, and can maintain functional integrity in a chimera (Kallen et al., J. Biol. Chem. 274:11859-

11867, 1999). Therefore, the helical domains of zcytor17lig will be useful for preparing chimeric fusion molecules, particularly with other short-helix form cytokines to determine and modulate receptor binding specificity. Of particular interest are fusion proteins engineered with helix A and/or helix D, and fusion proteins that combine helical and loop domains from other short-form cytokines such as IL-2, IL-4, IL-15, Lif, IL-12, IL-3 and GM-CSF.

The polynucleotide sequence for human IL-2 is shown in SEQ ID NO:161 and the corresponding amino acid sequence is shown in SEQ ID NO:162. The secretory signal sequence is comprised of amino acid residues 1 (Met) to 20 (Ser) of SEQ ID NO:162; nucleotides 48 to 107 of SEQ ID NO:161. The mature polypeptide is comprised of amino acid residues 21 (Ala) to 156 (Thr) of SEQ ID NO:162; nucleotides 108 to 515 of SEQ ID NO:161. Helix A of human IL-2 is comprised of amino acid residues 27 (Thr) to 48 (Leu) of SEQ ID NO:162; nucleotides 126 to 191 of SEQ ID NO:161. Helix B of human IL-2 comprises Helix B1 and Helix B2. Helix B1 of human IL-2 is comprised of amino acid residues 73 (Ala) to 80 (Gln) of SEQ ID NO:162; nucleotides 264 to 287 of SEQ ID NO:161. Helix B2 of human IL-2 is comprised of amino acid residues 83 (Glu) to 92 (Val) of SEQ ID NO:162; nucleotides 294 to 323 of SEQ ID NO:161. Thus, Helix B (comprising Helices B1 and B2) of IL-2 is represented by the amino acid sequence of SEQ ID NO:168 (nucleotide sequence of SEQ ID NO:167) wherein amino acid residues 9 and 10 can be any amino acid. SEQ ID NO:168 is identical to amino acids 73 (Ala) to 92 (Val) of SEQ ID NO:162 wherein amino acids 81 and 82 are any amino acid. In a preferred form, Helix B of IL-2 comprises amino acids 73 (Ala) to 92 (Val) of SEQ ID NO:162; nucleotides 264 to 323 of SEQ ID NO:161. Helix C of human IL-2 is comprised of amino acid residues 102 (His) to 116 (Val) of SEQ ID NO:162 nucleotides 351 to 395 of SEQ ID NO:161. Helix D of human IL-2 is comprised of amino acid residues 134 (Thr) to 149 (Gln) of SEQ ID NO:162; nucleotides 447 to 494 of SEQ ID NO:161.

The polynucleotide sequence for human IL-4 is shown in SEQ ID NO:163 and the corresponding amino acid sequence is shown in SEQ ID NO:164. The secretory signal sequence is comprised of amino acid residues 1 (Met) to 24 (Gly) of SEQ ID NO:164; nucleotides 64 to 135 of SEQ ID NO:163. The mature polypeptide is

comprised of amino acid residues 25 (His) to 153 (Ser) of SEQ ID NO:164; nucleotides 136 to 522 of SEQ ID NO:163. Helix A of human IL-4 is comprised of amino acid residues 30 (Thr) to 42 (Thr) of SEQ ID NO:164; nucleotides 151 to 189 of SEQ ID NO:163. Helix B of human IL-4 is comprised of amino acid residues 65 (Glu) to 83 (His) of SEQ ID NO:164; nucleotides 256 to 312 of SEQ ID NO:163. Helix C of human IL-4 is comprised of amino acid residues 94 (Ala) to 118 (Ala) of SEQ ID NO:164; nucleotides 343 to 417 of SEQ ID NO:163. Helix D of human IL-4 is comprised of amino acid residues 133 (Leu) to 151 (Cys) of SEQ ID NO:164; nucleotides 460 to 516 of SEQ ID NO:163.

The polynucleotide sequence for human GM-CSF is shown in SEQ ID NO:165 and the corresponding amino acid sequence is shown in SEQ ID NO:166. The secretory signal sequence is comprised of amino acid residues 1 (Met) to 17 (Ser) of SEQ ID NO:166; nucleotides 9 to 59 of SEQ ID NO:165. The mature polypeptide is comprised of amino acid residues 18 (Ala) to 144 (Glu) of SEQ ID NO:166; nucleotides 60 to 440 of SEQ ID NO:165. Helix A of human GM-CSF is comprised of amino acid residues 30 (Trp) to 44 (Asn) of SEQ ID NO:166; nucleotides 96 to 140 of SEQ ID NO:165. Helix B of human GM-CSF is comprised of amino acid residues 72 (Leu) to 81 (Gln) of SEQ ID NO:166; nucleotides 222 to 251 of SEQ ID NO:165. Helix C of human GM-CSF is comprised of amino acid residues 85 (Gly) to 103 (Gln) of SEQ ID NO:166; nucleotides 261 to 317 of SEQ ID NO:165. Helix D of human GM-CSF is comprised of amino acid residues 120 (Phe) to 131 (Leu) of SEQ ID NO:166; nucleotides 366 to 401 of SEQ ID NO:165.

The amino acid residues comprising helices A, B, C, and D, for human zcytor17lig, IL-3, IL-2, IL-4, and GM-CSF are shown in Table 1.

Table 1

	Helix A	Helix B	Helix C	Helix D	
zcytor17lig	38-52	83-98	104-117	137-152	of SEQ ID NO:2
IL-3	35-45	73-86	91-103	123-141	of SEQ ID NO: 102
IL-2	27-48	73-92	102-116	134-149	of SEQ ID NO:162; or Helix B as described in SEQ ID NO:168

IL-4	30-42	65-83	94-118	133-151	of SEQ ID NO:164
GM-CSF	30-44	72-81	85-103	120-131	of SEQ ID NO:166

The present invention provides polynucleotide molecules, including DNA and RNA molecules, that encode the zcytor17lig polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:3 is a degenerate DNA sequence that encompasses all DNAs that encode the zcytor17lig polypeptide, and fragments thereof, of SEQ ID NO:2. Those skilled in the art will recognize that the degenerate sequence of SEQ ID NO:3 also provides all RNA sequences encoding SEQ ID NO:2 by substituting U for T. Thus, zcytor17lig polypeptide-encoding polynucleotides comprising nucleotide 1 or 70 to nucleotide 492 of SEQ ID NO:3 and their RNA equivalents are contemplated by the present invention. Table 2 sets forth the one-letter codes used within SEQ ID NO:3 to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, with A being complementary to T, and G being complementary to C.

Table 2

Nucleotide	Resolution	Complement	Resolution
A	A	T	T
C	C	G	G
G	G	C	C
T	T	A	A
R	A|G	Y	C|T
Y	C|T	R	A|G
M	A|C	K	G|T
K	G|T	M	A|C
S	C|G	S	C|G
W	A|T	W	A|T
H	A|C|T	D	A|G|T
B	C|G|T	V	A|C|G
V	A|C|G	B	C|G|T
D	A|G|T	H	A|C|T
N	A|C|G|T	N	A|C|G|T

The degenerate codons used in SEQ ID NO:3, encompassing all possible codons for a given amino acid, are set forth in Table 3.

Table 3

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn|Asp	B		RAY
Glu|Gln	Z		SAR
Any	X		NNN

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding each amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequence of SEQ ID NO:2. Variant sequences can be readily tested for functionality as described herein.

One of ordinary skill in the art will also appreciate that different species can exhibit "preferential codon usage." In general, see, Grantham, et al., Nuc. Acids Res. 8:1893-912, 1980; Haas, et al. Curr. Biol. 6:315-24, 1996; Wain-Hobson, et al., Gene 13:355-64, 1981; Grosjean and Fiers, Gene 18:199-209, 1982; Holm, Nuc. Acids Res. 14:3075-87, 1986; Ikemura, J. Mol. Biol. 158:573-97, 1982. As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 3). For example, the amino acid Threonine (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different Thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequence disclosed in SEQ ID NO:3 serves as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for preparing DNA and RNA are well known in the art. In general, RNA is isolated from a tissue or cell that produces large amounts of zcytor17lig RNA. Such tissues and cells are identified by Northern blotting (Thomas, Proc. Natl. Acad. Sci. USA 77:5201, 1980), or by screening conditioned medium from various cell types for activity on target cells or tissue. Once the activity or RNA producing cell or tissue is identified, total RNA can be prepared using guanidinium isothiocyanate extraction followed by isolation by centrifugation in a CsCl gradient (Chirgwin et al., Biochemistry 18:52-94, 1979). Poly $(A)^+$ RNA is prepared from total RNA using the method of Aviv and Leder (Proc. Natl. Acad. Sci. USA 69:1408-12, 1972). Complementary DNA (cDNA) is prepared from poly$(A)^+$ RNA using known methods. In the alternative, genomic DNA can be isolated. Polynucleotides encoding zcytor17lig polypeptides are then identified and isolated by, for example, hybridization or PCR.

A full-length clone encoding zcytor17lig can be obtained by conventional cloning procedures. Complementary DNA (cDNA) clones are preferred, although for some applications (e.g., expression in transgenic animals) it may be preferable to use a genomic clone, or to modify a cDNA clone to include at least one genomic intron. Methods for preparing cDNA and genomic clones are well known and within the level of ordinary skill in the art, and include the use of the sequence disclosed herein, or parts thereof, for probing or priming a library. Expression libraries can be probed with antibodies to zcytor17lig fragments, zcytor17-comprising soluble receptors, or other specific binding partners.

Zcytor17lig polynucleotide sequences disclosed herein can also be used as probes or primers to clone 5' non-coding regions of a zcytor17lig gene. In view of the tissue-specific expression observed for zcytor17lig this gene region is expected to provide for hematopoietic- and lymphoid-specific expression. Promoter elements from a zcytor17lig gene could thus be used to direct the tissue-specific expression of heterologous genes in, for example, transgenic animals or patients treated with gene therapy. Cloning of 5' flanking sequences also facilitates production of zcytor17lig proteins by "gene activation" as disclosed in U.S. Patent No. 5,641,670. Briefly,

expression of an endogenous zcytor17lig gene in a cell is altered by introducing into the zcytor17lig locus a DNA construct comprising at least a targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site. The targeting sequence is a zcytor17lig 5' non-coding sequence that permits homologous recombination of the construct with the endogenous zcytor17lig locus, whereby the sequences within the construct become operably linked with the endogenous zcytor17lig coding sequence. In this way, an endogenous zcytor17lig promoter can be replaced or supplemented with other regulatory sequences to provide enhanced, tissue-specific, or otherwise regulated expression.

The present invention further provides counterpart polypeptides and polynucleotides from other species (orthologs). These species include, but are not limited to, mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of particular interest are zcytor17lig polypeptides from other mammalian species, including, for example, murine, porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides. Orthologs of human zcytor17lig can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses zcytor17lig as disclosed herein. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. A zcytor17lig-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent No. 4,683,202), using primers designed from the representative human zcytor17lig sequence disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to zcytor17lig polypeptide, binding studies or activity assays. Similar techniques can also be applied to the isolation of genomic clones.

The polynucleotide sequence for the mouse ortholog of zcytor17lig has been identified and is shown in SEQ ID NO:10 and SEQ ID NO:90 and the corresponding amino acid sequence shown in SEQ ID NO:11 and SEQ ID NO:91. The degenerate polynucleotide sequence encoding the polypeptide of SEQ ID NO:11 is shown in SEQ ID NO:12. For the zcytor17lig mouse cytokine amino acid sequence it is predicted that helix A is defined by amino acid residues 38-52; helix B by amino acid residues 85-98; helix C by amino acid residues 104-118; and helix D by amino acid residues 141-157; as shown in SEQ ID NO:11 and SEQ ID NO:91. There is 31% identity between the mouse and human sequences over the entire length of the amino acid sequences (SEQ ID NO:2 and SEQ ID NO:11) of zcytor17lig. Mature sequence for the mouse zcytor17lig putatively begins at Met_1, as shown in SEQ ID NO:11, which corresponds to Met_1, as shown in SEQ ID NO:2, in the human sequence. Tissue analysis revealed that expression of mouse zcytor17lig is found in testis, brain, CD90+ cells, prostate cells, salivary gland and skin. Further N-terminal sequencing analysis of purified zcytor17lig from 293T cells showed an N-terminus at residue 31 (Ala) as shown in SEQ ID NO:11 and SEQ ID NO:91, with the mature polypeptide comprised of amino acid residues 31 (Ala) to 163 (Cys) (as shown in SEQ ID NO: 11 and SEQ ID NO:91).

Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:1 represents a single allele of human zcytor17lig and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the DNA sequence shown in SEQ ID NO:1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NO:2. cDNAs generated from alternatively spliced mRNAs, which retain the properties of the zcytor17lig polypeptide, are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

The present invention also provides reagents which will find use in diagnostic applications. For example, the zcytor17lig gene, a probe comprising zcytor17lig DNA or RNA or a subsequence thereof, can be used to determine if the zcytor17lig gene is present on a human chromosome, such as chromosome 12, or if a gene mutation has occurred. Zcytor17lig is located at the 12q24.31 region of chromosome 12 (Example 13). Detectable chromosomal aberrations at the zcytor17lig gene locus include, but are not limited to, aneuploidy, gene copy number changes, loss of heterozygosity (LOH), translocations, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., ibid.; Ausubel et. al., ibid.; Marian, Chest 108:255-65, 1995).

The precise knowledge of a gene's position can be useful for a number of purposes, including: 1) determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) cross-referencing model organisms, such as mouse, which may aid in determining what function a particular gene might have.

One of skill in the art would recognize that the 12q24 region is frequently involved in gross genomic rearrangements, including translocations, deletions, inversions, and duplications, that are associated with various cancers. The Mitelman Database of Chromosomal Aberrations in Cancer, at the Cancer Genome Anatomy Project, National Insitutes of Health, Bethesda, Md located on the Internet lists 199 cases of cancers with genomic rearrangements involving 12q24. Of these, most are part of complex karyotypes with other rearrangements; however, in some cases the rearrangement involving 12q24 is the only genomic alteration. Given the expression of the receptor for zcytor17lig on cells of lymphoid and myeloid lineages, it is particularly significant to note that there are at least 4 cases of myeloid leukemia reported in the literature in which either translocation (2 cases: Yamagata et al, Cancer

Genet Cytogenet 97:90-93, 1997; Dunphy and Batanian, Cancer Genet Cytogenet 114:51-57, 1999) or duplication (2 cases: Bonomi et al, Cancer Genet Cytogenet 108:75-78, 1999) are the sole genomic alteration. This suggests that a gene or genes residing within 12q24 could be directly involved in the malignant transformation of these patients' cells. Inappropriate over expression of zcytor17lig could contribute to malignant transformation by promoting aberrant proliferation of receptor-bearing cells, through either autocrine or paracrine mechanisms. Inhibition of zcytor17lig activity could thus inhibit growth of such cells. Alternatively, a genomic rearrangement resulting in inactivation of the zcytor17lig gene may promote malignant transformation and/or metastasis by removing zcytor17lig immunoregulatory functions. Indeed, a gene suppressing metastasis in prostate cancer has been mapped to 12q24-qter (Ichikawa et al, Asian J Androl 2:167-171, 2000). If zcytor17lig is the gene within this region responsible for the suppression of metastasis, then zcytor17lig itself may have therapeutic value in the treatment of cancer.

A diagnostic could assist physicians in determining the type of disease and appropriate associated therapy, or assistance in genetic counseling. As such, the inventive anti-zcytor17lig antibodies, polynucleotides, and polypeptides can be used for the detection of zcytor17lig polypeptide, mRNA or anti-zcytor17lig antibodies, thus serving as markers and be directly used for detecting or genetic diseases or cancers, as described herein, using methods known in the art and described herein. Further, zcytor17lig polynucleotide probes can be used to detect abnormalities or genotypes associated with chromosome 12q24.3 deletions and translocations associated with human diseases, or other translocations involved with malignant progression of tumors or other 12q24.3 mutations, which are expected to be involved in chromosome rearrangements in malignancy; or in other cancers. Similarly, zcytor17lig polynucleotide probes can be used to detect abnormalities or genotypes associated with chromosome 12 trisomy and chromosome loss associated with human diseases or spontaneous abortion. Thus, zcytor17lig polynucleotide probes can be used to detect abnormalities or genotypes associated with these defects.

One of skill in the art would recognize that zcytor17lig polynucleotide probes are particularly useful for diagnosis of gross chromosomal abnormalities

associated with loss of heterogeneity (LOH), chromosome gain (e.g., trisomy), translocation, DNA amplification, and the like. Translocations within chromosomal locus 12q24.3 wherein the zcytor17lig gene is located are known to be associated with human disease. For example, 12q24 deletions and translocations, duplications and trisomy are associated with cancers as discussed above. Thus, since the zcytor17lig gene maps to this critical region, zcytor17lig polynucleotide probes of the present invention can be used to detect abnormalities or genotypes associated with 12q24 translocation, deletion and trisomy, and the like, described above.

As discussed above, defects in the zcytor17lig gene itself may result in a heritable human disease state. Molecules of the present invention, such as the polypeptides, antagonists, agonists, polynucleotides and antibodies of the present invention would aid in the detection, diagnosis prevention, and treatment associated with a zcytor17lig genetic defect. In addition, zcytor17lig polynucleotide probes can be used to detect allelic differences between diseased or non-diseased individuals at the zcytor17lig chromosomal locus. As such, the zcytor17lig sequences can be used as diagnostics in forensic DNA profiling.

In general, the diagnostic methods used in genetic linkage analysis, to detect a genetic abnormality or aberration in a patient, are known in the art. Analytical probes will be generally at least 20 nt in length, although somewhat shorter probes can be used (e.g., 14-17 nt). PCR primers are at least 5 nt in length, preferably 15 or more, more preferably 20-30 nt. For gross analysis of genes, or chromosomal DNA, a zcytor17lig polynucleotide probe may comprise an entire exon or more. Exons are readily determined by one of skill in the art by comparing zcytor17lig sequences (SEQ ID NO:1) with the genomic DNA for mouse zcytor17lig (SEQ ID NO:76). In general, the diagnostic methods used in genetic linkage analysis, to detect a genetic abnormality or aberration in a patient, are known in the art. Most diagnostic methods comprise the steps of (a) obtaining a genetic sample from a potentially diseased patient, diseased patient or potential non-diseased carrier of a recessive disease allele; (b) producing a first reaction product by incubating the genetic sample with a zcytor17lig polynucleotide probe wherein the polynucleotide will hybridize to complementary polynucleotide sequence, such as in RFLP analysis or by incubating the genetic sample

with sense and antisense primers in a PCR reaction under appropriate PCR reaction conditions; (iii) visualizing the first reaction product by gel electrophoresis and/or other known methods such as visualizing the first reaction product with a zcytor17lig polynucleotide probe wherein the polynucleotide will hybridize to the complementary polynucleotide sequence of the first reaction; and (iv) comparing the visualized first reaction product to a second control reaction product of a genetic sample from wild type patient, or a normal or control individual. A difference between the first reaction product and the control reaction product is indicative of a genetic abnormality in the diseased or potentially diseased patient, or the presence of a heterozygous recessive carrier phenotype for a non-diseased patient, or the presence of a genetic defect in a tumor from a diseased patient, or the presence of a genetic abnormality in a fetus or pre-implantation embryo. For example, a difference in restriction fragment pattern, length of PCR products, length of repetitive sequences at the zcytor17lig genetic locus, and the like, are indicative of a genetic abnormality, genetic aberration, or allelic difference in comparison to the normal wild type control. Controls can be from unaffected family members, or unrelated individuals, depending on the test and availability of samples. Genetic samples for use within the present invention include genomic DNA, mRNA, and cDNA isolated from any tissue or other biological sample from a patient, which includes, but is not limited to, blood, saliva, semen, embryonic cells, amniotic fluid, and the like. The polynucleotide probe or primer can be RNA or DNA, and will comprise a portion of SEQ ID NO:1, the complement of SEQ ID NO:1, or an RNA equivalent thereof. Such methods of showing genetic linkage analysis to human disease phenotypes are well known in the art. For reference to PCR based methods in diagnostics see generally, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), White (ed.), *PCR Protocols: Current Methods and Applications* (Humana Press, Inc. 1993), Cotter (ed.), *Molecular Diagnosis of Cancer* (Humana Press, Inc. 1996), Hanausek and Walaszek (eds.), *Tumor Marker Protocols* (Humana Press, Inc. 1998), Lo (ed.), *Clinical Applications of PCR* (Humana Press, Inc. 1998), and Meltzer (ed.), *PCR in Bioanalysis* (Humana Press, Inc. 1998).

Mutations associated with the zcytor17lig locus can be detected using nucleic acid molecules of the present invention by employing standard methods for

direct mutation analysis, such as restriction fragment length polymorphism analysis, short tandem repeat analysis employing PCR techniques, amplification-refractory mutation system analysis, single-strand conformation polymorphism detection, RNase cleavage methods, denaturing gradient gel electrophoresis, fluorescence-assisted mismatch analysis, and other genetic analysis techniques known in the art (see, for example, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), Marian, *Chest 108*:255 (1995), Coleman and Tsongalis, *Molecular Diagnostics* (Human Press, Inc. 1996), Elles (ed.) *Molecular Diagnosis of Genetic Diseases* (Humana Press, Inc. 1996), Landegren (ed.), *Laboratory Protocols for Mutation Detection* (Oxford University Press 1996), Birren *et al.* (eds.), *Genome Analysis, Vol. 2: Detecting Genes* (Cold Spring Harbor Laboratory Press 1998), Dracopoli *et al.* (eds.), *Current Protocols in Human Genetics* (John Wiley & Sons 1998), and Richards and Ward, "Molecular Diagnostic Testing," in *Principles of Molecular Medicine*, pages 83-88 (Humana Press, Inc. 1998). Direct analysis of an zcytor17lig gene for a mutation can be performed using a subject's genomic DNA. Methods for amplifying genomic DNA, obtained for example from peripheral blood lymphocytes, are well-known to those of skill in the art (see, for example, Dracopoli *et al.* (eds.), *Current Protocols in Human Genetics*, at pages 7.1.6 to 7.1.7 (John Wiley & Sons 1998)).

Positions of introns in the mouse zcytor17lig gene were determined by identification of genomic clones, followed by analysis the intron/exon junctions. The mouse genomic DNA is shown in SEQ ID NO:76. With reference to SEQ ID NO:76, three coding exons separated by introns are evident: the first coding exon lies between nucleic acid numbers 1104-1119 of SEQ ID NO:76, the second exon between nucleic acid numbers 1300-1451 of SEQ ID NO:76, and the third exon between nucleic acid numbers 2411-2998 of SEQ ID NO:76.

Within embodiments of the invention, isolated zcytor17lig-encoding nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules having the nucleotide sequence of SEQ ID NO:1, to nucleic acid molecules having the nucleotide sequence of nucleotides 28 to 519 of SEQ ID NO:1, or to nucleic acid molecules having a nucleotide sequence complementary to SEQ ID NO:1. In general, stringent conditions are selected to be about 5°C lower than the thermal

melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.

A pair of nucleic acid molecules, such as DNA-DNA, RNA-RNA and DNA-RNA, can hybridize if the nucleotide sequences have some degree of complementarity. Hybrids can tolerate mismatched base pairs in the double helix, but the stability of the hybrid is influenced by the degree of mismatch. The T_m of the mismatched hybrid decreases by 1°C for every 1-1.5% base pair mismatch. Varying the stringency of the hybridization conditions allows control over the degree of mismatch that will be present in the hybrid. The degree of stringency increases as the hybridization temperature increases and the ionic strength of the hybridization buffer decreases.

It is well within the abilities of one skilled in the art to adapt these conditions for use with a particular polynucleotide hybrid. The T_m for a specific target sequence is the temperature (under defined conditions) at which 50% of the target sequence will hybridize to a perfectly matched probe sequence. Those conditions which influence the T_m include, the size and base pair content of the polynucleotide probe, the ionic strength of the hybridization solution, and the presence of destabilizing agents in the hybridization solution. Numerous equations for calculating T_m are known in the art, and are specific for DNA, RNA and DNA-RNA hybrids and polynucleotide probe sequences of varying length (see, for example, Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Second Edition (Cold Spring Harbor Press 1989); Ausubel *et al.,* (eds.), *Current Protocols in Molecular Biology (*John Wiley and Sons, Inc. 1987); Berger and Kimmel (eds.), *Guide to Molecular Cloning Techniques*, (Academic Press, Inc. 1987); and Wetmur, *Crit. Rev. Biochem. Mol. Biol. 26*:227 (1990)). Sequence analysis software such as OLIGO 6.0 (LSR; Long Lake, MN) and *Primer Premier 4.0* (Premier Biosoft International; Palo Alto, CA), as well as sites on the Internet, are available tools for analyzing a given sequence and calculating T_m based on user defined criteria. Such programs can also analyze a given sequence under defined conditions and identify suitable probe sequences. Typically, hybridization of longer polynucleotide sequences, >50 base pairs, is performed at temperatures of about

20-25°C below the calculated T_m. For smaller probes, <50 base pairs, hybridization is typically carried out at the T_m or 5-10°C below the calculated T_m. This allows for the maximum rate of hybridization for DNA-DNA and DNA-RNA hybrids.

Following hybridization, the nucleic acid molecules can be washed to remove non-hybridized nucleic acid molecules under stringent conditions, or under highly stringent conditions. Typical stringent washing conditions include washing in a solution of 0.5x - 2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 55 - 65°C. That is, nucleic acid molecules encoding a variant zcytor17lig polypeptide hybridize with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C, including 0.5x SSC with 0.1% SDS at 55°C, or 2x SSC with 0.1% SDS at 65°C. One of skill in the art can readily devise equivalent conditions, for example, by substituting SSPE for SSC in the wash solution.

Typical highly stringent washing conditions include washing in a solution of 0.1x - 0.2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 50 - 65°C. In other words, nucleic acid molecules encoding a variant zcytor17lig polypeptide hybridize with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C, including 0.1x SSC with 0.1% SDS at 50°C, or 0.2x SSC with 0.1% SDS at 65°C.

The present invention also provides isolated zcytor17lig polypeptides that have a substantially similar sequence identity to the polypeptides of SEQ ID NO:2, or their orthologs. The term "substantially similar sequence identity" is used herein to denote polypeptides comprising at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or greater than 99% sequence identity to the sequences shown in SEQ ID NO:2, or their orthologs. The present invention also includes polypeptides that comprise an amino acid sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or greater than 99% sequence identity to the sequence of amino acid residues 1 to 162 or 33 to 162 of SEQ ID NO:2. The present invention further includes

nucleic acid molecules that encode such polypeptides. Methods for determining percent identity are described below.

The present invention also contemplates variant zcytor17lig nucleic acid molecules that can be identified using two criteria: a determination of the similarity between the encoded polypeptide with the amino acid sequence of SEQ ID NO:2, and/or a hybridization assay, as described above. Such zcytor17lig variants include nucleic acid molecules: (1) that hybridize with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55 - 65°C; or (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or greater than 99% identity to the amino acid sequence of SEQ ID NO:2. Alternatively, zcytor17lig variants can be characterized as nucleic acid molecules: (1) that hybridize with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C; and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or greater than 99% sequence identity to the amino acid sequence of SEQ ID NO:2.

Percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48:603 (1986), and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff (*ibid.*) as shown in Table 4 (amino acids are indicated by the standard one-letter codes).

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer}} \times 100$$

sequence in order to align the two sequences]

Table 4

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative variant zcytor17lig. The FASTA algorithm is described by Pearson and Lipman, Proc. Nat'l Acad. Sci. USA 85:2444 (1988), and by Pearson, Meth. Enzymol. 183:63 (1990).

Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, J. Mol. Biol. 48:444 (1970); Sellers, SIAM J. Appl. Math. 26:787 (1974)), which allows for amino acid insertions and deletions. Preferred parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, Meth. Enzymol. 183:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as default.

Variant zcytor17lig polypeptides or polypeptides with substantially similar sequence identity are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (as shown in Table 5 below) and other substitutions that do not significantly affect the folding or activity of the polypeptide; small deletions, typically of one to about 30 amino acids; and amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or an affinity tag. The present invention thus includes polypeptides of from about 108 to 216 amino acid residues that comprise a sequence that is at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or greater than 99% identical to the corresponding region of SEQ ID NO:2. Polypeptides comprising affinity tags can further comprise a proteolytic cleavage site between the zcytor17lig polypeptide and the affinity tag. Preferred such sites include thrombin cleavage sites and factor Xa cleavage sites.

Table 5

Conservative amino acid substitutions

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine
Hydrophobic:	leucine
	isoleucine
	valine
Aromatic:	phenylalanine
	tryptophan
	tyrosine
Small:	glycine
	alanine
	serine
	threonine
	methionine

Determination of amino acid residues that comprise regions or domains that are critical to maintaining structural integrity can be determined. Within these regions one can determine specific residues that will be more or less tolerant of change and maintain the overall tertiary structure of the molecule. Methods for analyzing sequence structure include, but are not limited to, alignment of multiple sequences with high amino acid or nucleotide identity, secondary structure propensities, binary patterns, complementary packing and buried polar interactions (Barton, Current Opin. Struct. Biol. 5:372-376, 1995 and Cordes et al., Current Opin. Struct. Biol. 6:3-10, 1996). In

general, when designing modifications to molecules or identifying specific fragments determination of structure will be accompanied by evaluating activity of modified molecules.

Amino acid sequence changes are made in zcytor17lig polypeptides so as to minimize disruption of higher order structure essential to biological activity. For example, where the zcytor17lig polypeptide comprises one or more helices, changes in amino acid residues will be made so as not to disrupt the helix geometry and other components of the molecule where changes in conformation abate some critical function, for example, binding of the molecule to its binding partners, e.g., A and D helices, residues 43 (Glu), 44 (Glu), and 136 (Phe) of SEQ ID NO:2. The effects of amino acid sequence changes can be predicted by, for example, computer modeling as disclosed above or determined by analysis of crystal structure (see, e.g., Lapthorn et al., Nat. Struct. Biol. 2:266-268, 1995). Other techniques that are well known in the art compare folding of a variant protein to a standard molecule (e.g., the native protein). For example, comparison of the cysteine pattern in a variant and standard molecules can be made. Mass spectrometry and chemical modification using reduction and alkylation provide methods for determining cysteine residues which are associated with disulfide bonds or are free of such associations (Bean et al., Anal. Biochem. 201:216-226, 1992; Gray, Protein Sci. 2:1732-1748, 1993; and Patterson et al., Anal. Chem. 66:3727-3732, 1994). It is generally believed that if a modified molecule does not have the same cysteine pattern as the standard molecule folding would be affected. Another well known and accepted method for measuring folding is circular dichrosism (CD). Measuring and comparing the CD spectra generated by a modified molecule and standard molecule is routine (Johnson, Proteins 7:205-214, 1990). Crystallography is another well known method for analyzing folding and structure. Nuclear magnetic resonance (NMR), digestive peptide mapping and epitope mapping are also known methods for analyzing folding and structurally similarities between proteins and polypeptides (Schaanan et al., Science 257:961-964, 1992).

A Hopp/Woods hydrophilicity profile of the zcytor17lig protein sequence as shown in SEQ ID NO:2 can be generated (Hopp et al., Proc. Natl. Acad. Sci.78:3824-3828, 1981; Hopp, J. Immun. Meth. 88:1-18, 1986 and Triquier et al.,

Protein Engineering 11:153-169, 1998). The profile is based on a sliding six-residue window. Buried G, S, and T residues and exposed H, Y, and W residues were ignored. For example, in human zcytor17lig, hydrophilic regions include amino acid residues 54-59 of SEQ ID NO:2, amino acid residues 129-134 of SEQ ID NO:2, amino acid residues 53-58 of SEQ ID NO:2, amino acid residues 35-40 of SEQ ID NO:2, and amino acid residues 33-38 of SEQ ID NO:2. For example, in mouse zcytor17lig, hydrophilic regions include amino acid residues 34-39 of SEQ ID NO:11, amino acid residues 46-51 of SEQ ID NO:11, amino acid residues 131-136 of SEQ ID NO:11, amino acid residues 158-163 of SEQ ID NO:11, and amino acid residues 157-162 of SEQ ID NO:11.

Those skilled in the art will recognize that hydrophilicity or hydrophobicity will be taken into account when designing modifications in the amino acid sequence of a zcytor17lig polypeptide, so as not to disrupt the overall structural and biological profile. Of particular interest for replacement are hydrophobic residues selected from the group consisting of Val, Leu and Ile or the group consisting of Met, Gly, Ser, Ala, Tyr and Trp. For example, residues tolerant of substitution could include Val, Leu and Ile or the group consisting of Met, Gly, Ser, Ala, Tyr and Trp residues as shown in SEQ ID NO:2. Conserved cysteine residues at positions within SEQ ID NO:2 and SEQ ID NO:11, will be relatively intolerant of substitution.

The identities of essential amino acids can also be inferred from analysis of sequence similarity between IL-3, Lif, IL12, IL-15, IL-2, IL-4 and GM-CSF with zcytor17lig. Using methods such as "FASTA" analysis described previously, regions of high similarity are identified within a family of proteins and used to analyze amino acid sequence for conserved regions. An alternative approach to identifying a variant zcytor17lig polynucleotide on the basis of structure is to determine whether a nucleic acid molecule encoding a potential variant zcytor17lig gene can hybridize to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, as discussed above.

Other methods of identifying essential amino acids in the polypeptides of the present invention are procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244:1081 (1989), Bass et al., Proc. Natl Acad. Sci. USA 88:4498 (1991), Coombs and

Corey, "Site-Directed Mutagenesis and Protein Engineering," in Proteins: Analysis and Design, Angeletti (ed.), pages 259-311 (Academic Press, Inc. 1998)). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological or biochemical activity as disclosed below to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al., J. Biol. Chem. 271*:4699 (1996).

The present invention also includes functional fragments of zcytor17lig polypeptides and nucleic acid molecules encoding such functional fragments. A "functional" zcytor17lig or fragment thereof as defined herein is characterized by its proliferative or differentiating activity, by its ability to induce or inhibit specialized cell functions, or by its ability to bind specifically to an anti- zcytor17lig antibody or zcytor17 receptor antibody or zcytor17, WSX-1, or OSMRbeta receptor or heterodimers (e.g., zcytor17/WSX-1 or zcytor17/OSMRbeta) or multimers (e.g., zcytor17/WSX-1/OSMRbeta) of these receptors (either soluble or immobilized). As previously described herein, zcytor17lig is characterized by a four-helical-bundle structure comprising helix A (amino acid residues 38-52), helix B (amino acid residues 83-98), helix C (amino acid residues 104-117) and helix D (amino acid residues 137-152), as shown in SEQ ID NO:2. Thus, the present invention further provides fusion proteins encompassing: (a) polypeptide molecules comprising one or more of the helices described above; and (b) functional fragments comprising one or more of these helices. The other polypeptide portion of the fusion protein may be contributed by another four-helical-bundle cytokine, such as IL-15, IL-2, IL-4 and GM-CSF, or by a non-native and/or an unrelated secretory signal peptide that facilitates secretion of the fusion protein.

Thus the present invention provides fusion proteins comprising at least four polypeptides, wherein the order of polypeptides from N-terminus to C-terminus are: a first polypeptide comprises amino acids selected from a group consisting of: (a) IL-2 helix A amino acid residues 27-48 of SEQ ID NO:162; (b) IL-3 helix A amino acid residues 35-45 of SEQ ID NO:102; (c) IL-4 helix A amino acid residues 30-42 of SEQ ID NO:164; (d) GM-CSF helix A amino acid residues 30-44 of SEQ ID NO:166; and (e) amino acids residues 38 to 52 of SEQ ID NO:2; a first spacer of 6-27 amino

acids; and a second polypeptide that comprises amino acid residues selected from the group consisting of: (a) IL-2 helix B amino acid residues of SEQ ID NO:168; (b) IL-4 helix B amino acid residues 65-83 of SEQ ID NO:164; (c) IL-3 helix B amino acid residues 73-86 of SEQ ID NO:102; (d) GM-CSF helix B amino acid residues 72-81 of SEQ ID NO:166; and (e) amino acid residues 83-98 of SEQ ID NO:2; a second spacer of 5-11 amino acid residues; a third polypeptide that comprises a sequence of amino acid residues selected from the group consisting of: (a) IL-2 helix C residues 102-116 of SEQ ID NO:162; (b) IL-4 helix C residues 94-118 of SEQ ID NO:164; (c) IL-3 helix C residues 91-103 of SEQ ID NO:102; (d) GM-CSF helix C residues 85-103 of SEQ ID NO:166; and (e) amino acid residues 104-117 of SEQ ID NO:2; a third spacer of 3-29 amino acid residues; and a fourth polypeptide that comprises amino acid residues selected from the group consisting of: (a) IL-2 helix D amino acid residues 134-149 of SEQ ID NO:162; (b) IL-3 helix D amino acid residues 123-141 of SEQ ID NO:102; (c) IL-4 helix D amino acid residues 133-151 of SEQ ID NO:164; (d) GM-CSF helix D amino acid residues 120-131 of SEQ ID NO:166; and (e) amino acid residues 137-152 of SEQ ID NO:2, wherein at least one of the four polypeptides is from zcytor17lig. In other embodiments that the spacer peptides will be selected from the A/B, B/C and C/D loops of zcytor17lig, and IL-3, as shown in Table 1.

Routine deletion analyses of nucleic acid molecules can be performed to obtain functional fragments of a nucleic acid molecule that encodes a zcytor17lig polypeptide. As an illustration, DNA molecules having the nucleotide sequence of SEQ ID NO:1 or fragments thereof, can be digested with *Bal*31 nuclease to obtain a series of nested deletions. These DNA fragments are then inserted into expression vectors in proper reading frame, and the expressed polypeptides are isolated and tested for zcytor17lig activity, or for the ability to bind anti-zcytor17lig antibodies or zcytor17 receptor. One alternative to exonuclease digestion is to use oligonucleotide-directed mutagenesis to introduce deletions or stop codons to specify production of a desired zcytor17lig fragment. Alternatively, particular fragments of a zcytor17lig gene can be synthesized using the polymerase chain reaction.

Standard methods for identifying functional domains are well-known to those of skill in the art. For example, studies on the truncation at either or both termini

of interferons have been summarized by Horisberger and Di Marco, Pharmac. Ther. 66:507 (1995). Moreover, standard techniques for functional analysis of proteins are described by, for example, Treuter et al., Molec. Gen. Genet. 240:113 (1993); Content et al., "Expression and preliminary deletion analysis of the 42 kDa 2-5A synthetase induced by human interferon," in Biological Interferon Systems, Proceedings of ISIR-TNO Meeting on Interferon Systems, Cantell (ed.), pages 65-72 (Nijhoff 1987); Herschman, "The EGF Receptor," in Control of Animal Cell Proliferation 1, Boynton et al., (eds.) pages 169-199 (Academic Press 1985); Coumailleau et al., J. Biol. Chem. 270:29270 (1995); Fukunaga et al., J. Biol. Chem. 270:25291 (1995); Yamaguchi et al., Biochem. Pharmacol. 50:1295 (1995); and Meisel et al., Plant Molec. Biol. 30:1 (1996).

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53 (1988)) or Bowie and Sauer (Proc. Nat'l Acad. Sci. USA 86:2152 (1989)). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (*e.g.*, Lowman *et al.*, Biochem. 30:10832 (1991), Ladner et al., U.S. Patent No. 5,223,409, Huse, international publication No. WO 92/06204), and region-directed mutagenesis (Derbyshire et al., Gene 46:145 (1986), and Ner et al., DNA 7:127, (1988)).

Variants of the disclosed zcytor17lig nucleotide and polypeptide sequences can also be generated through DNA shuffling as disclosed by Stemmer, Nature 370:389 (1994), Stemmer, Proc. Natl Acad. Sci. USA 91:10747 (1994), and international publication No. WO 97/20078. Briefly, variant DNA molecules are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNA molecules, such as allelic variants or DNA molecules from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid

"evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode biologically active polypeptides, or polypeptides that bind with anti-zcytor17lig antibodies or soluble zcytor17 receptor, or soluble WSX-1 or soluble OSMR or heterodimers or multimers of these soluble receptors as described herein can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

In addition, the proteins of the present invention (or polypeptide fragments thereof) can be joined to other bioactive molecules, particularly other cytokines, to provide multi-functional molecules. For example, one or more helices from zcytor17lig can be joined to other cytokines to enhance their biological properties or efficiency of production.

The present invention thus provides a series of novel, hybrid molecules in which a segment comprising one or more of the helices of zcytor17lig is fused to another polypeptide. Fusion is preferably done by splicing at the DNA level to allow expression of chimeric molecules in recombinant production systems. The resultant molecules are then assayed for such properties as improved solubility, improved stability, prolonged clearance half-life, improved expression and secretion levels, and pharmacodynamics. Such hybrid molecules may further comprise additional amino acid residues (e.g., a polypeptide linker) between the component proteins or polypeptides.

Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-

fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is typically carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722 (1991), Ellman et al., Methods Enzymol. 202:301 (1991), Chung et al., Science 259:806 (1993), and Chung et al., Proc. Nat'l Acad. Sci. USA 90:10145 (1993).

In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991 (1996)). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (*e.g.*, phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (*e.g.*, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide et al., Biochem. 33:7470 (1994). Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395 (1993). It may be advantageous to stabilize zcytor17lig to extend the half-life of the molecule, particularly for extending metabolic persistence in an active state. To achieve extended half-life, zcytor17lig molecules can be chemically modified using methods described herein. PEGylation is one method commonly used that has been demonstrated to increase plasma half-life, increased solubility, and decreased antigenicity and immunogenicity (Nucci et al., Advanced Drug Delivery Reviews 6:133-155, 1991 and Lu et al., Int. J. Peptide Protein Res. 43:127-138, 1994).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for zcytor17lig amino acid residues.

The present invention also provides polypeptide fragments or peptides comprising an epitope-bearing portion of a zcytor17lig polypeptide described herein. Such fragments or peptides may comprise an "immunogenic epitope," which is a part of a protein that elicits an antibody response when the entire protein is used as an immunogen. Immunogenic epitope-bearing peptides can be identified using standard methods (see, for example, Geysen et al., Proc. Nat'l Acad. Sci. USA 81:3998 (1983)).

In contrast, polypeptide fragments or peptides may comprise an "antigenic epitope," which is a region of a protein molecule to which an antibody can specifically bind. Certain epitopes consist of a linear or contiguous stretch of amino acids, and the antigenicity of such an epitope is not disrupted by denaturing agents. It is known in the art that relatively short synthetic peptides that can mimic epitopes of a protein can be used to stimulate the production of antibodies against the protein (see, for example, Sutcliffe et al., Science 219:660 (1983)). Accordingly, antigenic epitope-bearing peptides and polypeptides of the present invention are useful to raise antibodies (e.g., neutralizing antibodies) that bind with the polypeptides described herein. Hopp/Woods hydrophilicity profiles can be used to determine regions that have the most antigenic potential (Hopp et al., 1981, *ibid.* and Hopp, 1986, *ibid.*). For example, in human zcytor17lig, hydrophilic regions include amino acid residues 54-59 of SEQ ID NO:2, amino acid residues 129-134 of SEQ ID NO:2, amino acid residues 53-58 of SEQ ID NO:2, amino acid residues 35-40 of SEQ ID NO:2, and amino acid residues 33-38 of SEQ ID NO:2. For example, in mouse zcytor17lig, hydrophilic regions include amino acid residues 34-39 of SEQ ID NO:11, amino acid residues 46-51 of SEQ ID NO:11, amino acid residues 131-136 of SEQ ID NO:11, amino acid residues 158-163 of SEQ ID NO:11, and amino acid residues 157-162 of SEQ ID NO:11.

Antigenic epitope-bearing peptides and polypeptides preferably contain at least four to ten amino acids, at least ten to fourteen amino acids, or about fourteen to about thirty amino acids of SEQ ID NO:2 or SEQ ID NO:11. Such epitope-bearing peptides and polypeptides can be produced by fragmenting a zcytor17lig polypeptide, or

by chemical peptide synthesis, as described herein. Moreover, epitopes can be selected by phage display of random peptide libraries (see, for example, Lane and Stephen, Curr. Opin. Immunol. 5:268 (1993); and Cortese *et al.*, Curr. Opin. Biotechnol. 7:616 (1996)). Standard methods for identifying epitopes and producing antibodies from small peptides that comprise an epitope are described, for example, by Mole, "Epitope Mapping," in Methods in Molecular Biology, Vol. 10, Manson (ed.), pages 105-116 (The Humana Press, Inc. 1992); Price, "Production and Characterization of Synthetic Peptide-Derived Antibodies," in Monoclonal Antibodies: Production, Engineering, and Clinical Application, Ritter and Ladyman (eds.), pages 60-84 (Cambridge University Press 1995), and Coligan *et al.* (eds.), Current Protocols in Immunology, pages 9.3.1 - 9.3.5 and pages 9.4.1 - 9.4.11 (John Wiley & Sons 1997).

Regardless of the particular nucleotide sequence of a variant zcytor17lig polynucleotide, the polynucleotide encodes a polypeptide that is characterized by its proliferative or differentiating activity, its ability to induce or inhibit specialized cell functions, or by the ability to bind specifically to an anti-zcytor17lig antibody or zcytor17 receptor. More specifically, variant zcytor17lig polynucleotides will encode polypeptides which exhibit at least 50% and preferably, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or greater than 99%, of the activity of the polypeptide as shown in SEQ ID NO:2.

For any zcytor17lig polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 1 and 2 above.

The present invention further provides a variety of other polypeptide fusions (and related multimeric proteins comprising one or more polypeptide fusions). For example, a zcytor17lig polypeptide can be prepared as a fusion to a dimerizing protein as disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Preferred dimerizing proteins in this regard include immunoglobulin constant region domains. Immunoglobulin- zcytor17lig polypeptide fusions can be expressed in genetically engineered cells (to produce a variety of multimeric zcytor17lig analogs). Auxiliary domains can be fused to zcytor17lig polypeptides to target them to specific cells, tissues, or macromolecules. For example, a zcytor17lig polypeptide or protein could be

targeted to a predetermined cell type by fusing a zcytor17lig polypeptide to a ligand that specifically binds to a receptor on the surface of that target cell. In this way, polypeptides and proteins can be targeted for therapeutic or diagnostic purposes. A zcytor17lig polypeptide can be fused to two or more moieties, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, Tuan et al., Connective Tissue Research 34:1-9, 1996.

Using the methods discussed herein, one of ordinary skill in the art can identify and/or prepare a variety of polypeptides that have substantially similar sequence identity to residues 1-164 or 24-164 of SEQ ID NO:2, or functional fragments and fusions thereof, such as helices A-D (residues 38-152 of SEQ ID NO:2) wherein such polypeptides or fragments or fusions retain the properties of the wild-type protein such as the ability to stimulate proliferation, differentiation, induce specialized cell function or bind the zcytor17 receptor or zcytor17lig antibodies.

The zcytor17lig polypeptides of the present invention, including full-length polypeptides, functional fragments, and fusion polypeptides, can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, and Ausubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987.

In general, a DNA sequence encoding a zcytor17lig polypeptide can be operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the

exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a zcytor17lig polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of zcytor17lig, or may be derived from another secreted protein (e.g., t-PA) or synthesized *de novo*. The secretory signal sequence is operably linked to the zcytor17lig DNA sequence, i.e., the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

Alternatively, the secretory signal sequence contained in the polypeptides of the present invention is used to direct other polypeptides into the secretory pathway. The present invention provides for such fusion polypeptides. A signal fusion polypeptide can be made wherein a secretory signal sequence derived from amino acid residue 1-23 of SEQ ID NO:2 or residues 1-23 SEQ ID NO:11 is be operably linked to a DNA sequence encoding another polypeptide using methods known in the art and disclosed herein. The secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein. Such fusions may be used *in vivo* or *in vitro* to direct peptides through the secretory pathway.

Cultured mammalian cells are suitable hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler et al., Cell 14:725, 1978; Corsaro and

Pearson, Somatic Cell Genetics 7:603, 1981: Graham and Van der Eb, Virology 52:456, 1973), electroporation (Neumann et al., EMBO J. 1:841-5, 1982), DEAE-dextran mediated transfection (Ausubel et al., ibid.), and liposome-mediated transfection (Hawley-Nelson et al., Focus 15:73, 1993; Ciccarone et al., Focus 15:80, 1993, and viral vectors (Miller and Rosman, BioTechniques 7:980-90, 1989; Wang and Finer, Nature Med. 2:714-6, 1996). The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., J. Gen. Virol. 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Manassas, VA. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g., hygromycin resistance, multi-drug resistance, puromycin

acetyltransferase) can also be used. Alternative markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Other higher eukaryotic cells can also be used as hosts, including plant cells, insect cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., J. Biosci. (Bangalore) 11:47-58, 1987. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222 and WIPO publication No. WO 94/06463. Insect cells can be infected with recombinant baculovirus, commonly derived from *Autographa californica nuclear polyhedrosis virus* (AcNPV). See, King, L.A. and Possee, R.D., The Baculovirus Expression System: A Laboratory Guide, London, Chapman & Hall; O'Reilly, D.R. et al., Baculovirus Expression Vectors: A Laboratory Manual, New York, Oxford University Press., 1994; and, Richardson, C. D., Ed., Baculovirus Expression Protocols. Methods in Molecular Biology, Totowa, NJ, Humana Press, 1995. The second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, V.A, et al., J Virol 67:4566-79, 1993). This system is sold in the Bac-to-Bac kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the zcytor17lig polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." The pFastBac1™ transfer vector utilizes the AcNPV polyhedrin promoter to drive the expression of the gene of interest, in this case zcytor17lig. However, pFastBac1™ can be modified to a considerable degree. The polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as P*cor*, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins. See, Hill-Perkins, M.S. and Possee, R.D., J. Gen. Virol. 71:971-6, 1990; Bonning, B.C. et al., J. Gen. Virol. 75:1551-6, 1994; and, Chazenbalk, G.D., and Rapoport, B., J. Biol. Chem. 270:1543-9, 1995. In such transfer vector constructs, a

short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed which replace the native zcytor17lig secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen, Carlsbad, CA), or baculovirus gp67 (PharMingen, San Diego, CA) can be used in constructs to replace the native zcytor17lig secretory signal sequence. In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed zcytor17lig polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer, T. et al., Proc. Natl. Acad. Sci. 82:7952-4, 1985). Using techniques known in the art, a transfer vector containing zcytor17lig is transformed into *E. Coli*, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, e.g., Sf9 cells. Recombinant virus that expresses zcytor17lig is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

The recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *Spodoptera frugiperda*. See, in general, Glick and Pasternak, Molecular Biotechnology: Principles and Applications of Recombinant DNA, ASM Press, Washington, D.C., 1994. Another suitable cell line is the High FiveO™ cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent No. 5,300,435).

Fungal cells, including yeast cells, can also be used within the present invention. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient

(e.g., leucine). A preferred vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459-65, 1986 and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

The use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed in WIPO Publication Nos. WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, it is preferred that the promoter and terminator in the plasmid be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A preferred selectable marker for use in *Pichia methanolica* is a *P. methanolica ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, it is preferred to use host cells in

which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells deficient in vacuolar protease genes (*PEP4* and *PRB1*) are preferred. Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. It is preferred to transform *P. methanolica* cells by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (Ω) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art (see, e.g., Sambrook et al., ibid.). When expressing a zcytor17lig polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which

is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell. *P. methanolica* cells are cultured in a medium comprising adequate sources of carbon, nitrogen and trace nutrients at a temperature of about 25°C to 35°C. Liquid cultures are provided with sufficient aeration by conventional means, such as shaking of small flasks or sparging of fermentors. A preferred culture medium for *P. methanolica* is YEPD (2% D-glucose, 2% Bacto™ Peptone (Difco Laboratories, Detroit, MI), 1% Bacto™ yeast extract (Difco Laboratories), 0.004% adenine and 0.006% L-leucine).

It is preferred to purify the polypeptides of the present invention to ≥ 80% purity, more preferably to ≥90% purity, even more preferably ≥95% purity, and particularly preferred is a pharmaceutically pure state, that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, a purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

Expressed recombinant zcytor17lig polypeptides (or chimeric zcytor17lig polypeptides) can be purified using fractionation and/or conventional purification methods and media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling

chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, Affinity Chromatography: Principles & Methods, Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988.

The polypeptides of the present invention can be isolated by exploitation of their physical or biochemical properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, Trends in Biochem. 3:1-7, 1985). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (Methods in Enzymol., Vol. 182, "Guide to Protein Purification", M. Deutscher, (ed.), Acad. Press, San Diego, 1990, pp.529-39) and use of the soluble zcytor17 receptor. Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (e.g., maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Moreover, using methods described in the art, polypeptide fusions, or hybrid zcytor17lig proteins, are constructed using regions or domains of the inventive zcytor17lig in combination with those of other human cytokine family proteins (e.g. interleukins or GM-CSF), or heterologous proteins (Sambrook et al., ibid., Altschul et al., ibid., Picard, Cur. Opin. Biology, 5:511-5, 1994, and references therein). These methods allow the determination of the biological importance of larger domains or regions in a polypeptide of interest. Such hybrids may alter reaction kinetics, binding, constrict or expand the substrate specificity, or alter tissue and cellular localization of a polypeptide, and can be applied to polypeptides of unknown structure.

Fusion proteins can be prepared by methods known to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Alternatively, a polynucleotide encoding both components of the fusion protein in the proper reading frame can be generated using known techniques and expressed by the methods described herein. For example, part or all of a helix conferring a biological function may be swapped between zcytor17lig of the present invention with the functionally equivalent helices from another family member, such as IL-15, IL-2, IL-4 or GM-CSF. Such components include, but are not limited to, the secretory signal sequence; helices A, B, C, D; loops A/B, B/C, C/D; of four-helical-bundle cytokines. Such fusion proteins would be expected to have a biological functional profile that is the same or similar to polypeptides of the present invention or other known four-helical-bundle cytokine family proteins, depending on the fusion constructed. Moreover, such fusion proteins may exhibit other properties as disclosed herein.

Standard molecular biological and cloning techniques can be used to swap the equivalent domains between the zcytor17lig polypeptide and those polypeptides to which they are fused. Generally, a DNA segment that encodes a domain of interest, e.g., zcytor17lig helices A through D, or other domain described herein, is operably linked in frame to at least one other DNA segment encoding an additional polypeptide (for instance a domain or region from another cytokine, such as the IL-2, or the like), and inserted into an appropriate expression vector, as described herein. Generally DNA constructs are made such that the several DNA segments that encode the corresponding regions of a polypeptide are operably linked in frame to make a single construct that encodes the entire fusion protein, or a functional portion thereof. For example, a DNA construct would encode from N-terminus to C-terminus a fusion protein comprising a signal polypeptide followed by a mature four helical bundle cytokine fusion protein containing helix A, followed by helix B, followed by helix C, followed by helix D. Such fusion proteins can be expressed, isolated, and assayed for activity as described herein.

Zcytor17lig polypeptides or fragments thereof may also be prepared through chemical synthesis. zcytor17lig polypeptides may be monomers or multimers; glycosylated or non-glycosylated; pegylated or non-pegylated; and may or may not

include an initial methionine amino acid residue. For example, the polypeptides can be prepared by solid phase peptide synthesis, for example as described by Merrifield, J. Am. Chem. Soc. 85:2149, 1963.

The activity of molecules of the present invention can be measured using a variety of assays that measure proliferation of and/or binding to cells expressing the zcytor17 receptor. Of particular interest are changes in zcytor17lig-dependent cells. Suitable cell lines to be engineered to be zcytor17lig-dependent include the IL-3-dependent BaF3 cell line (Palacios and Steinmetz, Cell 41: 727-734, 1985; Mathey-Prevot et al., Mol. Cell. Biol. 6: 4133-4135, 1986), FDC-P1 (Hapel et al., Blood 64: 786-790, 1984), and MO7e (Kiss et al., Leukemia 7: 235-240, 1993). Growth factor-dependent cell lines can be established according to published methods (e.g. Greenberger et al., Leukemia Res. 8: 363-375, 1984; Dexter et al., in Baum et al. Eds., Experimental Hematology Today, 8th Ann. Mtg. Int. Soc. Exp. Hematol. 1979, 145-156, 1980).

Proteins of the present invention are useful for stimulating proliferation, activation, differentiation and/or induction or inhibition of specialized cell function of cells of the involved homeostasis of the hematopoiesis and immune function. In particular, zcytor17lig polypeptides are useful for stimulating proliferation, activation, differentiation, induction or inhibition of specialized cell functions of cells of the hematopoietic lineages, including, but not limited to, T cells, B cells, monocytes/macrophages, NK cells, neutrophils, endothelial cells, fibroblasts, eosinophils, chondrocytes, mast cells, langerhan cells, monocytes, and macrophages, as well as epithelial cells. Epithelial cells include, for example, ameloblasts, chief cells, chromatophores, enterochramaffin cells, enterochromaffin-like cells, goblet cells, granulosa cells, keratinocytes, dendritic cells, labyrinth supporting cells, melanocytes, merkel cells, paneth cells, parietal cells, sertoli cells, and the like. Proliferation and/or differentiation of hematopoietic cells can be measured *in vitro* using cultured cells or *in vivo* by administering molecules of the present invention to the appropriate animal model. Assays measuring cell proliferation or differentiation are well known in the art. For example, assays measuring proliferation include such assays as chemosensitivity to neutral red dye (Cavanaugh et al., Investigational New Drugs 8:347-354, 1990,

incorporated herein by reference), incorporation of radiolabelled nucleotides (Cook et al., Analytical Biochem. 179:1-7, 1989, incorporated herein by reference), incorporation of 5-bromo-2'-deoxyuridine (BrdU) in the DNA of proliferating cells (Porstmann et al., J. Immunol. Methods 82:169-179, 1985, incorporated herein by reference), and use of tetrazolium salts (Mosmann, J. Immunol. Methods 65:55-63, 1983; Alley et al., Cancer Res. 48:589-601, 1988; Marshall et al., Growth Reg. 5:69-84, 1995; and Scudiero et al., Cancer Res. 48:4827-4833, 1988; all incorporated herein by reference). Assays measuring differentiation include, for example, measuring cell-surface markers associated with stage-specific expression of a tissue, enzymatic activity, functional activity or morphological changes (Watt, FASEB, 5:281-284, 1991; Francis, Differentiation 57:63-75, 1994; Raes, Adv. Anim. Cell Biol. Technol. Bioprocesses, 161-171, 1989; all incorporated herein by reference).

The molecules of the present invention can be assayed *in vivo* using viral delivery systems. Exemplary viruses for this purpose include adenovirus, herpesvirus, retroviruses, vaccinia virus, and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for review, see T.C. Becker et al., Meth. Cell Biol. 43:161-89, 1994; and J.T. Douglas and D.T. Curiel, Science & Medicine 4:44-53, 1997).

As a ligand, the activity of zcytor17lig polypeptide can be measured by a silicon-based biosensor microphysiometer which measures the extracellular acidification rate or proton excretion associated with receptor binding and subsequent physiologic cellular responses. An exemplary device is the Cytosensor™ Microphysiometer manufactured by Molecular Devices, Sunnyvale, CA. A variety of cellular responses, such as cell proliferation, ion transport, energy production, inflammatory response, regulatory and receptor activation, and the like, can be measured by this method. See, for example, McConnell, H.M. et al., Science 257:1906-1912, 1992; Pitchford, S. et al., Meth. Enzymol. 228:84-108, 1997; Arimilli, S. et al., J. Immunol. Meth. 212:49-59, 1998; Van Liefde, I. et al., Eur. J. Pharmacol. 346:87-95, 1998.

Moreover, zcytor17lig can be used to identify cells, tissues, or cell lines which respond to a zcytor17lig-stimulated pathway. The microphysiometer, described

above, can be used to rapidly identify ligand-responsive cells, such as cells responsive to zcytor17lig of the present invention. Cells can be cultured in the presence or absence of zcytor17lig polypeptide. Those cells which elicit a measurable change in extracellular acidification in the presence of zcytor17lig are responsive to zcytor17lig. Such cells or cell lines, can be used to identify antagonists and agonists of zcytor17lig polypeptide as described above.

In view of the tissue distribution observed for zcytor17 receptor agonists (including the natural zcytor17lig/ substrate/ cofactor/ etc.) and/or antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as zcytor17lig agonists are useful for expansion, proliferation, activation, differentiation, and/or induction or inhibition of specialized cell functions of cells involved in homeostasis of hematopoiesis and immune function. For example, zcytor17lig and agonist compounds are useful as components of defined cell culture media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Agonists are thus useful in specifically promoting the growth and/or development of T-cells, B-cells, monocytes/macrophages, NK cells, cytotoxic lymphocytes, and other cells of the lymphoid and myeloid lineages in culture.

Antagonists are also useful as research reagents for characterizing sites of ligand-receptor interaction. Antagonists are useful to inhibit expansion, proliferation, activation, and/or differentiation of cells involved in regulating hematopoiesis. Inhibitors of zcytor17lig activity (zcytor17lig antagonists) include anti-zcytor17lig antibodies and soluble zcytor17lig receptors, as well as other peptidic and non-peptidic agents (including ribozymes).

Zcytor17lig can also be used to identify inhibitors (antagonists) of its activity. Test compounds are added to the assays disclosed herein to identify compounds that inhibit the activity of zcytor17lig. In addition to those assays disclosed herein, samples can be tested for inhibition of zcytor17lig activity within a variety of assays designed to measure receptor binding, the stimulation/inhibition of zcytor17lig-dependent cellular responses or proliferation of zcytor17 receptor-expressing cells.

A zcytor17lig polypeptide can be expressed as a fusion with an immunoglobulin heavy chain constant region, typically an F_c fragment, which contains

two constant region domains and lacks the variable region. Methods for preparing such fusions are disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Such fusions are typically secreted as multimeric molecules wherein the Fc portions are disulfide bonded to each other and two non-Ig polypeptides are arrayed in closed proximity to each other. Fusions of this type can be used for example, for dimerization, increasing stability and *in vivo* half-life, to affinity purify ligand, as *in vitro* assay tool or antagonist. For use in assays, the chimeras are bound to a support via the F_C region and used in an ELISA format.

A zcytor17lig-binding polypeptide can also be used for purification of ligand. The polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting medium will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then eluted using changes in salt concentration, chaotropic agents (guanidine HCl), or pH to disrupt ligand-receptor binding.

An assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIAcore, Pharmacia Biosensor, Piscataway, NJ) may be advantageously employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, J. Immunol. Methods 145:229-40, 1991 and Cunningham and Wells, J. Mol. Biol. 234:554-63, 1993. A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the

refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding. Alternatively, ligand/receptor binding can be analyzed using SELDI(TM) technology (Ciphergen, Inc., Palo Alto, CA).

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (see Scatchard, Ann. NY Acad. Sci. 51: 660-72, 1949) and calorimetric assays (Cunningham et al., Science 253:545-48, 1991; Cunningham et al., Science 245:821-25, 1991).

Zcytor17lig polypeptides can also be used to prepare antibodies that bind to zcytor17lig epitopes, peptides or polypeptides. The zcytor17lig polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal and elicit an immune response. Such antibodies can be used to block the biological action of pro-inflammatory zcytor17lig and are useful as anti-inflammatory therapeutics in a variety of diseases as described herein. One of skill in the art would recognize that antigenic, epitope-bearing polypeptides contain a sequence of at least 6. preferably at least 9, and more preferably at least 15 to about 30 contiguous amino acid residues of a zcytor17lig polypeptide (e.g., SEQ ID NO:2). Polypeptides comprising a larger portion of a zcytor17lig polypeptide, i.e., from 30 to 100 residues up to the entire length of the amino acid sequence are included. Antigens or immunogenic epitopes can also include attached tags, adjuvants, vehicles and carriers, as described herein. Suitable antigens include the zcytor17lig polypeptide encoded by SEQ ID NO:2 from amino acid number 24 to amino acid number 164, or a contiguous 9 to 141 amino acid fragment thereof. Other suitable antigens include, the full length and the mature zcytor17lig, helices A-D, and individual or multiple helices A, B, C, and D, of the zcytor17lig four-helical-bundle structure, as described herein. Preferred peptides to use as antigens are hydrophilic peptides such as those predicted by one of skill in the art from a hydrophobicity plot, as described herein, for example, amino acid residues 114-119, 101-105, 126-131, 113-118, and 158-162 of SEQ ID NO:2; and amino acid residues 34-39, 46-51, 131-136, 158-163 and 157-162 of SEQ ID NO:11. Moreover, zcytor17lig antigenic epitopes as

predicted by a Jameson-Wolf plot, e.g., using DNASTAR Protean program (DNASTAR, Inc., Madison, WI) serve as preferred antigens, and are readily determined by one of skill in the art.

Antibodies from an immune response generated by inoculation of an animal with these antigens can be isolated and purified as described herein. Methods for preparing and isolating polyclonal and monoclonal antibodies are well known in the art. See, for example, Current Protocols in Immunology, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995; Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL, 1982.

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from inoculating a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats with a zcytor17lig polypeptide or a fragment thereof. The immunogenicity of a zcytor17lig polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of zcytor17lig or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as $F(ab')_2$ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by

replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced. Moreover, human antibodies can be produced in transgenic, non-human animals that have been engineered to contain human immunoglobulin genes as disclosed in WIPO Publication No. WO 98/24893. It is preferred that the endogenous immunoglobulin genes in these animals be inactivated or eliminated, such as by homologous recombination.

Antibodies are considered to be specifically binding if: 1) they exhibit a threshold level of binding activity, and 2) they do not significantly cross-react with related polypeptide molecules. A threshold level of binding is determined if anti-zcytor17lig antibodies herein bind to a zcytor17lig polypeptide, peptide or epitope with an affinity at least 10-fold greater than the binding affinity to control (non-zcytor17lig) polypeptide. It is preferred that the antibodies exhibit a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, G., Ann. NY Acad. Sci. 51: 660-672, 1949).

Whether anti-zcytor17lig antibodies do not significantly cross-react with related polypeptide molecules is shown, for example, by the antibody detecting zcytor17lig polypeptide but not known related polypeptides using a standard Western blot analysis (Ausubel et al., ibid.). Examples of known related polypeptides are those disclosed in the prior art, such as known orthologs, and paralogs, and similar known members of a protein family. Screening can also be done using non-human zcytor17lig, and zcytor17lig mutant polypeptides. Moreover, antibodies can be "screened against" known related polypeptides, to isolate a population that specifically binds to the zcytor17lig polypeptides. For example, antibodies raised to zcytor17lig are adsorbed to related polypeptides adhered to insoluble matrix; antibodies specific to zcytor17lig will flow through the matrix under the proper buffer conditions. Screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to known closely related

polypeptides (Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; Current Protocols in Immunology, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995). Screening and isolation of specific antibodies is well known in the art. See, Fundamental Immunology, Paul (eds.), Raven Press, 1993; Getzoff et al., *Adv. in Immunol.* 43: 1-98, 1988; Monoclonal Antibodies: Principles and Practice, Goding, J.W. (eds.), Academic Press Ltd., 1996; Benjamin et al., Ann. Rev. Immunol. 2: 67-101, 1984. Specifically binding anti-zcytor17lig antibodies can be detected by a number of methods in the art, and disclosed below.

A variety of assays known to those skilled in the art can be utilized to detect antibodies which bind to zcytor17lig proteins or polypeptides. Exemplary assays are described in detail in Antibodies: A Laboratory Manual, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-precipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant zcytor17lig protein or polypeptide.

Antibodies to zcytor17lig may be used for tagging cells that express zcytor17lig; for isolating zcytor17lig by affinity purification; for diagnostic assays for determining circulating levels of zcytor17lig polypeptides; for detecting or quantitating soluble zcytor17lig as a marker of underlying pathology or disease; in analytical methods employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block zcytor17lig activity *in vitro* and *in vivo*. Suitable direct tags or labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Antibodies herein may also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. Moreover, antibodies to zcytor17lig or fragments thereof may

be used *in vitro* to detect denatured zcytor17lig or fragments thereof in assays, for example, Western Blots or other assays known in the art.

Suitable detectable molecules may be directly or indirectly attached to the polypeptide or antibody, and include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like. Suitable cytotoxic molecules may be directly or indirectly attached to the polypeptide or antibody, and include bacterial or plant toxins (for instance, diphtheria, toxin, saporin, *Pseudomonas* exotoxin, ricin, abrin and the like), as well as therapeutic radionuclides, such as iodine-131, rhenium-188 or yttrium-90 (either directly attached to the polypeptide or antibody, or indirectly attached through means of a chelating moiety, for instance). Polypeptides or antibodies may also be conjugated to cytotoxic drugs, such as adriamycin. For indirect attachment of a detectable or cytotoxic molecule, the detectable or cytotoxic molecule can be conjugated with a member of a complementary/anticomplementary pair, where the other member is bound to the polypeptide or antibody portion. For these purposes, biotin/streptavidin is an exemplary complementary/ anticomplementary pair.

Binding polypeptides can also act as zcytor17lig "antagonists" to block zcytor17lig binding and signal transduction *in vitro* and *in vivo*. These anti-zcytor17lig binding polypeptides would be useful for inhibiting zcytor17lig activity or protein-binding.

Polypeptide-toxin fusion proteins or antibody-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation (for instance, to treat cancer cells or tissues). Alternatively, if the polypeptide has multiple functional domains (i.e., an activation domain or a receptor binding domain, plus a targeting domain), a fusion protein including only the targeting domain may be suitable for directing a detectable molecule, a cytotoxic molecule or a complementary molecule to a cell or tissue type of interest. In instances where the domain only fusion protein includes a complementary molecule, the anti-complementary molecule can be conjugated to a detectable or cytotoxic molecule. Such domain-complementary molecule fusion proteins thus represent a generic targeting carrier or vehicle for cell/tissue-specific delivery of generic anti-complementary-detectable/ cytotoxic molecule conjugates.

In another embodiment, zcytor17lig cytokine fusion proteins or antibody-cytokine fusion proteins can be used for *in vivo* killing of target tissues (for example, leukemia, lymphoma, lung cancer, colon cancer, melanoma, pancreatic cancer, ovanian cancer, skin, blood and bone marrow cancers, or other cancers wherein zcytor17lig receptors ar expressed) (See, generally, Hornick et al., Blood 89:4437-47, 1997). The described fusion proteins enable targeting of a cytokine to a desired site of action, thereby providing an elevated local concentration of cytokine. Suitable zcytor17lig polypeptides or anti-zcytor17lig antibodies target an undesirable cell or tissue (i.e., a tumor or a leukemia), and the fused cytokine mediated improved target cell lysis by effector cells. Suitable cytokines for this purpose include interleukin 2 and granulocyte-macrophage colony-stimulating factor (GM-CSF), for instance.

In yet another embodiment, if the zcytor17lig polypeptide or anti-zcytor17lig antibody targets vascular cells or tissues, such polypeptide or antibody may be conjugated with a radionuclide, and particularly with a beta-emitting radionuclide, to reduce restenosis. Such therapeutic approaches pose less danger to clinicians who administer the radioactive therapy. For instance, iridium-192 impregnated ribbons placed into stented vessels of patients until the required radiation dose was delivered showed decreased tissue growth in the vessel and greater luminal diameter than the control group, which received placebo ribbons. Further, revascularisation and stent thrombosis were significantly lower in the treatment group. Similar results are predicted with targeting of a bioactive conjugate containing a radionuclide, as described herein.

The bioactive polypeptide or antibody conjugates described herein can be delivered intravenously, intraarterially or intraductally, or may be introduced locally at the intended site of action.

Moreover, inflammation is a protective response by an organism to fend off an invading agent. Inflammation is a cascading event that involves many cellular and humoral mediators. On one hand, suppression of inflammatory responses can leave a host immunocompromised; however, if left unchecked, inflammation can lead to serious complications including chronic inflammatory diseases (e.g., rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and the like), septic shock and

multiple organ failure. Importantly, these diverse disease states share common inflammatory mediators. The collective diseases that are characterized by inflammation have a large impact on human morbidity and mortality. Therefore it is clear that anti-inflammatory antibodies and binding polypeptides, such as anti-zcytor17lig antibodies and binding polypeptides described herein, could have crucial therapeutic potential for a vast number of human and animal diseases, from asthma and allergy to autoimmunity and septic shock. As such, use of anti-inflammatory anti zcytor17lig antibodies and binding polypeptides described herein can be used therapeutically as zcytor17lig antagonists described herein, particularly in diseases such as arthritis, endotoxemia, inflammatory bowel disease, psoriasis, related disease and the like.

1. Arthritis

Arthritis, including osteoarthritis, rheumatoid arthritis, arthritic joints as a result of injury, and the like, are common inflammatory conditions which would benefit from the therapeutic use of anti-inflammatory antibodies and binding polypeptides, such as anti-zcytor17lig antibodies and binding polypeptides of the present invention. For Example, rheumatoid arthritis (RA) is a systemic disease that affects the entire body and is one of the most common forms of arthritis. It is characterized by the inflammation of the membrane lining the joint, which causes pain, stiffness, warmth, redness and swelling. Inflammatory cells release enzymes that may digest bone and cartilage. As a result of rheumatoid arthritis, the inflamed joint lining, the synovium, can invade and damage bone and cartilage leading to joint deterioration and severe pain amongst other physiologic effects. The involved joint can lose its shape and alignment, resulting in pain and loss of movement.

Rheumatoid arthritis (RA) is an immune-mediated disease particularly characterized by inflammation and subsequent tissue damage leading to severe disability and increased mortality. A variety of cytokines are produced locally in the rheumatoid joints. Numerous studies have demonstrated that IL-1 and TNF-alpha, two prototypic pro-inflammatory cytokines, play an important role in the mechanisms involved in synovial inflammation and in progressive joint destruction. Indeed, the administration of TNF-alpha and IL-1 inhibitors in patients with RA has led to a dramatic improvement of clinical and biological signs of inflammation and a reduction

of radiological signs of bone erosion and cartilage destruction. However, despite these encouraging results, a significant percentage of patients do not respond to these agents, suggesting that other mediators are also involved in the pathophysiology of arthritis (Gabay, Expert. Opin. Biol. Ther. 2(2):135-149, 2002). One of those mediators could be zcytor17lig, and as such a molecule that binds or inhibits zcytor17lig, such as anti zcytor17lig antibodies or binding partners, could serve as a valuable therapeutic to reduce inflammation in rheumatoid arthritis, and other arthritic diseases.

There are several animal models for rheumatoid arthritis known in the art. For example, in the collagen-induced arthritis (CIA) model, mice develop chronic inflammatory arthritis that closely resembles human rheumatoid arthritis. Since CIA shares similar immunological and pathological features with RA, this makes it an ideal model for screening potential human anti-inflammatory compounds. The CIA model is a well-known model in mice that depends on both an immune response, and an inflammatory response, in order to occur. The immune response comprises the interaction of B-cells and CD4+ T-cells in response to collagen, which is given as antigen, and leads to the production of anti-collagen antibodies. The inflammatory phase is the result of tissue responses from mediators of inflammation, as a consequence of some of these antibodies cross-reacting to the mouse's native collagen and activating the complement cascade. An advantage in using the CIA model is that the basic mechanisms of pathogenesis are known. The relevant T-cell and B-cell epitopes on type II collagen have been identified, and various immunological (e.g., delayed-type hypersensitivity and anti-collagen antibody) and inflammatory (e.g., cytokines, chemokines, and matrix-degrading enzymes) parameters relating to immune-mediated arthritis have been determined, and can thus be used to assess test compound efficacy in the CIA model (Wooley, Curr. Opin. Rheum. 3:407-20, 1999; Williams et al., Immunol. 89:9784-788, 1992; Myers et al., Life Sci. 61:1861-78, 1997; and Wang et al., Immunol. 92:8955-959, 1995).

The administration of soluble zcytor17 comprising polypeptides (including heterodimeric and multimeric receptors described herein), such as zcytor17-Fc4 or other zcytor17 soluble and fusion proteins to these CIA model mice was used to evaluate the use of zcytor17 to ameliorate symptoms and alter the course of disease. As

a molecule that modulates immune and inflammatory response, zcytor17lig, may induce production of SAA, which is implicated in the pathogenesis of rheumatoid arthritis, zcytor17lig antagonists may reduce SAA activity *in vitro* and *in vivo*, the systemic or local administration of zcytor17lig antagonists such as anti-zcytor17lig antibodies or binding partners, zcytor17 comprising polypeptides (including heterodimeric and multimeric receptors described herein), such as zcytor17-Fc4 or other zcytor17 soluble and fusion proteins can potentially suppress the inflammatory response in RA. Other potential therapeutics include zcytor17 polypeptides, soluble heterodimeric and multimeric receptor polypeptides, or anti zcytor17lig antibodies or binding partners of the present invention, and the like.

2. Endotoxemia

Endotoxemia is a severe condition commonly resulting from infectious agents such as bacteria and other infectious disease agents, sepsis, toxic shock syndrome, or in immunocompromised patients subjected to opportunistic infections, and the like. Therapeutically useful of anti-inflammatory antibodies and binding polypeptides, such as anti-zcytor17lig antibodies and binding polypeptides of the present invention, could aid in preventing and treating endotoxemia in humans and animals. Other potential therapeutics include zcytor17 polypeptides, soluble heterodimeric and multimeric receptor polypeptides, or anti zcytor17lig antibodies or binding partners of the present invention, and the like, could serve as a valuable therapeutic to reduce inflammation and pathological effects in endotoxemia.

Lipopolysaccharide (LPS) induced endotoxemia engages many of the proinflammatory mediators that produce pathological effects in the infectious diseases and LPS induced endotoxemia in rodents is a widely used and acceptable model for studying the pharmacological effects of potential pro-inflammatory or immunomodulating agents. LPS, produced in gram-negative bacteria, is a major causative agent in the pathogenesis of septic shock (Glausner et al., Lancet 338:732, 1991). A shock-like state can indeed be induced experimentally by a single injection of LPS into animals. Molecules produced by cells responding to LPS can target pathogens directly or indirectly. Although these biological responses protect the host against invading pathogens, they may also cause harm. Thus, massive stimulation of innate

immunity, occurring as a result of severe Gram-negative bacterial infection, leads to excess production of cytokines and other molecules, and the development of a fatal syndrome, septic shock syndrome, which is characterized by fever, hypotension, disseminated intravascular coagulation, and multiple organ failure (Dumitru et al. Cell 103:1071-1083, 2000).

These toxic effects of LPS are mostly related to macrophage activation leading to the release of multiple inflammatory mediators. Among these mediators, TNF appears to play a crucial role, as indicated by the prevention of LPS toxicity by the administration of neutralizing anti-TNF antibodies (Beutler et al., Science 229:869, 1985). It is well established that 1ug injection of *E. coli* LPS into a C57Bl/6 mouse will result in significant increases in circulating IL-6, TNF-alpha, IL-1, and acute phase proteins (for example, SAA) approximately 2 hours post injection. The toxicity of LPS appears to be mediated by these cytokines as passive immunization against these mediators can result in decreased mortality (Beutler et al., Science 229:869, 1985). The potential immunointervention strategies for the prevention and/or treatment of septic shock include anti-TNF mAb, IL-1 receptor antagonist, LIF, IL-10, and G-CSF. Since LPS induces the production of pro- inflammatory factors possibly contributing to the pathology of endotoxemia, the neutralization of zcytor17lig activity, SAA or other pro-inflammatory factors by antagonizing zcytor17lig polypeptide can be used to reduce the symptoms of endotoxemia, such as seen in endotoxic shock. Other potential therapeutics include zcytor17 polypeptides, soluble heterodimeric and multimeric receptor polypeptides, or anti-zcytor17lig antibodies or binding partners of the present invention, and the like.

3 Inflammatory Bowel Disease. IBD

In the United States approximately 500,000 people suffer from Inflammatory Bowel Disease (IBD) which can affect either colon and rectum (Ulcerative colitis) or both, small and large intestine (Crohn's Disease). The pathogenesis of these diseases is unclear, but they involve chronic inflammation of the affected tissues. Potential therapeutics include zcytor17 polypeptides, soluble heterodimeric and multimeric receptor polypeptides, or anti-zcytor17lig antibodies or binding partners of the present invention, and the like., could serve as a valuable

therapeutic to reduce inflammation and pathological effects in IBD and related diseases.

Ulcerative colitis (UC) is an inflammatory disease of the large intestine, commonly called the colon, characterized by inflammation and ulceration of the mucosa or innermost lining of the colon. This inflammation causes the colon to empty frequently, resulting in diarrhea. Symptoms include loosening of the stool and associated abdominal cramping, fever and weight loss. Although the exact cause of UC is unknown, recent research suggests that the body's natural defenses are operating against proteins in the body which the body thinks are foreign (an "autoimmune reaction"). Perhaps because they resemble bacterial proteins in the gut, these proteins may either instigate or stimulate the inflammatory process that begins to destroy the lining of the colon. As the lining of the colon is destroyed, ulcers form releasing mucus, pus and blood. The disease usually begins in the rectal area and may eventually extend through the entire large bowel. Repeated episodes of inflammation lead to thickening of the wall of the intestine and rectum with scar tissue. Death of colon tissue or sepsis may occur with severe disease. The symptoms of ulcerative colitis vary in severity and their onset may be gradual or sudden. Attacks may be provoked by many factors, including respiratory infections or stress.

Although there is currently no cure for UC available, treatments are focused on suppressing the abnormal inflammatory process in the colon lining. Treatments including corticosteroids immunosuppressives (eg. azathioprine, mercaptopurine, and methotrexate) and aminosalicytates are available to treat the disease. However, the long-term use of immunosuppressives such as corticosteroids and azathioprine can result in serious side effects including thinning of bones, cataracts, infection, and liver and bone marrow effects. In the patients in whom current therapies are not successful, surgery is an option. The surgery involves the removal of the entire colon and the rectum.

There are several animal models that can partially mimic chronic ulcerative colitis. The most widely used model is the 2,4,6-trinitrobenesulfonic acid/ethanol (TNBS) induced colitis model, which induces chronic inflammation and ulceration in the colon. When TNBS is introduced into the colon of susceptible mice via intra-rectal instillation, it induces T-cell mediated immune response in the colonic

mucosa, in this case leading to a massive mucosal inflammation characterized by the dense infiltration of T-cells and macrophages throughout the entire wall of the large bowel. Moreover, this histopathologic picture is accompanies by the clinical picture of progressive weight loss (wasting), bloody diarrhea, rectal prolapse, and large bowel wall thickening (Neurath et al. Intern. Rev. Immunol. 19:51-62, 2000).

Another colitis model uses dextran sulfate sodium (DSS), which induces an acute colitis manifested by bloody diarrhea, weight loss, shortening of the colon and mucosal ulceration with neutrophil infiltration. DSS-induced colitis is characterized histologically by infiltration of inflammatory cells into the lamina propria, with lymphoid hyperplasia, focal crypt damage, and epithelial ulceration. These changes are thought to develop due to a toxic effect of DSS on the epithelium and by phagocytosis of lamina propria cells and production of TNF-alpha and IFN-gamma. Despite its common use, several issues regarding the mechanisms of DSS about the relevance to the human disease remain unresolved. DSS is regarded as a T cell-independent model because it is observed in T cell-deficient animals such as SCID mice.

The administration of anti-zcytor17lig antibodies or binding partners, soluble zcytor17 comprising polypeptides (including heterodimeric and multimeric receptors), such as zcytor17-Fc4 or other zcytor17 soluble and fusion proteins to these TNBS or DSS models can be used to evaluate the use of zcytor17lig antagonists to ameliorate symptoms and alter the course of gastrointestinal disease. Zcytor17lig may play a role in the inflammatory response in colitis, and the neutralization of zcytor17lig activity by administrating zcytor17lig antagonists is a potential therapeutic approach for IBD. Other potential therapeutics include zcytor17 polypeptides, soluble heterodimeric and multimeric receptor polypeptides, or anti-zcytor17lig antibodies or binding partners of the present invention, and the like.

4. Psoriasis

Psoriasis is a chronic skin condition that affects more than seven million Americans. Psoriasis occurs when new skin cells grow abnormally, resulting in inflamed, swollen, and scaly patches of skin where the old skin has not shed quickly enough. Plaque psoriasis, the most common form, is characterized by inflamed patches of skin ("lesions") topped with silvery white scales. Psoriasis may be limited to a few

plaques or involve moderate to extensive areas of skin, appearing most commonly on the scalp, knees, elbows and trunk. Although it is highly visible, psoriasis is not a contagious disease. The pathogenesis of the diseases involves chronic inflammation of the affected tissues. Zcytor17 polypeptides, soluble heterodimeric and multimeric receptor polypeptides, or anti-zcytor17lig antibodies or binding partners of the present invention, and the like, could serve as a valuable therapeutic to reduce inflammation and pathological effects in psoriasis, other inflammatory skin diseases, skin and mucosal allergies, and related diseases.

Psoriasis is a T-cell mediated inflammatory disorder of the skin that can cause considerable discomfort. It is a disease for which there is no cure and affects people of all ages. Psoriasis affects approximately two percent of the populations of European and North America. Although individuals with mild psoriasis can often control their disease with topical agents, more than one million patients worldwide require ultraviolet or systemic immunosuppressive therapy. Unfortunately, the inconvenience and risks of ultraviolet radiation and the toxicities of many therapies limit their long-term use. Moreover, patients usually have recurrence of psoriasis, and in some cases rebound, shortly after stopping immunosuppressive therapy.

Differentiation is a progressive and dynamic process, beginning with pluripotent stem cells and ending with terminally differentiated cells. Pluripotent stem cells that can regenerate without commitment to a lineage express a set of differentiation markers that are lost when commitment to a cell lineage is made. Progenitor cells express a set of differentiation markers that may or may not continue to be expressed as the cells progress down the cell lineage pathway toward maturation. Differentiation markers that are expressed exclusively by mature cells are usually functional properties such as cell products, enzymes to produce cell products, and receptors. The stage of a cell population's differentiation is monitored by identification of markers present in the cell population.

There is evidence to suggest that factors that stimulate specific cell types down a pathway towards terminal differentiation or dedifferentiation affect the entire cell population originating from a common precursor or stem cell. Thus, the present

invention includes stimulating or inhibiting the proliferation of lymphoid cells, hematopoietic cells and epithelial cells.

Zcytor17lig was isolated from tissue known to have important immunological function and which contain cells that play a role in the immune system. Zcytor17lig is expressed in CD3+ selected, activated peripheral blood cells, and it has been shown that zcytor17lig expression increases after T cell activation. Moreover, results of experiments described in the Examples section herein suggest that polypeptides of the present invention can have an effect on the growth/expansion of monocytes/macrophages, T-cells, B-cells, NK cells and/or differentiated state of monocytes/macrophages, T-cells, B-cells, NK cells or these cells' progenitors. Factors that both stimulate proliferation of hematopoietic progenitors and activate mature cells are generally known, however, proliferation and activation can also require additional growth factors. For example, it has been shown that IL-7 and Steel Factor (c-kit ligand) were required for colony formation of NK progenitors. IL-15 + IL-2 in combination with IL-7 and Steel Factor was more effective (Mrózek et al., Blood 87:2632-2640, 1996). However, unidentified cytokines may be necessary for proliferation of specific subsets of NK cells and/or NK progenitors (Robertson et. al., Blood 76:2451-2438, 1990). Similarly, zcytor17lig may act alone or in concert or synergy with other cytokines to enhance growth, proliferation expansion and modification of differentiation of monocytes/macrophages, T-cells, B-cells or NK cells.

Assays measuring differentiation include, for example, measuring cell markers associated with stage-specific expression of a tissue, enzymatic activity, functional activity or morphological changes (Watt, FASEB, 5:281-284, 1991; Francis, Differentiation 57:63-75, 1994; Raes, Adv. Anim. Cell Biol. Technol. Bioprocesses, 161-171, 1989; all incorporated herein by reference). Alternatively, zcytor17lig polypeptide itself can serve as an additional cell-surface or secreted marker associated with stage-specific expression of a tissue. As such, direct measurement of zcytor17lig polypeptide, or its loss of expression in a tissue as it differentiates, can serve as a marker for differentiation of tissues.

Similarly, direct measurement of zcytor17lig polypeptide, or its loss of expression in a tissue can be determined in a tissue or in cells as they undergo tumor

progression. Increases in invasiveness and motility of cells, or the gain or loss of expression of zcytor17lig in a pre-cancerous or cancerous condition, in comparison to normal tissue, can serve as a diagnostic for transformation, invasion and metastasis in tumor progression. As such, knowledge of a tumor's stage of progression or metastasis will aid the physician in choosing the most proper therapy, or aggressiveness of treatment, for a given individual cancer patient. Methods of measuring gain and loss of expression (of either mRNA or protein) are well known in the art and described herein and can be applied to zcytor17lig expression. For example, appearance or disappearance of polypeptides that regulate cell motility can be used to aid diagnosis and prognosis of prostate cancer (Banyard, J. and Zetter, B.R., Cancer and Metast. Rev. 17:449-458, 1999). As an effector of cell motility, zcytor17lig gain or loss of expression may serve as a diagnostic for lymphoid, B-cell, epithelial, hematopoietic and other cancers.

Moreover, the activity and effect of zcytor17lig on tumor progression and metastasis can be measured *in vivo*. Several syngeneic mouse models have been developed to study the influence of polypeptides, compounds or other treatments on tumor progression. In these models, tumor cells passaged in culture are implanted into mice of the same strain as the tumor donor. The cells will develop into tumors having similar characteristics in the recipient mice; and metastasis will also occur in some of the models. Appropriate tumor models for our studies include the Lewis lung carcinoma (ATCC No. CRL-1642) and B16 melanoma (ATCC No. CRL-6323), amongst others. These are both commonly used tumor lines, syngeneic to the C57BL6/J mouse, that are readily cultured and manipulated *in vitro*. Tumors resulting from implantation of either of these cell lines are capable of metastasis to the lung in C57BL6/J mice. The Lewis lung carcinoma model has recently been used in mice to identify an inhibitor of angiogenesis (O'Reilly MS, et al. Cell 79: 315-328, 1994). C57BL6/J mice are treated with an experimental agent either through daily injection of recombinant protein, agonist or antagonist or a one time injection of recombinant adenovirus. Three days following this treatment, 10^5 to 10^6 cells are implanted under the dorsal skin. Alternatively, the cells themselves may be infected with recombinant adenovirus, such as one expressing zcytor17lig, before implantation so that the protein

is synthesized at the tumor site or intracellularly, rather than systemically. The mice normally develop visible tumors within 5 days. The tumors are allowed to grow for a period of up to 3 weeks, during which time they may reach a size of 1500 - 1800 mm^3 in the control treated group. Tumor size and body weight are carefully monitored throughout the experiment. At the time of sacrifice, the tumor is removed and weighed along with the lungs and the liver. The lung weight has been shown to correlate well with metastatic tumor burden. As an additional measure, lung surface metastases are counted. The resected tumor, lungs and liver are prepared for histopathological examination, immunohistochemistry, and *in situ* hybridization, using methods known in the art and described herein. The influence of the expressed polypeptide in question, e.g., zcytor17lig, on the ability of the tumor to recruit vasculature and undergo metastasis can thus be assessed. In addition, aside from using adenovirus, the implanted cells can be transiently transfected with zcytor17lig. Use of stable zcytor17lig transfectants as well as use of induceable promoters to activate zcytor17lig expression *in vivo* are known in the art and can be used in this system to assess zcytor17lig induction of metastasis. Moreover, purified zcytor17lig or zcytor17lig conditioned media can be directly injected in to this mouse model, and hence be used in this system. For general reference see, O'Reilly MS, et al. Cell 79:315-328, 1994; and Rusciano D, et al. Murine Models of Liver Metastasis. Invasion Metastasis 14:349-361, 1995.

Zcytor17lig or antibodies thereto will be useful in treating tumorgenesis, and therefore would be useful in the treatment of cancer. Zcytor17lig is expressed in activated T-cells, monocytes and macrophages, and is linked to a region of the human chromosome wherein translocations are common in leukemias. Moreover, the zcytor17lig is shown to act through a cytokine receptor, zcytor17, which is also expressed in activated T-cells, monocytes and macrophages. Over stimulation of activated T-cells, monocytes and macrophages by zcytor17lig could result in a human disease state such as, for instance, an immune cell cancer or other cancers. As such, identifying zcytor17lig expression, polypeptides (e.g., by anti-zcytor17lig antibodies, zcytor17 soluble receptors (e.g., zcytor17 receptor, heterodimers (e.g., zcytor17/OSMRbeta, zcytor17/WSX-1), multimers (e.g., zcytor17/OSMRbeta/WSX-

1)), or other zcytor17lig binding partners) can serve as a diagnostic, and can serve as antagonists of zcytor17lig proliferative activity. The ligand could be administered in combination with other agents already in use including both conventional chemotherapeutic agents as well as immune modulators such as interferon alpha. Alpha/beta interferons have been shown to be effective in treating some leukemias and animal disease models, and the growth inhibitory effects of interferon-alpha and zcytor17lig may be additive.

NK cells are thought to play a major role in elimination of metastatic tumor cells and patients with both metastases and solid tumors have decreased levels of NK cell activity (Whiteside et. al., Curr. Top. Microbiol. Immunol. 230:221-244, 1998). An agent that stimulates NK cells would be useful in the elimination of tumors.

The present invention provides a method of reducing proliferation of a neoplastic monocytes/macrophages comprising administering to a mammal with a monocyte/macrophage neoplasm an amount of a composition of zcytor17lig or anti-zcytor17lig sufficient to reduce proliferation of the neoplastic monocytes/macrophages. In other embodiments, the composition can comprise at least one other cytokine. A second cytokine may be selected from the group consisting of IL-2, IL-3, IL-12, IL-21, IL-22, IL-15, IL-4, GM-CSF, Flt3 ligand or stem cell factor.

The present invention provides a method for inhibiting activation or differentiation of monocytes/macrophages. Monocytes are incompletely differentiated cells that migrate to various tissues where they mature and become macrophages. Macrophages play a central role in the immune response by presenting antigen to lymphocytes and play a supportive role as accessory cells to lymphocytes by secreting numerous cytokines. Macrophages can internalize extracellular molecules and upon activation have an increased ability to kill intracellular microorganisms and tumor cells. Activated macrophages are also involved in stimulating acute or local inflammation.

In another aspect, the present invention provides a method of reducing proliferation of a neoplastic B or T-cells comprising administering to a mammal with a B or T cell neoplasm an amount of a composition of zcytor17lig antagonist sufficient to reducing proliferation of the neoplastic monocytes/macrophages. In other embodiments, the composition can comprise at least one other cytokine, wherein the

cytokine may be selected from the group consisting of IL-2, IL-3, IL-12, IL-21, IL-22, IL-15, IL-4, GM-CSF, Flt3 ligand or stem cell factor. Furthermore, the zcytor17lig antagonist can be a ligand/toxin fusion protein.

A zcytor17lig-saporin fusion toxin may be employed against a similar set of leukemias and lymphomas, extending the range of leukemias that can be treated with zcytor17lig. For example, such leukemias can be those that over-express zcytor17 receptors (e.g., zcytor17 receptor, heterodimers (e.g., zcytor17/OSMRbeta, zcytor17/WSX-1), multimers (e.g., zcytor17/OSMRbeta/WSX)). Fusion toxin mediated activation of the zcytor17 receptor, zcytor17 receptor heterodimers or multimers (e.g., zcytor19/OSMRbeta, zcytor17/WSX-1 or zcytor19/WSX-1/OSMR) provides two independent means to inhibit the growth of the target cells, the first being identical to the effects seen by the ligand alone, and the second due to delivery of the toxin through receptor internalization. The lymphoid and monocyte restricted expression pattern of the zcytor17 receptor suggests that the ligand-saporin conjugate can be tolerated by patients.

When treatment for malignancies includes allogeneic bone marrow or stem cell transplantation, zcytor17lig may be valuable in enhancement of the graft-vs-tumor effect. Zcytor17lig may stimulate the generation of lytic NK cells from marrow progenitors and can stimulate the proliferation of monocytes and macrophages following activation of the antigen receptors. Therefore, when patients receive allogeneic marrow transplants, zcytor17lig will enhance the generation of anti-tumor responses, with or without the infusion of donor lymphocytes.

The tissue distribution of receptors for a given cytokine offers a strong indication of the potential sites of action of that cytokine. Expression of zcytor17 was seen in monocytes and B-cells, with a dramatic increase of expression upon activation for CD3+, CD4+, and CD8+ T-cells. In addition, two monocytic cell lines, THP-1 (Tsuchiya et al., Int. J. Cancer 26:171-176, 1980) and U937 (Sundstrom et al., Int. J. Cancer 17:565-577, 1976), were also positive for zcytor17 expression.

Northern analysis of WSX-1 receptor revealed transcripts in all tissues examined, with increased levels of expression in human spleen, thymus, lymph node,

bone marrow, and peripheral blood leukocytes. Also, expression levels of WSX-1 increased upon activation of T-cells.

Expression of OSMR is reported to be very broad (Mosley et al, JBC 271:32635-32643, 1996). This distribution of zcytor17, WSX-1, and OSM receptors supports a role for zcytor17lig in immune responses, especially expansion of T-cells upon activation or a role in the monocyte/macrophage arm of the immune system.

Thus, particular embodiments of the present invention are directed toward use of soluble zcytor17/WSX-1/OSMR, and zcytor17/OSMR heterodimers as antagonists in inflammatory and immune diseases or conditions such as pancreatitis, type I diabetes (IDDM), pancreatic cancer, pancreatitis, Graves Disease, inflammatory bowel disease (IBD), Crohn's Disease, colon and intestinal cancer, diverticulosis, autoimmune disease, sepsis, organ or bone marrow transplant; inflammation due to trauma, surgery or infection; amyloidosis; splenomegaly; graft versus host disease; and where inhibition of inflammation, immune suppression, reduction of proliferation of hematopoietic, immune, inflammatory or lymphoid cells, macrophages, T-cells (including Th1 and Th2 cells, CD4+ and CD8+ cells), suppression of immune response to a pathogen or antigen. Moreover the presence of zcytor17 expression in activated immune cells such as activated CD4+ and CD19+ cells showed that zcytor17 receptor may be involved in the body's immune defensive reactions against foreign invaders: such as microorganisms and cell debris, and could play a role in immune responses during inflammation and cancer formation. As such, antibodies and binding partners of the present invention that are agonistic or antagonistic to zcytor17 receptor function, such as zcytor17lig, can be used to modify immune response and inflammation.

The zcytor17lig structure and tissue expression suggests a role in early hematopoietic or thymocyte development and immune response regulation or inflammation. These processes involve stimulation of cell proliferation and differentiation in response to the binding of one or more cytokines to their cognate receptors. In view of the tissue distribution observed for this zcytor17lig, agonists (including the natural receptor(s)) and antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as zcytor17lig agonists are useful for stimulating proliferation and development of target cells *in vitro* and *in vivo*. For

example, agonist compounds, zcytor17lig, or anti-zcytor17lig antibodies, are useful as components of defined cell culture media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Agonists are thus useful in specifically promoting the growth and/or development or activation of monocytes, T-cells, B-cells, and other cells of the lymphoid and myeloid lineages, and hematopoietic cells in culture.

Zcytor17lig may be useful in stimulating cell-mediated immunity and for stimulating lymphocyte proliferation, such as in the treatment of infections involving immunosuppression, including certain viral infections. Additional uses include tumor suppression, where malignant transformation results in tumor cells that are antigenic. zcytor17lig could be used to induce cytotoxicity, which may be mediated through activation of effector cells such as T-cells, NK (natural killer) cells, or LAK (lymphoid activated killer) cells, or induced directly through apoptotic pathways. Zcytor17lig may also be useful in treating leukopenias by increasing the levels of the affected cell type, and for enhancing the regeneration of the T-cell repertoire after bone marrow transplantation; or for enhancing monocyte proliferation or activation, and for diagnostic and other uses described herein.

Zcytor17lig may find utility in the suppression of the immune system, such as in the treatment of autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, diabetes mellitis, inflammatory bowel disease, Crohn's disease, etc. Immune suppression can also be used to reduce rejection of tissue or organ transplants and grafts and to treat T-cell, B-cell or monocyte-specific leukemias or lymphomas, and other cancers, by inhibiting proliferation of the affected cell type. Moreover zcytor17lig can be used to detect monocytes, macrophages, and activated T-cells and aid in the diagnosis of such autoimmuine disease, particularly in disease states where monocytes are elevated or activated.

Zcytor17lig polypeptides, peptides, antibodies, and the like may also be used within diagnostic systems for the detection of circulating levels of zcytor17lig. Within a related embodiment, antibodies or other agents that specifically bind to zcytor17lig polypeptides can be used to detect circulating zcytor17lig polypeptides. Elevated or depressed levels of ligand polypeptides may be indicative of pathological

conditions, including cancer. Zcytor17lig polypeptides may contribute to pathologic processes and can be an indirect marker of an underlying disease.

Also, the zcytor17lig can be used to detect or target its receptor(s) in certain disease states. For example, elevated levels of soluble IL-2 receptor in human serum have been associated with a wide variety of inflammatory and neoplastic conditions, such as myocardial infarction, asthma, myasthenia gravis, rheumatoid arthritis, acute T-cell leukemia, B-cell lymphomas, chronic lymphocytic leukemia, colon cancer, breast cancer, and ovarian cancer (Heaney et al., Blood 87:847-857, 1996). Similarly, zcytor17 receptor is elevated in activated monocytes, and hence zcytor17 receptor and/or its soluble receptors may be associated with or serve as a marker for inflammatory and neoplastic conditions associated therewith. The zcytor17lig, including cytotoxic conjugates, hence can be used to detect or target such tissues, and disease states.

The molecules of the present invention have particular use in the monocyte/macrophage arm of the immune system. Methods are known that can assess such activity. For example, interferon gamma (IFNγ) is a potent activator of mononuclear phagocytes. For example, an increase in expression of zcytor17 upon activation of THP-1 cells (ATCC No. TIB-202) with interferon gamma could suggest that this receptor is involved in monocyte activation. Monocytes are incompletely differentiated cells that migrate to various tissues where they mature and become macrophages. Macrophages play a central role in the immune response by presenting antigen to lymphocytes and play a supportive role as accessory cells to lymphocytes by secreting numerous cytokines. Macrophages can internalize extracellular molecules and upon activation have an increased ability to kill intracellular microorganisms and tumor cells. Activated macrophages are also involved in stimulating acute or local inflammation. Moreover, monocyte-macrophage function has been shown to be abnormal in a variety of diseased states. For example see, Johnston, RB, New Eng. J. Med. 318:747-752, 1998.

One of skill in the art would recognize that agonists of zcytor17 receptor, such as zcytor17lig, are useful. For example, depressed migration of monocytes has been reported in populations with a predisposition to infection, such as newborn

infants, patients receiving corticosteroid or other immunosuppressive therapy, and patients with diabetes mellitus, burns, or AIDS. Agonists for zcytor17, such as zcytor17lig, could result in an increase in the ability of monocytes to migrate and possibly prevent infection in these populations. There is also a profound defect of phagocytic killing by mononuclear phagocytes from patients with chronic granulomatous disease. This results in the formation of subcutaneous abscesses, as well as abscesses in the liver, lungs, spleen, and lymph nodes. An agonist of zcytor17 receptor such as zcytor17lig, could correct or improve this phagocytic defect. In addition, defective monocyte cytotoxicity has been reported in patients with cancer and Wiskott-Aldrich syndrome (eczema, thrombocytopenia, and recurrent infections). Activation of monocytes by agonists of zcytor17 receptor such as zcytor17lig, could aid in treatment of these conditions. The monocyte-macrophage system is prominently involved in several lipid-storage diseases (sphingolipidoses) such as Gaucher's disease. Resistance to infection can be impaired because of a defect in macrophage function, which could be treated by agonists to zcytor17 receptor such as zcytor17lig.

Moreover, one of skill in the art would recognize that antagonists of zcytor17lig are useful. For example, in atherosclerotic lesions, one of the first abnormalities is localization of monocyte/macrophages to endothelial cells. These lesions could be prevented by use of antagonists to zcytor17lig. Anti-zcytor17lig antibodies (e.g., zcytor17lig neutralizing antibody), zcytor17 soluble receptors, heterodimers and multimers, and zcytor17lig binding partners can also be used as antagonists to the zcytor17lig. Moreover, monoblastic leukemia is associated with a variety of clinical abnormalities that reflect the release of the biologic products of the macrophage, examples include high levels of lysozyme in the serum and urine and high fevers. Moreover, such leukemias exhibit an abnormal increase of monocytic cells. These effects could possibly be prevented by antagonists to zcytor17lig, such as described herein. Moreover, anti- zcytor17lig can be conjugated to molecules such as toxic moieties and cytokines, as described herein to direct the killing of leukemia monocytic cells.

Using methods known in the art, and disclosed herein, one of skill could readily assess the activity of zcytor17lig agonists and antagonists in the disease states

disclosed herein, inflammation, immune (e.g., autoimmune), cancer, or infection as well as other disease states involving monocytic cells. In addition, as zcytor17lig is expressed in a T-cell, macrophage and monocyte-specific manner, and these diseases involve abnormalities in monocytic cells, such as cell proliferation, function, localization, and activation, the polynucleotides, polypeptides, and antibodies of the present invention can be used to as diagnostics to detect such monocytic cell abnormalities, and indicate the presence of disease. Such methods involve taking a biological sample from a patient, such as blood, saliva, or biopsy, and comparing it to a normal control sample. Histological, cytological, flow cytometric, biochemical and other methods can be used to determine the relative levels or localization of zcytor17lig, or cells expressing zcytor17lig, i.e., monocytes, in the patient sample compared to the normal control. A change in the level (increase or decrease) of zcytor17lig expression, or a change in number or localization of monocytes (e.g., increase or infiltration of monocytic cells in tissues where they are not normally present) compared to a control would be indicative of disease. Such diagnostic methods can also include using radiometric, fluorescent, and colorimetric tags attached to polynucleotides, polypeptides or antibodies of the present invention. Such methods are well known in the art and disclosed herein.

Amino acid sequences having zcytor17lig activity can be used to modulate the immune system by binding zcytor17 receptor, and thus, preventing the binding of zcytor17lig with endogenous zcytor17lig receptor. Zcytor17lig antagonists, such as anti- zcytor17lig antibodies, can also be used to modulate the immune system by inhibiting the binding of Zcytor17lig with the endogenous zcytor17lig receptor. Accordingly, the present invention includes the use of proteins, polypeptides, and peptides having zcytor17lig activity (such as zcytor17lig polypeptides, zcytor17lig analogs (*e.g.*, anti- zcytor17lig anti-idiotype antibodies), and zcytor17lig fusion proteins) to a subject which lacks an adequate amount of this polypeptide, or which produces an excess of zcytor17 comprising receptor(s). Zcytor17 antagonists (*e.g.*, anti-Zcytor17 antibodies) can be also used to treat a subject which produces an excess of either zcytor17lig or Zcytor17 comprising receptor(s). Suitable subjects include mammals, such as humans.

Zcytor17lig has been shown to be expressed in activated mononuclear cells, and may be involved in regulating inflammation. As such, polypeptides of the present invention can be assayed and used for their ability to modify inflammation, or can be used as a marker for inflammation. Methods to determine proinflammatory and antiinflammatory qualities of zcytor17lig are known in the art and discussed herein. Moreover, it may be involved in up-regulating the production of acute phase reactants, such as serum amyloid A (SAA), α1-antichymotrypsin, and haptoglobin, and that expression of zcytor17 receptor ligand may be increased upon injection of lipopolysaccharide (LPS) *in vivo* that are involved in inflammatory response (Dumoutier, L. et al., Proc. Nat'l. Acad. Sci. 97:10144-10149, 2000). Production of acute phase proteins, such as SAA, is considered s short-term survival mechanism where inflammation is beneficial; however, maintenance of acute phase proteins for longer periods contributes to chronic inflammation and can be harmful to human health. For review, see Uhlar, CM and Whitehead, AS, Eur. J. Biochem. 265:501-523, 1999, and Baumann H. and Gauldie, J. Immunology Today 15:74-80, 1994. Moreover, the acute phase protein SAA is implicated in the pathogenesis of several chronic inflammatory diseases, is implicated in atherosclerosis and rheumatoid arthritis, and is the precursor to the amyloid A protein deposited in amyloidosis (Uhlar, CM and Whitehead, supra.). Thus, where a ligand such as zcytor17lig that acts as a pro-inflammatory molecule and induces production of SAA, antagonists would be useful in treating inflammatory disease and other diseases associated with acute phase response proteins induced by the ligand. Such antagonists are provided by the present invention. For example, a method of reducing inflammation comprises administering to a mammal with inflammation an amount of a composition of zcytor17lig, or anti- zcytor17lig antibody (e.g., neutralizing antibody) that is sufficient to reduce inflammation. Moreover, a method of suppressing an inflammatory response in a mammal with inflammation can comprise: (1) determining a level of serum amyloid A protein; (2) administering a composition comprising a zcytor17lig polypeptide or anti- zcytor17lig antibody as described herein in an acceptable pharmaceutical carrier; (3) determining a post administration level of serum amyloid A protein; (4) comparing the level of serum amyloid A protein in step (1) to the level of serum amyloid A protein in step (3),

wherein a lack of increase or a decrease in serum amyloid A protein level is indicative of suppressing an inflammatory response.

The receptors that bind zcytor17lig of the present invention include at least one zcytor17 receptor subunit. A second receptor polypeptide included in the heterodimeric soluble receptor belongs to the receptor subfamily that includes class I cytokine receptor subunits, and more specifically OSMRbeta and WSX-1. According to the present invention, in addition to a monomeric or homodimeric zcytor17 receptor polypeptide, a heterodimeric soluble zcytor17 receptor, as exemplified by an embodiment comprising a soluble zcytor17 receptor + soluble Class I receptor heterodimeric component, such as OSMRbeta or WSX-1, can act as an antagonist of the zcytor17lig. Other embodiments include soluble multimeric receptors comprising zcytor17, such as zcytor17 receptor + soluble Class I receptor multimeric component, such as OSMRbeta and WSX-1.

Like zcytor17lig, analysis of the tissue distribution of the mRNA corresponding it's zcytor17 receptor cDNA showed that mRNA level was highest in monocytes and prostate cells, and is elevated in activated monocytes, and activated CD4+, activated CD8+, and activated CD3+ cells. Hence, zcytor17 receptor is also implicated in inducing inflammatory and immune response. Thus, particular embodiments of the present invention are directed toward use of zcytor17lig-antibodies, and zcytor17lig, as well as soluble zcytor17 receptor heterodimers as antagonists in inflammatory and immune diseases or conditions such as, pancreatitis, type I diabetes (IDDM), pancreatic cancer, pancreatitis, Graves Disease, inflammatory bowel disease (IBD), Crohn's Disease, colon and intestinal cancer, diverticulosis, autoimmune disease, sepsis, organ or bone marrow transplant; inflammation due to trauma, sugery or infection; amyloidosis; splenomegaly; graft versus host disease; and where inhibition of inflammation, immune suppression, reduction of proliferation of hematopoietic, immune, inflammatory or lymphoid cells, macrophages, T-cells (including Th1 and Th2 cells, CD4+ and CD8+ cells), suppression of immune response to a pathogen or antigen. Moreover the presence of zcytor17 receptor and zcytor17lig expression in activated immune cells such as activated CD3+, monocytes, CD4+ and CD19+ cells showed that zcytor17 receptor may be involved in the body's immune defensive

reactions against foreign invaders: such as microorganisms and cell debris, and could play a role in immune responses during inflammation and cancer formation. As such, zcytor17lig and zcytor17lig-antibodies of the present invention that are agonistic or antagonistic to zcytor17 receptor function, can be used to modify immune response and inflammation.

Moreover, zcytor17lig polypeptides that bind zcytor17 receptor polypeptides, and antibodies thereto are useful to:

1) Antagonize or block signaling via zcytor17-comprising receptors in the treatment of acute inflammation, inflammation as a result of trauma, tissue injury, surgery, sepsis or infection, and chronic inflammatory diseases such as asthma, inflammatory bowel disease (IBD), chronic colitis, splenomegaly, rheumatoid arthritis, recurrent acute inflammatory episodes (e.g., tuberculosis), and treatment of amyloidosis, and atherosclerosis, Castleman's Disease, asthma, and other diseases associated with the induction of acute-phase response.

2) Antagonize or block signaling via the zcytor17 receptor receptors in the treatment of autoimmune diseases such as IDDM, multiple sclerosis (MS), systemic Lupus erythematosus (SLE), myasthenia gravis, rheumatoid arthritis, and IBD to prevent or inhibit signaling in immune cells (e.g. lymphocytes, monocytes, leukocytes) via zcytor17 receptor (Hughes C et al., J. Immunol 153: 3319-3325, 1994). Alternatively antibodies, such as monoclonal antibodies (MAb) to zcytor17lig, can also be used as an antagonist to deplete unwanted immune cells to treat autoimmune disease. Asthma, allergy and other atopic disease may be treated with an MAb against, for example, anti-zcytor17lig antibodies, soluble zcytor17 receptor soluble receptors or zcytor17/CRF2-4 heterodimers, to inhibit the immune response or to deplete offending cells. Blocking or inhibiting signaling via zcytor17, using the polypeptides and antibodies of the present invention, may also benefit diseases of the pancreas, kidney, pituitary and neuronal cells. IDDM, NIDDM, pancreatitis, and pancreatic carcinoma may benefit. Zcytor17 may serve as a target for MAb therapy of cancer where an antagonizing MAb inhibits cancer growth and targets immune-mediated killing. (Holliger P, and Hoogenboom, H: Nature Biotech. 16: 1015-1016, 1998). Mabs to soluble zcytor17 receptor monomers, homodimers, heterodimers and multimers may

also be useful to treat nephropathies such as glomerulosclerosis, membranous neuropathy, amyloidosis (which also affects the kidney among other tissues), renal arteriosclerosis, glomerulonephritis of various origins, fibroproliferative diseases of the kidney, as well as kidney dysfunction associated with SLE, IDDM, type II diabetes (NIDDM), renal tumors and other diseases.

3) Agonize or initiate signaling via the zcytor17 receptors in the treatment of autoimmune diseases such as IDDM, MS, SLE, myasthenia gravis, rheumatoid arthritis, and IBD. zcytor17lig may signal lymphocytes or other immune cells to differentiate, alter proliferation, or change production of cytokines or cell surface proteins that ameliorate autoimmunity. Specifically, modulation of a T-helper cell response to an alternate pattern of cytokine secretion may deviate an autoimmune response to ameliorate disease (Smith JA et al., J. Immunol. 160:4841-4849, 1998). Similarly, zcytor17lig may be used to signal, deplete and deviate immune cells involved in asthma, allergy and atopoic disease. Signaling via zcytor17 receptor may also benefit diseases of the pancreas, kidney, pituitary and neuronal cells. IDDM, NIDDM, pancreatitis, and pancreatic carcinoma may benefit. Zcytor17 may serve as a target for MAb therapy of pancreatic cancer where a signaling MAb inhibits cancer growth and targets immune-mediated killing (Tutt, AL et al., J Immunol. 161: 3175-3185, 1998). Similarly T-cell specific leukemias, lymphomas, plasma cell dyscrasia (e.g., multiple myeloma), and carcinoma may be treated with monoclonal antibodies (e.g., neutralizing antibody) to zcytor17-comprising soluble receptors of the present invention.

Anti-zcytor17lig antibodies, soluble zcytor17 receptor monomeric, homodimeric, heterodimeric and multimeric polypeptides described herein can be used to neutralize/block zcytor17 receptor ligand activity in the treatment of autoimmune disease, atopic disease, NIDDM, pancreatitis and kidney dysfunction as described above. A soluble form of zcytor17 receptor may be used to promote an antibody response mediated by T cells and/or to promote the production of IL-4 or other cytokines by lymphocytes or other immune cells.

Anti-zcytor17lig antibodies, and soluble zcytor17-comprising receptors are useful as antagonists of zcytor17lig. Such antagonistic effects can be achieved by direct neutralization or binding of its natural ligand. In addition to antagonistic uses,

the soluble receptors can bind zcytor17lig and act as carrier or carrier proteins, in order to transport zcytor17lig to different tissues, organs, and cells within the body. As such, the soluble receptors can be fused or coupled to molecules, polypeptides or chemical moieties that direct the soluble-receptor-Ligand complex to a specific site, such as a tissue, specific immune cell, monocytes, or tumor. For example, in acute infection or some cancers, benefit may result from induction of inflammation and local acute phase response proteins. Thus, the soluble receptors described herein or antibodies of the present invention can be used to specifically direct the action of a pro-inflammatory zcytor17lig ligand. See, Cosman, D. Cytokine 5: 95-106, 1993; and Fernandez-Botran, R. Exp. Opin. Invest. Drugs 9:497-513, 2000.

Moreover, the soluble receptors can be used to stabilize the zcytor17lig, to increase the bioavailability, therapeutic longevity, and/or efficacy of the Ligand by stabilizing the Ligand from degradation or clearance, or by targeting the ligand to a site of action within the body. For example the naturally occurring IL-6/soluble IL-6R complex stabilizes IL-6 and can signal through the gp130 receptor. See, Cosman, D. supra., and Fernandez-Botran, R. supra.. Moreover, Zcytor17 may be combined with a cognate ligand such as its ligand to comprise a ligand/soluble receptor complex. Such complexes may be used to stimulate responses from cells presenting a companion receptor subunit. The cell specificity of zcytor17 receptor/zcytor17lig complexes may differ from that seen for the ligand administered alone. Furthermore the complexes may have distinct pharmacokinetic properties such as affecting half-life, dose/response and organ or tissue specificity. Zcytor17/ligand complexes thus may have agonist activity to enhance an immune response or stimulate mesangial cells or to stimulate hepatic cells. Alternatively only tissues expressing a signaling subunit the heterodimerizes with the complex may be affected analogous to the response to IL6/IL6R complexes (Hirota H. et al., Proc. Nat'l. Acad. Sci. 92:4862-4866, 1995; Hirano, T. in Thomason, A. (Ed.) "*The Cytokine Handbook*", 3rd Ed., p. 208-209). Soluble receptor/cytokine complexes for IL12 and CNTF display similar activities.

Zcytor17lig may also be used within diagnostic systems for the detection of circulating levels of ligand, and in the detection of acute phase inflammatory response. Within a related embodiment, antibodies or other agents that specifically

bind to zcytor17lig can be used to detect circulating zcytor17lig polypeptides; conversely, zcytor17lig itself can be used to detect circulating or locally-acting receptor polypeptides. Elevated or depressed levels of ligand or receptor polypeptides may be indicative of pathological conditions, including inflammation or cancer. Moreover, detection of acute phase proteins or molecules such as zcytor17lig can be indicative of a chronic inflammatory condition in certain disease states (e.g., rheumatoid arthritis). Detection of such conditions serves to aid in disease diagnosis as well as help a physician in choosing proper therapy.

The polypeptides and proteins of the present invention can also be used *ex vivo*, such as in autologous marrow culture. Briefly, bone marrow is removed from a patient prior to chemotherapy or organ transplant and treated with zcytor17lig, optionally in combination with one or more other cytokines. The treated marrow is then returned to the patient after chemotherapy to speed the recovery of the marrow or after transplant to suppress graft vs. host disease. In addition, the proteins of the present invention can also be used for the *ex vivo* expansion of monocytes/macrophages marrow or peripheral blood progenitor (PBPC) cells. Prior to treatment, marrow can be stimulated with stem cell factor (SCF) to release early progenitor cells into peripheral circulation. These progenitors can be collected and concentrated from peripheral blood and then treated in culture with zcytor17lig, optionally in combination with one or more other cytokines, including but not limited to SCF, IL-2, IL-4, IL-7, Lif, IL-3, IL-12, IL-21, or IL-15, to differentiate and proliferate into high-density lymphoid cultures, which can then be returned to the patient following chemotherapy or transplantation.

The present invention provides a method for expansion of hematopoietic cells and hematopoietic cell progenitors comprising culturing bone marrow or peripheral blood cells with a composition comprising an amount of zcytor17lig sufficient to produce an increase in the number of lymphoid cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of zcytor17lig. In other embodiments, the hematopoietic cells and hematopoietic progenitor cells are lymphoid cells. In another embodiment, the lymphoid cells are NK cells or cytotoxic T cells. Furthermore, the composition can

also comprise at least one other cytokine selected from the group consisting of IL-2, IL-15, IL-4, Lif, IL-3, IL-12, IL-21, GM-CSF, Flt3 ligand and stem cell factor.

Alternatively, zcytor17lig may activate the immune system which would be important in boosting immunity to infectious diseases, treating immunocompromised patients, such as HIV+ patients, cancer patients, or in improving vaccines. In particular, zcytor17lig stimulation or expansion of monocytes/macrophages, T-cells, B-cells, NK cells, or their progenitors, would provide therapeutic value in treatment of viral infection, and as an anti-neoplastic factor. Similarly, zcytor17lig stimulation of the immune response against viral and non-viral pathogenic agents (including bacteria, protozoa, and fungi) would provide therapeutic value in treatment of such infections by inhibiting the growth of such infections agents. Determining directly or indirectly the levels of a pathogen or antigen, such as a tumor cell, present in the body can be achieved by a number of methods known in the art and described herein.

The present invention include a method of stimulating an immune response in a mammal exposed to an antigen or pathogen comprising the steps of: (1) determining directly or indirectly the level of antigen or pathogen present in said mammal; (2) administering a composition comprising zcytor17lig polypeptide in an acceptable pharmaceutical carrier; (3) determining directly or indirectly the level of antigen or pathogen in said mammal; and (4) comparing the level of the antigen or pathogen in step 1 to the antigen or pathogen level in step 3, wherein a change in the level is indicative of stimulating an immune response. In another embodiment the zcytor17lig composition is re-administered. In other embodiments, the antigen is a B cell tumor; a virus; a parasite or a bacterium.

In another aspect, the present invention provides a method of stimulating an immune response in a mammal exposed to an antigen or pathogen comprising: (1) determining a level of an antigen- or pathogen-specific antibody; (2) administering a composition comprising zcytor17lig polypeptide in an acceptable pharmaceutical carrier; (3) determining a post administration level of antigen- or pathogen-specific antibody; (4) comparing the level of antibody in step (1) to the level of antibody in step

(3), wherein an increase in antibody level is indicative of stimulating an immune response.

Polynucleotides encoding zcytor17lig polypeptides are useful within gene therapy applications where it is desired to increase or inhibit zcytor17lig activity. If a mammal has a mutated or absent zcytor17lig gene, the zcytor17lig gene can be introduced into the cells of the mammal. In one embodiment, a gene encoding a zcytor17lig polypeptide is introduced *in vivo* in a viral vector. Such vectors include an attenuated or defective DNA virus, such as, but not limited to, herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. A defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Examples of particular vectors include, but are not limited to, a defective herpes simplex virus 1 (HSV1) vector (Kaplitt et al., Molec. Cell. Neurosci. 2:320-30, 1991); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., J. Clin. Invest. 90:626-30, 1992; and a defective adeno-associated virus vector (Samulski et al., J. Virol. 61:3096-101, 1987; Samulski et al., J. Virol. 63:3822-8, 1989).

A zcytor17lig gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent No. 5,399,346; Mann et al. Cell 33:153, 1983; Temin et al., U.S. Patent No. 4,650,764; Temin et al., U.S. Patent No. 4,980,289; Markowitz et al., J. Virol. 62:1120, 1988; Temin et al., U.S. Patent No. 5,124,263; International Patent Publication No. WO 95/07358, published March 16, 1995 by Dougherty et al.; and Kuo et al., Blood 82:845, 1993. Alternatively, the vector can be introduced by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7, 1987; Mackey et al., Proc. Natl. Acad. Sci. USA 85:8027-31, 1988). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. More particularly, directing transfection to particular cells represents one area of benefit. For instance, directing

transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the immune system, pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

It is possible to remove the target cells from the body; to introduce the vector as a naked DNA plasmid; and then to re-implant the transformed cells into the body. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter. See, e.g., Wu et al., J. Biol. Chem. 267:963-7, 1992; Wu et al., J. Biol. Chem. 263:14621-4, 1988.

Antisense methodology can be used to inhibit zcytor17lig gene transcription, such as to inhibit cell proliferation *in vivo*. Polynucleotides that are complementary to a segment of a zcytor17lig-encoding polynucleotide (e.g., a polynucleotide as set forth in SEQ ID NO:1) are designed to bind to zcytor17lig-encoding mRNA and to inhibit translation of such mRNA. Such antisense polynucleotides are used to inhibit expression of zcytor17lig polypeptide-encoding genes in cell culture or in a subject.

Mice engineered to express the zcytor17lig gene, referred to as "transgenic mice," and mice that exhibit a complete absence of zcytor17lig gene function, referred to as "knockout mice," may also be generated (Snouwaert et al., Science 257:1083, 1992; Lowell et al., Nature 366:740-42, 1993; Capecchi, M.R., Science 244: 1288-1292, 1989; Palmiter, R.D. et al. Annu Rev Genet. 20: 465-499, 1986). For example, transgenic mice that over-express zcytor17lig, either ubiquitously or under a tissue-specific or tissue-restricted promoter can be used to ask whether over-expression causes a phenotype. For example, over-expression of a wild-type zcytor17lig polypeptide, polypeptide fragment or a mutant thereof may alter normal cellular processes, resulting in a phenotype that identifies a tissue in which zcytor17lig expression is functionally relevant and may indicate a therapeutic target for the zcytor17lig, its agonists or antagonists. For example, a preferred transgenic mouse to

engineer is one that over-expresses the zcytor17lig (amino acid residues 23-164 of SEQ ID NO:2; or 24-163 of SEQ ID NO:11). Moreover, such over-expression may result in a phenotype that shows similarity with human diseases. Similarly, knockout zcytor17lig mice can be used to determine where zcytor17lig is absolutely required *in vivo*. The phenotype of knockout mice is predictive of the *in vivo* effects of that a zcytor17lig antagonist, such as those described herein, may have. The human or mouse zcytor17lig cDNA described herein can be used to generate knockout mice. These mice may be employed to study the zcytor17lig gene and the protein encoded thereby in an *in vivo* system, and can be used as *in vivo* models for corresponding human diseases. Moreover, transgenic mice expression of zcytor17lig antisense polynucleotides or ribozymes directed against zcytor17lig, described herein, can be used analogously to transgenic mice described above. Studies may be carried out by administration of purified zcytor17lig protein, as well.

Experimental evidence suggests a role for zcytor17lig in the progression of diseases that involve the skin or epithelium of internal surfaces, such as, for instance, large intestine, small intestine, pancrease, lung, prostate, uterus, and the like. First, as disclosed herein, zcytor17 receptors, including both OSM receptor beta and zcytor17, are expressed in several cell types located in epithelial surfaces including cell lines derived from lung epithelium, lung fibroblast, prostate, colon, breast, liver epithelium, bone and skin epithelium, bone fibroblast, and the like. Moreover, as disclosed herein, examples from each of these cell types also responded to zcytor17lig activation of a STAT reporter construct. In addition, several cell lines responded to zcytor17lig stimulation by producing increased levels of IL-6, IL-8, MCP-1 (a chemotactic factor) as described herein. In whole, these data suggest a role for zcytor17lig in diseases that involve the epithelium such as, for instance, atopic dermatitis; dermatitis; psoriasis; psoriatic arthritis; eczema; gingivitis; peridontal disease; inflammatory bowel diseases (IBD) (e.g., ulcerative colitis, Crohn's disease); reproductive disorders, such as, for instance, cervical dysplasia, cervical cancer; other skin diseases like cancers: sarcomas; canrcinomas; melanoma, etc. i.e., not just inflammatory diseases, since immune system is involved in activating/curing cancers; diseases involving barrier dysfunction, such as, for instance, graft-versus-host disease (GVHD) and irritable bowel syndrome (IBS); and

diseases that involve lung epithelium, such as asthma, emphysema, and the like. In addition, the release of cytokines IL-6, IL-8, and MCP-1 by cells exposed to zcytor17lig suggests that zcytor17lig is involved in inflammation. Therefore, regulation of zcytor17lig can be useful in the treatment of autoimmune, inflammatory, or cancerous diseases associated with the tissues that express receptor. These diseases include, for example, prostatitis, hepatitis, osteoarthritis, and the like. Zcytor17lig may positively or negatively directly or indirectly regulate these diseases. Therefore, the administration of zcytor17lig can be used to treat diseases as described herein directly or with molecules that inhibit zcytor17lig activity including, for example, both monoclonal antibodies to zcytor17lig or monoclonal antibodies to zcytor17, or monoclonal antibodies that recognize the zcytor17 and OSM receptor beta complex.

Data also suggests that zcytor17lig may be involved in the regulation of TH2 T cell mediated diseases. First, zcytor17lig is made by the TH2 subset of activated T cells. TH2 cells express more zcytor17lig as compared to TH1 cells. In addition, at least two lung epithelial cell lines (SK-LU-1, A549) were stimulated to increase IL13 receptor alpha-2 mRNA in response to zcyto17 ligand stimulation as described herein. There is an association of IL-13 receptor alpha2 chain and tumorigenicity of human breast and pancreatic tumors. This suggests that zcytor17lig may play a role in regulating tumorigenicity of these types of cancers, as well as other cancers. Therefore, the administration of a zcytor17lig antagonist or direct use of zcytor17lig may be useful in treatment of these types of cancers, benign or malignant and at various grades (grades I-IV) and stages (e.g., TNM or AJC staging methods) of tumor development, in mammals, preferably humans.

It is well-known in the art that IL13 is involved in the generation of activated TH2 cells and in TH2 mediated diseases, such as asthma, atopic dermatitis, and the like. Zcytor17lig or zcytor17lig antagonists may be useful in the treatment of diseases that involved TH2 T cells. This would include diseases such as, for instance, atopic dermatitis, asthma, as well as other diseases that are exacerbated by activated TH2 cells. The involvement of zcytor17lig in diseases, such as, for instance, atopic dermatitis, is also supported by the phenotype of the transgenic mice that overexpress zcytor17lig and develop symptoms of atopic dermatitis as described herein.

Despite the preferential expression of zcytor17lig by TH2 cells, there is still some expression of zcytor17lig in TH1 cells and in CD8+ T cells. Therefore, zcytor17lig or its antagonists may be useful in treating diseases that involve immune modulation of activated T cells including, for example, viral infection, cancers, graft rejection, and the like.

Zcytor17lig may also be involved in the development of cancer. There is expression of the zcytor17 and OSM receptor beta receptors in human bone fibroblast osteosarcomas, human skin fibroblast melanoma, colon epithelial carcinoma, adenocarcinoma, breast epithelial adenocarcinoma, prostate epithelial adenosarcoma, and lung epithelial adenocarcinoma and carcinoma. Therefore, it may be useful to treat tumors of epithelial origin with either zcytor17lig, fragments thereof, or zcytor17lig antagonists which include, but are not limited to, carcinoma, adenocarcinoma, and melanoma. Notwithstanding, zcytor17lig or a zcytor17lig antagonist may be used to treat a cancer, or reduce one or more symptoms of a cancer, from a cancer including but not limited to squamous cell or epidermoid carcinoma, basal cell carcinoma, adenocarcinoma, papillary carcinoma, cystadenocarcinoma, bronchogenic carcinoma, bronchial adenoma, melanoma, renal cell carcinoma, hepatocellular carcinoma, transitional cell carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, malignant mixed tumor of salivary gland origin, Wilms' tumor, immature teratoma, teratocarcinoma, and other tumors comprising at least some cells of epithelial origin.

Generally, the dosage of administered zcytor17lig polypeptide (or zcytor17 analog or fusion protein) will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of zcytor17lig polypeptide which is in the range of from about 1 pg/kg to 10 mg/kg (amount of agent/body weight of patient), although a lower or higher dosage also may be administered as circumstances dictate. One skilled in the art can readily determine such dosages, and adjustments thereto, using methods known in the art.

Administration of a Zcytor17lig polypeptide to a subject can be topical, inhalant, intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion through a regional catheter, or by direct

intralesional injection. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses.

Additional routes of administration include oral, mucosal-membrane, pulmonary, and transcutaneous. Oral delivery is suitable for polyester microspheres, zein microspheres, proteinoid microspheres, polycyanoacrylate microspheres, and lipid-based systems (see, for example, DiBase and Morrel, "Oral Delivery of Microencapsulated Proteins," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 255-288 (Plenum Press 1997)). The feasibility of an intranasal delivery is exemplified by such a mode of insulin administration (see, for example, Hinchcliffe and Illum, *Adv.* Drug Deliv. Rev. 35:199 (1999)). Dry or liquid particles comprising Zcytor17lig can be prepared and inhaled with the aid of dry-powder dispersers, liquid aerosol generators, or nebulizers (*e.g.*, Pettit and Gombotz, *TIBTECH 16*:343 (1998); Patton *et al.*, Adv. Drug Deliv. Rev. 35:235 (1999)). This approach is illustrated by the AERX diabetes management system, which is a hand-held electronic inhaler that delivers aerosolized insulin into the lungs. Studies have shown that proteins as large as 48,000 kDa have been delivered across skin at therapeutic concentrations with the aid of low-frequency ultrasound, which illustrates the feasibility of trascutaneous administration (Mitragotri *et al.*, Science 269:850 (1995)). Transdermal delivery using electroporation provides another means to administer a molecule having Zcytor17lig binding activity (Potts *et al.*, Pharm. Biotechnol. 10:213 (1997)).

A pharmaceutical composition comprising a protein, polypeptide, or peptide having Zcytor17lig binding activity can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic proteins are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. See, for example, Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995).

For purposes of therapy, molecules having Zcytor17lig binding activity

and a pharmaceutically acceptable carrier are administered to a patient in a therapeutically effective amount. A combination of a protein, polypeptide, or peptide having Zcytor17lig binding activity and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates at least a portion of the inflammatory response.

A pharmaceutical composition comprising Zcytor17lig (or Zcytor17lig analog or fusion protein) can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions, aerosols, droplets, topological solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants (Bremer *et al.*, Pharm. Biotechnol. 10:239-(1997); Ranade, "Implants in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 95-123 (CRC Press 1995); Bremer *et al.*, "Protein Delivery with Infusion Pumps," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 239-254 (Plenum Press 1997); Yewey *et al.*, "Delivery of Proteins from a Controlled Release Injectable Implant," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 93-117 (Plenum Press 1997)). Other solid forms include creams, pastes, other topological applications, and the like.

Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments (see, generally, Bakker-Woudenberg *et al., Eur. J. Clin. Microbiol. Infect. Dis. 12 (Suppl. 1)*:S61 (1993), Kim, *Drugs 46*:618 (1993), and Ranade, "Site-Specific Drug Delivery Using Liposomes as Carriers," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 3-24 (CRC Press 1995)). Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may

be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 μm to greater than 10 μm. A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s) (see, for example, Machy *et al., Liposomes In Cell Biology And Pharmacology* (John Libbey 1987), and Ostro *et al., American J. Hosp. Pharm. 46*:1576 (1989)). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents (Scherphof *et al., Ann. N.Y. Acad. Sci. 446*:368 (1985)). After intravenous administration, small liposomes (0.1 to 1.0 μm) are typically taken up by cells of the reticuloendothelial system, located principally in the liver and spleen, whereas liposomes larger than 3.0 μm are deposited in the lung. This preferential uptake of smaller liposomes by the cells of the reticuloendothelial system has been used to deliver chemotherapeutic agents to macrophages and to tumors of the liver.

The reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means (Claassen *et al., Biochim. Biophys. Acta 802*:428 (1984)). In addition, incorporation of glycolipid- or polyethelene glycol-derivatized phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system (Allen *et al., Biochim. Biophys. Acta 1068*:133 (1991); Allen *et al., Biochim. Biophys. Acta 1150*:9 (1993)).

Liposomes can also be prepared to target particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the liposomes. For example, liposomes, prepared with a high content of a nonionic surfactant, have been used to target the liver (Hayakawa *et al.*, Japanese Patent 04-244,018; Kato *et al., Biol. Pharm. Bull. 16*:960 (1993)). These formulations were prepared by mixing soybean phospatidylcholine, α-tocopherol, and ethoxylated

hydrogenated castor oil (HCO-60) in methanol, concentrating the mixture under vacuum, and then reconstituting the mixture with water. A liposomal formulation of dipalmitoylphosphatidylcholine (DPPC) with a soybean-derived sterylglucoside mixture (SG) and cholesterol (Ch) has also been shown to target the liver (Shimizu *et al., Biol. Pharm. Bull. 20*:881 (1997)).

Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies, antibody fragments, carbohydrates, vitamins, and transport proteins. For example, liposomes can be modified with branched type galactosyllipid derivatives to target asialoglycoprotein (galactose) receptors, which are exclusively expressed on the surface of liver cells (Kato and Sugiyama, Crit. Rev. Ther. Drug Carrier Syst. 14:287 (1997); Murahashi *et al.*, Biol. Pharm. Bull. 20:259 (1997)). Similarly, Wu *et al.*, Hepatology 27:772 (1998), have shown that labeling liposomes with asialofetuin led to a shortened liposome plasma half-life and greatly enhanced uptake of asialofetuin-labeled liposome by hepatocytes. On the other hand, hepatic accumulation of liposomes comprising branched type galactosyllipid derivatives can be inhibited by preinjection of asialofetuin (Murahashi *et al.*, Biol. Pharm. Bull. 20:259 (1997)). Polyaconitylated human serum albumin liposomes provide another approach for targeting liposomes to liver cells (Kamps *et al.*, Proc. Nat'l Acad. Sci. USA 94:11681 (1997)). Moreover, Geho, *et al.* U.S. Patent No. 4,603,044, describe a hepatocyte-directed liposome vesicle delivery system, which has specificity for hepatobiliary receptors associated with the specialized metabolic cells of the liver.

In a more general approach to tissue targeting, target cells are prelabeled with biotinylated antibodies specific for a ligand expressed by the target cell (Harasym *et al.*, Adv. Drug Deliv. Rev. 32:99 (1998)). After plasma elimination of free antibody, streptavidin-conjugated liposomes are administered. In another approach, targeting antibodies are directly attached to liposomes (Harasym *et al.*, Adv. Drug Deliv. Rev. 32:99 (1998)).

Polypeptides having Zcytor17lig binding activity can be encapsulated within liposomes using standard techniques of protein microencapsulation (see, for example, Anderson *et al.*, Infect. Immun. 31:1099 (1981), Anderson *et al.*, Cancer Res. 50:1853 (1990), and Cohen *et al.*, Biochim. Biophys. Acta 1063:95 (1991), Alving *et*

al. "Preparation and Use of Liposomes in Immunological Studies," in *Liposome Technology,* 2nd Edition, Vol. III, Gregoriadis (ed.), page 317 (CRC Press 1993), Wassef *et al., Meth. Enzymol. 149*:124 (1987)). As noted above, therapeutically useful liposomes may contain a variety of components. For example, liposomes may comprise lipid derivatives of poly(ethylene glycol) (Allen *et al.,* Biochim. Biophys. Acta 1150:9 (1993)).

Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as poly(lactide-co-glycolide) (PLG), polyanhydrides, poly (ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the polymer (Gombotz and Pettit, Bioconjugate Chem. 6:332 (1995); Ranade, "Role of Polymers in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 51-93 (CRC Press 1995); Roskos and Maskiewicz, "Degradable Controlled Release Systems Useful for Protein Delivery," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 45-92 (Plenum Press 1997); Bartus *et al.,* Science 281:1161 (1998); Putney and Burke, Nature Biotechnology 16:153 (1998); Putney, Curr. Opin. Chem. Biol. 2:548 (1998)). Polyethylene glycol (PEG)-coated nanospheres can also provide carriers for intravenous administration of therapeutic proteins (see, for example, Gref *et al.,* Pharm. Biotechnol. 10:167 (1997)).

The present invention also contemplates chemically modified polypeptides having zcytor17lig activity, such as a zcytor17lig polypeptide, zcytor17lig agonists, and Zcytor17lig antagonists, for example anti-zcytor17lig antibodies, which a polypeptide is linked with a polymer, as discussed above.

Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 5th Edition (Lea & Febiger 1990), Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995), and by Ranade and Hollinger, *Drug Delivery Systems* (CRC Press 1996).

As an illustration, pharmaceutical compositions may be supplied as a kit comprising a container that comprises a zcytor17lig polypeptide or a zcytor17lig antagonist (*e.g.*, an antibody or antibody fragment that binds a Zcytor17lig polypeptide).

Therapeutic polypeptides can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a therapeutic polypeptide. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition. Moreover, such information may include a statement that the Zcytor17lig composition is contraindicated in patients with known hypersensitivity to Zcytor17lig.

Within one aspect the present invention provides an isolated polypeptide comprising a sequence of amino acid residues that is at least 90% identical to the sequence of amino acid residues selected from the group consisting of: (a) the polypeptide shown from residues 38 (Val) to 152 (Leu) as shown in SEQ ID NO:2; (b) the polypeptide shown from residues 27 (Leu) to 164 (Thr) as shown in SEQ ID NO:2; (c) the polypeptide shown from residues 24 (Thr) to 164 (Thr) as shown in SEQ ID NO:2; and (d) the polypeptide shown from residues 1 (Met) to 164 (Thr) as shown in SEQ ID NO:2. In one embodiment, the isolated polypeptide is as disclosed above, wherein amino acid residues 73, 133 and 147 are cysteine. In another embodiment, the isolated polypeptide is as disclosed above, wherein the polypeptide binds the zcytor17 receptor as shown in SEQ ID NO:5 or SEQ ID NO:71. In another embodiment, the isolated polypeptide comprises at least 14 contiguous amino acid residues of SEQ ID NO:2 or SEQ ID NO:11. In another embodiment, the isolated polypeptide is as disclosed above, wherein the amino acid residues are selected from the group consisting of:(a) amino acid residues 38-52 of SEQ ID NO:2; (b) amino acid residues 83-98 of SEQ ID NO:2; (c) amino acid residues 104-117 of SEQ ID NO:2; and (d) amino acid residues 137-152 of SEQ ID NO:2.

Within a second aspect the present invention provides a fusion protein comprising at least four polypeptides, wherein the order of polypeptides from N-terminus to C-terminus are: a first polypeptide that comprises a sequence of amino acid residues from 38-52 of SEQ ID NO:2; a first spacer of 6-27 amino acid residues; a second polypeptide that comprises a sequence of amino acid residues selected from the group consisting of: (a) IL-2 helix B amino acid residues of SEQ ID NO:168; (b) IL-4 helix B residues 65-83 of SEQ ID NO:164; (c) IL-3 helix B residues 73-86 of SEQ ID

NO:102; (d) GM-CSF helix B residues 72-81 of SEQ ID NO:166; and (e) amino acid residues 83-98 of SEQ ID NO:2; a second spacer of 5-11 amino acid residues; a third polypeptide that comprises a sequence of amino acid residues selected from the group consisting of: (a) IL-2 helix C residues 102-116 of SEQ ID NO:162; (b) IL-4 helix C residues 94-118 of SEQ ID NO:164; (c) IL-3 helix C residues 91-103 of SEQ ID NO:102; (d) GM-CSF helix C residues 85-103 of SEQ ID NO:166; and (e) amino acid residues 104-117 of SEQ ID NO:2; a third spacer of 3-29 amino acid residues; and a fourth polypeptide that comprises a sequence of amino acid residues selected from the group consisting of: (a) IL-2 helix D residues 134-149 of SEQ ID NO:162; (b) IL-3 helix D residues 123-141 of SEQ ID NO:102; (c) IL-4 helix D residues 133-151 of SEQ ID NO:164; (d) GM-CSF helix D residues 120-131 of SEQ ID NO:166; and (e) amino acid residues 137-152 of SEQ ID NO:2.

Within a third aspect the present invention provides a fusion protein comprising at least four polypeptides, wherein the order of polypeptides from N-terminus to C-terminus are:a first polypeptide that comprises a sequence of amino acid residues selected from a group consisting of: (a) IL-2 helix A residues 27-48 of SEQ ID NO:162; (b) IL-4 helix A residues 30-42 of SEQ ID NO:164; (c) IL-3 helix A residues 35-45 of SEQ ID NO:102; (d) GM-CSF helix A residues 30-44 of SEQ ID NO:166; and (e) amino acids residues 38-52 of SEQ ID NO:2; a first spacer of 6-27 amino acid residues; a second polypeptide that comprises a sequence of amino acid residues selected from the group consisting of: (a) IL-2 helix B amino acid residues of SEQ ID NO:168; (b) IL-4 helix B residues 65-83 of SEQ ID NO:164; (c) IL-3 helix B residues 73-86 of SEQ ID NO:102; (d) GM-CSF helix B residues 72-81 of SEQ ID NO:166; and (e) amino acid residues 83-98 of SEQ ID NO:2; a second spacer of 5-11 amino acid residues; a third polypeptide that comprises a sequence of amino acid residues selected from the group consisting of: (a) IL-2 helix C residues 102-116 of SEQ ID NO:162; (b) IL-4 helix C residues 94-118 of SEQ ID NO:164; (c) IL-3 helix C residues 91-103 of SEQ ID NO:102; (d) GM-CSF helix C residues 85-103 of SEQ ID NO:166; and (e) amino acid residues 104-117 of SEQ ID NO:2; a third spacer of 3-29 amino acid residues; and a fourth polypeptide that comprises a sequence of amino acid residues from 137-152 of SEQ ID NO:2. In another embodiment, the fusion protein is as

disclosed above, wherein the fourth polypeptide comprises amino acid residues 137-152 of SEQ ID NO:2.

Within another aspect the present invention provides an isolated polynucleotide molecule comprising a sequence of nucleotides that encode the polypeptide as disclosed above. In one embodiment, the isolated polynucleotide is as disclosed above, wherein the nucleotides are selected from the group consisting of: (a) a polynucleotide as shown in SEQ ID NO: 1 from nucleotide 139 to nucleotide 483; (b) a polynucleotide as shown in SEQ ID NO: 1 from nucleotide 106 to nucleotide 519; (c) a polynucleotide as shown in SEQ ID NO: 1 from nucleotide 97 to nucleotide 519; and (d) a polynucleotide as shown in SEQ ID NO: 1 from nucleotide 28 to nucleotide 519.

Within another aspect the present invention provides an isolated polynucleotide molecule comprising a sequence of nucleotides that encode for the polypeptide as disclosed herein.

Within another aspect the present invention provides an expression vector comprising the following operably linked elements: (a) a transcription promoter; (b) a DNA segment encoding a polypeptide comprising a sequence of amino acid residues selected from the group consisting of: (i) amino acid residues 38-52 of SEQ ID NO:2; (ii) amino acid residues 83-98 of SEQ ID NO:2; (iii) amino acid residues 104-117 of SEQ ID NO:2; (iv) amino acid residues 137-152 of SEQ ID NO:2; and (v) combinations thereof; and (c) a transcription terminator.

Within another aspect the present invention provides an expression vector comprising the following operably linked elements: (a) a transcription promoter; (b) a DNA segment encoding a polypeptide comprising a sequence of amino acid residues that is at least 90% identical to residues 38 (Val) to 152 (Leu) as shown in SEQ ID NO:2; and (c) a transcription terminator. In one embodiment, the expression vector is as disclosed above, comprising the following operably linked elements: (a) a transcription promoter; (b) a DNA segment encoding a polypeptide comprising amino acid residues 38 (Val) to 152 (Leu) of SEQ ID NO:2; and (c) a transcription terminator.

Within another aspect the present invention provides a cultured cell comprising the expression vector as disclosed above.

Within another aspect the present invention provides a method of producing a protein comprising: culturing a cell as disclosed above under conditions wherein the DNA segment is expressed; and recovering the protein encoded by the DNA segment.

Within another aspect the present invention provides a method of producing an antibody to a zcytor17lig polypeptide comprising: inoculating an animal with a polypeptide selected from the group consisting of: (a) a polypeptide consisting of 9 to 141 amino acids, wherein the polypeptide is identical to a contiguous sequence of amino acid residues in SEQ ID NO:2 from amino acid number 24 (Ser) to amino acid number 164 (Thr); a polypeptide as disclosed above; (c) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 38-52; (d) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 83-98; (e) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 104-117; (f) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 137-152; (g) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 38-152; (h) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 24-164; (c) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 38-52; (d) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 85-98; (e) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 104-118; (f) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 141-157; (g) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 38-157; (h) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 24-163; (i) a polypeptide comprising an antigenic epitope according to a Hopp/Woods hydrophilicity profile of SEQ ID NO:2 or SEQ I NO 11, wherein the profile is based on a sliding six-residue window. Buried G, S, and T residues and exposed H, Y, and W residues ignored; and wherein the polypeptide elicits an immune response in the animal to produce the antibody; and isolating the antibody from the animal.

Within another aspect the present invention provides an antibody (e.g., neutralizing antibody) produced by the method as disclosed above, wherein the antibody binds to a polypeptide of SEQ IDN NO:2 or SEQ ID NO:11. In one embodiment, the antibody disclosed above specifically binds to a polypeptide shown in SEQ ID NO:2 or SEQ ID NO:11.

Within another aspect the present invention provides a method of stimulating an immune response in a mammal exposed to an antigen or pathogen comprising the steps of: (1) determining directly or indirectly the level of antigen or pathogen present in said mammal; (2) administering a composition comprising zcytor17lig polypeptide in an acceptable pharmaceutical carrier; (3) determining directly or indirectly the level of antigen or pathogen in said mammal; and (4) comparing the level of the antigen or pathogen in step 1 to the antigen or pathogen level in step 3, wherein a change in the level is indicative of stimulating an immune response. In one embodiment, the method of stimulating an immune response in a mammal disclosed above further comprises: (5) re-administering a composition comprising zcytor17lig polypeptide in an acceptable pharmaceutical carrier; (6) determining directly or indirectly the level of antigen or pathogen in said mammal; and; (7) comparing the number of comparing the antigen or pathogen level in step 1 to the antigen level in step 6, wherein a change in the level is indicative of stimulating an immune response.

Within another aspect the present invention provides a method for expansion of hematopoietic cells and hematopoietic cell progenitors comprising culturing bone marrow or peripheral blood cells with a composition comprising an amount of zcytor17lig sufficient to produce an increase in the number of lymphoid cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of zcytor17lig. In one embodiment, the method for expansion of hematopoietic cells and hematopoietic cell progenitors is as disclosed above, wherein the hematopoietic cells and hemopoietic progenitor cells are lymphoid cells. In another embodiment, the method for expansion of hematopoietic cells and hematopoietic cell progenitors is as disclosed above, wherein the lymphoid cells are monocytic cells, macrophages or T cells.

Within another aspect the present invention provides method of stimulating an immune response in a mammal exposed to an antigen or pathogen comprising: (1) determining a level of an antigen- or pathogen-specific antibody; (2) administering a composition comprising zcytor17lig polypeptide in an acceptable pharmaceutical carrier; (3) determining a post administration level of antigen- or pathogen-specific antibody; (4) comparing the level of antibody in step (1) to the level of antibody in step (3), wherein an increase in antibody level is indicative of stimulating an immune response.

Within another aspect the present invention provides a method of detecting the presence of zcytor17lig RNA in a biological sample, comprising the steps of: (a) contacting a zcytor17lig nucleic acid probe under hybridizing conditions with either (i) test RNA molecules isolated from the biological sample, or (ii) nucleic acid molecules synthesized from the isolated RNA molecules, wherein the probe has a nucleotide sequence comprising either a portion of the nucleotide sequence of the nucleic acid molecule as disclosed above, or its complement, and (b) detecting the formation of hybrids of the nucleic acid probe and either the test RNA molecules or the synthesized nucleic acid molecules, wherein the presence of the hybrids indicates the presence of zcytor17lig RNA in the biological sample.

Within another aspect the present invention provides a method of detecting the presence of zcytor17lig in a biological sample, comprising the steps of: (a) contacting the biological sample with an antibody, or an antibody fragment as disclosed above, wherein the contacting is performed under conditions that allow the binding of the antibody or antibody fragment to the biological sample, and (b) detecting any of the bound antibody or bound antibody fragment.

Within another aspect, the present invention provides a method of killing cancer cells comprising, obtaining *ex vivo* a tissue or biological sample containing cancer cells from a patient, or identifying cancer cells *in vivo*; producing a polypeptide by the method as disclosed herein; formulating the polypeptide in a pharmaceutically acceptable vehicle; and administering to the patient or exposing the cancer cells to the polypeptide; wherein the polypeptide kills the cells. In one embodiment the method of killing cancer cells is as disclosed above, wherein the polypeptide is further conjugated

to a toxin. In one embodiment the antibody is as disclosed above, wherein the antibody is selected from the group consisting of: (a) polyclonal antibody, (b) murine monoclonal antibody, (c) humanized antibody derived from (b), (d) an antibody fragment, and (e) human monoclonal antibody.

Within another aspect, the present invention provides an antibody or antibody fragment that specifically binds to a polypeptide of comprising a sequence of amino acid residues selected from the group consisting of: (a) the polypeptide shown from residues 38 (Val) to 152 (Leu) as shown in SEQ ID NO:2; (b) the polypeptide shown from residues 27 (Leu) to 164 (Thr) as shown in SEQ ID NO:2; (c) the polypeptide shown from residues 24 (Thr) to 164 (Thr) as shown in SEQ ID NO:2; and (d) the polypeptide shown from residues 1 (Met) to 164 (Thr) as shown in SEQ ID NO:2. In another embodiment the antibody is as disclosed above, wherein the antibody further comprises a radionuclide, enzyme, substrate, cofactor, fluorescent marker, chemiluminescent marker, peptide tag, magnetic particle, drug, or toxin.

Within another aspect, the present invention provides a method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic cell progenitors comprising culturing bone marrow or peripheral blood cells with a composition comprising an amount of an antibody as disclosed herein sufficient to reduce proliferation or differentiation of the hematopoietic cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of soluble cytokine receptor. In one embodiment the method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic cell progenitors is as disclosed above, wherein the hematopoietic cells and hematopoietic progenitor cells are lymphoid cells. In another embodiment the method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic cell progenitors is as disclosed above, wherein the lymphoid cells are macrophages or T cells.

Within another aspect, the present invention provides a method of reducing zcytor17lig-induced induced inflammation comprising administering to a mammal with inflammation an amount of a composition of a an antibody as disclosed herein sufficient to reduce inflammation.

Within another aspect, the present invention provides a method of suppressing an inflammatory response in a mammal with inflammation comprising: (1) determining a level of an inflammatory molecule; (2) administering a composition comprising an antibody as disclosed herein in an acceptable pharmaceutical vehicle; (3) determining a post administration level of the inflammatory molecule; (4) comparing the level of the inflammatory molecule in step (1) to the level of the inflammatory molecule in step (3), wherein a lack of increase or a decrease the inflammatory molecule level is indicative of suppressing an inflammatory response. In one embodiment, the antoibody is as disclosed above, wherein the antibody further comprises a radionuclide, enzyme, substrate, cofactor, fluorescent marker, chemiluminescent marker, peptide tag, magnetic particle, drug, or toxin.

Within another aspect, the present invention provides a method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic cell progenitors comprising culturing bone marrow or peripheral blood cells with a composition comprising an amount of an antibody as disclosed herein sufficient to reduce proliferation or differentiation of the hematopoietic cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of soluble cytokine receptor. In one embodiment the method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic cell progenitors is as disclosed above, wherein the hematopoietic cells and hematopoietic progenitor cells are lymphoid cells. In another embodiment the method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic cell progenitors is as disclosed above, wherein the lymphoid cells are macrophages or T cells.

Within another aspect, the present invention provides a method of reducing zcytor17lig-induced induced inflammation comprising administering to a mammal with inflammation an amount of a composition of a an antibody as disclosed herein sufficient to reduce inflammation.

\Within another aspect, the present invention provides a method of suppressing an inflammatory response in a mammal with inflammation comprising: (1) determining a level of an inflammatory molecule; (2) administering a composition

comprising an antibody as disclosed herein in an acceptable pharmaceutical vehicle; (3) determining a post administration level of the inflammatory molecule; (4) comparing the level of the inflammatory molecule in step (1) to the level of the inflammatory molecule in step (3), wherein a lack of increase or a decrease in the inflammatory molecule level is indicative of suppressing an inflammatory response.

Within another aspect, the present invention provides a method of treating a mammal afflicted with an inflammatory disease in which zcytor17lig plays a role, comprising: administering an antagonist of zcytor17lig to the mammal such that the inflammation is reduced, wherein the antagonist is selected from the group consisting of an antibody or binding polypeptide that specifically binds a polypeptide or polypeptide fragment of zcytor17lig (SEQ ID NO:2). In one embodiment, the method of treating a mammal afflicted with an inflammatory disease is as disclosed above, wherein the disease is a chronic inflammatory disease. In another embodiment, the method of treating a mammal afflicted with an inflammatory disease is as disclosed above, wherein the disease is a chronic inflammatory disease selected from the group consisting of: inflammatory bowel disease; ulcerative colitis; Crohn's disease; atopic dermatitis; eczema; and psoriasis. In antoher embodiment, the method of treating a mammal afflicted with an inflammatory disease is as disclosed above, wherein the disease is an acute inflammatory disease. In another embodiment, the method of treating a mammal afflicted with an inflammatory disease is as disclosed above, wherein the disease is an acute inflammatory disease selected from the group consisting of: endotoxemia; septicemia; toxic shock syndrome; and infectious disease. In another embodiment, the method of treating a mammal afflicted with an inflammatory disease is as disclosed above, wherein the antibody further comprises a radionuclide, enzyme, substrate, cofactor, fluorescent marker, chemiluminescent marker, peptide tag, magnetic particle, drug, or toxin.

Within another aspect, the present invention provides a method for detecting inflammation in a patient, comprising: obtaining a tissue or biological sample from a patient; incubating the tissue or biological sample with an antibody as disclosed herein under conditions wherein the antibody binds to its complementary polypeptide in the tissue or biological sample; visualizing the antibody bound in the tissue or

biological sample; and comparing levels of antibody bound in the tissue or biological sample from the patient to a normal control tissue or biological sample, wherein an increase in the level of antibody bound to the patient tissue or biological sample relative to the normal control tissue or biological sample is indicative of inflammation in the patient.

Within another aspect, the present invention provides a method for detecting inflammation in a patient, comprising: obtaining a tissue or biological sample from a patient; labeling a polynucleotide comprising at least 14 contiguous nucleotides of SEQ ID NO:1 or the complement of SEQ ID NO:1; incubating the tissue or biological sample with under conditions wherein the polynucleotide will hybridize to complementary polynucleotide sequence; visualizing the labeled polynucleotide in the tissue or biological sample; and comparing the level of labeled polynucleotide hybridization in the tissue or biological sample from the patient to a normal control tissue or biological sample, wherein an increase in the labeled polynucleotide hybridization to the patient tissue or biological sample relative to the normal control tissue or biological sample is indicative of inflammation in the patient.

The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1

Construction of MPL-zcytor17 Polypeptide Chimera: MPL Extracellular and TM Domain Fused to the zcytor17 Intracellular Signaling Domain

The 5' extracellular domain of the murine MPL receptor was isolated from a plasmid containing the murine MPL receptor (PHZ1/MPL plasmid) by digestion with EcoRI and BamHI generating a 1164 bp fragment. The digestion was run on a 1% agarose gel and the fragment was isolated using the Qiaquick gel extraction kit (Qiagen) as per manufacturer's instructions. The rest of the MPL extracellular domain and transmembrane domain were generated using PCR with primers ZC6,673 (SEQ ID NO:13) and ZC29,082 (SEQ ID NO:14). The reaction conditions were as follows: 15 cycles at 94°C for 1 min., 55°C for 1 min., 72°C for 2 min.; followed by 72°C for 7 min.; then a 4°C soak. The PCR product was run on a 1% agarose gel and the approximately 400bp MPL receptor fragment was isolated using Qiaquick™ gel extraction kit (Qiagen) as per manufacturer's instructions.

The intracellular domain of human zcytor17 was isolated from a plasmid containing zcytor17 receptor cDNA (#23/pCAP) using PCR with primers ZC29,083 (SEQ ID NO:15) and ZC29,145 (SEQ ID NO:16). The polynucleotide sequence that corresponds to the zcytor17 receptor coding sequence is shown in SEQ ID NO:5. The reaction conditions were as per above. The PCR product was run on a 1% agarose gel and the approximately 320 bp zcytor17 fragment isolated using Qiaquick gel extraction kit as per manufacturer's instructions.

Each of the isolated PCR fragments described above were mixed at a 1:1 volumetric ratio and used in a PCR reaction using ZC6673 (SEQ ID NO:13) and ZC29145 (SEQ ID NO:16) to create all but the 5' MPL portion of the MPL-zcytor17 chimera. The reaction conditions were as follows: 15 cycles at 94°C for 1 min., 55°C for 1 min., 72°C for 2 min.; followed by 72°C for 7 min.; then a 4°C soak. The entire PCR product was run on a 1% agarose gel and the approximately 700bp MPL-zcytor17 chimera fragment isolated using Qiaquick gel extraction kit (Qiagen) as per manufacturer's instructions. The MPL-zcytor17 chimera fragment was digested with

BamHI (BRL) and XbaI (Boerhinger Mannheim) as per manufacturer's instructions. The entire digest was run on a 1% agarose gel and the cleaved MPL-zcytor17 chimera isolated using Qiaquick™ gel extraction kit (Qiagen) as per manufacturer's instructions. The resultant cleaved MPL-zvytor17 chimera plus 5' MPL EcoRI/BamHI fragment described above were inserted into an expression vector to generate the full MPL-zcytor17 chimeric receptor as described below.

Recipient expression vector pZP-7 was digested with EcoRI (BRL) and Xba1 (BRL) as per manufacturer's instructions, and gel purified as described above. This vector fragment was combined with the EcoRI and XbaI cleaved MPL-zcytor17 PCR chimera isolated above and the EcoRI and BamHI 5' MPL fragment isolated above in a ligation reaction. The ligation was run using T4 Ligase (Epicentre Technologies), at room temperature for 1 hour as per manufacturer's instructions. A sample of the ligation was electroporated into DH10B ElectroMAX™ electrocompetent E. coli cells (25μF, 200Ω, 1.8V). Transformants were plated on LB+Ampicillin plates and single colonies screened by miniprep (Qiagen) and digestion with EcoRI to check for the MPL-zcytor17 chimera. EcoRI digestion of correct clones yield about a 2kb fragment. Confirmation of the MPL-zcytor17 chimera sequence was made by sequence analyses. The insert was approximately 3.1 kb, and was full-length.

Example 2

MPL-zcytor17 Chimera Based Proliferation in BAF3 Assay Using Alamar Blue

A. Construction of BaF3 Cells Expressing MPL-zcytor17 chimera

BaF3, an interleukin-3 (IL-3) dependent pre-lymphoid cell line derived from murine bone marrow (Palacios and Steinmetz, Cell 41: 727-734, 1985; Mathey-Prevot et al., Mol. Cell. Biol. 6: 4133-4135, 1986), was maintained in complete media (RPMI medium, JRH Bioscience Inc., Lenexa, KS) supplemented with 10% heat-inactivated fetal calf serum, 1-2 ng/ml murine IL-3 (mIL-3) (R & D, Minneapolis, MN), 2mM L-glutaMax-1™ (Gibco BRL), 1 mM Sodium Pyruvate (Gibco BRL), and PSN antibiotics (GIBCO BRL)). Prior to electroporation, pZP-7/MPL-zcytor17 plasmid DNA (Example 1) was prepared and purified using a Qiagen Maxi Prep kit (Qiagen) as per manufacturer's instructions. BaF3 cells for electroporation were washed twice in

RPMI media and then resuspended in RPMI media at a cell density of 10^7 cells/ml. One ml of resuspended BaF3 cells was mixed with 30 μg of the pZP-7/MPL-zcytor17 plasmid DNA and transferred to separate disposable electroporation chambers (GIBCO BRL). At room temperature cells were given 5x.1msec shocks at 800 volts followed by 5x2ms shocks at 600 volts delivered by an electroporation apparatus (Cyto-Pulse). Alternatively, cells were electroporated with two serial pulses (800 μFAD/300 V; followed by 1180 μFAD/300 V) delivered by a Cell-Porator (GibcoBRL) electroporation apparatus. The electroporated cells were transferred to 50 ml of complete media and placed in an incubator for 15-24 hours (37°C, 5% CO_2). Then Geneticin™ (Gibco) selection (1mg/ml G418) was added to the cells in a T-162 flask to isolate the G418-resistant pool. Pools of the transfected BaF3 cells, hereinafter called BaF3/MPL-zcytor17 cells, were assayed for signaling capability as described below.

B. Testing the signaling capability of the BaF3/MPL-zcytor17 cells using an Alamar Blue Proliferation Assay

BaF3/MPL-zcytor17 cells were spun down and washed in the complete media, described above, but without mIL-3 (hereinafter referred to as "mIL-3 free media"). The cells were spun and washed 3 times to ensure the removal of the mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at 5000 cells per well in a volume of 100 μl per well using the mIL-3 free media.

Proliferation of the BaF3/MPL-zcytor17 cells was assessed using murine thrombopoietin (mTPO) diluted with mIL-3 free media to 200 ng/ml, 100ng/ml, 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.1ng/ml, 1.5 ng/ml concentrations. One hundred microliters of the diluted mTPO was added to the BaF3/MPL-zcytor17 cells. The total assay volume was 200 μl. Negative controls were run in parallel using mIL-3 free media only, without the addition of mTPO. The assay plates were incubated at 37°C, 5% CO_2 for 3 days at which time Alamar Blue (Accumed, Chicago, IL) was added at 20μl/well. Alamar Blue gives a fluorometric readout based on the metabolic activity of cells, and is thus a direct measurement of cell proliferation in comparison to a negative control. Plates were again incubated at 37°C, 5% CO_2 for 24 hours. Plates were read on the Fmax™ plate reader (Molecular Devices Sunnyvale, CA) using the

SoftMax™ Pro program, at wavelengths 544 (Excitation) and 590 (Emission), or a Wallac Victor 2 plate reader (PerkinElmer Life Sciences, Boston, MA).

Results confirmed the signaling capability of the intracellular portion of the zcytor17 receptor, as the thrombopoietin induced proliferation at approximately 9-13 fold over background at mTPO concentrations of 50ng/ml and greater.

Example 3

Construction of Expression Vector Expressing Full-length zcytor17: pZp7pX/zcytor17

A. Cloning of full length zcytor17 cDNA for expression:

To obtain a full-length zcytor17 cDNA, 5' and 3' PCR products were isolated and joined using an internal PstI site. The PCR primers were designed using the nucleotide sequence SEQ ID NO:4 and include BamHI and Xho I restriction sites for cloning purposes.

A 5' PCR product was generated using a WI-38 cDNA library as a template and oligonucleotides ZC29,359 (SEQ ID NO:18) and ZC27,899 (SEQ ID NO:19) as primers. WI-38 is an in-house cDNA library generated from a human embryonic lung cell line (ATCC CRL-2221). This 5' PCR reaction was run as follows: 30 cycles at 94°C for 1 minute, 65°C for 1 minute, 72°C for 2 minutes, then 72°C for 7 minutes; 10°C soak. The PCR reaction used approximately 3 μg of plasmid prepared from the cDNA library, 20 pmoles of each oligonucleotide, and five units of PWO DNA polymerase (Roche). About 90% of the 5' PCR product was ethanol precipitated, digested with BamHI and PstI and gel purified on a 1.0% agarose gel. The approximately 600 bp band was excised and used for ligation to the cloning vector pUC18 digested with BamHI and PstI. The resulting transformants were sequenced to confirm the zcytor17 cDNA sequence. For one of these transformants, plasmid DNA was prepared and digested with BamHI and PstI. The resulting approximately 600 bp band was gel purified and used for a ligation below to form a full-length cDNA.

A 3' PCR product was generated using a human testes in-house cDNA library as a template and oligonucleotides ZC27,895 (SEQ ID NO:20) and ZC29,122 (SEQ ID NO:21) as primers. This 3' PCR reaction was run as follows: 30 cycles at 94° C for 45 seconds, 65°C for 45 seconds, 72°C for 2 minutes, then 72°C for 7 minutes; 10

°C soak. The entire 3' PCR reaction was gel purified on a 1.0% agarose gel and the major 1500 bp band excised. This band was cloned into the PCR Blunt II TOPO vector using the Zeroblunt TOPO kit (Invitrogen). The resulting transformants were sequenced to confirm the zcytor17 cDNA sequence. For one of these transformants, plasmid DNA was prepared and digested with PstI and XhoI. The resulting approximately 1500 bp band was gel purified. A three-part ligation was performed with the 5' BamHI to Pst I fragment above, the 3' PstI to XhoI fragment, and the expression vector pZp7pX digested with BamHI and XhoI. This generated a pZp7pX plasmid containing a full-length cDNA for zcytor17 (SEQ ID NO:4), designated pZp7p/zcytor17. The full length zcytor17 cDNA in pZp7p/zcytor17 has a silent mutation that changes the T to G at position 1888 of SEQ ID NO:4 (encoding a Gly residue at residue 464 of SEQ ID NO:5). As this mutation was silent, the zcytor17 cDNA in pZp7p/zcytor17 encodes the polypeptide as shown in SEQ ID NO:5. Plasmid pZp7pX is a mammalian expression vector containing an expression cassette having the CMV promoter, intron A, multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a puromycin resistance gene and the SV40 terminator.

B. Construction of Expression Vector Expressing Full-Length WSX-1

The entire WSX-1 receptor (SEQ ID NO:9) was isolated from a plasmid containing the WSX-1 receptor cDNA (SEQ ID NO:8) (U.S. Patent No. 5,925,735). hWSX-1/pBluescript SK(+) plasmid DNA (Stratagene, La Jolla, CA) was digested with EcoRI and XhoI to generate a 1075 bp fragment, and also digested with XhoI and XbaI to generate a 900 bp fragment. Both digests were run on a 1% agarose gel and the cleaved WSX-1 fragments isolated.

Recipient expression vector pZp7Z was digested with EcoRI and XbaI and gel purified as described above. This vector fragment was combined with the two cleaved zcytor17 fragments isolated above in a ligation reaction using T4 Ligase (BRL). The ligation was incubated at room temperature overnight. A sample of the ligation

was electroporated in to DH10B electroMAX™ electrocompetent *E. coli* cells (25μF, 200Ω, 2.3V). Six colonies were grown in culture and miniprepped DNA was prepared and digested to confirm the correct WSX-1 full-length insert of 2.0 kb. The resulting plasmid is pZPZ7Z/WSX-1.

Example 4

Zcytor17 based proliferation in BAF3 assay using Alamar Blue

A. Construction of BaF3 Cells Expressing zcytor17 receptor, WSX-1 receptor and OSMR

BaF3 cells expressing the full-length zcytor17 receptor were constructed as per Example 2A above, using 30μg of the zcytor17 expression vector, described in Example 3A. One exception is that in place of Geneticin selection, 2μg/ml of Puromycin (ClonTech) was added to the transfected cells in a T-162 flask to isolate the puromycin-resistant pool. The BaF3 cells expressing the zcytor17 receptor mRNA were designated as BaF3/zcytor17. To obtain clones, Baf3/zcytor17 cells were counted in a hemocytometer and plated at 1 cell/well, 0.5 cell/well, 0.1 cell/well, and 0.01 cell/well in 96-well dishes. Fifteen clones were scaled up to T75 flasks, and five clones were assayed for zcytor17 expression. Total RNA was isolated from cell pellets using a S.N.A.P.™ total RNA Isolation Kit (InVitrogen). First-strand cDNA was synthesized using the proSTAR™ First Strand RT-PCR kit, and then PCR with zcytor17 specific primers ZC29,180 (SEQ ID N0:22) and ZC29,122 (SEQ ID NO:23) was performed to screen the clones for expression of zcytor17. One clone, BaF3/zcytor17#15 was chosen to expand and transfect with the WSX-1 expression vector.

BaF3 cells expressing zcytor17 and full-length WSX-1 were constructed as per Example 2A above, using 30 ug of the WSX-1 expression vector WSX-1/pZp7Z (Example 3B) to electroporate the BaF3/zcytor17#15 cells. One exception is that in place of Geneticin selection, 200 μg/ml Zeocin (InVitrogen) was added to the transfected cells in a T-162 flask to isolate the zeocin-resistant pool. The BaF3 cells expressing zcytor17 and WSX-1 were designated BaF3/zcytor17/hWSX-1. To obtain clones, pools of Baf3/zcytor17/hWSX-1 cells were plated at limiting dilution in 96-well plates. The resulting clones were expanded and total RNA was isolated using a

S.N.A.P.™ total RNA Isolation Kit (InVitrogen). First-strand cDNA was synthesized using the proSTAR™ First Strand RT-PCR kit, and then PCR with WSX-1 specific primers ZC9791 (SEQ ID NO:24) and ZC9793 (SEQ ID NO:25) was used to screen the clones for expression of WSX-1. One clone, BaF3/zcytor17/hWSX-1#5 was chosen to expand further and transfect with the OSMRbeta expression vector.

BaF3 cells expressing zcytor17, WSX-1 and full-length OSMRbeta were constructed as per Example 2A above, using 30 ug of the OSMRbeta expression vector OSMR/pZp7NX as described in Example 29 to electroporate the BaF3/zcytor17/hWSX-1#5 cells. The BaF3 cells expressing zcytor17, WSX-1, and OSMRbeta mRNA were designated BaF3/zcytor17/WSX-1/OSMR. To obtain clones, pools of BaF3/zcytor17/WSX-1/OSMRbeta cells were plated at limiting dilution in 96-well plates. Individual clones were expanded and total RNA was isolated using a S.N.A.P.™ total RNA Isolation Kit (InVitrogen). First-strand cDNA was synthesized using the proSTAR™ First Strand RT-PCR kit, and then PCR with OSMRbeta specific primers ZC40109 (SEQ ID NO:26) and ZC40112 (SEQ ID NO:27) was used to screen the clones for expression of zcytor17, WSX-1, and OSMR. One clone, BaF3/zcytor17/WSX-1/OSMR#5 was selected and these cells were used to screen for zcytor17lig as described below in Examples 5 and 6.

B. Construction of BaF3 Cells Expressing zcytor17 receptor and OSMR

BaF3 cells expressing the full-length zcytor17 receptor were constructed as per Example 2A above, using 30μg of the zcytor17 expression vector, described in Example 3A. One exception is that in place of Geneticin selection, 2ug/ml of Puromycin (ClonTech) was added to the transfected cells in a T-162 flask to isolate the puromycin-resistant pool. The BaF3 cells expressing the zcytor17 receptor mRNA were designated as BaF3/zcytor17. To obtain clones, pools of Baf3/zcytor17 cells were plated at limiting dilution in 96-well plates. These clones were expanded in culture and total RNA was isolated using a S.N.A.P.™ total RNA Isolation Kit (InVitrogen). First-strand cDNA was synthesized using the proSTAR™ First Strand RT-PCR kit, and then PCR was used to screen the clones for expression of zcytor17. One clone, BaF3/zcytor17 #15 was chosen to expand and transfect with the OSMRbeta expression

vector.

BaF3 cells expressing zcytor17 and full-length OSMRbeta were constructed as per Example 2A above, using 30 ug of the OSMRbeta expression vector OSMR/pZp7NX (Example 29) to electroporate the BaF3/zcytor17#15 cells. The BaF3 cells expressing zcytor17 and OSMRbeta mRNA were designated BaF3/zcytor17/OSMR. These cells were used to screen for zcytor17lig as described below in Example 5.

Example 5

Screening for zcytor17lig using BaF3/Zcytor17/WSX-1/OSMRbeta cells using an Alamar Blue Proliferation Assay

A. Activation of CCRF-CEM and CCRF-HSB2 cells to test for presence of zcytor17lig

CCRF-CEM and CCRF-HSB2 cells were obtained from ATCC and stimulated in culture to produce conditioned media to test for the presence of zcytor17lig activity as described below. The suspension cells were seeded at 2 x 10^5 cells/ml or 5 x 10^5 cells/ml in RPMI-1640 media supplemented with 10% FBS, 2 mM L-glutamine (GibcoBRL), 1X PSN (GibcoBRL), and activated with 10 ng/ml Phorbol-12-myristate-13-acetate (PMA) (Calbiochem, San Diego, CA) and 0.5ug/ml Ionomycin™ (Calbiochem) for 24 or 48hrs. The supernatant from the stimulated cells was used to assay proliferation of the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells as described below.

B. Screening for zcytor17lig using BaF3/Zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells using an Alamar Blue Proliferation Assay

BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells cells were spun down and washed in mIL-3 free media. The cells were spun and washed 3 times to ensure the removal of the mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at 5000 cells per well in a volume of 100 µl per well using the mIL-3 free media.

Proliferation of the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells was assessed using conditioned media from activated

CCRFCEM and CCRF-HSB2 cells (see Example 5A). Conditioned media was diluted with mIL-3 free media to 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.75%, and 0.375% concentrations. One hundred microliters of the diluted conditioned media was added to the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells. The total assay volume was 200 µl. The assay plates were incubated at 37°C, 5% CO_2 for 3-5 days at which time Alamar Blue (Accumed, Chicago, IL) was added at 20 µl/well. Plates were again incubated at 37°C, 5% CO_2 for 24 hours. Plates were read on the Fmax™ plate reader (Molecular devices) as described above (Example 2).

Results confirmed the proliferative response of the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells to a factor present in the activated CCRF-CEM and CCRF-HSB2 conditioned media. The response, as measured, was approximately 10-fold over background at the 25% concentration. The untransfected BaF3 cells did not proliferate in response to this factor, nor did BaF3 cells transfected with zcytor17 and WSX-1 (BaF3/zcytor17/WXS-1 cells), showing that this factor was specific for Zcytor17/OSMRbeta or zcytor17/OSMRbeta/WSX-1 receptors. Moreover, soluble zcytor17 receptor diminished this proliferative activity of zcytor17lig in the BaF3/zcytor17/WSX-1/OSMRbeta cells (see, Example 11). Similar results are expected in BaF3/zcytor17/OSMRbeta cells.

C. Human Primary Source used to isolate zcytor17lig

One hundred milliliter blood draws were taken from each of six donors. The blood was drawn using 10X 10 ml vacutainer tubes containing heparin. Blood was pooled from six donors (600ml), diluted 1:1 in PBS, and separated using a Ficoll-Paque® PLUS (Pharmacia Biotech). The isolated primary human cell yield after separation on the ficoll gradient was $1.2X10^9$ cells.

Cells were suspended in 9.6 ml MACS buffer (PBS, 0.5% EDTA, 2mM EDTA). 1.6 ml of cell suspension was removed and 0.4 ml CD3 microbeads (Miltenyi Biotec, Auburn, CA) added. The mixture was incubated for 15 min. at 4°C. These cells labeled with CD3 beads were washed with 30 ml MACS buffer, and then resuspended in 2 ml MACS buffer.

A VS+ column (Miltenyi) was prepared according to the manufacturer's instructions. The VS+ column was then placed in a VarioMACS™ magnetic field (Miltenyi). The column was equilibrated with 5 ml MACS buffer. The isolated primary human cells were then applied to the column. The CD3 negative cells were allowed to pass through. The column was rinsed with 9 ml (3 X 3 ml) MACS buffer. The column was then removed from the magnet and placed over a 15 ml falcon tube. CD3+ cells were eluted by adding 5 ml MACS buffer to the column and bound cells flushed out using the plunger provided by the manufacturer. The incubation of the cells with the CD3 magnetic beads, washes, and VS+ column steps (incubation through elution) above were repeated five more times. The resulting CD3+ fractions from the six column separations were pooled. The yield of CD3+ selected human cells were $3X10^8$ total cells.

A sample of the pooled CD3+ selected human cells was removed for staining and sorting on a fluorescent antibody cell sorter (FACS) to assess their purity. The human CD3+ selected cells were 91% CD3+ cells.

The human CD3+ selected cells were activated by incubating in RPMI + 5% FBS + PMA 10 ng/ml and Ionomycin 0.5 μg/ml (Calbiochem) for 13 hours 37°C. The supernatant from these activated CD3+ selected human cells was tested for zcytor17lig activity as described below. Moreover, the activated CD3+ selected human cells were used to prepare a cDNA library, as described in Example 6, below.

D. Testing supernatant from activated CD3+ selected human cells for zcytor17lig using BaF3/Zcytor17/WSX-1/OSMRbeta cells and an Alamar Blue Proliferation Assay

BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells were spun down and washed in mIL-3 free media. The cells were spun and washed 3 times to ensure the removal of the mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at 5000 cells per well in a volume of 100 μl per well using the mIL-3 free media.

Proliferation of the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells were assessed using conditioned media from activated CD3+ selected human cells (see Example 5C) diluted with mIL-3 free media to 25%,

12.5%, 6.25%, 3.125%, 1.5%, 0.75%, 0.375% and 0.187% concentrations. One hundred microliters of the diluted conditioned media was added to the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells. The total assay volume was 200 µl. The assay plates were incubated and assayed as described in Example 5B.

Results confirmed the proliferative response of the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells to a factor present in the activated CD3+ selected human Cell conditioned media. The response, as measured, was approximately 15-fold over background at the 25% concentration. The untransfected BaF3 cells did not proliferate in response to this factor, nor did BaF3 cells transfected with zcytor17 and WSX-1 (BaF3/zcytor17/WXS-1 cells), showing that this factor was specific for Zcytor17/OSMRbeta or zcytor17/OSMRbeta/WSX-1 receptors.

Example 6

Cloning of human zcytor17lig from a human CD3+ selected cell Library

Screening of a primary human activated CD3+ selected cell cDNA library revealed an isolated cDNA that is a novel member of the four-helix bundle cytokine family. This cDNA encoded the zcytor17lig. The cDNA was identified by screening for activity of the zcytor17lig using the zcytor17/WSX-1/OSM receptors.

A. The vector for CD3+ selected library construction

The vector for CD3+ selected library construction was pZP7NX. The pZP7NX vector was constructed as follows: The coding region for the DHFR selective marker in vector pZP7 was removed by DNA digestion with NcoI and PstI restriction enzymes (Boehringer Mannheim). The digested DNA was run on 1% agarose gel, cut out and gel purified using the Qiagen Gel Extraction Kit (Qiagen) as per manufacturer's instructions. A DNA fragment representing the coding region of Zeocin selective marker was amplified by PCR method with primers ZC13,946 (SEQ ID NO:28) and ZC13,945 (SEQ ID NO:29), and pZeoSV2(+) as a template. There are additional PstI and BclI restriction sites in primer ZC13,946 (SEQ ID NO:28), and additional NcoI and

SfuI sites in primer ZC13,945 (SEQ ID NO:29). The PCR fragment was cut with PstI and NcoI restriction enzymes and cloned into pZP7 vector prepared by cleaving with the same two enzymes and subsequent gel purification. This vector was named pZP7Z. Then the Zeocin coding region was removed by DNA digestion of vector pZP7Z with BclI and SfuI restriction enzymes. The digested DNA was run on 1% agarose gel, cut out and gel purified, and then ligated with a DNA fragment of Neomycin coding region cut from pZem228 vector (deposited at the American Type Culture Collection (ATCC), Manassas, VA; ATCC Deposit No. 69446) with the same restriction enzymes (BclI and SfuI).

This new vector was named pZP7N, in which the coding region for DHFR selective marker was replaced by the coding region for a Neomycin selective marker from vector pZem228. A stuffer fragment including an Xho1 site was added to pZP7N to create a vector suitable for high efficiency directional cloning of cDNA; this new vector was called pZP7NX. To prepare the vector for cDNA, 20μg of pZP7NX was digested with 20 units of EcoR1 (Life Technologies Gaithersberg,MD) and 20 units of Xho1 (Boehringer Mannheim Indianapolis,IN) for 5 hours at 37°C, then 68°C for 15 minutes. The digest was then run on a 0.8% low melt agarose 1XTAE gel to separate the stuffer from the vector. The vector band was excised and digested with "beta-Agarase" (New England Biolabs, Beverly, MA) following the manufacturer's recommendations. After ethanol precipitation the digested vector was resuspended in water to 45ng/ml in preparation for ligation of CD3+ selected cDNA library described below.

B. Preparation of primary human activated CD3+ selected cell cDNA library

Approximately $1.5X10^8$ primary human CD3+ selected cells stimulated in ionomycin/PMA were isolated by centrifugation after culturing at 37°C for 13 hours (Example 5C). Total RNA was isolated from the cell pellet using the "RNeasy Midi" kit from Qiagen, Inc. (Valencia, CA). mRNA was isolated from 225 micrograms of total RNA using the "MPG mRNA purification kit" from CPG Inc. (Lincoln Park, NJ). 3.4 micrograms of mRNA was isolated and converted to double stranded cDNA using the following procedure.

First strand cDNA from stimulated human CD3+ selected cells was synthesized as follows. Nine µl Oligo d(T)-selected poly(A) CD3+ RNA at a concentration of 0.34 µg/ µl and 1.0 µl of 1 µg /µl first strand primer ZC18,698 (SEQ ID NO:30) containing an *Xho*I restriction site were mixed and heated at 65°C for 4 minutes and cooled by chilling on ice. First strand cDNA synthesis was initiated by the addition of 9 µl of first strand buffer (5x SUPERSCRIPT® buffer; (Life Technologies), 4 µl of 100 mM dithiothreitol and 2 µl of a deoxynucleotide triphosphate solution containing 10 mM each of dATP, dGTP, dTTP and 5-methyl-dCTP (Pharmacia Biotech Inc.) to the RNA-primer mixture. The reaction mixture was incubated at 45°C for 4 minutes followed by the addition of 8 µl of 200 U/µl SuperscriptII®, RNase H- reverse transcriptase (Life technologies). The reaction was incubated at 45°C for 45 minutes followed by an incubation ramp of 1°C every 2 minutes to 50°C where the reaction was held for 10 minutes. To denature any secondary structure and allow for additional extension of the cDNA the reaction was then heated to 70° C for 2 minutes then dropped to 55° C for 4 minutes after which 2 µl of SuperscriptII® RT was added and incubated an additional 15 minutes followed by a ramp up to 70°C 1minute/1°C. Unincorporated nucleotides were removed from the cDNA by twice precipitating in the presence of 2 µg of glycogen carrier, 2.0 M ammonium acetate and 2.5 volume ethanol, followed by a 100 µl wash with 70% ethanol. The cDNA was resuspended in 98 µl water for use in second strand synthesis.

Second strand synthesis was performed on the first strand cDNA under conditions that promoted first strand priming of second strand synthesis resulting in DNA hairpin formation. The second strand reaction contained 98 µl of the first strand cDNA, 30 µl of 5x polymerase I buffer (100 mM Tris: HCl, pH 7.5, 500 mM KCl, 25 mM $MgCl_2$, 50 mM $(NH_4)_2SO_4$), 2 µl of 100 mM dithiothreitol, 6 µl of a solution containing 10 mM of each deoxynucleotide triphosphate, 5 µl of 5 mM b-NAD, 1 µl of 3 U/µl *E. coli* DNA ligase (New England Biolabs Inc.) and 4 µl of 10 U/µl *E. coli* DNA polymerase I (New England Biolabs Inc.). The reaction was assembled at room temperature and was incubated at room temperature for 2 minutes followed by the addition of 4 µl of 3.8 U/µl RNase H (Life Technologies). The reaction was incubated at 15° C for two hours followed by a 15 minute incubation at room temperature. Ten

microliters of 1M TRIS pH7.4 was added to the reaction and extracted twice with phenol/chloroform and once with chloroform, the organic phases were then back extracted with 50 µl of TE (10mM TRIS pH 7.4, 1mM EDTA), pooled with the other aqueous and ethanol precipitated in the presence of 0.3 M sodium acetate. The pellet was washed with 100 µl 70% ethanol air dried and resuspended in 40 µl water.

The single-stranded DNA of the hairpin structure was cleaved using mung bean nuclease. The reaction mixture contained 40 µl of second strand cDNA, 5 µl of 10x mung bean nuclease buffer (Life technologies), 5 µl of mung bean nuclease (Pharmacia Biotech Corp.) diluted to 1U/µl in 1X mung bean nuclease buffer. The reaction was incubated at 37°C for 45 minutes. The reaction was terminated by the addition of 10 µl of 1 M Tris: HCl, pH 7.4 followed by sequential phenol/chloroform and chloroform extractions as described above. Following the extractions, the cDNA was ethanol precipitated in the presence of 0.3 M sodium acetate. The pellet was washed with 100 µl 70% ethanol air dried and resuspended in 38 µl water.

The resuspended cDNA was blunt-ended with T4 DNA polymerase. The cDNA, which was resuspended in 38 µl of water, was mixed with 12 µl 5x T4 DNA polymerase buffer (250 mM Tris:HCl, pH 8.0, 250 mM KCl, 25 mM $MgCl_2$), 2 µl 0.1 M dithiothreitol, 6 µl of a solution containing 10 mM of each deoxynucleotide triphosphate and 2 µl of 1 U/µl T4 DNA polymerase (Boehringer Mannheim Corp.). After an incubation of 45 minutes at 15°C, the reaction was terminated by the addition of 30 µl TE followed by sequential phenol/chloroform and chloroform extractions and back extracted with 20 µl TE as described above. The DNA was ethanol precipitated in the presence of 2 µl Pellet Paint™ (Novagen) carrier and 0.3 M sodium acetate and was resuspended 11 µl of water.

Eco RI adapters were ligated onto the 5' ends of the cDNA described above to enable cloning into an expression vector. 11 µl of cDNA and 4 µl of 65 pmole/µl of *Eco* RI hemiphophorylated adaptor (Pharmacia Biotech Corp) were mixed with 5 µl 5x ligase buffer (Life Technologies), 2 µl of 10 mM ATP and 3 µl of 1 U/µl T4 DNA ligase (Life Technologies), 1 µl 10X ligation buffer (Promega Corp), 9 µl water. The extra dilution with 1X buffer was to prevent the pellet paint from precipitating. The reaction was incubated 9 hours in a water bath temperature ramp

from 10°C to 22°C over 9 hours, followed by 45 minutes at 25°C. The reaction was terminated by incubation at 68°C for 15 minutes.

To facilitate the directional cloning of the cDNA into an expression vector, the cDNA was digested with *Xho*I, resulting in a cDNA having a 5' *Eco* RI cohesive end and a 3' *Xho*I cohesive end. The *Xho*I restriction site at the 3' end of the cDNA had been previously introduced using the ZC18698 (SEQ ID NO:30) primer. Restriction enzyme digestion was carried out in a reaction mixture containing 35 µl of the ligation mix described above, 6 µl of 10x H buffer (Boehringer Mannheim Corp.), 1 µl of 2mg/ml BSA (Biolabs Corp.), 17 µl water and 1.0 µl of 40 U/µl *Xho*I (Boehringer Mannheim). Digestion was carried out at 37°C for 1 hour. The reaction was terminated by incubation at 68°C for 15 minutes followed by ethanol precipitation, washing drying as described above and resuspension in 30 µl water.

The resuspended cDNA was heated to 65° C for 5 minutes and cooled on ice, 4 µl of 5X gel loading dye (Research Genetics Corp.) was added, the cDNA was loaded onto a 0.8% low melt agarose 1X TAE gel (SEA PLAQUE GTG™ low melt agarose; FMC Corp.) and electrophoresed. The contaminating adapters and cDNA below 0.6 Kb in length were excised from the gel. The electrodes were reversed, molten agarose was added to fill in the wells, the buffer was changed and the cDNA was electrophoresed until concentrated near the lane origin. The area of the gel containing the concentrated cDNA was excised and placed in a microfuge tube, and the agarose was melted by heating to 65°C for 15 minutes. Following equilibration of the sample to 45°C, 2 µl of 1 U/µl Beta-agarase I (Biolabs, Inc.) was added, and the mixture was incubated for 90 min. at 45°C to digest the agarose. After incubation, 1 tenth volume of 3 M Na acetate was added to the sample, and the mixture was incubated on ice for 15 minutes. The sample was centrifuged at 14,000 x g for 15 minutes at room temperature to remove undigested agarose, the cDNA was ethanol precipitated, washed in 70% ethanol, air-dried and resuspended in 40 µl water.

To determine the optimum ratio of cDNA to vector several ligations were assembled and electroporated. Briefly, 2 µl of 5X T4 ligase buffer (Life Technologies), 1 µl of 10mM ATP, 1 µl pZP7NX digested with EcoR1-Xho1, 1 µl T4 DNA ligase diluted to 0.25u/µl (Life Technologies) water to 10 µl and 0.5, 1, 2 or 3 µl

of cDNA were mixed in 4 separate ligations, incubated at 22°C for 4 hours, 68°C for 20 minutes, sodium acetate-ethanol precipitated, washed, dried and resuspended in 10 μl. A single microliter of each ligation was electroporated into 40 μl DH10b ElectroMax™ electrocompetent bacteria (Life Technologies) using a 0.1cm cuvette (Biorad) and a Genepulser, pulse controller™ (Biorad) set to 2.5KV, 25lF, 200ohms. These cells were immediately resuspended in 1 ml. SOC broth (Manniatis et al. supra.) followed by 500μl of 50% glycerol-SOC as a preservative. These "glycerol stocks " were frozen in several aliquots at -70°C. An aliquot of each was thawed and plated serially on LB-agar plates supplemented with ampicillin at 100 μg/ml. Colony numbers indicated that the optimum ratio of CD3+ cDNA to pZP7NX vector was 1 μl to 45 ng; such a ligation yielded 4.5 million primary clones.

For the purpose of screening the library using a BaF3-based proliferation assay (Example 5) glycerol stocks from above were diluted into liquid cultures of 100 or 250 clones per pool in deep well microtiter plates, grown 24 hours at 37°C with shaking and plasmid isolated using a Qiagen kit following the manufacturer's instructions. Such DNA was subsequently transfected into BHK cells, media conditioned 72 hours, harvested and stored at -80°C, and subsequently placed on 5K BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells for 72 hours after which proliferation was assessed using an "Alamar blue" fluorescence assay (Example 5B and Example 2B).

Example 7

Expression Cloning of human zcytor17lig

The glycerol stocks from the activated human CD3+ selected cell library (Example 6) were added to Super Broth II™ (Becton Dickinson, Cockeysville, MD) + 0.1 mg/ml ampicillin (amp) at a concentration of 250 cells per 800 microliters. The *E. coli* were allowed to equilibrate for 24 hours at room temperature. At the time of inoculation, 400 microliters was plated on LB + amp plates to determine the actual titer of the inoculation. After 24 hours the plates were counted and then the final concentration of the SuperBrothII™ + *E. coli* was adjusted so that the final concentration was 250 cells per 1.2 ml. Three times 2 liters were inoculated for a total

of 6 liters. The media were then plated into 96-well deep well blocks (Qiagen). Plating was done on the 8-channel Q-Fill2™ dispenser (Genetix, Christchurch, Dorset, UK). The *E. coli* were grown overnight at 37°C shaking at 250 rotations/min. on a New Brunswick Scientific Innova 4900 multi-tier environment shaker. The *E. coli* were spun out of solution at 3000 rpm, using a Beckman GS-6KR centrifuge. These *E. coli* pellets were frozen at -20°C or used fresh before miniprepping the plasmid DNA. Each pellet contains approximately 250 cDNA clones from the human CD3+ selected cell library.

These pools of 250 cDNA clones were then mini-prepped using QIAprep™ 96 Turbo Miniprep kit (Qiagen). Plasmid DNA was eluted using 125 µl of TE (10 mM Tris pH 8, 1 mM EDTA). This plasmid DNA was then used to transfect BHK cells.

BHK transfection

BHK cells were plated in 96-well tissue culture plates at a density of 12,000 cells per well in a volume of 100 µl per well. Culture media was DMEM (GibcoBRL), 5% heat-inactivated fetal bovine serum, 2 mM L-glutamine (GibcoBRL), 1X PSN (GibcoBRL), 1 mM NaPyruvate (GibcoBRL).

The following day, BHK cells were washed once with 100 µl SFA. SFA is serum-free media which is DMEM/F12 or DMEM (Gibco/BRL), 2 mM GlutaMax™ (Gibco/BRL), 1 mM NaPyruvate, 10 µg/ml transferrin, 5 µg/ml insulin, 10 µg/ml fetuin, 2 µg/ml selenium, 25mM HEPES (Gibco/BRL), 100 µM non-essential amino acids (Gibco/BRL).

A DNA/Lipofectamine™ mix was made as follows: 2.2 µl Lipofectamine™ reagent (Gibco/BRL) was combined with 102.8 µl SFA at room temperature; approximately 5 µl of the plasmid DNA (200 ng/µl) was then added to the Lipofectamine™/SFA to form the DNA/Lipofectamine™ mixture, which was incubated at room temperature for 30 minutes. The SFA was removed from the BHK cells and the cells were incubated with 50 µl of the DNA/lipofectamine™ mix for 5 hours at 37°C with 5% CO_2. Fifty µl of the DNA/Lipofectamine™ mixture was added to each of two wells of the BHK cells, so that transfections were done in duplicate.

After BHK cells were incubated with DNA/Lipofectamine™ mix for 5 hours, the DNA/Lipofectamine™ mix was removed and 100 µl culture media was added. Cells were incubated overnight, the media was removed and replaced with 100 µl culture media. After culturing cells for 48-72 hours, conditioned media was removed, frozen at -80°C for a minimum of 20 minutes, thawed, and then 50 µl was assayed in the Baf3 proliferation assay, described in Example 5, to identify pools of 250 clones with ligand activity.

Twenty 96-well plates were screened in a single assay. This represented approximately 250 cDNAs/well or 480,000 cDNAs total. Of these, conditioned media from approximately 60 wells (representing 250 cDNAs per well) tested positive in the proliferation assay. One of these positive pools was chosen to break down and isolate a single cDNA that would encode the zcytor17lig. This was pool 62A12.

For pool 62A12, 1 µl of DNA was used to transform ElectroMax™ DH10B cells (Gibco/BRL) by electroporation. The transformants were plated on LB + amp (100 µg/ml) plates to give single colonies. From the electroporated pool, 672 individual colonies were selected by toothpick into seven 96-well plates containing 1.2 ml of SuperBrothII™ per well. These plates were numbered #62.1 through #62.7. These were cultured overnight and the plasmid DNA miniprepped as above. For all seven plates, plasmid DNA from the breakdown plates was transfected into BHK cells and assayed by proliferation as above, except that transfections were not done in duplicate.

Two positive clones 62.6C7 and 62.6E9 were identified by activity from a total of 672 clones. Plasmid DNA miniprepped from clone 62.6E9 was sequenced and a tentative identification was obtained, but a mixed sequence was obtained from this positive clones. To further isolate the zcytor17lig cDNA to a single clone, 1 µl of DNA from pool 62.6E9 was used to electroporate DH10B cells and the transformants plated on LB + amp (100 µg/ml) plates to give single colonies. Plasmid DNA miniprepped from several colonies was sequenced to give the exact DNA sequence. The polynucleotide sequence of zcytor17lig was full-length (SEQ ID NO:1) and its corresponding amino acid sequence is shown (SEQ ID NO:2).

Example 8

Construction of Mammalian Expression Vectors That Express zcytor17 Soluble Receptors: zcytor17CEE, zcytor17CFLG, zcytor17CHIS and zcytor17-Fc4

A. Construction of zcytor17 Mammalian Expression Vector containing zcytor17CEE, zcytor17CFLG and zcytor17CHIS

An expression vector was prepared for the expression of the soluble, extracellular domain of the zcytor17 polypeptide, pZp9zcytor17CEE, where the construct was designed to express a zcytor17 polypeptide comprised of the predicted initiating methionine and truncated adjacent to the predicted transmembrane domain, and with a C-terminal Glu-Glu tag (SEQ ID NO:32).

An approximately 1500 bp PCR product was generated using ZC29,451 (SEQ ID NO:33) and ZC29,124 (SEQ ID NO:34) as PCR primers to add EcoRI and BamHI restriction sites. A human HPVS in-house cDNA library was used as a template and PCR amplification was performed as follows: 30 cycles at 94°C for 1 minute, 65°C for 1 minute, 72°C for 1.5 minutes, then 72°C for 7 minutes; 10°C soak. The PCR reaction was ethanol precipitated and digested with EcoRI and BamHI restriction enzymes. The digested PCR product was gel purified on a 1.0% agarose gel and the approximately 1500 bp band excised. This band was then re-amplified using identical primers with the following cycling: 30 cycles at 94°C for 1 minute, 65°C for 1 minute, 72°C for 3 minutes, then 72°C for 7 minutes; 10°C soak. The PCR reaction was ethanol precipitated and digested with EcoRI and BamHI restriction enzymes. The digested PCR product was gel purified on a 1.0% agarose gel and the approximately 1500 bp band excised. The excised DNA was subcloned into plasmid CEEpZp9 that had been cut with EcoRI and BamHI, to generate plasmid with a GLU-GLU C-terminally tagged soluble receptor for zcytor17, zcytor17CEEpZp9. The extracellular domain in the zcytor17CEE cDNA in zcytor17CEEpZp9 has a silent mutation that changes the T to C at position 1705 of SEQ ID NO:4 (encoding a Pro residue at residue 403 of SEQ ID NO:5). As this mutation was silent, the zcytor17 cDNA in zcytor17CEEpZp9 encodes the polypeptide as shown in SEQ ID NO:5. Moreover, because of the construct used, a Gly-Ser residue pair was inserted C-terminal to the end of the soluble, extracellular domain of zcytor17 and prior to the C-terminal Glu-Glu

Tag (SEQ ID NO:32). As such, the tag at the C-terminus of the zcytor17 extracellular domain, was a Glu-Glu tag as shown in (SEQ ID NO:17). Plasmid CEEpZp9 is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 promoter, multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator. Using standard molecular biological techniques zcytor17CEEpZp9 was electroporated into DH10B competent cells (GIBCO BRL, Gaithersburg, MD) according to manufacturer's direction and plated onto LB plates containing 100 μg/ml ampicillin, and incubated overnight. Colonies were screened by restriction analysis, or PCR from DNA prepared from individual colonies. The insert sequence of positive clones was verified by sequence analysis. A large scale plasmid preparation was done using a QIAGEN® Maxi prep kit (Qiagen) according to manufacturer's instructions.

The same process was used to prepare the zcytor17 soluble receptors with a C-terminal His tag, composed of 6 His residues in a row; and a C-terminal FLAG® tag (SEQ ID NO:36), zcytor17CFLAG. To construct these constructs, the aforementioned vector has either the HIS or the FLAG® tag in place of the glu-glu tag (e.g., SEQ ID NO:17; SEQ ID NO;32 or SEQ ID NO:35).

B. Mammalian Expression Construction of Soluble human zcytor17 receptor: zcytor17-Fc4

An expression vector, pEZE-2 hzcytor17/Fc4, was prepared to express a C-terminally Fc4 tagged soluble version of hzcytor17 (human zcytor17-Fc4) in PF CHO cells. PF CHO cells are an in house CHO cell line adapted for growth in protein-free medium (ExCell 325 PF medium; JRH Biosciences). The in house CHO cell line was originally derived from CHO DG44 cells (G. Urlaub, J. Mitchell, E. Kas, L.A. Chasin, V.L. Funanage, T.T. Myoda and J.L. Hamlin, "The Effect Of Gamma Rays at the Dihydrofolate Reductase Locus: Deletions and Inversions," Somatic Cell and Molec. Genet., 12: 555-566 (1986). A fragment of zcytor17 cDNA that includes the polynucleotide sequence from extracellular domain of the zcytor17 receptor was fused

in frame to the Fc4 polynucleotide sequence (SEQ ID NO:37) to generate a zcytor17-Fc4 fusion (SEQ ID NO:38 and SEQ ID NO:39). The pEZE-2 vector is a mammalian expression vector that contains the Fc4 polynucleotide sequence and a cloning site that allows rapid construction of C-terminal Fc4 fusions using standard molecular biology techniques.

A 1566 base pair fragment was generated by PCR, containing the extracellular domain of human zcytor17 and the first two amino acids of Fc4 (Glu and Pro) with FseI and BglII sites coded on the 5' and 3' ends, respectively. This PCR fragment was generated using primers ZC29,157 (SEQ ID NO:40) and ZC29,150 (SEQ ID NO:41) by amplification from a plasmid containing the extracellular domain of human zcytor17 (pZp9zcytor17CEE) (Example 8A). The PCR reaction conditions were as follows: 25 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes; 1 cycle at 72°C for 10 minutes; followed by a 4°C soak. The fragment was digested with FseI and BglII restriction endonucleases and subsequently purified by 1% gel electrophoresis and band purification using QiaQuick gel extraction kit (Qiagen). The resulting purified DNA was ligated for 5 hours at room temperature into a pEZE-2 vector previously digested with FseI and BglII containing Fc4 3' of the FseI and BglII sites.

Two µl of the ligation mix was electroporated in 37 µl DH10B electrocompetent *E. coli* (Gibco) according to the manufacturer's directions. The transformed cells were diluted in 400 µl of LB media and plated onto LB plates containing 100 µg/ml ampicillin. Clones were analyzed by restriction digests and positive clones were sent for DNA sequencing to confirm the sequence of the fusion construct. One microliter of a positive clone was transformed into 37 µl of DH10B electrocompetent *E. coli* and streaked on a LB/amp plate. A single colony was picked from this streaked plate to start a 250 ml LB/amp culture that was then grown overnight at 37°C with shaking at 250 rpm. This culture was used to generate 750 µg of purified DNA using a Qiagen plasmid Maxi kit (Qiagen).

Example 9

Transfection And Expression Of Zcytor17 Soluble Receptor Polypeptides

BHK 570 cells (ATCC No. CRL-10314), DG-44 CHO, or other mammalian cells are plated at about $1.2X10^6$ cells/well (6-well plate) in 800 μl of appropriate serum free (SF) media (e.g., DMEM, Gibco/BRL High Glucose) (Gibco BRL, Gaithersburg, MD). The cells are transfected with expression plasmids containing zcytor17CEE, zcytor17CFLG, zcytor17CHIS or zcytor17-Fc4 (Example 8), using Lipofectin™ (Gibco BRL), in serum free (SF) media according to manufacturer's instruction. Single clones expressing the soluble receptors are isolated, screened and grown up in cell culture media, and purified using standard techniques.

A. Mammalian expression of soluble human zcytor17CEE receptor

BHK 570 cells (ATCC NO: CRL-10314) were plated in T-75 tissue culture flasks and allowed to grow to approximately 50 to 70% confluence at 37°C, 5% CO_2, in DMEM/FBS media (DMEM, Gibco/BRL High Glucose, (Gibco BRL, Gaithersburg, MD), 5% fetal bovine serum, 1 mM L-glutamine (JRH Biosciences, Lenea, KS), 1 mM sodium pyruvate (Gibco BRL)). The cells were then transfected with the plasmid containing zcytor17CEE (Example 8A) using Lipofectamine™ (Gibco BRL), in serum free (SF) media formulation (DMEM, 10 mg/ml transferrin, 5 mg/ml insulin, 2 mg/ml fetuin, 1% L-glutamine and 1% sodium pyruvate). Ten micrograms of the plasmid DNA pZp9zcytor17CEE (Example 8A) was diluted into a 15ml tube to a total final volume of 500 µl with SF media. Fifty microliters of Lipofectamine was mixed with 450 µl of SF medium. The Lipofectamine mix was added to the DNA mix and allowed to incubate approximately 30 minutes at room temperature. Four ml of SF media was added to the DNA:Lipofectamine mixture. The cells were rinsed once with 5 ml of SF media, aspirated, and the DNA:Lipofectamine mixture was added. The cells were incubated at 37°C for five hours, and then 5 ml of DMEM/10%FBS media was added. The flask was incubated at 37°C overnight after which time the cells were split into the selection media (DMEM/FBS media from above with the addition of 1 µM methotrexate or 10 µM Methotrexate (Sigma Chemical Co., St. Louis, Mo.) in 150 mm plates at 1:2, 1:10, and 1:50. Approximately 10 days post-transfection, one 150 mm plate of 1 µM methotrexate resistant colonies was trypsinized, the cells were pooled, and one-half of the cells were replated in 10 µM methotrexate; to further amplify

expression of the zcytor17CEE protein. A conditioned-media sample from this pool of amplified cells was tested for expression levels using SDS-PAGE and Western analysis.

B. Mammalian expression of soluble human zcytor17-Fc4 receptor

Five replicates of 200 µg of pEZE-2hzcytor17Fc4 plasmid DNA (Example 8B) were linearized by restriction digestion with FspI, a restriction enzyme that cuts once within the vector and does not disturb genes necessary for expression. 200 µg of CHO cell genomic DNA was added to each replicate as carrier DNA and then the DNA was precipitated by addition of 0.1 volumes of 3M Sodium Acetate pH 5.2 and 2.2 volumes ethanol followed by a 15 minute ice incubation and microcentrifugation at 4°C. The resulting DNA pellets were washed in 70% ethanol and air dried before being resuspended in 100 µl protein free (PF) CHO non-selection growth media (21 g/L PF CHO Ex Cell 325 /200 mM L-glutamine (Gibco)/100 mM sodium pyruvate (Gibco)/1x HT Supplement (Gibco). Ten million PF CHO passage 61 cells were added to the DNA in 600 µl of PF CHO non-selection growth media and then electroporated in a Gene Pulser II Electroporation system (BioRad) using 950 µF capacitance and 300 Kv using a 0.4 cm gap Gene Pulser (BioRad) electroporation cuvette. All 5 replicates of the electroporated cells were pooled and directly selected in –HT media (21 g/L PF CHO Ex Cell 325/ 200 mM L-glutamine (Gibco)/100 mM sodium pyruvate (Gibco). Cells were selected for 15 days in –HT media before being passaged at 4 x 10^5 ml into 50 nm MTX selection. Eight days later cells were seeded at 3.5X10^5 cells/ml into 200 mM MTX selection. After one week, cells were seeded at 4X10^5 cells/ml into 1 µM MTX selection. After two weeks at 1 µM MTX, cells were seeded at 1X10^6 cells/ml into 50 ml to generate conditioned medium. The resulting 72 hour conditioned media was analyzed by probing western blots with an antibody generated against human Ig. The cells produced hzcytor17/Fc4 protein at approximately 1 mg/L.

C. Larger-scale mammalian expression of soluble human zcytor17-Fc4 receptor

Two hundred µg of pEZE-2hzcytor17Fc4 plasmid DNA (Example 8B) was linearized by restriction digestion with FspI, a restriction enzyme that cuts once

within the pEZE-2 vector and does not disturb genes necessary for expression. Two hundred micrograms of CHO genomic DNA (prepared in-house) was added as carrier DNA and then the DNA was precipitated by addition of 0.1 volumes of 3M Sodium Acetate pH 5.2 and 2.5 volumes ethanol followed by microcentrifugation at Room temperature. Five replicate DNA pellets were made and transformed. The resulting DNA pellet was washed in 70% ethanol and air dried before being resuspended in 100 µl PF CHO non-selection growth media (21 g/L PF CHO Ex Cell 325 /200 mM L-glutamine (Gibco)/100 mM sodium pyruvate (Gibco)/1x HT Supplement (Gibco). Ten million PF CHO cells were added to the DNA in 600 µl of PF CHO non-selection growth media and then electroporated in a Gene Pulser II Electroporation system (BioRad) using 950 µF capacitance and 300 volts using a 0.4 cm gap Gene Pulser (BioRad) electroporation cuvette. The electroporated cells were pooled and put directly into selection in –HT media (21 g/L PF CHO Ex Cell 325/ 200 mM L-glutamine (Gibco)/100 mM sodium pyruvate (Gibco). Cells were selected for 14 days in –HT media before being passaged at 4 x 10^5/ml into 50 nm MTX selection. Cells were amplified to 200nM MTX and then to 1uM MTX. The –HT, 50nM, and 1uM pools were seeded at 1 x 10^6 c/ml for 48 hours, and the resulting conditioned media was analyzed by probing western blots with an antibody generated against human Ig.

Example 10

Purification of zcytor17 soluble receptors from BHK 570 and CHO cells

A. Transient mammalian expression and purification of soluble human zcytor17-Fc4 receptor

pEZE-2hzcytor17Fc4 plasmid DNA (Example 8B) was introduced into 40 maxi plates of BHK cells using Lipofectamine (Gibco BRL) as described herein and in manufacturer's instructions. Cells were allowed to recover overnight, then were rinsed and refed with serum-free medium (SL7V4, made in-house). After 72 hours, the media was collected and filtered, and cells were refed with serum-free medium. After 72 hours, the media was again collected and filtered.

The serum-free conditioned media (2 x 1.5 L batches) from transiently transfected BHK cells was pumped over a 1.5 ml Protein A-agarose column in 20 mM

Tris, pH 7.5, 0.5 M NaCl. The column was washed extensively with this buffer and then the bound protein was eluted with 1 ml of 0.2 M glycine, pH 2.5, 0.5 M NaCl. The eluted protein was collected into 0.1 ml of 2 M Tris, pH 8.5. Aliquots were collected for SDS-polyacrylamide gel electrophoresis and the bulk zcytor17-Fc was dialyzed overnight against PBS. The soluble receptor was sterile filtered and placed in aliquots at –80°C.

B. Purification of zcytor17-Fc4

Recombinant carboxyl terminal Fc4 tagged zcytor17 (Example 8 and Example 9) was produced from transfected CHO cells. The CHO transfection was performed using methods known in the art. Approximately five-liters of conditioned media were harvested and sterile filtered using Nalgene 0.2μm filters.

Protein was purified from the filtered media by a combination of Poros 50 protein A affinity chromatography (PerSeptive Biosystems, 1-5559-01, Framingham, MA) and Superdex 200 gel exclusion chromatography column (Amersham Pharmacia Biotech, Piscataway, NJ). Culture medium was directly loaded onto a 10x70mm (5.5-ml bed volume) protein A affinity column at a flow of about 3-10 ml/minute. Following column washing for ten column volumes of PBS, bound protein was eluted by five column volumes of 0.1 M glycine, pH 3.0 at 10 ml/minute). Fractions of 2 ml each were collected into tubes containing 100μl of 2.0 M Tris, pH 8.0, in order to neutralize the eluted proteins. Samples from the affinity column were analyzed by SDS-PAGE with coomassie staining and Western blotting for the presence of zcytor17-Fc4 using human Ig-HRP. Zcytor17-Fc4-containing fractions were pooled and concentrated to 1-2 ml using Biomax-30 concentrator (Millipore), and loaded onto a 20x580 mm Superdex 200 gel filtration column. The fractions containing purified zcytor17-Fc4 were pooled, filtered through 0.2 μm filter, aliquoted into 100 μl each, and frozen at –80°C. The concentration of the final purified protein was determined by BCA assay (Pierce, Rockford, IL).

C. SDS-PAGE and Western blotting analysis of zcytor17/Fc4

Recombinant zcytor17-Fc4 was analyzed by SDS-PAGE (Nupage 4-

12%, Invitrogen, Carlsbad, CA) with coomassie staining method and Western blotting using human Ig-HRP. Either the conditioned media or purified protein was electrophoresed using an Invitrogen Novex's Xcell II mini-cell, and transferred to nitrocellulose (0.2 mm; Invitrogen, Carlsbad, CA) at room temperature using Novex's Xcell II blot module with stirring according to directions provided in the instrument manual. The transfer was run at 500 mA for one hour in a buffer containing 25 mM Tris base, 200 mM glycine, and 20% methanol. The filters were then blocked with 10% non-fat dry milk in PBS for 10 minutes at room temperature. The nitrocellulose was quickly rinsed, then the human Ig-HRP antibody (1:2000) was added in PBS containing 2.5% non-fat dry milk. The blots were incubated for two hours at room temperature, or overnight at 4°C, with gentle shaking. Following the incubation, the blots were washed three times for 10 minutes each in PBS, then quickly rinsed in H_2O. The blots were developed using commercially available chemiluminescent substrate reagents (SuperSignal® ULTRA reagents 1 and 2 mixed 1:1; reagents obtained from Pierce, Rockford, IL), and the signal was captured using Lumi-Imager's Lumi Analyst 3.0 software (Boehringer Mannheim GmbH, Germany) for exposure times ranging from 10 second to 5 minutes or as necessary.

The purified zcytor17-Fc4 appeared as a single band with either the coomassie or silver staining at about 220 kDa under non-reducing conditions, and at about 120 kDa under reducing conditions, suggesting the dimeric form of zcytor17-Fc4 under non-reducing conditions as expected.

Example 11

Assay using zcytor17 soluble receptor zcytor17-Fc4 Soluble receptor in competitive inhibition assay

BaF3/zcytor17/WSX-1/OSMRbeta cells and BaF3/zcytor17/OSMRbeta cells were spun down and washed in mIL-3 free media. The cells were spun and washed 3 times to ensure the removal of the mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at 5000 cells per well in a volume of 100 µl per well using the mIL-3 free media.

Both conditioned media from the CCRF-CEM and CCRF-HSB2 cell

activation and the human CD3+ selected cells, described in Example 5, were added in separate experiments at 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.75%, 0.375%, and 0.187 % concentrations, with or without zcytor17 soluble receptors (Zcytor17-Fc4; See, Example 9 and Example 10) at 1-10 μg/ml. The total assay volume was 200 μl.

The assay plates were incubated at 37°C, 5% CO_2 for 3-5 days at which time Alamar Blue (Accumed) was added at 20 μl/well. Plates were again incubated at 37°C, 5% CO_2 for 16-24 hours. Plates were read on the Fmax™ plate reader (Molecular Devices) as described in Example 2. Results demonstrated partial inhibition of cell growth with zcytor17-Fc4 soluble receptor at 10 μg/ml, confirming that the factor in each sample was specific for the zcytor17 receptor.

Titration curves, diluting out the soluble receptor, or soluble receptor heterodimers comprising zcytor17/OSMR and zcytor17/WSX-1 were also ran using the above stated assay to determine whether zcytor17 receptors are able to completely inhibit growth, for example, at low or physiologic concentrations.

Similar competitive inhibition assays were carried out using purified human zcytor17lig (Example 35) and soluble receptors in luciferase assays (Example 20). The results show that both homodimeric zcytor17 and heterodimeric zcytor17/OSMR are capable of inhibiting the activity of zcytor17lig.

Example 12

Secretion trap assay

A secretion trap assay was used to test the binding of the zcytor17lig to receptors comprising zcytor17 receptor, such as the zcytor17 receptor or receptor heterodimers comprising zcytor17/OSMR and zcytor17/WSX-1. Zcytor17lig plasmid DNA was transfected into COS cells, and used to assess binding of the zcytor17lig to receptors comprising zcytor17 receptor by secretion trap as described below.

A. COS Cell Transfections

The COS cell transfection was performed as follows: 800 ng of zcytor17lig cDNA and 4 μl Lipofectamine™ were mixed in 80 μl serum free DMEM media (55mg sodium pyruvate, 146mg L-glutamine, 5mg transferrin, 2.5mg insulin,

1μg selenium and 5mg fetuin in 500ml DMEM), and incubated at room temperature for 30 minutes. Then 320 μl serum free DMEM media was added. This 500 μl mixture was added onto $2x10^5$ COS cells/well plated on 12-well tissue culture plate and incubated for 5 hours at 37°C. Then 500μl 20% FBS DMEM media (100 ml FBS, 55 mg sodium pyruvate and 146mg L-glutamine in 500ml DMEM) was added, and cells were incubated overnight.

B. Secretion Trap Assay

The secretion trap was performed as follows: Media was rinsed off cells with PBS, then cells were fixed for 15 minutes with 1.8% Formaldehyde in PBS. Cells were then washed with PBS/0.1%BSA and permeabilized with 0.1% Triton-X in PBS for 15 minutes, and again washed with PBS/0.1%BSA. Cells were blocked for 1 hour with PBS/0.1%BSA. Depending on which soluble receptor was used, the cells were incubated for 1 hour in TNB with: (A) 1-3 μg/ml zcytor17 soluble receptor zcytor17-Fc4 fusion protein (Example 10); or (B) 1-3 μg/ml zcytor17/OSMRbeta soluble receptor protein. Cells were then washed with TNT. Depending on which soluble receptor was used (e.g., if labeled with an Fc4 tag (SEQ ID NO:37), C-terminal FLAG tag (SEQ ID NO:26), or CEE tag (SEQ ID NO:32; SEQ ID NO:35)), cells were incubated for another hour with: (A) 1:200 diluted goat-anti-human Ig-HRP (Fc specific); (B) 1:1000 diluted M2-HRP; (C) 1:1000 diluted anti-GluGlu antibody-HRP; or (D) 1:300 diluted streptavidin-HRP (NEN kit) in TNB, for example. Again cells were washed with TNT.

To detect positive binding fluorescein tyramide reagent was diluted 1:50 in dilution buffer (NEN kit) and incubated for 4-6 minutes, and washed with TNT. Cells were preserved with Vectashield Mounting Media (Vector Labs Burlingame, CA) diluted 1:5 in TNT. Cells were visualized using a FITC filter on fluorescent microscope. The results of this assay showed that human zcytor17lig does not bind to any of the soluble receptors. These data suggest that the structure of zcytor17lig was sensitive to the fixation step in this protocol, as it was clearly capable of binding to cell-surface receptors (see, for example, the flow cytometry data presented below in Example 39.

Example 13

Chromosomal Assignment and Placement of the gene sequence for the zcytor17lig

The zcytor17lig gene sequence was mapped to human chromosome 12 using the commercially available version of the "Stanford G3 Radiation Hybrid Mapping Panel" (Research Genetics, Inc., Huntsville, AL). The "Stanford G3 RH Panel" contains DNA from each of 83 radiation hybrid clones of the whole human genome, plus two control DNAs (the RM donor and the A3 recipient). A publicly available WWW server located on the Internet at www.stanford.edu allows chromosomal localization of markers and genes.

For the mapping of the zcytor17lig gene sequence with the "Stanford G3 RH Panel", 20 μl reactions were set up in a 96-well microtiter plate compatible for PCR (Stratagene, La Jolla, CA) and used in a "RoboCycler Gradient 96" thermal cycler (Stratagene). Each of the 95 PCR reactions consisted of 2 μl 10X PCR reaction buffer (Qiagen, Inc., Valencia, CA), 1.6 μl dNTPs mix (2.5 mM each, PERKIN-ELMER, Foster City, CA), 1 μl sense primer, ZC41,458 (SEQ ID NO:42), 1 μl antisense primer, ZC41,457 (SEQ ID NO:43), 2 μl "RediLoad" (Research Genetics, Inc., Huntsville, AL), 0.1 μl Qiagen HotStarTaq DNA Polymerase (5 units/μl), 25 ng of DNA from an individual hybrid clone or control and distilled water for a total volume of 20 μl. The reactions were overlaid with an equal amount of mineral oil and sealed. The PCR cycler conditions were as follows: an initial 1 cycle 15 minute denaturation at 95°C, 35 cycles of a 45 second denaturation at 95°C, 1 minute annealing at 53°C and 1 minute and 15 seconds extension at 72°C, followed by a final 1 cycle extension of 7 minutes at 72°C. The reactions were separated by electrophoresis on a 2% agarose gel (EM Science, Gibbstown, NJ) and visualized by staining with ethidium bromide.

The results showed linkage of the zcytor17lig gene sequence to the chromosome 12 marker SHGC-83339 with a LOD score of >11 and at a distance of 17 cR_10000 from the marker. This marker positions zcytor17lig gene in the 12q24.31 chromosomal region.

Example 14

Identification and cloning of murine zcytor17lig

A. Identification of full length murine zcytor17lig

Using the human zcytor17lig peptide sequence (SEQ ID NO:2) to query an in house DNA database, a murine cDNA, Genbank Accession No. AK005939, was identified as a potential partial sequence for the murine zcytor17lig. The AK005939 cDNA sequence was used to query a database containing murine genomic fragments. A genomic contig of the murine zcytor17lig was assembled (SEQ ID NO:76). Prediction of coding potential on this genomic fragment with the program Genscan revealed a likely cDNA sequence, with the same gene structure as the human zcytor17lig. A murine cDNA sequence is represented in SEQ ID NO:10, and corresponding polypeptide sequence is shown in SEQ ID NO:11.

B. Cloning of mouse zcytor17lig from a mouse testis cDNA library by PCR.

Based on the genomic sequence (SEQ ID NO:76), two PCR primers were designed and used to identify a cDNA source of mouse zcytor17lig by PCR. These Primers ZC41498 (SEQ ID NO:86) and ZC41496 (SEQ ID NO:87) were designed to the putative 5' and 3' untranslated regions of the mouse sequences (SEQ ID NO:76 and SEQ ID NO:10). Several cDNA sources were screened by PCR, including Marathon-ready cDNAs (Clontech) and aliquots of locally made cDNA libraries. Products were visualized on 1% agarose gels. Bands of the expected size were observed in reactions utilizing a mouse testis cDNA library template. These PCR reactions were successfully performed in approximately 50 μl volumes with or without 10% DMSO, using pfu turbo polymerase (Stratagene) according to the manufacturer's recommendations; with an additional application of a wax hot-start employing hot start 50s (Molecular Bioproducts, Inc. San Diego, CA). PCR thermocycling was performed with a single cycle of 94°C for 4 min; followed by 40 cycles of 94°C: 30 seconds, 48°C: 30 seconds, 72°C: 50 seconds; with additional final 72°C extension for 7 minutes. The two PCR reactions were pooled and purified using low melt agarose and Gelase agarose digesting enzyme (Epicenter, Inc. Madison, WI) according to the manufacturer's recommendations.

DNA sequence determination of these PCR products revealed a murine

zcytor17 cDNA sequence (SEQ ID NO:90) which comprised an ORF identical to SEQ ID NO:10, confirming that SEQ ID NO:10 encoded the mouse zcytor17lig polypeptide. PCR primers, ZC41583 (SEQ ID NO:88) and ZC41584 (SEQ ID NO:89), were then used to add FseI and AscI restriction sites and a partial Kozak sequence to the mcytor17lig open reading frame and termination codon (SEQ ID NO:92). A Robocycler 40 thermocycler (Stratagene) was used to run a temperature gradient of annealing temperatures and cycling as follows. Pfu turbo polymerase (Stratagene) was applied as described above, but only in 10% DMSO. Cycling was performed with a single cycle of 94°C for 4 min; followed by 20 cycles of 94°C: 30 seconds, 65°C to 51°C gradient: 30 seconds, 72°C: 1 minute; and a single 72°C extension for 7 minutes. The template for this second thermocycling reaction was 1 μl of the initial gel-purified mcytor17lig PCR product, above. Resulting PCR product from the three lowest temperature reactions were pooled and gel purified using the Gelase (Epicenter) method described above. This purified mzcytor17lig was digested with FseI and AscI and ligated into a pZP7X vector modified to have FseI and AscI sites in its cloning site. Plasmid pZP7X is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 (MT-1) promoter, multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selective marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene, and the SV40 terminator. The cloned murine cDNA sequence is represented in SEQ ID NO:90, and corresponding polypeptide sequence is shown in SEQ ID NO:91 (which is identical to SEQ ID NO:11).

Example 15

Isolation of mouse zcytor17lig cDNA clone from an activated mouse spleen library

A. Murine Primary Source used to isolate mouse zcytor17lig

Mouse spleens from Balb/C mice, are collected and mashed between frosted-end slides to create a cell suspension. The isolated primary mouse cell yield is expected to be about $6.4X10^8$ cells prior to selection described below.

The spleen cells are suspended in 9.6 ml MACS buffer (PBS, 0.5%

EDTA, 2mM EDTA). 1.6 ml of cell suspension is removed and 0.4 ml CD90 (Thy1.2) microbeads (Miltenyi Biotec) added. The mixture is incubated for 15 min. at 4°C. These cells labeled with CD90 beads are washed with 30 ml MACS buffer, and then resuspended in 2 ml MACS buffer.

A VS+ column (Miltenyi) is prepared according to the manufacturer's instructions. The VS+ column is then placed in a VarioMACS™ magnetic field (Miltenyi). The column is equilibrated with 5 ml MACS buffer. The isolated primary mouse cells are then applied to the column. The CD90 negative cells are allowed to pass through. The column is rinsed with 9 ml (3 X 3 ml) MACS buffer. The column is then removed from the magnet and placed over a 15 ml falcon tube. CD90+ cells are eluted by adding 5 ml MACS buffer to the column and bound cells flushed out using the plunger provided by the manufacturer. The incubation of the cells with the CD90 magnetic beads, washes, and VS+ column steps (incubation through elution) above are repeated once more. The resulting CD90+ fractions from the 2 column separations are pooled. The yield of CD90+ selected mouse spleen cells are expected to be about $1X10^8$ total cells.

A sample of the pooled CD90+ selected mouse cells is removed for staining and sorting on a fluorescent antibody cell sorter (FACS) to assess their purity. A PE-conjugated hamster anti-mouse CD3ε antibody (PharMingen) is used for staining and sorting the CD90+ selected cells. The mouse CD90+ selected cells should be about 93% CD3+ cells, suggesting the cells are 93% T-cells.

The murine CD90+ selected cells are activated by incubating $3X10^6$ cells/ml in RPMI + 5% FBS + PMA 10 ng/ml and Ionomycin 0.5 µg/ml (Calbiochem) for overnight at 37°C. The supernatant from these activated CD90+ selected mouse cells is tested for zcytor17lig activity as described below. Moreover, the activated CD90+ selected mouse cells are used to prepare a cDNA library, as described in Example 16, below.

Example 16

Cloning of mouse zcytor17lig from a mouse CD90+ selected cell library

Screening of a primary mouse activated CD90+ selected cell cDNA

library can reveal isolated cDNA that is a novel member of the four-helix bundle cytokine family that would encode the mouse ortholog of the human zcytor17lig. The cDNA is identified by hybridization screening.

A. The vector for CD90+ selected library construction

The vector, pZP7N is used for CD3+ selected library construction (See Example 6A).

B. Preparation of primary mouse activated CD90+ selected cell cDNA library

Approximately $1.5X10^8$ primary mouse CD90+ selected cells stimulated in ionomycin/PMA (Example 15) are isolated by centrifugation. Total RNA is isolated from the cell pellet, and converted to double stranded cDNA as described in Example 6B. This DNA is subsequently transfected into BHK cells, as described in Example 6B, and proliferation is assessed using an "Alamar blue" fluorescence assay (Example 2B).

For the purpose of screening the library by secretion trap cloning, a complex, amplified form of the library is needed to transfect COS-7 cells. 4.8 million clones are plated on 110 15cm LB-agar plates supplemented with 100 μg/ml ampicillin, 10 μg/ml methicillin. After growing the plates overnight at 37°C the bacteria are harvested by scraping and pelleted. Plasmid DNA is extracted from the pelleted bacteria using a Nucleobond-giga™ (Clonetech) following the manufacturer's instructions. This plasmid is then used to transfect COS-7 cells on slides and screened using the secretion trap technique described below (Example 17).

C. Screening the activated mouse cDNA library

Approximately $5X10^5$ clones are plated on 10 LB/Amp Maxi plates. The colonies are lifted, denatured, neutralized, and cross-linked using the standard procedure (Sambrook, J. et al. supra.). Fifty nanograms of the 300 bp 5' RACE PCR fragment (Example 14) is labeled with ^{32}P using Prime-Itr RmT random primer labeling kit (Stratagene). The 10 filters are hybridized with this labeled probe at 65°C overnight using ExpressHyb™ Hybridization Solution (Clontech). The filters are then washed sequentially at 60°C for 1 hour three times with 0.2xSSC (30 mM NaCl, 3 mM sodium

citrate, pH 7.0), 0.1% SDS; and then at 65°C for 1 hour. The filters are exposed at -80°C overnight, and the X-ray film are developed. Agar plugs containing the positive colonies are pulled, and the clones plated on 10-cm LB/Amp plates. The colonies are then filter-lifted and hybridized again following the same procedure described above. Single DNA clones are isolated and sequenced using standard methods, to identify the mouse cDNA.

Example 17

Mouse zcytor17lig does not bind to human zcytor17 soluble receptor in secretion trap assay

The DNA for mouse clone mzcytor17lig/pZP7 was transfected into COS cells, and the binding of zcytor17 comprising soluble receptors (human zcytor17 soluble receptor zcytor17-Fc4 (Example 10), or soluble receptor heterodimers (zcytor17/WSX-1 or BaF3/zcytor17/OSMRbeta), to the transfected COS cells were tested by a secretion trap assay (Example 12). The assay confirmed that the mouse zcytor17lig does not bind to human zcytor17 soluble receptor.

The COS cell transfection was performed as per Example 12 using about 0.7 μg mouse zcytor17lig cDNA (Example 16) in 3 μl.

The secretion trap was performed as per example 12 using, for example, 1 μg/ml zcytor17 soluble receptor Fc4 fusion protein (Example 10) (or zcytor17 comprising soluble receptor heterodimers as described herein) in TNB, and 1:200 diluted goat-anti-human Ig-HRP (Fc specific) in TNB for the detectable antibody. Positive binding of the soluble human zcytor17 receptor to the prepared fixed cells was not detected with fluorescein tyramide reagent as per Example 12. Cells were preserved and visualized according to Example 12.

Results indicated that the mouse zcytor17lig does not bind to human zcytor17 soluble receptor (or zcytor17 comprising soluble receptor heterodimers as described herein).

Example 18

Expression of mouse zcytor17lig in mammalian cells

Mammalian expression of mouse zcytor17lig

BHK 570 cells (ATCC No: CRL-10314) were plated in 10 cm tissue culture dishes and allowed to grow to approximately 20% confluence overnight at 37°C, 5% CO_2, in DMEM/FBS media (DMEM, Gibco/BRL High Glucose media; Gibco BRL, Gaithersburg, MD), 5% fetal bovine serum (Hyclone, Logan, UT), 1 mM L-glutamine (JRH Biosciences, Lenexa, KS), 1 mM sodium pyruvate (Gibco BRL). The cells were then transfected with the plasmid mzcytor17lig/pZP7X (Example 14) using a mammalian stable Lipofectamine (GibcoBRL) transfection kit according to the manufacturer's instructions.

One day after transfection, the cells were split 1:10 and 1:20 into the selection media (DMEM/FBS media with the addition of 1 μM methotrexate (Sigma Chemical Co., St. Louis, MO)) in 150 mm plates. The media on the cells was replaced with fresh selection media at day 5 post-transfection. Approximately 10 days post-transfection, methotrexate resistant colonies were trypsinized and the cells pooled and plated into large-scale culture flasks. Once the cells were grown to approximately 90% confluence, they were rinsed with PBS three times, and cultured with serum-free ESTEP2 media (DMEM (Gibco BRL), 0.11 g/l Na Pyruvate, 3.7 g/l $NaHCO_3$, 2.5 mg/l insulin, 5 mg/l transferrin, pH7.0) conditioned media. The conditioned media was collected three days later, and put into a BaF3 proliferation assay using Alamar Blue, described in Example 19 below.

Example 19

Mouse zcytor17lig does not activate human zcytor17 receptor in BaF3 assay using Alamar Blue

Proliferation of BaF3/zcytor17, BaF3/zcytor17/OSMRbeta and BaF3/zcytor17/WSX-1 cells (Example 4, and 5B) was assessed using serum-free conditioned media from BHK cells expressing mouse zcytor17lig (Example 18).

BaF3/Zcytor17, BaF3/zcytor17/OSMRbeta and BaF3/zcytor17/WSX-1 cells were spun down, washed and plated in mIL-3 free media as described in Example 5B. Conditioned media from BHK cells expressing mouse zcytor17lig (Example 18) was diluted with mIL-3 free media to 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.75%

and 0.375% concentrations. The proliferation assay was performed as per Example 5B. The results of this assay were negative, indicating that mouse zcytor17lig does not activate human zcytor17, zcytor17/OSMRbeta, or zcytor17/WSX-1 receptor complexes.

Example 20

Human zcytor17lig activates human, zcytor17/OSMRbeta receptor, in luciferase assay

A. Construction of BaF3/KZ134/zcytor17 cell line

The KZ134 plasmid was constructed with complementary oligonucleotides ZC12,749 (SEQ ID NO:44) and ZC12,748 (SEQ ID NO:45) that contain STAT transcription factor binding elements from 4 genes, which includes a modified c-fos Sis inducible element (m67SIE, or hSIE) (Sadowski, H. et al., Science 261:1739-1744, 1993), the p21 SIE1 from the p21 WAF1 gene (Chin, Y. et al., Science 272:719-722, 1996), the mammary gland response element of the β-casein gene (Schmitt-Ney, M. et al., Mol. Cell. Biol. 11:3745-3755, 1991), and a STAT inducible element of the Fcg RI gene, (Seidel, H. et al., Proc. Natl. Acad. Sci. 92:3041-3045, 1995). These oligonucleotides contain Asp718-XhoI compatible ends and were ligated, using standard methods, into a recipient firefly luciferase reporter vector with a c-fos promoter (Poulsen, L.K. et al., J. Biol. Chem. 273:6229-6232, 1998) digested with the same enzymes and containing a neomycin selectable marker. The KZ134 plasmid was used to stably transfect BaF3 cells, using standard transfection and selection methods, to make the BaF3/KZ134 cell line.

A stable BaF3/KZ134 indicator cell line, expressing the full-length zcytor17 receptor or zcytor17/OSMRbeta receptor was constructed as per Example 4. Clones were diluted, plated and selected using standard techniques. Clones were screened by luciferase assay (see Example 20B, below) using the human zcytor17lig conditioned media or purified zcytor17lig protein (see Example 35, below) as an inducer. Clones with the highest luciferase response (via STAT luciferase) and the lowest background were selected. Stable transfectant cell lines were selected. The cell lines were called BaF3/KZ134/zcytor17 or BaF3/KZ134/zcytor17/OSMRbeta depending on the receptors transfected into the cell line.

Similarly, BHK cell lines were also constructed using the method

described herein, and were used in luciferase assays described herein. The cell lines were called BHK/KZ134/zcytor17 or BHK/KZ134/zcytor17/OSMRbeta depending on the receptors transfected into the cell line.

B. Human zcytor17lig activates human zcytor17 receptor in BaF3/KZ134/zcytor17/OSMRbeta or BHK/KZ134/zcytor17/OSMRbeta luciferase assay

BaF3/KZ134/zcytor17 and BaF3/KZ134/zcytor17/OSMRbeta cells were spun down and washed in mIL-3 free media. The cells were spun and washed 3 times to ensure removal of mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at about 30,000 cells per well in a volume of 100 µl per well using the mIL-3 free media. The same procedure was used for untransfected BaF3/KZ134 cells for use as a control in the subsequent assay. BHK/KZ134/zcytor17 or BHK/KZ134/zcytor17/OSMRbeta cells were plated in a 96-well format at 15,000 cells per well in 100 µl media. Parental BHK/KZ134 cells were used as a control.

STAT activation of the BaF3/KZ134/Zcytor17, BaF3/KZ134/zcytor17/OSMRbeta, BHK/KZ134/zcytor17, or BHK/KZ134/zcytor17/OSMRbeta cells was assessed using (1) conditioned media from BHK570 cells transfected with the human zcytor17lig (Example 7), (2) conditioned media from BHK570 cells transfected with the mouse zcytor17lig (Example 18), (3) purified human zcytor17lig (Example 35), or (4) mIL-3 free media to measure media-only control response. Conditioned media was diluted with RPMI mIL-3 free media to 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.75% and 0.375% concentrations. Purified human zcytor17lig was diluted to a concentration of 1200, 600, 300, 150, 75, 37.5, 18.75, or 9.4 pM. One hundred microliters of the diluted conditioned media or protein was added to the BaF3/KZ134/Zcytor17, BaF3/KZ134/zcytor17/OSMRbeta, BHK/KZ134/zcytor17, or BHK/KZ134/zcytor17/OSMRbeta cells. The assay using the conditioned media was done in parallel on untransfected BaF3/KZ134 or BHK/KZ134 cells as a control. The total assay volume was 200 µl. The assay plates were incubated at 37°C, 5% CO_2 for 24 hours at which time the BaF3 cells were pelleted by centrifugation at 2000 rpm for 10 min., and the media was aspirated and 25 µl of lysis

buffer (Promega) was added. For the BHK cell lines, the centrifugation step was not necessary as the cells are adherant. After 10 minutes at room temperature, the plates were measured for activation of the STAT reporter construct by reading them on a luminometer (Labsystems Luminoskan, model RS) which added 40 µl of luciferase assay substrate (Promega) at a five second integration.

The results of this assay confirmed that the STAT reporter response of the BaF3/KZ134/zcytor17/OSMRbeta and BHK/KZ134/zcytor17/OSMRbeta cells to the human zcytor17lig when compared to either the BaF3/KZ134/zcytor17 cells, the BHK/KZ134/zcytor17 cells or the untransfected BaF3/KZ134 or BHK/KZ134 control cells, showed that the response was mediated through the zcytor17/OSMRbeta receptors. The results also showed that the mouse zcytor17lig does not activate the STAT reporter assay through the human receptor complex.

Example 21

Mouse zcytor17lig is active in mouse bone marrow assay

A. Isolation of Non-adherent Low Density Marrow Cells:

Fresh mouse femur aspirate (marrow) is obtained from 6-10 week old male Balb/C or C57BL/6 mice. The marrow is then washed with RPMI+10% FBS (JRH, Lenexa KS; Hyclone, Logan UT) and suspended in RPMI+10% FBS as a whole marrow cell suspension. The whole marrow cell suspension is then subjected to a density gradient (Nycoprep, 1.077, Animal; Gibco BRL) to enrich for low density, mostly mononuclear, cells as follows: The whole marrow cell suspension (About 8 ml) is carefully pipeted on top of about 5 ml Nycoprep gradient solution in a 15 ml conical tube, and then centrifuged at 600X g for 20 minutes. The interface layer, containing the low density mononuclear cells, is then removed, washed with excess RPMI+10% FBS, and pelleted by centrifugation at 400X g for 5-10 minutes. This pellet is resuspended in RPMI +10% FBS and plated in a T-75 flask at approximately 10^6 cells/ml, and incubated at 37°C 5% CO_2 for approximately 2 hours. The resulting cells in suspension are Non-Adherent Low Density (NA LD) Marrow Cells.

B. 96-Well Assay

NA LD Mouse Marrow Cells are plated at 25,000 to 45,000 cells/well in 96 well tissue culture plates in RPMI +10% FBS + 1ng/mL mouse Stem Cell Factor (mSCF) (R&D Systems, Minneapolis, MN), plus 5% conditioned medium from one of the following: (1) BHK 570 cells expressing mouse zcytor17lig (Example 18), (2) BHK 570 cells expressing human zcytor17lig (Example 7), or (3) control BHK 570 cells containing vector and not expressing either Ligand. These cells are then subjected to a variety of cytokine treatments to test for expansion or differentiation of hematopoietic cells from the marrow. For testing, the plated NA LD mouse marrow cells are subjected to human Interleukin-15 (hIL-15) (R&D Systems), or one of a panel of other cytokines (R&D Systems). Serial dilution of hIl-15, or the other cytokines, are tested, with 2-fold serial dilution from about 50 ng/ml down to about 0.5 ng/ml concentration. After 8 to 12 days the 96-well assays are scored for cell proliferation by Alamar blue assay as described in Example 5B.

C. Results from the 96-well NA LD Mouse Marrow assay

Conditioned media from the BHK cells expressing both mouse and human zcytor17lig can promote the expansion of a population of hematopoietic cells either alone or in synergy with other cytokines in the NA LD mouse marrow in comparison to control BHK conditioned medium. The population hematopoietic cells expanded by the mouse zcytor17lig with or without other cytokines, and those hematopoietic cells expanded by the human zcytor17lig with or without other cytokines, are further propagated in cell culture. These hematopoietic cells are stained with a Phycoerythrin labeled anti-Pan NK cell antibody (PharMingen) and subjected to flow cytometry analysis, which demonstrated that the expanded cells stained positively for this natural killer (NK) cell marker. Similarly, other specific hematopoietic cell markers can be used to determine expansion of, for example, CD4+ or CD8+ T-cells, other T-cell populations, B-cells, and other immune cell markers.

The same 96-well assay is run, using fresh human marrow cells bought from Poietic Technologies, Gaithersburg, MD. Again, a positive result shows that zcytor17lig alone or in synergy with other cytokines, the mouse and human zcytor17lig can expand a hematopoietic cell population that is stained positively for specific cell

markers as disclosed above.

Example 22

Constructs for generating zcytor17lig Transgenic Mice

A. Construct for expressing human zcytor17lig from the MT-1 promoter

Oligonucleotides were designed to generate a PCR fragment containing a consensus Kozak sequence and the human zcytor17lig coding region. These oligonucleotides were designed with an FseI site at the 5' end and an AscI site at the 3' end to facilitate cloning into (a) pMT12-8, standard transgenic vector, or (b) pKFO51, a lymphoid-specific transgenic vector (Example 22B).

PCR reactions are carried out with about 200 ng human zcytor17lig template (SEQ ID NO:1) and oligonucleotides designed to amplify the full-length or active portion of the zcytor17lig. PCR reaction conditions are determined using methods known in the art. PCR products are separated by agarose gel electrophoresis and purified using a QiaQuick™ (Qiagen) gel extraction kit. The isolated, correct sized DNA fragment is digested with FseI and AscI (Boerhinger-Mannheim), ethanol precipitated and ligated into pMT12-8 previously digested with FseI and AscI. The pMT12-8 plasmid, designed for expressing a gene of interest in liver and other tissues in transgenic mice, contains an expression cassette flanked by 10 kb of MT-1 5' DNA and 7 kb of MT-1 3' DNA. The expression cassette comprises the MT-1 promoter, the rat insulin II intron, a polylinker for the insertion of the desired clone, and the human growth hormone (hGH) poly A sequence.

About one microliter of each ligation reaction is electroporated into DH10B ElectroMax™ competent cells (GIBCO BRL, Gaithersburg, MD) according to manufacturer's direction and plated onto LB plates containing 100 μg/ml ampicillin, and incubated overnight. Colonies are picked and grown in LB media containing 100 μ g/ml ampicillin. Miniprep DNA is prepared from the picked clones and screened for the human zcytor17lig insert by restriction digestion with EcoRI alone, or FseI and AscI combined, and subsequent agarose gel electrophoresis. Maxipreps of the correct pMT-human zcytor17lig are performed. A SalI fragment containing with 5' and 3' flanking sequences, the MT-1 promoter, the rat insulin II intron, human zcytor17lig cDNA and

the hGH poly A sequence is prepared to be used for microinjection into fertilized murine oocytes. Microinjection and production of transgenic mice are produced as described in Hogan, B. et al. Manipulating the Mouse Embryo, 2nd ed., Cold Spring Harbor Laboratory Press, NY, 1994.

B. Construct for expressing human zcytor17lig from the lymphoid-specific EμLCK promoter

Oligonucleotides are designed to generate a PCR fragment containing a consensus Kozak sequence and the human zcytor17lig coding region. These oligonucleotides are designed with an FseI site at the 5' end and an AscI site at the 3' end to facilitate cloning into pKFO51, a lymphoid-specific transgenic vector.

PCR reactions are carried out with about 200 ng human zcytor17lig template (SEQ ID NO:1) and oligonucleotides designed to amplify the full-length or active portion of the zcytor17lig. A PCR reaction is performed using methods known in the art. The isolated, correct sized DNA fragment is digested with FseI and AscI (Boerhinger-Mannheim), ethanol precipitated and ligated into pKFO51 previously digested with FseI and AscI. The pKFO51 transgenic vector is derived from p1026X (Iritani, B.M., et al., EMBO J. 16:7019-31, 1997) and contains the T cell-specific lck proximal promoter, the B/T cell-specific immunoglobulin μ heavy chain enhancer, a polylinker for the insertion of the desired clone, and a mutated hGH gene that encodes an inactive growth hormone protein (providing 3' introns and a polyadenylation signal).

About one microliter of each ligation reaction is electroporated, plated, clones picked and screened for the human zcytor17lig insert by restriction digestion as described above. A correct clone of pKFO51-zcytor17lig is verified by sequencing, and a maxiprep of this clone is performed. A NotI fragment, containing the lck proximal promoter and immunoglobulin μ enhancer (EμLCK), zcytor17lig cDNA, and the mutated hGH gene is prepared to be used for microinjection into fertilized murine oocytes.

C. Construct for expressing mouse zcytor17lig from the EF1alpha promoter

Primers ZC41,498 (SEQ ID NO:86) and ZC41,496 (SEQ ID NO:87)

were used to PCR a mouse testis cDNA library template. These PCR reactions were successfully performed in approximately 50 μl volumes with or without 10% DMSO, using pfu turbo polymerase (Stratagene) according to the manufacturer's recommendations; with an additional application of a wax hot-start employing hot start 50s (Molecular Bioproducts, Inc. San Diego, CA). PCR thermocycling was performed with a single cycle of 94°C for 4 min; followed by 40 cycles of 94°C: 30 seconds, 48°C: 30 seconds, 72°C: 50 seconds; with additional final 72°C extension for 7 minutes. The two PCR reactions were pooled and purified using low melt agarose and Gelase agarose digesting enzyme (Epicenter, Inc. Madison, WI) according to the manufacturer's recommendations.

DNA sequenced PCR products revealed a murine zcytor17 cDNA sequence (SEQ ID NO:90) which comprised an ORF identical to SEQ ID NO:10, confirming that SEQ ID NO:10 encoded the mouse zcytor17lig polypeptide. PCR primers, ZC41583 (SEQ ID NO:88) and ZC41584 (SEQ ID NO:89), were then used to add FseI and AscI restriction sites and a partial Kozak sequence to the mcytor17lig open reading frame and termination codon (SEQ ID NO:92). A Robocycler 40 thermocycler (Stratagene) was used to run a temperature gradient of annealing temperatures and cycling as follows. Pfu turbo polymerase (Stratagene) was applied as described above, but only in 10% DMSO. Cycling was performed with a single cycle of 94°C for 4 min; followed by 20 cycles of 94°C: 30 seconds, 65°C to 51°C gradient: 30 seconds, 72°C: 1 minute; and a single 72°C extension for 7 minutes. The template for this second thermocycling reaction was 1 μl of the initial gel-purified mcytor17lig PCR product, above. Resulting PCR product from the three lowest temperature reactions were pooled and gel purified using the Gelase (Epicenter) method described above. This purified fragment was then digested with FseI and AscI and ligated into a pZP7X vector modified to have FseI and AscI sites in its cloning site. This was sent to sequencing to confirm the correct sequence. The cloned murine cDNA sequence is represented in SEQ ID NO:90, and corresponding polypeptide sequence is shown in SEQ ID NO:91 (which is identical to SEQ ID NO:11).

The isolated, correct sized DNA fragment digested with FseI and AscI (Boerhinger-Mannheim) was subcloned into a plasmid containing EF1alpha promoter

previously digested with FseI and AscI. Maxipreps of the correct EF1alpha mouse zcytor17lig were performed. The expression cassette contains the EF1alpha promoter (with a deleted FseI site), the EF1alpha intron, SUR IRES like site to facilitate expression, a polylinker flanked with rat insulin II sites on the 5'end which adds FseI PmeI AscI sites for insertion of the desired clone, and the human growth hormone (hGH) poly A sequence. A 7.5kb NotI fragment containing the EF1alpha promoter expression cassette and mouse zcytor17lig was prepared to be used for microinjection into fertilized murine oocytes. The EF1alpha plsdmid was obtained from Louis-Marie of the Laboratoire de Differenciation Cellulaire, as described in Taboit-Dameron et al., 1999, Transgenic Research 8:223-235.

D. Construct for expressing mouse zcytor17lig from the lymphoid-specific EμLCK promoter

Oligonucleotides were designed to generate a PCR fragment containing a consensus Kozak sequence and the mouse zcytor17lig coding region. These oligonucleotides were designed with an FseI site at the 5' end and an AscI site at the 3' end to facilitate cloning into pKFO51 (see Example 22B, above).

The isolated, correct sized zcytor17lig DNÁ fragment used in EF1alpha constructs, digested with FseI and AscI (Boerhinger-Mannheim), was subcloned into a plasmid containing pKFO51, a lymphoid-specific transgenic vector. The pKFO51 transgenic vector is derived from p1026X (Iritani, B.M., et al., EMBO J. 16:7019-31, 1997) and contains the T cell-specific lck proximal promoter, the B/T cell-specific immunoglobulin μ heavy chain enhancer, a polylinker for the insertion of the desired clone, and a mutated hGH gene that encodes an inactive growth hormone protein (providing 3' introns and a polyadenylation signal). A 6.5kb NotI fragment, containing the lck proximal promoter and immunoglobulin μ enhancer (EμLCK), mouse zcytor17lig cDNA, and the mutated hGH gene was prepared to be used for microinjection into fertilized murine oocytes (Example 41).

Example 23

Construction of mammalian expression vectors that express zcytor17lig-CEE

A. Construction of zCytor17Lig-CEE/pZMP21

An expression plasmid containing all or part of a polynucleotide encoding human zCytor17lig was constructed via homologous recombination. The plasmid was called zCytor17Lig-CEE/pZMP21.

The construction of zCytor17Lig-CEE/pZMP21 was accomplished by generating a zCytor17Lig-CEE fragment (SEQ ID NO:95) (its corresponding amion acid sequence is shown in SEQ ID NO:96) using PCR amplification. The DNA template used for the production of the zCytor17Lig-CEE fragment was zCytor17Lig/pZP7nx. The primers used for the production of the zCytor17Lig-CEE fragment were: (1) ZC41607 (SEQ ID NO:97) (sense sequence), which includes from the 5' to the 3' end: 28bp of the vector flanking sequence (5' of the insert) and 21 bp corresponding to the 5' sequence of zCytor17Lig; and (2) ZC41605 (SEQ ID NO:98) (anti-sense sequence), which includes from the 5' to the 3' end: 37 bp of the vector flanking sequence (3' of the insert), 3 bp of the stop codon, 21 bp encoding a C-terminal EE tag, and 21 bp corresponding to the 3' end of zCytor17Lig sequence. The fragment resulting from the above PCR amplification is a copy of the template zCytor17Lig with the addition of a C-terminal EE tag, yielding a final product zCytor17Lig-CEE.

PCR reactions were run as follows: To a 100μl final volume was added: 10μl of 10x Taq Polymerase Reaction Buffer with 15mM MgCl (Gibco), 1μl of Taq DNA Polymerase (5 units/μl, Gibco), 3μl of 10mM dNTPs, 78μl dH2O, 3μl of a 20 pmol/μl stock of primer ZC41607 (SEQ ID NO:97) 3μl of a 20 pmol/μl stock of primer ZC41605 (SEQ ID NO:98), and 2μl of a 0.13μg/μl stock of zCytor17lig template DNA. A volume equal to 50μl of mineral oil was added to the mixture. The reaction was heated to 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute; 55°C for 2 minutes; 72°C for 3 minutes; followed by a 10 minute extension at 72°C and held at 4°C until the reaction was collected.

The plasmid pZMP21 was restriction digested with BglII enzyme, cleaned with a QiaQuick PCR Purification Kit (Qiagen) using a microcentrifuge protocol, and used for recombination with the PCR fragment. Plasmid pZMP21 was constructed from pZMP20 which was constructed from pZP9 (deposited at the

American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, and is designated No. 98668) with the yeast genetic elements taken from pRS316 (deposited at the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, and designated No. 77145), an IRES element from poliovirus, and the extracellular domain of CD8, truncated at the carboxyl terminal end of the transmembrane domain. PZMP21 is a mammalian expression vector containing an expression cassette having the MPSV promoter, immunoglobulin signal peptide intron, multiple restriction sites for insertion of coding sequences, a stop codon and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene, the SV40 terminator, as well as the URA3 and CEN-ARS sequences required for selection and replication in *S. cerevisiae*.

Fifty microliters of competent yeast cells (*S. cerevisiae*) were independently combined with 100 ng of cut plasmid, 5 μl of previously described PCR mixture, and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV (5 kV/cm), infinite ohms, 25 μF. Each cuvette had 600 μl of 1.2 M sorbitol added, and the yeast was plated in one 100 μl aliquot and one 300 μl aliquot onto two URA-D plates and incubated at 30°C. After about 72 hours, the Ura+ yeast transformants from a single plate were resuspended in 1 ml H_2O and spun briefly to pellet the yeast cells. The cell pellet was resuspended in 500 μl of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). The 500 μl of the lysis mixture was added to an Eppendorf tube containing 300 μl acid washed 600μm glass beads and 300 μl phenol-chloroform, vortexed for 1 minute intervals two or three times, followed by a 5 minute spin in a Eppendorf centrifuge at maximum speed. Three hundred microliters of the aqueous phase was transferred to a fresh tube, and the DNA precipitated with 600 μl 100% ethanol (EtOH), followed by centrifugation for 10 minutes at 4°C. The DNA pellet was then washed with 500μl 70% EtOH, followed by centrifugation for 1 minute at 4°C. The DNA pellet was resuspended in 30 μl H_2O.

Transformation of electrocompetent *E. coli* cells (MC1061) was done with 5 μl of the yeast DNA prep and 50 μl of MC1061 cells. The cells were

electropulsed at 2.0 kV, 25 μF and 400 ohms(Ω). Following electroporation, 600μl SOC (2% Bacto' Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose) was added. The electroporated *E.coli* cells were plated in a 200μl and a 50μl aliquot on two LB AMP plates (LB broth (Lennox), 1.8% Bacto Agar (Difco), 100 mg/L Ampicillin). The plates were incubated upside down for about 24 hours at 37°C. Three Ampicillin-resistant colonies were selected at random and submitted for sequence analysis of the insert. Large scale plasmid DNA was isolated from a sequence-confirmed clone using the Qiagen Maxi kit (Qiagen) according to manufacturer's instructions.

B. Mammalian Expression of human zcytor17lig

Full-length zCytor17Lig protein was produced in BHK cells transfected with zCytor17Lig-CEE/pZMP21 (Example 23A). BHK 570 cells (ATCC CRL-10314) were plated in T75 tissue culture flasks and allowed to grow to approximately 50 to 70% confluence at 37°C, 5% CO_2, in growth media (SL7V4, 5%FBS, 1% pen/strep). The cells were then transfected with zCytor17Lig-CEE/pZMP21 by liposome-mediated transfection (using Lipofectamine™; Life Technologies), in serum free (SF) media (SL7V4). The plasmid (16μg) was diluted into 1.5 ml tubes to a total final volume of 640 μl with SF media. Thirty-five microliters of the lipid mixture was mixed with 605 μl of SF medium, and the resulting mixture was allowed to incubate approximately 15 minutes at room temperature. Five milliliters of SF media was then added to the DNA:lipid mixture. The cells were rinsed once with 10 ml of PBS, the PBS was decanted, and the DNA:lipid mixture was added. The cells are incubated at 37°C for five hours, then 15 ml of media (SL7V4, 5% FBS, 1% pen/strep) was added to each plate. The plates were incubated at 37°C overnight, and the DNA:lipid media mixture was replaced with selection media (SL7V4, 5% FBS, 1% pen/strep, 1μM methotrexate) the next day. Approximately 10 days post-transfection, methotrexate-resistant colonies from the T75 transfection flask were trypsinized, and the cells were pooled and plated into a T-162 flask and transferred to large-scale culture.

Example 24

Expression of zcytor17 Soluble Receptor in *E. coli*

A. Construction of expression vector pCMH01 that expresses huzcytor17/MBP-6H fusion polypeptide

An expression plasmid containing a polynucleotide encoding a zcytor17 soluble receptor fused C-terminally to maltose binding protein (MBP) was constructed via homologous recombination. The fusion polypeptide contains an N-terminal approximately 388 amino acid MBP portion fused to any of the zcytor17 soluble receptors described herein. A fragment of zcytor17 cDNA (SEQ ID NO:4) was isolated using PCR as described herein. Two primers were used in the production of the zcytor17 fragment in a standard PCR reaction: (1) one containing about 40 bp of the vector flanking sequence and about 25 bp corresponding to the amino terminus of the zcytor17, and (2) another containing about 40 bp of the 3' end corresponding to the flanking vector sequence and about 25 bp corresponding to the carboxyl terminus of the zcytor17. Two μl of the 100 μl PCR reaction was run on a 1.0% agarose gel with 1 x TBE buffer for analysis, and the expected approximately fragment was seen. The remaining PCR reaction was combined with the second PCR tube and precipitated with 400 μl of absolute ethanol. The precipitated DNA used for recombining into the Sma1 cut recipient vector pTAP170 to produce the construct encoding the MBP-zcytor17 fusion, as described below.

Plasmid pTAP170 was derived from the plasmids pRS316 and pMAL-c2. The plasmid pRS316 is a *Saccharomyces cerevisiae* shuttle vector (Hieter P. and Sikorski, R., Genetics 122:19-27, 1989). pMAL-C2 (NEB) is an *E. coli* expression plasmid. It carries the *tac* promoter driving *MalE* (gene encoding MBP) followed by a His tag, a thrombin cleavage site, a cloning site, and the *rrnB* terminator. The vector pTAP170 was constructed using yeast homologous recombination. 100ng of EcoR1 cut pMAL-c2 was recombined with 1µg Pvu1 cut pRS316, 1µg linker, and 1µg Sca1/EcoR1 cut pRS316. The linker consisted of oligos zc19,372 (SEQ ID NO:157) (100pmole): zc19,351 (SEQ ID NO:158) (1pmole): zc19,352 (SEQ ID NO:159) (1pmole), and zc19,371 (SEQ ID NO:160) (100pmole) combined in a PCR reaction. Conditions were as follows: 10 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and

72°C for 30 seconds; followed by 4°C soak. PCR products were concentrated via 100% ethanol precipitation.

One hundred microliters of competent yeast cells (*S. cerevisiae*) were combined with 10 μl of a mixture containing approximately 1 μg of the human zcytor17 insert, and 100 ng of SmaI digested pTAP170 vector, and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV (5 kV/cm), infinite ohms, 25 μF. To each cuvette was added 600 μl of 1.2 M sorbitol. The yeast was then plated in two 300 μl aliquots onto two -URA D plates and incubated at 30°C.

After about 48 hours, the Ura+ yeast transformants from a single plate were picked, DNA was isolated, and transformed into electrocompetent *E. coli* cells (e.g., MC1061, Casadaban et. al. J. Mol. Biol. 138, 179-207), and plated on MM/CA +KAN 25 μg/L plates (Pryor and Leiting, Protein Expression and Purification 10:309-319, 1997) using standard procedures. Cells were grown in MM/CA with 25 μg/ml Kanomyacin for two hours, shaking, at 37°C. One ml of the culture was induced with 1mM IPTG. Two to four hours later the 250 μl of each culture was mixed with 250 μl acid washed glass beads and 250 μl Thorner buffer with 5% βME and dye (8M urea, 100 mM Tris pH7.0, 10% glycerol, 2mM EDTA, 5% SDS). Samples were vortexed for one minute and heated to 65°C for 10 minutes. 20 μl were loaded per lane on a 4%-12% PAGE gel (NOVEX). Gels were run in 1XMES buffer. The positive clones were designated pCMH01 and subjected to sequence analysis.

One microliter of sequencing DNA was used to transform strain BL21. The cells were electropulsed at 2.0 kV, 25 μF and 400 ohms. Following electroporation, 0.6 ml MM/CA with 25 μg/L Kanomyacin. Cells were grown in MM/CA and induced with ITPG as described above. The positive clones were used to grow up for protein purification of the huzcytor17/MBP-6H fusion protein using standard techniques.

B. Purification of huzcytor17/MBP-6H soluble receptor from E.coli fermentation

Unless otherwise noted, all operations were carried out at 4°C. The following procedure was used for the purification of recombinant huzcytor17/MBP-6H

soluble receptor polypeptide. *E. coli* cells containing the pCMH01 construct and expressing huzcytor17/MBP-6H soluble receptor polypeptide were constructed using standard molecular biology methods and cultured in SuperBroth II (12 g/L Casien, 24 g/L Yeast Extract, 11.4 g/L di-potassium phosphate, 1.7 g/L Mono-potassium phosphate; Becton Dickenson, Cockeysville, MD). The resulting cells were harvested and frozen in 0.5% glycerol. Twenty grams of the frozen cells were used for protein purification.

Thawed cells were resuspended in 500mL Amylose Equilibration buffer (20mM Tris, 100mM NaCl, pH 8.0). A French Press cell breaking system (Constant Systems Ltd., Warwick, UK) with a temperature setting of -7°C to -10°C and 30K PSI was used to lyse the cells. The resuspended cells were checked for breakage by A_{600} readings before and after cycling through the French Press. The lysed cell suspension was pelleted at 10,000G for 30 minutes. Supernatant was harvested from the debris pellet for protein purification.

Twenty-five milliliters of Amylose resin (New England Biolabs, Beverly, MA) was poured into a Bio-Rad, 2.5 cm D x 10 cm H glass column. The column was packed and equilibrated by gravity with 10 column volumes (CVs) of Amylose Equilibration buffer. The harvested cell supernatant was batch loaded to the Amylose resin, overnight with rocking. The loaded resin was returned to the glass column, washed with 10 CVs Amylose Equilibration buffer, and eluted by gravity with ~2 CVs Amylose Elution buffer (Amylose Equilibration buffer, 10 mM Maltose, Fluka Biochemical, Switzerland). Ten 5 ml fractions were collected over the elution profile and assayed for absorbance at 280 and 320 nM. The Amylose resin was regenerated with 1 CV of distilled H_2O, 5 CVs of 0.1% (w/v) SDS (Sigma), 5 CVs of distilled H_2O, 5 CVs of Amylose Equilibration buffer, and finally 1 CV of Amylose Storage buffer (Amylose Equilibration buffer, 0.02% (w/v) Sodium Azide). The regenerated resin was stored at 4°C.

Elution profile fractions of interest were pooled and dialyzed in a 10K dialysis chamber (Slide-A-Lyzer, Pierce Immunochemical) against 4 changes of 4L PBS pH 7.4 (Sigma) over an 8 hour time period. Following dialysis, the material harvested represented the purified huzcytor17/MBP-6H polypeptide. The purified

huzcytor17/MBP-6H polypeptide was filter sterilized and analyzed via SDS-PAGE Coomassie staining for an appropriate molecular weight product. The concentration of the huzcytor17/MBP-6H polypeptide was determined by BCA analysis to be 0.76 mg/ml.

Purified huzcytor17/MBP-6H polypeptide was appropriately formulated for the immunizaton of rabbits and sent to R & R Research and Development (Stanwood, WA) for polyclonal antibody production (Example 25, below).

Example 25

Human zcytor17 Soluble Receptor Polyclonal Antibody

A. Preparation and Purification

Polyclonal antibodies were prepared by immunizing 2 female New Zealand white rabbits with the purified recombinant protein huzcytor17/MBP-6H (Example 24). The rabbits were each given an initial intraperitoneal (IP) injection of 200 μg of purified protein in Complete Freund's Adjuvant followed by booster IP injections of 100 μg protein in Incomplete Freund's Adjuvant every three weeks. Seven to ten days after the administration of the second booster injection (3 total injections), the animals were bled and the serum was collected. The animals were then boosted and bled every three weeks.

The huzcytor17/MBP-6H specific rabbit serum was pre-adsorbed of anti-MBP antibodies using a CNBr-SEPHAROSE 4B protein column (Pharmacia LKB) that was prepared using 10 mg of non-specific purified recombinant MBP-fusion protein per gram of CNBr-SEPHAROSE. The huzcytor17/MBP-6H-specific polyclonal antibodies were affinity purified from the pre-adsorbed rabbit serum using a CNBr-SEPHAROSE 4B protein column (Pharmacia LKB) that was prepared using 10 mg of the specific antigen purified recombinant protein huzcytor17/MBP-6H. Following purification, the polyclonal antibodies were dialyzed with 4 changes of 20 times the antibody volume of PBS over a time period of at least 8 hours. Huzcytor17-specific antibodies were characterized by ELISA using 500 ng/ml of the purified recombinant protein huzcytor17/MBP-6H as antibody target. The lower limit of detection (LLD) of the rabbit anti-huzcytor17/MBP-6H affinity purified antibody was

500 pg/ml on its specific purified recombinant antigen huzcytor17/MBP-6H.

B. SDS-PAGE and Western blotting analysis of Rabbit Anti-human ZcytoR17 MBP-6H antibody

Rabbit anti-human ZcytoR17 MBP-6H antibody was tested by SDS-PAGE (NuPage 4-12%, Invitrogen, Carlsbad, CA) with coomassie staining method and Western blotting using goat anti-rabbit IgG-HRP. Either purified protein (200-25 ng) or conditioned media containing zcytor17 was electrophoresed using an Invitrogen Novex's Xcell II mini-cell, and transferred to nitrocellulose (0.2 mm; Invitrogen, Carlsbad, CA) at room temperature using Novex's Xcell blot module with stirring according to directions provided in the instrument manual. The transfer was run at 300 mA for one hour in a buffer containing 25 mM Tris base, 200 mM glycine, and 20% methanol. The filter was then blocked with Western A buffer (in house, 50 mM Tris, pH 7.4, 5 mM EDTA, pH 8.0, 0.05% Igepal CA-630, 150 mM NaCl, and 0.25% gelatin) overnight with gentle rocking at 4°C. The nitrocellulose was quickly rinsed, then the rabbit anti-human zcytoR17 MBP-6H (1:1000) was added in Western A buffer. The blot was incubated for 1.5 hours at room temperature with gentle rocking. The blot was rinsed 3 times for 5 minutes each in Western A, then goat anti-rabbit IgG HRP antibody (1:1000) was added in Western A buffer. The blot was incubated for 1.25 hours at room temperature with gentle rocking. The blot was rinsed 3 times for 5 minutes each in Western A, then quickly rinsed in H_20. The blot was developed using commercially available chemiluminescent substrate reagents (ECLWestern blotting detection reagents 1 and 2 mixed 1:1; reagents obtained from Amersham Pharmacia Biotech, Buckinghamshire, England) and the blot was exposed to X-ray film for up to15 minutes.

The rabbit anti-human zcytoR17 MBP-6H was able to detect human zcytor17 present in conditioned media as well as zcytoR17 purified protein as a band at 120 kDa under reducing conditions.

Example 26

Tissue Distribution of Mouse zcytor17 in Tissue Panels Using PCR

A panel of cDNAs from murine tissues was screened for mouse zcytor17 expression using PCR. The panel was made in-house and contained 94 marathon cDNA and cDNA samples from various normal and cancerous murine tissues and cell lines are shown in Table 6, below. The cDNAs came from in-house libraries or marathon cDNAs from in-house RNA preps, Clontech RNA, or Invitrogen RNA. The mouse marathon cDNAs were made using the marathon-Ready™ kit (Clontech, Palo Alto, CA) and QC tested with mouse transferrin receptor primers ZC10,651 (SEQ ID NO:46) and ZC10,565 (SEQ ID NO:47) and then diluted based on the intensity of the transferrin band. To assure quality of the amplified library samples in the panel, three tests for quality control (QC) were run: (1) To assess the RNA quality used for the libraries, the in-house cDNAs were tested for average insert size by PCR with vector oligos that were specific for the vector sequences for an individual cDNA library; (2) Standardization of the concentration of the cDNA in panel samples was achieved using standard PCR methods to amplify full length alpha tubulin or G3PDH cDNA using a 5' vector oligo: ZC14,063 (SEQ ID NO:48) and 3' alpha tubulin specific oligo primer ZC17,574 (SEQ ID NO:49) or 3' G3PDH specific oligo primer ZC17,600 (SEQ ID NO:50); and (3) a sample was sent to sequencing to check for possible ribosomal or mitochondrial DNA contamination. The panel was set up in a 96-well format that included a mouse genomic DNA (Clontech, Palo Alto, CA) positive control sample. Each well contained approximately 0.2-100 pg/µl of cDNA. The PCR was set up using oligos ZC38,065 (SEQ ID NO:51) and ZC38,068 (SEQ ID NO:52), TaKaRa Ex Taq™ (TAKARA Shuzo Co LTD, Biomedicals Group, Japan), and Rediload dye (Research Genetics, Inc., Huntsville, AL). The amplification was carried out as follows: 1 cycle at 94°C for 5 minutes; 5 cycles of 94 for 30 seconds, 68°C for 30 seconds; 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 30 seconds, followed by 1 cycle at 72°C for 5 minutes. About 10 µl of the PCR reaction product was subjected to standard Agarose gel electrophoresis using a 4% agarose gel. The correct predicted DNA fragment size was observed in brain, CD90+ cells, dendritic, embryo, MEWt#2, Tuvak-prostate cell line, salivary gland, skin and testis.

The DNA fragment for skin and testis were excised and purified using a Gel Extraction Kit (Qiagen, Chatsworth, CA) according to manufacturer's instructions.

Fragments were confirmed by sequencing to show that they were indeed mouse zcytor17.

Table 6

Tissue/Cell line	#samples		Tissue/Cell line	#samples
229	1			
7F2	1			
Adipocytes-Amplified	1			
aTC1.9	1			
Brain	4			
CCC4	1			
CD90+ Amplified	1			
OC10B	1			
Dentritic	1			
Embyro	1			
Heart	2			
Kidney	3			
Liver	2			
Lung	2			
MEWt#2	1			
P388D1	1			
Pancreas	1			
Placenta	2			
Jakotay-Prostate Cell Line	1			
Nelix-Prostate Cell Line	1			
Paris-Prostate Cell Line	1			
Torres-Prostate Cell Line	1			
Tuvak-Prostate Cell Line	1			
Salivary Gland	2			
Skeletal Muscle	1			
Skin	2			
Small Intestine	1			
Smooth Muscle	2			
Spleen	2			
Stomach	1			
Testis	3			
Thymus	1			

Example 27

Human Zcytor17 Expression in Various Tissues Using Real-Time Quantitative RT/PCR

A. Primers and Probes for Human Zcytor17, OSMRbeta and Zcytor17lig for Conventional and Quantitative RT-PCR

Real-time quantitative RT-PCR using the ABI PRISM 7900 Sequence Detection System (PE Applied Biosystems, Inc., Foster City, CA) has been previously described (See, Heid, C.A. et al., Genome Research 6:986-994, 1996; Gibson, U.E.M. et al., Genome Research 6:995-1001, 1996; Sundaresan, S. et al., Endocrinology 139:4756-4764, 1998). This method incorporates use of a gene specific probe containing both reporter and quencher fluorescent dyes. When the probe is intact the reporter dye emission is negated due to the close proximity of the quencher dye. During PCR extension using additional gene-specific forward and reverse primers, the probe is cleaved by 5' nuclease activity of Taq polymerase which releases the reporter dye from the probe resulting in an increase in fluorescent emission.

The primers and probes used for real-time quantitative RT-PCR analyses of human Zcytor17, OSMRbeta and Zcytor17lig expression were designed using the primer design software Primer Express™ (PE Applied Biosystems, Foster City, CA). Primers for human Zcytor17 were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC37,877 (SEQ ID NO:53) and the reverse primer, ZC37,876 (SEQ ID NO:54) were used in a PCR reaction at a 200 nM concentration to synthesize a 73 bp product. The corresponding Zcytor17 TaqMan® probe, designated ZC37,776 (SEQ ID NO:55) was synthesized and labeled by PE Applied Biosystems and used in each PCR reaction at a concentration of 200nM. The ZC37,776 (SEQ ID NO:55) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied Biosystems) and at the 3' end with a fluorescent quencher dye (6-carboxy-tetramethyl-rhodamine) (TAMRA) (Epoch Biosciences, Bothell, WA).

Primers for human OSMRbeta were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC43,891 (SEQ ID NO:122) and the reverse primer, ZC43,900 (SEQ ID NO:123) were used in a PCR reaction (below) at a 200 nM concentration. The corresponding OSMRbeta TaqMan® probe, designated ZC43,896 (SEQ ID NO:124) was synthesized and labeled by PE Applied Biosystems and used in each PCR reaction at a concentration of 200nM. The ZC43,896 (SEQ ID NO:124) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied

Biosystems) and at the 3' end with a non-fluorescent quencher dye (ECLIPSE) (Epoch Biosciences).

Primers for human Zcytor17lig were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC43,280 (SEQ ID NO:125) and the reverse primer, ZC43,281 (SEQ ID NO:126) were used in a PCR reaction (below) at about 200 nM concentration. The corresponding Zcytor17lig TaqMan® probe, designated ZC43,275 (SEQ ID NO:127) was synthesized and labeled by PE Applied Biosystems and used in each PCR reaction at a concentration of 200nM. The ZC43,275 (SEQ ID NO:127) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied Biosystems) and at the 3' end with a non-fluorescent quencher dye (ECLIPSE) (Epoch Biosciences).

As a control to test the integrity and quality of RNA samples tested, all RNA samples were screened for either rRNA or GUS using primer and probe sets either ordered from PE Applied Biosystems (rRNA kit) or designed in-house (GUS). The rRNA kit contained the forward primer (SEQ ID NO:56), the rRNA reverse primer (SEQ ID NO:57), and the rRNA TaqMan® probe (SEQ ID NO:58). The rRNA probe was labeled at the 5'end with a reporter fluorescent dye VIC (PE Applied Biosystems) and at the 3' end with the quencher fluorescent dye TAMRA (PE Applied Biosystems). The GUS primers and probe were generated in-house and used in each PCR reaction at 200nM and 100nM, respectively. The forward primer was ZC40,574 (SEQ ID NO:128) and the reverse primer was ZC40,575 (SEQ ID NO:129). The GUS probe ZC43,017 (SEQ ID NO:130) was labeled at the 5'end with a reporter fluorescent dye (Yakima-Yellow) (Epoch Biosciences) and at the 3'end with a non-fluorescent quencher dye (ECLIPSE) (Epoch Biosciences). The rRNA and GUS results also serve as an endogenous control and allow for the normalization of the Zcytor17 mRNA expression results seen in the test samples.

For conventional non-quantitative RT-PCR, primers were designed using the primer design software Primer Express™ (PE Applied Biosystems, Foster City, CA). The human zcytor17 primers generate an approximately 1000 base pair product and are as follows: forward primer ZC28,917 (SEQ ID NO:83), and reverse

primer ZC28,480 (SEQ ID NO:131). The human OSMRbeta primers generate a 202 base pair product and are as follows: forward primer ZC41,653(SEQ ID NO:132) and reverse primer ZC41,655 (SEQ ID NO:133). The human Zcytor17lig primers generate a 305 base pair product and are as follows: forward primer ZC41,703 (SEQ ID NO:134) and reverse primer ZC41,704 (SEQ ID NO:135).

B. Primers and Probes for Murine Zcytor17, OSMRbeta and Zcytor17lig for Conventional and Quantitative RT-PCR

The primers and probes used for real-time quantitative RT-PCR analyses of murine Zcytor17, OSMRbeta and Zcytor17lig expression were designed using the primer design software Primer Express™ (PE Applied Biosystems, Foster City, CA). Primers for murine Zcytor17 were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC43,272 (SEQ ID NO:136) and the reverse primer, ZC43,273 (SEQ ID NO:137) were used in the PCR reactions (below) at 300 nM concentration. The corresponding Zcytor17 TaqMan® probe, designated ZC43,478 (SEQ ID NO:138) was synthesized and labeled by PE Applied Biosystems. The ZC43,478 (SEQ ID NO:138) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied Biosystems) and at the 3' end with a quencher fluorescent dye (6-carboxy-tetramethyl-rhodamine) (TAMRA) (PE Applied Biosystems). The ZC43,478 (SEQ ID NO:138) probe was used in the PCR reactions at a concentration of 100nM.

Primers for murine Zcytor17lig were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC43,278 (SEQ ID NO:139) and the reverse primer, ZC43,279 (SEQ ID NO:140) were used in the PCR reactions at 500 nM concentration. The corresponding Zcytor17lig TaqMan® probe, designated ZC43,276 (SEQ ID NO:141) was synthesized and labeled by PE Applied Biosystems. The ZC43,478 (SEQ ID NO:138) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied Biosystems) and at the 3' end with a non-fluorescent quencher dye (ECLIPSE) (Epoch Biosciences). The ZC43,276 (SEQ ID NO:141) probe was used in the PCR reactions (below) at a concentration of 200nM.

Primers for murine OSMRbeta were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC43,045 (SEQ ID NO:142) and the reverse primer, ZC43,046 (SEQ ID NO:143) were used in the PCR reactions at a 300 nM concentration. The corresponding OSMRbeta TaqMan® probe, designated ZC43,141(SEQ ID NO:144) was synthesized and labeled by Epoch Biosciences. The ZC43,141 (SEQ ID NO:144) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied Biosystems) and at the 3' end with a non-fluorescent quencher dye (ECLIPSE) (Epoch Biosciences). The ZC43,141 (SEQ ID NO:144) probe was used in the PCR reactions (below) at a concentration of 100nM.

As a control to test the integrity and quality of RNA samples tested, all RNA samples were screened for either murine GUS or transferrin receptor using primers and probes designed using the primer design program Primer Express™ (PE Applied Biosystems Inc., Foster City, CA). The murine GUS primers are as foliows: forward primer, ZC43,004 (SEQ ID NO:145), reverse primer, ZC43,005 (SEQ ID NO:146), and TaqMan® probe ZC43,018 (SEQ ID NO:147). The murine GUS probe ZC43,018 (SEQ ID NO:147) was labeled at the 5'end with a reporter fluorescent dye Yakima-Yellow (Epoch Biosciences) and at the 3' end with the non-fluorescent quencher dye ECLIPSE (Epoch Biosciences). The murine GUS primers were used in the PCR reactions at 300 nM and the probe, ZC43,018 (SEQ ID NO:147), was used at 100nM. In some cases murine Transferrin Receptor was used instead of GUS as the endogenous control. The transferrin receptor forward primer, ZC40,269 (SEQ ID NO:148) and the reverse primer, ZC40,268 (SEQ ID NO:149) were used at 300nM. The transferrin receptor probe, ZC40,298 (SEQ ID NO:150) was used in PCR at 100nM and was labeled at the 5'end with a reporter fluorescent dye VIC (PE Applied Biosystems) and at the 3'end with a fluorescent quencher dye (TAMRA) (PE Applied Biosystems). The murine GUS and transferrin receptor results also serve as an endogenous control and allow for the normalization of the Zcytor17, OSMRbeta and Zcytor17lig mRNA expression results seen in the test samples.

For conventional semi-quantitative RT-PCR, primers were designed using the primer design software Primer Express™ (PE Applied Biosystems). The

murine Zcytor17 primers generate a 276 base pair product and are as follows: forward primer ZC43,140 (SEQ ID NO:151), and reverse primer ZC43,139 (SEQ ID NO:152). The murine OSMRbeta primers generate a 575 base pair product and are as follows: forward primer ZC41,608 (SEQ ID NO:153) and reverse primer ZC41,609 (SEQ ID NO:154). The murine Zcytor17lig primers generate a 657bp product and are as follows: forward primer ZC41,502 (SEQ ID NO:155) and reverse primer ZC41,500 (SEQ ID NO:156).

C. Protocols for Realtime Quantitative RT-PCR and Conventional Semi-quantitative RT-PCR

Relative levels of Zcytor17, OSMRbeta and Zcytor17lig mRNA were determined by analyzing total RNA samples using the one-step RT-PCR method (PE Applied Biosystems). Total RNA from Zcytor17- and OSMRbeta-transfected BAF cells (human) or BHK cells (murine) was isolated by standard methods and used to generate a standard curve used for quantitation of Zcytor17 and OSMRbeta. The curve consisted of 10-fold serial dilutions ranging from 100-0.01ng/μl with each standard curve point analyzed in triplicate. Similarly, for Zcytor17lig, activated CD4+ T cell RNA (previously shown to make Zcytor17lig) was used to generate a standard curve in the same 100-0.01ng/μl range. Total RNA from human or murine cells was analyzed in triplicate for either human or murine Zcytor17, OSMRbeta and Zcytor17lig transcript levels and for one of the following endogenous control genes: rRNA, GUS or transferrin receptor. In a total volume of 10 μl, each RNA sample was subjected to a One-Step RT-PCR reaction containing: approximately 50-100ng of total RNA in a preformulated 2X master mix containing an internal control dye (ROX)(carboxy-x-rhodamine) and Thermo-Start® DNA Polymerase (Abgene, Surrey, UK); appropriate primers for the gene of interest (see parts A and B of current example); the appropriate probe (see parts A and B for concentration); Superscript® reverse transcriptase (50 U/μl) (PE Applied Biosystems), and an appropriate volume of RNase-free water. PCR thermal cycling conditions were as follows: an initial reverse transcription (RT) step of one cycle at 48°C for 30 minutes; followed by a Thermo-Start® enzyme activation step of one cycle at 95°C for 10 minutes; followed by 40 cycles of amplification at 95°C for

15 seconds and 60°C for 1 minute. Relative Zcytor17, OSMRbeta and Zcytor17lig RNA levels were determined by using the Standard Curve Method as described by the manufacturer, PE Biosystems (User Bulletin #2: ABI Prism 7700 Sequence Detection System, Relative Quantitation of Gene Expression, December 11, 1997). The rRNA, GUS or Transferrin Receptor measurements were used to normalize the levels of the gene of interest.

The semi-quantitative RT-PCR reactions used the 'Superscript One-Step RT-PCR System with Platinum Taq' (Invitrogen, Carlsbad, CA). Each 25μl reaction consisted of the following: 12.5μl of 2X Reaction Buffer, 0.5μl (20pmol/μl) of forward primer, 0.5μl (20pmol/μl) of reverse primer, 0.4μl RT/Taq polymerase mix, 5.0μl of Rediload Gel Loading Buffer (Invitrogen), 5.1μl RNase-free water, and 1.0μl total RNA (100ng/μl). The amplification was carried out as follows: one cycle at 45°C for 30 minutes followed by 35-38 cycles of 94°C, 20 seconds; Variable annealing temp (See Table 7 below), 20 seconds; 72°C, 45 seconds; then ended with a final extension at 72°C for 5 minutes. Eight to ten microliters of the PCR reaction product was subjected to standard agarose gel electrophoresis using a 2% agarose gel.

Table 7

Murine Zcytor17	58°C anneal temp
Murine OSMRbeta	60°C anneal temp
Murine Zcytor17lig	52°C anneal temp
Human Zcytor17	55°C anneal temp
Human OSMRbeta	59°C anneal temp
Human Zcytor17lig	59°C anneal temp

D. Isolation of RNA from Human and Murine PBMC Subsets and Cell Lines

Blood was drawn from several anonymous donors and peripheral blood mononuclear cells (PBMC) isolated using standard Ficoll gradient methodology. Monocytes were then isolated using the Monocyte Isolation Kit and the Magnetic Cell Separation System (Miltenyi Biotec, Auburn, CA). The monocytes were then plated onto ultra-low adherence 24-well plates in endotoxin-free media. They were either unstimulated or treated with recombinant human IFNg (R&D Systems Inc.) at 10ng/ml.

Cells were collected at 24 and 48 hours. In similar manner, CD4+ and CD8+ T cells were isolated from PBMC's using the anti-CD4 or anti-CD8 magnetic beads from Miltenyi Biotec. Cells were then activated for 4 or 16 hours in tissue culture plates coated with 0.5μg/ml anti-CD3 antibodies in media containing 5μg/ml anti-CD28 antibodies. NK cells were also isolated from PBMC's using Miltenyi's anti-CD56 coated magnetic beads. Some of the NK cells were collected at time zero for RNA and the others were plated in media containing Phorbol Myristate Acetate (PMA) (5ng/ml) and ionomycin (0.5μg/ml) for 24 hours. Additionally, several human monocyte-like cell lines, U937, THP-1 and HL-60, were collected in either their resting or activated states. U937 cells were activated overnight with PMA (10ng/ml). HL-60's were activated overnight with PMA (10ng/ml) or for 72 and 96 hours with IFNg (10ng/ml) to drive them down a monocytic pathway. THP-1 cells were activated overnight with a combination of LPS (10ng/ml) and IFNg (10ng/ml). RNA was prepared from all primary cells using the RNeasy Midiprep™ Kit (Qiagen, Valencia, CA) as per manufacturer's instructions. Carryover DNA was removed using the DNA-*Free*™ kit (Ambion, Inc., Austin, TX). RNA concentration was determined using standard spectrophotometry and RNA quality determined using the Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA).

Murine T Cell RNA was collected using a variety of methods well-known in the art. Primary splenic CD4+ and CD8+ T cells were isolated from the spleens of C57Bl/6 mice using antibody-coated magnetic beads and the Magnetic Cell Separation System from Miltenyi Biotec. The CD4+ and CD8+ T cells were then activated by culturing the cells in 24-well plates coated with anti-CD3 antibodies (500ng/ml) in media containing anti-CD28 antibodies at 5μg/ml. Cells were harvested for RNA at 0, 4 and 16 hours. Similarly, CD4+ T cells were isolated and then skewed towards a Th1 or Th2 phenotype using the following protocol. Since C57Bl/6 T cells are already skewed in the Th1 direction, all that was required was to activate for 6 hours with 0.5μg/ml PMA and 10ng/ml ionomycin. 'Th2' skewing was obtained by plating naïve CD4+ T cells with 2.5μg/ml anti-CD28, 10ng/ml mIL-2 (R&D Systems Inc.) and 25ng/ml mIL-4 (R&D Systems) into plates coated with 0.5μg/ml anti-CD3. After 2 days in culture, cells were resuspended in media containing 10ng/ml mIL-2 (R&D

Systems) and 25ng/ml mIL-4. Cells were cultured for an additional three days then activated with PMA and ionomycin for 6 hours.

One additional set of Th1 and Th2 skewed T cells was derived using the T Cell Receptor Transgenic DO11.10 T cell line. All cells were plated into anti-CD3 and anti-CD28 coated plates. The 'Th1' cells were plated in media containing mIL-12 (1ng/ml) and anti-IL-4 (10μg/ml). The 'Th2' cells were plated in media containing mIL-4 (10ng/ml) and anti-IFNg (10μg/ml). After 24 hours, all cultures were given mIL-2 (10ng/ml). After two more days, the media on the cells was changed and new media containing the aforementioned cytokines was added and cells were cultured an additional 4 days before being harvested.

All of the murine T cell RNA was prepared using the RNeasy Midiprep™ Kit (Qiagen) and contaminating DNA was removed using the DNA-*free*™ kit from Ambion.

E. Isolation of RNA from the Murine Models of Pancreatitis and Irritable Bowel Disease

To induce a condition similar to human Irritable Bowel Disease (IBD), the hybrid mouse strain C57Bl6/129S6F1 was used. Mice were divided into 4 groups with an average size of six mice per group. Group 1 was given no Dextran Sulfate Sodium (DSS) and was sacrificed on day 14. Group 2 received 2% DSS for two days prior to being sacrificed. Group 3 received 2% DSS for seven days prior to sacrifice. Group 4 received 2% DSS for seven days then allowed to recover for seven days and was sacrificed on day 14. On the day of sacrifice, the distal colon sections were removed and placed in RNA*later*™ (Ambion). The colon sections were homogenized using standard techniques and RNA was isolated using the RNeasy Midiprep™ Kit (Qiagen). Contaminating DNA was removed by DNA-*free*™ (Ambion) treatment as per manufacturer's instructions.

In a different study, acute pancreatitis was induced in male CD-1 mice by caerulein injection. Mice were divided into three groups (n= 8 mice/group). Group 1 animals were given seven i.p. injections (1 injection per hour) with Vehicle (saline), and then sacrificed at 12 and 24 hours following the first injection. Groups 2 and 3

were given seven i.p. injections of caerulein (Sigma) (Catalog#C-9026) at a dose of 50μg/kg/hr for six hours (1 injection per hour). Group 2 was sacrificed at 12 hrs after the first injection and Group 3 was sacrificed at 24 hrs following the first injection. Pancreases were removed at the time of sacrifice and snap frozen for RNA isolation. Tissues were homogenized and RNA was isolated using the Qiagen RNeasy Midiprep™ Kit.

In yet another study, murine Zcytor17lig transgenic mice were generated and observed for phenotypic changes (see Example 41). Piloerection and hair loss was observed in many of the transgenic mice. Four transgenic mice were sacrificed and skin samples from both normal and hairless areas were removed and snap frozen for later RNA isolation. Skin sections from two non-transgenic control mice were collected as well. Skin samples were homogenized and then digested with Proteinase K (Qiagen) (Catalog# 19133) for 20 minutes at 60°C. RNA was then isolated using the Qiagen RNeasy Midiprep™ Kit following manufacturer's instructions. Carryover DNA was removed using DNA-*free*™ kit from Ambion.

F. Results of Quantitative and Semi-Quantitative RT-PCR for Human Zcytor17, OSMRbeta and Zcytor17lig

Zcytor17 and OSMRbeta expression was examined by quantitative RT-PCR in four sets of primary human monocytes that were either in their resting state or activated with IFNg for 24 or 48 hours. Zcytor17 expression was below detection in the unstimulated cells but increased dramatically after the 24-hour activation with IFNg, and was the highest after 48 hours of activation. In all cases OSMRbeta was below detection. Zcytor17lig was not tested in these samples.

In the primary T cells, Zcytor17 was below detection in both the resting CD4+ and CD8+ subsets. After a four-hour activation, however, expression of Zcytor17 went up in both subsets and then decreased to a slightly lower level at the 16 hour time point. OSMRbeta was below detection in these samples. Zcytor17lig expression was examined using semi-quantitative RT-PCR. No expression was detected in the unstimulated CD4+ and CD8+ T cells. However, after the four hour activation, high levels of Zcytor17lig were detected. This level dropped somewhat at

the 16 hour time point.

Expression of Zcytor17 was not examined in NK cells. OSMRb was below detection in these samples. Zcytor17lig expression was below detection in the resting NK cells, however there was a faint signal generated by the activated NK cells suggesting that these cells may make Zcytor17lig under certain conditions.

In the human monocyte–like cell lines, U937, THP-1 and HL-60, OSMRbeta expression was below detection in all of the resting and activated samples except for activated THP-1 samples where a faint signal was detected. Zcytor17 expression was high in both the U937 and THP-1 resting cell lines and showed a strong upregulation following activation. Expression in U937's was the highest of any cell type. In the HL-60's, Zcytor17 was expressed at moderate levels in the unstimulated cells and decreased upon stimulation with PMA. However, the expression of Zcytor17 was dramatically upregulated in the HL-60's when stimulated with IFNg for 72 and 96 hours. All of the human expression data is summarized in Table 8 below.

Table 8

Primary Human Monocytes	Activation Status	Zcytor17	OSMRbeta	Zcytor17lig
Human Monocytes	Unstim	-	-	
Human Monocytes	Act. 24hr IFNg	+	-	
Human Monocytes	Act. 48hr IFNg	++	-	
Human CD4+	Unstim	-	-	-
Human CD4+	Act 4hr	++	-	++
Human CD4+	Act. 16hr	+	-	+
Human CD8+	Unstim	-	-	-
Human CD8+	Act 4hr	++	-	++
Human CD8+	Act. 16hr	+	-	+
Human NK Cells	Unstim		-	-
Human NK Cells	Act 24hr		-	+
U937	Unstim	++	-	-
U937	Act. 16hr	+++	-	-
THP-1	Unstim	++	-	-
THP-1	Act. 16hr	+++	+	-
HL-60	Unstim	++	-	-
HL-60	Act. 16hr PMA	+	-	-

HL-60	Act. 72hr IFNg	+++	-	-
HL-60	Act. 96hr IFNg	+++	-	-

G. Results of Quantitative and Semi-Quantitative RT-PCR for Murine Zcytor17, OSMRbeta and Zcytor17lig

Murine Zcytor17, OSMRbeta and Zcytor17lig expression levels were examined in several murine T cells populations and the results are summarized in Table 9 below. Murine Zcytor17 expression was tested by semi-quantitative RT-PCR and shown to be at low levels on both resting and activated primary CD4+ T cells. Expression of Zcytor17 was detected on resting CD8+ T cells and then seemed to drop upon activation with anti-CD3 and anti-CD28 antibodies at both the 4- and 16-hour time points. OSMRbeta expression was measured by quantitative RT-PCR and shown to be expressed in resting and activated CD4+ and CD8+ T cells. The expression of OSMRbeta went up after a 4-hour activation and then returned to the unstimulated levels by 16 hours in both the CD4+ and CD8+ T cells. Zcytor17lig was detected by quantitative RT-PCR and shown to be expressed at very low levels in unstimulated CD4+ T cells. However, following a 4-hour activation, Zcytor17lig expression was dramatically upregulated and then dropped slightly by the 16-hour time point. In CD8+ T cells, no Zcytor17lig was detected in the unstimulated cells. There was some Zcytor17lig expression at the 4-hour time point, but by 16 hours expression levels had dropped back below detection.

In the DO11.10 T cells, Zcytor17 expression was detected in the naïve and Th2 skewed cells, but not in the Th1 skewed cells. OSMRbeta expression was at low levels in the naïve DO11.10 cells. There was a dramatic increase in OSMRbeta expression levels in the Th1 skewed cells and a moderate increase of expression in the Th2-skewed cells. The Zcytor17lig expression in these cells was shown to be predominantly by the Th2 skewed subset. Low levels were detected in the Th1 subset and no expression was detected in the naïve cells. These results are summarized in the Table 9 below.

In the primary CD4+ T cells that were skewed in either the Th1 or Th2 direction, Zcyto17 wasn't examined. OSMRbeta expression was detected in all three samples with the highest levels found in the Th2 sample. Similar to the DO11.10 results, Zcytor17lig expression was detected at high levels in the Th2 skewed subset,

with a small amount detected in the Th1 subset and levels were below detection in the unstimulated cells. These results are summarized in the Table 9 below.

Table 9

Murine T Cells	Zcytor17	OSMRbeta	Zcytor17lig
CD4+ T Cells Unstimulated	+	+	+/-
CD4+ T Cells 4hr Activation	+	++	++
CD4+ T Cells 16hr Activation	+	+	+
CD8+ T Cells Unstimulated	+	+	-
CD8+ T Cells 4hr Activation	+/-	++	+
CD8+ T Cells 16hr Activation	-	+	-
DO11.10 Naïve	+	+	-
DO11.10 Th1	-	+++	+
DO11.10 Th2	+	++	++
CD4+ T Cells Unstimulated		++	-
CD4+ T Cells - Th1 Skewed		+++	+
CD4+ T Cells - Th2 Skewed		++	+++

In the Zcytor17lig transgenic skin samples, Zcytor17, OSMRbeta and Zcytor17lig expression levels were determined using quantitative RT-PCR. Zcytor17 was shown to be present in all samples at roughly equivalent levels. There were dramatically higher levels of OSMRbeta expression in the non-transgenic control animals than the transgenic samples. Zcytor17lig expression was below detection in the non-transgenic control animals with moderate to high levels of expression in the transgenic animals. The results are summarized in Table 10 below.

Table 10

Murine Zcytor17lig Transgenic Skin	*Skin* Phenotype	Zcytor17	OSMRbeta	Zcytor17lig
Wild Type Mouse	Normal	+	+++	-
Wild Type Mouse	Normal	+	+++	-
Transgenic #1	Normal	+	+	+
Transgenic #1	Hair Loss	+	+	+
Transgenic #2	Normal	+	+	+

Transgenic #2	Hair Loss	+	+	+
Transgenic #3	Normal	+	+	+
Transgenic #3	Hair Loss	+	+	+
Transgenic #4	Normal	+	+	+++
Transgenic #4	Hair Loss	+	+	+++

In a different experiment, Zcytor17, OSMRbeta and Zcytor17lig expression levels were measured by quantitative RT-PCR in the pancreases of mice subjected to acute pancreatitis. Zcytor17 expression was below detection in all of the samples. OSMRbeta expression was seen at low levels in the normal control samples (Group 1), but showed a strong upregulation at the 12-hour time point (Group 2) and slightly lower levels at the 24-hour time point (Group 3). Zcytor17lig expression was below detection in the control animals, but showed high levels in both of the caerulein injected groups. The data is summarized in Table 11 below.

Table 11

Pancreatitis Model	Description	Zcytor17	OSMRbeta	Zcytor17lig
Group 1	Normal Control	-	+	-
Group 2	12hr Post Injection	-	+++	++
Group 3	24hr Post Injection	-	++	++

In another experiment, Zcytor17 and OSMRbeta expression levels were examined in the distal colons of mice subjected to DSS treatment. In this murine model of Inflammatory Bowel Disease, expression levels of both genes were determined by quantitative RT-PCR and are summarized in Table 12 below. Zcytor17 expression levels increased with the severity of the disease, with low levels of expression in the Group1 normal animals and increasing amounts seen Groups 2 and 3. In the Group 4 animals, the Zcytor17 levels had returned to more normal levels. Unlike Zcytor17 expression, OSMRbeta levels were the highest in the control animals and levels actually decreased in all three DSS treated groups.

Table 12

IBD Model	Description	SAC Day		Zcytor17	OSMRbeta
Group 1	Normal Control	14		+	++
Group 2	DSS-Treated 2 days	2		++	+
Group 3	DSS-Treated 7 days	7		+++	+
Group 4	DSS-Treated 7 days	14		+	+

Example 28

Human Zcytor17lig Tissue Distribution Expression based on RT-PCR Analysis of Multiple Tissue First-Strand cDNAs

Gene expression of the zcytor17lig was examined using commercially available normalized multiple tissue first-strand cDNA panels (OriGene Technologies, Inc. Rockville, MD; BD Biosciences Clontech, Palo Alto, CA). These included the OriGene "Human Tissue Rapid-Scan™ Panel" (Cat. #CHSCA-101, containing 22 different tissues, bone marrow, and plasma blood leucocytes) and the BD Biosciences Clontech "Human Blood Fractions MTC™ Panel" (Cat. #K1428-1, containing 9 different blood fractions).

PCR reactions were set up using the zcytor17lig specific oligo primers ZC41,458 (SEQ ID NO:60), and ZC41,457 (SEQ ID NO:61), which yield a 139 bp product, and ZC41,459 (SEQ ID NO:62), and ZC41,460 (SEQ ID NO:63), which yield a 92 bp product, Qiagen HotStarTaq DNA polymerase and buffer (Qiagen, Inc., Valencia, CA), dH_2O, and *Redi*Load™ dye (Research Genetics, Inc., Huntville, AL). The PCR cycler conditions were as follows: an initial 1 cycle 15 minute denaturation at 95°C, 35 cycles of a 45 second denaturation at 95°C, 1 minute annealing at 53°C or 56°C and 1 minute and 15 seconds extension at 72°C, followed by a final 1 cycle extension of 7 minutes at 72°C. The reactions were separated by electrophoresis on a 2% agarose gel (EM Science, Gibbstown, NJ) and visualized by staining with ethidium bromide.

A DNA fragment of the correct size was observed in the following human adult tissues using the OriGene "Human Tissue Rapid-Scan™ Panel": testis, plasma blood leucocytes (PBL), and bone marrow.

A DNA fragment of the correct size was observed in the following

human blood fractions using the BD Biosciences Clontech "Human Blood Fractions MTC™ Panel": activated mononuclear cells (B- & T-cells and monocytes), activated CD8+ cells (T-suppressor/cytotoxic), activated CD4+ cells (T-helper/inducer) and faintly in resting CD8+ cells.

Example 29

Cloning the human Oncostatin M receptor

The OncostatinM beta receptor (OSMRbeta) is a type I cytokine receptor with structural similarity to IL12R-B2. ZcytoR17 has structural similarity to IL12R - B1. The OSMRbeta and zcytor17 were tested to see whether they could interact as subunits in a cytokine signaling complex, and whether together they could act as a signaling receptor, or soluble receptor antagonist, for zcytor17lig.

To isolate OSMRbeta, oligonucleotide PCR primers ZC39982 (SEQ ID NO:64) and ZC39983 (SEQ ID NO:65) were designed to amplify the full length coding region of the human OncostatinM beta cDNA sequence (SEQ ID NO:6) (Genbank Accession No. U60805; Mosley B, JBC Volume 271, Number 50, Issue of December 20, 1996 pp. 32635-32643).

PCR reactions were run on an array of cDNA library templates using a robust polymerase, Advantage II (Clonetech, PaloAlto, CA), in order to identify a source of the cDNA. The template DNA used was from amplified cDNA plasmid libraries each containing 5 million independent cDNA clones. Reactions were assembled as per manufacturer's instructions using 400 fmol/µl of each oligonucleotide and 2-20 ng/µl purified plasmid library DNA as template. The cDNA libraries were derived from the following human tissues and cell lines: fetal brain, prostate smooth muscle, bone marrow, RPMI1588, thyroid, WI-38, testis, stimulated peripheral blood mononuclear cells, stimulated CD3+ cells, THP-1, activated tonsil, HACAT and fetal liver. Reactions were performed on a thermocycler machine using the following conditions: 30 cycles of 95°C for 20 seconds, 68°C for 3 minutes. At the conclusion of 30 cycles an additional single extension cycle of 8 minutes at 68°C was run. PCR products were visualized by TAE agarose, gel electrophoresis in the presence of

ethidium bromide followed by UV illumination. The most abundant product was found to be from a prostate smooth muscle cDNA library. The PCR reaction using prostate smooth muscle template and oligonucleotides ZC39982 (SEQ ID NO:64) and ZC39983 (SEQ ID NO:65) was repeated using a less robust but higher fidelity thermostable DNA polymerase "turboPFu", (Stratagene, La Jolla, CA). Thirty amplification cycles were run with the following conditions: denaturing at 94°C, 30 seconds, annealing at 63°C 45 seconds, extension at 72°C 3.5 minutes. A single band product was gel purified on a 0.8% TAE, agarose gel.

This DNA was then amplified again using primers ZC39980 (SEQ ID NO:66) and ZC39981 (SEQ ID NO:67) designed to include restriction enzyme recognition sequences to allow the cloning of this cDNA into a mammalian expression vector.

The PCR reaction was performed using "TurboPfu" and the purified PCR product for 15 cycles of: 95°C 1 minute, 64°C 1 minute 20 seconds, 72°C 4.5 minutes. The PCR reaction was then digested with EcoR1 and Xho1 (Invitrogen, Carlsbad, CA) and gel purified as described above. A mammalian expression vector, pZ7NX, was prepared by digesting with EcoR1 and Xho1 and the PCR product was ligated to this vector and electroporated into *E. coli* DH10b cells. Several bacterial colonies were isolated and sequenced. One clone was correct with the exception of a single non-conservative mutation. In order to change this base to match the expected sequence, an oligonucleotide spanning mutation and a neighboring Pst1 restriction site was used in a PCR reaction with "TurboPfu" using the pZP7Nx-h. OncostatinM R plasmid previously sequenced as a template. The PCR amplified DNA was digested with Pst1 and Xho1 and cloned back into the pZP7Nx-h OncostatinM R plasmid in place of the Pst1/Xho1 fragment containing the offending mutation. This new plasmid was sequenced over the recently amplified Pst1 to Xho1 region to confirm the correction and make sure no other errors were created in the amplification process. This analysis confirmed sequence that matched the expected sequence over the coding region. The sequence is shown in SEQ ID NO:6, and corresponding amino acid sequence shown in SEQ ID NO:7.

Example 30

Constructs for Generating a Human Zcytor17 / OncostatinM receptor (OSMRbeta) Heterodimer

A system for construction, expression and purification of such soluble heterodimeric receptors is known in the art, and has been adapted to the receptor pair, human oncostatin M receptor (OSMRbeta) and human zcytor17. For this construct, the polynucleotide for the soluble receptor for OSMRbeta is shown in SEQ ID NO:68 and corresponding polypeptide is shown in SEQ ID NO:69; and the polynucleotide for the soluble receptor for human zcytor17 is shown in SEQ ID NO:70 and corresponding polypeptide is shown in SEQ ID NO:71.

To construct a cell line expressing a secreted soluble hzcytor17/human OSMRbeta heterodimer, a construct was made so that the resulting heterodimeric soluble receptor comprises the extracellular domain of human OSMRbeta fused to the heavy chain of IgG gamma1 (Fc4) (SEQ ID NO:37) with a Glu-Glu tag (SEQ ID NO:35) at the C-terminus; while the extracellular domain of zcytoR17 was fused to Fc4 (SEQ ID NO:37) with a His tag (SEQ ID NO:72) at the C-terminus. For both of the hzcytor17 and human OSMRbeta arms of the heterodimer a Gly-Ser spacer of 12 amino acids (SEQ ID NO:73) was engineered between the extracellular portion of the receptor and the N-terminus of Fc4.

A. Construction of human soluble OSMRbeta/Fc4-CEE

For construction of the human soluble OSMRbeta/Fc4-CEE portion of the heterodimer the extracellular portion of human OSMRbeta was isolated using PCR with oligos ZC14063 (SEQ ID NO:48) and ZC41557 (SEQ ID NO:74) under PCR reaction conditions as follows: 30 cycles of 95°C for 60 sec., 57°C for 30 sec., and 72°C for 100 sec.; and 72°C for 7 min. PCR products were purified using QIAquick PCR Purification Kit (Qiagen), digested with EcoR1 and BglII (Boerhinger-Mannheim), separated by gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen).

The expression cassette, plasmid backbone and Fc4-GluGlu tag portion of the chimera were contained within a previously made in house plasmid vector. The

plasmid vector was digested with EcoR1 and BamH1 (Boerhinger-Mannheim), separated by gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen). The digested and purified fragments of human OSMRbeta and Fc4-cEE containing plasmid were ligated together using T4 DNA Ligase (Life Technologies, Bethesda, MD) using standard ligation methods. Minipreps of the resulting ligation were screened for an EcoRI/Sma1 insert of the correct size (772bp) for the soluble OSMRbeta and positive minipreps were sequenced to confirm accuracy of the PCR reaction. This new plasmid construction is termed pZP9-ONCOMR-Fc4CEE.

B. Construction of human soluble Zcytor17 / Fc4-CHIS

For construction of the hzcytor17/Fc4-CHIS portion of the heterodimer, the extracellular portion of human zcytor17 was isolated by digestion of a plasmid previously containing Zcytor17-Fc4 soluble receptor. The plasmid was first digested with Sal1 (New England Biolabs, Beverly, MA) after which the reaction was serially phenol chloroform extracted and ethanol precipitated. The digested DNA was then treated with T4 DNA Polymerase (Boerhinger-Mannheim), to fill in the 5' overhangs created by the Sal1 digestion, leaving the DNA ends blunt, after which the reaction was serially phenol chloroform extracted and ethanol precipitated. The blunted DNA was then further digested with BglII to cut at the 3' end.), separated by gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen) as per manufacturer's instruction. The resulting DNA fragment containing the sequence coding for the extracellular domain of zcytoR17 was ligated into an Fc4-CHIS tag containing mammalian expression vector prepared as follows.

The expression cassette, plasmid backbone and Fc4-CHIS tag portion of the chimera were contained within a previously made in house plasmid vector. This plasmid vector was digested with EcoR1(Boerhinger-Mannheim) after which the reaction was serially phenol chloroform extracted and ethanol precipitated. The digested DNA was then treated with T4 DNA Polymerase (Boerhinger-Mannheim), to fill in the 5' overhangs created by the EcoR1 digestion, leaving the DNA ends blunt, after which the reaction was serially phenol chloroform extracted and ethanol precipitated. The blunted DNA was then further digested with BamH1 (Boerhinger-

Mannheim) to cut at the 3' end, separated by gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen). The digested and purified fragments of human zcytor17 and Fc4-CHIS containing plasmid were ligated together using T4 DNA Ligase (Life Technologies, Bethesda, MD) using standard ligation methods.

Minipreps of the resulting ligation were screened by PCR using the zcytor17 specific sense primer ZC29180 (SEQ ID NO:22) and the Fc4 specific antisense primer ZC29232 (SEQ ID NO:75) with the following PCR reaction conditions: 30 cycles of 94°C for 60 sec., 68°C for 150 sec; and 72°C for 7 min. An expected product size of 848 bp confirmed the correct assembly of the plasmid termed pZEM228 hzcytor17/Fc4HIS.

A second zcytor17-Fc4 construction was created for use in generating homodimer protein from COS cells. Briefly the coding region for the full fusion protein was isolated by digestion of a plasmid previously containing Zcytor17-Fc4 soluble receptor with Sal1 (Boerhinger-Mannheim). The reaction was serially phenol chloroform extracted and ethanol precipitated. The digested DNA was then treated with T4 DNA Polymerase (Boerhinger-Mannheim), to fill in the 5' overhangs created by the EcoR1 digestion, leaving the DNA ends blunt, after which the reaction was serially phenol chloroform extracted and ethanol precipitated. The blunted DNA was then further digested with Not1 (Boerhinger-Mannheim) to cut at the 3' end, separated by gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen). A mammalian expression vector containing a CMV driven expression cassette was digested to generate compatible ends and the 2 fragments were ligated together. Minipreps of the resulting ligation were screened by PCR using the vector specific sense primer ZC14063 (SEQ ID NO:48) and the zcytor17 specific antisense primer ZC27899 (SEQ ID NO:19) with the following PCR reaction conditions: 30 cycles of 94°C for 30 sec., 64°C for 30 sec; 70°C for 90 sec; and 72°C for 7 min. An expected product size of approximately 1000 bp confirmed the correct assembly of the plasmid termed pZP7NX-hzcytor17-Fc4. This plasmid was subsequently transfected into COS cells using Lipofectamine (Gibco/BRL), as per manufacturer's instructions. The cells were conditioned for 60 hours in DMEM + 5%FBS (Gibco/BRL) after which the protein was purified over a protein G-sepharose 4B chromatography column and made

available for *in vitro* bioassays, for example, such as those described herein.

C. Generating a Human Zcytor17 / OncostatinM receptor (OSMRbeta)

About 16 μg each of the pZP9-ONCOMR-Fc4CEE and pZEM228 hzcytor17/Fc4HIS were co-transfected into BHK-570 (ATCC No. CRL-10314) cells using lipofectamine (Gibco/BRL), as per manufacturer's instructions. The transfected cells were selected for 10 days in DMEM + 5%FBS (Gibco/BRL) containing 0.5 mg/ml G418 (Gibco/BRL) and 250 nM methyltrexate (MTX)(Sigma, St. Louis, MO) for 10 days.

The resulting pool of doubly-selected cells was used to generate the heterodimeric protein. Three cell Factories (Nunc, Denmark) of this pool were used to generate 10 L of serum free conditioned medium. This conditioned media was passed over a 1 ml protein-A column and eluted in (10) 750 microliter fractions. Four of these fractions found to have the highest concentration were pooled and dialyzed (10 kD MW cutoff) against PBS. The desired heterodimeric soluble zcytor17/OSMRbeta protein complex was isolated from other media components by passing the pool over a Nickel column and washing the column with various concentrations of Imidazole. The soluble zcytor17/OSMRbeta protein eluted at intermediate concentrations of Imidazole, while hzcytor17/Fc4HIS homodimer eluted at higher concentrations of Imidazole.

Example 31

Tissue Distribution of Human zcytor17 in Tissue Panels Using Northern Blot and PCR

A. Human zcytor17 Tissue Distribution using Northern Blot

Human Multiple Tissue Northern Blots (Human 12-lane MTN Blot I and II, and Human Immune System MTN Blot II; Human Endocrine MTN, Human Fetal MTN Blot II, Human Multiple Tissue Array) (Clontech) as well as in house blots containing various tissues were probed to determine the tissue distribution of human zcytor17 expression. The in-house prepared blots included the following tissue and cell line mRNA: SK-Hep-1 cells, THP1 cells, Adrenal gland (Clontech); Kidney (Clontech), Liver (Clontech and Invitrogen); Spinal cord (Clontech), Testis (Clontech), Human CD4+ T-cells, Human CD8+ T-cells, Human CD19+ T-cells, human mixed

lymphocyte reaction (MLR), THP1 cell line (ATCC No. TIB-202), U937 cell line, p388D1 mouse lymphoblast cell line (ATCC No. CCL-46) with or without stimulation by Ionomycin; and WI-38 human embryonic lung cell line (ATCC No. CRL-2221) with or without stimulation by Ionomycin.

An approximately 500 bp PCR derived probe for zcytor17 (SE ID NO:4) was amplified using oligonucleotides ZC28,575 (SEQ ID NO:77) and ZC27,899 (SEQ ID NO:19) as primers. The PCR amplification was carried out as follows: 30 cycles of 94°C for 1 minute, 65°C for 1 minute, and 72°C for 1 minute; followed by 1 cycle at 72°C for 7 minutes. The PCR product was visualized by agarose gel electrophoresis and the approximately 500 bp PCR product was gel purified as described herein. The probe was radioactively labeled using the PRIME IT II™ Random Primer Labeling Kit (Stratagene) according to the manufacturer's instructions. The probe was purified using a NUCTRAP™ push column (Stratagene). EXPRESSHYB™ (Clontech) solution was used for the prehybridization and as a hybridizing solution for the Northern blots. Prehybridization was carried out at 68°C for 2 hours. Hybridization took place overnight at 68°C with about $1.5X10^6$ cpm/ml of labeled probe. The blots were washed three times at room temperature in 2X SSC, 0.05% SDS, followed by 1 wash for 10 minutes in 2X SSC, 0.1% SDS at 50°C. Several faint bands were seen after several days exposure. An approximately 9 kb transcript was seen in trachea, skeletal muscle and thymus; an approximately 2 kb transcript was seen in PBL, HPV, U937 and THP-1 cells; and about a 1.2 kb transcript was seen in placenta, bone marrow and thyroid, and HPV and U937 cells. In all the tissues listed above, the signal intensity was faint. There appeared to be little expression in most normal tissues, suggesting that zcytor17 expression may be dependent on activation of the cell or tissues in which it is expressed.

B. Tissue Distribution in tissue panels using PCR

A panel of cDNAs from human tissues was screened for zcytor17 expression using PCR. The panel was made in-house and contained 94 marathon cDNA and cDNA samples from various normal and cancerous human tissues and cell lines is shown in Table 13, below. The cDNAs came from in-house libraries or

marathon cDNAs from in-house RNA preps, Clontech RNA, or Invitrogen RNA. The marathon cDNAs were made using the marathon-Ready™ kit (Clontech, Palo Alto, CA) and QC tested with clathrin primers ZC21195 (SEQ ID NO:78) and ZC21196 (SEQ ID NO:79) and then diluted based on the intensity of the clathrin band. To assure quality of the panel samples, three tests for quality control (QC) were run: (1) To assess the RNA quality used for the libraries, the in-house cDNAs were tested for average insert size by PCR with vector oligos that were specific for the vector sequences for an individual cDNA library; (2) Standardization of the concentration of the cDNA in panel samples was achieved using standard PCR methods to amplify full length alpha tubulin or G3PDH cDNA using a 5' vector oligo ZC14,063 (SEQ ID NO:48) and 3' alpha tubulin specific oligo primer ZC17,574 (SEQ ID NO:49) or 3' G3PDH specific oligo primer ZC17,600 (SEQ ID NO:50); and (3) a sample was sent to sequencing to check for possible ribosomal or mitochondrial DNA contamination. The panel was set up in a 96-well format that included a human genomic DNA (Clontech, Palo Alto, CA) positive control sample. Each well contained approximately 0.2-100 pg/µl of cDNA. The PCR reactions were set up using oligos ZC26,358 (SEQ ID NO:80) and ZC26,359 (SEQ ID NO:81), TaKaRa Ex Taq™ (TAKARA Shuzo Co. LTD, Biomedicals Group, Japan), and Rediload dye (Research Genetics, Inc., Huntsville, AL). The amplification was carried out as follows: 1 cycle at 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 66.3°C for 30 seconds and 72°C for 30 seconds, followed by 1 cycle at 72°C for 5 minutes. About 10 µl of the PCR reaction product was subjected to standard agarose gel electrophoresis using a 4% agarose gel. The correct predicted DNA fragment size was observed in lymph node, prostate, thyroid, HPV (prostate epithelia), HPVS (prostate epithelia, selected), lung tumor, uterus tumor reactions, along with the genomic DNA reaction.

The DNA fragment for prostate tissue (2 samples), HPV (prostate epithelia), HPVS (prostate epithelia, selected), and genomic were excised and purified using a Gel Extraction Kit (Qiagen, Chatsworth, CA) according to manufacturer's instructions. Fragments were confirmed by sequencing to show that they were indeed zcytor17.

Table 13

Tissue/Cell line	#samples		Tissue/Cell line	#samples
Adrenal gland	1		Bone marrow	3
Bladder	1		Fetal brain	3
Bone Marrow	1		Islet	2
Brain	1		Prostate	3
Cervix	1		RPMI #1788 (ATCC # CCL-156)	2
Colon	1		Testis	4
Fetal brain	1		Thyroid	2
Fetal heart	1		WI38 (ATCC # CCL-75	2
Fetal kidney	1		ARIP (ATCC # CRL-1674 - rat)	1
Fetal liver	1		HaCat - human keratinocytes	1
Fetal lung	1		HPV (ATCC # CRL-2221)	1
Fetal muscle	1		Adrenal gland	1
Fetal skin	1		Prostate SM	2
Heart	2		CD3+ selected PBMC's Ionomycin + PMA stimulated	1
K562 (ATCC # CCL-243)	1		HPVS (ATCC # CRL-2221) - selected	1
Kidney	1		Heart	1
Liver	1		Pituitary	1
Lung	1		Placenta	2
Lymph node	1		Salivary gland	1
Melanoma	1		HL60 (ATCC # CCL-240)	3
Pancreas	1		Platelet	1
Pituitary	1		HBL-100	1
Placenta	1		Renal mesangial	1
Prostate	1		T-cell	1
Rectum	1		Neutrophil	1
Salivary Gland	1		MPC	1

Skeletal muscle	1		Hut-102 (ATCC # TIB-162)	1
Small intestine	1		Endothelial	1
Spinal cord	1		HepG2 (ATCC # HB-8065)	1
Spleen	1		Fibroblast	1
Stomach	1		E. Histo	1
Testis	2			
Thymus	1			
Thyroid	1			
Trachea	1			
Uterus	1			
Esophagus tumor	1			
Gastric tumor	1			
Kidney tumor	1			
Liver tumor	1			
Lung tumor	1			
Ovarian tumor	1			
Rectal tumor	1			
Uterus tumor	1			

C. Expression analysis of zcytoR17 by PCR and Northern

Annotation of the cell types and growth conditions that affect expression of the receptor is a useful means of elucidating its function and predicting a source of ligand. To that end a wide variety of tissue and cell types were surveyed by PCR. The thermostable polymerase Advantage II™ (Clontech, La Jolla, CA) was used with the oligonucleotide primers ZC29,180 (SEQ ID NO:22) and ZC29,179 (SEQ ID NO:82) and 1-10ng of the various cDNA templates listed below for 30 amplification cycles of (94°C, 30 sec.; 66°C, 20 sec.; 68°C, 1 min. 30 sec.). Following this, 20% of each reaction was run out on 0.8% agarose, TAE /ethidium bromide gels and visualized with UV light. Samples were then scored on the basis of band intensity. See Table 14

below.

Table 14

Cells and Conditions	Score 0-5
Hel stimulated with PMA	0
U937	3
MCF-7	0
HuH7	1
Human follicle	0
HT-29	0
HEPG2	0
HepG2 stimulated with IL6	0
Human dermal endothelial	0
Human venous endothelial	0
Human CD4+	0
BEWO	0
Human CD19+	1
Human PBMC stimulated with PHA, PMA, Ionomycin, IL2, IL4, TNFα 24 hours	0
Human PBMC stimulated with LPS, PWM, IFNγ, TNFα, 24 hours	0
Human PBMC all of the above conditions for 48 hours	4
HUVEC p.2	4
RPMI1788	0
TF1	0
Monkey spleen T cells stimulated with PMA, Ionomycin	0
Human prostate epithelia HPV transformed	5
Human tonsils, inflamed	0
HACAT	0
Human chondrocyte	1
Human synoviacyte	1
THP1	5
REH	0

Of the strong positive PCR signals, two were from the human monocyte cell lines U937 and THP1.

These two cell lines along with a prostate epithelia line were selected for further analysis by Northern blot. Previous attempts at visualizing a transcript by northern analysis using mRNA from various tissues yielded weak and diffuse signals in the surprisingly large size range of 7-10kb making this data difficult to interpret. A denaturing formaldehyde/MOPS/0.8%agarose gel was prepared (RNA Methodologies, Farrell, RE Academic Press) and 2 μg of polyA+ mRNA was run for each sample along side an RNA ladder (Life Technologies, Bethesda, MD). The gel was then transferred to Hybond nylon (Amersham, Buckinghamshire, UK), UV crosslinked, and hybridized in ExpressHyb solution (Clontech, LaJolla, CA) at 68°C overnight using a probe to human zcytoR17 generated by PCR with the oligos ZC28,575 (SEQ ID NO:77), and ZC27,899 (SEQ ID NO:19) and labeled with a Megaprime ^{32}P kit (Amersham). The northern blot was subsequently washed with 0.2xSSC+0.1%SDS at 65C for 15 minutes and exposed to film for 7 days with intensifying screens. A prominent 8kb band was seen in both the prostate epithelia and U937 lanes while a fainter band was present in the THP1 lane.

To optimize the cDNA used as a hybridization probe, four different regions of the full-length human zcytoR17 sequence were amplified by PCR, labeled and hybridized as described above to southern blots containing genomic and amplified cDNA library DNA. The four probes, herein designated probes A-D, were amplified using the following primer pairs: (A) ZC28,575 (SEQ ID NO:77), ZC27,899 (SEQ ID NO:19); (B) ZC27,895 (SEQ ID NO:20), ZC28,917 (SEQ ID NO:83); (C) ZC28,916 (SEQ ID NO:84), ZC28,918 (SEQ ID NO:85); and (D) ZC28,916 (SEQ ID NO:84), ZC29,122 (SEQ ID NO:21). Human genomic DNA along with amplified cDNA libraries demonstrated to contain zcytor17 by PCR were digested with EcoR1 and Xho1 to liberate inserts and run out on duplicate TAE/0.8%agarose gels, denatured with 0.5M NaOH, 1.5 M NaCl, blotted to Hybond, UV crosslinked and each hybridized with a distinct probe. Probe B was found to have the least nonspecific binding and strongest signal. Thus, Probe B was used for all subsequent hybridizations.

Given that the THP1 cells are an excellent model of circulating monocytes and expressed zcytor17 at low levels we treated them with a variety of compounds in an effort to increase expression of zcytoR17. The cells were grown to a density of 2e5/ml, washed and resuspended in various stimulating media, grown for four or thirty hours, and harvested for RNA preparations. Each media was supplemented with one of the following drugs or pairs of cytokines: LPS 2ug/ml (Sigma Chemicals, St. Louis, MO), hTNFα 2 ng/ml (R&D Systems, Minneapolis, MN), hGM-CSF 2ng/ml (R&D Systems, Minneapolis, MN), hIFNγ 50 ng/ml (R&D Systems, Minneapolis, MN), hMCSF 1ng/ml (R&D Systems, Minneapolis, MN), hIL6 1ng/ml (R&D Systems, Minneapolis, MN), hIL1β 2ng/ml (R&D Systems, Minneapolis, MN), hIFNγ 50ng/ml+hIL4 0.5ng/ml (R&D Systems, Minneapolis, MN), hIFNγ 50ng/ml+hIL10 1ng/ml (R&D Systems, Minneapolis, MN), PMA 10ng/ml (Calbiochem, SanDiego, CA) and an untreated control. At the end of the culture period Total RNA was prepared using an RNAeasy Midi-kit (Qiagen, Valencia, CA). Poly A+ RNA was selected from the total RNA using an MPG kit (CPG, Lincoln Park, NJ). 2 ug of polyA+ RNA from each condition was run on formaldehyde/MOPS/agarose gels, transferred to nylon and UV crosslinked as described above. These northern blots were then hybridized, as above, to probe B at 68°C overnight, washed at high stringency with 0.2XSSC, 0.1%SDS at 65C, exposed to film overnight then exposed to phosphor screens for signal quantitation. A dominant 8 kb mRNA as well a relatively weaker 2.8 kb band were seen in all lanes. A 20-fold increase in zcytor17 mRNA was seen in RNA from cells treated with hIFNγ for 30 hours, this effect was slightly muted with simultaneous treatment with IL4. Minor 3 fold increases in mRNA were seen in RNA from cells treated with LPS, TNFα and GM-CSF while MCSF, IL6, and IL1β had no effect on zcytor17 mRNA levels. This data suggests a role for the zcytor17 receptor and its ligand in monocyte macrophage biology and by extension any number of disease processes in which these cells participate.

Example 32

Tissue Distribution of human zcytor17lig in Tissue Panels Using Northern Blot and PCR

A human zcytor17lig cDNA fragment was obtained using PCR with gene specific primers: Sense primer ZC41438 (SEQ ID NO:93) and antisense primer ZC41437 (SEQ ID NO:94) and template human zcytor17lig cDNA (SEQ ID NO:90) This fragment was purified using standard methods and about 25ng labeled with ^{32}P alpha dCTP using the Prime-It RmT random primer labeling kit (Stratagene) and hybridized in Ultrahyb, (Ambion) and used to expose Biomax film/intensifying screens per the manufacturer's recommendations in each case. New, previously unused blots Including the Clontech Human 12 lane MTN, the human brain MTN II, and the human brain MTN blot IV, the human immune system MTN II, and the human MTE array II, from Clontech were hybridized overnight at 42°C per the Ambion ultrahyb method. Non-specific radioactive counts were washed off using .1SSC/.5% SDS at 55°C. The positive blots included the human 12 lane MTN (Clontech). Of the 12 tissues examined, only placenta was positive for an approximately 1.2 KB transcript.

Example 33

Construction of mammalian expression vectors that express human zcytor17lig-CEE,

A. Construction of zCytor17Lig-CEE/pZMP21

An expression plasmid containing all or part of a polynucleotide encoding zCytor17Lig-CEE (SEQ ID NO:95) was constructed via homologous recombination. The plasmid was called zCytor17Lig-CEE/pZMP21.

The construction of zCytor17Lig-CEE/pZMP21 was accomplished by generating a zCytor17Lig-CEE fragment using PCR amplification. The DNA template used for the production of the zCytor17Lig-CEE fragment was zCytor17Lig/pZP7nx. The primers used for the production of the zCytor17Lig-CEE fragment were: (1) ZC41,607 (SEQ ID NO:97) (sense sequence), which includes from the 5' to the 3' end: 28bp of the vector flanking sequence (5' of the insert) and 21 bp corresponding to the 5' sequence of zCytor17Lig; and (2) ZC41,605 (SEQ ID NO:98) (anti-sense sequence), which includes from the 5' to the 3' end: 37 bp of the vector flanking sequence (3' of the insert), 3 bp of the stop codon, 21 bp encoding a C-terminal EE tag, and 21 bp corresponding to the 3' end of zCytor17Lig sequence. The fragment resulting from the above PCR amplification was a copy of the template zCytor17Lig with the addition of a

C-terminal EE tag, yielding a final product zCytor17Lig-CEE.

PCR reactions were run as follows: To a 100μl final volume was added: 10μl of 10x Taq Polymerase Reaction Buffer with 15mM MgCl (Gibco), 1μl of Taq DNA Polymerase (5 units/μl, Gibco), 3μl of 10mM dNTPs, 78μl dH_2O, 3μl of a 20 pmol/μl stock of primer ZC41,607 (SEQ ID NO:97) 3μl of a 20 pmol/μl stock of primer ZC41,605 (SEQ ID NO:98), and 2μl of a 0.13μg/μl stock of zCytor17lig template DNA. A volume equal to 50μl of mineral oil was added to the mixture. The reaction was heated to 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute; 55°C for 2 minutes; 72°C for 3 minutes; followed by a 10 minute extension at 72°C and held at 4°C until the reaction was collected.

The plasmid pZMP21 was restriction digested with BglII enzyme, cleaned with a QiaQuick PCR Purification Kit (Qiagen) using a microcentrifuge protocol, and used for recombination with the PCR fragment. Plasmid pZMP21 was constructed from pZMP20 which was constructed from pZP9 (deposited at the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, and is designated No. 98668) with the yeast genetic elements from pRS316 (deposited at the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, and designated No. 77145), an IRES element from poliovirus, and the extracellular domain of CD8, truncated at the carboxyl terminal end of the transmembrane domain. PZMP21 is a mammalian expression vector containing an expression cassette having the MPSV promoter, immunoglobulin signal peptide intron, multiple restriction sites for insertion of coding sequences, a stop codon and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene, the SV40 terminator, as well as the URA3 and CEN-ARS sequences required for selection and replication in *S. cerevisiae*.

Fifty microliters of competent yeast cells (*S. cerevisiae*) were independently combined with 100 ng of cut plasmid, 5 μl of previously described PCR mixture, and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV (5 kV/cm), ∞ ohms, 25 μF. Each cuvette had 600 μl of 1.2 M sorbitol added, and the yeast was plated in one 100 μl aliquot and one 300 μl

aliquot onto two URA-D plates and incubated at 30°C. After about 72 hours, the Ura+ yeast transformants from a single plate were resuspended in 1 ml H_2O and spun briefly to pellet the yeast cells. The cell pellet was resuspended in 500 μl of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). The 500 μl of the lysis mixture was added to an Eppendorf tube containing 300 μl acid washed 600μm glass beads and 300 μl phenol-chloroform, vortexed for 1 minute intervals two or three times, followed by a 5 minute spin in a Eppendorf centrifuge at maximum speed. Three hundred microliters of the aqueous phase was transferred to a fresh tube, and the DNA precipitated with 600 μl 100% ethanol (EtOH), followed by centrifugation for 10 minutes at 4°C. The DNA pellet was then washed with 500μl 70% EtOH, followed by centrifugation for 1 minute at 4°C. The DNA pellet was resuspended in 30 μl H_2O.

Transformation of electrocompetent *E. coli* cells (MC1061) was done with 5 μl of the yeast DNA prep and 50 μl of MC1061 cells. The cells were electropulsed at 2.0 kV, 25 μF and 400 ohms(Ω). Following electroporation, 600μl SOC (2% Bacto‘ Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose) was added. The electroporated *E.coli* cells were plated in a 200μl and a 50μl aliquot on two LB AMP plates (LB broth (Lennox), 1.8% Bacto Agar (Difco), 100 mg/L Ampicillin). The plates were incubated upside down for about 24 hours at 37°C. Three Ampicillin-resistant colonies were selected at random and submitted for sequence analysis of the insert. Large-scale plasmid DNA was isolated from a sequence-confirmed clone using the Qiagen Maxi kit (Qiagen) according to manufacturer’s instructions.

B. Construction of mouse zCytor17Lig(m)-CEE/pZMP21

An expression plasmid containing the entire polynucleotide encoding murine zCytor17Lig-CEE (SEQ ID NO:104 and SEQ ID NO:105) was also constructed via homologous recombination using the method described in Example 33A above. The primers used were: (1) ZC41643 (SEQ ID NO:106) (forward, 5' to 3' sense) having a 28bp vector overlap 5' of the insertion point; 21 bp of the 5' end of zcytor17lig(m) and (2) ZC41641 (SEQ ID NO:107) (reverse, 5' to 3' anti-sense) having a 37bp vector

overlap 3' of the insertion point; 3 bp stop codon; 21 bp C-terminal EE tag; 24 bp of the 3' end of zCytor17Lig(m)-CEE. The plasmid was called zcytor17lig(m)-CEE/pZMP21. The polynucleotide sequence of zcytor17lig(m)-CEE is shown in SEQ ID NO:104, and corresponding polypeptide sequence is shown in SEQ ID NO:105.

Example 34

Transfection And Expression Of zcytor17lig-CEE Polypeptides

A. Expression of human zcytor17lig-CEE/pZMP21 in 293T cells

ZCytor17Lig-CEE was expressed transiently in 293T cells (Stanford University School of Medicine, Stanford, CA, ATCC (SD-3515)) to generate initial purified protein. The day before the transfection, 293T cells were seeded at $6.5x10^4$ cells/cm^2 in 30 T162 culture flasks with a total volume of 30ml of culture media (SL7V4 +5%FBS +1% Pen/Strep) per flask. The cells were allowed to incubate for 24 hours at 37°C.

A DNA/Liposome mixture was prepared as follows: Two 50 ml conical tubes were filled with 25 mLs of transfection media (SL7V4 +1%Pen/Strep) and 1.13 mg of zCytor17Lig-CEE/pZMP21 (Example 33) was added to each. A separate set of two 50 ml conical tubes were filled with 22 ml of transfection media (above) and 3 ml of liposomes (Lipofectamine, Gibco) was added to each. For each set of tubes, one tube of DNA was added to one tube of liposomes and the DNA/liposome mix was incubated for 30 minutes. The two 50 ml conical tubes containing the DNA/liposome mixtures were pooled (about 100 ml) and 300 ml of transfection media was added.

The 30 flasks of the 293T cells were decanted, washed 1x with about 15 ml of PBS, and 12.5 ml of the diluted DNA/liposome mixture was added to each flask. The flasks were incubated for 3 hours at 37°C. After the incubation period, 25 ml of culture media (above) were added to each T162 flask. The transfection media was harvested after approximately 96 hours and was used for protein purification (Example 35).

B. Expression of human zcytor17lig-CEE/pZMP21 in BHK cells

Full-length zCytor17Lig protein was produced in BHK cells transfected with zCytor17Lig-CEE/pZMP21 (see Example 33 above). BHK 570 cells (ATCC CRL-10314) were plated in T75 tissue culture flasks and allowed to grow to approximately 50 to 70% confluence at 37°C, 5% CO_2, in growth media (SL7V4, 5%FBS, 1% pen/strep). The cells were then transfected with zCytor17Lig-CEE/pZMP21 by liposome-mediated transfection (using Lipofectamine™; Life Technologies), in serum free (SF) media (SL7V4). The plasmid (16μg) was diluted into 1.5 ml tubes to a total final volume of 640 μl with SF media. Thirty-five microliters the lipid mixture was mixed with 605 μl of SF medium, and the resulting mixture was allowed to incubate approximately 15 minutes at room temperature. Five milliliters of SF media was then added to the DNA:lipid mixture. The cells were rinsed once with 10 ml of PBS, the PBS was decanted, and the DNA:lipid mixture was added. The cells are incubated at 37°C for five hours, then 15 ml of media (SL7V4, 5% FBS, 1% pen/strep) was added to each plate. The plates were incubated at 37°C overnight, and the DNA:lipid media mixture was replaced with selection media (SL7V4, 5% FBS, 1% pen/strep, 1μM methotrexate) the next day. Approximately 10 days post-transfection, methotrexate-resistant colonies from the T75 transfection flask were trypsinized, and the cells were pooled and plated into a T-162 flask and transferred to large-scale culture.

C. Expression of mouse zcytor17lig-CEE(m)/pZMP21 in 293T cells

Mouse zcytor17lig(m)-CEE was expressed transiently in 293T cells as described in Example 34A and cultured media was used for protein purification (Example 35).

Example 35

Purification of Zcytor17lig-CEE from 293T cells

Unless otherwise noted, all operations were carried out at 4°C. The following procedure was used for purifying both mouse and human Zcytor17lig containing C-terminal Glu-Glu (EE) tags (SEQ ID NO:103). Conditioned media from 293T cells expressing Zcytor17lig-CEE (Example 34) was purified. Total target

protein concentrations of the conditioned media were determined via SDS-PAGE and Western blot analysis with the anti-EE antibody.

A 5.5 ml column of anti-EE Poros 50 A (PE BioSystems, Framingham, MA) (prepared as described below) was poured in a Waters AP-1, 1 cm x 7cm glass column (Waters, Milford, MA). The column was flow packed and equilibrated on a BioCad Sprint (PE BioSystems, Framingham, MA) with phosphate buffered saline (PBS) pH 7.4. The conditioned media was adjusted with NaCl to 0.3 M and the pH adjusted to 7.2. The conditioned media was then loaded on the column overnight with about 3 ml/minute flow rate. The column was washed with 10 column volumes (CVs) of PBS pH 7.4, and again washed with 3CVs 5X Sigma PBS pH 7.4. It was step eluted with 0.5 M Acetate, 0.5 M NaCl, pH 2.5 at 3 ml/minute. The fraction tubes contained 1ml Tris base (no pH adjustment) to neutralize the elution immediately. The column was again washed for 2CVs with 5X Sigma PBS, pH 7.4 to neutralize the column and then equilibrated in PBS (pH 7.4). Two ml fractions were collected over the entire elution chromatography and absorbance at 280 and 215 nM were monitored; the pass through and wash pools were also saved and analyzed. The 5X PBS and the acid elution peak fractions were analyzed for the target protein via SDS-PAGE Silver staining and Western Blotting with the primary antibody anti-EE and secondary antibody, anti mouse-HRP conjugated. The acid elution fractions of interest were pooled and concentrated from 38 ml to 0.8ml using a 5000 Dalton molecular weight cutoff membrane spin concentrator (Millipore, Bedford, MA) according to the manufacturer's instructions.

To separate Zcytor17lig-CEE from aggregated material and any other contaminating co-purifying proteins, the pooled concentrated fractions were subjected to size exclusion chromatography on a 1.6 x 60 cm (120 ml) Superdex 75 (Pharmacia, Piscataway, NJ) column equilibrated and loaded in PBS at a flow rate of 1.0 ml/min using a BioCad Sprint. Three ml fractions were collected across the entire chromatography and the absorbance at 280 and 215 nM were monitored. The peak fractions were characterized via SDS-PAGE Silver staining, and only the most pure fractions were pooled. This material represented purified Zcytor17lig -CEE protein.

On Western blotted, Coomassie Blue and Silver stained SDS-PAGE

gels, the Zcytor17lig-CEE was one major band. The protein concentration of the purified material was performed by BCA analysis (Pierce, Rockford, IL) and the protein was aliquoted, and stored at -80°C according to standard procedures.

To prepare PorosA50 anti-EE, a 65 ml bed volume of Poros A50 (PE Biosystems) was washed with 100 ml of water and then 0.1 M triethanolamine, pH 8.2 (TEA, ICN, Aurora, Ohio), 1 M Na_2S0_4, pH 8.8 containing 0.02% sodium azide using a vacuum flask filter unit. The EE monoclonal antibody solution, at a concentration of 2 mg/ml in a volume of 300 ml, was mixed with the washed resin in a volume of 250 ml. After an overnight incubation at room temperature, the unbound antibody was removed by washing the resin with 5 volumes of 200 mM TEA, 1 M Na_2S0_4, pH 8.8 containing 0.02% sodium azide as described above. The resin was resuspended in 2 volumes of TEA, 1 M Na_2S0_4, pH 8.8 containing 0.02% sodium azide and transferred to a suitable container. Three ml of 25 mg/ml (68mM) Disuccinimidyl suberate (in DMSO supplied by Pierce, Rockford, IL) was added and the solution was incubated for three hours at room temperature. Nonspecific sites on the resin were then blocked by incubating for 10 min at room temperature with 5 volumes of 20 mM ethanolamine (Sigma, St. Louis, MO) in 200 mM TEA, pH 8.8 using the vacuum flask filter unit. The resin was washed with PBS, pH 7.4, followed by 0.1 M Glycine, pH 3 and then neutralized with 10X PBS. After washing with distilled water, the final coupled anti-EE Poros-A 50 resin was stored at 4°C in 20% Ethanol.

Example 36

N-terminal sequencing of human and mouse Zcytor17lig

A. N-terminal sequencing of human Zcytor17lig

Standard automated N-terminal polypeptide sequencing (Edman degradation) was performed using reagents from Applied Biosystems. N-terminal sequence analysis was performed on a Model 494 Protein Sequencer System (Applied Biosystems, Inc., Foster City, CA). Data analysis was performed with Model 610A Data Analysis System for Protein Sequencing, version 2.1a (Applied Biosystems).

A purified human zcytor17lig-CEE sample (Example 35) was supplied. The sample was loaded onto a prepared glass fiber filter for n-terminal sequencing. The

glass fiber filter was prepared by precycling it with Biobrene™.

N-terminal sequence analysis of the secreted human zcytor17lig polypeptide did not verify the predicted cleavage site of the signal sequence but resulted in a mature start at residue 27(Leu) in SEQ ID NO:2 of the human zcytor17lig precursor sequence.

B. N-terminal sequencing of mouse Zcytor17lig

Standard automated N-terminal polypeptide sequencing (Edman degradation) was performed using reagents from Applied Biosystems. N-terminal sequence analysis was performed on a Model 494 Protein Sequencer System (Applied Biosystems, Inc., Foster City, CA). Data analysis was performed with Model 610A Data Analysis System for Protein Sequencing, version 2.1a (Applied Biosystems).

A purified mouse zcytor17lig-CEE sample was supplied as captured on Protein G Sepharose/anti-EE beads (Example 35). The beads were placed in reducing SDS PAGE sample buffer and on a boiling water bath before running on SDS PAGE, using a Novex SDS PAGE system (4-12% Bis-Tris MES NuPAGE; Invitrogen) as per manufacturer's instructions. The gel was electrotransferred to a Novex PVDF membrane (Invitrogen), and Coomassie blue stained (Sigma, St. Louis, MO) using standard methods. Corresponding anti-EE Western blots were performed to identify the zcytor17lig band for N-terminal protein sequencing. The mouse anti-EE IgG HRP conjugated antibody used was produced in house.

N-terminal sequence analysis of the secreted mouse zcytor17lig polypeptide verified the predicted cleavage site of the signal sequence resulting in a mature start at 31 (Ala) in reference to SEQ ID NO:11 and SEQ ID NO:91 of the mouse zcytor17lig precursor sequence.

Example 37

Cos cell binding assay

A binding assay was used to test the binding of the zcytor17lig to receptors comprising zcytor17 receptor, such as the zcytor17 receptor or receptor heterodimers and trimers comprising zcytor17 receptor (e.g., zcytor17/OSMR,

zcytor17/WSX-1, or zcytor17/OSMR/WSX-1, or other Class I cytokine receptor subunits). Zcytor17 receptor plasmid DNA was transfected into COS cells and transfected COS cells were used to assess binding of the zcytor17lig to receptors comprising zcytor17 receptor as described below.

A. COS Cell Transfections

The COS cell transfection was performed as follows: Mix 800 ng receptor plasmid DNA in the following combinations: pZp7pX/zcytor17 alone; pZp7Z/WSX-1 alone; pZp7NX/OSMR alone; pZp7pX/zcytor17 + pZp7NX/OSMR; pZp7pX/zcytor17 + pZp7Z/WSX-1; pZp7NX/OSMR + pZp7Z/WSX-1; pZp7pX/zcytor17 + pZp7NX/OSMR + pZp7Z/WSX-1) and 4ul Lipofectamine™ in 80 ul serum free DMEM media (55mg sodium pyruvate, 146mg L-glutamine, 5 mg transferrin, 2.5 mg insulin, 1 µg selenium and 5 mg fetuin in 500 ml DMEM), incubate at room temperature for 30 minutes and then add 320 µl serum free DMEM media. Add this 400 μl mixture onto 2 x10^5 COS cells/well plated on 12-well tissue culture plate (fibronectin-coated) and incubate for 5 hours at 37°C. Add 500ul 20% FBS DMEM media (100 ml FBS, 55 mg sodium pyruvate and 146mg L-glutamine in 500 ml DMEM) and incubate overnight.

B. Binding Assay

The binding assay was performed as follows: media was rinsed off cells with PBS + 0.1% BSA, and then cells were blocked for 60 minutes with the same solution. The cells were then incubated for 1 hour in PBS + 0.1% BSA with 1.0 µg/ml zcytor17ligCEE purified protein. Cells were then washed with PBS + 0.1% BSA and incubated for another hour with 1:1000 diluted mouse anti-GluGlu antibody. Again cells were washed with PBS + 0.1% BSA, then incubated for 1 hour with 1:200 diluted goat anti-mouse-HRP conjugated antibody.

Positive binding was detected with fluorescein tyramide reagent diluted 1:50 in dilution buffer (NEN kit) and incubated for 4-6 minutes, and washed with PBS + 0.1% BSA. Cells were fixed for 15 minutes with 1.8% Formaldehyde in PBS, then washed with PBS + 0.1%BSA. Cells were preserved with Vectashield Mounting Media

(Vector Labs Burlingame, CA) diluted 1:5 in PBS. Cells were visualized using a FITC filter on fluorescent microscope.

Positive binding was detected for cells transfected with zcytor17 only, zcytor17+OSMRbeta, zcytor17+WSX-1, and zcytor17+OSMRbeta+WSX-1. No binding was detected for cells transfected with WSX-1 + OSMRbeta, with OSMRbeta only, or with WSX-1 only.

Example 38

Mouse zcytor17lig activates mouse zcytor17/OSMRbeta receptor-in luciferase assay

A. Cloning of full-length mouse zcytor17 and mouse OSMRbeta for expression

A mouse testes cDNA library was screened for a full-length clone of mouse zcytoR17. The library was plated at 65,500 cfu/plate on 24 LB + Amp plates. Filter lifts were prepared using Hybond N (Amersham-Pharmacia Biotech, Inc., Piscataway, NJ) on a total of approximately 1.6 million colonies. The filters were marked with a hot needle for orientation and then denatured for 6 minutes in 0.5 M NaOH and 1.5 M Tris-HCl, pH 7.2. The filters were then neutralized in 1.5 M NaCl and 0.5 M Tris-HCl, pH 7.2 for 6 minutes. The DNA was affixed to the filters using a UV crosslinker (Stratalinker®, Stratagene, La Jolla, CA) at 1200 joules. The filters were then left to dry overnight at room temperature.

The next day, the filters were pre-washed at 65°C in pre-wash buffer consisting of 0.25X SSC, 0.25% SDS and 1mM EDTA. Cell debris was manually removed using Kimwipes® (Kimberly-Clark) and the solution was changed 3 times over a period of 1 hour. Filters were air dried and stored at room temperature until needed. The filters were then prehybridized for approximately 3 hours at 63°C in 20 ml of ExpressHyb™Hybridization Solution (Clontech, Palo Alto, CA).

Probe B (Example 31) was generated by PCR from human zcytoR17 template using oligonucleotide primers ZC27,895 (SEQ ID NO:20) and ZC28,917 (SEQ ID NO:83) and was radioactively labeled with ^{32}P using a commercially available kit (Megaprime DNA Labeling System; Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions. The probe was purified using a Stratagene™ push column (NucTrap® column; Stratagene, La Jolla, CA). The probe

was denatured at 100°C for 15 min and added to ExpressHyb™. Filters were hybridized in 15 ml hybridizing solution containing 1.6 x 10^6 cpm/ml of probe at 63°C overnight. Filters were washed at 55°C in 2X SSC, 0.1 % SDS and 1 mM EDTA and exposed to X-ray film at -80°C for 4 1/2 days. Thirteen positives were picked from the plates as plugs and placed in 1 ml LB +amp in 1.7 ml tubes. Tubes were placed at 4°C overnight. These 13 positives were subjected to two further rounds of purification. The tertiary plates were outgrown at 37°C after filter lifts were taken and single colonies were picked and sent to sequencing. Three of these were determined to contain sequence of the mouse ortholog of zcytoR17.

In addition, a PCR product was generated using CTLL-2 cDNA as a template and oligonucleotides ZC38,239 (SEQ ID NO:108) and ZC38,245 (SEQ ID NO:109) as primers. CTLL-2 is a mouse cytotoxic T lymphocyte cell line (ATCC No. TIB-214). This PCR reaction was run as follows: 1 cycle at 95°C for 1 minute, 30 cycles at 95°C for 15 seconds, 68°C for 3 minutes, then 68°C for 10 minutes; 4°C soak. The PCR reaction used approximately 0.5 ng of cDNA, 20 pmoles of each oligonucleotide, and 1 µl of Advantage II polymerase mix (ClonTech). About 6% of the PCR product was used as a template in a new PCR reaction, as above, except with oligonucleotides ZC38,239 (SEQ ID NO:108) and ZC38,238 (SEQ ID NO:110). This PCR reaction was run as follows: 30 cycles at 94°C for 45 seconds, 65°C for 45 seconds, 72°C for 1 minute, then 72°C for 7 minutes; 10°C soak. Most of the PCR reaction was loaded on a 1.0% agarose gel and the predominant band at approximately 360 bp was excised, the DNA fragment was eluted, and DNA sequencing was performed.

The sequence of the mouse zcytor17 polynucleotide is shown in SEQ ID NO:111 and the corresponding amino acid sequence shown in SEQ ID NO:112. In addition, a truncated soluble form of the mouse zcytor17 polynucleotide is shown in SEQ ID NO:113 and the corresponding amino acid sequence shown in SEQ ID NO:114.

To obtain a full-length mouse OSMRbeta cDNA, 5' and 3' PCR products were isolated and joined using an internal BamHI site. The PCR primers were designed using the nucleotide sequence SEQ ID NO:119 and include EcoRI and XbaI restriction

sites for cloning purposes. The genomic mouse OSMRbeta nucleic acid sequence is shown in SEQ ID NO:119, wherein the coding sequence encompasses residues 780 to 3692 encoding a mouse OSMRbeta 970 amino acid polypeptide, which is shown in SEQ ID NO:120. A degenerate nucleic acid sequence which encodes the polypeptide of SEQ ID NO:120 is shown in SEQ ID NO:121.

A 5' PCR product was generated using an in-house 3T3-L1 (differentiated mouse adipocyte) cDNA library as a template and oligonucleotides ZC41,764 (SEQ ID NO:115) and ZC41,598 (SEQ ID NO:116) as primers. This 5' PCR reaction was run as follows: 30 cycles at 95°C for 45 seconds, 55°C for 45 seconds, 72°C for 1 minute 30 seconds, then 72°C for 7 minutes; 4°C soak. The PCR reaction used approximately 3 μg of plasmid prepared from the cDNA library, 20 pmoles of each oligonucleotide, and five units of *Pwo* DNA polymerase (Roche). About 90% of the 5' PCR product was digested with EcoRI and BamHI and gel purified on a 1.0% agarose gel. The approximately 1446 bp band was excised and used for ligation (see below).

A 3' PCR product was generated using a mouse placenta in-house cDNA library as a template and oligonucleotides ZC41,948 (SEQ ID NO:117) and ZC41,766 (SEQ ID NO:118) as primers. This 3' PCR reaction was run as follows: 30 cycles at 95°C for 45 seconds, 55°C for 45 seconds, 72°C for 1 minute 30 seconds, then 72°C for 7 minutes; 4°C soak. The PCR reaction used approximately 3 μg of plasmid prepared from the cDNA library, 20 pmoles of each oligonucleotide, and five units of *Pwo* DNA polymerase (Roche). About 90% of the 3' PCR product was digested with BamHI and XbaI and gel purified on a 1.0% agarose gel. The approximately 2200 bp band was excised and used for ligation along with the 5' PCR product (described above) to the expression vector pZP-5Z digested with EcoRI and XbaI. The three-part ligation was performed with the 5' EcoRI to BamHI fragment above, the 3' BamHI to XbaI fragment, and the expression vector pZP-5Z digested with EcoRI and XbaI. This generated a pZP-5Z plasmid containing a full-length cDNA for mouse OSMRbeta (nucleotides 780 to 3692 of SEQ ID NO:119), designated pZP-5Z/OSMRbeta. The full length mouse OSMRbeta cDNA in pZP5Z/OSMRbeta has two amino acid insertions from SEQ ID NO:120. There is a duplication of amino acid Glycine at position 370 and a duplication

of amino acid Glutamic Acid at position 526. Plasmid pZP-5Z is a mammalian expression vector containing an expression cassette having the CMV promoter, multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a zeocin resistance gene and the SV40 terminator.

The resulting transformants were sequenced to confirm the mouse OSMRbeta cDNA sequence.

B. Construction of BaF3/KZ134/zcytor17m, BaF3/KZ134/zcytor17m/OSMRbetam, BHK/KZ134/zcytor17m, and BHK/KZ134/zcytor17m/OSMRbetam cell lines

Stable BaF3/KZ134 and BHK/KZ134 cell lines (Example 20) were transfected with an expression plasmid encoding full-length mouse zcytor17, pZP-7P/zcytor17m (Example 38A), to create BaF3/KZ134/zcytor17m and BHK/KZ134/zcytor17m cells, respectively. The mouse OSMRbeta expression plasmid, pZP-5Z/OSMRbetam (Example 38A), was then transfected into these cells to create BaF3/KZ134/zcytor17m/OSMRbetam and BHK/KZ134/zcytor17m/OSMRbetam cell lines, respectively. Methods were as described in Example 4 with the exception that Baf3/KZ134/zcytor17m and BHK/KZ134/zcytor17m were selected with, in addition to Geneticin, 2ug/ml puromycin while Baf3/KZ134/zcytor17m/OSMRbetam and BHK/KZ134/zcytor17m/OSMRbetam were selected with, in addition to Geneticin, 2ug/ml puromycin and 200 ug/ml zeocin.

Clones were diluted, plated and selected using standard techniques. Clones were screened by luciferase assay (see Example 20, above) using the mouse zcytor17lig conditioned media or purified mouse zcytor17lig protein (Example 35) as an inducer. Clones with the highest luciferase response (via STAT luciferase) and the lowest background were selected. Stable transfectant cell lines were selected.

C. Mouse Zcytor17lig activates mouse zcytor17 receptor in BaF3/KZ134/zcytor17m/OSMRbetam or BHK/KZ134/zcytor17m/OSMRbetam luciferase assay

Cell lines were plated for luciferase assays as described in Example 20 above. STAT activation of the BaF3/KZ134/Zcytor17m, BaF3/KZ134/zcytor17m/OSMRbetam, BHK/KZ134/zcytor17m, or BHK/KZ134/zcytor17m/OSMRbetam cells was assessed using (1) conditioned media from BHK570 cells transfected with the human zcytor17lig (Example 7), (2) conditioned media from BHK570 cells transfected with the mouse zcytor17lig (Example 18), (3) purified mouse and human zcytor17lig (Example 35), and (4) mIL-3 free media to measure media-only control response. Luciferase assays were performed as described in Example 20.

The results of this assay confirm the STAT reporter response of the BaF3/KZ134/zcytor17m/OSMRbetam and BHK/KZ134/zcytor17m/OSMRbetam cells to the mouse zcytor17lig when compared to either the BaF3/KZ134/zcytor17m cells, the BHK/KZ134/zcytor17m cells or the untransfected BaF3/KZ134 or BHK/KZ134 control cells, and show that the response is mediated through the mouse zcytor17/OSMRbeta receptors. The results also show that the human zcytor17lig does not activate the STAT reporter assay through the mouse receptor complex.

Example 39

Human zcytor17lig binding to zcytor17 and zcytor17/OSMRbeta by Flow Cytometry

The biotinylation of human zcytor17L was done as follows: 100μL of zcytor17 at 5.26mg/mL was combined with 30μL of 10mg/mL EZ-link Sulfo-NHS-LC-biotin (Pierce, Rockford, IL) dissolved in ddH_2O. This solution was incubated on a rocker for 30 minutes at room temperature. After biotinylation the solution was dialyzed in PBS using a Slide-A-Lyzer dialysis cassette.

To test the binding properties of human zcytor17lig to different receptor combinations both BHK and BAF3 cells were transfected with expression plasmids using standard techniques well-known in the art. These plasmids were transfected into both cell lines in the following combinations: zcytor17 alone, OSMRbeta alone, and both zcytor17 and OSMRbeta. Transfection was performed as detailed above. Untransfected BHK and BAF3 cells were used as controls. Cells were stained by FACS as follows: 2E5 cells were stained with either: 2.0μg/mL, 100ng/mL, 10ng/mL,

1.0ng/mL, 100pg/mL, 10pg/mL, 1.0pg/mL of biotinylated zcytor17L or left unstained for 30 minutes on ice in FACS buffer (PBS + 2%BSA + 2%NHS (Gemini) + 2%NGS). Cells were washed 1.5 times and then stained with SA-PE (Jackson Immuno Laboratories) at 1:250 for 30 minutes on ice. Cells were then washed 1.5 times with FACS buffer and resuspended in FACS buffer and analyzed by FACS on a BD FACSCaliber using CellQuest software (Becton Dickinson, Mountain View, CA).

Both BHK and BAF3 cells showed that zcytor17lig bound to both zcytor17 alone and in combination with OSMRbeta with the binding to the zcytor17/OSMRbeta heterodimer being slightly stronger. No binding was seen in either cell lines expressing OSMRbeta alone. The zcytor17lig bound in a concentration dependent manner. The mean fluorescent intensity (MFI) values for the BHK binding are shown below in Table 15.

Table 15

zcytor17 μg/mL	2.0	0.100	0.010	0.001	0.0001	0.00001	0.000001	0.0
BHK C17+OSMRbeta	3780	2126	328	53	17	15	14	13
BHK-C17	3032	1600	244	39	16	15	14	15
BHK-OSMRbeta	13	X	X	X	X	X	X	0
BHK-WT	15	14	13	X	X	X	X	13

zcytor17 μg/mL	10.0	3.33	1.11	0.37	0.12	0.04	0.00
BAF3-C17+OSMRbeta	531	508	489	441	364	247	7
BAF3-OSMRbeta	6	5	5	5	5	5	11
BAF3-WT	13	13	12	12	12	12	13

zcytor17 ng/mL	100.0	10.0	1.0	0.0
BAF3-C17	347	72	17	7

Example 40

Gene Expression Array Analysis of Human Zcytor17lig Treated Cells

RNA was isolated from human zcytor17lig treated A549 cells, zcytor17lig treated SK-LU-1 cells, and untreated control cells using a RNeasy Midi Kit (Qiagen, Valencia, CA) according to the manufactures instructions.

Gene expression profiling of the cells treated with zcytor17lig and the

respective control cells was carried out using GEArray Q series cDNA expression arrays (SuperArray Inc., Bethesda, MD). The Q Series cDNA expression arrays contain up to 96 cDNA fragments associated with a specific biological pathway, or genes with similar functions or structural features. Comparison of arrays from treated and control cells allows for a determination of the up and down regulation of specific genes. Probe labeling, hybridization and detection were carried out according to the manufactures instructions. Chemiluminscent signal detection and data acquisition was carried out on a Lumi-Imager workstation (Roche, Indianapolis, IN). The resulting image data was analyzed using ImageQuant 5.2 (Amersham Biosciences, Inc., Piscataway, NJ) and GEArray Analyzer 1.2 (SuperArray Inc., Bethesda, MD) software.

Analysis of the results from the Human Interleukin and Receptor Q series HS-014N arrays, showed, after normalization, an approximate 4.7 fold increase of IL13RA2 signal in the zcytor17lig treated human SK-LU-1 cells and an approximate 2.2 fold increase of the IL13RA2 signal in the zcytor17lig treated human A549 cells.

These results indicate that zcytor17lig significantly up regulated IL13RA2 in the SK-LU-1 and A549 cells. Both of these are established cell lines derived from human lung carcinomas (Blobel et al., Virchows Arch B Cell Pathol Incl Mol Pathol., 1984;45(4):407-29). More specifically, A549 is characterized as a human pulmonary epithelial cell line (Lin, et al., J Pharm Pharmacol., 2002 Sep;54(9):1271-8; Martinez et al., Toxicol Sci., 2002 Oct;69(2):409-23).

Interleukin-13 (IL13), a cytokine secreted by activated T lymphocytes, has been demonstrated to be both necessary and sufficient for the expression of allergic asthma and for use in experimental models of asthma, which include airway hyperresponsiveness, eosinophil recruitment, and mucus overproduction (Wills-Karp et al., Science, 1998;282:2258-2261). It has been shown, that selective neutralization of IL13 will ameliorate the asthma phenotype (Grunig et al., Science, 1998; 282:2261-2263). It has also been reported that IL13 is involved in the up regulation of mucin gene MUC8 expression in human nasal polyp epithelium and cultured nasal epithelium (Kimm et al., Acta Otolaryngol., 2002; Sep;122(6):638-643; Seong et al., Acta Otolaryngol., 2002; Jun;122(4):401-407). MUC8, a major airway mucin glycoprotein,

is implicated as playing a role in the pathogenesis of mucus hypersecretion in chronic sinusitis with polps (Seong et al., Acta Otolaryngol., 2002; Jun;122(4):401-407).

Functionally, IL13 signals through a receptor complex consisting of the interleukin-13 receptor alpha-1 chain (IL13RA1) and IL-4 receptor alpha (IL4RA) (Daines and Hershey, J Biol Chem., 2002; 22(12):10387-10393). It has also been shown, that the interleukin-13 receptor alpha-2 (IL13RA2) binds IL13 with high affinity, but by itself (Daines and Hershey, J Biol Chem., 2002; 22(12):10387-10393). This receptor lacks, however, the cytoplasmic domain necessary for signaling and, therefore, is considered to be a decoy receptor. It has been shown that IL13RA2 is predominately an intracellular molecule that can be quickly mobilized from intracellular stores and surface expressed following cellular treatment with interferon (IFN)-gamma. The surface expression of IL13RA2 after IFN-gamma treatment does not involve protein synthesis and results in diminished IL13 signaling (Daines and Hershey, J Biol Chem., 2002; 22(12):10387-10393).

The results of the gene expression array analysis for zcytor17lig indicate the action of zcytor17lig to be novel to that of IFN-gamma in that the zcytor17lig treatment of lung epithelial derived cell lines resulted in a significant increase of IL13RA2 gene expression. Thus, zcytor17lig treatment can be beneficial in cases where long-term up regulation of IL13RA2 expression and down regulation of IL13 is desired such as in asthma, airway hyperactivity (AHR), and mucin regulation, including chronic sinusitis with polps.

Example 41

Murine zcytor17lig Trangenic Mice

To evaluate the *in vivo* effects of zcytor17lig overexpression, multiple founders of transgenic mice expressing the murine form of the gene were generated, driven by two different promoters: the lymphocyte-specific promoter Eμ/lck, and the ubiquitous promoter, EF1α (Example 22). Serum protein levels range from approximately 20-300 ng/ml. The Eμ/lck promoter generated mice with higher levels of serum protein than those in the EF1α-zcytor17lig transgenic mice.

The zcytor17lig transgenic mice developed a skin phenotype around 4-8 weeks of age. The fur of the transgenic mice became "ruffled," with obvious piloerection and mild to severe hair loss, usually on their backs, sides of the torso, and around their eyes. This phenotype was consistently found in mice with detectable levels of zcytor17lig protein in their serum. Among the founders, 100% incidence rate among the mice expressing the Eμ/lck -driven gene, and a 50% incidence in the EF1α-zcytor17lig transgenic mice was noted, correlating well with the relative levels of zcytor17lig that was detected in their serum. The transgenic skin appeared to be pruritic, as evidenced by the scratching behavior of the mice, sometimes excessive enough to induce excoriation and lesions of the skin, which usually became infected (with at least *Staphylococcus aureus*). The mice were originally identified with metal ear tags, but in most cases, the ear tags were forcibly removed by the mice themselves. This often resulted in severe damage to the external ear. These wounded ears often did not heal properly, as reflected in the presence of long-lasting pustules and crusting, and a seeping, expanding wound would that developed in many of the animals, behind and between their ears. Some of the transgenic mice also developed scabby wounds on their shoulders and neck. Skin lesions were observed in a subset of the animals, generally evolving on areas of skin where hair loss had already been apparent, and were often exacerbated by the scratching behavior of the mice.

RealTime quantitative RT-PCR was used to detect zcytor17lig RNA transcripts in transgenic (but not non-transgenic) skin samples, with the Eμ/lck transgenic skin expressing more zcytor17lig RNA than skin from EF1α-zcytor17lig transgenic mice. The genes encoding the zcytor17 receptor subunits, zcytor17 and OSM-Rbeta were expressed in the skin of both non-transgenic and zcytor17lig-transgenic mice.

An examination of the lymphoid tissues from a subset of the Eμ/lck-transgenic founders by flow cytometry revealed a significant increase in the proportion of activated T cells in the spleen and lymph nodes of these mice. Two of the four mice analyzed had severely enlarged cervical lymph nodes, possibly due to the presence of lesions on their necks. A subtle increase in spleen weight and a slight increase in monocytes and neutrophils circulating in the blood of the transgenic mice was

observed. There was no increase in a variety of cytokines tested, nor were there changes in the circulating serum amyloid A levels in these mice. The effects on the immune cells in the transgenic mice may be a direct or an indirect result of zcytor17lig, or are secondary effects of the skin lesions.

Histopathology was performed on many tissues other than skin, including liver, thymus, spleen, kidney, and testes, and no significant abnormalities in these organs were noted. Analysis of the transgenic skin, however, did reveal a number of alterations, which varied greatly depending upon the source and location of skin (e.g., normal, hairless, or lesional). In many cases, the ears of the transgenic mice had a thickened epidermis as compared to the non-transgenic controls (e.g., approximately 4 layers versus 2 layers), and the underlying tissues contained low to moderate numbers of inflammatory cells, which were primarily mononuclear with occasional neutrophils. The epidermis over the abdomen appeared multifocally slightly thicker in the transgenic, but there was no apparent increase in inflammatory cells in the underlying dermis or subcutis. In the hairless portions of skin from these mice, there were dilated hair follicles that contained some debris but no hair shafts (e.g., the hairs fell out by the roots). In the lesioned areas, there was severe thickening of the epidermis (acanthosis), increased keratin on the surface of the skin (hyperkeratosis), scattered ulcers of varying size and significant numbers of inflammatory cells in the dermis (mainly neutrophils, with varying numbers of macrophages and lymphocytes). The dermis also contained numerous mast cells bordering the lesions. Some of the hair shafts in the lesioned areas of the transgenic skin were in the active stage (anagen), in contrast to many of the hair shafts in "normal" areas which were in the involuting (catagen) to inactive (telogen) stage.

The phenotype of the zcytor17lig transgenic mice strongly resembles that of atopic dermatitis (AD) patients, and mouse models of AD. AD is a common chronic inflammatory disease that is characterized by hyperactivated cytokines of the helper T cell subset 2 (Th2). Zcytor17lig is preferentially expressed by Th2 vs. Th1 cells, which lends further credence to this comparison. Although the exact etiology of AD is unknown, multiple factors have been implicated, including hyperactive Th2 immune responses, autoimmunity, infection, allergens, and genetic predisposition. Key

features of the disease include xerosis (dryness of the skin), pruritus (itchiness of the skin), conjunctivitis, inflammatory skin lesions, *Staphylococcus aureus* infection, elevated blood eosinophilia, elevation of serum IgE and IgG1, and chronic dermatitis with T cell, mast cell, macrophage and eosinophil infiltration. Colonization or infection with *S. aureus* has been recognized to exacerbate AD and perpetuate chronicity of this skin disease.

AD is often found in patients with asthma and allergic rhinitis, and is frequently the initial manifestation of allergic disease. About 20% of the population in Western countries suffer from these allergic diseases, and the incidence of AD in developed countries is rising for unknown reasons. AD typically begins in childhood and can often persist through adolescence into adulthood. Current treatments for AD include topical corticosteroids, oral cyclosporin A, non-corticosteroid immunosuppressants such as tacrolimus (FK506 in ointment form), and interferon-gamma. Despite the variety of treatments for AD, many patients' symptoms do not improve, or they have adverse reactions to medications, requiring the search for other, more effective therapeutic agents.

Epithelial cells, which express the heterodimeric receptor for zcytor17lig (zcytoR17 and OSM-Rbeta), are located at the sites (e.g., skin, gut, lung, etc.) of allergen entry into the body and interact closely with dendritic cells (professional antigen presenting cells) *in situ*. Dendritic cells play an important role in the pathogenesis of allergic diseases, and zcytor17lig may interact with its receptor on epithelial cells in the skin and lung and influence immune responses in these organs. Zcytor17lig and its receptor(s) may therefore contribute to the pathogenesis of allergic diseases such as AD and asthma. Furthermore, the phenotype of the zcytor17lig transgenic mice suggests that this ligand may play a role in wound healing, since the mice seem unable to repair damage to their ears, and often bear long-lasting lesions on their backs and sides. An antagonist of zcytor17lig might therefore represent a viable therapeutic for these and other indications.

Example 42

Luciferase Assay on Human Transformed Epithelial Cell Lines via Transient Infection with an Adenoviral STAT/SRE Reporter Gene

A wide variety of human transformed epithelial cell lines (see Table 16 below) were seeded in 96-well flat-bottom plates at 10,000 cell/well in regular growth media as specified for each cell type. The following day, the cells were infected with an adenovirus reporter construct, KZ136, at a multiplicity of infection of 5000. The KZ136 reporter contains the STAT elements in addition to a serum response element. The total volume was 100 ul/well using DMEM supplemented with 2 mM L-glutamine (GibcoBRL), 1 mM Sodium Pyruvate (GibcoBRL) and 1x Insulin-Transferrin-Selenium supplement (GibcoBRL) (hereinafter referred to as serum-free media). Cells were cultured overnight.

The following day, the media was removed and replaced with 100 μl of induction media. The induction media was human zcytor17lig diluted in serum-free media at 100ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.125 ng/ml and 1.56 ng/ml. A positive control of 20% FBS was used to validate the assay and to ensure the infection by adenovirus was successful. The cells were induced for 5 hours at which time the media was aspirated. The cells were then washed in 50 μl/well of PBS, and subsequently lysed in 30 μl/well of 1X cell lysis buffer (Promega). After a 10-minute incubation at room temperature, 25 μl/well of lysate was transferred to opaque white 96-well plates. The plates were then read on the Luminometer using 5-second integration with 40 μl/well injection of luciferase substrate (Promega).

The results revealed the ability of multiple epithelial cell lines to respond to zcytor17lig as shown in Table 16 below.

Table 16

Cell Line	Species	Tissue	Morphology	Disease	Fold Induction
A549	Human	Lung	Epithelial	Carcinoma	2x
Sk-Lu-1	Human	Lung	Epithelial	Adenocarcinoma	6x
WI-38	Human	Embryonic Lung	Fibroblast		Negative
MRC-5	Human	Lung	Fibroblast		Negative
DU 145	Human	Prostate	Epithelial	Carcinoma	10x
PZ-HPV-7	Human	Prostate	Epithelial	Transformed with HPV	5x
PC-3	Human	Prostate	Epithelial	Adenocarcinoma	Negative
U2OS	Human	Bone	Epithelial	Osteosarcoma	15.5x
SaOS2	Human	Bone	Epithelial	Osteosarcoma	22x
MG-63	Human	Bone	Fibroblast	Osteosarcoma	Negative
143B	Human	Bone	Fibroblast	Osteosarcoma	3.5x
HOS	Human	Bone	Fibroblast and Epithelial		8x
TRBMeC	Human	Vascular Bone Marrow	Epithelial		2x
HT144	Human	Skin	Fibroblast	Melanoma	5x
C32	Human	Skin		Melanoma	Negative
Sk-Mel-2	Human	Skin	Polygonal	Melanoma	2.7x
WM-115	Human	Skin	Epithelial	Melanoma	2x
HCT-116	Human	Colon	Epithelial	Carcinoma	Negative
HT-29	Human	Colon	Epithelial	Carcinoma	Negative

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CaCo2	Human	Colon	Epithelial	Adenocarcinoma	3x
HBL-100	Human	Breast	Epithelial		1.5x
ME-180	Human	Cervix	Epithelial	Carcinoma	Negative
HeLa 299	Human	Cervix	Epithelial	Adenocarcinoma	Negative
SK-N-SH	Human	Brain	Epithelial	Neuroblastoma	Negative
U138 MG	Human	Brain	Polygonal	Glioblastoma	Negative
HepG2	Human	Liver	Epithelial	Carcinoma	Negative
Chang liver	Human	Liver	Epithelial		Negative
Sk-Hep-1	Human	Liver	Epithelial	Adenocarcinoma	4x
Int 407	Human	Intestine	Epithelial		Negative
3a-Sub E	Human	Placenta			Negative

Example 43

Cytokine production by human epithelial cell lines cultured with human zcytor17lig

Human disease-state epithelial cell lines (A549, human lung epithelial carcinoma; SkLu1, human lung epithelial adenocarcinoma; DU145, human prostate epithelial carcinoma; PZ-HPV-7, human prostate epithelial HPV transformed; U20S, human bone epithelial osteosarcoma) were screened for cytokine production in response to zcytor17lig *in vitro*. These cell lines have both zcytor17 and OSMR-beta, identified by RT-PCR, and respond to human zcytor17lig when assayed with the adenoviral luciferase reporter construct, KZ136 (Example 42). Cytokine production by these cell lines was determined in response to human zcytor17lig in a series of three experiments.

A. Cytokine production by human disease-state epithelial cell lines cultured with human zcytor17lig

Cells were plated at a density of 4.5 x 10^5 cells per well in a 6 well plate (Costar) and cultured in respective growth media. The cells were cultured with test reagents; 100ng/mL zcytor17lig, 10ng/mL Interferon gamma (IFN gamma) (R&D Systems, Minneapolis, MN), 10ng/mL Tumor Necrosis Factor alpha (TNF alpha) (R&D Systems, Minneapolis, MN), 10ng/mL IL-1beta (R&D Systems, Minneapolis, MN) or 100ug/mL Lipopolysaccharide (LPS) (Sigma). Supernatants were harvested at 24 and 48 hours and assayed for cytokines; GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor), IL-1b, IL-6, IL-8, MCP-1 (Macrophage Chemoattractant Protein-1) and TNFa. Multiplex Antibody Bead kits from BioSource International (Camarillo, CA) were used to measure cytokines in samples. Assays were read on a Luminex-100 instrument (Luminex, Austin, TX) and data was analyzed using MasterPlex software (MiraiBio, Alameda, CA). Cytokine production (pg/mL) for each cell line in the 24-hour samples is shown below in Table 17.

Table 17

GM-CSF pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	18.80	10.26	16.19	13.26	14.10
IFN-g	16.19	13.36	11.56	16.26	11.81
IL-1b	104.60	126.44	76.77	338.25	27.32
TNFa	106.67	33.20	58.50	107.09	33.79
LPS	17.64	10.62	11.81	25.47	18.34
control	14.81	8.56	13.26	21.67	13.96

IL-1b pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	26.90	30.17	28.77	29.07	28.00
IFN-g	29.07	35.33	21.96	26.90	26.73
IL-1b	1332.88	1256.17	979.02	1107.35	998.60
TNFa	31.11	33.28	35.33	31.24	25.66
LPS	33.28	28.77	29.07	31.11	31.24
control	28.77	28.77	26.73	31.24	29.07

IL-6 pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	20.09	26.89	193.05	19.37	17.30
IFN-g	17.52	33.64	217.58	27.02	17.63
IL-1b	175.44	5920.19	2375.29	304.08	18.44
TNFa	354.16	1002.51	1612.17	103.58	18.33
LPS	18.06	35.65	162.18	22.42	17.30
control	17.63	27.80	71.23	19.32	17.19

IL-8 pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	86.33	150.81	150.61	45.92	6.81
IFN-g	24.07	72.82	163.31	81.78	1.35
IL-1b	1726.24	4083.12	4407.79	5308.83	124.17
TNFa	3068.68	3811.75	2539.39	3324.02	69.65
LPS	20.28	167.13	230.39	115.08	7.95
control	14.92	109.78	107.27	93.44	9.49

MCP-1 pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	8.97	187.29	26.84	105.15	7.20
IFN-g	7.30	267.99	17.05	88.68	7.71
IL-1b	8.11	8039.84	88.78	3723.81	4.70

TNFa	8.50	7100.37	153.26	3826.80	2.80
LPS	9.40	185.83	22.65	61.62	5.61
control	8.16	167.93	13.68	47.78	5.61

TNFa pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	16.23	17.52	16.67	15.80	17.09
IFN-g	15.80	17.09	15.80	16.65	15.80
IL-1b	16.66	17.09	15.80	17.95	16.23
TNFa	1639.92	1648.83	2975.07	1348.33	3554.82
LPS	16.87	15.80	15.37	17.09	17.52
control	16.23	15.80	15.80	17.09	16.66

All cell lines tested produced GM-CSF and IL-8 in response to stimulation with control cytokines IL-1b and TNFa. Most cell lines produced IL-6 and MCP-1in response to IL-1b and TNFa stimulation. Zcytor17lig stimulated IL-6 production in the DU145 cell line compared to control (193pg/mL vs. 71pg/mL). Zcytor17lig stimulated 3 of 5 cell lines to produce IL-8 with the greatest effect seen in A549 cells (5 fold), and reduced IL-8 production in U2OS cells by 2 fold. There was a slight effect on MCP-1 production by DU145 and U2OS cells when cultured with zcytor17lig.

B. Cytokine production by normal human epithelial cell lines cultured with human zcytor17lig

In addition to the human epithelial cell lines, normal human bronchial epithelial cells (NHBE, Clonetics) were also tested. Cells were plated at a density of 1 x 10^5 cells per well in a 24 well plate and cultured with test reagents; 1000ng/mL, 100ng/mL and 10ng/mL zcytor17lig (A760F), 10ng/mL TNFa, 10ng/mL OSM, 10ng/mL IFNa, 10ng/mL TGFb or 10ng/mL Lymphotactin. Supernatants were harvested at 24 and 48 hours and assayed for cytokines; IL-6, IL-8, MCP-1, MIP-1a, RANTES and Eotaxin. Cytokines were assayed as previously described. Cytokine production (pg/mL) for each cell line in the 48-hour samples is shown below in Table 18.

Table 18

IL-6 pg/ml

	A549	DU145	SkLu1	U2OS	NHBE
r17L 1000ng/ml	24.5	56.3	32.1	25.2	64.5
r17lL 100ng/ml	25.0	65.0	31.0	25.4	50.2
r17L 10ng/ml	24.8	51.8	30.2	25.3	54.3
TNFa	272.9	355.4	437.5	36.1	299.3
OSM	26.4	73.5	112.4	25.6	80.4
IFNa	24.6	109.3	33.7	26.4	52.4
TGFb	24.4	102.6	42.7	27.8	268.9
control	24.5	36.3	29.9	25.2	47.9

IL-8 pg/ml

	A549	DU145	SkLu1	U2OS	NHBE
r17L 1000ng/ml	35.0	243.3	45.6	18.6	402.0
r17lL 100ng/ml	31.0	290.7	40.1	21.3	296.0
r17L 10ng/ml	30.4	240.4	33.4	18.9	361.8
TNFa	2809.3	2520.9	1385.2	784.9	1486.3
OSM	37.8	60.6	68.0	22.5	494.6
IFNa	18.9	315.3	39.5	33.1	231.6
TGFb	9.9	77.5	19.6	88.9	246.9
control	10.9	238.0	38.0	39.7	315.8

MCP-1 pg/ml

	A549	DU145	SkLu1	U2OS	NHBE
r17L 1000ng/ml	nd	nd	149.1	81.0	nd
r17lL 100ng/ml	nd	nd	130.6	81.9	nd
r17L 10ng/ml	nd	nd	111.7	49.1	nd
TNFa	nd	22.1	2862.6	1104.7	nd
OSM	nd	17.2	448.2	85.8	nd
IFNa	nd	nd	131.7	10.5	nd
TGFb	nd	1.7	54.5	27.6	nd
control	nd	nd	113.0	1.7	nd

nd = not detected

DU145 cells produced IL-6 in response to zcytor17lig, repeating the previous results in Example 43A. However, only A549 and U2OS had similar IL-8 responses as seen Example 43A. SkLu1 and U2OS cells both produced MCP-1 in response to zcytor17lig. Cytokine production by NHBE cells was marginal compared to controls.

C. Cytokine production by human disease-state epithelial cell lines co-cultured with human zcytor17lig and IFN gamma

Cells were plated at a density of 2 x 10^5 cells per well in 24 well plate and co-cultured with 10ng/mL IFN gamma +/- zcytor17lig at 100ng/mL, 10ng/mL or 1ng/mL. Supernatants were collected at 24 and 48 hours and assayed for IL-8 and MCP-1 as described above. Cytokine production (pg/mL) for each cell line in the 24-hour samples is shown below in Table 19.

Table 19

		IL-8 pg/ml	MCP-1 pg/ml
A549	10ng/mL IFNg+100ng/mL r17L	86.7	nd
	10ng/mL IFNg+10ng/mL r17L	75.1	nd
	10ng/mL IFNg+1ng/mL r17L	63.6	nd
	10ng/ml IFNg	35.4	nd
	control	36.6	nd
DU145	10ng/mL IFNg+100ng/mL r17L	102.3	nd
	10ng/mL IFNg+10ng/mL r17L	92.9	nd
	10ng/mL IFNg+1ng/mL r17L	79.9	nd
	10ng/ml IFNg	70.7	nd
	control	79.4	nd
SkLu1	10ng/mL IFNg+100ng/mL r17L	152.2	604.9
	10ng/mL IFNg+10ng/mL r17L	194.4	870.7
	10ng/mL IFNg+1ng/mL r17L	138.7	585.4
	10ng/ml IFNg	170.8	652.6
	control	203.0	292.3
U2OS	10ng/mL IFNg+100ng/mL r17L	106.8	357.0
	10ng/mL IFNg+10ng/mL r17L	108.2	347.7
	10ng/mL IFNg+1ng/mL r17L	109.9	293.3
	10ng/ml IFNg	118.8	159.8
	control	146.8	7.0

A549 cells produced IL-8 in response to zcytor17lig, however there was no effect of co-culturing cells with the addition of IFN gamma. U2OS cells made 20 fold more MCP-1 when cultured with IFNg and 50 fold more MCP-1 when cultured with IFN gamma + zcytor17lig.

Example 44

Zcytor17lig Effects on ^{3}H-TdR Incorporation in DU145 Prostate Epithelial Carcinoma Cells

Cells were seeded in 96-well tissue clusters (Falcon) at a density of 25,000/well in MEM (Life Technologies) growth medium supplemented with glutamine, pyruvate, non-essential amino acids (Life Technologies) and 10% fetal bovine serum (Hyclone). At confluence (24 hours later), cells were switched to growth arrest media by substituting 0.1% BSA (Life Technologies) for serum. After 48 hours to achieve cell synchronization, the growth-arrest medium was replaced with fresh medium. Then, human recombinant zcytor17lig (test reagent) was added at various concentrations (from 0.24 to 60 ng/mL) (see Table 16 below), to test for the effect of the protein on basal DNA replication. Some wells received 2.5% FBS (Hyclone) in addition to zcytor17lig, in order to test effect of the protein on elevated levels of TdR incorporation. FBS 10% and 20ng/ml Platelet Derived Growth Factor-BB (PDGF-BB) (R&D) were used as positive control.

Eighteen hours following addition of zcytor17lig and the rest of the test reagents, cells were pulsed with 250 nCi/mL [^{3}H]-thymidine (NEN) for 4 hours. Following the 4-hour pulse, media were discarded and 100 μL trypsin solution (Life Technologies) was added in each well to dislodge the cells. The radioactivity incorporated by DU145 was determined by harvesting the cells with a Packard Filtermate 196 cell harvester and by counting the incorporated label using a Packard TopCount NXT microplate scintillation counter.

As can be seen in Table 20 below, zcytor17lig induced thymidine incorporation in quiescent cells (in 0.1% BSA) in a concentration-dependent manner. This effect reached 2.5-fold of the BSA control at the highest concentration used, 60 ng/mL. In addition, this effect of zcytor17lig was also detectable when the baseline incorporation was elevated by the addition of 2.5% FBS (in this series as potent a mitogen as 10% FBS). These results therefore indicate that under both basal and stimulated conditions zcytor17lig can act as a mitogenic factor for the DU145 carcinoma cells.

Table 16 shows the effects of zcytor17lig on thymidine incorporation by DU145 cells. Results are expressed in cpm/well and numbers are the mean±st.dev of triplicate wells.

Table 20

	0.1%BSA	2.5%FBS
BSA Control	1139±336	4228±600
Zcytor17lig (0.24ng/mL)	1430±136	4894±1037
Zcytor17lig (0.74ng/mL)	1657±32	5038±810
Zcytor17lig (2.22ng/mL)	1646±57	5162±808
Zcytor17lig (6.67ng/mL)	2226±189	6385±1613
Zcytor17lig (20ng/mL)	2168±108	5880±1085
Zcytor17lig (60ng/mL)	2512±111	6165±417
PDGF-BB (20ng/mL)	4094±202	5927±360

Example 45

Expression of huzcytor17lig in *E. coli*

A. Construction of expression vector pRPS01 that expresses huzcytor17Lig/MBP-6H fusion polypeptide

An expression plasmid containing a polynucleotide encoding a huzcytor17lig fused C-terminally to Maltose Binding Protein (MBP) was constructed via homologous recombination. The fusion polypeptide contains an N-terminal approximately 388 amino acid MBP portion fused to the huzcytor17Lig described herein. A fragment of huzcytor17lig cDNA was isolated using the PCR method as described herein. Two primers were used in the production of the zcytor17lig fragment in a standard PCR reaction: (1) one containing 40 bp of the vector flanking sequence and 20 bp corresponding to the amino terminus of the huzcytor17lig, and (2) another containing 40 bp of the 3' end corresponding to the flanking vector sequence and 20 bp corresponding to the carboxyl terminus of the huzcytor17lig. Two microliters of the 100 μl PCR reaction was run on a 1.0% agarose gel with 1 x TBE buffer for analysis, and the expected molecular weight fragment was observed. The remaining PCR reaction was combined with the second PCR tube and precipitated with 400 μl of

absolute ethanol. The precipitated DNA was used for recombination into the Sma1 cut recipient vector pTAP98 to produce the construct encoding the MBP-huzcytor17lig fusion, as described below.

The vector pTAP98 was constructed using yeast homologous recombination. One hundred nanograms of EcoR1 cut pMAL-c2 was recombined with 1μg Pvu1 cut pRS316, 1μg linker, and 1μg Sca1/EcoR1 cut pRS316 were combined in a PCR reaction. PCR products were concentrated via 100% ethanol precipitation. The competent yeast cell (*S. cerevisiae*) strain, SF838-9Dα, was combined with10 μl of a mixture containing approximately 1 μg of the huzcytor17lig PCR product (above) and 100 ng of SmaI digested pTAP98 vector, and electroporated at 0.75 kV, 25 μF and ∞ ohms. The resulting reaction mixture was plated onto URA-D plates and incubated at 30°C.

After 48 hours, the Ura+ yeast transformants from a single plate were selected. DNA was isolated and transformed into electrocompetent *E. coli* cells (e.g., MC1061, Casadaban et. al. J. Mol. Biol. 138, 179-207). The resulting *E. coli* cells were plated on MM/CA +AMP 100 mg/L plates (Pryor and Leiting, Protein Expression and Purification 10:309-319, 1997) using standard procedures. Four individual clones were harvested from the plates and inoculated into MM/CA with 100 μg/ml Ampicillin for two hours at 37°C. One milliliter of each of the culture was induced with 1mM IPTG. Approximately 2-4 hours later, 250 μl of each induced culture was mixed with 250 μl acid washed glass beads and 250 μl Thorner buffer with 5% βME and dye (8M urea, 100 mM Tris pH7.0, 10% glycerol, 2mM EDTA, 5% SDS). Samples were vortexed for one minute and heated to 65°C for 10 minutes. Twenty microliters of each sample was loaded per lane on a 4%-12% PAGE gel (NOVEX). Gels were run in 1XMES buffer. The positive clones were designated pRPS01 and subjected to sequence analysis.

One microliter of sequencing DNA was used to transform electrocompetent *E. coli* cell strain MC1061. The cells were electropulsed at 2.0 kV, 25 μF and 400 ohms. Following electroporation, cells were rescued 0.6 ml SOC and grown on LB+Amp plates at 37°C overnight, with 100 mg/L Ampicillin. Four cultures were induced with ITPG and screened for positives as described above. The positive clones were expanded for protein purification of the huzcytor17lig/MBP-6H fusion

protein using standard techniques.

B. Purification of huzcytor17Lig/MBP-6H from *E. coli* fermentation

Unless otherwise noted, all operations were carried out at 4°C. The following procedure was used to purify recombinant huzcytor17Lig/MBP-6H polypeptide. *E. coli* cells containing the pRPS01 construct and expressing huzcytor17Lig/MBP-6H, were constructed using standard molecular biology methods and cultured in 50.0 g/L SuperBroth II (12 g/L Casien, 24 g/L Yeast Extract, 11.4 g/L di-potassium phosphate, 1.7 g/L Mono-potassium phosphate; Becton Dickenson, Cockeysville, MD), 5 g/L glycerol and 5 mL/L 1M Magnesium Sulfate. Twenty grams of cells were harvested and frozen for protein purification.

The thawed cells were resuspended in 500 mL Amylose Equilibration buffer (20mM Tris, 100mM NaCl, pH 8.0). A French Press cell breaking system (Constant Systems Ltd., Warwick, UK) with a temperature setting of -7°C to -10°C and 30K PSI was used to lyse the cells. The resuspended cells were assayed for breakage by A_{600} readings before and after cycling through the French Press. The processed cell suspension was pelleted at 10,000G for 30 minutes to remove the cellular debris and the supernatant was harvested for protein purification.

A 25 ml column of Amylose resin (New England Biolabs, Beverly, MA) (prepared as described below) was poured into a Bio-Rad, 2.5 cm D x 10 cm H glass column. The column was packed and equilibrated by gravity with 10 column volumes (CVs) of Amylose Equilibration buffer. The processed cell supernatant was batch loaded to the Amylose resin overnight, with rocking. The resin was returned to the Bio-Rad column and washed with 10 CV's of Amylose Equilibration buffer by gravity. The column was eluted with ~2 CV of Amylose Elution buffer (Amylose Equilibration buffer + 10 mM Maltose, Fluka Biochemical, Switzerland) by gravity. Ten 5 mL fractions were collected over the elution profile and assayed for Absorbance at 280 and 320 nM. The Amylose resin was regenerated with 1 CV of distilled H_2O, 5 CVs of 0.1% (w/v) SDS (Sigma), 5 CVs of distilled H_2O, 5 CVs of Amylose Equilibration buffer and finally 1 CV of Amylose Storage buffer (Amylose Equilibration buffer + 0.02% Sodium Azide). The regenerated column was stored at 4°C.

Elution profile fractions of interest were pooled and dialyzed in a 10K dialysis chamber (Slide-A-Lyzer, Pierce Immunochemicals) against 4 x 4L PBS pH 7.4 (Sigma) over an 8 hour time period to remove low molecular weight contaminants, buffer exchange and desalt. Following dialysis, the material harvested represented the purified huzcytor17Lig/MBP-6H polypeptide. The purified huzcytor17Lig/MBP-6H polypeptide was filter sterilized and analyzed via SDS-PAGE Coomassie staining for an appropriate molecular weight product. The concentration of the huzcytor17Lig/MBP-6H polypeptide was determined by BCA analysis to be 1.28 mg/mL.

Example 46

Human zcytor17lig Polyclonal Antibody

A. Preparation and Purification

Polyclonal antibodies were prepared by immunizing 2 female New Zealand white rabbits with the purified recombinant protein hzcytor17L/MBP-6H (Example 45). The rabbits were each given an initial intraperitoneal (IP) injection of 200 μg of purified protein in Complete Freund's Adjuvant followed by booster IP injections of 100 μg protein in Incomplete Freund's Adjuvant every three weeks. Seven to ten days after the administration of the second booster injection (3 total injections), the animals were bled and the serum was collected. The animals were then boosted and bled every three weeks.

The hzcytor17L/MBP-6H specific rabbit serum was pre-adsorbed of anti-MBP antibodies using a CNBr-SEPHAROSE 4B protein column (Pharmacia LKB) that was prepared using 10 mg of non-specific purified recombinant MBP-fusion protein per gram of CNBr-SEPHAROSE. The hzcytor17L/MBP-6H-specific polyclonal antibodies were affinity purified from the pre-adsorbed rabbit serum using a CNBr-SEPHAROSE 4B protein column (Pharmacia LKB) that was prepared using 10 mg of the specific antigen purified recombinant protein hzcytor17L/MBP-6H. Following purification, the polyclonal antibodies were dialyzed with 4 changes of 20 times the antibody volume of PBS over a time period of at least 8 hours. Hzcytor17-Ligand-specific antibodies were characterized by ELISA using 500 ng/ml of the purified recombinant proteins hzcytor17L/MBP-6H or hzcytor17L-CEE produced in a

baculovirus expression system as antibody targets. The lower limit of detection (LLD) of the rabbit anti-hzcytor17L/MBP-6H affinity purified antibody was 100 pg/ml on its specific purified recombinant antigen hzcytor17L/MBP-6H and 500 pg/ml on purified recombinant hzcytor17L-CEE produced in a baculovirus expression system.

B. SDS-PAGE and Western blotting analysis of Rabbit Anti-human ZcytoR17lig MBP-6H antibody

Rabbit Anti-human ZcytoR17lig MBP-6H antibody was tested by SDS-PAGE (NuPage 4-12%, Invitrogen, Carlsbad, CA) with coomassie staining method and Western blotting using goat anti-rabbit IgG-HRP. Human and mouse zcytor17lig purified protein (200-25 ng) was electrophoresed using an Invitrogen Novex's Xcell II mini-cell, and transferred to nitrocellulose (0.2 mm; Invitrogen, Carlsbad, CA) at room temperature using Novex's Xcell blot module with stirring according to directions provided in the instrument manual. The transfer was run at 300 mA for one hour in a buffer containing 25 mM Tris base, 200 mM glycine, and 20% methanol. The filter was then blocked with Western A buffer (in house, 50 mM Tris, pH 7.4, 5 mM EDTA, pH 8.0, 0.05% Igepal CA-630, 150 mM NaCl, and 0.25% gelatin) overnight with gentle rocking at 4°C. The nitrocellulose was quickly rinsed, then the rabbit anti-human zcytoR17lig MBP-6H (1:1000) was added in Western A buffer. The blot was incubated for 1.5 hours at room temperature with gentle rocking. The blot was rinsed 3 times for 5 minutes each in Western A, then goat anti-rabbit IgG HRP antibody (1:5000) was added in Western A buffer. The blot was incubated for 1 hour at room temperature with gentle rocking. The blot was rinsed 3 times for 5 minutes each in Western A, then quickly rinsed in H_20. The blot was developed using commercially available chemiluminescent substrate reagents (ECLWestern blotting detection reagents 1 and 2 mixed 1:1; reagents obtained from Amersham Pharmacia Biotech, Buckinghamshire, England) and the blot was exposed to x-ray film for up to 5 minutes.

The purified human zcytor17lig appeared as a large band at about 30 kDa and a smaller band at about 20 kDa under reduced conditions. The mouse zcytor17lig was not detected by the rabbit anti-human zcytor17lig antibody.

Example 47

Zcytor17lig Effects on U937 Monocyte Adhesion

to Transformed Bone Marrow Endothelial Cell (TRBMEC) Monolayer

Transformed Bone Marrow Endothelial Cells (TRBMEC) were seeded in 96-well tissue clusters (Falcon) at a density of 25,000/well in medium M131 (Cascade Biologics) supplemented with Microvascular Growth Supplement (MVGS) (Cascade Biologics). At confluence (24 hours later), cells were switched to M199 (Gibco-Life Technologies) supplemented with 1% Fetal Bovine Serum (Hyclone). Human recombinant zcytor17lig (test reagent) was added at various concentrations (from 0.4 to 10 ng/mL) (see Table 21 below), to test for the effect of the protein on immune cell-endothelial cell interactions resulting in adhesion. Some wells received 0.3ng/ml Tumor Necrosis Factor (TNFalpha R&D Systems), a known pro-inflammatory cytokine, in addition to zcytor17lig, to test an effect of the protein on endothelial cells under inflammatory conditions. TNFalpha at 0.3ng/ml alone was used as positive control and the concentration used represents approximately 70% of the maximal TNFalpha effect in this system, i.e., it does not induce maximal adherence of U937 cells (a human monocyte-like cell line) to the endothelium. For this reason, this setup can detect both upregulation and downregulation of the TNFalpha effects. Basal levels of adhesion both with and without TNFalpha were used as baseline to assess effect of test reagents.

After overnight incubation of the endothelial cells with the test reagents (zcytor17ligand ± TNFalpha), U937 cells, stained with 5μM Calcein-AM fluorescent marker (Molecular Probes), the cells were suspended in RPMI 1640 (no phenol-red) supplemented with 1% FBS and plated at 100,000 cells/well on the rinsed TRBMEC monolayer. Fluorescence levels at excitation/emission wavelengths of 485/538nm (Molecular Devices micro-plate reader, CytoFluor application) were measured 30 minutes later, before and after rinsing the well three times with warm RPMI 1640 (no phenol-red), to remove non-adherent U937. Pre-rinse (total) and post-rinse (adherence-specific) fluorescence levels were used to determine percent adherence (net adherent/net total x 100 = % adherence).

As can be seen in Table 21 below, zcytor17lig when added alone affected the basal adherence of U937 cells to the endothelial monolayers at the concentration range used (less than 2-fold increases, $p<0.01$ by ANOVA test). By itself, the positive control, 0.3ng/mL TNFalpha, increased the adherence of U937 cells from a basal 5.8% to 35% (6-fold). In the presence of TNFalpha, zcytor17lig synergized with TNFalpha and further enhanced U937 adhesion in a concentration-dependent manner between 0.4 and 10ng/mL ($p<0.01$ by ANOVA test). At 10ng/mL, zcytor17lig enhanced the effect of TNFalpha by 62%. These results indicate that zcytor17lig may by itself be a pro-inflammatory agent. Zcytor17lig was able to synergize with sub-maximal concentrations of TNFalpha to increase monocyte adherence to endothelial cells. These results also show that endothelial cells, especially when exposed to pro-inflammatory cytokines such as TNFalpha, are a likely target tissue of zcytor17lig action. The consequence of zcytor17ligand on endothelial cells may be to heighten monocyte or macrophage adhesion to a site of proinflammatory activity. Activated monocytes and macrophages are important in many inflammatory diseases. Therefore inhibition of monocyte/macrophage adhesions may provide a therapeutic rationale for zcytor17ligand antagonists. This data would support the use of zcytor17 ligand antagonists for treatment lung diseases, vascular diseases, autoimmunity, tumor metastasis, disease involving allergic reactions, wound healing and diseases of the skin including contact, allergic or non-allergic dermatistic or psoriasis and inflammatory bowel disease. Table 21 shows the effects of zcytor17lig on U937 monocyte adhesion to TRBMEC endothelial monolayers. Results are expressed in percent adhesion and numbers are the mean±st.dev of triplicate wells.

Table 21

	Basal	*0.3ng/mL TNFalpha*
Basal	5.8±1.2	35±5.5
zcytor17lig 0.4 ng/mL	9±0.7	44.7±2.5
zcytor17lig 1.1 ng/mL	10.4±0.8	45.2±0.6
zcytor17lig 3.3 ng/mL	7.9±1.7	51.1±4
zcytor17lig 10 ng/mL	9.5±0.5	56.6±3.9

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SEQUENCE LISTING

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Ser Val Leu Phe Leu Phe Cys Cys Leu Gly Gly Trp Leu Ala Ser His
 10                  15                  20                  25

acg ttg ccc gtc cgt tta cta cga cca agt gat gat gta cag aaa ata   150
Thr Leu Pro Val Arg Leu Leu Arg Pro Ser Asp Asp Val Gln Lys Ile
                 30                  35                  40

gtc gag gaa tta cag tcc ctc tcg aag atg ctt ttg aaa gat gtg gag   198
Val Glu Glu Leu Gln Ser Leu Ser Lys Met Leu Leu Lys Asp Val Glu
             45                  50                  55

gaa gag aag ggc gtg ctc gtg tcc cag aat tac acg ctg ccg tgt ctc   246
Glu Glu Lys Gly Val Leu Val Ser Gln Asn Tyr Thr Leu Pro Cys Leu
         60                  65                  70

agc cct gac gcc cag ccg cca aac aac atc cac agc cca gcc atc cgg   294
Ser Pro Asp Ala Gln Pro Pro Asn Asn Ile His Ser Pro Ala Ile Arg
     75                  80                  85

gca tat ctc aag aca atc aga cag cta gac aac aaa tct gtt att gat   342
Ala Tyr Leu Lys Thr Ile Arg Gln Leu Asp Asn Lys Ser Val Ile Asp
 90                  95                 100                 105

gag atc ata gag cac ctc gac aaa ctc ata ttt caa gat gca cca gaa   390
Glu Ile Ile Glu His Leu Asp Lys Leu Ile Phe Gln Asp Ala Pro Glu
                110                 115                 120

aca aac att tct gtg cca aca gac acc cat gaa tgt aaa cgc ttc atc   438
Thr Asn Ile Ser Val Pro Thr Asp Thr His Glu Cys Lys Arg Phe Ile
            125                 130                 135

ctg act att tct caa cag ttt tca gag tgc atg gac ctc gca cta aaa   486
Leu Thr Ile Ser Gln Gln Phe Ser Glu Cys Met Asp Leu Ala Leu Lys
        140                 145                 150

tca ttg acc tct gga gcc caa cag gcc acc act taaggccatc tcttcctttc 539
Ser Leu Thr Ser Gly Ala Gln Gln Ala Thr Thr
    155                 160

ggattggcag gaacttaagg agccttaaaa agatgaccga cagctaagtg tgggaactct 599
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gccgtgattc cttaagtaca tttttccaat gaataatctc agggacccct catatgggct 659
agtcccggga gggctgagat gtgaatttgt gaattacctt gaaaaacatt aggttattgt 719
tattagtctt ggtatttatg gaatgctttt cttctgcagg cttaagtctt acttattata 779
ccctcgtgag ggtgggaggt ggcagctatg ttaatttatt gatatttatt gtactaagag 839
ttgtcaatgc tccctggggg agccctcgga atctatttaa taaattatat tgaatttttc 899
tcata 904

<210> 2
<211> 164
<212> PRT
<213> Homo sapiens

<400> 2

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

<210> 3
<211> 492
<212> DNA
<213> Artificial Sequence

<220>

<223> human zcytor17lig degenerate polynucleotide of SEQ ID NO:2

<221> misc_feature
<222> (1)...(492)
<223> n = A,T,C or G

<400> 3
atggcnwsnc aywsnggncc nwsnacnwsn gtnytnttyy tnttytgytg yytnggnggn 60
tggytngcnw sncayacnyt nccngtnmgn ytnytnmgnc cnwsngayga ygtncaraar 120
athgtngarg arytncarws nytnwsnaar atgytnytna argaygtnga rgargaraar 180
ggngtnytng tnwsncaraa ytayacnytn ccntgyytnw snccngaygc ncarccnccn 240
aayaayathc aywsnccngc nathmgngcn tayytnaara cnathmgnca rytngayaay 300
aarwsngtna thgaygarat hathgarcay ytngayaary tnathttyca rgaygcnccn 360
garacnaaya thwsngtncc nacngayacn caygartgya armgnttyat hytnacnath 420
wsncarcart tywsngartg yatggayytn gcnytnaarw snytnacnws nggngcncar 480
cargcnacna cn 492

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<211> 2903
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gaatgtccgc aaaacattag tttcactctt gtcgccaggt tggagtacaa tggcacgatc 180
ttggctcact gcaacctctg cctcccgggt tcaagcgatt ctcctgcctc agcctcccga 240
gtagctggga ttacagttaa caataatgca atccatttcc cagcataagt gggtaagtgc 300
cactttgact tgggctgggc ttaaaagcac aagaaaagct cgcagacaat cagagtggaa 360
acactcccac atcttagtgt ggataaatta aagtccagat tgttcttcct gtcctgactt 420
gtgctgtggg aggtggagtt gcctttgatg caaatccttt gagccagcag aacatctgtg 480
gaacatcccc tgatac atg aag ctc tct ccc cag cct tca tgt gtt aac ctg 532
Met Lys Leu Ser Pro Gln Pro Ser Cys Val Asn Leu
1 5 10

ggg atg atg tgg acc tgg gca ctg tgg atg ctc cct tca ctc tgc aaa 580
Gly Met Met Trp Thr Trp Ala Leu Trp Met Leu Pro Ser Leu Cys Lys
15 20 25

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ttc agc ctg gca gct ctg cca gct aag cct gag aac att tcc tgt gtc   628
Phe Ser Leu Ala Ala Leu Pro Ala Lys Pro Glu Asn Ile Ser Cys Val
     30                  35                  40

tac tac tat agg aaa aat tta acc tgc act tgg agt cca gga aag gaa   676
Tyr Tyr Tyr Arg Lys Asn Leu Thr Cys Thr Trp Ser Pro Gly Lys Glu
 45                  50                  55                  60

acc agt tat acc cag tac aca gtt aag aga act tac gct ttt gga gaa   724
Thr Ser Tyr Thr Gln Tyr Thr Val Lys Arg Thr Tyr Ala Phe Gly Glu
                 65                  70                  75

aaa cat gat aat tgt aca acc aat agt tct aca agt gaa aat cgt gct   772
Lys His Asp Asn Cys Thr Thr Asn Ser Ser Thr Ser Glu Asn Arg Ala
             80                  85                  90

tcg tgc tct ttt ttc ctt cca aga ata acg atc cca gat aat tat acc   820
Ser Cys Ser Phe Phe Leu Pro Arg Ile Thr Ile Pro Asp Asn Tyr Thr
         95                 100                 105

att gag gtg gaa gct gaa aat gga gat ggt gta att aaa tct cat atg   868
Ile Glu Val Glu Ala Glu Asn Gly Asp Gly Val Ile Lys Ser His Met
    110                 115                 120

aca tac tgg aga tta gag aac ata gcg aaa act gaa cca cct aag att   916
Thr Tyr Trp Arg Leu Glu Asn Ile Ala Lys Thr Glu Pro Pro Lys Ile
125                 130                 135                 140

ttc cgt gtg aaa cca gtt ttg ggc atc aaa cga atg att caa att gaa   964
Phe Arg Val Lys Pro Val Leu Gly Ile Lys Arg Met Ile Gln Ile Glu
                145                 150                 155

tgg ata aag cct gag ttg gcg cct gtt tca tct gat tta aaa tac aca   1012
Trp Ile Lys Pro Glu Leu Ala Pro Val Ser Ser Asp Leu Lys Tyr Thr
            160                 165                 170

ctt cga ttc agg aca gtc aac agt acc agc tgg atg gaa gtc aac ttc   1060
Leu Arg Phe Arg Thr Val Asn Ser Thr Ser Trp Met Glu Val Asn Phe
        175                 180                 185

gct aag aac cgt aag gat aaa aac caa acg tac aac ctc acg ggg ctg   1108
Ala Lys Asn Arg Lys Asp Lys Asn Gln Thr Tyr Asn Leu Thr Gly Leu
```

190 195 200

cag cct ttt aca gaa tat gtc ata gct ctg cga tgt gcg gtc aag gag 1156
Gln Pro Phe Thr Glu Tyr Val Ile Ala Leu Arg Cys Ala Val Lys Glu
205 210 215 220

tca aag ttc tgg agt gac tgg agc caa gaa aaa atg gga atg act gag 1204
Ser Lys Phe Trp Ser Asp Trp Ser Gln Glu Lys Met Gly Met Thr Glu
225 230 235

gaa gaa gct cca tgt ggc ctg gaa ctg tgg aga gtc ctg aaa cca gct 1252
Glu Glu Ala Pro Cys Gly Leu Glu Leu Trp Arg Val Leu Lys Pro Ala
240 245 250

gag gcg gat gga aga agg cca gtg cgg ttg tta tgg aag aag gca aga 1300
Glu Ala Asp Gly Arg Arg Pro Val Arg Leu Leu Trp Lys Lys Ala Arg
255 260 265

gga gcc cca gtc cta gag aaa aca ctt ggc tac aac ata tgg tac tat 1348
Gly Ala Pro Val Leu Glu Lys Thr Leu Gly Tyr Asn Ile Trp Tyr Tyr
270 275 280

cca gaa agc aac act aac ctc aca gaa aca atg aac act act aac cag 1396
Pro Glu Ser Asn Thr Asn Leu Thr Glu Thr Met Asn Thr Thr Asn Gln
285 290 295 300

cag ctt gaa ctg cat ctg gga ggc gag agc ttt tgg gtg tct atg att 1444
Gln Leu Glu Leu His Leu Gly Gly Glu Ser Phe Trp Val Ser Met Ile
305 310 315

tct tat aat tct ctt ggg aag tct cca gtg gcc acc ctg agg att cca 1492
Ser Tyr Asn Ser Leu Gly Lys Ser Pro Val Ala Thr Leu Arg Ile Pro
320 325 330

gct att caa gaa aaa tca ttt cag tgc att gag gtc atg cag gcc tgc 1540
Ala Ile Gln Glu Lys Ser Phe Gln Cys Ile Glu Val Met Gln Ala Cys
335 340 345

gtt gct gag gac cag cta gtg gtg aag tgg caa agc tct gct cta gac 1588
Val Ala Glu Asp Gln Leu Val Val Lys Trp Gln Ser Ser Ala Leu Asp
350 355 360

gtg aac act tgg atg att gaa tgg ttt ccg gat gtg gac tca gag ccc 1636

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Val Asn Thr Trp Met Ile Glu Trp Phe Pro Asp Val Asp Ser Glu Pro
365                 370                 375                 380

acc acc ctt tcc tgg gaa tct gtg tct cag gcc acg aac tgg acg atc   1684
Thr Thr Leu Ser Trp Glu Ser Val Ser Gln Ala Thr Asn Trp Thr Ile
            385                 390                 395

cag caa gat aaa tta aaa cct ttc tgg tgc tat aac atc tct gtg tat   1732
Gln Gln Asp Lys Leu Lys Pro Phe Trp Cys Tyr Asn Ile Ser Val Tyr
        400                 405                 410

cca atg ttg cat gac aaa gtt ggc gag cca tat tcc atc cag gct tat   1780
Pro Met Leu His Asp Lys Val Gly Glu Pro Tyr Ser Ile Gln Ala Tyr
    415                 420                 425

gcc aaa gaa ggc gtt cca tca gaa ggt cct gag acc aag gtg gag aac   1828
Ala Lys Glu Gly Val Pro Ser Glu Gly Pro Glu Thr Lys Val Glu Asn
430                 435                 440

att ggc gtg aag acg gtc acg atc aca tgg aaa gag att ccc aag agt   1876
Ile Gly Val Lys Thr Val Thr Ile Thr Trp Lys Glu Ile Pro Lys Ser
445                 450                 455                 460

gag aga aag ggt atc atc tgc aac tac acc atc ttt tac caa gct gaa   1924
Glu Arg Lys Gly Ile Ile Cys Asn Tyr Thr Ile Phe Tyr Gln Ala Glu
                465                 470                 475

ggt gga aaa gga ttc tcc aag aca gtc aat tcc agc atc ttg cag tac   1972
Gly Gly Lys Gly Phe Ser Lys Thr Val Asn Ser Ser Ile Leu Gln Tyr
            480                 485                 490

ggc ctg gag tcc ctg aaa cga aag acc tct tac att gtt cag gtc atg   2020
Gly Leu Glu Ser Leu Lys Arg Lys Thr Ser Tyr Ile Val Gln Val Met
        495                 500                 505

gcc agc acc agt gct ggg gga acc aac ggg acc agc ata aat ttc aag   2068
Ala Ser Thr Ser Ala Gly Gly Thr Asn Gly Thr Ser Ile Asn Phe Lys
    510                 515                 520

aca ttg tca ttc agt gtc ttt gag att atc ctc ata act tct ctg att   2116
Thr Leu Ser Phe Ser Val Phe Glu Ile Ile Leu Ile Thr Ser Leu Ile
525                 530                 535                 540
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ggt gga ggc ctt ctt att ctc att atc ctg aca gtg gca tat ggt ctc   2164
Gly Gly Gly Leu Leu Ile Leu Ile Ile Leu Thr Val Ala Tyr Gly Leu
                545                 550                 555

aaa aaa ccc aac aaa ttg act cat ctg tgt tgg ccc acc gtt ccc aac   2212
Lys Lys Pro Asn Lys Leu Thr His Leu Cys Trp Pro Thr Val Pro Asn
            560                 565                 570

cct gct gaa agt agt ata gcc aca tgg cat gga gat gat ttc aag gat   2260
Pro Ala Glu Ser Ser Ile Ala Thr Trp His Gly Asp Asp Phe Lys Asp
        575                 580                 585

aag cta aac ctg aag gag tct gat gac tct gtg aac aca gaa gac agg   2308
Lys Leu Asn Leu Lys Glu Ser Asp Asp Ser Val Asn Thr Glu Asp Arg
    590                 595                 600

atc tta aaa cca tgt tcc acc ccc agt gac aag ttg gtg att gac aag   2356
Ile Leu Lys Pro Cys Ser Thr Pro Ser Asp Lys Leu Val Ile Asp Lys
605                 610                 615                 620

ttg gtg gtg aac ttt ggg aat gtt ctg caa gaa att ttc aca gat gaa   2404
Leu Val Val Asn Phe Gly Asn Val Leu Gln Glu Ile Phe Thr Asp Glu
                625                 630                 635

gcc aga acg ggt cag gaa aac aat tta gga ggg gaa aag aat ggg act   2452
Ala Arg Thr Gly Gln Glu Asn Asn Leu Gly Gly Glu Lys Asn Gly Thr
            640                 645                 650

aga att ctg tct tcc tgc cca act tca ata taagtgtgga ctaaaatgcg     2502
Arg Ile Leu Ser Ser Cys Pro Thr Ser Ile
        655                 660

agaaaggtgt cctgtggtct atgcaaatta gaaaggacat gcagagtttt ccaactagga 2562
agactgaatc tgtggcccca agagaaccat ctctgaagac tgggtatgtg gtcttttcca 2622
cacatggacc acctacggat gcaatctgta atgcatgtgc atgagaagtc tgttattaag 2682
tagagtgtga aaacatggtt atggtaatag gaacagcttt taaaatgctt ttgtatttgg 2742
gcctttcata caaaaaagcc ataataccat tttcatgtaa tgctatactt ctatactatt 2802
ttcatgtaat actatacttc tatactattt tcatgtaata ctatacttct atactatttt 2862
catgtaatac tatacttcta tattaaagtt ttacccactc a                     2903
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<211> 662
<212> PRT

<213> Homo sapiens

<400> 5

Met Lys Leu Ser Pro Gln Pro Ser Cys Val Asn Leu Gly Met Met Trp
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Thr Trp Ala Leu Trp Met Leu Pro Ser Leu Cys Lys Phe Ser Leu Ala
20 25 30
Ala Leu Pro Ala Lys Pro Glu Asn Ile Ser Cys Val Tyr Tyr Tyr Arg
35 40 45
Lys Asn Leu Thr Cys Thr Trp Ser Pro Gly Lys Glu Thr Ser Tyr Thr
50 55 60
Gln Tyr Thr Val Lys Arg Thr Tyr Ala Phe Gly Glu Lys His Asp Asn
65 70 75 80
Cys Thr Thr Asn Ser Ser Thr Ser Glu Asn Arg Ala Ser Cys Ser Phe
85 90 95
Phe Leu Pro Arg Ile Thr Ile Pro Asp Asn Tyr Thr Ile Glu Val Glu
100 105 110
Ala Glu Asn Gly Asp Gly Val Ile Lys Ser His Met Thr Tyr Trp Arg
115 120 125
Leu Glu Asn Ile Ala Lys Thr Glu Pro Pro Lys Ile Phe Arg Val Lys
130 135 140
Pro Val Leu Gly Ile Lys Arg Met Ile Gln Ile Glu Trp Ile Lys Pro
145 150 155 160
Glu Leu Ala Pro Val Ser Ser Asp Leu Lys Tyr Thr Leu Arg Phe Arg
165 170 175
Thr Val Asn Ser Thr Ser Trp Met Glu Val Asn Phe Ala Lys Asn Arg
180 185 190
Lys Asp Lys Asn Gln Thr Tyr Asn Leu Thr Gly Leu Gln Pro Phe Thr
195 200 205
Glu Tyr Val Ile Ala Leu Arg Cys Ala Val Lys Glu Ser Lys Phe Trp
210 215 220
Ser Asp Trp Ser Gln Glu Lys Met Gly Met Thr Glu Glu Glu Ala Pro
225 230 235 240
Cys Gly Leu Glu Leu Trp Arg Val Leu Lys Pro Ala Glu Ala Asp Gly
245 250 255
Arg Arg Pro Val Arg Leu Leu Trp Lys Lys Ala Arg Gly Ala Pro Val
260 265 270
Leu Glu Lys Thr Leu Gly Tyr Asn Ile Trp Tyr Tyr Pro Glu Ser Asn
275 280 285
Thr Asn Leu Thr Glu Thr Met Asn Thr Thr Asn Gln Gln Leu Glu Leu
290 295 300
His Leu Gly Gly Glu Ser Phe Trp Val Ser Met Ile Ser Tyr Asn Ser
305 310 315 320

Leu Gly Lys Ser Pro Val Ala Thr Leu Arg Ile Pro Ala Ile Gln Glu
325 330 335
Lys Ser Phe Gln Cys Ile Glu Val Met Gln Ala Cys Val Ala Glu Asp
340 345 350
Gln Leu Val Val Lys Trp Gln Ser Ser Ala Leu Asp Val Asn Thr Trp
355 360 365
Met Ile Glu Trp Phe Pro Asp Val Asp Ser Glu Pro Thr Thr Leu Ser
370 375 380
Trp Glu Ser Val Ser Gln Ala Thr Asn Trp Thr Ile Gln Gln Asp Lys
385 390 395 400
Leu Lys Pro Phe Trp Cys Tyr Asn Ile Ser Val Tyr Pro Met Leu His
405 410 415
Asp Lys Val Gly Glu Pro Tyr Ser Ile Gln Ala Tyr Ala Lys Glu Gly
420 425 430
Val Pro Ser Glu Gly Pro Glu Thr Lys Val Glu Asn Ile Gly Val Lys
435 440 445
Thr Val Thr Ile Thr Trp Lys Glu Ile Pro Lys Ser Glu Arg Lys Gly
450 455 460
Ile Ile Cys Asn Tyr Thr Ile Phe Tyr Gln Ala Glu Gly Gly Lys Gly
465 470 475 480
Phe Ser Lys Thr Val Asn Ser Ser Ile Leu Gln Tyr Gly Leu Glu Ser
485 490 495
Leu Lys Arg Lys Thr Ser Tyr Ile Val Gln Val Met Ala Ser Thr Ser
500 505 510
Ala Gly Gly Thr Asn Gly Thr Ser Ile Asn Phe Lys Thr Leu Ser Phe
515 520 525
Ser Val Phe Glu Ile Ile Leu Ile Thr Ser Leu Ile Gly Gly Gly Leu
530 535 540
Leu Ile Leu Ile Ile Leu Thr Val Ala Tyr Gly Leu Lys Lys Pro Asn
545 550 555 560
Lys Leu Thr His Leu Cys Trp Pro Thr Val Pro Asn Pro Ala Glu Ser
565 570 575
Ser Ile Ala Thr Trp His Gly Asp Asp Phe Lys Asp Lys Leu Asn Leu
580 585 590
Lys Glu Ser Asp Asp Ser Val Asn Thr Glu Asp Arg Ile Leu Lys Pro
595 600 605
Cys Ser Thr Pro Ser Asp Lys Leu Val Ile Asp Lys Leu Val Val Asn
610 615 620
Phe Gly Asn Val Leu Gln Glu Ile Phe Thr Asp Glu Ala Arg Thr Gly
625 630 635 640
Gln Glu Asn Asn Leu Gly Gly Glu Lys Asn Gly Thr Arg Ile Leu Ser
645 650 655
Ser Cys Pro Thr Ser Ile

660

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<212> DNA
<213> Homo sapiens

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<221> CDS
<222> (13)...(2949)

<400> 6

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                1               5                  10

aca ttg ctg tcc ttg agg act tac cag agt gaa gtc ttg gct gaa cgt   99
Thr Leu Leu Ser Leu Arg Thr Tyr Gln Ser Glu Val Leu Ala Glu Arg
     15                  20                  25

tta cca ttg act cct gta tca ctt aaa gtt tcc acc aat tct acg cgt   147
Leu Pro Leu Thr Pro Val Ser Leu Lys Val Ser Thr Asn Ser Thr Arg
 30                  35                  40                  45

cag agt ttg cac tta caa tgg act gtc cac aac ctt cct tat cat cag   195
Gln Ser Leu His Leu Gln Trp Thr Val His Asn Leu Pro Tyr His Gln
                50                  55                  60

gaa ttg aaa atg gta ttt cag atc cag atc agt agg att gaa aca tcc   243
Glu Leu Lys Met Val Phe Gln Ile Gln Ile Ser Arg Ile Glu Thr Ser
            65                  70                  75

aat gtc atc tgg gtg ggg aat tac agc acc act gtg aag tgg aac cag   291
Asn Val Ile Trp Val Gly Asn Tyr Ser Thr Thr Val Lys Trp Asn Gln
        80                  85                  90

gtt ctg cat tgg agc tgg gaa tct gag ctc cct ttg gaa tgt gcc aca   339
Val Leu His Trp Ser Trp Glu Ser Glu Leu Pro Leu Glu Cys Ala Thr
     95                  100                 105

cac ttt gta aga ata aag agt ttg gtg gac gat gcc aag ttc cct gag   387
His Phe Val Arg Ile Lys Ser Leu Val Asp Asp Ala Lys Phe Pro Glu
```

110 115 120 125

cca aat ttc tgg agc aac tgg agt tcc tgg gag gaa gtc agt gta caa 435
Pro Asn Phe Trp Ser Asn Trp Ser Ser Trp Glu Glu Val Ser Val Gln
130 135 140

gat tct act gga cag gat ata ttg ttc gtt ttc cct aaa gat aag ctg 483
Asp Ser Thr Gly Gln Asp Ile Leu Phe Val Phe Pro Lys Asp Lys Leu
145 150 155

gtg gaa gaa ggc acc aat gtt acc att tgt tac gtt tct agg aac att 531
Val Glu Glu Gly Thr Asn Val Thr Ile Cys Tyr Val Ser Arg Asn Ile
160 165 170

caa aat aat gta tcc tgt tat ttg gaa ggg aaa cag att cat gga gaa 579
Gln Asn Asn Val Ser Cys Tyr Leu Glu Gly Lys Gln Ile His Gly Glu
175 180 185

caa ctt gat cca cat gta act gca ttc aac ttg aat agt gtg cct ttc 627
Gln Leu Asp Pro His Val Thr Ala Phe Asn Leu Asn Ser Val Pro Phe
190 195 200 205

att agg aat aaa ggg aca aat atc tat tgt gag gca agt caa gga aat 675
Ile Arg Asn Lys Gly Thr Asn Ile Tyr Cys Glu Ala Ser Gln Gly Asn
210 215 220

gtc agt gaa ggc atg aaa ggc atc gtt ctt ttt gtc tca aaa gta ctt 723
Val Ser Glu Gly Met Lys Gly Ile Val Leu Phe Val Ser Lys Val Leu
225 230 235

gag gag ccc aag gac ttt tct tgt gaa acc gag gac ttc aag act ttg 771
Glu Glu Pro Lys Asp Phe Ser Cys Glu Thr Glu Asp Phe Lys Thr Leu
240 245 250

cac tgt act tgg gat cct ggg acg gac act gcc ttg ggg tgg tct aaa 819
His Cys Thr Trp Asp Pro Gly Thr Asp Thr Ala Leu Gly Trp Ser Lys
255 260 265

caa cct tcc caa agc tac act tta ttt gaa tca ttt tct ggg gaa aag 867
Gln Pro Ser Gln Ser Tyr Thr Leu Phe Glu Ser Phe Ser Gly Glu Lys
270 275 280 285

aaa ctt tgt aca cac aaa aac tgg tgt aat tgg caa ata act caa gac 915

```
Lys Leu Cys Thr His Lys Asn Trp Cys Asn Trp Gln Ile Thr Gln Asp
                290                 295                 300

tca caa gaa acc tat aac ttc aca ctc ata gct gaa aat tac tta agg   963
Ser Gln Glu Thr Tyr Asn Phe Thr Leu Ile Ala Glu Asn Tyr Leu Arg
        305                 310                 315

aag aga agt gtc aat atc ctt ttt aac ctg act cat cga gtt tat tta   1011
Lys Arg Ser Val Asn Ile Leu Phe Asn Leu Thr His Arg Val Tyr Leu
        320                 325                 330

atg aat cct ttt agt gtc aac ttt gaa aat gta aat gcc aca aat gcc   1059
Met Asn Pro Phe Ser Val Asn Phe Glu Asn Val Asn Ala Thr Asn Ala
    335                 340                 345

atc atg acc tgg aag gtg cac tcc ata agg aat aat ttc aca tat ttg   1107
Ile Met Thr Trp Lys Val His Ser Ile Arg Asn Asn Phe Thr Tyr Leu
350                 355                 360                 365

tgt cag att gaa ctc cat ggt gaa gga aaa atg atg caa tac aat gtt   1155
Cys Gln Ile Glu Leu His Gly Glu Gly Lys Met Met Gln Tyr Asn Val
                370                 375                 380

tcc atc aag gtg aac ggt gag tac ttc tta agt gaa ctg gaa cct gcc   1203
Ser Ile Lys Val Asn Gly Glu Tyr Phe Leu Ser Glu Leu Glu Pro Ala
            385                 390                 395

aca gag tac atg gcg cga gta cgg tgt gct gat gcc agc cac ttc tgg   1251
Thr Glu Tyr Met Ala Arg Val Arg Cys Ala Asp Ala Ser His Phe Trp
        400                 405                 410

aaa tgg agt gaa tgg agt ggt cag aac ttc acc aca ctt gaa gct gct   1299
Lys Trp Ser Glu Trp Ser Gly Gln Asn Phe Thr Thr Leu Glu Ala Ala
    415                 420                 425

ccc tca gag gcc cct gat gtc tgg aga att gtg agc ttg gag cca gga   1347
Pro Ser Glu Ala Pro Asp Val Trp Arg Ile Val Ser Leu Glu Pro Gly
430                 435                 440                 445

aat cat act gtg acc tta ttc tgg aag cca tta tca aaa ctg cat gcc   1395
Asn His Thr Val Thr Leu Phe Trp Lys Pro Leu Ser Lys Leu His Ala
                450                 455                 460
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aat gga aag atc ctg ttc tat aat gta gtt gta gaa aac cta gac aaa 1443
Asn Gly Lys Ile Leu Phe Tyr Asn Val Val Val Glu Asn Leu Asp Lys
465 470 475

cca tcc agt tca gag ctc cat tcc att cca gca cca gcc aac agc aca 1491
Pro Ser Ser Ser Glu Leu His Ser Ile Pro Ala Pro Ala Asn Ser Thr
480 485 490

aaa cta atc ctt gac agg tgt tcc tac caa atc tgc gtc ata gcc aac 1539
Lys Leu Ile Leu Asp Arg Cys Ser Tyr Gln Ile Cys Val Ile Ala Asn
495 500 505

aac agt gtg ggt gct tct cct gct tct gta ata gtc atc tct gca gac 1587
Asn Ser Val Gly Ala Ser Pro Ala Ser Val Ile Val Ile Ser Ala Asp
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ccc gaa aac aaa gag gtt gag gaa gaa aga att gca ggc aca gag ggt 1635
Pro Glu Asn Lys Glu Val Glu Glu Glu Arg Ile Ala Gly Thr Glu Gly
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gga ttc tct ctg tct tgg aaa ccc caa cct gga gat gtt ata ggc tat 1683
Gly Phe Ser Leu Ser Trp Lys Pro Gln Pro Gly Asp Val Ile Gly Tyr
545 550 555

gtt gtg gac tgg tgt gac cat acc cag gat gtg ctc ggt gat ttc cag 1731
Val Val Asp Trp Cys Asp His Thr Gln Asp Val Leu Gly Asp Phe Gln
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tgg aag aat gta ggt ccc aat acc aca agc aca gtc att agc aca gat 1779
Trp Lys Asn Val Gly Pro Asn Thr Thr Ser Thr Val Ile Ser Thr Asp
575 580 585

gct ttt agg cca gga gtt cga tat gac ttc aga att tat ggg tta tct 1827
Ala Phe Arg Pro Gly Val Arg Tyr Asp Phe Arg Ile Tyr Gly Leu Ser
590 595 600 605

aca aaa agg att gct tgt tta tta gag aaa aaa aca gga tac tct cag 1875
Thr Lys Arg Ile Ala Cys Leu Leu Glu Lys Lys Thr Gly Tyr Ser Gln
610 615 620

gaa ctt gct cct tca gac aac cct cac gtg ctg gtg gat aca ttg aca 1923
Glu Leu Ala Pro Ser Asp Asn Pro His Val Leu Val Asp Thr Leu Thr
625 630 635

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tcc cac tcc ttc act ctg agt tgg aaa gat tac tct act gaa tct caa   1971
Ser His Ser Phe Thr Leu Ser Trp Lys Asp Tyr Ser Thr Glu Ser Gln
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cct ggt ttt ata caa ggg tac cat gtc tat ctg aaa tcc aag gcg agg   2019
Pro Gly Phe Ile Gln Gly Tyr His Val Tyr Leu Lys Ser Lys Ala Arg
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cag tgc cac cca cga ttt gaa aag gca gtt ctt tca gat ggt tca gaa   2067
Gln Cys His Pro Arg Phe Glu Lys Ala Val Leu Ser Asp Gly Ser Glu
670                 675                 680                 685

tgt tgc aaa tac aaa att gac aac ccg gaa gaa aag gca ttg att gtg   2115
Cys Cys Lys Tyr Lys Ile Asp Asn Pro Glu Glu Lys Ala Leu Ile Val
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gac aac cta aag cca gaa tcc ttc tat gag ttt ttc atc act cca ttc   2163
Asp Asn Leu Lys Pro Glu Ser Phe Tyr Glu Phe Phe Ile Thr Pro Phe
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act agt gct ggt gaa ggc ccc agt gct acg ttc acg aag gtc acg act   2211
Thr Ser Ala Gly Glu Gly Pro Ser Ala Thr Phe Thr Lys Val Thr Thr
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ccg gat gaa cac tcc tcg atg ctg att cat atc cta ctg ccc atg gtt   2259
Pro Asp Glu His Ser Ser Met Leu Ile His Ile Leu Leu Pro Met Val
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Phe Cys Val Leu Leu Ile Met Val Met Cys Tyr Leu Lys Ser Gln Trp
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atc aag gag acc tgt tat cct gac atc cct gac cct tac aag agc agc   2355
Ile Lys Glu Thr Cys Tyr Pro Asp Ile Pro Asp Pro Tyr Lys Ser Ser
                770                 775                 780

atc ctg tca tta ata aaa ttc aag gag aac cct cac cta ata ata atg   2403
Ile Leu Ser Leu Ile Lys Phe Lys Glu Asn Pro His Leu Ile Ile Met
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aat gtc agt gac tgt atc cca gat gct att gaa gtt gta agc aag cca   2451
Asn Val Ser Asp Cys Ile Pro Asp Ala Ile Glu Val Val Ser Lys Pro
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Glu Gly Thr Lys Ile Gln Phe Leu Gly Thr Arg Lys Ser Leu Thr Glu
815 820 825

acc gag ttg act aag cct aac tac ctt tat ctc ctt cca aca gaa aag 2547
Thr Glu Leu Thr Lys Pro Asn Tyr Leu Tyr Leu Leu Pro Thr Glu Lys
830 835 840 845

aat cac tct ggc cct ggc ccc tgc atc tgt ttt gag aac ttg acc tat 2595
Asn His Ser Gly Pro Gly Pro Cys Ile Cys Phe Glu Asn Leu Thr Tyr
850 855 860

aac cag gca gct tct gac tct ggc tct tgt ggc cat gtt cca gta tcc 2643
Asn Gln Ala Ala Ser Asp Ser Gly Ser Cys Gly His Val Pro Val Ser
865 870 875

cca aaa gcc cca agt atg ctg gga cta atg acc tca cct gaa aat gta 2691
Pro Lys Ala Pro Ser Met Leu Gly Leu Met Thr Ser Pro Glu Asn Val
880 885 890

cta aag gca cta gaa aaa aac tac atg aac tcc ctg gga gaa atc cca 2739
Leu Lys Ala Leu Glu Lys Asn Tyr Met Asn Ser Leu Gly Glu Ile Pro
895 900 905

gct gga gaa aca agt ttg aat tat gtg tcc cag ttg gct tca ccc atg 2787
Ala Gly Glu Thr Ser Leu Asn Tyr Val Ser Gln Leu Ala Ser Pro Met
910 915 920 925

ttt gga gac aag gac agt ctc cca aca aac cca gta gag gca cca cac 2835
Phe Gly Asp Lys Asp Ser Leu Pro Thr Asn Pro Val Glu Ala Pro His
930 935 940

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Cys Ser Glu Tyr Lys Met Gln Met Ala Val Ser Leu Arg Leu Ala Leu
945 950 955

cct ccc ccg acc gag aat agc agc ctc tcc tca att acc ctt tta gat 2931
Pro Pro Pro Thr Glu Asn Ser Ser Leu Ser Ser Ile Thr Leu Leu Asp
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Thr	Pro	Val	Ser	Leu	Lys	Val	Ser	Thr	Asn	Ser	Thr	Arg	Gln	Ser	Leu				
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His	Leu	Gln	Trp	Thr	Val	His	Asn	Leu	Pro	Tyr	His	Gln	Glu	Leu	Lys				
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Arg	Ile	Lys	Ser	Leu	Val	Asp	Asp	Ala	Lys	Phe	Pro	Glu	Pro	Asn	Phe				
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Trp	Ser	Asn	Trp	Ser	Ser	Trp	Glu	Glu	Val	Ser	Val	Gln	Asp	Ser	Thr				
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Gly	Gln	Asp	Ile	Leu	Phe	Val	Phe	Pro	Lys	Asp	Lys	Leu	Val	Glu	Glu				
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Val	Ser	Cys	Tyr	Leu	Glu	Gly	Lys	Gln	Ile	His	Gly	Glu	Gln	Leu	Asp				
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Pro	His	Val	Thr	Ala	Phe	Asn	Leu	Asn	Ser	Val	Pro	Phe	Ile	Arg	Asn				
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Gly	Met	Lys	Gly	Ile	Val	Leu	Phe	Val	Ser	Lys	Val	Leu	Glu	Glu	Pro				
225					230					235					240				
Lys	Asp	Phe	Ser	Cys	Glu	Thr	Glu	Asp	Phe	Lys	Thr	Leu	His	Cys	Thr				
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Trp	Asp	Pro	Gly	Thr	Asp	Thr	Ala	Leu	Gly	Trp	Ser	Lys	Gln	Pro	Ser				

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305 310 315 320
Val Asn Ile Leu Phe Asn Leu Thr His Arg Val Tyr Leu Met Asn Pro
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Phe Ser Val Asn Phe Glu Asn Val Asn Ala Thr Asn Ala Ile Met Thr
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Glu Leu His Gly Glu Gly Lys Met Met Gln Tyr Asn Val Ser Ile Lys
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Ala Pro Asp Val Trp Arg Ile Val Ser Leu Glu Pro Gly Asn His Thr
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Val Gly Pro Asn Thr Thr Ser Thr Val Ile Ser Thr Asp Ala Phe Arg
580 585 590
Pro Gly Val Arg Tyr Asp Phe Arg Ile Tyr Gly Leu Ser Thr Lys Arg
595 600 605

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Pro Ser Asp Asn Pro His Val Leu Val Asp Thr Leu Thr Ser His Ser
625 630 635 640
Phe Thr Leu Ser Trp Lys Asp Tyr Ser Thr Glu Ser Gln Pro Gly Phe
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Ile Gln Gly Tyr His Val Tyr Leu Lys Ser Lys Ala Arg Gln Cys His
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Pro Arg Phe Glu Lys Ala Val Leu Ser Asp Gly Ser Glu Cys Cys Lys
675 680 685
Tyr Lys Ile Asp Asn Pro Glu Glu Lys Ala Leu Ile Val Asp Asn Leu
690 695 700
Lys Pro Glu Ser Phe Tyr Glu Phe Phe Ile Thr Pro Phe Thr Ser Ala
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Gly Glu Gly Pro Ser Ala Thr Phe Thr Lys Val Thr Thr Pro Asp Glu
725 730 735
His Ser Ser Met Leu Ile His Ile Leu Leu Pro Met Val Phe Cys Val
740 745 750
Leu Leu Ile Met Val Met Cys Tyr Leu Lys Ser Gln Trp Ile Lys Glu
755 760 765
Thr Cys Tyr Pro Asp Ile Pro Asp Pro Tyr Lys Ser Ser Ile Leu Ser
770 775 780
Leu Ile Lys Phe Lys Glu Asn Pro His Leu Ile Ile Met Asn Val Ser
785 790 795 800
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Lys Ile Gln Phe Leu Gly Thr Arg Lys Ser Leu Thr Glu Thr Glu Leu
820 825 830
Thr Lys Pro Asn Tyr Leu Tyr Leu Leu Pro Thr Glu Lys Asn His Ser
835 840 845
Gly Pro Gly Pro Cys Ile Cys Phe Glu Asn Leu Thr Tyr Asn Gln Ala
850 855 860
Ala Ser Asp Ser Gly Ser Cys Gly His Val Pro Val Ser Pro Lys Ala
865 870 875 880
Pro Ser Met Leu Gly Leu Met Thr Ser Pro Glu Asn Val Leu Lys Ala
885 890 895
Leu Glu Lys Asn Tyr Met Asn Ser Leu Gly Glu Ile Pro Ala Gly Glu
900 905 910
Thr Ser Leu Asn Tyr Val Ser Gln Leu Ala Ser Pro Met Phe Gly Asp
915 920 925
Lys Asp Ser Leu Pro Thr Asn Pro Val Glu Ala Pro His Cys Ser Glu
930 935 940
Tyr Lys Met Gln Met Ala Val Ser Leu Arg Leu Ala Leu Pro Pro Pro

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ccgagggacg cc atg cgg gga ggc agg ggc gcc cct ttc tgg ctg tgg ccg 171
Met Arg Gly Gly Arg Gly Ala Pro Phe Trp Leu Trp Pro
1 5 10

ctg ccc aag ctg gcg ctg ctg cct ctg ttg tgg gtg ctt ttc cag cgg 219
Leu Pro Lys Leu Ala Leu Leu Pro Leu Leu Trp Val Leu Phe Gln Arg
15 20 25

acg cgt ccc cag ggc agc gcc ggg cca ctg cag tgc tac gga gtt gga 267
Thr Arg Pro Gln Gly Ser Ala Gly Pro Leu Gln Cys Tyr Gly Val Gly
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ccc ttg ggc gac ttg aac tgc tcg tgg gag cct ctt ggg gac ctg gga 315
Pro Leu Gly Asp Leu Asn Cys Ser Trp Glu Pro Leu Gly Asp Leu Gly
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gcc ccc tcc gag tta cac ctc cag agc caa aag tac cgt tcc aac aaa 363
Ala Pro Ser Glu Leu His Leu Gln Ser Gln Lys Tyr Arg Ser Asn Lys
65 70 75

acc cag act gtg gca gtg gca gcc gga cgg agc tgg gtg gcc att cct 411
Thr Gln Thr Val Ala Val Ala Ala Gly Arg Ser Trp Val Ala Ile Pro
80 85 90

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cgg gaa cag ctc acc atg tct gac aaa ctc ctt gtc tgg ggc act aag   459
Arg Glu Gln Leu Thr Met Ser Asp Lys Leu Leu Val Trp Gly Thr Lys
 95                  100                 105

gca ggc cag cct ctc tgg ccc ccc gtc ttc gtg aac cta gaa acc caa   507
Ala Gly Gln Pro Leu Trp Pro Pro Val Phe Val Asn Leu Glu Thr Gln
110                 115                 120                 125

atg aag cca aac gcc ccc cgg ctg ggc cct gac gtg gac ttt tcc gag   555
Met Lys Pro Asn Ala Pro Arg Leu Gly Pro Asp Val Asp Phe Ser Glu
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gat gac ccc ctg gag gcc act gtc cat tgg gcc cca cct aca tgg cca   603
Asp Asp Pro Leu Glu Ala Thr Val His Trp Ala Pro Pro Thr Trp Pro
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tct cat aaa gtt ctg atc tgc cag ttc cac tac cga aga tgt cag gag   651
Ser His Lys Val Leu Ile Cys Gln Phe His Tyr Arg Arg Cys Gln Glu
        160                 165                 170

gcg gcc tgg acc ctg ctg gaa ccg gag ctg aag acc ata ccc ctg acc   699
Ala Ala Trp Thr Leu Leu Glu Pro Glu Leu Lys Thr Ile Pro Leu Thr
 175                 180                 185

cct gtt gag atc caa gat ttg gag cta gcc act ggc tac aaa gtg tat   747
Pro Val Glu Ile Gln Asp Leu Glu Leu Ala Thr Gly Tyr Lys Val Tyr
190                 195                 200                 205

ggc cgc tgc cgg atg gag aaa gaa gag gat ttg tgg ggc gag tgg agc   795
Gly Arg Cys Arg Met Glu Lys Glu Glu Asp Leu Trp Gly Glu Trp Ser
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ccc att ttg tcc ttc cag aca ccg cct tct gct cca aaa gat gtg tgg   843
Pro Ile Leu Ser Phe Gln Thr Pro Pro Ser Ala Pro Lys Asp Val Trp
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gta tca ggg aac ctc tgt ggg acg cct gga gga gag gaa cct ttg ctt   891
Val Ser Gly Asn Leu Cys Gly Thr Pro Gly Gly Glu Glu Pro Leu Leu
        240                 245                 250

cta tgg aag gcc cca ggg ccc tgt gtg cag gtg agc tac aaa gtc tgg   939
Leu Trp Lys Ala Pro Gly Pro Cys Val Gln Val Ser Tyr Lys Val Trp
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Phe Trp Val Gly Gly Arg Glu Leu Ser Pro Glu Gly Ile Thr Cys Cys
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tgc tcc cta att ccc agt ggg gcg gag tgg gcc agg gtg tcc gct gtc   1035
Cys Ser Leu Ile Pro Ser Gly Ala Glu Trp Ala Arg Val Ser Ala Val
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aac gcc aca agc tgg gag cct ctc acc aac ctc tct ttg gtc tgc ttg   1083
Asn Ala Thr Ser Trp Glu Pro Leu Thr Asn Leu Ser Leu Val Cys Leu
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gat tca gcc tct gcc ccc cgt agc gtg gca gtc agc agc atc gct ggg   1131
Asp Ser Ala Ser Ala Pro Arg Ser Val Ala Val Ser Ser Ile Ala Gly
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agc acg gag cta ctg gtg acc tgg caa ccg ggg cct ggg gaa cca ctg   1179
Ser Thr Glu Leu Leu Val Thr Trp Gln Pro Gly Pro Gly Glu Pro Leu
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gag cat gta gtg gac tgg gct cga gat ggg gac ccc ctg gag aaa ctc   1227
Glu His Val Val Asp Trp Ala Arg Asp Gly Asp Pro Leu Glu Lys Leu
350                 355                 360                 365

aac tgg gtc cgg ctt ccc cct ggg aac ctc agt gct ctg tta cca ggg   1275
Asn Trp Val Arg Leu Pro Pro Gly Asn Leu Ser Ala Leu Leu Pro Gly
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aat ttc act gtc ggg gtc ccc tat cga atc act gtg acc gca gtc tct   1323
Asn Phe Thr Val Gly Val Pro Tyr Arg Ile Thr Val Thr Ala Val Ser
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gct tca ggc ttg gcc tct gca tcc tcc gtc tgg ggg ttc agg gag gaa   1371
Ala Ser Gly Leu Ala Ser Ala Ser Ser Val Trp Gly Phe Arg Glu Glu
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tta gca ccc cta gtg ggg cca acg ctt tgg cga ctc caa gat gcc cct   1419
Leu Ala Pro Leu Val Gly Pro Thr Leu Trp Arg Leu Gln Asp Ala Pro
    415                 420                 425

cca ggg acc ccc gcc ata gcg tgg gga gag gtc cca agg cac cag ctt   1467
Pro Gly Thr Pro Ala Ile Ala Trp Gly Glu Val Pro Arg His Gln Leu
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ccc tcc gtc tgc atg aat gtg agt ggc aac aca cag agt gtc acc ctg   1563
Pro Ser Val Cys Met Asn Val Ser Gly Asn Thr Gln Ser Val Thr Leu
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cct gac ctt cct tgg ggt ccc tgt gag ctg tgg gtg aca gca tct acc   1611
Pro Asp Leu Pro Trp Gly Pro Cys Glu Leu Trp Val Thr Ala Ser Thr
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atc gct gga cag ggc cct cct ggt ccc atc ctc cgg ctt cat cta cca   1659
Ile Ala Gly Gln Gly Pro Pro Gly Pro Ile Leu Arg Leu His Leu Pro
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gat aac acc ctg agg tgg aaa gtt ctg cca ggc atc cta ttc ttg tgg   1707
Asp Asn Thr Leu Arg Trp Lys Val Leu Pro Gly Ile Leu Phe Leu Trp
510                 515                 520                 525

ggc ttg ttc ctg ttg ggg tgt ggc ctg agc ctg gcc acc tct gga agg   1755
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Cys Tyr His Leu Arg His Lys Val Leu Pro Arg Trp Val Trp Glu Lys
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gtt cct gat cct gcc aac agc agt tca ggc cag ccc cac atg gag caa   1851
Val Pro Asp Pro Ala Asn Ser Ser Ser Gly Gln Pro His Met Glu Gln
        560                 565                 570

gta cct gag gcc cag ccc ctt ggg gac ttg ccc atc ctg gaa gtg gag   1899
Val Pro Glu Ala Gln Pro Leu Gly Asp Leu Pro Ile Leu Glu Val Glu
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gag atg gag ccc ccg ccg gtt atg gag tcc tcc cag ccc gcc cag gcc   1947
Glu Met Glu Pro Pro Pro Val Met Glu Ser Ser Gln Pro Ala Gln Ala
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acc gcc ccg ctt gac tct ggg tat gag aag cac ttc ctg ccc aca cct   1995
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Thr Ala Pro Leu Asp Ser Gly Tyr Glu Lys His Phe Leu Pro Thr Pro
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gag gag ctg ggc ctt ctg ggg ccc ccc agg cca cag gtt ctg gcc 2040
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625 630 635

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Gln Gly Ser Ala Gly Pro Leu Gln Cys Tyr Gly Val Gly Pro Leu Gly
35 40 45
Asp Leu Asn Cys Ser Trp Glu Pro Leu Gly Asp Leu Gly Ala Pro Ser
50 55 60
Glu Leu His Leu Gln Ser Gln Lys Tyr Arg Ser Asn Lys Thr Gln Thr
65 70 75 80
Val Ala Val Ala Ala Gly Arg Ser Trp Val Ala Ile Pro Arg Glu Gln
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100 105 110
Pro Leu Trp Pro Pro Val Phe Val Asn Leu Glu Thr Gln Met Lys Pro
115 120 125
Asn Ala Pro Arg Leu Gly Pro Asp Val Asp Phe Ser Glu Asp Asp Pro
130 135 140

Leu Glu Ala Thr Val His Trp Ala Pro Pro Thr Trp Pro Ser His Lys
145 150 155 160
Val Leu Ile Cys Gln Phe His Tyr Arg Arg Cys Gln Glu Ala Ala Trp
165 170 175
Thr Leu Leu Glu Pro Glu Leu Lys Thr Ile Pro Leu Thr Pro Val Glu
180 185 190
Ile Gln Asp Leu Glu Leu Ala Thr Gly Tyr Lys Val Tyr Gly Arg Cys
195 200 205
Arg Met Glu Lys Glu Glu Asp Leu Trp Gly Glu Trp Ser Pro Ile Leu
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Ser Phe Gln Thr Pro Pro Ser Ala Pro Lys Asp Val Trp Val Ser Gly
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Asn Leu Cys Gly Thr Pro Gly Gly Glu Glu Pro Leu Leu Leu Trp Lys
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Ala Pro Gly Pro Cys Val Gln Val Ser Tyr Lys Val Trp Phe Trp Val
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385 390 395 400
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Pro Ala Ile Ala Trp Gly Glu Val Pro Arg His Gln Leu Arg Gly His
435 440 445
Leu Thr His Tyr Thr Leu Cys Ala Gln Ser Gly Thr Ser Pro Ser Val
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Cys Met Asn Val Ser Gly Asn Thr Gln Ser Val Thr Leu Pro Asp Leu
465 470 475 480
Pro Trp Gly Pro Cys Glu Leu Trp Val Thr Ala Ser Thr Ile Ala Gly

485 490 495

Gln Gly Pro Pro Gly Pro Ile Leu Arg Leu His Leu Pro Asp Asn Thr

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Leu Arg Trp Lys Val Leu Pro Gly Ile Leu Phe Leu Trp Gly Leu Phe

515 520 525

Leu Leu Gly Cys Gly Leu Ser Leu Ala Thr Ser Gly Arg Cys Tyr His

530 535 540

Leu Arg His Lys Val Leu Pro Arg Trp Val Trp Glu Lys Val Pro Asp

545 550 555 560

Pro Ala Asn Ser Ser Ser Gly Gln Pro His Met Glu Gln Val Pro Glu

565 570 575

Ala Gln Pro Leu Gly Asp Leu Pro Ile Leu Glu Val Glu Glu Met Glu

580 585 590

Pro Pro Pro Val Met Glu Ser Ser Gln Pro Ala Gln Ala Thr Ala Pro

595 600 605

Leu Asp Ser Gly Tyr Glu Lys His Phe Leu Pro Thr Pro Glu Glu Leu

610 615 620

Gly Leu Leu Gly Pro Pro Arg Pro Gln Val Leu Ala

625 630 635

<210> 10
<211> 755
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(489)

<400> 10

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Met Ile Phe His Thr Gly Thr Thr Lys Pro Thr Leu Val Leu Leu Cys
1 5 10 15

tgt ata gga acc tgg ctg gcc acc tgc agc ttg tcc ttc ggt gcc cca 96
Cys Ile Gly Thr Trp Leu Ala Thr Cys Ser Leu Ser Phe Gly Ala Pro
20 25 30

ata tcg aag gaa gac tta aga act aca att gac ctc ttg aaa caa gag 144
Ile Ser Lys Glu Asp Leu Arg Thr Thr Ile Asp Leu Leu Lys Gln Glu
35 40 45

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tct cag gat ctt tat aac aac tat agc ata aag cag gca tct ggg atg   192
Ser Gln Asp Leu Tyr Asn Asn Tyr Ser Ile Lys Gln Ala Ser Gly Met
     50                  55                  60

tca gca gac gaa tca ata cag ctg ccg tgt ttc agc ctg gac cgg gaa   240
Ser Ala Asp Glu Ser Ile Gln Leu Pro Cys Phe Ser Leu Asp Arg Glu
 65                  70                  75                  80

gca tta acc aac atc tcg gtc atc ata gca cat ctg gag aaa gtc aaa   288
Ala Leu Thr Asn Ile Ser Val Ile Ile Ala His Leu Glu Lys Val Lys
                 85                  90                  95

gtg ttg agc gag aac aca gta gat act tct tgg gtg ata aga tgg cta   336
Val Leu Ser Glu Asn Thr Val Asp Thr Ser Trp Val Ile Arg Trp Leu
            100                 105                 110

aca aac atc agc tgt ttc aac cca ctg aat tta aac att tct gtg cct   384
Thr Asn Ile Ser Cys Phe Asn Pro Leu Asn Leu Asn Ile Ser Val Pro
        115                 120                 125

gga aat act gat gaa tcc tat gat tgt aaa gtg ttc gtg ctt acg gtt   432
Gly Asn Thr Asp Glu Ser Tyr Asp Cys Lys Val Phe Val Leu Thr Val
    130                 135                 140

tta aag cag ttc tca aac tgc atg gca gaa ctg cag gct aag gac aat   480
Leu Lys Gln Phe Ser Asn Cys Met Ala Glu Leu Gln Ala Lys Asp Asn
145                 150                 155                 160

act aca tgc tgagtgatgg gggggggggg ggtgcagtgt cctcagcagt          529
Thr Thr Cys

gcctgtcctt cgagggctga gcttgcaacc caggacttaa ctccaaaggg actgtgcggt 589
cattactagt catgttattt atgtttttat tttgtccact gaaatcttgt tctgctaccc 649
tgtagggact ggaagtggca gctatattta tttatttatg tactgagttt gttaacgctc 709
catggaggag ccttcagagt ctatttaata aattatattg acatga                755

      <210> 11
      <211> 163
      <212> PRT
      <213> Mus musculus

      <400> 11
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Met Ile Phe His Thr Gly Thr Thr Lys Pro Thr Leu Val Leu Leu Cys
1 5 10 15
Cys Ile Gly Thr Trp Leu Ala Thr Cys Ser Leu Ser Phe Gly Ala Pro
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Ile Ser Lys Glu Asp Leu Arg Thr Thr Ile Asp Leu Leu Lys Gln Glu
35 40 45
Ser Gln Asp Leu Tyr Asn Asn Tyr Ser Ile Lys Gln Ala Ser Gly Met
50 55 60
Ser Ala Asp Glu Ser Ile Gln Leu Pro Cys Phe Ser Leu Asp Arg Glu
65 70 75 80
Ala Leu Thr Asn Ile Ser Val Ile Ile Ala His Leu Glu Lys Val Lys
85 90 95
Val Leu Ser Glu Asn Thr Val Asp Thr Ser Trp Val Ile Arg Trp Leu
100 105 110
Thr Asn Ile Ser Cys Phe Asn Pro Leu Asn Leu Asn Ile Ser Val Pro
115 120 125
Gly Asn Thr Asp Glu Ser Tyr Asp Cys Lys Val Phe Val Leu Thr Val
130 135 140
Leu Lys Gln Phe Ser Asn Cys Met Ala Glu Leu Gln Ala Lys Asp Asn
145 150 155 160
Thr Thr Cys

<210> 12
<211> 489
<212> DNA
<213> Artificial Sequence

<220>
<223> mouse zcytor17lig degenerate polynucleotide of SEQ ID NO:11

<221> misc_feature
<222> (1)...(489)
<223> n = A,T,C or G

<400> 12
atgathttyc ayacnggnac nacnaarccn acnytngtny tnytntgytg yathggnacn 60
tggytngcna cntgywsnyt nwsnttyggn gcnccnathw snaargarga yytnmgnacn 120
acnathgayy tnytnaarca rgarwsncar gayytntaya ayaaytayws nathaarcar 180
gcnwsnggna tgwsngcnga ygarwsnath carytnccnt gyttywsnyt ngaymgngar 240
gcnytnacna ayathwsngt nathathgcn cayytngara argtnaargt nytnwsngar 300

aayacngtng ayacnwsntg ggtnathmgn tggytnacna ayathwsntg yttyaayccn 360
ytnaayytna ayathwsngt nccnggnaay acngaygarw sntaygaytg yaargtntty 420
gtnytnacng tnytnaarca rttywsnaay tgyatggcng arytncargc naargayaay 480
acnacntgy 489

<210> 13
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC6673

<400> 13
gcgcaaggtg ccgttcacag c 21

<210> 14
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29082

<400> 14
caatttgttg ggtttttta gcagcagtag gcccag 36

<210> 15
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29083

<400> 15
ctgggcctac tgctgctaaa aaaacccaac aaattg 36

<210> 16
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29145

<400> 16
gcgtctagag ggttatattg aagttgggca ggaaga 36

<210> 17
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Gly-Ser Modified Glu-Glu (CEE) peptide tag

<400> 17
Gly Ser Glu Tyr Met Pro Met Glu
1 5

<210> 18
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29359

<400> 18
gcgggatcca tgaagctctc tccccagcct tca 33

<210> 19
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC27899

<400> 19
ccagaacttt gactccttga ccg 23

<210> 20
<211> 23

<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC27895

<400> 20
gaagtcaact tcgctaagaa ccg 23

<210> 21
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29122

<400> 21
ccgctcgagt tatattgaag ttgggcagga agac 34

<210> 22
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29180

<400> 22
cctggagtcc ctgaaacgaa ag 22

<210> 23
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29122

<400> 23
ccgctcgagt tatattgaag ttgggcagga agac 34

<210> 24

<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC9791

<400> 24
cgttccaaca aaacccagac 20

<210> 25
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC9793

<400> 25
tggcgttgac agcggacac 19

<210> 26
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC40109

<400> 26
ccattccagc accagccaac 20

<210> 27
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC40112

<400> 27
tacaacttca atagcatctg gg 22

<210> 28
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC13496

<400> 28
ccctgcagtg atcaacatgg ccaagttgac cagtgccgtt 40

<210> 29
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC13945

<400> 29
gcccatggac tagtttcgaa aggtcgagtg tcagtcctgc tcctc 45

<210> 30
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC18698

<400> 30
ttttttctc gagacttttt tttttttttt tttt 34

<210> 31

<220>
<223> Skipped Sequence

<400> 31
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<210> 32
<211> 10

<212> PRT
<213> Artificial Sequence

<220>
<223> Glu-Glu (CEE) peptide tag with Gly-Ser residue pair

<400> 32
Gly Ser Gly Gly Glu Tyr Met Pro Met Glu
1 5 10

<210> 33
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29451

<400> 33
ccggaattcc cctgatacat gaagctctct ccc 33

<210> 34
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29124

<400> 34
cgcggatccc tcaaagacac tgaatgacaa tgt 33

<210> 35
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Glu-Glu (CEE) peptide tag without Gly-Ser residue pair

<400> 35
Glu Tyr Met Pro Met Glu
1 5

<210> 36
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> C-terminal FLAG peptide sequence

<400> 36
Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> 37
<211> 684
<212> DNA
<213> Homo sapiens

<400> 37
tcagacaaaa ctcacacatg cccaccgtgc ccagcacctg aagccgaggg ggcaccgtca 60
gtcttcctct tccccccaaa acccaaggac accctcatga tctcccggac ccctgaggtc 120
acatgcgtgg tggtggacgt gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg 180
gacggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagta caacagcacg 240
taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtac 300
aagtgcaagg tctccaacaa agccctccca tcctccatcg agaaaaccat ctccaaagcc 360
aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga tgagctgacc 420
aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg 480
gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc cgtgctggac 540
tccgacggct ccttcttcct ctacagcaag ctcaccgtgg acaagagcag gtggcagcag 600
gggaacgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacgcagaag 660
agcctctccc tgtctccggg taaa 684

<210> 38
<211> 2295
<212> DNA
<213> Artificial Sequence

<220>

<223> human zcytor17-Fc4 fusion polynucleotide

<221> CDS
<222> (1)...(2295)

<400> 38

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Met Lys Leu Ser Pro Gln Pro Ser Cys Val Asn Leu Gly Met Met Trp
 1               5                  10                  15

acc tgg gca ctg tgg atg ctc cct tca ctc tgc aaa ttc agc ctg gca   96
Thr Trp Ala Leu Trp Met Leu Pro Ser Leu Cys Lys Phe Ser Leu Ala
            20                  25                  30

gct ctg cca gct aag cct gag aac att tcc tgt gtc tac tac tat agg   144
Ala Leu Pro Ala Lys Pro Glu Asn Ile Ser Cys Val Tyr Tyr Tyr Arg
        35                  40                  45

aaa aat tta acc tgc act tgg agt cca gga aag gaa acc agt tat acc   192
Lys Asn Leu Thr Cys Thr Trp Ser Pro Gly Lys Glu Thr Ser Tyr Thr
    50                  55                  60

cag tac aca gtt aag aga act tac gct ttt gga gaa aaa cat gat aat   240
Gln Tyr Thr Val Lys Arg Thr Tyr Ala Phe Gly Glu Lys His Asp Asn
65                  70                  75                  80

tgt aca acc aat agt tct aca agt gaa aat cgt gct tcg tgc tct ttt   288
Cys Thr Thr Asn Ser Ser Thr Ser Glu Asn Arg Ala Ser Cys Ser Phe
                85                  90                  95

ttc ctt cca aga ata acg atc cca gat aat tat acc att gag gtg gaa   336
Phe Leu Pro Arg Ile Thr Ile Pro Asp Asn Tyr Thr Ile Glu Val Glu
            100                 105                 110

gct gaa aat gga gat ggt gta att aaa tct cat atg aca tac tgg aga   384
Ala Glu Asn Gly Asp Gly Val Ile Lys Ser His Met Thr Tyr Trp Arg
        115                 120                 125

tta gag aac ata gcg aaa act gaa cca cct aag att ttc cgt gtg aaa   432
Leu Glu Asn Ile Ala Lys Thr Glu Pro Pro Lys Ile Phe Arg Val Lys
    130                 135                 140

cca gtt ttg ggc atc aaa cga atg att caa att gaa tgg ata aag cct   480
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Pro Val Leu Gly Ile Lys Arg Met Ile Gln Ile Glu Trp Ile Lys Pro
145 150 155 160

gag ttg gcg cct gtt tca tct gat tta aaa tac aca ctt cga ttc agg 528
Glu Leu Ala Pro Val Ser Ser Asp Leu Lys Tyr Thr Leu Arg Phe Arg
165 170 175

aca gtc aac agt acc agc tgg atg gaa gtc aac ttc gct aag aac cgt 576
Thr Val Asn Ser Thr Ser Trp Met Glu Val Asn Phe Ala Lys Asn Arg
180 185 190

aag gat aaa aac caa acg tac aac ctc acg ggg ctg cag cct ttt aca 624
Lys Asp Lys Asn Gln Thr Tyr Asn Leu Thr Gly Leu Gln Pro Phe Thr
195 200 205

gaa tat gtc ata gct ctg cga tgt gcg gtc aag gag tca aag ttc tgg 672
Glu Tyr Val Ile Ala Leu Arg Cys Ala Val Lys Glu Ser Lys Phe Trp
210 215 220

agt gac tgg agc caa gaa aaa atg gga atg act gag gaa gaa gct cca 720
Ser Asp Trp Ser Gln Glu Lys Met Gly Met Thr Glu Glu Glu Ala Pro
225 230 235 240

tgt ggc ctg gaa ctg tgg aga gtc ctg aaa cca gct gag gcg gat gga 768
Cys Gly Leu Glu Leu Trp Arg Val Leu Lys Pro Ala Glu Ala Asp Gly
245 250 255

aga agg cca gtg cgg ttg tta tgg aag aag gca aga gga gcc cca gtc 816
Arg Arg Pro Val Arg Leu Leu Trp Lys Lys Ala Arg Gly Ala Pro Val
260 265 270

cta gag aaa aca ctt ggc tac aac ata tgg tac tat cca gaa agc aac 864
Leu Glu Lys Thr Leu Gly Tyr Asn Ile Trp Tyr Tyr Pro Glu Ser Asn
275 280 285

act aac ctc aca gaa aca atg aac act act aac cag cag ctt gaa ctg 912
Thr Asn Leu Thr Glu Thr Met Asn Thr Thr Asn Gln Gln Leu Glu Leu
290 295 300

cat ctg gga ggc gag agc ttt tgg gtg tct atg att tct tat aat tct 960
His Leu Gly Gly Glu Ser Phe Trp Val Ser Met Ile Ser Tyr Asn Ser
305 310 315 320

ctt ggg aag tct cca gtg gcc acc ctg agg att cca gct att caa gaa 1008
Leu Gly Lys Ser Pro Val Ala Thr Leu Arg Ile Pro Ala Ile Gln Glu
325 330 335

aaa tca ttt cag tgc att gag gtc atg cag gcc tgc gtt gct gag gac 1056
Lys Ser Phe Gln Cys Ile Glu Val Met Gln Ala Cys Val Ala Glu Asp
340 345 350

cag cta gtg gtg aag tgg caa agc tct gct cta gac gtg aac act tgg 1104
Gln Leu Val Val Lys Trp Gln Ser Ser Ala Leu Asp Val Asn Thr Trp
355 360 365

atg att gaa tgg ttt ccg gat gtg gac tca gag ccc acc acc ctt tcc 1152
Met Ile Glu Trp Phe Pro Asp Val Asp Ser Glu Pro Thr Thr Leu Ser
370 375 380

tgg gaa tct gtg tct cag gcc acg aac tgg acg atc cag caa gat aaa 1200
Trp Glu Ser Val Ser Gln Ala Thr Asn Trp Thr Ile Gln Gln Asp Lys
385 390 395 400

tta aaa ccc ttc tgg tgc tat aac atc tct gtg tat cca atg ttg cat 1248
Leu Lys Pro Phe Trp Cys Tyr Asn Ile Ser Val Tyr Pro Met Leu His
405 410 415

gac aaa gtt ggc gag cca tat tcc atc cag gct tat gcc aaa gaa ggc 1296
Asp Lys Val Gly Glu Pro Tyr Ser Ile Gln Ala Tyr Ala Lys Glu Gly
420 425 430

gtt cca tca gaa ggt cct gag acc aag gtg gag aac att ggc gtg aag 1344
Val Pro Ser Glu Gly Pro Glu Thr Lys Val Glu Asn Ile Gly Val Lys
435 440 445

acg gtc acg atc aca tgg aaa gag att ccc aag agt gag aga aag ggt 1392
Thr Val Thr Ile Thr Trp Lys Glu Ile Pro Lys Ser Glu Arg Lys Gly
450 455 460

atc atc tgc aac tac acc atc ttt tac caa gct gaa ggt gga aaa gga 1440
Ile Ile Cys Asn Tyr Thr Ile Phe Tyr Gln Ala Glu Gly Gly Lys Gly
465 470 475 480

ttc tcc aag aca gtc aat tcc agc atc ttg cag tac ggc ctg gag tcc 1488
Phe Ser Lys Thr Val Asn Ser Ser Ile Leu Gln Tyr Gly Leu Glu Ser
485 490 495

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ctg aaa cga aag acc tct tac att gtt cag gtc atg gcc agc acc agt   1536
Leu Lys Arg Lys Thr Ser Tyr Ile Val Gln Val Met Ala Ser Thr Ser
            500                 505                 510

gct ggg gga acc aac ggg acc agc ata aat ttc aag aca ttg tca ttc   1584
Ala Gly Gly Thr Asn Gly Thr Ser Ile Asn Phe Lys Thr Leu Ser Phe
        515                 520                 525

agt gtc ttt gag gag ccc aga tct tca gac aaa act cac aca tgc cca   1632
Ser Val Phe Glu Glu Pro Arg Ser Ser Asp Lys Thr His Thr Cys Pro
    530                 535                 540

ccg tgc cca gca cct gaa gcc gag ggg gca ccg tca gtc ttc ctc ttc   1680
Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe
545                 550                 555                 560

ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc   1728
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
                565                 570                 575

aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc   1776
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
            580                 585                 590

aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg   1824
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
        595                 600                 605

cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc   1872
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
    610                 615                 620

gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc   1920
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
625                 630                 635                 640

tcc aac aaa gcc ctc cca tcc tcc atc gag aaa acc atc tcc aaa gcc   1968
Ser Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
                645                 650                 655

aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg   2016
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
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660 665 670

gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc 2064
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
675 680 685

ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg 2112
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
690 695 700

gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc 2160
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
705 710 715 720

ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag 2208
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
725 730 735

ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac 2256
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
740 745 750

tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa taa 2295
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys *
755 760

<210> 39
<211> 764
<212> PRT
<213> Artificial Sequence

<220>
<223> human zcytor17-Fc4 fusion polypeptide

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Thr Trp Ala Leu Trp Met Leu Pro Ser Leu Cys Lys Phe Ser Leu Ala
20 25 30
Ala Leu Pro Ala Lys Pro Glu Asn Ile Ser Cys Val Tyr Tyr Tyr Arg
35 40 45
Lys Asn Leu Thr Cys Thr Trp Ser Pro Gly Lys Glu Thr Ser Tyr Thr

50 55 60
Gln Tyr Thr Val Lys Arg Thr Tyr Ala Phe Gly Glu Lys His Asp Asn
65 70 75 80
Cys Thr Thr Asn Ser Ser Thr Ser Glu Asn Arg Ala Ser Cys Ser Phe
85 90 95
Phe Leu Pro Arg Ile Thr Ile Pro Asp Asn Tyr Thr Ile Glu Val Glu
100 105 110
Ala Glu Asn Gly Asp Gly Val Ile Lys Ser His Met Thr Tyr Trp Arg
115 120 125
Leu Glu Asn Ile Ala Lys Thr Glu Pro Pro Lys Ile Phe Arg Val Lys
130 135 140
Pro Val Leu Gly Ile Lys Arg Met Ile Gln Ile Glu Trp Ile Lys Pro
145 150 155 160
Glu Leu Ala Pro Val Ser Ser Asp Leu Lys Tyr Thr Leu Arg Phe Arg
165 170 175
Thr Val Asn Ser Thr Ser Trp Met Glu Val Asn Phe Ala Lys Asn Arg
180 185 190
Lys Asp Lys Asn Gln Thr Tyr Asn Leu Thr Gly Leu Gln Pro Phe Thr
195 200 205
Glu Tyr Val Ile Ala Leu Arg Cys Ala Val Lys Glu Ser Lys Phe Trp
210 215 220
Ser Asp Trp Ser Gln Glu Lys Met Gly Met Thr Glu Glu Glu Ala Pro
225 230 235 240
Cys Gly Leu Glu Leu Trp Arg Val Leu Lys Pro Ala Glu Ala Asp Gly
245 250 255
Arg Arg Pro Val Arg Leu Leu Trp Lys Lys Ala Arg Gly Ala Pro Val
260 265 270
Leu Glu Lys Thr Leu Gly Tyr Asn Ile Trp Tyr Tyr Pro Glu Ser Asn
275 280 285
Thr Asn Leu Thr Glu Thr Met Asn Thr Thr Asn Gln Gln Leu Glu Leu
290 295 300
His Leu Gly Gly Glu Ser Phe Trp Val Ser Met Ile Ser Tyr Asn Ser
305 310 315 320
Leu Gly Lys Ser Pro Val Ala Thr Leu Arg Ile Pro Ala Ile Gln Glu
325 330 335
Lys Ser Phe Gln Cys Ile Glu Val Met Gln Ala Cys Val Ala Glu Asp
340 345 350
Gln Leu Val Val Lys Trp Gln Ser Ser Ala Leu Asp Val Asn Thr Trp
355 360 365
Met Ile Glu Trp Phe Pro Asp Val Asp Ser Glu Pro Thr Thr Leu Ser
370 375 380
Trp Glu Ser Val Ser Gln Ala Thr Asn Trp Thr Ile Gln Gln Asp Lys
385 390 395 400

Leu Lys Pro Phe Trp Cys Tyr Asn Ile Ser Val Tyr Pro Met Leu His
405 410 415
Asp Lys Val Gly Glu Pro Tyr Ser Ile Gln Ala Tyr Ala Lys Glu Gly
420 425 430
Val Pro Ser Glu Gly Pro Glu Thr Lys Val Glu Asn Ile Gly Val Lys
435 440 445
Thr Val Thr Ile Thr Trp Lys Glu Ile Pro Lys Ser Glu Arg Lys Gly
450 455 460
Ile Ile Cys Asn Tyr Thr Ile Phe Tyr Gln Ala Glu Gly Gly Lys Gly
465 470 475 480
Phe Ser Lys Thr Val Asn Ser Ser Ile Leu Gln Tyr Gly Leu Glu Ser
485 490 495
Leu Lys Arg Lys Thr Ser Tyr Ile Val Gln Val Met Ala Ser Thr Ser
500 505 510
Ala Gly Gly Thr Asn Gly Thr Ser Ile Asn Phe Lys Thr Leu Ser Phe
515 520 525
Ser Val Phe Glu Glu Pro Arg Ser Ser Asp Lys Thr His Thr Cys Pro
530 535 540
Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe
545 550 555 560
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
565 570 575
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
580 585 590
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
595 600 605
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
610 615 620
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
625 630 635 640
Ser Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
645 650 655
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
660 665 670
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
675 680 685
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
690 695 700
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
705 710 715 720
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
725 730 735
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His

740 745 750
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
755 760

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<210> 63

<211> 18
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<400> 63
ccggatggct gggctgtg 18

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<210> 65
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1 5 10 15

tcc ttg agg act tac cag agt gaa gtc ttg gct gaa cgt tta cca ttg 96
Ser Leu Arg Thr Tyr Gln Ser Glu Val Leu Ala Glu Arg Leu Pro Leu
20 25 30

act cct gta tca ctt aaa gtt tcc acc aat tct acg cgt cag agt ttg 144
Thr Pro Val Ser Leu Lys Val Ser Thr Asn Ser Thr Arg Gln Ser Leu
35 40 45

cac tta caa tgg act gtc cac aac ctt cct tat cat cag gaa ttg aaa 192
His Leu Gln Trp Thr Val His Asn Leu Pro Tyr His Gln Glu Leu Lys
50 55 60

atg gta ttt cag atc cag atc agt agg att gaa aca tcc aat gtc atc 240
Met Val Phe Gln Ile Gln Ile Ser Arg Ile Glu Thr Ser Asn Val Ile
65 70 75 80

tgg gtg ggg aat tac agc acc act gtg aag tgg aac cag gtt ctg cat 288
Trp Val Gly Asn Tyr Ser Thr Thr Val Lys Trp Asn Gln Val Leu His

85 90 95

tgg agc tgg gaa tct gag ctc cct ttg gaa tgt gcc aca cac ttt gta 336
Trp Ser Trp Glu Ser Glu Leu Pro Leu Glu Cys Ala Thr His Phe Val
100 105 110

aga ata aag agt ttg gtg gac gat gcc aag ttc cct gag cca aat ttc 384
Arg Ile Lys Ser Leu Val Asp Asp Ala Lys Phe Pro Glu Pro Asn Phe
115 120 125

tgg agc aac tgg agt tcc tgg gag gaa gtc agt gta caa gat tct act 432
Trp Ser Asn Trp Ser Ser Trp Glu Glu Val Ser Val Gln Asp Ser Thr
130 135 140

gga cag gat ata ttg ttc gtt ttc cct aaa gat aag ctg gtg gaa gaa 480
Gly Gln Asp Ile Leu Phe Val Phe Pro Lys Asp Lys Leu Val Glu Glu
145 150 155 160

ggc acc aat gtt acc att tgt tac gtt tct agg aac att caa aat aat 528
Gly Thr Asn Val Thr Ile Cys Tyr Val Ser Arg Asn Ile Gln Asn Asn
165 170 175

gta tcc tgt tat ttg gaa ggg aaa cag att cat gga gaa caa ctt gat 576
Val Ser Cys Tyr Leu Glu Gly Lys Gln Ile His Gly Glu Gln Leu Asp
180 185 190

cca cat gta act gca ttc aac ttg aat agt gtg cct ttc att agg aat 624
Pro His Val Thr Ala Phe Asn Leu Asn Ser Val Pro Phe Ile Arg Asn
195 200 205

aaa ggg aca aat atc tat tgt gag gca agt caa gga aat gtc agt gaa 672
Lys Gly Thr Asn Ile Tyr Cys Glu Ala Ser Gln Gly Asn Val Ser Glu
210 215 220

ggc atg aaa ggc atc gtt ctt ttt gtc tca aaa gta ctt gag gag ccc 720
Gly Met Lys Gly Ile Val Leu Phe Val Ser Lys Val Leu Glu Glu Pro
225 230 235 240

aag gac ttt tct tgt gaa acc gag gac ttc aag act ttg cac tgt act 768
Lys Asp Phe Ser Cys Glu Thr Glu Asp Phe Lys Thr Leu His Cys Thr
245 250 255

tgg gat cct ggg acg gac act gcc ttg ggg tgg tct aaa caa cct tcc 816

Trp Asp Pro Gly Thr Asp Thr Ala Leu Gly Trp Ser Lys Gln Pro Ser
260 265 270

caa agc tac act tta ttt gaa tca ttt tct ggg gaa aag aaa ctt tgt 864
Gln Ser Tyr Thr Leu Phe Glu Ser Phe Ser Gly Glu Lys Lys Leu Cys
275 280 285

aca cac aaa aac tgg tgt aat tgg caa ata act caa gac tca caa gaa 912
Thr His Lys Asn Trp Cys Asn Trp Gln Ile Thr Gln Asp Ser Gln Glu
290 295 300

acc tat aac ttc aca ctc ata gct gaa aat tac tta agg aag aga agt 960
Thr Tyr Asn Phe Thr Leu Ile Ala Glu Asn Tyr Leu Arg Lys Arg Ser
305 310 315 320

gtc aat atc ctt ttt aac ctg act cat cga gtt tat tta atg aat cct 1008
Val Asn Ile Leu Phe Asn Leu Thr His Arg Val Tyr Leu Met Asn Pro
325 330 335

ttt agt gtc aac ttt gaa aat gta aat gcc aca aat gcc atc atg acc 1056
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340 345 350

tgg aag gtg cac tcc ata agg aat aat ttc aca tat ttg tgt cag att 1104
Trp Lys Val His Ser Ile Arg Asn Asn Phe Thr Tyr Leu Cys Gln Ile
355 360 365

gaa ctc cat ggt gaa gga aaa atg atg caa tac aat gtt tcc atc aag 1152
Glu Leu His Gly Glu Gly Lys Met Met Gln Tyr Asn Val Ser Ile Lys
370 375 380

gtg aac ggt gag tac ttc tta agt gaa ctg gaa cct gcc aca gag tac 1200
Val Asn Gly Glu Tyr Phe Leu Ser Glu Leu Glu Pro Ala Thr Glu Tyr
385 390 395 400

atg gcg cga gta cgg tgt gct gat gcc agc cac ttc tgg aaa tgg agt 1248
Met Ala Arg Val Arg Cys Ala Asp Ala Ser His Phe Trp Lys Trp Ser
405 410 415

gaa tgg agt ggt cag aac ttc acc aca ctt gaa gct gct ccc tca gag 1296
Glu Trp Ser Gly Gln Asn Phe Thr Thr Leu Glu Ala Ala Pro Ser Glu
420 425 430

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gcc cct gat gtc tgg aga att gtg agc ttg gag cca gga aat cat act   1344
Ala Pro Asp Val Trp Arg Ile Val Ser Leu Glu Pro Gly Asn His Thr
        435                 440                 445

gtg acc tta ttc tgg aag cca tta tca aaa ctg cat gcc aat gga aag   1392
Val Thr Leu Phe Trp Lys Pro Leu Ser Lys Leu His Ala Asn Gly Lys
    450                 455                 460

atc ctg ttc tat aat gta gtt gta gaa aac cta gac aaa cca tcc agt   1440
Ile Leu Phe Tyr Asn Val Val Val Glu Asn Leu Asp Lys Pro Ser Ser
465                 470                 475                 480

tca gag ctc cat tcc att cca gca cca gcc aac agc aca aaa cta atc   1488
Ser Glu Leu His Ser Ile Pro Ala Pro Ala Asn Ser Thr Lys Leu Ile
                485                 490                 495

ctt gac agg tgt tcc tac caa atc tgc gtc ata gcc aac aac agt gtg   1536
Leu Asp Arg Cys Ser Tyr Gln Ile Cys Val Ile Ala Asn Asn Ser Val
            500                 505                 510

ggt gct tct cct gct tct gta ata gtc atc tct gca gac ccc gaa aac   1584
Gly Ala Ser Pro Ala Ser Val Ile Val Ile Ser Ala Asp Pro Glu Asn
        515                 520                 525

aaa gag gtt gag gaa gaa aga att gca ggc aca gag ggt gga ttc tct   1632
Lys Glu Val Glu Glu Glu Arg Ile Ala Gly Thr Glu Gly Gly Phe Ser
    530                 535                 540

ctg tct tgg aaa ccc caa cct gga gat gtt ata ggc tat gtt gtg gac   1680
Leu Ser Trp Lys Pro Gln Pro Gly Asp Val Ile Gly Tyr Val Val Asp
545                 550                 555                 560

tgg tgt gac cat acc cag gat gtg ctc ggt gat ttc cag tgg aag aat   1728
Trp Cys Asp His Thr Gln Asp Val Leu Gly Asp Phe Gln Trp Lys Asn
                565                 570                 575

gta ggt ccc aat acc aca agc aca gtc att agc aca gat gct ttt agg   1776
Val Gly Pro Asn Thr Thr Ser Thr Val Ile Ser Thr Asp Ala Phe Arg
            580                 585                 590

cca gga gtt cga tat gac ttc aga att tat ggg tta tct aca aaa agg   1824
Pro Gly Val Arg Tyr Asp Phe Arg Ile Tyr Gly Leu Ser Thr Lys Arg
        595                 600                 605
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att gct tgt tta tta gag aaa aaa aca gga tac tct cag gaa ctt gct   1872
Ile Ala Cys Leu Leu Glu Lys Lys Thr Gly Tyr Ser Gln Glu Leu Ala
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cct tca gac aac cct cac gtg ctg gtg gat aca ttg aca tcc cac tcc   1920
Pro Ser Asp Asn Pro His Val Leu Val Asp Thr Leu Thr Ser His Ser
625                 630                 635                 640

ttc act ctg agt tgg aaa gat tac tct act gaa tct caa cct ggt ttt   1968
Phe Thr Leu Ser Trp Lys Asp Tyr Ser Thr Glu Ser Gln Pro Gly Phe
                645                 650                 655

ata caa ggg tac cat gtc tat ctg aaa tcc aag gcg agg cag tgc cac   2016
Ile Gln Gly Tyr His Val Tyr Leu Lys Ser Lys Ala Arg Gln Cys His
            660                 665                 670

cca cga ttt gaa aag gca gtt ctt tca gat ggt tca gaa tgt tgc aaa   2064
Pro Arg Phe Glu Lys Ala Val Leu Ser Asp Gly Ser Glu Cys Cys Lys
        675                 680                 685

tac aaa att gac aac ccg gaa gaa aag gca ttg att gtg gac aac cta   2112
Tyr Lys Ile Asp Asn Pro Glu Glu Lys Ala Leu Ile Val Asp Asn Leu
    690                 695                 700

aag cca gaa tcc ttc tat gag ttt ttc atc act cca ttc act agt gct   2160
Lys Pro Glu Ser Phe Tyr Glu Phe Phe Ile Thr Pro Phe Thr Ser Ala
705                 710                 715                 720

ggt gaa ggc ccc agt gct acg ttc acg aag gtc acg act ccg gat gaa   2208
Gly Glu Gly Pro Ser Ala Thr Phe Thr Lys Val Thr Thr Pro Asp Glu
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His Ser Ser
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Thr Pro Val Ser Leu Lys Val Ser Thr Asn Ser Thr Arg Gln Ser Leu
35 40 45
His Leu Gln Trp Thr Val His Asn Leu Pro Tyr His Gln Glu Leu Lys
50 55 60
Met Val Phe Gln Ile Gln Ile Ser Arg Ile Glu Thr Ser Asn Val Ile
65 70 75 80
Trp Val Gly Asn Tyr Ser Thr Thr Val Lys Trp Asn Gln Val Leu His
85 90 95
Trp Ser Trp Glu Ser Glu Leu Pro Leu Glu Cys Ala Thr His Phe Val
100 105 110
Arg Ile Lys Ser Leu Val Asp Asp Ala Lys Phe Pro Glu Pro Asn Phe
115 120 125
Trp Ser Asn Trp Ser Ser Trp Glu Glu Val Ser Val Gln Asp Ser Thr
130 135 140
Gly Gln Asp Ile Leu Phe Val Phe Pro Lys Asp Lys Leu Val Glu Glu
145 150 155 160
Gly Thr Asn Val Thr Ile Cys Tyr Val Ser Arg Asn Ile Gln Asn Asn
165 170 175
Val Ser Cys Tyr Leu Glu Gly Lys Gln Ile His Gly Glu Gln Leu Asp
180 185 190
Pro His Val Thr Ala Phe Asn Leu Asn Ser Val Pro Phe Ile Arg Asn
195 200 205
Lys Gly Thr Asn Ile Tyr Cys Glu Ala Ser Gln Gly Asn Val Ser Glu
210 215 220
Gly Met Lys Gly Ile Val Leu Phe Val Ser Lys Val Leu Glu Glu Pro
225 230 235 240
Lys Asp Phe Ser Cys Glu Thr Glu Asp Phe Lys Thr Leu His Cys Thr
245 250 255
Trp Asp Pro Gly Thr Asp Thr Ala Leu Gly Trp Ser Lys Gln Pro Ser
260 265 270
Gln Ser Tyr Thr Leu Phe Glu Ser Phe Ser Gly Glu Lys Lys Leu Cys
275 280 285
Thr His Lys Asn Trp Cys Asn Trp Gln Ile Thr Gln Asp Ser Gln Glu
290 295 300
Thr Tyr Asn Phe Thr Leu Ile Ala Glu Asn Tyr Leu Arg Lys Arg Ser
305 310 315 320
Val Asn Ile Leu Phe Asn Leu Thr His Arg Val Tyr Leu Met Asn Pro
325 330 335

Phe Ser Val Asn Phe Glu Asn Val Asn Ala Thr Asn Ala Ile Met Thr
340 345 350
Trp Lys Val His Ser Ile Arg Asn Asn Phe Thr Tyr Leu Cys Gln Ile
355 360 365
Glu Leu His Gly Glu Gly Lys Met Met Gln Tyr Asn Val Ser Ile Lys
370 375 380
Val Asn Gly Glu Tyr Phe Leu Ser Glu Leu Glu Pro Ala Thr Glu Tyr
385 390 395 400
Met Ala Arg Val Arg Cys Ala Asp Ala Ser His Phe Trp Lys Trp Ser
405 410 415
Glu Trp Ser Gly Gln Asn Phe Thr Thr Leu Glu Ala Ala Pro Ser Glu
420 425 430
Ala Pro Asp Val Trp Arg Ile Val Ser Leu Glu Pro Gly Asn His Thr
435 440 445
Val Thr Leu Phe Trp Lys Pro Leu Ser Lys Leu His Ala Asn Gly Lys
450 455 460
Ile Leu Phe Tyr Asn Val Val Val Glu Asn Leu Asp Lys Pro Ser Ser
465 470 475 480
Ser Glu Leu His Ser Ile Pro Ala Pro Ala Asn Ser Thr Lys Leu Ile
485 490 495
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500 505 510
Gly Ala Ser Pro Ala Ser Val Ile Val Ile Ser Ala Asp Pro Glu Asn
515 520 525
Lys Glu Val Glu Glu Glu Arg Ile Ala Gly Thr Glu Gly Gly Phe Ser
530 535 540
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545 550 555 560
Trp Cys Asp His Thr Gln Asp Val Leu Gly Asp Phe Gln Trp Lys Asn
565 570 575
Val Gly Pro Asn Thr Thr Ser Thr Val Ile Ser Thr Asp Ala Phe Arg
580 585 590
Pro Gly Val Arg Tyr Asp Phe Arg Ile Tyr Gly Leu Ser Thr Lys Arg
595 600 605
Ile Ala Cys Leu Leu Glu Lys Lys Thr Gly Tyr Ser Gln Glu Leu Ala
610 615 620
Pro Ser Asp Asn Pro His Val Leu Val Asp Thr Leu Thr Ser His Ser
625 630 635 640
Phe Thr Leu Ser Trp Lys Asp Tyr Ser Thr Glu Ser Gln Pro Gly Phe
645 650 655
Ile Gln Gly Tyr His Val Tyr Leu Lys Ser Lys Ala Arg Gln Cys His
660 665 670
Pro Arg Phe Glu Lys Ala Val Leu Ser Asp Gly Ser Glu Cys Cys Lys

675 680 685
Tyr Lys Ile Asp Asn Pro Glu Glu Lys Ala Leu Ile Val Asp Asn Leu
690 695 700
Lys Pro Glu Ser Phe Tyr Glu Phe Phe Ile Thr Pro Phe Thr Ser Ala
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His Ser Ser

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agc ctg gca gct ctg cca gct aag cct gag aac att tcc tgt gtc tac 96
Ser Leu Ala Ala Leu Pro Ala Lys Pro Glu Asn Ile Ser Cys Val Tyr
20 25 30

tac tat agg aaa aat tta acc tgc act tgg agt cca gga aag gaa acc 144
Tyr Tyr Arg Lys Asn Leu Thr Cys Thr Trp Ser Pro Gly Lys Glu Thr
35 40 45

agt tat acc cag tac aca gtt aag aga act tac gct ttt gga gaa aaa 192
Ser Tyr Thr Gln Tyr Thr Val Lys Arg Thr Tyr Ala Phe Gly Glu Lys
50 55 60

cat gat aat tgt aca acc aat agt tct aca agt gaa aat cgt gct tcg 240
His Asp Asn Cys Thr Thr Asn Ser Ser Thr Ser Glu Asn Arg Ala Ser
65 70 75 80

tgc tct ttt ttc ctt cca aga ata acg atc cca gat aat tat acc att 288
Cys Ser Phe Phe Leu Pro Arg Ile Thr Ile Pro Asp Asn Tyr Thr Ile

85 90 95

gag gtg gaa gct gaa aat gga gat ggt gta att aaa tct cat atg aca 336
Glu Val Glu Ala Glu Asn Gly Asp Gly Val Ile Lys Ser His Met Thr
100 105 110

tac tgg aga tta gag aac ata gcg aaa act gaa cca cct aag att ttc 384
Tyr Trp Arg Leu Glu Asn Ile Ala Lys Thr Glu Pro Pro Lys Ile Phe
115 120 125

cgt gtg aaa cca gtt ttg ggc atc aaa cga atg att caa att gaa tgg 432
Arg Val Lys Pro Val Leu Gly Ile Lys Arg Met Ile Gln Ile Glu Trp
130 135 140

ata aag cct gag ttg gcg cct gtt tca tct gat tta aaa tac aca ctt 480
Ile Lys Pro Glu Leu Ala Pro Val Ser Ser Asp Leu Lys Tyr Thr Leu
145 150 155 160

cga ttc agg aca gtc aac agt acc agc tgg atg gaa gtc aac ttc gct 528
Arg Phe Arg Thr Val Asn Ser Thr Ser Trp Met Glu Val Asn Phe Ala
165 170 175

aag aac cgt aag gat aaa aac caa acg tac aac ctc acg ggg ctg cag 576
Lys Asn Arg Lys Asp Lys Asn Gln Thr Tyr Asn Leu Thr Gly Leu Gln
180 185 190

cct ttt aca gaa tat gtc ata gct ctg cga tgt gcg gtc aag gag tca 624
Pro Phe Thr Glu Tyr Val Ile Ala Leu Arg Cys Ala Val Lys Glu Ser
195 200 205

aag ttc tgg agt gac tgg agc caa gaa aaa atg gga atg act gag gaa 672
Lys Phe Trp Ser Asp Trp Ser Gln Glu Lys Met Gly Met Thr Glu Glu
210 215 220

gaa gct cca tgt ggc ctg gaa ctg tgg aga gtc ctg aaa cca gct gag 720
Glu Ala Pro Cys Gly Leu Glu Leu Trp Arg Val Leu Lys Pro Ala Glu
225 230 235 240

gcg gat gga aga agg cca gtg cgg ttg tta tgg aag aag gca aga gga 768
Ala Asp Gly Arg Arg Pro Val Arg Leu Leu Trp Lys Lys Ala Arg Gly
245 250 255

gcc cca gtc cta gag aaa aca ctt ggc tac aac ata tgg tac tat cca 816

Ala Pro Val Leu Glu Lys Thr Leu Gly Tyr Asn Ile Trp Tyr Tyr Pro
260 265 270

gaa agc aac act aac ctc aca gaa aca atg aac act act aac cag cag 864
Glu Ser Asn Thr Asn Leu Thr Glu Thr Met Asn Thr Thr Asn Gln Gln
275 280 285

ctt gaa ctg cat ctg gga ggc gag agc ttt tgg gtg tct atg att tct 912
Leu Glu Leu His Leu Gly Gly Glu Ser Phe Trp Val Ser Met Ile Ser
290 295 300

tat aat tct ctt ggg aag tct cca gtg gcc acc ctg agg att cca gct 960
Tyr Asn Ser Leu Gly Lys Ser Pro Val Ala Thr Leu Arg Ile Pro Ala
305 310 315 320

att caa gaa aaa tca ttt cag tgc att gag gtc atg cag gcc tgc gtt 1008
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325 330 335

gct gag gac cag cta gtg gtg aag tgg caa agc tct gct cta gac gtg 1056
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340 345 350

aac act tgg atg att gaa tgg ttt ccg gat gtg gac tca gag ccc acc 1104
Asn Thr Trp Met Ile Glu Trp Phe Pro Asp Val Asp Ser Glu Pro Thr
355 360 365

acc ctt tcc tgg gaa tct gtg tct cag gcc acg aac tgg acg atc cag 1152
Thr Leu Ser Trp Glu Ser Val Ser Gln Ala Thr Asn Trp Thr Ile Gln
370 375 380

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Gln Asp Lys Leu Lys Pro Phe Trp Cys Tyr Asn Ile Ser Val Tyr Pro
385 390 395 400

atg ttg cat gac aaa gtt ggc gag cca tat tcc atc cag gct tat gcc 1248
Met Leu His Asp Lys Val Gly Glu Pro Tyr Ser Ile Gln Ala Tyr Ala
405 410 415

aaa gaa ggc gtt cca tca gaa ggt cct gag acc aag gtg gag aac att 1296
Lys Glu Gly Val Pro Ser Glu Gly Pro Glu Thr Lys Val Glu Asn Ile
420 425 430

ggc gtg aag acg gtc acg atc aca tgg aaa gag att ccc aag agt gag 1344
Gly Val Lys Thr Val Thr Ile Thr Trp Lys Glu Ile Pro Lys Ser Glu
435 440 445

aga aag ggt atc atc tgc aac tac acc atc ttt tac caa gct gaa ggt 1392
Arg Lys Gly Ile Ile Cys Asn Tyr Thr Ile Phe Tyr Gln Ala Glu Gly
450 455 460

gga aaa gga ttc tcc aag aca gtc aat tcc agc atc ttg cag tac ggc 1440
Gly Lys Gly Phe Ser Lys Thr Val Asn Ser Ser Ile Leu Gln Tyr Gly
465 470 475 480

ctg gag tcc ctg aaa cga aag acc tct tac att gtt cag gtc atg gcc 1488
Leu Glu Ser Leu Lys Arg Lys Thr Ser Tyr Ile Val Gln Val Met Ala
485 490 495

agc acc agt gct ggg gga acc aac ggg acc agc ata aat ttc aag aca 1536
Ser Thr Ser Ala Gly Gly Thr Asn Gly Thr Ser Ile Asn Phe Lys Thr
500 505 510

ttg tca ttc agt gtc ttt gag 1557
Leu Ser Phe Ser Val Phe Glu
515

<210> 71
<211> 519
<212> PRT
<213> Homo sapiens

<400> 71
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1 5 10 15
Ser Leu Ala Ala Leu Pro Ala Lys Pro Glu Asn Ile Ser Cys Val Tyr
20 25 30
Tyr Tyr Arg Lys Asn Leu Thr Cys Thr Trp Ser Pro Gly Lys Glu Thr
35 40 45
Ser Tyr Thr Gln Tyr Thr Val Lys Arg Thr Tyr Ala Phe Gly Glu Lys
50 55 60
His Asp Asn Cys Thr Thr Asn Ser Ser Thr Ser Glu Asn Arg Ala Ser
65 70 75 80
Cys Ser Phe Phe Leu Pro Arg Ile Thr Ile Pro Asp Asn Tyr Thr Ile
85 90 95

Glu Val Glu Ala Glu Asn Gly Asp Gly Val Ile Lys Ser His Met Thr
100 105 110
Tyr Trp Arg Leu Glu Asn Ile Ala Lys Thr Glu Pro Pro Lys Ile Phe
115 120 125
Arg Val Lys Pro Val Leu Gly Ile Lys Arg Met Ile Gln Ile Glu Trp
130 135 140
Ile Lys Pro Glu Leu Ala Pro Val Ser Ser Asp Leu Lys Tyr Thr Leu
145 150 155 160
Arg Phe Arg Thr Val Asn Ser Thr Ser Trp Met Glu Val Asn Phe Ala
165 170 175
Lys Asn Arg Lys Asp Lys Asn Gln Thr Tyr Asn Leu Thr Gly Leu Gln
180 185 190
Pro Phe Thr Glu Tyr Val Ile Ala Leu Arg Cys Ala Val Lys Glu Ser
195 200 205
Lys Phe Trp Ser Asp Trp Ser Gln Glu Lys Met Gly Met Thr Glu Glu
210 215 220
Glu Ala Pro Cys Gly Leu Glu Leu Trp Arg Val Leu Lys Pro Ala Glu
225 230 235 240
Ala Asp Gly Arg Arg Pro Val Arg Leu Leu Trp Lys Lys Ala Arg Gly
245 250 255
Ala Pro Val Leu Glu Lys Thr Leu Gly Tyr Asn Ile Trp Tyr Tyr Pro
260 265 270
Glu Ser Asn Thr Asn Leu Thr Glu Thr Met Asn Thr Thr Asn Gln Gln
275 280 285
Leu Glu Leu His Leu Gly Gly Glu Ser Phe Trp Val Ser Met Ile Ser
290 295 300
Tyr Asn Ser Leu Gly Lys Ser Pro Val Ala Thr Leu Arg Ile Pro Ala
305 310 315 320
Ile Gln Glu Lys Ser Phe Gln Cys Ile Glu Val Met Gln Ala Cys Val
325 330 335
Ala Glu Asp Gln Leu Val Val Lys Trp Gln Ser Ser Ala Leu Asp Val
340 345 350
Asn Thr Trp Met Ile Glu Trp Phe Pro Asp Val Asp Ser Glu Pro Thr
355 360 365
Thr Leu Ser Trp Glu Ser Val Ser Gln Ala Thr Asn Trp Thr Ile Gln
370 375 380
Gln Asp Lys Leu Lys Pro Phe Trp Cys Tyr Asn Ile Ser Val Tyr Pro
385 390 395 400
Met Leu His Asp Lys Val Gly Glu Pro Tyr Ser Ile Gln Ala Tyr Ala
405 410 415
Lys Glu Gly Val Pro Ser Glu Gly Pro Glu Thr Lys Val Glu Asn Ile
420 425 430
Gly Val Lys Thr Val Thr Ile Thr Trp Lys Glu Ile Pro Lys Ser Glu

435 440 445

Arg Lys Gly Ile Ile Cys Asn Tyr Thr Ile Phe Tyr Gln Ala Glu Gly
450 455 460

Gly Lys Gly Phe Ser Lys Thr Val Asn Ser Ser Ile Leu Gln Tyr Gly
465 470 475 480

Leu Glu Ser Leu Lys Arg Lys Thr Ser Tyr Ile Val Gln Val Met Ala
485 490 495

Ser Thr Ser Ala Gly Gly Thr Asn Gly Thr Ser Ile Asn Phe Lys Thr
500 505 510

Leu Ser Phe Ser Val Phe Glu
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<210> 72
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> C-Terminal His peptide tag

<400> 72
Gly Ser Gly Gly His His His His His His
1 5 10

<210> 73
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Gly-Ser spacer of 12 amino acids

<400> 73
Gly Ser Gly Ser Gly Ser Gly Ser Glu Pro Arg Ser
1 5 10

<210> 74
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC41557

<400> 74
ttatagatct cgaggagtgt tcatccggag t 31

<210> 75
<211> 65
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29232

<400> 75
cgactgactc gagtcagtga tggtgatggt gatggccacc tgatccttta cccggagaca 60
gggag 65

<210> 76
<211> 3196
<212> DNA
<213> Mus musculus

<400> 76
tgtatgtctg cacagtttgt gtatgcctgg tgcccacaga ggctcgagag tgtcagattc 60
cccccaaaac tggagttaca gttttgagcc gccccatgct tgatagcaat caaacctggg 120
tcctctgaaa gagcatccag tgcatgtaac cactgaacca tctctccaaa ccatgaacat 180
cactttaatt ttttttaata gttaaaggat attttgattc taaagatgta aaagaacgtc 240
tcacctattt tgaaatttgg taataaatgt ttcttcaaag cttaaaaaaa ttagttcagg 300
ttttttttt ttttcagtca gtgatttgct aagctgccca aactggctta gaatttgtga 360
ccctcttgtc tcagcatact gagtgttaag attacaagtg cacccccctac ccagttccca 420
taattaactg atccaccccc acccccatcc caccccactc ccattgcctg ggcaagtaac 480
tcttgagccc cattctggtt ctagagtctg aagtcacaaa ggtgcaggtg agaacgcaag 540
gacaagggca ggccctggag cacagatgcc ttctccttat gccttccctg tgttcactag 600
agccatcccc ctgcctccgg aattcccaca gatggatcgc tctgtggctt cttaaaactt 660
ccctgcaggg cactgaccct cagcccctct aagtcacttc ttccccagtg attgtacttt 720
tcaatcgggc ttcaaacttt cctctcatta aatcagcaag cactttccaa gaaaagagag 780
atgctcaaga tgccttcctg tgtggtatgt gtatgcgttt gtgtgtgtgc acgcatgtgt 840
gtgcatgtga ctcaatcttc tgccttgcct tgagggtaac ctcagcattt ccttccagcc 900
ctgctttccc caggccgagc cgaggctggc aacctttga aaatgtttc tggagaaaag 960
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atattgcctg gtggccatgg cgaacacacc aggctccaga gaccacaggc aaagcgggcc 1080

ttcctcactc tcttaccgtc gccatgatct tccacacagg taccgctggc tccacacgca 1140
gctcagcatg gcttcagctc catggctctt atcatgttag gggaaggagc cgggaatggc 1200
tgcctcaggt ggttgctgga cagaggctgt tgtaactgaa gctgggatgg gcaggggcat 1260
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tgctgctttg ctgtatagga acctggctgg ccacctgcag cttgtccttc ggtgccccaa 1380
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ataacaacta tgtaagtgcc cttgagattg ttttttctta accatttctt taaaatgtct 1500
tattttgcta tctaagcaca gctatccttt ctcgatataa agccagctat ggaagccaga 1560
gaggcatggg gaaacattgg aattcggttg gggtgaaatg tttccaaggg ggtaaatgca 1620
ctagcagaag aggcagaggc agactggtcc agggactgaa accttggcag cttacgaaac 1680
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cccccaagag caatgagagc cagtttcagc agccaaagat gtcctagtgg aagcagggtg 1860
tgaggatctt cctttgggtc tccgttgact aactaggcaa ctgtctgtgt gttcttggag 1920
catcctggag ggccctctgc ctggccagag cctggcacag gtacagcaca ggacccagaa 1980
agtgtgaata cttcatttcc ttgggaccgc ttagataact tcagttgaag caagtaacag 2040
ggaaactgat ggagacacag ataacctccc tgcccctctc acttcagtca ctgagcctcc 2100
gagaacaggt tgcagatggc taggggcagc ctcagccaga taggcggagg cagactgggt 2160
agaagcatcc ttaggaacca cggccaacct gggtgggtat gccatgtctt ctagctcata 2220
agccaactag accttcgatt cctgtagaca cagagttagt gatggcccaa gcttcagaag 2280
gttgttgtac caattagata aggtctgagg caggctagac acagaggaag ccctggaaat 2340
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gaaagtcaaa gtgttgagcg agaacacagt agatacttct tgggtgataa gatggctaac 2580
aaacatcagc tgtttcaacc cactgaattt aaacatttct gtgcctggaa atactgatga 2640
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agaactgcag gctaaggaca atactacatg ctgagtgatg gggggggggg ggtgcagtgt 2760
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tctgctaccc tgtagggact ggaagtggca gctatattta tttatttatg tactgagttt 2940
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ataatcagtt ttggaatttg tgatggggtt gaaatcaaag attaggaatg ttctggaaat 3060
agtttatgct accctctccc tccattagac agactcatga gcaaataatc ccagcagcat 3120
cacgtgcatg ataaacatct ttgttccagg tcataagtac aatcactgtc cttttggtat 3180
gtaggctgga aactaa 3196

<210> 77
<211> 24
<212> DNA
<213> Artificial Sequence

<220>

<223> Oligonucleotide primer ZC28575

<400> 77
ccaggaaagg aaaccagtta tacc 24

<210> 78
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC21195

<400> 78
gaggagacca taacccccga cag 23

<210> 79
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC21196

<400> 79
catagctccc accacacgat ttt 23

<210> 80
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC26358

<400> 80
aaaaccaaac gtacaacctc acggg 25

<210> 81
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC26359

<400> 81
gagcagccat acaccagagc agaca 25

<210> 82
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC29179

<400> 82
gcagggttgg gaacggtgg 19

<210> 83
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC28917

<400> 83
tgcaagatgc tggaattgac 20

<210> 84
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC28916

<400> 84
agtcaattcc agcatcttgc 20

<210> 85
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC28918

<400> 85
tcacagagtc atcagactcc 20

<210> 86
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC41498

<400> 86
ggctccagag accacagg 18

<210> 87
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC41496

<400> 87
atgactagta atgaccgcac ag 22

<210> 88
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC41583

<400> 88
cgtacgggcc ggccaccatg atcttccaca caggaacaa 39

<210> 89
<211> 36
<212> DNA

<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC41584

<400> 89
tgacgaggcg cgcctcagca tgtagtattg tcctta 36

<210> 90
<211> 650
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (53)...(542)

<400> 90
ggctccagag accacaggca aagcgggcct tcctcactct cttaccgtcg cc atg atc 58
Met Ile
1

ttc cac aca gga aca acg aag cct acc ctg gtg ctg ctt tgc tgt ata 106
Phe His Thr Gly Thr Thr Lys Pro Thr Leu Val Leu Leu Cys Cys Ile
5 10 15

gga acc tgg ctg gcc acc tgc agc ttg tcc ttc ggt gcc cca ata tcg 154
Gly Thr Trp Leu Ala Thr Cys Ser Leu Ser Phe Gly Ala Pro Ile Ser
20 25 30

aag gaa gac tta aga act aca att gac ctc ttg aaa caa gag tct cag 202
Lys Glu Asp Leu Arg Thr Thr Ile Asp Leu Leu Lys Gln Glu Ser Gln
35 40 45 50

gat ctt tat aac aac tat agc ata aag cag gca tct ggg atg tca gca 250
Asp Leu Tyr Asn Asn Tyr Ser Ile Lys Gln Ala Ser Gly Met Ser Ala
55 60 65

gac gaa tca ata cag ctg ccg tgt ttc agc ctg gac cgg gaa gca tta 298
Asp Glu Ser Ile Gln Leu Pro Cys Phe Ser Leu Asp Arg Glu Ala Leu
70 75 80

acc aac atc tcg gtc atc ata gca cat ctg gag aaa gtc aaa gtg ttg 346

Thr Asn Ile Ser Val Ile Ile Ala His Leu Glu Lys Val Lys Val Leu
85 90 95

agc gag aac aca gta gat act tct tgg gtg ata aga tgg cta aca aac 394
Ser Glu Asn Thr Val Asp Thr Ser Trp Val Ile Arg Trp Leu Thr Asn
100 105 110

atc agc tgt ttc aac cca ctg aat tta aac att tct gtg cct gga aat 442
Ile Ser Cys Phe Asn Pro Leu Asn Leu Asn Ile Ser Val Pro Gly Asn
115 120 125 130

act gat gaa tcc tat gat tgt aaa gtg ttc gtg ctt acg gtt tta aag 490
Thr Asp Glu Ser Tyr Asp Cys Lys Val Phe Val Leu Thr Val Leu Lys
135 140 145

cag ttc tca aac tgc atg gca gaa ctg cag gct aag gac aat act aca 538
Gln Phe Ser Asn Cys Met Ala Glu Leu Gln Ala Lys Asp Asn Thr Thr
150 155 160

tgc t gagtgatggg gggggggtgc agtgtcctca gcagtgcctg tccttcgagg 592
Cys

gctgagcttg caacccagga cttaactcca aagggactgt gcggtcatta ctagtcat 650

<210> 91
<211> 163
<212> PRT
<213> Mus musculus

<400> 91
Met Ile Phe His Thr Gly Thr Thr Lys Pro Thr Leu Val Leu Leu Cys
1 5 10 15
Cys Ile Gly Thr Trp Leu Ala Thr Cys Ser Leu Ser Phe Gly Ala Pro
20 25 30
Ile Ser Lys Glu Asp Leu Arg Thr Thr Ile Asp Leu Leu Lys Gln Glu
35 40 45
Ser Gln Asp Leu Tyr Asn Asn Tyr Ser Ile Lys Gln Ala Ser Gly Met
50 55 60
Ser Ala Asp Glu Ser Ile Gln Leu Pro Cys Phe Ser Leu Asp Arg Glu
65 70 75 80
Ala Leu Thr Asn Ile Ser Val Ile Ile Ala His Leu Glu Lys Val Lys
85 90 95

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

<210> 92
<211> 511
<212> DNA
<213> Artificial Sequence

<220>
<223> Cloned murine cDNA sequence (SEQ ID NO:90) w/ FseI and AscI restriction sites and a partial Kozak sequence to the mcytor17lig open reading frame and termination codon

<400> 92
ggccggccac catgatcttc cacacaggaa caacgaagcc taccctggtg ctgctttgct 60
gtataggaac ctggctggcc acctgcagct tgtccttcgg tgccccaata tcgaaggaag 120
acttaagaac tacaattgac ctcttgaaac aagagtctca ggatctttat aacaactata 180
gcataaagca ggcatctggg atgtcagcag acgaatcaat acagctgccg tgtttcagcc 240
tggaccggga agcattaacc aacatctcgg tcatcatagc acatctggag aaagtcaaag 300
tgttgagcga gaacacagta gatacttctt gggtgataag atggctaaca aacatcagct 360
gtttcaaccc actgaattta aacatttctg tgcctggaaa tactgatgaa tcctatgatt 420
gtaaagtgtt cgtgcttacg gttttaaagc agttctcaaa ctgcatggca gaactgcagg 480
ctaaggacaa tactacatgc tgaggcgcgc c 511

<210> 93
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC41438

<400> 93

gccatggcct ctcactcagg c 21

<210> 94
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC41437

<400> 94
ccagggagca ttgacaactc ttag 24

<210> 95
<211> 516
<212> DNA
<213> Artificial Sequence

<220>
<223> human zCytor17Lig-CEE polynucleotide

<221> CDS
<222> (1)...(516)

<400> 95
atg gcc tct cac tca ggc ccc tcg acg tct gtg ctc ttt ctg ttc tgc 48
Met Ala Ser His Ser Gly Pro Ser Thr Ser Val Leu Phe Leu Phe Cys
1 5 10 15

tgc ctg gga ggc tgg ctg gcc tcc cac acg ttg ccc gtc cgt tta cta 96
Cys Leu Gly Gly Trp Leu Ala Ser His Thr Leu Pro Val Arg Leu Leu
20 25 30

cga cca agt gat gat gta cag aaa ata gtc gag gaa tta cag tcc ctc 144
Arg Pro Ser Asp Asp Val Gln Lys Ile Val Glu Glu Leu Gln Ser Leu
35 40 45

tcg aag atg ctt ttg aaa gat gtg gag gaa gag aag ggc gtg ctc gtg 192
Ser Lys Met Leu Leu Lys Asp Val Glu Glu Glu Lys Gly Val Leu Val
50 55 60

tcc cag aat tac acg ctg ccg tgt ctc agc cct gac gcc cag ccg cca 240
Ser Gln Asn Tyr Thr Leu Pro Cys Leu Ser Pro Asp Ala Gln Pro Pro

65 70 75 80

aac aac atc cac agc cca gcc atc cgg gca tat ctc aag aca atc aga 288
Asn Asn Ile His Ser Pro Ala Ile Arg Ala Tyr Leu Lys Thr Ile Arg
85 90 95

cag cta gac aac aaa tct gtt att gat gag atc ata gag cac ctc gac 336
Gln Leu Asp Asn Lys Ser Val Ile Asp Glu Ile Ile Glu His Leu Asp
100 105 110

aaa ctc ata ttt caa gat gca cca gaa aca aac att tct gtg cca aca 384
Lys Leu Ile Phe Gln Asp Ala Pro Glu Thr Asn Ile Ser Val Pro Thr
115 120 125

gac acc cat gaa tgt aaa cgc ttc atc ctg act att tct caa cag ttt 432
Asp Thr His Glu Cys Lys Arg Phe Ile Leu Thr Ile Ser Gln Gln Phe
130 135 140

tca gag tgc atg gac ctc gca cta aaa tca ttg acc tct gga gcc caa 480
Ser Glu Cys Met Asp Leu Ala Leu Lys Ser Leu Thr Ser Gly Ala Gln
145 150 155 160

cag gcc acc act gaa gaa tac atg ccg atg gaa taa 516
Gln Ala Thr Thr Glu Glu Tyr Met Pro Met Glu *
165 170

<210> 96
<211> 171
<212> PRT
<213> Artificial Sequence

<220>
<223> human zCytor17Lig-CEE polypeptide

<400> 96
Met Ala Ser His Ser Gly Pro Ser Thr Ser Val Leu Phe Leu Phe Cys
1 5 10 15
Cys Leu Gly Gly Trp Leu Ala Ser His Thr Leu Pro Val Arg Leu Leu
20 25 30
Arg Pro Ser Asp Asp Val Gln Lys Ile Val Glu Glu Leu Gln Ser Leu
35 40 45
Ser Lys Met Leu Leu Lys Asp Val Glu Glu Glu Lys Gly Val Leu Val

50 55 60
Ser Gln Asn Tyr Thr Leu Pro Cys Leu Ser Pro Asp Ala Gln Pro Pro
65 70 75 80
Asn Asn Ile His Ser Pro Ala Ile Arg Ala Tyr Leu Lys Thr Ile Arg
85 90 95
Gln Leu Asp Asn Lys Ser Val Ile Asp Glu Ile Ile Glu His Leu Asp
100 105 110
Lys Leu Ile Phe Gln Asp Ala Pro Glu Thr Asn Ile Ser Val Pro Thr
115 120 125
Asp Thr His Glu Cys Lys Arg Phe Ile Leu Thr Ile Ser Gln Gln Phe
130 135 140
Ser Glu Cys Met Asp Leu Ala Leu Lys Ser Leu Thr Ser Gly Ala Gln
145 150 155 160
Gln Ala Thr Thr Glu Glu Tyr Met Pro Met Glu
165 170

<210> 97
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZCZC41607

<400> 97
tccagggaat tcatataggc cggccaccat ggcctctcac tcaggcccc 49

<210> 98
<211> 82
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZCZC41605

<400> 98
caaccccaga gctgttttaa ggcgcgcctc tagattatta ttccatcggc atgtattctt 60
cagtggtggc ctgttgggct cc 82

<210> 99
<211> 629
<212> DNA

<213> Mus musculus

<220>
<221> CDS
<222> (28)...(528)

<400> 99

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aacccttgg aggaccagaa cgagaca atg gtt ctt gcc agc tct acc acc agc 54
                               Met Val Leu Ala Ser Ser Thr Thr Ser
                                1               5

atc cac acc atg ctg ctc ctg ctc ctg atg ctc ttc cac ctg gga ctc  102
Ile His Thr Met Leu Leu Leu Leu Leu Met Leu Phe His Leu Gly Leu
 10                  15                  20                  25

caa gct tca atc agt ggc cgg gat acc cac cgt tta acc aga acg ttg  150
Gln Ala Ser Ile Ser Gly Arg Asp Thr His Arg Leu Thr Arg Thr Leu
                30                  35                  40

aat tgc agc tct att gtc aag gag att ata ggg aag ctc cca gaa cct  198
Asn Cys Ser Ser Ile Val Lys Glu Ile Ile Gly Lys Leu Pro Glu Pro
            45                  50                  55

gaa ctc aaa act gat gat gaa gga ccc tct ctg agg aat aag agc ttt  246
Glu Leu Lys Thr Asp Asp Glu Gly Pro Ser Leu Arg Asn Lys Ser Phe
        60                  65                  70

cgg aga gta aac ctg tcc aaa ttc gtg gaa agc caa gga gaa gtg gat  294
Arg Arg Val Asn Leu Ser Lys Phe Val Glu Ser Gln Gly Glu Val Asp
    75                  80                  85

cct gag gac aga tac gtt atc aag tcc aat ctt cag aaa ctt aac tgt  342
Pro Glu Asp Arg Tyr Val Ile Lys Ser Asn Leu Gln Lys Leu Asn Cys
90                  95                  100                 105

tgc ctg cct aca tct gcg aat gac tct gcg ctg cca ggg gtc ttc att  390
Cys Leu Pro Thr Ser Ala Asn Asp Ser Ala Leu Pro Gly Val Phe Ile
                110                 115                 120

cga gat ctg gat gac ttt cgg aag aaa ctg aga ttc tac atg gtc cac  438
Arg Asp Leu Asp Asp Phe Arg Lys Lys Leu Arg Phe Tyr Met Val His
            125                 130                 135
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ctt aac gat ctg gag aca gtg cta acc tct aga cca cct cag ccc gca   486
Leu Asn Asp Leu Glu Thr Val Leu Thr Ser Arg Pro Pro Gln Pro Ala
        140                 145                 150

tct ggc tcc gtc tct cct aac cgt gga acc gtg gaa tgt taa           528
Ser Gly Ser Val Ser Pro Asn Arg Gly Thr Val Glu Cys  *
    155                 160                 165

aacagcaggc agagcaccta aagtctgaat gttcctcatg gcccatggtc aaaaggattt 588
tacattcctt tatgccatca aatgtcttat caatttatct a                     629
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<210> 100
<211> 166
<212> PRT
<213> Mus musculus

<400> 100

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Met Val Leu Ala Ser Ser Thr Thr Ser Ile His Thr Met Leu Leu Leu
 1               5                  10                  15
Leu Leu Met Leu Phe His Leu Gly Leu Gln Ala Ser Ile Ser Gly Arg
            20                  25                  30
Asp Thr His Arg Leu Thr Arg Thr Leu Asn Cys Ser Ser Ile Val Lys
        35                  40                  45
Glu Ile Ile Gly Lys Leu Pro Glu Pro Glu Leu Lys Thr Asp Asp Glu
    50                  55                  60
Gly Pro Ser Leu Arg Asn Lys Ser Phe Arg Arg Val Asn Leu Ser Lys
65                  70                  75                  80
Phe Val Glu Ser Gln Gly Glu Val Asp Pro Glu Asp Arg Tyr Val Ile
                85                  90                  95
Lys Ser Asn Leu Gln Lys Leu Asn Cys Cys Leu Pro Thr Ser Ala Asn
            100                 105                 110
Asp Ser Ala Leu Pro Gly Val Phe Ile Arg Asp Leu Asp Asp Phe Arg
        115                 120                 125
Lys Lys Leu Arg Phe Tyr Met Val His Leu Asn Asp Leu Glu Thr Val
    130                 135                 140
Leu Thr Ser Arg Pro Pro Gln Pro Ala Ser Gly Ser Val Ser Pro Asn
145                 150                 155                 160
Arg Gly Thr Val Glu Cys
                165
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<210> 101
<211> 674

<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (10)...(464)

<400> 101

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gatccaaac atg agc cgc ctg ccc gtc ctg ctc ctg ctc caa ctc ctg gtc  51
          Met Ser Arg Leu Pro Val Leu Leu Leu Leu Gln Leu Leu Val
           1               5                  10

cgc ccc gga ctc caa gct ccc atg acc cag aca acg tcc ttg aag aca   99
Arg Pro Gly Leu Gln Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr
 15                  20                  25                  30

agc tgg gtt aac tgc tct aac atg atc gat gaa att ata aca cac tta  147
Ser Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu
                 35                  40                  45

aag cag cca cct ttg cct ttg ctg gac ttc aac aac ctc aat ggg gaa  195
Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu
             50                  55                  60

gac caa gac att ctg atg gaa aat aac ctt cga agg cca aac ctg gag  243
Asp Gln Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu
         65                  70                  75

gca ttc aac agg gct gtc aag agt tta cag aac gca tca gca att gag  291
Ala Phe Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu
     80                  85                  90

agc att ctt aaa aat ctc ctg cca tgt ctg ccc ctg gcc acg gcc gca  339
Ser Ile Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala
 95                 100                 105                 110

ccc acg cga cat cca atc cat atc aag gac ggt gac tgg aat gaa ttc  387
Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe
                115                 120                 125

cgg agg aaa ctg acg ttc tat ctg aaa acc ctt gag aat gcg cag gct  435
Arg Arg Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala
            130                 135                 140
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caa cag acg act ttg agc ctc gcg atc tt ttagtccaac gtccagctcg 484
Gln Gln Thr Thr Leu Ser Leu Ala Ile
145 150

ttctctgggc cttctcacca cagcgcctcg ggacatcaaa aacagcagaa cttctgaaac 544
ctctgggtca tctctcacac attccaggac cagaagcatt tcacctttc ctgcggcatc 604
agatgaattg ttaattatct aatttctgaa atgtgcagct cccatttggc cttgtgcggt 664
tgtgttctca 674

<210> 102
<211> 151
<212> PRT
<213> Homo sapiens

<400> 102
Met Ser Arg Leu Pro Val Leu Leu Leu Leu Gln Leu Leu Val Arg Pro
1 5 10 15
Gly Leu Gln Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp
20 25 30
Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln
35 40 45
Pro Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln
50 55 60
Asp Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe
65 70 75 80
Asn Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile
85 90 95
Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr
100 105 110
Arg His Pro Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg
115 120 125
Lys Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln
130 135 140
Thr Thr Leu Ser Leu Ala Ile
145 150

<210> 103
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Alternative Glu-Glu (CEE) peptide tag without Gly-Ser residue pair

<400> 103
Glu Glu Tyr Met Pro Met Glu
1 5

<210> 104
<211> 513
<212> DNA
<213> Artificial Sequence

<220>
<223> Mouse zCytor17Lig(m)-CEE polynucleotide

<221> CDS
<222> (1)...(513)

<400> 104
atg atc ttc cac aca gga aca acg aag cct acc ctg gtg ctg ctt tgc 48
Met Ile Phe His Thr Gly Thr Thr Lys Pro Thr Leu Val Leu Leu Cys
1 5 10 15

tgt ata gga acc tgg ctg gcc acc tgc agc ttg tcc ttc ggt gcc cca 96
Cys Ile Gly Thr Trp Leu Ala Thr Cys Ser Leu Ser Phe Gly Ala Pro
20 25 30

ata tcg aag gaa gac tta aga act aca att gac ctc ttg aaa caa gag 144
Ile Ser Lys Glu Asp Leu Arg Thr Thr Ile Asp Leu Leu Lys Gln Glu
35 40 45

tct cag gat ctt tat aac aac tat agc ata aag cag gca tct ggg atg 192
Ser Gln Asp Leu Tyr Asn Asn Tyr Ser Ile Lys Gln Ala Ser Gly Met
50 55 60

tca gca gac gaa tca ata cag ctg ccg tgt ttc agc ctg gac cgg gaa 240
Ser Ala Asp Glu Ser Ile Gln Leu Pro Cys Phe Ser Leu Asp Arg Glu
65 70 75 80

gca tta acc aac atc tcg gtc atc ata gca cat ctg gag aaa gtc aaa 288
Ala Leu Thr Asn Ile Ser Val Ile Ile Ala His Leu Glu Lys Val Lys

85 90 95

gtg ttg agc gag aac aca gta gat act tct tgg gtg ata aga tgg cta 336
Val Leu Ser Glu Asn Thr Val Asp Thr Ser Trp Val Ile Arg Trp Leu
100 105 110

aca aac atc agc tgt ttc aac cca ctg aat tta aac att tct gtg cct 384
Thr Asn Ile Ser Cys Phe Asn Pro Leu Asn Leu Asn Ile Ser Val Pro
115 120 125

gga aat act gat gaa tcc tat gat tgt aaa gtg ttc gtg ctt acg gtt 432
Gly Asn Thr Asp Glu Ser Tyr Asp Cys Lys Val Phe Val Leu Thr Val
130 135 140

tta aag cag ttc tca aac tgc atg gca gaa ctg cag gct aag gac aat 480
Leu Lys Gln Phe Ser Asn Cys Met Ala Glu Leu Gln Ala Lys Asp Asn
145 150 155 160

act aca tgc gaa gaa tac atg ccg atg gaa tga 513
Thr Thr Cys Glu Glu Tyr Met Pro Met Glu *
165 170

<210> 105
<211> 170
<212> PRT
<213> Artificial Sequence

<220>
<223> Mouse zCytor17Lig(m)-CEE polypeptide

<400> 105
Met Ile Phe His Thr Gly Thr Thr Lys Pro Thr Leu Val Leu Leu Cys
1 5 10 15
Cys Ile Gly Thr Trp Leu Ala Thr Cys Ser Leu Ser Phe Gly Ala Pro
20 25 30
Ile Ser Lys Glu Asp Leu Arg Thr Thr Ile Asp Leu Leu Lys Gln Glu
35 40 45
Ser Gln Asp Leu Tyr Asn Asn Tyr Ser Ile Lys Gln Ala Ser Gly Met
50 55 60
Ser Ala Asp Glu Ser Ile Gln Leu Pro Cys Phe Ser Leu Asp Arg Glu
65 70 75 80
Ala Leu Thr Asn Ile Ser Val Ile Ile Ala His Leu Glu Lys Val Lys

85 90 95
Val Leu Ser Glu Asn Thr Val Asp Thr Ser Trp Val Ile Arg Trp Leu
100 105 110
Thr Asn Ile Ser Cys Phe Asn Pro Leu Asn Leu Asn Ile Ser Val Pro
115 120 125
Gly Asn Thr Asp Glu Ser Tyr Asp Cys Lys Val Phe Val Leu Thr Val
130 135 140
Leu Lys Gln Phe Ser Asn Cys Met Ala Glu Leu Gln Ala Lys Asp Asn
145 150 155 160
Thr Thr Cys Glu Glu Tyr Met Pro Met Glu
165 170

<210> 106
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC41643

<400> 106
tccagggaat tcatataggc cggccaccat gatcttccac acaggaaca 49

<210> 107
<211> 85
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC41641

<400> 107
caaccccaga gctgttttaa ggcgcgcctc tagattatca ttccatcggc atgtattctt 60
cgcatgtagt attgtcctta gcctg 85

<210> 108
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC38,239

<400> 108
gccgactaag ccagagaac 19

<210> 109
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC38,245

<400> 109
ctgttgacag ttctgaaccg 20

<210> 110
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC38,238

<400> 110
cgcggtttcc attgtatctg 20

<210> 111
<211> 2748
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (237)...(2222)

<400> 111
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ttcatctggt catgctgaat atactctcaa gatgtgctgg agaaggtgct gctgtccggg 120
ctctcagaga aggcagtgct ggaggcgttc ctggcccggg tctcctccta ctgttcctgg 180
tagcccagcc ttctcggggt ggaaggagaa gctggccagg tgagctctga ggaagc atg 239
Met
1

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ctg agc agc cag aag gga tcc tgc agc cag gaa cca ggg gca gcc cac   287
Leu Ser Ser Gln Lys Gly Ser Cys Ser Gln Glu Pro Gly Ala Ala His
                5                  10                  15

gtc cag cct ctg ggt gtg aac gct gga ata atg tgg acc ttg gca ctg   335
Val Gln Pro Leu Gly Val Asn Ala Gly Ile Met Trp Thr Leu Ala Leu
            20                  25                  30

tgg gca ttc tct ttc ctc tgc aaa ttc agc ctg gca gtc ctg ccg act   383
Trp Ala Phe Ser Phe Leu Cys Lys Phe Ser Leu Ala Val Leu Pro Thr
        35                  40                  45

aag cca gag aac att tcc tgc gtc ttt tac ttc gac aga aat ctg act   431
Lys Pro Glu Asn Ile Ser Cys Val Phe Tyr Phe Asp Arg Asn Leu Thr
    50                  55                  60                  65

tgc act tgg aga cca gag aag gaa acc aat gat acc agc tac att gtg   479
Cys Thr Trp Arg Pro Glu Lys Glu Thr Asn Asp Thr Ser Tyr Ile Val
                70                  75                  80

act ttg act tac tcc tat gga aaa agc aat tat agt gac aat gct aca   527
Thr Leu Thr Tyr Ser Tyr Gly Lys Ser Asn Tyr Ser Asp Asn Ala Thr
            85                  90                  95

gag gct tca tat tct ttt ccc cgt tcc tgt gca atg ccc cca gac atc   575
Glu Ala Ser Tyr Ser Phe Pro Arg Ser Cys Ala Met Pro Pro Asp Ile
        100                 105                 110

tgc agt gtt gaa gta caa gct caa aat gga gat ggt aaa gtt aaa tct   623
Cys Ser Val Glu Val Gln Ala Gln Asn Gly Asp Gly Lys Val Lys Ser
    115                 120                 125

gac atc aca tat tgg cat tta atc tcc ata gca aaa acc gaa cca cct   671
Asp Ile Thr Tyr Trp His Leu Ile Ser Ile Ala Lys Thr Glu Pro Pro
130                 135                 140                 145

ata att tta agt gtg aat cca att tgt aat aga atg ttc cag ata caa   719
Ile Ile Leu Ser Val Asn Pro Ile Cys Asn Arg Met Phe Gln Ile Gln
                150                 155                 160

tgg aaa ccg cgt gaa aag act cgt ggg ttt cct tta gta tgc atg ctt   767
Trp Lys Pro Arg Glu Lys Thr Arg Gly Phe Pro Leu Val Cys Met Leu
            165                 170                 175
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cgg ttc aga act gtc aac agt agc cgc tgg acg gaa gtc aat ttt gaa   815
Arg Phe Arg Thr Val Asn Ser Ser Arg Trp Thr Glu Val Asn Phe Glu
        180                 185                 190

aac tgt aaa cag gtc tgc aac ctc aca gga ctt cag gct ttc aca gaa   863
Asn Cys Lys Gln Val Cys Asn Leu Thr Gly Leu Gln Ala Phe Thr Glu
    195                 200                 205

tat gtc ctg gct cta cga ttc agg ttc aat gac tca aga tat tgg agc   911
Tyr Val Leu Ala Leu Arg Phe Arg Phe Asn Asp Ser Arg Tyr Trp Ser
210                 215                 220                 225

aag tgg agc aaa gaa gaa acc aga gtg act atg gag gaa gtt cca cat   959
Lys Trp Ser Lys Glu Glu Thr Arg Val Thr Met Glu Glu Val Pro His
                230                 235                 240

gtc ctg gac ctg tgg aga att ctg gaa cca gca gac atg aac gga gac   1007
Val Leu Asp Leu Trp Arg Ile Leu Glu Pro Ala Asp Met Asn Gly Asp
            245                 250                 255

agg aag gtg cga ttg ctg tgg aag aag gca aga gga gcc ccc gtc ttg   1055
Arg Lys Val Arg Leu Leu Trp Lys Lys Ala Arg Gly Ala Pro Val Leu
        260                 265                 270

gag aaa aca ttt ggc tac cac ata cag tac ttt gca gag aac agc act   1103
Glu Lys Thr Phe Gly Tyr His Ile Gln Tyr Phe Ala Glu Asn Ser Thr
    275                 280                 285

aac ctc aca gag ata aac aac atc acc acc cag cag tat gaa ctg ctt   1151
Asn Leu Thr Glu Ile Asn Asn Ile Thr Thr Gln Gln Tyr Glu Leu Leu
290                 295                 300                 305

ctg atg agc cag gca cac tct gtg tcc gtg act tct ttt aat tct ctt   1199
Leu Met Ser Gln Ala His Ser Val Ser Val Thr Ser Phe Asn Ser Leu
                310                 315                 320

ggc aag tcc caa gag acc atc ctg agg atc cca gat gtc cat gag aag   1247
Gly Lys Ser Gln Glu Thr Ile Leu Arg Ile Pro Asp Val His Glu Lys
            325                 330                 335

acc ttc cag tac att aag agc atg cag gcc tac ata gcc gag ccc ctg   1295
Thr Phe Gln Tyr Ile Lys Ser Met Gln Ala Tyr Ile Ala Glu Pro Leu
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340 345 350

ttg gtg gtg aac tgg caa agc tcc att cct gcg gtg gac act tgg ata 1343
Leu Val Val Asn Trp Gln Ser Ser Ile Pro Ala Val Asp Thr Trp Ile
355 360 365

gtg gag tgg ctc cca gaa gct gcc atg tcg aag ttc cct gcc ctt tcc 1391
Val Glu Trp Leu Pro Glu Ala Ala Met Ser Lys Phe Pro Ala Leu Ser
370 375 380 385

tgg gaa tct gtg tct cag gtc acg aac tgg acc atc gag caa gat aaa 1439
Trp Glu Ser Val Ser Gln Val Thr Asn Trp Thr Ile Glu Gln Asp Lys
390 395 400

cta aaa cct ttc aca tgc tat aat ata tca gtg tat cca gtg ttg gga 1487
Leu Lys Pro Phe Thr Cys Tyr Asn Ile Ser Val Tyr Pro Val Leu Gly
405 410 415

cac cga gtt gga gag ccg tat tca atc caa gct tat gcc aaa gaa gga 1535
His Arg Val Gly Glu Pro Tyr Ser Ile Gln Ala Tyr Ala Lys Glu Gly
420 425 430

act cca tta aaa ggt cct gag acc agg gtg gag aac atc ggt ctg agg 1583
Thr Pro Leu Lys Gly Pro Glu Thr Arg Val Glu Asn Ile Gly Leu Arg
435 440 445

aca gcc acg atc aca tgg aag gag att cct aag agt gct agg aat gga 1631
Thr Ala Thr Ile Thr Trp Lys Glu Ile Pro Lys Ser Ala Arg Asn Gly
450 455 460 465

ttt atc aac aat tac act gta ttt tac caa gct gaa ggt gga aaa gaa 1679
Phe Ile Asn Asn Tyr Thr Val Phe Tyr Gln Ala Glu Gly Gly Lys Glu
470 475 480

ctc tcc aag act gtt aac tct cat gcc ctg cag tgt gac ctg gag tct 1727
Leu Ser Lys Thr Val Asn Ser His Ala Leu Gln Cys Asp Leu Glu Ser
485 490 495

ctg aca cga agg acc tct tat act gtt tgg gtc atg gcc agc acc aga 1775
Leu Thr Arg Arg Thr Ser Tyr Thr Val Trp Val Met Ala Ser Thr Arg
500 505 510

gct gga ggt acc aac ggg gtg aga ata aac ttc aag aca ttg tca atc 1823

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Ala Gly Gly Thr Asn Gly Val Arg Ile Asn Phe Lys Thr Leu Ser Ile
    515                 520                 525

agt gtg ttt gaa att gtc ctt cta aca tct cta gtt gga gga ggc ctt   1871
Ser Val Phe Glu Ile Val Leu Leu Thr Ser Leu Val Gly Gly Gly Leu
530                 535                 540                 545

ctt cta ctt agc atc aaa aca gtg act ttt ggc ctc aga aag cca aac   1919
Leu Leu Leu Ser Ile Lys Thr Val Thr Phe Gly Leu Arg Lys Pro Asn
                550                 555                 560

cgg ttg act ccc ctg tgt tgt cct gat gtt ccc aac cct gct gaa agt   1967
Arg Leu Thr Pro Leu Cys Cys Pro Asp Val Pro Asn Pro Ala Glu Ser
            565                 570                 575

agt tta gcc aca tgg ctc gga gat ggt ttc aag aag tca aat atg aag   2015
Ser Leu Ala Thr Trp Leu Gly Asp Gly Phe Lys Lys Ser Asn Met Lys
        580                 585                 590

gag act gga aac tct ggg aac aca gaa gac gtg gtc cta aaa cca tgt   2063
Glu Thr Gly Asn Ser Gly Asn Thr Glu Asp Val Val Leu Lys Pro Cys
    595                 600                 605

ccc gtc ccc gcg gat ctc att gac aag ctg gta gtg aac ttt gag aat   2111
Pro Val Pro Ala Asp Leu Ile Asp Lys Leu Val Val Asn Phe Glu Asn
610                 615                 620                 625

ttt ctg gaa gta gtt ttg aca gag gaa gct gga aag ggt cag gcg agc   2159
Phe Leu Glu Val Val Leu Thr Glu Glu Ala Gly Lys Gly Gln Ala Ser
                630                 635                 640

att ttg gga gga gaa gcg aat gag tat atc tta tcc cag gaa cca agc   2207
Ile Leu Gly Gly Glu Ala Asn Glu Tyr Ile Leu Ser Gln Glu Pro Ser
            645                 650                 655

tgt cct ggc cat tgc tgaagctacc ctcagggtcc aggacagctg tcttgttggc   2262
Cys Pro Gly His Cys
        660

acttgactct ggcaggaacc tgatctctac ttttcttctc cctgtctccg gacactttct 2322
ctccttcatg cagagaccag gactagagcg gattcctcat ggtttgccag gctcctcagt 2382
ccttgctcgg gctcaggatc ttcaacaatg ccctttctgg gacactccat catccactta 2442
tatttatttt ttgcaacatt gtggattgaa cccagggact tgtttatgcg cgcaacttca 2502
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gtaactgtgg cagagactta ggaatggaga tctgaccctt tgcagaaggt ttctggacat 2562
ccgtccctgt gtgagcctca gacagcattg tctttacttt gaatcagctt ccaagttaat 2622
aaaagaaaaa cagagaggtg gcataacagc tcctgcttcc tgacctgctt gagttccagt 2682
tctgacttcc tttggtgatg aacagcaatg tgggaagtgt aagctgaata aacccttcc 2742
tcccca 2748

<210> 112
<211> 662
<212> PRT
<213> Mus musculus

<400> 112

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

245 250 255
Asp Arg Lys Val Arg Leu Leu Trp Lys Lys Ala Arg Gly Ala Pro Val
260 265 270
Leu Glu Lys Thr Phe Gly Tyr His Ile Gln Tyr Phe Ala Glu Asn Ser
275 280 285
Thr Asn Leu Thr Glu Ile Asn Asn Ile Thr Thr Gln Gln Tyr Glu Leu
290 295 300
Leu Leu Met Ser Gln Ala His Ser Val Ser Val Thr Ser Phe Asn Ser
305 310 315 320
Leu Gly Lys Ser Gln Glu Thr Ile Leu Arg Ile Pro Asp Val His Glu
325 330 335
Lys Thr Phe Gln Tyr Ile Lys Ser Met Gln Ala Tyr Ile Ala Glu Pro
340 345 350
Leu Leu Val Val Asn Trp Gln Ser Ser Ile Pro Ala Val Asp Thr Trp
355 360 365
Ile Val Glu Trp Leu Pro Glu Ala Ala Met Ser Lys Phe Pro Ala Leu
370 375 380
Ser Trp Glu Ser Val Ser Gln Val Thr Asn Trp Thr Ile Glu Gln Asp
385 390 395 400
Lys Leu Lys Pro Phe Thr Cys Tyr Asn Ile Ser Val Tyr Pro Val Leu
405 410 415
Gly His Arg Val Gly Glu Pro Tyr Ser Ile Gln Ala Tyr Ala Lys Glu
420 425 430
Gly Thr Pro Leu Lys Gly Pro Glu Thr Arg Val Glu Asn Ile Gly Leu
435 440 445
Arg Thr Ala Thr Ile Thr Trp Lys Glu Ile Pro Lys Ser Ala Arg Asn
450 455 460
Gly Phe Ile Asn Asn Tyr Thr Val Phe Tyr Gln Ala Glu Gly Gly Lys
465 470 475 480
Glu Leu Ser Lys Thr Val Asn Ser His Ala Leu Gln Cys Asp Leu Glu
485 490 495
Ser Leu Thr Arg Arg Thr Ser Tyr Thr Val Trp Val Met Ala Ser Thr
500 505 510
Arg Ala Gly Gly Thr Asn Gly Val Arg Ile Asn Phe Lys Thr Leu Ser
515 520 525
Ile Ser Val Phe Glu Ile Val Leu Leu Thr Ser Leu Val Gly Gly Gly
530 535 540
Leu Leu Leu Leu Ser Ile Lys Thr Val Thr Phe Gly Leu Arg Lys Pro
545 550 555 560
Asn Arg Leu Thr Pro Leu Cys Cys Pro Asp Val Pro Asn Pro Ala Glu
565 570 575
Ser Ser Leu Ala Thr Trp Leu Gly Asp Gly Phe Lys Lys Ser Asn Met
580 585 590

Lys Glu Thr Gly Asn Ser Gly Asn Thr Glu Asp Val Val Leu Lys Pro
595 600 605
Cys Pro Val Pro Ala Asp Leu Ile Asp Lys Leu Val Val Asn Phe Glu
610 615 620
Asn Phe Leu Glu Val Val Leu Thr Glu Glu Ala Gly Lys Gly Gln Ala
625 630 635 640
Ser Ile Leu Gly Gly Glu Ala Asn Glu Tyr Ile Leu Ser Gln Glu Pro
645 650 655
Ser Cys Pro Gly His Cys
660

<210> 113
<211> 2728
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (237)...(1877)

<400> 113
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ttcatctggt catgctgaat atactctcaa gatgtgctgg agaaggtgct gctgtccggg 120
ctctcagaga aggcagtgct ggaggcgttc ctggcccggg tctcctccta ctgttcctgg 180
tagcccagcc ttctcggggt ggaaggagaa gctggccagg tgagctctga ggaagc atg 239
Met
1

ctg agc agc cag aag gga tcc tgc agc cag gaa cca ggg gca gcc cac 287
Leu Ser Ser Gln Lys Gly Ser Cys Ser Gln Glu Pro Gly Ala Ala His
5 10 15

gtc cag cct ctg ggt gtg aac gct gga ata atg tgg acc ttg gca ctg 335
Val Gln Pro Leu Gly Val Asn Ala Gly Ile Met Trp Thr Leu Ala Leu
20 25 30

tgg gca ttc tct ttc ctc tgc aaa ttc agc ctg gca gtc ctg ccg act 383
Trp Ala Phe Ser Phe Leu Cys Lys Phe Ser Leu Ala Val Leu Pro Thr
35 40 45

aag cca gag aac att tcc tgc gtc ttt tac ttc gac aga aat ctg act 431
Lys Pro Glu Asn Ile Ser Cys Val Phe Tyr Phe Asp Arg Asn Leu Thr

50 55 60 65

tgc act tgg aga cca gag aag gaa acc aat gat acc agc tac att gtg 479
Cys Thr Trp Arg Pro Glu Lys Glu Thr Asn Asp Thr Ser Tyr Ile Val
70 75 80

act ttg act tac tcc tat gga aaa agc aat tat agt gac aat gct aca 527
Thr Leu Thr Tyr Ser Tyr Gly Lys Ser Asn Tyr Ser Asp Asn Ala Thr
85 90 95

gag gct tca tat tct ttt ccc cgt tcc tgt gca atg ccc cca gac atc 575
Glu Ala Ser Tyr Ser Phe Pro Arg Ser Cys Ala Met Pro Pro Asp Ile
100 105 110

tgc agt gtt gaa gta caa gct caa aat gga gat ggt aaa gtt aaa tct 623
Cys Ser Val Glu Val Gln Ala Gln Asn Gly Asp Gly Lys Val Lys Ser
115 120 125

gac atc aca tat tgg cat tta atc tcc ata gca aaa acc gaa cca cct 671
Asp Ile Thr Tyr Trp His Leu Ile Ser Ile Ala Lys Thr Glu Pro Pro
130 135 140 145

ata att tta agt gtg aat cca att tgt aat aga atg ttc cag ata caa 719
Ile Ile Leu Ser Val Asn Pro Ile Cys Asn Arg Met Phe Gln Ile Gln
150 155 160

tgg aaa ccg cgt gaa aag act cgt ggg ttt cct tta gta tgc atg ctt 767
Trp Lys Pro Arg Glu Lys Thr Arg Gly Phe Pro Leu Val Cys Met Leu
165 170 175

cgg ttc aga act gtc aac agt agc cgc tgg acg gaa gtc aat ttt gaa 815
Arg Phe Arg Thr Val Asn Ser Ser Arg Trp Thr Glu Val Asn Phe Glu
180 185 190

aac tgt aaa cag gtc tgc aac ctc aca gga ctt cag gct ttc aca gaa 863
Asn Cys Lys Gln Val Cys Asn Leu Thr Gly Leu Gln Ala Phe Thr Glu
195 200 205

tat gtc ctg gct cta cga ttc agg ttc aat gac tca aga tat tgg agc 911
Tyr Val Leu Ala Leu Arg Phe Arg Phe Asn Asp Ser Arg Tyr Trp Ser
210 215 220 225

aag tgg agc aaa gaa gaa acc aga gtg act atg gag gaa gtt cca cat 959

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Lys Trp Ser Lys Glu Glu Thr Arg Val Thr Met Glu Glu Val Pro His
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gtc ctg gac ctg tgg aga att ctg gaa cca gca gac atg aac gga gac   1007
Val Leu Asp Leu Trp Arg Ile Leu Glu Pro Ala Asp Met Asn Gly Asp
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agg aag gtg cga ttg ctg tgg aag aag gca aga gga gcc ccc gtc ttg   1055
Arg Lys Val Arg Leu Leu Trp Lys Lys Ala Arg Gly Ala Pro Val Leu
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gag aaa aca ttt ggc tac cac ata cag tac ttt gca gag aac agc act   1103
Glu Lys Thr Phe Gly Tyr His Ile Gln Tyr Phe Ala Glu Asn Ser Thr
    275                 280                 285

aac ctc aca gag ata aac aac atc acc acc cag cag tat gaa ctg ctt   1151
Asn Leu Thr Glu Ile Asn Asn Ile Thr Thr Gln Gln Tyr Glu Leu Leu
290                 295                 300                 305

ctg atg agc cag gca cac tct gtg tcc gtg act tct ttt aat tct ctt   1199
Leu Met Ser Gln Ala His Ser Val Ser Val Thr Ser Phe Asn Ser Leu
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ggc aag tcc caa gag acc atc ctg agg atc cca gat gtc cat gag aag   1247
Gly Lys Ser Gln Glu Thr Ile Leu Arg Ile Pro Asp Val His Glu Lys
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acc ttc cag tac att aag agc atg cag gcc tac ata gcc gag ccc ctg   1295
Thr Phe Gln Tyr Ile Lys Ser Met Gln Ala Tyr Ile Ala Glu Pro Leu
        340                 345                 350

ttg gtg gtg aac tgg caa agc tcc att cct gcg gtg gac act tgg ata   1343
Leu Val Val Asn Trp Gln Ser Ser Ile Pro Ala Val Asp Thr Trp Ile
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gtg gag tgg ctc cca gaa gct gcc atg tcg aag ttc cct gcc ctt tcc   1391
Val Glu Trp Leu Pro Glu Ala Ala Met Ser Lys Phe Pro Ala Leu Ser
370                 375                 380                 385

tgg gaa tct gtg tct cag gtc acg aac tgg acc atc gag caa gat aaa   1439
Trp Glu Ser Val Ser Gln Val Thr Asn Trp Thr Ile Glu Gln Asp Lys
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cac cga gtt gga gag ccg tat tca atc caa gct tat gcc aaa gaa gga   1535
His Arg Val Gly Glu Pro Tyr Ser Ile Gln Ala Tyr Ala Lys Glu Gly
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act cca tta aaa ggt cct gag acc agg gtg gag aac atc ggt ctg agg   1583
Thr Pro Leu Lys Gly Pro Glu Thr Arg Val Glu Asn Ile Gly Leu Arg
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aca gcc acg atc aca tgg aag gag att cct aag agt gct agg aat gga   1631
Thr Ala Thr Ile Thr Trp Lys Glu Ile Pro Lys Ser Ala Arg Asn Gly
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ttt atc aac aat tac act gta ttt tac caa gct gaa ggt gga aaa gaa   1679
Phe Ile Asn Asn Tyr Thr Val Phe Tyr Gln Ala Glu Gly Gly Lys Glu
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ctc tcc aag act gtt aac tct cat gcc ctg cag tgt gac ctg gag tct   1727
Leu Ser Lys Thr Val Asn Ser His Ala Leu Gln Cys Asp Leu Glu Ser
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ctg aca cga agg acc tct tat act gtt tgg gtc atg gcc agc acc aga   1775
Leu Thr Arg Arg Thr Ser Tyr Thr Val Trp Val Met Ala Ser Thr Arg
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gct gga ggt acc aac ggg gtg aga ata aac ttc aag aca ttg tca atc   1823
Ala Gly Gly Thr Asn Gly Val Arg Ile Asn Phe Lys Thr Leu Ser Ile
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agt gag tac tgg ctt cag gcc tca ttc tgg agt tta ctt cgg gtt gga   1871
Ser Glu Tyr Trp Leu Gln Ala Ser Phe Trp Ser Leu Leu Arg Val Gly
530                 535                 540                 545

aat gtt tgacaggagc aaggagagcc agcagagggc agcagagcat ggcttctcct    1927
Asn Val

gctctctctg gctcactcac ctcccaggag ttactgagga gctggcaaag ggagggctga 1987
gttagaccaa caggccattt tgatccttgc tggtaagcag ccacaaataa tcttaagatg 2047
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35 40 45
Thr Lys Pro Glu Asn Ile Ser Cys Val Phe Tyr Phe Asp Arg Asn Leu
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Thr Cys Thr Trp Arg Pro Glu Lys Glu Thr Asn Asp Thr Ser Tyr Ile
65 70 75 80
Val Thr Leu Thr Tyr Ser Tyr Gly Lys Ser Asn Tyr Ser Asp Asn Ala
85 90 95
Thr Glu Ala Ser Tyr Ser Phe Pro Arg Ser Cys Ala Met Pro Pro Asp
100 105 110
Ile Cys Ser Val Glu Val Gln Ala Gln Asn Gly Asp Gly Lys Val Lys
115 120 125
Ser Asp Ile Thr Tyr Trp His Leu Ile Ser Ile Ala Lys Thr Glu Pro
130 135 140
Pro Ile Ile Leu Ser Val Asn Pro Ile Cys Asn Arg Met Phe Gln Ile
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Gln Trp Lys Pro Arg Glu Lys Thr Arg Gly Phe Pro Leu Val Cys Met
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Glu Asn Cys Lys Gln Val Cys Asn Leu Thr Gly Leu Gln Ala Phe Thr

195 200 205
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Ser Lys Trp Ser Lys Glu Glu Thr Arg Val Thr Met Glu Glu Val Pro
225 230 235 240
His Val Leu Asp Leu Trp Arg Ile Leu Glu Pro Ala Asp Met Asn Gly
245 250 255
Asp Arg Lys Val Arg Leu Leu Trp Lys Lys Ala Arg Gly Ala Pro Val
260 265 270
Leu Glu Lys Thr Phe Gly Tyr His Ile Gln Tyr Phe Ala Glu Asn Ser
275 280 285
Thr Asn Leu Thr Glu Ile Asn Asn Ile Thr Thr Gln Gln Tyr Glu Leu
290 295 300
Leu Leu Met Ser Gln Ala His Ser Val Ser Val Thr Ser Phe Asn Ser
305 310 315 320
Leu Gly Lys Ser Gln Glu Thr Ile Leu Arg Ile Pro Asp Val His Glu
325 330 335
Lys Thr Phe Gln Tyr Ile Lys Ser Met Gln Ala Tyr Ile Ala Glu Pro
340 345 350
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Glu Leu Ser Lys Thr Val Asn Ser His Ala Leu Gln Cys Asp Leu Glu
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Ser Leu Thr Arg Arg Thr Ser Tyr Thr Val Trp Val Met Ala Ser Thr
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Arg Ala Gly Gly Thr Asn Gly Val Arg Ile Asn Phe Lys Thr Leu Ser
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Gly Asn Val
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ctgtgaggcc gaggaccggc aaggctccgg agcaggtcgc caggcgggta atcagacca 779
atg gct ttc tct gtg gtc ctt cat cca gcc ttc ctc ctg gca gtg ctg 827
Met Ala Phe Ser Val Val Leu His Pro Ala Phe Leu Leu Ala Val Leu
1 5 10 15

tcc ctg agg gca tcc cga agc gaa gtc ttg gag gag cct tta cca ttg 875
Ser Leu Arg Ala Ser Arg Ser Glu Val Leu Glu Glu Pro Leu Pro Leu
20 25 30

act cct gag ata cat aaa gtt tct ttt caa ttg aaa ctt caa gaa gtg 923
Thr Pro Glu Ile His Lys Val Ser Phe Gln Leu Lys Leu Gln Glu Val
35 40 45

aat tta gaa tgg act gtc cca gcc ctt act cat gaa gaa tta aac atg 971
Asn Leu Glu Trp Thr Val Pro Ala Leu Thr His Glu Glu Leu Asn Met
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ata ttt cag ata gag atc agt aga ctg aac ata tcc aac acc atc tgg   1019
Ile Phe Gln Ile Glu Ile Ser Arg Leu Asn Ile Ser Asn Thr Ile Trp
 65                  70                  75                  80

gtg gag aat tat agc acc act gtg aag cgt gaa gaa gct gtg cgt tgg   1067
Val Glu Asn Tyr Ser Thr Thr Val Lys Arg Glu Glu Ala Val Arg Trp
                85                  90                  95

aac tgg acg tct gat atc cct ttg gag tgt gtc aaa cat ttc ata aga   1115
Asn Trp Thr Ser Asp Ile Pro Leu Glu Cys Val Lys His Phe Ile Arg
            100                 105                 110

atc agg gct ctg gta gat gac acc aag tcc ctt cca cag agt tcc tgg   1163
Ile Arg Ala Leu Val Asp Asp Thr Lys Ser Leu Pro Gln Ser Ser Trp
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ggc aac tgg agt tcc tgg aaa gaa gtt aat gca aag gtt tcc gtt gaa   1211
Gly Asn Trp Ser Ser Trp Lys Glu Val Asn Ala Lys Val Ser Val Glu
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cct gat aaa tca tta ata ttt cct aaa gac aaa gtg ttg gaa gaa ggc   1259
Pro Asp Lys Ser Leu Ile Phe Pro Lys Asp Lys Val Leu Glu Glu Gly
145                 150                 155                 160

tcc aat gtc acc atc tgt ctg atg tat ggg cag aat gta tat aat gta   1307
Ser Asn Val Thr Ile Cys Leu Met Tyr Gly Gln Asn Val Tyr Asn Val
                165                 170                 175

tcc tgt aag ttg caa gat gag cca atc cat gga gaa caa ctt gat tcc   1355
Ser Cys Lys Leu Gln Asp Glu Pro Ile His Gly Glu Gln Leu Asp Ser
            180                 185                 190

cac gtg tca tta tta aaa ttg aac aat gta gtt ttc ctt agt gac aca   1403
His Val Ser Leu Leu Lys Leu Asn Asn Val Val Phe Leu Ser Asp Thr
        195                 200                 205

ggg aca aac atc aat tgt caa gcc acg aag ggt cct aaa aga ata ttt   1451
Gly Thr Asn Ile Asn Cys Gln Ala Thr Lys Gly Pro Lys Arg Ile Phe
    210                 215                 220

ggt act gtt ctc ttt gtc tcg aaa gtg ctc gag gaa cct aag aat gtt   1499
Gly Thr Val Leu Phe Val Ser Lys Val Leu Glu Glu Pro Lys Asn Val
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225 230 235 240

tcc tgt gaa acc cga gac ttt aag act ttg gac tgt tca tgg gaa cct 1547
Ser Cys Glu Thr Arg Asp Phe Lys Thr Leu Asp Cys Ser Trp Glu Pro
245 250 255

ggg gta gat acg act ttg act tgg cgt aaa caa aga ttc caa aac tac 1595
Gly Val Asp Thr Thr Leu Thr Trp Arg Lys Gln Arg Phe Gln Asn Tyr
260 265 270

act tta tgt gaa tcg ttc tct aag aga tgt gag gtt tct aac tac agg 1643
Thr Leu Cys Glu Ser Phe Ser Lys Arg Cys Glu Val Ser Asn Tyr Arg
275 280 285

aac tcc tat acc tgg caa atc act gaa ggc tca cag gaa atg tat aac 1691
Asn Ser Tyr Thr Trp Gln Ile Thr Glu Gly Ser Gln Glu Met Tyr Asn
290 295 300

ttt act ctc aca gct gaa aac caa cta agg aaa aga agt gtc aac att 1739
Phe Thr Leu Thr Ala Glu Asn Gln Leu Arg Lys Arg Ser Val Asn Ile
305 310 315 320

aat ttt aac ctg acc cat aga gtt cat cca aag gct ccg cag gac gtc 1787
Asn Phe Asn Leu Thr His Arg Val His Pro Lys Ala Pro Gln Asp Val
325 330 335

acc ctt aaa att ata ggt gct aca aaa gcc aac atg act tgg aag gtt 1835
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340 345 350

cac tcc cat gga aac aac tac aca ctt ttg tgt cag gtt aaa ctc caa 1883
His Ser His Gly Asn Asn Tyr Thr Leu Leu Cys Gln Val Lys Leu Gln
355 360 365

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Tyr Gly Glu Val Ile His Glu His Asn Val Ser Val His Met Ser Ala
370 375 380

aac tac ctc ttc agt gat ctg gat cca gac aca aag tac aag gct ttt 1979
Asn Tyr Leu Phe Ser Asp Leu Asp Pro Asp Thr Lys Tyr Lys Ala Phe
385 390 395 400

gtg cgc tgt gca agt gcc aac cac ttc tgg aaa tgg agc gac tgg acc 2027

Val Arg Cys Ala Ser Ala Asn His Phe Trp Lys Trp Ser Asp Trp Thr
405 410 415

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Gln Lys Glu Phe Ser Thr Pro Glu Thr Ala Pro Ser Gln Ala Leu Asp
420 425 430

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435 440 445

ttc tgg aag cca cta tta aaa tca cag gcc aat ggc aaa atc ata tcc 2171
Phe Trp Lys Pro Leu Leu Lys Ser Gln Ala Asn Gly Lys Ile Ile Ser
450 455 460

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Tyr Asn Ile Val Val Glu Asn Glu Ala Lys Pro Thr Glu Ser Glu His
465 470 475 480

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500 505 510

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Pro Glu Ser Leu Met Val Leu Ser Asn Asp Ser Gly His Glu Val Lys
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Pro Val Ser Gly Asp Thr Met Gly Tyr Val Val Asp Trp Cys Ala His
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565 570 575

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Thr Thr Ser Thr Thr Ile Thr Ser Asp Asp Phe Lys Pro Gly Val Arg
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Tyr Asn Phe Arg Ile Phe Glu Arg Ser Val Glu His Lys Ala Arg Leu
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gta gag aaa caa aga gga tac acc cag gaa ctg gct cct ttg gtg aat   2651
Val Glu Lys Gln Arg Gly Tyr Thr Gln Glu Leu Ala Pro Leu Val Asn
    610                 615                 620

cca aaa gtg gag att cct tac tcg acc cct aac tcc ttc gtt cta aga   2699
Pro Lys Val Glu Ile Pro Tyr Ser Thr Pro Asn Ser Phe Val Leu Arg
625                 630                 635                 640

tgg cca gat tat gac agc gac ttc cag gct ggt ttt ata aaa ggg tac   2747
Trp Pro Asp Tyr Asp Ser Asp Phe Gln Ala Gly Phe Ile Lys Gly Tyr
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agg acc ctc ctt cca gat aat tca gtc ctc tgt aaa tac gac atc aat   2843
Arg Thr Leu Leu Pro Asp Asn Ser Val Leu Cys Lys Tyr Asp Ile Asn
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ctc tat gag ttt ttc gtc act ccg tac acc agc gct ggc cca gga ccc   2939
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ctg ctg cag atc ata cta ccc atg acc ctc tgc gtc ttg ctc agc atc   3035
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gac att ccc aat ccg tac aag agc agc att ctg tca ctc ata aaa tcc   3131
Asp Ile Pro Asn Pro Tyr Lys Ser Ser Ile Leu Ser Leu Ile Lys Ser
    770                 775                 780

aag aag aat cct cac tta ata atg aat gtc aaa gac tgc att cca gat   3179
Lys Lys Asn Pro His Leu Ile Met Asn Val Lys Asp Cys Ile Pro Asp
785                 790                 795                 800

gtc ctt gaa gtg ata aac aaa gca gaa ggc agc aag aca cag tgt gta   3227
Val Leu Glu Val Ile Asn Lys Ala Glu Gly Ser Lys Thr Gln Cys Val
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ggc tct ggg aaa ctt cac att gaa gat gta ccc act aag ccg cca atc   3275
Gly Ser Gly Lys Leu His Ile Glu Asp Val Pro Thr Lys Pro Pro Ile
            820                 825                 830

gtg cca aca gaa aag gat tcc tca ggg cct gtg ccc tgc atc ttc ttt   3323
Val Pro Thr Glu Lys Asp Ser Ser Gly Pro Val Pro Cys Ile Phe Phe
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gag aat ttt act tac gat cag tca gct ttt gac tct ggt tcc cat ggc   3371
Glu Asn Phe Thr Tyr Asp Gln Ser Ala Phe Asp Ser Gly Ser His Gly
    850                 855                 860

ctc att cca ggt ccc cta aaa gac aca gca cac caa ctt gga cta ttg   3419
Leu Ile Pro Gly Pro Leu Lys Asp Thr Ala His Gln Leu Gly Leu Leu
865                 870                 875                 880

gct cca cct aac aag ttc cag aac gta tta aaa aat gac tac atg aag   3467
Ala Pro Pro Asn Lys Phe Gln Asn Val Leu Lys Asn Asp Tyr Met Lys
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Pro Leu Val Glu Ser Pro Thr Glu Glu Thr Ser Leu Ile Tyr Val Ser
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cag ctg gct tca ccc atg tgc gga gac aag gac acg ctt gcc aca gaa   3563
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Met Ala Phe Ser Val Val Leu His Pro Ala Phe Leu Leu Ala Val Leu
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Ser Leu Arg Ala Ser Arg Ser Glu Val Leu Glu Glu Pro Leu Pro Leu
20 25 30
Thr Pro Glu Ile His Lys Val Ser Phe Gln Leu Lys Leu Gln Glu Val
35 40 45
Asn Leu Glu Trp Thr Val Pro Ala Leu Thr His Glu Glu Leu Asn Met
50 55 60
Ile Phe Gln Ile Glu Ile Ser Arg Leu Asn Ile Ser Asn Thr Ile Trp
65 70 75 80
Val Glu Asn Tyr Ser Thr Thr Val Lys Arg Glu Glu Ala Val Arg Trp
85 90 95
Asn Trp Thr Ser Asp Ile Pro Leu Glu Cys Val Lys His Phe Ile Arg
100 105 110
Ile Arg Ala Leu Val Asp Asp Thr Lys Ser Leu Pro Gln Ser Ser Trp
115 120 125

Gly Asn Trp Ser Ser Trp Lys Glu Val Asn Ala Lys Val Ser Val Glu
130 135 140
Pro Asp Lys Ser Leu Ile Phe Pro Lys Asp Lys Val Leu Glu Glu Gly
145 150 155 160
Ser Asn Val Thr Ile Cys Leu Met Tyr Gly Gln Asn Val Tyr Asn Val
165 170 175
Ser Cys Lys Leu Gln Asp Glu Pro Ile His Gly Glu Gln Leu Asp Ser
180 185 190
His Val Ser Leu Leu Lys Leu Asn Asn Val Val Phe Leu Ser Asp Thr
195 200 205
Gly Thr Asn Ile Asn Cys Gln Ala Thr Lys Gly Pro Lys Arg Ile Phe
210 215 220
Gly Thr Val Leu Phe Val Ser Lys Val Leu Glu Glu Pro Lys Asn Val
225 230 235 240
Ser Cys Glu Thr Arg Asp Phe Lys Thr Leu Asp Cys Ser Trp Glu Pro
245 250 255
Gly Val Asp Thr Thr Leu Thr Trp Arg Lys Gln Arg Phe Gln Asn Tyr
260 265 270
Thr Leu Cys Glu Ser Phe Ser Lys Arg Cys Glu Val Ser Asn Tyr Arg
275 280 285
Asn Ser Tyr Thr Trp Gln Ile Thr Glu Gly Ser Gln Glu Met Tyr Asn
290 295 300
Phe Thr Leu Thr Ala Glu Asn Gln Leu Arg Lys Arg Ser Val Asn Ile
305 310 315 320
Asn Phe Asn Leu Thr His Arg Val His Pro Lys Ala Pro Gln Asp Val
325 330 335
Thr Leu Lys Ile Ile Gly Ala Thr Lys Ala Asn Met Thr Trp Lys Val
340 345 350
His Ser His Gly Asn Asn Tyr Thr Leu Leu Cys Gln Val Lys Leu Gln
355 360 365
Tyr Gly Glu Val Ile His Glu His Asn Val Ser Val His Met Ser Ala
370 375 380
Asn Tyr Leu Phe Ser Asp Leu Asp Pro Asp Thr Lys Tyr Lys Ala Phe
385 390 395 400
Val Arg Cys Ala Ser Ala Asn His Phe Trp Lys Trp Ser Asp Trp Thr
405 410 415
Gln Lys Glu Phe Ser Thr Pro Glu Thr Ala Pro Ser Gln Ala Leu Asp
420 425 430
Val Trp Arg Gln Val Trp Ser Glu Asn Gly Arg Arg Ile Val Thr Leu
435 440 445
Phe Trp Lys Pro Leu Leu Lys Ser Gln Ala Asn Gly Lys Ile Ile Ser
450 455 460
Tyr Asn Ile Val Val Glu Asn Glu Ala Lys Pro Thr Glu Ser Glu His

465 470 475 480
Tyr Cys Val Trp Ala Pro Ala Leu Ser Thr Asn Leu Ser Leu Asp Leu
485 490 495
Gln Pro Tyr Lys Ile Arg Ile Thr Ala Asn Asn Ser Met Gly Ala Ser
500 505 510
Pro Glu Ser Leu Met Val Leu Ser Asn Asp Ser Gly His Glu Val Lys
515 520 525
Glu Lys Thr Ile Lys Gly Ile Lys Asp Ala Phe Asn Ile Ser Trp Glu
530 535 540
Pro Val Ser Gly Asp Thr Met Gly Tyr Val Val Asp Trp Cys Ala His
545 550 555 560
Ser Gln Asp Gln Arg Cys Asp Leu Gln Trp Lys Asn Leu Gly Pro Asn
565 570 575
Thr Thr Ser Thr Thr Ile Thr Ser Asp Asp Phe Lys Pro Gly Val Arg
580 585 590
Tyr Asn Phe Arg Ile Phe Glu Arg Ser Val Glu His Lys Ala Arg Leu
595 600 605
Val Glu Lys Gln Arg Gly Tyr Thr Gln Glu Leu Ala Pro Leu Val Asn
610 615 620
Pro Lys Val Glu Ile Pro Tyr Ser Thr Pro Asn Ser Phe Val Leu Arg
625 630 635 640
Trp Pro Asp Tyr Asp Ser Asp Phe Gln Ala Gly Phe Ile Lys Gly Tyr
645 650 655
Leu Val Tyr Val Lys Ser Lys Glu Met Gln Cys Asn Gln Pro Trp Glu
660 665 670
Arg Thr Leu Leu Pro Asp Asn Ser Val Leu Cys Lys Tyr Asp Ile Asn
675 680 685
Gly Ser Glu Thr Lys Thr Leu Thr Val Glu Asn Leu Gln Pro Glu Ser
690 695 700
Leu Tyr Glu Phe Phe Val Thr Pro Tyr Thr Ser Ala Gly Pro Gly Pro
705 710 715 720
Asn Glu Thr Phe Thr Lys Val Thr Thr Pro Asp Ala Arg Ser His Met
725 730 735
Leu Leu Gln Ile Ile Leu Pro Met Thr Leu Cys Val Leu Leu Ser Ile
740 745 750
Ile Val Cys Tyr Trp Lys Ser Gln Trp Val Lys Glu Lys Cys Tyr Pro
755 760 765
Asp Ile Pro Asn Pro Tyr Lys Ser Ser Ile Leu Ser Leu Ile Lys Ser
770 775 780
Lys Lys Asn Pro His Leu Ile Met Asn Val Lys Asp Cys Ile Pro Asp
785 790 795 800
Val Leu Glu Val Ile Asn Lys Ala Glu Gly Ser Lys Thr Gln Cys Val
805 810 815

Gly Ser Gly Lys Leu His Ile Glu Asp Val Pro Thr Lys Pro Pro Ile
820 825 830
Val Pro Thr Glu Lys Asp Ser Ser Gly Pro Val Pro Cys Ile Phe Phe
835 840 845
Glu Asn Phe Thr Tyr Asp Gln Ser Ala Phe Asp Ser Gly Ser His Gly
850 855 860
Leu Ile Pro Gly Pro Leu Lys Asp Thr Ala His Gln Leu Gly Leu Leu
865 870 875 880
Ala Pro Pro Asn Lys Phe Gln Asn Val Leu Lys Asn Asp Tyr Met Lys
885 890 895
Pro Leu Val Glu Ser Pro Thr Glu Glu Thr Ser Leu Ile Tyr Val Ser
900 905 910
Gln Leu Ala Ser Pro Met Cys Gly Asp Lys Asp Thr Leu Ala Thr Glu
915 920 925
Pro Pro Val Pro Val His Gly Ser Glu Tyr Lys Arg Gln Met Val Val
930 935 940
Pro Gly Ser Leu Ala Ser Pro Ser Leu Lys Glu Asp Asn Ser Leu Thr
945 950 955 960
Ser Thr Val Leu Leu Gly Gln Gly Glu Gln
965 970

<210> 121
<211> 2910
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate polynucleotide encoding the polypeptide of SEQ ID NO:120.

<221> misc_feature
<222> (1)...(2910)
<223> N = A, T, G, or C

<221> misc_feature
<222> (1)...(2910)
<223> n = A,T,C or G

<400> 121
atggcnttyw sngtngtnyt ncayccngcn ttyytnytng cngtnytnws nytnmgngcn 60
wsnmgnwsng argtnytnga rgarccnytn ccnytnacnc cngarathca yaargtnwsn 120
ttycarytna arytncarga rgtnaayytn gartggacng tnccngcnyt nacncaygar 180

garytnaaya tgathttyca rathgarath wsnmgnytna ayathwsnaa yacnathtgg 240
gtngaraayt aywsnacnac ngtnaarmgn gargargcng tnmgntggaa ytggacnwsn 300
gayathccny tngartgygt naarcaytty athmgnathm gngcnytngt ngaygayacn 360
aarwsnytnc cncarwsnws ntggggnaay tggwsnwsnt ggaargargt naaygcnaar 420
gtnwsngtng arccngayaa rwsnytnath ttyccnaarg ayaargtnyt ngargarggn 480
wsnaaygtna cnathtgyyt natgtayggn caraaygtnt ayaaygtnws ntgyaarytn 540
cargaygarc cnathcaygg ngarcarytn gaywsncayg tnwsnytnyt naarytnaay 600
aaygtngtnt tyytnwsnga yacnggnacn aayathaayt gycargcnac naarggnccn 660
aarmgnatht tyggnacngt nytnttygtn wsnaargtny tngargarcc naaraaygtn 720
wsntgygara cnmgngaytt yaaracnytn gaytgywsnt gggarccngg ngtngayacn 780
acnytnacnt ggmgnaarca rmgnttycar aaytayacny tntgygarws nttywsnaar 840
mgntgygarg tnwsnaayta ymgnaaywsn tayacntggc arathacnga rggnwsncar 900
garatgtaya ayttyacnyt nacngcngar aaycarytnm gnaarmgnws ngtnaayath 960
aayttyaayy tnacncaymg ngtncayccn aargcnccnc argaygtnac nytnaarath 1020
athggngcna cnaargcnaa yatgacntgg aargtncayw sncayggnaa yaaytayacn 1080
ytnytntgyc argtnaaryt ncartayggn gargtnathc aygarcayaa ygtnwsngtn 1140
cayatgwsng cnaaytayyt nttywsngay ytngayccng ayacnaarta yaargcntty 1200
gtnmgntgyg cnwsngcnaa ycayttytgg aartggwsng aytggacnca raargartty 1260
wsnacnccng aracngcncc nwsncargcn ytngaygtnt ggmgncargt ntggwsngar 1320
aayggnmgnm gnathgtnac nytnttytgg aarccnytny tnaarwsnca rgcnaayggn 1380
aarathathw sntayaayat hgtngtngar aaygargcna arccnacnga rwsngarcay 1440
taytgygtnt gggcnccngc nytnwsnacn aayytnwsny tngayytnca rccntayaar 1500
athmgnatha cngcnaayaa ywsnatgggn gcnwsnccng arwsnytnat ggtnytnwsn 1560
aaygaywsng gncaygargt naargaraar acnathaarg gnathaarga ygcnttyaay 1620
athwsntggg arccngtnws nggngayacn atgggntayg tngtngaytg gtgygcncay 1680
wsncargayc armgntgyga yytncartgg aaraayytng gnccnaayac nacnwsnacn 1740
acnathacnw sngaygaytt yaarccnggn gtnmgntaya ayttymgnat httygarmgn 1800
wsngtngarc ayaargcnmg nytngtngar aarcarmgng gntayacnca rgarytngcn 1860
ccnytngtna ayccnaargt ngarathccn taywsnacnc cnaaywsntt ygtnytnmgn 1920
tggccngayt aygaywsnga yttycargcn ggnttyatha arggntayyt ngtntaygtn 1980
aarwsnaarg aratgcartg yaaycarccn tgggarmgna cnytnytncc ngayaaywsn 2040
gtnytntgya artaygayat haayggnwsn garacnaara cnytnacngt ngaraayytn 2100
carccngarw snytntayga rttyttygtn acnccntaya cnwsngcngg nccnggnccn 2160
aaygaracnt tyacnaargt nacnacnccn gaygcnmgnw sncayatgyt nytncarath 2220
athytnccna tgacnytntg ygtnytnytn wsnathathg tntgytaytg gaarwsncar 2280
tgggtnaarg araartgyta yccngayath ccnaayccnt ayaarwsnws nathytnwsn 2340
ytnathaarw snaaraaraa yccncayytn athatgaayg tnaargaytg yathccngay 2400
gtnytngarg tnathaayaa rgcngarggn wsnaaracnc artgygtngg nwsnggnaar 2460
ytncayathg argaygtncc nacnaarccn ccnathgtnc cnacngaraa rgaywsnwsn 2520
ggnccngtnc cntgyathtt yttygaraay ttyacntayg aycarwsngc nttygaywsn 2580
ggnwsncayg gnytnathcc nggnccnytn aargayacng cncaycaryt nggnytnytn 2640
gcnccnccna ayaarttyca raaygtnytn aaraaygayt ayatgaarcc nytngtngar 2700
wsnccnacng argaracnws nytnathtay gtnwsncary tngcnwsncc natgtgyggn 2760

gayaargaya cnytngcnac ngarccnccn gtnccngtnc ayggnwsnga rtayaarmgn 2820
caratggtng tnccnggnws nytngcnwsn ccnwsnytna argargayaa ywsnytnacn 2880
wsnacngtny tnytnggnca rggngarcar 2910

<210> 122
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43891

<400> 122
gggcagtagg atatgaatca gcat 24

<210> 123
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43900

<400> 123
gaaggcccca gtgctacgt 19

<210> 124
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> OSMRbeta probe ZC43896

<400> 124
tcatccggag tcgtgacctt cgtg 24

<210> 125
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Primer ZC43280

<400> 125
gagcacgccc ttctcttcct 20

<210> 126
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43281

<400> 126
cgttgcccgt ccgtttacta 20

<210> 127
<211> 40
<212> DNA
<213> qArtificial Sequence

<220>
<223> zcytor17lig probe ZC43275

<400> 127
ctgtaattcc tcgactattt tctgtacatc atcacttggt 40

<210> 128
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC40574

<400> 128
ctcatttgga attttgccga tt 22

<210> 129
<211> 22
<212> DNA
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<220>
<223> Primer ZC40575

<400> 129
ccgagtgaag atccccttt ta 22

<210> 130
<211> 26
<212> DNA
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<220>
<223> GUS probe ZC43017

<400> 130
tgaacagtca ccgacgagag tgctgg 26

<210> 131
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC28480

<400> 131
cgattcagga cagtcaacag tacc 24

<210> 132
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC41653

<400> 132
ccttcgtgaa cgtagcactg g 21

<210> 133
<211> 19
<212> DNA
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<220>
<223> Primer ZC41655

<400> 133
ctgaaatcca aggcgaggc 19

<210> 134
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC41703

<400> 134
tgaagctggc cttgctctct 20

<210> 135
<211> 19
<212> DNA
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<220>
<223> Primer ZC41704

<400> 135
gagatatgcc cggatggct 19

<210> 136
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43272

<400> 136
gctggtatca ttggtttcct tctc 24

<210> 137
<211> 24
<212> DNA

<213> Artificial Sequence

<220>
<223> Primer ZC43273

<400> 137
cattctcttt cctctgcaaa ttca 24

<210> 138
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> zcytor17 probe ZC43478

<400> 138
tagagaacat ttcctgcgtc ttttacttcg acag 34

<210> 139
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43278

<400> 139
aaacaagagt ctcaggatct ttataacaac 30

<210> 140
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43279

<400> 140
acggcagctg tattgattcg t 21

<210> 141
<211> 26

<212> DNA
<213> Artificial Sequence

<220>
<223> zcytor17lig probe ZC43276

<400> 141
taaagcaggc atctgggatg tcagca 26

<210> 142
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43045

<400> 142
aaacatgata tttcagatag agatcagtag act 33

<210> 143
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43046

<400> 143
cttatgaaat gtttgacaca ctccaa 26

<210> 144
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> OSMRbeta probe ZC43141

<400> 144
ctgtgcgttg gaactggacg tctgatatc 29

<210> 145

<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43004

<400> 145
gaaacccgcc gcatattact t 21

<210> 146
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43005

<400> 146
tggcgttgct cacaaaggt 19

<210> 147
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Murine GUS probe ZC43018

<400> 147
acccacacca aagccctgga cctc 24

<210> 148
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC40269

<400> 148
gccagtttca cacactcctc ttt 23

<210> 149
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC40268

<400> 149
gcagctattg cactagtcat tttctt 26

<210> 150
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Transferrin receptor probe ZC40298

<400> 150
cccaggtagc cactcatgaa tccaatcaa 29

<210> 151
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43140

<400> 151
ttcttccaca gcaatcgcac 20

<210> 152
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC43139

<400> 152
atgcatgctt cggttcagaa c 21

<210> 153
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC41608

<400> 153
gcagacaatg atgctgagca agac 24

<210> 154
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC41609

<400> 154
caacgctgtg atttgcagtg gaag 24

<210> 155
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer ZC41502

<400> 155
agcgggcctt cctcactc 18

<210> 156
<211> 24
<212> DNA
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<220>
<223> Primer ZC41500

<400> 156

ggacaaaata aaaacataaa taac 24

<210> 157
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Linker

<400> 157
tgtcgatgaa gccctgaaag acgcgcagac taattcgagc 40

<210> 158
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Linker

<400> 158
acgcgcagac taattcgagc tcccaccatc accatcacca cgcgaattcg gtaccgctgg 60

<210> 159
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Linker

<400> 159
actcactata gggcgaattg cccgggggat ccacgcggaa ccagcggtac cgaattcgcg 60

<210> 160
<211> 42
<212> DNA
<213> Artificial Sequence

<220>

<223> Linker

<400> 160
acggccagtg aattgtaata cgactcacta tagggcgaat tg 42

<210> 161
<211> 825
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (48)...(518)

<400> 161
atcactctct ttaatcacta ctcacattaa cctcaactcc tgccaca atg tac agg 56
Met Tyr Arg
1

atg caa ctc ctg tct tgc att gca cta att ctt gca ctt gtc aca aac 104
Met Gln Leu Leu Ser Cys Ile Ala Leu Ile Leu Ala Leu Val Thr Asn
5 10 15

agt gca cct act tca agt tcg aca aag aaa aca aag aaa aca cag cta 152
Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Lys Lys Thr Gln Leu
20 25 30 35

caa ctg gag cat tta ctg ctg gat tta cag atg att ttg aat gga att 200
Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
40 45 50

aat aat tac aag aat ccc aaa ctc acc agg atg ctc aca ttt aag ttt 248
Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
55 60 65

tac atg ccc aag aag gcc aca gaa ctg aaa cag ctt cag tgt cta gaa 296
Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys Gln Leu Gln Cys Leu Glu
70 75 80

gaa gaa ctc aaa cct ctg gag gaa gtg ctg aat tta gct caa agc aaa 344
Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

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aac ttt cac tta aga ccc agg gac tta atc agc aat atc aac gta ata   392
Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100                 105                 110                 115

gtt ctg gaa cta aag gga tct gaa aca aca ttc atg tgt gaa tat gca   440
Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
                120                 125                 130

gat gag aca gca acc att gta gaa ttt ctg aac aga tgg att acc ttt   488
Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
            135                 140                 145

tgt caa agc atc atc tca aca cta act tga taattaagtg cttcccactt     538
Cys Gln Ser Ile Ile Ser Thr Leu Thr  *
        150                 155

aaaacatatc aggccttcta tttatttatt taaatattta aattttatat ttattgttga 598
atgtatggtt gctacctatt gtaactatta ttcttaatct taaaactata aatatggatc 658
ttttatgatt ctttttgtaa gccctagggg ctctaaaatg gtttacctta tttatcccaa 718
aaatatttat tattatgttg aatgttaaat atagtatcta tgtagattgg ttagtaaaac 778
tatttaataa atttgataaa tataaaaaaa aaaaacaaaa aaaaaaa               825

      <210> 162
      <211> 156
      <212> PRT
      <213> Homo sapiens

      <400> 162
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ile Leu Ala Leu
 1               5                  10                  15
Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Lys Lys
            20                  25                  30
Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu
        35                  40                  45
Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr
    50                  55                  60
Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys Gln Leu Gln
65                  70                  75                  80
Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala
                85                  90                  95
Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile
            100                 105                 110
Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys
```

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        115                 120                 125
Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp
    130                 135                 140
Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Thr
145                 150                 155
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<210> 163
<211> 614
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (64)...(525)

<400> 163

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gatcgttagc ttctcctgat aaactaattg cctcacattg tcactgcaaa tcgacaccta 60
tta atg ggt ctc acc tcc caa ctg ctt ccc cct ctg ttc ttc ctg cta   108
   Met Gly Leu Thr Ser Gln Leu Leu Pro Pro Leu Phe Phe Leu Leu
    1               5                  10                  15

gca tgt gcc ggc aac ttt gtc cac gga cac aag tgc gat atc acc tta   156
Ala Cys Ala Gly Asn Phe Val His Gly His Lys Cys Asp Ile Thr Leu
                20                  25                  30

cag gag atc atc aaa act ttg aac agc ctc aca gag cag aag act ctg   204
Gln Glu Ile Ile Lys Thr Leu Asn Ser Leu Thr Glu Gln Lys Thr Leu
            35                  40                  45

tgc acc gag ttg acc gta aca gac atc ttt gct gcc tcc aag aac aca   252
Cys Thr Glu Leu Thr Val Thr Asp Ile Phe Ala Ala Ser Lys Asn Thr
        50                  55                  60

act gag aag gaa acc ttc tgc agg gct gcg act gtg ctc cgg cag ttc   300
Thr Glu Lys Glu Thr Phe Cys Arg Ala Ala Thr Val Leu Arg Gln Phe
    65                  70                  75

tac agc cac cat gag aag gac act cgc tgc ctg ggt gcg act gca cag   348
Tyr Ser His His Glu Lys Asp Thr Arg Cys Leu Gly Ala Thr Ala Gln
80                  85                  90                  95

cag ttc cac agg cac aag cag ctg atc cga ttc ctg aaa cgg ctc gac   396
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Gln Phe His Arg His Lys Gln Leu Ile Arg Phe Leu Lys Arg Leu Asp
                100                 105                 110

agg aac ctc tgg ggc ctg gcg ggc ttg aat tcc tgt cct gtg aag gaa   444
Arg Asn Leu Trp Gly Leu Ala Gly Leu Asn Ser Cys Pro Val Lys Glu
        115                 120                 125

gcc aac cag agt acg ttg gaa aac ttc ttg gaa agg cta aag acg atc   492
Ala Asn Gln Ser Thr Leu Glu Asn Phe Leu Glu Arg Leu Lys Thr Ile
    130                 135                 140

atg aga gag aaa tat tca aag tgt tcg agc tga atattttaat ttatgagttt 545
Met Arg Glu Lys Tyr Ser Lys Cys Ser Ser  *
145                 150

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atagaatct                                                        614

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      <400> 164
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 1               5                  10                  15
Cys Ala Gly Asn Phe Val His Gly His Lys Cys Asp Ile Thr Leu Gln
            20                  25                  30
Glu Ile Ile Lys Thr Leu Asn Ser Leu Thr Glu Gln Lys Thr Leu Cys
        35                  40                  45
Thr Glu Leu Thr Val Thr Asp Ile Phe Ala Ala Ser Lys Asn Thr Thr
    50                  55                  60
Glu Lys Glu Thr Phe Cys Arg Ala Ala Thr Val Leu Arg Gln Phe Tyr
65                  70                  75                  80
Ser His His Glu Lys Asp Thr Arg Cys Leu Gly Ala Thr Ala Gln Gln
                85                  90                  95
Phe His Arg His Lys Gln Leu Ile Arg Phe Leu Lys Arg Leu Asp Arg
            100                 105                 110
Asn Leu Trp Gly Leu Ala Gly Leu Asn Ser Cys Pro Val Lys Glu Ala
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145 150

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         1               5                  10

agc atc tct gca ccc gcc cgc tcg ccc agc ccc agc acg cag ccc tgg   98
Ser Ile Ser Ala Pro Ala Arg Ser Pro Ser Pro Ser Thr Gln Pro Trp
 15                  20                  25                  30

gag cat gtg aat gcc atc cag gag gcc cgg cgt ctc ctg aac ctg agt   146
Glu His Val Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser
                35                  40                  45

aga gac act gct gct gag atg aat gaa aca gta gaa gtc atc tca gaa   194
Arg Asp Thr Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu
            50                  55                  60

atg ttt gac ctc cag gag ccg acc tgc cta cag acc cgc ctg gag ctg   242
Met Phe Asp Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu
        65                  70                  75

tac aag cag ggc ctg cgg ggc agc ctc acc aag ctc aag ggc ccc ttg   290
Tyr Lys Gln Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu
    80                  85                  90

acc atg atg gcc agc cac tac aag cag cac tgc cct cca acc ccg gaa   338
Thr Met Met Ala Ser His Tyr Lys Gln His Cys Pro Pro Thr Pro Glu
 95                  100                 105                 110

act tcc tgt gca acc cag act atc acc ttt gaa agt ttc aaa gag aac   386
Thr Ser Cys Ala Thr Gln Thr Ile Thr Phe Glu Ser Phe Lys Glu Asn
```

115 120 125

ctg aag gac ttt ctg ctt gtc atc ccc ttt gac tgc tgg gag cca gtc 434
Leu Lys Asp Phe Leu Leu Val Ile Pro Phe Asp Cys Trp Glu Pro Val
130 135 140

cag gag tga gaccggccag atgaggctgg ccaagccggg gagctgctct 483
Gln Glu *

ctcatgaaac aagagctaga aactcaggat ggtcatcttg gagggaccaa ggggtgggcc 543
acagccatgg tgggagtggc ctggacctgc cctgggccac actgaccctg atacaggcat 603
ggcagaagaa tgggaatatt ttatactgac agaaatcagt aatatttata tatttatatt 663
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<210> 166
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1 5 10 15
Ser Ala Pro Ala Arg Ser Pro Ser Pro Ser Thr Gln Pro Trp Glu His
20 25 30
Val Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser Arg Asp
35 40 45
Thr Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu Met Phe
50 55 60
Asp Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys
65 70 75 80
Gln Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu Thr Met
85 90 95
Met Ala Ser His Tyr Lys Gln His Cys Pro Pro Thr Pro Glu Thr Ser
100 105 110
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gcc aca gaa ctg aaa cag ctt cag nnn nnn gaa gaa gaa ctc aaa cct 48
Ala Thr Glu Leu Lys Gln Leu Gln Xaa Xaa Glu Glu Glu Leu Lys Pro
1 5 10 15

ctg gag gaa gtg 60
Leu Glu Glu Val
20

<210> 168
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<220>
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<223> Any amino acid

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<400> 168
Ala Thr Glu Leu Lys Gln Leu Gln Xaa Xaa Glu Glu Glu Leu Lys Pro
1 5 10 15

Leu Glu Glu Val
20

CLAIMS

We claim:

1. An isolated polypeptide comprising a sequence of amino acid residues that is at least 90% identical to the sequence of amino acid residues from the group of:

(a) the polypeptide shown from residues 38 (Val) to 152 (Leu) as shown in SEQ ID NO:2;

(b) the polypeptide shown from residues 27 (Leu) to 164 (Thr) as shown in SEQ ID NO:2;

(c) the polypeptide shown from residues 24 (Thr) to 164 (Thr) as shown in SEQ ID NO:2; and

(d) the polypeptide shown from residues 1 (Met) to 164 (Thr) as shown in SEQ ID NO:2.

2. The isolated polypeptide of claim 1, wherein amino acid residues 73, 133 and 147 are cysteine.

3. The isolated polypeptide of claim 1, wherein the polypeptide binds the zcytor17 receptor as shown in SEQ ID NO:5 or SEQ ID NO:71.

4. An isolated polypeptide comprising at least 14 contiguous amino acid residues of SEQ ID NO:2 or SEQ ID NO:11.

5. The isolated polypeptide of claim 4, wherein the amino acid residues are from the group of:

(a) amino acid residues 38-52 of SEQ ID NO:2;

(b) amino acid residues 83-98 of SEQ ID NO:2;

(c) amino acid residues 104-117 of SEQ ID NO:2; and

(d) amino acid residues 137-152 of SEQ ID NO:2.

6. A fusion protein comprising at least four polypeptides, wherein the order of polypeptides from N-terminus to C-terminus are:

a first polypeptide that comprises a sequence of amino acid residues from 38-52 of SEQ ID NO:2;

a first spacer of 6-27 amino acid residues;

a second polypeptide that comprises a sequence of amino acid residues from the group of:

(a) IL-2 helix B amino acid residues of SEQ ID NO:168;

(b) IL-4 helix B residues 65-83 of SEQ ID NO:164;

(c) IL-3 helix B residues 73-86 of SEQ ID NO:102;

(d) GM-CSF helix B residues 72-81 of SEQ ID NO:166; and

(e) amino acid residues 83-98 of SEQ ID NO:2;

a second spacer of 5-11 amino acid residues;

a third polypeptide that comprises a sequence of amino acid residues from the group of:

(a) IL-2 helix C residues 102-116 of SEQ ID NO:162;

(b) IL-4 helix C residues 94-118 of SEQ ID NO:164;

(c) IL-3 helix C residues 91-103 of SEQ ID NO:102;

(d) GM-CSF helix C residues 85-103 of SEQ ID NO:166; and

(e) amino acid residues 104-117 of SEQ ID NO:2;

a third spacer of 3-29 amino acid residues; and

a fourth polypeptide that comprises a sequence of amino acid residues from the group of:

(a) IL-2 helix D residues 134-149 of SEQ ID NO:162 ;

(b) IL-3 helix D residues 123-141 of SEQ ID NO:102;

(c) IL-4 helix D residues 133-151 of SEQ ID NO:164;

(d) GM-CSF helix D residues 120-131 of SEQ ID NO:166; and

(e) amino acid residues 137-152 of SEQ ID NO:2.

7. A fusion protein comprising at least four polypeptides, wherein the order of polypeptides from N-terminus to C-terminus are:

a first polypeptide that comprises a sequence of amino acid residues selected from a group consisting of:

(a) IL-2 helix A residues 27-48 of SEQ ID NO:162;

(b) IL-4 helix A residues 30-42 of SEQ ID NO:164;

(c) IL-3 helix A residues 35-45 of SEQ ID NO:102;

(d) GM-CSF helix A residues 30-44 of SEQ ID NO:166; and

(e) amino acids residues 38-52 of SEQ ID NO:2;

a first spacer of 6-27 amino acid residues;

a second polypeptide that comprises a sequence of amino acid residues from the group of:

(a) IL-2 helix B residues of SEQ ID NO:168;

(b); IL-4 helix B residues 65-83 of SEQ ID NO:164;

(c) IL-3 helix B residues 73-86 of SEQ ID NO:102;

(d) GM-CSF helix B residues 72-81 of SEQ ID NO:166; and

(e) amino acid residues 83-98 of SEQ ID NO:2;

a second spacer of 5-11 amino acid residues;

a third polypeptide that comprises a sequence of amino acid residues from the group of:

(a) IL-2 helix C residues 102-116 of SEQ ID NO:162;

(b) IL-4 helix C residues 94-118 of SEQ ID NO:164;

(c) IL-3 helix C residues 91-103 of SEQ ID NO:102;

(d) GM-CSF helix C residues 85-103 of SEQ ID NO:166; and

(e) amino acid residues 104-117 of SEQ ID NO:2;

a third spacer of 3-29 amino acid residues; and

a fourth polypeptide that comprises a sequence of amino acid residues from 137-152 of SEQ ID NO:2.

8. A fusion protein according to claim 6, wherein the fourth polypeptide comprises amino acid residues 137-152 of SEQ ID NO:2.

9. An isolated polynucleotide molecule comprising a sequence of nucleotides that encode the polypeptide of claim 1.

10. The isolated polynucleotide molecule of claim 9, wherein the nucleotides are from the group of:

(a) a polynucleotide as shown in SEQ ID NO: 1 from nucleotide 139 to nucleotide 483;

(b) a polynucleotide as shown in SEQ ID NO: 1 from nucleotide 106 to nucleotide 519;

(c) a polynucleotide as shown in SEQ ID NO: 1 from nucleotide 97 to nucleotide 519; and

(d) a polynucleotide as shown in SEQ ID NO: 1 from nucleotide 28 to nucleotide 519.

11. An isolated polynucleotide molecule comprising a sequence of nucleotides that encode for the polypeptide of claim 4.

12. An expression vector comprising the following operably linked elements:

(a) a transcription promoter;

(b) a DNA segment encoding a polypeptide comprising a sequence of amino acid residues from the group of:

(a) amino acid residues 38-52 of SEQ ID NO:2;

(b) amino acid residues 83-98 of SEQ ID NO:2;

(c) amino acid residues 104-117 of SEQ ID NO:2; and

(d) amino acid residues 137-152 of SEQ ID NO:2; and

(c) a transcription terminator.

13. An expression vector comprising the following operably linked elements:

(a) a transcription promoter;

(b) a DNA segment encoding a polypeptide comprising a sequence of amino acid residues that is at least 90% identical to residues 38 (Val) to 152 (Leu) as shown in SEQ ID NO:2; and

(c) a transcription terminator.

14. An expression vector according to claim 13, comprising the following operably linked elements:

(a) a transcription promoter;

(b) a DNA segment encoding a polypeptide comprising amino acid residues 38 (Val) to 152 (Leu) of SEQ ID NO:2; and

(c) a transcription terminator.

15. A cultured cell comprising the expression vector according claim 13.

16. A method of producing a protein comprising:

culturing a cell according to claim 15 under conditions wherein the DNA segment is expressed; and

recovering the protein encoded by the DNA segment.

17. A method of producing an antibody to a zcytor17lig polypeptide comprising:

inoculating an animal with a polypeptide from the group of:

(a) a polypeptide consisting of 9 to 141 amino acids, wherein the polypeptide is identical to a contiguous sequence of amino acid residues in SEQ ID NO:2 from amino acid number 24 (Ala) to amino acid number 164 (Thr);

(b) a polypeptide according to claim 1;

(c) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 38-52;

(d) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 83-98;

(e) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 104-117;

(f) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 137-152;

(g) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 38-152;

(h) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 from amino acid number 24-164;

(c) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 38-52;

(d) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 85-98;

(e) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 104-118;

(f) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 141-157;

(g) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 38-157;

(h) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 from amino acid number 24-163;

(i) a polypeptide comprising an antigenic epitope according to a Hopp/Woods hydrophilicity profile of SEQ ID NO:2 or SEQ ID NO 11, wherein the profile is based on a sliding six-residue window. Buried G, S, and T residues and exposed H, Y, and W residues ignored; and

wherein the polypeptide elicits an immune response in the animal to produce the antibody; and

isolating the antibody from the animal.

18. An antibody produced by the method of claim 17, wherein the antibody binds to a polypeptide of SEQ ID NO:2 or SEQ ID NO:11.

19. An antibody which specifically binds to a polypeptide shown in SEQ ID NO:2 or SEQ ID NO:11.

20. A method of stimulating an immune response in a mammal exposed to an antigen or pathogen comprising the steps of:

(1) determining directly or indirectly the level of antigen or pathogen present in said mammal;

(2) administering a composition comprising zcytor17lig polypeptide in an acceptable pharmaceutical carrier;

(3) determining directly or indirectly the level of antigen or pathogen in said mammal; and

(4) comparing the level of the antigen or pathogen in step 1 to the antigen or pathogen level in step 3, wherein a change in the level is indicative of stimulating an immune response.

21. The method of claim 20, further comprising:

(5) re-administering a composition comprising zcytor17lig polypeptide in an acceptable pharmaceutical carrier;

(6) determining directly or indirectly the level of antigen or pathogen in said mammal; and;

(7) comparing the number of comparing the antigen or pathogen level in step 1 to the antigen level in step 6, wherein a change in the level is indicative of stimulating an immune response.

22. A method for expansion of hematopoietic cells and hematopoietic cell progenitors comprising culturing bone marrow or peripheral blood cells with a composition comprising an amount of zcytor17lig sufficient to produce an increase in the number of lymphoid cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of zcytor17lig.

23 The method of claim 22, wherein the hematopoietic cells and hemopoietic progenitor cells are lymphoid cells.

24. The method of claim 23, wherein the lymphoid cells are monocytic cells, macrophages or T cells.

25. A method of stimulating an immune response in a mammal exposed to an antigen or pathogen comprising:

(1) determining a level of an antigen- or pathogen-specific antibody;

(2) administering a composition comprising zcytor17lig polypeptide in an acceptable pharmaceutical carrier;

(3) determining a post administration level of antigen- or pathogen-specific antibody;

(4) comparing the level of antibody in step (1) to the level of antibody in step (3), wherein an increase in antibody level is indicative of stimulating an immune response.

26. A method of detecting the presence of zcytor17lig RNA in a biological sample, comprising the steps of :

(a) contacting a zcytor17lig nucleic acid probe under hybridizing conditions with either (i) test RNA molecules isolated from the biological sample, or (ii) nucleic acid molecules synthesized from the isolated RNA molecules, wherein the probe has a nucleotide sequence comprising either a portion of the nucleotide sequence of the nucleic acid molecule of claim 10, or its complement, and

(b) detecting the formation of hybrids of the nucleic acid probe and either the test RNA molecules or the synthesized nucleic acid molecules,

wherein the presence of the hybrids indicates the presence of zcytor17lig RNA in the biological sample.

27. A method of detecting the presence of zcytor17lig in a biological sample, comprising the steps of:

(a) contacting the biological sample with an antibody, or an antibody fragment, of claim 19, wherein the contacting is performed under conditions that allow the binding of the antibody or antibody fragment to the biological sample, and

(b) detecting any of the bound antibody or bound antibody fragment.

28. A method of killing cancer cells comprising,

obtaining *ex vivo* a tissue or biological sample containing cancer cells from a patient, or identifying cancer cells *in vivo*;

producing a polypeptide by the method of claim 16;

formulating the polypeptide in a pharmaceutically acceptable vehicle; and

administering to the patient or exposing the cancer cells to the polypeptide;

wherein the polypeptide kills the cells.

29. A method of killing cancer cells of claim 28, wherein the polypeptide is further conjugated to a toxin.

30. The antibody of claim 19, wherein the antibody is from the group of: (a) polyclonal antibody, (b) murine monoclonal antibody, (c) humanized antibody derived from (b), (d) an antibody fragment, and (e) human monoclonal antibody.

31. An antibody or antibody fragment that specifically binds to a polypeptide of comprising a sequence of amino acid residues from the group of:

(a) the polypeptide shown from residues 38 (Val) to 152 (Leu) as shown in SEQ ID NO:2;

(b) the polypeptide shown from residues 27 (Leu) to 164 (Thr) as shown in SEQ ID NO:2;

(c) the polypeptide shown from residues 24 (Thr) to 164 (Thr) as shown in SEQ ID NO:2; and

(d) the polypeptide shown from residues 1 (Met) to 164 (Thr) as shown in SEQ ID NO:2.

32. The antibody of claim 19, wherein the antibody further comprises a radionuclide, enzyme, substrate, cofactor, fluorescent marker, chemiluminescent marker, peptide tag, magnetic particle, drug, or toxin.

33. A method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic cell progenitors comprising culturing bone marrow or peripheral blood cells with a composition comprising an amount of an antibody according to claim 19 sufficient to reduce proliferation or differentiation of the hematopoietic cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of soluble cytokine receptor.

34. The method of claim 33, wherein the hematopoietic cells and hematopoietic progenitor cells are lymphoid cells.

35. The method of claim 34, wherein the lymphoid cells are macrophages or T cells.

36. A method of reducing zcytor17lig-induced induced inflammation comprising administering to a mammal with inflammation an amount of a composition of a an antibody according to claim 19 sufficient to reduce inflammation.

37. A method of suppressing an inflammatory response in a mammal with inflammation comprising:

(1) determining a level of an inflammatory molecule;

(2) administering a composition comprising an antibody according to claim 19 in an acceptable pharmaceutical vehicle;

(3) determining a post administration level of the inflammatory molecule;

(4) comparing the level of the inflammatory molecule in step (1) to the level of the inflammatory molecule in step (3), wherein a lack of increase or a decrease the inflammatory molecule level is indicative of suppressing an inflammatory response.

38. The antibody of claim 31, wherein the antibody further comprises a radionuclide, enzyme, substrate, cofactor, fluorescent marker, chemiluminescent marker, peptide tag, magnetic particle, drug, or toxin.

39. A method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic cell progenitors comprising culturing bone marrow or peripheral blood cells with a composition comprising an amount of an antibody according to claim 31 sufficient to reduce proliferation or differentiation of the hematopoietic cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of soluble cytokine receptor.

40. The method of claim 39, wherein the hematopoietic cells and hematopoietic progenitor cells are lymphoid cells.

41. The method of claim 40, wherein the lymphoid cells are macrophages or T cells.

42. A method of reducing zcytor17lig-induced induced inflammation comprising administering to a mammal with inflammation an amount of a composition of a an antibody according to claim 31 sufficient to reduce inflammation.

43. A method of suppressing an inflammatory response in a mammal with inflammation comprising:

(1) determining a level of an inflammatory molecule;

(2) administering a composition comprising an antibody according to claim 31 in an acceptable pharmaceutical vehicle;

(3) determining a post administration level of the inflammatory molecule;

(4) comparing the level of the inflammatory molecule in step (1) to the level of the inflammatory molecule in step (3), wherein a lack of increase or a decrease in the inflammatory molecule level is indicative of suppressing an inflammatory response.

44. A method of treating a mammal afflicted with an inflammatory disease in which zcytor17lig plays a role, comprising:

administering an antagonist of zcytor17lig to the mammal such that the inflammation is reduced, wherein the antagonist is from the group of an antibody or binding polypeptide that specifically binds a polypeptide or polypeptide fragment of zcytor17lig (SEQ ID NO:2).

45. A method of claim 44, wherein the disease is a chronic inflammatory disease.

46. A method of claim 45, wherein the disease is a chronic inflammatory disease from the group of:

(a) inflammatory bowel disease;

(b) ulcerative colitis;

(c) Crohn's disease;

(d) atopic dermatitis;

(e) eczema; and

(f) psoriasis.

47. A method of claim 44, wherein the disease is an acute inflammatory disease.

48. A method of claim 47, wherein the disease is an acute inflammatory disease from the group of:

(a) endotoxemia;

(b) septicemia;

(c) toxic shock syndrome; and

(d) infectious disease.

49. A method of claim 44, wherein the antibody further comprises a radionuclide, enzyme, substrate, cofactor, fluorescent marker, chemiluminescent marker, peptide tag, magnetic particle, drug, or toxin.

50. A method for detecting inflammation in a patient, comprising:

obtaining a tissue or biological sample from a patient;

incubating the tissue or biological sample with an antibody of claim 19 under conditions wherein the antibody binds to its complementary polypeptide in the tissue or biological sample;

visualizing the antibody bound in the tissue or biological sample; and

comparing levels of antibody bound in the tissue or biological sample from the patient to a normal control tissue or biological sample,

wherein an increase in the level of antibody bound to the patient tissue or biological sample relative to the normal control tissue or biological sample is indicative of inflammation in the patient.

51. A method for detecting inflammation in a patient, comprising:

obtaining a tissue or biological sample from a patient;

labeling a polynucleotide comprising at least 14 contiguous nucleotides of SEQ ID NO:1 or the complement of SEQ ID NO:1;

incubating the tissue or biological sample with under conditions wherein the polynucleotide will hybridize to complementary polynucleotide sequence;

visualizing the labeled polynucleotide in the tissue or biological sample; and

comparing the level of labeled polynucleotide hybridization in the tissue or biological sample from the patient to a normal control tissue or biological sample,

wherein an increase in the labeled polynucleotide hybridization to the patient tissue or biological sample relative to the normal control tissue or biological sample is indicative of inflammation in the patient.

```
                1               15 16              30 31              45
1 zcytor17lig   ---ASHTLPVRLLRP SDDVQKIVEELQSLS KMLLKD--VEEEKGV      40
2 mzcytor17lig  ---ATCSLSFGAPIS KEDLRTTIDLLKQES QDLYNNYSIKQASGM      42
3 mIL-3         ASISGRDTHRLTRTL NCS-SIVKEIIGKL- --PEP----ELKT--      35
4 hIL-3         APMTQTTPLKTSW-V NCS-NMIDEIITHLK QPPLP--LLDFNNLN      41

                46              60 61              75 76              90
1 zcytor17lig   LVSQNYTLPCLSPDA QPPNNIHSPAIRAYL KTIRQLDNKSVIDEI      85
2 mzcytor17lig  SADESIQLPCFSLDR EALTNISVIIAHLEK VKVLSE-NTVDTSWV      86
3 mIL-3         DDEGPSLRNKS---- -----FRRVNLSKFV ESQGEVDPEDRYVIK      71
4 hIL-3         GEDQDILMENN---- -----LRRPNLEAFN RAVKSL--QNASAIE      75

                91             105 106             120 121             135
1 zcytor17lig   IEHLDKLIFQDAPET NISVPTDTHE---CK RFILTISQQFSECMD      127
2 mzcytor17lig  IRWLTNISCFNPLNL NISVPGNTDESYDCK VFVLTVLKQFSNCMA      131
3 mIL-3         SNLQKLNCCLPTSAN DSALPGVFIRDLD-- DFRKKLRFYMVH-LN      113
4 hIL-3         SILKNLLPCLPLATA APTRHPIHIKDGDWN EFRRKLTFY----LK      116

                136             150 151             165 166             180
1 zcytor17lig   LALKSLTSGAQQATT                                      142
2 mzcytor17lig  ELQAKDNTTC                                          141
3 mIL-3         DLETVLTSRPPQPAS GSVSPNRGTVEC                        140
4 hIL-3         TLENA----QAQQTT LSLAIF                              133
```

FIG. 1

```
                10        20        30        40        50
ZCYTOR MASHSGPSTSVLFLFCCLGGWLASHTLPVRLLRPSDDVQKIVEELQSLSKMLLKD--VEE
       :  :.:....: :.:.:.:::. .:.    ..:..... :.  :. : .. ...
M17RL- MIFHTGTTKPTLVLLCCIGTWLATCSLSFGAPISKEDLRTTIDLLKQESQDLYNNYSIKQ
               10        20        30        40        50        60

       60        70        80        90        100       110
ZCYTOR EKGVLVSQNYTLPCLSPDAQPPNNIHSPAIRAYLKTIRQLDNKSVIDE-IIEHLDKLIFQ
       ..:. ....  :::.: : .. .::  ..: :.:.... :....: .. .:  :...
M17RL- ASGMSADESIQLPCFSLDREALTNI--SVIIAHLEKVKVLSENTVDTSWVIRWLTNISCF
               70    .   80        90        100       110

       120       130          140       150       160
ZCYTOR DAPETNISVPTDTHE---CKRFILTISQQFSECM-DLALKSLTSGAQQATT
       .. . :::::.:.:   X: :.::. .::::X .:. :. :.
M17RL- NPLNLNISVPGNTDESYDCKVFVLTVLKQFSNCMAELQAKDNTTC
     120       130       140       150       160
```

FIG. 2

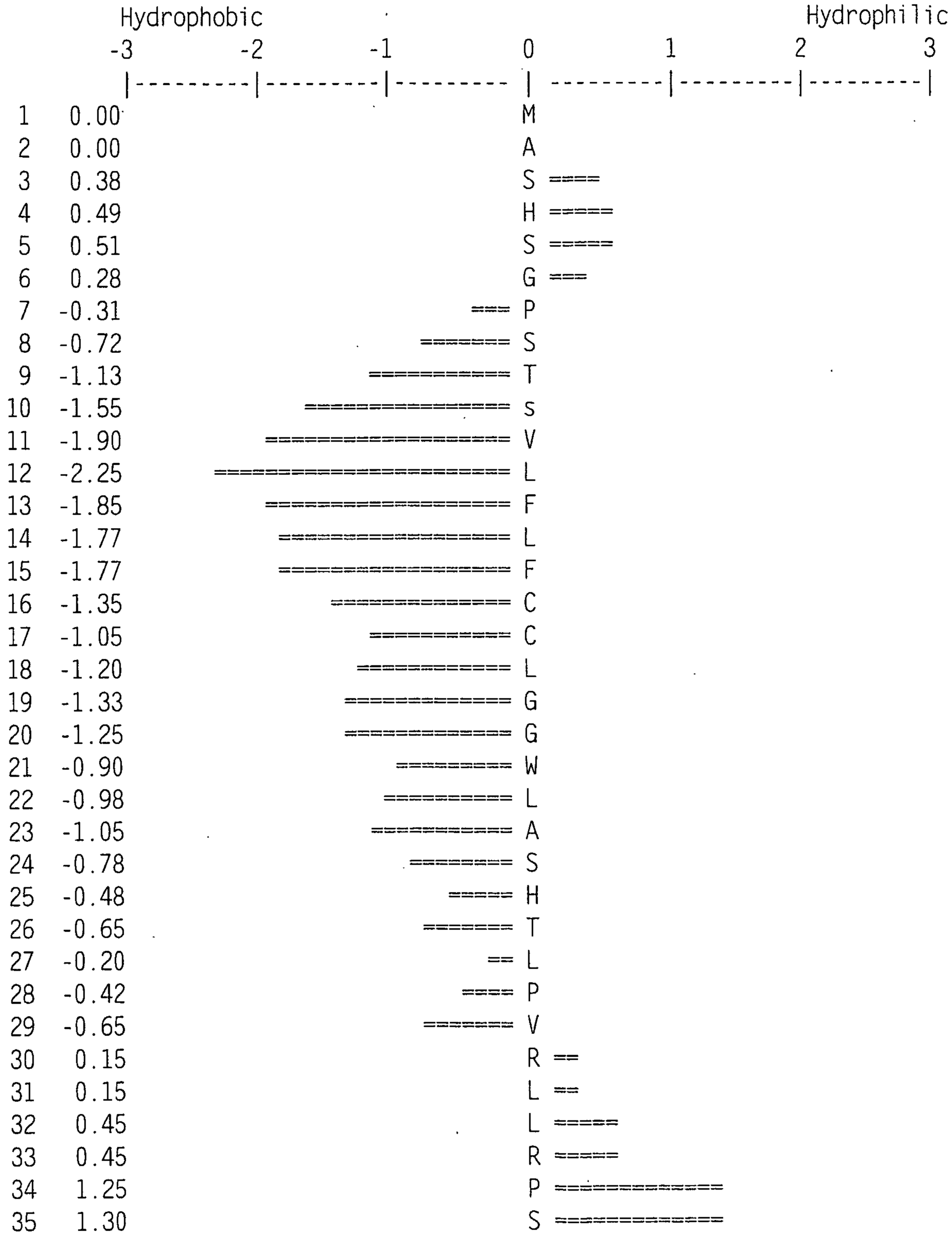

Hydrophobic
Hydrophilic
-3 -2 -1 0 1 2 3
1 0.00 M
2 0.00 A
3 0.38 S ====
4 0.49 H =====
5 0.51 S =====
6 0.28 G ===
7 -0.31 === P
8 -0.72 ======= S
9 -1.13 ========== T
10 -1.55 ================ s
11 -1.90 ================== V
12 -2.25 ====================== L
13 -1.85 ================== F
14 -1.77 ================= L
15 -1.77 ================== F
16 -1.35 ============= C
17 -1.05 ========== C
18 -1.20 ========== L
19 -1.33 ============ G
20 -1.25 ============ G
21 -0.90 ========= W
22 -0.98 ========== L
23 -1.05 ========== A
24 -0.78 ======= S
25 -0.48 ===== H
26 -0.65 ======= T
27 -0.20 == L
28 -0.42 ==== P
29 -0.65 ======= V
30 0.15 R ==
31 0.15 L ==
32 0.45 L =====
33 0.45 R =====
34 1.25 P ==========
35 1.30 S ==========

FIG. 3A

```
36   0.83                                    D ========
37   1.33                                    D ============
38   0.98                                    V ==========
39   0.23                                    Q ==
40   0.23                                    K ==
41   0.98                                    I ==========
42   0.65                                    V =======
43   0.18                                    E ==
44   0.53                                    E =====
45   0.48                                    L =====
46   0.03                                    Q
47   0.03                                    S
48   0.12                                    L =
49  -0.22                                 == S
50  -0.57                                 ====== K
51   0.23                                    M ==
52   0.68                                    L =======
53  -0.07                                  = L
54   0.65                                    K =======
55   1.45                                    D ==============
56   2.25                                    V ========================
57   2.25                                    E ========================
58   1.75                                    E ==================
59   1.75                                    E ==================
60   0.95                                    K =========
61   0.20                                    G ==
62  -0.25                                 === V
63  -0.72                                 ======= L
64  -0.68                                 ======= V
65  -0.42                                 ==== S
66  -0.18                                  == Q
67  -0.23                                  == N
68  -0.28                                  === y
69  -0.48                                  ===== T
70  -0.82                                 ======== L
71  -0.78                                 ======== P
72  -0.72                                  ====== C
73   0.08                                    L =
74   0.00                                    S
```

FIG. 3B

```
75    0.20                                    P ==
76    0.50                                    D =====
77    0.45                                    A =====
78    0.48                                    Q =====
79    0.02                                    P
80   -0.20                                 == P
81   -0.32                                 === N
82   -0.27                                 === N
83   -0.27                                 === I
84   -0.38                                 ==== H
85   -0.72                                 ======= S
86    0.08                                     P =
87    0.08                                     A =
88   -0.35                                 ==== I
89   -0.65                                 ======= R
90   -0.07                                   = A
91    0.17                                     Y ==
92   -0.63                                 ====== L
93   -0.05                                    K
94    0.37                                    T ====
95    0.37                                    I ====
96    0.37                                    R ====
97    0.47                                    Q =====
98    1.27                                    L ============
99    0.82                                    D ========
100   0.53                                    N =====
101   0.53                                    K =====
102   0.53                                    S =====
103   1.00                                    V ==========
104   0.20                                    I ==
105  -0.15                                 = D
106   0.60                                    E ======
107   0.82                                    I ========
108   0.02                                    I
109   0.02                                    E
110   0.82                                    H ========
111   0.82                                    L ========
112   0.02                                    D
```

FIG. 3C

```
113   -0.32                                    === K
114    0.02                                      L
115    0.02                                      I
116   -0.57                                ====== F
117   -0.27                                    === Q
118    0.53                                      D =====
119    0.88                                      A =========
120    0.88                                      P ========
121    0.08                                      E =
122    0.22                                      T ==
123   -0.03                                      N
124   -0.53                                 ===== I
125   -0.53                                 ===== S
126   -0.07                                     = V
127    0.17                                      P ==
128    0.43                                      T ====
129    1.18                                      D ==========
130    1.02                                      T =========
131    1.58                                      h ===============
132    1.58                                      E ===============
133    1.23                                      C ==========
134    0.62                                      K ======
135   -0.18                                   == R
136   -0.58                                ====== F
137   -1.38                             ========== I
138   -1.83                          ============== L
139   -1.38                             ========== t
140   -1.05                              ========= I
141   -1.17                             ========== S
142   -0.55                                ====== Q
143    0.25                                      Q ===
144    0.03                                      F
145   -0.22                                   == S
146    0.25                                      E ===
147    0.37                                      C ====
148    0.23                                    M ==
149   -0.57                                ====== D
150    0.10                                      L =
```

FIG. 3D

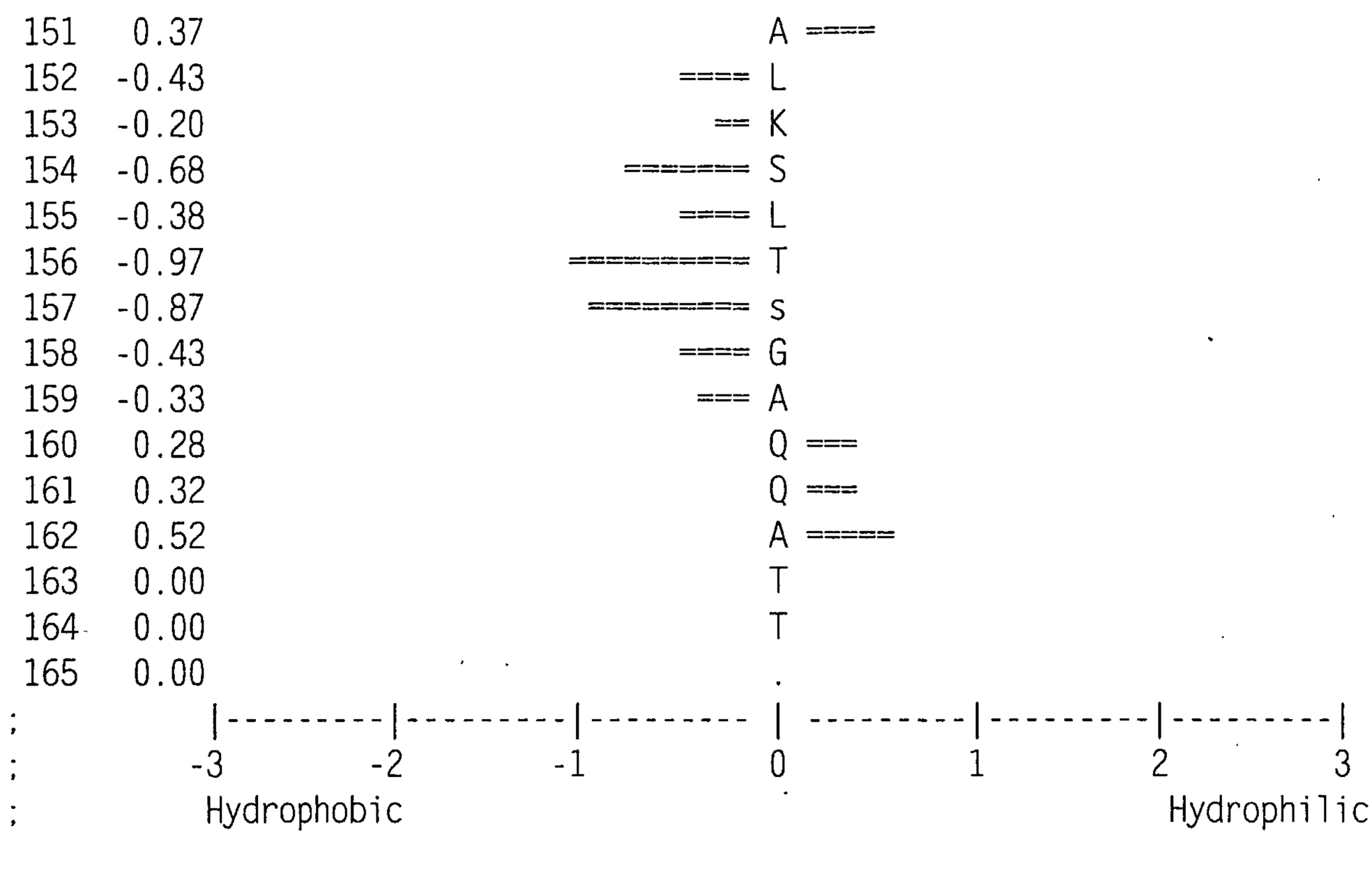

151 0.37 A ====
152 -0.43 ==== L
153 -0.20 == K
154 -0.68 ======= S
155 -0.38 ==== L
156 -0.97 ========== T
157 -0.87 ========= s
158 -0.43 ==== G
159 -0.33 === A
160 0.28 Q ===
161 0.32 Q ===
162 0.52 A =====
163 0.00 T
164 0.00 T
165 0.00 .
-3 -2 -1 0 1 2 3
Hydrophobic
Hydrophilic

FIG. 3E