



(86) Date de dépôt PCT/PCT Filing Date: 2000/03/03
(87) Date publication PCT/PCT Publication Date: 2000/09/08
(45) Date de délivrance/Issue Date: 2014/06/17
(85) Entrée phase nationale/National Entry: 2001/09/13
(86) N° demande PCT/PCT Application No.: US 2000/005452
(87) N° publication PCT/PCT Publication No.: 2000/052047
(30) Priorité/Priority: 1999/03/05 (US09/263,022)

(51) Cl.Int./Int.Cl. *C12N 15/12* (2006.01),
A61K 38/17 (2006.01), *C07K 14/47* (2006.01),
C07K 16/18 (2006.01), *C12P 21/00* (2006.01),
C12Q 1/68 (2006.01), *G01N 33/50* (2006.01),
G01N 33/53 (2006.01), *G01N 33/566* (2006.01),
A61K 38/00 (2006.01)
(72) Inventeur/Inventor:
MCCARTHY, SEAN, US
(73) Propriétaire/Owner:
MILLENNIUM PHARMACEUTICALS, INC., US
(74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : PROTEINE HUMAINE ET MOLECULES D'ACIDE NUCLEIQUE ASSOCIEES A DICKKOPF ET LEURS
UTILISATIONS
(54) Title: HUMAN DICKKOPF-RELATED PROTEIN AND NUCLEIC ACID MOLECULES AND USES THEREFOR

(57) **Abrégé/Abstract:**

Novel Dkk and Dkk-related polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated, full-length Dkk and Dkk-related proteins, the invention further provides isolated fusion proteins, antigenic peptides and antibodies. The invention also provides Dkk and Dkk-related nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a Dkk and Dkk-related gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.



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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 September 2000 (08.09.2000)

PCT

(10) International Publication Number
WO 00/52047 A3

- (51) International Patent Classification⁷: C07K 14/47, C12N 15/12, C07K 16/18, G01N 33/50, 33/53, C12Q 1/68, A61K 38/17
- (74) Agents: MANDRAGOURAS, Amy, E.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 et al. (US).
- (21) International Application Number: PCT/US00/05452
- (81) Designated States (*national*): AU, CA, JP.
- (22) International Filing Date: 3 March 2000 (03.03.2000)
- (84) Designated States (*regional*): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
09/263,022 5 March 1999 (05.03.1999) US
- (71) Applicant: MILLENNIUM PHARMACEUTICALS, INC. [US/US]; 75 Sidney Street, Cambridge, MA 02139 (US).
- (88) Date of publication of the international search report:
1 February 2001
- (72) Inventor: MCCARTHY, Sean; 62 Commonwealth Avenue, Apartment #4, Boston, MA 02116 (US).
- Published:
— With international search report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 00/52047 A3

(54) Title: HUMAN DICKKOPF-RELATED PROTEIN AND NUCLEIC ACID MOLECULES AND USES THEREFOR

(57) Abstract: Novel Dkk and Dkk-related polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated, full-length Dkk and Dkk-related proteins, the invention further provides isolated fusion proteins, antigenic peptides and antibodies. The invention also provides Dkk and Dkk-related nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a Dkk and Dkk-related gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

HUMAN DICKKOPF-RELATED PROTEIN AND NUCLEIC ACID MOLECULES AND USES THEREFOR

Background of the Invention

5 Secreted proteins play an integral role in the formation, differentiation, and maintenance of cells in multicellular organisms. For instance, secretory proteins are known in the art to be involved in signaling between cells which are not in direct contact. Such secreted signaling molecules are particularly important in the development of vertebrate tissue during embryogenesis as well as in the maintenance of
10 the differentiated state of adult tissues. For example, inductive interactions that occur between neighboring cell layers and tissues in the developing embryo are largely dependent on the existence and regulation of secreted signaling molecules. In inductive interactions, biochemical signals secreted by one cell population influence the developmental fate of a second cell population, typically by altering the fate of the
15 second cell population. For example, the Wnt proteins are now recognized as one of the major families of developmentally important signaling molecules in organisms ranging from *Drosophila* to mice.

 The Wnt gene family encode a large class of secreted proteins related to the *Int1/Wnt1* proto-oncogene and *Drosophila* wingless ("Wg"), a *Drosophila* Wnt1
20 homologue, (Cadigan *et al.* (1997) *Genes & Development* 11:3286-3305). Wnts are expressed in a variety of tissues and organs and are required for many developmental processes, including segmentation in *Drosophila*, endoderm development in *Caenorhabditis elegans*, establishment of limb polarity, neural crest differentiation, kidney morphogenesis, sex determination, and brain development in mammals
25 (reviewed in Parr and McMahon (1994) *Curr. Opinion Genetics & Devel.* 4:523-528; Cadigan and Nusse, *supra*).

 Recent studies in diverse organisms have led to identification of several components of the Wnt signal transduction pathway in responding cells (Cadigan and Nusse, *supra*). Wnt signals are transduced by the Frizzled ("Fz") family of seven
30 transmembrane domain receptors (Bhanot *et al.* (1996) *Nature* 382:225-230). The resulting signal leads to the activation of the cytoplasmic protein Dishevelled (Dsh) and

- 2 -

stabilization of Armadillo/ β -catenin (Perrimon (1994) *Cell* 76:781-784). Negative regulators of the Wnt pathway include glycogen synthase kinase 3 (GSK3)/shaggy (Perrimon, *supra*), the tumor suppressor gene product adenomatous polyposis coli (APC) (Gumbiner (1997) *Curr. Biol.* 7:R443-436) and a novel protein, called Axin
5 (Zeng *et al.* (1997) *Cell* 90:181-192). In the absence of a Wnt ligand, these proteins promote phosphorylation and then degradation of β -catenin, whereas Wnt signaling inactivates GSK3, thus preventing β -catenin degradation. As a result, β -catenin is translocated to the nucleus, where it forms a complex with TCF transcription factors and activates target gene expression (Cadigan and Nusse, *supra*). Deregulation of this
10 pathway can lead to carcinogenesis (reviewed by Gumbiner, *supra*), emphasizing the long-recognized connection between Wnts, normal development and cancer. This connection has been further established recently with the identification the c-Myc proto-oncogene as a target of Wnt signaling (He *et al.* (1998) *Science* 281:1509-3512).

While the outcome of Wnt signaling may be influenced by multiple intracellular
15 regulatory mechanisms, recent studies have identified several classes of secreted factors which can modulate Wnt action outside of the cell. These include Cerberus, a secreted Wnt inhibitor implicated in head development (Bouwmeester *et al.* (1996) *Nature* 382:595-601), and a family of proteins related to the extracellular domain of Frizzled. These Frizzled-related proteins ("FRPs") (Rattner *et al.* (1997) *Proc. Natl. Acad. Sci.*
20 *USA* 94:2859-2863), also known as secreted apoptosis-related proteins ("SARPs"), are encoded by several independently discovered genes including FrzA/FRP1, SDF5/FRP2, FrzB/FRP3, FRP4 and Sizzled (Melkonyan *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94:13636-13641; Finch *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94:6770-6775; Wang *et al.* (1997) *Cell* 88:747-766; Leyns *et al.* (1997) *Cell* 88:747-756; Mayr *et al.* (1997)
25 *Mech. Dev.* 63:109-325; and Salic *et al.* (1997) *Development* 124:4739-4748). These proteins inhibit the ability of Xwnt8 to induce a secondary axis in frog embryos (for review see Zorn (1997) *Curr. Biol.* 7:R501-504), and are thought to compete for binding of Wnt ligands to the Frizzled receptors. Data on binding of certain FRPs to Xwnt8 (Wang *et al.*, (1997) *Biochem. Biophys. Res. Comm.* 236:502-504; and Leyns *et al.*,
30 *supra*) and Wg corroborate this notion (Rattner *et al.*, *supra*).

- 3 -

It is now recognized that many of these families of signaling molecules have a dual role to play in both the development of an organism as well as in promoting or maintaining the differentiated state of tissues in the adult animal. Furthermore, major families of signaling molecules have been implicated in controlling proliferation of cells in mature adult tissue, for example, during normal cell turnover in the adult organism as well as in tissue regeneration activated as a result of damage to the adult tissue. Given the important role of these signalling molecules such as the Wnts and FRPs in both developing and adult tissues, there exists a need for identifying novel modulators of such molecules for use in regulating a variety of cellular processes.

Summary of the Invention

The present invention is based, at least in part, on the discovery of nucleic acid molecules which encode a novel family of secreted human proteins, referred to herein as the human Dickkopf proteins or "hDkks" (formerly referred to as the "Cysteine-Rich Secreted Proteins", "CRSPs", "CRISPYs", or "CRSP proteins). The Dkk molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding Dkk proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of Dkk-encoding nucleic acids. In another aspect, this invention provides isolated nucleic acid molecules encoding Dkk-related proteins (*e.g.*, Soggy proteins) or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of Dkk- or Soggy-encoding nucleic acids.

In one embodiment, a Dkk nucleic acid molecule is 60% homologous to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452 or complement thereof. In yet another embodiment, a Dkk nucleic acid molecule is 80% homologous to the nucleotide sequence shown in SEQ ID NO:4, SEQ ID NO:6, or a complement thereof. In yet another embodiment, a Dkk nucleic acid molecule is 60% homologous to the nucleotide sequence shown in SEQ ID NO:7, SEQ ID NO:9, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as

- 4 -

Accession Number 98633, or a complement thereof. In yet another embodiment, a Dkk nucleic acid molecule is 85% homologous to the nucleotide sequence shown in SEQ ID NO:7, SEQ ID NO:9, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633, or a complement thereof. In yet
5 another embodiment, a Dkk nucleic acid molecule is 70% homologous to the nucleotide sequence shown in SEQ ID NO:20, SEQ ID NO:22 or a complement thereof. In yet another embodiment, a nucleic acid molecule of the present invention (*e.g.*, a Dkk-related nucleic acid molecule) is 90% homologous to the nucleotide sequence shown in SEQ ID NO:13, SEQ ID NO:15, or a complement thereof.

10

In a preferred embodiment, an isolated Dkk nucleic acid molecule has the nucleotide sequence shown SEQ ID NO:3, or a complement thereof. In another embodiment, a Dkk nucleic acid molecule further comprises nucleotides 1-37 of SEQ ID NO:1. In yet another preferred embodiment, a Dkk nucleic acid molecule further
15 comprises nucleotides 1088-2479 of SEQ ID NO:1. In another preferred embodiment, an isolated Dkk nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:1.

In another preferred embodiment, an isolated Dkk nucleic acid molecule has the nucleotide sequence shown SEQ ID NO:6, or a complement thereof. In another
20 embodiment, a Dkk nucleic acid molecule further comprises nucleotides 1-124 of SEQ ID NO:4. In yet another preferred embodiment, a Dkk nucleic acid molecule further comprises nucleotides 797-848 of SEQ ID NO:4. In another preferred embodiment, an isolated Dkk nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:4.

In another preferred embodiment, an isolated Dkk nucleic acid molecule has the
25 nucleotide sequence shown SEQ ID NO:9, or a complement thereof. In another embodiment, a Dkk nucleic acid molecule further comprises nucleotides 1-108 of SEQ ID NO:7. In yet another preferred embodiment, a Dkk nucleic acid molecule further comprises nucleotides 907-1536 of SEQ ID NO:7. In another preferred embodiment, an isolated Dkk nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:7.

- 5 -

In another preferred embodiment, an isolated Dkk nucleic acid molecule has the nucleotide sequence shown SEQ ID NO:22, or a complement thereof. In another embodiment, a Dkk nucleic acid molecule further comprises nucleotides 1-723 of SEQ ID NO:20. In yet another preferred embodiment, a Dkk nucleic acid molecule further
5 comprises nucleotides 1501-3687 of SEQ ID NO:20. In yet another preferred embodiment, an isolated Dkk nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:20.

In another preferred embodiment, an isolated nucleic acid molecule of the present invention (*e.g.*, a Dkk-related nucleic acid molecule) has the nucleotide sequence
10 shown SEQ ID NO:15, or a complement thereof. In another embodiment, a nucleic acid molecule further comprises nucleotides 1-74 of SEQ ID NO:13. In yet another preferred embodiment, a nucleic acid molecule further comprises nucleotides 801-928 of SEQ ID NO:13. In yet another preferred embodiment, an isolated nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:13.

15 In another embodiment, a Dkk nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:21. In another preferred embodiment, a Dkk nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 60%
20 homologous to the amino acid sequence of SEQ ID NO:2. In yet another preferred embodiment, a Dkk nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 60% homologous to the amino acid sequence of SEQ ID NO:5. In yet another preferred embodiment, a Dkk nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid
25 sequence at least 60% homologous to the amino acid sequence of SEQ ID NO:8. In yet another preferred embodiment, a Dkk nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 75% homologous to the amino acid sequence of SEQ ID NO:8. In yet another preferred embodiment, a Dkk nucleic acid molecule includes a nucleotide sequence encoding a protein having an
30 amino acid sequence at least 65% homologous to the amino acid sequence of SEQ ID NO:21. In another embodiment, a nucleic acid molecule of the present invention (*e.g.*, a

- 6 -

Dkk-related nucleic acid molecule) includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:14 (*e.g.*, encodes a protein having an amino acid sequence which is 60% homologous to the amino acid sequence of SEQ ID NO:14).

5 In another embodiment, an isolated nucleic acid molecule of the present invention encodes a Dkk protein which includes a signal sequence and at least one cysteine-rich region, and is secreted. In another embodiment, an isolated nucleic acid molecule of the present invention encodes a Dkk protein which includes a signal
10 sequence and a cysteine-rich region, wherein the cysteine-rich region comprises at least one cysteine-rich domain, and is secreted. In yet another embodiment, a Dkk nucleic acid molecule encodes a Dkk protein and is a naturally occurring nucleotide sequence.

 In another embodiment, an isolated nucleic acid molecule of the present invention encodes a Dkk-related protein (*e.g.*, a Soggy protein) which includes a signal sequence, lacks cysteine-rich domains, and is secreted. In another embodiment, an
15 isolated nucleic acid molecule of the present invention encodes a Dkk-related protein (*e.g.*, a Soggy protein) which includes a signal sequence and a Soggy domain, and is secreted. In yet another embodiment, a nucleic acid molecule of the present invention encodes a Dkk-related protein and is a naturally occurring nucleotide sequence.

 Another embodiment of the invention features nucleic acid molecules which
20 specifically detect Dkk nucleic acid molecules relative to nucleic acid molecules encoding non-Dkk proteins (or specifically detect Dkk-related nucleic acid molecules). For example, in one embodiment, a nucleic acid molecule hybridizes under stringent conditions to a nucleic acid molecule consisting of nucleotides 470-2479 of nucleotide sequence shown in SEQ ID NO:1, to nucleotides 1-475 of nucleotide sequence shown in
25 SEQ ID NO:4, or to nucleotides 1-600 of nucleotide sequence shown in SEQ ID NO:7, or hybridizes under stringent conditions to the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or to the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633.

30 In another embodiment, the nucleic acid molecule is at least 500 nucleotides in length and hybridizes under stringent conditions to a

- 7 -

nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20 or a complement thereof.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to the coding strand of a Dkk nucleic acid or Dkk-related nucleic acid. Another embodiment of the invention provides an isolated nucleic acid molecules in a form suitable for expression of mRNA. In another embodiment, the isolated nucleic acid molecules are in a form suitable for expression of protein. In yet another embodiment, the isolated nucleic acid molecules are free from vector sequences.

Another aspect of the invention provides a vector comprising a Dkk nucleic acid molecule or Dkk-related nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. The invention also provides a method for producing a Dkk protein or Dkk-related protein by culturing in a suitable medium, a host cell of the invention containing a recombinant expression vector such that a Dkk protein or Dkk-related protein is produced.

Another aspect of this invention features isolated or recombinant Dkk proteins and polypeptides or Dkk-related proteins and polypeptides. In one embodiment, an isolated Dkk protein has a signal sequence and a cysteine-rich region which comprises two cysteine-rich domains, and is secreted. In another embodiment, an isolated Dkk protein has an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:21. In a preferred embodiment, a Dkk protein has an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:2. In another preferred embodiment, a Dkk protein has an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:5. In another preferred embodiment, a Dkk protein has an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:8. In another preferred embodiment, a Dkk protein has an amino acid sequence at least about 75% homologous to the amino acid sequence of SEQ ID NO:8. In another preferred embodiment, a Dkk protein has an amino acid sequence at least about 65% homologous to the amino acid sequence of SEQ ID NO:21. In another embodiment, a Dkk protein has the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID

- 8 -

NO:8, or SEQ ID NO:21. In another preferred embodiment, a protein of the present invention has an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:14. In another embodiment, a protein has the amino acid sequence of SEQ ID NO:14.

5 Another embodiment of the invention features an isolated Dkk protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 60% homologous to a nucleotide sequence of SEQ ID NO:1, or a complement thereof. Another embodiment of the invention features an isolated Dkk protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 80% homologous
10 to a nucleotide sequence of SEQ ID NO:4, or a complement thereof. Another embodiment of the invention features an isolated Dkk protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 60% homologous to a nucleotide sequence of SEQ ID NO:7, or a complement thereof. Another embodiment of the invention features an isolated Dkk protein which is encoded by a nucleic acid
15 molecule having a nucleotide sequence at least about 85% homologous to a nucleotide sequence of SEQ ID NO:7, or a complement thereof. Another embodiment of the invention features an isolated Dkk protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 70% homologous to a nucleotide sequence of SEQ ID NO:20, or a complement thereof. Another embodiment of the invention
20 features an isolated protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 90% homologous to a nucleotide sequence of SEQ ID NO:13, or a complement thereof. This invention further features an isolated protein which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule
25 comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, or a complement thereof.

The proteins of the present invention, or biologically active portions thereof, can be operatively linked to a non-Dkk polypeptide or non-Dkk-related polypeptide to form fusion proteins. The invention further features antibodies that specifically bind Dkk or
30 Dkk-related proteins, such as monoclonal or polyclonal antibodies. In addition, the

- 9 -

proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting Dkk expression (or the expression of a Dkk-related molecule) in a biological sample by
5 contacting the biological sample with an agent capable of detecting a nucleic acid molecule, protein or polypeptide of the present invention such that the presence of a Dkk (of Dkk-related) nucleic acid molecule, protein or polypeptide is detected in the biological sample.

In another aspect, the present invention provides a method for detecting the
10 presence of a Dkk activity (or Dkk-related activity) in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of Dkk activity (or Dkk-related activity) such that the presence of the activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating Dkk activity
15 (or Dkk-related activity) comprising contacting the cell with an agent that modulates the activity such that the activity in the cell is modulated. In one embodiment, the agent inhibits Dkk activity (or Dkk-related activity). In another embodiment, the agent stimulates Dkk activity (or Dkk-related activity). In one embodiment, the agent is an antibody that specifically binds to a Dkk (or Dkk-related) protein. In another
20 embodiment, the agent modulates expression of a protein (*e.g.*, a Dkk or a Dkk-related protein) by modulating transcription of a gene or translation of a mRNA of the present invention. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of a mRNA or gene of the present invention.

25 In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant expression or activity of a protein or nucleic acid of the invention by administering to the subject an agent which is a modulator of Dkk or a Dkk-related molecule. In one embodiment, the modulator is a Dkk or Dkk-related protein. In another embodiment the modulator is a Dkk or Dkk-
30 related nucleic acid molecule. In yet another embodiment, the modulator is an antibody peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the

- 10 -

disorder characterized by aberrant protein or nucleic acid expression is a developmental, differentiative, or proliferative disorder.

The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant
5 modification or mutation of a gene encoding a Dkk or Dkk-related protein; (ii) mis-regulation of said gene; and (iii) aberrant post-translational modification of a Dkk or Dkk-related protein, wherein a wild-type form of said gene encodes an protein with a Dkk or Dkk-related activity.

In another aspect the invention provides a method for identifying a compound
10 that binds to or modulates the activity of a Dkk or Dkk-related protein, by providing a indicator composition comprising a Dkk or Dkk-related protein having a biological activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on the activity in the indicator composition to identify a compound that modulates the activity of a Dkk or Dkk-related protein.

15 Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1A-B depicts the cDNA sequence and predicted amino acid sequence of
20 human Dkk-3. The nucleotide sequence corresponds to nucleic acids 1 to 2479 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 350 of SEQ ID NO:2.

Figure 2 depicts the cDNA sequence and predicted amino acid sequence of
human Dkk-4. The nucleotide sequence corresponds to nucleic acids 1 to 848 of SEQ
25 ID NO:4. The amino acid sequence corresponds to amino acids 1 to 224 of SEQ ID NO:5.

Figure 3 depicts the cDNA sequence and predicted amino acid sequence of
human Dkk-1. The nucleotide sequence corresponds to nucleic acids 1 to 1536 of SEQ
ID NO:7. The amino acid sequence corresponds to amino acids 1 to 266 of SEQ ID
30 NO:8.

Figure 4A-B depicts the cDNA sequence and predicted amino acid sequence of full-length human Dkk-2. The nucleotide sequence corresponds to nucleic acids 1 to 3687 of SEQ ID NO:20. The amino acid sequence corresponds to amino acids 1 to 259 of SEQ ID NO:21.

5 *Figure 5A-B* depicts the cDNA sequence and predicted amino acid sequence of murine Dkk-3. The nucleotide sequence corresponds to nucleic acids 1 to 2380 of SEQ ID NO:16. The amino acid sequence corresponds to amino acids 1 to 349 of SEQ ID NO:17.

10 *Figure 6* depicts a multiple sequence alignment of the amino acid sequences of hDkk-1 (corresponding the SEQ ID NO:8), mDkk-1 (having Accession No. AF030433), *Xenopus* Dkk-1 ("xDkk-1") (having Accession No. AF030434), hDkk-2 (corresponding to SEQ ID NO:21), hDkk-3 (corresponding to SEQ ID NO:2), mDkk-3 (corresponding to SEQ ID NO:17), chicken Dkk-3 ("cDkk-3") (having Accession No. D26311), and hDkk-4 (corresponding to SEQ ID NO:5). The alignment was performed using the
15 ClustalW^{*} algorithm as implemented in the GCG program PILEUP. The alignment provides information regarding the relationship between the Dkk proteins of the instant invention. Predicted signal peptides are underlined, N-glycosylation sites are indicated by a thick bar, CRD-1 by an open box, CRD-2 by a shaded box. The proteolytic cleavage site within hDkk4 is indicated by an arrow.

20 *Figure 7* depicts the cDNA sequence and predicted amino acid sequence of human Soggy. The nucleotide sequence corresponds to nucleic acids 1 to 928 of SEQ ID NO:13. The amino acid sequence corresponds to amino acids 1 to 242 of SEQ ID NO:14.

25 *Figure 8* depicts the cDNA sequence and predicted amino acid sequence of murine Soggy-1. The nucleotide sequence corresponds to nucleic acids 1 to 835 of SEQ ID NO:26. The amino acid sequence corresponds to amino acids 1 to 230 of SEQ ID NO:27.

30 *Figure 9* is a schematic diagram illustrating the Dkk and Dkk-related proteins of the instant invention. The figure depicts the structural domains of the human Dkks and Soggy. Signal peptides are indicated by darkened boxes. The cysteine-rich domains of

* Trademark

- 12 -

a Dkk cysteine-rich region are depicted as CRD-1 and CRD-2. Branches indicate sites of N-glycosylation.

Figure 10 depicts a multiple sequence alignment of hSoggy-1 (corresponding to SEQ ID NO:14), murine Soggy-1 (corresponding to SEQ ID NO:27), hDkk-3 (corresponding to SEQ ID NO:2), and mDkk-3 (corresponding to SEQ ID NO:17). The alignment was generated as described in the legend to Figure 6. The alignment provides details regarding the relationship between the Dkk-3 and Soggy-1 proteins of the instant invention. Predicted signal peptides are underlined, N-glycosylation sites are indicated by a thick bar. CRD-1 and CRD-2 within Dkk-3 are indicated for reference by open and shaded boxes.

Figure 11 depicts a multiple sequence alignment of the carboxy-terminal cysteine-rich domains of hDkk-1, hDkk-2, hDkk-3, hDkk-4 with human colipase (having accession No. J02883). The carboxy-terminal cysteine-rich domains of the Dkk proteins are indicated by an open box. The alignment was generated using PILEUP (gap penalties of 12 for opening and 12 for extending). A minor adjustment was necessary since PILEUP inserts a single gap in hDkk-1 and hDkk-2 between Gly56 and Ser57, even with a gap opening penalty of 15. The conserved residues are indicated. The disulfide-bonding pattern typical for the colipase family and predicted for the Dkk family is indicated below the alignment.

Figure 12 is a schematic diagram depicting the relationship between the hDkk-3 nucleotide sequence (corresponding to SEQ ID NO:1) and those of RIG and RIG-like 7-1 (Accession Nos. U32331 and AF034208, respectively). Thick bars indicate regions of sequence identity between hDkk-3 and RIG or RIG-like 7-1 mRNAs. As between RIG and hDkk-3, there exists a short region of identity within the 3' untranslated regions of the mRNAs when the mRNAs are aligned in reverse orientation. As between hDkk-3 and RIG-like 7-1, there exists a longer region of identity, however, RIG-like 7-1 lacks a signal sequence and, accordingly, is not predicted to be secreted.

Detailed Description of the Invention

The present invention is based on the discovery of novel molecules, referred to herein as Dkk protein and nucleic acid molecules, which comprise a family of molecules

- 13 -

having certain conserved structural and functional features. The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally-occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin. Members of a family may also have common functional characteristics.

In one embodiment, a Dkk family member is identified based on the presence of at least one "cysteine-rich domain" in the protein molecule or corresponding amino acid sequence. As defined herein, a "cysteine-rich domain" refers to a portion of a Dkk protein (*e.g.*, hDkk-3) which is rich in cysteine residues. In a preferred embodiment, a "cysteine-rich domain" is a protein domain having an amino acid sequence of about 45-85 amino acids of which preferably 10 amino acids are cysteine residues located at the same relative amino acid position as the cysteine residues in human Dkk-3 having SEQ ID NO:2 (*e.g.*, amino acid residues 147-195 of SEQ ID NO:2). In another embodiment, a "cysteine-rich domain" has 30-100 amino acids, preferably about 35-95 amino acids, more preferably about 40-90 amino acids, more preferably about 50-80 amino acids, even more preferably about 55-75, 60-70, or 65 amino acids, of which at least about 3-20, preferably about 5-15, or more preferably about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids are cysteine residues.

A preferred Dkk protein of the present invention has a first cysteine-rich domain ("CRD-1") referred to herein as an "amino-terminal cysteine-rich domain" or "N-terminal cysteine-rich domain" and a second cysteine-rich domain ("CRD-2"), referred to herein as a "carboxy-terminal cysteine-rich domain" or "C-terminal cysteine-rich domain". As defined herein, an "amino-terminal cysteine-rich domain" is a protein domain having an amino acid sequence of about 45-55 amino acids of which preferably 10 amino acids are cysteine residues located at the same relative position as the cysteine residues in an amino-terminal cysteine-rich domain of human Dkk-3 having SEQ ID NO:2 (*e.g.*, amino acid residues 147-195 of SEQ ID NO:2). In another embodiment, an "amino-terminal cysteine-rich domain" has 30-70, preferably 35-65, more preferably

- 14 -

about 40-60, and even more preferably about 46, 47, 48, 49, 50, 51, 52, 53, or 54 amino acids, of which at least about 3-20, preferably about 5-15, or more preferably about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids are cysteine residues. In a preferred embodiment, an amino-terminal cysteine-rich domain has the following

5 consensus sequence: C - X(2) - D - X(2) - C - X(5) - C - X(8-13) - C - X(2) - C - X(6) - C - X(5) - C - C - X(4) - C - X(4) - C (SEQ ID NO:23). The consensus sequences described herein are described according to standard Prosite^{*} Signature designation (*e.g.*, all amino acids are indicated according to their universal single letter designation; X designates any amino acid; X(n) designates any n amino acids, *e.g.*, X (2) designates any

10 2 amino acids; and [LIVM] indicates any one of the amino acids appearing within the brackets, *e.g.*, any one of L, I, V, or M, in the alternative, any one of Leu, Ile, Val, or Met.)

As defined herein, a "carboxy-terminal cysteine-rich domain" is a protein domain having an amino acid sequence of about 80-85 amino acids of which preferably 10

15 amino acids are cysteine residues located at the same relative position as the cysteine residues in a carboxy-terminal cysteine-rich domain of human Dkk-3 having SEQ ID NO:2 (*e.g.*, amino acid residues 201-284 of SEQ ID NO:2). In another embodiment, a "carboxy-terminal cysteine-rich domain" has 65-100, preferably 70-95, more preferably about 75-90, and even more preferably about 81, 82, 83, or 84 amino acids, of which at

20 least about 3-20, preferably about 5-15, or more preferably about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids are cysteine residues. In a preferred embodiment, a carboxy-terminal cysteine-rich domain has the following consensus sequence: C - X(4) - D - C - X(2) - G - X - C - C - X(8-10) - C - X - P - X(4) - G - X(2) - C - X(16-24) - C - X - C - X(2) - P - X(4) - G - X(2) - C - X(16-24) - C - X - C - X(2) -

25 G - L - X - C - X(10-17) - C (SEQ ID NO:24).

A preferred protein of the present invention is a hDkk-3 protein (human Dkk-3) containing an amino-terminal cysteine-rich domain including about amino acids 147-195 of SEQ ID NO:2, having 10 cysteine residues, and a carboxy-terminal cysteine-rich domain including about amino acids 201-284 of SEQ ID NO:2, having 10 cysteine

30 residues (the positions of the cysteine residues are depicted in Figure 6). In another embodiment, a hDkk-4 (human Dkk-4) protein contains an amino-terminal cysteine-rich

* Trademark

- 15 -

domain including about amino acids 41-90 of SEQ ID NO:5, having 10 cysteine residues, and a carboxy-terminal cysteine-rich domain including about amino acids 138-218 of SEQ ID NO:5, having 10 cysteine residues (the positions of the cysteine residues are depicted in Figure 6). In another embodiment, a hDkk-1 protein (human Dkk-1) contains an amino-terminal cysteine-rich domain including about amino acids 85-138 of SEQ ID NO:8, having 10 cysteine residues, and a carboxy-terminal cysteine-rich domain including about amino acids 182-263 of SEQ ID NO:8, having 10 cysteine residues (the positions of the cysteine residues are depicted in Figure 6). In another embodiment, a hDkk-2 protein (human Dkk-2) contains an amino-terminal cysteine-rich domain including about amino acids 78-127 of SEQ ID NO:21, having 10 cysteine residues, and a carboxy-terminal cysteine-rich domain including about amino acids 176-256 of SEQ ID NO:21, having 10 cysteine residues (the positions of the cysteine residues are depicted in Figure 6).

Alignment of the human Dkk proteins with human colipase (having Accession No. J02883) indicates that the carboxy-terminal cysteine-rich domains of the human Dkk proteins have a pattern of cysteines typical of colipase (Figure 11 and Avarind and Koonin, *supra*). Within colipase, these cysteine residues are involved in disulfide bonding which gives rise to a structure termed the "colipase fold". The "colipase fold" is typical of a range of small proteins which are involved in protein-protein interactions including, but not limited to the colipases, snake and scorpion toxins and protease inhibitors (Hubbard *et al.* (1997) *Nucleic Acids Res.* 25:236-239. These proteins have a series of short β strands with large connecting loops, which are held together by disulfide bonds. The disulfide-bonding pattern typical for colipase and predicted for the Dkk family is indicated below the alignment of Figure 11. Conserved hydrophobic residues between the Dkks and human colipase suggest that the Dkks, like the colipases, interact with lipids (*e.g.*, Leu51 of human colipase, SEQ ID NO:25 which corresponds to Leu271 of hDkk-3 (SEQ ID NO:2); Leu200 of hDkk-4 (SEQ ID NO:5); Leu243 of hDkk-1 (SEQ ID NO:8); and Leu237 of hDkk-2 (SEQ ID NO:21). The carboxy-terminal cysteine-rich domain of the Dkk family, may function in the membrane association of Dkk, which in turn may be required for the inhibition of Wnt secretion or Wnt:7 transmembrane receptor interaction. In addition, inhibition of Wnt function by

- 16 -

the Dkk family may be closely associated with the cell membrane and the carboxy-terminal cysteine-rich domain of the Dkk family may mediate this association.

Furthermore, the amino-terminal cysteine-rich domain of the Dkk family may directly interact with Wnt or its receptor. Accordingly, a preferred Dkk protein of the present invention comprises a carboxy-terminal cysteine-rich domain. In one embodiment, a Dkk protein comprising a carboxy-terminal cysteine-rich domain lacks the amino-terminal cysteine-rich domain.

In a preferred embodiment, the cysteine residues of a cysteine-rich domain are located at the same relative amino acid position as the cysteine residues in human Dkk-3 having SEQ ID NO:2. In another preferred embodiment, the cysteine residues of a cysteine-rich domain are located at the same relative position as the cysteine residues in a cysteine-rich domain of human Dkk-3 having SEQ ID NO:2. For example, as shown in Figure 6, human Dkk-4 has at least about 10 cysteine residues located at the same relative amino acid position as the cysteine residues in human Dkk-3 having SEQ ID NO:2 (e.g., cys151 in Dkk-4, SEQ ID NO:5, is located at the same relative amino acid position as cys214 in Dkk-3, SEQ ID NO:2; cys156 in Dkk-4, SEQ ID NO:5, is located at the same relative amino acid position as cys219 in Dkk-3, SEQ ID NO:2; and cys157 in Dkk-4, SEQ ID NO:5, is located at the same relative amino acid position as cys220 in Dkk-3, SEQ ID NO:2). Similarly, as shown in Figure 6, Dkk-1 has at least about 10 cysteine residues located at the same relative amino acid position as the cysteine residues in human Dkk-3 having SEQ ID NO:2. As also shown in Figure 6, Dkk-2 has at least about 10 cysteine residues located at the same relative amino acid position as the cysteine residues in human Dkk-3 having SEQ ID NO:2. Table I sets forth at least 20 cysteine residues in each of hDkk-4, hDkk-1, and hDkk-2 which are located in the same relative position as 20 cysteine residues in hDkk-3.

- 17 -

Table I:

cysteine	aa position in hDkk-3	aa position in hDkk-4	aa position in hDkk-1	aa position in hDkk-2
1	147	41	85	78
2	153	47	91	84
3	159	53	97	90
4	168	63	111	100
5	171	66	114	103
6	178	73	121	110
7	184	79	127	116
8	185	80	128	117
9	190	85	133	122
10	195	90	138	127
11	208	145	189	183
12	214	151	195	189
13	219	156	200	194
14	220	157	201	195
15	231	166	210	204
16	241	176	220	214
17	265	194	237	231
18	267	196	239	233
19	273	202	245	239
20	284	218	263	256

The first 10 rows of Table I contain 10 cysteine residues that are included within
5 the first, or amino-terminal, cysteine-rich domain of each of hDkks-3, -4, -1, and -2.
The last 10 rows of Table I contain 10 cysteine residues that are included within the
second, or carboxy-terminal, cysteine-rich domain of each of hDkks-3, -4, -1, and -2.

- 18 -

Preferred Dkk proteins have more than one cysteine-rich domain, more preferably have at least two cysteine-rich domains and, thus, have a cysteine-rich region. As used herein, the term "cysteine-rich region" refers to a protein domain which includes at least two cysteine-rich domains and has an amino acid sequence of about 120-200 amino acid residues of which at least about 20 of the amino acids are cysteine residues. In another embodiment, a "cysteine-rich region" has preferably about 140-180 amino acid residues, and even more preferably at least about 135-175 amino acids of which at least about 10-30, preferably about 15-20, and more preferably about 16, 17, 18, or 19 of the amino acids are cysteine residues. In a preferred embodiment, a cysteine-rich region is located in the C-terminal region of a Dkk protein. For example, in one embodiment, a hDkk-3 protein contains a cysteine rich region containing about amino acids 147-284 of SEQ ID NO:2, having 20 cysteine residues at the positions indicated in Figure 6. In another embodiment, a hDkk-4 protein contains a cysteine rich region containing about amino acids 41-218 of SEQ ID NO:5, having 20 cysteine residues at the positions indicated in Figure 6. In another embodiment, a hDkk-1 protein contains a cysteine rich region containing about amino acids 85-263 of SEQ ID NO:8, having 20 cysteine residues at the positions indicated in Figure 6. In another embodiment, a hDkk-2 protein contains a cysteine rich region containing about amino acids 78-256 of SEQ ID NO:21, having 20 cysteine residues at the positions indicated in Figure 6.

In another embodiment, in addition to cysteine-rich domains, the cysteine-rich region contains a spacer region which separates the first and second cysteine-rich domains. As used herein, the "spacer region" refers to amino acid residues which are located between the first and second cysteine-rich domains of a cysteine-rich region and includes amino acid residues located C-terminal to the first cysteine-rich domain and N-terminal to the second cysteine-rich domain. As defined herein, a "spacer region" refers to a protein domain of about 5-70 amino acids, preferably about 10-65 amino acids, more preferably about 15-60 amino acids, even more preferably about 20-55 amino acids, and even more preferably about 25-50, 30-45 or 35-40 amino acids. For example, hDkk-3 protein contains a spacer region of about amino acids 196-200 of SEQ ID NO:2; hDkk-4 protein contains a spacer region of about amino acids 91-137 of SEQ ID NO:5; hDkk-1 protein contains a spacer region of about amino acids 139-181 of SEQ ID NO:8;

- 19 -

and hDkk-2 protein contains a spacer region of about amino acids 128-175 of SEQ ID NO:21. The spacer regions of hDkk-1, hDkk-2 and hDkk-4 are remarkably conserved in length (*e.g.*, the spacer region of hDkk-1 consists of 43 amino acid residues, the spacer region of hDkk-2 consists of 48 amino acid residues and the spacer region of hDkk-4 consists of 47 amino acid residues, suggesting that the close proximity of CRD-1 and CRD-2 is important in Dkk function. Accordingly, in one embodiment, the spacer region functions to spatially restrict the separation of CRD-1 from CRD-2.

In another embodiment of the invention, the Dkk protein has at least one cysteine-rich domain, preferably a cysteine-rich region, and a signal sequence. As used herein, a "signal sequence" refers to a peptide containing about 18-24 amino acids which occurs at the N-terminus of secretory and integral membrane proteins and which contains at least about 40-70% hydrophobic amino acid residues (*e.g.*, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, or proline). In another embodiment, a signal sequence contains at least about 8-34, 9-33, 10-32, 11-31, 12-30, 13-29, 14-28 amino acid residues, preferably about 15-27 amino acid residues, more preferably about 16-26 amino acid residues, more preferably about 17-25 amino acid residues, and more preferably about 18-24, 19-23, 20-22, or 21 amino acid residues, and has at least about 50-65%, and more preferably about 55-60% hydrophobic amino acid residues (*e.g.*, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, or proline). Such a "signal sequence", also referred to in the art as a "signal peptide", serves to direct a protein containing such a sequence to a lipid bilayer. For example, in one embodiment, a hDkk-3 protein contains a signal sequence of about amino acids 1-23 of SEQ ID NO:2. In another embodiment, a hDkk-4 protein contains a signal sequence of about amino acids 1-19 of SEQ ID NO:5. In another embodiment, a hDkk-1 protein contains a signal sequence of about amino acids 1-20 of SEQ ID NO:8. In another embodiment, a hDkk-2 protein contains a signal sequence of about amino acids 1-33 of SEQ ID NO:21. A preferred Dkk protein of the present invention is a human protein (*e.g.*, encoded by a nucleotide sequence corresponding to a naturally-occurring human gene).

- 20 -

Accordingly, one embodiment of the invention features a Dkk protein having at least one cysteine-rich domain, preferably at least one cysteine-rich region. Another embodiment features a Dkk protein having at least one cysteine-rich region, wherein the cysteine-rich region includes at least one cysteine-rich domain. Another embodiment
5 features a Dkk protein having at least one cysteine-rich region, wherein the cysteine-rich region includes at least two cysteine-rich domains. Another embodiment features a protein or domain within a protein having 20, 30, 40, 50, 60, 70, 80, 90, 95, or 99% homology to a cysteine-rich domain of a Dkk protein of the invention (*e.g.*, hDkk-3, hDkk-4, hDkk-1, or hDkk-2).

10 Yet another embodiment of the invention features a Dkk protein having at least one cysteine-rich domain, preferably at least one cysteine-rich region and a signal peptide. Another embodiment features a Dkk protein having at least one cysteine-rich domain, preferably at least one cysteine-rich region and a signal peptide, wherein the cysteine-rich region includes at least two cysteine-rich domains. Another embodiment
15 features a Dkk protein having at least one cysteine-rich domain, preferably at least one cysteine-rich region and a signal peptide, wherein the cysteine-rich region includes at least two cysteine-rich domains and a spacer.

Yet another aspect of the invention features Dkk proteins having domains and/or regions which are conserved among a subset of Dkk proteins but are not necessarily
20 conserved among all Dkk family members. In one embodiment, a Dkk protein (*e.g.*, Dkk-3) has an "extended N-terminal region" which is extended in length as compared to, for example, the "N-terminal regions" of other Dkk family members (*e.g.*, Dkk-4, Dkk-1, and Dkk-2). As defined herein, an "N-terminal region" of a Dkk proteins consists of amino acid residues found between the signal peptide and CRD-1 of a Dkk protein.
25 Preferably, the first amino acid residue of an N-terminal region of Dkk is the first residue of a mature Dkk protein and the last residue of an N-terminal region of Dkk is the residue preceeding the first cysteine residue of CRD-1. In a preferred embodiment, an N-terminal region is about 1-20 amino acid residues in length, preferably about 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, 91-100, 101-110, 111-120, 121-130, 131-
30 140, 141-150, 151-160 or more amino acid residues in length. In contrast, an "extended N-terminal region" is at least about 71-80, 81-90, 91-100, 101-110, 111-120, 121-130,

- 21 -

131-140, 141-150, 151-160 or more amino acid residues in length. For example, in one embodiment, a hDkk-4 protein includes an "N-terminal region" of about amino acids 20-40 of SEQ ID NO:5 (21 amino acid residues in length). In another embodiment, a hDkk-1 protein includes an N-terminal region of about amino acids 21-84 of SEQ ID NO:8 (64 amino acid residues in length). In another embodiment, a hDkk-2 protein includes an "N-terminal region" of about amino acids 34-77 of SEQ ID NO:21 (44 amino acid residues in length). In another embodiment, a hDkk-3 protein has an "extended N-terminal region" of about amino acids 23-146 of SEQ ID NO:2 (124 amino acid residues in length).

10 In another embodiment, a Dkk protein (*e.g.*, Dkk-3) has an "acidic C-terminal region" which includes amino acid residues found C-terminal to CRD-2 of a Dkk protein. Preferably, the first amino acid residue of an acidic C-terminal region is the residue following the last cysteine of CRD-2 and the last residue of an acidic C-terminal region is the last residue of a Dkk protein. In a preferred embodiment, an acidic C-terminal
15 region is about 65-66 amino acid residues in length and has about 27-25% acidic amino acid residues (*e.g.*, glutamic acid or aspartic acid). In another preferred embodiment, an acidic C-terminal region is about 55-80 amino acid residues in length, preferably about 60-75 amino acid residues in length, and more preferably about 64-70 amino acid residues in length and has about 21-35% acidic amino acid residues, preferably about
20 23-33% acidic amino acid residues, and more preferably about 25-31% acidic amino acid residues. Preferably, an acidic C-terminal region is involved in protein-protein interactions. For example, in one embodiment, a hDkk-3 protein has an acidic C-terminal region from about amino acids 285-350 of SEQ ID NO:2.

Preferred Dkk molecules of the present invention have an amino acid sequence
25 sufficiently homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:21. As used herein, the term "sufficiently homologous" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or
30 nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains and/or a common functional activity. For example,

- 22 -

amino acid or nucleotide sequences which share common structural domains have at least about 40% homology, preferably 50% homology, more preferably 60%-70% homology across the amino acid sequences of the domains and contain at least one, preferably two, more preferably three, and even more preferably four, five or six structural domains, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 40%, preferably 50%, more preferably 60, 70, or 80% homology and share a common functional activity are defined herein as sufficiently homologous.

As used interchangeably herein, a "Dkk activity", "biological activity of Dkk" or "functional activity of Dkk", refers to an activity exerted by a Dkk protein, polypeptide or nucleic acid molecule (*e.g.*, an activity on a Dkk responsive cell) as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a Dkk activity is a direct activity, such as an association with a Dkk-target molecule. As used herein, a "target molecule" is a molecule with which a Dkk protein binds or interacts in nature, such that Dkk-mediated function is achieved. A Dkk target molecule can be a non-Dkk molecule or a Dkk protein or polypeptide of the present invention. In an exemplary embodiment, a Dkk target molecule is a membrane-bound protein (*e.g.*, a cell-surface receptor or "Dkk receptor") or a modified form of such a protein which has been altered such that the protein is soluble (*e.g.*, recombinantly produced such that the protein does not express a membrane-binding domain). In another embodiment, a Dkk target is a second soluble protein molecule (*e.g.*, a "Dkk binding partner" or "Dkk substrate"). In such an exemplary embodiment, a Dkk binding partner can be a second soluble non-Dkk protein or a second Dkk protein molecule of the present invention. Alternatively, a Dkk activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the Dkk protein with a second protein (*e.g.*, a Dkk receptor). As used herein, the term "Dkk receptor" refers to a protein or protein complex, to which a Dkk protein, *e.g.*, human Dkk, can bind. A receptor can be a cell surface receptor, *e.g.*, a peptide, growth factor, or nuclear hormone receptor. Dkk receptors can be isolated by methods known in the art and further described herein. Interaction of a Dkk protein with a Dkk receptor can result in transduction of a signal from the cell surface to the nucleus. The signal transduced can be, an increase in intracellular calcium, an increase in

- 23 -

phosphatidylinositol or other molecule, and can result in, *e.g.*, in phosphorylation of specific proteins, a modulation of gene transcription and any of the other biological activities set forth herein.

In a preferred embodiment, a Dkk activity is at least one or more of the following activities: (i) interaction of a Dkk protein with and/or binding to a second molecule, (*e.g.*, a protein, such as a Dkk receptor, a soluble form of a Dkk receptor, a receptor for a member of the *wnt* family of signaling proteins, or a non-Dkk signaling molecule, for example, a lipid included in a cell membrane); (ii) interaction of a Dkk protein with an intracellular protein *via* a membrane-bound Dkk receptor; (iii) complex formation between a soluble Dkk protein and a second soluble Dkk binding partner (*e.g.*, a non-Dkk protein molecule or a second Dkk protein molecule); (iv) interaction with other extracellular proteins (*e.g.*, regulation of *wnt*-dependent cellular adhesion to extracellular matrix components); (v) binding to and eliminating an undesirable molecule (*e.g.*, a detoxifying activity or defense function); and/or (vi) an enzymatic activity. In yet another preferred embodiment, a Dkk activity is at least one or more of the following activities: (1) modulation of cellular signal transduction, either *in vitro* or *in vivo* (*e.g.*, modulation, *e.g.*, antagonism, of the activity of members of the *wnt* family of secreted proteins or suppression of *wnt*-dependent signal transduction, for example suppression of Wnt 2b, Wnt3 and/or Wnt8-dependent signal transduction by hDkk-1 and/or hDkk-4); (2) regulation of communication between cells (*e.g.*, regulation of *wnt*-dependent cell-cell interactions); (3) regulation of expression of genes whose expression is modulated by binding of Dkk (*e.g.*, hDkk-3) to a receptor; (4) regulation of gene transcription in a cell involved in development or differentiation, either *in vitro* or *in vivo* (*e.g.*, induction of cellular differentiation); (5) regulation of gene transcription in a cell involved in development or differentiation, wherein at least one gene encodes a differentiation-specific protein; (6) regulation of gene transcription in a cell involved in development or differentiation, wherein at least one gene encodes a second secreted protein; (7) regulation of gene transcription in a cell involved in development or differentiation, wherein at least one gene encodes a signal transduction molecule; (8) regulation of cellular proliferation, either *in vitro* or *in vivo* (*e.g.*, induction of cellular proliferation or inhibition of proliferation as in the case of suppression of tumorigenesis (*e.g.*, suppression

- 24 -

of glial cell tumor growth, for example, glioblastoma growth)); (9) formation and maintenance of ordered spatial arrangements of differentiated tissues in vertebrates, both adult and embryonic (*e.g.*, induction of head formation during vertebrate development or maintenance of hematopoietic progenitor cells); (10) modulation of cell death, such as stimulation of cell survival; (11) regulating cell migration; and/or (12) immune modulation.

As referred to herein, "differentiation-specific proteins" include proteins involved in the transition of a cell from the undifferentiated to the differentiated phenotype. For example, such proteins can be differentiation specific structural proteins or differentiation-specific transcription factors. Such differentiation-specific proteins are generally expressed at higher levels in cells which are making the transition from the undifferentiated to the differentiated phenotype (*e.g.*, during embryonic development or during regeneration of mature tissue in the adult animal), or are expressed at higher levels in fully-differentiated or terminally-differentiated cells as compared to their undifferentiated counterparts. Also, as referred to herein, "differentiation-specific genes" include nucleic acid molecules which encode differentiation-specific proteins.

Accordingly, another embodiment of the invention features isolated Dkk proteins and polypeptides having a Dkk activity. Preferred Dkk proteins have at least one cysteine-rich region and a Dkk activity. In another preferred embodiment, the Dkk protein has at least one cysteine-rich region, wherein the cysteine-rich region comprises at least one cysteine-rich domain, and a Dkk activity. In another preferred embodiment, the Dkk protein has at least one cysteine-rich region, wherein the cysteine-rich region comprises at least two cysteine-rich domains, and a Dkk activity. In yet another preferred embodiment, a Dkk protein further comprises a signal sequence. In still another preferred embodiment, a Dkk protein has a cysteine-rich region, a Dkk activity, and an amino acid sequence sufficiently homologous to an amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:21.

A preferred Dkk fragment comprises a carboxy-terminal cysteine-rich domain. In one embodiment, a Dkk fragment comprises a carboxy-terminal cysteine-rich domain and retains a biological activity of a Dkk protein. In yet another embodiment, a Dkk fragment lacks an amino-terminal cysteine-rich domain.

- 25 -

The human Dkk-3 cDNA, which is approximately 2479 nucleotides in length, encodes a protein which is approximately 350 amino acid residues in length. The human Dkk-3 protein contains an N-terminal signal sequence and a cysteine-rich region comprising two cysteine-rich domains. A Dkk cysteine-rich region can be found at least, for example, from about amino acids 147-284 of SEQ ID NO:2. The hDkk-3 cysteine-rich region comprises an amino-terminal cysteine-rich domain from about amino acids 147-195 of SEQ ID NO:2 and a carboxy-terminal cysteine-rich domain from about amino acids 201-284 of SEQ ID NO:2. The human Dkk-3 protein is a secreted protein which further contains a signal sequence at about amino acids 1-21, 1-22, 1-23, or 1-24 of SEQ ID NO:2. Accordingly, a mature human Dkk-3 protein begins at about amino acid residue 22, 23, 24, or 25 of SEQ ID NO:2 and is about 329, 328, 327, or 326 amino acids in length. The prediction of such a signal peptide can be made, for example, utilizing the computer algorithm SIGNALP (Nielsen, *et al.*, (1997) *Protein Engineering* 10:1-6).

The human Dkk-4 cDNA, which is approximately 848 nucleotides in length, encodes a protein which is approximately 224 amino acid residues in length. The human Dkk-4 protein contains an N-terminal signal sequence and a cysteine-rich region comprising two cysteine-rich domains. A Dkk cysteine-rich region can be found at least, for example, from about amino acids 41-218 of SEQ ID NO:5. The hDkk-4 cysteine-rich region comprises an amino-terminal cysteine-rich domain from about amino acids 41-90 of SEQ ID NO:5 and a carboxy-terminal cysteine-rich domain from about amino acids 138-218 of SEQ ID NO:5. The human Dkk-4 protein is a secreted protein which further contains a signal sequence at about amino acids 1-17, 1-18, 1-19, or 1-20 of SEQ ID NO:5. Accordingly, a mature human Dkk-4 protein begins at about amino acid residue 18, 19, 20, or 21 of SEQ ID NO:5 and is about 207, 206, 205, or 204 amino acids in length. A preferred fragment of hDkk-4 comprises amino acid residues 134-224 of SEQ ID NO:5. In another embodiment, a preferred fragment of hDkk-4 consists of amino acid residues 134-224 of SEQ ID NO:5.

The human Dkk-1 cDNA, which is approximately 1536 nucleotides in length, encodes a protein which is approximately 266 amino acid residues in length. The human Dkk-1 protein contains an N-terminal signal sequence and a cysteine-rich region

- 26 -

comprising two cysteine-rich domains. A Dkk cysteine-rich region can be found at least, for example, from about amino acids 85-263 of SEQ ID NO:8. The hDkk-1 cysteine-rich region comprises an amino-terminal cysteine-rich domain from about amino acids 85-138 of SEQ ID NO:8 and a carboxy-terminal cysteine-rich domain from about amino acids 182-263 of SEQ ID NO:8. The human Dkk-1 protein is a secreted protein which further contains a signal sequence at about amino acids 1-18, 1-19, 1-20, or 1-21 of SEQ ID NO:8. Accordingly, a mature human Dkk-1 protein begins at about amino acid residue 19, 20, 21, or 22 of SEQ ID NO:8 and is about 248, 247, 246, or 245 amino acids in length.

10 The human Dkk-2 cDNA, which is approximately 3687 nucleotides in length, encodes a protein which is approximately 259 amino acid residues in length. The human Dkk-2 protein contains a cysteine-rich region comprising two cysteine-rich domains. A Dkk cysteine-rich region can be found at least, for example, from about amino acids 78-256 of SEQ ID NO:21. The hDkk-2 cysteine-rich region comprises an amino-terminal cysteine-rich domain from about amino acids 78-127 of SEQ ID NO:21 and a carboxy-terminal cysteine-rich domain from about amino acids 176-256 of SEQ ID NO:21. The human Dkk-2 protein is a secreted protein which further contains a signal sequence at about amino acids 1-31, 1-32, 1-33, or 1-34 of SEQ ID NO:21. Accordingly, a mature human Dkk-2 protein begins at about amino acid residue 32, 33, 34, or 35 of SEQ ID NO:21 and is about 228, 227, 226, or 225 amino acids in length.

Dkk proteins of the present invention can be used to identify additional Dkk-related proteins or family members. For example, a protein having homology to hDkk-3 was identified using the nucleotide sequence encoding the N-terminal unique region of hDkk-3 to search a nucleotide sequence database. A human cDNA clone (Accession No.: AA397836) was identified from the dBEST database as having homology to hDkk-3 and was fully sequenced. The encoded protein is referred to herein as human "Soggy-1" or "Dkk-like-N". The nucleotide and predicted amino acid sequence of human Soggy-1 are depicted in Figure 7. The nucleotide sequence of human Soggy-1 (SEQ ID NO:13) encodes a protein having 242 amino acids (SEQ ID NO:14). The nucleotide sequence of human Soggy-1 includes a 5' untranslated region containing nucleotides 1-74 of SEQ ID NO:13, a coding region containing nucleotides 75-800 of SEQ ID NO:13

- 27 -

(corresponding to nucleotides 1-726 of SEQ ID NO:15), and a 3' untranslated region containing nucleotides 801-928 of SEQ ID NO:13. The Soggy-1 protein (amino acid residues 32-132) has 25% identity to an N-terminal domain of human Dkk-3 (consisting of amino acid residues 22-140) as determined by ALIGN, Myers and Miller, (1989)

5 *CABIOS*, utilizing a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4.

Two murine cDNA clones were further identified from the database and fully sequenced. Combining the sequence information from these two clones resulted in a full-length sequence for murine Soggy-1. The nucleotide sequence and predicted amino
10 acid sequence of murine Soggy-1 are depicted in Figure 8. The nucleotide sequence of murine Soggy-1 (SEQ ID NO:26) encodes a protein having 230 amino acids (SEQ ID NO:27). The nucleotide sequence of murine Soggy-1 includes a 5' untranslated region containing nucleotides 1-56 of SEQ ID NO:26, a coding region containing nucleotides 57-746 of SEQ ID NO:26 (corresponding to SEQ ID NO:26), and a 3' untranslated
15 region containing nucleotides 747-835 of SEQ ID NO:26. Human and murine Soggy-1 proteins display 59% overall identity. An alignment of human and murine Soggy proteins to human and murine Dkk-3 proteins is depicted in Figure 10.

In one embodiment, a Soggy protein is identified based on the presence of at least one soggy domain or "SGY" domain in the protein or corresponding nucleic acid
20 molecule. As defined herein, a "SGY domain" includes a protein domain of a Soggy protein (*e.g.*, hSoggy-1) having an amino acid sequence of about 45-56 amino acids and having at least about 25-40% identity with amino acid residues 90-140 of hDkk-3 (leu90-glu140 of SEQ ID NO:2). In another embodiment, a "SGY domain" has 46-55, preferably 47-54, more preferably about 48-53, and even more preferably about 49-52 or
25 50-51 amino acids, and has at least about 27-38%, preferably about 28-37%, more preferably about 29-36%, even more preferably about 30-35%, and even more preferably about 31-34%, or 32-33% identity with amino acid residues 90-140 of hDkk-3 (Leu90-Glu140 of SEQ ID NO:2). In yet another embodiment, a "SGY domain" has the following consensus sequence: L - P - X(3) - H - X - E - X(7) - G - N - X - T - X(3) - H -
30 X(4) - K - X - T - X - N - X(2) - G - X(4) - S - E - X - V - X(2) - S - X(4) - E (SEQ ID NO:29). For example, human Soggy-1 has a SGY domain from about amino acid

- 28 -

residues 81-131 (Leu 81-Glu131 of SEQ ID NO:14) having 33% identity with amino acid residues 90-140 of hDkk-3 (leu90-glu140 of SEQ ID NO:2). Likewise, murine Soggy-1 has a SGY domain from about amino acid residues 71-120 (Leu 71-Glu120 of SEQ ID NO:27) having 33% identity with amino acid residues 90-140 of hDkk-3 (leu90-glu140 of SEQ ID NO:2). The SGY domains of human and murine Soggy-1 are depicted by shaded boxes in Figure 10.

In another embodiment of the invention, a Soggy protein has at least one SGY domain and a signal sequence. For example, in one embodiment, a hSoggy-1 protein contains a signal sequence of about amino acids 1-29, 1-30, 1-31, or 1-32 of SEQ ID NO:14. Accordingly, a mature hSoggy-1 protein begins at about amino acid residue 30, 31, 32, or 33 of SEQ ID NO:14 and is about 213, 212, 211, or 210 amino acids in length. In another embodiment, a mSoggy-1 protein contains a signal sequence of about amino acids 1-19, 1-20, 1-21, or 1-22 of SEQ ID NO:27. Accordingly, a mature mSoggy-1 protein begins at about amino acid residue 211, 210, 209, or 208 of SEQ ID NO:28 and is about 213, 212, 211, or 210 amino acids in length.

Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode Dkk proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify Dkk-encoding nucleic acids (*e.g.*, Dkk mRNA) and fragments for use as PCR primers for the amplification or mutation of Dkk nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*,

- 29 -

sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated Dkk nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. An isolated chromosome is not an isolated nucleic acid molecule as defined herein. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633, as

a hybridization probe, Dkk nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as

- 30 -

Accession Number 98452 or, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633, can be

isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers
5 designed based upon the sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633.

10

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis.

15 Furthermore, oligonucleotides corresponding to Dkk nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the human Dkk-3 cDNA. This cDNA comprises sequences
20 encoding the human Dkk-3 protein (*i.e.*, "the coding region", from nucleotides 38-1087), as well as 5' untranslated sequences (nucleotides 1 to 37) and 3' untranslated sequences (nucleotides 1088 to 2479). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 38 to 1087, corresponding to SEQ ID NO:3). A plasmid containing the full-length nucleotide sequence encoding hDkk-3
25 was deposited with the American Type Culture Collection (ATCC), presently in Manassas Virginia, on June 11, 1997 and assigned Accession Number 98452.

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:4. The sequence of SEQ ID NO:4 corresponds to the human Dkk-4 cDNA. This cDNA comprises
30 sequences encoding the human Dkk-4 protein (*i.e.*, "the coding region", from nucleotides 125-796), as well as 5' untranslated sequences (nucleotides 1 to 124) and 3'

- 31 -

untranslated sequences (nucleotides 797 to 848). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:4 (*e.g.*, nucleotides 125 to 796, corresponding to SEQ ID NO:6).

5 In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:7. The sequence of SEQ ID NO:7 corresponds to the human Dkk-1 cDNA. This cDNA comprises sequences encoding the human Dkk-1 protein (*i.e.*, "the coding region", from nucleotides 109-906), as well as 5' untranslated sequences (nucleotides 1 to 108) and 3' untranslated sequences (nucleotides 907-1536). Alternatively, the nucleic acid molecule
10 can comprise only the coding region of SEQ ID NO:7 (*e.g.*, nucleotides 109-906, corresponding to SEQ ID NO:9). A plasmid containing the full-length nucleotide sequence encoding hDkk-1 was deposited with the American Type Culture Collection (ATCC), presently in Manassas Virginia, on January 16, 1998 and assigned Accession Number 98633.

15 In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:20. The sequence of SEQ ID NO:20 corresponds to the human Dkk-2 cDNA. This cDNA comprises sequences encoding the human Dkk-2 protein (*i.e.*, "the coding region", from nucleotides 724-1500), 5' untranslated sequences (nucleotides 1-723), as well as 3' untranslated sequences (nucleotides 1501-3687). Alternatively, the nucleic acid
20 molecule can comprise only the coding region of SEQ ID NO:20 (*e.g.*, nucleotides 724 to 1500, corresponding to SEQ ID NO:22). A plasmid, clone fthul33, containing the full-length nucleotide sequence encoding hDkk-2 was deposited with the American Type Culture Collection (ATCC), presently in Manassas Virginia, on March 2, 1999.

25

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:13. The sequence of SEQ ID NO:13 corresponds to the human Soggy cDNA. This cDNA comprises sequences encoding the human Soggy protein (*i.e.*, "the coding region", from
30 nucleotides 75 to 800), as well as 5' untranslated sequences (nucleotides 1 to 74) and 3' untranslated sequences (nucleotides 801 to 928). Alternatively, the nucleic acid

- 32 -

molecule can comprise only the coding region of SEQ ID NO:13 (*e.g.*, nucleotides 75 to 800, corresponding to SEQ ID NO:15).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide
5 sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

10 or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as
15 Accession Number 98633, is one which is sufficiently

complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide
20 sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20, or the nucleotide sequence of the DNA insert of the plasmid deposited
25 with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the
30 present invention comprises a nucleotide sequence which is at least about 30-35%, preferably about 40-45%, more preferably about 50-55%, even more preferably about

- 33 -

60-65%, and even more preferably at least about 70-75%, 80-85%, 90-95% or more homologous to the nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:20, or SEQ ID NO:22, the nucleotide sequence of the DNA insert of the
5 plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633, or a portion of any of these nucleotide sequences.

In one aspect, the present invention features isolated nucleic acid molecules
10 which are linear (*e.g.*, linear fragments of double-stranded DNA, linear strands of single-stranded DNA, single-stranded RNA molecules, and oligonucleotides). Another aspect of the present invention features circular nucleic acid molecules (*e.g.*, double-stranded DNA molecules, for example, plasmid molecules including the nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7,
15 SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:20, or SEQ ID NO:22, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633, or a portion of any of these nucleotide sequences).

20

In one embodiment, the isolated nucleic acid molecules of the present invention are DNA molecules which are in a form suitable for expression (*e.g.*, suitable for expression of corresponding messenger RNA or mRNA). In another embodiment, the isolated nucleic acid molecules are DNA molecules which are in a form suitable for
25 expression of corresponding protein (*e.g.*, in a form, for example, in a vector, which is capable of expressing protein, *e.g.*, in the appropriate orientation for expression from regulatory elements and/or in-frame with appropriate regulatory elements). In another embodiment, the isolated nucleic acids are in a form suitable for determination of nucleic acid sequence (*e.g.*, in a form suitable for sequencing, for example, is a
30 sequencing vector including a M13, T7, T3 and SP6 promoter. Examples of sequencing vectors include, but are not limited to pBluescript^{*} (StratageneTM), pT7T3D (PharmaciaTM)

* Trademark

- 34 -

and pCR2.1 (InVitrogen). In yet another embodiment, the isolated nucleic acid molecules are free from vector sequences. In a preferred embodiment, an isolated nucleic acid molecule is free from sequencing vector sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion
5 of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

10 for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a Dkk protein or Dkk-related protein. The nucleotide sequence determined from the cloning of the human Dkk genes allows for the generation of probes and primers designed for use in identifying and/or cloning Dkk homologues in other cell types, *e.g.*, from other tissues, as well as
15 Dkk homologues from other mammals and Dkk-related proteins. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 40, 50 or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ
20 ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

of an anti-sense sequence of SEQ ID NO:1, SEQ ID NO:4,
25 SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

or of a naturally occurring mutant of SEQ ID
30 NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number

- 35 -

98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633.

In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide
5 sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule consisting of nucleotides 470-2479 of SEQ ID NO:1 or to a nucleic acid molecule consisting of nucleotides 1-475 of SEQ ID NO:4.

Probes based on human nucleotide sequences (*e.g.*, the human Dkk nucleotide sequence) can be used to detect transcripts or genomic sequences encoding the same or
10 homologous proteins. For instance, primers based on the nucleic acid represented in SEQ ID NOs:1 or 3 can be used in PCR reactions to clone Dkk homologs (*e.g.*, hDkk-3 homologues). In a preferred embodiment of the invention, Dkk homologs are cloned by PCR amplification (*e.g.*, RT-PCR) using primers hybridizing to a portion of the nucleotide sequence encoding the Dkk cysteine rich domain. Likewise, probes based on
15 the subject Dkk sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as
20 a part of a diagnostic test kit for identifying cells or tissue which misexpress a Dkk protein, such as by measuring a level of a Dkk-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting Dkk mRNA levels or determining whether a genomic Dkk gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a Dkk or Dkk-related protein" can be prepared by isolating a portion of SEQ ID NO:1, SEQ ID NO:4,
25 SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633, which encodes a polypeptide having a biological activity (the biological activities of the Dkk and Dkk-related proteins have previously

- 36 -

been described), expressing the encoded portion of the protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the protein.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

due to degeneracy of the genetic code and thus encode the same proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633.

In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO: 5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21.

In addition to the human nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the Dkk or Dkk-related proteins may exist within a population (e.g., the human population). Such genetic polymorphism in the Dkk or Dkk-related genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a protein, preferably a mammalian Dkk or Dkk-related protein. Such natural allelic variations can typically result in 1-5% variance

- 37 -

in the nucleotide sequence of a Dkk or Dkk-related gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in genes that are the result of natural allelic variation and that do not alter the functional activity of a Dkk or Dkk-related protein are intended to be within the scope of the invention.

5 Moreover, nucleic acid molecules encoding Dkk or Dkk-related proteins from other species, and thus which have a nucleotide sequence which differs from the human sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid
10 deposited with ATCC as Accession Number 98633, are

intended to be within the scope of the invention. For example, a murine Dkk-3 cDNA has been identified based of the nucleotide sequence of human Dkk-3. The nucleotide sequence of murine Dkk-3 (SEQ ID NO:16) encodes a hDkk-3 protein having 349
15 amino acids. The nucleotide and amino acid sequences of murine Dkk-3 are depicted in Figure 5. The coding region of murine Dkk-3 is represented by SEQ ID NO:18. A plasmid containing the full-length nucleotide sequence encoding mDkk-3 was deposited with the American Type Culture Collection (ATCC), presently in Manassas, Virginia, on January 16, 1998 and assigned Accession Number 98634. Likewise, a murine Dkk-
20 related protein (Soggy-1) has been identified based of the nucleotide sequence of human Dkk-3. The nucleotide sequence of murine Soggy-1 (SEQ ID NO:26) encodes a protein having 230 amino acids (SEQ ID NO:27). The nucleotide and amino acid sequences of murine Soggy-1 are depicted in Figure 8. The coding region of murine Soggy-1 is represented by SEQ ID NO:28.

25 Nucleic acid molecules corresponding to natural allelic variants and homologues of the Dkk or Dkk-related cDNAs of the invention can be isolated based on their homology to the human nucleic acids disclosed herein using the human cDNA, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Examples of tissues and/or libraries suitable
30 for isolation of the subject nucleic acids include brain, spinal chord and heart tissue. cDNA encoding a Dkk protein (e.g., a hDkk-3 protein) can be obtained by isolating total

- 38 -

mRNA from a cell, *e.g.*, a vertebrate cell, a mammalian cell, or a human cell, including embryonic cells. Double stranded cDNAs can then be prepared from the total mRNA, and subsequently inserted into a suitable plasmid or bacteriophage vector using any one of a number of known techniques. The gene encoding a hDkk-3 protein can also be
5 cloned using established polymerase chain reaction techniques in accordance with the nucleotide sequence information provided by the invention. The nucleic acid of the invention can be DNA or RNA or analogs thereof.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15 nucleotides in length and hybridizes under stringent conditions to
10 the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633.

15 In another embodiment, the nucleic acid is at least 30, 50, 100, 250, 300, 400 or 500 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other. Preferably, the conditions are
20 such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization
25 conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an
30 RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

- 39 -

In addition to naturally-occurring allelic variants of the Dkk or Dkk-related sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence
5 of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

thereby leading to changes in the amino acid sequence of the encoded Dkk proteins, without altering the functional ability
10 of the Dkk proteins. For example, nucleotide substitutions leading to amino acid substitutions (particularly conservative amino acid substitutions) at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence
15 of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633.

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of Dkk (or wild-type Dkk-related sequence) (*e.g.*, the sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ
20 ID NO:21) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the Dkk or Dkk-related proteins of the present invention (*e.g.*, cysteine residues within cysteine-rich domains), are predicted to be particularly unamenable to alteration. Furthermore, amino acid residues that are conserved between Dkk protein
25 and other proteins having cysteine-rich domains are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding Dkk or Dkk-related proteins that contain changes in amino acid residues that are not essential for activity. Such proteins differ in amino acid sequence from SEQ ID
30 NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21 yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a

- 40 -

nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8,, SEQ ID NO:14, or SEQ ID NO:21. Preferably, the protein encoded by the nucleic acid molecule is at least about 65-70% homologous to
5 SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21, more preferably at least about 75-80% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21, even more preferably at least about 85-90% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21, and most preferably at least about 95% homologous to SEQ ID NO:2, SEQ
10 ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21.

An isolated nucleic acid molecule encoding a Dkk protein homologous to the protein of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7,
15 SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

such that one or more amino acid substitutions, additions or
20 deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633, by

25 standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar
30 side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g.,

- 41 -

lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*,
5 threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, for example, a predicted nonessential amino acid residue in a Dkk protein (*e.g.*, one not located in a cysteine-rich domain) is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a Dkk or
10 Dkk-related coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the nucleotide sequence of the DNA insert of
15 the plasmid deposited with ATCC as Accession Number 98633,

the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant Dkk or Dkk-related protein can be assayed
20 for intracellular calcium, an increase in phosphatidylinositol or other molecule, and can result, *e.g.*, in phosphorylation of specific proteins, a modulation of gene transcription and any of the other biological activities set forth herein.

In a preferred embodiment, a mutant Dkk or Dkk-related protein can also be assayed for the ability to (1) modulate cellular signal transduction, either *in vitro* or *in vivo*;
25 *vivo*; (2) regulate communication between cells; (3) regulate expression of genes whose expression is modulated by binding of Dkk (*e.g.*, hDkk-3) to a receptor; (4) regulate gene transcription in a cell involved in development or differentiation, either *in vitro* or *in vivo*; (5) regulate cellular proliferation, either *in vitro* or *in vivo*; (6) form and/or maintain ordered spatial arrangements of differentiated tissues in vertebrates; (7)
30 modulate cell death (*e.g.* cell survival); (8) regulate cell migration; and/or (9) modulate immune system function.

- 42 -

In addition to the nucleic acid molecules encoding Dkk or Dkk-related proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire Dkk coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding Dkk. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of human Dkk-3 corresponds to SEQ ID NO:3, the coding region of human Dkk-4 corresponds to SEQ ID NO:6, the coding region of human Dkk-1 corresponds to SEQ ID NO:9, the coding region of human Dkk-2 corresponds to SEQ ID NO:22, and the coding region of human Soggy corresponds to SEQ ID NO:15). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding a Dkk or Dkk-related protein. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences disclosed herein (*e.g.*, SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:15, or SEQ ID NO:22), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of a Dkk or Dkk-related mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of the mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of Dkk mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, or 90 nucleotides in length. In a preferred embodiment, an oligonucleotide is about 30-90, preferably about 40-80, more preferably about 50-70 nucleotides in length and is antisense to a portion of

- 43 -

SEQ ID NO:1 from about nucleotides 1-150. In another embodiment, an oligonucleotide is antisense to a portion of SEQ ID NO:4 from about nucleotides 25-225. In another embodiment, an oligonucleotide is antisense to a portion of SEQ ID NO:7 from about nucleotides 1-200. In another embodiment, an oligonucleotide is
 5 antisense to a portion of SEQ ID NO:20 from about nucleotides 625-825. In yet another embodiment, an oligonucleotide is antisense to a portion of SEQ ID NO:13 from about nucleotides 1-175.

An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For
 10 example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives, acridine substituted nucleotides, can be used.
 15 Alternatively, the antisense nucleic acid molecule can be synthesized to increase transport across cellular membranes, *e.g.*, methylphosphonate derivatives. The antisense molecules can include a 3'-terminal cap (*e.g.*, a 3'-aminopropyl modification), a biotin moiety, or even a 3'-3' terminal linkage.

Examples of modified nucleotides which can be used to generate the antisense
 20 nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-
 25 methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-
 30 oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the

- 44 -

antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

5 The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a Dkk or Dkk-related protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable
10 duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically.
15 For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular
20 concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

 In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms
25 specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.*, (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-*o*-methylribonucleotide (Inoue *et al.*, (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.*, (1987) *FEBS Lett.* 215:327-330).

- 45 -

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes
5 (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave Dkk or Dkk-related mRNA transcripts to thereby inhibit translation of Dkk or Dkk-related mRNA. A ribozyme having specificity for a Dkk- or Dkk-related-encoding nucleic acid can be designed based upon the nucleotide sequence of a Dkk or Dkk-related cDNA disclosed herein (*i.e.*, SEQ ID NO:1, SEQ ID NO:4, SEQ ID
10 NO:7, SEQ ID NO:13, SEQ ID NO:20, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98633). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be
15 cleaved in a Dkk-encoding mRNA. See, *e.g.*, Cech *et al.*, U.S. Patent No. 4,987,071; and Cech *et al.*, U.S. Patent No. 5,116,742. Alternatively, Dkk (or Dkk-related) mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

20 Alternatively, gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the Dkk or Dkk-related gene (*e.g.*, the promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.*, (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In yet another embodiment, the nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to
30 generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs"

- 46 -

refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis
5 of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* PNAS 93: 14670-675.

PNAs of Dkk or Dkk-related nucleic acid molecules can be used therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents
10 for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of Dkk or Dkk-related nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*));
15 or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of Dkk can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of
20 drug delivery known in the art. For example, PNA-DNA chimeras of Dkk nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of
25 appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and
30 modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et*

- 47 -

al. (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. US.* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134, published April 25, 1988). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *BioTechniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

II. Isolated Dkk Proteins and Anti-Dkk Antibodies

One aspect of the invention pertains to isolated Dkk proteins, Dkk-related proteins and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise antibodies. In one embodiment, native Dkk or Dkk-related proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a Dkk or Dkk-related protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the Dkk or Dkk-related protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of protein in

- 48 -

which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of Dkk or Dkk-related protein having less than about 30% (by dry weight) of non-Dkk protein or non-Dkk-related protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-Dkk protein or non-Dkk-related protein, still more preferably less than about 10% of non-Dkk protein or non-Dkk-related protein, and most preferably less than about 5% non-Dkk protein or non-Dkk-related protein. When the Dkk or Dkk-related protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of Dkk or Dkk-related protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of Dkk protein having less than about 30% (by dry weight) of chemical precursors, non-Dkk chemicals, or non-Dkk-related chemicals, more preferably less than about 20% chemical precursors, non-Dkk chemicals, or non-Dkk-related chemicals, still more preferably less than about 10% chemical precursors, non-Dkk chemicals, or non-Dkk-related chemicals, and most preferably less than about 5% chemical precursors, non-Dkk chemicals, or non-Dkk-related chemicals.

Biologically active portions of a Dkk or Dkk-related protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the Dkk or Dkk-related protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21, which include less amino acids than the full length proteins, and exhibit at least one activity of a Dkk or Dkk-related protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the Dkk or Dkk-related protein. A biologically active portion of a protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

- 49 -

In one embodiment, a biologically active portion of a Dkk protein comprises at least a cysteine-rich region. In another embodiment, a biologically active portion of a Dkk protein comprises at least a cysteine-rich region, wherein the cysteine-rich region includes at least one cysteine-rich domain. In yet another embodiment, a biologically
5 active portion of a Dkk protein comprises at least a signal sequence.

In another embodiment, a biologically active portion of a Dkk-related protein (*e.g.*, a Soggy protein) comprises at least a Soggy domain. In yet another embodiment, a biologically active portion of a Dkk-related protein comprises at least a signal sequence.

In an alternative embodiment, a biologically active portion of a Dkk or Dkk-
10 related protein comprises an amino acid sequence lacking a signal sequence.

It is to be understood that a preferred biologically active portion of a Dkk or Dkk-related protein of the present invention may contain at least one of the above-identified structural domains. A more preferred biologically active portion of a Dkk or Dkk-related protein may contain at least two of the above-identified structural domains.
15 An even more preferred biologically active portion of a protein may contain at least three of the above-identified structural domains. A particularly preferred biologically active portion of a protein of the present invention may contain at least four of the above-identified structural domains.

Moreover, other biologically active portions, in which other regions of the
20 protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native Dkk or Dkk-related protein.

In a preferred embodiment, the Dkk protein has an amino acid sequence shown in SEQ ID NO:2 or an amino acid sequence at least about 55% homologous to SEQ ID NO:2. In another preferred embodiment, the Dkk protein has an amino acid sequence
25 shown in SEQ ID NO:5 or an amino acid sequence at least about 35% homologous to SEQ ID NO:5. In another preferred embodiment, the Dkk protein has an amino acid sequence shown in SEQ ID NO:8 or an amino acid sequence at least about 85% homologous to SEQ ID NO:8. In another preferred embodiment, the Dkk protein has an amino acid sequence shown in SEQ ID NO:21 or an amino acid sequence at least about
30 35% homologous to SEQ ID NO:21. In another preferred embodiment, the protein has an amino acid sequence shown in SEQ ID NO:14 or an amino acid sequence at least

- 50 -

about 60% homologous to SEQ ID NO:14. In still another preferred embodiment, a protein of the present invention comprises an amino acid sequence which is at least about 30-35%, preferably about 40-45%, more preferably about 50-55%, even more preferably about 60-65%, and even more preferably at least about 70-75%, 80-85%, 90-
5 95% or more homologous to the amino acid sequences shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21.

In other embodiments, the protein is substantially homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21, and, preferably, retains the functional activity of the protein of SEQ ID NO:2, SEQ ID NO:5, SEQ ID
10 NO:8, SEQ ID NO:14, or SEQ ID NO:21, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the protein is a protein which comprises an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21 and, preferably,
15 retains the functional activity of the proteins of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:11, respectively. Preferably, the protein is at least about 70% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21, more preferably at least about 80% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21, even more
20 preferably at least about 90% homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21, and most preferably at least about 95% or more homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21.

To determine the percent homology of two amino acid sequences or of two
25 nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence and non-homologous sequences can be disregarded for comparison purposes). In one embodiment, an alignment is a global alignment, *e.g.*, an overall sequence alignment. In another embodiment, an
30 alignment is a local alignment. In a preferred embodiment, the length of a sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more

- 51 -

preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence to which it is aligned (*e.g.*, when aligning a second sequence to the Dkk amino acid sequence of SEQ ID NO:2, at least 105, preferably at least 145, more preferably at least 175, even more preferably at least 210, and even more preferably at least 245, 280 or 315 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (Accelrys Software Inc., San Diego, CA), using either a Blossom* 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, 5, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (Accelrys Software Inc., San Deigo, CA), using a NWSgapdna* CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

* Trademark

- 52 -

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to Dkk nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to Dkk protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

The invention also provides Dkk or Dkk-related chimeric or fusion proteins. As used herein, a "chimeric protein" or "fusion protein" comprises a Dkk or Dkk-related polypeptide operatively linked to a non-Dkk polypeptide or non-Dkk-related polypeptide. A "Dkk polypeptide" or "Dkk-related polypeptide" refers to a polypeptide having an amino acid sequence corresponding to Dkk or a Dkk-related protein, whereas a "non-Dkk polypeptide" or "non-Dkk-related polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the Dkk or Dkk-related protein, *e.g.*, a protein which is different from the Dkk or Dkk-related protein and which is derived from the same or a different organism. Within a Dkk or Dkk-related fusion protein the Dkk or Dkk-related polypeptide can correspond to all or a portion of a Dkk or Dkk-related protein. In a preferred embodiment, a Dkk or Dkk-related fusion protein comprises at least one biologically active portion of a Dkk protein. In another preferred embodiment, a Dkk or Dkk-related fusion protein comprises at least two biologically active portions of a Dkk or Dkk-related protein. In another preferred embodiment, a Dkk or Dkk-related fusion protein comprises at least three biologically active portions of a Dkk or Dkk-related protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the Dkk or Dkk-related polypeptide and the non-Dkk or non-Dkk-related polypeptide are

- 53 -

fused in-frame to each other. The non-Dkk or non-Dkk-related polypeptide can be fused to the N-terminus or C-terminus of the Dkk or Dkk-related polypeptide.

For example, in one embodiment, the fusion protein is a GST-Dkk fusion protein in which the Dkk sequences are fused to the C-terminus of the GST sequences. Such
5 fusion proteins can facilitate the purification of recombinant Dkk.

In another embodiment, the fusion protein is a Dkk or Dkk-related protein containing a heterologous signal sequence at its N-terminus. For example, the native Dkk signal sequence (i.e., about amino acids 1 to 23 of SEQ ID NO:2) can be removed and replaced with a signal sequence from another protein. In certain host cells (*e.g.*,
10 mammalian host cells), expression and/or secretion of Dkk or Dkk-related proteins can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is a Dkk-immunoglobulin fusion protein in which the Dkk sequences comprising primarily the Dkk cysteine-rich regions are fused to sequences derived from a member of the immunoglobulin protein family.
15 Soluble derivatives have also been made of cell surface glycoproteins in the immunoglobulin gene superfamily consisting of an extracellular domain of the cell surface glycoprotein fused to an immunoglobulin constant (Fc) region (see *e.g.*, Capon, *et al.* (1989) *Nature* 337:525-531 and Capon U.S. Patents 5,116,964 and 5,428,130 [CD4-IgG1 constructs]; Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 173:721-730 [a CD28-IgG1 construct and a B7-1-IgG1 construct]; and Linsley, P.S. *et al.* (1991) *J. Exp. Med.* 174:561-569 and U.S. Patent 5,434,131[a CTLA4-IgG1]). Such fusion proteins have proven useful for modulating receptor-ligand interactions. Soluble derivatives of cell surface proteins of the tumor necrosis factor receptor (TNFR) superfamily proteins have been made consisting of an extracellular domain of the cell surface receptor fused to an
20 immunoglobulin constant (Fc) region (See for example Moreland *et al.* (1997) *N. Engl. J. Med.* 337(3):141-147; van der Poll *et al.* (1997) *Blood* 89(10):3727-3734; and Ammann *et al.* (1997) *J. Clin. Invest.* 99(7):1699-1703.)

The Dkk-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction
30 between a Dkk ligand and a Dkk receptor on the surface of a cell, to thereby suppress Dkk-mediated signal transduction *in vivo*. The Dkk-immunoglobulin fusion proteins

- 54 -

can be used to affect the bioavailability of a Dkk cognate receptor. Inhibition of the Dkk ligand/Dkk interaction may be useful therapeutically for both the treatment of differentiative or proliferative disorders, as well as modulating (*e.g.*, promoting or inhibiting) developmental responses, cell adhesion, and/or cell fate. Moreover, the Dkk-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-Dkk antibodies in a subject, to purify Dkk ligands and in screening assays to identify molecules which inhibit the interaction of Dkk with a Dkk ligand.

Preferably, a Dkk or Dkk-related chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.*, John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A Dkk-encoding nucleic acid or nucleic acid encoding a Dkk-related protein can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the protein.

The present invention also pertains to variants of the Dkk or Dkk-related proteins which function as either agonists (mimetics) or as antagonists. Variants of the Dkk or Dkk-related proteins can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a Dkk or Dkk-related protein. An agonist of the Dkk or Dkk-related proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a Dkk or Dkk-related protein. An antagonist of a Dkk or Dkk-related protein can inhibit one or more of the activities of the naturally occurring

- 55 -

form of the protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the Dkk or Dkk-related protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of
5 the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the Dkk or Dkk-related protein.

In one embodiment, variants of a Dkk or Dkk-related protein which function as either agonists (mimetics) or as antagonists can be identified by screening combinatorial
10 libraries of mutants, *e.g.*, truncation mutants, of a Dkk or Dkk-related protein for protein agonist or antagonist activity. In one embodiment, a variegated library of Dkk or Dkk-related variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of Dkk or Dkk-related variants can be produced by, for example, enzymatically ligating a mixture of synthetic
15 oligonucleotides into gene sequences such that a degenerate set of potential Dkk or Dkk-related sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of Dkk or Dkk-related sequences therein. There are a variety of methods which can be used to produce libraries of potential Dkk or Dkk-related variants from a degenerate oligonucleotide
20 sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential Dkk or Dkk-related sequences. Methods for synthesizing degenerate oligonucleotides are known in the art
25 (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.*, (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.*, (1984) *Science* 198:1056; Ike *et al.*, (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of a Dkk or Dkk-related protein coding sequence can be used to generate a variegated population of Dkk or Dkk-related
30 fragments for screening and subsequent selection of variants of a Dkk or Dkk-related protein. In one embodiment, a library of coding sequence fragments can be generated by

- 56 -

treating a double stranded PCR fragment of a Dkk coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded
5 portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the Dkk protein.

Several techniques are known in the art for screening gene products of
10 combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of Dkk or Dkk-related proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the
15 gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in
20 combination with the screening assays to identify Dkk variants (Arkin and Yourvan (1992) *PNAS* 89:7811-7815; Delgrave *et al.*, (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated Dkk or Dkk-related library. For example, a library of expression vectors can be transfected into a cell line which ordinarily responds to a particular ligand in a Dkk-
25 dependent manner. The transfected cells are then contacted with the ligand and the effect of expression of the mutant on signaling by the ligand can be detected, *e.g.*, by measuring any of a number of immune cell responses. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of ligand induction, and the individual clones further characterized.

- 57 -

An isolated Dkk protein, Dkk-related protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind Dkk or Dkk-related proteins using standard techniques for polyclonal and monoclonal antibody preparation. A full-length Dkk or Dkk-related protein can be used or, alternatively, the invention
5 provides antigenic peptide fragments for use as immunogens. The antigenic peptide of Dkk comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, or SEQ ID NO:21 and encompasses an epitope of Dkk or Dkk-related protein such that an antibody raised
10 against the peptide forms a specific immune complex with the protein. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of Dkk or Dkk-related proteins that are located on the surface of the protein, *e.g.*, hydrophilic
15 regions.

A Dkk or Dkk-related immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed Dkk or Dkk-related protein or a chemically synthesized Dkk or
20 Dkk-related polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic Dkk preparation, for example, induces a polyclonal anti-Dkk antibody response.

Accordingly, another aspect of the invention pertains to anti-Dkk antibodies as
25 well as antibodies to Dkk-related proteins. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as Dkk or Dkk-related antigens. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and
30 F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind Dkk

- 58 -

or Dkk-related polypeptides. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of Dkk or a or Dkk-related protein. A monoclonal antibody composition thus
5 typically displays a single binding affinity for a particular Dkk or Dkk-related protein with which it immunoreacts.

Polyclonal antibodies can be prepared as described above by immunizing a suitable subject with a Dkk or Dkk-related immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an
10 enzyme linked immunosorbent assay (ELISA) using immobilized Dkk or Dkk-related protein. If desired, the antibody molecules directed against Dkk or Dkk-related protein can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the antibody titers are highest, antibody-
15 producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.*, (1981) *J. Immunol.* 127:539-46; Brown *et al.*, (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.*, (1976) *PNAS* 76:2927-31; and Yeh *et al.*, (1982) *Int. J. Cancer* 29:269-75), the more
20 recent human B cell hybridoma technique (Kozbor *et al.*, (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.*, (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum
25 Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M.L. Gefter *et al.*, (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a Dkk or Dkk-related immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a
30 hybridoma producing a monoclonal antibody that binds Dkk or Dkk-related protein.

- 59 -

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating a monoclonal antibody (see, *e.g.*, G. Galfre *et al.*, (1977) *Nature* 266:55052; Gefter *et al.*, *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind Dkk or Dkk-related protein, *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with Dkk or Dkk-related protein to thereby isolate immunoglobulin library members that bind Dkk or Dkk-related protein. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.*, U.S. Patent No. 5,223,409; Kang *et al.*, PCT International Publication No.

- 60 -

WO 92/18619; Dower *et al.*, PCT International Publication No. WO 91/17271; Winter *et al.*, PCT International Publication WO 92/20791; Markland *et al.*, PCT International Publication No. WO 92/15679; Breitling *et al.*, PCT International Publication WO 93/01288; McCafferty *et al.*, PCT International Publication No. WO 92/01047; Garrard *et al.*, PCT International Publication No. WO 92/09690; Ladner *et al.*, PCT International Publication No. WO 90/02809; Fuchs *et al.*, (1991) *Bio/Technology* 9:1370-1372; Hay *et al.*, (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.*, (1989) *Science* 246:1275-1281; Griffiths *et al.*, (1993) *EMBO J* 12:725-734; Hawkins *et al.*, (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.*, (1991) *Nature* 352:624-628; Gram *et al.*, (1992) *PNAS* 89:3576-3580; Garrard *et al.*, (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.*, (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.*, (1991) *PNAS* 88:7978-7982; and McCafferty *et al.*, *Nature* (1990) 348:552-554.

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.*, International Application No. PCT/US86/02269; Akira, *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, PCT International Publication No. WO 86/01533; Cabilly *et al.*, U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.*, (1988) *Science* 240:1041-1043; Liu *et al.*, (1987) *PNAS* 84:3439-3443; Liu *et al.*, (1987) *J. Immunol.* 139:3521-3526; Sun *et al.*, (1987) *PNAS* 84:214-218; Nishimura *et al.*, (1987) *Canc. Res.* 47:999-1005; Wood *et al.*, (1985) *Nature* 314:446-449; and Shaw *et al.*, (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.*, (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.*, (1986) *Nature* 321:552-525; Verhoeyan *et al.*, (1988) *Science* 239:1534; and Beidler *et al.*, (1988) *J. Immunol.* 141:4053-4060.

- 61 -

An antibody (*e.g.*, monoclonal antibody) can be used to isolate Dkk or Dkk-related protein by standard techniques, such as affinity chromatography or immunoprecipitation. An antibody can facilitate the purification of natural Dkk or Dkk-related protein from cells and of recombinantly produced Dkk or Dkk-related protein expressed in host cells. Moreover, an antibody can be used to detect Dkk or Dkk-related protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the Dkk or Dkk-related protein. Antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding Dkk or a nucleic acid encoding a Dkk-related protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal

- 62 -

mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as

- 63 -

described herein (*e.g.*, Dkk proteins, Dkk-related proteins, mutant forms of Dkk or Dkk-related proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of Dkk or Dkk-related proteins in prokaryotic or eukaryotic cells. For example, Dkk can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in activity assays, in ligand binding (*e.g.*, direct assays or competitive assays described in detail below), to generate antibodies specific for Dkk or Dkk-related proteins, as examples. In a preferred embodiment, a Dkk or Dkk-related fusion expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently

- 64 -

transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the Dkk or Dkk-related expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, Dkk or Dkk-related protein can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith *et al.*, (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

- 65 -

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.*, (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.*, (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.*, (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.*, (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an

- 66 -

RNA molecule which is antisense to Dkk mRNA or a Dkk-related mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory
5 sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion
10 of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and
15 "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used
20 herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, Dkk protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

25 Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated
30 transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.*, (*Molecular Cloning: A*

- 67 -

Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may
5 integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be
10 introduced into a host cell on the same vector as that encoding Dkk or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in
15 culture, can be used to produce (*i.e.*, express) a Dkk or Dkk-related protein. Accordingly, the invention further provides methods for producing Dkk or Dkk-related proteins using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding Dkk or a Dkk-related protein has been introduced) in a suitable medium such
20 that protein is produced. In another embodiment, the method further comprises isolating Dkk or a Dkk-related protein from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which Dkk-coding sequences (or Dkk-related
25 coding sequences) have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous Dkk sequences (or Dkk-related sequences) have been introduced into their genome or homologous recombinant animals in which endogenous Dkk sequences (or Dkk-related sequences) have been altered. Such animals are useful for studying the function and/or activity of Dkk or Dkk-related
30 proteins and for identifying and/or evaluating modulators of Dkk or Dkk-related protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a

- 68 -

mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous Dkk or Dkk-related gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created, for example, by introducing Dkk-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human Dkk cDNA sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, or SEQ ID NO:20 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human Dkk gene, such as a mouse Dkk gene, can be isolated based on hybridization to the human Dkk cDNA (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the Dkk transgene to direct expression of Dkk protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.*, and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the Dkk transgene in its genome and/or expression of Dkk mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional

- 69 -

animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding Dkk can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a Dkk gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the Dkk gene. The Dkk gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:3, SEQ ID NO: 6, SEQ ID NO:9 or SEQ ID NO:22), but more preferably, is a non-human homologue of a human Dkk gene. For example, a mouse Dkk gene of SEQ ID NO:16 can be used to construct a homologous recombination vector suitable for altering an endogenous Dkk gene in the mouse genome. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous Dkk gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous Dkk gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous Dkk protein). In the homologous recombination vector, the altered portion of the Dkk gene is flanked at its 5' and 3' ends by additional nucleic acid of the Dkk gene to allow for homologous recombination to occur between the exogenous Dkk gene carried by the vector and an endogenous Dkk gene in an embryonic stem cell. The additional flanking Dkk nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced Dkk gene has homologously recombined with the endogenous Dkk gene are selected (see *e.g.*, Li, E. *et al.*, (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the

- 70 -

homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, A.

5 (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.* It is also within the scope of the present invention to practice the above-described transgenic methodology utilizing nucleic acid molecules which encode Dkk-related proteins.

10 In another embodiment, transgenic non-humans animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.*, (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase
15 system of *Saccharomyces cerevisiae* (O'Gorman *et al.*, (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a
20 selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.*, (1997) *Nature* 385:810-813. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can
25 then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is
30 isolated.

IV. Pharmaceutical Compositions

The Dkk and Dkk-related nucleic acid molecules, Dkk and Dkk-related proteins, and anti-Dkk or anti-Dkk-related protein antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions
5 suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration.
10 The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible
15 with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils,
20 polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases,
25 such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the
30 extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic

- 72 -

water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as

5 bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of

10 surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be

15 brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a Dkk protein, Dkk-related protein or antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as

20 required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a

25 powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and

30 used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is

- 73 -

applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as

5 microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel*, or corn starch; a lubricant such as magnesium stearate or Sterotes*; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

10 For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be
15 permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in
20 the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will
25 protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.

30 The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected

* Trademark

- 74 -

cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine

- 75 -

useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for
5 example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.*, (1994) *PNAS* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector
10 can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

15

V. Uses and Methods of the Invention

The molecules of the present invention (*e.g.*, nucleic acid molecules, proteins, protein homologues, and antibodies described herein) can be used in one or more of the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic assays,
20 prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (*e.g.*, therapeutic and prophylactic). As described herein, a Dkk protein of the invention has one or more of the following activities: intracellular calcium, an increase in phosphatidylinositol or other molecule, and can result, *e.g.*, in phosphorylation of specific proteins, a modulation of gene transcription and any of the other biological
25 activities set forth herein.

In a preferred embodiment, a Dkk activity is at least one or more of the following activities: (i) interaction of a Dkk protein with and/or binding to a second molecule, (*e.g.*, a protein, such as a Dkk (*e.g.*, hDkk-3) receptor, a soluble form of a Dkk receptor, a receptor for a member of the *wnt* family of signaling proteins, or a non-Dkk signaling
30 molecule); (ii) interaction of a Dkk protein with an intracellular protein via a membrane-bound Dkk receptor; (iii) complex formation between a soluble Dkk protein and a

- 76 -

second soluble Dkk binding partner (*e.g.*, a non-Dkk protein molecule or a second Dkk protein molecule); (iv) interaction with other extracellular proteins (*e.g.*, regulation of *wnt*-dependent cellular adhesion to extracellular matrix components); (v) binding to and eliminating an undesirable molecule (*e.g.*, a detoxifying activity or defense function);
5 and/or (vi) an enzymatic activity, and can thus be used in, for example, (1) modulation of cellular signal transduction, either *in vitro* or *in vivo* (*e.g.*, antagonism of the activity of members of the *wnt* family of secreted proteins or suppression of *wnt*-dependent signal transduction); (2) regulation of communication between cells (*e.g.*, regulation of *wnt*-dependent cell-cell interactions); (3) regulation of expression of genes
10 whose expression is modulated by binding of Dkk (*e.g.*, hDkk-3) to a receptor; (4) regulation of gene transcription in a cell involved in development or differentiation, either *in vitro* or *in vivo* (*e.g.*, induction of cellular differentiation); (5) regulation of gene transcription in a cell involved in development or differentiation, wherein at least one gene encodes a differentiation-specific protein; (6) regulation of gene transcription
15 in a cell involved in development or differentiation, wherein at least one gene encodes a second secreted protein; (7) regulation of gene transcription in a cell involved in development or differentiation, wherein at least one gene encodes a signal transduction molecule; (8) regulation of cellular proliferation, either *in vitro* or *in vivo* (*e.g.*, induction of cellular proliferation or inhibition of proliferation, for example, inhibition of
20 tumorigenesis (*e.g.*, inhibition of glioblastoma proliferation)); (9) formation and maintenance of ordered spatial arrangements of differentiated tissues in vertebrates, both adult and embryonic (*e.g.*, induction of head formation during vertebrate development or maintenance of hematopoietic progenitor cells); (10) modulation of cell death, such as stimulation of cell survival; (11) regulating cell migration; and/or (12) immune
25 modulation.

Accordingly one embodiment of the present invention involves a method of use (*e.g.*, a diagnostic assay, prognostic assay, or a prophylactic/therapeutic method of treatment) wherein a molecule of the present invention (*e.g.*, a Dkk protein, Dkk nucleic acid, or a Dkk modulator) is used, for example, to diagnose, prognose and/or treat a
30 disease and/or condition in which any of the aforementioned activities (*i.e.*, activities (i) - (vi) and (1) - (12) in the above paragraph) is indicated. In another embodiment, the

- 77 -

present invention involves a method of use (*e.g.*, a diagnostic assay, prognostic assay, or a prophylactic/therapeutic method of treatment) wherein a molecule of the present invention (*e.g.*, a Dkk protein, Dkk nucleic acid, or a Dkk modulator) is used, for example, for the diagnosis, prognosis, and/or treatment of subjects, preferably a human subject, in which any of the aforementioned activities is pathologically perturbed. In a preferred embodiment, the methods of use (*e.g.*, diagnostic assays, prognostic assays, or prophylactic/therapeutic methods of treatment) involve administering to a subject, preferably a human subject, a molecule of the present invention (*e.g.*, a Dkk protein, Dkk nucleic acid, or a Dkk modulator) for the diagnosis, prognosis, and/or therapeutic treatment. In another embodiment, the methods of use (*e.g.*, diagnostic assays, prognostic assays, or prophylactic/therapeutic methods of treatment) involve administering to a human subject a molecule of the present invention (*e.g.*, a Dkk protein, Dkk nucleic acid, or a Dkk modulator).

Other embodiments of the invention pertain to the use of isolated nucleic acid molecules of the invention can be used, for example, to express Dkk or Dkk-related protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect Dkk or Dkk-related mRNA (*e.g.*, in a biological sample) or a genetic alteration in a Dkk or Dkk-related gene, and to modulate Dkk or Dkk-related activity, as described further below. In addition, the Dkk or Dkk-related proteins can be used to screen drugs or compounds which modulate the Dkk activity as well as to treat disorders characterized by insufficient or excessive production of Dkk or Dkk-related protein or production of Dkk or Dkk-related protein forms which have decreased or aberrant activity compared to Dkk or Dkk-related wild type protein (*e.g.*, developmental disorders or proliferative diseases such as cancer as well as diseases, ocular disorders (*e.g.*, blindness) conditions or disorders characterized by abnormal cell differentiation and/or survival, an abnormal extracellular structure, or an abnormality in a defense mechanism). Moreover, the antibodies of the invention can be used to detect and isolate Dkk or Dkk-related proteins, regulate the bioavailability of Dkk or Dkk-related proteins, and modulate Dkk or Dkk-related activity. The term "an aberrant activity", as applied to an activity of a protein such as Dkk (*e.g.*, hDkk-3), refers to an activity which differs from the activity of the wild-type or native protein or which differs from the activity of

- 78 -

the protein in a healthy subject. An activity of a protein can be aberrant because it is stronger than the activity of its native counterpart. Alternatively, an activity can be aberrant because it is weaker or absent related to the activity of its native counterpart. An aberrant activity can also be a change in an activity. For example an aberrant protein
5 can interact with a different protein relative to its native counterpart. A cell can have an aberrant Dkk (*e.g.*, hDkk-3) activity due to overexpression or underexpression of the gene encoding Dkk.

A. Screening Assays:

10 The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to Dkk or Dkk-related proteins or have a stimulatory or inhibitory effect on, for example, Dkk or Dkk-related expression or activity. Modulators can include, for example, agonists and/or
15 antagonists. The term "agonist", as used herein, is meant to refer to an agent that mimics or upregulates (*e.g.* potentiates or supplements) a Dkk or Dkk-related (*e.g.*, hDkk-3) bioactivity. An agonist can be a compound which mimics a bioactivity of a Dkk or Dkk-related protein, such as transduction of a signal from a Dkk receptor, by, *e.g.*, interacting with a hDkk-3 receptor. An agonist can also be a compound that upregulates
20 expression of a Dkk or Dkk-related gene. An agonist can also be a compound which modulates the expression or activity of a protein which is located downstream, for example, of a Dkk receptor, thereby mimicking or enhancing the effect of binding of Dkk to a Dkk receptor.

"Antagonist" as used herein is meant to refer to an agent that inhibits, decreases
25 or suppresses a bioactivity (*e.g.*, hDkk-3). An antagonist can be a compound which decreases signalling from a Dkk or Dkk-related protein, *e.g.*, a compound that is capable of binding to hDkk-3 or to a hDkk-3 receptor. A preferred antagonist inhibits the interaction between a Dkk or Dkk-related protein and another molecule, such as a Dkk receptor. Alternatively, an antagonist can be a compound that downregulates expression
30 of a Dkk or Dkk-related gene. An antagonist can also be a compound which modulates

- 79 -

the expression or activity of a protein which is located downstream of a Dkk receptor, thereby antagonizing the effect of binding of Dkk to a Dkk receptor.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a Dkk or Dkk-related protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a Dkk receptor. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.*, (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a Dkk receptor on the cell surface is contacted with a test compound and the ability of the test compound to bind to a Dkk receptor determined. The cell, for

- 80 -

example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to a Dkk receptor can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the Dkk receptor can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a test compound to interact with a Dkk receptor without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a test compound with a Dkk receptor without the labeling of either the test compound or the receptor. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between ligand and receptor.

In a preferred embodiment, the assay comprises contacting a cell which expresses a Dkk receptor on the cell surface with a Dkk protein or biologically-active portion thereof, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a Dkk receptor, wherein determining the ability of the test compound to interact with a Dkk receptor comprises determining the ability of the test compound to preferentially bind to the Dkk receptor as compared to the ability of Dkk, or a biologically active portion thereof, to bind to the receptor.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a Dkk target molecule with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the Dkk target molecule. Determining the ability of the test compound to modulate the activity of a

- 81 -

Dkk target molecule can be accomplished, for example, by determining the ability of the Dkk protein to bind to or interact with the Dkk target molecule.

Determining the ability of the Dkk protein to bind to or interact with a Dkk target molecule can be accomplished by one of the methods described above for determining
5 direct binding. In a preferred embodiment, determining the ability of the Dkk protein to bind to or interact with a Dkk target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+} , diacylglycerol, IP_3 , etc.), detecting catalytic/enzymatic activity of
10 the target an appropriate substrate, detecting the induction of a reporter gene (comprising a Dkk-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, development, differentiation or rate of proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay
15 in which a Dkk or Dkk-related protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the Dkk or Dkk-related protein or biologically active portion thereof is determined. Binding of the test compound to the Dkk or Dkk-related protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting
20 the Dkk or Dkk-related protein or biologically active portion thereof with a known compound which binds Dkk or the Dkk-related protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a Dkk or Dkk-related protein, wherein determining the ability of the test compound to interact with a Dkk or Dkk-related protein comprises
25 determining the ability of the test compound to preferentially bind to Dkk or a Dkk-related protein or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a Dkk or Dkk-related protein or biologically active portion thereof is contacted with a test compound
30 and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the Dkk or Dkk-related protein or biologically active portion thereof is determined.

- 82 -

Determining the ability of the test compound to modulate the activity of a Dkk or Dkk-related protein can be accomplished, for example, by determining the ability of the Dkk or Dkk-related protein to bind to a target molecule (*e.g.*, a Dkk-target molecule) by one of the methods described above for determining direct binding. Determining the ability
5 of the Dkk or Dkk-related protein to bind to a target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA).

Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.*

(1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants

10 (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a Dkk or Dkk-related protein can be accomplished by determining the ability of the Dkk or Dkk-related protein to further modulate the activity
15 of a target molecule (*e.g.*, a Dkk-target molecule). For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a Dkk or Dkk-related protein or biologically active portion thereof with a known compound which
20 binds the Dkk or Dkk-related protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the Dkk or Dkk-related protein, wherein determining the ability of the test compound to interact with the Dkk or Dkk-related protein comprises determining the ability of the Dkk or Dkk-related protein to preferentially bind to or modulate the
25 activity of a target molecule (*e.g.*, a Dkk target molecule).

In many drug screening programs which test libraries of compounds and natural extracts, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often
30 preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by

- 83 -

a test compound. Moreover, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in the *in vitro* system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity with upstream or downstream elements. Accordingly, in
5 an exemplary screening assay of the present invention, the compound of interest is contacted with a Dkk (*e.g.*, hDkk-3) protein or a Dkk (*e.g.*, hDkk-3) binding partner, *e.g.*, a receptor. The receptor can be soluble or the receptor can be present on a cell surface. To the mixture of the compound and the Dkk protein or Dkk binding partner is then added a composition containing a Dkk binding partner or a Dkk protein,
10 respectively. Detection and quantification of complexes of Dkk proteins and Dkk binding partners provide a means for determining a compound's efficacy at inhibiting (or potentiating) complex formation between Dkk and a binding partner. The efficacy of the compound can be assessed by generating dose response curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also
15 be performed to provide a baseline for comparison. In the control assay, isolated and purified Dkk polypeptide or binding partner is added to a composition containing the Dkk binding partner or Dkk polypeptide, and the formation of a complex is quantitated in the absence of the test compound.

The cell-free assays of the present invention are amenable to use of both soluble
20 and/or membrane-bound forms of isolated proteins (*e.g.* Dkk proteins or biologically active portions thereof or Dkk target molecules). In the case of cell-free assays in which a membrane-bound form an isolated protein is used (*e.g.*, a Dkk target molecule or receptor) it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the isolated protein is maintained in solution. Examples of such
25 solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate
30 (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

- 84 -

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either Dkk, a Dkk-related protein or a target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a Dkk or Dkk-related protein, or interaction of a Dkk or Dkk-related protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/Dkk fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose^{*} beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or Dkk protein, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of Dkk binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a Dkk protein, Dkk-related protein, or a Dkk target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with Dkk, Dkk-related protein, or target molecules but which do not interfere with binding of the protein to its target molecule can be derivatized to the wells of the plate, and unbound target, Dkk, or Dkk-related protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized

* Trademark

- 85 -

complexes, include immunodetection of complexes using antibodies reactive with the Dkk or Dkk-related protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the Dkk or Dkk-related protein or target molecule.

5 In another embodiment, modulators of Dkk or Dkk-related expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of Dkk or Dkk-related mRNA or protein in the cell is determined. The level of expression of mRNA or protein in the presence of the candidate compound is compared to the level of expression of mRNA or protein in the absence of the candidate
10 compound. The candidate compound can then be identified as a modulator of Dkk or Dkk-related expression based on this comparison. For example, when expression of Dkk mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of Dkk mRNA or protein expression. Alternatively, when expression of Dkk
15 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of Dkk mRNA or protein expression. The level of Dkk or Dkk-related mRNA or protein expression in the cells can be determined by methods described herein for detecting Dkk mRNA or protein.

20 In yet another aspect of the invention, the Dkk or Dkk-related proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.*, (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins,
25 which bind to or interact with Dkk or Dkk-related proteins ("binding proteins" or "bp") and modulate Dkk or Dkk-related activity. Such binding proteins are also likely to be involved in the propagation of signals by the Dkk or Dkk-related proteins as, for example, downstream elements of a Dkk-mediated signaling pathway. Alternatively, such binding proteins are likely to be cell-surface molecules associated with non-Dkk
30 expressing cells, wherein such binding proteins are involved in signal transduction.

- 86 -

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a Dkk protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a Dkk-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the Dkk or Dkk-related protein.

This invention further pertains to novel agents identified by the above-described screening assays and to processes for producing such agents by use of these assays. Accordingly, in one embodiment, the present invention includes a compound or agent obtainable by a method comprising the steps of any one of the aforementioned screening assays (*e.g.*, cell-based assays or cell-free assays). For example, in one embodiment, the invention includes a compound or agent obtainable by a method comprising contacting a cell which expresses a target molecule with a test compound and the determining the ability of the test compound to bind to, or modulate the activity of, the target molecule. In another embodiment, the invention includes a compound or agent obtainable by a method comprising contacting a cell which expresses a target molecule with a Dkk or Dkk-related protein or biologically-active portion thereof, to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with, or modulate the activity of, the target molecule. In another embodiment, the invention includes a compound or agent obtainable by a method comprising contacting a Dkk or Dkk-related protein or biologically active portion thereof with a test compound and determining the ability of the test compound to

- 87 -

bind to, or modulate (*e.g.*, stimulate or inhibit) the activity of, the Dkk or Dkk-related protein or biologically active portion thereof. In yet another embodiment, the present invention includes a compound or agent obtainable by a method comprising contacting a Dkk or Dkk-related protein or biologically active portion thereof with a known
5 compound which binds the Dkk or Dkk-related protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with, or modulate the activity of the Dkk or Dkk-related protein.

Accordingly, it is within the scope of this invention to further use an agent
10 identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a Dkk modulating agent, an antisense Dkk nucleic acid molecule, a Dkk-specific antibody, or a Dkk-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal
15 model to determine the mechanism of action of such an agent.

The present invention also pertains to uses of novel agents identified by the above-described screening assays for diagnoses, prognoses, and treatments as described herein. Accordingly, it is within the scope of the present invention to use such agents in the design, formulation, synthesis, manufacture, and/or production of a drug or
20 pharmaceutical composition for use in diagnosis, prognosis, or treatment, as described herein. For example, in one embodiment, the present invention includes a method of synthesizing or producing a drug or pharmaceutical composition by reference to the structure and/or properties of a compound obtainable by one of the above-described screening assays. For example, a drug or pharmaceutical composition can be
25 synthesized based on the structure and/or properties of a compound obtained by a method in which a cell which expresses a target molecule (*e.g.*, a Dkk target molecule) is contacted with a test compound and the ability of the test compound to bind to, or modulate the activity of, the target molecule is determined. In another exemplary embodiment, the present invention includes a method of synthesizing or producing a
30 drug or pharmaceutical composition based on the structure and/or properties of a compound obtainable by a method in which a Dkk or Dkk-related protein or biologically

- 88 -

active portion thereof is contacted with a test compound and the ability of the test compound to bind to, or modulate (*e.g.*, stimulate or inhibit) the activity of, the Dkk or Dkk-related protein or biologically active portion thereof is determined.

5 B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with
10 genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

15 Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the Dkk or Dkk-related nucleotide sequences, described herein, can be used to map the location of the Dkk or Dkk-related genes on a chromosome. The mapping of the Dkk or Dkk-related
20 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, Dkk or Dkk-related genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the Dkk or Dkk-related nucleotide sequences. Computer analysis of the Dkk or Dkk-related sequences can be used to
25 predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the Dkk or Dkk-related sequences will yield an amplified fragment.

- 89 -

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.*, (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the Dkk or Dkk-related nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a 90, 1p, or 1v sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.*, (1990) *PNAS*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will

- 90 -

suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single
5 chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

10 Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can
15 then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with a Dkk or Dkk-related gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any
20 unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several
25 individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The Dkk or Dkk-related sequences of the present invention can also be used to
30 identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for

- 91 -

identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification
5 difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the Dkk or Dkk-related nucleotide
10 sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a
15 unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The Dkk or Dkk-related nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It
20 is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The
25 noncoding sequences of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20, can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:15 or SEQ ID NO:22 are used, a more appropriate
30 number of primers for positive individual identification would be 500-2,000.

- 92 -

If a panel of reagents from Dkk or Dkk-related nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be
5 made from extremely small tissue samples.

3. Use of Partial Dkk or Dkk-related Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence
10 found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the
15 origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular
20 individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NOs:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions,
25 making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the Dkk nucleotide sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, or SEQ ID NO:20, having a length of at least 20 bases, preferably at least 30 bases.

- 93 -

The Dkk or Dkk-related nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is presented
5 with a tissue of unknown origin. Panels of such Dkk or Dkk-related probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, Dkk or Dkk-related primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

10

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically.
15 Accordingly, one aspect of the present invention relates to diagnostic assays for determining Dkk or Dkk-related protein and/or nucleic acid expression as well as Dkk or Dkk-related activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant Dkk or Dkk-related
20 expression or activity, such as aberrant cell proliferation, differentiation, and/or survival resulting for example in a neurodegenerative disease (*e.g.*, Alzheimer's disease, Parkinson's disease, Huntington's chorea, amyotrophic lateral sclerosis and the like, as well as spinocerebellar degenerations) or cancer (for example, cancers of the epithelia (*e.g.*, carcinomas of the pancreas, stomach, liver, secretory glands (*e.g.*,
25 adenocarcinoma) bladder, lung, breast, skin (*e.g.*, malignant melanoma), reproductive tract including prostate gland, ovary, cervix and uterus); cancers of the hematopoietic and immune system (*e.g.*, leukemias and lymphomas); cancers of the central nervous, brain system and eye (*e.g.*, gliomas, glioblastoma, neuroblastoma and retinoblastoma); and cancers of connective tissues, bone, muscles and vasculature (*e.g.*, sarcomas)). The
30 invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with Dkk or Dkk-related protein,

- 94 -

nucleic acid expression or activity. For example, mutations in a Dkk or Dkk-related gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with Dkk or Dkk-related protein, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of Dkk or Dkk-related in clinical trials.

These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of Dkk or Dkk-related protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting Dkk or Dkk-related protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes Dkk or Dkk-related protein such that the presence of Dkk or Dkk-related protein or nucleic acid is detected in the biological sample. A preferred agent for detecting Dkk or Dkk-related mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to Dkk or Dkk-related mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length Dkk nucleic acid, such as the nucleic acid of SEQ ID NO: 1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:20, the DNA insert of the plasmid deposited with ATCC as Accession Number 98452, or the DNA insert of the plasmid deposited with ATCC as Accession Number 98633,

or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to Dkk or Dkk-related mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

- 95 -

A preferred agent for detecting Dkk or Dkk-related protein is an antibody capable of binding to the protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect Dkk or Dkk-related mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of Dkk or Dkk-related mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of Dkk or Dkk-related protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of Dkk or Dkk-related genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of Dkk or Dkk-related protein include introducing into a subject a labeled antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting Dkk or Dkk-related protein, mRNA, or genomic DNA, such that the presence of Dkk or Dkk-related protein, mRNA or genomic DNA is

- 96 -

detected in the biological sample, and comparing the presence of Dkk or Dkk-related protein, mRNA or genomic DNA in the control sample with the presence of Dkk or Dkk-related protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of Dkk or a Dkk-related protein in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting Dkk or Dkk-related protein or mRNA in a biological sample; means for determining the amount of Dkk or Dkk-related protein or mRNA in the sample; and means for comparing the amount of Dkk or Dkk-related protein or mRNA in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect Dkk or Dkk-related protein or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant Dkk expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with Dkk or Dkk-related protein, nucleic acid expression or activity such as a proliferative disorder, a differentiative or developmental disorder, a hematopoietic disorder as well as diseases, conditions or disorders characterized by abnormal cell survival, abnormal extracellular structure, or an abnormality in a defense mechanism. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a differentiative or proliferative disease (*e.g.*, cancer). Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant Dkk or Dkk-related expression or activity in which a test sample is obtained from a subject and Dkk or Dkk-related protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of Dkk or Dkk-related protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant Dkk or Dkk-related expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a

- 97 -

subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant Dkk or Dkk-related expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder, such as a proliferative disorder, a differentiative or developmental disorder, a hematopoietic disorder, as well disorders characterized by abnormal cell survival, an abnormal extracellular structure, or an abnormality in a defense mechanism. Alternatively, such methods can be used to determine whether a subject can be effectively treated with an agent for a differentiative or proliferative disease (*e.g.*, cancer). Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant Dkk or Dkk-related expression or activity in which a test sample is obtained and Dkk or Dkk-related protein or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of Dkk or Dkk-related protein or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant Dkk or Dkk-related expression or activity.)

The methods of the invention can also be used to detect genetic alterations in a Dkk or Dkk-related gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by aberrant development, aberrant cellular differentiation, aberrant cellular proliferation or an aberrant hematopoietic response. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a Dkk or Dkk-related-protein, or the mis-expression of the Dkk or Dkk-related gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a Dkk or Dkk-related gene; 2) an addition of one or more nucleotides to a Dkk or Dkk-related gene; 3) a substitution of one or more nucleotides of a Dkk or Dkk-related gene, 4) a chromosomal rearrangement of a Dkk or Dkk-related gene; 5) an

- 98 -

alteration in the level of a messenger RNA transcript of a Dkk or Dkk-related gene, 6) aberrant modification of a Dkk or Dkk-related gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a Dkk or Dkk-related gene, 8) a non-wild type level of a Dkk or Dkk-related-protein, 9) allelic loss of a Dkk or Dkk-related gene, and 10) inappropriate post-translational modification of a Dkk or Dkk-related-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting alterations in a Dkk or Dkk-related gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

10 In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly
15 useful for detecting point mutations in the Dkk or Dkk-related-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a Dkk or Dkk-related gene under conditions such
20 that hybridization and amplification of the Dkk or Dkk-related-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described
25 herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, 1989, *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase^{*} (Lizardi, P.M. *et al.*, 1988, *Bio/Technology* 6:1197), or any
30 other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection

* Trademark

- 99 -

schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a Dkk or Dkk-related gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in a Dkk or Dkk-related gene can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in Dkk can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the Dkk or Dkk-related gene and detect mutations by comparing the sequence of the sample Dkk or Dkk-related sequence with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *PNAS* 74:560) or Sanger ((1977) *PNAS* 74:5463). It is also contemplated that any of a variety

- 100 -

of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-
5 159).

Other methods for detecting mutations in the Dkk or Dkk-related gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of
10 formed by hybridizing (labeled) RNA or DNA containing the wild-type Dkk or Dkk-related sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with
15 RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the
20 site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called
25 "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in Dkk cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a Dkk
30 sequence, *e.g.*, a wild-type Dkk sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme,

- 101 -

and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in Dkk or Dkk-related genes. For example, single strand
5 conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; and Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA fragments of sample and control Dkk or Dkk-related nucleic acids will be denatured and allowed to renature. The
10 secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred
15 embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing
20 gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control
25 and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions
30 which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific

- 102 -

oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a Dkk gene.

Furthermore, any cell type or tissue in which Dkk or a Dkk-related sequence is expressed may be utilized in the prognostic assays described herein.

3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of Dkk or Dkk-related molecule (*e.g.*, modulation of cellular signal transduction, regulation of gene transcription in a cell involved in development or differentiation, regulation of cellular proliferation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a

- 103 -

screening assay as described herein to increase Dkk or Dkk-related gene expression, protein levels, or upregulate Dkk or Dkk-related activity, can be monitored in clinical trials of subjects exhibiting decreased Dkk or Dkk-related gene expression, protein levels, or downregulated Dkk or Dkk-related activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease Dkk or Dkk-related gene expression, protein levels, or downregulate Dkk or Dkk-related activity, can be monitored in clinical trials of subjects exhibiting increased Dkk or Dkk-related gene expression, protein levels, or upregulated Dkk or Dkk-related activity. In such clinical trials, the expression or activity of Dkk or Dkk-related and, preferably, other genes that have been implicated in, for example, a proliferative disorder can be used as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including Dkk and Dkk-related genes, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates Dkk or Dkk-related activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on proliferative disorders, developmental or differentiative disorder, hematopoietic disorder as well disorders characterized by abnormal cell differentiation and/or survival, an abnormal extracellular structure, or an abnormality in a defense mechanism, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of Dkk, Dkk-related, and other genes implicated in the proliferative disorder, developmental or differentiative disorder, hematopoietic disorder as well as disorders characterized by abnormal cell differentiation and/or survival, an abnormal extracellular structure, or an abnormality in a defense mechanism, respectively. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of Dkk, Dkk-related, or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

- 104 -

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a Dkk or Dkk-related protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the Dkk or Dkk-related protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the Dkk or Dkk-related protein, mRNA, or genomic DNA in the pre-administration sample with the Dkk or Dkk-related protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of Dkk or Dkk-related nucleic acid or protein to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of Dkk or Dkk-related nucleic acid or protein to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, Dkk or Dkk-related expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

C. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant Dkk or Dkk-related expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of

- 105 -

how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the Dkk or Dkk-related molecules of the present invention or Dkk or Dkk-related modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

10

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant Dkk or Dkk-related expression or activity, by administering to the subject an agent which modulates Dkk or Dkk-related expression or at least one Dkk or Dkk-related activity. Subjects at risk for a disease which is caused or contributed to by aberrant Dkk or Dkk-related expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the Dkk or Dkk-related aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of Dkk or Dkk-related aberrancy, for example, an agonist or antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the present invention are further discussed in the following subsections.

25

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating Dkk or Dkk-related expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of Dkk or Dkk-related protein activity associated with the cell. An agent that modulates Dkk or Dkk-related protein activity can be an agent as described herein, such

30

- 106 -

as a nucleic acid or a protein, a naturally-occurring target molecule of a Dkk or Dkk-related protein, a peptide, a Dkk or Dkk-related peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more Dkk or Dkk-related protein activity. Examples of such stimulatory agents include active Dkk or Dkk-related protein and a nucleic acid molecule encoding Dkk or Dkk-related that has been introduced into the cell. In another embodiment, the agent inhibits one or more Dkk or Dkk-related protein activity. Examples of such inhibitory agents include antisense Dkk or Dkk-related nucleic acid molecules and antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a Dkk or Dkk-related protein or nucleic acid molecule. The present invention also provides methods of modulating the function, morphology, proliferation, and/or differentiation of cells in the tissues in which a Dkk or Dkk-related protein or nucleic acid molecule is expressed. Alternatively, Dkk or Dkk-related polypeptides, nucleic acids, and modulators thereof, can be used to treat disorders associated with abnormal or aberrant metabolism or function of cells in the tissues in which the Dkk or Dkk-related protein or nucleic acid molecule is expressed.

For example, tissues in which Dkk-3 is expressed include embryonic eye, bone, and cartilage, fetal brain, lung, and kidney, and adult heart (in particular, atrioventricular valves and atrial myocytes), eye (in particular, the integrating bipolar and ganglion cells of the retina, the ciliary body, and lens epithelium), brain (in particular, neurons of the cortex and hippocampus), placenta, lung, and skeletal muscle. Accordingly, Dkk-3 polypeptides, nucleic acids, or modulators thereof, can be used to treat cardiovascular disorders, such as ischemic heart disease (e.g., angina pectoris, myocardial infarction, and chronic ischemic heart disease), hypertensive heart disease, pulmonary heart disease, valvular heart disease (e.g., rheumatic fever and rheumatic heart disease, endocarditis, mitral valve prolapse, and aortic valve stenosis), congenital heart disease (e.g., valvular and vascular obstructive lesions, atrial or ventricular septal defect, and patent ductus arteriosus), or myocardial disease (e.g., myocarditis, congestive cardiomyopathy, and hypertrophic cardiomyopathy).

- 107 -

In another embodiment, Dkk-3 polypeptides, nucleic acids, or modulators thereof, can be used to treat optic disorders such as diseases associated with amaurosis (*e.g.*, a. fugax and a. albuminuric) diseases associated with amblyopia, glaucoma, optic neuropathy (*e.g.*, ischemic neuropathy, optic neuritis, and infiltrative neuropathy),
5 ophthalmia (*e.g.*, o. catarrhal, trachoma, o. neuroparalytic, and conjunctiva), visual disorders resulting from systemic disease or disorders of other tissues (*e.g.*, diabetes mellitus, hyperthyroidism, and vitamin A or riboflavin deficiency), or tumors, neoplasms, and metastases.

In another embodiment, Dkk-3 polypeptides, nucleic acids, or modulators
10 thereof, can be used to treat disorders of the brain, such as cerebral edema, senile dementia of the Alzheimer type, epilepsy, amnesia, hydrocephalus, brain herniations, iatrogenic disease (due to, *e.g.*, infection, toxins, or drugs), inflammations (*e.g.*, bacterial and viral meningitis, encephalitis, and cerebral toxoplasmosis), cerebrovascular diseases (*e.g.*, hypoxia, ischemia, and infarction, intracranial hemorrhage and vascular
15 malformations, and hypertensive encephalopathy), and tumors (*e.g.*, neuroglial tumors, neuronal tumors, tumors of pineal cells, meningeal tumors, primary and secondary lymphomas, intracranial tumors, and medulloblastoma), and to treat injury or trauma to the brain.

In another embodiment, Dkk-3 polypeptides, nucleic acids, or modulators
20 thereof, can be used to treat placental disorders, such as toxemia of pregnancy (*e.g.*, preeclampsia and eclampsia), placentitis, or spontaneous abortion.

In another embodiment, Dkk-3 polypeptides, nucleic acids, or modulators thereof, can be used to treat pulmonary disorders, such as atelectasis, pulmonary congestion or edema, chronic obstructive airway disease (*e.g.*, emphysema, chronic
25 bronchitis, bronchial asthma, and bronchiectasis), diffuse interstitial diseases (*e.g.*, sarcoidosis, pneumoconiosis, hypersensitivity pneumonitis, Goodpasture's syndrome, idiopathic pulmonary hemosiderosis, pulmonary alveolar proteinosis, desquamative interstitial pneumonitis, chronic interstitial pneumonia, fibrosing alveolitis, hamman-rich syndrome, pulmonary eosinophilia, diffuse interstitial fibrosis, Wegener's
30 granulomatosis, lymphomatoid granulomatosis, and lipid pneumonia), or tumors (*e.g.*, bronchogenic carcinoma, bronchioloalveolar carcinoma, bronchial carcinoid,

- 108 -

hamartoma, and mesenchymal tumors).

In another embodiment, Dkk-3 polypeptides, nucleic acids, or modulators thereof, can be used to treat disorders of skeletal muscle, such as muscular atrophy (due to, *e.g.*, denervation, malnutrition, loss of blood supply, or neuromuscular disease, *e.g.*, amyotonia congenita, amyotrophic lateral sclerosis of Charcot, and progressive muscular atrophy of Aran-Duchenne), myositis (due to, *e.g.*, bacterial, viral, fungal or parasitic infection), muscular dystrophies (*e.g.*, Duchenne type, Becker type, facioscapulohumeral, limb-girdle, myotonic dystrophy, and ocular myopathy), myasthenia gravis, or tumors and tumor-like lesions of muscles (*e.g.*, traumatic myositis ossificans, desmoids, musculoaponeurotic fibromatosis, Dupuytren's contracture, nodular (pseudosarcomatous) fasciitis, rhabdomyoma, rhabdomyosarcoma, and granular cell myoblastomas).

Tissues in which Dkk-4 is expressed include cerebellum, activated human T-lymphocytes, lung, and esophagus. Accordingly, in one embodiment, Dkk-4 polypeptides, nucleic acids, or modulators thereof, can be used to treat disorders of the cerebellum, such as disturbances of synergy (*e.g.*, asynergia or limb ataxia, dysmetria, decomposition of movement, hypermetria, hypometria, dysdiadochokinesia, hypotonia, tremor, dysarthria, nystagmus), disturbances of equilibrium (due to, *e.g.*, a lesion involving the vestibulocerebellum), disturbances of gait stance, or tone (due to, *e.g.*, a lesion or degeneration of the spinocerebellum), or tumors (*e.g.*, astrocytoma and medulloblastoma).

In another embodiment, Dkk-4 polypeptides, nucleic acids, or modulators thereof, can be used to treat lymphocytic disorders, such as lymphopenia, lymphocytosis, acute and chronic lymphadenitis, malignant lymphomas (*e.g.*, Non-Hodgkin's lymphomas, Hodgkin's lymphomas, leukemias, multiple myeloma, histiocytoses, and angioimmunoblastic lymphadenopathy).

In another embodiment, Dkk-4 polypeptides, nucleic acids, or modulators thereof, can be used to treat pulmonary disorders, such as atelectasis, pulmonary congestion or edema, chronic obstructive airway disease (*e.g.*, emphysema, chronic bronchitis, bronchial asthma, and bronchiectasis), diffuse interstitial diseases (*e.g.*, sarcoidosis, pneumoconiosis, hypersensitivity pneumonitis, Goodpasture's syndrome,

- 109 -

idiopathic pulmonary hemosiderosis, pulmonary alveolar proteinosis, desquamative interstitial pneumonitis, chronic interstitial pneumonia, fibrosing alveolitis, hamman-rich syndrome, pulmonary eosinophilia, diffuse interstitial fibrosis, Wegener's granulomatosis, lymphomatoid granulomatosis, and lipid pneumonia), or tumors (*e.g.*,
5 bronchogenic carcinoma, bronchioloalveolar carcinoma, bronchial carcinoid, hamartoma, and mesenchymal tumors).

In another embodiment, Dkk-4 polypeptides, nucleic acids, or modulators thereof, can be used to treat esophageal disorders, such as neuromuscular disturbances (*e.g.*, achalasia, annular narrowings, Schatzki's rings, hiatal hernia, Mallory-Weiss
10 syndrome), esophagitis (due to *e.g.*, bacteremia, viremia, fungal infections, uremia, graft-versus-host disease, chemotherapy, radiation, and prolonged gastric intubation), diverticula (*e.g.*, Zenker's diverticulum), systemic sclerosis, varices (due to, *e.g.*, portal hypertension, systemic amyloidosis and sarcoidosis), or tumors or neoplasms (*e.g.*, leiomyoma, fibromas, lipomas, hemangiomas, lymphangiomas, squamous papillomas,
15 adenocarcinomas and undifferentiated carcinomas, and sarcomas).

Dkk-1 is highly expressed, for example, in placenta. Accordingly, Dkk-1 polypeptides, nucleic acids, or modulators thereof, can be used to treat placental disorders, such as toxemia of pregnancy (*e.g.*, preeclampsia and eclampsia), placentitis, or spontaneous abortion.

20 Tissues in which Dkk-2 is expressed include, for example, heart, brain, placenta, lung, and skeletal muscle. Accordingly, Dkk-2 polypeptides, nucleic acids, or modulators thereof, can be used to treat cardiovascular disorders, such as ischemic heart disease (*e.g.*, angina pectoris, myocardial infarction, and chronic ischemic heart disease), hypertensive heart disease, pulmonary heart disease, valvular heart disease (*e.g.*,
25 rheumatic fever and rheumatic heart disease, endocarditis, mitral valve prolapse, and aortic valve stenosis), congenital heart disease (*e.g.*, valvular and vascular obstructive lesions, atrial or ventricular septal defect, and patent ductus arteriosus), or myocardial disease (*e.g.*, myocarditis, congestive cardiomyopathy, and hypertrophic cardiomyopathy).

- 110 -

In another embodiment, Dkk-2 polypeptides, nucleic acids, or modulators thereof, can be used to treat disorders of the brain, such as cerebral edema, senile dementia of the Alzheimer type, epilepsy, amnesia, hydrocephalus, brain herniations, iatrogenic disease (due to, *e.g.*, infection, toxins, or drugs), inflammations (*e.g.*, bacterial and viral meningitis, encephalitis, and cerebral toxoplasmosis), cerebrovascular diseases (*e.g.*, hypoxia, ischemia, and infarction, intracranial hemorrhage and vascular malformations, and hypertensive encephalopathy), and tumors (*e.g.*, neuroglial tumors, neuronal tumors, tumors of pineal cells, meningeal tumors, primary and secondary lymphomas, intracranial tumors, and medulloblastoma), and to treat injury or trauma to the brain.

In another embodiment, Dkk-2 polypeptides, nucleic acids, or modulators thereof, can be used to treat placental disorders, such as toxemia of pregnancy (*e.g.*, preeclampsia and eclampsia), placentitis, or spontaneous abortion.

In another embodiment, Dkk-2 polypeptides, nucleic acids, or modulators thereof, can be used to treat pulmonary disorders, such as atelectasis, pulmonary congestion or edema, chronic obstructive airway disease (*e.g.*, emphysema, chronic bronchitis, bronchial asthma, and bronchiectasis), diffuse interstitial diseases (*e.g.*, sarcoidosis, pneumoconiosis, hypersensitivity pneumonitis, Goodpasture's syndrome, idiopathic pulmonary hemosiderosis, pulmonary alveolar proteinosis, desquamative interstitial pneumonitis, chronic interstitial pneumonia, fibrosing alveolitis, hamman-rich syndrome, pulmonary eosinophilia, diffuse interstitial fibrosis, Wegener's granulomatosis, lymphomatoid granulomatosis, and lipid pneumonia), or tumors (*e.g.*, bronchogenic carcinoma, bronchioloalveolar carcinoma, bronchial carcinoid, hamartoma, and mesenchymal tumors).

In another embodiment, Dkk-2 polypeptides, nucleic acids, or modulators thereof, can be used to treat disorders of skeletal muscle, such as muscular atrophy (due to, *e.g.*, denervation, malnutrition, loss of blood supply, or neuromuscular disease, *e.g.*, amyotonia congenita, amyotrophic lateral sclerosis of Charcot, and progressive muscular atrophy of Aran-Duchenne), myositis (due to, *e.g.*, bacterial, viral, fungal or parasitic infection), muscular dystrophies (*e.g.*, Duchenne type, Becker type, facioscapulohumeral, limb-girdle, myotonic dystrophy, and ocular myopathy),

- 111 -

myasthenia gravis, or tumors and tumor-like lesions of muscles (*e.g.*, traumatic myositis ossificans, desmoids, musculoaponeurotic fibromatosis, Dupuytren's contracture, nodular (pseudosarcomatous) fasciitis, rhabdomyoma, rhabdomyosarcoma, and granular cell myoblastomas).

5 Soggy-1 is expressed in, for example, testis (*e.g.*, spermatogenic epithelium of the seminiferous tubules, spermatogonia) and in embryonic developing dorsal root ganglia, cartilage primordium of the nasal septum, and the eye. Accordingly, Soggy-1 polypeptides, nucleic acids, or modulators thereof, can be used to treat testicular disorders, such as unilateral testicular enlargement (*e.g.*, nontuberculous, granulomatous
10 orchitis), inflammatory diseases resulting in testicular dysfunction (*e.g.*, gonorrhea and mumps), and tumors (*e.g.*, germ cell tumors, interstitial cell tumors, androblastoma, testicular lymphoma and adenomatoid tumors). In another embodiment, Soggy-1 polypeptides, nucleic acids, or modulators thereof, can be used to treat infertility due to, for example, spermatogenetic failure.

15 In one aspect, the above-described methods involve administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) Dkk or Dkk-related expression or activity. In another embodiment, the method involves administering a Dkk or Dkk-related protein or nucleic acid molecule as therapy to compensate for reduced or aberrant
20 Dkk or Dkk-related expression or activity.

A preferred embodiment of the present invention involves a method for treatment of a disease or disorder associated with a Dkk or Dkk-related protein which includes the step of administering a therapeutically effective amount of an antibody to a Dkk or Dkk-related protein to a subject. As defined herein, a therapeutically effective amount of
25 antibody (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including
30 but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a

- 112 -

subject with a therapeutically effective amount of an antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result from the results of diagnostic assays as described herein.

Stimulation of Dkk or Dkk-related activity is desirable in situations in which Dkk or Dkk-related activity is abnormally downregulated and/or in which increased Dkk or Dkk-related activity is likely to have a beneficial effect. Likewise, inhibition of Dkk or Dkk-related activity is desirable in situations in which Dkk or Dkk-related activity is abnormally upregulated and/or in which decreased Dkk or Dkk-related activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant development or cellular differentiation. Another example of such a situation is where the subject has a proliferative disease (*e.g.*, cancer) or a neurogenerative disorder. Yet another example of such a situation is where it is desirable to achieve tissue regeneration in a subject (*e.g.*, where a subject has undergone brain or spinal cord injury and it is desirable to regenerate neuronal tissue in a regulated manner.)

Accordingly, in one embodiment, the disease is a disease characterized by an abnormal cell proliferation, differentiation, and/or survival. For example, the disease can be a hyper-or hypoproliferative disease. The invention also provides methods for treating diseases characterized by an abnormal cell proliferation, differentiation, and/or survival in a subject, which are not characterized by an abnormal Dkk or Dkk-related activity (*e.g.*, hDkk-3 activity). In fact, since Dkk is likely to be capable of modulating the proliferative state of a cell (*i.e.*, state of proliferation, differentiation, and or survival of a cell), Dkk can regulate disease wherein the abnormal proliferative state of a cell results from a defect other than an abnormal Dkk activity.

- 113 -

Hyperproliferative diseases can be treated with Dkk or Dkk-related (*e.g.*, hDkk-3) therapeutics include neoplastic and hyperplastic diseases, such as various forms of cancers and leukemias, and fibroproliferative disorders. Other hyperproliferative diseases that can be treated or prevented with the subject Dkk or Dkk-related therapeutics (*e.g.* hDkk-3 therapeutics) include malignant conditions, premalignant conditions, and benign conditions. The condition to be treated or prevented can be a solid tumor, such as a tumor arising in an epithelial tissue. Accordingly, treatment of such a cancer could comprise administration to the subject of a Dkk or Dkk-related therapeutic decreasing the interaction of Dkk with a Dkk receptor. Other cancers that can be treated or prevented with a Dkk or Dkk-related protein include cancers of the epithelia (*e.g.*, carcinomas of the pancreas, kidney, stomach, colon, esophagus liver, secretory glands (*e.g.*, adenocarcinoma) bladder, lung, breast, skin (*e.g.*, malignant melanoma, seminoma squamous adenocarcinoma), reproductive tract including prostate gland, testis, ovary, cervix and uterus); cancers of the hematopoietic and immune system (*e.g.*, leukemias and lymphomas); cancers of the central nervous, brain system and eye (*e.g.*, malignant astrocytoma, gliomas, neuroblastoma and retinoblastoma); and cancers of connective tissues, bone, heart, muscles and vasculature (*e.g.*, sarcomas, for example, osteosarcoma). Additional solid tumors within the scope of the invention include those that can be found in a medical textbook.

The condition to be treated or prevented can also be a soluble tumor, such as leukemia, either chronic or acute, including chronic or acute myelogenous leukemia, chronic or acute lymphocytic leukemia, promyelocytic leukemia, monocytic leukemia, myelomonocytic leukemia, and erythroleukemia. Yet other proliferative disorders that can be treated with a Dkk or Dkk-related therapeutic of the invention include heavy chain disease, multiple myeloma, lymphoma, *e.g.*, Hodgkin's lymphoma and non-Hodgkin's lymphoma, and Waldenstroem's macroglobulemia.

Diseases or conditions characterized by a solid or soluble tumor can be treated by administering a Dkk or Dkk-related therapeutic either locally or systemically, such that aberrant cell proliferation is inhibited or decreased. Methods for administering the compounds of the invention are further described below.

- 114 -

The invention also provides methods for preventing the formation and/or development of tumors. For example, the development of a tumor can be preceded by the presence of a specific lesion, such as a pre-neoplastic lesion, *e.g.*, hyperplasia, metaplasia, and dysplasia, which can be detected, *e.g.*, by cytologic methods. Such lesions can be found, *e.g.*, in epithelial tissue. Thus, the invention provides a method for inhibiting progression of such a lesion into a neoplastic lesion, comprising administering to the subject having a preneoplastic lesion an amount of a Dkk or Dkk-related therapeutic sufficient to inhibit progression of the preneoplastic lesion into a neoplastic lesion.

The invention also provides for methods for treating or preventing diseases or conditions in which proliferation of cells is desired. For example, Dkk or Dkk-related therapeutics can be used to stimulate tissue repair or wound healing, such as after surgery or to stimulate tissue healing from burns. Other diseases in which proliferation of cells is desired are hypoproliferative diseases, *i.e.*, diseases characterized by an abnormally low proliferation of certain cells.

In yet another embodiment, the invention provides a method for treating or preventing diseases or conditions characterized by aberrant cell differentiation. Accordingly, the invention provides methods for stimulating cellular differentiation in conditions characterized by an inhibition of normal cell differentiation which may or may not be accompanied by excessive proliferation. Alternatively, Dkk or Dkk-related therapeutics can be used to inhibit differentiation of specific cells.

In a preferred method, the aberrantly proliferating and/or differentiating cell is a cell present in the nervous system. A role for Dkk in the nervous system is suggested at least in part from the fact that human Dkk-3 is expressed in human fetal brain.

Accordingly, the invention provides methods for treating diseases or conditions associated with a central or peripheral nervous system. For example, the invention provides methods for treating lesions of the nervous system associated with an aberrant proliferation, differentiation or survival of any of the following cells: cells of the central nervous system including neurons and glial cells (*e.g.*, astrocytes and oligodendrocytes) and supporting cells of peripheral neurons (*e.g.*, Schwann cells and satellite cells).

Disorders of the nervous system include, but are not limited to: spinal cord injuries,

- 115 -

brain injuries, brain tumors (*e.g.*, astrocytic tumors, for example, astrocytomas and glioblastomas), lesions associated with surgery, ischemic lesions, malignant lesions, infectious lesions, degenerative lesions (*e.g.*, Parkinson's disease, Alzheimer's disease, Huntington's chorea, amyotrophic lateral sclerosis), demyelinating diseases (*e.g.*,
5 multiple sclerosis, human immunodeficiency associated myelopathy, transverse myelopathy, progressive multifocal leukoencephalopathy, pontine myelinolysis), motor neuron injuries, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (*i.e.*, Fazio-Londe syndrome), poliomyelitis, and hereditary motorsensory
10 neuropathy (*i.e.*, Charcot-Marie-Tooth disease).

In another embodiment, the invention provides a method for enhancing the survival and/or stimulating proliferation and/or differentiation of cells and tissues *in vitro*. In a preferred embodiment, Dkk or Dkk-related therapeutics are used to promote tissue regeneration and/or repair (*e.g.*, to treat nerve injury). For example, tissues from a
15 subject can be obtained and grown *in vitro* in the presence of a Dkk or Dkk-related therapeutic, such that the tissue cells are stimulated to proliferate and/or differentiate. The tissue can then be readministered to the subject.

Among the approaches which may be used to ameliorate disease symptoms involving an aberrant Dkk or Dkk-related activity and/or an abnormal cell proliferation,
20 differentiation, and/or survival, are, for example, antisense, ribozyme, and triple helix molecules described above. Examples of suitable compounds include the antagonists, agonists or homologues described in detail above.

Yet other Dkk or Dkk-related therapeutics consist of a first peptide comprising a Dkk or Dkk-related peptide capable of binding to a Dkk receptor, and a second peptide
25 which is cytotoxic. Such therapeutics can be used to specifically target and lyse cells expressing or overexpressing a receptor for Dkk.

3. Pharmacogenomics

The Dkk or Dkk-related molecules of the present invention, as well as agents, or
30 modulators which have a stimulatory or inhibitory effect on Dkk or Dkk-related activity (*e.g.*, Dkk or Dkk-related gene expression) as identified by a screening assay described

- 116 -

herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (*e.g.*, proliferative or developmental disorders) associated with aberrant Dkk or Dkk-related activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a Dkk or Dkk-related molecule or Dkk or Dkk-related modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a Dkk or Dkk-related molecule or Dkk or Dkk-related modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, M., *Clin Exp Pharmacol Physiol*, 1996, 23(10-11):983-985 and Linder, M.W., *Clin Chem*, 1997, 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers

- 117 -

associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (*e.g.*, a Dkk protein or Dkk receptor of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed

- 118 -

metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to
5 identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a Dkk molecule or Dkk modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics
10 approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a Dkk molecule or Dkk or Dkk-related modulator, such as a modulator identified by one of the
15 exemplary screening assays described herein.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

20

EXAMPLES

The invention is based, at least in part, on the discovery of a family of genes encoding human cysteine-rich secreted proteins which are related to *Xenopus* Dickkopf (Dkk) proteins. This family includes hDkk-1, hDkk-2, hDkk-3, and hDkk-4. hDkks 1-4
25 contain two highly conserved cysteine-rich domains (CRDs), the most C-terminal of which demonstrates similarity to the colipase protein family. The invention is based also in part on the discovery of a family of Dkk-related proteins, referred to as Soggy proteins, as well as the genes encoding Soggy proteins. Soggy-1 is a novel secreted protein which is related to the N-terminal region of Dkk-3 but lacks CRDs. The
30 following examples illustrate the structure and function of each of these novel human secreted proteins.

Example 1: Isolation And Characterization of Human hDkk-3 cDNA

In this example, the isolation and characterization of the gene encoding human Dkk-3 (also referred to as "hDkk-3", "Cysteine Rich Secreted Protein-1", "CRSP-1"
5 "CRISPY-1" or "TANGO 59") is described.

Isolation of a Human Dkk-3 cDNA

The invention is based at least in part on the discovery of a human gene encoding a secreted protein, referred to herein as human Dickkopf-3 (hDkk-3). A partial cDNA
10 was isolated using a Signal Sequence Trap^{*} method. This methodology takes advantage of the fact that molecules such as Dkk have an amino terminal signal sequence which directs certain secreted and membrane-bound proteins through the cellular secretory apparatus.

Briefly, a randomly primed cDNA library using mRNA prepared from human
15 fetal brain tissue (Clontech, Palo Alto CA) was made by using the Stratagene-ZAP-cDNA Synthesis[™] kit, (catalog #20041). The cDNA was ligated into the mammalian expression vector pTrap^{*} adjacent to a cDNA encoding placental alkaline phosphatase lacking a secretory signal. The plasmids were transformed into *E. coli* and DNA was prepared using the Wizard[™] DNA purification kit (Promega). DNA was transfected into
20 COS-7 cells with lipofectamine[™] (Gibco-BRL). After 48 hours incubation the COS cell supernatants were assayed for alkaline phosphatase on a Wallac Micro-Beta scintillation counter using the Phospha-Light[™] kit (Tropix Inc. Catalog #BP300). The individual plasmid DNAs scoring positive in the COS cell Alkaline Phosphatase secretion assay were further analyzed by DNA sequencing using standard procedures.

25 Using a partial cDNA isolated by the above-described method (clone Amhb3c2), a full length cDNA encoding human Dkk-3 was isolated from a lambda Ziplox[™] human fetal brain cDNA library using conventional hybridization techniques (Sambrook *et al.*, *supra*). The nucleotide sequence encoding the full length human Dkk-3 protein is shown in Figure 1 and is set forth as SEQ ID NO: 1. The full length protein encoded by
30 this nucleic acid is comprised of about 350 amino acids and has the amino acid sequence

* Trademark

- 120 -

shown in Figure 1 and set forth as SEQ ID NO:2. The coding portion (open reading frame) of SEQ ID NO:1 is set forth as SEQ ID NO:3. DNA for the clone Fmhb059 was deposited with the ATCC as Accession No. 98452.

5 Analysis of Human hDkk-3

Determination of the hydrophobicity profile of human Dkk-3 having the amino acid sequence set forth in SEQ ID NO:2 indicated the presence of a hydrophobic region from about amino acid 1 to about amino acid 22 of SEQ ID NO:2. Further analysis of the amino acid sequence SEQ ID NO:2 using a signal peptide prediction program
10 predicted the presence of a signal peptide from about amino acid 1 to about amino acid 22 of SEQ ID NO:2. Accordingly, the mature hDkk-3 protein includes about 328 amino acids spanning from about amino acid 23 to about amino acid 350 of SEQ ID NO:2. The presence of the signal sequence, in addition to the fact that hDkk-3 has been identified using a Signal Sequence Trap system, indicates that hDkk-3 is a secreted
15 protein. Furthermore, the prediction of such a signal peptide and signal peptide cleavage site can be made, for example, utilizing the computer algorithm SIGNALP (Nielsen, *et al.*, (1997) *Protein Engineering* 10:1-6).

Examination of the cDNA sequence depicted in Figure 1 shows that human Dkk-3 is particularly rich in cysteine residues. As shown in Figure 1, hDkk-3 contains 20
20 cysteine residues located between amino acid 147 and amino acid 284 of SEQ ID NO: 2. This region has been termed the cysteine-rich region. These cysteine residues can form 10 disulfide bridges.

A BLAST search (Altschul *et al.*, (1990) *J. Mol. Biol.* 215:403) of the nucleotide and the amino acid sequences of hDkk-3 has revealed that hDkk-3 is similar to a chicken
25 cDNA encoding a protein of unknown function having GenBank^{*} Accession No. D26311. This cDNA was isolated from a chicken lens cDNA library and was shown to be expressed in lens fibers and lens epithelium, but not in neural retina nor in liver cells. (Sawada *et al.*, (1996) *Int. J. Dev. Biol.* 40:531). hDkk-3 and the chicken protein have 56% amino acid sequence identity and 72% amino acid sequence similarity. The amino
30 acid sequence similarity between the chicken protein and human Dkk-3 is particularly high in the cysteine-rich domain of hDkk-3 which is located between amino acids 147

* Trademark

- 121 -

and 284 of SEQ ID NO: 2. In particular, the 20 cysteine residues of hDkk-3 located in this region are present in the chicken protein.

Two genes recently identified in a screen for suppressors of glioblastoma formation (Ligon *et al.* (1997) *Oncogene* 14:1075-1081) also show homology to hDkk-3. These genes, RIG ("Regulated In Glioblastoma") and RIG-like 7-1 (GenBank Accession Nos. U32331 and AF034208, respectively) were identified in a differential screen for mRNAs regulated by the introduction of a normal copy of chromosome 10 into a glioblastoma cell line harboring a deletion in chromosome 10 that promotes tumorigenesis. A schematic diagram summarizing the relationship between the sequences of the hDkk-3 and the RIG genes is presented as Figure 12. The indicated region of identity between hDkk-3 and RIG comprises a short portion of the 3' UTR of the human Dkk-3 mRNA (*e.g.*, RIG mRNA is ~100% identical to residues 2479 to 2153 of SEQ ID NO:1). RIG-like 7-1 is homologous to hDkk-3 across a longer region (*e.g.*, 97% identical from about nucleotides 316 to 2438 of SEQ ID NO:1) although the encoded RIG-like 7-1 protein lacks the Dkk N-terminal signal sequence and is not therefore predicted to be a secreted protein. These data associate hDkk-3 with human glioblastoma and suggest that hDkk-3 may be important in the suppression of the tumorigenic phenotype. A role in glioblastoma is also consistent with the high level of hDkk-3 mRNA expression observed in human brain tissue. In addition, the co-localization of the hDkk-3, RIG and RIG-like genes to a region of chromosome 11 (11p15.1) implicated in the development of human malignant astrocytoma (Ligon *et al.*, *supra*) further indicates a role for these genes in tumorigenesis.

Human hDkk-3 protein has also some amino acid sequence similarity to metallothionein, particularly in the cysteine-rich domain.

Tissue Distribution of hDkk-3 mRNA

For Northern blots, all hybridizations were to Clontech Multiple Tissue Northern Blots and were performed in ExpressHyb^{*} solution (Clontech) for 1-20 hours. All probes were prepared by random primed radiolabelling (Prime-It^{*}, Stratagene). Blots were washed sequentially to a final stringency of 0.2x SSC/0.2% SDS and exposed to autoradiographic film. Hybridizations of a control β -actin cDNA probe consistently

* Trademark

- 122 -

demonstrated even loading of the Northern blots. The results of hybridization of the probe to various mRNA samples are described below.

Hybridization of a Clontech Fetal Multiple Tissue Northern (MTN) blot (Clontech, LaJolla, CA) containing RNA from fetal brain, lung, liver, and kidney
5 indicated the presence of high levels of hDkk-3 mRNA (~2.5kb) in fetal brain, lung, and slightly lower levels of hDkk-3 mRNA in fetal kidney. However, no significant level of hDkk-3 mRNA was found in fetal liver.

Hybridization of a Clontech human Multiple Tissue Northern (MTN) blot (Clontech, LaJolla, CA) containing RNA from adult heart, brain, placenta, lung, liver,
10 skeletal muscle, kidney, and pancreas with a human Dkk-3 probe indicated the presence of high levels of hDkk-3 mRNA in heart, slightly lower levels in brain, and much lower levels in placenta and lung. Some hDkk-3 mRNA was also found in adult skeletal muscle. However, no significant levels of hDkk-3 mRNA was observed in adult liver,
15 kidney, or pancreas. Interestingly, the chicken gene which is homologous to hDkk-3 was not expressed at detectable levels in liver either (Sawada *et al.*, (1996) *Int. J. Dev. Biol.* 40:531).

Further hybridization of a Clontech human Multiple Tissue Northern (MTN) blot (Clontech, LaJolla, CA) including RNA from bone marrow, adrenal gland, trachea, lymph node, spinal cord, thyroid, and stomach revealed high levels of expression of
20 hDkk-3 in mRNA isolated from adult spinal cord, and lower level expression in adrenal gland, trachea, thyroid, and stomach.

Thus, hDkk-3 is expressed in a tissue specific manner, with the strongest expression observed in brain, heart, and spinal cord.

25 **Example 2: Isolation And Characterization of mDkk-3 cDNA**

In this example, the isolation and characterization of the gene encoding murine Dkk-3 (also referred to as "mDkk-3", "murine Cysteine Rich Secreted Protein-1", "murine CRSP-1" or "murine CRISPY-1") is described.

- 123 -

Identification of a Murine Dkk-3 cDNA

A full length mDkk-3 cDNA was identified by comparison of the hDkk-3 sequence to a proprietary EST Database using the BLAST-X algorithm. A single clone identified in a adult mouse brain cDNA library was obtained and sequenced fully. DNA for the clone Fmmb059s was deposited with the ATCC as Accession No. 98634. mDkk-3 is predicted to have a signal peptide from residues 1 to 23 of SEQ ID NO:17, cleavage of which results in a mature protein having 326 amino acids in length corresponding to amino acids 24 to 349 of SEQ ID NO:17.

10 Tissue Distribution of mDkk-3 mRNA

To determine the expression pattern of mDkk-3, *in situ* hybridization was performed as follows. Normal mouse embryos and adult mouse tissues were collected from C57BL/6 mice, embedded in TissueTek™ O.C.T Compound (Sakura Finetek U.S.A., Inc., Torrance, CA), frozen on dry ice, and stored at -80°C. Cryostat serial sections (8µm) were thaw mounted on Superfrost Plus™ slides (VWR Scientific, West Chester, PA.) and air dried on a slide warmer at 40°C for 20 minutes. Sections were then fixed with 4% formaldehyde in DEPC treated 0.1 M phosphate-buffered saline (PBS, pH 7.5) at room temperature for 10 minutes and rinsed twice in DEPC-PBS. Sections were rinsed in 0.1 M triethanolamine-HCl (TEA, pH 8.0), incubated in 0.25% acetic anhydride-TEA for 10 minutes and rinsed in DEPC-2X SSC (standard sodium citrate). Sections were dehydrated through a series of graded ethanols, incubated in 100% chloroform for 5 minutes, rinsed in 100% and 95% ethanol for 1 minutes and air dried.

Antisense and sense RNA transcripts were prepared by *in vitro* transcription (Riboprobe Gemini System™, Promega) of PCR amplified cDNA templates. Template amplification primers were as follows;

mDkk-3 forward 5'-CAGTGAGTGCTGTGGAGACC-3' (SEQ ID NO:30), and

reverse 5'-TCTTCAGTCAGGCTCCTCTC-3' (SEQ ID NO:31).

Probes were labeled with ³⁵S-UTP (NEN) and purified on G-25 spin columns (Pharmacia). The hybridization cocktail contained: 50% formamide, 10% dextran

- 124 -

sulfate, 0.1% sodium dodecyl sulfate (SDS), 0.1% sodium thiosulfate, 1X Denhardt's solution, 0.6 M NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA, 100 mM dithiothreitol (DTT), 0.1 mg/ml sheared salmon sperm, 50 µg/ml yeast tRNA, 0.5 mg/ml yeast total RNA, and ³⁵S-UTP labeled probe at a concentration of 5 X 10⁷ c.p.m./100µl of final
5 hybridization solution; 100 µl of hybridization solution was put on each section. The sections were then covered with a glass coverslip and incubated in a humidified chamber at 55°C for 18 h. After hybridization, slides were washed with 2 X SSC. Sections were then sequentially incubated at 37°C in TNE (a solution containing 10 mM Tris-HCl (pH 7.6), 500 mM NaCl, and 1 mM EDTA), for 10 minutes, in TNE with 10ug/ml RNase A
10 for 30 minutes, and finally in TNE for 10 minutes. Slides were then rinsed with 2 X SSC at room temperature, washed in 2 X SSC at 50°C for 1 h, 0.2 X SSC at 55°C for 1h, and 0.2 X SSC at 60°C for 1 h. Sections were dehydrated with a series of graded concentrations of ethanol 0.3 M ammonium acetate, air dried and exposed to Kodak Biomax MR™ scientific imaging film for 6 days at room temperature.

15 mDkk-3 expression in the brain was found to be highly localized to the cortex and hippocampus but was not observed in the dentate gyrus. Higher power magnification confirmed the mDkk-3 mRNA was localized to neurons within these structures. In the adult eye, mDkk-3 mRNA was found to be highly expressed in the retina, ciliary body, and lens epithelium. Expression in the retina was localized to the
20 integrating bipolar and ganglion cells. In adult heart, mDkk-3 was detected in the atrioventricular valves and also in myocytes of the atria. Expression was highly restricted to the atria and noticeably absent from ventricular tissue. High level expression of mDkk3 mRNA was also observed in developing eye, bone and cartilage in day 14 embryos. These findings corroborate and extend the northern analysis of hDkk-3
25 mRNA expression in human tissues and also suggest that Dkk-3 may play a role in bone and ocular physiology in addition to functions in neural and cardiac tissues.

Example 3: Secretion and Post-Translational Modification of Dkk-3

This example describes the secretion and post-translational modification (*e.g.*, glycosylation and processing) of hDkk-3 as well as methods for small and large scale purification of hDkk-3.

5

hDkk-3 Expression Constructs

Expression constructs for two forms of hDkk-3 were prepared using the mammalian expression vector pMET-stop. Form-1 comprised a cDNA incorporating the complete 350aa hDkk-3 protein coding sequence (hDkk-3flag.long) and form-2
10 comprised the entire hDkk-3 protein coding sequence except for the final 18 amino acids (hDkk-3flag.short). A C-terminal sequence encoding the FLAG epitope (DYKDDDDK) (SEQ ID NO:19) was added to both hDkk-3 forms for ease of detection and purification. hDkk-3flag cDNAs were generated by PCR from a full length hDkk-3 cDNA template and ligated into pMET-stop using EcoR1 and Sal1 restriction sites.

15

Trial Transfection - Small Scale Expression

Expression constructs for hDkk-3flag.long and hDkk-3flag.short were transfected into 293T cells using 10 µl of lipofectamine* (GIBCO/BRL) and 2 µg of DNA per well of a 6-well plate of cells which were 70-80% confluent. After 5 hours at
20 37°C, cells were fed with 1ml of 20%FCS/DMEM. After incubation overnight at 37°C, cells were conditioned in 1ml OptiMEM* for 48 hours at 37°C. Samples of supernatant and cell pellets were solubilized in boiling SDS-PAGE gel buffer, run out on a 4-20% SDS-PAGE gel, transferred to a nylon membrane and probed with the anti-FLAG monoclonal antibody M2. Samples from both supernatant and pellet samples showed
25 significant immunoreactivity within a molecular weight range of 40-65 kDa on autoradiographic film using a HRP conjugated secondary antibody and ECL detection reagents. Thus, both forms of hDkk-3 tested are secreted from 293T cells thereby confirming experimentally that hDkk-3 is a secreted protein. It should be noted that the molecular weights of both forms of hDkk-3 tested are greater than predicted from the
30 amino acid sequence, suggesting that the hDkk-3 proteins secreted by 293T cells may be glycosylated. This is consistent with the presence of four potential sites for N-linked

* Trademark

- 126 -

glycosylation in the hDkk-3 protein (*e.g.*, at about amino acids 96-99, 106-109, 121-124, and 204-207 of SEQ ID NO:2).

Deglycosylation of hDkk-3

5 Given the heterogenous nature of secreted human Dkk-3, the effect of N-Glycanase treatment on the mobility of secreted flag-tagged hDkk-3 was studied. Briefly, 1mL samples of 293T cell supernatants collected 72 hours after transfection with the appropriate constructs were incubated with 50 μ L anti-flag M2 agarose beads (Sigma) for 16hrs at 4°C. Beads were washed with PBS (pH7.4) containing,
10 sequentially, 0.1%, 0.05% and 0.01% Triton X-100. The beads were resuspended in 20 μ L of 20mM sodium phosphate, pH 7.5, 50mM EDTA, 0.02% sodium azide, (incubation buffer) together with 0.5% SDS, 5% 2-mercaptoethanol and boiled for 2 minutes. The supernatant was split into equal 10 μ L aliquots which were diluted with 10 μ L incubation buffer, 5 μ L 5% NP-40^{*} and then with either 5 μ L N-Glycanase (Oxford Glycosystems) in
15 enzyme buffer (20mM Tris-HCl, 1mM EDTA, 50mM NaCl, 0.02% sodium azide pH 7.5) or with enzyme buffer alone as control. After 18 hours at 37°C, samples were boiled in equal volumes of SDS-PAGE buffer and analyzed by SDS-PAGE and Western blotting. For western analysis, samples were electroblotted onto PVDF (Novex) after SDS-PAGE on 4-20% gradient gels, probed with M2 anti-flag antibody (1:500, Sigma)
20 followed by HRP conjugated sheep anti-mouse IgG (1:5000, Amersham), developed with chemiluminescent reagents (Renaissance, Dupont) and exposed to autoradiography film (Biomax^{*} MR2 film, Kodak).

Utilizing the above-described methodology, it was determined that hDkk-3 protein displayed a significant increase in mobility following N-Glycanase treatment.
25 The major 45-65 kD form of soluble hDkk-3 was observed as two species of 45-55 and 40 kD following deglycosylation. This finding is consistent with the presence of multiple potential sites of N-linked glycosylation in the hDkk-3 protein. The reason for the heterogeneity of deglycosylated hDkk-3 reflects either proteolytic processing or incomplete removal of carbohydrate from one or more attachment sites. A 30 kD hDkk3
30 species was also observed in these experiments, the mobility of which was unaltered by

* Trademark

- 127 -

N-Glycanase treatment. This form was only observed after overnight incubation of the samples and may be a non-specific degradation product.

Large Scale hDkk-3 Protein Production

5 For scale-up of hDkk-3flag.long protein expression, 30 x 150mm plates of 293T cells at 70-80% confluence were transfected with 27 µg DNA, 100 µl lipofectamine in 18ml OptiMEM for 5 hours at 37°C. 18 ml of 10%FCS/DMEM was added to each plate and incubated overnight at 37°C. 24 hours after the start of transfection, transfection supernatant was aspirated and 35 mls OptiMEM was added to each plate and the plates
10 incubated at 37°C for 72 hours. Conditioned medium was harvested, spun at 4000 rpm for 30 min. at 4°C, and filtered through a 0.45 micron filter unit. 1100 ml was passed over a 1.6 x 10 cm anti-FLAG M2 affinity column pre-equilibrated in PBS pH7.4 buffer at a flow rate of 2.0 ml per minute. After washing with 200 ml of PBS pH 7.4 buffer, bound material was eluted by a step of 200 mM Glycine pH 3.0 buffer and 0.5 ml
15 fractions collected. Upon elution, a significant protein peak was detected by absorbance at 280nm. Samples corresponding to conditioned medium, flow through and eluted fractions were analyzed by Coomassie blue and silver stained SDS-PAGE and by western blot analysis as described above. Significant immunoreactivity within a molecular weight range of 40-65 kDa was detected in conditioned medium and eluted
20 fractions but not in the flow through sample, indicating that the secreted hDkk-3flag.long protein bound to the affinity column specifically and was eluted efficiently by the described conditions. Coomassie blue staining of SDS-PAGE gels suggested that the predominant immunoreactive protein constituted >90% of the protein present in the bound and eluted protein peak. Peak fractions of eluted protein were pooled and
25 dialysed against Phosphate Buffered Saline resulting in a 4 ml volume of recombinant hDkk-3flag.long protein at a concentration of approximately 1mg/ml.

Example 4: Isolation and Characterization of hDkk-4

In this example, the isolation and characterization of the gene encoding human
30 Dkk-4 (also referred to as "hDkk-4", "Cysteine Rich Secreted Protein-2", "CRSP-2" or "CRISPY-2") is described.

Isolation and Analysis of a Human Dkk-4 cDNA

To identify novel proteins related to hDkk-3, the human Dkk-3 amino acid sequence was used to search the dbEST database using TBLASTN (WashUversion, 2.0, BLOSUM62 search matrix). A dbEST clone with accession number AA565546 was identified as having homology to a portion of the hDkk-3 cDNA. This clone was obtained from the IMAGE consortium and sequenced fully to define the entire hDkk-4 sequence depicted in Figure 2.

Determination of the hydrophobicity profile of human Dkk-4 having the amino acid sequence set forth in SEQ ID NO:5 indicated the presence of a hydrophobic region from about amino acid 1 to about amino acid 19 of SEQ ID NO:5. Further analysis of the amino acid sequence SEQ ID NO:5 using a signal peptide prediction program predicted the presence of a signal peptide from about amino acid 1 to about amino acid 19 of SEQ ID NO:5. Accordingly, the mature hDkk-4 protein includes about 205 amino acids spanning from about amino acid 20 to about amino acid 224 of SEQ ID NO:5.

Tissue Distribution of hDkk-4

hDkk-4 mRNA was undetectable by Northern analysis in all adult and fetal human tissues examined. Accordingly, a survey was performed of a cDNA library panel by PCR with hDkk-4 specific PCR primers. Using such primers, products were identified in libraries prepared from cerebellum, activated human T-lymphocytes, lung and esophagus.

Secretion and Post-Translational Modification of human Dkk-4

Flag epitope-tagged human Dkk-4 protein was transiently overexpressed in 293T cells and analyzed as described previously for hDkk-3. Soluble hDkk-4 was consistently detected as three major immunoreactive species of approximately 40 kD [form (i)], 30-32 kD [form (ii)] and 15-17 kD [form (iii)]. Neither form (i), (ii) or (iii) was significantly affected by N-glycanase treatment, consistent with the absence of N-glycosylation sites from the protein.

- 129 -

To determine the possible cause of heterogeneity in the size of secreted hDkk-4, Edman N-terminal sequencing of anti-flag affinity purified material corresponding to bands (i), (ii) and (iii) was performed. Briefly, flag-tagged Dkk-4 protein was isolated by passing the conditioned media over an M2-biotin (Sigma)/streptavidin Poros^{*} column (2.1 X 30 mm, PE Biosystems); the column was then washed with PBS, pH 7.4 and flag-tagged protein eluted with 200 mM glycine, pH 3.0. Eluted fractions with 280nm absorbance greater than background were analyzed by SDS-PAGE and western blot. Purified Dkk-4 protein bound to PVDF membrane after SDS-PAGE and electroblotting was sequenced for N-terminal amino acid analysis on a PE Applied Biosystems Model 494 Procise instrument using Edman-based chemistry protein sequencing. The amino acid residues were analyzed by HPLC (Spherogel^{*} micro PTH 3-micron column) and determined by separation and peak height as compared to standards.

The N-terminal sequence of band (i) was found to be XVLDFNNIRS (SEQ ID NO:34) which corresponds exactly to the predicted signal peptide cleavage site (between Ala-18 and Leu-19). Because the same band is identified by anti-flag antibodies, which recognize the C-terminal epitope tag, band (i) was thus identified as the full length, mature hDkk-4 protein. The band (iii) N-terminal sequence was found to be SQGRKGQEGS (SEQ ID NO:35) which corresponds to CRD-2 cleaved at the dibasic site Lys132/Lys133 (e.g., Lys113/Lys114 of the mature protein following cleavage of the a 19 amino acid signal sequence or Lys 114/Lys 115 following cleavage of a 18 amino acid signal sequence). These data obtained for bands (i) and (iii) indicate clearly that hDkk4 is proteolytically processed by 293T cells, resulting in the release of CRD-2 (a 91 amino acid biologically-active fragment) from the full length protein.

Moreover, the three major species migrated similarly on SDS-PAGE conducted under either reducing or non-reducing conditions. Thus, each of the major C-terminal (anti-flag immunoreactive) hDkk-4 species exist as independent proteolytic fragments that are not covalently linked via disulfide bonds to other subunits or complex components when secreted from 293T cells.

* Trademark

- 130 -

Example 5: Isolation and Characterization of hDkk-1

In this example, the isolation and characterization of the gene encoding human Dkk-1 (also referred to as "hDkk-1", "Cysteine Rich Secreted Protein-3", "CRSP-3" or "CRISPY-3") is described.

5

Identification of a Human Dkk-1 cDNA

Searching a proprietary database of EST information using the sequence of hDkk-3, an hDkk-1 partial sequence was found corresponding to a clone from a human fetal kidney cDNA library having the identification code jthKb075a10. This clone was
10 sequenced further and to define the entire hDkk-1 sequence depicted in Figure 3. DNA for the clone jthKb075a10 was deposited with the ATCC as Accession No. 98633. hDkk-1 has a predicted signal peptide from about amino acid residue 1 to 20 of SEQ ID NO:8, cleavage of which results in a mature protein having 246 amino acid residues in length and corresponding to amino acid residues 21 to 266 of SEQ ID NO:8.

15

Tissue Distribution of hDkk-1

Northern blot analysis of various tissues including heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas was performed as previously described using a probe specific for hDkk-1. A ~1.8 kb hDkk1 mRNA was detected at high levels
20 in human placenta, but not in other tissues tested.

Secretion and Post-Translational Modification of hDkk-1

Flag epitope-tagged human Dkk-1 protein was transiently overexpressed in 293T cells and analyzed as described previously. hDkk-1 was efficiently secreted from
25 mammalian cells and was readily detected in conditioned medium of transfected cells. Mature secreted hDkk-1 migrated with a molecular weight of approximately 42-50 kD. Treatment with N-Glycanase had no significant effect on the mobility of soluble hDkk-1. Although hDkk-1 contains one potential site of N-linked glycosylation at its extreme C-terminus (e.g., at amino acids 256-259 of SEQ ID NO8), this site is not conserved in
30 *Xenopus* Dkk-1 (Glinka *et al.*, *supra*) and appears not to be a major site of carbohydrate addition in 293T cells.

Example 6: Isolation and Characterization of hDkk-2

In this example, the isolation and characterization of the gene encoding human Dkk-2 (also referred to as "hDkk-2", "Cysteine Rich Secreted Protein-4", "CRSP-4" or "CRISPY-4") is described.

5

Isolation of a Human Dkk-2 cDNA

Using the hDkk-3 sequence to query the dbEST database, a clone having similarity to a portion of hDkk-3 was identified having Accession No. W55979. This clone was subsequently obtained from the IMAGE consortium and sequenced to define a partial hDkk-2 sequence set forth as SEQ ID NO:10. This cDNA comprises a coding region from nucleotides 1-537, as well as 3' untranslated sequences (nucleotides 538 to 702). The coding region alone is set forth as SEQ ID NO:12. The predicted amino acid sequence corresponds to amino acids 1 to 179 of SEQ ID NO:11. A cDNA encoding full length hDkk-2 was isolated from a human fetal lung lambda Ziplox libraries by conventional plaque hybridization (Sambrook *et al.*, 1989) and fully sequenced. The full-length nucleotide sequence is set forth as SEQ ID NO:20 and the predicted amino acid sequence is set forth as SEQ ID NO:21. The coding region alone is set forth as SEQ ID NO:22. The predicted amino acid sequence corresponds to amino acids 1 to 259 of SEQ ID NO:21. DNA for the clone fthul33 was deposited with the ATCC.

hDkk-2 has a predicted signal peptide from about amino acid residue 1 to 33 of SEQ ID NO:21, cleavage of which results in a mature protein having 226 amino acid residues in length and corresponding to amino acid residues 34 to 259 of SEQ ID NO:21.

Tissue Distribution of hDkk-2

Northern blot analysis of various tissues (*e.g.*, heart, brain, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, and peripheral blood leukocytes) was performed as previously described using a probe specific for hDkk-2. Of the tissues tested, hDkk-2 mRNA expression was highest in heart, brain, placenta, lung, and skeletal muscle. hDkk-2 transcripts of approximately 4.0 and 4.5 kb were observed.

- 132 -

Secretion and Post-translational Modification of hDkk-2

Flag epitope-tagged human Dkk-2 protein was transiently overexpressed in 293T cells and analyzed as described previously. Soluble hDkk-2 was detected as a major species of 15-17 kD, closely similar in size to form (iii) of hDkk-4. Additional minor forms of hDkk-2 were also observed in certain experiments in the range of 20-21kD. Deglycosylation of hDkk-2 was not studied since the protein sequence lacks potential N-glycosylation sites. By comparison with the data presented in Example 4 regarding the dibasic proteolytic cleavage site in the hDkk-4 protein sequences, it is predicted that the major 15-17 kD form of hDkk-2 detected in these experiments corresponds to CRD-2, as was the case for hDkk-4.

Example 7: Isolation of Soggy proteins

In this example, the isolation and characterization of the gene encoding human and murine Soggy-1 (also referred to as "Cysteine Rich Secreted Protein-N" or "CRISP-N") is described.

Identification of a Human and Murine Soggy-1 cDNAs

Human Soggy-1 was identified as a novel protein with similarity to the N-terminal domain of hDkk3. A human partial sequence was identified in the dbEST database for a clone having the accession number AA397836. This clone was obtained from the IMAGE collection and sequenced fully to define the entire human Soggy-1 sequence depicted in Figure 7. Two murine partial sequences were likewise identified in the dBEST database. The clones were obtained from the IMAGE consortium and sequenced. The entire murine Soggy-1 sequence is depicted in Figure 8. Human and murine Soggy cDNAs encode proteins of 242aa and 230aa, respectively, and are predicted to be secreted owing to the presence of N-terminal signal peptides. hSoggy-1 has a predicted signal peptide from about amino acid residue 1 to 30 of SEQ ID NO:14, cleavage of which results in a mature protein having 194 amino acid residues in length and corresponding to amino acid residues 31 to 224 of SEQ ID NO:14. mSoggy-1 has a predicted signal peptide from about amino acid residue 1 to 20 of SEQ ID NO:27,

- 133 -

cleavage of which results in a mature protein having 210 amino acid residues in length and corresponding to amino acid residues 21 to 230 of SEQ ID NO:27. Human and murine Soggy proteins display 59% overall identity although significant amino acid identities are seen beyond this domain that extend into the CRDs of Dkk-3 (Figure 10).

5 However, cysteine residues are not conserved within these domains and the residues shared by Soggy and Dkk-3 are poorly conserved in other Dkks indicating that the sequence relationship between these proteins is unique. Homology is most obvious within a 51 amino acid region in which 33% identity is observed between hSoggy, mSoggy, hDkk-3 and mDkk-3. This 51 amino acid domain is referred to herein as an

10 SGY domain. Human and mouse Soggy-1 proteins each possess 2 sites of potential N-linked glycosylation which are within the SGY domain and are also conserved with Dkk3. (e.g., NNLT, corresponding to amino acid residues 97-100 of SEQ ID NO:14 and NKTG corresponding to amino acid residues 112-115 of SEQ ID NO:14). In contrast to other Dkks, the C-terminal domain of Soggy-1 shows no similarity to other protein

15 sequences in the public databases nor does it contain any cysteine residues. Soggy was so named in view of its lack of CRDs compared to hDkk-3, which had been previously designated Cysteine Rich Secreted Protein-1 ("CRISPY-1").

Tissue Distribution of Soggy-1

20 To investigate Soggy-1 mRNA expression, a mouse cDNA probe was used on murine Northern blots. A 1kb mSoggy-1 mRNA was expressed at very high levels in testis and, interestingly, demonstrated transient expression during mouse embryogenesis. Soggy-1 mRNA, which was undetectable at day 7 of gestation, was transiently expressed at day 11 and day 15, after which the expression level declined to

25 undetectable levels. Thus, mSoggy-1 displays a developmentally regulated pattern of expression.

In situ analysis was performed as described in Example 1. For detection of murine Soggy-1, the following primers were used:

mSoggy forward 5'-ACCTGCAATGTGTCGACTGAG-3' (SEQ ID NO:32), and

30 *reverse* 5'-CACTTACAGCTGTTGGGATG-3' (SEQ ID NO:33).

- 134 -

Consistent with the Northern analysis, very high level expression of Soggy-1 mRNA was observed by *in situ* analysis in adult testis. Upon high magnification, Soggy-1 mRNA was found to be expressed at high levels in the spermatogenic epithelium of the seminiferous tubules and in the spermatogonia at various stages of development. A series of sagittal sections of mouse embryos from E13.5 - E 17.5 and post-natal day 1.5 pups were also analyzed. In E15.5 embryos, Soggy-1 mRNA transcripts were localized to the developing dorsal root ganglia (DRGs) and also found in the cartilage primordium of the nasal septum. Soggy-1 expression was also seen in the eye from E13.5 to E16.5, as observed for mDkk-3. Expression of Soggy-1 mRNA at various stages of development is consistent with the northern analysis described above and suggests that Soggy-1 may play a role in multiple stages of development.

Secretion and Post-Translational Modification of Soggy Proteins

Flag epitope-tagged human Soggy-1 protein was transiently overexpressed in 293T cells and analysed as previously described. hSoggy was efficiently secreted from transfected 293T cells and migrated with a molecular weight of approximately 40-50 kD. Given the heterogenous nature of secreted human Soggy-1, the effect of N-Glycanase treatment on the mobility of secreted flag-tagged hSoggy-1 was studied. hSoggy displayed a 5-10 kD decrease in apparent molecular weight after N-Glycanase treatment, consistent with the presence of 2 potential sites of N-glycosylation in the protein.

Example 8: Structure of the Dkk Family proteins and Dkk-Related Proteins

The amino acid and nucleotide homology between Dkk family members and Dkk-related proteins is set forth in the following tables. Where indicated, mDkk-1 and xDkk-1 correspond to a murine and *Xenopus* proteins set forth in Glinks *et al.*, *supra*, and having Accession Nos: AF030433 and AF030434, respectively. Likewise cDkk-3 has Accession No. D26311

- 135 -

Table II sets forth overall sequence identities as determined using the ALIGN program, (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4:

	hDkk-3	hDkk-4	hDkk-1	hDkk-2	mDkk-1	xDkk-1	CLFEST
hDkk-3	100	16.0	18.6	15.1	18.5	16.5	53.0
hDkk-4		100	33.7	35.2	32.6	33.7	16.2
hDkk-1			100	33.1	80.2	53.5	17.4
hDkk-2				100	30.5	33.7	12.5

5

Table III sets forth nucleic acid identities as determined using the using the Wilbur Lipman DNA alignment program, Ktuple: 3; Gap Penalty: 3; Window: 20:

	hDkk-3	hDkk-4	hDkk-1	hDkk-2	mDkk-1	xDkk-1	CLFEST
hDkk-3	100	30.0	37.2	34.7	31.5	45.4	58.8
hDkk-4		100	43.0	35.9	38.8	38.4	36.7
hDkk-1			100	59.3	66.4	53.7	32.1
hDkk-2				100	38.8	38.4	36.7

- 136 -

Table IV sets forth local amino acid identities as determined using the Smith-Waterman algorithm as implemented in the program Bestfit* of the GCG package, with gap penalties of 8 for opening and 1 for extending:

hDkk-1								
mDkk-1	82							
xDkk-1	64	63						
hDkk-2	50	48	47					
hDkk-3	39	37	37	37				
mDkk-3	36	33	38	40	83			
cDkk-3	34	31	35	36	61	60		
hDkk-4	45	43	47	46	40	39	34	
	hDkk-1	mDkk-1	xDkk-1	hDkk-2	hDkk-3	mDkk-3	cDkk-3	hDkk-4

5

A multiple alignment of the amino acid sequences of hDkk-1, hDkk-2, hDkk-3, hDkk-4, mDkk-1, mDkk-3, xDkk-1, and cDkk-3 is shown in Figure 6. Predicted signal peptides are underlined, N-glycosylation sites are indicated by a thick bar, CRD-1 by an open box and CRD-2 by a shaded box. The proteolytic cleavage site within hDkk4 is indicated by a double asterisk. The domain structure of the full length human Dkk proteins of the present invention as well as human Soggy are schematically illustrated in Figure 9. Signal peptides (darkened boxes), Cysteine Rich Domain 1 ("CRD-1") (also referred to as the "amino-terminal cysteine-rich domain"), Cysteine Rich Domain 2 ("CRD-2") (also referred to as the "carboxy-terminal cysteine-rich domain"), the soggy domain (SGY) within hDkk-3 and hSoggy-1, and sites of N-glycosylation are indicated.

15

As demonstrated at least in Figures 6 and 9, human Dkks 1 through 4 each possess an N-terminal signal peptide and contain two conserved cysteine-rich domains (CRDs) separated by a linker or spacer region. Each CRD possesses 10 conserved cysteine residues. The second CRD has elsewhere been described as a colipase-like domain because the positions of the ten conserved cysteines in this domain have been shown to be closely similar to those in proteins of the colipase family (Aravind and Koonin, *supra*). Conservation of CRD-1 and CRD-2 suggests important functions for

20

* Trademark

- 137 -

these domains. In contrast to the CRDs, the linker or spacer region that joins CRD-1 and CRD-2 is highly variable between hDkks, being notably larger in hDkk-1, -2 and -4 (50-55aa) as compared to Dkk-3 (12aa). Four potential sites of N-linked glycosylation are present in hDkk3 and are conserved in chicken and mouse Dkk-3. These sites are not conserved in other Dkk family members. hDkk1 possesses one potential N-glycosylation site located close to the C-terminus of the protein which is conserved in murine Dkk-1 but not in *Xenopus* Dkk-1 (Fig. 6). In addition, each hDkk possesses several potential dibasic proteolytic cleavage sites, suggesting the proteins may be subject to post-translational processing. hDkk3 is the most divergent of the four human Dkks and possesses an extended N-terminal unique region which precedes CRD-1 and an extended C-terminal unique region which is highly acidic.

Example 9: Effects of hDkks and Soggy on Wnt-induced axis duplication in *Xenopus* embryos

This Example describes the functional activities of the hDkk and Soggy proteins of the present invention.

Xenopus embryo culture and RNA microinjections

Eggs were obtained from *Xenopus* females injected with 700 units of human chorionic gonadotropin, fertilized *in vitro* and cultured in 0.1 x MMR (Newport and Kirschner (1982) *Cell* 30:675-686). Embryonic stages were determined according to Nieuwkoop and Faber (1967) *Normal table of Xenopus laevis* (Daudin) Amsterdam: North Holland Publ. All cDNAs were subcloned into pCS2 vector (Rupp *et al.* (1984) *Genes & Development* 8:1311-1323), and capped mRNAs were synthesized *in vitro* as described (Krieg and Melton (1984) *Nucleic Acids Res.* 12:7057-7070, using the Message Machine^{*} kit (Ambion). The following plasmids were used as templates for mRNA synthesis: hDkk-1-pCS2, hDkk-2-pCS2, hDkk-3-pCS2, hDkk-4-pCS2, hSoggy-pCS2, Xwnt8 (Christian *et al.*, (1991) *Development* 111:1045-1055), Xwnt2B (Landesman and Sokol (1997) *Mech. Dev.* 61:1199-209), Xwnt3a (Wolda *et al.* (1993) *Dev. Biol.* 155:46-5), Xfz8-pXT7 (Itoh *et al.* (1998) *Mech. Devel.* 74:145-157), Xdsh-pXT7 (Sokol, *et al.* (1995) *Mech. Devel.* 74:145-157). Protein expression from all

* Trademark

- 138 -

pCS2-Dkk constructs was confirmed by *in vitro* transcription and translation (TNT, Promega). For secondary axis induction, a single ventral blastomere of 4- or 8-cell embryos was injected with 10nl of a solution containing 2-4pg of Xwnt8 mRNA, 2.5-5pg of Xwnt3a mRNA or 10pg of Xwnt2B mRNA as described (Itoh *et al.* (1995) *supra.*). The effect of Dkk RNAs was tested by coinjecting Wnt mRNAs with 2.5ng of hDkk mRNAs. For studies of Frizzled and Dhshevelled, 5ng Fz8 and 1ng Xdsh mRNAs were injected as indicated. After injections, embryos were cultured in 3% Ficoll 400 (Pharmacia), 0.5xMMR. Secondary axes were scored at stage 35 as complete, when they contained anterior neuroectodermal derivatives including pronounced cement gland and eyes, and as partial, when the secondary neural tube with melanocytes, but without head structures, was apparent.

Inhibition of Secondary Axis Induction by hDkk-1 and hDkk-4 in *Xenopus* Embryos

hDkk-1 or hDkk-2 mRNAs were coinjected with Xwnt8 mRNA into single ventral blastomeres of 4- or 8-cell embryos. Injected embryos were cultured for 2 days and secondary axes were scored based on external morphology. Xwnt8 injected embryos displayed complete axis duplication, which was inhibited by co-injection with mRNAs encoding hDkk-1 and hDkk-4. To determine whether hDkks interacted with specific Wnt ligands, several different Wnts were assayed in combination with hDkk-1 or hDkk-4 for secondary axis formation. hDkk-1 and hDkk-4 inhibited axis duplication in response to Xwnt3a and Xwnt2b in addition to Xwnt8. hDkk-1 consistently demonstrated stronger inhibition of Wnt signaling than hDkk-4. Thus, hDkk-1 and hDkk -4 do not show any clear selectivity for the Wnt ligands used in this study. This compares to the FRPs, which also show little specificity with respect to their ability to inhibit Wnts (Leyns *et al.* (1997) *supra*; Wang *et al.* (1997) *supra*; Salic *et al.* (1997) *supra*; Mayr *et al.* (1997) *supra*; Finch *et al.* (1997) *supra*).

To investigate the mechanism by which hDkk-1 and hDkk-4 inhibit Wnt signaling, Dkk mRNAs were coinjected with Xdsh, a downstream component of the Wnt signaling pathway (Itoh *et al.* (1998) *supra*). hDkks-1 and -4 did not block secondary axis formation by Xdsh, indicating that Dkks function upstream of, or parallel with, Xdsh signaling. Similar findings have been reported previously for xDkk-1 (Glinka *et*

- 139 -

al. (1998) *supra*). It was also determined whether hDkks could antagonize signaling by *Xenopus* Frizzled-8 (Xfz8), which can also induce a secondary axis through Wnt signaling (Itoh *et al.* (1998) *supra*). Neither hDkk-1 or hDkk-4 inhibited the axis-inducing activity of Xfz8 mRNA. This data, taken together with the fact that hDkk-1 and hDkk-4 are secreted, indicate that Dkks antagonize Wnt signaling at a point upstream of Wnt receptors.

Assay for Inhibition of Secondary Axis Induction by hDkk-2, hDkk-3 and hSoggy-1 in *Xenopus* Embryos

hDkk-2, hDkk-3 or Soggy mRNAs were coinjected with Xwnt8 mRNA into single ventral blastomeres of 4- or 8-cell embryos and secondary axes were scored after two days as described for hDkk-1 and hDkk-4. Injection of mRNAs encoding hDkk-2, hDkk-3 or hSoggy-1 had no effect on Xwnt8-induced axis duplication. The ability of hDkk-2, hDkk-3 and hSoggy-1 to interact with specific Wnt ligands was also determined as described previously. hDkk-2, hDkk-3 and hSoggy-1 were inactive against each of the three Wnts tested. The lack of activity of hDkk-2, hDkk-3 and hSoggy-1 suggests that these proteins antagonize other members of the Wnt superfamily not tested here, or that they perform functions distinct from Wnt inhibition.

Example 10: Preparation of Antibodies Specific for hDkk and hSoggy Proteins

This example describes the making of polyclonal antibodies specific for hDkk-1, hDkk-4, hDkk-1, hDkk-2, and hSoggy-1.

Peptides were synthesized using Fmoc solid phase methodology utilizing MAP resin technology which increases the antigenic response (Tarn (1988) *Proc. Natl. Acad. Sci. USA* 85:5409-5413. For each protein, the peptides used for immunization are listed below:

hDkk-3

peptide #44 FREVEELMEDTQHKL

peptide #46 GSFMEEVQRQELEDLE

hDkk-4

peptide #91 HAEGTTGHPVQENQP

- 140 -

hDkk-1

peptide #93 GNKYQTIDNYQPYPYPC

hDkk-2

peptide #56 GHYSNHDLGWQNLGR

5 hSoggy-1

peptide #58 LQAIRDGLRKGTHKD

Peptides were designed to meet at least the following criteria: (1) not included within the cysteine-rich domain; (2) not including an N-glycosylation site; and (3)

10 hydrophilic (*e.g.*, solvent exposed).

Antibodies were generated in New Zealand white rabbits over a 10-week period. The immunogen includes KLH-peptide emulsified by mixing with an equal volume of Freund's Adjuvant, and injected into three subcutaneous dorsal sites, for a total of 0.1mg peptide per immunization. Animals were bled from the articular artery.

15 The blood was allowed to clot and the serum collected by centrifugation. The serum is stored at -20°C.

For purification, peptide antigens were immobilized on an activated support. Antisera was passed through the sera column and then washed. Specific antibodies were eluted *via* a pH gradient, collected and stored in a borate buffer (0.125M total

20 borate) at ~0.25mg/ml. The anti-peptide titers were determined using ELISA methodology with free peptide bound in solid phase (1µg/well). Detection was obtained using biotinylated anti-rabbit IgG, HRP-SA conjugate, and ABTS.

All antibodies performed well in ELISA assays. Anti-peptide #44, #46, and #58 are particularly useful for detection of hDkk-3 and hSoggy-1, respectively, as

25 determined by western blotting of supernatants from hDkk-3- and hSoggy-1-transfected 293T cells.

The Dkk family comprises a novel family of secreted proteins which to date

30 includes hDkk-1, hDkk-2, hDkk-3, hDkk-4, xDkk-1, mDkk-1 and cDkk-1.

Structurally, Dkks 1-4 are related by several conserved features. Firstly, all four

- 141 -

proteins are secreted proteins. Secondly, Dkks 1-4 each possess two distinct cysteine rich domains. Each domain contains 10 conserved cysteine residues, and these domains are highly conserved between family members. The C-terminal cysteine rich domain, referred to as CRD-2, bears significant similarity to proteins of the colipase family and sequence conservation among the Dkks is greatest within CRD-2 (Aravind and Koonin, *supra*). This may reflect a need for Dkks to interact with lipids in order to regulate Wnt function, since Wnt proteins remain tightly associated with the cell surface.

Despite the similarities between Dkks 1-4, notable differences between these family members appear with regard to their mRNA expression patterns. In adult human tissues hDkk-1 and hDkk-4 showed highly restricted mRNA expression patterns while hDkk-2 and hDkk-3 are more widely expressed. Murine Dkk-3 mRNA was found to be restricted to the myocytes of the atria in the heart, neurons of the cortex and hippocampus in the brain and also to the retinal neurons and lens epithelium in the eye. Such specific expression patterns reflect localized action of the Dkks as regulators of Wnt activity and/or that of other signaling molecules. Different Wnt family members have been shown to have divergent patterns of mRNA expression in adult and embryonic mammalian tissues. For example, murine Wnts 4, 7a and 7b are expressed in brain and lung, whereas Wnt6 is highly expressed in testis (Gavin *et al.*, 1990). Wnts 5b and 13 are more broadly expressed (Gavin *et al.* (1990) *supra*; Katoh *et al.* (1996) *supra*). Although Wnts have been studied mostly in the context of their roles in embryonic development and tumorigenesis, the expression of many family members in normal adult human and mouse tissues, together with their regulators such as the Dkks, suggests that these signaling proteins play important roles in normal tissue homeostasis.

Marked differences in the post-translational processing of different human Dkk proteins was also observed. hDkk-3 is secreted from 293T cells as a heterogeneously glycosylated protein, whereas Dkk-1, 2 and 4 proteins show no evidence of glycosylation. This is consistent with sequence analysis that identifies 4 potential sites of N-glycosylation in the hDkk-3 protein but no sites in either hDkk-2 or hDkk-4. A single putative site in hDkk-1 does not appear to be utilized in 293T cells and may

- 142 -

well not be a significant site for N-linked carbohydrate addition in view of its C-terminal location and lack of conservation with xDkk-1. C-terminal proteolysis of hDkk4 in 293T cells was also characterized. Dkk proteins contain multiple potential sites of proteolytic processing. Secreted hDkk-4 was consistently detected as three major C-terminal fragments. N-terminal sequencing identified two of these as mature, full length hDkk4 and CRD-2, which was derived from the full length protein by a specific proteolytic event at lysines 132 and 133. Thus, the hDkk-4 CRD-2 is released from the full length protein upon expression in 293T cells. Similar processing of hDkk4 in COS cells has been observed.

Within the Dkk family, Dkks 1, 2 and 4 display closest similarity whereas Dkk-3 is more distantly related. For example, Dkk-3 contains a linker region connecting CRD-1 and CRD-2 which is shorter than in other Dkks. Dkk-3 also possesses extended N- and C-terminal regions compared to other Dkks. Within the Dkk-3 N-terminal unique region, a distinct domain has been identified that is also found in Soggy (the SGY domain). The SGY domains of human and mouse Soggy-1 and Dkk-3 proteins contain two conserved sites of N-linked glycosylation. Protein expression studies confirm that, like hDkk3, hSoggy is secreted as a glycoprotein. Murine Soggy-1 is highly expressed in adult testis and also displays a highly restricted mRNA expression in E15 -E16 mouse embryos, being localized mainly to the cartilage primordia within the developing vertebrae/developing dorsal root ganglia. Soggy mRNA was also detected at high levels in the developing eye, similar to Dkk-3. This developmentally regulated pattern of Soggy expression overlaps with that of Dkk-3 suggesting that Soggy may play a role in the regulation of Dkk-3 function.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

<110> MILLENNIUM PHARMACEUTICALS, INC.

<120> HUMAN DICKKOPF-RELATED PROTEIN AND NUCLEIC ACID
MOLECULES AND USES THEREFOR

<130> 08-892661CA

<140>

<141> 2000-03-03

<150> 09/263,022

<151> 1999-03-05

<160> 38

<170> PatentIn Ver. 2.0

<210> 1

<211> 2479

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (38)..(1087)

<220>

<223> 'n' at position 1146 may be any nucleotide

<400> 1

```

ggcacgaggg ggcggcggct gcgggcgcag agcggag atg cag cgg ctt ggg gcc 55
                                   Met Gln Arg Leu Gly Ala
                                   1           5

acc ctg ctg tgc ctg ctg ctg gcg gcg gcg gtc ccc acg gcc ccc gcg 103
Thr Leu Leu Cys Leu Leu Leu Ala Ala Ala Val Pro Thr Ala Pro Ala
           10           15           20

ccc gct ccg acg gcg acc tcg gct cca gtc aag ccc ggc ccg gct ctc 151
Pro Ala Pro Thr Ala Thr Ser Ala Pro Val Lys Pro Gly Pro Ala Leu
           25           30           35

agc tac ccg cag gag gag gcc acc ctc aat gag atg ttc cgc gag gtt 199
Ser Tyr Pro Gln Glu Glu Ala Thr Leu Asn Glu Met Phe Arg Glu Val
           40           45           50

gag gaa ctg atg gag gac acg cag cac aaa ttg cgc agc gcg gtg gaa 247
Glu Glu Leu Met Glu Asp Thr Gln His Lys Leu Arg Ser Ala Val Glu
           55           60           65           70

gag atg gag gca gaa gaa gct gct gct aaa gca tca tca gaa gtg aac 295
Glu Met Glu Ala Glu Glu Ala Ala Ala Lys Ala Ser Ser Glu Val Asn
           75           80           85

ctg gca aac tta cct ccc agc tat cac aat gag acc aac aca gac acg 343
Leu Ala Asn Leu Pro Pro Ser Tyr His Asn Glu Thr Asn Thr Asp Thr
           90           95           100

aac gtt gga aat aat acc atc cat gtg cac cga gaa att cac aag ata 391

```

- 2 -

Asn	Val	Gly	Asn	Asn	Thr	Ile	His	Val	His	Arg	Glu	Ile	His	Lys	Ile		
		105					110					115					
acc	aac	aac	cag	act	gga	caa	atg	gtc	ttt	tca	gag	aca	gtt	atc	aca	439	
Thr	Asn	Asn	Gln	Thr	Gly	Gln	Met	Val	Phe	Ser	Glu	Thr	Val	Ile	Thr		
	120					125					130						
tct	gtg	gga	gac	gaa	gaa	ggc	aga	agg	agc	cac	gag	tgc	atc	atc	gac	487	
Ser	Val	Gly	Asp	Glu	Glu	Gly	Arg	Arg	Ser	His	Glu	Cys	Ile	Ile	Asp		
135					140					145					150		
gag	gac	tgt	ggg	ccc	agc	atg	tac	tgc	cag	ttt	gcc	agc	ttc	cag	tac	535	
Glu	Asp	Cys	Gly	Pro	Ser	Met	Tyr	Cys	Gln	Phe	Ala	Ser	Phe	Gln	Tyr		
				155					160					165			
acc	tgc	cag	cca	tgc	cgg	ggc	cag	agg	atg	ctc	tgc	acc	cgg	gac	agt	583	
Thr	Cys	Gln	Pro	Cys	Arg	Gly	Gln	Arg	Met	Leu	Cys	Thr	Arg	Asp	Ser		
			170					175					180				
gag	tgc	tgt	gga	gac	cag	ctg	tgt	gtc	tgg	ggc	cac	tgc	acc	aaa	atg	631	
Glu	Cys	Cys	Gly	Asp	Gln	Leu	Cys	Val	Trp	Gly	His	Cys	Thr	Lys	Met		
		185					190					195					
gcc	acc	agg	ggc	agc	aat	ggg	acc	atc	tgt	gac	aac	cag	agg	gac	tgc	679	
Ala	Thr	Arg	Gly	Ser	Asn	Gly	Thr	Ile	Cys	Asp	Asn	Gln	Arg	Asp	Cys		
	200					205					210						
cag	ccg	ggg	ctg	tgc	tgt	gcc	ttc	cag	aga	ggc	ctg	ctg	ttc	cct	gtg	727	
Gln	Pro	Gly	Leu	Cys	Cys	Ala	Phe	Gln	Arg	Gly	Leu	Leu	Phe	Pro	Val		
215					220					225					230		
tgc	aca	ccc	ctg	ccc	gtg	gag	ggc	gag	ctt	tgc	cat	gac	ccc	gcc	agc	775	
Cys	Thr	Pro	Leu	Pro	Val	Glu	Gly	Glu	Leu	Cys	His	Asp	Pro	Ala	Ser		
				235					240					245			
cgg	ctt	ctg	gac	ctc	atc	acc	tgg	gag	cta	gag	cct	gat	gga	gcc	ttg	823	
Arg	Leu	Leu	Asp	Leu	Ile	Thr	Trp	Glu	Leu	Glu	Pro	Asp	Gly	Ala	Leu		
			250					255					260				
gac	cga	tgc	cct	tgt	gcc	agt	ggc	ctc	ctc	tgc	cag	ccc	cac	agc	cac	871	
Asp	Arg	Cys	Pro	Cys	Ala	Ser	Gly	Leu	Leu	Cys	Gln	Pro	His	Ser	His		
		265					270					275					
agc	ctg	gtg	tat	gtg	tgc	aag	ccg	acc	ttc	gtg	ggg	agc	cgt	gac	caa	919	
Ser	Leu	Val	Tyr	Val	Cys	Lys	Pro	Thr	Phe	Val	Gly	Ser	Arg	Asp	Gln		
	280					285					290						
gat	ggg	gag	atc	ctg	ctg	ccc	aga	gag	gtc	ccc	gat	gag	tat	gaa	gtt	967	
Asp	Gly	Glu	Ile	Leu	Leu	Pro	Arg	Glu	Val	Pro	Asp	Glu	Tyr	Glu	Val		
295					300					305					310		
ggc	agc	ttc	atg	gag	gag	gtg	cgc	cag	gag	ctg	gag	gac	ctg	gag	agg	1015	
Gly	Ser	Phe	Met	Glu	Glu	Val	Arg	Gln	Glu	Leu	Glu	Asp	Leu	Glu	Arg		
				315					320					325			
agc	ctg	act	gaa	gag	atg	gcg	ctg	agg	gag	cct	gcg	gct	gcc	gcc	gct	1063	
Ser	Leu	Thr	Glu	Glu	Met	Ala	Leu	Arg	Glu	Pro	Ala	Ala	Ala	Ala	Ala		
			330					335					340				

- 3 -

gca ctg ctg gga agg gaa gag att tagatctgga ccaggctgtg ggtagatgtg 1117
Ala Leu Leu Gly Arg Glu Glu Ile
345 350

caatagaaat	agctaattta	tttccccang	tgtgtgcttt	aagcgtgggc	tgaccagggt	1177
tcttcctaca	tcttcttccc	agtaagtttc	ccctctgggt	tgacagcatg	aggtgttgtg	1237
catttgttca	gctccccag	gctgttctcc	aggcttcaca	gtctgggtgct	tgggagagtc	1297
aggcagggtt	aaactgcagg	agcagtttgc	caccctgtgc	cagattattg	gctgctttgc	1357
ctctaccagt	tggcagacag	ccgtttgttc	tacatggctt	tgataattgt	ttgaggggag	1417
gagatggaaa	caatgtggag	tctccctctg	attggttttg	gggaaatgtg	gagaagagtg	1477
ccctgctttg	caaacatcaa	cctggcaaaa	atgcaacaaa	tgaattttcc	acgcagttct	1537
ttccatgggc	ataggtaagc	tgtgccttca	gctgttgcag	atgaaatggt	ctgttcaccc	1597
tgcattacat	gtgtttattc	atccagcagt	gttgctcagc	tcctacctct	gtgccagggc	1657
agcattttca	tatccaagat	caattccctc	tctcagcaca	gcctggggag	ggggtcattg	1717
ttctcctcgt	ccatcaggga	tttcagaggc	tcagagactg	caagctgctt	gcccgaagtca	1777
cacagctagt	gaagaccaga	gcagtttcat	ctggttgtga	ctctaagctc	agtgctctct	1837
ccactacccc	acaccagcct	tggtgccacc	aaaagtgctc	ccaaaaggga	aggagaatgg	1897
gatttttctt	ttgaggcatg	cacatctgga	attaagggtca	aactaattct	cacatccctc	1957
taaaagtaaa	ctactgttag	gaacagcagt	gttctcacag	tgtggggcag	ccgtccttct	2017
aatgaagaca	atgatattga	cactgtccct	ctttggcagt	tgcattagta	actttgaaag	2077
gtatatgact	gagcgtagca	tacaggttaa	cctgcagaaa	cagtacttag	gtaattgtag	2137
ggcgaggatt	ataaatgaaa	tttgcaaaat	cacttagcag	caactgaaga	caattatcaa	2197
ccacgtggag	aaaatcaaac	cgagcagggc	tgtgtgaaac	atggttgtaa	tatgcgactg	2257
cgaacactga	actctacgcc	actccacaaa	tgatgttttc	aggtgtcatg	gactgttgcc	2317
accatgtatt	catccagagt	tcttaaagtt	taaagttgca	catgattgta	taagcatgct	2377
ttctttgagt	tttaaattat	gtataaacat	aagttgcatt	tagaaatcaa	gcataaatca	2437
cttcaactgc	taaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aa		2479

```
<210> 2
<211> 350
<212> PRT
<213> Homo sapiens
```

```
<400> 2
Met  Gln  Arg  Leu  Gly  Ala  Thr  Leu  Leu  Cys  Leu  Leu  Leu  Ala  Ala  Ala
   1              5              10              15
```

- 4 -

Val	Pro	Thr	Ala	Pro	Ala	Pro	Ala	Pro	Thr	Ala	Thr	Ser	Ala	Pro	Val
			20					25					30		
Lys	Pro	Gly	Pro	Ala	Leu	Ser	Tyr	Pro	Gln	Glu	Glu	Ala	Thr	Leu	Asn
		35					40					45			
Glu	Met	Phe	Arg	Glu	Val	Glu	Glu	Leu	Met	Glu	Asp	Thr	Gln	His	Lys
	50					55					60				
Leu	Arg	Ser	Ala	Val	Glu	Glu	Met	Glu	Ala	Glu	Glu	Ala	Ala	Ala	Lys
65					70					75					80
Ala	Ser	Ser	Glu	Val	Asn	Leu	Ala	Asn	Leu	Pro	Pro	Ser	Tyr	His	Asn
				85					90					95	
Glu	Thr	Asn	Thr	Asp	Thr	Asn	Val	Gly	Asn	Asn	Thr	Ile	His	Val	His
			100					105					110		
Arg	Glu	Ile	His	Lys	Ile	Thr	Asn	Asn	Gln	Thr	Gly	Gln	Met	Val	Phe
		115					120					125			
Ser	Glu	Thr	Val	Ile	Thr	Ser	Val	Gly	Asp	Glu	Glu	Gly	Arg	Arg	Ser
	130					135					140				
His	Glu	Cys	Ile	Ile	Asp	Glu	Asp	Cys	Gly	Pro	Ser	Met	Tyr	Cys	Gln
145					150					155					160
Phe	Ala	Ser	Phe	Gln	Tyr	Thr	Cys	Gln	Pro	Cys	Arg	Gly	Gln	Arg	Met
				165					170					175	
Leu	Cys	Thr	Arg	Asp	Ser	Glu	Cys	Cys	Gly	Asp	Gln	Leu	Cys	Val	Trp
			180					185					190		
Gly	His	Cys	Thr	Lys	Met	Ala	Thr	Arg	Gly	Ser	Asn	Gly	Thr	Ile	Cys
		195					200					205			
Asp	Asn	Gln	Arg	Asp	Cys	Gln	Pro	Gly	Leu	Cys	Cys	Ala	Phe	Gln	Arg
	210					215					220				
Gly	Leu	Leu	Phe	Pro	Val	Cys	Thr	Pro	Leu	Pro	Val	Glu	Gly	Glu	Leu
225					230					235					240
Cys	His	Asp	Pro	Ala	Ser	Arg	Leu	Leu	Asp	Leu	Ile	Thr	Trp	Glu	Leu
				245					250					255	
Glu	Pro	Asp	Gly	Ala	Leu	Asp	Arg	Cys	Pro	Cys	Ala	Ser	Gly	Leu	Leu
			260					265					270		
Cys	Gln	Pro	His	Ser	His	Ser	Leu	Val	Tyr	Val	Cys	Lys	Pro	Thr	Phe
		275					280					285			
Val	Gly	Ser	Arg	Asp	Gln	Asp	Gly	Glu	Ile	Leu	Leu	Pro	Arg	Glu	Val
	290					295					300				
Pro	Asp	Glu	Tyr	Glu	Val	Gly	Ser	Phe	Met	Glu	Glu	Val	Arg	Gln	Glu
305					310					315					320
Leu	Glu	Asp	Leu	Glu	Arg	Ser	Leu	Thr	Glu	Glu	Met	Ala	Leu	Arg	Glu

- 5 -

	325		330		335	
Pro	Ala	Ala	Ala	Ala	Ala	Ala
	340		345			Ile
						350

<210> 3
 <211> 1050
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(1050)

<400> 3

atg	cag	cgg	ctt	ggg	gcc	acc	ctg	ctg	tgc	ctg	ctg	ctg	gcg	gcg	gcg	48
Met	Gln	Arg	Leu	Gly	Ala	Thr	Leu	Leu	Cys	Leu	Leu	Leu	Ala	Ala	Ala	
1				5					10					15		

gtc	ccc	acg	gcc	ccc	gcg	ccc	gct	ccg	acg	gcg	acc	tgc	gct	cca	gtc	96
Val	Pro	Thr	Ala	Pro	Ala	Pro	Ala	Pro	Thr	Ala	Thr	Ser	Ala	Pro	Val	
			20					25					30			

aag	ccc	ggc	ccg	gct	ctc	agc	tac	ccg	cag	gag	gag	gcc	acc	ctc	aat	144
Lys	Pro	Gly	Pro	Ala	Leu	Ser	Tyr	Pro	Gln	Glu	Glu	Ala	Thr	Leu	Asn	
		35					40					45				

gag	atg	ttc	cgc	gag	gtt	gag	gaa	ctg	atg	gag	gac	acg	cag	cac	aaa	192
Glu	Met	Phe	Arg	Glu	Val	Glu	Glu	Leu	Met	Glu	Asp	Thr	Gln	His	Lys	
	50					55					60					

ttg	cgc	agc	gcg	gtg	gaa	gag	atg	gag	gca	gaa	gaa	gct	gct	gct	aaa	240
Leu	Arg	Ser	Ala	Val	Glu	Glu	Met	Glu	Ala	Glu	Glu	Ala	Ala	Ala	Lys	
65					70					75					80	

gca	tca	tca	gaa	gtg	aac	ctg	gca	aac	tta	cct	ccc	agc	tat	cac	aat	288
Ala	Ser	Ser	Glu	Val	Asn	Leu	Ala	Asn	Leu	Pro	Pro	Ser	Tyr	His	Asn	
				85					90					95		

gag	acc	aac	aca	gac	acg	aac	gtt	gga	aat	aat	acc	atc	cat	gtg	cac	336
Glu	Thr	Asn	Thr	Asp	Thr	Asn	Val	Gly	Asn	Asn	Thr	Ile	His	Val	His	
			100					105					110			

cga	gaa	att	cac	aag	ata	acc	aac	aac	cag	act	gga	caa	atg	gtc	ttt	384
Arg	Glu	Ile	His	Lys	Ile	Thr	Asn	Asn	Gln	Thr	Gly	Gln	Met	Val	Phe	
		115					120					125				

tca	gag	aca	gtt	atc	aca	tct	gtg	gga	gac	gaa	gaa	ggc	aga	agg	agc	432
Ser	Glu	Thr	Val	Ile	Thr	Ser	Val	Gly	Asp	Glu	Glu	Gly	Arg	Arg	Ser	
	130					135						140				

cac	gag	tgc	atc	atc	gac	gag	gac	tgt	ggg	ccc	agc	atg	tac	tgc	cag	480
His	Glu	Cys	Ile	Ile	Asp	Glu	Asp	Cys	Gly	Pro	Ser	Met	Tyr	Cys	Gln	
145					150					155					160	

ttt	gcc	agc	ttc	cag	tac	acc	tgc	cag	cca	tgc	cgg	ggc	cag	agg	atg	528
Phe	Ala	Ser	Phe	Gln	Tyr	Thr	Cys	Gln	Pro	Cys	Arg	Gly	Gln	Arg	Met	
				165					170					175		

- 6 -

```

ctc tgc acc cgg gac agt gag tgc tgt gga gac cag ctg tgt gtc tgg 576
Leu Cys Thr Arg Asp Ser Glu Cys Cys Gly Asp Gln Leu Cys Val Trp
      180                      185                      190

ggt cac tgc acc aaa atg gcc acc agg ggc agc aat ggg acc atc tgt 624
Gly His Cys Thr Lys Met Ala Thr Arg Gly Ser Asn Gly Thr Ile Cys
      195                      200                      205

gac aac cag agg gac tgc cag ccg ggg ctg tgc tgt gcc ttc cag aga 672
Asp Asn Gln Arg Asp Cys Gln Pro Gly Leu Cys Cys Ala Phe Gln Arg
      210                      215                      220

ggc ctg ctg ttc cct gtg tgc aca ccc ctg ccc gtg gag ggc gag ctt 720
Gly Leu Leu Phe Pro Val Cys Thr Pro Leu Pro Val Glu Gly Glu Leu
      225                      230                      235                      240

tgc cat gac ccc gcc agc cgg ctt ctg gac ctc atc acc tgg gag cta 768
Cys His Asp Pro Ala Ser Arg Leu Leu Asp Leu Ile Thr Trp Glu Leu
      245                      250                      255

gag cct gat gga gcc ttg gac cga tgc cct tgt gcc agt ggc ctc ctc 816
Glu Pro Asp Gly Ala Leu Asp Arg Cys Pro Cys Ala Ser Gly Leu Leu
      260                      265                      270

tgc cag ccc cac agc cac agc ctg gtg tat gtg tgc aag ccg acc ttc 864
Cys Gln Pro His Ser His Ser Leu Val Tyr Val Cys Lys Pro Thr Phe
      275                      280                      285

gtg ggg agc cgt gac caa gat ggg gag atc ctg ctg ccc aga gag gtc 912
Val Gly Ser Arg Asp Gln Asp Gly Glu Ile Leu Leu Pro Arg Glu Val
      290                      295                      300

ccc gat gag tat gaa gtt ggc agc ttc atg gag gag gtg cgc cag gag 960
Pro Asp Glu Tyr Glu Val Gly Ser Phe Met Glu Glu Val Arg Gln Glu
      305                      310                      315                      320

ctg gag gac ctg gag agg agc ctg act gaa gag atg gcg ctg agg gag 1008
Leu Glu Asp Leu Glu Arg Ser Leu Thr Glu Glu Met Ala Leu Arg Glu
      325                      330                      335

cct gcg gct gcc gcc gct gca ctg ctg gga agg gaa gag att 1050
Pro Ala Ala Ala Ala Ala Ala Leu Leu Gly Arg Glu Glu Ile
      340                      345                      350

```

<210> 4
 <211> 848
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (125)..(796)

<400> 4
 gaattcggca cgagagacga cgtgctgagc tgccagctta gtggaagctc tgctctgggt 60
 ggagagcagc ctcgcttttg tgacgcacag tgctgggacc ctccaggagc cccgggattg 120

- 7 -

aagg atg gtg gcg gcc gtc ctg ctg ggg ctg agc tgg ctc tgc tct ccc	169
Met Val Ala Ala Val Leu Leu Gly Leu Ser Trp Leu Cys Ser Pro	
1 5 10 15	
ctg gga gct ctg gtc ctg gac ttc aac aac atc agg agc tct gct gac	217
Leu Gly Ala Leu Val Leu Asp Phe Asn Asn Ile Arg Ser Ser Ala Asp	
20 25 30	
ctg cat ggg gcc cgg aag ggc tca cag tgc ctg tct gac acg gac tgc	265
Leu His Gly Ala Arg Lys Gly Ser Gln Cys Leu Ser Asp Thr Asp Cys	
35 40 45	
aat acc aga aag ttc tgc ctc cag ccc cgc gat gag aag ccg ttc tgt	313
Asn Thr Arg Lys Phe Cys Leu Gln Pro Arg Asp Glu Lys Pro Phe Cys	
50 55 60	
gct aca tgt cgt ggg ttg cgg agg agg tgc cag cga gat gcc atg tgc	361
Ala Thr Cys Arg Gly Leu Arg Arg Arg Cys Gln Arg Asp Ala Met Cys	
65 70 75	
tgc cct ggg aca ctc tgt gtg aac gat gtt tgt act acg atg gaa gat	409
Cys Pro Gly Thr Leu Cys Val Asn Asp Val Cys Thr Thr Met Glu Asp	
80 85 90 95	
gca acc cca ata tta gaa agg cag ctt gat gag caa gat ggc aca cat	457
Ala Thr Pro Ile Leu Glu Arg Gln Leu Asp Glu Gln Asp Gly Thr His	
100 105 110	
gca gaa gga aca act ggg cac cca gtc cag gaa aac caa ccc aaa agg	505
Ala Glu Gly Thr Thr Gly His Pro Val Gln Glu Asn Gln Pro Lys Arg	
115 120 125	
aag cca agt att aag aaa tca caa ggc agg aag gga caa gag gga gaa	553
Lys Pro Ser Ile Lys Lys Ser Gln Gly Arg Lys Gly Gln Glu Gly Glu	
130 135 140	
agt tgt ctg aga act ttt gac tgt ggc cct gga ctt tgc tgt gct cgt	601
Ser Cys Leu Arg Thr Phe Asp Cys Gly Pro Gly Leu Cys Cys Ala Arg	
145 150 155	
cat ttt tgg acg aaa att tgt aag cca gtc ctt ttg gag gga cag gtc	649
His Phe Trp Thr Lys Ile Cys Lys Pro Val Leu Leu Glu Gly Gln Val	
160 165 170 175	
tgc tcc aga aga ggg cat aaa gac act gct caa gct cca gaa atc ttc	697
Cys Ser Arg Arg Gly His Lys Asp Thr Ala Gln Ala Pro Glu Ile Phe	
180 185 190	
cag cgt tgc gac tgt ggc cct gga cta ctg tgt cga agc caa ttg acc	745
Gln Arg Cys Asp Cys Gly Pro Gly Leu Leu Cys Arg Ser Gln Leu Thr	
195 200 205	
agc aat cgg cag cat gct cga tta aga gta tgc caa aaa ata gaa aag	793
Ser Asn Arg Gln His Ala Arg Leu Arg Val Cys Gln Lys Ile Glu Lys	
210 215 220	
cta taaatatttc aaaataaaga agaatccaca ttgcaaaaaa aaaaaaaaaa aa	848
Leu	

- 8 -

<210> 5
 <211> 224
 <212> PRT
 <213> Homo sapiens

<400> 5

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

<210> 6
 <211> 672
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(672)

- 9 -

<400> 6

atg	gtg	gcg	gcc	gtc	ctg	ctg	ggg	ctg	agc	tgg	ctc	tgc	tct	ccc	ctg	48
Met	Val	Ala	Ala	Val	Leu	Leu	Gly	Leu	Ser	Trp	Leu	Cys	Ser	Pro	Leu	
1				5					10					15		
gga	gct	ctg	gtc	ctg	gac	ttc	aac	aac	atc	agg	agc	tct	gct	gac	ctg	96
Gly	Ala	Leu	Val	Leu	Asp	Phe	Asn	Asn	Ile	Arg	Ser	Ser	Ala	Asp	Leu	
			20					25					30			
cat	ggg	gcc	cgg	aag	ggc	tca	cag	tgc	ctg	tct	gac	acg	gac	tgc	aat	144
His	Gly	Ala	Arg	Lys	Gly	Ser	Gln	Cys	Leu	Ser	Asp	Thr	Asp	Cys	Asn	
		35					40					45				
acc	aga	aag	ttc	tgc	ctc	cag	ccc	cgc	gat	gag	aag	ccg	ttc	tgt	gct	192
Thr	Arg	Lys	Phe	Cys	Leu	Gln	Pro	Arg	Asp	Glu	Lys	Pro	Phe	Cys	Ala	
	50					55					60					
aca	tgt	cgt	ggg	ttg	cgg	agg	agg	tgc	cag	cga	gat	gcc	atg	tgc	tgc	240
Thr	Cys	Arg	Gly	Leu	Arg	Arg	Arg	Cys	Gln	Arg	Asp	Ala	Met	Cys	Cys	
65					70				75						80	
cct	ggg	aca	ctc	tgt	gtg	aac	gat	gtt	tgt	act	acg	atg	gaa	gat	gca	288
Pro	Gly	Thr	Leu	Cys	Val	Asn	Asp	Val	Cys	Thr	Thr	Met	Glu	Asp	Ala	
				85					90					95		
acc	cca	ata	tta	gaa	agg	cag	ctt	gat	gag	caa	gat	ggc	aca	cat	gca	336
Thr	Pro	Ile	Leu	Glu	Arg	Gln	Leu	Asp	Glu	Gln	Asp	Gly	Thr	His	Ala	
			100					105					110			
gaa	gga	aca	act	ggg	cac	cca	gtc	cag	gaa	aac	caa	ccc	aaa	agg	aag	384
Glu	Gly	Thr	Thr	Gly	His	Pro	Val	Gln	Glu	Asn	Gln	Pro	Lys	Arg	Lys	
		115					120					125				
cca	agt	att	aag	aaa	tca	caa	ggc	agg	aag	gga	caa	gag	gga	gaa	agt	432
Pro	Ser	Ile	Lys	Lys	Ser	Gln	Gly	Arg	Lys	Gly	Gln	Glu	Gly	Glu	Ser	
	130					135					140					
tgt	ctg	aga	act	ttt	gac	tgt	ggc	cct	gga	ctt	tgc	tgt	gct	cgt	cat	480
Cys	Leu	Arg	Thr	Phe	Asp	Cys	Gly	Pro	Gly	Leu	Cys	Cys	Ala	Arg	His	
145					150					155					160	
ttt	tgg	acg	aaa	att	tgt	aag	cca	gtc	ctt	ttg	gag	gga	cag	gtc	tgc	528
Phe	Trp	Thr	Lys	Ile	Cys	Lys	Pro	Val	Leu	Leu	Glu	Gly	Gln	Val	Cys	
				165					170					175		
tcc	aga	aga	ggg	cat	aaa	gac	act	gct	caa	gct	cca	gaa	atc	ttc	cag	576
Ser	Arg	Arg	Gly	His	Lys	Asp	Thr	Ala	Gln	Ala	Pro	Glu	Ile	Phe	Gln	
			180					185					190			
cgt	tgc	gac	tgt	ggc	cct	gga	cta	ctg	tgt	cga	agc	caa	ttg	acc	agc	624
Arg	Cys	Asp	Cys	Gly	Pro	Gly	Leu	Leu	Cys	Arg	Ser	Gln	Leu	Thr	Ser	
		195					200					205				
aat	cgg	cag	cat	gct	cga	tta	aga	gta	tgc	caa	aaa	ata	gaa	aag	cta	672
Asn	Arg	Gln	His	Ala	Arg	Leu	Arg	Val	Cys	Gln	Lys	Ile	Glu	Lys	Leu	
	210					215					220					

- 10 -

<210> 7
 <211> 1536
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (109)..(906)

<400> 7
 gtcgacccac gcgtccgcgg acgcgtgggc ggcacggttt cgtggggacc caggcttgca 60
 aagtgacggt cattttctct ttctttctcc ctcttgagtc cttctgag atg atg gct 117
 Met Met Ala
 1
 ctg ggc gca gcg gga gct acc cgg gtc ttt gtc gcg atg gta gcg gcg 165
 Leu Gly Ala Ala Gly Ala Thr Arg Val Phe Val Ala Met Val Ala Ala
 5 10 15
 gct ctc ggc ggc cac cct ctg ctg gga gtg agc gcc acc ttg aac tcg 213
 Ala Leu Gly Gly His Pro Leu Leu Gly Val Ser Ala Thr Leu Asn Ser
 20 25 30 35
 gtt ctc aat tcc aac gct atc aag aac ctg ccc cca ccg ctg ggc ggc 261
 Val Leu Asn Ser Asn Ala Ile Lys Asn Leu Pro Pro Pro Leu Gly Gly
 40 45 50
 gct gcg ggg cac cca ggc tct gca gtc agc gcc gcg ccg gga atc ctg 309
 Ala Ala Gly His Pro Gly Ser Ala Val Ser Ala Ala Pro Gly Ile Leu
 55 60 65
 tac ccg ggc ggc aat aag tac cag acc att gac aac tac cag ccg tac 357
 Tyr Pro Gly Gly Asn Lys Tyr Gln Thr Ile Asp Asn Tyr Gln Pro Tyr
 70 75 80
 ccg tgc gca gag gac gag gag tgc ggc act gat gag tac tgc gct agt 405
 Pro Cys Ala Glu Asp Glu Glu Cys Gly Thr Asp Glu Tyr Cys Ala Ser
 85 90 95
 ccc acc cgc gga ggc gac gca ggc gtg caa atc tgt ctc gcc tgc agg 453
 Pro Thr Arg Gly Gly Asp Ala Gly Val Gln Ile Cys Leu Ala Cys Arg
 100 105 110 115
 aag cgc cga aaa cgc tgc atg cgt cac gct atg tgc tgc ccc ggc aat 501
 Lys Arg Arg Lys Arg Cys Met Arg His Ala Met Cys Cys Pro Gly Asn
 120 125 130
 tac tgc aaa aat gga ata tgt gtg tct tct gat caa aat cat ttc cga 549
 Tyr Cys Lys Asn Gly Ile Cys Val Ser Ser Asp Gln Asn His Phe Arg
 135 140 145
 gga gaa att gag gaa acc atc act gaa agc ttt ggt aat gat cat agc 597
 Gly Glu Ile Glu Glu Thr Ile Thr Glu Ser Phe Gly Asn Asp His Ser
 150 155 160
 acc ttg gat ggc tat tcc aga aga acc acc ttg tct tca aaa atg tat 645
 Thr Leu Asp Gly Tyr Ser Arg Arg Thr Thr Leu Ser Ser Lys Met Tyr
 165 170 175

- 11 -

cac acc aaa gga caa gaa ggt tct gtt tgt ctc cgg tca tca gac tgt 693
 His Thr Lys Gly Gln Glu Gly Ser Val Cys Leu Arg Ser Ser Asp Cys
 180 185 190 195

gcc tca gga ttg tgt tgt gct aga cac ttc tgg tcc aag atc tgt aaa 741
 Ala Ser Gly Leu Cys Cys Ala Arg His Phe Trp Ser Lys Ile Cys Lys
 200 205 210

cct gtc ctg aaa gaa ggt caa gtg tgt acc aag cat agg aga aaa ggc 789
 Pro Val Leu Lys Glu Gly Gln Val Cys Thr Lys His Arg Arg Lys Gly
 215 220 225

tct cat gga cta gaa ata ttc cag cgt tgt tac tgt gga gaa ggt ctg 837
 Ser His Gly Leu Glu Ile Phe Gln Arg Cys Tyr Cys Gly Glu Gly Leu
 230 235 240

tct tgc cgg ata cag aaa gat cac cat caa gcc agt aat tct tct agg 885
 Ser Cys Arg Ile Gln Lys Asp His His Gln Ala Ser Asn Ser Ser Arg
 245 250 255

ctt cac act tgt cag aga cac taaaccagct atccaaatgc agtgaactcc 936
 Leu His Thr Cys Gln Arg His
 260 265

ttttatataa tagatgctat gaaaaccttt tatgaccttc atcaactcaa tcctaaggat 996
 atacaagttc tgtgggtttca gttaagcatt ccaataacac cttccaaaaa cctggagtgt 1056
 aagagctttg tttcttttatg gaactcccct gtgattgcag taaattactg tattgtaaat 1116
 tctcagtgtg gcacttacct gttaaagcaa tgaaactttt aattattttt cttaaagggtgc 1176
 tgcactgcct atttttcctc ttgttatgta aatttttgta cacattgatt gttatcttga 1236
 ctgacaaata ttctatatattg aactgaagta aatcattttca gcttatagtt cttaaaagca 1296
 taacccttta ccccatTTaa ttctagagtc tagaacgcaa ggatctcttg gaatgacaaa 1356
 tgataggtac ctaaaatgta acatgaaaat actagcttat tttctgaaat gtactatctt 1416
 aatgcttaaa ttatatTTcc ctttaggctg tgatagtttt tgaaataaaa tttaacattt 1476
 aatatcatga aatgTTataa gtagacataa aaaaaaaaaa aaaaaaaaaa gggcggccgc 1536

<210> 8

<211> 266

<212> PRT

<213> Homo sapiens

<400> 8

Met Met Ala Leu Gly Ala Ala Gly Ala Thr Arg Val Phe Val Ala Met
 1 5 10 15

Val Ala Ala Ala Leu Gly Gly His Pro Leu Leu Gly Val Ser Ala Thr
 20 25 30

Leu Asn Ser Val Leu Asn Ser Asn Ala Ile Lys Asn Leu Pro Pro Pro

- 12 -

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

<210> 9
 <211> 798
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(798)

<400> 9
 atg atg gct ctg ggc gca gcg gga gct acc cgg gtc ttt gtc gcg atg 48
 Met Met Ala Leu Gly Ala Ala Gly Ala Thr Arg Val Phe Val Ala Met
 1 5 10 15

- 13 -

gta	gcg	gcg	gct	ctc	ggc	ggc	cac	cct	ctg	ctg	gga	gtg	agc	gcc	acc	96
Val	Ala	Ala	Ala	Leu	Gly	Gly	His	Pro	Leu	Leu	Gly	Val	Ser	Ala	Thr	
			20					25					30			
ttg	aac	tcg	gtt	ctc	aat	tcc	aac	gct	atc	aag	aac	ctg	ccc	cca	ccg	144
Leu	Asn	Ser	Val	Leu	Asn	Ser	Asn	Ala	Ile	Lys	Asn	Leu	Pro	Pro	Pro	
		35					40					45				
ctg	ggc	ggc	gct	gcg	ggg	cac	cca	ggc	tct	gca	gtc	agc	gcc	gcg	ccg	192
Leu	Gly	Gly	Ala	Ala	Gly	His	Pro	Gly	Ser	Ala	Val	Ser	Ala	Ala	Pro	
	50					55					60					
gga	atc	ctg	tac	ccg	ggc	ggg	aat	aag	tac	cag	acc	att	gac	aac	tac	240
Gly	Ile	Leu	Tyr	Pro	Gly	Gly	Asn	Lys	Tyr	Gln	Thr	Ile	Asp	Asn	Tyr	
65					70					75					80	
cag	ccg	tac	ccg	tgc	gca	gag	gac	gag	gag	tgc	ggc	act	gat	gag	tac	288
Gln	Pro	Tyr	Pro	Cys	Ala	Glu	Asp	Glu	Glu	Cys	Gly	Thr	Asp	Glu	Tyr	
				85					90					95		
tgc	gct	agt	ccc	acc	cgc	gga	ggg	gac	gca	ggc	gtg	caa	atc	tgt	ctc	336
Cys	Ala	Ser	Pro	Thr	Arg	Gly	Gly	Asp	Ala	Gly	Val	Gln	Ile	Cys	Leu	
			100					105						110		
gcc	tgc	agg	aag	cgc	cga	aaa	cgc	tgc	atg	cgt	cac	gct	atg	tgc	tgc	384
Ala	Cys	Arg	Lys	Arg	Arg	Lys	Arg	Cys	Met	Arg	His	Ala	Met	Cys	Cys	
		115					120					125				
ccc	ggg	aat	tac	tgc	aaa	aat	gga	ata	tgt	gtg	tct	tct	gat	caa	aat	432
Pro	Gly	Asn	Tyr	Cys	Lys	Asn	Gly	Ile	Cys	Val	Ser	Ser	Asp	Gln	Asn	
	130					135					140					
cat	ttc	cga	gga	gaa	att	gag	gaa	acc	atc	act	gaa	agc	ttt	ggt	aat	480
His	Phe	Arg	Gly	Glu	Ile	Glu	Glu	Thr	Ile	Thr	Glu	Ser	Phe	Gly	Asn	
145					150					155					160	
gat	cat	agc	acc	ttg	gat	ggg	tat	tcc	aga	aga	acc	acc	ttg	tct	tca	528
Asp	His	Ser	Thr	Leu	Asp	Gly	Tyr	Ser	Arg	Arg	Thr	Thr	Leu	Ser	Ser	
				165					170					175		
aaa	atg	tat	cac	acc	aaa	gga	caa	gaa	ggt	tct	gtt	tgt	ctc	cgg	tca	576
Lys	Met	Tyr	His	Thr	Lys	Gly	Gln	Glu	Gly	Ser	Val	Cys	Leu	Arg	Ser	
			180				185						190			
tca	gac	tgt	gcc	tca	gga	ttg	tgt	tgt	gct	aga	cac	ttc	tgg	tcc	aag	624
Ser	Asp	Cys	Ala	Ser	Gly	Leu	Cys	Cys	Ala	Arg	His	Phe	Trp	Ser	Lys	
		195				200						205				
atc	tgt	aaa	cct	gtc	ctg	aaa	gaa	ggt	caa	gtg	tgt	acc	aag	cat	agg	672
Ile	Cys	Lys	Pro	Val	Leu	Lys	Glu	Gly	Gln	Val	Cys	Thr	Lys	His	Arg	
	210					215					220					
aga	aaa	ggc	tct	cat	gga	cta	gaa	ata	ttc	cag	cgt	tgt	tac	tgt	gga	720
Arg	Lys	Gly	Ser	His	Gly	Leu	Glu	Ile	Phe	Gln	Arg	Cys	Tyr	Cys	Gly	
225					230					235					240	
gaa	ggt	ctg	tct	tgc	cgg	ata	cag	aaa	gat	cac	cat	caa	gcc	agt	aat	768
Glu	Gly	Leu	Ser	Cys	Arg	Ile	Gln	Lys	Asp	His	His	Gln	Ala	Ser	Asn	
				245					250					255		

- 14 -

tct tct agg ctt cac act tgt cag aga cac	798
Ser Ser Arg Leu His Thr Cys Gln Arg His	
260 265	

<210> 10
 <211> 702
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(537)

<400> 10	
gaa ttc ggc acg agg gtt ggg agg tat tgc cac agt ccc cac caa gga	48
Glu Phe Gly Thr Arg Val Gly Arg Tyr Cys His Ser Pro His Gln Gly	
1 5 10 15	
tca tcg gcc tgc atg gtg tgt cgg aga aaa aag aag cgc tgc cac cga	96
Ser Ser Ala Cys Met Val Cys Arg Arg Lys Lys Lys Arg Cys His Arg	
20 25 30	
gat ggc atg tgc tgc ccc agt acc cgc tgc aat aat ggc atc tgt atc	144
Asp Gly Met Cys Cys Pro Ser Thr Arg Cys Asn Asn Gly Ile Cys Ile	
35 40 45	
cca gtt act gaa agc atc tta acc cct cac atc ccg gct ctg gat ggt	192
Pro Val Thr Glu Ser Ile Leu Thr Pro His Ile Pro Ala Leu Asp Gly	
50 55 60	
act cgg cac aga gat cga aac cac ggt cat tac tca aac cat gac ttg	240
Thr Arg His Arg Asp Arg Asn His Gly His Tyr Ser Asn His Asp Leu	
65 70 75 80	
gga tgg cag aat cta gga aga cca cac act aag atg tca cat ata aaa	288
Gly Trp Gln Asn Leu Gly Arg Pro His Thr Lys Met Ser His Ile Lys	
85 90 95	
ggg cat gaa gga gac ccc tgc cta cga tca tca gac tgc att gaa ggg	336
Gly His Glu Gly Asp Pro Cys Leu Arg Ser Ser Asp Cys Ile Glu Gly	
100 105 110	
ttt tgc tgt gct cgt cat ttc tgg acc aaa atc tgc aaa cca gtg ctc	384
Phe Cys Cys Ala Arg His Phe Trp Thr Lys Ile Cys Lys Pro Val Leu	
115 120 125	
cat cag ggg gaa gtc tgt acc aaa caa cgc aag aag ggt tct cat ggg	432
His Gln Gly Glu Val Cys Thr Lys Gln Arg Lys Lys Gly Ser His Gly	
130 135 140	
ctg gaa att ttc cag cgt tgc gac tgt gcg aag ggc ctg tct tgc aaa	480
Leu Glu Ile Phe Gln Arg Cys Asp Cys Ala Lys Gly Leu Ser Cys Lys	
145 150 155 160	
gta tgg aaa gat gcc acc tac tcc tcc aaa gcc aga ctc cat gtg tgt	528
Val Trp Lys Asp Ala Thr Tyr Ser Ser Lys Ala Arg Leu His Val Cys	
165 170 175	

- 15 -

cag aaa att tgatcaccat tgaggaacat catcaattgc agactgtgaa 577
Gln Lys Ile

gttgtgtatt taatgcatta tagcatgggtg gaaaataagg ttcagatgca gaagaatggc 637

taaaataaga aacgtgataa gaatatagat gatcacaaaa aaaaaaaaaa aaaagatgcg 697

gccgc 702

<210> 11

<211> 179

<212> PRT

<213> Homo sapiens

<400> 11

Glu Phe Gly Thr Arg Val Gly Arg Tyr Cys His Ser Pro His Gln Gly
1 5 10 15

Ser Ser Ala Cys Met Val Cys Arg Arg Lys Lys Lys Arg Cys His Arg
20 25 30

Asp Gly Met Cys Cys Pro Ser Thr Arg Cys Asn Asn Gly Ile Cys Ile
35 40 45

Pro Val Thr Glu Ser Ile Leu Thr Pro His Ile Pro Ala Leu Asp Gly
50 55 60

Thr Arg His Arg Asp Arg Asn His Gly His Tyr Ser Asn His Asp Leu
65 70 75 80

Gly Trp Gln Asn Leu Gly Arg Pro His Thr Lys Met Ser His Ile Lys
85 90 95

Gly His Glu Gly Asp Pro Cys Leu Arg Ser Ser Asp Cys Ile Glu Gly
100 105 110

Phe Cys Cys Ala Arg His Phe Trp Thr Lys Ile Cys Lys Pro Val Leu
115 120 125

His Gln Gly Glu Val Cys Thr Lys Gln Arg Lys Lys Gly Ser His Gly
130 135 140

Leu Glu Ile Phe Gln Arg Cys Asp Cys Ala Lys Gly Leu Ser Cys Lys
145 150 155 160

Val Trp Lys Asp Ala Thr Tyr Ser Ser Lys Ala Arg Leu His Val Cys
165 170 175

Gln Lys Ile

<210> 12

<211> 537

<212> DNA

<213> Homo sapiens

- 16 -

<220>

<221> CDS

<222> (1)..(537)

<400> 12

gaa ttc ggc acg agg gtt ggg agg tat tgc cac agt ccc cac caa gga	48
Glu Phe Gly Thr Arg Val Gly Arg Tyr Cys His Ser Pro His Gln Gly	
1 5 10 15	
tca tcg gcc tgc atg gtg tgt cgg aga aaa aag aag cgc tgc cac cga	96
Ser Ser Ala Cys Met Val Cys Arg Arg Lys Lys Lys Arg Cys His Arg	
20 25 30	
gat ggc atg tgc tgc ccc agt acc cgc tgc aat aat ggc atc tgt atc	144
Asp Gly Met Cys Cys Pro Ser Thr Arg Cys Asn Asn Gly Ile Cys Ile	
35 40 45	
cca gtt act gaa agc atc tta acc cct cac atc ccg gct ctg gat ggt	192
Pro Val Thr Glu Ser Ile Leu Thr Pro His Ile Pro Ala Leu Asp Gly	
50 55 60	
act cgg cac aga gat cga aac cac ggt cat tac tca aac cat gac ttg	240
Thr Arg His Arg Asp Arg Asn His Gly His Tyr Ser Asn His Asp Leu	
65 70 75 80	
gga tgg cag aat cta gga aga cca cac act aag atg tca cat ata aaa	288
Gly Trp Gln Asn Leu Gly Arg Pro His Thr Lys Met Ser His Ile Lys	
85 90 95	
ggg cat gaa gga gac ccc tgc cta cga tca tca gac tgc att gaa ggg	336
Gly His Glu Gly Asp Pro Cys Leu Arg Ser Ser Asp Cys Ile Glu Gly	
100 105 110	
ttt tgc tgt gct cgt cat ttc tgg acc aaa atc tgc aaa cca gtg ctc	384
Phe Cys Cys Ala Arg His Phe Trp Thr Lys Ile Cys Lys Pro Val Leu	
115 120 125	
cat cag ggg gaa gtc tgt acc aaa caa cgc aag aag ggt tct cat ggg	432
His Gln Gly Glu Val Cys Thr Lys Gln Arg Lys Lys Gly Ser His Gly	
130 135 140	
ctg gaa att ttc cag cgt tgc gac tgt gcg aag ggc ctg tct tgc aaa	480
Leu Glu Ile Phe Gln Arg Cys Asp Cys Ala Lys Gly Leu Ser Cys Lys	
145 150 155 160	
gta tgg aaa gat gcc acc tac tcc tcc aaa gcc aga ctc cat gtg tgt	528
Val Trp Lys Asp Ala Thr Tyr Ser Ser Lys Ala Arg Leu His Val Cys	
165 170 175	
cag aaa att	537
Gln Lys Ile	

<210> 13

<211> 928

<212> DNA

<213> Homo sapiens

<220>

- 17 -

<221> CDS

<222> (75)..(800)

<400> 13

ctcgaggcca aaattcggca cgaggccggg ctgtggtcta gcataaaggc ggagcccaga 60

agaagggg	cg	gggt	atg	gga	gaa	gcc	tcc	cca	cct	gcc	ccc	gca	agg	cgg	110
			Met	Gly	Glu	Ala	Ser	Pro	Pro	Ala	Pro	Ala	Arg	Arg	
			1				5					10			

cat	ctg	ctg	gtc	ctg	ctg	ctg	ctc	ctc	tct	acc	ctg	gtg	atc	ccc	tcc	158
His	Leu	Leu	Val	Leu	Leu	Leu	Leu	Leu	Ser	Thr	Leu	Val	Ile	Pro	Ser	
		15					20					25				

gct	gca	gct	cct	atc	cat	gat	gct	gac	gcc	caa	gag	agc	tcc	ttg	ggc	206
Ala	Ala	Ala	Pro	Ile	His	Asp	Ala	Asp	Ala	Gln	Glu	Ser	Ser	Leu	Gly	
	30					35					40					

ctc	aca	ggc	ctc	cag	agc	cta	ctc	caa	ggc	ttc	agc	cga	ctt	ttc	ctg	254
Leu	Thr	Gly	Leu	Gln	Ser	Leu	Leu	Gln	Gly	Phe	Ser	Arg	Leu	Phe	Leu	
45					50					55					60	

aaa	ggt	aac	ctg	ctt	cgg	ggc	ata	gac	agc	tta	ttc	tct	gcc	ccc	atg	302
Lys	Gly	Asn	Leu	Leu	Arg	Gly	Ile	Asp	Ser	Leu	Phe	Ser	Ala	Pro	Met	
				65					70					75		

gac	ttc	cgg	ggc	ctc	cct	ggg	aac	tac	cac	aaa	gag	gag	aac	cag	gag	350
Asp	Phe	Arg	Gly	Leu	Pro	Gly	Asn	Tyr	His	Lys	Glu	Glu	Asn	Gln	Glu	
			80					85					90			

cac	cag	ctg	ggg	aac	aac	acc	ctc	tcc	agc	cac	ctc	cag	atc	gac	aag	398
His	Gln	Leu	Gly	Asn	Asn	Thr	Leu	Ser	Ser	His	Leu	Gln	Ile	Asp	Lys	
		95					100					105				

atg	acc	gac	aac	aag	aca	gga	gag	gtg	ctg	atc	tcc	gag	aat	gtg	gtg	446
Met	Thr	Asp	Asn	Lys	Thr	Gly	Glu	Val	Leu	Ile	Ser	Glu	Asn	Val	Val	
	110					115					120					

gca	tcc	att	caa	cca	gcg	gag	ggg	agc	ttc	gag	ggc	gat	ttg	aag	gta	494
Ala	Ser	Ile	Gln	Pro	Ala	Glu	Gly	Ser	Phe	Glu	Gly	Asp	Leu	Lys	Val	
125					130					135					140	

ccc	agg	atg	gag	gag	aag	gag	gcc	ctg	gta	ccc	atc	cag	aag	gcc	acg	542
Pro	Arg	Met	Glu	Glu	Lys	Glu	Ala	Leu	Val	Pro	Ile	Gln	Lys	Ala	Thr	
				145					150					155		

gac	agc	ttc	cac	aca	gaa	ctc	cat	ccc	cgg	gtg	gcc	ttc	tgg	atc	att	590
Asp	Ser	Phe	His	Thr	Glu	Leu	His	Pro	Arg	Val	Ala	Phe	Trp	Ile	Ile	
			160					165					170			

aag	ctg	cca	cgg	cgg	agg	tcc	cac	cag	gat	gcc	ctg	gag	ggc	ggc	cac	638
Lys	Leu	Pro	Arg	Arg	Arg	Ser	His	Gln	Asp	Ala	Leu	Glu	Gly	Gly	His	
		175					180					185				

tgg	ctc	agc	gag	aag	cga	cac	cgc	ctg	cag	gcc	atc	cgg	gat	gga	ctc	686
Trp	Leu	Ser	Glu	Lys	Arg	His	Arg	Leu	Gln	Ala	Ile	Arg	Asp	Gly	Leu	
	190					195				200						

cgc	aag	ggg	acc	cac	aag	gac	gtc	cta	gaa	gag	ggg	acc	gag	agc	tcc	734
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

- 18 -

Arg Lys Gly Thr His Lys Asp Val Leu Glu Glu Gly Thr Glu Ser Ser
 205 210 215 220
 tcc cac tcc agg ctg tcc ccc cga aag acc cac tta ctg tac atc ctc 782
 Ser His Ser Arg Leu Ser Pro Arg Lys Thr His Leu Leu Tyr Ile Leu
 225 230 235
 agg ccc tct cgg cag ctg taggggtggg gaccggggag cacctgcctg 830
 Arg Pro Ser Arg Gln Leu
 240
 tagcccccat cagaccctgc cccaagcacc atatggaaat aaagttcttt cttacatcta 890
 aaaaaaaaaa aaaaaaaaaa aaaaaaattg gcggccgc 928

<210> 14
 <211> 242
 <212> PRT
 <213> Homo sapiens

<400> 14
 Met Gly Glu Ala Ser Pro Pro Ala Pro Ala Arg Arg His Leu Leu Val
 1 5 10 15
 Leu Leu Leu Leu Leu Ser Thr Leu Val Ile Pro Ser Ala Ala Ala Pro
 20 25 30
 Ile His Asp Ala Asp Ala Gln Glu Ser Ser Leu Gly Leu Thr Gly Leu
 35 40 45
 Gln Ser Leu Leu Gln Gly Phe Ser Arg Leu Phe Leu Lys Gly Asn Leu
 50 55 60
 Leu Arg Gly Ile Asp Ser Leu Phe Ser Ala Pro Met Asp Phe Arg Gly
 65 70 75 80
 Leu Pro Gly Asn Tyr His Lys Glu Glu Asn Gln Glu His Gln Leu Gly
 85 90 95
 Asn Asn Thr Leu Ser Ser His Leu Gln Ile Asp Lys Met Thr Asp Asn
 100 105 110
 Lys Thr Gly Glu Val Leu Ile Ser Glu Asn Val Val Ala Ser Ile Gln
 115 120 125
 Pro Ala Glu Gly Ser Phe Glu Gly Asp Leu Lys Val Pro Arg Met Glu
 130 135 140
 Glu Lys Glu Ala Leu Val Pro Ile Gln Lys Ala Thr Asp Ser Phe His
 145 150 155 160
 Thr Glu Leu His Pro Arg Val Ala Phe Trp Ile Ile Lys Leu Pro Arg
 165 170 175
 Arg Arg Ser His Gln Asp Ala Leu Glu Gly Gly His Trp Leu Ser Glu
 180 185 190
 Lys Arg His Arg Leu Gln Ala Ile Arg Asp Gly Leu Arg Lys Gly Thr

- 19 -

195	200	205
His Lys Asp Val Leu Glu Glu Gly Thr Glu Ser Ser Ser His Ser Arg		
210	215	220
Leu Ser Pro Arg Lys Thr His Leu Leu Tyr Ile Leu Arg Pro Ser Arg		
225	230	235 240
Gln Leu		

<210> 15
 <211> 726
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(726)

<400> 15

atg gga gaa gcc tcc cca cct gcc ccc gca agg cgg cat ctg ctg gtc	48
Met Gly Glu Ala Ser Pro Pro Ala Pro Ala Arg Arg His Leu Leu Val	
1 5 10 15	
ctg ctg ctg ctc ctc tct acc ctg gtg atc ccc tcc gct gca gct cct	96
Leu Leu Leu Leu Leu Ser Thr Leu Val Ile Pro Ser Ala Ala Ala Pro	
20 25 30	
atc cat gat gct gac gcc caa gag agc tcc ttg ggt ctc aca ggc ctc	144
Ile His Asp Ala Asp Ala Gln Glu Ser Ser Leu Gly Leu Thr Gly Leu	
35 40 45	
cag agc cta ctc caa ggc ttc agc cga ctt ttc ctg aaa ggt aac ctg	192
Gln Ser Leu Leu Gln Gly Phe Ser Arg Leu Phe Leu Lys Gly Asn Leu	
50 55 60	
ctt cgg ggc ata gac agc tta ttc tct gcc ccc atg gac ttc cgg ggc	240
Leu Arg Gly Ile Asp Ser Leu Phe Ser Ala Pro Met Asp Phe Arg Gly	
65 70 75 80	
ctc cct ggg aac tac cac aaa gag gag aac cag gag cac cag ctg ggg	288
Leu Pro Gly Asn Tyr His Lys Glu Glu Asn Gln Glu His Gln Leu Gly	
85 90 95	
aac aac acc ctc tcc agc cac ctc cag atc gac aag atg acc gac aac	336
Asn Asn Thr Leu Ser Ser His Leu Gln Ile Asp Lys Met Thr Asp Asn	
100 105 110	
aag aca gga gag gtg ctg atc tcc gag aat gtg gtg gca tcc att caa	384
Lys Thr Gly Glu Val Leu Ile Ser Glu Asn Val Val Ala Ser Ile Gln	
115 120 125	
cca gcg gag ggg agc ttc gag ggt gat ttg aag gta ccc agg atg gag	432
Pro Ala Glu Gly Ser Phe Glu Gly Asp Leu Lys Val Pro Arg Met Glu	
130 135 140	
gag aag gag gcc ctg gta ccc atc cag aag gcc acg gac agc ttc cac	480

- 20 -

Glu	Lys	Glu	Ala	Leu	Val	Pro	Ile	Gln	Lys	Ala	Thr	Asp	Ser	Phe	His	
145					150					155					160	
aca	gaa	ctc	cat	ccc	cgg	gtg	gcc	ttc	tgg	atc	att	aag	ctg	cca	cgg	528
Thr	Glu	Leu	His	Pro	Arg	Val	Ala	Phe	Trp	Ile	Ile	Lys	Leu	Pro	Arg	
				165					170					175		
cgg	agg	tcc	cac	cag	gat	gcc	ctg	gag	ggc	ggc	cac	tgg	ctc	agc	gag	576
Arg	Arg	Ser	His	Gln	Asp	Ala	Leu	Glu	Gly	Gly	His	Trp	Leu	Ser	Glu	
			180					185					190			
aag	cga	cac	cgc	ctg	cag	gcc	atc	cgg	gat	gga	ctc	cgc	aag	ggg	acc	624
Lys	Arg	His	Arg	Leu	Gln	Ala	Ile	Arg	Asp	Gly	Leu	Arg	Lys	Gly	Thr	
		195				200						205				
cac	aag	gac	gtc	cta	gaa	gag	ggg	acc	gag	agc	tcc	tcc	cac	tcc	agg	672
His	Lys	Asp	Val	Leu	Glu	Glu	Gly	Thr	Glu	Ser	Ser	Ser	His	Ser	Arg	
	210					215					220					
ctg	tcc	ccc	cga	aag	acc	cac	tta	ctg	tac	atc	ctc	agg	ccc	tct	cgg	720
Leu	Ser	Pro	Arg	Lys	Thr	His	Leu	Leu	Tyr	Ile	Leu	Arg	Pro	Ser	Arg	
225					230					235					240	
cag	ctg															726
Gln	Leu															
<210> 16																
<211> 2380																
<212> DNA																
<213> Homo sapiens																
<220>																
<221> CDS																
<222> (109)..(1155)																
<400> 26																
gtcgacccac gcgtccgctg tggcagccca gctaccggtc gtgaccagat ccagcttgca 60																
gctcagcttt gttcattcga attgggcggc ggccagcgcg gaacaaac atg cag cgg 117																
Met Gln Arg																
1																
ctc	ggg	ggt	att	ttg	ctg	tgt	aca	ctg	ctg	gcg	gcg	gcg	gtc	ccc	act	165
Leu	Gly	Gly	Ile	Leu	Leu	Cys	Thr	Leu	Leu	Ala	Ala	Ala	Val	Pro	Thr	
	5					10				15						
gct	cct	gct	cct	tcc	ccg	acg	gtc	act	tgg	act	ccg	gcg	gag	ccg	ggc	213
Ala	Pro	Ala	Pro	Ser	Pro	Thr	Val	Thr	Trp	Thr	Pro	Ala	Glu	Pro	Gly	
	20				25				30					35		
cca	gct	ctc	aac	tac	cct	cag	gag	gaa	gct	acg	ctc	aat	gag	atg	ttt	261
Pro	Ala	Leu	Asn	Tyr	Pro	Gln	Glu	Glu	Ala	Thr	Leu	Asn	Glu	Met	Phe	
			40					45					50			
cga	gag	gtg	gag	gag	ctg	atg	gaa	gac	act	cag	cac	aaa	ctg	cgc	agt	309
Arg	Glu	Val	Glu	Glu	Leu	Met	Glu	Asp	Thr	Gln	His	Lys	Leu	Arg	Ser	
			55					60					65			

- 21 -

gcc	gtg	gag	gag	atg	gag	gcg	gaa	gaa	gca	gct	gct	aaa	acg	tcc	tct	357
Ala	Val	Glu	Glu	Met	Glu	Ala	Glu	Glu	Ala	Ala	Ala	Lys	Thr	Ser	Ser	
		70					75					80				
gag	gtg	aac	ctg	gca	agc	tta	cct	ccc	aac	tat	cac	aat	gag	acc	agc	405
Glu	Val	Asn	Leu	Ala	Ser	Leu	Pro	Pro	Asn	Tyr	His	Asn	Glu	Thr	Ser	
	85					90					95					
acg	gag	acc	agg	gtg	gga	aat	aac	aca	gtc	cat	gtg	cac	cag	gaa	gtt	453
Thr	Glu	Thr	Arg	Val	Gly	Asn	Asn	Thr	Val	His	Val	His	Gln	Glu	Val	
100					105					110					115	
cac	aag	ata	acc	aac	aac	cag	agt	gga	cag	gtg	gtc	ttt	tct	gag	aca	501
His	Lys	Ile	Thr	Asn	Asn	Gln	Ser	Gly	Gln	Val	Val	Phe	Ser	Glu	Thr	
				120					125					130		
gtc	att	aca	tct	gta	ggg	gat	gaa	gaa	ggc	aag	agg	agc	cat	gaa	tgt	549
Val	Ile	Thr	Ser	Val	Gly	Asp	Glu	Glu	Gly	Lys	Arg	Ser	His	Glu	Cys	
			135					140					145			
atc	att	gat	gaa	gac	tgt	ggg	ccc	acc	agg	tac	tgc	cag	ttc	tcc	agc	597
Ile	Ile	Asp	Glu	Asp	Cys	Gly	Pro	Thr	Arg	Tyr	Cys	Gln	Phe	Ser	Ser	
		150					155					160				
ttc	aag	tac	acc	tgc	cag	cca	tgc	cgg	gac	cag	cag	atg	cta	tgc	acc	645
Phe	Lys	Tyr	Thr	Cys	Gln	Pro	Cys	Arg	Asp	Gln	Gln	Met	Leu	Cys	Thr	
	165					170					175					
cga	gac	agt	gag	tgc	tgt	gga	gac	cag	ctg	tgt	gcc	tgg	ggc	cac	tgc	693
Arg	Asp	Ser	Glu	Cys	Cys	Gly	Asp	Gln	Leu	Cys	Ala	Trp	Gly	His	Cys	
180					185					190					195	
acc	caa	aag	gcc	acc	aaa	ggc	ggc	aat	ggg	acc	atc	tgt	gac	aac	cag	741
Thr	Gln	Lys	Ala	Thr	Lys	Gly	Gly	Asn	Gly	Thr	Ile	Cys	Asp	Asn	Gln	
				200					205					210		
agg	gat	tgc	cag	cct	ggc	ctg	tgt	tgt	gcc	ttc	caa	aga	ggc	ctg	ctg	789
Arg	Asp	Cys	Gln	Pro	Gly	Leu	Cys	Cys	Ala	Phe	Gln	Arg	Gly	Leu	Leu	
			215				220						225			
ttc	ccc	gtg	tgc	aca	ccc	ctg	ccc	gtg	gag	gga	gag	ctc	tgc	cat	gac	837
Phe	Pro	Val	Cys	Thr	Pro	Leu	Pro	Val	Glu	Gly	Glu	Leu	Cys	His	Asp	
		230					235					240				
ccc	acc	agc	cag	ctg	ctg	gat	ctc	atc	acc	tgg	gaa	ctg	gag	cct	gaa	885
Pro	Thr	Ser	Gln	Leu	Leu	Asp	Leu	Ile	Thr	Trp	Glu	Leu	Glu	Pro	Glu	
	245					250					255					
gga	gct	ttg	gac	cga	tgc	ccc	tgc	gcc	agt	ggc	ctc	cta	tgc	cag	cca	933
Gly	Ala	Leu	Asp	Arg	Cys	Pro	Cys	Ala	Ser	Gly	Leu	Leu	Cys	Gln	Pro	
260					265					270					275	
cac	agc	cac	agt	ctg	gtg	tac	atg	tgc	aag	cca	gcc	ttc	gtg	ggc	agc	981
His	Ser	His	Ser	Leu	Val	Tyr	Met	Cys	Lys	Pro	Ala	Phe	Val	Gly	Ser	
				280					285					290		
cat	gac	cac	agt	gag	gag	agc	cag	ctg	ccc	agg	gag	gcc	ccg	gat	gag	1029
His	Asp	His	Ser	Glu	Glu	Ser	Gln	Leu	Pro	Arg	Glu	Ala	Pro	Asp	Glu	
			295					300					305			

- 22 -

tac gaa gat gtt ggc ttc ata ggg gaa gtg cgc cag gag ctg gaa gac 1077
 Tyr Glu Asp Val Gly Phe Ile Gly Glu Val Arg Gln Glu Leu Glu Asp
 310 315 320

ctg gag cgg agc cta gcc cag gag atg gca ttt gag ggg cct gcc cct 1125
 Leu Glu Arg Ser Leu Ala Gln Glu Met Ala Phe Glu Gly Pro Ala Pro
 325 330 335

gtg gag tca cta ggc gga gag gag gag att taggcccaga cccagctgag 1175
 Val Glu Ser Leu Gly Gly Glu Glu Glu Ile
 340 345

tcaactggtag atgtgcaata gaaatggcta atttatatttc ccaggagtgt ccccaagtgt 1235
 ggaatggccg cagctccttc ccagtagctt ttcctctggc ttgacaaggt acagtgcagt 1295
 acatttcttc cagccgccct gcttctctga cttgggaaag acaggcatgg cgggtaaggg 1355
 cagcggtgag tcgtccctcg ctgttgctag aaacgctgtc ttgttcttca tggatggaag 1415
 atttgtttga agggagagga tgggaagggg tgaagtctgc tcatgatgga tttgggggat 1475
 acagggagga ggatgcctgc cttgcagacg tggacttggc aaaatgtaac ctttgctttt 1535
 gtcttgcgcc gctcccatgg gctgaggcag tggctacaca agagctatgc tgctctgtgg 1595
 cctcccatat attcatccct gtgtttcagc tcctacctca ctgtcagcac agcccttcat 1655
 agccacgccc cctcttgctc accacagcct aggaggggac cagaggggac ttctctcaga 1715
 gcccctatgct ctctctctca accccatacc agcctctgtg ccagcgacag tccttccaaa 1775
 tggagggagt gaaatccttt ggtttaatta ttttctcctt caaggcacgc ctgccactaa 1835
 ggtcaggctg acttgcatgt ccctctaacg ttcgtagcag tgtggtggac actgtcttcc 1895
 accgactgct tcaatacctc tgaaagccag tgctcggagt gcagttcgtg taaattaatt 1955
 tgcaggaagt atacttggct aattgtaggg ctaggattgt gaatgaaatt tgcaaagtcg 2015
 cttagcaaca atggaaagcc tttctcagtc acaccgagaa gtcacaacca agccaggttg 2075
 tgtagagtac agctgtgaca tacagacaga agaaggctgg gctggatgtc aggcctcaga 2135
 tgacggtttc aggtgccagg aactattacc attctgtatc tatccagagt tattaaaatt 2195
 gaaagttgca cacatttgta taagcatgcc tttctcctga gtttttaaatt atatgtatac 2255
 acaaacatgt ggccctcaaa gatcatgcac aaaccactac tctttgctaa ttcttggact 2315
 tttctctttg attttcaata aatacaaata cccttcatgc aaaaaaaaaa aaaaagggcg 2375
 gccgc 2380

<210> 17
 <211> 349
 <212> PRT

- 23 -

<213> Homo sapiens

<400> 17

Met	Gln	Arg	Leu	Gly	Gly	Ile	Leu	Leu	Cys	Thr	Leu	Leu	Ala	Ala	Ala	1	5	10	15
Val	Pro	Thr	Ala	Pro	Ala	Pro	Ser	Pro	Thr	Val	Thr	Trp	Thr	Pro	Ala	20	25	30	
Glu	Pro	Gly	Pro	Ala	Leu	Asn	Tyr	Pro	Gln	Glu	Glu	Ala	Thr	Leu	Asn	35	40	45	
Glu	Met	Phe	Arg	Glu	Val	Glu	Glu	Leu	Met	Glu	Asp	Thr	Gln	His	Lys	50	55	60	
Leu	Arg	Ser	Ala	Val	Glu	Glu	Met	Glu	Ala	Glu	Glu	Ala	Ala	Ala	Lys	65	70	75	80
Thr	Ser	Ser	Glu	Val	Asn	Leu	Ala	Ser	Leu	Pro	Pro	Asn	Tyr	His	Asn	85	90	95	
Glu	Thr	Ser	Thr	Glu	Thr	Arg	Val	Gly	Asn	Asn	Thr	Val	His	Val	His	100	105	110	
Gln	Glu	Val	His	Lys	Ile	Thr	Asn	Asn	Gln	Ser	Gly	Gln	Val	Val	Phe	115	120	125	
Ser	Glu	Thr	Val	Ile	Thr	Ser	Val	Gly	Asp	Glu	Glu	Gly	Lys	Arg	Ser	130	135	140	
His	Glu	Cys	Ile	Ile	Asp	Glu	Asp	Cys	Gly	Pro	Thr	Arg	Tyr	Cys	Gln	145	150	155	160
Phe	Ser	Ser	Phe	Lys	Tyr	Thr	Cys	Gln	Pro	Cys	Arg	Asp	Gln	Gln	Met	165	170	175	
Leu	Cys	Thr	Arg	Asp	Ser	Glu	Cys	Cys	Gly	Asp	Gln	Leu	Cys	Ala	Trp	180	185	190	
Gly	His	Cys	Thr	Gln	Lys	Ala	Thr	Lys	Gly	Gly	Asn	Gly	Thr	Ile	Cys	195	200	205	
Asp	Asn	Gln	Arg	Asp	Cys	Gln	Pro	Gly	Leu	Cys	Cys	Ala	Phe	Gln	Arg	210	215	220	
Gly	Leu	Leu	Phe	Pro	Val	Cys	Thr	Pro	Leu	Pro	Val	Glu	Gly	Glu	Leu	225	230	235	240
Cys	His	Asp	Pro	Thr	Ser	Gln	Leu	Leu	Asp	Leu	Ile	Thr	Trp	Glu	Leu	245	250	255	
Glu	Pro	Glu	Gly	Ala	Leu	Asp	Arg	Cys	Pro	Cys	Ala	Ser	Gly	Leu	Leu	260	265	270	
Cys	Gln	Pro	His	Ser	His	Ser	Leu	Val	Tyr	Met	Cys	Lys	Pro	Ala	Phe	275	280	285	
Val	Gly	Ser	His	Asp	His	Ser	Glu	Glu	Ser	Gln	Leu	Pro	Arg	Glu	Ala	290	295	300	

- 24 -

Pro Asp Glu Tyr Glu Asp Val Gly Phe Ile Gly Glu Val Arg Gln Glu
305 310 315 320

Leu Glu Asp Leu Glu Arg Ser Leu Ala Gln Glu Met Ala Phe Glu Gly
325 330 335

Pro Ala Pro Val Glu Ser Leu Gly Gly Glu Glu Glu Ile
340 345

<210> 18

<211> 1047

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)..(1047)

<400> 18

atg cag cgg ctc ggg ggt att ttg ctg tgt aca ctg ctg gcg gcg gcg 48
Met Gln Arg Leu Gly Gly Ile Leu Leu Cys Thr Leu Leu Ala Ala Ala
1 5 10 15

gtc ccc act gct cct gct cct tcc ccg acg gtc act tgg act ccg gcg 96
Val Pro Thr Ala Pro Ala Pro Ser Pro Thr Val Thr Trp Thr Pro Ala
20 25 30

gag ccg ggc cca gct ctc aac tac cct cag gag gaa gct acg ctc aat 144
Glu Pro Gly Pro Ala Leu Asn Tyr Pro Gln Glu Glu Ala Thr Leu Asn
35 40 45

gag atg ttt cga gag gtg gag gag ctg atg gaa gac act cag cac aaa 192
Glu Met Phe Arg Glu Val Glu Glu Leu Met Glu Asp Thr Gln His Lys
50 55 60

ctg cgc agt gcc gtg gag gag atg gag gcg gaa gaa gca gct gct aaa 240
Leu Arg Ser Ala Val Glu Glu Met Glu Ala Glu Glu Ala Ala Ala Lys
65 70 75 80

acg tcc tct gag gtg aac ctg gca agc tta cct ccc aac tat cac aat 288
Thr Ser Ser Glu Val Asn Leu Ala Ser Leu Pro Pro Asn Tyr His Asn
85 90 95

gag acc agc acg gag acc agg gtg gga aat aac aca gtc cat gtg cac 336
Glu Thr Ser Thr Glu Thr Arg Val Gly Asn Asn Thr Val His Val His
100 105 110

cag gaa gtt cac aag ata acc aac aac cag agt gga cag gtg gtc ttt 384
Gln Glu Val His Lys Ile Thr Asn Asn Gln Ser Gly Gln Val Val Phe
115 120 125

tct gag aca gtc att aca tct gta ggg gat gaa gaa ggc aag agg agc 432
Ser Glu Thr Val Ile Thr Ser Val Gly Asp Glu Glu Gly Lys Arg Ser
130 135 140

cat gaa tgt atc att gat gaa gac tgt ggg ccc acc agg tac tgc cag 480
His Glu Cys Ile Ile Asp Glu Asp Cys Gly Pro Thr Arg Tyr Cys Gln

- 25 -

145		150		155		160	
ttc tcc agc ttc aag tac acc tgc cag cca tgc cgg gac cag cag atg							528
Phe Ser Ser Phe Lys Tyr Thr Cys Gln Pro Cys Arg Asp Gln Gln Met							
		165		170		175	
cta tgc acc cga gac agt gag tgc tgt gga gac cag ctg tgt gcc tgg							576
Leu Cys Thr Arg Asp Ser Glu Cys Cys Gly Asp Gln Leu Cys Ala Trp							
		180		185		190	
ggt cac tgc acc caa aag gcc acc aaa ggt ggc aat ggg acc atc tgt							624
Gly His Cys Thr Gln Lys Ala Thr Lys Gly Gly Asn Gly Thr Ile Cys							
		195		200		205	
gac aac cag agg gat tgc cag cct ggc ctg tgt tgt gcc ttc caa aga							672
Asp Asn Gln Arg Asp Cys Gln Pro Gly Leu Cys Cys Ala Phe Gln Arg							
		210		215		220	
ggc ctg ctg ttc ccc gtg tgc aca ccc ctg ccc gtg gag gga gag ctc							720
Gly Leu Leu Phe Pro Val Cys Thr Pro Leu Pro Val Glu Gly Glu Leu							
		225		230		235	240
tgc cat gac ccc acc agc cag ctg ctg gat ctc atc acc tgg gaa ctg							768
Cys His Asp Pro Thr Ser Gln Leu Leu Asp Leu Ile Thr Trp Glu Leu							
		245		250		255	
gag cct gaa gga gct ttg gac cga tgc ccc tgc gcc agt ggc ctc cta							816
Glu Pro Glu Gly Ala Leu Asp Arg Cys Pro Cys Ala Ser Gly Leu Leu							
		260		265		270	
tgc cag cca cac agc cac agt ctg gtg tac atg tgc aag cca gcc ttc							864
Cys Gln Pro His Ser His Ser Leu Val Tyr Met Cys Lys Pro Ala Phe							
		275		280		285	
gtg ggc agc cat gac cac agt gag gag agc cag ctg ccc agg gag gcc							912
Val Gly Ser His Asp His Ser Glu Glu Ser Gln Leu Pro Arg Glu Ala							
		290		295		300	
ccg gat gag tac gaa gat gtt ggc ttc ata ggg gaa gtg cgc cag gag							960
Pro Asp Glu Tyr Glu Asp Val Gly Phe Ile Gly Glu Val Arg Gln Glu							
		305		310		315	320
ctg gaa gac ctg gag cgg agc cta gcc cag gag atg gca ttt gag ggg							1008
Leu Glu Asp Leu Glu Arg Ser Leu Ala Gln Glu Met Ala Phe Glu Gly							
		325		330		335	
cct gcc cct gtg gag tca cta ggc gga gag gag gag att							1047
Pro Ala Pro Val Glu Ser Leu Gly Gly Glu Glu Glu Ile							
		340		345			

<210> 19

<211> 8

<212> PRT

<213> synthtic construct

<400> 19

Asp Tyr Lys Asp Asp Asp Asp Lys

1

5

- 26 -

```
<210> 20
<211> 3696
<212> DNA
<213> Homo sapiens
```

```
<220>
<221> CDS
<222> (712)..(1500)
```

```

<400> 20
gtcgacccac gcg tccggcg ggagcccgcg gcgagcgtag cgcaagtccg ctccctaggc 60
atcgctgcgc tggcagcgat tcgctgtctc ttgtgagtca ggggacaacg cttcggggca 120
actgtgagtg cgcgtgtggg ggacctcgat tctcttcaga tctcgaggat tcgggtccggg 180
gacgtctcct gatcccctac taaagcgcct gctaactttg aaaaggagca ctgtgtcctg 240
caaagtttga cacataaagg ataggaaaag agaggagaga aaagcaactg agttgaagga 300
gaaggagctg atgcgggcct cctgatcaat taagaggaga gttaaaccgc cgagatcccg 360
gcgggaccaa ggaggtgcgg ggcaagaagg aacggaagcg gtgcgatcca cagggctggg 420
ttttcttgca ccttgggtca cgcctccttg gcgagaaagc gcctcgcatt tgattgcttc 480
cagttattgc agaacttcct gtcttgggtg agaagcgggt ctcgcttggg ttccgctaata 540
ttctgtcctg aggcgtgaga ctgagttcat agggtccttg gtccccgaac caggaagggt 600
tgagggaaca caatctgcaa gccccgcga cccaagtgag gggccccgtg ttgggggtcct 660
ccctcccttt gcattcccac ccctccgggc tttgcgtctt cctggggacc c cct cgc 717
                                     Pro Arg
                                     1

cgg gag atg gcc gcg ttg atg cgg agc aag gat tcg tcc tgc tgc ctg 765
Arg Glu Met Ala Ala Leu Met Arg Ser Lys Asp Ser Ser Cys Cys Leu
          5                      10                      15

ctc cta ctg gcc gcg gtg ctg atg gtg gag agc tca cag atc ggc agt 813
Leu Leu Leu Ala Ala Val Leu Met Val Glu Ser Ser Gln Ile Gly Ser
          20                      25                      30

tcg cgg gcc aaa ctc aac tcc atc aag tcc tct ctg ggc ggg gag acg 861
Ser Arg Ala Lys Leu Asn Ser Ile Lys Ser Ser Leu Gly Gly Glu Thr
          35                      40                      45                      50

cct ggt cag gcc gcc aat cga tct gcg ggc atg tac caa gga ctg gca 909
Pro Gly Gln Ala Ala Asn Arg Ser Ala Gly Met Tyr Gln Gly Leu Ala
          55                      60                      65

ttc ggc ggc agt aag aag ggc aaa aac ctg ggg cag gcc tac cct tgt 957
Phe Gly Gly Ser Lys Lys Gly Lys Asn Leu Gly Gln Ala Tyr Pro Cys
          70                      75                      80

agc agt gat aag gag tgt gaa gtt ggg agg tat tgc cac agt ccc cac 1005

```


- 28 -

gttagagttt aacaaaatac tcctagaata acttggttata caatagggttc taaaaataaa 1960
 attgctaaac aagaaatgaa aacatggagc attgttaatt tacaacagaa aattaccttt 2020
 tgatttgtaa cactacttct gctgttcaat caagagtctt ggtagataag aaaaaaatca 2080
 gtcaatatatt ccaaataatt gcaaaataat ggccagttgt ttaggaaggc ctttaggaag 2140
 acaaataaat aacaaacaaa cagccacaaa tacttttttt tcaaaatttt agttttacct 2200
 gtaattaata agaactgata caagacaaaa acagttcctt cagattctac ggaatgacag 2260
 tatatctctc tttatcctat gtgattcctg ctctgaatgc attatatatt ccaaagtata 2320
 ccataaatt gtgactagta aaatacttac acagagcaga attttcacag atggcaaaaa 2380
 aatttaaaga tgtccaatat atgtgggaaa agagctaaca gagagatcat tatttcttaa 2440
 agattggcca taacctgtat tttgatagaa ttagattggt aaatacatgt attcatacat 2500
 actctgtggt aatagagact tgagctggat ctgtactgca ctggagtaag caagaaaatt 2560
 gggaaaactt tttcgtttgt tcaggttttg gcaacacata gatcatatgt ctgaggcaca 2620
 agttggctgt tcatctttga aaccagggga tgcacagtct aaatgaatat ctgcatggga 2680
 tttgtatcat aatatttact atgcagatga attcagtgtg aggtcctgtg tccgtactat 2740
 cctcaaatta tttattttat agtgctgaga tcctcaaata atctcaattt caggagggtt 2800
 cacaaaatgg actcctgaag tagacagagt agtgagggtt cattgccctc tataagcttc 2860
 tgactagcca atggcatcat ccaattttct tcccaaacct ctgcagcatc tgctttattg 2920
 ccaaagggtt agtttcggtt ttctgcagcc attgcggtta aaaaatataa gtaggataac 2980
 ttgtaaaacc tgcataattgc taatctatag acaccacagt ttctaaattc tttgaaacca 3040
 ctttactact ttttttaaac ttaactcagt tctaaatact ttgtctggag cacaaaacaa 3100
 taaaagggtt tcttatagtt gtgactttaa actttttagt accacaattc actttttagt 3160
 tttcttttac ttaaattcca tctgcagtct caaatttaag ttctcccagt agagattgag 3220
 tttgagcctg tatatctatt aaaaatttca acttcccaca tatatttact aagatgatta 3280
 agacttacat tttctgcaca ggtctgcaaa aacaaaaatt ataaactagt ccatccaaga 3340
 accaaagttt gtataaacag gttgctataa gcttggtgaa atgaaaatgg aacatttcaa 3400
 tcaaacattt cctatataac aattattata tttacaattt ggtttctgca atatttttct 3460
 tatgtccacc cttttaaaaa ttattatttg aagtaattta ttacaggaa atgttaatga 3520
 gatgtatttt cttatagaga tatttcttac agaaagcttt gtagcagaat atatttgcag 3580
 ctattgactt tgtaatttag gaaaaatgta taataagata aaatctatta aatttttctc 3640
 ctctaaaaac tgaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaagggc ggccgc 3696

Leu His Val Cys Gln Lys Ile
260

- 30 -

<210> 22
 <211> 789
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(789)

<400> 22

cct	cg	cgc	gag	atg	gcc	gcg	ttg	atg	cgg	agc	aag	gat	tcg	tcc	tgc	48
Pro	Arg	Arg	Glu	Met	Ala	Ala	Leu	Met	Arg	Ser	Lys	Asp	Ser	Ser	Cys	
1				5					10					15		
tgc	ctg	ctc	cta	ctg	gcc	gcg	gtg	ctg	atg	gtg	gag	agc	tca	cag	atc	96
Cys	Leu	Leu	Leu	Leu	Ala	Ala	Val	Leu	Met	Val	Glu	Ser	Ser	Gln	Ile	
			20					25					30			
ggc	agt	tcg	cgg	gcc	aaa	ctc	aac	tcc	atc	aag	tcc	tct	ctg	ggc	ggg	144
Gly	Ser	Ser	Arg	Ala	Lys	Leu	Asn	Ser	Ile	Lys	Ser	Ser	Leu	Gly	Gly	
		35					40					45				
gag	acg	cct	ggt	cag	gcc	gcc	aat	cga	tct	gcg	ggc	atg	tac	caa	gga	192
Glu	Thr	Pro	Gly	Gln	Ala	Ala	Asn	Arg	Ser	Ala	Gly	Met	Tyr	Gln	Gly	
	50					55					60					
ctg	gca	ttc	ggc	ggc	agt	aag	aag	ggc	aaa	aac	ctg	ggg	cag	gcc	tac	240
Leu	Ala	Phe	Gly	Gly	Ser	Lys	Lys	Gly	Lys	Asn	Leu	Gly	Gln	Ala	Tyr	
65					70					75					80	
cct	tgt	agc	agt	gat	aag	gag	tgt	gaa	gtt	ggg	agg	tat	tgc	cac	agt	288
Pro	Cys	Ser	Ser	Asp	Lys	Glu	Cys	Glu	Val	Gly	Arg	Tyr	Cys	His	Ser	
				85					90					95		
ccc	cac	caa	gga	tca	tcg	gcc	tgc	atg	gtg	tgt	cgg	aga	aaa	aag	aag	336
Pro	His	Gln	Gly	Ser	Ser	Ala	Cys	Met	Val	Cys	Arg	Arg	Lys	Lys	Lys	
			100					105					110			
cgc	tgc	cac	cga	gat	ggc	atg	tgc	tgc	ccc	agt	acc	cgc	tgc	aat	aat	384
Arg	Cys	His	Arg	Asp	Gly	Met	Cys	Cys	Pro	Ser	Thr	Arg	Cys	Asn	Asn	
		115					120					125				
ggc	atc	tgt	atc	cca	gtt	act	gaa	agc	atc	tta	acc	cct	cac	atc	ccg	432
Gly	Ile	Cys	Ile	Pro	Val	Thr	Glu	Ser	Ile	Leu	Thr	Pro	His	Ile	Pro	
	130					135					140					
gct	ctg	gat	ggt	act	cgg	cac	aga	gat	cga	aac	cac	ggt	cat	tac	tca	480
Ala	Leu	Asp	Gly	Thr	Arg	His	Arg	Asp	Arg	Asn	His	Gly	His	Tyr	Ser	
145					150					155					160	
aac	cat	gac	ttg	gga	tgg	cag	aat	cta	gga	aga	cca	cac	act	aag	atg	528
Asn	His	Asp	Leu	Gly	Trp	Gln	Asn	Leu	Gly	Arg	Pro	His	Thr	Lys	Met	
				165					170					175		
tca	cat	ata	aaa	ggg	cat	gaa	gga	gac	ccc	tgc	cta	cga	tca	tca	gac	576
Ser	His	Ile	Lys	Gly	His	Glu	Gly	Asp	Pro	Cys	Leu	Arg	Ser	Ser	Asp	
			180					185					190			

- 31 -

tgc att gaa ggg ttt tgc tgt gct cgt cat ttc tgg acc aaa atc tgc	624
Cys Ile Glu Gly Phe Cys Cys Ala Arg His Phe Trp Thr Lys Ile Cys	
195 200 205	
aaa cca gtg ctc cat cag ggg gaa gtc tgt acc aaa caa cgc aag aag	672
Lys Pro Val Leu His Gln Gly Glu Val Cys Thr Lys Gln Arg Lys Lys	
210 215 220	
ggt tct cat ggg ctg gaa att ttc cag cgt tgc gac tgt gcg aag ggc	720
Gly Ser His Gly Leu Glu Ile Phe Gln Arg Cys Asp Cys Ala Lys Gly	
225 230 235 240	
ctg tct tgc aaa gta tgg aaa gat gcc acc tac tcc tcc aaa gcc aga	768
Leu Ser Cys Lys Val Trp Lys Asp Ala Thr Tyr Ser Ser Lys Ala Arg	
245 250 255	
ctc cat gtg tgt cag aaa att	789
Leu His Val Cys Gln Lys Ile	
260	

<210> 23

<211> 54

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: consensus sequence

<220>

<223> Xaa's at positions
2,3,5,6,8-12,14-26,28,29,31-36,38-42,45-48, and
50-53 may be any amino acid

<220>

<223> Xaa's at postions 22-26 may be absent

<400> 23

Cys Xaa Xaa Asp Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Cys Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa Xaa Xaa
35 40 45

Cys Xaa Xaa Xaa Xaa Cys
50

<210> 24

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: consensus

- 32 -

sequence

<220>

<223> Xaa's at positions

2,5,8,9,11,14-23,25,27-30,32,33,35-53,60,62,63,65,
68,70,71,73-96,98,100,101,104, and 106-122 may be
any amino acid

<220>

<223> Xaa's at positions 22,23,51-58,89-96 and 116-122
may be absent

<400> 24

Cys	Xaa	Xaa	Xaa	Xaa	Asp	Cys	Xaa	Xaa	Gly	Xaa	Cys	Cys	Xaa	Xaa	Xaa	
1					5				10						15	
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Gly	Xaa	
			20					25						30		
Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	
		35					40					45				
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Xaa	Cys	Xaa	Xaa	Pro	
	50						55				60					
Xaa	Xaa	Xaa	Xaa	Gly	Xaa	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	
65					70				75						80	
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	
				85					90						95	
Cys	Xaa	Cys	Xaa	Xaa	Gly	Leu	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	
			100					105						110		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys					
			115					120								

<210> 25

<211> 87

<212> PRT

<213> Homo sapiens

<400> 25

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

- 33 -

Asp Ala Gly Arg Ser Lys Gln
85

<210> 26

<211> 835

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (57)..(746)

<400> 25

gaattcggca cgaggcagaa ggcgcgaatg aaggcaaagc ctcccaccca cctgca atg 59
Met
1

tgt cga ctg agg gtc ttg ctg ctg ctg ctc ccc ttg gcc ttc gtg tcc 107
Cys Arg Leu Arg Val Leu Leu Leu Leu Leu Pro Leu Ala Phe Val Ser
5 10 15

tcc tct gct ctc ccc atc cat gat gtc gac tct cag cag aac acc tcc 155
Ser Ser Ala Leu Pro Ile His Asp Val Asp Ser Gln Gln Asn Thr Ser
20 25 30

ggg ttc ctg ggc ctt cag agg ctt ctc caa agc ttt agt cga ctg ttc 203
Gly Phe Leu Gly Leu Gln Arg Leu Leu Gln Ser Phe Ser Arg Leu Phe
35 40 45

cta aaa aat gac ctg cta cga gac ctg gac aac ttc ttc tcc tcc ccc 251
Leu Lys Asn Asp Leu Leu Arg Asp Leu Asp Asn Phe Phe Ser Ser Pro
50 55 60 65

atg gac ttc cga gac ctt cct agg aac ttc cat cag gaa gag aac cag 299
Met Asp Phe Arg Asp Leu Pro Arg Asn Phe His Gln Glu Glu Asn Gln
70 75 80

gag cac aga atg ggc aac cat acc ctc tcc agc cac cta cag ata gac 347
Glu His Arg Met Gly Asn His Thr Leu Ser Ser His Leu Gln Ile Asp
85 90 95

aag gtg act gac aac cag aca ggg gag gtg cac atc tcg gag aaa gtc 395
Lys Val Thr Asp Asn Gln Thr Gly Glu Val His Ile Ser Glu Lys Val
100 105 110

gag gcc tcc att gag cca gaa cgg aac ccg gaa ggg gac tgg aag gtt 443
Glu Ala Ser Ile Glu Pro Glu Arg Asn Pro Glu Gly Asp Trp Lys Val
115 120 125

ccc aaa gta gaa gca aaa gag ccc ccg gtg cct gtg cag aag gtc acc 491
Pro Lys Val Glu Ala Lys Glu Pro Pro Val Pro Val Gln Lys Val Thr
130 135 140 145

gac agc ttg cac cca gag ccc cgg cag gtg gct ttc tgg atc atg aag 539
Asp Ser Leu His Pro Glu Pro Arg Gln Val Ala Phe Trp Ile Met Lys
150 155 160

atg cca agg cgg agg acc cag ccc gat gtc cag gat gga ggc cgc tgg 587

- 34 -

Met Pro Arg Arg Arg Thr Gln Pro Asp Val Gln Asp Gly Gly Arg Trp
 165 170 175

ctc ata gaa aag cga cat cgc atg cag gcc atc cgg gat ggg ctc cgt 635
 Leu Ile Glu Lys Arg His Arg Met Gln Ala Ile Arg Asp Gly Leu Arg
 180 185 190

gga ggc gcc cgt gag gac agc ctg gag gat ggg gtc cat atc ccc caa 683
 Gly Gly Ala Arg Glu Asp Ser Leu Glu Asp Gly Val His Ile Pro Gln
 195 200 205

cac gcc aag ctg cct gtc aga aag aca cac ttt ctc tac atc ctc agg 731
 His Ala Lys Leu Pro Val Arg Lys Thr His Phe Leu Tyr Ile Leu Arg
 210 215 220 225

cca tcc caa cag ctg taagtgggga ccagatgtcc cacaccctac cccaacacca 786
 Pro Ser Gln Gln Leu
 230

tatggaaata aagggttttct tacatctaaa aaaaaaaaaa aaaaaaaaaa 835

<210> 27
 <211> 230
 <212> PRT
 <213> Mus musculus

<400> 27

Met Cys Arg Leu Arg Val Leu Leu Leu Leu Leu Pro Leu Ala Phe Val
 1 5 10 15

Ser Ser Ser Ala Leu Pro Ile His Asp Val Asp Ser Gln Gln Asn Thr
 20 25 30

Ser Gly Phe Leu Gly Leu Gln Arg Leu Leu Gln Ser Phe Ser Arg Leu
 35 40 45

Phe Leu Lys Asn Asp Leu Leu Arg Asp Leu Asp Asn Phe Phe Ser Ser
 50 55 60

Pro Met Asp Phe Arg Asp Leu Pro Arg Asn Phe His Gln Glu Glu Asn
 65 70 75 80

Gln Glu His Arg Met Gly Asn His Thr Leu Ser Ser His Leu Gln Ile
 85 90 95

Asp Lys Val Thr Asp Asn Gln Thr Gly Glu Val His Ile Ser Glu Lys
 100 105 110

Val Glu Ala Ser Ile Glu Pro Glu Arg Asn Pro Glu Gly Asp Trp Lys
 115 120 125

Val Pro Lys Val Glu Ala Lys Glu Pro Pro Val Pro Val Gln Lys Val
 130 135 140

Thr Asp Ser Leu His Pro Glu Pro Arg Gln Val Ala Phe Trp Ile Met
 145 150 155 160

Lys Met Pro Arg Arg Arg Thr Gln Pro Asp Val Gln Asp Gly Gly Arg

- 35 -

	165		170		175											
Trp	Leu	Ile	Glu	Lys	Arg	His	Arg	Met	Gln	Ala	Ile	Arg	Asp	Gly	Leu	
	180							185					190			
Arg	Gly	Gly	Ala	Arg	Glu	Asp	Ser	Leu	Glu	Asp	Gly	Val	His	Ile	Pro	
	195						200					205				
Gln	His	Ala	Lys	Leu	Pro	Val	Arg	Lys	Thr	His	Phe	Leu	Tyr	Ile	Leu	
	210					215					220					
Arg	Pro	Ser	Gln	Gln	Leu											
225					230											

<210> 28
 <211> 690
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)..(690)

<400> 28

atg	tgt	cga	ctg	agg	gtc	ttg	ctg	ctg	ctg	ctc	ccc	ttg	gcc	ttc	gtg	48
Met	Cys	Arg	Leu	Arg	Val	Leu	Leu	Leu	Leu	Leu	Pro	Leu	Ala	Phe	Val	
1				5					10					15		
tcc	tcc	tct	gct	ctc	ccc	atc	cat	gat	gtc	gac	tct	cag	cag	aac	acc	96
Ser	Ser	Ser	Ala	Leu	Pro	Ile	His	Asp	Val	Asp	Ser	Gln	Gln	Asn	Thr	
			20					25					30			
tcc	ggg	ttc	ctg	ggc	ctt	cag	agg	ctt	ctc	caa	agc	ttt	agt	cga	ctg	144
Ser	Gly	Phe	Leu	Gly	Leu	Gln	Arg	Leu	Leu	Gln	Ser	Phe	Ser	Arg	Leu	
		35					40					45				
ttc	cta	aaa	aat	gac	ctg	cta	cga	gac	ctg	gac	aac	ttc	ttc	tcc	tcc	192
Phe	Leu	Lys	Asn	Asp	Leu	Leu	Arg	Asp	Leu	Asp	Asn	Phe	Phe	Ser	Ser	
	50					55					60					
ccc	atg	gac	ttc	cga	gac	ctt	cct	agg	aac	ttc	cat	cag	gaa	gag	aac	240
Pro	Met	Asp	Phe	Arg	Asp	Leu	Pro	Arg	Asn	Phe	His	Gln	Glu	Glu	Asn	
65					70					75					80	
cag	gag	cac	aga	atg	ggc	aac	cat	acc	ctc	tcc	agc	cac	cta	cag	ata	288
Gln	Glu	His	Arg	Met	Gly	Asn	His	Thr	Leu	Ser	Ser	His	Leu	Gln	Ile	
				85					90					95		
gac	aag	gtg	act	gac	aac	cag	aca	ggg	gag	gtg	cac	atc	tcg	gag	aaa	336
Asp	Lys	Val	Thr	Asp	Asn	Gln	Thr	Gly	Glu	Val	His	Ile	Ser	Glu	Lys	
			100					105					110			
gtc	gag	gcc	tcc	att	gag	cca	gaa	cgg	aac	ccg	gaa	ggg	gac	tgg	aag	384
Val	Glu	Ala	Ser	Ile	Glu	Pro	Glu	Arg	Asn	Pro	Glu	Gly	Asp	Trp	Lys	
		115					120					125				
gtt	ccc	aaa	gta	gaa	gca	aaa	gag	ccc	ccg	gtg	cct	gtg	cag	aag	gtc	432
Val	Pro	Lys	Val	Glu	Ala	Lys	Glu	Pro	Pro	Val	Pro	Val	Gln	Lys	Val	

- 36 -

130	135	140	
acc gac agc ttg cac cca gag ccc cgg cag gtg gct ttc tgg atc atg			480
Thr Asp Ser Leu His Pro Glu Pro Arg Gln Val Ala Phe Trp Ile Met			
145	150	155	160
aag atg cca agg cgg agg acc cag ccc gat gtc cag gat gga ggc cgc			528
Lys Met Pro Arg Arg Arg Thr Gln Pro Asp Val Gln Asp Gly Gly Arg			
	165	170	175
tgg ctc ata gaa aag cga cat cgc atg cag gcc atc cgg gat ggg ctc			576
Trp Leu Ile Glu Lys Arg His Arg Met Gln Ala Ile Arg Asp Gly Leu			
	180	185	190
cgt gga ggc gcc cgt gag gac agc ctg gag gat ggg gtc cat atc ccc			624
Arg Gly Gly Ala Arg Glu Asp Ser Leu Glu Asp Gly Val His Ile Pro			
	195	200	205
caa cac gcc aag ctg cct gtc aga aag aca cac ttt ctc tac atc ctc			672
Gln His Ala Lys Leu Pro Val Arg Lys Thr His Phe Leu Tyr Ile Leu			
	210	215	220
agg cca tcc caa cag ctg			690
Arg Pro Ser Gln Gln Leu			
225	230		

<210> 29

<211> 51

<212> PRT

<213> Artificial Sequence

<220>

<223> Xaa at positions

3-5, 7, 9-15, 18, 20-22, 24-27, 29, 31, 33, 34, 36-39, 42, 44,
45, and 47-50 may be any amino acid

<220>

<223> Description of Artificial Sequence: consensus sequence

<400> 29

Leu	Pro	Xaa	Xaa	Xaa	His	Xaa	Glu	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Gly
1				5					10					15	

Asn	Xaa	Thr	Xaa	Xaa	Xaa	His	Xaa	Xaa	Xaa	Xaa	Lys	Xaa	Thr	Xaa	Asn
			20					25					30		

Xaa	Xaa	Gly	Xaa	Xaa	Xaa	Xaa	Ser	Glu	Xaa	Val	Xaa	Xaa	Ser	Xaa	Xaa
		35					40					45			

Xaa	Xaa	Glu
		50

<210> 30

<211> 20

<212> DNA

<213> Artificial Sequence

- 37 -

<220>

<223> Description of Artificial Sequence: primer

<400> 30

cagtgagtgc tgtggagacc

20

<210> 31

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 31

tcttcagtca ggctcctctc

20

<210> 32

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 32

acctgcaatg tgtcgactga g

21

<210> 33

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 33

cacttacagc tggtgggatg

20

<210> 34

<211> 10

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa at position 1 may be any amino acid

<400> 34

Xaa	Val	Leu	Asp	Phe	Asn	Asn	Ile	Arg	Ser
1					5				10

<210> 35

<211> 10

- 38 -

<212> PRT

<213> Homo sapiens

<400> 35

Ser	Gln	Gly	Arg	Lys	Gly	Gln	Glu	Gly	Ser
1				5					10

<210> 36

<211> 272

<212> PRT

<213> Mus musculus

<400> 36

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

Lys Gly Glu Phe Thr Thr Val Pro Lys Thr Ser Arg Leu His Thr Cys

WO 00/52047

PCT/US00/05452

- 40 -

245

250

255

Gln Arg His

<210> 38

<211> 350

<212> PRT

<213> Gallus gallus

<400> 38

Met Arg Arg Gly Glu Gly Pro Ala Pro Arg Arg Arg Trp Leu Leu Leu
 1 5 10 15

Leu Ala Val Leu Ala Ala Leu Cys Cys Ala Ala Ala Gly Ser Gly Gly
 20 25 30

Arg Arg Arg Ala Ala Ser Leu Gly Glu Met Leu Arg Glu Val Glu Ala
 35 40 45

Leu Met Glu Asp Thr Gln His Lys Leu Arg Asn Ala Val Gln Glu Met
 50 55 60

Glu Ala Glu Glu Glu Gly Ala Lys Lys Leu Ser Glu Val Asn Phe Glu
 65 70 75 80

Asn Leu Pro Pro Thr Tyr His Asn Glu Ser Asn Thr Glu Thr Arg Ile
 85 90 95

Gly Asn Lys Thr Val Gln Thr His Gln Glu Ile Asp Lys Val Thr Asp
 100 105 110

Asn Arg Thr Gly Ser Thr Ile Phe Ser Glu Thr Ile Ile Thr Ser Ile
 115 120 125

Lys Gly Gly Glu Asn Lys Arg Asn His Glu Cys Ile Ile Asp Glu Asp
 130 135 140

Cys Glu Thr Gly Lys Tyr Cys Gln Phe Ser Thr Phe Glu Tyr Lys Cys
 145 150 155 160

Gln Pro Cys Lys Thr Gln His Thr His Cys Ser Arg Asp Val Glu Cys
 165 170 175

Cys Gly Asp Gln Leu Cys Val Trp Gly Glu Cys Arg Lys Ala Thr Ser
 180 185 190

Arg Gly Glu Asn Gly Thr Ile Cys Glu Asn Gln His Asp Cys Asn Pro
 195 200 205

Gly Thr Cys Cys Ala Phe Gln Lys Glu Leu Leu Phe Pro Val Cys Thr
 210 215 220

Pro Leu Pro Glu Glu Gly Glu Pro Cys His Asp Pro Ser Asn Arg Leu
 225 230 235 240

Leu Asn Leu Ile Thr Trp Glu Leu Glu Pro Asp Gly Val Leu Glu Arg
 245 250 255

WO 00/52047

PCT/US00/05452

- 41 -

Cys	Pro	Cys	Ala	Ser	Gly	Leu	Ile	Cys	Gln	Pro	Gln	Ser	Ser	His	Ser
			260					265					270		
Thr	Thr	Ser	Val	Cys	Glu	Leu	Ser	Ser	Asn	Glu	Thr	Arg	Lys	Asn	Glu
		275					280					285			
Lys	Glu	Asp	Pro	Leu	Asn	Met	Asp	Glu	Met	Pro	Phe	Ile	Ser	Leu	Ile
	290					295					300				
Pro	Arg	Asp	Ile	Leu	Ser	Asp	Tyr	Glu	Glu	Ser	Ser	Val	Ile	Gln	Glu
305					310					315					320
Val	Arg	Lys	Glu	Leu	Glu	Ser	Leu	Glu	Asp	Gln	Ala	Gly	Val	Lys	Ser
				325					330					335	
Glu	His	Asp	Pro	Ala	His	Asp	Leu	Phe	Leu	Gly	Asp	Glu	Ile		
			340					345					350		

What is claimed:

1. An isolated antibody or immunological active portion thereof that selectively binds a peptide consisting of an amino acid sequence selected from the group consisting of amino acids 51-65 of SEQ ID NO:2, amino acids 311-325 of SEQ ID NO:2, amino acids 111-125 of SEQ ID NO:5, amino acids 71-85 of SEQ ID NO:8, and amino acids 73-87 of SEQ ID NO:11.
2. The antibody or immunologically active portion thereof of claim 1, wherein said antibody or immunologically active portion thereof is a monoclonal antibody or an immunologically active portion of a monoclonal antibody.
3. The antibody or immunologically active portion thereof of claim 1 or 2, wherein said antibody or immunologically active portion thereof is an immunologically active portion of said antibody.
4. The antibody or immunologically active portion thereof of any one of claims 1-3, wherein said immunologically active portion is selected from the group consisting of an Fab fragment and an F(ab')₂ fragment.
5. The antibody or immunologically active portion thereof of any one of claims 1-4, wherein said antibody or immunologically active portion thereof is coupled to a detectable label.
6. The antibody or immunologically active portion thereof of claim 5, wherein said detectable label is an enzyme, a prosthetic group, a fluorescent material, a luminescent material, a bioluminescent material or a radioactive material.
7. A kit comprising the antibody or immunologically active portion thereof of any one of claims 1-6 and instructions for use.
8. A use of a kit for detecting in a sample, a polypeptide selected from the group consisting of SEQ ID NO:2, 5, 8 and 11, said kit comprising the antibody or

immunologically active portion thereof as defined in any one of claims 1-6 and instructions for use.

9. A pharmaceutical composition comprising the antibody or immunologically active portion thereof of any one of claims 1-6 and a pharmaceutically acceptable carrier.
10. A method of detecting in a biological sample, a polypeptide selected from the group consisting of SEQ ID NO:2, 5, 8 and 11 comprising contacting a biological sample with the antibody or immunologically active portion thereof of any one of claims 1-6, and determining whether the antibody or immunologically active portion thereof binds to a polypeptide in the sample to thereby detect a polypeptide in the sample.
11. The isolated antibody or immunologically active portion thereof of any one of claims 1-3, wherein said antibody or immunologically active portion selectively binds a peptide consisting of the amino acid sequence of amino acids 71-85 of SEQ ID NO:8.

WO 00/52047

PCT/US00/05452

FIG. 1A-1

1/29

GGCACGAGGGGCGGCTCGGCGCAGAGCGGAG ATG CAG CGG CTT GGG GCC ACC CTG CTG TGC											10	
											67	
L	L	L	A	A	A	V	P	T	A	P	A	30
CTG	CTG	CTG	GCG	GCG	GCG	GTC	CCC	ACG	GCC	CCC	GCT	127
P	V	K	P	G	P	A	L	S	Y	P	Q	50
CCA	GTC	AAG	CCC	GGC	CCG	GCT	CTC	AGC	TAC	CCG	CAG	187
F	R	E	V	E	E	L	M	E	D	T	Q	70
TTC	CGC	GAG	GTT	GAG	GAA	CTG	ATG	GAG	GAC	ACG	CAG	247
E	M	E	A	E	E	A	A	A	K	A	S	90
GAG	ATG	GAG	GCA	GAA	GAA	GCT	GCT	GCT	AAA	GCA	TCA	307
P	P	S	Y	H	N	E	T	N	T	D	T	110
CCT	CCC	AGC	TAT	CAC	AAT	GAG	ACC	AAC	ACA	GAC	ACG	367
V	H	R	E	I	H	K	I	T	N	N	Q	130
GTG	CAC	CGA	GAA	ATT	CAC	AAG	ATA	ACC	AAC	AAC	CAG	427
T	V	I	T	S	V	G	D	E	E	G	R	150
ACA	GTT	ATC	ACA	TCT	GTG	GGA	GAC	GAA	GAA	GGC	AGA	487
E	D	C	G	P	S	M	Y	C	Q	F	A	170
GAG	GAC	TGT	GGG	CCC	AGC	ATG	TAC	TGC	CAG	TTT	GCC	547
C	R	G	Q	R	M	L	C	T	R	D	S	190
TGC	CGG	GGC	CAG	AGG	ATG	CTC	TGC	ACC	CGG	GAC	AGT	607

WO 00/52047

PCT/US00/05452

2/29

FIG. 1A-2

V	W	G	H	C	T	K	M	A	T	R	G	S	N	G	T	I	C	D	N	210
GTC	TGG	GGT	CAC	TGC	ACC	AAA	ATG	GCC	ACC	AGG	GGC	AGC	AAT	GGG	ACC	ATC	TGT	GAC	AAC	667
Q	R	D	C	Q	P	G	L	C	C	A	F	Q	R	G	L	L	F	P	V	230
CAG	AGG	GAC	TGC	CAG	CCG	GGG	CTG	TGC	TGT	GCC	TTC	CAG	AGA	GGC	CTG	CTG	TTC	CCT	GTG	727
C	T	P	L	P	V	E	G	E	L	C	H	D	P	A	S	R	L	L	D	250
TGC	ACA	CCC	CTG	CCC	GTG	GAG	GGC	GAG	CTT	TGC	CAT	GAC	CCC	GCC	AGC	CGG	CTT	CTG	GAC	787
L	I	T	W	E	L	E	P	D	G	A	L	D	R	C	P	C	A	S	G	270
CTC	ATC	ACC	TGG	GAG	CTA	GAG	CCT	GAT	GGA	GCC	TTG	GAC	CGA	TGC	CCT	TGT	GCC	AGT	GGC	847
L	L	C	Q	P	H	S	H	S	L	V	Y	V	C	K	P	T	F	V	G	290
CTC	CTC	TGC	CAG	CCC	CAC	AGC	CAC	AGC	CTG	GTG	TAT	GTG	TGC	AAG	CCG	ACC	TTC	GTG	GGG	907
S	R	D	Q	D	G	E	I	L	L	P	R	E	V	P	D	E	Y	E	V	310
AGC	CGT	GAC	CAA	GAT	GGG	GAG	ATC	CTG	CTG	CCC	AGA	GAG	GTC	CCC	GAT	GAG	TAT	GAA	GTT	967
G	S	F	M	E	E	V	R	Q	E	L	E	D	L	E	R	S	L	T	E	330
GGC	AGC	TTC	ATG	GAG	GAG	GTG	CGC	CAG	GAG	CTG	GAG	GAC	CTG	GAG	AGG	AGC	CTG	ACT	GAA	1027
E	M	A	L	G	E	P	A	A	A	A	A	A	L	L	G	G	E	E	I	350
GAG	ATG	GCG	CTG	GGG	GAG	CCT	GCG	GCT	GCC	GCC	GCT	GCA	CTG	CTG	GGA	GGG	GAA	GAG	ATT	1087
*																				351
TAG																				1090

WO 00/52047

PCT/US00/05452

FIG. 1B-1

3/29

ATCTGGACCAGGCTGTGGGTAGATGTGCAATAGAAATAGCTAATTATTTCCCCANGTGTGTGCTTTAAAGCGTGGGCTG 1169
ACCAGGCTTCTCCTACATCTTCTTCCCAGTAAGTTTCCCCCTCTGGCTTGACAGCATGAGGTGTTGTGCAATTGTGTTCAG 1248
CTCCCCCAGGCTGTTCTCCAGGCTTCACAGTCTGGTGCTTGGGAGAGTCAGGCAGGGTTAAACTGCAGGAGCAGTTTGC 1327
CACCCCTGTCCAGATTATTGCGTGCCTTGTGCCCTCTACCAGTTGGCAGACAGCCGTTTGTCTCTACATGGCTTTTGATAATTG 1406
TTTGAGGGGAGGAGATGGAAACAAATGTGGAGTCTCCCTCTGATTGGTTTTGGGGAAATGTGGAGAAGAGTGCCCTGCTT 1485
TGCAAAACATCAACCTGGCAAATAATGCAACAAATGAATTTTCCACGCAGTCTTTCATGGGCATAGGTAAGCTGTGCCT 1564
TCAGCTGTTGCAGATGAATGTCTGTTCACCCCTGCATTACATGTGTTTATTATCCAGCAGTGTGTTGCTCAGCTCCTAC 1643
CTCTGTGCCAGGCAGCATTTTTCATATCCAAAGATCAATTCCCTCTCTCAGCACAGCCTGGGGAGGGGTCAATTGTTCTC 1722
CTCGTCCATCAGGGATTTCAGAGGCTCAGAGACTGCAAGCTGCTTGCCCCAAGTCACACAGCTAGTGAAGACCAGAGCAG 1801
TTTTCATCTGGTTGTGACTCTAAGCTCAGTGCTCTCTCCACTACCCCAACAGCCTTGGTGCCACCAAAAGTGCTCCCC 1880
AAAGGAAGGAGAAIGGGATTTTCTTTTGAGGCATGCACATCTGGAATTAAGGTCAAACATAATCTCACAATCCCCCTTA 1959
AAAGTAAACTACTGTTAGGAACAGCAGTGTTCTCACAGTGTGGGGCAGCCGTCCTTCTAATGAAGACAAATGATATTGAC 2038
ACTGTCCCTCTTTTGGCAGTTGCATTAGTAACCTTTGAAAGGTATATGACTGAGCGTAGCATACAGTTAACCTGCAGAAA 2117
CAGTACTTAGGTAATTGTAGGGCGAGGATTATAAATGAATTTGCAAAATCACTTAGCAGCAACTGAAGACAAATTATCA 2196

WO 00/52047

4/29

PCT/US00/05452

FIG. 1B-2

ACCACGTGGAGAAATCAAAACCGAGCAGGCTGTGTGAACATGGTTGTAAATATGCGACTGCGAACACTGAACTCTACG	2275
CCACTCCACAAATGATGTTTTCAGGTGTCATGGACTGTTGCCACCATGTATTTCATCCAGAGTTCCTTAAAGTTTAAAGTT	2354
GCACATGATTGTATAAGCATGCTTCTTTGAGTTTAAATTATGTATAAACATAAGTTGCATTTAGAAATCAAGCATAA	2433
ATCACTTCAACTGCTAAATAAAAAAAAAAAAAAAAAAAAAA	2479

WO 00/52047

PCT/US00/05452

5/29

FIG. 2A

GAATTCGGCACGAGACGACGTGCTGAGCTGCCAGCTTAGTGGAAGCTCTGCTCTGGTGGAGAGCAGCCTCGCTTTG																79
M V A A V L L G																8
GTGACGCACAGTGTGGACCTCCAGGAGCCCGGATTGAAGG ATG GTG GCG GCC GTC CTG CTG GGG																148
L S W L C S P L G A L V L D F N N I R S	28															
CTG AGC TGG CTC TGC TCT CCC CTG GGA GCT CTG GTC CTG GAC TTC AAC AAC ATC AGG AGC	208															
S A D L L H G A R K G S Q C L S D T D C N	48															
TCT GCT GAC CTG CAT GGG GCC CGG AAG GGC TCA CAG TGC CTG TCT GAC ACG GAC TGC AAT	268															
T R K F C L Q P R D E K P F C A T C R G	68															
ACC AGA AAG TTC TGC CTC CAG CCC CGC GAT GAG AAG CCG TTC TGT GCT ACA TGT CGT GGG	328															
L R R R C Q R D A M C C P G T L C V N D	88															
TTG CGG AGG AGG TGC CAG CGA GAT GCC ATG TGC TGC CCT GGC ACA CTC TGT GTG AAC GAT	388															
V C T T M E D A T P I L E R Q L D E Q D	108															
GTT TGT ACT ACG ATG GAA GAT GCA ACC CCA ATA TTA GAA AGG CAG CTT GAT GAG CAA GAT	448															
G T H A E G T T G H P V Q E N Q P K R K	128															
GGC ACA CAT GCA GAA GGA ACA ACT GGG CAC CCA GTC CAG GAA AAC CAA CCC AAA AGG AAG	508															
P S I K K S Q G R K G Q E G E S C L R T	148															
CCA AGT ATT AAG AAA TCA CAA GGC AAG AAG GGA GAA AGT TGT CTG AGA ACT	568															

WO 00/52047

PCT/US00/05452

6/29

FIG. 2B

F	D	C	G	P	G	L	C	C	A	R	H	F	W	T	K	I	C	K	P	168
TTT	GAC	TGT	GGC	CCT	GGA	CTT	TGC	TGT	GCT	CGT	CAT	TTT	TGG	ACG	AAA	ATT	TGT	AAG	CCA	628
V	L	L	E	G	Q	V	C	S	R	R	G	H	K	D	T	A	Q	A	P	188
GTC	CTT	TTG	GAG	GGA	CAG	GTC	TGC	TCC	AGA	AGA	GAG	CAT	AAA	GAC	ACT	GCT	CAA	GCT	CCA	688
E	I	F	Q	R	C	D	C	G	P	G	L	L	C	R	S	Q	L	T	S	208
GAA	ATC	TTC	CAG	CGT	TGC	GAC	TGT	GGC	CCT	GGA	CTA	CTG	TGT	CGA	AGC	CAA	TTG	ACC	AGC	748
N	R	Q	H	A	R	L	R	V	C	Q	K	I	E	K	L	*				225
AAT	CGG	CAG	CAT	GCT	CGA	TTA	AGA	GTA	TGC	CAA	AAA	ATA	GAA	AAG	CTA	TAA				799
ATATTTCAAAATAAAGAGAAATCCACATTGCAAAAAATAAAAAAATAA																				848

FIG. 3A

GTCGACCCACGCGTCCGGACGCGGTGGCGGCACGGTTTCGTGGGACCCAGGCTTGCAAGTGACGGTCAATTTCTC																				79
TTTCTTTCTCCCTCTTGAGTCCTTCTGAG ATG ATG GCT CTG GGC GCA GCG GGA GCT ACC CGG GTC																				12 144
F	V	A	M	V	A	A	A	L	G	G	H	P	L	L	G	V	S	A	T	32
TTT	GTC	GCG	ATG	GTA	GCG	GCT	CTC	GGC	GGC	CAC	CCT	CTG	CTG	CTG	GGA	GTG	AGC	GCC	ACC	204
L	N	S	V	L	N	S	N	A	I	K	N	L	P	P	P	L	G	G	A	52
TTG	AAC	TCG	GTT	CTC	AAT	TCC	AAC	GCT	ATC	AAG	AAC	CTG	CCC	CCA	CCG	CTG	GGC	GGC	GCT	264
A	G	H	P	G	S	A	V	S	A	A	P	G	I	L	Y	P	G	G	N	72
GCG	GGG	CAC	CCA	GCG	TCT	GCA	GTC	AGC	GCC	GCG	CCG	GGA	ATC	CTG	TAC	CCG	GGC	GGG	AAT	324
K	Y	Q	T	I	D	N	Y	Q	P	Y	P	C	A	E	D	E	E	C	G	92
AAG	TAC	CAG	ACC	ATT	GAC	AAC	TAC	CAG	CCG	TAC	CCG	TGC	GCA	GAG	GAC	GAG	GAG	TGC	GGC	384
T	D	E	Y	C	A	S	P	T	R	G	G	D	A	G	V	Q	I	C	L	112
ACT	GAT	GAG	TAC	TGC	GCT	AGT	CCC	ACC	CGC	GGA	GGG	GAC	GCA	GGC	GTG	CAA	ATC	TGT	CTC	444
A	C	R	K	R	R	K	R	C	M	R	H	A	M	C	C	P	G	N	Y	132
GCC	TGC	AGG	AAG	CGC	CGA	AAA	CGC	TGC	ATG	CGT	CAC	GCT	ATG	TGC	TGC	CCC	GGG	AAT	TAC	504
C	K	N	G	I	C	V	S	S	D	Q	N	H	F	R	G	E	I	E	E	152
TGC	AAA	AAT	GGA	ATA	TGT	GTG	TCT	TCT	GAT	CAA	AAT	CAT	TTC	CGA	GGA	GAA	ATT	GAG	GAA	564
T	I	T	E	S	F	G	N	D	H	S	T	L	D	G	Y	S	R	R	T	172
ACC	ATC	ACT	GAA	AGC	TTT	GGT	AAT	GAT	CAT	AGC	ACC	TTG	GAT	GGG	TAT	TCC	AGA	AGA	ACC	624

WO 00/52047

PCT/US00/05452

8/29

FIG. 3B

T L S S K M Y H T K G Q E G S V C L R S 192
ACC TTG TCT TCA AAA ATG TAT CAC ACC AAA GGA CAA GAA GGT TCT TCT GTT TGT CTC CGG TCA 684

S D C A S G L C C A R H F W S K I C K P 212
TCA GAC TGT GCC TCA GGA TTG TGT TGT GCT AGA CAC TTC TGG TCC AAG ATC TGT AAA CCT 744

V L K E G Q V C T K H R R K G S H G L E 232
GTC CTG AAA GAA GGT CAA GTG TGT ACC AAG CAT AGG AGA AAA GGC TCT CAT GGA CTA GAA 804

I F Q R C Y C G E G L S C R I Q K D H H 252
ATA TTC CAG CGT TGT TAC TGT GGA GAA GGT CTG TCT TGC CGG ATA CAG AAA GAT CAC CAT 864

Q A S N S S R L H T C Q R H * 267
CAA GCC AGT AAT TCT TCT TCT AGG CTT CAC ACT TGT CAG AGA CAC TAA 909

ACCAGCTATCCAAATGCAGTGAACTCCTTTTATATAATAGATGCTATGAAAACCTTTTATGACCTTCATCAACTCAATC 988

CTAAGGATATACAAGTCTGTGTTTCAGTTAAGCATTCCTCAATAACACCTTCCAAAAACCTGGAGTGTAAGAGCTTTGT 1067

TTCTTTATGGAACTCCCCCTGTGATTGCAGTAAATTACTGTATTGTAAATTCTCAGTGTGGCAGCTTACCTGTAAATGCAA 1146

TGAAACTTTTAAATTATTTTCTAAAGGTGCTGCACCTGCCTATTTTTCCTCTTGTATGTAAATTTTGTACACATTGAT 1225

TGTTATCTTGACTGACAAATATTCTATATTGAACTGAAGTAAATCATTTTCAGCTTATAGTTCTTAAAGCATAAACCCTT 1304

TACCCCATTTAATTCTAGAGCTAGAACGCAAGGATCTCTTGGAATGACAAATGATAGGTACCTAAATGTAACATGAA 1383

AATACTAGCTTATTTTCTGAAATGTACTATCTTAATGCTTAAATTAATTTCCCTTTAGGCTGTGATAGTTTGTGAAAT 1462

AAAAATTTAACATTTAATATCATGAAATGTTATAAGTAGACATAAAAAAAAAGGGGGCCGC 1536

FIG. 4A-1

GTCGACCCACGCGTCCGGCGGAGCCCGCGGAGCGTAGCGCAAGTCCGCTCCCTAGGCATCGCTGCGCTGGCAGCGA 79
TTCGCTGTCTCTGTGAGTCAGGGACAAACGCTTCGGGGCAACTGTGAGTGCGCGTGTGGGGACCTCGATTCTCTTCA 158
GATCTCGAGGATTCGGTCCGGGACGTCTCCTGATCCCCCTACTAAAGCGCCTGCTAACTTTGAAAAGGACACTGTGTC 237
CTGCAAAGTTTGACACATAAAGGATAGGAAAAGAGAGGAGAGAAAGCAACTGAGTTGAAGAGAGGAGCTGATCGG 316
GCCTCCTGATCAATTAAGAGGAGATTAAACCGCCGAGATCCCGGCGGACCAAGGAGTGCGGGCAAGAAAGAACGG 395
AAGCGGTGCGATCCACAGGCTGGGTTTCTTGACCTTGGGTACGCCCTCCTTGCGGAGAAAGCGCCTCGCATTTGAT 474
TGCTTCCAGTTATTGCAGAACTTCCCTGTCTGGTGAGAACGGGTCTCGCTTGGGTTCCGCTAATTTCTGTCTGAGG 553
CGTGAGACTGAGTTCATAGGGTCCCTGGTCCCCGAAACCAGGAAGGTTGAGGGAACACAATCTGCAAGCCCCCGGACC 632
CAAGTGAGGGCCCCGTGTGGGTCCCTCCCTTTGCAATCCCAACCCCTCCGGCTTTGCGTCTTCTCTGGGACCC 711
M A A L M R S K D S S C L L L L L 17
CCTCGCCGGGAG ATG GCC GCG TTG ATG CGG AGC AAG GAT TCG TCC TGC TGC CTG CTC CTA CTG 774
A A V L M V E S S Q I G S S R A K L N S 37
GCC GCG GTG CTG ATG GTG GAG AGC TCA CAG ATC GGC AGT TCG CGG GCC AAA CTC AAC TCC 834
I K S S L G G E T P G Q A A N R S A G M 57
ATC AAG TCC TCT CTG GGC GGG GAG ACG CCT GGT CAG GCC GCG AAT CGA TCT GCG GGC ATG 894

WO 00/52047

PCT/US00/05452

10/29

FIG. 4A-2

Y	Q	G	L	A	F	G	G	S	K	K	G	K	N	L	G	Q	A	Y	P	77
TAC	CAA	GGA	CTG	GCA	TTC	GGC	GGC	AGT	AAG	AAG	GGC	AAA	AAC	CTG	GGG	CAG	GCC	TAC	CCT	954
C	S	S	D	K	E	C	E	V	G	R	Y	C	H	S	P	H	Q	G	S	97
TGT	AGC	AGT	GAT	AAG	GAG	TGT	GAA	GTT	GGG	AGG	TAT	TGC	CAC	AGT	CCC	CAC	CAA	GGA	TCA	1014
S	A	C	M	V	C	R	R	K	K	K	R	C	H	R	D	G	M	C	C	117
TCG	GCC	TGC	ATG	GTG	TGT	CGG	AGA	AAA	AAG	AAG	CGC	TGC	CAC	CGA	GAT	GGC	ATG	TGC	TGC	1074
P	S	T	R	C	N	N	G	I	C	I	P	V	T	E	S	I	L	T	P	137
CCC	AGT	ACC	CGC	TGC	AAT	AAT	GGC	ATC	TGT	ATC	CCA	GTT	ACT	GAA	AGC	ATC	TTA	ACC	CCT	1134
H	I	P	A	L	D	G	T	R	H	R	D	R	N	H	G	H	Y	S	N	157
CAC	ATC	CCG	GCT	CTG	GAT	GGT	ACT	CGG	CAC	AGA	GAT	CGA	AAC	CAC	GGT	CAT	TAC	TCA	AAC	1194
H	D	L	G	W	Q	N	L	G	R	P	H	T	K	M	S	H	I	K	G	177
CAT	GAC	TTG	GGA	TGG	CAG	AAT	CTA	GGA	AGA	CCA	CAC	ACT	AAG	ATG	TCA	CAT	ATA	AAA	GGG	1254
H	E	G	D	P	C	L	R	S	S	D	C	I	E	G	F	C	C	A	R	197
CAT	GAA	GGA	GAC	CCC	TGC	CTA	CGA	TCA	TCA	GAC	TGC	ATT	GAA	GGG	TTT	TGC	TGT	GCT	CGT	1314
H	F	W	T	K	I	C	K	P	V	L	H	Q	G	E	V	C	T	K	Q	217
CAT	TTC	TGG	ACC	AAA	ATC	TGC	AAA	CCA	GTG	CTC	CAT	CAG	GGG	GAA	GTC	TGT	ACC	AAA	CAA	1374
R	K	K	G	S	H	G	L	E	I	F	Q	R	C	D	C	A	K	G	L	237
CGC	AAG	AAG	GGT	TCT	CAT	GGG	CTG	GAA	ATT	TTC	CAG	CGT	TGC	GAC	TGT	GCG	AAG	GGC	CTG	1434
S	C	K	V	W	K	D	A	T	Y	S	S	K	A	R	L	H	V	C	Q	257
TCT	TGC	AAA	GTA	TGG	AAA	GAT	GCC	ACC	TAC	TCC	TCC	AAA	GCC	AGA	CTC	CAT	GTG	TGT	CAG	1494

WO 00/52047

PCT/US00/05452

11/29

FIG. 4A-3

K I * 260
AAA ATT TGA 1503

TCACCAATTGAGGAACATCATCAATTGCAGACTGTGAAGTTGTGTATTTAATGCATTATAGCATGGTGGAATAAGGTT 1582
CAGATGCCAGAAAGAAATGGCTAAATAAAGAAACCGTGAATAAGAAATATAGATGATCACAAAAGGGAGAGAAAGAAACATGAAC 1661
TGAATAGATTAGAAATGGGTGACAAATGCAGTGCAGCCAGTGTGTTCCATTATATGCACACTTGTCTATGTAAATAATGTACAC 1740

WO 00/52047

PCT/US00/05452

12/29

FIG. 4B-1

ATTGTGGAAAATGCTATTATTAAAGAAACAAGCACACAGTGGAAATTACTGATGAGTAGCATGTGACTTTCCTCAAGAGT 1819
TTAGGTTGTGCTGGAGGAGAGGTTTCCTTCAGATTGCTGATTGCTTATACAAATAACCTACATGCCAGATTTCATTCA 1898
ACGTTAGAGTTTAACAAAAATACTCCTAGAAATAACTTGTATACAAATAGGTTCTAAAAATAAAATTGCTAAACAAGAAAT 1977
GAAACATGGAGCATTGTTAATTACAAACAGAAAATTACCTTTTGATTGTGAACACTACTTCTGCTGTTCAATCAAGAG 2056
TCTTGGTAGATAAGAAAAAATCAGTCAATATTCCAAATAATTGC AAAATAATGGCCAGTTGTTTAGGAAGGCCCTTAA 2135
GGAAGACAAATAAATAACAAACAACAGCCACAAATACTTTTTTTTCAAAAATTTTAGTTTACCTGTAAATAAAGAA 2214
CTGATACAGACAAAACAGTTCCCTTCAGATTCTACGGAATGACAGTATATCTCTCTTTATCCCTATGTGATTCCTGCTC 2293
TGAATGCATTATATTTTCCAAAGTATACCCATAAAATTGTGACTAGTAAATACTTACACAGAGCAGAAATTTTCACAGAT 2372
GGCAAAAAAATTTAAAGATGTCCAATATATGTGGGAAAAGAGCTAACACAGAGAGATCATTTATTTCTTAAAGATTGGCCAT 2451
AACCTGTATTTTGATAGAAATTAGATTGGTAAATACATGTATTACATACACTCTGTGGTAAATAGAGACTTGAGCTGGAT 2530
CTGTACTGCACTGGAGTAAGCAAGAAAATTGGGAAAACCTTTTCGTTTGTTCAGGTTTGGCAACACATAGATCATATG 2609
TCTGAGGCACAAAGTTGGCTGTTTCATCTTTGAAACCAGGGGATGCACAGTCTAAATGAATATCTGCATGGGATTGCTAT 2688
CATAATNTTTCCTATGCNNGNTGAATTCNGTGTGAGGTCCTGTGTCCTATCCCTCAAAATTATTTATTTTATAGTGCT 2767
GAGATCCTCAAAATAATCTCAATTTTCGGAGGTTTCACAAAAATGGACTCCTGAAGTAGACAGAGTAGTGAGGTTTCATTGC 2846

WO 00/52047

PCT/US00/05452

FIG. 4B-2

13/29

CCTCTATAAGCTTCTGACTAGCCAAATGGCATCATCCAATTTTCTCTCCAAACCTCTGCAGCATCTGCTTTATTGCCCAA 2925

GGGCTAGTTTCGGGTTTCTGCCAGCCATTGCCGTTAAAAAATAAAGTAGATAAAGTGTAAACCTGCATATTGCTAA 3004

TCTATAGACACACAGTTTCTAAATTCTTTTGAAACCACTTTTACTACTTTTTTTAACTTAACTCAGTTCTAAATACTTT 3083

GTCTGGAGCACAAACAATAAAAGTTATCTTATAGTTGTGACTTTTAAACTTTTGTAGACCACAATTCTACTTTTGTAGTT 3162

TTC'TTTTACTTAAATCCCATCTGCAGTCTCAAATTTAAGTTCTCCAGTAGAGATTGAGTTTGAGCCTGTATATCTATT 3241

AAAAATTTCAACTTCCCACATATATTACTAAGATGATTAAGACTTACATTTTCTGCACAGGCTCGCAAAAACAATAAT 3320

TATAAACTAGTCCATCCAAGAACCAAGTTTGTATAAACAGGTTGCTATAAGCTTGGTGAAATGAAAAATGGAACATTTC 3399

AATCAAACATTTCCCTATATAACAATTAATATATTGCTTCTGCAATATTTTCTTATGTCCACCCTTTTAA 3478

AAATTATATTGGAAGTAATTATTTACAGGAAATGTTAATGAGATGTATTTTCTTATAGAGATAATTCTTACAGAAAG 3557

CTTTGTAGCAGATAATTTGCAGCTATTGACTTTGTAAATTTAGGAAAAATGTATAATAAGATAAAATCTATTAAATTT 3636

TTCTCCTCTAAAAACTGAAAAAATAAAAAAAGGGCGCGCCG 3687

FIG. 5A-1

14/29

FGTCGACCCACGCGTCCGCTGTGGCAGCCAGCTACCGGTGTCGTGACCATCCAGCTTGCAGCTCAGCTTTGTTTCATTC	79
GAATTGGCGCGCGCAGCGCGGAACAAC	12
ATG CAG CGG CTC GGG GGT ATT TTG CTG TGT ACA CTG	145
L A A A V P T A P A P S P T V T W T P A	32
CTG GCG GCG GCG GTC CCC ACT GCT CCT GCT CCT TCC CCG ACG GTC ACT TGG ACT CCG GCG	205
E P G P A L N Y P Q E E A T L N E M F R	52
GAG CCG GCG CCA GCT CTC AAC TAC CCT CAG GAG GAA GCT ACG CTC AAT GAG ATG TTT CGA	265
E V E E L M E D T Q H K L R S A V E E M	72
GAG GTG GAG GAG CTG ATG GAA GAC ACT CAG CAC AAA CTG CGC AGT GCC GTG GAG GAG ATG	325
E A E E A A K T S S E V N L A S L P P	92
GAG GCG GAA GAA GCA GCT GCT AAA ACG TCC TCT GAG GTG AAC CTG GCA AGC TTA CCT CCC	385
N Y H N E T S T E T R V G N N T V H V H	112
AAC TAT CAC AAT GAG ACC AGC ACG GAG ACC AGG GTG GGA AAT AAC ACA GTC CAT GTG CAC	445
Q E V H K I T N N Q S G Q V V F S E T V	132
CAG GAA GTT CAC AAG ATA ACC AAC AAC CAG AGT GGA CAG GTG GTC TTT TCT GAG ACA GTC	505
I T S V G D E E G K R S H E C I I D E D	152
ATT ACA TCT GTA GGG GAT GAA GAA GGC AAG AGG AGC CAT GAA TGT ATC ATT GAT GAA GAC	565
C G P T R Y C Q F S S F K Y T C Q P C R	172
TGT GGG CCC ACC AGG TAC TGC CAG TTC TCC AGC TTC AAG TAC ACC TGC CAG CCA TGC CGG	625

WO 00/52047

PCT/US00/05452

15/29

FIG. 5A-2

D	Q	Q	M	L	C	T	R	D	S	E	C	C	G	D	Q	L	C	A	W	192
GAC	CAG	CAG	ATG	CTA	TGC	ACC	CGA	GAC	AGT	GAG	TGC	TGT	GGA	GAC	CAG	CTG	TGT	GCC	TGG	685
G	H	C	T	Q	K	A	T	K	G	G	N	G	T	I	C	D	N	Q	R	212
GGT	CAC	TGC	ACC	CAA	AAG	GCC	ACC	AAA	GGT	GGC	AAT	GGG	ACC	ATC	TGT	GAC	AAC	CAG	AGG	745
D	C	Q	P	G	L	C	C	A	F	Q	R	G	L	L	F	P	V	C	T	232
GAT	TGC	CAG	CCT	GGC	CTG	TGT	TGT	GCC	TTC	CAA	AGA	GGC	CTG	CTG	TTC	CCC	GTG	TGC	ACA	805
P	L	P	V	E	G	E	L	C	H	D	P	T	S	Q	L	L	D	L	I	252
CCC	CTG	CCC	GTG	GAG	GGA	GAG	CTC	TGC	CAT	GAC	CCC	ACC	AGC	CAG	CTG	CTG	GAT	CTC	ATC	865
T	W	E	L	E	P	E	G	A	L	D	R	C	P	C	A	S	G	L	L	272
ACC	TGG	GAA	CTG	GAG	CCT	GAA	GGA	GCT	TTG	GAC	CGA	TGC	CCC	TGC	GCC	AGT	GGC	CTC	CTA	925
C	Q	P	H	S	H	S	L	V	Y	M	C	K	P	A	F	V	G	S	H	292
TGC	CAG	CCA	CAC	AGC	CAC	AGT	CTG	GTG	TAC	ATG	TGC	AAG	CCA	GCC	TTC	GTG	GGC	AGC	CAT	985
D	H	S	E	E	S	Q	L	P	R	E	A	P	D	E	Y	E	D	V	G	312
GAC	CAC	AGT	GAG	GAG	AGC	CAG	CTG	CCC	AGG	GAG	GCC	CCG	GAT	GAG	TAC	GAA	GAT	GTT	GGC	1045
F	I	G	E	V	R	Q	E	L	E	D	L	E	R	S	L	A	Q	E	M	332
TTC	ATA	GGG	GAA	GTG	CGC	CAG	GAG	CTG	GAA	GAC	CTG	GAG	CGG	AGC	CTA	GCC	CAG	GAG	ATG	1105

WO 00/52047

PCT/US00/05452

16/29

FIG. 5B-1

A	F	E	G	P	A	P	V	E	S	L	G	G	E	E	E	I	*	350
GCA	TTT	GAG	GGG	CCT	GCC	CCT	GTG	GAG	TCA	CTA	GGC	GGA	GAG	GAG	GAG	ATT	TAG	1159
GCCCAGACCCAGCTGAGTCAC	TGGTAGATGTGCAATAGAAATGGCTAATTTATTTTCCAGGAGTG	TCCCCCAAGTGTGG																1238
AATGGCCGAGCTCCTTCCAGTAGCTTTTCCCTCTGGCTTGACAAGGTACAGTGCAGTACATTTCTTCCAGCCGCCCTG																		1317
CTTCTCTGACTTGGGAAAGACAGGCATGGCCGGGTAAAGGCAGCGGTAGTGGTCCCTCGCTGTTGCTAGAAACGCTGTC																		1396
TTGTTCTTCATGGATGGAAGATTGTTTGAAGGAGAGGATGGGAAGGGTGAAAGTCTGCTCATGATGGATTTGGGGGA																		1475
TACAGGAGGAGGATGCCTGCTTGCAGACGTGGACTTGGCAAAATGTAAACCTTTTGCTTTTGTCTTGCGCCGCTCCCAT																		1554
GGGCTGAGGCAGTGGCTACACAAGAGCTATGCTGCTCTGTGGCCTCCACATATTCATCCCTGTGTGTTTCAGCTCCTACC																		1633
TCACTGTCAGCACAGCCCTTCATAGCCACGCCCTCTTGGCTCACCCACAGCCTAGGAGGGGACCAGAGGGGACTTCTCT																		1712
CAGAGCCCATGCTCTCTCTCAACCCCATACACGCCCTCTGTGCCAGCGACAGTCCCTTCCAAATGGAGGGAGTGAAAT																		1791
CCTTTGGTTTAAATTATTTTCTCCTTCAAGGCACGCCCTGCCACTAAGGTCAGGCTGACTTGCAATGTCCCTCTAACGTTCTG																		1870
TAGCAGTGTGGTGGACACTGTCTTCCACCGACTGCTTCAATACCTCTGAAAGCCAGTGCTCGGAGTGCGAGTTCGTGTAA																		1949
ATTAAATTGCAAGGAAGTATACTTGGCTAATTGTAGGGCTAGGATGTGAAATGAAATTGTGCAAAAGTCGCTTAGCAACAAT																		2028
GGAAAGCCCTTCTCAGTCACACCGAGAAGTCAACAACCAAGCCAGGTTGTGTAGAGTACAGCTGTGACATACAGACAGAA																		2107

WO 00/52047

PCT/US00/05452

FIG. 5B-2

GAAGGCTGGGCTGGATGTCAGGCCCTCAGATGACGGTTTCAGGTGCCAGGAACCTATTACCATTCCTGTATCTATCCAGAGT	2186
TATTAAATTGAAAGTTGCACACATTTGTATAAGCATGCCCTTCTCCTGAGTTTAAATTATATGTATACACAAACATG	2265
TGGCCCTCAAAGATCATGCACAAACCACCTACTCTTTTGCTAAATTCCTTGGACTTTTCTCTTTGATTTTCAATAATACAAA	2344
TCCCCCTTCATGCAAAAAAATAAGGGCGCCGC	2381

06

120

180

121	100
hdkk-1	GSAV... SA <u>APGILYPG</u> ..
mdkk-1	GSAV... SV <u>APGVLYEG</u> ..
xdkk-1	YPV... SV <u>SPDSLYDI</u> ..
hdkk-2	GQAA... NR <u>SAG.MYQGLA</u>
hdkk-3	HREIHKITNN <u>QTGQMVFSET</u>
mdkk-3	HQEVHKITNN <u>QSGQVVFSET</u>
cdkk-3	HQEIDKVTDN <u>RTGSTIFSET</u>
hdkk-4	SWLC... SP <u>LGALVLDENN</u>

19/29

FIG. 6B

hdkk-1	181	VQICLACRKR	RKRCMRHAMC	CPGNYCKNGI	CVS..SDQNH	F..RGEIET	240	ITESFGN.DH
mdkk-1		VQICLACRKR	RKRCMTHAMC	CPGNYCKNGI	OMP..SDHSH	FP..RGEIEES		IIENLGN.DH
xdkk-1		.LVCLACRKR	RKRCCLRDAMC	CTGNYCSNGI	CVPVEQDQER	FQHQGYLEET		I LENYNNADH
hdkk-2		...CMVCRRK	KKRCHRDGMC	CPSTRCNNGI	CIPV.TESIL	TPHIPALDGT		RHRD.RNHGH
hdkk-3		QYTCQPCRQ	RMLCTRDSEC	CGDQLCVWGH	CTKMAT			
mdkk-3		KYTCQPCRQ	QMLCTRDSEC	CGDQLCAWGH	CTQKAT			
cdkk-3		EYKCQPCKTQ	HTHCSRDEVC	CGDQLCVWGE	CRKATS			
hdkk-4		..FCATCRGL	RRRCQORDAMC	CPGTLCVNDV	CTTME.DATP	ILERQLDEQD		GTHAEGTTGH

hdkk-1	241	STL..DGYSR	RTTLSSKMYH	TKQEGSVCL	RSSDCASGLC	CA..RHEWSK	300	ICKPVLKEGQ
mdkk-1		NAAAGDGYPR	RTTLTSKIYH	TKQEGSVCL	RSSDCAAGLC	CA..RHEWSK		ICKPVLKEGQ
xdkk-1		ATM..DTHSK	LTTSPSGMQP	FKGRDGDVCL	RSTDCAPGLC	CA..RHEWSK		ICKPVLDEGQ
hdkk-2		YSNHDLGWQN	LGRPHTKMSH	IKGHEGDPCL	RSSDCIEGFC	CA..RHEWTK		ICKPVLHQGE
hdkk-3				..RGSNGTICD	NQDCCQPGLC	CAFQRGLLFP		VCTPLPVEGE
mdkk-3				..KGGNGTICD	NQDCCQPGLC	CAFQRGLLFP		VCTPLPVEGE
cdkk-3				..RGENGTICE	NQHD CNP GTC	CAFQKELLFP		VCTPLPEEGE
hdkk-4		PV..QENQPK	RKPSIKKSQG	RGQEGESCL	RTFDCGPGLC	CA..RHEWTK		ICKPVLLEGO

hdkk-1	301	VC...TKHRR	KG.SHGLE..	IFQRCYCCE	GLSCRIQK.D	HHQASNSSRL	360	HTCQRH~~~~
mdkk-1		VC...TKHRR	KG.SHGLE..	IFQRCYCCE	GLACRIQK.D	HHQASNSSRL		HTCQRH~~~~
xdkk-1		VC...TKHRR	KG.SHGLE..	IFQRCYCCE	GLSCRIQKGE	FTTVPKTSRL		HTCQRH~~~~
hdkk-2		VC...TKQRK	KG.SHGLE..	IFQRCDCAK	GLSCKVWKD.	..ATYSSKARL		HVCQKI~~~~
hdkk-3		LCHDPASRLI	DLITWELEPD	GALDRCPCAS	GLLCQPH.SH	SLVYVCKPTF		VGSRDQDGE.
mdkk-3		LCHDPTSQLL	DLITWELEPE	GALDRCPCAS	GLLCQPH.SH	SLVYMCKPAF		VGSHDHSEE.
cdkk-3		PCHDPSNRLL	NLITWELEPD	GVLERCPCAS	GLICQPQSSH	STTSVCELSS		NETRKNNEKED
hdkk-4		VC...SRRGH	KDTAQAPE..	IFQRCDCGP	GLLCRSQ LTS	NRQH...ARL		RVCQKIEKL~

20/29

FIG. 6C

	361		424
hdkk-1	~~~~~	~~~~~	~~~~~
mdkk-1	~~~~~	~~~~~	~~~~~
xdkk-1	~~~~~	~~~~~	~~~~~
hdkk-2	~~~~~	~~~~~	~~~~~
hdkk-3		ILLPREVPDE YEVGSFMEEV RQELEDLERS LTEEMALGEP AAAAAALLGGEET~	~~~~~
mdkk-3		SQLPREAPDE YEDVGFIGEV RQELEDLERS LAQEMAFEGP APVES..LGGEETI	~~~~~
cdkk-3	PLNMDEMPFI	SLIPRDILSD YEESSVIQEV RKELESLE..DQAGVKSEH DPAHDLFLGDEI~	~~~~~
hdkk-4	~~~~~	~~~~~	~~~~~

WO 00/52047

PCT/US00/05452

21/29

FIG. 7A

CTCGAGCCAAATTCGGCACGAGGCCGGCTGTGGTCTAGCATAAAGCGGAGCCAGAAAGGGCGGGGT																														ATG			77																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
G	E	A	S	P	P	A	P	A	R	R	H	L	L	V	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L

WO 00/52047

PCT/US00/05452

22/29

FIG. 7B

D	A	L	E	G	G	H	W	L	S	E	K	R	H	R	L	Q	A	I	R	201
GAT	GCC	CTG	GAG	GGC	GGC	CAC	TGG	CTC	AGC	GAG	AAG	CGA	CAC	CGC	CTG	CAG	GCC	ATC	CGG	677
D	G	L	R	K	G	T	H	K	D	V	L	E	E	G	T	E	S	S	S	221
GAT	GGA	CTC	CGC	AAG	GGG	ACC	CAC	AAG	GAC	GTC	CTA	GAA	GAG	GGG	ACC	GAG	AGC	TCC	TCC	737
H	S	R	L	S	P	R	K	T	H	L	L	Y	I	L	R	P	S	R	Q	241
CAC	TCC	AGG	CTG	TCC	CCC	CGA	AAG	ACC	CAC	TTA	CTG	TAC	ATC	CTC	AGG	CCC	TCT	CGG	CAG	797
L	*																			243
CTG	TAG																			803
																				882
																				928

WO 00/52047

PCT/US00/05452

23/29

FIG. 8A

M C R L R																	5			
GAATTTCGGCAGGCGAGGCGCGAATGAAGGCAAGCCTCCACCCACCTGCA ATG TGT CGA CTG AGG																		71		
V	L	L	L	L	L	P	L	A	F	V	S	S	A	L	P	I	H	D	25	
GTC	TTG	CTG	CTG	CTG	CTC	CCC	TTG	GCC	TTC	GTG	TCC	TCC	TCT	GCT	CTC	CCC	ATC	CAT	GAT	131
V	D	S	Q	Q	N	T	S	G	F	L	G	L	Q	R	L	L	Q	S	F	45
GTC	GAC	TCT	CAG	CAG	AAC	ACC	TCC	GGG	TTC	CTG	GGC	CTT	CAG	AGG	CTT	CTC	CAA	AGC	TTT	161
S	R	L	F	L	K	N	D	L	L	R	D	L	D	N	F	F	S	S	P	65
AGT	CGA	CTG	TTC	CTA	AAA	AAT	GAC	CTG	CTA	CGA	GAC	CTG	GAC	AAC	TTC	TTC	TCC	TCC	CCC	251
M	D	F	R	D	L	P	R	N	F	H	Q	E	E	N	Q	E	H	R	M	85
ATG	GAC	TTC	CGA	GAC	CTT	CCT	AGG	AAC	TTC	CAT	CAG	GAA	GAG	AAC	CAG	GAG	CAC	AGA	ATG	311
G	N	H	T	L	S	S	H	L	Q	I	D	K	V	T	D	N	Q	T	G	105
GGC	AAC	CAT	ACC	CTC	TCC	AGC	CAC	CTA	CAG	ATA	GAC	AAG	GTG	ACT	GAC	AAC	CAG	ACA	GGG	371
E	V	H	I	S	E	K	V	E	A	S	I	E	P	E	R	N	P	E	G	125
GAG	GTG	CAC	ATC	TCG	GAG	AAA	GTC	GAG	GCC	TCC	ATT	GAG	CCA	GAA	CGG	AAC	CCG	GAA	GGG	431
D	W	K	V	P	K	V	E	A	K	E	P	P	V	P	V	Q	K	V	T	145
GAC	TGG	AAG	GTT	CCC	AAA	GTA	GAA	GCA	AAA	GAG	CCC	CCG	GTG	CCT	GTG	CAG	AAG	GTC	ACC	491
D	S	L	H	P	E	P	R	Q	V	A	F	W	I	M	K	M	P	R	R	165
GAC	AGC	TTG	CAC	CCA	GAG	CCC	CGG	CAG	GTG	GCT	TTC	TGG	ATC	ATG	AAG	ATG	CCA	AGG	CGG	551

WO 00/52047

PCT/US00/05452

24/29

FIG. 8B

R	T	Q	P	D	V	Q	D	G	G	R	W	L	I	E	K	R	H	R	M	185
AGG	ACC	CAG	CCC	GAT	GTC	CAG	GAT	GGA	GGC	CGC	TGG	CTC	ATA	GAA	AAG	CGA	CAT	CGC	ATG	611
Q	A	I	R	D	G	L	R	G	G	A	R	E	D	S	L	E	D	G	V	205
CAG	GCC	ATC	CGG	GAT	GGG	CTC	CGT	GGA	GGC	GCC	CGT	GAG	GAC	AGC	CTG	GAG	GAT	GGG	GTC	671
H	I	P	Q	H	A	K	L	P	V	R	K	T	H	F	L	Y	I	L	R	225
CAT	ATC	CCC	CAA	CAC	GCC	AAG	CTG	CCT	GTC	AGA	AAG	ACA	CAC	TTT	CTC	TAC	ATC	CTC	AGG	731
P	S	Q	Q	L	*															231
CCA	TCC	CAA	CAG	CTG	TAA	GTGGG	GAC	CAG	ATGTCC	CAAC	CCTAC	CCCA	CCCA	CCAT	ATGG	AAATA	AAAG	TTTTC		805
TTAC	ATCT	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	AAAA	835

FIG. 9

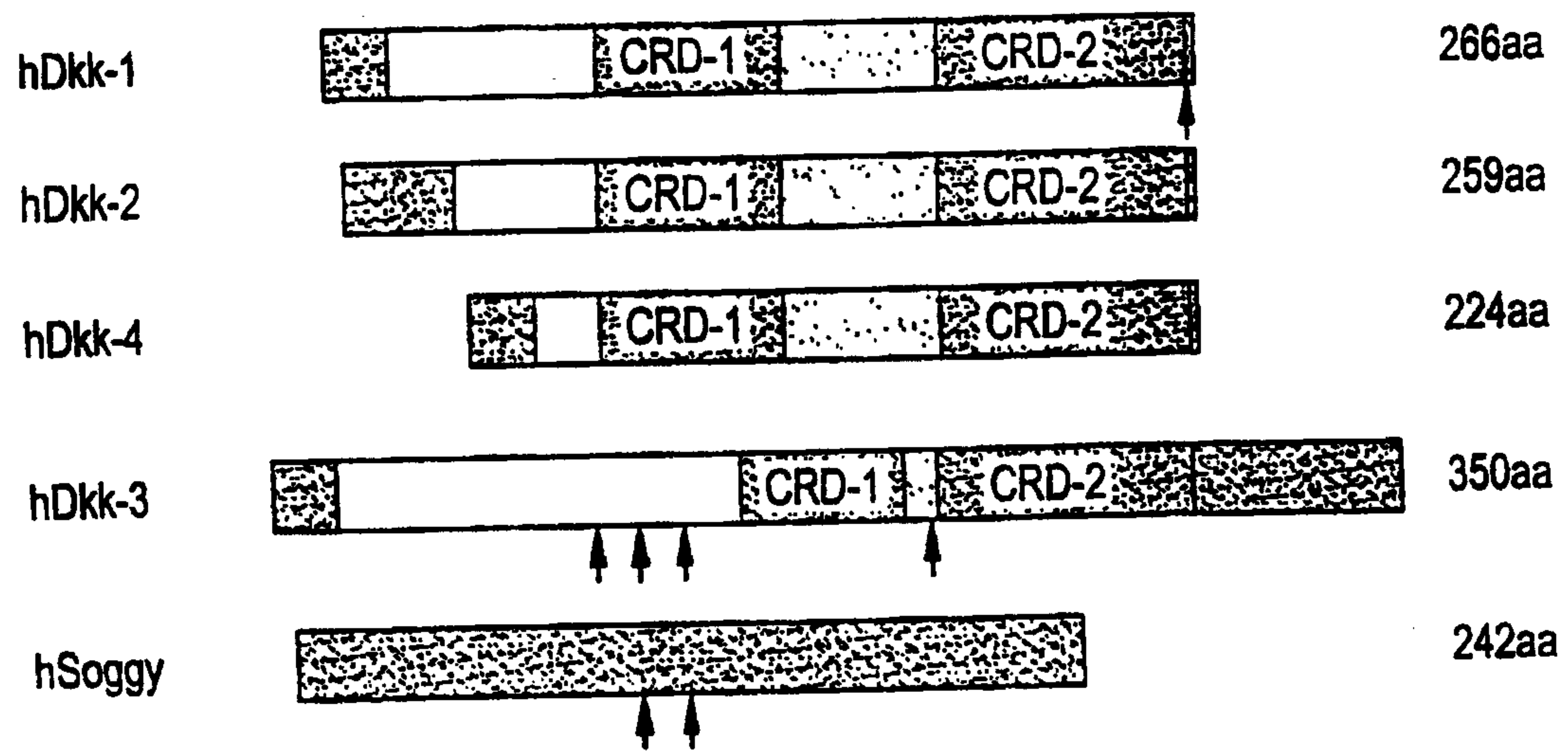


FIG. 10A

hsoggy msoggy hdkk-3 mdkk-3	1	MGEASPPAPA RRHL.LVLLL LLSTLVIPSA AAPIHDAQAQ	60
		~~~~~ MCRL.RVLLL LLPLAFVSSS ALPIHDVDSQ	...LTGLQSL
		~~~~~ MQLGATLLC LLAAAVPTA PAPAPTATSA	...FLGLQRL
		~~~~~ MQLGGILLC TLLAAAVPTA PAPSPVTWT	PVKPGPALSY PQEEATLNEM
			PQEEATLNEM *
hsoggy msoggy hdkk-3 mdkk-3	61	LQGSRLF.. LKGNLLRGID SL..... FSAPMDFRGL	120
		LQGSRLF.. LKNDLLRDLD NF..... FSFPMDFRDL	PNGYHKEENQ EHQLGNNTLS
		FREVEELMED TQHKLRSAVE EMEAEAAAK	PRNFHQEENQ EHRMGNHTLS
		FREVEELMED TQHKLRSAVE EMEAEAAAK	PPSYHNETNT DTKVGNNTIH
			PPNYHNETST ETRVGNNTVH *
hsoggy msoggy hdkk-3 mdkk-3	121	SHLQIDKMTD NKTGEVLISE NVVASIQPAE	180
		SHLQIDKMTD NKTGEVHISE KVEASIEP.E RNPEGDWKVP	RMEKEALVP IQKATDSFHT
		VHREIHKITN NQTGQMVFE TVITSVGDEE GR.....	KVEAKEPPVP VQKVTDLSHP
		VHQEVBHKITN NQSGQVVFSE TVITSVGDEE GK.....	..RSHECII. ....DEDCCP
			..RSHECII. ....DEDCCP *
hsoggy msoggy hdkk-3 mdkk-3	181	ELHPR.VAFW IIKLPRRRSH ....QDALEG	240
		E..PRQVAFW IMKMPRRRTQ ....PDVQDG	LQAIRDGLRK ...GTHKD..
		SMYCQFASFQ YTCQPCRGQR MLCTRDSECC	MQAIRDGLRG ...GARED..
		TRYCQFSSEK YTCQPCRDQQ MLCTRDSECC	TKMATRGNG TICDNQRDCQ
			TQKATKGNG TICDNQRDCQ *

FIG. 10B

hsoggy	241	.....VLEEG TESSHSRLS PRKTHLLYIL RPSRQL	300
msoggy		.....SLEDG VHQPQAKLP VRKTHFLYIL RPSQQL	
hdkk-3		PGLCCAFQRG LLEFVCTPLP V.EGELCH.. DPASRLLDLI TWELEPDGAL DRCPCASGLL	
mdkk-3		PGLCCAFQRG LLEFVCTPLP V.EGELCH.. DPTSQLLDLI TWELEPEGAL DRCPCASGLL	
		+	
		+	
hdkk-3	301	CQPHSHSLVY VCKPTFVGSR DQDGEILLPR EVPDEYEVGS FMEEVRQELE DLESLTEEM	360
mdkk-3		CQPHSHSLVY MCKPAFVGSH DHSEESQLPR EAPDEYEDVG FIGEVRQELE DLESLAQEM	
		+	
		+	
hdkk-3	361	ALGEPAAAAA ALLGGEET~	379
mdkk-3		AFEGPAPVES ..LGGEETI	

**FIG. 11**

1	TKGQEGSVCL	RSSDCASGLC	CA..RHEWSK	ICKPVLKEGQ	VCTKHRRK..	.....GSHGL	EIFORCYCGE	GLSCRIQKDH	HQASNSSRLH	TCQRH~~~~
hdkk-1	IKGHEGDPC	L RSSDCIEG	EC CA..RHEWTK	ICKPVLHQGE	VCTKQRKK..	.....GSHGL	EIFORCDCAK	GLSCKVWKD.	ATYSSKARLH	VCQKI~~~~
hdkk-2	TRGSNGTICD	NQORDCQPLC	CAFQRCGLLEP	VCTPLPVEGE	LCHDPASRL	DLITWELEPD	GALDRCPCAS	GLLC.....	.QPHSHSLVY	VCKPTFVGSR
hdkk-3	RKGQEGESCL	RTFDCGPGLC	CA..RHEWTK	ICKPVLLEGG	VCSSRRGHK..	....DTAQAP	EIFORCDCGP	GLLCRSQLTS	NR..QHARLR	VCQKIEKL~~
hdkk-4	INLENGELCM	NSAQCKSN.C	COHSSALGLA	RCTSMASENS	ECSVKTL...	.....Y	GIYKPCPER	GLTCEGDKTI	VGSITNTNFG	ICHDACRSKQ
colipase	G C	C C	C C	C	C		C C	GL C		C

**FIG. 12**

