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(54) Title: AW755252-INTERACTING PROTEINS AND USE THEREOF

(57) Abstract: Protein complexes are provided comprising AW755252 and one or more proteins selected from the group consisting of GROUP1. Methods of using the protein complexes in diagnosing diseases and disorders are also provided. In addition, the protein complexes are also useful in screening assays for identifying compounds effective in treating and/or preventing diseases and disorders associated with AW755252 and its interactors.



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AW755252-INTERACTING PROTEINS AND USE THEREOF

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Related Applications

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application 60/406,613, filed August 28, 2002 and U.S. Provisional Application
10 60/411,657 filed September 18, 2002, the entireties of which are incorporated herein by reference.

Field of the Invention

The present invention generally relates to protein-protein interactions,
15 particularly to protein complexes formed by protein-protein interactions and methods of use thereof.

Background of the Invention

The prolific output from numerous genomic sequencing efforts, including the Human Genome Project, is creating an ever-expanding foundation for large-scale
20 study of protein function. Indeed, this emerging field of proteomics can appropriately be viewed as a bridge that connects DNA sequence information to the physiology and pathology of intact organisms. As such, proteomics – the large-scale study of protein function – will likely be starting point for the development of many future pharmaceuticals. The efficiency of drug development will therefore depend
25 on the diversity and robustness of the methods used to elucidate protein function, i.e., the proteomic tools, that are available.

Several approaches are generally known in the art for studying protein function. One method is to analyze the DNA sequence of a particular gene and the amino acid sequence coded by the gene in the context of sequences of genes with
30 known functions. Generally, similar functions can be predicted based on sequence homologies. This “homology method” has been widely used, and powerful computer programs have been designed to facilitate homology analysis. *See, e.g., Altschul et al., Nucleic Acids Res., 25:3389-3402 (1997).* However, this method is

useful only when the function of a homologous protein is known.

Another useful approach is to interfere with the expression of a particular gene in a cell or organism and examine the consequent phenotypic effects. For example, Fire *et al.*, *Nature*, 391:806-811 (1998) disclose an "RNA interference" assay in which double-stranded RNA transcripts of a particular gene are injected into cells or organisms to determine the phenotypes caused by the exogenous RNA. Alternatively, transgenic technologies can be utilized to delete or "knock out" a particular gene in an organism and the effect of the gene knockout is determined. See e.g., Winzeler *et al.*, *Science*, 285:901-906 (1999); Zambrowicz *et al.*, *Nature*, 392:608-611 (1998). The phenotypic effects resulting from the disruption of expression of a particular gene can shed some light on the functions of the gene. However, the techniques involved are complex and the time required for a phenotype to appear can be long, especially in animals. In addition, in many cases disruption of a particular gene may not cause any detectable phenotypic effect.

Gene functions can also be uncovered by genetic linkage analysis. For example, genes responsible for certain diseases may be identified by positional cloning. Alternatively, gene function may be inferred by comparing genetic variations among individuals in a population and correlating particular phenotypes with the genetic variations. Such linkage analyses are powerful tools, particularly when genetic variations exist in a traceable population from which samples are readily obtainable. However, readily identifiable genetic diseases are rare and samples from a large population with genetic variations are not easily accessible. In addition, it is also possible that a gene identified in a linkage analysis does not contribute to the associated disease or symptom but rather is simply linked to unknown genetic variations that cause the phenotypic defects.

With the advance of bioinformatics and publication of the full genome sequence of many organisms, computational methods have also been developed to assign protein functions by comparative genome analysis. For example, Pellegrini *et al.*, *Proc. Natl. Acad. Sci. USA* 96:4285-4288 (1999) discloses a method that constructs a "phylogenetic profile," which summarizes the presence or absence of a particular protein across a number of organisms as determined by analyzing the genome sequences of the organisms. A protein's function is predicted to be linked to

another protein's function if the two proteins share the same phylogenetic profile. Another method, the Rosetta Stone method, is based on the theory that separate proteins in one organism are often expressed as separate domains of a fusion protein in another organism. Because the separate domains in the fusion protein are
5 predictably associated with the same function, it can be reasonably predicted that the separate proteins are associated with same functions. Therefore, by discovering separate proteins corresponding to a fusion protein, i.e., the "Rosetta Stone sequence," functional linkage between proteins can be established. See Marcotte *et al.*, *Science*, 285:751-753 (1999); Enright *et al.*, *Nature*, 402:86-90 (1999). Another
10 computational method is the "gene neighbor method." See Dandekar *et al.*, *Trends Biochem. Sci.*, 23:324-328 (1998); Overbeek *et al.*, *Proc. Natl. Acad. Sci. USA* 96:2896-2901 (1999). This method is based on the likelihood that if two genes are found to be neighbors in several different genomes, the proteins encoded by the genes share a common function.

15 While the methods described above are useful in analyzing protein functions, they are constrained by various practical limitations such as unavailability of suitable samples, inefficient assay procedures, and limited reliability. The computational methods are useful in linking proteins by function. However, they are only applicable to certain proteins, and the linkage maps established therewith are sketchy.
20 That is, the maps lack specific information that describes how proteins function in relation to each other within the functional network. Indeed, none of the methods places the identified protein functions in the context of protein-protein interactions.

In contrast with the traditional view of protein function, which focuses on the action of a single protein molecule, a modern expanded view of protein function
25 defines a protein as an element in an interaction network. See Eisenberg *et al.*, *Nature*, 405:823-826 (2000). That is, a full understanding of the functions of a protein will require knowledge of not only the characteristics of the protein itself, but also its interactions or connections with other proteins in the same interacting network. In essence, protein-protein interactions form the basis of almost all biological
30 processes, and each biological process is composed of a network of interacting proteins. For example, cellular structures such as cytoskeletons, nuclear pores, centrosomes, and kinetochores are formed by complex interactions among a multitude

of proteins. Many enzymatic reactions are associated with large protein complexes formed by interactions among enzymes, protein substrates, and protein modulators. In addition, protein-protein interactions are also part of the mechanisms for signal transduction and other basic cellular functions such as DNA replication, transcription, and translation. For example, the complex transcription initiation process generally requires protein-protein interactions among numerous transcription factors, RNA polymerase, and other proteins. *See e.g., Tjian and Maniatis, Cell, 77:5-8 (1994).*

Because most proteins function through their interactions with other proteins, if a test protein interacts with a known protein, one can reasonably predict that the test protein is associated with the functions of the known protein, e.g., in the same cellular structure or same cellular process as the known protein. Thus, interaction partners can provide an immediate and reliable understanding towards the functions of the interacting proteins. By identifying interacting proteins, a better understanding of disease pathways and the cellular processes that result in diseases may be achieved, and important regulators and potential drug targets in disease pathways can be identified.

There has been much interest in protein-protein interactions in the field of proteomics. A number of biochemical approaches have been used to identify interacting proteins. These approaches generally employ the affinities between interacting proteins to isolate proteins in a bound state. Examples of such methods include coimmunoprecipitation and copurification, optionally combined with cross-linking to stabilize the binding. Identities of the isolated protein interacting partners can be characterized by, e.g., mass spectrometry. *See e.g., Rout et al., J. Cell. Biol., 148:635-651 (2000); Houry et al., Nature, 402:147-154 (1999); Winter et al., Curr. Biol., 7:517-529 (1997).* A popular approach useful in large-scale screening is the phage display method, in which filamentous bacteriophage particles are made by recombinant DNA technologies to express a peptide or protein of interest fused to a capsid or coat protein of the bacteriophage. A whole library of peptides or proteins of interest can be expressed and a bait protein can be used to screening the library to identify peptides or proteins capable of binding to the bait protein. *See e.g., U.S. Patent Nos. 5,223,409; 5,403,484; 5,571,698; and 5,837,500.* Notably, the phage display method only identifies those proteins capable of interacting in an *in*

vitro environment, while the coimmunoprecipitation and copurification methods are not amenable to high throughput screening.

The yeast two-hybrid system is a genetic method that overcomes certain shortcomings of the above approaches. The yeast two-hybrid system has proven to
5 be a powerful method for the discovery of specific protein interactions *in vivo*. *See generally*, Bartel and Fields, eds., *The Yeast Two-Hybrid System*, Oxford University Press, New York, NY, 1997. The yeast two-hybrid technique is based on the fact that the DNA-binding domain and the transcriptional activation domain of a transcriptional activator contained in different fusion proteins can still activate gene
10 transcription when they are brought into proximity to each other. In a yeast two-hybrid system, two fusion proteins are expressed in yeast cells. One has a DNA-binding domain of a transcriptional activator fused to a test protein. The other, on the other hand, includes a transcriptional activating domain of the transcriptional activator fused to another test protein. If the two test proteins interact with each
15 other *in vivo*, the two domains of the transcriptional activator are brought together reconstituting the transcriptional activator and activating a reporter gene controlled by the transcriptional activator. *See, e.g.*, U.S. Patent No. 5,283,173.

Because of its simplicity, efficiency and reliability, the yeast two-hybrid system has gained tremendous popularity in many areas of research. In addition,
20 yeast cells are eukaryotic cells. The interactions between mammalian proteins detected in the yeast two-hybrid system typically are bona fide interactions that occur in mammalian cells under physiological conditions. As a matter of fact, numerous mammalian protein-protein interactions have been identified using the yeast two-hybrid system. The identified proteins have contributed significantly to the
25 understanding of many signal transduction pathways and other biological processes. For example, the yeast two-hybrid system has been successfully employed in identifying a large number of novel mammalian cell cycle regulators that are important in complex cell cycle regulations. Using known proteins that are important in cell cycle regulation as baits, other proteins involved in cell cycle control
30 were identified by virtue of their ability to interact with the baits. *See generally*, Hannon *et al.*, in *The Yeast Two-Hybrid System*, Bartel and Fields, eds., pages 183-196, Oxford University Press, New York, NY, 1997. Examples of mammalian cell cycle

regulators identified by the yeast two-hybrid system include CDK4/CDK6 inhibitors (e.g., p16, p15, p18 and p19), Rb family members (e.g., p130), Rb phosphatase (e.g., PP1- α 2), Rb-binding transcription factors (e.g., E2F-4 and E2F-5), General CDK inhibitors (e.g., p21 and p27), CAK cyclin (e.g., cyclin H), and CDK Thr161
5 phosphatase (e.g., KAP and CDI1). *See id* at page 192. "The two-hybrid approach promises to be a useful tool in our ongoing quest for new pieces of the cell cycle puzzle." *See id* at page 193.

The yeast two-hybrid system can be employed to identify proteins that interact with a specific known protein involved in a disease pathway, and thus provide
10 valuable understandings of the disease mechanism. The identified proteins and the protein-protein interactions they participate are potential drug targets for use in identifying new drugs for treating the disease.

Summary of the Invention

The inventor of the present invention has discovered using the yeast
15 two-hybrid system that AW755252 specifically interacts with GROUP1. The specific interactions between these proteins and AW755252 suggest that AW755252 and the AW755252-interacting proteins may be involved in the same biological processes. In addition, the interactions between such AW755252-interacting proteins and AW755252 may lead to the formation of protein complexes both in vitro and in
20 vivo, which contain AW755252 and one or more of the AW755252-interacting proteins. The protein complexes formed under physiological conditions may mediate the functions and biological activities of AW755252 and GROUP1 proteins. For example, they are believed to be involved in cardiac function. Thus, the AW755252-interacting proteins and the protein complexes are potential drug targets
25 for the development of drugs useful in treating or preventing diseases and disorders associated with the AW755252-containing protein complexes or a protein member thereof, or with abnormal cardiac function.

In accordance with a first aspect of the present invention, isolated protein complexes are provided comprising AW755252 and one or more
30 AW755252-interacting proteins selected from the group consisting of GROUP1. In addition, homologues, derivatives, and fragments of AW755252 and of the

AW755252-interacting proteins may also be used in forming protein complexes. In a specific embodiment, fragments of AW755252 and the AW755252-interacting proteins corresponding to the protein domains responsible for the interaction between AW755252 and the AW755252-interacting proteins are used in forming a protein
5 complex of the present invention. In yet another embodiment, a protein complex is provided from a hybrid protein, which comprises AW755252 or a homologue, derivative, or fragment thereof covalently linked, directly or through a linker, to a AW755252-interacting protein selected from the group consisting of GROUP1 or a homologue, derivative, or fragment thereof.

10 The protein complexes can be prepared by isolation or purification from tissues and cells or produced by recombinant expression of their protein members. The protein complexes can be incorporated into a protein microchip or microarray, which are useful in large-scale high throughput screening assays involving the protein complexes.

15 In accordance with a second aspect of the invention, antibodies are provided which are immunoreactive with a protein complex of the present invention. In one embodiment, an antibody is selectively immunoreactive with a protein complex of the present invention. In another embodiment, a bifunctional antibody is provided which has two different antigen binding sites, each being specific to a different
20 interacting protein member in a protein complex of the present invention. The antibodies of the present invention can take various forms including polyclonal antibodies, monoclonal antibodies, chimeric antibodies, antibody fragments such as Fv fragments, single-chain Fv fragments (scFv), Fab' fragments, and F(ab')₂ fragments. Preferably, the antibodies are partially or fully humanized antibodies.
25 The antibodies of the present invention can be readily prepared using procedures generally known in the art. For example, recombinant libraries such as phage display libraries and ribosome display libraries may be used to screen for antibodies with desirable specificities. In addition, various mutagenesis techniques such as site-directed mutagenesis and PCR diversification may be used in combination with
30 the screening assays.

The present invention also provides detection methods for determining whether there is any aberration in a patient with respect to a protein complex having

AW755252 and one or more AW755252-interacting protein selected from the group consisting of GROUP1. In one embodiment, the method comprises detecting an aberrant level of the protein complexes of the present invention. Alternatively, the levels of one or more interacting protein members (at protein or cDNA or mRNA
5 level) of a protein complex of the present invention are measured. In addition, the cellular localization, or tissue or organ distribution of a protein complex of the present invention is determined to detect any aberrant localization or distribution of the protein complex. In another embodiment, mutations in one or more interacting protein members of a protein complex of the present invention can be detected. In
10 particular, it is desirable to determine whether the interacting protein members have any mutations that will lead to, or in disequilibrium with, changes in the functional activity of the proteins or changes in their binding affinity to other interacting protein members in forming a protein complex of the present invention. In yet another embodiment, the binding constant of the interacting protein members of one or more
15 protein complexes is determined.

A kit may be used for conducting the detection methods of the present invention. Typically, the kit contains reagents useful in any of the above-described embodiments of the detection methods, including, e.g., antibodies specific to a protein complex of the present invention or interacting members thereof, and oligonucleotides
20 selectively hybridizable to the cDNAs or mRNAs encoding one or more interacting protein members of a protein complex. The detection methods may be useful in diagnosing a disease or disorder such as ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris, staging the disease or disorder, and identifying a predisposition to the disease or
25 disorder.

The present invention also provides screening methods for selecting modulators of a protein complex formed between AW755252 or a homologue, derivative or fragment thereof and an AW755252-interacting protein selected from the group consisting of GROUP1 or a homologue, derivative, or fragment thereof.
30 Screen methods are also provided for selecting modulators of an AW755252-interacting protein selected from the group consisting of GROUP1. The compounds identified in the screening methods of the present invention can be used in

modulating the functions or activities of AW755252, the AW755252-interacting proteins, or the protein complexes of the present invention. They may also be effective in modulating the cellular functions involving AW755252, AW755252-interacting proteins or AW755252-containing protein complexes, and in preventing or ameliorating diseases or disorders such as ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris. Thus, test compounds may be screened in an in vitro binding assay to identify compounds capable of binding a protein complex of the present invention or AW755252 or a AW755252-interacting protein identified in accordance with the present invention or a homologue, derivative or fragment thereof. In addition, in vitro dissociation assays may also be employed to select compounds capable of dissociating the protein complexes identified in accordance with the present invention. An in vitro screening assay may also be used to identify compounds that trigger or initiate the formation of, or stabilize, a protein complex of the present invention. In preferred embodiments, in vivo assays such as yeast two-hybrid assays and various derivatives thereof, preferably reverse two-hybrid assays, are utilized in identifying compounds that interfere with or disrupt protein-protein interactions between AW755252 or a homologue, derivative or fragment thereof and a AW755252-interacting protein or a homologue, derivative or fragment thereof. In addition, systems such as yeast two-hybrid assays are also useful in selecting compounds capable of triggering or initiating, enhancing or stabilizing protein-protein interactions between AW755252 or a homologue, derivative or fragment thereof and a AW755252-interacting protein selected from the group consisting of GROUP1 or a homologue, derivative or fragment thereof.

In accordance with yet another aspect of the present invention, methods are provided for modulating the functions and activities of a AW755252-containing protein complex of the present invention, or interacting protein members thereof. The methods may be used in treating or preventing diseases and disorders such as ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris. In one embodiment, the methods comprise reducing the protein complex level and/or inhibiting the functional activities of the protein complex. Alternatively, the level and/or activity of AW755252 or one of the AW755252-interacting proteins may be inhibited. Thus, the methods may include

administering to a patient an antibody specific to a protein complex or AW755252 or a AW755252-interacting protein, an antisense oligo or ribozyme selectively hybridizable to a gene or mRNA encoding AW755252 or a AW755252-interacting protein, or a compound identified in a screening assay of the present invention. In addition, gene therapy methods may also be used in reducing the expression of the gene encoding AW755252 or an AW755252-interacting protein.

In another embodiment, the method for modulating the functions and activities of a AW755252-containing protein complex of the present invention or interacting protein members thereof comprise increasing the protein complex level and/or activating the functional activities of the protein complex. Alternatively, the level and/or activity of one of the AW755252-interacting proteins or AW755252 may be increased. Thus, a particular AW755252-containing protein complex, AW755252 or a AW755252-interacting protein of the present invention may be administered directly to a patient. Or, exogenous genes encoding one or more protein members of an AW755252-containing protein complex may be introduced into a patient by gene therapy techniques. In addition, a patient needing treatment or prevention may also be administered with compounds identified in a screening assay of the present invention capable of triggering or initiating, enhancing or stabilizing protein-protein interactions between AW755252 or a homologue, derivative or fragment thereof and a AW755252-interacting protein selected from the group consisting of GROUP1, or a homologue, derivative or fragment thereof.

The present invention also provides cell and animal models in which one or more of the AW755252-containing protein complexes identified in the present invention are in an aberrant form, e.g., increased or decreased level of the protein complexes, altered interaction between interacting protein members of the protein complexes, and/or altered distribution or localization (e.g., in organs, tissues, cells, or cellular compartments) of the protein complexes. Such cell and animal models are useful tools for studying the disorders and diseases caused by the protein complex aberrations and for testing various methods for treating the diseases and disorders.

The foregoing and other advantages and features of the invention, and the manner in which the same are accomplished, will become more readily apparent upon consideration of the following detailed description of the invention taken in

conjunction with the accompanying examples, which illustrate preferred and exemplary embodiments.

Brief Description of the Drawings

- Figure 1-Northern blotting analysis of AW755252 RNA expression.
- 5 Figure 2- Partial Amino Acid Sequence (AW755252) (SEQ ID NO: 10)
- Figure 3-Full-length Amino Acid Sequence (mFHL2) (SEQ ID NO: 11)
- Figure 4-Partial Amino Acid Sequence (mPN34854) (SEQ ID NO: 7)
- Figure 5- Full-length Amino Acid Sequence (mPRPH1) (SEQ ID NO: 12)
- Figure 6-Full-length Amino Acid Sequence (mTCTEX-1) (SEQ ID NO: 9)
- 10 Figure 7- Partial Nucleotide Sequence (mPN34854) (SEQ ID NO: 13)
- Figure 8-Full-length Amino Acid Sequence (ACTN2) (SEQ ID NO: 14)
- Figure 9- Full-length Amino Acid Sequence (mACTN4) (SEQ ID NO: 15)
- Figure 10- Full-length Amino Acid Sequence (mMRJ) (SEQ ID NO: 16)

Detailed Description of the Invention

15 Definitions

The term "GROUP1" used herein means AW755252-interacting proteins including mFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ, which have been identified using yeast two-hybrid system in the present invention.

The term "PROTEIN2" used herein means any one of proteins in GROUP1.

- 20 The terms "polypeptide," "protein," and "peptide" are used herein interchangeably to refer to amino acid chains in which the amino acid residues are linked by peptide bonds. The amino acid chains can be of any length of at least two amino acids, including full-length proteins. Unless otherwise specified, the terms "polypeptide," "protein," and "peptide" also encompass various modified forms

thereof, including but not limited to glycosylated forms, phosphorylated forms, myristoylated forms, palmitoylated forms, ribosylated forms, etc.

As used herein, the term “interacting” or “interaction” means that two protein domains or complete proteins exhibit sufficient physical affinity to each other so as to bring the two “interacting” protein domains or proteins physically close to each other. An extreme case of interaction is the formation of a chemical bond that results in continual and stable proximity of the two domains. Interactions that are based solely on physical affinities, although usually more dynamic than chemically bonded interactions, can be equally effective in co-localizing two proteins. Examples of physical affinities and chemical bonds include but are not limited to, forces caused by electrical charge differences, hydrophobicity, hydrogen bonds, Vander-waals force, ionic force, covalent linkages, and combinations thereof. The state of proximity between the interacting domains or entities may be transient or permanent, reversible or irreversible. In any event, it is in contrast to and distinguishable from contact caused by natural random movement of two entities. Typically although not necessarily, an “interaction” is exhibited by the binding between the interacting domains or entities. Examples of interactions include specific interactions between antigen and antibody, ligand and receptor, enzyme and substrate, and the like.

An “interaction” between two protein domains or complete proteins can be determined by a number of methods. For example, an interaction can be determined by functional assays such as the two-hybrid systems. Protein-protein interactions can also be determined by various biochemical approaches based on the affinity binding between the two interacting partners. Such biochemical methods generally known in the art include, but are not limited to, protein affinity chromatography, affinity blotting, immunoprecipitation, and the like. The binding constant for two interacting proteins, which reflects the strength or quality of the interaction, can also be determined using methods known in the art. See Phizicky and Fields, *Microbiol. Rev.*, 59:94-123 (1995).

As used herein, the term “protein complex” means a composite unit that is a combination of two or more proteins formed by interaction between the proteins. Typically but not necessarily, a “protein complex” is formed by the binding of two or more proteins together through specific non-covalent binding affinities. However,

covalent bonds may also be present between the interacting partners. For instance, the two interacting partners can be covalently crosslinked so that the protein complex becomes more stable.

Isolated as used herein refers to that altered by the hand of a human being from
5 its natural state, i.e., it has been altered outside of its natural environment or removed from its original environment, or both. "Isolated" can refer to, without limitation, isolated host cells, isolated polynucleotides or isolated polypeptides.

For example, a polynucleotide or a polypeptide naturally present in a living organism is not isolated, but the same polynucleotide or polypeptide substantially
10 separated from the coexisting materials of its natural state is isolated. Moreover, a polynucleotide or a polynucleotide encoding a polypeptide, which polynucleotide is introduced into a cell (e.g., a bacterial cell) or an organism by transformation, genetic manipulation or by any other recombinant method is isolated even if it is still present in the cell or organism, which cell or organism may be naturally occurring.

15 The term "isolated" when used in reference to nucleic acids (which include gene sequences) of this invention is intended to mean that a nucleic acid molecule is present in a form other than found in nature in its original environment with respect to its association with other molecules. For example, since a naturally existing chromosome includes a long nucleic acid sequence, an "isolated nucleic acid" as used
20 herein means a nucleic acid molecule having only a portion of the nucleic acid sequence in the chromosome but not one or more other portions present on the same chromosome. Thus, for example, an isolated gene typically includes no more than 50 kb, preferably no more than 25 kb, more preferably no more than 10 kb naturally occurring nucleic acid sequence which immediately flanks the gene in the naturally
25 existing chromosome or genomic DNA. However, it is noted that an "isolated nucleic acid" as used herein is distinct from a clone in a conventional library such as genomic DNA library and cDNA library in that the clones in a library is still in admixture with almost all the other nucleic acids in a chromosome or a cell. An isolated nucleic acid can be in a vector. An isolated nucleic acid can also be part of
30 a composition so long as the composition is substantially different from the nucleic acid's original natural environment. In this respect, an isolated nucleic acid can be in a semi-purified state, i.e., in a composition having certain natural cellular

components, while it is substantially separated from other naturally occurring nucleic acids and can be readily detected and/or assayed by standard molecular biology techniques. Preferably, an "isolated nucleic acid" is separated from at least 50%, more preferably at least 75%, most preferably at least 90% of other naturally occurring nucleic acids.

The term "isolated nucleic acid" embraces "purified nucleic acid" which means a specified nucleic acid is in a substantially homogenous preparation of nucleic acid substantially free of other cellular components, other nucleic acids, viral materials, or culture medium, or chemical precursors or by-products associated with chemical reactions for chemical synthesis of nucleic acids. Typically, a "purified nucleic acid" can be obtained by standard nucleic acid purification methods. In a purified nucleic acid, preferably the specified nucleic acid molecule constitutes at least 75%, preferably at least 85, and more preferably at least 95 percent of the total nucleic acids in the preparation. The term "purified nucleic acid" also means nucleic acids prepared from a recombinant host cell (in which the nucleic acids have been recombinantly amplified and/or expressed) or chemically synthesized nucleic acids.

The term "isolated nucleic acid" also encompasses "recombinant nucleic acid" which is used herein to mean a hybrid nucleic acid produced by recombinant DNA technology having the specified nucleic acid molecule covalently linked to one or more nucleic acid molecules that are not the nucleic acids naturally flanking the specified nucleic acid. Typically, such one or more nucleic acid molecules flanking the specified nucleic acid are no more than 50 kb, preferably no more than 25 kb.

The term "isolated polypeptide" as used herein means a polypeptide molecule is present in a form other than found in nature in its original environment with respect to its association with other molecules. Typically, an "isolated polypeptide" is separated from at least 50%, more preferably at least 75%, most preferably at least 90% of other naturally co-existing polypeptides in a cell or organism.

The term "isolated polypeptide" encompasses a "purified polypeptide" which is used herein to mean a specified polypeptide is in a substantially homogenous preparation substantially free of other cellular components, other polypeptides, viral materials, or culture medium, or when the polypeptide is chemically synthesized,

chemical precursors or by-products associated with the chemical synthesis. Preferably, in a purified polypeptide, preferably the specified polypeptide molecule constitutes at least 75%, preferably at least 85, and more preferably at least 95 percent of the total polypeptide in the preparation. A "purified polypeptide" can be obtained
5 from natural or recombinant host cells by standard purification techniques, or by chemically synthesis.

The term "isolated polypeptide" also encompasses a "recombinant polypeptide" which is used herein to mean a hybrid polypeptide produced by recombinant DNA technology or chemical synthesis having a specified polypeptide
10 molecule covalently linked to one or more polypeptide molecules which do not naturally flank the specified polypeptide.

The term "isolated protein complex" means a protein complex present in a composition or environment that is different from that found in nature in its native or original cellular or body environment. Preferably, an "isolated protein complex" is
15 separated from at least 50%, more preferably at least 75%, most preferably at least 90% of other naturally co-existing cellular or tissue components. Thus, an "isolated protein complex" may also be a naturally existing protein complex in an artificial preparation or a non-native host cell. An "isolated protein complex" may also be a "purified protein complex", that is, a substantially purified form in a substantially
20 homogenous preparation substantially free of other cellular components, other polypeptides, viral materials, or culture medium, or when the protein components in the protein complex are chemically synthesized, chemical precursors or by-products associated with the chemical synthesis. A "purified protein complex" typically means a preparation containing preferably at least 75%, more preferably at least 85%,
25 and most preferably at least 95% a particular protein complex. A "purified protein complex" may be obtained from natural or recombinant host cells or other body samples by standard purification techniques, or by chemical synthesis.

The terms "hybrid protein," "hybrid polypeptide," "hybrid peptide," "fusion protein," "fusion polypeptide," and "fusion peptide" are used herein interchangeably
30 to mean a non-naturally occurring protein having a specified polypeptide molecule covalently linked to one or more polypeptide molecules which do not naturally link to the specified polypeptide. Thus, a "hybrid protein" may be two naturally occurring

proteins or fragments thereof linked together by a covalent linkage. A “hybrid protein” may also be a protein formed by covalently linking two artificial polypeptides together. Typically but not necessarily, the two or more polypeptide molecules are linked or “fused” together by a peptide bond forming a single
5 non-branched polypeptide chain.

The term “antibody” as used herein encompasses both monoclonal and polyclonal antibodies that fall within any antibody classes, e.g., IgG, IgM, IgA, or derivatives thereof. The term “antibody” also includes antibody fragments including, but not limited to, Fab, F(ab')₂, and conjugates of such fragments, and single-chain antibodies comprising an antigen recognition epitope. In addition, the term “antibody” also means humanized antibodies, including partially or fully humanized antibodies. An antibody may be obtained from an animal, or from a hybridoma cell line producing a monoclonal antibody, or obtained from cells or libraries recombinantly expressing a gene encoding a particular antibody.

The term “selectively immunoreactive” as used herein means that an antibody is reactive thus binds to a specific protein or protein complex, but not other similar proteins or fragments or components thereof.

The term “compound” as used herein encompasses all types of organic or
10 inorganic molecules, including but not limited proteins, peptides, polysaccharides, lipids, nucleic acids, small organic molecules, inorganic compounds, and derivatives thereof.

The term “small molecule” as used herein refers to acids (for example acetic acid, salicylic acid, ascorbic acid) bases, formamide, amino acids and their derivatives
15 (for example protoheme, cytochrome heme) inorganic molecules (for example phosphoric acid), acetylcholine, sugars, prosthetic groups, cofactors and inhibitors (for example, Flavin adenine dinucleotide, riboflavin, NAD, NDP⁺, NADPH, folic acid, methotrexate) aspirin, palmitic acid, caffeine, beta-mercaptoethanol, urea, minerals or vitamins.

20

2. Protein Complexes

Novel protein-protein interactions have been discovered and confirmed using yeast two-hybrid system described herein. In particular, after studying the

interacting ability of AW755252 (bait) with random polypeptides expressed by anonymous cDNA libraries, it has been discovered that AW755252 specifically interacts with proteins including GROUP1 (preys). Different fragments or domains of bait and prey proteins were also tested using yeast two-hybrid system to delineate domains or residues important for the interaction. Accordingly, this invention also discloses specific domains or fragments of AW755252 capable of interacting with the specific domains or fragments of GROUP1. These details are summarized in Table 1. The amino acid sequence of the bait fragments used in the yeast two-hybrid system described herein are presented in Table 2. The amino acid sequences of the isolated prey fragments are presented in Table 3.

The sequences for some or all of the interacting proteins in this disclosure are not novel and are available in public databases such as GenBank. See, Tables 1 and 3 for The GenBank Accession Nos. The start and end numbers of the bait and prey fragments indicated in Tables 1-3 are based on the sequences of the corresponding full-length proteins known to one skilled in the art or the corresponding novel proteins of the present invention. These protein sequences are provided in the Figures presented herein.

TABLE1: Binding Domains of AW755252 and Its Interactors								
AW755252 (GenBank Accession No. AW755252) 527 AA in total	Bait AA Number		Prey Protein	GB Accession No.	AA in total	Prey AA Number		cDNA library
	Start	End				Start	End	
	240	398	mFHL2	NM_010212	279	100	235	Mouse Embryo
	397	527	mPN34854	NA	486	1	486	Mouse Embryo
	1	155	mPRPH1	NM_013639	507	215	438	Mouse Embryo
	1	196						
	245	527	mTCTEX-1	M25825	113	1	113	Mouse Embryo
	397	527						
	240	398	ACTN2	NM_001103	894	514	894	Heart
	240	398	mACTN4	NM_021895	912	550	912	Mouse embryo
						651	912	
	353	527	mMRJ	NM_011847	242	32	176	Mouse embryo

TABLE2: Bait Sequences of AW755252			
Bait Sequences of AW755252		Sequence	SEQ ID NO:
Start	End		
1	155	MECLVADKQNFHKSCFRCHHCNSKLSLGNYASLHGQIYCKP HFKQLFKSKGNYDEGFGHKQHKDRWNCKNQSRVDFIPNE EPNMCKNIAENTLVPGDRNEHLDAGNSEGQRNDLRKLGERG KLKVIWPPSKEIPKKTLPFEEELKMSKPKWPPE	1
1	196	MECLVADKQNFHKSCFRCHHCNSKLSLGNYASLHGQIYCKPH FKQLFKSKGNYDEGFGHKQHKDRWNCKNQSRVDFIPNEEPN MCKNIAENTLVPGDRNEHLDAGNSEGQRNDLRKLGERGKLKV IWPPSKEIPKKTLPFEEELKMSKPKWPEMTTLLSPEFKSESLLD VRTPENKGQRQDHFPLQPYLQST	2
240	398	NRKSAMDLNDNNNVIVQSAEKEKNEKTNQTNNGAEVLQVTNT DDEMMPENHKENLNKNNNNNYVAVSYLNNCRQKTSILEFLD LLPLSSEANDTANEYEIEKLENTSRISELLGIFESEKTYSRNVLA MALKKQTDRAAAGSPVQPAPKPSLSRGLMVKG	3
245	527	MDLNDNNNVIVQSAEKEKNEKTNQTNNGAEVLQVTNTDDEM MPENHKENLNKNNNNNYVAVSYLNNCRQKTSILEFLDLLPLSS EANDTANEYEIEKLENTSRISELLGIFESEKTYSRNVLAMALKK QTDRAAAGSPVQPAPKPSLSRGLMVKGSGSSIISPDTNLLNIKGS HSKSKNLHFFFSNTVKITAFSKKNENIFNCDLIDSVDQIKNMPCL DLREFGKDVKPWHVETTEAARNNENTGFDALSHECTAKPLF PRVEVQSEQLTVEEQIKRNRCYSDTE	4
397	527	KGSSIIISPDTNLLNIKGSKSKNLHFFFSNTVKITAFSKKNENI FNCDLIDSVDQIKNMPCLDLREFGKDVKPWHVETTEAARNNE NTGFDALSHECTAKPLFPRVEVQSEQLTVEEQIKRNRCYSDTE	5

AA: amino acid

TABLE3: Prey Sequences						
Prey Protein	GB Accession No.	AA in total	Prey AA Number		Sequence	SEQ ID NO:
			Start	End		
mFHL2	NM_010212	279	100	235	KCQECKKTIMPGTRKMEYKGSSW HETCFTCQRCQQPIGTKSFIPKENQ NFCVPCYEKQYALQCVQCKKPITT GGVTYREQPWHKECFVCTACKKQ LSGQRFTARDEFPYCLTCFCDLYAK KCAGCTNPISGLGGTK	6
mPN34854	NA	486	1	486	SLTFTTSERVVLTCELSRVDFPATW YKDGQKVEEESLIVKTEGRKHRL ILPEAQVRDSGEFECRTEGVSAFFG VTVQDPPVHIVNPQEHVVFVHAITSE CVRLTCEVDREDTTVHWYKDGQE VEESDIIVLENKGPHHRLVLPAAARP SDGGEFQCVAGDERAYFTVTITDV FSWIVYPSSEVHVAAIRLERVLTTC ELCRPWAEVRWTKDGEEVVESPAL LLEKEDTIRRLVLPVQLEDSDGEYL CEIHDESASFTITVTEPPVRIHPQD EVTLHAVSLECVVLTCELSREDAP VRWYKDGLEVEESEALVLQSDGPR RRLVLPAAQPEDGGEFVCDAGDDS AFFTVTVTAPPERIVHPAARSLDLQ FGAPGHVELRCEVAPAGSQVRWY KDGLEVEVSDALQLGAEGPARTLT LPHAQPEDAGEYVCETRDEAVTFN VSLAELPVQFLAPEAAPNPLCVVP GEPVVLSCELSRASAQVFWSHNG	7
mPRPH1	NM_013639	507	215	438	RLELERKIESLMDEIEFLKKLHEEE LRDLQVSVESQQVQQVEVEATVKP ELTAALRDIRAQYENIAAKNLQEA EEWYKSKVREHWGNPGPRVGRH WEWRCASQPGLSATAQYADLSDA ANRNHEALRQAKQEMNESRRRIQ SLTCEVDGLRGTEALLRQLRELE EQFALEAGGYQAGAARLEELRQL KEEMARHLREYQELNVKMALDI EIATYRKLEEG	8
mTCTEX-1	M25825	113	1	113	MEDFQASEETAFFVDEVSSIVKEAI ESAIGGNAYQHSKVNQWTTNVLE QTLSQLTKLGRPFKYIVTCVIMQK NGAGLHSASSCFWDSSTDGSCTVR WENKTMYCIVSTFGLSI	9

TABLE3: Prey Sequences						
Prey Protein	GB Accession No.	AA in total	Prey AA Number		Sequence	SEQ ID NO:
			Start	End		
ACTN2	NM_001103	894	514	894	IDQLHLEFAKRAAPFNNWMEGAM EDLQDMFIVHSIEEIQSLITAHEQFK ATLPEADGERQSIMAIQNEVEKVIQ SYNIRISSSNPYSTVTMDELRTKWD KVKQLVPIRDQSLQEELARQHANE RLRRQFAAQANAIGPWIQNKMEEI ARSSIQTGALEDQMNQLKQYEHN IINYKNNIDKLEGDHQLIQEALVFD NKHTNYTMEHIRVGWELLTTIAR TINEVETQILTRDAKGITQEQMNEF RASFNHFDRRKNGLMDHEDFRAC LISMGYDLGEAEFARIMTLVDPNG QGTVTTFQSFIDFMTRETADTDTA QVIASFRILASDKPYILABELRRELP PDQAQYCIKRMPAYSGPGSVPGAL DYAAFSSALYGESDL	17
mACTN4	NM_021895	912	550	912	EDLQDMFIVHTIEEIEGLISAHDQF KSTLPDADREREAILAIHKEAQRIA ESNHIKLSGSNPYTTVTPQIINSKW EKVQQLVPKRDHALLEEQSKQQSN EHLRRQFASQANMVGWPWIQTKME EIGRISIEMNGTLEDQLSHLKQYER SIVDYKPSLDLLEQQHQLIQEALIF DNKHTNYTMEHIRVGWEQLTTIA RTINEVENQILTRDAKGISQEQMQE FRASFNHFDKDHGGALGPEEFKAC LISLGYDVENDRQGDAEFNRIMSV VDPNHSGLVTFQAFIDFMSRETTD TDTADQVIASFKVLADGNFITAEE LRRELPPDQAEYCIARMAPYQGPD AAPGALDYKSFSTALYGESDL	18
			651	912	LRRQFASQANMVGWPWIQTKMEEIG RISIEMNGTLEDQLSHLKQYERSIV DYKPSLDLLEQQHQLIQEALIFDNK HTNYTMEHIRVGWEQLTTIARTIN EVENQILTRDAKGISQEQMQEFRA SFNHFDKDHGGALGPEEFKACLIS LGYDVENDRQGDAEFNRIMSVVD PNHSGLVTFQAFIDFMSRETTDTDT ADQVIASFKVLADGNFITAEEELR RELPPDQAEYCIARMAPYQGPDA PGALDYKSFSTALYGESDL	19
MMRJ	NM_011847	242	32	176	PDKNPENKEEAERKFKQVAEAYEV LSDAKKRDIYDKYGKEGLNGGGG GGGIHFDSPFEFGFTFRNPDDVFRE FFGGRDPFSFDFFEDPFDDFFGNRR GPRGNRSRGAAFFSTFSGFPSFGS GFPAFDTGFTPFGLGHGGLTSF	20

AA: amino acid; NA: not applicable

2.1. Cellular Functions of AW755252 and The Interacting Proteins, and Disease Involvement

5 AW755252

Recently, novel rat genes, which increased the mRNA expression at heart failure phase on rat myocardial infarction model using differential display method were identified. (SEQ ID NOs: 13 and 25; WO 02/36763). These genes are expressed only in heart and skeletal muscle. Using *in situ* hybridization, these genes
10 are high-expressed in area at risk of infarction. The myocyte in this area cannot contract normally, because of abnormal sarcomere conformation. Therefore, one of this protein's role in heart failure may be to cause decreased heart function. Pathways including these genes are unknown. However, molecular interaction of the protein encoded by these genes and their binding proteins may have important role in
15 the pathology of heart disease. As a Result of Blast searches against public database, AW755252 was found as human ortholog of these genes.

The cDNA encoding AW755252 was isolated from a cardiac muscle cDNA expression library by Incyte Genomics, Inc. (GenBank accession number AW755252). Northern blot analysis demonstrates detectable AW755252 mRNA only in heart and
20 skeletal muscle (Fig.1).

The amino acid sequence of AW755252 (SEQ ID NO: 1-5 in Table2 and SEQ ID NO: 10 in Figure 2) was deduced from the nucleotide sequence in GenBank accession number AW755252 in which only the nucleotide sequence information is available. The amino acid sequence of AW755252 (SEQ ID NO: 10 in Figure 2)
25 corresponds to that of human 187-2B protein (c.f., SEQ ID NO: 34 of PCT/JP03/08666) shown in PCT/JP03/08666 filed on July 8, 2003. Preferred example of the fragments of AW755252 includes a fragment consisting of amino acids 245 to 527 (of 527 total amino acids) of AW755252, which corresponds to that of human 187-2A protein (c.f., SEQ ID NO: 33 of PCT/JP03/08666) shown in
30 PCT/JP03/08666 filed on July 8, 2003. Structural analysis suggests the presence of a LIM domain (amino acids 1 to 49) and no other significant features. LIM domains are zinc-binding domains that are thought to mediate protein-protein interactions (Freyd

et al., *Nature*. 1990 344(6269):876-9, Schmeichel et al., *Cell*. 1994 79(2):211-9, Schmeichel et al., *Mol Biol Cell*. 1997 8(2):219-30, reviewed in Sanchez-Garcia and Rabbitts *Trends Genet*. 1994 10(9):315-20.).

AW755252 interacts with mFHL2.

5 A bait comprising amino acids 240 to 398 (of 527 total amino acids) of AW755252 selected a single clone from a mouse embryo activation domain library encoding amino acids 100 to 235 (of 279 total amino acids) of mFHL2 (NM_010212).

 The fragments of the interacting bait and prey should contain the minimal binding domain of each protein. Since the bait fragment of AW755252 comprises
10 amino acids 240 to 398, the sequence having a truncation of up to 239 amino acids at N-terminus and/or up to 129 (which is obtained by subtracting 398 from 527, the total amino acids number of AW755252) amino acids at the C-terminus of the AW755252 sequence set forth in Figure 2 does not render it unable to interact with mFHL2. Likewise, since the prey fragment of mFHL2 comprises amino acids 100 to 235,
15 sequence having a truncation of up to 99 amino acids at N-terminus and/or up to 44 (which is obtained by subtracting 235 from 279, the total amino acids number of mFHL2) amino acids at the C-terminus of the mFHL2 sequence set forth in Figure 3 does not render it unable to interact with AW755252.

 mFHL2 also is known as skeletal muscle LIM-protein 3, SLIM 3,
20 LIM-domain protein DRAL, four and a half LIM domains protein 2, and FHL-2. The cDNA encoding mFHL2 was isolated based on its similarity to other LIM-domain containing proteins (Morgan and Madgwick, *Biochem Biophys Res Commun*. 1999 255(2):245-50). The mRNA encoding mFHL2 is expressed primarily in heart tissue in mice (Morgan and Madgwick, *Biochem Biophys Res Commun*. 1999 255(2):245-50)
25 similar to the pattern of expression of human FHL2 (DRAL) (NM_001450) (Chan *et al.*, *Gene*. 1998 210(2):345-50). Structural analysis of mFHL2 reveals the presence of four intact LIM domains (amino acids 39 to 92, 100 to 153, 161 to 212, and 220 to 275) as well as a partial LIM domain (amino acids 3 to 31). No other structural features are evident. LIM domains, in addition to mediating protein-protein
30 interactions, also are found in proteins that have a role in the nucleus, suggesting a possible nucleic acid binding function (Sanchez-Garcia and Rabbitts, *Trends Genet*. 1994 10(9):315-20.). mFHL2 is a member of a family of proteins, the four and one

half LIM domain family that, as its name implies, contains four and one half LIM domains as the only definable feature. These proteins initially were thought to be involved primarily in cytoskeletal roles in the cytoplasm (Li *et al.*, *Cell Motil Cytoskeleton*. 2001 48(1):11-23). However, they were shown more recently to play a
5 role in the nucleus as co-activators of transcription for the androgen receptor (Muller *et al.*, *EMBO J.* 2000 19(3):359-69, Muller *et al.*, *EMBO J.* 2002 Feb 15;21(4):736-48) as well as for other transcription activation pathways (Fimia *et al.*, *Mol Cell Biol.* 2000 20(22):8613-22.). Given the co-expression of AW755252 and mFHL2 in heart, these two proteins together may play an important role in heart
10 physiology.

AW755252 interacts with a novel protein mPN34854 similar to KIAA0657.

A bait comprising amino acids 397 to 527 (of 527 total amino acids) of AW755252 selected a single clone from a mouse embryo activation domain library spanning amino acids 1 to 486 (of 486 total amino acids) of a novel protein similar to
15 KIAA0657.

The fragments of the interacting bait and prey should contain the minimal binding domain of each protein. Since the bait fragment of AW755252 comprises amino acids 397 to 527, the sequence having a truncation of up to 396 amino acids at N-terminus of the AW755252 sequence set forth in Figure 2 does not render it unable
20 to interact with mPN34854.

Based on RT-PCR data, the mRNA encoding the presumed human ortholog of KIAA0657 (AB014577) is expressed at highest levels in heart tissue, with lower levels in all other tissues examined (Ishikawa *et al.*, *DNA Res.* 1998 5(3):169-76.). Structural analysis of mPN34854 reveals the presence of several immunoglobulin-like
25 domains (5 to 71, 96 to 162, 187 to 253, 278 to 344 and 370 to 436). No other structural features are evident. Since mPN34854 is only a partial sequence, other structural features that give a better idea of the protein's function may be present. Since AW755252 also is expressed highly in heart tissue, the interaction between AW755252 and mPN34854 may represent an interaction that is important for proper
30 heart function.

AW755252 interacts with mPRPH1.

Two overlapping baits of AW755252, comprising amino acids 1 to 155 and 1 to 196 respectively, (of 527 total amino acids) each selected a single clone from a mouse embryo activation domain library spanning amino acids 215 to 438 (of 507
5 total amino acids) of mPRPH1 (NM_013639).

The fragments of the interacting bait and prey should contain the minimal binding domain of each protein. Since the minimal bait fragment of AW755252 spans amino acids 1 to 155, the sequence having a truncation of up to 372 (which is obtained by subtracting 155 from 527, the total amino acids number of AW755252)
10 amino acids at the C-terminus of the AW755252 sequence set forth in Figure 2 does not render it unable to interact with mPRPH1. Likewise, since the prey fragment of mPRPH1 comprises amino acids 215 to 438, the sequence having a truncation of up to 214 amino acids at N-terminus and/or up to 69 (which is obtained by subtracting 438 from 507, the total amino acids number of mPRPH1) amino acids at the
15 C-terminus of the mPRPH1 sequence set forth in Figure 5 does not render it unable to interact with AW755252.

mPRPH1 also is known as peripherin. cDNA clones encoding mPRPH1 were isolated from a neuroblastoma cDNA library using an antiserum directed against mPRPH1 (Landon et al., EMBO J. 1989 8(6):1719-26.). Peripherin mRNA is
20 expressed in primarily in neuronal cells and in cell lines derived from neuroblastoma tumors (Landon et al., EMBO J. 1989 8(6):1719-26, Goldstein et al., J Neurosci Res. 1991 30(1):92-104.). Structural analysis of mPRPH1 reveals the presence of an intermediate filament domain (amino acids 100 to 442), consistent with the function of mPRPH1 as a neuronal intermediate filament protein. In addition, two
25 overlapping regions of potential coiled coil structure (amino acids 87 to 248 and 327 to 433) are present. No other structural features are evident. The interaction between AW755252 and mPRPH1 may be a representative of an interaction between AW755252 and a protein expressed in heart or muscle tissue that is structurally similar to mPRPH1. Alternatively, perhaps mPRPH1 interacts with a protein
30 structurally similar to AW755252 in neuronal tissue. This interaction may play important role in the regulation of cytoskeleton formation at cardiomyocyte.

AW755252 interacts with mTCTEX-1 (M25825)

Two overlapping baits, comprising amino acids 245 to 527 and 397 to 527 respectively, each selected a single clone from a mouse cardiac cell activation domain library spanning amino acids 1 to 113 (of 113 total amino acids) of mTCTEX-1
5 (M25825).

The fragments of the interacting bait and prey should contain the minimal binding domain of each protein. Since the minimal bait fragment of AW755252 spans amino acids 397 to 527, the sequence having a truncation of up to 396 amino acids at N-terminus of the AW755252 sequence set forth in Figure 2 does not render it unable
10 to interact with mTCTEX-1.

mTCTEX-1 also is known as cytoplasmic dynein light chain and T-complex testis-specific protein 1. The cDNA encoding mTCTEX-1 was isolated from a mouse testicular cell cDNA library using a subtractive hybridization strategy. mTCTEX-1 mRNA is expressed most highly in mouse testis, with much lower levels in heart, liver,
15 brain, spleen, and kidney (Lander *et al.*, *Cell*. 1989 58(5):969-79.). However, publicly available EST data suggest a much broader range of expression. Structural analysis of mTCTEX-1 reveals no significant features. mTCTEX-1 appears to function as a dynein light chain, and as such is involved in transport of rhodopsin in rod photoreceptor cells (Tai *et al.*, *Cell*. 1999 97(7):877-87). This function is consistent
20 with the more broad expression pattern observed in public EST data. The interaction between AW755252 and a dynein light chain protein suggests a role for AW755252 in the cellular transport machinery or in other cellular mechanical processes. For example, the transportation from cytoplasm to nuclear, or from membrane to cytoplasm and so on.

25 AW755252 interacts with ACTN2

A bait comprising amino acids 240 to 398 (of 527 total amino acids) of AW755252 selected a single clone from a heart activation domain library spanning amino acids 514 to 894 (of 894 total amino acids) of ACTN2. The interacting fragments of the bait and prey should contain the minimal binding domain of each
30 protein. Since the bait fragment of AW755252 comprises amino acids 240 to 398, the sequence having a truncation of up to 239 amino acids at N-terminus and/or up to

129 (which is obtained by subtracting 398 from 527, the total amino acids number of AW755252) amino acids at the C-terminus of the AW755252 sequence set forth in Figure 2 does not render it unable to interact with ACTN2. Likewise, since the prey fragment of ACTN2 comprises amino acids 514 to 894, the sequence having a truncation of up to 513 amino acids at N-terminus of the ACTN2 sequence set forth in Figure 8 does not render it unable to interact with AW755252. ACTN2 also is known as alpha-actinin 2, alpha actinin skeletal muscle isoform 2, and F-actin cross linking protein. The cDNA encoding ACTN2 was isolated based on its similarity to other alpha-actinin sequences (Beggs et al., *J. Biol. Chem.* 267:9281-8 (1992)). The mRNA encoding ACTN2 is expressed both in cardiac muscle and in skeletal muscle (Beggs et al., *J. Biol. Chem.* 267:9281-8 (1992)). Structural analysis of ACTN2 reveals the presence of two CH (calponin homology) domains (amino acids 40 to 140 and 153 to 252), and four spectrin repeats (amino acids 284 to 390, 404 to 505, 519 to 626, and 640 to 739). Four EF hand calcium-binding domains comprise the C-terminus of ACTN2 (amino acids 757 to 894). The CH domains are actin binding domains found in numerous actin binding proteins. The region of ACTN2 shown here to interact with AW755252 includes one of the spectrin repeats and all of the EF hand domains. ACTN2 is thought to play a role in myofibril organization, and clearly plays a role in mediating protein-protein interactions in both cardiac and skeletal muscle (reviewed in Nag and Lee, *Tsitologia* 39:907-12 (1997), Viel, *FEBS Lett.* 460:391-4 (1999), Watkins et al., *Microsc. Res. Tech.* 48:131-41 (2000)). Further, decreases in ACTN2 have been observed in cases of cardiac hypertrophy and failure (reviewed in Hein et al., *Cardiovasc. Res.* 45:273-8 (2000)). Taken together, these observations suggest a role for AW755252 and ACTN2 in allowing proper myofibril formation or in maintaining proper myofibrillar structure.

AW755252 interacts with mACTN4.

A bait comprising amino acids 240 to 398 (of 527 total amino acids) of AW755252 selected two clones from a mouse embryo activation domain library comprising amino acids 550 to 912 and 651 to 912 (of 912 total amino acids) of mACTN4. The interacting fragments of the bait and prey should contain the minimal binding domain of each protein. Since the bait fragment of AW755252 comprises amino acids 240 to 398, the sequence having a truncation of up to 239 amino acids at N-terminus and/or up to 129 (which is obtained by subtracting 398

from 527, the total amino acids number of AW755252) amino acids at the C-terminus of the AW755252 sequence set forth in Figure 2 does not render it unable to interact with mACTN4. Likewise, since the minimal prey fragment of mACTN4 which interacts with AW755252 spans amino acids 651 to 912, the sequence having a truncation of up to 650 amino acids at N-terminus of the mACTN4 sequence set forth in Figure 9 does not render it unable to interact with AW755252. mACTN4 also is known as mouse alpha-actinin 4, non-muscle alpha-actinin 4, and F-actin cross linking protein. The gene encoding mACTN4 was isolated based on its overlap with the gene encoding mCAPN12 on mouse chromosome 7 (Dear et al., *Genomics* 68:152-60 (2000)).

Based on publicly available EST data the mRNA encoding mACTN4 is expressed in a broad variety of tissues and cell types. Structural analysis of mACTN4 reveals the presence of two CH (calponin homology) domains (amino acids 53 to 153 and 166 to 265), and four spectrin repeats (amino acids 297 to 403, 417 to 518, 532 to 639, and 653 to 752). Four EF hand calcium-binding domains comprise the C-terminus of mACTN4 (amino acids 770 to 912). The CH domains are actin binding domains found in numerous actin binding proteins. The region of mACTN4 shown here to interact with AW755252 includes one full and part of a second spectrin repeat and all of the EF hand domains. mACTN4 is thought to play a role, through interactions with the cytoskeleton, in cell migration and to be involved in later stages of tumorigenesis (Honda et al., *J. Cell Biol.* 140:1383-93 (1998), Nikolopoulos et al., *Oncogene* 19:380-6 (2000)). In addition, mutations in the human ortholog of mACTN4 have been shown to be causal for autosomal dominant focal and segmental glomerulosclerosis (FSGS) (Kaplan et al., *Nat. Genet.* 24:251-6 (2000)). This effect is thought to be mediated by the tighter binding of ACTN4 to filamentous actin, compared to wild type ACTN4 (Kaplan et al., *Nat. Genet.* 24:251-6 (2000)). Since mACTN4 is a member of a protein family that includes muscle and non-muscle proteins, it is possible that the interaction between AW755252 reflects an interaction between a domain or domains found in mACTN4 that also is found in muscle forms of alpha actinin. Alternatively, this interaction may suggest a role for AW755252 in the cytoskeleton of cardiac and skeletal muscle tissues which are its primary sites of expression.

The two interactions described in the preceding paragraphs involving AW755252 and two different alpha actinin proteins strongly suggest that AW755252 plays a role in cytoskeletal organization. The regions of ACTN2 and mACTN4 that interact with AW755252 each contain an intact spectrin repeat as well as the four EF
5 hand calcium-binding domains found in the C-terminus of the proteins. In the region common to all three interacting clones, ACTN2 and mACTN4 exhibit 86% amino acid similarity, and identical domain structures. Thus, these two interactions could represent either direct functional interactions between AW755252 and the two ACTN
10 ACTN isoform with a similar domain structure.

AW755252 interacts with mMRJ.

A bait comprising amino acids 353 to 527 (of 527 total amino acids) of AW755252 selected a single clone from a mouse embryo activation domain library spanning amino acids 32 to 176 (of 242 total amino acids) of mMRJ. The interacting
15 fragments of the bait and prey should contain the minimal binding domain of each protein. Since the bait fragment of AW755252 comprises amino acids 353 to 527, the sequence having a truncation of up to 352 amino acids at N-terminus of the AW755252 sequence set forth in Figure 2 does not render it unable to interact with mMRJ. Likewise, since the prey fragment of mMRJ which interacts with
20 AW755252 comprises amino acids 32 to 176, the sequence having a truncation of up to 31 amino acids at N-terminus and/or up to 66 (which is obtained by subtracting 176 from 242, the total amino acids number of mMRJ) amino acids at the C-terminus of the mMRJ sequence set forth in Figure 10 does not render it unable to interact with AW755252. mMRJ also is known as DnaJ homolog subfamily B member 6, heat
25 shock protein J2, HSJ-2, and mDj4. The gene encoding mMRJ was isolated in a gene trap screen for genes involved in placental development (Hunter et al., *Development* 126:1247-58 (1999)). mMRJ mRNA is expressed at all stages of embryonic development, and is particularly high in trophoblast cells of the placenta. In addition, the gene trap procedure itself disrupted the mMRJ gene, resulting in a
30 mouse line disrupted in placental development at about embryonic day 8.5 (Hunter et al., *Development* 126:1247-58 (1999)). This suggests a role for mMRJ in development, in particular in the placenta. Structural analysis of mMRJ reveals the presence of a DnaJ chaperone domain (amino acids 2 to 61) and no other structural

features. Since chaperone proteins are involved in mediating proper protein folding, the interaction between mMRJ and AW755252 suggests a role for mMRJ in ensuring proper AW755252 structure.

2.2. Protein Complexes

5 Accordingly, the present invention provides protein complexes formed between AW755252 and one or more AW755252-interacting proteins selected from the group consisting of GROUP1. The present invention also provides a protein complex formed from the interaction between a homologue, derivative or fragment of AW755252 and one or more of the AW755252-interacting proteins in accordance with
10 the present invention. In addition, the present invention further encompasses a protein complex having AW755252 and a homologue, derivative or fragment of one or more of the AW755252-interacting proteins in accordance with the present invention. In yet another embodiment, a protein complex is provided having a homologue, derivative or fragment of AW755252 and a homologue, derivative or
15 fragment of one or more of the AW755252-interacting proteins in accordance with the present invention. In other words, one or more of the interacting protein members of a protein complex of the present invention may be a native protein or a homologue, derivative or fragment of a native protein.

As described above, individual protein domains involved in the specific
20 protein-protein interactions have been discovered and summarized in Table 1. Accordingly, protein fragments consisting of the amino acid sequence of the identified interaction domains or homologues or derivatives thereof can be used in forming the protein complexes of the present invention. In addition, as will be apparent to a skilled artisan, a hybrid protein containing such an interaction domain may also be
25 used as an interacting partner in the protein complex of the present invention.

As used herein, the term "homologue" means a polypeptide that exhibits an amino acid sequence homology and/or structural resemblance to one of the above interacting native proteins, preferably native human proteins, or to one of the interaction domains of the native proteins such that it is capable of interacting with an
30 interacting partner of the native protein or a homologue thereof, either in the presence or absence of a compound capable of modulating the interaction between the

polypeptide and the interacting partner of the native protein or the homologue thereof. For example, a protein homologue may have an amino acid sequence that is at least 50%, preferably at least 75%, more preferably at least 85%, even more preferably at least 90%, and most preferably 95% identical to one of the above native interacting
5 proteins or an interaction domain thereof. Homologues may be the counterpart proteins of other species including animals, plants, yeast, bacteria, and the like. Homologues may also be selected by, e.g., mutagenesis in AW755252 and its interacting partners. Homologues may be identified by site-specific mutagenesis in combination with assay systems for detecting protein-protein interactions, e.g., the
10 yeast two-hybrid system described below.

Homology as used herein may refer to its precise meaning in biology of having a common evolutionary origin (such as mouse and human AW755252 proteins) and/or to structural resemblances. Structural resemblance is expressed in terms of identity or similarities. Identity or similarity as known in the art, is a relationship between two or
15 more polypeptide sequences (or two or more polynucleotide sequences, as the case may be) as determined by comparing the sequences. Identity also means the degree of sequence relatedness between polypeptide sequences or polynucleotide sequences, as determined by the match between strings of such sequences from the amino end to the carboxyl end or 5' to 3' end for polynucleotides. "Identity" can be readily calculated
20 by art known methods. See e.g., Altschul *et al.*, *Nucleic Acids Res.*, 25:3389-3402 (1997). Thus, homologues in the present invention include isolated polypeptides or polynucleotides having at least a 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99 or 100% identity to a specific polypeptide or polynucleotide sequence disclosed (also referred to herein as a reference sequence, i.e., the sequence having a SEQ ID NO that is disclosed
25 herein) in this application.

The expression of "% identity" as used herein can be understood by considering the following description: A polypeptide sequence of the present invention may be identical to the reference sequence (i.e., the sequence having a SEQ ID NO that is disclosed herein) in that it may be 100% identical, or it may include up to a certain
30 number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations (also referred to as mutations or point mutations) are at least one amino acid deletion, substitution

(conservative and/or non-conservative substitution) or insertion. For example, by a polypeptide sequence having at least 90% identity to a reference polypeptide sequence of SEQ ID NO: 10 (which is a bait polypeptide sequence disclosed in this application), it is meant that the polypeptide sequence is identical to the reference sequence except
 5 that the polypeptide sequence may include up to 10 mutations per 100 amino acids of the reference sequence of SEQ ID NO: 10. Similarly, if a polypeptide has at least 91% identity or 92% or 93% or 95% or 96% or 97% or 98% or 99% to a reference sequence, then the polypeptide has up to 9 or 8 or 7 or 5 or 4 or 3 or 2 or 1 amino acid alterations, respectively, per 100 amino acids of the reference sequence.

10 The alterations may occur at the NH₂- or COOH-terminal positions of the reference sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. In the case of polynucleotides, the alterations may occur at the 5' or 3'-terminal positions of the reference sequence or
 15 anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference polynucleotide sequence or in one or more contiguous groups within the reference sequence.

The number of amino acid alterations (Aa) for a given % identity is determined by first multiplying (x) the total number of amino acids (Ta) in the
 20 reference sequence by a number (n) which is obtained by dividing the percent identity by 100 (for example 0.80 for 80%, 0.90 for 90%, 0.92 for 92%, 0.95 for 95%, 0.97 for 97% and so on) and then subtracting that product from said total number of amino acids (Ta) in the reference sequence. After this calculation, any non-integer value may be rounded off to the nearest integer to obtain the approximate number with out
 25 decimal values. For purposes of clarity, only the first decimal number is rounded off, to approximate the number of amino acid alterations to an integer to obtain a polypeptide of a given % identity. If the first decimal number is 5 or greater than 5, then the number preceding the decimal point is increased by "one" and all the decimal numbers are dropped (rounded up). If the first decimal number is less than 5, then
 30 the number preceding the decimal point is unchanged and all the decimal numbers are dropped (rounded down). The calculation is summarized in the following formula:

$$Aa \cong Ta - (Ta \times n)$$

For example, the number of amino acid alterations needed to obtain a polypeptide that is at least 95% identical to the reference sequence of SEQ ID NO:10 is determined by first multiplying 527 (the total number of amino acids in the reference sequence) by 0.95 (which is obtained by dividing 95 by 100), and then subtracting that product from 527 (the total number of amino acids in the reference sequence), i.e., $527 \times 0.95 = 500.65$ and then $527 - 500.65 = 26.35$. After this calculation, the value of 26.35 is rounded down to 26, i.e., up to 26 amino acid alterations are needed over the entire length of the 527 amino acids of SEQ ID NO:10 to obtain a polypeptide that is at least 95% identical to the reference sequence of SEQ ID NO:10.

Although the above description is provided only with reference to polypeptides and certain percent identities, it should be noted that calculations for polynucleotides and other percent identities can be made by following that exemplary description.

The term "derivative" refers to a derivative or modified form of a protein. Examples of modified forms include glycosylated forms, phosphorylated forms, myristylated forms, ribosylated forms, and the like. Derivatives also include hybrid or fusion proteins containing one of the above native interacting proteins or a homologue or fragment thereof. In addition, derivatives also encompass artificial proteins having substituted non-naturally occurring amino acids, e.g., D-amino acids.

A fragment of a polypeptide according to the present invention is also a variant polypeptide having an amino acid sequence that is entirely the same as part but not all of any amino acid sequence of any specific polypeptide disclosed herein.

Preferred fragments include, for example, truncation polypeptides having a portion of an amino acid sequence of SEQ ID NOs:1-12. Further preferred are fragments characterized by structural or functional attributes such as fragments having alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet forming regions, beta-turn and beta-turn forming regions, coiled-coil and coiled-coil forming regions and other known in the art.

Particularly preferred fragments include an isolated polypeptide comprising an amino acid sequence having 1 or more or at least 15, 20, 30, 40, 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 contiguous amino acids truncated or deleted from the either amino- or carboxy-terminus of the amino acid sequences of

SEQ ID NO: 1-12 disclosed herein. Preferred are fragments are those fragments that mediate activities of or retain properties for protein interactions including those with a similar activity/property or an improved activity/property, or with a decreased undesirable activity or property.

5 In a specific embodiment of the protein complex of the present invention, two or more interacting partners (AW755252 and one or more proteins selected from the group consisting of GROUP1, or homologue, derivative or fragment thereof) are directly fused together, or covalently linked together through a peptide linker, forming a hybrid protein having a single unbranched polypeptide chain. Thus, the protein
10 complex may be formed by "intramolecular" interactions between two portions of the hybrid protein. Again, one or both of the fused or linked interacting partners in this protein complex may be a native protein or a homologue, derivative or fragment of a native protein.

A variant polypeptide is a polypeptide that differs from a reference
15 polypeptide but retains its essential properties (e.g., retains ability to interact with other protein(s) of the present protein-protein interaction).

By way of example, a variant of AW755252 can have a sequence consisting of the amino acids identical to that set forth in SEQ ID NO:10 (a reference polypeptide in this case) except that, over the entire length corresponding to the amino acid
20 sequence of SEQ ID NO:10, the amino acid sequence of the variant can have one or more conservative amino acid substitutions, whereby an amino acid residue is replaced by another with like properties. Typical conservative amino acid substitutions are among Ala, Val, Leu and Ile; among Thr and Ser; among the acidic residues Glu and Asp; among Asn and Gln; and among basic residues Lys and Arg; or
25 aromatic residues Phe and Tyr. A variant of AW755252 can also have a sequence consisting of the amino acids identical to that set forth in SEQ ID NO:10, the reference polypeptide, except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO:10, the amino acid sequence of the variant can have one or more non-conservative amino acid substitutions, deletions or insertions at such
30 positions of the amino acid sequence which do not alter its essential properties, such as for example, its interacting ability or activity with other polypeptides. A variant and reference polypeptides may differ in amino acid sequence by one or more

substitutions, additions, deletions in any combination. Substitutions, additions, deletions are also sometimes referred to as mutations. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. Particularly preferred are variants in which 5-10, 1-5, 1-4, 1-3, 1-2 or 1 amino acids are substituted, deleted or added in any combination for every 100 amino acids. A variant may be induced or naturally occurring such as an allelic variant. Variants may be created by mutagenesis or by direct synthesis or other methods known to skilled workers in this art.

The protein complexes of the present invention can also be in a modified form. For example, an antibody selectively immunoreactive with the protein complex can be bound to the protein complex. In another example, a non-antibody modulator capable of enhancing the interaction between the interacting partners in the protein complex may be included. Alternatively, the protein members in the protein complex may be cross-linked for purposes of stabilization. Various crosslinking methods may be used. For example, a bifunctional reagent in the form of R-S-S-R' may be used in which the R and R' groups can react with certain amino acid side chains in the protein complex forming covalent linkages. See, e.g., Traut *et al.*, in Creighton ed., *Protein Function: A Practical Approach*, IRL Press, Oxford, 1989; Baird *et al.*, *J. Biol. Chem.*, 251:6953-6962 (1976). Other useful crosslinking agents include, e.g., Denny-Jaffee reagent, a heterbifunctional photoactivable moiety cleavable through an azo linkage (See, Denny *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:5286-5290 (1984)), and 125I-{S-[N-(3-iodo-4-azidosalicyl)cysteaminy]-2-thiopyridine}, a cysteine-specific photocrosslinking reagent (see Chen *et al.*, *Science*, 265:90-92 (1994)).

The above-described protein complexes may further include any additional components, e.g., other proteins, nucleic acids, lipid molecules, monosaccharides or polysaccharides, ions, etc.

2.3. Methods of Preparing Protein Complexes

The protein complex of the present invention can be prepared by a variety of methods. Specifically, a protein complex can be isolated directly from an animal

tissue sample, preferably a human tissue sample containing the protein complex. Alternatively, a protein complex can be purified from host cells that recombinantly express the members of the protein complex. As will be apparent to a skilled artisan, a protein complex can be prepared from a tissue sample or recombinant host cell by
5 coimmunoprecipitation using an antibody immunoreactive with an interacting protein partner, or preferably an antibody selectively immunoreactive with the protein complex as will be discussed in detail below.

The antibodies can be monoclonal or polyclonal. Coimmunoprecipitation is a commonly used method in the art for isolating or detecting bound proteins. In this
10 procedure, generally a serum sample or tissue or cell lysate is admixed with a suitable antibody. The protein complex bound to the antibody is precipitated and washed. The bound protein complexes are then eluted.

Alternatively, immunoaffinity chromatography and immunoblotting techniques may also be used in isolating the protein complexes from native tissue
15 samples or recombinant host cells using an antibody immunoreactive with an interacting protein partner, or preferably an antibody selectively immunoreactive with the protein complex. For example, in protein immunoaffinity chromatography, the antibody may be covalently or non-covalently coupled to a matrix such as Sepharose in, e.g., a column. The tissue sample or cell lysate from the recombinant cells can
20 then be contacted with the antibody on the matrix. The column is then washed with a low-salt solution to wash off the unbound components. The protein complexes that are retained in the column can be then eluted from the column using a high-salt solution, a competitive antigen of the antibody, a chaotropic solvent, or sodium dodecyl sulfate (SDS), or the like. In immunoblotting, crude proteins samples from
25 a tissue sample or recombinant host cell lysate can be fractionated on a polyacrylamide gel electrophoresis (PAGE) and then transferred to, e.g., a nitrocellulose membrane. The location of the protein complex on the membrane may be identified using a specific antibody, and the protein complex is subsequently isolated.

30 In another embodiment, individual interacting protein partners may be isolated or purified independently from tissue samples or recombinant host cells using similar methods as described above. The individual interacting protein partners are

then contacted with each other under conditions conducive to the interaction therebetween thus forming a protein complex of the present invention. It is noted that different protein-protein interactions may require different conditions. As a starting point, for example, a buffer having 20 mM Tris-HCl, pH 7.0 and 500 mM NaCl may be used. Several different parameters may be varied, including temperature, pH, salt concentration, reducing agent, and the like. Some minor degree of experimentation may be required to determine the optimum incubation condition, this being well within the capability of one skilled in the art once apprised of the present disclosure.

10 In yet another embodiment, the protein complex of the present invention may be prepared from tissue samples or recombinant host cells or other suitable sources by protein affinity chromatography or affinity blotting. That is, one of the interacting protein partners is used to isolate the other interacting protein partner(s) by binding affinity thus forming protein complexes. Thus, an interacting protein partner
15 prepared by purification from tissue samples or by recombinant expression or chemical synthesis may be bound covalently or non-covalently to a matrix such as Sepharose in, e.g., a chromatography column. The tissue sample or cell lysate from the recombinant cells can then be contacted with the bound protein on the matrix. A low-salt solution is used to wash off the unbound components, and a high-salt solution
20 is then employed to elute the bound protein complexes in the column. In affinity blotting, crude protein samples from a tissue sample or recombinant host cell lysate can be fractionated on a polyacrylamide gel electrophoresis (PAGE) and then transferred to, e.g., a nitrocellulose membrane. The purified interacting protein member is then bound to its interacting protein partner(s) on the membrane forming
25 protein complexes, which are then isolated from the membrane.

It will be apparent to skilled artisans that any recombinant expression methods may be used in the present invention for purposes of recombinantly expressing the protein complexes or individual interacting proteins. Generally, a nucleic acid encoding an interacting protein member can be introduced into a suitable
30 host cell. For purposes of recombinantly forming a protein complex within a host cell, nucleic acids encoding two or more interacting protein members should be introduced into the host cell.

Typically, the nucleic acids, preferably in the form of DNA, are incorporated into a vector to form expression vectors capable of expressing the interacting protein member(s) once introduced into a host cell. Many types of vectors can be used for the present invention. Methods for the construction of an expression vector for
5 purposes of this invention should be apparent to skilled artisans apprised of the present disclosure. *See generally, Current Protocols in Molecular Biology*, Vol. 2, Ed. Ausubel, et al., Greene Publish. Assoc. & Wiley Interscience, Ch. 13, 1988; Glover, *DNA Cloning*, Vol. II, IRL Press, Wash., D.C., Ch. 3, 1986; Bitter, et al., in *Methods in Enzymology* 153:516-544 (1987); *The Molecular Biology of the Yeast*
10 *Saccharomyces*, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II, 1982; and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, 1989.

Generally, the expression vectors may include a promoter operably linked to a DNA encoding an interacting protein member, an origin of DNA replication for the
15 replication of the vectors in host cells. Preferably, the expression vectors also include a replication origin for the amplification of the vectors in, e.g., *E. coli*, and selection marker(s) for selecting and maintaining only those host cells harboring the expression vectors. Additionally, the expression vectors preferably also contain inducible elements, which function to control the transcription from the DNA
20 encoding an interacting protein member. Other regulatory sequences such as transcriptional enhancer sequences and translation regulation sequences (e.g., Shine-Dalgarno sequence) can also be operably included. Termination sequences such as the polyadenylation signals from bovine growth hormone, SV40, lacZ and AcMNPV polyhedral protein genes may also be operably linked to the DNA encoding
25 an interacting protein member. An epitope tag coding sequence for detection and/or purification of the expressed protein can also be operably incorporated into the expression vectors. Examples of useful epitope tags include, but are not limited to, influenza virus hemagglutinin (HA), Simian Virus 5 (V5), polyhistidine (6xHis), c-myc, lacZ, GST, and the like. Proteins with polyhistidine tags can be easily
30 detected and/or purified with Ni affinity columns, while specific antibodies immunoreactive with many epitope tags are generally commercially available. The expression vectors may also contain components that direct the expressed protein extracellularly or to a particular intracellular compartment. Signal peptides, nuclear

localization sequences, endoplasmic reticulum retention signals, mitochondrial localization sequences, myristoylation signals, palmitoylation signals, and transmembrane sequences are example of optional vector components that can determine the destination of expressed proteins. When it is desirable to express two or more interacting protein members in a single host cell, the DNA fragments encoding the interacting protein members may be incorporated into a single vector or different vectors.

The thus constructed expression vectors can be introduced into the host cells by any techniques known in the art, e.g., by direct DNA transformation, microinjection, electroporation, viral infection, lipofection, gene gun, and the like. The expression of the interacting protein members may be transient or stable. The expression vectors can be maintained in host cells in an extrachromosomal state, i.e., as self-replicating plasmids or viruses. Alternatively, the expression vectors can be integrated into chromosomes of the host cells by conventional techniques such as selection of stable cell lines or site-specific recombination. The vector construct can be designed to be suitable for expression in various host cells, including but not limited to bacteria, yeast cells, plant cells, insect cells, and mammalian and human cells. Methods for preparing expression vectors for expression in different host cells should be apparent to a skilled artisan.

Homologues and fragments of the native interacting protein members can also be easily expressed using the recombinant methods described above. For example, to express a protein fragment, the DNA fragment incorporated into the expression vector can be selected such that it only encodes the protein fragment. Likewise, a specific hybrid protein can be expressed using a recombinant DNA encoding the hybrid protein. Similarly, a homologue protein may be expressed from a DNA sequence encoding the homologue protein. A homologue-encoding DNA sequence may be obtained by manipulating the native protein-encoding sequence using recombinant DNA techniques. For this purpose, random or site-directed mutagenesis can be conducted using techniques generally known in the art. To make protein derivatives, for example, the amino acid sequence of a native interacting protein member may be changed in predetermined manners by site-directed DNA mutagenesis to create or remove consensus sequences for, e.g., phosphorylation by

protein kinases, glycosylation, ribosylation, myristoylation, palmytoylation, and the like. Alternatively, non-natural amino acids can be incorporated into an interacting protein member during the synthesis of the protein in recombinant host cells. For example, photoreactive lysine derivatives can be incorporated into an interacting
5 protein member during translation by using a modified lysyl-tRNA. See, e.g., Wiedmann *et al.*, *Nature*, 328:830-833 (1989); Musch *et al.*, *Cell*, 69:343-352 (1992). Other photoreactive amino acid derivatives can also be incorporated in a similar manner. See, e.g., High *et al.*, *J. Biol. Chem.*, 368:28745-28751 (1993). Indeed, the photoreactive amino acid derivatives thus incorporated into an interacting protein
10 member can function to cross-link the protein to its interacting protein partner in a protein complex under predetermined conditions.

In addition, derivatives of the native interacting protein members of the present invention can also be prepared by chemically linking certain moieties to amino acid side chains of the native proteins.

15 If desired, the homologues and derivatives thus generated can be tested to determine whether they are capable of interacting with their intended interacting partners to form protein complexes. Testing can be conducted by e.g., the yeast two-hybrid system or other methods known in the art for detecting protein-protein interaction.

20 A hybrid protein as described above having AW755252 or a homologue, derivative, or fragment thereof covalently linked by a peptide bond or a peptide linker to a protein selected from the group consisting of GROUP1 or a homologue, derivative, or fragment thereof, can be expressed recombinantly from a chimeric nucleic acid, e.g., a DNA or mRNA fragment encoding the fusion protein.
25 Accordingly, the present invention also provides a nucleic acid encoding the hybrid protein of the present invention. In addition, an expression vector having incorporated therein a nucleic acid encoding the hybrid protein of the present invention is also provided. The methods for making such chimeric nucleic acids and expression vectors containing them should be apparent to skilled artisans apprised of
30 the present disclosure.

2.4. Protein Microchip

In accordance with another embodiment of the present invention, a protein microchip or microarray is provided having one or more of the protein complexes of the present invention. Protein microarrays are becoming increasingly important in both proteomics research and protein-based detection and diagnosis of diseases. The protein microarrays in accordance with this embodiment of the present invention will be useful in a variety of applications including, e.g., large-scale or high-throughput screening for compounds capable of binding to the protein complexes or modulating the interactions between the interacting protein members in the protein complexes.

The protein microarray of the present invention can be prepared in a number of methods known in the art. An example of a suitable method is that disclosed in MacBeath and Schreiber, *Science*, 289:1760-1763 (2000). Essentially, glass microscope slides are treated with an aldehyde-containing silane reagent (SuperAldehyde Substrates purchased from TeleChem International, Cupertino, California). Nanoliter volumes of protein samples in a phosphate-buffered saline with 40% glycerol are then spotted onto the treated slides using a high-precision contact-printing robot. After incubation, the slides are immersed in a bovine serum albumin (BSA)-containing buffer to quench the unreacted aldehydes and to form a BSA layer which functions to prevent non-specific protein binding in subsequent applications of the microchip. Alternatively, as disclosed in MacBeath and Schreiber, proteins or protein complexes of the present invention can be attached to a BSA-NHS slide by covalent linkages. BSA-NHS slides are fabricated by first attaching a molecular layer of BSA to the surface of glass slides and then activating the BSA with N,N'-disuccinimidyl carbonate. As a result, the amino groups of the lysine, aspartate, and glutamate residues on the BSA are activated and can form covalent urea or amide linkages with protein samples spotted on the slides. See MacBeath and Schreiber, *Science*, 289:1760-1763 (2000).

Another example of useful method for preparing the protein microchip of the present invention is that disclosed in PCT Publication Nos. WO 00/4389A2 and WO 00/04382, both of which are assigned to Zyomyx and are incorporated herein by reference. First, a substrate or chip base is covered with one or more layers of thin organic film to eliminate any surface defects, insulate proteins from the base materials,

and to ensure uniform protein array. Next, a plurality of protein-capturing agents (e.g., antibodies, peptides, etc.) are arrayed and attached to the base that is covered with the thin film. Proteins or protein complexes can then be bound to the capturing agents forming a protein microarray. The protein microchips are kept in flow
5 chambers with an aqueous solution.

The protein microarray of the present invention can also be made by the method disclosed in PCT Publication No. WO 99/36576 assigned to Packard Bioscience Company, which is incorporated herein by reference. For example, a three-dimensional hydrophilic polymer matrix, i.e., a gel, is first disposed on a solid
10 substrate such as a glass slide. The polymer matrix gel is capable of expanding or contracting and contains a coupling reagent that reacts with amine groups. Thus, proteins and protein complexes can be contacted with the matrix gel in an expanded aqueous and porous state to allow reactions between the amine groups on the protein or protein complexes with the coupling reagents thus immobilizing the proteins and
15 protein complexes on the substrate. Thereafter, the gel is contracted to embed the attached proteins and protein complexes in the matrix gel.

Alternatively, the proteins and protein complexes of the present invention can be incorporated into a commercially available protein microchip, e.g., the ProteinChip System from CIPHERGEN Biosystems Inc., Palo Alto, CA. The ProteinChip System
20 comprises metal chips having a treated surface, which interact with proteins. Basically, a metal chip surface is coated with a silicon dioxide film. The molecules of interest such as proteins and protein complexes can then be attached covalently to the chip surface via a silane coupling agent.

The protein microchips of the present invention can also be prepared with
25 other methods known in the art, e.g., those disclosed in U.S. Patent Nos. 6,087,102, 6,139,831, 6,087,103; PCT Publication Nos. WO 99/60156, WO 99/39210, WO 00/54046, WO 00/53625, WO 99/51773, WO 99/35289, WO 97/42507, WO 01/01142, WO 00/63694, WO 00/61806, WO 99/61148, WO 99/40434, all of which are incorporated herein by reference.

30

3. Antibodies

In accordance with another aspect of the present invention, an antibody immunoreactive against a protein complex of the present invention is provided. In one embodiment, the antibody is selectively immunoreactive with a protein complex of the present invention. Specifically, the phrase “selectively immunoreactive with a protein complex” as used herein means that the immunoreactivity of the antibody of the present invention with the protein complex is substantially higher than that with the individual interacting members of the protein complex so that the binding of the antibody to the protein complex is readily distinguishable from the binding of the antibody to the individual interacting member proteins based on the strength of the binding affinities. Preferably, the binding constant differs by a magnitude of at least 2 fold, more preferably at least 5 fold, even more preferably at least 10 fold, and most preferably at least 100 fold. In a specific embodiment, the antibody is not substantially immunoreactive with the interacting protein members of the protein complex.

The antibody of the present invention can be readily prepared using procedures generally known in the art. *See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, 1988. Typically, the protein complex against which the antibody to be generated will be immunoreactive is used as the antigen for the purpose of producing immune response in a host animal. In one embodiment, the protein complex used consists the native proteins. Preferably, the protein complex includes only the binding domains of AW755252 and one or more proteins selected from the group consisting of GROUP1, respectively. As a result, a greater portion of the total antibodies may be selectively immunoreactive with the protein complexes. The binding domains can be selected from, e.g., those summarized in Table 1. In addition, various techniques known in the art for predicting epitopes may also be employed to design antigenic peptides based on the interacting protein members in a protein complex of the present invention to increase the possibility of producing an antibody selectively immunoreactive with the protein complex. Suitable epitope-prediction computer programs include, e.g., MacVector from International Biotechnologies, Inc. and Protean from DNASTar.

In a specific embodiment, a hybrid protein as described above in Section 2.1 is used as an antigen which has AW755252 or a homologues, derivative, or fragment thereof covalently linked by a peptide bond or a peptide linker to a protein selected from the group consisting of GROUP1 or a homologue, derivative, or fragment thereof. In a preferred embodiment, the hybrid protein consists of two interacting binding domains selected from Table 1, or homologues or derivatives thereof, covalently linked together by a peptide bond or a linker molecule.

The antibody of the present invention can be a polyclonal antibody to a protein complex of the present invention. To produce the polyclonal antibody, various animal hosts can be employed, including, e.g., mice, rats, rabbits, goats, guinea pigs, hamsters, etc. A suitable antigen which is a protein complex of the present invention or a derivative thereof as described above can be administered directly to a host animal to illicit immune reactions. Alternatively, it can be administered together with a carrier such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin, and Tetanus toxoid. Optionally, the antigen is conjugated to a carrier by a coupling agent such as carbodiimide, glutaraldehyde, and MBS. Any conventional adjuvants may be used to boost the immune response of the host animal to the protein complex antigen. Suitable adjuvants known in the art include but are not limited to Complete Freund's Adjuvant (which contains killed mycobacterial cells and mineral oil), incomplete Freund's Adjuvant (which lacks the cellular components), aluminum salts, MF59 from Biocine, monophospholipid, synthetic trehalose dicorynomycolate (TDM) and cell wall skeleton (CWS) both from RIBI ImmunoChem Research Inc., Hamilton, MT, non-ionic surfactant vesicles (NISV) from Proteus International PLC, Cheshire, U.K., and saponins. The antigen preparation can be administered to a host animal by subcutaneous, intramuscular, intravenous, intradermal, or intraperitoneal injection, or by injection into a lymphoid organ.

The antibodies of the present invention may also be monoclonal. Such monoclonal antibodies may be developed using any conventional techniques known in the art. For example, the popular hybridoma method disclosed in Kohler and Milstein, *Nature*, 256:495-497 (1975) is now a well-developed technique that can be used in the present invention. See U.S. Patent No. 4,376,110, which is incorporated

herein by reference. Essentially, B-lymphocytes producing a polyclonal antibody against a protein complex of the present invention can be fused with myeloma cells to generate a library of hybridoma clones. The hybridoma population is then screened for antigen binding specificity and also for immunoglobulin class (isotype). In this manner, pure hybridoma clones producing specific homogenous antibodies can be selected. See generally, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, 1988. Alternatively, other techniques known in the art may also be used to prepare monoclonal antibodies, which include but are not limited to the EBV hybridoma technique, the human N-cell hybridoma technique, and the trioma technique.

In addition, antibodies selectively immunoreactive with a protein complex of the present invention may also be recombinantly produced. For example, cDNAs prepared by PCR amplification from activated B-lymphocytes or hybridomas may be cloned into an expression vector to form a cDNA library, which is then introduced into a host cell for recombinant expression. The cDNA encoding a specific desired protein may then be isolated from the library. The isolated cDNA can be introduced into a suitable host cell for the expression of the protein. Thus, recombinant techniques can be used to recombinantly produce specific native antibodies, hybrid antibodies capable of simultaneous reaction with more than one antigen, chimeric antibodies (e.g., the constant and variable regions are derived from different sources), univalent antibodies which comprise one heavy and light chain pair coupled with the Fc region of a third (heavy) chain, Fab proteins, and the like. See U.S. Patent No. 4,816,567; European Patent Publication No. 0088994; Munro, *Nature*, 312:597 (1984); Morrison, *Science*, 229:1202 (1985); Oi *et al.*, *BioTechniques*, 4:214 (1986); and Wood *et al.*, *Nature*, 314:446-449 (1985), all of which are incorporated herein by reference. Antibody fragments such as Fv fragments, single-chain Fv fragments (scFv), Fab' fragments, and F(ab')₂ fragments can also be recombinantly produced by methods disclosed in, e.g., U.S. Patent No. 4,946,778; Skerra & Plückthun, *Science*, 240:1038-1041(1988); Better *et al.*, *Science*, 240:1041-1043 (1988); and Bird, *et al.*, *Science*, 242:423-426 (1988), all of which are incorporated herein by reference.

In a preferred embodiment, the antibodies provided in accordance with the present invention are partially or fully humanized antibodies. For this purpose, any

methods known in the art may be used. For example, partially humanized chimeric antibodies having V regions derived from the tumor-specific mouse monoclonal antibody, but human C regions are disclosed in Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1989). In addition, fully humanized antibodies can be made using

5 transgenic non-human animals. For example, transgenic non-human animals such as transgenic mice can be produced in which endogenous immunoglobulin genes are suppressed or deleted, while heterologous antibodies are encoded entirely by exogenous immunoglobulin genes, preferably human immunoglobulin genes, recombinantly introduced into the genome. See e.g., U.S. Patent Nos. 5,530,101;

10 5,545,806; 6,075,181; PCT Publication No. WO 94/02602; Green *et al.*, *Nat. Genetics*, 7: 13-21 (1994); and Lonberg *et al.*, *Nature* 368: 856-859 (1994), all of which are incorporated herein by reference. The transgenic non-human host animal may be immunized with suitable antigens such as a protein complex of the present invention or one or more of the interacting protein members thereof to illicit specific

15 immune response thus producing humanized antibodies. In addition, cell lines producing specific humanized antibodies can also be derived from the immunized transgenic non-human animals. For example, mature B-lymphocytes obtained from a transgenic animal producing humanized antibodies can be fused to myeloma cells and the resulting hybridoma clones may be selected for specific humanized antibodies

20 with desired binding specificities. Alternatively, cDNAs may be extracted from mature B-lymphocytes and used in establishing a library which is subsequently screened for clones encoding humanized antibodies with desired binding specificities.

In yet another embodiment, a bifunctional antibody is provided which has two different antigen binding sites, each being specific to a different interacting

25 protein member in a protein complex of the present invention. The bifunctional antibody may be produced using a variety of methods known in the art. For example, two different monoclonal antibody-producing hybridomas can be fused together. One of the two hybridomas may produce a monoclonal antibody specific against an interacting protein member of a protein complex of the present invention, while the

30 other hybridoma generates a monoclonal antibody immunoreactive with another interacting protein member of the protein complex. The thus formed new hybridoma produces different antibodies including a desired bifunctional antibody, i.e., an antibody immunoreactive with both of the interacting protein members. The

bifunctional antibody can be readily purified. See Milstein and Cuello, *Nature*, 305:537-540 (1983).

Alternatively, a bifunctional antibody may also be produced using heterobifunctional crosslinkers to chemically link two different monoclonal antibodies, each being immunoreactive with a different interacting protein member of a protein complex. Therefore, the aggregate will bind to two interacting protein members of the protein complex. See Staerz *et al*, *Nature*, 314:628-631(1985); Perez *et al*, *Nature*, 316:354-356 (1985).

In addition, bifunctional antibodies can also be produced by recombinantly expressing light and heavy chain genes in a hybridoma that itself produces a monoclonal antibody. As a result, a mixture of antibodies including a bifunctional antibody is produced. See DeMonte *et al*, *Proc. Natl. Acad. Sci., USA*, 87:2941-2945 (1990); Lenz and Weidle, *Gene*, 87:213-218 (1990).

Preferably, a bifunctional antibody in accordance with the present invention is produced by the method disclosed in U.S. Patent No. 5,582,996, which is incorporated herein by reference. For example, two different Fabs can be provided and mixed together. The first Fab can bind to an interacting protein member of a protein complex, and has a heavy chain constant region having a first complementary domain not naturally present in the Fab but capable of binding a second complementary domain. The second Fab is capable of binding another interacting protein member of the protein complex, and has a heavy chain constant region comprising a second complementary domain not naturally present in the Fab but capable of binding to the first complementary domain. Each of the two complementary domains is capable of stably binding to the other but not to itself. For example, the leucine zipper regions of c-fos and c-jun oncogenes may be used as the first and second complementary domains. As a result, the first and second complementary domains interact with each other to form a leucine zipper thus associating the two different Fabs into a single antibody construct capable of binding to two antigenic sites.

Other suitable methods known in the art for producing bifunctional antibodies may also be used, which include those disclosed in Holliger *et al*, *Proc. Nat'l Acad. Sci. USA*, 90:6444-6448 (1993); de Kruif *et al*, *J. Biol. Chem.*, 271:7630-7634

(1996); Coloma and Morrison, *Nat. Biotechnol.*, 15:159-163 (1997); Muller et al., *FEBS Lett.*, 422:259-264 (1998); and Muller et al., *FEBS Lett.*, 432:45-49 (1998), all of which are incorporated herein by reference.

4. Methods of Detecting Protein Complex and Diagnosis

5 Another aspect of the present invention relates to methods for detecting the protein complexes of the present invention, particularly for determining the level of a specific protein complex in a patient sample.

In one embodiment, the level of a protein complex having AW755252 and one or more proteins selected from the group consisting of GROUP1 in a cell, tissue, or organ of a patient is determined. An aberrant level is thus detected. For example,
10 the protein complex can be isolated or purified from a patient sample obtained from a cell, tissue, or organ of the patient and the amount thereof is determined. As described above, the protein complex can be prepared from a cell, tissue or organ sample by coimmunoprecipitation using an antibody immunoreactive with an
15 interacting protein member, a bifunctional antibody that is immunoreactive with two or more interacting protein members of the protein complex, or preferably an antibody selectively immunoreactive with the protein complex. When bifunctional antibodies or antibodies immunoreactive with only free interacting protein members are used, individual interacting protein members not complexed with other proteins
20 may also be isolated along with the protein complex containing such individual proteins. However, they can be readily separated from the protein complex using methods known in the art, e.g., size-based separation methods such as gel filtration, or by subtracting the protein complex from the mixture using an antibody specific against another individual interacting protein member. Additionally, proteins in a
25 sample can be separated in a gel such as polyacrylamide gel and subsequently immunoblotted using an antibody immunoreactive with the protein complex.

Alternatively, the level of the protein complex can be determined in a sample without separation, isolation or purification. For this purpose, it is preferred that an antibody selectively immunoreactive with the specific protein complex is used in an immunoassay. For example, immunocytochemical methods can be used. Other well known antibody-based techniques can also be used including, e.g.,

enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoradiometric assays (IRMA), fluorescent immunoassays, protein A immunoassays, and immunoenzymatic assays (IEMA). *See e.g.*, U.S. Patent Nos. 4,376,110 and 4,486,530, both of which are incorporated herein by reference.

In addition, since a specific protein complex is formed from its interacting protein members, if one of the interacting protein members is at a relatively low level in a patient, it may be reasonably expected that the level of the protein complex in the patient may also be low. Therefore, the level of an individual interacting protein member of a specific protein complex can be determined in a patient sample which can be used as a reasonably accurate indicator of the level of the protein complex in the sample. For this purpose, antibodies against an individual interacting protein member of a specific complex can be used in any one of the methods described above. In a preferred embodiment, the level of each of the interacting protein members of a protein complex is determined in a patient sample and the relative level of the protein complex is then deduced.

In addition, the relative protein complex level in a patient can also be determined by determining the level of the mRNA encoding an interacting protein member of the protein complex. Preferably, each interacting protein member's mRNA level in a patient sample is determined. For this purpose, methods for determining mRNA level generally known in the art may all be used. Examples of such methods include, e.g., Northern blot assay, dot blot assay, PCR assay (preferably quantitative PCR assay), *in situ* hybridization assay, and the like.

As discussed above, the interactions between AW755252 and the proteins GROUP1 suggest that these proteins and/or the protein complexes formed by such proteins may be involved in the same biological processes and disease pathways. In addition, the interactions between AW755252 and GROUP1 under physiological conditions may lead to the formation of protein complexes *in vivo*, which contain AW755252 and one or more of the AW755252-interacting proteins. The protein complexes are expected to mediate the functions and biological activities of AW755252 and GROUP1. For example, AW755252 and the AW755252-interacting proteins may be involved in cardiac function and associated with diseases and disorders such as ischemic heart disease, myocardial infarction,

cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris. Thus, aberrations in the level and/or activity of the protein complexes and/or the proteins such as AW755252 and the AW755252-interacting proteins may result in diseases or disorders such as ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris. Thus, the aberration in the protein complexes or the individual proteins and the degree of the aberration may be indicators for the diseases or disorders. They may be used as parameters for classifying and/or staging one of the above-described diseases. In addition, they may also be indicators for patients' response to a drug therapy.

10 Association between a physiological state (e.g., physiological disorder, predisposition to the disorder, a disease state, response to a drug therapy, or other physiological phenomena or phenotypes) and a specific aberration in a protein complex of the present invention or an individual interacting member thereof can be readily determined by comparative analysis of the protein complex and/or the
15 interacting members thereof in a normal population and an abnormal or affected population. Thus, for example, one can study the level, localization and distribution of a particular protein complex, mutations in the interacting protein members of the protein complex, and/or the binding affinity between the interacting protein members in both a normal population and a population affected with a particular physiological
20 disorder described above. The study results can be compared and analyzed by statistical means. Any detected statistically significant difference in the two populations would indicate an association. For example, if the level of the protein complex is statistically significantly higher in the affected population than in the normal population, then it can be reasonably concluded that higher level of the protein
25 complex is associated with the physiological disorder.

Thus, once an association is established between a particular type of aberration in a particular protein complex of the present invention or in an interacting protein member thereof and a physiological disorder or disease or predisposition to the physiological disorder or disease, then the particular physiological disorder or disease
30 or predisposition to the physiological disorder or disease can be diagnosed or detected by determining whether a patient has the particular aberration.

Accordingly, the present invention also provides a method for diagnosing a disease or physiological disorder or a predisposition to the disease or disorder such as ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris in a patient by determining whether there is
5 any aberration in the patient with respect to a protein complex having a first protein which is AW755252 interacting with a second protein selected from the group consisting of GROUP1. The same protein complex is analyzed in a normal individual and is compared with the results obtained in the patient. In this manner, any protein complex aberration in the patient can be detected. As used herein, the
10 term "aberration" when used in the context of protein complexes of the present invention means any alterations of a protein complex including increased or decreased level of the protein complex in a particular cell or tissue or organ or the total body, altered localization of the protein complex in cellular compartments or in locations of a tissue or organ, changes in binding affinity of an interacting protein member of the
15 protein complex, mutations in an interacting protein member or the gene encoding the protein, and the like. As will be apparent to a skilled artisan, the term "aberration" is used in a relative sense. That is, an aberration is relative to a normal individual or population of normal individuals.

As used herein, the term "diagnosis" means detecting a disease or disorder or
20 determining the stage or degree of a disease or disorder. The term "diagnosis" also encompasses detecting a predisposition to a disease or disorder, determining the therapeutic effect of a drug therapy, or predicting the pattern of response to a drug therapy or xenobiotics. The diagnosis methods of the present invention may be used independently, or in combination with other diagnosing and/or staging methods
25 known in the medical art for a particular disease or disorder.

Thus, in one embodiment, the method of diagnosis is conducted by detecting, in a patient, the levels of one or more protein complexes of the present invention using any one of the methods described above, and determining whether the patient has an aberrant level of the protein complexes.

30 The diagnosis may also be based on the determination of the levels of one or more interacting protein members (at protein or cDNA or mRNA level) of a protein complex of the present invention. An aberrant level of an interacting protein

member may indicate a physiological disorder or a predisposition to a physiological disorder.

In another embodiment, the method of diagnosis comprises determining, in a patient, the cellular localization, or tissue or organ distribution of a protein complex of the present invention and determining whether the patient has an aberrant localization or distribution of the protein complex. For example, immunocytochemical or immunohistochemical assays can be performed on a cell, tissue or organ sample from a patient using an antibody selectively immunoreactive with a protein complex of the present invention. Antibodies immunoreactive with both an individual interacting protein member and a protein complex containing the protein member may also be used, in which case it is preferred that antibodies immunoreactive with other interacting protein members are also used in the assay. In addition, nucleic acid probes may also be used in *in situ* hybridization assays to detect the localization or distribution of the mRNAs encoding the interacting protein members of a protein complex. Preferably, the mRNA encoding each interacting protein member of a protein complex is detected concurrently.

In yet another embodiment, the method of diagnosis of the present invention comprises detecting any mutations in one or more interacting protein members of a protein complex of the present invention. In particular, it is desirable to determine whether the interacting protein members have any mutations that will lead to, or in disequilibrium with, changes in the functional activity of the proteins or changes in their binding affinity to other interacting protein members in forming a protein complex of the present invention. Examples of such mutations include but are not limited to, e.g., deletions, insertions and rearrangements in the genes encoding the protein members, and nucleotide or amino acid substitutions and the like. In a preferred embodiment, the binding domains of the interacting protein members responsible for the protein-protein interactions in forming a protein complex are screened to detect any mutations therein. For example, genomic DNA or cDNA encoding an interacting protein member can be prepared from a patient sample, and sequenced. The thus obtained sequence may be compared with known wild-type sequences to identify any mutations. Alternatively, an interacting protein member may be purified from a patient sample and analyzed by protein sequencing or mass

spectrometry to detect any amino acid sequence changes. Any methods known in the art for detecting mutations may be used, as will be apparent to skilled artisans apprised of the present disclosure.

In another embodiment, the method of diagnosis includes determining the
5 binding constant of the interacting protein members of one or more protein complexes. For example, the interacting protein members can be obtained from a patient by direct purification or by recombinant expression from genomic DNAs or cDNAs prepared from a patient sample encoding the interacting protein members. Binding constants represent the strength of the protein-protein interaction between the
10 interacting protein members in a protein complex. Thus, by measuring binding constant, subtle aberration in binding affinity may be detected.

A number of methods known in the art for estimating and determining binding constants in protein-protein interactions are reviewed in Phizicky and Fields, *et al.*, *Microbiol. Rev.*, 59:94-123 (1995), which is incorporated herein by reference. For
15 example, protein affinity chromatography may be used. First, columns are prepared with different concentrations of an interacting protein member which is covalently bound to the columns. Then a preparation of an interacting protein partner is run through the column and washed with buffer. The interacting protein partner bound to the interacting protein member linked to the column is then eluted. The binding
20 constant is then estimated based on the concentrations of the bound protein and the eluted protein. Alternatively, the method of sedimentation through gradients monitors the rate of sedimentation of a mixture of proteins through gradients of glycerol or sucrose. At concentrations above the binding constant, proteins sediment as a protein complex. Thus, binding constant can be calculated based on the
25 concentrations. Other suitable methods known in the art for estimating binding constant include but are not limited to gel filtration column such as nonequilibrium "small-zone" gel filtration columns (*See e.g.*, Gill *et al.*, *J. Mol. Biol.*, 220:307-324 (1991)), the Hummel-Dreyer method of equilibrium gel filtration (*See e.g.*, Hummel and Dreyer, *Biochim. Biophys. Acta*, 63:530-532 (1962)) and large-zone equilibrium
30 gel filtration (*See e.g.*, Gilbert and Kellett, *J. Biol. Chem.*, 246:6079-6086 (1971)), sedimentation equilibrium (*See e.g.*, Rivas and Minton, *Trends Biochem.*, 18:284-287 (1993)), fluorescence methods such as fluorescence spectrum (*See e.g.*, Otto-Bruc *et*

al, *Biochemistry*, 32:8632-8645 (1993)) and fluorescence polarization or anisotropy with tagged molecules (*See e.g.*, Weiel and Hershey, *Biochemistry*, 20:5859-5865 (1981)), solution equilibrium measured with immobilized binding protein (*See e.g.*, Nelson and Long, *Biochemistry*, 30:2384-2390 (1991)), and surface plasmon
5 resonance (*See e.g.*, Panayotou *et al.*, *Mol. Cell. Biol.*, 13:3567-3576 (1993)).

In another embodiment, the diagnosis method of the present invention comprises detecting protein-protein interactions in functional assay systems such as the yeast two-hybrid system. Accordingly, to determine the protein-protein interaction between two interacting protein members that normally form a protein
10 complex in normal individuals, cDNAs encoding the interacting protein members can be isolated from a patient to be diagnosed. The thus cloned cDNAs or fragments thereof can be subcloned into vectors for use in yeast two-hybrid system. Preferably a reverse yeast two-hybrid system is used such that failure of interaction between the proteins may be positively detected. The use of yeast two-hybrid system or other
15 systems for detecting protein-protein interactions is known in the art and is described below in Section 5.3.1.

A kit may be used for conducting the diagnosis methods of the present invention. Typically, the kit should contain, in a carrier or compartmentalized container, reagents useful in any of the above-described embodiments of the diagnosis
20 method. The carrier can be a container or support, in the form of, e.g., bag, box, tube, rack, and is optionally compartmentalized. The carrier may define an enclosed confinement for safety purposes during shipment and storage. In one embodiment, the kit includes an antibody selectively immunoreactive with a protein complex of the present invention. In addition, antibodies against individual interacting protein
25 members of the protein complexes may also be included. The antibodies may be labeled with a detectable marker such as radioactive isotopes, and enzymatic or fluorescence markers. Alternatively secondary antibodies such as labeled anti-IgG and the like may be included for detection purposes. Optionally, the kit can include one or more of the protein complexes of the present invention prepared or purified
30 from a normal individual or an individual afflicted with a physiological disorder associated with an aberration in the protein complexes or an interacting protein member thereof. In addition, the kit may further include one or more of the

interacting protein members of the protein complexes of the present invention prepared or purified from a normal individual or an individual afflicted with a physiological disorder associated with an aberration in the protein complexes or an interacting protein member thereof. Suitable oligonucleotide primers useful in the
5 amplification of the genes or cDNAs for the interacting protein members may also be provided in the kit. In particular, in a preferred embodiment, the kit includes a first oligonucleotide selectively hybridizable to the mRNA or cDNA encoding AW755252 and a second oligonucleotide selectively hybridizable to the mRNA or cDNA encoding a protein selected from the group consisting of GROUP1. Additional
10 oligos hybridizing to AW755252 and its interacting partners as identified in the present invention may also be included. Such oligos may be used as PCR primers for, e.g., quantitative PCR amplification of mRNAs encoding AW755252 and an interacting partner thereof, or as hybridizing probes for detecting the mRNAs. The oligonucleotides may have a length of from about 8 nucleotides to about 100
15 nucleotides, preferably from about 12 to about 50 nucleotides, and more preferably from about 15 to about 30 nucleotides. In addition, the kit may also contain oligonucleotides that can be used as hybridization probes for detecting the cDNAs or mRNAs encoding the interacting protein members. Preferably, instructions for using the kit or reagents contained therein are also included in the kit.

**5. Use of Protein Complexes or Interacting Protein Members thereof
in Screening Assays**

The protein complexes of the present invention, AW755252 and AW755252-interacting proteins such as GROUP1 can also be used in screening assays to identify modulators of the protein complexes, AW755252, and/or the AW755252-interacting proteins. In addition, homologues, derivatives and fragments of AW755252 and the AW755252-interacting proteins may also be used in such screening assays. As used herein, the term “modulator” encompasses any compounds that can cause any forms of alteration of the biological activities or functions of the proteins or protein complexes, including, e.g., enhancing or reducing their biological activities, increasing or decreasing their stability, altering their affinity or specificity to certain other biological molecules, etc. In addition, the term “modulator” as used herein also includes any compounds that simply bind AW755252,

AW755252-interacting proteins, and/or the proteins complexes of the present invention. For example, a modulator can be a dissociator capable of interfering with or disrupting or dissociating protein-protein interaction between AW755252 or a homologue or derivative thereof and a protein selected from the group consisting of
5 GROUP1 or a homologue or derivative thereof. A modulator can also be an enhancer or initiator that initiates or strengthens the interaction between the protein members of a protein complex of the present invention.

Accordingly, the present invention provides screening methods for selecting modulators of AW755252, a AW755252-interacting protein selected from the group
10 consisting of GROUP1, or a protein complex formed between AW755252 and one or more of the AW755252-interacting proteins. Screening methods are also provided for selecting modulators of AW755252 homologues, derivatives or fragments, or homologues, derivatives or fragments of a AW755252-interacting protein, or a protein complex formed between a AW755252 homologue, derivative or fragment and a
15 homologue or derivative or fragment of a AW755252-interacting protein.

The modulators selected in accordance with the screen methods of the present invention can be effective in modulating the functions or activities of AW755252, a AW755252-interacting protein, or the protein complexes of the present invention. For example, compounds capable of binding to the protein complexes may be capable
20 of modulating the functions of the protein complexes. Additionally, compounds that interfere with, weaken, dissociate or disrupt, or alternatively, initiate, facilitate or stabilize the protein-protein interaction between the interacting protein members of the protein complexes can also be effective in modulating the functions or activities of the protein complexes. Thus, the compounds identified in the screening methods of
25 the present invention can be made into therapeutically or prophylactically effective drugs for preventing or ameliorating diseases, disorders or symptoms caused by or associated with aberration in the protein complexes or AW755252 or the AW755252-interacting proteins of the present invention. Alternatively, they may be used as leads to aid the design and identification of therapeutically or prophylactically
30 effective compounds for diseases, disorders or symptoms caused by or associated with aberration in the protein complexes or AW755252 or the AW755252-interacting proteins of the present invention. The protein complexes and/or interacting protein

members thereof in accordance with the present invention can be used in any of a variety of drug screening techniques. Drug screening can be performed as described herein or using well-known techniques, such as those described in U.S. Patent Nos. 5,800,998 and 5,891,628, both of which are incorporated herein by reference.

5

5.1. Test Compounds

Any test compounds may be screened in the screening assays of the present invention to select modulators of AW755252, a AW755252-containing protein complex and/or a AW755252-interacting protein of the present invention. By the term "selecting" or "select" compounds it is intended to encompass both (a) choosing
10 compounds from a group previously unknown to be modulators of AW755252, a AW755252-containing protein complex and/or a AW755252-interacting protein of the present invention, and (b) testing compounds that are known to be capable of binding, or modulating the functions and activities of, AW755252, a AW755252-containing protein complex and/or a AW755252-interacting protein of the present invention.
15 Both types of compounds are generally referred to herein as "test compounds." The test compounds may include, by way of example, proteins (e.g., antibodies, small peptides, artificial or natural proteins), nucleic acids, and derivatives, mimetics and analogs thereof, and small organic molecules having a molecular weight of no greater than 10,000 dalton, more preferably less than 5,000 dalton. Preferably, the test
20 compounds are provided in library formats known in the art, e.g., in chemically synthesized libraries, recombinantly expressed libraries (e.g., phage display libraries), and in vitro translation-based libraries (e.g., ribosome display libraries).

For example, the screening assays of the present invention can be used in the antibody production processes described in Section 3 to select antibodies with
25 desirable specificities. Various forms antibodies or derivatives thereof may be screened, including but not limited to, polyclonal antibodies, monoclonal antibodies, bifunctional antibodies, chimeric antibodies, single chain antibodies, antibody fragments such as Fv fragments, single-chain Fv fragments (scFv), Fab' fragments, and F(ab')₂ fragments, and various modified forms of antibodies such as catalytic
30 antibodies, and antibodies conjugated to toxins or drugs, and the like. The antibodies can be of any types such as IgG, IgE, IgA, or IgM. Humanized antibodies are particularly preferred. Preferably, the various antibodies and antibody fragments

may be provided in libraries to allow large-scale high throughput screening. For example, expression libraries expressing antibodies or antibody fragments may be constructed by a method disclosed, e.g., in Huse *et al.*, *Science*, 246:1275-1281 (1989), which is incorporated herein by reference. Single-chain Fv (scFv) antibodies
5 are of particular interest in diagnostic and therapeutic applications. Methods for providing antibody libraries are also provided in U.S. Patent Nos. 6,096,551; 5,844,093; 5,837,460; 5,789,208; and 5,667,988, all of which are incorporated herein by reference.

Peptidic test compounds may be peptides having L-amino acids and/or
10 D-amino acids, phosphopeptides, and other types of peptides. The screened peptides can be of any size, but preferably have less than about 50 amino acids. Smaller peptides are easier to deliver into a patient's body. Various forms of modified peptides may also be screened. Like antibodies, peptides can also be provided in, e.g., combinatorial libraries. *See generally*, Gallop *et al.*, *J. Med. Chem.*,
15 37:1233-1251 (1994). Methods for making random peptide libraries are disclosed in, e.g., Devlin *et al.*, *Science*, 249:404-406 (1990). Other suitable methods for constructing peptide libraries and screening peptides therefrom are disclosed in, e.g., Scott and Smith, *Science*, 249:386-390 (1990); Moran *et al.*, *J. Am. Chem. Soc.*, 117:10787-10788 (1995) (a library of electronically tagged synthetic peptides);
20 Stachelhaus *et al.*, *Science*, 269:69-72 (1995); U.S. Patent Nos. 6,156,511; 6,107,059; 6,015,561; 5,750,344; 5,834,318; 5,750,344, all of which are incorporated herein by reference. For example, random-sequence peptide phage display libraries may be generated by cloning synthetic oligonucleotides into the gene III or gene VIII of an *E. coli*. filamentous phage. The thus generated phage can propagate in *E. coli*. and
25 express peptides encoded by the oligonucleotides as fusion proteins on the surface of the phage. Scott and Smith, *Science*, 249:368-390 (1990). Alternatively, the "peptides on plasmids" method may also be used to form peptide libraries. In this method, random peptides may be fused to the C-terminus of the *E. coli*. Lac repressor by recombinant technologies and expressed from a plasmid that also contains Lac
30 repressor-binding sites. As a result, the peptide fusions bind to the same plasmid that encodes them.

Small organic or inorganic non-peptide non-nucleotide compounds are

preferred test compounds for the screening assays of the present invention. They too can be provided in a library format. *See generally*, Gordan *et al.* *J. Med. Chem.*, 37:1385-1401 (1994). For example, benzodiazepine libraries are provided in Bunin and Ellman, *J. Am. Chem. Soc.*, 114:10997-10998 (1992), which is incorporated herein
5 by reference. A method for constructing and screening peptoid libraries are disclosed in Simon *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:9367-9371 (1992). Methods for the biosynthesis of novel polyketides in a library format are described in McDaniel *et al.*, *Science*, 262:1546-1550 (1993) and Kao *et al.*, *Science*, 265:509-512 (1994). Various libraries of small organic molecules and methods of construction
10 thereof are disclosed in U.S. Patent Nos. 6,162,926 (multiply-substituted fullerene derivatives); 6,093,798 (hydroxamic acid derivatives); 5,962,337 (combinatorial 1,4-benzodiazepin-2, 5-dione library); 5,877,278 (Synthesis of N-substituted oligomers); 5,866,341 (compositions and methods for screening drug libraries); 5,792,821 (polymerizable cyclodextrin derivatives); 5,766,963 (hydroxypropylamine
15 library); and 5,698,685 (morpholino-subunit combinatorial library), all of which are incorporated herein by reference.

Other compounds such as oligonucleotides and peptide nucleic acids (PNA), and analogs and derivatives thereof may also be screened to identify clinically useful compounds. Combinatorial libraries of oligos are also known in the art. *See* Gold
20 *et al.*, *J. Biol. Chem.*, 270:13581-13584 (1995).

5.2. *In vitro* Assays

The test compounds may be screened in an *in vitro* assay to identify compounds capable of binding the protein complexes or interacting protein members thereof in accordance with the present invention. For this purpose, a test compound is contacted with a protein complex or an interacting protein member thereof under conditions and for a time sufficient to allow specific interaction between the test compound and the target components to occur and thus binding of the compound to the target forming a complex. Subsequently, the binding event is detected.

Agonists as used herein are those compounds that enhance the desired activities or properties for protein interactions. Antagonists are those compounds that interfere with or block the desired activities or properties for protein interactions.

Various screening techniques known in the art may be used in the present invention. The protein complexes and the interacting protein members thereof may be prepared by any suitable methods, e.g., by recombinant expression and purification. The protein complexes and/or interacting protein members thereof (both are referred
5 to as "target" hereinafter in this section) may be free in solution. A test compound may be mixed with a target forming a liquid mixture. The compound may be labeled with a detectable marker. Upon mixing under suitable conditions, the binding complex having the compound and the target may be co-immunoprecipitated and washed. The compound in the precipitated complex may be detected based on the
10 marker on the compound.

In a preferred embodiment, the target is immobilized on a solid support or on a cell surface. Preferably, the target can be arrayed into a protein microchip in a method described in Section 2.3. For example, a target may be immobilized directly onto a microchip substrate such as glass slides or onto a multi-well plates using non-neutralizing antibodies, i.e., antibodies capable of binding to the target but do not substantially affect its biological activities. To effect the screening, test compounds can be contacted with the immobilized target to allow binding to occur to form complexes under standard binding assay conditions. Either the targets or test compounds are labeled with a detectable marker using well-known labeling techniques. For example, U.S. Patent No. 5,741,713 discloses combinatorial libraries of biochemical compounds labeled with NMR active isotopes. To identify binding compounds, one may measure the formation of the target-test compound complexes or kinetics for the formation thereof. When combinatorial libraries of organic non-peptide non-nucleic acid compound are screened, it is preferred that labeled or encoded (or "tagged") combinatorial libraries are used to allow rapid decoding of lead structures. This is especially important because, unlike biological libraries, individual compounds found in chemical libraries cannot be amplified by self-amplification. Tagged combinatorial libraries are provided in, e.g., Borchardt and Still, *J. Am. Chem. Soc.*, 116:373-374 (1994) and Moran *et al.*, *J. Am. Chem. Soc.*, 117:10787-10788 (1995), both of which are incorporated herein by reference.

Alternatively, the test compounds can be immobilized on a solid support, e.g., forming a microarray of test compounds. The target protein or protein complex is

then contacted with the test compounds. The target may be labeled with any suitable detection marker. For example, the target may be labeled with radioactive isotopes or fluorescence marker before binding reaction occurs. Alternatively, after the binding reactions, antibodies that are immunoreactive with the target and are labeled with radioactive materials, fluorescence markers, enzymes, or labeled secondary anti-Ig antibodies may be used to detect any bound target thus identifying the binding compound. One example of this embodiment is the protein probing method. That is, the target provided in accordance with the present invention is used as a probe to screen expression libraries of proteins or random peptides. The expression libraries can be phage display libraries, in vitro translation-based libraries, or ordinary expression cDNA libraries. The libraries may be immobilized on a solid support such as nitrocellulose filters. See e.g., Sikela and Hahn, *Proc. Natl. Acad. Sci. USA*, 84:3038-3042 (1987). The probe may be labeled by a radioactive isotope or a fluorescence marker. Alternatively, the probe can be biotinylated and detected with a streptavidin-alkaline phosphatase conjugate. More conveniently, the bound probe may be detected with an antibody.

In yet another embodiment, a known ligand capable of binding to the target can be used in competitive binding assays. Complexes between the known ligand and the target can be formed and then contacted with test compounds. The ability of a test compound to interfere with the interaction between the target and the known ligand is measured. One exemplary ligand is an antibody capable of specifically binding the target. Particularly, such an antibody is especially useful for identifying peptides that share one or more antigenic determinants of the target protein complex or interacting protein members thereof.

In a specific embodiment, a protein complex used in the screening assay includes a hybrid protein as described in Section 2.1, which is formed by fusion of two interacting protein members or fragments or domains thereof. The hybrid protein may also be designed such that it contains a detectable epitope tag fused thereto. Suitable examples of such epitope tags include sequences derived from, e.g., influenza virus hemagglutinin (HA), Simian Virus 5 (V5), polyhistidine (6xHis), *c-myc*, *lacZ*, GST, and the like.

Test compounds may be also screened in an *in vitro* assay to identify

compounds capable of dissociating the protein complexes identified in accordance with the present invention. Thus, for example, a AW755252-containing protein complex can be contacted with a test compound and the protein complex can be detected. Conversely, test compounds may also be screened to identify compounds capable of enhancing the interaction between AW755252 and a AW755252-interacting protein or stabilizing the protein complex formed by the two proteins.

The assay can be conducted in similar manners as the binding assays described above. For example, the presence or absence of a particular protein complex can be detected by an antibody selectively immunoreactive with the protein complex. Thus, after incubation of the protein complex with a test compound, immunoprecipitation assay can be conducted with the antibody. If the test compound disrupts the protein complex, then the amount of immunoprecipitated protein complex in this assay will be significantly less than that in a control assay in which the same protein complex is not contacted with the test compound. Similarly, two proteins the interaction between which is to be enhanced may be incubated together with a test compound. Thereafter, protein complex may be detected by the selectively immunoreactive antibody. The amount of protein complex may be compared to that formed in the absence of the test compound. Various other detection methods may be suitable in the dissociation assay, as will be apparent to skilled artisan apprised of the present disclosure.

5.3. *In vivo* Screening Assay

Test compounds can also be screened in any *in vivo* assays select modulators of the protein complexes or interacting protein members thereof in accordance with the present invention. For example, any *in vivo* assays known in the art useful in
5 identifying compounds capable of strengthening or interfering with the stability of the protein complexes of the present invention may be used.

5.3.1. Two-Hybrid Assay

In a preferred embodiment, one of the yeast two-hybrid systems or their analogous or derivative forms is used. Examples of suitable two-hybrid systems
10 known in the art include, but are not limited to, those disclosed in U.S. Patent Nos.

5,283,173; 5,525,490; 5,585,245; 5,637,463; 5,695,941; 5,733,726; 5,776,689; 5,885,779; 5,905,025; 6,037,136; 6,057,101; 6,114,111; and Bartel and Fields, eds., *The Yeast Two-Hybrid System*, Oxford University Press, New York, NY, 1997, all of which are incorporated herein by reference.

5 Typically, in a classic transcription-based two-hybrid assay, two chimeric genes are prepared encoding two fusion proteins: one contains a transcription activation domain fused to an interacting protein member of a protein complex of the present invention or an interacting domain of the interacting protein member, while the other fusion protein includes a DNA binding domain fused to another interacting
10 protein member of the protein complex or an interacting domain thereof. For the purpose of convenience, the two interacting protein members or interacting domains thereof are referred to as "bait fusion protein" and "prey fusion protein," respectively. The chimeric genes encoding the fusion proteins are termed "bait chimeric gene" and "prey chimeric gene," respectively. Typically, a "bait vector" and a "prey vector" are
15 provided for the expression of a bait chimeric gene and a prey chimeric gene, respectively.

5.3.1.1. Vectors

Many types of vectors can be used in a transcription-based two-hybrid assay. Methods for the construction of bait vectors and prey vectors should be apparent to
20 skilled artisans in the art apprised of the present disclosure. *See generally, Current Protocols in Molecular Biology*, Vol. 2, Ed. Ausubel, *et al.*, Greene Publish. Assoc. & Wiley Interscience, Ch. 13, 1988; Glover, *DNA Cloning*, Vol. II, IRL Press, Wash., D.C., Ch. 3, 1986; Bitter, *et al.*, in *Methods in Enzymology* 153:516-544 (1987); *The Molecular Biology of the Yeast Saccharomyces*, Eds. Strathern *et al.*, Cold Spring
25 Harbor Press, Vols. I and II, 1982; and Rothstein in *DNA Cloning: A Practical Approach*, Vol. 11, Ed. DM Glover, IRL Press, Wash., D.C., 1986.

Generally, the bait and prey vectors may include a promoter operably linked to a chimeric gene for the transcription of the chimeric gene, an origin of DNA replication for the replication of the vectors in host cells and a replication origin for
30 the amplification of the vectors in, e.g., *E. coli*, and selection marker(s) for selecting and maintaining only those host cells harboring the vectors. Additionally, the

vectors preferably also contain inducible elements, which function to control the expression of a chimeric gene. Making the expression of the chimeric genes inducible and controllable is especially important in the event that the fusion proteins or components thereof are toxic to the host cells. Other regulatory sequences such as transcriptional enhancer sequences and translation regulation sequences (e.g., Shine-Dalgarno sequence) can also be included. Termination sequences such as the bovine growth hormone, SV40, lacZ and AcMNPV polyhedral polyadenylation signals may also be operably linked to a chimeric gene. An epitope tag coding sequence for detection and/or purification of the fusion proteins can also be incorporated into the expression vectors. Examples of useful epitope tags include, but are not limited to, influenza virus hemagglutinin (HA), Simian Virus 5 (V5), polyhistidine (6xHis), *c-myc*, lacZ, GST, and the like. Proteins with polyhistidine tags can be easily detected and/or purified with Ni affinity columns, while specific antibodies to many epitope tags are generally commercially available. The vectors can be introduced into the host cells by any techniques known in the art, e.g., by direct DNA transformation, microinjection, electroporation, viral infection, lipofection, gene gun, and the like. The bait and prey vectors can be maintained in host cells in an extrachromosomal state, i.e., as self-replicating plasmids or viruses. Alternatively, one or both vectors can be integrated into chromosomes of the host cells by conventional techniques such as selection of stable cell lines or site-specific recombination.

The *in vivo* assays of the present invention can be conducted in many different host cells, including but not limited to bacteria, yeast cells, plant cells, insect cells, and mammalian cells. A skilled artisan will recognize that the designs of the vectors can vary with the host cells used. In one embodiment, the assay is conducted in prokaryotic cells such as *Escherichia coli*, *Salmonella*, *Klebsiella*, *Pseudomonas*, *Caulobacter*, and *Rhizobium*. Suitable origins of replication for the expression vectors useful in this embodiment of the present invention include, e.g., the ColE1, pSC101, and M13 origins of replication. Examples of suitable promoters include, for example, the T7 promoter, the lacZ promoter, and the like. In addition, inducible promoters are also useful in modulating the expression of the chimeric genes. For example, the lac operon from bacteriophage lambda plac5 is well known in the art and is inducible by the addition of IPTG to the growth medium. Other known inducible

promoters useful in a bacteria expression system include pL of bacteriophage lambda, the trp promoter, and hybrid promoters such as the tac promoter, and the like.

In addition, selection marker sequences for selecting and maintaining only those prokaryotic cells expressing the desirable fusion proteins should also be incorporated into the expression vectors. Numerous selection markers including auxotrophic markers and antibiotic resistance markers are known in the art and can all be useful for purposes of this invention. For example, the *bla* gene which confers ampicillin resistance is the most commonly used selection marker in prokaryotic expression vectors. Other suitable markers include genes that confer neomycin, kanamycin, or hygromycin resistance to the host cells. In fact, many vectors are commercially available from vendors such as Invitrogen Corp. of San Diego, Calif., Clontech Corp. of Palo Alto, Calif., BRL of Bethesda, Maryland, and Promega Corp. of Madison, Wisconsin. These commercially available vectors, e.g., pBR322, pSPORT, pBluescriptIISK, pcDNAI, and pcDNAII all have a multiple cloning site into which the chimeric genes of the present invention can be conveniently inserted using conventional recombinant techniques. The constructed expression vectors can be introduced into host cells by various transformation or transfection techniques generally known in the art.

In another embodiment, mammalian cells are used as host cells for the expression of the fusion proteins and detection of protein-protein interactions. For this purpose, virtually any mammalian cells can be used including normal tissue cells, stable cell lines, and transformed tumor cells. Conveniently, mammalian cell lines such as CHO cells, Jurkat T cells, NIH 3T3 cells, HEK-293 cells, CV-1 cells, COS-1 cells, HeLa cells, VERO cells, MDCK cells, WI38 cells, and the like are used. Mammalian expression vectors are well known in the art and many are commercially available. Examples of suitable promoters for the transcription of the chimeric genes in mammalian cells include viral transcription promoters derived from adenovirus, simian virus 40 (SV40) (e.g., the early and late promoters of SV40), Rous sarcoma virus (RSV), and cytomegalovirus (CMV) (e.g., CMV immediate-early promoter), human immunodeficiency virus (HIV) (e.g., long terminal repeat (LTR)), vaccinia virus (e.g., 7.5K promoter), and herpes simplex virus (HSV) (e.g., thymidine kinase promoter). Inducible promoters can also be used. Suitable inducible promoters

include, for example, the tetracycline responsive element (TRE) (*See Gossen et al., Proc. Natl. Acad. Sci. USA*, 89:5547-5551 (1992)), metallothionein IIA promoter, ecdysone-responsive promoter, and heat shock promoters. Suitable origins of replication for the replication and maintenance of the expression vectors in mammalian cells include, e.g., the Epstein Barr origin of replication in the presence of the Epstein Barr nuclear antigen (*see Sugden et al., Mol. Cell. Biol.*, 5:410-413 (1985)) and the SV40 origin of replication in the presence of the SV40 T antigen (which is present in COS-1 and COS-7 cells) (*see Margolskee et al., Mol. Cell. Biol.*, 8:2837 (1988)). Suitable selection markers include, but are not limited to, genes conferring resistance to neomycin, hygromycin, zeocin, and the like. Many commercially available mammalian expression vectors may be useful for the present invention, including, e.g., pCEP4, pcDNA1, pIND, pSecTag2, pVAX1, pcDNA3.1, and pBI-EGFP, and pDisplay. The vectors can be introduced into mammalian cells using any known techniques such as calcium phosphate precipitation, lipofection, electroporation, and the like. The bait vector and prey vector can be co-transformed into the same cell or, alternatively, introduced into two different cells which are subsequently fused together by cell fusion or other suitable techniques.

Viral expression vectors, which permit introduction of recombinant genes into cells by viral infection, can also be used for the expression of the fusion proteins. Viral expression vectors generally known in the art include viral vectors based on adenovirus, bovine papilloma virus, murine stem cell virus (MSCV), MFG virus, and retrovirus. *See Sarver, et al., Mol. Cell. Biol.*, 1: 486 (1981); Logan & Shenk, *Proc. Natl. Acad. Sci. USA*, 81:3655-3659 (1984); Mackett, *et al., Proc. Natl. Acad. Sci. USA*, 79:7415-7419 (1982); Mackett, *et al., J. Virol.*, 49:857-864 (1984); Panicali, *et al., Proc. Natl. Acad. Sci. USA*, 79:4927-4931 (1982); Cone & Mulligan, *Proc. Natl. Acad. Sci. USA*, 81:6349-6353 (1984); Mann *et al., Cell*, 33:153-159 (1993); Pear *et al., Proc. Natl. Acad. Sci. USA*, 90:8392-8396 (1993); Kitamura *et al., Proc. Natl. Acad. Sci. USA*, 92:9146-9150 (1995); Kinsella *et al., Human Gene Therapy*, 7:1405-1413 (1996); Hofmann *et al., Proc. Natl. Acad. Sci. USA*, 93:5185-5190 (1996); Choate *et al., Human Gene Therapy*, 7:2247 (1996); WO 94/19478; Hawley *et al., Gene Therapy*, 1:136 (1994) and Rivere *et al., Genetics*, 92:6733 (1995), all of which are incorporated by reference.

Generally, to construct a viral vector, a chimeric gene according to the present invention can be operably linked to a suitable promoter. The promoter-chimeric gene construct is then inserted into a non-essential region of the viral vector, typically a modified viral genome. This results in a viable recombinant virus capable of
5 expressing the fusion protein encoded by the chimeric gene in infected host cells. Once in the host cell, the recombinant virus typically is integrated into the genome of the host cell. However, recombinant bovine papilloma viruses typically replicate and remain as extrachromosomal elements.

In another embodiment, the detection assays of the present invention are
10 conducted in plant cell systems. Methods for expressing exogenous proteins in plant cells are well known in the art. *See generally*, Weissbach & Weissbach, *Methods for Plant Molecular Biology*, Academic Press, NY, 1988; Grierson & Corey, *Plant Molecular Biology*, 2d Ed., Blackie, London, 1988. Recombinant virus expression vectors based on, e.g., cauliflower mosaic virus (CaMV) or tobacco mosaic virus
15 (TMV) can all be used. Alternatively, recombinant plasmid expression vectors such as Ti plasmid vectors and Ri plasmid vectors are also useful. The chimeric genes encoding the fusion proteins of the present invention can be conveniently cloned into the expression vectors and placed under control of a viral promoter such as the 35S RNA and 19S RNA promoters of CaMV or the coat protein promoter of TMV, or of a
20 plant promoter, e.g., the promoter of the small subunit of RUBISCO and heat shock promoters (e.g., soybean hsp17.5-E or hsp17.3-B promoters).

In addition, the *in vivo* assay of the present invention can also be conducted in insect cells, e.g., *Spodoptera frugiperda* cells, using a baculovirus expression system. Expression vectors and host cells useful in this system are well known in the art and
25 are generally available from various commercial vendors. For example, the chimeric genes of the present invention can be conveniently cloned into a non-essential region (e.g., the polyhedrin gene) of an *Autographa californica* nuclear polyhedrosis virus (AcNPV) vector and placed under control of an AcNPV promoter (e.g., the polyhedrin promoter). The non-occluded recombinant viruses thus generated can be used to
30 infect host cells such as *Spodoptera frugiperda* cells in which the chimeric genes are expressed. *See* U.S. Patent No. 4,215,051.

In a preferred embodiment of the present invention, the fusion proteins are

expressed in a yeast expression system using yeasts such as *Saccharomyces cerevisiae*, *Hansenula polymorpha*, *Pichia pastoris*, and *Schizosaccharomyces pombe* as host cells. The expression of recombinant proteins in yeasts is a well-developed field, and the techniques useful in this respect are disclosed in detail in *The Molecular*
5 *Biology of the Yeast Saccharomyces*, Eds. Strathern *et al.*, Vols. I and II, Cold Spring Harbor Press, 1982; Ausubel *et al.*, *Current Protocols in Molecular Biology*, New York, Wiley, 1994; and Guthrie and Fink, *Guide to Yeast Genetics and Molecular Biology*, in *Methods in Enzymology*, Vol. 194, 1991, all of which are incorporated herein by reference. Sudbery, *Curr. Opin. Biotech.*, 7:517-524 (1996) reviews the
10 success in the art in expressing recombinant proteins in various yeast species; the entire content and references cited therein are incorporated herein by reference. In addition, Bartel and Fields, eds., *The Yeast Two-Hybrid System*, Oxford University Press, New York, NY, 1997 contains extensive discussions of recombinant expression of fusion proteins in yeasts in the context of various yeast two-hybrid systems, and
15 cites numerous relevant references. These and other methods known in the art can all be used for purposes of the present invention. The application of such methods to the present invention should be apparent to a skilled artisan apprised of the present disclosure.

Generally, each of the two chimeric genes is included in a separate expression
20 vector (bait vector and prey vector). Both vectors can be co-transformed into a single yeast host cell. As will be apparent to a skilled artisan, it is also possible to express both chimeric genes from a single vector. In a preferred embodiment, the bait vector and prey vector are introduced into two haploid yeast cells of opposite mating types, e.g., a-type and α -type, respectively. The two haploid cells can be
25 mated at a desired time to form a diploid cell expressing both chimeric genes.

Generally, the bait and prey vectors for recombinant expression in yeast include a yeast replication origin such as the 2 μ origin or the *ARSH4* sequence for the replication and maintenance of the vectors in yeast cells. Preferably, the vectors also have a bacteria origin of replication (e.g., ColE1) and a bacteria selection marker (e.g.,
30 amp^R marker, i.e., *bla* gene). Optionally, the *CEN6* centromeric sequence is included to control the replication of the vectors in yeast cells. Any constitutive or inducible promoters capable of driving gene transcription in yeast cells may be employed to

control the expression of the chimeric genes. Such promoters are operably linked to the chimeric genes. Examples of suitable constitutive promoters include but are not limited to the yeast *ADHI*, *PGK1*, *TEF2*, *GPD1*, *HIS3*, and *CYC1* promoters. Example of suitable inducible promoters include but are not limited to the yeast *GALI* (inducible by galactose), CUP1 (inducible by Cu^{++}), and FUS1 (inducible by pheromone) promoters; the AOX/MOX promoter from *H. polymorpha* and *P. Pastoris* (repressed by glucose or ethanol and induced by methanol); chimeric promoters such as those that contain LexA operators (inducible by LexA-containing transcription factors); and the like. Inducible promoters are preferred when the fusion proteins encoded by the chimeric genes are toxic to the host cells. If it is desirable, certain transcription repressing sequences such as the upstream repressing sequence (URS) from SPO13 promoter can be operably linked to the promoter sequence, e.g., to the 5' end of the promoter region. Such upstream repressing sequences function to fine-tune the expression level of the chimeric genes.

Preferably, a transcriptional termination signal is operably linked to the chimeric genes in the vectors. Generally, transcriptional termination signal sequences derived from, e.g., the *CYC1* and *ADHI* genes can be used.

Additionally, it is preferred that the bait vector and prey vector contain one or more selectable markers for the selection and maintenance of only those yeast cells that harbor a chimeric gene. Any selectable markers known in the art can be used for purposes of this invention so long as yeast cells expressing the chimeric gene(s) can be positively identified or negatively selected. Examples of markers that can be positively identified are those based on color assays, including the lacZ gene which encodes beta-galactosidase, the firefly luciferase gene, secreted alkaline phosphatase, horseradish peroxidase, the blue fluorescent protein (BFP), and the green fluorescent protein (GFP) gene (see Cubitt *et al.*, *Trends Biochem. Sci.*, 20:448-455 (1995)). Other markers emitting fluorescence, chemiluminescence, UV absorption, infrared radiation, and the like can also be used. Among the markers that can be selected are auxotrophic markers including, but not limited to, *URA3*, *HIS3*, *TRP1*, *LEU2*, *LYS2*, *ADE2*, and the like. Typically, for purposes of auxotrophic selection, the yeast host cells transformed with bait vector and/or prey vector are cultured in a medium lacking a particular nutrient. Other selectable markers are not based on auxotrophies, but

rather on resistance or sensitivity to an antibiotic or other xenobiotic. Examples of such markers include but are not limited to chloramphenicol acetyl transferase (CAT) gene, which confers resistance to chloramphenicol; *CAN1* gene, which encodes an arginine permease and thereby renders cells sensitive to canavanine (*see Sikorski et al., Meth. Enzymol.*, 194:302-318 (1991)); the bacterial kanamycin resistance gene (kan^R), which renders eucaryotic cells resistant to the aminoglycoside G418 (*see Wach et al., Yeast*, 10:1793-1808 (1994)); and *CYH2* gene, which confers sensitivity to cycloheximide (*see Sikorski et al., Meth. Enzymol.*, 194:302-318 (1991)). In addition, the *CUP1* gene, which encodes metallothionein and thereby confers resistance to copper, is also a suitable selection marker. Each of the above selection markers may be used alone or in combination. One or more selection markers can be included in a particular bait or prey vector. The bait vector and prey vector may have the same or different selection markers. In addition, the selection pressure can be placed on the transformed host cells either before or after mating the haploid yeast cells.

As will be apparent, the selection markers used should complement the host strains in which the bait and/or prey vectors are expressed. In other words, when a gene is used as a selection marker gene, a yeast strain lacking the selection marker gene (or having mutation in the corresponding gene) should be used as host cells. Numerous yeast strains or derivative strains corresponding to various selection markers are known in the art. Many of them have been developed specifically for certain yeast two-hybrid systems. The application and optional modification of such strains with respect to the present invention should be apparent to a skilled artisan apprised of the present disclosure. Methods for genetically manipulating yeast strains using genetic crossing or recombinant mutagenesis are well known in the art. *See e.g.*, Rothstein, *Meth. Enzymol.*, 101:202-211 (1983). By way of example, the following yeast strains are well known in the art, and can be used in the present invention upon necessary modifications and adjustment:

L40 strain which has the genotype *MATa his3 delta200 trp1-901 leu2-3,112 ade2 LYS2:: (lexAop)4-HIS3 URA3:: (lexAop)8-lacZ*;

EGY48 strain which has the genotype *MATalpha trp1 his3 ura3 6ops-LEU2*; and MaV103 strain which has the genotype *MATalpha ura3-52 leu2-3,112 trp1-901*

his3 delta200 ade2-101 gal4delta gal80delta SPAL10::URA3 GAL1::HIS3::lys2
(see Kumar *et al.*, *J. Biol. Chem.* 272:13548-13554 (1997); Vidal *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:10315-10320 (1996)). Such strains are generally available in the research community, and can also be obtained by simple yeast genetic manipulation.
5 See, e.g., *The Yeast Two-Hybrid System*, Bartel and Fields, eds., pages 173-182, Oxford University Press, New York, NY, 1997.

In addition, the following yeast strains are commercially available:

Y190 strain which is available from Clontech, Palo Alto, California and has the genotype *MATalpha gal4 gal80 his3delta200 trp1-901 ade2-101 ura3-52 leu2-3*,
10 *112 URA3::GAL1-lacZ LYS2::GAL1-HIS3 cyh^r*; and

YRG-2 Strain which is available from Stratagene, La Jolla, California and has the genotype *MATalpha ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3, 112 gal4-542 gal80-538 LYS2::GAL1-HIS3 URA3::GAL1/CYC1-lacZ*.

In fact, different versions of vectors and host strains specially designed for
15 yeast two-hybrid system analysis are available in kits from commercial vendors such as Clontech, Palo Alto, California and Stratagene, La Jolla, California, all of which can be modified for use in the present invention.

5.3.1.2. Reporters

Generally, in a transcription-based two-hybrid assay, the interaction between a
20 bait fusion protein and a prey fusion protein brings the DNA-binding domain and the transcription-activation domain into proximity forming a functional transcriptional factor, which acts on a specific promoter to drive the expression of a reporter protein. The transcription activation domain and the DNA-binding domain may be selected from various known transcriptional activators, e.g., GAL4, GCN4, ARD1, the human
25 estrogen receptor, *E. coli* LexA protein, herpes simplex virus VP16 (Triezenberg *et al.*, *Genes Dev.* 2:718-729 (1988)), the *E. coli* B42 protein (acid blob, see Gyuris *et al.*, *Cell*, 75:791-803 (1993)), NF-kB p65, and the like. The reporter gene and the promoter driving its transcription typically are incorporated into a separate reporter vector. Alternatively, the host cells are engineered to contain such a
30 promoter-reporter gene sequence in their chromosomes. Thus, the interaction or lack

of interaction between two interacting protein members of a protein complex can be determined by detecting or measuring changes in the reporter in the assay system. Although the reporters and selection markers can be of similar types and used in a similar manner in the present invention, the reporters and selection markers should be
5 carefully selected in a particular detection assay such that they are distinguishable from each other and do not interfere with each other's function.

Many different types reporters are useful in the screening assays. For example, a reporter protein may be a fusion protein having an epitope tag fused to a protein. Commonly used and commercially available epitope tags include sequences
10 derived from, e.g., influenza virus hemagglutinin (HA), Simian Virus 5 (V5), polyhistidine (6xHis), *c-myc*, lacZ, GST, and the like. Antibodies specific to these epitope tags are generally commercially available. Thus, the expressed reporter can be detected using an epitope-specific antibody in an immunoassay.

In another embodiment, the reporter is selected such that it can be detected by
15 a color-based assay. Examples of such reporters include, e.g., the lacZ protein (beta-galactosidase), the green fluorescent protein (GFP), which can be detected by fluorescence assay and sorted by flow-activated cell sorting (FACS) (*See* Cubitt *et al.*, *Trends Biochem. Sci.*, 20:448-455 (1995)), secreted alkaline phosphatase, horseradish peroxidase, the blue fluorescent protein (BFP), and luciferase photoproteins such as
20 aequorin, obelin, mnemiopsin, and berovin (*See* U.S. Patent No. 6,087,476, which is incorporated herein by reference).

Alternatively, an auxotrophic factor is used as a reporter in a host strain deficient in the auxotrophic factor. Thus, suitable auxotrophic reporter genes include, but are not limited to, *URA3*, *HIS3*, *TRP1*, *LEU2*, *LYS2*, *ADE2*, and the like. For
25 example, yeast cells containing a mutant *URA3* gene can be used as host cells (Ura⁻ phenotype). Such cells lack *URA3*-encoded functional orotidine-5'-phosphate decarboxylase, an enzyme required by yeast cells for the biosynthesis of uracil. As a result, the cells are unable to grow on a medium lacking uracil. However, wild-type orotidine-5'-phosphate decarboxylase catalyzes the conversion of a non-toxic
30 compound 5-fluoroorotic acid (5-FOA) to a toxic product, 5-fluorouracil. Thus, yeast cells containing a wild-type *URA3* gene are sensitive to 5-FOA and cannot grow on a medium containing 5-FOA. Therefore, when the interaction between the

interacting protein members in the fusion proteins results in the expression of active orotidine-5'-phosphate decarboxylase, the Ura⁻ (Foa^R) yeast cells will be able to grow on a uracil deficient medium (SC-Ura plates). However, such cells will not survive on a medium containing 5-FOA. Thus, protein-protein interactions can be detected
5 based on cardiac function.

Additionally, antibiotic resistance reporters can also be employed in a similar manner. In this respect, host cells sensitive to a particular antibiotics is used. Antibiotics resistance reporters include, for example, chloramphenicol acetyl transferase (CAT) gene and the kan^R gene, which confers resistance to G418 in
10 eukaryotes and to kanamycin in prokaryotes.

5.3.1.3. Screening Assay for Dissociators

The screening assay of the present invention is useful in identifying compounds capable of interfering with or disrupting or dissociating protein-protein interaction between AW755252 or a homologue or derivative thereof and a protein
15 selected from the group consisting of GROUP1 or a homologue or derivative thereof. For example, AW755252 and its interacting partners are believed to play a role in cardiac function, and thus are involved in ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris. It may be possible to ameliorate or alleviate the diseases or disorders in a
20 patient by interfering with or dissociating normal interactions between AW755252 and one of GROUP1. Alternatively, if the disease or disorder is associated with increased expression of AW755252 and/or one of the AW755252-interacting proteins in accordance with the present invention, then the disease may be treated or prevented
25 by weakening or dissociating the interaction between AW755252 and the member in a patient. In addition, if a disease or disorder is associated with mutant forms of AW755252 and/or one of the AW755252-interacting proteins that lead to strengthened protein-protein interaction therebetween, then the disease or disorder may be treated with a compound that weakens or interferes with the interaction between the mutant
form of AW755252 and the member.

30 In a screening assay for a dissociator, AW755252, a mutant form or a binding domain thereof, and a AW755252-interacting protein, or a mutant form or a binding

domain thereof, are used as test proteins expressed in the form of fusion proteins as described above for purposes of a two-hybrid assay. The fusion proteins are expressed in a host cell and allowed to interact with each other in the presence of one or more test compounds.

5 In a preferred embodiment, a counterselectable marker is used as a reporter such that a detectable signal (e.g., appearance of color or fluorescence, or cell survival) is present only when the test compound is capable of interfering with the interaction between the two test proteins. In this respect, the reporters used in various "reverse two-hybrid systems" known in the art may be employed. Reverse
10 two-hybrid systems are disclosed in, e.g., U.S. Patent Nos. 5,525,490; 5,733,726; 5,885,779; Vidal *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:10315-10320 (1996); and Vidal *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:10321-10326 (1996), all of which are incorporated herein by reference.

 Examples of suitable counterselectable reporters useful in a yeast system
15 include the *URA3* gene (encoding orotidine-5'-decarboxylase, which converts 5-fluoroorotic acid (5-FOA) to the toxic metabolite 5-fluorouracil), the *CAN1* gene (encoding arginine permease, which transports toxic arginine analog canavanine into yeast cells), the *GAL1* gene (encoding galactokinase, which catalyzes the conversion of 2-deoxygalactose to toxic 2-deoxygalactose-1-phosphate), the *LYS2* gene (encoding
20 alpha-aminoadipate reductase, which renders yeast cells unable to grow on a medium containing alpha-aminoadipate as the sole nitrogen source), the *MET15* gene (encoding O-acetylhomoserine sulphydrylase, which confers on yeast cells sensitivity to methyl mercury), and the *CYH2* gene (encoding L29 ribosomal protein, which confers sensitivity to cycloheximide). In addition, any known cytotoxic agents
25 including cytotoxic proteins such as the diphtheria toxin (DTA) catalytic domain can also be used as counterselectable reporters. See U.S. Patent No. 5,733,726. DTA causes the ADP-ribosylation of elongation factor-2 and thus inhibits protein synthesis and causes cell death. Other examples of cytotoxic agents include ricin, Shiga toxin, and exotoxin A of *Pseudomonas aeruginosa*.

30 For example, when the *URA3* gene is used as a counterselectable reporter gene, yeast cells containing a mutant *URA3* gene can be used as host cells (Ura⁻ Foa^R phenotype) for the *in vivo* assay. Such cells lack *URA3*-encoded functional

orotidine-5'-phosphate decarboxylase, an enzyme required for the biosynthesis of uracil. As a result, the cells are unable to grow on media lacking uracil. However, because of the absence of a wild-type orotidine-5'-phosphate decarboxylase, the yeast cells cannot convert non-toxic 5-fluoroorotic acid (5-FOA) to a toxic product, 5-fluorouracil. Thus, such yeast cells are resistant to 5-FOA and can grow on a medium containing 5-FOA. Therefore, for example, to screen for a compound capable of disrupting interaction between AW755252 and PROTEIN2, AW755252 can be expressed as a fusion protein with a DNA-binding domain of a suitable transcription activator while PROTEIN2 is expressed as a fusion protein with a transcription activation domain of a suitable transcription activator. In the host strain, the reporter *URA3* gene may be operably linked to a promoter specifically responsive to the association of the transcription activation domain and the DNA-binding domain. After the fusion proteins are expressed in the *Ura⁻ Foa^R* yeast cells, an *in vivo* screening assay can be conducted in the presence of a test compound with the yeast cells being cultured on a medium containing uracil and 5-FOA. If the test compound does not disrupt the interaction between AW755252 and PROTEIN2, active *URA3* gene product, i.e., orotidine-5'-decarboxylase, which converts 5-FOA to toxic 5-fluorouracil, is expressed. As a result, the yeast cells cannot grow. On the other hand, when the test compound disrupts the interaction between AW755252 and PROTEIN2, no active orotidine-5'-decarboxylase is produced in the host yeast cells. Consequently, the yeast cells will survive and grow on the 5-FOA-containing medium. Therefore, compounds capable of interfering with or dissociating the interaction between AW755252 and PROTEIN2 can thus be identified based on colony formation.

As will be apparent, the screening assay of the present invention can be applied in a format appropriate for large-scale screening. For example, combinatorial technologies can be employed to construct combinatorial libraries of small organic molecules or small peptides. See generally, e.g., Kenan *et al.*, *Trends Biochem. Sc.*, 19:57-64 (1994); Gallop *et al.*, *J. Med. Chem.*, 37:1233-1251 (1994); Gordon *et al.*, *J. Med. Chem.*, 37:1385-1401 (1994); Ecker *et al.*, *Biotechnology*, 13:351-360 (1995). Such combinatorial libraries of compounds can be applied to the screening assay of the present invention to isolate specific modulators of particular protein-protein interactions. In the case of random peptide libraries, the random

peptides can be co-expressed with the fusion proteins of the present invention in host cells and assayed *in vivo*. See e.g., Yang *et al.*, *Nucl. Acids Res.*, 23:1152-1156 (1995). Alternatively, they can be added to the culture medium for uptake by the host cells.

5 Conveniently, yeast mating is used in an *in vivo* screening assay. For example, haploid cells of alpha-mating type expressing one fusion protein as described above is mated with haploid cells of a-mating type expressing the other fusion protein. Upon mating, the diploid cells are spread on a suitable medium to form a lawn. Drops of test compounds can be deposited onto different areas of the
10 lawn. After culturing the lawn for an appropriate period of time, drops containing a compound capable of modulating the interaction between the particular test proteins in the fusion proteins can be identified by stimulation or inhibition of growth in the vicinity of the drops.

 The screening assays of the present invention for identifying compounds
15 capable of modulating protein-protein interactions can also be fine-tuned by various techniques to adjust the thresholds or sensitivity of the positive and negative selections. Mutations can be introduced into the reporter proteins to adjust their activities. The uptake of test compounds by the host cells can also be adjusted. For example, yeast high uptake mutants such as the *erg6* mutant strains can facilitate yeast
20 uptake of the test compounds. See Gaber *et al.*, *Mol. Cell. Biol.*, 9:3447-3456 (1989). Likewise, the uptake of the selection compounds such as 5-FOA, 2-deoxygalactose, cycloheximide, alpha-aminoadipate, and the like can also be fine-tuned.

5.3.1.4. Screening Assay for Enhancers

 The screening assay of the present invention can also be used in identifying
25 compounds that trigger or initiate, enhance or stabilize protein-protein interaction between AW755252 or a mutant thereof and a protein selected from the group consisting of GROUP1 or a mutant thereof. For example, if a disease or disorder is associated with decreased expression of AW755252 and/or a member of selected from the group of GROUP1, then the disease or disorder may be treated or prevented by
30 strengthening or stabilizing the interaction between AW755252 and the AW755252-interacting member in a patient. Alternatively, if a disease or disorder is

associated with mutant forms of AW755252 and/or a AW755252-interacting protein that lead to weakened or abolished protein-protein interaction therebetween, then the disease or disorder may be treated with a compound that initiates or stabilizes the interaction between the mutant forms of AW755252 and/or the AW755252-interacting
5 protein.

Thus, a screening assay can be performed in the same manner as described above, except that a positively selectable marker is used. For example, AW755252 or a mutant form or a binding domain thereof, and a protein selected from the group consisting of GROUP1, or a mutant form or a binding domain thereof, are used as test
10 proteins expressed in the form of fusion proteins as described above for purposes of a two-hybrid assay. The fusion proteins are expressed in a host cell and allowed to interact with each other in the presence of one or more test compounds.

A gene encoding a positively selectable marker such as the lacZ protein may be used as a reporter gene such that when a test compound enables or enhances the
15 interaction between AW755252, or a mutant form or a binding domain thereof, and a protein selected from the group consisting of GROUP1 or a mutant form or a binding domain thereof, the lacZ protein, i.e., beta-galactosidase is expressed. As a result, the compound may be identified based on the appearance of a blue color when the host cells are cultured in a medium containing X-Gal.

20 Optionally, a control assay is performed in which the above screening assay is conducted in the absence of the test compound. The result is then compared with that obtained in the presence of the test compound.

5.4. Optimization of the Identified Compounds

Once an effective compound is identified, structural analogs or mimetics
25 thereof can be produced based on rational drug design with the aim of improving drug efficacy and stability, and reducing side effects. Methods known in the art for rational drug design can be used in the present invention. *See, e.g., Hodgson et al., Bio/Technology*, 9:19-21 (1991); U.S. Patent Nos. 5,800,998 and 5,891,628, all of which are incorporated herein by reference. An example of rational drug design is
30 the development of HIV protease inhibitors. *See Erickson et al., Science*, 249:527-533 (1990).

Preferably, structural information on the protein-protein interaction to be modulated is obtained. For example, each of the interacting pair can be expressed and purified. The purified interacting protein pairs are then allowed to interact with each other *in vitro* under appropriate conditions. Optionally, the interacting protein
5 complex can be stabilized by crosslinking or other techniques. The interacting complex can be studied using various biophysics techniques including, e.g., X-ray crystallography, NMR, computer modeling, mass spectrometry, and the like. Likewise, structural information can also be obtained from protein complexes formed by interacting proteins and a compound that initiates or stabilizes the interaction of
10 the proteins.

In addition, understanding of the interaction between the proteins of interest in the presence or absence of a modulator can also be derived from mutagenesis analysis using yeast two-hybrid system or other methods for detection protein-protein interaction. In this respect, various mutations can be introduced into the interacting
15 proteins and the effect of the mutations on protein-protein interaction is examined by a suitable method such as the yeast two-hybrid system.

Various mutations including amino acid substitutions, deletions and insertions can be introduced into a protein sequence using conventional recombinant DNA technologies. Generally, it is particularly desirable to decipher the protein
20 binding sites. Thus, it is important that the mutations introduced only affect protein-protein interaction and cause minimal structural disturbances. Mutations are preferably designed based on knowledge of the three-dimensional structure of the interacting proteins. Preferably, mutations are introduced to alter charged amino acids or hydrophobic amino acids exposed on the surface of the proteins, since ionic
25 interactions and hydrophobic interactions are often involved in protein-protein interactions. Alternatively, the "alanine scanning mutagenesis" technique is used. See Wells, *et al.*, *Methods Enzymol.*, 202:301-306 (1991); Bass *et al.*, *Proc. Natl. Acad. Sci. USA*, 88:4498-4502 (1991); Bennet *et al.*, *J. Biol. Chem.*, 266:5191-5201 (1991); Diamond *et al.*, *J. Virol.*, 68:863-876 (1994). Using this technique, charged or
30 hydrophobic amino acid residues of the interacting proteins are replaced by alanine, and the effect on the interaction between the proteins is analyzed using e.g., the yeast two-hybrid system. For example, the entire protein sequence can be scanned in a

window of five amino acids. When two or more charged or hydrophobic amino acids appear in a window, the charged or hydrophobic amino acids are changed to alanine using standard recombinant DNA techniques. The thus mutated proteins are used as "test proteins" in the above-described two-hybrid assay to examine the effect of the mutations on protein-protein interaction. Preferably, the mutagenesis analysis is conducted both in the presence and in the absence of an identified modulator compound. In this manner, the domains or residues of the proteins important to protein-protein interaction and/or the interaction between the modulator compound and the proteins can be identified.

Based on the structural information obtained, structural relationships between the interacting proteins as well as between the identified compound and the interacting proteins are elucidated. The moieties and the three-dimensional structure of the identified compound, i.e., lead compound, critical to its modulating effect on the interaction of the proteins of interest are revealed. Medicinal chemists can then design analog compounds having similar moieties and structures.

In addition, an identified peptide compound capable of modulating particular protein-protein interactions can also be analyzed by the alanine scanning technique and/or the two-hybrid assay to determine the domains or residues of the peptide important to its modulating effect on particular protein-protein interactions. The peptide compound can be used as a lead molecule for rational design of small organic molecules or peptide mimetics. See Huber *et al.*, *Curr. Med. Chem.*, 1:13-34 (1994).

The residues or domains critical to the modulating effect of the identified compound constitute the active region of the compound known as its "pharmacophore."

Once the pharmacophore has been elucidated, a structural model can be established by a modeling process that may incorporate data from NMR analysis, X-ray diffraction data, alanine scanning, spectroscopic techniques and the like. Various techniques including computational analysis, similarity mapping and the like can all be used in this modeling process. See *e.g.*, Perry *et al.*, in *OSAR: Quantitative Structure-Activity Relationships in Drug Design*, pp.189-193, Alan R. Liss, Inc., 1989; Rotivinen *et al.*, *Acta Pharmaceutica Fennica*, 97:159-166 (1988);

Lewis *et al.*, *Proc. R. Soc. Lond.*, 236:125-140 (1989); McKinaly *et al.*, *Annu. Rev. Pharmacol. Toxicol.*, 29:111-122 (1989). Commercial molecular modeling systems available from Polygen Corporation, Waltham, MA, include the CHARMM program, which performs the energy minimization and molecular dynamics functions, and
5 QUANTA program which performs the construction, graphic modeling and analysis of molecular structure. Such programs allow interactive construction, visualization and modification of molecules. Other computer modeling programs are also available from BioDesign, Inc. (Pasadena, CA.), Hypercube, Inc. (Cambridge, Ontario), and Allelix, Inc. (Mississauga, Ontario, Canada).

10 A template can be formed based on the established model. Various compounds can then be designed by linking various chemical groups or moieties to the template. Various moieties of the template can also be replaced. In addition, in the case of a peptide lead compound, the peptide or mimetics thereof can be cyclized, e.g., by linking the N-terminus and C-terminus together, to increase its stability.
15 These rationally designed compounds are further tested. In this manner, pharmacologically acceptable and stable compounds with improved efficacy and reduced side effect can be developed. The compounds identified in accordance with the present invention can be incorporated into a pharmaceutical formulation suitable for administration to an individual.

20 6. Therapeutic Applications

As described above, the interactions between AW755252 and the AW755252-interacting proteins suggest that these proteins and/or the protein complexes formed by such proteins may be involved in the same biological processes and disease pathways. Thus, one may modulate such biological processes by
25 modulating the functions and activities of AW755252, a AW755252-interacting protein, and a protein complex formed by the proteins. As used herein, modulating the functions or activities of AW755252, a AW755252-interacting protein, and a protein complex formed by the proteins means causing any forms of alteration of the properties, biological activities or functions of the proteins or protein complexes,
30 including, e.g., increasing the levels of AW755252, a AW755252-interacting protein or a protein complex formed by the proteins, enhancing or reducing their biological activities, increasing or decreasing their stability, altering their affinity or specificity

to certain other biological molecules, etc. For example, an AW755252-containing protein complex of the present invention or its members thereof may be involved in cardiac function. Thus, assays such as those described in Section 4 may be used in determining the effect of an aberration in a particular AW755252-containing complex
5 or an interacting member thereof on cardiac function. In addition, it is also possible to determine, using the same assay methods, the presence or absence of an association between a AW755252-containing complex or an interacting member thereof and a physiological disorder or disease such as ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina
10 pectoris or predisposition to the physiological disorder or disease.

Once such associations are established, the diagnostic methods as described in Section 4 can be used in diagnosing the disease or disorder. In addition, various *in vitro* and *in vivo* assays may be employed to test the therapeutic or prophylactic efficacies of the various therapeutic approaches described in Sections 6.2 and 6.3
15 which are aimed to modulate the functions and activities of a particular AW755252-containing complex of the present invention or an interacting member thereof. Similar assays can also be used to test whether the therapeutic approaches described in Sections 6.2 and 6.3 result in the modulation of cardiac function. The cell model or transgenic animal model described in Section 7 may be employed in the
20 *in vitro* and *in vivo* assays.

6.1. Applicable Diseases

The method for modulating the function and activities of AW755252-containing protein complexes of the present invention or interacting members thereof may be employed to modulate cardiac function.

25 In addition, the methods may also be used in the treatment or prevention of ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris.

6.2. Inhibiting Protein Complex or Interacting Protein Members Thereof

30 In one aspect of the present invention, methods are provided for reducing in a

patient the level and/or activity of a protein complex identified in accordance with the present invention which comprises AW755252 and a member of the GROUP1. In addition, methods are also provided for reducing in a patient the level and/or activity of a AW755252-interacting protein selected from the GROUP1. By reducing the
5 protein complex and/or the AW755252-interacting protein level and/or inhibiting the functional activities of the protein complex and/or the AW755252-interacting protein, the diseases involving such protein complex or AW755252-interacting protein may be treated or prevented.

6.2.1. Antibody Therapy

10 In one embodiment, an antibody may be administered to a patient. The antibody administered may be immunoreactive with AW755252 or a member of the GROUP1. Suitable antibodies may be monoclonal or polyclonal that fall within any antibody classes, e.g., IgG, IgM, IgA, etc. The antibody suitable for this invention may also take a form of various antibody fragments including, but not limited to, Fab
15 and F(ab')₂, single-chain fragments (scFv), and the like. In one embodiment, an antibody selectively immunoreactive with the protein complex formed from AW755252 and a AW755252-interacting protein in accordance with the present invention is administered to a patient. In another embodiment, an antibody specific to a AW755252-interacting protein selected from the GROUP1 is administered to a
20 patient. Methods for making the antibodies of the present invention should be apparent to a person of skill in the art, especially in view of the discussions in Section 3 above. The antibodies can be administered in any suitable form and route as described in Section 8 below. Preferably, the antibodies are administered in a pharmaceutical composition together with a pharmaceutically acceptable carrier.

25 Alternatively, the antibodies may be delivered by a gene-therapy approach. That is, nucleic acids encoding the antibodies, particularly single-chain fragments (scFv), may be introduced into a patient such that desirable antibodies may be produced recombinantly *in vivo* from the nucleic acids. For this purpose, the nucleic acids with appropriate transcriptional and translation regulatory sequences can be
30 directly administered into the patient. Alternatively, the nucleic acids can be incorporated into a suitable vector as described in Sections 2.2 and 5.3.1.1 and delivered into a patient along with the vector. The expression vector containing the

nucleic acids can be administered directly to a patient. It can also be introduced into cells, preferably cells derived from a patient to be treated, and subsequently delivered into the patient by cell transplantation. See Section 6.3.2 below.

6.2.2. Antisense Therapy

5 In another embodiment, antisense compounds specific to nucleic acids encoding one or more interacting protein members of a protein complex identified in the present invention is administered to a patient to be therapeutically or prophylactically treated. The antisense compounds should specifically inhibit the expression of the one or more interacting protein members. As is known in the art,
10 antisense drugs generally act by hybridizing to a particular target nucleic acid thus blocking gene expression. Methods for designing antisense compounds and using such compounds in treating diseases are well known and well developed in the art. For example, the antisense drug Vitravene[®] (fomivirsen), a 21-base long oligonucleotide, has been successfully developed and marketed by Isis
15 Pharmaceuticals, Inc. for treating cytomegalovirus (CMV)-induced retinitis.

Any methods for designing and making antisense compounds may be used for purpose of the present invention. See generally, Sanghvi *et al.*, eds., *Antisense Research and Applications*, CRC Press, Boca Raton, 1993. Typically, antisense compounds are oligonucleotides designed based on the nucleotide sequence of the mRNA or gene of one or more of the interacting protein members of a particular protein complex of the present invention. In particular, antisense compounds can be designed to specifically hybridize to a particular region of the gene sequence or mRNA of one or more of the interacting protein members to modulate (increase or decrease), replication, transcription, or translation. As used herein, the term “specifically hybridize” or paraphrases thereof means a sufficient degree of complementarity or pairing between an antisense oligo and a target DNA or mRNA such that stable and specific binding occurs therebetween. In particular, 100% complementary or pairing is not required. Specific hybridization takes place when sufficient hybridization occurs between the antisense compound and its intended target nucleic acids in substantially absence of non-specific binding of the antisense compound to non-target sequences under predetermined conditions, e.g., for purposes of in vivo treatment, preferably under physiological conditions. Preferably, specific

hybridization results in the interference with normal expression of the target DNA or mRNA.

For example, an antisense oligo can be designed to specifically hybridize to the replication or transcription regulatory regions of a target gene, or the translation regulatory regions such as translation initiation region and exon/intron junctions, or the coding regions of a target mRNA.

As is generally known in the art, commonly used oligonucleotides are oligomers or polymers of ribonucleic acid or deoxyribonucleic acid having a combination of naturally-occurring nucleoside bases, sugars and covalent linkages between nucleoside bases and sugars including a phosphate group. However, it is noted that the term "oligonucleotides" also encompasses various non-naturally occurring mimetics and derivatives, i.e., modified forms, of naturally-occurring oligonucleotides as described below. Typically an antisense compound of the present invention is an oligonucleotide having from about 6 to about 200, preferably from about 8 to about 30 nucleoside bases.

The antisense compounds preferably contain modified backbones or non-natural internucleoside linkages, including but not limited to, modified phosphorous-containing backbones and non-phosphorous backbones such as morpholino backbones; siloxane, sulfide, sulfoxide, sulfone, sulfonate, sulfonamide, and sulfamate backbones; formacetyl and thioformacetyl backbones; alkene-containing backbones; methyleneimino and methylenehydrazino backbones; amide backbones, and the like.

Examples of modified phosphorous-containing backbones include, but are not limited to phosphorothioates, phosphorodithioates, chiral phosphorothioates, phosphotriesters, aminoalkylphosphotriesters, alkyl phosphonates, thionoalkylphosphonates, phosphinates, phosphoramidates, thionophosphoramidates, thionoalkylphosphotriesters, and boranophosphates and various salt forms thereof. *See e.g.*, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821;

5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

Examples of the non-phosphorous containing backbones described above are disclosed in, e.g., U.S. Pat. Nos. 5,034,506; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

Another useful modified oligonucleotide is peptide nucleic acid (PNA), in which the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, e.g., an aminoethylglycine backbone. See U.S. Patent Nos. 5,539,082 and 5,714,331; and Nielsen *et al.*, *Science*, 254, 1497-1500 (1991), all of which are incorporated herein by reference. PNA antisense compounds are resistant to RNase H digest and thus exhibit longer half-life. In addition, various modifications may be made in PNA backbones to impart desirable drug profiles such as better stability, increased drug uptake, higher affinity to target nucleic acid, etc.

Alternatively, the antisense compounds are oligonucleotides containing modified nucleosides, i.e., modified purine or pyrimidine bases, e.g., 5-substituted pyrimidines, 6-azapyrimidines, and N-2, N-6 and O-substituted purines, and the like. See e.g., U.S. Pat. Nos. 3,687,808; 4,845,205; 5,130,302; 5,175,273; 5,367,066; 5,432,272; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,587,469; 5,594,121; 5,596,091; 5,681,941; and 5,750,692, each of which is incorporated herein by reference in its entirety.

In addition, oligonucleotides with substituted or modified sugar moieties may also be used. For example, an antisense compound may have one or more 2'-O-methoxyethyl sugar moieties. See e.g., U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,567,811; 5,576,427; 5,591,722; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference.

Other types of oligonucleotide modifications are also useful including linking an oligonucleotide to a lipid, phospholipid or cholesterol moiety, cholic acid,

thioether, aliphatic chain, polyamine, polyethylene glycol (PEG), or a protein or peptide. The modified oligonucleotides may exhibit increased uptake into cells, improved stability, i.e., resistance to nuclease digestion and other biodegradations. *See e.g.*, U.S. Patent No. 4,522,811; Burnham, *Am. J. Hosp. Pharm.*, 15:210-218 (1994).

Antisense compounds can be synthesized using any suitable methods known in the art. In fact, antisense compounds may be custom made by commercial suppliers. Alternatively, antisense compounds may be prepared using DNA synthesizers commercially from various vendors, e.g., Applied Biosystems Group of Norwalk, CT.

The antisense compounds can be formulated into a pharmaceutical composition with suitable carriers and administered into a patient using any suitable route of administration. Alternatively, the antisense compounds may also be used in a "gene-therapy" approach. That is, the oligonucleotide is subcloned into a suitable vector and transformed into human cells. The antisense oligonucleotide is then produced *in vivo* through transcription. Methods for gene therapy are disclosed in Section 6.3.2 below.

6.2.3. Ribozyme Therapy

In another embodiment, an enzymatic RNA or ribozyme is designed to target the nucleic acids encoding one or more of the interacting protein members of the protein complex of the present invention. Ribozymes are RNA molecules, which have an enzymatic activity and are capable of repeatedly cleaving other separate RNA molecules in a nucleotide base sequence specific manner. *See Kim et al.*, *Proc. Natl. Acad. of Sci. USA*, 84:8788 (1987); Haseloff and Gerlach, *Nature*, 334:585 (1988); and Jefferies *et al.*, *Nucleic Acid Res.*, 17:1371 (1989). A ribozyme typically has two portions: a catalytic portion and a binding sequence that guides the binding of ribozymes to a target RNA through complementary base-pairing. Once the ribozyme is bound to a target RNA, it enzymatically cleaves the target RNA, typically destroying its ability to direct translation of an encoded protein. After a ribozyme has cleaved its RNA target, it is released from that target RNA and thereafter can bind and cleave another target. That is, a single ribozyme molecule can repeatedly bind

and cleave new targets. Therefore, one advantage of ribozyme treatment is that a lower amount of exogenous RNA is required as compared to conventional antisense therapies. In addition, ribozymes exhibit less affinity to mRNA targets than DNA-based antisense oligos, and therefore are less prone to bind to wrong targets.

In accordance with the present invention, a ribozyme may target any portions of the mRNA of one or more interacting protein members including AW755252, and GROUP 1 proteins. Methods for selecting a ribozyme target sequence and designing and making ribozymes are generally known in the art. *See e.g.*, U.S. Patent Nos. 4,987,071; 5,496,698; 5,525,468; 5,631,359; 5,646,020; 5,672,511; and 6,140,491, each of which is incorporated herein by reference in its entirety. For example, suitable ribozymes may be designed in various configurations such as hammerhead motifs, hairpin motifs, hepatitis delta virus motifs, group I intron motifs, or RNase P RNA motifs. *See e.g.*, U.S. Patent Nos. 4,987,071; 5,496,698; 5,525,468; 5,631,359; 5,646,020; 5,672,511; and 6,140,491; Rossi *et al.*, *AIDS Res. Human Retroviruses* 8:183 (1992); Hampel and Tritz, *Biochemistry* 28:4929 (1989); Hampel *et al.*, *Nucleic Acids Res.*, 18:299 (1990); Perrotta and Been, *Biochemistry* 31:16 (1992); and Guerrier-Takada *et al.*, *Cell*, 35:849 (1983).

Ribozymes can be synthesized by the same methods used for normal RNA synthesis. For example, such methods are disclosed in Usman *et al.*, *J. Am. Chem. Soc.*, 109:7845-7854 (1987) and Scaringe *et al.*, *Nucleic Acids Res.*, 18:5433-5441 (1990). Modified ribozymes may be synthesized by the methods disclosed in, e.g., U.S. Pat. No. 5,652,094; International Publication Nos. WO 91/03162; WO 92/07065 and WO 93/15187; European Patent Application No. 92110298.4; Perrault *et al.*, *Nature*, 344:565 (1990); Pieken *et al.*, *Science*, 253:314 (1991); and Usman and Cedergren, *Trends in Biochem. Sci.*, 17:334 (1992).

Ribozymes of the present invention may be administered to cells by any known methods, e.g., disclosed in International Publication No. WO 94/02595. For example, they can be administered directly to a patient through any suitable route, e.g., intravenous injection. Alternatively, they may be delivered in encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. In addition, they may also be delivered by gene therapy approach, using a DNA

vector from which the ribozyme RNA can be transcribed directly. Gene therapy methods are disclosed in detail below in Section 6.3.2.

6.2.4. Other Methods

The patient level and activity of a particular protein complex and the interacting protein members thereof identified in accordance with the present invention may also be inhibited by various other methods. For example, compounds identified in accordance with the methods described in Section 5 that are capable of interfering with or dissociating protein-protein interactions between the interacting protein members of a protein complex may be administered to a patient. Compounds identified in *in vitro* binding assays described in Section 5.2 that bind to the AW755252-containing protein complex or the interacting members thereof may also be used in the treatment. In addition, useful agents also include incomplete proteins, i.e., fragments of the interacting protein members that are capable of binding to their respective binding partners in a protein complex but are defective of its normal cellular functions. For example, binding domains of the interacting member proteins of a protein complex may be used as competitive inhibitors of the activities of the protein complex. As will be apparent to skilled artisans, derivatives or homologues of the binding domains may also be used.

In yet another embodiment, the gene therapy methods discussed in Section 6.2.2 below are used to “knock out” the gene encoding an interacting protein member of a protein complex, or to reduce the gene expression level. For example, the gene may be replaced with a different gene sequence or a non-functional sequence or simply deleted by homologous recombination. In another gene therapy embodiment, the method disclosed in U.S. Patent No. 5,641,670, which is incorporated herein by reference, may be used to reduce the expression of the genes for the interacting protein members. Essentially, an exogenous DNA having at least a regulatory sequence, an exon and a splice donor site can be introduced into an endogenous gene encoding an interacting protein member by homologous recombination such that the regulatory sequence, the exon and the splice donor site present in the DNA construct become operatively linked to the endogenous gene. As a result, the expression of the endogenous gene is controlled by the newly introduced exogenous regulatory sequence. Therefore, when the exogenous regulatory sequence is a strong gene

expression repressor, the expression of the endogenous gene encoding the interacting protein member is reduced or blocked. *See* U.S. Patent No. 5,641,670.

6.3. Activating Protein Complex or Interacting Protein Members Thereof

The present invention also provides methods for increasing in a patient the level and/or activity of a protein complex or of an individual protein member thereof identified in accordance with the present invention. Such methods can be particularly useful in instances where a reduced level and/or activity of a protein
5 complex or a protein member thereof are associated with a particular disease or disorder to be treated, or where an increased level and/or activity of a protein complex or a protein member thereof would be beneficial to the improvement of a cellular function or disease state. By increasing the level of the protein complex or a protein member thereof, and/or stimulating the functional activities of the protein complex or
10 a protein member thereof, the disease or disorder may be treated or prevented.

6.3.1. Administration of Protein Complex or Protein Members Thereof

Where the level or activity of a particular AW755252-containing protein complex or a AW755252-interacting protein of the present invention in a patient is determined to be low or is desired to be increased, the protein complex or the AW755252-interacting protein may be administered directly to the patient to increase the level and/or activity of the protein complex or the AW755252-interacting protein. For this purpose, protein complexes prepared by any one of the methods described in Section 2.2 may be administered to the patient, preferably in a pharmaceutical composition as described below. Alternatively, one or more individual interacting protein members of the protein complex may also be administered to the patient in need of treatment. For example, one or more proteins such as AW755252, GROUP1 may be given to a patient. Proteins isolated or purified from normal individuals or recombinantly produced can all be used in this respect. Preferably, two or more interacting protein members of a protein complex are administered. The proteins or protein complexes may be administered to a patient needing treatment in any methods described in Section 8.

6.3.2. Gene Therapy

In another embodiment, the patient level and/or activity of a particular AW755252-containing protein complex or a AW755252-interacting protein member thereof (selected from the group of GROUP1) is increased or restored by the gene therapy approach. For example, nucleic acids encoding one or more protein members of a AW755252-containing protein complex of the present invention, or portions or fragments of the protein members are introduced into tissue cells of a patient needing treatment such that the one or more protein members are expressed from the introduced nucleic acids. For this purposes, nucleic acids encoding one or more of AW755252, GROUP1, or fragments, homologues or derivatives thereof can be used in the gene therapy in accordance with the present invention. For example, if a disease-causing mutation exists in one of the protein members of a patient, then a nucleic acid encoding a wild-type protein can be introduced into tissue cells of the patient. The exogenous nucleic acid can be used to replace the corresponding endogenous defective gene by, e.g., homologous recombination. See U.S. Patent No. 6,010,908, which is incorporated herein by reference. Alternatively, if the disease-causing mutation is a recessive mutation, the exogenous nucleic acid is simply used to express a wild-type protein in addition to the endogenous mutant protein. In another approach, the method disclosed in U.S. Patent No. 6,077,705 may be employed in gene therapy. That is, the patient is administered both a nucleic acid construct encoding a ribozyme and a nucleic acid construct comprising a ribozyme resistant gene encoding a wild type form of the gene product. As a result, undesirable expression of the endogenous gene is inhibited and a desirable wild-type exogenous gene is introduced. In yet another embodiment, if the endogenous gene is of wild-type and the level of expression of the protein encoded thereby is desired to be increased, additional copies of wild-type exogenous genes may be introduced into the patient by gene therapy, or alternatively, a gene activation method such as that disclosed in U.S. Patent No. 5,641,670 may be used.

Various gene therapy methods are well known in the art. Successes in gene therapy have been reported recently. See e.g., Kay *et al.*, *Nature Genet.*, 24:257-61 (2000); Cavazzana-Calvo *et al.*, *Science*, 288:669 (2000); and Blaese *et al.*, *Science*, 270: 475 (1995); Kantoff, *et al.*, *J. Exp. Med.* 166:219 (1987).

Any suitable gene therapy method may be used for purposes of the present invention. Generally, a nucleic acid encoding a desirable protein, e.g., one selected from AW755252, GROUP1 is incorporated into a suitable expression vector and is operably linked to a promoter in the vector. Suitable promoters include but are not limited to viral transcription promoters derived from adenovirus, simian virus 40 (SV40) (e.g., the early and late promoters of SV40), Rous sarcoma virus (RSV), and cytomegalovirus (CMV) (e.g., CMV immediate-early promoter), human immunodeficiency virus (HIV) (e.g., long terminal repeat (LTR)), vaccinia virus (e.g., 7.5K promoter), and herpes simplex virus (HSV) (e.g., thymidine kinase promoter). Where tissue-specific expression of the exogenous gene is desirable, tissue-specific promoters may be operably linked to the exogenous gene. In addition, selection markers may also be included in the vector for purposes of selecting, *in vitro*, those cells that contain the exogenous gene. Various selection markers known in the art may be used including, but not limited to, e.g., genes conferring resistance to neomycin, hygromycin, zeocin, and the like.

In one embodiment, the exogenous nucleic acid (gene) is incorporated into a plasmid DNA vector. Many commercially available expression vectors may be useful for the present invention, including, e.g., pCEP4, pcDNA1, pIND, pSecTag2, pVAX1, pcDNA3.1, and pBI-EGFP, and pDisplay.

Various viral vectors may also be used. Typically, in a viral vector, the viral genome is engineered to eliminate the disease-causing capability, e.g., the ability to replicate in the host cells. The exogenous nucleic acid to be introduced into a patient may be incorporated into the engineered viral genome, e.g., by inserting it into a viral gene that is non-essential to the viral infectivity. Viral vectors are convenient to use as they can be easily introduced into tissue cells by way of infection. Once in the host cell, the recombinant virus typically is integrated into the genome of the host cell. In rare instances, the recombinant virus may also replicate and remain as extrachromosomal elements.

A large number of retroviral vectors have been developed for gene therapy. These include vectors derived from adenovirus, oncoretroviruses (e.g., MLV), lentiviruses (e.g., HIV and SIV) and other retroviruses. For example, gene therapy vectors have been developed based on murine leukemia virus (*See, Cepko, et al., Cell,*

37:1053-1062 (1984), Cone and Mulligan, *Proc. Natl. Acad. Sci. U.S.A.*, 81:6349-6353 (1984)), mouse mammary tumor virus (*See*, Salmons *et al.*, *Biochem. Biophys. Res. Commun.*, 159:1191-1198 (1984)), gibbon ape leukemia virus (*See*, Miller *et al.*, *J. Virology*, 65:2220-2224 (1991)), HIV, (*See* Shimada *et al.*, *J. Clin. Invest.*, 88:1043-1047 (1991)), and avian retroviruses (*See* Cosset *et al.*, *J. Virology*, 64:1070-1078 (1990)). In addition, various retroviral vectors are also described in U.S. Patent Nos. 6,168,916; 6,140,111; 6,096,534; 5,985,655; 5,911,983; 4,980,286; and 4,868,116, all of which are incorporated herein by reference.

Adeno-associated virus (AAV) vectors have been successfully tested in clinical trials. *See e.g.*, Kay *et al.*, *Nature Genet.* 24:257-61 (2000). AAV is a naturally occurring defective virus that requires other viruses such as adenoviruses or herpes viruses as helper viruses. *See* Muzyczka, *Curr. Top. Microbiol. Immun.*, 158:97 (1992). A recombinant AAV virus useful as a gene therapy vector is disclosed in U.S. Patent No. 6,153,436, which is incorporated herein by reference.

Adenoviral vectors can also be useful for purposes of gene therapy in accordance with the present invention. For example, U.S. Patent No. 6,001,816 discloses an adenoviral vector, which is used to deliver a leptin gene intravenously to a mammal to treat obesity. Other recombinant adenoviral vectors may also be used, which include those disclosed in U.S. Patent Nos. 6,171,855; 6,140,087; 6,063,622; 6,033,908; and 5,932,210, and Rosenfeld *et al.*, *Science*, 252:431-434 (1991); and Rosenfeld *et al.*, *Cell*, 68:143-155 (1992).

Other useful viral vectors include recombinant hepatitis viral vectors (*See, e.g.*, U.S. Patent No. 5,981,274), and recombinant entomopox vectors (*See, e.g.*, U.S. Patent Nos. 5,721,352 and 5,753,258).

Other non-traditional vectors may also be used for purposes of this invention. For example, International Publication No. WO 94/18834 discloses a method of delivering DNA into mammalian cells by conjugating the DNA to be delivered with a polyelectrolyte to form a complex. The complex may be microinjected into or uptaken by cells.

The exogenous gene fragment or plasmid DNA vector containing the exogenous gene may also be introduced into cells by way of receptor-mediated

endocytosis. *See e.g.*, U.S. Patent No. 6,090,619; Wu and Wu, *J. Biol. Chem.*, 263:14621 (1988); Curiel *et al.*, *Proc. Natl. Acad. Sci. USA*, 88:8850 (1991). For example, U.S. Patent No. 6,083,741 discloses introducing an exogenous nucleic acid into mammalian cells by associating the nucleic acid to a polycation moiety (e.g., poly-L-lysine having 3-100 lysine residues), which is itself coupled to an integrin receptor binding moiety (e.g., a cyclic peptide having the sequence RGD).

Alternatively, the exogenous nucleic acid or vectors containing it can also be delivered into cells via amphiphiles. *See e.g.*, U.S. Patent No. 6,071,890. Typically, the exogenous nucleic acid or a vector containing the nucleic acid forms a complex with the cationic amphiphile. Mammalian cells contacted with the complex can readily take the complex up.

The exogenous gene can be introduced into a patient for purposes of gene therapy by various methods known in the art. For example, the exogenous gene sequences alone or in a conjugated or complex form described above, or incorporated into viral or DNA vectors, may be administered directly by injection into an appropriate tissue or organ of a patient. Alternatively, catheters or like devices may be used for delivery into a target organ or tissue. Suitable catheters are disclosed in, e.g., U.S. Patent Nos. 4,186,745; 5,397,307; 5,547,472; 5,674,192; and 6,129,705, all of which are incorporated herein by reference.

It is preferred that these vectors be administered in a pharmaceutically acceptable carrier for injection such as a sterile aqueous solution or dispersion, preferably isotonic. Dose and duration of treatment is determined individually depending on the degree and rate of improvement. Such determinations are performed routinely by physicians in the art.

In addition, the exogenous gene or vectors containing the gene can be introduced into isolated cells using any known techniques such as calcium phosphate precipitation, microinjection, lipofection, electroporation, gene gun, receptor-mediated endocytosis, and the like. Cells expressing the exogenous gene may be selected and redelivered back to the patient by, e.g., injection or cell transplantation. The appropriate amount of cells delivered to a patient will vary with patient conditions, and desired effect, which can be determined by a skilled artisan.

See e.g., U.S. Patent Nos. 6,054,288; 6,048,524; and 6,048,729. Preferably, the cells used are autologous, i.e., cells obtained from the patient being treated.

6.3.3. Small Organic Compounds

Defective conditions or disorders in a patient associated with decreased level or activity of a AW755252-containing protein complex or a AW755252-interacting protein identified in accordance with the present invention can also be ameliorated by administering to the patient a compound identified by the methods described in Sections 5.3.1.4, 5.2, and Section 5.4, which is capable of modulating the functions of the protein complex or the AW755252-interacting protein, e.g., by triggering or initiating, enhancing or stabilizing protein-protein interaction between the interacting protein members of the protein complex, or the mutant forms of such interacting protein members found in the patient.

7. Cell and Animal Models

In another aspect of the present invention, cell and animal models are provided in which one or more of the AW755252-containing protein complexes identified in the present invention are in an aberrant form, e.g., increased or decreased level of the protein complexes, altered interaction between interacting protein members of the protein complexes, and/or altered distribution or localization (e.g., in organs, tissues, cells, or cellular compartments) of the protein complexes. Such cell and animal models are useful tools for studying the disorders and diseases caused by the protein complex aberration and for testing various methods for treating the diseases and disorders.

7.1. Cell Models

Cell models having an aberrant form of one or more of the protein complexes of the present invention are provided in accordance with the present invention.

The cell models may be established by isolating, from a patient, cells having an aberrant form of one or more of the protein complexes of the present invention. The isolated cells may be cultured *in vitro* as a primary cell culture. Alternatively, the cells obtained from the primary cell culture or directly from the patient may be

immortalized to establish a human cell line. Any methods for constructing immortalized human cell lines may be used in this respect. *See generally* Yeager and Reddel, *Curr. Opin. Biotech.*, 10:465-469 (1999). For example, the human cells may be immortalized by transfection of plasmids expressing the SV40 early region genes (*See e.g.*, Jha *et al.*, *Exp. Cell Res.*, 245:1-7 (1998)), introduction of the HPV E6 and E7 oncogenes (*See e.g.*, Reznikoff *et al.*, *Genes Dev.*, 8:2227-2240 (1994)), and infection with Epstein-Barr virus (*See e.g.*, Tahara *et al.*, *Oncogene*, 15:1911-1920 (1997)). Alternatively, the human cells may be immortalized by recombinantly expressing the gene for the human telomerase catalytic subunit hTERT in the human cells. *See* Bodnar *et al.*, *Science*, 279:349-352 (1998).

In alternative embodiments, cell models are provided by recombinantly manipulating appropriate host cells. The host cells may be bacteria cells, yeast cells, insect cells, plant cells, animal cells, and the like. Preferably, the cells are derived from mammals, preferably humans. The host cells may be obtained directly from an individual, or a primary cell culture, or preferably an immortal stable human cell line. In a preferred embodiment, human embryonic stem cells or pluripotent cell lines derived from human stem cells are used as host cells. Methods for obtaining such cells are disclosed in, e.g., Shamblott, *et al.*, *Proc. Natl. Acad. Sci. USA*, 95:13726-13731 (1998) and Thomson *et al.*, *Science*, 282:1145-1147 (1998).

In one embodiment, a cell model is provided by recombinantly expressing one or more of the protein complexes of the present invention in cells that do not normally express such protein complexes. For example, cells that do not contain a particular protein complex may be engineered to express the protein complex. In a specific embodiment, a particular human protein complex is expressed in non-human cells. The cell model may be prepared by introducing into host cells nucleic acids encoding all interacting protein members required for the formation of a particular protein complex, and expressing the protein members in the host cells. For this purpose, the recombination expression methods described in Section 2.2 may be used. In addition, the methods for introducing nucleic acids into host cells disclosed in the context of gene therapy in Section 6.2.2 may also be used.

In another embodiment, a cell model over-expressing one or more of the protein complexes of the present invention is provided. The cell model may be

established by increasing the expression level of one or more of the interacting protein members of the protein complexes. In a specific embodiment, all interacting protein members of a particular protein complex are over-expressed. The over-expression may be achieved by introducing into host cells exogenous nucleic acids encoding the proteins to be over-expressed, and selecting those cells that over-express the proteins. The expression of the exogenous nucleic acids may be transient or, preferably stable. The recombinant expression methods described in Section 2.2, and the methods for introducing nucleic acids into host cells disclosed in the context of gene therapy in Section 6.2.2 may be used. Alternatively, the gene activation method disclosed in U.S. Patent No. 5,641,670 can be used. Any host cells may be employed for establishing the cell model. Preferably, human cells lacking a protein complex to be over-expressed or having a normal level of the protein complex are used as host cells. The host cells may be obtained directly from an individual, or a primary cell culture, or preferably an immortal stable human cell line. In a preferred embodiment, human embryonic stem cells or pluripotent cell lines derived from human stem cells are used as host cells. Methods for obtaining such cells are disclosed in, e.g., Shamblott, *et al.*, *Proc. Natl. Acad. Sci. USA*, 95:13726-13731 (1998), and Thomson *et al.*, *Science*, 282:1145-1147 (1998).

In yet another embodiment, a cell model expressing an abnormally low level of one or more of the protein complexes of the present invention is provided. Typically, the cell model is established by genetically manipulating cells that express a normal and detectable level of a protein complex identified in accordance with the present invention. Generally the expression level of one or more of the interacting protein members of the protein complex is reduced by recombinant methods. In a specific embodiment, the expression of all interacting protein members of a particular protein complex is reduced. The reduced expression may be achieved by "knocking out" the genes encoding one or more interacting protein members. Alternatively, mutations that can cause reduced expression level (e.g., reduced transcription and/or translation efficiency, and decreased mRNA stability) may also be introduced into the gene by homologous recombination. A gene encoding a ribozyme or antisense compound specific to the mRNA encoding an interacting protein member may also be introduced into the host cells, preferably stably integrated into the genome of the host cells. In addition, a gene encoding an antibody or fragment thereof specific to an

interacting protein member may also be introduced into the host cells. The recombination expression methods described in Sections 2.2, 6.1 and 6.2 can all be used for purposes of manipulating the host cells.

The present invention also contemplates a cell model provided by recombinant DNA techniques that exhibits aberrant interactions between the interacting protein members of a protein complex identified in the present invention. For example, variants of the interacting protein members of a particular protein complex exhibiting altered protein-protein interaction properties and the nucleic acid variants encoding such variant proteins may be obtained by random or site-directed mutagenesis in combination with a protein-protein interaction assay system, particularly the yeast two-hybrid system described in Section 5.3.1. Essentially, the genes encoding one or more interacting protein members of a particular protein complex may be subject to random or site-specific mutagenesis and the mutated gene sequences are used in yeast two-hybrid system to test the protein-protein interaction characteristics of the protein variants encoded by the gene variants. In this manner, variants of the interacting protein members of the protein complex may be identified that exhibit altered protein-protein interaction properties in forming the protein complex, e.g., increased or decreased binding affinity, and the like. The nucleic acid variants encoding such protein variants may be introduced into host cells by the methods described above, preferably into host cells that normally do not express the interacting proteins.

7.2. Cell-Based Assays

The cell models of the present invention containing an aberrant form of a AW755252-containing protein complex of the present invention are useful in screening assays for identifying compounds useful in treating diseases and disorders involving ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris. In addition, they may also be used in *in vitro* pre-clinical assays for testing compounds, such as those identified in the screening assays of the present invention. A variety of parameters relevant to particularly physiological disorders or diseases may be analyzed.

For example, in one aspect of the invention, a method for screening for compounds that selectively inhibit abnormal cardiac function may be employed.

The method has following steps: (a) delivering a compound to be screened to a cell population of a first kind such as cell growth, apoptosis, cell viability, beating (using a primary cardiomyocyte) and cell size, wherein the first kind of the cell population is known to show abnormal cardiac function under a set of culture conditions sufficient for other cell population to show normal cardiac function and wherein said abnormal cardiac function is due to an aberration in a protein complex or an interaction of thereof between AW755252 and a protein selected from GROUP1 or a homologue or derivative or fragment thereof; (b) delivering the compound to a cell population of a second kind that is known to show normal cardiac function under said conditions and not known to have said aberration, wherein the compound does not affect the normal cardiac function of the second kind of the cell population; (c) comparing the cardiac function of the first and second kinds of cell populations; and (d) selecting the compound that inhibits the abnormal cardiac function of the first kind of cell population comparable to that of the second kind of cell population.

The first kind of cell populations may be those derived from ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophy cordis and angina pectoris tissues.

7.3. Transgenic Animals

In another aspect of the present invention, transgenic non-human animals are provided expressing an aberrant form of one or more of the AW755252-containing protein complexes of the present invention. Animals of any species may be used to generate the transgenic animal models, including but not limited to, mice, rats, hamsters, sheep, pigs, rabbits, guinea pigs, preferably non-human primates such as monkeys, chimpanzees, baboons, and the like.

In one embodiment, the transgenic animals are produced to over-express one or more protein complexes formed from AW755252 or a derivative or homologue thereof (including the animal counterpart of AW755252) and a AW755252-interacting protein selected from the group of GROUP1, or a derivative or homologue thereof (including an animal counterpart thereof). Over-expression may be exhibited in a tissue or cell that normally express the animal counterparts of such protein complexes. That is the level the protein complexes is elevated and is

higher than the normal level. Alternatively, the one or more protein complexes are expressed in tissues or cells that do not normally express such protein complexes (including the animal counterpart of the human protein complexes). In a specific embodiment, human AW755252 and at least one human protein selected from the group of GROUP1 are expressed in the transgenic animals.

To achieve over-expression in transgenic animals, the transgenic animals are made such that they contain and express exogenous genes encoding AW755252 or a homologue or derivative thereof and one or more of the AW755252-interacting proteins or a homologue or derivative thereof. Preferably, both exogenous genes are human genes. Such exogenous genes may be operably linked to a native or non-native promoter, preferably a non-native promoter. For example, an exogenous AW755252 gene may be operably linked to a promoter that is not the native AW755252 promoter. If the expression of the exogenous gene is desired to be limited to a particular tissue, an appropriate tissue-specific promoter may be used.

Over-expression may also be achieved by manipulating the native promoter to create mutations that lead to gene over-expression, or by a gene activation method such as that disclosed in U.S. Patent No. 5,641,670 as described above.

In another embodiment, the transgenic animal expresses an abnormally low level of one or more of protein complexes comprising AW755252 and a protein selected from the group of GROUP1. In a specific embodiment, the transgenic animal is a "knockout" animal wherein the endogenous gene encoding the animal homologue of AW755252 and/or an endogenous gene encoding an animal homologue of a AW755252-interacting protein are knocked out. In a specific embodiment, the expression of all interacting protein members of a particular protein complex comprising an animal homologues of AW755252 and an animal homologues of a protein selected from the group of GROUP1 is reduced or knocked out. The reduced expression may be achieved by knocking out the genes encoding one or more interacting protein members, typically by homologous recombination. Alternatively, mutations that can cause reduced expression level (e.g., reduced transcription and/or translation efficiency, and decreased mRNA stability) may also be introduced into the endogenous genes by homologous recombination. Genes encoding ribozymes or antisense compounds specific to the mRNAs encoding the interacting protein

members may also be introduced into the transgenic animal. In addition, genes encoding antibodies or fragments thereof specific to the interacting protein members may also be introduced into the transgenic animal.

In an alternate embodiment, the transgenic animal endogenous genes encoding the animal homologues of AW755252 and the animal homologues of a AW755252-interacting protein are both knocked out. Instead, the transgenic animal expresses a human version of AW755252 and a protein selected from the group of proteins in GROUP1.

Unique approaches have been developed and reported in the art, which approaches combine gene knocked out of the endogenous gene of a non-human mammal and gene transfer of a human homologue into the early embryo of a non-human mammal to generate an animal model for drug screening and development studies. For example, a transgenic mouse can be generated which can be knock out for the endogenous AW755252 (AW755252-null) but expresses a wild type human AW755252 gene. Because of the homology human AW755252 gene can compensate for the endogenous AW755252 gene. These animals are useful in the study of the progression of AW755252 related disorders and the development of strategies to cure such disorders by therapeutic drugs or by somatic cell therapy. Production of Transgenic animals such as, example, mice, rats, pigs, rabbits, cows, goats and monkeys can achieved by embryonic stem cell technology. The expression of the transgenes can be directed to specific tissues by using tissue specific promoters or sequences such as the locus control regions known in the art. The transgenic AW755252-null animals expressing a human AW755252 gene containing specific mutations similar to that observed in AW755252 of human patients, which mutations cause AW755252 related disorder in these patients, can be generated. Alternatively, these specific mutations can be introduced directly into the transgenic animals expressing a wild type human AW755252 gene via homologous recombination in embryonic stem cells. The transgenic animal with specific mutations in the human AW755252 transgene provide an excellent test model to predict onset and progression of the ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris and to design and test drug formulations for treatment of AW755252 related disorders

resulting from a specific mutation in humans.

In yet another embodiment, the transgenic animal of this invention exhibits aberrant interactions between AW755252 and a AW755252-interacting protein selected from the group of GROUP1. For this purpose, variants of AW755252 and
5 its interaction partners exhibiting altered protein-protein interaction properties and the nucleic acid variants encoding such variant proteins may be obtained by random or site-specific mutagenesis in combination with a protein-protein interaction assay system, particularly the yeast two-hybrid system described in Section 5.3.1. For example, variants of AW755252 and its interaction partners exhibiting increased or
10 decreased or abolished binding affinity to each other may be identified and isolated. The transgenic animal of the present invention may be made to express such protein variants by modifying the endogenous genes. Alternatively, the nucleic acid variants may be introduced exogenously into the transgenic animal genome to express the protein variants therein. In a specific embodiment, the exogenous nucleic acid
15 variants are derived from human and the corresponding endogenous genes are knocked out.

Any techniques known in the art for making transgenic animals may be used for purposes of the present invention. For example, the transgenic animals of the present invention may be provided by methods described in, e.g., Jaenisch, *Science*, 240:1468-1474 (1988); Capecchi, *et al.*, *Science*, 244:1288-1291 (1989); Hasty *et al.*, *Nature*, 350:243 (1991); Shinkai *et al.*, *Cell*, 68:855 (1992); Mombaerts *et al.*, *Cell*, 68:869 (1992); Philpott *et al.*, *Science*, 256:1448 (1992); Snouwaert *et al.*, *Science*, 257:1083 (1992); Donehower *et al.*, *Nature*, 356:215 (1992); Hogan *et al.*, *Manipulating the Mouse Embryo; A Laboratory Manual*, 2nd edition, Cold Spring Harbor Laboratory Press, 1994; and U.S. Patent Nos. 4,873,191; 5,800,998; 5,891,628, all of which are incorporated herein by reference.

Generally, the founder lines may be established by introducing appropriate exogenous nucleic acids into, or modifying an endogenous gene in, germ lines, embryonic stem cells, embryos, or sperms which are then in producing a transgenic animal. The gene introduction may be conducted by various methods including those described in Sections 2.2, 6.1 and 6.2. *See also*, Van der Putten *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:6148-6152 (1985); Thompson *et al.*, *Cell*, 56:313-321

(1989); Lo, *Mol. Cell. Biol.*, 3:1803-1814 (1983); Gordon, *Transgenic Animals, Intl. Rev. Cytol.* 115:171-229 (1989); and Lavitrano *et al.*, *Cell*, 57:717-723 (1989). In a specific embodiment, the exogenous gene is incorporated into an appropriate vector, such as those described in Sections 2.2 and 6.2, and is transformed into embryonic stem (ES) cells. The transformed ES cells are then injected into a blastocyst. The blastocyst with the transformed ES cells is then implanted into a surrogate mother animal. In this manner, a chimeric founder line animal containing the exogenous nucleic acid (transgene) may be produced.

Preferably, site-specific recombination is employed to integrate the exogenous gene into a specific predetermined site in the animal genome, or to replace an endogenous gene or a portion thereof with the exogenous sequence. Various site-specific recombination systems may be used including those disclosed in Sauer, *Curr. Opin. Biotechnol.*, 5:521-527 (1994); and Capecchi, *et al.*, *Science*, 244:1288-1291 (1989). and Gu *et al.*, *Science*, 265:103-106 (1994). Specifically, the Cre/lox site-specific recombination system known in the art may be conveniently used which employs the bacteriophage P1 protein Cre recombinase and its recognition sequence *loxP*. See Rajewsky *et al.*, *J. Clin. Invest.*, 98:600-603 (1996); Sauer, *Methods*, 14:381-392 (1998); Gu *et al.*, *Cell*, 73:1155-1164 (1993); Araki *et al.*, *Proc. Natl. Acad. Sci. USA*, 92:160-164 (1995); Lakso *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:6232-6236 (1992); and Orban *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:6861-6865 (1992).

The transgenic animals of the present invention may be transgenic animals that carry a transgene in all cells or mosaic transgenic animals carrying a transgene only in certain cells, e.g., somatic cells. The transgenic animals may have a single copy or multiple copies of a particular transgene.

The founder transgenic animals thus produced may be bred to produce various offspring. For example, they can be inbred, outbred, and crossbred to establish homozygous lines, heterozygous lines, and compound homozygous or heterozygous lines.

7.4. Myocardial Infarction Models

Animal models used for the present invention may include myocardial

infarction models, which are, for example, prepared by the following method:

Another group of male Wistar rats (250 -300 g) was anesthetized with sodium pentobarbital, and the chest was opened. After the heart was exposed, the left anterior descending artery was ligated for an hour, and then the ligature was released. The heart was excised 24 or 48 hours after reperfusion, and the left ventricle was divided into six slices perpendicular to the apex-base axis. These were then placed into 1% triphenyltetrazolium cchloride solution (37°C) for 10 minutes to dye the normal region. The undyed infarct region was cut out and weighed; the myocardial infarct size was expressed as a percentage of the weight of the left ventricle. (*Circulation Research*, vol. 69, 370-377 (1991)).

8. Pharmaceutical Compositions and Formulations

In another aspect of the present invention, pharmaceutical compositions are also provided containing one or more of the therapeutic agents provided in the present invention as described in Section 6. The compositions are prepared as a pharmaceutical formulation suitable for administration into a patient. Accordingly, the present invention also extends to pharmaceutical compositions, medicaments, drugs or other compositions containing one or more of the therapeutic agent in accordance with the present invention.

In the pharmaceutical composition, an active compound identified in accordance with the present invention can be in any pharmaceutically acceptable salt form. As used herein, the term "pharmaceutically acceptable salts" refers to the relatively non-toxic, organic or inorganic salts of the compounds of the present invention, including inorganic or organic acid addition salts of the compound. Examples of such salts include, but are not limited to, hydrochloride salts, sulfate salts, bisulfate salts, borate salts, nitrate salts, acetate salts, phosphate salts, hydrobromide salts, laurylsulfonate salts, glucoheptonate salts, oxalate salts, oleate salts, laurate salts, stearate salts, palmitate salts, valerate salts, benzoate salts, naththylate salts, mesylate salts, tosylate salts, citrate salts, lactate salts, maleate salts, succinate salts, tartrate salts, fumarate salts, and the like. *See, e.g., Berge, et al., J. Pharm. Sci.*, 66:1-19 (1977).

For oral delivery, the active compounds can be incorporated into a formulation

that includes pharmaceutically acceptable carriers such as binders (e.g., gelatin, cellulose, gum tragacanth), excipients (e.g., starch, lactose), lubricants (e.g., magnesium stearate, silicon dioxide), disintegrating agents (e.g., alginate, Primogel, and corn starch), and sweetening or flavoring agents (e.g., glucose, sucrose, saccharin, methyl salicylate, and peppermint). The formulation can be orally delivered in the form of enclosed gelatin capsules or compressed tablets. Capsules and tablets can be prepared in any conventional techniques. The capsules and tablets can also be coated with various coatings known in the art to modify the flavors, tastes, colors, and shapes of the capsules and tablets. In addition, liquid carriers such as fatty oil can also be included in capsules.

Suitable oral formulations can also be in the form of suspension, syrup, chewing gum, wafer, elixir, and the like. If desired, conventional agents for modifying flavors, tastes, colors, and shapes of the special forms can also be included. In addition, for convenient administration by enteral feeding tube in patients unable to swallow, the active compounds can be dissolved in an acceptable lipophilic vegetable oil vehicle such as olive oil, corn oil and safflower oil.

The active compounds can also be administered parenterally in the form of solution or suspension, or in lyophilized form capable of conversion into a solution or suspension form before use. In such formulations, diluents or pharmaceutically acceptable carriers such as sterile water and physiological saline buffer can be used. Other conventional solvents, pH buffers, stabilizers, anti-bacteria agents, surfactants, and antioxidants can all be included. For example, useful components include sodium chloride, acetates, citrates or phosphates buffers, glycerin, dextrose, fixed oils, methyl parabens, polyethylene glycol, propylene glycol, sodium bisulfate, benzyl alcohol, ascorbic acid, and the like. The parenteral formulations can be stored in any conventional containers such as vials and ampoules.

Routes of topical administration include nasal, buccal, mucosal, rectal, or vaginal applications. For topical administration, the active compounds can be formulated into lotions, creams, ointments, gels, powders, pastes, sprays, suspensions, drops and aerosols. Thus, one or more thickening agents, humectants, and stabilizing agents can be included in the formulations. Examples of such agents include, but are not limited to, polyethylene glycol, sorbitol, xanthan gum, petrolatum,

beeswax, or mineral oil, lanolin, squalene, and the like. A special form of topical administration is delivery by a transdermal patch. Methods for preparing transdermal patches are disclosed, e.g., in Brown, *et al.*, *Annual Review of Medicine*, 39:221-229 (1988), which is incorporated herein by reference.

Subcutaneous implantation for sustained release of the active compounds may also be a suitable route of administration. This entails surgical procedures for implanting an active compound in any suitable formulation into a subcutaneous space, e.g., beneath the anterior abdominal wall. *See, e.g.*, Wilson *et al.*, *J. Clin. Psych.* 45:242-247 (1984). Hydrogels can be used as a carrier for the sustained release of the active compounds. Hydrogels are generally known in the art. They are typically made by crosslinking high molecular weight biocompatible polymers into a network, which swells in water to form a gel like material. Preferably, hydrogels are biodegradable or biosorbable. For purposes of this invention, hydrogels made of polyethylene glycols, collagen, or poly(glycolic-co-L-lactic acid) may be useful. *See, e.g.*, Phillips *et al.*, *J. Pharmaceut. Sci.* 73:1718-1720 (1984).

The active compounds can also be conjugated, to a water soluble non-immunogenic non-peptidic high molecular weight polymer to form a polymer conjugate. For example, an active compound is covalently linked to polyethylene glycol to form a conjugate. Typically, such a conjugate exhibits improved solubility, stability, and reduced toxicity and immunogenicity. Thus, when administered to a patient, the active compound in the conjugate can have a longer half-life in the body, and exhibit better efficacy. *See generally*, Burnham, *Am. J. Hosp. Pharm.*, 15:210-218 (1994). PEGylated proteins are currently being used in protein replacement therapies and for other therapeutic uses. For example, PEGylated interferon (PEG-INTRON A[®]) is clinically used for treating Hepatitis B. PEGylated adenosine deaminase (ADAGEN[®]) is being used to treat severe combined immunodeficiency disease (SCIDS). PEGylated L-asparaginase (ONCAPSPAR[®]) is being used to treat acute lymphoblastic leukemia (ALL). It is preferred that the covalent linkage between the polymer and the active compound and/or the polymer itself is hydrolytically degradable under physiological conditions. Such conjugates known as "prodrugs" can readily release the active compound inside the body. Controlled release of an active compound can also be achieved by incorporating the

active ingredient into microcapsules, nanocapsules, or hydrogels generally known in the art.

Liposomes can also be used as carriers for the active compounds of the present invention. Liposomes are micelles made of various lipids such as cholesterol, phospholipids, fatty acids, and derivatives thereof. Various modified lipids can also be used. Liposomes can reduce the toxicity of the active compounds, and increase their stability. Methods for preparing liposomal suspensions containing active ingredients therein are generally known in the art. *See, e.g.,* U.S. Patent No. 4,522,811; Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.Y. (1976).

The active compounds can also be administered in combination with another active agent that synergistically treats or prevents the same symptoms or is effective for another disease or symptom in the patient treated so long as the other active agent does not interfere with or adversely affect the effects of the active compounds of this invention. Such other active agents include but are not limited to anti-inflammation agents, antiviral agents, antibiotics, antifungal agents, antithrombotic agents, cardiovascular drugs, cholesterol lowering agents, anti-cancer drugs, hypertension drugs, and the like.

Generally, the toxicity profile and therapeutic efficacy of the therapeutic agents can be determined by standard pharmaceutical procedures in cell models or animal models, e.g., those provided in Section 7. As is known in the art, the LD₅₀ represents the dose lethal to about 50% of a tested population. The ED₅₀ is a
5 parameter indicating the dose therapeutically effective in about 50% of a tested population. Both LD₅₀ and ED₅₀ can be determined in cell models and animal models. In addition, the IC₅₀ may also be obtained in cell models and animal models, which stands for the circulating plasma concentration that is effective in achieving about 50% of the maximal inhibition of the symptoms of a disease or disorder. Such
10 data may be used in designing a dosage range for clinical trials in humans. Typically, as will be apparent to skilled artisans, the dosage range for human use should be designed such that the range centers around the ED₅₀ and/or IC₅₀, but significantly below the LD₅₀ obtained from cell or animal models.

It will be apparent to skilled artisans that therapeutically effective amount for each active compound to be included in a pharmaceutical composition of the present invention can vary with factors including but not limited to the activity of the compound used, stability of the active compound in the patient's body, the severity of the conditions to be alleviated, the total weight of the patient treated, the route of administration, the ease of absorption, distribution, and excretion of the active compound by the body, the age and sensitivity of the patient to be treated, and the like. The amount of administration can also be adjusted as the various factors change over time.

10

9. Isolated Nucleic Acids

The present invention also provides for isolated nucleic acid molecules and their fragments encoding one or more interacting protein members of a protein complex identified in the present invention or portions of these polypeptides that are capable of interacting with other protein(s) of the present protein-protein interactions.

15 The term "nucleic acid" is intended to include both DNA (e.g., cDNA or genomic DNA) and RNA (e.g., mRNA). This aspect of the invention also pertains to isolated nucleic acid fragments sufficient for use as hybridization probes to identify nucleic acids encoding polypeptides capable of interacting with other protein(s) of the protein-protein interactions disclosed herein, and to isolated nucleic acid fragments for use as PCR primers for the amplification or mutation of nucleic acids encoding polypeptides capable of interacting with the other proteins.

The nucleic acid fragment encoding a polypeptide capable of interacting with other protein(s) of the protein-protein interactions disclosed herein can be prepared by isolating a fragment, sequencing the fragment (optional), expressing the fragment (e.g., by recombinant expression *in vitro*) and assessing the protein interacting property of the encoded polypeptide.

The isolated polynucleotide, over its entire length, may be 100% identical or less than 100% identical to a reference sequence (i.e., a specific nucleic acid sequence disclosed herein) or to a fragment of the reference sequence depending on the number of nucleotide alterations or variations in the isolated polynucleotide. The isolated polynucleotide which, over its entire length, is less than 100% identical to the

reference sequence or to the fragment of the reference sequence is a variant nucleic acid. The number of nucleotide alterations or variations (A_{nt}) needed for a given % identity is determined by first multiplying (x) the total number of nucleotides (T_{nt}) in the reference sequence by a number (n) which is obtained by dividing the percent
 5 identity by 100 (for example 0.80 for 80%, 0.90 for 90% 0.92 for 92%, 0.95 for 95%, 0.97 for 97% and so on) and then subtracting that product from said total number of nucleotides (T_{nt}) in the reference sequence. After this calculation, any non-integer value may be rounded off to the nearest integer to obtain the approximate number without decimal values. For purposes of clarity, only the first decimal number is
 10 rounded off, to approximate the number of nucleic acid alterations to an integer to obtain a polynucleotide of a given % identity. If the first decimal number is 5 or greater than 5, then the number preceding the decimal point is increased by "one" and all the decimal numbers are dropped (rounded up). If the first decimal number is less than 5, then the number preceding the decimal point is unchanged and all the
 15 decimal numbers are dropped (rounded down). The calculation is summarized in the following formula:

$$A_{nt} \cong T_{nt} - (T_{nt} \times n)$$

Accordingly, in another aspect of the invention, the isolated nucleic acids of the present invention encompass variant nucleic acids, which are variants of the
 20 full-length nucleic acids disclosed herein or variants of the fragments of the full-length nucleic acids. The variant nucleic acid may encode a polypeptide the same as a specific polypeptide disclosed herein or a variant polypeptide as that term is used herein (See, 2.2. Protein Complexes). For example, the variant nucleic acid may encode the same polypeptide because of degeneracy of the code (i.e., a given
 25 amino acid may be specified by more than one codon). Under certain circumstances, an isolated variant nucleic acid may encode a variant polypeptide instead. For example, nucleic acid sequence polymorphisms leading to changes in the amino acid sequences of SEQ ID NO: 7 or their homologues of these sequences may exist within a given population (e.g., the human population). Such genetic polymorphisms may
 30 exist among individuals within a population due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphism that may be the result of variation, natural or induced allelic variation and that do not alter the

functional properties of interest herein are contemplated by the present invention.

A variant nucleic acid encoding a polypeptide capable of interacting with other protein(s) of the protein-protein interactions disclosed herein can be prepared by isolating a nucleic acid, determining the sequence identities with the reference
5 sequences or fragments thereof, expressing the variant nucleic acid (e.g., by recombinant expression in vitro) and assessing the protein interacting property of the encoded polypeptide.

The isolated nucleic acids, their fragments or variants encoding polypeptides of the present invention may be mouse sequences or their homologues (e.g. human
10 proteins).

The nucleic acid sequences of the present invention, e.g., a nucleic acid molecule having the sequence of SEQ ID NO: 13, or a fragment thereof, can be isolated from an appropriate biological source or library using methods known to one skilled in the art and the sequence information disclosed herein. For example, using
15 all or a portion of a nucleic acid sequence disclosed herein as a hybridization probe, the nucleic acid such as a cDNA clone is isolated from a cDNA library of human origin. Further, utilizing the sequence information provided by the cDNA sequence, human genomic clones encoding a polypeptide identical to that set forth herein or variants thereof can be isolated.

20 The nucleic acids having the appropriate level of sequence relatedness with the reference polynucleotide sequences may be identified by using hybridization and washing conditions of appropriate stringency. The terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 90% preferably at least 95% and more preferably at least 97% and most preferably 100%
25 identity between the sequences. It is well known that during nucleic acid hybridizations, conditions can be set up so that hybridizations only occur between the probe and the target nucleic acid of interest that is highly complementary to the probe. The T_m (melting temperature; a measure of the stability of a nucleic acid duplex) of perfect hybrids formed by DNA, RNA or oligonucleotide probes can be determined
30 according to the art known formula which is as follows:

$$T_m (^{\circ}\text{C}) = 81.5^* + 16.6(\log M [\text{Na}^+]) + 0.41(\% \text{G+C}) - 0.72(\% \text{fomamide}).$$

(*The value of 81.5 in the above formula is for DNA-DNA hybrids; For DNA-RNA, RNA-RNA, oligo-DNA or oligo-RNA hybrids this value is different and is known in the art).

For mammalian genomes, with a base composition of about 40% GC, the DNA
5 denatures with a T_m of about 87°C. A specific example of stringent hybridization
conditions is as follows: an overnight incubation at 42°C in a solution having: 5x SSC
(150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x
Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured,
sheared salmon sperm DNA, 50% formamide, followed by washing the hybridization
10 support in 0.1x SSC at about 65°C. The $[Na^+]$ M of different strengths of SSC are as
follows: For 20X, 10X, 5X, 2X, 1X, 0.1X are 3.3, 1.65, 0.825, 0.33, 0.165 and 0.0165,
respectively. Hybridization and wash conditions are well known and exemplified in
laboratory manuals. See, Sambrook, *et al.*, Molecular Cloning: A Laboratory
Manual, particularly Chapter 10 (third edition) therein. Solution hybridization may
15 also be used with the polynucleotide sequences provided by the invention.

The novel polynucleotides of the present invention may also be obtained from
an appropriate library or nucleic acid containing samples (e.g. cell samples) by
selective amplification of target sequences using PCR. The sequence information
disclosed in the present application can be used to design oligonucleotide primers.
20 Primers corresponding to regions immediately upstream and downstream of the
nucleic encoding a given polypeptide can be used to amplify the sequences encoding
one or more interacting protein members of a protein complex identified in the
present invention or portions of these polypeptides that are capable of interacting with
other protein(s) of the present protein-protein interactions. The oligonucleotide
25 primers can be from 15 to about 25 nucleotides long. In preferred embodiments,
primers of about 20 nucleotides long are used. By keeping the stringency of
annealing between the primer and the target very high, the formation of spurious
products (i.e., those that do not encode polypeptides capable of interacting with bait
polypeptides) can be avoided. To this end, strategies known in the prior art (e.g.,
30 choosing suitable length of primers, avoiding substantial tandem repeats of one or
more nucleotides in the primer, avoiding sequences prone to secondary structure,
nested primers etc.) may be applied to achieve specificity.

The PCR primers specific to a given nucleic acid, can be used to isolate polynucleotides (e.g., from mouse and/or other mammalian samples including humans), the polynucleotides encoding polypeptides identical to the disclosed polypeptides and variants thereof. The polynucleotides may then be subject to
5 various prior art known techniques for elucidation of the polynucleotide sequence. In this way, variants of (or mutations in) the polynucleotide sequence can be detected. This information can be used in the protein-protein interaction of the invention.

Thus, probes and primers based on the nucleic acid sequences disclosed herein can be used to detect and isolate transcripts or genomic sequences encoding
10 polypeptides of interest from mouse samples or homologous polypeptides from human samples.

In a preferred embodiment, an isolated nucleic acid molecule of the invention consists essentially of nucleotide sequence shown in SEQ ID NO: 13.

In another preferred embodiment, an isolated nucleic acid molecule of the
15 invention consists essentially of a nucleic acid molecule which is a complement of the nucleic acid sequence shown in SEQ ID NO: 13 or a portion of any of these nucleic acid sequences. An isolated nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO: 13 is either fully complementary or sufficiently complementary to the nucleotide sequence shown in SEQ ID NO: 13,
20 respectively, so that it can hybridize to the nucleotide sequence shown in SEQ ID NO: 13, respectively, under stringent hybridization conditions.

In still another preferred embodiment, the nucleic acid fragments of the invention consist essentially of contiguous nucleotides 1 to 1458 set forth in SEQ ID NO: 13. The hybridization probe used to detect and isolate these fragments can be a segment
25 of 15-mer to 30-mer, 50-mer, 100-mer or more of the nucleic acid set forth in SEQ ID NO:13.

EXAMPLES

1. Yeast Two-Hybrid System

The principles and methods of the yeast two-hybrid system have been described in detail (Bartel and Fields, 1997). The following is thus a description of the particular procedure that we used, which was applied to all proteins.

5 The cDNA encoding the bait protein was generated by PCR from cDNA prepared from a desired tissue. The cDNA product was then introduced by recombination into the yeast expression vector pGBT.Q, which is a close derivative of pGBT.C (See Bartel et al., *Nat Genet.*, 12:72-77 (1996)) in which the polylinker site has been modified to include M13 sequencing sites. The new construct was selected
10 directly in the yeast strain PNY200 for its ability to drive tryptophane synthesis (genotype of this strain: MATalpha trp1-901 leu2-3,112 ura3-52 his3-200 ade2 gal4delta gal80). In these yeast cells, the bait was produced as a C-terminal fusion protein with the DNA binding domain of the transcription factor Gal4 (amino acids 1 to 147). Prey libraries were transformed into the yeast strain BK100 (genotype of
15 this strain: MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4delta gal80 LYS2::GAL-HIS3 GAL2-ADE2 met2::GAL7-lacZ), and selected for the ability to drive leucine synthesis. In these yeast cells, each cDNA was expressed as a fusion protein with the transcription activation domain of the transcription factor Gal4 (amino acids 768 to 881) and a 9 amino acid hemagglutinin epitope tag. PNY200
20 cells (MATalpha mating type), expressing the bait, were then mated with BK100 cells (MATa mating type), expressing prey proteins from a prey library. The resulting diploid yeast cells expressing proteins interacting with the bait protein were selected for the ability to synthesize tryptophan, leucine, histidine, and adenine. DNA was prepared from each clone, transformed by electroporation into *E. coli* strain KC8
25 (Clontech KC8 electrocompetent cells, Catalog No. C2023-1), and the cells were selected on ampicillin-containing plates in the absence of either tryptophane (selection for the bait plasmid) or leucine (selection for the library plasmid). DNA for both plasmids was prepared and sequenced by the dideoxynucleotide chain termination method. The identity of the bait cDNA insert was confirmed and the cDNA insert
30 from the prey library plasmid was identified using the BLAST program to search against public nucleotide and protein databases. Plasmids from the prey library were

then individually transformed into yeast cells together with a plasmid driving the synthesis of lamin and 5 other test proteins, respectively, fused to the Gal4 DNA binding domain. Clones that gave a positive signal in the beta-galactosidase assay were considered false-positives and discarded. Plasmids for the remaining clones
5 were transformed into yeast cells together with the original bait plasmid. Clones that gave a positive signal in the beta-galactosidase assay were considered true positives.

2. Production of Antibodies Selectively Immunoreactive with Protein Complex

The AW755252-interacting domain of PROTEIN2 and the PROTEIN2-interacting domain of AW755252 are indicated in Table I in Section 2. Both interacting domains are recombinantly expressed in *E. coli*. and isolated and
10 purified. A protein complex is formed by mixing the two purified interacting domains. A protein complex is also formed by mixing recombinantly expressed intact complete AW755252 and PROTEIN2. The two protein complexes are used as antigens in immunizing a mouse. mRNA is isolated from the immunized mouse spleen cells, and first-strand cDNA is synthesized based on the mRNA. The V_H and
15 V_K genes are amplified from the thus synthesized cDNAs by PCR using appropriate primers.

The amplified V_H and V_K genes are ligated together and subcloned into a phagemid vector for the construction of a phage display library. *E. coli*. cells are transformed with the ligation mixtures, and thus a phage display library is established.
20 Alternatively, the ligated V_H and V_K genes are subcloned into a vector suitable for ribosome display in which the V_H-V_K sequence is under the control of a T7 promoter. See Schaffitzel *et al.*, *J. Immun. Meth.*, 231:119-135 (1999).

The libraries are screened with the AW755252-PROTEIN2 complex and individual AW755252 and PROTEIN2. Several rounds of screening are preferably
25 performed. Clones corresponding to scFv fragments that bind the AW755252-PROTEIN2 complex, but not the individual AW755252 and PROTEIN2 are selected and purified. A single purified clone is used to prepare an antibody selectively immunoreactive with the AW755252-PROTEIN2 complex. The antibody is then verified by an immunochemistry method such as RIA and ELISA.

30 In addition, the clones corresponding to scFv fragments that bind the

AW755252-PROTEIN2 complex and also binds AW755252 and/or PROTEIN2 may be selected. The scFv genes in the clones are diversified by mutagenesis methods such as oligonucleotide-directed mutagenesis, error-prone PCR (*See Lin-Goerke et al., Biotechniques*, 23:409 (1997)), dNTP analogues (*See Zaccolo et al., J. Mol. Biol.*,
5 255:589 (1996)), and other methods. The diversified clones are further screened in phage display or ribosome display libraries. In this manner, scFv fragments selectively immunoreactive with the AW755252-PROTEIN2 complex may be obtained.

10 All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

15 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated protein complex comprising a first protein which comprises AW755252 or a homologue or derivative or fragment thereof interacting with a
5 second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ, or a homologue or derivative or fragment thereof.
2. The isolated protein complex of Claim 1, wherein said first protein comprise
10 AW755252.
3. The isolated protein complex of Claim 1, wherein said second protein is selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.
15
4. The isolated protein complex of Claim 1, wherein said first protein is AW755252, and said second protein is selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.
- 20 5. The isolated protein complex of Claim 1, wherein said first protein comprises an amino acid sequence selected from the group consisting of:
 - (a) a first sequence consisting of the amino acids as set forth in Figure 2;
 - (b) a second sequence consisting of the amino acids as set forth in SEQ
25 ID NO: 1, 2, 3, 4 or 5;
 - (c) a third sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 1, 2, 3, 4 or 5 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4 or 5, the third sequence has one or more conservative amino acid substitutions;
 - 30 (d) a fourth sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 1, 2, 3, 4 or 5 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4 or 5, the fourth sequence has one or

more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not alter its interacting property with the second protein;

(e) a fifth sequence consisting of the amino acids identical to that set forth in Figure 2 except that, over the entire length corresponding to the amino acid sequence of Figure 2, the fifth sequence has one or more conservative amino acid substitutions;

(f) a sixth sequence consisting of the amino acids identical to that set forth in Figure 2 except that, over the entire length corresponding to the amino acid sequence of Figure 2, the sixth sequence has one or more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not alter its interacting property with the second protein;

6. The isolated protein complex of Claim 1, wherein said second protein comprises an amino acid sequence selected from the group consisting of:

(a) a first sequence consisting of the amino acids as set forth in Figure 3, 4, 5 or 6;

(b) a second sequence consisting of the amino acids as set forth SEQ ID NO: 6, 7, 8 or 9;

(c) a third sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 6, 7, 8 or 9 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 6, 7, 8 or 9, the third sequence has one or more conservative amino acid substitutions;

(d) a fourth sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 6, 7, 8 or 9 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 6, 7, 8 or 9, the fourth sequence has one or more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not render it unable to interact with the first protein;

(e) a fifth sequence consisting of the amino acids identical to that set forth in Figure 3, 4, 5 or 6 except that, over the entire length corresponding to the amino acid sequence of Figure 3, 4, 5 or 6, the fifth sequence has one or more conservative amino acid substitutions; and

(f) a sixth sequence consisting of the amino acids identical to that set

forth in Figure 3, 4, 5 or 6 except that, over the entire length corresponding to the amino acid sequence of Figure 3, 4, 5 or 6, the sixth sequence has one or more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not render it unable to interact with the first
5 protein.

7. The isolated protein complex of Claim 1, wherein said second protein comprises an amino acid sequence selected from the group consisting of:

(a) a first sequence consisting of the amino acids as set forth in Figure 8,
10 9 or 10;

(b) a second sequence consisting of the amino acids as set forth SEQ ID NO: 17, 18, 19 or 20;

(c) a third sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 17, 18, 19 or 20 except that, over the entire length
15 corresponding to the amino acid sequence of SEQ ID NO: 17, 18, 19 or 20, the third sequence has one or more conservative amino acid substitutions;

(d) a fourth sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 17, 18, 19 or 20 except that, over the entire length
20 corresponding to the amino acid sequence of SEQ ID NO: 17, 18, 19 or 20, the fourth sequence has one or more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not render it unable to interact with the first protein;

(e) a fifth sequence consisting of the amino acids identical to that set forth in Figure 8, 9 or 10 except that, over the entire length corresponding to the
25 amino acid sequence of Figure 8, 9 or 10, the fifth sequence has one or more conservative amino acid substitutions; and

(f) a sixth sequence consisting of the amino acids identical to that set forth in Figure 8, 9 or 10 except that, over the entire length corresponding to the amino acid sequence of Figure 8, 9 or 10, the sixth sequence has one or more
30 non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not render it unable to interact with the first protein.

8. The isolated protein complex of Claim 1, wherein said first protein is
116

AW755252 comprising:

- (a) an amino acid sequence set forth in Figure 2; or
- (b) a sequence having a truncation of up to 239 amino acids at the N-terminus or up to 129 amino acids at the C-terminus of the sequence set forth in
5 Figure 2, such that the truncation does not render it unable to interact with mFHL2; or
- (c) a sequence having a truncation of up to 239 amino acids at the N-terminus and up to 129 amino acids at the C-terminus of the sequence set forth in Figure 2, such that the truncation does not render it unable to interact with mFHL2,
and wherein said second protein is mFHL2 comprising:
10 (d) an amino acid sequence set forth in Figure 3; or
- (e) a sequence having a truncation of up to 99 amino acids at the N-terminus or up to 44 amino acids at the C-terminus of the sequence set forth in Figure 3, such that the truncation does not render it unable to interact with AW755252; or
15 (f) a sequence having a truncation of up to 99 amino acids at the N-terminus and up to 44 amino acids at the C-terminus of the sequence set forth in Figure 3, such that the truncation does not render it unable to interact with AW755252.

20 9. The isolated protein complex of Claim 1, wherein said first protein is AW755252 comprising:

- (b) amino acid sequence set forth in Figure 2; or
- (b) a sequence having a truncation of up to 396 amino acids at the N-terminus of the sequence set forth in Figure 2, such that the truncation does not
25 render it unable to interact with mPN34854,
and wherein said second protein is mPN34854 comprising:
(c) an amino acid sequence set forth in Figure 4.

10. The isolated protein complex of Claim 1, wherein said first protein is
30 AW755252 comprising:

- (c) an amino acid sequence set forth in Figure 2; or
- (b) a sequence having a truncation of up to 372 amino acids at the C-terminus of the sequence set forth in Figure 2, such that the truncation does not render it unable to interact with mPRPH1,

and wherein said second protein is mPRPH1 comprising:

- (c) an amino acid sequence set forth in Figure 5, or
- (d) a sequence having a truncation of up to 214 amino acids at the N-terminus or up to 69 amino acids at the C-terminus of the sequence set forth in Figure 5, such that the truncation does not render it unable to interact with AW755252; or
- (e) a sequence having a truncation of up to 214 amino acids at the N-terminus and up to 69 amino acids at the C-terminus of the sequence set forth in Figure 5, such that the truncation does not render it unable to interact with AW755252.

11. The isolated protein complex of Claim 1, wherein said first protein is AW755252 comprising:

- (d) an amino acid sequence set forth in Figure 2; or
- (b) a sequence having a truncation of up to 396 amino acids at the N-terminus of the sequence set forth in Figure 2, such that the truncation does not render it unable to interact with mTCTEX-1,

and wherein said second protein is mTCTEX-1 comprising:

- (c) an amino acid sequence set forth in Figure 6.

12. The isolated protein complex of Claim 1, wherein said first protein is AW755252 comprising:

- (a) an amino acid sequence set forth in Figure 2; or
 - (b) a sequence having a truncation of up to 239 amino acids at the N-terminus or up to 129 amino acids at the C-terminus of the sequence set forth in Figure 2, such that the truncation does not render it unable to interact with ACTN2; or
 - (c) a sequence having a truncation of up to 239 amino acids at the N-terminus and up to 129 amino acids at the C-terminus of the sequence set forth in Figure 2, such that the truncation does not render it unable to interact with ACTN2,
- and wherein said second protein is ACTN2 comprising:
- (d) an amino acid sequence set forth in Figure 3; or

- (e) a sequence having a truncation of up to 513 amino acids at the N-terminus of the sequence set forth in Figure 3, such that the truncation does not render it unable to interact with AW755252.
- 5 13. The isolated protein complex of Claim 1, wherein said first protein is AW755252 comprising:
- (a) amino acid sequence set forth in Figure 2; or
 - (b) a sequence having a truncation of up to 239 amino acids at the N-terminus or up to 129 amino acids at the C-terminus of the sequence set forth in
10 Figure 2, such that the truncation does not render it unable to interact with mACTN4;
or
 - (c) a sequence having a truncation of up to 239 amino acids at the N-terminus and up to 129 amino acids at the C-terminus of the sequence set forth in
15 Figure 2, such that the truncation does not render it unable to interact with mACTN4,
and wherein said second protein is mACTN4 comprising:
- (d) an amino acid sequence set forth in Figure 4; or
 - (e) a sequence having a truncation of up to 650 amino acids at the N-terminus of the sequence set forth in Figure 4, such that the truncation does not render it
20 unable to interact with AW755252.
14. The isolated protein complex of Claim 1, wherein said first protein is AW755252 comprising:
- (a) amino acid sequence set forth in Figure 2; or
 - (b) a sequence having a truncation of up to 239 amino acids at the
25 N-terminus or up to 129 amino acids at the C-terminus of the sequence set forth in
Figure 2, such that the truncation does not render it unable to interact with mACTN4;
or
 - (c) a sequence having a truncation of up to 239 amino acids at the N-terminus and up to 129 amino acids at the C-terminus of the sequence set forth in
30 Figure 2, such that the truncation does not render it unable to interact with mACTN4,
and wherein said second protein is mACTN4 comprising:
- (d) an amino acid sequence set forth in Figure 4; or

- (e) a sequence having a truncation of up to 650 amino acids at the N-terminus of the sequence set forth in Figure 4, such that the truncation does not render it unable to interact with AW755252.

5 15. The isolated protein complex of Claim 1, wherein said first protein consists of an amino acid sequence set forth in SEQ ID NO: 1, 2, 3, 4 or 5, said second protein consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 7, 8 9, 17, 18, 19, and 20.

10 16. The isolated protein complex of Claim 1, wherein said first protein is a hybrid protein containing the complete amino acid sequence of AW755252.

17. The isolated protein complex of Claim 1, wherein said second protein is a hybrid protein containing the complete amino acid sequence of a protein selected
15 from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.

18. The isolated protein complex of Claim 1, wherein said first protein comprises an amino acid sequence set forth in SEQ ID NO: 1, 2, 3, 4 or 5.

20

19. The isolated protein complex of Claim 1, wherein said second protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 7, 8 9, 17, 18, 19 and 20.

25 20 A method for making the protein complex of any one of Claims 1-15, comprising the step of providing said first protein and said second protein under conditions such that said first and second proteins contact each other.

21. An antibody specific to the protein complex of any one of Claims 1-19.

30

22. The antibody of Claim 21, wherein said antibody is an scFv antibody fragment.

23. An isolated protein having a first polypeptide covalently linked to a second
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polypeptide, the first polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 1, 2, 3, 4 or 5 or a variant thereof, the second polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 7, 8, 9, 17, 18, 19, 20 or a variant thereof,

5 wherein the first polypeptide or the variant thereof is capable of interacting with the second polypeptide or the variant thereof.

24. The isolated protein of claim 23, wherein the variant of the first polypeptide comprises an amino acid sequence selected from the group consisting of:

10 (a) a first sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 1, 2, 3, 4 or 5 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4 or 5, the first sequence has one or more conservative amino acid substitutions; and

 (b) a second sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 1, 2, 3, 4 or 5 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4 or 5, the second sequence has one or more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not alter its interacting property with the second polypeptide.

20

25. The isolated protein of claim 23, wherein the variant of the second polypeptide comprises an amino acid sequence selected from the group consisting of:

 (a) a first sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 6, 7, 8, 9, 17, 18, 19 or 20 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 6, 7, 8, 9, 17, 18, 19 or 20 the first sequence has one or more conservative amino acid substitutions; and

 (b) a second sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 6, 7, 8, 9, 17, 18, 19 or 20 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 6, 7, 8, 9, 17, 18, 19 or 20 the second sequence has one or more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not alter its interacting property with the first polypeptide.

26. The isolated protein of Claim 23, wherein said first polypeptide comprises an

amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4 or 5, and said second polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 7, 8, 9 17, 18, 19 and 20.

- 5 27. An isolated protein having a first polypeptide and a second polypeptide, the first polypeptide that is AW755252 or a variant thereof covalently linked to the second polypeptide selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a variant thereof,
wherein the first polypeptide or the variant thereof is capable of interacting
10 with the second polypeptide or the variant thereof.

28. An isolated protein having a first polypeptide and a second polypeptide, the first polypeptide comprising an amino acid sequence as set forth in Figure 2 or a variant thereof covalently linked to the second polypeptide comprising an amino acid
15 sequence selected from the group consisting of SEQ ID NO: 6, 7, 8 9, 17, 18, 19 and 20 or a variant thereof,
wherein the first polypeptide or the variant thereof is capable of interacting with the second polypeptide or the variant thereof.

- 20 29. An isolated protein having a first polypeptide and a second polypeptide, the first polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4 or 5 or a variant thereof covalently linked to the second polypeptide comprising an amino acid sequence of a protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4
25 and mMRJ or a variant thereof,
wherein the first polypeptide or the variant thereof is capable of interacting with the second polypeptide or the variant thereof.

30. A nucleic acid encoding the protein of any one of Claims 23-29.
30

31. An antibody specific to the isolated protein of any one of Claims 23-29.

32. A bifunctional antibody specific to AW755252 and a protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2,

mACTN4 and mMRJ.

33. A protein microarray comprising a protein complex having a first protein which is AW755252 or a homologue or derivative or fragment thereof interacting with
5 a second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof.

34. The protein microarray of Claim 33, wherein said first protein is AW755252
10 and said second protein is selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.

35. A method for detecting, in a sample, a protein complex containing AW755252 and a polypeptide selected from the group consisting of MFHL2,
15 mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ comprising:

contacting said sample with an antibody selected from the group consisting of an antibody specific to said protein complex, an antibody specific to AW755252 and an antibody specific to a protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.

20

36. A method for selecting modulators of a protein complex formed between a first protein which is AW755252 or a homologue or derivative or fragment thereof and a second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or a derivative
25 or a fragment thereof, comprising:

providing the protein complex;
contacting said protein complex with a test compound; and
determining binding of the test compound with said protein complex.

30 37. The method of Claim 36, wherein said test compound is provided in a phage display library.

38. The method of Claim 36, wherein said test compound is provided in a combinatorial library.

39. The method of Claim 36, wherein at least one of said first and second proteins are provided in the protein complex as a hybrid protein having a detectable tag fused thereto.

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40. The method of Claim 36, wherein said first protein is AW755252 and said second protein is selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.

10 41. A method for selecting modulators of a protein complex formed from a first protein which is AW755252 or a homologue or derivative or fragment thereof, and a second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof, comprising:

15 contacting said first protein with said second protein in the presence of a test compound; and

 determining the interaction between said first protein and said second protein.

20 42. The method of claim 41, wherein the test compound is an agonist or an antagonist.

43. The method of claim 42, wherein the agonist or an antagonist comprises a peptide, a small molecule or an organic molecule.

25 44. The method of claim 42, wherein the antagonist binds to a binding site of the first protein or the second protein, thereby preventing the interaction between the first and the second protein.

30 45. The method of claim 42, wherein the agonist binds to a binding site of the first protein or the second protein, thereby promoting the interaction between the first and the second protein.

46. The method of Claim 41, wherein said test compound is provided in a phage display library.

47. The method of Claim 41, wherein said test compound is provided in a combinatorial library.
- 5 48. The method of Claim 41, wherein said first protein is a hybrid protein containing AW755252 or a fragment thereof, said second protein is a hybrid protein containing a protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a fragment thereof.
- 10 49. The method of Claim 41, wherein said interaction between said first protein and said second protein is determined in a cell.
50. The method of Claim 49, wherein said cell is a yeast cell.
- 15 51. The method of Claim 41, wherein said step of determining the interaction between said first protein and said second protein is conducted in a substantially cell free environment.
52. A method for screening to identify compounds that promote or that inhibit a
20 binding affinity of a protein complex formed between a first protein which is AW755252 or a homologue or derivative or fragment thereof and a second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or a derivative or a fragment thereof, the method comprising:
- 25 (a) contacting a composition comprising said protein complex with a test compound to be screened under conditions to permit interaction between the compound and said protein complex; and
- (b) determining the binding affinity of said protein complex after step (a).
- 30 53. The method of claim 52, wherein the test compound is an agonist or an antagonist.
54. The method of claim 52, wherein the agonist or an antagonist comprises a peptide, a small molecule or an organic molecule.

55. The method of claim 53, wherein the antagonist binds to a binding site of the protein complex, thereby inhibiting normal biological activity of the protein complex.

5 56. The method of claim 53, wherein the agonist binds to a binding site of the protein complex, thereby promoting normal biological activity of the protein complex.

57. A method for screening to identify compounds that activate or that inhibit a activity of a protein complex formed between a first protein which is AW755252 or a
10 homologue or derivative or fragment thereof and a second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or a derivative or a fragment thereof, the method comprising:

- 15 (a) measuring the activity of said protein complex in the presence of a candidate compound;
- (b) measuring the activity of said protein complex in the absence of the candidate compound; and
- (c) detecting the effect of the candidate compound by comparing the activity in (a) and (b).

20

58. The method of Claim 57, wherein said candidate compound is provided in a phage display library.

59. The method of Claim 57, wherein said candidate compound is provided in a
25 combinatorial library.

60. The method of Claim 57, wherein at least one of said first and second proteins are provided in the protein complex as a hybrid protein having a detectable tag fused thereto.

30

61. The method of Claim 57, wherein said first protein is AW755252 and said second protein is selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.

62. A method for determining whether a compound is capable of modulating an interaction between a first polypeptide and a second polypeptide, said first polypeptide being AW755252 or a homologue or derivative or fragment thereof and said second polypeptide being selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof, said method comprising:

- (a) expressing in an isolated host cell in the presence of a test compound, a first hybrid protein having a DNA binding domain fused to said first polypeptide, a second hybrid protein having a transcription-activating domain fused to said second polypeptide and a reporter gene, wherein the expression of the reporter gene is dependent on the interaction between the first polypeptide and the second polypeptide; and
- (b) detecting the expression of said reporter gene.

63. A method for determining whether a compound is capable of modulating an interaction between a first polypeptide and a second polypeptide, said first polypeptide being AW755252 or a homologue or derivative or fragment thereof and said second polypeptide being selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof, said method comprising:

- (a) expressing in an isolated host cell in the presence of a test compound, a first hybrid protein having a transcription-activating domain fused to said first polypeptide, a second hybrid protein having a DNA binding domain fused to said second polypeptide and a reporter gene, wherein the expression of the reporter gene is dependent on the interaction between the first polypeptide and the second polypeptide; and
- (b) detecting the expression of said reporter gene.

64. A composition comprising:

a first expression vector having a nucleic acid encoding AW755252 or a homologue or derivative or fragment thereof; and

a second expression vector having a nucleic acid encoding a protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof.

65. An isolated host cell comprising:

a first expression vector having a first nucleic acid encoding a first protein which is AW755252 or a homologue or derivative or fragment thereof; and

5 a second expression vector having a second nucleic acid encoding a second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof.

10 66. The isolated host cell of Claim 65, wherein said first protein is AW755252.

67. The isolated host cell of Claim 65, wherein said second protein is selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.

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68. The isolated host cell of Claim 65, wherein said first protein is AW755252, and said second protein is selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.

20 69. The isolated host cell of Claim 65, wherein said first protein comprises an amino acid sequence selected from the group consisting of:

(a) a first sequence consisting of the amino acids as set forth in Figure 2;

(b) a second sequence consisting of the amino acids as set forth in SEQ ID NO: 1, 2, 3, 4 or 5;

(c) a third sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 1, 2, 3, 4 or 5 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4 or 5, the third sequence has one or more conservative amino acid substitutions;

30 (d) a fourth sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 1, 2, 3, 4 or 5 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 1, 2, 3, 4 or 5, the fourth sequence has one or more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not alter its interacting property with

the second protein;

(e) a fifth sequence consisting of the amino acids identical to that set forth in Figure 2 except that, over the entire length corresponding to the amino acid sequence of Figure 2, the fifth sequence has one or more conservative amino acid
5 substitutions; and

(g) a sixth sequence consisting of the amino acids identical to that set forth in Figure 2 except that, over the entire length corresponding to the amino acid sequence of Figure 2, the sixth sequence has one or more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence
10 which do not alter its interacting property with the second protein.

70. The isolated host cell of Claim 65, wherein said second protein comprises an amino acid sequence selected from the group consisting of:

(a) a first sequence consisting of the amino acids as set forth in Figure 3,
15 4, 5 or 6;

(b) a second sequence consisting of the amino acids as set forth in SEQ ID NO: 6, 7, 8 or 9;

(c) a third sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 6, 7, 8 or 9 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 6, 7, 8 or 9, the third sequence has one or
20 more conservative amino acid substitutions;

(d) a fourth sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 6, 7, 8 or 9 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 6, 7, 8 or 9, the fourth sequence has one or
25 more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not render it unable to interact with the first protein;

(e) a fifth sequence consisting of the amino acids identical to that set forth in Figure 3, 4, 5 or 6 except that, over the entire length corresponding to the amino acid sequence of Figure 3, 4, 5 or 6, the fifth sequence has one or more
30 conservative amino acid substitutions; and

(f) a sixth sequence consisting of the amino acids identical to that set forth in Figure 3, 4, 5 or 6 except that, over the entire length corresponding to the amino acid sequence of Figure 3, 4, 5 or 6, the sixth sequence has one or more

non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not render it unable to interact with the first protein.

5 71. (Corresponds to claim 65 in 58219-P)

The isolated host cell of Claim 60, wherein said second protein comprises an amino acid sequence selected from the group consisting of:

- (a) a first sequence consisting of the amino acids as set forth in Figure 8, 9 or 10;
- 10 (b) a second sequence consisting of the amino acids as set forth in SEQ ID NO: 17, 18, 19 or 20;
- (c) a third sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 17, 18, 19 or 20 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 17, 18, 19 or 20, the third sequence has
15 one or more conservative amino acid substitutions;
- (d) a fourth sequence consisting of the amino acids identical to that set forth in SEQ ID NO: 17, 18, 19 or 20 except that, over the entire length corresponding to the amino acid sequence of SEQ ID NO: 17, 18, 19 or 20, the fourth sequence has
20 positions of the amino acid sequence which do not render it unable to interact with the first protein;
- (e) a fifth sequence consisting of the amino acids identical to that set forth in Figure 8, 9 or 10 except that, over the entire length corresponding to the amino acid sequence of Figure 8, 9 or 10, the fifth sequence has one or more
25 conservative amino acid substitutions; and
- (f) a sixth sequence consisting of the amino acids identical to that set forth in Figure 8, 9 or 10 except that, over the entire length corresponding to the amino acid sequence of Figure 8, 9 or 10, the sixth sequence has one or more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid
30 sequence which do not render it unable to interact with the first protein.

72. The isolated host cell of Claim 65, wherein said first protein consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4 or 5, said second protein consists of an amino acid sequence selected from the group

consisting of SEQ ID NO: 6, 7, 8, 9, 17, 18, 19 and 20.

73. The isolated host cell of Claim 65, wherein said first protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4 or
5 5.

74. The isolated host cell of Claim 65, wherein said second protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 7, 8, 9, 17, 18, 19 and 20.
10

75. The isolated host cell of Claim 65, wherein said cell is a yeast cell.

76. The isolated host cell of Claim 65, wherein said cell is a mammalian cell.

15 77. The isolated host cell of Claim 65, wherein one of said first and second nucleic acids is linked to a nucleic acid encoding a DNA binding domain, and the other of said first and second nucleic acids is linked to a nucleic acid encoding a transcription-activation domain, whereby two hybrid proteins can be produced in said cell.

20 78. The isolated host cell of Claim 77, further comprising a reporter gene, wherein the transcription of the reporter gene is dependent on the interaction between the first polypeptide and the second polypeptide.

25 79. The isolated host cell of Claim 78, wherein said reporter gene encodes an auxotrophic factor.

80. The isolated host cell of Claim 78, wherein the reporter gene encodes a protein that inhibits the growth of said isolated host cell under a predetermined
30 condition.

81. The isolated host cell of Claim 78, wherein said reporter gene encodes a cytotoxic toxin.

82. A method for modulating the function or activity of a protein complex in cells of a specific tissue of a mammal, said protein complex having a first protein which is AW755252 or a homologue or derivative or fragment thereof interacting with a second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof, said method comprising:

delivering to the specific tissue, a selected compound for modulating the function or activity of said protein complex.

83. The method of Claim 82, wherein said compound is an antibody.

84. The method of Claim 82, wherein said antibody is specific to the protein complex.

85. The method of Claim 82, wherein said compound interferes with the interaction between said first protein and said second protein.

86. The method of Claim 82, wherein said compound enhances the interaction between said first protein and said second protein.

87. A method for screening for compounds that selectively inhibit abnormal cardiac function, the method comprising:

(a) delivering a compound to be screened to a cell population of a first kind selecting from the group consisting of cell growth, apoptosis, cell viability, beating and cell size, wherein the first kind of the cell population shows abnormal cardiac function under a set of culture conditions sufficient for other cell population to show normal cardiac function wherein said abnormal cardiac function is due to an aberration in a protein complex or an interaction of thereof between AW755252 and a protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4, mMRJ, and homologues, derivatives, or fragments thereof;

(b) delivering the compound to a cell population of a second kind that is known to show normal cardiac function under said conditions and not known to have said aberration, wherein the compound does not affect the normal cardiac function

ability of the second kind of the cell population;

(c) comparing the cardiac function of the first and second kinds of cell populations; and

(d) selecting the compound that inhibit the abnormal cardiac function of
5 the first kind of cell population comparable to that of the second kind of cell population.

88. The method of claim 87 further comprising administering the selected compound after step (d) to a subject in need of said compound wherein the subject is
10 selected from the group consisting of mammals, tissue explants, and individual cells.

89. A method of screening for potential therapeutic compounds that selectively inhibit abnormal cardiac function, the method comprising:

(a) administering a compound to be screened to a transgenic
15 AW755252-null non-human animal model expressing a mutated human AW755252 protein that fails to interact with or interacts aberrantly with a protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof leading to symptoms associated with ischemic heart disease, myocardial infarction, cardiac
20 failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris; and

(b) selecting the compound that effectuated recovery of the transgenic animal from symptoms associated with ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris.

25 90. The method of claim 89, wherein the compound is administered by intra-tissue infusion.

91. A method for screening for potential therapeutic compounds that selectively inhibit abnormal cardiac function, the method comprising:

30 (a) administering a compound to be screened to a first transgenic AW755252-null non-human animal model expressing a mutated human AW755252 protein that fails to interact with or interacts aberrantly with a protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof leading to

symptoms associated with ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris;

(b) administering the compound to a second transgenic AW755252-null non-human animal model expressing a wild-type human AW755252 protein that
5 interacts with the protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof, wherein said compound does not affect normal cardiac function in the second transgenic animal model; and

(c) selecting the compound that effectuated recovery of the first
10 transgenic animal from symptoms associated with ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris as revealed by the comparisons between the first and second transgenic animals.

15 92. A method for modulating cardiac function in a patient, comprising:
administering to the patient a selected compound for modulating the function or activity of a protein complex having a first protein which is AW755252 or a homologue or derivative or fragment thereof interacting with a second protein selected from the group consisting of SEQ ID NO: 6, 7, 8, 9, 17, 18, 19, 20 or a
20 homologue or derivative or fragment thereof.

93. The method of Claim 92, wherein said compound is an antibody.

94. The method of Claim 93, wherein said antibody is specific to the protein
25 complex.

95. The method of Claim 92, wherein said compound interferes with the interaction between said first protein and said second protein.

30 96. The method of Claim 92, wherein said compound enhances the interaction between said first protein and said second protein.

97. A method for modulating cardiac function in a patient, comprising:
modulating the function or activity of a protein complex having a first protein which

is AW755252 or a homologue or derivative or fragment thereof interacting with a second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof.

5

98. The method of Claim 97, wherein said modulating step comprises increasing the protein complex level.

99. The method of Claim 98, wherein the level of the protein complex is increased by administering to the patient a therapeutically effective amount of said protein complex.

100. The method of Claim 98, wherein said increasing step comprises administering to the patient one of the first protein and the second protein or both.

15

101. The method of Claim 98, wherein said increasing step comprises administering to the patient a nucleic acid encoding said first protein, or a nucleic acid encoding said second protein, or both.

102. The method of Claim 98, wherein said increasing step comprises administering to the patient a plurality of cells expressing said protein complex.

103. The method of Claim 97, wherein said modulating step comprises reducing the protein complex level.

25

104. The method of Claim 102, wherein said reducing step comprises administering to the patient an antibody against said first protein or an antibody against said second protein, or both, or an antibody specific to the protein complex.

105. The method of Claim 103, wherein said reducing step comprises administering to the patient an antisense compound capable of interfering with the expression of said first or second protein or both.

106. The method of Claim 103, wherein said reducing step comprises

administering to the patient a ribozyme specific against an mRNA encoding said first protein or an mRNA encoding said second protein, or both.

107. The method of Claim 106, wherein said reducing step comprises
5 administering to the patient a peptide nucleic acid (PNA) capable of specifically binding to an mRNA encoding said first protein or an mRNA encoding said second protein, or both.

108. The method of Claims 87-107, which is used to treat ischemic heart disease,
10 myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris.

109. A method for the treatment of a patient having a need to inhibit the interaction of AW755252 or a homologue or derivative or fragment thereof with a
15 protein required to form a protein complex in ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris of the patient, the method comprising administering to the patient a therapeutically effective amount of an antagonist targeted to the ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia
20 cordis and angina pectoris, wherein the antagonist binds to AW755252 or a homologue or derivative or fragment thereof, the second protein or the protein complex and inhibits the interaction, wherein the second protein is selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ or a homologue or derivative or fragment thereof.

25

110. The method of claim 109, wherein the antagonist binds to a binding site of the first protein or the second protein, thereby preventing the interaction between the first and the second protein.

30 111. The method of claim 109, wherein the agonist or an antagonist comprises a peptide or an organic molecule.

112. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a protein complex having a human AW755252

interacting with a human protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.

113. A pharmaceutical composition comprising AW755252 and a protein selected
5 from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ, in a combined amount that is therapeutically or prophylactically effective, and a pharmaceutically acceptable carrier.

114. A pharmaceutical composition comprising an antibody specific to AW755252
10 and an antibody specific to a protein selected from the group consisting of SEQ ID NO: 6, 7, 8, 9, 17, 18, 19 and 20, in a combined amount that is therapeutically or prophylactically effective, and a pharmaceutically acceptable carrier.

115. A method of isolating, from a sample, AW755252, comprising:
15 contacting said sample with a second protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 7, 8, 9, 17, 18, 19 and 20; and
isolating a protein complex comprising AW755252 and said second protein.

20 116. A method of detecting, in a sample, AW755252 comprising:
contacting said sample with a second protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 7, 8, 9, 17, 18, 19 and 20; and
detecting a protein complex comprising the AW755252 and said second
25 protein.

117. A method of isolating, from a sample, a first protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ comprising:
30 contacting said sample with a second protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4 or 5; and
isolating a protein complex comprising said first protein and said second protein.

118. A method of detecting, in a sample, a first protein selected from the group

consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ comprising:

contacting said sample with a second protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4 or 5; and

5 detecting a protein complex comprising the first protein and said second protein.

119. A method for diagnosing a physiological disorder associated with ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris or a predisposition to the disorder in a patient,
10 comprising:

detecting, in said patient, any aberration of a protein complex having a first protein which is AW755252 interacting with a second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and
15 mMRJ.

120. The method of Claim 119, wherein said detecting step comprises detecting an aberrant level of said protein complex in a tissue sample from said patient.

20 121. The method of Claim 119, wherein said detecting step comprises detecting an aberrant binding affinity between said first protein and said second protein.

122. The method of Claim 119, wherein said detecting step comprises determining the level of said first protein and/or said second protein in a tissue sample from said
25 patient.

123. The method of Claim 119, wherein said detecting step comprises determining the level of the mRNAs encoding said first protein and/or said second protein, in a tissue sample from said patient.

30

124. The method of Claim 119, wherein said detecting step comprises detecting an aberrant localization of said protein complex in a cell from said patient.

125. The method of Claim 119, wherein said detecting step comprises detecting a

mutation in a gene encoding said first or second protein.

126. A kit for use in diagnosing a physiological disorder associated with ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris in a patient, comprising, in a compartmentalized carrier, an antibody specific to a protein complex having a first protein which is AW755252 interacting with a second protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.

10

127. A kit for use in diagnosing a physiological disorder associated with ischemic heart disease, myocardial infarction, cardiac failure, dilated cardiomyopathy, hypertrophia cordis and angina pectoris in a patient, comprising, in a compartmentalized carrier, a first oligonucleotide selectively hybridizable to the mRNA encoding AW755252 and a second oligonucleotide selectively hybridizable to the mRNA encoding a protein selected from the group consisting of MFHL2, mPN34854, mPRPH1, mTCTEX-1, ACTN2, mACTN4 and mMRJ.

15

128. An isolated polypeptide selected from the group consisting of:

(a) a first polypeptide comprising the amino acid sequence set forth in Figure 4;

(b) a second polypeptide which is the amino acid sequence set forth in Figure 4;

(c) a third polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 7;

(d) a fourth polypeptide which is the amino acid sequence set forth in SEQ ID NO: 7;

(e) a fifth polypeptide which is encoded by a polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 13.

(f) a sixth polypeptide consisting of the amino acids identical to that set forth in Figure 4 except that, over the entire length corresponding to the amino acid sequence in Figure 4, the sixth sequence has one or more conservative amino acid substitutions;

20

25

30

(g) a seventh polypeptide consisting of the amino acids identical to that

set forth in Figure 4 except that, over the entire length corresponding to the amino acid sequence in Figure 4, the seventh sequence has one or more non-conservative amino acid substitutions, deletions or insertions at such positions of the amino acid sequence which do not alter its interacting property with a protein which is
5 AW755252 or a homologue or derivative or fragment thereof.

129. An isolated polynucleotide segment, comprising a polynucleotide sequence selected from the group consisting of:

- (a) a first sequence having nucleotides 1 to 1458 of SEQ ID NO: 13;
- 10 (b) a second sequence consisting of the nucleotides identical to that set forth in SEQ ID NO: 13 except that, over the entire length corresponding to the nucleotide sequence of SEQ ID NO: 13, the second sequence has one or more nucleotide substitutions, deletions or insertions;
- (c) a third sequence obtained by screening an appropriate library under
15 stringent hybridization conditions with a probe which is a segment of 15 or more nucleotides of SEQ ID NO: 13 wherein the third sequence encodes a polypeptide identical to mPN34854;
- (d) a fourth sequence that encodes a polypeptide sequence set forth in Figure 4; and
- 20 (e) a fifth sequence that encodes a truncated polypeptide of the polypeptide sequence set forth in Figure 4
wherein the polypeptide or the truncated polypeptide encoded by the polynucleotide sequence interacts with AW755252.

25

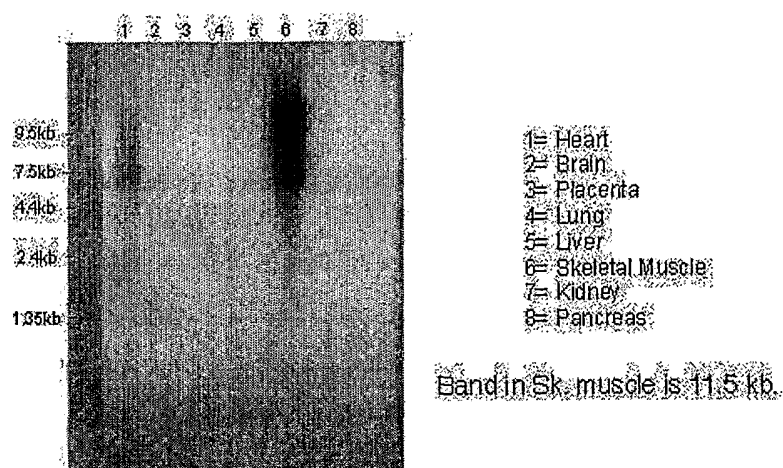


Fig.1. Northern blotting result of AW755252

NH₂-
MECLVADKQNFHKSCFRCHHCNSKLSLGNYSLHGQIYCKPHFKQLFKSKGN
YDEGFGHKQHKDRWNCKNQSRSDVIPNEEPMCKNIAENTLVPGRNEHL
DAGNSEGQRNDLRKLGERGKLKVIWPPSKEIPKKTLPFEEELKMSKPKWPPE
5 MTTLLSPEFKSESLEDVRTPENKGQRQDHFPLQPYLQSTHVCQKEDVIGIKE
MKMPEGRKDEKKEGRKNVQDRPSEAEDTKSNRKSAMDNDNNNVIVQSAE
KEKNEKTNQTNGAEVLQVTNTDDEMMPENHKENLNKNNNNNYVAVSYLNN
CRQKTSILEFLDLLPLSSEANDTANEYEIEKLENTSRISELLGIFESEKTYSRNVL
AMALKKQTDRAAAGSPVQPAPKPSLSRGLMVKGGSSIISPDTNLLNIKGSHSK
10 SKNLHFFSNTVKITAFSKKNENIFNCDLIDSVDQIKNMPCLDLREFGKDVKP
WHVETTEAARNNENTGFDALSHECTAKPLFPRVEVQSEQLTVEEQIKRNCYS
DTE-COOH

Figure 2- Partial Amino Acid Sequence (AW755252) (SEQ ID NO:10)

15

NH₂-
MTERFDCHHCNESLYGKKYILKEENPHCVACFEELYANTCEECPGTPIGCDCKD
LSYKDRHWHEGCFHCSRCSLVDKPFAAKEEQLLCTDCYSNEYSSKCQECK
KTIMPGTRKMEYKGSSWHETCFTCQRCQQPIGTKSFIPKENQNFVPCYEKQY
5 ALQCVQCKKPITGGVITYREQPWHKECFVCTACKKQLSGQRFTARDEFPYCL
TCFCDLYAKKCAGCTNPISGLGGTKYISFEERQWHNDCFNCKKCSLSLVGRGF
LTERDDILCPDCGKDI-COOH

Figure 3-Full-length Amino Acid Sequence (mFHL2) (SEQ ID NO:11)

10

NH₂-
SLTFTTSERVVLTCELSRVDFPATWYKDGQKVEEESLIVKTEGRKHLILPEA
QVRDSGEFECRTEGVSAFFGVTVQDPPVHIVNPQEHVFVHAITSECVRLTCEV
DREDDTVHWHYKDGQEVEESDIIVLENKGPHHRLVLPAAARPSDGGFQCVAGD
5 ERAYFTVTITDVFSWIVYPSSEVHVAAIRLERVVLTCELCRPWAEVRWTKDG
EEVVESPALLEKEDTIRRLVLPVQLEDSEYLCIHDSEASFTITVTEPPVRRI
YPQDEVTLHAVSLECVVLTCELSREDAPVRWYKDGLEVEESEALVLQSDGPR
RRLVLPAAQPEDGGFVCDAGDDSAFFTVTVTAPPERIVHPAARSLDLQFGAP
GHVELRCEVAPAGSQVRWYKDGLEVEVSDALQLGAEGPARTLTLPHAQPED
10 AGEYVCETRDEAVTFNVSLAELPVQFLAPEAAPNPLCVVPGEPPVLSCELSRA
SAQVFWSHNG -COOH

Figure 4-Partial Amino Acid Sequence (mPN34854) (SEQ ID NO:7)

15

NH₂-
MPSSASMSHHHSSGLRSSISSTSYRRTFGPPPSLSPGAFSYSSSSRFSSSRLLGSG
SPSSSARLGsFRAPRAGALRLPserLDfSMAEALNQEFLaTRsNEKQELQELN
DRFANFIEKVRFLEQQNAALRGELSQARGQEPARADQLCQQELRELRLRELELL
5 GRERDRVQVERDGLAEDLAALKQRLEEETRKREDAEHNLVLFrKdVDDATLS
RLELERKIESLMDEIEFLKKLHEEELRDLQVSVESQQVQQVEVEATVKPELTA
ALRDIRAQYENIAAKNLQEAEEWYKSKVREHWGNPggPRVGRHWEWRCAS
QPGLSATAQYADLSDAANRNHEALRQAKQEMNESRRQIQSLTCEVDGLRGTN
EALLRQLRELEEQFALEAGGYQAGAAARLEEELRQLKEEMARHLREYQELLNv
10 KMALDIEIATYRKLLGEESRISVPVHSFASLSLKTTVPemePLQDSHsKKMVL
IRTiETRDGEKVVtESQKEQHSDLDKSSiHSY-COOH

Figure 5- Full-length Amino Acid Sequence (mPRPH1) (SEQ ID NO:12)

15

NH₂-

MEDFQASEETAFVVDEVSSIVKEAIESAIGGNAYQHSKVNQWTTNVLEQTLSQ
LTKLGRPFKYIVTCVIMQKNGAGLHSASSCFWDSSTDGSCTVRWENKTMYCI
VSTFGLSI-COOH

5

Figure 6-Full-length Amino Acid Sequence (mTCTEX-1) (SEQ ID NO:9)

5'-
TCACTGACCTTCACGACCTCTGAACGGGTAGTGCTGACCTGTGAGCTCTCA
AGGGTGGATTTTCCTGCAACCTGGTACAAGGATGGGCAGAAGGTGGAGGA
GAGCGAGTCGCTCATAGTGAAGACAGAGGGCCGAAACACCGACTGATCC
5 TGCCTGAGGCTCAAGTCCGAGACAGCGGTGAATTTGAGTGCAGAACGGAA
GGGGTCTCGGCCTTCTTTGGAGTCACTGTTCAAGATCCCCCAGTGCACATC
GTGAACCCCCAAGAGCATGTGTTTGTCCATGCCATCACCTCCGAGTGTGTC
AGGCTGACCTGTGAGGTAGACCGAGAGGACACAACCTGTACACTGGTACAA
GGATGGGCAGGAGGTGGAGGAGAGTGACATCATCGTATTAGAAAATAAAG
10 GGCCCCATCACCGCCTGGTGCTACCTGCAGCCCGGCCCTCCGACGGGGGC
GAGTTCCAGTGTGTGCGCAGGAGATGAACGTGCCTACTTCACAGTTACCATC
ACAGATGTCTTCTCGTGGATCGTCTACCCAGTAGCGAAGTGCATGTGGCA
GCCATACGCCTAGAGCGTGTGGTGCTGACCTGTGAGCTGTGCCGACCCTGG
GCTGAGGTGCGCTGGACCAAAGATGGGGAGGAGGTAGTGGAGAGCCCAG
15 CACTGCTCCTGGAGAAGGAAGACACCATCCGCCGCCTGGTGCTGCCCTCT
GTCCAGCTTGAGGATTCTGGCGAGTACCTGTGTGAAATCCATGATGAGTCG
GCTTCCTTCACCATCACCGTCACAGAGCCCCCTGTGCGGATCATATACCCCC
AGGACGAGGTGACCTTACACGCCGTGAGTTTGGAATGTGTGGTGCTCACCT
GTGAGCTGTCTAGAGAGGATGCTCCTGTACGCTGGTACAAGGATGGGTTAG
20 AGGTGGAGGAGAGTGAAAGCCCTGGTGCTCCAGAGCGATGGGCCTCGTCGC
CGTCTGGTGTTGCCTGCTGCCCAGCCAGAGGACGGGGGCGAGTTTGTGTG
TGATGCTGGGGATGATTCAGCCTTCTTCACTGTCACTGTCACAGCTCCACC
AGAAAGGATTGTGCACCCAGCGGCCCGATCCCTGGATTGTCAGTTTGGGGC
TCCAGGACACGTGGAGCTACGCTGCGAAGTGGCCCCGGCTGGGTCTCAGG
25 TGCCTGGTACAAGGATGGCCTAGAGGTAGAGGTGTCAGATGCGCTGCAG
CTGGGTGCTGAGGGGCCTGCCCCGACTCTCACCTGCCCCACGCCAGCCT
GAGGATGCCGGGGAGTATGTATGTGAGACCCGAGATGAGGCTGTCACCTTC
AACGTCAGCCTGGCTGAGCTTCCGGTGCAGTTTCTGGCTCCAGAGGCAGC
CCCAAATCCGCTCTGCGTGGTTCCTGGGGAGCCCGTGGTGCTGAGCTGTGA
30 GCTGTCCCGAGCAAGCGCACAAAGTGTCTGGAGCCACAATGGG-3'

Figure 7- Nucleotide Sequence (mPN34854) (SEQ ID NO:13) (1458 nucleotides in total)

NH₂-

MNQIEPGVQYNYVYDEDEYMIQEEEWDRDLLLLDPAWEKQQRKTFTA
WCNSHLRKAGTQIENIEEDFRNGLKMLLLEVISGERLPKPDRGKMRFHKIAN
VNKALDYIASKGVKLVSIGAEIIVDGNVKMTLGMWTHLRFAIQDISVEETSA
5 KEGLLLWCQRKTAPYRNVNIQNFHTSWKDGLGLCALIHRHRPDLIDYSKLNK
DDPIGNINLAMEIAEKHLDIPKMLDAEDIVNTPKPDERAIMTYVSCFYHAFAG
AEQAETAANRICKVLAVNQENERLMEEYERLASELLEWIRRTIPWLENRTPEK
TMQAMQKKLEDFRDYRRKHKPPKVQEKQLEINFNTLQTKLRISNRPAFMPS
EGKMVSDIAGAWQRLEQAEKGYEEWLLNEIRRLERLEHLAEKFRQKASTHET
10 WAYGKEQILLQKDYESASLTEVRALLRKHEAFESDLAAHQDRVEQIAAIAQEL
NELDYHDAVNVNDRCQKICDQWDRLGTLTQKRREALERMEKLLETIDQLHL
EFAKRAAPFNNWMEGAMEDLQDMFIVHSIEEIQSLITAHEQFKATLPEADGER
QSIMAIQNEVEKVIQSYNIRISSSNPYSTVTMDELRTKWVKQLVPIRDQSLQ
EELARQHANERLRRQFAAQANAIGPWIQNKMEEIARSSIQITGALEDQMNQL
15 KQYEHNIINYKNNIDKLEGDHQLIQEALVFDNKHTNYTMEHIRVGWELLTTI
ARTINEVETQILTRDAKGITQEQMNEFRASFNHFDRRKNGLMDHEDFRACLIS
MGYDLGEAEFARIMTLVDPNGQGTVTTFQSFIDFMRETADTDTAEQVIASFRIL
ASDKPYILAEELRRELPPDQAQYCIKRMPAYSGPGSVPGALDYAAFSSALYGES
DL-COOH

20

Figure 8-Full-length Amino Acid Sequence (ACTN2) (SEQ ID NO:14)

NH₂-

MVDYHAANQAYQYGPNSGGGNGAGGGGSMGDYMAQEDDWDRDLL
LDPAWEKQQRKTFTAWCNSHLRKAGTQIENIDEDFRDGLKLMLLLEVISGER
LPKPERGKMRVHKINNVNKALDFIASKGVKLVSIGAEIIVDGNAMTLGMIW
5 TIILRFAIQDISVEETSAKEGLLLWCQRKTAPYKNVNVQNFHISWKDGLAFNA
LIHRHRPELIEYDKLRKDDPVTNLNNAFEVAEKYLDIPKMLDAEDIVNTARPD
EKAIMTYVSSFYHAFSGAQKAETAANRICKVLAVNQENEHLMEDYERLASDL
LEWIRRTIPWLED RVPQKTIQEMQQKLEDFRDYRRVHKPPKVQEKCCQLEINFN
TLQTKLRLSNRPAFMPSEGRMVSDINNGWQHLEQAEKGYEEWLLNEIRRLER
10 LDHLAEKFRQKASIHEAWTDGKEAMLKQRDYETATLSDIKALIRKHEAFESD
LAAHQDRVEQIAAIAQELNELDYD SHNVNTRCQKICDQWDNLGSLTHSRRE
ALEKTEKQLETIDQLHLEYAKRAAPFNNWMESAMEDLQDMFIVHTIEEIEGLI
SAHDQFKSTLPDADREREAILAIHKEAQRIAESNHIKLSGSPYTTVTPQIINSK
WEKVQQLVPKRDHALLEEQSKQQSNEHLRRQFASQANMVGPWIQTKMEEIG
15 RISIEMNGTLEDQLSHLKQYERSIVDYKPSLDLLEQQHQLIQEALIFDNKHTNY
TMEHIRVGWEQLLTTIARTINEVENQILTRDAKGISQEQMQEFRASFNHFDKD
HGGALGPEEFKACLISLGYDVENDRQGDAEFNRIMSVVDPNHSGLVTFQAFID
FMSRETTDTDTADQVIASFVKVLAGDKNFITAEELRRELPPDQAEYCIARMAPY
QGPDAAPGALDYKSFSTALYGESDL-COOH

20

Figure 9-Partial Amino Acid Sequence (mACTN4) (SEQ ID NO: 15)

NH₂-

MVDYYEVLGVQRHASPEDIKKAYRKQALKWHPDKNPENKEEAERKF
KQVAEAYEVLSDAKKRDIYDKYGKEGLNGGGGGGGIHFDSPFEGFTFRNPD
DVFREFFGGRDPFSFDFFEDPFDDFFGNRRGPRGNRSRGAAPFFSTFSGFPSFG
5 SGFPAFDTGFTPFGLGHGGLTSFSSTSFGGSGMGNFKSISTSTKIVNGKKITTK
RIVENGQERVEVEEDGQLKPLTINGKEHLLRLDNK-COOH

Figure 10- Full-length Amino Acid Sequence (mMDJ) (SEQ ID NO:16)