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Conditional-siRNAS and uses thereof in treating cardiac hypertrophy

Abstract

Disclosed herein are conditional siRNAs activatable by pro-hypertrophic RNA sequences and use thereof for treating conditions such as cardiac hypertrophy. The conditional siRNAs target calcineurin or HDAC2.

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Background/Summary

PRIORITY CLAIM (1) This application is a U.S. National Phase Application of International Application No. PCT/US2018/046379, filed Aug. 10, 2018, which claims priority to U.S. Provisional Patent Application No. 62/543,882, filed Aug. 10, 2017, the subject matter of which is hereby incorporated by reference in its entirety, as if fully set forth herein.

SEQUENCE LISTING
(1) This disclosure includes a sequence listing, which is submitted in ASCII format via EFS-Web, and is hereby incorporated by reference in its entirety. The ASCII copy, created Nov. 29, 2021, is named SubstituteSequenceListing.txt and is 252 kilobytes in size.
BACKGROUND
(2) RNA interference (RNAi) is a sequence-specific mRNA degradation pathway mediated by siRNA duplexes, key for cellular immunity and developmental regulation. Researchers have utilized synthetic RNAi triggers for therapeutics by inhibiting a specific gene product found to be essential in disease driving pathways but non-essential for normal functioning.
(3) Consider however that some genes essential in disease progression may have vital functions in normal cells and are dangerous to target. Meanwhile other upregulated genes are not essential for disease progression, but serve as effective indicators. Therefore, there is a need in the art to develop effective therapies to exploit this differential expression in various indications. The conditionally active siRNA complexes described below are candidates for investigation of treatments for those indications, such as cardiac hypertrophy.
(4) Heart Failure (HF) is a chronic cardiac condition, affecting millions of people worldwide, and considered a major contributor to healthcare expenditure in the US. Compensatory cardiac hypertrophy is one of the initial hallmarks of pathological ventricular remodeling, which is

characterized by an upregulation of a variety of genes and miRNA that mediate and regulate myocardial hypertrophy, and ultimately HF. Even though important advances have been done in the treatment of HF, no cardiac specific therapies with lack of adverse effects have been developed to date. Therefore, there is a need in the art to develop an effective therapy for HF.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

(1) The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

(2) FIG. **1** shows a comparison of secondary and tertiary structure (from full atomistic MD simulations) of a Cond-siRNA construct according to one embodiment. Black arrows show corresponding features between the 2D and 3D representations.

(3) FIG. **2** is a diagram showing the RNAi pathway.

(4) FIG. **3** shows toehold mediated strand displacement process of conditional siRNA. In step I, c-siRNA meets RNA transcript with correct activation sequence (Input). In step II, an Input RNA binds to the toehold. Step III shows toehold mediated strand displacement. Step IV shows the sensor strand and input forming a waste duplex that separates from the pro-siRNA. In step V, XRN1, exosome and other cytosolic RNAses rapidly degrade unprotected overhangs, turning pro-siRNA into efficient Dicer substrate. In step VI, siRNA is processed by Dicer for incorporation into RISC. The basic biophysical process of toehold mediated strand displacement includes a fast 1 D random walk: uS to mS for each of N{circumflex over ( )}2 steps. This results in sequence specificity from both toehold and duplexes. Thermodynamically stable chemical modifications are confined to sensor strand to avoid kinetic traps.

(5) FIGS. **4**A-C: A) General construct design of cond-siRNA with green sensor strand designed reverse comp. to signal gene mRNA, red core strand with nick either 11 or 12 bp from toehold end on sensor side designed comp. to sensor and guide, and yellow guide strand designed reverse comp. to target gene mRNA. B) Model of cond-siRNA. C) Molecular simulation of cond-siRNA.

(6) FIG. **5** shows an overview of the design process for Cond-siRNAs according to one embodiment.

(7) FIG. **6** shows a hypothetical sensor duplex for mRNA used to check for thermodynamic stability of the sensor according to one embodiment.

(8) FIG. **7** shows a structure calculation showing sensor strand with low internal secondary structure according to one embodiment.

(9) FIG. **8** shows a histogram showing a 97% predicted formation of the hypothetical sensor duplex and correct secondary structure according to one embodiment.

(10) FIG. **9** shows a hypothetical sensor duplex for miRNA used to check for thermodynamic stability of the sensor according to one embodiment.

(11) FIG. **10** shows a structure of the RNAi targeting duplex according to one embodiment.

(12) FIG. **11** is a schematic diagram of a signaling pathway involved in cardiac gene program regulating hypertrophy.

(13) FIGS. **12**A-B show schematic depicting in vivo and in vitro screening approaches.

(14) FIGS. **13**A-B: A) NuPack generated secondary structure of selected NPPB 31 bp sensor strand. Minimal secondary structure with approximately 40-50% probability of folding onto itself. B) NPPB sensor strand bound to 5′ and 3′ core overhangs 99% of the time.

(15) FIGS. **14**A-B: A) NuPack generated secondary structure of selected MYH7 31 bp sensor strand. Minimal secondary structure with approximately 30% probability folding onto itself. B) NPPB sensor strand bound to 5′ and 3′ core overhangs 97% of the time.

(16) FIGS. **15**A-B: A) NuPack generated secondary structure of HDAC2 25 bp guide strand. Significant secondary structure indicated by the equilibrium probabilities of binding. B) NuPack generated secondary structure of HDAC2 guide bound to core strand with the 5′ and 3′ overhangs that bind to the appropriate sensor strand. Although guide strand has significant and strong secondary structure, when placed with core strand, guide binds to core 100%.
(17) FIG. **16** shows test constructs detecting murine ANP mRNA and targeting murine Calcineurin according to certain embodiments.
(18) FIG. **17** shows test constructs detecting murine and human mir-23a-3p and targeting murine Calcineurin according to certain embodiments.
(19) FIG. **18** is a schematic depicting a site of injury that can be targeted for treatment according to methods of the present invention.
(20) FIG. **19** is a series of bar graphs depicting experimental results of differential gene expression in tissues of wild-type mice in homeostasis.
(21) FIG. **20**: Gene expression in NRVM under hypoxia.
(22) FIG. **21**: Differential miRNA expression in NRVM under hypoxia.
(23) FIG. **22**: Gene expression in NRVM after PE treatment.
(24) FIG. **23**: Differential miRNA expression in NRVM after treatment with PE.
(25) FIG. **24**: Gene expression in mice with non-ischemic (TAC) and ischemic (I/R) HF.
(26) FIG. **25**: miRNA expression in mice with non-ischemic (TAC) and ischemic (I/R) HF.
(27) FIG. **26** shows an equilibrium probability for an MFE structure.
(28) FIG. **27** shows an equilibrium probability for an MFE structure.
(29) FIG. **28** shows an equilibrium probability for an MFE structure.
(30) FIG. **29** shows an equilibrium probability for an MFE structure.
(31) FIG. **30** shows (ANP:calcineurin) and (mir-23a-3p:calcineurin) Cond-siRNAs on 10% non-denaturing PAGE gel in TBE. The correct assemblies are indicated in the green boxes.
(32) FIG. **31** shows results of a dual luciferase assay of mir-23a-3p calcineurin Cond-siRNA according to certain embodiments.
(33) FIG. **32** is a bar graph showing RNAi activity against Calcineurin in NRVM cells under PE stimulation according to one embodiment.
(34) FIG. **33** shows images of NRVM cells with and without PE stimulation when treated with scrambled siRNA (negative control), (ANP:calcineurin) Cond-siRNA, and commercial calcineurin siRNA (positive control) according to one embodiment.
(35) FIG. **34** shows results of cell size quantitation according to one embodiment.
(36) FIG. **35**A shows Post-MI cardiac remodeling and left ventricular enlargement.
(37) FIG. **35**B is a bar graph showing experimental results where patients with any of the patterns of LV remodeling post-MI had a greater risk of the composite of cardiovascular (CV) death, MI, heart failure (HF), stroke, or resuscitated cardiac arrest.
(38) FIG. **36** is a flowchart accompanied by a corresponding schematic showing the RNA interference pathway starting with pri-miRNA processing in the nucleus.
(39) FIG. **37** is a schematic showing post-MI remodeling.
(40) FIG. **38** is a series of bar graphs depicting experimental results of differential miRNA expression in tissues of wild-type mice in homeostasis.
(41) FIG. **39** is a table including candidate sensor strands for the 3′ UTR of the human myh7 gene. Column abbreviations are as follows: BS is Bad Segments; 3LN is 3-Letteredness; NBP is Number Bad Points; P is Position.
(42) FIG. **40** is a table including candidate sensor strands for the 3′ UTR of the rat myh7 gene. Column abbreviations are as follows: BS is Bad Segments; 3LN is 3-Letteredness; NBP is Number Bad Points; P is Position.
(43) FIG. **41** is a table including candidate sensor strands for the 3′ UTR of the human nppa gene. Column abbreviations are as follows: BS is Bad Segments; 3LN is 3-Letteredness; NBP is Number

Bad Points; P is Position.
(44) FIG. **42** is a table including candidate sensor strands for the 3′ UTR of the rat nppa gene. Column abbreviations are as follows: BS is Bad Segments; 3LN is 3-Letteredness; NBP is Number Bad Points; P is Position.
(45) FIG. **43** is a table including candidate sensor strands for the 3′ UTR of the human nppb gene. Column abbreviations are as follows: BS is Bad Segments; 3LN is 3-Letteredness; NBP is Number Bad Points; P is Position.
(46) FIG. **44** is a table including candidate sensor strands for the 3′ UTR of the rat nppb gene. Column abbreviations are as follows: BS is Bad Segments; 3LN is 3-Letteredness; NBP is Number Bad Points; P is Position.
(47) FIG. **45** shows a top view of a 3D schematic of a Cond-siRNA construct according to one embodiment.
(48) FIG. **46** shows the design of sensor miR-23-a-3p gene sequence.
(49) FIG. **47** illustrates NCBI check for sensor strand.
(50) FIG. **48** illustrates NCBI check for calcineurin and HDAC2 guide strand sequences.
(51) FIG. **49** shows the secondary structure and MFE structure at 37° C. of the full miR-23a-3p sensor strand with toehold for calcineurin.
(52) FIGS. **50**A-**50**D show that NuPack analyses were performed on core (FIG. **50**A), guide (FIG. **50**B), Sensor with two small overhangs of core: 97% (FIG. **50**C), and calcineurin guide with core: 100% (FIG. **50**D).
(53) FIG. **51** shows NCBI check for calcineurin guide strand vs. human constructs.
(54) FIGS. **52**A-**52**D show that NuPack analyses of miR-23a-3p sensor strand for HDAC2 were performed on core (FIG. **52**A), guide (FIG. **52**B), HDAC2 guide with core: 100% (FIG. **52**C), and sensor with core overhangs: 97% (FIG. **52**D).
(55) FIG. **53** shows NCBI check for HDAC2 guide strand vs. human transcripts.
(56) FIGS. **54**A-**54**B illustrate the check of guide vs. NCBI human transcripts and sequence alignment, respectively.
(57) FIG. **55** shows the MFE structure of SEQ ID NO: 4.
(58) FIG. **56** shows the BNP sensor sequence (SEQ ID NO: 4) together with core and guide sequences.
(59) FIGS. **57**A and **57**B show Nupack analyses of BNP sensor (SEQ ID NO: 4) with overhangs, and guide with core, respectively.
(60) FIG. **58** shows NCBI check of BNP sensor first candidate vs. human transcripts.
(61) FIG. **59** shows the MFE structure of SEQ ID NO: 5.
(62) FIG. **60** shows the BNP sensor sequence (SEQ ID NO: 5) together with core and guide sequences.
(63) FIGS. **61**A and **61**B show Nupack analyses of BNP sensor (SEQ ID NO: 5) with overhangs, and guide with core, respectively.
(64) FIG. **62** shows NCBI check of BNP sensor second candidate vs. human transcripts.
(65) FIG. **63** shows the MFE structure of SEQ ID NO: 6.
(66) FIG. **64** shows the BNP sensor sequence (SEQ ID NO: 6) together with core and guide sequences.
(67) FIG. **65** shows Nupack analysis of guide with core for the third BNP candidate.
(68) FIG. **66** shows NCBI check of BNP sensor third candidate vs. human transcripts.
(69) FIG. **67** shows the MFE structure of SEQ ID NO: 7.
(70) FIG. **68** shows the MYH7 sensor sequence (SEQ ID NO: 7) together with core and guide sequences.
(71) FIGS. **69**A and **69**B show Nupack analyses of MYH7 sensor (SEQ ID NO: 7) with overhangs, and guide with core, respectively.
(72) FIG. **70** shows NCBI check of MYH7 sensor vs. human transcripts.

(73) FIG. **71** shows the MFE structure of NPPA HDAC2 construct #1.
(74) FIG. **72** shows the MFE structure of NPPA HDAC2 construct #3.
(75) FIG. **73** shows the MFE structure of NPPA calcineurin construct #1.
DETAILED DESCRIPTION
(76) Overview of Conditional-siRNA
(77) Described herein are conditional siRNA complexes (also referred to herein as Cond-siRNA, a conditional RNA-sensor, or an RNA-sensor) that include a therapeutic component (e.g., siRNA molecule) associated with a molecular sensor via a core molecule. The conditional siRNA complexes are inactive under normal conditions, but are activated upon interaction between the molecular sensor and a biomarker. Such molecules are synthetic riboswitch molecules that allow an input gene or RNA molecule to "switch on" an RNAi pathway against a target output gene.
(78) An RNA-sensor molecule or complex includes sensor strand, a guide strand, and a core strand that bind to each other to form a multi-strand molecular complex having a dual duplex structure shown in FIGS. **1**, **45**. In certain embodiments, those three strands (core, sensor and guide) form two parallel oligonucleotide duplexes connected in a double crossover configuration. [14] (See FIG. **1**). In some aspects, the length of each of the oligonucleotide duplexes is sufficient to operate within the RNA interference (RNAi) pathway (See FIGS. **2**, **36**). For example, the duplexes may be between about 15 and 30 base pairs in length. In some embodiments, the duplexes are between 15 and 20 base pairs in length, between 20 and 25 base pairs in length, between 25 and 30 base pairs in length. In other embodiments, the duplexes are about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 25, 26, 27, 28, 29, 30, or more than 30 base pairs in length.
(79) The double crossover configuration as shown in FIG. **1** represents the inactive or "OFF" state of the RNA-sensor complex wherein the sensor duplex inhibits RNAi loading of the siRNA duplex, serving as a "lock" on RNAi activity. In the OFF state, the guide strand binds a first portion (or "passenger" segment) of the core strand to form an siRNA duplex that serves as a pro-RNA molecule. The pro-RNA molecule operates in the RNAi pathway of a target cell to alter expression of a target gene or target RNA molecule associated with a pathological condition (i.e., the "therapeutic target molecule"). The second duplex is formed by the sensor strand binding to a second portion (or "protection" segment) of the core strand to form the sensor duplex. In some embodiments, the core strand has a third portion (or "protection" segment) that binds the sensor strand. In certain such embodiments, the core strand includes the passenger strand (P) that is joined to first and second protection segments (A, B) at each end by a linker (L1, L2) in the following configuration:
5′ B-L2-P-L1-A 3′
(80) The sequence of the core strand is determined by the sequences of the sensor and guide strands, and may be fully or complementary to the sensor strand, the guide strand, or both. Any suitable linker can be used in accordance with the embodiments described herein, including, but not limited to, an internal C3 spacer, a C6 linker, a tri-ethylene glycol linker.
(81) The RNA-sensor complex is activated to the "ON" state upon interaction with a biomarker in the cell expressing a phenotype associated with the pathological condition targeted by the guide strand of the siRNA duplex. This activation is primarily due to the design of the sensor strand, which serves as the activation signal for RNAi activity. When this is the case, the RNA-sensor complex is said to detect the biomarker.
(82) The sensor strand includes a nucleotide sequence designed to bind the biomarker associated with the pathological condition (i.e., "pathological biomarker"). Binding to the biomarker is initiated by the binding of at least one toehold segment (single stranded) to an input RNA strand that encodes at least a portion of the pathological biomarker, as shown in FIG. **3**. Upon displacement of the sensor strand, the sensor and input strands from a waste duplex that separates from the pro-siRNA molecule, allowing the pro-siRNA to be processed by the target cell's RNAi system. The structure and binding dynamics of the conditional-siRNAs described herein is

explained further in U.S. Pat. No. 9,725,715, the content of which is incorporated herein by reference in its entirety.

(83) The sequence of the sensor strand can be fully or partially complementary to an RNA sequence present in the pathological biomarker. In certain embodiments, the sensor strand is 100% complementary to the RNA sequence present in the pathological biomarker. Other embodiments may include a sensor strand that is largely complementary to the RNA sequence present in the pathological biomarker, for example, the sensor strand may be greater than 70% complementary, greater than 75% complementary, greater than 80% complementary, greater than 85% complementary, greater than 90% complementary, greater than 95% complementary, greater than 96% complementary, greater than 97% complementary, greater than 98% complementary, or greater than 99% complementary to the RNA sequence present in the pathological biomarker.

(84) In some embodiments, the pathological biomarker is an RNA sequence that forms or encodes a molecule that is associated with the pathologic condition. In some aspects, the pathological biomarker is an RNA sequence that is present in the target cell under pathological conditions, but is substantially absent under normal conditions. Alternatively, the pathological biomarker is an RNA sequence that is upregulated in the target cell under pathological conditions as compared to normal conditions.

(85) The guide strand includes a Dicer cleavage site near the 3′ end. The sequence between the Dicer cleavage site and the 3′ terminus of the guide strand is either fully or partially complementary to a nucleotide sequence found in the therapeutic target molecule (e.g., target gene, target mRNA or target miRNA). When this is the case, the Cond-siRNA is said to target the gene or RNA molecule. In certain embodiments, the guide strand is 100% complementary to the nucleotide sequence found in the therapeutic target molecule. Other embodiments may include a guide strand that is largely complementary to the nucleotide sequence found in the therapeutic target molecule, for example, the guide strand may be greater than 70% complementary, greater than 75% complementary, greater than 80% complementary, greater than 85% complementary, greater than 90% complementary, greater than 95% complementary, greater than 96% complementary, greater than 97% complementary, greater than 98% complementary, or greater than 99% complementary to the nucleotide sequence found in the therapeutic target molecule.

(86) A challenge of using oligonucleotides in vivo lies in preventing nuclease degradation of RNA nucleotides. Several chemical modifications in the sensor strand can be used to overcome this challenge. For example, Locked Nucleic Acids (LNAs) include a modification of RNA nucleotides with an extra bridge between the 2′ O and 4′ C increases thermal stability of RNA duplexes and allows for resistance to nucleases. 2′ O-Methyl modifications confer stability, increase binding affinity to RNA nucleotides and prevent degradation by nucleases. And, phosphorothioate: modification by replacing one of the non-bridging oxygens in the phosphate linkage between bases with a sulfur that reduces nucleolytic degradation; however also lowers binding affinity.

(87) Thus, in certain embodiments, the RNA-sensor complex includes one or more modifications to the nucleotide sequence of the sensor strand, the core strand, and/or the guide strand. Exemplary modifications that may be used include, but are not limited to, locked nucleic acids (LNA), peptide nucleic acids (PNA), 2′-O-methyl modifications, morpholino modifications, phosphorothioate modifications, terminal modifications, and other linker or backbone modifications or connections. Additional chemical modifications may be chosen according to methods described in U.S. Pat. No. 9,725,715B2, the disclosure of which is hereby fully incorporated herein.

(88) The approach of designing a cond-siRNA sensor complex for use in treating a disease or other pathological condition using the cond-siRNA sensor complexes is advantageous in that it allows the complex to become biologically active only in diseased cells AND remain OFF in healthy cells. In addition, the approach allows for increased disease cell specificity and prevents toxicity from delivery to unintended off-targets. Further, the approach combines disease specificity from one gene with treatment efficacy from a second gene to create therapeutics that are precisely tailored to

specific gene expression patterns. Still further, the approach is advantageous due to steric hindrance of the two RNA duplexes positioned in a parallel configuration (FIG. **4**). The sensor strand inhibits RNAi loading of siRNA and will only displace when activated in disease cells.

(89) Overview of Methods for Designing a Conditional siRNA Complex

(90) An siRNA complex is designed based on biomarkers and therapeutic target molecules that are specific to each cell type, pathological condition, and/or indication. According to certain embodiments, methods for designing and testing each conditional siRNA complex includes several steps, as described below.

(91) FIG. **5** shows an overview of the design process. In certain embodiments methods for designing a conditional siRNA complex (the "design method") includes a step of determining a biomarker that will serve as an input for activation and a therapeutic target for RNAi inhibition. This step may include a determining one or more factors that are differentially expressed (i.e., upregulated or present in a diseased cell as compared to a normal cell) using methods known in the art.

(92) The design method further includes a step of generating a list of candidate target segments of the biomarker (i.e., target mRNA sequence or target miRNA sequence) that can serve as a biomarker segment for binding the sensor strand, and then designing sensor strands for each biomarker.

(93) The design method further includes a step of estimating the thermodynamic stability of the resulting sensor strand-biomarker duplexes (the sensor duplex) generated by the target segments and sensor strands by using secondary structure prediction tools used in the art [15].

(94) The design method further includes a step of checking for the uniqueness of the binding site for the most stable sensor duplexes against the known transcriptome of the animal to which the conditional siRNA complex will be tested against.

(95) The design method further includes a step of generating a list of guide strand sequences by using a protocol that may include, but is not limited to, standard siRNA design tools, literature references, or heuristic rules.

(96) The design method further includes a step of creating a Dicer substrate from the chosen guide strand sequences.

(97) The design method further includes a step of generating sequences for the core strand that connect the sensor strands to the guide strands.

(98) The design method further includes a step of checking that the sensor: guide pairing does not create unwanted interactions.

(99) The design method further includes a step of selecting a pattern of suitable chemical modifications as described herein, and optionally simulating the constructs using molecular simulation methods used in the art [16] to simulate the constructs (optional).

(100) The design method may also include a method of synthesizing or purchasing the sensor, core, and guide strands from commercial vendors such as Qiagen, Dharmacon, or IDT, the constructs of which are then assembled, characterized, and purified using gel electrophoresis.

(101) The design method further includes a step of conducting preliminary biological testing and validation of the construct function, and then optionally test in in vitro and in vivo models of pathological conditions, including, but not limited to, MI induced maladaptive hypertrophy as described below.

(102) Additional embodiments related to designing the guide, the sensor and the core strands are explained below.

(103) Method for Designing Sensor Strands for mRNA Biomarker

(104) According to certain embodiments, methods for designing and testing sensor strands that target an mRNA biomarker includes an algorithm that includes several steps, as described below.

(105) In certain embodiments, a method for designing a sensor strand for an mRNA biomarker (the "mRNA sensor design method") includes a step of identifying the 3′ UTR for each messenger RNA

biomarker.
(106) The mRNA sensor design method further includes a step of generating all possible consecutive 31 base sequences for each 3′ UTR identified above.
(107) The mRNA sensor design method further includes a step of obtaining the prospective sensor strand sequence for each sequence segment from the previous step by identifying the reverse complement (full or partial) of each sequence
(108) The mRNA sensor design method further includes a step of checking each sensor strand sequence for the following undesirable features: (i) three or more consecutive Gs, and (ii) four or more consecutive A or U bases.
(109) The mRNA sensor design method further includes a step of checking each sensor strand sequence for the following desirable features: (i) higher than 50% G/C bases—this correlates with thermodynamic stability, (ii) "three letteredness," (iii) The first base at the 5′ end of the sensor strand is a C or a G; and (iv) the 9th base from the 3′ end of the sensor strand is a C or a G. According to the embodiments described herein, "three letteredness" is defined as the proportion of the sequence comprising of the three most numerous bases (e.g., the extent to which sequence is mostly made of A, U, C; or C, G, A; or A, U, G). A higher three letteredness score correlates with lower internal secondary structure. Exemplar ranking tables can be seen in FIGS. **39**-**44**, which correspond to the genes or nucleotide sequences in Appendices C-H, submitted herewith.
(110) The mRNA sensor design method further includes a step of ranking all possible sensor strands. Strands with the least number of features from 4 and the highest scores from 5 are ranked highest
(111) The mRNA sensor design method further includes a step of generating hypothetical sensor duplexes using the pattern shown in FIG. **6**, starting from the highest ranked strands.
(112) The RNA sensor design method further includes a step of using Nupack or similar secondary structure prediction codes to calculate the following, starting from the highest ranked strands: (i) the internal secondary structure of the sensor strand (lower amounts of internal secondary structure are desirable (FIG. **7**), (ii) the thermodynamic stability of the hypothetical duplex from 7. Ideally, at 1 nM strand concentration, Nupack should predict that >90% or >95% of component strands should form the hypothetical sensor duplex (FIG. **8**); and (iii) if sensor duplex is not stable, can adjust 1 to 5 bases at the 5′ terminus of the sensor sequence to increase stability at the cost of reducing complementarity to the corresponding binding site on the putative biomarker.
(113) The RNA sensor design method further includes a step of screening the sensor strand for thermodynamically stable duplexes using NCBI BLAST according to the following parameters: (i) use the "somewhat similar" search option, (ii) for sensor sequences, the 8 bases at the 3′ terminus (constituting the 3′ toehold) should have no more than 5 bases complementary to known transcripts in the target animal (eg, human or mouse) other than the intended biomarker, and (iii) if the first two criteria not met, broaden sequences considered in 1 to the coding region or the entirety of the mRNA.
(114) Method for Designing Sensors for miRNA Biomarker
(115) According to certain embodiments, methods for designing and testing sensor strands that target an miRNA biomarker includes an algorithm that includes several steps, as described below.
(116) In certain embodiments, a method for designing a sensor strand for an miRNA biomarker (the "miRNA sensor design method") includes a step of identifying a guide sequence for each miRNA biomarker, to which the sensor strand is designed to bind (typically approximately 21 bases according to one aspect)
(117) The miRNA sensor design method further includes a step of obtaining the reverse complement (full or partial) of the miRNA guide sequence.
(118) The miRNA sensor design method further includes a step of adding 8 more bases to the 5′ end of the sequence from the prior step.
(119) The miRNA sensor design method further includes a step of generating hypothetical sensor

duplexes using the pattern shown in FIG. **9**, starting from the sequence developed in the prior step.
(120) The miRNA sensor design method further includes a step of using Nupack or similar secondary structure prediction codes to calculate the following: (i) the thermodynamic stability of the hypothetical duplex from the prior step. Ideally, at 1 nM strand concentration, Nupack should predict that >90% or >95% of component strands should form the hypothetical sensor duplex. (ii) if sensor duplex is not stable or the secondary structure is incorrect, determine whether the 8 terminal bases at the 5′ end of the sensor strand, or the length of strand A or strand B can be altered or modified to optimize thermodynamic stability.
(121) The miRNA sensor design method further includes a step of screening the sensor strand for thermodynamically stable duplexes in NCBI BLAST according to the following parameters: (i) use the "somewhat similar" search option, (ii) for sensor sequences, the 8 bases added at the 5′ end of the sensor should not increase complementarity to transcripts other than the intended miRNA. If they do, adjust the sequence and start over from 4.
(122) Methods for Designing a Guide Strand Sequence Against a Therapeutic Target Molecule
(123) According to certain embodiments, methods for designing a guide strand sequence against a therapeutic target gene or RNA molecule (e.g., mRNA or miRNA) includes several steps, as described below.
(124) In certain embodiments, a method for designing a guide strand sequence against a therapeutic target (the "guide strand design method") includes a step of obtaining one or more prospective guide strand sequences using at least one of the following methods: (i) find a published guide strand sequence for the intended target; (ii) find a known miRNA target site on the target gene, or (iii) use a published algorithm or design tool known in the art [17, 18].
(125) The guide strand design method further includes a step of checking the guide sequence to make sure that the 6 bases at the 5′ domain (FIG. **10**) is more AU rich than the 6 bases in the 3′ domain (FIG. **10**). This will improve probability for correct strand loading [19]. Ideally, the 3′ domain should be CG rich, and terminate in a CG base-pair.
(126) The guide strand design method further includes a step of adding four terminal bases to the 5′ end of the guide strand to complete the duplex. Those should be CG rich to improve thermodynamic stability.
(127) The guide strand design method further includes a step of constructing the hypothetical RNAi targeting duplex as shown in FIG. **10**.
(128) The guide strand design method further includes a step of checking that the guide strand has weak internal secondary structure and minimal tendency to bind to itself (no more than 10% at 1 nM strand concentration) using Nupack or similar standard secondary structure calculation tool. Adjust bases added in 3 as necessary.
(129) Methods for Designing a Core Strand Sequence and Checking Compatibility of Pairing Sensor to Guide
(130) According to certain embodiments, methods for designing a core strand sequence and checking compatibility of pairing sensor to guide includes several steps, as described below.
(131) In certain embodiments, a method for designing a guide strand sequence against a therapeutic target (the "core strand design method") includes a step of choosing a suitable combination of sensor and guide strands, methods for designing those strands are discussed above and in the working examples, according to the embodiments described herein.
(132) The core strand design method further includes a step of constructing the core strand by constructing a strand of the form 5′-B-C3-P-C3 A-3′ where A and B are the sequence of complementary strand B from the hypothetical sensor duplex (FIG. **6** or **9**), P is the sequence of the passenger strand from the hypothetical RNAi duplex (FIG. **10**) and C3 are C3 linkers.
(133) The core strand design method further includes a step of using Nupack or similar standard secondary structure calculation tool to check that the guide strand and core strand base-pairing has the following properties: (i) >95% of strands are base-paired in the correct duplex at 1 nM strand

concentration, (ii) the guide strand duplex has the correct conformation, with a ˜23 base-pair duplex, a two base 3′ guide strand overhang, and 10-12 base 5′ and 3′ core overhangs with minimal secondary structures, and (iii) If above criteria not met, choose new sensor or guide pairing.

(134) Conditional siRNA Complexes for Treating Cardiac Hypertrophy

(135) Disclosed herein is a therapeutic strategy that targets molecular pathways involved in cardiac hypertrophy (that often results after a myocardial infarction), with minimal off-target effects. A myocardial infarction (MI) is a heart attack. MIs can occur when a blood clot blocks a coronary artery, interrupting blood flow to the heart—a condition known as cardiac ischemia.

(136) Heart muscles downstream of the blockage lose oxygen, leading to injury and death of the muscle cells. Post-MI, the hypoxia and tissue damage induces left ventricular remodeling (FIGS. **35**A, **37**). Cardiac reperfusion results in inflammation and oxidative damage. The injuries to cardiomyocytes during MI can cause a cascade of biological signaling events that leads cardiomyocytes to increase in volume and undergo proliferation in a specific way that compromises the functioning of the heart. This hypertrophic response is driven by a complex interplay of factors including a maladaptive regeneration gene program. During maladaptive regeneration, the heart undergoes changes that induce detrimental conditions to the patient (FIG. **35**B). The affected ventricle increases its volume, but the walls of the ventricle become thinner, and the ability of the heart to pump blood decreases over time. This can lead to a variety of serious problems, including heart failure, a second heart attack, or sudden death. Thus, this process is called maladaptive hypertrophy (as opposed to adaptive hypertrophy, which strengthens the heart in reaction to stimuli such as exercise).

(137) Maladaptive cardiac hypertrophy can be ameliorated by drugs that inhibit calcineurin and histone deacetylase 2 (HDAC2), for example. However, these drugs can cause serious side effects in non-cardiac tissues. Therefore, it's necessary to have a method to restrict drug activity to the heart.

(138) The specificity and versatility of the conditional small interfering RNAs (cond-siRNA) described herein offers a new class of therapeutics for a variety of diseases and cancers by hijacking the RNA interference (RNAi) pathway. Although current treatment options for post-MI cardiac hypertrophy alleviate the severity of the condition, it is necessary to target the internal maladaptive gene program that drives the hypertrophic responses. In particular, cond-siRNAs (FIG. **4**) are sequenced with a signal and target strand that due to steric hindrance, will only activate when the appropriate cardiac hypertrophy signal is present-thus only targeting disease cells through toehold-mediated strand displacement.

(139) The Cond-siRNAs described herein can achieve this by using cardiac RNA biomarkers of MI as activation signals to switch ON RNAi silencing against calcineurin or HDAC2. Using this approach, RNAi silencing of the target genes are restricted to cardiomyocytes that express MI associated RNA biomarkers. This means that RNAi activity will NOT occur in other organs and tissues where inhibition of the targets can cause serious side effects. In one aspect, disclosed herein is a strand-displacement operated, programmable conditional-siRNA complex that can be activated by specific mRNA and miRNA transcripts expressed in the hypertrophied myocardium, to target unrelated pro-hypertrophic pathways by RNAi knockdown.

(140) To design an effective Cond-siRNA for treating Cardiac Hypertrophy, in vivo and/or in vitro screening approaches for measuring relevant gene expression may be used in accordance with the embodiments described herein.

(141) Certain genes are upregulated under pathological cardiac hypertrophic conditions, which may be candidate pathological biomarkers to guide a Cond-siRNA molecule to a population of target cells, and which can be used to displace the sensor strand of the Cond-siRNA. For example, certain signaling cascades are activated under hypertrophic stimulating conditions (see FIG. **11**). For example, genes and miRNAs that are upregulated in pathological cardiac hypertrophy were screened for differential gene expression in wild type (wt) mice as well as under various

hypertrophic conditions to determine which genes are suitable candidates for use as a target for designing a sensor strand. See working examples below

(142) In vitro screening approaches may include the use of a cardiomyocyte cell line (e.g., neonatal rat ventricular myocytes (NRVM), human cardiac myocytes (HCM)) cultured under hypoxic conditions or treated with phenylephrine. FIG. **12**A. In vivo approaches may also be used including, but not limited to, rat models for ischemic heart failure (HF) (e.g., ischemia/reperfusion model), or rat models for non-ischemic HF (e.g., thoracic aortic constriction (TAC) model). See FIG. **12**B.

(143) Selection of RNA pathological biomarkers (input signals) for activation of Cond-siRNAs an important process for designing the cond-siRNA complexes described herein. The purpose of using pathological RNA biomarkers specific to the condition of cardiac hypertrophy for conditional RNAi activation is to ensure that RNAi activity is only active in cardiac tissues. Ideally, these biomarkers should be highly overexpressed in cardiac tissues affected by MI and not expressed in other tissues of the body. By comparing data gathered from in vitro and in vivo experiments on NRVM cell cultures and mice models with known organism wide expression patterns for the tested mRNAs and miRNAs (see working examples below), it was determined that at least three mRNAs and three miRNAs fit the criteria, including mRNAs that encode atrial naturetic peptide (ANP), B-type natriuretic peptide (BNP), and myosin heavy chain β (MHCP), and miRNAs that encode mir-23a-3p, mir-125-5p, and mir-199b-5p.

(144) Thus therapeutic biomarkers that may be used to activate the conditional siRNA complexes in accordance with the embodiments described herein include, but are not limited to, mRNA biomarkers for MI affected cardiomyocytes, such as those described below.

(145) In certain embodiments, a Cond-siRNA for treating cardiac hypertrophy includes a sensor strand designed to target a biomarker that is present and/or upregulated in heart cells (e.g., cardiac myocytes). Such biomarkers may include, but are not limited to, atrial naturetic peptide (ANP), B-type natriuretic peptide (BNP), myosin heavy chain β (MHCP), mir-23a-3p, mir-125-5p, and mir-199b-5p. In some aspects, the sensor strand detects an mRNA or an miRNA sequence that encodes the biomarker. And, in certain aspects, the sensor strand detects an mRNA sequence that encodes the biomarker by binding to the 3′ UTR of the mRNA. Additional information regarding exemplary biomarkers is discussed below.

(146) ANP (nppa) signal sensor strands. nppa (Natruiretic Peptide A) encodes for ANP protein that is overexpressed in hypertrophic conditions, thus is suitable as a biomarker for hypertrophy.

(147) BNP (nppb) signal sensor strands. nppb (Natruiretic Peptide B) encodes for BNP protein that functions as a cardiac hormone and regulates natruiresis, diuresis, vasorelaxation and cardiovascular homeostasis. Low levels of BNP naturally found in the bloodstream in healthy individuals; high levels from cardiac ventricles. High concentrations of BNP in bloodstream indicates heart failure, and is a biomarker for hypertrophy. Thus, significant upregulation of BNP in induced cardiac hypertrophic conditions is indicative of effective sensor strand gene selection.

(148) In some embodiments, a NPPB RH SSS v2.0 sensor was designed, wherein 31 bp sequence windows checked by hand through screening in entire 3′ UTR of *Homo sapiens* mRNA based on A-U richness, hairpins, poly G tracts, and 8 bp toehold region. Three areas were found; NCBI Blast check for matches with other mRNAs and % yield of binding narrowed down options to chosen sensor (see NPPB sensor structures, FIG. **13**).

(149) MHC3 (myh7) signal sensor strands. MYH7 (myosin heavy chain 7) encodes for the p-heavy chain subunit of cardiac myosin. Varying amounts of the encoded protein correlate with cardiac muscle fiber contractile velocity. myh7 is predominantly expressed in the ventricle and type I muscle fibers. Gene mutations are associated with hypertrophic cardiomyopathy, and mybh7 is upregulated in pathological cardiac hypertrophy. A significant upregulation of myh7 is seen in induced cardiac hypertrophic conditions, indicating that myh7 is another potential sensor strand gene selection.

(150) In some embodiments, a myh7 RH SSS v2.0 sensor was designed, wherein 31 bp sequence windows checked by hand through screening of entire *Homo sapiens* mRNA. Because 3′ UTR of mRNA is short with numerous poly G tracts, screening went into the coding region; however those contained secondary structure. Only 1 area found and chosen; checked NCBI Blast for matches with other mRNAs (see, e.g., myh7 sensor structures, FIG. **14**).

(151) In some embodiments, the sensor strand is fully complementary to any of the biomarkers discussed above (i.e., 100% complementary). In some embodiments the sensor strand is partially complementary to any of the biomarkers discussed above. For example, the sensor strand may be at least 70% complementary to the biomarker, at least 70% complementary to the biomarker, at least 75% complementary to the biomarker, at least 80% complementary to the biomarker, at least 85% complementary to the biomarker, at least 90% complementary to the biomarker, at least 95% complementary to the biomarker, at least 96% complementary to the biomarker, at least 97% complementary to the biomarker, at least 98% complementary to the biomarker, or at least 99% complementary to the biomarker.

(152) Further, the complementarity of the sensor strand to the biomarker may be matched to any 19-40 base segment of any variant of the mRNA sequence that encodes ANP (nppa), BNP (nppb), or MHCβ (myh7).

(153) In other embodiments, the sensor strand includes one of the sequences in Table 1 below:

(154) TABLE-US-00001 SEQ ID BIO- NO STRAND MARKER SEQUENCE 1 SENSOR MIR-5′ CGAAGAACGGAAAUCCCUGGCAAUGTGAT 23A- 3′ 3P 2 SENSOR MIR- 5′ CGAAGAACGGAAAUCCCTGGCAATGTGAU 23A- 3′ 3P 3 SENSOR MIR- 5′ GGAGAAGAACGGAAAUCCCUGGC**AAUGUGAU** 23A- 3′ 3P 4 SENSOR BNP 5′ AUCAGAAGCAGGUGUCUGCAGCC**AGGACUUC** 3′ (used with HDAC2 - 2) + 2 U 5 SENSOR BNP 5′ CUUGUGGAAUCAGAAGCAGGUGU**CUGCAGCC** 3′ 6 SENSOR BNP 5′ CAAAGGCGGCCACAGGGUUGAGG**AAAAAGCC** 3′ 7 SENSOR MHCβ/ 5′ AUCUUGAUCUGCUCAGCCCUGGA**GGUGCCAG** myh7 3′ 8 SENSOR ANP 5′ CAACAAGAUGACACAAAUGCAGCAGAGACCC 3′ 9 SENSOR ANP 5′ AUGACACAAAUGCAGCAGAGACCCCAGGGGA 3′ 10 SENSOR ANP 5′ CTUCACCACCUCUCAGTGGCAAUGCGACCAA 3′

(155) In certain embodiments, a Cond-siRNA for treating cardiac hypertrophy includes a guide strand designed to target a therapeutic RNAi target that is present and/or upregulated in heart cells (e.g., cardiac myocytes) and known to the field to ameliorate post-MI maladaptive hypertrophy, but whose systemic inhibition or expression may lead to unwanted side effects. In certain embodiments, therapeutic targets that may be used to design guide strands of Cond-siRNAs include, but are not limited to, Calcineurin [7-10] (or a subunit thereof, e.g., PPP3Ca, PPP3CB, PPP3CC, PPP3R1, PPP3R2), and HDAC2 [11, 12] or HDAC2 [11, 12]. In some aspects, the guide strand binds to an mRNA or an miRNA sequence that encodes the therapeutic target.

(156) HDAC2 guide strands. HDAC2 (Histone deacetylase 2) functions as a central regulator in transcriptional regulation, cell cycle progression and developmental pathways by modifying chromatin structure. HDAC2 inhibition represses the maladaptive regeneration program through a pathway involving GSK33, inhibiting the hypertrophic response. HDAC2 serves as a key cardiac hypertrophic regulator and a potential therapeutic target.

(157) In some embodiments, an HDAC2 RH TGS v2.1 guide strand was designed, wherein a 23 base-pair sequence was taken from the HDAC2 *Homo sapiens* mRNA based on past research utilizing HDAC2-targeted siRNAs. Two U base pairs added to the 3′ end. Four base pairs at 5′ end purposefully changed to mismatch the mRNA in order to prevent potential improper Dicer cleavage and RISC complex loading incorporation. An NCBI Blast check was also done to check for matches with other mRNAs. See HDAC2 guide strand structures, FIG. **15**

(158) Calcineurin guide strands. Calcineurin is a major promoter of cardiac hypertrophy, and inhibition of Calcineurin has been found to reduce hypertrophy. And, since it's always present in

ischemic cells, calcineurin is a good therapeutic target for the present invention.

(159) In some aspects, the RNAi targeting segment of the guide strand (i.e., bases 1-21 from the 3′ terminus) is fully complementary to any of the therapeutic targets discussed above (i.e., 100% complementary). In some aspects, the RNAi targeting segment of the guide strand (i.e., bases 1-21 from the 3′ terminus) is partially complementary to any of the therapeutic targets discussed above. For example, the sensor strand may be at least 70% complementary to the biomarker, at least 70% complementary to the biomarker, at least 75% complementary to the biomarker, at least 80% complementary to the biomarker, at least 85% complementary to the biomarker, at least 90% complementary to the biomarker, at least 95% complementary to the biomarker, at least 96% complementary to the biomarker, at least 97% complementary to the biomarker, at least 98% complementary to the biomarker, or at least 99% complementary to any of the therapeutic targets discussed above. In other embodiments, bases 14-20 from the 3′ terminus (the putative seed region of the guide strand) has at least 90% complementarity to the 3′ UTR of an mRNA sequence that encodes at least a portion of a subunit of calcineurin (e.g., PPP3Ca, PPP3CB, PPP3CC, PPP3R1, PPP3R2) or HDAC2.

(160) In certain embodiments, Cond-siRNAs that inhibit maladaptive hypertrophy are Cond-siRNAs that detect any biomarker from list A and target any member of list B in Table 2 below

(161) TABLE-US-00002 TABLE 2 List A-cardiomyocytes List B-anti-maladaptive biomarkers hypertrophy targets ANP (nppa) Calcineurin BNP (nppb) HDAC2 MHCβ (myh7) mir-23a-3p mir-125-5p mir-199b-5p

(162) Thus, according to some embodiments, the Cond-siRNA described herein may have a sensor strand that is mostly or completely complementary to a sequence of the RNA transcripts corresponding to biomarkers listed in A, and a guide strand that targets a member of list B in the manner described above.

(163) In other embodiments, the sensor strand includes one of the sequences in Table 3 below:

(164) TABLE-US-00003 SEQ THERA- ID PEUTIC NO STRAND TARGET SEQUENCE 11 GUIDE CALCI- 5′ CGA**G**UGUUGUUUGGCUUUUCCUGUU 3′ NEURIN (green: change from C to G) 12 GUIDE CALCI- 5′ CGAGUGUUGUUUGGCUUUUCCUGUU 3′ NEURIN 13 GUIDE HDAC2 5′ GCACUUAGAUUGAAACAACCCAGUU 3′ 14 GUIDE HDAC2 5′ UGUUAUCUGGUGU UAUUGACCGU 3′ 15 GUIDE HDAC2 5′ **CGAG**AUCUGGUGU UAUUGACCGU 3′ (4 bp of 5′ guide purposefully mismatched?)(used with BNP Sensor) 16 GUIDE HDAC2 5′ GCUCUUAGAUUGAAACAACCCAGUU3′

(165) In other embodiments, a Cond-siRNA for treating cardiac hypertrophy includes a core strand designed to connect the sensor to the guide strand according a method described above. IN certain aspects the core strand includes one of the sequences in Table 4 below:

(166) TABLE-US-00004 SEQ ID THERAPEUTIC NO STRAND TARGET SEQUENCE 17 CORE CALCINEURIN 5′ **CGUUCUUCUC C-linker-** CAGGAAAAGCCAAACAACA**C**UCG-linker-**GCCAGGGAUU UC** 3′ 18 CORE CALCINEURIN 5′ GUCAUCUUGUUG-linker- CAGGAAAAGCCAAACAACACUCG-linker- GCUGCAUUUGU 3′ 19 CORE CALCINEURIN 5′ AGGUGGUGAAG-linker- CAGGAAAAGCCAAACAACACUCG-linker- AUUGCCACUGAG 3′ 20 CORE HDAC2 5′ CGUUC UUCUC C CUGGGUUGUUUCAAUCUAAGUGC GCCAG GGAUU UC 3′ 21 CORE HDAC2 5′ CCUGCUUCUGAU-linker- ACGGUCAAUAACACCAGAUCUCG-linker- GGCUGCAGACA 3′ (used with BNP Sensor) 22 CORE HDAC2 5′ GAUUCCACAAG-linker- ACGGUCAAUAACACCAGAUCUCG- linker- ACACCUGCUUCU 3′ 23 CORE HDAC2 5′ GGCCGCCUUUG-linker- ACGGUCAAUAACACCAGAUCUCG-linker- CCUCAACCCUGU 3′ 24 CORE HDAC2 5′ GCAGAUCAAGAU-linker- ACGGUCAAUAACACCAGAUCUCG-linker- UCCAGGGCUGA 3′ 25 CORE HDAC2 5′

GUCAUCUUGUUGCUGGGUUGUUUCAAUCUAAGAGCGC UGCAUUUGU 3′ 26 CORE HDAC2 5′ CAUUUGUGUCAUUGUUAGAUUGAAACAACCCAGGGUCU CUGCUG 3′

(167) In some embodiments, the core strands above include 03 spacer linkers, where indicated, but any suitable linker may be used.

(168) In certain embodiments, a Cond-siRNA for treating cardiac hypertrophy is a construct that includes a guide strand, core strand, and sensor strand as indicated in Table 5 below (also see FIGS. **16**-**17**):

(169) TABLE-US-00005 SENSOR CORE GUIDE CONSTRUCT 1 BNP HDAC2 HDAC2 (SEQ ID (SEQ ID (SEQ ID NO: 4) NO 21) NO 15) CONSTRUCT 2 BNP HDAC2 HDAC2 (SEQ ID (SEQ ID (SEQ ID NO: 5) NO 22) NO 15) CONSTRUCT 3 BNP HDAC2 HDAC2 (SEQ ID (SEQ ID (SEQ ID NO 6) NO 23) NO 15) CONSTRUCT 4 MHCp/myh7 HDAC2 HDAC2 (SEQ ID (SEQ ID (SEQ ID NO 7) NO 24) NO 15) CONSTRUCT 5 ANP HDAC2 HDAC2 (SEQ ID (SEQ ID (SEQ ID NO 8) NO 25) NO 16) CONSTRUCT 6 ANP HDAC2 HDAC2 (SEQ ID (SEQ ID (SEQ ID NO 9) NO 26) NO 16) CONSTRUCT 7 ANP Calcineurin Calcineurin (SEQ ID (SEQ ID (SEQ ID NO 8) NO 18) NO 11) CONSTRUCT 8 ANP Calcineurin Calcineurin (SEQ ID (SEQ ID (SEQ ID NO 10) NO 19) NO 12) CONSTRUCT 9 MIR-23A-3P Calcineurin Calcineurin (SEQ ID (SEQ ID (SEQ ID NO: 1) NO 19) NO 12) CONSTRUCT 10 MIR-23A-3P Calcineurin Calcineurin (SEQ ID (SEQ ID (SEQ ID NO. 2) NO 19) NO 12))

(170) In other embodiments, the conditional siRNA complex may comprise any combination of one sensor strand, one guide strand, and one core strand selected from Tables 1, 3, and 4, respectively. Additional embodiments of the conditional siRNA complex showing the full complex may be found in Appendix A, which is attached hereto.

(171) Further, as described in the examples below, several mRNA and miRNA transcripts were screened under different pro-hypertrophic conditions both in vivo and in vitro, to assist with designing cond-siRNA complex strands.

(172) Methods of Treatment

(173) The cond-siRNA complexes described above may be used in methods to treat cardiac hypertrophy. Thus, in some embodiments, a method for treating cardiac hypertrophy is disclosed herein, wherein the method includes a step of administering to a subject a therapeutically effective amount of one or more of the cardiac hypertrophy-related cond-siRNAs described above. As disclosed herein, the subject may be any human or other animal suffering from post-MI cardiac hypertrophy, or any other type of cardiac hypertrophy.

(174) “Treating” or “treatment” of a condition may refer to preventing the condition, slowing the onset or rate of development of the condition, reducing the risk of developing the condition, preventing or delaying the development of symptoms associated with the condition, reducing or ending symptoms associated with the condition, generating a complete or partial regression of the condition, or some combination thereof.

(175) The methods for treating cardiac hypertrophy include administering a therapeutically effective amount of a therapeutic composition. An “effective amount,” “therapeutically effective amount” or “effective dose” is an amount of a composition (e.g., a therapeutic composition or agent) that produces a desired therapeutic effect in a subject, such as preventing or treating a target condition or alleviating symptoms associated with the condition. The precise therapeutically effective amount is an amount of the composition that will yield the most effective results in terms of efficacy of treatment in a given subject. This amount will vary depending upon a variety of factors, including but not limited to the characteristics of the therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine

experimentation, namely by monitoring a subject's response to administration of a compound and adjusting the dosage accordingly. For additional guidance, see Remington: The Science and Practice of Pharmacy 21st Edition, Univ. of Sciences in Philadelphia (USIP), Lippincott Williams & Wilkins, Philadelphia, PA, 2005.

(176) In some embodiments, one or more cond-siRNAs may be used alone or as part of a pharmaceutical composition for treating cardiac hypertrophy. Thus, in some embodiments, a pharmaceutical composition comprising any one or more of the cardiac hypertrophy-related Cond-siRNAs described above is disclosed. The therapeutic compositions may also include one or more pharmaceutically acceptable carriers. A “pharmaceutically acceptable carrier” refers to a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or some combination thereof. Each component of the carrier must be “pharmaceutically acceptable” in that it must be compatible with the other ingredients of the formulation. It also must be suitable for contact with any tissue, organ, or portion of the body that it may encounter, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

(177) The therapeutic compositions described herein may be administered by any suitable route of administration. A route of administration may refer to any administration pathway known in the art, including but not limited to aerosol, enteral, nasal, ophthalmic, oral, parenteral, rectal, transdermal (e.g., topical cream or ointment, patch), or vaginal. “Transdermal” administration may be accomplished using a topical cream or ointment or by means of a transdermal patch. “Parenteral” refers to a route of administration that is generally associated with injection, including infraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. In one embodiment the cardiac hypertrophy-related cond-siRNAs or therapeutic compositions thereof is administered by intracardial injection (FIG. **18**) to ensure local delivery to the heart tissue.

(178) Having described the invention with reference to the embodiments and illustrative examples, those in the art may appreciate modifications to the invention as described and illustrated that do not depart from the spirit and scope of the invention as disclosed in the specification. The examples are set forth to aid in understanding the invention but are not intended to, and should not be construed to limit its scope in any way. The examples do not include detailed descriptions of conventional methods. Such methods are well known to those of ordinary skill in the art and are described in numerous publications. Further, all references cited above and in the examples below are hereby incorporated by reference in their entirety, as if fully set forth herein. All appendices A-E submitted herewith constitute part of the complete disclosure.

EXAMPLES

Example 1: Prohypertrophic Gene and miRNA Expression Screening for Selection of Sensor Candidates for Treatment of Cardiac Hypertrophy

(179) Genes and miRNA that are upregulated under pathological cardiac hypertrophic conditions were screened for differential expression in mice and in NRVM under various conditions to determine which molecules are suitable candidates for use as a biomarker target for designing a sensor strand.

(180) Differential gene expression in tissues of wild type mice in homeostasis. Suitable pathological biomarker candidates should be differentially expressed in the heart as compared to other tissues to minimize off target effects. Several genes that are upregulated in pathological cardiac hypertrophy (DDiT4L, MYH7, ANP, BNP), as well as transcription factors that control their expression (MEF2C, Myocardin), were measured in normal heart, liver, lung, kidney, skeletal

muscle, and brains of wt mice. FIG. **19**. Several miRNA were also measured, as shown in 38
(181) Differential gene and miRNA expression in NRVM under hypoxic conditions. Suitable pathological biomarker candidates (ANP, BNP, MYH7, MEF2C, Myocardin, DDiT4L, and miRNAs) were screened in NRVM for differential expression under hypoxic conditions as shown in FIGS. **20**-**21**.
(182) This experiment mimics oxygen deprivation (hypoxia) during myocardial infarction (MI). NRVM cells were prepared cultured on standard petri dish substrates using techniques known to those who are skilled in the art [1,2]. Cultured NRVM cells are then exposed to hypoxic conditions (0.2% 02 atmosphere) for 24 hours at 37 C. This was followed by incubation for 12 hours under normal oxygen conditions (95% ambient air/5% CO2).
(183) Following incubation, NRVM cells were harvested and total RNA was extracted using protocols and kits standard in the art [3]. The messenger RNAs ANP, BNP, Myh7, MEF2C, Myocardin, DDiT4L, and the microRNAs mir-23a-3p, mir-125b-5p, mir-199b-5p, mir-208 and mir-195 were quantified by quantitative RT-PCR using standard methods appropriate for mRNAs4 and miRNAs [5].
(184) Results are presented in FIGS. **20**-**21** as fold change in copy numbers of mRNA or miRNA present in NRVM cells exposed to hypoxia compared to those incubated for 36 hours under normal oxygen conditions.
(185) The results of this experiment show that ANP, BNP, DDiT4L, mir-23a-3p, and mir-199b-5p, were over expressed by more than 4× under hypoxic conditions.
(186) Differential gene and miRNA expression in NRVM after phenylephrine treatment. Expression of miRNAs, and of ANP, BNP, MYH7, MEF2C, Myocardin, DDiT4L was measured in phenylephrine (PE) treated as compared to untreated NRVM.
(187) Phenylephrine stimulation is a standard method for studying hypertrophy in cardiomyocytes [6,7]. In this experiment, NRVM cells were prepared by standard protocols. Phenylephrine was then added to the culture media to 50 μM concentration for 24 hours. After 24 hours, cells were harvested for RNA isolation and analysis as described above.
(188) The results of the experiment show that ANP, BNP, Myh7, myocardin mRNA were overexpressed by more than 10× after PE stimulation (FIG. **22**), and that miRNAs, mir-23a-3p, mir-125b-5p, and mir-199b-5p were overexpressed by more than 4× after PE stimulation (FIG. **23**).
(189) Differential gene expression in mouse models of heart failure. Expression of ANP, BNP, MYH7, MEF2C, Myocardin, DDiT4L was measured in heart tissue of mice with non-ischemic heart failure in a thoracic aortic constriction (TAC) model and of mice with ischemic heart failure in an ischemia/reperfusion (I/R) model as compared to sham-treated mice.
(190) In experiments related to the ischemia/reperfusion (I/R) model, mice underwent procedures to simulate ischemic (deprivation of blood flow) heart failure. At day zero, mice were subject to ischemic heart failure via surgical clamping of a coronary artery for 20 min, followed by reperfusion. After 28 days, the experimental mice were sacrificed. Heart tissue was harvested and RNA was isolated using standard protocols as described above. mRNA and miRNAs were quantified using RT-PCR as described above. The mRNA and miRNA in the treated mice were compared with those found in control mice who were subjected to a sham procedure that did not involve clamping of the coronary artery to induce ischemia/reperfusion.
(191) The results show that all mRNAs tested were overexpressed by more than 10× in mice with ischemic HF (FIG. **24**). ANP and myh7 were overexpressed by more than 1000× (FIG. **24**). And for miRNAs, only mir-23a-3p was significantly overexpressed, by ˜15× (FIG. **25**).
(192) In experiments related to the non-ischemic HF model (TAC), mice underwent thoracic aortic constriction (TAC) procedures to induce non-ischemic heart failure. Briefly the upper thorax of mice constricted to reduce blood flow through the aorta for 28 days. This induced non-ischemic heart failure. After 28 days, the animals were sacrificed and heart tissue was harvested. RNA was isolated using standard protocols as described above. mRNA and miRNAs were quantified using

RT-PCR as described above. The mRNA and miRNA in the treated mice were compared with those found in control mice who were subjected to a sham procedure that did not involve aortic constriction.

(193) The results show that all mRNAs tested were overexpressed by more than 20× in the constricted mice (FIG. **24**). ANP and myh7 were overexpressed by more than 1000× (FIG. **24**). For miRNAs, all tested miRNAs were overexpressed by more than 10×. (FIG. **25**). And, mir-23a-3p was overexpressed by ˜80× (FIG. **25**).

Example 2: Design of ANP:Calcineurin Cond-siRNA

(194) The 5′ UTR and coding sequences of messenger RNAs are frequently occupied by mRNA binding proteins or transiting ribosomes. In mammals, miRNAs commonly bind at 3′ UTR sites to regulate mRNAs. Thus, binding sites in the 3′ UTR may be more accessible than sites in the 5′ UTR and the coding sequence of region of mRNAs.

(195) When designing sensors to detect mRNAs, it is desirable to start with sites in the 3′ UTR. If such sites cannot be found or there are other reasons (such as the need to detect a particular important sequence in the coding region or the 5′ UTR), then sensors can be designed to those sites.

(196) ANP 3′UTR sequencing results. Murine models were used to test the ability of ANP:Calcineurin Cond-siRNAs to inhibit phenylephrine induced cardiomyocytes hypertrophy. Thus, an ANP sensor was designed to target rat ANP.

(197) To design the ANP sensor, the 3′ UTR of ANP mRNA found in neonatal rat ventricular cardiomyocytes cells was sequenced by extracting RNA using standard procedures, amplifying the 3′ UTR via RT-PCR, and submitting the amplified DNA for sequencing by the Massachusetts General Hospital's DNA sequencing core.

(198) The sequenced DNA is as follows (N indicates indetermined base) ANP 3′UTR sequencing results from CCIB DNA Core (MGH) 293387-293389_D10_1_074_nppa_3_UTR_Ane.seq this is the forward sequence:

(199) TABLE-US-00006 (SEQ ID NO: 27)

TCAGCCANNNNNNNNNGAGCAGATCGCAAAAGATCCCAAGGCCTTGCG
GTGTGTCACACAGCTTGGTCGCATTGCCACTGAGAGGTGGTGAATACCC
TCCTGGAGCTGCAGCTTCCTGTCTTCATCTATCACGATCGATGTTAAGTG
TAGATGAGTGGTTTAGTGAGGCCTTACCTCTCCCACTCTGCATATTAAGG
TAGATCCTCACCCNNNNANNANNNNCNNNNNNNNNNNNNNNNNNNNN NNNNNNNN

(200) Using NCBI BLAST, it was determined that the central sequence

(201) TABLE-US-00007 (SEQ ID NO: 28)

GAGCAGATCGCAAAAGATCCCAAGGCCTTGCGGTGTGTCACACAGC**TTGG**
TCGCATTGCCACTGAGAGGTGGTGAATACCCTCCTGGAGCTGCAGCTTCC
TGTCTTCATCTATCACGATCGATGTTAAGTGTAGATGAGTGGTTTAGTGA
GGCCTTACCTCTCCCACTCTGCATATTAAGGTAGATCCTCACCC

(202) Is 99% homologous to *Rattus norvegicus* nppa mRNA 3′ UTR and 92% homologous to *Mus musculus* nppa mRNA 3′ UTR.

(203) Generation of Sensor Candidates:

(204) To generate candidate sensor strands, the reverse complement of the above sequence was determined.

(205) TABLE-US-00008 (SEQ ID NO: 29)

NNNNNNNNNNNNNNNNNNNNNNNNNNNNNGNNNNTNNTNNNNGGGTGAG
GATCTACCTTAATATGCAGAGTGGGAGAGGTAAGGCCTCACTAAACCACT
CATCTACACTTAACATCGATCGTGATAGATGAAGACAGGAAGCTGCAGCT
CCAGGAGGGTATTCACCACCTCTCAGTGGCAATGCGACCAAGCTGTGTGA
CACACCGCAAGG[custom character]
CTTGGGATCTTTTGCGATCTGCTCNNNNNNNNNTGGC TGA

(206) Then, a Python script was used to generate all possible consecutive 31 nt segments (8 nt for

toehold, plus 23 nt for duplex region) of the central bold sequence (rat nppa 3 prime utr sensor.xlsx). These are the initial possible sensor sequences for this region (T needs to be converted to U). The python script is attached herewith as Appendix B.

(207) The Python code that was generated performed following analyses for each sensor sequence: (i) add one demerit point for each occurrence of three or more consecutive Gs (eg: GGG, GGGG, GGGGG); (ii) add one demerit point for each occurrence of four or more consecutive A/Ts (eg: ATAT, AAAA, TTTT, TTAT, etc), (iii) calculate the percent of the sequence composed of G or Cs; and (iv) calculate the percent of the sequence accounted for by the most numerous three bases (e.g., out of A, G, C, T, if A, G and T are the most numerous in the sequence, what percent of bases are A, G or T).

(208) Then the list of possible sensors were ranked by the following criteria, in order of importance: (i) least number of demerit points, preferably 0′ (ii) highest 3 letteredness; and (iii) highest GC content (see, e.g., FIGS. **41**-**42**)

(209) The sequences on the ranked list were screened one by one for two qualities: (i) the hypothetical sensor duplex has high stability and correctness according to standard RNA secondary structure prediction codes; and (ii) the sensor strand has few significant matches to RNA transcripts other than nppa mRNA in rats and mice that extends from the toehold region (8 bases at 3′) into more than 50% of the duplex region (bases 9 to 31 from the 3′).

(210) The following sensor sequence was identified as favorable:

(211) TABLE-US-00009 (SEQ ID NO: 30)

A**TTCACCACCTCTCAGTGGCAATGCGACCAA**

(212) To further improve the thermodynamic stability of the sensor, the first base was changed at the 5′ from an A to a C. This gives the following sensor sequence:

(213) TABLE-US-00010 Sensor (mutated base underlined): (SEQ ID NO: 31)

CUUCACCACCUCUCAGUGGCAAUGCGACCAA

(214) On nupack, the hypothetical sensor duplex constructed from this sequence showed 8 thermodynamic stability, with an equilibrium concentration of 0.97 nM. Also see FIG. **26**.

(215) NCBI BLAST of the sensor sequence using “somewhat similar” settings showed no significant sequence matches other than to mouse and rat ANP (nppa) mRNA.

(216) Calcineurin is a heterodimer composed of one of three catalytic isozymes (PPP3CA, PPP3CB, PPP3CC) and one of two regulatory subunits (PPP3R1 and PPP3R2). To target Calcineurin, a guide sequence against the PPP3CA subunit of Calcineurin was identified that targets a widely conserved target site present in human, rat, and mice: UGUUGU UUGGCUU UUCCUG UU (SEQ ID NO:32)

(217) The segment CGAG was then added to the 5′ end to create a 23 nt guide strand, and then generated the core strand according to the previous stated rules. Those sequences are shown below:

(218) TABLE-US-00011 Guide: (SEQ ID NO: 11) CG AG UGUUGU UUGGC UU UUCCUG UU Sensor (mutated base underlined): (SEQ ID NO: 33) CUUCACCACCU CUCAGUGGCAAU GCGACCAA Core: (SEQ ID NO: 19) AGGUGGUGAAG-linker-CAGGAAAAGCCAAACAACACUCG-linker- AUUGCCACUGAG

(219) The guide strand plus the core strand showed good thermodynamic stability as predicted by Nupack (FIG. **27**)

(220) Then, chemical modifications were added according to schemes previously disclosed in U.S. Pat. No. 9,725,715, the subject matter of which is incorporated by reference herein. The final sequences are shown below:

(221) TABLE-US-00012 Sensor: (SEQ ID NO: 34) /5Sp9/mC*+T*mU*mC*+A*mC*mC*+A*mC*+C*mU*mC*mU*+C*mA *mG*+T*mG*+G*mC*mA*+A*mU*mG*mC*+G*mA*mC*mC*+A*mA*/ 3AmMO/ Guide: (SEQ ID NO: 35) /5AmMC6/+C*+G rArG rUrGrUrUrGrU rUrUrGrGrC rUrU rUrUrCrCrUrG rUrU Core: (SEQ ID NO: 36)

mArGmGrUrGrGrUrGrArArG/iSpC3/mC*+A*mGrGrArArArArGr CrCrArArArCrArArCrArCrUrC*mG/iSpC3/rArUrUrGrCrCrAr CrUrGrAmG
(222) The nucleotides and modifications are indicated as follows: (1)+A, +T, +C, +G are LNA; (2) mA, mU, mC, mG are 2′-O-methyl; (3) rA, rU, rC, rG are RNA; (4) * denotes phosphorothioate backbone connection; (5) /5Sp9/ is a tri-ethylene glycol linker; (6) /iSpC3/ is an internal C3 spacer; (7) /5AmMC6/ is a 5′ primary amine modification on a C6 linker; (8) /3AmMO/ is a 3′ primary amine modification.
Example 3: Design of mir-23a-3p:Calcineurin Cond-siRNA
(223) An mir-23a-3p sensor was designed as follows. The mir-23a entry for miRbase is found at the following URL: http://www.mirbase.org/cgi-bin/mirna entry.pl?acc=M10000079
(224) The sequence of mir-23a-3P sequence, 5′-3′ is:
(225) TABLE-US-00013 >hsa-miR-23a-3p MIMAT0000078 (SEQ ID NO: 37) AUCACAUUGCCAGGGAUUUCC
(226) The reverse complement of mir-23a-3p is GGAAAUCCCUGGCAAUGUGAU (SEQ ID NO:38)
(227) The Cond-siRNA sensor to sense a microRNA input is that the microRNA guide strand is usually only 21 nt long, whereas the Cond-siRNA sensor's duplex region is usually 23 nucleotides long and the toehold is usually 5 to 8 nucleotides long. This means that a microRNA guide strand is not long enough to completely displace the sensor strand from the core strand.
(228) This issue was solved by configuring the sensor strand so that the guide strand will displace the sensor from base-pairing with the 3′ overhang of the core strand and the last few bases at the 5′ terminus of 5′ overhang of the core strand.
(229) This way, the 3′ overhang of the core strand becomes unprotected and is degraded. The 5′ terminus of the 5′ overhang also becomes unprotected and subject to degradation, leading to eventual degradation of the entire 5′ overhang. This then allows the sensor strand to completely dissociate from the RNAi region.
(230) Thus, assuming a 21 nt miRNA guide strand, some of the possible geometries for the sensor strand, starting from the 3′ end, are shown in Table 6 below:
(231) TABLE-US-00014 Length bound Length bound Toehold to 3′ core to 5′ core Scheme length strand overhang strand overhang A 6 11 12 B 7 11 12 C 7 10 13 D 6 10 13 E 6 10 12 F 7 11 11
(232) In Table 6, scheme A gives a 23 bp sensor duplex and allows the miRNA to displace up to 4 terminal bases of the 5′ core strand segment, scheme B, 23 bp sensor, 3 terminal bases displaced, scheme C, 23 bp sensor, 4 terminal bases displaced, scheme D, 23 bp sensor, 5 terminal bases displaced, scheme E, 22 bp sensor, 5 terminal bases displaced, and scheme F, 22 bp sensor, 3 terminal bases displaced
(233) Calcineurin Sensor Design:
(234) Scheme F from Table 6 was used to design the following calcineurin sensor:
(235) TABLE-US-00015 (SEQ ID NO: 39) 5’
C**GAAGAAC**<u>**GGAAAUCCCUGGCAAUGUGAU**</u> 3’
(236) A sequence: CGAAGAAC (SEQ ID NO:40) is added to the 5′ of the sensor. This sequence is designed to improve thermodynamic stability of the sensor duplex, minimize secondary structure in the sensor strand, and minimize overlap with non-mir23a-3p transcripts.
(237) According to NCBI BLAST, the sensor has no significant unintended matches to human RNA transcripts, and there are only a few significant unintended matches to mouse RNA transcripts.
(238) The same Calcineurin PPP3CA siRNA guide identified for the ANP: Calcineurin construct above was used and the core strand sequence was generated using the algorithms described herein.
(239) TABLE-US-00016 Core: (SEQ ID NO: 41) UCCGUUCUUCG-linker-CAGGAAAAGCCAAACAACACUCG-linker- UGCCAGGGAUU
(240) The guide strand plus the core strand showed good thermodynamic stability as predicted by

Nupack (FIG. **28**), as did the hypothetical sensor duplex (FIG. **29**).
(241) Thus, the final fully modified sequences are as follows:
(242) TABLE-US-00017 Calcineurin guide: (SEQ ID NO: 42) C6Amine+C*+GrArG rUrGrUrUrGrU rUrUrGrGrC rUrU rUrUrCrCrUrG rUrU Mir-23a-3p sensor using a 22 bp sensor duplex with LNA pattern: (SEQ ID NO: 43) /5Sp9/mC*+G*mA*+A*mG*mA+A*mC *+G*mG*mA*+A*mA*mU* mC*mC*+C*mU*mG*+G*mC*mA*+A*mU*mG*+T*mG*+A*+T*/ 3AmMO/ Core strand: (SEQ ID NO: 44) mUrCrCrGrUrUrCrUrUrCrG/iSpC3/mC*+A*mGrGrArArArArGr CrCrArArArCrArArCrArCrUrC*mG/iSpC3/rUrGrCrCrArGrGr G mA rU mU
(243) The nucleotides and modifications are indicated as follows: (1)+A, +T, +C, +G are LNA; (2) mA, mU, mC, mG are 2′-O-methyl; (3) rA, rU, rC, rG are RNA; (3) * denotes phosphorothioate backbone connection; (4) /5Sp9/ is a tri-ethylene glycol linker; (5) /iSpC3/ is an internal C3 spacer; (6) /5AmMC6/ is a 5′ primary amine modification on a C6 linker; (7) /3AmMO/ is a 3′ primary amine modification.
Example 4: Synthesis and Testing of Cond-siRNA Constructs
(244) To demonstrate use of Cond-siRNAs to inhibit hypertrophy of cardiomyocytes, Cond-siRNAs were designed and synthesized to detect murine ANP or mir-23a-3p and inhibit calcineurin. The constructs are shown in FIGS. **16** and **17**. All strands were purchased from a commercial oligonucleotide vendor (Exiqon Inc, now a part of Qiagen).
(245) Assembly and purification. Sensor, core, and guide strands were mixed at 1.0:1.1:1.0 ratios at 50 nM to 1 uM strand concentrations and underwent thermal annealing in 1×PBS buffer (80 C for 30 seconds followed by constant temperature incubation at 50 C to 60 C for −1 hour followed by cooling to room temperature).
(246) Where purification was desired, constructs were annealed at 500 nM, loaded at 20 uL per well in 10% non-denaturing PAGE. Run in 1×TBE buffer at 120V for 90 min. The correct bands were excised. The Cond-siRNA constructs were then extracted via the crush and soak method using standard RNA isolation kits.
(247) FIG. **30** shows an example gel where Cond-siRNAs were assembled and purified.
(248) Dual luciferase assays of mir-23a-3p:calcineurin Cond-siRNA (FIG. **31**). For this assay, the Cond-siRNA described above was assessed for its ability of to keep RNAi activity OFF in the absence of the correct biomarker and switching RNAi ON in the presence of RNA transcripts bearing the mir-23a-3p sequence. For this test, unpurified mir-23a-3p:calcineurin Cond-siRNAs was co-transfected at the indicated concentrations into human Hek 293 cells, along activator and dual luciferase plasmids.
(249) The activator plasmids expressed either a null transcript, a transcript with an incorrect activator, the 21 base mir-23a-3p sequence, or a longer sequence that was complementary to the entire sensor strand.
(250) The dual luciferase plasmid encoded Firefly luciferase as the control and a Renilla luciferase with the calcineurin target site in its 3′ UTR as the target of RNAi.
(251) The results show that this Cond-siRNA had significantly increased RNAi activity against the calcineurin target when either the mir-23a-3p sequence or the fully matching sequence was expressed. FIG. **31**. Thus, this construct should be able to activation RNAi activity against calcineurin in the presence of mir-23a-3p.
(252) in vitro experiment for purified ANP:calcineurin Cond-siRNA in NRVM cells under PE stimulation. For this experiment, it was tested whether the murine ANP calcineurin Cond-siRNA could detect overexpression of ANP upon phenylephrine (PE) stimulation, and activate RNAi knockdown against calcineurin.
(253) The biological effects of ANP:calcineurin against murine biomarkers and targets is tested because there are no suitable human models to test against. The biological effect of this murine oriented Cond-siRNA should be representative of biological effects that Cond-siRNA configured

for humans would have.
(254) For this experiment, NRVM cells were incubated using standard protocols under normal conditions (95% air, 5% CO2, 37 C). The purified ANP: calcineurin Cond-siRNA was transfected at 20 nM concentration into NRVM cells using RNAiMax. The transfected cells were incubated for 24 hours. PE was then added to the media to 50 μM final concentration. After a further 48 hours, cells were harvested and stained or processed for RNA isolation.
(255) The results for RT-PCR quantitation of calcineurin mRNA are shown in FIG. **32**. For each cohort (untreated and PE treated cells), the level of calcineurin mRNA observed in cells transfected with scrambled siRNA (negative control) was normalized to 1.0. In the untreated cohort, cells transfected with ANP:Calcineurin Cond-siRNA had no detectable knockdown of calcineurin. This means that, as intended, the Cond-siRNA has very little RNAi activity in normal cells.
(256) In cells treated with PE, the Cond-siRNA activated RNAi, and reduced calcineurin mRNA levels by ˜ 50% compared with levels seen in cells transfected with the scrambled siRNA control. This shows that the ANP:calcineurin Cond-siRNA can detect overexpression of ANP mRNA, and respond with RNAi inhibition of calcineurin as intended.
(257) The results of imaging of the treated and untreated cells by fluorescence microscopy are shown in FIG. **33**. The results show that cells treated with purified ANP:calcineurin Cond-siRNAs underwent less hypertrophy than cells treated with scrambled siRNA. Furthermore, the effects of treatment with Cond-siRNAs was similar to treatment with the positive control (commercial, non-conditional calcineurin siRNA).
(258) The results of cell size quantitation using fluorescence microscopy are shown in FIG. **34**. The results show that PE stimulation resulting in the increase in the average cell size from ˜750 μm2 to ˜1300 μm2 in cells treated with the negative control scrambled siRNA. However, in cells treated with the Cond-siRNA, average cell size increased from ˜750 μm2 to ˜900 μm2, and was not statistically significant. This result is similar to the non-conditional, commercial calcineurin siRNA.
(259) The above results show that the ANP:Calcineurin Cond-siRNA has low background RNAi activity, can detect and respond to PE stimulation of NRVM cells, and has significant biological effects on reducing hypertrophy of NRVM cells.
Example 5: Exemplary Guide and Sensor Strand Sequences
(260) Below are examples of automatically generating core strand sequences from guide and sensor strand sequences. These have 23 bp sensor duplexes with 8 base toeholds
(261) Example 5a, randomly chosen human/rat PPP3CA mRNA guide paired with randomly chosen human NPPA sensor
(262) TABLE-US-00018 Cond-siRNA Guide: (SEQ ID NO: 45) CCAC UUUACCAGCAUCUCAGUCAUU Cond-siRNA Sensor: (SEQ ID NO: 46) GGAGAGGCGAGGAAGUCACCAUCAAACCACU Core is: (SEQ ID NO: 47) CUCGCCUCUCC UGACUGAGAUGCUGGUAAAGUGGGAUGGUGACUUC
(263) Example 5b, human/rat PPP3CA mRNA guide above paired with randomly chosen human NPPB sensor
(264) TABLE-US-00019 Cond-siRNA Guide: (SEQ ID NO: 45) CCAC UUUACCAGCAUCUCAGUCAUU Cond-siRNA Sensor: (SEQ ID NO: 48) GGAAUCAGAAGCAGGUGUCUGCAGCCAGGAC Core is: (SEQ ID NO: 49) CUUCUGAUUCC UGACUGAGAUGCUGGUAAAGUGGUGCAGACACCUG
(265) Example 5c, human/rat PPP3CA mRNA guide above paired with randomly chosen human Myh7 sensor
(266) TABLE-US-00020 Cond-siRNA Guide: (SEQ ID NO: 45) CCAC UUUACCAGCAUCUCAGUCAUU Cond-siRNA Sensor: (SEQ ID NO: 50) CCAAGGAGCUGUUACACAGGCUCCAGCAUGG Core is: (SEQ ID NO: 51) CAGCUCCUUGG UGACUGAGAUGCUGGUAAAGUGGGAGCCUGUGUAA

(267) Example 5d, randomly chosen human/rat HDAC2 mRNA guide paired with human NPPA sensor from example 1

(268) TABLE-US-00021 Cond-siRNA Guide: (SEQ ID NO: 52) CCAC UUCAUCACAAGCUAUCCGCUU Cond-siRNA Sensor: (SEQ ID NO: 46) GGAGAGGCGAGGAAGUCACCAUCAAACCACU Core is: (SEQ ID NO: 53) CUCGCCUCUCC GCGGAUAGCUUGUGAUGAAGUGGGAUGGUGACUUC

(269) Example 5e, randomly chosen human/rat HDAC2 mRNA guide above paired with human NPPB sensor from example 2

(270) TABLE-US-00022 Cond-siRNA Guide: (SEQ ID NO: 52) CCAC UUCAUCACAAGCUAUCCGCUU Cond-siRNA Sensor: (SEQ ID NO: 48) GGAAUCAGAAGCAGGUGUCUGCAGCCAGGAC Core is: (SEQ ID NO: 54) CUUCUGAUUCC GCGGAUAGCUUGUGAUGAAGUGGUGCAGACACCUG

(271) Example 5f, randomly chosen human/rat HDAC2 mRNA guide above paired with human Myh7 sensor from example 3

(272) TABLE-US-00023 Cond-siRNA Guide: (SEQ ID NO: 52) CCAC UUCAUCACAAGCUAUCCGCUU Cond-siRNA Sensor: (SEQ ID NO: 50) CCAAGGAGCUGUUACACAGGCUCCAGCAUGG Core is: (SEQ ID NO: 55) CAGCUCCUUGG GCGGAUAGCUUGUGAUGAAGUGG GAGCCUGUGUAA

(273) In the next examples, we reuse the guide from examples 1-6, but choose miRNA sensors. The sensors are configured as 22 bp duplexes with 7 base overhangs and symmetric 11 base core strand overhangs

(274) Example 5g, randomly chosen human/rat PPP3CA mRNA guide paired with mir-23a-3p sensor

(275) TABLE-US-00024 Cond-siRNA Guide: (SEQ ID NO: 45) CCAC UUUACCAGCAUCUCAGUCAUU Cond-siRNA Sensor: (SEQ ID NO: 39) CGAAGAAC GGAAAUCCCUGGCAAUGUGAU Core is: (SEQ ID NO: 56) UCCGUUCUUCG UGACUGAGAUGCUGGUAAAGUGG UGCCAGGGAUU

(276) Example 5h, human/rat PPP3CA mRNA guide above paired with mir-125b-5p sensor

(277) TABLE-US-00025 Cond-siRNA Guide: (SEQ ID NO: 45) CCAC UUUACCAGCAUCUCAGUCAUU Cond-siRNA Sensor: (SEQ ID NO: 57) CGACAGU UCACAAGUUAGGGUCUCAGGGA Core is: (SEQ ID NO: 58) GUGAACUGUCG UGACUGAGAUGCUGGUAAAGUGG GACCCUAACUU

(278) Example 5i, human/rat PPP3CA mRNA guide above paired with mir-195b-5p sensor

(279) TABLE-US-00026 Cond-siRNA Guide: (SEQ ID NO: 45) CCAC UUUACCAGCAUCUCAGUCAUU Cond-siRNA Sensor: (SEQ ID NO: 59) CCUGAA GAACAGAUAGUCUAAACACUGGG Core is: (SEQ ID NO: 60) UGUUCUUCAGG UGACUGAGAUGCUGGUAAAGUGG UUUAGACUAUC

(280) Example 5j, randomly chosen human/rat HDAC2 mRNA guide paired with mir-23a-3p sensor

(281) TABLE-US-00027 Cond-siRNA Guide: (SEQ ID NO: 52) CCAC UUCAUCACAAGCUAUCCGCUU Cond-siRNA Sensor: (SEQ ID NO: 39) CGAAGAAC GGAAAUCCCUGGCAAUGUGAU Core is: (SEQ ID NO: 61) AACAGCUCCUUGG GCGGAUAGCUUGUGAUGAAGUGG GGAGCCUGUGU

(282) Example 5k, randomly chosen human/rat HDAC2 mRNA guide above paired with mir-125b-5p sensor

(283) TABLE-US-00028 Cond-siRNA Guide: (SEQ ID NO: 52) CCAC UUCAUCACAAGCUAUCCGCUU Cond-siRNA Sensor: (SEQ ID NO: 57) CGACAGU UCACAAGUUAGGGUCUCAGGGA Core is: (SEQ ID NO: 62) UCCGUUCUUCG GCGGAUAGCUUGUGAUGAAGUGG UGCCAGGGAUU

(284) Example 5l, randomly chosen human/rat HDAC2 mRNA guide above paired with mir-195b-

5p sensor
(285) TABLE-US-00029 Cond-siRNA Guide: (SEQ ID NO: 52) CCAC UUCAUCACAAGCUAUCCGCUU Cond-siRNA Sensor: (SEQ ID NO: 59) CCUGAA GAACAGAUAGUCUAAACACUGGG Core is: (SEQ ID NO: 63) UGUUCUUCAGG GCGGAUAGCUUGUGAUGAAGUGG UUUAGACUAUC
Example 6: Exemplar siRNAs Designed by a Commercial siRNA Automated Design Website
(286) From: http://dharmacon.horizondiscovery.com/design-center/
(287) For each category below, the design tool was tasked with designing siRNAs targeting the protein coding region of both the human and the rat mRNA. The top three candidates are shown. Cond-siRNA guides are made by adding 4 G/C rich bases to the 5′ of the antisense strand.
(288) Candidate siRNA guide strands sequences (antisense) and corresponding target sites (sense) targeting both Human (NM_000944) and Rat (NM_017041) PPP3CA mRNA
(289) TABLE-US-00030 Sense: (SEQ ID NO: 64) 5′ G.A.A.C.A.A.G.A.U.C.C.G.A.G.C.A.A.U.A.U.U 3′ Antisense: (SEQ ID NO: 65) 5′ U.A.U.U.G.C.U.C.G.G.A.U.C.U.U.G.U.U.C.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 66) 5′ CGACU.A.U.U.G.C.U.C.G.G.A.U.C.U.U.G.U.U.C.U.U 3′ Sense: (SEQ ID NO: 67) 5′ U.G.A.C.U.G.A.G.A.U.G.C.U.G.G.U.A.A.A.U.U 3′ Antisense: (SEQ ID NO: 68) 5′ U.U.U.A.C.C.A.G.C.A.U.C.U.C.A.G.U.C.A.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 69) 5′ CGACU.U.U.A.C.C.A.G.C.A.U.C.U.C.A.G.U.C.A.U.U 3′ Sense: (SEQ ID NO: 70) 5′ G.G.U.C.A.G.A.A.G.A.A.G.A.U.G.G.A.U.U.U.U 3′ Antisense: (SEQ ID NO: 71) 5′ A.A.U.C.C.A.U.C.U.U.C.U.U.C.U.G.A.C.C.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 72) 5′ CCACA.A.U.C.C.A.U.C.U.U.C.U.U.C.U.G.A.C.C.U.U 3′
(290) Candidate siRNA guide strands sequences (antisense) and corresponding target sites (sense) targeting both Human (NM_001142353) and Rat (NM_017042) PPP3CB mRNA
(291) TABLE-US-00031 Sense: (SEQ ID NO: 73) 5′ G.C.U.A.U.A.G.A.A.U.G.U.A.C.A.G.A.A.A.U.U 3′ Antisense: (SEQ ID NO: 74) 5′ U.U.U.C.U.G.U.A.C.A.U.U.C.U.A.U.A.G.C.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 75) 5′ CGACU.U.U.C.U.G.U.A.C.A.U.U.C.U.A.U.A.G.C.U.U Sense: (SEQ ID NO: 76) 5′ C.C.U.U.U.A.A.G.C.A.G.G.A.A.U.G.U.A.A.U.U 3′ Antisense: (SEQ ID NO: 77) 5′ U.U.A.C.A.U.U.C.C.U.G.C.U.U.A.A.A.G.G.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 78) 5′ GGACU.U.A.C.A.U.U.C.C.U.G.C.U.U.A.A.A.G.G.U.U Sense: (SEQ ID NO: 79) 5′ G.C.A.A.U.U.G.G.C.A.A.G.A.U.G.G.C.A.A.U.U 3′ Antisense: (SEQ ID NO: 80) 5′ U.U.G.C.C.A.U.C.U.U.G.C.C.A.A.U.U.G.C.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 81) 5′ CCACU.U.G.C.C.A.U.C.U.U.G.C.C.A.A.U.U.G.C.U.U
(292) Candidate siRNA guide strands sequences (antisense) and corresponding target sites (sense) targeting both Human (NM_001243974) and Rat (NM_134367) PPP3CB mRNA
(293) TABLE-US-00032 Sense: (SEQ ID NO: 82) 5′ G.U.A.U.A.G.A.G.U.G.U.G.U.G.C.U.G.U.A.U.U 3′ Antisense: (SEQ ID NO: 83) 5′ U.A.C.A.G.C.A.C.A.C.A.C.U.C.U.A.U.A.C.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 84) 5′ CCACU.A.C.A.G.C.A.C.A.C.A.C.U.C.U.A.U.A.C.U.U 3′ Sense: (SEQ ID NO: 85) 5′ A.G.U.A.U.U.U.G.A.G.A.A.U.G.G.G.A.A.A.U.U 3′ Antisense: (SEQ ID NO: 86) 5′ U.U.U.C.C.C.A.U.U.C.U.C.A.A.A.U.A.C.U.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 87) 5′ CCACU.U.U.C.C.C.A.U.U.C.U.C.A.A.A.U.A.C.U.U.U 3′ Sense: (SEQ ID NO: 88) 5′ C.U.A.U.G.U.G.G.A.C.A.G.A.G.G.C.U.A.U.U.U 3′ Antisense: (SEQ ID NO: 89) 5′ A.U.A.G.C.C.U.C.U.G.U.C.C.A.C.A.U.A.G.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 90) 5′ CCACA.U.A.G.C.C.U.C.U.G.U.C.C.A.C.A.U.A.G.U.U 3′
(294) Candidate siRNA guide strands sequences (antisense) and corresponding target sites (sense) targeting both Human (NM_001527) and Rat (NM_053447) HDAC2 mRNA
(295) TABLE-US-00033 Sense: (SEQ ID NO: 91) 5′ G.C.G.G.A.U.A.G.C.U.U.G.U.G.A.U.G.A.A.U.U 3′ Antisense: (SEQ ID NO: 92) 5′

U.U.C.A.U.C.A.C.A.A.G.C.U.A.U.C.C.G.C.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 52) 5′ CCACU.U.C.A.U.C.A.C.A.A.G.C.U.A.U.C.C.G.C.U.U 3′ Sense: (SEQ ID NO: 93) 5′ G.G.A.U.A.U.U.G.G.U.G.C.U.G.G.A.A.A.A.U.U 3′ Antisense: (SEQ ID NO: 94) 5′ U.U.U.U.C.C.A.G.C.A.C.C.A.A.U.A.U.C.C.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 95) 5′ CCACU.U.U.U.C.C.A.G.C.A.C.C.A.A.U.A.U.C.C.U.U 3′ Sense: (SEQ ID NO: 96) 5′ A.A.G.C.A.G.A.U.G.C.A.G.A.G.A.U.U.U.A.U.U 3′ Antisense: (SEQ ID NO: 97) 5′ U.A.A.A.U.C.U.C.U.G.C.A.U.C.U.G.C.U.U.U.U 3′ Cond-siRNA Guide: (SEQ ID NO: 98) 5′ CCACU.A.A.A.U.C.U.C.U.G.C.A.U.C.U.G.C.U.U.U.U 3′

Example 7: Example Designs for miRNA Sensor Strands

(296) Example designs for miRNA sensor strands are shown below.

(297) miRNAs have highly conserved sequences across mammalian species. Therefore, we can design a single miRNA sensor for all test animals, including humans.

(298) For each sequence, we first take the reverse complement of the guide sequence, then add 8 bases to create a 29 nt sensor.

(299) TABLE-US-00034 >hsa-miR-23a-3p MIMAT0000078 (SEQ ID NO: 37) AUCACAUUGCCAGGGAUUUCC > reverse complement (SEQ ID NO: 38) GGAAAUCCCUGGCAAUGUGAU > Sensor, add 8 bases to make 29 mer (SEQ ID NO: 39) CGAAGAAC GGAAAUCCCUGGCAAUGUGAU

(300) Nupack shows minimum secondary structure and no self-self base-pairing accept

(301) TABLE-US-00035 >hsa-miR-125b-5p MIMAT0000423 (SEQ ID NO: 99) UCCCUGAGACCCUAACUUGUGA > reverse complement (SEQ ID NO: 100) UCACAAGUUAGGGUCUCAGGGA > Sensor, add 7 bases to make 29 mer, (SEQ ID NO: 57) CGACAGU UCACAAGUUAGGGUCUCAGGGA

(302) Use secondary structure prediction codes make sure that the secondary structure is relatively open. If not, change the added bases and try again

(303) TABLE-US-00036 >hsa-miR-199b-5p MIMAT0000263 (SEQ ID NO: 101) CCCAGUGUUUAGACUAUCUGUUC > reverse complement (SEQ ID NO: 102) GAACAGAUAGUCUAAACACUGGG > Sensor, add 6 bases to make 29 mer, (SEQ ID NO: 59) CCUGAA GAACAGAUAGUCUAAACACUGGG

Acceptably Low Secondary Structure

Example 8: miRNAs

(304) Additional miRNAs are shown below:

(305) TABLE-US-00037 >rno-miR-23a-3p MIMAT0000792 (SEQ ID NO: 37) AUCACAUUGCCAGGGAUUUCC >hsa-miR-23a-3p MIMAT0000078 (SEQ ID NO: 37) AUCACAUUGCCAGGGAUUUCC >rno-miR-23a-3p MIMAT0000792 (SEQ ID NO: 37) AUCACAUUGCCAGGGAUUUCC >mmu-miR-125b-5p MIMAT0000136 (SEQ ID NO: 99) UCCCUGAGACCCUAACUUGUGA >hsa-miR-125b-5p MIMAT0000423 (SEQ ID NO: 99) UCCCUGAGACCCUAACUUGUGA >rno-miR-125b-5p MIMAT0000830 (SEQ ID NO: 99) UCCCUGAGACCCUAACUUGUGA >hsa-miR-199b-5p MIMAT0000263 (SEQ ID NO: 101) CCCAGUGUUUAGACUAUCUGUUC >mmu-miR-199b-5p MIMAT0000672 (SEQ ID NO: 103) CCCAGUGUUUAGACUACCUGUUC

Example 9: Design Review for Sensor Strand miR-23a-3p with Calcineurin and HDAC2 Targets

(306) FIG. **46** illustrates the design of sensor miR-23a-3p. FIG. **47** illustrates that the sequence of the sensor strand was checked against NCBI by blast. FIG. **48** illustrates that calcineurin and HDAC2 guide strand sequences are checked against NCBI by blast as well. FIG. **49** illustrates the secondary structure and MFE structure at 37° C. of the full miR-23a-3p sensor strand with toehold for calcineurin or HDAC2. The sensor has the following sequence, with toe hold shown in bold and underlined:

(307) TABLE-US-00038 (SEQ ID NO: 3) 5′ GGAGA AGAAC G (nick) GAAA UCCCU GGC**AA UGUGAU** 3′ (31 bp).

The Nupack analysis was performed on the sensor strand. The exiqon code with LNA modifications is shown as follows:

(308) TABLE-US-00039 (SEQ ID NO: 109) 5′ G + GA + GA + AG + AA + C G G + AA + A TC + CCT + GGC + AA + TGT + G + A + T 3′.

(309) Calcineurin is a protein phosphatase and is composed of two subunits: PPP3CA (catalytic) and PPP3R1 (regulatory). Thermo Fisher has an siRNA for this protein (PPP3CA) beginning at base pair 1549 (www.thermofisher.com/order/genome-database/browse/sirna/keyword/s72075). The guide and core strand sequences are as follows:

(310) Guide (calcineurin target of 19 bp), starting from 1549: 5′ CGAG UGUUG UUUGG CUUUU CCUG UU 3′ (SEQ ID NO: 11, mutation from C to G is shown in bold and underlined);

(311) TABLE-US-00040 Core strand: (SEQ ID NO: 17) 5′ CGUUC UUCUC C CAGGA AAAGC CAAAC AACAC UCG GCCAG GGAUU UC 3′.

(312) FIG. **50** shows that NuPack analyses of miR-23a-3p sensor strand for calcineurin were performed on core (FIG. **50**A), guide (FIG. **50**B), Sensor with two small overhangs of core: 97% (FIG. **50**C), and calcineurin guide with core: 100% (FIG. **50**D).

(313) FIG. **51** shows NCBI check for calcineurin guide strand vs. human constructs. The matches of primary concern include: *Homo sapiens* poly(ADP-ribose) polymerase family member 14 (PARP14), transcript variant X2, mRNA (20/21 plus/minus match); *Homo sapiens* uncharacterized LOC105374732 (LOC105374732), ncRNA (15 bp plus/minus match); *Homo sapiens* zinc finger FYVE-type containing 16 (ZFYVE16), transcript variant X19, mRNA (15 bp plus/minus match); and *Homo sapiens* GC-rich promoter binding protein 1 (GPBP1), transcript variant X11, misc_RNA (15 bp plus/minus match).

(314) The guide of HDAC2 was from S100434959 (www.qiagen.com/us/shop/rnai/flexitube-sirna/?catno=S100434952#orderinginformation), having the following sequence: 5′ GC ACUUA GAUUG AAACA ACCCA GUU 3′ (25 bp) (SEQ ID NO: 13). The core with HDAC2 target has the following sequence, with short overhangs shown in bold and underlined: 5′ CGUUC UUCUC C CUGGGUUGUUUCAAUCUAAGUGC GCCAG GGAUU UC 3′ (SEQ ID NO: 20).

(315) FIG. **52** shows that NuPack analyses of miR-23a-3p sensor strand for HDAC2 were performed on core (FIG. **52**A), guide (FIG. **52**B), HDAC2 guide with core: 100% (FIG. **52**C), and sensor with core overhangs: 97% (FIG. **52**D).

(316) FIG. **53** shows NCBI check for HDAC2 guide strand vs. human transcripts. The matches of primary concern include: *Homo sapiens* solute carrier family 35 member F5 (SLC35F5), transcript variant X6, mRNA (15 bp plus/minus match); and PREDICTED: *Homo sapiens* aquaporin 12B (AQP12B), transcript variant X16, misc_RNA (14 bp plus/minus match).

Example 10: Design Review for HDAC2 Targeted Conditional siRNA Constructs with Signals of BNP and MYH7

(317) HDAC2 target guide sequence was designed as follows. HDAC2 siRNA was disclosed in published literature: www.nature.com/cddis/journal/v8/n3/extref/cddis201749x1.docx. The sequence was checked against the HDAC2 mRNA sequence from NCBI: www.ncbi.nlm.nih.gov/nuccore/NM_001527.3. A given DNA sequence was made into an RNA sequence, and then taken the reverse complement for the guide strand, starting at bp 518 on HDAC2 mRNA:

(318) TABLE-US-00041 (SEQ ID NO: 111) 5′ ACG GTCAATAAGA CCAGATAACA 3′; (SEQ ID NO: 112) 5′ ACG GUCAAUAACA CCAGAUAACA 3′;

(319) Guide target HDAC2: 5′ UGU UAUCUGGUGU UAUUGACCGU 3′ (SEQ ID NO: 14); and then 4 bp of 5′ guide were purposefully mismatched: 5′ CGAG AUCUGGUGU UAUUGACCGU 3′ (SEQ ID NO: 15).

(320) FIG. **54** illustrates the check of guide vs. NCBI human transcripts and sequence alignment.

(321) Three BNP candidates were selected. The first BNP candidate had an mRNA sequence source from www.ncbi.nlm.nih.gov/nuccore/83700236. The sequence starting from 3′ UTR of BNP mRNA is as follows, with the 31 bp sequence used for reference for sensor strand shown in bold and underlined:

(322) TABLE-US-00042 (SEQ ID NO: 121)

GAG**GAAGUCCUGGCUGCAGACACUGCUUCUGAU**UCCACAAGGGGCUUUUU CCUCAACCCUGUGCCGCCUUUGAAGUGACUCAUUUUUUUUAAUGUAUUUAU GAUUUAUUUGAUUGUUUUAUAUAAGAUGGUUUCUUACCUUUGAGCACAAA AUUUCCACGGGAAAUAAAGUCAACAUUAU AAGCUUUAAAAAAAAAAA.

(323) The BNP sensor was designed by taking the reverse complement of the bold and underlined portion of SEQ ID NO: 121. The sequence is as follows, with the 8 bp toehold shown in bold and underlined:

(324) TABLE-US-00043 (SEQ ID NO: 4)

AUCAGAAGCAGGUGUCUGCAGCC**AGGACUUC**.

The Nupack assessment was performed (www.nupack.org/partition/histogram_detail/1166536?token=PcTqQEaZRt&strand_id=0) and the MFE structure of SEQ ID NO: 4 is shown in FIG. **55**.

(325) FIG. **56** shows the BNP sensor sequence (SEQ ID NO: 4) together with core and guide sequences. FIGS. **57**A and **57**B show Nupack analyses of BNP sensor (SEQ ID NO: 4) with overhangs, and guide with core, respectively. The revised guide strand with the first 4 bp of 5′ end with CGAG to provide mismatching in case of incorrect Dicer cleavage and RISC complex loading. The modified guide has 2 U overhang: 5′ CGAG AUCUGGUGUU AUUGACCGUUU 3′ (SEQ ID NO: 4). The modified core has the following sequence: CCUGCUUCUGAUACGGUCAAUAACACCAGAUCUCGGGCUGCAGACA (SEQ ID NO: 122). FIG. **58** shows NCBI check of BNP sensor vs. human transcripts.

(326) The design of the second BNP candidate was similar to the first except that the 31 bp sequence used for reference for sensor strand was a different portion from SEQ ID NO: 121 shown in bold and underlined:

(327) TABLE-US-00044 (SEQ ID NO: 121)

GAGGAAGUCCU**GGCUGCAGACACUGCUUCUGAUUCCACAAG**GGGCUUUUU CCUCAACCCUGUGCCGCCUUUGAAGUGACUCAUUUUUUUUAAUGUAUUUAU GAUUUAUUUGAUUGUUUUAUAUAAGAUGGUUUCUUACCUUUGAGCACAAA AUUUCCACGGGAAAUAAAGUCAACAUUAU AAGCUUUAAAAAAAAAAA.

(328) The sequence of the second BNP sensor is as follows, with the 8 bp toehold shown in bold and underlined:

(329) TABLE-US-00045 (SEQ ID NO: 5)

CUUGUGGAAUCAGAAGCAGGUGU**CUGCAGCC**.

The Nupack assessment was performed (www.nupack.org/partition/histogram_detail/1166628?token=wqLsVGJXbN&strand_id=0) and the MFE structure of SEQ ID NO: 5 is shown in FIG. **59**. FIG. **60** shows the BNP sensor sequence (SEQ ID NO: 5) together with core and guide sequences. FIGS. **61**A and **61**B show Nupack analyses of BNP sensor (SEQ ID NO: 5) with overhangs, and guide with core, respectively. FIG. **62** shows NCBI check of BNP sensor second candidate vs. human transcripts.

(330) Likewise, the design of the third BNP candidate was similar to the first and second except that the 31 bp sequence used for reference for sensor strand was a different portion from SEQ ID NO: 121 shown in bold and underlined:

(331) TABLE-US-00046 (SEQ ID NO: 121)

GAGGAAGUCCUGGCUGCAGACACUGCUUCUGAUUCCACAAGG**GGCUUUUU CCUCAACCCUGUGGCCGCCUUUG**AAGUGACUCAUUUUUUUUAAUGUAUUUA UGAUUUAUUUGAUUGUUUUAUAUAAGAUGGUUUCUUACCUUUGAGCACAA AAUUUCCACGGGAAAUAAAGUCAACAUUAU AAGCUUUAAAAAAAAAAA.

(332) The sequence of the third BNP sensor is as follows, with the 8 bp toehold shown in bold and underlined:

(333) TABLE-US-00047 (SEQ ID NO: 6)

CAAAGGCGGCCACAGGGUUGAGG**AAAAAGCC**.

The Nupack assessment was performed (www.nupack.org/partition/histogram_detail/1166638?token=wZSopNPdBt&strand_id=0) and the MFE structure of SEQ ID NO: 6 is shown in FIG. **63**. FIG. **64** shows the BNP sensor sequence (SEQ ID NO: 6) together with core and guide sequences. FIG. **65** shows Nupack analysis of guide with core for the third BNP candidate. FIG. **66** shows NCBI check of BNP sensor third candidate vs. human transcripts.

(334) Myosin heavy chain 7 (MYH7) encodes for a heavy chain subunit of cardiac myosin-contractile velocity of cardiac muscle. Myosin has 2 heavy chains, 2 alkali light chains, and 2 regulatory light chains. It is expressed in normal human ventricles as well as type 1 (slow twitch) muscle fibers. Mutations in this gene result in hypertrophic cardiomyopathy, myosin storage myopathy, and numerous other cardiac diseases. See www.genecards.org/cgi-bin/carddisp.pl?gene=MYH7.

(335) The MYH7 candidate had an mRNA sequence source from www.ncbi.nlm.nih.gov/nuccore/NM_000257.3. The sequence starting from 3′ UTR of MYH7 mRNA is as follows, with the 31 bp sequence used for reference for sensor strand shown in bold and underlined:

(336) TABLE-US-00048 (SEQ ID NO: 143) UUUUUUUUUU UUUUUCUCGG CUUCAAGGAA AAUUGCUUUA UUCUGCUUCC UCCCAAGGAG CUGUUACACA GGCUCCAGCA UGGGGCUUUG **CUGGCACCUC CAGGGCUGAG CAGAUCAAGA U**GUGGCAAAG.

(337) The MYH7 sensor was designed by taking the reverse complement of the bold and underlined portion of SEQ ID NO: 143. The sequence is as follows, with the 8 bp toehold shown in bold and underlined:

(338) TABLE-US-00049 (SEQ ID NO: 7)

AUCUUGAUCUGCUCAGCCCUGGA**GGUGCCAG**.

The Nupack assessment was performed (www.nupack.org/partition/histogram_detail/1167009?token=OyDl4ywh0J&strand_id=0) and the MFE structure of SEQ ID NO: 7 is shown in FIG. **67**.

(339) FIG. **68** shows the MYH7 sensor sequence (SEQ ID NO: 7) together with core and guide sequences. FIGS. **69**A and **69**B show Nupack analyses of MYH7 sensor (SEQ ID NO: 7) with overhangs, and guide with core, respectively. FIG. **70** shows NCBI check of MYH7 sensor vs. human transcripts.

Example 11: Design Review of Conditional siRNAs in Cardiac Ischemia

(340) NPPA (aka: ANP, ANF, ANH, or CDD), if overexpressed in heart cells, inhibits, maladaptive cardiac hypertrophy. High levels of NPPB (aka: BNP) serve as a biomarker for heart failure in ischemic patients. Overexpression or mutation of MYH7 (aka: CMD1S, C1, MYHCB, SPMD, or SPMM) can cause cells to die prematurely and increase cardiac fibrosis. This design uses NPPA, NPPB or MYH7 as sensor, and HDAC2 and calcineurin as targets. Inhibition of HDAC2 or calcineurin causes reduced cardiac hypertrophy.

(341) Examples of NPPA HDAC2 designs are illustrated as follows. Construct #1 (best, MPE structure shown in FIG. **71**) (www.nupack.org/partition/histogram_detail/1157307?temperature=37.0&token=gYZWv2FATz&permutation_id=2&complex_id=23) was designed with the following sequences:

(342) TABLE-US-00050 Core: 5′ GUCAUCUUGUUGCUGGGUUGUUUCAAUCUAAGAGCGCUGCAUUUGU 3′ (SEQ ID NO: 25); Sensor: 5′ CAACAAGAUGACACAAAUGCAGCAGAGACCC 3′ (SEQ ID NO: 8) Modified sensor (SEQ ID NO: 147): CA + ACA + AG + ATG + AC + ACA + AA + TGC + AGC + AG + AGA + C +

C + C s1-s1: 28 S1: 24 DNA ™: 87 RNA ™: 92; and Guide: 5′ GCUCUUAGAUUGAAACAACCCAGUU 3′ (SEQ ID NO: 16)

(343) Construct #3 (MPE structure shown in FIG. **72**) (www.nupack.org/partition/show/1169058?time_refresh=1.0&token=OXLbgX6bBo) was designed with the following sequences:

(344) TABLE-US-00051 Core: 5′ CAUUUGUGUCAUUGUUAGAUUGAAACAACCCAGGGUCUCUGCUG 3′ (SEQ ID NO: 26); Sensor: 5′ AUGACACAAAUGCAGCAGAGACCCCAGGGGA 3′ (SEQ ID NO: 9) Modified sensor (SEQ ID NO: 148): A + TG + AC + ACA + AA + TGC + AGC + A + G + A + G + ACCCC + AG + GGG + A s1-s1: 40 s1: 33 DNA ™: 95 RNA ™: 100 and Guide: 5′ GCUCUUAGAUUGAAACAACCCAGUU 3′ (SEQ ID NO: 16).

(345) An example of NPPA calcineurin design is illustrated as follows. Construct #1 (best, MPE structure shown in FIG. **73**) (www. www.nupack.org/partition/show/1169063?time_refresh=1.0&token=RnEIROmvsz) was designed with the following sequences:

(346) TABLE-US-00052 Core: 5′ GUCAUCUUGUUGCAGGAAAAGCCAAACAACACUCGGCUGCAUUUGU 3′ (SEQ ID NO: 149); Sensor: 5′ CAACAAGAUGACACAAAUGCAGCAGAGACCC 3′ (SEQ ID NO: 8) Modified sensor (SEQ ID NO: 147): Modified sensor: CA + ACA + AG + ATG + AC + ACA + AA + TGC + AGC + AG + AGA + C + C + C s1-s1: 28 s1: 24 DNA ™: 87 RNA ™: 92 and Guide: 5′ CGAGUGUUGUUUGGCUUUUCCUGUU 3′ (SEQ ID NO: 12).

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APPENDIX B

(348) TABLE-US-00053 import re def check_sequence(seq, n, filename, exclude = [‘CCC’, ‘GGG’, ‘[A, U, T]{4}’]): ‘“This will check through a sequence and see if it meets a set of requirements”’ nseq = len(seq) assert nseq >= n excludereg = [ ] for i in exclude: exclude_reg.append(re.compile(i)) # make everything upper case seq = seq.upper( ) lines = [ ] for i in range(nseq−n+1): bad=0 excluded = [ ] seg = seq[i:i+n] gcau = (seg.count(‘G’), seg.count(‘C’), seg.count(‘A’), seg.count(‘U’)+seg.count(‘T’)) assert (gcau[0]+gcau[l]+ gcau[2] + gcau[3]) == n gc_percent = float((gcau[0]+gcau[1]))/n for pat in exclude reg: x = pat.findall(seg) bad += len(x) excluded, append(x) threeletter = l-float(min(gcau))/n lines.append((seg, excluded, gc_percent, threeletter, bad, i)) f = open(filename, ‘w’) f.write(‘Sequence \t Bad Segments\t GCness\t 3-letteredness\t Number bad points\t position\n’) for i in lines: for j in i: fwrite(repr(j)+‘\f’) f.write(‘\n’) f.close() return lines def reverse_complement_RNA(input): output =“ input=input.upper() for i in range(len(input)): x = input[i] if x== ‘A’: output += U elif x == ‘a’ output += ‘u’ elif x == U or x=T‘: output += ‘A’

elif x == ‘u’: output += ‘a’ elif x == ‘G’: output += ‘C’ elif x == ‘g’: output += ‘c’ elif x == ‘C’: output += ‘G’ elif x == ‘c’: output += ‘g’ return output[::−1] # generate 31 nt sensor candidates for human myh7 3′ utr hmyh7 = ‘gctttgccacatcttgatctgctcagccctggaggtgccagcaa agccccatgctggagcctgtgtaacagctccttgggaggaagca gaataaagcaattttccttgaagccgag’ (SEQ ID NO: 150) chmyh7 = reverse_complement_RNA(hmyh7) print(chmyh7) print(‘\n’) check_sequence(chmyh7, 31, ‘myh7 human.tsv’) # generate 31 nt sensor candidates for human nppa 3′ utr hnppa =‘“agata acagccaggg aggacaagca gggctgggcc tagggacaga ctgcaagagg ctcctgtccc ctggggtctc tgctgcattt gtgtcatctt gttgccatgg agttgtgatc atcccatcta agctgcagct tcctgtcaac acttctcaca tcttatgcta actgtagata aagtggtttg atggtgactt cctcgcctct cccacccccat gcattaaatt ttaaggtaga acctcacctg ttactgaaag tggtttgaaa gtgaataaac ttcagcacca tggacagaag ac”’ (SEQ ID NO: 151) chnppa = reverse_complement_RNA(hnppa) print(chnppa) printin’) check_sequence(chnppa, 31, ‘nppa human.tsv’) # generate 31 nt sensor candidates for human nppb 3′ utr hnppb = ‘“gag gaagtcctgg ctgcagacac ctgcttctga ttccacaagg ggctttttcc tcaaccctgt ggccgccttt gaagtgactc atttttttaa tgtatttatg tatttatttg attgttttat ataagatggt ttcttacctt tgagcacaaa atttccacgg tgaaataaag tcaacattat aagcttf”’ (SEQ ID NO: 152) chnppb = reversecomplementRNA(hnppb) print(chnppb) print(‘\n’) check_sequence(chnppb, 31, ‘nppb human.tsv’) # generate 31 nt sensor candidates for rat myh7 3′ utr rmyh7 = ‘“atct tgtgctaccc aaccctaagg atgcctgtga agccctgaga cctggagcct ttgaaacagc accttaggca gaaacacaat aaagcaattt tccttcaagc c”’ (SEQ ID NO: 153) crmyh7 = reverse_complement_RNA(rmyh7) print(crmyh7) print(‘\n’) check_sequence(crmyh7, 31, ‘myh7 rat.tsv’) # generate 31 nt sensor candidates for rat nppa 3′ utr rnppa = ‘“cagcc aaatctgctc gagcagatcg caaaagatcc caagcccttg cggtgtgtca cacagcttgg tcgcattgcc actgagaggt ggtgaatacc ctcctggagc tgcagcttcc tgtcttcatc tatcacgatc gatgttaagt gtagatgagt ggtttagtga ggccttacct ctcccactct gcatattaag gtagatcctc acccctttca gaaagcagtt ggaaaaaaat aaatccgaat aaacttcagc accacggaca gacgctgagg cctg”’ (SEQ ID NO: 154) crnppa = reversecomplementRNA(rnppa) print(crnppa) print(‘\n’) check_sequence(crnppa, 31, ‘nppa rat.tsv’) # generate 31 nt sensor candidates for rat nppa 3′ utr rnppb = ‘“gaagacc tcctggctgc agactccggc ttctgactct gcctgcggct cttctttccc cagctctggg accacctctc aagtgatcct gtttatttat ttgtttattt atttatttttt atgttgctga ttttctacaa gactgtttct tatcttccag cacaaacttg ccacagtgta ataaacatag cctatttctt gcttttgg”’ (SEQ ID NO: 155) crnppb = reversecomplementRNA(rnppb) print(crnppb) print(‘\n’) check_sequence(crnppa, 31, ‘nppb rat.tsv’)

APPENDIX C

(349) TABLE-US-00054 *Homo sapiens* myosin heavy chain 7 (MYH7), mRNA NCBI Reference Sequence: NM_000257.3 FASTA Graphics Go to: LOCUS NM_0002576069 bp mRNA linear PRI 17-JUN-2018 DEFINITION *Homo sapiens* myosin heavy chain 7 (MYH7), mRNA. ACCESSION NM_000257 XM_005267696 VERSION NM_000257.3 KEYWORDS RefSeq. SOURCE *Homo sapiens* (human) ORGANISM *Homo sapiens Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo.* REFERENCE 1 (bases 1 to 6069) AUTHORS Feng X, He T, Wang JG and Zhao P. TITLE Asn391Thr Mutation of beta-Myosin Heavy Chain in a Hypertrophic Cardiomyopathy Family JOURNAL Int Heart J 59 (3), 596-600 (2018) PUBMED 29743414 REMARK GeneRIF: Asn391Thr mutation of MYH7 is a malignant mutation for hypertrophic cardiomyopathy and that mutation carriers should get effective treatment to prevent sudden death. REFERENCE 2 (bases 1 to 6069) AUTHORS Viswanathan SK, Sanders HK,

McNamara JW, Jagadeesan A, Jahangir A, Tajik AJ and Sadayappan S. TITLE Hypertrophic cardiomyopathy clinical phenotype is independent of gene mutation and mutation dosage JOURNAL PLoS ONE 12 (11), eO 187948 (2017) PUBMED 29121657 REMARK GeneRIF: Data provide evidence that MYH7 mutations contributed to 24.4% MYBPC3 mutations of hypertrophic cardiomyopathy (HCM) cases, that MYBPC3 constitute the preeminent cause of HCM and that both mutations are phenotypically indistinguishable. Publication Status: Online-Only REFERENCE 3 (bases 1 to 6069) AUTHORS Wang B, Guo R, Zuo L, Shao H, Liu Y, Wang Y, Ju Y, Sun C, Wang L, Zhang Y and Liu L. TITLE [Analysis of genotype and phenotype correlation of MYH7-V878A mutation among ethnic Han Chinese pedigrees affected with hypertrophic cardiomyopathy] JOURNAL Zhonghua Yi Xue Yi Chuan Xue Za Zhi 34 (4), 514-518 (2017) PUBMED 28777849 REMARK GeneRIF: MYH7-V878A is a hot spot among ethnic Han Chinese with a high penetrance. REFERENCE 4 (bases 1 to 6069) AUTHORS OldforsA. TITLE Hereditary myosin myopathies JOURNAL Neuromuscul. Disord. 17 (5), 355-367 (2007) PUBMED 17434305 REMARK Review article REFERENCE 5 (bases 1 to 6069) AUTHORS Cirino, A.L. and Ho, C. TITLE Hypertrophic Cardiomyopathy Overview JOURNAL (in) Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K and Amemiya A (Eds.); GENEREVIEW S((R)); (1993) PUBMED 20301725 REFERENCE 6 (bases 1 to 6069) AUTHORS Lamont, P. and Laing, N.G. TITLE Laing Distal Myopathy JOURNAL (in) Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K and Amemiya A (Eds.); GENEREVIEW S((R)); (1993) PUBMED 20301606 REFERENCE 7 (bases 1 to 6069) AUTHORS Hershberger, R.E. and Morales, A. TITLE Dilated Cardiomyopathy Overview JOURNAL (in) Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K and Amemiya A (Eds.); GENEREVIEW S((R)); (1993) PUBMED 20301486 REFERENCE 8 (bases 1 to 6069) AUTHORS DeChene, E.T., Kang, P.B. and Beggs, A.H. TITLE Congenital Fiber-Type Disproportion JOURNAL (in) Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K and Amemiya A (Eds.); GENEREVIEW S((R)); (1993) PUBMED 20301436 REFERENCE 9 (bases 1 to 6069) AUTHORS Warlick CA, Ramachandra S, Mishra S and Donis-Keller H. TITLE Dinucleotide repeat polymorphism at the human cardiac beta-myosin heavy chain gene (HMSYHCO1) locus JOURNAL Hum. Mol. Genet. 1 (2), 136 (1992) PUBMED 1301151 REFERENCE 10 (bases 1 to 6069) AUTHORS Fougerousse F, Dufour C, Roudaut C and Beckmann JS. TITLE Dinucleotide repeat polymorphism at the human gene for cardiac beta-myosin heavy chain (MYH6) JOURNAL Hum. Mol. Genet. 1 (1), 64 (1992) PUBMED 1301139 COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff The reference sequence was derived from BF834726.1, EU747717.1, M58018.1 andBC112173.L On or before Jun. 20, 2014 this sequence version replaced XM_005267696.1, NM_000257.2. Summary: Muscle myosin is a hexameric protein containing 2 heavy chain subunits, 2 alkali light chain subunits, and 2 regulatory light chain subunits. This gene encodes the beta (or slow) heavy chain subunit of cardiac myosin. It is expressed predominantly in normal human ventricle. It is also expressed in skeletal muscle tissues rich in slow-twitch type I muscle fibers. Changes in the relative abundance of this protein and the alpha (or fast) heavy subunit of cardiac myosin correlate with the contractile velocity of cardiac muscle. Its expression is also altered during thyroid hormone depletion and hemodynamic overloading. Mutations in this gene are associated with familial

hypertrophic cardiomyopathy, myosin storage myopathy, dilated cardiomyopathy, and Laing early-onset distal myopathy, [provided by RefSeq, Jul 2008]. Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Gene record to access additional publications. ##Evidence-Data-START ## Transcript exon combination :: EU747717.1, M58018.1 [ECO:0000332] RNAseq introns:: mixed/partial sample support SAMEA1965299, SAMEAl968540 [ECO:0000350] ##Evidence-Data-END## COMPLETENESS: complete on the 3′ end. PRIMARY REFSEQ_ PRIMARY IDENTIFIER COMP SPAN PRIMARY SPAN 1-25 BF834726.1 60-84 26-320 EU747717.1 1-295 321-1831 M58018.1 276-1786 1832-3501 BC112173.1 1756-3425 3502-6053 M58018.1 3457-6008 6054-6069 EU747717.1 6029-6044 FEATURES Location/Qualifiers source 1 . . . 6069 /organism="*Homo sapiens*" /mol_type="mRNA" /db_xref="taxon:9606" /chromosome="14" /map="14qll.2" gene 1 . . . 6069 /gene="MYH7" /gene_synonym="CMD1S; CMH1; MPD1; MYHCB; SPMD; SPMM" /note="myosin heavy chain 7" /db_xref="GeneID:4625" exon 1 . . . 67 /gene="MYH7" /gene_synonym="CMD1S; CMH1; MPD1; MYHCB; SPMD; SPMM" /inference="alignment: Splign:2.1.0" exon 68 . . . 123 /gene="MYH7" /gene_synonym="CMD1S; CMH1; MPD1; MYHCB; SPMD; SPMM" /inference="alignment: Splign:2.1.0" misc_feature 102 . . . 104 /gene="MYH7" /gene_synonym="CMD1S; CMH1; MPD1; MYHCB; SPMD; SPMM" /note="upstream in-frame stop codon" exon 124 . . . 332 /gene="MYH7" /gene_synonym="CMD1S; CMH1; MPD1; MYHCB; SPMD; SPMM" /inference="alignment: Splign:2.1.0" CDS 132 . . . 5939 /gene="MYH7" /gene_synonym=″CMD 1S; CMH1; MPDI; MYHCB, SPMD; SPMM" /note=″myosin, heavy polypeptide 7, cardiac muscle, beta; myhc-slow; myopathy, distal 1; cardiac muscle myosin heavy chain 7 beta; rhabdomyosarcoma antigen MU-RMS-40.7A; myHC-beta; myosin heavy chain slow isoform; myosin heavy chain, cardiac muscle beta isoform; myosin 7; myosin, heavy chain 7, cardiac muscle, beta; myosin heavy chain beta-subunit" /codon_start=1 /product="myosin-7" /protein_id="NP_000248.2" /db_xref="CCDS:CCDS9601.1" /db_xref="GeneID:4625" /db_xref="HGNC:HGNC:7577" /db xref="MIM: 160760" /gene_synonym="CMD 1S; CMH1; MPDI; MYHCB, SPMD; SPMM" /note="myosin, heavy polypeptide 7, cardiac muscle, beta; myhc-slow; myopathy, distal 1; cardiac muscle myosin heavy chain 7 beta; rhabdomyosarcoma antigen MU-RMS-40.7A; myHC-beta; myosin heavy chain slow isoform; myosin heavy chain, cardiac muscle beta isoform; myosin 7; myosin, heavy chain 7, cardiac muscle, beta; myosin heavy chain beta-subunit" /codon_start=1 /product="myosin-7" /protein_id="NP_000248.2" /db_xref="CCDS:CCDS9601.1" /db_xref="GeneID:4625" /db_xref="HGNC:HGNC:7577" /db xref="MIM: 160760" /translation= "MGDSEMAVFGAAAPYLRKSEKERLEAQTRPFDLKKDVFVPDDKQ
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KRSEAPPHIFSISDNAYQYMLTDRENQSILITGESGAGKTVNTKRVIQYF
AVIAAIGDRSKKDQSPGKGTLEDQIIQANPALEAFGNAKTVRNDNSSRFG
KFIRIHFGATGKLASADIETYLLEKSRVIFQLKAERDYHIFYQILSNKKP
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KNSMYKLTGAIMHFGNMKFKLKQREEQAEPDGTEEADKSAYLMGLNSADL
LKGLCHPRVKVGNEYVTKGQNVQQVIYATGALAKAVYERMFNWMVTRINA
TLETKQPRQYFIGVLDIAGFEIFDFNSFEQLCINFTNEKLQQFFNHHMFV
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MTFKAKLFDNHLGKSANFQKPRNIKGKPEAHFSLIHYAGIVDYNIIGWLQ
KNKDPLNETVVGLYQKSSLKLLSTLFANYAGADAPIEKGKGKAKKGSSFQ
TVSALHRENLNKLMTNLRSTHPHFVRCIIPNETKSPGVMDNPLVMHQLRC
NGVLEGIRICRKGFPNRILYGDFRQRYRILNPAAIPEGQFIDSRKGAEKL
LSSLDIDHNQYKFGHTKVFFKAGLLGLLEEMRDERLSRIITRIQAQSRGV
LARMEYKKLLERRDSLLVIQWNIRAFMGVKNWPWMKLYFKIKPLLKSAER
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APPENDIX D

(350) TABLE-US-00055 *Homo sapiens* natriuretic peptide A (NPPA), mRNA NCBI Reference Sequence: NM_006172.3 FASTA Graphics Go to: LOCUS NM_006172 858 bp mRNA linear PRI 29-JUL.-2018 DEFINITION *Homo sapiens* natriuretic peptide A (NPPA), mRNA. ACCESSION NM_006172 VERSION NM_006172.3 KEYWORDS RefSeq. SOURCE *Homo sapiens* (human) ORGANISM *Homo sapiens* Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo. REFERENCE 1 (bases 1 to 858) AUTHORS Cannone V., Scott C. G., Decker P. A., Larson N. B., Palmas W, Taylor K. D., Wang T. J., Gupta D. K., Bielinski S. J. and Burnett J. C. Jr. TITLE A favorable cardiometabolic profile is associated with the G allele of the genetic variant rs5068 in African Americans: The Multi-Ethnic Study of Atherosclerosis (MESA) JOURNAL PLoS ONE 12 (12), e0189858 (2017) PUBMED 29253899 REMARK GeneRIF: the G allele of the genetic variant rs5068 in African Americans is associated with lower prevalence of metabolic syndrome and lower triglycerides values Publication Status: Online-Only REFERENCE 2 (bases 1 to 858) AUTHORS Salo, P. P., Havulinna, A. S., Tukiainen, T., Raitakari, O., Lehtimaki, T., Kahonen, M., Kettunen, J., Mannikko, M., Eriksson, J. G., Jula, A., Blankenberg, S., Zeller, T., Salomaa, V., Kristiansson, K. and Perola, M. TITLE Genome-Wide Association Study Implicates Atrial Natriuretic Peptide Rather Than B-Type Natriuretic Peptide in the Regulation of Blood Pressure in the General Population JOURNAL Circ Cardiovasc Genet 10 (6) (2017) PUBMED 29237677 REMARK GeneRIF: Data indicate the blood pressure-lowering effect of atrial natriuretic peptide (ANP) in the general population. REFERENCE 3 (bases 1 to 858) AUTHORS Wakula P., Neumann B., Kienemund J., Thon-Gutschi E., Stojakovic T., Manninger M., Scherr D., Schamagl H., Kapi M., Pieske B. and Heinzel F. R. TITLE CHA2DS2-VASc score and blood biomarkers to identify patients with atrial high-rate episodes and paroxysmal atrial fibrillation JOURNAL Europace 19 (4), 544-551 (2017) PUBMED 28431065 REMARK GeneRIF: TIMP-4, NT-proANP, NT-proBNP were strongest associated with PAF and AHRE. The discriminatory performance of CHADS2-VASc for PAF was increased by addition of selected biomarkers. REFERENCE 4 (bases 1 to 858) AUTHORS Bartus K., Podolec J., Lee R. J., Kapelak B., Sadowski J., Bartus M., Oles K., Ceranowicz P., Trabka R. and Litwinowicz R. TITLE Atrial natriuretic peptide and brain natriuretic peptide changes after epicardial percutaneous left atrial appendage suture ligation using LARIAT device JOURNAL J. Physiol. Pharmacol. 68 (1), 117-123 (2017) PUBMED 28456775 REMARK GeneRIF: In summary, there were no significant differences in ANP and BNP levels after percutaneous epicardial left atrial appendage suture ligation using LARIAT device 3 months after procedure. REFERENCE 5 (bases 1 to 858) AUTHORS Suga S., Nakao K., Hosoda K., Mukoyama M., Ogawa Y., Shirakami G., Arai H., Saito Y., Kambayashi Y., Inouye K. et al. TITLE Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide JOURNAL Endocrinology 130 (1), 229-239 (1992) PUBMED 1309330 REFERENCE 6 (bases 1 to 858) AUTHORS Bennett B. D., Bennett G. L., Vitangcol R. V., Jewett J. R., Burnier J., Henzel W. and Lowe D. G. TITLE Extracellular domain-IgG fusion proteins for three human natriuretic peptide receptors. Hormone pharmacology and application to solid phase

screening of synthetic peptide antisera JOURNAL J. Biol. Chern. 266 (34), 23060-23067 (1991) PUBMED 1660465 REFERENCE 7 (bases 1 to 858) AUTHORS Koller K. J, Lowe D. G., Bennett G. L., Minamino N., Kangawa K., Matsuo H. and Goeddel D. V. TITLE Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP) JOURNAL Science 252 (5002), 120-123 (1991) PUBMED 1672777 REFERENCE 8 (bases 1 to 858) AUTHORS Yang-Feng, T. L., Floyd-Smith, G., Nemer, M., Drouin, J. and Francke, U. TITLE The pronatriodilatin gene is located on the distal short arm of human chromosome 1 and on mouse chromosome 4 JOURNAL Am. J. Hum. Genet. 37 (6), 1117-1128 (1985) PUBMED 2934979 REFERENCE 9 (bases 1 to 858) AUTHORS Zivin, R. A., Condra, J. H., Dixon, R. A., Seidah, N. G., Chretien, M., Nemer, M., Chamberland, M. and Drouin, J. TITLE Molecular cloning and characterization of DNA sequences encoding rat and human atrial natriuretic factors JOURNAL Proc. Natl. Acad. Sci. U.S.A. 81 (20), 6325-6329 (1984) PUBMED 6238331 REFERENCE 10 (bases 1 to 858) AUTHORS Oikawa, S., Imai, M., Ueno, A., Tanaka, S., Noguchi, T., Nakazato, H., Kangawa, K., Fukuda, A. and Matsuo, H. TITLE Cloning and sequence analysis of cDNA encoding a precursor for human atrial natriuretic polypeptide JOURNAL Nature 309 (5970), 724-726 (1984) PUBMED 6203042 COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff The reference sequence was derived from BC005893.1 and AA779538.1. This sequence is a reference standard in the RefSeqGene project. On Jun. 13, 2009 this sequence version replaced NM_006172.2. Summary: The protein encoded by this gene belongs to the natriuretic peptide family. Natriuretic peptides are implicated in the control of extracellular fluid volume and electrolyte homeostasis. This protein is synthesized as a large precursor (containing a signal peptide), which is processed to release a peptide from the N-terminus with similarity to vasoactive peptide, cardiodilatin, and another peptide from the C-terminus with natriuretic-diuretic activity. Mutations in this gene have been associated with atrial fibrillation familial type 6. This gene is located adjacent to another member of the natriuretic family of peptides on chromosome 1. [provided by RefSeq, October 2015]. Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Gene record to access additional publications. ##Evidence-Data-START ## Transcript exon combination :: BC005893.1, ERR279837.2678.1 [ECO: 0000332] RNAseq introns :: single sample supports all introns SAMEA2154361, SAMEA2155550 [ECO: 0000348] ##Evidence-Data-END## COMPLETENESS: complete on the 3′end. PRIMARY COMP REFSEQ_SPAN PRIMARY_IDENTIFIER PRIMARY_SPAN 1-552 BC005893.1 1-552 553-858 AA779538.1 1-306 c FEATURES Location/Qualifiers source 1 . . . 858 /organism = “*Homo sapiens*” /mol_type = “mRNA” /db_xref = “taxon: 9606” /chromosome = “1” /map = “1p36.22” gene 1 . . . 858 /gene = “NPPA” /gene_synonym = “ANF; ANP; ATFB6; ATRST2; CDD; CDD-ANF; CDP; PND” /note = “natriuretic peptide A” /db_xref = “GeneID: 4878” /db_xref = “HGNC :HGNC: 7939” /db_xref = “MIM: 108780” exon 1 . . . 222 /gene = “NPPA” /gene_synonym = “ANF; ANP; ATFB6; ATRST2; CDD; CDD-ANF; CDP; PND” /inference = “alignment: Splign: 2.1.0” CDS 100 . . . 555 /gene = “NPPA” /gene_synonym = “ANF; ANP; ATFB6; ATRST2; CDD; CDD-ANF; CDP; PND” /note = “cardiodilatin-related peptide; cardionatrin; atriopeptin; prepronatriodilatin; natriuretic peptide precursor A variant 1” /codon_start = 1

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ttactgaaag tggtttgaaa gtgaataaac ttcagcacca 841 tggacagaag acaaaaaa (SEQ ID NO: 159)

APPENDIX E

(351) TABLE-US-00056 *Homo sapiens* natriuretic peptide B (NPPB), mRNA NCBI Reference Sequence: NM_002521.2 FASTA Graphics Go to: LOCUS NM_002521 708 bp mRNA linear PRI 22-JUL.-2018 DEFINITION *Homo sapiens* natriuretic peptide B (NPPB), mRNA. ACCESSION NM_002521 VERSION NM_002521.2 KEYWORDS RefSeq. SOURCE *Homo sapiens* (human) ORGANISM *Homo sapiens* Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo. REFERENCE 1 (bases 1 to 708) AUTHORS Hex C., Smeets M., Penders J., Van Hoof V., Verbakel J., Buntinx F. and Vaes B. TITLE Accuracy, user-friendliness and usefulness of the Cobas h232 point-of-care test for NT-proBNP in primary care JOURNAL J. Clin. Pathol. 71 (6), 539-545 (2018) PUBMED 29263170 REMARK GeneRIF: Report usefulness of point-of-care test for NT-proBNP in primary care for the diagnosis of heart failure. REFERENCE 2 (bases 1 to 708) AUTHORS Drozdz T., Kwinta P., Kordon Z., Sztefko K., Rudzinski A., Zachwieja K., Miklaszewska M., Czarnecka D. and Drozdz D. TITLE [B-type natriuretic peptide as a marker of cardiac dysfunction in children with chronic kidney disease] JOURNAL Pol. Merkur. Lekarski 44 (262), 171-176 (2018) PUBMED 29775443 REMARK GeneRIF: In children with chronic kidney disease, BNP is an indicator of heart failure correlating with renal function parameters and left ventricular mass index. REFERENCE 3 (bases 1 to 708) AUTHORS Fernandez-Susavila H, Rodriguez-Yanez M., Dopico-Lopez A., Arias S., Santamaria M., Avila-Gomez P., Doval-Garcia J. M., Sobrino T., Iglesias-Rey R., Castillo J. and Campos F. TITLE Heads and Tails of Natriuretic Peptides: Neuroprotective Role of Brain Natriuretic Peptide JOURNAL J Am Heart Assoc 6 (12), e007329 (2017) PUBMED 29203579 REMARK GeneRIF: Suggest potential role for BNP as a protective endogenous factor against cerebral ischemia. Publication Status: Online-Only REFERENCE 4 (bases 1 to 708) AUTHORS Legaz-Arrese A., Carranza-Garcia L. E., Navarro-Orocio R., Valadez-Lira A., Mayolas-Pi C., Munguia-Izquierdo D., Reverter-Masia J. and George K. TITLE Cardiac Biomarker Release after Endurance Exercise in Male and Female Adults and Adolescents JOURNAL J. Pediatr. 191, 96-102 (2017) PUBMED 29173327 REMARK GeneRIF: An exercise-associated increase in hs-cTnT and NT-proBNP occurred in response to a 60-minute maximal swimming test that was independent of pubertal status/adolescent vs adults. The present data also suggests that baseline and postexercise hs-cTnT values are higher in male compared with female, with no sex differences in NT-proBNP values. REFERENCE 5 (bases 1 to 708) AUTHORS Krause A., Liepke C., Meyer M., Adermann K., Forssmann W. G. and Maronde E. TITLE Human natriuretic peptides exhibit antimicrobial activity JOURNAL Eur. J. Med. Res. 6 (5), 215-218 (2001) PUBMED 11410403 REMARK GeneRIF: Brain-type natriuretic peptide (hBNP-32) is an antimicrobial peptide active against Gram-positive and Gram-negative bacteria and yeast. REFERENCE 6 (bases 1 to 708) AUTHORS Arden K. C., Viars C. S., Weiss S., Argentin S. and Nemer M. TITLE Localization of the human B-type natriuretic peptide precursor (NPPB) gene to chromosome lp36 JOURNAL Genomics 26 (2), 385-389 (1995) PUBMED 7601467 REFERENCE 7 (bases 1 to 708) AUTHORS Suga S., Nakao K., Hosoda K., Mukoyama M., Ogawa Y., Shirakami G., Arai H., Saito Y., Kambayashi Y.,

Inouye K. et al. TITLE Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide JOURNAL Endocrinology 130 (1), 229-239 (1992) PUBMED 1309330 REFERENCE 8 (bases 1 to 708) AUTHORS Bennett B. D., Bennett G. L., Vitangcol R. V., Jewett J. R., Burnier J., Henzel W. and Lowe D. G.. TITLE Extracellular domain-IgG fusion proteins for three human natriuretic peptide receptors. Hormone pharmacology and application to solid phase screening of synthetic peptide antisera JOURNAL J. Biol. Chern. 266 (34), 23060-23067 (1991) PUBMED 1660465 REFERENCE 9 (bases 1 to 708) AUTHORS Koller K. J., Lowe D. G., Bennett G. L., Minamino N., Kangawa K., Matsuo H. and Goeddel D. V.. TITLE Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP) JOURNAL Science 252 (5002), 120-123 (1991) PUBMED 1672777 REFERENCE 10 (bases 1 to 708) AUTHORS Sudoh T., Maekawa K., Kojima M., Minamino N., Kangawa K. and Matsuo H. TITLE Cloning and sequence analysis of cDNA encoding a precursor for human brain natriuretic peptide JOURNAL Biochem. Biophys. Res. Commun. 159 (3), 1427-1434 (1989) PUBMED 2522777 COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from AJ708502.1, M25296.1 and BC025785.1. On Dec. 16, 2005 this sequence version replaced NM_002521.1. Summary: This gene is a member of the natriuretic peptide family and encodes a secreted protein which functions as a cardiac hormone. The protein undergoes two cleavage events, one within the cell and a second after secretion into the blood. The protein's biological actions include natriuresis, diuresis, vasorelaxation, inhibition of renin and aldosterone secretion, and a key role in cardiovascular homeostasis. A high concentration of this protein in the bloodstream is indicative of heart failure. The protein also acts as an antimicrobial peptide with antibacterial and antifungal activity. Mutations in this gene have been associated with postmenopausal osteoporosis, [provided by RefSeq, November 2014]. Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Gene record to access additional publications. ##Evidence-Data-START## Transcript exon combination :: BC025785.1, ERR279856.3578.1 [ECO: 0000332] RNAseq introns :: single sample supports all introns SAMEA2148093, SAMEA2151741 [ECO: 0000348] ##Evidence-Data-END## ##RefSeq-Attributes-START## Protein has antimicrobial activity :: PMID: 11410403 ##RefSeq-Attributes-END## COMPLETENESS: complete on the 3′end. PRIMARY COMP REFSEQ_SPAN PRIMARY_IDENTIFIER PRIMARY_SPAN 1-4 AJ708502.1 19-22 5-695 M25296.1 2-692 696-708 BC025785.1 683-695 FEATURES Location/Qualifiers source 1 . . . 708 /organism = “*Homo sapiens*” /mol_type = “mRNA” /db_xref = “taxon: 9606” /chromosome = “1” /map = “1p36.22” gene 1 . . . 708 /gene = “NPPB” /gene_synonym = “BNP” /note = “natriuretic peptide B” /db_xref = “GeneID: 4879” /db_xref = “HGNC: HGNC: 7940” /db_xref = “MIM: 600295” exon 1 . . . 234 /gene = “NPPB” /gene_synonym = “BNP” /inference = “alignment: Splign: 2.1.0” CDS 103 . . . 507 /gene = “NPPB” /gene_sy nony m = “BNP” /note = “natriuretic peptide precursor B; brain type natriuretic peptide; natriuretic peptides B; natriuretic protein; gamma-brain natriuretic peptide” /codon_start = 1 /product = “natriuretic peptides B preproprotein” /protein_id = “NP_002512.1” /db_xref = “CCDS: CCDS140.1” /db_xref = “GeneID: 4879” /db_xref = “HGNC HGNC: 7940” /db_xref = “MIM: 600295” /translation

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APPENDIX F

(352) TABLE-US-00057 *Rattus norvegicus* myosin heavy chain 7 (Myh7), mRNA NCBI Reference Sequence: NM_017240.2 FASTA Graphics Go to: LOCUS NM_0172405923 bp mRNA linear ROD 31-MAY.-2018 DEFINITION *Rattus norvegicus* myosin heavy chain 7 (Myh7), mRNA. ACCESSION NM_017240 VERSION NM_017240.2 KEYWORDS RefSeq. SOURCE *Rattus norvegicus* (Norway rat) ORGANISM *Rattus norvegicus* Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Rattus. REFERENCE 1 (bases 1 to 5923) AUTHORS Tomita-Mitchell A., Stamm K. D., Mahnke D. K., Kim M. S., Hidestrand P. M., Liang H. L., Goetsch M. A., Hidestrand M., Simpson P., Pelech A. N., Tweddell J. S., Benson D. W., Lough J. W. and Mitchell M.E. TITLE Impact of MYH6 variants in hypoplastic left heart syndrome JOURNAL Physiol. Genomics 48 (12), 912-921 (2016) PUBMED 27789736 REFERENCE 2 (bases 1 to 5923) AUTHORS Chandra V., Gollapudi S. K. and Chandra M. TITLE Rat cardiac troponin T mutation (F72L)-mediated impact on thin filament cooperativity is divergently modulated by alpha- and beta-myosin heavy chain isoforms JOURNAL Am. J. Physiol. Heart Circ. Physiol. 309 (8), H1260-H1270 (2015) PUBMED 26342069 REMARK GeneRIF: TnT mutation F72L leads to contractile changes that are linked to dilated cardiomyopathy in the presence of MYH6 and hypertrophic cardiomyopathy in the presence of MYH7. REFERENCE 3 (bases 1 to 5923) AUTHORS Kralova E., Doka G., Pivackova L., Srankova J., Kuracinova K., Janega P., Babal P., Klimas J. and Krenek P. TITLE 1-Arginine Attenuates Cardiac Dysfunction, But Further Down-Regulates alpha-Myosin Heavy Chain

Expression in Isoproterenol-Induced Cardiomyopathy JOURNAL Basic Clin. Pharmacol. Toxicol. 117 (4), 251-260 (2015) PUBMED 25865156 REFERENCE 4 (bases 1 to 5923) AUTHORS Taylor K. C., Buvoli M., Korkmaz E. N., Buvoli A., Zheng Y., Heinze N. T., Cui Q., Leinwand L. A. and Rayment I. TITLE Skip residues modulate the structural properties of the myosin rod and guide thick filament assembly JOURNAL Proc. Natl. Acad. Sci. U.S.A. 112 (29), E3806-E3815 (2015) PUBMED 26150528 REFERENCE 5 (bases 1 to 5923) AUTHORS Zhang P., Shan T., Liang X., Deng C. and Kuang S. TITLE Mammalian target of rapamycin is essential for cardiomyocyte survival and heart development in mice JOURNAL Biochem. Biophys. Res. Commun. 452 (1), 53-59 (2014) PUBMED 25139234 REFERENCE 6 (bases 1 to 5923) AUTHORS O'Neill L., Holbrook N. J., Fargnoli J. and Lakatta E. G. TITLE Progressive changes from young adult age to senescence in mRNA for rat cardiac myosin heavy chain genes JOURNAL Cardioscience 2 (1), 1-5 (1991) PUBMED 1888877 REFERENCE 7 (bases 1 to 5923) AUTHORS Schuyler G. T. and Yarbrough L. R. TITLE Changes in myosin and creatine kinase mRNA levels with cardiac hypertrophy and hypothyroidism JOURNAL Basic Res. Cardiol. 85 (5), 481-494 (1990) PUBMED 1703406 REFERENCE 8 (bases 1 to 5923) AUTHORS McNally E. M., Kraft R., Bravo-Zehnder M., Taylor D. A. and Leinwand L. A. TITLE Full-length rat alpha and beta cardiac myosin heavy chain sequences. Comparisons suggest a molecular basis for functional differences JOURNAL J. Mol. Biol. 210 (3), 665-671 (1989) PUBMED 2614840 REFERENCE 9 (bases 1 to 5923) AUTHORS Kraft R., Bravo-Zehnder M., Taylor D. A. and Leinwand L. A. TITLE Complete nucleotide sequence of full length cDNA for rat beta cardiac myosin heavy chain JOURNAL Nucleic Acids Res. 17 (18), 7529-7530 (1989) PUBMED 2798112 REFERENCE 10 (bases 1 to 5923) AUTHORS Izumo, S., Lompre, A. M., Matsuoka, R., Koren, G., Schwartz, K., Nadal-Ginard, B. and Mahdavi, V. TITLE Myosin heavy chain messenger RNA and protein isoform transitions during cardiac hypertrophy. Interaction between hemodynamic and thyroid hormone-induced signals JOURNAL J. Clin. Invest. 79 (3), 970-977 (1987) PUBMED 2950137 COMMENT PROVISIONAL REFSEQ: This record has not yet been subject to final NCBI review. The reference sequence was derived from X15939.1. On Feb. 21, 2013 this sequence version replaced NM_017240.1. Summary: heavy chain of myosin; involved in muscle contraction [RGD, February 2006]. Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Gene record to access additional publications. ##Evidence-Data-START## Transcript exon combination :: X15939.1 [ECO: 0000332] RNAseq introns :: mixed/partial sample support SAMD00052296, SAMD00052297 [ECO: 0000350] ##Evidence-Data-END## PRIMARY COMP REFSEQ_SPAN PRIMARY_IDENTIFIER PRIMARY_ SPAN 1-5923 X15939.1 3-5925 FEATURES Location/Qualifiers source 1 . . . 5923 /organism = “*Rattus norvegicus*” /mol_type = “mRNA” /db xref = “taxon: 10116” /chromosome = “15” /map = “15p13” gene 1 . . . 5923 /gene = “Myh7” /gene_synonym = “Bmyo; myHC-beta; myHC-slow; Myhcb” /note = “myosin heavy chain 7” /db_xref = “GeneID: 29557” /db_xref = “RGD: 62030” exon 1 . . . 209 /gene = “Myh7” /gene_synonym = “Bmyo; myHC-beta; myHC-slow; Myhcb” /inference = “alignment: Splign: 2.0.8” CDS 9 . . . 5816 /gene = “Myh7” /gene_synonym = “Bmyo; myHC-beta; myHC-slow; Myhcb” /note = “myosin heavy chain, cardiac muscle, fetal; beta myosin heavy chain; myosin heavy

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APPENDIX G

(353) TABLE-US-00058 *Rattus norvegicus* natriuretic peptide A (Nppa), mRNA NCBI Reference Sequence: NM_012612.2 FASTA Graphics Go to: LOCUS

NM_012612831 bp mRNA linear ROD 10-JUN-2018 DEFINITION *Rattus norvegicus* natriuretic peptide A (Nppa), mRNA. ACCESSION NM_012612 VERSION NM_012612.2 KEYWORDS RefSeq. SOURCE *Rattus norvegicus* (Norway rat) ORGANISM *Rattus norvegicus* Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Rattus. REFERENCE 1 (bases 1 to 831) AUTHORS Barallobre-Barreiro J, Gupta SK, Zoccarato A, Kitazume-Taneike R, Fava M, Yin X, Werner T, Hirt MN, Zampetaki A, Viviano A, Chong M, Bern M, Kourliouros A, Domenech N, Willeit P, Shah AM, Jahangiri M, Schaefer L, Fischer JW, lozzo RV, Viner R, Thum T, Heineke J, Ki chi er A, Otsu K and Mayr M. TITLE Glycoproteomics Reveals Decorin Peptides With Anti-Myostatin Activity in Human Atrial Fibrillation JOURNAL Circulation 134 (11), 817-832 (2016) PUBMED 27559042 REFERENCE 2 (bases 1 to 831) AUTHORS Yuan K, Park BM, Choi YT, Kim JH, Cho KW and Kim SH. TITLE Effects of endothelin family on ANP secretion JOURNAL Peptides 82, 12-19 (2016) PUBMED 27208702 REMARK GeneRIF: we suggest that the order of secretagogue effect of ET family on ANP secretion was ET-1>/ = ET-2>>ET-3>s6C and ET-1-induced atrial natriuretic peptide secretion negatively regulates the pressor effect of ET-1. REFERENCE 3 (bases 1 to 831) AUTHORS Lee CH, Ha GW, Kim JH and Kim SH. TITLE Modulation in Natriuretic Peptides System in Experimental Colitis in Rats JOURNAL Dig. Dis. Sci. 61 (4), 1060-1068 (2016) PUBMED 26660905 REMARK GeneRIF: augmentation of inhibitory effect on basal motility by ANP in experimental colitis may be due an increased expression of colonic natriuretic peptide receptor-A mRNA REFERENCE 4 (bases 1 to 831) AUTHORS Bugrova, M.L. TITLE [ATRIAL AND BRAIN NATRIURETIC PEPTIDES OF CARDIAC MUSCLE CELLS IN POSTREPERFUSION PERIOD IN RATS] JOURNAL Tsitologiia 58 (2), 129-134 (2016) PUBMED 27228659 REMARK GeneRIF: This is due to the fact that ANP is the main hormone of the natriuretic peptide system involved in the regulation of blood pressure in normal conditions, while BNP is the principal regulator of pressure in cardiovascular pathology REFERENCE 5 (bases 1 to 831) AUTHORS Pang A, Hu Y, Zhou P, Long G, Tian X, Men L, Shen Y, Liu Y and Cui Y. TITLE Corin is down-regulated and exerts cardioprotective action via activating pro-atrial natriuretic peptide pathway in diabetic cardiomyopathy JOURNAL Cardiovasc Diabetol 14, 134 (2015) PUBMED 26446774 REMARK GeneRIF: ANP mRNA and protein are decreased in diabetic cardiomyopathy. Publication Status: Online-Only REFERENCE 6 (bases 1 to 831) AUTHORS Bennett BD, Bennett GL, Vitangcol RV, Jewett JR, Burnier J, Henzel W and Lowe DG. TITLE Extracellular domain-IgG fusion proteins for three human natriuretic peptide receptors. Hormone pharmacology and application to solid phase screening of synthetic peptide antisera JOURNAL J. Biol. Chern. 266 (34), 23060-23067 (1991) PUBMED 1660465 REFERENCE 7 (bases 1 to 831) AUTHORS Levin ER and Frank HJ. TITLE Natriuretic peptides inhibit rat astroglial proliferation: mediation by C receptor JOURNAL Am. J. Physiol. 261 (2 Pt 2), R453-R457 (1991) PUBMED 1652217 REFERENCE 8 (bases 1 to 831) AUTHORS Koller KJ, Lowe DG, Bennett GL, Minamino N, Kangawa K, Matsuo H and Goeddel DV. TITLE Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP) JOURNAL Science 252 (5002), 120-123 (1991) PUBMED 1672777 REFERENCE 9 (bases 1 to 831) AUTHORS Mukoyama,M., Nakao,K., Saito,Y., Ogawa,Y., Hosoda,K., Suga,S., Shirakami,G., Jougasaki,M. and Imura,H. TITLE Increased human

brain natriuretic peptide in congestive heart failure JOURNAL N. Engl. J. Med. 323 (11), 757-758 (1990) PUBMED 2143809 REFERENCE 10 (bases 1 to 831) AUTHORS Jin H, Yang RH, Chen YF, Jackson RM and Oparil S. TITLE Atrial natriuretic peptide attenuates the development of pulmonary hypertension in rats adapted to chronic hypoxia JOURNAL J. Clin. Invest. 85 (1), 115-120 (1990) PUBMED 2136863 COMMENT This record has undergone validation or VALIDATED preliminary review. The reference sequence was derived from REFSEQ: CB724799.1, X00665.1 and AI602287.1. On Oct 17, 2007 this sequence version replaced NM_012612.1. Summary: peptide involved in the control of fluid volume and vascular function [RGD, Feb 2006]. Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Gene record to access additional publications. ##Evidence-Data-START## Transcript exon combination:: EV765126.1, BC158590.1 [ECO:0000332] RNAseq introns:: single sample supports all introns SAMD00052296, SAMD00052297 [ECO:0000348] ##Evidence-Data-END## PRIMARY COMP REFSEQ_SPAN PRIMARY IDENTIFIER PRIMARY SPAN 1-8 CB724799.1 128-135 9-576 X00665.1 1-568 577-831 AI602287.1 1-255 c FEATURES Location/Qualifiers source 1..831 /organism = ″*Rattus norvegicus*″ /mol_type = ″mRNA″ /strain = ″Sprague-Dawley″ /db_xref = ″taxon: 10116″ /chromosome = ″ 5″ /map = ″5q36″ gene 1..831 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /note = ″natriuretic peptide A″ /db_xref = ″GeneID:24602″ /db_xref = ″RGD:3193″ exon 1..196 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /inference = ″alignment: Splign: 2.0.8″ unsure 9..14 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /note = ″pot. cloning artefact″ STS 73..481 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /standard_name = ″PMC 123178P1″ /db_xref = ″UniSTS:270444″ CDS 77..535 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /note = ″Natriuretic peptide precursor A (pronatriodilatin, also Anf, Pnd); atrial natriuretic factor; natriuretic peptides A; prepronatriodilatin; atrial natriuretic peptide; natriuretic peptide precursor type A″ /codon_start = 1 /product = ″natriuretic peptides A precursor″ /protein_id = ″NP_036744.1″ /db_xref = ″GeneID:24602″ /db_xref = ″RGD:3193″ /translation = ″MGSFSITKGFFLFLAFWLPGHIGANPVYSAVSNTDLMDFKNLLD HLEEKMPVEDEVMPPQALSEQTDEAGAALSSLSEVPPWTGEVNPSQRDGGALGRGP WD PSDRSALLKSKLRALLAGPRSLRRSSCFGGRIDRIGAQSGLGCNSFRYRR″ (SEQ ID NO: 164) sig_peptide 77..148 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /inference = ″COORDINATES: ab initio prediction:SignalP:4.0″ misc_feature 440..445 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /experiment = ″experimental evidence, no additional details recorded″ /note = ″Cleavage, by CORIN. {ECO:0000250UniProtKB:P01160); propagated from UniProtKB/Swiss-Prot (P01161.1); cleavage site″ miscfeature 461..466 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /experiment = ″experimental evidence, no additional details recorded″ /note = ″Cleavage, by MME. {ECO:0000269|PubMed:2966343}; propagated from UniProtKB/Swiss-Prot (P01161.1); cleavage site″ STS 131..520 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /standard_name = ″PMC3 16718P1″ /db_xref = ″UniSTS:273041″ prim_transcript 149.. 532 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /note = ″AFN″ exon 197..523 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /inference = ″alignment: Splign: 2.0.8″ STS 197..413 /gene = ″Nppa″ /gene_synonym = ″ANF; ANP; Pnd; RATANF″ /standard_name = ″NoName″

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APPENDIX H

(354) TABLE-US-00059 *Rattus norvegicus* natriuretic peptide B (Nppb), mRNA NCBI Reference Sequence: NM_031545.1 FASTA Graphics Go to: LOCUS NM_031545628 bp mRNA linear ROD 21-JUL-2018 DEFINITION *Rattus norvegicus* natriuretic peptide B (Nppb), mRNA. ACCESSION NM_031545 VERSION NM_031545.1 KEYWORDS RefSeq. SOURCE *Rattus norvegicus* (Norway rat) ORGANISM *Rattus norvegicus* Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Rattus. REFERENCE 1 (bases 1 to 628) AUTHORS Saklani R, Gupta SK, Mohanty IR, Kumar B, Srivastava S and Mathur R. TITLE Cardioprotective effects of rutin via alteration in TNF-alpha, CRP, and BNP levels coupled with antioxidant effect in STZ-induced diabetic rats JOURNAL Mol. Cell. Biochem. 420 (1-2), 65-72 (2016) PUBMED 27443845 REMARK GeneRIF: Cardioprotective effects of rutin via alteration in TNF-alpha, CRP, and BNP levels coupled with antioxidant effect in STZ-induced diabetic rats. REFERENCE 2 (bases 1 to 628) AUTHORS Holditch SJ, Schreiber CA, Burnett JC and Ikeda Y. TITLE Arterial Remodeling in B-Type Natriuretic Peptide Knock-Out Females JOURNAL Sci Rep 6, 25623 (2016) PUBMED 27162120 REMARK GeneRIF: Data show that approximately 60% of natriuretic peptide precursor type B (Nppb)-/- females developed mesenteric polyarteritis-nodosa (PAN)-like vasculitis in their life span, some as early as 4 months of age. Publication Status: Online-Only REFERENCE 3 (bases 1 to 628) AUTHORS Terse PS, Joshi PS, Bordelon NR, Brys AM, Patton KM, Arndt TP and Sutula TP. TITLE 2-Deoxy-d-Glucose (2-DG)-Induced Cardiac Toxicity in Rat: NT-proBNP and BNP as Potential Early Cardiac Safety Biomarkers JOURNAL Int. J. Toxicol. 35 (3), 284-293 (2016) PUBMED 26838190 REMARK GeneRIF: NT-proBNP and BNP are potential early biomarkers for 2-DG-induced cardiac toxicity that can be useful

to monitor 2-DG therapy in clinical trials. REFERENCE 4 (bases 1 to 628) AUTHORS Bugrova,ML. TITLE [ATRIAL AND BRAIN NATRIURETIC PEPTIDES OF CARDIAC MUSCLE CELLS IN POSTREPERFUSION PERIOD IN RATS] JOURNAL Tsitologiia 58 (2), 129-134 (2016) PUBMED 27228659 REMARK GeneRIF: This is due to the fact that ANP is the main hormone of the natriuretic peptide system involved in the regulation of blood pressure in normal conditions, while BNP is the principal regulator of pressure in cardiovascular pathology REFERENCE 5 (bases 1 to 628) AUTHORS Dogan H, Sarikaya S, Neijmann ST, Uysal E, Yucel N, Ozucelik DN, Okuturlar Y, Solak S, Sever N and Ayan C. TITLE N-terminal pro-B-type natriuretic peptide as a marker of blunt cardiac contusion in trauma JOURNAL Int J Clin Exp Pathol 8 (6), 6786-6792 (2015) PUBMED 26261563 REMARK GeneRIF: Serum NT-proBNP levels significantly increased after 5 hours of the blunt chest trauma. Publication Status: Online-Only REFERENCE 6 (bases 1 to 628) AUTHORS Bennett BD, Bennett GL, Vitangcol RV, Jewett JR, Burnier J, Henzel W and Lowe DG. TITLE Extracellular domain-IgG fusion proteins for three human natriuretic peptide receptors. Hormone pharmacology and application to solid phase screening of synthetic peptide antisera JOURNAL J. Biol. Chern. 266 (34), 23060-23067 (1991) PUBMED 1660465 REFERENCE 7 (bases 1 to 628) AUTHORS Dagnino L, Drouin J and Nemer M. TITLE Differential expression of natriuretic peptide genes in cardiac and extracardiac tissues JOURNAL Mol. Endocrinol. 5 (9), 1292-1300 (1991) PUBMED 1837590 REFERENCE 8 (bases 1 to 628) AUTHORS Levin ER and Frank HJ. TITLE Natriuretic peptides inhibit rat astroglial proliferation: mediation by C receptor JOURNAL Am. J. Physiol. 261 (2 Pt 2), R453-R457 (1991) PUBMED 1652217 REFERENCE 9 (bases 1 to 628) AUTHORS Hoffman A, Grossman E and Keiser HR. TITLE Increased plasma levels and blunted effects of brain natriuretic peptide in rats with congestive heart failure JOURNAL Am. J. Hypertens. 4 (7 Pt 1), 597-601 (1991) PUBMED 1831369 REFERENCE 10 (bases 1 to 628) AUTHORS Koller KJ, Lowe DG, Bennett GL, Minamino N, Kangawa K, Matsuo H and Goeddel DV. TITLE Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP) JOURNAL Science 252 (5002), 120-123 (1991) PUBMED 1672777 COMMENT This record has not yet been subject to final PROVISIONAL NCBI review. The reference sequence was derived from M25297.1. REFSEQ: Summary: hormone produced primarily by the atrium and ventricle of the heart [RGD, Feb 2006]. Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Gene record to access additional publications. ##Evidence-Data-START## Transcript exon combination:: M25297.1, FQ228997.1 [ECO:0000332] RNAseq introns:: single sample supports all introns SAMEA2689596, SAMEA2689600 [ECO:0000348] ##Evidence-Data-END## FEATURES Location/Qualifiers source 1..628 /organism = ″*Rattus norvegicus*″ /mol_type = ″mRNA″ /db_xref = ″taxon: 10116″ /chromosome = ″5″ /map = ″5q36″ gene 1..628 /gene = ″Nppb″ /gene_synonym = ″Bnf; BNP″ /note = ″natriuretic peptide B″ /db_xref = ″GeneID:25105″ /db_xref = ″RGD:3194″ exon 1..183 /gene = ″Nppb″ /gene_synonym = ″Bnf; BNP″ /inference = ″alignment: Splign: 2.0.8″ CDS 58..423 /gene = ″Nppb″ /gene_synonym = ″Bnf; BNP″ /note = ″natriuretic peptides B; brain natriuretic peptide; natriuretic peptide precursor B; iso-ANP; gamma-brain natriuretic peptide; Brain natriuretic factor; natriuretic peptide precursor type B″ /codon_start = 1 /product = ″natriuretic peptides B precursor″ /protein _id = ″NP_1 13733.1″ /db_xref = ″GeneID:25105″

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Claims

1. A conditional RNA-sensor complex comprising: a sensor strand comprising at least one toehold segment, wherein the toehold segment capable of binding a pathological biomarker present in or overexpressed in a target cell, wherein the pathological biomarker comprises a molecule that encodes ANP, BNP, MHCβ, mir-23a-3p, mir-125-6p, or mir-199b-5p; and a double stranded pro-RNA molecule comprising a guide strand comprising an RNA molecule capable of binding a therapeutic target molecule in the target cell, wherein the therapeutic target molecule is an RNA molecule that encodes calcineurin or histone deacetylase 2 (HDAC2); and a core strand comprising a first portion comprising a passenger strand that is fully or partially complimentary to and binds the guide strand, a second portion comprising a first protection segment that is fully or partially complimentary to and binds the sensor strand, and a first linker that joins a first end of the passenger strand to the first protection segment.
2. The conditional RNA-sensor complex of claim 1, wherein the core strand further comprises a third portion comprising a second protection segment that is fully or partially complimentary to and binds the sensor strand, and a second linker that joins a second end of the passenger strand to the second protection segment.
3. The conditional RNA-sensor complex of claim 1, wherein the toehold segment is an aptamer.
4. The conditional RNA-sensor complex of claim 1, wherein the sensor strand is displaced from the double stranded pro-RNA molecule when the pathological biomarker binds the toehold segment and the resulting double stranded pro-RNA molecule is a substrate for Dicer.
5. The conditional RNA-sensor complex of claim 1, wherein the target cell is a cardiac myocyte.
6. The conditional RNA-sensor complex of claim 1, wherein the molecule that encodes ANP, BNP, or MHCβ is an mRNA molecule and the molecule that encodes mir-23a-3p, mir-125-6p, or mir-

199b-5p is an miRNA molecule.
7. The conditional RNA-sensor complex of claim 6, wherein the molecule that encodes ANP comprises a sequence selected from SEQ ID Nos: 8-10, the molecule that encodes BNP comprises a sequence selected from SEQ ID Nos: 4-6, the molecule that encodes MHCβ comprises a sequence of SEQ ID NO: 7, and the molecule that encodes mir-23a-3p comprises a sequence selected from SEQ ID Nos: 1-3.
8. The conditional RNA-sensor complex of claim 7, wherein the sensor strand further comprises one or more chemical modifications to the RNA sequence, wherein the one or more chemical modifications are selected from a locked nucleic acid (LNA) modification, a peptide nucleic acid (PNA) modification, a 2′-O-methyl modification, morpholino modification, a phosphorothioate modification, a terminal modification, or a linker modification.
9. The conditional RNA-sensor complex of claim 5, wherein the double stranded pro-RNA molecule is an RNA interference (RNAi) molecule.
10. The conditional RNA-sensor complex of claim 9, wherein the guide strand comprises a sequence selected from SEQ ID NOS: 11-16.
11. The conditional RNA-sensor complex of claim 2, wherein the first linker, the second linker, or both the first and second linkers is a C3 spacer.
12. The conditional RNA-sensor complex of claim 9, wherein the guide strand further comprises one or more chemical modifications to the RNA sequence, wherein the one or more chemical modifications are selected from a locked nucleic acid (LNA) modification, a peptide nucleic acid (PNA) modification, a 2′-O-methyl modification, morpholino modification, a phosphorothioate modification, a terminal modification, or a linker modification.
13. The conditional RNA-sensor complex of claim 2, wherein the core strand comprises a passenger strand; a first linker that joins a 3′ end of the passenger strand to the first protection segment; and a second linker that joins a 5′ end of the passenger strand to the second protection segment.
14. The conditional RNA-sensor complex of claim 13, wherein the core strand comprises a sequence selected from SEQ ID NOS: 17-26.
15. The conditional RNA-sensor complex of claim 13, wherein the core strand further comprises one or more chemical modifications to the RNA sequence, wherein the one or more chemical modifications are selected from a locked nucleic acid (LNA) modification, a peptide nucleic acid (PNA) modification, a 2′-O-methyl modification, morpholino modification, a phosphorothioate modification, a terminal modification, or a linker modification.
16. The conditional RNA-sensor complex of claim 1, wherein: (a) the sensor strand comprises a sequence having at least 95% homology to SEQ ID NO:4, the core strand comprises a sequence having at least 95% homology to SEQ ID NO:21, SEQ ID NO: 49, and SEQ ID NO: 50, and the guide strand comprises a sequence having at least 95% homology to SEQ ID NO:15; (b) the sensor strand comprises a sequence having at least 95% homology to SEQ ID NO:5, the core strand comprises a sequence having at least 95% homology to SEQ ID NO:22, SEQ ID NO: 49, and SEQ ID NO: 51, and the guide strand comprises a sequence having at least 95% homology to SEQ ID NO:15; (c) the sensor strand comprises a sequence having at least 95% homology to SEQ ID NO:6, the core strand comprises a sequence having at least 95% homology to SEQ ID NO:23, SEQ ID NO: 49, and SEQ ID NO: 52, and the guide strand comprises a sequence having at least 95% homology to SEQ ID NO:15; (d) the sensor strand comprises a sequence having at least 95% homology to SEQ ID NO:7, the core strand comprises a sequence having at least 95% homology to SEQ ID NO:24, SEQ ID NO: 49, and SEQ ID NO: 53, and the guide strand comprises a sequence having at least 95% homology to SEQ ID NO:15; (e) the sensor strand comprises a sequence having at least 95% homology to SEQ ID NO:8, the core strand comprises a sequence having at least 95% homology to SEQ ID NO:25, and the guide strand comprises a sequence having at least 95% homology to SEQ ID NO:16; (f) the sensor strand comprises a sequence having at least 95%

homology to SEQ ID NO:8, the core strand comprises a sequence having at least 95% homology to SEQ ID NO:18, SEQ ID NO: 45, and SEQ ID NO: 47, and the guide strand comprises a sequence having at least 95% homology to SEQ ID NO:11; (g) the sensor strand comprises a sequence having at least 95% homology to SEQ ID NO:10, the core strand comprises a sequence having at least 95% homology to SEQ ID NO:19, SEQ ID NO: 45, and SEQ ID NO: 48, and the guide strand comprises a sequence having at least 95% homology to SEQ ID NO:12; (h) the sensor strand comprises a sequence having at least 95% homology to SEQ ID NO:1, the core strand comprises a sequence having at least 95% homology to SEQ ID NO: 41, SEQ ID NO: 45, and SEQ ID NO: 56, and the guide strand comprises a sequence having at least 95% homology to SEQ ID NO:12; (i) the sensor strand comprises a sequence having at least 95% homology to SEQ ID NO:2, the core strand comprises a sequence having at least 95% homology to SEQ ID NO: 41, SEQ ID NO: 45, and SEQ ID NO: 56, and the guide strand comprises a sequence having at least 95% homology to SEQ ID NO: 12; or (j) the sensor strand comprises a sequence having at least 95% homology to SEQ ID NO:39, the core strand comprises a sequence having at least 95% homology to SEQ ID NO: 41, SEQ ID NO: 45, and SEQ ID NO: 56, and the guide strand comprises a sequence having at least 95% homology to SEQ ID NO:12.

17. The conditional RNA-sensor complex of claim 16, wherein the sensor strand, the guide strand and/or the core strand further comprises one or more chemical modifications to the RNA sequence, wherein the one or more chemical modifications are selected from a locked nucleic acid (LNA) modification, a peptide nucleic acid (PNA) modification, a 2′-O-methyl modification, morpholino modification, a phosphorothioate modification, a terminal modification, or a linker modification.

18. A pharmaceutical composition comprising: a conditional RNA-sensor complex of claim 1; and a pharmaceutically acceptable carrier or excipient.

19. A method of treating a pathological condition comprising administering a therapeutically effective amount of a conditional RNA-sensor complex of claim 1 to a subject suffering from the pathological condition, wherein the pathological condition is myocardial infarction (MI), or cardiac hypertrophy.

20. The method of claim 19, wherein administering a therapeutically effective amount comprises an intramyocardial injection of the conditional RNA-sensor complex or the pharmaceutical composition after detection of MI.

21. The conditional RNA-sensor complex of claim 1, wherein the passenger strand bound to the guide strand forms a duplex between 15 and 30 base pairs in length.