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(54) Title: BIOMARKERS OF CARDIOVASCULAR STATUS AND USES THEROF

(57) Abstract: Disclosed are biomarkers and their use for determining or predicting a cardiovascuiar status (e.g., adverse cardiac (e.g., left ventricular) remodeling) following a cardiovascuiar event (e.g., injury or trauma, such as, e.g., myocardial Infarction).

BIOMARKERS OF CARDIOVASCULAR STATUS AND USES THEREOF

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was made with government support under grant number TR000901 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

The invention pertains to methods of using biomarkers to assess, determine, or predict a cardiovascular condition in a subject.

BACKGROUND

Cardiac injuries and diseases associated with cardiac remodeling and subsequent development of heart failure (HF) can detrimentally impact function and quality of life. There is a need for new technologies to determine, assess, predict, or infer various cardiovascular conditions, in particular as they relate to cardiac remodeling.

SUMMARY OF THE INVENTION

In one aspect, disclosed is a method for assessing, determining, predicting, or inferring a cardiovascular condition in a subject (e.g., a human), the method comprising determining, in at least one sample from the subject, expression of at least one biomarker (e.g., one, two, three, four, five, ten, twenty, thirty, forty, fifty, sixty, or seventy-four biomarkers, such as a biomarker having the sequence of any one of SEQ ID NOs 1-74, or a complement thereof, and variants thereof). The biomarker(s) may have at least 80% (e.g., 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to any one of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, or 74, or a complement thereof; e.g., the biomarker(s) may be one or more of the biomarkers of PC1, PC2, and/or PC3 (see Figure 5) or one or more of SEQ ID NOs: 1-12). Also featured are methods of treating a cardiovascular condition in a subject (e.g., heart failure or myocardial infarction (MI)) by administering a cardiac therapy to the subject, in which the subject has been determined to be in need of the cardiac therapy according to the diagnostic methods described herein.

The subject may be one that has experienced cardiovascular injury or disease (e.g., vascular cardiovascular injury or disease, traumatic cardiovascular injury or disease, infectious cardiovascular injury or disease, autoimmune cardiovascular injury or disease, metabolic cardiovascular injury or disease, inflammatory cardiovascular injury or disease, degenerative cardiovascular injury or disease, or neoplastic cardiovascular injury or disease). In another embodiment, the method further comprises collecting at least one sample from the subject (e.g., two or more samples (e.g., 2, 3, 4, 5, 6, or more samples, which may be collected at the same time or at different times and/or from the same tissue or different tissues). In another embodiment, the method further comprises computationally predicting or

determining a cardiovascular status of the subject based on the expression level of one or more of the biomarkers.

Also featured is a method for detecting a level of a biomarker (e.g., one, two, three, four, five, ten, twenty, thirty, forty, fifty, sixty, or seventy-four of the biomarkers of SEQ ID NOs: 1-74, or a complement thereof, and variants thereof having at least 80% (e.g., 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to any one of SEQ ID NOs: 1-74, or a complement thereof; e.g., the biomarker(s) detected may be one or more of the biomarkers of PC1, PC2, and/or PC3 (see Figure 5) or one or more of SEQ ID NOs: 1-12)) in a sample from a subject having a cardiovascular disease or injury, such as a subject who has had a myocardial infarction (MI) or a subject with heart failure. The method includes (a) contacting the sample (e.g., a blood sample) from the subject including one or more nucleic acid molecules with: i) one or more single-stranded nucleic acid molecules (e.g., single-stranded nucleic acid molecules having a length in the range of 1 to 200 (e.g., 1-100, 1-50, 1-30, 1-25, 1-20, or 1-15) nucleotides) capable of specifically hybridizing with nucleotides of one or more of the biomarkers selected from any one or more of SEQ ID NOs: 1-74, or a complement thereof (e.g., the single-stranded nucleic acid molecules may be affixed to, e.g., a solid support, e.g., a device, such as a microarray); and (b) detecting a level of the one or more biomarkers or the complement thereof in the sample by detecting hybridization between the one or more single-stranded nucleic acid molecules and the one or more nucleic acid molecules of the sample (e.g., by performing microarray analysis or quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)). The expression level of the biomarker(s) may be detected by determining the level of a messenger RNA (mRNA) corresponding to the biomarker(s); or a complementary DNA (cDNA) thereof. In another embodiment, the method comprises collecting two samples at two distinct times and/or from one or more tissue sources. In another embodiment, the method comprises determining expression of the biomarker(s) based on RNA sequencing or by measurement on a platform, e.g., Fireplex (ABCAM). In an embodiment, the level of expression of one or more of the biomarkers (e.g., an increase in the level of expression of one or more of the biomarkers (e.g., a two-fold or more (e.g., 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 50-, 100-, or 1000-fold) increase relative to a reference level) indicates the cardiovascular status of the subject (e.g., a human), e.g., whether the subject will experience beneficial or adverse cardiac (e.g., LV) remodeling (e.g., after a cardiovascular event (e.g., MI), the likely response of a subject to a treatment (e.g., for a cardiovascular condition), the presence of a disease condition (e.g., a cardiovascular disease or condition), likely recovery from a disease condition (e.g., a cardiovascular disease or condition), an improvement in a prior determination of a cardiovascular status, or combinations thereof).

A second aspect of the invention features a method of determining cardiovascular status of a subject (e.g., a human), e.g., whether the subject will experience beneficial or adverse cardiac (e.g., LV) remodeling (e.g., after a cardiovascular event (e.g., MI), the likely response of a subject to a treatment (e.g., for a cardiovascular condition), the presence of a disease condition (e.g., a cardiovascular disease or condition), likely recovery from a disease condition (e.g., a cardiovascular disease or condition), an improvement in a prior determination of a cardiovascular status, or combinations thereof). The method includes: i) measuring (e.g., the expression level of) one or more biomarkers selected from SEQ ID NOs: 1-74 (e.g., biomarkers of SEQ ID NOs: 1-74 or variants thereof having at least 80% (e.g., 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to SEQ ID NOs: 1-74, or a complement thereof; e.g., one or more of the biomarkers of PC1, PC2, and/or PC3 (see Figure 5) or one or more of SEQ ID NOs: 1-12)); ii)

measuring cardiac function in the subject, e.g., using imaging (e.g., magnetic resonance imaging (CMR)) and/or ventricular ejection fraction (e.g., left ventricular ejection fraction); and/or iii) measuring cardiac structure, e.g., using imaging (e.g., CMR, e.g., segmentation of ventricular myocardium). In another embodiment, the method includes computationally predicting or determining cardiovascular status in the subject by: i) evaluating medication information; ii) evaluating in a sample from the subject for a change in expression of one or more of the biomarkers relative to one or more control samples (e.g., showing a two-fold or more (e.g., 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 50-, 100-, or 1000-fold) increase in expression of the biomarker(s) in a sample from the subject relative to a control sample); iii) using a statistical model to predict or determine the cardiovascular status; using a principal component analysis; and/or analyzing one or more cardiac images of the subject. The method of the second aspect can further include determining an elevation of biomarker level. The method of the second aspect can further include determining a cardiovascular status of the subject (e.g., whether the subject will experience or has had beneficial or adverse cardiac (e.g., LV) remodeling (e.g., after a cardiovascular event (e.g., MI)) at a current time (e.g., substantially concurrent with a cardiac event (e.g., an MI)), or at a future time (e.g., subsequent to a cardiovascular event (e.g., 1-4 days, weeks, months, or years after a cardiac event (e.g., an MI)), following a treatment (e.g., administration of an anti-inflammatory agent), after development of a disease condition (e.g., a cardiovascular condition), following recovery from a disease condition (e.g., a cardiovascular condition), or following an improvement in a prior determination of a cardiovascular status in the subject, or combinations thereof. The method of the second aspect can further comprise analyzing the CMR of the subject for one or more or all of the following: segmenting a myocardial region, segmenting a ventricular region, quantifying an ejection volume, quantifying an infarct size, or quantifying a mass index.

A third aspect features a method for using measured cardiovascular status to determine a cardiac therapy for the subject (e.g., an anti-inflammatory agent, such as an interleukin 1-beta receptor antagonist (e.g. anakinra), a beta blocker, an angiotensin-converting enzyme inhibitor, or an angiotensin-2 receptor blocker) or the need of the subject for a cardiac therapy. The cardiovascular status can be measured according to a method of the first or second aspect of the invention.

A fourth aspect of the invention features a method for determining a response of the subject (e.g., a positive or negative response of the subject) to a cardiac therapy (e.g., an anti-inflammatory agent, such as an interleukin 1-beta receptor antagonist (e.g. anakinra), a beta blocker, an angiotensin-converting enzyme inhibitor, or an angiotensin-2 receptor blocker) or the need of the subject for a cardiac therapy. The method includes i) measuring one or more biomarkers selected from SEQ ID NOs: 1-74, or a complement or variant thereof; ii) measuring cardiac function, e.g., using magnetic resonance imaging (CMR) (e.g., left ventricular ejection fraction); and/or iii) measuring cardiac structure, e.g., using CMR (e.g. segmentation of ventricular myocardium). The method may be performed before a treatment, after a treatment or a course of treatment, or combinations thereof. The cardiovascular status can be measured according to a method of the first or second aspect of the invention.

A fifth aspect of the invention features a kit (e.g., for determining a cardiovascular status of a subject) with one or more reagents for collecting nucleic acid molecules from a sample from a subject, one or more reagents for amplifying the nucleic acid molecules to produce an amplified sample, and one or more reagents for measuring expression of a biomarker, e.g., a biomarker having at least 80% (e.g.,

85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to the sequence of any one of SEQ ID NOs: 1-74, or a complement thereof; e.g., reagents for measuring one or more of the biomarkers of PC1, PC2, and/or PC3 (see Figure 5) or one or more of SEQ ID NOs: 1-12). In an embodiment, the kit may be used to assess at least one sample or multiple samples, e.g., two samples may be collected, e.g., at the same time or at two or more different times. Implementations of the kit may include computer readable (e.g., non-transitory computer readable) instructions for predicting or determining a cardiovascular status of a subject based on, e.g., the level of expression of one or more of the biomarker(s). Determining expression of at least one of the biomarker(s) may comprise extracting RNA from the at least one sample. Determining expression of at least one of the biomarkers may comprise constructing RNA libraries from the at least one sample. Determining expression of at least one of the biomarkers may comprise performing gel electrophoresis. Determining expression of at least one of the biomarker may comprise determining expression of two or more biomarkers. Determining expression of at least one of the biomarkers may be based on RNA sequencing (e.g., RNA-Seq). Determining expression of at least one of the biomarkers may be based on quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and/or by measurement on a platform, e.g., Fireplex (ABCAM). Determining expression of at least one of the biomarkers may comprise the use of a device, e.g. a microarray, to which one or more single-stranded nucleic acid molecules are attached (e.g., nucleic acid molecules having a length in the range of 1 to 1000 nucleotides, e.g., 1-15, 1-20, 10-20, 20-40, 40-60, 60-100, 100-150, 150-200, 200-350, 350-500, 500-650, 650-800, or 800-1000 nucleotides), in which the single-stranded nucleic acid molecules specifically hybridize to at least one of the biomarkers of SEQ ID NOs: 1-74, or a complement thereof or a variant thereof having at least 80% (e.g., 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to the sequence of any one of SEQ ID NOs: 1-74; e.g., the single-stranded nucleic acid molecules specifically hybridize to at least one of the biomarkers of PC1, PC2, and/or PC3 (see Figure 5) or one or more of SEQ ID NOs: 1-12). The single-stranded nucleic acid molecules of the device may be labeled or immobilized on a solid substrate. The cardiovascular status of the subject includes whether the subject will experience beneficial or adverse cardiac (e.g., LV) remodeling (e.g., after a cardiovascular event (e.g., MI), the likely response of a subject to a treatment (e.g., for a cardiovascular condition), the presence of a disease condition (e.g., a cardiovascular disease or condition), likely recovery from a disease condition (e.g., a cardiovascular disease or condition), an improvement in a prior determination of a cardiovascular status, or combinations thereof).

In some embodiments, a biomarker (e.g., a biomarker having the sequence of any one of SEQ ID NOs: 1-74, or a complement thereof or a variant thereof having at least 80% (e.g., 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to the sequence of any one of SEQ ID NOs: 1-74) may be in a form of a nucleic acid molecule, such as DNA, RNA, non-coding RNA, extracellular RNA, or miRNA, or a protein, or combinations thereof. The biomarker may have a nucleic acid sequence with at least 80% (e.g., 80%, 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, or 74, or a complement thereof.

In some embodiments, a biomarker may comprise a RNA having at least 80% (e.g., 80%, 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13,

14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, or 74, or a complement thereof.

In some embodiments, a biomarker may comprise a nucleic acid (e.g., RNA) having at least 80% (e.g., 80%, 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 70, 71, 72, 73, or 74, or a complement thereof.

In some embodiments, a biomarker may be in a form of non-coding RNA, extracellular RNA, miRNA, or a protein, or combinations thereof. A biomarker may comprise a nucleic acid having at least 80% (e.g., 80%, 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to at least one sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, or 74, or a complement thereof.

Implementations of computationally predicting or determining a cardiovascular status of a subject (whether the subject will experience beneficial or adverse cardiac (e.g., LV) remodeling (e.g., after a cardiovascular event (e.g., MI), the likely response of a subject to a treatment (e.g., for a cardiovascular condition), the presence of a disease condition (e.g., a cardiovascular disease or condition), likely recovery from a disease condition (e.g., a cardiovascular disease or condition), an improvement in a prior determination of a cardiovascular status, or combinations thereof) may comprise using a demographic profile. In some applications, computationally predicting a cardiovascular status may comprise using medication information (e.g., of the subject). In some cases, computationally predicting or determining a cardiovascular status of the subject may comprise evaluating in a sample from the subject a change in the expression level of a biomarker relative to the expression level of the biomarker in one or more control samples. In an embodiment, the biomarker has the sequence of any one of SEQ ID NOs: 1-74, or a complement thereof or a variant thereof having at least 80% (e.g., 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to the sequence of any one of SEQ ID NOs: 1-74; e.g., the biomarker has the sequence of one or more of the miRNAs of PC1, PC2, and/or PC3 (see Figure 5), such as the sequence of one or more of SEQ ID NOs: 1-12)). In some embodiments, computationally predicting or determining a cardiovascular status of the subject may comprise using a statistical model to predict the subject's cardiovascular status. Implementations of computationally predicting or determining a cardiovascular status may comprise using a principal component analysis. Implementations of computationally predicting or determining a cardiovascular status may comprise analyzing one or more cardiac images from the subject (e.g., a human). Implementations of analyzing one or more cardiac images may comprise one or more or all of the following: segmenting a myocardial region, segmenting a ventricular region, quantifying an ejection volume, quantifying an infarct size, or quantifying a mass index.

In some cases, the cardiovascular status may include a cardiovascular status of the subject (e.g., whether the subject will experience or has had beneficial or adverse cardiac (e.g., LV) remodeling (e.g., after a cardiovascular event (e.g., MI)) at a current time (e.g., substantially concurrent with a cardiac event (e.g., an MI)), at a future time (e.g., subsequent to a cardiovascular event (e.g., 1-4 days, weeks, months, or years after a cardiac event (e.g., an MI)), following a treatment (e.g., administration of an anti-inflammatory agent), after development of a disease condition (e.g., a cardiovascular condition), following

recovery from a disease condition (e.g., a cardiovascular condition), or following an improvement in a prior determination of a cardiovascular status in the subject, or combinations thereof. In some embodiments, the treatment may include a cardiac therapy. The response of the subject may be a positive response of the subject or a negative response of the subject.

The kit may be used for evaluating cardiovascular status of the subject before a treatment, after a treatment or a course of a treatment, or combinations thereof.

In an embodiment of all aspects of the invention, the subject is a human. In another embodiment of all aspects of the invention, the methods may include determining the expression level of one or more (e.g., all) of the PC1 biomarkers, the PC2 biomarkers, or the PC3 biomarkers (see, e.g., Fig. 5) or the kit may include reagents for determining the expression level of one or more (e.g., all) of the PC1 biomarkers, the PC2 biomarkers, or the PC3 biomarkers.

DEFINITIONS

The term "treatment" as used herein refers to, for example, administering to a subject (e.g., a human) a therapy (e.g., cardiac resynchronization therapy, a compound or a drug (e.g., for treating a cardiac disease or condition, such as, e.g., an anti-inflammatory agent, such as an interleukin 1-beta receptor antagonist (e.g. anakinra), a beta blocker, an angiotensin-converting enzyme inhibitor, or an angiotensin-2 receptor blocker), or combinations thereof) or a form of medical intervention to treat a cardiac disease or symptom thereof of the cardiovascular system (e.g., a cardiac disease or symptom thereof that affects the heart (e.g., an MI)).

The term "cardiovascular injury or disease" refers to a pathological process or condition of any etiology (e.g., vascular cardiovascular injury or disease, traumatic cardiovascular injury or disease, infectious cardiovascular injury or disease, autoimmune cardiovascular injury or disease, metabolic cardiovascular injury or disease, inflammatory cardiovascular injury or disease, degenerative cardiovascular injury or disease, or neoplastic cardiovascular injury or disease).

The term "sample" as used herein refers to a subject's biological sample (e.g., tissues, biofluids, bloods, plasmas, urines, discharges, sputa, or genetic materials, or combinations of them)

The term "biomarker" as used herein refers to, for example, a biomolecule or a portion of a subject's biological sample that is expressed in a form being measured (e.g., a nucleic acid (e.g., a DNA, an RNA, a non-coding RNA, a mRNA, a miRNA, and a precursor-miRNA), a protein, and combinations thereof, whose presence, absence, or expression level indicates an association with a current or future status of a heart condition (e.g., beneficial or adverse cardiac (e.g., LV) remodeling). The heart may or may not have undergone a cardiac therapy.

The term "cardiac remodeling" as used herein refers to changes that occur in the heart after cardiac injury or disease. Beneficial cardiac remodeling refers to changes in the heart (e.g., the left ventricle) that may not cause disease or have an adverse effect on (e.g., a reduction in) cardiac function (e.g., a reduction in LVEF). Adverse cardiac remodeling refers to changes in the heart (e.g., the left ventricle) that may cause disease or have an adverse effect on (e.g., a reduction in) cardiac function (e.g., a reduction in LVEF).

The term "computational prediction" refers to the use of computer-based analysis of one or more large data sets.

The term "cardiovascular condition" refers to the state of a subject's cardiovascular system (e.g., the presence or absence of a cardiovascular disease or injury, such as the presence or absence of beneficial or adverse cardiac (e.g., LV) remodeling).

The term "cardiovascular status" refers to a condition of the subject's cardiovascular system (e.g., the heart), e.g., at a particular point in time, such as substantially currently with the occurrence of a cardiovascular injury or disease (e.g., an MI) or after a cardiovascular injury or disease (e.g., an MI)., for 1-4 hours, days, weeks, months, or years after the cardiovascular injury or disease. For example, the subject's cardiovascular status indicates, e.g., the presence or absence of heart failure, e.g. left ventricular failure.

The term "post-MI time course" refers to a period following an MI, e.g., an acute period, a subacute period, or a chronic period.

The term "positive response" refers to a response of a subject (e.g., a human) that improves the function or structure of the subject's heart, as determined by, e.g., clinical assessment, imaging, or laboratory studies (e.g., a determination of the expression level of one of more of the biomarkers described herein).

The term "negative response" refers to a response of the subject (e.g., a human) that worsens the function or structure of the subject's heart, as determined by, e.g., clinical assessment, imaging, or laboratory studies (e.g., a determination of the expression level of one of more of the biomarkers described herein).

The term "control" or "control sample" as used herein refers to, for example, a sample from a subject (e.g., human, animal, or cell lines) who is normal (e.g., a subject with no clinical symptom(s) of a cardiac disease or condition, such as MI) or who has undergone a cardiac therapy that results in any combination of alleviation of symptoms of the cardiac disease, reduction in risk of mortality, or delay in mortality.

The term "reference level" as used herein refers to the level of expression of one or more of the biomarkers described herein in a population of subjects following a cardiovascular event (e.g., an MI) who have undergone adverse or beneficial cardiac (e.g., LV) remodeling or in a population of normal subjects (e.g., without a history of a cardiovascular event (e.g., an MI)).

The term "level of expression" or "expression level" or "expression" as used herein refers to, for example, an amount of a biomarker described herein present in a sample (e.g., a sample from a subject, such as a human, e.g., following a cardiovascular event (e.g., an MI)) relative to the amount of that biomarker in a control sample (e.g., a sample from a normal subject (e.g., a subject without a history of a cardiovascular event (e.g., an MI)) or relative to a cutoff value (e.g., a cutoff value established based on the expression level in a population of normal subjects, e.g., subjects without a history of a cardiovascular event (e.g., an MI)). An "increase" in a "level of expression" of a biomarker means an increased amount of a biomarker present in a sample relative to the amount of that biomarker in a control sample or relative to a cutoff value for the reference level. A "decrease" in a "level of expression" means a decreased amount of a biomarker present in a sample relative to the amount of that biomarker in a control sample or relative to a cutoff value for reference level.

The terms "miRNA" and "miR" as used interchangeably herein refer to a small non-coding RNA (e.g., an RNA that is incorporated into RNA induced silencing complexes and plays an important role in

post-transcriptional gene regulation either by transcriptional degradation or translational repression of mRNA).

The term "miR-1" as used in this document indicates a mature miR-1 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 1 (UGGAAUGUAAAGAAGUAUGUAU)) or a miR-1 precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 2 (UGGGAAACAUACUUCUUUAUAUGCCCAUAUGGACCUGCUAAGCUAUGGAAUGUAAAGAAGUAUG UAUCUCA)).

The term "miR-100-5p" as used in this document indicates a mature miR-100-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 3 (AACCCGUAGAUCCGAACUUGUG)) or a miR-100-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 4 (CCUGUUGCCACAAACCCGUAGAUCCGAACUUGUGGUAUUAGUCCGCACAAGCUUGUAUCUAUAG GUAUGUGUCUGUUAGG)).

The term "miR-133a-5p" as used in this document indicates a mature miR-133a-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 5 (AGCUGGUAAAAUGGAACCAAAU)) or a miR-133a-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 6 (ACAAUGCUUUGCUAGAGCUGGUAAAAUGGAACCAAAUCGCCUCUUCAAUGGAUUUGGUCCCCUU CAACCAGCUGUAGCUAUGCAUUGA)).

The term "miR-146a-5p" as used in this document indicates a mature miR-146a-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 7 (UGAGAACUGAAUUCCAUGGGUU)) or a miR-146a-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 8 (CCGAUGUGUAUCCUCAGCUUUGAGAACUGAAUUCCAUGGGUUGUGUCAGUGUCAGACCUCUGAA AUUCAGUUCUUCAGCUGGGAUAUCUCUGUCAUCGU)).

The term "miR-146b-5p" as used in this document indicates a mature miR-146b-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 9 (UGAGAACUGAAUUCCAUAGGCU)) or a miR-146b-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 10 (CCUGGCACUGAGAACUGAAUUCCAUAGGCUGUGAGCUCUAGCAAUGCCCUGUGGACUCAGUUCU GGUGCCCGG)).

The term "miR-150-5p" as used in this document indicates a mature miR-150-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 11 (UCUCCCAACCCUUGUACCAGUG)) or a miR-150-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 12 (CUCCCCAUGGCCCUGUCUCCCAACCCUUGUACCAGUGCUGGGCUCAGACCCUGGUACAGGCCU GGGGGACAGGGACCUGGGGAC)).

The term "miR-155-5p" as used in this document indicates a mature miR-155-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 13 (UUAAUGCUAAUCGUGAUAGGGGU)) or a miR-155-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 14 (CUGUUAAUGCUAAUCGUGAUAGGGGUUUUUGCCUCCAACUGACUCCUACAUAUUAGCAUUAACAG)).

The term "miR-15a-5p" as used in this document indicates a mature miR-15a-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 15 (UAGCAGCACAUAAUGGUUUGUG)) or a miR-15a-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 16 (CCUUGGAGUAAAGUAGCAGCACAUAAUGGUUUGUGGAUUUUGAAAAGGUGCAGGCCAUAUUGUGCUGCCUCAAAAAUACAAGG)).

The term "miR-193b-5p" as used in this document indicates a mature miR-193b-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 17 (CGGGGUUUUGAGGGCGAGAUGA)) or a miR-193b-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 18 (GUGGUCUCAGAAUCGGGGUUUUGAGGGCGAGAUGAGUUUAUGUUUUAUCCAACUGGCCCUCAAAGUCCCGCUUUUGGGGUCAU)).

The term "miR-194-5p" as used in this document indicates a mature miR-194-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 19 (UGUAACAGCAACUCCAUGUGGA)) or a miR-194-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 20 (AUGGUGUUAUCAAGUGUAACAGCAACUCCAUGUGGACUGUGUACCAAUUUCCAGUGGAGAUGCUGUUACUUUUGAUGGUUACCAA)).

The term "miR-200a-3p" as used in this document indicates a mature miR-200a-3p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 21 (UAACACUGUCUGGUAACGAUGU)) or a miR-200a-3p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 22 (CCGGGCCCCUGUGAGCAUCUUACCGGACAGUGCUGGAUUUCCCAGCUUGACUCUAACACUGUCUGGUAACGAUGUUCAAAGGUGACCCGC)).

The term "miR-208a-3p" as used in this document indicates a mature miR-208a (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 23 (AUAAGACGAGCAAAAAGCUUGU)) or a miR-208a precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 24 (UGACGGGCGAGCUUUUGGCCCGGGUUAUACCUGAUGCUCACGUAUAAGACGAGCAAAAAGCUUGUUGGUCA)).

The term "miR-208b-3p" as used in this document indicates a mature miR-208b-3p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 25 (AUAAGACGAACAAAAGGUUUGU)) or a miR-208b-3p precursor (e.g., a nucleic acid

having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 26 (CCUCUCAGGGAAGCUUUUUGCUCGAAUUAUGUUUCUGAUCCGAAUAUAAGACGAACAAAAGGUUUGUCUGAGGGCAG)).

The term "miR-21-5p" as used in this document indicates a mature miR-21-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 27 (UAGCUUAUCAGACUGAUGUUGA)) or a miR-21-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 28 (UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCUGUCUGACA)).

The term "miR-215-5p" as used in this document indicates a mature miR-215-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 29 (AUGACCUAUGAAUUGACAGAC)) or a miR-215-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 30 (AUCAUUCAGAAAUGGUAUACAGGAAAAUGACCUAUGAAUUGACAGACAAUAUAGCUGAGUUUGUCUGUCAUUUCUUUAGGCCAAUAUUCUGUAUGACUGUGCUACUUCAA)).

The term "miR-223-3p" as used in this document indicates a mature miR-223-3p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 31 (UGUCAGUUUGUCAAAUACCCCA)) or a miR-223-3p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 32; CCUGGCCUCCUGCAGUGCCACGCUCCGUGUAUUUGACAAGCUGAGUUGGACACUCCAUGUGGUAGAGUGUCAGUUUGUCAAAUACCCCAAGUGCGGCACAUGCUUACCAG).

The term "miR-29a-3p" as used in this document indicates a mature miR-29a-3p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 33 (UAGCACCAUCUGAAAUCGGUUA)) or a miR-29a-3p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 34; AUGACUGAUUUCUUUUGGUGUUCAGAGUCAAUAUAAUUUUCUAGCACCAUCUGAAAUCGGUUAU).

The term "miR-29a-5p" as used in this document indicates a mature miR-29a-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 35 (ACUGAUUUCUUUUGGUGUUCAG)) or a miR-29a-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 36; AUGACUGAUUUCUUUUGGUGUUCAGAGUCAAUAUAAUUUUCUAGCACCAUCUGAAAUCGGUUAU).

The term "miR-29b-3p" as used in this document indicates a mature miR-29b-3p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 37 (UAGCACCAUUUGAAAUCAGUGUU)) or a miR-29b-3p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 38; CUUCAGGAAGCUGGUUUCAUAUGGUGGUUUAGAUUUAAAUAGUGAUUGUCUAGCACCAUUUGAAAUCAGUGUUCUUGGGGG).

The term "miR-29c-3p" as used in this document indicates a mature miR-29c-3p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 39 (UAGCACCAUUUGAAAUCGGUUA)) or a miR-29c-3p precursor (e.g., a nucleic acid having a

sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 40; AUCUCUUACACAGGCUGACCGAUUUCUCCUGGUGUUCAGAGUCUGUUUUUGUCUAGCACCAUUU GAAAUCGGUUAUGAUGUAGGGGGA).

The term "miR-30a-5p" as used in this document indicates a mature miR-30a-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 41 (UGUAAACAUCCUCGACUGGAAG)) or a miR-30a-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 42 (GCGACUGUAAACAUCCUCGACUGGAAGCUGUGAAGCCACAGAUGGGCUUUCAGUCGGAUGUUUG CAGCUGC)).

The term "miR-30d-5p" as used in this document indicates a mature miR-30d-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 43 (UGUAAACAUCCCCGACUGGAAG)) or a miR-30d-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 44 (GUUGUUGUAAACAUCCCCGACUGGAAGCUGUAAGACACAGCUAAGCUUUCAGUCAGAUGUUUGC UGCUAC)).

The term "miR-3168" as used in this document indicates a mature miR-3168 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 45 (GAGUUCUACAGUCAGAC)) or a miR-3168 precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 46 (AAGAUCAUGAGUUCUACAGUCAGACAGCCUGAGUUGGAGGCUCAUCUUCACUUCUUGCUGUGUG ACCCUGGGCCAGUGACUU)).

The term "miR-378c" as used in this document indicates a mature miR-378c (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 47 (ACUGGACUUGGAGUCAGAAGAGUGG)) or a miR-378c precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 48 (GGAGGCCAUCACUGGACUUGGAGUCAGAAGAGUGGAGUCGGGUCAGACUUCAACUCUGACUUU GAAGGUGGUGAGUGCCUC)).

The term "miR-378e" as used in this document indicates a mature miR-378e (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 49 (ACUGGACUUGGAGUCAGGA)) or a miR-378e precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 50 (CUGACUCCAGUGUCCAGGCCAGGGGCAGACAGUGGACAGAGAACAGUGCCCAAGACCACUGGAC UUGGAGUCAGGACAU)).

The term "miR-381-3p" as used in this document indicates a mature miR-381-3p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 51 (UAUACAAGGGCAAGCUCUCUGU)) or a miR-381-3p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 52 (UACUUAAAGCGAGGUUGCCCUUUGUAUAUUCGGUUUAUUGACAUGGAAUAUACAAGGGCAAGCU CUCUGUGAGUA)).

The term "miR-423-5p" as used in this document indicates a mature miR-423-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ

ID NO: 53 (UGAGGGGCAGAGAGCGAGACUUU)) or a miR-423-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 54 (AUAAAGGAAGUUAGGCUGAGGGGCAGAGAGCGAGACUUUUCUAUUUUCCAAAAGCUCGGUCUGA GGCCCCUCAGUCUUGCUUCCUAACCCGCGC)).

The term "miR-4306" as used in this document indicates a mature miR-4306 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 55 (UGGAGAGAAAGGCAGUA)) or a miR-4306 precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 56 (AAGCUGCUUAGUGUCCUUAGAGUCUCCAGAGGCAUCCCUAACCCAGAAUCUUUUGACUGUCCUC UGGAGAGAAAGGCAGUAGGUCUGUACC)).

The term "miR-4326" as used in this document indicates a mature miR-4326 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 57 (UGUUCCUCUGUCUCCCAGAC)) or a miR-4326 precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 58 (GCUGCUCUGCUGUUCCUCUGUCUCCCAGACUCUGGGUGGAUGGAGCAGGUCGGGGGCCA)).

The term "miR-4443" as used in this document indicates a mature miR-4443 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 59 (UUGGAGGCGUGGGUUUU)) or a miR-4443 precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 60 (GGUGGGGGUUGGAGGCGUGGGUUUUAGAACCUAUCCCUUUCUAGCCCUGAGCA)).

The term "miR-499a-5p" as used in this document indicates a mature miR-499a-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 61 (UUAAGACUUGCAGUGAUGUUU)) or a miR-499a-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 62 (GCCCUGUCCCCUGUGCCUUGGGCGGGCGGCUGUUAAGACUUGCAGUGAUGUUUAACUCCUCUC CACGUGAACAUCACAGCAAGUCUGUGCUGCUUCCCGUCCCUACGCUGCCUGGGCAGGGU)).

The term "miR-658" as used in this document indicates a mature miR-658 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 63 (GGCGGAGGGAAGUAGGUCCGUUGGU)) or a miR-658precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 64 (GCUCGGUUGCCGUGGUUGCGGGCCCUGCCCGCCCGCCAGCUCGCUGACAGCACGACUCAGGGC GGAGGGAAGUAGGUCCGUUGGUCGGUCGGGAACGAGG)).

The term "miR-744-5p" as used in this document indicates a mature miR-744-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 65 (UGCGGGGCUAGGGCUAACAGCA)) or a miR-744-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 66 (UUGGGCAAGGUGCGGGGCUAGGGCUAACAGCAGUCUUACUGAAGGUUUCCUGGAAACCACGCA CAUGCUGUUGCCACUAACCUCAACCUUACUCGGUC)).

The term "miR-98-5p" as used in this document indicates a mature miR-98-5p (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ

ID NO: 67 (UGAGGUAGUAAGUUGUAUUGUU)) or a miR-98-5p precursor (e.g., a nucleic acid having a sequence at least 80% (e.g.,85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 68 (AGGAUUCUGCUCAUGCCAGGGUGAGGUAGUAAGUUGUAUUGUUGUGGGGUAGGGAUAUUAGGC CCCAAUUAGAAGAUAACUAUACAACUUACUACUUUCCCUGGUGUGUGGCAUAUUCA)).

The term "PIR57322" as used in this document indicates a mature PIR57322 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 69 (GACAACAACGGCGGCCGTGACTATGC)).

The term "PIR51124" as used in this document indicates a mature PIR51124 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 70 (TGGTCGTGGTTGTAGTCCGTGCGAGA)).

The term "PIR43376" as used in this document indicates a mature PIR43376 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 71 (CAGAGTGTAGCTTAACACAAAGCACCCAACT)).

The term "PIR57584" as used in this document indicates a mature PIR57584 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 72 (GAGAAAGGAAAACACCGTGAGCCTCAGAC)).

The term "plasma" as used herein refers to, for example, the straw-colored/pale-yellow liquid component of blood that normally contains blood cells in whole blood in suspension. Blood plasma may be prepared by spinning a tube of fresh blood containing an anti-coagulant in a centrifuge until the blood cells fall to the bottom of the tube; the blood plasma may then be poured or drawn off.

The term "response" as used herein refers to, for example, an outcome in a subject following administration of a therapy. For instances of cardiac therapy, a response can be a positive response resulting in an improvement in cardiovascular function or structure (e.g., heart function or structure), such as an improvement in LV ejection fraction by >10%, e.g., at a certain period in time (e.g., at least or at most 1 week, 2 weeks, 4 weeks, 3 months, 6 months, 9 months, or 1 year)) post-therapy. A response can also be a negative response resulting in a decline in cardiovascular function or structure (e.g., heart function or structure), such as a decrease in LV ejection fraction to less than 50%, e.g., at a certain period in time (e.g., at least or at most 1 week, 2 weeks, 4 weeks, 3 months, 6 months, 9 months, or 1 year)) post-therapy.

The term "RNY1-201" as used in this document indicates a mature RNY1-201 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 74 (GGCTGGTCCGAAGGTAGTGAGTTATCTCAATTGATTGTTCACAGTCAGTTACAGATCGAACTCCTTG TTCTACTCTTTCCCCCCTTCTCACTACTGCACTTGACTAGTCTTTT)).

The term "sequence identity" as used herein refers to, for example, a molecular sequence (e.g., nucleic acid sequence) that has the same molecular sequence as a reference sequence, or has a specified percentage of molecular sequences that are the same at the corresponding location within a reference sequence when the two sequences are optimally aligned. For instance, a nucleic acid sequence may have at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the reference nucleic acid sequence, or a complement thereof. A length of comparison sequences may generally be at least 5 contiguous nucleotides, preferably at least 10, 11, 12, 13, 14, 15,

16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 contiguous nucleotides, and most preferably the full-length nucleotide sequence. Sequence identity may be measured using sequence analysis software on the default setting (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705). Such software may match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. In some embodiments, sequence identify may be determined with respect to a reference sequence or with respect to a complement of the reference sequence.

The term "SNO1291" as used in this document indicates a mature SNO1291 (e.g., a nucleic acid having a sequence at least 80% (e.g., 85%, 90%, 95%, 98%, 100%) identity to the sequence of SEQ ID NO: 73 (GCTGTACATGATGACAACTGGCTCCCTCTACTGAACTGCCATGAGGAAACTGCCATGTCACCCTTCTGATTACAG)).

BRIEF DESCRIPTION OF THE DRAWINGS

The features of the disclosed technologies are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the technologies are utilized, and the accompanying drawings of which:

Figure 1 shows graphs of RNA levels of cell-specific markers.

Figure 2 is a histogram showing the percent change in LVESV and LVM.

Figure 3 is a graph showing Top RNA species detected by sequencing.

Figure 4 is a flow chart showing an exemplary analysis flow.

Figure 5 is a Venn diagram showing the miRNAs associated with the top principal components.

Figure 6 shows graphs of statistically significant associations between principal components and myocardial structure/function.

Figure 7 shows a Venn diagram of miRNA targets.

Figures 8A, **8B**, and **8C** show the top three principal component groups: Fig. 8A (PC1), Fig. 8B (PC2), and Fig. 8C (PC3).

Figures 9A and **9B** are heat maps showing temporally distinct expression patterns of miRNA.

Figure 10 is a heat map showing miRNA expression in non-cardiomyocytes, endothelial cells, cardiomyocytes, and fibroblasts.

Figure 11 shows graphs of miRNA expression levels in EVs released in the media.

Figure 12 is a graph showing a principle components analysis plot of miRNAs in the post-MI cohort and healthy controls.

Figure 13 shows box-plot graphs of differentially expressed non-microRNA exRNAs in "adverse" vs "beneficial" remodelers. Data represents mean ± SEM, p values stated below plots.

DETAILED DESCRIPTION

Although early mortality following acute myocardial infarction (MI) has declined due to revascularization, subsequent cardiac remodeling and the development of heart failure remains a significant long-term complication in post-MI subjects. Post-MI cardiac remodeling is a complex process

and the molecular mechanisms and markers of differential remodeling have not been fully characterized. Small non-coding RNAs that modulate networks of gene expression have been implicated in cardiac remodeling, specifically myocardial fibrosis and inflammation. The technologies disclosed in this document associate one or more cardiovascular conditions with one or more biomarkers (e.g., a biomarker signature) derived from one or more of the following: plasma extracellular small non-coding RNAs (exRNAs), cardiac magnetic resonance imaging, and signaling networks. The signature exRNAs may temporally regulate, or be regulated, or both, during a period (e.g., a post-MI course).

Cardiac remodeling (e.g., post-MI cardiac remodeling) and heart failure remain a significant source of morbidity. Cardiac remodeling in any portion of the myocardium, such as the left ventricle (LV), is a multifaceted and dynamic process that may be the product of the complex interplay between infarct size, genetic and epigenetic influences on cell biology, and effects of neurohormonal antagonism.

Early identification of pathological LV remodeling is a significant clinical challenge in the treatment of post-MI subjects. Natriuretic peptides may be used as a biomarker for heart failure; however, their limitations include that: levels are elevated once there is a change in the LV function but significant remodeling has already occurred. There may be an inverse relationship between LV ejection fraction and likelihood of adverse remodeling, but many subjects with a preserved LV ejection fraction post-MI can still go on to develop adverse remodeling. The majority of post-MI heart failure cases in the modern era may occur with preserved LV function and may not be captured by alterations in natriuretic peptides. Finally, natriuretic peptides have not been shown to identify or predict post-MI heart failure or arrhythmias in large population studies.

Cardiac diseases (e.g., myocardial infarction and post-MI LV remodeling) may include distinct phases involving multiple cell types in the heart including cardiomyocytes, fibroblasts, endothelial cells, and leukocytes. The acute post-MI phase may be hallmarked by cardiomyocyte death and subsequent recruitment of inflammatory cells to remove dead cells and begin the repair process. Distinct from this period may be a sub-acute phase where the inflammatory response is likely resolved. In some cases of the sub-acute phase, fibroblast proliferation and secretion of extracellular matrix proteins may lead to the formation of scar. Chronically, continuation of these processes and the global impact of molecular changes on cardiac function may lead to what is termed "beneficial" or "adverse" LV remodeling. Differences in cellular response to events, timing of the resolution of inflammatory response, and the degree of fibrosis all contribute to driving LV remodeling down either beneficial or adverse paths. The technologies described in this document may characterize molecular mechanisms and markers of these stages and processes, and will offer the opportunity to identify potential early markers of adverse LV remodeling and to prevent the development of post-MI heart failure or other cardiovascular conditions.

The technologies disclosed in this document include the use of non-coding RNAs and/or information regarding the expression level (e.g., exact or relative or estimated expression levels, exact or relative or estimated abundance levels, complementary bases, intermediate products, or associated enzymes, or combinations of them) of non-coding RNAs to predict or determine the presence of or the amelioration of a cardiovascular disease and/or the beneficial or adverse cardiac (e.g., LV) remodeling in a subject (e.g., a subject that has experienced a cardiovascular event (e.g., MI). Non-coding RNAs disclosed in this document may include small non-coding RNAs, which are short regulatory transcripts (e.g., less than 10, 20, 30, 40, 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nt). Non-

coding RNAs may include other RNAs, such as microRNAs (miRNA), piwi-interacting RNA (piRNA), small nucleolar RNA (snoRNA), tRNA fragments, and Y RNA fragments. These RNA species may be capable of affecting the expression of gene networks relevant to cardiovascular diseases, for example, myocardial fibrosis, coronary atherosclerosis, and cardiac arrhythmias. Non-coding RNAs may be stably expressed in the circulation (extracellular RNA; exRNA) as well as in tissues, leading to the rise of their utility as diagnostic and prognostic biomarkers of cardiovascular diseases (e.g., cardiac (e.g., LV) remodeling).

The technologies disclosed in this document may include plasma exRNAs as biomarkers for various cardiovascular diseases, such as acute coronary syndrome, coronary artery disease, and cardiac ischemia. The technologies may include examining exRNAs in human circulation by RNA sequencing (RNA-Seq) to determine whether changes in plasma exRNAs associated with cardiovascular diseases; for instance, examining the temporal, structural, and functional aspects of cardiac remodeling post-MI. In some implementations, the cellular origin and temporal regulation of candidate exRNAs may be examined in animal and cell models. Cell models may be representations of some cardiovascular diseases.

Biomarkers

The one or more biomarkers detected in the methods described herein may include one or more non-coding RNAs (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to the sequence of SEQ ID NOs: 1-74 or a complement thereof). Non-limiting examples of non-coding RNAs include short regulatory transcripts (e.g., less than 10, 20, 30, 40, 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nt), microRNAs (miRNA), a miRNA precursor, piwi-interacting RNA (piRNA), small nucleolar RNA (snoRNA), tRNA fragments, Y RNA fragments, or a combination thereof. These RNA species may be capable of affecting the expression of gene networks relevant to cardiovascular diseases, for example, myocardial fibrosis, coronary atherosclerosis, and cardiac arrhythmias. Non-coding RNAs may be stably or transiently expressed in the circulation (extracellular RNA; exRNA), as well as in tissues. For example, the biomarkers presently disclosed may include plasma exRNAs (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof) as biomarkers for a cardiovascular disease, such as acute coronary syndrome, coronary artery disease, and cardiac ischemia, or for the presence of adverse cardiac (e.g., LV) remodeling. In certain instances, the methods described herein may include determining expression of exRNAs in human circulation, such as by RNA sequencing (e.g., RNA-Seq) or other methodologies known in the art (e.g., as outlined below). The methods can be used to determine a subject's risk of cardiovascular disease (e.g., post-MI and/or during cardiac remodeling).

In particular, the methods disclosed herein may involve detection of one or more biomarkers (e.g., 1 biomarker or 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 60, 70, or all 74 biomarkers) having at least 80% (e.g., 80%, 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, or 74. The biomarkers described herein may be assessed either singly or in combination. For example, the determination step of the methods described herein may include determining the expression level of one biomarker having at least

80% (e.g., 80%, 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to any one of SEQ ID NO: 1-74. Alternatively, the determination step may include determining the expression level of multiple (e.g., two or more) biomarkers having at least 80% (e.g., 80%, 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to any multiple of (e.g., any two of) SEQ ID NOs: 1-74 or a complement thereof. In instances where multiple (e.g., two or more) biomarkers are examined, the biomarkers may be examined in aggregate as a biomarker panel, profile, or signature, as further described herein. The process by which the biomarkers may be assessed is described in further detail below. The methods may include determining the expression level of one or more (e.g., all) of the PC1 biomarkers, the PC2 biomarkers, or the PC3 biomarkers (see, e.g., Fig. 5).

Sample collection

The sample (e.g., a plasma sample or tissue sample) collected from a subject may be acquired or collected via any method known in the art. Samples may be collected from one or more subjects for diagnostic testing, to serve as control samples, or both. Accordingly, in some instances, samples are collected from one or more human subjects, animal subjects, or both. Samples may be collected from subjects who are considered clinically normal (e.g., healthy; e.g., for the purpose of establishing a reference level or control cutoff value), abnormal (e.g., subjects who have or have had a cardiovascular disease, for example, myocardial fibrosis, coronary atherosclerosis, and cardiac arrhythmias), or both. Sample collection may be double-blind. Sample collection may be placebo-controlled designed to test the effects of a therapy (e.g., an anti-inflammatory agent; such as omega-3 fatty acid treatment (e.g., 4 grams/day for 6 months)), for example, after acute MI, to assess, e.g., an effect on adverse LV remodeling.

In some instances, more than one sample may be collected from the same subject. For example, in certain instances, sample collection may take place at multiple sites from a subject (e.g., a plasma sample and a tissue sample may be collected from a subject). Further, samples may be collected from a subject at one or more time points (e.g., 1 time point, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 time points). For example, samples may be collected as a time series, such as at an initial timepoint as a baseline, and at one or more times after the baseline. For example, the sample may be collected at a time point at, at least, or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 24, 28, or 30 days after the baseline. In some instances, the sample may be collected at a time point at, at least, or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,17, 18, 19, 20, 24, 28, 32, 36, 40, 44, 48, or 52 weeks after the baseline. In some instances, the sample may be collected at a time point at, at least, or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months after the baseline.

In particular, a sample may be collected from a subject during or immediately after a cardiovascular event (e.g., after acute coronary syndrome, coronary artery disease, and cardiac ischemia) and assessed for the level of one or more of the biomarkers of SEQ ID NOs: 1-74 or a subset thereof (e.g., one or more of the biomarkers of PC1, PC2, and/or PC3 (see Figure 5) or one or more of SEQ ID NOs: 1-12). During an acute phase of the cardiovascular event, one or more samples may be taken every hour, every two, three, four, five, or six hours, every 12, 24, or 36 hours, or one or more times per day, every other day, every third day, every fourth day, every fifth day, every sixth day, or every seventh day (e.g., for one, two, three, four, five, six, seven, or eight weeks or more following the

cardiovascular event). Samples may continue to be collected from the subject during treatment for the cardiovascular event (e.g., during an acute phase or chronic phase of the cardiovascular event) and/or subsequent to treatment for the cardiovascular event (e.g., after resolution of the acute phase of the cardiovascular event). For example, one or more samples may be taken from the subject during a chronic phase of the cardiovascular one or more times per month, bi-monthly, quarterly, semi-annually, or yearly (e.g., for one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, twenty-four, thirty-six, forty-eight, or sixty months or more following the cardiovascular event).

In some instances, the sample from the individual is a tissue sample, a whole blood sample, a plasma sample, a serum sample, or a combination thereof. In some instances, the sample is a tissue sample. For example, the tissue sample may be a cardiac tissue sample. Samples may additionally or alternatively include blood, venous blood, or plasma, or combinations thereof. Samples may include one or more cell types. The sample may include cardiomyocytes or non-cardiomyocytes, such as non-cardiomyocyte cardiac cells (e.g., cardiac immune cells), fibroblasts, and endothelial cells.

The sample may undergo processing. For example, blood may be centrifuged at 2000 xg for a period (e.g., 10 min) for plasma separation, and immediately stored at a low temperature (e.g., -80°C). The sample may be frozen, fresh, fixed (e.g., formalin fixed), centrifuged, and/or embedded (e.g., paraffin embedded), etc. The cell sample can be subjected to a variety of well-known post-collection preparative and storage techniques (e.g., nucleic acid and/or protein extraction, fixation, storage, freezing, ultrafiltration, concentration, evaporation, centrifugation, etc.) prior to assessing the amount of the biomarker in the sample. Likewise, biopsies may also be subjected to post-collection preparative and storage techniques, e.g., fixation, such as formalin fixation.

In some implementations, the cellular origin and temporal regulation of the one or more biomarkers may be detected in samples from an animal and cell model of a cardiovascular disease (e.g., acute coronary syndrome, coronary artery disease, and cardiac ischemia) to inform the diagnostic or treatment criteria described herein. For example, the biomarker may be detected in cardiomyocytes. Alternatively, the biomarker may be detected in non-cardiomyocytes, such as non-cardiomyocyte cardiac cells (e.g., cardiac immune cells), fibroblasts, and endothelial cells.

Sample collection may include collecting cardiac images of subjects using, for example, cardiac magnetic resonance imaging (CMR) or echocardiography or both, to determine cardiac status. Cardiac imaging may be used at the time of baseline or at one or more times after the baseline; e.g., a time point may be at, or at least, or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 24, 28, 32, 36, 40, 44, 48, or 52 weeks after the baseline.

Sample collections may include phenotyping. Examples of phenotyping tools include cardiac imaging, physician evaluation, or an existing diagnostic tool, or combinations of them. In some instances, phenotypes may include LV end-systolic volume index (LVESVI), LV ejection fraction, myocardial mass, and extracellular volume fraction (ECV; a validated surrogate of myocardial interstitial expansion).

In some instances, the subjects may undergo post-treatment follow-up; e.g., CMR for serial comparison for LV remodeling. Infarct size by late gadolinium enhancement may be quantified using, for example, at least one, two, three, or four standard deviations beyond mean remote myocardial signal intensity and full width half maximum method. Baseline characteristics may be compared via chi-squared (categorical) or Wilcoxon tests (continuous).

Biomarker detection

The presence and/or expression level or amount of one or more of the biomarkers described herein (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to the sequence of SEQ ID NOs: 1-74 or a complement thereof) can be determined qualitatively and/or quantitatively using material that includes, but is not limited to, DNA, mRNA, cDNA, proteins, protein fragments, and/or gene copy number. For example, the detection of the biomarkers described herein can involve a determination of the presence or expression level of one or more biomarkers based on, e.g., an exact expression level, an expression level relative to a reference level, or an estimated expression level of a non-coding RNA, or complementary sequence thereto. Alternatively or additionally, the biomarker may be detected based on an exact, relative, or estimated abundance; the levels of intermediate products; levels of proteins that can associate with the biomarker, or combinations thereof.

In some instances, nucleic acid expression levels of the biomarkers described herein may be measured by polymerase chain reaction (PCR)-based assays, e.g., quantitative PCR, real-time PCR, quantitative real-time PCR (qRT-PCR), reverse transcriptase PCR (RT-PCR), and reverse transcriptase quantitative PCR (RT-qPCR). Platforms for performing quantitative PCR assays include Fluidigm (e.g., BIOMARK™ HD System). Other amplification-based methods include, for example, transcript-mediated amplification (TMA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), and signal amplification methods, such as bDNA.

In some instances, the biomarkers described herein may be measured by sequencing-based techniques, such as, for example, RNA-Seq, serial analysis of gene expression (SAGE), high-throughput sequencing technologies (e.g., massively parallel sequencing), and Sequenom MassARRAY® technology. Biomarker expression also may be measured by, for example, NanoString nCounter, and high-coverage expression profiling (HiCEP). Additional protocols for evaluating the status of genes and gene products are found, for example in Ausubel et al., eds., 1995, *Current Protocols In Molecular Biology*, Units 2 (Northern Blotting), 4 (Southern Blotting), 15 (Immunoblotting), and 18 (PCR Analysis).

Other methods for detecting the biomarkers described herein include protocols which examine or detect mRNAs, such as target mRNAs, in a tissue or cell sample by microarray technologies. Using nucleic acid microarrays, test and control mRNA samples from test and control tissue samples are reverse transcribed and labeled to generate cDNA probes. The probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene.

Primers and probes may be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator, or enzyme. Such probes and primers can be used to detect the presence of the biomarker. As will be understood by the skilled artisan, many different primers and probes may be prepared based on the sequences provided herein (or, in the case of genomic DNA, their adjacent sequences) and used effectively to amplify, clone, and/or determine the presence and/or expression levels of the genes described herein.

Methodologies for detection of RNA expression levels are well-known in the art, and a skilled artisan will appreciate that any such method may be used in the present methods. Non-limiting examples of methodologies (e.g., RNA-Seq) that may be used to detect the presence or level of the biomarkers described herein are further described in the sub-sections below.

RNA-Seq

The methods described herein may include RNA-Seq for identifying one or more of the biomarkers described herein in a sample(s) from a subject. To identify exRNAs (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof) associated with cardiovascular status (e.g., LV remodeling) by plasma RNA-Seq, samples may be selected for running RNA-Seq. The subjects from which the samples are obtained may be assessed for the presence of beneficial cardiac remodeling (e.g., beneficial LV remodeling; e.g., a decrease in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%), or adverse cardiac remodeling (e.g., adverse LV remodeling; e.g., an increase in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%). The subjects in different conditions (e.g., beneficial vs. adverse cardiac remodeling) may be matched for age, sex, diabetes status, LVEF, or infarct size by CMR, or combinations thereof.

RNA may be extracted from plasma (e.g., at least 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, 0.6 mL, 0.7 mL, 0.8 mL, 0.9 mL, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 mL, or 10 mL). Then, RNA libraries may be constructed from the plasma. For example, the plasma RNA may be isolated with a protocol, and the libraries may be constructed and amplified from approximately or at least or at most 1 ng, 2 ng, 3 ng, 4 ng, 5 ng, 6 ng, 7 ng, 8 ng, 9 ng, 10 ng, 15 ng, 20 ng, 25 ng, 30 ng, 35 ng, 40 ng, 45 ng, 50 ng, 60 ng, 70 ng, 80 ng, 90 ng, or 100 ng RNA. Size selection of libraries may be performed by gel electrophoresis on a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, or 15% gel with excision of certain nucleotide bands (e.g., at least 50, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, or 300; or at most 50, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, or 300; or combinations of them). The bands may correspond to at least 2, 3, 4, 5, 6, 7, 8, 9, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 nucleotide RNA fragments. Libraries may be diluted to a final concentration (e.g., at least or at most 0.1 nM, 0.2 nM, 0.3 nM, 0.4 nM, 0.5 nM, 1 nM, 2 nM, 3 nM, 4 nM, or 5 nM), pooled, and sequenced.

Plasma exRNA from control samples may be isolated; for example, using a protocol (e.g., the mirVana PARIS protocol) with sequential phenol–chloroform extractions. RNA may be concentrated, and libraries may be prepared. Sample pools may be created, denatured, and clustered. The flowcells may be run for multiple (e.g., at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100) cycles with an indexing read.

The outputs from RNA-Seq may be de-multiplexed and the adaptor sequences within the read sequences may be trimmed. The processed sequences may be filtered for small RNAs greater than, for example, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, or 50 nucleotides in length. The sequences may be then aligned, quantified, and annotated. In some implementations, the analysis may use a hierarchical sequence mapping strategy that first maps and removes spike-in library, contaminants, and rRNA. Next, RNA species may be sequentially mapped to known mature miRNA, tRNA, snoRNA, piRNA, and Y RNA on the human genome sequence (hg19) using

sequence alignment tools (e.g., Bowtie, MUMmer, BLAST or Vmatch) with parameters that allow for 1 or 2 or 3 or 4 or 5 mismatches in seed alignment (-N 1), try two set of seeds (-R 2), and set the length of seed substrings to be 16 (-L 16). Mapped small RNA species may be quantified to read counts and normalized to reads per kilobase per million reads mapped (RPKM). Differential expression analysis may be performed for all contrasts. The p-values may be adjusted for multiple-test correction using False Discovery Rate (FDR).

Data analysis

The methods disclosed herein may include data analysis using statistical machine learning algorithms. For example, principal components analysis (PCA), an unsupervised learning technique that statistically groups correlated miRNAs (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof) together into principle components, may be employed for analyzing various types of data (e.g., RNA-Seq data, qPCR validation data, and cardiac images).

In some instances of analyzing differences in miRNA profiles between test subjects (e.g., post-MI, or experiencing one or more cardiovascular conditions (e.g., cardiovascular diseases, such as acute coronary syndrome, coronary artery disease, cardiac ischemia, myocardial fibrosis, coronary atherosclerosis, and cardiac arrhythmias)) and normal controls, miRNA reads per million (RPM) for all subjects may be computed and may be mean centered, standardized, and log-transformed before performing the analysis. In the analysis of PCA, the first few (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) principal components may be considered for all subjects. A similar approach may be applied to the validation data (e.g., data from qPCR or high-throughput qPCR). In the validation data, miRNA values may be centered and standardized (e.g., mean 0 and variance 1) before running PCA.

In some implementations, top few (e.g., at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) principal components may be considered. The consideration may be based on (1) examination of an eigenvalue plot, or (2) inclusion of principal components that explain up to a certain percentage (e.g., at least 50%, 60%, 70%, 80%, or 90%) of population variance, or both (1) and (2). Varimax rotation may be used to determine final PC loadings. PC scores based on loadings and miRNA expression values may be included in linear regression models for CMR outcome variables (described below).

Data analysis may include quantitative analysis of cardiac status (e.g., LV remodeling) by other information, such as cardiac images (e.g., CMR or echocardiography data). Examples of cardiac image analysis include serial quantification of LV volumes (end-diastole and end-systole), ECV in the non-infarct myocardium as a surrogate measure of interstitial fibrosis, and infarct size by late gadolinium enhancement. A map of segments (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 segments) on myocardial regions or ventricular regions, or both, may be used in quantification of ECV. In some implementations, infarct size and segments with late gadolinium enhancement may, or may not be, included in the measurement of non-infarct ECV.

In some instances, the quantitative analysis may include a linear regression analysis; for example, linear regression models may be used for several cross-sectional (e.g., at the time of the blood draw, or baseline at least 1, 2, 3, 4, 5, 6, 7, or 8 weeks post-MI) and longitudinal phenotypes (e.g.,

changes in LV phenotypes from baseline to at least 1, 2, 3, 4, 5, 6, 6, 7, 8, 9, 10, 11, or 12 months post-MI), such as LVESVI, LVEF, or LV mass index, or combinations thereof.

In some instances, the quantitative analysis may examine the association of baseline non-infarct myocardial fibrosis by ECV with each PC. The association may be based on a statistical model (e.g., machine learning, regression, or Bayesian inference, or combinations of them). The association may specify the CMR parameters (e.g., LVESVI, LVEF, or LV mass index) as outcomes, and include all PC scores, as well as adjustment for age, sex, race, presence of diabetes, a binary variable denoting randomization (e.g., a therapy vs. placebo, or a therapy vs. another therapy), and a binary variable representing use of any LV remodeling medication (e.g., beta-blockade, angiotensin converting enzyme inhibition, or angiotensin-II receptor blockade, or combinations of them). For the exRNAs other than miRNAs (yRNAs, snoRNAs and piRNAs), the association may be carried out for mean centered and standardized Ct values as a function of changes in LV phenotypes from baseline to a period (e.g., at least 1, 2, 3, 4, 5, 6, 6, 7, 8, 9, 10, 11, or 12 months) post-MI (LVESVI, LV Mass, or LVEF, or combinations thereof) after adjustment for all the variables used in the association analysis.

The technologies disclosed herein may include a pathway analysis. For example, mRNA targets of the miRNAs from top principal components (e.g., top 3 principal components, which individually denoted as PC1, PC2, and PC3; see Figure 5) that had a loading greater than a certain percentage (e.g., at least 50%, 60%, 70%, 80%, or 90%) may be obtained through pathway analysis on miRNA. In some instances, only those mRNAs that are experimentally observed targets of the miRNAs from each PC may be included in the core pathway analysis.

Analysis based on animal and cell models

A murine model may be used to identify or validate one or more biomarkers (e.g., the biomarkers of SEQ ID NOs: 1-74 and variants thereof having at least 80% or more sequence identity thereto) for use in the methods described herein (e.g., methods for determining cardiovascular status of a subject, such as the presence of beneficial or adverse cardiac (e.g., LV) remodeling). In some instances, adult wild-type C57BL/6 mice may be subjected to ischemia reperfusion (I/R) including a period (e.g., 10, 20, 30, 40, 50, 60, 90, or 120 minutes) of left anterior coronary artery ligation followed by reperfusion. Blood and heart tissue may be collected from the animals 24 hrs, 1 week, or 4 weeks, or a longer period, after I/R. In addition, hearts may be collected from healthy adult wild-type mice to isolate cardiomyocytes and non-cardiomyocytes, and primary endothelial cells and fibroblasts may be cultured from hearts of wild-type mice. Isolated cardiomyocytes, non-cardiomyocytes, and cultured primary endothelial cells may be confirmed by qPCR analysis cell-specific markers including cTnI, CD31, and Col1a1, as shown in Figure 1.

A cell model of hypoxia/reoxygenation may be used to identify or validate one or more biomarkers (e.g., the biomarkers of SEQ ID NOs: 1-74 and variants thereof having at least 80% or more sequence identity thereto) for use in the methods described herein (e.g., methods for determining cardiovascular status of a subject, such as the presence of beneficial or adverse cardiac (e.g., LV) remodeling). For example, neonatal rat ventricular myocytes (NRVMs) may be isolated from 1 day postnatal Sprague-Dawley pups and subjected to 24 hrs hypoxia (2% oxygen) followed by 12 hrs re-oxygenation. Cells may

be harvested in lysis buffer and media may be collected and sequentially centrifuged at 2,000 xg for 10 minutes and 3,000 xg for 10 minutes.

RNA extraction and qPCR may be used to determine or validate the expression or level of expression of one or more biomarkers (e.g., the biomarkers of SEQ ID NOs: 1-74 and variants thereof having at least 80% or more sequence identity thereto) for use in the methods described herein (e.g., methods for determining cardiovascular status of a subject, such as the presence of beneficial or adverse cardiac (e.g., LV) remodeling). RNA from cultured or isolated murine cells and mouse tissue samples may be isolated. Plasma samples may be 'spiked' with at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 pmol/mL exogenous cel-miR-39-3p and RNA may be extracted. In some embodiments of using pig plasma samples, RNA may be incubated with 1 unit of heparinase I at 25°C for 2 hrs to prevent heparin-mediated inhibition of downstream reactions. Extracellular vesicle (EV) associated RNA may be extracted from NRVM culture media and cellular RNA may be extracted as well. All RNA samples may be reverse transcribed and qPCR may be performed for specific miRNA species.

The methods described herein may also include a statistical analysis. U6 (mouse tissue and NRVM cells), cel-miR-39-3p (plasma), or hsa-miR-191-5p (isolated murine cells) may be used for qPCR normalization. In some instances, due to a lack of reliable housekeeper genes, culture media EV miRNA may be normalized to cell number and media volume input. Statistical analysis may be performed on ΔCt values. Data from the mouse model may be analyzed by a statistical analysis, such as one-way ANOVA with Tukey post-hoc analysis; cell data may be analyzed by another statistical analysis, such as Student's t-test.

In some instances, the qPCR ΔCt values for mouse tissue, plasma, and cell data may be analyzed by a clustering algorithm. In some implementations, the data may be exponentiated as 2-1/Ct, scaled, and centered before unsupervised agglomerative hierarchical clustering with a distance measure (e.g., Manhattan distance or Euclidean distance or Viterbi distance), followed by a clustering algorithm (e.g., hierarchical cluster analysis, Ward method). The clustering results can be visualized, such as a heatmap.

Determining Cardiovascular Status of a Subject Based on Biomarker Expression Level

The expression level of the one or more biomarkers described herein (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof) can be compared to a reference or control level or a cutoff value to predict or assess a subject's cardiovascular status (e.g., the presence of beneficial or adverse cardiac (e.g., LV) remodeling). For example, a change in the expression of the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof) relative to a reference level may indicate that the subject is likely to experience beneficial LV remodeling (e.g., a decrease in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%), or adverse LV remodeling (e.g., an increase in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%). The method may assess a change in the expression of one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5).

In some instances, an increase in the expression level of the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) in a sample (e.g., a tissue and/or plasma sample) relative to a reference or control level (e.g., relative to the expression level of the one or more biomarkers in a subject who experiences beneficial LV remodeling) or cutoff value indicates that the subject is likely to experience adverse LV remodeling (e.g., an increase in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%). Alternatively, in some instances, a decrease in the expression level of the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) in a sample (e.g., a tissue and/or plasma sample) relative to a reference or control level (e.g., relative to the expression level of the one or more biomarkers in a subject who experiences adverse LV remodeling) or cutoff value indicates that the subject is likely to experience beneficial LV remodeling (e.g., a decrease in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%).

For example, in some instances, an expression level of the one or more biomarkers in about the top 99th percentile (equal to, or higher than, about the 1% prevalence level), about the top 95th percentile (equal to, or higher than, about the 5% prevalence level), about the top 90th percentile (equal to, or higher than, about the 10% prevalence level), about the top 85th percentile (equal to, or higher than, about the 15% prevalence level), about the top 80th percentile (equal to, or higher than, about the 20% prevalence level), about the top 75th percentile (equal to, or higher than, about the 25% prevalence level), about the top 70th percentile (equal to, or higher than, about the 30% prevalence level), about the top 65th percentile (equal to, or higher than, about the 35% prevalence level), about the top 60th percentile (equal to, or higher than, about the 40% prevalence level), about the top 55th percentile (equal to, or higher than, about the 10% prevalence level), about the top 50th percentile (equal to, or higher than, about the 50% prevalence level), about the top 45th percentile (equal to, or higher than, about the 55% prevalence level), about the top 40th percentile (equal to, or higher than, about the 60% prevalence level), about the top 35th percentile (equal to, or higher than, about the 65% prevalence level), about the top 30th percentile (equal to, or higher than, about the 70% prevalence level), about the top 25th percentile (equal to, or higher than, about the 75% prevalence level), about the top 20th percentile (equal to, or higher than, about the 80% prevalence level), about the top 15th percentile (equal to, or higher than, about the 85% prevalence level), about the top 10th percentile (equal to, or higher than, about the 90% prevalence level), about the top 5th percentile (equal to, or higher than, about the 95% prevalence level), or about the top 1st percentile (equal to, or higher than, about the 99% prevalence level) of the expression of the one or more biomarkers in a reference population (e.g., a population of subjects who experience beneficial LV remodeling) identifies the individual as one who is likely to experience adverse LV remodeling (e.g., an increase in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%).

In some instances, an expression level of the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) that is higher than a reference or control level (e.g., relative

to one or more subjects who experience beneficial LV remodeling) or cutoff value refers to an overall increase of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% or greater in the expression level of the one or more biomarkers, detected by standard art-known methods, such as those described herein, as compared to the expression level of the one or more biomarkers in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue (e.g., as compared to the expression of the one or more biomarkers in a subject who experiences beneficial LV remodeling). In certain instances, an expression level of the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) that is higher than a reference expression level refers to an increase in the expression level of the one or more biomarkers in the sample, wherein the increase is at least about 1.5x, 1.75x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 25x, 50x, 75x, or 100x the expression level of the one or more biomarkers in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue (e.g., relative to the expression of the one or more biomarkers in a subject who experiences beneficial LV remodeling). In some instances, an expression level of the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) that is higher than a reference expression level refers to an overall increase in the expression level of the one or more biomarkers that is greater than about 1.5-fold, about 1.75-fold, about 2-fold, about 2.25-fold, about 2.5-fold, about 2.75-fold, about 3.0-fold, or about 3.25-fold as compared to the expression level of the one or more biomarkers in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue (e.g., as compared to the expression of the one or more biomarkers in a subject who experiences beneficial LV remodeling).

In some instances, an expression level of the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) that is higher than a reference expression level refers to an overall increase of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% or greater in the expression level of the one or more biomarkers, detected by standard art-known methods, such as those described herein, as compared to a pre-assigned expression level of the one or more biomarkers. In certain instances, an expression level for the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) that is higher than a reference expression level refers to an increase in the expression level of the one or more biomarkers in the sample, wherein the increase is at least about 1.5x, 1.75x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 25x, 50x, 75x, or 100x a pre-assigned expression level of the one or more biomarkers. In some instances, an expression level for the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) that is higher

than a reference expression level refers to an overall increase in the expression level of the one or more biomarkers that is greater than about 1.5-fold, about 1.75-fold, about 2-fold, about 2.25-fold, about 2.5-fold, about 2.75-fold, about 3.0-fold, or about 3.25-fold as compared to a pre-assigned expression level of the one or more biomarkers.

As an example, an expression level for the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to the sequence of SEQ ID NOs: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) can be assessed by comparison to a control gene or mRNA, such as a housekeeper gene or mRNA, such as a miRNA (e.g., for qPCR normalization), or to the expression level of the one or more biomarkers in control cell (e.g., a non-cardiac cell or a normal cardiac cell that has not been exposed to a cardiovascular insult or trauma) . An example of a control mRNA is, e.g., hsa-miR-191-5p.

Reference Expression Level

A reference expression level can be used to assess an expression level of one or more biomarkers described herein (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) in order, e.g., to make a diagnostic, predictive, prognostic, and/or therapeutic determination regarding the cardiovascular status (e.g., the presence of beneficial or adverse cardiac (e.g., LV) remodeling) of a subject (e.g., a human). For example, the reference expression level may be derived from expression levels in a reference sample from the same or a different subject undergoing testing and/or treatment, a reference population, an internal control (e.g., housekeeping gene), and/or a pre-assigned value. In certain instances, the reference expression level may be a pre-determined value (e.g., expression level, percentile, or other cut-off value) that was previously determined to significantly (e.g., statistically significantly) separate different subsets of individuals, e.g., a cut-off value that significantly separates a subset of subjects who experience beneficial cardiac (e.g., LV) remodeling (e.g., a decrease in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%) and a subset of subjects who experience adverse cardiac (e.g., LV) remodeling (e.g., an increase in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%). An appropriate numerical value for the reference expression level can be established based on the indication, the methodology used to detect expression levels (e.g., RNA-Seq or RT-qPCR), the statistical methods used to analyze the expression level, and/or the specific combinations of biomarkers examined.

Molecular signature

The methods described herein may involve determining the expression of two or more biomarkers (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 60, 70, or all 74 biomarkers, e.g., non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof, or, e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)). In instances where the expression of multiple (e.g., two or more) biomarkers is detected, the biomarkers may be assessed as a panel, profile, or signature, as further described herein. In some instances, the signature is based on two or more of the

biomarkers of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, and 74, such as, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 60, 70, or all 74 of the biomarkers described herein, e.g., non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity or a complement thereof) to any one of SEQ ID NO: 1-74 or based on, e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5). The expression level of two or more biomarkers can be evaluated in aggregate in accordance with methods standard in the art and/or expressed as a single value, e.g., as a biomarker signature value or score.

For example, the methods disclosed herein may include identifying signatures that associate plasma circulating exRNA profiles with one or more cardiovascular conditions (e.g., post-MI LV remodeling phenotypes). The profiles of non-control subjects may be distinct from controls. One or more signature miRNAs may not increase abundance or expression level in plasma at acute time-points. In some cases, a statistical analysis (e.g., PCA, probabilistic inference analysis, or Bayesian network analysis, or combinations of them) may identify that one or more signature miRNAs exhibit temporal expression of miRNAs differing in tissue and plasma.

For example, in studies of post-MI LV remodeling phenotypes, **Table 1** summarizes signatures associated with top principal components based on the PCA analysis. **Table 2** shows corresponding statistics of the signature miRNAs. The signatures may include, e.g., one or more or all of the following: SEQ ID NOS: 27-28 (miR-21-5p), SEQ ID NOS: 33-34 (miR-29a-3p), SEQ ID NOS: 37-38 (miR-29b-3p), SEQ ID NOS: 39-40 (miR-29c-3p), SEQ ID NOS: 41-42 (miR-30a-5p), SEQ ID NOS: 43-44 (miR-30d-5p), SEQ ID NOS: 3-4 (miR-100-5p), SEQ ID NOS: 7-8 (miR-146a-5p), SEQ ID NOS: 9-10 (miR-146b-5p), SEQ ID NOS: 11-12 (miR-150-5p), SEQ ID NOS: 19-20 (miR-194-5p), SEQ ID NOS: 31-32 (miR-223-3p), SEQ ID NOS: 47-48 (miR-378c), SEQ ID NOS: 53-54 (miR-423-5p), and SEQ ID NOS: 65-66 (miR-744-5p). Signatures may be used, individually or collectively, for predicting a cardiovascular condition. For instance, referring to **Table 1**, signatures SEQ ID NOS: 27-28 (miR-21-5p), SEQ ID NOS: 41-42 (miR-30a-5p), SEQ ID NOS: 43-44 (miR-30d-5p), SEQ ID NOS: 3-4 (miR-100-5p), SEQ ID NOS: 7-8 (miR-146a-5p), SEQ ID NOS: 9-10 (miR-146b-5p), SEQ ID NOS: 31-32 (miR-223-3p), SEQ ID NOS: 53-54 (miR-423-5p), and SEQ ID NOS: 65-66 (miR-744-5p) belong to PC1, and they may individually or collectively be used to predict a cardiovascular condition (e.g., ΔLV). Similarly, SEQ ID NOS: 27-28 (miR-21-5p), SEQ ID NOS: 33-34 (miR-29a-3p), of SEQ ID NOS: 37-38 (miR-29b-3p), SEQ ID NOS: 39-40 (miR-29c-3p), SEQ ID NOS: 41-42 (miR-30a-5p), SEQ ID NOS: 43-44 (miR-30d-5p), SEQ ID NOS: 11-12 (miR-150-5p), and SEQ ID NOS: 19-20 (miR-194-5p) belong to PC2, and they may individually or collectively be used predict a cardiovascular condition (e.g., LVEF). Further, SEQ ID NOS: 47-48 (miR-378c) belongs to PC3, and it may be used to predict or assess a cardiovascular condition (e.g., adverse cardiac remodeling, as determined, e.g., by a reduction in left ventricular ejection fraction (LVEF); such as an LVEF of less than 50%).

Table 1

	RNA-Seq of beneficial vs. adverse remodelers		qPCR validation (331 patients)		
	Fold change	p-value	PC1 (ΔLV)	PC2 (LVEF)	PC3 (ECV)
miR-21-5p	1.96	0.13	X	X	
miR-29a-3p	0.49	0.09		X	
miR-29b-3p	0.23	0.09		X	
miR-29c-3p	0.24	0.05		X	
miR-30a-5p	0.4	0.05	X	X	
miR-30d-5p	0.94	0.86	X	X	
miR-100-5p	0.4	0.09	X		
miR-146a-5p	2.36	0.04	X		
miR-146b-5p	2.36	0.08	X		
miR-150-5p	0.08	0.04		X	
miR-194-5p	0.18	0.04		X	
miR-223-3p	0.6	0.58	X		
miR-378c	0.26	0.09			X
miR-423-5p	0.91	0.85	X		
miR-744-5p	2.11	0.06	X		
	In relation to poor remodelers (i.e., positive number= increased in poor remodeler)				

Table 2

	Mouse model I/R						In vitro hypox/reox	Cell type expression
	LV tissue			Plasma				
	24 hr	1 week	4 weeks	24 hr	1 week	4 weeks	Media EVs	Predominant cell type
miR-21-5p		7.2****			8.5*	16.3****	10.3*	FB/EC
miR-29a-3p	10.4***							FB/EC
miR-29b-3p							4.7*	CM/EC
miR-29c-3p	374.1****					52.8****		CM
miR-30a-5p	162.0****			20.2**		15.8*		CM
miR-30d-5p				92.3****		10.8**	8.7*	CM
miR-100-5p					9.0***		6.7*	CM/FB
miR-146a-5p	133.0****					18.3***		Non-CM
miR-146b-5p	377.3****					15.2*		Non-CM

miR-150-5p	165.2****					8.3		Non-CM
miR-194-5p	14.8****	2.6*						CM/non-CM
miR-223-3p	325.4***			3.9**				Non-CM
miR-378c	78.4****					31.3**		CM
miR-423-5p	9.7****	4.3*						FB/EC
miR-744-5p	169.2***							EC
	Fold change vs. baseline, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, Student's t-test							

Additional diagnostic information (e.g., molecular, clinical, demographic, or cardiac images or combinations of them) may be combined with the signatures shown in **Table 1** for predicting a cardiovascular condition. For example, one or more or all signatures shown in **Table 6** or **Table 7** or both may be used along with one or more or all signatures of **Table 1** to predict a cardiovascular condition. Further details are described below.

In some instances, a variety of cell types in the myocardium are affected during a disease condition (e.g., acute ischemic injury), so the expression of miRNAs may be cell-specific; in other words, cells responsible for "myocardial" miRNA expression may not be exclusively cardiomyocytes. In some embodiments, where cardiomyocytes are exposed to hypoxia/reoxygenation (a cellular model of reperfused MI), several of the feature miRNAs may release into the media and associate with EVs, suggesting that cardiomyocytes may remain an important source of non-coding RNA expression in response to acute injury. The signature miRNAs involving various cell types may indicate the complex interplay between different cell types in orchestrating the process of cardiac remodeling.

Due to different roles of miRNAs in communication across cell types and organs, miRNAs may be novel biomarkers or therapeutics or targets, or combinations of them, in cardiovascular diseases. In some cases, miRNAs may play crucial roles in pathway discovery or in pathway treating cardiovascular diseases.

In some instances, given their dual role as a measurable, circulating biomarker and function in gene silencing in target cells, exRNAs may be mediators of a cardiovascular condition, such as early myocardial remodeling (e.g., fibrosis and hypertrophy), complementary to natriuretic and other peptide-based biomarkers. In some instances, the association of miRNA clusters with clinical CMR measures may be related to myocardial extracellular matrix expansion and hypertrophy, suggesting that plasma miRNAs expressed in the sub-acute post-MI phase (e.g., samples collected 2-4 weeks post-MI) may have correlation to chronic LV remodeling.

In some instances, the miRNAs that appear to be part of principal components may be significantly associated with cellular pathways including apoptosis signaling, immune cell function and signaling, and fibrotic pathways. That means, they have functional relevance in post-MI LV remodeling beyond application as passive markers of change. Of interest is the relationship between each of the principal components and the inflammatory signaling pathways. Each principal component is related to NF-kB signaling which ultimately leads to increased IL-1β activity. This pathway is of particular interest because IL-1β pathway antagonist may be used to treat post-MI patients. The IL-1β receptor antagonist,

anakinra, can reduce adverse post-MI remodeling, and decrease the risk of heart failure hospitalization in a post-MI population. Similarly, there are associations between each of the principal components and IL-6 signaling, which is downstream of IL-1β activity. These findings underscore the potential role of inflammation in post-MI remodeling, and suggest that specific miRNAs may exert their effect on post-MI remodeling through the IL-1β cytokine pathway and that dynamic changes in these miRNAs in response to anti-inflammatory therapies may serve as novel markers for assessing therapeutic response in subjects treated for a cardiac condition, such as MI.

The methods described herein may use a holistic approach (RNA-Seq) on samples (e.g., post-MI plasma) to demonstrate a wide array of RNAs in circulation. In addition to miRNA, several other exRNA species (4 piRNAs, and 1 snoRNA) may be identified as differentially expressed in individuals with adverse vs. beneficial remodeling post-MI. In some instances, a fragment of hY1 may be also differentially expressed in the RNA-Seq cohort (trending towards statistical significance, p=0.15) and significantly correlated with change in LV mass in a qPCR validation cohort, with higher levels associated with adverse remodeling. In some instances, a functional role for yRNA fragments may be related to inflammation; specifically, secretion of a 5' fragment of hY4 in EVs from cardiosphere-derived cells and its cardioprotective role in a rat model of I/R. Some embodiments also show perturbation of yRNA fragment expression in human cardiovascular disease.

In some instances, to characterize exRNA candidates associated with a cardiovascular condition (e.g., LV remodeling phenotypes), a mouse model of I/R may be utilized to investigate the temporal regulation of miRNA in plasma and tissue. In some instances, analyses may show that: distinct clustering of time-points by miRNA expression; baseline and acute (e.g., 24 hour) samples clustered distinctly in tissue, whereas baseline and chronic (e.g., 4 week) samples exhibit particularly strong separation in plasma. In some instances, 11 of the 15 miRNAs measured may be significantly up-regulated in tissue at the 24-hr time-point with only 3 up-regulated in plasma. In some cases, during the sub-acute period (1 week) 3 miRNA may be up-regulated in tissue and 2 in plasma, with one species (miR-21-5p) up-regulated in both tissue and plasma. In some instances, no miRNA may be up-regulated in tissue at the chronic time point but 8 of the miRNA candidates may be up-regulated in plasma. Some embodiments demonstrate a highly complex temporal regulation of miRNA expression in some cardiovascular conditions (e.g., post-MI). Some embodiments also show the usefulness of temporal data collection and analysis. On the other hand, the patient samples used in the studies align well with the sub-acute time-point (collected 2-4 weeks post-MI). In some instances, the post-MI miRNA expression levels may be compared with baseline expression levels in order to describe the temporal pattern of post-MI miRNA expression.

In some instances, the expression levels in tissue and plasma may be correlated. In some instances, the expression levels in tissue and plasma may not be correlated. In the case of lacking correlation, the implications may be a significant proportion of the miRNA dysregulated (e.g., post-MI) does not originate from cardiac tissue, or there is a delay in their release, or both. In some instances, expression of the candidate miRNA may show cell-type specificity. In some instances, miRNAs from each PC group may be expressed across different cell types, highlighting the importance of multiple cell types in the cardiac remodeling processes. In some cases, miRNA with high expression in the "non-cardiomyocyte" group include those associated with inflammation and inflammatory cells (e.g., SEQ ID

NOS: 7-8 (miR-146a-5p), SEQ ID NOS: 9-10 (miR-146b-5p), or SEQ ID NOS: 11-12 (miR-150-5p) or combinations of them). In some instances, cardiomyocytes may release EV-associated miRNA in response to hypoxia/reoxygenation *in vitro*, suggesting that stressors can trigger the release of remodeling-associated exRNAs.

Various examples disclosed in this document suggest that multiple cell types contribute to the post-MI exRNA milieu. Use of these exRNAs may assemble a tool for characterizing (e.g., diagnosing, staging, or prognosing, or combinations thereof) cardiovascular diseases, or therapeutic compounds, or targets for treating cardiovascular disease, or combinations thereof.

The present disclosure identifies differentially expressed miRNA candidates from the discovery cohort. The statistical analysis (e.g., PCA) can group similar miRNAs to limit the type-1 error in regression. The data disclosed herein demonstrate that: (1) exRNA biomarkers (e.g., either singly or in combination), including the small RNAs disclosed herein, are associated with CMR-defined LV remodeling phenotypes; (2) plasma and tissue levels of the candidate remodeling-associated miRNA are dynamically regulated in a murine model of I/R, and ,thus, these miRNAs (e.g., human miRNAs) can be used as biomarkers to assess or predict cardiac remodeling outcomes in a subject, such as a human; and (3) distinct cell types in the heart contribute to these miRNAs pointing to the complex intercellular interactions in the pathogenesis of LV remodeling. Overall, these data demonstrate plasma miRNAs as functional mediators of cardiac remodeling processes and phenotypes. Thus, the level of expression of these miRNAs can be used to assess or predict cardiac (e.g., LV) remodeling outcomes (e.g., beneficial or adverse cardiac remodeling).

Predicting response to a cardiac therapy

The methods disclosed herein may be used to predict a subject's responsiveness to a cardiac therapy (e.g., a therapy selected from an anti-inflammatory agent, such as those described herein or known in the art for treating cardiovascular conditions, such as MI). The expression level of the one or more biomarkers described herein (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) can be compared to a reference or control level or a cutoff value to predict or assess a subject's cardiovascular status (e.g., the presence of beneficial or adverse cardiac (e.g., LV) remodeling) and predict the effect of therapy on promoting or increasing beneficial, as opposed to adverse, cardiac remodeling. For example, a change in the expression of the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) relative to a reference level may indicate that the subject is likely to experience beneficial LV remodeling (e.g., a decrease in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%), or adverse LV remodeling (e.g., an increase in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%), in response to a cardiac therapy described herein.

In some instances, an increase in the expression level of the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all)

of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) in a sample (e.g., a tissue and/or plasma sample) relative to a reference or control level (e.g., relative to the expression level of the one or more biomarkers in a subject indicates that the subject is more likely to benefit from a cardiac therapy described herein. In certain instances, an increase in the expression level of the one or more biomarkers (e.g., one or more non-coding RNAs having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 97%, 99%, or 100% sequence identity) to SEQ ID NO: 1-74 or a complement thereof; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5)) in a sample (e.g., a tissue and/or plasma sample) relative to a reference or control level (e.g., relative to the expression level of the one or more biomarkers in a subject who experiences beneficial LV remodeling) or cutoff value indicates that the subject is likely to experience adverse LV remodeling (e.g., an increase in LVESVI of ≥10%, 15%, 20%, 30%, 40%, or 50%) and is more likely to benefit from a cardiac therapy described herein.

Kits

Also provided are kits that can be used in accordance with the methods described herein. For example, the kit may include reagents for collecting nucleic acids from a patient sample (e.g., blood sample or a plasma sample); reagents for amplifying the nucleic acid molecules to produce an amplified sample; and reagents for measuring the level of expression of a biomarker having at least 80% (e.g., 80%, 85%, 90%, 95%, 97%, 99%, or 100%) sequence identity to one or more or all sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, or 74; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5). In some instances, the kit may include reagents for measuring the level of expression of one or more or all sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, or 74; e.g., one or more (or all) of the PC1, PC2, and/or PC3 biomarkers (see Figure 5). The kit may also include machine-readable instructions for predicting response to a cardiac therapy, e.g., surgery, drug treatment, or cardiac resynchronization therapy or combinations thereof.

EXAMPLES

The following examples are to illustrate the invention. They are not meant to limit the invention in any way.

The practice of this invention may employ, unless otherwise indicated, conventional techniques of molecular biology and cell biology, which are within the skill of the person skilled in the art (see, e.g., Green and Sambrook. *Molecular Cloning: A Laboratory Manuel, 4th edition, 2012*; Ausubel, et al. *Current Protocols in Molecular Biology*, 1987; *Methods in Enzymology*. Academic Press, Inc.; and MacPherson et al. *PCR2: A Practical Approach*, 1995).

Example 1. OMEGA-REMODEL trial methodology

Patient Population

The Omega-3 Acid Ethyl Esters on LV Remodeling After Acute MI (OMEGA-REMODEL) trial was a prospective, multicenter, double-blind, placebo-controlled trial designed to test the effects of 4 grams/day of omega-3 fatty acid treatment for 6 months after acute MI on adverse LV remodeling by cardiac magnetic resonance imaging (CMR). The Institutional Review Boards of the respective institutions approved all protocols involving human patients and all participants provided written informed consent. Blood samples collected in the OMEGA-REMODEL trial were used in this study. Venous blood was collected in EDTA vacutainers from 331 individuals 2-4 weeks after MI at the time of baseline CMR imaging. Blood was centrifuged at 2000 x g for 10 min for plasma separation, and immediately stored at -80°C. CMR phenotyping included LV end-systolic volume index (LVESVI), left ventricular ejection fraction (LVEF), myocardial mass, and extracellular volume fraction (ECV; a validated surrogate of myocardial interstitial expansion. Of the 331 subjects, 238 underwent post-treatment follow-up CMR for serial comparison for LV remodeling. Infarct size by late gadolinium enhancement was quantified using ≥2 standard deviations beyond mean remote myocardial signal intensity and full width half maximum method. Baseline characteristics were compared via chi-squared (categorical) or Wilcoxon tests (continuous).

RNASeq of plasma samples

To identify exRNAs associated with LV remodeling by plasma RNAseq, we selected 11 individuals with "beneficial" LV remodeling (decrease in LVESVI ≥20%) and 11 individuals with "adverse" LV remodeling (increase in LVESVI ≥15%), matched for age, sex, diabetes status, LVEF, and infarct size by CMR. RNA was extracted from 1 mL plasma and libraries constructed according to previously published methods. Briefly, plasma RNA was isolated using the miRCURY RNA Isolation kit for Biofluids (Exiqon) with modified protocol and libraries were constructed and amplified from approximately 10 ng RNA using the NEBNext small RNA library prep set for Illumina (NEB). Size selection of libraries was performed by gel electrophoresis on a 10% Novex TBE gel with excision of the 140 to 160 nucleotide bands (corresponding to 21-40 nucleotide RNA fragments). Libraries were diluted to a final concentration of 2 nM, pooled, and sequenced on an Illumina HiSeq 2000 for single read 50 cycles at the Center for Cancer Computational Biology at Dana-Farber Cancer Institute.

For healthy controls, plasma exRNA from 26 subjects was isolated using a modified mirVana PARIS protocol (AM1556; Life Technologies) with sequential phenol–chloroform extractions (Burgos et al., 2013). RNA was concentrated using the Zymo RNA Clean & Concentrator kit (Zymo Research) and libraries were prepared using the NEXTflex Small RNA Sequencing Kit v2 by Bioo-Scientific. Pools of 15 samples were created, denatured and clustered on either a single read Illumina V3 flowcell (GD-401-3001; Illumina) or a single read rapid Illumina V2 flowcell (GD-402-4002; Illumina). The flowcells were run on the Illumina HiSeq 2500 (Illumina) for 50 cycles with a 7-cycle indexing read.

Sequence Analysis

The BCL files were de-multiplexed using CASAVA v1.82, and the adaptor sequences within the read sequences were trimmed by FastX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit). The processed sequences were filtered for small RNAs greater than 16 nucleotides in length. The sequences were then aligned, quantified, and annotated using sRNABench 1.0 pipeline (Barturen et al., In Methods in Next

Generation Sequencing, 2014). Briefly, the pipeline implemented hierarchical sequence mapping strategy that first mapped and removed spike-in library, contaminants, and rRNA. Next, RNA species were sequentially mapped to known mature miRNA, tRNA, snoRNA, piRNA, and Y RNA on the human genome sequence (hg19) using Bowtie2 (Langmead et al., 2009) with parameters that allow for 1 mismatch in seed alignment (-N 1), try two set of seeds (-R 2), and set the length of seed substrings to be 16 (-L 16). Mapped small RNA species were quantified to read counts and normalized to reads per kilobase per million reads mapped (RPKM). Differential expression analysis was performed using edgeR (Robinson et al., *Bioinformatics* 26:139-140, 2010) from Bioconductor (Gentleman et al., Bioconductor: open software development for computational biology and bioinformatics. Genome Biology 5, 2004) for all contrasts. The p-values were adjusted for multiple-test correction using False Discovery Rate (FDR) (Benjamini and Hochberg, Journal of the Royal Statistical Society Series B (Methodological) 57: 289-300, 1995). All fastq files have been deposited into the exRNA Atlas (exrna.org/resources/data/) as part of Extracellular RNA Communication Consortium data sharing, and is widely available.

High throughput RT qPCR

RNA was extracted from plasma (331 patients, OMEGA-REMODEL trial) using miRCURY RNA Isolation Kit – Biofluids (Exiqon) and reverse transcribed using miScript II RT Kit (Qiagen, Fredrick, MD, USA). cDNA samples were pre-amplified with miScript Microfluidics PreAMP Kit (Qiagen, Fredrick, MD, USA) and qPCR miRNA profiling analyses performed on BioMark System (Fluidigm Corp. South San Francisco, CA) using miScript miRNA Assays (Qiagen, Fredrick, MD, USA) and Dynamic Array 96.96 (Fluidigm Corp. South San Francisco, CA). A Ct value of 23 was set as the cut-off point for detectable miRNA species.

Principal components analysis (PCA)

We utilized a PCA, an unsupervised learning technique that statistically groups correlated miRNAs together into components, for analysis of both RNAseq data and qPCR validation data. For analysis of differences in miRNA profiles between post-MI patients and healthy controls, miRNA reads per million (RPM) for all subjects were computed and entered into a PC analysis after they were mean centered, standardized, and log-transformed; the first two principal components were plotted for all subjects. For the validation data, miRNA values from high-throughput qPCR were centered and standardized (mean 0, variance 1) and entered into the PCA. We included 4 principal components (PCs) based on (1) examination of an eigenvalue scree plot, and (2) inclusion of PCs that explain up to 60% of population variance (61.3% for 4 PCs). Varimax rotation was used to determine final PC loadings. PC scores based on loadings and miRNA expression values were included in linear regression models for CMR outcome variables (see below).

Ingenuity Pathway Analysis (IPA)

mRNA targets of the miRNAs from PC1, PC2, and PC3 that had a loading greater than 60% were obtained through IPA microRNA Target Filter. Only those mRNAs that were experimentally observed targets of the miRNAs from each PC were chosen to be included in the core pathway analysis form IPA.

Quantitative Evidence of LV Remodeling by CMR

We performed serial quantitation of LV volumes (end-diastole and end-systole), ECV in the non-infarct myocardium as a surrogate measure of interstitial fibrosis, and infarct size by late gadolinium enhancement (Heydari et al., *Circulation* 134:378, 2016). A 16-segmental map was used in quantitation of ECV and infarct size and segments with late gadolinium enhancement were not included in the measurement of non-infarct ECV. We estimated linear regression models for several cross-sectional (at the time of the blood draw, baseline at 2-4 weeks post-MI) and longitudinal LV phenotypes (changes in LV phenotypes from baseline to 6 months post-MI): (1) LVESVI; (2) LVEF; (3) LV mass index. In addition, we examined the association of baseline non-infarct myocardial fibrosis by ECV with each PC. Each regression model was specified with each of the 3 CMR parameters as the outcome (dependent variable), included all 4 PC scores, as well as adjustment for age, sex, race, presence of diabetes, a binary variable denoting randomization (to omega-3 fatty acid therapy vs. placebo), and a binary variable representing use of any LV remodeling medication (beta-blockade, angiotensin converting enzyme inhibition, or angiotensin-II receptor blockade). For the exRNAs other than miRNAs (yRNAs, snoRNAs and piRNAs), linear regression was carried out for mean centered and standardized Ct values as a function of changes in LV phenotypes from baseline to 6 months post-MI (LVESVI, LV Mass, LVEF) after adjustment for all the variables used in the PC regression analysis.

Animal and cell models of ischemia and hypoxia

Adult wild-type C57BL/6 mice were subjected to ischemia reperfusion (I/R) consisting of 30 min left anterior coronary artery (LAD) ligation followed by reperfusion, as previously described (Matsui et al., 2002). Animals were survived for 24 hrs, 1 week, or 4 weeks prior to sacrifice and blood and heart tissue was collected. In addition, hearts were collected from healthy adult wild-type mice to isolate cardiomyocytes and non-cardiomyocytes (Liu et al., *Cell Metabolism* 21:584-595, 2015), and primary endothelial cells and fibroblasts were cultured from hearts of wild type mice as previously described (Ashida et al., *Nature Communications* 2, 2011). Isolated cardiomyocytes, non-cardiomyocytes, and cultured primary endothelial cells were confirmed by qPCR analysis cell-specific markers including cTnI, CD31, and Col1a1 (**Figure 1**).

Cell model of hypoxia/reoxygenation: Neonatal rat ventricular myocytes (NRVMs) were isolated from 1 day postnatal Sprague-Dawley pups and subjected to 24 hrs hypoxia (2% oxygen) followed by 12 hours re-oxygenation. Cells were harvested in lysis buffer (miRVANA PARIS kit) and media was collected and sequentially centrifuged at 2,000 x g for 10 min. and 3,000 x g for 10 min.

RNA extraction and qPCR

RNA from cultured or isolated murine cells and mouse tissue samples were isolated using TRIzol following the manufactures' protocol. Plasma samples were 'spiked' with 5 pmol/mL exogenous cel-miR-39-3p and RNA was extracted using the miRCURY RNA Isolation kit for Biofluids (Exiqon) according to

manufacturer's protocol. For pig plasma samples, RNA was incubated with 1 unit of heparinase I (Sigma-Aldrich) at 25°C for 2 hours to prevent heparin-mediated inhibition of downstream reactions. Extracellular vesicle (EV) associated RNA was extracted from NRVM culture media using the exoRNEASY kit (Qiagen) and cellular RNA extracted with the miRVANA PARIS kit according to manufacturer's protocol (Ambion). All RNA samples were reverse transcribed by Universal cDNA Synthesis kit (Exiqon) and qPCR performed for specific miRNA species using the LNA-based Exiqon platform.

Statistical analysis

U6 (mouse tissue and NRVM cells), cel-miR-39-3p (plasma), or hsa-miR-191-5p (isolated murine cells) was used for qPCR normalization; due to a lack of reliable housekeeper genes, culture media EV miRNA was normalized to cell number and media volume input. Statistical analysis was performed on ΔCt values using Graphpad Prism software. Data from the mouse model was analyzed by one-way ANOVA with Tukey post-hoc analysis, and cell data by Student's t-test. Additionally, the qPCR ΔCt values for mouse tissue, plasma, and cell data were exponentiated as 2-1/Ct, scaled, and centered before unsupervised agglomerative hierarchical clustering with the Manhattan distance measure and the Ward method of clustering was performed. The clustering results were plotted as a heatmap using a modified version of *heatmap.2* in R.

Example 2. Identification of baseline characteristics of subjects

Three hundred and thirty-one subjects who suffered an acute myocardial infarction 2-4 weeks prior,with a mean age of 58.9±10.1, were included in this study; baseline characteristics are presented in **Table 3**. Blood was collected from subjects at time of initial CMR (2-4 weeks post-MI) and 238 underwent post-treatment follow-up CMR 6 months later (**Table 4**). The vast majority of subjects in this cohort had beneficial LVRm and preserved LVEF with a mean of 56.6±7.9%. Histograms of the percent change in LVESV and LVM reveal a Gaussian-like distribution centered on zero percent change (Figure 2) indicating that the majority had little change in these variables and, more importantly, very few had adverse LVRm as seen in the right-tail of the distribution in these histograms. From this cohort we selected n=11 subjects with beneficial and n=11 subjects with adverse LVRm for RNAseq analysis (**Table 5**). Subjects from these 2 groups were matched for age, sex, diabetes status, fish oil consumption, LVEF, and infarct size by CMR.

Table 3: Baseline clinical and biochemical characteristics of individuals in the cohort including 331 subjects. Values for continuous covariates expressed as mean ± standard deviation; categorical covariates are expressed as a fraction of the overall cohort.

Variable	N	Value
Age (years)	331	58.9±10.1
Female, %	331	64 (19%)
White race, %	331	268 (81%)
Diabetes, %	331	86 (26%)
Current or prior smoking, %	331	166 (50%)
Hypertension, %	331	213 (64%)
Hyperlipidemia, %	331	234 (71%)
Prior myocardial infarction, %	331	196 (59%)
Body mass index (kg/m^2)	331	29.0±5.4
Systolic blood pressure (mmHg)	329	121±16
Diastolic blood pressure (mmHg)	329	70±10
ST-elevation myocardial infarction, %	331	196 (60%)
Aspirin use	331	325 (98%)
Beta blocker use	331	303 (92%)
Angiotensin converting enzyme inhibitor use	331	244 (74%)
Statin use	331	320 (97%)
Randomized to omega-3 fatty acid, %	331	162 (49%)
Serum total cholesterol, mg/dl	305	138±67
Serum high density lipoprotein, mg/dl	305	37±27
Serum low density lipoprotein, mg/dl	304	80±47
Serum triglycerides, mg/dl	305	121±95

Table 4. Baseline (2-4 week) and final (6 month) CMR imaging characteristics. All morphologic parameters are indexed to body surface area, calculated at the time of CMR. The "Δ" (change) indices are calculated as final (6-month) value minus baseline (4-week) value divided by initial value. Late gadolinium enhancement was measured by the full-width half-maximum technique.

CMR index	Baseline (N≤331)		Follow-up (N≤238. 72%)	
	N	Mean (or N)	N	Mean (or N)
LVESVI (ml/m^2)	331	39.4±16.5	238	36.8±13.1
LV mass index (g/m^2)	319	60.0±14.5	226	57.5±13.0
LV ejection fraction, %	331	53.9±9.4	238	56.6±7.9
RV ejection fraction, %	331	53.3±7.1	239	52.6±7.7
Late gadolinium enhancement, g	327	17.4±17.2	244	14.0±13.9
Average extracellular volume fraction	255	0.34±0.05	192	0.35±0.05
ΔLVESVI	-		238	-2±19%
ΔLV mass index	-		219	-2±17%
ΔLV ejection fraction	-		238	3±12%

Table 5. Baseline clinical, biochemical, and CMR imaging characteristics of sequencing cohort. "Favorable" and "unfavorable" remodeling is defined in text. Values for continuous covariates expressed as mean ± standard deviation; categorical covariates are expressed as a fraction of the overall cohort. P-values correspond to comparison of favorable versus unfavorable remodeling (Kruskal-Wallis for continuous; chi-squared for categorical). Abbreviations: kg, kilogram; m, meters; ACEI, angiotensin converting enzyme inhibitor; LGE, late gadolinium enhancement; BP, blood pressure.

Variable	Beneficial Remodeling (N=11)	Adverse Remodeling (N=11)	P-value
Age (years)	59.9±11.9	59.7±8.9	0.97
Female, %	3 (27%)	4 (36%)	0.65
White race, %	7 (64%)	8 (73%)	0.65
Diabetes, %	3 (277%)	3 (27%)	1.00
Current or prior smoking, %	6(55%)	6(55%)	1.00
Hypertension, %	8 (73%)	8 (73%)	1.00
Hyperlipidemia, %	9 (82%)	7 (64%)	0.34
Prior myocardial infarction, %	4 (36%)	7 (64%)	0.20
Body mass index, (kg/m^2)	28.6±7.0	30.4±4.6	0.25
Systolic BP (mmHg)	126±20	125±15	0.84
Diastolic BP (mmHg)	69±17	72±8	0.55
ST-elevation myocardial infarction, %	7 (64%)	6 (55%)	0.66
Aspirin use	10 (91%)	11 (100%)	0.31
Beta blocker use	9 (82%)	10 (91%)	0.53
ACEI use	10 (91%)	7 (64%)	0.31
Statin use	11 (100%)	11 (100%)	1.00
Randomized to omega-3 fatty acid, %	4 (36%)	2 (18%)	0.34
Baseline			
LVEF, %	58.1±5.7	58.2±4.9	0.97
LVESVI, ml/m^2	35.3±7.7	32.2±7.1	0.38
LVMI, g/m^2	67.1±20.2	57.6±18.6	0.29
LGE size, g	10.8±11.1	11.5±10.0	0.97
Change to follow up			
%ΔLVEF	+9.3±9.8%	-8.2±8.9%	0.0007
%ΔLVESVI	-29.1±12.2%	+37.0±11.8%	<0.0001
%ΔLVMI	-7.1±11.9%	+0.3±15.0%	0.29

Example 3. RNAseq and exRNA diversity

RNAseq was performed on plasma from 22 subjects (n=11 beneficial and n=11 adverse LVRm). Top RNA species detected by sequencing included miRNA, piRNA, yRNA fragments, tRNA fragments,

and snoRNA (**Figure 3**), and the percentage of reads assigned to each species was not significantly different between the two groups. In this study we primarily focused on miRNA, of which 188 different species were expressed in at least 50% of all samples, with 42 different species expressed across all samples. To identify miRNA candidates differentially expressed between beneficial and adverse LVRm, we performed differential expression analysis between the two subgroups. To focus on miRNAs ubiquitously abundant in plasma and suitable as biomarker candidates, we selected miRNA candidates with a fold change in expression of ≥2 (or ≤0.5), a nominal p value of ≤0.1, and expression (RPKM>0) across ≥50% of samples. This yielded 21 miRNA candidates from the discovery cohort for validation (miR-30a-5p, miR-100-5p, miR-146a-5p, miR-146b-5p, miR-744-5p, miR-98-5p, miR-194-5p, miR-29b-3p, miR-29c-3p, miR-378c, miR-378e, miR-381-3p, miR-658, miR-15a-5p, miR-193b-5p, miR-200a-3p, miR-215-5p, miR-3168, miR-4306, miR-4326, and miR-4443). We included 12 additional miRNAs candidates, miR-21-5p, miR-30d-5p, miR-1, miR-423-5p, miR-150-5p, miR-223-3p, miR-29a-3p, miR-29a-5p, miR-155-5p, miR-208b-3p, miR- 208a, miR-133a-5p, and miR-499a-5p. A diagram outlining the analysis approach utilized in this study is shown in **Figure 4.** To determine how miRNA expression differed between post-MI subjects and healthy controls, this data was compared to RNAseq data from a cohort of 26 healthy controls (age in years: 44±11.8; female: 38%). PC analysis on miRNA expression for these three groups shows robust separation between healthy controls and post-MI subjects, while the beneficial and adverse LVRm groups show some separation (**Figure 12**). In addition to miRNA, statistically significant differential expression was detected for 4 annotated piRNAs, 1 snoRNA, and differential expression trending towards significance for 1 yRNA fragment (corresponding to the 5' fragment of hY1; **Figure 13**). We considered non-miRNA exRNAs as exploratory given their limited and variable annotation and unclear function, and thus focused on miRNA candidates for further analysis. Nonetheless, we did assess the association of these exRNAs with cardiac remodeling phenotypes in the validation cohort.

Example 4. Association of candidate RNAs with baseline and serial LV phenotypes by CMR

The miRNA candidates selected above were measured in 331 participants of the OMEGA-REMODEL study by high-throughput qPCR (**Table 6**). Due to the vast majority of these subjects exhibiting relatively favorable LVRm, differential expression analysis between beneficial and adverse remodelers was not an appropriate analysis method. We instead chose to analyze results by PC regression to eliminate co-linearity of miRNA species and reduce dimensionality of the data. This allowed us to examine the correlation of PC factors consisting of distinct sets of miRNA correlated with distinct CMR measures and biological processes. PCA demonstrated 4 PCs accounting for 61.3% of total variance. We used PC loading, which indicates the amount of variation explained by a variable, as a measurement of influence by a specific miRNA in a given PC. miRNAs with ≥60% loading in a given PC and with >60% expression across samples (14 total miRNAs) are shown in **Figure 5** and **Table 7**. In linear models for association of PCs with baseline and follow-up CMR parameters (**Table 8**), we found statistically significant associations between several PCs and myocardial structure/function (**Figures 5 and 6**): PC1 vs. change in LV Mass; PC2 vs. VESVI, LVEF, and LVmass; PC3 vs. ECV (Haaf et al., *J. Cardiovasc. Mag. Res.* 18:89, 2017).

Table 6. Ct values from high throughput qPCR for candidate miRNA measured in 331 patients

Variable	Number of study participants expressing	Fraction of study participants expressing	Minimum Ct value	Maximum Ct value	Mean	SD
miR-1	100	0.30	15.41	21.98	20.03	1.35
miR-208a	4	0.01	20.93	21.81	21.32	0.36
miR-423-5p	284	0.86	10.81	21.91	17.06	2.17
miR-100-5p	280	0.85	14.90	21.94	18.89	1.46
miR-133a-5p	220	0.66	19.52	21.98	20.87	0.60
miR-146a-5p	281	0.85	9.06	21.92	17.73	2.63
miR-146b-5p	244	0.74	12.18	21.93	18.69	2.05
miR-150-5p	325	0.98	9.96	20.98	14.80	1.69
miR-155-5p	46	0.14	18.24	21.94	20.84	0.82
miR-15a-5p	180	0.54	15.25	21.98	20.09	1.22
miR-193b-5p	159	0.48	16.73	21.99	20.57	0.93
miR-194-5p	293	0.89	14.68	21.88	19.13	1.33
miR-200a-3p	19	0.06	19.33	22.00	21.14	0.68
miR-208b-3p	30	0.09	19.14	21.92	21.14	0.62
miR-21-5p	327	0.99	7.44	21.22	13.45	2.16
miR-215-5p	90	0.27	17.82	21.98	20.56	1.08
miR-223-3p	325	0.98	6.02	20.19	13.38	2.68
miR-29a-3p	329	0.99	10.03	21.82	17.06	1.61
miR-29a-5p	6	0.02	20.93	21.90	21.47	0.36
miR-29b-3p	266	0.80	13.39	21.99	19.40	1.49
miR-29c-3p	317	0.96	9.69	21.98	18.17	1.80
miR-30a-5p	327	0.99	10.65	21.97	17.19	1.85
miR-30d-5p	322	0.97	11.04	21.62	17.63	1.93
miR-3168	2	0.01	21.56	21.80	21.68	0.17
miR-378c	280	0.85	12.67	21.93	18.58	1.95
miR-378e	62	0.19	18.30	21.99	20.68	0.89
miR-381-3p	83	0.25	16.67	21.98	20.52	1.14
miR-4306	126	0.38	16.81	21.99	20.50	1.05
miR-4326	21	0.06	19.91	21.96	21.04	0.66
miR-4443	2	0.01	20.63	21.32	20.97	0.49
miR-499a-5p	28	0.08	19.31	21.99	21.18	0.75
miR-658	85	0.26	17.63	21.94	20.48	1.04
miR-744-5p	191	0.58	13.69	21.95	19.16	2.11
miR-98-5p	82	0.25	16.45	21.99	20.29	1.24

Table 7. Principal components analysis (PCA) of 34 candidate miRNAs across 331 study participants. RNAs (expressed in Ct value) were mean-centered and standardized for PCA. Based on an examination of the scree plot, 4 PCs (accounting for 61.3% of total variance) were selected. Each column displays the loading for each miRNA on the respective PC (scaled out of 100). * (and red color) represents those miRNAs that had a loading of greater than 60 with detectable expression in >60% of samples (14 total miRNAs).

miRNA	% Detectable expression	PC 1	PC 2	PC 3	PC 4
miR-21-5p	0.99	64*	63*	-12	5
miR-30a-5p	0.99	60*	68*	24	11
miR-30d-5p	0.97	63*	66*	18	9
miR-1	0.30	81	17	9	11
miR-423-5p	0.86	73*	40	6	3
miR-100-5p	0.85	68*	55	-5	10
miR-146a-5p	0.85	85*	40	2	8
miR-146b-5p	0.74	79*	41	19	14
miR-223-3p	0.98	74*	53	-9	-5
miR-744-5p	0.58	88	20	13	7
miR-98-5p	0.25	82	11	4	13
miR-150-5p	0.98	45	68*	-5	1
miR-194-5p	0.89	27	80*	23	23
miR-29a-3p	0.99	28	69*	47	13
miR-29b-3p	0.80	41	75*	15	22
miR-29c-3p	0.96	41	77*	14	23
miR-155-5p	0.14	28	5	63	35
miR-208b-3p	0.09	-4	8	73	8
miR-378c	0.85	-13	29	73*	-16
miR-378e	0.19	-6	16	78	-2
miR-381-3p	0.25	-5	19	82	5
miR-658	0.26	9	19	79	7
miR-208a	0.01	12	-5	37	-7
miR-133a-5p	0.66	17	0	0	-20
miR-15a-5p	0.54	28	59	37	27
miR-193b-5p	0.48	32	46	4	28
miR-200a-3p	0.06	4	10	26	61
miR-215-5p	0.27	26	40	3	45
miR-29a-5p	0.02	-1	27	14	-7
miR-3168	0.01	-6	4	-10	60

miR-4306	0.38	49	48	11	22
miR-4326	0.06	17	-2	58	28
miR-4443	0.01	12	6	2	41
miR-499a-5p	0.08	30	18	10	56

* indicates those miRNAs that had a loading of greater than 60 with detectable expression in >60% of samples (14 total miRNAs).

Table 8. Linear regression models for CMR parameters as a function of RNA PC score. Each model was adjusted for age, sex, race, presence of diabetes, parent study randomization (omega-3 fatty acids versus placebo), and a binary variable representing the use of any remodeling medication (beta-blocker, ACE-I or ARB). All PCs were included simultaneously in each model. Red cells represent significant P values at nominal P<0.05 level. ECV, extracellular volume fraction; LVESVI, left ventricular end systolic volume index; LVEF, left ventricular ejection fraction; LV, left ventricular.

	ECV (log-transformed) N=255		LVESVI (log-transformed) N=331		LVEF N=331		LV mass index (log-transformed) N=319		%ΔLV Mass Index N=219		%ΔLVESVI N=238		%ΔLVEF N=238	
PC	β	P	β	P	β	P	β	P	β	P	β	P	β	P
PC 1	-0.002	0.79	0.017	0.39	0.058	0.91	0.004	0.74	-0.027	0.02	-0.011	0.39	0.011	0.15
PC 2	0.001	0.90	0.044	0.02	-1.15	0.02	0.037	0.005	-0.014	0.24	-0.015	0.22	0.011	0.17
PC 3	-0.021	0.03	-0.008	0.67	0.207	0.69	0.004	0.77	-0.015	0.18	-0.003	0.76	0.002	0.75
PC 4	-0.013	0.26	-0.009	0.63	0.259	0.61	0.016	0.20	-0.019	0.08	0.007	0.57	-0.001	0.88

Using Ingenuity Pathway Analysis, we generated predicted functional pathways associated with validated mRNA targets of the miRNAs in each of these PC groups (**Figures 7 and 8**). Top pathway hits included interleukin signaling, PTEN signaling, and toll-like receptor signaling, with a strong overall theme of inflammatory and fibrotic pathways represented. Furthermore, apoptosis and death receptor signaling were amongst the significant target pathways for PC2, the PC group associated with LVEF. While there was some overlap in mRNA targets between groups, a greater proportion of the mRNA targets identified were unique to each PC group, suggesting that these represent clusters of miRNA with distinct functionality that may contribute to the observed CMR parameter correlations.

Finally, of the other exRNAs assessed in the 331 subjects, the hY1 fragment had a significant correlation with change in LV Mass, with a β coefficient of -0.043 and a Benjamini-Hochberg adjusted P value of 0.007. Here, a negative β coefficient means greater increase in LVM over time is associated with higher circulating hY1 fragment concentration.

Example 5: Temporal regulation of candidate miRNA in animal models

To determine the temporal regulation of exRNA, candidate miRNAs that were highly loaded on each PC and highly expressed were selected for analysis in the murine I/R model. Time-points chosen were pre-ischemic (baseline), acute to sub-acute phase (24 hrs and 1 week) and chronic post-infarct phase (4 weeks). Both plasma and tissue had temporally distinct expression of miRNA (**Figures 9A and 9B**; **Tables 1 and 2**). At 24 hours, miR-223-3p and miR-30a-5p alone increased in plasma, while 13 of the 14 miRNA measured were upregulated in tissue (miR-100-5p, miR-146a-5p, miR-150-5p, miR-146b-5p, miR-194-5p, miR-223-3p, miR-29a-3p, miR-29c-3p, miR-30a-5p, miR-30d-5p, miR-378c, miR-423-5p, miR-744-5p). Conversely, the majority of plasma changes were seen at the chronic time-point (upregulation of miR-146a-5p, miR-150-5p, miR-146b-5p, miR-21-5p, miR-29c-3p, miR-30a-5p, miR-30d-5p, and miR-378c) with no significant alterations in tissue miRNA expression at this time. Fewer changes were observed at the sub-acute (1 week) time-point: miR-21-5p increased in plasma and miR-194-5p, miR-21-5p, and miR-423-5p were up-regulated in tissue.

Example 6: Cell type expression of miRNA and hypoxia/reoxygenation model

We assessed cell type sources of these miRNAs in dissociated cells and found distinct clustering of cardiomyocytes vs non-cardiomyocytes (**Figure 10**). Specifically, miR-378c, and members of the miR-30 and miR-29 family were highly expressed in cardiomyocytes compared to noncardiomyocytes; miR-423-5p, miR-21-5p, and miR-744-5p were highly expressed in fibroblasts and endothelial cells; and miR-146b-5p, miR-150-5p, miR-223-3p, and miR146a-5p were expressed highest in the non-cardiomyocyte group (representing all non-cardiomyocyte cardiac cells, including immune cells). Stimulation of expression and release of miRNA by hypoxia/reoxygenation in cardiomyocytes was investigated in NRVMs. miR-100-5p, miR-30d-5p, miR-21-5p, and miR-29b-3p were upregulated in EVs released in the media (**Figure 11**).

OTHER EMBODIMENTS

Although the embodiments have been described with reference to the presently preferred embodiments, various modifications can be made without departing from the invention. Unless otherwise

apparent from the context any step, element, embodiment, feature or aspect of the embodiments can be used with any other.

While preferred embodiments have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the embodiments. It should be understood that various alternatives to the embodiments described herein may be employed in practicing the described methods. It is intended that the following claims define the scope of the embodiments and that methods and structures within the scope of these claims and their equivalents be covered thereby.

INCORPORATION BY REFERENCE

All publications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

CLAIMS

1. A method comprising:
    a) contacting a sample comprising one or more nucleic acid molecules from a subject having a cardiovascular injury or disease with one or more single-stranded nucleic acid molecules capable of specifically hybridizing with nucleotides of one or more biomarkers selected from the group consisting of SEQ ID NOs: 1-74 or a complement thereof; and
    b) detecting a level of expression of said one or more biomarkers or the complement thereof by detecting hybridization between the one or more single-stranded nucleic acid molecules and the one or more nucleic acid molecules of the sample.

2. The method of claim 1, wherein the single-stranded nucleic acid molecules are present in or on a device.

3. The method of claim 2, wherein the device comprises at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, or more single-stranded nucleic acid molecules capable of specifically hybridizing with nucleotides of one or more of the biomarkers.

4. The method of claim 3, wherein the device is a microarray or is for performing a quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) reaction.

5. The method of any one of claims 1 to 4, wherein the one or more single-stranded nucleic acid molecules have a length in the range of 1 to 200 nucleotides, wherein optionally the one or more of the single-stranded nucleic acid molecules have a length in the range of 20 to 60 nucleotides.

6. The method of any one of claims 1 to 5, wherein the one or more single-stranded nucleic acid molecules are labeled or immobilized on a solid substrate.

7. The method of any one of claims 1 to 6, wherein the level of the one or more biomarkers or the complement thereof are detected by performing microarray analysis or qRT-PCR.

8. The method of any one of claims 1 to 7, wherein said method comprises RNA sequencing.

9. The method of any one of claims 1 to 8, wherein one or more of said biomarkers is a nucleic acid molecule.

10. The method of any one of claims 1 to 9, wherein one or more of said biomarker is DNA or RNA.

11. The method of claim 10, wherein said RNA is non-coding RNA, extracellular RNA, or miRNA.

12. The method of claim 11, wherein said extracellular RNA is piRNA, tRNA, snoRNA, or yRNA, or a fragment thereof.

13. The method of any one of claims 1 to 12, wherein the nucleic acid molecules of the sample comprise mRNA or a cDNA thereof.

14. The method of any one of claims 1 to 13, wherein said method comprises extracting RNA from the sample.

15. The method of any one of claims 1 to 14, wherein said method comprises constructing an RNA library from the sample.

16. The method of any one of claims 1 to 15, wherein the method is for determining or predicting cardiovascular status in a subject.

17. The method of claim 16, wherein the cardiovascular status of the subject relates to cardiac remodeling.

18. The method of claim 17, wherein the subject has adverse cardiac remodeling if the level of said biomarkers or the complement thereof is substantially higher than the level of said biomarkers relative to a control sample or relative to a cutoff value of a normal reference level of the biomarkers.

19. The method of claim 18, wherein the adverse cardiac remodeling is characterized by reduced ventricular function, wherein preferably the ventricular function is left ventricular function.

20. The method of any one of claims 1 to 19, wherein said subject has a cardiovascular disease or injury.

21. The method of any one of claims 1 to 20, wherein the method further comprises administering a treatment to the subject.

22. The methods of claim 21, wherein said treatment is a cardiac therapy, wherein preferably the cardiac therapy is an anti-inflammatory agent.

23. The method of any one of claims 1 to 22, wherein more than one said sample may be collected from the subject.

24. The method of claim 23, wherein more than one said sample is collected from the subject over time.

25. The method of any one of claims 1 to 24, wherein the subject is a human.

26. The method of any one of claims 1 to 25, wherein the subject has experience myocardial infarction.

27. The method of claim 26, wherein the method is performed immediately after the myocardial infarction or within 1 to 4 hours, days, weeks, months, or years after the myocardial infarction.

28. The method of any one of claims 1 to 27, wherein the method further comprises computationally predicting the subject's cardiovascular status comprising performing one or more of the following:
    a) analyzing cardiac imaging data of the subject, wherein preferably said cardiac imaging data comprises cardiac magnetic resonance imaging or echocardiography and/or comprises an analysis of cardiac structure and/or function, for example, said analysis comprises any one or more of the following:
        i) segmenting a myocardial region;
        ii) segmenting a ventricular region;
        iii) quantifying an ejection volume;
        iv) quantifying an infarct size; and/or
        v) quantifying a mass index; and.or
    b) obtaining medication information of the subject; and/or
    c) obtaining demographic profiles of different cardiac subjects; and/or
    d) determining a statistical model to predict the cardiovascular status of the subject; and/or
    e) performing principal component analysis.

29. The method of any one of claims 1 to 28, wherein said method establishes the subject's cardiovascular status at a present time or at a future time.

30. The method of claim 29, wherein said cardiovascular status comprises a recovery from said disease condition.

31. The method of claim 29 or 30, wherein said cardiovascular status comprises an improvement from a prior determination of a cardiovascular status of the subject.

32. The method of any one of claims 29 to 31, wherein said cardiovascular status comprises cardiac remodeling.

33. The method of claim 32, wherein said cardiac remodeling comprises beneficial cardiac remodeling.

34. The method of claim 32, wherein said cardiac remodeling comprises adverse cardiac remodeling.

35. The method of any one of claims 29 to 34, wherein said cardiovascular status comprises a disease condition.

36. The method of any one of claims 1 to 35, wherein said method determines or predicts the subject's response to a treatment.

37. The method of claim 36, wherein said treatment comprises a cardiac therapy.

38. The method of claim 37, wherein said method determines or predicts a positive response of said subject to said treatment.

39. The method of claim 37, wherein said method determines or predicts a negative response of said subject to said treatment.

40. The method of any one of claims 1 to 39, wherein the sample of the subject is collected after the subject has experienced said cardiac injury or disease, wherein preferably the method predicts or determines the nature of cardiac remodeling of the subject.

41. The method of claim 21, wherein said treatment comprises an interleukin-1 beta receptor antagonist.

42. The method of 41, wherein the cardiovascular status of the subject is determined before said treatment.

43. The method of claim 41, wherein the cardiovascular status of the subject is determined after said treatment.

44. The method of claim 41, wherein the cardiovascular status of the subject is determined during a course of said treatment.

45. The method of any one of claims 1 to 40, wherein the biomarkers comprise SEQ ID NOs: 3-4, SEQ ID NOs: 7-10, SEQ ID NOs: 7-12, SEQ ID NOs: 11-12, SEQ ID NOs: 19-20, SEQ ID NOs: 27-28, SEQ ID NOs: 31-32, SEQ ID NOs: 33-34, SEQ ID NOs: 37-44, SEQ ID NOs: 41-44, SEQ ID NOs: 53-54, SEQ ID NOs: 65-66, or SEQ ID NOs: 1-12 or wherein the biomarkers are selected from one or more biomarkers of principal component 1 (PC1), PC2, PC3, or a combination thereof .

46. A method of treating a subject in need of treatment for adverse cardiac remodeling comprising administering a cardiac therapy to said subject, wherein said subject is identified as in need of said treatment according to the method of any one of claims 1 to 45.

47. A kit comprising one or more reagents for measuring a level of expression of one or more biomarkers having at least 80% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1-74; and, optionally:
    a) one or more reagents for collecting nucleic acid molecules from a sample from a subject; and/or
    b) one or more reagents for amplifying said nucleic acid molecules to produce an amplified sample; and/or
    c) instructions for determining or predicting a cardiovascular status of the subject based on the level of expression of said one or more biomarkers.

48. The method of claim 1, wherein the one or more single-stranded nucleic acid molecules have a length in the range of 1 to 200 nucleotides, wherein optionally the one or more of the single-stranded nucleic acid molecules have a length in the range of 20 to 60 nucleotides.

49. The method of claim 1, wherein the one or more single-stranded nucleic acid molecules are labeled or immobilized on a solid substrate.

50. The method of claim 1, wherein the level of the one or more biomarkers or the complement thereof are detected by performing microarray analysis or qRT-PCR.

51. The method of claim 1, wherein said method comprises RNA sequencing.

52. The method of claim 1, wherein one or more of said biomarkers is a nucleic acid molecule.

53. The method of claim 1, wherein one or more of said biomarker is DNA or RNA.

54. The method of claim 53, wherein said RNA is non-coding RNA, extracellular RNA, or miRNA.

55. The method of claim 54, wherein said extracellular RNA is piRNA, tRNA, snoRNA, or yRNA, or a fragment thereof.

56. The method of claim 1, wherein the nucleic acid molecules of the sample comprise mRNA or a cDNA thereof.

57. The method of claim 1, wherein said method comprises extracting RNA from the sample.

58. The method of claim 1, wherein said method comprises constructing an RNA library from the sample.

59. The method of claim 1, wherein the method is for determining or predicting cardiovascular status in a subject.

60. The method of claim 59, wherein the cardiovascular status of the subject relates to cardiac remodeling.

61. The method of claim 60, wherein the subject has adverse cardiac remodeling if the level of said biomarkers or the complement thereof is substantially higher than the level of said biomarkers relative to a control sample or relative to a cutoff value of a normal reference level of the biomarkers.

62. The method of claim 61, wherein the adverse cardiac remodeling is characterized by reduced ventricular function, wherein preferably the ventricular function is left ventricular function.

63. The method of claim 1, wherein said subject has a cardiovascular disease or injury.

64. The method of claim 1, wherein the method further comprises administering a treatment to the subject.

65. The methods of claim 64, wherein said treatment is a cardiac therapy, wherein preferably the cardiac therapy is an anti-inflammatory agent.

66. The method of claim 1, wherein more than one said sample may be collected from the subject.

67. The method of claim 66, wherein more than one said sample is collected from the subject over time.

68. The method of claim 1, wherein the subject is a human.

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69. The method of claim 1, wherein the subject has experience myocardial infarction.

70. The method of claim 69, wherein the method is performed immediately after the myocardial infarction or within 1 to 4 hours, days, weeks, months, or years after the myocardial infarction.

71. The method of claim 1, wherein the method further comprises computationally predicting the subject's cardiovascular status comprising performing one or more of the following:
    a) analyzing cardiac imaging data of the subject, wherein preferably said cardiac imaging data comprises cardiac magnetic resonance imaging or echocardiography and/or comprises an analysis of cardiac structure and/or function, for example, said analysis comprises any one or more of the following:
        i) segmenting a myocardial region;

ii) segmenting a ventricular region;
iii) quantifying an ejection volume;
iv) quantifying an infarct size; and/or
v) quantifying a mass index; and.or

b) obtaining medication information of the subject; and/or
c) obtaining demographic profiles of different cardiac subjects; and/or
d) determining a statistical model to predict the cardiovascular status of the subject; and/or
e) performing principal component analysis.

72. The method of claim 1, wherein said method establishes the subject's cardiovascular status at a present time or at a future time.

73. The method of claim 72, wherein said cardiovascular status comprises a recovery from said disease condition.

74. The method of claim 72, wherein said cardiovascular status comprises an improvement from a prior determination of a cardiovascular status of the subject.

75. The method of claim 72, wherein said cardiovascular status comprises cardiac remodeling.

76. The method of claim 75, wherein said cardiac remodeling comprises beneficial cardiac remodeling.

77. The method of claim 75, wherein said cardiac remodeling comprises adverse cardiac remodeling.

78. The method of claim 72, wherein said cardiovascular status comprises a disease condition.

79. The method of claim 1, wherein said method determines or predicts the subject's response to a treatment.

80. The method of claim 79, wherein said treatment comprises a cardiac therapy.

81. The method of claim 80, wherein said method determines or predicts a positive response of said subject to said treatment.

82. The method of claim 80, wherein said method determines or predicts a negative response of said subject to said treatment.

83. The method of claim 1, wherein the sample of the subject is collected after the subject has experienced said cardiac injury or disease, wherein preferably the method predicts or determines the nature of cardiac remodeling of the subject.

84. The method of claim 64, wherein said treatment comprises an interleukin-1 beta receptor antagonist.

85. The method of 84, wherein the cardiovascular status of the subject is determined before said treatment.

86. The method of claim 84, wherein the cardiovascular status of the subject is determined after said treatment.

87. The method of claim 84, wherein the cardiovascular status of the subject is determined during a course of said treatment.

88. The method of claim 1, wherein the biomarkers comprise SEQ ID NOs: 3-4, SEQ ID NOs: 7-10, SEQ ID NOs: 7-12, SEQ ID NOs: 11-12, SEQ ID NOs: 19-20, SEQ ID NOs: 27-28, SEQ ID NOs: 31-32, SEQ ID NOs: 33-34, SEQ ID NOs: 37-44, SEQ ID NOs: 41-44, SEQ ID NOs: 53-54, SEQ ID NOs: 65-66, or SEQ ID NOs: 1-12 or wherein the biomarkers are selected from one or more biomarkers of principal component 1 (PC1), PC2, PC3, or a combination thereof .

89. A method of treating a subject in need of treatment for adverse cardiac remodeling comprising administering a cardiac therapy to said subject, wherein said subject is identified as in need of said treatment according to the method of claim 1.

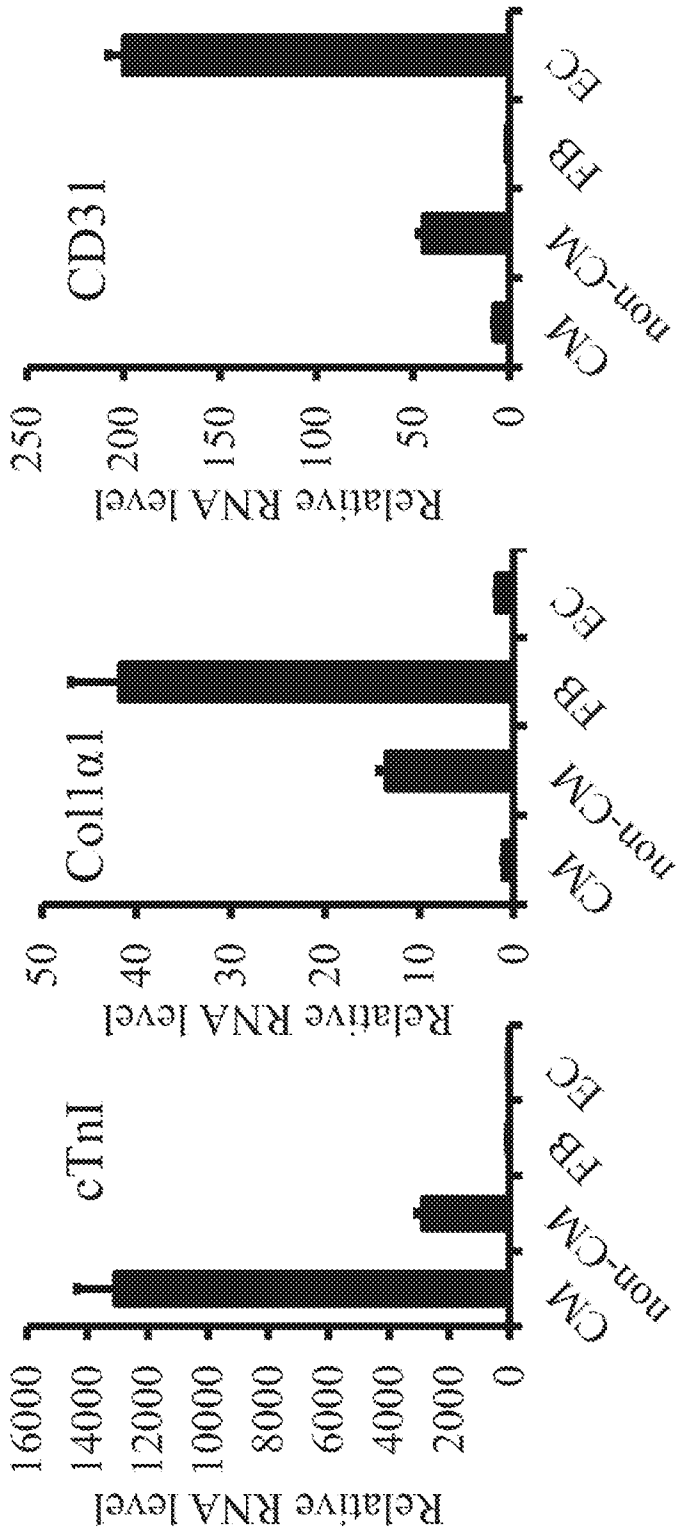

cTnI
Relative RNA level
16000
14000
12000
10000
8000
6000
4000
2000
0
CM
non-CM
FB
EC
Col1α1
Relative RNA level
50
40
30
20
10
0
CM
non-CM
FB
EC
CD31
Relative RNA level
250
200
150
100
50
0
CM
non-CM
FB
EC

FIG. 1

FIG. 2

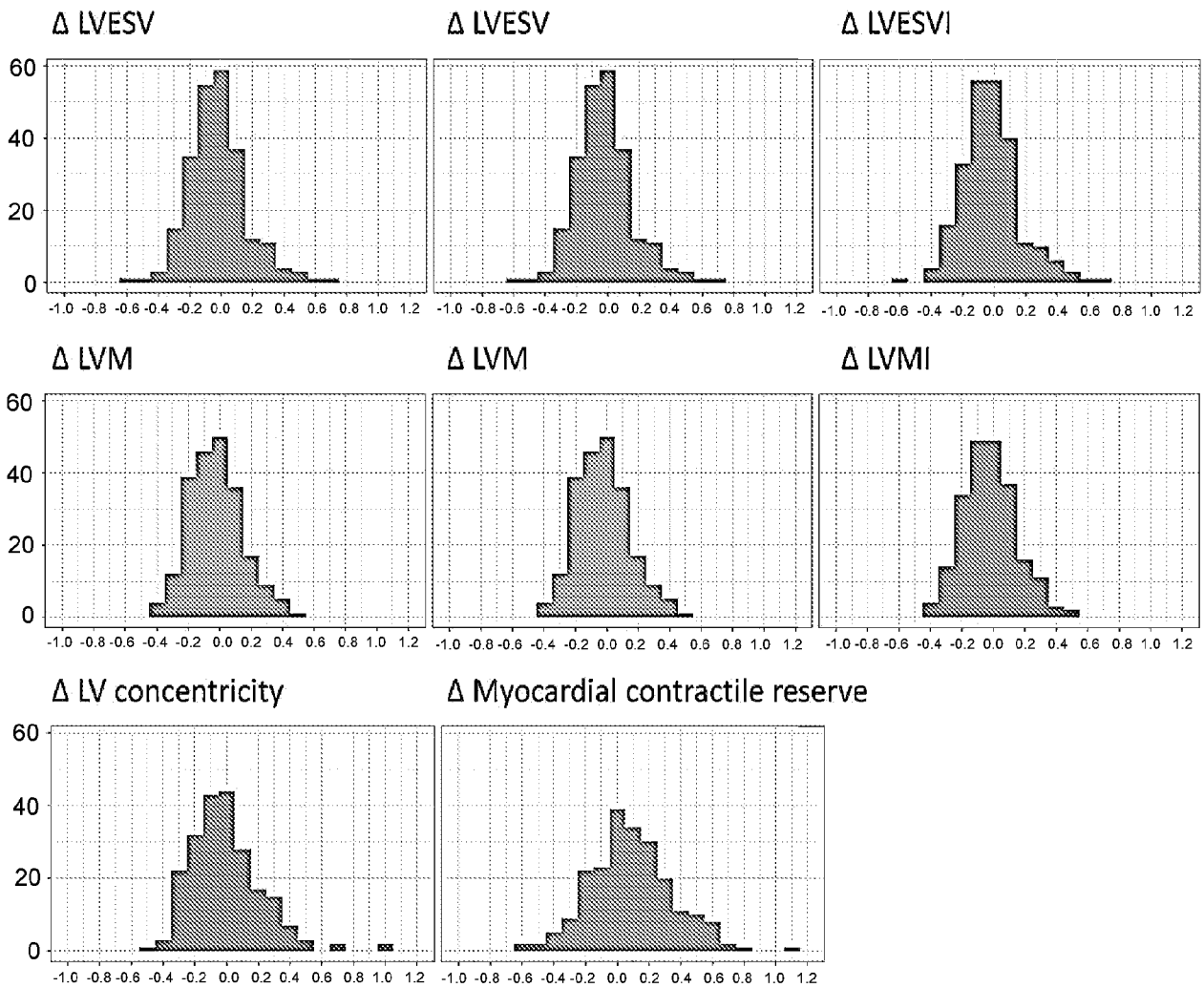

Δ LVESV
Δ LVESV
Δ LVESVI
Δ LVM
Δ LVM
Δ LVMI
Δ LV concentricity
Δ Myocardial contractile reserve
60
40
20
0
-1.0 -0.8 -0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8 1.0 1.2

FIG. 2

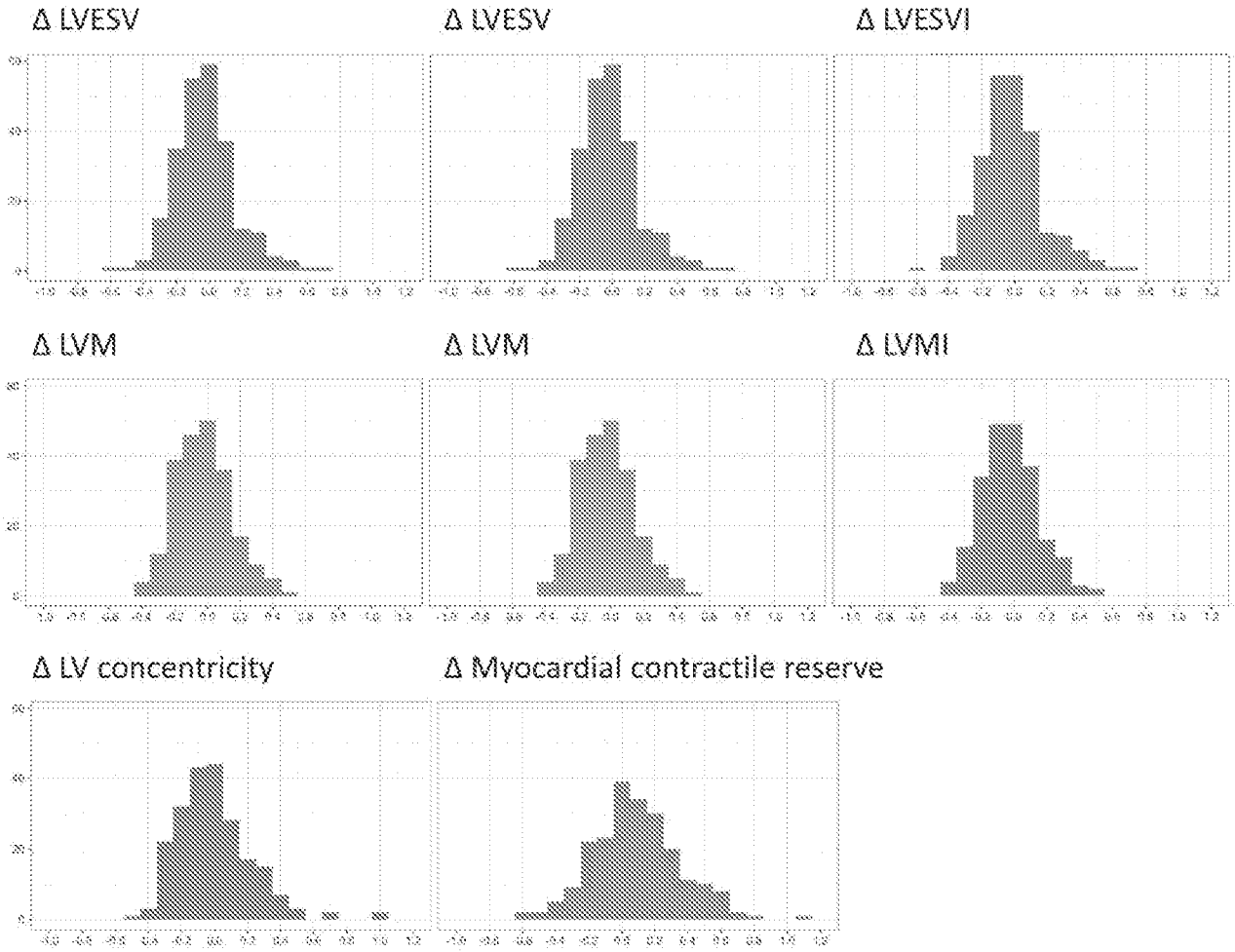

Δ LVESV
Δ LVESV
Δ LVESVI
Δ LVM
Δ LVM
Δ LVMI
Δ LV concentricity
Δ Myocardial contractile reserve

FIG. 3

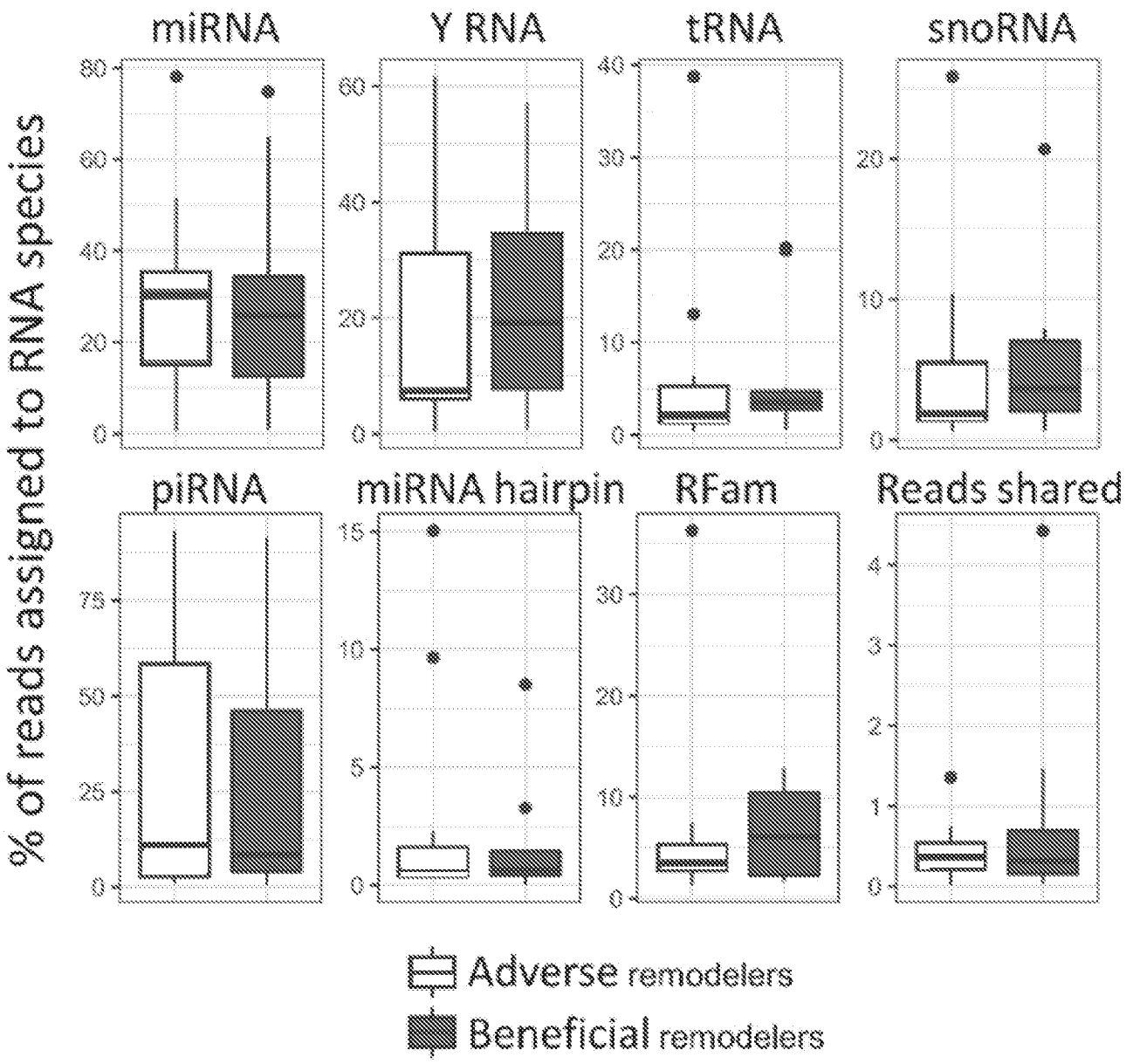

miRNA
Y RNA
tRNA
snoRNA
piRNA
miRNA hairpin
RFam
Reads shared
% of reads assigned to RNA species
Adverse remodelers
Beneficial remodelers

FIG. 4

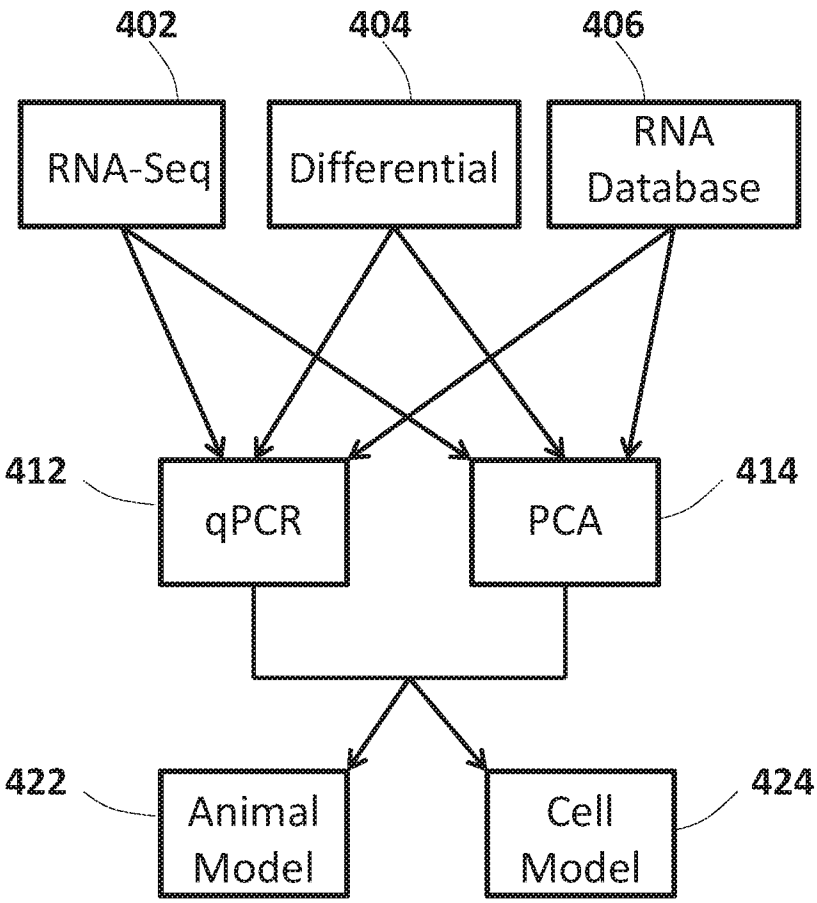

402
404
406
RNA-Seq
Differential
RNA Database
412
qPCR
PCA
414
422
Animal Model
Cell Model
424

FIG. 5

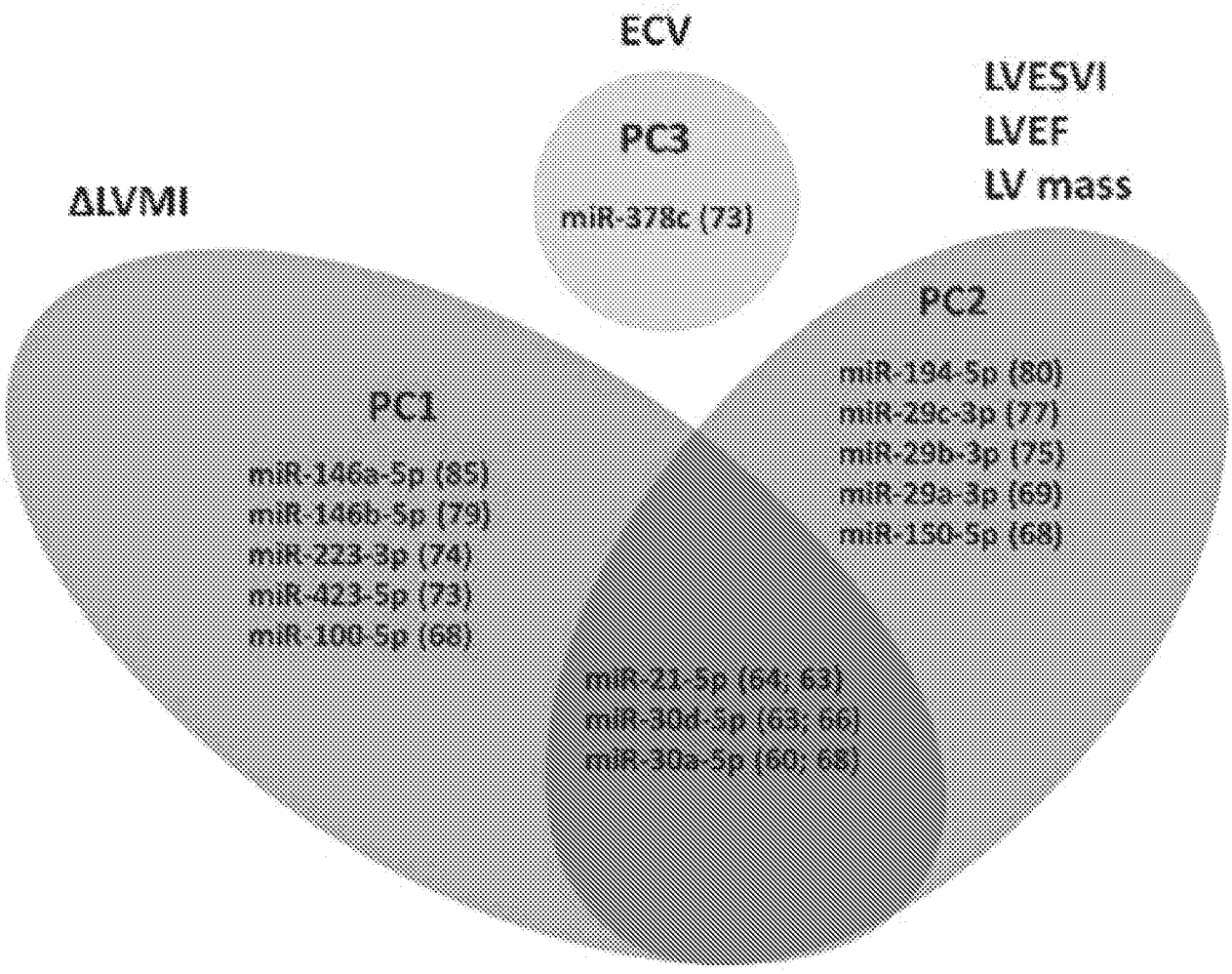

ECV
LVESVI
LVEF
LV mass
PC3
miR-378c (73)
ΔLVMI
PC2
miR-194-5p (80)
miR-29c-3p (77)
miR-29b-3p (75)
miR-29a-3p (69)
miR-150-5p (68)
PC1
miR-146a-5p (85)
miR-146b-5p (79)
miR-223-3p (74)
miR-423-5p (73)
miR-100-5p (68)
miR-21-5p (64; 63)
miR-30d-5p (63; 66)
miR-30a-5p (60; 68)

FIG. 6

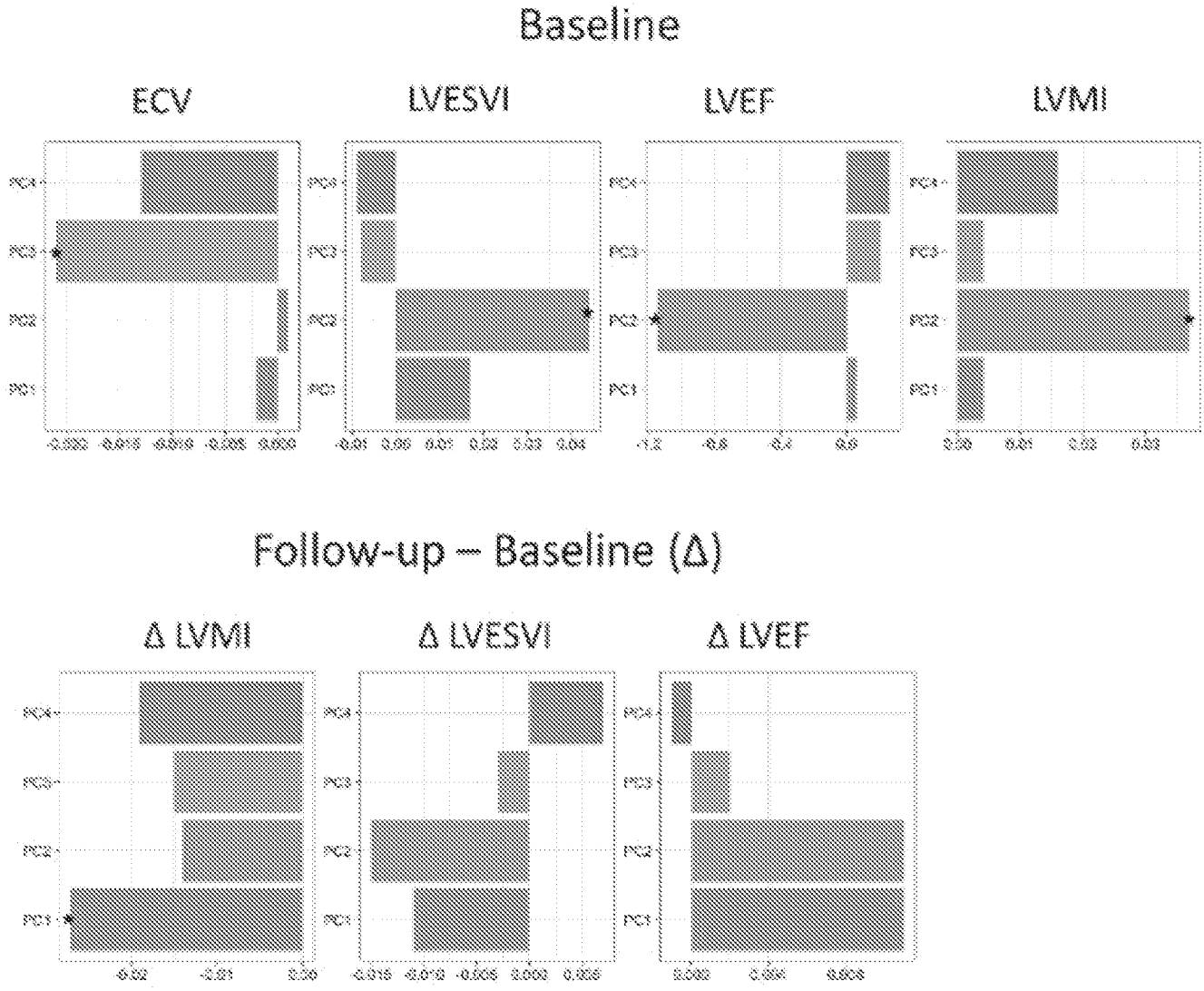

Baseline
ECV
LVESVI
LVEF
LVMI
PC4
PC3
PC2
PC1
Follow-up – Baseline (Δ)
Δ LVMI
Δ LVESVI
Δ LVEF

FIG. 7

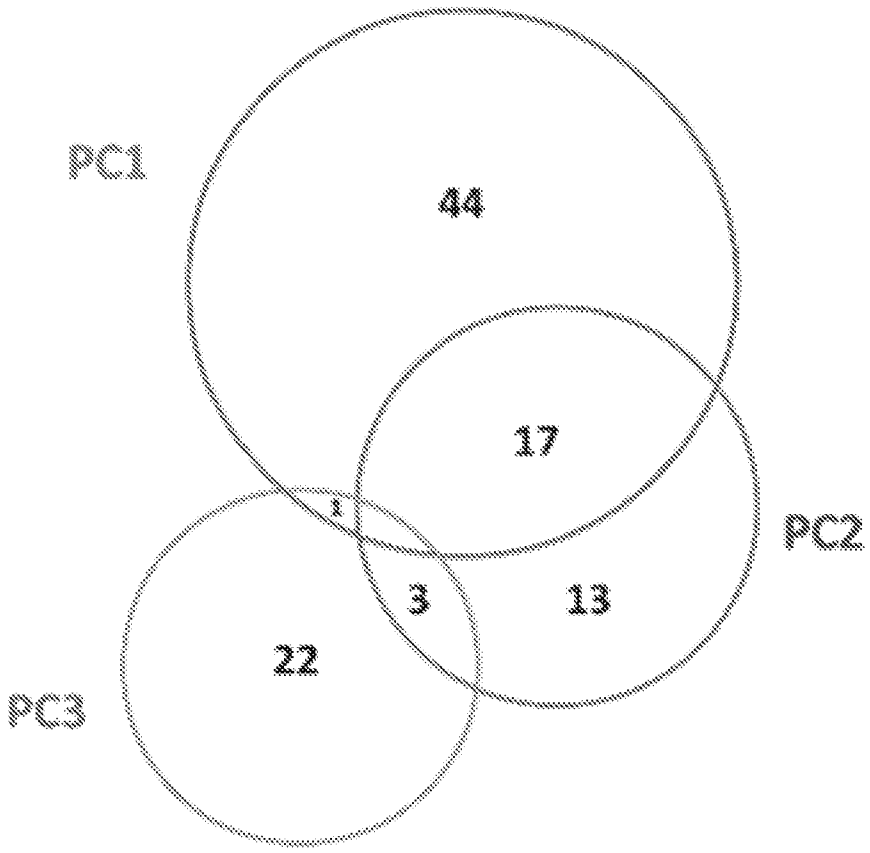


FIG. 8A

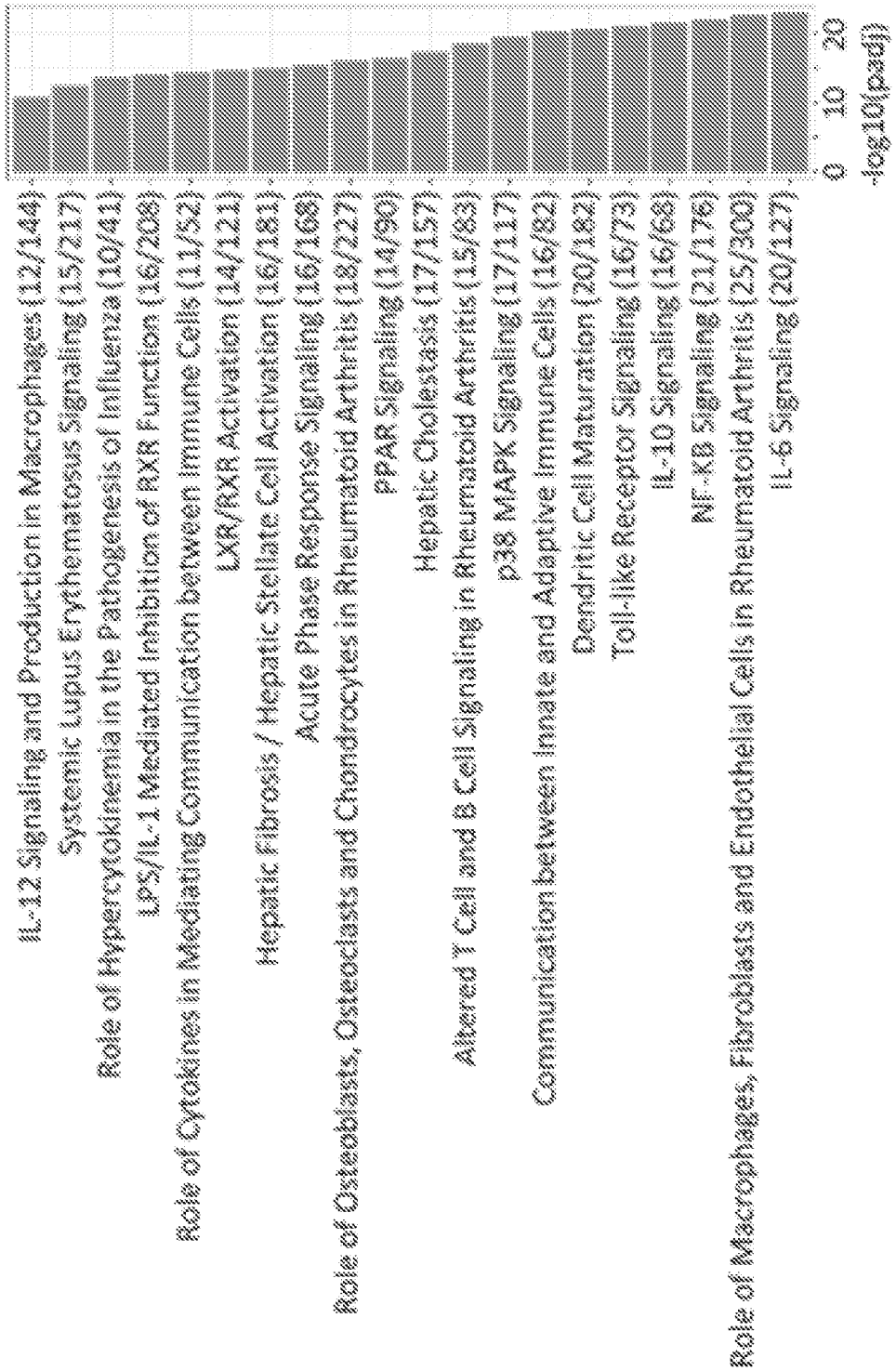

PC1
IL-12 Signaling and Production in Macrophages (12/144)
Systemic Lupus Erythematosus Signaling (15/217)
Role of Hypercytokinemia in the Pathogenesis of Influenza (10/41)
LPS/IL-1 Mediated Inhibition of RXR Function (16/208)
Role of Cytokines in Mediating Communication between Immune Cells (11/52)
LXR/RXR Activation (14/121)
Hepatic Fibrosis / Hepatic Stellate Cell Activation (16/181)
Acute Phase Response Signaling (16/168)
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis (18/227)
PPAR Signaling (14/90)
Hepatic Cholestasis (17/157)
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis (15/83)
p38 MAPK Signaling (17/117)
Communication between Innate and Adaptive Immune Cells (16/82)
Dendritic Cell Maturation (20/182)
Toll-like Receptor Signaling (16/73)
IL-10 Signaling (16/68)
NF-κB Signaling (21/176)
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis (25/300)
IL-6 Signaling (20/127)
0
10
20
-log10(padj)

FIG. 8B

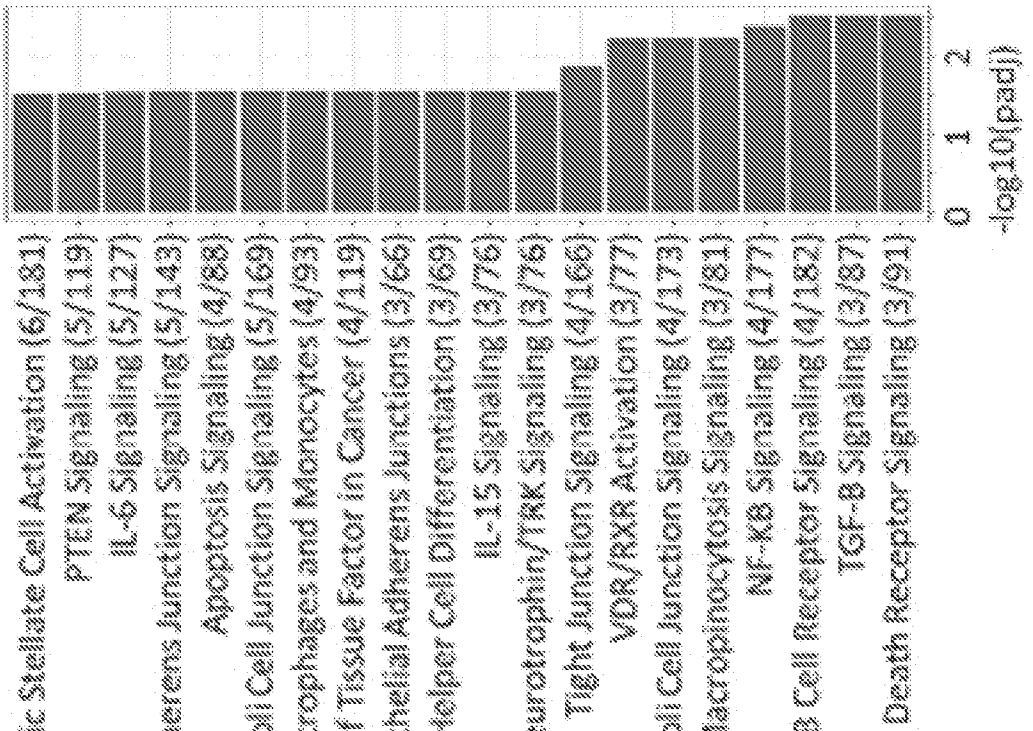

PC2
Hepatic Fibrosis / Hepatic Stellate Cell Activation (6/181)
PTEN Signaling (5/119)
IL-6 Signaling (5/127)
Epithelial Adherens Junction Signaling (5/143)
Apoptosis Signaling (4/88)
Germ Cell-Sertoli Cell Junction Signaling (5/169)
Fc_ Receptor-mediated Phagocytosis in Macrophages and Monocytes (4/93)
Role of Tissue Factor in Cancer (4/119)
Remodeling of Epithelial Adherens Junctions (3/66)
T Helper Cell Differentiation (3/69)
IL-15 Signaling (3/76)
Neurotrophin/TRK Signaling (3/76)
Tight Junction Signaling (4/166)
VDR/RXR Activation (3/77)
Sertoli Cell-Sertoli Cell Junction Signaling (4/173)
Macropinocytosis Signaling (3/81)
NF-κB Signaling (4/177)
B Cell Receptor Signaling (4/182)
TGF-β Signaling (3/87)
Death Receptor Signaling (3/91)
0
1
2
-log10(padj)

FIG. 8C

PC3
IL-12 Signaling and Production in Macrophages (4/144)
NF-κB Signaling (4/177)
Hepatic Fibrosis / Hepatic Stellate Cell Activation (4/181)
Toll-like Receptor Signaling (3/73)
IL-4 Signaling (3/86)
Acute Myeloid Leukemia Signaling (3/90)
IL-6 Signaling (3/127)
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis (4/301)
iNOS Signaling (2/43)
Acute Phase Response Signaling (3/168)
Regulation of the Epithelial-Mesenchymal Transition Pathway (3/188)
Eicosanoid Signaling (2/63)
cAMP-mediated signaling (3/217)
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis (3/227)
STAT3 Pathway (2/73)
VDR/RXR Activation (2/77)
Growth Hormone Signaling (2/81)
Macropinocytosis Signaling (2/81)
JAK/Stat Signaling (2/83)
Prolactin Signaling (2/83)
0
1
2
-log10(padj)

FIG. 9A

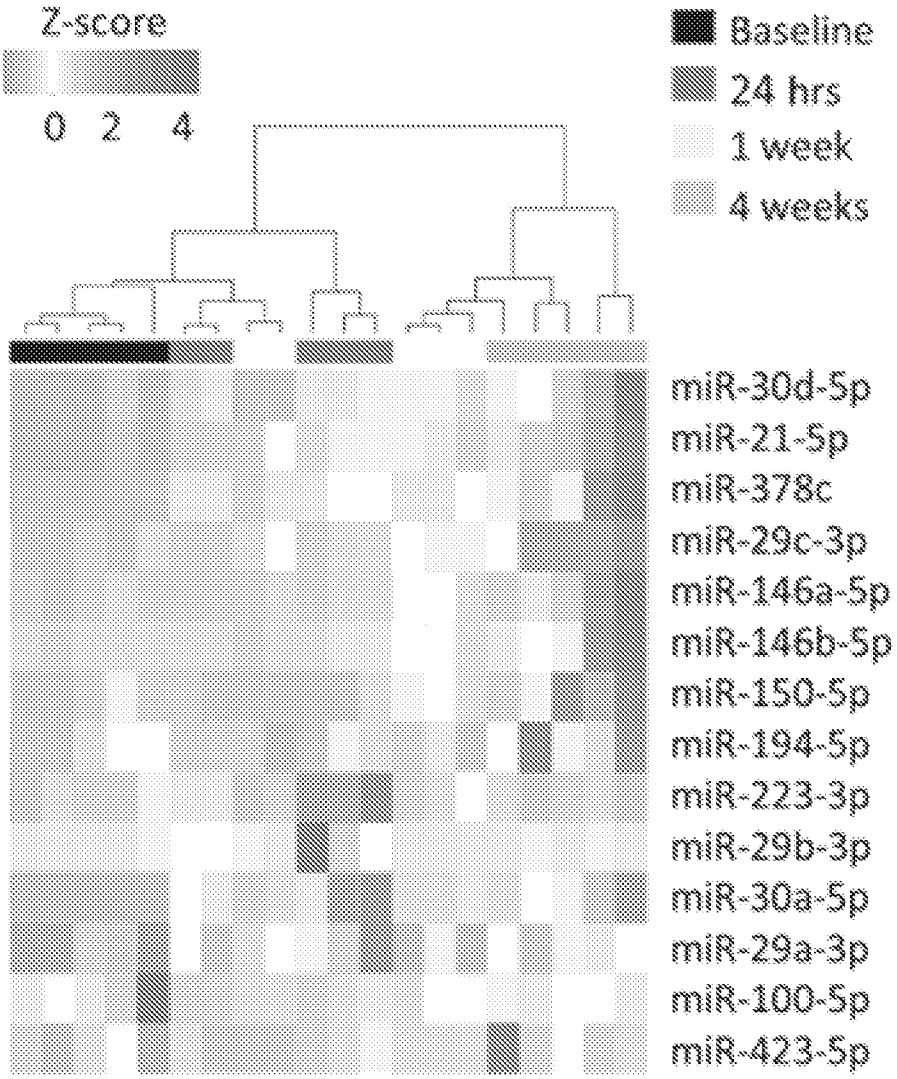

Z-score
0 2 4
Baseline
24 hrs
1 week
4 weeks
miR-30d-5p
miR-21-5p
miR-378c
miR-29c-3p
miR-146a-5p
miR-146b-5p
miR-150-5p
miR-194-5p
miR-223-3p
miR-29b-3p
miR-30a-5p
miR-29a-3p
miR-100-5p
miR-423-5p

FIG. 9B

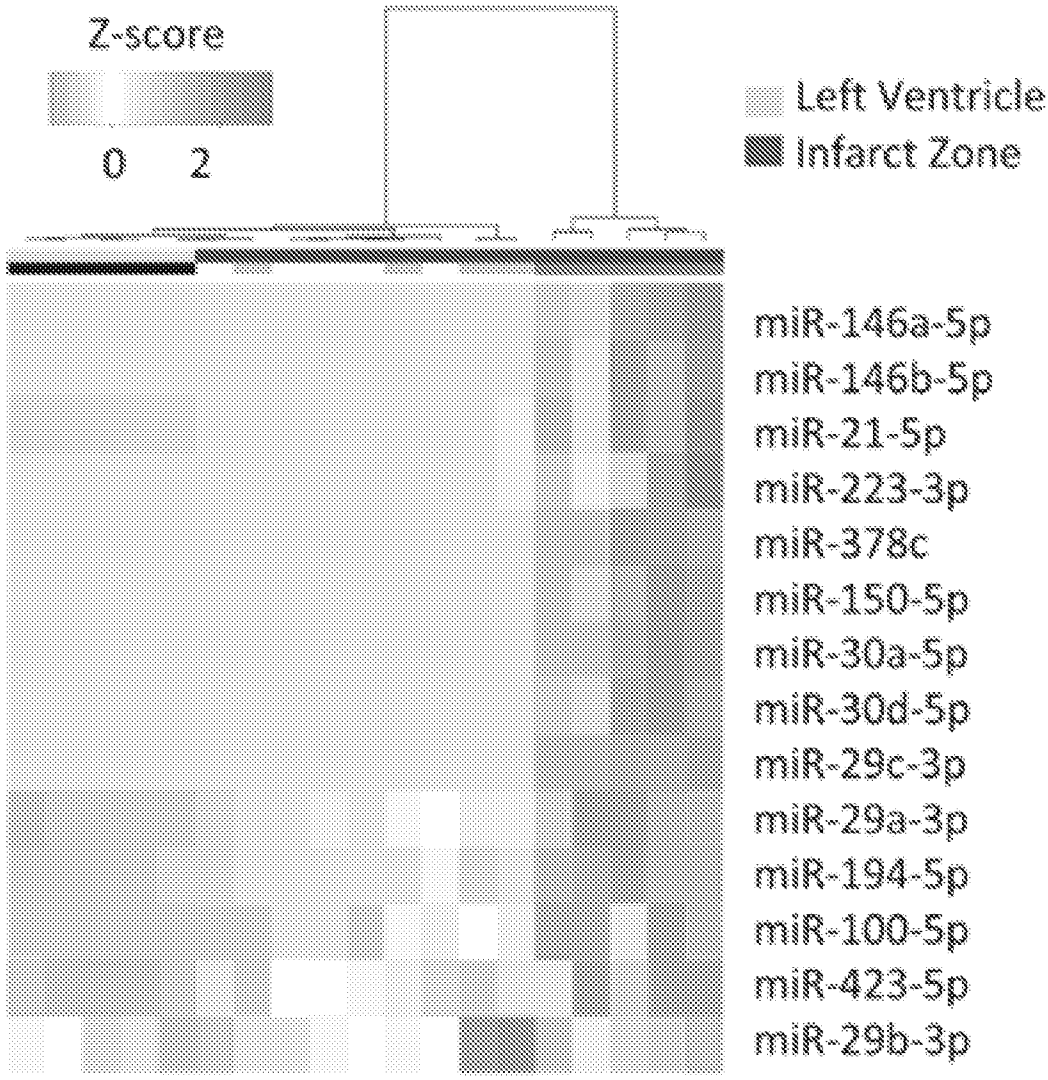

Z-score
0
2
Left Ventricle
Infarct Zone
miR-146a-5p
miR-146b-5p
miR-21-5p
miR-223-3p
miR-378c
miR-150-5p
miR-30a-5p
miR-30d-5p
miR-29c-3p
miR-29a-3p
miR-194-5p
miR-100-5p
miR-423-5p
miR-29b-3p

FIG. 10

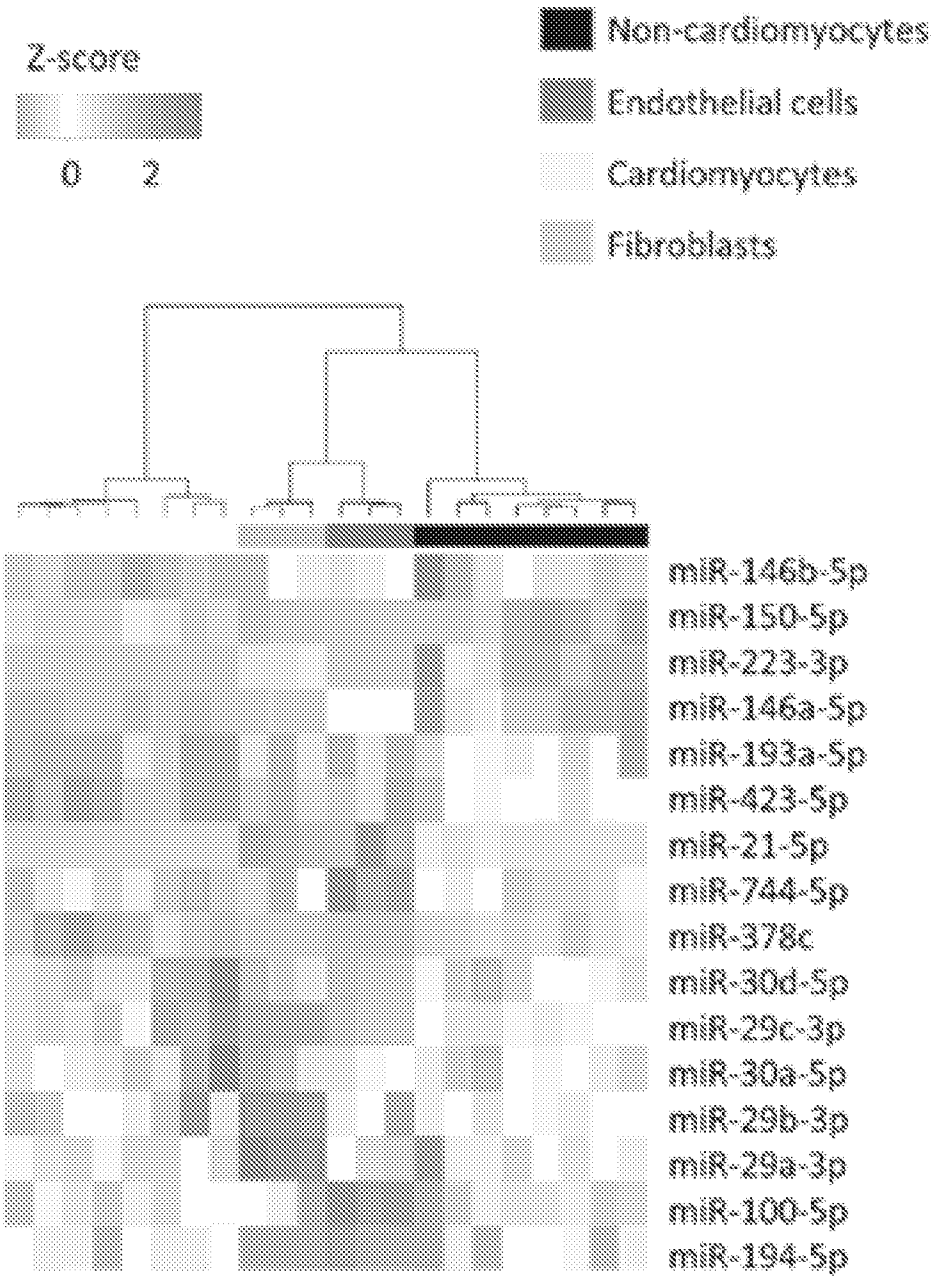
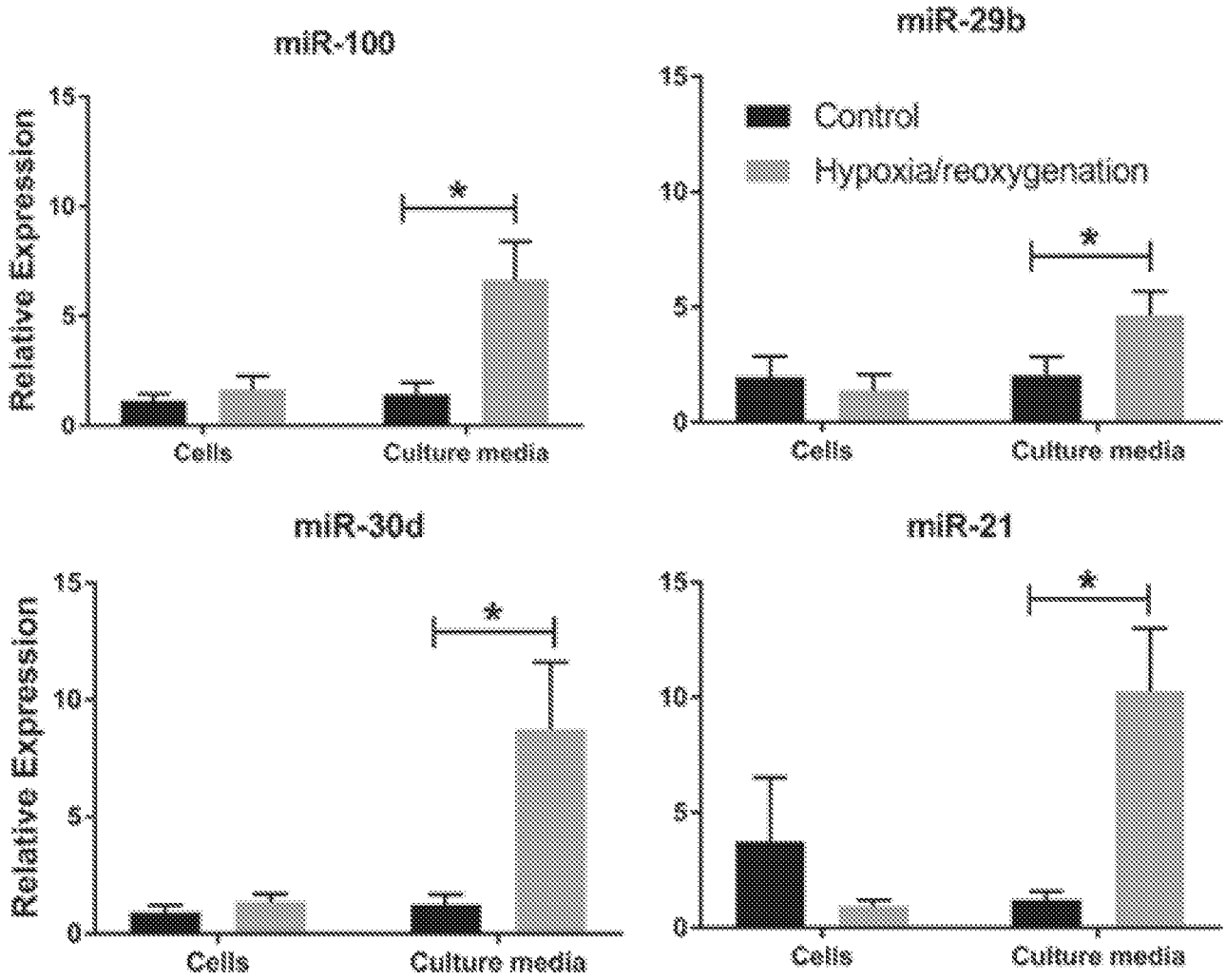

Z-score
0
2
Non-cardiomyocytes
Endothelial cells
Cardiomyocytes
Fibroblasts
miR-146b-5p
miR-150-5p
miR-223-3p
miR-146a-5p
miR-193a-5p
miR-423-5p
miR-21-5p
miR-744-5p
miR-378c
miR-30d-5p
miR-29c-3p
miR-30a-5p
miR-29b-3p
miR-29a-3p
miR-100-5p
miR-194-5p

FIG. 11


miR-100
miR-29b
miR-30d
miR-21
Relative Expression
Control
Hypoxia/reoxygenation
Cells
Culture media
0
5
10
15
*

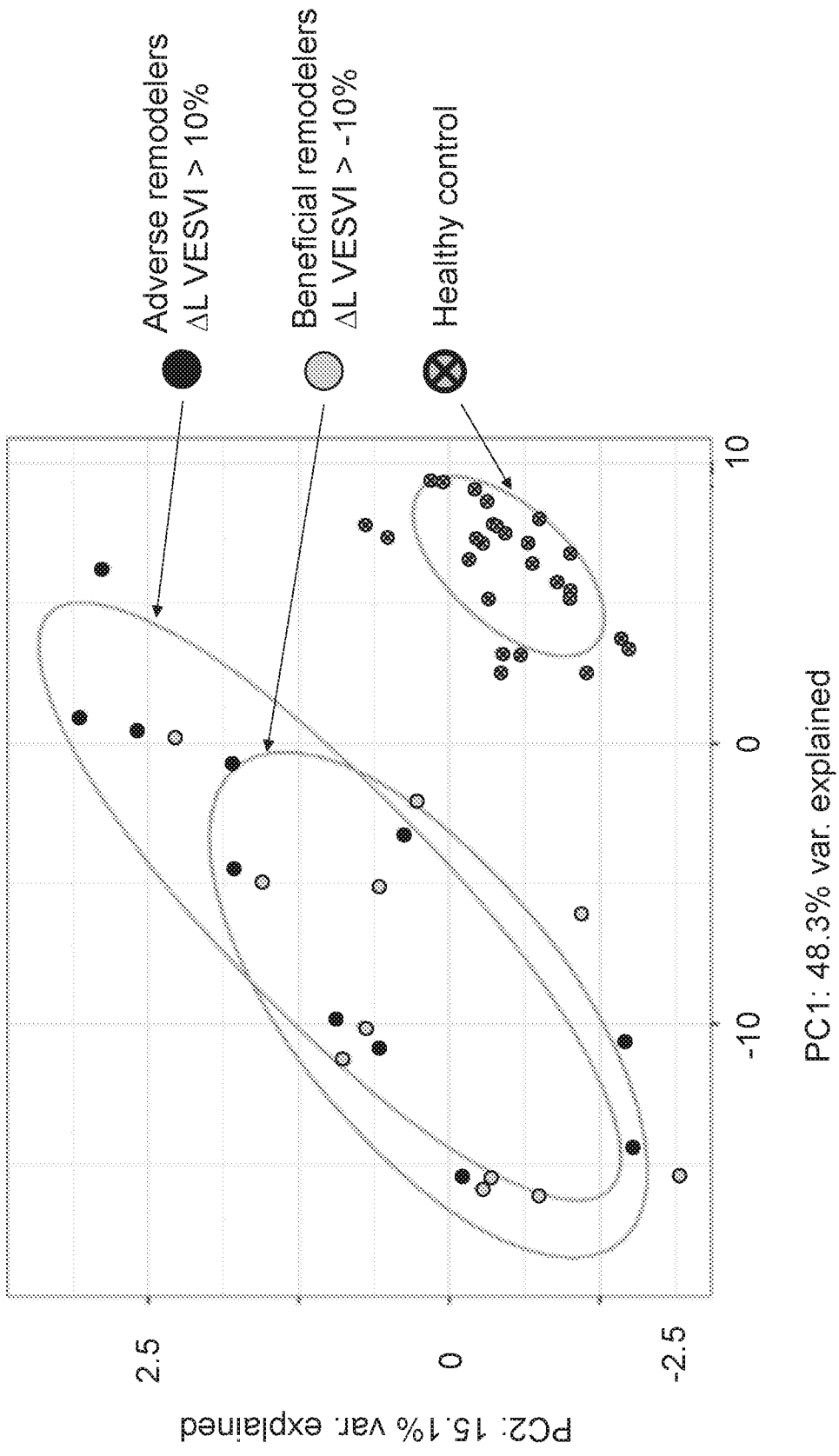
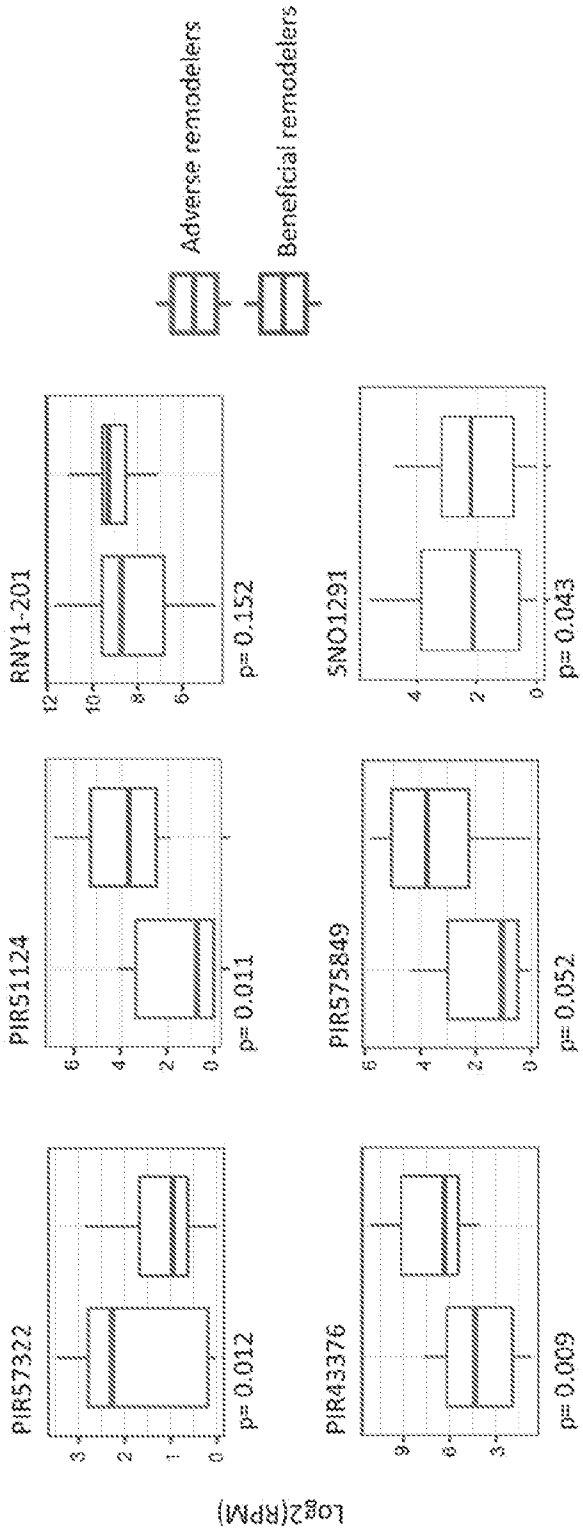

Adverse remodelers
ΔL VESVI > 10%
Beneficial remodelers
ΔL VESVI > -10%
Healthy control
2.5
0
-2.5
10
0
-10
PC2: 15.1% var. explained
PC1: 48.3% var. explained

FIG. 12

FIG. 13


Adverse remodelers
Beneficial remodelers
RNY1-201
p= 0.152
SNO1291
p= 0.043
PIR51124
p= 0.011
PIR575849
p= 0.052
PIR57322
p= 0.012
PIR43376
p= 0.009
log2(RPM)