

CARDIAC CELL REPROGRAMMING WITH MYOCARDIN AND ASCL1

FIELD OF THE INVENTION

[0001] The present disclosure relates generally to the fields of gene therapy, cellular reprogramming, and cellular therapy for diseases or disorders of the heart.

RELATED APPLICATIONS

[0002] This application claims priority to U.S. Provisional Patent Appl. No. 62/852,746, filed May 24, 2019; U.S. Provisional Patent Appl. No. 62/788,479, filed January 4, 2019; and U.S. Provisional Patent Appl. No. 62/725,168, filed August 30, 2018, each of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0003] This application is being filed electronically via EFS-Web and includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled "TENA_007_02US_SeqList_ST25.txt" created on August 30, 2019 and having a size of ~ 277 kilobytes. The sequence listing contained in this .txt file is part of the specification and is incorporated herein by reference in its entirety.

BACKGROUND

[0004] Heart failure is a major cause of death worldwide. *Braunwald's Heart Disease*, 11th ed. (2015). It has an estimated prevalence of 38 million patients worldwide, a number that is increasing with the ageing of the population. Braunwald, E. The War against Heart Failure. *Lancet* 385:812-824 (2015). The prognosis of heart failure is worse than that of most cancers. Various treatments for heart failure have been proposed, including: (1) enhancing myofilament sensitivity to Ca^{2+} , *e.g.* transfer of the gene for SERCA2a (the protein that pumps calcium into the sarcoplasmic reticulum of the cardiomyocyte); (2) gene therapy to correct abnormal calcium-handling proteins; (3) treatment with antagomirs to block the function of selected microRNAs; (4) cell therapy, *e.g.*, with bone marrow-derived mononuclear cells or mesenchymal cells; and (5) long-term ventricular assistance devices, in some cases leading to myocardial recovery and explantation of the device.

[0005] An alternative approach is cell-based therapy with induced cardiomyocytes (iCM). Cardiomyocytes have an extremely limited capacity for cell division, thus limiting the capacity of

the heart to regenerate new muscle and repair itself. One strategy for generating cardiomyocytes is *ex vivo* induction of fibroblast cells to form induced pluripotent stem cells (iPSCs) and then subsequent differentiation of iPSCs into iCMs. For example, the factors OCT4, SOX2, KLF4, and c-MYC generate iPSCs (Takahasi et al. *Cell*. 2006;126:663–76), which can be differentiated into iCMs with various method including modified temporal modulation of TGF-beta signaling and Wnt signaling using various cytokines and small molecule inhibitors (Lian et al. *Nat. Protoc.* 8:162-75 (2013); Fonoudi et al. *Stem Cells Transl. Med.* 4:1482-94 (2015)).

[0006] Direct cardiac reprogramming has emerged as a strategy to create new cardiomyocytes, leading to improved heart function following injury to the heart, such as from myocardial infarction (MI). Srivastava and DeWitt. *Cell* 166:1386-96 (2016). Direct cardiac reprogramming involves conversion of cells into cardiomyocytes without induction of a pluripotent phenotype. In one application of direct cardiac reprogramming, resident support cells within damaged organs are converted into the desired cell type *in situ*. For example, a combination of three cardiac developmental transcription factors—GATA4, MEF2C, and TBX5 (GMT)—can be used to reprogram dermal or cardiac fibroblasts to iCM-like cells in mice. Ieda et al. *Cell*. 142:375-86 (2010).

[0007] Various other combinations of genetic, as well as chemical approaches, to convert fibroblasts to cardiac progenitor or cardiomyocyte state have been proposed. HAND2, NKX2.5, JAK or TGF- β enhance such reprogramming. GATA4, MEF2C, TBX5, MESP1, and MYOCD (GMTMM) when expressed together as a cocktail of factors change cell morphology from a spindle-like shape to a rod-like shape and causes cells to exhibit spontaneous Ca^{2+} oscillation. In humans, supplementation of GMT with ETS2 and MESP1 induces cardiac-specific gene expression and sarcomere formation. Other combinations of factors for direct reprogramming are known.

SUMMARY OF THE DISCLOSURE

[0008] There remains a long-felt and unmet need for compositions and methods for generating induced cardiomyocyte cells and for the treatment of heart conditions (including heart injury, such as MI, congenic heart disease, heart disease associated with aging, and other heart failure). The present disclosure provides such compositions and methods, and more. Reprogramming factor

cocktails for generating cardiomyocytes generally require the use of at least three reprogramming factors. The inventors have surprisingly found that *MYOCD* and either one of the factors *ASCL1* and *MYF6* achieve, in human cells, direct reprogramming of non-cardiomyocytes into cardiomyocytes. Embodiments of the disclosure include compositions and methods that use *MYOCD* and *ASCL1* and/or *MYF6*, optionally with other factors, to directly reprogram non-cardiomyocytes (*e.g.*, human cardiac fibroblasts) into cardiomyocytes. Advantageously, fewer than five factors are used to reprogram human cardiac fibroblasts into cardiomyocytes. Yet more advantageously, only two factors are used to reprogram human cardiac fibroblasts into cardiomyocytes.

[0009] The disclosure provides vectors comprising combinations of a *MYOCD* polynucleotide; an *ASCL1* polynucleotide and/or a *MYF6* polynucleotide; and, optionally, a *MEF2C* polynucleotide and/or a *TBX5* polynucleotide. These protein-coding polynucleotides can be arranged in the vector in any 5' to 3' order and on the same or different polynucleotide strands within the vector. The disclosure further provides vector systems made up of more than one vector. Some vectors are polycistronic vectors—for example, 2A-linked polycistronic vectors, such as, without limitation, vectors comprising *MYOCD-2A-ASCL1*, *ASCL1-2A-MYOCD*, *MYOCD-2A-MYF6*, or *MYF6-2A-MYOCD* polynucleotides. In some cases, vector systems include a first polycistronic vector selected from the foregoing and a complementary second polycistronic vector providing *MEF2C* and *TBX5* polynucleotides.

[0010] The vectors include viral and non-viral vectors, such as, without limitation, a lipid nanoparticle, a transposon, an adeno-associated virus (AAV) vector, an adenovirus, a retrovirus, an integrating lentiviral vector (LVV), and a non-integrating LVV. Each of the polynucleotides optionally share sequence identity to a native, human polynucleotide sequence for the corresponding gene, or have a heterologous sequence encoding a protein identical to or sharing sequence identity to the corresponding native, human protein. In some embodiments, the *MYOCD* encoded by the *MYOCD* polynucleotide is an engineered myocardin. For example, myocardin may be engineered to include an internal deletion that reduces its size but preserves its function.

[0011] The disclosure further provides methods of using the foregoing vectors and vector systems. Methods of use include methods of inducing a cardiomyocyte phenotype in differentiated cells (*in vivo* or *in vitro*) and methods of treating a heart condition in a subject suffering from, or at risk for, a heart condition. In some embodiments, the method of inducing a cardiomyocyte

phenotype converts a non-cardiomyocyte cell into an induced cardiomyocyte cell. The methods of the disclosure further include methods of converting a non-cardiomyocyte cell into an induced cardiomyocyte cell. In some embodiments, the non-cardiomyocyte cell is a differentiated non-cardiomyocyte cell. In some embodiments, the non-cardiomyocyte cell is a non-cardiomyocyte cardiac cell, or cardiac fibroblast cell. Cardiomyocyte phenotype may be defined by increased expression of cTnT and/or α -actinin, or by expression of other markers for cardiomyocyte phenotype known in the art or prospectively identified. The disclosure further provides kits comprising vectors and vector systems with instructions for use in treating a heart condition.

[0012] In an aspect, the disclosure provides a vector, comprising a *MYOCD* polynucleotide and either or both of an *ASCL1* polynucleotide or a *MYF6* polynucleotide; each polynucleotide operatively linked to at least one promoter. In some embodiments, vector comprises a *MYOCD* polynucleotide and an *ASCL1* polynucleotide. In some embodiments, the vector comprises a *MYOCD* polynucleotide and a *MYF6* polynucleotide. In some embodiments, the vector comprises a *MYOCD* polynucleotide; either or both of a *MEF2C* polynucleotide and a *TBX5* polynucleotide; and either or both of an *ASCL1* polynucleotide and a *MYF6* polynucleotide. In some embodiments, the vector comprises a *MYOCD* polynucleotide, an *ASCL1* polynucleotide, a *MEF2C* polynucleotide, and a *TBX5* polynucleotide. In some embodiments, the vector comprises a *MYOCD* polynucleotide, a *MYF6* polynucleotide, a *MEF2C* polynucleotide, and a *TBX5* polynucleotide. In some embodiments, the *MYOCD* is an engineered myocardin. In some embodiments, the vector comprises no reprogramming factor polynucleotide other than a *MYOCD* polynucleotide, an *ASCL1* polynucleotide, a *MYF6* polynucleotide, a *MEF2C* polynucleotide, or a *TBX5* polynucleotide. In some embodiments, the vector comprises no other other protein-coding gene. In some embodiments, the vector is a polycistronic vector. In some embodiments, the vector is a viral vector. In some embodiments, the vector is an AAV vector. In some embodiments, the vector is lentiviral vector. In some embodiments, the *ASCL1* polynucleotide, if present, or the *MYF6* polynucleotide, if present, and the *MYOCD* polynucleotide are operatively linked to the same promoter and are co-translationally expressed. In some embodiments, the vector comprises a *MYOCD-2A-ASCL1* polynucleotide or an *ASCL1-2A-MYOCD* polynucleotide, operatively linked to a single promoter. In some embodiments, the vector comprises a *MYOCD-2A-MYF6* polynucleotide or an *ASCL1-2A-MYF6* polynucleotide, operatively linked to a single promoter.

[0013] In some embodiments, the *MYOCD* polynucleotide comprises the nucleotide sequence of human *MYOCD* (SEQ ID NO: 4) or a codon variant thereof. In some embodiments, the *MYOCD* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *MYOCD* (SEQ ID NO: 4). In some embodiments, the *MYOCD* polynucleotide encodes human MYOCD (SEQ ID NO: 3) or a functional variant thereof. In some embodiments, the *MYOCD* polynucleotide encodes a polypeptide that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to human MYOCD (SEQ ID NO: 3). In some embodiments, the *MYOCD* polynucleotide comprises the nucleotide sequence of *MyΔ3* (SEQ ID NO: 72) or a codon variant thereof. In some embodiments, the *MYOCD* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of *MyΔ3* (SEQ ID NO: 72). In some embodiments, the *MYOCD* polynucleotide encodes MyΔ3 (SEQ ID NO: 16) or a functional variant thereof. In some embodiments, the *MYOCD* polynucleotide encodes a polypeptide that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to MyΔ3 (SEQ ID NO: 16) or a codon variant thereof. In some embodiments, the *ASCL1* polynucleotide comprises the nucleotide sequence of human *ASCL1* (SEQ ID NO: 2) or a codon variant thereof. In some embodiments, the *ASCL1* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *ASCL1* (SEQ ID NO: 2) or a codon variant thereof. In some embodiments, the *ASCL1* polynucleotide encodes a polypeptide that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to human *ASCL1* (SEQ ID NO: 1). In some embodiments, the *ASCL1* polynucleotide encodes human ASCL1 (SEQ ID NO: 1). In some embodiments, the *MYF6* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *MYF6* (SEQ ID NO: 56).

[0014] In some embodiments, the vector comprises an expression cassette, the expression cassette comprising: the *MYOCD* polynucleotide, wherein the *MYOCD* polynucleotide comprises *MyΔ3* (SEQ ID NO: 72) or a codon variant thereof, and/or encodes MyΔ3 (SEQ ID NO: 16) or a functional variant thereof, the *MYOCD* polynucleotide operatively linked to a first promoter; and the *ASCL1* polynucleotide, wherein the *ASCL1* polynucleotide comprises *ASCL1* (SEQ ID NO: 2) or a codon variant thereof, and/or encodes ASCL1 (SEQ ID NO: 1) or a functional variant thereof, the *ASCL1* polynucleotide operatively linked to a second promoter.

[0015] In some embodiments, the first promoter shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a CAG promoter (SEQ ID NO: 67) and/or the second promoter shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a SCP promoter (SEQ ID NO: 68).

[0016] In some embodiments, the vector comprises an expression cassette, the expression cassette comprising a *MYOCD-2A-ASCL1* polynucleotide, operatively linked to a single promoter.

[0017] In some embodiments, the *MYOCD-2A-ASCL1* polynucleotide comprises SEQ ID NO: 37 or a codon variant thereof. In some embodiments, the *MYOCD-2A-ASCL1* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 37.

[0018] In some embodiments, the *MYOCD-2A-ASCL1* polynucleotide encodes SEQ ID NO: 59 or a functional variant thereof. In some embodiments, the *MYOCD-2A-ASCL1* polynucleotide encodes a polypeptide that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 59. In some embodiments, the expression cassette comprises one or more of an SV40 intron (SEQ ID NO: 73), a short polyA signal (SEQ ID NO: 74) and a WPRE (SEQ ID NO: 75).

[0019] In some embodiments, the expression cassette is flanked by inverted terminal repeats, optionally AAV2 ITRs (SEQ ID NO: 76). In some embodiments, the vector comprises an AAV5 capsid protein, optionally comprising SEQ ID NO: 71 or a functional variant thereof. In some embodiments, the vector is capable of reprogramming differentiated cells into induced cardiomyocyte (iCM) cells. In some embodiments, at least 2.5%, at least 5%, at least 10%, at least 15%, or at least 20% of iCM cells are α -actinin positive. In some embodiments, at least 2%, at least 5%, or at least 8% of iCM cells are cTnT positive.

[0020] In another aspect, the disclosure provides a vector system, comprising a first vector encoding a *MYOCD* polynucleotide and either or both of an *ASCL1* polynucleotide or a *MYF6* polynucleotide; and a second vector comprising a *MEF2C* polynucleotide and a *TBX5* polynucleotide. In some embodiments, the first vector comprises a *MYOCD-2A-ASCL1* polynucleotide or an *ASCL1-2A-MYOCD* polynucleotide. In some embodiments, the first vector comprises a *MYOCD-2A-MYF6* polynucleotide or an *ASCL1-2A-MYF6* polynucleotide. In some embodiments, vector system the vector system comprises a first vector encoding a *MYOCD* polynucleotide and a second vector encoding an *ASCL1* polynucleotide or a *MYF6* polynucleotide.

[0021] In some embodiments, the first vector and the second vector are each independently selected from a lipid nanoparticle, a transposon, an adeno-associated virus (AAV) vector, an adenovirus, a retrovirus, an integrating lentiviral vector (LVV), and a non-integrating LVV.

[0022] In some embodiments, the *ASCL1* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *ASCL1* (SEQ ID NO: 2).

[0023] The vector system of any one of claims 43 to 48, wherein the *MYOCD* polynucleotide encodes human MYOCD (SEQ ID NO: 3) or a functional variant thereof or My Δ 3 (SEQ ID NO: 16) or a functional variant thereof.

[0024] In another aspect, the disclosure provides method of inducing a cardiomyocyte phenotype in differentiated cells, comprising contacting the differentiated cells with a vector or vector system of the disclosure. In some embodiments, the differentiated cells are cardiac fibroblast cells. In some embodiments, the differentiated cells are *in vitro* cells during the contacting step. In some embodiments, the differentiated cells are *in vivo* cells in a subject suffering from or at risk for a heart condition.

[0025] In another aspect, the disclosure provides a method of treating a heart condition in a subject suffering from or at risk for the heart condition, comprising contacting the differentiated cells *in vitro* with a vector or vector system to generate iCM cells, and administering the iCM cells to the subject. In some embodiments, the heart condition is dilated cardiomyopathy.

[0026] In another aspect, the disclosure provides a method of treating a heart condition in a subject suffering from or at risk for a heart condition, comprising administering the a vector or vector system of the disclosure to the subject. In some embodiments, the heart condition is a myocardial infarction. In some embodiments, the heart condition is an acute myocardial infarction.

[0027] In some embodiments the heart condition is a heart failure. In some embodiments, the heart condition is a chronic ischemic heart failure.

[0028] In another aspect, the disclosure provides a kit comprising a vector or vector system of the disclosure, and instructions for use in treating a heart condition.

[0029] In another aspect, the disclosure provides a method of converting a differentiated non-cardiomyocyte cell into a cardiomyocyte, comprising comprising contacting the differentiated cells with a vector or vector system of the disclosure. In some embodiments, the differentiated non-cardiomyocyte cell is a differentiated non-cardiomyocyte cell. In some embodiments, the differentiated non-cardiomyocyte cell is a human differentiated non-cardiomyocyte cell. In some

embodiments, the differentiated non-cardiomyocyte cell is an *in vivo* differentiated non-cardiomyocyte cell. In some embodiments, the differentiated non-cardiomyocyte cell is an *in vitro* differentiated non-cardiomyocyte cell. In some embodiments, the differentiated non-cardiomyocyte cell is a cardiac cell.

[0030] Furthermore, there also remains a long-felt and unmet need for compositions and methods for generating induced cardiomyocyte cells and for the treatment of heart injury, such as MI. The present disclosure provides such compositions and methods, and more.

[0031] Myocardin has the domain architecture depicted in **FIG. 9A**. The present inventors have discovered that the myocardin protein retains its function when an internal deletion is made, such as an internal deletion between the SAP domain and the TAD domain. The second Gata4 interaction domain is dispensable for function in some cases. The leucine zipper (LZ) domain within the second Gata4 interaction domain is deleted in some embodiments, although some embodiments retain this LZ domain. Moreover, the present inventors have discovered that some embodiments of myocardin with an internal deletion have increased potency compared to full-length myocardin.

[0032] Various embodiments of the functional engineered myocardin proteins are depicted in **FIGs. 9B – 9D**. This disclosure provides these engineered myocardin proteins and others, isolated polynucleotides encoding such engineered myocardin proteins and others, vectors for delivering such polynucleotides alone or in combination with other nucleic acids, and methods of using such polynucleotides and vectors for gene therapy, cellular reprogramming, and other uses. Also provided are kits, pharmaceutical compositions, compositions for use, and other useful embodiments of the present invention.

[0033] In one aspect, the disclosure provides an isolated polynucleotide, comprising an engineered MYOCD polynucleotide encoding an engineered myocardin protein having a length of at most 850 amino acids, wherein the engineered myocardin protein comprises an SRF interaction domain, an SAP domain, and a TAD domain.

[0034] In some embodiments, the engineered myocardin protein comprises an Mef2c interaction domain. In some embodiments, the Mef2c interaction domain shares at least 85% identity to SEQ ID NO: 17, the SRF domain shares at least 85% identity to SEQ ID NO: 18, the SAP domain shares at least 85% identity to SEQ ID NO: 19, and the TAD domain shares at least 85% identity

to SEQ ID NO: 11. In some embodiments, the engineered myocardin protein comprises an LZ domain. In some embodiments, the LZ domain shares at least 85% identity to SEQ ID NO: 20.

[0035] In some embodiments, the engineered myocardin protein comprises a first polypeptide that shares at least 85% identity to residues 5-413 of human myocardin (SEQ ID NO: 10), and second polypeptide that shares at least 85% identity to residues 764-986 of human myocardin (SEQ ID NO: 11), wherein the first polypeptide and the second polypeptide are linked by a linker comprising a peptide bond or a polypeptide linker of 1-50 amino acid residues.

[0036] In some embodiments, the engineered myocardin protein comprises a first polypeptide that shares at least 85% identity to residues 5-438 of human myocardin (SEQ ID NO: 12), and second polypeptide that shares at least 85% identity to residues 764-938 of human myocardin (SEQ ID NO: 11), wherein the first polypeptide and the second polypeptide are linked by a linker comprising a peptide bond or a polypeptide linker of 1-50 amino acid residues.

[0037] In some embodiments, the engineered myocardin protein comprises a first polypeptide that shares at least 85% identity to residues 5-559 of human myocardin (SEQ ID NO: 13), and second polypeptide that shares at least 85% identity to residues 764-938 of human myocardin (SEQ ID NO: 11), wherein the first polypeptide and the second polypeptide are linked by a linker comprising a peptide bond or a polypeptide linker of 1-50 amino acid residues.

[0038] In some embodiments, the linker consists of a peptide bond. In some embodiments, the linker is a polypeptide selected from G, GG, GGG, GSG, GSS, GGS, GGSGGS (SEQ ID NO: 30), GSSGGS (SEQ ID NO: 31), GGSGSS (SEQ ID NO: 32), GGSGGSGGS (SEQ ID NO: 33), GGSGGSGGSGGS (SEQ ID NO: 34).

[0039] In some embodiments, the engineered myocardin protein comprises a sequence selected from SEQ ID NOs: 14-16.

[0040] In another aspect, the disclosure provides an isolated polynucleotide, comprising an engineered MYOCD polynucleotide encoding an engineered myocardin protein, wherein the engineered myocardin protein comprises a deletion of at least 50 amino acids in the region corresponding to amino acids 414-764 of the native myocardin (SEQ ID NO: 3).

[0041] In some embodiments, the engineered myocardin protein comprises a deletion of amino acids from about 414 to about 763 of the native myocardin (SEQ ID NO: 3).

[0042] In some embodiments, the engineered myocardin protein comprises a sequence at least 85% identical to SEQ ID NO: 14. In some embodiments, the engineered myocardin protein

consists of sequence identical to SEQ ID NO: 14. In some embodiments, the engineered myocardin protein comprises a deletion of amino acids from about 439 to about 763 of the native myocardin (SEQ ID NO: 3).

[0043] In some embodiments, the engineered myocardin protein comprises a sequence at least 85% identical to SEQ ID NO: 15. In some embodiments, the engineered myocardin protein consists of sequence identical to SEQ ID NO: 15. In some embodiments, the engineered myocardin protein comprises a deletion of amino acids from about 560 to about 763 of the native myocardin (SEQ ID NO: 3).

[0044] In some embodiments, the engineered myocardin protein comprises a sequence at least 85% identical to SEQ ID NO: 16. In some embodiments, the engineered myocardin protein consists of sequence identical to SEQ ID NO: 16. In some embodiments, the engineered myocardin protein is a functional engineered myocardin protein.

[0045] In some embodiments, the functional engineered myocardin protein is expressed at at least 10% of the level of native MYOCD in the same expression system. In some embodiments, the functional engineered myocardin protein is capable of inducing increased expression of at least one marker of cardiomyocyte phenotype either (a) when expressed in human cardiac fibroblasts with MEF2C and TBX5 or TBX5 in the presence of a TGF β inhibitor, optionally SB431542, and a Wnt inhibitor, optionally XAV939, or (b) when expressed in human cardiac fibroblasts with ASCL1.

[0046] In some embodiments, the polynucleotide is operatively linked to a promoter. In some embodiments, the polynucleotide comprises a MEF2C polynucleotide and a TBX5 polynucleotide. In some embodiments, the polynucleotide comprises an TBX5 polynucleotide. In some embodiments, the polynucleotide comprises an ASCL1 polynucleotide. In some embodiments, the polynucleotide is flanked by inverted terminal repeats (ITRs) or the polynucleotide is flanked by long terminal repeats (LTRs).

[0047] In another aspect, the disclosure provides a recombinant virus comprising any of the polynucleotides of the disclosure. In an embodiment, the recombinant virus is a recombinant adeno-associated virus (rAAV). In some embodiments, the recombinant virus is a lentivirus.

[0048] In another aspect, the disclosure provides a population of cells comprising any of the polynucleotides of the disclosure.

[0049] In some embodiments, the percentage of cells in the population that exhibit calcium transients (CaT) in an in vitro CaT assay is at least 2-fold greater than the percentage of cells in a control population of cells not comprising the polynucleotide in the same in vitro CaT assay.

[0050] In another aspect, the disclosure provides an induced cardiomyocyte (iCM), wherein the iCM comprises any of the polynucleotides of the disclosure.

[0051] In another aspect, the disclosure provides a pharmaceutical composition comprising any of the polynucleotides of the disclosure and a non-viral delivery system.

[0052] In another aspect, the disclosure provides a pharmaceutical composition comprising any of the recombinant viruses of the disclosure.

[0053] In another aspect, the disclosure provides a method of generating an induced cardiomyocyte (iCM), comprising contacting a mammalian fibroblast with a polynucleotide of the disclosure, a recombinant virus of the disclosure, or pharmaceutical composition of the disclosure.

[0054] In another aspect, the disclosure provides a method of treating a heart condition in a subject, comprising administering a pharmaceutical composition of the disclosure.

[0055] In some embodiments, the recombinant virus is administered via intracardiac catheterization. In some embodiments, the recombinant virus is administered via intracardiac injection. In another aspect, the disclosure provides a recombinant adeno-associated virus (rAAV), comprising an expression cassette (as depicted in FIG. 13B or FIG. 13C), wherein the expression cassette comprises in 5' to 3' order a 5' inverted terminal repeat (ITR), a CAG promoter, an SV40 intron, a polynucleotide encoding one or more proteins, a short polyadenylation signal, and a 3' ITR, and wherein the polynucleotide encoding one or more proteins comprises in 5' to 3' order a polynucleotide encoding My Δ 3, a polynucleotide encoding a P2A linker, and a polynucleotide encoding ASCL1.

[0056] In some embodiments, the expression cassette comprises a WPRE. In some embodiments, the My Δ 3 comprises SEQ ID NO: 16. In some embodiments, the 2A linker comprises ATNFSLLKQAGDVEENPGP (SEQ ID NO: 23). In some embodiments, the ASCL1 comprises SEQ ID NO: 1. In some embodiments, the rAAV comprises a polynucleotide at least 95% identical to SEQ ID NO: 35 (My Δ 3A AAV).

[0057] In another aspect, the disclosure provides a pharmaceutical composition comprises a rAAV of the disclosure.

[0058] In another aspect, the disclosure provides a method of treating a heart condition in a subject, comprising administering a pharmaceutical composition of the disclosure.

[0059] These aspects and other features and advantages of the invention are described below in more detail. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following disclosure and claims.

BRIEF DESCRIPTION OF FIGURES

[0060] **FIG. 1A – FIG. 1C illustrate identification of enhancers of human cardiac reprogramming from a human ORF cDNA screen.** FIG. 1A shows a schematic of the screening strategy for activators of MyMT– or MyMT+SB/XAV mediated human cardiac reprogramming in a human cardiac fibroblast (HCF) cell line. My: MYOCD, M: MEF2C, T: TBX5, SB: SB431542, XAV: XAV-939. FIG. 1B shows six enhancers (ASCL1, DLX3, DLX6, GATA2, GATA5 and MYF6) of human cardiac reprogramming identified from the screen. The graph shows the fold change of α -actinin positive cells from each reprogramming combination relative to the activity of MyMT+SB/XAV. FIG. 1C shows cardiac gene expression analysis for reprogrammed cells. Adult human cardiac fibroblasts (AHCFs) were infected with retroviruses that encoded different reprogramming factors as indicated in the graph. Reprogrammed cells were cultured for 3 weeks. The transcript levels of cardiac marker genes (MYH6, TNNT2, TNNC1, NPPA, TNNI3 and RYR2) were determined by q-PCR.

[0061] **FIG. 2A – FIG. 2D illustrate ASCL1 and MYF6 enhance cardiac reprogramming in both adult human and pig cardiac fibroblasts.** FIG. 2A and FIG. 2B show representative immunocytochemistry images (FIG. 2A) and analyses (FIG. 2B) of reprogrammed adult human cardiac fibroblasts (APCFs) 3 weeks post-infection with indicated retroviruses. Two cardiac proteins α -actinin (upper row) and cTnT (lower row) were used as markers to quantify reprogramming efficiency. The red fluorescent signal is indicative of protein expression in both cases. DAPI was used to stain nuclei (blue). FIG. 2C and FIG. 2D show representative immunocytochemistry images (FIG. 2C) and analyses (FIG. 2D) of reprogrammed adult pig cardiac fibroblasts (AHPFs) 3 weeks post-infection with indicated retroviruses. Two cardiac proteins α -actinin (upper row, red) and cTnT (lower row, red) were used as markers to qualify reprogramming efficiency. DAPI of the nucelei staining is shown (blue).

[0062] FIG. 3A – FIG. 3E illustrate dose response of reprogramming factors in AHCFs. FIG. 3A – FIG. 3D show representative immunocytochemistry images (FIG. 3A-3D) and analyses (FIG. 3E) to show dose responses of reprogramming factors. AHCFs were infected with indicated retroviruses and were cultured for 3 weeks before immunostaining. Two cardiac proteins α -actinin (upper row, red) and cTnT (lower row, red) were used as markers to qualify reprogramming efficiency. DAPI (blue).

[0063] FIG. 4A – FIG. 4C illustrate human cardiac reprogramming by two-in-one polycistronic vectors. FIG. 4A – FIG. 4B show representative immunocytochemistry images (FIG. 4A) and analyses (FIG. 4B) to determine the optimal combination of two-in-one polycistronic vectors for cardiac reprogramming. AHCFs were infected with indicated retroviruses and were cultured for 3 weeks before immunostaining. Two cardiac proteins α -actinin (up, red) and cTnT (down, red) were used as markers to qualify reprogramming efficiency. DAPI (blue). FIG. 4C shows cardiac gene expression analysis for reprogrammed cells by different combination of two-in-one polycistronic vectors. AHCFs were infected with indicated retroviruses and were cultured for 3 weeks before RNA extraction. The transcript levels of cardiac markers were determined by q-PCR.

[0064] FIG. 5A – FIG. 5D illustrate human cardiac reprogramming by a single polycistronic vector. FIG. 5A – FIG. 5B show representative immunocytochemistry images (FIG. 5A) and analyses (FIG. 5B) of AHCFs 3 weeks post-infection with GFP, My+A+M+T or My+A retroviruses to show that My+A are enough to induce cardiac reprogramming in human fibroblasts. α -actinin (up, red), cTnT (down, red), DAPI (blue). FIG. 5C – FIG. 5D show representative immunocytochemistry images (FIG. 5C) and analyses (FIG. 5D) of AHCFs 3 weeks post-infection with GFP, My+A+M+T, My-P2A-A and A-P2A-My retroviruses to show that a single polycistronic vector is enough to induce cardiac reprogramming in human fibroblasts. α -actinin (up, red), cTnT (down, red), DAPI (blue).

[0065] FIG. 6A – FIG. 6C illustrate human cardiac reprogramming by a polycistronic vector expressing four genes. FIG. 6A – FIG. 6B show representative immunocytochemistry analyses (FIG. 6A) and images (FIG. 6B) of AHCFs 3 weeks post-infection with GFP and 24 different four-in-one polycistronic retroviruses. α -actinin (up, red), cTnT (down, red), DAPI

(blue). FIG. 6C shows a factor position score matrix showing different reprogramming factors prefer different positions in the four-in-one polycistronic vector.

[0066] FIG. 7A – FIG. 7D illustrate *in vivo* delivery of reprogramming cocktails promotes heart repair after myocardial infarction. FIG. 7A shows a schematic of an *in vivo* study of different reprogramming cocktails to repair mouse heart after myocardial infarction. Mice were subjected to myocardial infarction (MI) ligation followed by intramyocardial injection of GFP, ASCL1, MyMT, MyMTA or MyA retroviruses. Reprogramming factors were in a separate virus. Cardiac function was evaluated at various time points by echocardiography. FIG. 7B – FIG. 7D show ejection fraction (FIG. 7B), end systolic volume (FIG. 7C), and end diastolic volume (FIG. 7D), of the left ventricle as quantified by echocardiography at various time points shown. MyMT, MyMTA or MyA improved cardiac function after MI. (n=11-13 for each group. n.s. $p>0.05$; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$).

[0067] FIG. 8A – FIG. 8E show design and comparative testing of bicistronic expression MyA vectors. FIG. 8A shows the design of a non-integrating lentiviral (NIL) construct encoding native myocardin (MYOCD) and Achaete-scute homolog 1 (ASCL1) proteins. FIG. 8B shows the design of an AAV construct encoding native MYOCD and ASCL1 proteins. FIG. 8C shows expression of MYOCD from the NIL construct compared to expression from a monocistronic NIL construct encoding MYOCD alone. FIG. 8D shows expression of MYOCD from the AAV construct compared to expression from a monocistronic AAV construct encoding MYOCD alone. FIG. 8E shows percentage of α -actinin positive cells after transduction with bicistronic AAV or NIL.

[0068] FIG. 9A – FIG. 9D show domain schematics of the native human myocardin (FIG. 1A) and three embodiments of engineered myocardin proteins with internal deletions: My Δ 1 (FIG. 1B), My Δ 2 (FIG. 1C), and My Δ 3 (FIG. 1D).

[0069] FIG. 10A and FIG. 10B show protein expression (FIG. 10A) and localization (FIG. 10B) of My Δ 1, My Δ 2, and My Δ 3.

[0070] FIG. 11 show calcium transients for cells transduced with MEF2C, TBX5 and native or engineered myocardin in the presence of small molecules SB431542 and XAV939.

[0071] FIG. 12A and FIG. 12B show gene expression by q-PCR (FIG. 12A), and morphology (FIG. 12B) for cells transduced with TBX5 and native or engineered myocardin in the presence of small molecules SB431542 and XAV939.

[0072] **FIGs. 13A – FIG. 13C** show design of AAV vectors for expression of native or engineered myocardin proteins with ASCL1. FIG. 13A shows MyA Δ WPRE short A. FIG. 13B shows My Δ 3A Δ WPRE short A. FIG. 13C shows My Δ 3A WPRE short A.

[0073] **FIGs. 14A – FIG. 14D** show testing of bicistronic AAV expression vectors encoding My Δ 3 and ASCL1. Protein expression levels for myocardin (FIG. 14A) and ASCL1 (FIG. 14B) are shown. Percentage of cells expressing markers of cardiac phenotype α -actinin (FIG. 14C) and cTNT (FIG. 14D) are shown.

[0074] **FIG. 15** shows percentage of cells expressing markers of cardiac phenotype α -actinin and cTNT after transduction with bicistronic NIL or AAV vectors.

[0075] **FIGs. 16A – FIG. 16B** show testing of MYOCD in combination with ASCL1 and MYF6 for direct reprogramming of human cardiac fibroblasts into cardiomyocytes. MYOCD in combination with either ASCL1 or MYF6 demonstrated robust reprogramming as measured by percentage of cTnT⁺, α -actinin⁺, or double positive cells (FIG. 16A). Sixteen other factors showed some but lower reprogramming efficiency. Representative immunofluorescence micrographs are shown in FIG. 16B.

[0076] **FIGs. 17A – FIG. 17B** show *in vivo* testing of an AAV-delivered myocardin and ASCL1 in a bicistronic format (AAV5:My Δ 3/A). FIG. 17A shows a schematic of the construct design for bicistronic AAV5:My Δ 3A. FIG. 17B shows that bicistronic AAV5: My Δ 3/A improves ejection fraction in a mouse model of myocardial infarction (MI). Mice MI model was generated by ligation of the left anterior descending (LAD) artery. AAV5:GFP or AAV5: My Δ 3/A (delivered in a single viral vector in which My Δ 3 and ASCL1 transcripts were driven from separate promoters (CAG and SCP)) at a dose of 1.2×10^{11} genome copies (GC) was intramyocardially injected to animals minutes after the ligation procedure. Cardiac function (ejection fraction) was evaluated by echocardiography at 2, 4 and 7 weeks post-MI. Imaging revealed that mice injected with AAV5:My Δ 3A showed a statistically significant improvement in ejection fraction compared to the mice injected with AAV5:GFP at 4 and 7 weeks post-MI. (n=6-13 for each group. ** p<0.01).

[0077] **FIGs. 18A – FIG. 18B** show *in vivo* testing of an AAV-delivered myocardin and ASCL1 in a monocistronic format (AAV5:My Δ 3A). FIG. 18A shows a schematic of the construct design for monocistronic AAV5:My Δ 3A. AAV5:My Δ 3A improves ejection fraction in a rat model of myocardial infarction (MI). Hank's Balanced Salt Solution (HBSS) or AAV5:My Δ 3A at dose

1.2×10^{11} GC (My Δ 3 and ASCL1 were expressed from a single transcript driven by the CAG promoter with the coding sequences separated by a P2A peptide) was delivered by intramyocardial injection immediately after permanent coronary ligation. Heart function was followed up by echocardiography every two weeks until 8 weeks post-MI. Imaging revealed cardiac function (ejection fraction) in rats injected with vehicle (HBSS) continued to decline after MI. Rats injected with AAV5:My Δ 3A showed a statistically significant improvement in ejection fraction 4-8 weeks post-MI. (n=8 for each group. * $p < 0.05$ *** $p < 0.001$).

[0078] **FIGs. 19A – FIG. 19B** show *in vivo* testing of an AAV-delivered myocardin and ASCL1 in a monocistronic format (AAV5:My Δ 3A). AAV5:My Δ 3A improves ejection fraction in a rat model of chronic heart failure due to myocardial infarction (MI). HBSS or AAV5:My Δ 3A at dose 5×10^{11} GC (My Δ 3 and ASCL1 transcripts were expressed from a single transcript including a 2A peptide) was delivered by intramyocardial injection at 2 weeks post permanent coronary ligation. **FIG. 19A** shows heart function in treated (AAV5:My Δ 3A) and vehicle control (HBSS) groups assessed by echocardiography every two weeks until 10 weeks post-MI. Echocardiography revealed that cardiac function in rats injected with vehicle (HBSS) continued to decline after MI. AAV5:My Δ 3A halted this decline in heart function. **FIG. 19B** shows that AAV5:My Δ 3A treatment caused a statistically significant improvement in ejection fraction 6-10 weeks post-MI compared to animals with vehicle. (n=10 for each group. * $p < 0.05$ ** $p < 0.01$).

DETAILED DESCRIPTION

[0079] There remains a long-felt and unmet need for compositions and methods suited for, without limitation, generating induced cardiomyocyte cells; direct reprogramming of cardiac fibroblasts into cardiomyocytes, preferably *in vivo*; treatment of various forms of heart failure, preferably dilated cardiomyopathy; and treatment of heart injury, such as MI. The present disclosure provides such compositions and methods, and more.

[0080] The present disclosure provides methods and compositions relation to the generation of induced cardiomyocyte (iCM) cells (*in vivo*, *in vitro*, or *ex vivo*) by reprogramming other cell types. In particular, the present inventors have discovered that differentiated cells, for example, fibroblasts, can be reprogrammed into cardiomyocytes by expression of Achaete-scute homolog 1 (ASCL1) and/or Myogenic Factor 6 (MYF6). ASCL1 is known primarily for its role in nervous

system, neuronal, and neuroendocrine development. Mao et al. Functional and Physical Interactions between Mammalian Achaete-Scute Homolog 1 and Myocyte Enhancer Factor 2A. *J. Bio. Chem.* 271:14371-75 (1996); Borges et al. An achaete-scute homologue essential for neuroendocrine differentiation in the lung. *Nature*. 386 (6627):852-55 (1997). In the context of cellular reprogramming, ASCL1 is known in the art as a factor associated with conversion of nonneuronal cells into functional neurons. Chanda et al. Generation of Induced Neuronal Cells by the Single Reprogramming Factor ASCL1. *Stem Cell Report* 3:282-96 (2014); Wapinski et al. Rapid Chromatin Switch in the Direct Reprogramming of Fibroblasts to Neurons. *Cell Reports* 20:3236-47 (2017); Wapinski et al. Hierarchical mechanisms for transcription factor-mediated reprogramming of fibroblasts to neurons. *Cell* 155:621-35 (2013). Indeed, expression of ASCL1 in conjunction with other reprogramming factors has been used in the art to convert human-induced pluripotent stem cells (hiPSCs) from a cardiomyocyte phenotype to a neuronal (Tuj1+cTnT⁻) or neuronal-like phenotype (Tuj1+cTnT⁺)—a contrary effect of reprogramming cardiomyocytes. Chuang et al. Partial Reprogramming of Pluripotent Stem Cell-Derived Cardiomyocytes into Neurons. *Sci Rep.* 7:44840 (2017). In contrast, the present disclosure provides compositions and methods from generating induced cardiomyocyte (iCM) cells from fibroblasts using ASCL1.

[0081] MYF6 is a myogenic factor associated with muscle differentiation. It induces fibroblasts to differentiate into myoblasts, and mutations in MYF6 are associated with congenital muscle disease. There is no suggestion that MYF6 is involved in cardiomyocyte differentiation. In contrast, the present disclosure provides compositions and methods from generating induced cardiomyocyte (iCM) cells using MYF6.

[0082] Furthermore, the present disclosure provides polynucleotides encoding engineered myocardin proteins with an internal deletion, engineered myocardin proteins with an internal deletion, and methods of use thereof. The present disclosure provides vectors comprising such polynucleotides and, in some embodiments, one or more additional nucleic acids encoding other proteins. The disclosed polynucleotides are useful, for example, for transduction of mammalian fibroblasts with polycistronic adeno-associated virus (AAV) vectors to generate induced cardiomyocytes. The present disclosure further provides methods and compositions relation to the generation of induced cardiomyocyte (iCM) cells by reprogramming of other cell types. In particular, the present inventors have discovered that differentiated cells, for example, fibroblasts,

can be reprogrammed into induced cardiomyocytes by expression of Achaete-scute homolog 1 (ASCL1); that co-expression of myocardin (MYOCD) with ASCL1 is effective for such reprogramming; that engineered myocardin proteins with an internal deletion in some embodiments enhance the function of the vector in gene expression, reprogramming, or other functions, or at least retain the same level of function as vectors with the native myocardin protein. Some embodiments of AAV vectors encoding such an engineered myocardin and ASCL1 are surprisingly improved compared to AAV vector encoding native myocardin and ASCL1. Other disclosed embodiments include, without limitation, retroviral (*e.g.*, lentiviral) vectors, vectors for co-expression of engineered myocardin with other factors in addition to or instead of ASCL1, non-viral delivery of the polynucleotides of the disclosure, *in vivo* and *ex vivo* methods of applying these embodiments, and compositions and methods for treatment of heart disease, including methods involving administration of one or more vectors and, in some embodiments, one or more small molecules. The invention is limited only by the claims, and the following detailed description provides diverse embodiments beyond those described in this paragraph.

[0083] In an aspect, the disclosure relates to engineered variants of myocardin (MYOCD). MYOCD is a large, polyfunctional transcription factor. In addition to showing that expression of ASCL1 in combination with MYOCD was sufficient to induce a cardiomyocyte phenotype in mammalian fibroblasts with or without other reprogramming factors the present inventors recognized that a viral vector encoding ASCL1 and MYOCD generates induced cardiomyocytes. The disclosure provides viral vectors that encode both MYOCD and ASCL1, including, for example, lentiviral and AAV vectors. The present inventors further recognized that the lentiviral vectors of disclosure, in some embodiments, induced cardiomyocytes more effectively than the AAV vectors of the disclosure.

[0084] The present inventors also surprisingly find that MYOCD comprising an internal deletion retains the expression and function of myocardin and MYOCD comprising an internal deletion can be used alone or in combination with other reprogramming factors (*e.g.*, for generating cardiomyocytes from fibroblasts); and furthermore that viral vectors comprising such engineered MYOCD were, in some embodiments, as effective or more effective than viral vectors comprising the native MYOCD. The present disclosure therefore also provides various engineered MYOCD polynucleotides, viral vectors, gene delivery systems, and method of use thereof.

I. Definitions

[0085] As used herein, the term “functional cardiomyocyte” refers to a differentiated cardiomyocyte that is able to send or receive electrical signals. In some embodiments, a cardiomyocyte is said to be a functional cardiomyocyte if it exhibits electrophysiological properties such as action potentials and/or Ca^{2+} transients.

[0086] As used herein, a “differentiated non-cardiac cell” can refer to a cell that is not able to differentiate into all cell types of an adult organism (*i.e.*, is not a pluripotent cell), and which is of a cellular lineage other than a cardiac lineage (*e.g.*, a neuronal lineage or a connective tissue lineage). Differentiated cells include, but are not limited to, multipotent cells, oligopotent cells, unipotent cells, progenitor cells, and terminally differentiated cells. In particular embodiments, a less potent cell is considered “differentiated” in reference to a more potent cell.

[0087] As used herein, “protein-coding gene” means, when referring to a component of a vector, a polynucleotide that encodes a protein, other than a gene associated with the function of the vector. For example, the term protein-coding gene would encompass a polynucleotide encoding a human protein, or functional variant thereof, with reprogramming activity. It is intended that the phrase “the vector comprising no other protein-coding gene” in reference to a vector means that the vector comprises a polynucleotide(s) encoding the protein of interest(s) that is listed, but no polynucleotide encoding another protein that has reprogramming activity—such as other proteins known in the art to promote either a pluripotent or a cardiomyocyte phenotype. The phrase “the vector comprising no other protein-coding gene” does not exclude polynucleotides encoding proteins required for function of the vector, which optionally may be present, nor does the phrase exclude polynucleotides that do not encode proteins. Such vectors will include non-coding polynucleotide sequences and may include polynucleotides encoding RNA molecules (such as microRNAs). Conversely, when only certain protein-coding genes are listed, it is implied that other protein-coding genes may additionally be present, such as protein-coding genes that encode proteins that have reprogramming activity. further promote reprogramming.

[0088] A “somatic cell” is a cell forming the body of an organism. Somatic cells include cells making up organs, skin, blood, bones and connective tissue in an organism, but not germ cells.

[0089] The terms “cardiac pathology” or “cardiac dysfunction” are used interchangeably and refer to any impairment in the heart’s pumping function. This includes, for example, impairments

in contractility, impairments in the ability to relax (sometimes referred to as diastolic dysfunction), abnormal or improper functioning of the heart's valves, diseases of the heart muscle (sometimes referred to as cardiomyopathies), diseases such as angina pectoris, myocardial ischemia and/or infarction characterized by inadequate blood supply to the heart muscle, infiltrative diseases such as amyloidosis and hemochromatosis, global or regional hypertrophy (such as may occur in some kinds of cardiomyopathy or systemic hypertension), and abnormal communications between chambers of the heart.

[0090] As used herein, the term “cardiomyopathy” refers to any disease or dysfunction of the myocardium (heart muscle) in which the heart is abnormally enlarged, thickened and/or stiffened. As a result, the heart muscle's ability to pump blood is usually weakened. The etiology of the disease or disorder may be, for example, inflammatory, metabolic, toxic, infiltrative, fibroplastic, hematological, genetic, or unknown in origin. There are two general types of cardiomyopathies: ischemic (resulting from a lack of oxygen) and non-ischemic.

[0091] “Heart failure (HF) is a complex clinical syndrome that can result from any structural or functional cardiovascular disorder causing systemic perfusion inadequate to meet the body's metabolic demands without excessively increasing left ventricular filling pressures. It is characterized by specific symptoms, such as dyspnea and fatigue, and signs, such as fluid retention. As used herein, “chronic heart failure” or “congestive heart failure” or “CHF” refer, interchangeably, to an ongoing or persistent forms of heart failure. Common risk factors for CHF include old age, diabetes, high blood pressure and being overweight. CHF is broadly classified according to the systolic function of the left ventricle as HF with reduced or preserved ejection fraction (HFrEF and HFpEF). The term “heart failure” does not mean that the heart has stopped or is failing completely, but that it is weaker than is normal in a healthy person. In some cases, the condition can be mild, causing symptoms that may only be noticeable when exercising, in others, the condition may be more severe, causing symptoms that may be life-threatening, even while at rest. The most common symptoms of chronic heart failure include shortness of breath, tiredness, swelling of the legs and ankles, chest pain and a cough. In some embodiments, the methods of the disclosure decrease, prevent, or ameliorate one or more symptoms of CHF (*e.g.*, HFrEF) in a subject suffering from or at risk for CHF (*e.g.*, HFrEF). In some embodiments, the disclosure provides methods of treating CHF and conditions that can lead to CHF.

[0092] As used herein “acute heart failure” or “decompensated heart failure” refer, interchangeably, to a syndrome of the worsening of signs and symptoms reflecting an inability of the heart to pump blood at a rate commensurate to the needs of the body at normal filling pressure. AHF typically develops gradually over the course of days to weeks and then decompensates requiring urgent or emergent therapy due to the severity of these signs or symptoms. AHF may be the result of a primary disturbance in the systolic or diastolic function of the heart or of abnormal venous or arterial vasoconstriction, but generally represents an interaction of multiple factors, including volume overload. The majority of patients with AHF have decompensation of chronic heart failure (CHF) and consequently much of the discussion of the pathophysiology, presentation, and diagnosis of CHF is directly relevant to an understanding of AHF. In other cases, AHF results from an insult to the heart or an event that impairs heart function, such as an acute myocardial infarction, severe hypertension, damage to a heart valve, abnormal heart rhythms, inflammation or infection of the heart, toxins and medications. In some embodiments, the methods of the disclosure decrease, prevent, or ameliorate one or more symptoms of AHF in a subject suffering from or at risk for AHF. In some embodiments, the disclosure provides methods of treating AHF and conditions that can lead to AHF. AHF may be the result of ischemia associated with myocardial infarction.

[0093] In some embodiments, the methods of the disclosure (*e.g.*, reprogramming therapy) treat one or more heart conditions (*e.g.* heart conditions that can lead to acute and chronic heart failure in some subjects). Illustrative conditions treatable according to the methods and compositions of the disclosure include acute myocardial infarction (MI), ischemic heart disease or ischemic cardiomyopathy (CM) (forms of chronic MI), dilated CM, hypertensive CM, familial CM, genetic CM, idiopathic CM, CM resulting from valvular heart disease, medication and toxin-induced CMs, CM due to myocarditis, and peripartum CM. In some embodiments, the compositions and methods disclosure herein treat congenital heart disease. In some embodiments, the methods of the disclosure comprise administering a composition (*e.g.* a viral vector) to a subject having, exhibiting symptoms of, or at risk for a condition that has progressed to heart failure or has yet to progress to heart failure. Reprogramming could be used either to treat or to prevent heart failure in patients with these conditions. Chronic heart failure due to all of these conditions fall into the general term “heart failure with reduced ejection fraction” (HFrEF).

[0094] As used herein, the term “gene of interest” refers to a reprogramming factor or to nucleic acid encoding the reprogramming factor. For example, when the reprogramming factor is a protein, the gene of interest is either – as apparent from context – a protein or the corresponding protein-coding polynucleotide sequence. Introduction, administration, or other use of gene of interest should be understood to refer to any means of increasing the expression of, or increasing the activity of, a gene, gene product, or functional variant of a gene product. Thus, in some embodiments, the disclosure provides methods of generating iCM cells comprising introducing a polynucleotide of interest, *e.g.* ASCL1 and/or MYF6, as a nucleic acid (*e.g.* deoxyribonucleotide (DNA) or ribonucleotide (RNA)) into a target cell as a polynucleotide (*e.g.* deoxyribonucleotide (DNA) or ribonucleotide (RNA)). The polynucleotide may be introduced into a cell in any of the various means known in the art, including without limitation in a viral, non-viral vector, by contacting the cell with naked polynucleotide or polynucleotide in complex with a transfection reagent, or by electroporation. Use of a gene of interest as a nucleic acid may also include indirect alteration of the expression or activity of the gene of interest, such as gene-editing of the locus encoding the endogenous gene, expression of transcription or regulatory factors, contacting cells with a small-molecule activator of the gene of interest, or use of gene-editing methods, including DNA- or RNA-based methods, to alter the expression or activity of the gene of interest as a nucleic acid. In some embodiments, the methods of the disclosure include de-repressing transcription of a gene of interest by editing regulatory regions (*e.g.* enhancers or promoters), altering splice sites, removing or inserting microRNA recognition sites, administering an antagomir to repress a microRNA, administering a microRNA mimetic, or any other various means of modulating expression or activity of the gene of interest.

[0095] As used herein, “gene product” means the product of expression of a polynucleotide sequence. For example, a protein-coding sequence is expressed by translation of the sequence into a protein gene product, or a RNA-coding sequence is expression by transcription of the DNA sequence into the corresponding RNA.

[0096] As used herein, the term “totipotent” means the ability of a cell to form all cell lineages of an organism. For example, in mammals, only the zygote and the first cleavage stage blastomeres are totipotent.

[0097] As used herein, the term “pluripotent” means the ability of a cell to form all lineages of the body or soma. For example, embryonic stem cells are a type of pluripotent stem cells that are

able to form cells from each of the three germs layers, the ectoderm, the mesoderm, and the endoderm. Pluripotent cells can be recognized by their expression of markers such as Nanog and Rex1.

[0098] As used herein, the term “multipotent” refers to the ability of an adult stem cell to form multiple cell types of one lineage. For example, hematopoietic stem cells are capable of forming all cells of the blood cell lineage, *e.g.*, lymphoid and myeloid cells.

[0099] As used herein, the term “oligopotent” refers to the ability of an adult stem cell to differentiate into only a few different cell types. For example, lymphoid or myeloid stem cells are capable of forming cells of either the lymphoid or myeloid lineages, respectively.

[00100] As used herein, the term “unipotent” means the ability of a cell to form a single cell type. For example, spermatogonial stem cells are only capable of forming sperm cells.

[00101] As used herein, the terms “subject” or “patient” refers to any animal, such as a domesticated animal, a zoo animal, or a human. The “subject” or “patient” can be a mammal like a dog, cat, horse, livestock, a zoo animal, or a human. The subject or patient can also be any domesticated animal such as a bird, a pet, or a farm animal. Specific examples of “subjects” and “patients” include, but are not limited to, individuals with a cardiac disease or disorder, and individuals with cardiac disorder-related characteristics or symptoms.

[00102] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of tissue culture, immunology, molecular biology, cell biology and recombinant DNA, which are within the skill of the art. *See, e.g.*, Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition; the series Ausubel et al. eds. (2007) *Current Protocols in Molecular Biology*; the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson et al. (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson et al. (1995) *PCR 2: A Practical Approach*; Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual*; Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition; Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Pat. No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization*; Anderson (1999) *Nucleic Acid Hybridization*; Hames and Higgins eds. (1984) *Transcription and Translation*; IRL Press (1986) *Immobilized Cells and Enzymes*; Perbal (1984) *A Practical Guide to Molecular Cloning*; Miller and Calos eds. (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and

Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Herzenberg et al. eds (1996) *Weir's Handbook of Experimental Immunology; Manipulating the Mouse Embryo: A Laboratory Manual*, 3rd edition (2002) Cold Spring Harbor Laboratory Press; Sohail (2004) *Gene Silencing by RNA Interference: Technology and Application* (CRC Press); Sell (2013) *Stem Cells Handbook*.

[00103] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the disclosure also contemplates that in some embodiments, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[00104] All numerical designations, *e.g.*, pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 1.0 or 0.1, as appropriate, or alternatively by a variation of +/- 15 %, or alternatively 10%, or alternatively 5%, or alternatively 2%. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term “about”. It is to be understood that such range format is used for convenience and brevity and should be understood flexibly to include numerical values explicitly specified as limits of a range, but also to include all individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly specified. For example, a ratio in the range of about 1 to about 200 should be understood to include the explicitly recited limits of about 1 and about 200, but also to include individual ratios such as about 2, about 3, and about 4, and sub-ranges such as about 10 to about 50, about 20 to about 100, and so forth. It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[00105] It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a cardiomyocyte” includes a plurality of cardiomyocytes.

[00106] Also as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[00107] “Administration,” “administering” and the like, when used in connection with a composition of the invention refer both to direct administration, which may be administration to non-cardiomyocytes *in vitro*, administration to non-cardiomyocytes *in vivo*, administration to a subject by a medical professional or by self-administration by the subject and/or to indirect administration, which may be the act of prescribing a composition of the invention. When used herein in reference to a cell, it refers to introducing a composition to the cell. Typically, an effective amount is administered, which amount can be determined by one of skill in the art. Any method of administration may be used. Small molecules may be administered to the cells by, for example, addition of the small molecules to the cell culture media or injection *in vivo* to the site of cardiac injury. Administration to a subject can be achieved by, for example, intravascular injection, intramyocardial delivery, and the like.

[00108] As used herein the term “cardiac cell” refers to any cell present in the heart that provides a cardiac function, such as heart contraction or blood supply, or otherwise serves to maintain the structure of the heart. Cardiac cells as used herein encompass cells that exist in the epicardium, myocardium or endocardium of the heart. Cardiac cells also include, for example, cardiac muscle cells or cardiomyocytes, and cells of the cardiac vasculatures, such as cells of a coronary artery or vein. Other non-limiting examples of cardiac cells include epithelial cells, endothelial cells, fibroblasts, cardiac stem or progenitor cells, cardiac conducting cells and cardiac pacemaking cells that constitute the cardiac muscle, blood vessels and cardiac cell supporting structure. Cardiac cells may be derived from stem cells, including, for example, embryonic stem cells or induced pluripotent stem cells.

[00109] The term “cardiomyocyte” or “cardiomyocytes” as used herein refers to sarcomere-containing striated muscle cells, naturally found in the mammalian heart, as opposed to skeletal muscle cells. Cardiomyocytes are characterized by the expression of specialized molecules *e.g.*, proteins like myosin heavy chain, myosin light chain, cardiac α -actinin. The term “cardiomyocyte” as used herein is an umbrella term comprising any cardiomyocyte subpopulation or cardiomyocyte subtype, *e.g.*, atrial, ventricular and pacemaker cardiomyocytes.

[00110] The term “cardiomyocyte-like cells” is intended to mean cells sharing features with cardiomyocytes, but which may not share all features. For example, a cardiomyocyte-like cell may differ from a cardiomyocyte in expression of certain cardiac genes.

[00111] The term “culture” or “cell culture” means the maintenance of cells in an artificial, *in vitro* environment. A “cell culture system” is used herein to refer to culture conditions in which a population of cells may be grown as monolayers or in suspension. “Culture medium” is used herein to refer to a nutrient solution for the culturing, growth, or proliferation of cells. Culture medium may be characterized by functional properties such as, but not limited to, the ability to maintain cells in a particular state (*e.g.*, a pluripotent state, a quiescent state, etc.), or to mature cells, such as, in some embodiments, to promote the differentiation of progenitor cells into cells of a particular lineage (*e.g.*, a cardiomyocyte).

[00112] As used herein, the term “expression” or “express” refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell. The expression level of a gene may be determined by measuring the amount of mRNA or protein in a cell or tissue sample.

[00113] As used herein, an “expression cassette” is a DNA polynucleotide comprising one or more polynucleotide encoding protein(s) or nucleic acid(s) that is configured to express the polynucleotide in a host cell. Typically, expression of the polynucleotide(s) is placed under the control of certain regulatory elements, including constitutive or inducible promoters, tissue-specific regulatory elements, and enhancers. Such polynucleotides are said to be “operably linked to” or “operatively linked to” the regulatory elements (*e.g.*, a promoter).

[00114] The term “induced cardiomyocyte” or the abbreviation “iCM” refers to a non-cardiomyocyte (and its progeny) that has been transformed into a cardiomyocyte (and/or cardiomyocyte-like cell). The methods of the present disclosure can be used in conjunction with any methods now known or later discovered for generating induced cardiomyocytes, for example, to enhance other techniques.

[00115] The term “non-cardiomyocyte” as used herein refers to any cell or population of cells in a cell preparation not fulfilling the criteria of a “cardiomyocyte” as defined and used herein. Non-limiting examples of non-cardiomyocytes include somatic cells, cardiac fibroblasts, non-cardiac fibroblasts, cardiac progenitor cells, and stem cells.

[00116] The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound

medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00117] The terms “regenerate,” “regeneration” and the like as used herein in the context of injured cardiac tissue shall be given their ordinary meanings and shall also refer to the process of growing and/or developing new cardiac tissue in a heart or cardiac tissue that has been injured, for example, injured due to ischemia, infarction, reperfusion, or other disease. In some embodiments, cardiac tissue regeneration comprises generation of cardiomyocytes.

[00118] As used herein, the term “reprogramming” or “transdifferentiation” refers to the generation of a cell of a certain lineage (*e.g.*, a cardiac cell) from a different type of cell (*e.g.*, a fibroblast cell) without an intermediate process of de-differentiating the cell into a cell exhibiting pluripotent stem cell characteristics. As used herein “reprogramming” includes transdifferentiation, dedifferentiation and the like.

[00119] As used herein, “reprogramming activity” refers to the ability of a protein or polynucleotide having reprogramming activity to induce or to promote reprogramming of a cell into a cardiomyocyte or cardiomyocyte-like cell when it is introduced into or expressed by the cell, alone or in combination with other proteins or polynucleotides having reprogramming activity. For example, a first protein has reprogramming activity if expression of the first protein in a cell with no other factors induces or promotes reprogramming of the cell; but the first protein also has reprogramming activity, as the term is used herein, if the first protein promotes reprogramming in combination with a second protein—that is, when both the first protein and the second protein are expressed together.

[00120] As used herein, the term “reprogramming efficiency” refers to the number of cells in a sample that are successfully reprogrammed to cardiomyocytes relative to the total number of cells in the sample.

[00121] The term “reprogramming factor” as used herein includes a factor that is introduced for expression in a cell to assist in the reprogramming of the cell into an induced cardiomyocyte. Reprogramming factors include proteins and nucleic acids (*e.g.*, RNAs such as microRNAs, siRNA, or shRNAs).

[00122] The term “stem cells” refer to cells that have the capacity to self-renew and to generate differentiated progeny. The term “pluripotent stem cells” refers to stem cells that can give rise to

cells of all three germ layers (endoderm, mesoderm and ectoderm), but do not have the capacity to give rise to a complete organism.

[00123] “Treatment,” “treating,” and “treat” are defined as acting upon a disease, disorder, or condition with an agent to reduce or ameliorate harmful or any other undesired effects of the disease, disorder, condition and/or their symptoms.

[00124] As used herein, the term “effective amount” and the like refers to an amount that is sufficient to induce a desired physiologic outcome (*e.g.*, reprogramming of a cell or treatment of a disease). An effective amount can be administered in one or more administrations, applications or dosages. Such delivery is dependent on a number of variables including the time period which the individual dosage unit is to be used, the bioavailability of the composition, the route of administration, etc. It is understood, however, that specific amounts of the compositions (*e.g.*, reprogramming factors) for any particular subject depends upon a variety of factors including the activity of the specific agent employed, the age, body weight, general health, sex, and diet of the subject, the time of administration, the rate of excretion, the composition combination, severity of the particular disease being treated and form of administration.

[00125] As used herein, the term “equivalents thereof” in reference to a polypeptide or nucleic acid sequence refers to a polypeptide or nucleic acid that differs from a reference polypeptide or nucleic acid sequence, but retains essential properties (*e.g.*, biological activity). A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, deletions, additions, fusions and truncations in the polypeptide encoded by the reference sequence. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical.

[00126] The term “isolated” means separated from constituents, cellular and otherwise, in which the cell, tissue, polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, which are normally associated in nature. For example, an isolated cell is a cell that is separated from tissue or cells of dissimilar phenotype or genotype. As is apparent to those of skill in the art, a non-naturally occurring polynucleotide, peptide, polypeptide, protein, or cell does not require “isolation” to distinguish it from its naturally occurring counterpart.

[00127] As used herein, the term “nucleic acid” and “polynucleotide” are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting examples of polynucleotides include linear and circular nucleic acids, messenger RNA (mRNA), cDNA, recombinant polynucleotides, vectors, probes, and primers.

[00128] The terms “polypeptide,” “peptide,” and “protein,” are used interchangeably herein and refer to a polymeric form of amino acids of any length, which can include genetically coded and non-genetically coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues, immunologically tagged proteins, and the like.

[00129] As used herein, the word “polynucleotide” preceded by a gene name (for example, “*MYOCD* polynucleotide”) refers to a polynucleotide sequence encoding the corresponding protein (for example, a “*MYOCD* protein”).

[00130] As used herein, the word “protein” preceded by a gene name (for example, “*MYOCD* protein”) refers to either the native protein or a functional variant thereof. A “native protein” is a protein encoded by a genomic copy of a gene of an organism, preferably the organism for which the vector is intended (*e.g.*, a human, a rodent, a primate, or an animal of veterinary interest), in any of the gene’s functional isoforms or functional allelic variations.

[00131] As used herein, a “functional variant” or “variant” of a protein is a variant with any number of amino acid substitutions, insertions, truncations, or internal deletions that retains the functional attributes of the protein, including, *e.g.*, the protein’s ability to induce, in combination with other factors, the reprogramming of cells into cardiomyocytes. Functional variants can be identified computationally, such as variants having only conservative substitutions, or experimentally using *in vitro* or *in vivo* assays.

[00132] As used herein, a “codon variant” of a polynucleotide sequence is polynucleotide sequence that encodes the same protein as a reference polynucleotide sequence having one or more synonymous codon substitutions. Selection of synonymous codons is within the skill of those in the art, the coding as the genetic code being known. Codon optimization is a known technical that can be performed using a variety of computational tools (such the GENSMART™ Codon

Optimization tool available at www.genscript.com). Generally codon optimization is used to increase the expression of protein in a heterologous system, for instance when a human coding sequence is expressed in a bacterial system. The term “codon variant” is intended to encompass both sequences that are optimized in this manner and sequences that are optimized for other purposes, such as removal of CpG islands and/or cryptic start sites.

[00133] As used herein, the term “progenitor cell” refers to a cell that is committed to differentiate into a specific type of cell or to form a specific type of tissue. A progenitor cell, like a stem cell, can further differentiate into one or more kinds of cells, but is more mature than a stem cell such that it has a more limited/restricted differentiation capacity.

[00134] The term “vector” refers to a macromolecule or complex of molecules comprising a polynucleotide or protein to be delivered to a host cell, either *in vitro* or *in vivo*. A vector can be a modified RNA, a lipid nanoparticle (encapsulating either DNA or RNA), a transposon, an adeno-associated virus (AAV) vector, an adenovirus, a retrovirus, an integrating lentiviral vector (LVV), or a non-integrating LVV. Thus, as used herein “vectors” include naked polynucleotides used for transformation (*e.g.* plasmids) as well as any other composition used to deliver a polynucleotide to a cell, included vectors capable of transducing cells and vectors useful for transfection of cells. “Vector systems” refers to combinations of one, two, three, or more vectors used to delivery one, two, three, or more polynucleotides. For example, in some embodiments, two viral vectors (*e.g.* two AAV vectors) may be used to delivery two polynucleotides or three viral vectors (*e.g.* two AAV vectors) may be used to delivery three polynucleotides. For example one AAV may encode the MYOCD or a variant thereof and another AAV may encode ASCL1 or a variant thereof. Alternatively, multiple vectors may be used to delivery a single polynucleotide through post-transfection recombination. Dual vector systems for delivery of large genes are described, for example, in McClements et al. *Yale J. Biol. Med.* 90:611-23 (2017), which is incorporated herein by reference in its entirety.

[00135] As used herein, the term “viral vector” refers either to a nucleic acid molecule that includes virus-derived nucleic acid elements that typically facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will typically include various viral components and sometimes also cell components in addition to nucleic acid(s).

[00136] The term “genetic modification” refers to a permanent or transient genetic change induced in a cell following introduction of new nucleic acid (*i.e.*, nucleic acid exogenous to the cell). Genetic change can be accomplished by incorporation of the new nucleic acid into the genome of the cardiac cell, or by transient or stable maintenance of the new nucleic acid as an extrachromosomal element. Where the cell is a eukaryotic cell, a permanent genetic change can be achieved by introduction of the nucleic acid into the genome of the cell. Suitable methods of genetic modification include viral infection, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate precipitation, direct microinjection, and the like.

[00137] The term “stem cells” refer to cells that have the capacity to self-renew and to generate differentiated progeny. The term “pluripotent stem cells” refers to stem cells that can give rise to cells of all three germ layers (endoderm, mesoderm and ectoderm), but do not have the capacity to give rise to a complete organism. In some embodiments, the compositions for inducing cardiomyocyte phenotype can be used on a population of cells to induce reprogramming. In other embodiments, the compositions induce a cardiomyocyte phenotype.

[00138] The term “induced pluripotent stem cells” shall be given its ordinary meaning and shall also refer to differentiated mammalian somatic cells (*e.g.*, adult somatic cells, such as skin) that have been reprogrammed to exhibit at least one characteristic of pluripotency. *See*, for example, Takahashi et al. (2007) Cell 131(5):861-872, Kim et al. (2011) Proc. Natl. Acad. Sci. 108(19): 7838-7843, Sell (2013) Stem Cells Handbook.

[00139] Unless stated otherwise, the abbreviations used throughout the specification have the following meanings: **AHCF**, adult human cardiac fibroblast; **APCF**, adult pig cardiac fibroblast; **a-MHC-GFP**, alpha-myosin heavy chain green fluorescence protein; **CAG**, CMV early enhancer/chicken beta actin (promoter); **CF**, cardiac fibroblast; **cm**, centimeter; **CO**, cardiac output; **EF**, ejection fraction; **FACS**, fluorescence activated cell sorting; **GFP**, green fluorescence protein; **GMT**, Gata4, Mef2c and Tbx5; **GMTc**, Gata4, Mef2c, Tbx5, TGF- β i, WNTi; **GO**, gene ontology; **HCF**, human cardiac fibroblast; **iCM**, induced cardiomyocyte; **LAD**, left anterior descending (artery); **kg**, kilogram; **μ g**, microgram; **μ l**, microliter; **mg**, milligram; **ml**, milliliter; **MI**, myocardial infarction; **msec**, millisecond; **min**, minute; **MyAMT**, Myocardin, Ascl1, Mef2c and Tbx5; **MyA**, Myocardin and Ascl1; **MyMT**, Myocardin, Mef2c and Tbx5; **MyMTc**, Myocardin, Mef2c, Tbx5, TGF- β i, WNTi; **MRI**, magnetic resonance imaging; **PBS**, phosphate

buffered saline; **PBST**, phosphate buffered saline, triton; **PFA**, paraformaldehyde; **qPCR**, quantitative polymerase chain reaction; **qRT-PCR**, quantitative reverse transcriptase polymerase chain reaction; **RNA**, ribonucleic acid; **RNA-seq**, RNA sequencing; **RT-PCR**, reverse transcriptase polymerase chain reaction; **sec**, second; **SV**, stroke volume; **TGF- β** , transforming growth factor beta; **TGF- β i**, transforming growth factor beta inhibitor; **WNT**, wntless-Int; **WNTi**, wntless-Int inhibitor; **YFP**, yellow fluorescence protein; **SCP**, super core promoter; **4F**, Gata4, Mef2c, TBX5, and Myocardin; **4Fc**, Gata4, Mef2c, TBX5, and Myocardin + TGF- β i and WNTi; **7F**, Gata4, Mef2c, and Tbx5, Esrrg, Myocardin, Zfp281, and Mesp1; **7Fc**, Gata4, Mef2c, and Tbx5, Esrrg, Myocardin, Zfp281, and Mesp1 + TGF- β i and WNTi.

[00140] The detailed description of the disclosure is divided into various sections only for the reader's convenience and disclosure found in any section may be combined with that in another section. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

II. Reprogramming Factors

[00141] In some embodiments, the present disclosure provides reprogramming factors and compositions thereof that are capable of modulating the expression of one or more genes such as polynucleotides or proteins of interest. The present inventors have surprisingly discovered that differentiated cells can be reprogrammed into induced cardiomyocyte (iCM) cells using one or more reprogramming factors that modulate the expression of one or more genes such as polynucleotides or proteins of interest, such as Achaete-scute homolog 1 (ASCL1), Myogenic Factor 6 (MYF6), Myocardin (MYOCD), myocyte-specific enhancer factor 2C (MEF2C), and/or T-box transcription factor 5 (TBX5). In some embodiments, the one or more reprogramming factors are provided as a polynucleotide (*e.g.*, an RNA, an mRNA, or a DNA polynucleotide) that encode one or more genes such as polynucleotides or proteins of interest. In some embodiments, the one or more reprogramming factors are provided as a protein.

[00142] In some embodiments, the reprogramming factors are microRNAs or microRNA antagonists, siRNAs, or small molecules that are capable of increasing the expression of one or more genes such as polynucleotides or proteins of interest of interest. In some embodiments, expression of a gene of interest is decreased by expression of a microRNA or a microRNA antagonist that targets a microRNA target site in the transcript from which a gene of interest is expressed. Alternatively, in some embodiments, expression of a gene of interest is increased by expression of a microRNA or a microRNA antagonist. For example, endogenous expression of an Oct4 polypeptide can be indirectly increased by introduction of microRNA-302 (miR-302), or by increased expression of miR-302, which targets a negative regulatory of Oct4. *See, e.g.,* Hu et al., *Stem Cells* 31(2): 259-68 (2013), which is incorporated herein by reference in its entirety. Hence, miRNA-302 can be an indirect inducer of endogenous Oct polypeptide expression. The miRNA-302 can be introduced alone or with a nucleic acid that encodes the Oct polypeptide.

[00143] In some embodiments, the reprogramming factor is a small molecule selected from the group consisting of SB431542, LDN-193189, dexamethasone, LY364947, D4476, myricetin, IWR1, XAV939, docosahexaenoic acid (DHA), S-Nitroso-TV- acetylpenicillamine (SNAP), Hh-Agl.5, alprostadil, cromakalim, MNITMT, A769662, retinoic acid p-hydroxylanlide, decamethonium dibromide, nifedipine, piroxicam, bacitracin, aztreonam, harmalol hydrochloride, amide-C2 (A7), Ph-C12 (CIO), mCF3-C-7 (J5), G856-7272 (A473), 5475707, or any combination thereof.

A. Genes of Interest (Polynucleotides and Proteins of Interest)

[00144] In some embodiments, the one or more reprogramming factors provided herein modulate (*e.g.,* increase or decrease) the expression of one or more genes of interest. In some embodiments, the one or more target genes are known to be involved in cardiomyocyte differentiation, proliferation, and/or function. In some embodiments, the one or more target genes are selected from the group consisting of *MYOCD*, *MEF2C*, and *TBX5*. In some embodiments, the one or more target genes have not been previously described to be involved in cardiomyocyte differentiation, proliferation, and/or function, or have been previously described to be involved in the differentiation, proliferation, and/or function of a non-cardiac cell lineage, such as *ASCL1*. Illustrative gene sequences useful in the compositions and methods of the present disclosure are provided in **Table 1**. Where more than one isoform of a given gene of interest is known, it will be

understood that embodiments of the the present disclosure include compositions and methods that comprise the alternative isoforms of each gene of interest. The compositions and methods of the disclosure are not limited to the disclosed sequences, which are provided for example and illustration and are non-limiting.

[00145] In some embodiments, the present disclosure provides a reprogramming factor that modulates the expression of one or more genes of interest selected from *ASCL1*, *MYOCD*, *MEF2C*, and *TBX5*. In some embodiments, the reprogramming factors disclosed herein modulate the expression of one or more genes of interest selected from *ASCL1*, *MYOCD*, *MEF2C*, *AND* *TBX5*, *CCNB1*, *CCND1*, *CDK1*, *CDK4*, *AURKB*, *OCT4*, *BAF60C*, *ESRRG*, *GATA4*, *GATA6*, *HAND2*, *IRX4*, *ISLL*, *MESP1*, *MESP2*, *NKX2.5*, *SRF*, *TBX20*, and *ZFPM2*.

[00146] In some embodiments, the reprogramming factors disclosed herein modulate the expression of one or more genes of interest selected from *GATA4*, *MEF2C*, and *TBX5* (*i.e.*, GMT). In some embodiments, the reprogramming factors disclosed herein modulate the expression of one or more genes of interest selected from *MYOCD*, *MEF2C*, and *TBX5* (*i.e.*, MyMT). In some embodiments, the reprogramming factors disclosed herein modulate the expression of one or more genes of interest selected from *MYOCD*, *ASCL1*, *MEF2C*, and *TBX5* (*i.e.*, MyAMT). In some embodiments, the reprogramming factors disclosed herein modulate the expression of one or more genes of interest selected from *MYOCD* and *ASCL1* (*i.e.*, MyA). In some embodiments, the reprogramming factors disclosed herein modulate the expression of one or more genes of interest selected from *GATA4*, *MEF2C*, *TBX5*, and *MYOCD* (*i.e.*, 4F). In other embodiments, the reprogramming factors disclosed herein modulate the expression of one or more genes of interest selected from *GATA4*, *MEF2C*, *TBX5*, *ESRRG*, *MYOCD*, *ZFPM2*, and *MESP1* (*i.e.*, 7F).

[00147] In some embodiments, the present disclosure provides a reprogramming factor that modulates the expression of one or more genes of interest selected from *ASCL1*, *MYOCD*, *MEF2C*, *TBX5*, *DLX3*, *DLX6*, *GATA2*, and *GATA5*.

Table 1: Representative Sequences

	Protein	Nucleotide (Open Reading Frame)
ASCL1	MESSAKMESGGAGQQPQPQPQQPFLPPA ACFFATAAAAAAAAAAAAAQSAQQQQQQ QQQQQQAPQLRPAADGQPSGGGHKSAPK QVKRQRSSPELMRCKRRLNFSGFYSL PQQQPAAVARRNERERNRVKLVNLGFAT LREHVPNGAANKKMSKVETLRSAVEYIR	ATGGAAAGCTCTGCCAAGATGGAGAGCGGCGGCGCCG GCCAGCAGCCCCAGCCGAGCCCCAGAGCCCTTCCT GCCGCCCGCAGCCTGTTTCTTTGCCACGGCCGCAGCC GCGGCGGCCGAGCCGCCGAGCGGCAGCGCAGAGCG CGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCA GGCGCCGAGCTGAGACCGGCGGCCGACGGCCAGCCC

	ALQQLLDEHDAVSAAFQAGVLSPTISPN YSNDLNSMAGSPVSSYSSDEGSYDPLSP EEQEELLDFTNWF (SEQ ID NO: 1)	TCAGGGGGCGGTCACAAGTCAGCGCCCAAGCAAGTCA AGCGACAGCGCTCGTCTTCGCCCCGAAGTATGCGCTG CAAACGCCGGCTCAACTTCAGCGGCTTTGGCTACAGC CTGCCGAGCAGCAGCGCGCCGCGCTGGCGCGCCGCA ACGAGCGCGAGCGCAACCGCGTCAAGTTGGTCAACCT GGGCTTTGCCACCCTTCGGGAGCACGTCCCCAACGGC GCGGCCAACAAAGAAGATGAGTAAGGTGGAGACACTGC GCTCGGCGGTTCAGTACATCCGCGCGCTGCAGCAGCT GCTGGACGAGCATGACGCGGTGAGCGCCGCTTCCAG GCAGGCGTCTGTGCGCCACCCTCTCCCCAACTACT CCAACGACTTGAACCTCCATGGCCGGCTCGCCGGTCTC ATCCTACTCGTTCGACGAGGGCTCTTACGACCCGCTC AGCCCGAGGAGCAGGAGCTTCTCGACTTCACCAACT GGTTCTGA (SEQ ID NO: 2)
DLX3	MSGSFDRKLSSILTDISSLSCHAGSKD SPTLPESVTDLGYYSAPQHDYYSQPY GQTVNPTYHYHQFNLNLAGTGAYSPKS EYTYGASYRQYGAYREQPLPAQDPVSVK EEPEAEVRMVNGKPKVRKPRTIYSSYQ LAALQRRFQKAQYLALPERAELAAQLGL TQTQVKIWFQNRSSKFKKLYKNGEVPLE HSPNNSDSMACNSPPSPALWDTSSHSTP APARSQLPPLPYASPSYLDPTNSWY HAQNLSGPHLQQQPPQPATLHHASPGPP PNPGAVY (SEQ ID NO: 43)	ATGAGTGGCTCCTTCGATCGCAAGCTCAGCA GCATCCTCACCGACATCTCCAGCTCCCTTAGCTGCCA TGCGGGCTCCAAGGACTCGCCTACCCTGCCCGAGTCT TCTGTCACTGACCTGGGCTACTACAGCGCTCCCCAGC ACGATTACTACTCGGGCCAGCCCTATGGCCAGACGGT GAACCCCTACACCTACCACCACCAATTCAATCTCAAT GGGCTTGACGGCAGCGGCGCTTACTCGCCCAAGTCGG AATATACCTACGGAGCCTCCTACCGGCAATACGGGGC GTATCGGGAGCAGCCGCTGCCAGCCAGGACCCAGTGC TCGGTGAAGGAGGAGCGCGGAAGCAGAGGTGCGCATGG TGAATGGGAAGCCCAAGAAGGTCCGAAAGCCGCGTAC GATCTACTCCAGCTACCAGCTGGCCGCCCTGCAGCGC CGCTTCCAGAAGGCCAGTACCTGGCGCTGCCCGAGC GCGCCGAGCTGGCCGCGCAGCTGGCCCTCACGCAGAC ACAGGTGAAAATCTGGTTCAGAACCGCCGTTCCAAG TTCAAGAACTCTACAAGAACGGGGAGGTGCCGCTGG AGCACAGTCCCAATAACAGTGATTCCATGGCCTGCAA CTCACCACCATCACCCGCCCTCTGGGACACCTCTTCC CACTCCACTCCGGCCCTGCCCGCAGTCACTGCCCC CGCCGCTCCCATACAGTGCCTCCCCAGCTACCTGGA CGACCCCACTCCTGGTATCACGCACAGAACCTG AGTGGACCCCACTTACAGCAGCAGCCGCTCAGCCAG CCACCCTGCACCATGCCTCTCCCGGGCCCCCGCCCAA CCCTGGGGCTGTGTACTGA (SEQ ID NO: 44)
DLX6	MMTMTMDAGLEGQDSSKSAFMEFGQQQ QQQQQQQQQQQQQQPPPPPPPPPPQPH SQSSSPAMAGAHYPLHCLHSAAAAAAG SHHHHHQHSHHGSFYASGGGNSYNHRS LAAPYMSHSQHSFYLSYHNSAAAQT RGDDTDQQKTTVIENGIRFNGKGGKIR KPRTIYSSLQLQALNHRFQQTYLALPE RAELAASLGLTQTQVKIWFQNRSKFKK LLKQGSNPHESDPLQGSAAALSPRSPALP PVWDVSASAKGVSMPNPSYMPGYSHWYS SPHQDTMQRPQMM (SEQ ID NO: 45)	ATGATGACCATGACTACGATGGCTGACGGCTTGGAA GCCAGGACTCGTCCAAATCCGCCTTCATGGAGTTCGG GCAGCAGCAGCAGCAGCAGCAGCAACAGCAGCAGCAG CAGCAGCAGCAACAGCAACAGCCGCCGCCGCCGCCG CGCCGCCGCCGAGCCGCACTCGCAGCAGAGCTCCCC GGCCATGGCAGGCGCGCACTACCTCTGCACTGCCTG CACTCGGCGGCGGCGGCGGCGAGCGCGGCTCGCACC ACCACCACCACCAGCACCACCACCAGGCTCGCC CTACGCGTTCGGGCGGAGGGAACCTTACAACCACCGC TCGCTCGCCGCTACCCCTACATGAGCCACTCGCAGC ACAGCCCTTACCTCCAGTCTTACCACAACAGCAGCGC AGCCGCCCAGACGCGAGGGGACGACACAGATCAACAA AAAATACAGTGATTGAAAACGGGGAAATCAGGTTCA ATGGAAAAGGGAAGATTCGGAAGCCTCGGACCAT TTATTCCAGCTGCAGCTCCAGGCTTAAACCATCGC

		TTTCAGCAGACACAGTATCTGGCCCTTCCAGAGAGAG CCGAACCTGGCAGCTTCTTAGGACTGACACAAACACA GGTGAAGATATGGTTTCAGAACAAACGCTCTAAGTTT AAGAACTGCTGAAGCAGGGCAGTAATCCTCATGAGA GCGACCCCTCCAGGGCTCGGCGGCCCTGTGCGCCACG CTCGCCAGCGCTGCCTCCAGTCTGGGACGTTTCTGCC TCGGCCAAGGGTGTCTAGTATGCCCCCAACAGCTACA TGCCTGGCTATTCTCACTGGTACTCTCTCCACACCA GGACACGATGCAGAGACCACAGATGATGTGA (SEQ ID NO: 46)
ESRRG	MDSVELCLPESFSLHYEEELLCRMSNKD RHIDSSCSSFIKTEPSSPASLTDSVNH SPGSSDASGSYSSTMNGHQGLDSPPL YPSAPILGGSGPVRKLYDDCSSTIVEDP QTKCEYMLNSMPKRLCLVCGDIASGYHY GVASCEACKAFFKRTIQGNI EYSCPATN ECEITKRRRKSCQACRFMKCLKVGMLKE GVRLLDRVRGGRQKYKRRIDAENSPYLN QLVQPAKKPYNKIVSHLLVAEPEKIYAM PDPTVPDSIKALTTLCDLADRELVI I GWAKHIPGFSTLSLADQMSLLQSAWMEI LILGVYRSLSFEDEL VYADDYIMDEDQ SKLAGLLDLNNAI LQLVKKYKSMKLEKE EFVTLKAIALANSDSMHIEDVEAVQKLQ DVLHEALQDYEAGQHMEDPRRAGKMLMT LPLLRQTSTKAVQHFYNIKLEGKVP MHK LFLEMLEAKV (SEQ ID NO: 47)	ATGGATTTCGGTAGAACTTTGCCTTCCTGAATCTTTT CCCTGCACTACGAGGAAGAGCTTCTCTGCAGAATGTC AAACAAAGATCGACACATTGATTCAGCTGTTCTGTC TTCATCAAGACGGAACCTTCCAGCCAGCCTCCCTGA CGGACAGCGTCAACCACCACAGCCCTGGTGGCTCTTC AGACGCCAGTGGGAGCTACAGTTCAACCATGAATGGC CATCAGAACGGACTTGACTCGCCACCTCTCTACCTT CTGCTCCTATCCTGGGAGGTAGTGGGCCTGTCTAGGAA ACTGTATGATGACTGCTCCAGCACCATTTGTTGAAGAT CCCCAGACCAAGTGTGAATACATGCTCAACTCGATGC CCAAGAGACTGTGTTTAGTGTGTGGTGACATCGCTTC TGGGTACCACTATGGGGTAGCATCTGTGAAGCCTGC AAGGCATTCTTCAAGAGGACAATTCAAGGCAATATAG AATACAGCTGCCCTGCCACGAATGAATGTGAAATCAC AAAGCGCAGACGTAAATCCTGCCAGGCTTGCCGCTTC ATGAAGTGTTTAAAGTGGGCATGCTGAAAGAAGGGG TGCGTCTTGACAGAGTACGTGGAGGTGGCAGAAAGTA CAAGCGCAGGATAGATGCGGAGAACAGCCCATACCTG AACCTCAGCTGGTTTCAGCCAGCCAAAAAGCCATATA ACAAGATTGTCTCACATTTGTTGGTGGCTGAACCGGA GAAGATCTATGCCATGCCGTGACCTACTGTCCCCGAC AGTGACATCAAAGCCCTCACTACACTGTGTGACTTGG CCGACCGAGAGTTGGTGGTTATCATTGGATGGGCGAA GCATATTCAGGCTTCTCCACGCTGTCCCTGGCGGAC CAGATGAGCCTTCTGCAGAGTGCTTGGATGGAATTT TGATCCTTGGTGTCTGATACCGGTCTCTTTCGTTTGA GGATGAACTTGTCTATGCAGACGATTATATAATGGAC GAAGACCAGTCCAAATTAGCAGGCCTTCTTGATCTAA ATAATGCTATCCTGCAGCTGGTAAAGAAATACAAGAG CATGAAGCTGGAAAAAGAAGATTTGTCAACCTCAAA GCTATAGCTCTTGCTAATTCACTCCATGCACATAG AAGATGTTGAAGCCGTTGAGAAGCTTCAGGATGTCTT ACATGAAGCGCTGCAGGATTATGAAGCTGGCCAGCAC ATGGAAGACCCTCGTCGAGCTGGCAAGATGCTGATGA CACTGCCACTCCTGAGGCAGACCTTACCAAGGCCGT GCAGCATTTCTACAACATCAACTAGAAGGCAAAGTC CCAATGCACAACTTTTTTTTGAAATGTTGGAGGCCA AGGTCTGA (SEQ ID NO: 48)
GATA2	MEVAPEQPRWMAHPAVLNAQHPSHHPG LAHNYMEPAQLLPDEVDVFFNHLDSQG NPYYANPAHARARVSYS PAHARLTGGQM CRPHLLHSPGLPWLDGGKAALSAAAHH HNPWTVSPFSKTP LHPSAAGGPGGPLSV YPGAGGSGSGSGSSVASLTPTAAHSGS	ATGGAGGTGGCGCCCCGAGCAGCCGCGCTGGATGGCGC ACCCGGCCGTGCTGAATGCGCAGCACCCCGACTCACA CCACCCGGGCTGGCGCACAACTACATGGAACCCGCG CAGCTGCTGCCTCCAGACGAGGTGGACGTCTTCTTCA ATCACCTCGACTCGCAGGGCAACCCCTACTATGCCAA CCCCGCTCACGCGGGGCGCGCGTCTCTACAGCCCC

	HLFGFPPTPPKEVSPDPSTTGAASSPASS SAGGSAARGEDKDGVKYQVSLTESMKME SGSPLRPGLATMGTPATHHPIPTYPSY VPAAAHDYSSGLFHPGGFLGGPASSFTP KQRSKARSCSEGRECVNCGATATPLWRR DGTGHYLCNACGLYHKMNGQNRPLIKPK RRLSAARRAGTCCANCQTTTTTLWRRNA NGDPVCNACGLYKLNHNVRPLTMKKEG IQTRNRKMSNKS KSKSGAECEFEELSKC MQEKSSPFSAAALAGHMAPVGHLPFFSH SGHILPTPTPIHPSSSLSFHHPHSSMV TAMG (SEQ ID NO: 49)	GCGCACGCCCGCCTGACCGGAGGCCAGATGTGCCGCC CACACTTGTTGCACAGCCCGGGTTTGCCCTGGCTGGA CGGGGGCAAAGCAGCCCTCTCTGCCCTGCGGCCAC CACCACAACCCCTGGACCGTGAGCCCTTCTCCAAGA CGCCACTGCACCCCTCAGCTGCTGGAGGCCCTGGAGG CCCCTCTCTGTGTACCCAGGGGCTGGGGGTGGGAGC GGGGGAGGCAGCGGGAGCTCAGTGGCCTCCCTCACCC CTACAGCAGCCCACTCTGGCTCCACCTTTTCGGCTT CCCACCCACGCCACCCAAAGAAGTGTCTCCTGACCCCT AGCACACGGGGGCTGCGTCTCCAGCCTCATCTTCG CGGGGGGTAGTGCAGCCCGAGGAGAGGACAAGGACGG CGTCAAGTACCAGGTGTCACTGACGGAGAGCATGAAG ATGGAAAGTGGCAGTCCCTGCGCCAGGCCTAGCTA CTATGGGCACCCAGCCTGCTACACACCACCCCATCCC CACCTACCCCTCCTATGTGCCGGCGGCTGCCACGAC TACAGCAGCGGACTCTTCCACCCCGAGGCTTCCTGG GGGACCGGCCTCCAGCTTACCCCTAAGCAGCGCAG CAAGGCTCGTTCCTGTTTCAAGGCCGGGAGTGTGT AACTGTGGGGCCACAGCCACCCCTCTCTGGCGCGGG ACGGCACCGGCCACTACCTGTGCAATGCCTGTGGCT CTACCACAAGATGAATGGGCAGAACCGACCACTCATC AAGCCCAAGCGAAGACTGTGCGCCGCCAGAAGAGCCG GCACCTGTTGTGCAAAATGTGACAGCACAACCCAC CTTATGGCGCCGAAACGCCAACGGGACCCCTGTCTGC AACGCTGTGGCCTCTACTACAAGCTGCACAATGTTA ACAGGCCACTGACCATGAAGAAGGAAGGGATCCAGAC TCGGAACCGGAAGATGTCCAACAAGTCCAAGAAGAGC AAGAAAGGGGCGGAGTGCTTCGAGGAGCTGTCAAAGT GCATGCAGGAGAAGTCATCCCCCTTCAGTGCAGCTGC CCTGGCTGGACACATGGCACCTGTGGGCCACCTCCCG CCCTTCAGCCACTCCGGACACATCCTGCCCACTCCGA CGCCCATCCACCCCTCCTCCAGCCTCTCCTTCGGCCA CCCCACCCGTCAGCATGGTGACCGCCATGGGCTAG (SEQ ID NO: 50)
GATA4	MYQSLAMAANHGPPPGAYEAGGPGAFMH GAGAASSPVYVTPRPVSSVLGLSYLQG GGAGSASGGASGGSSGGAASGAGPGTQ GSPGWSQAGADGAAYTPPPVSPRFSFPG TTGSLAAAAAAAAREAAAYSSGGGAAG AGLAGREQYGRAGFAGSYSSPYPAYMAD VGASWAAAAAASAGPFDSPVLHSLPGRA NPAARHPNLVDMFDDFSEGRECVNCGAM STPLWRRDGTGHYLCNACGLYHKMNGIN RPLIKPQRRLSASRRVGLSCANCQTTTT TLWRRNAEGEPVCNACGLYMKLHGVPRP LAMRKEGIQTRKRKPKNLNKS KTPAAPS GSESLPPASGASSNSSNATTSSEEMRP IKTEPGLSSHYGHSSSVSQTFVSAMSG HGPSIHPVLSALKLSQGYASPVQSPO TSSKQDSWNSLVADSHGDIITA (SEQ ID NO: 51)	ATGTATCAGAGCTTGCCCATGGCCGCCAACACGGGC CGCCCCCGGTGCTTACGAGGCGGGCGGCCCGGCGC CTTCATGCACGGCGCGGGCGCCGCTCTCGCCAGTC TACGTGCCACACCGCGGGTGCCCTCCTCCGTGCTGG GCCTGTCTTACCTCCAGGGCGGAGCGCGGGCTCTGC GTCCGGAGGCGCCTCGGGCGGCAGCTCCGGTGGGGCC GCGTCTGGTGCGGGGCCCCGGGACCCAGCAGGGCAGCC CGGGATGGAGCCAGCGGGGAGCCGACGGAGCCGCTTA CACCCCGCCGCGGTGTGCGCGCGCTTCTCCTTCCCG GGGACCACCGGGTCCCTGGCGGCCCGCCGCGCGCTG CCGCGGCCCGGAAGCTGCGGCCTACAGCAGTGGCGG CGGAGCGCGGGGTGCGGGCCTGGCGGGCCGCGAGCAG TACGGGCGCGCGGCTTCCGCGGCTCCTACTCCAGCC CCTACCCGGCTTACATGGCCGACGTGGGCGGCTCCTG GGCCGACCCGCGCGCCCTCCGCGGGCCCTTCGAC AGCCCGGTCTGCACAGCCTGCCCGGCCGGGCCAACCC CGGCCGCGGACACCCCAATCTCGTAGATATGTTTGA CGACTTCTCAGAAGGCAGAGAGTGTGTCAACTGTGGG GCTATGTCCACCCCGCTCTGGAGGCAGATGGGACGG GTCATATCTGTGCAACGCCTGCGGCCTTACCCAA GATGAACGGCATCAACCGGCCGCTCATCAAGCCTCAG

		CGCCGGCTGTCCGCCTCCCGCCGAGTGGGCCTCTCCT GTGCCAACTGCCAGACCACCACCACGCTGTGGCG CCGCAATGCGGAGGGCGAGCCTGTGTGCAATGCCTGC GGCCTCTACATGAAGCTCCACGGGTCCCCAGGCCTC TTGCAATGCGGAAAGAGGGGATCCAAACCAGAAAACG GAAGCCCAAGAACCTGAATAAATCTAAGACACCAGCA GCTCCTTCAGGCAGTGAGAGCCTTCCTCCCGCCAGCG GTGCTTCCAGCAACTCCAGCAACGCCACCACCAGCAG CAGCGAGGAGATGCGTCCCATCAAGACGGAGCCTGGC CTGTCTCTCACTACGGGCACAGCAGCTCCGTGTCCC AGACGTTCTCAGTCAGTGCGATGTCTGGCCATGGGCC CTCCATCCACCCTGTCTCTCGGCCCTGAAGCTCTCC CCACAAGGCTATGCGTCTCCGTCAGCCAGTCTCCAC AGACCAGCTCCAAGCAGGACTCTTGAACAGCCTGGT CTTGGCCGACAGTCACGGGGACATAATCACTGCGTAA (SEQ ID NO: 52)
MESPI	MAQPLCPPLSESWMLSAAWGPTRRPPPS DKDCGRSLVSSPDSWGSTPADSPVASPA RPGTLRDPRAPSVGRRGARSSRLGSGQR QSASEREKLRMRTLARALHELRRFLPPS VAPAGQSLTKIETLRLAIRYIGHLSAVL GLSEESLQRRRCRQRGDAGSPRGCPICPD DCPAQMQRTRQAEGQGQGRGLGLVSAVR AGASWGSPPACPGARAAPEPRDPPALFA EAACPEGQAMEPSPPSPLLPGDVLALLE TWMPLSPLEWLPPEPK (SEQ ID NO: 53)	ATGGCCAGCCCTGTGCCCGCCGCTCTCCGAGTCCCT GGATGCTCTCTGCGGCCTGGGGCCCACTCGGCGGCC GCCGCCCTCCGACAAGGACTGCGGCCGCTCCCTCGTC TCGTCCCCAGACTCATGGGGCAGCACCCAGCCGACA GCCCCGTGGCGAGCCCCGCGCGGCCAGGCACCTCCG GGACCCCGCGCCCCCTCCGTAGGTAGGCGCGCGCGG CGCAGCAGCCGCTGGGCAGCGGGCAGAGGCAGAGCG CCAGTGAGCGGGAGAACTGCGCATGCGCACGCTGGC CCGCGCCCTGCACGAGCTGCGCCGCTTCTACCGCCG TCCGTGGCGCCCGCGGGCCAGAGCCTGACCAAGATCG AGACGCTGCGCCTGGCTATCCGCTATATCGGCCACCT GTCGGCCGTGCTAGGCCTCAGCGAGGAGAGTCTCCAG CGCCGGTGCCGGCAGCGCGGTGACGCGGGGTCCCTC GGGGCTGCCCGCTGTGCCCGGACGACTGCCCGCGCA GATGCAGACACGGACGAGGCTGAGGGGCAGGGGCAG GGGCGCGGGCTGGGCCTGGTATCCGCCGTCCGCGCCG GGGCGTCTGGGGATCCCCGCTGCCTGCCCGGAGC CCGAGCTGCACCCGAGCCGCGGACCCGCTGCGCTG TTCGCCGAGGCGGCGTGCCCTGAAGGGCAGGCGATGG AGCCAAGCCACCGTCCCCGCTCCTTCCGGGCGACGT GCTGGCTCTGTTGGAGACCTGGATGCCCTCTCGCCT CTGGAGTGGCTGCCTGAGGAGCCCAAGTGA (SEQ ID NO: 54)
MYF6	MMMDLFETGSYFFYLDGENVTLQPLEVA EGSPLYPGSDGTLSPCQDQMPPEAGSDS SGEEHVLAPPGLQPPHCPGQCLIWACKT CKRKSAPTDRRKAATLRERRRLKINEA FEALKRRTVANPNQRLPKVEILRSISY IERLQDLLHRLDQQEKMQELGVDFFSYR PKQENLEGADFLRTCSSQWPSVSDHSRG LVITAKEGGASIDSSASSSLRCLSSIVD SISSEERKLPCVEEVVEK (SEQ ID NO: 55)	ATGATGATGGACCTTTTGAACTGGCTCCTATTCT TCTACTTGGATGGGGAAAATGTTACTCTGCAGCCATT AGAAGTGGCAGAAGGCTCTCCTTTGTATCCAGGGAGT GATGGTACCTTGTCCTCCCTGCCAGGACCAATGCCCC CGGAAGCGGGGAGCGACAGCAGCGGAGAGGAACATGT CCTGGCGCCCCCGGGCCTGCAGCCTCCACACTGCCCC GGCCAGTGTCTGATCTGGGCTTGCAAGACCTGCAAGA GAAAATCTGCCCCACTGACCGGCGAAAAGCCGCCAC CCTGCGCGAAAGGAGGAGGCTAAAGAAAATCAACGAG GCCTTCGAGGCACTGAAGCGGCGAACTGTGGCCAACC CCAACCAGAGGCTGCCCAAGGTGGAGATTCTGCGGAG CGCCATCAGCTATATTGAGCGGCTGCAGGACCTGCTG CACC GGCTGGATCAGCAGGAGAAGATGCAGGAGCTGG GGGTGGACCCCTTCAGCTACAGACCCAAACAAGAAAA TCTTGAGGGTGCGGATTCTCTGCGACCTGCAGCTCC CAGTGGCCAAGTGTTCGGATCATTCAGGGGGCTCG

		TGATAACGGCTAAGGAAGGAGGAGCAAGTATTGATTC GTGAGCCTCGAGTAGCCTTCGATGCCTTTCTCCATC GTGGACAGTATTTCTCGGAGGAACGCAAACCTCCCT GCGTGGAGGAAGTGGTGGAGAAGTAA (SEQ ID NO: 56)
MYOCD	MTLLGSEHSLIRSKFRSVLQLRLQRR TQEQLANQGIIPPLKRPAEFHEQRKHL SDKAKNSLKRKARNRCSADLVNMHILQ ASTAERSIPTAQMKLKRARLADDLNEKI ALRPGPLELVEKNILPVDSAVKEAIKGN QVSFSKSTDAFAFEEDSSSDGLSPDQTR SEDPQNSAGSPDAKASDTPSTGSLGTN QDLASGSENDNRNDSASQPSHQSDAGKQG LGPPSTPIAVHAAVKSKSLGDSKNRHKK PKDKPKVKKKLYHQYIPPDQKAEKSP PMDSAYARLLQQQLFLQLQLLSQQQQQ QQHRSYLGMHQAQLKEPNEQMRNPNS SSTPLSNTPLSPVKNSFSQGTVSSFKP GPLPPLNDDLKVSELRLQLRIRGLPVSG TKTALMDRLRPFQDCSGNPVPNFGDITT VTFPVTPTNLPNYQSSSSTSALSNGFYH FGSTSSSPPISSPSSDLVAGSLPDFTN DASPSFGLHPSVHVCTEESLMSLNGG SVPSELDGLDSEKDKMLVEKQKVINELT WKLQEQRQVEELRMQLQKQKRNNCSEK KPLPFLAASIKQEEAVSSCPFASQVPVK RQSSSECHPPACEAAQLQPLGNAHCVE SSDQTNVLSSTFLSPQCSPPHSPGLGAVK SPQHISLPPSPNNPHFLPSSSGAQGEH RVSSPFISSQVCTAQNSGAHDGHPPSFSF HSSSLHPPFSGAQADSSHGAGNPNCPKS PCVQQKMAGLHSSDKVGPKFSPSPSPTFS KSSSAISEVTQPPSYEDAVKQQMTRSQQ MDELDDVLIIESGEMPADAREDHSLQKV PKIPRSSRSPTAVLTKPSASFEQASSGS QIPFDPYATDSDEHLEVLLNSQSPLGKM SDVTLKIGSEEPHFDGIMDGFSGKAAE DLFNAHEILPGPLSPMQTFSPSSVDSN GLQLSFTESPWETMEWLDLTPPNSTPGF SALTSSPSIFNIDFLDVTDLNLNSSMD LHLQQW (SEQ ID NO: 3)	ATGACACTCCTGGGGTCTGAGCATTCTTGTGATTA GGAGCAAGTTCAGATCAGTTTTACAGTTAAGACTTCA ACAAGAAGGACCCAGGAACAAGTGGCTAACCAGGC ATAATACCACCACTGAAACGTCCAGCTGAATTCATG AGCAAAGAAAACATTTGGATAGTGACAAGGCTAAAA TTCCCTGAAGCGCAAAGCCAGAAACAGGTGCAACAGT GCCGACTTGGTTAATATGCACATACTCCAAGCTTCCA CTGCAGAGAGGTCCATTCCAAGTCTCAGATGAAGCT GAAAAGAGCCCGACTCGCCGATGATCTCAATGAAAA ATTGCTCTACGACCAGGGCCACTGGAGCTGGTGGAAA AAAACATTCTTCTGTGGATTCTGTGTGAAAGAGGC CATAAAAGGTAACCAGGTGAGTTTCTCCAAATCCACG GATGCTTTTGCCTTTGAAGAGGACAGCAGCAGCGATG GGCTTTCTCCGATCAGACTCGAAGTGAAGACCCCA AAACCTCAGCGGATCCCCGCCAGAGCTAAAGCCTCA GATACCCCTTCGACAGGTTCTCTGGGGACAAACCAGG ATCTTGCTTCTGGCTCAGAAAATGACAGAAATGACTC AGCCTCAGAGCCAGCCACAGTCAGATGCGGGGAGG CAGGGGCTTGGCCCCCAGCAGCCCCATAGCCGTGC ATGCTGCTGTAAAGTCCAAATCCTTGGGTGACAGTAA GAACCGCCACAAAAAGCCCAAGGACCCCAAGCCAAAG GTGAAGAAGCTTAAATATCACCAGTACATTCCCCCAG ACCAGAAGGAGAGAAAGTCCCTCCACCTATGGACTC AGCCTACGCTCGGCTGCTCCAGCAACAGCAGCTGTTT CTGCAGCTCCAAATCCTCAGCCAGCAGCAGCAGCAGC AGCAACACCGATTGAGTACCTAGGGATGCACCAAGC TCAGCTTAAGGAACCAATGAACAGATGGTCAGAAAT CCAAACCTCTTCTCAACGCCACTGAGCAATACCCCTT TGTCTCCTGTCAAAAACAGTTTTTCTGGACAAACTGG TGTCTCTTCTTCAAAACAGGCCACTCCACCTAAC CTGGATGATCTGAAGGTCTCTGAATTAAGACAACAGC TTCGAATTGCGGGCTTGCCTGTGTGAGGCACCAAAAC GGCTCTCATGGACCGGCTTCGACCTTCCAGGACTGC TCTGGCAACCCAGTGCCGAATTTGGGGATATAACGA CTGTCACTTTTCTGTGACACCCACACGCTGCCCAA TTACCAGTCTTCTCTTCTACCAGTGCCCTGTCCAAC GGCTTCTACCACTTTGGCAGCACCAGCTCCAGCCCC CGATCTCCCCAGCCTCCTCTGACCTGTGAGTCGCTGG GTCCCTGCCGGACACCTTCAATGATGCCTCCCCCTCC TTCGGCCTGCACCCGTCCCCAGTCCACGTGTGCACGG AGGAAAGTCTCATGAGCAGCCTGAATGGGGGCTCTGT TCCTTCTGAGCTGGATGGGCTGGACTCCGAGAAGGAC AAGATGCTGGTGGAGAAGCAGAAGGTGATCAATGAAC TCACCTGGAAACTCCAGCAAGAGCAGAGGCAGGTGGA GGAGCTGAGGATGCAGCTTCAGAAGCAGAAAAGGAAT AACTGTTGAGAGAAGAGCCGCTGCCTTTCTGGCTG CCTCCATCAAGCAGGAAGAGGCTGTCTCCAGCTGTCC TTTTGCATCCCAAGTACCTGTGAAAAGACAAAGCAGC AGCTCAGAGTGTACCCACCGCTTGTGAAGCTGCTC AACTCCAGCTCTTGGAAATGCTCATTGTGTGGAGTC

		CTCAGATCAAACCAATGTACTTTCTTCCACATTTCTC AGCCCCCAGTGTTCCCTCAGCATTCACCGCTGGGG CTGTGAAAAGCCCACAGCACATCAGTTTGGCCCCATC ACCCAACAACCTCCTTCTGCCCCATCCTCCGGG GCCCAGGGAGAAGGGCACAGGGTCTCCTCGCCCATCA GCAGCCAGGTGTGCACTGCACAGAACTCAGGAGCACA CGATGGCCATCCTCCAAGCTTCTCTCCCATTCTTCC AGCCTCCACCCGCCCTTCTCTGGAGCCCAAGCAGACA GCAGTCATGGTGCCGGGGGAAACCTTGTCCCAAAAG CCCATGTGTACAGCAAAAGATGGCTGGTTTACACTCT TCTGATAAGGTGGGGCCAAAGTTTTCAATTCCATCCC CAACTTTTTCTAAGTCAAGTTCAGCAATTTTACAGGTT AACACAGCCTCCATCCTATGAAGATGCCGTAAAGCAG CAAATGACCCGGAGTCAGCAGATGGATGAACCTCTGG ACGTGCTTATTGAAAGCGGAGAAATGCCAGCAGACGC TAGAGAGGATCACTCATGTCTTCAAAAAGTCCCAAG ATACCCAGATCTTCCCGAAGTCCAACTGCTGTCTCA CCAAGCCCTCGGCTTCTTTGAACAAGCCTCTTCAGG CAGCCAGATCCCCTTTGATCCCTATGCCACCGACAGT GATGAGCATCTTGAAGTCTTATTAAATTCCCAGAGCC CCCTAGGAAAGATGAGTGATGTCACCTTCTAAAAAT TGGGAGCGAAGAGCCTCACTTTGATGGGATAATGGAT GGATTCTCTGGGAAGGCTGCAGAAGACCTCTTCAATG CACATGAGATCTTGCCAGGCCCTCTCTCCAATGCA GACACAGTTTTTACCCTCTTCTGTGGACAGCAATGGG CTGCAGTTAAGCTTCACTGAATCTCCCTGGGAAACCA TGGAGTGGCTGGACCTCACTCCGCCAAATTCACACC AGGCTTTAGCGCCCTCACCACCAGCAGCCCCAGCATC TTCAACATCGATTTCTCGGATGTCACTGATCTCAATT TGAATTCTCCATGGACCTTCACTTGCAGCAGTGGTA G (SEQ ID NO: 4)
MEF2C	MGRKKIQITRIMDERNRQVTFTRKRFGL MKKAYELSVLCDCEIALIIIFNSTNKLFLQ YASTDMDKVLKYTEYNEPHESRTNSDI VEALNKKENKGCESPDSSYALTPRTE EKYKKINEEFDNMIKSHKIPAVPPNFE MPVSI PVSSHNSLVYSNPVSSLGNPNLL PLAHPSLQRNSMSGVTHRPPSAGNTGG LMGGDLTSGAGTSAGNGYGNPRNSPGLL VSPGNLKNMQAKSPPPMNLGMNRPD LRVLIPPGSKNTMPSVNQRINNSQSAQS LATPVVSVATPTLPQGGMGYPSAISTT YGTEYSLSSADLSSLSGFNTASALHLGS VTGWQQQHLHNMPPSALSQLGACTSTHL SQSSNLSLPSTQSLNIKSEPVSPPRDRT TTPSRYPQHTRHEAGRSPVDSLSSCSSS YDGS DREDHRNEFHSPIGLTRPSDERE SPSVKRMRLSEGWAT (SEQ ID NO: 5)	ATGGGGAGAAAAAGATTGAGATTACGAGGATTATGG ATGAACGTAAACAGACAGGTGACATTACAAAGAGGAA ATTTGGGTTGATGAAGAAGGCTTATGAGCTGAGCGTG CTGTGTGACTGTGAGATTGCGCTGATCATCTTCAACA GCACCAACAAGCTGTTCCAGTATGCCAGCACCAGCAT GGACAAAGTGCTTCTCAAGTACACGGAGTACAACGAG CCGCATGAGAGCCGGACAACTCAGACATCGTGGAGG CATTGAACAAGAAAGAAAACAAAGGCTGTGAAAGCCC CGATCCCGACTCCTCTTATGCACTACCCCCACGCACT GAAGAAAAATACAAAAAATTAATGAAGAATTGATA ATATGATCAAGAGTCATAAAATTCCTGCTGTTCCACC TCCCACTTCGAGATGCCAGTCTCCATCCCAGTGTCC AGCCACAACAGTTTGGTGACAGCAACCTGTGAGCT CACTGGGAAACCCCAACCTATTGCCACTGGCTCACC TTCTCTGCAGAGGAATAGTATGTCTCCTGGTGTAA CATCGACCTCCAAGTGCAGGTAACACAGGTGGTCTGA TGGGTGGAGACCTCACGTCTGGTGCAGGCACCAGTGC AGGGAACGGGTATGGCAATCCCCGAACTCACCAGGT CTGCTGGTCTCACCTGGTAACCTGAACAAGAATATGC AAGCAAAATCTCCTCCCCAATGAATTTAGGAATGAA TAACCGTAAACCAGATCTCCGAGTTCTTATCCACCA GGCAGCAAGAATACGATGCCATCAGTGAATCAAAGGA TAAATAACTCCCAGTCGGCTCAGTCATTGGCTACCCC

		AGTGGTTTCCGTAGCAACTCCTACTTTACCAGGACAA GGAATGGGAGGATATCCATCAGCCATTTCAACAACAT ATGGTACCGAGTACTCTCTGAGTAGTGACAGCTGTC ATCTCTGTCTGGGTTTAACACCGCCAGCGCTCTTCAC CTTGGTTCAGTAACCTGGCTGGCAACAGCAACACCTAC ATAACATGCCACCATCTGCCCTCAGTCAGTTGGGAGC TTGCACTAGCACTCATTTATCTCAGAGTTCAAATCTC TCCCTGCCTTCTACTCAAAGCCTCAACATCAAGTCAG AACCTGTTTCTCCTCCTAGAGACCGTACCACCACCCC TTCGAGATACCCACAACACACGCGCCACGAGGCGGGG AGATCTCCTGTTGACAGCTTGAGCAGCTGTAGCAGTT CGTACGACGGGAGCGACCGAGAGGATCACCGGAACGA ATTCCACTCCCCATTGGACTCACCAGACCTTCGCCG GACGAAAGGGAAAGTCCCTCAGTCAAGCGCATGCGAC TTTCTGAAGGATGGGCAACATGA (SEQ ID NO: 6)
TBX5	MADADEGFGLAHTPLEPDAKDLPCDSKP ESALGAPSKSPSPQAAFTQQGMEGIKV FLHERELWLKFHEVGTETMIITKAGRME PSYKVKVTGLNPKTKYILLMDIVPADDH RYKFADNKWSVTGKAEPAMPGRLYVHPD SPATGAHWMRQLVSFQKLKLTNNHLDPF GHIIILNSMHKYQPRHLHIVKADENNFGFS KNTAFCTHVFPEAFIAVTSYQNHKITQ LKIENNPFAKGFRGSDDMELHRMSRMS KEYPVVPRSTVRQKVASNHSPFSSSRA LSTSSNLGSQYQCENGVSQDILLPPP NPYPLPQEHSTYHCTKRKEEECSSTDH PYKKPYMETSPSEEDSFYRSSYPQQOGL GASYRTESAQRQACMYASSAPPSEPVPS LEDISCNTWPSMPSYSSCTVTVPMDR LPYQHFSAHFTSGPLVPRLAGMANHGSP QLGEGMFQHQTSVAHQPVVRQCGPQTGL QSPGTLQPPEFLYSHGVPRTLSPHQYHS VHGVGMVPEWSDNS (SEQ ID NO: 7)	ATGGCCGACGACGAGGAGGGCTTTGGCCTGGCGCACA CGCCTCTGGAGCCTGACGCAAAAGACCTGCCCTGCGA TTCGAAACCCGAGAGCGCGCTCGGGGCCCCAGCAAG TCCCCGTCGTCCCCGAGGCGCGCTTACCCAGCAGG GCATGGAGGGAATCAAAGTGTCTTCCATGAAAGAGA ACTGTGGCTAAAATTCCACGAAGTGGGCACGGAAATG ATCATAACCAAGGCTGGAAGGCGGATGTTTCCCAGTT ACAAAGTGAAGGTGACGGGCCTTAATCCCAAAACGAA GTACATTCTTCTCATGGACATTGTACCTGCCGACGAT CACAGATACAAATTCGACGATAATAAATGGTCTGTGA CGGGCAAAGCTGAGCCCGCCATGCCTGGCCGCTGTA CGTGACCCAGACTCCCCGCCACCGGGGCGCATTTGG ATGAGGCAGCTCGTCTCCTTCCAGAACTCAAGCTCA CCAACAACACCTGGACCCATTTGGGCATATTATTTCT AAATTCCATGCACAAATACAGCCTAGATTACACATC GTGAAAGCGGATGAAAATAATGGATTTGGCTCAAAAA ATACAGCGTTCTGCACTCACGTCTTCTGAGACTGC GTTTATAGCAGTGACTTCCTACCAGAACCACAAGATC ACGCAATTAAAGATTGAGAATAATCCCTTTGCCAAAG GATTTTCGGGCGAGTGATGACATGGAGCTGCACAGAAT GTCAAGAATGCAAAGTAAAGAATATCCCGTGGTCCCC AGGAGCACCGTGAGGCAAAAAGTGGCCTCCAACCACA GTCCTTTACGACGCGAGTCTCGAGCTCTCTCCACCTC ATCCAATTTGGGGTCCAATACCAGTGTGAGAATGGT GTTTCCGGCCCCCTCCAGGACCTCCTGCCTCCACCCA ACCCATACCCACTGCCCCAGGAGCATAGCCAAATTTA CCATTGTACCAAGAGGAAAGAGGAAGAATGTTCCACC ACAGACCATCCCTATAAGAAGCCCTACATGGAGACAT CACCCAGTGAAGAAGATTCTTCTACCGCTCTAGCTA TCCACAGCAGCAGGGCCTGGGTGCCTCCTACAGGACA GAGTCGGCACAGCGGCAAGCTTGATGTATGCCAGCT CTGCGCCCCCAGCGAGCCTGTGCCCAGCCTAGAGGA CATCAGCTGCAACACGTGGCCAAGCATGCCTTCCTAC AGCAGCTGCACCGTCAACACCGTGCAGCCCATGGACA GGCTACCCCTACCAGCACTTCTCCGCTCACTTACCTC GGGGCCCCCTGGTCCCTCGGCTGGCTGGCATGGCCAAC CATGGCTCCCCACAGCTGGGAGAGGGAATGTTCCAGC ACCAGACCTCCGTGGGCCACAGCCTGTGGTCAGGCA GTGTGGGCTCAGACTGGCCTGCAGTCCCCTGGCACC

		CTTCAGCCCCCTGAGTTCCTCTACTCTCATGGCGTGC CAAGGACTCTATCCCCTCATCAGTACCACTCTGTGCA CGGAGTTGGCATGGTGCCAGAGTGGAGCGACAATAGC TAA (SEQ ID NO: 8)
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Engineered Myocardins

[00148] In another aspect, the disclosure relates to engineered variants of myocardin (MYOCD), such as an engineered MYOCD expressed from a smaller open reading frame, as described in U.S. Provisional Pat. Appl. No. 62/788,479. Applicant has found that *MYOCD* comprising an internal deletion retains the expression and function of myocardin and MYOCD comprising an internal deletion can be used alone or in combination with other reprogramming factors (e.g., for generating cardiomyocytes from fibroblasts). In some embodiments of the present disclosure, the engineered myocardin protein comprises a deletion of at least 50 amino acids in the region corresponding to amino acids 414-764 of the native myocardin (FIG. 9A; SEQ ID NO: 3). In some embodiments, the engineered MYOCD is selected from one or three myocardin variants with internal deletions: MyΔ1 having a deletion of residues 414 to 763 (FIG. 9B; SEQ ID NO: 14); MyΔ2 having a deletion of residues 439 to 763 (FIG. 9C; SEQ ID NO: 15); and preferably MyΔ3 having a deletion of residues 560 to 763 (FIG. 9D; SEQ ID NO: 16).

[00149] In some embodiments, the *MYOCD* polynucleotide is an engineered MYOCD polynucleotide. “MYOCD” or “myocardin” refers to either an engineered MYOCD protein or preferably a native MYOCD. In some embodiments, the engineered MYOCD polynucleotide encodes an engineered myocardin protein having a length of at most 500, 550, 600, 650, 700, 750, 800, 850 or any number therebetween of amino acids. In some embodiments, the engineered myocardin protein comprises an SRF interaction domain, an SAP domain, and a TAD domain. In some embodiments, the engineered myocardin protein further comprises a Mef2C interaction domain. In some embodiments, the engineered MYOCD polynucleotide encodes an engineered myocardin protein, with a deletion of at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, or 350 amino acids in the region corresponding to amino acids 414-764 of the native myocardin (SEQ ID NO: 3). Various sequences used in the engineering of myocardin are provided in Table 2. In some embodiments, about four N-terminal residues of MYOCD are omitted

or altered as inter-species conservation of MYOCD begins at residue 5. In some embodiments, further residues from the N terminus of MYOCD are omitted or altered.

Table 2: Sequences Used in Engineering of Myocardin

Native MYOCD	MTLLGSEHSLIRSKFRSVLQLRLQQRRTQEQLANQGIIPPLKRPAEFHEQRKHLSDSKAKNSLKRKARNRNCNSADLVNMHILQASTAERSIPTAQMKLKRARLADDLNEKIALRPGPLELVEKNILPVDSAVKEAIKGNQVSFSKSTDAFAFEEDSSSDGLSPDQTRSEDPQNSAGSPDDAKASDTPSTGSLGTNQDLASGSENDNRNDASQPSHQSDAGKQGLGPPSTPIAVHAAVKSLSLGD SKNRHKKPKDPKPKVKKLKYHQYIPPDQKAEKSPPMDSAYARLLQQQQLFLQLQLLSQQQQQQQHRFSYLG MHQAQLKEPNEQMVARNPNSSSTPLSNTPLSPVKNSFSGQTGVSSFKPGPLPPNLDLKVSELRQQLRIRGLPVSGTKTALMDRLRPFQDCSGNPVNFPGDITTVTFPVT PNTLPNYQSSSSTSALNGFYHFGSTSSSPPI SPASSDL SVAGSLPDTFNDASPSFGLHPSPVHVCTEESLMSSLNNGSVPSELDGLDSEKDKMLVEKQKVINELTWKLOQEQRQVEELRMQLQKQKRNNCSEKKPLPFLAASIKQEEAVSSCPFASQVPVKRQSSSSECHPPACEAAQLQLPLGNAHCVESSDQTNVLSSTFLSPQCSPQHSP LGAVKSPQHISLPPSPNNPHFLPSSSGAQGEGHRVSSPISSQVCTAQNSGAHDGHPSPFSPHSSSLHPPFSGAQADSSHGAGGNPCPKSPCVQQKMAGLHSSDKVGPKFSIPSPTFKSSSAISEVTQPPSYEDAVKQQMTRSQQMDELLDVLIESGEMPADAREDHSLQKVPKIPRSSRSPTAVLT KPSASFEQASSGSQIPFDPYATDSDEHLEVLLNSQSPLGKMSDVTLLKIGSEEPHFDGIMDGFSGKAAEDLFNAHEILPGPLSPMQTQFSPSSVDSNGLQLSFTESPWETMEWLDLTPPNSTPGFSALTSSPSIFNIDFLDVTDLNLSMMDLHLQQW
	(SEQ ID NO: 3)
MYOCD 5-413	GSEHSLIRSKFRSVLQLRLQQRRTQEQLANQGIIPPLKRPAEFHEQRKHLSDSKAKNSLKRKARNRNCNSADLVNMHILQASTAERSIPTAQMKLKRARLADDLNEKIALRPGPLELVEKNILPVDSAVKEAIKGNQVSFSKSTDAFAFEEDSSSDGLSPDQTRSEDPQNSAGSPDDAKASDTPSTGSLGTNQDLASGSENDNRNDASQPSHQSDAGKQGLGPPSTPIAVHAAVKSLSLGD SKNRHKKPKDPKPKVKKLKYHQYIPPDQKAEKSPPMDSAYARLLQQQQLFLQLQLLSQQQQQQQHRFSYLG MHQAQLKEPNEQMVARNPNSSSTPLSNTPLSPVKNSFSGQTGVSSFKPGPLPPNLDLKVSELRQQLRIRGLPVSGTKTALMDRLRPFQDCSGNPV
	(SEQ ID NO: 10)
MYOCD 764-986	EVTQPPSYEDAVKQQMTRSQQMDELLDVLIESGEMPADAREDHSLQKVPKIPRSSRSPTAVLT KPSASFEQASSGSQIPFDPYATDSDEHLEVLLNSQSPLGKMSDVTLLKIGSEEPHFDGIMDGFSGKAAEDLFNAHEILPGPLSPMQTQFSPSSVDSNGLQLSFTESPWETMEWLDLTPPNSTPGFSALTSSPSIFNIDFLDVTDLNLSMMDLHLQQW
	(SEQ ID NO: 11)
MYOCD 5-438	GSEHSLIRSKFRSVLQLRLQQRRTQEQLANQGIIPPLKRPAEFHEQRKHLSDSKAKNSLKRKARNRNCNSADLVNMHILQASTAERSIPTAQMKLKRARLADDLNEKIALRPGPLELVEKNILPVDSAVKEAIKGNQVSFSKSTDAFAFEEDSSSDGLSPDQTRSEDPQNSAGSPDDAKASDTPSTGSLGTNQDLASGSENDNRNDASQPSHQSDAGKQGLGPPSTPIAVHAAVKSLSLGD SKNRHKKPKDPKPKVKKLKYHQYIPPDQKAEKSPPMDSAYARLLQQQQLFLQLQLLSQQQQQQQHRFSYLG MHQAQLKEPNEQMVARNPNSSSTPLSNTPLSPVKNSFSGQTGVSSFKPGPLPPNLDLKVSELRQQLRIRGLPVSGTKTALMDRLRPFQDCSGNPVNFPGDITTVTFPVT PNTLPNYQSSS
	(SEQ ID NO: 12)
MYOCD 1-559	GSEHSLIRSKFRSVLQLRLQQRRTQEQLANQGIIPPLKRPAEFHEQRKHLSDSKAKNSLKRKARNRNCNSADLVNMHILQASTAERSIPTAQMKLKRARLADDLNEKIALRPGPLELVEKNILPVDSAVKEAIKGNQVSFSKSTDAFAFEEDSSSDGLSPDQTRSEDPQNSAGSPDDAKASDTPSTGSLGTNQDLASGSENDNRNDASQPSHQSDAGKQGLGPPSTPIAVHAAVKSLSLGD SKNRHKKPKDPKPKVKKLKYHQYIPPDQKAEKSPPMDSAYARLLQQQQLFLQLQLLSQQQQQQQHRFSYLG MHQAQLK

	EPNEQMVRNPNSSTPLSNTPLSPVKNSFSGQTGVSSFKPGPLPPNLDDLKVSELRQQLRIRGL PVSGTKTALMDRLRPFQDCSGNPVNFVDITTVTFPVTPTNLPNYQSSSSTALSNGFYHFGST SSSPISPASSDLVAGSLPDTFNDASPSFGLHPSPVHVCTEESLMSSLNNGSVPSELDGLDSE KDKMLVEKQKVINELTWKLQQEQRQVEELRMQLQKQKRNNCSE (SEQ ID NO: 13)
MYOCD 1-413, 764-986 (MyΔ1)	MTLLGSEHSLLIRSKFRSVLQRLRQQRRTQEQLANQGIIPPLKRPAEFHEQRKHLDS KAKNSLKRKARNRCNSADLVNMHILQASTAERSIPTAQMKLKRARLADDLNEKIALRP GPLELVEKNILPVDSAVKEAIKGNQVSFSKSTDAFAFEEDSSSDGLSPDQTRSEDPQN SAGSPDPAKASDTPSTGSLGTNQDLASGSENDERNDASQPSHQSDAGKQGLGPPSTPI AVHAAVKSKSLGDSKNRHKKPKDPKPKVKKLYHQYIPPDQKAEKSPPPMDSAYARLL QQQQLFLQLQILSQQQQQQQHRFSYLGMLHQAQLKEPNEQMVRNPNSSTPLSNTPLSP VKNSFSGQTGVSSFKPGPLPPNLDDLKVSELRQQLRIRGLPVSGTKTALMDRLRPFQD CSGNPVPNEVTQPPSYEDAVKQMQMTRSQQMDELLDVLIESGEMPADAREDHSCQKVPK IPRSSRSPTAVLTKPSASFEQASSGSQIPFDPYATDSDEHLEVLLNSQSPLGKMSDVT LLKIGSEEPHFDGIMDGFSGKAAEDLFNAHEILPGPLSPMQTQFSPSSVDSNGLQLSF TESPWETMEWLDLTPPNSTPGFSALTTSSPSIFNIDFLDVTDLNLSMDLHLQQW (SEQ ID NO: 14)
MYOCD 1-438, 764-986 (MyΔ2)	MTLLGSEHSLLIRSKFRSVLQRLRQQRRTQEQLANQGIIPPLKRPAEFHEQRKHLDS KAKNSLKRKARNRCNSADLVNMHILQASTAERSIPTAQMKLKRARLADDLNEKIALRP GPLELVEKNILPVDSAVKEAIKGNQVSFSKSTDAFAFEEDSSSDGLSPDQTRSEDPQN SAGSPDPAKASDTPSTGSLGTNQDLASGSENDERNDASQPSHQSDAGKQGLGPPSTPI AVHAAVKSKSLGDSKNRHKKPKDPKPKVKKLYHQYIPPDQKAEKSPPPMDSAYARLL QQQQLFLQLQILSQQQQQQQHRFSYLGMLHQAQLKEPNEQMVRNPNSSTPLSNTPLSP VKNSFSGQTGVSSFKPGPLPPNLDDLKVSELRQQLRIRGLPVSGTKTALMDRLRPFQD CSGNPVPNEVDITTVTFPVTPTNLPNYQSSSEVTQPPSYEDAVKQMQMTRSQQMDELL DVLIESGEMPADAREDHSCQKVPKIPRSSRSPTAVLTKPSASFEQASSGSQIPFDPY ATDSDEHLEVLLNSQSPLGKMSDVTLLKIGSEEPHFDGIMDGFSGKAAEDLFNAHEIL PGPLSPMQTQFSPSSVDSNGLQLSFTESPWETMEWLDLTPPNSTPGFSALTTSSPSIF NIDFLDVTDLNLSMDLHLQQW (SEQ ID NO: 15)
MYOCD 1-559, 764-986 (MyΔ3)	MTLLGSEHSLLIRSKFRSVLQRLRQQRRTQEQLANQGIIPPLKRPAEFHEQRKHLDS KAKNSLKRKARNRCNSADLVNMHILQASTAERSIPTAQMKLKRARLADDLNEKIALRP GPLELVEKNILPVDSAVKEAIKGNQVSFSKSTDAFAFEEDSSSDGLSPDQTRSEDPQN SAGSPDPAKASDTPSTGSLGTNQDLASGSENDERNDASQPSHQSDAGKQGLGPPSTPI AVHAAVKSKSLGDSKNRHKKPKDPKPKVKKLYHQYIPPDQKAEKSPPPMDSAYARLL QQQQLFLQLQILSQQQQQQQHRFSYLGMLHQAQLKEPNEQMVRNPNSSTPLSNTPLSP VKNSFSGQTGVSSFKPGPLPPNLDDLKVSELRQQLRIRGLPVSGTKTALMDRLRPFQD CSGNPVPNEVDITTVTFPVTPTNLPNYQSSSSTALSNGFYHFGSTSSSPISPASSD LSVAGSLPDTFNDASPSFGLHPSPVHVCTEESLMSSLNNGSVPSELDGLDSEKDKMLV EKQKVINELTWKLQQEQRQVEELRMQLQKQKRNNCSEVTQPPSYEDAVKQMQMTRSQQ MDELLDVLIESGEMPADAREDHSCQKVPKIPRSSRSPTAVLTKPSASFEQASSGSQI PFDPYATDSDEHLEVLLNSQSPLGKMSDVTLLKIGSEEPHFDGIMDGFSGKAAEDLFN AHEILPGPLSPMQTQFSPSSVDSNGLQLSFTESPWETMEWLDLTPPNSTPGFSALTTS SPSIFNIDFLDVTDLNLSMDLHLQQW (SEQ ID NO: 16)

Mef2c interac tion domain (5-120)	GSEHSLIRSKFRSVLQLRLQRRRTQEQLANQGIIPPLKRPAEFHEQRKHLSDSKAKNSLKRKA RNRNSADLVNMHILQASTAERSIPTAQMKLKRARLADDLNEKIALRPGPLE (SEQ ID NO: 17)
SRF domain (210- 320)	SASQPSHQSDAGKQGLGPPSTPIAVHAAVKSKSLGDSKNRHHKPKDPKPKVKKLYHQYIPPDQ KAEKSPPPMDSAYARLLQQQQLFLQLQLLSQQQQQQQHRFSYLGMHQ (SEQ ID NO: 18)
SAP domain (360- 413)	SSFKPGPLPPNLDLKVSELRQQLRIRGLPVSGTKTALMDRLRPFQDCSGNPVP (SEQ ID NO: 19)
LZ domain (510- 550)	LDGLDSEKDKMLVEKQKVINELTWKLQQEQRQVEELRMQLQ (SEQ ID NO: 20)
TAD domain (764- 986)	EVTQPPSYEDAVKQQMTRSQQMDELLDVLIESGEMPADAREDHSCLOKVPKIPRSSRSPTAVLT KPSASFQASSGSQIPFDPYATDSDEHLEVLLNSQSPLGKMSDVTLLKIGSEEPHFDGIMDGFS GKAAEDLFNAHEILPGPLSPMQTQFSPSSVDSNGLQLSFTESPWETMEWLDLTPPNSTPGFSAL TTSSPSIFNIDFLDVTDLNLNLSMDLHLQW (SEQ ID NO: 11)

[00150] In some embodiments, the engineered myocardin protein comprises one or more of an Mef2c interaction domain, an SRF domain, a SAP domain, an LZ domain, and a TAD domain. In some embodiments, the engineered myocardin protein comprises an Mef2c interaction domain, an SRF domain, a SAP domain, an LZ domain, and a TAD domain. In some embodiments, the engineered myocardin protein comprises an Mef2c interaction domain, an SRF domain, a SAP domain, and a TAD domain. In some embodiments, the engineered myocardin protein comprises an SRF domain, a SAP domain, an LZ domain, and a TAD domain. In some embodiments, the engineered myocardin protein comprises an SRF domain, a SAP domain, and a TAD domain.

[00151] In some embodiments, the engineered MYOCD is provided as a polynucleotide encoding the engineered MYOCD and, optionally, one or more other proteins of interest. In some embodiments, the polynucleotides are RNA, DNA, or mRNA polynucleotides. In some embodiments, the MYOCD polynucleotide shares identity with any of the isoforms of MYOCD. In some embodiments, the MYOCD polynucleotide encodes an engineered MYOCD protein that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%

identical to a sequence selected from My Δ 1 (SEQ ID NO: 14), My Δ 2 (SEQ ID NO: 15), and My Δ 3 (SEQ ID NO: 16). In some embodiments, the engineered MYOCD protein comprises at least 2, 3, 4, 5, of a Mef2c interaction domain, a SRF domain, a SAP domain, an LZ domain, and a TAD domain. In some embodiments, the Mef2c interaction domain is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 17. In some embodiments, the SRF domain is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 18. In some embodiments, the SAP domain is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19. In some embodiments, the LZ domain is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 20. In some embodiments, the TAD domain is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 11.

[00152] In some embodiments, the engineered myocardin protein comprises two or more fragments of the native MYOCD linked by linkers. In general, a linker refers either to a peptide bond or a polypeptide sequence. In some embodiments other linkers are used, such as any of various chemical linkers used in peptide chemistry known in the art. Reference to a “peptide bond” means that two sequences are joined together to generate a composite sequence without any intervening amino acid residues. For example, My Δ 3 (SEQ ID NO: 16) comprises MYOCD 1-559 (SEQ ID NO: 13) joined by a peptide bond to MYOCD 764-986 (SEQ ID NO: 11). In some embodiments, the linker is any of various polypeptides used as linkers in the art; for example, without limitation, glycine-serine linkers such as G, GG, GGG, GSS, GGS, GGSGGS (SEQ ID NO: 30), GSSGGS (SEQ ID NO: 31), GGSGSS (SEQ ID NO: 32), GGSGGSGGS (SEQ ID NO: 33), GGSGGSGGSGGS (SEQ ID NO: 34). In some embodiments, the linker is a domain of a protein other than MYOCD.

[00153] Throughout the disclosure, expression of a polynucleotide may refer to any means known in the art to increase the expression of a gene of interest. In some embodiments, the gene of interest

is encoded in the messenger RNA (mRNA). The mRNA may be synthetic or naturally occurring. In some embodiments, the mRNA is chemically modified in various ways known in the art. For example, modified RNAs may be used, such as described in Warren, L. et al. *Cell Stem Cell* 7:618–30 (2010); WO2014081507A1; WO2012019168; WO2012045082; WO2012045075; WO2013052523; WO2013090648; US9572896B2. In some embodiments, expression of the gene of interest is increased by delivery of a polynucleotide to a cell. In some embodiments, the polynucleotide encoding the gene of interest is delivered by a viral or non-viral vector. In some embodiments, the gene of interest is encoded in the DNA polynucleotide, optionally delivered by any viral or non-viral method known in the art. In some embodiments, the disclosure provides methods comprising contacting cells with a lipid nanoparticle comprising a DNA or mRNA encoding a gene of interest. In some embodiments, the methods of the disclosure comprise contacting cells with a virus comprising a DNA or RNA (e.g., a DNA genome, a negative-sense RNA genome, a positive-sense RNA genome, or a double-stranded RNA genome) encoding a gene of interest. In some embodiments, the virus is selected from a retrovirus, adenovirus, AAV, non-integrating lentiviral vector (LVV), and an integrating LVV. In some embodiments, the cells are transfected with a plasmid. In some embodiments, the plasmid comprises a polynucleotide encoding a reprogramming factor. In some embodiments, the plasmid comprises a transposon comprising a reprogramming factor

B. Polynucleotides Encoding Proteins of Interest

[00154] In some embodiments, the reprogramming factors are provided as a polynucleotide encoding the one or more proteins of interest. In some embodiments, the polynucleotides are RNA, DNA, or mRNA polynucleotides. In some embodiments, the polynucleotides comprise a nucleic acid sequence that comprises at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *ASCL1* (SEQ ID NO: 2) across at least 100, 200, 300, 400, or 500 nucleotides. In some embodiments, the *ASCL1* polynucleotide shares identity with any of the isoforms of *ASCL1*. In some embodiments, the *ASCL1* polynucleotide encodes an ASCL1 protein that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of human *ASCL1* (SEQ ID NO: 1).

[00155] In some embodiments, the reprogramming factors are provided as a polynucleotide encoding the one or more proteins of interest. In some embodiments, the polynucleotides are RNA, DNA, or mRNA polynucleotides. In some embodiments, the polynucleotides comprise a nucleic acid sequence that comprises at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *MYF6* (SEQ ID NO: 56) across at least 100, 200, 300, 400, or 500 nucleotides. In some embodiments, the *MYF6* polynucleotide shares identity with any of the isoforms of *MYF6*. In some embodiments, the *MYF6* polynucleotide encodes a MYF6 protein that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of human MYF6 (SEQ ID NO: 55).

[00156] In some embodiments, the compositions and methods of the disclosure provide iCM cells comprising, or methods comprising administering, one or more of a myocardin (*MYOCD*) polynucleotide, a myocyte-specific enhancer factor 2C (*MEF2C*) polynucleotide, and a T-box transcription factor (*TBX5*) polynucleotide.

[00157] In some embodiments, the compositions and methods of the disclosure provide recombinant viruses comprising, or methods comprising administering, a myocardin (*MYOCD*) polynucleotide and an *ASCL1* polynucleotide. In some embodiments, the compositions and methods of the disclosure provide recombinant viruses comprising, or methods comprising administering, an engineered *MYOCD* polynucleotide and an *ASCL1* polynucleotide.

[00158] In some embodiments, the compositions and methods of the disclosure provide iCM cells or recombinant virus or non-viral vectors comprising, or methods comprising administering, an *MYOCD* polynucleotide. In some embodiments, the *MYOCD* polynucleotide encodes a MYOCD protein that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of human *MYOCD* (SEQ ID NO: 3). In some embodiments, the *MYOCD* polynucleotide shares at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *MYOCD* (SEQ ID NO: 4)

across at least 100, 200, 300, 400, or 500 nucleotides. In some embodiments, the *MYOCD* polynucleotide shares identity with any of the isoforms of MYOCD.

[00159] In some embodiments, the engineered myocardin protein is provided as a polynucleotide encoding the engineered myocardin protein.

[00160] In some embodiments, the MYOCD polynucleotide encodes a MYOCD protein that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of an engineered MYOCD (e.g., SEQ ID NOs: 14).

[00161] In some embodiments, the MYOCD polynucleotide encodes a MYOCD protein that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of an engineered MYOCD (e.g., SEQ ID NOs: 15).

[00162] In some embodiments, the MYOCD polynucleotide encodes a MYOCD protein that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of an engineered MYOCD (e.g., SEQ ID NOs: 16).

[00163] In some embodiments, the compositions and methods of the disclosure provide iCM cells or recombinant viruses or non-viral vectors comprising, or methods comprising administering, an *MEF2C* polynucleotide. In some embodiments, the *MEF2C* polynucleotide encodes a MEF2C protein that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of human *MEF2C* (SEQ ID NO: 5). In some embodiments, the *MEF2C* polynucleotide shares at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *MEF2C* (SEQ ID NO: 6) across at least 100, 200, 300, 400, or 500 nucleotides. In some embodiments, the *MEF2C* polynucleotide shares identity with any of the isoforms of *MEF2C*.

[0100] In some embodiments, the compositions and methods of the disclosure provide iCM cells or recombinant viruses or non-viral vectors comprising, or methods comprising administering, an *TBX5* polynucleotide. In some embodiments, the *TBX5* polynucleotide encodes a TBX5 protein that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%,

84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence of human *TBX5* (SEQ ID NO: 7). In some embodiments, the *TBX5* polynucleotide shares at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *TBX5* (SEQ ID NO: 8) across at least 100, 200, 300, 400, or 500 nucleotides. In some embodiments, the *TBX5* polynucleotide shares identity with any of the isoforms of *TBX5*. The *TBX5* polypeptide contains a predicted nuclear localization sequence (NLS), KRKEEEECSTTDHPYKKPYME (SEQ ID NO: 9). In some embodiments, the NLS of the polypeptide encoded by the *TBX5* polynucleotide is a functional NLS.

[0101] In some embodiments, the polynucleotide encoding a protein of interest is a synthetic messenger RNA (mRNA). Synthetic mRNAs provide the genetic information for making proteins of interest and can be chemically modified to avoid triggering an immune response. Zangi et al. (2013) *Nature Biotech* 31 :898-907. Since mRNAs do not integrate into the host cell genome, the synthetic mRNA acts for a period of time and then disappears as the cell divides. In some embodiments the synthetic mRNAs are modified, for example, with pseudouridine and/or 5-methyl-cytidine, to reduce innate antiviral response to single-stranded RNA.

[0102] In some embodiments, the polynucleotides encoding the one or more proteins of interest may be codon-optimized or otherwise altered so long as the functional activity of the encoded gene is preserved. In some embodiments, the polynucleotides encode a modified or variant of the one or more genes of interest, including truncations, insertions, deletions, or fragments, so long as the functional activity of the encoded gene is preserved.

[0103] In some embodiments, the polynucleotides encoding the one or more proteins of interest are comprised in an expression cassette. In some embodiments, the expression cassette comprises one or more polynucleotides encoding one or more genes of interest. For example, in some embodiments, the expression cassette comprises 2, 3, 4, 5, 6, 7, 8, 9, or 10 polynucleotides encoding 2, 3, 4, 5, 6, 7, 8, 9, or 10 genes of interest.

[0104] It will be appreciated that where two or more proteins of interest are to be expressed in a cell, one or polynucleotides or expression cassettes can be used. For example, a polycistronic expression cassette can be used wherein one expression cassette can comprise multiple polynucleotides expressing multiple proteins. In some embodiments, the polycistronic expression

cassette comprises two or more polynucleotides in a single open reading frame, the polynucleotides linked together by the 2A region of aphthovirus foot-and-mouth disease virus (FMDV) polyprotein, such as described in Donnelly et al. *J. Gen. Virol.* 82:1013-15 (2001) and improvements thereof known in the art. The 2A region produces a ribosomal ‘skip’ from one codon to the next without the formation of a peptide bond. In some embodiments, the polynucleotide comprises an internal cleavage site, such that two or more peptides are generated by post-translational cleavage.

[0105] In some embodiments, multicistronic vectors of the present disclosure comprise a polynucleotide sequence encoding a plurality of polypeptides joined by linkers comprising peptides capable of inducing ribosome skipping or self-cleavage. In some embodiments, the linker comprises a 2A peptide. The term “2A peptide” as used herein refers to a class of ribosome skipping or self-cleaving peptides configured to generate two or more proteins from a single open reading frame. 2A peptides are 18–22 residue-long viral oligopeptides that mediate “cleavage” of polypeptides during translation in eukaryotic cells. “2A peptide” may refer to peptides with various amino acid sequences. In the present disclosure it will be understood that where a lentiviral vector comprises two or more 2A peptides, the 2A peptides may be identical to one another or different. Detailed methodology for design and use of 2A peptides is provided by Szymczak-Workman et al. Design and Construction of 2A Peptide-Linked Multicistronic Vectors. *Cold Spring Harb. Protoc.* 2012 Feb 1; 2012(2):199-204. In the literature, 2A peptides are often referred to as self-cleaving peptides, but mechanistic studies have shown that the “self-cleavage” observed is actually a consequence of the ribosome skipping the formation of the glycyl-prolyl peptide bond at the C terminus of the 2A peptide. Donnelly et al. *J Gen Virol.* 2001 May; 82(Pt 5):1027-41. The present invention is not bound by theory or limited to any particular mechanistic understanding of 2A peptide function.

[0106] Exemplary 2A peptides include, without limitation, those listed in Table 3.

Table 3: Exemplary 2A peptides

	Source	Nucleotide	Peptide
P2A	porcine teschovirus-1	GCC ACG AAC TTC TCT CTG TTA AAG CAA GCA GGA GAC GTG GAA GAA AAC CCC GGT CCT (SEQ ID NO: 21)	ATNFSLLKQAGDVEENPGP (SEQ ID NO: 23)

		- or - GCT ACT AAC TTC AGC CTG CTG AAG CAG GCT GGA GAC GTG GAG GAG AAC CCT GGA CCT (SEQ ID NO: 22)	
T2A	<i>Thoseaasigna</i> virus	GAG GGC AGA GGA AGT CTG CTA ACA TGC GGT GAC GTC GAG GAG AAT CCT GGA CCT (SEQ ID NO: 24)	EGRGSLTTCGDVEENPGP (SEQ ID NO: 25)
E2A	Equine rhinitis A virus (ERAV)	CAG TGT ACT AAT TAT GCT CTC TTG AAA TTG GCT GGA GAT GTT GAG AGC AAC CCT GGA CCT (SEQ ID NO: 26)	QCTNYALLKLAGDCESNPGP (SEQ ID NO: 27)
F2A	Foot-and-mouth disease virus (FMDV)	GTG AAA CAG ACT TTG AAT TTT GAC CTT CTC AAG TTG GCG GGA GAC GTG GAG TCC AAC CCT GGA CCT (SEQ ID NO: 28)	VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 29)

[0107] Optionally, one or more of the linkers further comprises a sequence encoding the residues Gly-Ser-Gly, which is in some embodiments N-terminal to the 2A peptide. N-terminal to the 2A peptide means that the sequence encoding the residues is upstream to the sequence encoding the 2A peptide. Generally, the Gly-Ser-Gly motif will be immediately N-terminal to the 2A peptide or 1 to 10 other amino acid residues are inserted between the motif and the 2A peptide. In some embodiments, the polynucleotide sequence encoding this motif is GGA AGC GGA. As with any peptide-encoding polynucleotide, the nucleotide sequence may be altered without changing the encoded peptide sequence. Substitution of amino acid residues is within the skill of those in the art, and it will be understood that the term 2A peptide refers to variants of the foregoing that retain the desired skipping/self-cleavage activity but, optionally, have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more substitutions relative to the reference 2A peptide sequence. Exemplary 2A peptides are described in Kim et al. PLOS ONE 6(4): e18556. In some embodiments, two or more different 2A peptides are used in the same construct. Varied 2A peptides have been reported to result in improved expression. *See* Liu et al. *Sci Rep.* 2017; 7:2193 In some embodiments, the polypeptide comprises a between 1 and 5, or more than 5, Gly or Ser residues N- and/or C-terminal to the 2A peptide (*e.g.* the P2A peptide). In some embodiments, the polypeptide comprises a Gly-Ser-Gly residues N-

and/or C-terminal to the 2A peptide (*e.g.* the P2A peptide). In some embodiments, the P2A peptide and N-terminal linker comprise SEQ ID NO: 84.

[0108] In some embodiments, the disclosure provides an expression cassette comprising, in 5' to 3' order, a promoter, a polynucleotide encoding MYOCD-2A-ASCL1, and a polyadenylation sequence. In some embodiments, the expression cassette comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35. In some embodiments, the disclosure provides a recombinant AAV (rAAV) comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rAAV particle comprising the expression cassette. In some embodiments, the rAAV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35. In some embodiments, the disclosure provides a recombinant lentivirus (rLV) comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rLV particle comprising the expression cassette. In some embodiments, the rLV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 35.

[0109] In some embodiments, the disclosure provides an expression cassette comprising, in 5' to 3' order, a promoter, a polynucleotide encoding MYOCD-2A-MYF6, and a polyadenylation sequence. In some embodiments, the expression cassette comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 36. In some embodiments, the disclosure provides a rAAV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rAAV particle comprising the expression cassette. In some embodiments, the rAAV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 36. In some embodiments, the disclosure provides a rLV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rLV particle comprising the expression cassette. In some embodiments, the rLV comprises a polynucleotide at least 70%, 71%,

72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 36.

[0110] In some embodiments, the disclosure provides an expression cassette comprising, in 5' to 3' order, a promoter, a polynucleotide encoding MyΔ3-2A-ASCL1, and a polyadenylation sequence. In some embodiments, the expression cassette comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 37. In some embodiments, the disclosure provides a rAAV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rAAV particle comprising the expression cassette. In some embodiments, the rAAV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 37. In some embodiments, the disclosure provides a rLV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rLV particle comprising the expression cassette. In some embodiments, the rLV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 37.

[0111] In some embodiments, the disclosure provides an expression cassette comprising, in 5' to 3' order, a promoter, a polynucleotide encoding MyΔ3-2A-MYF6, and a polyadenylation sequence. In some embodiments, the expression cassette comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 38. In some embodiments, the disclosure provides a rAAV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rAAV particle comprising the expression cassette. In some embodiments, the rAAV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 38. In some embodiments, the disclosure provides a rLV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rLV particle comprising the

expression cassette. In some embodiments, the rLV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 38.

[0112] In some embodiments, the disclosure provides an expression cassette comprising, in 5' to 3' order, a promoter, a polynucleotide encoding ASCL1-2A-MYOC, and a polyadenylation sequence. In some embodiments, the expression cassette comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 39. In some embodiments, the disclosure provides a rAAV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rAAV particle comprising the expression cassette. In some embodiments, the rAAV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 39. In some embodiments, the disclosure provides a rLV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rLV particle comprising the expression cassette. In some embodiments, the rLV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 39.

[0113] In some embodiments, the disclosure provides an expression cassette comprising, in 5' to 3' order, a promoter, a polynucleotide encoding MYF6-2A-MYOC, and a polyadenylation sequence. In some embodiments, the expression cassette comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 40. In some embodiments, the disclosure provides a rAAV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rAAV particle comprising the expression cassette. In some embodiments, the rAAV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 40. In some embodiments, the disclosure provides a rLV comprising the expression

cassette, a transfer plasmid comprising the expression cassette, or a rLV particle comprising the expression cassette. In some embodiments, the rLV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 40.

[0114] In some embodiments, the disclosure provides an expression cassette comprising, in 5' to 3' order, a promoter, a polynucleotide encoding ASCL1-2A-MyΔ3, and a polyadenylation sequence. In some embodiments, the expression cassette comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 41. In some embodiments, the disclosure provides a rAAV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rAAV particle comprising the expression cassette. In some embodiments, the rAAV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 41. In some embodiments, the disclosure provides a rLV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rLV particle comprising the expression cassette. In some embodiments, the rLV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 41.

[0115] In some embodiments, the disclosure provides an expression cassette comprising, in 5' to 3' order, a promoter, a polynucleotide encoding MYF6-2A-MyΔ3, and a polyadenylation sequence. In some embodiments, the expression cassette comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 42. In some embodiments, the disclosure provides a rAAV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rAAV particle comprising the expression cassette. In some embodiments, the rAAV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ

ID NO: 42. In some embodiments, the disclosure provides a rLV comprising the expression cassette, a transfer plasmid comprising the expression cassette, or a rLV particle comprising the expression cassette. In some embodiments, the rLV comprises a polynucleotide at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 42.

[0116] In some embodiments, the disclosure provides an expression cassette comprising a polynucleotide encoding a protein sequence at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 57-64 (*i.e.*, any one of MYOCD-2A-ASCL1, MYOCD-2A-MYF6, My Δ 3-2A-ASCL1, My Δ 3-2A-MYF6, ASCL1-2A-MYOCD, MYF6-2A-MYOCD, ASCL1-2A-My Δ 3, and MYF6-2A-My Δ 3).

III. Vectors

[0117] In some embodiments, the reprogramming factors employed to reprogram cells to the cardiac lineage can be introduced into a selected cell or a selected population of cells by a vector. In some embodiments, the vector is a nucleic acid vector, such as a plasmids (*e.g.*, DNA plasmids or RNA plasmids), transposons, cosmids, bacterial or yeast artificial chromosomes, or viral vectors.. In some embodiments, the vector is a non-nucleic acid vector, such as a nanoparticle. In some embodiments, the vectors described herein comprise a peptide, such as cell-penetrating peptides or cellular internalization sequences. Cell-penetrating peptides are small peptides that are capable of translocating across plasma membranes. Exemplary cell-penetrating peptides include, but are not limited to, Antennapedia sequences, TAT, HIV-Tat, Penetratin, Antp-3A (Antp mutant), Buforin II, Transportan, MAP (model amphipathic peptide), K-FGF, Ku70, Prion, pVEC, Pep-1, SynB1, Pep-7, I-IN-1, BGSC (Bis-Guanidinium-Spermidine-Cholesterol, and BGTC (Bis-Guanidinium-Tren-Cholesterol).

[0118] Techniques in the field of recombinant genetics can be used for such recombinant expression. Basic texts disclosing general methods of recombinant genetics include Sambrook et al., *Molecular Cloning, A Laboratory Manual* (3rd ed. 2001); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel et al., eds., 1994)). In some embodiments, the vectors do not contain a mammalian origin of

replication. In some embodiments, the expression vector is not integrated into the genome and/or is introduced via a vector that does not contain a mammalian origin of replication.

[0119] In some cases, the expression vector(s) encodes or comprises, in addition to one or more reprogramming factors, a marker gene that facilitates identification or selection of cells that have been transfected, transduced or infected. Examples of marker genes include, but are not limited to, genes encoding fluorescent proteins, *e.g.*, enhanced green fluorescent protein, Ds-Red (DsRed: *Discosoma* sp. red fluorescent protein (RFP); Bevis et al. (2002) *Nat. Biotechnol.* 20(11):83-87), yellow fluorescent protein, mCherry, and cyanofluorescent protein; and genes encoding proteins conferring resistance to a selection agent, *e.g.*, a neomycin resistance gene, a puromycin resistance gene, a blasticidin resistance gene, and the like.

[0120] In one embodiment, the expression vector further comprises a suicide gene. Expression of the suicide gene may be regulated by the same or different promoter that expresses at least one proliferation and/or cell cycle reentry factor polypeptide-encoding nucleotide. A suicide gene is one that allows for negative selection of the cells. In the methods described herein, a suicide gene is used as a safety system, allowing the cells expressing the gene to be killed by introduction of a selective agent. This is desirable in case the recombinant gene causes a mutation leading to uncontrolled cell growth. A number of suicide gene systems have been identified, including the herpes simplex virus thymidine kinase (tk or TK) gene, the cytosine deaminase gene, the varicella-zoster virus thymidine kinase gene, the nitroreductase gene, the *Escherichia coli* (*E. coli*) gpt gene, and the *E. coli* Deo gene (also see, for example, Yazawa K, Fisher W E, Brunicardi F C: Current progress in suicide gene therapy for cancer. *World J. Surg.* (2002) 26(7):783-9). In one embodiment, the suicide gene is the TK gene. In one aspect, the TK gene is a wild-type TK gene. In other aspects, the TK gene is a mutated form of the gene, *e.g.*, sr23tk. Cells expressing the TK protein can be killed using ganciclovir. In another embodiment, the nucleic acid encoding the tetracycline activator protein and the suicide gene are regulated by one promoter.

A. Nucleic Acid Vectors

1. Viral Vectors

[0121] Suitable viral vectors include, but are not limited to, viral vectors (*e.g.* viral vectors based on vaccinia virus; poliovirus; adenovirus (*e.g.*, Li et al. (1994) *Invest Ophthalmol Vis Sci* 35:2543-2549; Borrás et al. (1999) *Gene Ther* 6:515-524; Li and Davidson, (1995) *Proc. Natl. Acad. Sci.*

92:7700-7704; Sakamoto et al. (1999) Hum Gene Ther 5: 1088-1097; WO 94/12649; WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (*e.g.*, Ali et al. (1998) Hum Gene Ther 9(1):81-86, 1998, Flannery et al. (1997) Proc. Natl. Acad. Sci. 94:6916-6921; Bennett et al. (1997) Invest Ophthalmol Vis Sci 38:2857-2863; Jomary et al. (1997) Gene Ther 4:683-690; Rolling et al. (1999), Hum Gene Ther 10:641-648; Ali et al. (1996) Hum Mol Genet. 5:591-594; WO 93/09239, Samulski et al. (1989) J. Vir. 63 :3822-3828; Mendelson et al. (1988) Virol. 166: 154-165; and Flotte et al. (1993) Proc. Natl. Acad. Sci. 90: 10613-10617; SV40; herpes simplex virus; human immunodeficiency virus (*e.g.*, Miyoshi et al. (1997) Proc. Natl. Acad. Sci. 94: 10319-10323; Takahashi et al. (1999) J Virol 73 :7812-7816); a retroviral vector (*e.g.*, Murine-Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like. Numerous suitable expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example; for eukaryotic cells: pXTI, pSG5 (Stratagene), pSVK3, pBPV, pMSG, pSVLSV40 (Pharmacia), and pAd (Life Technologies). However, any other vector may be used so long as it is compatible with the cells of the present disclosure.

[0122] The ability of certain viruses to infect cells or enter cells via receptor-mediated endocytosis, and express viral genes stably and efficiently have made them attractive candidates for the transfer of foreign nucleic acids into cells (*e.g.*, mammalian cells). Viral vectors can include control sequences such as promoters for expression of the polypeptide of interest. Although many viral vectors integrate into the host cell genome, if desired, the segments that allow such integration can be removed or altered to prevent such integration. Moreover, in some embodiments, the vectors do not contain a mammalian origin of replication. Non-limiting examples of virus vectors are described below that can be used to deliver nucleic acids encoding a transcription factor into a selected cell. In some embodiments, the viral vector is derived from a replication-deficient virus.

[0123] In general, other useful viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the polypeptide of interest. Non-cytopathic viruses include certain retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. In general, the retroviruses are replication-deficient (*e.g.*, capable of directing synthesis of the desired

transcripts, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of polynucleotide *in vivo*.

[0124] In some embodiments, a polynucleotide encoding a reprogramming factor can be housed within an infective virus that has been engineered to express a specific binding ligand. The virus particle will thus bind with specificity to the cognate receptors of the target cell and deliver the contents to the cell. In some embodiments, the virus is modified to impart particular viral tropism, *e.g.*, the virus preferentially infects fibroblasts, heart cells, or more particularly cardiac fibroblasts (CFs). For AAV, capsid proteins can be mutated to alter the tropism of the viral vector. For lentivirus, tropism can be modified by using different envelope proteins; this is known as “pseudotyping.”

a. Retroviral Vectors

[0125] In some embodiments, the viral vector is a retroviral vector. Retroviruses can integrate their genes into the host genome, transfer a large amount of foreign genetic material, infect a broad spectrum of species and cell types, and can be packaged in special cell-lines (Miller et al., *Am. J. Clin. Oncol.*, 15(3):216-221, 1992). In some embodiments, a retroviral vector is altered so that it does not integrate into the host cell genome.

[0126] The recombinant retrovirus may comprise a viral polypeptide (*e.g.*, retroviral env) to aid entry into the target cell. Such viral polypeptides are well-established in the art, for example, U.S. Pat. No. 5,449,614. The viral polypeptide may be an amphotropic viral polypeptide, for example, amphotropic env, which aids entry into cells derived from multiple species, including cells outside of the original host species. The viral polypeptide may be a xenotropic viral polypeptide that aids entry into cells outside of the original host species. In some embodiments, the viral polypeptide is an ecotropic viral polypeptide, for example, ecotropic env, which aids entry into cells of the original host species.

[0127] Examples of viral polypeptides capable of aiding entry of retroviruses into cells include, but are not limited to: MMLV amphotropic env, MMLV ecotropic env, MMLV xenotropic env, vesicular stomatitis virus-g protein (VSV-g), HIV-1 env, Gibbon Ape Leukemia Virus (GALV) env, RD114, FeLV-C, FeLV-B, MLV 10A1 env gene, and variants thereof, including chimeras. Yee et al. (1994) *Methods Cell Biol*, Pt A:99-1 12 (VSV-G); U.S. Pat. No. 5,449,614. In some

cases, the viral polypeptide is genetically modified to promote expression or enhanced binding to a receptor.

[0128] The retroviral construct may be derived from a range of retroviruses, *e.g.*, MMLV, HIV-1, SIV, FIV, or other retrovirus described herein. The retroviral construct may encode all viral polypeptides necessary for more than one cycle of replication of a specific virus. In some cases, the efficiency of viral entry is improved by the addition of other factors or other viral polypeptides. In other cases, the viral polypeptides encoded by the retroviral construct do not support more than one cycle of replication, *e.g.*, U.S. Pat. No. 6,872,528. In such circumstances, the addition of other factors or other viral polypeptides can help facilitate viral entry. In an exemplary embodiment, the recombinant retrovirus is HIV-1 virus comprising a VSV-g polypeptide, but not comprising a HIV-1 env polypeptide.

[0129] The retroviral construct may comprise: a promoter, a multi-cloning site, and/or a resistance gene. Examples of promoters include but are not limited to CMV, SV40, EFla, β -actin; retroviral LTR promoters, and inducible promoters. The retroviral construct may also comprise a packaging signal (*e.g.*, a packaging signal derived from the MFG vector; a psi packaging signal). Examples of some retroviral constructs known in the art include but are not limited to: pMX, pBabeX or derivatives thereof. Onishi et al. (1996) *Experimental Hematology*, 24:324-329. In some cases, the retroviral construct is a self-inactivating lentiviral vector (SIN) vector. Miyoshi et al. (1998) *J. Virol* 72(10):8150- 8157. In some cases, the retroviral construct is LL-CG, LS-CG, CL-CG, CS-CG, CLG or MFG. Miyoshi et al. (1998) *J. Virol* 72(10):8150-8157; Onishi et al. (1996) *Experimental Hematology*, 24:324-329; Riviere et al. (1995) *Proc. Natl. Acad. Sci.*, 92:6733-6737.

[0130] A retroviral vector can be constructed by inserting a nucleic acid (*e.g.*, one encoding a polypeptide of interest or an RNA) into the viral genome in the place of some viral sequences to produce a virus that is replication-defective. To produce virions, a packaging cell line containing the gag, pol, and env genes, but without the LTR and packaging components, is constructed (Mann et al., *Cell* 33:153-159, 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into a special cell line (*e.g.*, by calcium phosphate precipitation), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubinstein, In: *Vectors: A survey of molecular cloning vectors and their uses*, Rodriguez and

Denhardt, eds., Stoneham: Butterworth, pp. 494-513, 1988; Temin, In: Gene Transfer, Kucherlapati (ed.), New York: Plenum Press, pp. 149-188, 1986; Mann et al., Cell, 33:153-159, 1983). The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression typically involves the division of host cells (Paskind et al., Virology, 67:242-248, 1975).

b. Adenoviral Vectors

[0131] In some embodiments, the viral vector is an adenoviral vector. The genetic organization of adenovirus includes an approximate 36 kb, linear, double-stranded DNA virus, which allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus et al., *Seminar in Virology* 200(2):535-546, 1992)). Reprogramming factors may be introduced into the cell using adenovirus assisted transfection. Increased transfection efficiencies have been reported in cell systems using adenovirus coupled systems (Kelleher and Vos, *Biotechniques*, 17(6):1110-7, 1994; Cotten et al., *Proc Natl Acad Sci USA*, 89(13):6094-6098, 1992; Curiel, *Nat Immun*, 13(2-3):141-64, 1994.).

c. Adeno-associated viral (AAV) Vectors

[0132] In some embodiments, the viral vector is an AAV vector. AAV is an attractive vector system as it has a low frequency of integration and it can infect non-dividing cells, thus making it useful for delivery of polynucleotides into mammalian cells, for example, in tissue culture (Muzyczka, *Curr Top Microbiol Immunol*, 158:97-129, 1992) or *in vivo*. Details concerning the generation and use of rAAV vectors are described in U.S. Patent Nos. 5,139,941 and 4,797,368, each incorporated herein by reference in its entirety.

[0133] AAV is a replication-deficient parvovirus, the single-stranded DNA genome of which is about 4.7 kb in length including two 145 nucleotide inverted terminal repeat (ITRs). There are multiple serotypes of AAV. The nucleotide sequences of the genomes of the AAV serotypes are known. For example, the complete genome of AAV-1 is provided in GenBank Accession No. NC_002077; the complete genome of AAV-2 is provided in GenBank Accession No. NC_001401 and Srivastava et al., J. Virol., 45: 555-564 (1983); the complete genome of AAV-3 is provided in GenBank Accession No. NC_1829; the complete genome of AAV-4 is provided in GenBank

Accession No. NC_001829; the AAV-5 genome is provided in GenBank Accession No. AF085716; the complete genome of AAV-6 is provided in GenBank Accession No. NC_001862; at least portions of AAV-7 and AAV-8 genomes are provided in GenBank Accession Nos. AX753246 and AX753249, respectively; the AAV-9 genome is provided in Gao et al., J. Virol., 78: 6381-6388 (2004); the AAV-10 genome is provided in Mol. Ther., 13(1): 67-76 (2006); and the AAV-11 genome is provided in Virology, 330(2): 375-383 (2004). The sequence of the AAV rh.74 genome is provided in U.S. Patent 9,434,928, incorporated herein by reference. Cis-acting sequences directing viral DNA replication (rep), encapsidation/packaging and host cell chromosome integration are contained within the AAV ITRs. Three AAV promoters (named p5, p19, and p40 for their relative map locations) drive the expression of the two AAV internal open reading frames encoding rep and cap genes. The two rep promoters (p5 and p19), coupled with the differential splicing of the single AAV intron (at nucleotides 2107 and 2227), result in the production of four rep proteins (rep 78, rep 68, rep 52, and rep 40) from the rep gene. Rep proteins possess multiple enzymatic properties that are ultimately responsible for replicating the viral genome. The cap gene is expressed from the p40 promoter and it encodes the three capsid proteins VP1, VP2, and VP3. Alternative splicing and non-consensus translational start sites are responsible for the production of the three related capsid proteins. A single consensus polyadenylation site is located at map position 95 of the AAV genome. The life cycle and genetics of AAV are reviewed in Muzyczka, Current Topics in Microbiology and Immunology, 158: 97-129 (1992).

[0134] AAV possesses unique features that make it attractive as a vector for delivering foreign DNA to cells, for example, in gene therapy. AAV infection of cells in culture is noncytopathic, and natural infection of humans and other animals is silent and asymptomatic. Moreover, AAV infects many mammalian cells allowing the possibility of targeting many different tissues *in vivo*. Moreover, AAV transduces slowly dividing and non-dividing cells, and can persist essentially for the lifetime of those cells as a transcriptionally active nuclear episome (extrachromosomal element). The AAV proviral genome is inserted as cloned DNA in plasmids, which makes construction of recombinant genomes feasible. Furthermore, because the signals directing AAV replication and genome encapsidation are contained within the ITRs of the AAV genome, some or all of the internal approximately 4.3 kb of the genome (encoding replication and structural capsid proteins, rep-cap) may be replaced with foreign DNA. To generate AAV vectors, the rep and cap proteins may be provided *in trans*. Another significant feature of AAV is that it is an extremely

stable and hearty virus. It easily withstands the conditions used to inactivate adenovirus (56° to 65°C for several hours), making cold preservation of AAV less critical. AAV may even be lyophilized. Finally, AAV-infected cells are not resistant to superinfection. The AAV vectors of the disclosure include self-complementary, duplexed AAV vectors, synthetic ITRs, and/or AAV vectors with increased packaging compacity. Illustrative methods are provided in US 8,784,799; US 8,999,678; US 9,169,494; US 9,447,433; and US 9,783,824, each of which is incorporated by reference in its entirety.

[0135] AAV DNA in the rAAV genomes may be from any AAV serotype for which a recombinant virus can be derived including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13 and AAV rh74. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. See, for example, Marsic et al., *Mol. Therapy*. 22):1900-09 (2014). The nucleotide sequences of the genomes of various AAV serotypes are known in the art. AAV vectors of the present disclosure include AAV vectors of serotypes AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV39, AAV43, AAV.rh74, and AAV.rh8. Illustrative AAV vectors are provided in US 7,105,345; US 15/782,980; US 7,259,151; US 6,962,815; US 7,718,424; US 6,984,517; US 7,718,424; US 6,156,303; US 8,524,446; US 7,790,449; US 7,906,111; US 9,737,618; US App 15/433,322; US 7,198,951, each of which is incorporated by reference in its entirety.

[0136] In some embodiments, the AAV expression vector is pseudotyped to enhance targeting. To promote gene transfer and sustain expression in fibroblasts, AAV5, AAV7, and AAV8, may be used. In some cases, the AAV2 genome is packaged into the capsid of producing pseudotyped vectors AAV2/5, AAV2/7, and AAV2/8 respectively, as described in Balaji et al. *J Surg Res*. 184:691-98 (2013). In some embodiments, an AAV9 may be used to target expression in myofibroblast-like lineages, as described in Piras et al. *Gene Therapy* 23:469–478 (2016). In some embodiments, AAV1, AAV6, or AAV9 is used, and in some embodiments, the AAV is engineered, as described in Asokari et al. *Hum Gene Ther*. 24:906-13 (2013); Pozsgai et al. *Mol Ther*. 25:855-69 (2017); Kotterman et al. *Nature Reviews Genetics* 15:445-51 (2014); and US20160340393A1 to Schaffer et al. In some embodiments, the viral vector is AAV engineered to increase target cell infectivity as described in US20180066285A1.

[0137] In some embodiments, the AAV vectors of the disclosure comprises a modified capsid, in particular as capsid engineered to enhance or promote *in vivo* or *ex vivo* transduction of cardiac cells, or more particularly cardiac fibroblasts; or that evade the subject's immune system; or that have improved biodistribution. Illustrative AAV capsids are provided in US 7,867,484; US 9,233,131; US 10,046,016; WO 2016/133917; WO 2018/222503; and WO 20019/060454, each of which is incorporated by reference in its entirety. In an AAV capsid (or in particular an AAV2 capsid), one or more substitutions at E67, S207, N551, and I698 may be used to increase infectivity towards cardiac fibroblasts. More particularly, the AAV vectors of the disclosure, optionally AAV2-based vectors, may comprise in their capsid proteins one or more substitutions selected from E67A, S207G, V229I, A490T, N551S, A581T, and I698V. In some embodiments, the AAV vectors of the disclosure comprise the AAV-A2 capsid and/or serotype, which is described in WO 2018/222503. In some embodiments, the AAV capsid comprises an insertion in the GH loop of the capsid protein, such as NKIQRTD (SEQ ID NO: 65) or NKTTNKD (SEQ ID NO: 66). It will be appreciated that these substitutions and insertions may be combined together to generate various capsid proteins useful in the present disclosure.

d. Lentiviral Vectors

[0138] In some embodiments, the viral vector is a lentiviral vector. Lentiviruses are complex retroviruses, which, in addition to the common retroviral genes gag, pol, and env, contain other genes with regulatory or structural function. Information on lentiviral vectors is available, for example, in Naldini et al., *Science* 272(5259):263-267, 1996; Zufferey et al., *Nat Biotechnol* 15(9):871-875, 1997; Blomer et al., *J Virol*. 71(9):6641-6649, 1997; U.S. Patent Nos. 6,013,516 and 5,994,136, each of which is incorporated herein by reference in its entirety. Some examples of lentivirus include the Human Immunodeficiency Viruses: HIV-1, HIV-2 and the Simian Immunodeficiency Virus: SIV. Lentiviral vectors have been generated by attenuating the HIV virulence genes, for example, the genes env, vif, vpr, vpu and nef are deleted to make the vector biologically safe. The lentivirus employed can also be replication and/or integration defective.

[0139] Recombinant lentiviral vectors are capable of infecting non-dividing cells and can be used for both *in vivo* and *ex vivo* gene transfer and expression of nucleic acid sequences. For example, recombinant lentivirus capable of infecting a non-dividing cell wherein a suitable host cell is transfected with two or more vectors carrying the packaging functions, namely gag, pol and

env, as well as rev and tat is described in U.S. Patent No. 5,994,136, which is incorporated herein by reference in its entirety. Those of skill in the art can target the recombinant virus by linkage of the envelope protein with an antibody or a particular ligand for targeting to a receptor of a particular cell type. For example, a target-specific vector can be generated by inserting a nucleic acid segment (including a regulatory region) of interest into the viral vector, along with another gene that encodes a ligand for a receptor on a specific target cell type.

[0140] Lentiviral vectors are known in the art, see Naldini et al., (1996 and 1998); Zufferey et al., (1997); Dull et al., 1998, U.S. Pat. Nos. 6,013,516; and 5,994,136 all incorporated herein by reference. In general, these vectors are plasmid-based or virus-based, and are configured to carry the essential sequences for incorporating foreign nucleic acid, for selection and for transfer of the nucleic acid into a host cell. In some cases, a lentiviral vector is introduced into a cell concurrently with one or more lentiviral packaging plasmids, which may include, without limitation, pMD2.G, pRSV-rev, pMDLG-pRRE, and pRRL-GOI. Introduction of a lentiviral vector alone or in combination with lentiviral packaging plasmids into a cell may cause the lentiviral vector to be packaged into a lentiviral particle. In some embodiments, the lentiviral vector is a non-integrating lentiviral (NIL) vector. Illustrative methods for generating NIL vectors, such as the D64V substitution in the integrase gene, are provided in US 8,119,119.

[0141]

2. Methods of Producing Viral Vectors

[0142] In general, a viral vector is produced by introducing a viral DNA or RNA construct into a producer cell. In some cases, the producer cell does not express exogenous genes. In other cases, the producer cell is a “packaging cell” comprising one or more exogenous genes, *e.g.*, genes encoding one or more gag, pol, or env polypeptides and/or one or more retroviral gag, pol, or env polypeptides. The retroviral packaging cell may comprise a gene encoding a viral polypeptide, *e.g.*, VSV-g, that aids entry into target cells. In some cases, the packaging cell comprises genes encoding one or more lentiviral proteins, *e.g.*, gag, pol, env, vpr, vpu, vpx, vif, tat, rev, or nef. In some cases, the packaging cell comprises genes encoding adenovirus proteins such as El A or El B or other adenoviral proteins. For example, proteins supplied by packaging cells may be retrovirus-derived proteins such as gag, pol, and env; lentivirus-derived proteins such as gag, pol, env, vpr, vpu, vpx, vif, tat, rev, and nef; and adenovirus-derived proteins such as El A and El B.

In many examples, the packaging cells supply proteins derived from a virus that differs from the virus from which the viral vector is derived. Methods of producing recombinant viruses from packaging cells and their uses are well established; *see, e.g.*, U.S. Pat. Nos. 5,834,256; 6,910,434; 5,591,624; 5,817,491; 7,070,994; and 6,995,009.

[0143] Packaging cell lines include but are not limited to any easily-transfectable cell line. Packaging cell lines can be based on 293T cells, NIH3T3, COS or HeLa cell lines. Packaging cells are often used to package virus vector plasmids deficient in at least one gene encoding a protein required for virus packaging. Any cells that can supply a protein or polypeptide lacking from the proteins encoded by such viral vectors or plasmids may be used as packaging cells. Examples of packaging cell lines include but are not limited to: Platinum-E (Plat-E), Platinum-A (Plat- A), BOSC 23 (ATCC CRL 11554) and Bing (ATCC CRL 11270). Morita et al. (2000) *Gene Therapy* 7(12): 1063-1066; Onishi et al. (1996) *Experimental Hematology*, 24:324-329; U.S. Pat. No. 6,995,009. Commercial packaging lines are also useful, *e.g.*, Ampho-Pak 293 cell line, Eco-Pak 2-293 cell line, RetroPack PT67 cell line, and Retro-X Universal Packaging System (all available from Clontech).

3. Plasmids

[0144] Virus vector plasmids (or constructs), include: pMXs, pMXs-IB, pMXs-puro, pMXs-neo (pMXs- IB is a vector carrying the blasticidin-resistant gene instead of the puromycin-resistant gene of pMXs-puro) Kimatura et al. (2003) *Experimental Hematology* 31 : 1007-1014; MFG Riviere et al. (1995) *Proc. Natl. Acad. Sci.*, 92:6733-6737; pBabePuro; Morgenstern et al. (1990) *Nucleic Acids Research* 18:3587-3596; LL-CG, CL-CG, CS-CG, CLG Miyoshi et al. (1998) *J. Vir.* 72:8150-8157 and the like as the retrovirus system, and pAdexl Kanegae et al. (1995) *Nucleic Acids Research* 23 :3816-3821 and the like as the adenovirus system. In exemplary embodiments, the retroviral construct comprises blasticidin (*e.g.*, pMXs-IB), puromycin (*e.g.*, pMXs-puro, pBabePuro), or neomycin (*e.g.*, pMXs-neo). Morgenstern et al. (1990) *Nucleic Acids Research* 18:3587-3596.

[0145] In some embodiments, the viral vector or plasmid comprises a transposon or a transposable element comprising a polynucleotide encoding a reprogramming factor. Delivery of polynucleotides via DNA transposons, such as piggyBac and Sleeping Beauty, offers advantages in ease of use, ability to delivery larger cargo, speed to clinic, and cost of production. The piggyBac

DNA transposon, in particular, offers potential advantages in giving long-term, high-level and stable expression of polynucleotides, and in being significantly less mutagenic, being non-oncogenic and being fully reversible.

4. *Direct Translation from Introduced RNA*

[0146] When the one or more genes of interest are expressed transiently in the selected cells, the gene(s) of interest can be introduced as an RNA molecule, which is translated to protein within the cell's cytoplasm. For example, the protein of interest can be translated from introduced RNA molecules that have the open reading frame (ORF) for the polypeptide flanked by a 5' untranslated region (UTR) containing a translational initiation signal (*e.g.*, a strong Kozak translational initiation signal) and a 3' untranslated region terminating with an oligo(dT) sequence for templated addition of a polyA tail. Such RNA molecules do not have the promoter sequences employed in most expression vectors and expression cassettes. The RNA molecules can be introduced into the selected cells by a variety of techniques, including electroporation or by endocytosis of the RNA complexed with a cationic vehicle. See, *e.g.*, Warren et al., *Cell Stem Cell* 7: 618-30 (2010), incorporated herein by reference in its entirety.

[0147] Protein translation can persist for several days, especially when the RNA molecules are stabilized by incorporation of modified ribonucleotides. For example, incorporation of 5-methylcytidine (5mC) for cytidine and/or pseudouridine (psi) for uridine can improve the half-life of the introduced RNA *in vivo*, and lead to increased protein translation. If high levels of expression are desired, or expression for more than a few days is desired, the RNA can be introduced repeatedly into the selected cells.

[0148] The RNA encoding the protein can also include a 5' cap, a nuclear localization signal, or a combination thereof. See, *e.g.*, Warren et al., *Cell Stem Cell* 7: 618-30 (2010).

[0149] Such RNA molecules can be made, for example, by *in vitro* transcription of a template for the polynucleotide of interest using a ribonucleoside blend that includes a 3'-O-Me-m7G(5')ppp(5')G ARCA cap analog, adenosine triphosphate and guanosine triphosphate, 5-methylcytidine triphosphate and pseudouridine triphosphate. The RNA molecules can also be treated with phosphatase to reduce cytotoxicity.

[0150] The RNA can be introduced alone or with a microRNA (*e.g.*, for Oct4 expression, miRNA-302), which can be an inducer of endogenous polypeptide expression. The microRNA

functions as a structural RNA that does not encode a protein. Hence, no translation is needed for microRNA to perform its function. The microRNA can be introduced directly into cells, for example, in a delivery vehicle such as a liposome, microvesicle, or exosome. Alternatively, the microRNA can be expressed from an expression cassette or expression vector that has been introduced into a cell or a cell population.

B. Non-Nucleic Acid Vectors

[0151] In certain embodiments, the vector comprises lipid particles as described in Kanasty R, Delivery materials for siRNA therapeutics *Nat Mater.* 12(11):967-77 (2013), which is hereby incorporated by reference. In some embodiments, the lipid-based vector is a lipid nanoparticle, which is a lipid particle between about 1 and about 100 nanometers in size.

[0152] In some embodiments, the lipid-based vector is a lipid or liposome. Liposomes are artificial spherical vesicles comprising a lipid bilayer.

[0153] In some embodiments, the lipid-based vector is a small nucleic acid-lipid particle (SNALP). SNALPs comprise small (less than 200nm in diameter) lipid-based nanoparticles that encapsulate a nucleic acid. In some embodiments, the SNALP is useful for delivery of an RNA molecule such as siRNA. In some embodiments, SNALP formulations deliver nucleic acids to a particular tissue in a subject, such as the heart.

[0154] In some embodiments, the one or more polynucleotides are delivered via polymeric vectors. In some embodiments, the polymeric vector is a polymer or polymerosome. Polymers encompass any long repeating chain of monomers and include, for example, linear polymers, branched polymers, dendrimers, and polysaccharides. Linear polymers comprise a single line of monomers, whereas branched polymers include side chains of monomers. Dendrimers are also branched molecules, which are arranged symmetrically around the core of the molecule. Polysaccharides are polymeric carbohydrate molecules, and are made up of long monosaccharide units linked together. Polymersomes are artificial vesicles made up of synthetic amphiphilic copolymers that form a vesicle membrane, and may have a hollow or aqueous core within the vesicle membrane.

[0155] Various polymer-based systems can be adapted as a vehicle for administering DNA or RNA encoding the one or more reprogramming factors. Exemplary polymeric materials include poly(D,L-lactic acid-co-glycolic acid) (PLGA), poly(caprolactone) (PCL), ethylene vinyl acetate

polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), PLGA-b-poly(ethylene glycol)-PLGA (PLGA-bPEG-PLGA), PLLA-bPEG-PLLA, PLGA-PEG-maleimide (PLGA-PEG-mal), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacrylate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) (polyacrylic acids), and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate), polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), trimethylene carbonate, polyvinylpyrrolidone, polyorthoesters, polyphosphazenes, Poly([beta]-amino esters (PBAE), and polyphosphoesters, and blends and/or block copolymers of two or more such polymers. Polymer-based systems may also include Cyclodextrin polymer (CDP)-based nanoparticles such as, for example, CDP-admantane (AD)-PEG conjugates and CDP-AD-PEG-transferrin conjugates.

[0156] Exemplary polymeric particle systems for delivery of drugs, including nucleic acids, include those described in US 5,543,158, US 6,007,845, US 6,254,890, US 6,998,115, US 7,727,969, US 7,427,394, US 8,323,698, US 8,071,082, US 8,105,652, US 2008/0268063, US 2009/0298710, US 2010/0303723, US 2011/0027172, US 2011/0065807, US 2012/0156135, US

2014/0093575, WO 2013/090861, each of which are hereby incorporated by reference in its entirety.

[0157] In some embodiments, the lipid-based vector comprises a lipid encapsulation system. The lipid encapsulation system can be designed to drive the desired tissue distribution and cellular entry properties, as well as to provide the requisite circulation time and biodegrading character. The lipid encapsulation may involve reverse micelles and/or further comprise polymeric matrices, for example as described in US 8,193,334, which is hereby incorporated by reference. In some embodiments, the particle includes a lipophilic delivery compound to enhance delivery of the particle to tissues, including in a preferential manner. Such compounds are disclosed in US 2013/0158021, which is hereby incorporated by reference in its entirety. Such compounds may generally include lipophilic groups and conjugated amino acids or peptides, including linear or cyclic peptides, and including isomers thereof. In some embodiments, the lipid encapsulation comprises one or more of a phospholipid, cholesterol, polyethylene glycol (PEG)-lipid, and a lipophilic compound.

[0158] The particles, whether lipid or polymeric or both, may include additional components useful for enhancing the properties for *in vivo* nucleic acid delivery (including compounds disclosed in US 8,450,298 and US 2012/0251560, which are each hereby incorporated by reference). The delivery vehicle may accumulate preferentially in certain tissues thereby providing a tissue targeting effect, but in some embodiments, the delivery vehicle further comprises at least one cell-targeting or tissue-targeting ligand. Functionalized particles, including exemplary targeting ligands, are disclosed in US 2010/0303723 and 2012/0156135, which are hereby incorporated by reference in their entireties.

[0159] A delivery vehicle can be designed to drive the desired tissue distribution and cellular entry properties of the delivery systems disclosed herein, as well as to provide the requisite circulation time and biodegrading character. For example, lipid particles can employ amino lipids as disclosed in US 2011/0009641, which is hereby incorporated by reference.

[0160] The lipid or polymeric particles may have a size (*e.g.*, an average size) in the range of about 50 nm to about 5 μ m. In some embodiments, the particles are in the range of about 10 nm to about 100 μ m, or about 20 nm to about 50 μ m, or about 50 nm to about 5 μ m, or about 70 nm to about 500 nm, or about 70 nm to about 200 nm, or about 50 nm to about 100 nm. Particles may

be selected so as to avoid rapid clearance by the immune system. Particles may be spherical, or non-spherical in certain embodiments.

C. Promoters and Enhancers

[0161] In some embodiments, a nucleic acid encoding a reprogramming factor can be operably linked to a promoter and/or enhancer to facilitate expression of the reprogramming factor. Depending on the host/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (*e.g.*, Bitter et al. (1987) *Methods in Enzymology*, 153 :516-544).

[0162] Separate promoters and/or enhancers can be employed for each of the polynucleotides. In some embodiments, the same promoter and/or enhancer is used for two or more polynucleotides in a single open reading frame. Vectors employing this configuration of genetic elements are termed “polycistronic.” An example of a polycistronic vector comprises an enhancer and a promoter operatively linked to a single open-reading frame comprising two or more polynucleotides linked by 2A region(s), whereby expression of the open-reading frame result in multiple polypeptides being generated co-translationally. The 2A region is believed to mediate generation of multiple polypeptide sequences through codon skipping; however, the present disclosure relates also to polycistronic vectors that employ post-translational cleavage to generate polypeptides for two or more genes of interest from the same polynucleotide. Illustrative 2A sequences, vectors, and associated methods are provided in US20040265955A1, which is incorporated herein by reference. Other polycistronic vectors of the disclosure employ internal promoter(s), splicing, reinitiation, internal ribosome entry site(s) (IRES), proteolytic cleavable site(s) (*e.g.* fusagen) and fusion of genes.

[0163] Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include CMV, CMV immediate early, HSV thymidine kinase, early and late SV40, long terminal repeats (LTRs) from retrovirus, and mouse metallothionein-I. In some embodiments, promoters that are capable of conferring cardiac-specific expression will be used. Non-limiting examples of suitable cardiac-specific promoters include desmin (Des), alpha-myosin heavy chain (a-MHC), myosin light chain 2 (MLC-2), cardiac troponin T (cTnT) and cardiac troponin C (cTnC). Non-limiting examples of suitable neuron specific promoters include synapsin I (SYN),

calcium/calmodulin-dependent protein kinase II, tubulin alpha I, neuron-specific enolase and platelet-derived growth factor beta chain promoters and hybrid promoters by fusing cytomegalovirus enhancer (E) to those neuron-specific promoters.

[0164] Examples of suitable promoters for driving expression reprogramming factors include, but are not limited to, retroviral long terminal repeat (LTR) elements; constitutive promoters such as CMV, HSV1-TK, SV40, EF-1a, β -actin, phosphoglycerol kinase (PGK); inducible promoters, such as those containing Tet-operator elements; cardiac-specific promoters, such as desmin (Des), alpha-myosin heavy chain (α -MHC), myosin light chain 2 (MLC-2), cardiac troponin T (cTnT) and cardiac troponin C (cTnC); neural-specific promoters, such as nestin, neuronal nuclei (NeuN), microtubule-associate protein 2 (MAP2), beta III tubulin, neuron-specific enolase (NSE), oligodendrocyte lineage (Olig1/2), and glial fibrillary acidic protein (GFAP); and pancreatic-specific promoters, such as Pax4, Nkx2.2, Ngn3, insulin, glucagon, and somatostatin.

[0165] In some embodiments, a polynucleotide is operably linked to a cell type-specific transcriptional regulator element (TRE), where TREs include promoters and enhancers. Suitable TREs include, but are not limited to, TREs derived from the following genes: myosin light chain-2, α -myosin heavy chain, AE3, cardiac troponin C, and cardiac actin. Franz et al. (1997) *Cardiovasc. Res.* 35:560-566; Robbins et al. (1995) *Ann. N. Y. Acad. Sci.* 752:492-505; Linn et al. (1995) *Circ. Res.* 76:584-591; Parmacek et al. (1994) *Cell. Biol.* 14: 1870-1885; Hunter et al. (1993) *Hypertension* 22:608-617; and Sartorelli et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:4047-4051.

[0166] The promoter can be one naturally associated with a gene or nucleic acid segment. Similarly, for RNAs (*e.g.*, microRNAs), the promoter can be one naturally associated with a microRNA gene (*e.g.*, an miRNA-302 gene). Such a naturally associated promoter can be referred to as the “natural promoter” and may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon. Similarly, an enhancer may be one naturally associated with a nucleic acid sequence. However, the enhancer can be located either downstream or upstream of that sequence.

[0167] Alternatively, certain advantages will be gained by positioning the coding nucleic acid segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a nucleic acid in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a nucleic acid

sequence in its natural environment. Such promoters or enhancers can include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not “naturally occurring,” *i.e.*, containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR™, in connection with the compositions disclosed herein (see U.S. Pat. No. 4,683,202, U.S. Pat. No. 5,928,906, each incorporated herein by reference).

[0168] The promoters employed may be constitutive, inducible, developmentally-specific, tissue-specific, and/or useful under the appropriate conditions to direct high level expression of the nucleic acid segment. For example, the promoter can be a constitutive promoter such as, a CMV promoter, a CMV cytomegalovirus immediate early promoter, a CAG promoter, an EF-1 α promoter, a HSV1-TK promoter, an SV40 promoter, a β -actin promoter, a PGK promoter, or a combination thereof. Examples of eukaryotic promoters that can be used include, but are not limited to, constitutive promoters, *e.g.*, viral promoters such as CMV, SV40 and RSV promoters, as well as regulatable promoters, *e.g.*, an inducible or repressible promoter such as the tet promoter, the hsp70 promoter and a synthetic promoter regulated by CRE. In some embodiments, the promoter comprises a CAG promoter (*i.e.* a CMV early enhancer element and chicken beta-actin promoter) (SEQ ID NO: 67). In some embodiments, the expression cassette comprises an SV40 intron (SEQ ID NO: 83). In some embodiments, the promoter comprises a CMV early enhancer element, chicken beta-actin promoter and a CMV intron (SEQ ID NO: 69). In some embodiments, one or more of the polynucleotides encoding a protein of interest is operatively linked to a CAG promoter (SEQ ID NO: 67). In some embodiments, one or more of the polynucleotides encoding a protein of interest is operatively linked to a super core promoter (SCP) (SEQ ID NO: 68), *see* US 7,968,698. Other examples of promoters that can be employed include a human EF1 α elongation factor promoter, a CMV cytomegalovirus immediate early promoter, a CAG chicken albumin promoter, a viral promoter associated with any of the viral vectors described herein, or a promoter that is homologous to any of the promoters described herein (*e.g.*, from another species). In some embodiments, the promoter is a ubiquitous promoter, optionally selected from the group consisting of a CMV, EF1A, EFS, CAG, CBh, SV40, mPGK, hPGK, and UBC promoters. In some embodiments, the promoter is an inducible promoter. In some embodiments, the promoter is

fibroblast-specific promoter, optionally selected from the group consisting of COL1A1, COL6A1, FN1 POSTN, COL1A2, MAP2K3, and PPAR γ promoters.

[0169] In some embodiments, an internal ribosome entry sites (IRES) element can be used to create multigene, or polycistronic, messages. IRES elements are able to bypass the ribosome scanning model of 5'-methylated Cap dependent translation and begin translation at internal sites (Pelletier and Sonenberg, *Nature* 334(6180):320-325 (1988)). IRES elements from two members of the picornavirus family (polio and encephalomyocarditis) have been described (Pelletier and Sonenberg, *Nature* 334(6180):320-325 (1988)), as well an IRES from a mammalian message (Macejak & Samow, *Nature* 353:90-94 (1991)). IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message (see U.S. Patent Nos. 5,925,565 and 5,935,819, herein incorporated by reference).

[0170] In some embodiments, the vectors of the disclosure include one or more polyA signals. Illustrative polyA signals useful in the vectors of the disclosure include the short polyA signal (SEQ ID NO: 74) and the bGH polyA signal (SEQ ID NO: 85).

D. Vector Delivery to Cells

[0171] The viral vector may be introduced into a host fibroblast by any method known in the art, including but not limited to: a calcium phosphate method, a lipofection method (*e.g.*, Feigner et al. (1987) *Proc. Natl. Acad. Sci.* 84:7413-7417), an electroporation method, microinjection, Eugene transfection, nucleofection and the like, and any method described herein.

[0172] Examples of procedures include, for example, those described by Stadtfeld and Hochedlinger, *Nature Methods* 6(5):329-330 (2009); Yusa et al., *Nat. Methods* 6:363-369 (2009); Woltjen, et al., *Nature* 458, 766-770 (9 Apr. 2009)). Such methods include, but are not limited to, direct delivery of DNA such as by *ex vivo* transfection (*e.g.*, Wilson et al., *Science*, 244:1344-1346, 1989, Nabel & Baltimore, *Nature* 326:711-713, 1987), optionally with Eugene6 (Roche) or Lipofectamine (Invitrogen); by injection (*e.g.*, U.S. Pat. Nos. 5,994,624, 5,981,274, 5,945,100, 5,780,448, 5,736,524, 5,702,932, 5,656,610, 5,589,466 and 5,580,859, each incorporated herein by reference), including microinjection (*e.g.*, Harland and Weintraub, *J. Cell Biol.*, 101:1094-

1099, 1985; U.S. Pat. No. 5,789,215, incorporated herein by reference in its entirety); by electroporation (*e.g.*, U.S. Pat. No. 5,384,253, incorporated herein by reference in its entirety, Turk-Kaspa et al., *Mol. Cell Biol.*, 6:716-718, 1986; Potter et al., *Proc. Nat'l Acad. Sci. USA*, 81:7161-7165, 1984); by calcium phosphate precipitation (*e.g.*, Graham & Van Der Eb, *Virology*, 52:456-467, 1973; Chen and Okayama, *Mol. Cell Biol.*, 7(8):2745-2752, 1987; Rippe et al., *Mol. Cell Biol.*, 10:689-695, 1990); by use of DEAE-dextran followed by polyethylene glycol (*e.g.*, Gopal, *Mol. Cell Biol.*, 5:1188-1190, 1985); by direct sonic loading (*e.g.*, Fechheimer et al., *Proc. Nat'l Acad. Sci. USA*, 84:8463-8467, 1987); by liposome-mediated transfection (*e.g.*, Nicolau & Sene, *Biochim. Biophys. Acta*, 721:185-190, 1982, Fraley et al., *Proc. Nat'l Acad. Sci. USA*, 76:3348-3352, 1979; Nicolau et al., *Methods Enzymol.*, 149:157-176, 1987, Wong et al., *Gene*, 10:87-94, 1980, Kaneda et al., *Science*, 243:375-378, 1989, Kato et al., *Biol. Chem.*, 266:3361-3364, 1991), receptor-mediated transfection (*e.g.*, Wu and Wu, *Biochemistry*, 27:887-892, 1988; Wu and Wu, *J. Biol. Chem.*, 262:4429-4432, 1987); by endocytosis of the RNA complexed with a cationic vehicle (Warren et al., *Cell Stem Cell* 7: 618-30 (2010)); and any combination of such methods. Each of the foregoing references is incorporated herein by reference in its entirety.

[0173] Various techniques may be employed for introducing nucleic acid molecules of the disclosure into cells, depending on whether the nucleic acid molecules are introduced *in vitro* or *in vivo* in a host. Such techniques include transfection of nucleic acid molecule-calcium phosphate precipitates, transfection of nucleic acid molecules associated with DEAE, transfection or infection with the foregoing viruses including the nucleic acid molecule of interest, liposome-mediated transfection, and the like. Other examples include: N-TER™ Nanoparticle Transfection System by Sigma-Aldrich, FECTOFLY™ transfection reagents for insect cells by Polyplus Transfection, Polyethylenimine “Max” by Polysciences, Inc., Unique, Non-Viral Transfection Tool by Cosmo Bio Co., Ltd., LIPOFECTAMINE™ LTX Transfection Reagent by Invitrogen, SATISFECTION™ Transfection Reagent by Stratagene, LIPOFECTAMINE™ Transfection Reagent by Invitrogen, FUGENE® HD Transfection Reagent by Roche Applied Science, GMP compliant *IN VIVO*-JETPEI™ transfection reagent by Polyplus Transfection, and Insect GENEJUICE® Transfection Reagent by Novagen.

IV. Reprogramming Factor Compositions

[0174] Reprogramming with one or more of ASCL1, MYOCD, MEF2C, and TBX5 can be combined with other reprogramming strategies in some cases with improved results. In some embodiments, the target tissues or starting cells express or are induced to express the Oct4 polypeptide. Target tissues or starting cells can be treated or incubated, respectively, with a reprogramming composition that contains one or more WNT agonists, GSK3 inhibitors, TGF-beta inhibitors, epigenetic modifiers, adenylyl cyclase agonists, Oct-4 expression activators, and any combination thereof. The composition can contain at least two of such agents, or at least three of such agents, or at least four of such agents, or at least five of such agents, or at least six of such agents. For example, the composition can include SB431542 (an ALK4/5/7 inhibitor), CHIR99021 (a GSK3 inhibitor), parnate (an LSD1/KDM1 inhibitor, also called tranlylcypromine) and forskolin (an adenylyl cyclase activator).

[0175] In certain embodiments, the reprogramming is enhanced by the administration of one or more anti-inflammatory agents, *e.g.*, an anti-inflammatory steroid or a nonsteroidal anti-inflammatory drug (NSAID).

[0176] Anti-inflammatory steroids for use in the invention include corticosteroids, and in particular those with glucocorticoid activity, *e.g.*, dexamethasone and prednisone. Nonsteroidal anti-inflammatory drugs (NSAIDs) for use in the invention generally act by blocking the production of prostaglandins that cause inflammation and pain, cyclooxygenase-1 (COX-1) and/or cyclooxygenase-2 (COX-2). Traditional NSAIDs work by blocking both COX-1 and COX-2. The COX-2 selective inhibitors block only the COX-2 enzyme. In certain embodiment, the NSAID is a COX-2 selective inhibitor, *e.g.*, celecoxib (CELEBREX[®]), rofecoxib (Vioxx), and valdecoxib (B extra). In certain embodiments, the anti-inflammatory is an NSAID prostaglandin inhibitor, *e.g.*, Piroxicam.

[0177] To prepare the composition, the vectors and/or the cells are generated, and the vectors or cells are purified as necessary or desired. The vectors, cells, and/or other agents can be suspended in a pharmaceutically acceptable carrier. If the composition contains only compounds, without cells, the composition can be lyophilized. These compounds and cells can be adjusted to an appropriate concentration, and optionally combined with other agents. The absolute weight of a

given compound and/or other agent included in a unit dose can vary widely. The dose and the number of administrations can be optimized by those skilled in the art.

[0178] For example, about 10^2 - 10^{10} vector genomes (vg) may be administered. In some embodiments, the dose be at least about 10^2 vg, about 10^3 vg, about 10^4 vg, about 10^5 vg, about 10^6 vg, about 10^7 vg, about 10^8 vg, about 10^9 vg, about 10^{10} vg, or more vector genomes. In some embodiments, the dose be about 10^2 vg, about 10^3 vg, about 10^4 vg, about 10^5 vg, about 10^6 vg, about 10^7 vg, about 10^8 vg, about 10^9 vg, about 10^{10} vg, or more vector genomes.

[0179] Daily doses of the compounds can vary as well. Such daily doses can range, for example, from at least about 10^2 vg/day, about 10^3 vg/day, about 10^4 vg/day, about 10^5 vg/day, about 10^6 vg/day, about 10^7 vg/day, about 10^8 vg/day, about 10^9 vg/day, about 10^{10} vg/day, or more vector genomes per day.

[0180] In some embodiments, the method of the disclosure comprise administering a vector or vector system of the disclosure (*e.g.* an rAAV vector) by intracardiac injection, intramyocardiac injection, intracardiac catheterization, or systemic administration. In some embodiments, the subject (*e.g.*, a human) is treated by administering between about 1×10^8 and about 1×10^{15} GC of a vector (*e.g.*, an AAV vector or lentiviral vector) by intracardiac injection, intramyocardiac injection, intracardiac catheterization, or systemic administration. In some embodiments, the subject is treated by administering between about 1×10^8 and about 1×10^{15} GC, between about 1×10^8 and about 1×10^{15} GC, between about 1×10^9 and about 1×10^{14} GC, between about 1×10^{10} and about 1×10^{13} GC, between about 1×10^{11} and about 1×10^{12} GC, or between about 1×10^{12} and about 1×10^{13} GC of vector. In some embodiments, the subject is treated by administering between about 1×10^8 and about 1×10^{10} GC, between about 1×10^9 and about 1×10^{11} GC, between about 1×10^{10} and about 1×10^{12} GC, between about 1×10^{11} and about 1×10^{13} GC, between about 1×10^{12} and about 1×10^{14} GC, or between about 1×10^{13} and about 1×10^{15} GC of vector. In some embodiments, the subject is treated by administering at least 1×10^8 , at least about 1×10^9 , at least about 1×10^{10} , at least about 1×10^{11} , at least about 1×10^{12} , at least about 1×10^{13} , or at least about 1×10^{15} GC of vector. In some embodiments, the subject is treated by administering at most 1×10^8 , at most about 1×10^9 , at most about 1×10^{10} , at most about 1×10^{11} , at most about 1×10^{12} , at most about 1×10^{13} , or at most about 1×10^{15} GC of vector. In some embodiments, the subject (*e.g.*, a human) is treated by administering between about 1×10^8 and about 1×10^{15} GC/kg of a vector (*e.g.*, an AAV vector or lentiviral vector) by intracardiac injection or systemically. In some embodiments, the subject is treated by

administering between about 1×10^8 and about 1×10^{15} GC/kg, between about 1×10^8 and about 1×10^{15} GC/kg, between about 1×10^9 and about 1×10^{14} GC/kg, between about 1×10^{10} and about 1×10^{13} GC/kg, between about 1×10^{11} and about 1×10^{12} GC/kg, or between about 1×10^{12} and about 1×10^{13} GC/kg of vector. In some embodiments, the subject is treated by administering between about 1×10^8 and about 1×10^{10} GC/kg, between about 1×10^9 and about 1×10^{11} GC/kg, between about 1×10^{10} and about 1×10^{12} GC/kg, between about 1×10^{11} and about 1×10^{13} GC/kg, between about 1×10^{12} and about 1×10^{14} GC/kg, or between about 1×10^{13} and about 1×10^{15} GC/kg of vector. In some embodiments, the subject is treated by administering at least 1×10^8 , at least about 1×10^9 , at least about 1×10^{10} , at least about 1×10^{11} , at least about 1×10^{12} , at least about 1×10^{13} , or at least about 1×10^{15} GC/kg of vector. In some embodiments, the subject is treated by administering at most 1×10^8 , at most about 1×10^9 , at most about 1×10^{10} , at most about 1×10^{11} , at most about 1×10^{12} , at most about 1×10^{13} , or at most about 1×10^{15} GC/kg of vector. It will be appreciated that the amount of vectors and cells for use in treatment will vary not only with the particular carrier selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient. Ultimately, the attendant health care provider may determine proper dosage. A pharmaceutical composition may be formulated with the appropriate ratio of each compound in a single unit dosage form for administration with or without cells. Cells or vectors can be separately provided and either mixed with a liquid solution of the compound composition, or administered separately.

[0181] The compositions can also be formulated for sustained release (for example, using microencapsulation, see WO 94/07529, and/or U.S. Patent No. 4,962,091). The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known to the pharmaceutical arts. Such methods may include the step of mixing the therapeutic agent with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system.

[0182] One or more suitable unit dosage forms containing the compounds and/or the reprogrammed cells can be administered by a variety of routes including parenteral (including subcutaneous, intravenous, intramuscular and intraperitoneal), intracranial, intraspinal, oral, rectal, dermal, transdermal, intrathoracic, intrapulmonary and intranasal (respiratory) routes.

[0183] The vectors or cells of the invention may be prepared in many forms that include aqueous solutions, suspensions, tablets, hard or soft gelatin capsules, and liposomes and other slow-release formulations, such as shaped polymeric gels. Administration of cells often involves parenteral or local administration in an aqueous solution. Similarly, compositions containing cells and/or compounds can be administered in a device, scaffold, or as a sustained release formulation. Different types of formulating procedures are described in U.S. Patent No. 6,306,434 and in the references contained therein.

[0184] Liquid pharmaceutical compositions may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, dry powders for reconstitution with water or other suitable vehicles before use. Such liquid pharmaceutical compositions may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

[0185] Vectors and/or cells can be formulated for parenteral administration (*e.g.*, by injection, for example, bolus injection or continuous infusion) and may be presented in unit dosage form in ampoules, prefilled syringes, small volume infusion containers or multi-dose containers with an added preservative. The pharmaceutical compositions can take the form of suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Suitable carriers include saline solution, phosphate buffered saline, and other materials commonly used in the art.

[0186] The compositions can also contain other ingredients such as agents useful for treatment of cardiac diseases, conditions and injuries, such as, for example, an anticoagulant (*e.g.*, dalteparin (fragmin), danaparoid (orgaran), enoxaparin (lovenox), heparin, tinzaparin (innohep), and/or warfarin (coumadin)), an antiplatelet agent (*e.g.*, aspirin, ticlopidine, clopidogrel, or dipyridamole), an angiotensin-converting enzyme inhibitor (*e.g.*, Benazepril (Lotensin), Captopril (Capoten), Enalapril (Vasotec), Fosinopril (Monopril), Lisinopril (Prinivil, Zestril), Moexipril (Univasc), Perindopril (Aceon), Quinapril (Accupril), Ramipril (Altace), and/or Trandolapril (Mavik)), angiotensin II receptor blockers (*e.g.*, Candesartan (Atacand), Eprosartan (Teveten), Irbesartan (Avapro), Losartan (Cozaar), Telmisartan (Micardis), and/or Valsartan (Diovan)), a beta blocker (*e.g.*, Acebutolol (Sectral), Atenolol (Tenormin), Betaxolol (Kerlone), Bisoprolol/hydrochlorothiazide (Ziac), Bisoprolol (Zebeta), Carteolol (Cartrol), Metoprolol (Lopressor, Toprol XL), Nadolol (Corgard), Propranolol (Inderal), Sotalol (Betapace), and/or

Timolol (Blocadren)), Calcium Channel Blockers (*e.g.*, Amlodipine (Norvasc, Lotrel), Bepridil (Vascor), Diltiazem (Cardizem, Tiazac), Felodipine (Plendil), Nifedipine (Adalat, Procardia), Nimodipine (Nimotop), Nisoldipine (Sular), Verapamil (Calan, Isoptin, Verelan), diuretics (*e.g.*, Amiloride (Midamor), Bumetanide (Bumex), Chlorothiazide (Diuril), Chlorthalidone (Hygroton), Furosemide (Lasix), Hydro-chlorothiazide (Esidrix, Hydrodiuril), Indapamide (Lozol) and/or Spironolactone (Aldactone)), vasodilators (*e.g.*, Isosorbide dinitrate (Isordil), Nesiritide (Natrecor), Hydralazine (Apresoline), Nitrates and/or Minoxidil), statins, nicotinic acid, gemfibrozil, clofibrate, Digoxin, Digitoxin, Lanoxin, or any combination thereof.

[0187] Additional agents can also be included such as antibacterial agents, antimicrobial agents, anti-viral agents, biological response modifiers, growth factors; immune modulators, monoclonal antibodies and/or preservatives. The compositions of the invention may also be used in conjunction with other forms of therapy.

[0188] The viral vectors and non-viral vectors described herein can be administered to a subject to treat a disease or disorder. Such a composition may be in a single dose, in multiple doses, in a continuous or intermittent manner, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is in response to traumatic injury or for more sustained therapeutic purposes, and other factors known to skilled practitioners. The administration of the compounds and compositions of the invention may be essentially continuous over a preselected period of time or may be in a series of spaced doses. Both local and systemic administration is contemplated. In some embodiments, localized delivery of a viral or non-viral vector is achieved. In some embodiments, localized delivery of cells and/or vectors is used to generate a population of cells within the heart. In some embodiments, such a localized population operates as "pacemaker cells" for the heart.

[0189] Supplementary factors can be included in the compositions and/or in a cell culture media containing any of the cells, compositions, compounds or agents described herein. Examples of such supplementary factors include bone morphogenic protein (BMP)-1, bone morphogenic protein-2, bone morphogenic protein-3, bone morphogenic protein-4, bone morphogenic protein-5, bone morphogenic protein-6, bone morphogenic protein-7, bone morphogenic protein-8, bone morphogenic protein-9, bone morphogenic protein-10, bone morphogenic protein-11, bone morphogenic protein-12, bone morphogenic protein-13, bone morphogenic protein-14, bone morphogenic protein-15, brain derived neurotrophic factor, ciliary neurotrophic factor, cytokine-

induced neutrophil chemotactic factor 1, cytokine-induced neutrophil chemotactic factor 2 α , cytokine-induced neutrophil chemotactic factor 2 β , β endothelial cell growth factor, endothelin 1, epidermal growth factor, epithelial-derived neutrophil attractant, fibroblast growth factor (FGF) 4, fibroblast growth factor 5, fibroblast growth factor 6, fibroblast growth factor 7, fibroblast growth factor 8, fibroblast growth factor 8b, fibroblast growth factor 8c, fibroblast growth factor 9, fibroblast growth factor 10, fibroblast growth factor (acidic), fibroblast growth factor (basic), growth related protein, growth related protein α , growth related protein β , growth related protein γ , heparin binding epidermal growth factor, hepatocyte growth factor, insulin-like growth factor I, insulin-like growth factor II, insulin-like growth factor binding protein, keratinocyte growth factor, leukemia inhibitory factor, neurotrophin-3, neurotrophin-4, placenta growth factor, placenta growth factor 2, platelet-derived endothelial cell growth factor, platelet derived growth factor, platelet derived growth factor A chain, platelet derived growth factor AA, platelet derived growth factor AB, platelet derived growth factor B chain, platelet derived growth factor BB, pre-B cell growth stimulating factor, stem cell factor, transforming growth factor a, transforming growth factor β , transforming growth factor β 1, transforming growth factor β 1.2, transforming growth factor β 1.3, transforming growth factor β 3, latent transforming growth factor β 1, transforming growth factor β binding protein I, transforming growth factor β binding protein II, transforming growth factor β binding protein III, and vascular endothelial growth factor.

[0190] Exemplary cytokines can be included such as interleukin (IL)-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, interferon (IFN), IFN- γ , tumor necrosis factor (TNF), TNF1, TNF2, TNF- α , macrophage colony stimulating factor (M-CSF), granulocyte-monocyte colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), megakaryocyte colony stimulating factor (Meg-CSF)-thrombopoietin, stem cell factor, and erythropoietin. Chemokines can also be included such as IP-10 and Stromal Cell-Derived Factor 1 α .

[0191] Exemplary hormones contemplated for inclusion in the compositions and/or cell culture media described herein can include, but are not limited to, steroid hormones and peptide hormones, such as insulin, somatostatin, growth hormone, hydrocortisone, dexamethasone, 3,3',5-Triiodo-L-thyronine, and L-Thyroxine..

V. Reprogramming Methods

[0192] As described herein, target cells (*e.g.*, non-cardiomyocyte cells) can be reprogrammed to the cardiac lineage (*e.g.*, cardiomyocyte lineage) *in vitro* by incubation of the target cells with the compositions described herein or *in vivo* by administration of a viral or non-viral vector to target tissues or cells. In some embodiments, the target cells are fibroblast cells. In some embodiments, the target cells are cardiac fibroblast (CF) cells. Non-cardiomyocytes cells can be differentiated into cardiomyocytes cells *in vitro* or *in vivo* using any method available to one of skill in the art. For example, see methods described in Ieda et al. (2010) *Cell* 142:375-386; Christoforou et al. (2013) *PLoS ONE* 8:e63577; Addis et al. (2013) *J. Mol. Cell Cardiol.* 60:97-106; Jayawardena et al. (2012) *Circ. Res.* 110: 1465-1473; Nam Y et al., *PNAS USA.* 2013; 110:5588-5593; Wada R et al. *PNAS USA.* 2013; 110: 12667-12672; and Fu J et al., *Stem Cell Reports.* 2013; 1:235-247. Such methods can therefore be used to generate a population of cardiac progenitor cells or cardiomyocytes that can be transplanted into a subject or used for experimentation.

A. Cells

[0193] Cardiomyocytes or cardiac myocytes are the muscle cells that make up the cardiac muscle. Each myocardial cell contains myofibrils, which are long chains of sarcomeres, the contractile units of muscle cells. Cardiomyocytes show striations similar to those on skeletal muscle cells, but unlike multinucleated skeletal cells, they contain only one nucleus. Cardiomyocytes have a high mitochondrial density, which allows them to produce ATP quickly, making them highly resistant to fatigue. Mature cardiomyocytes can express one or more of the following cardiac markers: α -actinin, MLC2v, cMHC, NKX2-5, GATA4, cTNT, cTNI, MEF2c, MLC2a, or any combination thereof. In some embodiments, the mature cardiomyocytes express NKX2-5, MEF2c or a combination thereof. In some embodiments, cardiac progenitor cells express early stage cardiac progenitor markers such as GATA4, ISL1 or a combination thereof.

[0194] The non-cardiomyocytes that are induced to cardiomyocytes can be from any of a variety of sources. Cells can be from, *e.g.*, human or non-human mammals. Exemplary non-human mammals include, but are not limited to, mice, rats, cats, dogs, rabbits, guinea pigs, hamsters, sheep, pigs, horses, bovines, and non-human primates. In some embodiments, a cell is from an adult human or non-human mammal. In some embodiments, a cell is from a neonatal human, an adult human, or non-human mammal. In some embodiments, the species of cell and the species of

the protein to be expressed are the same. For example, if a mouse cell is used, a mouse ortholog is introduced into the cell. If a human cell is used, a human ortholog is introduced into the cell.

[0195] Mammalian non-cardiomyocytes (*e.g.*, human or murine) can be used. In some embodiments, the cardiomyocytes are mammalian cardiomyocytes, and in specific embodiments the non-cardiomyocytes are human cells. In some embodiments, the non-cardiomyocytes can be derived from stem cells (*e.g.*, pluripotent stem cells, induced pluripotent stem cells, reprogrammed cardiac cells or cardiac stem cells) or progenitor cells (*e.g.*, cardiac progenitor cells). Cardiomyocytes can be derived from cardiac or non-cardiac cells. Cardiomyocytes can be from or derived from any of a variety of tissue sources. For example, cardiac fibroblasts, foreskin fibroblast, dermal fibroblasts, lung fibroblasts, etc. The non-cardiomyocytes can be embryonic, fetal, or post-natal (*e.g.*, adult) cells. In preferred embodiments, the non-cardiomyocytes are adult cells.

[0196] The non-cardiomyocyte for use in the present invention can be any non-cardiomyocyte cell type known to one of skill in the art. Non-limiting examples of a non-cardiomyocyte include, for example, a somatic cell, a cardiac fibroblast, a non-cardiac fibroblast, a cardiac progenitor cell, and a stem cell. The non-cardiomyocyte can be cardiac cells from the epicardium, myocardium or endocardium of the heart. Non-cardiomyocyte cardiac cells include, for example, smooth muscle and endothelial cells. Other non-limiting examples of cardiac cells include epithelial cells, endothelial cells, fibroblasts, cardiac stem or progenitor cells, cardiac conducting cells and cardiac pacemaking cells that constitute the cardiac muscle, blood vessels and cardiac cell supporting structure. The non-cardiomyocyte can, for example, be selected from one or more of hepatocytes, fibroblasts, endothelial cells, B cells, T cells, dendritic cells, keratinocytes, adipose cells, epithelial cells, epidermal cells, chondrocytes, cumulus cells, neural cells, glial cells, astrocytes, cardiac cells, esophageal cells, skeletal muscle cells, skeletal muscle satellite melanocytes, hematopoietic cells, osteocytes, macrophages, monocytes, mononuclear cells or stem cells including embryonic stem cells, embryonic germ cells, adult brain stem cells, epidermal stem cells, skin stem cells, pancreatic stem cells, kidney stem cells, liver stem cells, breast stem cells, lung stem cells, muscle stem cells, heart stem cells, eye stem cells, bone stem cells, spleen stem cells, immune system stem cells, cord blood stem cells, bone marrow stem cells and peripheral blood stem cells.

[0197] Where the cells for reprogramming are a population of non-cardiomyocytes, the population of cells is composed of at least about 30% non-cardiomyocytes, at least about 35% non-

cardiomyocytes, at least about 40% non-cardiomyocytes, at least about 45% non-cardiomyocytes, at least about 50% non-cardiomyocytes, at least about 55% non-cardiomyocytes, at least about 60% non-cardiomyocytes, at least about 65% non-cardiomyocytes, at least about 70% non-cardiomyocytes, at least about 75% non-cardiomyocytes, at least about 80% non-cardiomyocytes, at least about 85% non-cardiomyocytes, at least about 90% non-cardiomyocytes, at least about 95% non-cardiomyocytes, at least about 98% non-cardiomyocytes, at least about 99% non-cardiomyocytes, or greater than 99% non-cardiomyocytes.

[0198] In some embodiments, the starting cells are adult human cardiac fibroblasts (AHCfs) or adult pig cardiac fibroblasts (APCFs). AHCfs or APCFs can be obtained, for example, by digestion of tissue fragments from the left ventricle of a donor subject. Digestion can be performed with various methods known in the art—for example by subjecting the cells to 10 µg/ml Liberase TH, 10 µg/ml Liberase TM, 1 unit/ml DNase I, and 0.01% Polaxomer for 1 h in 37°C. Various alternative enzymes and digestion procedures are known in the art. The compositions and methods of the disclosure relate to both *in vivo* and *in vitro* (*ex vivo*) applications. The cells to be reprogrammed are referred to as “target cells” or “starting cells.” Target cells can be contacted or incubated with the compositions described herein. Such target cells are also referred to as starting cells or collectively as a starting population of cells. A starting population of cells can be derived from various source, and can be heterogeneous or homogeneous. In certain embodiments, the cells to be treated as described herein are adult cells, including any accessible adult cell type(s). In other embodiments, the cells used according to the invention are adult stem cells, progenitor cells, or somatic cells. In still other embodiments, the cells treated with any of the compositions and/or methods described herein include any type of cell from a newborn, including, but not limited to, newborn cord blood, newborn stem cells, progenitor cells, and tissue-derived cells (*e.g.*, somatic cells). Accordingly, a starting population of cells that is reprogrammed by the compositions and/or methods described herein, can be any live somatic cell type.

[0199] As illustrated herein, fibroblasts can be reprogrammed to cross lineage boundaries and to be directly converted to another cell type – *e.g.*, a cardiac progenitor cell or a cardiomyocyte cell type. Various cell types from all three germ layers have been shown to be suitable for somatic cell reprogramming by genetic manipulation, including, but not limited to liver and stomach (Aoi et al., *Science* 321(5889):699-702 (2008); pancreatic β cells (Stadtfeld et al., *Cell Stem Cell* 2: 230-

40 (2008); mature B lymphocytes (Hanna et al., *Cell* 133: 250-264 (2008); human dermal fibroblasts (Takahashi et al., *Cell* 131, 861-72 (2007); Yu et al., *Science* 318(5854) (2007); Lowry et al., *Proc Natl Acad Sci USA* 105, 2883-2888 (2008); Aasen et al., *Nat Biotechnol* 26(11): 1276-84 (2008); meningiocytes (Qin et al., *J Biol Chem* 283(48):33730-5 (2008); neural stem cells (DiSteffano et al., *Stem Cells Devel.* 18(5): (2009); and neural progenitor cells (Eminli et al., *Stem Cells* 26(10): 2467-74 (2008). Any such cells can be reprogrammed and/or programmed by use of the compositions and methods described herein.

[0200] The cells can be autologous or allogeneic cells (relative to a subject to be treated or who may receive the cells). Cells can be present in the subject or isolated from the subject. Immunosuppressive drugs are commonly used prior to, during, or after cell therapy. Immunosuppressive drugs may also be used prior to, during, or after viral or non-viral vector. In some cases, use of an immunosuppressive drugs may improve treatment outcomes. In some cases, use of an immunosuppressive drugs may diminish side effects of treatment, such as, without limitation, acute graft-versus-host disease, chronic graft-versus-host disease, and post-transplant lymphoproliferative disease. The present disclosure contemplates use of immunosuppressive drugs with any of the methods of treating or preventing a disease or condition of the present disclosure, including, without limitation, methods of the present disclosure in which the lentiviral vector confers resistance to an immunosuppressive drug to transduced cells.

[0201] In some embodiments the non-cardiomyocytes are endogenous cells within the subject and the methods of generating induced cardiomyocytes are by *in vivo* induction. In other embodiments, the non-cardiomyocytes are exogenous and are modified *in vitro*.

[0202] The non-cardiomyocytes can be obtained from a living subject. The cells can be obtained from tissue taken from a living subject. The cells can be obtained from a recently deceased subject who is considered a suitable tissue donor. In some embodiments, the subject is screened for various genetic disorders, viral infections, etc. to determine whether the subject is a suitable source of cells. In general, a cell that is suitable for use in the present invention is non-transformed (*e.g.*, exhibits normal cell proliferation) and is otherwise normal (*e.g.*, exhibits normal karyotype).

[0203] Cells can be derived from tissue of a non-embryonic subject, a neonatal infant, a child or an adult. Cells can be derived from neonatal or post-natal tissue collected from a subject within the period from birth, including cesarean birth, to death. For example, the non-cardiomyocytes can be from a subject who is greater than about 10 minutes old, greater than about 1 hour old, greater

than about 1 day old, greater than about 1 month old, greater than about 2 months old, greater than about 6 months old, greater than about 1 year old, greater than about 2 years old, greater than about 5 years old, greater than about 10 years old, greater than about 15 years old, greater than about 18 years old, greater than about 25 years old, greater than about 35 years old, >45 years old, >55 years old, >65 years old, >80 years old, <80 years old, <70 years old, <60 years old, <50 years old, <40 years old, <30 years old, <20 years old or <10 years old.

[0204] Methods of isolating non-cardiomyocytes cells from tissues are known in the art, and any known method can be used. As a non-limiting example, adult cardiac cells can be obtained from human heart atrial biopsy specimens obtained from patients undergoing cardiac surgery. Cardiac tissue can be minced and digested with collagenase and cardiac stem/progenitor cells expanded in c-kit⁺ progenitor cell expansion media using the methods of Choi et al. (2013) Transplantation Proceedings 45:420-426. In addition, cardiac fibroblasts can be obtained using the methods of Ieda et al. (2009) Dev. Cell 16(2):233-244. Foreskin fibroblasts can be obtained from foreskin tissue of a male individual. The fibroblasts can be obtained by mincing the foreskin tissue, then dissociating the tissue to single cells. Foreskin cell clumps can be dissociated by any means known in the art including physical de-clumping or enzymatic digestion using, for example, trypsin.

B. In vitro Reprogramming Methods

[0205] The disclosure provides methods for generating cardiomyocytes and/or cardiomyocyte-like cells *in vitro*. Selected starting cells are treated for a time and under conditions sufficient to convert the starting cells across lineage and/or differentiation boundaries to form cardiac progenitor cells and/or cardiomyocytes. In some embodiments, expression of the gene(s) of interest in the starting cells is initiated for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days before treatment with the induced cardiomyocyte cells (iCM cells) described herein. Reprogramming efficiency of the cells can be improved by expression of the genes of interest for at least two days, or at least three days, or at least four days, or at least five days prior to administrations of iCM cells or recombinant viruses or non-viral vectors to the subject. In some embodiments, the starting cells are fibroblast cells. In some embodiments, the starting cells express one or more markers indicative of a differentiated phenotype.

[0206] The starting cells can be dispersed in a cell culture medium that contains the reprogramming composition at a density that permits cell expansion. For example, about 1 to 10¹²

cells can be contacted with a viral or non-viral vector in a selected cell culture medium, especially when the cells are maintained at a cell density of about 1 to about 10^8 cells per milliliter, or at a density of about 100 to about 10^7 cells per milliliter, or at a density of about 1000 to about 10^6 cells per milliliter. In some embodiments, the methods of the disclosure comprise contacting at least 10^3 cells, 10^4 cells, 10^5 cells, 10^6 cells, 10^7 cells, 10^8 cells, 10^9 cells, 10^{10} cells, 10^{11} cells, 10^{12} cells, 10^{13} cells, 10^{14} cells, 10^{15} cells, or any number of cells therebetween with viral or non-viral vector(s), thereby inducing expression of the one or more genes of interest.

[0207] The time for conversion of starting cells into cardiac progenitor and cardiomyocyte cells can vary. For example, the starting cells can be incubated after treatment with one or more genes of interest until cardiac or cardiomyocyte cell markers are expressed. Such cardiac or cardiomyocyte cell markers can include any of the following markers: α -GATA4, TNNT2, MYH6, RYR2, NKX2-5, MEF2c, ANP, Actinin, MLC2v, cMHC, ISL1, cTNT, cTNI, MLC2a and any combination thereof.

[0208] In some embodiments, the induced cardiomyocyte cells are negative for one or more neuronal cells markers. Such neuronal cell markers can include any of the following markers: DCX, TUBB3, MAP2, and ENO2.

[0209] Incubation can proceed in any of the compositions described herein, for example, until cardiac progenitor markers are expressed by the starting cells. Such cardiac progenitor markers include Gata4, Tnnt2, Myh6, Ryr2, or a combination thereof. The cardiac progenitor markers such as Gata4, Tnnt2, Myh6, Ryr2, or a combination thereof can be expressed by about 8 days, or by about 9 days, or by about 10 days, or by about 11 days, or by about 12 days, or by about 14 days, or by about 15 days, or by about 16 days, or by about 17 days, or by about 18 days, or by about 19 days, or by about 20 days after starting incubation of cells in the compositions described herein.

[0210] Further incubation of the cells can be performed until expression of late stage cardiac progenitor markers such as NKX2-5, MEF2C or a combination thereof occurs. The late stage cardiac progenitor marker such as NKX2-5 and/or MEF2C can be expressed by about 15 days, or by about 16 days, or by about 17 days, or by about 18 days, by about 19 days, or by about 20 days, or by about 21 days, or by about 22 days, or by about 23 days, or by about 24 days, or by about 25 days of incubation of cells using the compositions and methods described herein.

[0211] Reprogramming efficiency may be measured as a function of cardiomyocyte markers. Such pluripotency markers include, but are not limited to, the expression of cardiomyocyte marker

proteins and mRNA, cardiomyocyte morphology and electrophysiological phenotype. Non-limiting examples of cardiomyocyte markers include, a-sarcoglycan, atrial natriuretic peptide (ANP), bone morphogenetic protein 4 (BMP4), connexin 37, connexin 40, crypto, desmin, GATA4, GATA6, MEF2C, MYH6, myosin heavy chain, NKX2-5, TBX5, and Troponin T. In some aspects, reprogramming efficiency is increased by about 5%, 10%, 20%, 30%, 40%, 50%, 50%, 70%, 80%, 90%, 1-fold, 1.1-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 50-fold, 100-fold or more higher relative to a control. Non-limiting examples of appropriate controls include a sample that has not been exposed to reprogramming factors.

[0212] The expression of various markers specific to cardiomyocytes may be detected by conventional biochemical or immunochemical methods (*e.g.*, enzyme-linked immunosorbent assay, immunohistochemical assay, and the like). Alternatively, expression of a nucleic acid encoding a cardiomyocyte-specific marker can be assessed. Expression of cardiomyocyte-specific marker-encoding nucleic acids in a cell can be confirmed by reverse transcriptase polymerase chain reaction (RT-PCR) or hybridization analysis, molecular biological methods which have been commonly used in the past for amplifying, detecting and analyzing mRNA coding for any marker proteins. Nucleic acid sequences coding for markers specific to cardiomyocytes are known and are available through public databases such as GenBank. Thus, marker-specific sequences needed for use as primers or probes are easily determined.

[0213] Cardiomyocytes exhibit some cardiac-specific electrophysiological properties. One electrical characteristic is an action potential, which is a short-lasting event in which the difference of potential between the interior and the exterior of each cardiac cell rises and falls following a consistent trajectory. Another electrophysiological characteristic of cardiomyocytes is the cyclic variations in the cytosolic-free Ca^{2+} concentration, named as Ca^{2+} transients, which are employed in the regulation of the contraction and relaxation of cardiomyocytes. These characteristics can be detected and evaluated to assess whether a population of cells has been reprogrammed into cardiomyocytes.

[0214] In some embodiments, the starting cells can be incubated with the reprogramming medium under cell culture conditions for about 1 day to about 30 days, or about 2 days to about 27 days, or about 3 days to about 25 days, or about 4 days to about 23 days, or about 5 days to about 20 days, or about 6 days to about 20 days, or about 10 days to about 20 days.

[0215] Cells in the culture media can express a gene of interest particularly during reprogramming. For example, the cells in the culture medium can transiently express the ASCL1 polypeptide. Cells selected for reprogramming do not require expression of heterologous Klf, Sox2, or Myc, and may not be in contact with a Klf, Myc or Sox2 polypeptide. In some embodiments, the expression of other transcription factors such as Myc, Sox2, Klf4 may not be directly or indirectly induced by the culture media.

[0216] However, in other embodiments, the cell culture medium can induce expression of endogenous Klf4 polypeptides, Myc polypeptides, Sox2 polypeptides or a combination thereof. For example, expression of endogenous Klf4 polypeptides, Myc polypeptides, and/or Sox2 polypeptides can occur upon exposure to a composition described herein, even when no exogenous Klf4, Myc, and/or Sox2 nucleic acids have been introduced.

1. Culture Conditions

[0217] The cells of the present disclosure can be cultured under any conditions known to one of skill in the art. In some embodiments, the cells (*e.g.*, non-cardiomyocytes, cardiomyocytes, and combinations thereof) are cultured in conditions of 1-20% oxygen (O₂) and 5% carbon dioxide (CO₂). In some embodiments, the cells of the present disclosure are cultured under hypoxic conditions (*e.g.*, in the presence of less than 10% O₂). In some embodiments, the cells of the present disclosure are cultured at about 37°C. In some embodiments, the cells of the present disclosure can be cultured at about 37°C, 5% CO₂ and 10-20% O₂. In some embodiments, the cells are cultured in hypoxic conditions for a period of time. For example, the cells may be cultured under normoxic conditions (~20% O₂) for a period of time and then switched to hypoxic conditions, for example ~5% O₂.

[0218] The advantage of *in vitro* or *ex vivo* differentiating of non-cardiomyocytes to cardiomyocytes is the ability to easily identify cells suitable for implantation or for discrimination of cells that are damaged or have not differentiated. *In vitro* or *ex vivo* differentiation allows induced cardiomyocytes to be purified or isolated from non-cardiomyocytes that have not differentiated.

[0219] After incubation of the starting cells in a reprogramming medium, the cells can then incubated in another media, for example, a maintenance media, an expansion media, or a cardiac induction media that can induce further maturation of the cells.

[0220] The base media employed to which the reprogramming agents or induction agents are added can be a convenient cell culture medium. The term “cell culture medium” (also referred to herein as a “culture medium” or “medium”) as referred to herein is a medium for culturing cells containing nutrients that maintain cell viability and support proliferation. The cell culture medium can contain any of the following in an appropriate combination: salt(s), buffer(s), amino acids, glucose or other sugar(s), antibiotics, serum or serum replacement, and other components such as peptide growth factors, etc. Cell culture media ordinarily used for particular cell types are available to those skilled in the art.

[0221] Examples of cell culture media that can be employed include MTESR-1[®] medium (StemCell Technologies, Inc., Vancouver, CA), or ESSENTIAL 8[®] medium (Life Technologies, Inc.) on a Matrigel substrate (BD Biosciences, NJ) or on a CORNING[®] Synthemax surface, or in Johansson and Wiles chemically defined media (CDM) supplemented with insulin, transferrin, lipids and polyvinyl alcohol (PVA) as substitute for Bovine Serum Albumin (BSA). Examples of commercially available media also include, but are not limited to, Dulbecco's Modified Eagle's Medium (DMEM), Minimal Essential Medium (MEM), Basal Medium Eagle (BME), RPMI 1640, Ham's F-10, Ham's F-12, α -Minimal Essential Medium (α MEM), Glasgow's Minimal Essential Medium (G-MEM), Iscove's Modified Dulbecco's Medium, or a general purpose media modified for use with pluripotent cells, such as X-VIVO (Lonza) or a hematopoietic base media. In some embodiments, mixtures of two or more cell culture media are used, such as 4 parts Dulbecco's Modified Eagle's Medium (DMEM) to 1 part GIBCO[®] Media 199. The media maybe supplemental with fetal bovine serum (FBS), amino acids, and/or antibiotics. In some embodiments, the media is mixed with GIBCO[®] RPMI 1640 (RPMI) + GIBCO[®] B-27 Supplement (B27). Growth of cells can be enhanced by additional of rhFGF, rhFGF-10, and rhVEGF (FFV).

[0222] The compositions and/or culture media can contain any of the agent(s) or compound(s) described herein in an amount sufficient to induce a cell to express cardiac or cardiomyocyte cell markers. Such cardiac or cardiomyocyte cell markers can include any of the following markers: α -actinin, MLC2v, cMHC, NKX2-5, MEF2c, GATA4, ISL1, cTNT, cTNI, MLC2a and any combination thereof. For example, the culture media can include a TGF- β inhibitor such as SB431542 (*e.g.*, at about 0.1-10 μ M), a WNT signaling activator such as CHIR99021 (*e.g.*, at

about 3-20 μ M), an LSD1/KDM1 inhibitor such as parnate (*e.g.*, at about 0.1-10 μ M), and an adenylyl cyclase activator such as forskolin (*e.g.*, at about 3-20 μ M).

[0223] Incubation can proceed in any of the compositions described herein, for example, until early stage cardiac progenitor markers are expressed by the starting cells. Such early stage cardiac progenitor markers include GATA4, ISL1 or a combination thereof. The early stage cardiac progenitor markers such as GATA4 and/or ISL1 can be expressed by about 6 days, or by about 8 days, or by about 9 days, or by about 10 days, or by about 11 days, or by about 12 days of incubation of cells using the compositions and methods described herein.

[0224] The culture media can contain any of the agent(s) or compound(s) described herein in an amount sufficient to reprogram at least 0.001%, or about 0.005%, or about 0.01%, or about 0.02%, or about 0.03% of the cells in a population of cells into a cardiac cell type.

C. In vivo Reprogramming Methods

[0225] In some embodiments, at least one reprogramming factor has been administered to the subject, for example, ASCL1, MYOCD, MEF2C, TBX5, BAF60C, ESRRG, GATA4, GATA6, HAND2, IRX4, ISLL, MEF2C, MESP1, MESP2, NKX2.5, SRF, TBX20, and ZFPM2, or any combination thereof. For example, the subject may be administered a viral or non-viral vector comprising a polynucleotide encoding at least one reprogramming factor. In preferred embodiments, a combination of two or more, and more preferably three or more, of the reprogramming factors are administered to the subject. In some embodiments, one or more of the above listed reprogramming factors is expressly excluded. In other embodiments, the reprogramming factors are selected from the group of ASCL1, MYOCD, MEF2C, TBX5, or any combination thereof. In other embodiments, the reprogramming factors are selected from the group of GATA4, MEF2C, TBX5, MYOCD, or any combination thereof. In specific embodiments, the reprogramming factors are Ascl1 and Myocd (MyA). In specific embodiments, the reprogramming factors are ASCL1, MYOCD, MEF2C and TBX5 (MyAMT). In other some embodiments, the reprogramming factors are GATA4, MEF2C, and TBX5 (GMT). In other specific embodiments, the reprogramming factors are Myocardin, MEF2C, AND TBX5 (*i.e.*, MyMT). In other specific embodiments, the reprogramming factors are GATA4, MEF2C, TBX5, and Myocardin (*i.e.*, 4F). In other embodiments, the reprogramming factors are GATA4, MEF2C, AND TBX5, ESRRG, Myocardin, ZFPM2, and MESP1 (*i.e.*, 7F).

VI. Compositions: Cells and Vectors

[0226] The present disclosure provides viral and non-viral vectors. The present disclosure also provides isolated induced cardiomyocytes generated according to the methods of the invention. The induced cardiomyocytes may express at least one cardiac gene at a level higher or a level lower than that found in a naturally occurring cardiomyocyte. The induced cardiomyocyte may be an isolated iCM or a iCM produced *in vivo* by administration of a composition to the subject.

[0227] In some embodiments, the cardiac gene expressed at a higher level than that found in the naturally occurring cardiomyocyte is selected from the group consisting of *TNNT2*, *ACTN2*, *ATP2A2*, *MYH6*, *RYR2*, *MYH7*, and *ACTCL*. In some embodiments, the cardiac gene expressed at a lower level than that found in the naturally occurring cardiomyocyte is selected from the group consisting of *MYBPC3*, *PIN*, *MB*, *LMOD2*, *MYL2*, *MYL3*, *COX6A2*, *ATP5AL*, *TTN*, *TNNI3*, *PDK4*, *MYCZ2*, *CACNALC*, *SCN5A*, *MYOCD*, and *NPPA*.

[0228] In another aspect, a substantially homogenous population of induced cardiomyocytes generated according to the methods of the invention are provided. In some embodiments, the induced cardiomyocytes of the substantially homogenous population express at least one cardiac gene at a higher level or a lower level than found in a naturally occurring cardiomyocyte.

[0229] In some embodiments, the composition comprises a population of isolated induced cardiomyocytes described herein and a carrier, optionally a pharmaceutically acceptable excipient. In some embodiments, the compositions further comprise a stabilizer and/or a preservative.

[0230] In some embodiments, the composition comprises a viral or non-viral vector described herein and a carrier, optionally a pharmaceutically acceptable excipient. In some embodiments, the compositions further comprise a stabilizer and/or a preservative.

[0231] The composition may comprise a pharmaceutically acceptable excipient, a pharmaceutically acceptable salt, diluents, carriers, vehicles and such other inactive agents well known to the skilled artisan. Vehicles and excipients commonly employed in pharmaceutical preparations include, for example, talc, gum Arabic, lactose, starch, magnesium stearate, cocoa butter, aqueous or non-aqueous solvents, oils, paraffin derivatives, glycols, etc. Solutions can be prepared using water or physiologically compatible organic solvents such as ethanol, 1,2-propylene glycol, polyglycols, dimethylsulfoxide, fatty alcohols, triglycerides, partial esters of glycerine and the like.

[0232] Parenteral compositions may be prepared using conventional techniques that may include sterile isotonic saline, water, 1,3-butanediol, ethanol, 1,2-propylene glycol, polyglycols mixed with water, Ringer' s solution, etc. In one aspect, a coloring agent is added to facilitate locating and properly placing the composition to the intended treatment site.

[0233] The composition can include agents that are administered using an implantable device. Suitable implantable devices contemplated by this disclosure include intravascular stents (*e.g.*, self-expandable stents, balloon-expandable stents, and stent-grafts), scaffolds, grafts, and the like. Such implantable devices can be coated on at least one surface, or impregnated, with a composition capable of generating an induced cardiomyocyte. The composition can also include agents that are contained within a reservoir in the implantable device. Where the agents are contained within a reservoir in the implantable device, the reservoir is structured so as to allow the agents to elute from the device. The agents of the composition administered from the implantable device may comprise a WNT inhibitor, the TGF- β inhibitor or both.

[0234] Pharmaceutical compositions can be provided in any form amenable to administration. Compositions may include a preservative and/or a stabilizer. Non-limiting examples of preservatives include methyl-, ethyl-, propyl- parabens, sodium benzoate, benzoic acid, sorbic acid, potassium sorbate, propionic acid, benzalkonium chloride, benzyl alcohol, thimerosal, phenylmercurate salts, chlorhexidine, phenol, 3-cresol, quaternary ammonium compounds (QACs), chlorbutanol, 2-ethoxyethanol, and imidurea.

[0235] To control tonicity, an aqueous pharmaceutical composition can comprise a physiological salt, such as a sodium salt. Sodium chloride (NaCl) is preferred, which may be present at between 1 and 20 mg/ml. Other salts that may be present include potassium chloride, potassium dihydrogen phosphate, disodium phosphate dehydrate, magnesium chloride and calcium chloride.

[0236] Compositions may include one or more buffers. Typical buffers include: a phosphate buffer; a Tris buffer; a borate buffer; a succinate buffer; a histidine buffer; or a citrate buffer. Buffers will typically be included at a concentration in the 5-20 mM range. The pH of a composition will generally be between 5 and 8, and more typically between 6 and 8 *e.g.* between 6.5 and 7.5, or between 7.0 and 7.8.

[0237] The composition is preferably sterile. The composition is preferably gluten free. The composition is preferably non-pyrogenic.

[0238] The pharmaceutical composition can be administered by any appropriate route, which will be apparent to the skilled person depending on the disease or condition to be treated. Typical routes of administration include oral, intravenous, intra-arterial, intramuscular, subcutaneous, intracranial, intranasal or intraperitoneal.

[0239] In some embodiments, a composition comprising cells may include a cryoprotectant agent. Non-limiting examples of cryoprotectant agents include a glycol (*e.g.*, ethylene glycol, propylene glycol, and glycerol), dimethyl sulfoxide (DMSO), formamide, sucrose, trehalose, dextrose, and any combinations thereof.

[0240] In some embodiments, one or more agents used in the methods of the invention is provided in a controlled release formulation. The term “controlled release formulation” includes sustained release and time-release formulations. Controlled release formulations are well-known in the art. These include excipients that allow for sustained, periodic, pulse, or delayed release of the composition. Controlled release formulations include, without limitation, embedding of the composition (a WNT inhibitor and/or TGF- β inhibitor) into a matrix; enteric coatings; micro-encapsulation; gels and hydrogels; implants; and any other formulation that allows for controlled release of a composition.

[0241] In some embodiments, a reprogrammed population of cells (at various stages of reprogramming) can be frozen at liquid nitrogen temperatures, stored for periods of time, and then thawed for use at a later date. If frozen, a population of reprogrammed cells can be stored in any suitable cryopreservation media, *e.g.*, 10% DMSO, 50% FCS, within 40% RPMI 1640 medium. Once thawed, the cells can be expanded by culturing the cells in an appropriate medium that can contain selected growth factors, vitamins, feeder cells, and other components selected by a person of skill in the art. Viral and non-viral vectors can also be frozen at liquid nitrogen temperatures, or often at higher temperatures, stored for periods of time, and then thawed for use at a later date.

[0242] In some embodiments, at least 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% of iCM cells are α -actinin positive. In some embodiments, at least 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25% of iCM cells are cTnT positive. In some embodiments, at least 3%, 4%, 5%, 6%, 7%,

8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25% of iCM cells are α -actinin and cTnT double-positive.

[0243] The population of reprogrammed cells generated by the methods described herein can include low percentages of non-cardiac cells (*e.g.*, fibroblasts). For example, a population of reprogrammed cells for use in compositions and for administration to subjects can have less than about 90% non-cardiac cells, less than about 85% non-cardiac cells, less than about 80% non-cardiac cells, less than about 75% non-cardiac cells, less than about 70% non-cardiac cells, less than about 65% non-cardiac cells, less than about 60% non-cardiac cells, less than about 55% non-cardiac cells, less than about 50% non-cardiac cells, less than about 45% non-cardiac cells, less than about 40% non-cardiac cells, less than about 35% non-cardiac cells, less than about 30% non-cardiac cells, less than about 25% non-cardiac cells, less than about 20% non-cardiac cells, less than about 15% non-cardiac cells, less than about 12% non-cardiac cells, less than about 10% non-cardiac cells, less than about 8% non-cardiac cells, less than about 6% non-cardiac cells, less than about 5% non-cardiac cells, less than about 4% non-cardiac cells, less than about 3% non-cardiac cells, less than about 2% non-cardiac cells, or less than about 1% non-cardiac cells of the total cells in the cell population.

[0244] Reprogrammed cells can be included in the compositions in varying amounts depending upon the disease or injury to be treated. For example, the compositions can be prepared in liquid form for local or systemic administration containing about 10^3 to about 10^{12} reprogrammed cells, or about 10^4 to about 10^{10} reprogrammed cells, or about 10^5 to about 10^8 reprogrammed cells.

[0245] One or more of the following types of compounds can also be present in the composition with the cells: a WNT agonist, a GSK3 inhibitor, a TGF-beta signaling inhibitor, an epigenetic modifier, LSD1 inhibitor, an adenylyl cyclase agonist, or any combination thereof. Any of the compounds described herein can be administered with the cells.

[0246] The disclosure also provides a kit of parts comprising the above-mentioned agents, compositions or formulations.

VII. Methods of Treatment

[0247] The reprogrammed cells and compositions that are described herein can also be employed in a method of treating a subject with a cardiac disease or condition. “Treating” or “treatment of a condition or subject in need thereof” refers to (1) taking steps to obtain beneficial

or desired results, including clinical results such as the reduction of symptoms; (2) preventing the disease, for example, causing the clinical symptoms of the disease not to develop in a patient that may be predisposed to the disease, but does not yet experience or display symptoms of the disease; (3) inhibiting the disease, for example, arresting or reducing the development of the disease or its clinical symptoms; (4) relieving the disease, for example, causing regression of the disease or its clinical symptoms; or (5) delaying the disease. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, generating an induced cardiomyocyte and/or promoting myocardial regeneration.

[0248] Subjects in need of treatment using the compositions, cells and methods of the present disclosure include, but are not limited to, individuals having a congenital heart defect, individuals suffering from a degenerative muscle disease, individuals suffering from a condition that results in ischemic heart tissue (*e.g.*, individuals with coronary artery disease), and the like. In some examples, a method is useful to treat a degenerative muscle disease or condition (*e.g.*, familial cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, or coronary artery disease with resultant ischemic cardiomyopathy). In some examples, a subject method is useful to treat individuals having a cardiac or cardiovascular disease or disorder, for example, cardiovascular disease, aneurysm, angina, arrhythmia, atherosclerosis, cerebrovascular accident (stroke), cerebrovascular disease, congenital heart disease, congestive heart failure, myocarditis, valve disease coronary, artery disease dilated, diastolic dysfunction, endocarditis, high blood pressure (hypertension), cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, coronary artery disease with resultant ischemic cardiomyopathy, mitral valve prolapse, myocardial infarction (heart attack), or venous thromboembolism.

[0249] Subjects who are suitable for treatment using the compositions, cells and methods of the present disclosure include individuals (*e.g.*, mammalian subjects, such as humans, non-human primates, domestic mammals, experimental non-human mammalian subjects such as mice, rats, etc.) having a cardiac condition including but limited to a condition that results in ischemic heart tissue (*e.g.*, individuals with coronary artery disease) and the like.

[0250] In some examples, an individual suitable for treatment suffers from a cardiac or cardiovascular disease or condition, *e.g.*, cardiovascular disease, aneurysm, angina, arrhythmia, atherosclerosis, cerebrovascular accident (stroke), cerebrovascular disease, congenital heart disease, congestive heart failure, myocarditis, valve disease coronary, artery disease dilated,

diastolic dysfunction, endocarditis, high blood pressure (hypertension), cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, coronary artery disease with resultant ischemic cardiomyopathy, mitral valve prolapse, myocardial infarction (heart attack), or venous thromboembolism. In some examples, individuals suitable for treatment with a subject method include individuals who have a degenerative muscle disease, *e.g.*, familial cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, or coronary artery disease with resultant ischemic cardiomyopathy.

[0251] Subjects in need of treatment using the compositions, cells and methods of the present disclosure include, but are not limited to, individuals having a congenital heart defect, individuals suffering from a degenerative muscle disease, individuals suffering from a condition that results in ischemic heart tissue (*e.g.*, individuals with coronary artery disease), and the like. In some examples, a method is useful to treat a degenerative muscle disease or condition (*e.g.*, familial cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, or coronary artery disease with resultant ischemic cardiomyopathy). In some examples, a subject method is useful to treat individuals having a cardiac or cardiovascular disease or disorder, for example, cardiovascular disease, aneurysm, angina, arrhythmia, atherosclerosis, cerebrovascular accident (stroke), cerebrovascular disease, congenital heart disease, congestive heart failure, myocarditis, valve disease coronary, artery disease dilated, diastolic dysfunction, endocarditis, high blood pressure (hypertension), cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, coronary artery disease with resultant ischemic cardiomyopathy, mitral valve prolapse, myocardial infarction (heart attack), or venous thromboembolism.

[0252] Subjects who are suitable for treatment using the compositions, cells and methods of the present disclosure include individuals (*e.g.*, mammalian subjects, such as humans, non-human primates, experimental non-human mammalian subjects such as mice, rats, etc.) having a cardiac condition including, but limited to, a condition that results in ischemic heart tissue (*e.g.*, individuals with coronary artery disease) and the like. In some examples, an individual suitable for treatment suffers from a cardiac or cardiovascular disease or condition, *e.g.*, cardiovascular disease, aneurysm, angina, arrhythmia, atherosclerosis, cerebrovascular accident (stroke), cerebrovascular disease, congenital heart disease, congestive heart failure, myocarditis, valve disease coronary, artery disease dilated, diastolic dysfunction, endocarditis, high blood pressure (hypertension), cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, coronary artery

disease with resultant ischemic cardiomyopathy, mitral valve prolapse, myocardial infarction (heart attack), or venous thromboembolism. In some examples, individuals suitable for treatment with a subject method include individuals who have a degenerative muscle disease, *e.g.*, familial cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, or coronary artery disease with resultant ischemic cardiomyopathy.

[0253] Examples of diseases and conditions that can be treated using the reprogrammed cells and/or compositions (containing any of the compounds described herein with or without reprogrammed cells) include any cardiac pathology or cardiac dysfunction. Diseases and conditions that can be treated include those that occur as a consequence of genetic defect, physical injury, environmental insult or conditioning, bad health, obesity and other disease risk.

[0254] Ischemic cardiomyopathy is a chronic disorder caused by coronary artery disease (a disease in which there is atherosclerotic narrowing or occlusion of the coronary arteries on the surface of the heart). Coronary artery disease often leads to episodes of cardiac ischemia, in which the heart muscle is not supplied with enough oxygen-rich blood.

[0255] Non-ischemic cardiomyopathy is generally classified into three groups based primarily on clinical and pathological characteristics: dilated cardiomyopathy, hypertrophic cardiomyopathy and restrictive and infiltrative cardiomyopathy.

[0256] In another embodiment, the cardiac pathology is a genetic disease such as Duchenne muscular dystrophy and Emery Dreiffuss dilated cardiomyopathy.

[0257] For example, the cardiac pathology can be selected from the group consisting of congestive heart failure, myocardial infarction, cardiac ischemia, myocarditis and arrhythmia. In some embodiments, the subject is diabetic. In some embodiments, the subject is non-diabetic. In some embodiments, the subject suffers from diabetic cardiomyopathy.

[0258] Reprogrammed cells generated as described herein can be employed for tissue reconstitution or regeneration in a human patient or other subjects in need of such treatment. The cells are administered in a manner that permits them to graft or migrate to a diseased or injured tissue site and to reconstitute or regenerate the functionally deficient area. Devices are available that can be adapted for administering cells, for example, to cardiac tissues.

[0259] For therapy, recombinant viruses, non-viral vectors, cardiac progenitor cells, cardiomyocytes and/or pharmaceutical compositions can be administered locally or systemically. A reprogrammed population of cells can be introduced by injection, catheter, implantable device,

or the like. A population of recombinant viruses or reprogrammed cells can be administered in any physiologically acceptable excipient or carrier that does not adversely affect the cells. For example, the recombinant viruses, non-viral vectors, cardiac progenitor cells, cardiomyocytes and/or pharmaceutical compositions can be administered intravenously or through an intracardiac route (*e.g.*, epicardially or intramyocardially). Methods of administering the recombinant viruses, non-viral vectors, cardiomyocytes and pharmaceutical compositions (*e.g.*, compositions comprising vectors) of the disclosure to subjects, particularly human subjects include injection, implantation, or infusion of the pharmaceutical compositions (*e.g.*, compositions comprising viral vectors) or cells into target sites in the subjects. Injection may include direct muscle injection and infusion may include intravascular infusion. The vectors, pharmaceutical compositions, or cells can be inserted into a delivery device which facilitates introduction by injection or implantation of the pharmaceutical compositions or cells into the subjects. Such delivery devices include tubes, *e.g.*, catheters, for injecting cells and fluids into the body of a recipient subject. The tubes can additionally include a needle, *e.g.*, a syringe, through which the cells of the invention can be introduced into the subject at a desired location. In some embodiments, the pharmaceutical compositions or cells are delivered by microneedle patch as described in, for example, Tang et al. Cardiac cell-integrated microneedle patch for treating myocardial infarction. *Science Advances* 28 Nov 2018: Vol. 4, no. 11, eaat9365.

[0260] The recombinant viruses, non-viral vectors, cardiac progenitor cells and cardiomyocytes can be inserted into such a delivery device, *e.g.*, a syringe, in different forms. A population of recombinant viruses, non-viral vectors, reprogrammed cells can be supplied in the form of a pharmaceutical composition. Such a composition can include an isotonic excipient prepared under sufficiently sterile conditions for human administration. For general principles in medicinal formulation, the reader is referred to *Cell Therapy: Stem Cell Transplantation, Gene Therapy, and Cellular Immunotherapy*, by G. Morstyn & W. Sheridan eds, Cambridge University Press, 1996; and *Hematopoietic Stem Cell Therapy*, E. D. Ball, J. Lister & P. Law, Churchill Livingstone, 2000. The choice of the cellular excipient and any accompanying constituents of the composition that includes a population of reprogrammed cells can be adapted to optimize administration by the route and/or device employed.

[0261] As used herein, the term “solution” includes a carrier or diluent in which the cardiomyocytes and cardiac cells of the invention remain viable or the viral vectors remain

biologically active. Carriers and diluents that can be used include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is preferably sterile and fluid to the extent that easy syringability exists. For transplantation, cardiomyocytes and/or cardiac cells are drawn up into a syringe and administered to anesthetized transplantation recipients. For direct injection, a needle, syringe, or catheter is inserted into the heart surgically, ideally in a minimally invasive setting. Multiple injections may be made using this procedure.

[0262] The cardiac progenitor cells, cardiac cells, and/or cardiomyocytes can also be embedded in a support matrix. A composition that includes a population of reprogrammed cells can also include or be accompanied by one or more other ingredients that facilitate engraftment or functional mobilization of the reprogrammed cells. Suitable ingredients include matrix proteins that support or promote adhesion of the reprogrammed cells, or complementary cell types, such as cardiac pacemaker cells, or cardiac cells at different stages of maturation. In another embodiment, the composition may include physiologically acceptable matrix scaffolds. Such physiologically acceptable matrix scaffolds can be resorbable and/or biodegradable.

A. *Methods of Treatment Using Recombinant viruses*

[0263] In some aspects, a viral particle of the present disclosure can be used to treat a subject in need thereof. In some embodiments, the recombinant viruses can be administered to the subject in need thereof, where administration into the subject of the recombinant viruses, treats a cardiovascular disease in the subject.

[0264] Recombinant viruses may be administered locally or systemically. Recombinant viruses may be engineered to target specific cell types by selecting an appropriate capsid protein or by pseudotyping the virus with a protein from another virus type. To determine the suitability of various therapeutic administration regimens and dosages of viral particle compositions, the recombinant viruses can first be tested in a suitable animal model. At one level, recombinant viruses are assessed for their ability to infect target cells *in vivo*. Recombinant viruses can also be assessed to ascertain whether they migrate to target tissues, whether they induce an immune response in the host, or to determine an appropriate number, or dosage, of recombinant viruses to be administered. It may be desirable or undesirable for the recombinant viruses to generate an immune response, depending on the disease to be treated. Generally, if repeated administration of

a viral particle is required, it will be advantageous if the viral particle is not immunogenic. For testing purposes, viral particle compositions can be administered to immunodeficient animals (such as nude mice, or animals rendered immunodeficient chemically or by irradiation). Target tissues or cells can be harvested after a period of infection and assessed to determine if the tissues or cells have been infected and if the desired phenotype (*e.g.* induced cardiomyocyte) has been induced in the target tissue or cells.

[0265] Recombinant viruses can be administered by various routes, including without limitation direct injection into the heart or cardiac catheterization. Alternatively, the recombinant viruses can be administered systemically such as by intravenous infusion. When direct injection is used, it may be performed either by open-heart surgery or by minimally invasive surgery. In some cases, the recombinant viruses are delivered to the pericardial space by injection or infusion. Injected or infused recombinant viruses can be traced by a variety of methods. For example, recombinant viruses labeled with or expressing a detectable label (such as green fluorescent protein, or beta-galactosidase) can readily be detected. The recombinant viruses may be engineered to cause the target cell to express a marker protein, such as a surface-expressed protein or a fluorescent protein. Alternatively, the infection of target cells with recombinant viruses can be detected by their expression of a cell marker that is not expressed by the animal employed for testing (for example, a human-specific antigen when injecting cells into an experimental animal). The presence and phenotype of the target cells can be assessed by fluorescence microscopy (*e.g.*, for green fluorescent protein, or beta-galactosidase), by immunohistochemistry (*e.g.*, using an antibody against a human antigen), by ELISA (using an antibody against a human antigen), or by RT-PCR analysis using primers and hybridization conditions that cause amplification to be specific for RNA indicative of a cardiac phenotype.

[0266] In some embodiments, the disclosure provides methods of treating myocardial infarction in subject (*e.g.*, a human) in need thereof. In some embodiments, the methods comprise administering an AAV vector encoding My Δ 3 and ASCL1. In some embodiments, the methods comprise administering to the subject an AAV vector comprising a polynucleotide encoding My Δ 3-2A-ASCL1. In some embodiments, the methods comprise administering to the subject an AAV vector comprising a polynucleotide encoding ASCL1-2A-My Δ 3. In some embodiments, the disclosure provides methods of treating myocardial infarction in subject (*e.g.*, a human) in need thereof, comprising administering to the subject an AAV vector comprising a polynucleotide

encoding My Δ 3-2A-ASCL1 (AAV:My Δ 3A), wherein the method results in partial, substantial, or complete reversal of heart failure and/or partial, substantial, or complete halt to progression of heart failure and/or partial, substantial, or complete prevention of heart failure in the subject. The AAV vector may be an AAV5 vector. The AAV vector may be a variant of AAV5. The AAV vector may be another AAV vector. The AAV vector may be capable of infection or transduction (*e.g.*, preferential infection or transduction) of non-cardiomyocyte cells (*e.g.*, heart fibroblast cells). In some embodiments, the methods of treatment of the disclosure result in increased left ventricular ejection fraction (LVEF) and/or maintained LVEF in the subject. In some embodiments, the subject suffers from or is at risk for myocardial infarction (MI). In some embodiments, the subject suffers from or is at risk for acute myocardial infarction (AMI). In some embodiments, the subject suffers from or is at risk for acute myocardial infarction (CMI). In some embodiments, the subject suffers from or is at risk for chronic heart failure (*e.g.*, due to MI or AMI or CMI). In some embodiments, the subject suffers from or is at risk for congestive heart failure (*e.g.*, due to MI or AMI or CMI). In various embodiments, the administering step comprises intracardiac or intramyocardial injection, intracoronary catheterization, or systemic administration (*e.g.*, intravenous administration). In some embodiments, AAV delivery of My Δ 3A provides functional benefit *in vivo* in either or both of acute myocardial infarction (AMI) and chronic myocardial infarction (CMI). In some embodiments, heart failure due to AMI is partially, substantially or completely reversed. In some embodiments, progression of heart failure due to CMI is partially, substantially or completely halted.

[0267] In some embodiments, the administration step comprises administering the AAV vector within about one hour, within about two hours, within about three hours, within about four hours, within about five hours, within about 12 hours, within about 18 hours, or within about 24 hours of a heart attack. In some embodiments, the administration step comprises administering the AAV vector within about one day, within about two days, within about three days, within about four days, within about five days, within about six days, within about seven days, or within about 24 hours of a heart attack. In some embodiments, the administration step comprises administering the AAV vector within about one week, within about two weeks, within about three weeks, or within about four weeks a heart attack.

[0268] In an aspect, the disclosure provides a method of treating a heart condition in a subject suffering from or at risk for a heart condition, comprising administering a vector or vector system of the disclosure to the subject. The vector may be a monocistronic, bicistronic, or polycistronic vector. In an embodiment, the disclosure provides a method of promoting, increasing, improving, or sustaining improvement in heart function. In some embodiments, the method increases the number of myocytes in the heart by at least about 5%, at least about 10%, at least about 15%, or at least about 20%. In some embodiments, the heart condition is a myocardial infarction. In some embodiments, the heart condition is an acute myocardial infarction. In some embodiments, the heart condition is a heart failure. In some embodiments, the heart condition is a chronic ischemic heart failure. In another aspect, the disclosure provides a kit comprising a vector or vector system of the disclosure, and optionally instructions for use in treating a heart condition.

[0269] In another aspect, the disclosure provides method of converting a differentiated non-cardiomyocyte cell into a cardiomyocyte, comprising contacting the differentiated cells with a vector or vector system of the disclosure. In some embodiments, the differentiated non-cardiomyocyte cell is a differentiated non-cardiomyocyte cell. In some embodiments, the differentiated non-cardiomyocyte cell is a human differentiated non-cardiomyocyte cell. In some embodiments, the differentiated non-cardiomyocyte cell is an *in vivo* differentiated non-cardiomyocyte cell. In some embodiments, the differentiated non-cardiomyocyte cell is an *in vitro* differentiated non-cardiomyocyte cell. In some embodiments, the differentiated non-cardiomyocyte cell is a cardiac cell.

[0270] In some embodiments, the methods of the disclosure improve or restore the ejection fraction of the heart. In some embodiments, the improvement comprises at least about 5%, at least about 10%, at least about 20%, at least about 30%, or at least about 40% improvement. In some embodiments, the method comprises increasing the ejection fraction of the heart of the subject to at least about 30%, at least about 40%, at least about 50%, or at least about 60%. In some embodiments, the improvement or restoration of ejection fraction occurs within at most about two, about three, about four, about five, or about six weeks. In some embodiments, the improvement or restoration of ejection fraction occurs within two, three, four, five, or six weeks. In some embodiments, the methods of the disclosure improve or restore the ejection fraction of the heart. In some embodiments, the improvement comprises about 10%, about 20%, about 30%, or about 40% improvement. In some embodiments, the method comprises increasing the ejection fraction

of the heart of the subject to about 30%, about 40%, about 50%, or about 60%. In some embodiments, the methods of the disclosure improve or restore the ejection fraction of the heart. In some embodiments, the improvement comprises about 5% to about 10%, about 10% to about 20%, about 20% to about 30%, or about 30% to about 40% improvement. In some embodiments, the method comprises increasing the ejection fraction of the heart of the subject to about 30%, about 40%, about 50%, or about 60%. In some embodiments, the improvement or restoration of ejection fraction occurs within about two, about three, about four, about five, or about six weeks. In some embodiments, the improvement or restoration of ejection fraction occurs within two, three, four, five, or six weeks.

B. Methods of Treatment Using Induced Cardiomyocytes

[0271] In some aspects, an induced cardiomyocyte of the present disclosure can be used to treat a subject in need thereof. In some embodiments, the induced cardiomyocytes can be administered to the subject in need thereof, where administration into the subject of the induced cardiomyocytes, treats a cardiovascular disease in the subject.

[0272] Many cell types are capable of migrating to an appropriate site for regeneration and differentiation within a subject. To determine the suitability of various therapeutic administration regimens and dosages of cell compositions, the cells can first be tested in a suitable animal model. At one level, cells are assessed for their ability to survive and maintain their phenotype *in vivo*. Cells can also be assessed to ascertain whether they migrate to diseased or injured sites *in vivo*, or to determine an appropriate number, or dosage, of cells to be administered. Cell compositions can be administered to immunodeficient animals (such as nude mice, or animals rendered immunodeficient chemically or by irradiation). Tissues can be harvested after a period of regrowth, and assessed as to whether the administered cells or progeny thereof are still present, are alive, and/or have migrated to desired or undesired locations.

[0273] Injected cells can be traced by a variety of methods. For example, cells containing or expressing a detectable label (such as green fluorescent protein, or beta-galactosidase) can readily be detected. The cells can be pre-labeled, for example, with BrdU or [³H]-thymidine, or by introduction of an expression cassette that can express green fluorescent protein, or beta-galactosidase. Alternatively, the reprogrammed cells can be detected by their expression of a cell marker that is not expressed by the animal employed for testing (for example, a human-specific

antigen when injecting cells into an experimental animal). The presence and phenotype of the administered population of reprogrammed cells can be assessed by fluorescence microscopy (*e.g.*, for green fluorescent protein, or beta-galactosidase), by immunohistochemistry (*e.g.*, using an antibody against a human antigen), by ELISA (using an antibody against a human antigen), or by RT-PCR analysis using primers and hybridization conditions that cause amplification to be specific for RNA indicative of a cardiac phenotype.

C. Methods of Treatment Using Reprogramming Factors and Compositions

[0274] In other embodiments, a method of treating cardiovascular disease involves administering to the subject in need thereof an effective amount of a reprogramming composition capable of increasing the expression of ASCL1, MYOCD, MEF2C, TBX5, or combinations thereof. In other embodiments, a method of treating cardiovascular disease involves administering to the subject in need thereof an effective amount of a reprogramming composition capable of increasing the expression of ASCL1, MYOCD, MEF2C, TBX5, or combinations thereof.

[0275] The invention provides methods of treating a cardiovascular disease comprising administering to a subject in need thereof an effective amount of an induced cardiomyocyte produced by the methods described herein.

[0276] In some aspects, an induced cardiomyocyte of the present disclosure can be used to treat a subject in need thereof. In some embodiments, the induced cardiomyocyte can be administered to the subject in need thereof, where administration into the subject of the induced cardiomyocyte, treats a cardiovascular disease in the subject. Thus, in some embodiments, a method of treating cardiovascular disease involves administering to a subject in need thereof a population of induced cardiomyocytes. In other embodiments, a method of treating cardiovascular disease involves administering to the subject in need thereof an effective amount of a composition comprising a WNT inhibitor, a TGF- β inhibitor or both.

[0277] In some embodiments, the non-cardiomyocyte is cultured in the presence of the TGF- β inhibitor for about 6 hours, about 12 hours, about 18 hours, about 24 hours, about 30 hours, about 36 hours, about 42 hours, or about 48 hours prior to addition of the WNT inhibitor. In one preferred embodiment, the non-cardiomyocyte is cultured in the presence of the TGF- β inhibitor for about 24 hours prior to addition of the WNT inhibitor.

VIII. Kits

[0278] A variety of kits are described herein that include any of the compositions, compounds and/or agents described herein. The kit can include, for example, a culture media in concentrated or non-concentrated form. The kit can include any of the compounds described herein, either mixed together or individually packaged, and in dry or hydrated form. The compounds and/or agents described herein can be packaged separately into discrete vials, bottles or other containers. Alternatively, any of the compounds and/or agents described herein can be packaged together as a single composition, or as two or more compositions that can be used together or separately. The compounds and/or agents described herein can be packaged in appropriate ratios and/or amounts to facilitate conversion of selected cells across differentiation boundaries to form cardiac progenitor cells and/or cardiomyocytes.

[0279] The kit can also include an expression cassette, an expression vector, or a combination thereof that includes a polynucleotide segment encoding a protein of interest operably linked to a promoter and other optional regulatory elements. The expression cassette or vector can be provided in dehydrated form or in a ready to use solution.

[0280] A kit is described herein for culture of cells *in vitro* that can include any of the compositions, compounds, expression cassettes, expression vectors, and/or agents described herein, as well as instructions for using those compositions, compounds, expression cassettes, expression vectors, and/or agents.

[0281] Some kits can include a cell culture or cell media that includes any of the compositions, compounds and/or agents described herein. The kits can include one or more sterile cell collection devices such as a swab, skin scrapping device, a needle, a syringe, and/or a scalpel. The kits can also include antibodies for detection of cardiac progenitor and/or cardiomyocyte cell markers such as antibodies against any of the following markers: α -actinin, MLC2v, cMHC, NKX2-5, GATA4, ISL1, MEF2C, cTNT, cTNI, MLC2a, and any combination thereof. The antibodies can be labeled so that a detectable signal can be observed when the antibodies form a complex with the cardiac progenitor cell and/or cardiomyocytes cell marker(s).

[0282] The instructions can include guidance for introducing a nucleic acid into selected cells (*e.g.*, a selected starting cell or selected cells). Such a nucleic acid can be an RNA, an expression cassette, or an expression vector that encodes a polynucleotide or protein of interest, and culturing

the cells for a time and under conditions sufficient to express the polynucleotide or protein of interest. The instructions can also include instructions for converting the cells across differentiation boundaries and into the cardiac lineage using any of the compositions and methods disclosed herein. For example, the instructions can describe amounts of the compositions, compounds and/or agents described herein to add to cell culture media, times sufficient to convert cells to the cardiac lineage, maintenance of appropriate cell densities for optimal conversion, and the like. For example, the instructions can describe procedures for rehydration or dilution of the compositions, compounds and/or agents described herein. When a kit provides a cell culture medium containing some of the compositions, compounds and/or agents described herein, the instructions can describe how to add other compounds and/or agents. The instructions can also describe how to convert the selected cells to cardiac progenitor cells or to cardiomyocytes.

[0283] The instructions can also describe procedures for detecting cardiac progenitor and/or cardiomyocyte cell markers by use of antibodies against those markers so that the extent of conversion and/or differentiation can be assessed.

[0284] Another kit is also described herein that includes any of the compositions, compounds and/or agents described herein for therapeutic treatment of a subject. The kit can include any of the compositions, compounds and/or agents described herein, as well as instructions for administering those compositions, compounds and/or agents. Such instructions can provide the information described throughout this application.

[0285] The kit can also include cells. For example, the kit can include chemically induced cardiac progenitor cells and/or cardiomyocytes that have been treated by the compositions and/or methods described herein and that are ready for administration.

[0286] The recombinant viruses, non-viral vectors, cells, compositions and/or compounds can be provided within any of the kits in the form of a delivery device. Alternatively a delivery device can be separately included in the kit(s), and the instructions can describe how to assemble the delivery device prior to administration to a subject. The delivery device can provide a scaffold for cell growth and/or a matrix for controlled release of any of the compositions, compounds or agents described herein.

[0287] Any of the kits can also include syringes, catheters, scalpels, sterile containers for sample or cell collection, diluents, pharmaceutically acceptable carriers, and the like.

[0288] The kits can provide other factors such as any of the supplementary factors or drugs described herein for the compositions in the preceding section or other parts of the application.

ENUMERATED EMBODIMENTS

[0289] The invention may be defined by reference to the following enumerated embodiments:

Embodiments Set 1:

- Clause 1-1. Induced cardiomyocyte (iCM) cells, wherein the cells comprise an *ASCL1* polynucleotide.
- Clause 1-2. The iCM cells of clause 1-1, wherein the cells comprise a *MYOCD* polynucleotide, a *MEF2C* polynucleotide, and a *TBX5* polynucleotide.
- Clause 1-3. The iCM cells of clause 1-2, wherein the *ASCL1* polynucleotide and the *MYOCD* polynucleotide are expressed from a first polycistronic vector; and wherein the *MEF2C* polynucleotide and the *TBX5* polynucleotide are expressed from a second polycistronic vector.
- Clause 1-4. The iCM cells of clause 1-3, wherein the first polycistronic vector and the second polycistronic vector are each independently selected from a lipid nanoparticle, a transposon, an adeno-associated virus (AAV) vector, an adenovirus, a retrovirus, an integrating lentiviral vector (LVV), and a non-integrating LVV.
- Clause 1-5. The iCM cells of any of clauses 1-2 – 4, wherein at least 25% of iCM cells are α -actinin positive.
- Clause 1-6. The iCM cells of any of clauses 1-2 – 5, wherein at least 2% of iCM cells are cTnT positive.
- Clause 1-7. The iCM cells of clause 1-1, wherein the cells comprise a polynucleotide encoding MYOCD polypeptide.
- Clause 1-8. The iCM cells of clause 1-2, wherein the *ASCL1* polynucleotide and the *MYOCD* polynucleotide are co-translationally expressed from a polycistronic vector.

- Clause 1-9. The iCM cells of clause 1-8, wherein the polycistronic vector is a vector comprising a *MYOCD-2A-ASCL1* polynucleotide and wherein the vector is selected from a lipid nanoparticle, a transposon, an adeno-associated virus (AAV) vector, an adenovirus, a retrovirus, an integrating lentiviral vector (LVV), and a non-integrating LVV.
- Clause 1-10. The iCM cells of any of clauses 1-7 – 9, wherein at least 25% of iCM cells are α -actinin positive.
- Clause 1-11. The iCM cells of any of clauses 1-7 – 10, wherein at least 5% of iCM cells are cTnT positive.
- Clause 1-12. The iCM cells of clause 1-1, wherein the cells are generated from primary adult human cardiac fibroblasts (AHCfs).
- Clause 1-13. A method of inducing a cardiomyocyte phenotype in differentiated cells, comprising contacting the differentiated cells with a retroviral expression vector comprising an *ASCL1* polynucleotide, thereby generating a population of induced cardiomyocyte (iCM) cells.
- Clause 1-14. The method of clause 1-1, wherein the retroviral expression vector comprises a *MYOCD* polynucleotide.
- Clause 1-15. The method of clause 1-14, wherein the *ASCL1* polynucleotide and the *MYOCD* polynucleotide are expressed from a *MYOCD-2A-ASCL1* polynucleotide.
- Clause 1-16. The method of clause 1-1, wherein the method comprises contacting the source cells with a second retroviral expression vector comprising one or both of a *MEF2C* polynucleotide and the *TBX5* polynucleotide.
- Clause 1-17. The method of clause 1-16, wherein the *MEF2C* polynucleotide and the *TBX5* polynucleotide are expressed from a *MEF2C-2A-TBX5* polynucleotide or a *TBX5-2A-MEF2C* polynucleotide.

- Clause 1-18. The method of any of clauses 1-13 – 17, wherein at least 25% of iCM cells are α -actinin positive.
- Clause 1-19. The method of any of clauses 1-13 – 18, wherein at least 5% of iCM cells are cTnT positive.
- Clause 1-20. The method of clause 1-13, wherein the differentiated cells comprise primary adult human cardiac fibroblasts (AHCfs).
- Clause 1-21. A method of treating a heart condition in a subject, comprising administering one or more nucleic acids encoding an *ASCL1* polynucleotide and optionally one or more of a *MYOCD* polynucleotide, a *MEF2C* polynucleotide, and a *TBX5* polynucleotide into the subject, thereby inducing transdifferentiation of fibroblast cells into induced cardiomyocyte (iCM) cells in the subject *in vivo*, thereby treating the subject.
- Clause 1-22. The method of clause 1-21, wherein the administering step comprises a route of administration selected from direct injection into the heart of the subject, intravenous injection, and delivery into the subject by catheter.
- Clause 1-23. The method of clause 1-21, wherein the one or more nucleic acids are provided in one or more retroviral vectors.
- Clause 1-24. The method of clause 1-21, wherein the one or more nucleic acids are provided in one or more adeno-associated virus (AAV) vectors.
- Clause 1-25. A method of treating a heart condition in a subject, comprising introducing into differentiated cells one or more nucleic acids encoding an *ASCL1* polynucleotide and optionally one or more of a *MYOCD* polynucleotide, a *MEF2C* polynucleotide, and a *TBX5* polynucleotide in cell culture, thereby generating induced cardiomyocyte (iCM) cells *ex vivo*; and administering the iCM cells to the subject, thereby treating the subject.
- Clause 1-26. The method of clause 1-25, wherein the one or more nucleic acids are provided in one or more retroviral vectors.

- Clause 1-27. The method of clause 1-25, wherein the one or more nucleic acids are provided in one or more adeno-associated virus (AAV) vectors.
- Clause 1-28. The method of clause 1-25, wherein the iCM cells are administered on a biocompatible scaffold.
- Clause 1-29. The method of clause 1-25, wherein the differentiated cells comprise primary adult human cardiac fibroblasts (AHCfs).
- Clause 1-30. The method of clause 1-29, wherein the AHCfs are autologous cells derived from the subject.
- Clause 1-31. The method of clause 1-29, wherein the AHCfs are allogeneic cells derived from a donor other than the subject.
- Clause 1-32. A vector, comprising a polynucleotide selected from a *MEF2C-2A-TBX5* polynucleotide, a *TBX5-2A-MEF2C* polynucleotide, and a *MYOCD-2A-ASCL1* polynucleotide, wherein the vector is selected from a lipid nanoparticle, a transposon, an adeno-associated virus (AAV) vector, an adenovirus, a retrovirus, an integrating lentiviral vector (LVV), and a non-integrating LVV

Embodiments Set 2:

- Clause 2-1. A vector, comprising, in any 5' to 3' order and on the same or different polynucleotide strands, a *MYOCD* polynucleotide and either or both of an *ASCL1* polynucleotide or a *MYF6* polynucleotide; each polynucleotide operatively linked to at least one promoter.
- Clause 2-2. The vector of clause 2-1, wherein the vector comprises, in any 5' to 3' order and on the same or different polynucleotide strands, a *MYOCD* polynucleotide and an *ASCL1* polynucleotide.
- Clause 2-3. The vector of clause 2-1, wherein the vector comprises, in any 5' to 3' order and on the same or different polynucleotide strands, a *MYOCD* polynucleotide and a *MYF6* polynucleotide.

- Clause 2-4. The vector of clause 2-1, wherein the vector comprises, in any 5' to 3' order and on the same or different polynucleotide strands, a *MYOCD* polynucleotide; either or both of a *MEF2C* polynucleotide and a *TBX5* polynucleotide; and either or both of an *ASCL1* polynucleotide and a *MYF6* polynucleotide.
- Clause 2-5. The vector of clause 2-4, wherein the vector comprises, in any 5' to 3' order and on the same or different polynucleotide strands, a *MYOCD* polynucleotide, an *ASCL1* polynucleotide, a *MEF2C* polynucleotide, and a *TBX5* polynucleotide.
- Clause 2-6. The vector of clause 2-4, wherein the vector comprises, in any 5' to 3' order and on the same or different polynucleotide strands, a *MYOCD* polynucleotide, a *MYF6* polynucleotide, a *MEF2C* polynucleotide, and a *TBX5* polynucleotide.
- Clause 2-7. The vector of any one of clauses 2-1 to 7, wherein the *MYOCD* is an engineered myocardin.
- Clause 2-8. The vector of any one of clauses 2-1 to 7, wherein the vector comprises no reprogramming factor polynucleotide other than a *MYOCD* polynucleotide, an *ASCL1* polynucleotide, a *MYF6* polynucleotide, a *MEF2C* polynucleotide, or a *TBX5* polynucleotide.
- Clause 2-9. The vector of any one of clauses 2-1 to 7, wherein the vector comprises no other other protein-coding gene.
- Clause 2-10. The vector of any one of clauses 2-1 to 9, wherein the vector is a polycistronic vector.
- Clause 2-11. The vector of any one of clauses 2-1 to 10, wherein the vector is selected from a lipid nanoparticle, a transposon, an adeno-associated virus (AAV) vector, an adenovirus, a retrovirus, an integrating lentiviral vector (LVV), and a non-integrating LVV.
- Clause 2-12. The vector of any one of clauses 2-1 to 10, wherein the vector is an AAV vector.
- Clause 2-13. The vector of any one of clauses 2-1 to 10, wherein the vector is an integrating LVV or a non-integrating LVV.

- Clause 2-14. The vector of any one of clauses 2-1 to 13, wherein the vector is capable of reprogramming differentiated cells into induced cardiomyocyte (iCM) cells.
- Clause 2-15. The vector of clause 2-14, wherein at least 10%, at least 15%, or at least 20% of iCM cells are α -actinin positive.
- Clause 2-16. The vector of clause 2-14 or 15, wherein at least 2%, at least 5%, or at least 8% of iCM cells are cTnT positive.
- Clause 2-17. The vector of any one of clauses 2-1 to 16, wherein the *ASCL1* polynucleotide, if present, or the *MYF6* polynucleotide, if present, and the *MYOCD* polynucleotide are operatively linked to the same promoter and are co-translationally expressed.
- Clause 2-18. The vector of clause 2-2, wherein the vector comprises a *MYOCD-2A-ASCL1* polynucleotide or an *ASCL1-2A-MYOCD* polynucleotide, operatively linked to the same promoter.
- Clause 2-19. The vector of clause 2-3, wherein the vector comprises a *MYOCD-2A-MYF6* polynucleotide or an *ASCL1-2A-MYF6* polynucleotide, operatively linked to the same promoter.
- Clause 2-20. The vector of any one of clauses 2-1 to 19, wherein the *ASCL1* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *ASCL1* (SEQ ID NO: 2).
- Clause 2-21. The vector of any one of clauses 2-1 to 19, wherein the *MYF6* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *MYF6* (SEQ ID NO: 56).
- Clause 2-22. A vector system, comprising a first polycistronic vector encoding a *MYOCD* polynucleotide and either or both of an *ASCL1* polynucleotide or a *MYF6* polynucleotide; and a second polycistronic vector comprising a *MEF2C* polynucleotide and a *TBX5* polynucleotide.

- Clause 2-23. The vector system of clause 2-22, wherein the first polycistronic vector comprises a *MYOCD-2A-ASCL1* polynucleotide or an *ASCL1-2A-MYOCD* polynucleotide.
- Clause 2-24. The vector system of clause 2-22, wherein the first polycistronic vector comprises a *MYOCD-2A-MYF6* polynucleotide or an *ASCL1-2A-MYF6* polynucleotide.
- Clause 2-25. The vector system of any one of clauses 2-22 to 24, wherein the first polycistronic vector and the second polycistronic vector are each independently selected from a lipid nanoparticle, a transposon, an adeno-associated virus (AAV) vector, an adenovirus, a retrovirus, an integrating lentiviral vector (LVV), and a non-integrating LVV.
- Clause 2-26. The vector system of any one of clauses 2-22 to 25, wherein the *ASCL1* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *ASCL1* (SEQ ID NO: 2).
- Clause 2-27. The vector system of any one of clauses 2-22 to 25, wherein the *MYF6* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *MYF6* (SEQ ID NO: 56).
- Clause 2-28. A method of inducing a cardiomyocyte phenotype in differentiated cells, comprising contacting the differentiated cells with the vector of any one of clauses 2-1 to 21.
- Clause 2-29. A method of inducing a cardiomyocyte phenotype in differentiated cells, comprising contacting the differentiated cells with the vector system of any one of clauses 2-22 to 27.
- Clause 2-30. The method of clause 2-28 or 29, wherein the differentiated cells are *in vitro* cells during the contacting step.
- Clause 2-31. The method of clause 2-28 or 29, wherein the differentiated cells are *in vivo* cells in a subject suffering from or at risk for a heart condition.
- Clause 2-32. A method of treating a heart condition in a subject suffering from or at risk for a heart condition, comprising contacting the differentiated cells *in vitro* with the vector of any

one of clauses 2-1 to 21 to generate iCM cells, and administering the iCM cells to the subject.

Clause 2-33. A method of treating a heart condition in a subject suffering from or at risk for a heart condition, comprising contacting the differentiated cells *in vitro* with the vector system of any one of clauses 2-22 to 27 to generate iCM cells, and administering the iCM cells to the subject.

Clause 2-34. A method of treating a heart condition in a subject suffering from or at risk for a heart condition, comprising administering the vector of any one of clauses 2-1 to 21 to the subject.

Clause 2-35. A method of treating a heart condition in a subject suffering from or at risk for a heart condition, comprising administering the vector system of any one of clauses 2-22 to 27 to the subject.

Clause 2-36. A kit comprising the vector of any one of clauses 2-1 to 21, and instructions for use in treating a heart condition.

Clause 2-37. A kit comprising the vector system of any one of clauses 2-22 to 27, and instructions for use in treating a heart condition.

Embodiments Set 3:

Clause 3-1. An isolated polynucleotide, comprising an engineered *MYOCD* polynucleotide encoding an engineered myocardin protein having a length of at most 850 amino acids, wherein the engineered myocardin protein comprises an SRF interaction domain, an SAP domain, and a TAD domain.

Clause 3-2. The polynucleotide of clause 3-1, wherein the engineered myocardin protein comprises an Mef2c interaction domain.

Clause 3-3. The polynucleotide of clause 3-2, wherein:

- Clause 3-4. the Mef2c interaction domain shares at least 85% identity to SEQ ID NO: 17,
- Clause 3-5. the SRF domain shares at least 85% identity to SEQ ID NO: 18,
- Clause 3-6. the SAP domain shares at least 85% identity to SEQ ID NO: 19, and
- Clause 3-7. the TAD domain shares at least 85% identity to SEQ ID NO: 11.
- Clause 3-8. The polynucleotide of clause 3-1 or 2, wherein the engineered myocardin protein comprises an LZ domain.
- Clause 3-9. The polynucleotide of clause 3-4, wherein the LZ domain shares at least 85% identity to SEQ ID NO: 20.
- Clause 3-10. The polynucleotide of any of clauses 3-1 to 5, wherein the engineered myocardin protein comprises:
- Clause 3-11. a first polypeptide that shares at least 85% identity to residues 5-413 of human myocardin (SEQ ID NO: 10), and
- Clause 3-12. second polypeptide that shares at least 85% identity to residues 764-986 of human myocardin (SEQ ID NO: 11),
- Clause 3-13. wherein the first polypeptide and the second polypeptide are linked by a linker comprising a peptide bond or a polypeptide linker of 1-50 amino acid residues.
- Clause 3-14. The polynucleotide of any of clauses 3-1 to 5, wherein the engineered myocardin protein comprises:
- Clause 3-15. a first polypeptide that shares at least 85% identity to residues 5-438 of human myocardin (SEQ ID NO: 12), and
- Clause 3-16. second polypeptide that shares at least 85% identity to residues 764-938 of human myocardin (SEQ ID NO: 11),

- Clause 3-17. wherein the first polypeptide and the second polypeptide are linked by a linker comprising a peptide bond or a polypeptide linker of 1-50 amino acid residues.
- Clause 3-18. The polynucleotide of any of clauses 3-1 to 5, wherein the engineered myocardin protein comprises:
- Clause 3-19. a first polypeptide that shares at least 85% identity to residues 5-559 of human myocardin (SEQ ID NO: 13), and
- Clause 3-20. second polypeptide that shares at least 85% identity to residues 764-938 of human myocardin (SEQ ID NO: 11),
- Clause 3-21. wherein the first polypeptide and the second polypeptide are linked by a linker comprising a peptide bond or a polypeptide linker of 1-50 amino acid residues.
- Clause 3-22. The polynucleotide of any of clauses 3-6 to 8, wherein the linker consists of a peptide bond.
- Clause 3-23. The polynucleotide of any of clauses 3-6 to 8, wherein the linker is a polypeptide selected from G, GG, GGG, GSG, GSS, GGS, GGSGGS (SEQ ID NO: 30), GSSGGS (SEQ ID NO: 31), GGSGSS (SEQ ID NO: 32), GGSGGSGGS (SEQ ID NO: 33), GGSGGSGGSGGS (SEQ ID NO: 34).
- Clause 3-24. The polynucleotide of clause 3-9, wherein the engineered myocardin protein comprises a sequence selected from SEQ ID NOs: 14-16.
- Clause 3-25. An isolated polynucleotide, comprising an engineered *MYOCD* polynucleotide encoding an engineered myocardin protein, wherein the engineered myocardin protein comprises a deletion of at least 50 amino acids in the region corresponding to amino acids 414-764 of the native myocardin (SEQ ID NO: 3).
- Clause 3-26. The polynucleotide of clause 3-12, wherein the engineered myocardin protein comprises a deletion of amino acids from about 414 to about 763 of the native myocardin (SEQ ID NO: 3).

- Clause 3-27. The polynucleotide of clause 3-13, wherein the engineered myocardin protein comprises a sequence at least 85% identical to SEQ ID NO: 14.
- Clause 3-28. The polynucleotide of clause 3-14, wherein the engineered myocardin protein consists of sequence identical to SEQ ID NO: 14.
- Clause 3-29. The polynucleotide of clause 3-12, wherein the engineered myocardin protein comprises a deletion of amino acids from about 439 to about 763 of the native myocardin (SEQ ID NO: 3).
- Clause 3-30. The polynucleotide of clause 3-16, wherein the engineered myocardin protein comprises a sequence at least 85% identical to SEQ ID NO: 15.
- Clause 3-31. The polynucleotide of clause 3-17, wherein the engineered myocardin protein consists of sequence identical to SEQ ID NO: 15.
- Clause 3-32. The polynucleotide of clause 3-12, wherein the engineered myocardin protein comprises a deletion of amino acids from about 560 to about 763 of the native myocardin (SEQ ID NO: 3).
- Clause 3-33. The polynucleotide of clause 3-19, wherein the engineered myocardin protein comprises a sequence at least 85% identical to SEQ ID NO: 16.
- Clause 3-34. The polynucleotide of clause 3-20, wherein the engineered myocardin protein consists of sequence identical to SEQ ID NO: 16.
- Clause 3-35. The polynucleotide of any of clauses 3-1 to 21, wherein the engineered myocardin protein is a functional engineered myocardin protein.
- Clause 3-36. The polynucleotide of clause 3-22, wherein the functional engineered myocardin protein is expressed at at least 10% of the level of native *MYOCD* in the same expression system.
- Clause 3-37. The polynucleotide of clause 3-22 or 23, wherein the functional engineered myocardin protein is capable of inducing increased expression of at least one marker of

cardiomyocyte phenotype either (a) when expressed in human cardiac fibroblasts with MEF2C and TBX5 or TBX5 in the presence of a TGF β inhibitor, optionally SB431542, and a Wnt inhibitor, optionally XAV939, or (b) when expressed in human cardiac fibroblasts with ASCL1.

Clause 3-38. The polynucleotide of any of clause 3-1 to 24, wherein the polynucleotide is operatively linked to a promoter.

Clause 3-39. The polynucleotide of any of clause 3-1 to 25, wherein the polynucleotide comprises a *MEF2C* polynucleotide and a *TBX5* polynucleotide.

Clause 3-40. The polynucleotide of any of clause 3-1 to 25, wherein the polynucleotide comprises an *TBX5* polynucleotide.

Clause 3-41. The polynucleotide of any of clause 3-1 to 25, wherein the polynucleotide comprises an *ASCL1* polynucleotide.

Clause 3-42. The polynucleotide of any of clause 3-1 to 28, wherein the polynucleotide is flanked by inverted terminal repeats (ITRs) or the polynucleotide is flanked by long terminal repeats (LTRs).

Clause 3-43. A recombinant virus comprising the polynucleotide of any of clauses 3-1 to 42.

Clause 3-44. The recombinant virus of clause 3-30, wherein the recombinant virus is a recombinant adeno-associated virus (rAAV).

Clause 3-45. The recombinant virus of clause 3-30, wherein the recombinant virus is a lentivirus.

Clause 3-46. A population of cells comprising the polynucleotide of any of clause 3-1 to 29.

Clause 3-47. The population of cells of clause 3-46, wherein the percentage of cells in the population that exhibit calcium transients (CaT) in an *in vitro* CaT assay is at least 2-

fold greater than the percentage of cells in a control population of cells not comprising the polynucleotide in the same *in vitro* CaT assay.

Clause 3-48. An induced cardiomyocyte (iCM), wherein the iCM comprises the polynucleotide of any of clauses 3-1 to 42.

Clause 3-49. A pharmaceutical composition comprising the polynucleotide of any of clauses 3-1 to 42 and a non-viral delivery system.

Clause 3-50. A pharmaceutical composition comprising the recombinant virus of any of clauses 3-43 to 45.

Clause 3-51. A method of generating an induced cardiomyocyte (iCM), comprising contacting a mammalian fibroblast with the polynucleotide of any of clauses 3-1 to 42, the recombinant virus of any of clauses 3-43 to 45, or the pharmaceutical composition of clause 3-49 or clause 3-37.

Clause 3-52. A method of treating a heart condition in a subject, comprising administering the pharmaceutical composition of clause 3-50 to the subject.

Clause 3-53. The method of clause 3-52, wherein the recombinant virus is administered via intracardiac catheterization.

Clause 3-54. The method of clause 3-52, wherein the recombinant virus is administered via intracardiac injection.

Clause 3-55. A recombinant adeno-associated virus (rAAV), comprising an expression cassette (as depicted in FIG. 13B or FIG. 13C), wherein the expression cassette comprises in 5' to 3' order a 5' inverted terminal repeat (ITR), a CAG promoter, an SV40 intron, a polynucleotide encoding one or more proteins, a short polyadenylation signal, and a 3' ITR, and wherein the polynucleotide encoding one or more proteins comprises in 5' to 3' order a polynucleotide encoding MyΔ3, a polynucleotide encoding a P2A linker, and a polynucleotide encoding ASCL1.

- Clause 3-56. The rAAV of clause 3-55, wherein the expression cassette comprises a WPRE.
- Clause 3-57. The rAAV of clause 3-55 or 56, wherein the MyΔ3 comprises SEQ ID NO: 16.
- Clause 3-58. The rAAV of any of clauses 3-55 to 57, wherein the 2A linker comprises ATNFSLLKQAGDVEENPGP (SEQ ID NO: 23).
- Clause 3-59. The rAAV of any of clauses 3-55 to 57, wherein the ASCL1 comprises SEQ ID NO: 1.
- Clause 3-60. The rAAV of clause 3-59, wherein the rAAV comprises a polynucleotide at least 95% identical to SEQ ID NO: 35 (MyΔ3A AAV).
- Clause 3-61. A pharmaceutical composition comprises the rAAV of any of clauses 3-55 to 60.
- Clause 3-62. A method of treating a heart condition in a subject, comprising administering the pharmaceutical composition of clause 3-61 to the subject.

[0290] The following non-limiting Examples illustrate some of the experimental work involved in developing the invention.

EXAMPLES

Example 1: Identification of enhancers of human cardiac reprogramming

[0291] We developed a screening system in which factors that are able to induce human cardiac reprogramming were identified by quantitatively analyzing cardiac marker expression. After three weeks of adding reprogramming factors, a high-throughput cell analyzer system was used to image and quantify cardiac reprogramming efficiency based on cardiac marker α -actinin expression. We used this screening system to screen a retroviral expression library consisting of 1,052 open reading frame (ORF) cDNAs encoding human transcription factors (including nuclear receptors),

cytokines, and epigenetic regulators. (Zhou et al., 2017). We added retroviruses encoding individual human ORFs together with MyMT to the HCFs. Positive hits were defined as genes that increase the percentage of α -actinin positive (α -actinin+) cells in combination with either MyMT or MyMT+SB/XAV (FIG. 1A) by a factor of two-fold or greater. The primary screen identified ASCL1, MYF6, DLX3, DLX6, GATA2 and GATA5 as enhancers of human cardiac reprogramming (FIG. 1B). The activity of those six factors were further confirmed by a secondary screen in which additional cardiac markers (MYH6, TNNT2, TNNC1, NPPA, TNNI3 and RYR2) were examined. ASCL1 and MYF6 were identified as the two strongest hits for enhancing cardiac reprogramming from the secondary screen (FIG. 1C).

Example 2: ASCL1 and MYF6 enhance human and pig cardiac reprogramming

[0292] Because ASCL1 and MYF6 were the two strongest hits for enhancing cardiac reprogramming (FIG. 1C), we focused our attention on ASCL1 and MYF6 in the following studies. We used primary adult human cardiac fibroblasts (AHCFs) to assess the reprogramming efficiency of adding ASCL1 or MYF6 in combination with different reprogramming factors. Immunocytochemistry showed that addition ASCL1 or MYF6 on top of MyMT generated ~15% α -actinin+, ~50% cTnT+ induced cardiomyocytes (iCMs) after three weeks of reprogramming (FIG. 2A-2B). This reprogramming efficiency is very significant relative to that of MyMT alone. Similar reprogramming efficiency was also achieved using MyMT plus ASCL1 or MYF6 on primary adult pig cardiac fibroblasts (APCFs) (FIG. 2C&D).

Example 3: Optimal stoichiometry of reprogramming factors enhances reprogramming efficiency

[0293] It has been shown that the relative ratio (or stoichiometry) of reprogramming factors influence the efficiency and quality of cardiac reprogramming. (Wang et al., 2015). An optimal ratio of reprogramming factors correlated with higher reprogramming efficiency and more mature iCMs. To determine the optimal ratio of MYOCD, ASCL1, MEF2C and TBX5 (MyAMT) for reprogramming, we manipulated the relative levels of My, A, M and T protein expression by mixing various amounts of retrovirus that express one of four reprogramming factors and fixing the ratio of the rest of the three reprogramming factors (FIG. 3A-3E). We transduced AHCFs with those retroviruses cocktails and reprogrammed the cells for three weeks. Cardiac markers α -actinin

and cTnT were used to indicate reprogramming efficiency. Immunocytochemistry analysis of reprogrammed AHCs with antibodies against α -actinin and cTnT showed that cardiac reprogramming efficiency was most sensitive to the expression level of MYOCD and ASCL1. The data indicated that more expression of MYOCD or ASCL1 led to higher levels of reprogramming efficiency. Reprogramming efficiency dropped to almost zero when there was no MYOCD or ASCL1 (FIG. 3A, 3B, 3E). Dosing MEF2C showed a similar pattern of reprogramming efficiency as MYOCD and ASCL1. However, a significant amount of α -actinin+ and cTnT+ iCMs can still be detected even without adding MEF2C, indicating cardiac reprogramming is less sensitive to the expression level of MEF2C (FIG. 3C, 3E). Unlike MYOCD, ASCL1 and MEF2C, dosing of TBX5 showed a distinct pattern of reprogramming efficiency, in which the reprogramming efficiency was maximized at a lower level of TBX5 expression (FIG. 3D&E). Collectively, our results demonstrated that an optimal My, A, M and T stoichiometry, defined by high protein level of MYOCD and ASCL1, medium levels of MEF2C and a low level of TBX5, significantly increases cardiac reprogramming efficiency.

Example 4: Cardiac reprogramming by reprogramming factors in two-in-one polycistronic vectors

[0294] Gene therapy using separate vectors to express many different genes is not practical for clinical development. AAVs are the most popular gene therapy vectors that have been used in many clinic trials to date [Dunbar, 2018]. However, the packaging capacity of AAVs is only ~4.7 kb, thus, fitting multiple reprogramming factors into the fewest AAV vectors is a major challenge for cardiac reprogramming using AAV. We took advantage of the 2A peptide polycistronic system to put MyAMT reprogramming cocktails into two AAV vectors. Due to the size limitation of AAV and space required for the promoter and other regulatory elements, MYOCD (3.0kb) can only be paired with ASCL1 (0.7 kb) to fit into one AAV vector, whereas MEF2C (1.4kb) and TBX5 (1.5kb) are too large to pair with MYOCD but together can fit into another AAV vector. It has been demonstrated that the order of factors in the 2A polycistronic polynucleotides determined the gene's expression level (Wang et al., 2015). We have demonstrated that the optimal My, A, M and T stoichiometry significantly increases cardiac reprogramming efficiency (FIG. 3A-3E). To achieve the optimal stoichiometry in the 2A polycistronic vectors, we generated all the possible combinations of My+A and M+T with same 2A sequences in a single open reading frame (My-

P2A-A, A-P2A-My, M-P2A-T and T-P2A-M). We transduced AHCFs with retroviral combinations that consist of all four possible combinations of polycistronic vectors, including My-P2A-A + M-P2A-T, My-P2A-A + T-P2A-M, A-P2A-My + M-P2A-T and A-P2A-My + T-P2A-M. We analyzed the expression of cardiac markers α -actinin and cTnT by immunocytochemistry after three weeks of reprogramming. We found that the My-P2A-A + M-P2A-T was the optimal combination and generated ~35% of α -actinin+ iCMs and ~4% of cTnT+ iCMs (FIG. 4A-4B). Q-PCR analysis of a panel of 21 cardiac markers also demonstrated that My-P2A-A + M-P2A-T is the optimal polycistronic vector combination based on its ability to induce the highest levels of expression of the most cardiac genes (FIG. 4C).

Example 5: Cardiac reprogramming by a single polycistronic vector

[0295] We sought to further simplify the reprogramming vectors by putting all reprogramming factors into a single polycistronic transcription unit. Because our stoichiometry experiment (FIG. 3A-3E) showed that MYOCD and ASCL1 are the most important reprogramming factors in the MyAMT cocktails, and iCMs were generated even without adding MEF2C or TBX5, albeit with lower reprogramming efficiency, we speculated that just MYOCD and ASCL1 (MyA) are able to reprogram AHCFs to iCMs. To test this hypothesis, we transduced MYOCD and ASCL1 retroviruses into AHCFs. Interestingly, ~10% of α -actinin+ iCMs and ~30% of cTnT+ iCMs were generated by MyA cocktails after three weeks of reprogramming (FIG. 5A-5B). Furthermore, we tested the polycistronic vectors that encode My and A with different orders (My-P2A-A and A-P2A-My), and My-P2A-A showed much better reprogramming efficiency than A-P2A-My (FIG. 5C-5D). Collectively, these data suggest that the MyA combination is able to reprogram human cardiac fibroblasts to iCMs with high efficiency.

Example 6: Cardiac reprogramming by a polycistronic vector expressing four genes

[0296] Our data show that the order of reprogramming factors significantly in a polycistronic vector with two factors can influence cardiac reprogramming efficiency (FIG. 3A-3E, FIG. 4A-4C). Since MyAMT showed better reprogramming capacity than MyA (FIG. 4A-4C, FIG. 5A-5D), we sought to further determine the optimal order of four factors, MyAMT, in a single polycistronic polynucleotide. We generated all the possible 24 combinations of My, A, M, T with different translation skipping sequences (P2A, T2A and E2A) in a single polynucleotide. We

transduced AHCFs with either GFP or one of 24 combinations of My, A, M, T polycistronic retroviruses and analyzed the expression of cardiac markers α -actinin and cTnT by immunocytochemistry after three weeks of reprogramming. We found that three of 24 polycistronic vectors, A-P2A-T-T2A-My-E2A-M, T-P2A-A-T2A-My-E2A-M and M-P2A-A-T2A-My-E2A-T, are the best combinations which generated ~15% of α -actinin+ iCMs and ~15% of cTnT+ iCMs (FIG. 6A-6B). The non-optimized polycistronic vectors, such as My-P2A-M-T2A-T-E2A-A, almost didn't reprogram fibroblasts to iCMs (FIG. 6A-6B), highlighting the importance of stoichiometry of reprogramming factors for cardiac reprogramming. By using factor position score matrix which reflects the average reprogramming efficiency for one particular reprogramming factor in one particular position, we found that MYOCD prefers to be at position 3, ASCL1 prefer to be at position 2, and both MEF2C and TBX5 prefer to be at position 1 or position 4.

Example 7: *In vivo* delivery of reprogramming cocktails promotes heart repair

[0297] We examined whether forced expression of reprogramming cocktails that we optimized above in non-cardiomyocytes could lead to measurable improvement in the function of injured hearts (FIG. 7A). Mice were subjected to left anterior descending artery (LAD) ligation followed by intramyocardial injection of GFP, ASCL1, MyMT, MyMTA or MyA retroviruses, each encoding a single factor. Cardiac function following MI was assessed in a blinded fashion by ejection fraction (EF), end systolic volume (ESV) and end diastolic volume (EDV) using echocardiography. Before MI surgery, cardiac function of each cohort of mice was equal by assessing EF, ESV and EDV by echocardiography. After MI surgery, cardiac function of GFP-injected mice declined and reached a stable value at two weeks post-MI. In contrast, infection of injured heart with MyMT, MyAMT or MyA retroviruses blunted worsening cardiac function two weeks post-MI. (FIG. 7B-D). The cardiac functional improvement was delayed and less complete with MyMT at five weeks post-MI (FIG. 7B-7D), consistent with the reduced efficiency of this transcription factor combination in reprogramming *in vitro* (FIG. 2A-2D). Injection of injured heart with ASCL1-expressing vector alone did not have any effect on cardiac function compared with GFP (FIG. 7B-7D). These data suggest MyMT, MyMTA and MyA reprogramming cocktails promote heart repair and adding ASCL1 in combination with Myocardin provides benefit for cardiac functional improvement *in vivo*.

MATERIALS AND METHODS

[0298] Isolation of primary adult human and pig cardiac fibroblasts. For isolation of adult human cardiac fibroblasts (AHCfs) or adult pig cardiac fibroblasts (APCFs), adult human or pig left ventricles were minced into small pieces and digested in cardiac fibroblast digestion medium (10 µg/ml Liberase TH, 10 µg/ml Liberase TM, 1 unit/ml DNase I, 0.01% Polaxomer) for 1 h in 37°C. After digestion, the cells were filtered through a 70 µm strainer into a 50 mL falcon tube. Cells were pelleted by spinning down for 5 min at 1200×g and placed in fibroblast growth medium. The medium was replaced every two days. Four days later, AHCfs or APCFs were frozen or re-plated for viral transduction.

[0299] Retrovirus production. For retrovirus production, PLATINUM-A™ (PLAT-A™) cells from Cell Biolabs, Inc. were seeded into culture dishes (5×10^4 cells/cm²) one day before transfection in DMEM supplemented with 10% FBS. Cells reached ~60% confluency on the day of transfection. DNA plasmids (based on the pMXs-ORF vector from Cell Biolabs, Inc.) were transfected into Platinum-A cells using Promega Corp. FUGENE® HD transfection reagent. Forty-eight hours after transfection, viral medium that was filtered through a 0.45-µm filter, and polybrene was added at a concentration of 8 µg/ml.

[0300] Cellular reprogramming. For *in vitro* cardiac reprogramming, AHCfs or APCFs were seeded into culture dishes or plates at a density of 5×10^3 /cm² in fibroblast growth medium (day -1). One day after plating cells (day 0), fibroblast growth medium was removed and virus medium was added. One day after viral transduction (day 1), virus medium was replaced by iCM medium that composed of 4 parts Dulbecco's Modified Eagle's Medium (DMEM) and 1 part GIBCO® Media 199, 10% FBS, 1% nonessential amino acids, 1% penicillin/streptomycin, for every two days until day 4. On day 4, medium was changed to 75% iCM media and 25% RPMI + B27. On day 7, medium was changed to 50% iCM and 50% RPMI + B27. On day 11, medium was changed to 25% iCM and 75% RPMI + B27. On day 14, medium was changed to RPMI + B27+FFV (10ng/ml rhFGF, 15ng/ml rhFGF-10, and 5ng/ml rhVEGF) for every day until day 21.

[0301] Immunocytochemistry. For immunocytochemistry, cells were fixed in 4% paraformaldehyde for 20 min and permeabilized with 0.1% Triton-X100 at room temperature for 30 min. Cells were washed with PBS three times followed by blocking with 1% bovine serum albumin (BSA) for 1 h. Cells then were incubated with mouse monoclonal anti-cardiac Troponin

T (cTnT) antibody (Thermo Scientific, MA5-12960) at 1:200 dilutions or mouse anti- α -actinin antibody (Sigma, A7811) at 1:200 dilutions in 1% BSA for 1 h. After washing with PBS three times, cells were then incubated with donkey anti-mouse Alexa Fluor 594 (Invitrogen, A21203) at 1:200 dilutions in 1% BSA for 1 h. Cells were then imaged and quantified using a cell imaging multi-mode reader, CYTATION™ 5 (BioTek).

[0302] Quantitative mRNA measurement. Total RNA was extracted using RNeasy Mini Kit (QIAGEN®) according to vender's protocol. RNAs were retrotranscribed to cDNA using iScript SUPERMIX™ (BIO-RAD®). qPCR was performed using TAQMAN® Gene Expression Master Mix (THERMO SCIENTIFIC®) mRNA levels were normalized by comparison to GAPDH mRNA.

[0303] Quantification and statistical analysis. All data are presented as mean with standard error of the mean (SEM) and have $n = 2-3$ per group. P values were calculated with either unpaired/two-way t test or one-way analysis of variance (ANOVA). Statistical analyses were performed using the GRAPHPAD PRISM® 7 software package (GRAPHPAD SOFTWARE™). A P value of < 0.05 was considered significant in all cases after corrections were made for multiple pairwise comparisons.

[0304] MI surgery, intramyocardial injection of retroviruses and echocardiography. Surgeries were performed on CHARLES RIVER® CD-1 IGS male mice of 9-10 weeks of age. Mice were anesthetized with 2.4% isoflurane/97.6% oxygen and placed in a supine position on a heating pad (37°C). Animals were intubated with a 20-gauge intravenous catheter and ventilated with a mouse ventilator (MINIVENT™, Harvard Apparatus, Inc.). MI was induced by permanent ligation of the left anterior descending artery (LAD) with a 7-0 prolene suture. Concentrated 10^{10} genome copies (GC) of retrovirus in 20 μ l of PBS (concentration = 5×10^{11} GC/ml) (GFP, ASCL1, MyMT, MyAMT, or MyA) were injected into the myocardium through an insulin syringe with an incorporated 29 gauge needle (BD). Injection with a full dosage was carried out along the boundary between the infarct zone (IZ) and border zone (BZ) based on the blanched infarct area after coronary artery occlusion. After injection, the chest was closed with sutures and the mouse was allowed to recover with the mouse ventilator and heating pad. All surgical procedures were performed under aseptic conditions. Cardiac function was evaluated by two-dimensional transthoracic echocardiography on conscious mice using a VISUALSONICS™ VEVO® 3100

imaging system. Ejection fraction (EF), end systolic volume (ESV) and end diastolic volume (EDV) were used as indices of cardiac contractile function.

Example 8: Comparison of lentiviral and adeno-associated viruses expressing MyA

[0305] We compared MYOCD expression from lentiviral or AAV vectors encoding MYOCD and a second protein. Polycistronic vectors with MYOCD and ASCL1 were generated in a non-integrating lentivirus (NIL) format using the expression cassette depicted in FIG. 8A. The lentivirus is non-integrating because the integrase included in the plasmid-based packaging system included a mutation to prevent integration of the gene construct into the genome of transduced cells. The same expression construct depicted in FIG. 8A is in some embodiments used to make an integrating lentivirus. In this lentiviral construct, MYOCD-2A-ASCL1 was placed after an SV40 intron ("SV40 Int") under control of a CAG promoter with a WPRE flanked by 5' and 3' long-terminal repeats (LTRs). The 3' LTR operates as a polyadenylation signal in this construct.

[0306] An analogous AAV construct (FIG. 8B) was generated by placing the same MYOCD-2A-ASCL1 polynucleotide into an AAV vector, in which the expression cassette is flanked by 5' and 3' inverted terminal repeats (ITR). Due to the packaging limit of the AAV vector, the WPRE was removed and the polyadenylation sequence selected was a short polyA signal ("A") (SEQ ID NO: 75). Control monocistronic constructs with only MYOCD (and not ASCL1) were also made (not shown). The control AAV included a WPRE and used the bGH polyA (SEQ ID NO: 85) in place of the short polyA of the polycistronic AAV.

[0307] Cells were transfected with the NIL (FIG. 8C) or AAV vector (FIG. 8D) and analyzed by immunoblotting using antibodies against MYOCD, ASCL1, or the loading control GAPDH. Monocistronic (My) and polycistronic (MyA) NIL vectors expressed MYOCD at the same level (FIG. 8C). MYOCD protein was expressed by cells transduced with monocistronic (M) and polycistronic (MyA) AAVs, but expression of MYOCD protein by the polycistronic MyA AAV vector was lower than expression of MYOCD protein by the polycistronic lentiviral MyA NIL (FIG. 8C), by the monocistronic lentiviral My NIL (FIG. 8C), or by monocistronic adeno-associated virus My AAV (FIG. 8D). A high molecular weight band attributed to uncleaved MYOCD-2A-ASCL1 was observed (black arrow in FIG. 8D).

[0308] The ability of AAV MyA or NIL MyA to induce a cardiomyocyte phenotype in transduced cells was assessed by quantitative immunofluorescence microscopic detection of α -

actinin (FIG. 8E), using the indicated primary antibody and an ALEXA FLUOR[®] 594 secondary antibody on a BIOTEK CYTATION™ 5 imaging reader, and mRNA was quantitated by quantitative polymerase chain reaction (qPCR) using gene-specific primers for TNNC1, TNNT2, PLN, NPPA, or MYH6 (Table 4).

Table 4 Expression of Genetic Markers

	TNNC1	TNNT2	PLN	NPPA	MYH6
No Inf	1.1	0.9	0.8	0.3	1.4
No Inf	0.9	1.1	1.2	3.8	0.7
NIL MyA - 1.5×10^9 GC	12.3	26.8	12.1	1114.7	47.8
NIL MyA - 1.5×10^9 GC	11.7	23.0	9.0	963.8	16.3
NIL MyA - 5×10^9 GC	80.0	102.6	30.0	4405.6	64.9
NIL MyA - 5×10^9 GC	74.3	120.2	30.1	5436.8	64.5
AAV MyA - 5×10^9 GC	5.1	11.7	4.4	322.6	16.1
AAV MyA - 5×10^9 GC	2.4	8.9	4.2	327.4	8.4
AAV MyA - 2.5×10^{10} GC	8.0	20.0	7.2	524.4	8.6
AAV MyA - 2.5×10^{10} GC	8.2	23.3	8.8	672.5	1.6

[0309] Control samples were not infected (No Inf). Transduction was performed with 1.5×10^9 , 5×10^9 , or 2.5×10^{10} genome copies (GC) per 30,000 cells (one well). FIG. 8E shows that NIL MyA (2.5×10^{10} GC not tested) induced α -actinin expression in a greater fraction of cells than AAV MyA (1.5×10^9 GC not tested). Table 4 shows that NIL MyA induced higher expression of markers associated with the cardiomyocyte phenotype than AAV MyA.

Example 9: Design and testing of engineered myocardin proteins with internal deletions

[0310] FIG. 9A shows a domain schematic of wild-type human MYOCD. The N-terminal RPEL domains (RPxxxEL) mediate MEF2C binding. The basic (++) and polyglutamine (Q) motifs are critical for SRF interaction, whereas GATA4 interacts with MYOCD via a domain labeled “Gata4 Interact I” that includes the SAP (SAF-A/B, Acinus and PIAS) domain and also with a second interaction domain labeled “Gata4 Interact II” containing the leucine zipper (LZ). The C-terminus contains a transactivation domain (TAD). The isoform of the native protein expressed in human cardiac tissue is 986 amino acids in length.

[0311] To increase the potency of MyA AAV, three myocardin variants with internal deletions were designed and tested in AAV. My Δ 1 has a deletion of residues 414 to 763 (FIG. 9B), which deletes most of the entire second GATA4 interaction domain. My Δ 2 has a deletion of residues 439 to 763 (FIG. 9C), which also deletes most of the entire second GATA4 interaction domain. My Δ 3 has a deletion of residues 560 to 763 (FIG. 9D), which also deletes most of the entire second GATA4 interaction domain but retains the LZ domain.

[0312] FIG. 10A shows by immunoblot that despite fusing diverse portions of the coding sequence together, the three internal deletion mutants generate stable myocardin protein. Lysates were made from transiently transfected HEK293T cells for 48 hours. FIG. 10B shows immunofluorescence microscopy of native and engineered myocardin proteins. My Δ 1, My Δ 2, and My Δ 3 are each localized to the nucleus of the cell properly, as is the native myocardin protein.

[0313] Retroviral vectors encoding native myocardin (MYOCD) or one of three engineered myocardin proteins with an internal deletion (My Δ 1, My Δ 2, or My Δ 3) were generated. Coinfection of a human cardiac fibroblast (HCF) cell line was used to induce expression of MYOCD, MEF2C, and TBX5 (MyMT); My Δ 1, MEF2C, and TBX5 (My Δ 1MT); My Δ 2, MEF2C, and TBX5 (My Δ 2MT); or My Δ 3, MEF2C, and TBX5 (My Δ 3MT). Coinfection was performed in the presence of two small molecules, TGF-beta inhibitor SB431542 and Wnt inhibitor XAV939, which have been shown to enhance cardiac reprogramming in mouse and human cells (MyMT+SB/XAV) (Mohamed et al., *Circulation*. 135:978-95 (2017); Int'l Patent Appl. No. PCT/US2017/025132).

[0314] Calcium transients were assessed as previously described. Qian, L., et al. (2012) *Nature* 485:593-598. Briefly, isolated myocytes were loaded with Fluo-4 for 30 min at room temperature before being transferred to the superfusion chamber. The loading solution contained a 1:10 mixture of 5 mM Fluo-4 AM in dry DMSO and PowerloadTM concentrate (Invitrogen), which was diluted 100-fold into extracellular Tyrode's solution containing suspended myocytes. Calcium transients were evoked by applying voltage pulses at 1 Hz between platinum wires placed on either side of the cell of interest and connected to a field stimulator (IonOptix, Myopacer). Fluo-4 fluorescence transients were recorded via a standard filter set (#49011 ET, Chroma Technology). For each replicate, eight fields of cells were imaged for twenty seconds.

[0315] FIG. 11 indicates that human cardiac fibroblasts transduced with MEF2C, TBX5 and native or engineered Myocardin all exhibit robust calcium transients after 1 week or 3 weeks of

reprogramming. We concluded that MyMT+SB/XAV generated induced cardiomyocytes (iCM) even when native MYOCD is replaced by My Δ 1, My Δ 2, or My Δ 3.

[0316] Similar experiments were performed with native or engineered myocardin and TBX5 in the presence of SB431542 and Wnt inhibitor XAV939, but without MEF2C. Coinfection was used to induce expression of MYOCD and TBX5 (MyT), My Δ 1 and TBX5 (My Δ 1T), My Δ 2 and TBX5 (My Δ 2T), or My Δ 3 and TBX5 (My Δ 3T) in the presence of small molecules (+ SB/XAV). Expression profiles were measured by qPCR as described in Example 7. FIG. 12A demonstrates that human cardiac fibroblasts transduced with polynucleotides encoding TBX5 and My Δ 3 exhibited a transcriptional profile comparable to those transduced with TBX5 and native MYOCD. FIG. 12B shows immunofluorescence microscopy of cells transduced with MyT or My Δ 3T in the presence of small molecules from the same experiment. Cardiac fibroblasts transduced with TBX5 and native MYOCD, TBX5 and My Δ 2, or TBX5 and My Δ 3 exhibit comparable α -actinin structures.

Example 10: Reprogramming of Human Cardiac Fibroblasts with AAV-based My Δ 3A

[0317] The AAV vector shown in FIG. 8B, which is reproduced as FIG. 13A (MyA Δ WPRE short A) was modified by substitution of native MYOCD with a polynucleotide encoding an engineered myocardin with an internal deletion of residues 560 to 763 (My Δ 3A Δ WPRE short A; FIG. 13B), or this substitution and addition of an WPRE (My Δ 3A WPRE short A; FIG. 13C). Control vectors with native MYOCD, WPRE, and bGH polyA signal (My; not shown); or native MYOCD and short polyA, but no WPRE (My Δ WPRE short A; not shown) were also generated.

[0318] Replacement of wild-type MYOCD with My Δ 3 in the AAV cassette boosted myocardin (FIG. 14A) and ASCL1 (FIG. 14B) protein expression based on immunoblotting with MYOCD or ASCL1-specific antibodies. AAV vector lacking WPRE expressed myocardin protein, though at a lower level than the control vector with WPRE (My compared to My Δ WPRE short A). The engineered myocardin with internal deletion (My Δ 3A Δ WPRE short A) is expressed at a higher level compared to native MYOCD in vectors lacking WPRE. Addition of a miniature WPRE (WPRE3) to the My Δ 3A AAV construct (My Δ 3A WPRE3 short A) did not have a robust additional effect. A higher molecular weight band corresponding to uncleaved My Δ 3-2A-ASCL1 is also observed (black arrow in FIG. 14A). Replacing native MYOCD with My Δ 3 similarly

increases expression of ASCL1 (FIG. 14B). The higher molecular weight band for My Δ 3-2A-ASCL1 is also observed in the immunoblot (black arrow in FIG. 14B).

[0319] Native MYOCD plus ASCL1 vector (MyA Δ WPRE short A; now labeled AAV MyA) and engineered myocardin plus ASCL1 vector (My Δ 3A Δ WPRE short A; now labeled My Δ 3A) were further tested for their ability to induce a α -actinin+ cTnT+ induced cardiomyocyte (iCM) phenotype in the HCF cell line by immunocytochemistry, as described in Example 7. **Table 5** demonstrates that My Δ 3A increased expression of various reprogramming factors compared to MyA at various multiplicities of infection (MOI).

Table 5: Expression of Reprogramming Factors

Vector (MOI)	TNNT2	MYH6	NPPA	PLN	TNNC1	ACTC1	CASQ2	CSPR3	MYH7
No Inf	1.0	1.0	2.2	0.9	0.9	1.2	1.4	1.7	0.2
No Inf	1.0	1.0	0.6	1.2	1.2	0.9	0.8	2.5	1.7
No Inf	0.8	0.9	1.8	1.0	0.9	1.0	0.9	0.2	2.7
AAV MyA 10k	12.5	2.6	987.0	4.6	7.0	32.0	55.2	6.1	2.3
AAV MyA 10k	14.0	15.7	1257.6	5.5	13.3	29.3	98.3	3.2	1.7
AAV MyA 10k	15.7	8.4	1085.4	6.1	13.6	41.5	66.2	1.7	0.1
AAV MyA 40k	21.9	13.1	4135.0	7.2	28.4	47.4	96.9	3.7	2.5
AAV MyA 40k	15.8	23.7	1237.6	4.7	16.5	35.7	74.1	4.4	2.8
AAV MyA 40k	19.9	21.5	2996.0	6.1	14.7	42.6	105.1	4.1	-
AAV MyA 160k	50.0	25.1	5979.8	12.4	45.6	132.8	206.9	4.4	4.3
AAV MyA 160k	46.6	19.1	7650.1	8.6	45.3	110.7	263.8	7.0	0.2
AAV MyA 160k	43.6	41.9	4964.5	12.1	27.5	106.9	239.4	7.5	3.2
AAV My Δ 3A 10k	94.5	147.9	14423.4	16.7	125.0	163.9	488.8	8.2	9.2
AAV My Δ 3A 10k	51.1	60.9	5759.0	10.5	56.3	101.5	277.1	8.0	7.0
AAV My Δ 3A 10k	55.0	74.4	11600.2	9.7	70.9	110.1	335.7	9.1	6.0
AAV My Δ 3A 40k	112.0	164.7	26318.9	18.9	210.4	244.7	642.5	10.5	17.3
AAV My Δ 3A 40k	114.1	171.1	23530.4	20.8	153.3	241.2	658.9	13.9	8.4
AAV My Δ 3A 40k	92.0	114.2	14123.5	17.9	136.8	152.8	573.1	7.8	12.8
AAV My Δ 3A 160k	236.3	330.2	47673.8	31.1	308.8	380.9	1147.3	17.4	25.7
AAV My Δ 3A 160k	150.8	408.3	32839.6	27.4	254.1	457.1	1082.5	17.8	9.4
AAV My Δ 3A 160k	333.0	438.6	77995.5	48.8	523.2	785.8	1641.8	14.1	22.2

[0320] This increased reprogramming factor expression correlated with increased cardiac fibroblast reprogramming capacity of My Δ 3A compared to MyA based on quantitative immunofluorescence measurement of α -actinin+ cells (FIG. 14C) or cTnT+ cells (FIG. 14D).

[0321] The enhanced reprogramming capacity of AAV-based reprogramming with My Δ 3A was further validated by head-to-head comparison of AAV to NIL. AAV-based expression of My Δ 3A outperformed NIL-based reprogramming based on immunofluorescent analysis of percentage of α -actinin+ cells or percentage of cTnT+ cells (FIG. 15). This contrasts with the lower performance of AAV compared to NIL observed with native MYOCD (FIG. 8E).

Example 11: Reprogramming of Human Cardiac Fibroblasts with AAV-based MyMyf6

[0322] A library of approximately 1,000 human protein-coding genes were screened for reprogramming activity in combination with *MYOCD* alone by transduction of retroviruses encoding MYOCD and the each of the ~1,000 genes into cardiac fibroblasts. The ~1,000 human protein coding genes were tested for their ability to reprogram human cardiac fibroblasts assayed by expression of cTnT and α -actinin. Results for the best eighteen factors are shown (FIG 16A). ASCL1 and MYF6 both show robust activity in combination with MYOCD and no other heterologous protein-coding gene. The expression of cTnT and of α -actinin were each quantified by Z-Score. FIG. 16B shows representative immunocytochemistry images of reprogrammed adult human cardiac fibroblasts (AHCfs) two weeks post-infection with retrovirus(es) expressing MYOCD (My); ASCL1 alone (A); MYOCD and ASCL1 (My/A); or MYOCD and MYF6 (My/Myf6). Table 6 shows cardiac gene expression analysis on reprogrammed cells using My/A or My/Myf6 each expressed from a single retroviral vector.

Table 6: Expression of Gene Markers

	MyA	MyMyf6
NPPA	21000	29
ACTC1	15000	8000
PLN	1000	380
TNNT2	160	49
CASQ2	77	30
TNNC1	49	23
MYH7	42	96

MYH6	26	2.3
SCN5A	24	4.0
NPPB	9.7	2.0
ACTN2	6.2	13

[0323] AHCFs were infected with retroviruses that encoded MYOCD-2A-ASCL1 (MyA) or MYOCD-2A-MYF6 (MyMyf). Reprogrammed cells were cultured for three weeks. The transcript levels of cardiac marker genes (NPPA, ACTC1, PLN, TNNT2, CAQ2, TNNC1, MYH6, MYH7, SCN5A, NPPB) were determined by q-PCR.

Example 12: Treatment of Myocardial Infarction

[0324] Viral vectors for expression of myocardin and ASCL1 were tested *in vivo* as a treatment for myocardial infarction (MI). Adeno-associated virus (AAV) vectors were constructed by co-transfection of HEK293T cells with a PACG5 plasmid encoding the *rep* gene of AAV2 and the *cap* gene of AAV5 and pHelper encoding adenovirus gene E4, E2a and VA along with, individually, each of the three plasmid having a test expression cassette flanked by the inverted terminal repeats (ITRs) of AAV2. This co-transfection generated AAV5 viral particles capable of delivering each of the three expression cassettes. The negative-control expression cassette encoded green fluorescence protein (GFP) under the control of a CAG promoter. Two expression cassettes for myocardin and ASCL1 were tested. In both tested expression cassettes, the engineered myocardin MyΔ3 was used to reduce the size of the expression cassette.

[0325] The first tested expression cassette was a bicistronic expression cassette (FIG. 17A), which placed a polynucleotide (SEQ ID NO: 72) encoding MyΔ3 (SEQ ID NO: 16) under the transcriptional control of a CAG promoter (SEQ ID NO: 67) with an intervening SV40 intron (SEQ ID NO: 73) and a bGH polyA signal (SEQ ID NO: 85). This first expression cassette further included 3' to the MyΔ3 polyA signal, a polynucleotide (SEQ ID NO: 2) encoding ASCL1 (SEQ ID NO: 1) under the transcriptional control of a SCP promoter (SEQ ID NO: 68) and a short polyA signal (SEQ ID NO: 74). No WPRE sequence was used in this construct.

[0326] The second tested expression cassette was a monocistronic expression cassette (FIG. 18A) similar to that in Example 10, which placed a polynucleotide (SEQ ID NO: 72) encoding MyΔ3 (SEQ ID NO: 16) followed by a polynucleotide sequence (SEQ ID NO: 22) encoding a 2A

peptide (SEQ ID NO: 23) and then a polynucleotide (SEQ ID NO: 2) encoding ASCL1 (SEQ ID NO: 1). The complete polynucleotide sequence of this open reading frame is SEQ ID NO: 35, which encodes the polypeptide sequence SEQ ID NO: 57. This second expression cassette placed this *MyΔ3-2A-ASCL1* polynucleotide sequence under the transcriptional control of a CAG promoter (SEQ ID NO: 67) with an intervening SV40 intron (SEQ ID NO: 73) and a 3' short polyA signal (SEQ ID NO: 74). No WPRE sequence was used.

[0327] As a model for MI, CD-1 mice was generated by ligation of the left anterior descending (LAD) artery, as described in Gao et al. *Cardiovasc Res.* 45:330-38 (2000) (mice); Litwin et al. *Circulation.* 89:345-54 (1994) (rat); and Hood et al. *Cardiovasc Drugs Ther.* 6:233-37 (1992) (dogs). Ligation deprived the heart of oxygen leading to immediate damage to the heart. This damage has been shown to accurately reflect the damage sustained by a human heart during MI. (same citations) In addition, this experimental model is known in the art to be predict of response to treatment for heart failure (*e.g.* chronic ischemic heart failure).

[0328] After ligation the heart, the mice were intramyocardially injected with the negative control vector (AAV5:GFP) or the bicistronic vector (labeled AAV5:MyΔ3A) at a dose of 1.2×10^{11} genome copies (GC). Systemic administration of a vector, in particular a vector capable of transcytosis (such as, without limitation, AAV8, AAV9, AAVrh10, or selected variants of any known AAV serotype), would be expected to achieve the same result as intracardiac injection, at an adjusted dose (such as by multiplying the dose for intracardiac injection by the weight of the subject in kilograms, or using a dose determined by dosage ranging studies). Cardiac function (ejection fraction) was evaluated by echocardiography at 2, 4 and 7 weeks post-MI. Imaging revealed that mice injected with AAV5:MyΔ3A showed a statistically significant improvement in ejection fraction compared to the mice injected with AAV5:GFP at 4 and 7 weeks post-MI. (n=6-13 for each group. ** $p < 0.01$). Results are shown in FIG. 17B.

[0329] The ligation procedure was repeated on a second cohort of mice. After ligation, the heart was immediately injectioned, intramyocardially, with the vehicle control vector (Hank's Balanced Salt Solution, HBSS) or the monocistronic vector (labeled AAV5:MyΔ3A) again at a dose of 1.2×10^{11} genome copies (GC). Heart function was followed up by echocardiography every two weeks until 8 weeks post-MI. Imaging revealed cardiac function (ejection fraction) in rats injected with vehicle (HBSS) continued to decline after MI. Rats injected with AAV5:MyΔ3A showed a

statistically significant improvement in ejection fraction 4-8 weeks post-MI. (n=8 for each group. * $p<0.05$ *** $p<0.001$). Results are shown in FIG. 18B. Given that the heart weight ratio of mice or rats to humans is about 1,000, a dose of between about 10^{13} GC to about 10^{14} GC is tested for efficacy in humans.

[0330] Additional testing was performed in a rat model of chronic myocardial infarction (CMI). The two week time point after LAD ligation for injection of viral vectors was chosen because this is after the phase of fibroblast proliferation post-MI (Fu et al. *J. Clin. Invest.* 128:2127–43 (2018)). **FIGs. 19A – FIG. 19B** show *in vivo* testing of an AAV-delivered myocardin and ASCL1 in a monocistronic format (AAV5:My Δ 3A). AAV5:My Δ 3A improves ejection fraction in a rat model of chronic heart failure due to myocardial infarction (MI). HBSS or AAV5:My Δ 3A at dose 5×10^{11} GC (My Δ 3 and ASCL1 transcripts were expressed from a single transcript including a 2A peptide) was delivered by intramyocardial injection at 2 weeks post permanent coronary ligation. **FIG. 19A** shows heart function in treated (AAV5:My Δ 3A) and vehicle control (HBSS) groups assessed by echocardiography every two weeks until 10 weeks post-MI. Echocardiography revealed that cardiac function in rats injected with vehicle (HBSS) continued to decline after MI. AAV5:My Δ 3A halted this decline in heart function. **FIG. 19B** shows that AAV5:My Δ 3A treatment caused a statistically significant improvement in ejection fraction 6-10 weeks post-MI compared to animals with vehicle. (n=10 for each group. * $p<0.05$ ** $p<0.01$).

[0331] These data from mice and rats demonstrate that AAV delivery of My Δ 3A can provide functional benefit *in vivo* in both acute myocardial infarction (AMI) and chronic myocardial infarction (CMI). Heart failure due to AMI is substantially reversed. Progression of heart failure due to CMI is halted.

CLAIMS

What is claimed is:

1. A vector, comprising a *MYOCD* polynucleotide and either or both of an *ASCL1* polynucleotide or a *MYF6* polynucleotide; each polynucleotide operatively linked to at least one promoter.
2. The vector of claim 1, wherein the vector comprises a *MYOCD* polynucleotide and an *ASCL1* polynucleotide.
3. The vector of claim 1 or claim 2, wherein the vector comprises a *MYOCD* polynucleotide and a *MYF6* polynucleotide.
4. The vector of claim 1, wherein the vector comprises a *MYOCD* polynucleotide; either or both of a *MEF2C* polynucleotide and a *TBX5* polynucleotide; and either or both of an *ASCL1* polynucleotide and a *MYF6* polynucleotide.
5. The vector of claim 4, wherein the vector comprises a *MYOCD* polynucleotide, an *ASCL1* polynucleotide, a *MEF2C* polynucleotide, and a *TBX5* polynucleotide.
6. The vector of claim 4, wherein the vector comprises a *MYOCD* polynucleotide, a *MYF6* polynucleotide, a *MEF2C* polynucleotide, and a *TBX5* polynucleotide.
7. The vector of any one of claims 1 to 6, wherein the *MYOCD* is an engineered myocardin.
8. The vector of any one of claims 1 to 7, wherein the vector comprises no reprogramming factor polynucleotide other than a *MYOCD* polynucleotide, an *ASCL1* polynucleotide, a *MYF6* polynucleotide, a *MEF2C* polynucleotide, or a *TBX5* polynucleotide.
9. The vector of any one of claims 1 to 8, wherein the vector comprises no other other protein-coding gene.
10. The vector of any one of claims 1 to 9, wherein the vector is a polycistronic vector.
11. The vector of any one of claims 1 to 10, wherein the vector is a viral vector.

12. The vector of claim 11, wherein the vector is an AAV vector.
13. The vector of claim 11, wherein the vector is lentiviral vector.
14. The vector of any one of claims 1 to 13, wherein the *ASCLI* polynucleotide, if present, or the *MYF6* polynucleotide, if present, and the *MYOCD* polynucleotide are operatively linked to the same promoter and are co-translationally expressed.
15. The vector of any one of claim 2 or claims 7 to 13, wherein the vector comprises a *MYOCD-2A-ASCLI* polynucleotide or an *ASCLI-2A-MYOCD* polynucleotide, operatively linked to a single promoter.
16. The vector of any one of claim 3 or claims 7 to 13, wherein the vector comprises a *MYOCD-2A-MYF6* polynucleotide or an *ASCLI-2A-MYF6* polynucleotide, operatively linked to a single promoter.
17. The vector of any one of claims 1 to 16, wherein the *MYOCD* polynucleotide comprises the nucleotide sequence of human *MYOCD* (SEQ ID NO: 4) or a codon variant thereof.
18. The vector of any one of claims 1 to 17, wherein the *MYOCD* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *MYOCD* (SEQ ID NO: 4).
19. The vector of any one of claims 1 to 18, wherein the *MYOCD* polynucleotide encodes human MYOCD (SEQ ID NO: 3) or a functional variant thereof .
20. The vector of any one of claims 1 to 19, wherein the *MYOCD* polynucleotide encodes a polypeptide that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to human MYOCD (SEQ ID NO: 3).
21. The vector of any one of claims 1 to 16, wherein the *MYOCD* polynucleotide comprises the nucleotide sequence of *Myh43* (SEQ ID NO: 72) or a codon variant thereof.

22. The vector of any one of claims 1-16 or 21, wherein the *MYOCD* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of *MyΔ3* (SEQ ID NO: 72).
23. The vector of any one of claims 1-16 or 21-22, wherein the *MYOCD* polynucleotide encodes MyΔ3 (SEQ ID NO: 16) or a functional variant thereof.
24. The vector of any one of claims 1-16 or 21-23, wherein the *MYOCD* polynucleotide encodes a polypeptide that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to MyΔ3 (SEQ ID NO: 16) or a codon variant thereof.
25. The vector of any one of claims 1 to 24, wherein the *ASCL1* polynucleotide comprises the nucleotide sequence of human *ASCL1* (SEQ ID NO: 2) or a codon variant thereof.
26. The vector of any one of claims 1 to 25, wherein the *ASCL1* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *ASCL1* (SEQ ID NO: 2) or a codon variant thereof.
27. The vector of any one of claims 1 to 25, wherein the *ASCL1* polynucleotide encodes a polypeptide that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to human *ASCL1* (SEQ ID NO: 1).
28. The vector of any one of claims 1 to 25, wherein the *ASCL1* polynucleotide encodes human ASCL1 (SEQ ID NO: 1).
29. The vector of any one of claims 1 to 24, wherein the *MYF6* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *MYF6* (SEQ ID NO: 56).
30. The vector of claim 11, wherein the vector comprises an expression cassette, the expression cassette comprising:
 - a. the *MYOCD* polynucleotide, wherein the *MYOCD* polynucleotide comprises *MyΔ3* (SEQ ID NO: 72) or a codon variant thereof, and/or encodes MyΔ3 (SEQ

- ID NO: 16) or a functional variant thereof, the *MYOCD* polynucleotide operatively linked to a first promoter; and
- b. the *ASCL1* polynucleotide, wherein the *ASCL1* polynucleotide comprises *ASCL1* (SEQ ID NO: 2) or a codon variant thereof, and/or encodes ASCL1 (SEQ ID NO: 1) or a functional variant thereof, the *ASCL1* polynucleotide operatively linked to a second promoter.
31. The vector of claim 30, wherein the first promoter shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a CAG promoter (SEQ ID NO: 67) and/or the second promoter shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a SCP promoter (SEQ ID NO: 68).
32. The vector of claim 11, wherein the vector comprises an expression cassette, the expression cassette comprising a *MYOCD-2A-ASCL1* polynucleotide, operatively linked to a single promoter.
33. The vector of claim 32, wherein the *MYOCD-2A-ASCL1* polynucleotide comprises SEQ ID NO: 37 or a codon variant thereof.
34. The vector of claim 32 or claim 33, wherein the *MYOCD-2A-ASCL1* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 37.
35. The vector of claim 32, wherein the *MYOCD-2A-ASCL1* polynucleotide encodes SEQ ID NO: 59 or a functional variant thereof.
36. The vector of claim 32, wherein the *MYOCD-2A-ASCL1* polynucleotide encodes a polypeptide that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 59.
37. The vector of any one of claims 32 to 36, wherein the expression cassette comprises one or more of an SV40 intron (SEQ ID NO: 73), a short polyA signal (SEQ ID NO: 74) and a WPRE (SEQ ID NO: 75).

38. The vector of any one of claims 32 to 37, wherein the expression cassette is flanked by inverted terminal repeats, optionally AAV2 ITRs (SEQ ID NO: 76).
39. The vector of any one of claims 32 to 38, wherein the vector comprises an AAV5 capsid protein, optionally comprising SEQ ID NO: 71 or a functional variant thereof.
40. The vector of any one of claims 1 to 39, wherein the vector is capable of reprogramming differentiated cells into induced cardiomyocyte (iCM) cells.
41. The vector of claim 40, wherein at least 2.5%, at least 5%, at least 10%, at least 15%, or at least 20% of iCM cells are α -actinin positive.
42. The vector of claim 41, wherein at least 2%, at least 5%, or at least 8% of iCM cells are cTnT positive.
43. A vector system, comprising a first vector encoding a *MYOCD* polynucleotide and either or both of an *ASCL1* polynucleotide or a *MYF6* polynucleotide; and a second vector comprising a *MEF2C* polynucleotide and a *TBX5* polynucleotide.
44. The vector system of claim 22, wherein the first vector comprises a *MYOCD-2A-ASCL1* polynucleotide or an *ASCL1-2A-MYOCD* polynucleotide.
45. The vector system of claim 22, wherein the first vector comprises a *MYOCD-2A-MYF6* polynucleotide or an *ASCL1-2A-MYF6* polynucleotide.
46. A vector system, comprising a first vector encoding a *MYOCD* polynucleotide and a second vector encoding an *ASCL1* polynucleotide or a *MYF6* polynucleotide.
47. The vector system of any one of claims 43 to 46, wherein the first vector and the second vector are each independently selected from a lipid nanoparticle, a transposon, an adeno-associated virus (AAV) vector, an adenovirus, a retrovirus, an integrating lentiviral vector (LVV), and a non-integrating LVV.

48. The vector system of any one of claims 43 to 47, wherein the *ASCL1* polynucleotide shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the nucleotide sequence of human *ASCL1* (SEQ ID NO: 2).
49. The vector system of any one of claims 43 to 48, wherein the *MYOCD* polynucleotide encodes human MYOCD (SEQ ID NO: 3) or a functional variant thereof or MyΔ3 (SEQ ID NO: 16) or a functional variant thereof.
50. A method of inducing a cardiomyocyte phenotype in differentiated cells, comprising contacting the differentiated cells with the vector of any one of claims 1 to 42 or the vector system of any one of claims 43 to 49.
51. The method of claim 50, wherein the differentiated cells are cardiac fibroblast cells.
52. The method of claim 50 or claim 51, wherein the differentiated cells are *in vitro* cells during the contacting step.
53. The method of any one of claims 50 to 52, wherein the differentiated cells are *in vivo* cells in a subject suffering from or at risk for a heart condition.
54. A method of treating a heart condition in a subject suffering from or at risk for the heart condition, comprising contacting the differentiated cells *in vitro* with the vector of any one of claims 1 to 42 or the vector system of any one of claims 43 to 49 to generate iCM cells, and administering the iCM cells to the subject.
55. The method of claims 54, wherein the heart condition is dilated cardiomyopathy.
56. A method of treating a heart condition in a subject suffering from or at risk for a heart condition, comprising administering the the vector of any one of claims 1 to 42 or the vector system of any one of claims 43 to 49 to the subject.
57. The method of claim 56, wherein the heart condition is a myocardial infarction.
58. The method of claim 56, wherein the heart condition is an acute myocardial infarction.

59. The method of claim 56, wherein the heart condition is a heart failure.
60. The method of claim 56, wherein the heart condition is a chronic ischemic heart failure.
61. A kit comprising the vector any one of claims 1 to 42 or the vector system of any one of claims 43 to 49, and instructions for use in treating a heart condition.
62. A method of converting a differentiated non-cardiomyocyte cell into a cardiomyocyte, comprising comprising contacting the differentiated cells with the vector any one of claims 1 to 42 or the vector system of any one of claims 43 to 49.
63. The method of claim 62, wherein the differentiated non-cardiomyocyte cell is a differentiated non-cardiomyocyte cell.
64. The method of claim 62 or claim 63, wherein the differentiated non-cardiomyocyte cell is a human differentiated non-cardiomyocyte cell.
65. The method of any one of claims 62 to 64, wherein the differentiated non-cardiomyocyte cell is an *in vivo* differentiated non-cardiomyocyte cell.
66. The method of any one of claims 62 to 64, wherein the differentiated non-cardiomyocyte cell is an *in vitro* differentiated non-cardiomyocyte cell.
67. The method of any one of claims 61 to 66, wherein the differentiated non-cardiomyocyte cell is a cardiac cell.
68. An isolated polynucleotide, comprising an engineered *MYOCD* polynucleotide encoding an engineered myocardin protein having a length of at most 850 amino acids, wherein the engineered myocardin protein comprises an SRF interaction domain, an SAP domain, and a TAD domain.
69. The polynucleotide of claim 68, wherein the engineered myocardin protein comprises an Mef2c interaction domain.
70. The polynucleotide of claim 69, wherein:

- a. the Mef2c interaction domain shares at least 85% identity to SEQ ID NO: 17,
 - b. the SRF domain shares at least 85% identity to SEQ ID NO: 18,
 - c. the SAP domain shares at least 85% identity to SEQ ID NO: 19, and
 - d. the TAD domain shares at least 85% identity to SEQ ID NO: 11.
71. The polynucleotide of claim 68 or 70, wherein the engineered myocardin protein comprises an LZ domain.
72. The polynucleotide of claim 71, wherein the LZ domain shares at least 85% identity to SEQ ID NO: 20.
73. The polynucleotide of any of claims 68 to 72, wherein the engineered myocardin protein comprises:
- a. a first polypeptide that shares at least 85% identity to residues 5-413 of human myocardin (SEQ ID NO: 10), and
 - b. second polypeptide that shares at least 85% identity to residues 764-986 of human myocardin (SEQ ID NO: 11),
- wherein the first polypeptide and the second polypeptide are linked by a linker comprising a peptide bond or a polypeptide linker of 1-50 amino acid residues.
74. The polynucleotide of any of claims 68 to 72, wherein the engineered myocardin protein comprises:
- a. a first polypeptide that shares at least 85% identity to residues 5-438 of human myocardin (SEQ ID NO: 12), and
 - b. second polypeptide that shares at least 85% identity to residues 764-938 of human myocardin (SEQ ID NO: 11),

wherein the first polypeptide and the second polypeptide are linked by a linker comprising a peptide bond or a polypeptide linker of 1-50 amino acid residues.

75. The polynucleotide of any of claims 68 to 72, wherein the engineered myocardin protein comprises:

- a. a first polypeptide that shares at least 85% identity to residues 5-559 of human myocardin (SEQ ID NO: 13), and
- b. second polypeptide that shares at least 85% identity to residues 764-938 of human myocardin (SEQ ID NO: 11),

wherein the first polypeptide and the second polypeptide are linked by a linker comprising a peptide bond or a polypeptide linker of 1-50 amino acid residues.

76. The polynucleotide of any of claims 73 to 75, wherein the linker consists of a peptide bond.

77. The polynucleotide of any of claims 73 to 75, wherein the linker is a polypeptide selected from G, GG, GGG, GSS, GGS, GGSGGS (SEQ ID NO: 30), GSSGGS (SEQ ID NO: 31), GGSGSS (SEQ ID NO: 32), GGSGGSGGS (SEQ ID NO: 33), GGSGGSGGSGGS (SEQ ID NO: 34).

78. The polynucleotide of claim 76, wherein the engineered myocardin protein comprises a sequence selected from SEQ ID NOs: 14-16.

79. An isolated polynucleotide, comprising an engineered *MYOCD* polynucleotide encoding an engineered myocardin protein, wherein the engineered myocardin protein comprises a deletion of at least 50 amino acids in the region corresponding to amino acids 414-764 of the native myocardin (SEQ ID NO: 3).

80. The polynucleotide of claim 79, wherein the engineered myocardin protein comprises a deletion of amino acids from about 414 to about 763 of the native myocardin (SEQ ID NO: 3).

81. The polynucleotide of claim 80, wherein the engineered myocardin protein comprises a sequence at least 85% identical to SEQ ID NO: 14.
82. The polynucleotide of claim 81, wherein the engineered myocardin protein consists of sequence identical to SEQ ID NO: 14.
83. The polynucleotide of claim 79, wherein the engineered myocardin protein comprises a deletion of amino acids from about 439 to about 763 of the native myocardin (SEQ ID NO: 3).
84. The polynucleotide of claim 83, wherein the engineered myocardin protein comprises a sequence at least 85% identical to SEQ ID NO: 15.
85. The polynucleotide of claim 74, wherein the engineered myocardin protein consists of sequence identical to SEQ ID NO: 15.
86. The polynucleotide of claim 79, wherein the engineered myocardin protein comprises a deletion of amino acids from about 560 to about 763 of the native myocardin (SEQ ID NO: 3).
87. The polynucleotide of claim 86, wherein the engineered myocardin protein comprises a sequence at least 85% identical to SEQ ID NO: 16.
88. The polynucleotide of claim 87, wherein the engineered myocardin protein consists of sequence identical to SEQ ID NO: 16.
89. The polynucleotide of any of claims 68 to 88, wherein the engineered myocardin protein is a functional engineered myocardin protein.
90. The polynucleotide of claim 89, wherein the functional engineered myocardin protein is expressed at at least 10% of the level of native *MYOCD* in the same expression system.
91. The polynucleotide of claim 89 or 90, wherein the functional engineered myocardin protein is capable of inducing increased expression of at least one marker of cardiomyocyte phenotype either (a) when expressed in human cardiac fibroblasts with MEF2C and TBX5

or *TBX5* in the presence of a TGF β inhibitor, optionally SB431542, and a Wnt inhibitor, optionally XAV939, or (b) when expressed in human cardiac fibroblasts with *ASCL1*.

92. The polynucleotide of any of claim 68 to 91, wherein the polynucleotide is operatively linked to a promoter.
93. The polynucleotide of any of claim 68 to 92, wherein the polynucleotide comprises a *MEF2C* polynucleotide and a *TBX5* polynucleotide.
94. The polynucleotide of any of claim 68 to 93, wherein the polynucleotide comprises an *TBX5* polynucleotide.
95. The polynucleotide of any of claim 68 to 93, wherein the polynucleotide comprises an *ASCL1* polynucleotide.
96. The polynucleotide of any of claim 68 to 95, wherein the polynucleotide is flanked by inverted terminal repeats (ITRs) or the polynucleotide is flanked by long terminal repeats (LTRs).
97. A recombinant virus comprising the polynucleotide of any of claims 68 to 96.
98. The recombinant virus of claim 97, wherein the recombinant virus is a recombinant adeno-associated virus (rAAV).
99. The recombinant virus of claim 97, wherein the recombinant virus is a lentivirus.
100. A population of cells comprising the polynucleotide of any of claim 68 to 96.
101. The population of cells of claim 100, wherein the percentage of cells in the population that exhibit calcium transients (CaT) in an *in vitro* CaT assay is at least 2-fold greater than the percentage of cells in a control population of cells not comprising the polynucleotide in the same *in vitro* CaT assay.
102. An induced cardiomyocyte (iCM), wherein the iCM comprises the polynucleotide of any of claims 68 to 96.

103. A pharmaceutical composition comprising the polynucleotide of any of claims 68 to 96 and a non-viral delivery system.
104. A pharmaceutical composition comprising the recombinant virus of any of claims 97 to 99.
105. A method of generating an induced cardiomyocyte (iCM), comprising contacting a mammalian fibroblast with the polynucleotide of any of claims 68 to 96, the recombinant virus of any of claims 97 to 99, or the pharmaceutical composition of claim 103 or claim 104.
106. A method of treating a heart condition in a subject, comprising administering the pharmaceutical composition of claim 105 to the subject.
107. The method of claim 106, wherein the recombinant virus is administered via intracardiac catheterization.
108. The method of claim 106, wherein the recombinant virus is administered via intracardiac injection.
109. A recombinant adeno-associated virus (rAAV), comprising an expression cassette, wherein the expression cassette comprises in 5' to 3' order a 5' inverted terminal repeat (ITR), a CAG promoter, an SV40 intron, a polynucleotide encoding one or more proteins, a short polyadenylation signal, and a 3' ITR, and wherein the polynucleotide encoding one or more proteins comprises in 5' to 3' order a polynucleotide encoding My Δ 3, a polynucleotide encoding a P2A linker, and a polynucleotide encoding ASCL1.
110. The rAAV of claim 109, wherein the expression cassette comprises a WPRE.
111. The rAAV of claim 109 or 110, wherein the My Δ 3 comprises SEQ ID NO: 16.
112. The rAAV of any of claims 109 to 111, wherein the 2A linker comprises ATNFSLLKQAGDVEENPGP (SEQ ID NO: 23).

113. The rAAV of any of claims 109 to 111, wherein the ASCL1 comprises SEQ ID NO: 1.
114. The rAAV of claim 113, wherein the rAAV comprises a polynucleotide at least 95% identical to SEQ ID NO: 35 (My Δ 3A AAV).
115. A pharmaceutical composition comprises the rAAV of any of claims 109 to 114.
116. A method of treating a heart condition in a subject, comprising administering the pharmaceutical composition of claim 115 to the subject.