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(54) Title: METHODS FOR GENERATING CARDIOMYOCYTES

(57) Abstract: The present disclosure provides method of generating cardiomyocytes from post-natal fibroblasts. The present disclosure further provides cells and compositions for use in generating cardiomyocytes.

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METHODS FOR GENERATING CARDIOMYOCYTES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/328,988, filed April 28, 2010, and of U.S. Provisional Patent Application No. 61/364,295, filed July 14, 2010, which applications are incorporated herein by reference in their entirety.

BACKGROUND

[0002] Heart disease is a leading cause of adult and childhood mortality in the western world. The underlying pathology is typically loss of cardiomyocytes that leads to heart failure, or improper development of cardiomyocytes during embryogenesis that leads to congenital heart malformations. Because cardiomyocytes have little or no regenerative capacity, current therapeutic approaches are limited. Embryonic stem cells possess clear cardiogenic potential, but efficiency of cardiac differentiation, risk of tumor formation, and issues of cellular rejection must be overcome.

[0003] There is a need in the art for methods of generating cardiomyocytes.

Literature

[0004] U.S. Patent Publication No. 2009/0208465; U.S. Patent No. 7,682,828; U.S. Patent Publication No. 2010/0075421; WO 2009/152484; WO 2009/152485; Takahashi and Yamanaka (2006) *Cell* 126:663.

SUMMARY OF THE INVENTION

[0005] The present disclosure provides method of generating cardiomyocytes from post-natal fibroblasts. The present disclosure further provides cells and compositions for use in generating cardiomyocytes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] Figures 1A-I depict the results of screening for cardiomyocyte-inducing factors.

[0007] Figures 2A-F depict the effect of various factors on cardiac gene expression in fibroblasts.

[0008] Figures 3A-J depict reprogramming of cardiac fibroblasts directly into cardiomyocytes.

[0009] Figures 4A-D depict reprogramming of gene expression in induced cardiomyocytes (iCMs).

[0010] Figures 5A-D depict spontaneous Ca^{2+} flux, electrical activity, and beating in iCMs.

[0011] Figures 6A-C depict *in vivo* reprogramming of cardiac fibroblasts into cardiomyocytes.

[0012] Figures 7A-G depict *in vivo* reprogramming of cardiac fibroblasts to cardiomyocyte-like cells.

[0013] Figures 8A-N depict single-cell analysis of cardiac reprogramming *in vivo*.

[0014] Figure 9 depicts intracellular recordings showing action potentials for additional *in vivo* reprogrammed iCMs.

[0015] Figures 10A-D depict determination of area at risk (AAR) and infarct size for dsRed or GMT (expression vector encoding three factors: Gata4-Mef2c-Tbx-5) injected hearts after coronary ligation and echocardiography data.

[0016] Figures 11A-D depict the effect of *in vivo* delivery of cardiac reprogramming factors on cardiac function after myocardial infarction.

[0017] Figures 12A-F and Figures 13A-E depict the effect of thymosin β4 on cardiac fibroblasts upon injury and on *in vivo* reprogramming.

DEFINITIONS

[0018] The terms "polypeptide," "peptide," and "protein", used interchangeably herein, 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.

[0019] The terms "nucleic acid" and "polynucleotide" are used interchangeably herein 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.

[0020] The term “operably linked” refers to functional linkage between molecules to provide a desired function. For example, “operably linked” in the context of nucleic acids refers to a functional linkage between nucleic acids to provide a desired function such as transcription, translation, and the like, e.g., a functional linkage between a nucleic acid expression control sequence (such as a promoter, signal sequence, or array of transcription factor binding sites) and a second polynucleotide, wherein the expression control sequence affects transcription and/or translation of the second polynucleotide.

[0021] As used herein the term “isolated” with reference to a cell, refers to a cell that is in an environment different from that in which the cell naturally occurs, e.g., where the cell naturally occurs in a multicellular organism, and the cell is removed from the multicellular organism, the cell is “isolated.” An isolated genetically modified host cell can be present in a mixed population of genetically modified host cells, or in a mixed population comprising genetically modified host cells and host cells that are not genetically modified. For example, an isolated genetically modified host cell can be present in a mixed population of genetically modified host cells *in vitro*, or in a mixed *in vitro* population comprising genetically modified host cells and host cells that are not genetically modified.

[0022] A “host cell,” as used herein, denotes an *in vivo* or *in vitro* cell (e.g., a eukaryotic cell cultured as a unicellular entity), which eukaryotic cell can be, or has been, used as recipients for a nucleic acid (e.g., an exogenous nucleic acid) or an exogenous polypeptide(s), and include the progeny of the original cell which has been modified by introduction of the exogenous polypeptide(s) or genetically modified by the nucleic acid. It is understood that the progeny of a single cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

[0023] The term “genetic modification” and 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 (“modification”) can be accomplished by incorporation of the new nucleic acid into the genome of the host 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.

[0024] As used herein, the term “exogenous nucleic acid” refers to a nucleic acid that is not normally or naturally found in and/or produced by a cell in nature, and/or that is introduced into the cell (e.g., by electroporation, transfection, infection, lipofection, or any other means of introducing a nucleic acid into a cell).

[0025] The terms “individual,” "subject," “host,” and "patient," used interchangeably herein, refer to a mammal, including, but not limited to, murines (rats, mice), non-human primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), etc. In some embodiments, the individual is a human. In some embodiments, the individual is a murine.

[0026] A "therapeutically effective amount" or "efficacious amount" means the amount of a compound or a number of cells that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the compound or the cell, the disease and its severity and the age, weight, etc., of the subject to be treated.

[0027] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0028] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0029] 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 herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0030] 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 “an induced cardiomyocyte” includes a plurality of such cardiomyocytes and reference to “the post-natal fibroblast” includes reference to one or more post-natal fibroblasts and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

[0031] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0032] The present disclosure provides method of generating cardiomyocytes from post-natal fibroblasts. The present disclosure further provides cells and compositions for use in generating cardiomyocytes.

METHODS OF GENERATING CARDIOMYOCYTES

[0033] The present disclosure provides method of generating cardiomyocytes from post-natal fibroblasts. The methods generally involve introducing into a post-natal fibroblast one or more reprogramming factors. In some cases, the polypeptides themselves are introduced into a post-natal fibroblast. In other cases, the post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding the re-programming factors.

[0034] In some embodiments, the methods involve introducing into a post-natal fibroblast one or more reprogramming factors selected from Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides. In some cases, the polypeptides themselves are introduced into a post-natal fibroblast. In other cases, the post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides.

[0035] In some embodiments, the methods involve introducing into a post-natal fibroblast three (and only three) reprogramming factors: Gata4, Mef2c, and Tbx5 polypeptides. In some cases, the polypeptides themselves are introduced into a post-natal fibroblast. In other cases, the post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides.

[0036] Cardiomyocytes generated directly from post-natal fibroblasts using a subject method are referred to herein as “induced cardiomyocytes.” Polypeptides such as Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf are also referred to collectively herein as “reprogramming factors,” or “reprogramming transcription factors.” A post-natal fibroblast into one or more reprogramming factors are introduced is reprogrammed directly into a differentiated cardiomyocyte, without first becoming a stem cell or a progenitor cell.

[0037] As noted above, in some cases, a subject method of generating a cardiomyocyte involves genetically modifying a post-natal fibroblast with one or more nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides. The reprogramming factors encoded by the nucleotide sequences are produced in the post-natal fibroblast and, as a result of the production of the one or more reprogramming factors, the genetically modified fibroblast is reprogrammed directly into a differentiated cardiomyocyte. The genetically modified fibroblast is reprogrammed directly into a differentiated cardiomyocyte, without first becoming a stem cell or progenitor cell.

[0038] As noted above, in some cases, a subject method of generating a cardiomyocyte involves genetically modifying a post-natal fibroblast with one or more nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides. The Gata4, Mef2c, and Tbx5 polypeptides are produced in the post-natal fibroblast and, as a result of the production of the Gata4, Mef2c, and Tbx5 polypeptides, the genetically modified fibroblast is reprogrammed directly into a differentiated cardiomyocyte. The genetically modified fibroblast is reprogrammed directly into a differentiated cardiomyocyte, without first becoming a stem cell or progenitor cell.

[0039] In some cases, a post-natal fibroblast is modified by introducing one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides themselves into a host post-natal fibroblast. A post-natal fibroblast into which one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides has been introduced (either by introducing the polypeptides themselves or by introducing one or more nucleic acids comprising nucleotide sequence encoding the one or more polypeptides) is referred to as a “modified fibroblast” or a “modified post-natal fibroblast.” A post-natal fibroblast into which one or more nucleic acids comprising nucleotide sequence encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides has been introduced is referred to as a “genetically modified fibroblast” or a “genetically modified post-natal fibroblast.”

[0040] In some cases, a post-natal fibroblast is modified by introducing Gata4, Mef2c, and Tbx5 polypeptides themselves into a host post-natal fibroblast. A post-natal fibroblast into which Gata4, Mef2c, and Tbx5 polypeptides have been introduced (either by introducing the polypeptides themselves or by introducing one or more nucleic acids comprising nucleotide sequence encoding Gata4, Mef2c, and Tbx5 polypeptides) is referred to as a “modified fibroblast” or a “modified post-natal fibroblast.” A post-natal fibroblast into which one or more nucleic acids comprising nucleotide sequence encoding Gata4, Mef2c, and Tbx5 polypeptides have been introduced is referred to as a “genetically modified fibroblast” or a “genetically modified post-natal fibroblast.”

[0041] As noted above, using a subject method, one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) are introduced into a post-natal fibroblast (e.g., the reprogramming factor polypeptide(s) themselves are introduced into a post-natal fibroblast; or a post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding reprogramming factor polypeptide(s)), and as a result, the modified fibroblast is reprogrammed directly into a differentiated cardiomyocyte, without first becoming a stem cell or progenitor cell. Thus, for example, the modified or genetically modified fibroblast does not produce detectable levels of an early cardiac progenitor marker. For example, the modified or genetically modified fibroblast does not produce detectable levels of Isl1, an early cardiac progenitor marker that is transiently expressed before cardiac differentiation.

[0042] In some embodiments, a post-natal fibroblast is genetically modified *in vitro* with one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides); or one or more reprogramming factor polypeptides themselves (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) are introduced *in vitro* into a post-natal fibroblast; where the modified or genetically modified fibroblasts become cardiomyocytes *in vitro*. Once the fibroblasts are reprogrammed directly into cardiomyocytes *in vitro*, generating induced cardiomyocytes, the induced cardiomyocytes can be introduced into an individual.

[0043] For example, in some embodiments, a subject method involves: a) genetically modifying a post-natal fibroblast *in vitro* with one or more nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, where production of the encoded polypeptides in the genetically modified fibroblasts results in reprogramming of the genetically modified fibroblast directly into a cardiomyocyte *in vitro*, thereby generating an induced cardiomyocyte; and b) introducing the induced cardiomyocyte(s) into an individual. In other embodiments, a subject method involves: a) introducing one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides into a post-natal fibroblast *in vitro*, generating a modified fibroblast, where the modified fibroblast, as a result of introduction of the one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, is reprogrammed directly into a cardiomyocyte *in vitro*, thereby generating an induced cardiomyocyte; and b) introducing the induced cardiomyocyte into an individual.

[0044] As another example, in some embodiments, a subject method involves: a) genetically modifying a post-natal fibroblast *in vitro* with one or more nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides, where production of the Gata4, Mef2c, and Tbx5 polypeptides in the genetically modified fibroblasts results in reprogramming of the genetically modified fibroblast directly into a cardiomyocyte *in vitro*, thereby generating an induced cardiomyocyte; and b) introducing the induced cardiomyocyte(s) into an individual. In other embodiments, a subject method involves: a) introducing Gata4, Mef2c, and Tbx5 polypeptides into a post-natal fibroblast *in vitro*, generating a modified fibroblast, where the modified fibroblast, as a result of introduction of the Gata4, Mef2c, and Tbx5 polypeptides, is reprogrammed directly into a cardiomyocyte *in vitro*, thereby generating an induced cardiomyocyte; and b) introducing the induced cardiomyocyte into an individual.

[0045] In other embodiments, a post-natal fibroblast is genetically modified *in vitro* with one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides); or one or more reprogramming factor polypeptides themselves (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) are introduced *in vitro* into a post-natal fibroblast; and the modified or genetically modified fibroblasts are introduced into an individual, where the modified or genetically modified fibroblasts are reprogrammed directly into cardiomyocytes *in vivo*.

[0046] Thus, for example, in some embodiments, a subject method involves: a) genetically modifying a post-natal fibroblast *in vitro* with one or more nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; polypeptides; and b) introducing the genetically modified fibroblasts into an individual, where production of the one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; polypeptides in the genetically modified fibroblasts results in reprogramming of the genetically modified fibroblast directly into a cardiomyocyte *in vivo*, thereby generating an induced cardiomyocyte in the individual. In other embodiments, a subject method involves: a) introducing one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; polypeptides into a post-natal fibroblast *in vitro*, generating a modified fibroblast; and b) introducing the modified fibroblast(s) into an individual, where the modified fibroblasts, as a result of introduction of the one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; polypeptides, are reprogrammed directly into a cardiomyocyte *in vivo*, thereby generating an induced cardiomyocyte in the individual.

[0047] As another example, in some embodiments, a subject method involves: a) genetically modifying a post-natal fibroblast *in vitro* with one or more nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides; and b) introducing the genetically modified fibroblasts into an individual, where production of the Gata4, Mef2c, and Tbx5 polypeptides in the genetically modified fibroblasts results in reprogramming of the genetically modified fibroblast directly into a cardiomyocyte *in vivo*, thereby generating an induced cardiomyocyte in the individual. In other embodiments, a subject method involves: a) introducing Gata4, Mef2c, and Tbx5 polypeptides into a post-natal fibroblast *in vitro*, generating a modified fibroblast; and b) introducing the modified fibroblast(s) into an individual, where the modified fibroblasts, as a result of introduction of the Gata4, Mef2c, and Tbx5 polypeptides, are reprogrammed directly into a cardiomyocyte *in vivo*, thereby generating an induced cardiomyocyte in the individual.

[0048] In other embodiments, a post-natal fibroblast is genetically modified *in vivo* with one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides), or one or more reprogramming factor polypeptides themselves (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) are introduced *in vivo* into a post-natal fibroblast; and the modified or genetically modified fibroblasts are reprogrammed directly into cardiomyocytes *in vivo*.

[0049] A post-natal fibroblast that is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides), or that is modified with one or more reprogramming factor polypeptides themselves (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides), is reprogrammed into a differentiated cardiomyocyte in a time period of from about 5 days to about 7 days, or from about 7 days to about 14 days. For example, where a population of post-natal fibroblasts is genetically modified or modified by introducing reprogramming factor polypeptides, as described above, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 50%, at least about 75%, or more than 75% (e.g., at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or more than 98%), of the population is reprogrammed into differentiated cardiomyocytes (induced cardiomyocytes) in a time period of from about 5 days to about 7 days, from about 7 days to about 14 days, or from about 2 weeks to about 4 weeks.

[0050] In some embodiments, where a population of post-natal fibroblasts is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides), or where a population of post-natal fibroblasts is modified by introducing one or more reprogramming factors themselves (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) into the fibroblasts, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 50%, at least about 75%, or more than 75% (e.g., at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or more than 98%), of the population is $cTnT^+$ (i.e., expresses cardiac troponin T) in a time period of from about 5 days to about 7 days, from about 7 days to about 14 days, or from about 2 weeks to about 4 weeks.

[0051] In some embodiments, a subject method of generating induced cardiomyocytes involves genetically modifying a host post-natal fibroblast (or a population of host post-natal fibroblasts) with one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides); generating a population of genetically modified post-natal fibroblasts, and, after a time (e.g., 5 days to 7 days, 1 week to 2 weeks, or 2 weeks to 4 weeks), sorting the population of genetically modified post-natal fibroblasts to enrich for cardiomyocytes. The population of genetically modified post-natal fibroblasts can be sorted for expression of a fibroblast-specific marker, to remove any remaining fibroblasts. The population of genetically modified post-natal fibroblasts can be sorted for expression of a cardiomyocyte-specific marker.

[0052] In some embodiments, a subject method of generating induced cardiomyocytes involves modifying a host post-natal fibroblast (or a population of host post-natal fibroblasts) by introducing one or more reprogramming factor polypeptides (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) into host post-natal fibroblasts; generating a population of modified post-natal fibroblasts, and, after a time (e.g., 5 days to 7 days, 1 week to 2 weeks, or 2 weeks to 4 weeks), sorting the population of modified post-natal fibroblasts to enrich for cardiomyocytes. The population of modified post-natal fibroblasts can be sorted for expression of a fibroblast-specific marker, to remove any remaining fibroblasts. The population of modified post-natal fibroblasts can be sorted for expression of a cardiomyocyte-specific marker.

[0053] In some embodiments, a host post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides), or is modified by introducing one or more reprogramming factor polypeptides (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) into host post-natal fibroblasts); and is also genetically modified with a nucleic acid comprising a nucleotide sequence encoding a detectable marker (e.g., a polypeptide that directly produces a detectable signal; an enzyme that produces a detectable signal upon acting on a substrate), where the detectable marker-encoding nucleotide sequence is operably linked to a cardiomyocyte-specific promoter. Suitable polypeptides that provide a direct detectable signal include a fluorescent protein such as a green fluorescent protein, a yellow fluorescent protein, a blue fluorescent protein, etc. Suitable enzymes that produce a detectable signal upon acting on a substrate include, e.g., luciferase (acting on the substrate luciferin), alkaline phosphatase, and the like. Cardiomyocyte-specific promoters include, e.g., an α-myosin heavy chain promoter; a cTnT promoter; and the like. Expression of the detectable marker can provide for detection of an induced cardiomyocyte; and can provide a means of sorting for induced cardiomyocytes.

[0054] The post-natal fibroblasts that serve as host cells for modification or genetic modification, as described above, can be from any of a variety of sources. Mammalian fibroblasts, e.g., human fibroblasts, murine (e.g., mouse) fibroblasts, rat fibroblasts, porcine fibroblasts, etc., can be used. In some embodiments, the fibroblasts are human fibroblasts. In other embodiments, the fibroblasts are mouse fibroblasts. In other embodiments, the fibroblasts are rat fibroblasts. Thus, a "post-natal fibroblast" refers to a fibroblast obtained from a post-natal mammal, or the progeny of a fibroblast obtained from a post-natal mammal.

[0055] The post-natal fibroblasts can be from any of a variety of tissue sources. For example, cardiac fibroblasts, foreskin fibroblasts, dermal fibroblasts, lung fibroblasts, etc.

[0056] The fibroblasts can be obtained from a living individual. The fibroblasts can be obtained from tissue taken from a living individual. The fibroblasts can be obtained from a recently deceased individual who is considered a suitable organ donor. In some embodiments, the individual is screened for various genetic disorders, viral infections, etc., to determine whether the individual is a suitable source of fibroblasts, where individuals may be excluded on the basis of one or more of a genetic disorder, a viral infection, etc.

[0057] Suitable fibroblasts express markers characteristic of fibroblasts, where such markers include, e.g., vimentin, prolyl-4-hydroxylase (an intracellular enzyme involved in collagen synthesis), fibroblast-specific protein-1 (see, e.g., Strutz et al. (1995) *J. Cell Biol.* 130:393), fibroblast surface antigen, and collagen type 1. In some embodiments, the fibroblasts used as host cells are cardiac fibroblasts, where cardiac fibroblasts can be characterized as Thy1$^+$, vimentin$^+$, and are also negative for c-kit or equivalent of c-kit.

[0058] In general, a fibroblast that is suitable for use as a host cell for modification or genetic modification in accordance with a subject method is non-transformed (e.g., exhibits normal cell proliferation), and is otherwise normal.

[0059] Where the host cells for modification or genetic modification is a population of fibroblasts, the population of fibroblasts are isolated, e.g., the population of fibroblasts is composed of at least about 75% fibroblasts, at least about 80% fibroblasts, at least about 85% fibroblasts, at least about 90% fibroblasts, at least about 95% fibroblasts, at least about 98% fibroblasts, at least about 99% fibroblasts, or greater than 99% fibroblasts.

[0060] Post-natal fibroblasts can be derived from tissue of a non-embryonic subject, a neonatal infant, a child, or an adult. Post-natal fibroblasts 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 post-natal fibroblasts used to generate induced 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.

[0061] Methods of isolating fibroblasts from tissues are known in the art, and any known method can be used. As a non-limiting example, cardiac fibroblasts can be obtained using the method of Ieda et al. (2009) *Dev. Cell* 16:233, or as described in Example 1. Foreskin fibroblasts can be obtained from foreskin tissue (i.e., the skin tissue covering the glans penis; preputium penis) of a male individual, e.g., from an 8-14 day old male individual. The fibroblasts can be obtained by mincing the foreskin tissue, then dissociating the tissue to single cells. Foreskin cell clamps can be dissociated by any means known in the art including physical de-clamping or enzymatic digestion using, for example trypsin.

[0062] As noted above, a post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides), or is modified by introducing one or more reprogramming factor polypeptides themselves (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) into the post-natal fibroblast. Amino acid sequences of such reprogramming factors are known in the art. Nucleotide sequences encoding reprogramming factors are known in the art.

<u>Reprogramming factors</u>

[0063] As discussed above, a post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides), or a post-natal fibroblast is modified by introducing one or more reprogramming factor polypeptides themselves (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) into the post-natal fibroblast.

[0064] In some embodiments, the one or more reprogramming factors includes 1, 2, 3, 4, 5, 6, 7, 8, or 9 of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf. In some embodiments, the one or more reprogramming factors includes all of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf. In some embodiments, the one or more reprogramming factors a subset of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf. Exemplary subsets include:

[0065] 1) Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf;

[0066] 2) Gata4, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf;

[0067] 3) Gata4, Mef2c, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf;

[0068] 4) Gata4, Mef2c, Tbx5, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf;

[0069] 5) Gata4, Mef2c, Tbx5, Mesp1, Isl-1, Myocd, Smyd1, and Srf;

[0070] 6) Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Myocd, Smyd1, and Srf;

[0071] 7) Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Smyd1, and Srf;

[0072] 8) Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, and Srf;

[0073] 9) Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, and Smyd1;

[0074] 10) Gata4, Mef2c, Mesp1, Myocd, Nkx2-5, and Tbx5;

[0075] 11) Mef2c, Mesp1, Myocd, Nkx2-5, and Tbx5;

[0076] 12) Gata4, Mesp1, Myocd, Nkx2-5, and Tbx5;

[0077] 13) Gata4, Mef2c, Myocd, Nkx2-5, and Tbx5;

[0078] 14) Gata4, Mef2c, Mesp1, Nkx2-5, and Tbx5;

[0079] 15) Gata4, Mef2c, Mesp1, Myocd, and Tbx5;

[0080] 16) Gata4, Mef2c, Mesp1, Myocd, and Nkx2-5;

[0081] 17) Mef2c, Mesp1, Myocd, and Tbx5;

[0082] 18) Gata4, Mesp1, Myocd, and Tbx5;

[0083] 19) Gata4, Mef2c, Myocd, and Tbx5;

[0084] 20) Gata4, Mef2c, Mesp1, and Tbx5;

[0085] 21) Gata4, Mef2c, Mesp1, and Myocd;

[0086] 22) Mef2c, Mesp1, and Tbx5;

[0087] 23) Gata4, Mef2c, and Tbx5.

[0088] As indicated above, in some embodiments, the subset of reprogramming factors is Gata4, Mef2c, and Tbx5.

Gata4

[0089] A Gata4 polypeptide is a member of the GATA family zinc-finger transcription factor that recognizes and binds a GATA motif (e.g., recognizes and binds the consensus sequence 5'-AGATAG-3') present in the promoter region of many genes. See, e.g., Huang et al. (1995) *Gene* 155:219. Amino acid sequences for Gata4 polypeptides, and nucleotide sequences encoding Gata4 polypeptides, from a variety of species are known in the art. See, e.g.: 1) GenBank Accession No. NP_002043.2 (*Homo sapiens* Gata4 amino acid sequence; and GenBank Accession No. NM_002052 (*Homo sapiens* Gata4-encoding nucleotide sequence; 2) GenBank Accession No. NP_0032118 (*Mus musculus* Gata4 amino acid sequence); and GenBank Accession No. NM_008092 (*Mus musculus* Gata4-encoding nucleotide sequence); 3) GenBank Accession No. NP_653331 (*Rattus norvegicus* Gata4 amino acid sequence); and GenBank Accession No. NM_144730 (*Rattus norvegicus* Gata4-encoding nucleotide sequence); 4) GenBank Accession No. ABI63575 (*Danio rerio* Gata4 amino acid sequence; and GenBank Accession No. DQ886664 (*Danio rerio* Gata4-encoding nucleotide sequence; and 5) GenBank Accession No. AAH71101.1 (*Xenopus laevis* Gata4 amino acid sequence); and GenBank Accession No. BC071107 (*Xenopus laevis* Gata4-encoding nucleotide sequence).

[0090] In some embodiments, a suitable Gata4 nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 900 nucleotides to about 1000 nucleotides (nt), from about 1000 nt to about 1100 nt, from about 1100 nt to about 1200 nt, or from about 1200 nt to 1329 nt, of the nucleotide sequence depicted in SEQ ID NO:14. In some embodiments, a suitable Gata4 nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 900 nucleotides to about 1000 nucleotides (nt), from about 1000 nt to about 1100 nt, from about 1100 nt to about 1200 nt, or from about 1200 nt to 1323 nt, of the nucleotide sequence depicted in SEQ ID NO:28.

[0091] A suitable Gata4 nucleic acid comprises a nucleotide sequence encoding a Gata4 polypeptide, where in some embodiments, a suitable Gata4 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids (aa) to about 400 aa, or from about 400 aa to 442 aa, of the amino acid sequence depicted in SEQ ID NO:13. In some embodiments, a suitable Gata4 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids (aa) to about 400 aa, or from about 400 aa to 441 aa, of the amino acid sequence depicted in SEQ ID NO:27. The encoded Gata4 polypeptide is biologically active, e.g., recognizes and binds a GATA motif (e.g., recognizes and binds the consensus sequence 5'-AGATAG-3') present in a promoter; and activates transcription of a gene operably linked to the promoter comprising the GATA motif.

[0092] In some embodiments, a polypeptide that is functionally equivalent to a Gata4 polypeptide (or a nucleotide sequence encoding such functional equivalent) is used. For example, in some embodiments, a Gata5 polypeptide (or a nucleotide sequence encoding a Gata5 polypeptide) is used. In other embodiments, a Gata6 polypeptide (or a nucleotide sequence encoding a Gata6 polypeptide) is used.

[0093] Amino acid sequences of Gata5 polypeptides, and nucleotide sequences encoding Gata5 polypeptides, are known in the art. See, e.g., GenBank Accession Nos.: 1) NP_536721 (*Homo sapiens* Gata5 amino acid sequence), and NM_080473 (nucleotide sequence encoding the NP_536721 amino acid sequence); 2) NP_032119 (*Mus musculus* Gata5 amino acid sequence), and NM_008093 (nucleotide sequence encoding the NP_032119 amino acid sequence); and 3) NP_001019487 (*Rattus norvegicus* Gata5 amino acid sequence), and NM_001024316 (nucleotide sequence encoding the NP_001019487 amino acid sequence).

[0094] Amino acid sequences of Gata6 polypeptides, and nucleotide sequences encoding Gata6 polypeptides, are known in the art. See, e.g., GenBank Accession Nos.: 1) NP_005248 (*Homo sapiens* Gata6 amino acid sequence), and NM_005257 (nucleotide sequence encoding the NP_005248 amino acid sequence); 2) NP_062058 (*Rattus norvegicus* Gata6 amino acid sequence) and NM_019185 (nucleotide sequence encoding the NP_062058 amino acid sequence); 3) NP_034388 (*Mus musculus* Gata6 amino acid sequence), and NM_010258 (nucleotide sequence encoding the NP_034388 amino acid sequence).

[0095] In some embodiments, a suitable functional equivalent of a Gata4 polypeptide is a polypeptide having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence of a Gata5 polypeptide or a Gata6 polypeptide.

[0096] In some embodiments, a suitable nucleotide sequence encoding a functional equivalent of a Gata4 polypeptide comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a nucleotide sequence encoding a Gata5 polypeptide or a Gata6 polypeptide.

Mef2c

[0097] Mef2c (myocyte-specific enhancer factor 2c) is a transcription activator that binds specifically to the MEF2 element (e.g., the consensus sequence: 5′-CT(A/t)(a/t)AAATAG-3′) (SEQ ID NO:10) present in the regulatory regions of many muscle-specific genes. See, e.g., Andrés et al. (1995) *J. Biol. Chem.* 270:23246. Mef2c can include one or more post-translational modifications, e.g., phosphorylation on Ser-59 and Ser-396; sumoylation on Lys-391; and acetylation on Lys-4.

[0098] Amino acid sequences of Mef2c polypeptides, and nucleotide sequences encoding Mef2c polypeptides, from a variety of species are known in the art. See, e.g.: 1) GenBank Accession No. XP_001056692 (*Rattus norvegicus* Mef2c amino acid sequence); and GenBank Accession No. XM_001056692 (*Rattus norvegicus* Mef2c-encoding nucleotide sequence); 2) GenBank Accession No. NP_079558.1 (*Mus musculus* Mef2c isoform 2 amino acid sequence); and GenBank Accession No. NM_025282 (*Mus musculus* Mef2c isoform 2-encoding nucleotide sequence); 3) GenBank Accession No. NP_001164008 (*Mus musculus* Mef2c isoform 1 amino acid sequence); and GenBank Accession No. NM_001170537 (*Mus musculus* Mef2c isoform 1-encoding nucleotide sequence); 4) GenBank Accession No. NP_001124477 (*Homo sapiens* Mef2c isoform 2 amino acid sequence); and GenBank Accession No. NM_001131005 (*Homo sapiens* Mef2c isoform 2-encoding nucleotide sequence); 5) GenBank Accession No. NP_002388 (*Homo sapiens* Mef2c isoform 1 amino acid sequence); and GenBank Accession No. NM_002397 (*Homo sapiens* Mef2c isoform 1-encoding nucleotide sequence).

[0099] In some embodiments, a suitable Mef2c nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 900 nucleotides to about 1000 nucleotides (nt), from about 1000 nt to about 1100 nt, from about 1100 nt to about 1200 nt, from about 1200 nt to 1300 nt, or from about 1300 nt to 1392 nt, of the nucleotide sequence depicted in SEQ ID NO:16.

[00100] In some embodiments, a suitable Mef2c nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 900 nucleotides to about 1000 nucleotides (nt), from about 1000 nt to about 1100 nt, from about 1100 nt to about 1200 nt, from about 1200 nt to 1300 nt, or from about 1300 nt to 1422 nt, of the nucleotide sequence depicted in SEQ ID NO:18. In some embodiments, a suitable Mef2c nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 900 nucleotides to about 1000 nucleotides (nt), from about 1000 nt to about 1100 nt, from about 1100 nt to about 1200 nt, or from about 1200 nt to 1296 nt, of the nucleotide sequence depicted in SEQ ID NO:23.

[00101] A suitable Mef2c nucleic acid comprises a nucleotide sequence encoding a Mef2c polypeptide, where in some embodiments a suitable Mef2c polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids (aa) to about 400 aa, or from about 400 aa to 463 aa, of the amino acid sequence depicted in SEQ ID NO:15. The encoded Mef2c polypeptide is biologically active, e.g., recognizes and binds a MEF2C element in a promoter; and activates transcription of a gene operably linked to the promoter.

[00102] In some embodiments, a suitable Mef2c polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids (aa) to about 400 aa, or from about 400 aa to 432 aa, of the amino acid sequence depicted in SEQ ID NO:24. The encoded Mef2c polypeptide is biologically active, e.g., recognizes and binds a MEF2C element in a promoter; and activates transcription of a gene operably linked to the promoter.

[00103] A suitable Mef2c nucleic acid comprises a nucleotide sequence encoding a Mef2c polypeptide, where a suitable Mef2c polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids (aa) to about 400 aa, or from about 400 aa to 473 aa, of the amino acid sequence depicted in SEQ ID NO:17. The encoded Mef2c polypeptide is biologically active, e.g., recognizes and binds a MEF2C element in a promoter; and activates transcription of a gene operably linked to the promoter.

[00104] In some embodiments, a polypeptide that is functionally equivalent to a Mef2c polypeptide (or a nucleotide sequence encoding such functional equivalent) is used. For example, in some embodiments, a Mef2a polypeptide (or a nucleotide sequence encoding a Mef2a polypeptide) is used. In other embodiments, a Mef2b polypeptide (or a nucleotide sequence encoding a Mef2b polypeptide) is used. In other embodiments, a Mef2d polypeptide (or a nucleotide sequence encoding a Mef2d polypeptide) is used.

[00105] Amino acid sequences of Mef2a, Me2b, and Mef2d polypeptides are known, as are nucleotide sequences encoding Mef2a, Me2b, and Mef2d polypeptides. See, e.g., GenBank Accession Nos.: 1) NP_005578.2 (*Homo sapiens* Mef2a isoform 1 amino acid sequence), and NM_005587 (nucleotide sequence encoding the NP_005578.2 amino acid sequence); 2) NP_001124398.1 (*Homo sapiens* Mef2a isoform 2 amino acid sequence), and NM_001130926 (nucleotide sequence encoding the NP_001124398.1 amino acid sequence); 3) NP_001124399.1 (*Homo sapiens* Mef2a isoform 3 amino acid sequence), and NM_001130927 (nucleotide sequence encoding the NP_001124399.1 amino acid sequence); 4) NP_001124400.1 (*Homo sapiens* Mef2a isoform 4 amino acid sequence), and NM_001130928 (nucleotide sequence encoding the NP_001124400.1 amino acid sequence); 5) NP_001139257.1 (*Homo sapiens* Mef2b isoform a amino acid sequence), and NM_001145785 (nucleotide sequence encoding the NP_001139257.1 amino acid sequence); 6) NP_005910.1 (*Homo sapiens* Mef2b isoform b amino acid sequence), and NM_005919 (nucleotide sequence encoding the NP_005910.1 amino acid sequence); 7) NP_032604.2 (*Mus musculus* Mef2b isoform 1 amino acid sequence), and NM_008578 (nucleotide sequence encoding the NP_032604.2 amino acid sequence); 8) NP_001038949.1 (*Mus musculus* Mef2b isoform 2 amino acid sequence), and NM_001045484 (nucleotide sequence encoding the NP_001038949.1 amino acid sequence); 9) NP_005911.1 (*Homo sapiens* Mef2d amino acid sequence), and NM_005920 (nucleotide sequence encoding the NP_005911.1 amino acid sequence); and 10) NP_598426.1 (*Mus musculus* Mef2d amino acid sequence), and NM_133665 (nucleotide sequence encoding the NP_598426.1 amino acid sequence).

[00106] In some embodiments, a suitable functional equivalent of a Mef2c polypeptide is a polypeptide having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence of a Mef2a polypeptide, a Mef2b polypeptide, or a Mef2d polypeptide.

[00107] In some embodiments, a suitable nucleotide sequence encoding a functional equivalent of a Mef2c polypeptide comprises a nucleotide sequence having at least about

80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a nucleotide sequence encoding a Mef2a polypeptide, a Mef2b polypeptide, or a Mef2d polypeptide.

Tbx5

[00108] Tbx5 (T-box transcription factor 5) is a transcription factor that binds to an recognizes a T-box (e.g., an element having the consensus sequence 5'-(A/G)GGTGT-3') in the promoter region of some genes; and activates transcription of genes operably linked to such promoters.

[00109] Amino acid sequences for Tbx5 polypeptides, and nucleotide sequences encoding Tbx5 polypeptides, from a variety of species are known in the art. See, e.g.: 1) GenBank Accession No. CAA70592.1 (*Homo sapiens* Tbx5 amino acid sequence); and GenBank Accession No. Y09445 (*Homo sapiens* Tbx5-encoding nucleotide sequence); 2) GenBank Accession No. NP_000183 (*Homo sapiens* Tbx5 amino acid sequence); and GenBank Accession No. NM_000192 (*Homo sapiens* Tbx5-encoding nucleotide sequence); 3) GenBank Accession No. NP_001009964.1 (*Rattus norvegicus* Tbx5 amino acid sequence); and GenBank Accession No. NM_001009964 (*Rattus norvegicus* Tbx5-encoding nucleotide sequence; 4) GenBank Accession No. NP_035667 (*Mus musculus* Tbx5 amino acid sequence); and NM_011537 (*Mus musculus* Tbx5-encoding nucleotide sequence); 5) GenBank Accession No. NP_001079170 (*Xenopus laevis* Tbx5 amino acid sequence); and GenBank Accession No. NM_001085701 (*Xenopus laevis* Tbx5-encoding nucleotide sequence).

[00110] In some embodiments, a suitable Tbx5 nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 900 nucleotides to about 1000 nucleotides (nt), from about 1200 nt to 1300 nt, from about 1300 nt to about 1400 nt, or from about 1400 nt to about 1500 nt, or from about 1500 nt to 1542 nt of the nucleotide sequence depicted in SEQ ID NO:20. In some embodiments, a suitable Tbx5 nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 900 nucleotides to about 1000 nucleotides (nt), from about 1200 nt to 1300 nt, from about 1300 nt to about

1400 nt, or from about 1400 nt to about 1500 nt, or from about 1500 nt to 1560 nt of the nucleotide sequence depicted in SEQ ID NO:25.

[00111] In some embodiments, a suitable Tbx5 nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 900 nucleotides to about 1000 nucleotides (nt), from about 1200 nt to 1300 nt, from about 1300 nt to about 1400 nt, or from about 1400 nt to about 1500 nt, or from about 1500 nt to 1557, of the nucleotide sequence depicted in SEQ ID NO:22.

[00112] A suitable Tbx5 nucleic acid comprises a nucleotide sequence encoding a Tbx5 polypeptide. In some embodiments, a suitable Tbx5 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids (aa) to about 400 aa, from about 400 aa to about 500 aa, or from about 500 aa to 513 aa, of the amino acid sequence depicted in SEQ ID NO:19. In some embodiments, a suitable Tbx5 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids (aa) to about 400 aa, from about 400 aa to about 500 aa, or from about 500 aa to 518 aa, of the amino acid sequence depicted in SEQ ID NO:26. The encoded Tbx5 polypeptide is biologically active, e.g., recognizes and binds a Tbx5 binding site (e.g., an element having the consensus sequence 5’-(A/G)GGTGT-3’) in a promoter; and activates transcription of a gene operably linked to the promoter.

[00113] A suitable Tbx5 nucleic acid comprises a nucleotide sequence encoding a Tbx5 polypeptide, where in some embodiments a suitable Tbx5 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 350 amino acids (aa) to about 400 aa, from about 400 aa to about 500 aa, or from about 500 aa to 518 aa, of the amino acid sequence depicted in SEQ ID NO:21. The encoded Tbx5 polypeptide is biologically active, e.g., recognizes and binds a Tbx5 binding site (e.g., an element having the consensus sequence 5'-(A/G)GGTGT-3') in a promoter; and activates transcription of a gene operably linked to the promoter.

Mesp1

[00114] In some embodiments, a suitable mesoderm posterior protein 1 (Mesp1) nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 600 nucleotides to about 800 nucleotides (nt), or 804 nt, of the nucleotide sequence depicted in SEQ ID NO:30.

[00115] A suitable Mesp1 nucleic acid comprises a nucleotide sequence encoding a Mesp1 polypeptide, where in some embodiments, a suitable Mesp1 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 200 amino acids (aa) to about 250 aa, or from about 250 aa to 268 aa, of the amino acid sequence depicted in SEQ ID NO:29. The encoded Mesp1 polypeptide is biologically active.

Nkx2-5

[00116] In some embodiments, a suitable Nkx2-5 nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 350 nucleotides to about 450 nucleotides (nt), or 456 nt, of the nucleotide sequence depicted in SEQ ID NO:32. In some embodiments, a suitable Nkx2-5 nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 850 nucleotides to about 950 nucleotides (nt), or 975 nt, of the nucleotide sequence depicted in SEQ ID NO:34. In some embodiments, a suitable Nkx2-5 nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 230 nucleotides to about 330 nucleotides (nt), or 339 nt, of the nucleotide sequence depicted in SEQ ID NO:36.

[00117] A suitable Nkx2-5 nucleic acid comprises a nucleotide sequence encoding a Nkx2-5 polypeptide, where in some embodiments, a suitable Nkx2-5 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 125 amino acids (aa) to about 150 aa, of the amino acid sequence depicted in SEQ ID NO:31. In some embodiments, a suitable Nkx2-5 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 275 amino acids (aa) to about 300 aa, or from about 300 aa to about 324 aa, of the amino acid sequence depicted in SEQ ID NO:33. In some embodiments, a suitable Nkx2-5 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 75 amino acids (aa) to about 100 aa, or from about 100 aa to about 112 aa, of the amino acid sequence depicted in SEQ ID NO:35. The encoded Nkx2-5 polypeptide is biologically active.

Isl-1

[00118] In some embodiments, a suitable islet-1 (Isl-1) nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 900 nucleotides to about 1000 nucleotides (nt), or 1050 nt, of the nucleotide sequence depicted in SEQ ID NO:38.

[00119] A suitable Isl-1 nucleic acid comprises a nucleotide sequence encoding a Isl-1 polypeptide, where in some embodiments, a suitable Isl-1 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids (aa) to about 325 aa, or from about 325 aa to 346 aa, of the amino acid sequence depicted in SEQ ID NO:37. The encoded Isl-1 polypeptide is biologically active.

Myocd

[00120] In some embodiments, a suitable myocardin (Myocd) nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 1500 nucleotides to about 2000 nucleotides (nt), or 2055 nt, of the nucleotide sequence depicted in SEQ ID NO:40. In some embodiments, a suitable Myocd nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 2500 nucleotides to about 2900 nucleotides (nt), or 2961 nt, of the nucleotide sequence depicted in SEQ ID NO:42. In some embodiments, a suitable Myocd nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 2500 nucleotides to about 2800 nucleotides (nt), or 2871 nt, of the nucleotide sequence depicted in SEQ ID NO:44.

[00121] A suitable Myocd nucleic acid comprises a nucleotide sequence encoding a Myocd polypeptide, where in some embodiments, a suitable Myocd polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 600 amino acids (aa) to about 650 aa, or from about 650 aa to 684 aa, of the amino acid sequence depicted in SEQ ID NO:39. In some embodiments, a suitable Myocd polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 900 amino acids (aa) to about 950 aa, or from about 950 aa to 986 aa, of the amino acid sequence depicted in SEQ ID NO:41. In some embodiments, a suitable Myocd polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 900 amino acids (aa) to about 925 aa, or

from about 925 aa to 938 aa, of the amino acid sequence depicted in SEQ ID NO:43. The encoded Myocd polypeptide is biologically active.

Smyd1

[00122] In some embodiments, a suitable Smyd1 nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 1400 nucleotides to about 1450 nucleotides (nt), or from about 1450 nt to 1473 nt, of the nucleotide sequence depicted in SEQ ID NO:46.

[00123] A suitable Smyd1 nucleic acid comprises a nucleotide sequence encoding a Smyd1 polypeptide, where in some embodiments, a suitable Smyd1 polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 400 amino acids (aa) to about 450 aa, or from about 450 aa to 490 aa, of the amino acid sequence depicted in SEQ ID NO:45. The encoded Smyd1 polypeptide is biologically active.

Srf

[00124] In some embodiments, a suitable Srf nucleic acid comprises a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a contiguous stretch of from about 1450 nucleotides to about 1500 nucleotides (nt), or from about 1500 nt to 1527 nt, of the nucleotide sequence depicted in SEQ ID NO:48.

[00125] A suitable Srf nucleic acid comprises a nucleotide sequence encoding a Srf polypeptide, where in some embodiments, a suitable Srf polypeptide comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 450 amino acids (aa) to about 500 aa, or from about 500 aa to 508 aa, of the amino acid sequence depicted in SEQ ID NO:47. The encoded Srf polypeptide is biologically active.

[00126] It has been found that introduction of one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of

Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) is sufficient to reprogram a post-natal fibroblast into a cardiomyocyte. Thus, a post-natal fibroblast can be reprogrammed to become a cardiomyocyte without the need for introducing an induction factor (e.g., any other exogenous polypeptide; any other nucleic acid encoding any other exogenous polypeptide) that would reprogram a fibroblast into a stem cell or progenitor cell into the post-natal fibroblast. For example, a subject method does not require and does not involve introducing into a post-natal fibroblast any of an exogenous Sox2 polypeptide, an exogenous Oct-3/4 polypeptide, an exogenous c-Myc polypeptide, an exogenous Klf4 polypeptide, an exogenous Nanog polypeptide, or an exogenous Lin28 polypeptide. A subject method does not require and does not involve introducing into a post-natal fibroblast a nucleic acid(s) comprising nucleotide sequences encoding any of Sox2, Oct-3/4, c-Myc, Klf4, Nanog, or any other polypeptide that would reprogram a fibroblast into a stem cell or progenitor cell.

[00127] As noted above, to generate an induced cardiomyocyte, a post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides). Induced cardiomyocytes express one or more cardiomyocyte-specific markers, where cardiomyocyte-specific markers include, but are not limited to, cardiac troponin I, cardiac troponin-C, tropomyosin, caveolin-3, myosin heavy chain, myosin light chain-2a, myosin light chain-2v, ryanodine receptor, sarcomeric α-actinin, Nkx2.5, connexin 43, and atrial natriuretic factor. Induced cardiomyocytes can also exhibit sarcomeric structures. Induced cardiomyocytes exhibit increased expression of cardiomyocyte-specific genes Actc1 (cardiac α-actin), Myh6 (α-myosin heavy chain), Ryr2 (ryanodine receptor 2), and Gja1 (connexin43). Expression of fibroblasts markers such as Col1a2 (collagen 1a2) is downregulated in induced cardiomyocytes, compared to fibroblasts from which the iCM is derived.

[00128] The expression of various markers specific to cardiomyocytes is detected by conventional biochemical or immunochemical methods (e.g., enzyme-linked immunosorbent assay; immunohistochemical assay; and the like). Alternatively, expression of 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 data bases such as GenBank; thus, marker-specific sequences needed for use as primers or probes is easily determined.

[00129] Induced cardiomyocytes can also exhibit spontaneous contraction. Whether an induced cardiomyocyte exhibits spontaneous contraction can be determined using standard electrophysiological methods (e.g., patch clamp); a suitable method is described in the Examples.

[00130] Induced cardiomyocytes can also exhibit spontaneous Ca^{2+} oscillations. Ca^{2+} oscillations can be detected using standard methods, e.g., using any of a variety of calcium-sensitive dyes. intracellular Ca^{2+} ion-detecting dyes include, but are not limited to, fura-2, bis-fura 2, indo-1, Quin-2, Quin-2 AM, Benzothiaza-1, Benzothiaza-2, indo-5F, Fura-FF, BTC, Mag-Fura-2, Mag-Fura-5, Mag-Indo-1, fluo-3, rhod-2, rhod-3, fura-4F, fura-5F, fura-6F, fluo-4, fluo-5F, fluo-5N, Oregon Green 488 BAPTA, Calcium Green, Calcein, Fura-C18, Calcium Green-C18, Calcium Orange, Calcium Crimson, Calcium Green-5N, Magnesium Green, Oregon Green 488 BAPTA-1, Oregon Green 488 BAPTA-2, X-rhod-1, Fura Red, Rhod-5F, Rhod-5N, X-Rhod-5N, Mag-Rhod-2, Mag-X-Rhod-1, Fluo-5N, Fluo-5F, Fluo-4FF, Mag-Fluo-4, Aequorin, dextran conjugates or any other derivatives of any of these dyes, and others (see, e.g., the catalog or Internet site for Molecular Probes, Eugene, see, also, Nuccitelli, ed., *Methods in Cell Biology, Volume* 40: *A Practical Guide to the Study of Calcium in Living Cells*, Academic Press (1994); Lambert, ed., *Calcium Signaling Protocols* (Methods in Molecular Biology Volume 114), Humana Press (1999); W. T. Mason, ed., *Fluorescent and Luminescent Probes for Biological Activity. A Practical Guide to Technology for Quantitative Real-Time Analysis*, Second Ed, Academic Press (1999); *Calcium Signaling Protocols* (Methods in Molecular Biology), 2005, D.G. Lamber, ed., Humana Press.).

Introduction of exogenous re-programming factor polypeptide into a post-natal fibroblast

[00131] In some embodiments, introduction of exogenous reprogramming factor polypeptides (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) into a post-natal fibroblast is achieved by contacting the post-natal fibroblast with exogenous reprogramming factor polypeptides, wherein the exogenous reprogramming factor polypeptides are taken up into the cell.

[00132] In some embodiments, each of an exogenous reprogramming factorpolypeptides comprises a protein transduction domain. As a non-limiting example, an exogenous Gata4 polypeptide, an exogenous Mef2C polypeptide, and a Tbx4 polypeptide is linked, covalently or non-covalently, to a protein transduction domain.

[00133] "Protein Transduction Domain" or PTD refers to a polypeptide, polynucleotide, carbohydrate, or organic or inorganic compound that facilitates traversing a lipid bilayer, micelle, cell membrane, organelle membrane, or vesicle membrane. A PTD attached to another molecule facilitates the molecule traversing a membrane, for example going from extracellular space to intracellular space, or cytosol to within an organelle. In some embodiments, a PTD is covalently linked to the amino terminus of a reprogramming factor polypeptide (e.g., a Gata4 polypeptide, a Mef2c polypeptide, or a Tbx5 polypeptide). In some embodiments, a PTD is covalently linked to the carboxyl terminus of a reprogramming factor polypeptide (e.g., a Gata4 polypeptide, a Mef2c polypeptide, or a Tbx5 polypeptide).

[00134] Exemplary protein transduction domains include but are not limited to a minimal undecapeptide protein transduction domain (corresponding to residues 47-57 of HIV-1 TAT comprising YGRKKRRQRRR; SEQ ID NO:1); a polyarginine sequence comprising a number of arginines sufficient to direct entry into a cell (e.g., 3, 4, 5, 6, 7, 8, 9, 10, or 10-50 arginines); a VP22 domain (Zender et al., Cancer Gene Ther. 2002 June; 9(6):489-96); an Drosophila Antennapedia protein transduction domain (Noguchi et al., Diabetes 2003; 52(7):1732-1737); a truncated human calcitonin peptide (Trehin et al. Pharm. Research, 21:1248-1256, 2004); polylysine (Wender et al., PNAS, Vol. 97:13003-13008); RRQRRTSKLMKR (SEQ ID NO:2); Transportan GWTLNSAGYLLGKINLKALAALAKKIL (SEQ ID NO:3); KALAWEAKLAKALAKALAKHLAKALAKALKCEA (SEQ ID NO:4); and

RQIKIWFQNRRMKWKK (SEQ ID NO:5). Exemplary PTDs include but are not limited to, YGRKKRRQRRR (SEQ ID NO:1), RKKRRQRRR (SEQ ID NO:6); an arginine homopolymer of from 3 arginine residues to 50 arginine residues; Exemplary PTD domain amino acid sequences include, but are not limited to, any of the following: YGRKKRRQRRR (SEQ ID NO:1); RKKRRQRR (SEQ ID NO:6); YARAAARQARA (SEQ ID NO:7); THRLPRRRRRR (SEQ ID NO:8); and GGRRARRRRRR (SEQ ID NO:9).

[00135] In some embodiments, an exogenous reprogramming factor polypeptide (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) comprises an arginine homopolymer of from 3 arginine residues to 50 arginine residues, e.g., from 3 to 6 arginine residues, from 6 to 10 arginine residues, from 10 to 20 arginine residues, from 20 to 30 arginine residues, from 30 to 40 arginine residues, or from 40 to 50 arginine residues. In some embodiments, an exogenous reprogramming factor polypeptide (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) comprises six Arg residues covalently linked (e.g., by a peptide bond) at the amino terminus of the reprogramming factor polypeptide. In some embodiments, an exogenous reprogramming factor polypeptide comprises six Arg residues covalently linked (e.g., by a peptide bond) at the carboxyl terminus of the reprogramming factor polypeptide.

[00136] Exogenous reprogramming factor polypeptides (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) that are introduced into a host post-natal fibroblast can be purified, e.g., at least about 75% pure, at least about 80% pure, at least about 85% pure, at least about 90% pure, at least about 95% pure, at least about 98% pure, at least about 99% pure, or more than 99% pure, e.g., free of proteins other than reprogramming factor(s) being introduced into the cell and free of macromolecules other than the reprogramming factor(s) being introduced into the cell.

Genetic modification of a post-natal fibroblast

[00137] In some embodiments, introduction of exogenous reprogramming factor polypeptides (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) into a post-natal fibroblast is achieved by genetic modification of the post-natal fibroblast with one or more exogenous nucleic acids comprising nucleotide sequences encoding reprogramming factor polypeptides (e.g., one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf; or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides). In the following discussion, one or more exogenous nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf (or a subset of reprogramming factors comprising Gata4, Mef2c, and Tbx5 polypeptides) are referred to generically as “one or more exogenous nucleic acids.”

[00138] The one or more exogenous nucleic acids comprising nucleotide sequences encoding the above-noted exogenous reprogramming factor polypeptides can be a recombinant expression vector, where suitable vectors include, e.g., recombinant retroviruses, lentiviruses, and adenoviruses; retroviral expression vectors, lentiviral expression vectors, nucleic acid expression vectors, and plasmid expression vectors. In some cases, the one or more exogenous nucleic acids is integrated into the genome of a host post-natal fibroblast and its progeny. In other cases, the one or more exogenous nucleic acids persists in an episomal state in the host post-natal fibroblast and its progeny. In some cases, an endogenous, natural version of the reprogramming factor-encoding nucleic acid may already exist in the cell but an additional "exogenous gene" is added to the host post-natal fibroblast to increase expression of the reprogramming factor. In other cases, the exogenous reprogramming factor-encoding nucleic acid encodes a reprogramming factor polypeptide having an amino acid sequence that differs by one or more amino acids from a polypeptide encoded by an endogenous reprogramming factor-encoding nucleic acid within the host post-natal fibroblast.

[00139] In some embodiments, a post-natal fibroblast is genetically modified with three separate expression constructs (expression vectors), each comprising a nucleotide sequence encoding one of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf. In some embodiments, an expression construct will comprise nucleotide sequences encoding two or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf.

[00140] In some embodiments, a post-natal fibroblast is genetically modified with three separate expression constructs (expression vectors), each comprising a nucleotide sequence encoding one of Gata4, Mef2c, and Tbx5. In some embodiments, an expression construct will comprise nucleotide sequences encoding both Gata4 and Mef2c, both Gata4 and Tbx5, or both Mef2c and Tbx5. In some embodiments, an expression construct will comprise nucleotide sequences encoding Gata4, Mef2c, and Tbx5.

[00141] In some embodiments, one or more exogenous nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf (or a subset, e.g., Gata4, Mef2c, and Tbx5) polypeptides is introduced into a single post-natal fibroblast (e.g., a single post-natal fibroblast host cell) *in vitro*. In other embodiments, one or more exogenous nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf (or a subset, e.g., Gata4, Mef2c, and Tbx5) polypeptides is introduced into a population of post-natal fibroblasts (e.g., a population of host post-natal fibroblasts) *in vitro*. In some embodiments, one or more exogenous nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf (or a subset, e.g., Gata4, Mef2c, and Tbx5) polypeptides is introduced into a post-natal fibroblast (e.g., a single post-natal fibroblast or a population of post-natal fibroblasts) *in vivo*.

[00142] Where a population of post-natal fibroblasts is genetically modified (*in vitro* or *in vivo*) with one or more exogenous nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf (or a subset, e.g., Gata4, Mef2c, and Tbx5) polypeptides, the one or more exogenous nucleic acids can be introduced into greater than 20% of the total population of post-natal fibroblasts, e.g., 25%, 30%, 35%, 40%, 44%, 50%, 57%, 62%, 70%, 74%, 75%, 80%, 90%, or other percent of cells greater than 20%.

[00143] In some embodiments, the one or more nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf (or a subset, e.g., Gata4, Mef2c, and Tbx5) polypeptides is/are an expression construct that provides for production of the one or more reprogramming factor polypeptides in the genetically modified host post-natal fibroblast cell. In some embodiments, the expression construct is a viral construct, e.g., a recombinant adeno-associated virus construct (see, e.g., U.S. Patent No. 7,078,387), a recombinant adenoviral construct, a recombinant lentiviral construct, etc.

[00144] Suitable expression vectors include, but are not limited to, viral vectors (e.g. viral vectors based on vaccinia virus; poliovirus; adenovirus (see, e.g., Li et al., Invest Opthalmol Vis Sci 35:2543 2549, 1994; Borras et al., Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto et al., H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, e.g., Ali et al., Hum Gene Ther 9:81 86, 1998, Flannery et al., PNAS 94:6916 6921, 1997; Bennett et al., Invest Opthalmol Vis Sci 38:2857 2863, 1997; Jomary et al., Gene Ther 4:683 690, 1997, Rolling et al., Hum Gene Ther 10:641 648, 1999; Ali et al., Hum Mol Genet 5:591 594, 1996; Srivastava in WO 93/09239, Samulski et al., J. Vir. (1989) 63:3822-3828; Mendelson et al., Virol. (1988) 166:154-165; and Flotte et al., PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., PNAS 94:10319 23, 1997; Takahashi et al., J Virol 73:7812 7816, 1999); 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.

[00145] 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 host cells: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, and pSVLSV40 (Pharmacia). However, any other vector may be used so long as it is compatible with the host cell.

[00146] 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 (see e.g., Bitter et al. (1987) *Methods in Enzymology*, 153:516-544).

[00147] In some embodiments, a reprogramming factor-encoding nucleotide sequence (e.g., a Gata4-encoding nucleotide sequence, an Mef2c-encoding nucleotide sequence, a Tbx5-encoding nucleotide sequence) is operably linked to a control element, e.g., a transcriptional control element, such as a promoter. The transcriptional control element is functional in a eukaryotic cell, e.g., a mammalian cell. Suitable transcriptional control elements include promoters and enhancers. In some embodiments, the promoter is constitutively active. In other embodiments, the promoter is inducible.

[00148] Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include CMV immediate early, HSV thymidine kinase, early and late SV40, long terminal repeats (LTRs) from retrovirus, and mouse metallothionein-I.

[00149] In some embodiments, a reprogramming factor-encoding nucleotide sequence is operably linked to a cardiac-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) Mol. 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.

[00150] Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression.

[00151] Examples of suitable mammalian expression vectors (expression vectors suitable for use in mammalian host cells) include, but are not limited to: recombinant viruses, nucleic acid vectors, such as plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, human artificial chromosomes, cDNA, cRNA, and polymerase chain reaction (PCR) product expression cassettes. Examples of suitable promoters for driving expression of a Gata4-, Mef2c-, or Tbx5-encoding nucleotide sequence include, but are not limited to, retroviral long terminal repeat (LTR) elements; constitutive promoters such as CMV, HSV1-TK, SV40, EF-1α, β-actin; phosphoglycerol kinase (PGK), and inducible promoters, such as those containing Tet-operator elements. In some cases, the mammalian expression vector(s) encodes, in addition to exogenous Gata4, Mef2c, and Tbx5 polypeptides, a marker gene that facilitates identification or selection of cells that have been transfected 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 and Glick (2002) *Nat. Biotechnol.* 20:83), yellow fluorescent protein, 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.

[00152] Examples of suitable viral vectors include, but are not limited, viral vectors based on retroviruses (including lentiviruses); adenoviruses; and adeno-associated viruses. An example of a suitable retrovirus-based vector is a vector based on murine moloney leukemia virus (MMLV); however, other recombinant retroviruses may also be used, e.g., Avian Leukosis Virus, Bovine Leukemia Virus, Murine Leukemia Virus (MLV), Mink-Cell focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis virus, Gibbon Abe Leukemia Virus, Mason Pfizer Monkey Virus, or Rous Sarcoma Virus, see, e.g., U.S. Pat. No. 6,333,195.

[00153] In other cases, the retrovirus-based vector is a lentivirus-based vector, (e.g., Human Immunodeficiency Virus-1 (HIV-1); Simian Immunodeficiency Virus (SIV); or Feline Immunodeficiency Virus (FIV)), See, e.g., Johnston et al., (1999), Journal of Virology, 73(6):4991-5000 (FIV); Negre D et al., (2002), Current Topics in Microbiology and Immunology, 261:53-74 (SIV); Naldini et al., (1996), Science, 272:263-267 (HIV).

[00154] 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, see, e.g., U.S. Pat. No. 5,449,614. The viral polypeptide may be an amphotropic viral polypeptide, e.g., 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, e.g., ecotropic env, which aids entry into cells of the original host species.

[00155] 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. See e.g., Yee et al., (1994), Methods Cell Biol., Pt A:99-112 (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.

[00156] In general, a recombinant virus 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 E1A or E1B 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 E1A and E1B. In many examples, the packaging cells supply proteins derived from a virus that differs from the virus from which the viral vector derives.

[00157] 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 virus vector plasmid 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), see, e.g., Morita et al., (2000), Gene Therapy, 7:1063-1066; 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).

[00158] 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.

[00159] 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, EF1α, 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. See e.g., Onishi et al., (1996), Experimental Hematology, 24:324-329. In some cases, the retroviral construct is a self-inactivating lentiviral vector (SIN) vector, see, e.g., 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), PNAS, 92:6733-6737. Virus vector plasmids (or constructs), include: pMXs, pMxs-IB, pMXs-puro, pMXs-neo (pMXs-IB is a vector carrying the blasticidin-resistant gene in stead 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. U.S.A., 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), Journal of Virology, 72:8150-8157 and the like as the retrovirus system, and pAdex1 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). See, e.g., Morgenstern et al., (1990), Nucleic Acids Research, 18:3587-3596.

[00160] 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. Many methods begin with the introduction of a viral construct into a packaging cell line. The viral construct 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 (Felgner et al. (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84:7413-7417), an electroporation method, microinjection, Fugene transfection, and the like, and any method described herein.

[00161] A nucleic acid construct can be introduced into a host cell using a variety of well known techniques, such as non-viral based transfection of the cell. In an exemplary aspect the construct is incorporated into a vector and introduced into a host cell. Introduction into the cell may be performed by any non-viral based transfection known in the art, such as, but not limited to, electroporation, calcium phosphate mediated transfer, nucleofection, sonoporation, heat shock, magnetofection, liposome mediated transfer, microinjection, microprojectile mediated transfer (nanoparticles), cationic polymer mediated transfer (DEAE-dextran, polyethylenimine, polyethylene glycol (PEG) and the like) or cell fusion. Other methods of transfection include transfection reagents such as Lipofectamine™, Dojindo Hilymax™, Fugene™, jetPEI™, Effectene™, and DreamFect™.

Additional polypeptides

[00162] In some embodiments, a subject method does not require and does not involve introducing into a post-natal fibroblast any of an exogenous Hopx polypeptide, an exogenous Nkx2-5 polypeptide, an exogenous Hrt2 polypeptide, an exogenous Pitx2 polypeptide, an exogenous Smyd1 polypeptide, an exogenous Myocd polypeptide, an exogenous Baf60c polypeptide, an exogenous Srf polypeptide, an exogenous Isl1 polypeptide, an exogenous Hand2 polypeptide, or an exogenous Mesp1 polypeptide. In some embodiments, a subject method does not require and does not involve introducing into a post-natal fibroblast a nucleic acid(s) comprising nucleotide sequences encoding any of Hopx, Nkx2-5, Hrt2, Pitx2, Smyd1, Myocd, Baf60c, Srf, Isl1, Hand2, or Mesp1. However, in other embodiments, a subject method can involve use of Gata4, Mef2c, and

Tbx5; and one or more additional polypeptides that can contribute to the reprogramming of a post-natal fibroblast directly into a cardiomyocyte.

[00163] A post-natal fibroblast can be modified (by introduction into the post-natal fibroblast of a polypeptide), or genetically modified as described above, where the post-natal fibroblast is modified with a Gata4 polypeptide, an Mef2c polypeptide, and a Tbx5 polypeptide, or where the post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding a Gata4 polypeptide, an Mef2c polypeptide, and a Tbx5 polypeptide. In some embodiments, one or more additional polypeptides (or nucleic acids comprising nucleotide sequences encoding same) are introduced into a post-natal fibroblast, where the one or more additional polypeptides is selected from Mesp1, Isl1, Myocd, Smyd1, Srf, Baf60c, Hand2, Hopx, Hrt2, Pitx2c, and Nkx2-5.

[00164] Thus, in some embodiments, a post-natal fibroblast is modified by introduction into the post-natal fibroblast of a Gata4 polypeptide, an Mef2c polypeptide, and a Tbx5 polypeptide; and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11, of a Mesp1 polypeptide, a Isl1 polypeptide, a Myocd polypeptide, a Smyd1 polypeptide, a Srf polypeptide, a Baf60c polypeptide, a Hand2 polypeptide, a Hopx polypeptide, a Hrt2 polypeptide, a Pitx2c polypeptide, and an Nkx2-5 polypeptide. In some embodiments, a post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding a Gata4 polypeptide, an Mef2c polypeptide, and a Tbx5 polypeptide; and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11, of a Mesp1 polypeptide, a Isl1 polypeptide, a Myocd polypeptide, a Smyd1 polypeptide, a Srf polypeptide, a Baf60c polypeptide, a Hand2 polypeptide, a Hopx polypeptide, a Hrt2 polypeptide, a Pitx2c polypeptide, and an Nkx2-5 polypeptide. The following are exemplary, non-limiting combinations.

[00165] In some embodiments, a post-natal fibroblast is modified by introduction into the post-natal fibroblast of a Gata4 polypeptide, an Mef2c polypeptide, a Tbx5 polypeptide, a Isl1 polypeptide, an Mesp1 polypeptide, a Myocd polypeptide, an Nkx2.5 polypeptide, a Smyd1 polypeptide, and a Srf polypeptide. In some embodiments, a post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding a Gata4 polypeptide, an Mef2c polypeptide, a Tbx5 polypeptide, a Isl1 polypeptide, an Mesp1 polypeptide, a Myocd polypeptide, an Nkx2.5 polypeptide, a Smyd1 polypeptide, and a Srf polypeptide.

[00166] In some embodiments, a post-natal fibroblast is modified by introduction into the post-natal fibroblast of a Gata4 polypeptide, an Mef2c polypeptide, a Tbx5 polypeptide, an Mesp1 polypetpide, a Myocd polypeptide, and an Nkx2.5 polypeptide. In some embodiments, a post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding a Gata4 polypeptide, an Mef2c polypeptide, a Tbx5 polypeptide, an Mesp1 polypetpide, a Myocd polypeptide, and an Nkx2.5 polypeptide.

[00167] In some embodiments, a post-natal fibroblast is modified by introduction into the post-natal fibroblast of a Gata4 polypeptide, an Mef2c polypeptide, a Tbx5 polypeptide, an Mesp1 polypeptide, and a Myocd polypeptide. In some embodiments, a post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding a Gata4 polypeptide, an Mef2c polypeptide, a Tbx5 polypeptide, an Mesp1 polypeptide, and a Myocd polypeptide.

[00168] In some embodiments, a post-natal fibroblast is modified by introduction into the post-natal fibroblast of a Gata4 polypeptide, an Mef2c polypeptide, a Tbx5 polypeptide, and an Mesp1 polypeptide. In some embodiments, a post-natal fibroblast is genetically modified with one or more nucleic acids comprising nucleotide sequences encoding a Gata4 polypeptide, an Mef2c polypeptide, a Tbx5 polypeptide, and an Mesp1 polypeptide.

[00169] Amino acid sequences of Mesp1, Isl1, Myocd, Smyd1, Srf, Baf60c, Hand2, Hopx, Hrt2, Pitx2c, and Nkx2-5 polypeptides are known in the art, as are nucleotide sequences encoding the polypeptides. See, e.g., GenBank Accession Nos: 1) AAR88511.1 (*Homo sapiens* BAF60c; amino acid sequence), and AY450431 (nucleotide sequence encoding the AAR88511.1 amino acid sequence); 2) AAR88510.1 (*Homo sapiens* BAF60c; amino acid sequence), and AY450430 (nucleotide sequence encoding the AAR88510.1 amino acid sequence); 3) NP_068808 (*Homo sapiens* Hand2 amino acid sequence), and NM_021973 (nucleotide sequence encoding the NP_068808 amino acid sequence); 4) NP_115884.4 (*Homo sapiens* Hopx amino acid sequence), and NM_032495 (nucleotide sequence encoding the NP_115884.4 amino acid sequence); 5) NP_631958.1 (*Homo sapiens* Hopx amino acid sequence), and NM_139212 (nucleotide sequence encoding the NP_631958.1 amino acid sequence); 6) AAG31157 (*Homo sapiens* Hrt2 amino acid sequence), and AF311884 (nucleotide sequence encoding the

AAG31157 amino acid sequence; 7) NP_000316 (*Homo sapiens* Pitx2c amino acid sequence), and NM_000325 (nucleotide sequence encoding the NP_000316 amino acid seuqence); 8) NP_061140.1 (*Homo sapiens* Mesp1 amino acid sequence), and NM_018670.3 (nucleotide sequence encoding the NP_061140.1 amino acid sequence); 9) XP_523151.2 (*Pan troglodytes* Mesp1 amino acid sequence), and XM_523151 (nucleotide sequence encoding the XP_523151.2 amino acid sequence; 10) BAA12041.1 (*Mus musculus* Mesp1 amino acid sequence), and BAA12041 (nucleotide sequence encoding the BAA12041.1 amino acid sequence); 11) NP_001101001.1 (*Rattus norvegicus* Mesp1 amino acid sequence), and NM_001107531 (nucleotide sequence encoding the NP_001101001.1 amino acid sequence; 12) NP_004378.1 (*Homo sapiens* NKX2-5 amino acid sequence), and NM_004387 (nucleotide sequence encoding the NP_004378.1 amino acid sequence; 13) NP_001159648.1 (*Homo sapiens* NKX2-5 amino acid sequence), and NM_001166176 (nucleotide sequence encoding the NP_001159648.1 amino acid sequence; 14) NP_001159647.1 (*Homo sapiens* Nkx2-5 amino acid sequence), and NM_001166175 (nucleotide sequence encoding the NP_001159647.1 amino acid sequence; 15) NP_002193.2 (*Homo sapiens* Isl1 amino acid sequence), and NM_002202 (nucleotide sequence encoding the NP_002193.2 amino acid sequence); 16) NP_059035 (*Rattus norvegicus* Isl1 amino acid), and NM_017339 (nucleotide sequence encoding the NP_059035 amino acid sequence); 17) BAC41153 (*Mus musculus* Isl1 amino acid sequence), and AK090263 (nucleotide sequence encoding the BAC41153 amino acid sequence); 18) NP_001139785.1 (*Homo sapiens* Myocd amino acid sequence), and NM_001146313 (nucleotide sequence encoding the NP_001139785.1 amino acid sequence); 19) NP_001139784.1 (*Homo sapiens* Myocd amino acid sequence), and NM_001146312 (nucleotide sequence encoding the NP_001139784.1 amino acid sequence); 20) NP_705832.1 (*Homo sapiens* Myocd amino acid sequence), and NM_153604 (nucleotide sequence encoding the NP_705832.1 amino acid sequence); 21) NP_938015.1 (*Homo sapiens* Smyd1 amino acid sequence), and NM_198274 (nucleotide sequence encoding the NP_938015.1 amino acid sequence); 22) NP_001100065.1 (*Rattus norvegicus* Smyd1 amino acid sequence), and NM_001106595 (nucleotide sequence encoding the NP_001100065.1 amino acid sequence); 23) NP_001153599.1 (*Mus musculus* Smyd1 amino acid sequence), and NM_001160127 (nucleotide sequence encoding the NP_001153599.1

amino acid sequence); 24) NP_003122.1 (*Homo sapiens* Srf amino acid sequence), and NM_003131 (nucleotide sequence encoding the NP_003122.1 amino acid sequence; 25) XP_518487.2 (*Pan troglodytes* Srf amino acid sequence), and XM_518487 (nucleotide sequence encoding the XP_518487.2 amino acid sequence; 26) NP_001102772.1 (*Rattus norvegicus* Srf amino acid sequence), and NM_001109302 (nucleotide sequence encoding the NP_001102772.1 amino acid sequence).

[00170] Suitable mino acid sequences of Mesp1, Isl1, Myocd, Smyd1, Srf, Baf60c, Hand2, Hopx, Hrt2, Pitx2c, and Nkx2-5 polypeptides include amino acid sequences having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to an amino acid sequence set forth in one of the aforementioned GenBank entries.

[00171] Suitable nucleotide sequences encoding Mesp1, Isl1, Myocd, Smyd1, Srf, Baf60c, Hand2, Hopx, Hrt2, Pitx2c, and Nkx2-5 polypeptides include nucleotide sequences having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, nucleotide sequence identity to a nucleotide sequence set forth in one of the aforementioned GenBank entries.

Additional factors

[00172] A post-natal fibroblast can be modified or genetically modified as described above; and can also be contacted with one or more additional factors which can be added to the culture system, *e.g..*, the one or more additional factors can be included as additives in the culture medium. Examples of such additional factors include, but are not limited to: histone deacetylase (HDAC) inhibitors, see, e.g. Huangfu et al. (2008) Nature Biotechnol. 26:795-797; Huangfu et al. (2008) Nature Biotechnol. 26: 1269-1275; DNA demethylating agents, see, e.g., Mikkelson et al (2008) Nature 454, 49-55; histone methyltransferase inhibitors, see, e.g., Shi et al. (2008) Cell Stem Cell 2:525-528; L-type calcium channel agonists, see, e.g., Shi et al. (2008) 3:568-574; Wnt3a, see, e.g., Marson et al. (2008) Cell 134:521-533; siRNA, see, e.g., Zhao et al. (2008) Cell Stem Cell 3: 475-479.

[00173] Histone deacetylases (HDAC) are a class of enzymes that remove acetyl groups from an [epsilon]-N-acetyl lysine amino acid on a histone. Exemplary HDACs include those Class I HDAC: HDAC1, HDAC2, HDAC3, HDAC8; and Class II HDACs: HDAC4, HDAC5, HDAC6, HDAC7A, HDAC9, HDAC10. Type I mammalian HDACs include: HDAC1, HDAC2, HDAC3, HDAC8, and HDACI1. Type II mammalian HDACs include: HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, and HDAC1. In some embodiments, an HDAC inhibitor selectively inhibits a Class I or a Class II HDAC.

[00174] Suitable concentrations of an HDAC inhibitor range from about 0.001 nM to about 10 mM, depending on the particular HDAC inhibitor to be used. The HDAC concentration can range from 0.01 nM to 1000 nM.

[00175] Suitable HDAC inhibitors include any agent that inhibits HDAC enzymatic activity in deacetylation of histone. Suitable HDAC inhibitors include, but are not limited to, carboxylate HDAC inhibitors; hydroxamic acid HDAC inhibitors; peptide (e.g., cyclic tetrapeptide) HDAC inhibitors; benzamide HDAC inhibitors; electrophilic ketone HDAC inhibitors; hybrid polar HDAC inhibitors; and short chain fatty acid HDAC inhibitors.

[00176] Suitable HDAC inhibitors include trichostatin A and its analogs, for example: trichostatin A (TSA); and trichostatin C (Koghe et al., (1998), Biochem. Pharmacol, 56:1359-1364).

[00177] Suitable peptide HDAC inhibitors include, for example: oxamflatin [(2E)-5-[3-[(phenylsulfonyl)aminophenyl]-pent-2-ene-4-inohydroxamic acid (Kim et al., (1999), Oncogene, 18:2461-2470); Trapoxin A (cyclo-(L-phenylalanyl-L-phenylalanyl-D-pipecolinyl-L-2-amino-8-oxo-9,10-epoxy-decanoyl) (Kijima et al., (1993), J. Biol. Chem. 268:22429-22435); FR901228, depsipeptide ((1*S*,4*S*,7*Z*,10*S*,16*E*,21*R*)-7-ethylidene-4,21-diisopropyl-2-oxa-12,13-dithia-5,8,20,23-tetrazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentone) (Nakajima et al., (1998). Ex. Cell Res., 241:126-133); apicidin, cyclic tetrapeptide [cyclo-(N-O-methyl-L-tryptophanyl-L-isoleucinyl-D-pipecolinyl-L-2-amino-8-oxodecanoyl)] (Darkin-Rattray et al., (1996), Proc. Natl. Acad. Sci. U.S.A., 93:13143-13147; apicidin Ia, apicidin Ib, apicidin Ic, apicidin IIa, and apicidin IIb (WO 97/11366); HC-toxin, cyclic tetrapeptide (Bosch et al. (1995), Plant Cell, 7:1941-1950); and chlamydocin (Bosch et al., *supra*).

[00178] Suitable HDAC inhibitors include hybrid polar compounds (HPC) based on hydroxamic acid, for example: salicyl hydroxamic acid (SBHA) (Andrews et al., (2000), International J. Parasitology, 30:761-8); suberoylanilide hydroxamic acid (SAHA) (Richon et al., (1998), Proc. Natl. Acad. Sci. U.S.A., 95: 3003-7); azelaic bishydroxamic acid (ABHA) (Andrews et al., supra); azelaic-1-hydroxamate-9-anilide (AAHA) (Qiu et al., (2000), Mol. Biol. Cell, 11:2069-83); M-carboxy cinnamic acid bishydroxamide (CBHA) (Richon et al., *supra*); 6-(3-chlorophenylureido) carpoic hydroxamic acid, 3-Cl-UCHA) (Richon et al., *supra*); MW2796 (Andrews et al., *supra*); MW2996 (Andrews et al., supra); the hydroxamic acid derivative NVP-LAQ-824 (Catley et al. (2003) *Blood* 102:2615; and Atadja et al. (2004) *Cancer Res.* 64:689); and CBHA (m-carboxycinnaminic acid bishydroxamic acid).

[00179] Suitable HDAC inhibitors include short chain fatty acid (SCFA) compounds, for example: sodium butyrate (Cousens et al., (1979), J. Biol. Chem., 254:1716-23); isovalerate (McBain et al., (1997), Biochem. Pharm., 53:1357-68); valproic acid; valerate (McBain et al., *supra*); 4-phenyl butyric acid (4-PBA) (Lea and Tulsyan, (1995), Anticancer Research, 15:879-3); phenyl butyric acid (PB) (Wang et al., (1999), Cancer Research 59: 2766-99); propinate (McBain et al., *supra*); butylamide (Lea and Tulsyan, *supra*); isobutylamide (Lea and Tulsyan, *supra*); phenyl acetate (Lea and Tulsyan, supra); 3-bromopropionate (Lea and Tulsyan, *supra*); tributyrin (Guan et al., (2000), Cancer Research, 60:749-55); arginine butyrate; isobutyl amide; and valproate.

[00180] Suitable HDAC inhibitors include benzamide derivatives, for example: MS-275 [N-(2-aminophenyl)-4-[N-(pyridine-3-yl-methoxycarbonyl)aminomethyl]benzam- ide] (Saito et al., (1999), Proc. Natl. Acad. Sci. U.S.A., 96:4592-7); and a 3'-amino derivative of MS-275 (Saito et al., *supra*); and CI-994.

[00181] Additional suitable HDAC inhibitors include: BML-210 (N-(2-aminophenyl)-N'-phenyl-octanediamide); Depudecin (e.g., (-)-Depudecin); Nullscript (4-(1,3-Dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-N-hydroxybutanamide); Scriptaid; Suramin Sodium; pivaloyloxymethyl butyrate (Pivanex, AN-9), Trapoxin B; CI-994 (i.e., N-acetyl dinaline); MGCD0103 (*N*-(2-Aminophenyl)-4-[[(4-pyridin-3-ylpyrimidin-2-yl)amino]methyl] benzamide); JNJ16241199 (R-306465; see, e.g., Arts et al. (2007) *Br. J. Cancer* 97:1344); Tubacin; A-161906; proxamide; oxamflatin; 3-C1-UCHA (6-(3-chlorophenylureido)caproic hydroxamic acid); and AOE (2-amino-8-oxo-9,10-epoxydecanoic acid).

[00182] Suitable DNA methylation inhibitors are inhibitors of DNA methyltransferase, and include, but are not limited to, 5-deoxy-azacytidine (DAC); 5-azacytidine (5-aza-CR) (Vidaza); 5-aza-2'-deoxycytidine (5-aza-CdR; decitabine); 1-[beta]-D-arabinofuranosyl-5-azacytosine; dihydro-5-azacytidine; zebularine ((1-(β-Dribofuranosyl)-1,2-dihydropyrimidin-2-one); Sinefungin (e.g., InSolution™ Sinefungin), and 5-fluoro-2'-deoxycyticine (FdCyd). Examples of suitable non-nucleoside DNA methyltransferse inhibitors (e.g., other than procaine) include: (-)-epigallocatechin-3-gallate (EGCG); hydralazine; procainamide; psammaplin A (N,N"-(dithiodi-2,1-ethanediyl)bis[3-bromo-4-hydroxy-a-(hydroxyimino)-benzenepropanamide); and RG108 (2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-3-(1H-indol-3-yl)propionic acid).

[00183] Suitable histone methyltransferase (HMT) inhibitors include, but are not limited to, SC-202651 (2-(Hexahydro-4-methyl-1H-1,4-diazepin-1-yl)-6,7-dimethoxy-N-(1-(phenylmethyl)-4-piperidinyl)-4-quinazolinamine); chaetocin (Grainer et al. (2005) *Nature Chem. Biol.* 1:143); BIX-01294 (2-(Hexahydro-4-methyl-1H-1,4-diazepin-1-yl)-6,7-dimethoxy-N-[1-(phenylmethyl)-4-piperidinyl]-4-quinazolinamine trihydrochloride); 3-deazaneplanocin (Glazer et al. (1986) *BBRC* 135:688); and the like.

GENETICALLY MODIFIED HOST CELLS

[00184] The present disclosure provides genetically modified host cells, including isolated genetically modified host cells, where a subject genetically modified host cell comprises (has been genetically modified with) one or more exogenous nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides. In some embodiments, a subject genetically modified host cell is *in vitro*. In some embodiments, a subject genetically modified host cell is a human cell or is derived from a human cell. In some embodiments, a subject genetically modified host cell is a rodent cell or is derived from a rodent cell. The present disclosure further provides progeny of a subject genetically modified host cell, where the progeny can comprise the same exogenous nucleic acid as the subject genetically modified host cell from which it was derived. The present disclosure further provides a composition comprising a subject genetically modified host cell.

[00185] The present disclosure provides genetically modified host cells, including isolated genetically modified host cells, where a subject genetically modified host cell comprises (has been genetically modified with) one or more exogenous nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides. In some embodiments, a subject genetically modified host cell is *in vitro*. In some embodiments, a subject genetically modified host cell is a human cell or is derived from a human cell. In some embodiments, a subject genetically modified host cell is a rodent cell or is derived from a rodent cell. The present disclosure further provides progeny of a subject genetically modified host cell, where the progeny can comprise the same exogenous nucleic acid as the subject genetically modified host cell from which it was derived. The present disclosure further provides a composition comprising a subject genetically modified host cell.

Genetically modified post-natal fibroblasts

[00186] In some embodiments, a subject genetically modified host cell is a genetically modified post-natal fibroblast. Thus, the present disclosure provides a genetically modified post-natal fibroblast that comprises (has been genetically modified with) one or more exogenous nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, or a subset (e.g., Gata4, Mef2c, and Tbx5 polypeptides). In some embodiments, a subject genetically modified post-natal fibroblast is *in vitro*. In some embodiments, a subject genetically modified post-natal fibroblast is a human cell or is derived from a human cell. In some embodiments, a subject genetically modified post-natal fibroblast is a rodent cell or is derived from a rodent cell. The present disclosure further provides progeny of a subject genetically modified post-natal fibroblast, where the progeny can comprise the same exogenous nucleic acid as the subject genetically modified post-natal fibroblast from which it was derived. The present disclosure further provides a composition comprising a subject genetically modified post-natal fibroblast.

Genetically modified induced cardiomyocytes

[00187] The present disclosure further provides cardiomyocytes (“induced cardiomyocytes”) derived from a subject genetically modified host cell. Because a subject induced cardiomyocyte is derived from a subject genetically modified post-natal fibroblast, a subject induced cardiomyocyte is also genetically modified. Thus, the present disclosure provides a genetically modified cardiomyocyte that comprises one or more exogenous nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, or a subset (e.g., Gata4, Mef2c, and Tbx5 polypeptides). In some embodiments, a subject genetically modified cardiomyocyte is *in vitro*. In some embodiments, a subject genetically modified cardiomyocyte is a human cell or is derived from a human cell. In some embodiments, a subject genetically modified cardiomyocyte is a rodent cell or is derived from a rodent cell. The present disclosure further provides progeny of a subject genetically modified cardiomyocyte, where the progeny can comprise the same exogenous nucleic acid as the subject genetically modified cardiomyocyte from which it was derived. The present disclosure further provides a composition comprising a subject genetically modified cardiomyocyte.

Compositions

[00188] The present disclosure provides a composition comprising a subject genetically modified host cell (e.g., a subject genetically modified post-natal fibroblast; progeny of a subject genetically modified post-natal fibroblast; a subject induced cardiomyocyte; progeny of a subject induced cardiomyocyte). A subject composition comprises a subject genetically modified host cell; and will in some embodiments comprise one or more further components, which components are selected based in part on the intended use of the genetically modified host cell. Suitable components include, but are not limited to, salts; buffers; stabilizers; protease-inhibiting agents; cell membrane- and/or cell wall-preserving compounds, e.g., glycerol, dimethylsulfoxide, etc.; nutritional media appropriate to the cell; and the like.

[00189] In some embodiments, a subject composition comprises a subject genetically modified host cell and a matrix (a “subject genetically modified cell/matrix composition”), where a subject genetically modified host cell is associated with the matrix. The term "matrix" refers to any suitable carrier material to which the genetically modified cells are able to attach themselves or adhere in order to form a cell composite. In some embodiments, the matrix or carrier material is present already in a three-dimensional form desired for later application. For example, bovine pericardial tissue is used as matrix which is crosslinked with collagen, decellularized and photofixed.

[00190] For example, a matrix (also referred to as a "biocompatible substrate") is a material that is suitable for implantation into a subject. A biocompatible substrate does not cause toxic or injurious effects once implanted in the subject. In one embodiment, the biocompatible substrate is a polymer with a surface that can be shaped into the desired structure that requires repairing or replacing. The polymer can also be shaped into a part of a structure that requires repairing or replacing. The biocompatible substrate can provide the supportive framework that allows cells to attach to it and grow on it.

[00191] Suitable matrix components include, e.g., collagen; gelatin; fibrin; fibrinogen; laminin; a glycosaminoglycan; elastin; hyaluronic acid; a proteoglycan; a glycan; poly(lactic acid); poly(vinyl alcohol); poly(vinyl pyrrolidone); poly(ethylene oxide); cellulose; a cellulose derivative; starch; a starch derivative; poly(caprolactone); poly(hydroxy butyric acid); mucin; and the like. In some embodiments, the matrix comprises one or more of collagen, gelatin, fibrin, fibrinogen, laminin, and elastin; and can further comprise a non-proteinaceous polymer, e.g., can further comprise one or more of poly(lactic acid), poly(vinyl alcohol), poly(vinyl pyrrolidone), poly(ethylene oxide), poly(caprolactone), poly(hydroxy butyric acid), cellulose, a cellulose derivative, starch, and a starch derivative. In some embodiments, the matrix comprises one or more of collagen, gelatin, fibrin, fibrinogen, laminin, and elastin; and can further comprise hyaluronic acid, a proteoglycan, a glycosaminoglycan, or a glycan. Where the matrix comprises collagen, the collagen can comprise type I collagen, type II collagen, type III collagen, type V collagen, type XI collagen, and combinations thereof.

[00192] The matrix can be a hydrogel. A suitable hydrogel is a polymer of two or more monomers, e.g., a homopolymer or a heteropolymer comprising multiple monomers. Suitable hydrogel monomers include the following: lactic acid, glycolic acid, acrylic acid, 1-hydroxyethyl methacrylate (HEMA), ethyl methacrylate (EMA), propylene glycol methacrylate (PEMA), acrylamide (AAM), N-vinylpyrrolidone, methyl methacrylate (MMA), glycidyl methacrylate (GDMA), glycol methacrylate (GMA), ethylene glycol, fumaric acid, and the like. Common cross linking agents include tetraethylene glycol dimethacrylate (TEGDMA) and N,N'-methylenebisacrylamide. The hydrogel can be homopolymeric, or can comprise co-polymers of two or more of the aforementioned polymers. Exemplary hydrogels include, but are not limited to, a copolymer of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO); Pluronic™ F-127 (a difunctional block copolymer of PEO and PPO of the nominal formula EO_{100}-PO_{65}-EO_{100}, where EO is ethylene oxide and PO is propylene oxide); poloxamer 407 (a tri-block copolymer consisting of a central block of poly(propylene glycol) flanked by two hydrophilic blocks of poly(ethylene glycol)); a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) co-polymer with a nominal molecular weight of 12,500 Daltons and a PEO:PPO ratio of 2:1); a poly(*N*-isopropylacrylamide)-base hydrogel (a

PNIPAAm-based hydrogel); a PNIPAAm-acrylic acid co-polymer (PNIPAAm-co-AAc); poly(2-hydroxyethyl methacrylate); poly(vinyl pyrrolidone); and the like.

[00193] A subject genetically modified cell/matrix composition can further comprise one or more additional components, where suitable additional components include, e.g., a growth factor; an antioxidant; a nutritional transporter (e.g., transferrin); a polyamine (e.g., glutathione, spermidine, etc.); and the like.

[00194] The cell density in a subject genetically modified cell/matrix composition can range from about 10^2 cells/mm^3 to about 10^9 cells/mm^3, e.g., from about 10^2 cells/mm^3 to about 10^4 cells/mm^3, from about 10^4 cells/mm^3 to about 10^6 cells/mm^3, from about 10^6 cells/mm^3 to about 10^7 cells/mm^3, from about 10^7 cells/mm^3 to about 10^8 cells/mm^3, or from about 10^8 cells/mm^3 to about 10^9 cells/mm^3.

[00195] The matrix can take any of a variety of forms, or can be relatively amorphous. For example, the matrix can be in the form of a sheet, a cylinder, a sphere, etc.

Implantable devices

[00196] The present disclosure provides an implantable device (such as an intravascular stent, a scaffold, a graft (e.g., an aortic graft), an artificial heart valve, a coronary shunt, a pacemaker electrode, an endocardial lead, etc.) that comprises a reprogramming composition comprising one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides. The present disclosure further provides an implantable device that comprises a reprogramming composition comprising one or more nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides. The reprogramming composition (comprising one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, or comprising one or more nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides) can be coated onto a surface of the implantable device, or can be contained within a reservoir in the implantable device. Where the reprogramming composition is contained within a reservoir in the implantable device, the reservoir is structured so as to allow the reprogramming composition to elute from the device.

[00197] The present disclosure provides an implantable device (such as an intravascular stent, a scaffold, a graft (e.g., an aortic graft), an artificial heart valve, a coronary shunt, a pacemaker electrode, an endocardial lead, etc.) that comprises a reprogramming composition comprising a Gata4 polypeptide, a Mef2c polypeptide, and a Tbx5 polypeptide. The present disclosure further provides an implantable device that comprises a reprogramming composition comprising one or more nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides. The reprogramming composition (comprising Gata4, Mef2c, and Tbx5 polypeptides, or comprising one or more nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides) can be coated onto a surface of the implantable device, or can be contained within a reservoir in the implantable device. Where the reprogramming composition is contained within a reservoir in the implantable device, the reservoir is structured so as to allow the reprogramming composition to elute from the device.

[00198] When the implantable device is at a site in an individual, the nucleic acids or the polypeptides in the reprogramming composition leave the implantable device, and the polypeptides or the nucleic acids enter into a fibroblast at or near the site of the implantable device. Thus, a subject implantable device, when implanted in an individual, can provide for introduction of reprogramming factors, or nucleic acids encoding same, into a fibroblast at or near the site of implant, and can thereby provide for reprogramming of the fibroblast into a cardiomyocyte. For example, where a subject implantable device is a stent, the stent can be implanted into a coronary artery, where the reprogramming factors, or nucleic acids encoding same, elute from the stent, enter fibroblasts in the coronary vascular bed, and reprogram the fibroblasts into cardiomyocytes.

[00199] The present disclosure provides a stent comprising a reprogramming composition. Intravascular stents include, e.g., self-expandable stents, balloon-expandable stents, and stent-grafts.

[00200] In some instances, a subject implantable device comprises: a reprogramming composition incorporated within a first polymeric material (a first layer) that is affixed to the surface of an implantable device (e.g., a stent); and a second polymeric material (e.g., a barrier layer) is affixed to the first polymeric material, where the second polymeric material controls the elution rate of the polypeptides or the nucleic acids present in the reprogramming composition. As an example, the first polymeric material can comprise a fluoropolymer; and the second polymeric material can comprise an acrylic.

[00201] In some instances, a subject implantable device comprises: a reprogramming composition incorporated into a polymeric layer (a first layer) that is coated onto a surface of an implantable device (e.g., a stent); and a barrier layer over at least a portion of the polymeric layer to reduce the rate of release of the polypeptides or nucleic acids contained within the reprogramming composition from the implantable device. The polymeric layer can comprise poly(methylmethacrylate) or poly(butylmethacrylate), and can further include poly(ethylene co-vinyl acetate). The barrier can comprise a polymer or an inorganic material.

[00202] Suitable polymer materials for first layer include, but are not limited to, polyurethanes, polyesterurethanes, silicone, fluoropolymers, ethylene vinyl acetate, polyethylene, polypropylene, polycarbonates, trimethylenecarbonate, polyphosphazene, polyhydroxybutyrate, polyhydroxyvalerate, polydioxanone, polyiminocarbonates, polyorthoesters, ethylene vinyl alcohol copolymer, L-polylactide, D,L-polylactide, polyglycolide, polycaprolactone, copolymers of lactide and glycolide, polymethylmethacrylate, poly(n-butyl)methacrylate, polyacrylates, polymethacrylates, elastomers, and mixtures thereof.

[00203] Representative elastomers include, but are not limited to, a thermoplastic elastomer material, polyether-amide thermoplastic elastomer, fluoroelastomers, fluorosilicone elastomer, sytrene-butadiene rubber, butadiene-styrene rubber, polyisoprene, neoprene (polychloroprene), ethylene-propylene elastomer, chloro-sulfonated polyethylene elastomer, butyl rubber, polysulfide elastomer, polyacrylate elastomer, nitrile, rubber, polyester, styrene, ethylene, propylene, butadiene and isoprene, polyester thermoplastic elastomer, and mixtures thereof.

[00204] The barrier layer is biocompatible (i.e., its presence does not elicit an adverse response from the body). The barrier layer can have a thickness ranging from about 50 angstroms to about 20,000 angstroms. The barrier can comprise mostly inorganic material. However, some organic compounds (e.g., polyacrylonitrile, polyvinylidene chloride, nylon 6-6, perfluoropolymers, polyethylene terephthalate, polyethylene 2,6-napthalene dicarboxylate, and polycarbonate) may be incorporated in the barrier. Suitable inorganic materials for use within the barrier include, but are not limited to, inorganic elements, such as pure metals including aluminum, chromium, gold, hafnium, iridium, niobium, palladium, platinum, tantalum, titanium, tungsten, zirconium, and alloys of these metals, and inorganic compounds, such as inorganic silicides, oxides, nitrides, and carbides. Generally, the solubility of the drug in the material of the barrier is significantly less than the solubility of the drug in the polymer carrier. Also, generally, the diffusivity of the drug in the material of the barrier is significantly lower than the diffusivity of the drug in the polymer carrier.

[00205] The barrier may or may not be biodegradable (i.e., capable of being broken down into harmless compounds by the action of the body). While non-biodegradable barrier materials may be used, some biodegradable materials may be used as barriers. For example, calcium phosphates such as hydroxyapatite, carbonated hydroxyapatite, tricalcium phosphate, beta-tricalcium phosphate, octacalcium phosphate, amorphous calcium phosphate, and calcium orthophosphate may be used. Certain calcium salts such as calcium phosphate (plaster of paris) may also be used. The biodegradability of the barrier may act as an additional mechanism for controlling drug release from the underlying first layer.

[00206] Methods of affixing the first layer onto the surface of an implantable device, and methods of affixing a barrier layer on the first layer, are known in the art. See, e.g., U.S. Patent Nos. 7,695,731 and 7,691,401.

[00207] As noted above, in some embodiments, a subject implantable device comprises a reservoir comprising a reprogramming composition. For example, in some embodiments, a subject implantable device has at least one surface for contacting a bodily tissue, organ or fluid, where the implantable device comprises: a substrate having a contacting surface; and a drug-eluting coating on at least a portion of the contacting surface, where the coating is comprised of a polymer having zeolites dispersed through the polymer, and where a porous structure of the zeolites includes reservoirs containing a release agent and a reprogramming composition. The release agent prevents the therapeutic material from exiting the reservoir until a triggering condition is met. A triggering condition can be contact of the release agent with a bodily fluid; a change in pH proximate to the release agent; and the like.

[00208] Biodegradable polymers, suitable for use alone or in combination, include, but are not limited to: poly(α-hydroxy acids), such as, polycapro lactone (PCL), poly(lactide-co-glycolide) (PLGA), polylactide (PLA), and polyglycolide (PGA), and combinations and blends thereof above at different ratios to fine-tune release rates, PLGA-PEG (polyethylene glycol), PLA-PEG, PLA-PEG-PLA, polyanhydrides, trimethylene carbonates, polyorthoesters, polyaspirins, polyphosphagenes, and tyrozine polycarbonates; natural and synthetic hydrogel materials, e.g., collagen, starch, chitosans, gelatin, alginates, dextrans, vinylpyrrolidone, polyvinyl alcohol (PVA), PVA-g-PLGA, PEGT-PBT copolymer (polyactive), methacrylates, poly(N-isopropylacrylamide), PEO-PPO-PEO (pluronics), PEO-PPO-PAA copolymers, and PLGA-PEO-PLGA. Polymer matrices according to embodiments of the present invention may include any of the following biostable polymers, alone or in combination: polyurethanes, polymethylmethacrylates copolymers, polyvinyl acetate (PVA), polyamides, and copolymers of polyurethane and silicone.

Reprogramming composition

[00209] The present disclosure provides reprogramming compositions.

[00210] In some embodiments, a subject reprogramming composition comprises either: 1) a mixture of two or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides; or 2) one or more nucleic acids comprising nucleotide sequences encoding two or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides. The reprogramming composition can comprise, in addition to the polypeptides or the nucleic acids, one or more of: a salt, e.g., NaCl, MgCl, KCl, $MgSO_4$, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; glycerol; and the like.

[00211] In some embodiments, a subject reprogramming composition comprises either: 1) a mixture of Gata4, Mef2c, and Tbx5 polypeptides; or 2) one or more nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides. The reprogramming composition can comprise, in addition to the polypeptides or the nucleic acids, one or more of: a salt, e.g., NaCl, MgCl, KCl, $MgSO_4$, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-

Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; glycerol; and the like.

[00212] A subject reprogramming composition can be included in a subject implantable device, as described above. A subject reprogramming composition can be administered directly into an individual. A subject reprogramming composition is useful for reprogramming a post-natal fibroblast into a cardiomyocyte, which reprogramming can be carried out *in vitro* or *in vivo*. Reprogramming a post-natal fibroblast into a cardiomyocyte can be used to treat various cardiac disorders, as described below.

[00213] A subject reprogramming composition can include a pharmaceutically acceptable excipient. Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985. The composition or formulation to be administered will, in any event, contain a quantity of a subject antibody adequate to achieve the desired state in the subject being treated.

[00214] The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

[00215] In some embodiments, a subject reprogramming composition is formulated as a controlled release formulation. Sustained-release preparations may be prepared using methods well known in the art. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody in which the matrices are in the form of shaped articles, e.g. films or microcapsules. Examples of sustained-release matrices include polyesters, copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, hydrogels, polylactides, degradable lactic acid-glycolic acid copolymers and poly-D-(-)-3-

hydroxybutyric acid. Possible loss of biological activity and possible changes in activity of a polypeptide or a nucleic acid comprised in sustained-release preparations may be prevented by using appropriate additives, by controlling moisture content and by developing specific polymer matrix compositions.

[00216] A subject reprogramming composition can further comprise one or more therapeutic agents. Therapeutic agents that can be included in a subject reprogramming composition can include, e.g., digitalis, a statin, an anti-platelet agent, an anti-coagulant, a calcium channel blocker, an angiotensin-converting enzyme inhibitor, a vasodilator, an angiotensin II receptor blocker, a beta blocker, and the like.

UTILITY

[00217] A subject method of reprogramming a post-natal fibroblast is useful for generating a population of induced cardiomyocytes, which induced cardiomyocytes can be used in analytical assays, for generating artificial heart tissue, and in treatment methods.

Analytical assays

[00218] A subject method can be used to generate cardiomyocytes for analytical assays. Analytical assays include, e.g., introduction of the cardiomyocytes into a non-human animal model of a disease (e.g., a cardiac disease) to determine efficacy of the cardiomyocytes in the treatment of the disease; use of the cardiomyocytes in screening methods to identify candidate agents suitable for use in treating cardiac disorders; and the like. In some cases, a cardiomyocyte generated using a subject method can be used to assess the toxicity of a test agent or for drug optimization.

Animal Models

[00219] In some embodiments, a cardiomyocyte generated using a subject method can be introduced into a non-human animal model of a cardiac disorder, and the effect of the cardiomyocyte on ameliorating the disorder can be tested in the non-human animal model (e.g., a rodent model such as a rat model, a guinea pig model, a mouse model, etc.; a non-human primate model; a lagomorph model; and the like). For example, the effect of a cardiomyocyte generated using a subject method on a cardiac disorder in a non-human animal model of the disorder can be tested by introducing the cardiomyocyte into, near, or around diseased cardiac tissue in the non-human animal model; and the effect, if any, of the introduced cardiomyocyte on cardiac function can be assessed.

Methods of assessing cardiac function are well known in the art; and any such method can be used.

Drug/Agent Screening or Identification

[00220] Cardiomyocytes generated using a subject method may be used to screen for drugs or test agents (e.g., solvents, small molecule drugs, peptides, oligonucleotides) or environmental conditions (e.g., culture conditions or manipulation) that affect the characteristics of such cells and/or their various progeny. See, e.g., United States Pat. No. 7425448. Drugs or test agents may be individual small molecules of choice (e.g., a lead compound from a previous drug screen) or in some cases, the drugs or test agents to be screened come from a combinatorial library, e.g., a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis by combining a number of chemical "building blocks." For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of amino acids in every possible way for a given compound length (e.g., the number of amino acids in a polypeptide compound). Millions of test agents (e.g., chemical compounds) can be synthesized through such combinatorial mixing of chemical building blocks. Indeed, theoretically, the systematic, combinatorial mixing of 100 interchangeable chemical building blocks results in the synthesis of 100 million tetrameric compounds or 10 billion pentameric compounds. See, e.g., Gallop et al. (1994), J. Med. Chem 37(9), 1233. Preparation and screening of combinatorial chemical libraries are well known in the art. Combinatorial chemical libraries include, but are not limited to: diversomers such as hydantoins, benzodiazepines, and dipeptides, as described in, e.g., Hobbs et al. (1993), Proc. Natl. Acad. Sci. U.S.A. 90, 6909; analogous organic syntheses of small compound libraries, as described in Chen et al. (1994), J. Amer. Chem. Soc., 116: 2661; Oligocarbamates, as described in Cho, et al. (1993), Science 261, 1303; peptidyl phosphonates, as described in Campbell et al. (1994), J. Org. Chem., 59: 658; and small organic molecule libraries containing, e.g., thiazolidinones and metathiazanones (U.S. Pat. No. 5,549,974), pyrrolidines (U.S. Pat. Nos. 5,525,735 and 5,519,134), benzodiazepines (U.S. Pat. No. 5,288,514).

[00221] Numerous combinatorial libraries are commercially available from, e.g., ComGenex (Princeton, NJ); Asinex (Moscow, Russia); Tripos, Inc. (St. Louis, MO);

ChemStar, Ltd. (Moscow, Russia); 3D Pharmaceuticals (Exton, PA); and Martek Biosciences (Columbia, MD).

[00222] In some embodiments, a cardiomyocyte generated using a subject method is contacted with a test agent, and the effect, if any, of the test agent on a biological activity of the cardiomyocyte is assessed, where a test agent that has an effect on a biological activity of the cardiomyocyte is a candidate agent for treating a cardiac disorder or condition. For example, a test agent of interest is one that increases a biological activity of the cardiomyocyte by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, or more than 10-fold, compared to the biological activity in the absence of the test agent. A test agent of interest is a candidate agent for treating a cardiac disorder or condition. In some embodiments, the contacting is carried out *in vitro*. In other embodiments, the contacting is carried out *in vivo*, e.g, in a non-human animal.

[00223] A “biological activity” includes, e.g., one or more of marker expression (e.g., cardiomyocyte-specific marker expression), receptor binding, ion channel activity, contractile activity, and electrophysiological activity.

[00224] For example, in some embodiments, the effect, if any, of the test agent on expression of a cardiomyocyte marker is assessed. Cardiomyocyte markers include, e.g., cardiac troponin I (cTnI), cardiac troponin T (cTnT), sarcomeric myosin heavy chain (MHC), GATA-4, Nkx2.5, N-cadherin, β-adrenoceptor (β1-AR), a member of the MEF-2 family of transcription factors, creatine kinase MB (CK-MB), myoglobin, and atrial natriuretic factor (ANF).

[00225] As another example, the effect, if any, of the test agent on electrophysiology of the cardiomyocyte is assessed. Electrophysiology can be studied by patch clamp analysis for cardiomyocyte-like action potentials. See Igelmund et al., Pflugers Arch. 437:669, 1999; Wobus et al., Ann. N.Y. Acad. Sci. 27:752, 1995; and Doevendans et al., J. Mol. Cell Cardiol. 32:839, 2000.

[00226] As another example, in some embodiments, the effect, if any, of the test agent on ligand-gated ion channel activity is assessed. As another example, in some embodiments, the effect, if any, of the test agent on voltage-gated ion channel activity is assessed. The effect of a test agent on ion channel activity is readily assessed using standard assays, e.g., by measuring the level of an intracellular ion (e.g., Na^+, Ca^{2+}, K^+, etc.). A change in the intracellular concentration of an ion can be detected using an indicator appropriate to the ion whose influx is controlled by the channel. For example, where the ion channel is a potassium ion channel, a potassium-detecting dye is used; where the ion channel is a calcium ion channel, a calcium-detecting dye is used; etc.

[00227] Suitable intracellular K^+ ion-detecting dyes include, but are not limited to, K^+-binding benzofuran isophthalate and the like. Suitable intracellular Ca^{2+} ion-detecting dyes are listed above.

[00228] The effect of a test agent in the assays described herein can be assessed using any standard assay to observe phenotype or activity of cardiomyocytes generated using a subject method, such as marker expression, receptor binding, contractile activity, or electrophysiology—either in *in vitro* cell culture or in vivo. See, e.g., United States Pat. No. 7425448. For example, pharmaceutical candidates are tested for their effect on contractile activity—such as whether they increase or decrease the extent or frequency of contraction, using any methods known in the art. Where an effect is observed, the concentration of the compound can be titrated to determine the median effective dose (ED50).

Test Agent/Drug Toxicity

[00229] A cardiomyocyte generated using a subject method can be used to assess the toxicity of a test agent, or drug, e.g., a test agent or drug designed to have a pharmacological effect on cardiomyocytes, e.g., a test agent or drug designed to have effects on cells other than cardiomyocytes but potentially affecting cardiomyocytes as an unintended consequence. In some embodiments, the disclosure provides methods for evaluating the toxic effects of a drug, test agent, or other factor, in a human or non-human (e.g., murine; lagomorph; non-human primate) subject, comprising contacting one or more cardiomyocytes generated using a subject method with a dose of a drug, test agent, or other factor and assaying the contacted cardiomyocytes for markers of toxicity or cardiotoxicity.

[00230] Any method known in the art may be used to evaluate the toxicity or adverse effects of a test agent or drug on cardiomyocytes generated using a subject method. Cytotoxicity or cardiotoxicity can be determined, e.g., by the effect on cell viability, survival, morphology, and the expression of certain markers and receptors. For example, biochemical markers of myocardial cell necrosis (e.g., cardiac troponin T and I (cTnT, cTnI)) may be used to assess drug-induced toxicity or adverse reactions in cardiomyocytes generated using a subject method, where the presence of such markers in extracellular fluid (e.g., cell culture medium) can indicate necrosis. See, e.g., Gaze and Collinson (2005) Expert Opin Drug Metab Toxicol 1(4):715-725. In another example, lactate dehydrogenase is used to assess drug-induced toxicity or adverse reactions in cardiomyocytes generated using a subject method. See, e.g., Inoue et al. (2007) AATEX 14, Special Issue: 457-462. In another example, the effects of a drug on chromosomal DNA can be determined by measuring DNA synthesis or repair and used to assess drug-induced toxicity or adverse reactions in cardiomyocytes generated using a subject method. In still another example, the rate, degree, and/or timing of [^{3}H]-thymidine or BrdU incorporation may be evaluated to assess drug-induced toxicity or adverse reactions in cardiomyocytes generated using a subject method. In yet another example, evaluating the rate or nature of sister chromatid exchange, determined by metaphase spread, can be used to assess drug-induced toxicity or adverse reactions in cardiomyocytes generated using a subject method. See, e.g., A. Vickers (pp 375-410 in In vitro Methods in Pharmaceutical Research, Academic Press, 1997). In yet another example, assays to measure electrophysiology or activity of ion-gated channels (e.g., Calcium-gated channels) can be used to assess drug-induced toxicity or adverse reactions in cardiomyocytes generated using a subject method. In still another example, contractile activity (e.g., frequency of contraction) can be used to assess drug-induced toxicity or adverse reactions in cardiomyocytes generated using a subject method.

[00231] In some embodiments, the present disclosure provides methods for reducing the risk of drug toxicity in a human or murine subject, comprising contacting one or more cardiomyocytes generated using a subject method with a dose of a drug, test agent, or pharmacological agent, assaying the contacted one or more differentiated cells for toxicity, and prescribing or administering the pharmacological agent to the subject if the assay is negative for toxicity in the contacted cells. In some embodiments, the present disclosure provides methods for reducing the risk of drug toxicity in a human or murine subject, comprising contacting one or more cardiomyocytes generated using a subject method with a dose of a pharmacological agent, assaying the contacted one or more differentiated cells for toxicity, and prescribing or administering the pharmacological agent to the subject if the assay indicates a low risk or no risk for toxicity in the contacted cells.

Treatment methods using cells

[00232] A subject modified or genetically modified fibroblast can be used to treat an individual in need of such treatment. Similarly, a subject induced cardiomyocyte can be used to treat an individual in need of such treatment. A subject modified or genetically modified fibroblast, or a subject induced cardiomyocyte, can be introduced into a recipient individual (an individual in need of treatment), where introduction into the recipient individual of a subject modified or genetically modified fibroblast, or a subject induced cardiomyocyte, treats a condition or disorder in the individual. Thus, in some embodiments, a subject treatment method involves administering to an individual in need thereof a population of subject modified or genetically modified fibroblasts. In some embodiments, a subject treatment method involves administering to an individual in need thereof a population of subject induced cardiomyocytes.

[00233] In some embodiments, the present disclosure provides a method for performing cell transplantation in a recipient individual in need thereof, the method generally involving: (i) generating an induced cardiomyocyte from a fibroblast obtained from a donor individual, wherein the donor individual is immunocompatible with the recipient individual; and (ii) transplanting one or more of the induced cardiomyocytes into the recipient individual. In some embodiments, the recipient individual and the donor individual are the same individual. In some embodiments, the recipient individual and the donor individual are not the same individuals.

[00234] In some embodiments, the present disclosure provides a method for performing cell transplantation in a recipient individual in need thereof, the method generally involving: (i) genetically modifying a host post-natal fibroblast with one or more nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides, where the host post-natal fibroblasts are obtained from a donor individual, wherein the donor individual is immunocompatible with the recipient individual; and (ii) transplanting one or more of the genetically modified post-natal fibroblasts into the recipient individual. In some embodiments, the recipient individual and the donor individual are the same individual. In some embodiments, the recipient individual and the donor individual are not the same individuals.

[00235] In some embodiments, the present disclosure provides a method for performing cell transplantation in a recipient individual in need thereof, the method generally involving: (i) modifying a host post-natal fibroblast by introducing into the host post-natal fibroblast one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, or a subset (e.g., Gata4, Mef2c, and Tbx5 polypeptides), where the host post-natal fibroblasts are obtained from a donor individual, wherein the donor individual is immunocompatible with the recipient individual; and (ii) transplanting one or more of the modified post-natal fibroblasts into the recipient individual. In some embodiments, the recipient individual and the donor individual are the same individual. In some embodiments, the recipient individual and the donor individual are not the same individuals.

[00236] A subject method of generating induced cardiomyocytes is useful for generating artificial heart tissue, e.g., for implanting into a mammalian subject in need thereof. In some embodiments, a subject treatment method involves administering to an individual in need thereof a subject artificial heart tissue.

[00237] A subject treatment method is useful for replacing damaged heart tissue (e.g., ischemic heart tissue). Where a subject method involves introducing (implanting) an induced cardiomyocyte into an individual, allogeneic or autologous transplantation can be carried out.

[00238] The present disclosure provides methods of treating a cardiac disorder in an individual, the method generally involving administering to an individual in need thereof a therapeutically effective amount of: a) a population of induced cardiomyocytes prepared using a subject method; b) a population of genetically modified post-natal fibroblasts prepared using a subject method; c) a population of modified post-natal fibroblasts prepared using a subject method; or d) an artificial heart tissue prepared using a subject method.

[00239] For example, in some embodiments, a subject method comprises: i) generating an induced cardiomyocyte *in vitro*, as described above; and ii) introducing the induced cardiomyocyte into an individual in need thereof. In other embodiments, a subject method comprises: i) genetically modifying a host post-natal fibroblast with one or more nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, or a subset (e.g., Gata4, Mef2c, and Tbx5 polypeptides); and ii) introducing the genetically modified post-natal fibroblasts into an individual in need thereof.

[00240] In other embodiments, a subject method comprises: i) modifying a host post-natal fibroblast by introducing into the host post-natal fibroblast one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, or a subset (e.g., Gata4, Mef2c, and Tbx5 polypeptides); and ii) introducing the modified post-natal fibroblasts into an individual in need thereof.

[00241] In other embodiments, a subject method comprises: i) generating artificial heart tissue by: a) generating an induced cardiomyocyte, as described above; and b) associating the induced cardiomyocyte with a matrix, to form artificial heart tissue; and ii) introducing the artificial heart tissue into an individual in need thereof. In other embodiments, a subject comprises: i) generating artificial heart tissue by: a) genetically modifying a host post-natal fibroblast with one or more nucleic acids comprising nucleotide sequences encoding one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, or a subset (e.g., Gata4, Mef2c, and Tbx5 polypeptides); and b) associating the genetically modified post-natal fibroblasts with a matrix, to form artificial heart tissue; and ii) introducing the artificial heart tissue into an individual in need thereof. In other embodiments, a subject comprises: i) generating artificial heart tissue by: a) modifying a host post-natal fibroblast by introducing into the host post-natal fibroblast one or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, or a subset (e.g., Gata4, Mef2c, and Tbx5 polypeptides); and b) associating the modified post-natal fibroblasts with a matrix, to form artificial heart tissue; and ii) introducing the artificial heart tissue into an individual in need thereof. The artificial heart tissue can be introduced into, on, or around existing heart tissue in the individual.

[00242] Individuals in need of treatment using a subject method and/or donor individuals 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 subject 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, 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.

[00243] Individuals who are suitable for treatment with a subject method 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.

[00244] For administration to a mammalian host, a population of induced cardiomyocytes, or a population of genetically modified post-natal fibroblasts, generated using a subject method can be formulated as a pharmaceutical composition. A pharmaceutical composition can be a sterile aqueous or non-aqueous solution, suspension or emulsion, which additionally comprises a physiologically acceptable carrier (i.e., a non-toxic material that does not interfere with the activity of the cardiomyocytes). Any suitable carrier known to those of ordinary skill in the art may be employed in a subject pharmaceutical composition. The selection of a carrier will depend, in part, on the nature of the substance (i.e., cells or chemical compounds) being administered. Representative carriers include physiological saline solutions, gelatin, water, alcohols, natural or synthetic oils, saccharide solutions, glycols, injectable organic esters such as ethyl oleate or a combination of such materials. Optionally, a pharmaceutical composition may additionally contain preservatives and/or other additives such as, for example, antimicrobial agents, anti-oxidants, chelating agents and/or inert gases, and/or other active ingredients.

[00245] In some embodiments, an induced cardiomyocyte population, a population of modified post-natal fibroblasts, or a population of genetically modified post-natal fibroblasts, is encapsulated, according to known encapsulation technologies, including microencapsulation (see, e.g., U.S. Pat. Nos. 4,352,883; 4,353,888; and 5,084,350). Where the cardiomyocytes, the modified post-natal fibroblasts, or the genetically modified post-natal fibroblasts are encapsulated, in some embodiments the cardiomyocytes, the modified post-natal fibroblasts, or the genetically modified post-natal fibroblasts are encapsulated by macroencapsulation, as described in U.S. Pat. Nos. 5,284,761; 5,158,881; 4,976,859; 4,968,733; 5,800,828 and published PCT patent application WO 95/05452.

[00246] In some embodiments, an induced cardiomyocyte population, a population of modified post-natal fibroblasts, or a population of genetically modified post-natal fibroblasts, is present in a matrix, as described below.

[00247] A unit dosage form of an induced cardiomyocyte population, a population of modified post-natal fibroblasts, or a population of genetically modified post-natal fibroblasts, can contain from about 10^3 cells to about 10^9 cells, e.g., from about 10^3 cells to about 10^4 cells, from about 10^4 cells to about 10^5 cells, from about 10^5 cells to about 10^6 cells, from about 10^6 cells to about 10^7 cells, from about 10^7 cells to about 10^8 cells, or from about 10^8 cells to about 10^9 cells.

[00248] An induced cardiomyocyte population, a population of modified post-natal fibroblasts, or a population of genetically modified post-natal fibroblasts, can be cryopreserved according to routine procedures. For example, cryopreservation can be carried out on from about one to ten million cells in "freeze" medium which can include a suitable proliferation medium, 10% BSA and 7.5% dimethylsulfoxide. Cells are centrifuged. Growth medium is aspirated and replaced with freeze medium. Cells are resuspended as spheres. Cells are slowly frozen, by, e.g., placing in a container at -80°C. Cells are thawed by swirling in a 37°C bath, resuspended in fresh proliferation medium, and grown as described above.

Artificial heart tissue

[00249] In some embodiments, a subject method comprises: a) reprogramming a population of post-natal fibroblasts into cardiomyocytes *in vitro*, e.g., where the post-natal fibroblasts are present in a matrix, wherein a population of induced cardiomyocytes is generated; and b) implanting the population of induced cardiomyocytes into or on an existing heart tissue in an individual. Thus, the present disclosure provides a method for generating artificial heart tissue *in vitro*; and implanting the artificial heart tissue *in vivo*. In some embodiments, a subject method comprises: a) reprogramming a population of post-natal fibroblasts into cardiomyocytes *in vitro*, generating a population of induced cardiomyocytes; b) associating the induced cardiomyocytes with a matrix, forming an artificial heart tissue; and c) implanting the artificial heart tissue into or on an existing heart tissue in an individual.

[00250] The artificial heart tissue can be used for allogeneic or autologous transplantation into an individual in need thereof. To produce artificial heart tissue, a matrix can be provided which is brought into contact with the post-natal fibroblasts, where the post-natal fibroblasts are reprogrammed into cardiomyocytes using a subject method, as described above. This means that this matrix is transferred into a suitable vessel and a layer of the cell-containing culture medium is placed on top (before or during the reprogramming of the post-natal fibroblasts). The term "matrix" should be understood in this connection to mean any suitable carrier material to which the cells are able to attach themselves or adhere in order to form the corresponding cell composite, i.e. the artificial tissue. In some embodiments, the matrix or carrier material, respectively, is present already in a three-dimensional form desired for later application. For example, bovine pericardial tissue is used as matrix which is crosslinked with collagen, decellularized and photofixed.

[00251] For example, a matrix (also referred to as a "biocompatible substrate") is a material that is suitable for implantation into a subject onto which a cell population can be deposited. A biocompatible substrate does not cause toxic or injurious effects once implanted in the subject. In one embodiment, the biocompatible substrate is a polymer with a surface that can be shaped into the desired structure that requires repairing or replacing. The polymer can also be shaped into a part of a structure that requires repairing or replacing. The biocompatible substrate provides the supportive framework that allows cells to attach to it, and grow on it. Cultured populations of cells can then be grown on the biocompatible substrate, which provides the appropriate interstitial distances required for cell-cell interaction.

Treatment methods using polypeptides or nucleic acids

[00252] The present disclosure provides methods of reprogramming a fibroblast into a cardiomyocyte *in vivo*.

[00253] In some embodiments, the methods generally involve contacting a fibroblast *in vivo* with a reprogramming composition. As discussed above, a reprogramming composition comprises either: 1) two or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides; or 2) one or more nucleic acids comprising nucleotide sequences encoding two or more of Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides. As described above, a subject reprogramming composition can comprise one or more additional components. A subject reprogramming composition can be administered to an individual at or near a treatment site, e.g., in or around the heart.

[00254] In some embodiments, the methods generally involve contacting a fibroblast *in vivo* with a reprogramming composition. As discussed above, a reprogramming composition comprises either: 1) a mixture of Gata4, Mef2c, and Tbx5 polypeptides; or 2) one or more nucleic acids comprising nucleotide sequences encoding Gata4, Mef2c, and Tbx5 polypeptides. As described above, a subject reprogramming composition can comprise one or more additional components. A subject reprogramming composition can be administered to an individual at or near a treatment site, e.g., in or around the heart.

[00255] In some embodiments, a reprogramming composition is introduced into an individual in need thereof in association with an implantable device. Thus, in some embodiments, the present disclosure provides methods of reprogramming a fibroblast into a cardiomyocyte *in vivo*, the methods generally involving introducing a subject implantable device (comprising a subject reprogramming composition) into an individual in need thereof, where the implantable device is introduced at or near a treatment site, e.g., in or around the heart.

[00256] Individuals in need of treatment using a subject method and/or donor individuals 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 subject 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, 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.

[00257] Individuals who are suitable for treatment with a subject method 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.

EXAMPLES

[00258] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1: Direct reprogramming of cardiac fibroblasts into functional cardiomyocytes by defined factors

MATERIALS AND METHODS

Generation of αMHC-GFP and Isl1-YFP mice

[00259] To generate α-myosin heavy chain-green fluorescent protein (αMHC-GFP) mice, enhanced green fluorescent protein-internal ribosome binding site-puromycin^R (EGFP-IRES-Puromycin) cDNA were subcloned into the expression vector containing α-myosin heavy chain (α-MHC) promoter (Gulick et al. (1991) J Biol Chem *266*, 9180-9185). Pronuclear microinjection and other procedures were performed according to the standard protocols (Ieda et al. (2007) Nat Med *13*, 604-612. The transgene was identified by polymerase chain reaction (PCR) analysis (the forward primer, 5'-ATGACAGACAGATCCCTCCT-3' (SEQ ID NO:11); the reverse primer, 5'-AAGTCGTGCTGCTTCATGTG-3' (SEQ ID NO:12)). Isl1-yellow fluorescent protein

(Isl1-YFP) mice were obtained by crossing Isl1–Cre mice and R26R-enhanced yellow fluorescent protein (R26R-EYFP) mice (Srinivas et al. (2001) BMC Dev Biol *1*, 4).

Cell Culture

[00260] For explant culture, isolated neonatal or adult mouse hearts were minced into small pieces less than 1 mm^3 in size. The explants were plated on gelatin-coated dishes, and cultured for 7 days in explant medium (IMDM/20%FBS) (Andersen et al. (2009) Stem Cells *27*, 1571-1581). Migrated cells were harvested and filtered with 40-μm cell strainers (BD) to avoid contamination of heart tissue fragments. αMHC-GFP^-/$Thy1^+$, Isl1-YFP^-/$Thy1^+$, αMHC-GFP^-/$Thy1^+$/c-kit^- or αMHC-GFP^-/$Thy1^+$/c-kit^+ live cells (as defined by the lack of propidium iodine staining) were isolated using fluorescence-activated cell sorting (FACS) Aria 2 (BD Biosciences). For conventional isolation of neonatal cardiac fibroblasts, hearts were digested with 0.1% trypsin and plated on plastic dishes (Ieda et al., (2009) Dev Cell 16:233). Attached fibroblasts were cultured for 7 days and sorted αMHC-GFP^-/$Thy1^+$ cells. Sorted cells were cultured in DMEM/M199 medium containing 10% fetal bovine serum (FBS) at a density of 10^4/cm^2. Cells were transduced by retroviruses after 24 h.

Isolation of Cardiomyocytes

[00261] To isolate cardiomyocytes, neonatal αMHC-GFP^+ ventricles were cut into small pieces and digested with collagenase type II solution (Ieda et al., (2009) *supra*). A single-cell suspension was obtained by gentle triturating and passing through a 40-μm cell strainer. αMHC-GFP^+ live cells were isolated by FACS Aria 2.

Molecular Cloning and Retroviral Infection

[00262] Retroviruses were generated as described (Kitamura et al., (2003) Exp. Hematol. 31:1007; Takahashi and Yamanaka, (2006) Cell 126:663). Briefly, to construct pMXs retroviral vectors, the coding regions of candidate genes were amplified by polymerase chain reaction (PCR) and subcloned into pMXs vector. The pMXs retroviral vectors were transfected into Plat-E cells with Fugene 6 (Roche) to generate viruses. Pool of virus-containing supernatants was used for transduction. After 24 h, the medium was replaced with DMEM/M199 medium and changed every 2–3 days. The pMXs-DsRed Express retrovirus infection in cardiac fibroblasts resulted in >95% transfection efficiency (Hong et al., (2009) Nature 460:1132).

[00263] Mouse nucleotide sequences of Mef2c, Tbx5, and Gata4 used in the constructs are set forth in SEQ ID NOs:23, 25, and 27, respectively. Amino acid sequences of the encoded Mef2c, Tbx5, and Gata4 polypeptides are set forth in SEQ ID NOs:24, 26, and 28, respectively.

FACS Analyses and Sorting

[00264] For green fluorescent protein (GFP) expression analyses, cells were harvested from cultured dishes and analyzed on a FACS Calibur (BD Biosciences) with FlowJo software. For αMHC-GFP/cTnT expression, cells were fixed with 4% PFA for 15 min, permealized with Saponin, and stained with anti-cTnT and anti-GFP antibodies, followed by secondary antibodies conjugated with Alexa 488 and 647 (Kattman et al., (2006) Dev. Cell 11:723).

[00265] For αMHC-GFP^{-}/Thy1^{+}and Isl1-YFP^{-}/Thy1^{+} cell sorting, cells were incubated with PECy7-conjugated anti-Thy1 antibody (eBioscience) and sorted by FACS Aria 2 (Ieda et al., (2009) *supra*). For αMHC-GFP^{-}/Thy1^{+}/c-kit^{-} and αMHC-GFP^{-}/Thy1^{+}/c-kit^{+} cell sorting, PECy7-conjugated anti-Thy1 and allophycocyanin (APC)-conjugated anti-c-kit antibodies (BD) were used. Bone marrow cells were used as a positive control for c-kit staining. PECy7 is a conjugate of phycoerythrin and Cy7 fluorescent dyes.

Cell Transplantation

[00266] Fibroblasts were harvested the next day after retroviral infection. A left thoracotomy was carried out in non-obese diabetic-severe combined immunodeficiency (NOD-SCID) mice, and 10^{6} cultured cells were injected into the left ventricle. After 1–2 weeks, the hearts were excised for immunohistochemistry.

Immunocytochemistry

[00267] Cells were fixed in 4% paraformaldehyde for 15 min at room temperature, blocked, and incubated with primary antibodies against sarcomeric α-actinin (Sigma Aldrich), vimentin (Progen), GFP (Invitrogen), Thy-1 (BD Biosciences), cardiac troponin T (cTnT) (Thermo Scientific), Nppa (Chemicon), RFP (Rockland), Nkx2.5 (Santa Cruz), with secondary antibodies conjugated with Alexa 488 or 594 (Molecular Probes), and DAPI (Invitrogen).

Histology

[00268] For immunohistochemical studies in cell-injected hearts, hearts were fixed in 0.4% paraformaldehyde overnight, embedded in OCT compound, and frozen in liquid

nitrogen (Ieda et al., (2007) *supra*; Ieda et al., (2009) *supra*). Hearts were cut vertically in 7-μm sections to show both ventricles. Sections were stained with primary antibodies against actinin, red fluorescent protein (RFP), green fluorescent protein (GFP), with secondary antibodies conjugated with Alexa 488 or 594, and 4',6'-diamino-2-phenylindole (DAPI). To analyze GFP expression pattern in αMHC-GFP hearts, hearts were cut longitudinally and stained with actinin, GFP and vimentin.

Quantitative RT-PCR

[00269] Total RNA was isolated from cells, and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed on an ABI 7900HT (Applied Biosystems) with TaqMan probes (Applied Biosystems): *Actc1* (Mm01333821_m1), *Col1a2* (Mm00483888_m1), *Myh6* (Mm00440354_m1), *Ryr2* (Mm00465877_m1), *Gja1* (Mm00439105_m1), *Tbx5* (Mm00803521_m1). The mRNA levels were normalized by comparison to *Gapdh* mRNA.

Microarray Analyses

[00270] Mouse genome-wide gene expression analyses were performed using Affymetrix Mouse Gene 1.0 ST Array. αMHC-GFP$^+$ cardiomyocytes were collected by FACS. Three-factor transduced GFP$^+$ cells and GFP$^-$ cells were collected by FACS after 4 weeks of culture. Cardiac fibroblasts were also collected after 4 weeks of culture. RNA was extracted using PicoPure RNA Isolation (Arcturus). Microarray analyses were performed in triplicate from independent biologic samples, according to the standard Affymetrix Genechip protocol. Data were analyzed using the Affymetrix Power Tool (APT, version 1.8.5). Linear models were fitted for each gene on the sample group to derive estimated group effects and their associated significance with the limma package (Smyth, 2004) in R/Bioconductor. Moderated t-statistics and the associated p-values were calculated. P-values were adjusted for multiple testing by controlling for false-discovery rate by the Benjamini-Hochberg method. Gene annotations were retrieved from Affymetrix (version Nov 12, 2007). Differential gene expression was defined using the statistics/threshold combination.

Ca^{2+} imaging

[00271] Ca^{2+} imaging was performed according to the standard protocol. Briefly, cells were labeled with Rhod-3 (Invitrogen) for 1 h at room temperature, washed, and incubated for an additional 1 h to allow de-esterification of the dye. Rhod-3 labeled cells were analyzed by Axio Observer (Zeiss) with MiCAM02 (SciMedia).

Electrophysiology

[00272] After 4-week transduction with three factors, the electrophysiological activities of induced cardiomyocytes were performed using extracellular electrode recording with an Axopatch 700B amplifier and the pClamp9.2 software (Axon Instruments). Induced cardiomyocytes were visually identified by GFP expression and spontaneous contraction. Glass patch pipettes, with typical resistances of 2–4 MΩ, were directly attached on single GFP$^+$ cells for extracellular recording in Tyrode's bath solution.

Statistical analyses

[00273] Differences between groups were examined for statistical significance using Student's t-test or ANOVA. *P* values of < 0.05 were regarded as significant.

RESULTS

Screening for Cardiomyocyte Inducing Factors

[00274] An assay system was developed in which the induction of mature cardiomyocytes from fibroblasts could be analyzed quantitatively by reporter-based fluorescence-activated cell sorting (FACS) (**Figure 1A**). To accomplish this, αMHC promoter-driven EGFP-IRES-Puromycin transgenic mice (αMHC-GFP) were generated, in which only mature cardiomyocytes expressed the green fluorescent protein (GFP) (Gulick et al., (1991) *supra*). It was confirmed that only cardiomyocytes, but not other cell types including cardiac fibroblasts, expressed GFP in the transgenic mouse hearts.

[00275] To have enough cardiac fibroblasts for FACS screening, GFP$^-$ cardiac fibroblasts were obtained from neonatal αMHC-GFP hearts by explant culture. Fibroblast-like cells migrated out from the explants after 2 days and were confluent after 1 week. The migrating cells did not express GFP, but expressed Thy1 and Vimentin, markers of cardiac fibroblasts (**Figure 1B**) (Hudon-David et al., (2007) J. Mol. Cell. Cardiol. 42:991; Ieda et al., (2009) *supra*). To avoid contamination of cardiomyocytes, the cells were filtered by cell strainers to remove heart tissue fragments and isolated Thy1$^+$/GFP$^-$ cells by FACS (**Figure 1C**). The purity of cardiac fibroblasts with Thy1 as a marker for FACS was previously shown (Ieda et al., (2009) *supra*). Using these procedures, no cardiomyocyte contamination was found in the fibroblast culture, and greater than twice

the number of cardiac fibroblasts could be generated than by conventional fibroblast isolation techniques (Ieda et al., (2009) *supra*).

[00276] To select potential cardiac reprogramming factors, microarray analyses were used to identify transcription factors and epigenetic remodeling factors with greater expression in mouse cardiomyocytes (CM) than in cardiac fibroblasts (CF) at embryonic day 12.5 (Ieda et al., (2009) *supra*). Among them, 13 factors were selected that exhibited severe developmental cardiac defects and embryonic lethality when mutated (**Table**).

Table

Gene	**Relative expression CM vs. CF (E12)**	**Gene**	**Relative expression CM vs. CF (E12)**
Hopx	33.1	Tbx5	3.0
Nkx2-5	30.7	Srf	2.5
Hrt2	29.6	Gata4	2.2
Pitx2	24.1	Isl1	2.1
Smyd1	20.6	Mef2c	2.0
Myocd	7.5	Hand2	1.8
Baf60c	3.9	Mesp1	ND

[00277] **Table**. Transcription factors upregulated in embryonic cardiomyocytes compared to cardiac fibroblasts by microarray are listed along with their fold enrichment ($n = 3$). Mesp1 expression was not detected (ND) in either cell type.

[00278] The cardiovascular mesoderm–specific transcription factor Mesp1 was also included because of its cardiac transdifferentiation effect in *Xenopus* (David et al., (2008) Nat. Cell Biol. 10:338). Individual retroviruses were generated, to efficiently express each gene in cardiac fibroblasts.

[00279] Thy1$^+$/GFP$^-$ neonatal mouse cardiac fibroblasts were transduced with a mixture of retroviruses expressing all 14 factors or with DsRed retrovirus (negative control) (Hong et al., (2009) *supra*). No GFP$^+$ cells in cardiac fibroblasts were observed 1 week after Ds-Red retrovirus infection or 1 week of culture without any viral infection. In contrast, transduction of all 14 factors into fibroblast cells resulted in the generation of a small number of GFP$^+$ cells (1.7%), indicating the successful activation of the cardiac-enriched *αMHC* gene in some cells (**Figure 1D and E**).

[00280] To determine which of the 14 factors were critical for activating cardiac gene expression, individual factors were serially removed from the pool of 14. Pools lacking five factors (Baf60c, Hand2, Hopx, Hrt2, and Pitx2c) produced an increased number of GFP^+ cells, suggesting they are dispensable (**Figure 1D and E**). Of note, removal of Gata4 decreased the percentage of GFP^+ cells to 0.5%, while removal of Pitx2c increased it to 5%. Three further rounds of single factor withdrawal were conducted from nine-, six-, and five-factor pools; it was found that four factors (Gata4, Mef2c, Mesp1, and Tbx5) were sufficient for efficient GFP^+ cell induction from cardiac fibroblasts (**Figure 1F-H**). The combination of these four factors dramatically increased the number of fibroblasts activating the *αMHC*-GFP reporter to over 20% (**Figure 1I**).

[00281] **Figures 1A-I**. **Screening for Cardiomyocyte Inducing Factors** (A) Schematic representation of the strategy to test candidate factors. (B) Morphology and characterization of fibroblast-like cells migrating from αMHC-GFP heart explants. Phase contrast (left), GFP (middle), and Thy-1 immunostaining (right). Insets are high-magnification views. (C) $Thy\text{-}1^+/GFP^-$ cells were FACS sorted from explant culture for reprogramming after filtration with cell strainers to remove myocytes. (D) Summary of FACS analyses for GFP^+ cells. Effect on GFP^+ cell induction with 14 factors or the removal of individual factors from the pool of 14 factors ($n = 3$).(E) FACS plots for analyses of GFP^+ cells. GFP^+ cells were analyzed 1 week after 14-factor transduction. The number of GFP^+ cells were reduced by removal of Gata4, but increased by removal of Pitx2c from 14 factors. (F–H) Effect on GFP^+ cell induction of the removal of individual factors from the pool of 9 (F), 6 (G), or 5 (H) factors ($n = 3$ in each case). (I) GFP^+ cells were induced from fibroblasts by the combination of four factors, Gata4, Mef2c, Mesp1, and Tbx5. Representative data are shown in each panel. All data are presented as means ± SD. PI, propidium iodine. *, $P<0.01$; **, $P<0.05$ vs relative control. Scale bars, 100 μm.

Gata4, Mef2c, and Tbx5 Are Necessary and Sufficient for Cardiomyocyte Induction

[00282] Next, expression of cardiac troponin T (cTnT), a specific sarcomeric marker of differentiated cardiomyocytes (Kattman et al., (2006) *supra*), was examined. It was found that 20% of GFP^+ cells expressed cTnT 1 week after the four-factor transduction. Again removing individual factors from the four-factor pool in transduction, it was found that Mesp1 was dispensable for cTnT expression (**Figure 2A and B**). In contrast, $cTnT^+$ or GFP^+ cells were not observed, when either Mef2c or Tbx5 was removed. Removal of Gata4 did not significantly affect the number of GFP^+ cells, but cTnT expression was completely abolished, suggesting Gata4 was also required. The combination of two factors, Mef2c and Tbx5, induced GFP expression but not cTnT. No other combination of two factors or single factor induced both GFP and cTnT expression in cardiac fibroblasts (**Figure 2C**). These data suggested that the combination of three factors, Gata4, Mef2c, and Tbx5, is necessary and sufficient to induce cardiac gene expression. To confirm the screening results, cardiac fibroblasts were transduced with three factors (Gata4, Mef2c, and Tbx5) plus Nkx2-5, a critical factor for cardiogenesis but excluded by the initial screening. Surprisingly, adding Nkx2-5 dramatically inhibited the expression of GFP and cTnT in cardiac fibroblasts, confirming the screening results (**Figure 2D**).

[00283] Next, immunocytochemistry was used to determine if other cardiac markers were expressed in GFP^+ cells. Most GFP^+ cells induced with the three factors expressed sarcomeric α-actinin (actinin) and had well-defined sarcomeric structures (**Figure 2E and F**). GFP^+ cells also expressed cTnT and ANF (atrial natriuretic factor), indicating GFP^+ cells expressed several cardiomyocyte-specific markers (**Figure 2F**).

[00284] **Figures 2A-F**. **Combination of Three Transcription Factors Induces Cardiac Gene Expression in Fibroblasts** (A) FACS analyses for α-MHC-GFP and cardiac Troponin T (cTnT) expression. Effects of the removal of individual factors from the pool of four factors on GFP^+ and $cTnT^+$ cell induction. Note that removal of Gata4 did not affect GFP^+, but cTnT expression was strongly inhibited. (B) Quantitative data of GFP^+ cells and $cTnT^+$ cells in (A) ($n = 3$). (C) Effect of the transduction of pools of three, two, and one factors on GFP^+ and $cTnT^+$ cell induction ($n = 3$). (D) FACS plot showing that Nkx2-5 inhibited reprogramming induced with three (GMT; Gata4, Mef2c and Tbx5) factors. (E) Immunofluorescent staining for GFP (green), actinin (red) and DAPI (blue). The combination of three factors, Gata4, Mef2c and Tbx5, induced abundant GFP expression in cardiac fibroblasts 2 weeks after transduction. Note that the majority of GFP^+ cells were positive for actinin. (F) Induced cardiomyocytes expressed several cardiac markers by immunocytochemistry with clear sarcomeric organization (actinin and Nppa, 2 weeks after transduction; cTnT, 4 weeks after transduction). Insets are high-

magnification views. All data are presented as means ± SD. *, $P<0.01$ vs relative control. Scale bars, 100 μm.

Induced Cardiomyocytes Are Directly Differentiated from Cardiac Fibroblasts

[00285] Next, neonatal cardiac fibroblasts were isolated by the conventional fibroblast isolation method in which hearts were digested with trypsin and plated on plastic dishes (Ieda et al., (2009) *supra*). More than 80% of the cells expressed Thy1, and Thy1^{+}/GFP^{-} cells were isolated by FACS to exclude cardiomyocyte contamination (**Figure 3A**). Fibroblasts transduced with Gata4/Mef2c/Tbx5, hereafter referred to as GMT, expressed GFP, cTnT and actinin after 1 week at the same level as fibroblasts isolated from explant cultures (**Figure 3B and C**). Similar results were obtained upon introduction of GMT into adult cardiac fibroblasts, with full formation of sarcomeric structures (**Figure 3D and E**).

[00286] To determine if the induced cardiomyocytes were arising from a subpopulation of stem-like cells, c-kit expression (Beltrami et al., (2003) Cell 114:763; Wu et al., (2006) Cell 127:1137) was analyzed in the Thy1^{+}/GFP^{-} cells. Most c-kit^{+} cells co-expressed Thy1, while 15% of Thy1^{+} cells expressed c-kit. GFP^{-}/Thy1^{+}/c-kit^{+} cells and GFP^{-}/Thy1^{+}/c-kit^{-} cells were isolated by FACS and transduced each population of cells with the three factors. The results showed 2–3-fold more cardiomyocyte induction from GFP^{-}/Thy1^{+}/c-kit^{-} cells than from GFP^{-}/Thy1^{+}/c-kit^{+} cells (**Figure 3F-H**). These results suggest that most of the induced cardiomyocytes originated from a c-kit negative population.

[00287] Next, it was investigated whether the reprogramming of cardiac fibroblasts to differentiated cardiomyocytes was a direct event or if the fibroblasts first passed through a cardiac progenitor cell fate before further differentiation. To distinguish between these two possibilities, Isl1-YFP mice were used, which were obtained by crossing Isl1-Cre mice and R26R-EYFP mice (Srinivas et al., (2001) *supra*). Isl1 is an early cardiac progenitor marker that is transiently expressed before cardiac differentiation. If cardiomyocytes generated from fibroblasts passed through a cardiac progenitor state, they and their descendents should permanently express YFP (Laugwitz et al., (2005) Nature 433:647). Isl1-YFP^{-} /Thy1^{+}cells were isolated from Isl1-YFP heart explants by FACS and transduced the cells with Gata4, Mef2c, and Tbx5. The resulting cTnT^{+} cells did not express YFP, suggesting that the induced cardiomyocytes (iCMs) were not first reprogrammed into cardiac progenitor cells. Moreover, these results indicated that iCMs did not originate from a rare population of cardiac progenitor cells, which might exist in neonatal hearts (**Figure 3I and J**).

[00288] **Figures 3A-J**. **Induced Cardiomyocytes Originate from Differentiated Cardiac Fibroblasts and are Directly Reprogrammed** (A) Cardiac fibroblasts isolated by the conventional isolation method. Most cells were positive for Thy1, and Thy-1$^+$/GFP$^-$ cells were sorted by FACS for transduction. (B) FACS analyses for GFP and cTnT expression in cardiac fibroblasts isolated in (A) one week after transduction by three factors (GMT). (C) Immunofluorescent staining for GFP (green), actinin (red) and DAPI (blue) in the three-factor induced cardiomyocytes originated from (A). (D) Cardiac fibroblasts isolated from adult αMHC-GFP hearts were transduced with three factors. (E) Immunofluorescent staining for GFP, actinin and DAPI in the induced cardiomyocytes originated from adult cardiac fibroblasts indicated in (D). (F) GFP$^-$/Thy1$^+$/c-kit$^+$ cells and GFP$^-$/Thy1$^+$/c-kit$^-$ cells were isolated by FACS, and transduced with 3 factors. (G) GFP$^-$/Thy1$^+$/c-kit$^-$ cells expressed more GFP and cTnT than GFP$^-$/Thy1$^+$/c-kit$^+$ cells by three-factor transduction. (H) Quantitative data of GFP$^+$ cells and cTnT$^+$ cells in (G) ($n = 3$). (I) Isl1-YFP$^-$/Thy1$^+$cells were sorted from Isl1-YFP heart explants and transduced with three factors. (J) The vast majority of cTnT$^+$ cells induced from Isl1-YFP$^-$/Thy1$^+$cells were negative for YFP. All data are presented as means ± SD. *, $P<0.01$ vs relative control. Scale bars, 100 μm.

Induced Cardiomyocytes Resemble Neonatal Cardiomyocytes in Global Gene Expression

[00289] The time course of cardiomyocyte induction was analyzed. GFP$^+$ cells were detected 3 days after induction and gradually increased in number up to 20% at day 10, and were still present after 4 weeks (**Figure 4A**). Importantly, the percentage of cTnT$^+$ cells and the intensity of cTnT expression in GFP$^+$ cells increased significantly over time (**Figure 4B and C**). GFP$^+$ cells were sorted at 7, 14, and 28 days after transduction with GMT and compared candidate gene expression with cardiac fibroblasts and neonatal cardiomyocytes. The cardiomyocyte-specifc genes, Actc1 (cardiac α-actin), Myh6 (α-myosin heavy chain), Ryr2 (ryanodine receptor 2), and Gja1 (connexin43), were significantly upregulated in a time-dependent manner in GFP$^+$ cells, but were not detected in cardiac fibroblasts by quantitative RT-PCR (qPCR). Col1a2 (collagen 1a2), a marker of fibroblasts, was dramatically downregulated in GFP^+ cells from 7-day culture to the same level as in cardiomyocytes. Expression of the three transduced genes was strongly upregulated in induced cardiomyocytes up to 4 weeks later, suggesting they were not silenced (**Figure 4D**). These data indicated that the three factors induced direct conversion of cardiac fibroblasts to cardiomyocytes rapidly and efficiently, but full maturation was a slow process.

[00290] The global gene expression pattern of iCMs, neonatal cardiomyocytes, and cardiac fibroblasts was compared by mRNA microarray analyses. GFP^+ cells and GFP^- cells were sorted 28 days after GMT transduction. The induced GFP^+ cells were strikingly similar to neonatal cardiomyocytes, but were distinct from GFP^- cells and cardiac fibroblasts in global gene expression pattern. These results demonstrate that iCMs are highly similar to neonatal cardiomyocytes, indicating that the reprogramming event was broadly reflected in global gene expression.

[00291] **Figures 4A-D**. **Gene Expression of Induced Cardiomyocytes is Globally Reprogrammed** (A) The percent of GFP^+ cells compared to the number of plated cells ($n = 3$). The number of GFP^+ cells was counted by FACS sorting at each time point. (B) FACS analyses of cTnT expression in GFP^+ cells. Note that $cTnT^+$ cell number and cTnT intensity were both increased over time ($n = 3$). (C) Quantitative data of cTnT intensity in (B) ($n = 4$). (D) Actc1, Myh6, Ryr2, Gja1, Col1a2 and Tbx5 mRNA expression in cardiac fibroblasts (CF), induced cardiomyocytes (GFP^+, 7 d, 14 d, 28 d after transduction) and neonatal cardiomyocytes (CM), determined by qPCR ($n = 3$).

Induced Cardiomyocytes Exhibit Spontaneous Contraction

[00292] To determine if iCMs possessed the functional properties characteristic of cardiomyocytes, intracellular Ca^{2+} flux was analyzed in iCMs after 4 weeks of culture. Around 30% of iCMs showed spontaneous Ca^{2+} oscillations that resembled those of neonatal cardiomyocytes (**Figure 5A**). Ca^{2+} oscillation frequency was variable among the cells (**Figure 5B**). In addition, spontaneous Ca^{2+} waves were observed in iCMs, similar to neonatal cardiomyocytes (**Figure 5C**).

[00293] In addition to the characteristic Ca^{2+} flux, iCMs showed spontaneous contractile activity after 4–5 weeks in culture. Single cell extracellular recording of electrical activity in beating cells revealed tracings similar to neonatal cardiomyocytes (**Figure 5D**) (Yeung et al., 2001). Thus, the reprogramming of cardiac fibroblast to iCMs was associated with global changes in gene expression and the functional properties characteristic of cardiomyocytes.

[00294] **Figures 5A-D. Induced Cardiomyocytes Exhibit Spontaneous Ca^{2+} Flux, Electrical Activity and Beating** (A) α-MHC-GFP^+ cells showed spontaneous Ca^{2+} oscillation. The pseudo-color image shows Rhod-3 fluorescence intensity in cells. Small squares indicate the Ca^{2+} measuring areas, and the inset is a high-magnification view (left panel). The Rhod-3 intensity trace (right panel) corresponds to the left panel. The arrow indicates the time point corresponding to the image on the left. (B) High frequency of Ca^{2+} oscillation was observed in induced cardiomyocytes. The arrow indicates the time point corresponding to the image on the left. (C) Spontaneous Ca^{2+} waves observed in the induced cardiomyocyte. GFP and Rhod-3 at Ca^{2+} max and min, are shown. The GFP^+ cell is outlined in white dots. (D) Spontaneously contracting cells had electrical activity measured by single cell extracellular electrodes. Representative data are shown in each panel (n = 10).

Cardiac Fibroblasts Convert to Cardiomyocytes by Three-Factor Transduction in Vivo

[00295] Next, to investigate whether Gata4 + Mef2c +Tbx5 (GMT)-transduced cardiac fibroblasts can be reprogrammed to cardiomyocytes in vivo, GFP^-/$Thy1^+$ cardiac fibroblasts were harvested at day 1 after viral transduction and injected them into non-obese diabetic-severe combined immunodeficiency (NOD-SCID) mouse hearts. GMT-infected cells did not express GFP at day 1 after transduction (**Figure 4A**). Cardiac fibroblasts were infected with either the mixture of the three factors and DsRed retroviruses or DsRed retrovirus (negative control) to be readily identified by fluorescence. Cardiac fibroblasts infected with DsRed did not express actinin or GFP, confirming cardiomyocyte conversion did not happen in the negative control (**Figure 6A and B**). Despite being injected into the heart only 1 day after viral infection, a subset of cardiac fibroblasts transduced with the three factors (GMT) and DsRed expressed GFP in the mouse heart within 2 weeks (**Figure 6B**). The GFP^+ cells expressed actinin and had clear sarcomeric structures (**Figure 6C**). These results suggested that Gata4, Mef2c, and Tbx5 were sufficient to convert cardiac fibroblasts to cardiomyocytes within two weeks *in vivo*.

[00296] **Figures 6A-C. Cardiac Fibroblasts Can Be Reprogrammed to Cardiomyocytes In Vivo** (A) DsRed infected cardiac fibroblasts (DsRed-cell) were transplanted into NOD-SCID mouse hearts 1 day after infection and sections of hearts analyzed by immunocytochemistry after 2 weeks. Transplanted fibroblasts marked with DsRed did not express actinin (green). (B) Cardiac fibroblasts infected with DsRed or Gata4/Mef2c/Tbx5 with DsRed (3F/DsRed-cell) were transplanted into NOD-SCID mouse hearts 1 day after infection. Note that a subset of 3F/DsRed cells (red) expressed α-MHC-GFP (green). Data were analyzed two weeks after transplantation. (C) Gata4/Mef2c/Tbx5-transduced cardiac fibroblasts (3F-cell) were transplanted into mouse hearts. A subset of induced GFP^+ cells expressed actinin (red) and had sarcomeric structures. Insets are high-magnification views (arrows). Data were analyzed two weeks after transplantation. Representative data are shown in each panel ($n = 4$ in each group). Scale bars, 100 μm.

Example 2: Direct reprogramming of cardiac fibroblasts into functional cardiomyocytes

[00297] Using methods essentially as described in Example 1, exogenous Gata4, Tbx5, and Mef2c were introduced into mouse post-natal tail tip fibroblasts. About 20% to 30% of the post-natal tail tip fibroblasts were reprogrammed to myosin heavy chain-GFP^+ cells (cardiomyocytes) following introduction of Gata4, Tbx5, and Mef2c (where Gata4, Tbx5, and Mef2c are collectively referred to as GMT).

Example 3: *In vivo* reprogramming of murine cardiac fibroblasts into cardiomyocytes

MATERIALS AND METHODS

[00298] **Retrovirus generation, concentration and titration.** Retroviruses were generated as described in Example 1. The pMXs retroviral vectors containing coding regions of Gata4, Mef2c, Tbx5, and dsRed were transfected into Plat-E cells with Fugene 6 (Roche) to generate viruses. Ultra-high titer virus ($>1X10^{10}$ plaque-forming units (p.f.u) per ml) was obtained by standard ultracentrifugation. Retroviral titration was performed using Retro-X qRT-PCR Titration Kit (Clontech).

[00299] **Animals, surgery, echocardiography and electrocardiography.** Postn-Cre;R26R-lacZ mice were obtained by crossing Periostin–Cre mice (Snider et al. (2009) Circulation Res. 105:934) and Rosa26-lacZ mice (Soriano (1999) *Nat. Genet.* 21:70). Postn-Cre;R26R-EYFP mice were obtained by crossing Periostin–Cre mice and Rosa26-

EYFP mice (Srinivas et al. (2001) *supra*). All surgeries and subsequent analyses were performed in a blinded fashion for genotype and intervention. Myocardial infarction (MI) was induced by permanent ligation of the left anterior descending artery (LAD) as described (Qian et al. (2011) J. Exp. Med. 208:549). A pool of concentrated virus (GMT (Gata-4, Mef2c, Tbx5); or GMTR (GMT plus DsRed)) was mixed, and 10 μl of mixed virus plus 10 μl of PBS or 40 ng/μl Thymosin β4 was injected along the boundary between infarct zone and border zone (based on the blanched infarct area) after coronary artery occlusion. Mouse echocardiography and surface electrocardiography were performed as described (Qian et al. (2011) *supra*).

[00300] **Immunohistochemistry and immunocytochemistry.** Immunohistochemistry and immunocytochemistry were performed as described (Qian et al. (2011) *supra*). Scar size was determined by Masson-Trichrome staining (Bock-Marquette et al. (2004) Nature 432:466; and Qian et al. (2011) *supra*). The area at risk (AAR) and myocardial infarct size were determined by Evans Blue/ triphenyltetrazolium chloride labeling technique (Kurrelmeyer et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:5456).

[00301] **Isolation of adult cardiomyocytes, single cell patch clamp, and cardiac fibroblast migration assay**. Adult cardiomyocyte isolation was performed as described with minor modifications (Xu et al. (1999) *J. Gen. Physiol.* 113:661). Single cell patch clamp recordings were performed as described (Knollmann et al. (2003) *Circulation Res.* 92:428; Le Guennec et al. (1994) *J. Physiol.* 478 Pt 3:493; Spencer et al. (2003) *Am. J. Physiol. Heart Circ. Physiol.* 285:H2552). Migration assay was performed according to the published explant culture protocol (Example 1; Bock-Marquette et al. (2004) *supra*; and Andersen et al. (2009) *supra*). In brief, isolated adult mouse hearts were minced into small pieces less than 1 mm^3 in size. The explants were plated on gelatin-coated dishes and cultured in explant medium (IMDM/20%FBS) until fibroblasts migrated out from minced tissue. The number of days when 10 heart pieces were identified with migratory fibroblasts was recorded.

[00302] **FACS and quantitative RT-PCR.** Dissociated cardiac cells from mouse hearts were stained with APC-conjugated anti-Thy1 antibody (eBioscience) for 30 minutes at room temperature. After washing with PBS twice, stained cells were sorted by FACSAria2 (BD). RNA was extracted by TRizol method (Invitrogen). RT-PCR was performed using the Superscript III first-strand synthesis system (Invitrogen). qPCR was

performed using the ABI 7900HT (TaqMan, Applied Biosystems), per the manufacturer's protocols. Optimized primers from Taqman Gene Expression Array were used.

[00303] **Statistics.** Differences between groups were examined for statistical significance using unpaired student's t-test or ANOVA. $p < 0.05$ was regarded as significant.

RESULTS

[00304] Example 1 describes direct reprogramming of fibroblasts into cardiomyocyte-like cells in vitro upon expression of the three transcription factors, Gata4, Mef2c, and Tbx5 (GMT). As observed in reprogramming to iPS cells, the percentage of fibroblast cells that became fully reprogrammed to beating cardiomyocytes *in vitro* was small, but far more were partially reprogrammed, much like pre-iPS cells that have potential to become fully pluripotent with additional stimuli. It was posited that cardiac fibroblasts may reprogram more fully *in vivo* in their native environment, which might promote their survival, maturation, and coupling with neighboring cells. If so, the vast endogenous pool of cardiac fibroblasts could serve as a potential source of new cardiomyocytes for regenerative therapy.

[00305] To efficiently deliver GMT at high levels *in vivo*, a retroviral system was used to express GMT, or dsRed as a marker, into hearts of 2-month-old male mice. 10 μl of ultra-high-titer retrovirus (~10^{10} copies/ml) that expressed each transcription factor and dsRed control was injected into the myocardial wall as a mixture. Two days after retrovirus injection, transverse sections across the injected area were prepared and co-stained for dsRed, the cardiomyocyte marker, α-Actinin, and Vimentin, which is enriched in the fibroblast population. While no markers are completely specific to cardiac fibroblasts, fibroblasts are characterized by expression of Vimentin and the surface markers Thy1 and DDR2 (Ieda et al. (2009) *supra*). At baseline, it was difficult to detect any α-Actinin- or Vimentin-positive cells that also expressed dsRed, suggesting minimal viral uptake. This was consistent with the observation that retroviruses only infect cells that are actively dividing (Byun et al. (2000) *J. Gene Med.* 2:2).

[00306] Fibroblasts, which have an embryologic origin distinct from cardiomyocytes, become activated after cardiac injury, such as myocardial infarction (MI), and migrate to the site of injury and proliferate. Cardiac injury was induced by coronary artery ligation and injected dsRed retrovirus into the myocardium bordering the infarct zone. While cells co-expressing dsRed and α-Actinin were still undetectable, many Vimentin-positive cells, that were also positive for dsRed, were found (**Figure 7A**). To quantify the percentage of heart cells that took up the virus, fluorescence activated cell sorting (FACS) was used to analyze cells dissociated from the infarct/border zone of injected hearts two days after injury. Cells stained with Thy1, a surface marker enriched in fibroblasts, were increased upon surgery, suggesting successful activation of cardiac fibroblasts (**Figure 7B**). Rare dsRed^{+}Thy1^{+} cells were detected by FACS from sham-operated mice; however, 4.2% of cells from the infarct/border zone of mice with MI were dsRed^{+}Thy1^{+}, suggesting successful delivery of virus into cardiac fibroblasts upon injury (**Figure 7C**). In agreement with this, dsRed^{+}Thy1^{+} sorted cells expressed 60 fold higher levels of Gata4, Mef2c, and Tbx5 than dsRed^{-}Thy1^{+} cells by quantitative PCR (qPCR) (**Figure 7D**).

[00307] To determine if cardiomyocyte conversion from a non-myocyte pool was occurring in vivo, lineage-tracing experiments were used to track the origin of putative induced cardiomyocytes. Cardiac fibroblasts were genetically labeled with a mouse line that expresses Cre-recombinase under the promoter of the fibroblast-enriched gene, Periostin (Snider et al. (2009) *supra*; and Snider et al. (2008) *Circulation Res.* 102:752). When intercrossed with the R26R-lacZ reporter line (Soriano et al. (1999) *supra*), which results in activation of β-galactosidase in Periostin-expressing cells and all their progeny (**Figure 7E**), β-galactosidase activity was found in the majority of cardiac fibroblasts and some endocardial and endothelial cells, as reported (Snider et al. (2009) *supra*; Snider et al. (2008) *supra*; and Takeda et al. (2010) *J. Clin. Invest.* 120:254). Most importantly, β-galactosidase activity was not detected in any cardiomyocytes, even after injury by coronary ligation, in agreement with reports (**Figure 7F**) (Snider et al. (2009) *supra*; Snider et al. (2008) *supra*; and Takeda et al. (2010) *supra*). However, 4 weeks after MI and retroviral delivery of GMT, many β-galactosidase^{+} cells that were also α-Actinin^{+} were found in the injured area of the heart, suggesting that they may be descendants of cells that once expressed Periostin (**Figure 7G**). These cells had well-formed sarcomeres and were shaped similar to β-galactosidase^{-} myocytes (**Figure 7G**).

[00308] **Figures 7A-G. Genetic lineage tracing demonstrates in vivo reprogramming of cardiac fibroblasts to cardiomyocyte-like cells. a,** Confocal images of immunofluourescent staining on hearts showing the integration of dsRed control virus (red) into Vimentin$^+$ cells (green) but not into αActinin$^+$ cells (blue) 2 days after MI. Arrows point to dsRed$^+$Vimentin$^+$ cells (yellow), two of which were scanned under high magnification and shown in the right three panels, with merged image at bottom. **b,** Quantification by FACS analyses of Thy-1 positive cells from hearts 2 days after sham-operation or myocardial infarction (MI). n=3, *p<0.05. **c,** FACS analyses of Thy-1$^+$dsRed$^+$ cells from sham-operated or MI mice with quantification (left) and representative FACS plots (right). n=3, *p<0.05. **d,** qPCR of Gata4, Mef2c and Tbx5 in Thy-1$^+$dsRed$^+$ cells compared to Thy-1$^+$dsRed$^-$ cells sorted two days after GMTR (Gata4, Mef2c, Tbx5 and dsRed) was injected into hearts post-MI. n=3 with technical quadruplicates. **e,** Schematic diagram showing the genetic fate mapping method to lineage trace cardiomyocyte-like cells reprogrammed from Postn-Cre;R26R-lacZ cells. **f,** Immunofluorescent staining for αActinin (green), βGal (red), and DAPI (blue) on Postn-Cre;R26R-lacZ mouse heart sections 4 weeks after sham operation or MI. Note absence of αActinin$^+$ βGal$^+$ double-positive cells even after MI. **g,** Immunofluorescent staining for αActinin (green), βGal (red), and DAPI (blue) on dsRed- or GMT-injected Postn-Cre;R26R-lacZ heart sections from border zone of mice 4 weeks post MI. The lowest panels are magnified pictures of boxed areas in the middle panels.

[00309] The extent and spectrum to which the β-galactosidase$^+$α-Actinin$^+$ cells had been reprogrammed was determined. To avoid the potential for false positive signals from overlaying cells due to the thickness of heart sections, adult cardiomyocytes were isolated at the single-cell level from the infarct/border zone of Periostin-Cre;R26R-lacZ hearts 4 weeks after coronary ligation and injection with GMT or dsRed control. No cardiomyocytes isolated from dsRed-injected hearts were β-galactosidase$^+$ by immunostaining (**Fig. 8A**, n=6 animals, 4~ 6 slides/animal); similar cells isolated from Periostin-Cre;R26R-EYFP mice showed no YFP$^+$ cardiomyocytes among thousands of cells visualized. In contrast, 35% of cells isolated in the cardiomyocyte preparation from the border/infarct zone were β-galactosidase$^+$ after GMT injection (**Figs. 8B and 8C**). Among the β-galactosidase$^+$ cells, 98% were α-Actinin+. To address the possibility that the β-galactosidase$^+$ cells might represent leaky expression of Periostin-Cre or ectopic activation of Cre in cardiomyocytes, hearts were co-injected with pooled retrovirus for GMT and dsRed (GMTR), providing a marker for non-myocyte cells that took up GMT retrovirus. It was found that the β-galactosidase$^+$ cells were also positive for dsRed, indicating they were infected by virus, consistent with their non-cardiomyocyte origin (**Figs. 8D-G**).

[00310] Morphologically, the majority of β-galactosidase$^+$ cells were large with a rod-shaped appearance and were binucleated, closely resembling endogenous cardiomyocytes that were β-galactosidase negative from the same isolation (**Figs. 8D-G**). Further analyses revealed that, in addition to α-Actinin (**Fig. 8H**), the β-galactosidase$^+$ cells expressed multiple sarcomeric markers, including Tropomyosin (**Fig. 8I**), cardiac muscle heavy chain (MHC) (**Fig. 8J**), and cardiac TroponinT (cTnT) (**Fig. 8K**). Examples of cells that showed nearly normal sarcomeric structures throughout the cell, representing ~50% of cells, are shown (**Figs. 8H-K**). The cells did not express Vimentin, smooth muscle α-actin, or SM22, suggesting the cells were no longer cardiac fibroblasts, nor did they become myofibroblasts or vascular smooth muscle cells. For simplicity, the β-galactosidase$^+$α-Actinin$^+$ cardiomyocyte-like cells are referred to as *in vivo* induced cardiomyocytes (iCMs), at least based on distinctive morphology, gene expression and sarcomeric structure.

[00311] To determine if the iCMs expressed proteins involved in cell-cell communication with endogenous cardiomyocytes, the expression level and pattern of N-Cadherin, a cell-surface Ca^{2+}-dependent adhesion molecule that is located in intercalated disks in the myocardium, was examined. Over 90% of iCMs expressed N-Cadherin; 61% had localized N-Cadherin at the cell border; and 5% fully resembled the endogenous cardiomyocyte localization (**Fig. 8L**). Expression of Connexin 43 (Cx43), the major gap junction protein in the heart that promotes electrical coupling of cells and synchronized contraction of myocytes throughout the ventricle, was also examined. About 90% of iCMs expressed Cx43, with half of those expressing Cx43 at high levels and in a pattern similar to endogenous cardiomyocytes with cell border localization (**Fig. 8M**); in 4%, Cx43 was localized in a pattern almost indistinguishable from that of an endogenous cardiomyocyte (**Fig. 8M**).

[00312] To determine if iCMs possessed the typical electrophysiological properties of mature cardiomyocytes, intracellular electrical recording was performed by standard patch clamp techniques. Recording from a single-cell suspension of cardiomyocytes from the border/infarct zone of Periostin-Cre;R26R-EYFP mice transduced with GMT, action potentials of YFP$^+$ cells (iCMs) and endogenous cardiomyocytes that were YFP$^-$

were compared (**Fig. 8N; and Fig. 9**). Many reprogrammed cells had a physiological resting membrane potential (-70 mV or less) and exhibited contraction in response to electrical stimulation but not at rest, similar to adult ventricular cardiomyocytes, which are normally quiescent in the absence of stimulation. Varying action potential morphologies were identified (**Fig. 8N; and Fig. 9**), and some spontaneously contracting cells were observed, but these had resting potentials around -50 mV. Taken together, many *in vivo* reprogrammed cardiomyocytes closely resembled adult ventricular cardiomyocytes 4 weeks after introduction of GMT, while others were broadly similar, differing mainly in their ability to maintain a hyperpolarized resting potential when evaluated as single cells in culture.

[00313] **Figures 8A-N. Single-cell analysis of cardiac reprogramming in vivo. a-c,** Immunofluorescent staining for βGal and DAPI on isolated cardiomyocytes from the infarct/border zone of Postn-Cre;R26R-lacZ hearts 4 weeks after dsRed (a) or GMT (b) injection with quantification in (c). **d-g,** Bright-field image of CMs isolated from GMTR-injected Postn-Cre;R26R-lacZ hearts 4 weeks after MI (d). Of these, (e) is βGal positive and (f,g) are co-stained with dsRed. **h-k,** Immunofluorescent staining for cardiac markers including αActinin, Tropomyosin, cardiac myosin heavy chain (MHC), and cardiac Troponin T (cTnT) co-labeled with βGal and DAPI, on isolated cardiomyocytes from the infarct/border zone of Postn-Cre;R26R-lacZ hearts 4 weeks after GMT injection. The pictures are representative examples of the induced cardiomyocytes next to an endogenous cardiomyocyte from the same preparation with quantification and sample size. High magnification images of boxed areas are shown in the far right panels. **l-m**, Immunofluorescent staining for N-Cadherin, or Connexin 43 co-labeled with βGal and DAPI, on isolated cardiomyocytes from the infarct/border zone of Postn-Cre;R26R-lacZ hearts 4 weeks after GMT injection. Left two panels are representative images with quantification and sample size; right two panels represent examples of the best-reprogrammed induced cardiomyocytes next to an endogenous cardiomyocyte from the same preparation with quantification and sample size. High magnification images of boxed areas are shown next to the merge pictures. **n,** Intracellular current clamp recording of multiple cardiomyocytes isolated from Postn-Cre;R26R-EYFP hearts 4 weeks after MI. iCMs that were YFP^+ displayed action potentials that resembled those of endogenous cardiomyocytes that were YFP^- from the

same preparation. Cells were isolated from Postn-Cre;Rosa-EYFP mice 8 weeks post MI and virus transduction.

[00314] **Figure 9**. Intracellular recordings showing action potentials for additional *in vivo* reprogrammed iCMs (YFP$^+$ cells isolated from Postn-Cre;Rosa-EYFP hearts 8 weeks after MI and infection with Gata4, Mef2c, and Tbx5).

[00315] Since *in vivo* reprogrammed iCMs had contractile potential and may electrically couple with viable endogenous cardiomyocytes, it was determined if converting endogenous cardiac fibroblasts into myocytes translates into partial restoration of heart function after MI. All studies were performed in blinded fashion, including retroviral injection, and de-coded after completion of measurements. Mice injected with GMT or dsRed alone underwent serial high-resolution echocardiography 1 day before and 3 days and 1, 4, 8, and 12 weeks after MI. Using Evans blue/TTC double staining, the area at risk (AAR), and the infarct size of myocardium 48 hours after coronary ligation, were assessed. GMT- and dsRed-injected mice showed no differences in AAR or infarct size (**Fig. 10A**), suggesting that the extent of initial cardiac injury post-MI was not significantly affected by GMT induction. All mice showed a reduction in left ventricular function after coronary artery ligation (**Fig. 11; and Fig. 10B**). 8 and 12 weeks after injection, the fraction of blood ejected from the left ventricle with each contraction (ejection fraction) and the fractional shortening of the ventricular chamber was significantly improved in mice injected with GMT, compared to controls injected with dsRed (**Fig. 11B; and Figs. 10B-D**). Stroke volume (volume of blood ejected with each heart beat) and cardiac output were improved after 8 weeks (**Fig. 10C**) and were close to normal after 12 weeks (**Fig. 11A**).

[00316] As a molecular readout of cardiac dysfunction, qPCR was performed to monitor the expression levels of atrial natriuretic factor (ANF), brain natriuretic peptide (BNP) and tenascin C (Tnc) from GMT-injected and control hearts in the area of injury. All were up-regulated after MI, as expected, but this upregulation was attenuated by injection of GMT in infarcted hearts (**Fig. 11B**). It was also found that the expression level of collagen genes, which was increased upon MI, was partially restored by injecting GMT (**Fig. 11C**). In agreement with the improvement of cardiac function, injection of GMT resulted in a smaller scar size 8 weeks after MI (**Fig. 11D**). ECG studies did not indicate evidence for arrhythmias with GMT injection compared to control dsRed injection, and no mice suffered sudden death.

[00317] **Figures 10A-D. Determination of area at risk (AAR) and infarct size for dsRed or GMT injected hearts after coronary ligation and additional echocardiography data. a,** Representative pictures of Evans blue TTC staining on four continuous slices of left ventricle from representative hearts of dsRed or GMT injected hearts 48 hours after myocardial infarction (MI). Scale bars: 500 μm. Histogram is the blinded quantification of the area at risk (AAR) and infarct size as described in Methods. There was no statistical difference between dsRed and GMT injected MI hearts. **b-c**, Fractional shortening (FS), ejection fraction (EF), stroke volume (SV), and cardiac output (CO) of the left ventricle are shown using high-resolution echocardiography on hearts injected with dsRed or GMT. Changes in these parameters 3 days (b) and 8weeks (c) after MI are shown. **d**, Heart rate during echocardiography is shown for each time point showing no difference between dsRed and GMT cohorts. All echo data in (b, c, d) were collected in blinded fashion. dsRed, n=9; GMT, n=10. $^*p<0.05$.

[00318] **Figures 11A-D. In vivo delivery of cardiac reprogramming factors improves cardiac function after myocardial infarction. a,** Fractional shortening (FS), ejection fraction (EF), stroke volume (SV), and cardiac output (CO) of the left ventricle were measured using high-resolution echocardiography. Changes in these parameters before and 12 weeks after MI were calculated. Data were collected in blinded fashion. dsRed, n=9; GMT (Gata4, Mef2c, Tbx5), n=10. $^*p<0.05$. **b,** qPCR of ANF (Atrial Natriuretic Factor), BNP (Brain Natriuretic Peptide) and Tnc (Tenascin C) on RNA extracted from the border zone of hearts 4 weeks after MI and injection of dsRed or GMT. **c,** qPCR of collagen type I alpha 1 (Col1a1), collagen type I alpha 2 (Col1a2), collagen type III alpha 1(Col3a1), elastin (Eln) on RNA extracted from the border zone of hearts 4 weeks after MI and injection of dsRed or GMT. Data in (b) and (c) are shown relative to dsRed injected sham-operated mice, indicated by dashed line. n=3 for each genotype with technical quadruplicates. **d,** Masson-Trichrome staining of heart sections 8 weeks post-MI injected with dsRed or GMT. Scale bars: 500 μm. Quantification of scar size was calculated by measurement of scar area in four sections for each heart in blinded fashion. dsRed, n=8; GMT, n=9. $^*p<0.05$.

[00319] While GMT delivery significantly affected cardiac repair after MI, it was hypothesized that increasing the number of Thy1$^+$ cells that were infected by the retrovirus might lead to an even greater functional improvement. Thymosin β4, a 43-amino-acid G-actin monomer-binding protein, promotes cell migration, cardiac cell survival and can activate epicardial cells to become more proliferative and give rise to more cardiac fibroblasts and endothelial cells. It was previously reported that Thymosin β4 improves cardiac function and decreases scar size after MI. To test the effects of Thymosin β4 on cardiac fibroblast migration, a cardiac explant migration assay was used. The average time for fibroblasts to migrate from adult heart explants was approximately 3 weeks; however, with Thymosin β4, equivalent fibroblast migration was observed after only 2 weeks and was even more accelerated after MI (**Fig. 12A**). Similarly, the proliferation of Vimentin$^+$ cells increased after MI, and increased even further with Thymosin β4, as marked by phosphohistone H3 (**Fig. 12B**). Consistent with the activation of fibroblasts by Thymosin β4, the percent of Thy1$^+$ (**Fig. 12C**) or Vimentin$^+$ (**Fig. 13A**) cells infected by retrovirus in the setting of MI more than doubled upon intramyocardial injection of Thymosin β4 (**Fig. 12C**). The improved delivery of GMT-expressing retrovirus by Thymosin β4 resulted in an increase in the percent of βGal$^+$ iCMs, compared to total cardiomyocytes, in single-cell cardiomyocyte culture from the infarct/border zone of Periostin-Cre;R26R-lacZ hearts (51% vs. 35%, $p<0.05$) (**Fig. 12D**). However, no change was observed in the *in vivo* reprogramming efficiency (iCMs/total cells infected with GMT virus), which remained ~12% (**Fig. 12D**), or the degree of reprogramming (**Fig. 13B**).

[00320] Injection of Thymosin β4 immediately after coronary ligation resulted in functional improvement of cardiac function, as reported. Co-injection of Thymosin β4 and GMT yielded further functional improvement in ejection fraction and cardiac output 8 weeks after infarction (**Fig. 13E; and Figs. 12C and 12D**). In agreement with this, co-injection of Thymosin β4 and GMT caused a greater reduction in scar size than either Thymosin β4 or GMT injection alone (**Fig. 12F**), despite the area at risk and initial infarct size being similar in both groups (**Fig. 13E**).

[00321] **Figures 12A-F. Thymosin β4 activates cardiac fibroblasts upon injury and enhances in vivo reprogramming. a,** Quantification for cardiac fibroblast migration assay performed on sham-operated or post-MI hearts with or without Tβ4 injection.

Days when 10 minced cardiac tissues were surrounded by migratory fibroblasts ("islands") were averaged from three injected hearts. **b,** Immunofluorescent staining for phosphohistone H3 (pH3, red), Vimentin (green) and DAPI (blue) on heart sections 48 hours after sham-operation, MI or MI with injection of Tβ4. At right, quantification for pH3$^+$Vimentin$^+$ cells. n=3 for each genotype. **c,** FACS analyses of Thy-1$^+$dsRed$^+$ cells from hearts 2 days after sham-operation, MI, or MI with injection of Tβ4 with quantification (left) and representative FACS plots (right). n=3. **d,** Upper panels show immunofluorescent staining for βGal and DAPI on isolated CMs from infarct/border zone of Postn-Cre;R26R-lacZ hearts 4 weeks after MI and GMT injection with or without Tβ4. Lower panels are bright field pictures for the same cells. Quantification of βGal$^+$ cardiomyocyte (CM)-like cells compared to total CMs or total dsRed$^+$ cells (virus-infected) from the border zone of hearts 4 weeks after MI and injection of GMT with or without Tβ4. n=3. **e,** Changes in ejection fraction (EF) and cardiac output (CO) of the left ventricle were determined using high-resolution echocardiography 8 weeks post-surgery. dsRed, n=9; GMT, n=10; dsRed+Tβ4, n=10; GMT+ Tβ4, n=8. **f,** Scar area calculated in blinded fashion from multiple heart sections 8 weeks post-MI after dsRed (n=8), GMT (n=9), dsRed + Tβ4 (n=7) or GMT + Tβ4 (n=8) injection. Representative Masson-Trichrome staining on heart sections is shown. Scale bars: 500 μm. Quantification of scar size was calculated by measurement of scar area in blinded fashion. *$p<0.05$; **$p<0.01$.

[00322] **Figures 13A-E. Thymosin β4 activates cardiac fibroblasts upon injury and enhances *in vivo* reprogramming. a,** Confocal images showing the integration of dsRed control virus into Vimentin$^+$ cells in hearts with or without Thymosin β4 (Tβ4) injection after myocardial infarction (MI). Arrows point to dsRed$^+$Vimentin$^+$ cells. **b,** Quantification of iCM phenotypes after single cell isolation from hearts injected with Tβ4 and GMT. **c,** Blinded quantification of the area at risk (AAR) and infarct size of hearts co-injected with Thymosin β4 and dsRed or GMT 48 hours after MI. **d**, Changes in fractional shortening (FS) and stroke volume (SV) of the left ventricle 8 weeks after MI using high-resolution echocardiography. dsRed, n=9; GMT, n=10; dsRed+Tβ4, n=10; GMT+ Tβ4, n=8. *$p<0.05$, **$p<0.01$. **e**, Heart rate was measured during echocardiography and no difference was found among the four groups. Echo data in (d, e) were collected in blinded fashion.

[00323] The above-described experiments show that resident cardiac fibroblasts can be converted into cardiomyocyte-like cells *in vivo* by local delivery of Gata4, Mef2c, and Tbx5 using retroviral-mediated gene transfer upon cardiac injury. Compared to *in vitro* conversion, *in vivo* cardiac reprogramming occurred with similar initial efficiency. Reprogrammed cells more closely resembled endogenous cardiomyocytes and were more fully reprogrammed.

[00324] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A method of generating an induced cardiomyocyte, the method comprising:

introducing into a post-natal fibroblast one or more reprogramming factors selected from Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides, wherein said introducing results in direct reprogramming of the post-natal fibroblast into a cardiomyocyte, thereby generating an induced cardiomyocyte.

2. The method of claim 1, wherein the reprogramming factors comprise Gata4, Mef2c, and Tbx5.

3. The method of claim 1, wherein the post-natal fibroblast is a human cell, a rodent cell, or a non-human primate cell.

4. The method of claim 1, wherein said introducing comprises genetically modifying the post-natal fibroblast with one or more nucleic acids comprising nucleotide sequences encoding the reprogramming factor polypeptides.

5. The method of claim 4, wherein said nucleic acid is a recombinant vector.

6. The method of claim 4, wherein the nucleotide sequences are operably linked to a transcription regulatory element.

7. The method of claim 6, wherein the transcription regulatory element is a constitutive promoter functional in the post-natal fibroblast.

8. The method of claim 1, wherein said introducing comprises introducing exogenous reprogramming factor polypeptides into the post-natal fibroblast.

9. The method of claim 7, wherein said exogenous reprogramming factor polypeptides comprise a heterologous protein transduction domain.

10. The method of claim 1, further comprising isolating the induced cardiomyocyte.

11. The method of claim 1, wherein said introducing is carried out *in vitro*.

12. The method of claim 1, wherein said introducing is carried out *in vivo*.

13. A genetically modified post-natal fibroblast, wherein the genetically modified post-natal fibroblast is genetically modified with one or more exogenous nucleic acids comprising nucleotide sequences encoding one or more reprogramming factors selected from Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides.

14. The genetically modified post-natal fibroblast of claim 13, wherein the reprogramming factors are Gata4, Mef2c, and Tbx5.

15. The genetically modified post-natal fibroblast of claim 13, wherein the genetically modified post-natal fibroblast is a human cell, a rodent cell, or a non-human primate cell.

16. The genetically modified post-natal fibroblast of claim 13, wherein the one or more exogenous nucleic acids is integrated into the genome of the fibroblast.

17. A composition comprising the genetically modified post-natal fibroblast of claim 13.

18. The composition of claim 17, wherein the composition comprises a liquid culture medium, a cryopreservative agent, or a matrix component.

19. The composition of claim 18, wherein the matrix comprises one or more of collagen, gelatin, fibrin, fibrinogen, laminin, a glycosaminoglycan, elastin, hyaluronic acid,

proteoglycan, a glycan, poly(lactic acid), poly(vinyl alcohol), poly(vinyl pyrrolidone), poly(ethylene oxide), cellulose; a cellulose derivative, starch, a starch derivative, poly(caprolactone), and poly(hydroxy butyric acid).

20. An induced cardiomyocyte derived from the genetically modified post-natal fibroblast of claim 13.

21. An induced cardiomyocyte generated using the method of claim 1.

22. A composition comprising the induced cardiomyocyte of claim 20 or claim 21.

23. The composition of claim 22, wherein the composition comprises a liquid culture medium, a cryopreservative agent, or a matrix component.

24. The composition of claim 23, wherein the matrix comprises one or more of collagen, gelatin, fibrin, fibrinogen, laminin, a glycosaminoglycan, elastin, hyaluronic acid, proteoglycan, a glycan, poly(lactic acid), poly(vinyl alcohol), poly(vinyl pyrrolidone), poly(ethylene oxide), cellulose; a cellulose derivative, starch, a starch derivative, poly(caprolactone), and poly(hydroxy butyric acid).

25. A reprogramming composition comprising:

a) one of:

i) two or more reprogramming factors selected from Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides; or

ii) one or more nucleic acids comprising nucleotide sequences encoding two or more reprogramming factors selected from Gata4, Mef2c, Tbx5, Mesp1, Nkx2-5, Isl-1, Myocd, Smyd1, and Srf polypeptides; and

b) a buffer.

26. The reprogramming composition of claim 25, wherein the two or more reprogramming factors are Gata4, Mef2c, and Tbx5.

27. The reprogramming composition of claim 25, further comprising a pharmaceutically acceptable excipient.

28. The reprogramming composition of claim 25, further comprising a therapeutic agent.

29. An implantable device comprising the reprogramming composition of claim 25.

30. The implantable device of claim 29, wherein the device is a stent.

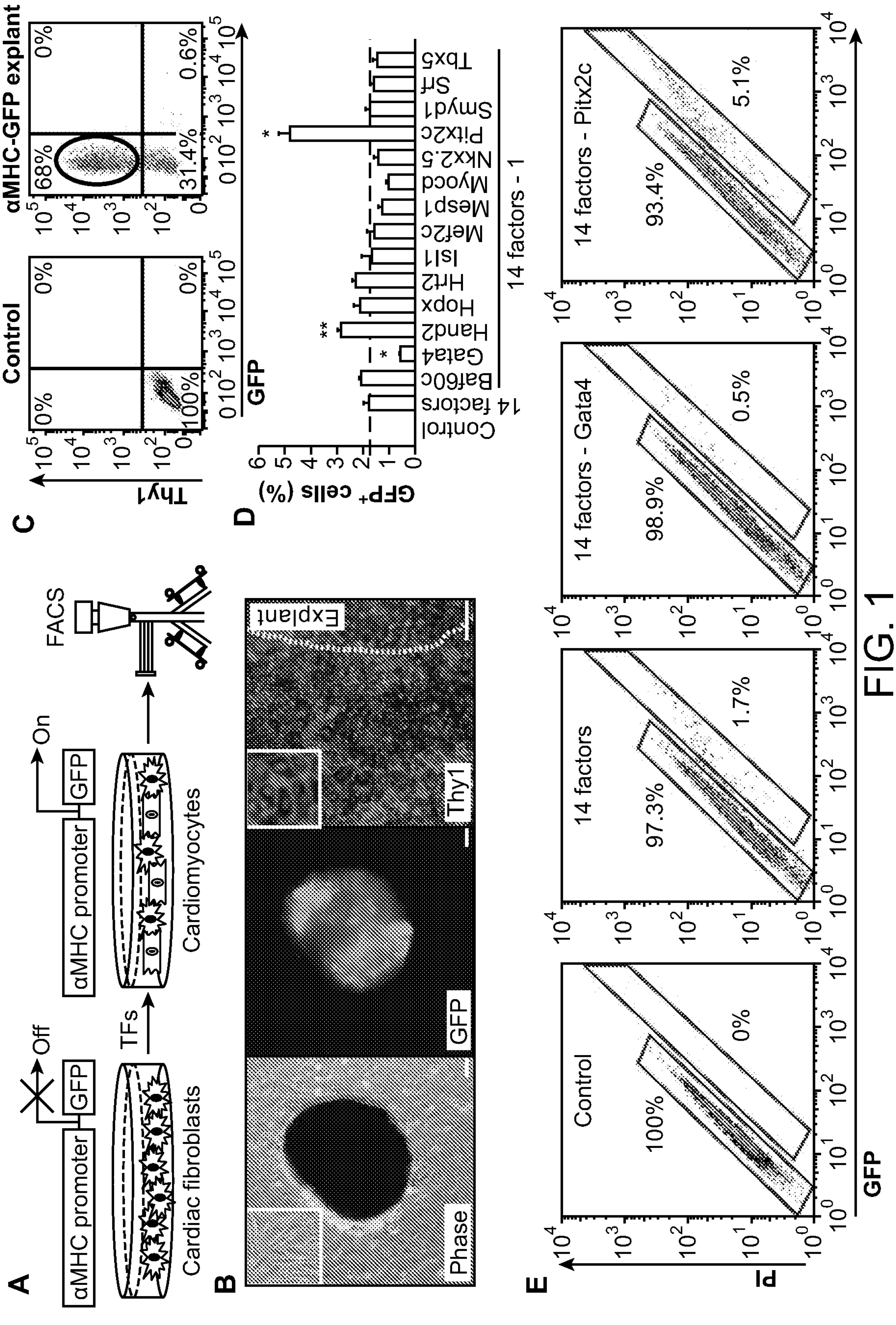
31. The implantable device of claim 29, wherein the reprogramming composition is a controlled-release composition.

32. A method for performing cell transplantation in a recipient individual in need thereof, the method comprising: (i) generating an induced cardiomyocyte from a post-natal fibroblast of a donor individual, wherein the donor individual is immunocompatible with the recipient individual; and (ii) transplanting the induced cardiomyocyte into the recipient individual.

33. The method of claim 32, wherein said recipient and said donor are the same individual.

34. The method of claim 32, wherein said recipient and said donor are different individuals.

35. The method of claim 32, wherein said induced cardiomyocyte is generated using the method of claim 1.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C
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GFP
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0%
100%
0%
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0%
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0.6%
D
GFP+ cells (%)
Control
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Gata4
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Hopx
Hrt2
Isl1
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Mesp1
Myocd
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Pitx2c
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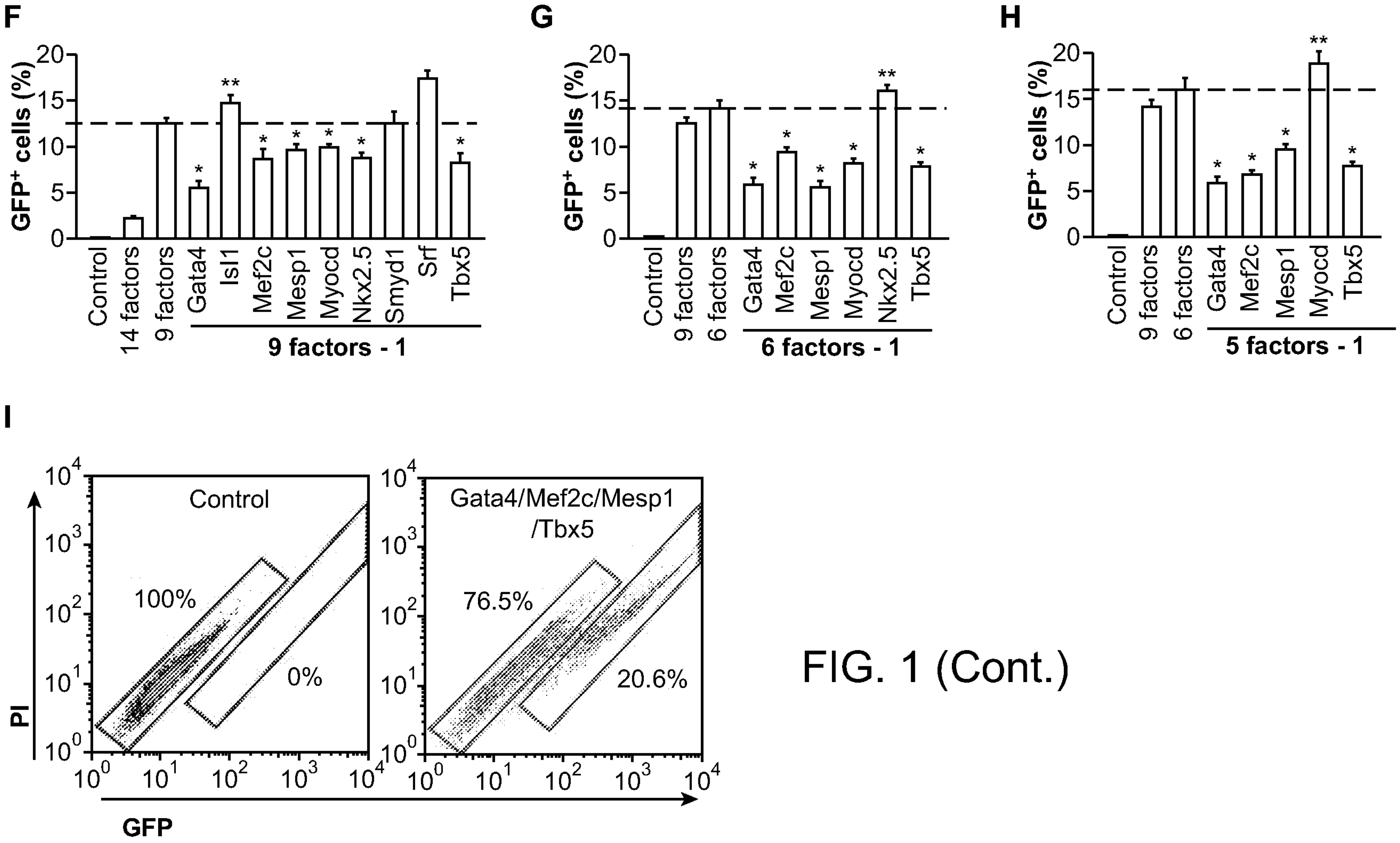
FIG. 1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Gata4
Mef2c
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Myocd
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Tbx5
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Mef2c
Mesp1
Myocd
Tbx5
5 factors - 1
I
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GFP
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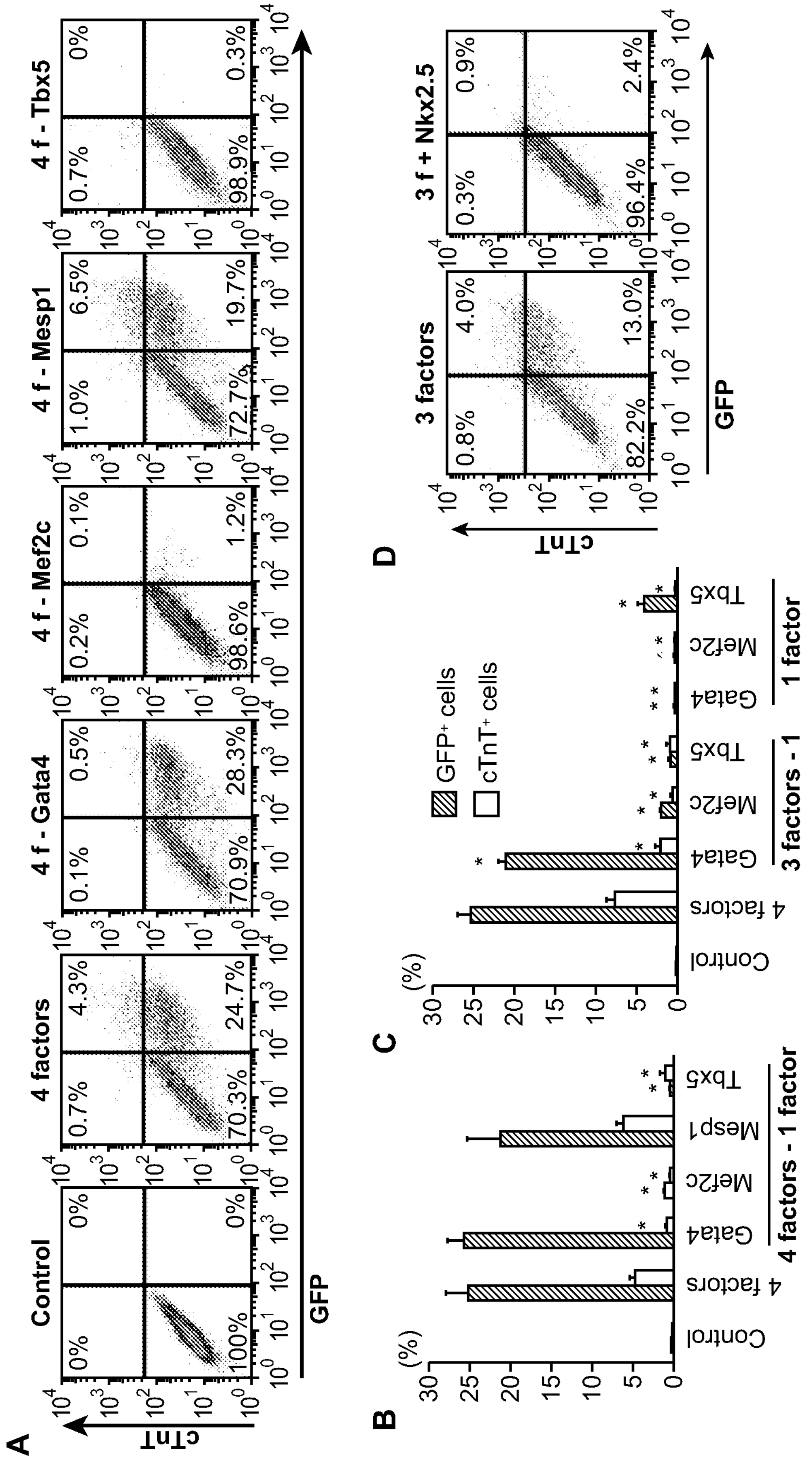
FIG. 1 (Cont.)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0%
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70.3%
24.7%
0.1%
0.5%
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0.1%
98.6%
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1.0%
6.5%
72.7%
19.7%
0.7%
0%
98.9%
0.3%
B
(%)
Control
4 factors
Gata4
Mef2c
Mesp1
Tbx5
4 factors - 1 factor
C
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4 factors
Gata4
Mef2c
Tbx5
3 factors - 1
Gata4
Mef2c
Tbx5
1 factor
GFP+ cells
cTnT+ cells
D
3 factors
3 f + Nkx2.5
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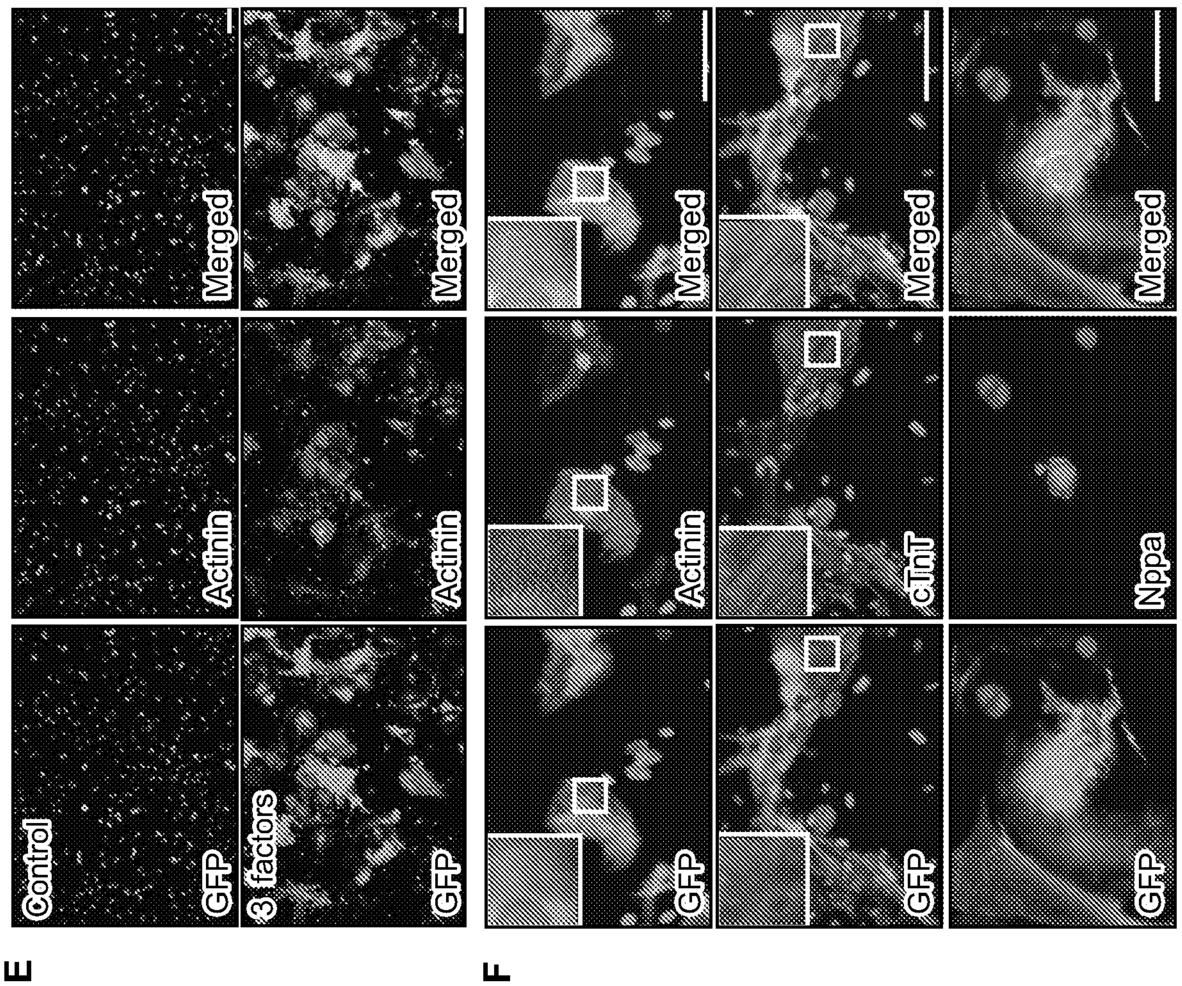
GFP

FIG. 2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Actinin
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Actinin
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Merged
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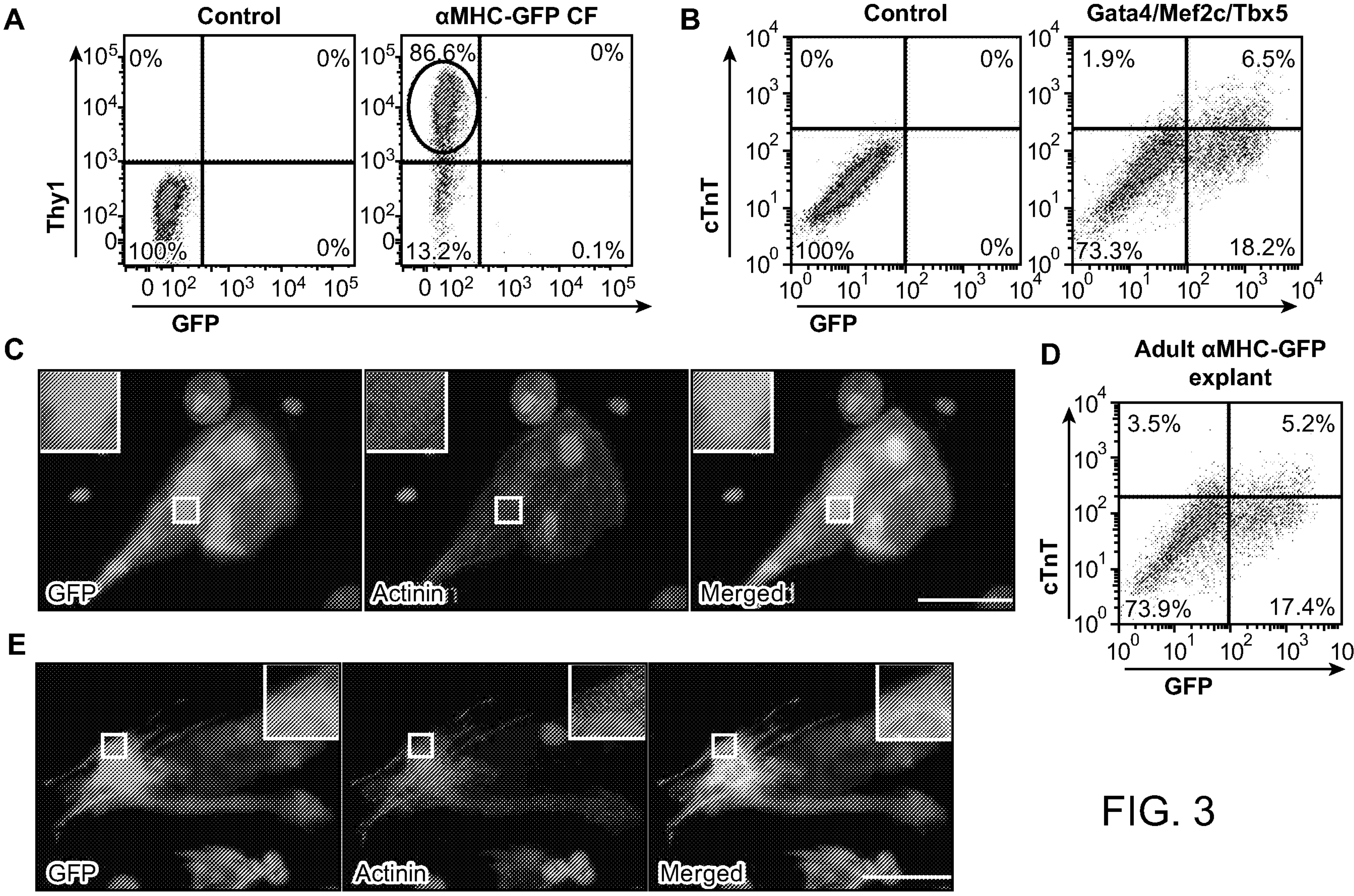
GFP
Nppa
Merged

FIG. 2 (Cont.)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0%
13.2%
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GFP
B
Control
Gata4/Mef2c/Tbx5
cTnT
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10^3
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100%
0%
1.9%
6.5%
73.3%
18.2%
10^0 10^1 10^2 10^3 10^4
GFP
C
GFP
Actinin
Merged
D
Adult αMHC-GFP explant
cTnT
3.5%
5.2%
73.9%
17.4%
10^0 10^1 10^2 10^3 10^4
GFP
E
GFP
Actinin
Merged

FIG. 3

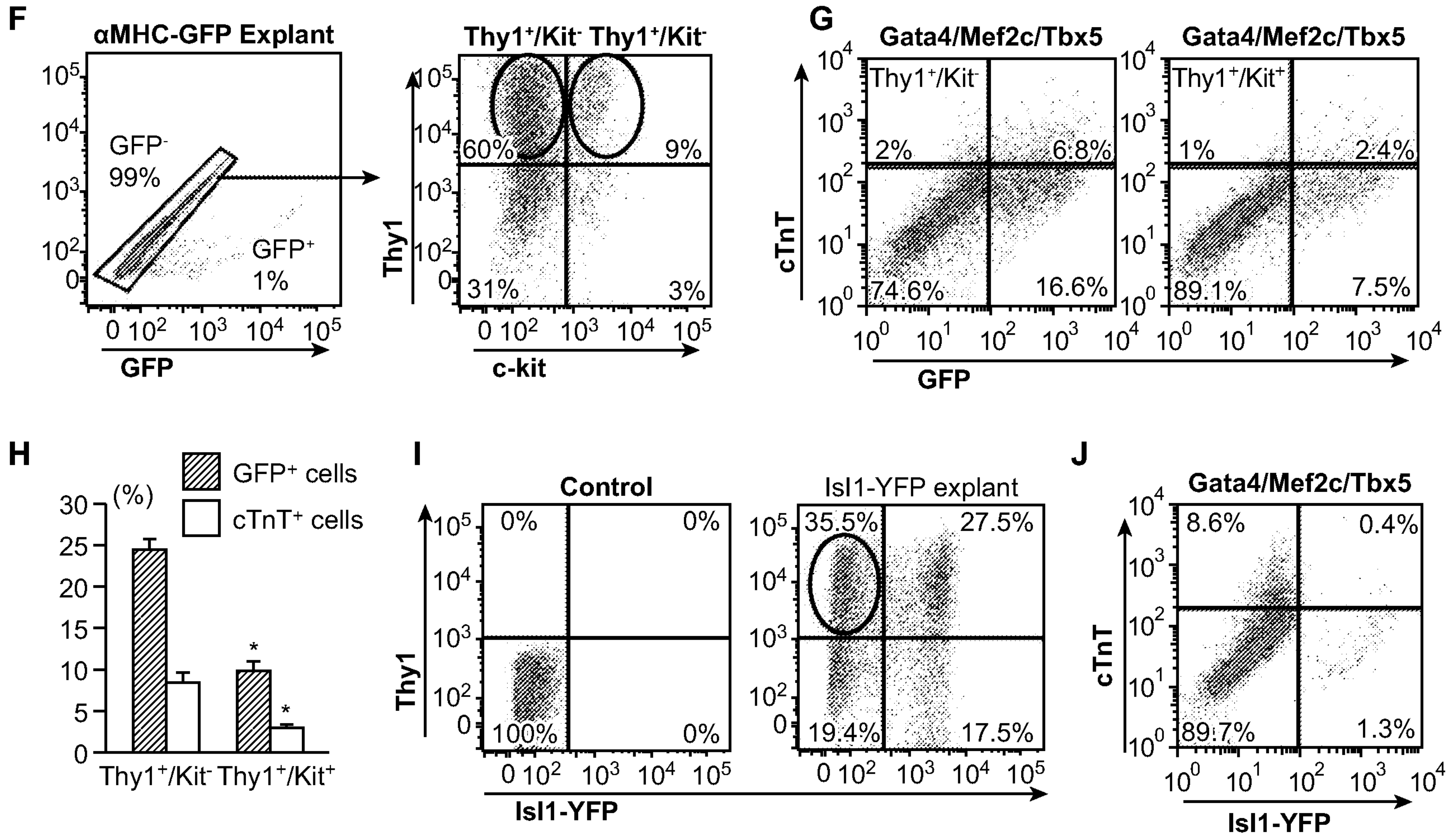

F
αMHC-GFP Explant
GFP-
99%
GFP+
1%
GFP
Thy1+/Kit- Thy1+/Kit-
Thy1
c-kit
60%
9%
31%
3%
G
Gata4/Mef2c/Tbx5
Thy1+/Kit-
2%
6.8%
74.6%
16.6%
Gata4/Mef2c/Tbx5
Thy1+/Kit+
1%
2.4%
89.1%
7.5%
cTnT
GFP
H
(%)
GFP+ cells
cTnT+ cells
Thy1+/Kit- Thy1+/Kit+
I
Control
0%
0%
100%
0%
Isl1-YFP explant
35.5%
27.5%
19.4%
17.5%
Thy1
Isl1-YFP
J
Gata4/Mef2c/Tbx5
8.6%
0.4%
89.7%
1.3%
cTnT
Isl1-YFP

FIG. 3 (Cont.)

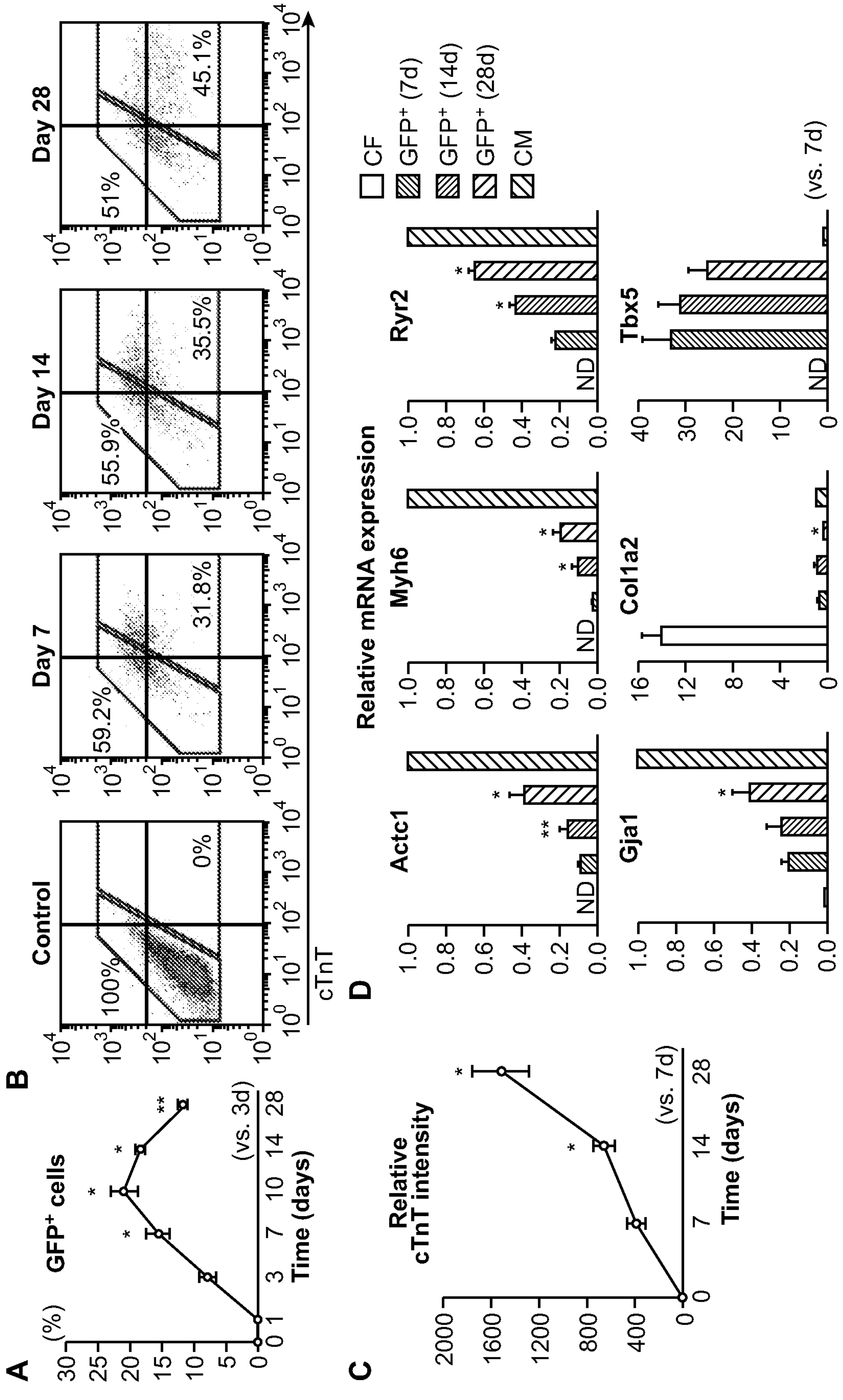

A
GFP⁺ cells
(%)
30
25
20
15
10
5
0
0 1 3 7 10 14 28
Time (days)
(vs. 3d)
*
*
*
**
B
Control
Day 7
Day 14
Day 28
100%
0%
59.2%
31.8%
55.9%
35.5%
51%
45.1%
10⁰ 10¹ 10² 10³ 10⁴
cTnT
C
Relative cTnT intensity
2000
1600
1200
800
400
0
0 7 14 28
Time (days)
(vs. 7d)
*
*
D
Relative mRNA expression
Actc1
Myh6
Ryr2
Gja1
Col1a2
Tbx5
1.0
0.8
0.6
0.4
0.2
0.0
16
12
8
4
0
40
30
20
10
0
ND
*
**
(vs. 7d)
□ CF
GFP⁺ (7d)
GFP⁺ (14d)
GFP⁺ (28d)
CM

FIG. 4

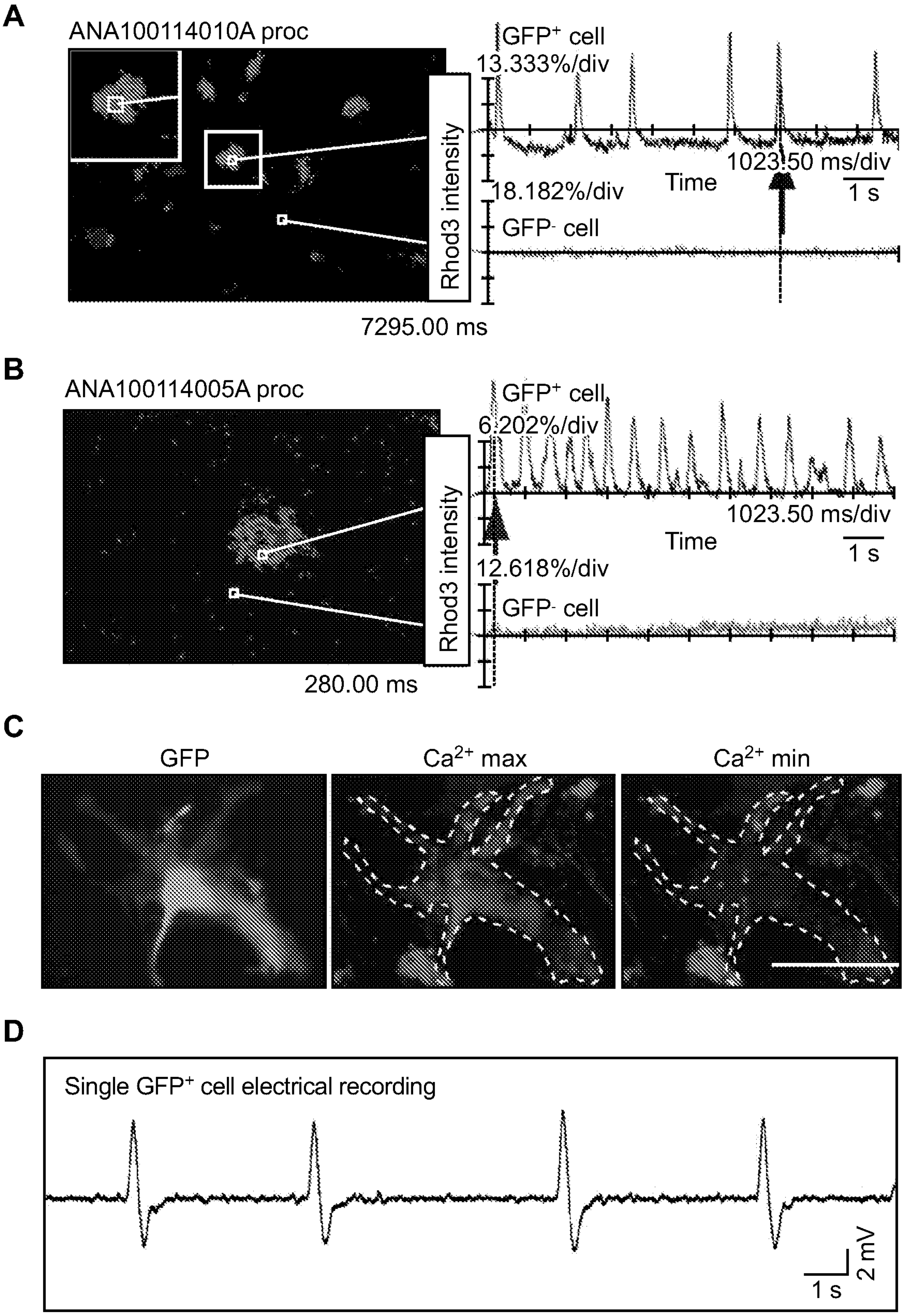

A
ANA100114010A proc
GFP⁺ cell
13.333%/div
Rhod3 intensity
18.182%/div
Time
1023.50 ms/div
1 s
GFP⁻ cell
7295.00 ms
B
ANA100114005A proc
GFP⁺ cell
6.202%/div
Rhod3 intensity
1023.50 ms/div
Time
1 s
12.618%/div
GFP⁻ cell
280.00 ms
C
GFP
Ca²⁺ max
Ca²⁺ min
D
Single GFP⁺ cell electrical recording
2 mV
1 s

FIG. 5

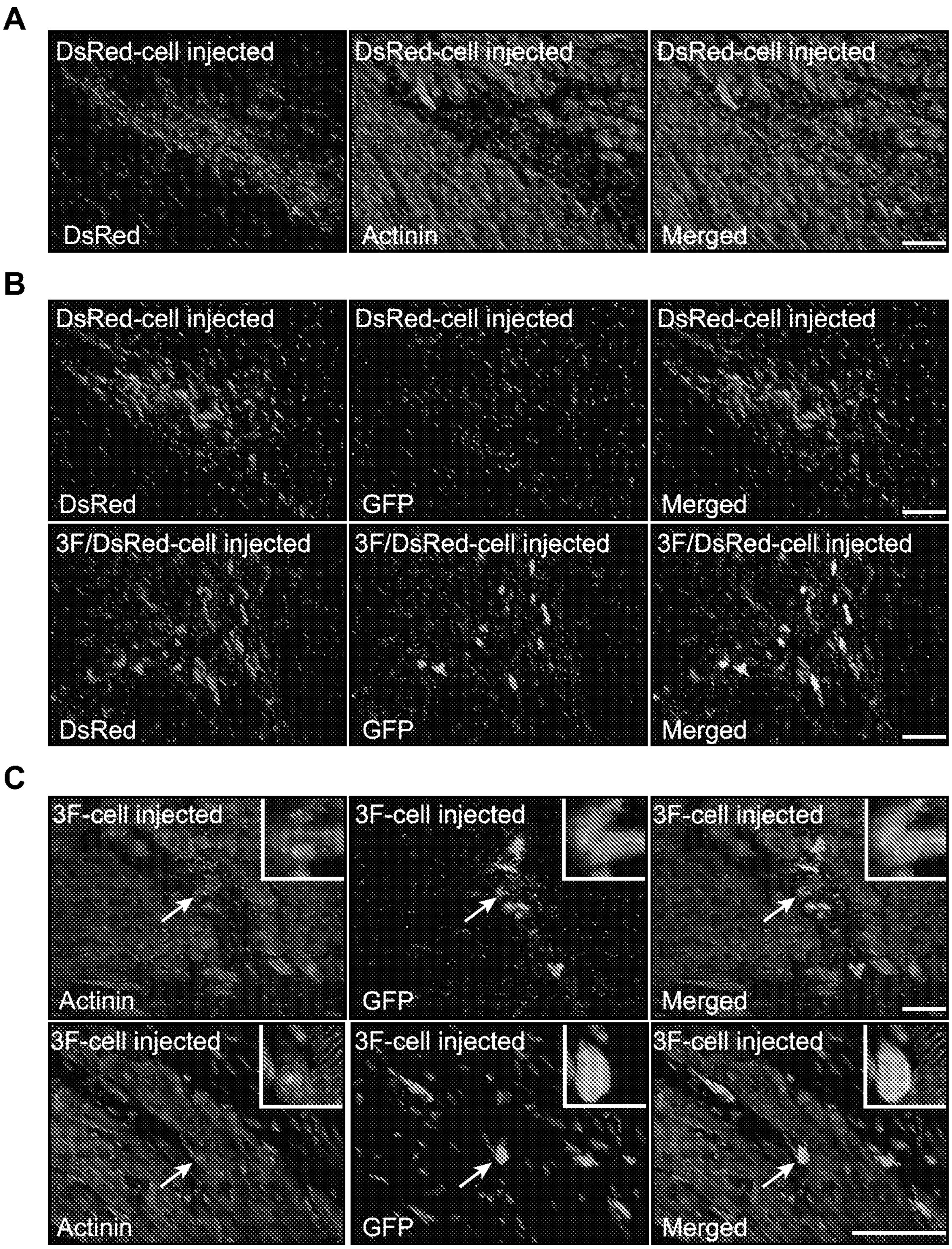

A
DsRed-cell injected
DsRed
DsRed-cell injected
Actinin
DsRed-cell injected
Merged
B
DsRed-cell injected
DsRed
DsRed-cell injected
GFP
DsRed-cell injected
Merged
3F/DsRed-cell injected
DsRed
3F/DsRed-cell injected
GFP
3F/DsRed-cell injected
Merged
C
3F-cell injected
Actinin
3F-cell injected
GFP
3F-cell injected
Merged
3F-cell injected
Actinin
3F-cell injected
GFP
3F-cell injected
Merged

FIG. 6

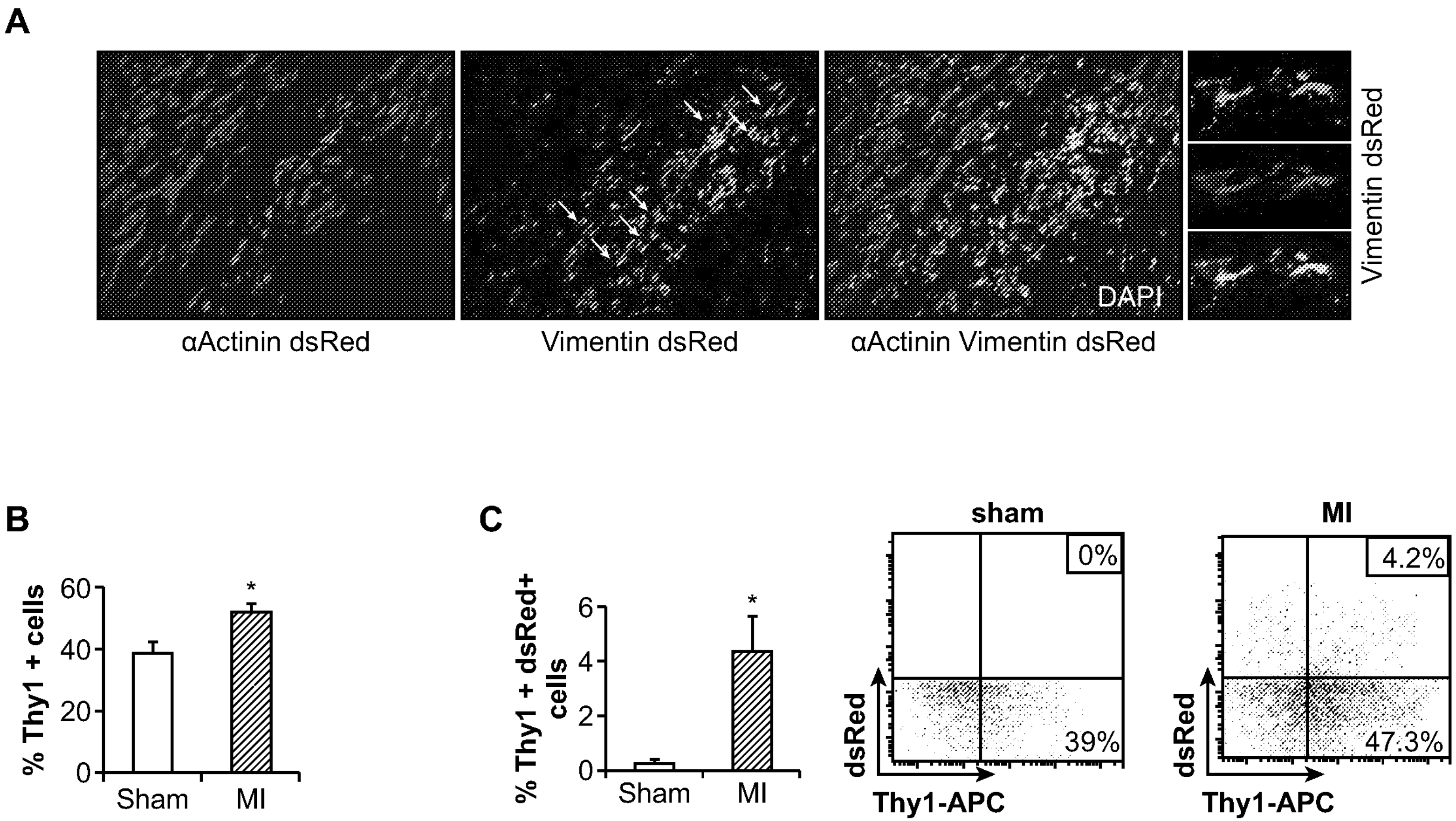

A
αActinin dsRed
Vimentin dsRed
αActinin Vimentin dsRed
DAPI
Vimentin dsRed
B
% Thy1 + cells
60
40
20
0
Sham
MI
*
C
% Thy1 + dsRed+ cells
6
4
2
0
Sham
MI
*
sham
0%
39%
dsRed
Thy1-APC
MI
4.2%
47.3%
dsRed
Thy1-APC

FIG. 7

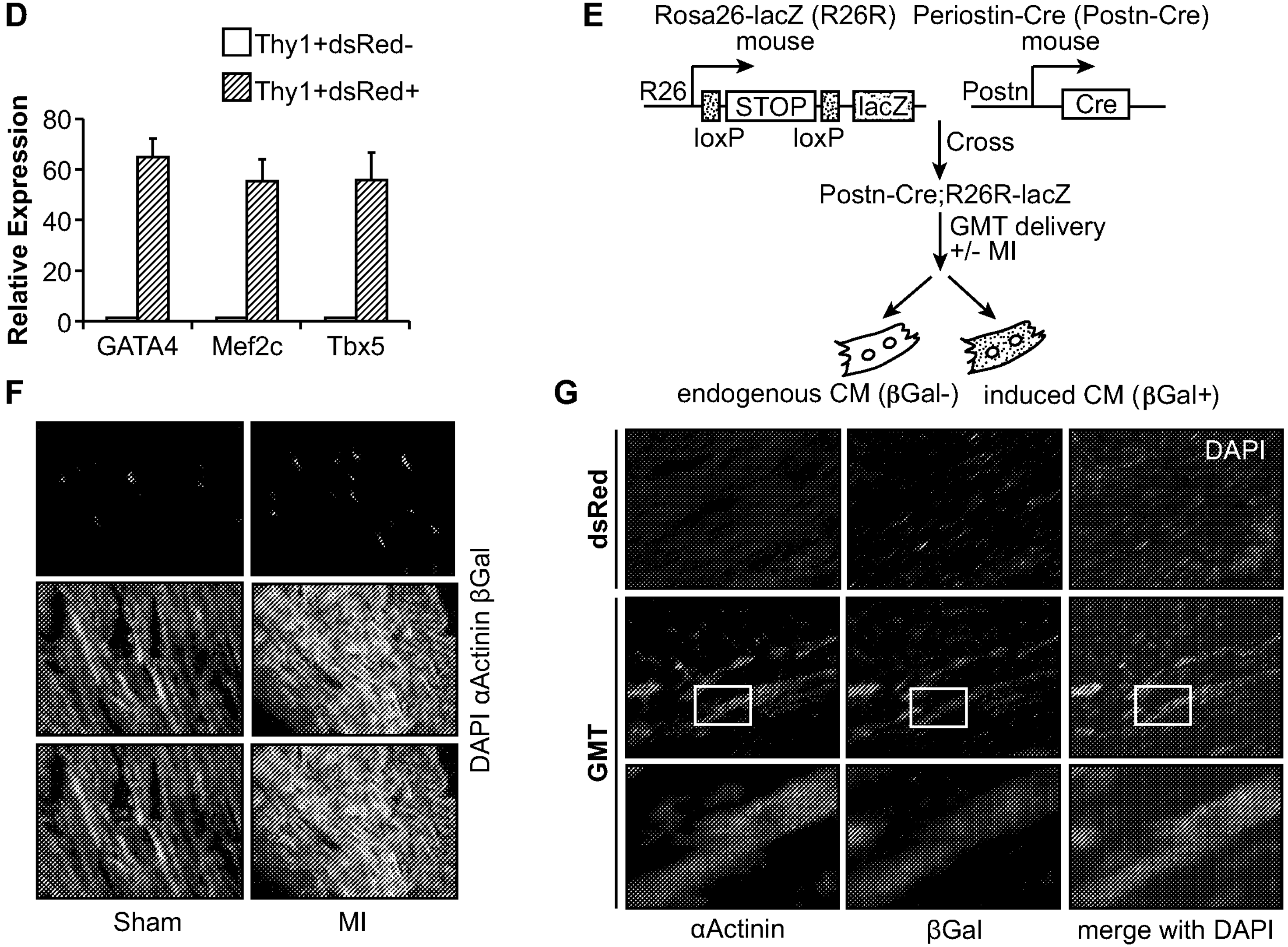

D
□ Thy1+dsRed-
▨ Thy1+dsRed+
Relative Expression
80
60
40
20
0
GATA4
Mef2c
Tbx5
E
Rosa26-lacZ (R26R) mouse
Periostin-Cre (Postn-Cre) mouse
R26
STOP
lacZ
loxP
loxP
Postn
Cre
Cross
Postn-Cre;R26R-lacZ
GMT delivery +/- MI
endogenous CM (βGal-)
induced CM (βGal+)
F
DAPI αActinin βGal
Sham
MI
G
dsRed
GMT
DAPI
αActinin
βGal
merge with DAPI

FIG. 7 (Cont.)

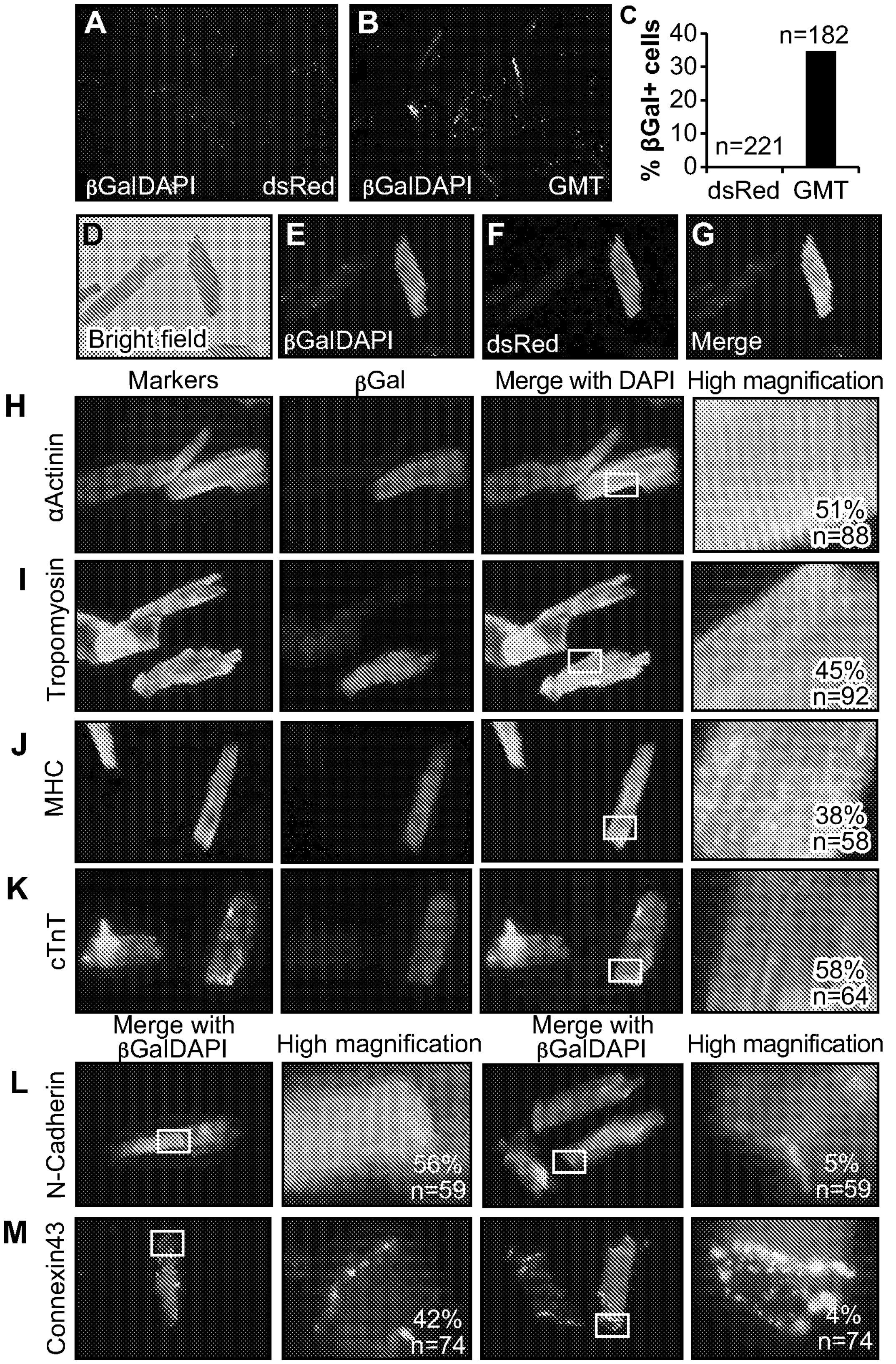

A
βGalDAPI
dsRed
B
βGalDAPI
GMT
C
% βGal+ cells
40
30
20
10
0
n=221
n=182
dsRed
GMT
D
Bright field
E
βGalDAPI
F
dsRed
G
Merge
Markers
βGal
Merge with DAPI
High magnification
H
αActinin
51%
n=88
I
Tropomyosin
45%
n=92
J
MHC
38%
n=58
K
cTnT
58%
n=64
Merge with βGalDAPI
High magnification
Merge with βGalDAPI
High magnification
L
N-Cadherin
56%
n=59
5%
n=59
M
Connexin43
42%
n=74
4%
n=74

FIG. 8

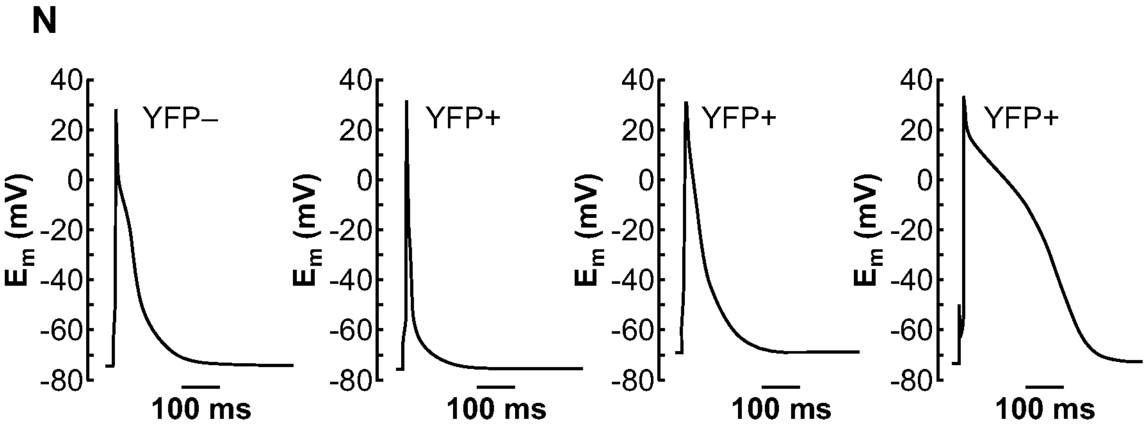

N
40
20
0
-20
-40
-60
-80
E_m (mV)
YFP–
YFP+
YFP+
YFP+
100 ms
100 ms
100 ms
100 ms

FIG. 8 (Cont.)

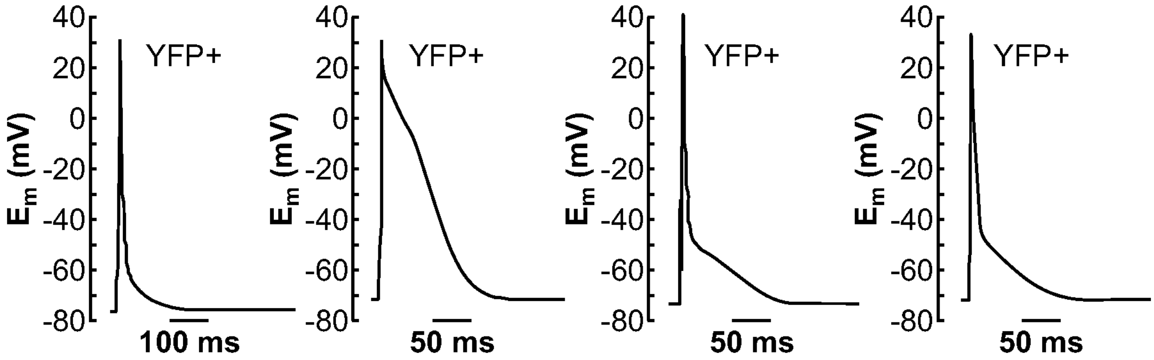


FIG. 9

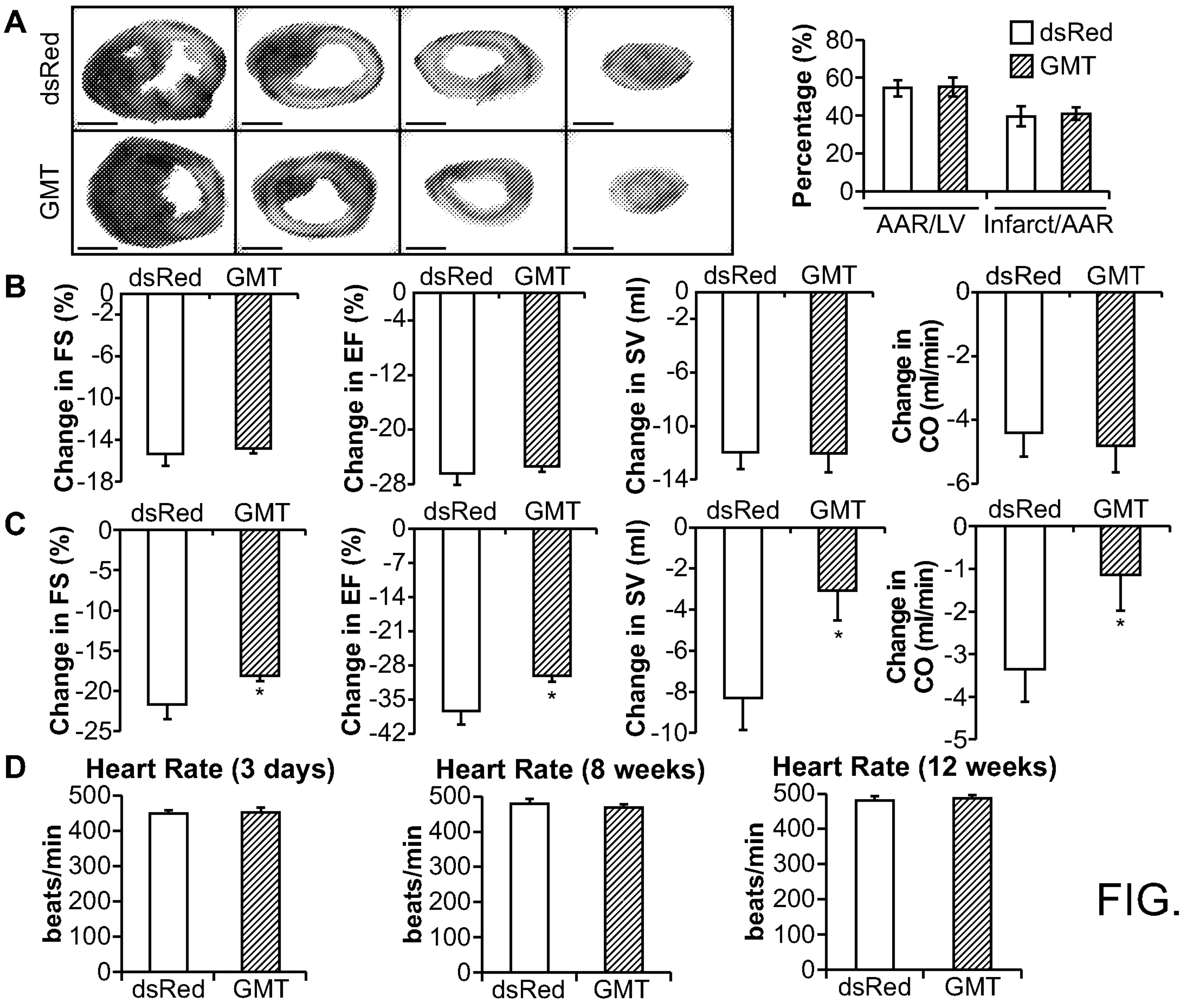

A
dsRed
GMT
Percentage (%)
80
60
40
20
0
AAR/LV
Infarct/AAR
dsRed
GMT
B
Change in FS (%)
0
-2
-6
-10
-14
-18
dsRed
GMT
Change in EF (%)
0
-4
-12
-20
-28
Change in SV (ml)
0
-2
-6
-10
-14
Change in CO (ml/min)
0
-2
-4
-6
C
Change in FS (%)
0
-5
-10
-15
-20
-25
*
Change in EF (%)
0
-7
-14
-21
-28
-35
-42
*
Change in SV (ml)
0
-2
-4
-6
-8
-10
*
Change in CO (ml/min)
0
-1
-2
-3
-4
-5
*
D
Heart Rate (3 days)
Heart Rate (8 weeks)
Heart Rate (12 weeks)
beats/min
500
400
300
200
100
0
dsRed
GMT

FIG. 10

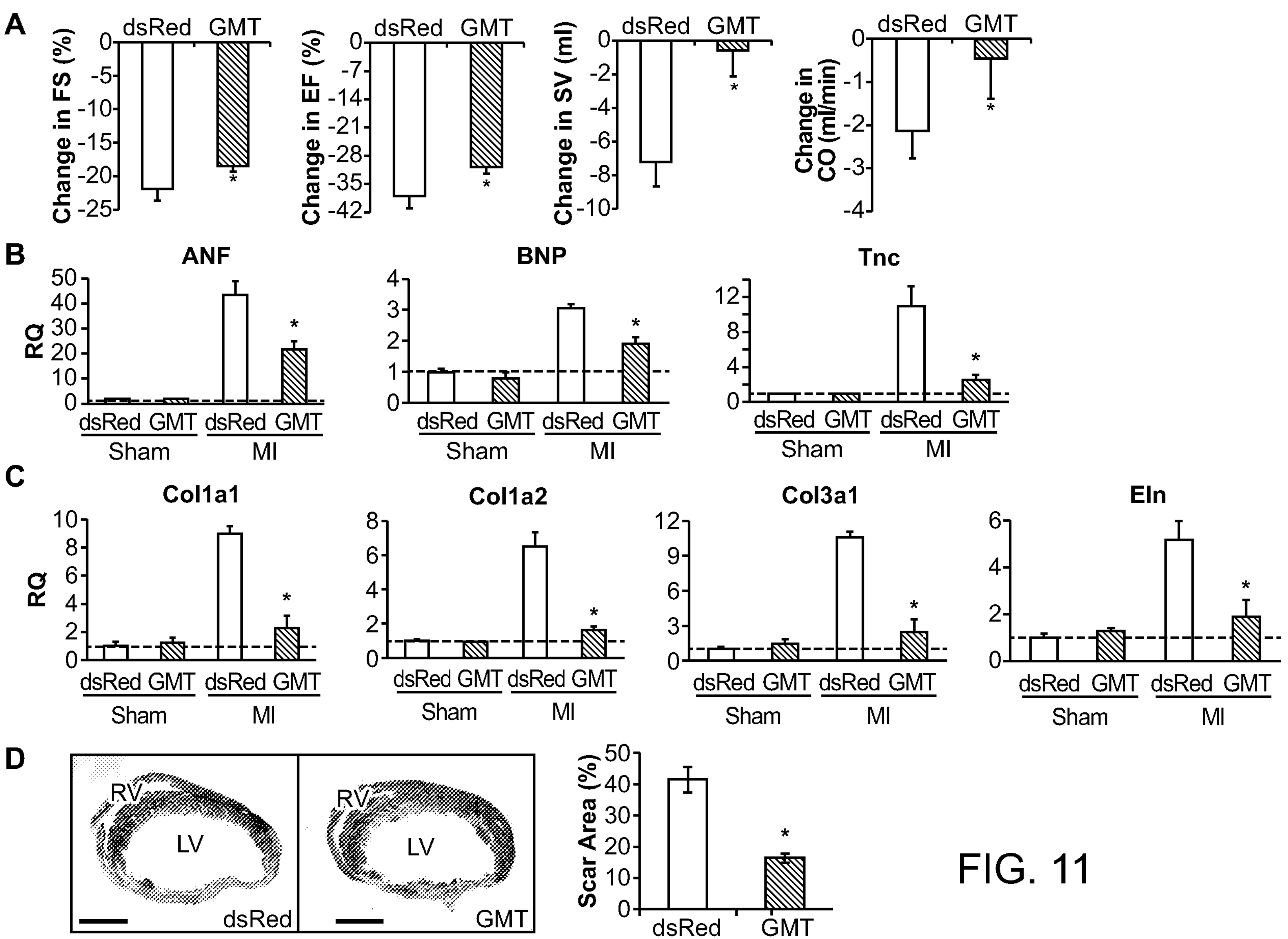

A
dsRed
GMT
Change in FS (%)
0
-5
-10
-15
-20
-25
*
Change in EF (%)
0
-7
-14
-21
-28
-35
-42
Change in SV (ml)
0
-2
-4
-6
-8
-10
Change in CO (ml/min)
0
-1
-2
-3
-4
B
ANF
BNP
Tnc
RQ
50
40
30
20
10
0
4
3
2
1
12
8
4
Sham
MI
C
Col1a1
Col1a2
Col3a1
Eln
10
8
6
4
2
12
9
6
3
D
RV
LV
Scar Area (%)
50
40
30
20
10
0

FIG. 11

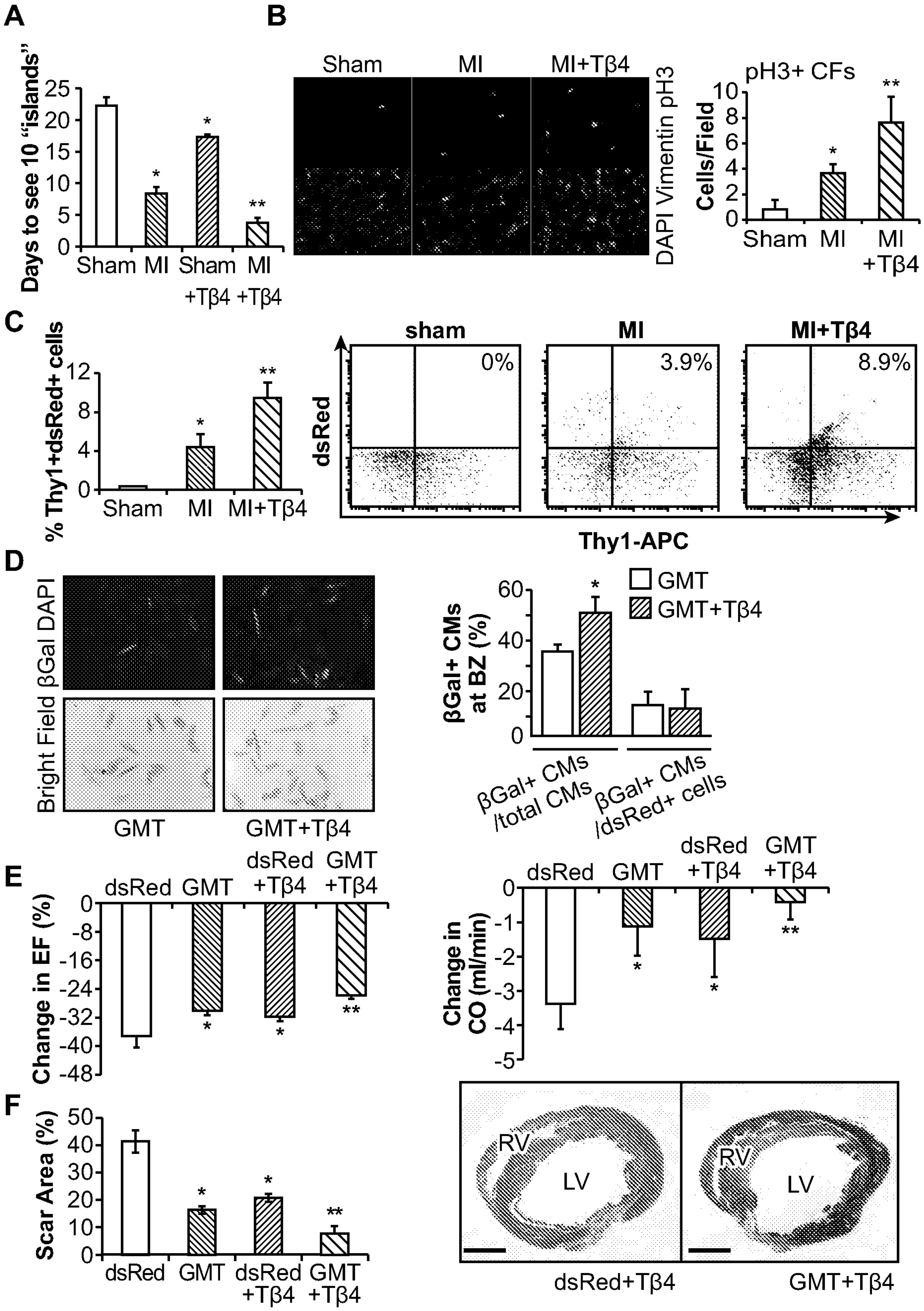
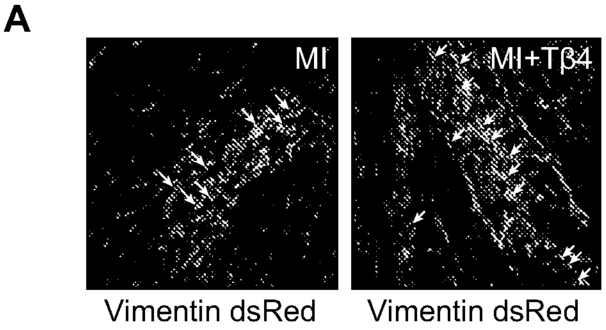

A
Days to see 10 "islands"
25
20
15
10
5
0
Sham
MI
Sham +Tβ4
MI +Tβ4
*
*
**
B
Sham
MI
MI+Tβ4
DAPI Vimentin pH3
pH3+ CFs
Cells/Field
10
8
6
4
2
0
Sham
MI
MI +Tβ4
*
**
C
% Thy1+dsRed+ cells
12
8
4
0
Sham
MI
MI+Tβ4
*
**
sham
MI
MI+Tβ4
0%
3.9%
8.9%
dsRed
Thy1-APC
D
βGal DAPI
Bright Field
GMT
GMT+Tβ4
βGal+ CMs at BZ (%)
60
40
20
0
GMT
GMT+Tβ4
*
βGal+ CMs /total CMs
βGal+ CMs /dsRed+ cells
E
dsRed
GMT
dsRed +Tβ4
GMT +Tβ4
Change in EF (%)
0
-8
-16
-24
-32
-40
-48
*
*
**
Change in CO (ml/min)
0
-1
-2
-3
-4
-5
*
*
**
F
Scar Area (%)
50
40
30
20
10
0
dsRed
GMT
dsRed +Tβ4
GMT +Tβ4
*
*
**
RV
LV
RV
LV
dsRed+Tβ4
GMT+Tβ4

FIG. 12



B

phenotype (%)	I	II	III	IV	n
αActinin	4.26	19.15	17.02	59.57	47
N-Cadherin	3.08	46.15	46.15	4.62	65
Connexin43	9.76	51.22	32.93	6.10	82

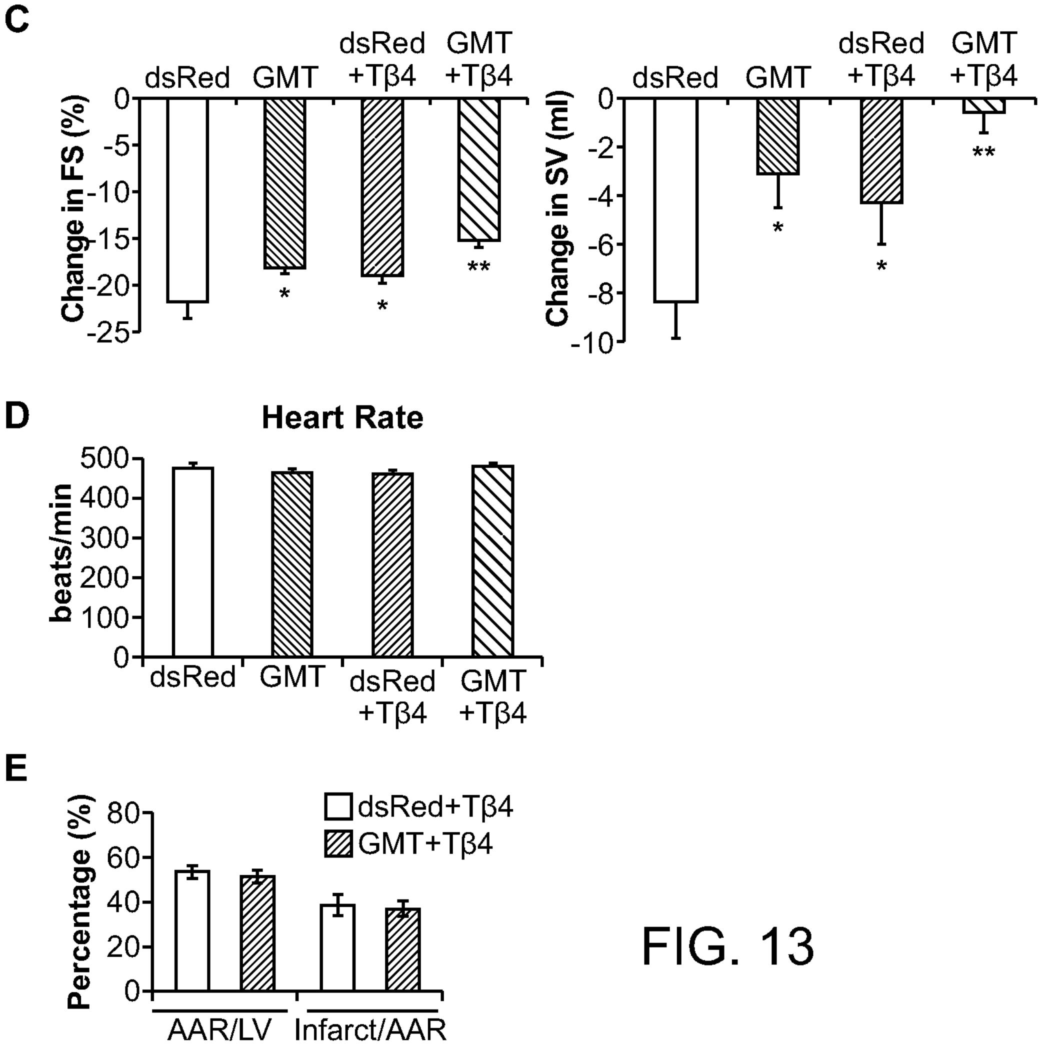


FIG. 13