

CLAIMS

What is claimed is:

1. A vector, comprising a polynucleotide comprising a polynucleotide sequence encoding one or more transgenes and a microRNA binding site for a microRNA, wherein the microRNA binding site is operatively linked to the polynucleotide sequence encoding the one or more transgenes, and wherein the microRNA is expressed at a higher level in cardiomyocytes or cardiomyocyte progenitors compared to cardiac fibroblasts.
2. The vector of claim 1, wherein the microRNA binding site promotes specific repression of expression of the one or more transgenes in a cardiomyocyte or cardiomyocyte progenitor compared to a cardiac fibroblast.
3. The vector of claim 1 or claim 2, wherein the microRNA is expressed at a lower level in cardiac fibroblasts and/or is expressed at a lower level in cardiac fibroblasts treated with the cardiomyocytes reprogramming factor for about 7 days or less, compared to a level of expression of the microRNA in cardiomyocytes and/or a level of expression of the microRNA in cardiac fibroblasts treated with the cardiomyocytes reprogramming factor for more than about 7 days.
4. The vector of any one of claims 1 to 3, wherein the microRNA is miR-208.
5. The vector of any one of claims 1 to 3, wherein the microRNA is miR-1.
6. The vector of any one of claims 1 to 3, wherein the microRNA is miR-133.
7. The vector of claim 4, wherein the microRNA is miR-208a.
8. The vector of claim 4, wherein the microRNA is miR-208b.
9. The vector of claim 8, wherein the microRNA is miR-208b-3p.
10. The vector of claim 9, wherein the microRNA binding site shares >70% identity to ACAAACCTTTTGTTCGTCTTAT (SEQ ID NO: 135) and no mismatches in the underlined seed region comprising the sequence CGTCTTA.

11. The vector of claim 9, wherein the microRNA binding site is
AAAATATATGTAATCGTCTTAA (SEQ ID NO: 136).
12. The vector of claim 9, wherein the microRNA binding site is
ACAAACCTTTTGTTTCGTCTTAT (SEQ ID NO: 135).
13. The vector of claim 9, wherein the microRNA binding site is
TGAAACCTTTTGTTTCGTCTTAT (SEQ ID NO: 137).
14. The vector of any one of claims 1 to 13, wherein the polynucleotide comprises at least two microRNA binding sites for the microRNA.
15. The vector of claim 14, wherein the polynucleotide comprises at least four microRNA binding sites for the microRNA.
16. The vector of claim 14 or claim 15, wherein the polynucleotide comprises at most six microRNA binding sites for the microRNA.
17. The vector of claim 16, wherein the polynucleotide comprises four microRNA binding sites for the microRNA.
18. The vector of any one of claim 1 to 17, wherein the one or more transgenes comprises one or more cardiomyocyte reprogramming factors.
19. The vector of claim 18, wherein the one or more cardiomyocyte reprogramming factors comprises two or more of MYOCD, ASCL1, GATA4, MEF2C, TBX5, miR-133, and MESP1.
20. The vector of claim 18, wherein the one or more cardiomyocyte reprogramming factors comprises three or more of MYOCD, ASCL1, GATA4, MEF2C, TBX5, miR-133, and MESP1.
21. The vector of claim 18, wherein the one or more cardiomyocyte reprogramming factors comprise MYOCD and ASCL1.

22. The vector of claim 21, wherein the polynucleotide sequence encodes a MYOCD-2A-ASCL1 protein.
23. The vector of any one of claims 20 to 22, wherein the MYOCD comprises an internal deletion.
24. The vector of claim 23, wherein the polynucleotide comprises, in 5' to 3' order, a promoter, a sequence encoding MYOCD and ASCL1, the microRNA binding site, and a polyadenylation sequence.
25. The vector of any one of claims 18 to 24, wherein the polynucleotide comprises a sequence encoding miR-133.
26. The vector of claim 24 or claim 25, wherein the polynucleotide comprises a sequence at least 95% identical to SEQ ID NO: 138, SEQ ID NO: 139 or SEQ ID NO: 140.
27. The vector of any one of claims 1 to 26, wherein the vector is a viral vector.
28. The vector of claim 27, wherein the viral vector is an adeno-associated virus (AAV) vector.
29. A method for reprogramming cardiac fibroblasts into cardiomyocytes, comprising:
 - a) selecting a microRNA specifically expressed in induced cardiomyocytes by treating cardiac fibroblasts with an effective amount of a composition that induces reprogramming of cardiac fibroblasts to cardiomyocytes and measuring the expression of one or more microRNAs in the cardiac fibroblasts, wherein the selected microRNA is expressed in the cardiac fibroblasts only after a predetermined time;
 - b) generating a vector comprising a polynucleotide comprising one or more microRNA binding sites for the selected microRNA operatively linked to a polynucleotide encoding one or more cardiomyocyte reprogramming factors; and
 - c) contacting a cardiac fibroblast with an effective amount of the vector.

30. The method of claim 29, wherein the microRNA binding site represses expression of the one or more cardiomyocyte reprogramming factors in cardiomyocyte cells.
31. The method of claim 29 or claim 30, wherein the microRNA binding site represses expression of the one or more cardiomyocyte reprogramming factors in skeletal muscle cells.
32. The method of any one of claims 29 to 31, wherein the microRNA binding site represses expression of the one or more cardiomyocyte reprogramming factors in cardiomyocyte progenitor cells.
33. The method of any one of claims 29 to 32, wherein the microRNA is miR-208.
34. The method of any one of claims 29 to 32, wherein the microRNA is miR-1.
35. The method of any one of claims 29 to 32, wherein the microRNA is miR-133.
36. The method of claim 33, wherein the microRNA is miR-208a.
37. The method of claim 33, wherein the microRNA is miR-208b.
38. The method of claim 37, wherein the microRNA is miR-208b-3p.
39. The method of claim 38, wherein the microRNA binding site shares >70% identity to ACAAACCTTTTGTTCGTCTTAT (SEQ ID NO: 135) and no mismatches in the underlined seed region comprising the sequence CGTCTTA.
40. The method of claim 38, wherein the microRNA binding site is AAAATATATGTAATCGTCTTAA (SEQ ID NO: 136).
41. The method of claim 38, wherein the microRNA binding site is ACAAACCTTTTGTTCGTCTTAT (SEQ ID NO: 135).
42. The method of claim 38, wherein the microRNA binding site is TGAAACCTTTTGTTCGTCTTAT (SEQ ID NO: 137).

43. The method of any one of claims 29 to 42, wherein the polynucleotide comprises at least two microRNA binding sites for the microRNA.
44. The method of claim 43, wherein the polynucleotide comprises at least four microRNA binding sites for the microRNA.
45. The method of claim 43, wherein the polynucleotide comprises at most six microRNA binding sites for the microRNA.
46. The method of claim 43, wherein the polynucleotide comprises four microRNA binding sites for the microRNA.
47. A method for reprogramming a cardiac fibroblast into a cardiomyocyte cell, comprises contacting the cardiac fibroblast with an effective amount of the vector of any one of claims 1 to 28.
48. The method of claim 47, wherein the method induces expression of at least one marker of cardiomyocyte phenotype in the cardiac fibroblast.
49. The method of claim 48, where at least one marker of cardiomyocyte phenotype is a messenger RNA level of ASCL1, MYOCD, CASQ2, NPPA, or TNNT2
50. A method of promoting formation of cardiomyocytes in a subject in need thereof, comprising administering the vector of any one of claims 1 to 28 to the subject.
51. A method of treating heart failure in a subject in need thereof, comprising administering the vector of any one of claims 1 to 28 to the subject.
52. A method of treating heart failure in a subject in need thereof, comprising administering to the subject an AAV vector comprising a polynucleotide comprising in 5' to 3' order, a promoter, a sequence encoding MYOCD and ASCL1, a microRNA binding site, and a polyadenylation sequence, wherein the microRNA binding site is a microRNA binding site for miR-1, miR-133, miR-208a, miR-208b, and/or miR-208b-3p.

53. The method of claim 52, wherein the microRNA binding site is a microRNA binding site for miR-1.
54. The method of claim 52, wherein the microRNA binding site is a microRNA binding site for miR-133.
55. The method of claim 52, wherein the microRNA binding site is a microRNA binding site for miR-208a.
56. The method of claim 52, wherein the microRNA binding site is a microRNA binding site for miR-208b.
57. The method of claim 52, wherein the microRNA binding site is a microRNA binding site for miR-208b-3p.
58. The method of any one of claims 52 to 57, wherein the polynucleotide comprises a sequence encoding miR-133.
59. The method of any one of claim 52 to 58, wherein the heart failure is due to myocardial infarction.
60. The method of any one of claim 52 to 59, wherein the heart failure is heart failure with reduced ejection fraction (HFrEF).
61. The method of any one of claim 52 to 60, wherein the method increases ejection fraction in the subject compared to the subject before administration.
62. The method of any one of claim 52 to 61, wherein the method increases ejection fraction in the subject compared to an untreated control subject.
63. The method of any one of claim 52 to 62, wherein the method increases ejection fraction in the subject to at least about 28%, 29%, 30%, 31%, or 32%.
64. The method of any one of claim 61 to 64, wherein ejection fraction is assessed after a predetermined time, optionally eight weeks after administration of the AAV vector.

65. The method of any one of claim 52 to 64, wherein the method decreases scar tissue formation in the subject compared to the subject before administration.
66. The method of any one of claim 52 to 65, wherein the method decreases scar tissue formation in the subject compared to an untreated control subject.
67. The method of any one of claim 65 or claim 66, wherein the method decreases scar tissue formation in the subject to at most about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20%.
68. The method of any one of claim 65 to 67, wherein scar tissue formation is assessed after a predetermined time, optionally eight weeks after administration of the AAV vector.