

CLAIMS

1. A method of producing a mature cardiomyocyte from an immature cardiomyocyte comprising contacting the immature cardiomyocyte with at least one cardiomyocyte maturation factor in three-dimensional culture.
2. The method of claim 1, wherein the at least one cardiomyocyte maturation factor comprises a p53 upregulator.
3. The method of claim 1, wherein the at least one cardiomyocyte maturation factor is selected from the group consisting of a senolytic, a MDM2 inhibitor, an mTOR inhibitor, and combinations thereof.
4. The method of claim 1, wherein the at least one cardiomyocyte maturation factor is selected from the group consisting of nutlin-3a, quercetin, Torin1, and combinations thereof.
5. The method of claim 1, wherein the at least one cardiomyocyte maturation factor is selected from the group consisting of nutlin-3a, quercetin, and combinations thereof.
6. The method of claim 1, wherein the at least one cardiomyocyte maturation factor comprises nutlin-3a.
7. The method of claim 1, wherein the at least one cardiomyocyte maturation factor comprises quercetin.
8. The method of claim 1, wherein the at least one cardiomyocyte maturation factor is contacted with the immature cardiomyocyte via pulse treatment.
9. The method of claim 1, wherein the at least one cardiomyocyte maturation factor is contacted with the immature cardiomyocyte via continuous treatment.
10. The method of claim 1, wherein the immature cardiomyocyte is derived from an iPS cell.
11. The method of claim 1, wherein the immature cardiomyocyte is derived from an ES cell.
12. The method of claim 1, wherein the immature cardiomyocyte is derived from a T cell.

13. The method of claim 1, wherein the immature cardiomyocyte is derived from a fibroblast.
14. The method of claim 1, wherein the immature cardiomyocyte resembles a fetal cardiomyocyte.
15. The method of claim 1, wherein the immature cardiomyocyte is contacted with the at least one cardiomyocyte maturation factor after the immature cardiomyocyte begins beating.
16. The method of claim 1, wherein the immature cardiomyocyte is contacted with the at least one cardiomyocyte maturation factor 1 to 3 days after the immature cardiomyocyte begins beating.
17. The method of claim 1, wherein the mature cardiomyocyte exhibits increased expression of one or more genes of maturation as compared to an immature cardiomyocyte, optionally wherein the one or more genes of maturation are selected from the group consisting of: *TNNI3*, *TNNT2*, *MYH6*, *MYH7*, *NPPB*, *HCN4*, *CACNA1c*, *SERCA2a*, *PPARGC1*, *KCNJ2*, *REST*, *RyR*, and *SCN5a*.
18. The method of claim 1, wherein the mature cardiomyocyte exhibits increased expression of one or more sarcomeric proteins as compared to an immature cardiomyocyte, optionally wherein the one or more sarcomeric proteins are selected from the group consisting of: *TNNT2*, *TNNI3*, *MYH6*, and *MYH7*.
19. The method of claim 1, wherein the mature cardiomyocyte exhibits increased expression of one or more ion channel genes as compared to an immature cardiomyocyte, optionally wherein the one or more ion channel genes are selected from the group consisting of: *KCNJ2*, *HCN4*, *SCN5a*, *RyR2*, *CACNA1c*, and *SERCA2a*.
20. The method of claim 1, wherein one or more of the following apply:
 - (a) the mature cardiomyocyte exhibits increased expression of *REST* and/or *GATA4* as compared to an immature cardiomyocyte;
 - (b) the mature cardiomyocyte exhibits a decreased beating rate as compared to an immature cardiomyocyte;

- (c) the mature cardiomyocyte exhibits a resting membrane potential of ≤ -70 mV;
 - (d) the mature cardiomyocyte exhibits a spontaneous beating rate of < 40 beats per minute;
 - (e) the mature cardiomyocyte exhibits an upstroke velocity of > 200 V/sec;
 - (f) the mature cardiomyocyte is an electrically mature cardiomyocyte;
 - (g) the mature cardiomyocyte is a contractility mature cardiomyocyte;
 - (h) the mature cardiomyocyte is a metabolically mature cardiomyocyte.
21. The method of claim 1, wherein the mature cardiomyocyte is an electrically, contractility, and metabolically mature cardiomyocyte.
 22. A non-naturally occurring cardiomyocyte produced by the methods of any one of claims 1-16.
 23. The non-naturally occurring cardiomyocyte of claim 22, wherein the non-naturally occurring cardiomyocyte exhibits increased expression of one or more genes of maturation as compared to an immature cardiomyocyte, optionally wherein the one or more genes of maturation are selected from the group consisting of: TNNT2, TNNI3, MYH6, MYH7, NPPB, HCN4, CACNA1c, SERCA2a, PPARGC1, KCNJ2, REST, RyR, and SCN5a.
 24. The non-naturally occurring cardiomyocyte of claim 22, wherein the non-naturally occurring cardiomyocyte exhibits increased expression of one or more sarcomeric proteins as compared to an immature cardiomyocyte, optionally wherein the one or more sarcomeric proteins are selected from the group consisting of: TNNT2, TNNI3, MYH6, and MYH7.
 25. The non-naturally occurring cardiomyocyte of claim 22, wherein the non-naturally occurring cardiomyocyte exhibits increased expression of one or more ion channel genes as compared to an immature cardiomyocyte, optionally wherein the one or more ion channel genes are selected from the group consisting of: KCNJ2, HCN4, SCN5a, RYR2, CACNA1c, and SERCA2a.

26. The non-naturally occurring cardiomyocyte of claim 22, wherein one or more of the following apply:
 - (a) the non-naturally occurring cardiomyocyte exhibits increased expression of one or more of TNNT2, TNNI3, KCNJ2, and p53;
 - (b) the non-naturally occurring cardiomyocyte exhibits a decreased beating rate as compared to an immature cardiomyocyte;
 - (c) the non-naturally occurring cardiomyocyte exhibits a resting membrane potential of ≤ -70 mV;
 - (d) the non-naturally occurring cardiomyocyte exhibits a spontaneous beating rate of < 40 beats per minute;
 - (e) the non-naturally occurring cardiomyocyte exhibits an upstroke velocity of > 200 V/sec;
 - (f) the non-naturally occurring cardiomyocyte is an electrically mature cardiomyocyte;
 - (g) the non-naturally occurring cardiomyocyte is a contractility mature cardiomyocyte; and
 - (h) the non-naturally occurring cardiomyocyte is a metabolically mature cardiomyocyte.
27. The non-naturally occurring cardiomyocyte of claim 22, wherein the non-naturally occurring cardiomyocyte is an electrically, contractility, and metabolically mature cardiomyocyte.
28. Use of a composition in the manufacture of a medicament for treatment of a heart condition, wherein the composition comprises at least one non-naturally occurring cardiomyocyte according to any one of claims 22 to 27.
29. The use of claim 28, wherein the subject has, or is at risk of developing, a ventricular arrhythmia, decreased systolic heart function, chronic heart failure, congenital heart disease, or other heart disease.
30. A method of inducing quiescence of a cardiomyocyte comprising contacting a senescent cardiomyocyte with a cardiomyocyte maturation factor in three-dimensional

culture, thereby inducing the senescent cardiomyocyte to transition into a quiescent cardiomyocyte.

31. The method of claim 30, wherein the cardiomyocyte maturation factor comprises one or more of: an upregulator of p53, an mTOR signaling pathway inhibitor, a senolytic, a MDM2 inhibitor, and combinations thereof.
32. The method of claim 30, wherein the cardiomyocyte maturation factor comprises an upregulator of p53 and an mTOR inhibitor.
33. The method of claim 30, wherein the cardiomyocyte maturation factor is selected from the group consisting of Torin1, nutlin-3a, quercetin, and combinations thereof.
34. The method of claim 33, wherein the cardiomyocyte maturation factor is selected from:
 - (a) Torin1;
 - (b) a combination of Torin1 and nutlin-3a;
 - (c) nutlin-3a;
 - (d) quercetin;
 - (e) a combination of nutlin-3a and quercetin.
35. The method of claim 30, wherein the cardiomyocyte maturation factor is not Torin1.
36. A method of producing a mature cardiomyocyte from an immature cardiomyocyte comprising contacting the immature cardiomyocyte with at least one cardiomyocyte maturation factor selected from the group consisting of nutlin-3a, quercetin, Torin1, and combinations thereof.
37. The method of claim 36, wherein one or more of the following apply:
 - (a) the cardiomyocyte maturation factor is selected from the group consisting of nutlin-3a, quercetin, and combinations thereof;
 - (b) the mature cardiomyocytes are produced in three-dimensional culture or the mature cardiomyocytes are produced in two-dimensional culture;

- (c) the immature cardiomyocytes are contacted with the at least one cardiomyocyte maturation factor via a pulse treatment or are contacted with the at least one cardiomyocyte maturation factor via a continuous treatment.